Synthesis and Structure–Activity Relationships of Peptidyl α-Keto Heterocycles as Novel Inhibitors of Prolyl Endopeptidase

Seiji Tsutsumi,*,† Tsuneo Okonogi,† Seiji Shibahara,† Shokichi Ohuchi,† Emiko Hatsushiba,† Arthur A. Patchett,‡ and Burton G. Christensen§

Pharmaceutical Research Laboratory, Meiji Seika Kaisha, Ltd., 760 Morooka-cho, Kohoku-ku, Yokohama 222, Japan, Merck Research Laboratories, P.O. Box 2000, Rahway, New Jersey 07065, and Microcide Pharmaceuticals, Inc., 4040 Campbell Avenue, Menlo Park, California 94025

Received March 11, 1994⊗

The preparation and in vitro prolyl endopeptidase (PEP) inhibitory activity of a series of α-keto heterocyclic compounds is described. The design is based on the introduction of α-keto heterocycles at the C-terminal end of substrate-like peptides. Many of the compounds including those substituted with thiazole, benzothiazole, benzoxazole, imidazole, and pyridine groups exhibit IC_{50} potencies of PEP inhibition at nanomolar levels. Structure–activity studies of the C-terminal heterocyclic groups indicate the importance of an sp^2 nitrogen atom at a β-position from the adjoining ketone carbonyl group. This heterocyclic nitrogen atom would provide a critical hydrogen-bond interaction with the histidine residue of the catalytic triad in PEP. Our inhibitors would extend the generality of the α-keto heterocycle design to another serine protease.

Introduction

Prolyl endopeptidase (PEP) (E.C. 3.4.21.26) is classified as a serine protease on the basis of studies with irreversible inhibitors[1] and by molecular cloning.[2] This enzyme degrades proline-containing neuropeptides, such as vasopressin and substance P.[3] Vasopressin has been suggested to be involved in learning and memory.[4] Substance P has been reported to exert a protective activity against the neurodegenerative effect of β-amyloid protein.[5] Recently, progress in Alzheimer's disease studies has revealed that the C-terminal portion of β-amyloid may injure neurons by stimulating the cell's signaling pathway.[6] Ishiura *et al.* suggest that PEP may be involved in processing the C-terminal portion of the amyloid precursor protein.[7] On the basis of these reports, PEP inhibitors may improve memory by blocking the metabolism of endogenous neuropeptides[8] and have possible potential as cognition-enhancing drugs.[9] Potent PEP inhibitors which are peptidyl aldehydes[10] and pyrrolidine derivatives[11] have been reported to ameliorate the experimental amnesia induced by scopolamine in rats.[12]

Progress in drug design has led to the development of small peptide enzyme inhibitors as therapeutic agents.[13,14] In the case of serine proteases, inhibitors have been developed by replacing the scissile amide bond of a protease substrate with a carbonyl group able to form a hemiketal that resembles the tetrahedral intermediate. A number of peptidyl carbonyl groups, such as aldehydes,[15] fluoro ketones,[16] keto esters,[17] and diketones,[18] have been successfully incorporated into hydrolytic enzyme inhibitors.

Recently, Edwards *et al.* determined by an X-ray crystal structure study[19] that a peptidyl α-keto benzoxazole derivative was an active site-directed elastase inhibitor in which the nitrogen atom of the benzoxazole participated in stabilizing the enzyme–inhibitor complex. PEP, like elastase, is a serine protease on the basis of its inactivation by both diisopropyl fluorophosphorofluoridate and the peptidyl chloromethyl ketone inhibitor Z-Gly-Pro-CH_2Cl.[1] Thus, a histidyl residue may also be located in the active site of PEP to act as a general acid–base catalyst to activate the serine residue.

In analogy with the evidence of Edwards *et al.*, we believe that the peptidyl α-keto heterocycle derivatives which stabilize the enzyme–inhibitor complex through formation of a hydrogen bond possess an inhibitory potency for PEP. In addition, to have oral potency and penetration into the central nervous system, we introduced the hydrophobic functionality "α-keto heterocycles" at the P_1' of peptidyl PEP inhibitors. We describe herein the design, synthesis, and biological activity of α-keto heterocyclic PEP inhibitors.

Synthesis

The compounds presented in Table 2 contain heterocycles at the P_1' position, L-proline at the P_1 position, and N-substituted L-proline derivatives in the P_2–P_3 area. These inhibitors were conveniently prepared as shown in Schemes 1–5.

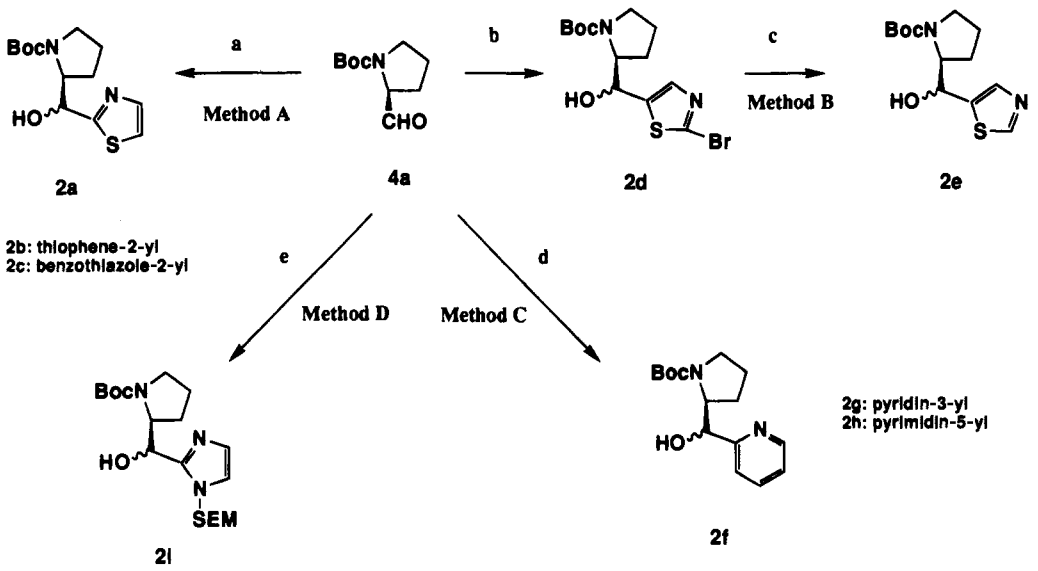
Synthetic methods for N-protected α-hydroxy heterocyclic derivatives **2a**–**m** are shown in Schemes 1–3. The first convergent approach involved the reaction of the appropriate lithiated heterocycles with *N*-(*tert*-butyloxycarbonyl)prolinal (*N*-Boc-prolinal) (**4a**).[20] Thiazole, thiophene, and benzothiazole derivatives **2a**–**c** were prepared via the direct preparation of lithiated heterocycles (method A). The alternative lithiation of 2-bromothiazole with LDA followed by removal of the 2-bromine atom provided the desired 5-substituted thiazole **2e** (method B).[21] 2-Bromopyridine was employed to direct metalation to the 2-position by treatment with *n*-butyllithium. The transmetalation of 6-membered heterocycles gave the desired pyridine[22] and pyrimidine[23] derivatives **2f**–**h** (method C).

In the case of imidazole, the acidic N-H proton on this heterocycle interferes with metalation by *n*-butyllithi-

† Meiji Seika Kaisha, Ltd.
‡ Merck Research Laboratories.
§ Microcide Pharmaceuticals, Inc.
⊗ Abstract published in *Advance ACS Abstracts*, September 1, 1994.

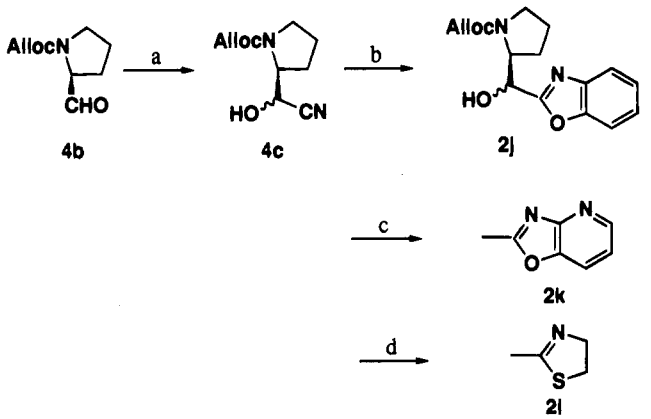
0022-2623/94/1837-3492$04.50/0

Scheme 1[a]



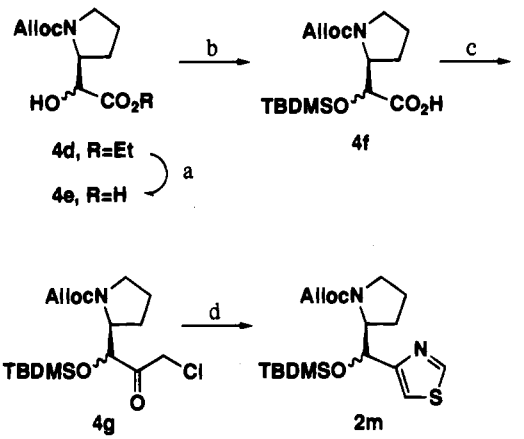
[a] Reagents and conditions: (a) thiazole, *n*-BuLi, THF; (b) 2-bromothiazole, diisopropylamine, *n*-BuLi, THF; (c) 2 equiv of *n*-BuLi, THF; (d) 2-bromopyridine, *n*-BuLi, THF; (e) 1-SEM-imidazole 1l, *n*-BuLi, THF.

Scheme 2. Method E[a]



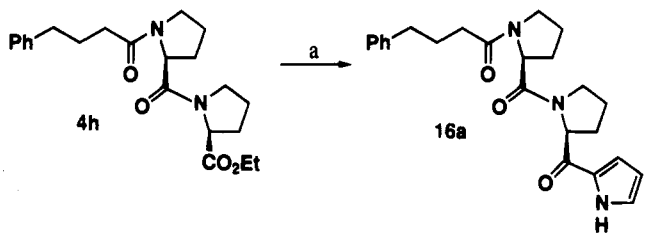
[a] Reagents: (a) acetone cyanohydrin, TEA, CH_2Cl_2; (b) (1) acetyl chloride, EtOH, $CHCl_3$, (2) 2-aminophenol, EtOH; (c) (1) acetyl chloride, EtOH, $CHCl_3$, (2) 2-amino-3-hydroxypyridine, EtOH; (d) (1) acetyl chloride, EtOH, $CHCl_3$, (2) 2-aminoethanethiol hydrochloride, Et_3N, EtOH.

Scheme 3. Method F[a]



[a] Reagents: (a) 2 N LiOH, THF; (b) (1) TBDMS-Cl, diisopropylethylamine, THF, (2) K_2CO_3, MeOH; (c) (1) $ClCO_2Et$, *N*-methylmorpholine, CH_2Cl_2, (2) CH_2N_2; (3) 4 N HCl–dioxane; (d) $HCSNH_2$, MeOH.

Scheme 4. Method G[a]



[a] Reagents: (a) pyrrole, MeMgBr, THF.

