Pergamon

Tetrahedron 58 (2002) 5741–5746

TETRAHEDRON

Solid and solution phase syntheses of the 2-cyanopyrrolidide DPP-IV inhibitor NVP-DPP728

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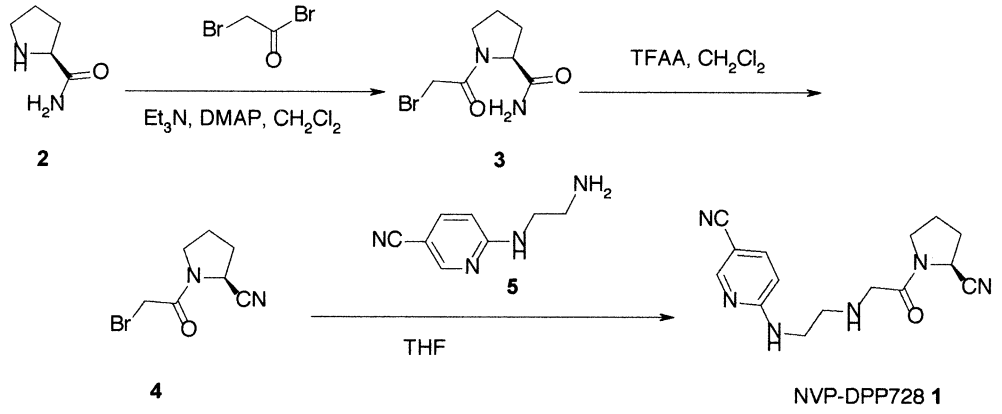
Received 22 January 2002; revised 22 April 2002; accepted 10 May 2002

Abstract—DPP-IV inhibitors have been suggested as potential new treatments for type-II diabetes and 2-cyanopyrrolidides have been reported as potent DPP-IV inhibitors. Alternative synthetic approaches to one such compound, NVP-DPP728, are investigated here. One strategy is based in solution phase and is amenable to scale-up. The other is based on solid phase and is appropriate for the rapid analoging of the structural series.

Dipeptidyl peptidase IV (DPP-IV, CD26, EC.3.4.14.5) is a membrane-bound and circulating serine protease. It catalyses the hydrolysis of peptides after a penultimate N-terminal proline or alanine residue.[1] One such peptide is glucagon-like peptide-1 (GLP-1). Hence, inhibition of DPP-IV has been suggested as a novel treatment for type-II diabetes.[2] A series of 2-cyanopyrrolidides has been reported by Ferring Research Limited as very potent inhibitors of DPP-IV.[3] More recently one 2-cyanopyrrolidide, NVP-DPP728 **1**, has been reported to increase plasma GLP-1 (7-36 amide) concentrations and improve oral glucose tolerance in obese Zucker rats.[4] This compound is in phase II clinical trials as a potential new drug therapy for type-II diabetes.

The preparation of NVP-DPP728 **1** is described in Scheme 1.[5] In our hands, this procedure gave a very poor yield. This is mainly due to the number of reactive amine functional groups present in both the starting substrate **5** and in the product **1**. Therefore, we investigated alternative routes that would avoid formation of by-products and/or allow easier preparation of analogues. We have developed two different strategies to the synthesis of NVP-DPP728 **1**. One makes use of an original protection and deprotection



Scheme 1. The solution phase synthesis of NVP-DPP728 as reported in patent application WO 98/19998.[5]

Keywords: NVP-DPP728; DPP-IV inhibitor; cyanopyrrolidide.

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0040–4020/02/$ - see front matter
PII: S0040-4020(02)00536-7

strategy. This is a solution phase synthesis and is suitable for scale-up. The other, performed on solid phase, is amenable to the fast preparation of structural analogues.[6]

