VASOPRESSIN ANALOGUES MODIFIED IN POSITIONS 2, 3 AND 8. SYNTHESIS AND BIOLOGICAL EFFECTS

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Four vasopressin analogues, modified in positions 2, 3 and 8 were prepared by solid phase as well as solution synthesis. Analogues, containing a D-amino acid in position 3, exhibit a low but markedly specific antidiuretic activity. Analogues with a D-substituent in position 2 show a more specific pressor activity.

In our previous communication[1] we have shown that a configurational change in the position 3 of [8-α,γ-diaminobutyric acid]vasopressin* affects its biological activity similarly as does a configurational change in position 8. Now, we are extending our investigation to the effect of simultaneous deamination in position 1 and configurational change in position 3. The present study concerns also the influence of a configurational change in position 2, as well as of simultaneous deamination in position 1 and configurational change in position 2, on biological activity of the same model system.

The following three vasopressin analogues have been prepared by solid-phase and solution synthesis: [1-β-mercaptopropionic acid, 3-D-phenylalanine, 8-α,γ--diaminobutyric acid]vasopressin (*VII*), [2-D-tyrosine, 8-α,γ-diaminobutyric acid] vasopressin (*XV*) and [1-β-mercaptopropionic acid, 2-D-tyrosine, 8-α,γ-diaminobutyric acid]vasopressin (*XXIII*). For comparison purposes we realized new synthesis of [3-D-phenylalanine, 8-α,γ-diaminobutyric acid]vasopressin (*XXIX*) both in solution and solid phase. The solid-phase syntheses were carried out according to the scheme elaborated previously[2]. Syntheses in solution were carried out according to the triad scheme[3]. The segments were condensed by N,N′-dicyclohexylcarbodiimide in the presence of N-hydroxybenzotriazole[4]. Preparation of the analogues from their precursors and study of their biological activities was performed by the usual procedures[1]. We isolated also dimeric forms of the analogues *VII*, *XV*,

* Symbols and abbreviations usual in peptide chemistry were employed. Further abbreviations: Mpr β-mercaptopropionic acid, Dab α,γ-diaminobutyric acid, VP vasopressin, AD antidiuretic activity, BP pressor activity.

XXIII and *XXIX* (in purification by continuous free-flow electrophoresis the dimeric forms showed higher mobility than the monomers) and determined their biological properties. The biological activities of the analogues and their dimers are summarized in Table I.

Results, obtained in the previous[1] and the present paper, show that a configurational change in position 3 affects the typical vasopressin activities much in the same way as does a change in position 8. However, the decrease of antidiuretic effect is greater whereas the pressor activity suppression is smaller. [D-Phe^3,Dab^8]VP (*XXIX*) exhibits a substantially lower and much less specific antidiuretic activity than [D-Dab^8]VP. 1-Deamination in [D-Phe^3,Dab^8]VP results in about the same reduction of antidiuretic and pressor activity. (1-Deamination in [D-Dab^8]VP enhances markedly the antidiuretic activity and its specificity.) Configurational change in position 2 has a different effect on both the typical vasopressin activities than configurational change in position 3 or 8. [D-Tyr^2,Dab^8]VP (*XV*) shows only a negligible antidiuretic effect and low pressor activity. Nevertheless, it is obvious that the analogue *XV* is a compound with more specific pressor activity. The 1-deamination enhances the effect of configurational change in position 2. [Mpr^1,D-Tyr^2,Dab^8]VP (*XXIII*) shows a significantly high and specific pressor activity.

Vasopressin analogues with enhanced specificity of the pressor activity are of certain practical significance. Literature describes[5] some vasopressin, and particularly vasotocin, analogues with more than 200× higher specificity of the pressor activity than that of the natural hormone, the pressor activity of these compounds being, however,

TABLE I
Biological activities of vasopressin analogues and their dimers

Compound		Activity, IU/mg		
		AD	BP	AD/BP
XXIX	[D-Phe^3,Dab^8]VP	35[a]	13	2·7
VII	[Mpr^1,D-Phe^3,Dab^8]VP	17[a]	7	2·4
XV	[D-Tyr^2,Dab^8]VP	[b]	2	—
XXIII	[Mpr^1,D-Tyr^2,Dab^8]VP	8	90	11·25[c]
Dimer	[D-Phe^3,Dab^8]VP	1·9	—	—
Dimer	[Mpr^1,D-Phe^3,Dab^8]VP	0·6	—	—
Dimer	[Mpr^1,D-Tyr^2,Dab^8]VP	—	0·5	—

[a] Mean from two values; [b] negligible; [c] BP/AD.

substantially lower than that of the natural hormones. It seems that the D-Tyr[2] substitution opens new possibilities in the region of analogues with enhanced specificity of the pressor activity.

EXPERIMENTAL

Solid-phase syntheses were carried out on chloromethylated polystyrene, cross-linked by 2% DVB (Calbiochem, USA; 0·96 mmol Cl/g) in a manually operated synthetizer according to the previously described scheme[2] (programme No 6). Instruments and analytical procedures used were the same as those described in our previous papers[6]. Purity of the compounds was checked by electrophoresis on Whatman 3MM paper in 6% acetic acid and by thin-layer chromatography on silica gel (Silufol, Kavalier, Czechoslovakia) in the following systems: A, n-butanol–acetic acid–water (4 : 1 : 1); B, n-butanol–tert-butanol–acetic acid–water (2 : 2 : 1 : 1); C, n-butanol–acetic acid–water (4 : 1 : 5); D, n-butanol–acetic acid–water–pyridine (15 : 3 : 10 : 12); E, chloroform–methanol (9 : 1).

