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Synthesis and biological evaluation of all eight stereoisomers of DPP-IV inhibitor saxagliptin

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ABSTRACT

All eight stereoisomers of saxagliptin have been synthesized and evaluated for their inhibitory activity against DPP-IV. It was unambiguously confirmed that the configuration of saxagliptin was critical to potent inhibition of DPP-IV. Docking study was performed to elucidate the configuration–activity relationship of saxagliptin stereoisomers. Tyr662 and Tyr470 have been suggested as the key residues of DPP-IV interacting with the inhibitors. This work provides valuable information for further inhibitor design against DPP-IV.

1. Introduction

Saxagliptin (Onglyza™, **1a**) is a recently approved drug for the treatment of type 2 diabetes mellitus.[1] It exerts glucose lowering effect by reversible inhibition of dipeptidyl peptidase IV (DPP-IV),[2–4] with the nitrile group covalently binding to DPP-IV.[5] Saxagliptin possesses four chiral carbon atoms, and theoretically has eight stereoisomers **1a**–**1h** (Fig. 1). To the best of our knowledge, there is so far no report on configuration–activity relationship of saxagliptin and its stereoisomers. Thus, it would be interesting to know how the configuration affects the DPP-IV inhibitory activity, which would provide valuable information for further drug design. Moreover, as potential impurities of saxagliptin API (Active Pharmaceutical Ingredient), the synthesis of stereoisomers **1b**–**1h** is highly desirable to provide the reference substances for drug quality control.[6–8] Herein, we describe the synthesis and biological evaluation of all eight stereoisomers of saxagliptin as DPP-IV inhibitors.

2. Results and discussion

2.1. Chemistry

Retrosynthetic analysis revealed that **1a**–**1h** could be assembled by two segments: *N*-Boc-hydroxyadamantylglycines (**8a** and **8b**) and 4,5-methanoproline amides (**15a**–**15d**). The chiral center of **8a** could be introduced by asymmetric Strecker amino acid synthesis.[1] As depicted in Scheme 1, adamantanealdehyde **2** was treated with NaCN and (*R*)-2-phenylglycinol (**3a**) to give (2*S*,2′*R*)-cyanoamine **4a**. Hydrolysis of **4a** with concentrated HCl afforded carboxylic acid **5a**. Hydrogenolysis of **5a** furnished enantiomerically pure (*S*)-adamantylglycine **6a**. Boc-protection of **6a** gave carboxylic acid **7a**, which was oxidized with $KMnO_4$ to give (*S*)-*N*-Boc-3-hydroxyadamantylglycine **8a** (ee >99%, determined by chiral HPLC). In the similar manner, (*R*)-*N*-Boc-3-hydroxyadamantylglycine **8b** (ee >99%, determined by chiral HPLC) was prepared with using (*S*)-2-phenylglycinol (**3b**) as the chiral auxiliary.

Following a reference procedure[9] with modifications, L-*cis*-4,5-methanoprolineamide methanesulfonic acid salt **15a** and D-*cis*-4,5-methanoprolineamide methanesulfonic acid salt **15b** were prepared (Scheme 2). Thus, Boc-protection of L-pyroglutamic acid ethyl ester **9a** gave ester **10a**. Reduction of **10a** with $LiEt_3BH$, followed by treatment with trifluoroacetic anhydride (TFAA) gave L-2,3-dihydroproline ester **11a**. Hydrolysis of **11a** with aqueous lithium hydroxide gave acid **12a** as a salt of diisopropylethylamine (DIPEA), which was converted to L-2,3-dihydroproline amide **13a** by treatment with mesyl chloride and ammonia. Stereoselective Simmons-Smith cyclopropanation of **13a** afforded *N*-Boc-L-*cis*-4,5-methanoprolineamide **14a** (ee >99%, determined by chiral HPLC. De >99%, determined by HPLC.). Deprotection of **14a** with methylsulfonic acid (MSA) gave **15a** in the form of methylsulfonic acid salt. Following the similar procedure, **15b** was prepared with using D-pyroglutamic acid ethyl

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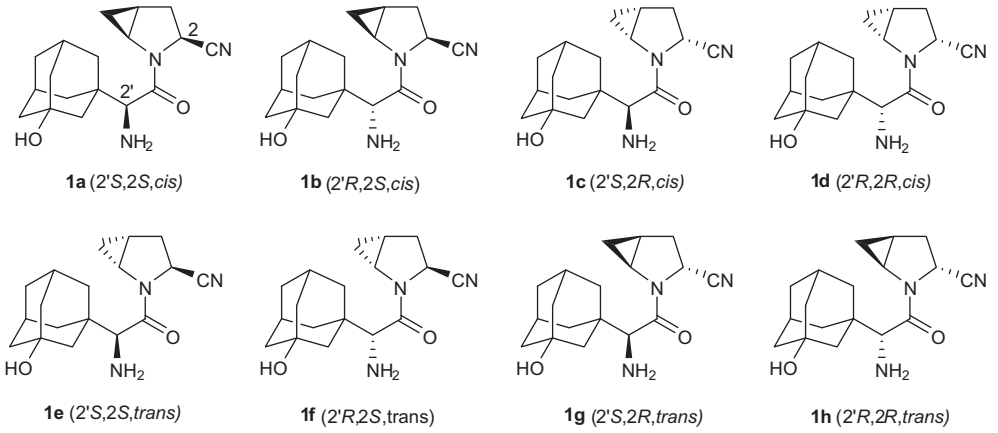


Figure 1. Structures of eight stereoisomers of saxagliptin (**1a**).

ester **9b** as the starting material. The high diastereoselectivity observed in the above Simmons-Smith reaction of amides **13a** and **13b** was interesting. Even with increasing the reaction temperature or changing the charging sequence, only *cis*-cyclopropanation products were obtained. Obviously, the amide function played a key role in achieving highly stereoselective *cis*-cyclopropanation through its strong complexation with the zinc ion of the cyclopropanation reagent.

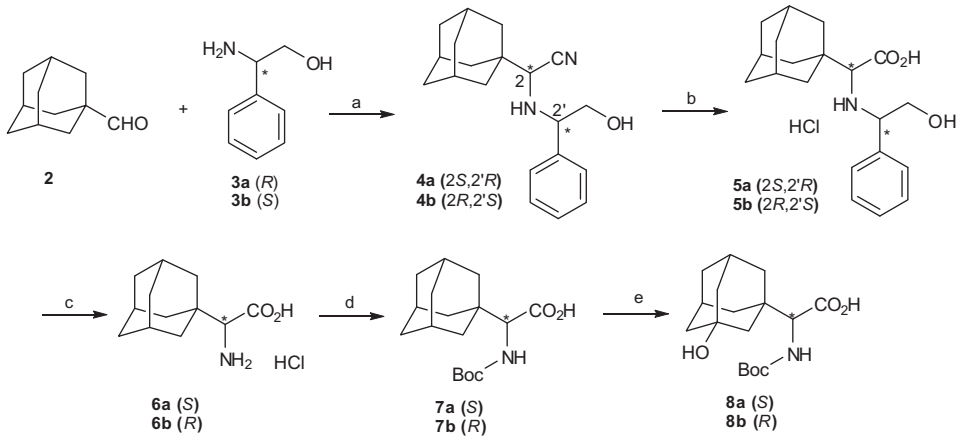
To prepare prolineamides **15c** and **15d** with *trans*-4,5-methano group, L-2,3-dihydroproline ester **11a** and D-2,3-dihydroproline ester **11b** were subjected to Simmons-Smith cyclopropanation, respectively (Scheme 3).[10] Thus, Simmons–Smith reaction of **11a** afforded *cis*-isomer **16a** together with *trans*-isomer **16b** (*cis*/*trans* ≈ 1:1), which could be separated by column chromatography. Hydrolysis of **16b**, followed by amidation and Boc-deprotection gave L-*trans*-4,5-methanoprolineamide **15c** in the form of methylsulfonic acid salt. In the similar manner, D-*trans*-4,5-methanoprolineamide **15d** was prepared starting with **11b**.

With all the building blocks in hand, eight stereoisomers of saxagliptin (**1a**–**1h**) were synthesized (Scheme 4). In brief, caboxylic acids **8a** and **8b** were coupled with amines **15a**–**15d** under the commonly used peptide coupling conditions to yield the dipeptide analogues **18a**–**18h**, respectively. Treatment of **18a**–**18h** with TFAA, followed by Boc-deprotection afforded **1a**–**1h** (ee >99%, determined by chiral HPLC).

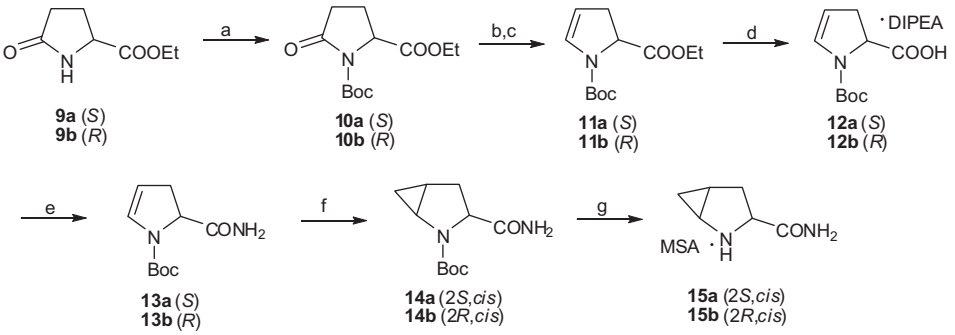
2.2. DPP-IV inhibitory activity and docking study

All the target compounds **1a**–**1h** were tested in vitro against the purified human DPP-IV.[11] The assay result (Table 1) showed that except **1a** (saxagliptin, IC_{50} = 30.3 nM), only **1e** exhibited certain inhibitory activity (IC_{50} = 4364 nM), and the others had no activity.

