# 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties

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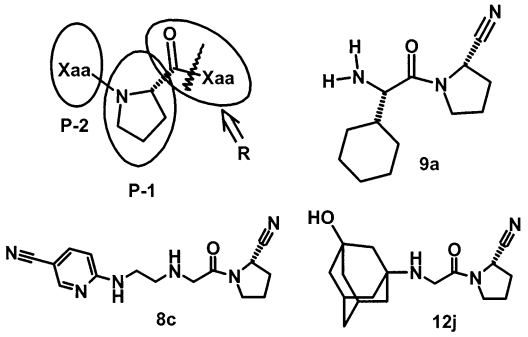
Dipeptidyl peptidase IV (DPP-IV) inhibition has the potential to become a valuable therapy for type 2 diabetes. The synthesis and structure−activity relationship of a new DPP-IV inhibitor class, N-substituted-glycyl-2-cyanopyrrolidines, are described as well as the path that led from clinical development compound 1-[2-[5-cyanopyridin-2-yl)amino]ethylamino]acetyl-2-cyano-(*S*)-pyrrolidine (NVP-DPP728, **8c**) to its follow-up, 1-[[(3-hydroxy-1-adamantyl) amino]acetyl]-2-cyano-(*S*)-pyrrolidine (NVP-LAF237, **12j**). The pharmacological profile of **12j** in obese Zucker fa/fa rats along with pharmacokinetic profile comparison of **8c** and **12j** in normal cynomolgus monkeys is discussed. The results suggest that **12j** is a potent, stable, selective DPP-IV inhibitor possessing excellent oral bioavailability and potent antihyperglycemic activity with potential for once-a-day administration.

## Introduction

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5) is a ubiquitous yet highly specific serine protease that cleaves N-terminal dipeptides from polypeptides with L-proline or L-alanine at the penultimate position.[1] The biological activities of many circulating regulatory peptides are altered or abolished by the action of DPP-IV in vitro.[2] However, in part because of the multiplicity of enzymes exhibiting DPP-IV-like activity,[3] the in vivo role of DPP-IV in mediating the cleavage of endogenous peptides and the consequences of its inhibition has yet to be established. One exception is with the incretin known as glucagon-like peptide-1 (GLP-1), the most potent insulinotropic hormone known.[4] Numerous studies with DPP-IV[5] and DPP-IV inhibitors[6−10] support a principal role of DPP-IV in the inactivation of GLP-1 in vivo. More importantly, the contribution of DPP-IV catalytic activity to blood glucose control through GLP-1 inactivation has recently been confirmed.[11] Because of multiple benefits of GLP-1 augmentation, DPP-IV inhibition has been recognized as a mechanistic approach of potential value in the treatment of type 2 diabetes.[12] Extending the duration of action of GLP-1 would stimulate insulin secretion, inhibit glucagon release,[13] and slow gastric emptying,[14] each a benefit in the control of glucose homeostasis in patients with type 2 diabetes or its predecessor syndromes. DPP-IV inhibition, through the preservation of active GLP-1 levels, has the potential to slow or even prevent the progression of type 2 diabetes by stimulating insulin gene expression and biosynthesis, increasing the expression of the

**Chart 1**




$\beta$-cell's glucose-sensing mechanism and promoting genes involved in the differentiation (neogenesis) of $\beta$-cells.[15] GLP-1 may play a role in acutely suppressing appetite in humans,[16] in mediating peripheral glucose uptake,[17] and may confer maximum glucose sensitivity to the hepatoportal glucose sensor.[18] As the glucose lowering effects of GLP-1 are dependent on elevated blood glucose and subside as glucose levels return to normal, the probability of hypoglycemia during treatment with a DPP-IV inhibitor is expected to be very low.[19] With few exceptions,[20−22] DPP-IV inhibitors resemble the P2−P1 dipeptidyl substrate cleavage product, where the P-1 site contains a proline mimic.[23,24] A straightforward replacement of the normally cleaved P-1 substrate amide (R in Chart 1) with an electrophile provides both irreversible (R = $P(O)(OPh)_2$, CONHOCOR′) and reversible (R = $B(OH)_2$, H, CN) inhibitors.[25] The nitrile is especially interesting and provides nanomolar inhibition and chemical stability adequate for oral administration ($X_{aa}$-(2*S*)-cyanopyrrolidines[26−28] and $X_{aa}$-(4*R*)-cyanothiazolidines[29]). Cyclohexylglycine-(2*S*)-cyanopyrrolidine (**9a**) is one of the more potent, selective, and stable representatives of this nitrile class ($K_i$ of 1.4 nM, >1000-fold selectivity over closely related peptidases and $t_{1/2}$ stability of > 48 h at pH 7.4).[27]

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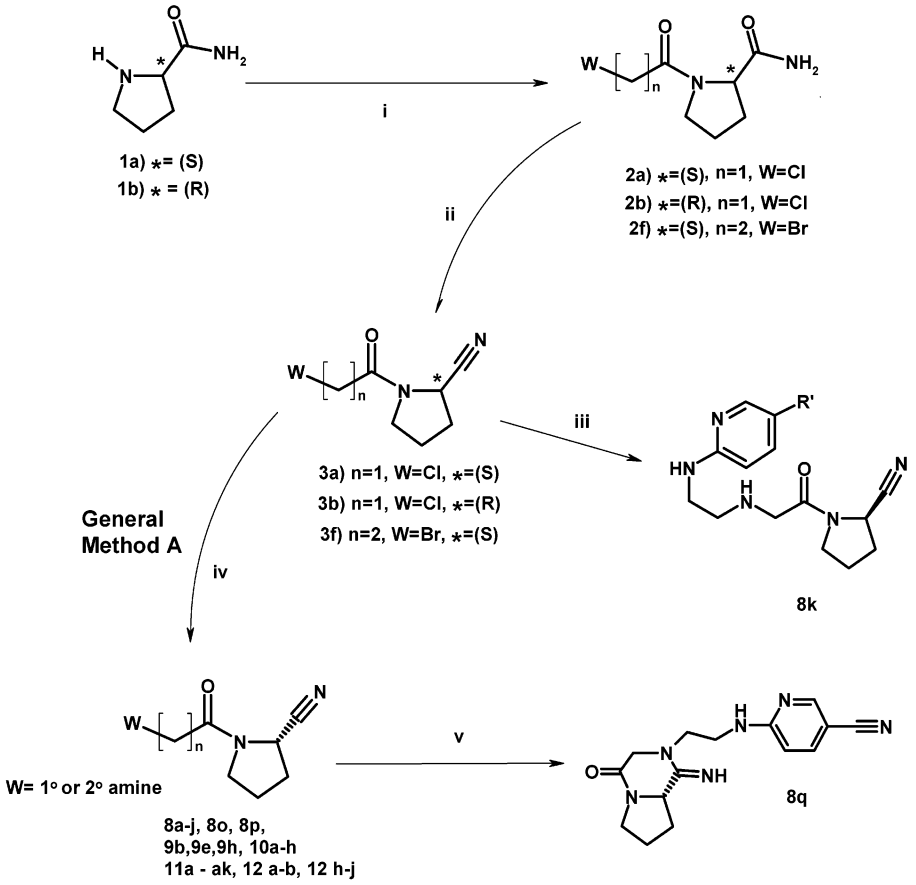
**Scheme 1**[a]



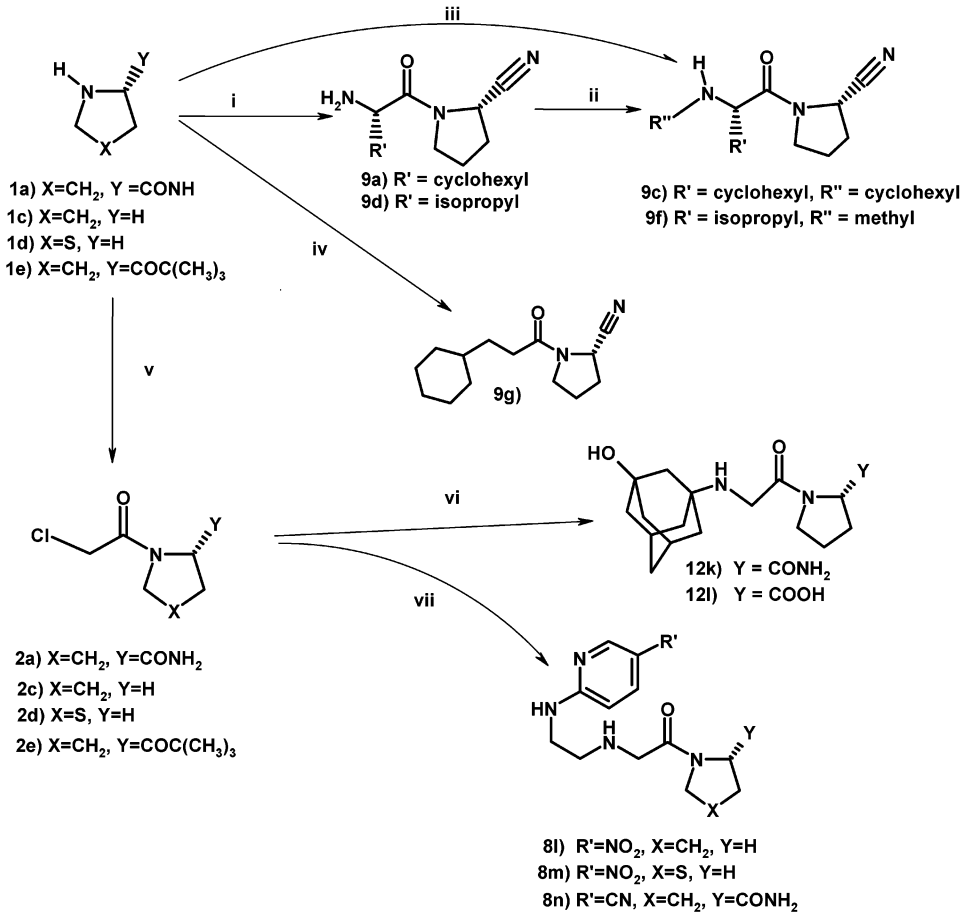

[a] Reagents: (i) **1a** → **2a** and **2f**, **1b** → **2b** respectively, $ClCH_2COCl$ for **2a** and **2b**, and $BrCH_2CH_2COCl$ for **2f**, THF or $CH_2Cl_2$, base, RT, 18 h; (ii) **2a**, **2b**, and **2f** for **3a**, **3b**, and **3f** respectively, TFAA, THF or $CH_2Cl_2$, RT; (iii) **3b**, 2-(2-aminoethylamino)-5-cyanopyridine, $K_2CO_3$, THF, RT; (iv) **3a** or **3f** (**3f** only for **8p**), THF, $K_2CO_3$, various amines, RT; then TFA/$CH_2Cl_2$ only for **11q**; (v) **8c**, 0.5 equiv of tributylamine, EtOAc, reflux, 96 h.

Until recently, a constant in DPP-IV inhibitor design had been an L-amino acid with a protonatable, N-terminal primary amine in the P-2 site (i.e., **9a**). The observation that substrates containing *N*-methylglycine in the P-2 site were cleaved[23] raised the possibility that structurally more complicated N-substituted glycines may be tolerated at the P-2 site. Through the previous use of a tandem resin-solution parallel synthesis,[30] a number of diverse P-2 site N-substituted glycines were prepared and found to provide potent inhibition when combined with a (2*S*)-cyanopyrrolidide in the P-1 site.[31−33] Herein is described the structure activity relationship of this class of DPP-IV inhibitors which has led to the selection of slow binding inhibitors NVP-DPP728 (**8c**) and NVP-LAF237 (**12j**) (Chart 1) as clinical development candidates for type 2 diabetes. In addition, a solution-based method for multigram synthesis of this P-2 site N-substituted glycine DPP-IV inhibitor class is described as well as the pharmacological profile of a selective DPP-IV inhibitor, **12j**, which exhibits excellent potency and oral bioavailability.

## Chemistry

Pyrrolidine and thiazolidine analogues **8a**−**q**, **9a**−**h**, **10a**−**h**, **11a**−**ak**, and **12a**,**b**,**h**−**l** were prepared as described in Schemes 1 and 2 and listed in Tables 1−3. As shown in Scheme 1, the addition of chloroacetyl chloride to **1a** and **1b** led to **2a** and **2b**, respectively, while the addition of 3-bromopropionyl chloride to **1a** led to **2f**. Dehydration of amides **2a**, **2b**, and **2f** with trifluoroacetic anhydride (TFAA) provided nitriles **3a**, **3b**, and **3f**, respectively, which were then coupled with various amines to provide the desired 2-cyanopyrrolidides **8a**−**k**, **8o**, **8p**, **9b**, **9e**, **9h**, **10a**−**h**, **11a-ak**, **12 a**−**b**, **12 h**−**j** either as the free base, monohydrochloride, dihydrochloride, or TFA salt. Amines used were either commercially available or were known and prepared from literature. These 2-cyanopyrrolidides are stable for months to years if kept as dry solids but will convert to the cyclic amidine with $t_{1/2}$ between 48 h to >70 days in buffered aqueous medium (pH 7.4). They appear as mixtures of cis and trans amide rotomers in solution according to NMR. Compounds **8c** and **12j** are trans amide rotomers in crystalline form as evidenced by the X-ray crystallographic analysis,[34] possessing a solubility of >100 mg/mL in distilled water. With minor modifications, the solution synthesis (general method A in Scheme 1) has provided **8c** and **12j** on the 100-kg scale. Intramolecular cyclization of **8c** with 0.5 equiv of tributylamine produced cyclic amidine **8q** after refluxing in EtOAc for 96 h. As shown in Scheme 2, the conversion of **1a** to nitriles **9a**, **9d**, **9f**, and **9g** was carried out through DIC coupling with *N*-t-boc-L-α-cyclohexylglycine, *N*-t-boc-L-valine, *N*-t-boc-3-methyl-2-methylaminobutyric acid, and cyclohexanepropionic acid, respectively, followed by amide dehydration using $POCl_3$ for

**Scheme 2**[a]




[a] Reagents: (i) **1a**, BocHNCHR′COOH, HOBt, $CH_2Cl_2$, NMM, DIC, RT, 18 h; then $POCl_3$, pyridine, imidazole, −30 °C, 1 h; then HCl(g), $Et_2O$, RT, 18 h; (ii) **9a** → **9c**, cyclohexanone, $NaB(O_2CCH_3)_3H$, $ClCH_2CH_2Cl$, RT, 18 h; (iii) **1a** → **9f**, *N*-t-boc-3-methyl-2-methylaminobutyric acid, HOBt, $CH_2Cl_2$, NMM, DIC, RT, 18 h; then $POCl_3$, pyridine, imidazole, −30 °C, 1 h; then HCl(g), $Et_20$, RT, 18 h; (iv) **1a**, 3-cyclohexanepropionic acid, $CH_2Cl_2$, DMAP, DIC, RT, 6 h, then TFAA, $CH_2Cl_2$, 0 °C, 2 h; (v) **1a**, **1c**, **1d**, and **1e** → **2a**, **2c**, **2d**, and **2e** respectively, $ClCH_2COCl$, THF or $CH_2Cl_2$, $K_2CO_3$, base, RT, 18 h; (vi) $K_2CO_3$, THF or $CH_2Cl_2$, RT, 3-hydroxy-1-aminoadamantane, **2a** for **12k** and **2e** for **12l**; then TFA, $CH_2Cl_2$, RT for **12l**; (vii) **2c**, **2d**, and **2a** for **8l**, **8m**, and **8n**, respectively, $K_2CO_3$, THF, 2-(2-aminoethylamino)-5-nitropyridine for **8l** and **8m** and 2-(2-aminoethylamino)-5-cyanopyridine for **8n**, RT.

