Recent Advances in the Medicinal Chemistry of α-Aminoboronic Acids, Amine-Carboxyboranes and Their Derivatives

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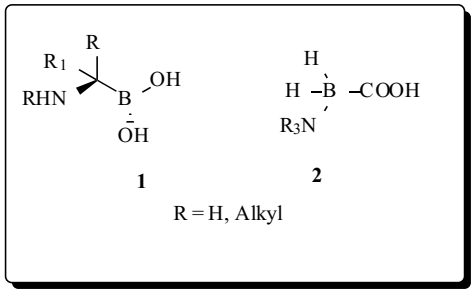
Abstract: This article describes recent developments in the synthesis and biological activity of α-aminoboronic acids, amine-carboxyboranes and their derivatives as potential therapeutic agents. α-Amino acid analogues are of considerable interest as inhibitors of enzymes involved in amino acid and peptide metabolism. In particular, α-amino alkylphosphonic acids and α-amino alkylboronic acids, in which the carboxyl group of amino acids is replaced by a phosphonic acid or boronic acid function, respectively, constitute a unique class of amino acid mimics from which a number of potent enzyme inhibitors have been synthesized. The inhibitory activity mainly stems from the fact that the tetrahedral phosphonic moiety or the tetrahedral adduct of electrophilic boronic acid is a good mimic of the putative tetrahedral transition state or intermediate encountered in the enzymatic hydrolysis or formation of peptides. Since the peptide hydrolysis and formation invariably involves the tetrahedral high energy species in the course of the reaction, these amino acid mimics serve as a general key element for inhibitors of a broad spectrum of proteases and peptide ligases. Serine protease inhibitors provide promising compounds having a P site binding moiety and a boronic acid chelating moiety. The compounds have been shown to have high inhibitory activity.

INTRODUCTION

In recent years there has been an increasing interest for new practical methods to prepare novel synthetic *D*-amino acid derivatives to serve as building blocks in combinatorial chemistry and drug discovery. As non-natural *D*-amino acid derivatives, boronic acids **1** and **2** have assumed great importance since they serve as transition state analogue of natural amino acids [1-4].

The bioorganic chemistry of boron-containing compounds is therefore an area of growing interest and has recently expanded to include purine nucleosides, psuedocryptands (which mimic the naturally occurring antibiotics boromycin and aplasmomycin), steroids, calixarenes, carbohydrates, fatty acids, porphyrins, and amino acids [1,5,6]. Boronic acids [$RB(OH)_2$] and boronate esters [$RB(OR_1)_2$] have been found to facilitate the transport of various ribonucleosides in and out of liposomes, an important attribute in the area of drug design [5,6]. The dipeptide boronic acid analogues are a potent and selective proteasome inhibitor in clinical trials for a variety of tumor types. In vitro and in vivo (murine xenograft) studies show that, for example, dipeptide boronic acid analogue PS-341 has activity against a variety of malignancies, including myeloma, chronic lymphocytic leukemia, prostate cancer, pancreatic cancer, breast cancer and colon cancer [2].

Simple aminoboron compounds have also found some utility in boron neutron capture therapy (BNCT) [7,8] and other forms of cancer therapy [2]. As a result, much effort has focused on the biological activity of boron-containing amino acid and peptide derivatives [1,9].



1. Serine Protease Inhibitors

Thrombin is a serine protease involved in blood coagulation and haemostasis. Like all serine proteases, it hydrolyses peptides and synthetic esters. In the first step of these hydrolytic reactions, there is a nucleophilic attack by the Oγ of the enzyme's catalytic serine residue on an electrophilic center of the substrate to form a tetrahedral intermediate. The enzyme/substrate complex collapses to the acyl intermediate. In turn, the ester bond undergoes nucleophilic attack by a water molecule activated by its interaction with His57 of the catalytic triad. This leads to the formation of another tetrahedral intermediate between the enzyme and the substrate, which collapses to give the carboxylate product and to regenerate the active enzyme [10]. α-Amino boronic acids were proposed by G.E. Leinhard as potential serine protease inhibitors which could bind these enzymes into configurations resembling their normal transition states. Serine proteases, a large and functionally diverse class of proteolytic enzymes, are prominent therapeutic targets because of their involvement in a host of physiological processes [11]. Most small molecule inhibitors of these enzymes form covalent adducts with the active site serine that mimic to some degree these tetrahedral intermediates. Peptide derivatives with electron-deficient ketones and aldehydes, boronic acids and phosphonylating

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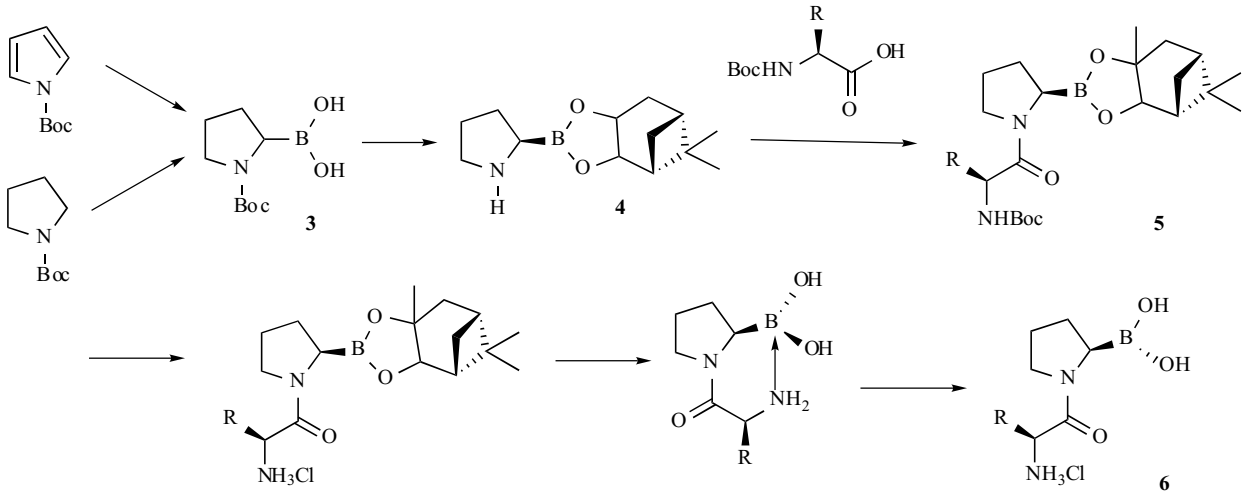
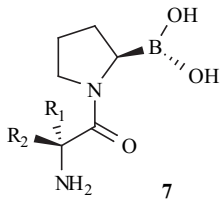


Scheme 1.

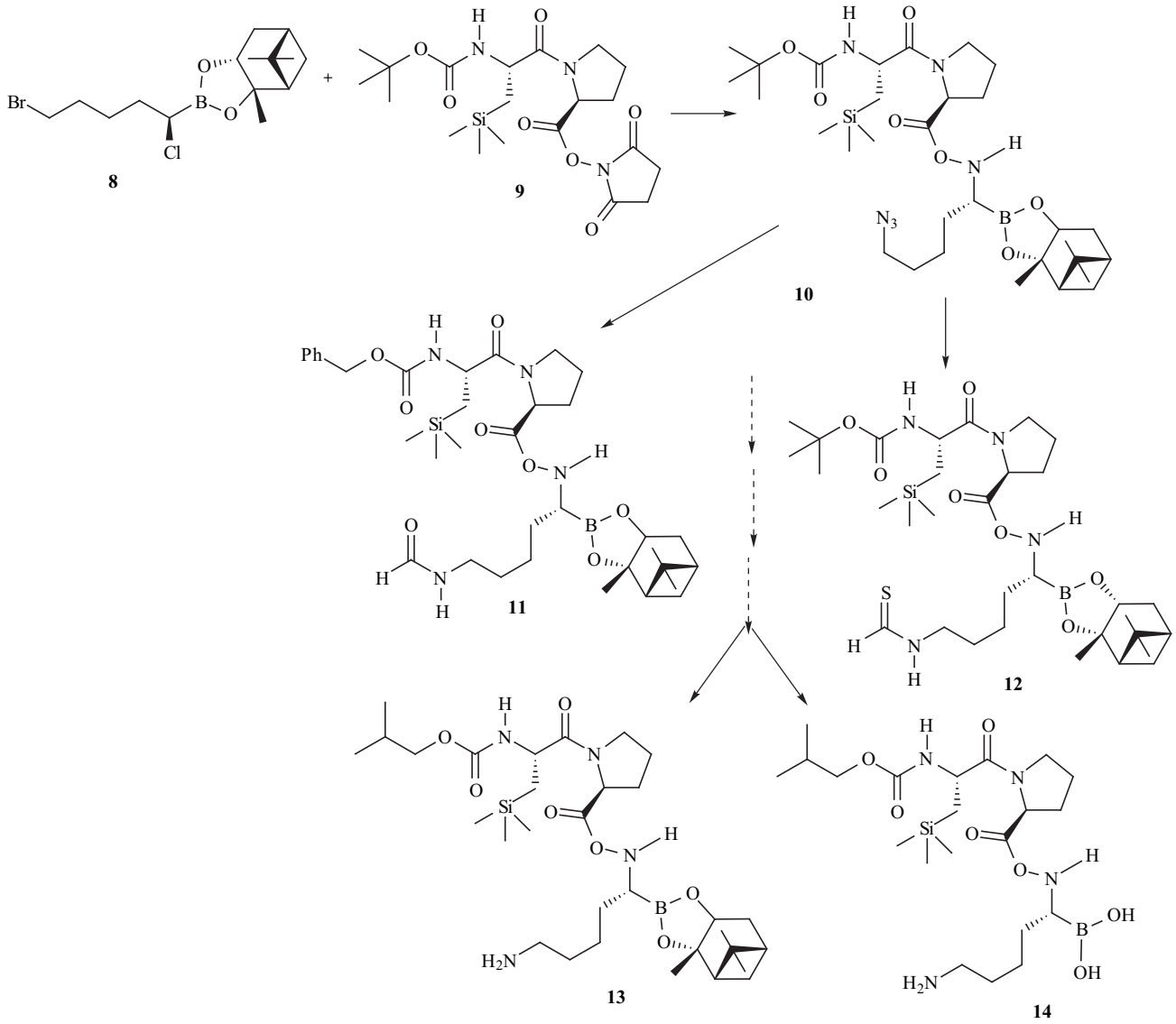
agents have been devised as analogues of the second tetrahedral intermediate [12] with their selectivity among the various proteases related to the substrate specificity these enzymes manifest at the S_1, S_2, and higher, binding sites [13].

