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Synthesis of (*2S,4S*)-4-phenylamino-5-oxoproline derivatives

I. A. Nizova, V. P. Krasnov, G. L. Levit, and **M. I. Kodess**

Institute of Organic Synthesis, Ural Division of Russian Academy of Sciences, Ekaterinburg, Russia

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Summary. The paper describes the synthesis of (*2S,4S*)-4-(N-Ts)- and (*2S,4S*)-4-(N-Boc)-phenylamino-5-oxoprolines (pyroglutamic acid). These derivatives have been shown to be useful for synthesis of their amides and peptides in spite of steric hindrances caused by bulky groups adjacent to the reaction centre. Under the conditions applied no lactam ring opening and no loss of stereochemical integrity of any of the chiral centres were observed, which has been confirmed by NMR techniques.

Keywords: Amino acids – Pyroglutamic acid – Amides – Peptides – Protecting groups – NMR spectroscopy

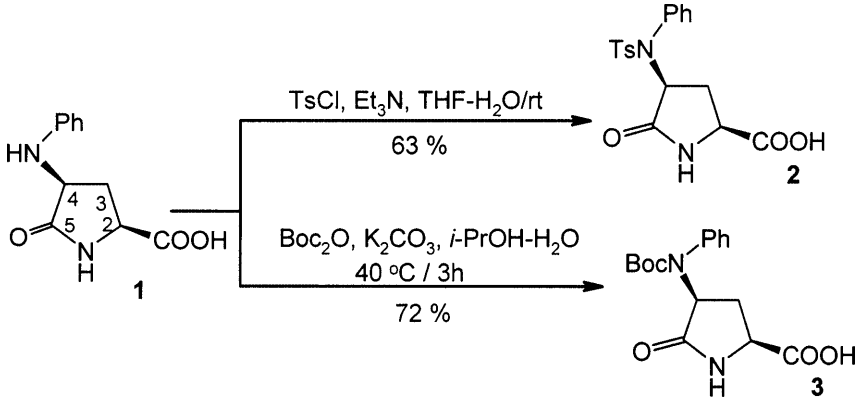
Introduction

C-4 Derivatives of glutamic acid are of considerable importance in metabolic processes. Recently, new approaches to stereoisomers of 4-aminoglutamic acid and its derivatives were reported by Mulzer et al. (1994), Funaki et al. (1996), Javidan et al. (1997), and Krasnov et al. (1997). In particular, we synthesized the stereoisomers of 4-arylamino-5-oxoprolines (Krasnov et al., 1993). These compounds being structurally similar to pyroglutamic acid could be used as synthons for the preparation of agents closely related to natural metabolites such as peptide hormones, neuropeptides and their fragments which participate in regulation of important physiological processes and exhibit nootropic activity. Modification of these compounds would allow to overcome such disadvantages of mediatory amino acids and natural neuropeptides as poor penetration through the blood-brain barrier, fast metabolism and undesirable effects on endocrine system.

In this paper we report a synthesis of (*2S,4S*)-4-phenylamino-5-oxoproline (**1**) derivatives with a selectively blocked phenylamino function to study their application for preparation of amides and peptides.

Results and discussion

Pyroglutamic acid is generally used in peptide synthesis without protection of α-NH-group. However, the presence of a rather reactive side chain, the phenylamino group, in acid **1** along with poor solubility in organic solvents makes it difficult to use it without additional protection. So, we have prepared 4-N-Ts- (**2**) and 4-N-Boc-blocked (**3**) derivatives of (*2S*,*4S*)-4-phenylamino-5-oxoproline (Scheme 1). Ts derivative **2** was obtained on treatment of acid **1** by