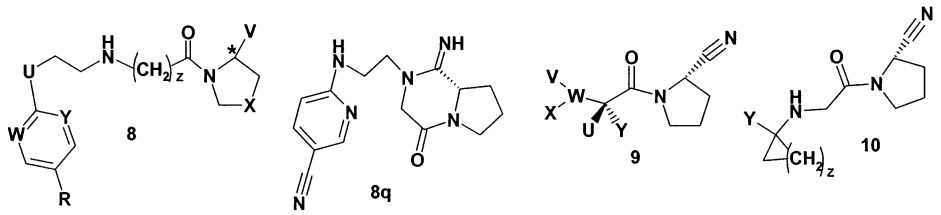
**9a**, **9d**, **9f** or TFAA for **9g**. Coupling of amine **9a** with cyclohexanone and reduction of the resulting imine using sodium triacetoxyborohydride provided amine **9c**. Coupling of **2a** and **2e** with 3-hydroxy-1-aminoadamantane[33] provided **12k** and the t-boc ester precursor of **12l**, respectively. Acid-catalyzed removal of the t-boc-ester using TFA in $CH_2Cl_2$ led to **12l**. Coupling of 2-(2-aminoethylamino)-5-nitropyridine with **2c** and **2d** provided **8l** and **8m**, respectively, while coupling of 2-(2-aminoethylamino)-5-cyanopyridine with **2f** provides **8n**.

Adamantyl analogues **12c**−**g** and **12m**−**r** were prepared as described in Scheme 3 and are listed in Table 3. The N-substituted glycine ester **4**, prepared by coupling 1-adamantaneamine and ethyl bromoacetate, was reacted with benzyl chloroformate followed by ester hydrolysis with LiOH to provide glycine derivative **5**. The free acid in **5** was DIC-coupled with various amines followed by CBZ-deprotection via palladium-catalyzed hydrogenation to provide *N*-adamantyl glycine amide derivatives **12c**−**g**. The hydroxyl group in carbamate **6**[33] was coupled with isocyanates or carbamoyl chlorides to provide bis-carbamates **7a**−**e** while coupling **6** with acetyl chloride provided ester **7f**. CBZ-deprotection of **7a**−**e** via palladium-catalyzed hydrogenation followed by coupling of the resulting adamantylamines with **3a** provided **12m**−**r**, respectively.

## Results and Discussion

The pyrrolidide, thiazolidide, and N-substituted glycine amide derivatives described above were tested for inhibition of DPP-IV derived from human colonic carcinoma cells (Caco-2),[35] and the results are included in Tables 1−3. An earlier manuscript[30] described the solid-phase synthesis of a library of ∼200 N-substituted glycine-2-cyano-(*S*)-pyrrolidides which provided **8a** as one of a few low nanomolar DPP-IV inhibitors. Starting with **8a**, a focused structure activity profiling effort was initiated by first introducing changes to the P-2 site amine, 2-(2-aminoethyamino)-5-nitropyridine. Replacement of the *p*-nitro group of **8a** with electrophilic groups, such as chloride (**8b**), cyano (**8c**), and trifluoromethyl (**8e**), provided modest decreases in inhibitory potency (4-, 3-, and 10-fold, respectively) while the *o*-trifluoromethyl analogue **8f** significantly reduced potency (>100-fold reduction). Electron-withdrawing group substitutions on the 2-aminopyridine ring do not appear to be essential since only a 4-fold reduction in inhibitory potency relative to the *p*-nitro analogue **8a** is obtained with the *p*-hydrogen substitute (**8g**, $IC_{50}$ = 33 nM).

**Table 1.** DPP-IV Inhibitor Data for Templates **8**−**10**[a]



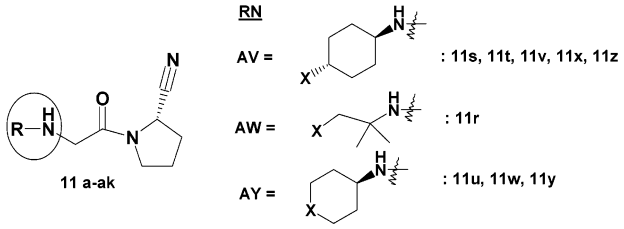
| compd | R | U | V | W | X | Y | Z | * | DPP−IV[b] |
|---|---|---|---|---|---|---|---|---|---|
| **8a** | $NO_2$ | NH | CN | CH | $CH_2$ | N | 1 | S | 8 ± 3 |
| **8b** | Cl | NH | CN | CH | $CH_2$ | N | 1 | S | 36 ± 5 |
| **8c** | CN | NH | CN | CH | $CH_2$ | N | 1 | S | 22 ± 4 |
| **8d** | H | NH | CN | N | $CH_2$ | N | 1 | S | 163 ± 39 |
| **8e** | $CF_3$ | NH | CN | CH | $CH_2$ | N | 1 | S | 83 ± 4 |
| **8f** | H | NH | CN | $CCF_3$ | $CH_2$ | N | 1 | S | 989 ± 218 |
| **8g** | H | NH | CN | CH | $CH_2$ | N | 1 | S | 33 ± 4 |
| **8h** | H | NH | CN | CH | $CH_2$ | CH | 1 | S | 298 ± 113 |
| **8i** | H | O | CN | CH | $CH_2$ | CH | 1 | S | 635 ± 112 |
| **8j** | H | $CH_2$ | CN | CH | $CH_2$ | CH | 1 | S | 491 ± 40 |
| **8k** | CN | NH | CN | CH | $CH_2$ | N | 1 | R | 1170 ± 21 |
| **8l** | $NO_2$ | NH | H | CH | $CH_2$ | N | 1 | | 7400 ± 1,500 |
| **8m** | $NO_2$ | NH | H | CH | S | N | 1 | | 3000 ± 600 |
| **8n** | CN | NH | $CONH_2$ | CH | $CH_2$ | N | 1 | S | 434 000 ± 20 000 |
| **8o** | CN | $NCH_2$ | CN | CH | $CH_2$ | N | 1 | S | 102 ± 10 |
| **8p** | $NO_2$ | NH | CN | CH | $CH_2$ | N | 2 | S | 18 700 ± 1600 |
| **8q** | | | | | | | | | 1540 ± 520 |
| **9a** | | H | H | N | H | c-$C_6H_{11}$ | | | 2 ± 0.5 |
| **9b** | | H | H | N | c-$C_6H_{11}$ | H | | | 64 ±33 |
| **9c** | | H | H | N | c-$C_6H_{11}$ | c-$C_6H_{11}$ | | | 18 600 ± 3900 |
| **9d** | | H | H | N | H | $CH(CH_3)_2$ | | | 2 ± 1 |
| **9e** | | H | H | N | $CH(CH_3)_2$ | H | | | 192 ± 112 |
| **9f** | | H | H | N | $CH_3$ | $CH(CH_3)_2$ | | | 9610 ± 3590 |
| **9g** | | H | H | CH | c-$C_6H_{11}$ | H | | | >500 000, $n = 2$ |
| **9h** | | H | $CH_3$ | N | c-$C_6H_{11}$ | H | | | 103 000 ± 8000 |
| **10a** | | | | | | H | 1 | | 612 ± 148 |
| **10b** | | | | | | H | 2 | | 80 ± 27 |
| **10c** | | | | | | H | 3 | | 54 ± 30 |
| **10d** | | | | | | $CH_2OH$ | 3 | | 26 ± 17 |
| **10e** | | | | | | $CH_2OH$ | 4 | | 104 ± 14 |
| **10f** | | | | | | H | 5 | | 63 ± 13 |
| **10g** | | | | | | H | 6 | | 53 ± 32 |
| **10h** | | | | | | H | 10 | | 338 ± 7 |

[a] Values are $IC_{50}$ (nM) expressed as the mean ± SD of three independent determinations unless otherwise noted. [b] Primary DPP−IV assay in human Caco-2 cells; procedure described in Experimental Section.

Replacement of the 2-aminopyridine ring in **8g**, with pyrimidine (**8d**), aniline (**8h**), phenyl (**8j**), and phenoxy (**8i**) provided increasingly less potent inhibition (5-, 9-, 15-, and 19-fold, respectively, relative to **8g**). Increasing from the ethyl diamine chain found in **8c** to the propyl diamine analogue **8o** provided a modest (4-fold) decrease in inhibitory potency while a dramatic reduction (2300-fold) was observed when the α-amino acid in **8a** was substituted with a *β*-amino acid (**8p**).

As expected, replacement of the P-1 site 2(*S*)-cyano group in **8c**, which plays a critical role as a transition-state mimetic[36] with a 2(*R*)-cyano group (**8k**), significantly reduced potency (53-fold). (Most of the inhibitory potency seen with **8k** can be attributed to ∼1% contamination with **8c** which is due to ∼1% L-prolinamide contamination in the commercial source of the D-prolinamide starting material. This contamination may also explain the previously reported identical dissociation kinetics between **8c** and **8k** ($k_{off}$ of $1.5 \times 10^{-3}$ $s^{-1}$).[36]) Replacement of the 2(*S*)-cyano group with either a hydrogen (**8l**) or a 2(*S*)-amide (**8n**) dramatically decreased inhibitory potency (900-fold compared to **8a** and 20 000-fold compared to **8c**, respectively). Both des-cyanopyrrolidide (**8l**) and des-cyanothiazolidide (**8m**) provided low *μ*M $IC_{50}$s, similar to what had been reported for such P-1 site carbon- and sulfur-containing rings in DPP-IV inhibitor classes possessing amino acid groups in the P-2 site.[37] Further evaluation of *p*-cyano analogue (**8c**) in various in vitro DPP-IV and selectivity assays (Table 4) led to its nomination as our first development compound. When compared with the *p*-nitro analogue **8a** and cyclohexylglycine-(2*S*)-cyanopyrrolidine (**9a**),[27] **8c** was of comparable activity against human plasma DPP-IV and was significantly more selective for DPP-IV over closely related peptidases, such as post-proline-cleaving enzyme (PPCE) and/or DPP-II.[38] Selectivity of **8c** for human plasma DPP-IV over PPCE and DPP-II was 27 000- and 16 000-fold, respectively. In addition, the in vitro specificity of **8c** was profiled in over 100 receptor and enzyme assays, and no significant binding was observed (10 *μ*M). Under neutral and basic aqueous conditions, the P-2 site amine can nucleophilically attack the carbon of the pyrrolidide-nitrile to form the 70-fold less active (attempts to convert **8c** to **8q** resulted in a 1% impurity of **8c**, which may account for most of the observed inhibitory potency seen for **8q**. Attempts to further purify cyclic amidine **8q** via HPLC were not pursued due to facile conversion to the ketopiperazine) cyclic amidine **8q**. Under in vitro assay conditions employed, this intramolecular cycliza-

**Table 2.** DPP-IV Inhibitor Data for Template **11**[a]

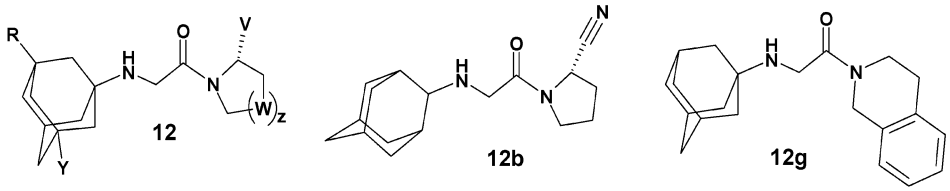



| compd | RN | DPP−IV[b] | compd | RN | DPP−IV[b] |
|---|---|---|---|---|---|
| **11a** | ethylamine | 444 ± 35 | **11p** | 2-amino-2-methyl-1,3-propanediol | 372 ± 92 |
| **11b** | propylamine | 475 ± 45 | **11q** | 2-amino-2-methylproprionic acid | 10 900 ± 6900 |
| **11c** | butylamine | 261 ± 132 | | **11r** to **11z** shown below | |
| **11d** | pentylamine | 266 ± 107 | | | |
| **11e** | hexylamine | 282 ± 15 | **11aa** | benzylamine | 988 ± 146 |
| **11f** | heptylamine | 291 ± 12 | **11ab** | 1-naphthalene-methylamine | 436 ± 37 |
| **11g** | 5-hydroxypentylamine | 346 ± 18 | **11ac** | phenethylamine | 305 ± 51 |
| **11h** | 3,3-dimethylbutylamine | 466 ± 18 | **11ad** | 1,1-dimethylphenethylamine | 24 ± 5 |
| **11i** | 1-ethylpropylamine | 398 ± 142 | **11ae** | 3,3-diphenylpropylamine | 377 ± 76 |
| **11jh** | (1*S*)-1-(hydroxymethyl)propylamine | 256 ± 132 | **11af** | 2-aminoindane | 133 ± 47 |
| **11k** | 1-isopropyl-2-methylpropylamine | 10 900 ± 5100 | **11ag** | 2-aminobenzimidazole | >1 000 000, $n = 2$ |
| **11l** | *tert*-butylamine | 35 ± 7 | **11ah** | *exo*-2-aminonorbornane | 15 ± 8 |
| **11m** | 1,1-dimethylpropylamine | 105 ± 16 | **11ai** | (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheylamine | 16 ± 5 |
| **11n** | 1,1,3,3-tetramethylbutylamine | 123 ± 30 | **11aj** | 1-adamantanemethylamine | 598 ± 204 |
| **11o** | 2-amino-2-methylpropan-1-ol | 218 ± 62 | **11ak** | (−)-*cis*-myrtanylamine | 242 ± 23 |

| compd | RN | X for AV, AW, or AY | DPP−IV[b] |
|---|---|---|---|
| **11r** | AW | 5-cyano-2-aminopyridine | 29 ± 2 |
| **11s** | AV | 5-cyano-2-aminopyridine | 5 ± 1 |
| **11t** | AV | 5-trifluoromethyl-2-aminopyridine | 7 ± 0 |
| **11u** | AY | 5-trifluoromethyl-2-aminopyridine | 296 ± 110 |
| **11v** | AV | 2-aminobenzothiazole | 10 ± 1 |
| **11w** | AY | 2-aminobenzothiazole | 228 ± 49 |
| **11x** | AV | c-$C_6H_{11}CONH$ | 18 ± 6 |
| **11y** | AY | c-$C_6H_{11}CONH$ | 23 ± 6 |
| **11z** | AV | *p*-Cl-$C_6H_4O$ | 14 ± 2 |

[a] Values are $IC_{50}$ (nM) expressed as the mean ± SD of three independent determinations. [b] Primary DPP−IV assay in human Caco-2 cells; procedure described in Experimental Section.

