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Design and synthesis of new potent dipeptidyl peptidase IV inhibitors with enhanced ex vivo duration

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Abstract—A series of 5β-methylprolyl-2-cyanopyrrolidine analogs were synthesized and evaluated as DPP-IV inhibitors, and the duration of their ex vivo activity was assessed. Comparison of their potency and duration of action was done among three different species. The mode of binding was investigated, and the effect on the plasma glucose level was evaluated. Structure–activity relationships are also presented.

1. Introduction

Dipeptidyl peptidase IV (DPP-IV, E.C. 3.4.14.5) is a serine protease that catalyzes the cleavage of dipeptides from the N-terminus of proteins with the sequence H-X-Pro-Y or H-X-Ala-Y- (where X, Y = any amino acid, Y ≠ Pro).[1] DPP-IV is widely distributed in mammalian tissues and is found in great abundance in the kidney, liver, intestinal epithelium, and placenta.[2] Inhibition of DPP-IV results in elevation of the circulating level of endogenous glucagon-like peptide-1 (GLP-1),[3] which is produced by L-cells of the small intestine in response to food intake.[4] GLP-1 stimulates the secretion of insulin in a glucose-dependent manner, inhibits glucagon release, slows gastric emptying, and induces satiety, with all of these actions promoting the control of glucose homeostasis in patients with type II diabetes. The active form of GLP-1 is rapidly inactivated by plasma DPP-IV, which cleaves the dipeptide from the N-terminus.[5,6] Thus, inhibition of DPP-IV could lead to the persistence of circulating GLP-1 levels, which would enhance insulin secretion and improve glucose tolerance. Accordingly, much attention has been paid to DPP-IV as a promising new target for drugs. In fact, a clinical investigation has already demonstrated the benefits of DPP-IV inhibition in type II diabetes.[7] Several DPP-IV inhibitors are currently under clinical development, including LAF237 (**1**),[8] MK-0431 (**2**),[9] and BMS-477118 (**3**)[10] (Fig. 1).

Sakashita et al. reported that (4-substituted)prolyl-2-cyanopyrrolidines increased DPP-IV inhibitory activity relative to the unsubstituted analog and (4-β-substituted)prolyl-2-cyanopyrrolidine showed 20-fold more potent activity than the corresponding 4-α-isomer.[11] Also Tsai et al. reported that (4β-carbamoyl)prolyl-2-cyanopyrrolidines showed enhanced inhibitory activity while (5,5-*gem*-dimethyl)prolyl-2-cyanopyrrolidine showed

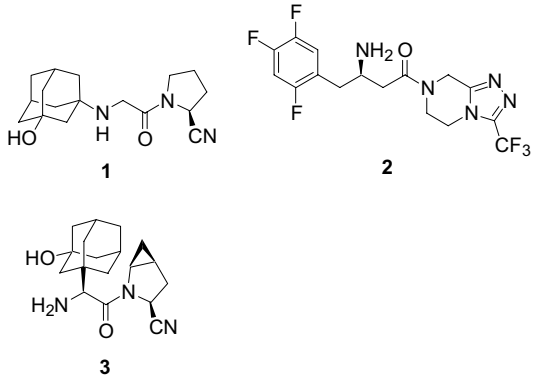

Figure 1. Long-acting inhibitors of DPP-IV.

Keywords: DPP-IV inhibitor; Prolyl-2-cyanopyrrolidine.
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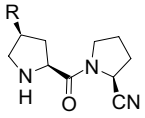
a 500-fold loss of DPP-IV inhibitory activity relative to the unsubstituted analog.[12] In an effort to develop long-acting DPP-IV inhibitors, we previously reported a series of (4*R*)-4-[2,6-disubstituted(hydroxyphenyl)]prolyl-2-cyanopyrrolidines, which showed a long duration of action as their corresponding phenol glucuronates.[13] We here report on the discovery of a series of (4-β-substituted-5-β-methyl)prolyl-2-cyanopyrrolidines possessing long duration of potent in vivo activity.

2. Chemistry

Synthesis of the compounds listed in Tables 1–3 is outlined in Schemes 3–8. Compound **8** was synthesized as described in Scheme 3. A nitrile ester **37** was prepared from a tosylate **36**, which was obtained by tosylation of the corresponding alcohol **35**. Conversion of the nitrile residue of **37** to an ethyl ester residue to produce **38** was carried out by treatment with trimethylsilyl chloride in EtOH, followed by reprotection of the deprotected pyrrolidine nitrogen. Removal of the benzyl moiety of **38** by catalytic hydrogenation afforded the half ester **39**, after which dehydrative condensation with L-prolineamide produced **40**. Lithium borohydride reduction of **40** gave the alcohol **41**. Dehydration of the amide of **41** resulted in the nitrile **42** and acidic deprotection of **42** led to the production of **8**.

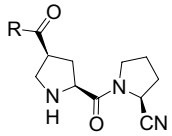
Compounds **11** and **14**–**26** were synthesized from **40**, as shown in Scheme 4. Alkaline hydrolysis of **40** afforded the carboxylic acid **43**, which was converted to a benzyl ester **44** by treatment with benzyl bromide in the presence of potassium carbonate. Dehydration of**44** with trifluoroacetic anhydride in the presence of pyridine provided the nitrile **45**, which was converted to the corresponding carboxylic acid **46** by catalytic hydrogenation. Reaction of **46** with the appropriate amines produced the amides **47b**–**e**, **47k**, **47l**, and **47n**. Other amide nitriles **47a**, **47f**–**47j**, and **m** were prepared from **43** by amidation with the appropriate amines, followed by dehydration with trifluoroacetic anhydride and pyridine. Acidic deprotection of **47a**–**n** produced **11**, and **14**–**26**, respectively.

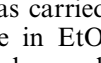
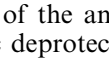
Table 1. In vitro inhibition for human DPP-IV and ex vivo plasma DPP-IV inhibition in normal rats



Compound	R	Human DPP-IV IC_{50} (nM)	Plasma DPP-IV inhibition (%) at 1 mg/kg po, normal rats (6 h)
4	H	20	20
6	Allyl	3.5	38
7	*n*-Propyl	3.4	50
8	CH_2OH	6.3	64
9	CH_2CH_2OH	3.8	64
10	CH_2COOH	16	58
11	$CONMe_2$	1.8	76
12	CH_2CONMe_2	4.5	66
13	$CH_2CH_2CONMe_2$	2.4	58

Table 2. In vitro inhibition for human DPP-IV and ex vivo plasma DPP-IV inhibition in normal rats



Compound	R	Human DPP-IV IC_{50} (nM)	Plasma DPP-IV inhibition (%) at 1 mg/kg po, normal rats	
			6 h	10 h
14	NH_2	7.7	47	20
15	NHMe	3.5	75	28
16	NHEt	4.4	60	19
17	NH^nPr	5.7	59	21
11	NMe_2	1.8	76	52
18	NMeEt	4.9	71	34
19	NEt_2	6.0	43	NT*
				
20	$n = 1$	4.0	77	46
21	$n = 2$	5.6	85	40
22	$n = 3$	4.9	84	50
23	$n = 4$	8.3	68	NT*
24	$n = 5$	11	63	NT*
25		4.3	73	44
26		4.8	72	19

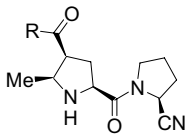
* Not tested.

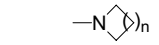
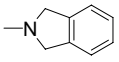
Synthesis of **10** and **12** is outlined in Scheme 5. Oxidation of protected L-hydroxyproline **48** afforded the corresponding ketone **49**, after which C-2 homologation using Peterson reaction resulted in **50**. Catalytic hydrogenation of **50**, followed by reprotection with Boc_2O, gave **51**.[14] Dehydrative condensation of **52** with (2*S*)-2-cyanopyrrolidine afforded **53**, acidic deprotection of which produced **10**. Then N-protection of **10** gave **54**, after which dehydrative condensation with *N*,*N*-dimethylamine led to **55**, and subsequent acidic deprotection produced **12**.

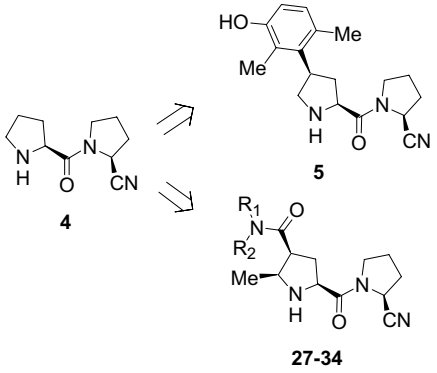
The synthesis of **9** is described in Scheme 6. Acidic deprotection of **51**, followed by N-protection with Boc_2O, afforded **56**. Sodium borohydride reduction of **56** by the acid anhydride method resulted in **57**, protection of which as a teterahydropyranyl ether provided **58**. Alkaline hydrolysis of **58** led to **59**, condensation of which with (2*S*)-2-cyanopyrrolidine gave **60**. Acidic deprotection of **60** produced **9**.

The synthesis of **13** is described in Scheme 7. Sonogashira cross-coupling reaction of the enol triflate **62**, which was prepared from the corresponding keto-derivative **61**, was done with 2-propyn-1-ol to

Table 3. In vitro inhibition for human DPP-IV and ex vivo plasma DPP-IV inhibition in normal rats



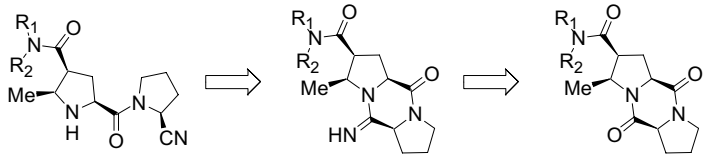
Compound	R	Human DPP-IV IC_{50} (nM)	Plasma DPP-IV inhibition (%) at 1 mg/kg po, normal rats	
			6 h	10 h
27	NMe_2	10	95	84
28	NMeEt	15	92	79
				
29	$n = 1$	13	94	88
30	$n = 2$	6.5	94	88
31	$n = 3$	13	91	81
32		20	94	85
33		6.2	92	85
34		5.6	86	77



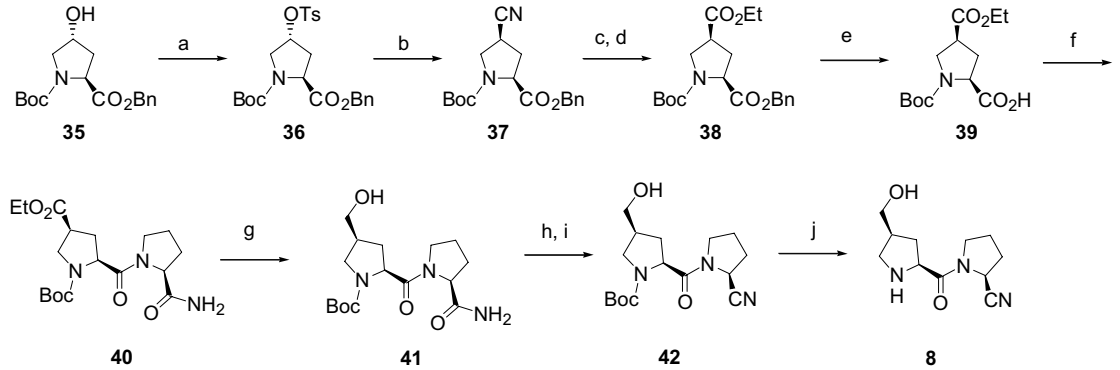
Scheme 1. Molecular design of long-acting inhibitors of DPP-IV.

afford **63**, after which catalytic hydrogenation gave **64**.[15] Oxidation of **64** with 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) provided **65** and then amide formation with *N*,*N*-dimethylamine gave **66**. Alkaline hydrolysis of **66** afforded **67**. Condensation of **67** with L-prolineamide, followed by dehydration with trifluoroacetic acid anhydride and pyridine, produced the nitrile **68**. Acidic deprotecti on of **68** resulted in **13** as a *p*-TsOH salt.

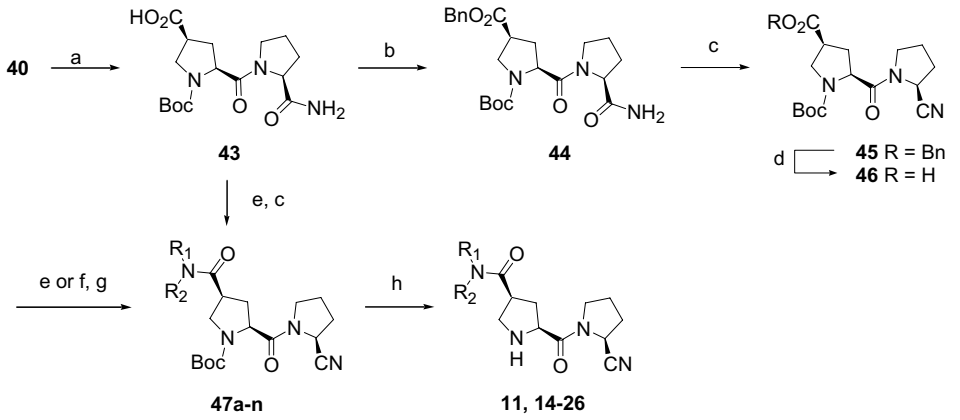
Synthesis of **27**–**34** is outlined in Scheme 8. The γ-carboxylic acid residue of an appropriately protected L-glutamic acid **69** was converted to the methyl ester residue by the usual method resulting in **70**. Acetylation of the



Scheme 2. Chemical instability of cyanopyrrolidines.

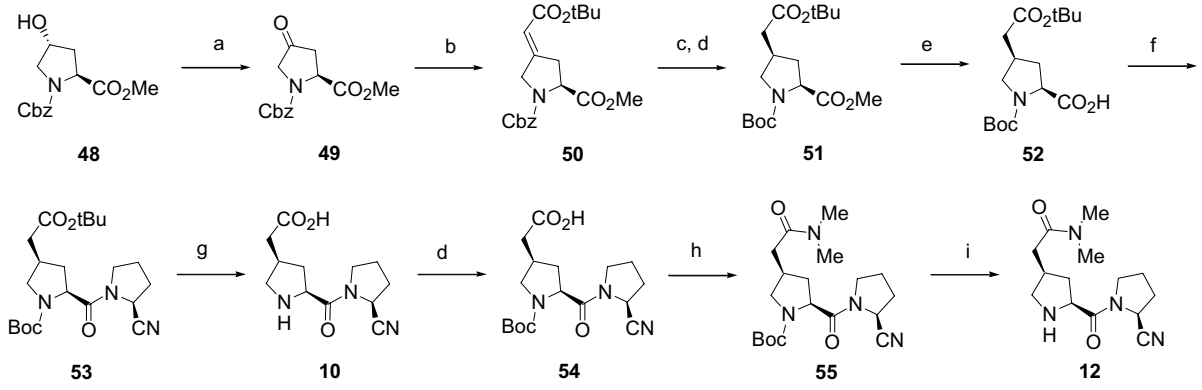


Scheme 3. Synthesis of **8**. Reagents: (a) TsCl, pyridine, CH_2Cl_2; (b) NaCN, DMSO; (c) TMSCl, EtOH; (d) Boc_2O, $NaHCO_3$ aq, THF, H_2O; (e) H_2, 10% Pd/C, MeOH; (f) L-ProNH_2, EDC, HOBt, CH_2Cl_2; (g) $LiBH_4$, THF; (h) TFAA, pyridine, CH_2Cl_2; (i) K_2CO_3, MeOH; (j) *p*-TsOH, EtOH.



