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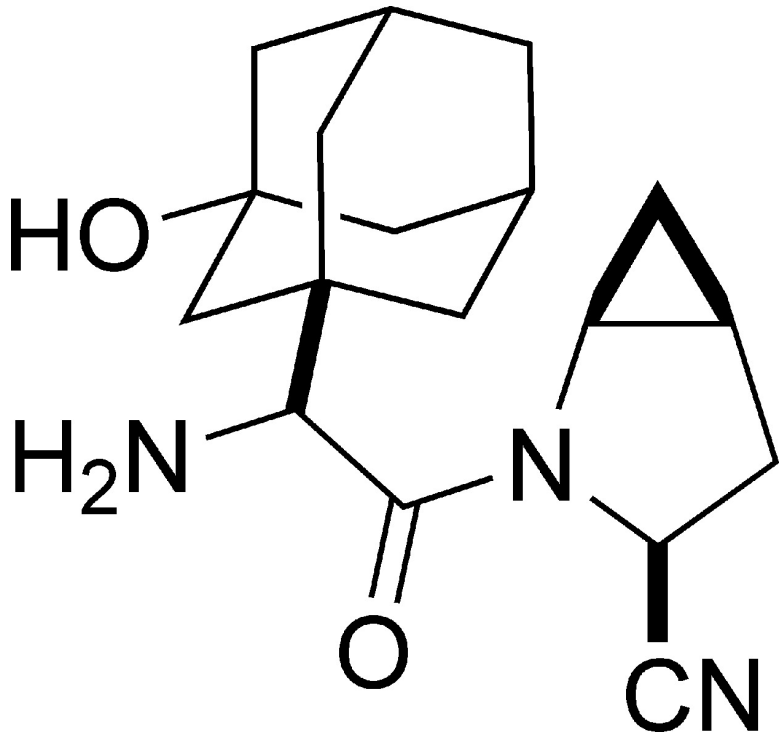

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Discovery and Preclinical Profile of Saxagliptin (BMS-477118): A Highly Potent, Long-Acting, Orally Active Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes

David J. Augeri,*[,†,‡] Jeffrey A. Robl,[†] David A. Betebenner,[†,§] David R. Magnin,[†] Ashish Khanna,[∥] James G. Robertson,[⊥] Aiying Wang,[⊥] Ligaya M. Simpkins,[†] Prakash Taunk,[†] Qi Huang,[⊥] Song-Ping Han,[⊥] Benoni Abboa-Offei,[⊥] Michael Cap,[⊥] Li Xin,[⊥] Li Tao,[#] Effie Tozzo,[⊥] Gustav E. Welzel,[⊥] Donald M. Egan,[⊥] Jovita Marcinkeviciene,[⊗] Shu Y. Chang,[∥] Scott A. Biller,[†,∞] Mark S. Kirby,[⊥] Rex A. Parker,[⊥] and Lawrence G. Hamann*[,†]

Departments of Discovery Chemistry, Metabolic Diseases, Pharmaceutical Candidate Optimization, Exploratory Pharmaceutics, Chemical Enzymology, Bristol-Myers Squibb, Pharmaceutical Research Institute, P.O. Box 5400, Princeton, New Jersey 08543-5400

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Efforts to further elucidate structure−activity relationships (SAR) within our previously disclosed series of β-quaternary amino acid linked L-*cis*-4,5-methanoprolinenitrile dipeptidyl peptidase IV (DPP-IV) inhibitors led to the investigation of vinyl substitution at the β-position of α-cycloalkyl-substituted glycines. Despite poor systemic exposure, vinyl-substituted compounds showed extended duration of action in acute rat ex vivo plasma DPP-IV inhibition models. Oxygenated putative metabolites were prepared and were shown to exhibit the potency and extended duration of action of their precursors in efficacy models measuring glucose clearance in Zucker$^{fa/fa}$ rats. Extension of this approach to adamantylglycine-derived inhibitors led to the discovery of highly potent inhibitors, including hydroxyadamantyl compound BMS-477118 (saxagliptin), a highly efficacious, stable, and long-acting DPP-IV inhibitor, which is currently undergoing clinical trials for treatment of type 2 diabetes.

Introduction

Primary defects in insulin secretion, along with development of insulin resistance, contribute to the etiology of type 2 diabetes mellitus. Diminished postprandial insulin secretion resulting from both functional defects and loss of survival of pancreatic β-cells progresses into hyperglycemia and declining insulin sensitivity. As lifestyle trends and dietary factors have contributed to an alarming rise in the incidence of type 2 diabetes,[1] the search for novel mechanistic approaches to control this chronic metabolic disease has intensified in parallel. To complement the currently available diabetes treatments,[2] approaches operating within the enteroinsular axis through the incretin hormone glucagon-like peptide 1 (GLP-1), alone or in combination with other agents, are beginning to show promise in the treatment of diabetes.[3] GLP-1 is a major component of the prandial nutrient-sensing mechanism regulating insulin secretion following meals.[4] Intact, active GLP-1(7−36) amide is secreted into the circulation from intestinal L-cells in response to dietary signals. Concentrations of GLP-1(7−36) amide sufficient to activate the GLP-1 receptor expressed on pancreatic β-cells result in increased insulin secretion, delayed glucose absorption, and reduced hepatic glucose production. All of these components work in concert to modulate blood glucose levels. Because GLP-1 release is nutrient stimulated, this mechanism promotes insulin secretion under prandial glycemia conditions, minimizing the potential for hypoglycemia. Recent reports have further demonstrated a beneficial effect of agents acting through the GLP-1 axis on the preservation and/or restoration of β-cell function in animals,[5] suggesting the exciting possibility that emerging drugs acting in this pathway may lead to improvement of the diabetic condition.

GLP-1 is rapidly truncated during its secretion in the ileum by the dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5) located on the capillary endothelium proximal to the L-cells where GLP-1 is secreted. The efficient cleavage by DPP-IV of the N-terminal dipeptide His-Ala from GLP-1(7−36) amide yields GLP-1(9−36) amide, a weak antagonist of the receptor,[6] and this cleavage has been demonstrated to be the primary physiological route of degradation of GLP-1(7−36) amide in both humans and animals.[7] The rapid cleavage by DPP-IV results in an apparent elimination half-life of only 60−90 s for GLP-1(7−36) amide, and peak circulating levels of intact GLP-1(7−36) amide typically do not exceed 5−10 pM, a range bracketing its K_a as GLP-1 receptor agonist. Inhibition of DPP-IV prevents the degradation of the incretin hormones GLP-1 and glucose-dependent insulinotropic peptide (GIP) and has been demonstrated to potentiate the levels of these peptides in multiple species.[8]

* Corresponding author. Telephone: 609-818-5526. fax: 609-818-3550. E-mail: lawrence.hamann@bms.com.
† Discovery Chemistry.
‡ Present address: Lexicon Pharmaceuticals, 350 Carter Road, Princeton, NJ 08540.
§ Present address: Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL 60064.
∥ Pharmaceutical Candidate Optimization.
⊥ Metabolic Diseases.
Exploratory Pharmaceutics.
⊗ Chemical Enzymology.
∞ Present address: Novartis Institute for BioMedical Research, 250 Massachusetts Avenue, Cambridge, MA 02139.

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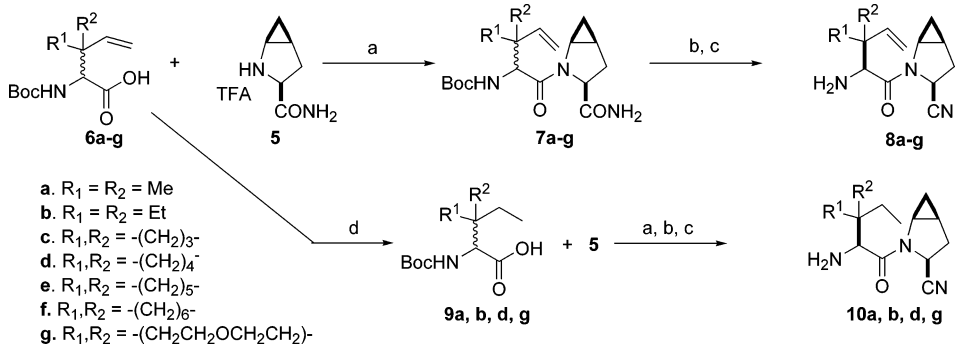
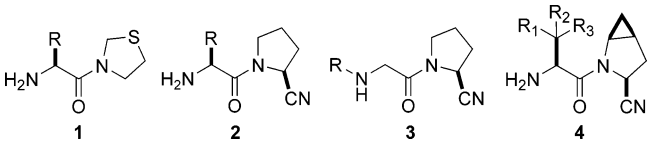
Scheme 1[a]



[a] (a) EDAC, HOBT, DMF; (b) $POCl_3$, pyridine, imidazole, −20 °C; (c) TFA, CH_2Cl_2, rt; (d) 5% Pd/C, H_2 1 atm, MeOH.

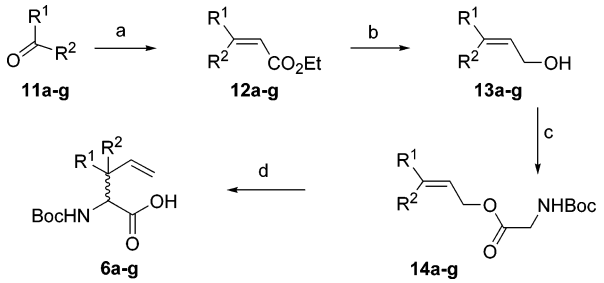
DPP-IV is a 240 kDa, 766 residue N-terminal dipeptidyl exopeptidase that is composed of two 110 kDa subunits[9] and exists as both a membrane-bound protein and as a soluble protein in plasma. It is a nonclassical serine protease that exhibits high specificity for peptides with proline or alanine in the P1 position. Any amino acid can occupy the P2 position so long as the P2−P1 peptide bond can adopt a trans configuration.[10] The membrane-bound form of DPP-IV is expressed in several tissues, including kidney, liver, the brush border membranes of intestinal enterocytes, on the pancreatic duct epithelia, and in vascular endothelial cells. In these tissues DPP-IV is N-terminally bound to the membrane with its catalytic activity located in the extracellular domain. The soluble, circulating form of DPP-IV is shed from cell surfaces by proteolytic cleavage releasing a fully active soluble form minus the 29 amino acids of the N-terminus.

Clinical evidence has shown that small molecule inhibitors of DPP-IV lower blood glucose levels, increase glucose tolerance, and improve insulin response to oral glucose in patients with type 2 diabetes.[11] Reversible small-molecule inhibitors of DPP-IV have been studied for the past several years, and a large body of structure−activity relationship (SAR) data has been generated.[3b,12] Until the very recent disclosures of several nonpeptidic chemotypes,[13,14] the known inhibitors had all been dipeptidomimetic in nature, bearing structural resemblance to the N-terminal dipeptide of the enzyme substrates. For this class of inhibitors, the penultimate N-terminal proline or proline mimetic, generally a thiazolidine (**1**),[15] a C-substituted or N-substituted



cyanopyrrolidine (**2**, **3**),[16,17] or a cyclopropanated cyanopyrrolidine (**4**),[18] is appended to an amino acid or an amino acid surrogate. Many inhibitors in this cyanopyrrolidine structural class have suffered from varying degrees of chemical instability which have hampered formulation efforts. In addition, many examples of this class exhibit limited pharmacodynamic duration of action. We report herein the discovery of highly efficacious long-acting inhibitors of DPP-IV that have led to the identification of compound **26** (BMS-477118, saxagliptin), which is currently undergoing clinical evaluation for the treatment of type 2 diabetes.