Table 1. Structure-Activity Relationships for H_2N-X_{aa}-boroPro Dipeptides vs DPPIV



Compound	Amino acid	R_1	R_2	Boron configuration	IC_{50} nM	±SE nM
7a	L-Val	H	*i*-Pr	*R*	26	1
7b	L-Val	H	*i*-Pr	*S*	4,000	600
7c	D-Val	*i*-Pr	H	*R*	116,000	15,000
7d	L-Ala	H	Me	*R*	15	3
7e	AiBu	Me	Me	*R*	30,000	8,000
7f	L-Gly	H	H	*R*	16,000	2,400
7g	L-Abu	H	Et	*R*	11	1
7h	L-Leu	H	*i*-Bu	*R*	44	2
7i	L-Ile	H	2-Bu	*R*	25	1
7j	L-tLeu	H	*t*-Bu	*R*	60	**7**
7k	L-Phe	H	CH_2Ph	*R*	**7**0	**7**
7l	L-Phg	H	Ph	*R*	63	5
7m	L-Tyr	H	CH_2(Ph-4-OH)*	*R*	32	1
7n	L-Lys	H	$(CH_2)_4NH_2$	*R*	95	19
7o	L-Thr	H	CH_3CHOH	*R*	190	13
7p	L-Pro	H	-$(CH_2)_3$-	*R*	20	5
7q	L-Azet	H	-$(CH_2)_2$-	*R*	250	13
7r	L-His	H	CH_2Im	*R*	17,000	1,800

*$CH_2C_6H_4OH$-4

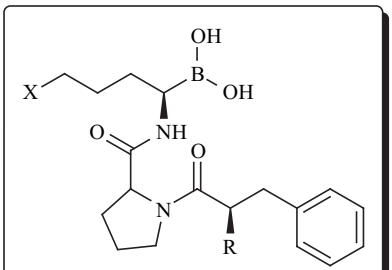


Scheme 2.

A series of prolineboronic acid (boroPro)-containing dipeptides was synthesised and assayed for their ability to inhibit the serine protease dipeptidyl peptidase IV (DPPIV) [14]. The synthesis of boroproline **3** was developed by Matteson's procedure [15,16] for the preparation of aminoboronic acids. The aminoboronic ester **4** was coupled with the desired Boc-amino acids in the presence of 1-(3-(dimethylamino)propyl)-3-ethyl-carbodiimide hydrochloride (EDC) to generate the fully-protected dipeptides **5**, and with HCl gave proline α−aminoboronic acids **6** via two intermediates (Scheme **1**). The abilities of the peptides to inhibit the enzyme [14] are shown in (Table **1**).

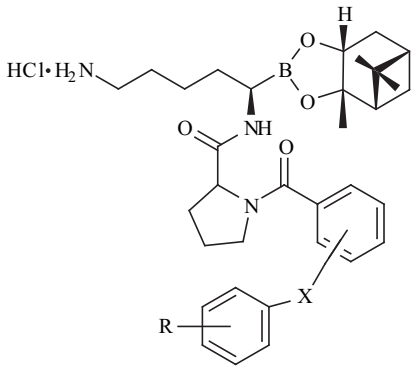
A series of Boc-D-trimethylsilylalanine-proline-boro-X pinanediol derivatives **11**-**14** which are active as thrombin inhibitors have been synthesised by Matteson *et al.* [17] (Scheme **2**). All of the thrombin inhibitors [18] were synthesised starting from the common intermediate **10** obtained from the reaction between **8** and **9**. The thioformamide **12** could be obtained by treatment of the corresponding formamide with Lawesson's reagent. Removal of the Boc protecting group followed by reaction with benzyl chloroformate led to **11**. Hydrolysis of **13** generated **14**. The X-ray structures of **11** and **14** have shown that these inhibitors bound to the active side of thrombin.

The synthesis of the thrombin inhibitor Du Pont 714 was achieved starting from 3-bromopropylboronic ester to form **15** [19]. This enzyme inhibitor was active in rabbits at dose levels of 0.1 mg kg^{-1} h^{-1} [20,21]. A methoxy group in place of guanidine on **15** also provides a potent thrombin inhibitor [19] (Fig. **1**).



15. Du Pont 714 = R = NHAc, X = NHC(NH)NH_2 HCl
16. R = NHAc, X = CH_2NH_2-HCl
17. R = H, X = CH_2NH_2-HCl

Table 2. Boropeptide Thrombin Inhibitors with Benzoic Acid-derived Residues



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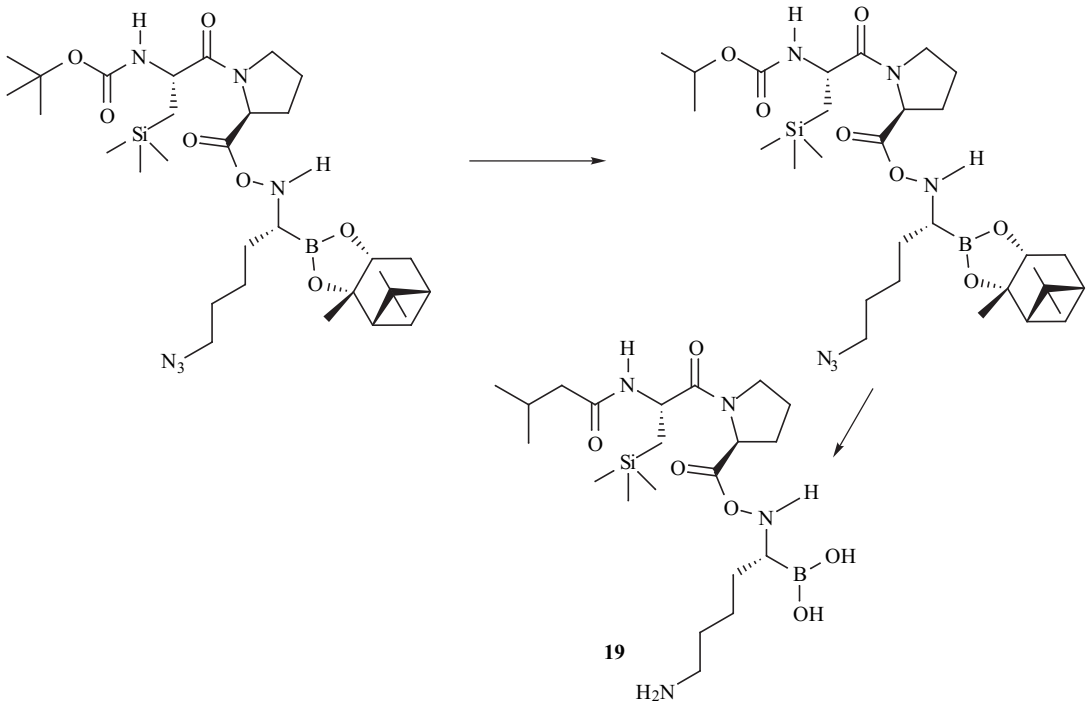
Compound	X	R	Position	K_inM
18a	CH_2	H	*ortho*	0.29
18b	CH_2	H	*meta*	0.19
18c	CH_2	H	*para*	1.80
18d	O	H	*ortho*	0.27
18e	O	H	*meta*	0.36
18f	CH_2	2-CF_3	*meta*	0.07
18g	CH_2	2-CH_3	*meta*	0.25
18h	CH_2	2-SCH_3	*meta*	0.50
18i	CH_2	2-Br	*meta*	0.23
18j	CH_2	3-F	*meta*	0.43
18k	CH_2	3-CF_3	*meta*	0.16
18l	CH_2	4-CF_3	*meta*	0.22
18m	CH_2	3,4-(-OCH_2O-)	*meta*	0.09
18n	S	H	*meta*	< 0.10
18o	S	2-CF_3	*meta*	0.45
18p	S	2-OCH_3	*meta*	0.19
18q	S	4-OCH_3	*meta*	0.42
18r	SO_2	H	*meta*	0.85
18s	SO_2	2-OCH_3	*meta*	0.58

A series of conformationally-restricted boropeptide thrombin inhibitors **16** and **17** has been synthesised [22]. The potent binding affinity of the resulting inhibitors **18**, such as **18**f, may be due in part to a unique mode of binding at the thrombin active site. The thrombin binding activity data for a series of inhibitors is shown in (Table **2**). The synthesis of these inhibitors involved the initial preparation of the P3 benzoic acids followed by elaboration to their corresponding boropeptides [22].

Thrombin, as the final serine protease in the blood coagulation cascade, is a promising target for the development of an anticoagulant agent. Therefore, there is a great deal of interest in the development of thrombin inhibitors. Boronic acid compounds were found to exhibit potent inhibition activities. Recently, through the examination of the X-ray crystal structure of boropeptide **11** bound to thrombin, it was found that the 3-phenylpropionyl chain attached to the proline residue forms a favorable edge-to-face interaction with the Trp-215 side chain located at the base of the S3 specificity pocket of thrombin. The X-ray crystal structures of **11** and **19** which bind human α-thrombin in a manner analogous to **14** and **15** are shown in (Fig. **1**). There are two principal classes of low molecular weight active site directed thrombin inhibitors: the first consists of inhibitors which do not form a covalent bond to the catalytic Ser 195 but bind non-covalently through hydrogen-bonding, hydrophobic, and electrostatic interactions within the S1-S3 specificity pockets. The second class of inhibitors form a covalent bond between the nucleophilic Ser 195 of the enzyme and an electrophilic center on the inhibitor. Many of these thrombin inhibitors have a basic P1 side-chain in order to form a salt bridge to Asp 189 at the bottom of the S1 pocket and thereby increase the inhibitory affinity for thrombin. However, it has been shown that a basic P1 side-chain is not a necessary prerequisite to obtain active thrombin inhibitors [17].



Scheme 3.

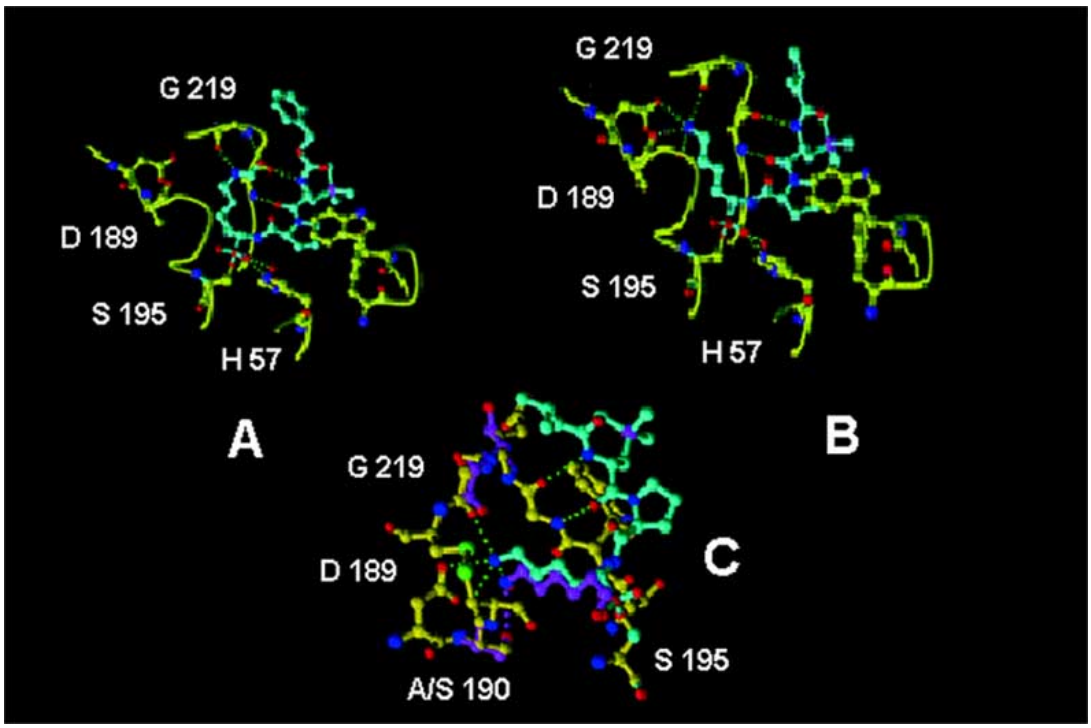


Fig. (1). Crystal structures of human α-thrombin tied with the boronic acid inhibitors **14** and **15**. **A**, the complex with the inhibitor **15**. B, the complex with the inhibitor **14**. **C**, Structures of thrombin and trypsin tied with inhibitor **14** and mung bean inhibitor, respectively [17]. Adapted by authors.