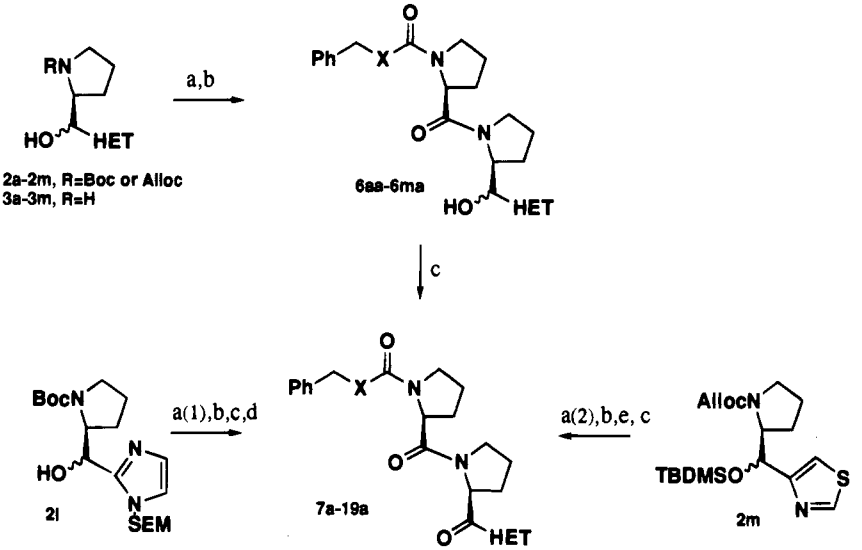
um. Therefore, we protected this active hydrogen with the [(trimethylsilyl)ethoxy]methyl (SEM) protective group as a solution to this problem (method D),[24] and in this way, the derivative **2i** was prepared.

Another useful synthetic method involved the formation of heterocycles from the cyanohydrin **4c** which was prepared from N-protected prolinal **4b** and an excess of acetone cyanohydrin and triethylamine. Treatment of **4c** with anhydrous HCl in ethanol provided the ethyl imidate hydrochloride which was cyclized with 2-aminophenol to afford the α-hydroxy benzoxazole derivative[19] **2j** (method E). In a similar way, the oxazolopyridine derivative **2k** and the thiazoline derivative **2l** were synthesized. The 4-substituted thiazole derivative **2m** was synthesized by using method F outlined in Scheme 3. An α-hydroxy acid derivative, **4e**, was prepared by hydrolysis of α-hydroxy ester **4d** which was obtained by the reaction of $NaBH_4$ and α-keto ester.[25] The protected α-hydroxy acid **4f** was converted into an acid anhydride with ethyl chloroformate and then treated with diazomethane and hydrogen chloride to give chloromethyl ketone **4g**. Cyclization of **4g** with thioformamide provided the desired 4-substituted thiazole derivative **2m** (method F).

Reaction of ethyl ester **4a** with pyrrolylmagnesium bromide provided the corresponding 2-ketopyrrole **16a** (method G).[26]

The N-Boc and -Alloc groups were removed by treatment with trifluoroacetic acid and $Pd(PPh_3)_4$, respectively. Treatment of proline derivatives with phenylbutanoyl chloride or benzyloxycarbonyl chloride provided the desired N-substituted proline derivatives **5a,b**. The N-substituted dipeptide inhibitors were prepared by

Scheme 5. Synthesis of α-Keto Heterocycle Derivatives[a]



[a] Reagents: (a) (1) TFA or (2) $Pd(PPh_3)_4$; (b) Z-proline (**5a**) or *N*-(4-phenylbutanoyl)proline (**5b**), WSCD, HOBt, CH_3CN; (c) $(COCl)_2$, DMSO, TEA, CH_2Cl_2; (d) 3 N HCl–MeOH, 50 °C; (e) Bu_4NF.

standard peptide coupling methods followed by oxidation as illustrated in Scheme 5. Condensation of the N-substituted proline derivatives **5a**,**b** with the amino alcohols **3a**–**m** gave the dipeptide derivatives **6aa**–**ma** as a mixture of diastereomers. The desired α-keto heterocycles **7a**–**19a** were prepared from the corresponding alcohols via Swern oxidation. In the case of the 4-substituted thiazole derivative **9a**, the O-protected TBDMS group was removed after the coupling reaction followed by oxidation. The SEM-protected intermediate was deprotected after Swern oxidation to give the desired compound **17b**.

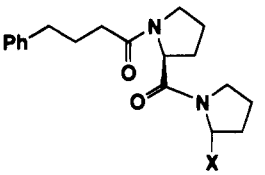
Enzyme Inhibitory Activities

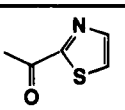
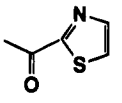
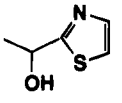
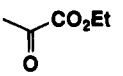
The peptidyl α-keto heterocycle derivative **7b** was a more potent inhibitor than the aldehyde **20** or the α-keto ester **21**. The epimeric isomer **7c**(***R***) and the alcohol analogue **6ab** were almost 1000-fold less potent than the corresponding ketone **7b** (Table 1).

Table 2 shows the inhibitory potencies of the novel peptidyl prolyl endopeptidase (PEP) inhibitors which contain a series of heterocycles at the P_1' position. On the N-terminal acyl group, most of the phenylbutanoyl derivative inhibitors (**7b** and **12b**) were more potent than the corresponding benzyloxycarbonyl derivatives (**7a** and **12a**).

The structure–activity relationships of a series of the substituted heterocycles at P_1' illustrate several interesting requirements for PEP inhibition. Among the active analogues, the one-ring type derivatives (**7b**, **10a**, **13a**, and **17b**) and the two-ring type derivatives (**12b**, **18a**, and **19a**) have a similar potency (IC_{50} = 3.8–9.0 nM). On the other hand, the thiophene derivatives (**11a**,**b**) had lost potency (IC_{50} = 2020 and 1260 nM, respectively). These results suggest that peptidyl heterocycles which possess a nitrogen atom are important for inhibitory activity. We hypothesized that PEP inhibitor potency depended on the position of the nitrogen atom relative to the adjoining ketone. To examine this hypothesis, we synthesized regioisomers of the thiazole and pyridine derivatives with different nitrogen atom positions. The inhibitory potency of the thiazole derivatives followed the trend: 2-thiazole **7b**

Table 1. Comparison of Electrophilic Carbonyl-Based PEP Inhibitors[a]

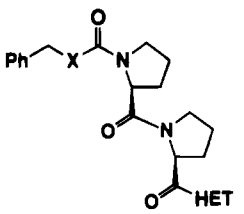


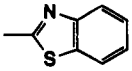
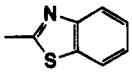
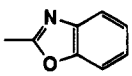
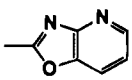
no.	X	IC_{50} (nM)[b]
7b(S)		5.0 (±0.3)
7c(R)		6020 (±710)
6ab		16100 (±1300)
20		8.7 (±0.8)[c]
21		10.3 (±0.8)

[a] See the Experimental Section for description of the method for determining the IC_{50} values of these inhibitors. [b] The values are the means ± SEM for the three independent experiments. [c] Literature[10a] IC_{50} = 8.7 nM.

(IC_{50} = 5.0 nM) ≥ 4-thiazole **9a** (IC_{50} = 6.2 nM) ≫ 5-thiazole **8a** (IC_{50} = 1090 nM). Comparison of 6-membered rings showed the following trend: 2-pyridine **13a** (IC_{50} = 6.9 nM) > 5-pyrimidine **15a** (IC_{50} = 61 nM) ≫ 3-pyridine **14a** (IC_{50} = 2290 nM). Of nearly equal importance to the choice of heterocycles was the pyrrole derivative (**16a**). The 2-pyrrole ketone **16a** (IC_{50} = 21.3 μM) with a nitrogen atom at a β-position had remarkably decreased inhibitory potency. This result suggests

Table 2. In Vitro PEP Inhibitory Activities of Peptidyl α-Keto Heterocycles[a]



no.	HET	X	Y	Z	Method	IC_{50}(nM)[b]
7a		O	H	H	A	8.5 (±0.8)
7b		CH_2CH_2	H	H	A	5.0 (±0.3)
8a		CH_2CH_2	H	H	B	1090 (±100)
9a		CH_2CH_2	H	H	F	6.2 (±0.3)
10a		CH_2CH_2	H	H	E	3.8 (±0.7)
11a		O	H	H	A	2020 (±100)
11b		CH_2CH_2	H	H	A	1260 (±120)
12a		O	H	H	A	5.0 (±0.8)
12b		CH_2CH_2	H	H	A	4.0 (±1.2)
13a		CH_2CH_2	H	H	C	6.9 (±1.0)
14a		CH_2CH_2	H	H	C	2290 (±320)
15a		CH_2CH_2	H	H	C	61 (±13)
16a		CH_2CH_2	H	H	G	21300 (±1200)
17b		CH_2CH_2	H	H	D	9.0 (±2.4)
18a		CH_2CH_2	H	H	E	5.6 (±1.0)
19a		CH_2CH_2	H	H	E	5.5 (±1.0)
20	H	CH_2CH_2	H	H		8.7 (±0.8)
21	CO_2Et	CH_2CH_2	H	H		10.3 (±0.8)

[a] See the Experimental Section for description of the method for determining the IC_{50} values of these inhibitors. [b] The values are the means ± SEM for the three independent experiments.

that the β-attachment was a necessary, but not sufficient, condition for inhibitory potency.

Discussion

Our starting point for these studies was the peptidyl α-keto heterocycle inhibitor **7b** and **9a** originally reported to be a potent PEP inhibitor.[27] This study showed that good PEP inhibitory activity requires a nitrogen atom at a β-position to the adjoining ketone moiety. This concept of inhibition has been extended to other heterocycles in this study. Among a series of 6-membered rings, inhibitory potency was found in the compound **13a**. Furthermore, PEP inhibitors are found in a wide variety of heterocyclic classes, one-ring types (**10a** and **13a**) and two-ring types (**12a**, **18a**, and **19a**). Although Edwards *et al.* reported that an α-keto heterocycle derivative would not be an effective inhibitor of serine protease inhibitor, except for α-keto benzoxazole and α-keto oxazoline derivatives,[19] our result showed that variations of the heterocycles were tolerated. The second significant structure–activity relationship for PEP inhibitory activity was discovered in the series containing imidazole substituents (**16a** and **17b**). The pyrrole derivative **16a** with a nitrogen atom at a β-position did not have inhibitory potency. These results suggest that high potency requires an sp^2 nitrogen atom which is able to accept a hydrogen bond at a β-position from the ketone moiety.