Scheme 2[a]



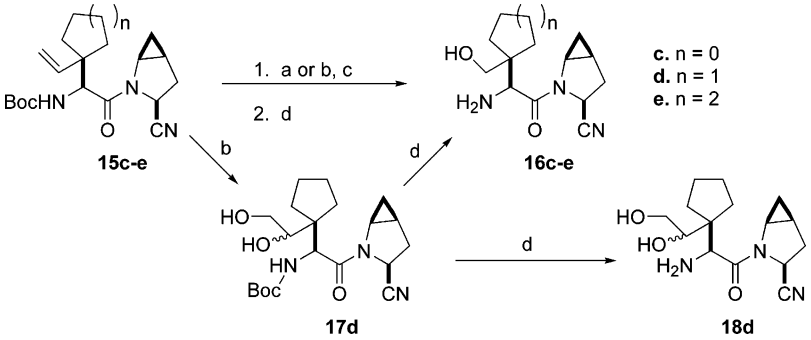
[a] (a) triethylphosphonoacetate, NaH, THF 0 °C to rt; (b) DIBAL-H, toluene, −78 °C to rt; (c) N−Boc glycine, DCC, DMAP, CH_2Cl_2, rt; (d) $ZnCl_2$, THF, LDA, −78 °C to rt.

Chemistry

To further our understanding of the SAR surrounding β-quaternary N-terminal amino acid-containing inhibitors, we focused on elaboration of our previously disclosed cyanomethanopyrrolidine-based scaffold[18] to produce long-acting inhibitors structurally related to the prototype scaffold **4**. A general synthesis route was chosen that incorporated at the β-position a vinyl substituent amenable to functionalization for further elucidation of SAR. Standard peptide coupling conditions[19] were employed to link enantiomerically pure L-methanoprolinamide core fragment **5** with various racemic vinyl-substituted amino acids **6a**−**g** to give dipeptides **7a**−**g** in yields of 85−95% (Scheme 1). Dehydration of the resultant amides using TFAA or $POCl_3$ gave the corresponding nitriles.[20] Chromatographic isolation of the bioactive L-isomer was generally carried out at the stage of the Boc-protected nitrile.[21] Finally, removal of the N-terminal Boc using TFA gave inhibitors **8a**−**g** in high yield. The vinyl groups of **6a**−**f** could be reduced (Pd/C, H_2) to afford the corresponding ethyl compounds, which were similarly elaborated to dipeptides **10a,b,d,g**.

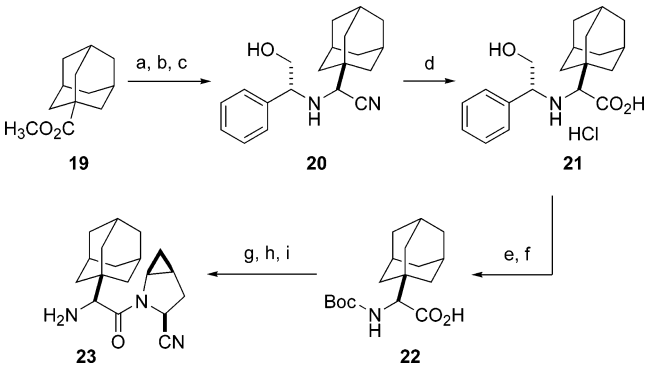
Amino acids possessing a β-quaternary vinyl group were prepared in a manner complementary to the malonate Knoevenagel/Michael addition sequence used previously.[18] Lewis acid-mediated ester enolate Claisen rearrangement of substituted glycinyl allylic esters[22] led directly to β-vinyl amino acids **6a**−**g** in 58−85% overall yields (Scheme 2). The requisite Claisen precursors were readily prepared in three steps from the appropriate ketones **11a**−**g**. Horner−Emmons olefination of ketones

Scheme 3[a]



[a] (a) O_3, MeOH/CH_2Cl_2 10:4, −78 °C; then $NaBH_4$, −78 to 0 °C, 60−79%; (b) OsO_4, NMNO, THF/H_2O 1:1, rt, 47−63%; (c) $NaIO_4$; workup, then $NaBH_4$, MeOH, rt, 56%; (d) TFA/CH_2Cl_2 1:2, 0 °C to rt.

Scheme 4[a]



[a] (a) LAH, THF, 0 °C to rt, 96%; (b) $(ClCO)_2$, DMSO, CH_2Cl_2, −78 °C, 98%; (c) (*R*)-(−)-2-phenylglycinol, $NaHSO_3$, KCN, 65%; (d) 12 M HCl, HOAc, 80 °C, 16 h, 78%; (e) 20% $Pd(OH)_2$, 50 psi H_2, MeOH/HOAc 5:1; (f) $(Boc)_2O$, K_2CO_3, DMF, 92%, two steps; (g) **5**, EDAC, HOBT, DMF, 92%; (h) $POCl_3$, pyridine, imidazole, −20 °C; (i) TFA, CH_2Cl_2, rt, quant.

11a−**g** with the ylide generated from triethylphosphonoacetate gave the α,β-unsaturated esters **12a**−**g** in 92−98% yield. Esters **12a**−**g** were then reduced with DIBAL to the corresponding allylic alcohols **13a**−**g** and condensed with *N*-Boc glycine using DCC/DMAP to give esters **14a**−**g** in 79−87% yield over two steps. $ZnCl_2$-mediated Claisen rearrangement of the LDA-generated enolate of glycine esters **14a**−**g** proceeded at low temperature to give the desired β-vinyl amino acids **6a**−**g** in 65−90% yield.

Further elaboration of vinyl-containing dipeptides **7c**−**e** was accomplished at the stage of the dehydrated cyano-containing compounds **15c**−**e** (Scheme 3). Oxidative cleavage of the vinyl substituent to prepare hydroxymethyl compounds **16c**−**e** was achieved either by ozonolysis/$NaBH_4$ reduction or by catalytic OsO_4−NMNO/$NaIO_4$/$NaBH_4$ conditions, followed by acidic deprotection of the Boc group. Additionally, **15d** was converted to the corresponding diol and deprotected to give **18d**.

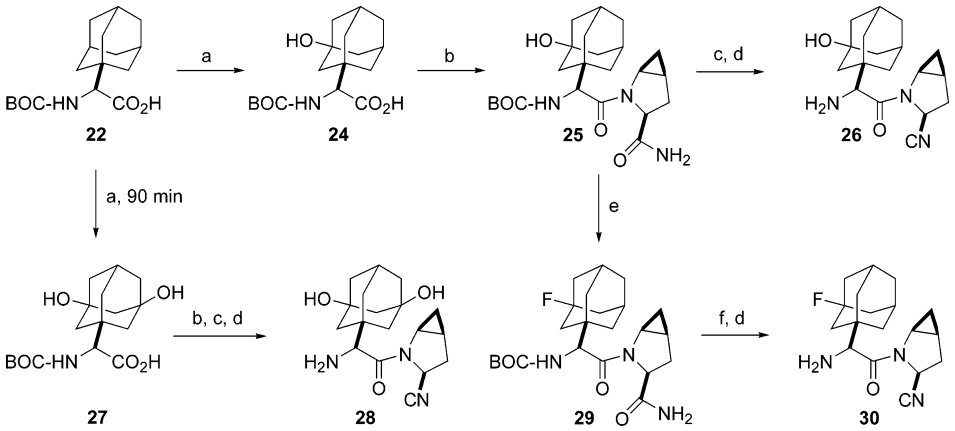
A logical extension of our previously observed SAR trends favoring β-branched P2 units led us to explore rigidly bridged polycyclic systems such as adamantyl. Analogues bearing an adamantyl ring at the *N*-terminal α-carbon were synthetically derived from a common homochiral adamantylglycine intermediate prepared using asymmetric Strecker chemistry (Scheme 4).[23] Reduction of commercially available adamantane carboxylic acid methyl ester **19** by LAH, followed by Swern oxidation, afforded the requisite aldehyde, which was then subjected to asymmetric Strecker conditions (condensation with (*R*)-(−)-2-phenylglycinol with addition of KCN) to give the desired homochiral *R,S* diastereomer **20** in 65% yield. Hydrolysis of the nitrile group to give acid **21**, followed by hydrogenolysis of the chiral auxiliary, afforded the enantiomerically pure amino acid **22**. Boc protection of the resulting primary amine, followed by coupling to methanoprolinamide core **5**, dehydration of the amide to nitrile, and deprotection, afforded the adamantylglycine containing inhibitor **23** in good overall yield.

Hydroxylation of *N*-Boc-adamantylglycine **22** at the bridgehead was accomplished using $KMnO_4$ in 2% aqueous KOH at elevated temperature to give *N*-Boc hydroxyadamantyl glycine **24** in 51% yield (Scheme 5).[24] Standard acylation conditions were used to couple **24** to methanoprolinamide core **5**, furnishing amide **25** in high yield. Amide **25** was subsequently elaborated to provide two additional analogues. Dehydration of amide **25** with TFAA, followed by in situ basic hydrolysis of the resulting trifluoroacetate and deprotection of the N-terminus, gave hydroxy derivative **26** in 87% yield over three steps. The hydroxy group of **25** was subjected to fluoride substitution using DAST,[24] and subsequent dehydration using $POCl_3$ in pyridine, followed by deprotection of the terminal nitrogen, provided fluoroadamantylglycine analogue **30** in 73% overall yield for three steps. Prolonged exposure of protected adamantylglycine **22** to $KMnO_4$ in 2% aqueous KOH provided the dihydroxyadamantylglycine derivative **27**. Coupling of **27** to **5**, followed by dehydration of the resultant prolineamide with TFAA, in situ basic hydrolysis of the bis-trifluoroacetate, and removal of the terminal Boc group using TFA, afforded dihydroxyadamantyl analogue **28** in 74% overall yield.

In Vitro and in Vivo Biological Activity. DPP-IV Inhibitory Activity in Vitro and ex Vivo. The DPP-IV inhibitory activity of analogues in the present series was measured against human DPP-IV using standard assays as described in the Experimental Section (Table 1). Many of the compounds in this series were potent inhibitors of DPP-IV in vitro, several with K_i's in the sub-nanomolar range. Additionally, several inhibitors in this series exhibited significant slow, tight-binding kinetics.[25]

A finer discrimination between the most potent compounds within this structurally related series of inhibitors with respect to pharmacodynamic effects and

Scheme 5[a]



[a] (a) $KMnO_4$, 2% aq KOH, 60 to 90 °C, 60 min, 51%; (b) **5**, EDAC, HOBT, DMF, 77−85%; (c) $(CF_3CO)_2O$, pyridine, THF 0 °C to rt, then 10% aq K_2CO_3 in MeOH, 89−92%; (d) TFA, CH_2Cl_2, rt, 89−95%; (e) DAST, CH_2Cl_2, −78 °C, 94%; (f) $POCl_3$, pyridine, imidazole, 82%.

Table 1. In Vitro Inhibition Constants for Human DPP-IV and ex Vivo Plasma DPP-IV Inhibition in Normal Rats

		% plasma DPP-IV inhibn at 4 μmol/kg po, normal rats	
compd	human DPP-IV K_i (nM)[a]	30 min	4 h
8a	57 ± 8	13	10
8b	25 ± 4	39	20
8c	12 ± 0.9	42	32
8d	3.9 ± 0.6	71	64
8e	1.4 ± 0.06	76	60
8f	10 ± 3	77	66
8g	10 ± 2	nd[b]	nd
10a	7.1 ± 0.7	0	0
10b	31 ± 2	nd	nd
10d	5.5 ± 0.7	40	44
10g	21 ± 0.6	nd	nd
16c	42 ± 4	36	17
16d	7.4 ± 1.1	69	56
16e	8.0 ± 0.4	17	8
18d	143 ± 15	nd	nd
23	0.9 ± 0.32	84	83
26	0.6 ± 0.06[c]	87	87
28	2.1 ± 0.3	62	57
30	1.8 ± 0.5	80	61

[a] Values represent the mean ± SEM and are at least triplicate determinations. [b] nd = not determined. [c] Compound **26** did not show any significant inhibition of dipeptidyl peptidase II (DPP-II) at concentrations up to 30 μM.

duration of action required utilization of a medium-throughput acute efficacy model measuring a surrogate biomarker expected to be predictive of downstream antihyperglycemic effects. As DPP-IV is found in plasma and on the surfaces of blood and tissue cells, it was reasoned that measurement of inhibition of the circulating enzyme in plasma might provide a convenient biomarker for the degree of preservation of plasma incretin hormone levels. Though the relative contribution of these enzyme loci to the physiological degradation of GLP-1(7−36) amide important for antihyperglycemic effects is not fully understood, it was further envisioned that plasma enzyme inhibition measured ex vivo after an oral dose of test compound might be used to develop pharmacokinetic−pharmacodynamic relationships and provide information regarding duration of action. Compounds were administered orally in water vehicle at 4 μmol/kg to normal Sprague−Dawley rats, and blood samples were taken at 30 min and 4 h postdose to assay plasma (prepared with EDTA) DPP-IV activity in vitro using the fluorogenic DPP-IV-specific substrate Ala-Pro-AFC. Plasma DPP-IV activity determinations were calculated by linear regression from plots of product vs time (initial 20 min). Data were calculated as mean percent inhibition vs controls receiving water vehicle. Maximal inhibition of plasma DPP-IV under the conditions of this assay reached 85−90% (Table 1). In a dose−relationship mode, ED_{50}'s were determined for select compounds at multiple time points of 0.5, 2, 4, and 6 h postdose (Table 2).

