SYNTHESIS OF NEW L-PROLINE AMIDES WITH ANTICONVULSIVE EFFECT

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Series of heterocyclic L-proline amides were prepared from BOC-L-proline and heterocyclic amines (mostly substituted piperazines and morpholines) via active ester with hydroxysuccinimide. 4-(4-Fluorobenzoyl)piperidine afforded L-proline 4-(4-(4-(4-fluorobenzoyl)piperidin-1-yl)benzoyl)piperidine (**7b**) simultaneously with expected L-proline 4-(4-fluorobenzoyl)piperidide (**7a**). D-Proline *N*-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)amide (**2**) was prepared starting from D-proline. The amides were tested by methods of biochemical and behavioural pharmacology.
Key words: Amides preparation; Amino acids reaction; Amines reaction; Anticonvulsive effect.

In a recent paper[1] we described a series of basic amides of racemic proline for which neuroprotective effects and positive influence on learning and memory were expected. The object of the present paper is the synthesis of heterocyclic L-proline amides by a different method. Proline *N*-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)amide, prepared formerly in the racemic form has now been obtained in the form of the L- and D-enantiomers (**1b** and **2**).

Reaction of BOC-L-proline[2,3] with *N*-hydroxysuccinimide in dichloromethane in presence of *N*,*N*′-dicyclohexylcarbodiimide gave active ester (for method cf. ref.[4]) which was treated with the following heterocyclic amines: 1-(3-aminopropyl)-4-(2-chlorophenyl)piperazine[5], 1-(3-aminopropyl)-4-(3-chlorophenyl)piperazine[6], 1-(3-aminopropyl)-4-(4-chlorophenyl)piperazine[5], 4-(2-aminoethyl)morpholine, 4-(4-aminobutyl)-morpholine[7], 1-(2-aminoethyl)-4-(3-trifluoromethylphenyl)piperazine[8], 1-(4-aminobutyl)-4-(3-trifluoromethylphenyl)piperazine[9], 1-(2-fluorophenyl)piperazine, 1-(3-fluorophenyl)piperazine[10], 1-(4-fluorophenyl)piperazine, 1-(3-chlorophenyl)piperazine, 1-(3-trifluoromethylphenyl)piperazine and 1-(4-trifluoromethylphenyl)piperazine[11]: the obtained *N*-protected intermediates (in several cases there were purified and

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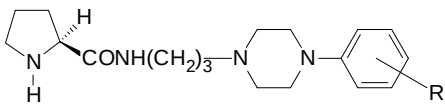
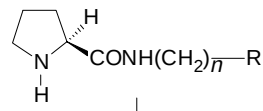
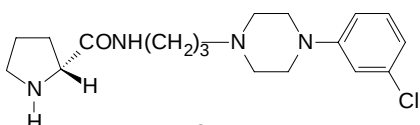
characterized) were processed in crude state by deprotection (removal of BOC) with ethanolic solutions of hydrogen chloride[12]. The resulting L-prolinamides **1a–1c, 3a**, **3b**, **4a**, **4b** and **5a–5f** were partly isolated directly in the form of crystalline hydrochlorides. In some cases, it was necessary to release the bases from the crude hydrochlorides and transform them to hydrogen oxalates for characterization and biological testing. The active ester which was similarly obtained from BOC-D-proline, gave by treatment with 1-(3-aminopropyl)-4-(3-chlorophenyl)piperazine[6] compound **2**, the mentioned enantiomer of **1b**.



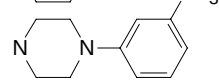
1	R
a	2-Cl
b	3-Cl
c	4-Cl

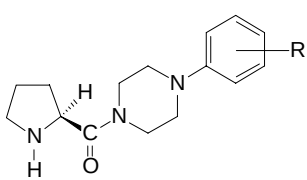


	n
3	2
4	4

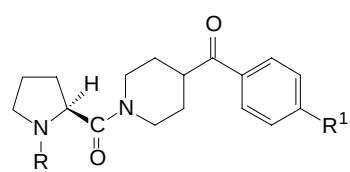


2

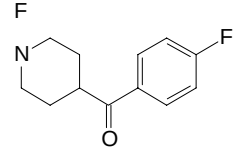
3, 4	R
a	
b	



5	R
a	2-F
b	3-F
c	4-F
d	3-Cl
e	3-CF_3
f	4-CF_3



	R
6	$COOC(CH_3)_3$
7	H

6, 7	R^1
a	F
b	

A similar sequence starting from the same activated BOC-L-proline ester and 4-(4-fluorobenzoyl)piperidine[13] was complicated by the simultaneously proceeding substitution reaction between two molecules of the starting piperidine derivative (the fluorine atom is strongly activated by the *para* carbonyl group) which resulted in 4-(4-(4-(4-fluorobenzoyl)piperidin-1-yl)benzoyl)piperidine: this by-product was not isolated but it participated in the coupling reaction. The crude product was thus separated to **6a** and **6b**. Usual deprotection of these products gave the desired compound **7a** and the unexpected **7b**.

