1-[2-[(5-Cyanopyridin-2-yl)amino]-ethylamino]acetyl-2-(*S*)-pyrrolidine-carbonitrile: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties

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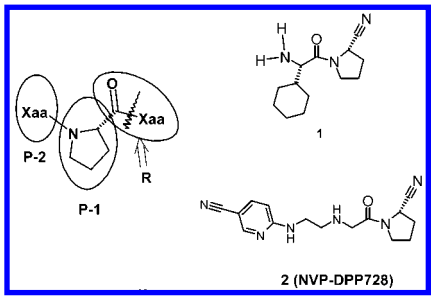
Abstract: Dipeptidyl peptidase IV (DPP-IV) inhibition has the potential to become a valuable therapy for type 2 diabetes. We report the first use of solid-phase synthesis in the discovery of a new DPP-IV inhibitor class and a solution-phase synthesis that is practical up to the multikilogram scale. One compound, NVP-DPP728 (**2**), is profiled as a potent, selective, and short-acting DPP-IV inhibitor that has excellent oral bioavailability and potent antihyperglycemic activity.

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 and determining the action of most substrates 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[6−9] 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.[10] 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.[11] By extending the duration of action of GLP-1, one would stimulate insulin secretion, inhibit glucagon release,[12] and slow gastric emptying;[13] each a benefit in the control of glucose homeostasis. 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 β-cell's glucose-sensing mechanism and promoting genes involved in the differentiation (neogenesis) of β-cells.[14] GLP-1 may play a role in acutely suppressing appetite in humans[15] and may play a role in mediating peripheral glucose uptake.[16] Since the blood glucose lowering effects of GLP-1 are dependent on elevated blood glucose and abate as glucose levels return to normal, the incidence of hypoglycemia during treatment with a DPP-IV inhibitor is expected to be very low.[17]

Chart 1



With few exceptions,[18−20] DPP-IV inhibitors resemble the P2−P1 dipeptidyl substrate cleavage product, where the P-1 site contains a proline mimic.[21] A straightforward replacement of the normally cleaved P-1 substrate amide (R in Chart 1) with an electrophile provides both irreversible (R = $P(O)(OPh)_2$, CO−NH−O−COR′) and reversible (R = $B(OH)_2$, H, CN) inhibitors.[22] Low nanomolar inhibition and chemical stability adequate for oral administration are obtained only with nitrile replacement of a substrate P-1 site amide (X_{aa}-(2*S*)-cyanopyrrolidines[23−25] and X_{aa}-(4*R*)-cyanothiazolidines[26]). Cyclohexylglycine-(2*S*)-cyanopyrrolidine **1** 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).[24]

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. Noticing that *N*-methylglycine was recognized in the substrate P-2 site,[21a] we were curious to investigate whether structurally more complicated N-substituted glycines would be tolerated at the P-2 site. We were gratified to find that a number of diverse P-2 site N-substituted glycines provided potent inhibition when combined with a (2*S*)-cyanopyrrolidide in the P-1 site (**8** in Scheme 1).[27] Intensive evaluation of this class[28] has led to the selection of the slow binding inhibitor **2** (NVP-DPP728)[29] as a clinical development candidate for type 2 diabetes. Herein, a tandem resin−solution parallel synthesis that led to the discovery of the P-2 site of **2** is described along with a solution-based method for multigram synthesis. Additionally, we report on the pharmacologic profile of this selective DPP-IV inhibitor, which exhibits excellent potency and oral bioavailability.

Chemistry. The preparation of a library of N-substituted 2-(*S*)-pyrrolidinecarbonitriles **8** has been carried out in a tandem five-step solid-phase and three step solution-phase sequence starting from commercially available Fmoc-protected Rink amide AM resin **4** as

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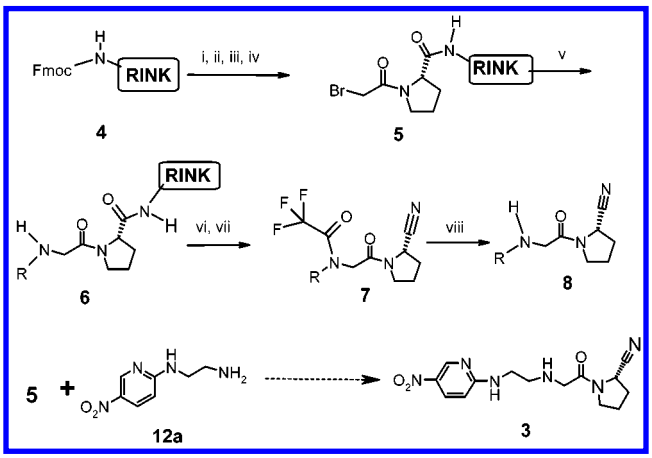
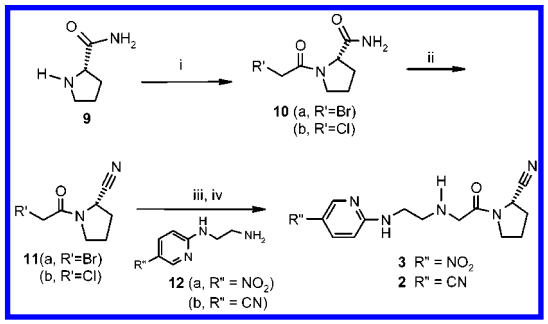
Scheme 1[a]