Table 2. Potency and Duration of Effect of Compounds **16d** and **26** in the ex Vivo Rat Plasma DPP-IV Inhibition Model

	ED_{50}, μmol/kg at time postdose[a]			
compd	0.5 h	2 h	4 h	6 h
16d	0.4 ± 0.15	3.2 ± 1.2	5.0 ± 1.9	11 ± 4.2
26	0.12 ± 0.04	0.2 ± 0.07	0.3 ± 0.10	0.5 ± 0.15

[a] Compounds dosed po to fasted normal SD rats at the indicated times postdose, plasma aliquots were isolated, and DPP-IV inhibition was assayed using the fluorogenic peptide assay. ED_{50} is the 50% inhibitory dose calculated from the plots of percent inhibition vs dose at each time point.

Results and Discussion

The SAR described in our previous account culminating in 4,5-methanoprolinenitrile analogues **4** revealed a strong preference for compounds with lipophilic N-terminal β-quaternary amino acids.[18] In the course of further studies exploring SAR around β-quaternary cycloalkylglycine-based inhibitors, we encountered unexpectedly potent activity and extended duration of action in ex vivo DPP-IV inhibition studies with compound **8d**, which contains a (vinylcyclopentyl)glycine amino acid fragment. However, metabolism and pharmacokinetic studies with **8d** revealed uncharacteristically poor oral bioavailability (F = 5.3%) and high rat liver microsomal turnover rate [0.55 nmol/min/mg protein for **8d** vs 0.32 for compound **4** (where R_1 and R_2 taken together = cyclopentyl, and R_3 = Me)]. Similar observations were made for other vinyl-containing analogues **8c,e,g**, and these results suggested conversion to an active metabolite in vivo. As the vinyl substituent seemed a likely site of metabolism, synthesis of oxygenated analogues (**16d** and **18d**) derived from chemical modification of the olefin moiety was under-

taken. Diol **18d** showed only weak inhibitory activity; however, hydroxymethyl analogue **16d** exhibited potency similar to that of the vinyl analogue in both in vitro and ex vivo assays, restored rat liver microsomal turnover rate to a more moderate level (0.16 nmol/min/mg protein), and restored oral bioavailability to within the range characteristic for other structurally related analogues in the series (F = 59%). Unequivocal characterization of **16d** as the active metabolite of **8d** was never established, though the behavior of **16d** mirrored that achieved upon administration of **8d**. Accordingly, DPP-IV inhibitors **16c** and **16e** were prepared. A similar trend toward reconnection of pharmacokinetic properties with pharmacodynamic measurements was observed for the homologous pairs of inhibitors **8c/16c** and **8e/16e**. Despite this latter observation, the five-membered ring compound **16d** stood out as significantly more effective in the rat ex vivo plasma DPP-IV inhibition assay.

A more striking observation of metabolic conversion was seen with the highly potent adamantylglycine-containing analogue **23** (K_i = 0.9 nM). Although this compound afforded potent plasma DPP-IV inhibition after oral administration to rats (84% at 0.5 h, 83% at 4 h), it exhibited poor absolute bioavailability (F = 2%) after oral dosing and rapid turnover in rat liver microsomes. Interestingly, compound **23** also weakly inhibited CYP3A4 with an IC_{50} of 20 μM, where previous closely related analogues were devoid of any CYP inhibitory activity. Preparation of the bridgehead-hydroxylated analogue **26** gave a compound with a virtually identical in vitro (K_i = 0.6 nM) and ex vivo (87% inhibition of plasma DPP-IV at 0.5 and 4 h) profile, a slow rat liver microsomal turnover rate, no CYP3A4 inhibition up to 100 μM, and good oral exposure (F = 75%, $t_{1/2}$ = 2.1 h). Two other substituted adamantyl-derived compounds were also synthesized and investigated. Dihydroxyadamantyl compound **28**, while still reasonably active in the ex vivo assay, exhibited extremely high aqueous solubility but exhibited low oral exposure in rats, presumably resulting from very poor absorption. Though fluoroadamantyl compound **30** was also effective ex vivo, it exhibited very low oral exposure and had a rat liver microsomal turnover rate indicative of extensive metabolism, similar to that of compound **23**. Due to its exceptional plasma inhibitory potency and pharmacodynamic duration of action in this preliminary ex vivo assay (ED_{50} for **26** at 6 h = 0.5 μmol/kg vs ED_{50} for **16d** at 6 h = 11 μmol/kg, Table 2), compound **26** was chosen for further study in acute efficacy models.

Oral Glucose Tolerance in Zucker$^{fa/fa}$ Rats. Zucker$^{fa/fa}$ rats are a well-established genetically modified rodent model of obesity-induced insulin resistance[26] and provide a background to measure the effects of DPP-IV inhibitors in a prediabetic animal.[27] The nutrient-induced incretin secretion component of the GLP-1-dependent mechanism makes this a suitable model with which to study postprandial glucose excursions after administration of an oral glucose tolerance test (oGTT). DPP-IV inhibitor **26** was chosen for further study in this animal model by virtue of its highly potent effects in vitro and ex vivo. Compound **26** was administered orally to Zucker$^{fa/fa}$ rats at 0.5 h prior to oGTT, consisting of a glucose challenge (2.0 g/kg), followed by blood sampling at intervals over the next 2 h for plasma glucose measurements (data not shown). Maximal responses in glucose excursion in this model were associated with plasma DPP-IV inhibition of approximately 60% vs control, and no additional antihyperglycemic effects were seen at higher percent inhibition. On the basis of these preliminary findings at a single dose, compound **26** was further evaluated in the Zucker$^{fa/fa}$ rat model with an oGTT performed 4 h after oral administration of test compound in a dose−response format. Postprandial plasma glucose and insulin levels were again measured at intervals over 2 h following the glucose challenge. Compound **26** was highly effective at eliciting marked dose-dependent enhancements in glucose clearance in the dose range 0.3−3 μmol/kg (0.13−1.3 mg/kg) in this model relative to controls (Figure 1).

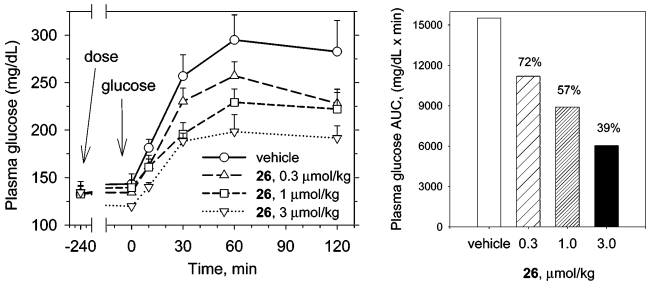


Figure 1. Effects of inhibitor **26** dosed at 0.3, 1, and 3 μmol/kg po versus vehicle control on plasma glucose clearance after an oGTT given 4 h postdose in Zucker$^{fa/fa}$ rats.

Oral Glucose Tolerance in *ob/ob* Mice. Evidence from both inhibitor studies and knock-out animals support that the mouse is also a suitable species in which to study the effects of DPP-IV inhibition on glucose clearance and insulin potentiation.[28] To this end, the effects of compound **26** on glucose clearance and enhancement of insulin secretion was studied in the *ob/ob* mouse. In this model the oGTT was performed at 1 h after oral administration of **26** at 1, 3, or 10 μmol/kg (Figure 2). The data show that compound **26** dose-dependently elevated plasma insulin significantly at 15 min post-oGTT, with concomitant improvement in the glucose clearance curves at 60 min post-oGTT.

Compound **26** exhibited robust glucose-lowering effects in a dose-relational manner in the Zucker$^{fa/fa}$ rat oGTT model, even when the glucose challenge was administered 4 h postdose of compound. Similarly outstanding efficacy was observed in reducing postprandial glucose AUC in *ob/ob* mice. This compound also proved quite effective in elevating insulin levels after an oGTT in *ob/ob* mice, further demonstrating the effectiveness of potentiating GLP-1-induced insulin secretion as a key component mediating the antihyperglycemic actions of this potent DPP-IV inhibitor. It is anticipated that compound **26**, given its extended pharmacodynamic response, will be amenable to once daily dosing in humans.

Conclusion

A series of β-quaternary cycloalkylglycine amino acid residues were incorporated into our previously disclosed 4,5-methanoprolinenitrile scaffold, and many of these compounds showed potent DPP-IV inhibitory activity. Several compounds containing a vinyl functionality also

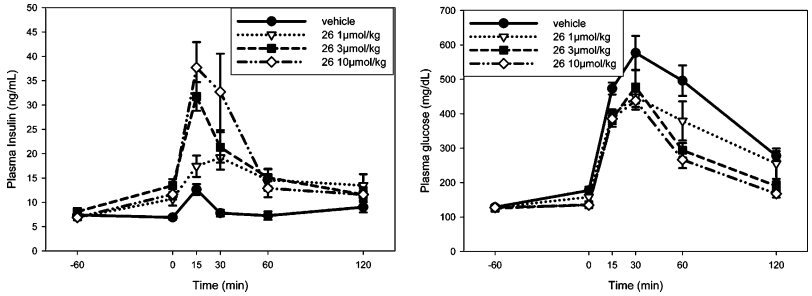


Figure 2. Effects of inhibitor **26** dosed at 1, 3, and 10 μmol/kg po versus vehicle control on plasma insulin (left panel) and plasma glucose (right panel) after an oGTT in *ob*/*ob* mice. Compound **26** significantly lowered plasma glucose levels (vs vehicle) at the 60 min time point at 3 and 10 μmol/kg ($p < 0.05$) and significantly increased plasma insulin levels (vs vehicle) at the 15 min time point at 10 μmol/kg ($p < 0.05$).

exhibited extended duration of action in an ex vivo plasma DPP-IV inhibition model in normal rats relative to closely related analogues lacking this moiety. These analogues, however, also showed markedly reduced systemic exposure after oral dosing and rapid rat liver microsomal turnover rates where related small alkyl-substituted analogues did not. Efforts to define the role of suspected metabolites resulted in the synthesis of several hydroxymethylcycloalkyl-based analogues that maintained in vitro and, for some, in vivo activity. These hydroxymethyl analogues also displayed favorable pharmacokinetic properties with a tighter correlation of pharmacokinetics to pharmacodynamics. Analogously, hydroxylation of a similarly disposed adamantylglycine-based inhibitor yielded a compound (**26**) with in vivo potency and duration of action superior to that of any compound from this series. Consequently, this compound was chosen for development and is currently under clinical investigation for the treatment of type 2 diabetes. The basis for the enhanced efficacy observed for the present compounds in animal models relative to other agents may be due to contributions from multiple factors, including exquisite enzyme inhibitory potency and compound distribution to the tissue compartment potentially critical for maximal antihyperglycemic effects ($V_{ss} = 5.2$ L/kg for compound **26** in the rat). Further studies are underway in these laboratories to more fully understand and quantitatively characterize the physicochemical basis for the observed findings.