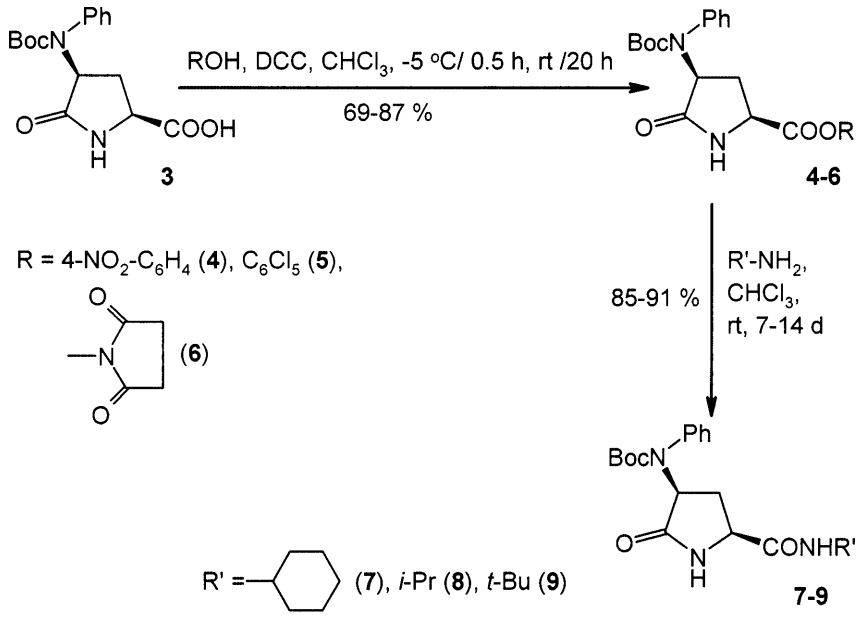
Scheme 1. Synthesis of (*2S*,*4S*)-4-(N-Ts)- (**2**) and (*2S*,*4S*)-4-(N-Boc)-phenylamino-5-oxoprolines (**3**)

tosyl chloride in aqueous THF in the presence of TEA. Introduction of Ts residue proceeded selectively, only at the 4-amino function and was not accompanied by lactam ring opening. Compound **2** incorporates all the advantages of Ts derivatives: it is easily obtained and crystallized, and is stable even in very acidic medium. However, the removal of Ts residue requires rather drastic conditions, which could result in a lactam ring opening. Along with compound **2** we obtained derivative **3** with an easily removable Boc group.

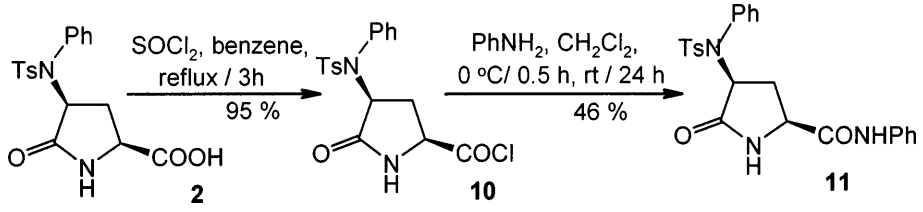
Introduction of the Boc residue was carried out by interaction of acid **1** with Boc_2O in aqueous propan-2-ol in the presence of K_2CO_3. Under these conditions the protection proceeded selectively, only at phenylamino function.

The position of the protecting groups in compounds **2**, **3** has been proved by ^1H NMR spectral data based on characteristic signals of the protons at C-4 and C-2 atoms, the spectra showed a double doublet at δ 4.72 and 4.82 ppm, respectively, due to the 4-H proton indicating splitting only on CH_2-3 protons, and a double double doublet at δ 4.27 and 4.41 ppm, respectively, due to the 2-H proton.

