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Synthesis of (2*S*,5*S*)-5-fluoromethylornithine; a potent inhibitor of ornithine aminotransferase

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Abstract: Only one of the four enantiomers of 5-fluoromethylornithine **1** was an irreversible inhibitor of ornithine aminotransferase. The active enantiomer **1a** was synthesized from diaminoadipic acid **2** with a chemical diastereomeric separation and an enantiomeric resolution by hydrolysis of a phenylacetamide with benzylpenicillinase.

Introduction

Hyperammonemic states are linked to acute liver failure, hepatogenic encephalopathy, bacterial infections of the neurogenic bladder, drug generated hyperammonia and liver failure and Alzheimer's disease.[1] Inhibitors of ornithine aminotransferase could potentiate the urea cycle through an increase of ornithine carbamoyltransferase, and thus favour ammonia elimination.[2] 5-Fluoromethylornithine **1**, a potent irreversible inhibitor of ornithine aminotransferase (OAT), achieved these goals.[3] This inhibitor, synthesized by Gerhart and collaborators in racemic form, is a mixture of two pairs of diastereomers. It has been found that only one of the four isomers was an irreversible inhibitor of OAT and therefore consumed by the enzyme.[4] From enzymatic considerations, it was anticipated that the 2*S*,5*S*-enantiomer was the active one.[5]

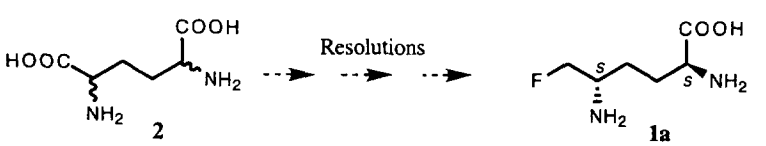
In the present publication, we will describe the synthesis of 5-fluoromethylornithine **1** as the biologically active enantiomer and the determination of its absolute configuration.

Results and discussion

If its two carboxylates and amines could be differentiated, diaminoadipic acid **2** would be a good starting material for synthesis of (*S*,*S*)-5-fluoromethylornithine **1a** via a diastereomeric separation and enantomeric resolutions (Scheme 1).

Racemic diaminoadipic acid **2** is a mixture of three isomers: (*R*,*R*)-, (*S*,*S*)- and meso-(*R*,*S*). These isomers have different chemical behaviours: the (*R*,*R*)- and (*S*,*S*)-enantiomers can lead to six-membered ring lactams **3a,b** (*SS*,*RR*) with cis substituents while the meso isomer gives the lactams **3c,d** (*RS*,*SR*) with trans substituents.[6] The lactams **3a,b**, having cis substituents, are able to form bicyclic di-lactams **4a,b** by reaction between the free carboxylate and the free amine[7] (Scheme 2).

This dilactamization would allow diastereomeric resolution of **2**. Then conversion of dilactams **4a,b** to 5-fluoromethylornithine through enantiomeric resolution would yield the desired active (*S*,*S*)-isomer.



Scheme 1.

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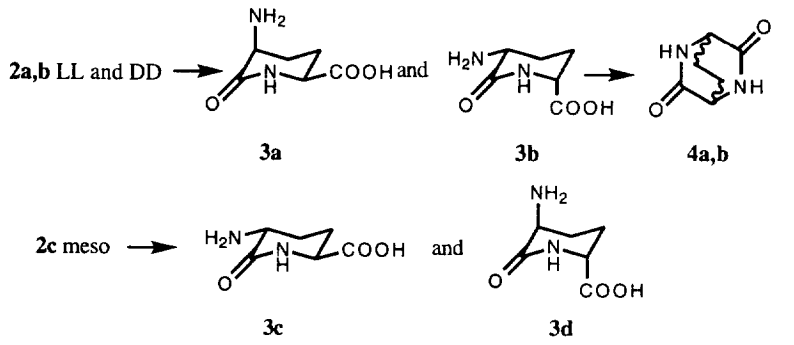
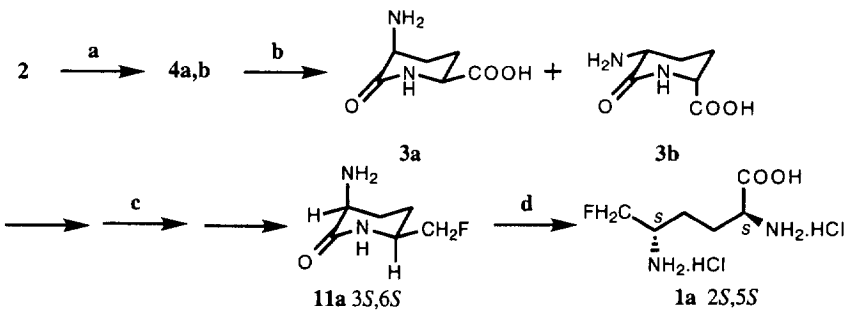


Scheme 2.