**Table 3.** DPP-IV Inhibitor Data for Adamantyl Template **12**[a]



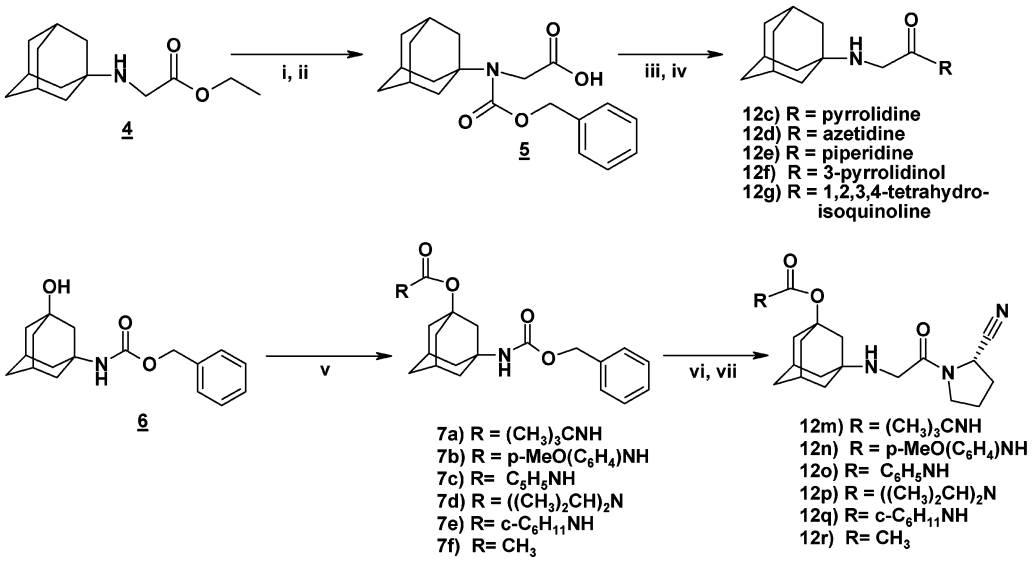

| compd | R | Y | V | W | Z | DPP−IV[b] |
|---|---|---|---|---|---|---|
| **12a** | H | H | CN | $CH_2$ | 1 | 3 ± 2 |
| **12b** | | | | | | 13 ± 6 |
| **12c** | H | H | H | $CH_2$ | 1 | 7000 ± 1300 |
| **12d** | H | H | H | $CH_2$ | 0 | 24 200 ± 5300 |
| **12e** | H | H | H | $CH_2$ | 2 | 52 600 ± 8500 |
| **12f** | H | H | H | CHOH | 1 | >1 000 000, $n = 2$ |
| **12g** | | | | | | 26 100 ± 4900 |
| **12h** | $CH_2CH_3$ | H | CN | $CH_2$ | 1 | 7 ± 1 |
| **12i** | $CH_3$ | $CH_3$ | CN | $CH_2$ | 1 | 17 ± 0 |
| **12j** | OH | H | CN | $CH_2$ | 1 | 3.5 ± 1.5 |
| **12k** | OH | H | $CONH_2$ | $CH_2$ | 1 | 931 000 ± 105 000 |
| **12l** | OH | H | COOH | $CH_2$ | 1 | 477 000 ± 89 000 |
| **12m** | $OCONHC(CH_3)_3$ | H | CN | $CH_2$ | 1 | 22 ± 7 |
| **12n** | $OCONH((C_6H_5)$ *p*-$CH_3O)$ | H | CN | $CH_2$ | 1 | 149 ± 15 |
| **12o** | $OCONHC_6H_5$ | H | CN | $CH_2$ | 1 | 62 ± 5 |
| **12p** | $OCON(CH(CH_3)_2)_2$ | H | CN | $CH_2$ | 1 | 183 ± 15 |
| **12q** | $OCONH(c\text{-}C_6H_{11})$ | H | CN | $CH_2$ | 1 | 64 ± 16 |
| **12r** | $OCOCH_3$ | H | CN | $CH_2$ | 1 | 70 ± 16 |

[a] Values are $IC_{50}$ (nM) expressed as the mean ± SD of three independent determinations. [b] Primary DPP−IV assay in human Caco-2 cells; procedure described in Experimental Section.

tion was slow ($t_{1/2}$ > 2 days), resulting in less than 1% of **8c** converting during the time frame of the experiments.

According to a recent crystal structure of human DPP-IV complexed with the known inhibitor (valine pyrrolidide),[39] the P-2 site valine points into a large cavity

**Scheme 3**[a]




[a] Reagents: (i) benzyl chloroformate, $CH_2Cl_2$, $K_2CO_3$, RT, 20 h; (ii) LiOH, THF/$H_2O$, 20 h; (iii) NMM, HOBt, DIC, $CH_2Cl_2$, various amines; (iv) 10% Pd/C, $H_2$ (1 atm), EtOAc, RT; (v) **6**, appropriate isocyante, TMSCl and either $CH_2Cl_2$, RT, 18 h or $ClCH_2CH_2Cl$, 50 °C, 18 h for **7a**−**c**,**e**; diisopropylcarbamoyl chloride for $ClCH_2CH_2Cl$, 50 °C, 18 h for **7d**; and acetyl chloride, pyridine, DMAP, $ClCH_2CH_2Cl$, RT, 24 h for **7f**; (vi) 10% Pd/C, $H_2$ (1 atm), EtOH, RT; (vii) **3a**, THF, $K_2CO_3$, RT, amines derived from **7a**−**f** to provide **12m**−**r**, respectively.

**Table 4.** DPP-IV Inhibition and Selectivity Assays[a]

| | Caco-2[b] | rat plasma[b] | human plasma[b] | PPCE[c] | DPP-II[d] |
|---|---|---|---|---|---|
| **8a** | 8.0 ± 3.0 | 17.3 ± 0.34 | 8.73 ± 0.79 | 16 000 ± 1200 | 12 000 ± 580 |
| **8c** | 22.0 ± 2.0 | 6.0 ± 1.0 | 7.0 ± 1.7 | 190 000 ± 46 000 | 110 000 ± 5800 |
| **9a** | 2.0 ± 0.3 | 2.8 ± 0.2 | 3.15 ± 0.19 | 41 000 ± 14 000 | 102 000 ± 20 000 |
| **12j** | 3.5 ± 1.5 | 2.3 ± 0.1 | 2.7 ± 0.1 | 210 000 ± 40 000 | >500 000 |

[a] Values are $IC_{50}$ (nM) expressed as the mean ± SEM of three independent determinations; procedures described in Experimental Section. [b] Primary DPP−IV assays. [c] Extract from human erythrocytes. [d] Extract from bovine kidney homogenate.

in the catalytic binding site and does not make specific contacts with DPP-IV. Due to the importance of the 2(*S*)-cyano group and the use of the P-1 site pyrrolidide ring in our N-substituted glycine series, one can assume that our inhibitors bind DPP-IV in a fashion similar to that observed for valine pyrrolidide. During our SAR effort around **8a**, we explored the steric limitations of DPP-IV's S-2 site cavity first with inhibitors that possess both P-2 site N- and C-substitutions. Whereas proline is a well-known N- and C-substituted cyclic amino acid for the P-2 site in DPP-IV inhibitors,[37] little is known about the effect of acyclic N- and C-substituted amino acids. Initial studies suggested that there are significant steric restrictions in this cavity when inhibitors possess both acyclic C- and N-substitutions. Whereas either a C-substituted (**9a**) or N-substituted (**9b**) cyclohexyl group provides low nM inhibition (Table 1), positioning cyclohexyl groups at both the C-and N-terminus (**9c**) significantly reduces potency (9300-fold less compared to **9a** and 290-fold less compared to **9b**). The steric restriction was also observed with significantly smaller lipophilic groups. Whereas a single isopropyl substitution is tolerated on either the P-2 site carbon (**9d**) or nitrogen (**9e**), isopropyl substitution at carbon and methyl at nitrogen (**9f**) was not well tolerated providing significantly reduced potency compared to C-substituted **9d** (4800-fold less). It was found that simple lipophilic and branched C-substitutions provided significantly greater inhibitory effect than N-substitution analogues (**9a**/**9d** vs **9b**/**9e**). One can speculate that the N- and C-substitution pattern plays a role in alignment of the basic terminal amine of inhibitors to form the required salt bridges with DPP-IV's glutamic acids, Glu 205 and Glu 206.[39] Inhibitor alignment between DPP-IV's salt bridge formed by Glu 205 and Glu 206, the oxyanion hole, and the S1-pocket may also explain the lack of potency observed with the P-2 site $\beta$-amino acid (**8p**) discussed above. As with DPP-IV substrates,[1,2] a basic, primary, or secondary amine is essential for inhibitory potency as replacement of the N-terminal amine in **9b** with carbon (**9g**) or *N*-methylation of **9b** to provide a tertiary amine (**9h**) dropped potency by >7800-fold and 1600-fold, respectively.

The study of P-2 site N-substituted glycines continued with the evaluation of various lipophilic ring-sizes (Table 1). The S-2 site pocket of DPP-IV appears to tolerate a large number of lipophilic rings, providing $IC_{50}$s ranging from 53 nM to 80 nM for four to eight members (**10b**, **10c**, **9b**, **10f**, **10g**) with only a modest drop in potency (5- to 10-fold) for the very small three-membered (**10a**) or rather large 12-membered (**10h**) rings (Table 1). Unlike the simpler ring series where the cyclopentyl (**10c**) and cyclohexyl (**9b**) rings are of similar potency, the hydroxymethylcyclohexyl analogue **10e** is 4-fold less active than the hydroxymethylcyclopentyl analogue **10d**.

As with the above ring systems, inhibitory potency did not vary dramatically ($IC_{50}$ = 261 to 475 nM, Table 2) between the simple N-terminal linear chain extensions (ethyl to heptyl, **11a**−**f**), the hydrophilic 5-hydroxypentyl **11g**, or with chain-end sterics found in 3,3-dimethylbutylamine **11h**. As linear chain extensions

were observed to be 4- to 8-fold less potent than ring systems branched near the terminal amine, acyclic chains branching α to the terminal amine were examined further (Table 2). Starting with the simplest branched analogue, isopropyl derivative **9e**, it was found that methylene extensions (1-ethylpropylamine, **11i**) or methylene and hydroxyl extensions (1-hydroxymethylpropylamine, **11j**) only slightly decreased potency. However, steric limits were quickly exceeded with the bis-*gem*-dimethyl extension (**11k**) which provided a 60-fold reduction in potency ($IC_{50}$ = 10.9 $\mu$M) compared to **9e**. Fully branched carbons adjacent to the P-2 site amine proved more interesting as demonstrated with the hydroxymethylcyclopentylamine (**10d**, $IC_{50}$ = 26 nM) and the *tert*-butylamine groups (**11l**, $IC_{50}$ = 35 nM). Simple lipophilic and hydrophilic chain extensions on the *tert*-butyl group of **11l** were well tolerated as shown with methyl (**11m**), *tert*-butyl (**11n**), monohydroxyl (**11o**), or bis-hydroxyl (**11p**) groups that only slightly decreased inhibitory potency relative to **11l** (3- to 10-fold). However, replacement of a methyl group from **11l** with a carboxylic acid (**11q**) dramatically reduced potency (300-fold) which may reflect hydrophobic and/or steric limitations.

Cyclic and acyclic branching on the P-2 site pharmacophore of **8c** and **8e** was well tolerated. Substitution of a *gem*-dimethyl group into the 1,2-diaminoethyl chain of **8c** provided the equipotent **11r**, while replacement with a *trans*-1,4-diaminocyclohexyl group provided increased potency as shown with **11s** (4-fold) and **11t** (12-fold), respectively. Substitutions on the *trans*-1,4-diaminocyclohexyl template were well tolerated as shown by the modest potency reductions through replacing the 5-cyano-2-aminopyridine of **11s** with a 2-aminobenzothiazole (**11v**) or a with a cyclohexylamide (**11x**) (2- and 3-fold reduction, respectively). However, replacements with 4-aminopiperidine provided a different profile than observed with the *trans*-1,4-diaminocyclohexyl group. Replacement with 4-aminopiperidine for the 1,2-diaminoethyl chain of **8e** (**11u**) and the *trans*-1,4-diaminocyclohexyl chain in **11t** and **11v** (**11w**) provided >20-fold decreased in inhibitory potency, whereas similar low nM potency was seen for 1,4-diaminocyclohexyl (**11x**) and 4-aminopiperidine (**11y**) analogues containing the cyclohexylamide groups. The advantage of geometrical restriction also carried over into the amino alcohol side chain **11z,** which provided a 45-fold increase in inhibitory potency over the 2-diaminoethane analogue **8i**.

As with the simple aliphatic chains described above, the inhibitory potency of phenyl-substituted aliphatic P-2 site amine extensions, such as benzylamine (**11aa**), 1-naphthalenylmethylamine (**11ab**), phenethylamine (**11ac**), 3-phenylpropylamine (**8j**), and 3,3-diphenylpropylamine (**11ae**), did not vary dramatically ($IC_{50}$ = 305 nM to 988 nM, Table 2). Numerous substitutions on benzylamine (**11aa**) and phenethylamine (**11ac**) were explored and showed little effect on potency compared to these unsubstituted analogues (data not shown). Compared to the simpler phenethyl derivative **11ac**, sterics in the form of a *gem*-dimethyl group adjacent to the P-2 site amine (**11ad**) and a conformational constrained ring analogue (**11af**) proved more potent (13- and 2-fold). The fact that the 2-aminobenzimidazole analogue **11ag** is >7500-fold less potent than the saturated 2-aminoindane analogue **11af** supports the importance of a basic, protonatable P-2 site amine in the N-substituted glycine template for DPP-IV inhibition.