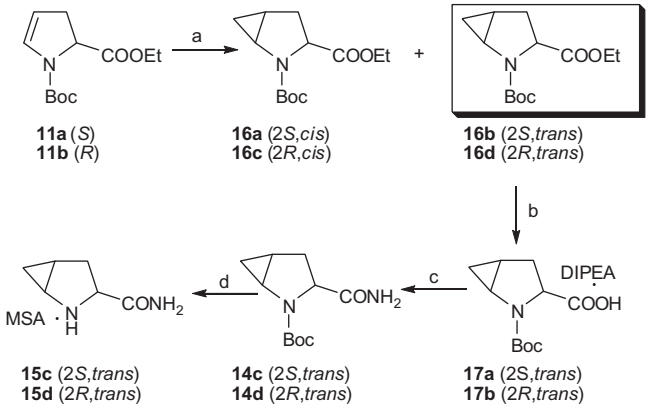
To elucidate why the configurations of **1a**–**1h** had such a profound effect on the potency, molecular docking of **1a**–**1h** into the DPP-IV binding site was performed based on the DPP-IV co-crystal structure (PDB code: 3BJM)[5] by the genetic algorithm of GOLD 3.0.1. The docking pose of **1a** almost overlapped with the crystal structure. The computational interaction mode (Fig. 2b) of **1a** was rather identical to that found in the DPP-IV co-crystal structure (Fig. 2a). The (2′*S*)-NH_2 of **1a** formed good H-bonding interactions with Glu205, Glu206 and Tyr662, and the carbonyl oxygen of **1a** did so with Arg125 and Asn710. On the other side, the adamantanol hydroxyl group of **1a** made H-bonding interactions with Tyr547 and H_2O806. With these interactions, the nitrile group of **1a** was rendered very close to Ser630. The distances between the nitrile carbon/nitrogen of **1a** and the Ser630 hydroxyl oxygen were so small (<2.5 Å) that the Ser630 hydroxyl group could readily share its electrons and proton with the electron-withdrawing nitrile group of **1a**, leading to a reversible carboximidate covalent bond between **1a** and DPP-IV. The newly generated imine structure could be stabilized by H-bonding interaction with Tyr547. Thus, the pose of **1a** was tightly clamped in the pocket.



Scheme 1. Reagents and conditions: (a) NaCN, $NaHSO_3$, 0–60 °C, 60% yield for **4a**, 61% yield for **4b**; (b) concd HCl, HOAc, 80 °C, 71% yield for **5a**, 77% yield for **5b**; (c) H_2, 10% Pd/C, HOAc, 90% yield for **6a**, 77% yield for **6b**; (d) $(Boc)_2O$, TEA, methanol, 80% yield for **7a**, 61% yield for **7b**; (e) $KMnO_4$, 2% KOH, 90 °C, 49% yield for **8a**, 42% yield for **8b**.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$, DMAP, 83% yield for **10a**, 80% yield for **10b**; (b) $LiEt_3BH$, toluene, −65 °C to −70 °C; (c) DIPEA, DMAP, TFAA; (d) LiOH, EtOH–H_2O, DIPEA; (e) MsCl, DIPEA, THF, NH_3, 3 steps 30% yield for **13a** and **13b**; (f) CH_2I_2, Et_2Zn, DME, DCM, −30 °C to 20 °C, 45% yield for **14a**, 44% yield for **14b**; (g) MSA, IPA, 60 °C, 80% yield for **15a**, 88% yield for **15b**.



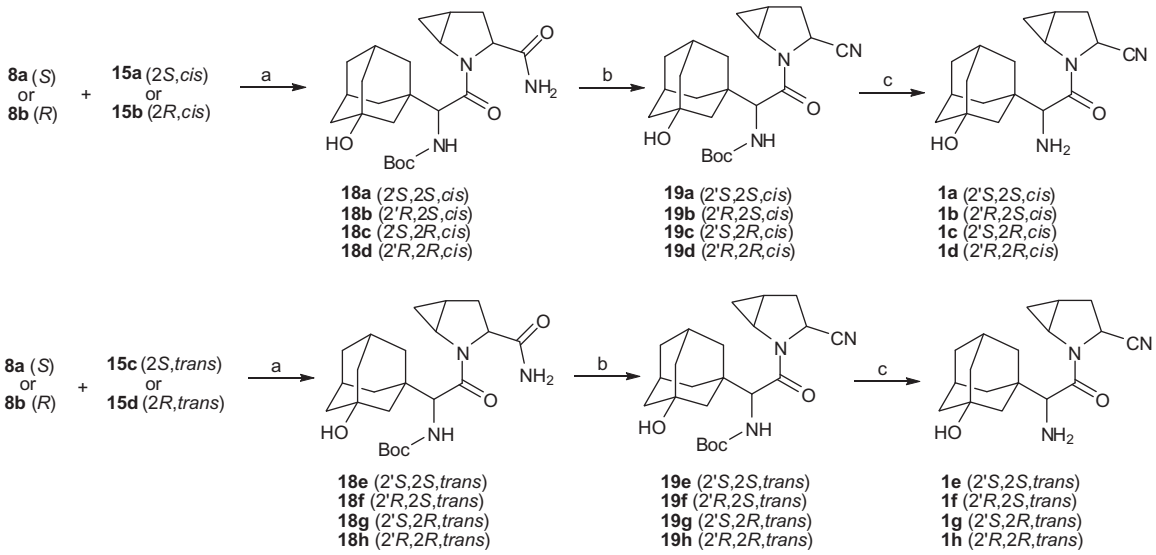
Scheme 3. Reagents and conditions: (a) CH_2I_2, Et_2Zn, DME, DCM, −30 °C to 20 °C, 11% yield for **16a** and 10% yield for **16b**, 14% yield for **16c** and 16% yield for **16d**; (b) LiOH, EtOH–H_2O, DIPEA, 100% yield for **17a** and **17b**; (c) MsCl, DIPEA, THF, NH_3, 42% yield for **14c**, 58% yield for **14d**; (d) MSA, IPA, 60 °C, 85% yield for **15c**, 83% yield for **15d**.

However, in the case of **1e**, the summit carbon of the *trans*-cyclopropyl group pointed to Tyr662. The pyrrolidine ring was forced to distort to avoid clash between the summit carbon of the *trans*-cyclopropyl group and Tyr662, causing weakened hydrophobic interactions with Tyr662. Besides, **1e** probably took a pose with its adamantanol hydroxyl group far away from Tyr547 (Fig. 3a), leading to the loss of H-bonding interaction between the adamantanol hydroxyl group and Tyr547. Thus, **1e** easily deviated from the best position required for covalently binding to Ser630 with the nitrile nitrogen of **1e** being 3.2 Å away from the Ser630 hydroxyl oxygen, ultimately resulting in reduced potency. As for isomers **1b**–**1d** and **1f**–**1h**, they even could not form effective H-bonding interactions with Glu205, Glu206 and Arg125 (Fig. 3b), thus leading to a complete loss of activity. Together, Tyr662 and

Table 1
DPP-IV inhibitory activity of **1a**–**1h**

Compound	IC_{50} (nM)
1a	30.3
1b	NA[a]
1c	NA
1d	NA
1e	4364
1f	NA
1g	NA
1h	NA

[a] NA means no activity at the concentration between 1 and 10,000 nM.



Scheme 4. Reagents and conditions: (a) EDC·HCl, HOBt, DIPEA, acetonitrile, ethyl acetate, 46–93% yield for **18a**–**18h**; (b) TFAA, pyridine, K_2CO_3, MeOH, 54–97% yield; (c) concd HCl, IPA, 60 °C, 75–91% yield.

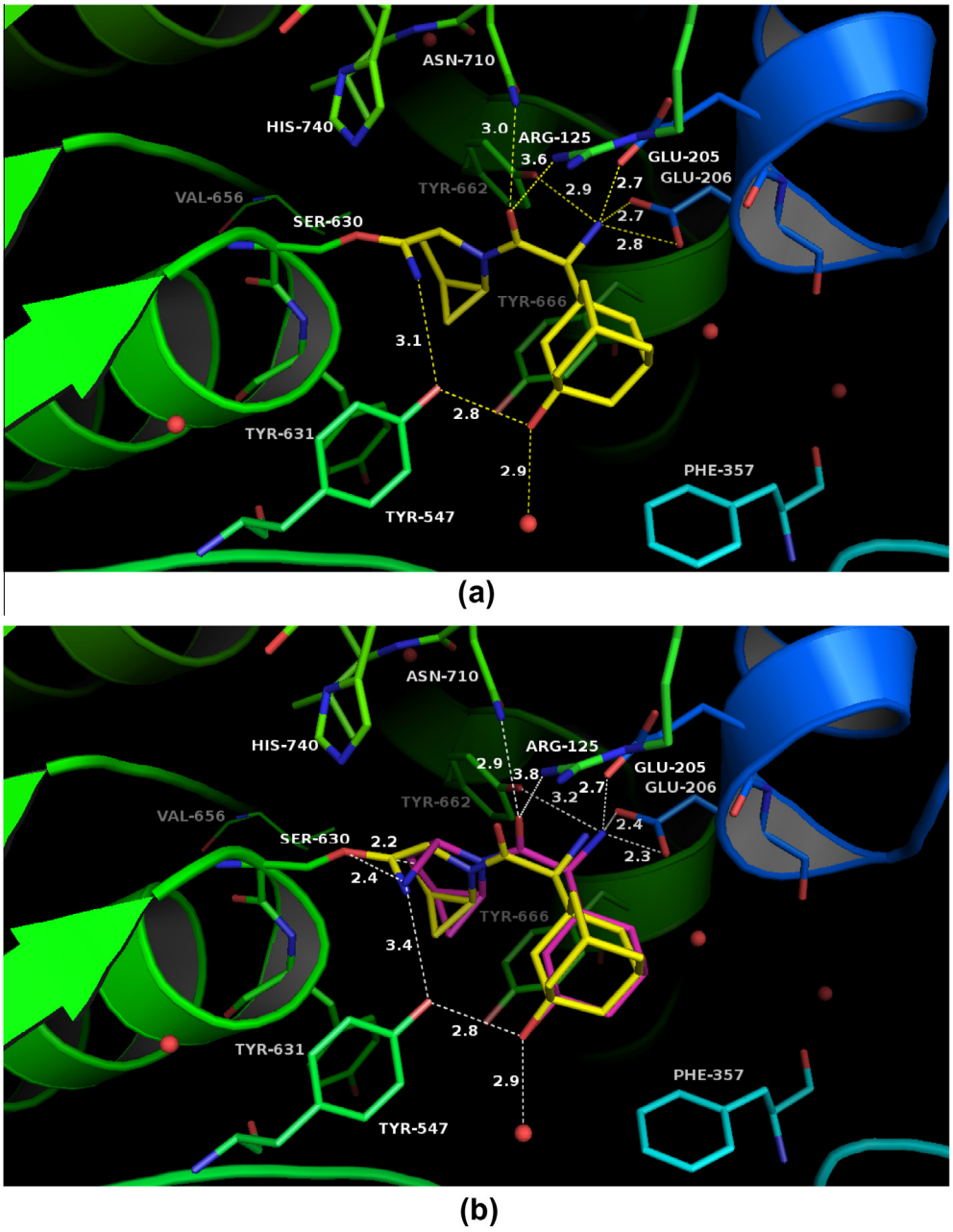


Figure 2. The proposed interaction modes of **1a**: (a) in the co-crystal structure (PDB code: 3BJM) (yellow); (b) computed by docking (purple).

