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Synthesis of (*S*)-, (*R*)-, and (*rac*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acids and an evaluation of the DPP IV inhibitory activity of Denagliptin diastereomers

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ABSTRACT

The non-proteinogenic amino acid (2*S*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acid [**(*S*)-1**] is a key intermediate required for the synthesis of Denagliptin (**2a**). Denagliptin is a dipeptidyl peptidase IV (DPP IV) inhibitor that is being developed for the treatment of type-2 diabetes mellitus. A diastereoselective, cost-efficient synthetic procedure for **(*S*)-1** was developed by alkylating a Ni(II) glycine equivalent derived from (*S*)-2-[(*N*-benzylprolyl) amino] benzophenone [(*S*)-BPB]. The alkylated product was then decomposed to isolate the target amino acid **(*S*)-1** (ee >99%) and ligand (*S*)-BPB, which can be reused in subsequent reactions. The enantiomer **(*R*)-1** and racemate **(*rac*)-1** were synthesized from their corresponding Ni(II) glycine equivalents. Denagliptin diastereomers (**2**), derived from the key intermediates **(*S*)-1**, **(*R*)-1**, and **(*rac*)-1** were synthesized, and their dipeptidyl peptidase IV inhibitory activities were investigated. These findings are important in the design and synthesis of DPP IV inhibitors.

1. Introduction

Recently, glucagon-like peptide-1 (GLP-1)[1] and dipeptidyl peptidase IV (DPP IV)[2] have been intensively researched for use in the treatment of type-2 diabetes mellitus. GLP-1 is an incretin hormone secreted by the intestinal L-cells in response to food ingestion. It is a novel pharmacological target with multiple antihyperglycemic actions. The glucoregulatory actions of GLP-1 include enhancement of glucose-dependent insulin secretion, inhibition of glucagon secretion, delayed gastric emptying, and reduction in food intake. However, GLP-1 is rapidly degraded in circulation at its N-terminus by DPP IV.[3] In order to potentiate the glucose-lowering actions of GLP-1, it is essential to inhibit DPP IV by inhibitors, which have now been of great importance in the treatment of type-2 diabetes.

The chiral amino acid **(*S*)-1** ((2*S*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acid, Figure 1) is a key intermediate in the synthesis of Denagliptin (**2a**),[4] a well-known DPP IV inhibitor as oral medication of type-2 diabetes being developed by GlaxoSmithKline. The original procedure used for the synthesis of **(*S*)-1**

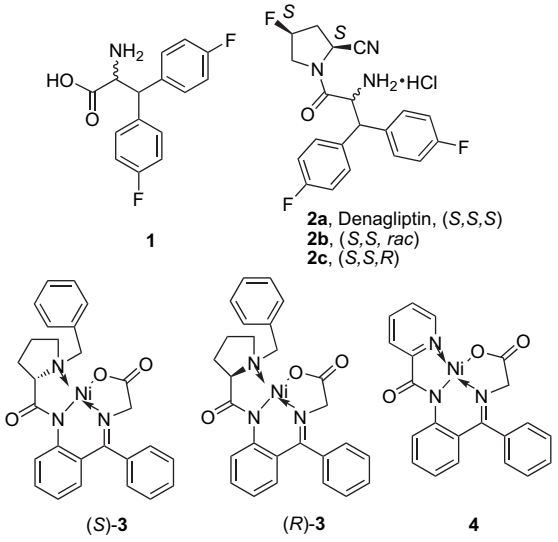

Figure 1. Structures of compounds **1**, **2** (Denagliptin), and Ni(II) complexes **3** and **4**.

