Synthesis and Structure–Activity Relationships of Potent 1-(2-Substituted-aminoacetyl)-4-fluoro-2-cyanopyrrolidine Dipeptidyl Peptidase IV Inhibitors

Hiroshi FUKUSHIMA,*[,a] Akira HIRATATE,[a] Masato TAKAHASHI,[a] Masako SAITO-HORI,[b] Eiji MUNETOMO,[b] Kiyokazu KITANO,[b] Hidetaka SAITO,[c] Yuji TAKAOKA,[d] and Koji YAMAMOTO[b]

[a] *Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd.;* [b] *Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd.;* [c] *Non-clinical Quality Assurance Section, Taisho Pharmaceutical Co., Ltd.; and* [d] *Research Computer System, Taisho Pharmaceutical Co., Ltd.; 1–403 Yoshino-cho, Kita-ku, Saitama, Saitama 331–9530, Japan.* Received February 14, 2008; accepted May 8, 2008; published online May 12, 2008

Dipeptidyl peptidase IV (DPP-IV) inhibitors have attracted attention as potential drugs for use in the treatment of type 2 diabetes because they prevent the degradation of glucagon-like peptide-1 (GLP-1) and extend its duration of action. We previously reported that 2-cyano-4-fluoropyrrolidines act as potent DPP-IV inhibitors and have been modifying the 1-position of pyrrolidine to obtain more useful inhibitors. An L-*tert*-butylglycine derivative was found to be a stable and potent DPP-IV inhibitor that exhibits a glucose lowering effect *in vivo*. Here, we report the synthesis of and biological data on the aforementioned derivatives.

Key words dipeptidyl peptidase IV; inhibitor; fluoropyrrolidine; diabetes

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5, CD26)[1,2] is a highly specific serine protease that cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position.[3] The natural substrates of DPP-IV have been described, and the inhibition of DPP-IV has been recognized as potentially useful for the modulation of glucagon-like peptide processing. The role of DPP-IV in glucagon-like peptide (GLP) processing is well-known.[4—6] Given the importance of the regulation of this process, DPP-IV inhibition has an obvious application for the treatment of diabetes.[7—10] Since the blood glucose-lowering effects of GLP-1 are dependent on an elevated blood glucose level and abate as glucose levels return to normal, the incidence of hypoglycemia during treatment with a DPP-IV inhibitor is expected to be very low.[11] Based on this concept, the clinical development of several DPP-IV inhibitors, including NVP-DPP728,[12] NVP-LAF237 (Vildagliptin),[13] BMS-477118 (Saxagliptin),[14] and MK-0431 (Sitagliptin),[15] has been initiated (Fig. 1).

We previously reported that the introduction of fluorine to the 4-position of 2-cyanopyrrolidine augmented DPP-IV inhibitory activity and the drug concentration after oral administration to rats.[16] While investigating the properties of 2-cyano-4-fluoropyrrolidine **1a**, we recognized the presence of decomposed products of **1a** in neutral aqueous solution. Some previous reports about the chemical instability of 2-cyanopyrrolidine derivatives have been published. Ashworth synthesized 2-cyanopyrrolidine derivatives using six kinds of amino acids and investigated the DPP-IV inhibitory activity and chemical stability.[17] Villhauer investigated the SAR of *N*-substituted-glycyl-2-cyanopyrrolidines and determined the structure of the decomposed compound to be a cyclic amidine.[13] And Magnin reported the synthesis of methanoprolinenitrile derivatives, their DPP-IV inhibitory activity and their chemical stability and found that the introduction of either a sterically bulky amino acid side chain on the N-terminal amino acid or a *cis*-4,5-methano bridge to the prolinenitrile moiety significantly enhanced solution stability.[18]

We thought that the chemical stability of **1a** should be further improved to make it more useful. Consequently, we have been exploring the synthesis of 2-cyano-4-fluoropyrrolidines in the hope of obtaining more effective DPP-IV inhibitors.

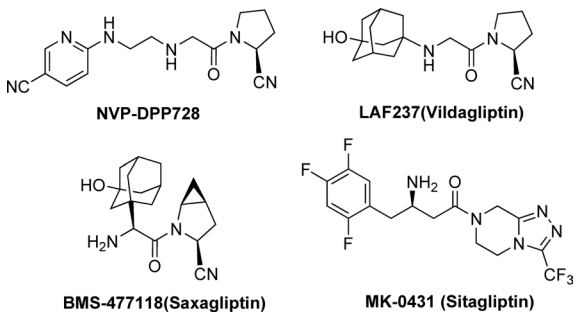


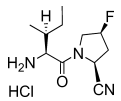
Fig. 1. DPP-IV Inhibitors

Chemistry

As the cause of the instability of 2-cyano-4-fluoropyrrolidine was undetermined, we investigated the structure of the decomposed product of **1a** (Table 1). Compound **1a** was considerably stable in a pH 1.2 acidic solution, but the residual amount decreased in a pH 6.8 neutral solution. The degraded compounds derived from **1a** were isolated and identified as the anticipated cyclic compounds.

The first step in the degradation process was thought to be the intramolecular attachment of a basic nitrogen to the

Table 1. Chemical Stability of **1a**

Compd.		Residual amount (%)[a)]	
		pH 1.2	pH 6.8
1a		>95	70

a) HPLC determination after 6 h incubation at 37 °C in aqueous solution.

* To whom correspondence should be addressed. e-mail: hiroshi.fukushima@po.rd.taisho.co.jp

cyano group to give cyclic amidine **2**, which was subsequently transformed into diketopiperadine **3**. Cyclic amidine **2** (retention time=4.7 min) was observed as a 67% peak area using reverse phase high-performance liquid chromatography (HPLC) after 2 h of the incubation of **1a** in a pH 6.8 aqueous buffer solution. After 10 h of incubation, compound **2** changed to diketopiperadine **3** (retention time=7.4 min), which was observed as an 86% peak area (Table 2).

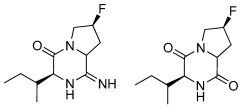
Cyclic amidine **2** was formed during the decomposition of **1a** in neutral solution, but it was difficult to isolate from aqueous solution. Since the cyclization of **4**, a free base of **1a**, was accelerated by the presence of carboxylic acid in an organic solvent, **4** was treated with a 1.1-molar equivalent of acetic acid in ethanol–*n*-hexane to obtain cyclic amidine **5** (acetic acid salt) as a precipitate. Diketopiperadine **3** was obtained by heating **5** in a pH 6.8 buffer solution at 80 °C (Chart 1). Spectral data for **5** and **3** supported their structures, and the HPLC retention times of **5** and **3** corresponded with those of the degradation products of **1a** in buffer solution.

Next, conversion at the P2 site was conducted in order to find a potent, stable and highly efficacious inhibitor. Magnin reported that the changes in stability caused by the conversion at the P2 site were affected by the substituent of the pyrrolidine ring.[18] Several derivatives converted at the P2 site were then synthesized, as in the case of 2-cyano-4-fluoropyrrolidine.

Table 2. Degradation Peaks of **1a**

	Retention time		
	4.7 min	7.4 min	8.7 min
Incubation time[a]			
0 h	Trace	0%	>97%
2 h	67%	12%	20%
10 h	13%	86%	0%
Estimated structure	**2**	**3**	**1a**

a) Compound **1a** was dissolved in pH 6.8 Britton–Robinson buffer solution and incubated at 60 °C.



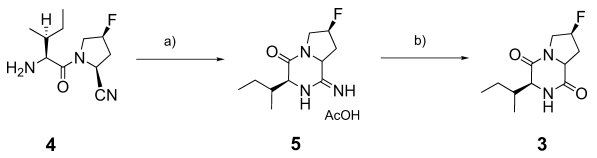
2 **3**

First, 2-aminocarbonyl-4-fluoropyrrolidine **7** was obtained in three steps from 4-hydroxide **6** and was then converted to amine hydrochloride **8** (Chart 2).[16,19]

Compound **9** was obtained *via* the dehydration of compound **7** with cyanuric chloride[20] in *N*,*N*-dimethylformamide (DMF) and was treated with hydrochloric acid to obtain intermediate **10**. Compound **8** was useful as a common intermediate, and cyano derivative **10** seemed to be more useful for the synthesis of a variety of derivatives.

Compound **8** was coupled with *N*-Boc or *N*-Fmoc α-amino acids using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenztriazole (HOBt) as coupling reagents to yield the dipeptides **11a**—**f**, **11k**, **11l**, **13i** and **13j**. Either the Fmoc group or the Boc group was used as an *N*-protecting group for of α-amino acids. In the same manner, cyanide **10** was converted to dipeptides **12g** and **14h** (Chart 3).

