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KINETIC RESOLUTION OF PIPERAZINE-2-CARBOXAMIDE BY LEUCINE AMINOPEPTIDASE. AN APPLICATION IN THE SYNTHESIS OF THE NUCLEOSIDE TRANSPORT BLOCKER (-) DRAFLAZINE

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ABSTRACT: The resolution of *N-t*-Boc-piperazine carboxamide **5** proceeded smoothly in the presence of leucine aminopeptidase to produce acid (S)-**1** and amide (R)-**5** with good optical purity. Sequential alkylation and functional group manipulation of carboxamide (S)-**5** provided the known nucleoside transport blocker draflazine (-) **2**.

The non-proteinogenic amino acid **1**, piperazine-2-carboxylic acid, and its derivatives have found widespread application in the syntheses of a number of pharmacologically active agents.[1a-e] Functionalized piperazine carboxylic acid derivatives exhibit neuroprotection in global models of cerebral ischemia and cardioprotection in myocardial infarction models. Examples of this class of compounds include the cardioprotective nucleoside transport blocker draflazine (**2**) and the HIV-1 protease inhibitor L-735,524 (**3**) which are in clinical trials[1d-e] and CPP (**4**), the high affinity competitive antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor.[1a] In our continuing interest in developing potential drug candidates that are enantiomerically pure, we were faced with the challenge of synthesizing one enantiomer of piperazine-2-carboxylic acid in a patentably distinct manner. Literature reports on the

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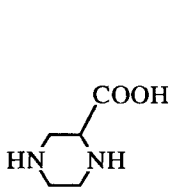
resolution[1e,2a-c] of piperazine-2-carboxylic acids involve conventional chemical derivatization and separation of the diastereomers or fractional crystallization of diastereomeric salts.



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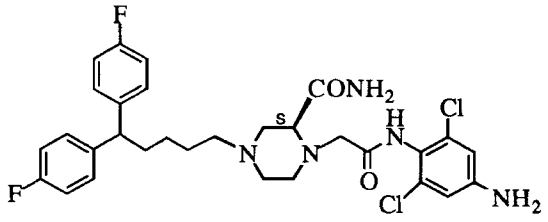

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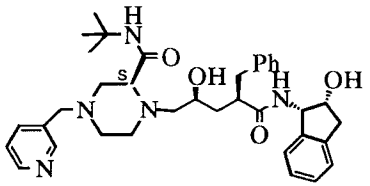
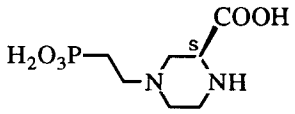