β-Benzylthiopropionyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide (*I*)

In the synthesis 2 g of the carrier were used (1 mmol of Gly). Ammonolysis afforded 1·17 g (87%) of the crude peptide, melting at 217–225°C. Two crystallizations from methanol–water gave 0·89 g (66%) of chromatographically homogeneous (systems B, C and D) compound, m.p. 228–229°C, $[\alpha]_D^{24}$ −28·9° (*c* 0·2, dimethylformamide). Amino acid composition: Tyr 1·00, Phe 1·09, Glu 0·97, Asp 1·05, Cys (Bzl) 1·00, Pro 0·93, Dab 1·03, Gly 0·99. For $C_{66}H_{80}N_{12}O_{14}S_2 . H_2O$ (1 348) calculated: 58·83% C, 6·13% H, 12·47% N; found: 58·86% C, 6·20% H, 12·39% N.

β-Benzylthiopropionyl-tyrosyl-D-phenylalanine Methyl Ester (*II*)

A 6·4M solution of HCl in dioxane (3·2 ml), followed (at −20°C) by butyl nitrite (2·06 g; 20 mmol) in dimethylformamide (5 ml), was added to a solution of β-benzylthiopropionyl-tyrosine hydrazide[7] (7·46 g; 20 mmol) in dimethylformamide (25 ml). The reaction was monitored by determination of nitrite content in the reaction mixture with Griess reagent[8]. After 15 min the mixture was adjusted to pH 7 with N-methylmorpholine and mixed with a solution of methyl D-phenylalaninate (5·28 g; 29·5 mmol) in dimethylformamide (15 ml). After standing at 0°C for 15 h and at room temperature for 5 h, the solvents were evaporated. Water (50 ml) was added to the residue and the separated crude tripeptide ester was dissolved in ethyl acetate (100 ml). The solution was washed with saturated sodium hydrogen carbonate solution (3 × 50 ml) and 5% hydrochloric acid (3 × 50 ml), dried over sodium sulfate and taken down. The residue was mixed with water (100 ml) and the separated solid (6·66 g; 64%) was filtered. The product was chromatographically homogeneous (systems A and E), m.p. 98–100°C, $[\alpha]_D^{24}$ −3·7° (*c* 1, dimethylformamide). An analytical sample was crystallized from dimethylformamide–water and melted at 101 to 102°C. For $C_{29}H_{32}N_2O_5S . H_2O$ (538·6) calculated: 64·66% C, 6·36% H, 5·20% N; found: 64·51% C, 6·31% H, 5·12% N.

β-Benzylthiopropionyl-tyrosyl-D-phenylalanine (*III*)

Compound *II* (4 g; 8 mmol) was dissolved in 1M-NaOH (20 ml). After standing for 40 min the solution was acidified with hydrochloric acid to pH 1–2. The separated compound (chromato-

graphically homogeneous in systems A and D) was collected on filter and dried, m.p. 92–95°C, $[\alpha]_D^{24}$ −11·3° (*c* 1, dimethylformamide); yield 3·62 g (93%). An analytical sample was crystallized from dimethylformamide–water. For $C_{28}H_{30}N_2O_5S.1/2\ H_2O$ (515·6) calculated: 65·22% C, 6·06% H, 6·43% N; found: 64·97% C, 6·31% H, 5·57% N.

β-Benzylthiopropionyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester (*IV*)

N,N'-Dicyclohexylcarbodiimide (1·62 g; 7·8 mmol) and 1-hydroxybenzotriazole (2·9 g; 21·3 mmol) were added at 0°C to a solution of compound *III* (3·6 g; 7·1 mmol) and glutaminyl-asparaginyl--S-benzylcysteine benzyl ester[9] (3·9 g; 7·1 mmol) in dimethylformamide (50 ml). After stirring at 0°C for 2 h, the solution was set aside overnight at 25°C. The separated N,N'-dicyclohexylurea was filtered, dimethylformamide was evaporated and the residue was mixed with water (100 ml). The product was filtered, three times triturated with 5% hydrochloric acid and then with a saturated sodium hydrogen carbonate solution. Crystallization from dimethylformamide–water afforded 6·58 g (89%) of the product, m.p. 206–209°C; $[\alpha]_D^{24}$ −6·3° (*c* 0·35, dimethylformamide). Amino acid composition: Tyr 0·93, Phe 1·05, Glu 1·04, Asp 1·05, Cys (Bzl) 0·93. For $C_{54}H_{61}.N_7O_{10}S_2.H_2O$ (1 050) calculated: 61·76% C, 6·05% H, 9·35% N; found: 61·85% C, 5·91% H, 9·12% N.