Tyr547 very likely acted as the key residues interacting with the inhibitors, and stereoselectively distinguished **1a** from the other stereoisomers in DPP-IV inhibition.

3. Conclusions

In summary, all eight stereoisomers of saxagliptin have been synthesized and evaluated as DPP-IV inhibitors. As expected, saxagliptin (**1a**) exhibited strong inhibitory activity against DPP-IV. For the other stereoisomers, only **1e** with (2′*S*,2*S*, *trans*)-configuration showed certain potency while **1b**–**1d** and **1f**–**1h** had no activity, implying that the configuration of saxagliptin is critical to its potency. Docking study was performed to elucidate the configuration–activity relationships. The docking results indicate that Tyr662 and Tyr547 of DPP-IV might act as the key residues that strictly and stereoselectively interact with the inhibitors.

4. Experimental

4.1. Chemistry

All commercially available solvents and reagents were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded at 300/500 and 75/125 MHz respectively. Chemical shifts were reported as values from an internal tetramethylsilane standard. Low-resolution and high-resolution mass spectra (LRMS and HRMS) were given with electron impact mode. The mass analyzer type used for the HRMS measurements was TOF. Reactions were monitored by TLC on silica gel 60 F254 plates (Qingdao Ocean Chemical Company, China). Column chromatography was carried out on silica gel (200–300 mesh, Qingdao Ocean Chemical Company, China). Optical rotation datas were recorded on Jasco p-1020 Polarimeter. The optical purity of the key compounds were

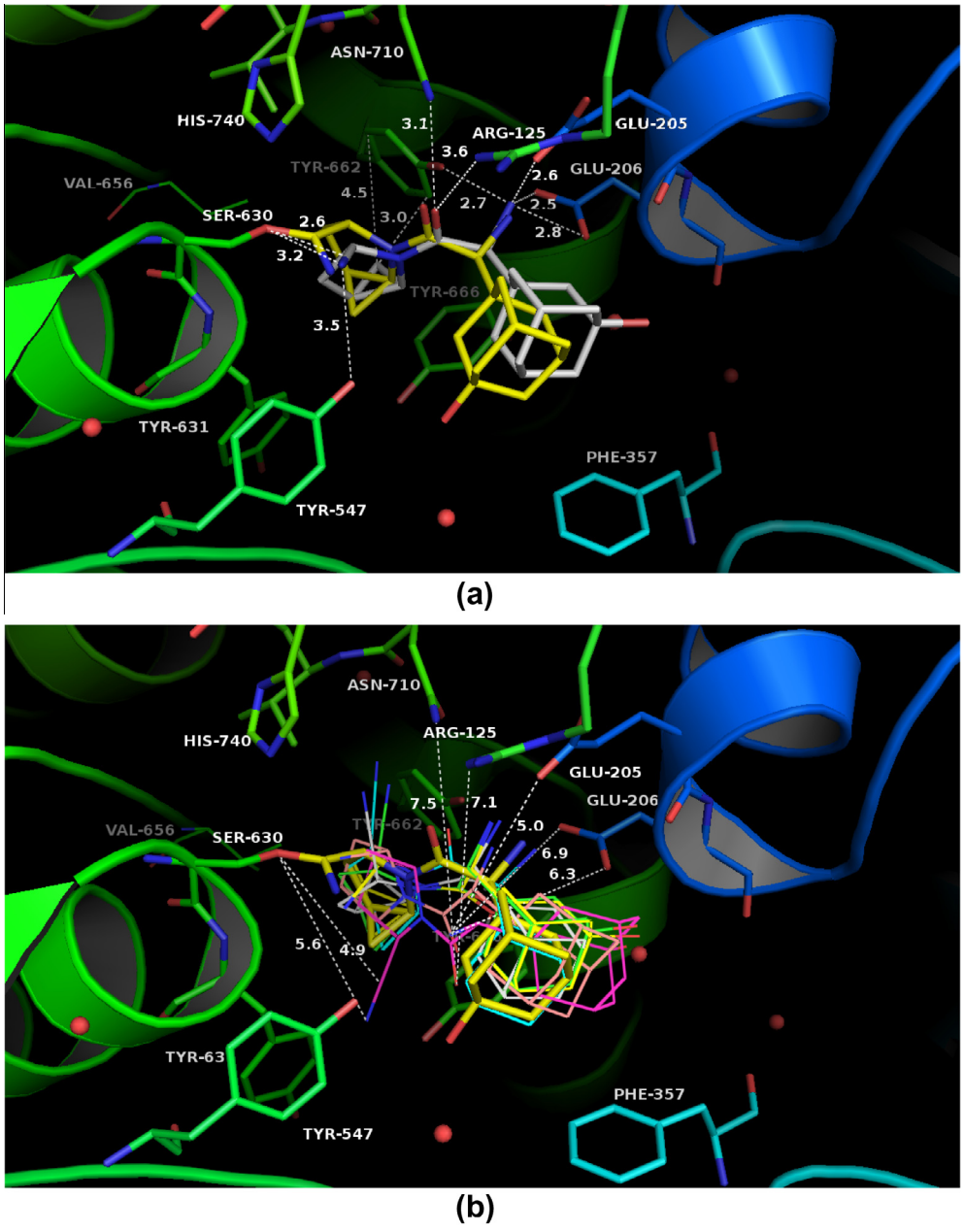


Figure 3. The computational interaction modes of **1e** (a, grey), **1b**–**1d** and **1f**–**1h** (b).

determined by analytical HPLC (Equipment: Agilent 1100 system with a VWD G1314A UV detector; Column: Chiralpak IC 4.6 mm × 250 mm).

4.1.1. 2-(*S*)-Adamantan-1-yl-(*R*)-(2-hydroxy-1-phenylethylamino)acetonitrile (4a)

Following a procedure described in the literature,[1] **4a** was prepared starting with **2** (45.0 g, 274 mmol) and (*R*)-2-phenylglycinol (37.5 g, 274 mmol): white crystalline solids, 50.0 g, 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56–1.77 (m, 13H), 1.95–2.12 (m, 3H), 2.87 (s, 1H), 3.47–3.57 (m, 1H), 3.79 (dd, J_1 = 11.0 Hz, J_2 = 3.5 Hz, 1H), 4.04–4.08 (m, 1H), 7.25–7.36 (m, 5H).

4.1.2. 2-(*R*)-Adamantan-1-yl-(*S*)-(2-hydroxy-1-phenylethylamino)acetonitrile (4b)

Following a procedure described in the literature,[1] **4b** was prepared starting with **2** (50.0 g, 305 mmol) and (*S*)-2-phenylglycinol (41.7 g, 305 mmol): white crystalline solids, 51.0 g, 61% yield, mp 118–120 °C. $[\alpha]_D^{25}$ 136.0 (*c* 0.25, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.56–1.78 (m, 13H), 1.95–2.05 (m, 3H), 2.87 (s, 1H), 3.49–3.64 (m, 1H), 3.79 (dd, J_1 = 11.0 Hz, J_2 = 3.5 Hz, 1H), 4.04–4.09 (m, 1H), 7.26–7.36 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.1, 35.8, 36.6, 38.7, 59.1, 63.6, 67.2, 118.6, 127.9, 128.4, 128.8, 137.9. ESI-MS *m*/*z* 333.2 $[M+Na]^+$. HRMS for $C_{20}H_{26}N_2ONa$ calcd 333.1943, found 333.1946 $[M+Na]^+$.

4.1.3. 2-(*S*)-Adamantan-1-yl-(*R*)-(2-hydroxy-1-phenylethylamino)acetic acid HCl salt (5a)

Following a procedure described in the literature,[1] **5a** was prepared starting with **4a** (16.0 g, 51.6 mmol): white solids, 13.5 g, 71% yield, mp 251–253 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 1.46–1.76 (m, 12H), 1.98 (br s, 3H), 3.00 (s, 1H), 3.90–3.92 (m, 1H), 4.00–4.10 (m, 2H), 7.48 (br s, 5H). ESI-MS *m*/*z* 330 $[M+H]^+$.

4.1.4. 2-(*R*)-Adamantan-1-yl-(*S*)-(2-hydroxy-1-phenylethylamino)acetic acid HCl salt (5b)

Following a procedure described in the literature,[1] **5b** was prepared starting with **4b** (16.0 g, 51.6 mmol): white solids, 33.7 g, 77% yield, mp 228–230 °C. $[\alpha]_D^{25}$ 100.9 (*c* 0.5, C_5H_5N). ^1H NMR (300 MHz, C_5H_5N): δ 1.58–2.15 (m, 15H), 3.14 (s, 1H), 4.07–4.15 (m, 2H), 4.31–4.35 (m, 1H), 7.28–7.73 (m, 5H). ^{13}C NMR (75 MHz, C_5H_5N): δ 28.8, 35.8, 37.1, 39.4, 65.2, 67.7, 69.5, 128.0, 128.8, 128.9, 141.1, 175.9. ESI-MS *m*/*z* 328.2 $[M-H]^-$. HRMS for $C_{20}H_{26}NO_3$ calcd 328.1913, found 333.1916 $[M-H]^-$.