Interaction of boronic acids **15**, **16**, and **18f** with Asp189 is shown in (Fig. **2**). The direct interaction of boroarginine side chains with Asp189 depicted in **15** is in contrast to the weaker interaction of borolysine side chains generally observed which occurs through a bridging molecule of water. The P1 region of compounds **18f** showed the borolysine side chain extends deeper into the S1 specificity pocket than has been observed for other borolysine inhibitors [22].

The synthesis of the pinanediol ester of prolineboronic acid was described by Kelly *et al.* (Scheme **4**) [23]. Boc-pyrrole, after treatment with tetramethylpiperidine gave boc-pyrrole-2-boronic acid **20**. Hydrogeno-lysis generated Boc-prolineboronic acid **22**, which was easily esterified with (1*S*,2*S*,3*R*,5*S*)-(+)-pinanediol to give **22** which was deprotected by HCl in EtOAc to form two diastereomers **23a** and **23b**.

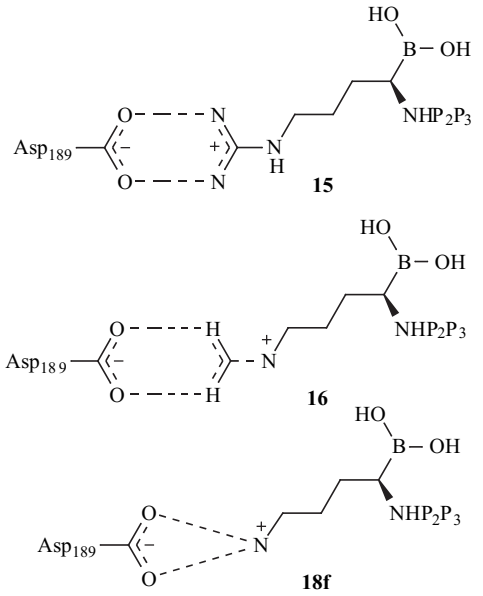


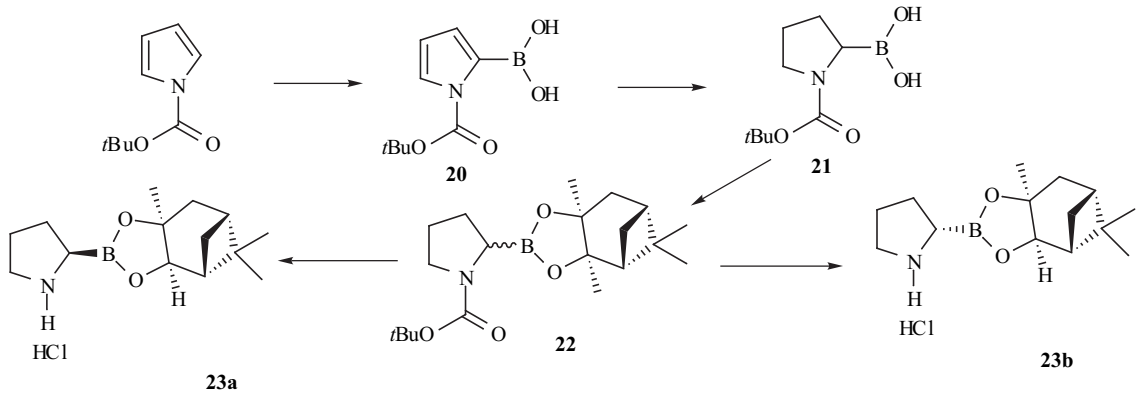
Fig. (2). Cartoon rendition of the P1 binding modes of Du Pont **15**, **16**, and **18f**. Dashed lines indicated hydrogen bonds and charge-charge interaction [22].

boronic acid analogues **24-26** of the *m*-cyanoborophenylalanine analogue **27** [24,25] (Scheme **5**). The free boronic acid **27** was isolated from the aqueous phase as a single component. The interaction of trypsin with a series of inhibitors **28** is shown in (Table **3**).

Potent and selective dipeptidyl boronic acid inhibitors have been demonstrated by Adams *et al.* [24]. The synthesis of the boronated dipeptide **29** was described in US Patent [26] (Scheme **6**). It was found that the boronic acid derivative **30** has inhibitor properties as indicated in (Table **4**).

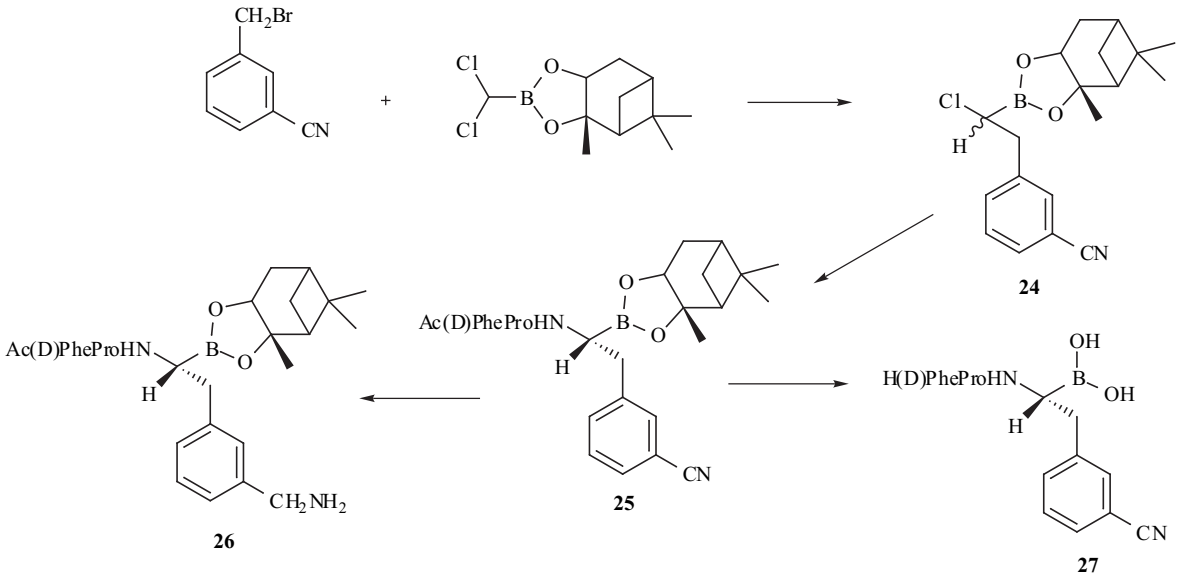
The dipeptidyl boronic acid **30** (bortezomib, Velcade(TM) Millennium Pharmaceuticals, Inc., formerly known as PS-341, LDP-341 and MLN-341), is a potent and specific inhibitor of the proteasome that holds promise as a potential human therapeutic. It is the first proteasome inhibitor to be examined in human clinical trials and according to preliminary Phase I and II data, the drug has exhibited both manageable toxicities and biological activity [25b-c].

Peptides such as Du Pont 714 **15**, containing boroarginine at the primary residue are potent thrombin inhibitors [27]. The synthesis of boropeptide, arginine α-



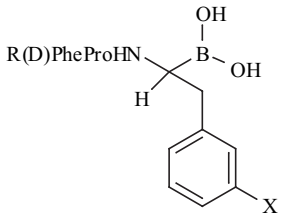
Scheme 4.

Novel highly effective thrombin inhibitors, alanine α-aminoboronic acids, have been obtained by preparing aminoboronic acid, **31** containing a basic α-aminoboronic ester started from the dichloromethylboronic ester with the



Scheme 5.

Table 3. Binding of Peptide Boronic Acids to Serine Proteases



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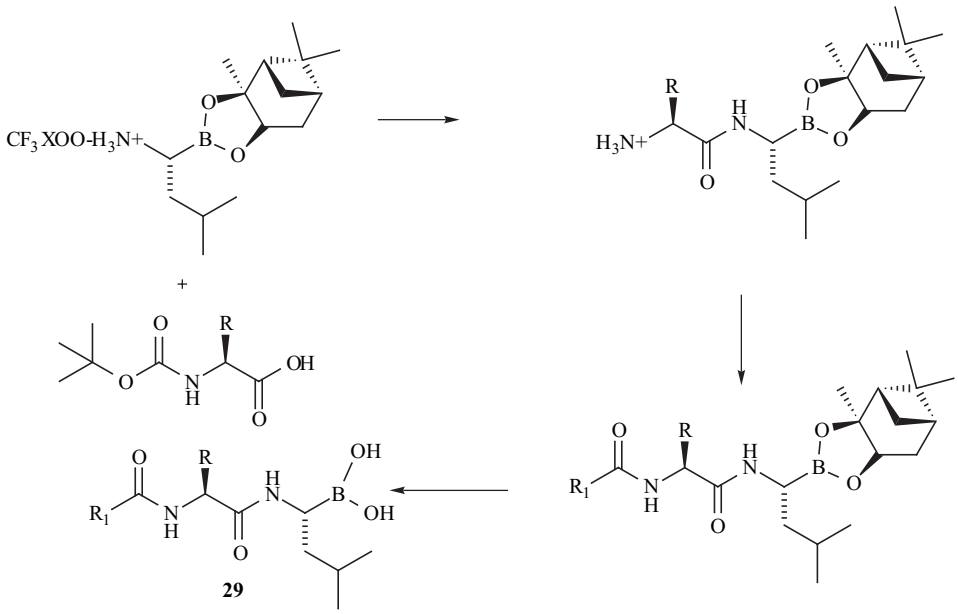
Protease	R	X X_1	or X_2	K_i for X_1 nM	K_i for X_2 nM
thrombin	Ac	CN	H	0.79	320
thrombin	Boc	CN	H	0.51	59
thrombin	H	CN	H	0.48	6.3
thrombin	Ac	CH_2NH_2		4.8	
trypsin	Ac	CN	H	130	11,000
trypsin	Boc	CN	H	73	5400
trypsin	H	CN	H	5.6	1800
trypsin	Ac	CH_2NH_2		5.6	
pancreatic	Ac	CN	H	8,600	39,000
chymotrypsin	Ac	CN	H	49	100