1. Solution phase strategy

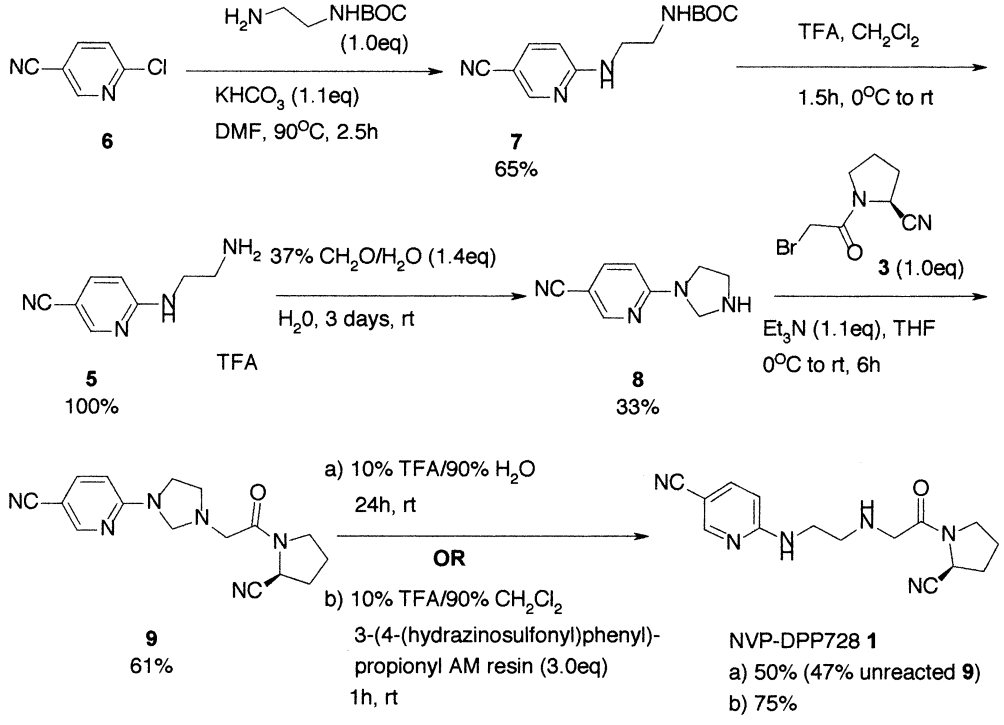
The major drawback of the existing synthesis is the presence of several nucleophilic groups during the alkylation step. In selecting an appropriate protection of the reactive amine functionalities, an important consideration is the susceptibility for cyclisation under strongly basic conditions. Nucleophilic attack by the secondary amine onto the nitrile in **1** affords a six-membered cyclic amidine that can hydrolyse to a diketopiperazine. Under non-basic conditions, the secondary amine is less prone to cyclisation. Depending on the example or the conditions employed, amino acid 2-cyanopyrrolidides demonstrate half-lives greater than 24 h.[3,7] Therefore, we were prompted to investigate protecting groups that may be removed under neutral or acidic conditions.

We were attracted by a strategy used for the selective protection of spermidine derivatives.[8] This involved the condensation of spermidine with formaldehyde yielding a cyclic product. In this way, the primary amino group is converted to a secondary amine, while the secondary amine is converted to a tertiary amine. Subsequent removal of the methylene group was carried out with malonic acid and pyridine in methanol. Scheme 2 shows the synthetic route to NVP-DPP728 **1** using a strategy similar to this. The amine·TFA salt **5** was prepared according to Scheme 2 and condensed with aqueous formaldehyde solution. This proceeded smoothly to yield the cyclic formaldehyde adduct **8**·TFA salt which was isolated by crystallisation from a 1:1 mixture of diethyl ether and ethanol. Although the yield of the crystallisation remains to be optimised, the chemistry is convenient to carry out and is amenable to scale-up. The free base of **8** was liberated and coupled to bromide **3** to give **9** in 61% yield. It was necessary to remove the formaldehyde group. The literature precedent was unsuitable because the high polarity of the product made isolation from malonic acid and pyridine difficult.[8] Instead, because of the reversibility of the formaldehyde condensation under aqueous conditions, it was hypothesised that simple acid catalysed hydrolysis under dilute conditions would be sufficient. Therefore, the formaldehyde adduct **9** was dissolved in a 10% TFA (aq.) solution and stirred for 24 h. Basic work-up and flash chromatography afforded the title compound **1** in a 50% yield. The remaining material was identified as unreacted **9**. There was no evidence of intramolecular cyclisation. Under these conditions, the final deprotection step is reversible and an equilibrium mixture is obtained. In order to drive the reaction to completion, high dilution conditions were considered. However, such conditions are not amenable to scale-up. Therefore, we decided to use a supported scavenging reagent. Excess 3-(4-(hydrazinosulfonyl)phenyl)propionyl AM resin was suspended in a solution of intermediate **9** in a mixture of 10% TFA/90% CH_2Cl_2 and stirred for an hour at room temperature.