3, L-735,524

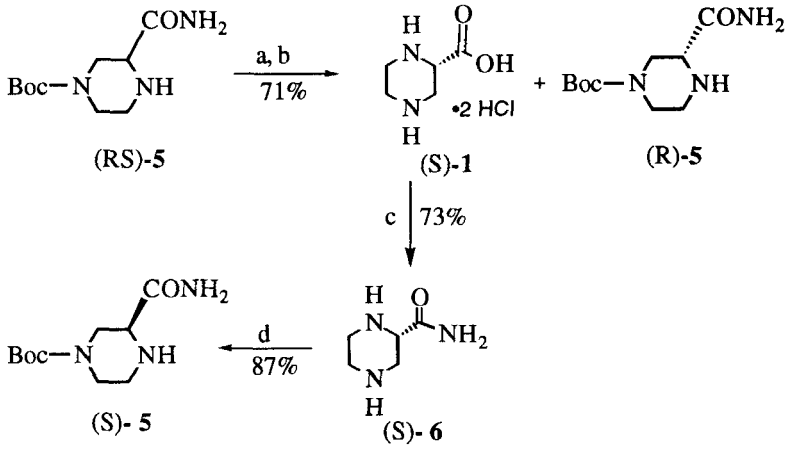


4, CPP

Enzyme based biocatalytic processes, in recent years, enjoy considerable favor as a versatile key reaction for the synthesis of natural and unnatural amino acids.[3a-e] Although numerous reports on the kinetic resolution of a-amino acids using hydrolase-catalysis[3c] or amino acid acylase from porcine kidney[3e] or bacterial aminopeptidase[3d] have appeared in the literature, none of these methods has yet been successful for piperazine-2-carboxylic acid. This may be due to the fact that some of these enzymes do necessitate a specific substitution pattern at the a-carbon and at the amine terminus. [3c,d] Also, most of these enzymes do not accept cyclic amino acids as substrates. Herein, we report our results on the successful application of L-leucine aminopeptidase-catalyzed resolution of *N-t*-Boc piperazine-2-carboxamide.

Initial review of the existing literature on aminopeptidases revealed that L-leucine aminopeptidase[4] has been successfully employed in the hydrolysis of cyclic amino acid proline amide. The unsubstituted piperazine-2-carboxamide, however, did not prove to be a useful substrate for hydrolysis. Modification of the substrate has often resulted in increased enantioselectivity in enzyme catalyzed resolutions;[5a]

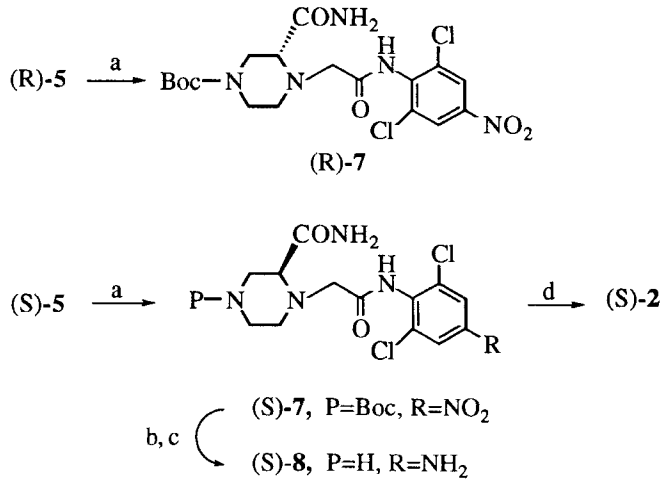
Scheme1



(a) L-leucine aminopeptidase, 0.1*M* Tris, pH 8.6. (b) Dowex 1X8 anion exchange resin. (c) NH_3, MeOH, Dowex 50W-X8 cation exchange resin. (d) $(Boc)_2O$, MeOH.

therefore, in an attempt to improve the substrate selectivity, we first examined the *N*-alkyl substituted derivatives of **5**. This effort was not successful. The *N-t*-Boc protected amide (RS)-**5**, however, served as a useful substrate in this process. The initial optimization experiments[5b] for enzyme activity in 0.1 *M* Tris buffer at pH 8.2 revealed an effective range from 0.1 to 2.0 mL enzyme suspension per mmol of substrate in 1 to 4 mL of buffer over a period of 72-144 h. Thus, when a solution of (RS)-**5** and 0.1 *M* Tris buffer containing L-leucine aminopeptidase suspension was stirred at room temperature over a period of seven days (an additional 4.0mL of enzyme suspension was added at the end of the third day), the R isomer of **5** remained as the amide and the S isomer was hydrolyzed to the free amino acid (S)-**1** (Scheme 1). The amide and the free (S)-acid were separated by passing the reaction mixture through a Dowex anion exchange resin, eluting the amide and Tris with water, and the acid with 1*N* HCl. The pure amide, (R)-**5**, was subsequently isolated in 72% yield after lyophilization and silica gel chromatography. The free

Scheme 2



(a) *N*-(2,6-dichloro-4-nitro)-2-bromoacetanilide, DMF, N(Et)3. (b) H_2, 5% Pt/C, 4% thiophene in MeOH, MeOH 50°C, 50psi. (c) HCl, Et_2O, MeOH. (d) 5,5-bis(4-fluorophenyl)pentyl bromide (**9**), NaI, *N*-methyl morpholine, DMF, 40-45°C, 2h.

acid, (S)-**1**, was directly converted to the *t*-Boc protected amide (S)-**5** in two steps. A resin-mediated amidation[6] followed by *t*-Boc protection provided (S)-**5** in 75% overall yield (from racemic **5**) after silica gel chromatography.