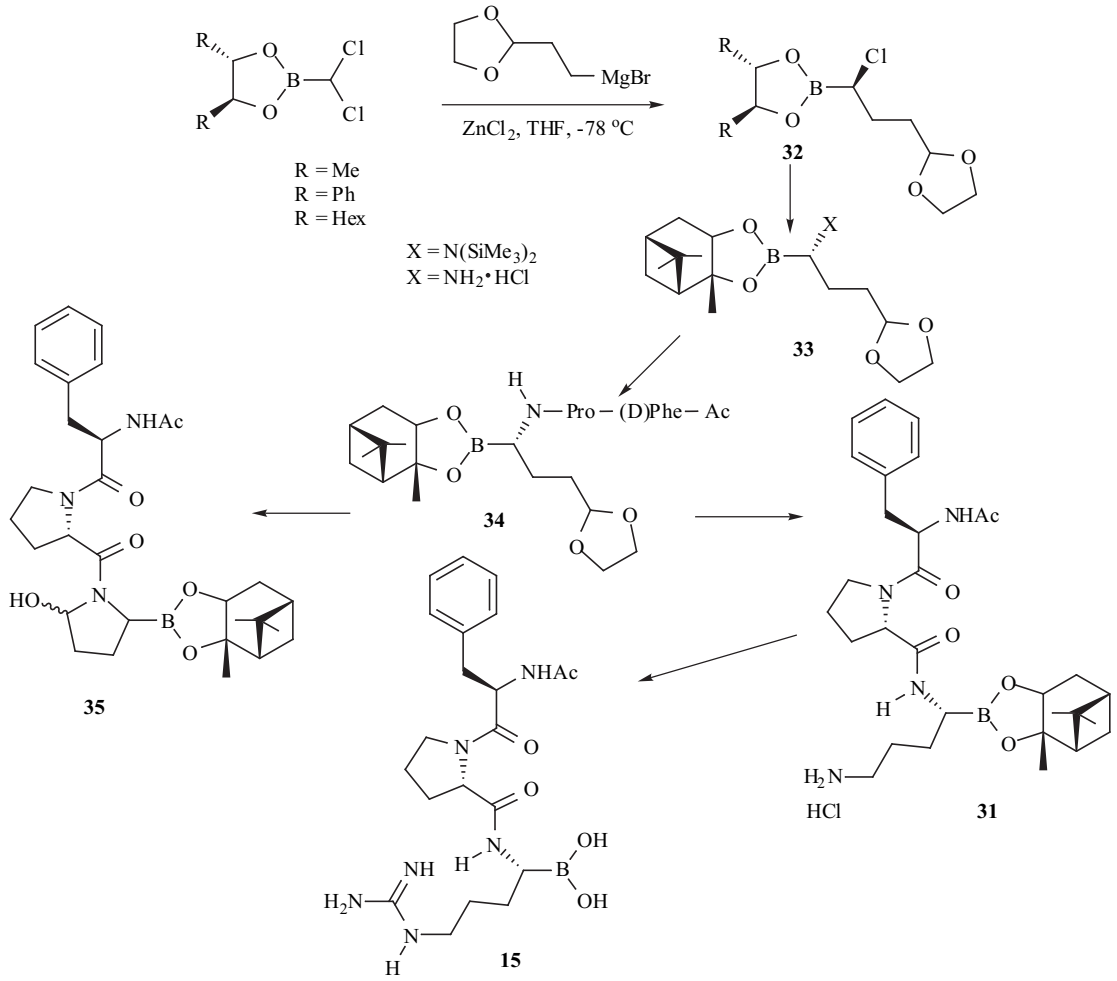
Grignard addition to give **32**. Reaction with (+)-pinanediol then provided the boronic ester **33**. The boropeptide **34** was obtained in 80% yield and it was transformed to **35** and **31** [28]. Du Pont 714 **62** obtained from **15** was stable in water (Scheme **7**).

Some novel arginine α−aminoboronic acids **36**–**39** which act as serine protease inhibitors have been synthesised [29] (Scheme **8**). The methodology affords α-aminoboronic acids **36**–**39** with the general formula R-NHCH(R)BO_2-pinanediol where R = CH_2CHF_2, CH_2CO_2tBu, and $(CH_2)_2COMe$.

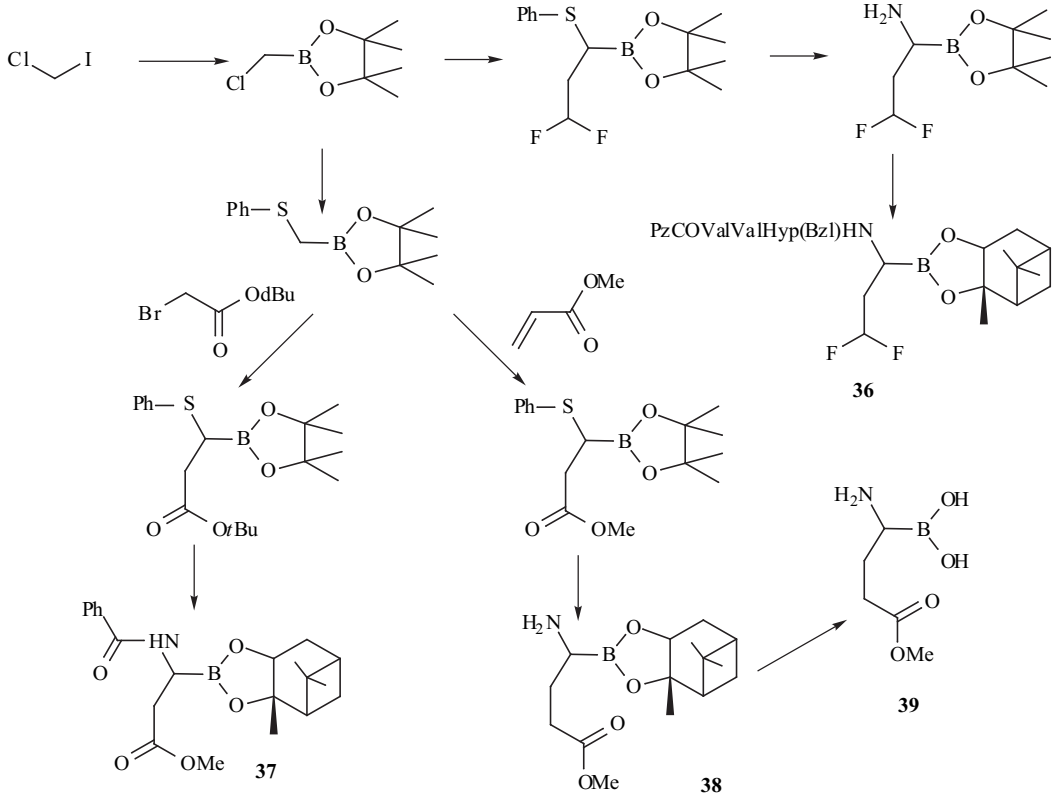
N-acetylphenylalanine, (*R*)-1-acetamido-2-phenylethane-1-boronic acid, **40**, a potential serine protease inhibitor has been synthesized from (+)-pinanediol phenylmethane-1-boronate (Scheme **9**) [30].



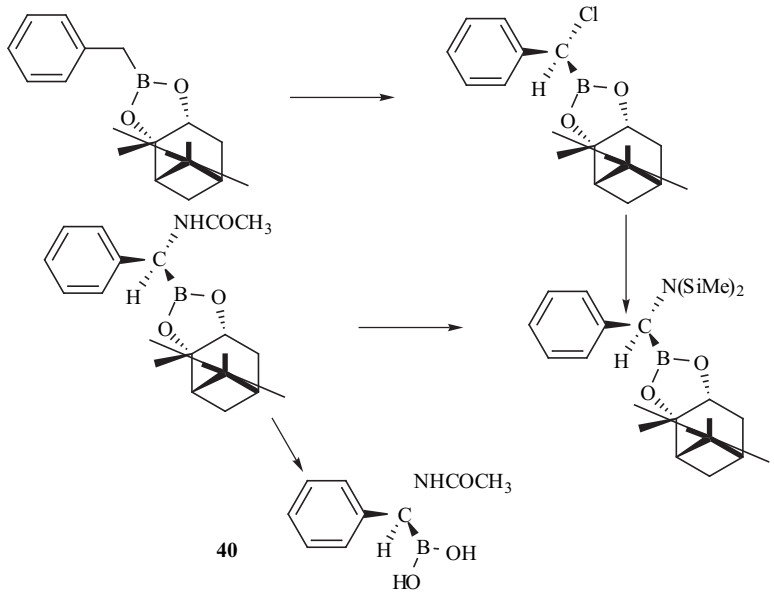
Scheme 6.



Scheme 7.

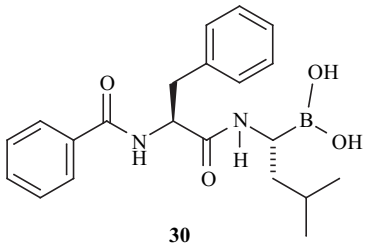


Scheme 8.



Scheme 9.

Table 4. Enzyme Inhibitory Profile of the Peptide Boronic Acid Derivative 30



30

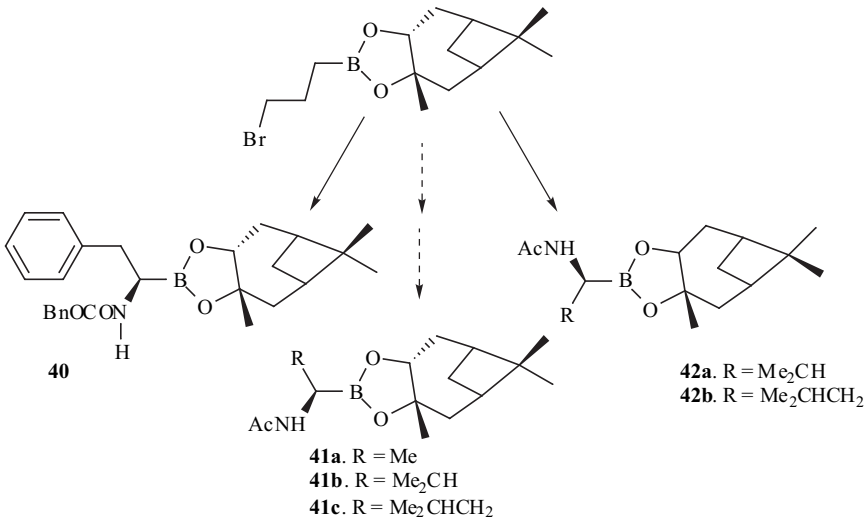
Enzyme	Ki nM
20S proteasome	0.62
human leukocyte elastase	2,300
human cathepsin G	630
human chymotrypsin	320
thrombin	13,000

2. α-Aminoboronic Acids as Potential Inhibitors Other Enzymes

Enzyme inhibition studies have shown that the D-amino acid analogue **42**b was an active inhibitor of *Bacillus cereus* β-lactamase, with K_i = 44 μM at pH 7 [31]. The pinane amidoboronic esters **40**, **41** and **42** could be synthesised by using Matteson's synthetic route (Scheme **10**) [15].