β-Benzylthiopropionyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine (*V*)

A 35% solution of hydrogen bromide in acetic acid (30 ml) was added to a solution of the ester *IV* (6·5 g; 6·3 mmol) in acetic acid (30 ml). The mixture was heated to 60°C for 15 min, taken down under reduced pressure and the residue was treated with water (200 ml). The separated compound was filtered and crystallized from dimethylformamide–water, affording 3·29 g (61%) of product, m.p. 204–207°C, $[\alpha]_D^{24}$ −12·1° (*c* 1, dimethylformamide). Amino acid composition: Tyr 0·95, Phe 1·06, Glu 1·02, Asp 0·97, Cys(Bzl) 1·00. For $C_{47}H_{55}N_7O_{10}S_2.1/2\ H_2O$ (951·1) calculated: 59·35% C, 5·93% H, 10·31% N; found: 59·61% C, 6·04% H, 10·39% N.

β-Benzylthiopropionyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl--prolyl-N$^\gamma$-tosyl-α,γ-diaminobutyryl-glycine Amide (*VI*)

N,N'-Dicyclohexylcarbodiimide (0·25 g; 1·2 mmol), followed by 1-hydroxybenzotriazole (0·4 g; 3 mmol) was added at 0°C to a solution of pentapeptide *V* (0·94 g; 1 mmol) and prolyl-N$^\gamma$-tosyl--α,γ-diaminobutyryl-glycine amide[10] (0·51 g; 1·2 mmol) in dimethylformamide (20 ml). After stirring for 2 h at 0°C the solution was set aside overnight. N,N'-Dicyclohexylurea was filtered, dimethylformamide evaporated and the residue mixed with 4% hydrochloric acid (50 ml). The separated compound was filtered, triturated with 4% hydrochloric acid (3 × 50 ml) and then with a saturated sodium hydrogen carbonate solution (3 × 50 ml), yielding 1·12 g (83%) of material melting at 212–218°C. Crystallization from dimethylformamide–methanol–water afforded 0·95 g (70%) of chromatographically homogeneous (systems A and D) product; m.p. 217–220°C, $[\alpha]_D^{24}$ −11·3° (*c* 0·3, dimethylformamide). Amino acid composition: Tyr 1·03, Phe 1·08, Glu 0·98, Asp 1·00, Cys(Bzl) 0·97, Pro 0·96, Dab 0·99, Gly 1·00. For $C_{65}H_{80}N_{12}O_{14}S_3.H_2O$ (1 368) calculated: 57·09% C, 6·04% H, 12·29% N; found: 57·35% C, 5·75% H, 12·05% N.

[1-β-Mercaptopropionic Acid, 3-D-Phenylalanine, 8-α,γ-Diaminobutyric Acid] vasopressin (*VII*)

a) The title compound *VII* was obtained from the protected peptide *I* (500 mg, 0·38 mmol) by successive reduction with sodium in liquid ammonia, oxidation with potassium ferricyanide,

desalting on Amberlite IR C-50 and lyophilization; yield 215 mg (60%) of crude product. Purification by continuous free-flow electrophoresis afforded two compounds. The electrophoretically more mobile monomer (85 mg; 24%) had $[\alpha]_D^{24}$ −90·9° (*c* 0·1; 1M-CH_3COOH). Amino acid composition: Tyr 0·98, Phe 1·05, Glu 0·96, Asp 1·03, Cys 1·00, Pro 0·95, Dab 1·03, Gly 1·00. For $C_{44}H_{60}N_{12}O_{12}S_2.2\,CH_3COOH.2\,H_2O$ (1 170) calculated: 49·31% C, 6·21% H, 14·37% N; found: 49·11% C, 5·99% H, 14·30% N. In addition to the main product 39 mg (11%) of the dimer was obtained; $[\alpha]_D^{24}$ −17·7° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Tyr 0·96, Phe 1·07, Glu 1·03, Asp 0·99, Cys 1·02, Pro 0·99, Dab 0·96, Gly 0·98. For $(C_{44}H_{60}N_{12}O_{12}S_2.2\,CH_3COOH.\frac{1}{2}H_2O)_2$ (2 284) calculated: 50·47% C, 6·09% H, 14·72% N; found: 50·72% C, 6·15% H, 14·12% N.

b) The protected octapeptide amide *VI* (380 mg; 0·28 mmol) was converted into the free octapeptide amide as described under *a*). Freeze-drying afforded 168 mg (59%) of crude material which was purified by continuous free-flow electrophoresis. The obtained product (67 mg; 23%) was chromatographically (systems A and D) as well as electrophoretically identical with the monomer prepared under *a*); $[\alpha]_D^{24}$ −88·8° (*c* 0·1, 1M-CH_3COOH). Amino acid composition: Tyr 0·95, Phe 1·06, Glu 1·01, Asp 1·04, Cys 0·97, Pro 1·00, Dab 0·95, Gly 1·02. A minor amount (16 mg; 6%) of the dimer was also obtained; $[\alpha]_D^{24}$ −16·9° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Tyr 0·88, Phe 1·11, Glu 1·05, Asp 0·97, Cys 0·95, Pro 1·02, Dab 0·95, Gly 1·07.