The compounds prepared were tested by methods of biochemical pharmacology in the following tests: inhibition of binding of [^{3}H]glycine (rat brain cortex membranes), [^{3}H]5,7-dichlorokynurenic acid (rat brain cortical membranes), [^{3}H]CGP-39653 (rat brain membranes), [^{3}H]AMPA (3-hydroxy-5-methylisoxazol-4-ylalanine) (membrane fraction from rat brain cortex) and [^{3}H]MK-801. In these tests, the compounds were practically inactive (some indications of effect in the inhibition of [^{3}H]glycine and [^{3}H]5,7-dichlorokynurenic acid binding).

Some of the products showed in behavioural tests antiischemic effects. Protection from acute anoxia induced by intravenous administration of KCN (i.p. dose of 20 mg/kg of the tested compound, time of survival in s): **2**, 31.4; **5a**, 30.3 (control, 25 s). Anticonvulsant effect in the electroshock test in female mice (i.p. dose of 20 mg/kg, effect in the number of animals out of the group given): **1c**, 5/6; **2**, 8/10; **5b**, 5/8; **5c**, 4/5. More detailed results of the testing (especially behavioural pharmacology) will be published in a separate paper.

EXPERIMENTAL

Melting points of analytical samples were determined in the Kofler block and they are not corrected; the samples were dried in vacuo of about 40 Pa at room temperature or at a suitably elevated temperature. IR spectra (ν in cm^{-1}) were recorded mostly in Nujol with Unicam SP 2000 or Perkin–Elmer 298 spectrophotometers; NMR spectra (bases in deuteriochloroform, salts in hexadeuteriodimethyl sulfoxide) on a Tesla BS 567A instrument (^{1}H at 100 MHz, ^{13}C at 25.14 MHz; chemical shifts are given in ppm (δ-scale), coupling constants (J) in MHz); mass spectra were measured on a Varian-MAT 44S (GC-MS) spectrometer. The homogeneity of the products and composition of the mixtures were checked by thin-layer chromatography (TLC) on silica gel (Silufol UV_{254}). Preparative chromatographic separations were carried out on a columns of silica gel (Fluka 60, 0.063–0.20 mm).

General Procedure for Preparation of the L-Proline Amides **1a–1c**, **3a**, **3b**, **4a**, **4b** and **5a–5f**

A stirred solution of BOC-L-proline[2,3] (3.4 g, 0.016 mol) in dichloromethane (60 ml) was treated at –10 °C with *N*-hydroxysuccinimide (1.8 g, 0.016 mol) and then at the same temperature dropwise with a solution of the *N*,*N*′-cyclohexylcarbodiimide (3.24 g, 0.016 mol) in dichloromethane (20 ml). The mixture was stirred for 1 h at –10 °C and then treated dropwise at –5 to 10 °C with a solution of the corresponding amine (0.016 mol) in dichloromethane (20 ml). After 1 h stirring, the cooling was discontinued and the mixture was allowed to stand overnight at room temperature. The separated *N*,*N*′-dicyclohexylurea was filtered off, washed with dichloromethane, the filtrate was washed three

times with 10% $KHSO_4$ solution, then with water, two times with 5% $NaHCO_3$ solution and finaly 2 × with water, dried with $MgSO_4$, and evaporated in vacuo. The crude BOC-protected intermediate (mostly oily) was dissolved in ethanol (25 ml), the solution was treated with ethanol which was saturated with HCl (11 ml) and the mixture was stirred for 1.5 h at room temperature. In some cases, homogeneous hydrochlorides separated by standing were used. In cases of inhomogeneous or hygroscopic hydrochlorides, the bases were released, sometimes used for measurement of spectra, and transformed by neutralization with oxalic acid to crystalline hydrogen oxalates.