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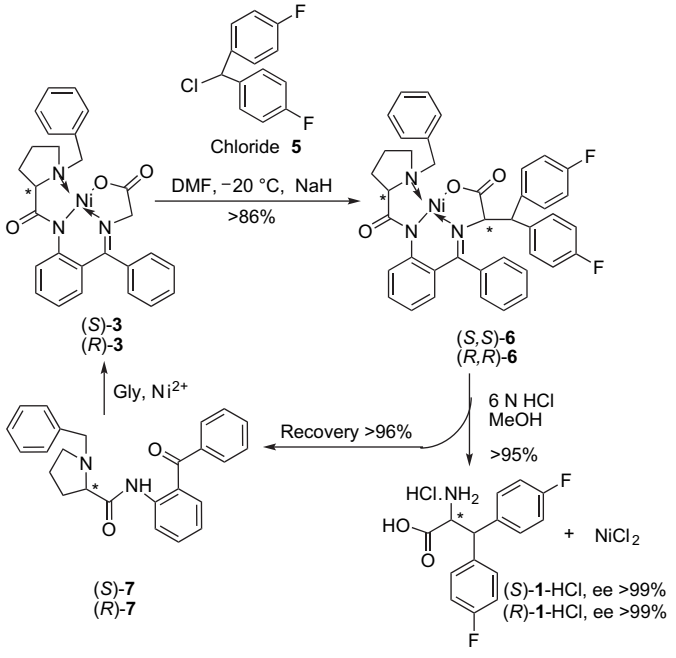
employed seven steps. In this procedure, asymmetric azidation was mediated by an oxazolidinone employed as a chiral auxiliary;[5] this procedure was considered unsatisfactory because of its duration as well as safety issues related to the stability of the azide intermediates generated. Patterson et al.[6] reported the synthesis of **(*S*)-1** by the asymmetric phase transfer catalytic (PTC) alkylation of *N*-(diphenylmethylene) glycerin *tert*-butyl ester; this reaction exhibited low enantioselectivity (ee=60% before crystallization). We aimed to carry out a structure–activity relationship (SAR) study of Denagliptin. For this study, this elucidation required key intermediate amino acids such as **(*S*)-1**, **(*R*)-1**, and **(*rac*)-1**. Therefore, it is necessary to develop strategies that are safe, inexpensive, and that produce minimal waste for the preparation of **(*S*)-1**, **(*R*)-1**, and **(*rac*)-1**. The Ni(II) complexes **(*S*)-3**[7] (commercially available), **(*R*)-3**,[8] and **4**[9] (Fig. 1) were selected as nucleophilic glycine equivalents for C-alkylation due to their preferred control to induce *S*- or *R*-configuration. In this study, we present an efficient protocol for the preparation of compound **(*S*)-1** and the synthesis of **(*R*)-1** and **(*rac*)-1** in two steps. In the first step, the Ni(II) complexes were alkylated by 4,4′-difluorobenzhydryl chloride (**5**) in the presence of NaH dissolved in the solvent DMF. In the second step, the alkylated products were decomposed with HCl to yield the desired amino acids (ee ≥99%; Scheme 1). These synthesized amino acids were subsequently utilized for the synthesis of Denagliptin diastereomers. The inhibitory activity of these diastereomers against DPP IV was then evaluated. Interestingly, the bioassay results revealed that Denagliptins **2a** and **2b** (derived from **(*S*)-1** and **(*rac*)-1**, respectively) exhibited high inhibitory activities; however, diastereomer **2c** (derived from **(*R*)-1**) exhibited extremely low DPP IV inhibitory activity.



Scheme 1. Synthesis of (2*S*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acid **(*S*)-1·HCl**.