Benzyloxycarbonyl-S-benzylcysteinyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-
-S-benzylcysteinyl-prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide (*VIII*)

The title compound was prepared using the same esterified resin (4 g; 2 mmol Gly) as used for compound *I*. Prior attachment of the last amino acid, the peptide on the carrier was divided into two halves; one was used for the next step, the second was employed in the synthesis of compound *XVI*. Ammonolysis afforded 1·33 g (89%) of crude product, m.p. 198–200°C. Two crystallizations from methanol–water gave 0·9 g (60%) of the nonapeptide amide, chromatographically homogeneous in systems A and D, m.p. 223–224°C, $[\alpha]_D^{24}$ −25·6° (*c* 0·2, dimethylformamide). Amino acid analysis: Cys(Bzl) 1·94, Tyr 0·95, Phe 1·07, Glu 1·06, Asp 1·05, Pro 1·01, Dab 0·93, Gly 0·99. For $C_{74}H_{87}N_{13}O_{16}S_2.1/2\,H_2O$ (1 488) calculated: 59·74% C, 5·96% H, 12·24% N; found: 59·52% C, 6·05% H, 11·99% N.

Tosyl-S-benzylcysteinyl-D-tyrosine Hydrazide (*IX*)

Tosyl-S-benzylcysteine (8·8 g; 24 mmol) was refluxed with thionyl chloride (17·5 ml) for 20 min. The reagent was distilled off under diminished pressure and the residue dissolved in ethyl acetate (13 ml). This solution was added in portions to a stirred mixture of D-tyrosine methyl ester hydrochloride (5·6 g; 24 mmol), sodium hydrogen carbonate (4·1 g; 48 mmol), water (13 ml) and ethyl acetate (13 ml). The stirring was continued for 10 min, the pH of the mixture being kept above 7·5 by addition of solid sodium hydrogen carbonate. The layers were separated and the aqueous one was washed with ethyl acetate. The combined organic layers were dried over sodium sulfate and taken down. The residue was dissolved in methanol (25 ml), and the solution refluxed with 100% hydrazine hydrate (2·5 ml; 52 mmol) for 2 h. The formed dipeptide hydrazide was precipitated with water (25 ml), filtered, washed with water and dried; yield 12 g (92%). Crystallization from ethanol–water afforded 10·7 g (82%) of chromatographically homogeneous (in systems B and D) product, m.p. 208–209°C; $[\alpha]_D^{24}$ −27·1° (*c* 0·5, dimethylformamide). For $C_{26}H_{30}N_4O_5S_2.H_2O$ (560·7) calculated: 55·70% C, 5·75% H, 9·99% N; found: 55·92% C, 5·61% H, 10·13% N.

Tosyl-S-benzylcysteinyl-D-tyrosyl-phenylalanine Methyl Ester (*X*)

This compound was prepared by azide synthesis from hydrazide *IX* (9·45 g; 17·5 mmol), 6·4M-HCl in dioxane (2·72 ml), butyl nitrite (1·80 g; 17·5 mmol) and phenylalanine methyl ester (4·39 g; 24·5 mmol). The reaction afforded 9·48 g (79%) of crude product, m.p. 88–92°C which was purified by two crystallizations from dimethylformamide–water; yield 8·31 g (69%), m.p. 91–93°C; chromatographically homogeneous (in systems A and E); $[\alpha]_D^{24}$ −6·4° (*c* 0·5, dimethylformamide). For $C_{36}H_{39}N_3O_7S_2.H_2O$ (707·8) calculated: 61·08% C, 5·84% H, 5·94% N; found: 60·99% C, 5·99% H, 5·71% N.

Tosyl-S-benzylcysteinyl-D-tyrosyl-phenylalanine (*XI*)

Tripeptide ester *X* (6·9 g; 10 mmol) in 1M-NaOH (25 ml) was set aside for 40 min and then acidified with hydrochloric acid to pH 1–2. The separated product was collected on filter and dried; yield 6·3 g (93%); m.p. 102–104°C; $[\alpha]_D^{24}$ +0·7° (*c* 1, dimethylformamide). For $C_{35}H_{37}.N_3O_7S_2.1/2\ H_2O$ (684·8) calculated: 61·39% C, 5·59% H, 6·15% N; found: 61·22% C, 5·73% H, 6·17% N.

Tosyl-S-benzylcysteinyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester (*XII*)

Compound *XII* was prepared by the same procedure as described for the ester *IV*. Reaction of tripeptide *XI* (3·38 g; 5 mmol), glutaminyl-asparaginyl-S-benzylcysteine benzyl ester[9] (2·7 g; 5 mmol), N,N′-dicyclohexylcarbodiimide (1·13 g; 5·5 mmol) and 1-hydroxybenzotriazole (2·03 g; 15 mmol) afforded 4·98 g (82%) of crude product, melting at 182–187°C. Chromatographically uniform (in systems D and E) compound (2·95 g; 48%) was obtained by two crystallizations from dimethylformamide–methanol–water; m.p. 190–192°C, $[\alpha]_D^{24}$ −11·0° (*c* 0·3, dimethylformamide). Amino acid composition: Cys(Bzl) 1·92, Tyr 0·99, Phe 1·08, Glu 1·02, Asp 1·02. For $C_{61}H_{68}.N_8O_{12}S_3.H_2O$ (1 219) calculated: 60·10% C, 5·79% H, 9·19% N; found: 60·30% C, 5·61% H, 8·90% N.

