Structure−Activity Relationships of Boronic Acid Inhibitors of Dipeptidyl Peptidase IV. 1. Variation of the P_2 Position of X_{aa}-boroPro Dipeptides

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A series of prolineboronic acid (boroPro) containing dipeptides were synthesized and assayed for their ability to inhibit the serine protease dipeptidyl peptidase IV (DPPIV). Inhibitory activity, which requires the (*R*)-stereoisomer of boroPro in the P_1 position, appears to tolerate a variety of L-amino acids in the P_2 position. Substitution at the P_2 position which is not tolerated include the D-amino acids, α,α-disubstituted amino acids, and glycine. Specificity against DPPII and proline specific endopeptidase is reported. A correlation between the ability to inhibit DPPIV in cell culture and in the human mixed lymphocyte reaction is demonstrated. A synthesis of prolineboronic acid is reported as well as conditions for generating the fully unprotected boronic acid dipeptides in either their cyclic or acyclic forms.

Introduction

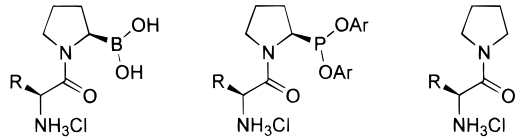
Inhibition of the serine protease dipeptidyl peptidase IV (DPPIV, CD26)[1] has been shown to cause the suppression of the T-cell-mediated immune response both *in vitro*[2] and *in vivo*.[3] The enzyme cleaves a dipeptide from the amino terminus of polypeptides where the penultimate residue is proline.[4] While the biological substrate and exact mechanism of immune-related function are not known, DPPIV has been reported to associate with several T-cell-related molecules including CD45[5] and adenosine deaminase.[6] There has also been a much-debated claim identifying DPPIV as the co-receptor for the human immunodeficiency virus.[7,8]

Inhibitors of this enzyme have been based on dipeptides which possess a proline or proline mimic in the P_1 position.[9] Recent classes of these compounds are demonstrated by the amino boronic acid dipeptides **1**,[10] the diphenyl phosphonates **2**,[11] and the pyrrolidides **3**.[12] In particular, the boronic acid dipeptides (**1**) have been shown by Bachovchin[10] to be exceptionally potent inhibitors. The empty P-orbital centered at boron is thought to interact with the catalytic serine to form a stable "ate" complex which mimics the transition state of amide hydrolysis.[13]

There are two complications which traditionally hamper the study of amino boronic acid dipeptides.[14,15] The first is that these molecules are usually tested as their protected boronate esters (*e.g.* **10**) which requires that the compounds undergo hydrolysis in order to be activated.[15] Often this hydrolysis is neither rapid nor complete, which can affect the accurate determination of potency. We therefore decided to develop a route to the fully deprotected amino boronic acid dipeptides and to study them exclusively.

The second concern when assaying the boronic acid dipeptides is that they lose activity in a time dependent manner upon exposure to aqueous buffer. We have recently demonstrated[14,15] that the loss in activity can be correlated to the position of a reversible intramolecular cyclization in which a dative B−N bond is formed to generate the boron analog of a diketopiperazine (*e.g.* **11**).

The current work details the structure−activity relationships associated with variations of the P_2 position of the dipeptide inhibitor as well as the synthetic protocols which generate the fully unprotected boronic acid dipeptides.



1 **2** **3**

Chemistry

At the outset of this project it became clear that an efficient route to prolineboronic acid (boroPro) was essential. The excellent procedure developed by Matteson[16] for the syntheses of amino boronic acids has been applied to the synthesis of boroproline (**6**)[17] but is limited due to the need for incorporation of the pyrrolidinyl ring. We have previously reported a procedure based on the lithiation−boronation−reduction of Boc-pyrrole (**4**, Scheme 1) which has generated multigram quantities of the >98% diastereomerically pure boroproline **7,** after resolution.[18] A second method has since been developed which involves the direct lithiation of Boc-pyrrolidine. Treatment of Boc-pyrrolidine (**5**) with *s*-BuLi generated the α-anion[19] which was subsequently quenched by the addition of $B(OMe)_3$. Hydrolytic workup produced the free boronic acid **6** in 81% yield, which was converted to **7** and resolved as described previously.[18]

The amino boronic ester **7** was coupled with the desired Boc-amino acids in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) to generate the fully protected dipeptides. As we had

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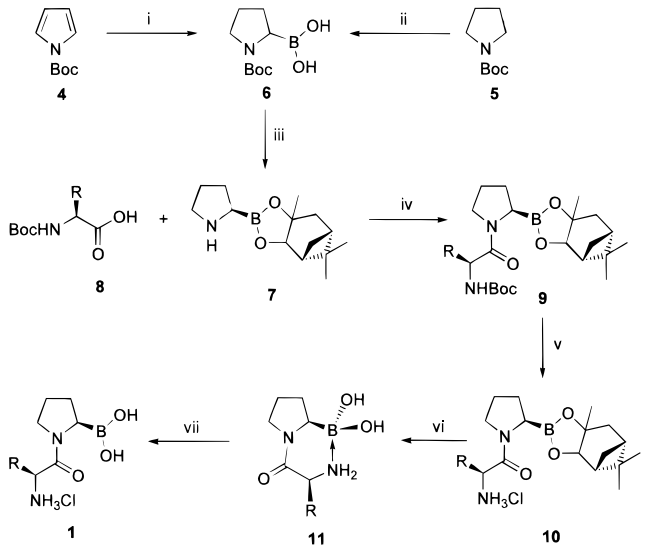
Scheme 1[a]



[a] Reagents: (i) (1) LiTMP, THF, TMEDA, −78 °C; (2) $(EtO)_3B$; (3) H_3O^+; (4) H_2, Pt−C, EtOAc; (ii) (1) *s*-BuLi, Et_2O, TMEDA, −40 °C; (2) $(EtO)_3B$; (3) H_3O^+; (iii) (1) (+)-pinanediol, Et_2O; (2) HCl, EtOAc; (3) recrystallization; (iv) (1) compound **8**, HOBT, EDC, CH_2Cl_2, 0 °C; (2) compound **7**, NMM; (v) HCl, Et_2O, 0 °C; (vi) H_2O (pH = 2), hexane, $PhB(OH)_2$; (vii) HCl or $MeSO_3H$.

elected to pursue the free boronic acids, the following deprotection protocol was developed. First, the Boc group was removed with HCl to produce the unprotected amines **10**. Deprotection of the boronic ester portion was then effected by transesterification of the pinanediol with phenylboric acid in a biphasic hexane−water (low pH) mixture.[20] Pinanediol phenylborate was recovered from the organic phase and the cyclic boronic acid dipeptides (**11**) were isolated from the aqueous phase by passage through a Dowex-50 cation exchange resin and elution with NH_4OH. The open forms of the dipeptides (**1**) were produced as their salts by treatment with MsOH or HCl.

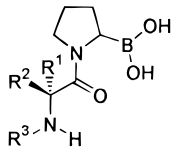
Results and Discussion

Structure Activity Relationships. The ability of the dipeptides to inhibit the enzyme was measured by a colormetric assay which used H_2N-Ala-Pro-4-methoxy-2-napthaleneamide as the substrate.[21] This is an end-point assay which measures the amount of substrate cleaved over 1 h. Results are shown in Table 1.

Compounds **1a** and **1b** demonstrate the necessity of having the boroPro in the proper configuration. Likewise, **1c** sets the requirement of having the P_2 position amino acid as the natural (L) stereoisomer. Reducing the size of the alkyl group at P_2 from *i*-Pr (Val **1a**) to Me (Ala **1d**) does not significantly affect the activity. Removal of the methyl group (Ala **1d** to Gly **1f**) ablates activity as does the insertion of a second methyl group at the P_2 position (α-aminoisobutyric acid **1e**).

Further exploration of the ability to bind small lipophilic amino acids at P_2 demonstrated that substituents such as Et (aminobutyric acid **1g**), *i*-Bu (leucine **1h**), *s*Bu (isoleucine **1i**), and *t*-Bu (*tert*-leucine **1j**) were all active with a slight preference for the smaller substituents. Replacement of these alkyl groups with aromatic residues (phenylalanine **1k**, phenylglycine **1l**, and tyrosine **1m**) also produced potent compounds. The two polar amino acids lysine (**1n**) and threonine (**1o**) were moderately less active then the simple alkyl-substituted derivatives.

