Synthesis of Novel Potent Dipeptidyl Peptidase IV Inhibitors with Enhanced Chemical Stability: Interplay between the N-Terminal Amino Acid Alkyl Side Chain and the Cyclopropyl Group of α-Aminoacyl-L-*cis*-4,5-methanoprolinenitrile-Based Inhibitors

David R. Magnin,[†] Jeffrey A. Robl,*[,†] Richard B. Sulsky,[†] David J. Augeri,[†,⊥] Yanting Huang,[†] Ligaya M. Simpkins,[†] Prakash C. Taunk,[†] David A. Betebenner,[†,#] James G. Robertson,[‡] Benoni E. Abboa-Offei,[‡] Aiying Wang,[‡] Michael Cap,[‡] Li Xin,[‡] Li Tao,[◆] Doree F. Sitkoff,[∞] Mary F. Malley,[○] Jack Z. Gougoutas,[○] Ashish Khanna,[§] Qi Huang,[‡] Song-Ping Han,[‡] Rex A. Parker,[‡] and Lawrence G. Hamann*[,†]

Departments of Discovery Chemistry, Metabolic Research, Exploratory Pharmaceutics, Computer-Assisted Drug Design, Solid State Chemistry, and Pharmaceutical Candidate Optimization, Bristol-Myers Squibb, Pharmaceutical Research Institute, P.O. Box 5400, Princeton, New Jersey 08543-5400

Received January 27, 2004

A series of methanoprolinenitrile-containing dipeptide mimetics were synthesized and assayed as inhibitors of the N-terminal sequence-specific serine protease dipeptidyl peptidase IV (DPP-IV). The catalytic action of DPP-IV is the principle means of degradation of glucagon-like peptide-1, a key mediator of glucose-stimulated insulin secretion, and DPP-IV inhibition shows clinical benefit as a novel mechanism for treatment of type 2 diabetes. However, many of the reversible inhibitors to date suffer from chemical instability stemming from an amine to nitrile intramolecular cyclization. Installation of a cyclopropyl moiety at either the 3,4- or 4,5-position of traditional 2-cyanopyrrolidide proline mimetics led to compounds with potent inhibitory activity against the enzyme. Additionally, *cis*-4,5-methanoprolinenitriles with *β*-branching in the N-terminal amino acid provided enhanced chemical stability and high inhibitory potency. This class of inhibitors also exhibited the ability to suppress prandial glucose elevations after an oral glucose challenge in male Zucker rats.

Introduction

With the spread of Western lifestyles, the prevalence of type 2 diabetes in the world's population is rising.[1] Current treatment strategies include reducing insulin resistance using glitazones,[2] supplementing insulin supplies with exogenous insulin,[3] increasing insulin secretion with sulfonylureas,[4] reducing hepatic glucose output with biguanides,[5] and limiting glucose absorption with glucosidase inhibitors.[6] Promising new targets for drug development are also emerging. Of particular interest is the pharmacology surrounding the incretin hormone glucagon-like peptide 1 (GLP-1).[7] GLP-1 is known to function as a mediator of glucose-stimulated insulin secretion, and several clinical studies have shown that administration of the peptide or its analogues results in antidiabetic action in subjects with type 2 diabetes.[8] Although GLP-1 is secreted as GLP-1 (7−36) amide from the small and large intestines in response to dietary signals, it is rapidly truncated to GLP-1 (9−36) by cleavage of the N-terminal dipeptide residues. The truncated metabolite has antagonist activity against the GLP-1 receptor both in vitro and in vivo.[9] The principle enzyme responsible for the cleavage of GLP-1 (7−36) amide to GLP-1 (9−36) amide is dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5), a nonclassical, sequence-specific 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).[10] Inhibition of DPP-IV has been shown to be effective at sustaining circulating levels of GLP-1 (7−36) and therefore offers a new therapeutic approach for the treatment of type 2 diabetes.[11]

Early reports of DPP-IV inhibitors included proline-based dipeptide mimics bearing boronic acid[12] (**1**) or diphenyl phosphonate substituents (**2**).[13] These compounds were irreversible inhibitors of DPP-IV or were slow to dissociate from the enzyme. Several first-generation dipeptide surrogates have been disclosed as reversible inhibitors of DPP-IV, including both C-substituted (**3**) and N-substituted (**4**) glycinylprolinenitrile dipeptide analogues.[14,15] These compound classes include many potent inhibitors of the enzyme, but all suffer from chemical instability whereby the N-terminal amine intramolecularly cyclizes onto the nitrile, forming inactive cyclic imidates and/or their diketopiperazine hydrolysis products. However, more recent publications have disclosed a series of more hindered *N*-alkylamines (**5**) that have much greater chemical stability.[16] Thia-

* To whom correspondence should be addressed. For J.A.R.: telephone, 609-818-5048; fax, 609-818-3550; e-mail, jeffrey.robl@bms.com. For L.G.H.: telephone, 609-818-5526; fax, 609-818-3550; e-mail: lawrence.hamann@bms.com.
[†] Department of Discovery Chemistry.
[⊥] Present address: Lexicon Pharmaceuticals, 350 Carter Road, Princeton, NJ 08540.
[#] Present address: Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.
[◆] Department of Exploratory Pharmaceutics.
[∞] Department of Computer-Assisted Drug Design.
[○] Department of Solid State Chemistry.
[§] Department of Pharmaceutical Candidate Optimization.
[‡] Department of Metabolic Research.

10.1021/jm049924d CCC: $27.50
Published on Web 04/13/2004

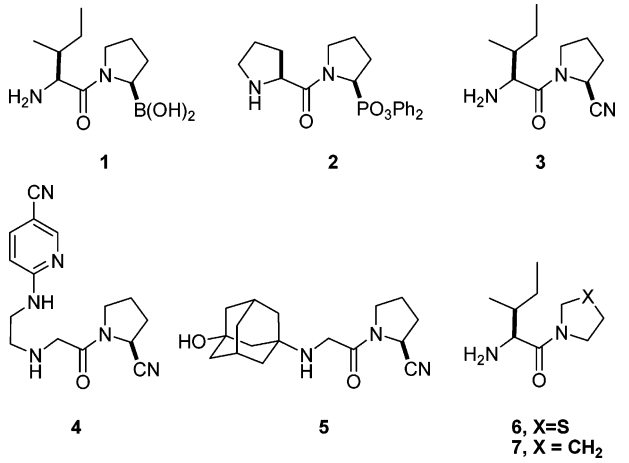


Figure 1. Known proline-derived dipeptidyl peptidase IV inhibitors.

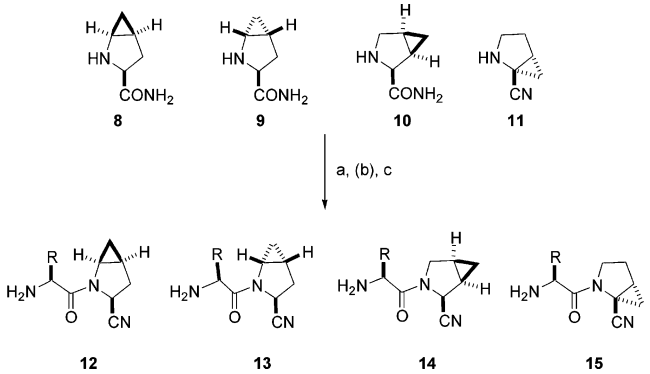
zolidide (**6**) and pyrrolidide (**7**) based inhibitors have also been disclosed that lack the nitrile moiety and thus possess greater stability, though at the cost of reduced potency[17a−c] with a few very recent notable exceptions.[17d−f] These compounds are shown in Figure 1.

We endeavored to synthesize novel dipeptide surrogates containing a proline mimic linked to an N-terminal amino acid that would act as reversible inhibitors of DPP-IV with maximum potency and enhanced solution stability. Hanessian has reported that installation of a 4,5-methano moiety into a proline residue has the effect of flattening the five-membered ring,[18] and one might extend a similar conformational argument to other cyclopropanated prolines as well. By inference, the cyclopropane bridge may occupy the space-filling region that would normally be given to the methylene group in the puckered or "envelope" conformation of a typical five-member ring. With this precedent we sought to establish whether a methanoproline derivative could serve as a viable proline surrogate in a DPP-IV inhibitor. Our strategy was to prepare dipeptide surrogates containing a cyclopropanated prolinenitrile derived from L-proline. The regio- and stereochemical disposition of the cyclopropane bridge was varied in order to identify a compound with maximal stability and potency. Herein, we report the discovery of L-*cis*-4,5-methanoprolinenitrile dipeptides as potent inhibitors of DPP-IV with increased chemical stability and high potency. In addition, we also present data demonstrating that these novel DPP-IV inhibitors effectively lower plasma glucose after a glucose challenge in rodent models.

Chemistry

Dipeptides (**12**−**15**) composed of N-terminal isoleucine appended to cyclopropylprolineamides or nitriles derived from L-proline (**8**−**11**) were targeted to probe the potential for this type of inhibitor scaffold. Isoleucine was selected as the N-terminal residue because it was the most potent natural amino acid reported in the 2-cyanopyrrolidide series.[14a] These inhibitors were expected to provide a dependable inhibitory benchmark for the differing methanoproline structures. The synthetic routes used to prepare the dipeptides derived from L-*cis*- and L-*trans*-4,5-methanoproline,[18b] L-*cis*-3,4-methanoproline,[19] and L-2,3-methanoproline[20] are shown in Scheme 1.

Scheme 1[a]



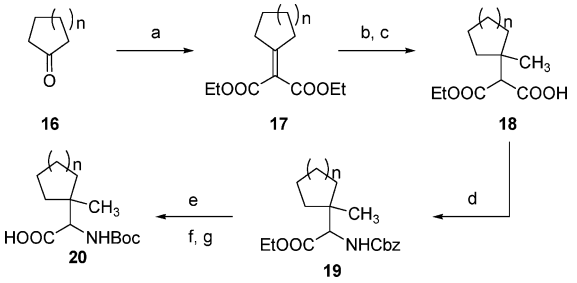
[a] (a) *N*-Boc-amino acid, EDAC, DMAP or PyBop, 50−90%; (b) (for intermediates **8**−**10**) $POCl_3$, pyridine, imidazole, 70−90%; (c) TFA, CH_2Cl_2, TFA or HCl in Et_2O or EtOAc, 70−90%.

Standard conditions were employed to couple the enantiomerically pure L-*cis*-4,5-cyclopropylprolineamide nucleus (**8**) to give the corresponding Boc-protected dipeptides in good to excellent yield.[21] Dehydration[14b,22] ($POCl_3$, pyridine, imidazole) of the amide and removal[23] of the N-terminal Boc protecting group (TFA or HCl) gave the proline dipeptide (**12**) in high yield. Similar protocols were used for the L-*cis*-3,4-methanoproline fragment **10** and the L-4,5-*trans*-methanoproline compound **9**. The known (−)-(2*S*,3*R*)-methanoprolinenitrile (**11**) was prepared according to the method of Hercout.[20a] In this instance, the dehydration reaction was performed prior to introduction of the isoleucine fragment.

Preparation of a library was undertaken to probe structure−activity relationships between the N-terminal amino acid residue and the *cis*-4,5-methanoprolinenitriles. These inhibitors were generated in a three-step sequence in parallel array format in a manner similar to that described in Scheme 1. Initial reaction of the Boc-protected amino acid with methanoprolineamide, EDAC, and DMAP in dichloromethane, and subsequent purification through an SCX ion exchange cartridge, gave good to excellent yields of coupled dipeptides. Dehydration and acid-promoted deprotection (TFA in dichloromethane) yielded the inhibitors as TFA salts. Further purification of the final products was easily accomplished by preparative reverse-phase HPLC or by trituration with Et_2O.

Holmberg has prepared *tert*-alkylmalonic acid derivatives through a $TiCl_4$-mediated Knoevenagel process and subsequent copper-assisted conjugate addition of a Grignard reagent or conjugate reduction. Following this method (Scheme 2), diesters can be converted to the protected amino acids in three steps.[24] This methodology provided an approach to the cycloalkylglycine amino acid derivatives **20**. First, the malonic acid diesters **17** were subjected to conjugate addition of a methyl group or hydride and then hydrolyzed to the corresponding monoacids **18**. Subsequent Curtius rearrangement of **18** by treatment with diphenylphosphoryl azide, followed by trapping of the intermediate isocyanate with benzyl alcohol, provided the Cbz adducts **19**. Finally, the esters were hydrolyzed to give the amino acids **20** in racemic form. The racemic Boc-protected acids **20** were then coupled to the L-*cis*-4,5-methanoprolineamide group.

Scheme 2[a]



[a] (a) $TiCl_4$, THF, CCl_4, diethyl malonate, 0 °C, then pyridine, 0 °C to room temp, 68%; (b) MeMgI, CuCl, Et_2O, 0 °C, 69%; (c) NaOH, THF, EtOH, 78%; (d) (i) diphenylphosphorylazide, NEt_3, PhH, reflux; (ii) BnOH, reflux, 18 h, 100%; (e) H_2, $Pd(OH)_2$, EtOAc, 100%; (f) $(Boc)_2O$, K_2CO_3, THF, H_2O, 92%, two steps; (g) NaOH, MeOH, THF, room temp, then aqueous HCl, 95%.