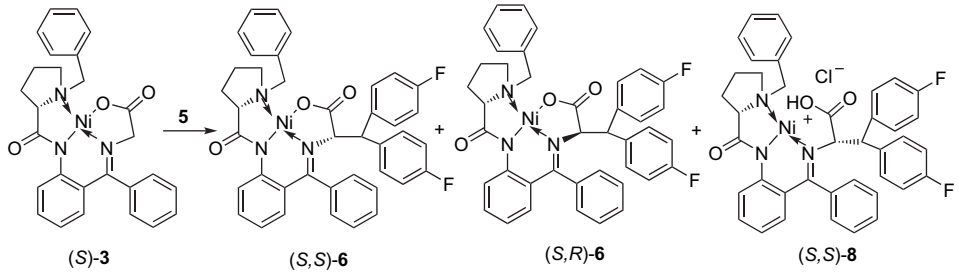
2. Results and discussion

The successful preparation of (*S*)-amino acids by the electronic re-side attack of the Ni (II) complex of **(*S*)-3** enolate double bond using simple halides was widely published by Belokon et al.;[8] however, the preparation of (*R*)-amino acids through the Ni(II) complex of **(*R*)-3** has been rarely reported.[8b] In this study, it was observed that the alkylation of the α-carbon atom of the complex **(*S*)-3** with 4,4′-difluorobenzhydryl chloride **5** was challenging due to steric hindrance, the bulk of the two individual compounds and the unexpected influence of the high electronegative F atoms on the aromatic ring of chloride **5**. Table 1 summarizes the interesting experimental results of the alkylation of **(*S*)-3** with chloride **5** with different bases in various solvents under a series of conditions. However, when previously described protocols were used,[7–9] 7 of the 12 solvent/base pairs (entries 1–7) yielded disappointing results since the desired alkylated product **6** was not obtained, even though strong inorganic bases such as lithium diisopropylamide (LDA) and KO^{t}Bu and organic bases such as 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) (entries 3, 6, and 7) were employed. Experiments were continuously carried out until the discovery of the DMF/NaH pair. With this pair, a moderate yield (35%) of the alkylated complex **6** was obtained (entry 8). Subsequently, when the reaction was allowed to run for a longer time period (entry 9) or at a higher temperature (entry 10), a significant decrease in product yield was observed. The product dr value was not different from that observed initially. Under these conditions (entries 9 and 10), compound **(*S*,*S*)-6** was gradually converted to a new product, which was identified to be derived by ring cleavage **8** by liquid chromatography-mass spectrometry (LC-MS) ($[M+H]^+$=701) and nuclear magnetic resonance (NMR) analysis. This byproduct **8** was identified to be responsible for the decreased yield (entries 9 and 10). Alkylation generated an excellent yield when the reaction was performed using 1 equiv of complex **(*S*)-3**, 1.3 equiv of chloride **5**, and 3 equiv of NaH at −20 °C. The reaction proceeded smoothly until its completion in 2.5 h (98:2 dr); purification using flash chromatography yielded the product **(*S*,*S*)-6** in >99:1 dr (86% combined yield; entry 11). By simulating the conditions under which optimum results were previously obtained, the experiment was successfully repeated with **(*R*)-3** as the starting material instead of **(*S*)-3** (entries 12 vs 11).

Thereafter, **(*S*,*S*)-6** and **(*R*,*R*)-6** were dissolved in methanol solution, followed by decomposition in 6 N HCl under reflux. The reaction was considered complete when the solution changed color from red to green. The reaction was completed in 30 min. The isolation of the desired amino acid **1** from the reaction mixture comprising water, $NiCl_2$, and ligand 2-[(*N*-benzylprolyl)amino]-benzophenone (BPB, **7**), is difficult due to the solubility of **1** both in water and in organic solvents. The classical methods elaborated by Belokon et al.[7,10] involving the use of Dowex resin or ethylene diamine tetraacetic acid (EDTA) were employed to isolate **1**. Unfortunately, these procedures yielded unacceptably low yields of product **1** (below 20%). Excellent yields of the products **(*S*)-1·HCl** ($[\alpha]_D^{23}$ +55.1; lit.[6] ee >99%[11]) and **(*R*)-1·HCl** ($[\alpha]_D^{23}$ −55.2; ee >99%) were successfully obtained by column chromatography performed using a reusable C_{18}-reversed phase (230–400 mesh) silica gel column, a widely used apparatus in the manufacturing industry. In this method, $NiCl_2$ was completely separated from the mixture using water as the eluent. Compound **(*S*)-1** was collected by 50% MeOH in water eluent, and BPB (**7**) was recycled using the MeOH eluent. The C_{18}-reversed phase silica gel could be washed with MeOH and reused. Further, the chiral ligand **(*S*)-7** was recovered (recovered **(*S*)-7** $[\alpha]_D^{23}$ −133.2, *c* 0.5 lit.[12]); this merits lower production costs.

Using similar protocols as those used for **(*S*)-1** and **(*R*)-1**, a high yield (95%) of **(*rac*)-1** was easily obtained by using Ni(II) complex **4** and chloride **5** (Scheme 2). Ni(II) complex **4** was prepared using a one-pot procedure in our published paper.[9a] The reaction was terminated by the addition of water saturated with NH_4Cl to form a red precipitate that was pure enough to be decomposed by 6 N HCl. By following the classical method developed by Belokon et al.[10] (yield, 71%) or by our established method that employed reusable C_{18}-reversed phase silica gel, **(*rac*)-1** and ligand 2-[*N*-(α-picolyl) amino]-benzophenone (PABP, **10**) could be easily separated (yield, 98%).