Scheme 3. a) Diastereomeric resolution; b) partial hydrolysis; c) several steps and enantiomeric separation; d) hydrolysis.

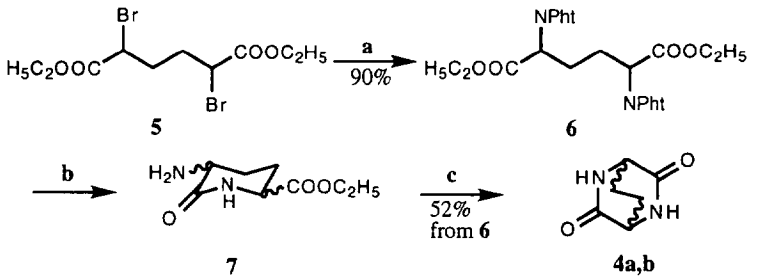
It is worthwhile noting that it might be possible to interconvert lactams **3c,d** to **3a,b** by epimerization. Thus, the diastereomeric resolution of diaminoadipic acid could be achieved without loss of product.

Based on the above considerations the synthetic scheme was designed as follows: preparation of dilactams **4a,b** followed by opening of one lactam ring to the piperidinones types **3a,b** which will be converted into 6-fluoromethyl-3-amino-2-piperidinone **11a** including an enantiomeric resolution step and finally hydrolysis of the lactam ring yielding 2*S*,5*S* 5-fluoromethylornithine **1a** (Scheme 3).

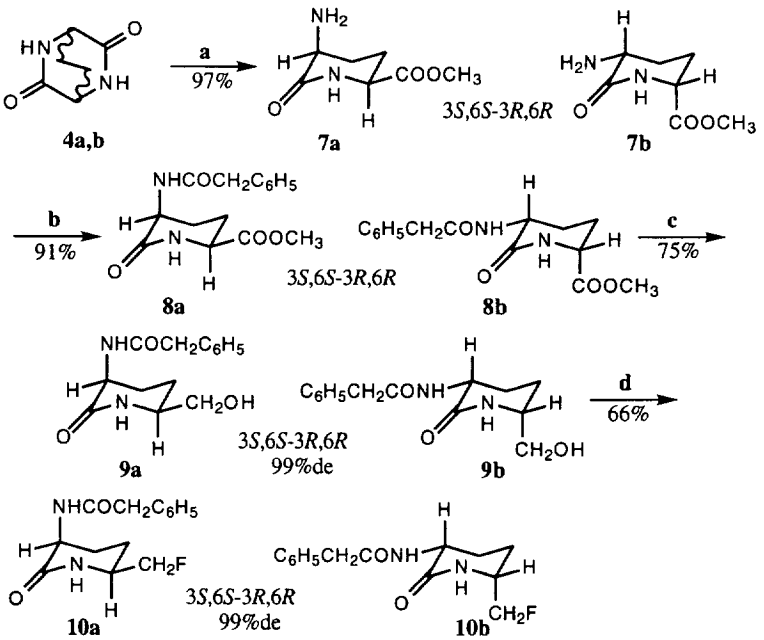
*1. Preparation of diastereomerically pure (3*S*,6*S*-3*R*,6*R*)-6-fluoromethyl-3-phenylacetamido-2-piperidinone **10ab***

To prepare dilactams **4a,b**, we repeated the synthesis described by Henry[7] with some modifications. Diethyl meso-2,5-dibromoadipate **5** treated in DMF with two equivalents of potassium phthalimide afforded racemic diethyl 2,5-diphthalimidoadipate (**6**) (90%). The phthalimido groups were cleaved using two equivalents of N-methylhydrazine in refluxing ethanol to afford racemic 3-amino-6-carboethoxy-2-piperidinone **7**. Treatment of **7** with one equivalent of sodium ethoxide in refluxing ethanol afforded only racemic dilactam **4a,b** (52% from **6**). This sequence achieved the diastereomeric resolution (Scheme 4).