Processing as previously described (Scheme 1) gave a mixture of diastereomers that were separated at the nitrile stage by silica gel flash chromatography. The later-eluting isomer in each case was consistently identified as the desired L,L-dipeptide.[25]

Results and Discussion

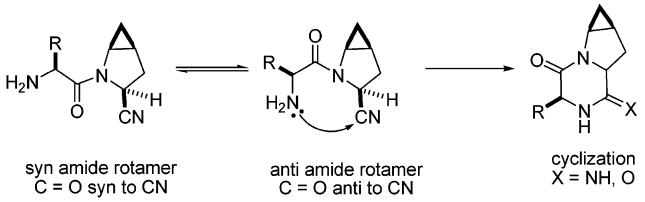
All compounds were tested in vitro against purified porcine DPP-IV. Inhibition was determined against the substrate H-Ala-Pro-*p*NA. Production of *p*-nitroaniline (*p*NA) was measured at 405 nm over 15 min. A comparison of methanoproline-derived isoleucine N-terminal dipeptides shows that the enzyme inhibitory activity is critically dependent on both the position and the orientation of the methano bridge (Table 1). The presence of the methano bridge on the trans side of the prolinenitrile (e.g., **22**, **23**) resulted in a significant loss of activity relative to the unsubstituted prolinenitrile **21**. This result was consistent with early reports where insertion of a methyl group at the 2-postion of 2-cyanopyrrolidides resulted in a 2000-fold decrease in activity.[26] In contrast, when the methano bridge was oriented cis to the nitrile at either the 4,5- or 3,4-position, inhibitory activity was only slightly diminished. This is clearly illustrated with *cis*-4,5-methano inhibitors **24** and **26** and *cis*-3,4-methano inhibitors **25** and **27**, where inhibitory potency resides in the 20 nM range. A more complete exploration of N-terminal amino acids reveals that increasing the degree of β-branching to that of *tert*-Leu (e.g., **29**) further increases potency in the 4,5-methano series to that of the prolinenitrile version (**28**). Replacement of these alkyl substituents with aromatic residues such as Phe (**31**) or Trp (**32**) in the N-terminal position significantly eroded potency. Alkyl substitution on the terminal amine was generally ill-tolerated in this series (e.g., **33**−**35**), though it would appear that the relatively more accessible N-terminus found in proline derivative **34** is capable of restoring a modest degree of inhibitory activity. Replacement of Leu and *tert*-Leu side chains with medium-ring cycloalkyl and methylcycloalkyl (**37**−**42**) generally led to inhibitors with comparable or slightly improved activity, though potency dropped steadily with increasing larger ring size (data not shown). The Cp-Gly and Me-Cp-Gly derived analogues **38** and **41** were among the most potent compound prepared in this series, exhibiting K_i values of 4 and 7 nM, respectively.

Table 1. In Vitro Inhibition Constants for Porcine DPP-IV and Solution Stability Half-Lives for Prolinenitrile DPP-4 Inhibitors

compd	N-terminal amino acid[a]	substituted prolinenitrile	K_i (nM)[b]	stability, $t_{1/2}$ (h)[c]
21	Ile	prolinenitrile	2 ± 0.5	5
22	Ile	*trans*-4,5-	1620 ± 80	
23	Ile	*trans*-2,3-	7500 ± 200	
24	Ile	*cis*-4,5-	25 ± 1	22
25	Ile	*cis*-3,4-	15 ± 1	4
26	Val	*cis*-4,5-	29 ± 1	28
27	Val	*cis*-3,4-	12 ± 1	2
28	*tert*-Leu	prolinenitrile	8 ± 0.5	27
29	*tert*-Leu	*cis*-4,5-	7 ± 0.5	42
30	*tert*-Leu	*cis*-3,4-	14 ± 1	4
31	Phe	*cis*-4,5-	65 ± 3	
32	Trp	*cis*-4,5-	230 ± 10	
33	*N*-Me-Val	*cis*-4,5-	1940 ± 80	
34	Pro	*cis*-4,5-	107 ± 5	
35	Pip	*cis*-4,5-	10000	
36	Met	*cis*-4,5-	135 ± 10	
37	Cb-Gly	*cis*-4,5-	12 ± 0.5	
38	Cp-Gly	*cis*-4,5-	4 ± 0.5	
39	Ch-Gly	*cis*-4,5-	15 ± 1	19
40	(1-Me-Cb-1-yl)-Gly	*cis*-4,5-	11 ± 0.5	
41	(1-Me-Cp-1-yl)-Gly	*cis*-4,5-	7 ± 0.5	24
42	(1-Me-Ch-1-yl)-Gly	*cis*-4,5-	8 ± 0.5	

[a] Cb = cyclobutyl; Cp = cyclopentyl; Ch = cyclohexyl. [b] Values represent the mean ± SEM and are at least triplicate determinations. [c] Solution stability data are measured at 39.5 °C and pH 7.2 in phosphate buffer.

Scheme 3



Solution Stability. After the discovery that the L-*cis*-4,5- and L-*cis*-3,4-methanoprolinenitriles were potent inhibitors of DPP-IV in vitro, a comparative study was initiated to investigate aqueous solution stability of these analogues. The N-terminal valine, isoleucine, and *tert*-leucine dipeptide nitriles were selected in order to make direct comparisons between methanoprolinenitrile and unsubstituted prolinenitrile compounds with respect to solution stability. Reaction rates were monitored by following the disappearance of starting material on reverse-phase HPLC at pH 7.2 and 39.5 °C in phosphate buffer. HPLC mass spectral analysis revealed that the two major products that formed during the stability experiments had either an identical mass or an M + 1 mass to the parent starting material. These data are consistent with the formation of intramolecular cyclization products, with the initial cyclic imidate (X = NH) surrendering to the diketopiperazine (X = O) upon hydrolysis (Scheme 3).

Two interesting aspects of the solution half-life data should be noted. The first is that there is increased solution stability associated with the *cis*-4,5-methanoprolinenitrile derivatives when compared to either the

cis-3,4-methanoprolinenitrile or the unadorned prolinenitrile compounds. For instance, in the case where the N-terminal amino acid is isoleucine, the isomeric 4,5-methano-substituted compound **24** has a solution half-life of 22 h, which is 5.5-fold longer than the analogous 3,4-methano-substituted compound **25** and approximately 4.5-fold longer than the unadorned prolinenitrile analogue **21**. The second feature is that there is a strong correlation between steric size at the β-position of the N-terminal amino acid and relative solution stability. Increasing the degree of branching at the β-position of the alkylglycine substituent increases solution stability. The most stable compounds within their respective prolinenitrile series have the *tert*-Leu N-terminal amino acid fragment in common. For example, unadorned prolinenitrile **28** ($t_{1/2}$ = 27 h) is more stable than its less branched isomer **21** ($t_{1/2}$ = 5 h). Similarly, *cis*-4,5-methanoprolinenitrile **29** ($t_{1/2}$ = 42 h) is more stable than the less branched dipeptidenitriles **24** and **39** ($t_{1/2}$ = 22 and 19 h, respectively), though the magnitude of the effect of increased β-branching in this series appears to be blunted by the inherent baseline stability imparted by the methano bridge. This observation is in agreement with data reported by Coutes[12a] and Snow[27] where the rates of cyclization for Xaa-boroproline dipeptides were shown to be Gly-BoroPro > Ala-BoroPro > Val-BoroPro.

Computational Analysis. Computational analysis was undertaken to more fully understand the relative stabilities of the methanoprolinenitriles. Ground-state conformations were generated for methanoprolinenitrile and prolinenitrile forms of the N-terminal *tert*-leucine dipeptide compounds. The calculated ground-state structure for the *tert*-leucine dipeptidenitrile is identical to the conformation observed through single-crystal X-ray structural analysis[28] of the TFA salt of **29** (rms = 0.1 Å for calculated and observed heavy atoms; see Figure 2, lower structure). Both have the same syn conformation around the amide bond, characterized by a small C(2)−N−C(8)═O torsional angle (−5° and +2° for the TFA structure). It is of additional interest that a similar conformation has been observed in several recently disclosed cocrystal structures of DPP-IV/inhibitor complexes.[29]

In addition to the syn conformation, there is a calculated local low-energy minimum where the reactive amine and nitrile are close to each other; in this anti conformation (Figure 2, upper structure), the C(2)−N−C(8)═O torsional angle is 180°. Moreover, the angle between the amine N and the C≡N group is 109° ± 1° and the distance between the these reactive partners is 2.95 Å. It is therefore reasonable to assume that the observed intramolecular cyclization is initiated from this conformation. The value of 109° between the amine group and the nitrile is in close agreement with the hypothetical angle of attack of at least 108° reported by Baxter and Connor.[30] It was envisioned that the relative energetic differences between the global minimum and the reactive local minimum would represent a means to evaluate the relative stabilities of compounds in solution.

The calculated conformations and their relative energies can be used to examine the basis for two aspects of the experimental compound stability: the increase in stability due to side chain bulk (specifically β-branching) and the increase in stability upon conversion from prolinenitrile to *cis*-4,5-methanoprolinenitrile. Calculated relative energies between the ground-state conformation and the local low-energy minimum conformation that brings the reactive amine and nitrile in proximity are presented in Table 2 for the methanoprolinenitrile and prolinenitrile forms of N-terminal *tert*-leucine and alanine dipeptidenitriles, as well as for the same prolinenitriles with a simple acetamide cap.

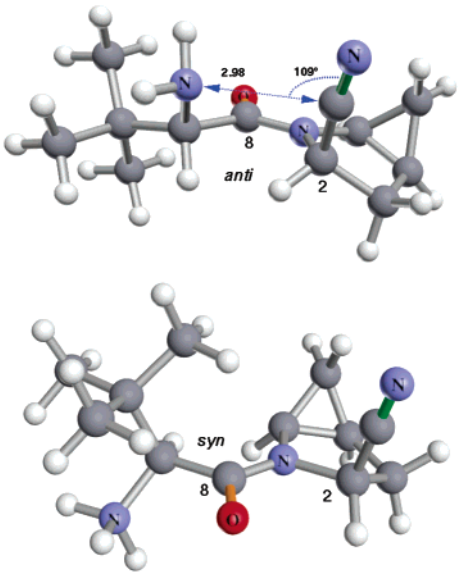
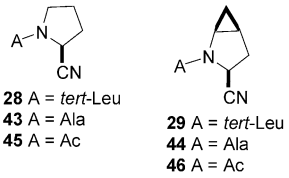


Figure 2. The upper structure depicts the local low-energy minimum for **29** where the reactive amine is close to the nitrile (anti conformation). The lower structure is the solid-state conformation observed in the X-ray crystallographic structure of the TFA salt of compound **29** (syn conformation). Two independent cations were observed in the crystal structure, though because these have identical conformations, only one is shown for purposes of clarity. Carbon atoms C(2) and C(8) are labeled.

Conformational Stability Due to Side Chain Bulk. The values in the first column of Table 2 compare the conformational energy differences between the ground state and the geometry where internal cyclization could occur for the unsubstituted prolinenitrile series. The ab initio (G98) results are expected to be considerably more accurate than the force field values and indicate that the energy required to assume the anti conformation increases with side chain bulk (e.g., 0.3, 1.9, 2.8 kcal/mol for no side chain, the alanine side chain, and the *tert*-leucine side chain, respectively). The force field energy results agree qualitatively with the ab initio values and suggest that the primary contribution is due to van der Waals interactions. Examination of the structures reveals extremely close contacts between two of the side chain methyl groups and the carbonyl oxygen in the anti conformation (approximately 3 Å each) that would increase the energy barrier for internal cyclization and thus lead to greater stability. A similar trend is observed for increasing side chain bulk in the methano-substituted series (column 2), and these values are in good agreement with the solution data where increased β-branching enhances stability.