	R_1	R_2		R_1 R_2
a	Me	Me	**h**	-$(CH_2)_3$-
b	H	H	**i**	-$(CH_2)_4$-
c	H	Me	**j**	-$(CH_2)_5$-
d	H	Et	**k**	-$(CH_2)_6$-
e	H	nPr	**l**	-$(CH_2)_7$-
f	Me	Et	**m**	-$CH_2CH_2OCH_2CH_2$-
g	Et	Et	**n**	-$CH_2CH_2SCH_2CH_2$-

Scheme 4. Synthesis of **11** and **14**–**26**. Reagents: (a) NaOH aq, MeOH; (b) BnBr, K_2CO_3, DMF; (c) TFAA, pyridine, THF, CH_2Cl_2; (d) H_2, $Pd(OH)_2$, EtOAc; (e) R_1R_2NH, EDC, HOBt, CH_2Cl_2; (f) $ClCO_2Et$, Et_3N, THF; (g) R_1R_2NH, THF; (h) *p*-TsOH, EtOH.



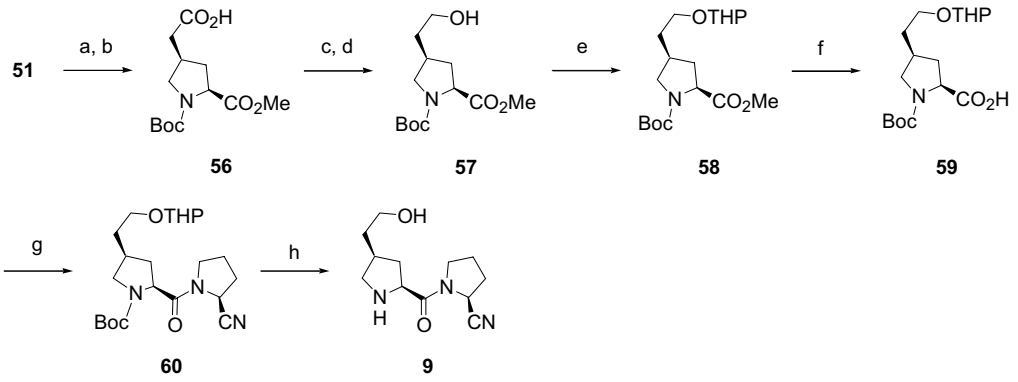
Scheme 5. Synthesis of **10** and **12**. Reagents: (a) SO_3–pyridine, Et_3N, DMSO; (b) $Me_3SiCH_2CO_2{}^tBu$, LDA, THF; (c) H_2, PtO_2, AcOH; (d) Boc_2O, $NaHCO_3$ aq, THF; (e) NaOH aq, MeOH, THF; (f) L-ProCN, EDC, HOBt, Et_3N, DMF; (g) *p*-TsOH, CH_3CN; (h) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (i) *p*-TsOH, EtOH.

γ-carbon of **70**, followed by treatment with acetic acid, produced **71**.[16] Stereoselective catalytic hydrogenation of **71** using platinum dioxide as the catalyst led to exclusive production of the 4β,5β-isomer **72**. Acidic deprotection of **72** with trifluoroacetic acid, followed by N-protection with Boc_2O, provided **73**. Condensation of **73** with L-prolineamide gave **74**, alkaline hydrolysis of which afforded **75**. Esterification of **75** with benzyl bromide in the presence of potassium carbonate led to the benzyl ester **76**, after which dehydration afforded **77**, and subsequent deprotection by catalytic hydrogenation led to the corresponding carboxylic acid **78**. Reaction of **78** with an appropriate amine in the presence of EDC gave amides **79a**–**h**, acidic deprotection of which resulted in **27**–**34**, respectively.

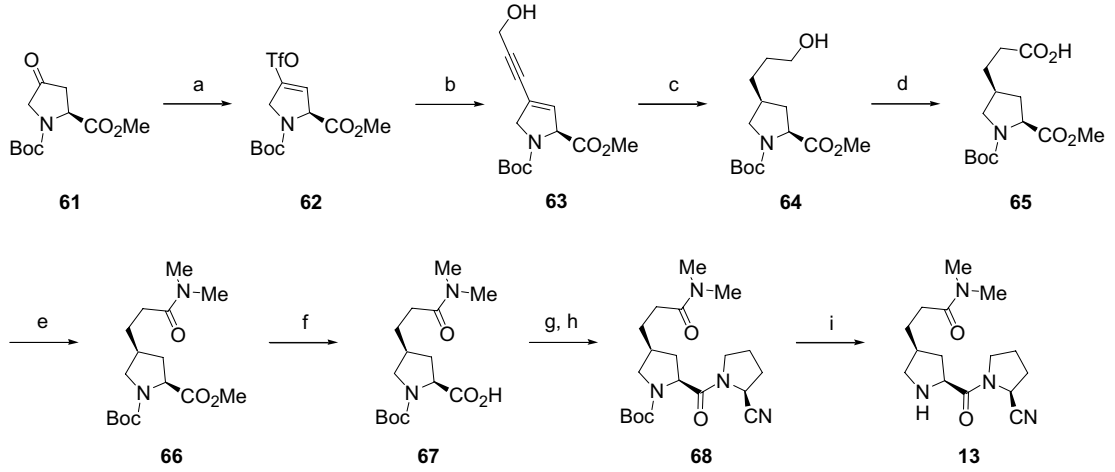
3. Results and discussion

The inhibitory activity of compounds listed in Tables 1–3 was tested in vitro using purified human DPP-IV enzyme and the synthetic substrate H-Gly-Pro-AMC.[17,18] Production of 7-amino-4-methyl coumarin (AMC) was measured at 460 nm over 15 min. Plasma DPP-IV inhibition (%) after oral dosing of each test compound (1 mg/kg) was monitored for 6 h and/or 10 h in normal rats (ex vivo experiment).

The previously reported phenolic inhibitors were found by molecular design of inhibitors with increased stability in solution.[13] The same concept was applied to drug design this time, as illustrated in Scheme 1.



Scheme 6. Synthesis of **9**. Reagents: (a) TFA, PhOMe; (b) Boc_2O, $NaHCO_3$ aq, THF; (c) $ClCO_2Et$, Et_3N, CH_2Cl_2; (d) $NaBH_4$, THF, H_2O; (e) DHP, PPTS, CH_2Cl_2; (f) NaOH aq, MeOH, THF; (g) L-ProCN, MsOBt, Et_3N, DMF; (h) (+)-CSA, EtOH.

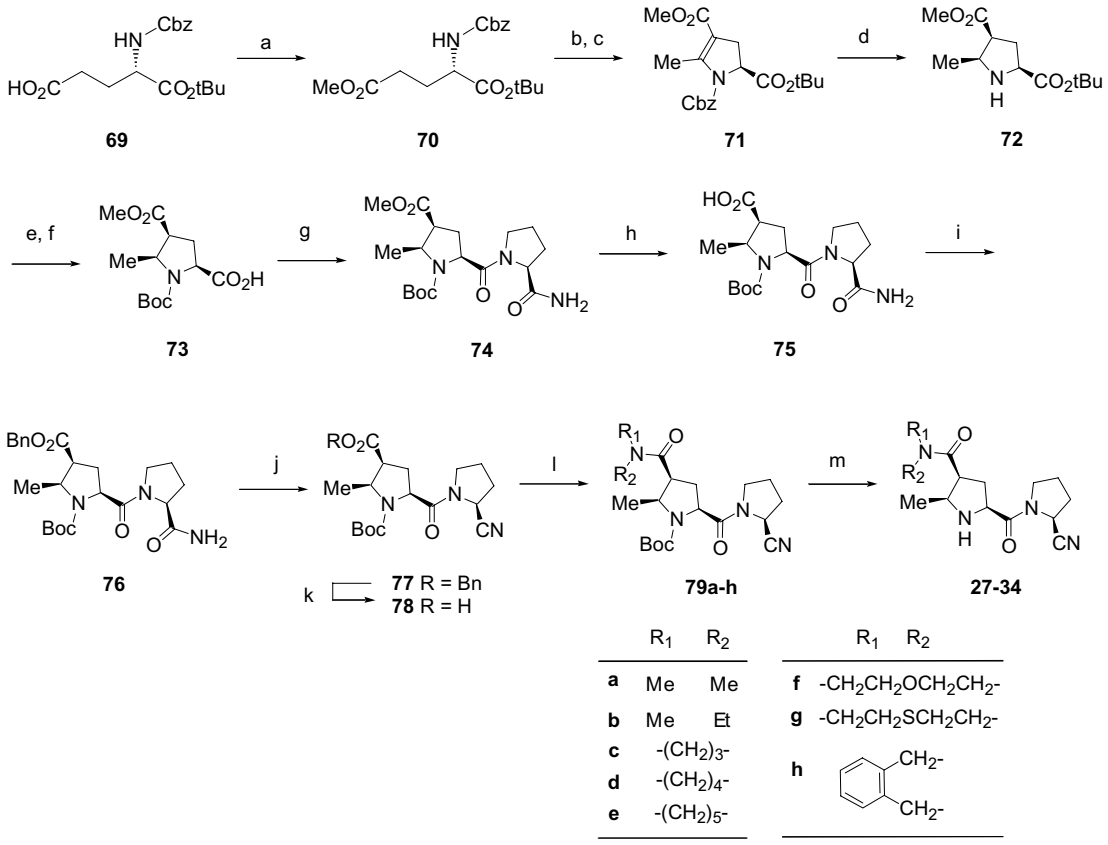


Scheme 7. Synthesis of **13**. Reagents: (a) NaHMDS, $PhNTf_2$, THF; (b) 2-propyn-1-ol, $PdCl_2(PPh_3)_2$, CuI, $^iPr_2NH$, THF; (c) H_2, 10% Pd–C, MeOH; (d) TEMPO, NaClO, $NaClO_2$, MeCN, sodium phosphate buffer; (e) Me_2NH, EDC, HOBt, Et_3N, CH_2Cl_2; (f) NaOH aq, MeOH, THF; (g) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (h) TFAA, pyridine, THF; (i) *p*-TsOH, EtOH.

The SAR described in our previous paper revealed that 4β-substitution of the prolyl moiety of **4** contributed to an increase of inhibitory activity relative to the corresponding 4α-substitution. In the course of further studies exploring the SAR 4β-substituted prolyl-2-cyanopyrrolidine-based inhibitors, we encountered unexpectedly strong activity and a long duration in ex vivo DPP-IV inhibition with compound **5**, which contained a 4β-(hydroxyphenyl)prolyl moiety. However, metabolic and pharmacokinetic (PK) studies of **5** revealed uncharacteristically poor oral bioavailability ($F = 3\%$). Similar results were obtained with other 4β-phenol residue-containing analogs, and these results led to the discovery of an active metabolite (the corresponding glucuronate) in vivo. Since the purpose of this project was to identify a drug candidate, we continued to search for other 4β substituents that increased the inhibitory activity and the duration of ex vivo DPP-IV inhibition. Our strategy was to prepare dipeptide analogs of prolyl-2-cyanopyrrolidine with improved PK and activity profiles. Accordingly, the design and synthesis of 4β-substituted prolyl-2-cyanopyrrolidines was undertaken.

As shown in Table 1, aliphatic substituents such as allyl and propyl groups were introduced at the 4β-position of the prolyl moiety of **4**, producing **6** and **7** that showed stronger inhibitory activity and a slight increase in their duration of action.[13] A further increase of ex vivo DPP-IV inhibition was obtained by the introduction of relatively polar hydroxyl substituents, as illustrated by compounds **8** and **9**. Introduction of an acetic acid residue as a more polar substituent relative to the alcohol residue led to **10**, which showed weaker inhibitory activity despite long-lasting ex vivo activity, presumably because of poor oral absorption. Introduction of a 4β-*N*,*N*-dimethylaminocarbonyl moiety into **4** afforded **11**, which showed an increase of inhibitory activity and ex vivo DPP-IV inhibition, while the *N*,*N*-dimethylaminocarbonylmethyl and *N*,*N*-dimethylaminocarbonylethyl analogs **12** and **13** both showed slightly less potent activity and a shorter duration of action relative to **11**. Therefore, the *N*,*N*-dimethylamide analog **11**, which showed the best activity profile among the compounds listed in Table 1, was selected as the chemical lead for further optimization.

To perform further optimization of 4β substituents, the 4β-amide analogs as shown in Table 2 were synthesized



Scheme 8. Synthesis of **27**–**34**. Reagents: (a) MeI, K_2CO_3, DMF; (b) AcCl, LiHMDS, THF; (c) AcOH; (d) H_2, PtO_2, AcOH; (e) TFA aq; (f) Boc_2O, $NaHCO_3$ aq, THF; (g) L-ProNH_2, EDC, HOBt, Et_3N, CH_2Cl_2; (h) LiOH aq, MeOH; (i) BnBr, K_2CO_3, DMF; (j) TFAA, pyridine, THF; (k) H_2, $Pd(OH)_2$, EtOAc; (l) R_1R_2NH, EDC, HOBt, NMM, CH_2Cl_2; (m) *p*-TsOH, EtOH.

and evaluated. The primary amide analog **14** and the *N*-alkyl amide analogs **15**–**17** tended to show a shorter duration of ex vivo activity and slightly weaker in vitro activity. The relatively more lipophilic *N*,*N*-dialkyl amide analogs **18** and **19** tended to show a shorter duration of action and slightly less potent in vitro activity relative to **11**. The cyclic amine-containing amides **20**–**26** were also synthesized and evaluated for in vitro activity and ex vivo DPP-IV inhibition. Among these compounds, analogs **20**–**22** showed a relatively longer duration of action while the more lipophilic amine-containing amide analogs **23** and **24** tended to have a shorter duration of action. The morpholine and thiomorpholine analogs **25** and **26** demonstrated nearly the same level of ex vivo activity as **11** after 6 h, but **25** showed more potent ex vivo inhibition than **26** after 10 h. As a result, the *N*,*N*-dialkyl amide analogs **11**, **18**–**22**, and **25** tended to be more potent ex vivo inhibitors than the *N*-alkyl amide analogs **15**–**17** after 10 h, while the thiomorpholine analog **26** was the only compound with unexpectedly weak ex vivo inhibitory activity after 10 h. It was assumed that more difficult metabolic inactivation of *N*,*N*-dialkyl amide analogs could be one of the reasons for their relatively longer duration of ex vivo activity.