Further explorations of sterics adjacent to the P-2 site amine concentrated on multiple cyclic rings and provided favorable results (Tables 2 and 3). Bicyclic ring systems containing 2-aminonorbornane (**11ah**) and isopinocampheylamine (**11ai**) or tricyclic ring systems containing 1- and 2-adamantylamine (**12a** and **12b**, respectively) all provided very potent inhibitory effect. With an $IC_{50}$ of 3 nM, **12a** was one of the most potent P-2, N-substituted glycine derivatives found. However, lipophilic extensions on **12a** as subtle as the 3-ethyl- (**12h**) and 3,5-dimethyl-1-adamantylamine (**12i**) analogues somewhat reduced inhibitory potency compared to **12a** (2- and 6-fold). The importance of steric ring bulk adjacent to the P-2 site amine is further supported by the dramatic potency decrease seen with methylene insertion between the steric group and the amine in the form of the 1-adamantanemethylamine group in **11aj** and (−)-*cis*-myrtanylamine group in **11ak** (Table 2). With a 1-adamantyl group as the optimal P-2 site substitution, P-1 site requirements were further evaluated starting with the des-cyano analogue of **12a** (**12c**). Although 2300-fold less potent as compared to its 2(*S*)-cyano counterpart, pyrrolidine (**12c**) was noticeably more potent than both azetidine (**12d**) and piperidine (**12e**) P-1 site ring analogues (4- and 8-fold, respectively). As expected, the narrow lipophilic pocket formed by the stacking of Tyr662 and Tyr666 in DPP-IV's catalytic S-1 site[39] did not tolerate the hydrophilic nature of the P-1 site 3-pyrrolinol in **12f** which was >330 000-fold less potent than its pyrrolidide counterpart (**12c**). Interestingly, the tetrahydroisoquinoline P-1 site analogue **12g** was as potent as the piperidine P-1 site counterpart **12e**, suggesting either the narrow pocket of DPP-IV's catalytic S-1 site can tolerate a phenyl-extended P-1 site analogue of **12e** or that **12g** binds DPP-IV differently than **12e**.

Examination of primary metabolites of 1-and 2-adamantylamine analogues **12a** and **12b** suggest that monohydroxylation on the adamantyl ring may be well-tolerated in DPP-IV's catalytic S-2 site. To study the effect of monohydroxylation and avoid incorporation of an additional chiral center in monohydroxylation, the 3-hydroxylated-1-aminoadamantane analogue **12j** was prepared. **12j** was noticeably more potent in the Caco-2, rat-, and human plasma DPP-IV assays (6-, 3-, and 3-fold) as well as showing superior efficacy in various in vitro selectivity assays (Table 4) when compared with both **8c** and literature's cyclohexylglycine-(2S)-cyanopyrrolidine (**9a**). **12j** was profiled in over 100 receptor and enzyme assays, and no significant binding was observed (10$\mu$M). Because of the sterics imposed by the adamamtyl group, the ability for **12j** to cyclize to an analogue of imidine **8q** was significantly reduced (>30-fold) compared to that of **8c** and **9a.** To date, **12j** is the most stable DPP-IV inhibitor, possessing a P-1 site transition-state mimetic. The dramatic reduction in inhibitory potency of 2(*S*)-amide (**12k**) and 2(*S*)-acid (**12l**) compared to **12j** (266 000- and 14 000-fold, respectively) provides further support that **12j** binds in the

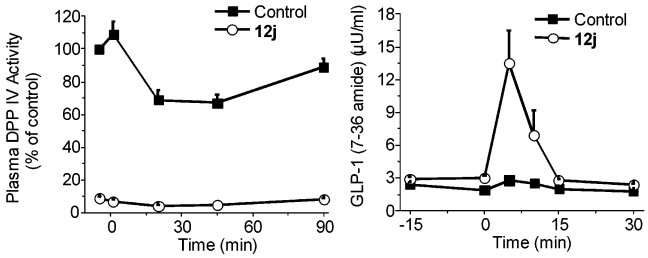



**Figure 1.** Obese male Zucker rats were orally dosed with CMC or **12j** (10 $\mu$mol/kg) immediately following the −15 min blood sample collection. Plasma DPP-IV activity (left) and levels of GLP-1 (7−36 amide) (right) were measured during the OGTT. Data were the mean ± SEM of nine rats/group; procedures are described in Experimental Section. For both DPP-IV activity and GLP-1 (7−36 amide) levels, values for the **12j**-treated rats were significantly different from control at all time points (Student's *t*-test, $p < 0.05$, except for time −15 min GLP-1 (7−36 amide) levels that were not significant). Plasma DPP-IV activity of −5 min control samples was 3.4 ± 0.9 mU/mL.

S1- and S2-catalytic sites of DPP-IV. Substitutions on the 3-hydroxyl group of **12j** in the form of carbamates (**12m**−**q**) and ester (**12r**) resulted in significantly reduced potency compared to **12j**. In the carbamate series, potency correlated with size as the diisopropyl analogue **12p** was 8-fold less potent than the *tert*-butyl analogue **12m** while the 4-methoxyphenyl analogue **12n** was 2-fold less potent than both the similarly sized phenyl and cyclohexyl analogues **12o** and **12q**, respectively.

Evaluation of our first development compound (**8c**) in rat,[9] monkey,[30] and human[40] had previously supported the connection between DPP-IV inhibition and improvement in oral glucose tolerance through an increase in active GLP-1 levels. In a recent chronic study, **8c** was shown to improve glucose tolerance in both normal and glucose-intolerant mice through improved islet function as judged by increased GLUT-2 expression, increased insulin secretion, and protection from increased islet size in insulin resistance.[41] In a continuing effort to explore the pharmacological potential of the N-substituted glycine-2-(*S*)-cyanopyrrolidide class of inhibitor, we evaluated the antidiabetic potential of **12j** in an oral glucose tolerance test (OGTT) with obese Zucker (fa/fa) rats which are profoundly insulin-resistant, are markedly glucose intolerant, and represent a rather severe model of Type II diabetes. The results shown in Figure 1 demonstrate that **12j** is a potent, orally active inhibitor of plasma DPP-IV activity that provides increased levels of GLP-1 (7−36 amide). Administration of **12j** (10 $\mu$mol/kg, po) 15 min prior to a glucose challenge inhibited plasma DPP-IV activity >90% within 10 min and throughout the 90 min study. The activity in CMC-treated plasma obtained at −5 min was 3.4 ± 0.9 mU/mL and was set as 100%. DPP-IV activity of rats dosed with CMC vehicle declined approximately 35% at 20 and 45 min postglucose challenge. This decline in the control group is attributed to both plasma volume expansion caused by increased osmolarity following the glucose load and to possible plasma dilution caused by frequent blood sampling and donor blood replacement. Prior to **12j** administration (−15 min) plasma levels of GLP-1 (7−36 amide) were no different (CMC vs **12j**; 2.4 ± 0.2 vs 2.9 ± 0.2 pM, (not statistically significant), Student's *t*-test). However, GLP-1 (7−36 amide) levels were 60% higher ($p < 0.001$,

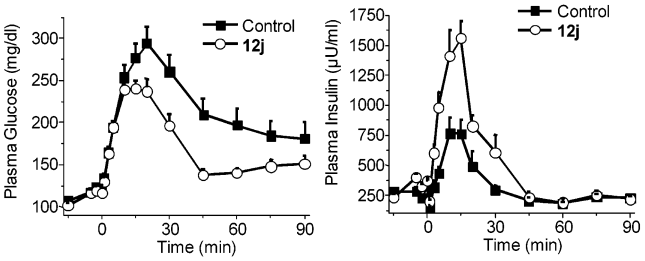



**Figure 2.** Obese male Zucker rats were orally dosed with CMC or 10 $\mu$mol/kg **12j** immediately following the −15 min blood sample collection. Glucose was administered orally (1 g/kg) immediately following the time 0 min blood collection. Data were the mean ± SEM of eight rats/group; procedure is described in Experimental Section. Analysis of the glucose and insulin AUC reveal statistically significant differences between the CMC and **12j** groups.

Student's *t*-test) in the **12j**-treated rats 15 min after drug administration and before the glucose challenge (0 min). Following the glucose challenge, GLP-1 (7−36 amide) in the drug-treated rats peaked at 5 min at levels that were close to 5 times those of CMC-treated rats (13.5 ± 3.0 vs 2.8 ± 0.2 pM, $p = 0.001$), and returned back to baseline by 15 min. This return to baseline of GLP-1 (7−36 amide) levels by 15 min while DPP-IV is completely inhibited is fully in line with the glucose-dependency of GLP-1 (7−36 amide) secretion from the intestinal L-cell. The results in Figure 2 support the ability of **12j** to both significantly decrease glucose excursions and stimulate insulin secretion. Obese animals receiving CMC were markedly glucose intolerant, with peak glucose levels of 294 ± 19 mg/dL at 20 min while **12j**-treatment decreased the glucose excursion with noticeable differences starting after 5 min and provided peak glucose levels that reached only 241 ± 8 mg/dL at 15 min. Interestingly, while insulin levels were no different between the two groups at the start of the experiment (−15 min), insulin levels were elevated 40% before the glucose challenge in the **12j**-treated group. This modest increase most likely resulted from **12j**-induced preservation of active GLP-1 (7−36 amide) described above. Upon glucose challenge, plasma insulin levels of **12j**-treated rats peaked at levels twice those of vehicle-treated rats and returned to baseline levels by 45 min. The 0 to 45 min insulin AUC was 275% higher in **12j**-treated rats with a corresponding 35% reduction of the 0−45 min glucose AUC. The glucose-stimulated insulin release index (insulin AUC 0−45/glucose AUC 0−45) was ∼5-fold greater in the **12j**-treated rats.

The pharmacokinetic profile of **12j** was compared to **8c** in normal cynomolgus monkeys. The absolute oral bioavailability of both **8c** and **12j** were both excellent (>90%) following a 1 $\mu$mol/kg oral dose while clearance from plasma was moderate ∼1.3 l/h/kg and ∼1.5 l/h/kg, respectively. The steady-state volumes of distribution are also moderate for both **8c** (0.8 L/kg) and **12j** (0.7 L/kg), suggesting that both are distributed principally in the body fluids. The $C_{max}$ for **12j** (293 nM at 72 min) is 2.7-fold lower than for **8c** (805 nM at 36 min) while the terminal elimination half-life of **12j** (90 min) is 2.6-fold longer than for **8c** (35 min). Differences seen in the pharmacokinetic profiles of **8c** and **12j** correlated well with observed DPP-IV inhibition pharmacodynamic differences. When comparing the effect on DPP-IV

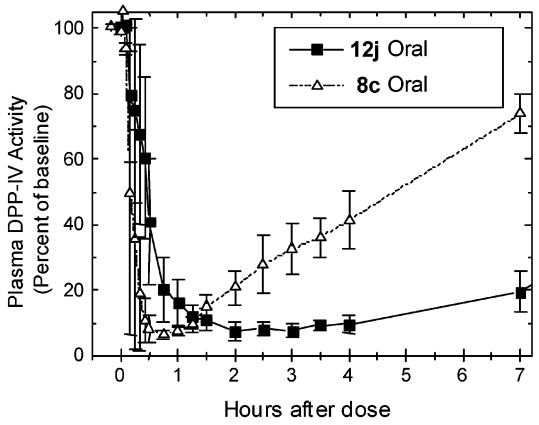



**Figure 3.** Effects of **12j** (black squares) and **8c** (open triangles) following oral administration of a single 1 $\mu$mol/kg dose. Values are means ± SEM of three monkeys **12j** or means ± range of two monkeys **8c**; procedure is described in Experimental Section.

inhibition in normal Cynomolgus monkeys (Figure 3), maximum inhibition of plasma DPP-IV activity (~95%) was observed approximately 2 h postdose of **12j** (1 $\mu$mol/kg, po) while ≥50% inhibition of DPP-IV was observed within 30 min postdose and persisted for ≥10h (not shown). Maximum inhibition of plasma DPP-IV activity (~95%) occurred within 30 min postdose with **8c** (1 $\mu$mol/kg, po) but inhibition of ≥50% persisted only for 4 to 5 h postdose. As expected with this glucose-dependent mechanism, no hypoglycemia was observed. As a reversible DPP-IV inhibitor with a relatively short half-life, development candidate **8c** might most effectively be taken with a meal when GLP-1 secretion is at maximal rate, while development candidate **12j,** with its longer half-life, provides a better profile for once-a-day treatment if desired. The results of these pharmacological studies suggest that **12j** is a potent, selective, and orally active inhibitor of DPP-IV that improves insulin secretion and glucose homeostasis with a profile wholly consistent with increased action of glucagon-like peptide-1.

## Experimental Section

**Chemistry.** All melting points (mp) were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR ($^1$H NMR) and carbon NMR ($^{13}$C NMR) spectra were obtained on a Bruker AC 300-MHz spectrometer. Chemical shifts were recorded in ppm ($\delta$) and were reported relative to the solvent peak or TMS. Chemical shifts recorded for carbon NMR spectra comprised both trans and cis amide rotomers without further delineation. Mass spectra were run on a Finnigan Mat 4600 spectrometer. Elemental analyses, performed by Robertson labs, are within 0.4% of theoretical values. Analytical results were within ±0.4% of the theoretical value. Fractional moles of water and solvent frequently found in some analytical samples were not removed despite 24−48 h of drying in a vacuum and were confirmed, where possible, by their presence in the $^1$H NMR spectrum. Optical rotation measurements were performed on a JASCO P-1030 polarimeter, 589 nm, cell length 1 dm, at 20 °C. Column chromatography separations were carried out using Merck silica gel 60 (mesh 230−400) employing a SIMS/Biotage apparatus. Reagents and solvents were purchased from common suppliers and were utilized as received. All reactions were conducted under a nitrogen atmosphere at room temperature with a calcium sulfate drying tube unless noted otherwise. Yields were of purified product and were not optimized. All starting materials and amines were commercially available unless otherwise indicated. Experimentals for **8b, 8d**−**j**, **8o**, **9b**, **9e**, **9h**, **10a**−**h**, **11a-ak**, **12a**−**b**, and **12h**−**i** follow General Method A and are found in the Supporting Information.