Treatment of dilactams **4a,b** with methanolic hydrochloric acid (0.27 M) opened one of the lactam rings and afforded 3-amino-6-carbomethoxy-2-piperidinones **7a,b**[8] which were, after neutralization with silver carbonate, acylated with phenylacetic acid using dicyclohexylcarbodimide in methylene chloride to afford 6-carbomethoxy-3-phenylacetamido-2-piperidinones **8a,b** (90% from **4a,b**). The methyl esters were reduced to alcohols **9a,b** using lithium borohydride with a catalytic amount of lithium triethylborohydride.[9] Some epimerization (~10%) occurred during the reduction. Thus,



Scheme 4. a) PhtNK (2 eq), DMF, 90°C, 2 h; b) CH_3NHNH_2 (2 eq), C_2H_5OH, reflux, 2 h; c) C_2H_5ONa (1 eq), C_2H_5OH, reflux 2 h, RT overnight.



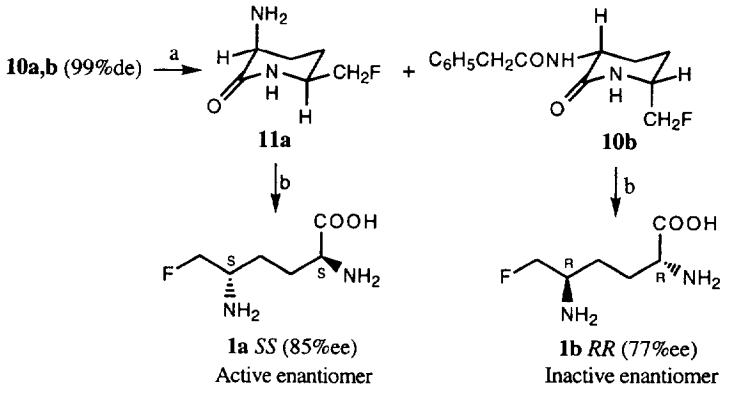
Scheme 5. a) HCl/CH_3OH (0.27 M), overnight; Ag_2CO_3 (1.1 eq); b) $C_6H_5CH_2COOH$ (1 eq), DCC (1 eq), pyridine (1.2 eq), DMAP (cat.), CH_2Cl_2, RT, 48 h; c) $LiBH_4$ (1 eq), $LiEt_3BH$ (0.1 eq), THF, reflux, overnight; d) DAST (1 eq), CH_2Cl_2, RT, overnight.

recrystallization from methanol afforded alcohols **9a,b** (75%, 99% de). Those alcohols were treated with one equivalent of DAST in methylene chloride to yield fluorinated lacatams **10a,b** (66%, 99% de) (Scheme 5).

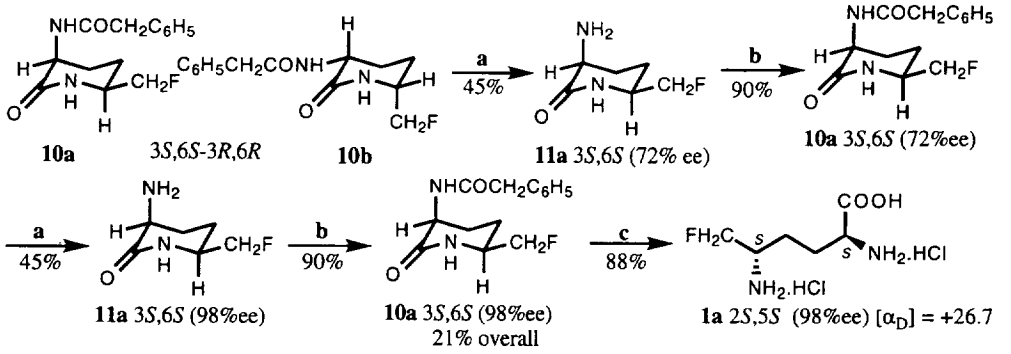
2. Enantiomeric separation and synthesis of 5-fluoromethylornithine active enantiomer ***1a***

Benzylpenicillinase at pH 7.8 and room temperature hydrolyzed faster than (*R*)-N-phenylacetylvinylgaba with a good selectivity[10]. We believed that similar selectivity could be found in the phenylacetamide of 2-piperidinones **8**, **9** or **10**.

Benzylpenicinillase at pH 7 (phosphate buffer) and room temperature hydrolysed more rapidly the phenylacetamide in one of the enantiomer of (*3S,6S/3R,6R*)-6-fluoromethyl-3-phenylacetamido-2-piperidinones **10a,b**. The 3-amino-6-fluoromethyl-2-piperidinone **11a** obtained gave, after acidic hydrolysis, the active isomer **1a** whilst the non hydrolyzed phenylacetamide gave an inactive enantiomer **1b** (Scheme 6).