L-Proline N-(3-(4-(2-chlorophenyl)piperazin-1-yl)propyl)amide (**1a**). The general procedure applied to 1-(3-aminopropyl)-4-(2-chlorophenyl)piperazine[5] (3.98 g, 0.016 mol) afforded 6.5 g (91%) of the oily BOC-**1a**. This compound (5.4 g) was deprotected using the general procedure which resulted in a hygroscopic hydrochloride. This was decomposed in chloroform by saturation with NH_3. The separated NH_4Cl was filtered off (a layer of celite), the filtrate was evaporated and the oily base **1a** (2.9 g, 51%) was transformed to the bis(hydrogen oxalate), m.p. 180–182.5 °C (methanol), $[\alpha]_D^{20}$ –16.8° (*c* 0.5, water). ^{13}C NMR spectrum: 168.44 s (CO), 164.78 s (oxalic acid), 147.82 s (C-1"), 130.42 d (C-3"), 128.25 d (C-5"), 127.58 s (C-2"), 124.59 d (C-6"), 121.00 d (C-4"), 58.93 d (C-1), 53.63 t (C-7), 51.47 t (C-2′, C-6′), 48.33 t (C-3′, C-5′), 45.34 t (C-4), 36.45 t (C-5), 29.43 t (C-6), 23.98 t (C-3), 23.68 t (C-2). For $C_{22}H_{31}ClN_4O_9$ (531.0) calculated: 49.77% C, 5.88% H, 6.67% Cl, 10.55% N; found: 49.59% C, 6.04% H, 6.99% Cl, 10.34% N.

L-Proline N-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)amide (**1b**). The use of 1-(3-aminopropyl)-4-(3-chlorophenyl)piperazine[6] (7.6 g, 0.03 mol) led (without deprotection) to 12.6 g of mixture which was separated by chromatography on silica gel. There were obtained first 2.28 g of unreacted *N*-BOC-L-proline *N*-hydroxysuccinimide ester, m.p. 136–138 °C (ref.[12] gave the m.p. 135–136 °C). This was followed by 9.56 g (76%) of homogeneous oily BOC-**1b**. ^{13}C NMR spectrum: 172.02 s (CO of BOC), 154.77 s (CO), 151.86 s (C-1"), 134.45 s (C-3"), 129.60 d (C-5"), 118.69 d (C-4"), 115.26 d (C-2"), 113.39 d (C-6"), 79.70 s (O–C– of BOC), 60.20 d (C-1), 56.02 t (C-7), 52.59 t (C-2′and C-6′), 48.10 t (C-3′and C-5′), 46.76 t (C-4), 37.87 t (C-5), 29.35 t (C-2), 28.01 q (CH_3 of BOC), 25.69 t (C-3), 23.75 t (C-6). Oily BOC-**1b** was deprotected by stirring for 1.5 h with ethanol saturated with HCl (23 ml). After the addition of ether, the separated product was isolated by decantation, stirred with propanol (10 ml) and filtered after standing overnight in a refrigerator. Yield 5.75 g (41%) of **1b** trihydrochloride, m.p. 137–140 °C (2-propanol–ethanol), $[\alpha]_D^{20}$ –20.0° (*c* 1, water). IR spectrum: 950, 1 255, 1 455 (CH_2); 1 568 (amide); 1 598 (arom.); 1 685 (C=O amide); 2 210–3 080 (NH^+); 2 935, 2 980 (aliph. C–H); 3 080 (arom.); 3 230 (NH). ^{13}C NMR spectrum: 167.99 s (CO), 163.81 s (oxalic acid), 151.18 s (C-1"), 133.71 s (C-3"), 130.19 d (C-5"), 118.47 d (C-4"), 114.73 d (C-2"), 113.69 d (C-6"), 58.93 d (C-1), 53.78 t (C-7), 50.94 t (C-2′and C-6′), 45.71 t (C-3′and C-5′), 45.27 t (C-4), 36.53 t (C-5), 29.06 t (C-2), 24.05 t (C-6), 23.23 t (C-3). For $C_{18}H_{30}Cl_4N_4O$ (460.2) calculated: 46.96% C, 6.57% H, 30.82% Cl, 12.17% N; found: 47.09% C, 6.63% H, 30.55% Cl, 12.03 % N.