It is well-known that DPP-IV inhibitors with 2-cyanopyrrolidine suffer from instability because the *P*2 amino group (either in a ring or an open chain) attacks the cyano group under neutral or basic conditions (Scheme 2), and the resulting cyclized imidate and diketopiperazine lose their activity as DPP-IV inhibitors.[8,19] Steric hindrance in proximity to the *P*2 amino group is known to modulate the rate of intramolecular cyclization and hence influences the stability.[8,19] Based on this information, we introduced a 5β-methyl group at the prolyl moiety of **11**, affording **27** with slightly weaker inhibitory activity, but enhanced ex vivo DPP-IV inhibition. The nonsymmetrical amide analog **28** also showed a similar profile of in vitro activity and similar duration of ex vivo activity. Other amide analogs containing cyclic amines such as **29**–**33** exhibited a long duration of action despite their different amine structures while the slightly more lipophilic isoindoline-containing amide analog **34** tended to show slightly less potent ex vivo DPP-IV inhibition. Thus, the 5β-methyl analogs **27**–**34** had a longer duration of action relative to **11**. The remaining percentages of **11** and **27** after their incubation in Tris buffer (pH 7.4, 100 mM) at room temperature for 24 h were 52% and 92%, respectively. Increased stability of these 5β-methyl analogs in solution was estimated to be one of the reasons for their enhanced duration of action.

In order to evaluate species differences of DPP-IV inhibition, the selected compounds (**11**, **27**–**28**, **30**, **32**, and **34**) were tested for ex vivo activity in three animal species (rat, beagle dog, and cynomolgus monkey). As shown in Table 4, compounds **27**, **28**, **30**, and **32** exhibited a long duration of action in all species tested,

Table 4. Plasma DPP-IV inhibition (%) of representative compounds (1 mg/kg, po) in the three species

Compound	12 h		
	Rat	Beagle dog	Cynomolgus monkey
11	24	45	44
27	81	98	93
28	74	89	95
30	82	95	95
32	85 (10 h)	98	90
34	71	16	NT*

* Not tested.

while the relatively lipophilic isoindoline analog **34** exhibited a difference in ex vivo activity between rats and beagle dogs. Analog **11**, which does not possess a 5β-methyl group, exhibited much weaker ex vivo activity after 12 h.

To investigate the effect of the 5β-methyl group on the binding of this series of analogs, the in vitro inhibitory activity of **11** and **27** was evaluated after preincubation of the enzyme with the test compound followed by addition of the synthetic substrate (Fig. 2). Compound **27** showed an increase of inhibitory activity in a preincubation time-dependent manner, while **11** demonstrated constant inhibitory activity regardless of the preincubation time.

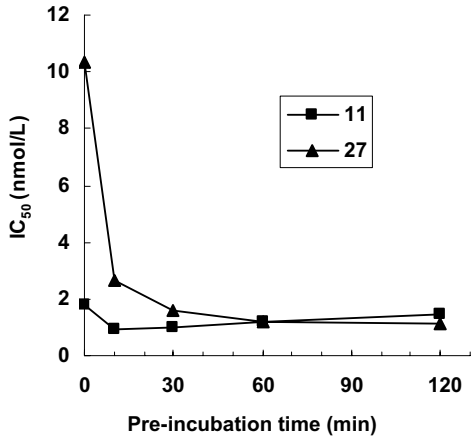


Figure 2. Binding modes of **11** and **27**.

To evaluate the effect of the inhibitor **27** in normal rats, a dose of 1 mg/kg was given orally at 30 min prior to glucose administration. The blood glucose level was evaluated while oral dosing of glucose (1 g/kg) was carried out over time (0, 6, and 12 h). As shown in Figure 3, significant suppression of the blood glucose level was observed at 0 and 6 h after oral dosing. Oral dosing of **27** also tended to suppress the plasma glucose level after 12 h.

In summary, a series of 5β-methyl-prolyl-2-cyanopyrrolidine analogs were identified as long-acting DPP-IV inhibitors, which exhibited enhanced inhibitory activity in a time-dependent manner. Compound **27** significantly suppressed the blood glucose level in normal rats at 0 and 6 h after oral dosing. The 5β-methyl residue was found to be effective for the improvement of chemical stability in solution.

4. Experimental

4.1. Chemistry

Analytical samples were homogeneous as confirmed by TLC and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. The chemical shift values are reported in parts per million (δ) and coupling constants (J) in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M-1200H spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained on a PerSeptive Biosystems VoyagerTM Elite spectrometer. Infrared spectra (IR) were measured in a JASCO FT/IR-430 spectrometer. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako gel C200 or Fuji Silysia FL60D]. Thin-layer chromatography was performed on silica gel (Merck TLC or HPTLC plates, silica gel

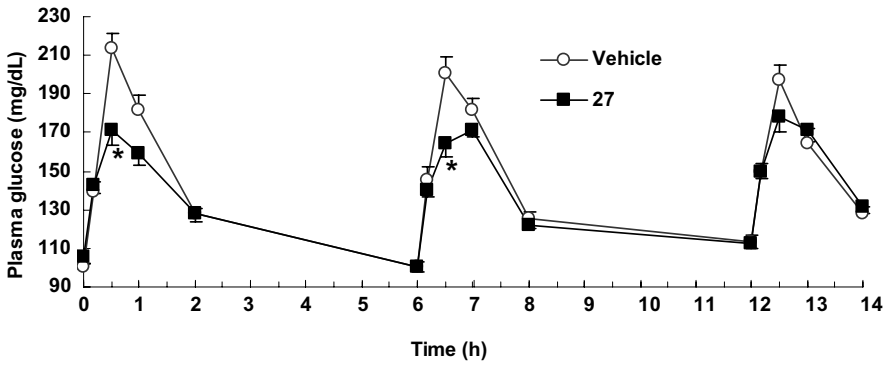


Figure 3. Effect of the inhibitor **27** on glucose excursion during multiple oral glucose tolerance tests in normal rats. All rats received 1 g/kg glucose orally at 0, 6 and 12 h. The compound (1 mg/kg) was orally administered to rats at −0.5 h. Data are expressed as means ± SEM ($n = 7$). $^*P < 0.05$; significantly different from the vehicle by Student's t test.

60 F254). The following abbreviations for solvents and reagents are used; tetrahydrofuran (THF), diethyl ether (Et_2O), diisopropyl ether ($^iPr_2O$), *tert*-butyl methyl ether (^{t}BuOMe), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), ethanol (EtOH), acetic acid (AcOH), and hydrochloric acid (HCl).

4.1.1. Benzyl (2*S*,4*R*)-(1-*tert*-butoxycarbonyl)-4-[(4-methylbenzenesulfonyl)oxy]-2-pyrrolidinecarboxylate (36). To a stirred solution of **35** (64.2 g, 200 mmol) in $CHCl_3$ (200 mL) were added pyridine (50 mL, 646 mmol) and *p*-toluenesulfonyl chloride (76.3 g, 400 mmol) at room temperature. After being stirred for 88 h, the reaction mixture was quenched with 1 M HCl and extracted with $CHCl_3$. The organic layer was successively washed with 1 M HCl, water, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:4) as an eluant. The resulting crystalline solid was washed with $^iPr_2O$ to yield **36** (77.7 g, 82%) as a white powder. TLC $R_f = 0.63$ (hexane/EtOAc, 1:1); MS (MALDI, pos. 20 V) *m*/*z* 498 $(M+Na)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 and 1.42 (s, 9H), 2.10–2.20 (m, 1H), 2.37–2.60 (m, 1H), 2.46 (s, 3H), 3.54–3.68 (m, 2H), 4.37–4.50 (m, 1H), 4.97–5.28 (m, 3H), 7.28–7.42 (m, 7H), 7.78 (d, $J = 7.5$ Hz, 2H).

4.1.2. Benzyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-cyano-2-pyrrolidinecarboxylate (37). To a stirred solution of **36** (77 g, 162 mmol) in DMSO (200 mL) was added NaCN (12 g, 244 mmol) at room temperature. After being stirred for 4 h at 80 °C, the reaction mixture was cooled to room temperature and diluted with ^{t}BuOMe. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:4) as an eluant to yield **37** (31.5 g, 58%) as a colorless oil. TLC $R_f = 0.32$ (EtOAc/hexane, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 and 1.47 (s, 9H), 2.21–2.37 (m, 1H), 2.60–2.77 (m, 1H), 3.03–3.17 (m, 1H), 3.60–3.73 (m, 1H), 3.84–4.03 (m, 1H), 4.30–4.50 (m, 1H), 5.05–5.37 (m, 2H), 7.30–7.42 (m, 5H).

4.1.3. 2-Benzyl 4-ethyl (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-2,4-pyrrolidinediicarboxylate (38). Trimethylsilyl chloride (150 mL, 1.18 mol) was added dropwise to EtOH (200 mL) at 0 °C. To the reaction mixture was added a solution of **37** (20.9 g, 63 mmol) in CH_2Cl_2 (120 mL). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was cooled to 0 °C and quenched with H_2O. The aqueous layer was neutralized with aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (1000 mL). To the organic layer was added di-*tert*-butyl-dicarbonate (13.7 g, 63 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:6) as an eluant to yield a mixture of **38** and the corresponding diethyl ester (20.0 g), which was used for the next reaction without further purification.

4.1.4. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(ethoxycarbonyl)-2-pyrrolidinecarboxylic acid (39). To a solution of **38** and corresponding diethyl ester (20.0 g) in MeOH (150 mL) was added 10% palladium on carbon (6.0 g). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc/hexane (1:1) and extracted with aqueous $NaHCO_3$. The aqueous layer was acidified with 2 M HCl and extracted with EtOAc. The organic layer was dried with Na_2SO_4 and concentrated in vacuo. The resulting crystalline solid was washed with $^iPr_2O$ to yield **39** (9.25 g, 51%) as a white powder. TLC $R_f = 0.40$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, $J = 7.5$ Hz, 3H), 1.44 and 1.50 (s, 9H), 2.30–2.67 (m, 2H), 3.00–3.12 (m, 1H), 3.60–3.92 (m, 2H), 4.14 (q, $J = 7.5$ Hz, 2H), 4.22–4.42 (m, 1H).

4.1.5. Ethyl (3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1- pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylate (40). To a stirred solution of **39** (4.31 g, 15 mmol) in CH_2Cl_2 (30 mL) were added L-prolineamide (2.57 g, 22 mmol), 1-hydroxybenzotriazole (2.30 g, 15 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.45 g, 18 mmol) at 0 °C. After being stirred for 4 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 5% $KHSO_4$, aq $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to give **40** as a colorless oil. TLC $R_f = 0.42$ (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22–1.30 (m, 3H), 1.40 and 1.47 (s, 9H), 1.70–2.60 (m, 6H), 3.00–3.18 (m, 1H), 3.48–3.94 (m, 4H), 4.10–4.20 (m, 2H), 4.22–4.70 (m, 2H), 5.35 and 5.59 (br s, 1H), 7.01 and 7.94 (br s, 1H).

4.1.6. (2*S*)-1-{[(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(hydroxymethyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarboxamide (41). To a stirred solution of **40** (550 mg, 1.43 mmol) in THF (6 mL) was added lithium borohydride (67 mg, 3.1 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was quenched with brine. The organic solvent was evaporated and the aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using CH_2Cl_2/MeOH (9/1) as an eluant to yield **41** (448 mg, 92%). TLC $R_f = 0.35$ (CH_2Cl_2/MeOH, 9:1); MS (MALDI, pos.) *m*/*z* 364 $(M+Na)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27–1.54 (m, 9H), 1.81–2.25 (m, 5H), 2.24–2.61 (m, 2H), 2.72 and 2.89 (s, 1H), 3.24–3.42 (m, 1H), 3.43–3.83 (m, 5H), 4.19–4.71 (m, 2H), 5.54 and 6.78 (s, 1H), 6.85 and 8.04 (s, 1H).

4.1.7. (2*S*)-1-{[(2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(hydroxymethyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile (42). To a stirred solution of **41** (435 mg, 1.28 mmol) in CH_2Cl_2 (3 mL) were added pyridine (0.82 mL, 10 mmol) and trifluoroacetic anhydride (0.45 mL, 3.2 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was quenched with aq $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was successively washed with 5% $KHSO_4$, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting

residue was used for the next reaction without further purification. To a stirred solution of the residue in MeOH (3 mL) was added K_2CO_3 (44 mg, 0.32 mmol) at room temperature. After 30 min, the reaction mixture was evaporated and diluted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using CH_2Cl_2/MeOH (25:1) as an eluant to yield **42** (350 mg, 85%). TLC $R_f = 0.17$ (CH_2Cl_2/MeOH, 9:1); MS (MALDI, pos.) *m/z* 346 $(M+Na)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30–1.51 (m, 9H), 1.73–1.88 (m, 1H), 1.99–2.34 (m, 5H), 2.33–2.58 (m, 2H), 3.27–3.41 (m, 1H), 3.48–3.88 (m, 5H), 4.29–4.57 (m, 1H), 4.72–4.94 (m, 1H).

4.1.8. (2*S*)-1-{[(2*S*,4*S*)-4-(Hydroxymethyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (8). A solution of **42** (340 mg, 1.05 mmol) and *p*-toluenesulfonic acid (234 mg, 1.23 mmol) in EtOH (6 mL) was refluxed for 5 h. The reaction mixture was evaporated. The resulting crystalline solid was washed with ^{t}BuOMe, collected by filtration, and dried under reduced pressure to yield **8** (410 mg, 98%) as a white powder. TLC $R_f = 0.16$ (EtOAc/AcOH/H_2O, 3:1:1); MS (MALDI, Pos.) *m/z* 224 $(M+H)^+$; IR (KBr) 3442, 3405, 2239, 1658, 1455, 1366, 1206, 1168, 1125, 1034, 1010, 685, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.45–1.60 (m, 1H), 1.93–2.10 (m, 2H), 2.09–2.31 (m, 3H), 2.28 (s, 3H), 2.42–2.63 (m, 1H), 2.99–3.14 (m, 1H), 3.20–3.48 (m, 4H), 3.49–3.62 (m, 2H), 4.40–4.56 (m, 1H), 4.82 (dd, $J = 7.8$, 4.7 Hz, 1H), 7.10 (d, $J = 8.3$ Hz, 2H), 7.46 (d, $J = 8.3$ Hz, 2H), 8.70 (s, 1H), 9.30 (s, 1H).

4.1.9. (3*S*,5*S*)-5-{[(2*S*)-2-(Aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylic acid (43). To a stirred solution of **40** (5.75 g, 15 mmol) in MeOH (40 mL) was added 1 M NaOH (20 mL) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was quenched with 1 M HCl (20 mL). The organic solvent was removed in vacuo. The resulting crystalline solid was collected by filtration, washed with H_2O, and dried in vacuo to yield **43** (4.20 g, 78%) as a white powder. TLC $R_f = 0.20$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.37 and 1.46 (s, 9H), 1.83–2.08 (m, 3H), 2.25–2.45 (m, 2H), 2.57–2.64 (m, 1H), 3.10–3.23 (m, 1H), 3.54–3.63 (m, 2H), 3.73–3.84 (m, 1H), 4.00–4.20 (m, 1H), 4.43–4.66 (m, 2H), 7.15 and 7.22 (s, 1H), 7.75 and 7.81 (s, 1H).