Peptidyl trifluoromethyl ketones, α-keto esters,[17] and α-diketones[18] of serine protease inhibitors were reported to be hydrated in DMSO with water from ^{13}C-NMR studies. These inhibitors can serve as transition analogues and react with a nucleophilic residue (serine hydroxy) to form a hemiketal that resembles the tetrahedral intermediate. We could not detect a new peak around 100 ppm which was consistent with a hydrated ketone (gem diol) in 10% D_2O/DMSO-d_6 (v/v) by the ^{13}C-NMR spectrum for **7b** and **12b**. This ^{13}C-NMR study suggests that participation in hydrogen-bond interactions by the heterocycles at the P_1' position was more more important for inhibitory activity of PEP than its hydrating capability. Recently, Patel *et al.* also suggested that the hydrophobic and/or hydrogen-bond interactions of the adjacent moiety contributed to the inhibitory potency of α-keto ester- and α-diketone-based renin inhibitors.[28]

In the course of this study, we found that the thiazole derivative **7a** was 300 times more potent than the thiophene derivative **11a**. The observation that the transition-state analogue **7a** binds to the PEP 3 orders of magnitude tighter than **11a** demonstrates the vital role the hydrogen bond plays in transition-state stabilization. The role of hydrogen bonding has been examined in several enzyme inhibitors.[29,30] Bartlett *et al.* determined that removal of hydrogen-bond capability in an inhibitor of the zinc endopeptidase thermolysin caused the loss of inhibitory potency, reflecting the loss of 4.0 kcal mol^{-1} in binding energy.[29] Applying the Gibbs–Helmholtz equation for the binding of our PEP inhibitor,[31] we obtained a difference in the free energy of binding between **7a** and **11a** and between **7b** and **11b** of 4.60 and 4.63 kcal mol^{-1}, respectively. We believe that the general agreement of these values with those of Bartlett *et al.* is in accord with hydrogen-bond formation to the heterocyclic nitrogen atom and enthalpy changes are likely to be the major contributor to the observed energy differences.

Structure–activity studies of the C-terminal heterocyclic groups indicate the importance of an sp^2 nitrogen atom at a β-position from the adjoining ketone carbonyl group. In analogy with the study of Edwards *et al.*, this heterocyclic nitrogen atom would provide a critical hydrogen-bond interaction with the histidine residue of the catalytic triad in PEP. These design features found in our inhibitors may well be capable of extension to other serine protease or cysteine protease inhibitors.

Experimental Section

The abbreviations which were used are as follows: HOBt, 1-hydroxybenzotriazole; WSCD, 1-ethyl-3-[3'-(dimethylamino)-propyl]carbodiimide; TFA, trifluoroacetic acid; TEA, triethylamine; THF, tetrahydrofuran; Boc, *tert*-butyloxycarbonyl; Alloc, allyloxycarbonyl; Z-proline, *N*-(benzyloxycarbonyl)proline; SEM-Cl, [2-(trimethylsilyl)ethoxy]methyl chloride; TBDMS-Cl, *tert*-butyldimethylsilyl chloride.

Chemistry. General Methods. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectras were recorded at ambient temperature on a JEOL JNM-GX 400 spectrometer or a Varian Gemini-300 spectrometer. All chemical shifts are reported in δ units (ppm) relative to tetramethylsilane (assigned to 0.0 ppm). Mass spectra and high-resolution mass spectra were determined on an HITACHI mass spectrometer M-80A or M-80B. Analytical results for compounds were determined by Toray Research Center, Inc., and the Meiji College of Pharamaceutical Science. Melting points were determined with a Yanaco micromelting point apparatus. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Thin-layer chromatography (TLC) analyses were performed on silica gel plates (0.25 mm; Merck) and visualized with UV, I_2, or a ninhydrin spray reagent. Flash chromatography was performed with silica gel (200–300 mesh; Merck). Purities of final products were checked by reverse-phase HPLC (YMC HPLC, C-18 column, 150 × 60 mm, 5 μm) at flow rates of 1 mL/min, eluting with CH_3CN and water (60:40 or 50:50), and detected by UV at 254 nm. Retention time, purity, and HPLC conditions are included in each individual experimental procedure.

Method A. Synthesis of 1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(thiazol-2-yl)methanol (2a). A solution of *n*-BuLi (3.3 mL of a 1.6 M solution in hexane, 5.5 mmol) was added dropwise to a solution of thiazole (470 mg, 5.5 mmol) in dry THF (10 mL) at −65 °C over 5 min under Ar. After the mixture was stirred for 30 min, a solution of *N*-Boc-prolinal (**4a**)[20] (1.0 g, 5.0 mmol) in dry THF (10 mL) was added and the mixture was stirred at −65 °C for 4 h. The reaction mixture was poured into water (5 mL), and the product was extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude material by flash chromatography (1:1 hexane/EtOAc) gave **2a** as a yellow solid (1.2 g, 83%): MS (EI) *m/z* 285 (M + 1); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.25–1.36 (m, 9 H), 1.70–1.90 (m, 1 H), 2.05–2.35 (m, 2 H), 2.76–2.83 (m, 1 H), 3.20–3.60 (m, 2 H), 4.38–4.42 (m, 1 H), 5.12–5.20 (m, 1 H), 6.70 (d, 1 H), 7.31 (d, 1 H, J = 3.3 Hz), 7.72 (d, 1 H, J = 3.3 Hz).

1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(thiophene-2-yl)methanol (2b). The title compound, **2b**, was prepared from 2-thienyllithium (5.5 mL of a 1.0 M solution in THF, 5.5 mmol) and **4a** (1.0 g, 5.0 mmol) using method A. The crude product was purified by chromatography (1:1 hexane/EtOAc) to afford **2b** (1.07 g, 76%) as a white solid: MS (SIMS) *m/z* 284 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.25–1.30 (m, 9 H), 1.70–2.05 (m, 4 H), 3.25–3.55 (m, 2 H), 4.05–4.30 (m, 1 H), 4.75–5.10 (m, 1 H), 5.85–6.05 (m, 1 H), 6.85–7.27 (m, 3 H).

1-(Benzothiazol-2-yl)-1-[1-(*tert*-butyloxycarbonyl)-2(*S*)-pyrrolidinyl]methanol (2c). The title compound, **2c**, was prepared from *n*-BuLi (33 mL of a 1.66 M solution in THF, 48 mmol), benzothiazole (6.8 g, 48 mmol), and **4a** (9.2 g, 46 mmol) using method A. The crude product was purified by chromatography (1:1 hexane/EtOAc) to afford **2c** (10.3 g, 67%) as a yellow solid: MS (EI) *m/z* 335 (M + 1); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.47–1.52 (m, 9 H), 1.70–2.86 (m, 4 H), 3.26–3.47 (m, 2 H), 4.24–4.48 (m, 1 H), 4.96–5.14 (m, 1 H), 6.64–6.90 (m, 1 H), 7.35–7.39 (m, 1 H), 7.44–7.52 (m, 1 H), 7.89–7.91 (m, 1 H), 7.97–7.99 (m, 1 H).

Method B. Synthesis of 1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(thiazol-5-yl)methanol (2e). A solution of lithium diisopropylamide (LDA) was prepared as follows: to diisopropylamine (770 μL, 5.5 mmol) in anhydrous THF (10 mL) at −60 °C was added 3.5 mL of *n*-BuLi (1.56 M solution in hexane, 5.5 mmol) under Ar. After stirring at 10 °C for 10 min, the reaction mixture was cooled at −70 °C. To the LDA solution was added 2-bromothiazole (900 mg, 5.5 mmol), and the mixture was stirred for 20 min. The resulting mixture was then treated with *N*-Boc-prolinal (**4a**) (1.0 g, 5.0 mmol) and stirred at −60 °C for 2 h. The reaction mixture was poured into aqueous saturated NH_4Cl solution at 0 °C. The product was extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude material by flash chromatography (40:1 CH_2Cl_2/EtOAc) gave **2d** (840 mg, 46%) as a brown oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.50–1.52 (m, 9 H), 1.78–2.14 (m, 3 H), 2.95–3.04 (m, 1 H), 3.27–3.49 (m, 2 H), 4.02–4.32 (m, 1 H), 4.82–5.00 (m, 1 H), 7.42 (s, 1 H). To the 2-bromothiazole derivative **2d** (820 mg, 2.3 mmol) in THF (10 mL) was added 3.2 mL of *n*-BuLi (1.56 M solution in hexane, 5.0 mmol) at −70 °C, and the reaction mixture was stirred for 15 min under Ar. The reaction mixture was poured into aqueous saturated NH_4Cl solution at 0 °C, and the product was extracted with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude material by flash chromatography (10:1 CH_2Cl_2/EtOAc) gave **2e** (430 mg, 66%) as a brown oil: MS (EI) *m/z* 285 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.50–1.52 (m, 9 H), 1.77–2.13 (m, 3 H), 2.95–3.00 (m, 1 H), 3.30–3.42 (m, 2 H), 4.36–4.38 (m, 1 H), 4.94–5.09 (m, 1 H), 7.77–7.78 (m, 1 H), 8.84 (s, 1 H).

Method C. Synthesis of 1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(pyridin-2-yl)methanol (2f). A solution of *n*-BuLi (3.5 mL of a 1.56 M solution in hexane, 5.5 mmol) was added to a solution of 2-bromopyridine (870 mg, 5.5 mmol) in dry THF (10 mL) at −65 °C over 5 min under Ar. After the solution was stirred for 60 min, a solution of *N*-Boc prolinal (**4a**) (1.0 g, 5 mmol) in dry THF (5 mL) was added and the resulting mixture was stirred at −65 °C for 2 h. The mixture was poured into water (5 mL), and then the product was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude material by flash chromatography (7:1 CH_2Cl_2/EtOAc) gave **2f** (970 mg, 70%) as a yellow oil: MS (FD) *m/z* 278 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.48–1.51 (m, 9 H), 1.70–2.05 (m, 4 H), 3.14–3.52 (m, 2 H), 4.00–4.20 (m, 1 H), 4.80–5.20 (m, 1 H), 7.22–7.51 (m, 2 H), 7.64–7.72 (m, 1 H), 8.75–8.77 (m, 1 H).

1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(pyridin-3-yl)methanol (2g). The title compound, **2g**, was prepared from *n*-BuLi (1.3 mL of a 1.56 M solution in THF, 2.0 mmol), 3-bromopyridine (316 mg, 2.0 mmol), and **4a** (400 mg, 2.0 mmol) using method C. The crude product was purified by chromatography (1:1 hexane/EtOAc) to afford **2g** (123 mg, 22%) as a yellow oil: MS (EI) *m/z* 279 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.51–1.52 (m, 9 H), 1.68–2.85 (m, 4 H), 3.28–3.55 (m, 2 H), 4.05–4.37 (m, 1 H), 4.59–4.88 (m, 1 H), 7.30–7.36 (m, 1 H), 7.68–7.81 (m, 1 H), 8.51–8.54 (m, 2 H).

1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(pyrimidin-5-yl)methanol (2h). The title compound, **2h**, was prepared from *n*-BuLi (1.3 mL of a 1.56 M solution in THF, 2.0 mmol), 5-bromopyrimidine (318 mg, 2.0 mmol), and **4a** (400 mg, 2.0 mmol) using method C. The crude product was purified by chromatography (1:1 hexane/EtOAc) to afford **2h** (153 mg, 28%) as a brown oil: MS (SIMS) *m/z* 280 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.51–1.52 (m, 9 H), 1.64–2.10

(m, 3 H), 2.78–2.90 (m, 1 H), 3.36–3.53 (m, 2 H), 4.10–4.38 (m, 1 H), 4.64–4.86 (m, 1 H), 8.69–8.76 (m, 2 H), 9.16–9.17 (m, 1 H).