L-Proline N-(3-(4-(4-chlorophenyl)piperazin-1-yl)propyl)amide (**1c**). The use of 1-(3-aminopropyl)-4-(4-chlorophenyl)piperazine[5] (6.3 g, 0.025 mol) resulted in 8.2 g (72%) of crystalline BOC-**1c**, m.p.134–136 °C (cyclohexane), $[\alpha]_D^{20}$ –30.43° (*c* 0.5, ethanol). ^1H NMR spectrum: 7.20 d, 2 H, J = 8.5 (H-3", H-5"); 6.85 d, 2 H, J = 8.5 (H-2", H-6"); 4.10 bm, 1 H (H-1); 3.40 bm, 4 H (2 × H-4, 2 × H-5); 3.18 bt, 4 H (2 × H-3′ and 2 × H-5′); 2.58 bt, 4 H (2 × H-2′ and 2 × H-6′); 2.46 t, 2 H (2 × H-7); 1.46 s, 9 H ($(CH_3)_3CO$). For $C_{23}H_{35}ClN_4O_3$ (451.0) calculated: 61.25% C, 7.82% H, 7.86% Cl, 12.42% N; found: 61.37% C, 8.05% H, 7.95% Cl, 12.10% N. Deprotection of BOC-**1c** (5.5 g) with ethanolic HCl gave 6.l g (53%) **1c** trihydrochloride, m.p. 154–158 °C (ethanol–methanol), $[\alpha]_D^{20}$ –20.12° (*c* 1, water). For $C_{18}H_{30}Cl_4N_4O$ (460.2) calculated: 46.96% C, 6.57% H, 30.82% Cl, 12.17% N; found: 46.87% C, 6.68% H, 30.65% Cl, 12.08% N.

D-Proline N-(3-(4-(3-chlorophenyl)piperazin-1-yl)propyl)amide (**2**). BOC-D-Proline (2.97 g, 0.014 mol) and 1-(3-aminopropyl)-4-(3-chlorophenyl)piperazine[6] (3.5 g, 0.014 mol) gave similarly 5.3 g of oily BOC-**2**, which was purified by chromatography on silica gel: 2.95 g (47%) of homogenous oily BOC-**2** whose ^{13}C NMR spectrum was identical with that of BOC-**1b**. Treatment of 2.75 g BOC-**2** with ethanolic HCl (6.2 ml) gave l.7 g of 2 trihydrochloride, m.p. 138–142 °C (2-propanol–ethanol). $[\alpha]_D^{20}$ +19.27° (*c* 1, water). IR spectrum: 950, 1 255, 1 455 (CH_2); 1 568 (amide); 1 598 (arom.); 1 685 (C=O of amide); 2 210–3 230 (NH^+); 2 935, 2 980 (aliph. C–H); 3 080 (arom.); 3 230 (NH). ^{13}C NMR spectrum: 168.21 s (C=O), 150.87 s (C-1"), 134.00 s (C-3"), 130.72 d (C-5"), 119.40 d (C-4"), 115.33 d (C-2"), 114 .28 d (C-6"), 58.93 d (C-1), 53.26 t (C-7), 50.42 t (C-2′, C-6′), 45.34 t (C-3′, C-5′), 44.82 t (C-4), 36.47 t (C-5), 29.58 t (C-6), 23.60 t (C-2), 23.30 t (C-3). Mass spectrum, *m/z* (%): 350 (M^+, $C_{18}H_{27}ClN_4O$, 0.3), 335 (1), 280 (1.8), 209 (5), 198 (6), 184 (31), 167 (10), 113 (8), 86 (9), 84 (13), 70 (100), 36 (HCl). For $C_{18}H_{30}Cl_4N_4O$ (460.2) calculated: 46.96% C, 6.57% H, 30.82% Cl, 12.17% N; found: 46.86% C, 6.55 % H, 30.55% Cl, 12.08% N.

L-Proline N-(2-(morpholin-4-yl)ethyl)amide (**3a**). From 4-(2-aminoethyl)morpholine (3.84 g, 0.03 mol), 8.16 g (83%) of crude BOC-**3a** was obtained and the deprotection with releasing the base gave 4.28 g (73%) of oily **3a**. Bis(hydrogen oxalate), m.p. 127–129 °C (ethanol), $[\alpha]_D^{20}$ –10.68° (*c* 1, water). ^{1}H NMR spectrum: 9.45 bs, 5 H (NH and oxalate); 8.65 bt, 1 H (CONH); 4.20 bt, l H (H-1); 3.70 bt, 4 H (2 × H-2′ and 2 × H-3′); 3.33 m, 4 H and 2.77 m, 6 H (2 × H-4, 2 × H-5, 2 × H-6, 2 × H-1′ and 2 × H-4′); 2.41–1.78 bm, 4 H (2 × H-2 and 2 × H-3). For $C_{15}H_{25}N_3O_{10}$ (407.4) calculated: 44.22% C, 6.18% H, 10.31% N; found: 43.94% C, 6.13% H, 10.30% N.