Tosyl-S-benzylcysteinyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine (*XIII*)

This compound was prepared from the hexapeptide ester *XII* (2·6 g; 2·1 mmol) and hydrogen bromide in acetic acid (15 ml) as described for compound *V*. The procedure gave 1·47 g (61%) of chromatographically (systems A and D) homogeneous product, m.p. 235–237°C, $[\alpha]_D^{24}$ −17·3° (*c* 0·5, dimethylformamide). For $C_{54}H_{62}N_8O_{12}S_3.H_2O$ (1 129) calculated: 57·43% C, 5·71% H, 9·92% N; found: 57·18% C, 6·00% H, 10·03% N.

Tosyl-S-benzylcysteinyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{γ}-tosyl-α,γ-diaminobutyryl-glycine Amide (*XIV*)

The title compound was prepared similarly as described for the peptide amide *VI*. Reaction of hexapeptide *XIII* (0·7 g; 0·63 mmol), prolyl-N^{γ}-tosyl-α,γ-diaminobutyryl-glycine amide[10] (0·32 g; 0·75 mmol), N,N′-dicyclohexylcarbodiimide (0·16 g; 0·75 mmol) and 1-hydroxybenzotriazole (0·25 g; 1·9 mmol) yielded 1·09 g (72%) of crude product, m.p. 179–182°C which was purified by crystallization from methanol–water, affording 0·84 g (55%) of chromatographically (in systems A, B and D) uniform compound, m.p. 182–183°C; $[\alpha]_D^{24}$ −9·5° (*c* 0·3, dimethylformamide). Amino acid composition: Cys(Bzl) 1·92, Tyr 0·96, Phe 1·08, Glu 1·03, Asp 1·04, Pro 1·02, Dab 0·96, Gly 1·01. For $C_{72}H_{87}N_{13}O_{16}S_4.3/2\ H_2O$ (1 546) calculated: 55·94% C, 5·87% H, 11·78% N; found: 56·19% C, 5·90% H, 11·49% N.

[2-D-Tyrosine, 8-α,γ-Diaminobutyric Acid]vasopressin (*XV*)

a) The protected nonapeptide amide *VIII* (500 mg; 0·33 mmol) was converted into 246 mg (72%) of compound *XV*. Purification by continuous free-flow electrophoresis afforded two compounds: 99 mg (29%) of the electrophoretically less mobile monomer and 36 mg (10%) of the more mobile dimer.

Monomer: $[\alpha]_D^{24}$ −57·4° (*c* 0·2, 1M-CH_3COOH); amino acid composition: Cys 1·93, Tyr 0·98, Phe 1·06, Glu 1·02, Asp 1·03, Pro 0·98, Dab 0·99, Gly 1·01. For $C_{44}H_{61}N_{13}O_{12}S_2.CH_3COOH.1/2\,H_2O$ (1097) calculated: 50·35% C, 6·06% H, 16·60% N; found: 50·50% C, 5·91% H, 16·45% N.

Dimer: $[\alpha]_D^{24}$ −52·1° (*c* 0·3, 1M-CH_3COOH); amino acid composition: Cys 1·82, Tyr 1·01, Phe 1·06, Glu 1·09, Asp 0·99, Pro 1·03, Dab 0·98, Gly 1·02.

b) Reduction of the protected nonapeptide amide *XIV* gave compound *XV* (287 mg; 85%) which was purified by continuous free-flow electrophoresis, affording 91 mg (27%) of the monomer (corresponding chromatographically and electrophoretically to the compound prepared by solid-phase synthesis) and 51 mg (15%) of the dimer.

Monomer: $[\alpha]_D^{24}$ −57·0° (*c* 0·24, 1M-CH_3COOH), amino acid analysis: Cys 1·88, Tyr 0·92, Phe 1·09, Glu 1·05, Asp 1·02, Pro 0·97, Dab 1·01, Gly 1·05. For $C_{44}H_{61}N_{13}O_{12}S_2.CH_3COOH.1/2\,H_2O$ (1097) calculated: 50·35% C, 6·06% H, 16·60% N; found: 50·47% C, 5·84% H, 16·47% N.

Dimer: $[\alpha]_D^{24}$ −53·3° (*c* 0·3, 1M-CH_3COOH); amino acid composition: Cys 1·90, Tyr 0·99, Phe 1·05, Glu 1·03, Asp 0·94, Pro 1·07, Dab 0·95, Gly 1·11. For $(C_{44}H_{61}N_{13}O_{12}S_2.CH_3COOH.H_2O)_2$ (2 212) calculated: 49·94% C, 6·10% H, 16·46% N; found: 49·85% C, 5·80% H, 16·31% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide (*XVI*)

In this synthesis the second half of the resin from the preparation of compound *VIII* was used. β-Benzylthiopropionic acid was attached to the amino-terminal tyrosine. After ammonolysis, 1·09 g (82%) of the crude product, m.p. 199–215°C, was obtained. Two crystallizations from methanol–water afforded 0·84 g (63%) of the octapeptide amide, chromatographically uniform in systems A and D; m.p. 218–220°C, $[\alpha]_D^{24}$ −39·7° (*c* 0·2, dimethylformamide). Amino acid analysis: Tyr 0·95, Phe 1·06, Glu 1·04, Asp 1·06, Cys(Bzl) 1·00, Pro 0·98, Dab 0·93, Gly 0·98. For $C_{66}H_{80}N_{12}O_{14}S_2.3/2\,H_2O$ (1 357) calculated: 58·44% C, 6·17% H, 12·37% N; found: 58·39% C, 5·89% H, 12·42% N.