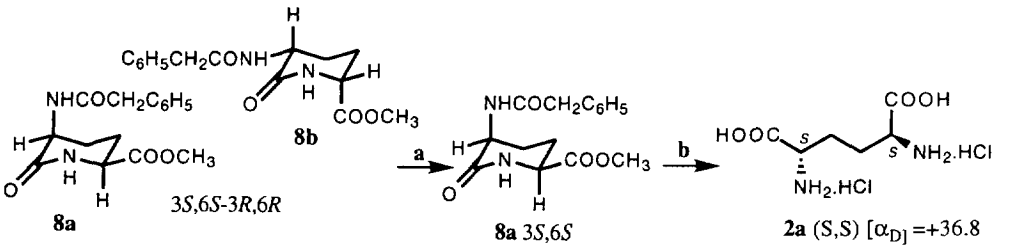
Scheme 6. a) Benzylpenicillinase; b) hydrolysis.



Scheme 7. a) Benzylpenicillinase, phosphate buffer (pH=7), RT, 10 min; b) $C_6H_5CH_2COOH$ (1 eq), DCC (1 eq), pyridine (1.2 eq), DMAP (cat.), CH_2Cl_2, 48 h; c) HCl, reflux, 4 h.

The selectivity of benzylpenicillinase towards phenylacetamides **8**, **9** or **10** was the same. With these phenylacetamides, we were able to obtain the free amine with 92–94% ee when the enzymatic reaction was stopped after 20% of hydrolysis. To obtain the pure enantiomer we decided to run first a complete enzymatic hydrolysis of one enantiomer, then to reprotect the amine and to carry out again an enzymatic hydrolysis. We decided to perform on a preparative scale the enzymatic separation on phenylacetamides **10 a,b**.

The mixture of *SS* and *RR* 6-fluoromethyl-3-phenylacetamido-2-piperidinones (**10a,b**) was treated with benzylpenicillinase at pH 7 (phosphate buffer) until 90% of *SS* isomer had been hydrolyzed. 3-Amino-6-fluoromethyl-2-pyridinone (**11a**) obtained had an enantiomeric purity of 72% ee (checked on 5-fluoromethylornithine obtained after acidic hydrolysis of **11a**). The amino group of **11a** was acylated with phenylacetic acid and DCC to yield **10a** (*SS*, 72% ee). This batch of **10a** was treated with benzylpenicillinase until 90% of the *SS* isomer has been hydrolyzed. The amine **11a** obtained had good enantiomeric purity (*SS* 98% ee checked on 5-fluoromethylornithine obtained from this amine after hydrolysis). To remove completely the salts it was decided to acylate back this amine with phenylacetic acid and DCC: (*3S,6S*)-6-fluoromethyl-3-phenylacetamido-3-piperidinone **10a** obtained had good enantiomeric purity (98% ee, 99% de). The hydrolysis of **10a** with 6N hydrochloric acid afforded pure isomer of 5-fluoromethylornithine **1a** (98% ee, 99% de, $[\alpha]_D$=+26.7) which was presumed to be the (*SS*)-isomer (Scheme 7).



Scheme 8. a) HPLC separation; b) HCl 6 N, reflux, 4 h.

3. Determination of ***1a*** *absolute configuration*

The three isomers of diaminoadipic acid have been characterized by their specific rotation. The enantiomer of 6-carbomethoxy-3-phenylacetamido-2-piperidinone **8a**, precursor of **10a** and **1a**, was purified by HPLC and hydrolyzed to diaminoadipic acid **2a**. The acid obtained ($[\alpha]_D$=+36.8) was correlated to (*S,S*)-isomer of diaminoadipic acid[11]. Thus, **1a** have the *SS* configuration (Scheme 8).

Conclusion

We have prepared the active enantiomer of 5-fluoromethylornithine **1a** and determined its absolute configuration by correlation with (*SS*)-diaminoadipic acid **2a**. Since it was not possible to reacylate enzymatically the free amine **1a** with phenylacetic acid, we had to reacylate chemically the free amine and to run a second time the enzymatic hydrolysis.