4.1.5. (*S*)-Adamantylglycine HCl salt (6a)

Following a procedure described in the literature,[1] **6a** was prepared starting with **5a** (2.0 g, 5.5 mmol): white solids, 1.2 g, 90% yield. $[\alpha]_D^{25}$ 26.5 (*c* 0.25, CH_3OH). ^1H NMR (300 MHz, CD_3OD): δ 1.58–2.01 (m, 13H), 2.63 (s, 3H), 2.95 (s, 1H). ESI-MS *m*/*z* 210 $[M+H]^+$.

4.1.6. (*R*)-Adamantylglycine HCl salt (6b)

Following a procedure described in the literature,[1] **6b** was prepared starting with **5b** (680 mg, 1.86 mmol): white solids, 350 mg, 77% yield. $[\alpha]_D^{25}$ −28.0 (*c* 0.25, CH_3OH). ^1H NMR (300 MHz, CD_3OD): δ 1.73–2.04 (m, 13H), 2.67 (s, 3H), 3.13 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 29.7, 35.1, 37.6, 39.6, 39.8, 66.2, 172.6. ESI-MS *m*/*z* 208.1 $[M-H]^-$. HRMS for $C_{12}H_{18}NO_2$ calcd 208.1338, found 208.1341 $[M-H]^-$.

4.1.7. (*S*)-*N*-Boc-adamantylglycine (7a)

Following a procedure described in the literature,[1] **7a** was prepared starting with **6a** (5.9 g, 28.5 mmol): white solids, 7.0 g, 80% yield. ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.55–1.73 (m, 12H), 2.00 (br s, 3H), 3.98, 5.12 (2d, 1H, rotamers).

4.1.8. (*R*)-*N*-Boc-adamantylglycine (7b)

Following a procedure described in the literature,[1] **7b** was prepared starting with **6b** (150 mg, 0.72 mmol): white solids, 140 mg, 61% yield. $[\alpha]_D^{25}$ −25.6 (*c* 0.25, CH_3OH). mp 176–178 °C. ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.55–1.73 (m, 12H), 2.00 (br s, 3H), 3.98, 5.12 (2d, 1H, rotamers). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.3, 28.4, 36.6, 38.6, 62.4, 71.0, 77.1, 157.1, 175.9. ESI-MS *m*/*z* 308.2 $[M-H]^-$. HRMS for $C_{17}H_{26}NO_4$ calcd 308.1862, found 308.1866 $[M-H]^-$.

4.1.9. (*S*)-*N*-Boc-3-hydroxyadamantylglycine (8a)

Following a procedure described in the literature,[1] **8a** was prepared starting with **7a** (2.5 g, 8.1 mmol): white foam, 1.3 g, 49% yield, ee >99%. $[\alpha]_D^{25}$ 24.04 (*c* 0.25, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H), 1.55–1.68 (m, 12H), 2.25 (br s, 2H), 4.06 and 5.15 (2d, 1H, rotamers). ESI-MS *m*/*z* 324 $[M+H]^+$.

4.1.10. (*R*)-*N*-Boc-3-hydroxyadamantylglycine (8b)

Following a procedure described in the literature,[1] **8b** was prepared starting with **7b** (50 mg, 0.16 mmol): white foam, 22 mg, 42% yield, ee >99%. $[\alpha]_D^{25}$ −24.0 (*c* 0.25, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.44 (s, 9H), 1.40–1.68 (m, 12H), 2.25 (br s, 2H), 4.09 (br d, 1H), 5.19 (br d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 28.3, 30.2, 35.1, 36.8, 37.8, 39.8, 44.1, 45.4, 45.9, 61.5, 69.4, 155.7, 174.6. ESI-MS *m*/*z* 324.2 $[M-H]^-$. HRMS for $C_{17}H_{26}NO_5$ calcd 324.1811, found 324.1814 $[M-H]^-$.

4.1.11. *N*-Boc-L-pyroglutamic acid ethyl ester (10a)

Following a procedure described in the literature,[9] **10a** was prepared starting with **9a** (1 kg): white solids, 1.4 kg, 83% yield. $[\alpha]_D^{25}$ −37.7 (*c* 0.63, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 1.25 (t, *J* = 5.0 Hz, 3H), 1.45 (s, 9H), 1.94–2.04 (m, 1H), 2.20–2.42 (m, 1H), 2.43–2.54 (m, 1H), 2.55–2.65 (m, 1H), 4.25 (q, *J* = 7.2 Hz, 1H), 4.57–4.58 (m, 1H).

4.1.12. *N*-Boc-D-pyroglutamic acid ethyl ester (10b)

Following a procedure described in the literature,[9] **10b** was prepared starting with **9b** (1 kg): white solids, 1.3 kg, 80% yield. $[\alpha]_D^{25}$ 32.5 (*c* 0.78, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$): δ 1.29 (t, *J* = 5.0 Hz, 3H), 1.50 (s, 9H), 2.00–2.31 (m, 1H), 2.31–2.36 (m, 1H), 2.46–2.53 (m, 1H), 2.59–2.66 (m, 1H), 4.22–4.26 (q, *J* = 5.0 Hz, 2H), 4.59–4.61 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1, 171.2, 149.1, 83.3, 61.4, 58.8, 31.0, 27.7, 21.4, 14.0. ESI-MS *m*/*z* 280 $[M+Na]^+$. HRMS for $C_{12}H_{19}NO_5Na$ calcd 280.1161, found 280.1163 $[M+Na]^+$.

4.1.13. (*S*)-1-*tert*-Butyl 2-ethyl 2,3-dihydro-1*H*-pyrrole-1,2-dicarboxylate (11a)

Following a procedure described in the literature,[9] **11a** was prepared starting with **10a** (257 g, 1.0 mol): yellow oil, 335 g, crude product. The analytical sample was obtained by flash chromatography. ^1H NMR (300 MHz, $CDCl_3$): δ 1.24–1.30 (m, 3H), 1.45 (s, 9H), 2.58–2.69 (m, 1H), 2.98–3.13 (m, 1H), 4.11–4.30 (m, 2H), 4.52–4.66 (m, 1H), 4.89–4.94 (m, 1H), 6.51–6.64 (m, 1H).

4.1.14. (*R*)-1-*tert*-Butyl 2-ethyl 2,3-dihydro-1*H*-pyrrole-1,2-dicarboxylate (11b)

Following a procedure described in the literature,[9] **11b** was prepared starting with **10b** (250 g, 1.0 mol): yellow oil, 230 g, crude product. The analytical sample was obtained by flash chromatography. $[\alpha]_D^{25}$ 71.4 (*c* 0.21, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$): δ 1.28–1.30 (m, 3H), 1.45 (s, 9H), 2.61–2.69 (m, 1H), 3.01–3.11 (m, 1H), 4.16–4.22 (m, 2H), 4.54–4.58 (m, 1H), 4.90–4.95 (m, 1H), 6.52–6.65 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.8, 151.4, 130.0, 105.0, 81.0, 61.0, 58.3, 57.9, 35.4, 34.2, 28.2, 14.1. ESI-MS *m*/*z* 242 $[M+H]^+$. HRMS for $C_{12}H_{20}NO_4$ calcd 242.1392, found 242.1394 $[M+H]^+$.

4.1.15. (*S*)-1-(*tert*-Butoxycarbonyl)-2,3-dihydro-1*H*-pyrrole-2-carboxylic acid DIPEA salt (12a)

Following a procedure described in the literature,[9] **12a** was prepared starting with **11a** (6.7 g): yellow oil, 8.0 g, crude product, which can be used without purification in next step.

4.1.16. (*R*)-1-(*tert*-Butoxycarbonyl)-2,3-dihydro-1*H*-pyrrole-2-carboxylic acid DIPEA salt (12b)

Following a procedure described in the literature,[9] **12b** was prepared starting with **11b** (350 g): yellow oil, 317 g, crude product, which can be used without purification in next step.

4.1.17. (*S*)-*tert*-Butyl 2-carbamoyl-2,3-dihydro-1*H*-pyrrole-1-carboxylate (13a)

Following a procedure described in the literature,[9] **13a** was prepared starting with **12a** (8.0 g): yellow oil, 1.5 g, 3 steps 34% yield. $[\alpha]_D^{25}$ −105.0 (*c* 0.71, CH_3OH). ^1H NMR (300 MHz, DMSO-d_6): δ 1.36 (s, 9H), 2.44–2.51 (m, 1H), 2.86–3.00 (m, 1H), 4.34–4.40 (m, 1H), 4.95 (br s, 1H), 6.48 (br d, 1H), 6.94 (br d, 1H), 7.36 (br d, 1H). ESI-MS *m*/*z* 235 $[M+Na]^+$.

4.1.18. (*R*)-*tert*-Butyl 2-carbamoyl-2,3-dihydro-1*H*-pyrrole-1-carboxylate (13b)

Following a procedure described in the literature,[9] **13b** was prepared starting with **12b** (300 g): yellow oil, 40 g, 3 steps 30% yield. ESI-MS *m*/*z* 213 $[M+H]^+$. HRMS for $C_{10}H_{17}N_2O_3$ calcd 213.1239, found 213.1241 $[M+H]^+$.

4.1.19. *N*-Boc-L-*cis*-4,5-methanoprolineamide (14a)

Following a procedure described in the literature,[9] **14a** was prepared starting with **13a** (5.0 g): white solids, 2.4 g, 45% yield, ee >99%, de >99%. $[\alpha]_D^{25}$ 35.2 (*c* 0.71, CH_3OH). ^1H NMR (300 MHz, CD_3OD): δ 0.68–0.72 (m, 1H), 0.87–1.00 (m, 1H), 1.48 (s, 9H), 1.52–1.59 (m, 1H), 1.94–1.99 (m, 1H), 2.63–2.70 (m, 1H), 3.43–3.48 (m, 1H), 4.48–4.57 (m, 1H). ESI-MS *m*/*z* 249 $[M+Na]^+$.