β-Benzylthiopropionyl-D-tyrosine Hydrazide (*XVII*)

Compound *XVII* was prepared in the same way as the hydrazide *IX*, starting from β-benzylthiopropionic acid (4·9 g; 25 mmol), thionyl chloride (17·5 ml), D-tyrosine methyl ester hydrochloride (5·8 g; 25 mmol), sodium hydrogen carbonate (4·2 g; 50 mmol) and hydrazine hydrate (2·5 ml; 52 mmol). The thus-obtained crude product (7·28 g; 88%) was crystallized from ethanol–water, affording 6·83 g (83%) of chromatographically (in system B and D) uniform compound, m.p. 192–193°C; $[\alpha]_D^{24}$ −23·6° (*c* 0·5, dimethylformamide). For $C_{19}H_{23}N_3O_3S.3/2\,H_2O$ (400·5) calculated: 56·98% C, 6·54% H, 10·49% N; found: 57·17% C, 6·41% H, 10·70% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanine Methyl Ester (*XVIII*)

This compound was prepared from hydrazide *XVII* (6·5 g; 17·5 mmol), 6·4M-HCl in dioxane (2·75 ml), butyl nitrite (1·8 g; 17·5 mmol) and phenylalanine methyl ester (4·4 g; 24·5 mmol) by the procedure described for compound *II*; yield 7·8 g (86%) of material m.p.133—136°C. Crystallization from dimethylformamide–water afforded 6·16 g (68%) of product, chromatographically pure in system A and E; m.p. 136—138°C; $[\alpha]_D^{24}$ —12·1° (*c* 0·5, dimethylformamide). For $C_{29}H_{32}N_2O_5S.1/2\ H_2O$ (529·6) calculated: 65·77% C, 6·28% H, 5·29% N; found: 65·71% C, 6·51% H, 5·40% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanine (*XIX*)

Tripeptide ester *XVIII* (5·2 g; 10 mmol) was saponified with 1M-NaOH in the same way as the ester *II*, yielding 4·6 g (91%) of product, m.p. 77—79°C; $[\alpha]_D^{24}$ —31·1° (*c* 0·5, dimethylformamide). Analytical sample was crystallized from dimethylformamide–water. For $C_{28}H_{30}N_2O_5S.1/2\ H_2O$ (515·6) calculated: 65·22% C, 6·06% H, 5·43% N; found: 64·99% C, 6·12% H, 5·41% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester (*XX*)

The title compound was prepared in the same manner as described for compound *IV*, starting from the dipeptide *XIX* (2·53 g; 5 mmol), glutaminyl-asparaginyl-S-benzylcysteine benzyl ester[9] (2·7 g; 5 mmol), N,N′-dicyclohexylcarbodiimide (1·13 g; 5·5 mmol) and 1-hydroxybenzotriazole (2·03 g; 15 mmol). The crude product (4·08 g; 79%), m.p. 220—224°C, was twice crystallized from a mixture of dimethylformamide, methanol and water, affording 2·63 g (51%) of compound, melting at 225—227°C, chromatographically homogeneous in systems D and E; $[\alpha]_D^{24}$ —8·5° (*c* 0·3, dimethylformamide). Amino acid composition: Tyr 0·95, Phe 1·06, Glu 1·05, Asp 1·05, Cys(Bzl) 0·91. For $C_{54}H_{61}N_7O_{10}S_2.H_2O$ (1 050) calculated: 61·76% C, 6·05% N, 9·34% N; found: 61·52% C, 6·30% H, 9·21% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine (*XXI*)

The benzyl ester group in compound *XX* (1·7 g; 1·6 mmol) was cleaved off with hydrogen bromide in acetic acid (10 ml). Crystallization from dimethylformamide–water afforded 1·25 g (80%) of product, m.p. 205—207°C, chromatographically uniform (in systems A and D); $[\alpha]_D^{24}$ —9·4° (*c* 0·5, dimethylformamide). For $C_{47}H_{55}N_7O_{10}S_2.1/2\ H_2O$ (951·1) calculated: 59·35% C, 5·93% H, 10·31%N; found: 59·28% C, 5·89% H, 10·50% N.