Method D. Synthesis of 1-[1-(*tert*-Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-[1-[[2-(trimethylsilyl)ethoxy]methyl]-imidazol-2-yl]methanol (2i). A solution of *n*-BuLi (6.56 mL of a 1.6 M solution in hexane, 10.5 mmol) was added to a solution of *N*-SEM-imidazole[24] (1.9 g, 9.6 mmol) in dry THF (10 mL) at −40 °C over 5 min under Ar. After the mixture was stirred for 60 min, a solution of *N*-Boc-prolinal (**4a**) (1.9 g, 9.6 mmol) in dry THF (10 mL) was added and the resulting mixture was stirred at −40 °C for 1 h and at 0 °C for 3 h. The mixture was poured into aqueous saturated NH_4Cl solution. The product was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the crude material by flash chromatography (3:1 hexane/EtOAc) gave **2i** (3.80 g, 99%) as a yellow oil: MS (EI) *m/z* 397 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.00 (s, 9 H), 0.92 (t, 2 H), 1.48–1.51 (m, 9 H), 1.75–2.10 (m, 4 H), 3.35–3.57 (m, 4 H), 4.14–4.38 (m, 1 H), 5.29–5.49 (m, 3 H), 6.99–7.06 (m, 2 H).

Method E. Synthesis of 1-[1-(Allyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(benzoxazol-2-yl)methanol (2j). A solution of *N*-Alloc-prolinal (**4b**) (5.5 g, 30 mmol) in CH_2Cl_2 (70 mL) was treated with acetone cyanohydrin (7.2 g, 85 mmol) followed by TEA (2.5 mL, 18 mmol) and stirred for 24 h at room temperature under Ar. The reaction mixture was concentrated under reduced pressure, and the residue was taken up in Et_2O. The Et_2O solution was washed with brine, dried over $MgSO_4$, and evaporated. The crude product was purified by chromatography (50:1 CH_2Cl_2/EtOAc) to afford cyanohydrin **4c** (6.2 g, 97%) as a colorless oil: MS (SIMS) *m/z* 211 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.80–2.28 (m, 4 H), 3.52–3.56 (m, 1 H), 3.68–3.74 (m, 1 H), 4.14–4.18 (m, 1 H), 4.54–4.70 (m, 3 H), 5.26–5.37 (m, 2 H), 5.91–6.01 (m, 1 H). Acetyl chloride (4.3 mL, 60 mmol) was added to a solution of $CHCl_3$ (4 mL) and EtOH (3 mL) at 0 °C over 15 min under Ar. Cyanohydrin **4c** (420 mg, 2 mmol) in $CHCl_3$ (4 mL) was added; the reaction mixture was stirred at 0 °C and at room temperature overnight. After evaporation, the crude imidate in EtOH (10 mL) was treated with 2-aminophenol (240 mg, 2.2 mmol) and heated at reflux for 6 h. The reaction mixture was concentrated under reduced pressure, and then the residue was taken up in EtOAc. The organic extract was washed with water and aqueous saturated $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated. The crude product was purified by chromatography (25:1 CH_2Cl_2/EtOAc) to afford **2j** (560 mg, 93%) as a yellow oil: MS (SIMS) *m/z* 303 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.80–1.88 (m, 4 H), 3.49–3.61 (m, 2 H), 4.20–4.93 (m, 4 H), 5.22–5.37 (m, 2 H), 5.94–5.98 (m, 1 H), 7.34–7.39 (m, 2 H), 7.53–7.57 (m, 1 H), 7.71–7.74 (m, 1 H).

1-[1-(Allyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(oxazolo-[4,5-*b*]pyridin-2-yl)methanol (2k). The title compound, **2k**, was prepared from **4c** (406 mg, 2.0 mmol) and 2-amino-3-hydroxypyridine (240 mg, 2.2 mmol) using method E. The crude product was purified by chromatography (10:1 CH_2Cl_2/EtOAc) to afford **2k** (90 mg, 15%) as a yellow oil: MS (SIMS) *m/z* 304 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.77–2.24 (m, 4 H), 3.14–3.55 (m, 2 H), 4.45–4.47 (m, 1 H), 4.61–4.64 (m, 2 H), 5.20–5.30 (m, 3 H), 5.81–5.90 (m, 1 H), 5.91–5.97 (m, 1 H), 7.31 (dd, 1 H, *J* = 4.7, 8.1 Hz), 7.84 (d, 1 H, *J* = 8.1 Hz), 8.57 (d, 1 H, *J* = 4.7 Hz).

1-[1-(Allyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-(thiazolin-2-yl)methanol (2l). The title compound, **2l**, was prepared from **4c** (406 mg, 2.0 mmol), TEA (610 μL, 4.4 mmol), and aminoethanethiol hydrochloride (250 mg, 2.2 mmol) using method E. The crude product was purified by chromatography (1:2 toluene/EtOAc) to afford **2l** (276 mg, 51%) as a yellow oil. MS (FD) *m/z* 270 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.72–2.10 (m, 4 H), 3.28–3.65 (m, 4 H), 4.15–4.34 (m, 3 H), 4.60–4.72 (m, 2 H), 4.85–4.95 (m, 1 H), 5.20–5.40 (m, 2 H), 5.90–6.04 (m, 1 H).

Method F. Synthesis of 1-[1-(Allyloxycarbonyl)-2(*S*)-pyrrolidinyl]-1-[(*tert*-butyldimethylsilyl)oxy]-1-(thiazol-4-yl)methane (2m). Ethyl 2-[1-(allyloxycarbonyl)-2(*S*)-pyrrolidinyl]-2-oxoacetate[25] (6.2 g, 25.8 mmol) in EtOH (80 mL) was reduced with $NaBH_4$ (380 mg, 10 mmol) at 0 °C for 30 min. After evaporation, the reaction mixture was diluted with EtOAc, washed with water and brine, and dried over $MgSO_4$. The organic layer was concentrated and the residue purified on silica gel to give the α-hydroxy ester derivative **4d** (4.7 g, 75.0%). A solution of the α-hydroxy ester **4d** (1.0 g, 3.89 mmol) in THF (10 mL) was treated with 2 N LiOH (2.05 mL) at room temperature for 2 h. The reaction mixture was then acidified with 2 N HCl and extracted with EtOAc to afford **4e** (715 mg, 80%) which was used without further purification. A solution of **4e** (710 mg, 3.1 mmol) in dry DMF (20 mL) was treated with diisopropylethylamine (1.7 mL, 9.9 mmol) and TBDMS-Cl (1.49 g, 9.9 mmol). The resulting mixture was stirred at room temperature for 15 h. The reaction mixture was poured into brine and extracted with EtOAc. The combined organic extracts were concentrated under reduced pressure. After the residue was dissolved in MeOH (55 mL) and THF (20 mL), 0.5 M K_2CO_3 solution (20 mL) was added and the solution was stirred at room temperature for 1 h. The reaction mixture was concentrated and neutralized to pH 4 by 1 N HCl. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography (EtOAc) to afford **4f** (870 mg, 82%) as a yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.00–0.32 (m, 6 H), 0.94 (s, 9 H), 1.78–2.12 (m, 4 H), 3.36–3.55 (m, 2 H), 4.06–4.14 (m, 1 H), 4.24–4.79 (m, 3 H), 5.20–5.38 (m, 2 H), 5.79–6.02 (m, 1 H). A solution of **4f** (870 mg, 2.65 mmol) in CH_2Cl_2 (15 mL) was treated with $ClCO_2Et$ (304 μL, 3.2 mmol) and *N*-methylmorpholine (378 μL, 3.4 mmol) at −10 °C for 30 min under Ar. To the reaction mixture was added diazomethane solution in Et_2O (10 mL, 5.0 mmol) at −10 °C. The reaction mixture was stirred at −10 °C for 30 min and treated with 1.3 mL of 4 N HCl/dioxane solution, and the mixture was stirred at 0 °C for 30 min. Aqueous $NaHCO_3$ solution was added, and the separated aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography (10:1 toluene/EtOAc) to afford **4g** (530 mg, 54%, two steps) as a yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.02–0.15 (m, 6 H), 0.90 (s, 9 H), 1.78–2.10 (m, 4 H), 3.35–3.60 (m, 2 H), 4.08–4.70 (m, 6 H), 5.20–5.36 (m, 2 H), 5.58–6.02 (m, 1 H). A solution of **4g** (400 mg, 1.06 mmol) in MeOH (4 mL) was treated with thioformamide (130 mg, 2.2 mmol) and stirred at 45 °C for 18 h. The reaction mixture was diluted with EtOAc and washed with water. The organic layer was concentrated under reduced pressure. The crude product was purified by chromatography (10:1 toluene/EtOAc) to afford **2m** (160 mg, 39%) as a yellow oil: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.00–0.20 (m, 6 H), 0.96 (s, 9 H), 1.56–2.12 (m, 4 H), 3.00–3.11 (m, 1 H), 3.28–3.46 (m, 1 H), 4.17–4.26 (m, 1 H), 4.58–4.72 (m, 2 H), 5.21–5.69 (m, 3 H), 5.88–6.04 (m, 1 H), 7.30 (d, 1 H), 8.85 (d, 1 H).

Method G. Synthesis of 2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]-pyrrole (16a). A solution of methylmagnesium bromide (1.78 mL of a 3 M solution in Et_2O, 5.34 mmol) in toluene (7 mL) was treated with pyrrole (557 mL, 8.0 mmol) at −40 °C, and the resulting mixture was then stirred at −10 °C for 10 min under Ar. A solution of this pyrrolylmagnesium bromide (4.5 mL) was added to a solution of the proline ethyl ester derivative **4h** (250 mg, 0.67 mmol) in toluene (1 mL) at −65 °C over 5 min. The mixture was stirred at −65 °C for 3 h and then at 0 °C for 3 h and at room temperature for 15 h. The reaction was quenched with aqueous saturated NH_4Cl. The product was extracted with EtOAc and dried over $MgSO_4$, and the solvent was removed under reduced pressure. Purification of the crude product by column chromatography (1:1 hexane/EtOAc) gave **16a** (150 mg, 55%) as a white foam: $[\alpha]_D$ −95.5° (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.89–2.20 (m, 9 H), 2.22–2.34 (m, 3 H), 2.64–2.68 (m, 2 H), 3.37–3.43 (m, 1 H), 3.54–3.60 (m, 1 H), 3.65–3.71 (m, 1 H), 3.93–3.99 (m, 1 H), 4.71–4.74 (m, 1 H), 5.27–5.31 (m, 1 H), 6.25–6.27 (m, 1 H), 6.98–7.01 (m, 2 H), 7.14–7.28 (m, 5 H), 9.50 (brs, 1 H); HPLC retention time 4.35 min (96.6%), eluting with 60% CH_3-

CN in water, 1.0 mL/min; MS (EI) *m/z* 407 (M); HRMS for $C_{24}H_{29}N_3O_3$ calcd 407.2207, found 407.2180. Anal. ($C_{24}H_{29}N_3O_3$) C, H, N.

Method H. General Deprotection Procedure. (1) The *N*-Boc-proline derivatives **2a**–**i** (0.2–0.4 mM) were dissolved in 90% trifluoroacetic acid in CH_2Cl_2 and stirred at 0 °C for 1 h. After removal of solvent, the pH of the residue was adjusted to 10 with 1 N NaOH and the mixture was extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$ to give the desired amines **3a**–**i** which were used without further purification.

(2) To a solution of the *N*-Alloc-proline derivatives **2j**–**m** (0.2–0.4 mM) in 50% EtOAc in CH_2Cl_2 was added dimedone (2 equiv) or pyrrolidine (5 equiv) at room temperature followed by tetrakis(triphenylphosphine)palladium (0.05 equiv). The mixture was stirred at room temperature for 2–4 h and then evaporated in vacuo. The crude product was purified by chromatography (CH_2Cl_2/MeOH) to afford the desired amines **3j**–**m** as a brown oil.