**1-Chloroacetyl-2-(*S*)-pyrrolidinecarboxamide (2a).** A solution of L-prolinamide (**1a**) (5.0 g, 43.9 mmol) in THF (100 mL) was added dropwise over 0.5 h to a stirred mixture of chloroacetyl chloride (3.50 mL, 43.9 mmol) and $K_2CO_3$ (24.0 g, 175 mmol) in THF (50 mL) at ambient temperature. The reaction mixture was then stirred at room temperature for 18 h and filtered and the filtrate concentrated to provide the desired amide **2a** as a clear, taffy (7.46 g, 89%); $^1$H NMR ($CDCl_3$) 1.94−2.45 (m, 4H), 3.54−3.65 (m, 1H), 3.65−3.75 (m, 1H), 4.08−4.18 (m, 2H), 4.57−4.67 (m, 1H), the two amide hydrogens spread from 6.1 to 7.2 with small broad peaks at 6.18 and 7.04; MS *m/z* (rel intensity, %) 191 ($M^+$, 100), 193 ($M^+$, 33).

**1-Chloroacetyl-2-cyano-(*S*)-pyrrolidine (3a).** A solution of L-prolinamide (**1a**) (10.0 g, 87.7 mmol) in THF (300 mL) was added dropwise over a period of 0.75 h to a mechanically stirred solution of chloroacetyl chloride (6.99 mL, 87.7 mmol) and $K_2CO_3$ (48.5 g, 351 mmol) in THF (100 mL). After another 2 h of being stirred, the reaction was filtered to remove potassium salts, and the filtrate was dried over $Na_2S0_4$ (30 g). This solution of **2a** was again filtered, and to the colorless filtrate was added trifluoroacetic anhydride (19.8 mL, 0.140 mmol) in one portion. The resulting clear, light amber reaction was stirred for 1 h at RT. EtOAc (30 mL) was added prior to rotovap concentration (water bath <45 °C) with two more EtOAc chasings (2×) once the concentrate was ~25 mL. The resulting clear, light-orange solution was then partitioned between EtOAc and saturated $NaHCO_3$, and the aqueous layer was washed twice with EtOAc. The combined organic layers were then successively washed with water and brine, dried over $Na_2SO_4$, filtered, and concentrated to obtain 1-chloroacetyl-2-(*S*)-pyrrolidinenitrile as clear, amber oil. This oil solidified quickly to provide the target product **3a** (7.82 g, 52%) as a yellow-white solid; mp 53−57 °C; $^1$H NMR ($CDCl_3$) (4 to 1 mixture of trans/cis amide rotomers): $\delta$ 2.10−2.40 (m, 4H), 3.55−3.66 (m, 1H), 3.66−3.79 (m, 1H), 4.03−4.21 (m, 0.4H, C*H*$_2$Cl), 4.09 (s, 1.6H, C*H*$_2$Cl), 4.76 (m, 0.8H, C*H*CN), 4.87 (dd, 0.2H, $J$ = 7.4 and 2.2 Hz, C*H*CN); $^{13}$C NMR ($CDCl_3$): $\delta$ 165.15, 117.72, 46.95, 46.82, 46.64, 46.37, 41.41, 32.32, 29.81, 25.05, 22.61; MS *m/z* (rel intensity, %) 173 ($M^+$, 100), 175 ($M^+$, 33).

**General Method A.** A 0.5 M solution of **3a**, **3b**, or **3f** (1 equiv) in THF was added dropwise over 0.5 h into an ice−water-cooled 0.3 M mixture of the appropriate amine (2 to 3 equiv) and $K_2CO_3$ (3 equiv) in THF. The resulting reaction was stirred at ice−water temperature for 2 h and then at room temperature for 1 to 3 days. The resulting mixture was then filtered to remove $K_2CO_3$, concentrated, and then partitioned between $CH_2Cl_2$ and saturated aqueous $NaHCO_3$. The aqueous layer was then washed with $CH_2Cl_2$ (2×), and the combined organic layers were washed with water, dried over $Na_2SO_4$, concentrated in vacuo, and chromotographed on silica gel with 5% $CH_3OH$ in $CH_2Cl_2$ or 20% EtOAc in hexane to provide the free base of the target product. Alternatively, the reaction may be carried out using $CH_2Cl_2$ as solvent in the reaction while EtOAc may be substituted for $CH_2Cl_2$ in the partitioning with water. The free base may be converted to the mono- or dihydrochloride salt by slowly passing HCl gas into a 0.1 M solution (THF or $Et_2O$) of the free base. The resulting solids are then filtered and dried in vacuo. The amine coupling step with **3a** or **3b** provided chromatographed yields that ranged between 30 to 50%.

**1-[[[2-[(5-Nitro-2-pyridinyl)-amino]ethyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine, Dihydrochloride (8a).** Method A: 2-(2-aminoethylamino)-5-nitropyridine[31] as amine and use of **3a.** Product was a light vanilla-colored solid: mp = 205−208 °C decomp; $^1$H NMR ($D_2O$) (trans and cis rotomers in a ratio of 9:1): $\delta$ 2.01−2.17 (m, 2H), 2.20−2.30 (q, 2H, $J$ = 5 Hz), 3.36−3.49 (m,3H), 3.55−3.64 (m, 1H), 3.82 (t, 2H, $J$ = 5 Hz), 4.10 (s,2H), 4.72 (t, 9/10H, $J$ = 5H, **H**CN), 4.90 (bd, 1/10H,

$J$ = 7 Hz, **H**CN), 6.81 (d,1H, $J$ = 9 Hz), 8.27 (bd, 1H, $J$ = 9 Hz), 8.90 (d, 1H, $J$ = 1 Hz); (isobutane/DCI) MH/e 318 ($MH^+$) of free base; Anal. ($C_{14}H_{18}N_6O_3$·2HCl·0.2$H_2O$·0.1THF) C, H, N, Cl.

**1-[[[2-[(5-Cyano-2-pyridinyl)amino]ethyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine, Dihydrochloride (8c).** Method A: 2-(2-aminoethylamino)-5-cyanopyridine[31] used as amine and use of **3a**. Product was an off-white solid; OR: [d] = −77.152, 12.25 mg/mL in MeOH; white solid; mp: 155−157 °C; $^1$H NMR ($D_2O$) (trans and cis amide rotomers in a ratio of 5:1): $\delta$ 2.15−2.30 (m, 2H), 2.35−2.43 (m, 2H), 3.50−3.60 (m, 1H), 3.63−3.75 (m, 1H), 3.80 (t, 2H, $J$ = 6 Hz), 3.90 (t, 2H, $J$ = 6 Hz), 4.19 (s, 2H), 4.85 (t, 5/6H, $J$ = 6 Hz, *H*CN), 5.00 (dd, 1/6H, $J$ = 8 and 1 Hz, *H*CN), 6.99 (d, 1H, $J$ = 9 Hz), 7.97 (dd, 1H, $J$ = 11 and 1 Hz), 8.50 (s, 1H). (isobutane/DCI) MH/e 299 ($MH^+$) of free base; Anal. ($C_{15}H_{18}N_6O$·2.0HCl·0.19$H_2O$·0.30THF) C, H, N.

**1-[[[2-[(5-Cyano-2-pyridinyl)amino]ethyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine, Dihydrochloride (8k).** D-Prolinamide (**1b**) (commercial source contaminated with ∼1% L-prolinamide) was converted to 1-chloroacetyl-2-cyano-(*R*)-pyrrolidine (**3b**) following the procedure of **3a**. The synthesis for dihydrochloride of **8c** was then followed. Product was a white solid; mp = 152−154 °C; $^1$H NMR ($D_2O$) (trans and cis amide rotomers in a ratio of 5:1): $\delta$ 2.17−2.27 (m, 2H), 2.35−2.45 (m, 2H), 3.48−3.60 (m, 2H), 3.67−3.75 (m, 1H), 3.79−3.87 (m, 1H), 3.93 (t, 2H, $J$ = 6 Hz), 4.22 (s, 2H), 4.87 (t, 5/6H, $J$ = 6 Hz, C*H*CN), 5.00 (dd, 1/6H, $J$ = 8 and 2 Hz, C*H*CN), 7.01 (d, 1H, $J$ = 9 Hz), 7.99 (dd, 1H, $J$ = 10 and 1 Hz), 8.48 (d, 1H, $J$ = 1 Hz); (isobutane/DCI) MH/e 299 ($MH^+$ of free base); Anal. ($C_{15}H_{18}N_6O_1$·1.95HCl·0.50$H_2O$·0.50THF) C, H, N.

**1-[2-(5-Nitropyridin-2-yl)amino]ethylamino]acetylpyrrolidine, Dihydrochloride (8l).** A solution of pyrrolidine **1c** (2.50 g, 35.0 mmol), triethylamine (3.90 mL, 38.7 mmol), and 20.0 mg of (dimethylamino)pyridine (DMAP) in $CH_2Cl_2$ (20.0 mL) was added, dropwise to an ice-cold solution of 3.08 mL (38.7 mmol) of chloroacetyl chloride in 20.0 mL of $CH_2Cl_2$, over a period of 60 min. The resulting solution was then stirred at ice−water temperature under a calcium sulfate drying tube for 2 h and then at room temperature for 16 h. The solution was poured into 150 mL of EtOAc and the resulting white precipitate filtered. The filtrate was washed with ethyl acetate and concentrated in vacuo to obtain 3.80 g of crude 1-chloroacetylpyrrolidine (**2c**). In a 200 mL flask was dissolved 14.3 g (79.0 mmol) of 2-[(5-nitropyridin-2-yl)amino]ethylamine into 50 mL of tetrahydrofuran, and the mixture was then ice−water cooled. To this cooled mixture was added a solution of 3.80 g (26.0 mmol) of 1-chloroacetylpyrrolidine (**2c**) in 25.0 mL of tetrahydrofuran, via addition funnel, over 40 min. The reaction was then stirred at ice−water temperature for 2 h under a calcium sulfate drying tube and then allowed to stir at room temperature for 18 h. The solvent was then removed in vacuo and the resulting oily paste partitioned between $CH_2Cl_2$ and water. The aqueous layer was then washed twice with methylene chloride, and the combined organic layers were washed successively with water and brine, dried over sodium sulfate, concentrated, and then purified on silica gel, employing a mixture of 5% methanol in methylene chloride as the eluent to yield the free base as a golden solid. The dihydrochloride salt was obtained following Method A to provide 5.42 g (57% yield) of **8l** a tan solid; mp 220−222 °C ; $^1$H NMR ($D_2O$): $\delta$ 1.92−2.12 (m, 4H), 3.51 (t, 2H, $J$ = 5.9 Hz), 3.58(t, 4H, $J$ = 6 Hz), 4.05 (t, 2H, $J$ = 6 Hz), 4.20 (s, 2H), 7.08 (d, 1H, $J$ = 10 Hz), 8.48 (d, 1H, $J$ = 10 Hz), 9.05 (d, 1H, $J$ = 1 Hz); (isobutane/DCI) MH/e 294 ($MH^+$ of free base); Anal. ($C_{13}H_{19}N_5O_1$·2HCl·0.2$H_2O$·0.2THF) C, H, N.

**1-[2-(5-Nitropyridin-2-yl)amino]ethylamino]acetylthiazolidine, Dihydrochloride (8m).** A solution of chloroacetyl chloride (2.5 g, 22.0 mmol) in 25 mL of tetrahydrofuran was added dropwise (over 20 min) into an ice−water cold solution containing 2.0 g (22.0 mmol) of thiazolidine (**1d**), 12.4 g (90.0 mmol) of $K_2CO_3$, and 50 mL of tetrahydrofuran. The reaction was then stirred at ice−water temperature under a calcium sulfate drying tube for 2h. The potassium salts were removed via filtration to yield a clear colorless solution of 1-chloroacetylthiazolidine (**2d**) which was immediately taken onto the next step and assumed quantitative (3.7 g). To a 500 flask was dissolved 12.1 g (67.0 mmol) of 2-(2-aminoethylamino)-5-nitropyridine into 50 mL of tetrahydrofuran, and the mixture was then cooled in an ice bath. To this cooled mixture was then added a solution of 3.7 g (22.0 mmol) of 1-chloroacetylthiazolidine in the tetrahydrofuran solution (from previous step) dropwise over 30 min. Following the procedure outlined in **8l**, the dihydrochloride salt of **8m** (1.80 g, 25%) was provided as an off-white solid; mp = 203 °C-205 °C; $^1$H NMR ($D_2O$): $\delta$ 3.01 (t, 1H, $J$ = 6.2 Hz), 3.09 (t, 1H, $J$ = 6.2 Hz), 3.37(t, 2H, $J$ = 5.2 Hz), 3.67(t, 1H, $J$ = 6.2 Hz), 3.71(t, 1H, $J$ = 6.2 Hz), 3.79 (t, 2H, $J$ = 5.9 Hz), 4.10 (d, 2H, 9.6 Hz), 4.43 (d, 2H, $J$ = 11.8 Hz), 6.76(d, 1H, $J$ = 9.2 Hz), 8.30 (dd, 1H, $J$ = 9.6, 2.6 Hz), 9.03 (d, 1H, $J$ = 2.9 Hz); (ESI-pos) MH/e 312 ($MH^+$ of free base); Anal. ($C_{12}H_{17}N_5O_3S_1$·2HCl), C, H, N.

***N*-[2-[(5-Cyano-2-pyridinyl)amino]ethyl]glycyl-L-prolinamide (8n).** A solution of **2a** (1.00 g, 5.24 mmol) in $CH_2Cl_2$ (20 mL) was added dropwise over 10 min to an ice−water-cooled mixture of 2-(2-aminoethylamino)-5-cyanopyridine (1.70 g, 10.5 mmol) and $K_2CO_3$ (2.90 g, 21.0 mmol) in $CH_2Cl_2$ (40 mL). The reaction was then stirred at ice−water temperature for 2 h and then room temperature for 18 h. The potassium salts were removed via filtration, and the filtrate was concentrated. The residue was chromatographed with 10% $CH_3OH$ in $CH_2Cl_2$ and then with a 90/10/1 mixture of $CH_2Cl_2$/$CH_3OH$/$NH_4OH$ which provided the desired product (558 mg, 34%) as a white solid; mp = 77−80 °C; $^1$H NMR ($CDCl_3$) (2 to 1 ratio of trans/cis amide rotomers): $\delta$ 1.86−2.10 (m, 2H), 2.10−2.23 (m, 1H), 2.23−2.32 (m, 1H), 2.82−3.03 (m, 2H), 3.31−3.61 (m, 4H), 3.48 (s, 1.33H, trans conformer), 3.48 (s, 0.66H, cis amide conformer), 3.65−3.57 (m, 1H), 4.47 (dd, 1/3 H, $J$ = 4.9 and 2.3 Hz, cis amide conformers), 4.52 (d,t 2/3H, $J$ = 8.3 and 2.3 Hz, trans amide comformer), 6.09 (bs, 1/3H, cis amide conformer), 6.34 (bs, 2/3H, trans amide conformer), 6.41−6.52 (m, 1H), 6.45 (d, 2/3H, $J$ = 9.0 Hz, trans amide conformer), 6.48 (s, 1/3H, $J$ = 7.9 Hz, cis amide conformer), 6.82 (bs, 1/3H, cis amide conformer), 6.93 (bs, 2/3H, trans amide conformer), 6.93 (bs, 2/3H, trans amide conformer), 7.43−7.56 (m, 1H), 8.33 (d, 1H, $J$ = 2.3 Hz); (ESI) MH/e 317 ($MH^+$ of free base); Anal. ($C_{17}H_{20}N_6O_2$·0.05$H_2O$) C, H, N.