Experimental Section

All reactions were carried out under a static atmosphere of argon or nitrogen and 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. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL GSX400 spectrometer using Me_4Si as an internal standard unless otherwise noted. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a JEOL JNM-ECP500 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, and assignment. All reactions were carried out using commercially available anhydrous solvents from Aldrich Chemical Co. or EM Science Chemical Co. unless otherwise noted. 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 gel plates (60 F_{254}) and were visualized with UV light, with 5% phosphomolybdic acid in 95% EtOH, or by a sequential treatment with 1 N HCl/MeOH followed by ninhydrin staining. LC/MS data were recorded on a Shimadzu LC-10AT equipped with a SIL-10A injector, a SPD-10AV detector, normally operating at 220 nm, and interfaced with a Micromass ZMD mass spectrometer. LC/MS or HPLC retention times, unless otherwise noted, are reported using a Phenomenex Luna C-18 4.6 mm × 50 mm column eluted with a 4 min gradient from 0 to 100% B, where A = 10% MeOH−90% H_2O−0.1% TFA and B = 90% MeOH−10% H_2O−0.1% TFA. All solvents were removed by rotary evaporation under vacuum using a standard rotovap equipped with a dry ice condenser. All filtrations were performed with a vacuum unless otherwise noted.

General Method A. Peptide Coupling to Enantiomerically Pure L-*cis*-4,5-Methanoprolinamide 5, Amide Dehydration, and Deprotection. Methanoprolinamide **5** was coupled to a variety of racemic quaternary protected amino acids using HOBT/EDC in DMF at room temperature to give a D/L mixture of diastereomers at the N-terminal amino acid. The desired L-diastereomer was most often chromatographically isolated as the *N*-Boc-protected nitrile, obtained by treatment of amide **7** with $POCl_3$/imidazole in pyridine at −20 °C. The final target compounds **8a**−**g** were obtained by deprotection using TFA in CH_2Cl_2.

(*S*)-2-(1-Ethenylcyclopent-1-yl)glycine-L-*cis*-4,5-methanoprolinamide (7d). 4,5-Methanoprolinamide **5** (877 mg, 3.65 mmol) and *N*-Boc cyclopentylvinylamino acid **6d** (1.13 g, 4.20 mmol), described in general method B, were dissolved in 20 mL of DMF and cooled to 0 °C and to this mixture were added EDAC (1.62 g, 8.4 mmol), HOBT hydrate (2.54 g, 12.6 mmol), and TEA (1.27 g, 12.6 mmol). The reaction was allowed to warm to room temperature and stirred for 24 h. The reaction mixture was taken up in 100 mL of EtOAc, washed with H_2O (3 × 20 mL), dried (Na_2SO_4), and purified by flash chromatography (100% EtOAc) to give 1.38 g (86%) of **7d** as a mixture of diastereomers: ^{1}H NMR (500 MHz, $CDCl_3$) 1.02−1.08 (m, 2H), 1.43 (s, 9H), 1.54−1.80 (m, 7H), 1.84−1.94 (m, 2H), 2.36 (dd, J = 13.6, 2.6, 1H), 2.58 (m, 1H), 3.90 (m, 1H), 4.63 (d, J = 9.7, 1H), 5.04 (dd, J = 10.5, 2.2, 1H), 5.14 (d, J = 17.6, 1H), 5.23 (d, J = 11, 1H), 5.97 (dd, J = 17.6, 11, 1H); MS *m/z* 378 $[M + H]^+$.

(*S*)-2-(1-Ethenylcyclopent-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile (8d). Diastereomeric amide **7d** (68 mg, 0.18 mmol, 1 equiv) and imidazole (26 mg, 0.38 mmol, 2.1 equiv) were dissolved in 2 mL of pyridine at −30 °C, to which $POCl_3$ (0.070 mL, 0.739 mmol, 4.10 equiv) was added. After stirring at −30 °C for 40 min, the solvent was removed and the residue thoroughly dried under vacuum. Purification by flash chromatography (10% EtOAc/CH_2Cl_2) afforded the desired slower eluting diastereomerically pure *N*-Boc-protected nitrile as a white solid (34 mg, 0.0946 mmol, 53%): ^{1}H NMR (500 MHz, $CDCl_3$) 1.02−1.07 (m, 2H), 1.42 (s, 9H), 1.55−1.75 (m, 7H), 1.83−1.93 (m, 2H), 2.36 (dd, J = 13.6, 2.6, 1H), 2.58 (m, 1H), 3.90 (m, 1H), 4.62 (d, J = 9.2, 1H), 5.03 (dd, J = 10.5, 2.2, 1H), 5.13 (d, J = 17.6, 1H), 5.23 (d, J = 11, 1H), 5.96 (dd, J = 17.6, 11, 1H); MS *m/z* 360 $[M + H]^+$. The *N*-Boc protected

nitrile (32.4 mg, 0.090 mmol) was dissolved in 1 mL of CH_2Cl_2 and treated with TFA (1 mL) at room temperature for 30 min. The solvent was evaporated, and the resulting oil was azeotroped to dryness with dry toluene and finally dried under vacuum. The residue was taken up in 10:90 H_2O/MeOH + 0.1% TFA and purified on a Shimadzu preparative HPLC (YMC S-5 ODS, 20 × 250 mm, 0−100% B, 18 min gradient at 20 mL/min, 220 nm). Lyophilization provided 22.1 mg (66%) of **8d** as a white lyophilate: ^{1}H NMR (500 MHz, D_2O) 0.99 (m, 1H), 1.17 (m, 1H), 1.54−1.83 (m, 6H), 1.90 (m, 1H), 1.97−2.10 (m, 2H), 2.41 (dd, J = 13.7, 2.7, 1H), 2.66 (m, 1H), 3.86 (m, 1H), 4.58 (s, 1H), 5.22 (dd, J = 11, 2.2, 1H), 5.30 (d, J = 17.6, 1H), 5.40 (d, J = 11, 1H), 5.76 (dd, J = 17.6, 11, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.34 min; MS *m/z* 260 $[M + H]^+$; HRMS calcd for $C_{15}H_{22}N_3O$ $[M + H]^+$ 260.1764, found 260.1768. Anal. ($C_{15}H_{21}N_3O$·1.15 CF_3CO_2H) C, H, N.

General Method B: Ethyl Cyclopentylideneacetate (12d). A suspension of NaH (2.55 g, 0.064 mol, 1.10 equiv) in THF (60 mL) was cooled to 0 °C. Triethylphosphonoacetate (12.8 mL, 0.064 mol, 1.10 equiv) was slowly added and the reaction stirred at 25 °C for 1 h. A solution of cyclopentanone (4.89 g, 0.058 mol, 1.00 equiv) in 5 mL of THF was added dropwise and the reaction stirred at room temperature for 2 h. The reaction was partitioned with Et_2O (100 mL) and H_2O (50 mL), and the organic phase was washed with H_2O and then brine, dried (Na_2SO_4), filtered, and concentrated to obtain **12d** as a colorless oil (8.81 g, 98%). R_f = 0.35 TLC (5% EtOAc/hexanes). ^{1}H NMR (500 MHz, $CDCl_3$) 1.26 (t, J = 7, 3H), 1.61−1.77 (m, 2H), 2.40−2.44 (m, 2H), 2.73−2.77 (m, 2H), 4.10−4.16 (m, 2H), 5.78 (m, 1H).

1,1-Cyclopentylidinemethanol (13d). Ethyl cyclopentylideneacetate **12d** (8.81 g, 0.057 mol, 1 equiv) was dissolved in 50 mL of toluene and cooled to −78 °C. DIBAL-H was added dropwise (1.5 M in toluene, 95 mL, 0.14 mol, 2.5 equiv), and the reaction was stirred at −78 °C for 1 h and then warmed to 0 °C for 30 min. The reaction was again cooled to −78 °C and carefully quenched with 15 mL of anhydrous MeOH. To the reaction was then added a 1 N aqueous solution of Rochelle's salt, and the reaction then stirred at room temperature for 1.5 h. The reaction was diluted with Et_2O, the layers were separated, and the organic phase was washed with brine, dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with 8% EtOAc/CH_2Cl_2 to afford 5.88 g (92%) of **13d** as a colorless oil: ^{1}H NMR (500 MHz, $CDCl_3$) 1.36 (s, 1H), 1.56−1.70 (m, 4H), 2.03−2.28 (m, 4H), 4.10−4.12 (m, 2H), 5.49 (m, 1H).

***N*-Boc-glycine Cyclopentylidenemethanoester (14d).** *N*-Boc-glycine (5.00 g, 0.028 mol, 1.00 equiv) was dissolved in 40 mL of CH_2Cl_2 and combined with a solution of **13d** (3.14 g, 0.028 mol, 1.00 equiv) in 10 mL of CH_2Cl_2. To the reaction was added DCC (6.12 g, 0.029 mol, 1.05 equiv) and DMAP (0.345 g, 0.0028 mol, 0.10 equiv), and the reaction was stirred for 16 h at room temperature. The mixture was filtered to remove insoluble urea, and the filtrate was concentrated and purified on a flash column (5 × 15 cm) with 5% EtOAc/CH_2Cl_2 to give **14d** as a colorless oil (6.79 g, 90%): TLC R_f = 0.39 (SiO_2, 5% EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) 1.43 (s, 9H), 1.57−1.71 (m, 4H), 2.26−2.30 (m, 4H), 3.90 (d, J = 4.8, 2H), 4.61 (d, J = 7.5, 2H), 5.00 (br s, 1H), 5.42 (m, 1H); MS (FAB) *m/z* 292 $[M + Na]^+$.

D/L-2-(*N*-Boc-1-ethenylcyclopent-1-yl)glycine (6d). An oven-dried flask was charged with $ZnCl_2$ (3.86 g, 0.028 mol, 1.10 equiv), dried by toluene azeotrope (3 × 10 mL), and then filled with argon. Compound **14d** (6.79 g, 0.025 mol, 1.00 equiv) was dried by toluene azeotrope (3 × 10 mL), dissolved in 60 mL of THF and added via cannula to the $ZnCl_2$, which was then cooled to −78 °C. LDA was freshly prepared under standard conditions [diisopropylamine (9.2 mL, 0.066 mol, 2.6 equiv), *n*-butyllithium (2.4 M in hexanes, 26.3 mL, 0.063 mol, 2.5 equiv) in 30 mL THF, cooled to −78 °C] and added via cannula to the mixture of **14d** and $ZnCl_2$. The reaction was stirred at −78 °C and was allowed to warm to room temperature overnight. After 17 h, the yellow reaction solution was diluted with Et_2O (100 mL) and washed with 1 N HCl (100 mL) and brine (50 mL). The organic phase was dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (3% MeOH/CH_2Cl_2 + 0.5% HOAc) to afford **6d** (4.80 g, 71%) as a white solid: TLC R_f = 0.41 (SiO_2, 5% MeOH/CH_2Cl_2 + 0.5% HOAc); ^{1}H NMR (500 MHz, DMSO-d_6) 1.38 (s, 9H), 1.40−1.73 (m, 8H), 3.95 (d, J = 9, 1H), 5.01 (d, J = 17.5, 1H), 5.05 (d, J = 11, 1H), 5.85 (dd, J = 17.5, 11, 1H), 6.62 (d, J = 9, 1H); MS (ESI-Q) *m/z* 268 $[M - H]^-$.

(*S*)-2-(1,1-Dimethylprop-2-en-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8a): ^{1}H NMR (500 MHz, D_2O) 0.98 (m, 1H), 1.20 (m, 1H), 1.27 (s, 3H), 1.29 (s, 3H), 2.07 (m, 1H), 2.43 (dd, J = 14, 2.6, 1H), 2.70 (m, 1H), 4.50 (s, 1h), 5.23 (dd, J = 10.7, 2.6, 1H), 5.29 (d, J = 17.6, 1H), 5.34 (d, J = 10.7, 1H), 5.98 (dd, J = 17.6, 10.7, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 1.96 min; MS (FAB) *m/z* 234 $[M + H]^+$; HRMS calcd for $C_{13}H_{20}N_3O$ $[M + H]^+$ 234.1608, found 234.1607.