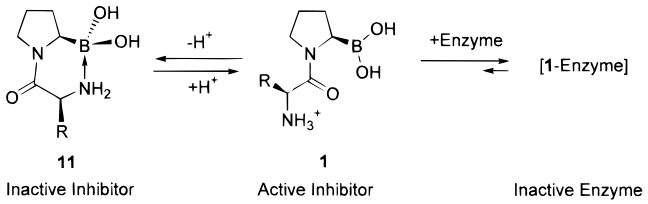
Table 1. Structure−Activity Relationships of H_2N-X_{aa}-boroPro Dipeptides vs DPPIV



compd	amino acid[a]	R^1	R^2	R^3	boron config	IC_{50}, nM	±SE, nM
1a	L-Val	H	*i*-Pr	H	*R*	26	1
1b	L-Val	H	*i*-Pr	H	*S*	4000	600
1c	D-Val	*i*-Pr	H	H	*R*	116000	15000
1d	L-Ala	H	Me	H	*R*	15	3
1e	AiBu	Me	Me	H	*R*	30000	8000
1f	Gly	H	H	H	*R*	16000	2400
1g	L-Abu	H	Et	H	*R*	11	1
1h	L-Leu	H	*i*-Bu	H	*R*	44	2
1i	L-Ile	H	2-Bu	H	*R*	25	1
1j	L-tLeu	H	*t*-Bu	H	*R*	60	7
1k	L-Phe	H	CH_2Ph	H	*R*	70	7
1l	L-Phg	H	Ph	H	*R*	63	5
1m	L-Tyr	H	CH_2(Ph-4-OH)	H	*R*	32	1
1n	L-Lys	H	$(CH_2)_4NH_2$	H	*R*	95	19
1o	L-Thr	H	CH_3CHOH	H	*R*	190	13
1p	L-Pro	H	$-(CH_2)_3-$		*R*	20	5
1q	L-Azet	H	$-(CH_2)_2-$		*R*	250	13
1r	L-His	H	CH_2Im	H	*R*	17000	1800

[a] Abbreviations: Aibu, α-aminoisobutyric acid; Abu, 2-aminobutyric acid; Phg, phenylglycine; tLeu, *tert*-leucine; Azet: azetidine-2-carboxylic acid.

Scheme 2



Inactive Inhibitor | Active Inhibitor | Inactive Enzyme

Pro-boroPro (**1p**) is known to be a potent inhibitor.[10] Further investigation showed that replacement of the pyrrolidine ring of the P_2 proline with the four-member azetidine ring (**1q**) decreased the activity by approximately 12-fold.

Two interesting aspects of the assay must be noted. The first is that the open form of these compounds equilibrates to the closed form and activity is lost *over the course of the assay*.[14,15] A corollary of this is that a compound which is a more potent inhibitor but which rapidly converts to its cyclic form may appear less active over the time course of this assay (Scheme 2). This may explain the weak activity for Gly-boroPro since H_2N-Gly-Pro-*p*-nitroanilide is known to be a good substrate for the enzyme.[22] This hypothesis is substantiated by recent data showing that Gly-boroPro cyclizes much more rapidly than Ala-boroPro, Pro-boroPro, or Val-boroPro.[15b] For more information, the reader is directed to a study of the relative rates of cyclization which already has been published.[15a]

The second issue is that the off rates for the bound inhibitors (Scheme 2) are very slow,[23] which produces a cumulative inhibition effect on the assay.[14] As such, cyclic compounds would still be expected to demonstrate some degree of inhibition if the equilibrium between **11** and **1** did not lie completely to the left and was reestablished during the course of the assay. Our earlier work demonstrated that the cyclic forms do

Scheme 3

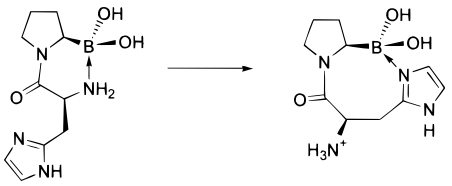


Table 2. Selectivity of X_{aa}-boroPro Peptides against Other Proline Specific Serine Proteases[a]

compd	dipeptidyl peptidase II[b] IC_{50} (nM)	prolylendopeptidase[c] IC_{50} (μM)
1a	15 ± 1	23 ± 3.1
1b	18000 ± 1500	(35%)[d]
1c	21 ± 3	61 ± 13.5
1d	60 ± 42	1 ± 0.06
1o	730 ± 118	6 ± 0.60
1p	770 ± 50	(63%)[d]

[a] See text for specificity against other serine proteases. [b] EC 3.4.14.2. [c] EC 3.4.21.26. [d] Percent inhibition at 100 μg/mL.

inhibit the enzyme via this mechanism but that the time course of this effect tends to be much slower than the time course of the assay. Therefore it is believed that the comparative activity of compounds in this assay is derived from a combination of binding ability and rates of cyclization rather than due to a large difference in off rates.

It is interesting to note the special case of histidine-boroPro (**1r**) which appears to convert from one inactive form to another (Scheme 3). ^{1}H NMR data support the hypothesis that the histadine-boroPro structure can adopt two conformations both of which contain a B−N bond. Upon initial deprotection the normal cyclic structure **11r** is generated which is expected to be inactive in the enzyme assay. Upon protonation of the amino group, the imidazolyl nitrogens are positioned to act as a Lewis base and to interact with the boron center, thus forming compound **1r** which is incapable of binding to the enzyme. Lysine-boroPro also has the potential to form a second cyclic structure but is initially prevented from doing so due to the protonation of the ϵ-amino group.

Enzymatic Specificity and *in Vitro* Activity. Boronic acid peptides are potent inhibitors of a variety of serine proteases. We were interested to gauge how specific the prolineboronic acid dipeptides were and hence tested a representative against a panel of enzymes. Val-boroPro (**1a**) showed no inhibition up to 100 μM against the following enzymes: trypsin, chymotrypsin, leukocyte elastase, thrombin, plasmin, plasma kallilkrein, or tryptase. Results against two proline specific peptidases are shown in Table 2.

Several compounds were tested against dipeptidylpeptidase II (DPPII) and proline specific endopeptidase (Table 2). DPPII is a lysozymal serine protease found mostly in thyroid, spleen, and kidney. It has very similar sequence specificity as DPPIV. Consequently, most of the inhibitors that are active against DPPIV are also active against this enzyme. Notably, both Thr-boroPro (**1o**) and Pro-boroPro (**1p**) are considerably less potent against DPPII which might point to an interesting specificity in the S_2 position of the enzyme.

Proline specific endopeptidase cleaves specifically the peptide bond on the carboxy side of proline residues.

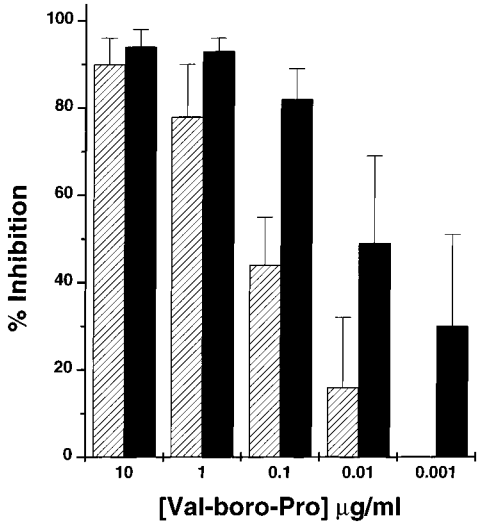


Figure 1. Correlation of DPPIV inhibition and mixed lymphocyte reaction of compound **1a**. The effect of varying concentrations of **1a** on the mixed lymphocyte reaction (MLR) was determined as described in the Experimental Section. In replicate cultures lymphocytes were incubated with varying concentrations of **1a** for 1 h, the cells were washed, and DPPIV activity was assayed as described in the Experimental Section. Data represent the mean ± standard deviation. DPPIV activity is the solid bars; MLR is the hashed bars.

Generally substrates have the structure Y-Pro-X, where Y is a peptide or N-protected amino acid and X can be an amino acid, peptide, or ester. Not surprisingly, the DPPIV inhibitors were only weakly active against this enzyme.

We also evaluated one of the best compounds, Val-boroPro (**1a**), in the human mixed lymphocyte reaction (MLR) and compared these results to the DPPIV activity in the lymphocyte culture. The results are shown in Figure 1 and demonstrate a correlation between the MLR and the activity of the enzyme. While one cannot be certain that the activities are linked, these results strongly suggest a relationship between the ability to inhibit DPPIV or DPPIV-like activity and the ability of T-cells to respond to a antigen specific challenge.

Conclusions

The synthesis and SAR of a number of boronic acid dipeptide inhibitors of DPPIV have been reported. Inhibitory activity requires a single stereoisomer of boroproline in the P_1 position. A number of substituents, both polar and nonpolar, are tolerated in the P_2 position; however, substitution at the P_2 position which is not tolerated include the unnatural amino acids and α,α-disubstituted amino acids. These data are consistent with what is known from substrate studies.[22]

The time course of the assay allows for some equilibration of the active species to its inactive cyclic form, prohibiting a true ranking of the binding ability of the inhibitors. Regardless, the net inhibition of DPPIV by these compounds demonstrates a potent effect which we have found to be an excellent predictor of the *in vitro* effects of these boronic acid dipeptides on immune function.

Experimental Section[24,25]

Synthesis of Protected Proline Boronate Ester (7). Two methods were employed to generate the intermediate **6**, which can be converted to the protected boroPro derivative **7**.