Conformational Stability Due to Methano Substitution. As shown in the third column of Table 2, the ab initio (G98) energy calculations indicate that the

Table 2. Calculated Energy[a] Differences between the Calculated Ground State and the Local Low-Energy Minimum Required for Cyclization



28 A = *tert*-Leu
43 A = Ala
45 A = Ac

29 A = *tert*-Leu
44 A = Ala
46 A = Ac

28 (Prolinenitrile) and **29** (Methanoprolinenitrile) (A = *tert*-Leu)

method interaction	ΔH(**28**) [b]	ΔH(**29**) [b]	$\Delta\Delta H$(**29** − **28**)
G98 ab initio	2.8	3.4	0.6
Insight CFF force field			
total nonbond	3.5	4.2	0.7
van der Waals	3.3	3.9	0.6
electrostatic	0.2	0.3	0.1

43 (Prolinenitrile) and **44** (Methanoprolinenitrile) (A = Ala)

method interaction	ΔH(**43**) [b]	ΔH(**44**) [b]	$\Delta\Delta H$ (**44** − **43**)
G98 ab initio	1.9	2.6	0.6
Insight CFF force field			
total nonbond	3.3	3.9	0.6
van der Waals	2.9	3.4	0.6
electrostatic	0.4	0.4	0.0

45 (Prolinenitrile) and **46** (Methanoprolinenitrile) (A = Ac)

method interaction	ΔH(**45**) [b]	ΔH(**46**) [b]	$\Delta\Delta H$ (**46** − **45**)
G98 ab initio	0.3	0.9	0.6
Insight CFF force field			
total nonbond	−0.2	0.0	0.2
van der Waals	−0.4	−0.2	0.2
electrostatic	0.2	0.3	0.1

[a] Energies are for the *cis*-4,5-methanoprolinenitrile and prolinenitrile versions of the compounds and are given in kcal/mol. [b] Energies are for the anti conformation (Scheme 3 and Figure 2 upper structure) relative to the global minimum conformation, which corresponds to the syn geometry in Scheme 3 and Figure 2 lower structure.

addition of the methano bridge increases the energy barrier toward adopting the conformation required for cyclization by approximately 0.6 kcal/mol for the N-terminal *tert*-leucine dipeptide compound as well as for the alanine dipeptide and acetamide compounds. The lack of dependence on the nature of the side chain suggests that the observed increase in stability of the *cis*-4,5-methano compounds is due to a local interaction between the methano bridge and the N-terminal amino acid that favors the ground state relative to the anti conformation where cyclization is initiated. The force field energy analysis (Insight CFF) results also agree qualitatively, implying that van der Waals interactions are primarily responsible for the increase in energy. These conclusions are supported by the cyclization rate differences between the isomeric isoleucine derivatives **24** ($t_{1/2}$ = 22 h) and **21** ($t_{1/2}$ = 5 h), where a 4.5-fold increase in stability is observed for the methanoprolinenitrile. This increase in stability is in fair agreement with the calculated value of 0.6 kcal/mol. The calculated $\Delta\Delta H$ and observed solution half-life results support contributions to stability from both β-branching and the presence of the 4,5-methano bridge on the pyrrolidine ring.

In Vivo Activity. In vivo evaluation of DPP-IV inhibitors has supported the connection between DPP-IV inhibition, increases in plasma insulin levels, and an improvement in glucose tolerance.[31] Compounds **29** and **41** were potent inhibitors of DPP-IV in vitro and demonstrated excellent solution stability. As such, these inhibitors were selected to determine the effects of DPP-IV inhibition in vivo on glucose tolerance and insulin secretion in Zucker$^{fa/fa}$ rats. An oral glucose tolerance test (oGTT) in the Zucker$^{fa/fa}$ rat is a frequently used model of hyperglycemia in type 2 diabetes and obesity research. Zucker$^{fa/fa}$ rats are severely hyperphagic, extremely obese, markedly insulin-resistant, and mildly hyperglycemic because of a mutation and loss of function of the leptin receptor gene.[32,33] Fasted male Zucker$^{fa/fa}$ rats were dosed orally with water or with inhibitors **29** and **41** (3 μmol/kg), and an oGTT was conducted 0.5 h after the dosing. Plasma DPP-IV activity, glucose, and insulin levels were then monitored over a 2 h period. Figures 3−5 show the ex vivo plasma DPP-IV activity, insulin response, and glucose excursion curves in response to an oral glucose challenge (2 g/kg). Animals in the control group reached peak plasma glucose levels 60 min after glucose administration, at which point the drug-treated animals exhibited a 30−35% decrease in glucose levels compared to controls (control animals, 356 mg/dL; compound **29** treated animals, 226 mg/dL; compound **41** treated animals, 245 mg/dL). Glucose levels were significantly reduced in the drug-treated animals from 30 min onward, with maximal reductions in glucose observed at 90 min (−34% to −38%). Plasma DPP-IV activity was maximally suppressed (60%) 30 min after dosing (Figure 3), and the effects of these inhibitors were sustained throughout the course of the experiment (60−35%). The insulin response to oral glucose was also enhanced by treatment with DPP-IV inhibitors (Figure 4), demonstrating the link between the glucose-lowering effects and DPP-IV inhibition of these compounds.

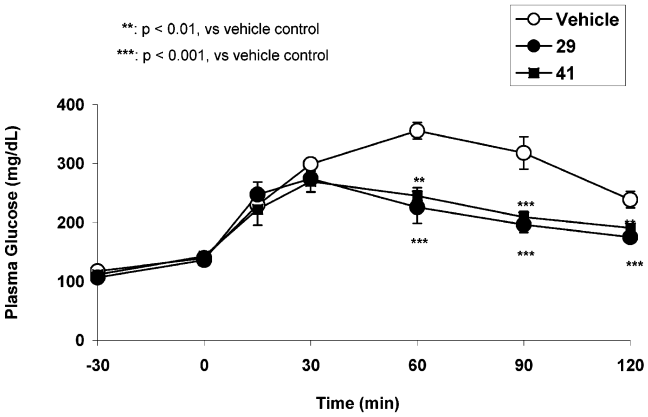


Figure 3. Effects of inhibitors **29** (●) and **41** (■) dosed at 3 μmol/kg po versus vehicle control (○) on plasma DPP-IV activity in Zucker$^{fa/fa}$ rats.

Interestingly, a significant decrease in plasma glucose levels occurred when DPP-IV activity in plasma was inhibited only by 35−60%. This finding suggests that it may not be necessary to completely suppress plasma DPP-IV activity in order to achieve antihyperglycemic efficacy in type 2 diabetics. However, differences may exist in the inhibition of the turnover of native substrates such as GLP-1 compared with that of the pseudosubstrate used in this assay, and potential assay

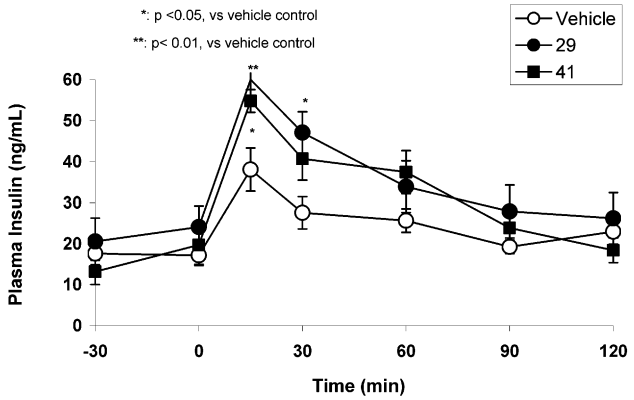


Figure 4. Effects of inhibitors **29** (●) and **41** (■) dosed at 3 μmol/kg po versus vehicle control (○) on plasma glucose after an oGTT in Zucker$^{fa/fa}$ rats.

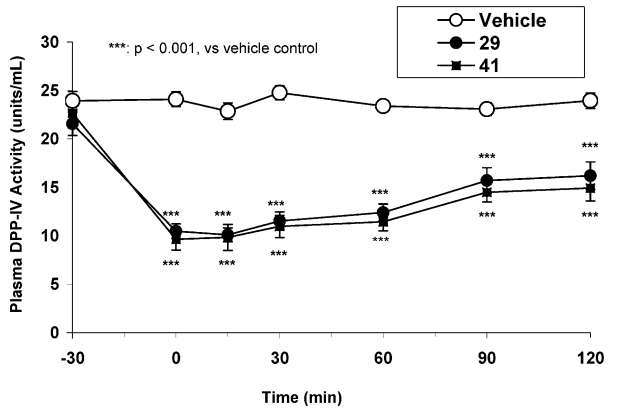


Figure 5. Effects of inhibitors **29** (●) and **41** (■) dosed at 3 μmol/kg po versus vehicle control (○) on plasma insulin after an oGTT in Zucker$^{fa/fa}$ rats.

artifacts relating to the kinetic aspects of certain inhibitors cannot be ruled out at present.

Conclusion

We have demonstrated that the prolinenitrile fragment of previously reported DPP-IV inhibitors can be replaced with either a *cis*-3,4-methano- or a *cis*-4,5-methanoprolinenitrile ring system to provide novel and highly potent DPP-IV inhibitors. Solution stability studies demonstrate that 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 enhances solution stability, minimizing a known intramolecular cyclization pathway. The greatest improvement in stability is observed when both of these structural features are present and at work in concert. This added stability has the potential to beneficially impact the chemical and formulation stability of cyanopyrrolidide-based pharmaceuticals. In many cases, the presence of a β-branched amino acid also provides increased inhibitory potency as well as solution stability. This class of inhibitors has been shown to be effective in suppression of prandial glucose elevations after an oral glucose challenge. These initial findings are being used as the basis for the development of DPP-IV inhibitors with even greater stability and in vivo efficacy for the treatment of diabetes and impaired glucose homeostasis through potentiation of meal-induced levels of GLP-1 (7−36) and insulin.

Experimental Section

General Chemical Procedures. All reactions were carried out using oven-dried or flame-dried round-bottomed (rb) flasks and glassware under a static atmosphere of argon or nitrogen, and the mixtures were stirred magnetically unless otherwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co., Sigma Chemical Co., Lancaster Chemical Co., or Acros Chemical Co. All reactions were carried out using commercially available anhydrous solvents from Aldrich Chemical Co. or EM Science Chemical Co. unless otherwise noted. "Brine" refers to a saturated aqueous solution of NaCl. Unless otherwise specified, solutions of common inorganic salts used in workups are aqueous solutions. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a JEOL JNM-ECP500 spectrometer, and ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were recorded on a JEOL GSX400 spectrometer. Chemical shifts are given in parts per million (ppm) downfield from internal reference tetramethylsilane in δ units, and coupling constants (J values) are given in hertz (Hz). Selected data are reported in the following manner: chemical shift; multiplicity; coupling constants; assignment. Elemental analyses were performed by the Analytical Chemistry department at Bristol-Myers Squibb. Melting points were taken on a Hoover Uni-melt melting point apparatus and are uncorrected. Boiling points are reported uncorrected. Kügelrohr distillations were performed using a Büchi GKR-51 apparatus, and reported boiling points correspond to uncorrected oven air bath temperatures. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter using a cell 1 dm in length and are reported as follows: $[\alpha]^{temp}_{wavelength}$ (concentration in g/100 mL, solvent). All flash chromatographic separations were performed using E. Merck silica gel (particle size, 0.040−0.063 mm). Reactions were monitored by TLC using 0.25 mm E. Merck silica gel plates (60 F_{254}) and were visualized using UV light and 5% phosphomolybdic acid in 95% EtOH, or by sequential treatment with 1 N HCl in CH_3OH followed by ninhydrin staining. LC/MS data were recorded on a Shimadzu LC-10AT equipped with an SIL-10A injector, an SPD-10AV detector normally operating at 220 nm and interfaced to a Micromass ZMD mass spectrometer. LC/MS or HPLC retention times are reported using a Phenomenex Luna C-18 4.6 mm × 50 mm column, with elution with a 4 min gradient of 0−100% solvent B, where solvent A is 10:90:0.1 CH_3OH-H_2O-TFA and solvent B is 90:10:0.1 CH_3OH-H_2O-TFA (HPLC, method 1). Other HPLC methods include the following: method 2, YMC S-5 C-18 4.6 mm × 50 mm, 0−100% B, elution with a 4 min gradient of 0−100% solvent B, where solvent A is 10:90:0.1 CH_3CN-H_2O-TFA and solvent B is 90:10:0.1 CH_3CN-H_2O-TFA; method 3, Zorbax SB C-18 4.6 mm × 75 mm column, elution with an 8 min gradient of 0−100% solvent B, where solvent A is 10:90:0.1 $CH_3OH-H_2O-H_3PO_4$ and solvent B is 90:10:0.1 $CH_3OH-H_2O-H_3PO_4$. All solvents were removed by rotary evaporation under vacuum using a standard rotary evaporator equipped with a dry ice condenser. All filtrations were performed using vacuum unless otherwise noted.

Representative Example of Preparation: General Method A. *N*-Boc-L-*cis*-4,5-methanoprolineamide. To a solution of *N*-Boc-4,5-methanoproline[18b] (1.20 g, 5.28 mmol) in THF (20 mL) at −15 °C was added 4-methylmorpholine (0.71 mL, 6.50 mmol) and then isobutyl chloroformate (0.78 mL, 6.00 mmol) over 5 min. The reaction mixture was stirred at −15 °C for 30 min, cooled to −30 °C, and then treated with a solution of NH_3 in dioxane (50 mL, 25 mmol). The reaction mixture was stirred at −30 °C for 0.5 h, warmed to room temperature, and stirred overnight. The reaction mixture was quenched with citric acid solution (pH 4) and extracted with Et_2O (3 × 50 mL). The combined organic fractions were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography on silica gel with EtOAc to give 1.00 g (84%) of the title compound. ^{1}H NMR and ^{13}C NMR signals were very broad and poorly defined as a result of carbamate rotamers. Anal. ($C_{11}H_{18}N_2O\cdot0.4H_2O$) C, H, N.

L-*cis*-4,5-Methanoprolineamide TFA Salt (8). To a solution of *N*-Boc-4,5-methanoprolineamide (0.90 g, 4.00 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added TFA (3 mL). The reaction mixture was stirred at 0 °C for 18 h and concentrated under reduced pressure to give 0.98 g (100%) of the title compound as an oil that solidified upon prolonged standing. Alternatively, the protected material can be treated with HCl(g) in EtOAc to give a white powder. Data for HCl salt: ^{1}H NMR (CD_3OD, 400 MHz) δ 0.78 (m, 1H), 0.95 (q, J = 8.0, 1H), 1.85 (m, 1H), 2.27 (dd, J = 14.1, 3.0, 1H), 2.75 (m, 1H), 3.42 (m, 1H), 4.57 (dd, J = 11.1, 3.0, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 10.7, 18.8, 33.3, 38.3, 62.2, 170.3. Anal. ($C_6H_{10}N_2O\cdot1.03HCl\cdot0.10H_2O$) C, H, N, Cl.