(*S*)-2-(1,1-Diethylprop-2-en-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8b): ^{1}H NMR (500 MHz, D_2O) 0.77 (t, J = 7.5, 3H), 0.81 (m, 1H), 0.91 (s, 3H), 0.92 (s, 3H), 1.01 (m, 1H), 1.26−1.42 (m, 2H), 1.88 (m, 1H), 2.25 (dd, J = 13.6, 2.6, 1H), 2.51 (m, 1H), 3.69 (m, 1H), 4.23 (s, 1H), 5.06 (dd, J = 11, 2.6, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.10 min; MS (FAB) *m/z* 236 $[M + H]^+$. Anal. ($C_{13}H_{21}N_3O$·CF_3CO_2H·0.25H_2O) C, H, N.

(*S*)-2-(1-Ethenylcyclobut-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8c): ^{1}H NMR (500 MHz, MeOH-d_4) 0.95 (m, 1H), 1.13 (m, 1H), 1.87−2.18 (m, 5H), 2.29−2.36 (m, 2H), 2.54−2.62 (m, 2H), 3.94 (m, 1H), 4.62 (d, J = 3.5, 1H), 5.20 (dd, J = 10.6, 2.2, 1H), 5.39−5.42 (m, 2H), 5.98 (dd, J = 17.1, 10.6, 1H); ^{13}C NMR (125 MHz, MeOH-d_4) 14.5, 16.5, 19.3, 29.3, 29.9, 31.4, 39.1, 47.0, 47.6, 58.1, 117.6, 120.4, 138.9, 167.2. Anal. ($C_{14}H_{19}N_3O$·CF_3CO_2H·0.50 H_2O) C, H, N.

(*S*)-2-(1-Ethenylcyclopent-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile (8d). See general method A.

(*S*)-2-(1-Ethenylcyclohex-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8e): ^{1}H NMR (500 MHz, MeOH-d_4) 0.97 (m, 1H), 1.10 (m, 1H), 1.16−1.82 (m, 9H), 2.00−2.09 (m, 2H), 2.35 (dd, J = 13.6, 2.2, 1H), 2.60 (m, 1H), 3.92 (m, 1H), 4.30 (s, 1H), 5.18 (dd, J = 10.5, 2.2, 1H), 5.30 (d, J = 17.6, 1H), 5.55−5.67 (m, 2H). Anal. ($C_{16}H_{23}N_3O$·CF_3CO_2H·0.50H_2O) C, H, N.

(*S*)-2-(1-Ethenylcyclohept-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8f): ^{1}H NMR (500 MHz, MeOH-d_4) 0.98 (m, 1H), 1.14 (m, 1H), 1.31 (s, 1H), 1.40−2.06 (m, 14H), 2.36 (dd, J = 14.1, 2.2, 1H), 2.60 (m, 1H), 3.93 (m, 1H), 4.36 (s, 1H), 5.19 (dd, J = 10.6, 2.2, 1H), 5.22 (d, J = 20.6, 1H), 5.43 (dd, J = 11.0, 2.7, 1H), 5.82 (dd, J = 17.6, 11.0, 1H). Anal. ($C_{17}H_{25}N_3O$·CF_3CO_2H) C, H, N.

(*S*)-2-(4-Ethenyltetrahydropyran-4-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (8g): ^{1}H NMR (500 MHz, D_2O) 0.96 (m, 1H), 1.17 (m, 1H), 1.69 (dd, J = 13.6, 2.2, 1H), 1.80 (m, 1H), 1.98−2.10 (m, 3H), 2.38 (dd, J = 13.6, 2.6, 1H), 2.64 (m, 1H), 3.53−3.64 (m, 2H), 3.80−3.86 (m, 3H), 4.44 (s, 1H), 5.18 (dd, J = 11, 2.6, 1H), 5.34 (m, 1H), 5.65−5.68 (m, 2H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 1.53 min; MS (FAB) *m/z* 276 $[M + H]^+$. Anal. ($C_{15}H_{21}N_3O_2$·CF_3CO_2H·0.4H_2O) C, H, N.

General Method C. (*S*)-2-(1-Ethylcyclopent-1-yl)-l-glycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (10d). Compound **6d** (2.23 g, 8.30 mmol) was dissolved in 50 mL of MeOH and placed in a hydrogenation vessel purged with argon. To this mixture was added 10% Pd−C (224 mg, 10% w/w) and the reaction stirred under 1 atm of H_2 at room temperature for 12 h. The reaction was filtered through Celite, concentrated, and purified by flash column chromatography on silica gel with 1:9 MeOH:CH_2Cl_2 to give *tert*-butyloxycarbonylamino(1-ethylcyclopent-1-yl)acetic acid **9d** (2.18 g, 97%) as a white solid (FAB $[M + H]^+$ 272). Coupling of **9d** to prolinamide core **5** and elaboration to **10d** was carried out using general method A: ^{1}H NMR (500 MHz, D_2O) 0.78 (t, J = 7.5, 3H), 0.82 (m, 1H), 1.02 (m, 1H), 1.27−1.60 (m, 9H), 1.72 (m, 1H), 1.88 (m, 1H), 2.25 (dd, J = 13.6, 2.6, 1H), 2.50 (m,

1H), 3.68 (m, 1H), 4.38 (s, 1H), 5.05 (dd, J = 11, 2.6, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.48 min; MS (FAB) m/z 262 $[M+H]^+$. Anal. ($C_{15}H_{23}N_3O$·CF_3CO_2H·0.4H_2O) C, H, N.

(*S*)-2-(1,1-Dimethylprop-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (10a): ^{1}H NMR (500 MHz, D_2O) 0.69 (t, J = 7.5, 3H), 0.82 (t, J = 7.5, 3H), 0.88 (m, 1H), 1.07 (m, 1H), 1.20 (m, 1H), 1.44−1.64 (m, 2H), 1.70−1.82 (m, 2H), 1.88−1.96 (m, 2H), 2.29 (dd, J = 13.8, 2.6, 1H), 2.56 (m, 1H), 3.76 (m, 1H), 4.37 (s, 1H), 5.08 (dd, J = 10.5, 2.6, 1H), 5.13 (d, J = 17.5, 1H), 5.33 (d, J = 11, 1H), 5.66 (dd, J = 17.5, 11, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.54 min; MS (FAB) m/z 262 $[M+H]^+$. Anal. ($C_{13}H_{21}N_3O$·CF_3CO_2H·0.1H_2O) C, H, N.

(*S*)-2-(1,1-Diethylprop-1-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (10b): ^{1}H NMR (500 MHz, D_2O) 0.72 (t, J = 7.5, 9H), 0.80 (m, 1H), 1.01 (m, 1H), 1.22−1.32 (m, 3H), 1.41−1.52 (m, 3H), 1.87 (m, 1H), 2.24 (dd, J = 13.8, 2.6, 1H), 2.50 (m, 1H), 3.68 (m, 1H), 4.21 (s, 1H), 5.07 (dd, J = 11, 2.6, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.60 min; MS (FAB) m/z 264 $[M+H]^+$. Anal. ($C_{15}H_{25}N_3O$·CF_3CO_2H·0.1H_2O) C, H, N.

(*S*)-2-(4-Ethyltetrahydropyran-4-yl)glycine-L-*cis*-4,5-methanoprolinenitrile TFA salt (10g): ^{1}H NMR (500 MHz, D_2O) 0.84 (t, J = 7.5, 3H), 0.87 (m, 1H), 1.06 (m, 1H), 1.33 (dd, J = 13.6, 2.2, 1H), 1.40−1.55 (m, 2H), 1.68 (dd, J = 13.8, 2.2, 1H), 1.80−2.00 (m, 3H), 2.30 (dd, J = 14, 2.6, 1H), 2.57 (m, 1H), 3.53−3.79 (m, 5H), 4.40 (s, 1H), 5.11 (dd, J = 11, 2.6, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 1.60 min; MS (FAB) m/z 278 $[M+H]^+$. Anal. ($C_{15}H_{23}N_3O_2$·CF_3CO_2H·0.3H_2O) C, H, N.

General Method D. Oxidative Cleavage of Vinyl Substituent by Ozonolysis. Protected vinyl nitriles **15** were treated with ozone and subjected to a reductive quench with $NaBH_4$ to furnish the hydroxymethyl analogues directly, which were then subsequently deprotected using TFA in CH_2Cl_2 at 0 °C to give target compounds **16**.

(*S*)-2-[1-(Hydroxymethyl)cyclopent-1-yl]glycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (16d). Vinyl nitrile **15d**, prepared from **7d** using general method A (1.28 g, 3.60 mmol), was dissolved in 56 mL of a 2:5 mixture of CH_2Cl_2/MeOH, cooled to −78 °C, and treated with a stream of ozone until the reaction solution took on a blue color, at which time $NaBH_4$ (566 mg, 15.0 mmol, 4.2 equiv) was added and the reaction warmed to 0 °C. After 30 min, the reaction was quenched with 2 mL of saturated $NaHCO_3$ and then warmed to room temperature. The reaction mixture was evaporated to dryness and taken up in EtOAc. A small amount of water was added to dissolve the inorganics, and the layers were separated. The EtOAc layer was dried (Na_2SO_4), filtered, and evaporated to an oil that was purified by flash column chromatography with EtOAc to give 922 mg (71%) of *N*-Boc hydroxymethyl compound: MS m/z 364 $[M+H]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) 1.08 (td, J = 6.3, 2.1, 2H), 1.22 (m, 1H), 1.44 (s, 9H), 1.51−1.96 (m, 8H), 2.38 (dd, J = 13.9, 2.3, 1H), 2.60 (m, 1H), 3.33 (d, J = 12.1, 1H), 3.57 (d, J = 12.1, 1H), 3.92 (m, 1H), 4.75 (d, J = 9.1, 1H), 5.06 (dd, J = 10.6, 2.3, 1H), 5.85 (d, J = 8.8, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 13.5, 17.8, 24.7, 24.9, 28.4, 30.6, 31.1, 33.0, 38.0, 45.5, 51.6, 55.8, 67.1, 80.3, 119.1, 171.4.

The *N*-Boc hydroxymethyl compound (900 mg, 2.48 mmol) was dissolved in 60 mL of CH_2Cl_2, cooled to 0 °C, and treated with 20 mL of freshly distilled TFA. The deprotection was complete after 80 min and the mixture was evaporated to dryness and purified by preparative HPLC (YMC S5 ODS 30 × 100 mm, 18 min gradient 80% A:B to 100% B; solvent A = 10% MeOH−90%H_2O−0.1% TFA, solvent B = 90% MeOH−10% H_2O−0.1% TFA, collected product from 5.1 to 6.5 min) to give, after lyophillization from water, 660 mg (71%) of the TFA salt of **16d** as a white lyophilate: ^{1}H NMR (500 MHz, MeOH-d_4) 0.87 (m, 1H), 1.00 (m, 1H), 1.30 (m, 2H), 1.54 (m, 1H), 1.58−1.80 (m, 6H), 2.00 (m, 2H), 2.36 (dd, J = 14.1, 2.6, 1H), 2.63 (m, 1H), 3.58 (d, J = 11.0, 1H), 3.64 (d, J = 11.0, 1H), 3.90 (m, 1H), 4.61 (s, 1H), 5.19 (dd, J = 10.9, 2.6, 1H); ^{13}C NMR (125 MHz, MeOH-d_4) 14.5, 19.5, 25.6, 25.7, 30.9, 31.9, 33.6, 39.6, 47.5, 51.2, 58.7, 68.2, 120.8, 168.5; MS m/z 264 $[M+H]^+$. Anal. ($C_{14}H_{21}N_3O_2$·1.20CF_3CO_2H) C, H, N.