The chiral purity of the isomers was assessed by chiral HPLC[7] and NMR spectroscopy determinations (see experimental section) of their chromophoric derivatives (Scheme 2). In a chiral solvent environment, we were able to separate and integrate selected signals for (R)-and (S)- **5**, as well as their aryl derivatives. In this manner, the optical purity for (R)-**5** isomer was estimated to be 82% ee. The 4-nitro-2,6-dichloro-acetanilide derivative (R)-**7** was also prepared and the high optical purity (88% ee by NMR) was further corroborated by chiral column chromatography (86% ee by HPLC). The corresponding (S)-**7**-isomer, prepared in three steps from the resolved free acid (S)-**1**, displayed an optical purity of 67% ee as determined by HPLC (Table I).

Table I: Enantiomeric Purity[a] Measurements of Piperazine-2-carboxamides

Compound	$[\alpha]_D^{23}$	^{1}H NMR[b] Dn[c]	HPLC
(R)-**5**	+18.58°(c=1.1, EtOH)	82% ee; 4.5 Hz	
(S)-**1**	-3.02° (c=1.2, H_2O)		
(S)-**6**	-23.11° (c=1.4, EtOH)		
(S)-**5**	-15.47° (c=1.3, EtOH)	64% ee; 3.7 Hz	
(S)-**7**	-28.70° (c=0.7, EtOH)	67.6% ee; 25.0 Hz	66.8% ee
(R)-**7**	+38.06° (c=1.0, EtOH)	88% ee 8.2 Hz	86% ee
(S)-**2**	-15.75° (c=1.6, EtOH)	63% ee; 18.7 Hz	

[a]The optical rotation was measured on a Perkin Elmer 241 polarimeter and the concentrations are expressed as mg/100mL of solvent. [b]The % ee's were calculated from the integration of the *t*-butyl signal in compounds (R)-**3**, (S)-**3** and (R)-**5** and from the aromatic signal in (S)-**5** and (S)-**2**. [c]The Dn's were calculated from the chemical shift differences of the above signals with the chiral solvating agent.

The racemic form of the intermediate (S)-**7** has been previously reported[8] in the synthesis of racemic draflazine (**2**). Thus, catalytic reduction of (S)-**7** with Pt/C followed by deprotection of the *t*-Boc group under acidic conditions produced (S)-**8** in good yield. Alkylation of (S)-**8** with bromide **9** gave draflazine (S)-**2** after silica gel chromatography. [9]

In summary, a simple and useful enzymatic resolution protocol for piperazine-2-carboxamide is described. An application of this intermediate in the synthesis of a cardioprotective nucleoside transport blocker is illustrated. Further elaboration of this enzymatic methodology and its application will be the subject of future reports.

EXPERIMENTAL

All new compounds were fully characterized by IR, MS, ^{1}H and ^{13}C NMR, elemental analysis and/or high resolution mass spectra. HPLC determinations were done using chiral column Chiralcel OD, 4.6x 250mm and a UV detector. All anhydrous reactions were performed under an atmosphere of nitrogen