Method I. General Procedure for Peptide-Bond Formation. To a solution containing an N-protected proline derivative, **5a** or **5b** (1 equiv, 0.02–0.05 mM), in CH_3CN were added a pyrrolidine methanol derivative (0.9–1.1 equiv), HOBt (1.0–1.2 equiv), and WSCD (1.0–1.2 equiv). In the case of a pyrrolidine derivative as an HCl salt, TEA was further added to the reaction mixture. The mixture was stirred overnight at 25 °C. After evaporation in vacuo, the residue was dissolved in EtOAc, washed with brine, dried over $MgSO_4$, and then concentrated. The product was purified by flash chromatography to give the desired carbinols **6aa**–**mb**.

Method J. General Procedure for the Preparation of α-Keto Heterocycles. DMSO (3.0–4.0 equiv) was added to a solution of $(COCl)_2$ (1.5–2.0 equiv) in dry CH_2Cl_2 at −60 °C, and the mixture was stirred at this temperature for 10 min under Ar. To the solution were added dropwise pyrrolidine methanol derivatives **6aa**–**mb** (0.1–0.2 mmol) in dry CH_2Cl_2, and the reaction mixture was stirred at −60 °C for 30 min. TEA (6.0–8.0 equiv) was added, and the mixture was allowed to warm to 0 °C and stirred for 1–6 h. The mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The product was purified by flash chromatography to afford the desired α-keto heterocycles **7a**–**19a**.

1-[1-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazol-2-yl)methanol (6aa). Compound **3a** was prepared by method H (1) starting from **2a**. Reaction of Z-proline (**5a**) (750 mg, 3.0 mmol) with the alcohol **3a** (560 mg, 3.0 mmol), HOBt (410 mg, 3.0 mmol), and WSCD (470 mg, 3.0 mmol) using method I gave **6aa** as an oil (1.0 g, 80%) which was purified by column chromatography (1:3 hexane/EtOAc): MS (EI) *m/z* 415 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.75–3.05 (m, 8 H), 3.38–3.80 (m, 4 H), 4.30–4.70 (m, 2 H), 4.90–5.25 (m, 3 H), 7.10–7.40 (m, 6 H), 7.66–7.75 (m, 1 H).

2-[[1-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiazole (7a). Reaction of the alcohol **6aa** (1.0 g, 2.40 mmol) with $(COCl)_2$ (320 μL, 3.65 mmol), DMSO (490 μL, 6.9 mmol), and TEA (2.1 mL, 15.2 mmol) using method J gave **7a** as a colorless foam (850 mg, 86%) which was purified by column chromatography (2:1 CH_2Cl_2/EtOAc): $[\alpha]_D$ −144.3° (*c* 1.0, $CHCl_3$); two rotamers [maj/min (3:2)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.86–2.46 (m, 8 H), 3.46–3.93 (m, 4 H), 4.48 (dd, *J* = 3.9, 8.5 Hz, min), 4.61 (dd, *J* = 3.9, 8.5 Hz, maj), 5.00–5.19 (m, 2H), 5.61 (dd, *J* = 4.6, 9.0 Hz, min), 5.78 (dd, *J* = 4.6, 9.0 Hz, maj), 7.28–7.39 (m, 5 H), 7.66 (d, *J* = 3.1 Hz, min), 7.68 (d, *J* = 3.1 Hz, maj), 8.00 (d, *J* = 3.1 Hz, maj), 8.02 (d, *J* = 3.1 Hz, min); HPLC retention time 5.65 min (93.5%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 413 (M); HRMS for $C_{21}H_{23}N_3O_4S_1$ calcd 413.1408, found 413.1450. Anal. ($C_{21}H_{23}N_3O_4S_1 \cdot 0.2H_2O$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazol-2-yl)methanol (6ab). Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (50 mg, 0.2 mmol) with the alcohol **3a** (36 mg, 0.2 mmol), HOBt (30 mg, 0.22 mmol), and WSCD (35 mg, 0.23 mmol) using method I gave **6ab** (64 mg, 75%) purified by column chromatography (15:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 427 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.75–2.05 (m, 9 H), 2.10–2.50 (m, 3 H), 2.60–2.70 (m, 2 H), 3.40–3.70 (m, 4 H), 4.20–4.62 (m, 2 H), 4.90–5.20 (m, 1 H), 7.15–7.35 (m, 6 H), 7.66–7.75 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiazole (7b). Reaction of the alcohol **6ab** (51 mg, 0.12 mmol) with $(COCl)_2$ (16 μL, 0.18 mmol), DMSO (24 μL, 0.34 mmol), and TEA (160 μL, 1.15 mmol) using method J gave **7b** as a colorless oil (39 mg, 76%) which was purified by column chromatography (30:1 CH_2Cl_2/MeOH): $[\alpha]_D$ −121.9° (*c* 0.8, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.90–2.21 (m, 9 H), 2.25–2.40 (m, 2 H), 2.45–2.55 (m, 1 H), 2.65–2.75 (m, 2 H), 3.40–3.50 (m, 1 H), 3.55–3.65 (m, 1 H), 3.72–3.80 (m, 1 H), 4.00–4.05 (m, 1 H), 4.73 (dd, 1 H, *J* = 3.6, 8.0 Hz), 5.75 (dd, 1 H, *J* = 4.7, 9.0 Hz), 7.15–7.30 (m, 5 H), 7.65 (d, 1 H, *J* = 3.1 Hz), 8.00 (d, 1 H, *J* = 3.1 Hz); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.2, 26.0, 28.6, 29.0, 33.6, 35.3, 47.2, 47.3, 57.6, 61.8, 125.9, 126.3, 128.4, 128.6, 141.9, 144.9, 165.1, 170.6, 171.6, 191.1; HPLC retention time 5.47 min (93.9%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 425 (M); HRMS for $C_{23}H_{27}N_3O_3S_1$ calcd 425.1772, found 425.1790.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*R*)-pyrrolidinyl]-1-(thiazol-2-yl)methanol (6ac). Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (75 mg, 3.0 mmol) with the alcohol **3a**(*R*) (56 mg, 3.0 mmol), HOBt (41 mg, 3.0 mmol), and WSCD (47 mg, 3.0 mmol) using method I gave **6ac** as an oil (105 mg, 82%) which was purified by column chromatography (20:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 427 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.85–2.21 (m, 8 H), 2.28–2.38 (m, 4 H), 2.63–2.70 (m, 2 H), 3.46–3.77 (m, 4 H), 4.50–4.63 (m, 2 H), 5.34 (d, 1 H), 5.82–5.85 (m, 1 H), 7.15–7.30 (m, 6 H), 7.73 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*R*)-pyrrolidinyl]carbonyl]thiazole (7c(*R*)). Reaction of the alcohol **6ac** (80 mg, 0.19 mmol) with $(COCl)_2$ (22 μL, 0.25 mmol), DMSO (34 μL, 0.48 mmol), and TEA (170 μL, 1.2 mmol) using method J gave **7c**(*R*) as a white foam (65 mg, 81%) which was purified by column chromatography (30:1 CH_2Cl_2/MeOH): $[\alpha]_D$ + 35.0° (*c* 1.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.80–2.09 (m, 8 H), 2.14–2.38 (m, 4 H), 2.62–2.79 (m, 2 H), 3.33–3.42 (m, 1 H), 3.45–3.77 (m, 3 H), 4.20–4.44 (m, 1 H), 5.64–6.20 (m, 1 H), 7.14–7.28 (m, 5 H), 7.62–7.76 (m, 1 H), 7.97–8.07 (m, 1 H); HPLC retention time 5.22 min (94.7%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 425 (M); HRMS for $C_{23}H_{27}N_3O_3S_1$ calcd 425.1772, found 425.1735. Anal. ($C_{23}H_{27}N_3O_3S_1$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazol-5-yl)methanol (6e). Compound **3e** was prepared by method H (1) starting from **2e**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (52 mg, 0.2 mmol) with the alcohol **3e** (40 mg, 0.22 mmol), HOBt (30 mg, 0.22 mmol), and WSCD (35 mg, 0.23 mmol) using method I gave **6e** as a yellow oil (63 mg, 74%) which was purified by column chromatography (50:1 CH_2Cl_2/MeOH): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.84–2.08 (m, 8 H), 2.23–2.41 (m, 4 H), 2.64–2.71 (m, 2 H), 3.09–3.13 (m, 1 H), 3.42–3.67 (m, 3 H), 4.60–4.71 (m, 2 H), 5.11–5.13 (m, 1 H), 7.14–7.30 (m, 5 H), 7.75–7.77 (m, 1 H), 8.76–8.80 (m, 1 H).

5-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiazole (8a). Reaction of the alcohol **6e** (64 mg, 0.15 mmol) with $(COCl)_2$ (26 μL, 0.3 mmol), DMSO (41 μL, 0.6 mmol), and TEA (125 μL, 0.9 mmol) using method J gave **8a** as a yellow solid (48 mg, 75%) which was purified by column chromatography (EtOAc): $[\alpha]_D$ −85.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.91–2.06 (m, 4 H), 2.09–2.24 (m, 4 H), 2.27–2.34 (m, 3 H), 2.62–2.68 (m, 3 H), 3.40–3.42 (m, 1 H), 3.54–3.56 (m, 1 H), 3.68–3.73 (m, 1 H), 3.94–4.00 (m, 1 H), 4.70 (dd, 1 H, *J* = 3.6, 8.0 Hz), 5.33 (dd, 1 H, *J* = 4.6, 8.7 Hz), 7.15–7.27 (m, 5 H), 8.54 (s, 1 H), 9.00 (s, 1 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.1, 25.8, 28.4, 29.0, 33.4, 35.1, 46.9, 47.1, 57.4, 62.6, 125.7, 128.2, 128.4, 128.6, 137.4, 141.7, 147.4, 159.0, 170.7, 171.5, 191.0; HPLC retention time 4.34 min (97.0%), eluting with 60% CH_3CN in water, 1.0

mL/min; MS (EI) *m/z* 425 (M); HRMS for $C_{23}H_{27}N_3O_3S_1$ calcd 425.1772, found 425.1717. Anal. ($C_{23}H_{27}N_3O_3S_1 \cdot 1.0H_2O$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazolin-2-yl)methanol (61). Compound **3l** was prepared by method H (2) starting from **2l**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (136 mg, 0.5 mmol) with the alcohol **3l** (88 mg, 0.47 mmol), HOBt (70 mg, 0.52 mmol), TEA (70 μL, 0.5 mmol), and WSCD·HCl (119 mg, 0.62 mmol) using method I gave **6l** as a yellow oil (120 mg, 60%) which was purified by column chromatography (1:1 acetone/EtOAc); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.85–2.34 (m, 12 H), 2.65–2.72 (m, 2 H), 3.20–3.30 (m, 2 H), 3.38–3.65 (m, 3 H), 3.90–3.96 (m, 1 H), 4.15–4.30 (m, 2 H), 4.45–4.80 (m, 3 H), 7.17–7.32 (m, 5 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiazoline (10a). Reaction of the alcohol **6l** (120 mg, 0.28 mmol) with $(COCl)_2$ (36 μL, 0.41 mmol), DMSO (59 μL, 0.83 mmol), and TEA (266 μL, 1.92 mmol) using method J gave **10a** as a colorless oil (105 mg, 88%) which was purified by column chromatography (1:2 acetone/EtOAc): $[\alpha]_D$ −90.4° (*c* 1.1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.89–2.18 (m, 10 H), 2.21–2.33 (m, 2 H), 2.64–2.68 (m, 2 H), 3.27–3.32 (m, 2 H), 3.36–3.42 (m, 1 H), 3.55–3.66 (m, 2 H), 3.88–3.94 (m, 1 H), 4.43–4.59 (m, 2 H), 4.69 (dd, 1 H, *J* = 3.6, 8.0 Hz), 5.47 (dd, 1 H, *J* = 5.0, 8.8 Hz), 7.15–7.28 (m, 5 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.0, 25.9, 28.4, 28.5, 32.4, 33.5, 35.2, 46.9, 47.2, 57.4, 61.6, 66.3, 125.8, 128.3, 128.5, 141.8, 168.7, 170.4, 171.5, 192.9; HPLC retention time 5.70 min (90.1%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 427 (M); HRMS for $C_{23}H_{29}N_3O_3S_1$ calcd 427.1928, found 427.1936. Anal. ($C_{23}H_{29}N_3O_3S_1 \cdot 0.3H_2O$) C, H, N.