4.1.10. Benzyl (3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)- 3-pyrrolidinecarboxylate (44). To a stirred solution of **43** (4.16 g, 11.7 mmol) in DMF (12 mL) were added K_2CO_3 (1.78 g, 12.9 mmol) and benzylbromide (1.4 mL, 12 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was successively washed with water and brine, dried over $MgSO_4$, and evaporated to yield **44** (4.97 g, 96%). TLC $R_f = 0.48$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 and 1.45 (s, 9H), 1.75–2.63 (m, 4H), 3.05–3.25 (m, 1H), 3.40–4.77 (m, 7H), 5.03–5.29 (m, 3H), 6.98 (s, 1H), 7.29–7.50 (m, 5H), 7.91 and 8.02 (s, 1H).

4.1.11. Benzyl (3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-3-pyrrolidinecarboxylate (45). To a stirred solution of **44** (4.97 g, 11.2 mmol) in THF (75 mL) were added pyridine (4.5 mL, 56 mmol) and trifluoroacetic anhydride (2.4 mL, 17 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was quenched with water, and extracted with hexane/EtOAc (1:1). The organic layer was successively washed with 0.5 M HCl, aq $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated to yield **45** (4.63 g, 97%). TLC $R_f = 0.67$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.35–1.50 (m, 9H), 2.07–2.68 (m, 6 H), 2.98–3.26 (m, 1H), 3.51–3.64 (m, 1H), 3.66–4.04 (m, 3H), 4.30–4.56 (m, 1H), 4.75–4.95 (m, 1H), 5.03–5.30 (m, 2H), 7.29–7.54 (m, 5H).

4.1.12. (3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinecarboxylic acid (46). To a solution of **45** (4.63 g, 10.8 mmol) in EtOAc (43 mL) was added 20% palladium hydroxide on carbon (460 mg). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **46** (3.68 g, 100%). TLC $R_f = 0.43$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.24–1.45 (m, 9H), 1.77–1.92 (m, 1H), 1.99–2.22 (m, 4H), 2.51–2.65 (m, 1H), 2.96–3.23 (m, 1H), 3.37–3.82 (m, 4H), 4.29–4.58 (m, 1H), 4.66-5.15 (m, 1H), 12.58 (s, 1H).

4.1.13. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(dimethylamino)carbonyl]-1-pyrrolidinecarboxylate (47a). To a stirred solution of **43** (300 mg, 0.85 mmol) in CH_2Cl_2 (2 mL) were added dimethylamine hydrochloride (104 mg, 1.27 mmol), triethylamine (0.36 mL, 2.5 mmol), 1-hydroxybenzotriazole (114 mg, 0.85 mmol), and 1-(3-dimetylaminopropyl)-3-ethylcarbodiimide hydrochloride (243 mg, 1.27 mmol) at 0 °C. After being stirred for 20 h at room temperature, the reaction mixture was poured into water, and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was used for the next reaction without further purification. To a solution of the residue in THF (3 mL) and CH_2Cl_2 (4 mL) were added pyridine (0.23 mL, 2.85 mmol) and trifluoroacetic anhydride (0.12 mL, 0.86 mmol) at 0 °C. After being stirred for 15 min, the reaction mixture was quenched with water, and extracted with CH_2Cl_2. The organic layer was successively washed with 1 M HCl, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crystalline solid was washed with ^{t}BuOMe and collected by filtration and dried under reduced pressure to yield **47a** (214 mg, 69%). TLC $R_f = 0.44$ (CH_2Cl_2/MeOH/AcOH, 10:1:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.21–1.45 (m, 9H), 1.61–2.57 (m, 6H), 2.82 (s, 3H), 3.02 (s, 3H), 3.16–3.76 (m, 5H), 4.28–4.47 (m, 1H), 4.75–4.85 (m, 1H), 4.92–5.05 (m, 1H).

4.1.14. *tert*-Butyl (2*S*,4*S*)-4-(aminocarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (47b). To a stirred solution of **46** (300 mg, 0.89 mmol) in THF (5 mL) were added triethylamine (0.14 mL,

1.0 mmol) and ethyl chloroformate (0.10 mL, 1.0 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was filtered and the filtrate was concentrated in vacuo. To a stirred solution of the residue in THF (5 mL) was added 28% aqueous NH_3 (2 mL) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2. The organic layer was successively washed with water, 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (20:1) as an eluant to yield **47b** (117 mg, 39%). TLC $R_f = 0.54$ ($CHCl_3$/MeOH, 4:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.24–1.40 (m, 9H), 1.73–1.93 (m, 1H), 1.97–2.31 (m, 4H), 2.37–2.46 (m, 1H), 2.80–2.99 (m, 1H), 3.08–3.23 (m, 1H), 3.36–3.64 (m, 2H), 3.64–3.78 (m, 1H), 4.28–4.46 (m, 1H), 4.72–5.00 (m, 1H), 7.01 (s, 1H), 7.44 (s, 1H).

According to the same procedures as described above, **47c** and **47d** were prepared from **46**.

4.1.15. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(methylamino)carbonyl]-1-pyrrolidinecarboxylate (47c). Yield 41%. A white powder. $R_f = 0.41$ (EtOAc/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.36 (s, 9 H) 1.87–2.00 (m, 1H) 2.02–2.11 (m, 2H) 2.12–2.22 (m, 2H), 2.44–2.48 (m, 1H), 2.61 (d, $J = 4.5$ Hz, 3H), 2.87–2.93 (m, 1H), 3.28 (t, $J = 10.0$ Hz, 1H), 3.50–3.64 (m, 2H), 3.73 (dd, $J = 10.0$, 8.2 Hz, 1H), 4.41 (dd, $J = 8.7$, 7.9 Hz, 1H), 4.73–4.83 (m, 1H), 7.10–7.99 (m, 1H).

4.1.16. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(ethylamino)carbonyl]-1-pyrrolidinecarboxylate (47d). Yield 59%. A white powder. $R_f = 0.64$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.01 (t, $J = 7.5$ Hz, 3H), 1.30 and 1.38 (s, 9H), 1.75–1.93 (m, 1H), 1.97–2.31 (m, 4H), 2.37–2.46 (m, 1H), 2.80–2.99 (m, 1H), 3.00–3.75 (m, 6H), 4.32–4.43 (m, 1H), 4.75–5.00 (m, 1H), 7.92–8.01 (m, 1H).

4.1.17. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(propylamino)carbonyl]-1-pyrrolidinecarboxylate (47e). To a stirred solution of **46** (300 mg, 0.89 mmol) in CH_2Cl_2 (2 mL) were added propyl amine (0.11 mL, 1.3 mmol), triethylamine (0.19 mL, 1.3 mmol), 1-hydroxybenzotriazole (142 mg, 1.05 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (243 mg, 1.3 mmol) at 0 °C. After being stirred for 16 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (50:1) as an eluant to yield **47e** (333 mg, 99%) as a white powder. TLC $R_f = 0.61$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos.) m/z 379 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 0.82 (t, $J = 7.5$ Hz, 3H), 1.25–1.44 (m, 2H), 1.28 and 1.36 (s, 9H), 1.88–2.20 (m, 5H), 2.40–2.50 (m, 1H), 2.84–3.06 (m, 3H), 3.10–3.24 (m, 1H), 3.40–3.76 (m, 3H), 4.33–4.43 (m, 1H), 4.77–4.82 (m, 1H), 7.93–8.00 (m, 1H).

According to the same procedures as described for the preparation of **47a** from **43**, **47f–j** were prepared from **43**.

4.1.18. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-{[ethyl(methyl)amino]carbonyl}-1-pyrrolidinecarboxylate (47f). Yield 66%. A white powder. $R_f = 0.42$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos.) m/z 379 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.07 (t, $J = 7.5$ Hz, 3H), 1.37 (s, 9H), 1.84–2.22 (m, 5H), 2.46–2.57 (m, 1H), 2.97 (s, 3H), 3.08–3.42 (m, 4H), 3.52–3.75 (m, 3H), 4.40–4.47 (m, 1H), 4.75–4.82 (m, 1H).

4.1.19. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[(diethylamino)carbonyl]-1-pyrrolidinecarboxylate (47g). Yield 58%. A white powder. $R_f = 0.61$ ($CHCl_3$/MeOH, 5:1); MS (APCI, pos.) m/z 393 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 0.99–1.18 (m, 6H), 1.36 (s, 9H), 1.85–2.12 (m, 3H), 2.12–2.23 (m, 2H), 2.49–2.55 (m, 1H), 3.22–3.43 (m, 6H), 3.49–3.64 (m, 2H), 3.66–3.76 (m, 1H), 4.43 (dd, $J = 9.2$, 7.7 Hz, 1H), 4.73–4.85 (m, 1H).

4.1.20. *tert*-Butyl (2*S*,4*S*)-4-(1-azetidinylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (47h). Yield 42%. A white powder. $R_f = 0.58$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos.) m/z 393 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.36 (s, 9H), 1.78–1.94 (m, 1H), 2.00–2.11 (m, 2H), 2.12–2.28 (m, 4H), 2.50–2.53 (m, 1H), 2.95–3.08 (m, 1H), 3.27 (t, $J = 10.4$ Hz, 1H), 3.49–3.61 (m, 2H), 3.64–3.77 (m, 1H), 3.83–4.24 (m, 4H), 4.40 (dd, $J = 9.0$, 7.7 Hz, 1H), 4.69–4.88 (m, 1H).

4.1.21. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (47i). Yield 77%. A white powder. $R_f = 0.46$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.23–1.40 (m, 9H), 1.66–1.96 (m, 5H), 1.96–2.18 (m, 4H), 2.49–2.60 (m, 1H), 3.08–3.31 (m, 5H), 3.48 (t, $J = 6.6$ Hz, 2 H), 3.53–3.76 (m, 2H), 4.25–4.46 (m, 1H), 4.65–5.06 (m, 1H).

4.1.22. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(1-piperidinylcarbonyl)-1-pyrrolidinecarboxylate (47j). Yield 41%. A white powder. $R_f = 0.55$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.24–1.38 (m, 9H), 1.39–1.64 (m, 7H), 1.78–1.90 (m, 1H), 1.93–2.31 (m, 4H), 3.29–3.53 (m, 7H), 3.53–3.74 (m, 2H), 4.27–4.46 (m, 1H), 4.65–5.08 (m, 1H).

According to the same procedures as described for the preparation of **47e** from **46**, **47k** and **47l** were prepared from **46**.

4.1.23. *tert*-Butyl (2*S*,4*S*)-4-(1-azepanylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (47k). Yield 78%. A white powder. $R_f = 0.56$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.21–1.40 (m, 9H), 1.40–1.71 (m, 8H), 1.72–1.93 (m, 1H), 1.97–2.31 (m, 4H), 2.36–2.62 (m, 1H), 3.15–3.78 (m, 9H), 4.21–4.48 (m, 1H), 4.57–5.17 (m, 1H).

4.1.24. *tert*-Butyl (2*S*,4*S*)-4-(1-azocanylcarbonyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (47l). Yield 83%. A white powder. $R_f = 0.49$ ($CHCl_3$/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.36 (s, 9H), 1.40–1.80 (m, 10H), 1.90–2.30 (m, 5H), 2.45–2.55 (m, 1H), 3.30–3.80 (m, 9H), 4.43 (dd, $J = 9.3$, 7.8 Hz, 1H), 4.74–4.84 (m, 1H).

4.1.25. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(4-morpholinylcarbonyl)-1-pyrrolidinecarboxylate (47m). Compound **47m** was obtained as a white powder in 61% yield from **43** according to the same procedures as described for the preparation of **47a** from **43**. $R_f = 0.63$ ($CHCl_3$/MeOH, 5/1); ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.35 (s, 9H), 1.82–1.96 (m, 1H), 1.99–2.12 (m, 2H), 2.13–2.28 (m, 2H), 2.50–2.59 (m, 1H), 3.24–3.43 (m, 2H), 3.44–3.61 (m, 10H), 3.64–3.78 (m, 1H), 4.32–4.53 (m, 1H), 4.65–4.88 (m, 1H).

4.1.26. *tert*-Butyl (2*S*,4*S*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-(4-thiomorpholinylcarbonyl)-1-pyrrolidinecarboxylate (47n). Compound **47n** was obtained as a white powder in 88% yield from **46** according to the same procedures as described for the preparation of **47e** from **46**. $R_f = 0.65$ ($CHCl_3$/MeOH, 5:1); ^1H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.37 (s, 9H), 1.84–2.20 (m, 5H), 2.45–2.63 (m, 5H), 2.12–2.28 (m, 4H), 3.30–3.42 (m, 1H), 3.52–3.62 (m, 1H), 3.66–3.80 (m, 5H), 4.40 (dd, $J = 9.0$, 7.8 Hz, 1H), 4.75–4.83 (m, 1H).

According to the same procedures as described for the preparation of **8** from **42**, **11** and **14–26** were prepared from **47a–n**, respectively.

4.1.27. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*-dimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (11). Yield 92%. A white powder. TLC $R_f = 0.13$ (CH_2Cl_2/MeOH, 5:1); MS (MALDI, Pos.) *m*/*z* 265 $(M+H)^+$; IR (KBr) 2243, 1647, 1452, 1219, 1171, 1123, 1034, 1010, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 2.02–2.17 (m, 6H), 2.36 (s, 3H), 2.64–2.77 (m, 1H), 2.89 (s, 3H), 2.93 (s, 3H), 3.33–3.45 (m, 1H), 3.45–3.67 (m, 2H), 3.68–3.79 (m, 1H), 4.79–4.86 (m, 1H), 5.02 (s, 1H), 7.18 (d, $J = 8.0$ Hz, 2H), 7.75 (d, $J = 8.0$ Hz, 2H), 8.28 (s, 1H), 9.59 (s, 1H); HRMS (FAB) calcd for $C_{13}H_{21}N_4O_2$: 265.1665. Found: 265.1668.

4.1.28. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (14). Yield 76%. A white powder. TLC $R_f = 0.11$ ($CHCl_3$/MeOH/AcOH, 3:1:1); MS (APCI, pos. 20 V) *m*/*z* 237 $(M+H)^+$; IR (KBr) 2239, 1693, 1661, 1379, 1230, 1171, 1126, 1038, 1014, 685, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.78–1.95 (m, 1H), 1.94–2.07 (m, 2H), 2.08–2.33 (m, 2H), 2.27 (s, 3H), 2.62–2.79 (m, 1H), 2.98–3.20 (m, 1H), 3.23–3.46 (m, 2H), 3.46–3.64 (m, 2H), 4.40–4.57 (m, 1H), 4.81 (dd, $J = 7.8$, 4.9 Hz, 1H), 7.09 (d, $J = 8.0$ Hz, 2H), 7.20 (s, 1H), 7.45 (d, $J = 8.0$ Hz, 2H), 7.61 (s, 1H), 9.09 (s, 2H); HRMS (FAB) calcd for $C_{11}H_{17}N_4O_2$: 237.1352. Found: 237.1356.