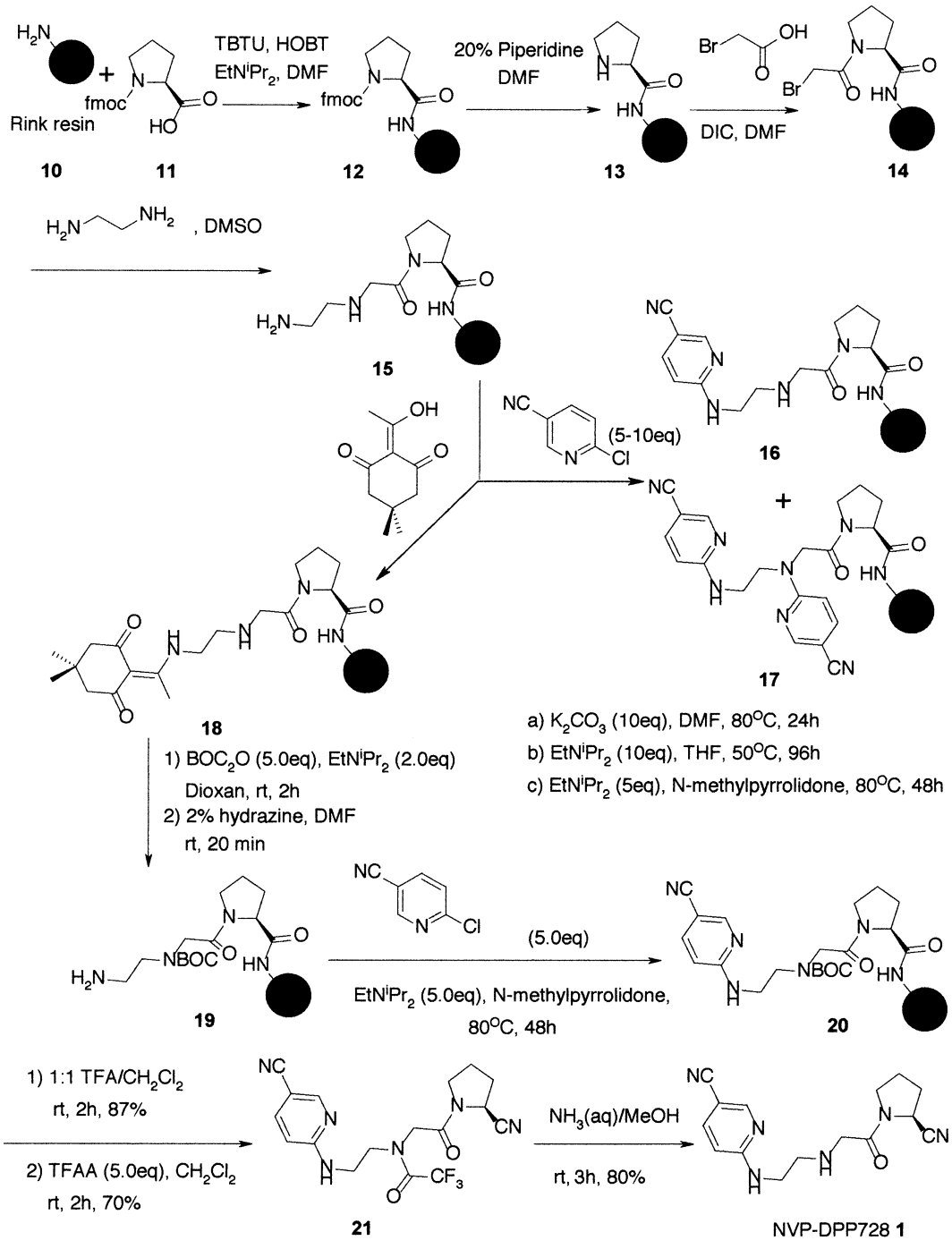
After work-up and chromatography the free base of NVP-DPP728 **1** was obtained in 75% yield. Thus the hydrazine scavenging reagent was successful at improving the yield of the final deprotection step, reducing the reaction time dramatically and eliminating the need for high-dilution conditions.

2. Solid phase strategy

First of all we prepared the Rink resin bound key



Scheme 2. The solution phase preparation of NVP-DPP728 **1** using a formaldehyde derived protecting group.