The first, which has already been described,[18] starts from Boc-pyrrole (**4**). A second procedure avoids the catalytic reduction by converting Boc-pyrrolidine (**5**) directly to the boronic acid via a procedure developed by Beak.[19] In this method Boc-pyrrolidine (1.71g, 10 mmol) was dissolved in a mixture of Et_2O (20 mL) and TMEDA (3 mL, 20 mmol) under a nitrogen atmosphere and cooled to −40 °C. A solution of *s*-BuLi (9.2 mL of a 1.3 M solution in cyclohexane, 12 mmol) was added at a rate wherein the temperature of the reaction mixture did not rise more than 5 °C. After complete addition of the *s*-BuLi the mixture was stirred for 3 h at −40 °C and then treated with $B(OMe)_3$ (3.11 g, 30 mmol). The cooling bath was then removed and the solution allowed to warm to room temperature. Once at room temperature, the reaction was quenched by the addition of H_2O and extracted into 2 N NaOH. The aqueous phase was acidified to pH = 3 using 2 N HCl and extracted with EtOAc. The extracts were dried over $MgSO_4$ and concentrated to produce compound **6** (1.75g, 81%) which was used without further purification to generate **7** as previously reported.[18]

General Method for Peptide Coupling (9). To an ice-cooled solution of the desired *N*-protected amino acid (**8**, 17.5 mmol) in CH_2Cl_2 (100 mL) was added hydroxybenzotriazole (2.37 g, 17.5 mmol) and EDC (4.32 g, 22.8 mmol). After 30 min, the pinanediol ester of proline boronic acid (**7**, 5.00 g, 17.5 mmol) and *N*-methylmorpholine (3.9 mL, 35.1 mmol) were added, and the solution was allowed to warm slowly to room temperature. After stirring overnight, the mixture was washed sequentially with water, 1 M $KHSO_4$, and Na_2CO_3 solutions. The organic layer was filtered through a plug of silica gel, eluting with EtOAc. Evaporation of the filtrate yielded the protected dipeptides **9** in 95−98% yield.

***N*-Boc-(*S*)-Val-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9a):** mp 128−130 °C; IR (film) 3350, 1710, 1640, 1460, 1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 0.91 (d, J = 7 Hz, 3 H), 0.97 (d, J = 7 Hz, 3 H), 1.27 (s, 3 H), 1.35−1.45 (m, 1 H), 1.39 (s, 3 H), 1.41 (s, 9 H), 1.72−2.14 (m, 9 H), 2.26−2.36 (m, 1 H), 3.15 (dd, J = 7, 10 Hz, 1 H), 3.43−3.51 (m, 1 H), 3.70−3.81 (m, 1 H), 4.19−4.28 (m, 2 H), 5.29 (d, J = 9 Hz, 1 H); ^{13}C NMR δ 17.3, 19.2, 24.0, 26.3, 27.1, 27.2, 27.4, 28.4, 28.6, 31.4, 33.9, 35.5, 38.2, 39.6, 46.7, 51.2, 56.6, 77.8, 79.2, 85.8, 155.9, 170.2; ^{11}B NMR δ 31.0; MS (CI) m/z 449 (MH^+, 100), 393 (50). Anal. Calcd for $C_{24}H_{41}BN_2O_5$: C, H, N.

***N*-Boc-(*S*)-Val-(*S*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9b):** ^1H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 0.91 (d, J = 7 Hz, 3 H), 0.96 (d, J = 7 Hz, 3 H), 1.27 (s, 3 H), 1.35−1.45 (m, 13 H), 1.7−2.4 (m, 10 H), 3.05 (dd, J = 7, 10 Hz, 1 H), 3.4−3.7 (m, 2 H), 4.19−4.28 (m, 2 H), 5.21 (d, J = 9 Hz, 1 H).

***N*-Boc-(*R*)-Val-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9c):** ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 0.91 (d, J = 7 Hz, 3 H), 0.97 (d, J =7 Hz, 3 H), 1.2−1.5 (m, 17 H), 1.7−2.2 (m, 8 H), 2.41 (m, 1 H), 3.05 (t, J = 10 Hz, 1 H), 3.45 (m, 1 H), 3.62 (m, 1 H), 4.15−4.25 (m, 2 H), 5.29 (d, J = 9 Hz, 1 H).

***N*-Boc-(*S*)-Ala-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9d):** oil; IR (film) 3350, 1715, 1635, 1460, 1390,1370 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.27 (s, 3 H), 1.29 (d, J = 7 Hz, 3 H), 1.30 (s, 3 H), 1.40−1.43 (m, 1 H), 1.42 (s, 9 H), 1.60−2.15 (m, 8 H), 2.25−2.40 (m, 1 H), 3.18 (dd, J = 7, 10 Hz, 1 H), 3.36−3.49 (m, 1 H), 3.62−3.75 (m, 1 H), 4.28 (dd, J = 2, 9 Hz, 1 H), 4.44 (dq, J = 7 Hz, 1 H), 5.48 (d, J = 7 Hz, 1 H); ^{13}C NMR δ 18.2, 23.9, 26.1, 27.1, 28.3, 28.4, 35.4, 38.0, 39.5, 44.4, 46.3, 47.1, 51.1, 77.7, 79.2, 84.2, 85.7, 155.1, 170.8; MS (CI) m/z 421 (MH^+); HRMS m/z calcd for $C_{22}H_{38}BN_2O_5$ 421.2874, found 421.2853.

***N*-Boc-Aibu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9e):** ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 1.2−1.6 (m, 23 H), 1.7−2.4 (m, 8 H), 3.12 (m, 1 H), 3.55 (m, 2 H), 4.22 (d, J = 13 Hz, 1 H), 5.20 (broad s, 1 H).

***N*-Boc-Gly-(*R*)-boroProl-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9f):** ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 1.2−1.3 (m, 4 H), 1.4−1.5 (m, 13 H), 1.7−2.4 (m, 8 H), 3.19 (m, 1 H), 3.45 (m, 2 H), 3.91 (m, 2 H), 4.34 (dd, J = 13, 1 Hz, 1 H), 5.55 (broad s, 1 H).

***N*-Boc-(*S*)-Abu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9g):** ^1H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 0.91 (t, J = 9 Hz, 3 H), 1.25 (s, 3 H), 1.2−1.5 (m, 13 H), 1.55−2.20 (m, 9 H), 2.30 (m, 1 H), 3.12 (m, 1 H), 3.43 (m, 1 H), 3.75 (m, 1 H), 4.2−4.4 (m, 2 H), 5.43 (d, J = 7 Hz, 1 H); MS (CI) m/z 435 (MH^+, 90), 379 (100).

***N*-Boc-(*S*)-Leu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9h):** ^1H NMR (DMSO-d_6) δ 0.83 (s, 3 H), 0.92 (d, J = 6 Hz, 3 H), 0.95 (d, J = 6 Hz, 3 H), 1.2−1.5 (m, 19 H), 1.6−2.2 (m, 8 H), 2.41 (m, 1 H), 3.15 (m, 1 H), 3.41 (m, 1 H), 3.75 (m, 1 H), 4.25 (m, 1 H), 4.45 (m, 1 H), 5.19 (d, J = 7 Hz, 1 H); MS (CI) m/z 463 (MH^+, 100).

***N*-Boc-(*S*)-Ile-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9i):** ^1H NMR (DMSO-d_6) δ 0.83 (s, 3 H), 0.92 (d, J = 7 Hz, 3 H), 0.95 (d, J = 7 Hz, 3 H), 1.28 (s, 3 H), 1.40 (m, 12 H), 1.5−2.2 (m, 12 H), 2.35 (m, 1 H), 3.20 (m, 1 H), 3.49 (m, 1 H), 3.80 (m, 1 H), 4.28 (m, 2 H), 5.21 (d, J = 7 Hz, 1 H).

***N*-Boc-(*S*)-*t*-Leu-(*R*)-boroPro-1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9j):** ^1H NMR (DMSO-d_6) δ 0.84 (s, 3 H), 1.0−1.5 (m, 26 H), 1.6−2.5 (m, 8 H), 3.15 (m, 1 H), 3.49 (m, 1 H), 3.75 (m, 1 H), 4.21 (m, 2 H), 5.42 (d, J = 7 Hz, 1 H); MS (CI) m/z 463 (MH^+, 100).

***N*-Boc-(*S*)-Phe-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9k):** ^1H NMR (DMSO-d_6) δ 0.84 (s, 3 H), 1.30 (s, 3 H), 1.3−1.5 (m, 11 H), 1.62 (m, 2 H), 1.80 (m, 2 H), 1.93 (m, 3 H), 2.05 (m, 1 H), 2.20 (m, 1 H), 2.3−2.6 (m 2 H), 2.85−3.15 (m, 4 H), 3.45 (m, 1 H), 4.35 (dd, J = 7, 1 Hz, 1 H), 4.52 (m, 1 H), 5.42 (d, J = 7 Hz, 1 H), 7.23 (m, 5 H); MS (CI) m/z 497 (MH^+, 100).