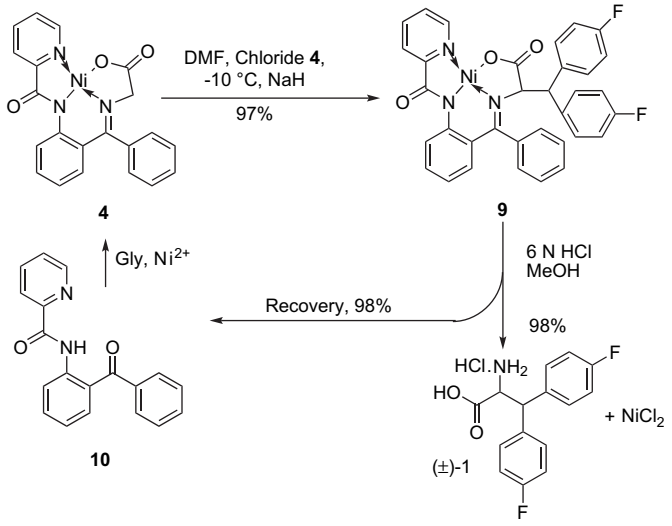
Table 1
Alkylation of Ni(II) complex **(*S*)-3** with chloride **5** under various conditions



Entry	Solvent/base	Temp (°C)	Time (h)	Yield[a] of **(*S*,*S*)-6**	dr **(*S*,*S*)-6**:**(*S*,*R*)-6**[b]
1	DCM/NaOH	rt	7	0	—
2	THF/BuLi	−78 to rt	1.5	0	—
3	THF/LDA	−78 to rt	1.5	0	—
4	MeCN/NaH	−10 to rt	3	0	—
5	DMF/NaOH	rt	0.1	0	—
6[c]	DMF/DBU	0	24	0	—
7[c]	DMF/KO^{t}Bu	−10	24	0	—
8[c]	DMF/NaH	0	2	35%	97:3
9[c]	DMF/NaH	0	5	20%	97:3
10[c]	DMF/NaH	rt	2	10%	95:5
11[d]	DMF/NaH	−20	2.5	70% (86%[e])	98:2
12[f]	DMF/NaH	−20	2.5	75 (89%[e])	98:2

[a] Yield of product **(*S*,*S*)-6** isolated by column chromatography.
[b] Determined by chiral Daicel optical density (OD)-H column analysis of the crude product **6** [80:20 hexane/isopropanol, 11.4 min (*S*,*S*), 12.7 min (*S*,*R*)] before purification. The configurations were assigned according to optical data and information from literature.[7,9b]
[c] Using a 1:1.3:2 mixture of Ni(II) complex **(*S*)-3**, chloride **5**, and base.
[d] Using a 1:1.3:3 mixture of Ni(II) complex **(*S*)-3**, chloride **5**, and base.
[e] Combined yield.
[f] Using **(*R*)-3** as the starting Ni(II) complex to yield **(*R*,*R*)-6**.

By following procedures similar to those described in a published patent application,[5] we synthesized Denagliptins **2a**, **2b**, and **2c** from **(*S*)-1**, **(*rac*)-1**, and **(*R*)-1**, respectively. These three compounds were assayed in vitro to determine the inhibitory activities of DPP IV. The results are shown in Table 2. The inhibition rates were observed to be 97.7%, 95.3%, and 13.1% for **2a**, **2b**, and **2c**, respectively; further, the median inhibition concentration (IC_{50}) value of **2b** was 0.33-fold less than that of **2a**. These results revealed that the absolute *S*-configuration of the amino acid residue is vital for the inhibitory activity of Denagliptin (Table 2). These findings have useful applications in the design and synthesis of DPP IV inhibitors.



Scheme 2. Preparation of **(*rac*)-1**.

3. Conclusion

In summary, an efficient protocol for the synthesis of **(*S*)-1** (ee >99%), **(*R*)-1** (ee >99%), and **(*rac*)-1** in two steps from chloride **5** and the corresponding Ni(II) complexes was established. Using this optimized protocol, the overall yields of the optically pure amino acids, namely, **(*S*)-1**, **(*R*)-1**, and **(*rac*)-1**, generated were 82%, 85%, and 95%, respectively. Further, the chiral auxiliary **7** and the achiral auxiliary **10** were recoverable and reusable for the further preparation of Ni (II) complexes. The biological evaluation of the Denagliptin diastereomers **2a**–**c** indicate that the *S*-configuration of the amino acid portion of Denagliptin is vital for its DPP IV inhibitory activity and the *R*-configuration results in low inhibitory activity. These findings have useful applications in the design and synthesis of DPP IV inhibitors.