4.1.29. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-methyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (15). Yield 77%. A white powder. TLC $R_f = 0.13$ (EtOAc/AcOH/H_2O, 3:1:1); MS (APCI, pos. 20 V) *m*/*z* 251 $(M+H)^+$; IR (KBr) 3105, 2964, 2783, 2246, 1663, 1567, 1455, 1186, 1124, 1035, 1010, 685, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.80–2.25 (m, 5H), 2.28 (s, 3H), 2.59 (d, $J = 4.2$ Hz, 3H), 2.64–2.75 (m, 1H), 2.98–3.17 (m, 1H), 3.24–3.36 (m, 1H), 3.38–3.62 (m, 3H), 4.45–4.59 (m, 1H), 4.82 (dd, $J = 7.8$, 4.9 Hz, 1H), 7.10 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 8.2$ Hz, 2H), 8.12 (d, $J = 4.2$ Hz, 1H), 8.73–8.94 (m, 1H), 9.32–9.53 (m, 1H); HRMS (FAB) calcd for $C_{12}H_{19}N_4O_2$: 251.1508. Found: 251.1510.

4.1.30. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (16). Yield 83%. A white powder. TLC $R_f = 0.31$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 265 $(M+H)^+$; IR (KBr) 3423, 2976, 2245, 1662, 1560, 1452, 1376, 1186, 1123, 1034, 1010, 684, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.01 (t, $J = 7.2$ Hz, 3H), 1.78–2.26 (m, 5H), 2.28 (s, 3H), 2.63–2.79 (m, 1H), 2.99–3.18 (m, 3H), 3.19–3.49 (m, 2H), 3.54–3.62 (m, 2H), 4.42–4.60 (m, 1H), 4.82 (dd, $J = 7.8$, 4.7 Hz, 1H), 7.10 (d, $J = 7.8$ Hz, 2H), 7.47 (d, $J = 7.8$ Hz, 2H), 8.08–8.24 (m, 1H), 8.68–8.97 (m, 1H), 9.33–9.57 (m, 1H); HRMS (FAB) calcd for $C_{13}H_{21}N_4O_2$: 265.1665. Found: 265.1668.

4.1.31. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-propyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (17). Yield 86%. A beige powder. TLC $R_f = 0.38$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 279 $(M+H)^+$; IR (KBr) 3434, 2247, 1661, 1189, 1124, 1035, 1011, 684 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.83 (t, $J = 7.4$ Hz, 3H), 1.31–1.49 (m, 2H), 1.75–1.94 (m, 1H), 1.95–2.09 (m, 2H), 2.09–2.26 (m, 2H), 2.28 (s, 3H), 2.36–2.47 (m, 1H), 2.64–2.84 (m, 1H), 2.94–3.18 (m, 3H), 3.20–3.38 (m, 1H), 3.55 (t, $J = 6.6$ Hz, 2H), 4.43–4.61 (m, 1H), 4.82 (dd, $J = 7.7$, 4.8 Hz, 1H), 7.10 (d, $J = 7.8$ Hz, 2H), 7.41–7.54 (m, 2H), 8.11–8.19 (m, 1H), 8.81 (s, 1H), 9.44 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1820.

4.1.32. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-*N*-methyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (18). Yield 98%. A beige powder. TLC $R_f = 0.30$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 279 $(M+H)^+$; IR (KBr) 3450, 2978, 2243, 1637, 1454, 1187, 1123, 1035, 1011, 684, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.92–1.20 (m, 3H), 1.64–2.26 (m, 5H), 2.28 (s, 3H), 2.71–3.04 (m, 4H), 3.13–3.71 (m, 7H), 4.43–4.61 (m, 1H), 4.82 (dd, $J = 7.6$, 4.6 Hz, 1H), 7.10 (d, $J = 7.8$ Hz, 2H), 7.47 (d, $J = 7.8$ Hz, 2H), 8.69–8.98 (m, 1H), 9.18–9.59 (m, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 279.1821. Found: 279.1818.

4.1.33. (3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*-diethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (19). Yield 97%. A white powder. TLC $R_f = 0.18$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V)

m/*z* 293 $(M+H)^+$; IR (KBr) 3568, 3449, 1663, 1655, 1646, 1638, 1451, 1214, 1190, 684, 569 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.95–1.06 (m, 3H), 1.07–1.18 (m, 3H), 1.69–1.84 (m, 1H), 1.97–2.07 (m, 2H), 2.09–2.25 (m, 2H), 2.28 (s, 3H), 2.73–2.88 (m, 1H), 3.17–3.50 (m, 7H), 3.51–3.64 (m, 2H), 4.43–4.60 (m, 1H), 4.83 (dd, J = 7.78, 4.8 Hz, 1H), 7.10 (d, J = 7.8 Hz, 2H), 7.40–7.54 (m, 2H), 8.84 (s, 1H), 9.37 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{23}N_4O_2$: 293.1978. Found: 293.1981.

4.1.34. (2*S*)-1-{[(2*S*,4*S*)-4-(1-Azetidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (20). Yield 98%. A white powder. TLC R_f = 0.19 ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 277 $(M+H)^+$; IR (KBr) 3439, 2242, 1654, 1648, 1446, 1188, 1123, 1034, 1010, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.67–1.86 (m, 1H), 1.95–2.08 (m, 2H), 2.11–2.26 (m, 4H), 2.28 (s, 3H), 2.66–2.87 (m, 1H), 3.07–3.22 (m, 1H), 3.22–3.36 (m, 2H), 3.49–3.65 (m, 2H), 3.86 (t, J = 7.6 Hz, 2H), 4.18 (t, J = 7.6 Hz, 2H), 4.38–4.59 (m, 1H), 4.83 (dd, J = 7.6, 4.7 Hz, 1H), 7.10 (d, J = 7.8 Hz, 2H), 7.42–7.50 (m, 2H), 8.88 (s, 1H), 9.40 (s, 1H); HRMS (FAB) calcd for $C_{14}H_{21}N_4O_2$: 277.1665. Found: 277.1667.

4.1.35. (2*S*)-1-{[(2*S*,4*S*)-4-(1-Pyrrolidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (21). Yield 96%. A white powder. TLC R_f = 0.67 ($CHCl_3$/MeOH/AcOH, 3:1:1); MS (APCI, pos. 20 V) *m*/*z* 291 $(M+H)^+$; IR (KBr) 3434, 3061, 2974, 2881, 1660, 1633, 1450, 1187, 1123, 1034, 1010, 683 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.68–2.08 (m, 7H), 2.09–2.26 (m, 2H), 2.27 (s, 3H), 2.50–2.61 (m, 1H), 2.77–2.93 (m, 1H), 3.28 (t, J = 6.7 Hz, 2H), 3.42–3.79 (m, 6H), 4.44–4.61 (m, 1H), 4.82 (dd, J = 7.8, 4.7 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.86 (s, 1H), 9.41 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{23}N_4O_2$: 291.1821. Found: 291.1821.

4.1.36. (2*S*)-1-{[(2*S*,4*S*)-4-(1-Piperidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (22). Yield 98%. A white powder. TLC R_f = 0.61 ($CHCl_3$/MeOH/AcOH, 3:1:1); MS (APCI, pos. 20 V) *m*/*z* 305 $(M+H)^+$; IR (KBr) 2938, 2241, 1662, 1636, 1448, 1218, 1186, 1123, 1033, 1010, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.37–1.69 (m, 7H), 1.94–2.23 (m, 4H), 2.36 (s, 3H), 2.61–2.80 (m, 1H), 3.22–3.82 (m, 9H), 4.76–4.88 (m, 1H), 4.93–5.10 (m, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 8.24 (s, 1H), 9.59 (s, 1H); HRMS (FAB) calcd for $C_{16}H_{25}N_4O_2$: 305.1978. Found: 305.1977.

4.1.37. (2*S*)-1-{[(2*S*,4*S*)-4-(1-Azepanylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile hydrochloride (23). Yield 97%. A white powder. TLC R_f = 0.22 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m*/*z* 319 $(M+H)^+$; IR (KBr) 3411, 2927, 2722, 1634, 1448, 1369, 1267, 1191, 1101, 1046, 730 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.39–1.72 (m, 8H), 1.72–1.88 (m, 1H), 1.91–2.06 (m, 2H), 2.08–2.32 (m, 2H), 2.36–2.60 (m, 1H), 2.67–2.86 (m, 1H), 3.33–3.82 (m, 8H), 4.43–4.58 (m, 1H), 4.81 (dd, J = 7.8, 4.7 Hz, 1H), 9.59 (s, 2H); HRMS (FAB) calcd for $C_{17}H_{27}N_4O_2$: 319.2134. Found: 319.2135.

4.1.38. (2*S*)-1-{[(2*S*,4*S*)-4-(1-Azocanylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (24). Yield 87%. A beige powder. TLC R_f = 0.22 ($CHCl_3$/MeOH, 9/1); MS (APCI, pos. 20 V) *m*/*z* 333 $(M+H)^+$; IR (KBr) 2927, 2242, 1662, 1636, 1450, 1214, 1174, 1123, 1033, 1010, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.34–1.72 (m, 10H), 1.74–1.90 (m, 1H), 1.93–2.09 (m, 2H), 2.09–2.25 (m, 2H), 2.27 (s, 3H), 2.38–2.62 (m, 1H), 2.67–2.92 (m, 1H), 3.27–3.66 (m, 8H), 4.44–4.57 (m, 1H), 4.82 (dd, J = 7.6, 4.9 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.85 (s, 1H), 9.38 (s, 1H); HRMS (FAB) calcd for $C_{18}H_{29}N_4O_2$: 333.2291. Found: 333.2288.

4.1.39. (2*S*)-1-{[(2*S*,4*S*)-4-(4-Morpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (25). Yield 97%. A white powder. TLC R_f = 0.19 ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 307 $(M+H)^+$; IR (KBr) 3449, 2243, 1654, 1647, 1450, 1217, 1188, 1121, 1034, 1010, 683, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.70–1.89 (m, 1H), 1.96–2.09 (m, 2H), 2.09–2.26 (m, 2H), 2.28 (s, 3H), 2.67–2.88 (m, 1H), 3.40–3.77 (m, 13H), 4.39–4.62 (m, 1H), 4.83 (dd, J = 7.7, 4.6 Hz, 1H), 7.06–7.15 (m, 2H), 7.43–7.50 (m, 2H), 8.88 (s, 1H), 9.41 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{23}N_4O_3$: 307.1770. Found: 307.1769.

4.1.40. (2*S*)-1-{[(2*S*,4*S*)-4-(4-Thiomorpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (26). Yield 97%. An ivory powder. TLC R_f = 0.38 ($CHCl_3$/MeOH, 5:1); MS (APCI, pos. 20 V) *m*/*z* 323 $(M+H)^+$; IR (KBr) 3449, 2243, 1655, 1451, 1222, 1195, 1122, 1033, 1009, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.73–1.87 (m, 1H), 1.94–2.08 (m, 2H), 2.09–2.25 (m, 2H), 2.28 (s, 3H), 2.52–2.59 (m, 2H), 2.60–2.69 (m, 2H), 2.70–2.83 (m, 1H), 3.40–3.90 (m, 9H), 4.40–4.57 (m, 1H), 4.83 (dd, J = 7.7, 4.6 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.87 (s, 1H), 9.39 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{23}N_4O_2S$: 323.1542. Found: 323.1545.

4.1.41. Methyl (2*S*)-1-(benzyloxycarbonyl)-4-oxo-2-pyrrolidinecarboxylate (49). To a stirred solution of **48** (15.5 g, 55 mmol) in EtOAc (40 mL) were added triethylamine (15.4 mL, 110 mmol), DMSO (20 mL), and sulfur trioxide–pyridine complex (17.5 g, 110 mmol) at 0 °C. After being stirred for 88 h at room temperature, the reaction mixture was quenched with water, and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:2) as an eluant to yield **49** (8.81 g, 58%) as a colorless oil. TLC R_f = 0.35 (hexane/EtOAc, 2:1); ^1H NMR (300 MHz, $CDCl_3$) δ 2.57–2.64 (m, 1H), 2.88–3.02 (m, 1H), 3.63 and 3.77 (s, 3H), 3.95 (s, 2H), 4.80–4.92 (m, 1H), 5.08–5.26 (m, 2H), 7.26–7.42 (m, 5H).

4.1.42. Methyl (2*S*,4*E*)-1-(benzyloxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethylidene)-2-pyrrolidinecarboxylate (50). To a stirred solution of diisopropylamine (72 mL, 510 mmol) in THF (1 L) was added a solution of *n*-butyllithum in hexane (325 mL, 1.58 M) at −10 °C. After being stirred for 30 min, the reaction mixture was cooled to −78 °C. To the reaction mixture was added (trimethylsilyl)acetic acid *tert*-butyl ester (96.7 g, 513 mmol) and stirred for 15 min. To the reaction mixture was added a solution of **49** (119 g, 428 mmol) in THF (500 mL). After being stirred for 3 h at −78 °C, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:3) as an eluant to yield **50** (62.5 g, 39%) as a colorless oil. TLC $R_f = 0.35$ (hexane/EtOAc, 3/1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44–1.46 (m, 9H), 2.76–2.84 (m, 1H), 3.07–3.20 (m, 1H), 3.58–3.75 (m, 3H), 4.30–4.60 (m, 3H), 5.03–5.24 (m, 2H), 5.57–5.74 (m, 1H), 7.26–7.42 (m, 5H).

4.1.43. Methyl (2*S*,4*R*)-1-(*tert*-butoxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethyl)-2-pyrrolidinecarboxylate (51). To a solution of **50** (2.66 g, 7.1 mmol) in AcOH (25 mL) was added platinum (IV) oxide (250 mg, 1.1 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 24 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To the resulting residue were added AcOH (25 mL) and 10% palladium on carbon (250 mg). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 3 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To a solution of the residue in THF (30 mL) were added aq $NaHCO_3$ and di-*tert*-butyl-dicarbonate (2.18 g, 10 mmol) at room temperature. After being stirred for 10 min, the reaction mixture was extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:3) as an eluant to yield **51** (2.02 g, 83%). TLC $R_f = 0.30$ (EtOAc/hexane, 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40–1.44 (m, 18H), 1.52–1.70 (m, 1H), 2.25–2.60 (m, 4H), 3.03–3.12 (m, 1H), 3.72 and 3.74 (s, 3H), 3.75–3.85 (m, 1H), 4.15–4.30 (m, 1H).

4.1.44. (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-*tert*-butoxy-2-oxoethyl)-2-pyrrolidinecarboxylic acid (52). To a stirred solution of **51** (12 g, 34.9 mmol) in MeOH (70 mL) and THF (35 mL) was added 1 M NaOH (70 mL) at 0 °C. After being stirred for 15 h at room temperature, the reaction mixture was quenched with 2 M HCl (35 mL). The organic solvent was removed by evaporation and the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield **52** (8.54 g), which was used for the next reaction without further purification.