Kinetic constants for the (*S,S*)-5-fluoromethylornithine **1a** (K_I=2.4±0.3μM; $\tau_{1/2}$=11±1min) were those expected in comparison of kinetic constants obtained with racemic 5-fluoromethylornithine **1** (K_I=11±2μM; $\tau_{1/2}$=14±2min).[12]

Experimental part

^{1}H NMR spectra, were recorded on a Bruker AM 360 or AM 200 spectrometer; the data are reported as follows: chemical shift in ppm from external Me_4Si on δ scale, multiplicity (b=broad, s=singlet, d=doublet, t=triplet, q=quartet, p=quintuplet, m=multiplet) and coupling constant (Hz). ^{19}F NMR spectra were recorded on a Bruker AM 360 or AM 200 spectrometer; the data are reported as follows: chemical shift in ppm from external C_6F_6 on δ scale, multiplicity and coupling constant (same as for ^{1}H). Melting points were determined on a Büchi apparatus and are uncorrected. The mass spectra were measured on a Finnigan SSQ 7000 spectrometer equipped with a thermospray ion source with mobile phase $CH_3COO^-NH_4^+$ (0.1 M): CH_3OH 40:60 and flow 1 mL/min. Elemental analysis are performed on a Carlo Erba elemental analyzer model 1106. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck Silica Gel $60F_{254}$ glass plates (0.25 mm) and the products visualized with phosphomolybdic acid spray. Column chromatography are carried out with the use of Merck Silicagel 60 (230–400 Mesh) as described by Still et al.[13] $[\alpha]_D$ are recorded on Perkin Elmer Polarimeter 241 and concentrations are given in gram for 100 mL.

Gas chromatography GC analyses *(Method A)* were carried using a Fisons HRCG apparatus with FID. Splitless injection was performed at 250°C. Helium, at 0.3 bar, was used as the carrier gas. The oven temperature was 150°C isothermal. Detection temperature was 250°C. Samples preparations were as follows: 0.5–1 mg of sample were mixed with 500 μL of ethanol cooled in an ice bath, and 5 droplets of thionyl chloride were added. This mixture was heated for 2 h at 80°C, evaporated under a stream of nitrogen, and 500 μL of trifluoroacetic anhydride (TFAA) were added. After 1 h at RT the TFAA and TFA were removed with nitrogen and the dry residue was dissolved in 2 mL of ethyl acetate. Aliquots of 0.5 μL were injected into the GC system.

HPLC analyses *(Method B)* were performed on a modular system WISP 710B autosamplers (Waters), a Waters pump controlled by a M680 control unit and a Waters 481 UV detector, set to 210 nM, equipped with chiralpack AD (5 μM) (25 cm × 4.6 mm ID) column thermostated at 21°C at 1 mL/min (960 PSI) flow rate. The mobile phase was 60/40 (v/v) mixture of ethanol and n-heptane. For separation, about 1 mg of sample (accurately weighed) was dissolved in 1 ml of methanol.

On unprotected aminoacid HPLC analyses *(Method C)* were performed on modular system WISP 717 auto sampler (Waters), a Varian pump 9012 and a Waters 486 UV detector, set to 254 nM, equipped with Merck Lichocart RP18 (15 cm × 4.0 m ID) column thermostated at 25°C at 1 mL/min (3200 PSI) flow rate. The mobile phase was a 57/43 (v/v) mixture of 0.2 M sodium acetate buffer (pH 4.52) and methanol. About 0.5 mg of sample, accurately weighted, were dissolved in 4 mL of water. An automated precolumn derivatization was carried out by mixing 30 μL of sample with 30 mL of OPA reagent.

Diethyl 2,2-diphthalimidoadipate ***6***

A mixture of diethyl meso-2,5-dibromoadipate **5** (100 g, 0.278 mole) and potassium phthalimide (114 g, 0,615 mole) was heated in dimethyl formamide (500 mL) at 90°C during 2 h. Dimethyl formamide was evaporated under reduced presssure (10 mbar). The solid residue was diluted with water (400 mL) and extracted three times with chloroform (3×150 mL). The organic layer was quickly washed with sodium hydroxide (0.2 N, 400 mL) and with water until neutral pH (2×300 mL). The organic layer was decanted, dried over sodium sulfate, filtered and concentrated under reduced pressure to afford diphthalimide derivative **6** as a white solid (135 g). Recrystallization in diethyl ether afforded pure racemic diethyl 2,5-diphthalimidoadipate **6** (111 g, 81%), mp=108–109°C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.17 (2t, J_{HH}=7.6 Hz, 6H), 2.17 to 2.43 (m, 4H), 4.16 (2q, J_{HH}=7.6 Hz, 4H), 4.76 to 5.00 (m, 2H), 7.71 to 7.94 (m, 8H). The chromatographic separation (*Method B*) of the stereoisomers of **6** shows the presence of 46.2% meso compound.