***N*-Boc-(*S*)-Phg-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9l):** ^1H NMR (DMSO-d_6) δ 0.84 (s, 3 H), 1.2−1.6 (m, 15 H), 1.6−2.1 (m, 8 H), 2.18 (m, 1 H), 2.32 (m, 1 H), 2.96 (m, 1 H), 3.26 (dd, J = 7, 6 Hz, 1 H), 3.55 (m, 1 H), 4.35 (m 1 H), 5.42 (d, J = 7 Hz, 1 H), 6.00 (d, J = 6 Hz, 1 H), 7.2−7.6 (m, 5 H); MS (CI) m/z 483 (MH^+, 100).

***N*-Boc-(*S*)-Tyr-(*O*-*t*-Bu)-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9m):** ^1H NMR ($CDCl_3$) δ 0.87 (s, 3 H), 1.2−1.8 (m, 28 H), 1.9−2.1 (m, 4 H), 2.15 (m, 1 H), 2.35 (m, 2 H), 2.91 (d, J = 7 Hz, 2 H), 3.04 (dd, J = 10, 7 Hz, 1 H), 3.42 (m, 1 H), 4.35 (dd, J = 9, 2 Hz, 1 H), 4.48 (q, J = 8 Hz, 1 H), 5.43 (d, J = 8 Hz, 1 H), 6.86 (d, J = 8 Hz, 2 H), 7.22 (d, J = 8 Hz, 2 H); MS (CI) m/z 569 (MH^+, 100).

***N*$^{\alpha}$-Fmoc-(*S*)-Lys-(*N*$^{\epsilon}$-Boc)-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9n):** ^1H NMR (DMSO-d_6) δ 0.81 (s, 3 H), 1.2−2.3 (m, 30 H), 2.38 (m, 1 H), 3.05−3.25 (m, 3 H), 3.45 (m, 1 H), 4.1−4.6 (m, 6 H), 4.75 (m, 1 H), 5.71 (d, J = 7 Hz, 1 H), 7.25−7.45 (m, 4 H), 7.58 (d, J = 6 Hz, 2 H), 7.80 (d, J = 6 Hz, 2 H); MS (CI) m/z 700 (MH^+, 100).

***N*-Boc-(*S*)-Thr-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9o):** ^1H NMR (DMSO-d_6) δ 0.83 (s, 3 H), 1.1−1.5 (m, 17 H), 1.58 (s, 3 H), 1.7−2.4 (m, 8 H), 3.25 (m, 1 H), 3.51 (m, 1 H), 3.57 (m, 2 H), 4.10 (m, 1 H), 4.31 (m, 1 H), 5.45 (d, J = 7 Hz, 1 H); MS (CI) m/z 451 (MH^+, 100).

***N*-Boc-(*S*)-Pro-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9p):** oil; IR (film) 1700, 1645, 1390, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.27 (s, 3 H), 1.38 and 1.39 (2 × s, 9 H, rotamers), 1.38−1.43 (m, 1 H), 1.43 (s, 3 H), 1.70−2.17 (m, 12 H), 2.26−2.38 (m, 1 H), 3.20 (ddd, J = 7, 10, 18 Hz, 1 H), 3.34−3.47 (m, 2 H), 3.48−3.66 (m, 1.6 H), 3.79−3.84 (m, 0.4 H), 4.25 (dt, J = 3, 9 Hz, 1 H), 4.34 (dd, J = 4, 7 Hz, 0.6 H), 4.49 (dd, J = 3, 8 Hz, 0.4 H); MS (CI) m/z 447 (MH^+, 100).

***N*-Boc-(*S*)-Azet-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9q):** ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.2−1.5 (m, 16 H), 1.6−2.5 (m, 11 H), 3.21 (m, 1 H), 3.42 (m, 1 H), 3.7−4.1 (m, 3 H), 4.32 (dd, J = 13, 1 Hz, 1 H), 4.81 (t, J = 5 Hz, 1 H); MS (CI) m/z 433 (MH^+, 40), 333 (100).

***N*-Boc-(*S*)-His-(*N*-Boc)-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (9r):** ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.2−1.5 (m, 16 H), 1.58 (s, 3 H), 1.65−2.20 (m, 14 H), 2.35 (m, 1 H), 2.75 (m, 1 H), 2.99 (m, 1 H), 3.18 (m, 1 H), 3.45 (m, 1 H), 3.69 (m, 1 H), 4.23 (dd, J = 9, 2 Hz, 1 H), 4.70 (m, 1 H), 5.43 (d, J = 8 Hz, 1 H), 7.20 (s, 1 H), 8.00 (s, 1 H); MS (CI) m/z 587 (MH^+, 85), 487 (100).

General Method for the Removal of the Boc Group (10). The protected dipeptide (3 mmol) was treated with a saturated solution of HCl in Et_2O (50 mL), with stirring at 0 °C. The solution was allowed to warm to room temperature over 3 h. The solvent was evaporated to produce the desired

hydrochlorides (**10**) in nearly quantitative yields. When noted, the HCl salt was exchanged for the maleate or MgOH salt.

H_2N-(*S*)-Val-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10a): mp 145−146 °C; $[\alpha]^{25}_D$ −48.3° (*c* 0.57, CH_2Cl_2); IR 1629, 1583, 1483 (B−O stretch) cm^{-1}; ^1H NMR δ 0.84 (s, 3 H), 1.08 (d, J = 7 Hz, 3 H), 1.13 (d, J = 7 Hz, 3 H), 1.26−1.31 (m, 2 H), 1.29 (s, 3 H), 1.38 (s, 3 H), 1.72−2.15 (m, 7 H), 2.24−2.38 (m, 2 H), 3.28 (dd, J = 7, 9 Hz, 1 H), 3.38−3.47 (m, 1 H), 3.73−3.78 (m, 1 H), 4.14 (d, J = 5 Hz, 1 H), 4.26 (d, J = 7 Hz, 1 H), 6.25 (s, 2 H), 7.5−9.0 (v br, 2 H); ^{13}C NMR δ 17.0, 18.4, 24.0, 26.3, 27.0, 27.1, 28.7, 30.0, 35.4, 38.2, 39.5, 47.3, 51.2, 56.6, 78.1, 86.2, 135.6, 166.3, 169.5; ^{11}B NMR δ 29.6; MS (CI) m/z 349 (MH^+, 100), 197 (18). Anal. Calcd for $C_{23}H_{37}BN_2O_7$: C, H, N.

***N*-Boc-(*S*)-Val-(*S*)-boroPro-OH (10b)** (note: the order of removal of the protecting groups was reversed for the synthesis of **11b**, *i.e.* the pinanediol group was removed first as described below before the Boc group was cleaved with HCl): ^1H NMR ($CDCl_3$) δ 0.95 (m, 6 H), 1.2−1.5 (m 10 H), 1.7−2.2 (m, 4 H), 2.95 (m, 1 H), 3.45 (m, 2 H), 4.20 (m, 1 H), 5.00 (d, J = 9 Hz, 1 H).

***cyclo*-H_2N-(*R*)-Val-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (10c)** (note that the free base (*i.e. cyclo* form) was generated by washing the CH_2Cl_2 with aqueous Na_2CO_3): ^1H NMR (DMSO-d_6) δ 0.86 (s, 3 H), 1.01 (d, J = 7 Hz, 6 H), 1.2−1.4 (m, 8 H), 1.7−2.1 (m, 9 H), 2.35 (m, 1 H), 2.58 (m, 1 H), 2.71 (dd, J = 6, 7 Hz, 1 H), 3.45 (m, 3 H), 4.13 (m, 1 H); MS (CI) m/z 349 (MH^+, 100).

H_2N-(*S*)-Ala-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, methanesulfonate (10d): mp 205−215 °C dec; $[\alpha]^{25}_D$ −46.4° (*c* 0.52, CH_2Cl_2); IR 3417, 3300−2800, 1638, 1391, 1375, 1250−1140, 1038 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 1.20 (d, J = 11 Hz, 1 H), 1.27 (s, 3 H), 1.39 (s, 3 H), 1.51 (d, J = 7 Hz, 3 H), 1.75−1.91 (m, 3 H), 1.95−2.18 (m, 4 H), 2.21−2.38 (m, 2 H), 2.75 (s, 3 H), 3.24−3.38 (m, 2 H), 3.74−3.85 (m, 1 H), 4.26 (dd, J = 2 and 9 Hz, 1 H), 4.25−4.40 (m, 1 H), 7.81 (br, 3 H); ^{13}C NMR δ 16.1, 24.0, 26.1, 27.01, 27.07, 27.13, 28.5, 35.4, 38.1, 39.2, 39.4, 44.4, 46.4, 47.8, 51.1, 77.8, 86.0, 167.6; ^{11}B NMR δ 32.3; MS (CI) m/z 321 (MH^+, 100), 169 (40); HRMS m/z calcd for $C_{17}H_{30}BN_2O_3$ 321.2350, found 321.2337. Anal. (MsOH salt). Calcd for $C_{18}H_{33}BN_2O_6S$: C, H, N, S.

H_2N-Aibu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10e): ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 1.2−1.4 (m, 14 H), 1.6−2.2 (m, 8 H), 3.30 (m, 1 H), 3.65 (m, 2 H), 4.22 (d, J = 13 Hz, 1 H), 8.60 (broad s, 3 H).