Amides Prepared from Known Carboxylic Acids by Method A. l-*trans*-4,5-Methanoprolineamide TFA Salt (9). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.89 (q, J = 6.0, 1H), 0.95 (m, 1H), 1.90 (m, 1H), 2.18 (td, J = 5.0, 11.4, 1H), 2.59 (dd, J = 8.1, 13.2, 1H), 3.47 (td, J = 2.5, 6.6, 1H), 4.01 (dd, J = 8.1, 10.8, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 7.1, 17.0, 32.0, 36.1, 58.3, 118.6 (q, J = 263.5), 163.0 (q, J = 35.4), 172.0. Anal. ($C_6H_{10}N_2O\cdot1.00TFA$) C H, N, F.

L-*cis*-3,4-Methanoprolineamide TFA Salt (10). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.60 (m, 1H), 0.75 (q, J = 7.5, 1H), 1.85 (m, 1H), 2.10 (m, 1H), 3.45 (d, J = 10.9, 1H), 3.49 (dd, J = 11.0, 3.5, 1H), 4.44 (d, J = 4.3, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 5.3, 17.5, 20.4, 62.5, 170.4 (TFA not observed). Anal. ($C_6H_{10}N_2O\cdot1.30TFA$) C, H, N, F.

L-2,3-Methanoprolinenitrile HCl Salt (11). ^{1}H NMR (CD_3OD, 400 MHz) δ 1.55 (m, 1H), 1.70 (m, 1H), 2.10 (m, 1H), 2.25 (m, 1H), 2.37 (m, 1H), 2.67 (m, 1H), 3.15 (m, 1H).

Representative Example of Preparation: General Method B. l-*tert*-Leucinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (29). An oven-dried 15 mL test tube was charged with the L-*cis*-4,5-methanoprolineamide TFA salt (56 mg, 0.22 mmol), *N*-*tert*-butyloxycarbonyl-L-*tert*-leucine (53 mg, 0.23 mmol), 4-(dimethylamino)pyridine (0.11 g, 0.88 mmol), and CH_2Cl_2 (4 mL). The mixture was treated with 1-[(3-(dimethyl)amino)propyl]-3-ethylcarbodiimide (84 mg, 0.44 mmol), and the tube was sealed under a nitrogen atmosphere. The tube was placed in a shaker and shaken overnight. The product was purified by solid-phase extraction using a United Technology SCX column (2 g of sorbent in a 6 mL column) by loading the material on an SCX ion exchange column and successively washing with CH_2Cl_2 (5 mL), 1:3 $CH_3OH-CH_2Cl_2$ (5 mL), 1:2 $CH_3OH-CH_2Cl_2$ (5 mL), and 1:1 $CH_3OH-CH_2Cl_2$ (10 mL). The product-containing fractions were concentrated under reduced pressure to give the desired amide. Further purification by reverse-phase preparative HPLC on a YMC S5 ODS 20 mm × 250 mm column gave the desired amide (50 mg, 68% yield). Purification conditions were the following: gradient elution from 30:70:0.1 CH_3OH-H_2O-TFA to 90:10:0.1 CH_3OH-H_2O−TFA over 15 min; flow rate, 20 mL/min; detection wavelength, 220 nm; retention time, 14 min. ^{1}H NMR (CD_3OD, 500 MHz) δ 0.87 (m, 1H), 1.08 (s + m, 10H), 1.44 (s, 9H), 1.75 (m, 1H), 2.01 (dd, J = 13.7, 3.5, 1H), 2.58 (m, 1H), 3.87 (m, 1H), 4.53 (s, 1H), 4.77 (dd, J = 11.8, 3.2, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 14.6, 18.4, 27.1, 28.7, 31.4, 36.4, 39.9, 60.4, 62.7, 80.7, 157.9, 172.4, 176.7.

An oven-dried 15 mL test tube was charged with the *N*-Boc-*tert*-leucine-*cis*-4,5-methanoprolineamide (50 mg, 0.15 mmol), imidazole (31 mg, 0.46 mmol), and pyridine (1 mL). The tube was sealed under nitrogen atmosphere and cooled to −30 °C. Slow addition of $POCl_3$ (141 mg, 88 μL, 0.92 mmol) gave after mixing a thick slurry. The tube was mixed at −30 °C for 1 h, and the volatiles were evaporated. The product was purified by solid-phase extraction using a United Technology silica extraction column (2 g of sorbent in a 6 mL column) by loading the material on a silica column and successively washing with CH_2Cl_2 (5 mL), 5:95 $CH_3OH-CH_2Cl_2$ (5 mL), 7:93 $CH_3OH-CH_2Cl_2$ (5 mL), and 12:88 $CH_3OH-CH_2Cl_2$ (10 mL). The product-containing fractions were pooled and concentrated under reduced pressure to give the *N*-Boc-protected nitrile compound (46 mg, 96%). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.88 (m, 1H), 1.08 (s + m, 10H), 1.43 (s, 9H), 1.94 (m, 1H), 2.32 (dd, J = 11.5, 2.5, 1H), 2.65 (m, 1H), 3.95 (m, 1H), 4.51 (s, 1H), 5.09 (dd, J = 11.2, 2.2, 1H).

An oven-dried 15 mL test tube was charged with the *N*-Boc-*tert*-leucine-4,5-methanoprolinenitrile (45 mg, 0.14 mmol), CH_2Cl_2 (1 mL), and TFA (1 mL). The reaction mixture was vortexed for 40 min at room temperature, diluted with toluene (4 mL), and concentrated under reduced pressure to a thick oil. The product was purified by reverse-phase preparative HPLC on a YMC S5 ODS 20 mm × 250 mm column to give 14 mg (35%) of the title compound. Purification conditions were the following: gradient elution from 10:90:0.1 CH_3OH-H_2O-TFA to 90:10:0.1 CH_3OH-H_2O−TFA over 18 min; flow rate, 20 mL/min; detection wavelength, 220 nm; retention time, 10 min. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.73 (m, 1H), 1.05 (s + m, 10H), 1.99 (m, 1H), 2.26 (dd, J = 13.5, 2.2, 1H), 2.50 (m, 1H), 4.11 (m, 1H), 4.37 (s, 1H), 5.26 (dd, J = 10.5, 2.2, 1H), 8.20 (s, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 12.7, 17.3, 25.9, 29.8, 34.2, 37.6, 45.0, 57.6, 118.0 (q, J = 280), 119.5, 157.5 (q, J = 38), 166.7.

L-*tert*-Leucinyl-L-*cis*-4,5-methanoprolinenitrile HCl Salt (29). An oven-dried 15 mL test tube was charged with *N*-Boc-*tert*-leucine-4,5-methanoprolinenitrile (0.45 mg, 0.14 mmol) and Et_2O (1 mL) and was treated with a 2 N HCl solution in Et_2O (1 mL). The reaction mixture was vortexed for 15 min at room temperature and concentrated under reduced pressure to a white powder. The product was purified by trituration with Et_2O, collected, and dried under vacuum to give the title compound (25 mg, 85%). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.91 (m, 1H), 1.13 (s + m, 10H), 2.00 (m, 1H), 2.32 (dd, J = 14.0, 2.2, 1H), 2.65 (m, 1H), 3.97 (m, 1H), 4.37 (s, 1H), 5.26 (dd, J = 10.5, 2.2, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.1, 19.1, 26.7, 31.4, 35.7, 39.4, 46.9, 60.1, 120.4, 168.1. Anal. ($C_{12}H_{20}ClN_3O\cdot0.25H_2O$) C, H, N.

Dipeptide Nitriles Prepared by General Method B. L-Isoleucine-L-prolinenitrile HCl Salt (21). ^{1}H NMR (400 MHz, CD_3OD) δ 0.99 (t, J = 7.2, 3H), 1.10 (d, J = 6.6, 3H), 1.20 (m, 2H), 1.70 (m, 1H), 1.90−2.45 (m, 4H), 3.70 (m, 2H), 4.11 (s, 1H), 4.81 (d, J = 8.0, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.7, 15.3, 25.1, 26.4, 30.9, 37.8, 48.0, 57.4, 119.2, 169.1. Anal. ($C_{11}H_{19}N_3O\cdot1.00HCl\cdot0.5H_2O$) C, H, N.

L-Isoleucinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (24). ^{1}H NMR (D_2O, 500 MHz) δ 0.90 (m, 1H), 0.95 (t, J = 7.3, 3H), 1.12 (d, J = 7.3, 3H), 1.17 (m, 1H), 1.25 (m, 1H), 1.47 (m, 1H), 2.05 (m, 1H), 2.32 (m, 1H), 2.55 (dd, J = 13.8, 2.8, 1H), 2.78 (m, 1H), 3.73 (td, J = 6.4, 2.8, 1H), 4.46 (d, J = 9.1, 1H), 5.32 (dd, J = 11, 2.8, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 10.9, 13.7, 14.9, 18.5, 23.6, 30.1, 35.5, 38.0, 47.0, 56.9, 116.6 (q, J = 290), 119.8, 163.2 (q, J = 35), 165.4. HPLC: method 3; t_R = 2.49 3.03 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{12}H_{20}N_3O$, 222.1606; found, 222.1606.

L-Valinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (26). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.93 (m, 1H), 1.07 (d, J = 7.1, 3H), 1.09 (m, 1H), 1.17 (d, J = 7.1, 3H), 2.01 (m, 1H), 2.35 (dd, J = 13.8, 3.0, 1H), 2.50 (m, 1H), 2.65 (m, 1H), 3.82 (td, J = 6.0, 2.5, 1H), 4.39 (d, J = 5.0, 1H), 5.16 (dd, J = 11.0, 2.2, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.3, 17.4, 19.4, 30.9, 31.8, 38.7, 47.6, 58.5, 120.8, 168.9. HPLC: method 2; t_R = 0.89 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{11}H_{17}N_3O$, 208.1450; found, 208.1447.

L-*tert*-Leucinyl-L-prolinenitrile TFA Salt (28). ^{1}H NMR (CD_3OD, 400 MHz) δ 1.13 (s, 9H), 1.90−2.45 (m, 4H), 3.72 (t, J = 6.3, 1H), 4.00 (s, 1H), 4.81 (d, J = 8.0, 1H); ^{13}C NMR (CD_3OD, 100 MHz) 26.7, 26.8, 31.2, 35.9, 48.2, 60.5, 119.4 (q, J = 248), 119.5, 163.2 (q, J = 46), 169.1. Anal. ($C_{11}H_{19}N_3O\cdot1.00TFA$) C, H, N, F.

L-Phenylalaninyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (31). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.31 (m, 1H), 0.66 (dd, J = 8.5, 6.5, 1H), 1.84 (m, 1H), 2.25 (dd, J = 13.8, 2.5, 1H), 2.57 (m, 1H), 3.25 (m, 1H), 3.30 (s, 2H), 3.65 (m, 1H), 4.73 (t, J = 6.6, 1H), 5.10 (dd, J = 10.2, 2.2, 1H), 7.32 (m, 5H); ^{13}C NMR (CD_3OD, 100 MHz) δ 13.7, 18.7, 31.9, 38.4, 38.7, 47.6, 54.3, 118.0 (q, J = 280), 120.0, 129.4, 130.7, 131.3, 135.1, 164.0 (q, J = 43), 168.0. HPLC: method 2; t_R = 1.33 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{15}H_{17}N_3O$, 256.1450; found, 256.1445.

L-Tryptophanyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (32). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.00 (m, 2H), 1.64 (m, 1H), 2.11 (dd, J = 13.8, 2.2 Hz, 1H), 2.44 (m, 1H), 3.23 (s, 2H), 3.35 (m, 1H), 4.71 (t, J = 6.6, 1H), 5.00 (dd, J = 10.5, 2.2, 1H), 7.00 (t, J = 7.2, 1H), 7.07 (t, J = 7.7, 1H), 7.10 (s, 1H), 7.32 (d, J = 8.2, 1H), 7.56 (d, J = 7.7, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 13.4, 18.6, 28.8, 31.8, 38.9, 47.4, 53.4, 107.7, 113.1, 119.2, 120.5, 120.9, 123.3, 126.2, 128.9, 138.9, 169.5. HPLC: method 2; t_R = 1.39 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{17}H_{18}N_4O$, 295.1559; found, 295.1558.

L-*N*-Methylvalinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (33). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.94 (m, 1H), 1.11 (m + d, J = 7.2, 4H), 1.18 (d, J = 7.2, 3H), 2.02 (m, 1H), 2.35 (dd, J = 13.8, 2.3, 1H), 2.50 (m, 1H), 2.65 (m, 1H), 2.70 (s, 3H), 3.85 (td, J = 6.0, 2.7, 1H), 4.42 (d, J = 5.0, 1H), 5.20 (dd, J = 10.5, 2.2, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.4, 18.3, 19.0, 19.5, 31.4, 31.8, 33.8, 38.8, 47.6, 66.8, 120.7, 167.5. HPLC: method 2; t_R = 0.87 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{12}H_{19}N_3O$, 222.1606; found, 222.1604.