We supposed that the most convenient synthetic approach to aliphatic amides of the modified pyroglutamic acid is the "active ester" method. Therefore, we have prepared 4-nitrophenyl (**4**), pentachlorophenyl (**5**), and N-hydroxysuccinimide (**6**) esters of Boc-protected acid **3** by DCC mediated coupling in EtOAc or $CHCl_3$ (Scheme 2). Aminolysis of active esters **4–6** gave



Scheme 2. Synthesis of (*2S*,*4S*)-4-(N-Boc)phenylamino-5-oxoproline amides (**7–9**)



Scheme 3. Synthesis of (*2S*,*4S*)-4-(N-Ts)phenylamino-5-oxoproline anilide (**11**)

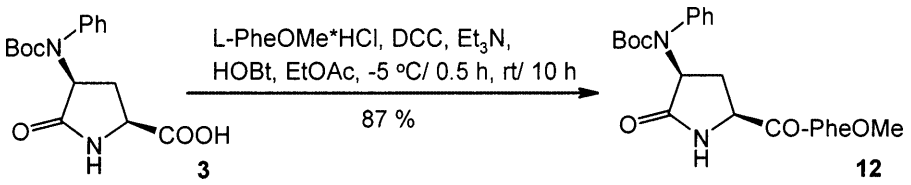
rise to the corresponding amides **7–9**. Because of the steric hindrance the nucleophilic substitution at the C atom of active esters with amines proceeded slowly within 7–14 days at room temperature.

The attempt to obtain anilide of compound **3** by this approach failed due to the weak nucleophilicity of aniline. Anilide **11** was obtained from acid chloride **10** and aniline in CH_2Cl_2 (Scheme 3). Acid chloride **10** was prepared from acid **2** and thionyl chloride under reflux in benzene.

No racemization was observed during the synthesis of compounds **2–11**. This conclusion was made using ^{1}H NMR spectral data based on the absence of additional signals of the *erythro* stereoisomer which could be formed due to loss of optical activity at any chiral centre.

The retention of *cis*-configuration in the obtained compounds was confirmed by 1D NOE-difference experiment for compound **11**. NOE enhancements of the H-2 (δ 4.37 ppm) and the H-4 (δ 4.83 ppm) were observed with the saturated proton H^B-3 (δ 1.75 ppm).

We have demonstrated the possibility to use compound **3** for the synthesis of peptides by its reaction with L-phenylalanine methyl ester by DCC medi-



Scheme 4. Synthesis of (*2S,4S*)-4-(N-Boc)phenylamino-5-oxoprolyl-(*S*)-phenylalanine methyl ester (**12**)

ated coupling in the presence of 1-hydroxybenzotriazole (HOBt) (Scheme 4) yielding peptide **12**.

In conclusion, 4-substituted 5-oxoprolines have been shown to be useful for synthesis of their amides and peptides in spite of steric hindrance caused by bulky groups adjacent to the reaction centre. The reactions described occur without loss of optical activity and lactam ring opening.

Materials and methods

All solvents used were distilled prior to use. Thin layer chromatography was carried out on Silufol UV 254 plates using the following eluents: A: benzene:acetone:acetic acid (12:1:0.01); B: chloroform:acetone (4:1). Column chromatography was performed on Chemapol silica gel 40/100 μ. Melting points (mps) were measured on a Boetius apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Tesla BS-567 A (100 MHz), Tesla BS-587 A (80 MHz) or Bruker DRX-400 (400 MHz) instruments using TMS as an internal standard. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. The optical rotations were measured on a A1 EPO polarimeter (Russia). Microanalyses were performed on a CHNS-O model EA-1102 elemental analyzer. 4-Phenylamino-5-oxoproline **1** was synthesized following the literature procedure (Krasnov et al., 1993).