β-Benzylthiopropionyl-D-tyrosyl-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl--prolyl -N^{γ}-tosyl-α,γ-diaminobutyryl-glycine Amide (*XXII*)

The compound *XXII* was prepared in the same way as described for compound *VI*. Reaction of pentapeptide *XXI* (0·94 g; 1 mmol), prolyl-N^{γ}-tosyl-α,γ-diaminobutyryl-glycine amide[10] (0·51 g; 1·2 mmol), N,N′-dicyclohexylcarbodiimide (0·25g; 1·2 mmol) and 1-hydroxybenzotriazole (0·4 g; 3 mmol) gave 1·09 g (79%) of crude octapeptide amide, m.p. 184—186°C. Crystallization from ethanol–water afforded 0·97 g (70%) of compound, chromatographically uniform in systems A, B and D; m.p. 186—188°C, $[\alpha]_D^{24}$ —15·8° (*c* 0·3, dimethylformamide). Amino acid composition: Tyr 0·95, Phe 0·99, Glu 1·03, Asp 1·04, Cys(Bzl) 0·96, Pro 1·02, Dab 1·01, Gly 1·00. For $C_{65}H_{80}N_{12}O_{14}S_3.H_2O$ (1 368) calculated: 57·09% C, 6·04% H, 12·29% N; found: 57·20% C, 6·12% H, 12·11% H.

[1-β-Mercaptopropionic Acid, 2-D-Tyrosine, 8-α,γ-Diaminobutyric Acid]vasopressin (*XXIII*)

a) The protected peptide *XVI* (500 mg; 0·38 mmol) was converted into 311 mg (83%) of crude product *XXIII*. Purification by continuous free-flow electrophoresis afforded 99 mg (26%) of the less mobile monomer and 41 mg (11%) of the dimer.

Monomer: $[\alpha]_D^{24}$ −69·7° (*c* 0·3, 1M-CH_3COOH). Amino acid composition: Tyr 0·96, Phe 1·05, Glu 1·02, Asp 1·05, Cys 1·03, Pro 0·97, Dab 0·95, Gly 0·98. For $C_{44}H_{60}N_{12}O_{12}S_2$. 3/2 CH_3.$COOH$.1/2 H_2O (1 112) calculated: 50·75% C, 6·07% H, 15·11% N; found: 50·80% C, 5·94% H, 15·01% N.

Dimer: $[\alpha]_D^{24}$ −50·9° (*c* 0·3, 1M-CH_3COOH); amino acid composition: Tyr 0·94, Phe 1·01, Glu 1·02, Asp 0·95, Cys 0·97, Pro 1·04, Dab 1·01, Gly 1·06.

b) Octapeptide amide *XXII* (510 mg; 0·42 mmol) was reduced to give 339 mg (79%) of the crude product *XXIII*. Purification by continuous free-flow electrophoresis gave 106 mg (26%) of monomer, chromatographically and electrophoretically identical with the compound prepared under *a*), and 50 mg (12%) of dimer.

Monomer: $[\alpha]_D^{24}$ −66·1° (*c* 0·3, 1M-CH_3COOH). Amino acid analysis: Tyr 0·97, Phe 1·10, Glu 1·01, Asp 1·05, Cys 0·95, Pro 0·91, Dab 0·97, Gly 1·03. For $C_{44}H_{60}N_{12}O_{12}S_2$.2 CH_3COOH.H_2O (1 151) calculated: 50·08% C, 6·13% H, 14·60% N; found: 49·89% C, 6·21% H, 14·45% N.

Dimer: $[\alpha]_D^{24}$ −54·8° (*c* 0·3, 1M-CH_3COOH). Amino acid analysis: Tyr 0·94, Phe 1·09, Glu 1·05, Asp 0·94, Cys 0·95, Pro 0·99, Dab 0·96, Gly 1·04. For $(C_{44}H_{60}N_{12}O_{12}S_2.CH_3COOH.2\ H_2O)_2$ (2 218) calculated: 49·81% C, 6·18% H, 15·15% N; found: 50·05% C, 6·45% H, 15·01% N.

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-N^{γ}-benzyloxycarbonyl-α,γ-diaminobutyryl-glycine Amide (*XXIV*)

The title compound was synthesized using 2 g of the glycine-esterified resin (1 mmol Gly). The peptide was liberated from the carrier by ammonolysis; yield 1·26 g (84%); m.p. 206–214°C. Two crystallizations from methanol–water afforded 0·98 g (65%) of product, homogeneous in systems B and D; m.p. 214–216°C; $[\alpha]_D^{24}$ −33·9° (*c* 0·2, dimethylformamide). Amino acid composition: Cys(Bzl) 1·83, Tyr 0·94, Phe 1·06, Glu 1·02, Asp 1·05, Pro 1·04, Dab 1·05, Gly 1·01. For $C_{73}H_{87}N_{13}O_{16}S_3.H_2O$ (1 517) calculated: 57·81% C, 5·91% H, 12·00% N; found: 57·56% C, 6·01% H, 11·96% N.

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine (*XXV*)

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanine methyl ester[1] (10·3 g; 15 mmol) was dissolved in 1M-NaOH (52 ml). After standing for 40 min at room temperature, the solution was acidified with conc. hydrochloric acid to pH 1–2 and the precipitated solid (homogeneous in systems A and D) was filtered and dried, yielding 8·7 g (86%) of product, m.p. 106–110°C; $[\alpha]_D^{24}$ −12·4° (*c* 1, dimethylformamide). For $C_{35}H_{37}N_3O_7S_2$.3/2 H_2O (702·8) calculated: 59·81% C, 5·74% H, 5·98% N; found: 59·53% C, 5·89% H, 6·01% N.