General Method E. (*S*)-2-[1-(Hydroxymethyl)cyclobut-1-yl]glycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (16c). *N*-Boc-protected vinylcyclobutyl compound **15c** (0.16 g, 0.46 mmol) was dissolved in 10 mL of a 1:1 mixture of THF:H_2O and treated with OsO_4 (12 mg, 0.05 mmol) and $NaIO_4$ (0.59 g, 2.76 mmol, 6 equiv). After 2 h, the reaction mixture was diluted with 50 mL of Et_2O and 10 mL of water. The layers were separated, and the organic layer was washed with $NaHCO_3$, dried ($MgSO_4$), and concentrated to give a dark oil. The oil was diluted with 10 mL of MeOH, and $NaBH_4$ (0.08 g, 2.0 mmol) was added at 25 °C. After 30 min, the mixture was diluted with Et_2O and the reaction was quenched with saturated $NaHCO_3$. The layers were separated, and the organic fraction was washed with 0.1 M HCl, saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated to give 90 mg of the Boc-protected hydroxymethyl compound as a dark oil. The crude intermediate (90 mg, 0.26 mmol) was dissolved in 3 mL of CH_2Cl_2, cooled to 0 °C, and treated with 3 mL of freshly distilled TFA. The deprotection was complete after 80 min and the mixture was evaporated to dryness and then purified by preparative HPLC (YMC S5 ODS 30 × 100 mm, 10 min gradient 100% A to 100% B; solvent A = 10% MeOH−90%H_2O−0.1% TFA; solvent B = 90% MeOH−10% H_2O−0.1% TFA, to give, after lyophilization, 50 mg (60%) of title compound **16c**: ^{1}H NMR (500 MHz, MeOH-d_4) 0.95 (m, 1H), 1.06 (q, J = 7.6, 1H), 1.30 (m, 2H), 1.54 (m, 1H), 1.75 (m, 1H), 1.90 (m, 1H), 2.00 (m, 3H), 2.20 (m, 1H), 2.35 (dd, J = 13.8, 2.2, 1H), 2.50 (m, 1H), 2.63 (m, 1H), 3.85 (s+m, 3H), 4.63 (s, 1H), 5.22 (dd, J = 10.7, 2.2, 1H); MS m/z 250 $[M+H]^+$. Anal. ($C_{13}H_{19}N_3O_2$·CF_3CO_2H·0.5H_2O): C, H, N.

(*S*)-2-[1-(Hydroxymethyl)cyclohex-1-yl]glycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (16e). This compound was prepared using general method D: ^{1}H NMR (500 MHz, MeOH-d_4) 0.92 (m, 1H), 1.10 (m, 1H), 1.26 (m, 1H), 1.35−1.76 (m, 10H), 1.95 (m, 1H), 2.01 (m, 1H), 2.36 (dd, J = 13.8, 2.2, 1H), 2.64 (m, 1H), 3.78 (d, J = 11.6, 1H), 3.90 (m, 1H), 4.48 (s, 1H), 5.22 (dd, J = 11, 2.2, 1H); ^{13}C NMR (125 MHz, MeOH-d_4) 14.2, 19.2, 22.1, 22.2, 26.6, 28.6, 31.1, 31.4, 39.3, 41.8, 47.1, 59.7, 64.7, 120.4, 167.9. Anal. ($C_{15}H_{23}N_3O_2$·CF_3CO_2H·0.4H_2O) C, H, N.

(2*S*)-2-[1-(1,2-Dihydroxyethyl)cyclopent-1-yl]glycine-L-*cis*-4,5-methanoprolinenitrile (18d). Vinyl nitrile **15d** (70 mg, 0.19 mmol) was dissolved in 5 mL of a 3:2 mixture of THF/*t*-BuOH, and *N*-methylmorpholine *N*-oxide (33 mg, 0.28 mmol) was added, followed by OsO_4 (0.01 mmol, 2.5 wt %/v in *t*-BuOH), and the reaction was stirred at room temperature for 16 h. The reaction was then quenched with 1 mL of 10% Na_2SO_3, and the mixture was taken up in EtOAc (10 mL), washed with brine, dried (Na_2SO_4), filtered, evaporated, and purified by flash chromatography to give 41 mg of the desired Boc-protected diol as a mixture of diastereomers, which was immediately deprotected with TFA/CH_2Cl_2 using general method A. Lyophilization of the product from water gave 27 mg (47%) of the desired diol **18d** as a diastereomeric mixture that was a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) 1.08 (m, 1H), 0.76 (m, 1H), 1.38−2.05 (m, 9H), 2.13 (dd, J = 13.6, 1.6, 1H), 2.28 (d, J = 3.1, 1H), 2.33 (m, 1H), 3.13 (m, 1H), 3.44 (m, 1H), 3.51−3.70 (m, 3H), 3.87 (m, 1H), 4.36 (dd, J = 11.0, 8.8, 1H), 4.57 (m, 1H), 4.84 (s, 1H), 5.24 (dd, J = 11.0, 2.7, 1H), 8.00 (br s, 2H); HRMS calcd for $C_{15}H_{23}N_3O_3$ $[M+H]^+$ 294.1818, found 294.1830.

1-(*S*)-Adamantan-1-yl-(*R*)-(2-hydroxy-1-phenylethylamino)acetonitrile (20). 1-Adamantanecarboxylic acid (10.0 g, 0.0549 mol) was dissolved in Et_2O (160 mL) and MeOH (40 mL), and trimethylsilyldiazomethane (2.0 M in hexane, 30 mL, 0.06 mol, 1.1 equiv) was added. After 3 h at room temperature, the reaction mixture was concentrated and purified by flash chromatography (5 × 15 cm silica column, eluted with 40% CH_2Cl_2/hexanes) to give the desired ester **19** as a white crystalline solid (10.7 g, 0.055 mol, quantitative):

^{1}H NMR (500 MHz, $CDCl_3$) 1.63 (br s, 6H), 1.81 (d, J = 2.6, 6H), 1.94 (br s, 3H), 3.58 (s, 3H).

Methyl 1-adamantanecarboxylate (**19**, 10.7 g, 0.0549 mol) was dissolved in THF (150 mL), cooled to 0 °C, and treated dropwise with a solution of $LiAlH_4$ (1.0M in THF, 69 mL, 0.069 mol, 1.25 equiv). The reaction was warmed to room temperature for 1.5 h and then cooled to 0 °C and quenched sequentially with 5.1 mL of H_2O, 5.1 mL of 15% NaOH, and 10.2 mL of H_2O. The reaction was stirred at room temperature for 15 min and filtered, and the aluminum salts were washed with EtOAc (2 × 100 mL). The filtrate was concentrated and the resulting solid purified by flash chromatography (10% EtOAc/CH_2Cl_2) to afford 1-hydroxymethyladamantane (8.74 g, 96%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 1.48 (s, 7H), 1.59−1.75 (m, 6H), 1.96 (br s, 3H), 3.17 (s, 2H).

An oven-dried three-neck flask equipped with 125-mL addition funnel was charged with 150 mL of CH_2Cl_2 and DMSO (10.3 mL, 0.145 mol, 2.5 equiv) and cooled to −78 °C. Oxalyl chloride (6.7 mL, 0.0768 mol, 1.32 equiv) was added dropwise and the reaction stirred for 15 min. A solution of 1-hydroxymethyladamantane (9.67 g, 0.058 mol, 1.00 equiv) in 75 mL of CH_2Cl_2 was added dropwise and the reaction was allowed to stir for 1 h. The resulting white mixture was then treated dropwise with triethylamine (40.5 mL, 0.29 mol, 5 equiv). After 30 min, the cooling bath was removed, and a cold solution of 20% KH_2PO_4 (25 mL) and cold H_2O (150 mL) were added, and the reaction was stirred at room temperature for 15 min. The mixture was diluted with Et_2O (400 mL), the layers separated, and the organic layer was washed with cold 10% KH_2PO_4 (3 × 150 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with CH_2Cl_2 to afford the desired aldehyde as a white solid (9.40 g, 0.057 mol, 98%): ^{1}H NMR (500 MHz, $CDCl_3$) 1.64−1.80 (m, 12H), 2.05 (br s, 3H), 9.29 (s, 1H).

Adamantane-1-carboxaldehyde (9.40 g, 0.057 mol, 1 equiv) was suspended in H_2O (145 mL) and cooled to 0 °C. $NaHSO_3$ (5.95 g, 0.057 mol, 1 equiv) was added followed by KCN (4.0 g, 0.059 mol, 1.04 equiv). A solution of (*R*)-(−)-phenylglycinol (8.01 g, 0.057 mol, 1 equiv) in MeOH was subsequently added dropwise. The resulting mixture was stirred at room temperature for 2 h and then heated to reflux for 16 h. The reaction was cooled to room temperature, diluted with EtOAc (200 mL), and stirred for 15 min. The layers were separated, and the aqueous layer was extracted with EtOAc (100 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with 20% EtOAc/hexanes to afford 11.6 g (65%) of the desired (*R,S*) diastereomer **20** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 1.43−1.80 (m, 12h), 2.03 (m, 3H), 2.86 (s, 1H), 2.55 (m, 1H), 3.79 (dd, J = 10.8, 3.7, 1H), 4.06 (m, 1H), 7.26−7.39 (m, 5H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 4.58 min; MS (FAB) *m/z* 311 $[M + H]^+$.

1-(*S*)-Adamantan-1-yl-(*R*)-(2-hydroxy-1-phenylethylamino)acetic Acid HCl Salt (21). Strecker nitrile adduct **20** (5.65 g, 0.018 mol) was heated in 120 mL of concentrated HCl and 30 mL of HOAc at 80 °C for 18 h and then cooled in an ice bath. Vacuum filtration of the resulting precipitate afforded **21** (5.21 g, 78%) as a white solid: ^{1}H NMR (500 MHz, MeOH-d_4) 1.55−1.88 (m, 12H), 2.10 (br s, 3H), 3.40 (s, 1H), 4.07 (m, 1H), 4.25−4.40 (m, 2H), 7.61 (br s, 5H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.28 min; MS (FAB) *m/z* 330 $[M + H]^+$.

(*S*)-*N*-Boc-Adamantylglycine (22). The hydrolyzed Strecker adduct **21** (5.21 g, 0.014 mol) was dissolved in 50 mL of MeOH and 10 mL of HOAc and hydrogenated with H_2 (50 psi) and 20% $Pd(OH)_2$−C (1.04 g, 20 wt %) for 18 h. The reaction was filtered and concentrated to afford the crude amino acid intermediate as a white solid which was triturated with Et_2O (3 × 25 mL) and dried in vacuo (4.9 g, quantitative): ^{1}H NMR (500 MHz, MeOH-d_4, NaOD) 1.50−1.70 (m, 12 H), 1.91 (br s, 3H), 2.72 (s, 1H). The solid thus obtained (4.90 g, 0.014 mol) was dissolved in 50 mL of DMF and treated with K_2CO_3 (5.90 g, 0.0427 mol, 3 equiv) and di-*tert*-butyl dicarbonate (3.14 g, 0.014 mol, 1 equiv). After 19 h, the DMF was removed under vacuum, 100 mL of H_2O and 100 mL Et_2O were added, and the layers were separated. The aqueous layer was washed with Et_2O (2 × 100 mL), cooled to 0 °C, diluted with EtOAc (200 mL), and the mixture was stirred vigorously while carefully acidifying to pH = 3 with 1 N HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (100 mL). The combined EtOAc extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography (5% MeOH/CH_2Cl_2 + 0.5% HOAc) to give **22** (4.07 g, 92% for two steps) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) 1.42 (s, 9H), 1.55−1.73 (m, 12H), 1.99 (br s, 3H), 3.98 and 5.07 (2d, 1H, rotamers); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, CH_3CN/H_2O/TFA) t_R = 2.70 min; MS (FAB) *m/z* 310 $[M + H]^+$.

(*S*)-Adamantylglycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (23). A DMF solution of compound **22** (129 mg, 0.416 mmol, 1 equiv) and **5** (100 mg, 0.416 mmol, 1 equiv) was treated with HOBT (169 mg, 1.25 mmol, 3 equiv), EDAC (163 mg, 0.833 mmol, 2 equiv), and TEA (0.17 mL, 1.25 mmol, 3 equiv) according to general method A. Purification using flash chromatography (silica gel, 2.5 × 13 cm, 50% EtOAc/CH_2Cl_2 and EtOAc) gave 156 mg (90%) of the desired product as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 0.78−0.84 (m, 2H), 1.42 (s, 9H), 1.50−1.75 (m, 12H), 1.97 (br s, 3H), 2.16 (m, 1H), 2.61 (d, J = 13.2, 1H), 3.67 (m, 1H), 4.41 (d, J = 9.7, 1H), 4.91 (d, J = 8.8, 1H), 5.33 (m, 1H), 7.10 (br s, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.13 min; MS *m/e* 418 $[M + H]^+$.