*1. (2S,4S)-4-(N-Tosyl)phenylamino-5-oxoproline (**2**)*

To a stirred solution of **1** (5.0 g, 22.7 mmol) and Et_3N (10 mL, 71.7 mmol) in a mixture of water (90 mL) and THF (45 mL) *p*-tosyl chloride (6.5 g, 34.1 mmol) was added portionwise over 30 min and the reaction mixture was stirred at r.t. for 2–5 h. THF was evaporated in vacuo, and the residue was diluted with water followed by filtration. pH of the filtrate was adjusted to 2 with 5% HCl. Filtration of the precipitate afforded 5.36 g (63%) of **2** with mp 241–243°C; TLC: R_f = 0.45 (A). $[\alpha]_D^{20}$-6.8° (c 1.0, acetone).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.27 (d, 1H, $J_{NH\text{-}2}$ = 8.7, NH), 7.76-7.14 (m, 9H, arom.), 4.72 (dd, 1H, $J_{4\text{-}3B}$ = 9.4, $J_{4\text{-}3A}$ = 7.0, H-4), 4.27 (ddd, 1H, $J_{2\text{-}3B}$ = 10.5, $J_{2\text{-}3A}$ = $J_{2\text{-}NH}$ = 8.7, H-2), 2.45 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.0, $J_{3A\text{-}2}$ = 8.7, $J_{3A\text{-}4}$ = 7.0, H^A-3), 2.38 (s, 3H, CH_3), 1.66 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.0, $J_{3B\text{-}2}$ = 10.5, $J_{3B\text{-}4}$ = 9.4, H^B-3).

Anal. Calcd for $C_{18}H_{18}N_2O_5S$: C 57.75; H 4.81; N 7.49; S 8.56. Found: C 57.90; H 4.85; N 7.49; S 8.42.

*2. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline (**3**)*

To a stirred solution of **1** (5.0 g, 22.7 mmol) and K_2CO_3 (3.41 g, 24.7 mmol) in water (22 mL) a solution of Boc_2O (6.27 g, 22.8 mmol) in *i*-PrOH (9 mL) was added and the reaction mixture was stirred at 40°C for 3 h. The reaction mixture was diluted with water

and extracted two times with hexane. The aqueous layer was acidified with citric acid to pH 4. The precipitate was filtered off and recrystallized from EtOH to give 5.24 g (72%) of **3**, mp 220–222°C (dec.). TLC: R_f = 0.50 (A). $[\alpha]_D^{20}$-29.5° (c 1.0, acetone).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.43-7.35 (m, 4H, arom.), 7.31 (d, 1H, $J_{NH\text{-}2}$ = 8.9, NH), 7.16 (tt, 1H, J = 7.0, 1.6, *p*-Harom), 4.82 (dd, 1H, $J_{4\text{-}3B}$ = 9.4, $J_{4\text{-}3A}$ = 7.0, H-4), 4.41 (ddd, 1H, $J_{2\text{-}3B}$ = 11.0, $J_{2\text{-}3A}$ = $J_{2\text{-}NH}$ = 8.9, H-2), 2.71 (ddd, 1H, $J_{3A\text{-}3B}$ = 11.2, $J_{3A\text{-}2}$ = 8.9, $J_{3A\text{-}4}$ = 7.0, H^A-3), 1.91 (ddd, 1H, $J_{3A\text{-}3B}$ = 11.2, $J_{3B\text{-}2}$ = 11.0, $J_{3B\text{-}4}$ = 9.4, H^B-3), 1.41 (s, 9H, $C(CH_3)$).

Anal. Calcd for $C_{16}H_{20}N_2O_5$: C 60.00; H 6.25; N 8.75. Found: C 60.17; H 6.27; N 8.45.

*3. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline 4-nitrophenyl ester (**4**)*

To a stirred mixture of **3** (0.91 g, 2.84 mmol) and *p*-nitrophenol (0.4 g, 2.88 mmol) in EtOAc (30 mL) a solution of DCC (0.59 g, 2.86 mmol) in EtOAc (10 mL) was added dropwise at −5–10°C and the reaction mixture was stirred at −50°C for 30 min, then at r.t. for 20 h. The precipitate was filtered off and the filtrate was washed carefully with 2 N Na_2CO_3 and water, and dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was recrystallized from EtOH to give 1.1 g (87%) of **4**, mp 182–185°C; TLC: R_f = 0.77 (B). $[\alpha]_D^{20}$-30.8° (c 1.0, acetone).