**1-[3-[[2-[(5-Nitro-2-pyridinyl)amino]ethyl]amino]-1-oxopropyl]-2-cyano-(*S*)-pyrrolidine, Dihydrochloride (8p).** Method A: 2-(2-aminoethylamino)-5-nitropyridine[31] as amine and **3f** (generated by replacing chloroacetyl chloride with 3-bromopropionyl chloride in the **3a** procedure). Product was a light yellow solid, mp: softens at 80 °C and foams 82−84 °C; $^1$H NMR ($CD_3OD$) (trans and cis amide rotomers in a ratio of 9:1): $\delta$ 2.10−2.22 (m, 2H), 2.22−2.32 (m, 2H), 2.90 (t, 2H, $J$ = 5.6 Hz), 3.32−3.43 (m, 4H), 3.48−3.60 (m, 1H), 3.65−3.78 (m, 1H), 3.87 (t, 2H, $J$ = 5.6 Hz), 4.80 (t, 9/10H, $J$ = 5.3 Hz, C*H*CN), 5.02 (t, 1/10H, $J$ = 6 Hz, C*H*CN), 6 0.83 (d, 1H, $J$ = 9.4 Hz), 8.30 (dd, 1H, $J$ = 9.4, 2.3 Hz), 9.04 (dd, 1H, $J$ = 7.9, 2.6 Hz); (isobutane/DCI) MH/e 299 ($MH^+$ of free base); Anal. ($C_{15}H_{20}N_6O_3$·2HCl·0.15$H_2O$·0.35THF) C, H, N.

**6-[[2-[(8a*S*)-hexahydro-1-imino-4-oxopyrrolo[1,2-*a*]pyrazin-2(1*H*)-yl]ethyl]amino]-3-pyridinecarbonitrile, Monohydrochloride (8q).** A cloudy, white mixture of **8c** as the monohydrochloride[30] (0.400 g, 1.20 mmol) and tributylamine (110 mg, 0.60 mmol) in EtOAc (5.00 mL) was heated at reflux for 4 days. The resulting cloudy, white reaction was then filtered from the reaction, washed with EtOAc (10 mL), and dried under reduced pressure to provide a 99/1 mixture of the target product (by HPLC) and starting material, respectively, as a white solid (0.150 g, 45%); mp = 263−265 °C decomp; $^1$H NMR ($D_2O$): $\delta$ 1.60−1.76 (m, 1H), 1.82−1.99 (m, 1H), 1.99−2.15 (m, 1H), 2.38−2.50 (m, 1H), 3.12−3.25 (m, 1H), 3.42−3.62 (m, 2H), 3.62−3.90 (m, 3H), 4.05 (d, 1H, $J_{ab}$ = 16.9 Hz), 4.37 (d,d 1H, $J_{ab}$ = 16.9, 2.2 Hz), 4.97−4.69 (m, 1H), 6.53 (d, 1H, $J$ = 9.2 Hz), 7.60 (d,d 1H, $J$ = 9.2,1.8 Hz), 8.21 (d, $J$ = 1.8 Hz, 1H); MS $m/z$ 299 ($MH^+$ of free base); Anal. ($C_{15}H_{18}N_6O_1$·1.0HCl) C, H, N.

**α-(*S*)-Cyclohexylglycine-2-cyano-(*S*)-pyrrolidine, Monohydrochloride (9a).** This compound was synthesized according to a reported general procedure[26] with slight modifications. To a stirring, opaque-white solution of *N*-t-boc-L-α-cyclohexylglycine (2.00 g, 7.77 mmol) and 1-hydroxybenzotriazole hydrate (1.03 g, 7.77 mmol) in $CH_2Cl_2$ (30 mL) was added *N*-methylmorpholine (2.36 g, 23.3 mmol) and then diisopropylcarbodiimide (2.45 g, 19.4 mmol). After DIC addition, the reaction became clear and then cloudy again after 10 min. At this time, the L-prolinamide (**1a**) (0.890 g, 7.77 mol) was added. The cloudy reaction was then stirred at room temperature for 18 h. The solids were then removed via filtration, and the filtrate was concentrated to provide a golden oil. Purification via chromatography using 3% MeOH/$CH_2Cl_2$ as eluent provided 1.90 g (69%) of α-(*S*)-*N*-t-boc-cyclohexylglycine-2-(*S*)-pyrrolidinecarboxamide. $POCl_3$ (1.31 mL, 14.0 mmol) was added over 3 min to a $CH_3CN$/dry ice bath cooled (−35 °C) solution of the above t-boc-protected amide (1.90 g, 5.38 mmol) and imidazole (0.475 g, 7.0 mmol) in dry pyridine (30.0 mL). The reaction was stirred at between −30 °C and −20 °C for 1 h, warmed to room temperature, and then concentrated via rotovap. Purification via chromatography using 3% MeOH in $CH_2Cl_2$ as eluent provided α-(*S*)-*N*-t-boc-cyclohexylglycine-2-(*S*)-pyrrolidinecarbonitrile (0.775 g, 43%) as a white solid; TLC $R_f$: 0.6 (10% MeOH/$CH_2Cl_2$). A saturated HCl/diethyl ether solution (2.00 mL) was added to a clear, colorless solution of the above t-boc protected nitrile (0.772 g, 2.31 mmol) in diethyl ether (20 mL). The resulting clear, colorless solution was stirred at room temperature for 18 h and then concentrated in vacuo to yield the target compound (0.600 g, 96%) as a white solid: mp = 188−190 °C; $^1$H NMR ($CD_3OD$) (10 to 1 mixture of trans to cis amide rotomers); δ 1.06−1.46 (m, 4H), 1.65−1.99 (m, 7H), 2.04−2.42 (m, 4H), 3.70 (t, 2H, *J* = 6.3 Hz), 4.04 (d, 10/11 H, *J* = 6.3 Hz), 4.26 (d, 1/11H, *J* = 5.1 Hz), 4.84 (dd, 10/11 H, *J* = 7.7 and 5.2 Hz, C*H*CN), 5.06 (dd, 1/11H, *J* = 6.3 and 3.7 Hz, C*H*CN); MS *m/z* 236 ($M^+$); Anal. ($C_{13}H_{21}N_3O_1$·1.0HCl·0.81$H_2O$·0.09 $Et_2O$) C, H, N.

**1-[(*S*)-Cyclohexyl(cyclohexylamino)acetyl]-2-cyano-(*S*)-pyrrolidine (9c).** Cyclohexanone (1.60 g, 15.0 mmol) was added to a partially dissolved mixture of **9a** (1.40 g, 5.1 mmol) in 1,2-dichloroethane (56.0 mL). To the ice−water cooled mixture was then added the sodium triacetoxyborohydride (2.18 g, 10.0 mmol) in portions over 10 min. The ice−water bath was then removed and the reaction stirred at room temperature for 18 h. The reaction was partitioned between ethyl acetate and water, and the ethyl acetate layer was dried over $Na_2SO_4$, filtered, and concentrated. Purification via chromotography using 2% $CH_3OH$ in $CH_2Cl_2$ as eluent provided (0.100 g, 6.3% yield) of the target as a white solid: mp = 78−80 °C; $^1$H NMR ($CD_3OD$) (5 to 1 mixture of trans/cis amide rotomers): δ 1.00−1.35 (m, 10 H), 1.55−1.85 (m, 10H), 1.88−1.95 (m, 1H), 2.09−2.22 (m, 2H), 2.22−2.38 (m, 2H), 2.38−2.52 (m, 1H), 3.56−3.67 (m, 2H), 3.69−3.80 (m, 1H), 4.82 (dd, 4/5H, *J* = 8.1 and 4.4 Hz, C*H*CN), 5.17 (dd, 1/5H, *J* = 7.4 and 1.4 Hz, C*H*CN); (ESI) MH/e 318 ($MH^+$ of free base); Anal. ($C_{19}H_{31}N_3O_1$·1.2$H_2O$·0.2 $CH_3OH$) C,H; N: calcd, 12.16; found, 11.53.

**1-[(2S)-2-Amino-3-methyl-1-oxobutyl]-2-cyano-(*S*)-pyrrolidine, Monohydrochloride (9d).** Same procedure as in **9a** with *N*-t-boc-L-valine as a replacement for *N*-t-boc-L-α-cyclohexylglycine. Product was a white solid; mp 162−164 °C; $^1$H NMR ($CD_3OD$) (trans and cis amide rotomers in a ratio of 9:1) δ 0.98 (d, 1/3H, *J* = 7.0 Hz), 1.08 (d, 3H, *J* = 7.0 Hz), 1.14 (d, 2.67H, *J* = 7.0 Hz), 2.07−2.42 (m, 5H), 3.72 (t, 2H, *J* = 7.0 Hz), 4.10 (d, 1H, *J* = 5.9 Hz), 4.84 (d,d, 9/10 H, *J* = 8.1, 4.8 Hz, C*H*CN), 5.08 (d,d, 1/10H, *J* = 6.2, 3.7 Hz, C*H*CN); (ESI) MH/e 196 ($MH^+$ of free base); Anal. ($C_{10}H_{17}N_3O_1$·1HCl·0.04$H_2O$·0.14 EtOAc), C, H, N.

**1-[(2S)-3-Methyl-2-(methylamino)-1-oxobutyl]-2-cyano-(*S*)-pyrrolidine, Monohydrochloride (9f).** Same procedure as in **9a** with *N*-t-boc-3-methyl-2-methylamino-butyric acid as a replacement for *N*-t-boc-L-α-cyclohexylglycine. Product was a white solid; mp = 223−225 °C; $^1$H NMR ($CD_3OD$) (trans and cis amide rotomers in a ratio of 9:1) δ 1.10 (d, 3H, *J* = 7.0 Hz), 1.16 (d, 3H, *J* = 7.0 Hz), 2.10 (m, 5H), 2.69(s, 2.7 H), 2.71 (s, 0.3H), 3.65−3.82 (m, 2H), 4.18 (d, 1H, *J* = 5.15 Hz), 4.89 (d,d, 0.9H, *J* = 8.1, 4.4 Hz, C*H*CN), 5.08(d,d, 0.1H, *J* = 7.0, 2.6 Hz, C*H*CN). $^{13}$CNMR ($CD_3OD$): δ 17.73, 18.59, 26.22, 30.73, 31.26, 33.29, 66.08, 119.14, 119.70, 167.53. (ESI) MH/e 210 ($MH^+$ of free base). Anal.($C_{11}H_{19}N_3O_1$·1HCl), C, H, N.

**1-(3-Cyclohexyl-1-oxopropyl)-2-cyano-(*S*)-pyrrolidine (9g).** To a cloudy solution of 3-cyclohexanepropionic acid (1.0 g, 6.40 mmol) and L-prolinamide (**1a**) (730 mg, 6.40 mmol) in $CH_2Cl_2$ (25 mL) were added DMAP (20 mg) and DIC (890 mg, 7.04 mmol). The initially clear reaction was stirred for a total of 6 h to provide a cloudy white mixture. The solids were removed via filtration, and the filtrate was concentrated to provide a white solid. Purification via chromotography using 3% $CH_3OH$ in $CH_2Cl_2$ as eluent provided 1-(3-cyclohexyl-1-oxopropyl)-(*S*)-prolinamide (1.37 g, 5.44 mmol) as a white solid. To an ice−water cooled solution of this amide (1.37 g, 5.44 mmol) in $CH_2Cl_2$ (25 mL) was slowly added TFAA (1.54 mL, 10.9 mmol) over 10 min and then stirred at ice−water temperature for 2 h. The reaction was then partitioned between methylene chloride and saturated aqueous sodium bicarbonate, and the aqueous layer was washed twice with methylene chloride. The combined organic layers were then washed successively with water and brine and then dried over sodium sulfate. Purification via chromatography using 40% EtOAc in hexane as eluent provided the desired product (871 mg, 69%) as a white solid; mp = 40−42 °C; $^1$H NMR ($CDCl_3$) (7 to 1 mixture of trans/cis amide rotomers): δ 0.82−1.02 (m, 2H), 1.11−1.33 (m, 4H), 1.51−1.63 (m, 2H), 1.63−1.77 (m, 5H), 2.07−2.25 (m, 2H), 2.25−2.40 (m, 4H), 3.41−3.52 (m, 1H), 3.57−3.70 (m, 1H), 4.59 (dd, 1/8 H, *J* = 8.1 and 1.8 Hz, C*H*CN), 4.75 (d, 7/8H, *J* = 7.7, 2.2 Hz, C*H*CN); (ESI) MH/e 235 ($MH^+$ of free base). Anal. ($C_{14}H_{22}N_2O_1$) C, H, N.