4.1.20. *N*-Boc-D-*cis*-4,5-methanoprolineamide (14b)

Following a procedure described in the literature,[9] **14b** was prepared starting with **13b** (10.0 g): white solids, 4.7 g, 44% yield, ee >99%, de >99%. $[\alpha]_D^{25}$ −36.4 (*c* 0.26, CH_3OH). ^1H NMR (500 MHz, CD_3OD): δ 0.69–0.71 (m, 1H), 0.87–1.0 (m, 1H), 1.48 (s, 9H), 1.54–1.58 (m, 1H), 1.96–2.05 (m, 1H), 2.53–2.63 (m, 1H), 3.47–3.52 (m, 1H), 4.49–4.50 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 178.3, 156.4, 81.6, 62.6, 61.7, 38.7, 33.6, 28.6, 15.5, 13.8. ESI-MS *m*/*z* 249 $[M+Na]^+$. HRMS for $C_{11}H_{18}N_2O_3Na$ calcd 249.1215, found 249.1217 $[M+Na]^+$.

4.1.21. L-*cis*-4,5-Methanoprolineamide methanesulfonic acid salt (15a)

Following a procedure described in the literature,[9] **15a** was prepared starting with **14a** (500 mg, 2.21 mmol): off-white solids, 390 mg, 80% yield. $[\alpha]_D^{25}$ −31.0 (*c* 0.71, CH_3OH). ^1H NMR (300 MHz, CD_3OD): δ 4.59 (dd, J_1 = 11.0 Hz, J_2 = 2.9 Hz, 1H), 3.40–3.42 (m, 1H), 2.74–2.82 (m, 1H), 2.72 (s, 3H), 2.28 (dd, J_1 = 13.8 Hz, J_2 = 2.9 Hz, 1H), 1.86–1.90 (m, 1H), 0.93–0.98 (m, 1H), 0.78–0.79 (m, 1H). ^{13}C NMR (300 MHz, CD_3OD): δ 172.66, 61.76, 39.47, 37.94, 32.89, 18.40, 10.22. ESI-MS *m*/*z* 127 $[M+H]^+$.

4.1.22. D-*cis*-4,5-Methanoprolineamide methanesulfonic acid salt (15b)

Following a procedure described in the literature,[9] **15b** was prepared starting with **14b** (4.4 g, 19.3 mmol): off-white solids, 3.8 g, 88% yield. $[\alpha]_D^{25}$ 40.8 (*c* 0.25, CH_3OH). ^1H NMR (500 MHz, DMSO-d_6): δ 7.93 (br s, 1H), 7.65 (br s, 1H), 4.44–4.47 (dd, J_1 = 10.0 Hz, J_2 = 5.0 Hz, 1H), 3.29–3.36 (m, 1H), 2.54–2.59 (m, 1H), 2.38 (s, 3H), 2.09–2.11 (m, 1H), 1.73–1.76 (m, 1H), 0.82–0.86 (m, 1H), 0.60–0.63 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 170.8, 59.5, 36.4, 31.6, 16.5, 9.1. ESI-MS *m*/*z* 127 $[M+H]^+$. HRMS for $C_6H_{11}N_2O$ calcd 127.0871, found 127.0872 $[M+H]^+$.

4.1.23. *N*-Boc-L-*cis*-4,5-methanoproline ethyl ester (16a) and *N*-Boc-L-*trans*-4,5-methanoproline ethyl ester (16b)

To methylene chloride (200 mL) cooled to -30 °C was added dimethoxy ethane (DME) (35 mL), followed by addition of 30% diethyl zinc toluene solution (2 mol/L, 206 mL) and diiodo methane (67 mL), the mixture was added to a solution of **11a** (35 g) dissolved in dry methylene chloride (200 mL) at 20 °C. Following completion of the reaction, saturated bicarbonate solution (200 mL) was added, the mixture was stirred for 1 h while a precipitate formed. The suspension was filtered. The filtrate was concentrated and purified by flash chromatography to afford **16a**: off-white solids, 4.0 g, 11% yield; **16b**: off-white solids, 3.8 g, 10% yield. **16a**: $[\alpha]_D^{25}$ 17.7 (*c* 0.21, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.56–4.60 and 4.46–4.51 (2 dd, 1H, J_1 = 11.7 Hz, J_2 = 3.3 Hz, rotamers), 4.11–4.21 (m, 1H), 3.44–3.56 (m, 1H), 2.52–2.64 (m, 1H), 2.01–2.04 (m, 1H), 1.40–1.48 (m, 1H), 1.42 and 1.51 (2 s, 9H, rotamers), 1.23–1.28 (m, 3H), 0.89–0.91 (m, 1H), 0.73–0.75 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 173.2, 154.3, 154.1, 79.9, 60.9, 59.8, 59.3, 37.2, 37.0, 31.7, 30.6, 28.3, 15.2, 14.1, 12.8, 12.3. ESI-MS *m*/*z* 256 $[M+H]^+$. HRMS for $C_{13}H_{22}NO_4$ calcd 256.1549, found 256.1552 $[M+H]^+$. **16b**: $[\alpha]_D^{25}$ 125.7 (*c* 0.18, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.16–4.20 (m, 1H), 3.99–4.01 (m, 1H), 3.47–3.51 (m, 1H), 2.20–2.36 (m, 2H), 1.55–1.60 (m, 1H), 1.42 (s, 9H), 1.26–1.29 (m, 13H), 0.78–0.82 (m, 1H), 0.45–0.47 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.1, 80.2, 60.9, 60.5, 37.5, 32.9, 29.6, 28.2, 15.5, 14.5, 14.1. ESI-MS *m*/*z* 256 $[M+H]^+$. HRMS for $C_{13}H_{22}NO_4$ calcd 256.1549, found 256.1551 $[M+H]^+$.

4.1.24. *N*-Boc-D-*cis*-4,5-methanoproline ethyl ester (16c) and *N*-Boc-D-*cis*-4,5-methanoproline ethyl ester (16d)

Following a procedure described for preparation of **16a** and **16b**, **16c** and **16d** were prepared starting with **11b** (54.3 g, 256 mmol). **16c**: off-white solids, 8.0 g, 14% yield; **16d**: off-white solids, 8.9 g, 16% yield. **16c**: $[\alpha]_D^{25}$ −11.0 (*c* 0.57, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$): δ 4.56–4.60 and 4.48–4.60 (2 dd, 1H, J_1 = 11.5 Hz, J_2 = 2.8 Hz, rotamers), 4.16–4.20 (m, 1H), 3.44–3.55 (m, 1H), 2.55–2.65 (m, 1H), 2.00–2.04 (m, 1H), 1.40–1.48 (m, 1H), 1.41 and 1.48 (2 s, 9H, rotamers), 1.23–1.28 (m, 3H), 0.89–0.92 (m, 1H), 0.66–0.68 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.4, 173.1, 154.0, 79.8, 60.8, 59.7, 59.2, 37.1, 36.9, 31.6, 30.5, 28.3, 15.0, 14.2, 12.7, 12.2. ESI-MS *m*/*z* 256 $[M+H]^+$. HRMS for $C_{13}H_{22}NO_4$ calcd 256.1549, found 256.1551. **16d**: $[\alpha]_D^{25}$ −146.0 (*c* 0.21, CH_3OH). ^1H NMR (500 MHz, $CDCl_3$): δ 4.16–4.20 (m, 1H), 3.99–4.01 (m, 1H), 3.46–3.51 (m, 1H), 2.32–2.36 (m, 1H), 2.20–2.22 (m, 1H), 1.49–1.60 (m, 1H), 1.44 (s, 9H), 1.26–1.44 (m, 13H), 0.80–0.82 (m, 1H), 0.45–0.47 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 80.1, 60.8, 60.4, 37.4, 32.9, 28.1 15.5, 14.4, 14.0. ESI-MS *m*/*z* 256 $[M+H]^+$. HRMS for $C_{13}H_{22}NO_4$ calcd 256.1549, found 256.1552 $[M+H]^+$.

4.1.25. *N*-Boc-L-*trans*-4,5-methanoproline DIPEA salt (17a)

A solution of **16b** (3.56 g) in ethanol (14.0 mL) was treated with a solution of lithium hydroxide hydrate (1.17 g) in water (12.5 mL). The reaction mixture was stirred for 2 h, and then diluted with ethyl acetate with stirring. The organic and aqueous phases were separated. The aqueous phase was collected and diluted with ethyl acetate. This mixture was adjusted to pH 2–3 with 2 N HCl. The organic and aqueous layers were separated. The organic layer was stored and charged with diisopropylethylamine (DIPEA) (3.65 mL, 1.5 equiv). The solution was then evaporated under vacuum to afford **17a**: yellow oil, 5.03 g. $[\alpha]_D^{25}$ −105.4 (*c* 0.16, CH_3OH). ESI-MS *m*/*z* 226 $[M-H]^-$. HRMS for $C_{11}H_{16}NO_4$ calcd 226.1079, found 226.1081 $[M-H]^-$.

4.1.26. *N*-Boc-D-*trans*-4,5-methanoproline DIPEA salt (17b)

Following a procedure described for preparation of **17a**, **17b** was prepared starting with **16d** (8.2 g): yellow oil, 10.5 g. $[\alpha]_D^{25}$ 99.3 (*c* 0.43, CH_3OH). ESI-MS *m*/*z* 226 $[M-H]^-$. HRMS for $C_{11}H_{16}NO_4$ calcd 226.1079, found 226.1082 $[M-H]^-$.

4.1.27. *N*-Boc-L-*trans*-4,5-methanoprolineamide (14c)

To the solution of **17a** (4.88 g, 13.7 mmol) dissolved in dry THF (40 mL), DIPEA (2.39 mL, 1 equiv) and mesyl chloride (1.57 mL, 1.5 equiv) was added slowly. The resulting suspension was stirred for 1 h, followed by saturation with ammonia. After saturation, the reaction mixture was stirred for 1 h. Following stirring, the reaction mixture was filtered to remove the solid. The filtrate was concentrated under vacuum and purified by flash chromatography to give pure product **14c**: white solids, 1.30 g, 42% yield, ee >99%, *de* 96.6%. $[\alpha]_D^{25}$ −164.3 (*c* 0.47, CH_3OH). ^1H NMR (500 MHz, CD_3OD): δ 3.93 (br s, 1H), 3.43 (br s, 1H), 2.37–2.41 (m, 1H), 2.18–2.22 (m, 1H), 1.62–1.65 (m, 1H), 1.45 (s, 9H), 0.77–0.81 (m, 1H), 0.42–0.45 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 177.7, 157.5, 81.9, 62.8, 38.7, 34.4, 28.6, 15.6. ESI-MS *m*/*z* 227 $[M+H]^+$. HRMS for $C_{11}H_{19}N_2O_3$ calcd 227.1396, found 227.1398 $[M+H]^+$.