using dry solvents from Aldrich Sure Seal bottles. Column chromatography was performed with silica gel 60 (E M Science, 230-400 mesh) using the mentioned solvent system as eluant. Thin-layer chromatography was conducted on Anatech GFLH or Whatman MK6F silica gel plates . Melting points were determined in an open capillary tube with a Thomas-Hoover melting point apparatus unless otherwise stated and are not corrected. Infared spectra were recorded on a Perkin-Elmer 1800 Fourier transform spectrophotometer as thin films or KBr pellets. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on either Bruker AM-300 or Bruker AC-300 instruments and are expressed as parts per million (ppm or δ) from the mentioned solvent as the internal standard. Coupling constants are in hertz and signals are quoted as apparent singlet (s), triplet (t), quartet (q), muliplet (m), and broad (br). Mass spectra were determined either on a Finnigan Model 4500 Quadrapole mass spectrometer by direct chemical ionization (DCI) with isobutane as the positive CI gas or by fast atom bombardment (FAB) on a Kratos MS-25 instrument . High resolution mass spectra (HRMS) were determined on a Kratos MS-50 mass spectrometer using the fast atom bombardment method with CsI in glycerol as the reference agent.

S-(-)-Piperazine-2-carboxylic acid Dihydrochloride (S)-1:

A solution of 4-(*tert*-butoxycarbonyl)piperazine-2-carboxamide (30.25 g, 0.132 mol) in 250 mL of 0.1 *M* TRIS buffer pH 8.2 containing L-leucine aminopeptidase suspension (13.0 mL, Sigma L-9876) was stirred at room temperature for 3 days, then an additional 4.0 mL of enzyme suspension was added, and stirring continued for 4 additional days. Progress of the reaction was monitored by ^{1}H NMR (D_2O), comparing the integrals of the methine protons of amide (d 2.97-3.01) and acid (d 3.20-3.25). The reaction mixture was passed through a 250-mL column of DOWEX 1X8, 200 mesh hydroxide form anion exchange resin. The amide (R)-**5** and TRIS were eluted with water, and the acid was eluted with 1 *N* HCl. A portion of the acidic eluant was dried azeotropically with n-propanol, resulting in removal of the Boc protective group, and yielding the title compound as a white solid, m.p. >235 °C (with decomposition); ^{1}H NMR (300 MHz, D_2O) δ 3.25-3.45 (m, 3 H), 3.53-3.66 (m, 2 H), 3.85-3.89 (m, 1 H), 4.31-4.37 (m, 1 H); ^{13}C NMR (75 MHz, D_2O) ppm 42.19, 42.48, 44.41, 55.65, 169.86; IR (KBr) 3700-2000, 1760, 1220 cm^{-1}; MS (DCI) *m/z* 131; $[a]_D^{20}$ -3.02 (c=1.2, H_2O).

3R-(-)-1,1-Dimethylethyl 3-Aminocarbonyl-1-piperazinecarboxylate (R)-5:

The basic fraction from the above experiment containing the amide and TRIS was lyophilized, then filtered through silica gel. The (R)-amide, (R)-**5**, was eluted with CH_2Cl_2:MeOH (90:10 to 75:25), and solvent removed *in vacuo* to yield the title compound as a white solid (10.8 g, 72% yield), m.p. 130-132 °C; ^1H NMR (300 MHz, DMSO-d_6): δ 1.38 (s, 9 H), 2.45-2.53 (m, 2 H), 2.71-2.85 (m, 3 H), 3.01-3.05 (m, 1 H), 3.59-3.63 (m, 1 H), 3.83 (br s, 1 H), 7.13 (s, 1 H), 7.27 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 28.06, 43.88, 57.77, 78.81, 153.85, 172.80; IR (KBr) 3380-3250, 1690, 1650, 1270 cm^{-1}; MS (DCI) *m/z* 230; $[a]_D^{20}$ -18.58 (c=1.1, EtOH). Anal. Calcd for $C_{10}H_{19}N_3O_3$: C, 52.39; H, 8.35; N, 18.33. Found: C, 52.41; H, 8.45; N, 18.25.