**1-[[(1-Adamantyl)amino]acetyl]pyrrolidine, Monohydrochloride (12c).** To an ice−water cooled mixture of 1-aminoadamantane (15.9 g, 105.0 mmol), $K_2CO_3$ (19.4 g, 140 mmol) and $CH_2Cl_2$ (168 mL) was added a solution of ethyl bromoacetate (5.17 mL, 140 mmol) and $CH_2Cl_2$ (50 mL) dropwise over 20 min. The resulting cloudy, yellow-white mixture was then stirred at ice−water temperature for 1.5 h, then at RT for 18 h and then filtered through a plug of Celite (50 g). The solids and Celite plug were then washed with 100 mL of $CH_2Cl_2$, and the combined organic fractions were concentrated in vacuo to provide a cloudy, yellow-white oil. Purification via chromatography (4/1 mixture of hexane/ethyl acetate as eluent) followed by concentration in vacuo provided 6.9 g (83% yield) of ester **4** as a white solid. To an ice−water cooled mixture of ester **4** (6.87 g, 28.9 mmol), $K_2CO_3$ (15.9 g, 115 mmol), and $CH_2Cl_2$ (80 mL) was added a solution of benzyl chloroformate (4.34 mL, 30.4 mmol) in $CH_2Cl_2$ (26 mL) over 15 min. The resulting cloudy, white mixture was then stirred at ice−water temperature for 1.5 h, then at RT for 18 h, filtered, and concentrated in vacuo to provide a white crunchy solid. Purification via chromatography (9/1 mixture of hexane/ethyl acetate as eluent) followed by concentration in vacuo provided 7.89 g (73.6% yield) of CBZ-protected **4** as a white solid. A solution of this CBZ-protected **4** (7.89 g, 21.3 mmol), THF/$H_2O$: 1/1 (85 mL), and LiOH·$H_2O$ (7.14 g, 170 mmol) was stirred at 50 °C for 22h, cooled to RT, and poured into a 303 mL mixture of EtOAc/$H_2O$/AcOH (200/100/3 ratio). The organic layer is then isolated, washed with 2 × 50 mL of $H_2O$, dried over $MgSO_4$, filtered, and concentrated in vacuo to provide (6.7 g, 92%) of acid **5** as an off-white solid. To a solution of acid **5** (0.500 g, 1.46 mmol) in $CH_2Cl_2$ (20 mL) were added HOBT (0.195 g, 1.46 mmol), NMM (0.440 g, 4.37 mmol), and finally DIC (0.460 g, 3.64 mmol). After stirring at room temperature for 10 min, pyrrolidine (0.195 g, 1.46 mmol) was added to the cloudy-white solution via syringe, and the reaction was stirred at RT for 18 h. A $CH_2Cl_2$/$NaHCO_3$(aq) workup and purification via chromatography (40% EtOAc in hexane as eluent) provided 0.428 g (74% yield) of the CBZ-protected **12c** as a white solid. Deprotection of this intermediate was accomplished by shaking 0.428 g (1.08 mmol) of the CBZ-protected **12c** in a solution of EtOAc (50 mL), 10% Pd/C (0.100 g) and $H_2$ (1atm) at RT for

18 h. The solution was filtered through Celite and concentrated in vacuo to yield the free base of **12c** as a clear oil. HCl gas was gently bubbled into a solution of this free base in THF (25 mL) for ~20 s stirred at RT for 15 min and then concentrated in vacuo to provide the monohydrochloride salt of **12c** as a white solid (63% yield from **5**); mp 242 °C, sublimes; $^1$H NMR ($D_2O$) $\delta$ 1.67 (bq, 6H, $J_{ab}$= 16.5 Hz), 1.89 (s, 6H), 1.80−2.00 (m, 4H), 2.16 (bs, 3H), 3.40 (q, 4H, $J$ = 6.3 Hz), 3.87 (s, 2H); (ESI) MH/e 263 (MH$^+$ of free base); Anal. ($C_{16}H_{26}N_2O_1$·1HCl·0.5$H_2O$·0.1THF) C, H, N.

**1-[(1-Adamantyl)amino]acetyl]azatidine (12d).** Following procedure for **12c** but replacing pyrrolidine with azetidine as the amine. Product was a white solid (67% yield from **5**): mp 65−67 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.52−1.72 (m, 10H), 1.85 (bs, 2H), 2.05 (bs, 3H), 2.22−2.35 (m, 2H), 3.18 (s, 2H), 4.06 (t, 2H, $J$ = 7.7 Hz), 4.12 (t, 2H, $J$ = 7.7 Hz); (ESI) MH/e 249 (MH$^+$ of free base); Anal. free base ($C_{15}H_{24}N_2O_1$·0.06$H_2O$) C, H, N.

**1-[[(1-Adamantyl)amino]acetyl]piperidine (12e).** Following procedure for **12c** but replacing pyrrolidine with piperidine as amine. Product was a white solid (68% yield from **5**); mp 80−82 °C; $^1$H NMR ($CDCl_3$): $\delta$ 1.48−1.70 (m, 18H), 2.03 (bs, 3H), 3.10(t, 2H, $J$ = 5.1 Hz), 3.40 (s, 2H), 3.52 (t, 2H, $J$ = 5.1 Hz); (ESI) MH/e 277 (MH$^+$ of free base); Anal. ($C_{17}H_{28}N_2O_1$·0.1EtOAc); C, H, N.

**1-[[(1-Adamantyl)amino]acetyl]-3-pyrrolidinol, Monohydrochloride (12f).** Following procedure for **12c** but replacing pyrrolidine with 3-pyrrolidinol as amine. Product was a white solid (51% yield from **5**); mp 230−232 °C; $^1$H NMR ($D_2O$): (3 to 2 ratio of amide rotomers: $\delta$ 1.65 (q, 6H, $J$ = 16.5 Hz), 1.87 (s, 6H), 1.91−2.10 (m, 2H), 2.15 (s, 3H), 3.37−3.63 (m, 3H), 3.83−3.97 (m, 2H), 4.47 (bs, 0.6H, OH of one amide rotomer), 4.54 (bs, 0.4H, OH of other amide rotomer); (ESI) MH/e 279 (MH$^+$ of free base); Anal. ($C_{16}H_{26}N_2O_2$·1.0HCl·0.95$H_2O$·0.35THF); C, H, N.

**1,2,3,4-Tetrahydro-2-[[(1-adamantyl)amino]acetyl]-isoquinoline, monohydrochloride (12g).** Following procedure for **12c** but replacing pyrrolidine with 1,2,3,4-tetrahydroisoquinoline as amine. Product was a white solid (53% yield from **5**); mp = 260−262 °C; $^1$H NMR ($D_2O$) (1 to 1 trans to cis amide rotomers) $\delta$ 1.65 (bq, 6H, $J$ = 15.3 Hz), 1.86 (s, 6H), 2.13 (bs, 3H), 2.84 (t, 1H, $J$ = 6 Hz), 2.91 (t, 1H, $J$ = 6 Hz), 3.62−3.75 (m, 2H), 4.05 (s, 2H, one rotomer), 4.06 (s, 2H, other rotomer), 4.59 (s, 1H, one amide rotomer), 4.63 (s, 1H, other amide rotomer), 7.15−7.25 (m, 4H); (ESI) MH/e 325 (MH$^+$ of free base); Anal. ($C_{21}H_{28}N_2O_1$·1HCl·0.896$H_2O$·0.14THF) C, H, N.

**1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-(*S*)-pyrrolidine (12j).** Method A: 3-hydroxy-1-aminoadamantane[32] as amine and use of **3a**. Product was a white solid; mp: 138−140 °C (when recrystallized from EtOAc and 2-propanol: mp: 148−150 °C); OR: [d] = −78.3° ($c$ = 9.73, MeOH); $^1$H NMR ($CDCl_3$) (5 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.47−1.79 (m, 12H), 1.67 (s, 3H), 2.05−2.25 (m, 2H), 2.25−2.38 (m, 4H), 3.40−3.54 (m, 1H), 3.45 (d, 2H, $J$ = 1.9 Hz), 3.57−3.67 (m, 1H), 4.77 (dd, 5/6H, $J$ = 7.5, 2.3 Hz, C*H*CN), 4.86 (dd, 1/6H, $J$ = 7.9, 1.9 H, C*H*CN); (ESI) MH/e 304 (MH$^+$ of free base), Anal. ($C_{17}H_{25}N_3O_2$) C, H, N.

***N*-(3-Hydroxy-1-adamantyl)glycyl-L-prolinamide (12k).** Following procedure for **8n** and using 3-hydroxy-1-aminoadamantane[32] as amine. Product was a white, hygroscopic solid; mp = 94−96 °C; $^1$H NMR (DMSO): (trans and cis amide rotomers in a ratio of 2:1) $\delta$ 1.24−1.54 (m, 10H), 1.54−1.63 (m, 3H), 1.70−2.01 (m, 4H), 2.11 (bs, 2H), 2.17 (bs, 1H), 3.17 (s, 4/3H), 3.32 (s, 2/3H), 3.36−3.47 (m,1H), 3.47−3.59 (m,1H), 4.18 (dd, 2/3H, $J$ = 9.2 and 2.9 Hz), 4.30 (dd, 1/3H, $J$ = 8.4 and 2.9), 6.92 (bs, 2/3H, amide), 7.18 (bs, 1/3H, amide), 7.28 (bs, 2/3H, amide), 7.57 (bs,1/3H, amide); IR (KBr): 1640.4, 1677.3 (cm$^{-1}$); (ESI) MH/e 322 (MH$^+$ of free base); Anal. ($C_{17}H_{27}N_3O_3$·1.5$H_2O$·0.22 $CH_3OH$) C, H, N.

***N*-(3-Hydroxy-1-adamantyl)glycyl-L-proline, Monotriflouroacetic Acid (12l).** Following the procedure for **2a**, L-proline-*tert*-butyl ester (**1e**) was coupled with chloroacetyl chloride to provide **2e** (golden oil) which was then coupled with 3 equiv of 3-hydroxy-1-aminoadamantane[32] following the procedure for **8n**. Purification with chromatography (4% MeOH in $CH_2Cl_2$ as eluent) provided a 42% yield over two steps of the t-boc ester of **12l** as a light yellow solid. Cleavage of the t-boc ester was obtained by stirring at room temperature with 2 equiv of TFA in 0.5 M solution of $CH_2Cl_2$ overnight. The resulting TFA salt of **12l** was obtained as a white, hygroscopic solid in a 92% yield upon concentration in vacuo; mp = 80−82 °C; $^1$H NMR ($CD_3OD$) (4 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.62 (s, 2H), 1.73 (s, 4H), 1.81−1.91-(m,2H),1.89 (s, 4H), 2.01−2.14 (m, 2H), 2.24−2.40 (m, 2H), 2.40(s, 2H), 3.54−3.73 (m,2H), 4.01(s,2H), 4.49(d,d, 4/5H, $J$ = 8.8, 3.3 Hz, C*H*CN), 4.69 (d,d,1/5H, $J$ = 8.5, 2.6 Hz, C*H*CN); (ESI) MH/e 435 (MH$^+$ of free base); Anal. ($C_{17}H_{26}N_2O_4$·1.3TFA·0.28$H_2O$) C, H, N. **Note:** An excess 0.3 equiv of TFA could not be removed even with several toluene chases, triturations with $Et_2O$, and high vacuum (~1 mm) over several days.

**1-[[[3-[[(*tert*-Butylamino)carbonyl]oxy]-1-adamantyl]-amino]acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12m).** To a mixture of 1-aminoadamantan-3-ol[32] (5.00 g, 30 mmol) and potassium carbonate (6.20 g; 45 mmol) in 150 mL of THF was added benzyl chloroformate (4.70 g, 33.0 mmol) in dropwise fashion over a 10 min period. The mixture was then stirred at RT for 2 h and then partitioned between ethyl acetate and water. The aqueous layer was washed twice with ethyl acetate (100 mL), and the combined organic layers were then washed successively with 100 mL of aqueous 2 N sodium hydroxide, water, and brine, dried over sodium sulfate, filtered, and concentrated in vacuo to provide (8.03 g, 85% yield) of 1-benzylcarbamoyladamantane-3-ol (**6**) as a white solid. To a clear solution of 1-benzylcarbamoyladamantane-3-ol (**6**) (1.00 g, 3.32 mmol) and *tert*-butyl isocyanate (380 $\mu$L, 3.32 mmol) in 30 mL of $CH_2Cl_2$ was then syringe-added TMSCl (20.0 $\mu$L, 0.17 mmol). This reaction was then stirred at RT for 18 h, concentrated in vacuo, and purified on silica gel with 20% ethyl acetate in hexane as eluent to yield 3-[[(*tert*-butlyamino)-carbonyl]oxy]-1-benzylcarbamoyladamantane (**7a**) as a white solid in quantitative yield. A mixture of 3-[[(*tert*-butylamino)-carbonyl]oxy]-1-benzylcarbamoyladamantane (1.50 g, 3.75 mmol) and 10% palladium on carbon (400 mg) in ethanol (150 mL) was then shaken under hydrogen (50 psi) for 24 h, filtered through Celite, and concentrated in vacuo to provide 3-[[(*tert*-butylamino)carbonyl]oxy]-1-aminoadamantane as a clear oil in 99% yield. Method A was then followed using 3-[[(*tert*-butylamino)carbonyl]oxy]-1-aminoadamantane as the amine to provide **12m** as a yellow solid; mp: 210−212 °C; $^1$H NMR ($CD_3OD$) (9 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.26 (s, 9H), 1.62−1.71 (m, 2H), 1.92 (s, 4H), 1.98−2.44 (m, 12H), 3.51−3.62 (m, 1H), 3.70−3.78 (m, 1H), 4.04 (d, 2H, $J$ = 6.6 Hz), 4.82 (t, 9/10H, $J$ = 5.5 Hz, C*H*CN), 5.07 (d,d, 1/10H, $J$ = 6.6, 2.9, C*H*CN); (ESI) MH/e 403 (MH$^+$ of free base); Anal. ($C_{22}H_{34}N_4O_3$·1.0HCl·0.67$H_2O$·0.40THF) C, H, N, Cl.

**1-[[[3-[[[(4-Methoxyphenyl)amino]carbonyl]oxy]-1-adamantyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12n).** The procedure for **12m** was followed except in the second step to form **7b** where an equivalent of 4-methoxyphenyl isocyanate replaces *tert*-butyl isocyanate, 1,2-dichloroethane was used as solvent instead of methylene chloride, and the reaction is stirred at 50 °C for 18 h. Product was provided as a yellow solid (55% yield from **7b**); mp: 212−214 °C; $^1$H NMR ($CD_3OD$) (9 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.63−1.79 (m, 2H), 1.94 (s, 4H), 2.08−2.34 (m, 8H), 2.46 (s, 4H), 3.51−3.62 (m, 2H), 3.75 (s, 3H), 4.07 (d, 2H, $J$ = 7.0 Hz), 4.82 (t, 9/10H, $J$ = 5.8 Hz, C*H*CN), 5.09 (d,d, 1/10H, $J$ = 7.0, 2.2, C*H*CN), 6.83 (d,d, 2H, $J$ = 8.8, 1.1 Hz), 7.27 (d, 2H, $J$ = 8.4 Hz); (ESI) MH/e 453 (MH$^+$ of free base); Anal. ($C_{25}H_{32}N_4O_4$·1.0HCl·1.15$H_2O$·0.55THF) C, H, N, Cl.

**1-[[[3-[[(Phenylamino)carbonyl]oxy]-1-adamantyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12o).** The procedure for **12m** was followed except in the second step to form **7c** where an equivalent of phenyl isocyanate replaces *tert*-butyl isocyanate, 1,2-dichloroethane was used as solvent instead of methylene chloride, and the reaction was stirred at 50 °C for 18 h. Product was a light yellow solid (48% yield from **7c**); mp = 205−207 °C; $^1$H NMR ($CD_3OD$) (9

to 1 mixture of trans to cis amide rotomers): $\delta$ 1.62−1.81 (m, 2H), 1.97 (s, 4H), 2.10−2.32 (m, 8H), 2.47 (s, 4H), 3.51−3.62 (m, 2H), 4.06 (d, 2H, $J$ = 7.0 Hz), 4.82 (t, 9/10H, $J$ = 5.9 Hz, C*H*CN), 5.08 (d,d, 1/10H, $J$ = 7.0 and 2.2 Hz, C*H*CN), 6.99 (d,d,d, 1H, $J$ = 7.0, 7.0, 1.1 Hz), 7.24 (d,d,d, 2H, $J$ = 7.4, 7.4, 2.2 Hz), 7.37 (d, 2H, $J$ = 8.8 Hz); (ESI) MH/e 423 ($MH^+$ of free base); Anal. ($C_{24}H_{30}N_4O_3$·1.0HCl·1.06$H_2O$·0.64THF) C, H, N.