Scheme 3. The synthesis of NVP-DPP728 **1** using a solid phase approach via the key BOC protected intermediate **8**.

intermediate **15** in high yield using similar chemistry to that already reported (Scheme 3).[6] This would allow rapid derivatisation of the primary amino group. Our initial attempts to exemplify this with the preparation of NVP-DPP728 were not successful. Scheme 3 illustrates three different sets of experimental conditions, all employing a large excess of 6-chloronicotinonitrile **6** (10 equiv.). Using potassium carbonate as base gave no reaction products. Using diisopropylethylamine in either THF or *N*-methylpyrrolidone yielded mixtures of the required product **16** and the double addition product **17** as well as other unidentified products. This led us to investigate a protecting group strategy that would allow specific addition of 6-chloronicotinonitrile **6** and other electrophiles to the terminal nitrogen under forcing conditions. Initial attempts to use formaldehyde, as in the solution phase synthesis, proved unsuccessful. Complex mixtures were obtained after cleavage from the resin. Accordingly, 2-acetyldimedone was added in DMF to the resin **15** to afford the Dde protected primary amine.[9] Addition of BOC anhydride followed by deprotection of the Dde group with hydrazine in DMF afforded the BOC protected compound **19**. 6-Chloronicotinitrile **6** was added to the terminal amine under forcing conditions (48 h at 80°C). After cleavage

from the resin and concomitant removal of the BOC group, the carboxamide was dehydrated under standard conditions. All steps in the solid phase synthesis proceeded cleanly such that ^{1}H NMR showed the crude material obtained upon cleavage from the resin and dehydration to be predominantly the required trifluoroacetamide **21**. After chromatography, treatment with methanolic ammonia afforded the free base of NVP-DPP728 **1**. This procedure illustrates an orthogonal protecting group strategy that affords the BOC protected resin bound intermediate **19**. This is a common intermediate that may be utilized for the rapid analoging of the terminal N-substituent using large sets of acylating and alkylating agents.

3. Summary

We have presented two new chemistries for the preparation of NVP-DPP728 **1**. The solid phase approach utilises an orthogonal protecting group strategy to afford a BOC protected intermediate. This may be used to prepare the title compound or may be used as a common intermediate for the rapid synthesis of a series of compounds for SAR analysis. Complementary to this, the solution phase approach utilises a new and efficient protecting group strategy that is amenable to scale-up. Considering the perceived importance of DPP-IV inhibitors in the field of diabetes these chemistries will be of benefit to any groups active in the field.

4. Experimental

4.1. Data for compounds

4.1.1. *tert*-Butyl (2-(5-cyano-2-pyridylamino)ethyl)carbamate (7). A solution of 6-chloronicotinonitrile (**6**) (21.7 g, 160 mmol), potassium hydrogen carbonate (17.6 g, 176 mmol) and *tert*-butyl (2-aminoethyl)carbamate (25.8 g, 160 mmol) in DMF (80 ml) was heated to 90°C under N_2 for 2.5 h. The mixture was cooled, added to saturated $NaHCO_3$ solution and extracted with EtOAc. The organic phase was dried over Na_2SO_4 and concentrated in vacuo until the product started to precipitate. Further product was precipitated by adding petroleum ether. The combined precipitates were collected and washed with cold EtOAc to afford *tert*-butyl (2-(5-cyano-2-pyridylamino)ethyl)carbamate **7** (27.2 g, 65%) as a white solid. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 1.41 (9H, s), 3.30–3.42 (2H, m), 3.44–3.56 (2H, m), 4.92 (1H, br s), 5.72 (1H, br s), 6.39 (1H, dd, J=8.9, 0.7 Hz), 7.51 (1H, dd, J=8.9, 2.2 Hz), 8.30–8.36 (1H, m) ppm.

4.1.2. 6-(2-Aminoethylamino)nicotinonitrile trifluoroacetate (5). Trifluoroacetic acid (125 ml) was added to an ice-cold stirred suspension of *tert*-butyl (2-(5-cyano-2-pyridylamino)ethyl)carbamate **7** (32.0 g, 120 mmol) in CH_2Cl_2 (125 ml) to give a clear solution. After gas evolution had ceased, the cooling bath was removed. After 1.5 h the mixture was concentrated in vacuo and azeotroped with toluene three times to afford 6-(2-aminoethylamino)-nicotinonitrile trifluoroacetate **5**. The yield was assumed to be quantitative and the material was used directly in the next step.