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine Benzyl Ester (*XXVI*)

The title compound was prepared from compound *XXV* (8·7 g; 12·9 mmol), glutaminyl-asparaginyl-S-benzylcysteine benzyl ester[9] (6·75 g; 12·9 mmol), N,N′-dicyclohexylcarbodiimide (3 g;

14·2 mmol) and 1-hydroxybenzotriazole (5·2 g; 36·6 mmol) in the same manner as described for compound *IV*. Crystallization from dimethylformamide–methanol–water gave 13·0 g (84%) of chromatographically uniform (in systems C, D and E) product, m.p. 234–236°C; $[\alpha]_D^{24}$ −3·1° (*c* 0·3, dimethylformamide). Amino acid composition: Cys(Bzl) 1·92, Tyr 0·97, Phe 1·06, Glu 1·05, Asp 1·00. For $C_{61}H_{68}N_8O_{12}S_3.1/2\,H_2O$ (1 210) calculated: 60·53% C, 5·75% H, 9·26% N; found: 60·48% C, 5·60% H, 9·44% N.

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteine (*XXVII*)

Compound *XXVII* was prepared from hexapeptide ester *XXVI* (10·2 g; 8·6 mmol) in acetic acid (45 ml) and 35% solution of hydrogen bromide in acetic acid (45 ml). Crystallization from dimethylformamide–water afforded 8·22 g (86%) of chromatographically uniform (systems A and D) product, m.p. 228–230°C; $[\alpha]_D^{24}$ −21·1° (*c* 1, dimethylformamide). Amino acid composition: Cys(Bzl) 1·90, Tyr 0·98, Phe 1·07, Glu 1·02, Asp 0·99. For $C_{54}H_{62}N_8O_{12}S_3.H_2O$ (1 129) calculated: 57·43% C, 5·71% H, 9·92% N; found: 57·57% C, 5·48% H, 10·03% N.

Tosyl-S-benzylcysteinyl-tyrosyl-D-phenylalanyl-glutaminyl-asparaginyl-S-benzylcysteinyl--prolyl-N$^{\gamma}$-tosyl-α,γ-diaminobutyryl-glycine Amide (*XXVIII*)

The title compound was prepared from hexapeptide *XXVII* (3·95 g; 3·55 mmol), prolyl-N$^{\gamma}$-tosyl--α,γ-diaminobutyryl-glycine amide[10] (1·8 g; 4 mmol), N,N′-dicyclohexylcarbodiimide (0·8 g; 4·3 mmol) and 1-hydroxybenzotriazole (1·41 g; 10·5 mmol) in the same manner as described for compound *VI*; yield 4·38 g (82%) of product, m.p. 171–180°C. Crystallization from methanol--water gave 3·31 g (60%) of chromatographically pure (system A and D) compound, m.p. 188 to 191°C; $[\alpha]_D^{24}$ −15·9° (*c* 0·38 g, dimethylformamide). Reported[1] m.p. 179–181°C, $[\alpha]_D^{25}$ −14·2° (*c* 0·21, dimethylformamide). Amino acid analysis: Cys(Bzl) 1·89, Tyr 0·94, Phe 1·06, Glu 1·06, Asp 1·01, Pro 0·98, Dab 0·99, Gly 1·07.

[3-D-Phenylalanine, 8-α,γ-Diaminobutyric Acid]vasopressin (*XXIX*)

a) The protected nonapeptide *XXIV* (500 mg; 0·33 mmol) was converted into 190 mg (55%) of crude product *XXIX*. Continuous free-flow electrophoresis afforded 88 mg (25·5%) of monomer and 41 mg (12%) of dimer.

Monomer: $[\alpha]_D^{24}$ −12·6° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Cys 1·97, Tyr 0·99 Phe 1·08, Glu 0·95, Asp 1·01, Pro 1·06, Dab 1·00, Gly 0·94. For $C_{44}H_{61}N_{13}O_{12}S_2.3\,CH_3COOH.H_2O$ (1 226) calculated: 48·98% C, 6·16% H, 14·85% N; found: 48·70% C, 5·94% H, 14·58% N.

Dimer: $[\alpha]_D^{24}$ −5·6° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Cys 1·86, Tyr 0·86, Phe 1·06, Glu 1·06, Asp 1·04, Pro 1·05, Dab 1·01, Gly 1·06.

b) Nonapeptide amide *XXVIII* (500 mg; 0·33 mmol) was reduced to give 235 mg (70%) of crude product *XXIX*. Purification by continuous free-flow electrophoresis yielded 73 mg (22%) of compound. The lyophylisate was identical with the monomer prepared under *a*) (chromatography in systems A and B, electrophoresis); $[\alpha]_D^{24}$ −12·0° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Cys 1·90, Tyr 0·96, Phe 1·07, Glu 1·04, Asp 1·05, Pro 1·03, Dab 0·95, Gly 1·00. In addition, 33 mg (10%) of dimer was isolated; $[\alpha]_D^{24}$ −4·8° (*c* 0·2, 1M-CH_3COOH). Amino acid composition: Cys 1·84, Tyr 0·95, Phe 1·11, Glu 1·05, Asp 0·97, Pro 1·08, Dab 0·93, Gly 1·02.

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