1-[1-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl-2(*S*)-pyrrolidinyl]-1-(thiophene-2-yl)methanol (6ba). Compound **3b** was prepared by method H (1) starting from **2b**. Reaction of Z-proline (**5a**) (620 mg, 2.5 mmol) with the alcohol **3b** (460 mg, 2.5 mmol), HOBt (365 mg, 2.7 mmol), and WSCD (420 mg, 2.7 mmol) using method I gave **6ba** as a colorless oil (1.0 g, 97%) which was purified by column chromatography (4:1 CH_2Cl_2/EtOAc): MS (EI) *m/z* 414 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.74–2.28 (m, 8 H), 3.30–3.90 (m, 4 H), 4.20–4.60 (m, 2 H), 4.70–5.23 (m, 3 H), 6.92–7.00 (m, 2 H), 7.24–7.30 (m, 6 H).

2-[[1-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiophene (11a). Reaction of the alcohol **6ba** (1.0 g, 2.4 mmol) with $(COCl)_2$ (420 μL, 4.8 mmol), DMSO (660 μL, 9.3 mmol), and TEA (2.1 mL, 15.2 mmol) using method J gave **11a** as a white solid (640 mg, 64%) which was purified by column chromatography (10:1 CH_2Cl_2/EtOAc): $[\alpha]_D$ −112.4° (*c* 1.0, $CHCl_3$); two rotamers [maj/min (3:2)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.90–2.30 (m, 8 H), 3.47–3.89 (m, 4 H), 4.48 (dd, *J* = 3.6, 8.3 Hz, min), 4.59 (dd, *J* = 3.6, 8.3 Hz, maj), 4.99–5.19 (m, 2 H), 5.44 (dd, 1 H, *J* = 4.2, 8.9 Hz), 7.12–7.14 (m, 1 H), 7.14–7.30 (m, 5 H), 7.63–7.65 (m, 1 H), 7.77–7.82 (m, 1 H); HPLC retention time 5.97 min (98.5%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 412 (M); HRMS for $C_{22}H_{24}N_2O_4S_1$ calcd 412.1455, found 412.1502. Anal. ($C_{22}H_{24}N_2O_4S_1$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiophene-2-yl)methanol (6bb). Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (260 mg, 1.0 mmol) with the alcohol **3b** (183 mg, 1.0 mmol), HOBt (150 mg, 1.1 mmol), and WSCD (180 mg, 1.2 mmol) using method I gave **6bb** as a yellow oil (310 mg, 73%) which was purified by column chromatography (1:1 CH_2Cl_2/EtOAc): MS (EI) *m/z* 426 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.90–2.08 (m, 9 H), 2.10–2.30 (m, 3 H), 2.65–2.75 (m, 2 H), 3.40–4.00 (m, 4 H), 4.45–5.24 (m, 3 H), 6.90–7.05 (m, 2 H), 7.21–7.30 (m, 6 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl-2(*S*)-pyrrolidinyl]carbonyl]thiophene (11b). Reaction of the alcohol **6bb** (300 mg, 0.72 mmol) with $(COCl)_2$ (126 μL, 1.44 mmol), DMSO (196 μL, 2.76 mmol), and TEA (650 μL, 4.7 mmol) using method J gave **11b** as a colorless oil (190 mg, 62%) which was purified by column chromatography (40:1 CH_2Cl_2/MeOH): $[\alpha]_D$ −84.7° (*c* 0.9, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.90–2.13 (m, 9 H), 2.20–2.32 (m, 3 H), 2.64–2.68 (m, 2 H), 3.39–3.56 (m, 2 H), 3.67–3.73 (m, 1 H), 3.91–3.97 (m, 1 H), 4.72 (dd, 1 H, *J* = 3.6, 7.8 Hz), 5.41 (dd, 1 H, *J* = 4.2, 8.8 Hz), 7.12 (dd, 1 H, *J* = 3.9, 5.0 Hz), 7.23–7.27 (m, 5 H), 7.63 (dd, 1 H, *J* = 1.1, 5.0 Hz), 7.80 (dd, 1 H, *J* = 1.1, 3.9 Hz); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.0, 25.9, 28.4, 29.3, 33.5, 35.2, 46.9, 47.2, 57.5, 61.8, 125.8, 128.2, 128.5, 132.4, 133.9, 141.7, 141.8, 170.6, 171.5, 191.1; HPLC retention time 6.05 min (94.7%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 424 (M); HRMS for $C_{24}H_{28}N_2O_3S_1$ calcd 424.1819, found 424.1850. Anal. ($C_{24}H_{28}N_2O_3S_1$) C, H, N.

1-(Benzothiazol-2-yl)-1-[1-[[1-(benzyloxycarbonyl)-2-(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]methanol (6ca). Compound **3c** was prepared by method H (1) starting from **2c**. Reaction of Z-proline (**5a**) (2.24 g, 9.0 mmol) with the alcohol **3c** (1.90 g, 8.1 mmol), HOBt (1.21 g, 9.0 mmol), and WSCD (1.39 g, 9.0 mmol) using method I gave **6ca** as a white foam (3.36 g, 90%) which was purified by column chromatography (1:2 hexane/EtOAc): MS (EI) *m/z* 465 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.83–2.10 (m, 5 H), 2.20–2.60 (m, 2 H), 2.90–3.12 (m, 1 H), 3.37–3.91 (m, 4 H), 4.27–4.72 (m, 2 H), 4.91–5.21 (m, 3 H), 7.27–7.38 (m, 7 H), 7.87–7.98 (m, 2 H).

2-[[1-[[1-(Benzyloxycarbonyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]benzothiazole (12a). Reaction of the alcohol **6ca** (3.87 g, 8.3 mmol) with $(COCl)_2$ (1.57 mL, 18 mmol), DMSO (2.45 mL, 35 mmol), and TEA (6.9 mL, 50 mmol) using method J gave **12a** as colorless crystals (2.42 g, 63%) which were purified by column chromatography (1:1 hexane/EtOAc): mp 142–144 °C; $[\alpha]_D$ −122.0° (*c* 1.0, $CHCl_3$); two rotamers [maj/min (3:2)]; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.83–2.48 (m, 8 H), 3.43–3.98 (m, 4 H), 4.48–4.63 (m, 1 H), 5.01–5.18 (m, 2 H), 5.68 (dd, *J* = 4.7, 8.9 Hz, min), 5.89 (dd, *J* = 4.7, 8.9 Hz, maj), 7.27–7.34 (m, 5 H), 7.49–7.59 (m, 2 H), 7.96–7.98 (m, 1 H), 8.16–8.20 (m, 1 H); HPLC retention time 10.7 min (99.6%), eluting with 50% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 463 (M); HRMS for $C_{25}H_{25}N_3O_4S_1$ calcd 463.1564, found 463.1746. Anal. ($C_{25}H_{25}N_3O_4S_1$) C, H, N.

1-(Benzothiazol-2-yl)-1-[1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]methanol (6cb). Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (1.3 g, 4.0 mmol) with the alcohol **3c** (940 mg, 4.0 mmol), HOBt (600 mg, 4.4 mmol), and WSCD (685 mg, 4.4 mmol) using method I gave **6cb** as a yellow foam (1.7 g, 89%) which was purified by column chromatography (4:1 CH_2Cl_2/EtOAc): MS (EI) *m/z* 477 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.80–2.20 (m, 8 H), 2.25–2.38 (m, 3 H), 2.51–2.56 (m, 1 H), 2.65–2.69 (m, 2 H), 3.06–3.86 (m, 4 H), 4.52–4.73 (m, 2 H), 5.18–5.21 (m, 1 H), 7.15–7.26 (m, 5 H), 7.34–7.47 (m, 2 H), 7.87–7.91 (m, 1 H), 7.94–7.98 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]benzothiazole (12b). Reaction of the alcohol **6cb** (200 mg, 0.42 mmol) with $(COCl)_2$ (88 μL, 1.0 mmol), DMSO (140 μL, 2.0 mmol), and TEA (0.82 mL, 5.9 mmol) using method J gave **12b** as a yellow solid (125 mg, 63%) which was purified by column chromatography (2:1 hexane/EtOAc): $[\alpha]_D$ −122.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.84–2.26 (m, 9 H), 2.15–2.25 (m, 2 H), 2.37–2.43 (m, 1 H), 2.56–2.60 (m, 2 H), 3.31–3.33 (m, 1 H), 3.45–3.49 (m, 1 H), 3.64–3.70 (m, 1 H), 3.94–3.98 (m, 1 H), 4.67–4.69 (m, 1 H), 5.80 (dd, 1 H, *J* = 5.0, 8.9 Hz), 7.10–7.24 (m, 5 H), 7.43–7.49 (m, 2 H), 7.87–7.91 (m, 1 H), 8.08–8.12 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.3, 25.9, 28.4, 29.0, 33.5, 35.2, 47.1, 57.5, 61.7, 122.3, 125.6, 125.8, 126.9, 128.3, 128.5, 137.2, 141.8, 153.6, 164.3, 170.5, 171.4, 192.7; HPLC retention time 10.5 min (93.4%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 475 (M); HRMS for $C_{27}H_{29}N_3O_3S_1$ calcd 475.1928, found 475.1960. Anal. ($C_{27}H_{29}N_3O_3S_1$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(pyridin-2-yl)methanol (6f). Compound **3f** was prepared by method H (1) starting from **2f**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (144 mg, 0.55 mmol) with the alcohol **3f** (88 mg, 0.53 mmol), HOBt (81 mg, 0.6 mmol), and WSCD (93 mg, 0.6 mmol) using method I gave **6f** as a yellow oil (165 mg, 74%) which was purified by column