4.1.28. *N*-Boc-D-*trans*-4,5-methanoprolineamide (14d)

Following a procedure described for preparation of **14c**, **14d** was prepared starting with **17b** (10.5 g, 29.5 mmol): white solids, 3.8 g, 58% yield, ee >99%, *de* 98.7%. $[\alpha]_D^{25}$ 169.1 (*c* 0.65, CH_3OH). ^1H

NMR (500 MHz, CD_3OD): δ 3.93 (br s, 1H), 3.43 (br s, 1H), 2.37–2.41 (m, 1H), 2.17–2.22 (m, 1H), 1.62–1.65 (m, 1H), 1.45 (s, 9H), 0.78–0.81 (m, 1H), 0.43–0.45 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 177.7, 157.7, 81.9, 62.9, 38.7, 34.3, 28.6, 15.6. ESI-MS *m*/*z* 227 $[M+H]^+$. HRMS for $C_{11}H_{19}N_2O_3$ calcd 227.1396, found 227.1399 $[M+H]^+$.

4.1.29. L-*trans*-4,5-Methanoprolineamide methanesulfonic acid salt (15c)

The slurry of **14c** (1.2 g, 5.3 mmol) and isopropyl alcohol (8.0 mL) was heated to 60 °C, then methanesulfonic acid (0.44 mL, 6.9 mmol) was added. The product began to crystallize out of solution with the reaction proceeding. The reaction was held at 60 °C for 3 h. The precipitate was collected by filtration, washed with isopropanol and dried to give the pure product of **15c**: off-white solids, 1.0 g, 85% yield. $[\alpha]_D^{25}$ −42.2 (*c* 0.5, CH_3OH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (br s, 1H), 7.61 (br s, 1H), 3.98–4.02 (m, 1H), 3.25–3.27 (m, 1H), 2.46–2.52 (m, 1H), 2.33 (s, 3H), 1.90–1.92 (m, 1H), 1.75–1.77 (m, 1H), 0.89–0.92 (m, 1H), 0.74–0.78 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.6, 55.9, 34.4, 30.1, 14.9, 5.6. ESI-MS *m*/*z* 127 $[M+H]^+$. HRMS for $C_6H_{11}N_2O$ calcd 127.0871, found 127.0872 $[M+H]^+$.

4.1.30. D-*trans*-4,5-Methanoprolineamide methanesulfonic acid salt (15d)

Following a procedure described for preparation of **15c**, **15d** was prepared starting with **14d** (3.0 g, 13.3 mmol): off-white solids, 2.5 g, 83% yield. $[\alpha]_D^{25}$ 43.2 (*c* 0.5, CH_3OH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.87 (br s, 1H), 7.61 (br s, 1H), 3.99–4.03 (m, 1H), 3.25–3.28 (m, 1H), 2.48–2.49 (m, 1H), 2.40 (s, 3H), 1.90–1.95 (m, 1H), 1.75–1.79 (m, 1H), 0.89–0.92 (m, 1H), 0.74–0.78 (m, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 168.6, 55.9, 34.3, 30.1, 14.9, 5.6. ESI-MS *m*/*z* 127 $[M+H]^+$. HRMS for $C_6H_{11}N_2O$ calcd 127.0871, found 127.0873 $[M+H]^+$.

4.2. General procedure for the preparation of 18a–h

To the mixture of **8a**–**b** (1.0 equiv), **15a**–**d** (1.0 equiv), HOBt (1.0 equiv) and EDC·HCl (1.0 equiv) was slowly added a homogeneous solution of DIPEA (3.0 equiv) in acetonitrile and ethyl acetate. The resulting solution was stirred for 3 h. Followed by addition of ethyl acetate and water with stirring. The seperated organic layer was washed with 1 N HCl, 1 N NaOH and saturated brine, dried over Na_2SO_4, purified by flash chromatography.

4.2.1. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinamide (18a)

Compound **18a** was prepared starting with **8a** (454 mg, 1.40 mmol) and **15a** (310 mg, 1.40 mmol): white solids, 260 mg, 46% yield. $[\alpha]_D^{25}$ 23.9 (*c* 0.71, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80–0.85 (m, 1H), 0.96–0.98 (m, 1H), 1.41 (s, 9H), 1.50–1.67 (m, 13H), 2.18 (br s, 1H), 2.31–2.40 (m, 2H), 3.65–3.67 (br m, 1H), 4.50 (d, *J* = 9.9 Hz, 1H), 4.82–4.86 (m, 1H), 5.47 (d, *J* = 9.9 Hz, 1H), 6.05 (br s, 1H), 6.94 (br s, 1H). ESI-MS *m*/*z* 434 $[M+H]^+$.

4.2.2. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinamide (18b)

Compound **18b** was prepared starting with **8b** (0.50 g, 1.54 mmol) and **15a** (0.36 g, 1.61 mmol): white solids, 0.50 g, 71% yield. $[\alpha]_D^{25}$ −6.8 (*c* 0.38, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83–0.85 (m, 1H), 0.98–0.99 (m, 1H), 1.43 (s, 9H), 1.44–1.76 (m, 13H), 2.25 (br s, 2H), 2.32–2.37 (m, 1H), 2.47–2.50 (m, 1H), 3.61–3.63 (m, 1H), 4.42 (d, *J* = 10.0 Hz, 1H), 4.80–4.83 (m, 1H), 5.36 (d, *J* = 10.0 Hz, 1H), 5.40 (br s, 1H), 6.97 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.3, 170.2, 156.3, 80.1, 68.5, 59.6, 46.2, 44.5, 40.2, 35.2, 30.3, 28.3, 16.2, 11.9. ESI-MS *m*/*z* 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2660 $[M+H]^+$.

4.2.3. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinamide (18c)

Compound **18c** was prepared starting with **8a** (1.39 g, 4.29 mmol) and **15b** (1.00 g, 4.50 mmol): white solids, 1.73 g, 93% yield. $[\alpha]_D^{25}$ 8.6 (*c* 0.25, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.82–0.85 (m, 1H), 1.00–1.01 (m, 1H), 1.43 (s, 9H), 1.44–1.71 (m, 13H), 2.24 (br s, 2H), 2.29–2.45 (m, 2H), 3.61–3.63 (m, 1H), 4.42 (d, *J* = 10.0 Hz, 1H), 4.79–4.82 (m, 1H), 5.46 (d, *J* = 10.0 Hz, 1H), 5.60 (br s, 1H), 6.98 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.4, 170.2, 156.3, 80.0, 68.4, 60.3, 59.8, 59.6, 46.0, 44.4, 44.31, 40.1, 35.2, 30.2, 30.1, 28.3, 16.2, 12.0. ESI-MS *m*/*z* 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2659 $[M+H]^+$.

4.2.4. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinamide (18d)

Compound **18d** was prepared starting with **8b** (0.50 g, 1.54 mmol) and **15b** (0.36 g, 1.61 mmol): white solids, 0.50 g, 71% yield. $[\alpha]_D^{25}$ −18.1 (*c* 0.73, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.03–1.07 (m, 1H), 1.19–1.21 (m, 1H), 1.65 (s, 9H), 1.70–1.89 (m, 13H), 2.42 (br s, 2H), 2.49–2.64 (m, 2H), 3.89–3.91 (br m, 1H), 4.73 (d, *J* = 10.0 Hz, 1H), 5.07 (dd, J_1 = 10.0 Hz, J_2 = 2.5 Hz, 1H), 5.68 (d, *J* = 10.0 Hz, 1H), 6.16 (br s, 1H), 7.15 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.9, 170.3, 155.8, 79.8, 68.5, 60.2, 58.8, 45.8, 44.4, 37.9, 37.3, 35.3, 30.2, 28.4, 27.8, 17.4, 13.0. ESI-MS *m*/*z* 434 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 434.2655, found 434.2659 $[M+H]^+$.

4.2.5. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinamide (18e)

Compound **18e** was prepared starting with **8a** (0.50 g, 1.54 mmol) and **15c** (0.36 g, 1.61 mmol): white solids, 0.55 g, 79% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79–0.82 (m, 1H), 1.35–1.37 (m, 1H), 1.73 (s, 9H), 1.79–1.85 (m, 12H), 2.08–2.10 (m, 1H), 2.18–2.20 (m, 1H), 2.44 (br s, 2H), 2.89–2.91 (m, 1H), 3.61–3.63 (m, 1H), 4.80 (dd, J_1 = 10.0 Hz, J_2 = 5.0 Hz, 1H), 4.87 (d, *J* = 10.0 Hz, 1H), 5.49 (d, *J* = 10.0 Hz, 1H), 5.99 (br s, 1H), 6.91 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.9, 171.1, 155.3, 79.8, 68.5, 64.4, 58.8, 45.9, 44.4, 41.0, 35.3, 30.2, 29.3, 28.3, 20.9, 19.2. ESI-MS *m*/*z* 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2660 $[M+H]^+$.

4.2.6. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinamide (18f)

Compound **18f** was prepared starting with **8b** (0.45 g, 1.38 mmol) and **15c** (0.32 g, 1.45 mmol): white solids, 0.36 g, 58% yield. $[\alpha]_D^{25}$ −90.4 (*c* 0.4, CH_3OH). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81–0.84 (m, 1H), 1.33–1.35 (m, 1H), 1.64 (s, 9H), 1.68–2.10 (m, 13H), 2.32–2.37 (m, 1H), 2.45 (br s, 2H), 2.76–2.80 (m, 1H), 4.03–4.05 (m, 1H), 4.57 (d, *J* = 10.0 Hz, 1H), 4.70 (dd, J_1 = 10.0 Hz, J_2 = 2.5 Hz, 1H), 5.52 (d, *J* = 10.0 Hz, 1H), 5.6 (br s, 1H), 7.1 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.5, 171.5, 156.5, 80.0, 68.0, 65.2, 59.5, 46.0, 44.1, 37.3, 34.8, 29.9, 27.9, 21.8, 18.0. ESI-MS *m*/*z* 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2661 $[M+H]^+$.