4.1.45. *tert*-Butyl (2*S*,4*R*)-4-(2-*tert*-butoxy-2-oxoethyl)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-pyrrolidinecarboxylate (53). To a stirred solution of **52** (8.54 g, 26 mmol) in DMF (30 mL) were added (2*S*)-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (6.98 g, 26 mmol), 1-hydroxybenzotriazole (3.18 g, 26 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.96 g, 26 mmol), and triethylamine (3.6 mL, 26 mmol) at 0 °C. After being stirred for 3 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 5% $KHSO_4$, aq $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel chromatography using acetone/hexane (1:2) as an eluant to yield **53** (8.65 g, 61%) as a colorless oil. TLC $R_f = 0.33$ (acetone/hexane, 1:2); ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.30–1.44 (m, 18H), 1.90–2.50 (m, 8H), 2.90–3.03 (m, 1H), 3.30–3.72 (m, 4H), 4.37–4.43 (m, 1H), 4.72–4.80 (m, 1H).

4.1.46. ((3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)acetic acid 4-methylbenzenesulfonate (10). A solution of **53** (106 mg, 0.26 mmol) and *p*-toluenesulfonic acid (74 mg, 0.39 mmol) in CH_3CN (2 mL) was refluxed for 5 h. The reaction mixture was evaporated. The resulting crystalline solid was washed with ^{t}BuOMe, collected by filtration, and dried under reduced pressure to yield **10** (104 mg, 95%) as an ivory powder. TLC $R_f = 0.17$ ($CHCl_3$/MeOH/AcOH, 5:1:1); MS (APCI, neg. 20 V) *m*/*z* 250 $(M-H)^-$; IR (KBr) 3063, 2983, 2244, 1728, 1662, 1455, 1372, 1221, 1156, 1122, 1033, 1008, 682, 566 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37-1.54 (m, 1H), 1.94-2.26 (m, 4H), 2.28 (s, 3H), 2.37–2.75 (m, 4H), 2.85–3.04 (m, 1H), 3.33–3.47 (m, 1H), 3.47–3.65 (m, 2H), 4.40–4.55 (m, 1H), 4.82 (dd, $J = 7.7$, 4.8 Hz, 1H), 7.10 (d, $J = 7.8$ Hz, 2H), 7.42–7.50 (m, 2H), 8.70 (s, 1H), 9.34 (s, 1H); HRMS (FAB) calcd for $C_{12}H_{18}N_3O_3$: 252.1348. Found: 252.1347.

4.1.47. ((3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)acetic acid (54). A solution of **53** (7.50 g, 18.4 mmol) and *p*-toluenesulfonic acid (5.25 g, 27.6 mmol) in CH_3CN (100 mL) was refluxed for 5 h. The reaction mixture was evaporated. To a solution of the residue in THF (50 mL) were added 1 M $NaHCO_3$ (50 mL) and di-*tert*-butyl-dicarbonate (5.89 g, 27 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with hexane (100 mL), and extracted with aq $NaHCO_3$. The aqueous layer was acidified with 1 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting crystalline solid was washed with EtOAc/hexane (1:1), collected by filtration, and dried under reduced pressure to yield **54** (4.48 g, 69%) as a white powder. TLC $R_f = 0.16$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos.) *m*/*z* 352 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.37 (s, 9H), 1.39–1.50 (m, 1H), 2.00–2.20 (m, 4H), 2.30–2.77 (m, 4H), 2.90–3.00 (m, 1H), 3.50–3.74 (m, 3H), 4.37–4.43 (m, 1H), 4.72–4.82 (m, 1H).

4.1.48. *tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[2-(dimethylamino)-2-oxoethyl]-1-pyrrolidinecarboxylate (55). To a stirred solution of **54** (250 mg, 0.71 mmol) in CH_2Cl_2 (7 mL) were added a solution of dimethylamine in THF (2 M, 0.53 mL), triethylamine (0.10 mL, 0.71 mmol), 1-hydroxybenzotriazole (96 mg, 0.71 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (164 mg, 0.85 mmol) at room temperature. After being stirred for 6 h, the reaction mixture was poured into water, and extracted with EtOAc. The organic layer was successively washed with 1 M HCl, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (40:1) as an eluant to yield **55** (174 mg, 64%) as a white powder. TLC R_f = 0.43 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 and 1.36 (s, 9H), 1.30–1.40 (m, 1H), 1.90–2.26 (m, 4H), 2.35–2.58 (m, 3H), 2.79 (s, 3H), 2.80–2.90 (m, 1H), 2.91 and 2.92 (s, 3H), 3.25–3.30 (m, 1H), 3.48–3.60 (m, 2H), 3.64–3.70 (m, 1H), 4.30–4.40 (m, 1H), 4.76–5.00 (m, 1H).

4.1.49. 2-((3*R*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)-*N*,*N*-dimethylacetamide 4-methylbenzenesulfonate (12). Compound **12** is obtained as a white powder in 77% yield from **55** according to the same procedures as described for the preparation of **8** from **42**. TLC R_f = 0.27 (EtOAc/AcOH/H_2O, 3:1:1); MS (FAB, Pos.) *m*/*z* 279 $(M+H)^+$; IR (KBr) 3447, 2959, 2244, 1662, 1649, 1456, 1373, 1221, 1170, 1122, 1035, 1011 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.37–1.54 (m, 1H), 1.93–2.07 (m, 2H), 2.08–2.33 (m, 3H), 2.28 (s, 3H), 2.46–2.57 (m, 1H), 2.57–2.73 (m, 2H), 2.80 (s, 3H), 2.83–2.99 (m, 1H), 2.91 (s, 3H), 3.33–3.67 (m, 3H), 4.41–4.54 (m, 1H), 4.82 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.10 (d, *J* = 7.8 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 2H), 8.66 (s, 1H), 9.30 (s, 1H).

4.1.50. [(3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)-3-pyrrolidinyl]acetic acid (56). A solution of **51** (1.37 g, 3.99 mmol) and anisole (0.8 mL) in trifluoroacetic acid (8 mL) was stirred for 1 h at room temperature. The reaction mixture was evaporated. To a stirred solution of the residue in THF (10 mL) were added aq $NaHCO_3$ (10 mL) and di-*tert*-butyl-dicarbonate (1.33 g, 6.10 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using $CHCl_3$/MeOH (9:1) as an eluant to yield **56** (1.16 g, 100%) as a colorless oil. TLC R_f = 0.38 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.44 (s, 9H), 1.60–1.70 (m, 1H), 2.40–2.63 (m, 4H), 3.07–3.14 (m, 1H), 3.73 and 3.74 (s, 3H), 3.75–3.85 (m, 1H), 4.20–4.34 (m, 1H).

4.1.51. Methyl (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-(2-hydroxyethyl)-1,2-pyrrolidinecarboxylate (57). To a stirred solution of **56** (575 mg, 2.00 mmol) in THF (10 mL) were added triethylamine (0.42 mL, 3.0 mmol) and ethyl chloroformate (0.23 mL, 2.4 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was filtered and the filtrate was concentrated in vacuo. To a stirred solution of the residue in THF (5 mL) was added a solution of sodium borohydride (378 mg, 10 mmol) in water (5 mL) at 0 °C. After being stirred for 30 min, the reaction mixture was extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and evaporated to yield **57** (546 mg, 100%) as a colorless oil. TLC R_f = 0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.57–1.72 (m, 3H), 2.20–2.51 (m, 2H), 3.05 (t, *J* = 11.2 Hz, 1H), 3.63–3.84 (m, 3H), 3.72 and 3.73 (s, 3H), 4.13–4.30 (m, 1H).

4.1.52. (2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-2-pyrrolidinecarboxylic acid (59). To a stirred solution of **57** (546 mg, 2 mmol) in CH_2Cl_2 (4 mL) were added 3,4-dihydro-2*H*-pyran (0.27 mL, 3.0 mmol) and pyridinium *p*-toluenesulfonate (50 mg, 0.20 mmol) at room temperature. After being stirred for 3 h, the reaction mixture was quenched with aqueous $NaHCO_3$. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to yield **58**, which was used for the next reaction without further purification. To a stirred solution of **58** in MeOH (4 mL) and THF (4 mL) was added 1 M NaOH (4 mL) at room temperature. After being stirred for 15 h, the reaction mixture was quenched with 1 M HCl (4 mL). The organic solvent was removed in vacuo and the aqueous layer was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield **59**, which was used for the next reaction without further purification.

4.1.53. (2*S*)-1-({(2*S*,4*R*)-1-(*tert*-Butoxycarbonyl)-4-[2-(tetrahydro-2*H*-pyran-2-yloxy)ethyl]-2-pyrrolidinyl}carbonyl)-2-pyrrolidinecarbonitrile (60). Compound **60** was obtained as a colorless oil in 65% yield from **59** according to the same procedures as described for the preparation of **53** from **52**. TLC R_f = 0.50 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 and 1.43 (s, 9H), 1.50–1.90 (m, 9H), 2.05–2.46 (m, 6H), 3.00–3.17 (m, 1H), 3.35–3.62 (m, 4H), 3.70–3.90 (m, 4H), 4.30–4.60 (m, 1H), 4.65–4.90 (m, 1H).

4.1.54. (2*S*)-1-{[(2*S*,4*R*)-4-(2-Hydroxyethyl)-2- pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile [(1*S*,4*R*)-7,7-dimethyl-2-oxobicyclo[2.2.1]hept-1-yl]methanesulfonate (9). A solution of **60** (6.0 g, 17.8 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (4.55 g, 19.6 mmol) in EtOH (20 mL) was refluxed for 4 h. After cooling to 0 °C, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **9** (6.80 g, 81%) as a white powder. TLC R_f = 0.22 ($CHCl_3$/MeOH/AcOH, 8:2:1); MS (APCI, pos. 20 V) *m*/*z* 238 $(M+H)^+$; IR (KBr) 3467, 3166, 2991, 2954, 2239, 1739, 1670, 1387, 1374, 1163, 1033 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.73 (s, 3H), 1.03 (s, 3H), 1.18–1.33 (m, 2H), 1.32–1.46 (m, 1H), 1.46–1.64 (m, 2H), 1.78 (d, *J* = 18.1 Hz, 1H), 1.82–1.89 (m, 1H), 1.92 (t, *J* = 4.4 Hz, 1H), 1.95–2.09 (m, 2H), 2.08–2.29 (m, 3H), 2.29–2.44 (m, 2H), 2.56–2.76 (m, 2H), 2.78–2.95 (m, 2H), 3.33–3.50 (m, 3H), 3.50–3.69 (m, 2H), 4.20–4.63 (m, 2H), 4.83 (dd, *J* = 7.8, 4.7 Hz, 1H), 8.66 (s, 1H), 9.33 (s, 1H); Anal. Calcd for $C_{22}H_{35}N_3O_6S$: C, 56.27; H, 7.51; N, 8.95. Found: C, 56.25; H, 7.45; N, 8.81.

4.1.55. Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)-4-{[(trifluoromethyl)sulfonyl]oxy}-2,5-dihydro-1*H*-pyrrole-2-carboxylate (62). To a stirred solution of sodium bis(trimethylsilyl)amide (2.02 g, 11 mmol) in THF (20 mL) was added dropwise a solution of **61** (2.43 g, 10 mmol) in THF (7 mL) at −78 °C. After being stirred for 15 min. *N*-Phenyl-bis(trifluoromethanesulfonimide) (3.57 g, 10 mmol) in THF (12 mL) was added and the reaction mixture was stirred at −78 °C for additional 3 h. The reaction mixture was quenched with aq $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:15) as an eluant to yield **62** (2.89 g, 77%) as a colorless oil. TLC R_f = 0.37 (EtOAc/hexane, 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 and 1.49 (s, 9H), 3.77 (s, 3H), 4.16–4.53 (m, 2H), 4.89–5.20 (m, 1H), 5.67–5.79 (m, 1H).

4.1.56. Methyl (2*S*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxy-1-propyn-1-yl)-2,5-dihydro-1*H*-pyrrole-2-carboxylate (63). To a mixture of **62** (750 mg, 2.0 mmol), diisopropylamine (1.6 mL, 11 mmol), copper(I) iodide (57 mg, 0.30 mmol), and bis(triphenylphosphine)palladium(II) dichloride (70 mg, 0.10 mmol) in THF (5 mL) was added 2-propyn-1-ol (0.12 mL, 4.0 mmol). The reaction mixture was stirred for 30 min under argon atmosphere. The reaction mixture was quenched with aq NH_4Cl and extracted with EtOAc. The organic layer was successively washed with water, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:3) as an eluent to yield **63** (579 mg, 100%). TLC R_f = 0.40 (EtOAc/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38–1.51 (m, 9H), 1.61–1.76 (m, 1H), 3.72–3.77 (m, 3H), 4.17–4.36 (m, 2H), 4.41 (dd, J = 6.3, 2.8 Hz, 2H), 4.98–5.13 (m, 1H), 5.84–6.05 (m, 1H).

4.1.57. Methyl (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-(3-hydroxypropyl)-2-pyrrolidinecarboxylate (64). To a solution of **63** (809 mg, 2.88 mmol) in MeOH (30 mL) was added 10% palladium on carbon (162 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 3 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:1) as an eluant to yield **64** (455 mg, 55%). TLC R_f = 0.50 (EtOAc/hexane, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37–1.49 (m, 9H), 1.50–1.67 (m, 6H), 2.06–2.27 (m, 1H), 2.32–2.50 (m, 1H), 3.02 (t, J = 10.1 Hz, 1H), 3.53–3.86 (m, 6H), 4.13–4.33 (m, 1H).

4.1.58. 3-[(3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)-3-pyrrolidinyl]propanoic acid (65). To a solution of **64** (497 mg, 1.73 mmol) in CH_3CN (9 mL) were added 2,2,6,6-tetramethylpiperidine 1-oxyl (19 mg, 0.12 mmol), and sodium phosphate buffer (pH 6.86, 6.5 mL). To the reaction mixture were added $NaClO_2$ (313 mg, 3.4 mmol) and 12% NaClO (0.3 mL) at 35 °C. The reaction mixture was vigorously stirred at 35 °C for 3 h, diluted with ^{t}BuOMe, and extracted with aq $NaHCO_3$. The aqueous layer was acidified with 1 M HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to yield **65** (451 mg, 85%). TLC R_f = 0.43 (EtOAc/hexane, 3:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.36 (s, 9H), 1.40–1.60 (m, 3H), 1.99–2.27 (m, 3H), 2.28–2.43 (m, 1H), 2.80–2.87 (m, 1H), 3.50–3.60 (m, 1H), 3.60 and 3.63 (s, 3H), 4.07–4.26 (m, 1H), 12.10 (s, 1H).

4.1.59. Methyl (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-[3-(dimethylamino)-3-oxopropyl]-2-pyrrolidinecarboxylate (66). Compound **66** was obtained as a colorless oil in 75% yield from **65** according to the same procedures as described for the preparation of **55** from **54**. TLC R_f = 0.63 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37–1.50 (m, 9H), 1.52–1.94 (m, 3H), 2.11–2.51 (m, 4H), 2.94 (s, 3H), 2.97–3.08 (m, 4H), 3.71–3.75 (m, 3H), 3.79 (dd, J = 10.2, 7.1 Hz, 1H), 4.14–4.31 (m, 1H).