4.1.3. 6-Imidazolidin-1-yl-nicotinonitrile (8). 6-(2-Aminoethylamino)nicotinonitrile trifluoroacetate **5** (120 mmol) was dissolved in water (1300 ml). Aqueous 37% formaldehyde solution (15.5 g, 12.5 ml, 168 mmol) was added and the mixture was stirred for 3 days. The mixture was concentrated then azeotroped with toluene twice and with petroleum ether once. The residue was taken up in Et_2O/EtOH (50:50, 200 ml) and scratched to initiate crystallisation. The mixture was cooled in an ice/water bath for 4 h and filtered to afford 6-imidazolidin-1-yl-nicotinonitrile trifluoroacetate (11.4 g) as a pale yellow crystalline solid. ^{1}H NMR (d_6-DMSO, 270 MHz): δ 3.58–3.78 (4H, m), 4.75 (2H, s), 6.78 (1H, d, J=8.9 Hz), 8.01 (1H, dd, J=2.2, 8.9 Hz), 8.58 (1H, d, J=2.2 Hz) ppm. This was dissolved in water and saturated $NaHCO_3$ solution was added until the solution was basic. The mixture was extracted with CH_2Cl_2 four times and the combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo to afford 6-imidazolidin-1-yl-nicotinonitrile free base **8** (6.7 g, 33%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 3.26–3.48 (4H, m), 4.46 (2H, s), 6.28 (1H, d, J=8.7 Hz), 7.57 (1H, dd, J=2.2, 8.7 Hz), 8.37 (1H, d, J=2.2 Hz) ppm.

4.1.4. 6-{3-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethyl]-imidazolidin-1-yl}-nicotinonitrile (9). A solution of (2*S*)-1-(bromoacetyl)pyrrolidine-2-carbonitrile **3**,[5] (3.94 g, 18.1 mmol) in THF (20 ml) was added to an ice-cold solution of 6-imidazolidin-1-yl-nicotinonitrile **8** (3.16 g, 18.1 mmol) and triethylamine (2.8 ml, 2.0 g, 20 mmol) in THF (20 ml) over 3 min. The resulting cloudy mixture was allowed to warm to room temperature and stirred for 6 h. The mixture was added to dilute $NaHCO_3$ solution and extracted with CH_2Cl_2 four times. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel (2.5% MeOH/97.5% CH_2Cl_2) afforded 6-{3-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-imidazolidin-1-yl}-nicotinonitrile **9** (3.4 g, 61%) as a white foam. ^{1}H NMR ($CDCl_3$, 270 MHz): δ 2.04–2.42 (4H, m), 3.18 (2H, t, J=6.7 Hz), 3.34–3.76 (6H, m) 4.26–4.42 (2H, m), 4.74–4.80 and 4.95–5.04 (total 1H in the ratio 3:1, each m), 6.30 (1H, d, J=8.9 Hz), 7.60 (1H, dd, J=2.0, 8.9 Hz), 8.38 (1H, d, J=2.0 Hz) ppm. MS (ESI) m/z 311.0 (MH^+).

4.1.5. (6-{2-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethyl-amino]-ethylamino}-nicotinonitrile (1), by removal of formaldehyde group using aqueous trifluoroacetic acid. A solution of 6-{3-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-imidazolidin-1-yl}-nicotinonitrile **9** (3.15 g, 10.2 mmol) in 10% trifluoroacetic acid (250 ml) was stirred for 24 h. The mixture was cooled in an ice/water bath and potassium carbonate was added cautiously until saturated, then extracted with CH_2Cl_2 seven times. The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography on silica gel (10% MeOH/90% CH_2Cl_2) afforded (6-{2-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile **1** (1.53 g, 50%) as a colourless gum. ^{1}H NMR at 25°C was consistent with the presence of two rotameric isomers in a ratio of ~85:15. ^{1}H NMR (d_6-DMSO, 270 MHz): δ 1.84–2.30

(4H, m), 2.71 (2H, t, J=6.2 Hz), 3.22–3.48 (5H, m), 3.50–3.66 (1H, m), 4.73 and 5.13 (1H, ratio ~85:15, dd, J=4.7, 6.4 Hz and dd, J=2.5, 6.7 Hz), 6.56 (1H, d, J=8.9 Hz), 7.50–7.72 (2H, m), 8.36 (1H, d, J=2.0 Hz) ppm. At higher temperatures, the NMR signals of the two compounds appeared to coalesce as is typical of rotameric isomers. ^{1}H NMR (d_6-DMSO, 270 MHz): δ 1.98–2.30 (4H, m), 2.77 (2H, t, J=6.2 Hz), 3.30–3.62 (6H, m), 4.62–4.88 (1H, m), 6.57 (1H, d, J=8.9 Hz), 7.26 (1H, br s), 7.61 (1H, dd, J=2.2, 8.9 Hz), 8.33 (1H, d, J=2.2 Hz) ppm.