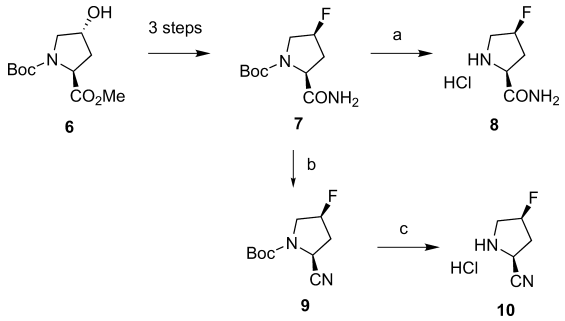
Natural amino acids (valine and proline), artificial amino acids (alloisoleucine, *tert*-butylglycine and cyclohexylglycine) and modified amino acids (*N*-benzyloxycarbonyl-ly-



4 **5** **3**

Reagents: (a) AcOH, EtOH, *n*-hexane; (b) pH 6.8 buffer.

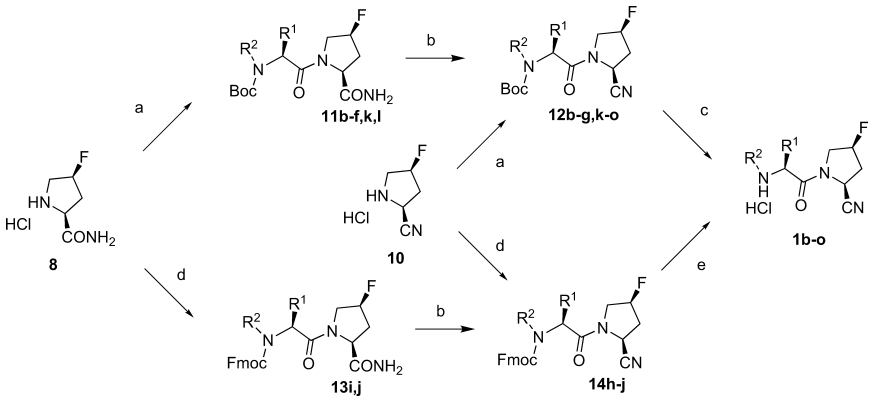
Chart 1



6 **7** **8** **9** **10**

Reagents: (a) 4 M-HCl/AcOEt; (b) cyanuric chloride/DMF; (c) 2 M–HCl/H_2O–MeOH.

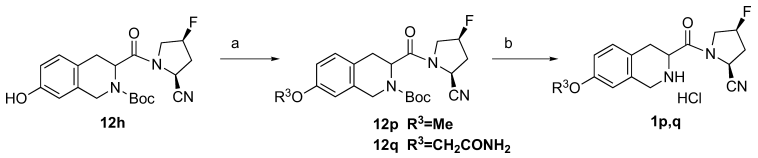
Chart 2



8 **11b-f,k,l** **12b-g,k-o** **10** **13i,j** **14h-j** **1b-o**

Reagents: (a) Boc$NR^2CH(R^1)CO_2H$, EDC, HOBt, DMF; (b) cyanuric chloride, DMF; or $(CF_3CO)_2$, *N*,*N*-diisopropylethylamine, THF; (c) 2 M-HCl/H_2O–MeOH; (d) Fmoc$NR^2CH(R^1)CO_2H$, EDC, HOBt, DMF; (e) Et_2NH, 1,2-dichloroethane; then HCl.

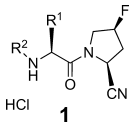
Chart 3

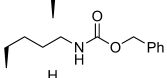
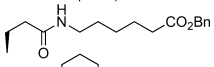


Reagents: (a) R3-X, K_2CO_3, DMF; (b) 2 M-HCl/H_2O–MeOH.

Chart 4

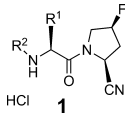
Table 3. DPP-IV Inhibitory Activity and Chemical Stability of **1a**—**k**

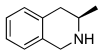
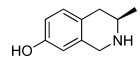
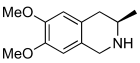
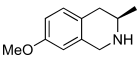
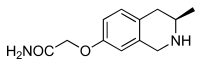


Compd.	R^1	R^2	IC_{50} (nM)[a]	Remaining amount (%)[b]
1a		H	0.6	70
1b		H	0.7	74
1c		H	1.2	65
1d		H	0.6	93
1e		H	1.4	52
1f		H	4.7	67
1g		H	2.1	73
1h		H	4.9	92
1i		H	0.7	41
1j		H	1.1	68
1k		Me	>100	—

a) DPP-IV inhibitory activity. *b*) Residual amount was measured after incubation at 37 °C for 6 h in pH 6.8 aqueous buffer solution.

Table 4. DPP-IV Inhibitory Activity of **1l**—**q**



Compd.		IC_{50} (nM)[a]
1l		0.8
1m		2.2
1n		1.2
1o		2.5
1p		1.7
1q		3.3

a) DPP-IV inhibitory activity.

sine, *O*-methylthreonine, *O*-benzylthreonine and *O*-*tert*-butylthreonine) were used at the P2 site.

The cyano compounds **12a**—**f**, **14i** and **14j** were obtained from carbamoyl compounds **11a**—**f**, **13i** and **13j** using cyanuric chloride in DMF or trifluoroacetic anhydride and *N*,*N*-diisopropyl ethylamine.

Boc-protected compounds **12a**—**g** and **12k**—**o** were deprotected using hydrochloric acid to produce **1a**—**g** and **1k**—**o**. On the other hand, Fmoc-protected compounds **14h**—**j** were converted to **1h**—**j** using diethylamine.

7-Methoxytetrahydroquinoline derivative **12p** and 7-carbamoylmethoxy derivative **12q** were obtained by alkylating the 7-hydroxy derivative **12h**; **12p** and **12q** were then treated with hydrochloric acid to produce **1p** and **1q**.

As the activities of stereoisomers appeared interesting, stereoisomers of **1d** were synthesized. The synthetic route was similar to that for **1d**. Four different hydroxy prolines (L-*trans*, L-*cis*, D-*trans* and D-*cis* forms) and L- and D-*N*-Boc-*tert*-butylglycine were used to produce seven stereoisomers **15**—**21**.

Results and Discussion

The synthesized compounds were evaluated for DPP-IV-inhibitory activity in human plasma using a fluorescence assay with Gly-Pro-4-methylcoumaryl-7-amide.

Various sorts of α-amino acids were introduced at the P2 site. Valine derivative **1b** and *tert*-butylglycine derivative **1d** exerted potent DPP-IV inhibitory activities (IC_{50}=0.7, 0.6 nM, respectively), similar to that of isoleucine derivative **1a** (IC_{50}=0.6 nM). Cyclohexylglycine derivative **1j** also maintained an inhibitory activity (IC_{50}=1.1 nM) (Table 3). On the other hand, threonine derivatives **1f**, **1g** and **1h** exhibited less potent activities (IC_{50}=4.7, 2.1, 4.9 nM, respectively).

X-ray crystallographic analysis of DPP-IV[21,22] has suggested that the pocket to which the P2 site binds is lipophilic. The oxygen atom on the threonine side chain seemed to have difficulty binding tightly to the pocket. However, a benzyl ether side chain (**1g**) resulted in notable activity, possibly because of the affinity of the phenyl group. Compounds **1e** and **1i** exhibited potent activities (IC_{50}=1.4, 0.7 nM, respectively), and the long side chains did not reduce the compounds' affinities.

Among the secondary amine derivatives, proline derivative **1l** had a potent inhibitory activity (IC_{50}=0.8 nM) and tetrahydroisoquinoline derivatives **1m**—**q** also had good potencies (IC_{50}=1.2—3.3 nM) (Table 4). However, the *N*-methyl isoleucine derivative **1k** had a very weak activity (IC_{50}> 100 nM).

Chemical stability is an important characteristics in the development of 2-cyanopyrrolidine DPP-IV inhibitors. Our lead compound **1a** was considerably stable in acidic solution, but the residual amount of compound **1a** decreased in neutral solution. The stabilities of the compounds listed in Table 3 varied widely in a manner that was correlated with their structures.

The degradation of compound **1a** under neutral conditions might result from the attachment of a basic nitrogen to the carbon atom of the cyano group, yielding a cyclic amidine. By constructing a sterically hindered environment near the nitrogen, this kind of degradation should be prevented.

tert-Butylglycine derivative **1d** and *O*-*tert*-butylthreonine **1h** were comparatively stable, as shown by their residual amounts (93%, 92%, respectively) after 6 h of incubation at 37 °C in a pH 6.8 buffer solution. Magnin reported that a *tert*-butylglycine derivative exhibited the same chemical stability as an isoleucine derivative in a *cis*-3,4-methanoprolinenitrile series and better chemical stability than an isoleucine derivative in a *cis*-4,5-methanoprolinenitrile series. Our result for fluoropyrrolidine was similar to that for the *cis*-4,5-methanoprolinenitrile series.

In contrast, compounds **1e** or **1i**, which are not branched at the carbon next to the α-position, yielded low residual amounts (52%, 41%, respectively). Proline derivative **1l** and tetrahydroisoquinoline derivatives **1m**—**o** also showed low residual amounts, with the compounds listed in Table 4 showing the residual amounts of less than 20% under the same condition. These compounds with cyclic side chains easily undergo cyclization, since their nitrogen atoms can come into proximity with the cyano group.