^{1}H NMR (100 MHz, $CDCl_3$): δ = 8.19 (d, 2H, J = 9.2, C_6H_4), 7.49-7.26 (m, 5H, C_6H_5), 6.97 (d, 2H, J = 9.2, C_6H_4), 5.33 (d, 1H, $J_{2\text{-}NH}$ = 5.2, NH), 5.03 (dd, 1H, $J_{4\text{-}3B}$ = 8.8, $J_{4\text{-}3A}$ = 7.7, H-4), 4.50 (ddd, 1H, $J_{2\text{-}3B}$ = 8.8, $J_{2\text{-}3A}$ = 7.7, $J_{2\text{-}NH}$ = 5.2, H-2), 3.24 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.7, $J_{3A\text{-}2}$ = $J_{3A\text{-}4}$ = 7.7, H^A-3), 2.28 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.7, $J_{3B\text{-}2}$ = $J_{3B\text{-}4}$ = 8.8, H^B-3), 1.48 (s, 9H, $C(CH_3)$).

Anal. Calcd for $C_{22}H_{23}N_3O_7$: C 59.87; H 5.22; N 9.52. Found: C 59.99; H 5.19; N 9.50.

*4. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline pentachlorophenyl ester (**5**)*

To a stirred solution of **3** (5.0 g, 15.6 mmol) and pentachlorophenol (4.15 g, 15.6 mmol) in $CHCl_3$ (120 mL) a solution of DCC (3.2 g, 15.6 mmol) in $CHCl_3$ (30 mL) was added dropwise at −5–10°C and the reaction mixture was stirred at −5°C for 30 min, at 0°C for 30 min, then at r.t. for 20 h. The reaction mixture was washed five times with 2 N Na_2CO_3. The organic layer was evaporated in vacuo and DMF (50 mL) was added to the residue. The precipitate was filtered off and the filtrate was poured into cold water (250 mL). Filtration of the precipitate afforded 6.75 g (78%) of **5**, mp 226–229°C; TLC: R_f = 0.85 (B).

^{1}H NMR (80 MHz, $CDCl_3$): δ = 7.62-7.22 (m, 5H, C_6H_5), 5.31 (d, 1H, $J_{NH\text{-}2}$ = 5.6, NH), 5.15 (dd, 1H, $J_{4\text{-}3B}$ = 7.9, $J_{4\text{-}3A}$ = 7.3, H-4), 4.52 (ddd, 1H, $J_{2\text{-}3B}$ = $J_{2\text{-}3A}$ = 8.5, $J_{2\text{-}NH}$ = 5.6, H-2), 3.30 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.9, $J_{3A\text{-}2}$ = 8.5, $J_{3A\text{-}4}$ = 7.3, H^A-3), 2.39 (ddd, 1H, $J_{3A\text{-}3B}$ = 12.9, $J_{3B\text{-}2}$ = 8.5, $J_{3B\text{-}4}$ = 7.9, H^B-3), 1.49 (s, 9H, $C(CH_3)$).

Anal. Calcd for $C_{22}H_{19}N_2O_5Cl$: C 46.48; H 3.35; N 4.93; Cl 31.17. Found: C 46.83; H 3.53; N 5.19; Cl 30.94.

*5. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline N-hydroxysuccinimide ester (**6**)*

To a stirred mixture of **3** (1.0 g, 3.13 mmol) and N-succinimide (0.36 g, 3.13 mmol) in $CHCl_3$ (20 mL) a solution of DCC (0.64 g, 3.13 mmol) in $CHCl_3$ (5 mL) was added

dropwise at −5°C and the reaction mixture was stirred at −5°C for 30 min, then at r.t. for 20 h. The solvent was evaporated in vacuo and the residue was treated with DMF (5 mL). The precipitate was filtered off and the filtrate was poured into cold water (50 mL). Filtration of the precipitate afforded 0.9 g (69%) of **6**, mp 195–197°C; TLC: R_f = 0.52 (B).