S-(-)-Piperazine-2-carboxamide (S)-6:

The acidic fraction containing (S)-**1** was passed through a 100 mL column of DOWEX 50X8-200 mesh, H^+ form cation exchange resin, and rinsed with water until the eluant reached a neutral pH. The resin was transferred to a round-bottom flask, and stirred in 300 mL of methanol for 30 minutes to remove any free water, then collected by filtration and resuspended in 300 mL of methanol and refluxed overnight with stirring, resulting in esterification of acid (S)-**1** and loss of the Boc protecting group. The mixture was then cooled to 0°C, and saturated with anhydrous NH_3 (17.0 g, 1.00 mol). The resulting mixture was sealed and stirred for 3 days. The mixture was then filtered, and solvent removed *in vacuo* from the filtrate. The resin was placed in a column and eluted with 3*N* aqueous NH_3 (100 mL). The eluant was combined with the residue from the methanolic filtrate, and this solution was passed through a 25 mL column of DOWEX 1X8-200 mesh, hydroxide form anion exchange resin to remove any unreacted carboxylic acid. The (S)-amide (S)-**6** was eluted with water and lyophilized, yielding the title compound as a white solid (7.60 g, 89% yield), m.p. 128-138 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 2.38-2.75 (m, 5 H), 2.83-2.88 (m, 1 H), 3.00-3.03 (m, 1 H), 6.98 (s, 1 H), 7.12 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 45.26, 45.99, 49.40, 59.11, 174.24; IR (KBr) 3600-2600, 1680 cm^{-1}; MS (DCI) *m/z* 130; $[a]_D^{20}$ -23.11 (c=1.4, EtOH). Anal. Calcd for $C_5H_{11}N_3O \cdot 0.1\ H_2O$: C, 45.86; H, 8.62; N, 32.09. Found: C, 46.06; H, 8.69; N, 32.08.

3S-(+)-1,1-Dimethylethyl 3-Aminocarbonyl-1-piperazinecarboxylate (S)-5:

To a stirred solution of (S)-**6** (7.40 g, 0.0574 mol) in 150 mL of MeOH at room temperature was added di-*t*-butyl dicarbonate (12.5 g, 0.0574 mol) in 2.5 g portions over a period of 1 hour. Solvent was then removed *in vacuo*, and the residue filtered through silica gel, eluting the diprotected piperazinecarboxamide with CH_2Cl_2:MeOH (96:4) and monoprotected piperazinecarboxamide with CH_2Cl_2:MeOH (95:5 to 85:15). Solvent was removed in vacuo to yield the title compound as a white solid (11.4 g, 87% yield), m.p. 125-128 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.38 (s, 9 H), 2.45-2.53 (m, 2 H), 2.71-2.85 (m, 3 H), 3.00-3.05 (m, 1 H), 3.59-3.63 (m, 1 H), 3.83 (br s, 1 H), 7.13 (s, 1 H), 7.27 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 28.05, 46.68, 57.77, 78.81, 153.85, 172.81; IR (KBr): 3380-3250, 1690, 1650, 1270 cm^{-1}; MS (DCI) *m/z* 230; $[a]_D^{20}$ +15.47 (c=1.3, EtOH). Anal. Calcd for $C_{10}H_{19}N_3O_3$: C, 52.39; H, 8.36; N, 18.33. Found: C, 52.39; H, 8.51; N, 18.34.

3R-(+)-1,1-Dimethylethyl 3-Aminocarbonyl-4-[[[(4-nitro-2,6-dichlorophenyl)amino]carbonyl]methyl]-1-piperazinecarboxylate (R)-7:

To a solution of (R)-**5** (0.50 g, 0.0022 mol) and triethylamine (0.35 mL, 0.0025 mol) in 10 mL of DMF was added *N*-(2,6-dichloro-4-nitro)-2-bromoacetanilide (0.715 g, 0.0022 mol), and the resulting mixture covered and stirred for 1.5 hours. The solution was then combined with 100 mL of ethyl acetate, and partitioned with 0.05 *M* potassium biphthalate buffer pH 5.0 (2X50 mL), followed by water (2X50 mL). The organic extract was then dried over Na_2SO_4 and solvent removed *in vacuo*. The residue was then dissolved in CH_2Cl_2 and passed through a silica gel plug and eluting the product with CH_2Cl_2:MeOH (90:10). Solvent was removed *in vacuo* to yield the title compound as an off-white solid (0.64 g, 61% yield, 86% ee), m.p. 98-115 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 1.39 (s, 9 H), 2.36-2.42 M, 1 H), 2.99-3.22 (m, 4 H), 3.34 (s, 2 H), 3.65-3.75 (m, 2 H), 7.34 (s, 1 H), 7.64 (s, 1 H), 8.40 (s, 2 H), 10.16 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 28.01, 58.12, 64.22, 79.13, 123.58, 134.27, 139.23, 146.28, 153.55, 168.49, 172.09; IR (KBr) 1700, 1680, 1540, 1345, 1250 cm^{-1}; MS (DCI) *m/z* 476; $[a]_D^{20}$ +38.06 (c=1.0, EtOH). Anal. calcd for $C_{18}H_{23}N_5O_6Cl_2 \cdot 0.6\ H_2O$: C,44.38; H, 5.01; N, 14.38. Found: C, 44.34; H, 4.78; N, 14.20.

3S-(-)-1,1-Dimethylethyl 3-Aminocarbonyl-4-[[[(4-nitro-2,6-dichlorophenyl)amino]carbonyl]methyl]-1-piperazinecarboxylate (S)-7:

Obtained in the same manner as (R)-**7**, yielding the title compound an off-white solid (0.67 g, 64% yield, 67% ee), m.p. 100-117 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 1.39 (s, 9 H), 2.36-2.42 (m, 1 H), 3.01-3.22 (m, 4 H), 3.33 (s, 2 H), 3.65-3.75 (m, 2 H), 7.34 (s, 1 H), 7.64 (s, 1 H), 8.40 (s, 2 H), 10.16 (s, 1 H); ^{13}C NMR (75 MHz, DMSO-d$_6$) ppm 28.02, 58.12, 64.21, 79.13, 123,58, 134.28, 139.23, 146.29, 153.55, 168.49, 172.08; IR (KBr) 1700, 1680, 1540, 1345, 1250 cm^{-1}; MS (DCI) *m/z* 476; [a]$_D^{20}$ -27.55 (c=0.8, EtOH). Anal. Calcd for $C_{18}H_{23}N_5O_6Cl_2 \cdot 0.6\ H_2O$: C, 44.38; H, 5.01; N, 14.38. Found: C, 44.57; H, 4.85; N, 14.34.

2S-(-)-2-Aminocarbonyl-N-(4-amino-2,6-dichlorophenyl)-1-piperazineacetamide Trihydrochloride Hydrate. (S)-8:

A suspension of (S)-**7** (4.22g, 8.85 mmol), 5% Pt/C (1.75g) and 4% methanolic thiophene (0.9 mL) in methanol (100 mL) was hydrogenated in a Parr apparatus at 50°C and 50 psi for 3 hours. The mixture was then filtered through Celite and the filtrate was concentrated down to dryness to yield 3.34g (85%) of 3S-(-) 1,1-dimethylethyl 3-aminocarbonyl-4-[[[(4-amino-2,6-dichlorophenyl)-amino]carbonyl]methyl]-1-piperazinecarboxy- late as a white foam after flash chromatography on silica gel (gradient elution with 5% methanol in chloroform followed by 10% methanol in chloroform), m.p. 166-186°C; ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 9.46 (s, 1H), 7.63 (s, 1H), 7.32 (s, 1H), 6.64 (s, 2H), 5.68 (s, 2H), 3.70-3.66 (m, 2H), 3.26-2.88 (series of m, 6H), 2.33-2.26 (m, 1H), 1.39 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d$_6$) ppm 172.22, 168.76, 153.54, 149.30, 133.88, 119.46, 112.49, 79.12, 64.83, 58.29, 50.16, 28.02; IR (KBr, cm^{-1}) 3450, 3352, 2978, 1682, 1598, 1504, 1426, 1368, 1272, 1246, 1168, 1126, 802; MS (FAB, MH$^+$) *m/z* 446; [a]$_D^{20}$ (EtOH, c=1.2) -25.90. Anal. Calcd for $C_{18}H_{25}Cl_2N_5O_4 \cdot 0.50H_2O$: C, 47.59; H, 5.55; N, 15.42. Found: C, 47.48; H, 5.49; N, 15.34.