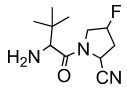
To investigate the correlation between stereochemistry and inhibitory activity, all the stereoisomers of **1d** were synthesized. Compound **1d** was selected for this investigation because it exhibited the best balance between stability and inhibitory activity.

We previously reported the structure–activity relationship (SAR) for the stereochemistry of 4-fluoride[16]; here, we report the SAR for that of 2-cyanide and α-amino acids at the P2 site as well as 4-fluoride. (4*R*)-Fluoride analogue **15** had a 400-fold lower potency (IC_{50}=246 nM) than **1d**, and (2*R*)-cyanide analogue **16** had a more than 500-fold lower potency (IC_{50}>300 nM) (Table 5). D-*tert*-Butylglycine analogue **18** had a 70-fold lower potency (IC_{50}=42 nM) than **1d**, but the genuine activity level of **18** was uncertain because the amount of commingled L-form **1d** could not be determined. The other isomers did not exhibit inhibitory activity. We concluded that all three chiral centers were important for activity because of the dramatic reductions in the activities of the stereoisomers of **1d**.

We obtained a crystal of **22**, the free base of **1d**, for X-ray crystallographic analysis and observed that the configuration of the cyano group and the fluorine atom was *cis*, while the conformation of the amide bond was *trans* (Fig. 2).

As **1d** exhibited the most favorable inhibitory activity and chemical stability profiles, the *in vivo* effect of **1d** was examined in Zucker fatty rats, a model of obesity and impaired glucose tolerance. Compound **1d** was orally administered at a dose of 1 mg/kg body weight at 30 min before glucose loading; the plasma glucose, insulin and DPP-IV activity levels were then monitored over time (Fig. 3).

Table 5. DPP-IV Inhibitory Activities of Stereoisomers of **1d**



Compd.	Streochemistry of *t*-Bu Gly	Stereochemistry of the pyrrolidine	IC_{50} (nM)[a]
1d (HCl)	L	(2*S*,4*S*)	0.6
15	L	(2*S*,4*R*)	246
16	L	(2*R*,4*S*)	>300
17	L	(2*R*,4*R*)	>300
18	D	(2*S*,4*S*)	42
19	D	(2*S*,4*R*)	>300
20	D	(2*R*,4*S*)	>300
21	D	(2*R*,4*R*)	>300

a) DPP-IV inhibitory activity.

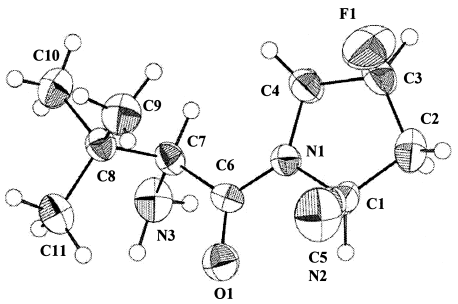


Fig. 2. X-Ray Crystal Structure of Compound **22** (Free Base of **1d**)

The plasma glucose level after glucose loading was significantly lower in the **1d**-treated group than in the vehicle-treated control group (Figs. 3A, B). DPP-IV activity was almost completely inhibited at 15 min after glucose loading, and this inhibitory effect was retained for 2 h (Fig. 3D). These results indicate that **1d** may be useful for the treatment of hyperglycemia, based on its inhibitory effect on plasma DPP-IV activity. Insulin secretion was significantly enhanced at 15 min after glucose loading (Fig. 3C). This finding supports the proposed mechanism that **1d** might prevent the inactivation of active GLP-1 *via* DPP-IV inhibition and that an increase in GLP-1 activity might stimulate insulin secretion by acting upon β-cells in the pancreas, resulting in the suppression of hyperglycemia after glucose loading.

Conclusion

We modified the P2 site of 2-cyano-4-fluoropyrrolidine derivative **1a**, which we previously reported, to obtain a more useful DPP-IV inhibitor. As a result, we obtained a stable and potent DPP-IV inhibitor, **1d**, which is expected to be useful as a therapeutic agent for lowering postprandial hyperglycemia and treating type 2 diabetes mellitus. Subsequent reports will describe the results of further investigations of 4-fluoro-2-cyanopyrrolidines.

Experimental

^{1}H-NMR spectroscopy was performed using a Varian VXR-300 or a JEOL GX500 spectrometer. Chemical shifts were reported in parts per million relative to tetramethylsilane as an internal standard (in NMR descriptions: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad peak). ^{13}C-NMR spectroscopy was performed using a JEOL GX500 spectrometer. ^{19}F-NMR spectroscopy was performed using a Varian VXR-300 spectrometer. ESI mass spectra were recorded using a Shimadzu/Kratos HV-300. High resolution spectra were recorded on a Micro-

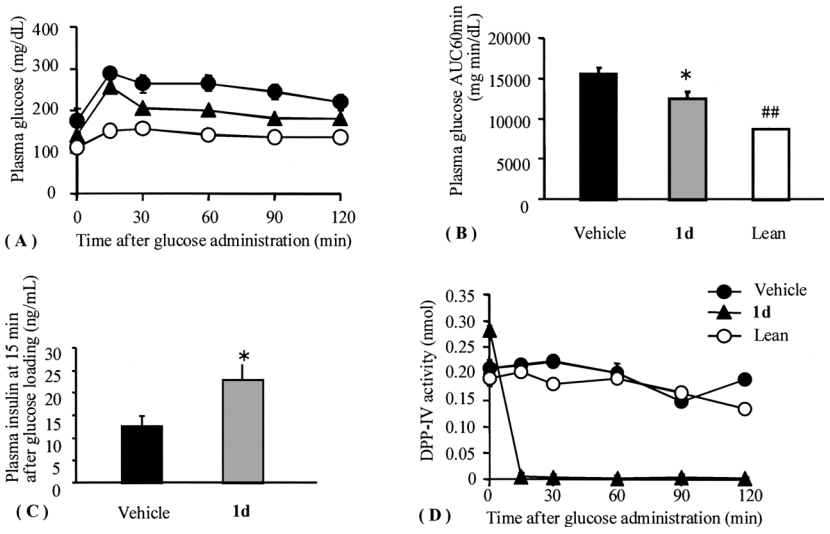


Fig. 3. Effects of Oral Administration of **1d** (1 mg/kg) on Plasma Glucose, Plasma Insulin and Plasma DPP-IV Activity during OGTT in Zucker Fatty Rats

Each point represents the mean±S.E. (n=6). $*p<0.05$ *vs.* vehicle, Dunnett's test. $^{\#\#}p<0.01$ *vs.* vehicle, Student's t-test.

mass Q-TOF2 instrument. Melting points were measured using a Buchi 535 melting point apparatus without correction. Infrared spectra were recorded using a Perkin-Elmer 1760 spectrometer. Elemental analyses were performed using a Perkin-Elmer 240C analyzer (for carbon, hydrogen, and nitrogen) or a Yokokawa-Denki IC7000P analyzer (for halogens and sulfur).

Analytical thin-layer chromatography was conducted on precoated silica gel 60 F254 plates (Merck). Chromatography was performed on 100- to 200-mesh silica gel C-200 (Wako Pure Chemical) using the solvent systems (volume ratios) indicated below.

Degradation of 1a and HPLC Analysis Compound **1a** (5 mg) was dissolved in pH 6.8 Britton–Robinson buffer solution (5 ml) and incubated at 60 °C. The solution was then analyzed using reverse phase HPLC using a CAPCELL PAK UG120 (5 μm particle size, ϕ 4.6×150 mm; SHISEIDO) and eluted at 1.0 ml/min with acetonitrile–H_2O (15 : 85 v/v, 10 mM ammonium acetate solution); its UV absorbance was monitored at 210 nm. To examine chemical stability, the residual amount was also analyzed using HPLC in a manner similar to the method described above.

(3*S*,7*S*)-7-Fluoro-1-imino-3-[(1*S*)-1-methylpropyl]hexahydropyrrolo[1,2-*a*]pyrazin-4(1*H*)-one Acetic Acid Salt (5) Compound **4** (free base of **1a**, 1.30 g, 5.72 mmol) was dissolved in EtOH (4.5 ml). AcOH (0.36 ml, 6.29 mmol) and *n*-hexane (12 ml) were then added and the solution was stirred at room temperature overnight. The resulting powder was collected using filtration and dried *in vacuo* to yield the desired product (0.73 g, 44%), which was a colorless powder. mp 143—146 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 5.34 (1H, dt like, J=53.1, 3.8 Hz, H-7), 4.35 (1H, dd, J=11.8, 5.7 Hz, H-8a), 3.87 (1H, ddd, J=38.4, 14.4, 4.3 Hz, H-6), 3.66 (1H, d, J=5.3 Hz, H-3), 3.41 (1H, dd, J=28.3, 14.4 Hz, H-6), 2.68 (1H, td like, J=15.1, 5.6 Hz, H-8), 2.04—1.88 (1H, m, H-8), 1.84 (3H, s, AcOH), 1.77—1.65 (1H, m), 1.52—1.42 (1H, m), 1.20—1.08 (1H, m), 0.87 (3H, d, J=6.7 Hz), 0.85 (3H, t, J=7.3 Hz); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 173.4, 167.2, 158.2, 90.5 (d, J_{C-F}=171.5 Hz), 64.1, 54.4, 51.7 (d, J_{C-F}=23.8 Hz), 39.4, 36.5 (d, J_{C-F}=20.7 Hz), 24.7, 22.0, 15.5, 11.2; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −173.4. MS (ESI pos.) m/z 228 $(M+H)^+$, 250 $(M+Na)^+$; (ESI neg.) m/z 226 $(M-H)^-$. *Anal.* Calcd for $C_{11}H_{18}FN_3O \cdot C_2H_4O_2 \cdot 0.5H_2O$: C, 52.69; H, 7.82; N, 14.18; F, 6.41. Found: C, 52.80; H, 7.93; N, 14.30; F, 6.45. $[\alpha]_D^{23}$ +99.4° (c=0.3, MeOH).