3-Amino-6-carboethoxy-2-piperidinone ***7***

A mixture of diphthalimido derivative **6** (123 g, 0.25 mole) and methylhydrazine (27 mL, 0.5 mole) was refluxed in ethanol (1 L) during 2 h. The reaction mixture was cooled to room remperature and concentrated to a half volume. Methylphthalhydrazide was filtered off. The mixture was put to dryness to afford **7** along with some methylphthalhydrazide as a white solid which was used without purification in the next step.

2,5-Diazabicyclo [2,2,2]octane-3,6-dione ***4a,b***

The crude 3-amino-6-carboethoxy-2-piperidinone **7** was dissolved in ethanol (600 mL) containing sodium ethylate (0.25 mole) prepared from sodium (5.75g) and the mixture was refluxed during 1 h and stirred overnight at room temperature. The reaction mixture was put to dryness. The residue was dissolved in the minimum amount of water (≈200 mL) and extracted with 2-butanone (10×100 mL). The organic layers were concentrated under reduced pressure to afford a white solid (27.9 g). Chromatography on aluminium oxide (neutral, activity III) and elution with graded mixtures of methylene chloride and methanol (95/5 to 80/20) afforded after crystallization from methanol pure dilactams **4a,b** (18.4 g 52%), mp=270°C; ^{1}H NMR (360 MHz, CD_3OD) δ 1.91 to 1.99 (m, 2H), 2.08 to 2.15 (m, 2H), 3.97 (dd, J_{HH}=1.5Hz, J_{HH}=3.5 Hz, 2H); MNH_4^+=158.

3-Amino-6-carbomethoxy-2-piperidinone ***7a,b***

Dilactams **4a,b** (10.75 g, 76.7 mmole) were dissolved in dry methanol (350 mL) and 0.65 M methanolic hydrochloric acid (250 mL) was added. The mixture was stirred overnight at room temperature. The reaction mixture was neutralized with silver carbonate (21.5 g, 46.4 mmole) and filtered. The filtrate was concentrated under reduced pressure to afford 3-amino-6-carbomethoxy-2-piperidinones **7a,b** (12.8 g, 97%): ^{1}H NMR (200 MHz, CD_3OD, on the hydrochloride) δ 1.47 to 1.74

(m, 1H), 1.96 to 2.28 (m, 3H), 3.48 (dd, J_{HH}=5.4 Hz, J_{HH}=10.8Hz, 1H), 3.78 (s, 3H), 4.18 (t, J_{HH}=4.8 Hz, 1H).

3R,6R-3S,6S-*6-Carbomethoxy-3-phenylacetamido-2-piperidinone* ***8a,b***

Dicyclohexylcarbodiimide (15.5 g, 75.1 mmole) was added to a mixture of phenylacetic acid (10.2 g, 74.9 mmole) and pyridine (7.2 mL, 89 mmole) in methylene chloride (350 mL). Then 4-dimethylaminopyridine (0.9 g, 7.3 mmole) and amines **7a,b** dissolved in methylene chloride (50 mL) were added and the reaction mixture was stirred at room temperature during 64 h. Dicyclohexylurea was filtered off and the filtrate was concentrated under reduced pressure to afford a white solid. Flash chromatography on silica gel and elution with graded mixtures of chloroform and methanol (95/5 to 90/10) afforded pure amides **8a,b** (19.6 g, de>99%, *Method B*, 91%), mp=136–137°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 to 1.62 (m, 1H), 2.01 to 2.31 (m, 2H), 2.31 to 2.51 (m, 1H), 3.57 (s, 2H), 3.73 (s, 3H), 4.02 to 4.16 (m, 1H), 4.32 (dt, J_{HH}=10.8 Hz, J_{HH}=5.9 Hz, 1H), 6.75 1 b, 2H), 7.16 to 7.4 (m, 5H).