4. Experimental section

4.1. General methods

All the reagents were used as obtained, unless otherwise stated. Solvents were evaporated under reduced pressure and below 50 °C if no description was noted. Melting points were measured in

Table 2
DPP IV inhibition study of compounds, **2a**, **2b**, and **2c**

Entry	Inhibition rate (%, 50 μM)	IC_{50} (nM)
2a	97.7	253[6]
2b	95.3	377
2c	13.1	>48,800
LAF-237[a]	ND[b]	38

[a] LAF-237[4] was used as a control.
[b] Not determined.

capillary tube without correction. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz instrument. Chemical shifts were reported in parts per million (ppm) upfield from TMS. Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). High resolution mass spectra (HRMS) were obtained on a Fourier Transform Mass Spectrometer. Optical rotations were measured on PE-341 polarimeter.

4.2. General procedure for the preparation of (*S*,*S*)-6, (*R*,*R*)-6, and 9 described as those for BPB-Ni(II)-(2*S*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acid (*S*,*S*)-6

To a stirred solution of nickel compound **(*S*)-3**[13] (8.936 g, 17.94 mmol) in DMF (60 ml) was added 4,4′-difluorobenzhydryl chloride **5** (4.708 g, 19.73 mmol) in one portion under an argon atmosphere. The mixture was cooled to −20 °C and NaH (2.15 g, 53.82 mmol, 60% suspension in oil) was added without stirring. The air was evacuated with a vacuum pump and the flask was filled with argon. Then the stirring was commenced and the reaction proceeded smoothly to completion in 2.5 h (98:2 dr). Then the reaction mixture was neutralized by slow addition of acetic acid (approximately 3.3 ml) under stirring and cooling. Finally, the reaction mixture was slowly poured into icewater (500 ml). After 12 h the precipitate was filtered. The crude red product was purified by silica gel chromatography with eluent (petroleum ether/ethyl acetate=1:1) to afford pure red product **(*S*,*S*)-6** 8.81 g (70% yield). The unreacted raw material **(*S*)-3** (2.2 g) obtained from the chromatography was reused repeating the procedure described above to afford an additional 2.0 g of product **(*S*,*S*)-6**. Totally, 10.81 g (86% yield) of pure product **(*S*,*S*)-6** was obtained in >99:1 dr (DAICEL OD-H HPLC COLUMN). R_f=0.23 (petroleum ether/ethyl acetate=1:1). Mp 213–215 °C. $[\alpha]_D^{23}$ +1991.9 (*c* 0.49, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.71–1.75 (m, 2H), 2.01–2.07 (m, 1H), 2.28–2.54 (m, 2H), 2.96–2.99 (m, 1H), 3.34–3.38 (m, 1H), 3.46 (d, *J*=12.9 Hz, 1H), 4.28 (d, *J*=12.6 Hz, 1H), 4.33 (d, *J*=3.0 Hz, 1H), 4.71 (d, *J*=3.6 Hz, 1H), 6.66–6.79 (m, 5H), 7.05–7.19 (m, 6H), 7.25–7.39 (m, 5H), 7.42–7.49 (m, 3H), 8.03 (d, *J*=7.2 Hz, 2H), 8.28 (d, *J*=8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=23.1, 31.0, 56.8, 57.3, 63.6, 70.6, 74.1, 114.7, 114.9, 123.1, 126.0, 127.0, 127.8, 128.7, 128.8, 128.9, 129.0, 129.5, 129.7, 129.8, 131.5, 131.7, 131.8, 132.6, 133.2, 133.7, 134.0, 135.2, 136.3, 143.0, 160.1, 161.3, 162.6, 163.7, 171.5, 176.9, 180.3. HRMS (ESI) $[M+Na]^+$ found *m*/*z* 722.1702, calcd for $[C_{40}H_{33}F_2N_3NiO_3+Na]^+$: 722.1741.