4.1.60. (2*S*,4*S*)-1-(*tert*-Butoxycarbonyl)-4-[3-(dimethylamino)-3-oxopropyl]-2-pyrrolidinecarboxylic acid (67). Compound **67** was obtained from **66** according to the same procedures as described for the preparation of **52** from **51**, which was used for the next reaction without further purification.

4.1.61. *tert*-Butyl (2*S*,4*R*)-2-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-4-[3-(dimethylamino)-3-oxopropyl]-1-pyrrolidinecarboxylate (68). To a stirred solution of **67** (285 mg, 0.91 mmol) in CH_2Cl_2 (2 mL) were added L-prolineamide (156 mg, 1.37 mmol), 1-hydroxybenzotriazole (209 mg, 1.37 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (262 mg, 1.37 mmol) at 0 °C. After being stirred for 4 h at room temperature, the reaction mixture was poured into water, and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aq $NaHCO_3$, brine, dried over $MgSO_4$, and evaporated. To a solution of the residue in THF (5 mL) were added pyridine (0.37 mL, 4.5 mmol) and trifluoroacetic anhydride (0.19 mL, 1.5 mmol) at 0 °C. After being stirred for 1 h at room temperature, the reaction mixture was quenched with water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aq $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (40:1) as an eluant to yield **68** (214 mg, 60%) as a white powder. TLC R_f = 0.44 ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.34 (s, 9H), 1.37–1.43 (m, 1H), 1.54–1.71 (m, 2H), 2.00–2.23 (m, 5H), 2.29 (t, J = 7.1 Hz, 2H), 2.39–2.46 (m, 1H), 2.70–2.93 (m, 7H), 3.46–3.73 (m, 3H), 4.36 (t, J = 8.1 Hz, 1H), 4.62–4.91 (m, 1H).

4.1.62. 3-((3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-3-pyrrolidinyl)-*N*,*N*-dimethylpropanamide 4-methylbenzenesulfonate (13). Compound **13** was obtained as a white powder in 88% yield from **68** according to the same procedures as described for the preparation of **8** from **42**. TLC R_f = 0.28 ($CHCl_3$/MeOH/AcOH, 3:1:1); MS (APCI, pos. 20 V) m/z 293 $(M+H)^+$; IR (KBr) 2924, 1658, 1650, 1643, 1634, 1454, 1186, 1122, 1034, 1010, 683 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.20–1.44 (m, 1H), 1.46–1.76 (m, 2H), 1.94–2.09 (m, 2H), 2.08–2.44 (m, 5H), 2.27

(s, 3H), 2.56–2.75 (m, 1H), 2.79 (s, 3H), 2.80–2.91 (m, 1H), 2.93 (s, 3H), 3.26–3.43 (m, 1H), 3.55 (t, J = 6.6 Hz, 2H), 4.32–4.55 (m, 1H), 4.82 (dd, J = 7.9, 4.6 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.69 (s, 1H), 9.28 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{25}N_4O_2$: 293.1978. Found: 293.1975.

4.1.63. 1-*tert*-Butyl 5-methyl (2*S*)-2-{[(benzyloxy)carbonyl]amino}pentanedioate (70). To a stirred solution of **69** (48 g, 142 mmol) in DMF (140 mL) were added K_2CO_3 (23.6 g, 171 mg) and methyl iodide (9.8 mL, 157 mmol) at room temperature. After being stirred for 20 h, the reaction mixture was poured into water and extracted with EtOAc/hexane (4:1). The organic layer was successively washed with water, brine, dried over $MgSO_4$, and evaporated to yield **70** (51.9 g, 100%) as a colorless oil. TLC R_f = 0.50 (hexane/EtOAc, 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.90–2.02 (m, 1H), 2.11–2.51 (m, 3H), 3.66 (s, 3H), 4.24–4.32 (m, 1H), 5.10 (s, 2H), 5.37 (d, J = 8.3 Hz, 1H), 7.30–7.38 (m, 5H).

4.1.64. 2-*tert*-Butyl 4-methyl (2*S*)-1-benzyloxycarbonyl-5-methyl-2,3-dihydro-1*H*-pyrrole-2,4-dicarboxylate (71). To a stirred solution of lithium bis(trimethylsilyl)amide in THF (400 mL, 1.0 M) was added dropwise a solution of **70** (51.9 g, 142 mmol) in THF (500 mL) at −78 °C. After being stirred for 30 min, acetyl chloride (31.4 mL, 441 mmol) was added and the reaction mixture was stirred at −78 °C for additional 1 h. The reaction mixture was quenched with AcOH (280 mL), warmed up to 50 °C, and stirred for 2 h. The reaction mixture was cooled to room temperature, diluted with EtOAc/hexane (1:1, 400 mL), and filtered. The filtrate was concentrated in vacuo and the resulting residue was diluted with EtOAc/hexane (1:1, 1000 mL). The organic layer was successively washed with 1 M HCl, aq $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:10) as an eluant to yield **71** (33.5 g, 60%) as a white powder. TLC R_f = 0.70 (EtOAc/hexane, 1:2); MS (APCI, pos. 20 V) m/z 376 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 2.65 (s, 3H), 2.66–2.75 (m, 1H), 3.03–3.17 (m, 1H), 3.71 (s, 3H), 4.59–4.66 (m, 1H), 5.17 (s, 2H), 7.30–7.38 (m, 5H).

4.1.65. 2-*tert*-Butyl 4-methyl (2*S*,4*S*,5*S*)-5-methyl-2,4-pyrrolidinedicarboxylate (72). To a solution of **71** (14.7 g, 39.2 mmol) in AcOH (80 mL) was added platinum (IV) oxide (890 mg, 3.92 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 8 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with EtOAc. The organic layer was washed with aq $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (20:1) as an eluant to yield **72** (8.50 g, 89%). TLC R_f = 0.36 (EtOAc/hexane, 2:1); MS (APCI, pos. 20 V) m/z 244 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 6.6 Hz, 3H), 1.48 (s, 9H), 2.14–2.37 (m, 2H), 2.90–2.99 (m, 1H), 3.32–3.43 (m, 1H), 3.66–3.70 (m, 1H), 3.67 (s, 3H).

4.1.66. (2*S*,4*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-4-(methoxycarbonyl)-5-methyl-2-pyrrolidinecarboxylic acid (73). A solution of **72** (9.68 g, 39.8 mmol) in trifluoroacetic acid (31 mL) and water (3 mL) was stirred for 19 h at room temperature. The reaction mixture was evaporated. To a stirred solution of the residue in THF (5 mL) and water (20 mL) were added $NaHCO_3$ (15 g, 178 mmol) and a solution of di-*tert*-butyl-dicarbonate (10.4 g, 47.8 mmol) in THF (15 mL) at room temperature. After being stirred for 3 h, the reaction mixture was quenched with 2 M HCl and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and evaporated to yield **73** (1.16 g, 100%). TLC R_f = 0.44 (EtOAc/MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 6.6 Hz, 3H), 1.48 (s, 9H), 2.38–2.78 (m, 2H), 3.11–3.21 (m, 1H), 3.73 (s, 3H), 4.20–4.40 (m, 2H).

4.1.67. Methyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (74). Compound **74** was obtained as a white powder in 85% yield from **73** according to the same procedures as described for the preparation of **40** from **39**. TLC R_f = 0.44 (EtOAc/MeOH, 9:1); MS (APCI, pos. 20 V) m/z 384 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18–1.27 (m, 3H), 1.39–1.46 (m, 9H), 1.60–2.60 (m, 7H), 3.10–3.70 (m, 3H), 3.72 (s, 3H), 4.20–4.72 (m, 2H), 5.28 and 5.51 (s, 1H), 7.02 and 7.98 (s, 1H).

4.1.68. (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(Aminocarbonyl)-1- pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylic acid (75). To a stirred solution of **74** (12.5 g, 32.5 mmol) in MeOH (65 mL) was added 1 M LiOH (36 mL) at 0 °C. After being stirred for 3 h at room temperature, the reaction mixture was quenched with 2 M HCl (18 mL). The organic solvent was removed in vacuo. The resulting residue was diluted with EtOH and filtered. The filtrate was evaporated to yield **75** (12.0 g), which was used for the next reaction without further purification.

4.1.69. Benzyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-(aminocarbonyl)-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (76). To a stirred solution of **75** (12.0 g, 32.5 mmol) in DMF (33 mL) were added K_2CO_3 (4.94 g, 35.8 mmol) and benzylbromide (4.3 mL, 36 mmol) at room temperature. After being stirred for 15 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to yield **76** (14.9 g), which was used for the next reaction without further purification.

4.1.70. Benzyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-1-(*tert*-butoxycarbonyl)-2-methyl-3-pyrrolidinecarboxylate (77). To a stirred solution of **76** (14.9 g, 32.5 mmol) in THF (100 mL) were added pyridine (7.9 mL, 98 mmol) and trifluoroacetic anhydride (5.5 mL, 39 mmol) at 0 °C. After being stirred for 30 min, the reaction mixture was quenched with water and extracted with EtOAc. The organic layer was successively washed with 1 M HCl, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane

(3:1) as an eluant to yield **77** (6.09 g, 42% from **74**). TLC $R_f = 0.29$ (EtOAc/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (d, $J = 7.5$ Hz, 3H), 1.34 and 1.42 (s, 9H), 2.05–2.50 (m, 6H), 3.10–3.22 (m, 1H), 3.53–3.83 (m, 2H), 4.22–4.41 (m, 2H), 4.77–4.84 (m, 1H), 5.12–5.22 (m, 2H), 7.30–7.42 (m, 5H).

4.1.71. (2*S*,3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2- methyl-3-pyrrolidinecarboxylic acid (78). To a solution of **77** (6.0 g, 13.6 mmol) in EtOAc (45 mL) was added 20% palladium hydroxide on carbon (1.2 g). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 20 min. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **78** (4.45 g, 93%). TLC $R_f = 0.20$ (EtOAc/hexane, 2:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, $J = 7.5$ Hz, 3H), 1.27 and 1.37 (s, 9H), 1.80–2.43 (m, 6H), 3.12–3.24 (m, 1H), 3.40–3.68 (m, 2H), 4.03–4.13 (m, 1H), 4.30–4.39 (m, 1H), 4.76–4.82 (m, 1H).

4.1.72. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-[(dimethylamino)carbonyl]-2-methyl-1-pyrrolidinecarboxylate (79a). To a stirred solution of **78** (200 mg, 0.57 mmol) in CH_2Cl_2 (2 mL) were added dimethyl amine hydrochloride (93 mg, 1.14 mmol), triethylamine (0.28 mL, 2.0 mmol), 1-hydroxybenzotriazole (87 mg, 0.57 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (164 mg, 0.85 mmol) at room temperature. After being stirred for 4 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was successively washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was recrystallized from EtOAc and hexane to yield **79a** (164 mg, 76%) as a white powder. TLC $R_f = 0.40$ ($CHCl_3$/MeOH, 10:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92 (d, $J = 7.5$ Hz, 3H), 1.27 and 1.36 (s, 9H), 1.98–2.28 (m, 6H), 2.82 (s, 3H), 3.01 (s, 3H), 3.38–3.68 (m, 3H), 4.08–4.16 (m, 2H), 4.77–4.83 (m, 1H).

According to the same procedures as described above, **79b–h** were prepared from **78**.

4.1.73. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-{[ethyl(methyl)amino]carbonyl}-2-methyl-1-pyrrolidinecarboxylate (79b). Yield 41%. A white powder. $R_f = 0.37$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.87–1.13 (m, 6H), 1.21–1.46 (m, 9H), 2.00–2.31 (m, 6H), 2.73–3.05 (m, 3H), 3.05–3.20 (m, 1H), 3.33–3.54 (m, 3H), 3.54–3.68 (m, 1H), 4.05–4.22 (m, 1H), 4.22–4.38 (m, 1H), 4.70–5.03 (m, 1H).

4.1.74. *tert*-Butyl (2*S*,3*S*,5*S*)-3-(1-azetidinylcarbonyl)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-1-pyrrolidinecarboxylate (79c). Yield 42%. A white powder. $R_f = 0.42$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.91–1.06 (m, 3H), 1.22–1.44 (m, 9H), 1.99–2.31 (m, 8H), 2.93–3.19 (m, 1H), 3.37–3.70 (m, 2H), 3.83 (t, $J = 7.7$ Hz, 2H), 3.95–4.23 (m, 3H), 4.28 (dd, $J = 10.1$, 7.6 Hz, 1H), 4.66–5.01 (m, 1H).

4.1.75. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (79d). Yield 72%. A white powder. $R_f = 0.32$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.88–1.02 (m, 3H), 1.23–1.42 (m, 9H), 1.67–1.95 (m, 4H), 2.00–2.31 (m, 6H), 3.21–3.70 (m, 7H), 4.12–4.22 (m, 1H), 4.23–4.37 (m, 1H), 4.64–5.07 (m, 1H).

4.1.76. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(1-piperidinylcarbonyl)-1-pyrrolidinecarboxylate (79e). Yield 96%. A white powder. $R_f = 0.53$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.89–1.00 (m, 3H), 1.23–1.38 (m, 9H), 1.37–1.68 (m, 6H), 1.99–2.32 (m, 6H), 3.24–3.73 (m, 7H), 4.02–4.16 (m, 1H), 4.20–4.36 (m, 1H), 4.71–5.01 (m, 1H).

4.1.77. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(4-morpholinylcarbonyl)-1-pyrrolidinecarboxylate (79f). Yield 84%. A white powder. $R_f = 0.36$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94–1.04 (m, 3H), 1.24–1.39 (m, 9H), 1.99–2.30 (m, 6H), 3.33–3.72 (m, 11H), 4.05–4.20 (m, 1H), 4.20–4.37 (m, 1H), 4.71–5.06 (m, 1H).

4.1.78. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-2-methyl-3-(4-thiomorpholinylcarbonyl)-1-pyrrolidinecarboxylate (79g). Yield 97%. A white powder. $R_f = 0.59$ ($CHCl_3$/MeOH, 9/1); ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.03 (d, $J = 6.4$ Hz, 3H), 1.35 (s, 9H), 1.97–2.39 (m, 6H), 2.50–2.53 (m, 1H), 2.51–2.65 (m, 4H), 3.36–3.47 (m, 1H), 3.52–3.64 (m, 2H), 3.78 (t, $J = 5.2$ Hz, 3H), 4.11–4.19 (m, 1H), 4.33 (t, $J = 8.7$ Hz, 1H), 4.70–4.85 (m, 1H).