3R,6R-3S,6S-*6-Hydroxymethyl-3-phenylacetamido-2-piperidinone* ***9a,b***

Amides **8a,b** (7.39 g, 25.48 mmole) were covered with tetrahydrofuran (60 mL) and diethylether (200 mL), lithium borohydride (555 mg, 25.48 mmole) and lithium triethylborohydride (1M solution in THF, 2.6 mL, 2.6 mmole) were added and the mixture was refluxed overnight. The reaction was cooled to 0°C and methanol (10 mL) was added, filtration on florisil followed by elution with methanol and concentration under reduced pressure afforded a white solid (7.58 g). Flash chromatography on silica gel and elution with graded mixtures of chloroform and methanol (95/5 to 90/10) afforded the hydroxy amides **9ab** (90.2% de; *Method B*). Recrystallization from methanol afforded **9a,b** (5g, 99% de, *Method B*, 75%), mp=162–163°C; ^{1}H NMR (360 MHz, $CDCl_3$/CD_3OD) δ 1.51 to 1.6 (m, 2H), 1.77 to 1.87 (m, 1H), 2.08 to 2.17 (m, 1H), 3.41 (d, J_{HH}=10.3 Hz, 1H), 3.43 to 3.48 (m, 1H), 3.51 (d, J_{HH}=6Hz, 1H), 3.54 (s, 2H), 4.15 (dd, J_{HH}=6Hz, J_{HH}=10.3 Hz, 1H), 7.2 to 7.28 (m, 5H). Anal. calcd for $C_{14}H_{18}N_2O_3$: C, 64.11; H, 6.92; N, 10.68. Found: C, 64.18; H, 6.88; N, 10.66.

3R,6R-3S,6S-*6-Fluoromethyl-3-phenylacetamido-2-piperidinone* ***10a,b***

Alcohols **9a,b** (5 g, 19 mmole) were suspended in methylene chloride (250 mL) and cooled to 0°C. DAST (2.5 mL, 19 mmole) was added dropwise. The reaction mixture was stirred at room temperature during 60 h. The mixture was poored in ice cold saturated aqueous sodium carbonate and extracted 3 times with methylene chloride. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to afford a yellowish solid. Flash chromatography on silica gel and elution with graded mixtures of ethylacetate and methanol (97/3 to 90/10) afforded fluorides **10a,b** (2.3 g, 99% de, *Method B*, 66%), mp=149–151°C; ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.51 (dq, J_{HH}=12.1 Hz, J_{HH}=4.9 Hz, 1H), 1.73 (dq, J_{HH}=14.5 Hz, J_{HH}=4.2 Hz, 1H), 1.99 to 2.10 (m, 1H), 2.46 (dq, J_{HH}=13.2 Hz, J_{HH}=5.1 Hz, 1H), 3.6 (s, 2H), 3.75 to 3.82 (m, 1H), 4.19 to 4.45 (m, 3H), 5.97 (s, 1H) 6.5 (d, J_{HH}=3Hz, 1H), 7.27 to 7.36 (m, 5H); ^{19}F NMR (338 MHz, $CDCl_3$) δ 62.7 (ddt, J_{HF}=1.8 Hz, J_{HF}=14.1 Hz, J_{HF}=46.8 Hz). Anal calcd for $C_{14}H_{17}FN_2O_2$: C, 63.62; H, 6.48; N, 10.60. Found: C, 63.37; H, 6.38; N, 10.41.

Enzymatic hydrolysis of phenylacetamides ***8, 9, 10****: determination of the enzyme stereoselectivity*

To 100 mg of the substrates **8, 9** or **10** dissolved at pH 7 in 10 mL phosphate buffer, was added immobilized penicillin-G amidase[14] (325 mg, 150 IU/g of wet enzyme). The mixture was stirred during suitable time (reaction monitored by HPLC with *Method B*) and the reaction was quenched by addition of methanol (10 mL). The resulting mixture was filtered and concentrated under reduced pressure. The residue was dissolved in water and the non hydrolyzed amide was extracted with a 8/2 mixture of ethylacetate and methanol. The organic layer was dried over sodium sulfate, filtrated and concentrated under reduced pressure and analyzed according to *Method B*. The aqueous layer was concentrated under reduced pressure, dissolved in 6 N aqueous hydrochloric acid and refluxed during 4 h. The mixture was concentrated under reduced pressure and analyzed according to *Method C*.

3S,6S-*3-Amino-6 fluoromethyl-2-piperidinone* ***11a***

Phenylacetamides **10a,b** (1.17 g, 4.43 mmole) were dissolved in pH=7 phosphate buffer (234 mL) and immobilized penicillin-G amidase[14] (3.5 g) was added. The mixture was stirred for 10 min at room temperature (*Method B* showed that 90% of **10a** was hydrolyzed). The reaction mixture was quenched with methanol (50 mL), filtered and concentrated under reduced pressure. The gummy residue was dissolved in water, and the non hydrolyzed amides were extracted with methylene chloride. The aqueous layer was concentrated under reduced pressure to give the crude amine **11a** (72% ee, *Method C*) which was reprotected to phenylacetamide without purification.