H_2N-Gly-(*R*)-boroProl-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10f): ^1H NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.1−1.5 (m, 6 H), 1.7−2.4 (m, 10 H), 3.25 (m, 1 H), 3.4−3.6 (m, 2 H), 3.90 (m, 1 H), 4.10 (m, 1 H), 4.30 (d, J = 13 Hz, 1 H), 8.45 (broad s, 3 H).

H_2N-(*S*)-Abu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10g): ^1H NMR ($CDCl_3$) δ 0.80 (s, 3 H), 1.05 (t, J = 9 Hz, 3 H), 1.2−1.3 (m, 4 H), 1.40 (s, 3 H), 1.7−2.2 (m, 10 H), 2.30 (m, 1 H), 3.30 (m, 1 H), 3.43 (m, 1 H), 3.80 (m, 1 H), 4.2−4.4 (m, 2 H), 8.42 (broad s, 3 H); MS (CI) m/z 335 (MH^+, 100).

H_2N-(*S*)-Leu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10h): mp 55−60 °C; ^1H NMR ($CDCl_3$) δ 0.82 (s, 3 H), 0.98 (d, J = 6 Hz, 6 H), 1.26 (s, 3 H), 1.37 (s, 3 H), 1.6−2.4 (m, 13 H), 3.33 (m, 2 H), 3.86 (m, 1 H), 4.27 (m, 2 H), 8.50 (broad s, 2 H); MS (CI) m/z 363 (MH^+, 100).

H_2N-(*S*)-Ile-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (10i), hydrochloride: ^1H NMR (DMSO-d_6) δ 0.81 (s, 3 H), 0.95 (m, 6 H), 1.12 (d, J = 9 Hz, 3 H), 1.2−1.5 (m, 9 H), 1.6−2.2 (m, 6 H), 2.80 (m, 1 H), 3.35 (m, 2 H), 3.85 (m, 1 H), 4.15 (m, 1 H), 4.21 (d, J = 6 Hz, 1 H), 8.34 (broad s, 2 H).

H_2N-(*S*)-*t*-Leu-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10j): ^1H NMR (DMSO-d_6) δ 0.85 (s, 3 H), 1.0−1.4 (m, 18 H), 1.6−2.2 (m, 7 H), 2.80 (m, 1 H), 3.2−3.5 (m, 2 H), 3.99 (m, 2 H), 4.25 (d, J = 6 Hz, 1 H), 8.35 (broad s, 3 H).

H_2N-(*S*)-Phe-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10k): ^1H NMR ($CDCl_3$) δ 0.85 (s, 3 H), 1.19−2.49 (m, 17 H), 3.17 (m, 2 H), 3.50 (m, 2 H), 4.32 (d, J = 8 Hz, 1 H), 4.42 (broad s, 1 H), 7.22 (m, 3 H), 7.48 (m, 2 H), 8.50 (broad s, 3 H); MS (CI) m/z 397 (MH^+, 100).

H_2N-(*S*)-Phg-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10l): ^1H NMR (DMSO-d_6) δ 0.84 (s, 3 H), 1.1−1.4 (m, 8 H), 1.6−2.1 (m, 6 H), 2.2−2.4 (m, 2 H), 2.45 (m, 1 H), 3.05−3.35 (m, 2 H), 3.66 (m, 1 H), 4.30 (dd, J = 6, 1 Hz, 1 H), 5.41 (m, 1 H), 7.38 (m, 3 H), 7.65 (m, 2 H), 8.75 (broad s, 3 H); MS (CI) m/z 383 (MH^+, 100).

***cyclo*-H_2N-(*S*)-Tyr-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester (10m)** (note: both the *O*-*t*-Bu and Boc groups were simultaneously removed by running this reaction in TFA in CH_2Cl_2 instead of HCl; also note that the free base (*i.e. cyclo* form) was generated by washing the CH_2Cl_2 with aqueous Na_2CO_3): ^1H NMR (DMSO-d_6) δ 0.81 (s, 3 H), 0.97 (s, 3 H), 1.14 (s, 3 H), 1.1−2.0 (m, 10 H), 2.12 (m, 1 H), 2.55 (m, 1 H), 2.75 (dd, J = 10, 3 Hz, 1 H), 3.17 (m, 2 H), 3.55 (m, 1 H), 3.92 (d, J = 8 Hz, 1 H), 4.82 (broad s, 2 H), 6.70 (d, J = 8 Hz, 2 H), 7.08 (d, J = 8 Hz, 2 H), 9.24 (s, 1 H); MS (CI) m/z 413 (MH^+, 100).

H_2N-(*S*)-Lys-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol Ester, Hydrochloride (10n) (note: the FMOC group was removed first by treatment of **9n** with piperidine in CH_2Cl_2 before treating with HCl to remove the Boc group). Compound **10n** was not isolated before conversion to **11n**.

H_2N-(*S*)-Thr-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10o): mp 100−105 °C; ^1H NMR (DMSO-d_6) δ 0.83 (s, 3 H), 1.05−1.32 (m, 11 H), 1.55−2.15 (m, 8), 2.30 (m, 1 H), 3.01 (dd, J = 7, 9 Hz, 1 H), 3.20−3.50 (m, 2 H), 3.75 (m, 2 H), 4.39 (d, J = 9 Hz, 1 H), 5.58 (broad s, 1 H), 8.30 (br s, 2 H); MS (CI) m/z 351 (MH^+, 100).

HN-(*S*)-Pro-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10p): mp 190 °C dec; $[\alpha]^{25}_D$ −114.2° (*c* 0.52, CH_2Cl_2); IR 2967, 2909, 2877, 2489, 1630, 1547, 1470, 1387, 1367 cm^{-1}; ^1H NMR δ 0.83 (s, 3 H), 1.19 (d, J = 11 Hz, 1 H), 1.28, (s, 3 H), 1.37 (s, 3 H), 1.78−2.16 (m, 11 H), 2.28−2.54 (m, 2 H), 3.32−3.42 (m, 3 H), 3.51−3.70 (m, 2 H), 4.27 (dd, J = 2 and 9 Hz, 1 H), 4.61 (br, 1 H), 7.20 (br, 2 H); ^{13}C NMR δ 23.8, 24.2, 25.9, 26.75, 26.79, 26.81, 28.4, 28.7, 35.1, 38.0, 39.2, 44.6, 46.4, 46.5, 50.9, 58.4, 77.8, 85.9, 165.7; ^{11}B NMR δ 33.3; MS (CI) m/z 347 (MH^+, 100). Anal. Calcd for $C_{19}H_{32}BClN_2O_3$: C, H, N, B, Cl.

H_2N-(*S*)-Azet-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, maleate (10q): mp 164 °C dec; ^1H NMR ($CDCl_3$) δ 0.83 (s, 3 H), 1.19 (m, 5 H), 1.29 (s, 2 H), 1.80−2.52 (m, 10 H), 2.91−3.62 (m, 4 H), 4.00 (m, 1 H), 4.33 (m, 2 H), 5.26 (t, J = 7 Hz, 1 H), 6.32 (s, 2 H), 11.64 (br s, 2 H); MS (CI) m/z 333 (MH^+, 100).

H_2N-(*S*)-His-(*R*)-boroPro-(1*S*,2*S*,3*R*,5*S*)-pinanediol ester, hydrochloride (10r) (note: imidazole protecting group is removed during this reaction): mp 155−60 °C; ^1H NMR ($CDCl_3$) δ 0.81 (s, 3 H), 1.1−1.5 (m, 7 H), 1.6−2.5 (m, 9 H), 3.36 (m, 3 H), 3.67 (m, 1 H), 4.07 (m, 1 H), 4.37 (m, 1 H), 4.80 (m, 1 H), 7.67 (s, 1 H), 8.37 (broad s, 3 H), 9.08 (s, 1 H); MS (CI) m/z 387 (MH^+, 85), 135 (100).

General Method for the Synthesis of Cyclic Peptides 11. A solution of the desired amine salt (**10**, 10 mmol) in H_2O (100 mL) was adjusted to pH = 2 by addition of dilute HCl. Hexane (100 mL) and phenyl boric acid (1.28 g, 10.5 mmol) were added, and the two-phase mixture was stirred vigorously. The hexane layer was replaced with fresh hexane after 30, 60, 90, and 120 min. After continuing stirring overnight, the aqueous layer was separated and applied to a Dowex 50-X2-100 ion exchange column (H^+ form) and eluted with water until the eluate was neutral. Elution was continued with aqueous ammonium hydroxide (1:50 dilution), and appropriate fractions lyophillized to yield the cyclic free boronic acid in 90−95% yield.