Treatment of 3S-(-)-1,1-dimethylethyl 3-aminocarbonyl-4-[[[(4-amino-2,6-dichlorophenyl)amino]carbonyl]methyl]-1-piperazinecarboxy-late (3.30g, 7.26 mmol) with cold (0°C) ethereal HCl (100 mL) in methanol (50 mL) for 4h afforded 3.01g (90%) of the title compound as a dense,white solid after suction-filtration,

m.p. 220-225°C (dec., sealed tube); ^{1}H NMR (300 MHz, D_2O) δ 7.50 (s, 2H), 3.77-3.69 (m, 1H), 3.60-3.26 (series of m, 7H), 2.98-2.89 (m, 1H); ^{13}C NMR (75 MHz, D_2O) ppm 175.32, 174.46, 137.69, 135.63, 133.36, 125.60, 62.85, 59.62, 49.63, 46.74, 44.97; IR (KBr, cm^{-1}) 3412, 2926, 2592, 1702, 1540, 1508, 1470, 1416, 970, 810, 596; MS (MH^+) *m/z* 346; [a]$_D^{20}$ (H_2O, c=1.1) -16.42. Anal. Calc'd for $C_{13}H_{17}Cl_2N_5O_2 \cdot 2.87HCl \cdot 0.145H_2O \cdot 0.1Et_2O$: C, 34.94; H, 4.63; N, 15.20; Cl, 37.44; H_2O, 0.57. Found: C, 34.96; H, 4.87; N, 14.88; Cl, 37.44; H_2O, 3.31.

2S-(-)-2-Aminocarbonyl-N-(4-amino-2,6-dichlorophenyl)-4-[5,5-bis(4-fluorophenyl)pentyl]-1-piperazineacetamide (S)-2:

Anhydrous sodium iodide (0.39g, 2.63 mmol) was added to a solution of 5,5-bis(4-fluorophenyl)pentyl bromide (0.89g, 2.63 mmol) in dry dimethylformamide (10 mL). The mixture was heated to reflux for 0.5h before it was cooled to ambient temperature and added to a suspension of (S)-**8** (1.20g, 2.63 mmol) and *N*-methylmorpholine (1.2 mL, 10.52 mmol) in anhydrous dimethylformamide (20 mL). The mixture was warmed to 45°C for 3h before it was diluted with ethyl acetate and water. The organic phase was then separated, washed with brine, dried and concentrated down to dryness. A portion of the residue was purified by flash chromatography on silica gel to obtain 0.13g of the title compound as a white foam, m.p. 82-115°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (s, 1H), 7.57 (s, 1H), 7.33-7.29 (m, 4H), 7.23 (s, 1H), 7.11-7.05 (m, 4H), 6.63 (s, 2H), 5.67-5.66 (m, 1.5H), 3.98-3.93 (m, 1H), 3.33 (s, 2H), 3.24-3.19 (m, 1H), 3.02-2.98 (m, 1H), 2.87-2.72 (2m, 1H), 2.64-2.60 (m, 1H), 2.30-2.14 (m, 5H), 2.01-1.93 (m, 2H), 1.42-1.40 (m, 2H), 1.22-1.10 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) ppm 173.09, 168.80, 162.20, 158.99, 149.27, 141.31, 133.85, 129.34, 129.24, 119.52, 115.20, 114.93, 112.50, 66.23, 58.95, 57.25, 55.41, 51.39, 51.22, 48.70, 34.76, 25.88, 25.21; IR (KBr, cm^{-1}) 3346, 2936, 2860, 2824, 1670, 1600, 1506, 1222, 1158, 834, 802; MS (FAB, MH^+) *m/z* 604; [a]$_D^{20}$ (EtOH, c=1.1) -15.75. Anal. Calcd for $C_{30}H_{33}Cl_2F_2N_5O_2 \cdot 0.50H_2O \cdot 0.20DMF$: C, 58.77; H, 5.71; N, 11.20. Found: C, 58.45; H, 5.45; N, 11.22.