L-Prolinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (34). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.93 (m, 1H), 1.10 (m, 1H), 2.00 (m, 1H), 2.11 (m, 3H), 2.38 (dd, J = 13.8, 2.2, 1H), 2.66 (m, 2H), 3.43 (m, 2H), 3.75 (td, J = 6.0, 2.7, 1H), 4.85 (t, J = 8.8, 1H), 5.11 (dd, J = 12.6, 2.2, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.1, 19.2, 25.4, 29.8, 30.0, 32.0, 38.3, 47.8, 61.1, 120.7, 168.4. HPLC: method 2; t_R = 0.48 min. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{11}H_{15}N_3O$, 206.1296; found, 206.1293.

L-Pipecolinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (35). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.93 (m, 1H), 1.12 (q, J = 6.5, 1H), 1.20 (m, 1H), 1.60−2.10 (m, 3H), 2.35 (dd, J = 14.0, 2.5, 1H), 2.65 (m, 1H), 3.10 (m, 1H), 2.66 (m, 1H), 3.45 (d, J = 11.0, 1H), 3.56 (t, J = 5.0, 1H), 3.68 (t, J = 4.5, 1H), 3.76 (m,-1H), 4.48 (dd, J = 12.5, 3.5, 1H), 5.06 (dd, J = 10.5, 2.5, 1H); ^{13}C NMR (CD_3OD, 125 MHz) δ 13.5, 16.5, 21.0, 21.7, 25.5, 29.1, 34.2, 40.5, 47.8, 61.4, 118.5 (q, J = 289), 120.7, 166.3 (q, J = 36), 171.1. HPLC: method 1; t_R = 0.85 min. MS m/z 220 $[M + H]^+$.

L-Methioninyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (36). ^{1}H NMR (CD_3OD, 500 MHz) δ 0.93 (m, 1H), 1.13 (q, J = 7.1, 1H), 2.03 (m, 1H), 2.25 (s, 3H), 2.29 (m, 2H), 2.36 (dd, J = 13.8, 2.0, 1H), 2.66 (m, 3H), 3.79 (td, J = 6.0, 3.0, 1H), 4.68 (dd, J = 7.0, 4.5, 1H), 5.15 (dd, J = 10.5, 2.0, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 13.9, 15.2, 19.0, 29.6, 30.3, 31.4, 38.1, 47.4, 52.4, 120.4, 168.4. HPLC: method 1; t_R = 1.18 min. MS m/z 240 $[M + H]^+$.

(*S*)-Cyclobutylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (37). ^{1}H NMR (D_2O, 400 MHz) δ 0.85 (m, 1H), 1.05 (m, 1H), 1.73 (m, 1H), 1.92 (m, 7H), 2.30 (dd, J = 14.1, 2.2, 1H), 2.56 (m, 1H), 2.89 (m, 1H), 3.70 (m, 1H), 4.45 (d, J = 8.4, 1H), 5.06 (dd, J = 10.8, 2.4, 1H); ^{13}C NMR (D_2O, 400 MHz) δ 13.8, 17.8, 18.3, 24.5, 24.7, 30.1, 38.0, 46.8, 55.1, 119.9, 168.3. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{12}H_{17}N_3O$, 220.145; found, 220.144. HPLC: method 1: t_R = 1.26 min. Anal. ($C_{12}H_{17}N_3O$·1.00TFA·0.67H_2O) C, H, N, F.

(*S*)-Cyclopentylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (38). ^{1}H NMR (D_2O, 400 MHz) δ 0.86 (m, 1H), 1.04 (q, J = 7.1, 1H), 1.25−1.80 (m, 8H), 1.93 (m, 1H), 2.32 (dd, J = 11.5, 2.6, 1H), 2.40 (m, 1H), 2.59 (m, 1H), 3.70 (td, J = 6.1, 2.6, 1H), 4.43 (d, J = 7.5, 1H), 5.09 (dd, J = 11.0, 2.6, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 13.5, 18.2, 24.3, 24.7, 27.9, 28.4, 30.2, 37.9, 41.3, 46.8, 55.1, 119.6, 169.1. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{13}H_{19}N_3O$, 234.1602; found, 234.1606. Anal. ($C_{13}H_{19}N_3O$·1.23TFA) C, H, N.

(*S*)-Cyclohexylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (39). ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (m, 1H), 1.05−1.35 (m, 6H), 1.70−1.90 (m, 5H), 2.00 (m, 1H), 2.15 (m, 1H), 2.37 (dd, J = 13.6, 1.8, 1H), 2.63 (m, 1H), 3.83 (m, 1H), 4.35 (d, J = 5.7, 1H), 5.17 (dd, J = 10.6, 2.2, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 13.9, 19.1, 26.7, 26.9, 27.1, 28.5, 30.0, 31.4, 38.4, 40.3, 47.1, 57.2, 120.5, 168.5. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{14}H_{21}N_3O$, 248.1768; found, 248.1763. Anal. ($C_{14}H_{21}N_3O$·1.00TFA) C, H, N.

(*S*)-1-Methylcyclobut-1-ylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (40). ^{1}H NMR (D_2O, 400 MHz) δ 0.99 (m, 1H), 1.19 (q, J = 8.8, 6.6, 1H), 1.37 (s, 3H), 1.89 (m, 3H), 2.12 (m, 2H), 2.24 (m, 1H), 2.47 (dd, J = 13.6, 2.6, 1H), 2.54 (m, 1H), 2.72 (m, 1H), 3.90 (m, 1H), 4.82 (s, 1H), 5.27 (dd, J = 10.8, 2.4, 1H); ^{13}C NMR (D_2O, 400 MHz) δ 14.1, 15.1, 18.5, 21.1, 30.0, 30.9, 31.0, 38.6, 40.1, 46.8, 59.0, 120.0, 167.8. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{13}H_{19}N_3O$, 234.1607; found, 234.1592. HPLC: method 3; t_R = 3.55 min. Anal. ($C_{13}H_{19}N_3O$·1.00TFA) C, H, N, F.

(*S*)-1-Methylcyclopent-1-ylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (41). ^{1}H NMR (D_2O, 400 MHz) δ 0.82 (m, 1H), 0.95 (s, 3H), 1.00 (q, J = 7.1, 1H), 1.25−1.75 (m, 8H), 1.90 (m, 1H), 2.28 (dd, J = 14.0, 2.7, 1H), 2.54 (m, 1H), 3.70 (td, J = 6.1, 3.1, 1H), 4.35 (s, 1H), 5.08 (dd, J = 8.5, 2.6, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 13.8, 14.5, 21.3, 23.2, 23.4, 30.0, 36.2, 36.7, 45.7, 46.7, 58.8, 119.8, 168.2. HRMS (FAB) m/z: $[M + H]^+$ calcd for $C_{14}H_{20}N_3O$, 248.1756; found, 248.1749. Anal. ($C_{14}H_{21}N_3O$·1.00TFA·0.89H_2O) C, H, N.

(*S*)-1-Methylcyclohex-1-ylglycinyl-L-*cis*-4,5-methanoprolinenitrile TFA Salt (42). ^{1}H NMR (D_2O, 400 MHz) δ 0.86 (m, 1H), 1.03 (s, 3H), 1.07 (m, 2H), 1.39 (m, 9H), 1.95 (m, 1H), 2.31 (dd, J = 13.6, 2.6, 1H), 2.58 (m, 1H), 3.76 (m, 1H), 4.26 (s, 1H), 5.12 (dd, J = 11, 2.6, 1H); ^{13}C NMR (D_2O, 400 MHz) δ 13.8, 18.2, 18.5, 20.9, 21.0, 25.3, 30.0, 33.5, 34.1, 37.9, 38.9, 46.7, 60,0, 119.8, 168.0. HRMS (ESI) m/z: $[M + H]^+$ calcd for $C_{15}H_{23}N_3O$, 262.1920; found, 262.1904. HPLC: method 3; t_R = 4.77 min. Anal. (C_{15},$H_{23}N_3O$·1.00TFA·0.50H_2O) C, H, N, F.

Representative Example of Preparation: General Method C. l-*tert*-Leucinyl-L-*cis*-3,4-methanoprolinenitrile TFA Salt (30). An oven-dried rb flask was charged with *cis*-3,4-methanoprolineamide TFA salt (50 mg, 0.21 mmol), *N*-*tert*-butyloxycarbonyl-L-*tert*-leucine (48 mg, 0.21 mmol), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (164 mg, 0.32 mmol), 4-methylmorpholine (70 μL, 0.63 mmol), and CH_2Cl_2 (4 mL). The flask was sealed under nitrogen atmosphere and stirred overnight. The product was purified by solid-phase extraction using a United Technology silica extraction column (2 g of sorbent in a 6 mL column) by loading the material on a silica column and successively washing with CH_2Cl_2 (5 mL), 5:95 $CH_3OH-CH_2Cl_2$ (5 mL), 7:93 $CH_3OH-CH_2Cl_2$ (5 mL), and 12:88 $CH_3OH-CH_2Cl_2$ (10 mL). The product-containing fractions were pooled and concentrated under reduced pressure to give the desired amide compound (64 mg, 90%). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.69 (m, 1H), 0.78 (q, J = 4.7, 1H), 1.00 (s, 9H), 1.42 (s, 9H), 1.74 (m, 1H), 1.93 (m, 1H), 3.82 (d, J = 9.2, 1H), 3.98 (m, 1H), 4.16 (s, 1H), 4.61 (d, J = 5.2, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 8.9, 17.7, 21.0, 26.8, 28.7, 36.1, 52.5, 60.2, 62.5, 80.7, 156.0, 174.0, 175.0. MS m/z 340 $[M + H]^+$.

An oven-dried flask was charged with *N*-Boc-*tert*-leucinyl-*cis*-4,5-methanoprolineamide (65 mg, 0.19 mmol), imidazole (31 mg, 0.46 mmol), and pyridine (2 mL). The flask was sealed under nitrogen atmosphere and cooled to −30 °C. Slow addition of $POCl_3$ (132 mg, 81 μL, 0.86 mmol) gave, after mixing, a thick slurry that was mixed at −30 °C for 1 h and concentrated. The product was purified by silica gel column chromatography using 1:5 EtOAc−CH_2Cl_2. The product-containing fractions were pooled and concentrated under reduced pressure to give the desired *N*-Boc-protected title compound (50 mg, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.65 (q, J = 4.7, 1H), 0.99 (s, 9H), 1.00 (m, 1H), 1.42 (s, 9H), 1.89 (m, 1H), 2.00 (m, 1H), 3.80 (d, J = 9.2, 1H), 3.99 (m, 1H), 4.15 (d, J = 9.2, 1H), 4.72 (d, J = 4.9, 1H), 5.18 (d, J = 9.6, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.1, 18.5, 19.1, 26.2, 28.3, 35.3, 50.0, 50.7, 58.6, 79.9, 116.9, 156.0, 173.0. MS m/z 322 $[M + H]^+$.

An oven-dried flask was charged with *N*-Boc-*tert*-leucinyl-3,4-methanoprolinenitrile (0.45 mg, 0.14 mmol), CH_2Cl_2 (1 mL), and TFA (1 mL). The reaction mixture was stirred for 1 h at room temperature, diluted with toluene (4 mL) and concentrated under reduced pressure. The product was purified by reverse-phase preparative HPLC on a YMC S5 ODS 20 mm × 250 mm column to give the title compound (20 mg, 45%). Purification conditions were the following: gradient

elution from 10:90:0.1 CH_3OH-H_2O-TFA to 90:10:0.1 CH_3OH-H_2O-TFA over 18 min; flow rate, 20 mL/min; detection wavelength, 220 nm; t_R, 10 min. ^{1}H NMR (CD_3OD, 400 MHz) δ 0.55 (q, $J = 5.7$, 1H), 1.10 (s + m, 10H), 1.95 (m, 1H), 2.13 (m, 1H), 3.80 (m, 2H), 3.93 (s, 1H), 4.84 (d, $J = 5.0$, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.3, 19.7, 20.1, 26.8, 35.8, 52.1, 52.3, 60.5, 117.3 (q, $J = 289$), 119.2, 161.6 (q, $J = 38$), 170.5. Anal. ($C_{12}H_{19}N_3O\cdot 1.00$TFA) C, H, N, F.

Dipeptide Nitriles Prepared by General Method C. l-Isoleucinyl-L-*trans*-4,5-methanoprolinenitrile TFA Salt (22). ^{1}H NMR (D_2O, 500 MHz) δ 0.72 (m, 1H), 0.92 (t, $J = 7.3$, 3H), 1.07 (d+m, $J = 7.3$, 4H), 1.25 (m, 1H), 1.55 (m, 1H), 2.19 (m, 2H), 2.43 (m, 1H), 2.60 (m, 1H), 3.63 (m, 1H), 4.39 (d, $J = 5.5$, 1H), 4.82 (q, $J = 9.1$, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 10.7, 14.6, 18.2, 18.8, 23.7, 31.6, 36.4, 37.1, 50.9, 56.6, 116.6 (q, $J = 290$), 118.5, 163.2 (q, $J = 35$), 170.2; HPLC: method 3; $t_R = 2.89$ min. HRMS (FAB) *m/z*: $[M + H]^+$ calcd for $C_{12}H_{19}N_3O$, 222.1606; found, 222.1601.