(3*S*,7*S*)-7-Fluoro-3-[(1*R*)-1-methylpropyl]hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (3) Compound **5** (240 mg, 1.28 mmol) was dissolved in pH 6.8 Britton–Robinson buffer solution (24 ml). The solution was then stirred at 80 °C for 5 h. The reaction solution was extracted with EtOAc, and the organic phase was successively washed with a saturated aqueous NaCl solution and dried over Na_2SO_4. The drying agent was removed by filtration, and the filtrate was concentrated under reduced pressure. EtOAc and *n*-hexane were added to the residue, and the resulting powder was collected using filtration and dried *in vacuo* to yield the desired product (128 mg, 44%), which was a colorless powder. mp 129—131 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.48 (1H, d, J=4.0 Hz), 5.31 (1H, dt like, J=53.0, 3.8 Hz, H-7), 4.36 (1H, dd, J=11.6, 6.1 Hz, H-8a), 3.83 (1H, ddd, J=38.4, 14.3, 4.0 Hz, H-6), 3.55—3.44 (2H, m, H-3, H-6), 2.38 (1H, td like, J=15.4, 5.9 Hz, H-8), 2.08 (1H, dddd, J=43.2, 15.3, 11.6, 3.7 Hz, H-8), 1.82—1.73 (1H, m), 1.57—1.47 (1H, m), 1.18—1.08 (1H, m), 0.89 (3H, d, J=7.0 Hz), 0.87 (3H, t, J=7.3 Hz); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 168.0, 165.0, 90.4 (d, J_{C-F}=171.5 Hz), 61.4, 55.8, 52.4 (d, J_{C-F}=23.8 Hz), 38.7, 35.9 (d, J_{C-F}=20.6 Hz), 24.6, 15.1, 10.9; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −172.7. MS (ESI pos.) m/z 251 $(M+Na)^+$; (ESI neg.) m/z 227 $(M-H)^-$. *Anal.* Calcd for $C_{11}H_{17}FN_2O_2$: C, 57.88; H, 7.51; N, 12.27; F, 8.32. Found: C, 57.86; H, 7.44; N, 12.26; F, 8.35. $[\alpha]_D^{23}$ +76.0° (c=0.3, MeOH).

***tert*-Butyl (2*S*,4*S*)-2-Cyano-4-fluoropyrrolidine-1-carboxylate (9)** *tert*-Butyl (2*S*,4*S*)-2-(aminocarbonyl)-4-fluoropyrrolidine-1-carboxylate **7** (697 mg, 3.00 mmol) was dissolved in DMF (3 ml), and cyanuric chloride (332 mg, 1.80 mmol) was added, followed by stirring at room temperature for 20 min. The reaction solution was taken up in water and extracted with EtOAc. The organic phase was washed with a saturated aqueous NaCl solution and dried over $MgSO_4$. The drying agent was removed by filtration, and the filtrate was concentrated under reduced pressure to yield the desired product (680 mg, quant.) as a light brown powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 5.37 (1H, br d, J=53.3 Hz, H-4), 4.85—4.80 (1H, m, H-2), 3.66—3.38 (2H, m, H-5), 2.58—2.36 (2H, m, H-3), 2.64—2.36 (2H, m), 1.44 (9H, s). MS (ESI pos.) m/z 237 $(M+Na)^+$; (ESI neg.) m/z 249 $(M+Cl)^-$.

(2*S*,4*S*)-4-Fluoropyrrolidine-2-carbonitrile Hydrochloride (10) Compound **9** (450 mg, 2.10 mmol) was dissolved in MeOH (3 ml), and 4 M HCl (3 ml) was added, followed by stirring at room temperature for 20 h. The reaction solution was concentrated *in vacuo* to yield the desired product (320 mg, quant.) as a light brown powder. This intermediate was used in the next reaction without purification. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 5.53 (1H, br d, J=52.4 Hz, H-4), 4.97 (1H, dd, J=8.2, 4.3 Hz, H-2), 3.59 (1H, dd, J=22.1, 13.7 Hz, H-5), 3.47 (1H, ddd, J=35.9, 13.7, 3.7 Hz, H-5), 2.64—2.43 (2H, m, H-3). MS (ESI pos.) m/z 115 $(M+H)^+$; (ESI neg.) m/z 149 $(M+Cl)^-$.

***N*-(*tert*-Butoxycarbonyl)-L-valyl-(4*S*)-4-fluoro-L-prolinamide (11b)** (4*S*)-4-Fluoro-L-prolinamide hydrochloride **8** (500 mg, 2.97 mmol) and *N*-(*tert*-butoxycarbonyl)-L-valine (645 mg, 2.97 mmol) were dissolved in DMF (10 ml), and 1-hydroxybenzotriazole monohydrate (455 mg, 2.97 mmol), 1-(3,3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (598 mg, 3.12 mmol) and *N*,*N*-diisopropylethylamine (0.54 ml) were added while cooling on ice. The temperature was then gradually increased, and the solution was stirred at room temperature overnight. The reaction solution was poured into a saturated aqueous NaCl solution and extracted with EtOAc. The organic phase was washed with a 10% aqueous $KHSO_4$ solution, a 5% aqueous $NaHCO_3$ solution and a saturated aqueous NaCl solution, successively, and then dried over $MgSO_4$. The drying agent was removed by filtration, and the filtrate was concentrated under reduced pressure to yield the desired product (840 mg, 85%), as a colorless amorphous powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.02 (1H, br s), 6.95—6.85 (2H, m), 5.33 (1H, br d, J=53.6 Hz, H-4), 4.43 (1H, dd, J=9.8, 1.9 Hz, H-2), 4.09—3.73 (3H, m),

2.50—2.10 (2H, m, H-3), 2.02—1.84 (1H, m), 1.37 (9H, s), 0.94 (3H, d, J=6.7 Hz), 0.88 (3H, d, J=6.7 Hz). MS (ESI pos.) m/z 354 $(M+Na)^+$; (ESI neg.) m/z 330 $(M-H)^-$.

(4*S*)-1-((2*S*)-2-Cyclohexyl-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]-amino}acetyl)-4-fluoro-L-prolinamide (13j) The title compound (880 mg, quant.) was obtained as a colorless amorphous powder from **8** (300 mg, 1.78 mmol) and (2*S*)-cyclohexyl{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}acetic acid (709 mg, 1.87 mmol) in a manner similar to method used to prepare **11b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.88 (2H, d, J=7.5 Hz), 7.73 (2H, d, J=7.4 Hz), 7.66 (1H, d, J=8.4 Hz), 7.41 (2H, t, J=7.4 Hz), 7.32 (2H, t, J=7.4 Hz), 7.06 (1H, br s), 6.89 (1H, br s), 5.32 (1H, br d, J=53.8 Hz, H-4), 4.44 (1H, dd, J=9.7, 2.1 Hz, H-2), 4.33—3.74 (6H, m), 2.50—2.10 (2H, m, H-3), 1.90—0.90 (11H, m). MS (ESI pos.) m/z 516 $(M+Na)^+$.

***tert*-Butyl ((1*S*)-1-{[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]carbonyl}-2-methylpropyl)carbamate (12b)** The title compound (656 mg, 91%) was obtained as a colorless amorphous powder from **11b** (760 mg, 2.29 mmol) in a manner similar to method used to prepare **9**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.17 (1H, br d, J=7.9 Hz), 5.49 (1H, br d, J=51.5 Hz, H-4), 5.01 (1H, dd, J=7.3, 2.0 Hz, H-2), 4.11—3.76 (3H, m), 2.60—2.25 (2H, m, H-3), 2.01—1.83 (1H, m), 1.36 (9H, s), 0.94 (3H, d, J=6.5 Hz), 0.88 (3H, d, J=6.8 Hz). MS (ESI pos.) m/z 336 $(M+Na)^+$; (ESI neg.) m/z 312 $(M-H)^-$.