The coupled amide (144 mg, 0.345 mmol) was dehydrated according to general method A and the product was purified using flash chromatography (3−5% EtOAc in CH_2Cl_2, gradient) to afford the corresponding nitrile (98 mg, 71%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) 1.00−1.08 (m, 2H), 1.41 (s, 9H), 1.50−1.88 (m, 12H), 2.00 (br s, 3H), 2.35 (m, 1H), 2.55 (m, 1H), 3.84 (m, 1H), 4.33 (d, J = 9.9, 1H), 5.05 (m, 1H), 5.26 (d, J = 9.9, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.24 min; MS *m/e* 400 $[M + H]^+$.

The nitrile (96 mg, 0.240 mmol) was deprotected according to general method A. The solvents were removed after 40 min, and a solid was obtained after being placed under high vacuum. Trituration with Et_2O afforded **23** (74 mg, 74%) as a white solid: ^{1}H NMR (500 MHz, MeOH-d_4) 0.94 (m, 1H), 1.10 (m, 1H), 1.65−1.90 (m, 12H), 1.99 (m, 1H), 2.07 (br s, 3H), 2.34 (dd, J = 14.3, 2.2, 1H), 2.60 (m, 1H), 3.91 (m, 1H), 4.17 (s, 1H), 5.19 (dd, J = 11.0, 2.2, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.84 min; MS *m/e* 300 $[M + H]^+$. Anal. ($C_{18}H_{25}N_3O\cdot CF_3CO_2H\cdot 0.1H_2O$) C, H, N.

(*S*)-*N*-Boc-3-hydroxyadamantylglycine (24). A solution of $KMnO_4$ (337 mg, 2.133 mmol, 1.1 equiv) in 2% KOH (6 mL) at 60 °C was treated portionwise with **22** (600 mg, 1.94 mmol, 1 equiv), and the temperature was then increased to 90 °C. After 1.5 h, the reaction was cooled to 0 °C, EtOAc (50 mL) was added, and the mixture was carefully acidified to pH 3 with 1 N HCl. The layers were separated, and the aqueous phase was extracted with EtOAc (50 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography using a gradient of MeOH in CH_2Cl_2 [2% (200 mL), 3% (200 mL), 4% (200 mL), and 5% (500 mL) MeOH/CH_2Cl_2 + 0.5% HOAc] to afford **24** (324 mg, 51%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 1.41 (s, 9H), 1.40−1.73 (m, 12H), 2.21 (br s, 2H), 4.05 and 5.19 (2 br d, 1H, rotamers), 7.09 (br s, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.42 min; MS (FAB) *m/z* 326 $[M + H]^+$.

(*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinamide (25). A coupling reaction between **24** (404 mg, 1.24 mmol, 1 equiv) and **5** (328 mg, 1.37 mmol, 1.1 equiv) was carried out using general method A (HOBT (520 mg, 3.85 mmol, 3.1 equiv), EDAC (510 mg, 2.61 mmol, 2.1 equiv), and TEA (0.54 mL, 3.85 mmol, 3.1 equiv). Purifica-

tion of the crude product using flash column chromatography with a gradient of 6% (200 mL), 7% (200 mL), and 8% (500 mL) MeOH/CH_2Cl_2 gave **25** (460 mg, 85%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 0.82 (dd, J = 15.0, 6.2, 1H), 0.91 (br m, 1H), 1.41 (s, 9H), 1.45−1.75 (m, 12H), 1.93 (br s, 1H), 2.17−2.28 (m, 3H), 2.49 (dd, J = 13.2, 1.8, 1H), 3.67 (br m, 1H), 4.50 (d, J = 9.7, 1H), 4.86 (dd, J = 10.5, 2.2, 1H), 5.32 (d, J = 38.7, 1H), 5.66 (br s, 1H), 6.91 (br s, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.53 min; MS (FAB) *m/z* 434 $[M + H]^+$.

(*S*)-3-Hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (26). Amide **25** (7.08 g, 0.0163 mol) was dissolved in 100 mL of THF and cooled to 0 °C. Pyridine (6.6 mL, 0.082 mol, 5.0 equiv) was added, followed by the dropwise addition of trifluoroacetic anhydride (5.8 mL, 0.0408 mol, 2.5 equiv). Complete consumption of starting material was observed by TLC (SiO_2, 7% MeOH/CH_2Cl_2) after 1 h. The solvent was reduced to a volume of approximately 10 mL and the intermediate trifluoroacetate nitrile was hydrolyzed by stirring with 10% K_2CO_3 (45 mL) in MeOH (100 mL) at room temperature for 18 h. The MeOH was removed and the aqueous layer extracted with EtOAc /Et_2O (1:1 v/v, 500 mL). The combined organic extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with a gradient of 50% (700 mL) and 60% (2 L) EtOAc/CH_2Cl_2 to afford the desired Boc-protected nitrile (6.24 g, 92%) as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$) 1.01−1.06 (m, 2H), 1.40 (s, 9H), 1.44−1.90 (m, 13H), 2.22 (br m, 2H), 2.34 (dd, J = 13.7, 2.2, 1H), 2.54 (ddd, J = 16.5, 11.0, 6.0, 1H), 3.81 (br dd, J = 10.4, 4.4, 1H), 4.43 (d, J = 9.9, 1H), 5.01 (dd, J = 10.4, 2.2 1H), 5.29 (dd, J = 9.9, 9.9, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.72 min; MS (FAB) *m/z* 416 $[M + H]^+$.

The nitrile thus obtained (6.986 g, 0.0168 mol) was deprotected using TFA according to general method A. The solvents were removed after 2.5 h, and the resulting oil was azeotroped with CH_2Cl_2/toluene (2×) to obtain an off-white solid. Trituration with Et_2O (3 × 25 mL) afforded hydroxyadamantyl compound **26** (6.89 g, 95%) as a white powder: ^{1}H NMR (500 MHz, MeOH-d_4) 0.97 (ddd, J = 11.6, 7.2, 2.8, 1H), 1.10 and 1.13 (ABq, J_{AB} = 6.6, 1H), 1.55−1.85 (m, 12H), 2.01 (ddd, J = 14.3, 11.6, 5.5, 1H), 2.28 (s, 2H), 2.35 (dd, J = 13.7, 2.2, 1H), 2.62 (ddd, J = 13.7, 11.0, 5.5, 1H), 3.92 (ddd, J = 8.8, 6.0, 2.8, 1H), 4.28 (s, 1H), 5.19 (dd, J = 11.0, 2.2, 1H); ^{13}C NMR (125 MHz, MeOH-d_4) 14.3, 19.2, 31.4, 31.4, 31.5, 36.0, 38.2, 39.3, 40.9, 44.8, 44.9, 46.6, 47.0, 60.0, 68.6, 120.4, 167.4; HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 1.99 min; MS (FAB) *m/z* 316 $[M + H]^+$ for $C_{18}H_{25}N_3O_2$. Anal. ($C_{18}H_{25}N_3O_2$·CF_3CO_2H·1.25H_2O) C, H, N. The structure of compound **26** was further confirmed by single-crystal X-ray analysis.[29]

(S)-*N*-Boc-3,5-Dihydroxyadamantylglycine (27). Refer to the procedure described for generating hydroxyadamantyl-*N*-*tert*-butyloxycarbonyl-L-glycine **24.** During the reaction to produce **24** from **22**, the diol **27** is formed as a lower R_f minor product. Prolonged reaction times (up to 90 min) gave up to 17% of **27** as a white solid after flash chromatography eluting with 15% MeOH−CH_2Cl_2−0.5% HOAc: ^{1}H NMR (500 MHz, MeOH-d_4) 1.41−1.73 (m, 21H), 2.29 (br s, 1H), 3.95 (s, 1H). ^{13}C NMR (125 MHz, MeOH-d_4) 28.7, 31.8, 37.5, 41.8, 43.9, 46.3, 48.3, 49.6, 52.5, 63.1, 70.9, 71.0, 80.6, 157.9, 174.2.

(*S*)-3,5-Dihydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (28). A coupling reaction between **27** (300 mg, 0.88 mmol, 1 equiv) and **5** (253 mg, 1.05 mmol, 1.2 equiv) was carried out using general method A (HOBT (356 mg, 2.64 mmol, 3.0 equiv), EDAC (340 mg, 1.76 mmol, 2.0 equiv), and TEA (0.37 mL, 2.64 mmol, 3.0 equiv). Purification using flash column chromatography with a gradient of 10−20% MeOH/CH_2Cl_2 gave the coupled amide, contaminated with HOBT, which was carried on immediately to the nitrile in two separate reactions. In each reaction, the amide (100 mg, 0.11 mmol) was dissolved in 1 mL of THF, cooled to 0 °C, and treated with pyridine (0.054 mL, 0.66 mmol, 6.00 equiv), followed by the addition of trifluoroacetic anhydride (0.056 mL, 0.39 mmol, 3.5 equiv). Complete consumption of starting material was observed by TLC (SiO_2, 7% MeOH/CH_2Cl_2) after 30 min. The solvent was removed and the intermediate trifluoroacetate nitrile was hydrolyzed by stirring with 10% K_2CO_3 (1 mL) in MeOH (2 mL) at room temperature for 18 h. The two reaction mixtures were combined, the MeOH was removed, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The extracts were dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with a gradient of 7−8% MeOH/CH_2Cl_2 to afford the desired nitrile (78 mg, 41% for two steps) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) 1.01−1.06 (m, 2H), 1.32−1.78 (m, 22H, includes *N*-Boc singlet), 1.88 (m, 1H), 2.06 (br s, 2H), 2.34−2.38 (m, 2H), 2.56 (m, 1H), 3.82 (m, 1H), 4.52 (d, J = 9.9, 1H), 5.0 (dd, J = 10.6, 2.2, 1H), 5.46 (d, J = 9.9, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) 13.7, 17.9, 28.4, 30.4, 36.3, 38.0, 42.6, 42.9, 43.1, 45.1, 45.2, 45.3, 51.9, 58.0, 70.4, 80.1, 119.2, 155.8, 169.8; MS (FAB) *m/z* 432 $[M + H]^+$.

The Boc group of the intermediate nitrile (64 mg, 0.15 mmol) was deprotected using TFA according to general method A. The solvents were removed after 2.5, and the resulting oil was azeotroped with CH_2Cl_2/toluene (2×) to obtain an off-white solid. Purification by preparative HPLC [YMC S5ODS 30 mm × 100 mm, 15 min gradient of 0 to 100% B, 25 mL/min, 220 nm; solvent A = 10% MeOH−90% H_2O−0.1% TFA and solvent B = 90% MeOH−10% H_2O−0.1% TFA, elution time 5−6 min) afforded, after lyophillization from H_2O, 34 mg (53%) of **28** as a white lyophilate: ^{1}H NMR (500 MHz, MeOH-d_4) 0.90 (m, 1H), 1.02 (m, 1H), 1.41−1.70 (m, 12H), 1.92 (m, 1H), 2.24−2.31 (m, 2H), 2.53 (m, 1H), 3.82 (m, 1H), 4.26 (s, 1H), 5.10 (dd, J = 10.0, 2.2, 1H). 3.92 (m, 1H), 4.28 (s, 1H), 5.19 (d, J = 10.7, 1H); ^{13}C NMR (125 MHz, MeOH-d_4) 14.5, 19.3, 31.4, 31.6, 37.1, 39.3, 42.1, 43.6, 43.7, 45.7, 45.8, 47.1, 52.4, 59.4, 70.5, 120.3, 167.2. HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 1.99 min; HRMS *m/z* calcd $[M + H]^+$ for $C_{18}H_{25}N_3O_3$ 332.1974, found 332.1981. Anal. ($C_{18}H_{25}N_3O_3$·1.15CF_3CO_2H·1.50H_2O) C, H, N.