[a] Reagents: (i) 20% piperidine/DMF; (ii) Fmoc-proline, DIC, DMF; (iii) 20% piperidine/DMF; (iv) $BrCH_2COOH$, DIC, DMF; (v) RNH_2, DMSO; (vi) 95% TFA/H_2O; (vii) TFAA, THF; (viii) NH_3/MeOH.

Scheme 2[a]



[a] Reagents: (i) $BrCH_2COBr$, Et_3N, CH_2Cl_2, and DMAP for **10a** while $ClCH_2COCl$, K_2CO_3, and THF for **10b**; (ii) **10a** for **11a** and **10b** for **11b**, TFAA, CH_2Cl_2; (iii) **11a**, THF, **12a** for **3** and **11b**, THF, **12b** for **2**; (iv) excess HCl/THF for di-HCl of **2** and di-HCl if **3** and 1 equiv of ethanolic HCl for mono-HCl of **2**.

described in Scheme 1.[30] Successive deprotection of **4** with piperidine, 1,3-diisopropylcarbodiimide (DIC) coupling with Fmoc-protected proline, deprotection with piperidine, and finally DIC coupling with bromoacetic acid provided the resin-bound α-bromoacetyl prolinamide **5**. Analogous to Zuckermann's synthesis for solid-phase peptoid libraries,[31] **5** was treated with a diverse array of over 200 primary aliphatic amines to provide a library of discreet, resin-bound N-substituted glycine-2-(*S*)-pyrrolidinecarboxamides (**6**). Trifluoroacetic acid (TFA) resin cleavage of **6** followed by amide dehydration with trifluoroacetic anhydride (TFAA) afforded N-substituted *N*-trifluoroacetylated-2-(*S*)-pyrrolidinecarbonitriles **7**. Deacetylation of **7** with ammonia in methanol provided the product library of N-substituted 2-(*S*)-pyrrolidinecarbonitriles (**8**) in a 1 to 1 mixture with trifluoroacetamide.[32] The commercially available 2-(2-aminoethylamino)-5-nitropyridine (**12a**) provided resin-derived **3** (IC_{50} of 20 ± 3 nM in the DPP-IV Caco-2 assay) as one of the few low nanomolar DPP-IV inhibitors from this library effort. Compound **3** served as our starting point for the structure−activity relationship (SAR) effort that led to the title compound **2**.[28] A solution-based preparation of **2** and **3** had been carried out in three steps beginning with L-prolinamide (**9**) as shown in Scheme 2. Coupling of **9** with either bromoacetyl bromide or chloroacetyl chloride provided **10a** or **10b**, respectively.

Table 1. DPP-IV Inhibition and Selectivity Assays[a]

	Caco-2[b]	rat plasma[b]	human plasma[b]	PPCE[c]	DPP-II[d]
1	2.0 ± 0.3	2.8 ± 0.2	3.2 ± 0.19	41 000 ± 14 000	102 000 ± 20 000
2	22.0 ± 2.0	6.0 ± 1.0	7.0 ± 1.7	190 000 ± 46 000	110 000 ± 5800
3	8.0 ± 3.0	17 ± 0.3	8.7 ± 0.8	16 000 ± 1200	12 000 ± 580

[a] Values are IC_{50} (nM) expressed as the mean ± SD of three independent determinations. Procedures are described in Supporting Information. [b] Primary DPP-IV assays. [c] Extract from human erythrocytes. [d] Extract from bovine kidney homogenate.

Amide dehydration of **10a** and **10b** with trifluoroacetic anhydride produced **11a** and **11b** as solids that were stable for months at room temperature. Coupling of bromide **11a** with an excess of **12a** provided **3**, which was isolated as the dihydrochloride salt. The coupling of commercially available 5-cyano-2-chloropyridine with excess ethylenediamine provided **12b**. Reaction of **11b** with excess **12b** provided **2**, which was isolated as either the mono- or the dihydrochloride salt. The monohydrochloride **2** possessed a solubility of >100 mg/mL in distilled water and crystallized as a hemihydrate trans-amide rotomer with (*S*) chirality, as evidenced by the X-ray crystallographic analysis.[33] In solution, **2** was a mixture of cis- and trans-amide rotomers according to NMR. With minor modifications, the present solution synthesis has provided **2** on the 100 kg scale.