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REFERENCES AND NOTES

1. (a) Bigge, C. F.; Johnson, G.; Ortwine, D. F.; Drummond, J. T.; Retz, D. M.; Brahce, L. J.; Marcoux, F. W.; Probert, A. W. Jr. *J. Med. Chem.* **1992**, *35*, 1371-84. (b) Demaine, D. A.; Smith, S.; Barraclough, P. *Synthesis* **1992**, 1065. (c) Phillips, G. B.; Morgan, T. K. Jr; Lumma, W. C. Jr; Gomez, R. P.; Lind, J. M.; Lis, R.; Argentieri, T.; Sullivan, M. E. *J. Med. Chem.* **1992**, 35, 743-50. (d) Van Belle, H.; Janssen, P. A. J. *Nucleoside and Nucleotides* **1991**, *10*, 975-82; (e) Askin, D.; Eng, K. K.; Rosen, K.; Purick, R. M.; Wells, K. M.; Volante, R. P.; Reider, P. J. *Tetraherdon Lett.* **1994**, *35*, 673-676.
2. (a) Aebischer, B.; Frey, P., Haerter, H. P.; Herrling, P. L.; Mueller, W.; Olverman, H. J.; Watkins, J. C. *Helv. Chim. Acta* **1989**, *72*, 1043-51. (b) Felder, E.; Maffei, S.; Pietra, S.; Pitre, D. *Helv. Chim. Acta* **1960**, *43*, 888-96. (c) Uozumi, Y.; Tanahashi, A.; Hayashi, T. *J. Org. Chem.* **1993**, *58*, 6826-32.
3. (a) Roos, E. C.; Mooiweer, H. H.; Hiemstra, H.; Speckamp, W. N.; Kaptein, B.; Boesten, W. H.J.; Kamphuis, J. *J. Org. Chem.* **1992**, *57*, 6769-78 and references cited therein. (b) Ng-Youn-Chen, M. C.; Serrequi, A. N.; Huang, Q.; Kazlauskas, R. *J. Org. Chem.* **1994**, *59*, 2075-81. (c) Williams, R. M. Synthesis of Optically Active a-Amino Acids; Pergamon: Oxford, **1989**, 257-279. (d) Schoemaker, H. E.; Boesten, W. H. J.; Kaptein, B.; Hermes, H. K. M.; Sonke, T.; Broxterman, Q. B.; van den Tweel, W. J. J.; Kamphuis, J. *Pure. Appl. Chem.* **1992**, *64*, 1171-75. (e) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. AM. Chem. Soc.* **1989**, *111*, 6354-6364.
4. (a) Smith, E. L. *Methods in Enzymology* **1955**, Vol. II, 88-93.
5. (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656-2665. (b) L-leucine aminopeptidase was purchased from Sigma (L-9876). No elaborate study was performed with respect to enzyme activation and pH.
6. Adapted from Roteman, R. *U. S. Patent 4,017,513*, **1977**.
7. All the optical purity measurements by NMR were done on a 500 MHz Bruker instrument in $CDCl_3$ with a few drops of (S)-trifluoromethybenzyl alcohol added as the chiral solvent. For a review see: Weisman, E. R.

"*Asymmetric Synthesis* " Morrison, J. D., Ed.; Academic Press, Inc., New York, **1983** ; Vol. I, 153-171.

8. Van Belle, H. *Cardiovasc. Res.* **1993**, *27*, 68-76.
9. (a) Alkylation procedures for a series of piperazine derivatives have been claimed: Van Daele, G. *U. S. Patent 4,766,125,* **1988.**

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