chromatography (100:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 421 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.83–2.17 (m, 10 H), 2.24–2.36 (m, 2 H), 2.65–2.72 (m, 2 H), 3.34–3.47 (m, 2 H), 3.59–3.63 (m, 1 H), 3.81–3.85 (m, 1 H), 4.40–4.66 (m, 2 H), 4.78–5.22 (m, 1 H), 7.16–7.31 (m, 6 H), 7.48–7.50 (m, 1 H), 7.71–7.74 (m, 1 H), 8.49–8.53 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]pyridine (13a). Reaction of the alcohol **6f** (160 mg, 0.38 mmol) with $(COCl)_2$ (67 μL, 0.76 mmol), DMSO (105 μL, 1.5 mmol), and TEA (346 μL, 2.5 mmol) using method J gave **13a** as a white oil (90 mg, 57%) which was purified by column chromatography (1:2 CH_2Cl_2/EtOAc): $[\alpha]_D$ −93.9° (*c* 1.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.93–2.21 (m, 9 H), 2.23–2.37 (m, 2 H), 2.40–2.48 (m, 1 H), 2.63–2.67 (m, 2 H), 3.37–3.43 (m, 1 H), 3.53–3.57 (m, 1 H), 3.68–3.74 (m, 1 H), 3.97–4.02 (m, 1 H), 4.75 (dd, 1 H, J = 3.3, 7.4 Hz), 5.98 (dd, 1 H, J = 4.9, 9.0 Hz), 7.14–7.29 (m, 5 H), 7.43 (ddd, 1 H, J = 0.8, 4.9, 7.7 Hz), 7.78 (ddd, 1 H, J = 1.56, 7.7, 7.7 Hz), 8.02 (d, 1 H, J = 7.7 Hz), 8.65 (d, 1 H, J = 4.9 Hz); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.1, 25.9, 28.5, 29.0, 33.5, 35.2, 47.2, 57.5, 60.9, 122.5, 125.7, 127.1, 128.2, 128.5, 136.8, 141.8, 148.8, 152.1, 170.3, 171.4, 198.6; HPLC retention time 5.42 min (93.3%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 419 (M); HRMS for $C_{25}H_{29}N_3O_3$ calcd 419.2207, found 419.2234. Anal. ($C_{25}H_{29}N_3O_3$·0.9H_2O) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(pyridin-3-yl)methanol (6g). Compound **3g** was prepared by method H (1) starting from **2g** (123 mg, 0.44 mmol). To a solution of the resulting TFA salt were added TEA (150 μL, 1.1 mmol), *N*-(4-phenylbutanoyl)proline (**5b**) (130 mg, 0.5 mmol), HOBt (68 mg, 0.5 mmol), and WSCD (75 mg, 0.5 mmol). The reaction using method I gave **6g** as a yellow oil (156 mg, 84%) which was purified by column chromatography (10:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 421 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.76–2.10 (m, 10 H), 2.17–2.39 (m, 2 H), 2.63–2.70 (m, 2 H), 3.00–3.82 (m, 4 H), 4.46–4.68 (m, 2 H), 4.79–5.21 (m, 1 H), 7.15–7.46 (m, 6 H), 7.75–7.91 (m, 1 H), 8.50–8.67 (m, 2 H).

3-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]pyridine (14a). Reaction of the alcohol **6g** (42 mg, 0.1 mmol) with $(COCl)_2$ (17.5 μL, 0.2 mmol), DMSO (27 μL, 0.4 mmol), and TEA (83 μL, 0.6 mmol) using method J gave **14a** as a colorless oil (24.3 mg, 58%) which was purified by column chromatography (25:1 CH_2Cl_2/MeOH): $[\alpha]_D$ −91.3° (*c* 1.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.87–2.20 (m, 9 H), 2.25–2.35 (m, 3 H), 2.65–2.68 (m, 2 H), 3.41–3.55 (m, 1 H), 3.54–3.59 (m, 1 H), 3.70–3.76 (m, 1 H), 3.96–4.02 (m, 1 H), 4.70 (dd, 1 H, J = 3.6, 7.7 Hz), 5.49 (dd, 1 H, J = 4.9, 9.0 Hz), 7.14–7.29 (m, 5 H), 7.46–7.48 (m, 1 H), 8.34 (m, 1 H), 8.78 (m, 1 H), 9.19 (brs, 1 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.7, 25.1, 25.9, 28.4, 28.5, 33.5, 35.1, 46.9, 47.2, 57.5, 61.0, 123.7, 125.8, 128.2, 128.5, 130.8, 136.0, 141.7, 149.5, 153.4, 170.6, 171.5, 197.4; HPLC retention time 4.25 min (95.1%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 419 (M); HRMS for $C_{25}H_{29}N_3O_3$ calcd 419.2207, found 419.2226. Anal. ($C_{25}H_{29}N_3O_3$·0.3H_2O) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(pyrimidin-5-yl)methanol (6h). Compound **3h** was prepared by method H (1) starting from **2h** (153 mg, 0.55 mmol). To a solution of the resulting TFA salt were added TEA (150 μL, 1.1 mmol), *N*-(4-phenylbutanoyl)proline (**5b**) (157 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol), and WSCD (93 mg, 0.6 mmol). The reaction using method I gave **6h** as a yellow oil (196 mg, 84%) which was purified by column chromatography (10:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 422 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.81–2.00 (m, 7 H), 2.10–2.37 (m, 4 H), 2.62–2.70 (m, 3 H), 2.94–3.00 (m, 1 H), 3.43–3.50 (m, 1 H), 3.60–3.67 (m, 1 H), 3.80–3.86 (m, 1 H), 4.56–4.66 (m, 2 H), 4.90–4.94 (m, 1 H), 7.16–7.85 (m, 5 H), 8.65–8.84 (m, 2 H), 9.13–9.16 (m, 1 H).

5-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]pyrimidine (15a). Reaction of the alcohol **6h** (42 mg, 0.1 mmol) with $(COCl)_2$ (17.5 μL, 0.2 mmol), DMSO (27 μL, 0.4 mmol), and TEA (83 μL, 0.6 mmol) using method J gave **15a** as a white solid (27.4 mg, 65%) which was purified by column chromatography (25:1 CH_2Cl_2/MeOH): $[\alpha]_D$ −76.3° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.87–2.21 (m, 9 H), 2.25–2.37 (m, 3 H), 2.64–2.68 (m, 2 H), 3.39–3.44 (m, 1 H), 3.53–3.59 (m, 1 H), 3.70–3.76 (m, 1 H), 3.98–4.04 (m, 1 H), 4.68–4.70 (m, 1 H), 5.43 (dd, 1H, J = 4.9, 8.7 Hz), 7.15–7.28 (m, 5 H), 9.26 (s, 2 H), 9.35 (s, 1 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.7, 25.3, 25.9, 28.4, 28.5, 33.5, 35.2, 47.0, 47.2, 57.5, 61.1, 125.8, 128.3, 128.5, 141.7, 156.8, 161.4, 170.8, 171.6, 196.4; MS (EI) *m/z* 420 (M); HPLC retention time 3.98 min (96.2%), eluting with 60% CH_3CN in water, 1.0 mL/min; HRMS for $C_{24}H_{28}N_4O_3$ calcd 420.2160, found 420.2263. Anal. ($C_{24}H_{28}N_4O_3$) C, H, N.

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-[1-[[2-(trimethylsilyl)ethoxy]methyl]]imidazol-2-yl]methanol (6i). Compound **3i** was prepared by method H (1) starting from **2i**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (780 mg, 3.0 mmol) with the alcohol **3i** (900 mg, 3.0 mmol), HOBt (480 mg, 3.5 mmol), and WSCD (543 mg, 3.5 mmol) using method I gave **6i** as a brown oil (960 mg, 59%) which was purified by column chromatography (30:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 540 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ −0.03 (s, 9 H), 0.86–0.95 (m, 2 H), 1.71–2.06 (m, 10 H), 2.20–2.67 (m, 2 H), 2.65–2.69 (m, 2 H), 3.37–3.45 (m, 1 H), 3.53–3.68 (m, 4 H), 3.86–3.91 (m, 1 H), 4.47–4.66 (m, 2 H), 5.00–5.18 (m, 1 H), 5.54–5.89 (m, 2 H), 7.17–7.29 (m, 6 H), 7.81–7.86 (m, 1 H).

1-[[2-(Trimethylsilyl)ethoxy]methyl]-2-[[1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]imidazole (17a). Reaction of the alcohol **6i** (1.0 g, 1.85 mmol) with $(COCl)_2$ (324 μL, 3.7 mmol), DMSO (505 μL, 7.1 mmol), and TEA (1.66 mL, 12.0 mmol) using method J gave **17a** as a yellow foam (720 mg, 72%) which was purified by column chromatography (50:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 538 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ −0.03 (s, 9 H), 0.91 (t, 2 H, J = 8.2 Hz), 1.93–2.13 (m, 9 H), 2.23–2.30 (m, 2 H), 2.38–2.45 (m, 1 H), 2.63–2.67 (m, 2 H), 3.36–3.40 (m, 1 H), 3.50–3.58 (m, 3 H), 3.65–3.71 (m, 1 H), 3.95–3.99 (m, 1 H), 4.74–4.76 (m, 1 H), 5.61 (d, 1 H, J = 10.5 Hz), 5.64 (dd, 1 H, J = 4.9, 9.0 Hz), 5.76 (d, 1 H, J = 10.5 Hz), 7.14–7.28 (m, 7 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]imidazole (17b). The SEM-protected derivative **17a** (720 mg, 1.34 mmol) was dissolved in 3 N HCl (30 mL) in EtOH (30 mL) and stirred at 50 °C until the reaction was judged complete by TLC analysis. The pH of the solution was adjusted to 8 with $NaHCO_3$, and the product was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and then concentrated. The product was purified by silica gel chromtography (10:1 CH_2Cl_2/MeOH) to give the title compound, **17b**, as a white foam (413 mg, 75%): $[\alpha]_D$ −117.0° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.92–2.10 (m, 8 H), 2.14–2.33 (m, 3 H), 2.42–2.45 (m, 1 H), 2.66–2.70 (m, 2 H), 3.39–3.45 (m, 1 H), 3.56–3.61 (m, 1 H), 3.70–3.74 (m, 1 H), 3.98–4.02 (m, 1 H), 4.86 (d, 1 H, J = 3.9 Hz), 5.64 (d, 1 H, J = 4.7 Hz), 7.07 (s, 1 H), 7.12 (s, 1 H), 7.15–7.32 (m, 5 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.5, 25.1, 25.9, 28.4, 29.1, 33.5, 35.1, 47.2, 57.6, 61.4, 120.6, 125.7, 128.2, 128.5, 131.0, 141.8, 143.3, 170.8, 171.5, 188.2; HPLC retention time 3.52 min (96.1%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 408 (M); HRMS for $C_{23}H_{28}N_4O_3$ calcd 408.2160, found 408.2175. Anal. ($C_{23}H_{28}N_4O_3$·0.1H_2O) C, H, N.