***cyclo*-(*S*)-Val-(*R*)-boroPro (11a):** mp 120−130 °C; $[\alpha]^{25}_D$ −81.0° (*c* 0.52, H_2O); IR 3400−3314, 3221−3108, 2961−2872, 1637, 1452−1369 cm^{-1}; ^1H NMR (D_2O) δ 0.97 (d, J = 7 Hz, 3 H), 1.06 (d, J = 7 Hz, 3 H), 1.59−1.80 (m, 2 H), 1.95−2.03 (m, 2 H), 2.41−2.51 (m, 1 H), 2.62−2.69 (m, 1 H), 3.23−3.32 (m, 1 H), 3.51−3.58 (m with overlapping doublet, J = 4 Hz, 2 H); ^{13}C NMR (D_2O) δ 19.0, 21.7, 27.3, 30.7, 29.9, 49.6, 61.0, 170.3; ^{11}B (D_2O) NMR δ 2.7; MS (CI) m/z 375 ($M_2H^+ - 3H_2O$, 90), 197 ($MH^+ - H_2O$, 100). Anal. Calcd for $C_9H_{19}BN_2O_3$: C, H, N, B.

***cyclo*-(*S*)-Val-(*S*)-boroPro (11b):** ^{1}H NMR (D_2O) δ 0.90 (d, J = 7 Hz, 3 H), 1.05 (d, J = 7 Hz, 3 H), 1.4−1.9 (m, 2 H), 2.00 (m, 2 H), 2.55 (m, 1 H), 2.65 (m, 1 H), 3.3−3.6 (m, 3 H).

***cyclo*-(*R*)-Val-(*R*)-boroPro (11c):** ^{1}H NMR (D_2O) δ 0.93 (d, J = 7 Hz, 3 H), 1.06 (d, J = 7 Hz, 3 H), 1.4−1.8 (m, 2 H), 2.00 (m, 2 H), 2.51 (m, 1 H), 2.65 (m, 1 H), 3.2−3.5 (m, 3 H).

***cyclo*-(*S*)-Ala-(*R*)-boroPro (11d):** mp 80−92 °C dec; $[\alpha]^{25}_D$ −59.3° (*c* 0.58, H_2O); IR 3437, 3211, 3099, 1622 cm^{-1}; ^{1}H NMR (D_2O) δ 1.44 (d, J = 7 Hz, 3 H), 1.54−1.78 (m, 2 H), 1.91−2.00 (m, 2 H), 2.61 (dd, J = 6 and 12 Hz, 1 H), 3.30 (dt, J = 8 and 9 Hz, 1 H), 3.50 (t, J = 10 Hz, 1 H), 3.81 (q, J = 7 Hz, 1 H); ^{13}C NMR (D_2O) δ 16.6, 24.5, 27.9, 47.3, 49.9, 53.2, 168.9; ^{11}B NMR (D_2O) δ 2.8; MS (CI) m/z 169 ($MH^+ - H_2O$, 100). HRMS (glycerol adduct) m/z calcd for $C_{10}H_{20}BN_2O_4$ 243.1516, found 243.1513. Anal. Calcd for $C_7H_{15}BN_2O_3 \cdot 0.3H_2O$: C, H, N.

***cyclo*-Aibu-(*R*)-boroPro (11e):** ^{1}H NMR (D_2O) δ 1.5−1.9 (m, 8 H), 2.01 (m, 2 H), 2.71 (m, 1 H), 3.3−3.6 (m, 2 H).

***cyclo*-Gly-(*R*)-boroPro (11f):** mp 245−50 °C dec; ^{1}H NMR (D_2O) δ 1.51−1.85 (m, 2 H), 1.95−2.10 (m, 2 H), 2.59−2.65 (m, 1 H), 3.34−3.68 (m, 4 H); MS (ethylene glycol adduct CI) m/z 199 (MH^+, 100).

***cyclo*-(*S*)-Abu-(*R*)-boroPro (11g):** MS (CI, ethylene glyocol adduct) m/z 227 (MH^+, 100).

***cyclo*-(*S*)-Leu-(*R*)-boroPro (11h):** mp 130 −2 °C; ^{1}H NMR (D_2O) δ 0.89 (d, J = 6 Hz, 3 H), 0.93 (d, J = 6 Hz, 3 H), 1.4−2.1 (m, 7 H), 2.60 (dd, J = 11, 6 Hz, 1 H), 3.2−3.8 (m, 3 H); MS (CI, ethylene glyocol adduct) m/z 255 (MH^+, 100).

***cyclo*-(*S*)-Ile-(*R*)-boroPro (11i):** ^{1}H NMR (D_2O) δ 0.95 (t, J = 9 Hz, 3 H), 1.05 (d, J = 7 Hz, 3 H), 1.20 (m, 1 H), 1.51 (m, 1 H), 1.75 (m, 2 H), 2.01 (m, 2 H), 2.20 (m, 1 H), 2.58 (m, 1 H), 3.30 (m, 1 H), 3.5−3.7 (m, 2 H).

***cyclo*-(*S*)-*t*-Leu-(*R*)-boroPro (11j):** ^{1}H NMR (D_2O) δ 1.0−1.32 (m, 9 H), 1.75 (m, 2 H), 2.10 (m, 2 H), 2.75 (m, 1 H), 3.25 (m, 1 H), 3.40 (m, 1 H), 3.60 (m, 1 H); MS (CI, ethylene glyocol adduct) m/z 255 (MH^+, 100).

***cyclo*-(*S*)-Phe-(*R*)-boroPro (11k):** mp:120−5 °C; ^{1}H NMR (D_2O) δ 1.59−1.79 (m, 2 H), 1.92−1.99 (m, 2 H), 2.62 (q, J = 6 Hz, 1 H) 3.03 (dd, J = 10, 4 Hz, 1 H), 3.26−3.57 (m, 3 H), 4.01 (dd, J = 6,5 Hz, 1 H), 7.32−7.46 (m, 5 H).

***cyclo*-(*S*)-Phg-(*R*)-boroPro (11l):** ^{1}H NMR (D_2O) δ 1.7−2.2 (m, 4 H), 2.85 (m, 1 H), 3.35−3.70 (m, 2 H), 4.90 (s, 1 H), 7.4−7.7 (m, 5 H); MS (CI, ethylene glyocol adduct) m/z 275 (MH^+, 100).

***cyclo*-(*S*)-Tyr-(*R*)-boroPro (11m):** ^{1}H NMR (D_2O) δ 1.5−2.1 (m, 4 H), 2.52 (m, 1 H), 2.94 (dd, J = 11, 9 Hz, 1 H), 3.35 (m, 2 H), 3.55 (m, 1 H), 3.91 (dd, J = 8, 6 Hz, 1 H), 6.91 (d, J = 8 Hz, 2 H), 7.21 (d, J = 8 Hz, 2 H).

***cyclo*-(*S*)-Lys-(*R*)-boroPro (11n):** mp 60−70 °C; ^{1}H NMR (D_2O) δ 1.31−1.82 (m, 8 H), 1.88−2.06 (m, 3 H), 2.63 (m, 1 H), 2.95 (m, 1 H), 3.25 (m, 1 H), 3.54 (m, 1 H), 3.62 (m, 1 H); MS (CI, ethylene glyocol adduct) m/z 270 (MH^+, 100).

***cyclo*-(*S*)-Thr-(*R*)-boroPro (11o):** ^{1}H NMR (appears to be a mixture of cyclic forms which exist in a 2:1 ratio, only the major peaks are reported, D_2O) δ 1.13 (m, 3 H), 1.5−2.1 (m, 4 H), 3.2−3.6 (m, 2 H), 3.8 (m, 1 H), 3.90 (d, J = 7 Hz, 1 H), 4.1 (m, 1 H).

***cyclo*-(*S*)-Pro-(*R*)-boroPro (11p):** mp 215−219 °C; $[\alpha]^{25}_D$ −84.2° (*c* 0.5, H_2O); IR 3429, 3387, 3123, 1612, 1486, 1393, 1325 cm^{-1}; ^{1}H NMR (D_2O) δ 1.56−2.03 (m, 7 H), 2.24−2.33 (m, 1 H), 2.66 (dd, J = 6 and 12 Hz, 1 H), 3.10−3.33 (m, 3 H), 3.49 (dd, J = 9 and 12 Hz, 1 H), 3.99 (t, J = 9 Hz, 1 H); ^{13}C NMR (D_2O) δ 25.6, 26.8, 30.2, 31.2, 46.0, 47.1, 49.7, 64.1, 170.0; ^{11}B NMR (D_2O) δ 2.0; MS (CI) m/z 239 (MH^+, 100); HRMS (glycerol adduct) m/z calcd for $C_{12}H_{22}BN_2O_4$ 269.1673, found 269.1657.

***cyclo*-(*S*)-Azet-(*R*)-boroPro (11q):** ^{1}H NMR (D_2O) δ 1.61−1.82 (m, 2 H), 1.90−2.04 (m, 2 H), 2.47−2.64 (m, 4 H), 3.26−3.37 (m, 1 H), 3.47−3.67 (m, 2 H), 3.85 (dd, J = 13, 1 Hz, 1 H); MS (CI, ethylene glyocol adduct) m/z 225 (MH^+, 100).

***cyclo*-(*S*)-His-(*R*)-boroPro (11r):** ^{1}H NMR (D_2O, 7:3 mixture of imidazole tautomers by ^{1}H NMR; only major isomer peaks are reported) δ 0.73−0.92 (m, 1 H), 1.55−2.11 (m, 3 H), 2.89 (dd, J = 15, 6 Hz, 1 H), 3.4−3.5 (m, 5 H), 4.30 (m, 1 H), 6.99 (s, 1 H), 7.95 (s, 1 H).