4.1.79. *tert*-Butyl (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-cyano-1-pyrrolidinyl]carbonyl}-3-(1,3-dihydro-2*H*-isoindol-2-ylcarbonyl)-2-methyl-1-pyrrolidinecarboxylate (79h). Yield 50%. A white powder. $R_f = 0.29$ (EtOAc/hexane, 9:1); MS (APCI, pos. 20 V) *m*/*z* 453 $(M+H)^+$; ^{1}H NMR (300 MHz, DMSO-d_6, 100 °C) δ 1.08 (d, $J = 6.4$ Hz, 3H), 1.38 (s, 9H), 2.00–2.39 (m, 6H), 3.40–3.50 (m, 1H), 3.56–3.64 (m, 2H), 4.17–4.23 (m, 2H), 4.60–5.03 (m, 5H), 7.28–7.38 (m, 4H).

4.1.80. (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*,*N*,2-trimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (27). A solution of **79a** (2.0 g, 5.29 mmol) and *p*-toluenesulfonic acid (1.21 g, 6.36 mmol) in EtOH (20 mL) and ^{i}PrOH (10 mL) was stirred at 90 °C for 2 h. After cooling to room temperature, the resulting precipitates were collected by filtration and dried under reduced pressure to yield **27** (1.91 g, 80%) as a white powder. TLC $R_f = 0.24$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 279$(M+H)^+$; IR (KBr) 3449, 2243, 1654, 1647, 1639, 1450, 1219, 1188, 1122, 1010, 682, 568 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, $J = 6.7$ Hz, 3H), 1.94–2.26 (m, 5H), 2.28 (s, 3H), 2.57–2.71 (m, 1H), 2.85 (s, 3H), 3.01 (s, 3H), 3.47–3.67 (m, 3H), 3.92 (s, 1H), 4.51 (s, 1H), 4.81 (dd, $J = 7.8$, 5.1 Hz, 1H), 7.10 (d, $J = 8.2$ Hz, 2H), 7.46 (d, $J = 7.8$ Hz, 2H), 8.10–8.44 (m, 1H), 9.53–9.80 (m, 1H); Anal. Calcd for $C_{21}H_{30}N_4O_5S$: C, 55.98; H, 6.71; N, 12.43. Found: C, 55.72; H, 6.76; N, 12.31.

According to the same procedures as described above, **28**–**34** were prepared from **79b**–**h**, respectively.

4.1.81. (2*S*,3*S*,5*S*)-5-{[(2*S*)-2-Cyano-1-pyrrolidinyl]carbonyl}-*N*-ethyl-*N*,2-dimethyl-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (28). Yield 95%. A white powder. TLC $R_f = 0.50$ ($CHCl_3$/MeOH, 5:1); MS (APCI, pos. 20 V) *m*/*z* 293 $(M+H)^+$; IR (KBr) 2980, 2244, 1663, 1560, 1496, 1452, 1222, 1173, 1122, 1010, 682, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.94–1.13 (m, 3H), 1.12–1.19 (m, 3H), 1.92–2.25 (m, 5H), 2.27 (s, 3H), 2.53–2.75 (m, 1H), 2.79–2.99 (m, 3H), 3.10–3.34 (m, 1H), 3.36–3.66 (m, 4H), 3.81–3.97 (m, 1H), 4.44–4.60 (m, 1H), 4.80 (dd, J = 7.7, 5.2 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.21 (s, 1H), 9.64 (s, 1H); HRMS (FAB) calcd for $C_{15}H_{25}N_4O_2S$: 293.1978. Found: 293.1975.

4.1.82. (2*S*)-1-{[(2*S*,4*S*,5*S*)-4-(1-Azetidinylcarbonyl)-5-methyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (29). Yield 68%. A white powder. TLC $R_f = 0.41$ (CH_2Cl_2/MeOH, 5:1); MS (APCI, pos. 20 V) *m*/*z* 291 $(M+H)^+$; IR (KBr) 2951, 1671, 1644, 1465, 1456, 1441, 1379, 1222, 1155, 1121, 1031, 681, 574 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.20 (d, J = 6.7 Hz, 3H), 1.91–2.25 (m, 7H), 2.27 (s, 3H), 2.52–2.73 (m, 1H), 3.14 (q, J = 7.6 Hz, 1H), 3.49–3.60 (m, 2H), 3.75–3.94 (m, 3H), 4.16 (t, J = 7.6 Hz, 2H), 4.43–4.57 (m, 1H), 4.79 (dd, J = 7.7, 5.2 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.21 (s, 1H), 9.67 (s, 1H).

4.1.83. (2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(1-pyrrolidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (30). Yield 93%. A white powder. TLC $R_f = 0.51$ ($CHCl_3$/MeOH, 5:1); MS (FAB, pos.) *m*/*z* 305$(M+H)^+$; IR (KBr) 2975, 2880, 2242, 1666, 1637, 1449, 1226, 1169, 1121, 1009, 681 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, J = 6.7 Hz, 3H), 1.70–2.25 (m, 9H), 2.27 (s, 3H), 2.54–2.73 (m, 1H), 3.21–3.37 (m, 2H), 3.36–3.60 (m, 5H), 3.84–4.01 (m, 1H), 4.40–4.61 (m, 1H), 4.80 (dd, J = 7.8, 5.3 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.20 (s, 1H), 9.68 (s, 1H); Anal. Calcd for $C_{23}H_{32}N_4O_5S$: C, 57.96; H, 6.77; N, 11.76. Found: C, 57.96; H, 6.75; N, 11.55.

4.1.84. (2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(1-piperidinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (31). Yield 87%. A pale yellow powder. TLC $R_f = 0.44$ ($CHCl_3$/MeOH, 4:1); MS (APCI, pos. 20 V) *m*/*z* 319 $(M+H)^+$; IR (KBr) 2939, 2242, 1665, 1644, 1561, 1447, 1370, 1248, 1227, 1167, 1122, 1032, 1009, 681, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, J = 6.7 Hz, 3H), 1.22–1.66 (m, 6H), 1.92–2.26 (m, 5H), 2.27 (s, 3H), 2.52–2.66 (m, 1H), 3.28–3.73 (m, 7H), 3.76–3.93 (m, 1H), 4.39–4.61 (m, 1H), 4.80 (dd, J = 7.8, 5.3 Hz, 1H), 7.09 (d, J = 8.0 Hz, 2H), 7.45 (d, J = 8.0 Hz, 2H), 8.24 (s, 1H), 9.57 (s, 1H); HRMS (FAB) calcd for $C_{17}H_{27}N_4O_2$: 319.2134. Found: 319.2130.

4.1.85. (2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(4-morpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (32). Yield 93%. A white powder. TLC $R_f = 0.43$ ($CHCl_3$/MeOH, 5:1); MS (APCI, pos. 20 V) *m*/*z* 321 $(M+H)^+$; IR (KBr) 2242, 1654, 1448, 1230, 1169, 1119, 1032, 1009, 681, 568 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.09–1.30 (m, 3H), 1.88–2.26 (m, 5H), 2.27 (s, 3H), 2.55–2.74 (m, 1H), 3.33–3.69 (m, 11H), 3.80–3.96 (m, 1H), 4.46–4.59 (m, 1H), 4.72–4.87 (m, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 8.27 (s, 1H), 9.63 (s, 1H).

4.1.86. (2*S*)-1-{[(2*S*,4*S*,5*S*)-5-Methyl-4-(4-thiomorpholinylcarbonyl)-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (33). Yield 99%. A white powder. TLC $R_f = 0.60$ ($CHCl_3$/MeOH, 4:1); MS (APCI, pos. 20 V) *m*/*z* 337 $(M+H)^+$; IR (KBr) 2242, 1652, 1450, 1221, 1198, 1168, 1122, 1032, 1009, 682 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.17 (d, J = 6.7 Hz, 3H), 1.91–2.10 (m, 2H), 2.11–2.26 (m, 2H), 2.28 (s, 3H), 2.52–2.75 (m, 3H), 3.47–3.87 (m, 11H), 4.40–4.61 (m, 1H), 4.81 (dd, J = 7.6, 5.1 Hz, 1H), 7.10 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 8.17–8.38 (m, 1H), 9.51–9.71 (m, 1H); HRMS (FAB) calcd for $C_{16}H_{25}N_4O_2S$: 337.1698. Found: 337.1689.

4.1.87. (2*S*)-1-{[(2*S*,4*S*,5*S*)-4-(1,3-Dihydro-2*H*-isoindol-2-ylcarbonyl)-5-methyl-2-pyrrolidinyl]carbonyl}-2-pyrrolidinecarbonitrile 4-methylbenzenesulfonate (34). Yield 98%. A white powder. TLC $R_f = 0.29$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 353 $(M+H)^+$; IR (KBr) 2980, 2243, 1656, 1449, 1370, 1227, 1166, 1121, 1032, 1008, 681, 566 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.23 (d, J = 6.7 Hz, 3H), 1.95–2.33 (m, 5H), 2.28 (s, 3H), 2.65–2.80 (m, 1H), 3.46–3.65 (m, 3H), 3.94–4.08 (m, 1H), 4.50–4.64 (m, 1H), 4.64–4.74 (m, 2H), 4.82 (dd, J = 7.8, 5.3 Hz, 1H), 4.86–5.01 (m, 2H), 7.10 (d, J = 8.2 Hz, 2H), 7.23–7.41 (m, 4H), 7.47 (d, J = 8.2 Hz, 2H), 8.13–8.50 (m, 1H), 9.57–9.96 (m, 1H); HRMS (FAB) calcd for $C_{20}H_{25}N_4O_2$: 353.1978. Found: 353.1975.

4.2. Biological method

4.2.1. Purification of human DPP-IV. Human DPP-IV was purified according to the published procedure with some modifications.[17] Briefly, the enzyme was prepared from pooled plasma obtained from healthy volunteers by ammonium sulfate precipitation (50–70%). After extensive dialysis against 25 mM Tris–HCl (pH 7.4), the material was mixed with DEAE cellulose, DE52 (Whatman Chemical Separation Inc., USA) for 60 min, and eluted with buffer containing 100 mM NaCl. Fractions of 10 mL were collected, and the fraction with maximal DPP-IV activity was dialyzed against 25 mM MES–NaOH (pH 6.0). DPP-IV-containing fractions were detected by the ability to hydrolyze Gly-Pro-7-amido-4-methyl-coumarin (Gly-Pro-AMC) (Sigma–Aldrich, USA) using the standard method described below. The DE52 elute was loaded onto a SP Sepharose Fast Flow column (GE Healthcare, Sweden), and the flow-through fraction containing DPP-IV was then applied to a DEAE cellulose column (Whatman DE52). Bounded proteins were eluted with 25 mM Tris–HCl (pH 7.8) containing 150 mM NaCl. Fractions of 10 mL were collected, and the fraction with

maximum DPP-IV activity was concentrated using polyethylene glycol 20000 (PEG20000). The concentrated material was applied to a Sephacryl S-300 High Resolution 26/60 column (GE Healthcare, Sweden) and was eluted at a flow rate of 0.1 mL/min. Fractions of 1 mL were collected, and the fractions containing DPP-IV activity were pooled.

4.2.2. Enzyme assays. Enzymatic activity was determined at 37 °C by the cleavage rate of a substrate, Gly-Pro-AMC (30 μM) (Sigma–Aldrich, USA).[18] Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μM Gly-Pro-AMC, 10 μL of 500 mM Tris–HCl (pH 7.4), 20 μL of distilled water, and 10 μL of a test compound. Then the change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/emission at 460 nm) (fmax, Molecular Devices, USA). The initial rate of DPP-IV activity was calculated over the first 15-min of the reaction and was defined as the rate of increase in the fluorescence intensity (arbitrary units 1 mL) under these conditions. The percent inhibition was calculated relative to the addition of the solvent alone and IC_{50} values were determined by logistic regression analysis. To study slow binding, the apparent inhibitory potency of compound **17** or **27** was determined as a function of the preincubation time in a standard IC_{50} experiment. Test compounds were preincubated with the enzyme for 0, 10, 30, 60 or 120 min, and then the reaction was initiated by adding the substrate.

4.2.3. DPP-IV inhibition in rats. Male Sprague–Dawley (SD) rats were purchased from Charles River Laboratories, Japan. The rats were housed in an air-conditioned animal room with controlled temperature (24 ± 2 °C), humidity (55 ± 5%), and lighting (12:12 h light/dark cycle), and were provided with standard pellet food for rodents (CRF-1, Oriental Yeast, Japan) and water ad libitum. All procedures were conducted according to the ONO Pharmaceutical Animal Care Committee guidelines. After fasting for at least 8 h, male SD rats (6–7 weeks old) were orally administered a test compound dissolved in 0.5% methyl cellulose as a single dose of 1 mg/kg. Blood samples were collected from the jugular vein before administration, and 0.25, 0.5, 1, 2, 4, 6 and 10 h after administration. In the study of species differences, blood samples were obtained at 12 h instead of 10 h. Each blood sample was immediately centrifuged to obtain plasma and the DPP-IV activity was determined. Briefly, 50 μL of plasma was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μM substrate. Then the initial rate of DPP-IV activity was measured using the method described above, and the percent inhibition relative to basal DPP-IV activity was calculated.

4.2.4. DPP-IV inhibition in beagle dogs and cynomolgus monkeys. On the basis of the extent of DPP-IV inhibition observed in rats, compounds **11**, **27**, **28**, **30**, **32**, and **34** were advanced to further assessment in beagle dogs and cynomolgus monkeys. Male beagle dogs weighing 11–15 kg (Kitayama-Labes Co., Ltd., Japan) and male cynomolgus monkeys weighing 3.5–6 kg (bred by Hamri Co., Ltd., Japan) were used. After fasting for 8 h, the dogs and monkeys were orally administrated test compounds dissolved in 0.5% methyl cellulose as a single dose of 1 mg/kg. Blood samples were collected via the femoral vein before administration and 12 h after administration. Each sample was immediately centrifuged to obtain plasma and the DPP-IV activity was determined. Then the percent inhibition relative to basal DPP-IV activity was calculated.

4.2.5. Multiple oral glucose tolerance tests in rats. The effect of inhibitor **27** on the outcome of multiple oral glucose tolerance tests was assessed in male SD rats (364–473 g). The rats were fasted for at least 20 h before the study and then were dosed orally with the vehicle (0.5% methyl cellulose) or with compound **27** (1 mg/kg) at −0.5 h. Blood samples (75 μL) were collected from the tail vein into heparinized tubes at −0.08 h. Glucose (1 g/kg) was administered orally at 0, 6, and 12 h. Additional blood samples (75 μl) were collected at 0.17, 0.5, 1, 2, 6, 6.17, 6.5, 7, 8, 12, 12.17, 12.5, 13, and 14 h after the first glucose load. Plasma was obtained from each sample by centrifugation and was stored at −80 °C until measurement of the glucose level, with a glucose oxidase peroxidase dye system (Diacolor GC, Toyobo, Japan).

References and notes

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15. Compound **64** was prepared by the following two methods. One of them is described in Section 2. The other is the same method as the reported one (Ezquerra et al. *J. Org. Chem.* **1995**, *60*, p 2925), which describes the established stereochemistry, starting with condensation reaction of the protected ethyl pyrroglutamate and 3-benzyloxypropionaldehyde. Products prepared by the above-described two methods showed an identical NMR. Based on the result, the stereochemistry of 4-position of **64** was determined to be 4β.
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