9*H*-Fluoren-9-ylmethyl ((1*S*,2*R*)-2-*tert*-Butoxy-1-{[(2*S*,4*S*)-2-cyano-4-fluoropyrrolidin-1-yl]carbonyl}propyl)carbamate (14h) The title compound (870 mg, 88%) was obtained as a colorless amorphous powder from **10** (301 mg, 2.0 mmol) and *O*-(*tert*-butyl)-*N*-[(9*H*-fluoren-9-ylmethoxy)carbonyl]-L-threonine (795 mg, 2.0 mmol) in a manner similar to method used to prepare **11b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.89 (2H, d, J=7.3 Hz), 7.74 (2H, dd, J=7.3, 2.2 Hz), 7.41 (2H, t, J=7.3 Hz), 7.37—7.28 (2H, m), 5.48 (1H, br d, J=51.6 Hz, H-4), 5.04—4.97 (1H, m, H-2), 4.36—4.16 (4H, m), 3.94—3.70 (2H, m, H-5), 2.60—2.30 (2H, m, H-3), 1.16 (9H, s), 1.09 (3H, d, J=6.2 Hz). MS (ESI pos.) m/z 516 $(M+Na)^+$.

9*H*-Fluoren-9-ylmethyl {(1*S*)-2-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-1-cyclohexyl-2-oxoethyl}carbamate (14j) Compound **13j** (860 mg, 1.74 mmol) was dissolved in THF (20 ml), and trifluoroacetic anhydride (0.49 ml, 3.48 mmol) was added while cooling on ice. The solution was then stirred on ice for 1 h. The reaction solution was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (developing solvent: hexane–EtOAc=3 : 1—1 : 2) to yield the desired product (760 mg, 92%) as a colorless amorphous powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.90—7.82 (3H, m), 7.72 (2H, d, J=7.3 Hz), 7.45—7.28 (5H, m), 5.49 (1H, br d, J=51.6 Hz, H-4), 5.04—4.97 (1H, m, H-2), 4.32—3.78 (6H, m), 2.60—2.25 (2H, m, H-3), 1.92—0.92 (11H, m). MS (ESI pos.) m/z 498 $(M+Na)^+$.

(2*S*,4*S*)-4-Fluoro-1-L-valylpyrrolidine-2-carbonitrile Hydrochloride (1b) MeOH (10.9 ml) and 4 M aqueous HCl solution (10.9 ml) were added to compound **12b** (1.70 g, 5.43 mmol) while cooling on ice, then stirred at room temperature overnight. Four molar aqueous NaOH solution (12 ml) and an excess amount of NaCl were added to the solution, and the resulting solution was extracted with EtOAc. The organic phase was washed with a saturated aqueous NaCl solution and dried over Na_2SO_4. After removing the drying agent using filtration, the filtrate was concentrated under reduced pressure to obtain (2*S*,4*S*)-4-fluoro-1-L-valylpyrrolidine-2-carbonitrile (800 mg, 70%), 600 mg of which was then dissolved in MeOH (1.2 ml) and 4 M HCl/EtOAc (0.77 ml) while cooling on ice, stirred for 1 h, and poured into diisopropyl ether (20 ml). The precipitated insoluble substance was collected using filtration to yield the desired product (750 mg) as a colorless powder. mp 256—259 °C (decomp.). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.57 (3H, br s), 5.55 (1H, br d, J=51.8 Hz, H-4), 5.06 (1H, d, J=9.2 Hz, H-2), 4.08—3.90 (2H, m, H-5), 3.83 (1H, d, J=7.3 Hz), 2.55—2.34 (2H, m, H-3), 2.17—2.07 (1H, m), 1.01 (3H, d, J=6.7 Hz), 0.98 (3H, d, J=6.7 Hz); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 168.2, 118.3, 92.9 (d, J_{C-F}=174.7 Hz), 55.2, 53.5 (d, J_{C-F}=21.7 Hz), 44.7, 35.4 (d, J_{C-F}=20.7 Hz), 29.9, 17.8, 17.7; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −175.2. MS (ESI pos.) m/z 214 $(M+H)^+$, 236 $(M+Na)^+$; (ESI neg.) m/z 248 $(M+Cl)^-$. HR-MS Calcd for $C_{10}H_{17}FN_3O$ $(M+H)^+$ 214.1356, Found (m/z) 214.1355. *Anal.* Calcd for $C_{10}H_{16}FN_3O$ HCl H_2O: C, 44.86; H, 7.15; N, 15.69; F, 7.10; Cl, 13.24. Found: C, 44.67; H, 7.09; N, 15.75; F, 6.97; Cl,12.90. $[\alpha]_D^{25}$ −68.2° (c=0.3, MeOH).

(2*S*,4*S*)-1-L-Alloisoleucyl-4-fluoropyrrolidine-2-carbonitrile Hydrochloride (1c) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.35 (3H, br s), 5.55 (1H, br d, J=51.0 Hz, H-4), 5.12—5.06 (1H, m, H-2), 4.02—3.80 (3H, m), 2.60—2.25 (2H, m, H-3), 1.92—1.80 (1H, m), 1.56—1.40 (1H, m), 1.34—1.15 (1H, m), 0.94 (3H, d, J=6.8 Hz), 0.91 (3H, t, J=7.3 Hz). MS (ESI pos.) m/z 228 $(M+H)^+$, 250 $(M+Na)^+$; (ESI neg.) m/z 262 $(M+Cl)^-$. HR-MS Calcd for $C_{11}H_{18}FN_3O$ $(M)^+$ 227.1434, Found (m/z) 227.1438.

(2*S*,4*S*)-4-Fluoro-1-(3-methyl-L-valyl)pyrrolidine-2-carbonitrile Hydrochloride (1d) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1b**. mp 265—269 °C (decomp.). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.54 (3H, br s), 5.55 (1H, br d, J=51.5 Hz, H-4), 5.07 (1H, d, J=9.8 Hz, H-2), 4.15—3.93 (2H, m, H-5), 3.79 (1H, s), 2.55—2.32 (2H, m, H-3), 1.05 (9H, s); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 167.8, 118.3, 92.9 (d, J_{C-F}=174.7 Hz), 57.5, 54.2 (d, J_{C-F}=22.8 Hz), 44.5, 35.4 (d, J_{C-F}=20.7 Hz), 34.0, 25.7; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −175.4. MS (ESI pos.) m/z 228 ($[M+H]^+$), 250 ($[M+Na]^+$); (ESI neg.) m/z 262 $(M+Cl)^-$. HR-MS Calcd for $C_{10}H_{16}FN_3O(M+H)^+$ 228.1512, found (m/z) 228.1513. *Anal.* Calcd for $C_{11}H_{18}FN_3O$ HCl H_2O: C, 46.89; H, 7.51; N, 14.91. Found: C, 46.74; H, 7.56; N, 14.78. $[\alpha]_D^{25}$ −44.4 (c=0.3, MeOH).

Benzyl {(5*S*)-5-Amino-6-[(2*S*,4*S*)-2-cyano-4-fluoropyrrolidin-1-yl]-6-oxohexyl}carbamate Hydrochloride (1e) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.42 (3H, br s), 7.42—7.21 (6H, m), 5.54 (1H, br d, J=51.0 Hz, H-4), 5.08 (1H, br d, J=8.2 Hz, H-2), 5.00 (2H, s), 4.06—3.66 (3H, m), 3.09—2.90 (2H, m), 2.62—2.26 (2H, m, H-3), 1.85—1.65 (2H, m), 1.50—1.25 (4H, m). MS (ESI pos.) m/z 399 $(M+Na)^+$; (ESI neg.) m/z 411 $(M+Cl)^-$, 375 $(M-H)^-$. HR-MS Calcd for $C_{19}H_{25}FN_4O_3$ $(M)^+$ 376.1911, Found (m/z) 376.1930.

(2*S*,4*S*)-4-Fluoro-1-(*O*-methyl-L-threonyl)pyrrolidine-2-carbonitrile Hydrochloride (1f) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1b**. mp 149—152 °C. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 8.62 (3H, br s), 5.54 (1H, br d, J=51.7 Hz, H-4), 5.07 (1H, d, J=8.8 Hz, H-2), 4.17—3.93 (3H, m), 3.70 (1H, quintet, J=6.4 Hz), 3.33 (3H, s), 2.55—2.33 (2H, m, H-3), 1.17 (3H, d, J=6.4 Hz); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 166.5, 118.2, 92.9 (d, J_{C-F}=174.7 Hz), 75.5, 56.5, 54.3, 53.4 (d, J_{C-F}=22.7 Hz), 44.9, 35.4 (d, J_{C-F}=19.6 Hz), 14.6; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −174.8. MS (ESI pos.) m/z 252 $(M+Na)^+$; (ESI neg.) m/z 264 $(M+Cl)^-$. HR-MS Calcd for $C_{10}H_{17}FN_3O_2$ $(M+H)^+$ 230.1305, Found (m/z) 230.1308. *Anal.* Calcd for $C_{10}H_{16}FN_3O_2$ HCl 1/3H_2O: C, 44.20; H, 6.55; N, 15.46; F, 6.99; Cl, 13.05. Found: C, 44.58; H, 6.77; N, 15.03; F, 6.87; Cl,12.71. $[\alpha]_D^{25}$ −80.8° (c=0.3, MeOH).