4.2.1. (2R)-BPB-Ni(II)-(2S)-2-amino-3,3-bis(4-fluorophenyl) propanoic acid (*R*,*R*)-6

Compound **(*R*,*R*)-6** was prepared from **(*R*)-3** using a procedure similar to that described for the preparation of **(*S*,*S*)-6** as a red solid. R_f=0.23 (petroleum ether/ethyl acetate=1:1). Mp 144–146 °C. $[\alpha]_D^{23}$ −2006 (*c* 0.57, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=1.70–1.74 (m, 1H), 1.98–2.09 (m, 1H), 2.23–2.57 (m, 2H), 2.88–3.01 (m, 1H), 3.34–3.38 (m, 1H), 3.46 (d, *J*=12.6 Hz, 1H), 4.28 (d, *J*=12.6 Hz, 1H), 4.33 (d, *J*=3.0 Hz, 1H), 4.71 (d, *J*=3.3 Hz, 1H), 6.65–6.79 (m, 5H), 7.05–7.19 (m, 6H), 7.24–7.33 (m, 5H), 7.40–7.48 (m, 3H), 8.03 (d, *J*=7.2 Hz, 2H), 8.28 (d, *J*=8.7 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=23.1, 31.0, 56.8, 57.3, 63.6, 70.6, 74.1, 114.7, 114.9, 123.1, 126.0, 127.0, 127.8, 128.7, 128.8, 129.0, 129.1, 129.5, 129.7, 129.8, 131.5, 131.7, 131.8, 132.6, 133.2, 133.7, 134.0, 135.2, 136.3, 142.9, 160.1, 161.3, 162.6, 163.7, 171.5, 177.0, 180.3. HRMS (ESI) $[M+Na]^+$ found *m*/*z* 722.1736, calcd for $[C_{40}H_{33}F_2N_3NiO_3+Na]^+$: 722.1741.

4.2.2. PABP-Ni(II)-2-amino-3,3-bis(4-fluorophenyl) propanoic acid **9**

Compound **9** was prepared from complex **4** using a procedure similar to that described for the preparation of **(*S*,*S*)-6** as a red solid. The reaction temperature was controlled at −10 °C. R_f=0.24 (petroleum ether/ethyl acetate=1:1). Mp >290 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ=4.27 (d, *J*=2.1 Hz, 1H), 4.80 (d, *J*=2.4 Hz, 1H), 6.80–6.89 (m, 6H), 7.07–7.11 (m, 3H), 7.34–7.36 (m, 3H), 7.47–52 (m, 3H), 7.63 (d, 3H), 7.85–89 (m, 1H), 7.95–8.01 (m, 1H), 8.77 (d, *J*=9.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=56.5, 76.0, 114.9, 115.0, 115.1, 115.2, 121.6, 123.7, 123.9, 126.7, 127.4, 127.5, 127.6, 129.4, 129.5, 129.8, 129.9, 130.2, 132.8, 132.9, 133.6, 133.7, 134.6, 134.7, 134.9, 140.4, 143.5, 146.6, 153.1, 160.4, 161.3, 162.8, 163.7, 169.4, 171.7, 176.4. HRMS (ESI) $[M+H]^+$ found *m*/*z* 619.1145, calcd for $[C_{40}H_{33}F_2N_3NiO_3+H]^+$: 619.1139.

4.3. General procedure for the preparation of (*S*)-1, (*R*)-1, and (*rac*)-1 described as those for (2*S*)-2-amino-3,3-bis(4-fluorophenyl)propanoic acid hydrochloride (*S*)-1·HCl

A one-neck flask was charged with **(*S*,*S*)-6** (10 g, 14.31 mmol) followed by MeOH (300 ml). The resulting slurry was reflux and then 25 ml 6 N HCl was added in one portion. The mixture was continued refluxing for 30 min while the red color disappeared and became green. The reaction was cooled to room temperature and then evaporated to dryness. Water (20 ml) was added to the residue to form a clear solution then this solution was separated by column chromatography on C_{18}-reversed phase (230–400 mesh) silica gel. Pure water as eluent was employed to remove the green $NiCl_2$ and excess HCl, then MeOH/water (1:1) was used to obtain optically pure product **(*S*)-1·HCl** (4.3 g, 95%). The ligand BPB (**7**) decomposed from **(*S*,*S*)-6** was recovered by MeOH eluent (5.8 g, 96%), and the column chromatography was washed by 100 ml MeOH for further use. R_f=0.36 (DCM/MeOH=5:1). Mp 162–163 °C (lit.[6] mp 149 °C). $[\alpha]_D^{23}$ +55.1 (*c* 2, MeOH, lit.[6] $[\alpha]_D^{23}$ +56.3), ee >99%. ^{1}H NMR (CD_3OD, 300 MHz): δ=4.43 (d, *J*=10.5 Hz, 1H), 4.80 (d, *J*=10.5 Hz, 1H), 7.05 (t, *J*=9.0 and 8.7 Hz, 2H), 7.14 (t, *J*=9.0 and 8.7 Hz, 2H), 7.39–7.43 (m, 2H), 7.50–7.54 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=53.7, 57.9, 116.9, 117.1, 117.6, 117.8, 131.7, 131.8, 131.9, 136.0, 136.6, 163.0, 165.4, 171.3. HRMS (ESI) $[M+H]^+$ found *m*/*z* 278.0993, calcd for $[C_{15}H_{13}F_2NO_2+H]^+$: 278.0993.