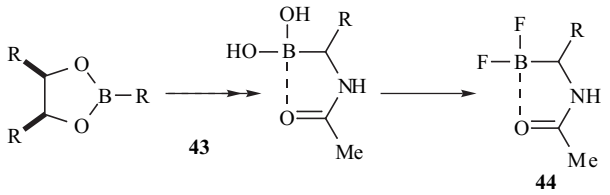
The racemic α-acetamidoboronic acids **43** have been obtained using similar chemistry. This reaction was used as the starting point for the corresponding *meso*-butanediol esters (Scheme **11**) and **43a-c** were found to inhibit elastase and chymotrypsin [32]. The fluoro-derivatives **44** could be obtained by the treatment of **43** with aqueous hydrofluoric acid [19].

Free α-aminoboronic acids were synthesised and tested as potential enzyme inhibitors. The racemic boraalanine **45** was obtained in solution by hydrolysis of the boronic ester (Scheme **12**). It is a good inhibitor of alanine racemase from *Bacillus stearothermophilus* with K_i = 20 mM (it was slow



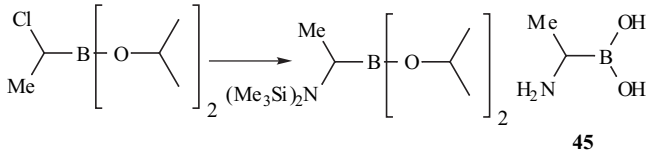
Scheme 10.

binding at K_i = 0.15 – 0.35 min^{-1}). For D-alanine:D-alanine ligase from *Salmonella typhimurium* two binding constants for different enzyme sites were found: K_i = 35 μM and K_i' = 18 Mm [33].



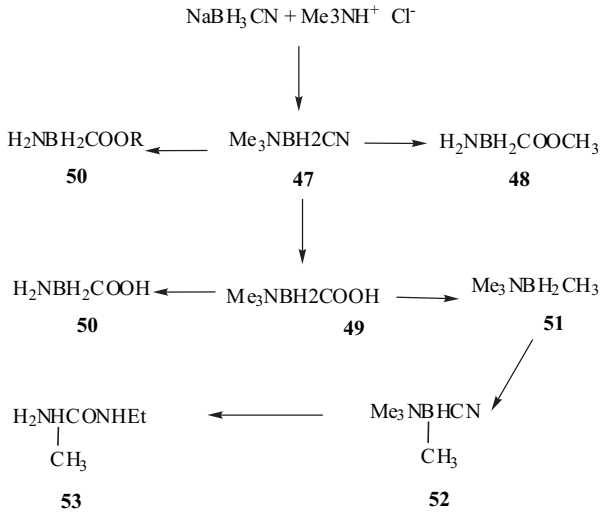
43a. R = (*S*)-EtMeCH
43b. R = Ph
43c. R = *n*-Pr

Scheme 11.



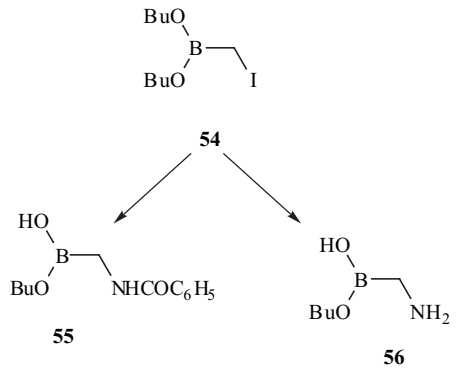
Scheme 12.

Several glycine α-aminoboronic acids **49**,**50** and derivatives **46**-**48**, **51**-**53** have been synthesized and show properties as potential chymotrypsin inhibitors. In the simplest amino acid, glycine, replacement of the central methylene by boron, as depicted in **50**, would give an isoelectronic and isostructural analogue. Upon displacement of the trimethylamine with a large excess of liquid ammonia, the desired glycine analogue **50** was isolated. The methyl ester **48** was formed from **47** in the presence of dicyclohexyl-carbodiimide which then was submitted to an amine exchange to give the glycine analogue **48** (Scheme **13**) [34,35].



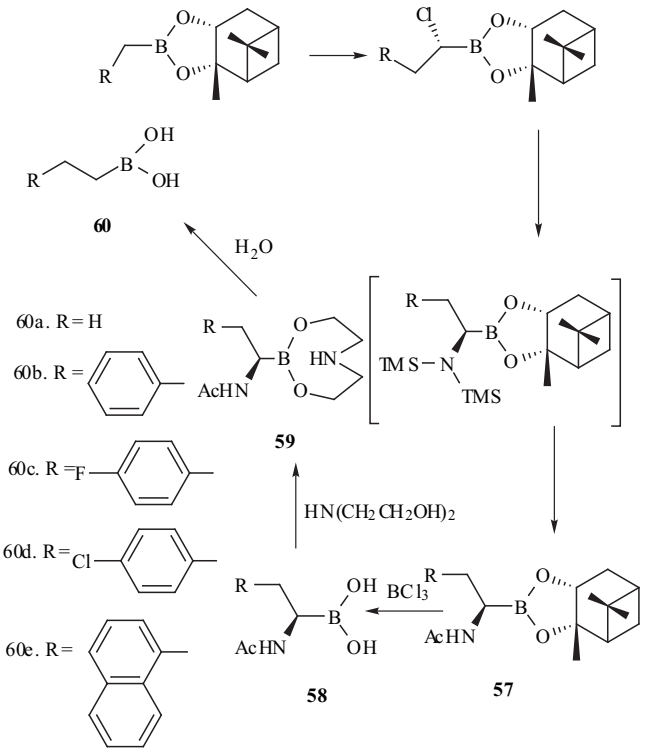
Scheme 13.

The initial synthetic efforts to obtain such amino acid-based inhibitors used N-acylated analogues of glycine **55** and **56**. In one example, dibutyl iodomethane-boronate **54** was alkylated with the sodium salt of benzamide to give **55** (Scheme **14**), which shown also to be a potent inhibitor of α-chymotrypsin. The reaction of **54** with $LiN(SiMe_3)_2$ gave **56**, which is also has inhibitor activity [36].



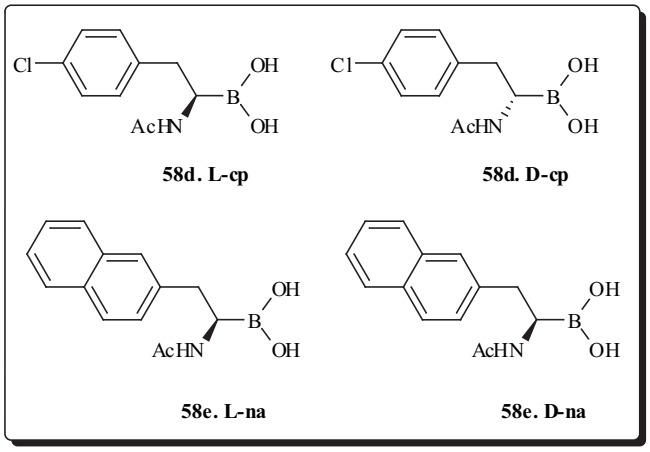
Scheme 14.

Enantiometric 1-acetamidoboronic acids, which are *N*-acetyl transition state inhibitor analogues of the L- and D-forms of the amino acids alanine, phenylalanine, *p*-fluoro-phenylalanine, *p*-chlorophenylalanine, and 1-naphtrylalanine were synthesised (Scheme **15**) and tested as inhibitors of the serine proteases subtilisin Carlsberg and α-chymotrypsin [37]. All L-(*R*)- and D-(*S*)-1-acetamidoboronic acids **57**-**60** were prepared according to the basic strategy developed by Matteson *et al.* [19,38]. Both the anhydride forms of **58** and the diethanolamine derivatives **59** are hydrolysed to the corresponding free boronic acids **60**a-e. All of the boronic acids **60**a-e are powerful competitive inhibitors of both enzymes.



Scheme 15.

A kinetic evaluation of the factors controlling the stereoselectivities of subtilisin Carlsberg and *R*-chymotrypsin was observed with a series of L-and D-boronic acid inhibitors **58**d,e. These included L-(1*R*)-(L-cp) and D-[(1*S*)-1-acetamido-2-(*p*-chlorophenyl)ethyl]boronic acid (D-cp) as well as L-(1*R*)-(L-na) and D-[(1*S*)-1-acetamido-2-(1-naphthyl)ethyl]boronic acid (D-na) [40]. Subtilisin



maintained its natural L-enantiomer preference for both the cp- and na-inhibitor series. The stereoselectivity of chymotrypsin, however, switched from L for the chlorophenyl compounds to D for the naphthyl-substituted inhibitors [40]. This unexpected reversal of stereochemical preference was examined by molecular modeling studies in an initialt attempt to identify the factors responsible. These computational studies on the enzyme-inhibitor complexes pointed to variations in the hydrogen-bonding patterns and to a reversal of the orientation of the naphthyl ring as potential determinants of the switch in stereo preference for chymotrypsin. Tsilikounas *et al.* [40b] proposed that the properties of the specificity pockets are responsible for placing those inhibitors which closely mimic natural substrates into a position favoring a covalent bond between boron and the catalytic serine residue. Inhibitors that deviate from native substrate-like structures may be forced into a binding mode that could lead to the formation of histidine-boron adducts. Studies have shown that a covalent bond from the serine Oγ to the boron atom can coexist with a long coordinate-covalent bond between boron and histidine $N\varepsilon_2$ [40].