L-Isoleucinyl-L-*trans*-2,3-methanoprolinenitrile TFA Salt (23). ^{1}H NMR (D_2O, 500 MHz) δ 0.84 (t, $J = 7.3$, 3H), 1.05 (d, $J = 7.3$, 3H), 1.14−1.30 (m 2H), 1.64 (m, 1H), 1.76 (m, 1H), 2.10 (m, 2H), 2.27 (m, 1H), 2.75 (m, 1H), 3.08 (m, 1H), 3.90 (m, 1H), 4.30 (d, $J = 2.3$, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 11.5, 15.0, 22.4, 23.6, 24.0, 28.1, 36.5, 42.0, 44.8, 60.7, 116.6 (q, $J = 290$), 117.7, 163.2 (q, $J = 35$), 165.6. HPLC: method 3; $t_R = 2.49$ min. HRMS (FAB) *m/z*: $[M + H]^+$ calcd for $C_{12}H_{19}N_3O$, 222.1606; found, 222.1611.

L-Isoleucinyl-L-*cis*-3,4-methanoprolinenitrile TFA Salt (25). ^{1}H NMR (500 MHz, D_2O) δ 0.53 (m, 1H), 0.90 (t, $J = 7.3$, 3H), 1.00 (d, $J = 6.9$, 3H), 1.08 (m, 1H), 1.18 (m, 1H), 1.48 (m, 1H), 2.00 (m, 2H), 2.20 (m, 1H), 3.83 (s, 2H), 4.10 (d, $J = 5.5$, 1H), 4.89 (d, $J = 5.0$, 1H); ^{13}C NMR (D_2O, 125 MHz) δ 10.1, 10.8, 14.6, 18.5, 23.8, 36.4, 50.7, 51.2, 56.4, 116.6 (q, $J = 290$), 118.1, 163.2 (q, $J = 35$), 170.4. HPLC: method 3; $t_R = 3.15$ min. HRMS (FAB) *m/z*: $[M + H]^+$ calcd for $C_{12}H_{19}N_3O$, 222.1606; found, 222.1605.

L-Valinyl-L-*cis*-3,4-methanoprolinenitrile TFA Salt (27). ^{1}H NMR (CD_3OD, 400 MHz) δ 0.55 (q, $J = 5.7$, 1H), 1.03 (d, $J = 6.5$, 3H), 1.08 (d + m, $J = 7.0$, 4H), 1.95 (m, 1H), 2.10 (m, 1H), 2.20 (m, 1H), 3.80 (d, $J = 9.5$, 1H), 3.86 (dd, $J = 9.5$, 4.8, 1H), 4.00 (d, $J = 5.7$, 1H), 4.84 (d, $J = 4.8$, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ 11.5, 17.8, 19.2, 19.8, 20.2, 31.7, 51.6, 52.1, 58.2, 118.4, 118.8 (q, $J = 289$), 162.0 (q, $J = 37$), 171.0. Anal. ($C_{11}H_{27}N_3O\cdot 1.30$TFA) C, H, N, F.

Representative Example of Preparation of Intermediates 19 and 20: General Method D. 2-Cyclopentylidenemalonic Acid Diethyl Ester (17b). A mixture of dry THF (200 mL) and dry CCl_4 (25 mL) was cooled to 0 °C and treated with $TiCl_4$ (11.0 mL, 0.1 mol). The resulting yellow suspension was stirred at 0 °C for 5 min, treated sequentially with cyclopentanone (4.4 mL, 0.05 mol) and freshly distilled diethyl malonate (7.6 mL, 0.05 mol), and then stirred at 0 °C for 0.5 h. The reaction mixture was then treated with a solution of dry pyridine (16 mL, 0.20 mol) in dry THF (30 mL) and stirred at 0 °C for 1.0 h and then at room temperature for 72 h. The reaction mixture was quenched with water and extracted with Et_2O (2 × 100 mL). The combined organic extracts were washed with brine and saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated under reduced pressure. The residue (12.1 g) was purified by flash column chromatography (EtOAc−hexanes, 5:95) to give the title compound (5.25 g, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30 (m, 6H), 1.73 (m, 4H), 2.68 (m, 4H), 4.24 (m, 4H). LC/MS *m/z*: 227 $[M + H]^+$.

2-(1-Methylcyclopent-1-yl)malonic Acid Monoethyl Ester (18b). A mixture of 3.0 M methylmagnesium iodide (3.1 mL, 9.4 mmol) and cuprous chloride (10.6 mg) was stirred at 0 °C, treated with a solution of 2-cyclopentylidenemalonic acid diethyl ester (**17b**) (1.41 g, 6.24 mmol) in dry Et_2O (2 mL) over 5 min, and stirred at 0 °C for 1 h and then at room temperature for 1 h. The reaction was then quenched by the dropwise addition of ice−water (15 mL) followed by 3 M HCl (3.7 mL). The mixture was then extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with 1% $Na_2S_2O_3$ and brine, dried ($MgSO_4$), and evaporated (1.57 g). The residue was purified by flash column chromatography on silica gel (5:95 Et_2O−hexanes) to give the diethyl ester compound (1.09 g, 68.7%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (s, 3H), 1.26 (t, $J = 7.0$, 6H), 1.57 (m, 2H), 1.55−1.70 (m, 6H), 3.34 (s, 1H), 4.20 (q, $J = 7.0$, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 23.7, 24.0, 38.6, 43.9, 60.7, 60.8, 168.7. LC/MS *m/z*: 243 $[M + H]^+$. A solution of 2-(1-methylcyclopentyl)malonic acid diethyl ester (736 mg, 3.04 mmol) in a mixture of EtOH (12 mL) and THF (6 mL) was treated with 1.0 M NaOH (3.04 mL, 3.04 mmol) and stirred at room temperature for 24 h. The reaction mixture was evaporated to a syrup, dissolved in water (10 mL), and extracted with Et_2O (15 mL). The aqueous phase was acidified with 1 M HCl to pH 2.0 and extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to give the title compound (0.87 g, 95%). $R_f = 0.56$ ($CH_2Cl_2-CH_3OH$, 9:1; PMA). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15 (s, 3H), 1.28 (t, $J = 7.0$, 3H), 1.55−1.70 (m, 8H), 3.75 (s, 1H), 4.20 (q, $J = 7.0$, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 23.2, 23.9, 38.5, 44.5, 60.4, 61.4, 169.7, 173.5. LC/MS *m/z*: 215 $[M + H]^+$.

Benzyloxycarbonylamino(1-methylcyclopent-1-yl)acetic Acid Ethyl Ester (19b). A solution of 2-(1-methylcyclopentyl)malonic acid monoethyl ester (0.48 g, 2.26 mmol) in dry benzene (2.85 mL) was treated with triethylamine (0.3 mL, 2.26 mmol) and diphenylphosphoryl azide (0.5 mL, 2.26 mmol), refluxed for 1 h, and cooled to room temperature. The solution was treated with benzyl alcohol (0.35 mL, 3.39 mmol), refluxed for 17 h, cooled, then partitioned between aqueous 10% citric acid solution (3.8 mL) and EtOAc (2 × 25 mL). The combined organic extracts were washed with 5% $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated. The remainder was purified by flash column chromatography (1:9 EtOAc−hexanes) to give the title compound (1.20 g, 100%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.92 (s + t, $J = 7.0$, 6H), 1.20−1.80 (m, 8H), 4.20 (m, 3H), 5.11 (s, 2H), 5.38 (m, 1H), 7.30−7.50 (m, 5H). LC/MS *m/z*: 342 $[M + Na]^+$.

***tert*-Butyloxycarbonylamino(1-methylcyclopent-1-yl)acetic Acid (20b).** A solution of benzyloxycarbonylamino(1-methylcyclopentyl)acetic acid ethyl ester (0.798 g, 2.26 mmol) in EtOAc (35 mL) was treated with 10% palladium hydroxide on carbon (190 mg) under an atmosphere of H_2 at room temperature for 20 h. The mixture was diluted with EtOAc and filtered through a Celite pad, washing the pad well with EtOAc (2 × 35 mL). The filtrate was evaporated to dryness to give the crude amine (0.42 g, 100%). The crude amine (0.42 g, 2.26 mmol) in a mixture of THF (9 mL) and water (9 mL) was treated with di-*tert*-butyl dicarbonate (0.73 g, 3.35 mmol) and K_2CO_3 (0.68 g, 4.54 mmol) and stirred at room temperature for 36 h. The reaction mixture was partitioned between water and Et_2O. The aqueous fraction was then extracted with Et_2O (3 × 30 mL), and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The remainder was purified by flash column chromatography (1:9 EtOAc−hexane) to give the desired *N*-Boc-protected ester (0.57 g, 92%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.95 (s, 3H), 1.28 (t, $J = 7.0$, 3H), 1.30 (m, 2H), 1.44 (s, 9H), 1.55−1.80 (m, 6H), 4.20 (q, $J = 7.0$, 2H), 5.11 (d, $J = 6.0$, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2, 22.5, 23.9, 24.3, 28.3, 36.8, 45.8, 60.8, 80.0, 155.7, 172.5. LC/MS *m/z*: 286 $[M + H]^+$. A solution of *tert*-butyloxycarbonylamino(1-methylcyclopentyl)acetic acid ethyl ester (0.55 g, 1.97 mmol) in MeOH (6.9 mL) and THF (6.9 mL) was treated with 1.0 M NaOH (2.9 mL, 2.9 mmol) and stirred at room temperature for 24 h. The reaction mixture was evaporated, dissolved in water (10 mL), and extracted with Et_2O. The aqueous phase was acidified to pH 2 with 1.0 M HCl (2.9 mL) and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to give the title compound (0.81 g, 96%): mp = 151−152 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.98 (s, 3H), 1.30 (m, 2H), 1.44 (s + m, 11H), 1.55−1.80 (m, 6H), 4.20 (d, $J = 7.0$, 2H), 5.11 (d, $J = 7.0$, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.5, 23.9, 24.3, 28.3, 36.9, 45.6, 60.8, 80.0, 155.7, 176.8. LC/MS *m/z*: 258 $[M + H]^+$.

The amino acids prepared according to this method were converted to the corresponding dipeptide nitriles using general method B.

Computational Assessment Methods. Conformations were generated for both methanoprolinenitrile and prolinenitrile forms of the N-terminal *tert*-leucine dipeptide and alanine dipeptides using Macromodel with OPLSAA parameters and GB/SA solvation model.[34] Two conformations were selected for energetic comparisons: the global minimum, which has the syn amide rotamer (Figure 2, lower structure), and an anti conformation, which brings the reactive amine and nitrile close to each other (Figure 2, upper structure). These conformations were reminimized using ab initio quantum mechanics with the B3LYP DFT method and the 6-31+G** basis set in Gaussian 98.0.[35] To approximate van der Waals and electrostatic contributions, relative energies of the conformations were also calculated using Insight's CFF force field.[36] Ab initio energetic minimizations and force field calculations were also performed on the proline and methanoproline compounds with a simple acetamide cap in both conformations.

In Vitro Assays. All compounds were tested in vitro against purified porcine DPP-IV as previously published.[37] Inhibition was determined using the substrate H-Gly-Pro-PNA. Production of *p*-nitroaniline was measured at 405 nm at 9 s intervals over 15 min. Reactions were initiated by addition of the enzyme, and data collection was started immediately. The reactions were run at 11 substrate concentrations (ranging from 60 to 3000 μM) and 7 inhibitor concentrations (ranging from 1 to 1000 nM). Enzyme reactions contained a final volume of 100 μL, ATE buffer (100 mM Aces, 52 mM Tris, 52 mM ethanolamine, pH 7.4), 4.5 nM porcine DPP-IV, and 1% DMSO.

In Vivo Assay Methods. Male Zucker$^{fa/fa}$ rats (Harlan) weighing between 400 and 450 g were housed in a room that was maintained on a 12 h light−dark cycle and were allowed free access to normal rodent chow and tap water. The day before the experiment, the rats were weighed and divided into control and treated groups of six. Rats were fasted 17 h prior to the start of the study. On the day of the experiment, animals were dosed orally with vehicle (water) or DPP-IV inhibitors (3 μmol/kg) at −30 min. Two blood samples were collected at −30 and 0 min by tail bleed. Glucose (2 g/kg) was administered orally at 0 min. Additional blood samples were collected at 15, 30, 60, 90, and 120 min. Blood samples were collected into EDTA containing tubes from Starstedt. Plasma glucose was determined by Cobas Mira (Roche Diagnostics) by the glucose oxidation method.

Rat plasma insulin was assayed using an ELISA kit from Crystal Chem Inc. Properly diluted plasma samples were added to the ELISA microplate. Insulin was then detected using guinea pig anti-insulin serum. DPP-IV activity in rat plasma was assayed ex vivo using Ala-Pro-AFC·TFA, a fluorescence-generating substrate from Enzyme Systems Products. A 20 μL plasma sample was mixed with 200 μL of reaction buffer, 50 mM Hepes, and 140 mM NaCl. The buffer contained 0.1 mM Ala-Pro-AFC·TFA. Fluorescence was then read for 20 min on a Perseptive Biosystem Cytofluor-II at 360 nm excitation wavelength and 530 nm emission wavelength. The initial rate of DPP-IV enzyme activity was calculated over the first 20 min of the reaction, with units/mL defined as the rate of increase of fluorescence intensity (arbitrary units) per milliliter of plasma. All in vivo data presented are the mean ± SE (n = 6). Data analysis was performed using ANOVA followed by Fisher Post-hoc.