General Method for the Synthesis of Open Boronic Acid Dipeptides (1). Methanesulfonate Salts. To a stirred suspension of the cyclic boronic acid **11** (25 mmol) in MeCN (190 mL) under nitrogen was added a solution of methanesulfonic acid (25 mmol) in MeCN (10 mL), dropwise over 5 min, and the mixture stirred at room temperature for 2 h. The product was collected by filtration, washed well with MeCN and Et_2O, and dried to afford the dipeptide salt in 80−90% yield. Alternatively, the reaction can be run in MeOH instead of MeCN in which case the solvent is removed by rotary evaporation and the salt solidified by trituration with Et_2O. Hydrochloride salts were generated in a similar manner using dry HCl in EtOAc.

H_2N-(*S*)-Val-(*R*)-boroPro-OH methanesulfonate (1a): mp 181−182 °C; $[\alpha]^{25}_D$ −42.4° (*c* 1.0, H_2O, pH = 2); IR 3387, 3000 (br), 2972, 2655, 1646, 1370 (B−O stretch), 1197 cm^{-1}; ^{1}H NMR (D_2O) δ 0.99 (d, J = 7 Hz, 3 H), 1.09 (d, J = 7 Hz, 3 H), 1.69−1.75 (m, 1 H), 1.90−1.99 (m, 1 H), 2.10−2.14 (m, 2 H), 2.28−2.35 (m, 1 H), 2.80 (s, 3 H), 3.07 (dd, J = 7, 11 Hz, 1 H), 3.46−3.51 (m, 1 H), 3.75 (t, J = 9 Hz, 1 H), 4.14 (d, J = 5 Hz, 1 H); the *cis* amide rotamer (ca. 3%) is also observed at 3.53−3.55 (m) and 3.83 (d, J = 6 Hz); ^{13}C NMR (D_2O) δ 16.2, 18.4, 26.9, 27.1, 29.0, 38.8, 47.9, 49.0, 57.2, 167.2; peaks due to the *cis* amide rotamer are observed at 16.8, 24.3, 29.9, 57.8, 167.5; ^{11}B NMR (D_2O) δ 31.5; MS (CI) (ethylene glycol adduct) m/z 241 (MH^+, 100). Anal. Calcd for $C_{10}H_{23}BN_2O_6S$: C, H, N, B, S.

H_2N-(*S*)-Val-(*S*)-boroPro-OH hydrochloride (1b): mp 207 °C dec; ^{1}H NMR (D_2O) δ 0.99 (m, 6 H), 1.60−1.71 (m, 1 H), 1.70−2.24 (m, 4 H), 3.05 (t, J = 16 Hz, 1 H), 3.41−3.70 (m, 2 H), 4.05 (d, J = 5 Hz, 1 H); MS (CI) (glycerol adduct) m/z 271 (MH^+, 100).

H_2N-(*R*)-Val-(*R*)-boroPro-OH hydrochloride (1c): mp 170−7 °C dec; ^{1}H NMR (D_2O) δ 1.04 (m, 6 H), 1.75−1.80 (m, 1 H), 1.99−2.14 (m, 3 H), 2.25−2.30 (m, 1 H), 3.12 (t, J = 16 Hz, 1 H), 3.56−3.69 (m, 2 H), 4.11 (d, J = 5 Hz, 1 H); ^{13}C NMR (D_2O) δ 16.7, 18.1, 26.7, 26.8, 47.7, 48.1, 56.7, 167.1; MS (CI) (glycerol adduct) m/z 271 (MH^+, 100).

H_2N-(*S*)-Ala-(*R*)-boroPro-OH, methanesulfonate (1d): mp 114−20 °C dec; $[\alpha]^{25}_D$ −47.5° (*c* 0.55, H_2O); IR 3400−2900 (br), 1642, 1513 1405, 1213, 1175, 1042 cm^{-1}; ^{1}H NMR (D_2O) δ 1.48 (d, J = 7 Hz, 3 H), 1.63−1.75 (m, 1 H), 1.84−2.01 (m, 1 H), 2.03−2.16 (m, 2 H), 2.78 (s, 3 H), 3.05 (ddd, J = 6, 8 and 10 Hz, 2 H), 3.69 (t, J = 8 Hz, 1 H), 4.31 (q, J = 7 Hz, 1 H); ^{13}C NMR (D_2O) δ 15.1, 27.0, 27.1, 38.7, 47.3, 48.1, 48.6, 168.2; ^{11}B NMR (D_2O) δ 31.4; MS (CI) (ethylene glycol adduct) m/z 213 (MH^+, 100), 142 (47); HRMS (glycerol adduct) m/z calcd for $C_{10}H_{20}BN_2O_4$ 243.1516, found 243.1527. Anal. (MsOH salt) Calcd for $C_8H_{19}BN_2O_6S$: C, H, N, S.

H_2N-Aibu-(*R*)-boroPro-OH hydrochloride (1e): mp 115−21 °C dec; ^{1}H NMR (D_2O) δ 1.44−1.52 (m, 1 H), 1.60 (s, 3 H), 1.63 (s, 3 H), 1.90−2.13 (m, 3 H), 2.99 (m, 1 H), 3.53−3.74 (m, 2 H); MS (CI) (ethylene glycol adduct) m/z 227 (MH^+, 15), 283 (100)

H_2N-Gly-(*R*)-boroProl-OH hydrochloride (1f): mp 130−5 °C dec; ^{1}H NMR (D_2O) δ 1.70−1.81 (m, 1 H), 1.93−2.16 (m, 3 H), 3.07−3.14 (m, 1 H), 3.39−3.60 (m, 2 H), 3.95 (s, 2 H); MS (CI) (ethylene glycol adduct) m/z 199 (MH^+, 35), 187 (100).

H_2N-(*S*)-Abu-(*R*)-boroPro-OH hydrochloride (1g): mp 231−5 °C; ^{1}H NMR (D_2O) δ 1.09 (t, J = 10 Hz, 3 H), 1.55−2.30 (m, 6 H), 3.14 (dd, J = 15, 1 Hz, 1 H), 3.59 (m, 1 H), 3.72 (t, J = 5 Hz, 1 H), 4.45 (t, J = 1 Hz, 1 H); ^{13}C NMR (D_2O) δ 8.3, 23.1, 26.8, 27.1, 47.6, 48.8 (broad), 53.0, 167.4; MS (CI) (ethylene glycol adduct) m/z 227 (MH^+, 100). Anal. Calcd for $C_8H_{23}BN_2O_3 \cdot 0.9HCl$: C, H, N.

H_2N-(*S*)-Leu-(*R*)-boroPro-OH hydrochloride (1h): mp 194−8 °C; ^{1}H NMR (D_2O) δ 0.98 (d, J = 4 Hz, 6 H), 1.70 (m, 4 H), 1.91−2.14 (m, 3 H), 3.07 (dd, J = 11,7 Hz, 1 H), 3.46 (m, 1 H), 3.75 (t, J = 9 Hz, 1 H), 4.26 (m, 1 H); MS (CI) (ethylene glycol adduct) m/z 255 (MH^+, 100).

H_2N-(*S*)-Ile-(*R*)-boroPro-OH hydrochloride (1i): ^{1}H NMR (D_2O) δ 0.9−1.4 (m, 7 H), 1.55 (m, 1 H), 1.75 (m, 1 H), 1.9−2.3 (m, 4 H), 3.15 (dd, J = 11, 7 Hz, 1 H), 3.55 (m, 1 H), 3.80 (m, 1 H), 4.21 (d, J = 7 Hz, 1 H); ^{13}C NMR (D_2O) δ 10.6, 14.5, 23.3, 26.9, 27.9, 35.5, 48.0, 48.9 (broad), 56.6, 167.2; MS (CI) (ethylene glycol adduct) m/z 255 (MH^+, 100). Anal. Calcd for $C_{10}H_{21}BN_2O_3 \cdot 0.25HCl$: C, H, N.

H_2N-(*S*)-*t*-Leu-(*R*)-boroPro-OH hydrochloride (1j): mp 243−7 °C; ^1H NMR (D_2O) δ 1.21 (s, 9 H), 1.77−1.95 (m, 1 H), 1.97−2.27 (m, 3 H), 3.18 (dd, J = 7,4 Hz, 1 H), 3.59 (m, 1 H), 3.92 (m, 1 H), 4.21 (s, 1 H); MS (CI) (ethylene glycol adduct) m/z 255 (MH^+, 100).

H_2N-(*S*)-Phe-(*R*)-boroPro-OH hydrochloride (1k): mp 108 °C; ^1H NMR (maleate salt, D_2O) δ 1.55−2.18 (m, 4 H), 2.90−3.42 (m, 4 H), 3.71−3.79 (m, 1 H), 4.50 (dd, J = 7, 6 Hz, 1 H), 6.45 (s, 2 H), 7.20−7.45 (m, 5 H); MS (CI) (ethylene glycol adduct) m/z 289 (MH^+, 15), 343 (100).