The four subtilisin structures all contained a covalent bond from Ser221 Oγ to boron, forming tetrahedral complexes. One of the boron hydroxyl groups interacts with the catalytic histidine, His64 $N\varepsilon_2$ and a water molecule, and the second hydroxyl occupies the oxyanion hole, with bonds to Ser221 NH and Asn155 $N\delta_2$. The aromatic rings of the inhibitors occupy the S_1 binding pocket of the enzyme (Fig. **3**). Five chymotrypsin structures were determined, one native structure including peptide bound in the active site and four inhibitor complexes. All four inhibitor complexes involve a 1.4 Å covalent bond from boron to the catalytic serine Ser195 Oγ, as for a natural peptide substrate. The aromatic ring groups all bind in the S_1 pocket (Fig. **3**). For the L-inhibitors, the boron forms a tetrahedral arrangement displacing a water molecule. The other binds in the oxyanion hole, with bonds to backbone amides of Gly193, Asp194, and Ser195. The 1-acetamido groups mimic a peptide backbone, hydrogen bonding (3.0and 3.5 Å, respectively) with Ser214 O. The aromatic rings make van der Waals contacts with residues Val213 Cγ and Gly216 CR of the S_1 pocket [40f].

The solid phase synthesis of the aminoboronic acids **79** and **80**, potent inhibitors of the hepatitis C virus NS3 proteinase, was demonstrated by Dunsdon *et al.* [39] (Scheme **16**) using well known peptide boronic acid derivatives **61**. Hepatitis C virus (HCV) is the cause of the majority of cases of transfusion-associated hepatitis. The

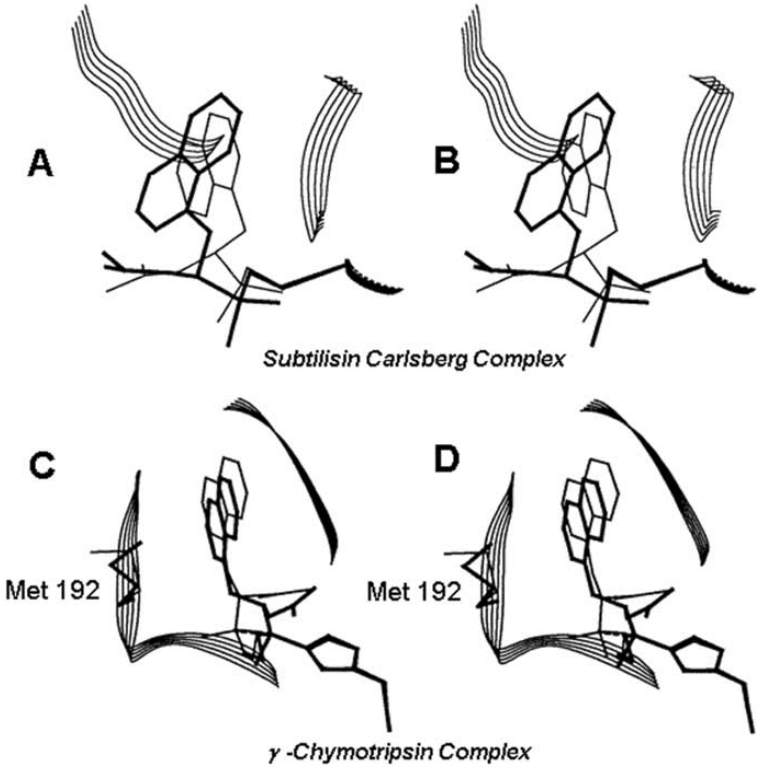
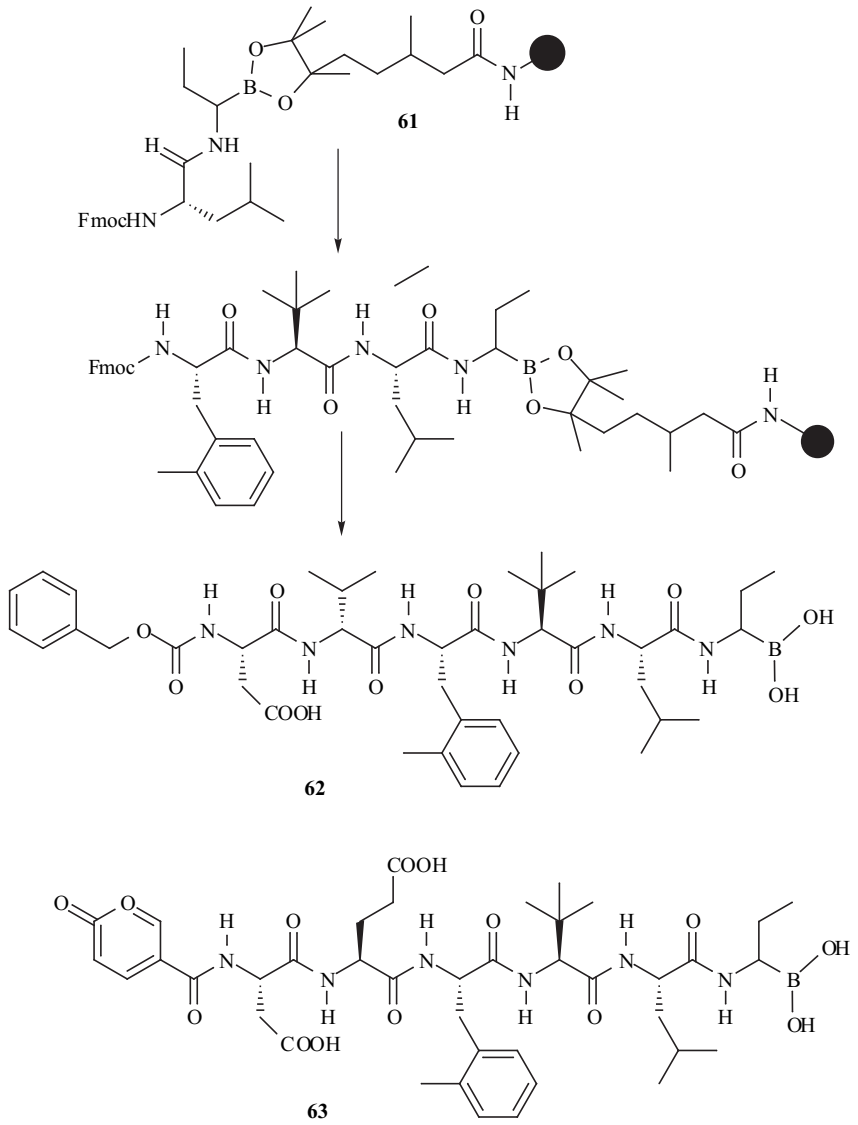


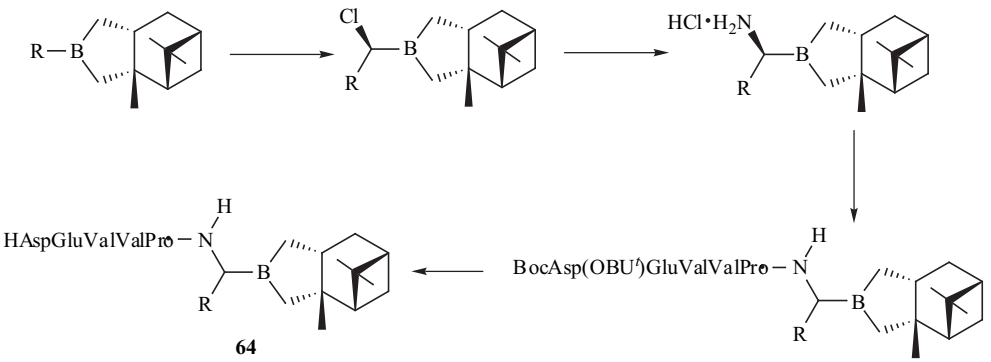
Fig. (3). Interaction of L-na (A, thin lines) and D-na (B) complexes with subtilisin Carlsberg, and L-na (A, thin lines) and D-na (B) complexes with γ-chymotrypsin [40f]. Adapted by authors.



Scheme 16.

target which requires new compounds for antiviral therapy against HCV is the NS3 serine proteinase [40]. The amidoboronic acids **62** and **63** were found to be highly potent inhibitors of the HCV NS3 proteinase [41].

A series of phenethyl peptide boronic acids containing extended, hydrophobic P1 residues have been synthesised to probe the shallow, hydrophobic S1 region of HCV NS3 protease [42]. Peptide boronic acid inhibitors were synthesized using the methodology for asymmetric homologation of boronic acid pinanediol esters developed by Matteson and co-workers [43,44]. As shown in (Scheme **17**), reaction of a Grignard reagent with triisopropyl borate, followed by esterification with (+)-pinanediol affords a boronic ester. Homologation with dichloromethyllithium diastereoselectively provides an (*S*)-α-chloroboronic ester.



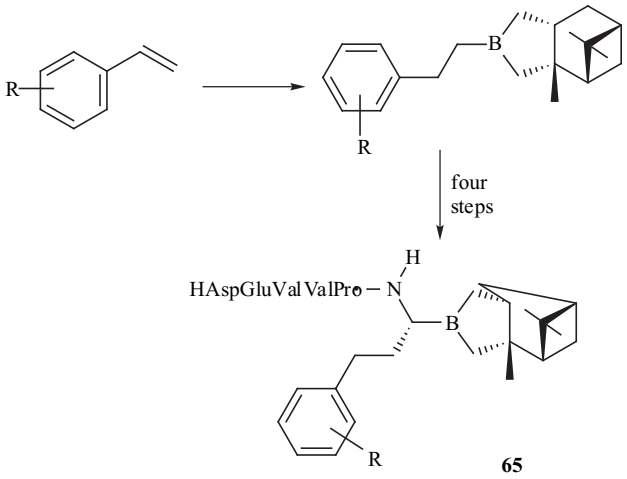
Scheme 17.

Displacement of chloride by lithium bis(trimethylsilyl)amide, followed by acid hydrolysis gives the (*R*)-α-aminoboronic ester as a stable hydrochloride salt. Coupling to the protected pentapeptide Boc-Asp(OBu^t)-Glu(OBu^t)-Val-Val-Pro-OH and deprotection with trifluoroacetic acid afforded the desired peptide boronic acids **64**a–l. The inhibition activity of peptide boronic acids **64**a-l has been demonstrated against NS3 protease, human leukocyte elastase, and human pancreatic chymotrypsin show in (Table **5**).

Table 5. Inhibition of NS3 Protease, Human Leukocyte Elastase, and Human Pancreatic Chymotrypsin by Peptide Boronic Acids 64a

No	R	NS3 K_i (μM)	Elastase IC_{50} (μM)	Chymotrypsin IC_{50} (μM)
64a	Ethyl	0.008	0.020	> 60
64b	*n*-Butyl	0.011	NT	2.1
64c	*n*-Pentyl	0.012	NT	0.38
64d	*n*-Hexyl	0.013	NT	0.42
64e	*i*-Butyl	0.008	0.060	NT
64f	*i*-Amyl	0.039	NT	0.30
64g	4-Methylpentyl	0.007	7.3	0.28
64h	Phenyl	0.9	NT	NT
64i	Benzyl	0.5	NT	0.070
64j	Phenethyl	0.008	3.5	0.075
64k	Phenpropyl	0.20	NT	NT
64l	Phenbutyl	0.010	0.4	1.9

A series of substituted phenethyl containing peptides was prepared as shown in (Scheme **18**). In this case, the required boronates were prepared by hydroboration of a substituted styrene with catecholborane, followed by transesterification with (+)-pinanediol [42]. Subsequent homologation, nitrogen substitution, and peptide coupling afforded hexapeptides **65**a-u. Inhibition of NS3 protease, human leukocyte elastase, and human pancreatic chymotrypsin by P1 phenethyl peptide boronic acids **65**a-u was observed [42],



Scheme 18.

and is shown in (Table **6**). Within the P1 phenethyl series, substantial effects on inhibitor potency and selectivity were observed with changes in the position and identity of the aromatic ring substituents, and the 4-trifluoromethylphenethyl **65**l P1 was identified as optimal with respect to inhibitor potency for NS3 and selectivity against elastase and chymotrypsin [42].