Acknowledgment. The authors thank Dr. Robert Zahler for helpful comments in the editing of this manuscript.

Supporting Information Available: Crystallographic data and atomic coordinate information for **29**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) King, H.; Aubert, R. E.; Herman, W. H. Global Burden of Diabetes, 1995−2025: Prevalence, Numerical Estimates and Projections. *Diabetes Care* **1998**, *21*, 1414−1431. (b) Wagman, A. S.; Nuss, J. M. Current Therapies and Emerging Targets for the Treatment of Diabetes. *Curr. Pharm. Des.* **2001**, *7*, 417−450. (c) Harris, M. I.; Hadden, W. C.; Knowler, W. C.; Bennet, P. H.; Prevalence of Diabetes and Impaired Glucose Tolerance and Plasma Glucose Levels in U.S. Population Aged 20−74 yr. *Diabetes* **1987**, *36*, 523−534.

(2) (a) Saltiel, A. R.; Olefsky, J. M. Thiazolidinediones in the Treatment of Insulin Resistance and Type II Diabetes. *Diabetes* **1996**, *45*, 1661−1669. (b) Schoonjans, K.; Martin, G.; Staels, B.; Auwerx, J. Peroxisome Proliferator-Activated Receptors, Orphans with Ligands and Functions. *Curr. Opin. Lipidol.* **1997**, *8*, 159−166. (c) Pittas, A. G.; Greenberg, A. S.; Thiazolidinediones in the Treatment of Type 2 Diabetes. *Expert Opin. Pharmacother.* **2002**, *3*, 529−540. (d) Rami, H. K.; Smith, S. A.; Synthetic Ligands for PPARγ—Review of Patent Literature 1994−1999. *Expert Opin. Ther. Pat.* **2000**, *10*, 623−634. (e) Van Gaal, L.; Scheen, A. J. Are All Glitazones the Same? *Diabetes Metab. Res. Rev.* **2002**, *18*, S1−S4. (f) Hauner, H. The Mode of Action of Thiazolidinediones. *Diabetes Metab. Res. Rev.* **2002**, *18*, S10−S15.

(3) (a) Rosenstock, J.; Schwartz, S. L.; Clark, C. M., Jr.; Park, G. D.; Donley, D. W.; Edwards, M. B. Basal Insulin Therapy in Type 2 Diabetes: 28-Week Comparison of Insulin Glargine (HOE 901) and NPH Insulin. *Diabetes Care* **2001**, *24*, 631−636. (b) Barnett, A. H.; Owens, D. R. Insulin Analogues. *Lancet* **1997**, *349*, 47−51.

(4) (a) Anonymous. United Kingdom Prospective Diabetes Study Group. United Kingdom Prospective Diabetes Study (UKPDS) 13: Relative Efficacy of Randomly Allocated Diet, Sulphonylurea, Insulin, or Metformin in Patients with Newly Diagnosed Non-Insulin Dependent Diabetes Followed for Three Years. *Br. Med. J.* **1995**, *310*, 83−88. (b) U.K. Prospective Diabetes Study Group. U.K. Prospective Diabetes Study 7: Response of Fasting Plasma Glucose to Diet Therapy in Newly Presenting Type II Diabetic Patients. *Metabolism* **1990**, *39*, 905−912. (c) Gerich, J. E. Oral Hypoglycemic Agents. *N. Engl. J. Med.* **1989**, *321*, 1231−1245.

(5) (a) Witters, L. The Blooming of the French Lilac. *J. Clin. Invest.* **2001**, *108*, 1105−1107. (b) Bailey, C. J.; Path, M. R. C.; Turner, R. C. Metformin *N. Engl. J. Med.* **1996**, *334*, 574−579.

(6) (a) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M**.** Recent Developments of Transition-State Analogue Glycosidase Inhibitors of Non-Natural Product Origin. *Chem. Rev.* **2002**, *102*, 515−553. (b) Berecibar, A.; Grandjean, C.; Siriwardena, A. Synthesis and Biological Activity of Natural Aminocyclopentitol Glycosidase Inhibitors: Mannostatins, Trehazolin, Allosamidins, and Their Analogs. *Chem. Rev.* **1999**, *99*, 779−844. (c) Jacob, G. S. Glycosylation Inhibitors in Biology and Medicine. *Curr. Opin. Struct. Biol.* **1995**, *5*, 605−611.

(7) (a) Holst, J. J. Glucagon-Like Peptide-1 (GLP-1) a Newly Discovered GI Hormone. *Gastroenterology* **1994**, *107*, 1048−1055. (b) Orsakov, C. Glucagon-Like Peptide-1, a New Hormone of the Enteroinsular Axis. *Diabetologia* **1992**, *35*, 701−711. (c) Drucker, D. J. Glucagon-Like Peptides. *Diabetes* **1998**, *47*, 159−169.

(8) (a) Nauck, M. A.; Kleine, N.; Orskov, C.; Holst, J. J.; Willms, B.; Creutzfeldt, W. Normalization of Fasting Hyperglycaemia by Exogenous Glucagon-Like Peptide 1 (7−36 amide) in Type 2 (non-insulin-dependent) Diabetic Patients. *Diabetologia* **1993**, *36*, 741−744. (b) Nauck, M. A.; Wollschlager, D.; Werner, J.; Holst, J. J.; Orskov, C.; Creutzfeldt, W.; Willms, B. Effects of Subcutaneous Glucagon-Like Peptide 1 (GLP-1 [7−36 amide] in Patients with NIDDM. *Diabetologia* **1996**, *39*, 1546−1553. (c) Toft-Nielsen, M. B.; Madsbad, S.; Holst, J. J. Continuous Subcutaneous Infusion of Glucagon-Like Peptide 1 (7−36) Amide Lowers Plasma Glucose and Reduces Appetite in Type 2 Diabetic Patients. *Diabetes Care* **1999**, *22*, 1137−1143. (d) Juhl, C. B.; Hollingdal, M.; Sturis, J.; Jakobsen, G.; Agerso, H.; Veldhuis, J.; Porksen, N.; Schmitz, O. Bedtime Administration of NN2211, a Long-Acting GLP-1 Derivative, Substantially Reduces Fasting and Postprandial Glycemia in Type 2 Diabetes. *Diabetes* **2002**, *51*, 424−429. (e) Kolterman, O. G.; Buse, J. B.; Fineman, M. S.; Gaines, E.; Heintz, S.; Bicsak, T. A.; Taylor, K.; Kim, D.; Aisporna, M.; Wang, Y.; Baron, A. D. Synthetic Exendin-4 (Exenatide) Significantly Reduces Postprandial and Fasting Plasma Glucose in Subjects with Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **2003**, *88*, 3082−3089.

(9) (a) Wettergren, A.; Wojdemann, M.; Holst, J. J. The Inhibitory Effect of Glucagon-Like Peptide-1 (7−36) Amide on Antral Motility Is Antagonized by its N-Terminally Truncated Primary Metabolite GLP-1 (9−36) Amide. *Peptides* **1998**, *19* (5), 877−882. (b) Knudsen, L B.; Pridal, L. Glucagon-Like Peptide-1 (9−

36) Amide Is a Major Metabolite of Glucagon-Like Peptide-1 (7−36) Amide after in Vivo administration to Dogs, and It Acts as an Antagonist on the Pancreatic Receptor. *European J. Pharmacol.* **1996**, *318*, 429−435.

(10) (a) Hansen, L.; Deacon, C. F.; Orskov, C.; Holst, J. J. Glucagon-Like Peptide-1 (7−36) Amide Is Transformed to Glucagon-Like Peptide-1 (9−36) Amide by Dipeptidyl Peptidase IV in the Capillaries Supplying the L-Cells of the Porcine Intestine. *Endocrinology* **1999**, *140*, 5356−5363. (b) Kieffer, T. J.; McIntosh, C. H. S.; Pederson, R. A. Degradation of Glucose-Dependent Insulinotropic Polypeptide and Truncated Glucagon-Like Peptide-1 in Vitro and in Vivo by Dipeptidyl Peptidase IV. *Endocrinology* **1995**, *136*, 3585−3597.

(11) (a) Drucker, D. J. Therapeutic Potential of Dipeptidyl Peptidase IV Inhibitors for the Treatment of Type 2 Diabetes. *Expert Opin. Invest. Drugs* **2003**, *12*, 87−96. (b) Augustyns, K.; Van der Veken, P.; Senten, K.; Haemers, A. Dipeptidyl Peptidase IV Inhibitors as New Therapeutic Agents for the Treatment of Type 2 Diabetes. *Expert Opin. Ther. Pat.* **2003**, *13*, 499−510. (c) Zhang, B. B.; Moller, D. E. New Approaches in the Treatment of Type 2 Diabetes. *Curr. Opin. Chem. Biol.* **2000**, *4*, 461−467. (d) Holst, J. J.; Deacon, C. F. Perspectives in Diabetes: Inhibition of the Activity of Dipeptidyl-Peptidase IV as a Treatment for Type 2 Diabetes. *Diabetes* **1998**, *47*, 1663−1670. (e) Hildebrandt, M.; Reutter, W.; Arck, P.; Rose, M.; Klapp, B. F. A Guardian Angel: The Involvement of Dipeptidyl Peptidase IV in Psychoneuroendocrine Function, Nutrition and Immune Defence. *Clin. Sci.* **2000**, *99*, 93−104. Additional studies in DPP-IV knockout animals have supported these findings: (f) Marguet, D.; Baggio, L.; Kobayashi, T.; Bernard, A.-M.; Pierres, M.; Nielsen, P. F.; Ribel, U.; Watanabe, T.; Drucker, D. J.; Wagtmann, N. Enhanced Insulin Secretion and Improved Glucose Tolerance in Mice Lacking CD26. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6864−6879. (g) Nagakura, T.; Yasuda, N.; Yamazaki, K.; Ikuta, H.; Yoshikawa, S.; Asano, O.; Tanaka, I. Improved Glucose Tolerance via Enhanced Glucose-Dependent Insulin Secretion in Dipeptidyl Peptidase IV-Deficient Fischer Rats. *Biochem. Biophys. Res. Commun.* **2001**, *284*, 501−506.

(12) (a) Coutts, S. J.; Kelly, T. A.; Snow, R. J.; Kennedy, C. A.; Barton, R. W.; Adams, J.; Krolikowski, D. A.; Freeman, D. M.; Campbell, S. J.; Ksiazek, J. F.; Bachovchin, W. W. Structure−Activity Relationships of Boronic Acid Inhibitors of Dipeptidyl Peptidase IV. 1. Variation of the P2 Position of Xaa-boroPro Dipeptides. *J. Med. Chem.* **1996**, *39*, 2087−2094. (b) Wallner, B. Inhibitors of Dipeptidyl Peptidase IV (DPP-IV) for Regulation of Substrate Activity and Therapeutic Use. PCT Int. Appl. . WO 0010549 A1, 2000 (38 pages). (c) Huber, B. T.; Schmitz, T. Underwood, R. Potentiation of the Immune Response through Delivery of Compounds Binding a Cytoplasmic Dipeptidase. PCT Int. Appl. WO 9850066 A1, 1998 (26 pages).

(13) (a) Senten, K.; Van der Veken, P.; Bal, G.; Haemers, A.; Augustyns, K. Polymer-Assisted Solution-Phase Parallel Synthesis of Dipeptide *p*-Nitroanilides and Dipeptide Diphenylphosphonates. *Tetrahedron Lett.* **2001**, *42*, 9135−9138. (b) Belyaev, A.; Borloo, M.; Augustyns, K.; Lambeir, A.-M.; De Meester, I.; Scharpe, S.; Blaton, N.; Peeters, O. M.; De Ranter, C.; Haemers, A. A New Synthetic Method for Proline Diphenylphosphonates. *Tetrahedron Lett.* **1995**, *36*, 3755−3758.

(14) (a) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. 2-Cyanopyrrolidides as Potent, Stable Inhibitors of Dipeptidyl Peptidase IV. *Bioorg. Med. Chem Lett.* **1996**, *6*, 1163−1166. (b) Jenkins, P. D.; Jones, D. M.; Szelke, M. Preparation of Peptide Analog DP-IV Serine Protease Inhibitors. PCT Int. Appl. WO 9515309 A1, 1995 (55 pages).

(15) (a) Villhauer, E. B. Preparation of 1-Glycyl-2-cyanopyrrolidines as Dipeptidyl Peptidase-IV Inhibitors**.** U.S. 6011155 A, 2000 (20 pages). (b) Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. NVP-DPP728 (1-[[[2-[(5-Cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine), a Slow-Binding Inhibitor of Dipeptidyl Peptidase IV. *Biochemistry* **1999**, *38*, 11597−11603. (c) 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. 1-[2-[(5-Cycanopyridine-2-yl)-amino]ethylamino]acetyl-2-(*S*)-pyrrolidine-carbonitrile: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties. *J. Med. Chem.* **2002**, *45*, 2362−2365.