L-Proline N-(2-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)ethyl)amide (**3b**). The use of 1-(2-aminoethyl)-4-((3-trifluoromethyl)phenyl)piperazine[8] (4.1 g, 0.015 mol) led to 7.02 g of crude BOC-**3b** which was subjected to treatment with ethanolic HCl. The hydrochloride obtained was hygroscopic, the base (3.88 g, 73 %) was released and transformed to the tris(hydrogen oxalate), m.p. 149–151 °C (ethanol); $[\alpha]_D^{20}$ –9.52° (water). ^{1}H NMR spectrum: 8.85 bs (oxalic acid, CONH); 7.56–7.04 m, 4 H (H-arom.); 4.21 m, 1 H (H-1); 3.60–1.76 m, 18 H (CH_2). Mass spectrum, *m/z* (%): 370 (M^+, $C_{18}H_{25}F_3N_4O$), 355, 301, 300, 256, 243, 229, 200, 170, 145, 113, 70, 45. For $C_{24}H_{31}FN_4O_{13}$ (640.5) calculated: 45.00% C, 4.88% H, 8.90% F, 8.75% N; found: 44.65% C, 4.88% H, 8.82% F, 8.44% N.

L-Proline N-(4-(morpholin-4-yl)butyl)amide (**4a**). 4-(4-Aminobutyl)morpholine[7] (4.68 g, 0.03 mol) gives similarly 10.48 g (98%) of BOC-**4a** which was treated with ethanolic HCl and afforded with NH_3 in chloroform 7.6 g (97%) of crude **4a**. Bis(hydrogen oxalate) hemihydrate, m.p. 142–143 °C (ethanol), $[\alpha]_D^{20}$ –23.5° (*c* 1, water). ^{1}H NMR spectrum: 8.53 bt, 1 H, *J* = 6.6 (CONH); 4.17 bt, 1 H (H-1); 3.77 bt, 4 H (2 × H-2′and 2 × H-3′); 3.37–2.71 m, 10 H (2 × H-4, 2 × H-5, 2 × H-8, 2 × H-1′, 2 × H-4′); 1.91 m, 4 H (2 × H-2, 2 × H-3); 1.56 m, 4 H (2 × H-6, 2 × H-7). For $C_{17}H_{29}N_3O_{10}$. 0.5 H_2O (444.4) calculated: 45.94% C, 6.80% H, 9.45% N; found: 45.92% C, 6.77% H, 9.37% N.

L-Proline N-(4-(4-(3-(trifluoromethyl)phenyl)piperazin-1-yl)butyl)amide (**4b**). 1-(4-Aminobutyl)-4-((3-trifluoromethyl)phenyl)piperazine[9] (6.03 g, 0.02 mol) gave similarly 9.35 g (96%) of crude BOC-**4b** which afforded by the deprotection procedure a hygroscopic hydrochloride. The released base **4b** was transformed to the bis(hydrogen oxalate), m.p. 149–150.5 °C (ethanol), $[\alpha]_D^{20}$ –18.14° (*c* 1, water). ^{1}H NMR spectrum: 8.51 bt, 1 H (CONH); 7.79 bs, 4 H (oxalic acid); 7.60–7.04 m, 4 H (H-arom.); 4.18 bt, 1 H (H-1); 3.59–1.42 m, 22 H (11 × CH_2). For $C_{24}H_{33}F_3N_4O_9$ (578.6) calculated: 49.82% C, 5.75% H, 9.85% F, 9.68% N; found: 49.88% C, 5.77% H, 9.83% F, 9.64% N.

L-Proline 4-(2-fluorophenyl)piperazide (**5a**). From 1-(2-fluorophenyl)piperazine (5.40 g, 0.03 mol), 12.06 g of crude BOC-**5a** (partly crystallizing yellow oil) was obtained which was subjected to the action of ethanolic HCl. The obtained **5a** hydrochloride proved inhomogeneous and the base, therefore, was released: homogeneous oil (TLC). ^{1}H NMR spectrum: 7.00 m, 4 H (H arom.); 3.60–4.05 bm, 5 H (H-1, 2 × H-1′, 2 × H-4′); 2.75–3.30 bm and 2.78 bs, 7 H (N-H, 2 × H-4, 2 × H-2′ and 2 × H-3′); 1.60–2.30 bm, 4 H (2 × H-2, 2 × H-3).

Hydrogen oxalate: m.p. 137.5–139 °C (2-propanol), $[\alpha]_D^{20}$ –59.3° (*c* 1, water). For $C_{17}H_{22}FN_3O_5$ (367.4) calculated: 55.58 % C, 6.04% H, 5.17% F, 11.44% N; found: 55.42% C, 6.08% H, 5.31% F, 11.37% N.