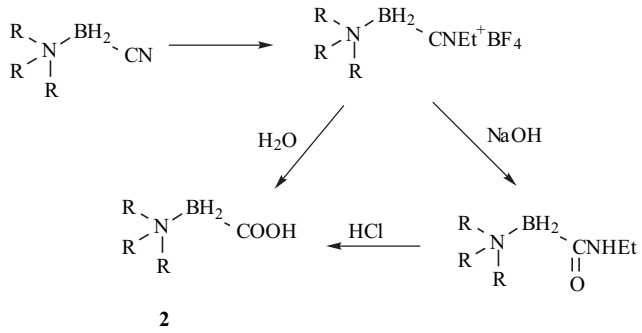
Table 6. Inhibition of NS3 Protease, Human Leukocyte Elastase, and Human Pancreatic Chymotrypsin by P1 Phenethyl Peptide Boronic Acids 65

No	R	NS3 K_i (μM)	Elastase IC_{50} (μM)	Chymotrypsin IC_{50} (μM)
65a	H	0.008	3.5	0.075
65b	2-Methyl	0.82	NT	NT
65c	3-Methyl	0.034	5.7	NT
65d	4-Methyl	0.017	5.0	3.7
65e	2,4-Dimethyl	0.53	NT	NT
65f	2,5-Dimethyl	1.0	NT	NT
65g	2-Fluoro	0.018	NT	NT
65h	3-Fluoro	0.009	NT	NT
65i	4-Fluoro	0.006	0.8	0.050
65j	2,6-Difluoro	0.930	NT	NT
65k	3-Trifluoromethyl	0.025	NT	NT
65l	4-Trifluoromethyl	0.002	1.8	16.0
65m	4-Chloro	0.002	1.4	0.065
65n	4-Bromo	0.004	1.6	NT
65o	4-Phenyl	0.007	0.9	48.0
65p	4-Isopropyl	0.005	0.45	> 60.0
65q	4-Cyclohexyl	0.003	0.40	> 60.0
65r	4-*tert*-Butyl	0.003	0.34	> 60.0
65s	4-Hydroxy	0.008	0.9	NT
65t	4-Methoxy	0.003	0.56	20.0
65u	4-Phenoxy	0.003	0.22	> 60.0

3. Wide Spectra Activities of Amine-carboxyboranes and their Derivatives

Amine-carboxyboranes with the common structure **2** can be regarded as isoelectronic analogues of protonated α-amino acids, or more correctly, aliphatic carboxylic acids. This resemblance has inspired extensive biological screening of these molecules and the promising early results led to the syntheses of a large number of ester, amide, peptide, hydroxamic acid and transition metal complex derivatives of amine-carboxyboranes (A-BH_2COX, X = OR, NR_1R_2, NHOH) containing a broad range substances, among other amineboranes, which have recently been reviewed [1]. Today, many of these molecules are known to possess remarkable antitumuor, anti-osteoporotic, anti-inflammatory, and hypolipidemic activities, and their mode of action is under inverstigation [47-49]. As boron has one less positive

charge on its nucleus than carbon, BH_2^- will be isoelectronic with CH_2 and consequently, the boron-analogue counterparts of the α-amino acids would exist in their protonated forms in the free state. Several routes for the synthesis of amine-carboxyboranes have been described. In one study, Das and Mukherjee [50] have demonstrated that the acid or base-catalysed hydrolysis of the aminecyanoboranes (Scheme **19**) always yields the acid **2**.



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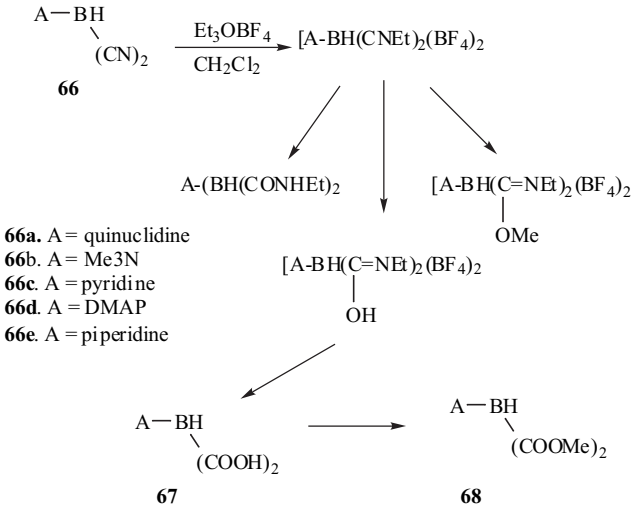
Scheme 19.

The aminedicarboxyboranes **67** and their dimethyl esters **68** have been synthesised [51]. The synthetic sequence is outlined in (Scheme **20**). The amine-dicyanoboranes **66a–d** were readily synthesized by base exchange [51].

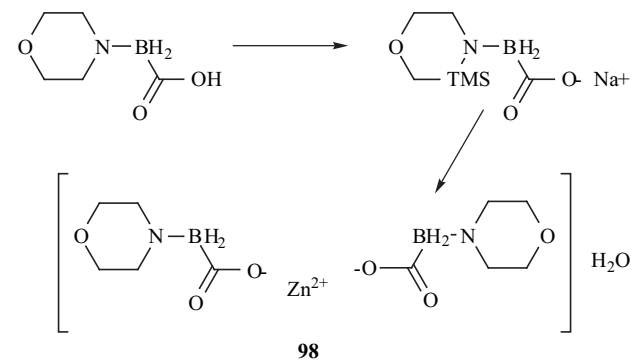


66a. A = quinuclidine
66b. A = Me3N
66c. A = pyridine
66d. A = DMAP
66e. A = piperidine

66 **67** **68**

Scheme 20.

These routes were used for the synthesis of acyclic amine-carboxyboranes which showed anti-inflammaory activity (see Table **7**). The heterocyclic amine derivatives as well as amine-carbamoylboranes, carboalkoxyboranes and cyanoboranes were generally less active. Those derivatives which demonstrated good anti-inflammatory activity **69**–**97** were effective inhibitors of hydrolytic lysosomal and proteolytic enzyme activities with IC_{50} values equal to 10^{-6} M in mouse macrophages, human leukocytes and Be Sal osteo-fibrolytic cells [52].



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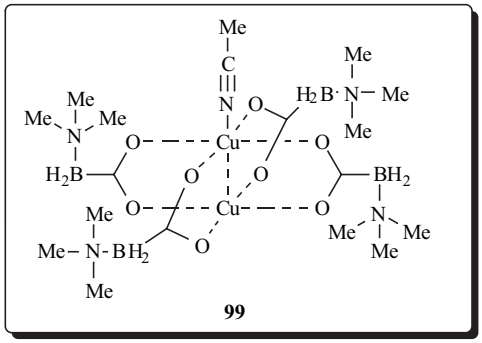
Scheme 21.

Table 7. Reaction Data for R-BH_2X Products and the Anti-inflammatory Activity of Boron Derivatives in CF-1 Mice at 8 mg/kg

No	Compound	Activity ED50
	Amine-BH_2-COOH derivatives	
69	H_3NBH_2COOH	
70	$MeNH_2BH_2COOH$	
71	Me_2NHBH_2COOH	
72	Me_3NBH_2COOH	
73	$EtNH_2BH_2COOH$	
74	$(n\text{-}C_3H_7)_2NHBH_2COOH$	
75	$C_{16}H_{33}NMe_2BH_2COOH$	
76	$C_{18}H_{37}NMe_2BH_2COOH$	
77	$H_2NNH_2BH_2COOH$	
78	$[CH_2NMe_2BH_2COOH]_2$	
79	$C_5H_5NBH_2COOH$	
	Amine-BH_2-COOMe derivatives	
80	H_3NBH_2COOMe	
81	$MeNH_2BH_2COOMe$	
82	Me_2NHBH_2COOMe	
83	Me_3NBH_2COOMe	58
	84 $CH_3(CH_2)_3NH_2BH_2COOMe$	
85	$C_{16}H_{33}NMe_2BH_2COOMe$	
	Amine-BH2-COOEt derivatives	
86	$C_{16}H_{33}NMe_2BH_2COOEt$	
87	Me_2NHBH_2COOEt	
88	$(BH_2CONHEt)_2$	
89	$H_3NBH_2CONHEt$	
90	$MeNH_2BH_2CONHEt$	
91	$Me_2NHBH_2CONHEt$	
92	$Me_3NBH_2CONHEt$	
	Amine-BH2-CONHR derivatives	
93	$Me_3NBH_2CONH(n\text{-}Pr)$	61
	94 $Me_3NBH_2CONH(n\text{-}Bu)$	
95	$Me_3NBH_2CONH(n\text{-}Oct)$	
96	$Me_3NBH_2CONHC_6H_5$	
97	$[Me_2NCH_2BH_2CONHC_2H_5]_2$	

A number of metal complexes of amine-carboxyborane adducts having antitumour activity have been synthesized. Bis-μ-(morpholine-boranecarboxylato)zinc dehydrate **98** demonstrated cytotoxic activity against human $Tmolt_3$, HeLa-S^3 and MB-9812 cell growth [53]. The synthesis of the compound **98** is shown in (Scheme **21**).



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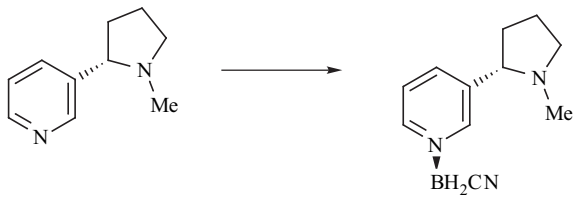

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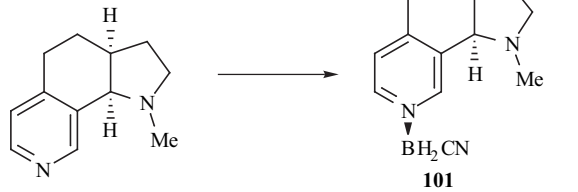

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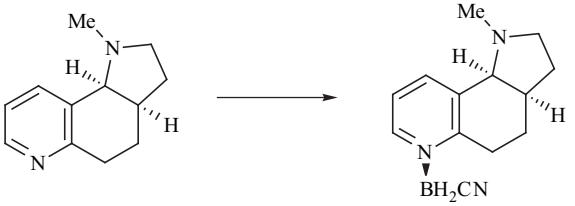

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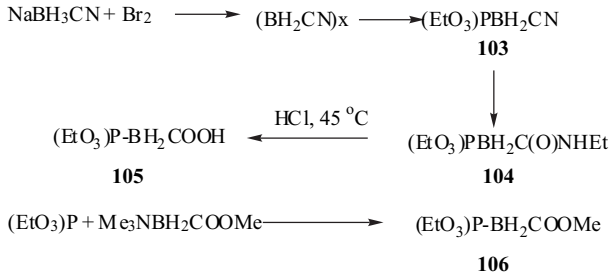
Scheme 22.