4.2.7. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinamide (18g)

Compound **18g** was prepared starting with **8a** (0.50 g, 1.54 mmol) and **15d** (0.36 g, 1.61 mmol): white solids, 0.50 g, 71% yield. $[\alpha]_D^{25}$ 86.4 (*c* 0.53, EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81–0.83 (m, 1H), 1.32–1.35 (m, 1H), 1.63 (s, 9H), 1.68–2.10 (m, 13H), 2.32–2.34 (m, 1H), 2.45 (br s, 2H), 2.74–2.77 (m, 1H), 4.03–4.05 (m, 1H), 4.56 (d, *J* = 10.0 Hz, 1H), 4.68 (dd, J_1 = 10.0 Hz,

J_2 = 2.5 Hz, 1H), 5.56 (d, J = 10.0 Hz, 1H), 5.7 (br s, 1H), 7.1 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.8, 171.9, 156.9, 80.3, 68.4, 65.5, 59.9, 46.3, 44.4, 37.6, 35.2, 30.2, 28.3, 22.0, 18.3. ESI-MS m/z 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2660 $[M+H]^+$.

4.2.8. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinamide (18h)

Compound **18h** was prepared starting with **8b** (0.45 g, 1.38 mmol) and **15d** (0.32 g, 1.45 mmol): white solids, 0.35 g, 57% yield. $[\alpha]_D^{25}$ 72.3 (c 0.34, EtOAc). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.59–0.61 (m, 1H), 1.14–1.17 (m, 1H), 1.50 (s, 9H), 1.59–1.65 (m, 12H), 1.88–1.90 (m, 1H), 1.99–2.01 (m, 1H), 2.24 (br s, 2H), 2.72–2.75 (m, 1H), 3.37–3.40 (m, 1H), 4.62–4.67 (m, 1H), 5.2 (d, J = 10.0 Hz, 1H), 5.6 (br s, 1H), 6.7 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.8, 170.2, 155.4, 79.8, 68.6, 64.6, 58.9, 46.0, 44.5, 37.5, 35.3, 30.2, 29.0, 28.3, 21.2, 19.4. ESI-MS m/z 434 $[M+H]^+$. HRMS for $C_{23}H_{36}N_3O_5$ calcd 434.2655, found 434.2659 $[M+H]^+$.

4.3. General procedure for the preparation of 19a–h

To a solution of **18a**–**h** (1.0 equiv), pyridine (5.0 equiv) in dry THF, trifluoroacetic anhydride (2.5 equiv) was added dropwise at 0 °C. After stirred for 0.5 h, TLC plate indicated the conversion was finished. The solvent was removed under vacuum. To the residue were added 10% aqueous K_2CO_3 solution and methol. The mixture was stirred for 18 h at room temperature. After removal of MeOH, the remaining aqueous layer was extracted with EtOAc. The combined organic extract was washed with 1 N HCl, 1 N NaOH and brine, dried over Na_2SO_4, purified by flash chromatography.

4.3.1. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (19a)

Compound **19a** was prepared starting with **18a** (264 mg, 0.64 mmol): white foam, 200 mg, 79% yield. $[\alpha]_D^{25}$ 1.8 (c 0.25, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05–1.09 (m, 2H), 1.44 (s, 9H), 1.44–1.93 (m, 13H), 2.26 (br s, 2H), 2.37 (dd, J_1 = 11.5 Hz, J_2 = 2.0 Hz, 1H), 2.58 (ddd, J_1 = 13.4 Hz, J_2 = 9.3 Hz, J_3 = 5.9 Hz, 1H), 3.82–3.83 (m, 1H), 4.46 (d, J = 9.8 Hz, 1H), 5.04 (dd, J_1 = 10.6 Hz, J_2 = 2.1 Hz, 1H), 5.30 (d, J = 9.8 Hz, 1H). ESI-MS m/z 416 $[M+H]^+$.

4.3.2. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (19b)

Compound **19b** was prepared starting with **18b** (250 mg, 0.60 mmol): white foam, 130 mg, 54% yield. $[\alpha]_D^{25}$ −9.5 (c 0.26, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97–0.99 (m, 1H), 1.13–1.15 (m, 1H), 1.41 (s, 9H), 1.41–1.86 (m, 13H), 2.23 (br s, 2H), 2.38–2.42 (m, 1H), 2.53–2.58 (m, 1H), 3.63–3.66 (m, 1H), 4.50 (d, J = 9.0 Hz, 1H), 4.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 5.29 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 155.2, 119.1, 68.7, 58.9, 46.1, 45.2, 44.5, 40.8, 35.1, 30.7, 30.2, 28.3, 27.3, 16.8, 12.7. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2553 $[M+H]^+$.

4.3.3. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinenitrile (19c)

Compound **19c** was prepared starting with **18c** (1.51 g, 3.5 mmol): white foam, 1.40 g, 97% yield. $[\alpha]_D^{25}$ 16.1 (c 0.54, EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96–0.99 (m, 1H), 1.11–1.13 (m, 1H), 1.41 (s, 9H), 1.41–1.86 (m, 13H), 2.23 (br s, 2H), 2.36–2.38 (m, 1H), 2.53–2.63 (m, 1H), 3.64–3.66 (m, 1H), 4.50 (d, J = 9.0 Hz, 1H), 4.88 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 5.29 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 155.5, 119.1, 68.9, 58.9, 45.8, 45.3, 44.3, 44.2, 40.8, 35.1, 30.2, 30.1, 28.3, 27.3, 16.8, 12.7. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2554 $[M+H]^+$.

4.3.4. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinenitrile (19d)

Compound **19d** was prepared starting with **18d** (340 mg, 0.79 mmol): white foam, 180 mg, 55% yield. $[\alpha]_D^{25}$ −1.0 (c 0.48, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05–1.09 (m, 2H), 1.44 (s, 9H), 1.44–1.69 (m, 13H), 2.26 (br s, 2H), 2.35–2.40 (m, 1H), 2.51–2.55 (m, 1H), 3.84–3.86 (m, 1H), 4.46 (d, J = 9.0 Hz, 1H), 5.04 (dd, J_1 = 10.0 Hz, J_2 = 3.0 Hz, 1H), 5.36 (d, J = 9.0 Hz, 1H): ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.5, 155.5, 119.2, 68.8, 58.7, 46.2, 45.2, 44.3, 41.2, 37.4, 35.1, 30.4, 30.2, 28.3, 17.8, 13.5. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2553 $[M+H]^+$.

4.3.5. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinenitrile (19e)

Compound **19e** was prepared starting with **18e** (430 mg, 1.0 mmol): white foam, 250 mg, 60% yield. $[\alpha]_D^{25}$ −90.3 (c 0.35, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67–0.72 (m, 1H), 1.23–1.25 (m, 1H), 1.43 (s, 9H), 1.43–1.74 (m, 12H), 2.00–2.05 (m, 1H), 2.27 (br s, 2H), 2.54–2.60 (m, 2H), 3.50–3.55 (m, 1H), 4.57 (d, J = 12.0 Hz, 1H), 4.83–4.87 (m, 1H), 5.24 (d, J = 12.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.2, 155.8, 118.2, 68.8, 58.8, 51.1, 46.0, 44.3, 37.3, 37.0, 35.1, 32.7, 30.2, 28.3, 27.3, 21.5, 18.9. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2552 $[M+H]^+$.

4.3.6. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinenitrile (19f)

Compound **19f** was prepared starting with **18f** (300 mg, 0.69 mmol): white foam, 200 mg, 70% yield. $[\alpha]_D^{25}$ −84.2 (c 0.41, CH_3OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.61–0.66 (m, 1H), 1.16–1.23 (m, 1H), 1.46 (s, 9H), 1.41–1.74 (m, 12H), 2.00–2.02 (m, 1H), 2.23 (br s, 2H), 2.31–2.33 (m, 1H), 2.58–2.66 (m, 1H), 3.82–3.84 (m, 1H), 4.47 (d, J = 9.0 Hz, 1H), 4.58 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 5.25 (d, J = 12.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 155.5, 117.8, 79.9, 68.5, 58.9, 51.1, 44.5, 37.3, 37.0, 35.1, 32.9, 30.2, 28.3, 21.6, 18.6. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2554 $[M+H]^+$.

4.3.7. (*S*)-*N*-Boc-3-hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinenitrile (19g)

Compound **19g** was prepared starting with **18g** (430 mg, 1.0 mmol): white foam, 250 mg, 61% yield. $[\alpha]_D^{25}$ 98.5 (c 0.33, EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.64–0.66 (m, 1H), 1.16–1.20 (m, 1H), 1.45 (s, 9H), 1.43–1.69 (m, 12H), 2.00–2.05 (m, 1H), 2.23 (br s, 2H), 2.36–2.38 (m, 1H), 2.55–2.59 (m, 1 H), 3.83–3.85 (m, 1H), 4.48 (d, J = 12.0 Hz, 1H), 4.58 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 5.30 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 155.9, 117.8, 80.3, 68.9, 58.9, 51.1, 44.3, 37.3, 37.0, 35.1, 32.8, 30.2, 28.3, 21.5, 18.6. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2553 $[M+H]^+$.

4.3.8. (*R*)-*N*-Boc-3-hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinenitrile (19h)

Compound **19h** was prepared starting with **18h** (280 mg, 0.69 mmol): white foam, 170 mg, 63% yield. $[\alpha]_D^{25}$ 76.2 (c 0.49, EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67–0.72 (m, 1H), 1.22–1.25 (m, 1H), 1.43 (s, 9H), 1.43–1.69 (m, 12H), 1.99–2.01 (m, 1H), 2.25 (br s, 2H), 2.24–2.27 (m, 1H), 2.54–2.59 (m, 1H), 3.51–3.56 (m, 1H), 4.57 (d, J = 9.0 Hz, 1H), 4.83–4.87 (m, 1H), 5.27 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.9, 155.5, 118.2, 68.9, 58.8, 51.1, 44.2, 40.9, 37.1, 32.7, 30.2, 28.3, 27.3, 21.4, 18.9. ESI-MS m/z 416 $[M+H]^+$. HRMS for $C_{23}H_{34}N_3O_4$ calcd 416.2549, found 416.2552 $[M+H]^+$.