3S,6S-*6-Fluoromethyl-3-phenylacetamide-2-piperidinone* ***10a***

Crude amine **11a** was suspended in methylene chloride (50 mL) then pyridine (0.21 mL, 2.6 mmole), 4-dimethylaminopyridine (27 mg, 0.22 mmole), phenylacetic acid (300 mg, 2.2 mmole) and dicyclohexylcarbodimide (501 mg, 2.4 mmole) were added and the mixture was stirred during 63 h at room temperature. The reaction mixture was filtered on celite and washed with water. The organic layer was decanted, dried on sodium sulfate, filtered and concentrated under reduced pressure to afford a white solid. Flash chromatography on silica gel and elution with graded mixtures of methanol and chloroform (5/95 to 10/90) afforded amide **10a** (509 mg, 43.5% from **10a,b**). Chromatographic analysis of the product showed 71% ee (*Method B*).

3S,6S-*3-Amino-6-fluoromethyl-2-piperidinone* ***11a***

The enzyme hydrolysis was carried as previously described using half quantitiy of buffer and enzyme. Crude amine obtained **11a** was 98% ee (*Method C*).

3S,6S-*6-Fluoromethyl-3-phenylacetamido-2-piperidinone* ***10a***

Crude amine **11a** was converted to amide **10a** exactly as described before in methylene chloride (50 mL) using pyridine (0.14 mL, 1.73 mmole) 4-dimethylaminopyridine (20 mg, 0.16 mmole), phenylacetic acid (190 mg, 1.4 mmole) dicyclohexylcarbodiimide (290 mg, 1.4 mmole). After purification **10a** (241 mg, 47% from **10a** 71% ee) were yielded with an ee of 98% after a second reprotection step.

2S,5S-*5-Fluoromethylornithine dihydrochloride* ***1a***

Amide **10a** (101 mg, 0.38 mmole) was dissolved in 6 N aqueous hydrochloric acid (20 mL) and refluxed during 4 h. The mixture was concentrated under reduced pressure. The residue was taken with water (10 mL) and extracted 3 times with ethylacetate. The aqueous layer was concentrated under reduced pressure to afford a white residue. Recrystallization from methanol/ether afforded pure *2S,5S*-5-fluoromethylornithine dihydrochloride **1a** (88 mg, 98%), $[\alpha]_D$=+26.7 (c=1, methanol), mp=210°C; ^{1}H NMR (360 MHz, D_2O) δ 1.75 to 1.85 (m, 1H), 1.90 to 2.10 (m, 3H), 3.58 to 3.72 (m, 1H), 3.99 (t, J_{HH}=6.1 Hz, 1H), 4.72 (ddq, J_{HF}=46.5 Hz, J_{HH}=5.4 Hz, J_{HH}=2.8 Hz, J_{HH}=7.86 Hz J_{HH}=10.9 Hz, 2H); ^{19}F NMR (338 MHz, D_2O,CF_3COOH external reference) δ 156.5 (dt, J_{HF}=23Hz, J_{HF}=46.5 Hz); MH^+=165. Anal. calcd for $C_6H_{15}FN_2O_2$ Cl_2, 0.5H_2O: C, 29.28; H, 6.55; N, 11.38; Found: C, 29.43; H, 6.35; N, 11.36. The chromatographic analysis of gave ee 98% (*Method C*).

2S,5S-*Diaminoadipic acid dihydrochloric salt* ***2a***

S,S-6-Carbomethoxy-3-phenylacetamide-2-piperidinone (23 mg, ee 96% $[\alpha]_D$=+44, c=1, methanol) purified by preparative HPLC (*Method B*) was dissolved in 6 N aqueous hydrochloric acid (10 mL) and refluxed during 4 h. The mixture was concentrated under reduced pressure. The residue was taken up with water (10 mL) and extracted 3 times with ethylacetate. The aqueous layer was concentrated under reduced pressure to afford *S,S*-diaminoadipic acid dihydrochloride **2a** (23 mg): $[\alpha]_D$=+37.8 (c=1 HCl 6N) (literature[11] $[\alpha]_D$=+26.5; c=5.92 HCl 6 N); ^{1}H NMR (200 MHz, CD_3OD) δ 1.92 to 2.32 (m, 4H), 4.04 (t, J_{HH}=6Hz, 2H); MH^+=177.

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