Another amine-carboxyborane metal complex, tetrakis-μ-(trimethylamine-boranecarboxylato)-acetonitrile dicopper **99** [53] and also **98** inhibited L_{1210} DNA, RNA and protein syntheses, with greatest inhibitory effects on DNA. The reduction in DNA synthesis correlates well with the inhibition of *de novo* purine synthesis and the key enzymes involved in this pathway, i.e. IMP dehydrogenase and PRPP amidotransferase.

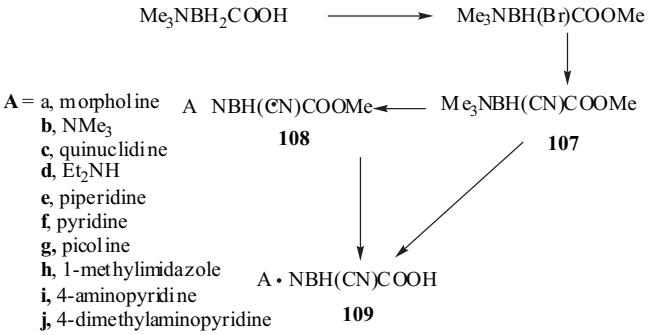
A series of boron-containing nicotine (NIC) analogues have been synthesised and evaluated for binding to α4β2 and α7 neuronal nicotinic receptors [54]. The boron-containing analogues **101**, **102** and **103** were synthesised by refluxing a suspension of $NaBH_3CN$ and the hydrochloride salts of the corresponding precursors in THF under N_2 overnight, according to (Scheme **22**). All three boron-containing analogues were found to be very stable in water. The compound **101** inhibited [^{3}H]-methyllyccaconitine binding to rat brain membranes with a similar potency compared to NIC (**100**).

Boron analogues of phosphonoacetates have been synthesised (Scheme **23**) and their antitumour and anti-inflammatory activity were studied [45,46]. Cytotoxicity data for the compounds **66** – **69** are shown in (Table **8**).



Scheme 23.

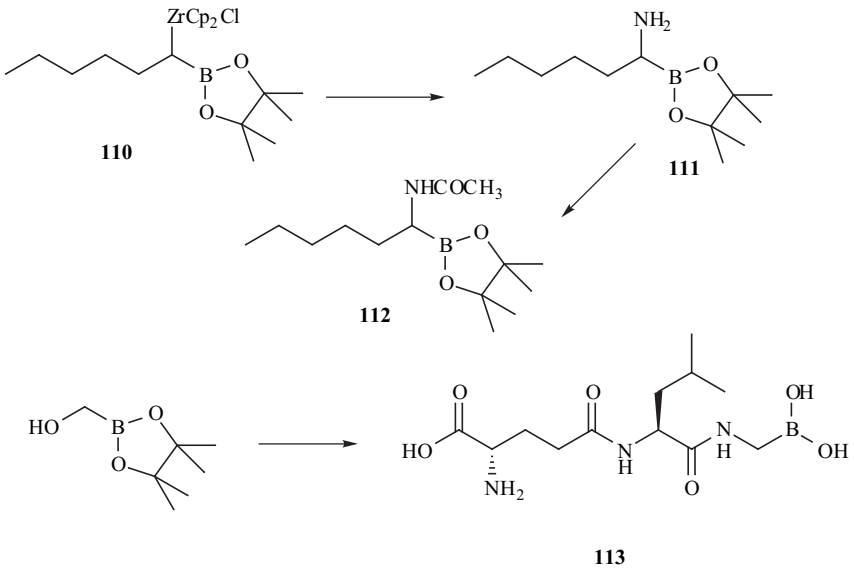
A number of aminecarboxyborane esters have been studied for their anti-hyperlipidemic activity [55] and hypolipidemic activity in rodents [56]. The synthesis of aminecyanocarboxyboranes – isoelectronic analogues of α-cyanocarboxylic acids - have been reported [57]. The synthetic sequence outlined in (Scheme **24**), employing activation and then nucleophilic substitution of the boron, subsequently resulted in the preparation of several novel compounds **107 - 109a – j.**



Scheme 24.

Table 8. Cytotoxicity of Boron Analogues of Phosphonoacetates (ED_{50}, μg/ml)

No	Ehrlich carcinoma (% inhibition)	Murine L1210	Murin e P388	Human HeLa	Human KB	Human glioma	Human osteosarcoma	Human lung	Human colon	Human $tmolt_3$
103	66	3.61		2.12	2.86	4.26	2.88	6.88	6.56	2.42
104	80	4.45	6.46							
105	39	3.47	7.26	4.41	3.87	5.29	4.18	4.48	2.65	6.16
106	97	4.15	4.98	3.96	1.75	1.60	7.25	4.80	6.96	7.64



Scheme 25.

α-Aminoboronic esters could be synthesised *via* zirconocene species [58,59]. The amination of *gem*-borazirconocene alkanes such as **110** with MSH gave compound the **111**, and subsequent treatment with Ac_2O formed the α-aminoboronic ester derivatives **112**, which are potential prodrug compounds (Scheme **25**).

The boronic acid peptide **113** was prepared from 2-bis(trimethylsilyl)aminomethyl-4,4,5,5-tetramethyl -1,3,2-dioxaborolane [60]. This peptide show inhibitory activity against glutathionylspermidine synthetase/amidase (IC_{50} = 17.2 μM) which is an essential enzyme in the biosynthesis and turnover of trypano-thione and represents an attractive target for the design of selective anti-parasitic drugs [61].

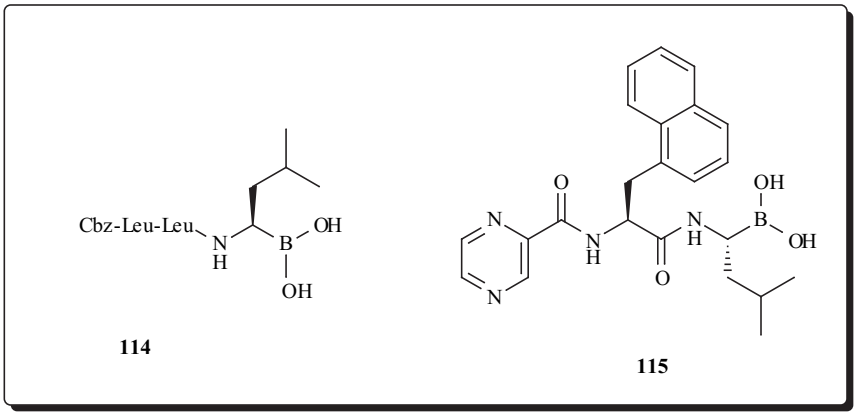
Compound **114** is strong inhibitor of cathepsin B with a K_i = 6.1 μM, representing a 200,000-fold selectivity for the proteasome, and **115** has K_i = 0.18 nM [2b,62].

CONCLUDING REMARKS

A remarkable diversity of reactions has been discussed in this brief review. This article has also described and summarized the development of a new boron-based methodology for applications in organic synthesis of α-amino-boronic acids, amine-carboxyboranes and their derivatives. These compounds are unique among boron-containing compounds and they have a high biological activity in different fields. The investigation of the chemistry of these compounds is continuing, especially in the areas of selective reactions, synthesis, catalysis and coordination chemistry [63-65].

In medicinal chemistry, the use of boronic acids as enzyme inhibitors to a large degree reflects the usefulness of boron as a carbon analog in the binding process, but not in terms of reactions, which is the essence of a good enzyme inhibitor. Boronic acids have been used for the development of enzyme inhibitors of peptidases/proteases, arginase, nitric oxide synthase, proteasomes, as well as trans-peptidases [1,2,9].

Recently, the activity of an extremely potent and selective proteasome inhibitor, bortezomib, **30** has been reported. In cell culture and animal models of cancer, it has potent tumoricidal effects and sensitizes cancer cells to conventional anticancer agents. In addition **30** effectively and specifically inhibits proteasome activity. In preclinical studies, bortezomib and other proteasome inhibitors have shown activity against a variety of B-cell malignancies, including multiple myeloma, diffuse large B-cell lymphoma, mantle cell lymphoma, and Hodgkin's



lymphoma. Bortezomib is the only proteasome inhibitor that has entered clinical trials in patients with cancer. With approximately 200 patients treated in phase I trials to date, bortezomib has been generally well tolerated at doses that achieve a desired degree of proteasome inhibition. Encouraging antitumor activity has been observed. These data served as the basis for phase II clinical trials of bortezomib in patients with a broad range of tumors and also for clinical studies of bortezomib plus chemotherapy. The early results of combination trials show that bortezomib was generally well tolerated at doses that resulted in a good level of proteasome inhibition when combined with chemotherapy in patients. No major overlapping toxicities have been observed to date and there was evidence of antitumor activity by many of the combinations tested in chemorefractory patients. Phase II or definitive phase III studies of bortezomib and chemotherapy will be considered after the completion of these initial trials and should serve to contribute to a further understanding of the potential role of bortezomib in the treatment of human malignancies in the near future. Preliminary data from the multiple myeloma phase II study indicate that a significant number of patients responded to therapy or exhibited stable disease and that the drug had manageable toxicities. These findings, along with extensive preclinical data, suggest that bortezomib and other proteasome inhibitors may have far-reaching potential in the treatment of various cancers, including B-cell malignancies [1,25,66,67].

In January 2003, Millennium submitted a New Drug Application (NDA) to the U.S. Food and Drug Administration (FDA) for approval to market VELCADE (bortezomib) as a treatment for relapsed and refractory multiple myeloma. Additional studies of Velcade as a single agent and in combination with other chemotherapeutic agents are continuing in a variety of different tumor types. Velcade is designed specifically to inhibit proteasomes, which are enzyme complexes in all cells responsible for breaking down a variety of proteins, including many that regulate the cell cycle. VELCADE has been granted fast-track designation by the FDA for refractory multiple myeloma. The FDA grants fast-track status to facilitate the development and expedite the review of an investigational drug if it is intended for the treatment of a serious life-threatening condition, and demonstrates the potential to address unmet medical needs for such a condition. VELCADE has also been granted Orphan Product Designation by the FDA [67c].

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