^{1}H NMR (80 MHz, $CDCl_3$): δ = 7.50-7.29 (m, 5H, C_6H_5), 5.25 (d, 1H, $J_{NH\text{-}2}$ = 6.4, NH), 5.04 (dd, 1H, $J_{4\text{-}3B} = J_{4\text{-}3A}$ = 7.7, H-4), 4.37 (ddd, 1H, $J_{2\text{-}3B} = J_{2\text{-}3A}$ = 8.3, $J_{2\text{-}NH}$ = 6.4, H-2), 3.25 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.0, $J_{3A\text{-}2}$ = 8.3, $J_{3A\text{-}4}$ = 7.7, H^A-3), 2.78 (s, 4H, CH_2—CH_2), 2.28 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.0, $J_{3B\text{-}2}$ = 8.3, $J_{3B\text{-}4}$ = 7.7, H^B-3), 1.48 (s, 9H, $C(CH_3)$).

Anal. Calcd for $C_{20}H_{23}N_3O_7$: C 57.55; H 5.52; N 10.07. Found: C 57.82; H 5.60; N 9.87.

*6. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline cyclohexylamide (**7**)*

To a solution of **5** (1.0 g, 1.76 mmol) in $CHCl_3$ (20 mL) cyclohexylamine (0.22 mL, 1.94 mmol) was added. The reaction mixture was allowed to stay at r.t. for 7 days, then washed carefully with 5% citric acid, water, 5% Na_2CO_3, water and dried ($MgSO_4$). Evaporation of the solvent and column chromatography of the crude product (EtOAc) afforded 0.6 g (85%) of **7**, mp 178–180°C; TLC: R_f = 0.45 (B).

^{1}H NMR (100 MHz, $CDCl_3$): δ = 7.55-7.17 (m, 5H, C_6H_5), 6.82 (m, 1H, amide NH), 5.63 (d, 1H, $J_{NH\text{-}2}$ = 6.8, lactam NH), 4.60 (dd, 1H, $J_{4\text{-}3A}$ = 8.5, $J_{4\text{-}3B}$ = 7.3, H-4), 4.15 (ddd, 1H, $J_{2\text{-}3A}$ = 10.1, $J_{2\text{-}3B}$ = 8.2, $J_{2\text{-}NH}$ = 6.8, H-2), 3.61 (m, 1H, cyclohexyl CH), 2.95 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.6, $J_{3A\text{-}2}$ = 10.1, $J_{3A\text{-}4}$ = 8.5, H^A-3), 2.12 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.6, $J_{3B\text{-}2}$ = 8.2, $J_{3B\text{-}4}$ = 7.3, H^B-3), 1.47 (s, 9H, $C(CH_3)$), 1.98–0.83 (m, 10H, cyclohexyl $(CH_2)_5$).

Anal. Calcd for $C_{22}H_{31}N_3O_4$: C 65.84; H 7.73; N 10.47. Found: C 65.78; H 7.88; N 10.48.

*7. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline i-propylamide (**8**)*

To a solution of **5** (0.35 g, 0.62 mmol) in $CHCl_3$ (10 mL) *i*-propylamine (0.045 mL, 0.68 mmol) was added. The reaction mixture was allowed to stay at r.t. for 11 days, then washed carefully with 5% citric acid, water, 5% Na_2CO_3, water and dried ($MgSO_4$). Evaporation of the solvent and column chromatography of the crude product (chloroform : acetone 4 : 1) afforded 0.20 g (91%) of **8**, mp 198–200°C; TLC: R_f = 0.75 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.73-7.53 (m, 4H, arom), 7.20 (t, 1H, J = 7.4, *p*-Harom), 6.78 (m, 1H, amide NH), 5.56 (d, 1H, $J_{NH\text{-}2}$ = 8.4, lactam NH), 4.59 (dd, 1H, $J_{4\text{-}3A}$ = 8.7, $J_{4\text{-}3B}$ = 7.2, H-4), 4.10 (ddd, 1H, $J_{2\text{-}3A}$ = 10.3, $J_{2\text{-}NH}$ = 8.4, $J_{2\text{-}3B}$ = 7.2, H-2), 3.88 (d sp, 1H, *i*-propyl CH), 2.97 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.7, $J_{3A\text{-}2}$ = 10.3, $J_{3A\text{-}4}$ = 8.7, H^A-3), 2.15 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.7, $J_{3B\text{-}2}$ = 7.2, H^B-3), 1.47 (s, 9H, $C(CH_3)$), 1.04 (d, 3H, J = 6.6, *i*-propyl CH_3), 0.80 (d, 3H, J = 6.6, *i*-propyl CH_3).