Results and Discussion. Compounds **1**−**3** were evaluated in vitro for their inhibition of DPP-IV extracted from Caco2 cells as well as from rat and human plasma (Table 1). Since under neutral and basic aqueous conditions the P-2 site amine can nucleophilically attack the carbon of the pyrrolidide-nitrile to form an inactive cyclic amidine,[28] the stability of **2** was examined under assay conditions. Under the assay conditions employed, this intramolecular cyclization was slow ($t_{1/2}$ > 2 days), resulting in less than 1% of **2** converting during the time frame of the experiment. As shown in Table 1, compound **2** potently inhibited both human and rat plasma DPP-IV and human epithelial cell-surface DPP-IV (IC_{50} = 7, 6, and 22 nM, respectively). Also, **2** was highly selective for DPP-IV over closely related peptidases, post-proline-cleaving enzyme (PPCE) and DPP-II[34] (Table 1). In addition, the in vitro specificity of **2** was profiled in over 100 receptor and enzyme assays and no significant binding was observed (10 μM).

In vivo evaluation of **2** in rat[9] and human[35] has supported the connection between DPP-IV inhibition and an improvement in oral glucose tolerance through an increase in active GLP-1 levels. We also found that **2** rapidly and effectively improved the metabolic profile in nonhuman primates. Oral administration of **2** (1 μmol/kg) significantly reduced plasma glucose levels (38% reduction in the 0−90 min glucose AUC, $p < 0.05$) in cynomologus monkey compared to the control during an oral glucose tolerance test (OGTT) (Figure 1). Additionally, peak glucose levels are significantly reduced in treated animals compared to control (98 ± 4 vs 88 ± 3 mg/dL, $p < 0.05$). When administered 30 min before an OGTT study, **2** maximally inhibited plasma DPP-IV activity (89%) 25 min postdose and provided a ≥70% DPP-IV inhibition throughout the study.

Pharmacokinetic evaluation of **2** was performed in male Sprague−Dawley rats and male cynomolgus mon-

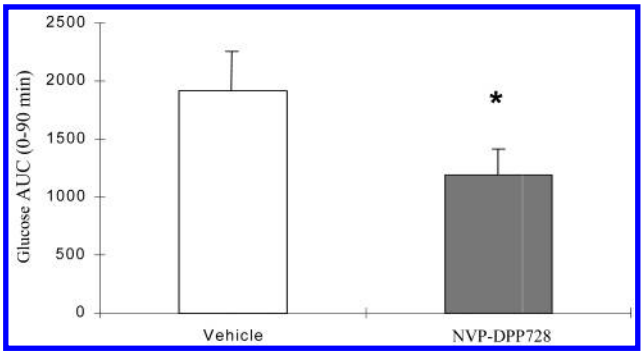


Figure 1. Incremental area under the glucose curve (from 0 to 90 min) during oral glucose tolerance tests (OGTTs) performed in seven anesthetized cynomolgus monkeys following oral administration of vehicle or NVP-DPP728 (**2**) (1 μmol/kg, po), mean ± SEM, (∗) $p < 0.05$. Vehicle is 0.5% carboxymethylcellulose in 0.2% Tween 80. Experimental procedure is detailed in Supporting Information.

keys. After an oral dose of 10 μmol/kg, C_{max} was 3.65 μM in rat and 7.56 μM in monkey. Absolute bioavailability was high in both rat and monkey at ≥74%. The steady-state volumes of distribution are similar in rat (670 mL/kg) and monkey (841 mL/kg), suggesting that **2** is distributed principally in the body fluids. Clearance of **2** from plasma is moderate at about 28 ± 1.5 mL min^{-1} kg^{-1} in the rat and 21.9 ± 3.1 mL min^{-1} kg^{-1} in the monkey. After an oral dose in monkey of 1 μmol/kg, **2** provided a half-life of 0.85 h and inhibited plasma DPP-IV activity by >50% for 4 h. A 100 mg oral dose of **2** in humans provided a similar half-live of 0.85 h, a >80% inhibition of plasma DPP-IV activity for ∼4 h, a significant increase in active GLP-1 levels, and an improvement in metabolic control.[35] As a reversible DPP-IV inhibitor possessing a relatively short half-life, **2** might most effectively be taken with a meal when GLP-1 secretion is at its maximal rate.

In summary, we report here the use of combined solid-phase and solution-phase chemistry to discover a potent, selective, and short-duration DPP-IV inhibitor that also has excellent oral bioavailability. The favorable pharmacokinetic profile for NVP-DPP728 (**2**) led to the selection of this compound for further study in a clinical setting for type 2 diabetes.[35] Profiling of this new class of DPP-IV inhibitors is under study and will be reported in due course.

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Supporting Information Available: Experimental procedures including characterization data for all compounds, biological methods, ORTEP drawing, and atomic coordinate information for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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