H_2N-(*S*)-Phg-(*R*)-boroPro-OH hydrochloride (1l): mp 157 °C; ^1H NMR (D_2O) δ 1.49−2.09 (m, 4 H), 2.79 (q, J = 9 Hz, 1 H), 3.13 (m, 1 H), 3.68 (m, 1 H), 5.36 (s, 1 H), 7.54 (s, 5 H); MS (CI) (ethylene glycol adduct) m/z 275 (MH^+, 100).

H_2N-(*S*)-Tyr-(*R*)-boroPro-OH hydrochloride (1m): mp 106−10 °C; ^1H NMR (D_2O) δ 1.65−1.71 (m, 1 H), 1.90−2.06 (m, 3 H), 2.92−3.25 (m, 4 H), 3.70 (m, 1 H), 4.56 (t, J = 10 Hz, 1 H), 6.87 (d, J = 5 Hz, 2 H), 7.20 (d, J = 5 Hz, 2 H); ^{13}C NMR (D_2O) δ 26.7, 26.9, 35.0, 47.6, 48.9 (broad), 53.4, 116.1, 125.6, 131.1, 131.4, 155.4, 166.8; MS (CI) (ethylene glycol adduct) m/z 305 (MH^+, 18), 195 (100). Anal. Calcd for $C_{13}H_{19}BN_2O_4 \cdot H_2O$: C, H, N.

H_2N-(*S*)-Lys-(*R*)-boroPro-OH dihydrochloride (1n): mp 205−10 °C; ^1H NMR (D_2O) δ 1.53 (m, 2 H), 1.76 (m, 3 H), 1.99 (m, 3 H), 2.15 (m, 2 H), 3.04−3.15 (m, 3 H), 3.50 (m, 1 H), 3.80 (m, 1 H), 4.37 (m, 1 H); MS (CI) (ethylene glycol adduct) m/z 270 (MH^+, 100).

H_2N-(*S*)-Thr-(*R*)-boroPro-OH hydrochloride (1o): mp 260−70 °C dec; ^1H NMR (D_2O) δ 1.33 (d, J = 4 Hz, 3 H), 1.73 (m, 1 H), 1.81−2.21 (m, 3 H), 3.09 (dd, J = 11,3 Hz, 1 H), 3.50 (m, 1 H), 3.79 (m, 1 H), 4.23 (m, 2 H).

HN-(*S*)-Pro-(*R*)-boroPro-OH methanesulfonate (1p): mp 138−147 °C dec; $[\alpha]^{25}_D$ −103.1° (*c* 2.0, H_2O); IR 3407, 3159−3149, 2878, 1645, 1407, 1283, 1202, 1176, 1045 cm^{-1}; ^1H NMR (D_2O) δ 1.76−1.89 (m, 1 H), 2.02−2.25 (m, 6 H), 2.56−2.66 (m, 1 H), 2.89 (s, 3 H), 3.17 (dd, J = 7 and 11 Hz, 1 H), 3.47−3.58 (m, 3 H), 3.79 (t, J = 9 Hz, 1 H), 4.68 (t, J = 7 Hz, 1 H); ^{13}C NMR (D_2O) δ 24.1, 26.9, 27.0, 28.4, 38.7, 46.7, 47.4, 48.7, 59.2, 167.0; ^{11}B NMR (D_2O) δ 30.7; MS (CI) (ethylene glycol adduct) m/z 239 (MH^+, 50), 97 (100). Anal. Calcd for $C_{10}H_{21}BN_2O_6S$: C, H, N, S.

H_2N-(*S*)-Azet-(*R*)-boroPro-OH hydrochloride (1q): mp oil; ^1H NMR (D_2O) δ 1.55−2.25 (m, 4 H), 2.66 (m, 1 H), 2.78 (m, 1 H), 3.05 (m, 1 H), 3.25 (m, 1 H), 3.45 (m, 1 H), 4.05 (m, 1 H), 4.15 (m, 1 H), 5.25 (m, 1 H); MS (CI) (glycerol adduct) m/z 225 (MH^+, 100).

H_2N-(*S*)-His-(*R*)-boroPro-OH hydrochloride (1r) (note: this compound exists as an eight-membered cyclic compound with a dative bond between an imidazoyl N and B): mp 280 °C dec; ^1H NMR (D_2O) δ 0.72−1.02 (m, 1 H), 1.65−2.21 (m, 3 H), 2.90−3.49 (m, 6 H), 4.40 (m, 1 H), 7.25 (s, 1 H), 8.40 (s, 1 H); ^{11}B NMR (D_2O) δ 0.

DPPIV Enzyme Assay. Dipeptidyl peptidase IV (DPPIV, EC 3.4.14.5) activity was assayed using the procedure previously described[21a] based upon the method of Smith and Van Frank.[21b] The DPPIV assay is a colormetric assay employing the substrate-specific dipeptide L-alanyl-L-proline-(4-methoxy-2-naphthylamide) (Ala-Pro-MNA) (Sigma Chemical Co., St. Louis, MO). Briefly, 50 μL of purified DPPIV was added to 60 μL of 0.91 mM Ala-Pro-MNA in a solution containing 0.1 M Tris·HCl pH 7.8, 1% Triton X-100, 0.01% NaN_3, (Sigma), 2.3% *N*,*N*-dimethylformamide (DMF) (EM Science, Cherry Hill, NJ) and varying concentrations of boronic acid dipeptides and incubated at 37 °C for 60 min. At the end of 60 min, 50 μL of 4-(dimethylamino)cinnamaldehyde, 3.3 mg/mL, was added, and the optical density at 570 nm was measured. (SLT 340ATTC plate reader, Hillsborough, NC).

Dipeptidyl Peptidase II Assay. Dipeptidyl peptidase II (DPPII, EC 3.4.14.2, Enzyme Systems Products, Dublin, CA) was diluted 1:500 into 20 mM sodium acetate pH 5.5 buffer and 50 μL was incubated at 37 °C with 50 μL of 1 mM Lys-Pro-MNA (Enzyme Systems Products) for 60 min at which time 50 μL of 4-(dimethylamino)cinnamaldehyde (3.3 mg/mL) was added and the resulting color measured at 570 nm on a SLT 340ATTC plate reader.

Proline Specific Endopeptidase Assay. Proline specific endopeptidase (EC 3.4.21.26, Seikagaku America Inc., Rockville MD) was diluted to 0.05 unit/mL in a solution of 0.1 M Tris·HCl pH 7.8 containing 1% Triton-X 100, 0.01% NaN_3. Next, 50 μL of diluted enzyme was incubated with 50 μL of 1 mM Z-Gly-Pro-MNA (BACHEM Bioscience Inc., King of Prussia, PA) for 60 min at 37 °C at which time 50 μL of 4-(dimethylamino)cinnamaldehyde (3.3 mg/mL) was added and the resulting color measured at 570 nm.

Mixed Lmphocyte Reaction (MLR). Peripheral blood was obtained from normal, healthy donors by venipuncture. The blood was collected in heparinized tubes, and 7.5 mL was layered over 7.5 mL of a Ficoll/Hypaque (Pharmacia, Piscataway, NJ) density gradient at room temperature and centrifuged at 1000*g* for 20 min. The interface was then collected and washed three times in RPMI-1640 (Gibco, Grand Island, NY). The resulting peripheral blood mononuclear cells (PBMC) were then counted and resuspended in RPMI-1640 containing 50 μg/mL gentamycin (Gibco), 1 mM L-glutamine (Gibco), and 5% heat inactivated human type AB sera (Flow Labs., Mclean, VA) culture medium (hereafter referred to as CM). PBMC were cultured in CM at 2.5 × 10^5 total cells/well in Linbro (Flow Labs, Mclean, VA) 96 well round bottom microtiter plates. Stimulator cells from separate donors were irradiated at 1000R and cultured with responder cells at equal concentrations in a total volume of 0.2 mL. In cultures receiving DPPIV inhibitors, aqueous solutions of inhibitors were prepared in RPMI-1640 just before use and added immediately. Responder cells and stimulator cells were also cultured alone as controls. The culture plates were incubated at 37 °C in a 5% CO_2-humidified incubator for 5 days and then pulsed with 0.5 μCi [^{3}H]thymidine (New England Nuclear, Boston, MA) for 18 h. The cells were then harvested onto glass fiber filters (Pharmacia, Turku, Finland) using an automated multiple sample harvester (Skatron, Sterling, VA). The filters were oven dried and counted on a Betaplate flatbed liquid scintillation counter (Pharmacia LKB Nuclear Inc., Gaithersburg, MD).

Supporting Information Available: ^1H NMR spectra of compounds **1a**−**r** (18 pages). Ordering information is given on any current masthead page.

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(24) For general experimental including details on the enzymatic assay, see ref 15.

(25) Abbreviations: Aibu, α-aminoisobutyric acid; Abu, 2-aminobutyric acid; Phg, phenylglycine; tLeu, *tert*-leucine; Azet, azetidine-2-carboxylic acid.

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