4.1.6. (6-{2-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile (1), by removal of formaldehyde group using solid supported reagent. 6-{3-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethyl]-imidazolidin-1-yl}-nicotinonitrile **9** (223 mg, 0.72 mmol) and 3-(4-(hydrazinosulfonyl)phenyl) propionyl AM resin (1.3 g, 1.9 mmol) were dissolved in CH_2Cl_2 (20 ml). Trifluoroacetic acid (3 ml) was added and the reaction was stirred at room temperature for 1 h. The organic layer was filtered and the resin was washed with CH_2Cl_2 (100 ml) and EtOH (200 ml). The combined organic layers were evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (100 ml) and washed with potassium carbonate solution (30 ml). The aqueous layer was extracted with CH_2Cl_2 three times and the combined organic layers were evaporated in vacuo. Flash chromatography on silica gel (90% CH_2Cl_2/10% MeOH and 80% CH_2Cl_2/20% MeOH) afforded (6-{2-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile **1** (162 mg, 75%) as a colourless gum. ^{1}H NMR data was identical to that obtained by the aqueous trifluoroacetic acid route above.

4.1.7. (6-{2-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile (1), hydrochloride salt. 4N HCl/dioxan (1.3 ml, 5.1 mmol) was added to a solution of (6-{2-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile **1** (1.53 g, 5.13 mmol) to afford a white precipitate. The solid was filtered, washed and triturated with Et_2O and dried in vacuo over P_2O_5 to (6-{2-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile **1** hydrochloride salt (1.40 g, 82%) as a white powder. ^{1}H NMR (d_6-DMSO, 270 MHz): δ 1.88–2.30 (4H, m), 3.13 (2H, s), 3.32–3.50 (1H, m), 3.52–3.98 (4H, m), 4.00–4.22 (1H, m), 4.84 (1H, t, J=5.6 Hz), 6.64 (1H, d, J=8.9 Hz), 7.76 (1H, dd, J=8.9, 2.1 Hz), 8.00 (1H, br s), 8.43 (1H, d, J=2.1 Hz), 9.25 (2H, br s) ppm. ^{13}C NMR (D_2O, 68 MHz): δ 24.7, 29.6, 38.2, 46.3, 47.0, 47.1, 48.2, 97.3, 111.5, 117.9, 118.7, 141.6, 149.5, 156.8, 165.0 ppm. MS (ESI) m/z 299 (MH^+, 100%). An analytical sample was recystallised from MeOH, mp 158–163°C (reported mp from WO98/19998, example no. 3=155–157°C).[4]

4.1.8. L-Pro-resin (13). Rink amide resin **10** (5 g, 0.47 mmol/g, 2.4 mmol) was shaken at room temperature with a 20% piperidine solution in DMF (50 ml) for 5 min. The mixture was filtered and shaken with 20% piperidine in DMF (50 ml) for another 20 min. The resin was then washed sequentially with DMF, CH_2Cl_2 and DMF. TBTU (1.9 g, 5.9 mmol), HOBT (0.79 g, 5.9 mmol) and EtN^iPr_2 (3.0 ml, 17 mmol) were added to a solution of Fmoc-L-Proline (2.0 g, 5.9 mmol) in DMF (30 ml). The resulting yellow solution was shaken at 0°C for 30 min then added to the resin. The reaction was shaken for 3 h at 20°C until a ninhydrin test was negative. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded Fmoc-L-Pro-resin **12**. Resin **12** was then shaken at room temperature with a 20% piperidine solution in DMF (50 ml) for 5 min. The mixture was filtered and shaken with 20% piperidine in DMF (50 ml) for another 20 min. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded L-Pro-resin **13** which was used directly in the next step. A few beads of the resin **13** were cleaved with trifluoroacetic acid to afford L-Pro-NH_2. MS (ESI) m/z 115 (MH^+, 100%).

4.1.9. *N*-Bromoacetyl-L-Pro-resin (14). A solution of bromoacetic acid (3.3 g, 24 mmol) in DMF (30 ml) was added to L-Pro-resin **13** (2.4 mmol) followed by a solution of DIC (4.8 ml, 3.9 g, 31 mmol) in DMF (5 ml) and the mixture was shaken at room temperature for 30 min. The resin was filtered and the procedure repeated once. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded *N*-bromoacetyl-L-Pro-resin **14** which was used directly in the next step. A few beads of the resin **14** were cleaved with trifluoroacetic acid to afford *N*-bromoacetyl-L-Pro-NH_2. MS (ESI) m/z 236 (MH^+, 100%).