4.3.1. (2R)-2-Amino-3,3-bis(4-fluorophenyl)propanoic acid hydrochloride (*R*)-1·HCl

Compound **(*R*)-1·HCl** was prepared from **(*R*,*R*)-6** using a procedure similar to that described for the preparation of **(*S*)-1·HCl** as a white solid. R_f=0.36 (DCM/MeOH=5:1). Mp 181–183 °C. $[\alpha]_D^{23}$ −55.2 (*c* 0.52, MeOH), ee >99%. ^{1}H NMR (CD_3OD, 300 MHz): δ=4.26 (d, *J*=9.6 Hz, 1H), 4.47 (d, *J*=9.3 Hz, 1H), 6.97–7.11 (m, 4H), 7.33–7.44 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=53.7, 59.2, 116.8, 117.0, 117.4, 117.7, 131.7, 131.8, 131.9, 132.0, 136.5, 137.2, 162.9, 165.3, 172.3. HRMS (ESI) $[M+H]^+$ found *m*/*z* 278.0993, calcd for $[C_{15}H_{13}F_2NO_2+H]^+$: 278.0995.

4.3.2. rac-2-Amino-3,3-bis(4-fluorophenyl)propanoic acid hydrochloride rac-1·HCl

Compound **(*rac*)-1·HCl** was prepared from **9** using a procedure similar to that described for the preparation of **(*S*)-1·HCl** as a white solid. R_f=0.36 (DCM/MeOH=5:1). Mp 186–187 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ=4.26 (d, *J*=9.6 Hz, 1H), 4.47 (d, *J*=9.0 Hz, 1H), 7.01 (t, *J*=8.7 and 9.0 Hz, 2H), 7.09 (t, *J*=9.0 and 8.7 Hz, 2H), 7.32–7.35 (m, 2H), 7.39–7.44 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=53.7, 60.6, 116.7, 116.8, 117.3, 117.5, 131.7, 131.8, 132.1, 132.2, 137.1, 138.0, 162.7, 165.2. 172.3. HRMS (ESI) $[M+H]^+$ found *m*/*z* 278.0993, calcd for $[C_{15}H_{13}F_2NO_2+H]^+$: 278.0993.

4.3.3. (2S,4S)-1-[(2S)-2-Amino-3,3-bis(4-fluorophenyl)propanoyl]-4-fluoropyrrolidine-2-carbonitrile hydrochloride (**2a**)

According to the procedures described in the published patent application,[5] **2a** was prepared as a white solid. R_f=0.47 (DCM/MeOH/acetic acid=10:1:1). Mp 183–184 °C. $[\alpha]_D^{23}$ +119 (*c* 0.51, MeOH). ^{1}H NMR (CD_3OD, 300 MHz): δ=2.23–2.53 (m, 2H), 3.06 (q, 1H), 3.75–3.86 (m, 1H), 4.53 (d, *J*=11.4 Hz, 1H), 4.86–4.90 (m, 2H),

5.32–35 (m, 1H), 7.01 (t, J=8.7 Hz, J=8.7 Hz, 2H), 7.22 (t, J=8.7 and 8.7 Hz, 2H), 7.34 (q, J=5.1, 3.6, and 5.4 Hz, 2H), 7.65 (q, J=5.4, 3.6, and 5.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=37.2, 46.8, 54.8, 54.3, 56.2, 92.7, 94.5, 117.6, 117.8, 118.0, 118.4, 132.0, 132.1, 132.3, 132.4, 134.8, 134.9, 163.2, 163.3, 165.6, 165.7, 169.4. HRMS (ESI) $[M+Na]^+$ found m/z 396.1285, calcd for $[C_{20}H_{18}F_3N_3O+Na]^+$: 396.1300.