1-(Benzoxazol-2-yl)-1-[1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]methanol (6j). Compound **3j** was prepared by method H (2) starting from **2j**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (80 mg, 0.3 mmol) with the alcohol **3j** (65 m g, 0.3 mmol), HOBt (45 mg, 0.33 mmol), and WSCD (52 mg, 0.33 mmol) using method I gave **6j** as a yellow oil (130 mg, 94%) which was purified by column chromatography (70:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 461 (M); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.88–2.09 (m, 7 H), 2.11–2.36 (m, 4 H), 2.65–2.70 (m, 3 H), 3.41–3.99 (m, 4 H), 4.66–5.30 (m, 3 H), 7.15–7.29 (m, 5 H), 7.34–7.47 (m, 2 H), 7.53–7.58 (m, 1 H), 7.70–7.76 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]benzoxazole (18a). Reaction

of the alcohol **6j** (130 mg, 0.28 mmol) with $(COCl)_2$ (49 μL, 0.56 mmol), DMSO (77 μL, 1.1 mmol), and TEA (250 μL, 1.8 mmol) using method J gave **18a** as a colorless oil (63.3 mg, 49%) which was purified by column chromatography (50:1 CH_2Cl_2/MeOH): $[\alpha]_D$ −86.7° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.83−2.17 (m, 9 H), 2.21−2.34 (m, 2 H), 2.42−2.49 (m, 1 H), 2.63−2.67 (m, 2 H), 3.35−3.40 (m, 1 H), 3.51−3.56 (m, 1 H), 3.75−3.79 (m, 1 H), 4.02−4.06 (m, 1 H), 4.72−4.75 (m, 1 H), 5.73 (dd, 1 H, *J* = 5.4, 8.5 Hz), 7.14−7.31 (m, 5 H), 7.42−7.46 (m, 1 H), 7.49−7.54 (m, 1 H), 7.62−7.64 (m, 1 H), 7.86−7.88 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.5, 25.4, 25.9, 28.4, 28.8, 33.5, 35.2, 47.1, 57.4, 62.1, 111.8, 122.4, 125.8, 128.2, 128.5, 140.5, 141.8, 150.7, 156.0, 170.6, 171.5, 188.1; HPLC retention time 7.71 min (95.6%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 459 (M); HRMS for $C_{27}H_{29}N_3O_4$ calcd 459.2156, found 459.2102. Anal. ($C_{27}H_{29}N_3O_4 \cdot 0.3H_2O$) C, H, N.

1-(Oxazolo[4,5-*b*]pyridin-2-yl)-1-[1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]methanol (6k). Compound **3k** was prepared by method H (2) starting from **2k**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (78 mg, 0.3 mmol) with the alcohol **3k** (55 mg, 0.25 mmol), HOBt (48 mg, 0.35 mmol), and WSCD (55 mg, 0.35 mmol) using method I gave **6k** as a yellow oil (100 mg, 87%) which was purified by column chromatography (50:1 CH_2Cl_2/MeOH): MS (EI) *m/z* 462 (M); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.83−2.07 (m, 6 H), 2.17−2.36 (m, 5 H), 2.64−2.69 (m, 3 H), 3.12−3.60 (m, 3 H), 3.84−3.90 (m, 1 H), 4.59−4.71 (m, 2 H), 4.87−5.15 (m, 1 H), 7.18−7.33 (m, 6 H), 7.80−7.84 (m, 1 H), 8.55−8.58 (m, 1 H).

2-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]oxazolo[4,5-*b*]pyridine (19a). Reaction of the alcohol **6k** (100 mg, 0.22 mmol) with $(COCl)_2$ (38.5 μL, 0.44 mmol), DMSO (60 μL, 0.85 mmol), and TEA (194 μL, 1.4 mmol) using method J gave **19a** as a white foam (34 mg, 34%) which was purified by column chromatography (30:1 CH_2Cl_2/MeOH); $[\alpha]_D$ −92.5° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.93−2.32 (m, 11 H), 2.46−2.67 (m, 3 H), 3.35−3.40 (m, 1 H), 3.51−3.56 (m, 1 H), 3.75−3.79 (m, 1 H), 4.02−4.06 (m, 1 H), 4.72 (dd, 1 H, *J* = 3.0, 8.4 Hz), 5.61 (dd, 1 H, *J* = 5.9, 8.2 Hz), 7.14−7.31 (m, 5 H), 7.48 (dd, 1 H, *J* = 4.9, 8.5 Hz), 7.98 (dd, 1 H, *J* = 1.5, 8.5 Hz), 8.74 (dd, 1 H, *J* = 1.5, 4.9 Hz); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.5, 25.6, 25.9, 28.2, 28.9, 33.5, 35.1, 47.1, 57.4, 62.9, 120.2, 123.1, 125.8, 128.2, 128.5, 141.8, 143.4, 148.7, 154.0, 157.4, 170.8, 171.5, 188.0; HPLC retention time 4.92 min (94.7%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 460 (M); HRMS for $C_{26}H_{28}N_4O_4$ calcd 406.2109, found 460.2119.

1-[(*tert*-Butyldimethylsilyl)oxy]-1-[1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazol-4-yl)methane (6ma). Compound **3m** was prepared by method H (2) starting from **2m**. Reaction of *N*-(4-phenylbutanoyl)proline (**5b**) (86 mg, 0.33 mmol) with the alcohol **3m** (90 mg, 0.3 mmol), HOBt (49 mg, 0.36 mmol), and WSCD·HCl (68 mg, 0.36 mmol) using method I gave **6ma** as a yellow oil (150 mg, 92%) which was purified by column chromatography (1:1 toluene/EtOAc); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.00 (s, 3 H), 0.18 (s, 3 H), 0.92 (m, 9 H), 1.88−2.10 (m, 8 H), 2.23−2.38 (m, 4 H), 2.65−2.73 (m, 2 H), 2.95−3.00 (m, 1 H), 3.38−3.45 (m, 1 H), 3.60−3.67 (m, 2 H), 4.42−4.48 (m, 1 H), 4.62 (dd, 1 H), 5.64 (d, 1 H), 7.14−7.30 (m, 5 H), 7.20 (s, 1 H), 8.68 (s, 1 H).

1-[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-1-(thiazol-4-yl)methanol (6mb). To a solution of **6ma** (150 mg, 0.28 mmol) in THF (2 mL) was added tetrabutylammonium fluoride (0.41 mL of a 1 M solution in THF, 0.41 mmol) at room temperature. After 1 h, the solution was evaporated and the residue was partitioned between EtOAc and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by chromatography (1:1 acetone/EtOAc) to give **6mb** as a yellow oil (93 mg, 79%); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.85−2.36 (m, 12 H), 2.65−2.72 (m, 2 H), 3.46−4.00 (m, 4 H), 4.38−5.25 (m, 3 H), 5.82−6.05 (m, 1 H), 7.14−7.30 (m, 5 H), 7.40 (m, 1 H), 8.72−8.80 (m, 1 H).

4-[[1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]carbonyl]thiazole (9a). Reaction of the alcohol **6mb** (93 mg, 0.22 mmol) with $(COCl)_2$ (28 μL, 0.32 mmol), DMSO (46 μL, 0.65 mmol), and TEA (206 μL, 1.49 mmol) using method J gave **9a** as a colorless foam (50 mg, 54%) which was purified by column chromatography (1:2 acetone/EtOAc): $[\alpha]_D$ −99.8° (*c* 1.0, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.90−2.24 (m, 9 H), 2.21−2.44 (m, 3 H), 2.64−2.68 (m, 2 H), 3.37−3.43 (m, 1 H), 3.54−3.59 (m, 1 H), 3.70−3.75 (m, 1 H), 3.89−4.01 (m, 1 H), 4.75 (dd, 1 H, *J* = 3.5, 7.8 Hz), 5.71 (dd, 1 H, *J* = 4.7, 9.0 Hz), 7.16−7.27 (m, 5 H), 8.25 (d, 1 H, *J* = 2.0 Hz), 8.88 (d, 1 H, *J* = 2.0 Hz); ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 24.6, 25.1, 25.9, 28.6, 33.5, 35.2, 47.1, 57.5, 62.7, 125.7, 126.2, 128.2, 128.5, 141.8, 152.8, 153.8, 170.4, 171.4, 192.4; HPLC retention time 5.29 min (94.2%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 425 (M); HRMS for $C_{23}H_{27}N_3O_3S_1$ calcd 425.1772, found 425.1751. Anal. ($C_{23}H_{27}N_3O_3S_1 \cdot 0.1H_2O$) C, H, N.

1-[[1-(4-Phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinecarboxaldehyde (20) was prepared by the method of Henning *et al.*[25] and purified by column chromatography (2:1 CH_2Cl_2/EtOAc) to give **20** as a white solid: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.85−2.48 (m, 12 H), 2.57−2.69 (m, 2 H), 3.24−3.85 (m, 4 H), 4.59−4.78 (m, 2 H), 7.16−7.29 (m, 5 H), 9.50 (d, 1 H, *J* = 1.1 Hz); HPLC retention time 4.17 min (93.7%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS (EI) *m/z* 342 (M); HRMS for $C_{20}H_{26}N_2O_3$ calcd 342.1942, found 342.1947.

Ethyl [1-[[1-(4-phenylbutanoyl)-2(*S*)-pyrrolidinyl]carbonyl]-2(*S*)-pyrrolidinyl]-2-oxoacetate (21) was prepared by the method of Henning *et al.*[25] and purified by column chromatography (2:1 toluene/EtOAc) to give **21** as a colorless oil: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.35 (t, 3 H, *J* = 7.2 Hz), 1.90−2.15 (m, 9 H), 2.22−2.36 (m, 3 H), 2.64−2.68 (m, 2 H), 3.38−3.42 (m, 1 H), 3.55−3.66 (m, 2 H), 3.91−3.97 (m, 1 H), 4.31 (q, 2 H, *J* = 7.2 Hz), 4.65 (dd, 1 H, *J* = 3.6, 8.2 Hz), 5.17 (dd, 1 H, *J* = 5.9, 8.8 Hz), 7.16−7.29 (m, 5 H); HPLC retention time 5.60 min (91.8%), eluting with 60% CH_3CN in water, 1.0 mL/min; MS *m/z* 414 (M); HRMS for $C_{23}H_{30}N_2O_5$ calcd 414.2153, found 414.2121.

Enzyme Assays for Prolyl Endopeptidase (PEP). The pig kidney prolyl endopeptidase used in assays of inhibitor potency was a partially purified preparation following the method of Yoshimoto *et al.*[32] Prolyl endopeptidase is present in all tissues, and the enzyme from different tissues is biologically and immunologically identical.[33] Inhibition assays of the text compounds (**7a**−**21**) were carried out with Z-Gly-Pro-*p*-nitroanilide as substrate. The inhibitor solution (3 μL of a DMSO solution of varying concentrations) was added to the substrate solution (0.26 mM in dioxane and 0.2 M phosphate buffer solution). To this solution was added 20 μL of the enzyme solution (0.05 units/mL), and this reaction mixture was incubated for 20 min at 37 °C. *p*-Nitroaniline formation was monitored spectrophotometrically at 405 nm. The IC_{50} value (n = 3−6) was estimated from the inhibitor concentration vs the activity curve.

For K_i determinations, PEP from *Flavobacterium meningosepticus* was purchased from Seikagaku Kougyo Co., Ltd., Tokyo. The substrate concentration was varied between 0.46 and 0.058 mM, the inhibitor concentration was varied between 0 and 0.75 μg, and the PEP concentration was 25 nM. K_i values were determined by Dixon plots. The average of triplicate assays, plotting $1/v$ vs inhibitor concentration, gave intersecting lines with a correlation coefficient ≥ 0.97. The **7a**,**b**, and **11a**,**b** PEP inhibitor K_i values are 0.25, 0.19, 440, and 350 nM, respectively. The calculated differences in binding energies were obtained from these measured K_i values for thiazole (**7a**,**b**) and thiophene (**11a**,**b**) derivatives.

Acknowledgment. We wish to acknowledge the contributions of Mr. Y. Arai for the preparation of some of the compounds. We also thank Ms. S. Miki for obtaining the high-resolution mass spectra and Ms. N. Ichihara and Dr. S. Gomi for analyzing the ^{1}H-NMR and ^{13}C-NMR spectra.

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