L-Proline 4-(3-fluorophenyl)piperazide (**5b**). 1-(3-Fluorophenyl)piperazine[10] (5.4 g, 0.03 mol) gave similarly 10.8 g (95%) of BOC-**5b** which was subjected to deprotection with ethanolic HCl. From the unstable hydrochloride, the oily base (6.55 g, 78%) was released. ^{1}H NMR spectrum: 7.18 m, 1 H (H-2"); 6.62 m, 3 H (H-4", H-5", H-6"); 2.92 m and 2.84 s, 2 H (H-1, N-H); 2.27–1.53 bm, 4 H (2 × H-2, 2 × H-3); 4.08–3.51 bm and 3.18 bt, 10 H (H of piperazine ring and 2 × H-4).

Hydrogen oxalate, m.p. 192–193.5 °C (ethanol–methanol), $[\alpha]_D^{20}$ –63.06° (*c* 1, water). Mass spectrum, *m/z* (%): 277 (M^+, $C_{15}H_{20}FN_3O$, 0.7), 165 (2), 152 (1), 138 (2.3), 122 (2), 70 (100). For $C_{17}H_{22}FN_3O_5$ (367.4) calculated: 55.58% C, 6.04% H, 5.17% F, 11.44% N; found: 55.48% C, 6.22% H, 5.36% F, 11.39% N.

L-Proline 4-(4-fluorophenyl)piperazide (**5c**). 1-(4-Fluorophenyl)piperazine (3.6 g, 0.02 mol) gave similarly 7.70 g of BOC-**5c** which afforded by treatment with ethanolic HCl an ustable **5c** hydrochloride. The released base **5c** was transformed to the hydrogen oxalate, m.p. 205–207 °C (ethanol–methanol), $[\alpha]_D^{20}$ –59.88° (*c* 1, water). Mass spectrum, *m/z* (%): 277 (M^+, $C_{15}H_{20}FN_3$, 0.2), 165 (3), 152 (2.5), 138 (3), 122 (2.5), 70 (100). For $C_{17}H_{22}FN_3O_5$ (367.4) calculated: 55.58% C, 6.04% H, 5.17% F, 11.44% N; found: 55.40% C, 6.13% H, 5.27% F, 11.27% N.

The homogeneous base **5c**, released from the oxalate, crystallized and melted at 109–112 °C (cyclohexane), $[\alpha]_D^{20}$ –77.24° (methanol). ^{1}H NMR spectrum: 6.95 m, 4 H (H-arom.); 3.50–4.00 nm, 5 H (H-1, 2 × H-1′, 2 × H-4′); 3.08 bt, 4 H (2 × H-2′, 2 × H-3′); 2.56 s, 1 H (N-H); 1.50–1.90 nm, 4 H (2 × H-2, 2 × H-3); 2.70–3.40 bm and 1.90–2.30 bm, 2 H (2 × H-4). For $C_{15}H_{20}FN_3O$ (277.3) calculated: 64.96% C, 7.27% H, 6.85% F, 15.15% N; found: 64.90% C, 7.37% H, 6.91% F, 14.93% N.

L-Proline 4-(3-chlorophenyl)piperazide (**5d**). l-(3-Chlorophenyl)piperazine (3.1 g, 0.016 mol) gave similarly 6.0 g of crude BOC-**5d** which was cleaved by treatment with ethanolic HCl. From the hygroscopic hydrochloride obtained, the base **5d** was released and transformed to the hydrogen oxalate, m.p. 189–190 °C (2-propanol–ethanol), $[\alpha]_D^{20}$ –60.93° (*c* 1, water). ^{1}H NMR spectrum: 6.80–7.40 m, 4 H (H-arom.); 4.65 bt, 1 H (H-1); 3.63 m and 3.25 bt, 8 H (H of piperazine); 2.20–2.40 bm, 2 H (2 × H-4); 1.90 m, 4 H (2 × H-2, 2 × H-3). For $C_{17}H_{22}ClN_3O_5$ (383.8) calculated: 53.20% C, 5.78% H, 9.28% Cl, 10.95% N; found: 53.01% C, 5.86% H, 9.36% Cl, 11.04 % N.