*4.3.4. (2S,4S)-**1**-[(±)-2-Amino-3,3-bis(4-fluorophenyl)propanoyl]-4-fluoropyrrolidine-2-carbonitrile hydrochloride (**2b**)*

Compound **2b** was prepared as a white solid using a procedure similar to **2a**. R_f=0.47 and 0.33 (DCM/MeOH/acetic acid=10:1:1). Mp 174–175 °C. $[\alpha]_D^{23}$ −31 (c 0.51, MeOH). ^{1}H NMR (CD_3OD, 300 MHz): δ=1.75–1.95 (m, 1H), 2.2–2.44 (m, 2H), 2.81–3.18 (m, 1H), 3.81–4.20 (m, 1H), 4.52 (t, J=11.1 and 11.1 Hz, 2H), 4.91–5.05 (m, 1H), 5.13–5.24 (m, 1H), 6.98–7.09 (m, 2H), 7.22 (t, J=8.7 and 8.4 Hz, 2H), 7.34 (q, J=4.8, 3.6, and 5.1 Hz, 2H), 7.66 (q, J=5.1, 3.3, and 5.1 Hz, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=37.5, 46.9, 54.2, 56.2, 92.4, 94.3, 117.1, 117.3, 117.7, 118.5, 132.0, 132.1, 132.2, 132.4, 134.5, 135.7, 163.2, 165.5, 169.3, 175.7. HRMS (ESI) $[M+H]^+$ found m/z 374.1501, calcd for $[C_{20}H_{18}F_3N_3O+H]^+$: 374.1480.

*4.3.5. (2S,4S)-**1**-[(2R)-2-Amino-3,3-bis(4-fluorophenyl)propanoyl]-4-fluoropyrrolidine-2-carbonitrile hydrochloride (**2c**)*

Compound **2c** was prepared using a procedure similar to **2a** as a white solid. R_f=0.33 (DCM/MeOH/acetic acid=10:1:1). Mp 175–176 °C. $[\alpha]_D^{23}$ −199 (c 0.51, MeOH). ^{1}H NMR (CD_3OD, 300 MHz): δ=1.78–1.92 (m, 2H), 2.22–2.44 (m, 1H), 2.62–2.95 (m, 1H), 4.04–4.11 (m, 1H), 4.32 (d, J=9.6 Hz, 2H), 4.50 (d, J=11.4 Hz, 1H), 4.97 (t, J=11.4 and 11.7 Hz, 1H), 5.09–5.26 (m, 1H), 7.05 (t, J=8.4 and 8.4 Hz, 2H), 7.21 (t, J=8.4 and 9.0 Hz, 2H), 7.33 (m, 2H), 7.65 (q, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ=37.5, 47.0, 54.0, 56.4, 60.7, 92.4, 94.3, 117.2, 117.4, 117.8, 118.9, 132.2, 132.3, 132.38, 132.41, 132.5, 134.4, 135.6, 163.2, 165.6, 169.3, 175.7. HRMS (ESI) $[M+Na]^+$ found m/z 396.1295, calcd for $[C_{20}H_{18}F_3N_3O+Na]^+$: 396.1300.

4.4. Determination of the optical purity of 1·HCl[11]

The **1·HCl** (0.1 mg) in methanol (20 μl) was added to the solution of Marfey's reagent (1 mg, 10 equiv) in acetone (0.1 ml) and 10 ml of Et_3N. The reaction mixture was heated for 2 h at 40 °C. Then the above solution was diluted to 0.8 ml, and 5 μl of this solution was injected on the HPLC column (ZORBAX SB-C18, 4.6×150 mm, 5 μm) at a flow rate of 1 ml/min, mobile phase 0.5% TFA in water; detection, 340 nm, 8 nm bandwidth; temperature 40 °C; retention times, desired diastereomer (S): 4.267 min, undesired (R): 6.278 min (the retention times were determined by the (***rac*)-1**).

4.5. Assay for DPP IV inhibition[14]

To measure the activity of DPP IV, a continuous fluorometric assay was employed using Gly-Pro–AMC, which is cleaved by the enzyme to release the fluorescent aminomethylcoumarin (AMC). A typical reaction contained 50 pmol/l enzyme, 50 μmol/l Gly-Pro–AMC, different concentrations of the compounds synthesized in this work, and buffer (100 mmol/l HEPES, pH 7.5, 0.1 mg/ml BSA) in a total reaction volume of 100 μl. Liberation of AMC was monitored using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. The enzyme used in these studies was soluble human protein produced in a baculovirus expression system (Bac-To-Bac; Life Technologies).

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