Anal. Calcd for $C_{19}H_{27}N_3O_4$: C 63.16; H 7.48; N 11.63. Found: C 62.86; H 7.72; N 11.43.

*8. (2S,4S)-4-(N-t-Butyloxycarbonyl)phenylamino-5-oxoproline t-butylamide (**9**)*

To a solution of **5** (1.0 g, 1.76 mmol) in $CHCl_3$ (20 mL) *t*-butylamine (0.21 mL, 1.94 mmol) was added. The reaction mixture was allowed to stay at r.t. for 14 days, then washed carefully with 5% citric acid, water, 5% Na_2CO_3, water and dried ($MgSO_4$). Evaporation

of the solvent and column chromatography of the crude product (EtOAc) afforded 0.58 g (87%) of **9**, mp 173–175°C; TLC: R_f = 0.80 (EtOAc).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.52-7.18 (m, 5H, C_6H_5), 6.64 (m, 1H, amide NH), 5.51 (m, 1H, lactam NH), 4.51 (dd, 1H, $J_{4\text{-}3A}$ = 8.6, $J_{4\text{-}3B}$ = 7.4, H-4), 4.08 (ddd, 1H, $J_{2\text{-}3A}$ = 10.2, $J_{2\text{-}NH}$ = 8.4, $J_{2\text{-}3B}$ = 7.7, H-2), 2.97 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.6, $J_{3A\text{-}2}$ = 10.2, $J_{3A\text{-}4}$ = 8.6, H^A-3), 2.15 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.6, $J_{3B\text{-}2}$ = 7.7, $J_{3B\text{-}4}$ = 7.4, H^B-3), 1.47 (s, 9H, $C(CH_3)$), 1.12 (s, 9H, N-$C(CH_3)$).

Anal. Calcd for $C_{20}H_{29}N_3O_4$: C 64.00; H 7.73; N 11.20. Found: C 64.05; H 7.95; N 11.01.

*9. (2S,4S)-4-(N-Tosyl)phenylamino-5-oxoprolyl chloride (**10**)*

A mixture of **2** (1.0 g, 2.67 mmol) and freshly distilled $SOCl_2$ (2 mL, 27.8 mmol) was refluxed in dry benzene (4 mL) for 3 h. The reaction mixture was evaporated in vacuo to dryness, the residue was dissolved in benzene (3 mL) and the solvent was evaporated in vacuo. The residue was treated with a mixture of benzene (3 mL) and hexane (20 mL). Filtration of the precipitate followed by washing with hexane afforded 0.99 g (95%) of **10** (crude product), mp 70–75°C.

Anal. Calcd for $C_{18}H_{17}ClN_2O_4S$: C 55.03; H 4.33; Cl 9.04, N 7.13; S 8.15. Found: C 55.39; H 4.52; Cl 9.31; N 7.19; S 8.34.

*10. (2S,4S)-4-(N-Tosyl)phenylamino-5-oxoprolyl aniline (**11**)*

To a stirred solution of aniline (0.37 mL, 4.08 mmol) in dry CH_2Cl_2 (5 mL) a solution of **10** (0.8 g, 2.04 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise at −5°C, the reaction mixture was stirred at 0°C for 30 min and then allowed to stay r.t. for 24 h. The precipitate was filtered off and treated with a mixture of EtOAc (50 mL) and water (10 mL). The organic layer was washed with 5% HCl and water, and dried ($MgSO_4$). Evaporation of the solvent afforded 0.42 g (46%) of **11**, mp 238–240°C; TLC: R_f = 0.48 (B).