4.4. General procedure for the preparation of 1a–h

To a solution of **19a** in a mixture of isopropyl alcohol and water, conc. HCl was added. The mixture was stirred at 65 °C for 1.5 h, followed by diluted by dichloromethane and water. The layer was separated. To the collected aqueous phase, dichloromethane and a solution of NaOH in water were added. The mixture was adjusted to pH 9 with 25% K_2CO_3 while being vigorously stirred. The aqueous phase was removed, and the organic phase was concentrated, purified by flash chromatography.

4.4.1. (*S*)-3-Hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (1a)

Compound **1a** was prepared starting with **19a** (0.12 g, 0.29 mmol): white solids, 0.07 g, 77% yield, mp 108–110 °C, ee >99%. $[\alpha]_D^{25}$ 17.6 (*c* 0.31, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 5.04 (dd, J_1 = 10.5 Hz, J_2 = 2.1 Hz, 1H), 3.57–3.62 (m, 1H), 3.43 (s, 1H), 2.47–2.57 (m, 1H), 2.35 (dd, J_1 = 13.5 Hz, J_2 = 2.1 Hz, 1H), 2.23 (br s, 2H), 1.35–1.95 (m, 17H), 1.09–1.15 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 119.4, 68.7, 60.7, 46.3, 44.9, 44.5, 41.3, 37.43, 37.2, 36.5, 35.3, 30.31, 17.5, 13.1. ESI-MS *m*/*z* 338 $[M+Na]^+$. HRMS for $C_{18}H_{25}N_3O_2Na$ calcd 338.1844, found 338.1850 $[M+Na]^+$.

4.4.2. (*R*)-3-Hydroxyadamantylglycine-L-*cis*-4,5-methanoprolinenitrile (1b)

Compound **1b** was prepared starting with **19b** (90 mg, 0.217 mmol): white solids, 59 mg, 86% yield, mp 100–102 °C, ee >99%. $[\alpha]_D^{25}$ −7.8 (*c* 0.19, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.91 (d, J = 9.0 Hz, 1H), 3.54–3.59 (m, 1H), 3.45 (s, 1H), 2.53–2.58 (m, 1H), 2.38–2.39 (m, 1H), 2.23 (br s, 2H), 1.54–1.84 (m, 17H), 1.10–1.13 (m, 1H), 0.95–1.00 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 119.5, 68.7, 60.8, 46.1, 45.4, 44.6, 44.6, 40.5, 38.0, 37.5, 35.3, 30.4, 16.9, 12.7. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2029 $[M+H]^+$.

4.4.3. (*S*)-3-Hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinenitrile (1c)

Compound **1c** was prepared starting with **19c** (1.2 g, 2.9 mmol): white solids, 0.75 g, 82% yield, mp 99–102 °C, ee >99%. $[\alpha]_D^{25}$ 10.5 (*c* 0.5, EtOAc). ^1H NMR (300 MHz, $CDCl_3$): δ 5.05 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 3.59–3.64 (m, 1H), 3.46 (s, 1H), 2.49–2.59 (m, 1H), 2.34–2.35 (m, 1H), 2.26 (br s, 2H), 1.43–1.84 (m, 17H), 1.03–1.09 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 119.5, 68.5, 60.7, 46.0, 45.4, 44.5, 44.5, 40.4, 37.9, 37.5, 35.4, 30.4, 16.9, 12.7. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2028 $[M+H]^+$.

4.4.4. (*R*)-3-Hydroxyadamantylglycine-D-*cis*-4,5-methanoprolinenitrile (1d)

Compound **1d** was prepared starting with **19d** (150 mg, 0.36 mmol): white solids, 85 mg, 75% yield, mp 109-110 °C, ee >99%. $[\alpha]_D^{25}$ −21.9 (*c* 0.24, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 5.05 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 3.58–3.63 (m, 1H), 3.45 (s, 1H), 2.49–2.54 (m, 1H), 2.37 (dd, J_1 = 12.0 Hz, J_2 = 3.0 Hz, 1H), 2.26 (br s, 2H), 1.44–1.80 (m, 17H), 1.03–1.12 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.7, 119.4, 68.6, 60.7, 46.2, 44.9, 44.5, 41.26, 37.4, 37.1, 35.3, 30.3, 17.5, 13.1. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2028 $[M+H]^+$.

4.4.5. (*S*)-3-Hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinenitrile (1e)

Compound **1e** was prepared starting with **19e** (200 mg, 0.48 mmol): white solids, 120 mg, 80% yield, mp 152–154 °C, ee >99%. $[\alpha]_D^{25}$ −11.8 (*c* 0.26, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.87 (dd, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 3.52 (s, 1H), 3.47–3.51 (m, 1H), 2.54–2.60 (m, 1H), 2.26–2.33 (m, 1H), 2.27 (br s, 2H), 1.99–2.01 (m, 1H), 1.48–1.79 (m, 17H), 1.13–1.19 (m, 1H), 0.69–0.71 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.4, 118.4, 68.6, 60.7, 51.0, 46.1, 44.5, 40.8, 37.9, 37.2, 35.3, 32.6, 30.3, 21.1, 18.9. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2027 $[M+H]^+$.

4.4.6. (*R*)-3-Hydroxyadamantylglycine-L-*trans*-4,5-methanoprolinenitrile (1f)

Compound **1f** was prepared starting with **19f** (150 mg, 0.36 mmol): white solids, 96 mg, 85% yield, mp 90–92 °C, ee >99%. $[\alpha]_D^{25}$ −138.5 (*c* 0.35, CH_3OH). ^1H NMR (300 MHz, $CDCl_3$): δ 4.20–4.22 (m, 1H), 3.76–3.81 (m, 1H), 3.76 (s, 1H), 2.35–2.41 (m, 1H), 2.10–2.22 (m, 2H), 2.22 (br s, 2H), 1.54–1.65 (m, 16H), 0.84–0.87 (m, 1H), 0.67–0.69 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 118.0, 69.9, 60.9, 51.0, 46.0, 44.5, 43.6, 40.4, 37.8, 37.5, 35.2, 32.7, 30.4, 18.6, 11.5. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2028 $[M+H]^+$.

4.4.7. (*S*)-3-Hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinenitrile (1g)

Compound **1g** was prepared starting with **19g** (200 mg, 0.48 mmol): white solids, 118 mg, 78% yield, mp 91–93 °C, ee >99%. $[\alpha]_D^{25}$ 159.0 (*c* 0.4, EtOAc). ^1H NMR (300 MHz, $CDCl_3$): δ 4.22–4.23 (m, 1H), 3.77–3.79 (m, 1H), 3.77 (s, 1H), 2.32–2.38 (m, 1H), 2.10–2.22 (m, 2H), 2.21 (br s, 2H), 1.50–1.64 (m, 16H), 0.83–0.86 (m, 1H), 0.66–0.69 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.4, 118.0, 68.7, 60.9, 51.0, 46.1, 44.7, 41.0, 37.4, 35.2, 32.7, 30.5, 18.6, 11.5. ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2029 $[M+H]^+$.

4.4.8. (*R*)-3-Hydroxyadamantylglycine-D-*trans*-4,5-methanoprolinenitrile (1h)

Compound **1h** was prepared starting with **19h** (110 mg, 0.26 mmol): white solids, 76 mg, 91% yield, mp 148–150 °C, ee >99%. $[\alpha]_D^{25}$ 29.6 (*c* 0.24, EtOAc). ^1H NMR (300 MHz, $CDCl_3$): δ 4.86–4.88 (m, 1H), 3.53 (s, 1H), 3.48–3.53 (m, 1H), 2.56–2.57 (m, 1H), 2.26–2.28 (m, 1H), 2.27 (br s, 2H), 1.99–2.01 (m, 1H), 1.47–1.69 (m, 17H), 1.16–1.20 (m, 1H), 0.68–0.70 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.4, 118.4, 68.7, 60.7, 51.0, 46.1, 44.5, 40.8, 35.3, 32.6, 30.3, 21.1, 18.9; ESI-MS *m*/*z* 316 $[M+H]^+$. HRMS for $C_{18}H_{26}N_3O_2$ calcd 316.2025, found 316.2028 $[M+H]^+$.

4.5. In vitro DPP-IV inhibition assay

The DPP-IV Drug Discovery Kit (Enzo Life Sciences International, Inc.) was used for the assay of inhibition of DPP-IV activity, which was determined by measuring the rate of hydrolysis of a surrogate substrate, H-Gly-Pro-7-amino-4-methylcoumarin (H-Gly-Pro-AMC). The DPP-IV inhibitor P32/98A was selected as the reference inhibitor. The 500 μM substrate solution was diluted with assay buffer (50 mM Tris, pH = 7.5) to give a 5 μM substrate solution. 10 μL of appropriately diluted solutions of the test compounds and 25 μL of assay buffer were added to 96-well microtiter plates, followed by addition of 15 μL of assay buffer containing 0.26 mU of recombinant human DPP-IV. The reaction was initiated by addition of 50 μL of 5 μM substrate solution. After incubation at room temperature for 10 min, fluorescence was measured using an excitation wavelength of 380 nm and an emission wavelength of 460 nm by a SpectraMax M5 microplate reader. A fluorescence standard curve for 7-amino-4-methylcoumarin (AMC) was generated using 0.12–15 μM of AMC buffer solutions. The inhibitory rate relative to the control without inhibitor was calculated and IC_{50} values were determined by nonlinear regression.

4.6. Molecular docking

The crystal structure of **1a** was extracted from the X-ray crystallographical structure of DPP-IV complexed with saxagliptin (PDB code: 3BJM) and used as the template to construct the 3D structures of the other stereoisomers **1b**–**1h** in Sybyl 6.9. After hydrogens were added and Gasteiger-Hückel charges were assigned, all these ligand structures were optimized with Tripos force field. The protein structure was prepared by removal of ligands and water except H_2O808 from the X-ray crystallographical structure of DPP-IV (PDB code: 3BJM) and protonated. Compounds **1a**–**1h** were docked into the inhibitor binding pocket of DPP-IV A chain with GOLD 3.0.1. The residues within a radius of 6 Å around the original ligand were selected to define the active site. Default parameters were set. Maximum 10 solutions were adapted for each compound and ranked according to Goldscore. Computational interaction modes of the best solutions and atom distances were pictured with PyMol 1.3.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.061.

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