L-Proline 4-(3-(trifluoromethyl)phenyl)piperazide (**5e**). From 1-(3-(trifluoromethyl)phenyl)piperazine (4.6 g, 0.02 mol), 7.5 g (87%) of crude BOC-**5e** were obtained which was transformed by treatment with ethanolic HCl to the crude **5e** hydrochloride. The released base **5e** crystallized on standing, m.p. 105.5–108 °C (cyclohexane), $[\alpha]_D^{20}$ –59.88° (*c* 1, water). ^{1}H NMR spectrum: 6.95–7.43 m, 4 H (H-arom.); 3.80 m, 6 H (2 × H-4, 2 × H-1′, 2 × H-4′); 3.25 bt, 4 H (2 × H-2′, 2 × H-3′); 2.90 m, 1 H (H-1); 2.56 s, 1 H (N-H); 1.80–2.30 m, 4 H (2 × H-2, 2 × H-3). For $C_{16}H_{20}F_3N_3O$ (327.4) calculated: 58.70% C, 6.16% H, 17.41% F, 12.84% N; found: 58.73% C, 6.31% H, 17.16% F, 12.57% N.

Hydrogen oxalate, m.p. 180–181 °C (ethanol), $[\alpha]_D^{20}$ –56.1° (*c* 1, water). Mass spectrum, *m/z* (%): 327 (M^+, $C_{16}H_{20}F_3N_3O$, 0.4), 288 (0.3), 215 (0.9), 200 (0.8), 188 (1.9), 172 (1.2), 145 (1.4), 70 (100). For $C_{18}H_{22}F_3N_3O_5$ (417.4) calculated: 51.80 % C, 5.31% H, 13.66% F, 10.07% N; found: 51.29% C, 5.33 % H, 13.51% F, 9.89% N.

L-Proline 4-(4-(trifluoromethyl)phenyl)piperazide (**5f**). From 1-(4-trifluoromethyl)phenyl)piperazine[11] (2.99 g, 0.013 mol) 5.56 g of oily BOC-**5f** were obtained which was subjected to the action of ethanolic HCl and from the crude **5f** hydrochloride, the base **5f** (3.08 g, 72%) was released, m.p. 127–130 °C (cyclohexane). ^{1}H NMR spectrum: 7.51 d, 2 H, *J* = 8.5 (H-3", H-5"); 6.93 d, 2 H, *J* = 8.5 (H-2", H-6"); 3.40–4.04 bm, 4 H (2 × H-1′, 2 × H-4′); 3.02–3.40 bm, 4 H (2 × H-2′, 2 × H-3′); 2.66 bs,

1 H (N-H); 2.70–3.00 bm and 1.50–2.30 bm, 7 H (H of pyrrolidine). For $C_{16}H_{20}F_3N_3O$ (327.4) calculated: 58.70% C, 6.16% H, 17.41% F, 12.84% N; found: 58.55% C, 6.09% H, 17.18% F, 12.66% N.

Hydrogen oxalate, m.p. 173–176 °C (2-propanol–ethanol), $[\alpha]_D^{20}$ –55.7° (*c* 1, water). ^{1}H NMR spectrum: 7.53 d, 2 H, *J* = 8.9 (H-3", H-5"); 7.09 d, 2 H, *J* = 8.9 (H-2", H-6"); 4.67 bt, 1 H (H-1); 3.64 m and 3.37 m, 8 H (H of piperazine); 2.40–1.72 bm, 6 H (2 × H-2, 2 × H-3, 2 × H-4). For $C_{18}H_{22}F_3N_3O_5$ (417.4) calculated: 51.80% C, 5.31% H, 13.66% F, 10.07% N; found: 51.47% C, 5.25% H, 13.54% F, 9.78% N.

1-(tert-Butyloxycarbonyl)-L-proline 4-(4-fluorobenzoyl)piperidide (**6a**). A similar sequence starting from 4-(4-fluorobenzoyl)piperidine[13] (4.7 g, 0.024 mol) resulted in a mixture which was separated by chromatography on silica gel (130 g). The first to be eluted with chloroform was the crystalline **6a** (3.8 g, 39%), m.p. 116.5–118 °C (ether–cyclohexane). ^{1}H NMR spectrum: 7.97 dd, 2 H, *J* = 8.5 (H-2 and H-6 of aryl); 7.15 t, 2 H, *J* = 8.5 (H-3 and H-5 of aryl); 1.44 s, 9 H ($(CH_3)_3C$). Mass spectrum, *m/z* (%): 404 (M^+, $C_{22}H_{29}FN_2O_4$, 1), 331 (3), 303 (1.2), 234 (3), 206 (3.2), 191 (2), 190 (2.4), 170 (21), 123 (21), 114 (88), 95 (8), 70 (100), 57 (70). For $C_{22}H_{29}FN_2O_4$ (404.5) calculated: 65.33% C, 7.23% H, 4.70% F, 6.93% N; found: 65.53% C, 7.30% H, 4.81% F, 6.83 % N.