(16) 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. 1-[[3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties. *J. Med. Chem.* **2003**, *46*, 2774−2789.

(17) (a) Sorbera, L. A.; Revel, L.; Castaner, J. P32/98: Antidiabetic Dipeptidyl-Peptidase IV Inhibitor. *Drugs Future* **2001**, *26*, 859−864. (b) Pauly, R. P.; Demuth, H.-U.; Rosche, F.; Schmidt, J.; White, H. A.; Lynn, F.; Mclntosh, C. H. S.; Pederson, R. A. Improved Glucose Tolerance in Rats Treated with the Dipeptidyl Peptidase IV (CD26) Inhibitor Ile-thiazolidide**.** *Metab., Clin. Exp.* **1999**, *48*, 385−389. (c) Pederson, R. A.; White, H. A.; Schlenzig, D.; Pauly, R. P.; McIntosh, C. H. S.; Demuth, H.-U. Improved Glucose Tolerance in Zucker Fatty Rats by Oral Administration of the Dipeptidyl Peptidase IV Inhibitor Isoleucine Thiazolidide. *Diabetes* **1998**, *47*, 1253−1258. Some very recent notable exceptions to this trend of reduced potency in dipeptide-based inhibitors lacking a nitrile moiety can be found in the following references from the Merck group: (d) Parmee, E. R.; He, J.; Mastracchio, A.; Edmondson, S. D.; Colwell, L.; Eiermann, G.; Feeney, W. P.; Habulihaz, B.; He, H.; Kilburn, R.; Leiting, B.; Lyons, K.; Marsilio, F.; Patel, R. A.; Petrov, A.; Di Salvo, J.; Wu, J. K.; Thornberry, N. A.; Weber, A. E. 4-Aminocyclohexylglycine Analogues as Potent Dipeptidyl Peptidase IV Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 43−46. (e) Ashton, W. T.; Dong, H.; Sisco, R. M.; Doss, G. A.; Leiting, B.; Patel, R. A.; Wu, J. K.; Marsilio, F.; Thornberry, N. A.; Weber, A. E. Diastereoselective Synthesis and Configuration-Dependent Activity of (3-Substitutedcycloalkyl)glycine Pyrrolidides and Thiazolidides as Dipeptidyl Peptidase IV Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 859−863. (f) Caldwell, C. G.; Chen, P.; He, J.; Parmee, E. R.; Leiting, B.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Eiermann, G. J.; Petrov, A.; He, H.; Lyons, K. A.; Thornberry, N. A.; Weber, A. E. Fluoropyrrolidine Amides as Dipeptidyl Peptidase IV Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1265−1268.

(18) (a) Hanessian, S.; Reinhold, U.; Saulnier, M.; Claridge, S. Probing the Importance of Spatial and Conformational Domains in Captopril Analogs for Angiotensin Converting Enzyme Activity. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2123−2128. (b) Hanessian, S.; Reinhold, U.; Gentile, G. The Synthesis of Enantiopure ω-Methanoprolines and ω-Methanopipecolic Acids by a Novel Cyclopropanation Reaction: The "Flattening" of Proline. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1881−1884.

(19) (a) Sasaki, N. A.; Sagnard, I.; Ciaroni, A.; Riche, C.; Potier, P. Enantioselective Synthesis of Cyclopropane α-Amino Acids: Synthesis of *N*-Boc-*cis*-(2*S*,3*R*,4*S*)-3,4-Methanoproline and *N*-Boc-(2*S*,3*R*,4*S*)-3,4-Methanoglutamic Acid. *Tetrahedron Lett.* **1995**, *36*, 3149−3152. (b) Sasaki, N. A.; Sagnard, I. A Novel Method for Chirospecific Synthesis of 2,5-Disubstituted Pyrrolides. *Tetrahedron* **1994**, *50*, 7093−7108. (c) Tverezovsky, V. V.; Baird, M. S.; Bolesov, I. G. Synthesis of (2*S*,3*R*,4*S*)-3,4-Methanoproline and Analogues by Cyclopropylidene Insertion. *Tetrahedron* **1997**, *53*, 14773−14792.

(20) (a) Hercouet, A.; Bessieres, B.; Le Corre, M. First Asymmetric Synthesis of (−)-(2*S*,3*R*)-Methanoproline. *Tetrahedron: Asymmetry* **1996**, *7*, 1267−1268. (b) Stammer, C. H.; Switzer, F. L.; Van Halbeek, H.; Holt, E. M. Synthesis of (±)-2,3-Methanoproline: A Novel Inhibitor of Ethylene Biosynthesis. *Tetrahedron* **1989**, *45*, 6091−6100.

(21) (a) Grayson, I. Water-Soluble Carbodiimide−An Efficient Coupling Agent for Synthesis. *Spec. Chem.* **2000**, *20*, 86−88. (b) Benoiton, N. L.; Kuroda, K**.** Studies on Racemization During Couplings Using a Series of Model Tripeptides Involving Activated Residues with Unfunctionalized Side Chains. *Int. J. Pept. Protein Res.* **1981**, *17*, 197−204. (c) Hoeg-Jensen, T.; Havsteen J. M.; Holm, A. A New Method for Rapid Solution Synthesis of Shorter Peptides by Use of (Benzotriazolyloxy)tripyrrolidinophosphonium hexafluorophosphate (Py BOP). *Tetrahedron Lett.* **1991**, *32*, 6387−6390. (d) Frerot, E.; Coste, J.; Pantaloni, A.; Dufour, M., N.; Jouin, P. PyBOP and PyBroP: Two Reagents for the Difficult Coupling of the α,α-Dialklylamino Acid, AIB**.** *Tetrahedron* **1991**, *47*, 259−270.

(22) Stuber, W.; Kosina, H.; Heimburger, N. Synthesis of a Tripeptide with a C-Terminal Nitrile Moiety and the Inhibition of Proteinases**.** *Int. J. Pept. Protein Res.* **1988**, *31*, 63−70.

(23) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley & Sons: New York, 1991; pp 315−347.

(24) (a) Lehnert W. Knovenagel-Kondensation Mit $TiCl_4$/Base-III. Umsetzen Von Ketonen und α-Halogenketonen Mit Malonester (Conversion from Ketones and α-Halogen Ketones with Malonic Ester). *Tetrahedron* **1973**, *29*, 635−638. (b) Holmberg, C. Synthese und Eigenschafter einiger *tert*-Alkylmalonsauredierivate (Synthesis of *tert*-Alkylmalonic Acid Derivatives). *Leibigs Ann. Chem.* **1981**, 748−760. (c) Yamada, S.-I.; Ninomiya, K.; Shioiri, T. Transfer of the Azido Function from Diphenylphophoryl Azide to Malonic Acid Half Esters. *Tetrahedron Lett.* **1973**, *26*, 2343−2346.

(25) In cases where racemic Boc-protected amino acids were coupled to the enantiomerically pure 4,5-methanoproline nitriles, the latter-eluting isomer on silica gel chromatography (10−40% EtOAc−hexanes) consistently provided the L,L-diastereomer. The stereochemistry was established in several cases through single-crystal X-ray crystallography, though because this stereochemistry has correlated with inhibitory activity against DPP-IV (in

this series and in prolinenitrile inhibitors published by others), the latter was typically used as confirmation of stereochemistry.

(26) Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, M. D.; Szelke, M. 4-Cyanothiazolidides as Very Potent, Stable Inhibitors of Dipeptidyl Peptidase IV. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2745−2748.

(27) Snow, R. J.; Bachovchin, W. W.; Barton, R. W.; Campbell, S. J.; Coutts, S. J.; Freeman, D. M.; Gutheil, W. G.; Kelly, T. A.; Kennedy, C. A.; Krolikowski, D. A.; Leonard, S. F.; Pargellis, C. A.; Tong, L.; Adams, J. Studies on Proline Boronic Acid Dipeptide Inhibitors of Dipeptidyl Peptidase IV: Identification of a Cyclic Species Containing a B−N Bond. *J. Am. Chem. Soc.* **1994**, *116*, 10860−10869.

(28) Crystallographic data (excluding structure factors) for the TFA salt of **29** have been deposited with the Cambridge Crystallographic Data Centre. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax, +44 1223 336033; e-mail, deposit@ccdc.cam.ac.uk).

(29) (a) Rasmussen, H. B.; Branner, S.; Wiberg, F. C.; Wagtmann, N. Crystal Structure of Human Dipeptidyl Peptidase IV/CD26 in Complex with a Substrate Analog. *Nat. Struct. Biol.* **2003**, *10*, 19−25. (b) Engel, M.; Torsten, H.; Wagner, L.; Wermann, M.; Heiser, U.; Kiefersauer, R.; Huber, R.; Bode, W.; Demuth, H.-U.; Brandstetter, H. The Crystal Structure of Dipeptidyl Peptidase IV (CD26) Reveals Its Functional Regulation and Enzymatic Mechanism. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5063−5068. (c) Thoma, R.; Löffler, B.; Stihle, M.; Huber, W.; Ruf, A.; Hennig, M. Structural Basis of Proline-Specific Exopeptidase Activity as Observed in Human Dipeptidyl Peptidase-IV. *Structure* **2003**, *11*, 947−959. (d) Oefner, C.; D'Arcy, A.; MacSweeney, A.; Pireau, S.; Gardiner, R.; Dale, G. E. High Resolution Structure of Human Apo Dipeptidyl Peptidase IV/CD26 and Its Complex with 1-[({2-[(5-Iodopyridin-2-yl)amino]ethyl}amino)-acetyl]-2-cyano-(*S*)-pyrrolidine. *Acta Crystallogr.* **2003**, *D59*, 1206−1212. (e) Hiramatsu, H.; Kyono, K.; Higashiyama, Y.; Fukushima, C.; Shima, H.; Sugiyama, S.; Inaka, K.; Yamamoto, A.; Shimizu, R. The Structure and Function of Human Dipeptidyl Peptidase IV, Possessing a Unique Eight-Bladed Beta-Propeller Fold. *Biochem. Biophys. Res. Commun.* **2003**, *302*, 849−854.

(30) Baxter, P. N. W.; Connor, J. A.; Povey, D. C.; Wallis, J. D. A Model for the Attack of a Nucleophile on a Nitrile Group: The X-ray Crystal Structure of 2,2′-Bipyridine-3,3′-dicarbonitrile. *J. Chem. Soc., Chem. Commun.* **1991**, *16*, 1135−1137. This angle is obtained from X-ray crystal structure data that advocate the favored direction of approach of a nucleophile to an sp carbon of a nitrile making an angle of at least 108°. Additionally, Aray and Murgich have reported a similar value of 103° based on analysis of charge density from ab initio calculations on CH_3-CN: Aray, Y.; Murgich, J. Nucleophilic Attack on the Cyano Group: A Description in Terms of the Laplacian of the Molecular Charge Density. *THEOCHEM* **1993**, *106*, 229−233.

(31) (a) Holst, J. J.; Deacon, C. F. Inhibition of the Activity of Dipeptidyl-Peptidase IV as a Treatment for Type 2 Diabetes. *Diabetes* **1998**, *47*, 1663−1670. (b) Balkan, B.; Kwansnik, L.; Miserendino, R.; Holst, J. J.; Li, X. Inhibition of Dipeptidyl Peptidase IV with NVP-DPP728 Increases Plasma GLP-1 (7−36) Amide Concentrations and Improves Oral Glucose Tolerance in Obese Zucker Rats. *Diabetologia* **1999**, *42* (11), 1324−1331. (c) Rothenberg, P.; Kalbag, J.; Smith, H. T.; Gingerich, R.; Nedelman, J.; Villhauer, E.; McLeod, J.; Hughes, T. Treatment with a DPP-IV Inhibitor, NVP-DPP728, Increases Prandial Intact GPL-1 Levels and Reduces Glucose Exposure in Humans. *Diabetes* **2000**, *49* (1), A39.

(32) Truett, G.; Bahary, N.; Friedman, J. M.; Leibel, R. L. The Zucker Rat Obesity Gene Fatty (fa) Maps to Chromosome 5 and Is a Homologue of the Mouse Diabetes (db) Gene. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7806−7809.

(33) Pederson, R. A.; White, H. A.; Schlenzig, D.; Pauly, R. P.; McIntosh, C. H.; Demuth, H. U. Improved Glucose Tolerance in Zucker Fatty Rats by Oral Administration of the Dipeptidyl Peptidase IV Inhibitor Isoleucine Thiazolidide. *Diabetes* **1998**, *47*, 1253−1258.

(34) *Maestro*, version 4.1.012, and *Mmshare*, version 1.0.067; Schrodinger, Inc.: Portland, OR, 1999−2001.

(35) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.6; Gaussian, Inc.: Pittsburgh, PA, 1998.

(36) *Insight II*, version 2000; Molecular Simulations Inc.: San Diego, CA, 2000.

(37) Wang, A.; Huang, Y.; Taunk, P.; Magnin, D. R.; Ghosh, K.; Robertson, J. G. Application of Robotics to Steady State Enzyme Kinetics: Analysis of Tight-Binding Inhibitors of Dipeptidyl Peptidase IV. *Anal. Biochem.* **2003**, *321*, 157−166.

JM049924D