(*S*)-*N*-Boc-3-fluoroadamantylglycine-L-*cis*-4,5-methanoprolinamide (29). An oven-dried flask purged with argon was charged with CH_2Cl_2 (3 mL) and cooled to −78 °C. To the solvent was added diethylaminosulfur trifluoride (0.060 mL, 0.453 mmol, 1.5 equiv), followed by a solution of **25** (131 mg, 0.302 mmol, 1 equiv) in CH_2Cl_2 (3 mL). After 15 min, the reaction mixture was poured directly into a separatory funnel containing saturated $NaHCO_3$ (25 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (25 mL), and the combined extracts were washed with brine (10 mL), dried (Na_2SO_4), filtered, concentrated, and purified by flash chromatography with 5% MeOH/CH_2Cl_2 to afford **29** (124 mg, 0.285 mmol, 94%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) 0.78−0.89 (m, 2H), 1.42 (s, 9H), 1.46−1.87 (m, 12H), 2.20 (m, 1H), 2.29 (br s, 2H), 2.57 (dd, J = 13.2, 2.2, 1H), 3.64 (m, 1H), 4.54 (d, J = 9.3, 1H), 4.89 (dd, J = 10.4, 2.2, 1H), 5.34 (d, J = 9.9, 1H), 6.95 (br s, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, CH_3CN/H_2O/TFA) t_R = 2.22 min; MS *m/e* 436 $[M + H]^+$.

(*S*)-3-Fluoroadamantylglycine-L-*cis*-4,5-methanoprolinenitrile TFA Salt (30). Amide **29** (161 mg, 0.370 mmol, 1 equiv) was dehydrated according to general method A using 4 mL of pyridine, imidazole (53.4 mg, 0.776 mmol, 2.1 equiv), and phosphorus oxychloride (0.143 mL, 1.52 mmol, 4.1 equiv) to give, after purification by flash chromatography (5% EtOAc/CH_2Cl_2), the corresponding nitrile (126 mg, 82%) as a white foam: ^{1}H NMR (500 MHz, $CDCl_3$) 1.03−1.07 (m, 2H), 1.41 (s, 9H), 1.43−1.98 (m, 12H), 2.31 (br s, 2H), 2.36 (dd, J = 13.7, 2.2, 1H), 2.56 (m, 1H), 3.80 (m, 1H), 4.46 (d, J = 9.9, 1H), 5.02 (dd, J = 10.4, 2.2, 1H), 5.27 (d, J = 11, 1H); HPLC (YMC S-5 C18, 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 3.97 min; MS *m/e* 418 $[M + H]^+$. The Boc group of the resultant nitrile (125 mg, 0.299 mmol) was deprotected using trifluoroacetic acid/CH_2Cl_2 (1:1 v/v, 2 mL) according to general method A. After 30 min, the solvents were removed, and the resulting

solid was dried under high vacuum. Trituration with Et_2O afforded **30** (93 mg, 72%) as a white solid: ^{1}H NMR (500 MHz, MeOH-d_4) 0.94 (m, 1H), 1.10 (m, 1H), 1.56−1.70 (m, 4H), 1.73−2.06 (m, 9H), 2.30−2.43 (m, 3H), 2.62 (m, 1H), 3.92 (m, 1H), 4.33 (s, 1H), 5.19 (dd, J = 10.4, 2.2, 1H); HPLC (YMC S-5 C18 4.6 × 50 mm, 0−100% B, MeOH/H_2O/H_3PO_4) t_R = 2.44 min; MS *m/e* 318 $[M + H]^+$. Anal. ($C_{18}H_{24}FN_3O\cdot CF_3CO_2H$) C, H, N.

Biological Assays. Cloning, Expression, and Purification of Human DPP-IV. To generate human DPP-IV, PCR (Red-tag polymerase, Sigma) was performed on human cDNA from placenta (Clontech) using two primers, ACGCCGACGATGAAGACA and AGGTAAAGAGAAACATTGTT, based on the nucleotide sequence of the human clone (accession number M74777). PCR products were cloned into the pcDN4/HisMax TOPO vector (Invitrogene). For stable transfection of CHO-DG44 cells, PCR of DPP-IV was redone using primers GGTACCAGCGCAGAGGCTT and CTCGAGCTAAGGTAAAGAGAAACATTG to generate *Kpn* I and *Xho* I sites. The *Kpn* I and *Xho* I sites were used to extract the N-terminal His-tagged gene. The His tag, which could be cleaved and removed by enterokinase, was included to allow purification using the TALON affinity column. The gene was then ligated into the *Kpn* I and *Xho* I sites of the pD16 vector for stable transfection. Stable cell lines were generated by transfecting the expression vector into Chinese hamster ovary (CHO-DG44) cells using electroporation. The CHO-DG44 cell line was grown in PFCHO media supplemented with HT (glycine, hypoxanthine, and thymidine; Invitrogene), glutamine, and Recombulin (ICN). Then 1×10^7 cells/mL were collected, transfected with 60 μg of DNA using electroporation at 300V, and then transferred to a T75 flask. On the third day following transfection, the HT supplement was removed and selection was initiated with methotrexate (MTX, 10 nM, ICN). After a further 10 days, the cells were plated into individual wells of 96-well plates. Every 10 days the concentration of MTX was increased 2−3-fold, up to a maximum of 400 nM. Final stable cell line selection was based on yield and activity of the expressed protein. Protein was further purified using conventional anion exchange (Sepharose Q), gel filtration (S-200) and high-resolution MonoQ columns. The final protein yielded a single band on SDS−PAGE gels. Amino acid sequence analysis indicated two populations of DPP-IV in the sample. One portion of the protein had 27 amino acids truncated from the N-terminus, while the other was lacking the N-terminal 37 amino acids, suggesting that during isolation the entire transmembrane domain (including the His tag) is removed by proteases present in the CHO cells. Total protein concentration was measured using the Bradford dye method, and the amount of the active DPP-IV was determined by titrating the enzyme with our previously reported inhibitor (compound **29** in ref 18). No biphasic behavior was observed during inhibition or catalysis, suggesting that both protein populations are functionally identical.

In Vitro DPP-IV Inhibition Assays. Inhibition of human DPP-IV activity was measured under steady-state conditions by following the absorbance increase at 405 nm upon the cleavage of the pseudosubstrate, Gly-Pro-pNA. Assays were performed in 96-well plates using a Thermomax plate reader. Typically reactions contained 100 μL of ATE buffer (100 mM Aces, 52 mM Tris, 52 mM ethanolamine, pH 7.4), 0.45 nM enzyme, either 120 or 1000 μM of substrate ($S < K_m$ and $S > K_m$, K_m = 180 μM) and variable concentration of the inhibitor. To ensure steady-state conditions for slow-binding inhibitors, enzyme was preincubated with the compound for 40 min prior to substrate addition. All serial inhibitor dilutions were in DMSO and final solvent concentration did not exceed 1%. Inhibitor potency was evaluated by fitting inhibition data to the binding isotherm: v_i/v = range/$[1 + (I/IC_{50})^n]$ + background, where v_i is the initial reaction velocity at different concentrations of inhibitor, I; v is the control velocity in the absence of inhibitor; range is the difference between the uninhibited velocity and background; background is the rate of spontaneous substrate hydrolysis in the absent of enzyme; n is the Hill coefficient. Calculated IC_{50}'s at each substrate concentration were converted to K_i's by assuming competitive inhibition according to the equation $K_i = IC_{50}/[1 + (S/K_m)]$. All inhibitors were competitive as judged by close agreement of K_i values obtained from assays at high and low substrate concentrations. In cases where IC_{50} at the low substrate concentration was close to the enzyme concentration used in the assay, the data were fit to the Morrison equation to account for the depletion of the free inhibitor.[30] IC_{50} values were further refined to determine K_i values to account for the substrate concentration in the assay using $K_i = IC_{50}/[1 + (S/K_m)]$.

Liver Microsomal Metabolic Rate Determination Methods. Rat liver microsomes were purchased from Xenotech Co. (Lenexa, KS). Incubations contained 50 mM potassium phosphate, ca. 1 mg/mL microsomal protein, 10 mM NADPH, and 10 μM test compound. Reactions were initiated by the addition of substrate and were carried out in a shaking water bath at 37 °C. Incubations were terminated by the addition of an equal volume of acetonitrile and centrifugation. The supernatants were analyzed by LC/MS with parent quantitation at 0 and 10 min. The percent change in concentration was used to calculate a rate of metabolism of parent compound.

Pharmacokinetic and Bioavailability Studies in Rats. All procedures were approved by the Bristol-Myers Squibb Institutional Animal Care and Use Committee. Rats were housed under standard conditions and had free access to water and standard rodent laboratory diet. Adult male Sprague Dawley rats (250−300 g, Harlan Sprague Dawley, Indianapolis, IN) were surgically prepared with indwelling jugular vein cannulae 1 day prior to drug administration. Rats were fasted overnight prior to dosing and were fed 8 h after dosing. The animals had free access to water and were conscious and unrestrained throughout the study. Each rat was given either a single intravenous (iv) or oral dose (10 mg/kg, n = 2, both routes). The iv doses were administered as a bolus through the jugular vein cannula and the oral doses were by gavage. The compounds were administered as a solution in water. Blood samples (250 μL) were collected at serial time points for 12 h after dose into heparin-containing tubes. Plasma was prepared immediately, frozen, and stored at −20 °C prior to analysis.

Plasma Sample Analysis. Plasma proteins were precipitated with one volume of acetonitrile and the supernantants evaporated to dryness under a stream of nitrogen. The samples were reconstituted in mobile phase, and the compound of interest was analyzed by ionspray LC/MS. Sample quantitation was completed by comparison to a standard curve prepared in plasma and quality control samples were run to ensure assay performance. AUC values were calculated by the trapezoidal rule (oral) or by mixed log−linear integration (iv) using the Kinetica software package (Innaphase, Philadelphia, PA). Oral bioavailability was estimated by dividing the average AUC value calculated for the oral route by the average AUC value calculated for the iv route.

Rat ex Vivo Plasma DPP-IV Inhibition. DPP-IV activity in rat plasma was assayed ex vivo using Ala-Pro-AFC·TFA, a fluorescence-generating substrate from Enzyme Systems Products. Plasma samples were collected from normal male Sprague−Dawley rats at various timepoints following an oral dose of test compound as previously described.[18] 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 mL plasma. All in vivo data presented are mean ± SE (n = 6). Data analysis was performed using ANOVA followed by Fisher Post-hoc.

Oral Glucose Tolerance Test in Zucker Rats. 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 (0.3, 1, or 3 μmol/kg) at −240 min. Two blood samples were collected at −240 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, 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.

Oral Glucose Tolerance Test in *ob/ob* Mice. Male 13−14 week-old *ob*/*ob* mice (Jackson Labs) were maintained under constant temperature and humidity conditions, a 12:12 light-dark cycle, and had free access to a 10% fat rodent diet (D1245B Research Diets) and tap water. After an overnight fasting period of 16 h, animals were dosed orally with vehicle (water) or DPP-IV inhibitor (1, 3, 10 μmol/kg) at −60 min. Two blood samples were collected at −60 and 0 min by tail bleed for glucose and insulin determinations. Glucose (2 g/kg) was administered orally at 0 min. Additional blood samples were collected at 15, 30, 60, 90, and 120 min for glucose and insulin determinations. Blood samples were collected into EDTA-containing tubes (Sarstedt). Plasma glucose was determined with a Accu-Chek Advantage (Roche) glucometer. Plasma insulin was assayed using a mouse insulin ELISA kit (ALPCO Diagnostics). Data represent the mean of 12−24 mice/group. Data analysis was performed using one way ANOVA followed by Dunnett's test. All procedures were performed according to BMS−IACUC guidelines.

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Supporting Information Available: Elemental analyses for compounds **8b**−**g**, **10a**−**g**, **16c**−**e**, **23**, **26**, **28**, and **30**, and HRMS data for compounds **8a** and **18d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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