(2*S*,4*S*)-1-(*O*-Benzyl-L-threonyl)-4-fluoropyrrolidine-2-carbonitrile Hydrochloride (1g) The title compound was obtained as a brown powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.57 (3H, br s), 7.42—7.23 (5H, m), 5.53 (1H, br d, J=52.1 Hz, H-4), 5.12 (1H, br d, J=8.4 Hz, H-2), 4.62 and 4.56 (2H, ABq, J=11.8 Hz), 4.20—3.85 (4H, m), 2.60—2.30 (2H, m, H-3), 1.23 (3H, d, J=6.4 Hz). MS (ESI pos.) m/z 306 $(M+H)^+$, 328 $(M+Na)^+$; (ESI neg.) m/z 340 $(M+Cl)^-$. HR-MS Calcd for $C_{16}H_{21}FN_3O_2$ $(M+H)^+$ 306.1618, Found (m/z) 306.1615.

(2*S*,4*S*)-4-Fluoro-1-(*N*-methyl-L-isoleucyl)pyrrolidine-2-carbonitrile Hydrochloride (1k) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, CD_3OD) δ 5.48 (1H, br d, J=51.9 Hz, H-4), 5.12 (1H, br d, J=8.2 Hz, H-2), 4.19—3.80 (3H, m), 2.71—2.33 (2H, m, H-3), 2.68 (3H, s), 2.10—1.93 (1H, m), 1.79—1.63 (1H, m), 1.37—1.17 (1H, m), 1.13 (3H, d, J=7.0 Hz), 1.02 (3H, t, J=7.3 Hz). MS (ESI pos.) m/z 242 $(M+H)^+$, 264 $(M+Na)^+$; (ESI neg.) m/z 276 $(M+Cl)^-$.

(2*S*,4*S*)-4-Fluoro-1-L-prolylpyrrolidine-2-carbonitrile Hydrochloride (1l) The title compound was obtained as a pale orange powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 5.56 (1H, br d, J=51.6 Hz, H-4), 5.12—5.05 (1H, m, H-2), 4.37 (1H, t, J=7.3 Hz), 3.96—3.74 (2H, m, H-5), 2.62—2.32 (2H, m), 2.00—1.87 (2H, m), 1.85—1.71 (1H, m). MS (ESI pos.) m/z 212 $(M+H)^+$, 234 $(M+Na)^+$; (ESI neg.) m/z 246 $(M+Cl)^-$. HR-MS Calcd for $C_{10}H_{14}FN_3O$ $(M)^+$ 211.1121, Found (m/z) 211.1129.

(2*S*,4*S*)-4-Fluoro-1-[(3*S*)-1,2,3,4-tetrahydroisoquinolin-3-ylcarbonyl]pyrrolidine-2-carbonitrile Hydrochloride (1m) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.94 (2H, br s), 7.34—7.24 (4H, m), 5.59 (1H, br d, J=52.7 Hz, H-4), 5.19—5.13 (1H, m, H-2), 4.50 (1H, dd, J=12.1, 4.4 Hz), 4.32 (2H, s), 4.18—3.87 (2H, m, H-5), 3.40 (1H, dd, J=16.6, 4.4 Hz), 3.01 (1H, dd, J=16.6, 12.1 Hz), 2.63—2.34 (2H, m, H-3). MS (ESI pos.) m/z 274 $(M+H)^+$, 296 $(M+Na)^+$; (ESI neg.) m/z 272 $(M-H)^-$, 308 $(M+Cl)^-$. HR-MS Calcd for $C_{15}H_{17}FN_3O$

$(M+H)^+$ 274.1356, Found (*m/z*) 274.1339.

(2*S*,4*S*)-4-Fluoro-1-{[(3*S*)-7-hydroxy-1,2,3,4-tetrahydroisoquinolin-3-yl]carbonyl}pyrrolidine-2-carbonitrile Hydrochloride (1n) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.81 (2H, br s), 9.57 (1H, s), 7.09 (1H, d, J=8.4 Hz), 6.72 (1H, dd, J=8.4, 2.4 Hz), 6.65 (1H, d, J=2.4 Hz), 5.57 (1H, br d, J=52.1 Hz, H-4), 5.18—5.12 (1H, m, H-2), 4.43 (1H, dd, J=12.1, 4.2 Hz), 4.22 (2H, s), 4.14—3.87 (2H, m, H-5), 3.27 (1H, dd, J=16.2, 4.2 Hz), 2.88 (1H, dd, J=16.2, 12.1 Hz), 2.62—2.32 (2H, m, H-3). MS (ESI pos.) *m/z* 290 $(M+H)^+$, 312 $(M+Na)^+$; (ESI neg.) *m/z* 288 $(M-H)^-$, 324 $(M+Cl)^-$. HR-MS Calcd for $C_{15}H_{17}FN_3O_2$ $(M+H)^+$ 290.1305, Found (*m/z*) 290.1304.

(2*S*,4*S*)-1-{[(3*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinolin-3-yl]-carbonyl}-4-fluoropyrrolidine-2-carbonitrile Hydrochloride (1o) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.86 (2H, br s), 6.91 (1H, s), 6.87 (1H, s), 5.56 (1H, br d, J=52.4 Hz, H-4), 5.19—5.13 (1H, m, H-2), 4.44 (1H, dd, J=12.4, 4.4 Hz), 4.21 (2H, s), 4.15—3.85 (2H, m, H-5), 3.733 (3H, s), 3.730 (3H, s), 3.35—3.25 (1H, m), 2.91 (1H, dd, J=16.1, 12.4 Hz), 2.63—2.34 (2H, m, H-3). MS (ESI pos.) *m/z* 334 $(M+H)^+$, 356 $(M+Na)^+$; (ESI neg.) *m/z* 368 $(M+Cl)^-$. HR-MS Calcd for $C_{17}H_{21}FN_3O_3$ $(M+H)^+$ 334.1567, Found (*m/z*) 334.1566.

(2*S*,4*S*)-1-[*O*-(*tert*-Butyl)-L-threonyl]-4-fluoropyrrolidine-2-carbonitrile Hydrochloride (1h) Compound **14h** (710 mg, 1.44 mmol) was dissolved in THF (7 ml), diethylamine (0.7 ml) was added, and the solution was continuously stirred at room temperature for 2 h. The solution was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (developing solvent: $CHCl_3$–MeOH=100 : 3—100 : 5). The resulting residue was dissolved in diethyl ether (20 ml) and, after the addition of 4-M HCl/EtOAc (0.36 ml), the resulting salt was collected using filtration and dried *in vacuo* to yield the desired product (278 mg, 59%) as a colorless powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.38 (3H, br s), 5.53 (1H, br d, J=52.4 Hz, H-4), 5.09 (1H, br d, J=8.2 Hz, H-2), 4.33—3.83 (4H, m), 2.58—2.25 (2H, m, H-3), 1.18 (9H, s) , 1.15 (3H, d, J=6.2 Hz). MS (ESI pos.) *m/z* 294 $(M+Na)^+$; (ESI neg.) *m/z* 306 $(M+Cl)^-$. HR-MS Calcd for $C_{13}H_{23}FN_3O_2$ $(M+H)^+$ 272.1774, Found (*m/z*) 272.1793.

Benzyl 6-({5-[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]-(4*S*)-4-amino-5-oxopentanoyl}amino)hexanoate (1i) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1h**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.49 (3H, br s), 8.06 (1H, t, J=5.6 Hz), 7.42—7.29 (5H, m), 5.54 (1H, br d, J=51.9 Hz, H-4), 5.08 (2H, s), 5.08—5.04 (1H, m, H-2), 4.13—3.66 (3H, m), 3.10—2.96 (2H, m), 2.55—2.18 (6H, m), 2.00—1.83 (2H, m), 1.60—1.48 (2H, m), 1.45—1.34 (2H, m), 1.32—1.20 (2H, m). MS (ESI pos.) *m/z* 469 $(M+Na)^+$; (ESI neg.) *m/z* 481 $(M+Cl)^-$. HR-MS Calcd for $C_{23}H_{31}FN_4O_4$ $(M)^+$ 446.2329, Found (*m/z*) 446.2328.

(2*S*,4*S*)-1-[(2*S*)-2-Amino-2-cyclohexylacetyl]-4-fluoropyrrolidine-2-carbonitrile Hydrochloride (1j) The title compound was obtained as a colorless powder in a manner similar to method used to prepare **1h**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.48 (3H, br s), 5.54 (1H, br d, J=51.6 Hz, H-4), 5.06 (1H, d, J=8.7 Hz, H-2), 4.10—3.78 (3H, m), 2.60—2.26 (2H, m, H-3), 1.86—1.51 (6H, m), 1.30—1.00 (5H, m). MS (ESI pos.) *m/z* 276 $(M+Na)^+$; (ESI neg.) *m/z* 288 $(M+Cl)^-$. HR-MS Calcd for $C_{13}H_{20}FN_3O$ $(M)^+$ 253.1590, Found (*m/z*) 253.1605.