4.1.10. *N*-(2-Aminoethyl)-Gly-L-Pro-resin (15). A solution of ethylenediamine (2.3 ml, 2.1 g, 35 mmol) in DMSO (30 ml) was added to the resin **14** (2.4 mmol) and the mixture was shaken for 2 h. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded *N*-(2-aminoethyl)-Gly-L-Pro-resin **15** which was used directly in the next step. A few beads of the resin **15** were cleaved with trifluoroacetic acid to afford *N*-(2-aminoethyl)-Gly-L-Pro-NH_2. MS (ESI) m/z 215 (MH^+, 100%).

4.1.11. *N*-(*N*-[1-{4,4-Dimethyl-2,6-dioxo-cyclohexylidene}-ethylamino]-2-aminoethyl)-Gly-L-Pro-resin (18). A solution of 2-acetyldimedone (0.69 g, 3.8 mmol) in DMF (10 ml) was added to a proportion of the resin **15** (5.1 g, 1.9 mmol) in DMF (20 ml). The mixture was shaken at room temperature for 3 h until a negative ninhydrin test. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded *N*-(*N*-[1-{4,4-dimethyl-2,6-dioxo-cyclohexylidene}-ethylamino]-2-aminoethyl)-Gly-L-Pro-resin **18** which was used directly in the next step. A few beads of the resin **18** were cleaved with trifluoroacetic acid to afford *N*-(*N*-[1-{4,4-dimethyl-2,6-dioxo-cyclohexylidene}-ethylamino]-2-aminoethyl)-Gly-L-Pro-NH_2 trifluoroacetate salt. ^{1}H NMR (d_6-DMSO, 300 MHz): δ 0.99 (6H, s), 1.82–2.06 (4H, m), 2.29 (4H, s), 2.50 (3H, s), 3.15–3.23 (2H, m), 3.40–3.50 (2H, m), 3.73–3.81 (2H, m), 4.03 (2H, s), 4.26 (1H, m), 4.50 (2H, s), 9.00 (2H, s), 13.13–13.21 (1H, m) ppm. MS (ESI) m/z 379 (MH^+, 100%).

4.1.12. *N*-(2-Aminoethyl)-Boc-Gly-L-Pro-resin (19). A solution of di-*tert*-butyl dicarbonate (2.0 g, 9.2 mmol) and EtN^iPr_2 (0.65 ml, 3.8 mmol) in dioxan (30 ml) was added to resin **18** (1.9 mmol) in dioxan (10 ml). The reaction was shaken for 2 h at room temperature. The resulting resin was washed sequentially with DMF, CH_2Cl_2 and DMF. The resin was treated with 2% hydrazine/DMF solution under continuous flow conditions (3 ml/min) for 20 min. Washing sequentially with DMF, CH_2Cl_2 and DMF

afforded *N*-(2-aminoethyl)-Boc-Gly-L-Pro-NH_2 resin **19** which was used directly in the next step.

4.1.13. *N*-(*N*-[5-Cyanopyridin-2-yl]-2-aminoethyl)-Boc-Gly-L-Pro-resin (20). 6-Chloronicotinonitrile (1.3 g, 9.5 mmol) and EtN^iPr_2 (1.6 ml, 1.2 g, 9.5 mmol) were added to the resin **19** (1.9 mmol) in *N*-methylpyrrolidinone (30 ml). The mixture was heated at 80°C and shaken for 48 h. Washing sequentially with DMF, CH_2Cl_2 and DMF afforded *N*-(*N*-[5-cyanopyridin-2-yl]-2-aminoethyl)-Boc-Gly-L-Pro-resin **20** which was used directly in the next step.