**1-[[[3-[[[(Diisopropyl)amino]carbonyl]-oxy]-1-adamantyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12p).** The procedure for **12m** was followed except in the second step to form **7d** where an equivalent of diisopropylcarbamoyl chloride replaces *tert*-butyl isocyanate, 1,2-dichloroethane was used as solvent instead of methylene chloride, and the reaction was stirred at 50 °C for 18 h. Product was a white solid (63% yield from **7d**); mp: 148−150 °C; $^1$H NMR ($CD_3OD$) (8 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.21 (bs, 12H), 1.61−1.78 (m, 2H), 1.95 (s,4H), 2.04−2.36 (11H, m), 2.36−2.48 (m, 4H), 3.50−3.61 (m, 1H), 4.06 (d, 2H, $J$ = 7.4 Hz), 4.82(t, 8/9H, $J$ = 5.9 Hz, C*H*CN), 5.09 (d,d, 1/9H, $J$ = 7.0, 2.6 Hz, C*H*CN); (ESI) MH/e 431 ($MH^+$ of free base); Anal. ($C_{24}H_{36}N_4O_3$·1.0HCl·1.2$H_2O$·0.40THF) C, H, N.

**1-[[[3-[[[(Cyclohexyl)amino]carbonyl]-oxy]-1-adamantyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12q).** The procedure for **12m** was followed except in the second step to form **7e** where an equivalent of cyclohexyl isocyanate replaces *tert*-butyl isocyanate, 1,2-dichloroethane was used as solvent instead of methylene chloride, and the reaction was stirred at 50 °C for 18 h. Product was provided as a light brown solid (41% yield from **7e**); mp: 155−157 °C; $^1$H NMR ($CD_3OD$) (9 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.08−1.40 (m, 6H), 1.56−1.78 (m, 5H), 1.78−1.98 (m, 6H), 1.98−2.47 (m, 12H), 3.43−3.61 (m, 2H), 4.04 (d, 2H, $J$ = 7.0 Hz), 4.83 (t, 9/10 H, $J$ = 5.9, Hz, C*H*CN), 5.09 (dd, 1/10H, $J$ = 7.5, 1.8, C*H*CN); (ESI) MH/e 429 ($MH^+$ of free base); Anal. ($C_{24}H_{36}N_4O_3$·1.0HCl·0.77$H_2O$·0.32THF) C, H, N.

**1-[[3-Acetyloxy-1-adamantyl)amino]-acetyl]-2-cyano-(*S*)-pyrrolidine; Monohydrochloride (12r).** The procedure for **12m** was followed except in the second step where a standard acylation of **6** is performed using 1.2 equiv of acetyl chloride, 3.0 equiv of pyridine, 0.1 equiv of DMAP, and 1,2-dichloroethane which are all stirred at room temperature for 24 h. Product obtained was a light yellow taffy (45% yield from **7f**); $^1$H NMR ($CD_3OD$) (6 to 1 mixture of trans to cis amide rotomers): $\delta$ 1.55−1.72 (6H, m), 1.94 (s, 3H), 1.98−2.34 (m, 13H), 3.42−3.58 (m, 1H), 3.44 (d, 1H, $J$ = 6.6 Hz), 3.62−3.75 (m, 1H), 4.77 (t, 6/7H, $J$ = 5.5 Hz, C*H*CN), 5.09 (d,d,1/7H, $J$ = 6.6 and 3.3 Hz, C*H*CN); (ESI) MH/e 346 ($MH^+$ of free base); Anal. ($C_{19}H_{27}N_3O_3$·0.38$H_2O$) C, H, N.

**In Vitro Studies. DPP-IV Inhibition Measurement in Vitro: Caco-2 Assay.** An extract from human colonic carcinoma cells (Caco-2; American Type Culture Collection; ATCC HTB 37) was used as the source of DPP-IV in the assay. The cells were differentiated to induce DPP-IV expression as described by previously.[42] Cell extract was prepared from cells solubilized in lysis buffer (10 mM Tris-HC1, 0.15 M NaC1, 0.04 T.I.U. (trypsin inhibitor unit) aprotinin, 0.5% nonidet-P40, pH 8.0) then centrifuged at 35 000*g* for 30 min at 4 °C to remove cell debris. The assay was conducted by adding 20 $\mu$g of solubilized Caco-2 protein, diluted to a final volume of 125 $\mu$L in assay buffer (25 mM Tris-HC1 pH 7.4, 140 mM NaC1, 10 mM KC1, 1% bovine serum albumin) to 96-well flat-bottom microtiter plates. The reaction was initiated by adding 25 $\mu$L of 1 mM substrate (H-Ala-Pro-pNA; pNA is *p*-nitroaniline). The reaction was run at room temperature for 10 min, and then 19 $\mu$L of 25% glacial acetic acid was added to stop the reaction. Fluorescence was measured using a CytoFluor II fluorometer (excitation 380 nm/ emission 460 nm). Test compounds and solvent controls were added as 30 $\mu$L additions, and the assay buffer volume was reduced to 95 $\mu$L. A standard curve of free *p*-nitroaniline was generated using 0−100 $\mu$M pNA in assay buffer. The curve generated, which was linear, was used for interpolation of substrate consumption (catalytic activity in nmoles substrate cleaved /min).

**DPP-IV Inhibition Measurement ex Vivo. Rat, Human, Monkey Plasma Assays.** Human, rat, or monkey plasma was used as the source of DPP-IV in the assay. The standard assay was modified from a previously published method.[43] Five $\mu$L of plasma was added to 96-well flat-bottom microtiter plates, followed by the addition of 5 $\mu$L of 80 mM $MgC1_2$ in assay buffer (25 mM HEPES, 140 mM NaC1, 1% RIA-grade BSA, pH 7.8). After a 5-min preincubation at room temperature, the reaction was initiated by the addition of 10 $\mu$L of assay buffer containing 0.1 mM substrate (H-Gly-Pro-AMC; AMC is 7-amino-4-methylcoumarin). The plates were covered with aluminum foil (or kept in the dark) and incubated at room temperature for 20 min. After incubation, fluorescence was measured using a CytoFluor II fluorometer (excitation 380 nm/ emission 460 nm). Test compounds and solvent controls were added as 2 $\mu$L additions, and the assay buffer volume was reduced to 13 $\mu$L. A standard curve of free AMC was generated using 0−50 $\mu$M solutions of AMC. The curve generated, which was linear, was used for interpolation of substrate consumption (catalytic activity in nmoles substrate cleaved /min).

**DPP-II Inhibition Measurement in Vitro.** An extract of bovine kidney homogenate, partially purified by ion-exchange and adenosine deaminase chromatography, was used as the source of DPP-II in the assay.[44−46] The standard assay was modified from a previously published method.[47] Twenty micrograms of DPP-II-containing fraction diluted to a final volume of 60 $\mu$L in assay buffer (0.2 M Borate, 0.05 M Citrate, pH 5.3) was added to 96-well flat-bottom microtiter plates, followed by the addition of 10 $\mu$L of 10 mM *o*-phenanthroline (to inhibit aminopeptidase activity) and 20 $\mu$L of 5 mM substrate (H-Lys-Ala-AMC; AMC is 7-amino-4-methylcoumarin). The plates were incubated at 37 °C for 30 min. After incubation, fluorescence was measured using a CytoFluor II fluorometer (excitation 380 nm/ emission 460 nm). Test compounds and solvent controls were added as 20 $\mu$L additions, and assay buffer volume is reduced to 50 $\mu$L. A standard curve of AMC was generated using 0 to 100 $\mu$M of AMC. The curve generated, which was linear, was used for interpolation of catalytic activity (in nmoles substrate cleaved/min).

**Post-Proline Cleaving Enzyme (PPCE) Inhibition Measurement in Vitro.** A cytosolic extract of human erythrocytes, partially purified by ion-exchange chromatography, was used as the source of PPCE in the assay. The standard assay is modified from a previously published method.[48] PPCE-containing fraction (350 ng protein) diluted to a final volume of 90 $\mu$L in assay buffer (20 mM NaPO4, 0.5 mM EDTA, 0.5 mM DTT, 1% BSA, pH 7.4) was added to 96-well flat-bottom microtiter plates, followed by the addition of 10 $\mu$L of 0.5 mM substrate (Z-Gly-Pro-AMC; AMC is 7-amino-4-methylcoumarin). The plates were incubated at room temperature for 30 min. After incubation, fluorescence was measured using a CytoFluor II fluorometer (excitation 380 nm/ emission 460 nm). Test compounds and solvent controls were added as 20 $\mu$L additions, and the assay buffer volume was reduced to 70 $\mu$L. A standard curve of free AMC was generated using 0 to 5 $\mu$M solutions of AMC. The curve generated, which was linear, was used for interpolation of catalytic activity (in nmoles substrate cleaved/min).

**In Vivo Obese Male (fa/fa) Zucker Rat Studies. Effect of 12j on DPP-IV Activity, Active GLP-1 Levels, and Glucose and Insulin Excursions.** Studies were performed on obese male Zucker (fa/fa) rats (Charles River Labs, Cambridge, MA); controls ($n$ = 9) and **12j**-treated ($n$ = 9). These rats were purchased at 7 weeks of age, cannulated at 7.5 weeks, and studied beginning at around 11 weeks of age. In the morning of the oral glucose tolerance test (OGTT), the rats were "fasted" by removing food before the lights were turned on, after which they were transferred to the experiment room at 8:00 a.m.. **12j** was dissolved in vehicle solution (0.5% carboxymethylcellulose (CMC) and 0.2% Tween 80). The cannulas were connected to sampling tubing (PE-100, 0.034 in. i.d. × 0.06 in. o.d.), which were filled with saline. After 30−40 min cage acclimation, a 0.5 mL baseline blood sample was taken at $t$ = −15 min, and the rats were then orally dosed

with CMC or **12j** (10 $\mu$mol/kg), after which additional baseline blood samples were taken at $t = -5$, $-2.5$, and 0 min. The animals were then administered an oral glucose solution (10% glucose, 1 g/kg) immediately after $t = 0'$. The rest of the samples were taken at 1, 3, 5, 10, 15, 20, 30, 45, 60, 75, and 90 min. Throughout the OGTT, an equal volume of donor blood was used to replace the blood withdrawn during sampling. Donor blood was obtained from donor rats through cardiac puncture. The collected blood samples (0.5 mL) were immediately transferred into chilled Eppendorf tubes containing 50 $\mu$L of EDTA: trasylol (25 mg/mL of 10 000 trasylol (FBA Pharmaceuticals)) and used for the measurement of glucose and insulin levels and DPP-IV activity. Larger blood samples (0.75 mL) were collected at $t = -15$, 0, 5, 10, 15, and 30 min for GLP-1 (7−36 amide) measurements. To these tubes, the DPP-IV inhibitor valine pyrrolidide[49] was added to yield a final concentration in the blood of 1 $\mu$M. Technical difficulties with obtaining blood samples after minute 20 for one rat in both the CMC and **12j** groups resulted in the inability to calculate glucose and insulin AUC data for those rats, leading to AUC data with an $n = 8$/group. Measurement of plasma glucose was made using a modification of a Sigma Diagnostics glucose oxidase kit. DPP-IV activity was measured in plasma samples obtained at −5, 0, 20, 45, and 90 min DPP-IV activity as previously described in the above ex vivo rat plasma experimental. Plasma levels of GLP-1 (7−36 amide) were measured using the GLP-1 (active) Elisa Kit (Linco Research Cat# EGLP-35K, St. Charles, MO). A double antibody RIA method using a rat-specific antiinsulin antibody from Linco Research (#1013, lot # ARI-02 (16T), St. Charles, MO) was used to measure plasma insulin. All data were expressed as the mean ± SEM for each experimental group of rats. Statistical analysis of data was performed using a two-tailed unpaired Student's *t*-test (Microsoft Excel 5.0), or a two-way repeated measures ANOVA with a Tukey post-ANOVA test (SPSS Inc. SigmaStat for windows, version 2.03).

**In Vivo Cynomolgus Monkey PK/PD Studies Using 8c and 12j.** Ketamine-anesthetized male healthy cynomolgus monkeys received either **8c** ($n = 2$) or **12j** ($n = 3$) (dissolved in CMC/Tween-80) by oral gavage (1.007 $\mu$mol/kg), and by intravenous administration (0.399 $\mu$mol/kg) (dissolved in saline). For iv study, compound was administered (0.4 mL/kg over 1 min) in 0.9% saline as vehicle. Different monkeys were used for each dosage regimen. Basal blood samples were collected at −10 min and immediately prior to administration of compound. Blood samples were collected at 0.03, 0.08, 0.17, 0.25, 0.33, 0.42, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 7, 12, and 25 h postdose for both routes of administration. Blood was obtained into heparin-coated syringes, transferred to microcentrifuge tubes, and centrifuged to separate the plasma. The plasma was stored at −80 °C in fresh microcentrifuge tubes until assay. DPP-IV activity was measured in a similar manner was as previously described in the above ex vivo rat and human plasma experimentals. Plasma DPP-IV activities were calculated and expressed as 'percent of baseline' to reduce variability due to individual differences in plasma enzyme activity. Area-under-curve (AUC) values for DPP-IV activity were calculated from time (hours after dose) vs effect (percent inhibition) curves from individual animals using the trapezoidal method. The ratio of dose-normalized effect AUC for oral/intravenous administration routes was taken as an estimate of effect bioavailability. Parent drug concentrations were determined using an HPLC/MS/MS method with a limit of quantification of 1 ng/mL. Pharmacokinetic parameters were calculated using noncompartment modeling, and the AUC was calculated using the linear trapezoidal method. Absolute oral bioavailability was calculated by ($AUC_{0-\infty}$po × 399)/($AUC_{0-\infty}$iv × 1007).

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**Supporting Information Available:** ORTEP drawing and atomic coordinate information for **12j** and experimental details for **8b, 8d−j**, **8o**, **9b**, **9e**, **9h**, **10a−h**, **11a−ak**, **12a**,**b**, and **12h−i**. This material is available free of charge via the Internet at http://pubs.acs.org.

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