***tert*-Butyl (3*S*)-3-{[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]carbonyl}-7-methoxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (12p)** Compound **12n** (630 mg, 1.62 mmol) was dissolved in DMF (6 ml) and K_2CO_3 (250 mg, 1.81 mmol) and methyl iodide (0.15 ml, 2.41 mmol) were added; the solution was then stirred at room temperature overnight. The reaction solution was diluted with EtOAc and washed with 0.5 M HCl aqueous solution, $NaHCO_3$ solution and a saturated aqueous NaCl solution, successively. The organic phase was then dried over Na_2SO_4. After the removal of the drying agent using filtration, the solvent was evaporated *in vacuo* and diisopropyl ether was added to the residue. The resulting precipitate was collected using filtration and dried *in vacuo* to yield the desired product (420 mg, 64%) as a pale yellow powder. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.22—7.10 (1H, m), 6.88—6.73 (2H, m), 5.56 (1H, br d, J=52.5 Hz, H-4), 5.06—3.78 (6H, m), 3.73 (3H, s), 3.20—2.25 (4H, m), 1.43, 1.37 and 1.33 (total 9H, each s). MS (ESI pos.) *m/z* 426 $(M+Na)^+$.

***tert*-Butyl (3*S*)-7-(2-Amino-2-oxoethoxy)-3-{[(2*S*,4*S*)-2-cyano-4-fluoropyrrolidin-1-yl]carbonyl}-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (12q)** The title compound (550 mg, 80%) was obtained as a colorless powder from **12n** (600 mg, 1.54 mmol) and 2-bromoacetamide (320 mg, 2.27 mmol) in a manner similar to method used to prepare **12p**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.47 (1H, br s), 7.36 (1H, br s), 7.23—7.11 (1H, m), 6.88—6.75 (2H, m), 5.55 (1H, br d, J=53.9 Hz, H-4), 5.07—4.46 (3H, m), 4.39 (2H, s), 4.35—3.60 (3H, m), 3.18—3.02 (1H, m), 2.94—2.70 (1H, m), 2.58—2.20 (2H, m), 1.43, 1.38 and 1.33 (total 9H, each s). MS (ESI pos.) *m/z* 469 $(M+Na)^+$; (ESI neg.) *m/z* 445 $(M-H)^-$.

(2*S*,4*S*)-4-Fluoro-1-{[(3*S*)-7-methoxy-1,2,3,4-tetrahydroisoquinolin-3-yl]carbonyl}pyrrolidine-2-carbonitrile Hydrochloride (1p) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.96 (2H, br s), 7.21 (1H, d, J=9.3 Hz), 6.90—6.85 (2H, m), 5.58 (1H, br d, J=52.3 Hz, H-4), 5.19—5.13 (1H, m, H-2), 4.46 (1H, dd, J=12.2, 4.3 Hz), 4.28 (2H, s), 4.16—3.87 (2H, m, H-5), 3.74 (3H, s), 3.34 (1H, dd, J=16.4, 4.3 Hz), 2.92 (1H, dd, J=16.4, 12.2 Hz), 2.63—2.33 (2H, m, H-3). MS (ESI pos.) *m/z* 304 $(M+H)^+$, 326 $(M+Na)^+$; (ESI neg.) *m/z* 338 $(M+Cl)^-$. HR-MS Calcd for $C_{16}H_{19}FN_3O_2$ $(M+H)^+$ 304.1461, Found (*m/z*) 304.1456.

2-[((3*S*)-3-{[(2*S*,4*S*)-2-Cyano-4-fluoropyrrolidin-1-yl]carbonyl}-1,2,3,4-tetrahydroisoquinolin-7-yl)oxy]acetamide Hydrochloride (1q) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1b**. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 9.78 (2H, br s), 7.52 (1H, br s), 7.39 (1H, br s), 7.23 (1H, d, J=8.4 Hz), 6.94—6.85 (2H, m), 5.58 (1H, br d, J=52.1 Hz, H-4), 5.19—5.13 (1H, m, H-2), 4.47 (1H, dd, J=11.8, 4.0 Hz), 4.42 (2H, s), 4.27 (2H, s), 4.16—3.84 (2H, m, H-5), 3.41—3.25 (1H, m), 2.92 (1H, dd, J=16.6, 12.4 Hz), 2.60—2.34 (2H, m, H-3). MS (ESI pos.) *m/z* 347 $(M+H)^+$, 369 $(M+Na)^+$; (ESI neg.) *m/z* 345 $(M-H)^-$, 381 $(M+Cl)^-$. HR-MS Calcd for $C_{17}H_{20}FN_4O_3$ $(M+H)^+$ 347.1519, Found (*m/z*) 347.1527.

(2*S*,4*R*)-4-Fluoro-1-(3-methyl-L-valyl)pyrrolidine-2-carbonitrile (15) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1d**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 5.37 (1H, br d, J=52.1 Hz, H-4), 4.75 (1H, dd, J=9.4, 7.9 Hz, H-2), 4.13 (1H, ddd, J=21.1, 12.7, 2.0 Hz, H-5), 3.72 (1H, ddd, J=38.5, 12.6, 2.8 Hz, H-5), 3.22 (1H, s), 2.73—2.63 (1H, m, H-3), 2.55—2.40 (1H, m, H-3), 1.63 (2H, br s), 0.91 (9H, s); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 174.2, 118.5, 91.8 (d, J_{C-F}=176.7 Hz), 59.2, 53.3 (d, J_{C-F}=21.7 Hz), 44.3, 35.4 (d, J_{C-F}=21.7 Hz), 35.2, 25.9; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −176.9. MS (ESI pos.) *m/z* 250 $(M+Na)^+$; (ESI neg.) *m/z* 226 $(M-H)^-$. HR-MS Calcd for $C_{11}H_{19}FN_3O$ $(M+H)^+$ 228.1512, Found (*m/z*) 228.1517. *Anal.* Calcd for $C_{11}H_{18}FN_3O$: C, 58.13; H, 7.98; N, 18.49; F, 8.36. Found: C, 57.93; H, 8.00; N, 18.34; F, 8.60. $[\alpha]_D^{25}$ −60.1° (c=0.3, MeOH).

(2*R*,4*R*)-4-Fluoro-1-(3-methyl-L-valyl)pyrrolidine-2-carbonitrile (17) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1d**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 5.45 (1H, br d, J=48.8 Hz, H-4), 4.96—4.88 (1H, m, H-2), 4.08 (1H, dd, J=25.0, 12.2 Hz, H-5), 3.73 (1H, ddd, J=38.8, 12.2, 3.4 Hz, H-5), 3.12 (1H, s), 2.58—2.35 (2H, m, H-3), 1.70 (2H, br s), 0.89 (9H, s); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 174.2, 118.8, 93.1 (d, J_{C-F}=174.3 Hz), 59.3, 53.2 (d, J_{C-F}=22.7 Hz), 44.4, 35.6 (d, J_{C-F}=20.6 Hz), 34.7, 26.0; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −174.3. MS (ESI pos.) *m/z* 250 $(M+Na)^+$; (ESI neg.) *m/z* 226 $(M-H)^-$. HR-MS Calcd for $C_{11}H_{19}FN_3O$ $(M+H)^+$ 228.1512, Found (*m/z*) 228.1515. *Anal.* Calcd for $C_{11}H_{18}FN_3O$: C, 58.13; H, 7.98; N, 18.49; F, 8.36. Found: C, 58.01; H, 8.04; N, 18.45; F, 8.35. $[\alpha]_D^{25}$ +163.4° (c=0.3, MeOH).

(2*S*,4*R*)-4-Fluoro-1-(3-methyl-D-valyl)pyrrolidine-2-carbonitrile (19) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1d**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 5.39 (1H, br d, J=52.1 Hz, H-4), 4.79 (1H, t, J=8.6 Hz, H-2), 4.08 (1H, ddd, J=20.3, 13.0, 1.8 Hz, H-5), 3.79 (1H, ddd, J=37.8, 12.8, 3.1 Hz, H-5), 3.19 (1H, s), 2.73—2.63 (1H, m, H-3), 2.53—2.38 (1H, m, H-3), 1.68 (2H, br s), 0.90 (9H, s); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 174.2, 118.7, 91.9 (d, J_{C-F}=175.7 Hz), 59.0, 52.9 (d, J_{C-F}=21.7 Hz), 44.3, 35.6 (d, J_{C-F}=21.7 Hz), 34.6, 25.9; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −178.0. MS (ESI pos.) *m/z* 250 $(M+Na)^+$; (ESI neg.) *m/z* 226 $(M-H)^-$. HR-MS Calcd for $C_{11}H_{19}FN_3O$ $(M+H)^+$ 228.1512, Found (*m/z*) 228.1514. *Anal.* Calcd for $C_{11}H_{18}FN_3O$: C, 58.13; H, 7.98; N, 18.49; F, 8.36. Found: C, 57.99; H, 8.05; N, 18.42; F, 8.39. $[\alpha]_D^{25}$ −190.0° (c=0.3, MeOH).