Continued elution with a mixture of chloroform and ethanol (20 : 1) gave 2.4 g (17%) of crystalline 1-(*tert*-butyloxycarbonyl)-L-proline 4-(4-(4-(4-fluorobenzoyl)piperidin-1-yl)benzoyl)piperidide (**6b**), m.p. 201–205 °C (benzene–cyclohexane). For $C_{34}H_{42}FN_3O_5$ (591.7) calculated: 69.01% C, 7.15% H, 3.21% F, 7.10% N; found: 68.72% C, 7.15% H, 2.80% F, 7.01% N.

L-Proline 4-(4-fluorobenzoyl)piperidide (**7a**). A solution of **6a** (2.3 g, 0.0056 mol) in ethanol (100 ml) was treated with 10% solution of HCl in ether (10 ml) and the mixture was allowed to stand for 3 days at room temperature. It was then evaporated in vacuo. The residue was made alkaline with 5% NaOH and the product was extracted with ether. After drying with K_2CO_3, the solution was evaporated giving 1.05 g (62%) of crude **7a** which was transformed to the hydrogen succinate, m.p. 111–114 °C (water). Mass spectrum, *m/z* (%): 305 (M + 1), 304 (M^+, $C_{17}H_{21}FN_2O_2$, chemical ionization), 303, 234, 206, 170, 123, 114, 95, 70, 57. For $C_{21}H_{27}FN_2O_6$ (422.5) calculated: 59.70% C, 6.44% H, 4.50% F, 6.63% N; found: 60.50% C, 6.11% H, 4.60% F, 6.78% N.

L-Proline 4-(4-(4-(4-fluorobenzoyl)piperidin-1-yl)benzoyl)piperidide (**7b**). A solution of **6b** (0.9 g, 0.0015 mol) in ethanol (100 ml) was treated with 10% solution of HCl in ether (2 ml), after standing for 2 days the mixture was evaporated in vacuo, and the residue was distributed by shaking between benzene and 5% NaOH. The organic layer was dried and evaporated in vacuo. Crystallization of the residue from a mixture of benzene and cyclohexane gave 0.69 g (90%) of **7b**, m.p. 163–167.5 °C. For $C_{21}H_{27}FN_2O_6$ (422.5) calculated: 71,55% C, 6.81% H, 3.77% F, 8.34% N; found: 71.06% C, 6.83% H, 3.60% F, 8.37% N.

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REFERENCES

1. Valenta V., Dobrovsky K., Krejci I., Polivka Z.: Collect. Czech. Chem. Commun. *61*, 1077 (1996).
2. Andersen G. W., McGregor A. C.: J. Am. Chem. Soc. *79*, 6180 (1957).

3. Schnabel E.: Justus Liebigs Ann. Chem. *702*, 188 (1967).
4. Bodanszky M. in: *The Peptides* (E. Gross and J. Meienhofer, Eds), Vol. 1, p. 128. Academic Press, New York 1979.
5. Hayao S., Schut R. N.: J. Org. Chem. *26*, 3414 (1961).
6. Wu Y. H. (Mead Johnson): U.S. 3,398,151; Chem. Abstr. *70*, 4143 (1969).
7. Utermohlen W. P., Hamilton C. S.: J. Am. Chem. Soc. *63*, 156 (1941).
8. De Antoni J., Eche R. (Laboratoires Bruneau): Fr. Addn. 93 884; Chem. Abstr. *72*, 79094 (1970).
9. Turconi M., Bietti G., Giraldo E., Borsini F., Bignotti M. (Boehringer Ingelheim Italia): Eur. Pat. Appl. 526 434; Chem. Abstr. *118*, 234092 (1993).
10. Janssen P. A. J.: U.S. 2,979,507; Chem. Abstr. *55*, 18785 (1961).
11. Bysouth P. T., Clarke R. W. (BDH Pharmaceuticals Ltd.): Ger. Offen. 2,024,826; Chem. Abstr. *74*, 53849 (1971).
12. Anderson G. W., Zimmerman J. E., Callahan F. N.: J. Am. Chem. Soc. *86*, 1839 (1964).
13. Duncan R. L., Helsley G. C., Welstead W. J., Da Vanzo J. P., Funderburk W. H., Lunsdorf C. D.: J. Med. Chem. *13*, 1 (1970).