4.1.14. *N*-[2-(5-Cyanopyridin-2-ylamino)-ethyl]-*N*-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-acetamide (21). The resin **20** (1.9 mmol) was added to a mixture of trifluoroacetic acid and CH_2Cl_2 (1:1) solution and stirred for 2 h at room temperature. The supernatant was added to Et_2O to precipitate 1-{2-[2-(5-cyanopyridin-2-ylamino)-ethylamino]-acetyl}-pyrrolidine-2-*S*-carboxylic acid amide trifluoroacetate salt (0.70 g, 87%). ^1H NMR (d_6-DMSO, 300 MHz): δ 1.82–2.05 (4H, m), 3.08–3.16 (2H, m), 3.40–3.50 (2H, m), 3.59–3.67 (2H, m), 4.02 (2H, s), 4.22–4.30 (1H, m), 5.40 (2H, s), 6.60 (1H, d, J=8.8 Hz), 7.74 (1H, dd, J=8.8, 2.1 Hz), 7.81 (1H, s), 8.42 (1H, d, J=2.1 Hz), 9.00 (2H, s) ppm. MS (ESI) m/z 317 (MH^+, 100%). This precipitate (0.54 g, 1.3 mmol) was dissolved in anhydrous THF (25 ml) at 0°C. Trifluoroacetic anhydride (0.88 ml, 6.3 mmol) was added. The reaction was stirred at 0°C for 2 h. The solvent was removed under vacuum. Flash chromatography on silica gel (2% MeOH/98% CH_2Cl_2 and 5% MeOH/95% CH_2Cl_2) afforded *N*-[2-(5-cyanopyridin-2-ylamino)-ethyl]-*N*-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-acetamide **21** as a white solid (0.35 g, 70%). ^1H NMR (d_6-DMSO, 300 MHz) δ 1.82–2.05 (4H, m), 3.40–3.50 (2H, m), 3.59–3.67 (2H, m), 4.06–4.14 (2H, m), 4.29 (2H, s), 4.68–4.76 (1H, m), 6.60 (1H, d, J=8.8 Hz), 7.74 (1H, dd, J=8.8, 2.1 Hz), 7.81 (1H, s), 8.42 (1H, d, J=2.1 Hz) ppm. MS (ESI) m/z 395 (MH^+, 100%).

4.1.15. (6-{2-[2-(2-*S*-Cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile (1), by removal of trifluoroacetyl group. Aqueous ammonia solution (33%, 0.50 ml) was added to a solution of *N*-[2-(5-cyanopyridin-2-ylamino)-ethyl]-*N*-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-acetamide **21** (20 mg, 0.05 mmol) in CH_2Cl_2 (2 ml). The reaction was stirred for 3 h. The solvent was removed under vacuum to give (6-{2-[2-(2-*S*-cyanopyrrolidin-1-yl)-2-oxo-ethylamino]-ethylamino}-nicotinonitrile **1** as a colourless gum (12 mg, 80%). Analytical data (^1H, ^{13}C NMR and MS) for this free base and after conversion to the hydrochloride salt were identical to those obtained from the solution phase synthesis.

References

1. Mentlein, R. *Regul. Pept.* **1999**, *85*, 9–24.
2. Deacon, C. F.; Nauck, M. A.; Toft-Nielsen, M-B.; Pridal, L.; Willms, B.; Holst, J. J. *Diabetes* **1995**, *44*, 1126–1131.
3. Ashworth, D. M.; Atrash, B.; Baker, G. R.; Baxter, A. J.; Jenkins, P. D.; Jones, D. M.; Szelke, M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1163–1166.
4. Balkan, B.; Kwasnik, L.; Miserendino, R.; Holst, J. J.; Li, X. *Diabetologia* **1999**, *42*, 1324–1331.
5. Villhauer, E. B. International Patent Application Number PCT/EP97/061255, published as WO98/19998 1998.
6. While this work was in progress an oral communication was presented that described the synthesis of NVP-DPP728 on solid phase. It differs from the one reported here in the order that the steps were carried out and in the choice of protecting groups. The published work is amenable to analoging of the ethylene sidechain while the one reported here is more suitable for analoging of the terminal aromatic group. Villhauer, E. B.; Anderson, R. C.; Balkan, B.; Barilla, D.; Brinkman, J. A.; Dunn, E.; Dunning, B.; Graham, E. D.; Gu, H. H.; Gutierrez, C. M.; Hamilton, B. H.; Kwasnik, L. A.; Li, X.; Mangold, B. L.; Maniara, W. M.; Miserendino-Molteni, R.; Mone, M.; Naderi, G. B.; Ramos, K. L.; Russell, M. E.; Rothenberg, P. L.; Tullman, R. H.; Valentin, M.; Walter, R. E.; Weldon, S. C.; Hughes, T. E. *Abstracts of Papers*, #343, 221st National Meeting of the American Chemical Society, San Diego, CA, 2001.
7. Hughes, T. E.; Mone, M. D.; Russell, M. E.; Weldon, S. C.; Villhauer, E. B. *Biochemistry* **1999**, *38*, 11597–11603.
8. Almeida, M. L. S.; Grehn, L.; Ragnarsson, U. *J. Chem. Soc., Perkin Trans. 1* **1988**, 1905–1911.
9. Nash, I.; Bycroft, B. W.; Chang, W. C. *Tetrahedron Lett.* **1996**, *37*, 2625–2628.