(2*R*,4*R*)-4-Fluoro-1-(3-methyl-D-valyl)pyrrolidine-2-carbonitrile (21) The title compound was obtained as a colorless amorphous powder in a manner similar to method used to prepare **1d**. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 5.47 (1H, br d, J=52.5 Hz, H-4), 4.98 (1H, br d, J=5.5 Hz, H-2), 3.98 (1H, dd like, J=25.7, 12.5 Hz, H-5), 3.82 (1H, ddd, J=39.6, 12.5, 3.3 Hz, H-5), 3.14 (1H, s), 2.48—2.33 (2H, m, H-3), 1.60 (2H, br s), 0.93 (9H, s); ^{13}C-NMR (125.4 MHz, DMSO-d_6) δ 174.1, 118.8, 93.2 (d, J_{C-F}=174.3 Hz), 58.9, 53.4 (d, J_{C-F}=21.7 Hz), 44.1, 35.3 (d, J_{C-F}=20.6 Hz), 35.0, 25.8; ^{19}F-NMR (282.2 MHz, DMSO-d_6) δ −174.5. MS (ESI pos.) *m/z* 250 $(M+Na)^+$;

(ESI neg.) m/z 226 $(M-H)^-$. HR-MS Calcd for $C_{11}H_{19}FN_3O$ $(M+H)^+$ 228.1512, Found (m/z) 228.1517. *Anal.* Calcd for $C_{11}H_{18}FN_3O$: C, 58.13; H, 7.98; N, 18.49; F, 8.36. Found: C, 57.78; H, 8.06; N, 18.42; F, 8.38. $[\alpha]_D^{25}$ +33.7° (c=0.3, MeOH).

X-Ray Crystal Analysis Single crystals of **22** (free base of **1d**) that were suitable for X-ray crystallography were grown by crystallization from chloroform/ethyl acetate. Data were collected at 288 K on a Mac Science/Bruker axs MXC18 four-circle automated diffractometer using Cu$K\alpha$ radiation (λ=1.54178). The compound with the chemical formula of $C_{11}H_{18}F_1N_3O_1$ crystallized in the monoclinic space group $P2_1$, a=9.642(2), b=10.662(2), c=6.1103(11) Å, β=106.793(13)°, V=601.4(2) Å^3, Z=2. The structure was solved by a direct method using the program *maXus* and was refined using the full-matrix least square method. All H-atom positions were located from a difference Fourier synthesis. All non-hydrogen atoms were refined anisotropically, and all H-atoms were refined isotropically. The final R and Rw values were 0.039 and 0.068, respectively.

DPP-IV Inhibitory Activity The inhibition of DPP-IV activity was tested using a method described by Deacon *et al.*[23] Plasma containing DPP-IV was prepared by centrifuging blood collected from healthy human volunteers. Enzyme reactions were carried out using 96-flat-bottom-well plates in a buffer solution of pH 7.8 containing 25 mM HEPES, 140 mM NaCl, and 1% BSA. To a mixture of 25 μl of 100 μM Gly-Pro-4-methylcoumaryl-7-amide solution (manufactured by Peptide Institute, Inc.), 7.5 μl of 133 mM $MgCl_2$ solution, 5 μl of the test compound, and 12.5 μl of plasma diluted to 1/100 with the above buffer solution were added. The solution was allowed to react at room temperature for 2 h, and 50 μl of 25% aqueous acetic acid solution was added to stop the reaction. The fluorescence intensity of the liberated 7-amino-4-methylcoumarin was determined using a fluorescence plate reader (1420 ARVO^{TM} Multilabel Counter manufactured by Wallac Oy; Excitation: 390 nm; Emission: 460 nm).

Oral Glucose Tolerance Test (OGTT) in Zucker Fatty Rats An OGTT in Zucker fatty rats was carried out based on the method described by Balkan *et al.*[24] Food was withheld overnight from male Zucker fatty and lean rats (10 weeks of age; n=6). Compound **1d** was then dissolved in distilled water and administered orally. After 30 min, a glucose solution (2 g/kg body weight) was orally administered. Blood samples were collected from the orbital venous sinus under ether anesthesia at the indicated times, and plasma samples were prepared. The plasma glucose concentration, plasma insulin concentration, and plasma DPP-IV activity were measured.

Acknowledgment The authors wish to thank the following individuals for their assistance with this work: Keita Matsumoto, Atsushi Okada, Noriko Takahashi, Ayumi Koda, and Miho Kawanishi.

References

1) Heins J., Weiker P., Schonlein C., Born I., Hartrodt B., Neubert K., Tsuru D., Barth A., *Biochim. Biophys. Acta*, **954**, 161—169 (1988).
2) Hegen M., Niedobitek G., Klein C. E., Stein H., Fleischer B., *J. Immunol.*, **144**, 2908—2914 (1990).
3) Yaron A., Nadier F., *Crit. Rev. Biochem. Mol. Biol.*, **28**, 31—81 (1993).
4) Drucker D., *J. Endocrinol.*, **142**, 521—527 (2001).
5) Lovshin J., Drucker D. J., *Regul. Pept.*, **90**, 27—32 (2000).
6) Holst J. J., *Curr. Med. Chem.*, **6**, 1005—1017 (1999).
7) Zhang B. B., Moller D. E., *Curr. Opin. Chem. Biol.*, **4**, 461—467 (2000).
8) Deacon C. F., Holst J. J., Carr R. D., *Drugs Today*, **35**, 159—170 (1999).
9) Holst J. J., Deacon C. F., *Diabetes*, **47**, 1663—1670 (1998).
10) Weber A. E., *J. Med. Chem.*, **47**, 4135—4141 (2004).
11) Juana F., Valdeolmillos M., *Diabetes*, **48**, 754—757 (1999).
12) Villhauer E. B., Brinkman J. A., Naderi G. B., Dunning B. E., Mangold B. L., Mone M. D., Russell M. E., Weldon S. C., Hughes T. E., *J. Med. Chem.*, **45**, 2362—2365 (2002).
13) Villhauer E. B., Brinkman J. A., Naderi G. B., Burkey B. F., Dunning B. E., Prasad K., Mangold B. L., Russell M. E., Hughes T. E., *J. Med. Chem.*, **46**, 2774—2789 (2003).
14) Augeri D. J., Robl J. A., Betebenner D. A., Magnin D. R., Khanna A., Robertson J. G., Wang A., Simpkins L. M., Taunk P., Huang Q., Han S. P., Abboa-Offei B., Cap M., Xin L., Tao L., Tozzo E., Welzel G. E., Egan D. M., Marcinkeviciene J., Chang S. Y., Biller S. A., Kirby M. S., Parker R. A., Hamann L. G., *J. Med. Chem.*, **48**, 5025—5037 (2005).
15) Kim D., Wang L., Beconi M., Eiermann G. J., Fisher M. H., He H., Hickey G. J., Kowalchick J. E., Leiting B., Lyons K., Marsilio F., McCann M. E., Patel R. A., Petrov A., Scapin G., Patel S. B., Roy R. S., Wu J. K., Wyvratt M. J., Zhang B. B., Zhu L., Thornberry N. A., Weber A. E., *J. Med. Chem.*, **48**, 141—151 (2005).
16) Fukushima H., Hiratate A., Takahashi M., Saito M., Munetomo E., Kitano K., Saito H., Takaoka Y., Yamamoto K., *Bioorg. Med. Chem.*, **12**, 6053—6061 (2004).
17) Ashworth D. M., Atrash B., Baker G. R., Baxter A. J., Jenkins P. D., Jones D. M., Szelke M., *Bioorg. Med. Chem. Lett.*, **6**, 1163—1166 (1996).
18) Magnin D. R., Robl J. A., Sulsky R. B., Augeri D. J., Huang Y., Simpkins L. M., Taunk P. C., Betebenner D. A., Robertson J. G., Abboa-Offei B. E., Wang A., Cap M., Xin L., Tao L., Sitkoff D. F., Malley M. F., Gougoutas J. Z., Khanna A., Huang Q., Han S., Parker R. A., Hamann L. G., *J. Med. Chem.*, **47**, 2587—2598 (2004).
19) Demange L., Menez A., Dugave C., *Tetrahedron Lett.*, **39**, 1169—1172 (1998).
20) Maetz P., Rodriguez M., *Tetrahedron Lett.*, **38**, 4221—4222 (1997).
21) Rasmussen H. B., Branner S., Wiberg F. C., Wagtmann N., *Nat. Struct. Biol.*, **10**, 19—25 (2003).
22) Hiramatsu H., Kyono K., Higashiyama Y., Fukushima C., Shima H., Sugiyama S., Inaka K., Yamamoto A., Shimizu R., *Biochem. Biophys. Res. Commun.*, **302**, 849—854 (2003).
23) Deacon C. F., Hughes T. E., Holst J. J., *Diabetes*, **47**, 764—769 (1998).
24) Balkan B., Kwasnik L., Miserendino R., Holst J. J., Li X., *Diabetologia*, **42**, 1324—1331 (1999).