^{1}H NMR (400 MHz, DMSO-d_6): δ = 10.37 (m, 1H, amide NH), 8.26 (m, 1H, lactam NH), 7.77 (m, 2H, arom), 7.48-7.02 (m, 12H, arom.), 4.83 (dd, 1H, $J_{4\text{-}3B}$ = 9.2, $J_{4\text{-}3A}$ = 6.7, H-4), 4.37 (ddd, 1H, $J_{2\text{-}3B}$ = 10.8, $J_{2\text{-}3A}$ = $J_{2\text{-}NH}$ = 8.7, H-2), 2.43 (ddd, 1H, $J_{3A\text{-}3B}$ = 11.9, $J_{3A\text{-}2}$ = 8.7, $J_{3A\text{-}4}$ = 6.7, H^A-3), 2.37 (s, 3H, CH_3), 1.75 (ddd, 1H, $J_{3A\text{-}3B}$ = 11.9, $J_{3B\text{-}2}$ = 10.8, $J_{3B\text{-}4}$ = 9.2, H^B-3).

Anal. Calcd for $C_{24}H_{23}N_3O_4S$: C 64.14; H 5.12; N 9.35; S 7.13. Found: C 64.08; H 4.99; N 9.37; S 7.40.

*11. N-[(2S,4S)-4-(N-Boc)phenylamino-5-oxoprolyl]-(S)-phenylalanine methyl ester (**12**)*

To a stirred mixture of **3** (0.5 g, 1.56 mmol), L-phenylalanine methyl ester hydrochloride (0.37 g, 1.72 mmol), HOBt (0.41 g, 1.74 mmol) and Et_3N (0.24 mL, 1.72 mmol) in EtOAc (40 mL) a solution of DCC (0.36 g, 1.74 mmol) in EtOAc (10 mL) was added dropwise at −5–10°C. The reaction mixture was stirred at −5°C for 30 min, then at r.t. for 10 h. The precipitate was filtered off and the filtrate was washed with 5% $NaHCO_3$ and water, and dried ($MgSO_4$). The solvent was evaporated in vacuo and the residue was recrystallized from EtOH to give 0.65g (87%) of **12**, mp 197–198°C; TLC: R_f = 0.62 (B). $[\alpha]_D^{20}$-12.0° (c 1.0, acetone).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.41-6.96 (m, 10H, arom), 6.79 (d, 1H, J = 8.0, amide NH), 5.46 (d, 1H, $J_{NH\text{-}2}$ = 8.0, NH), 4.73 (ddd, 1H, J = 8.0, J = 6.7, C_αH-Phe), 4.56 (dd, 1H,

$J_{4\text{-}3B}$ = 8.1, $J_{4\text{-}3A}$ = 7.1, H-4), 4.21 (ddd, 1H, $J_{2\text{-}3A}$ = 9.7, $J_{2\text{-}NH}$ = 8.0, $J_{2\text{-}3B}$ = 7.1, H-2), 3.53 (s, 3H, CH_3O), 3.06 (dd, 1H, J = 14.0, 5.8, C$\underline{H}^{A}$H^{B}-Phe), 2.98 (dd, 1H, J = 14.0, 6.7, CH$^{A}\underline{H}^{B}$-Phe), 2.88 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.5, $J_{3A\text{-}2}$ = 9.7, $J_{3A\text{-}4}$ = 8.1, H^{A}-3), 1.97 (ddd, 1H, $J_{3A\text{-}3B}$ = 13.5, $J_{3B\text{-}2}$ = $J_{3B\text{-}4}$ = 7.1, H^{B}-3), 1.48 (s, 9H, C(CH_3)).

Anal. Calcd for $C_{26}H_{31}N_3O_6$: C 64.88; H 6.54; N 8.58. Found: C 64.88; H 6.45; N 8.73.

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Authors' address: Prof. Dr. Victor P. Krasnov, Laboratory of Amino Acids Chemistry, Institute of Organic Synthesis, Ural Division, Russian Academy of Sciences, 20, S. Kovalevskoy St., Ekaterinburg, GSP-147, 620219, Russia, Fax +7(3432)7411 89, E-mail:ca@ios.uran.ru

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