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Synthesis and Some Pharmacological Properties of [1-β-Mercaptopropionic Acid, 2-(3,5-Dibromo-L-Tyrosine)]-8-Lysine-Vasopressin[1]

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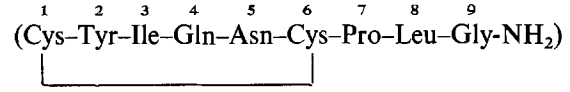
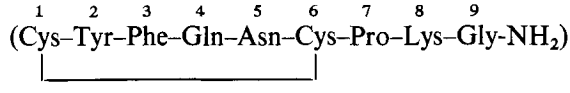
The title compound, [1-β-mercaptopropionic acid, 2-(3,5-dibromo-L-tyrosine)]-8-lysine-vasopressin (X), was synthesized by condensation of Pro-Lys(Boc)-Gly-NH_2 with the cyclic peptide [1-β-mercaptopropionic acid, 2-(3,5-dibromo-L-tyrosine)]-pressinoic acid. X has no oxytocic, avian vasodepressor, pressor, or antipressor activities, but is a weak inhibitor of the responses to oxytocin in the oxytocic and avian vasodepressor assays. Its pharmacological properties are qualitatively identical to those of the corresponding analog of oxytocin, although it is a less potent antagonist than the latter compound.

INTRODUCTION

Several types of chemical modification of the hormone oxytocin



produce analogs that have inhibitory properties or exhibit sharply decreased agonist activity in several assays. In many cases the same modifications of 8-lysine-vasopressin (LVP)



result in analogs with similar changes in pharmacological activities. For example, [Tyr(OMe)[2]]oxytocin (*1*) and [Tyr(OEt)[2]]oxytocin (*2*, *3*) have been found to inhibit, under appropriate conditions, the oxytocic, rat pressor, and other responses to oxytocin and vasopressin; the corresponding analogs of LVP, [Tyr(OMe)[2]]-LVP (*4*, *5*) and [Tyr(OEt)[2]]-LVP (*5*), also have antioxytocic and antipressor (or reduced pressor) properties. It has been proposed that the antagonistic characteristics of these analogs

[1] The following abbreviations are used: β-Mpa (β-mercaptopropionic acid); Dbt (3,5-dibromotyrosine); Ec (ethylcarbamoyl); DCC (*N*,*N*′-dicyclohexylcarbodiimide); DCU (*N*,*N*′-dicyclohexylurea); DMF (dimethylformamide); $HOPhCl_3$ (2,4,5-trichlorophenol). All optically active amino acids are of the L configuration.

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result from the bulky, lipophilic substituents on the aromatic ring rather than from the blocking or elimination of the phenolic functional groups (*2*, *5*, *6*).

Support for this hypothesis, as it relates to the antioxytocic activity of oxytocin analogs, has recently been provided by the potent inhibitors [2-*o*-iodotyrosine]oxytocin (*7*) and [1-β-mercaptopropionic acid, 2-(3,5-dibromotyrosine)]oxytocin (*8*), analogs containing the free phenolic function as well as bulky, lipophilic substituents in the 2 position. [2-*o*-Iodotyrosine]oxytocin inhibits the oxytocic response with a pA_2 value of 7.2[3] and also inhibits the pressor response. [1-β-Mercaptopropionic acid, 2-(3,5-dibromotyrosine)]oxytocin inhibits both the oxytocic and avian vasodepressor (AVD) responses to oxytocin with pA_2 values of 7.05 ($\bar{M} = 8.9 \times 10^{-8}$, $\sigma = 0.2 \times 10^{-8}$) and 7.44 ($\bar{M} = 3.6 \times 10^{-8}$, $\sigma = 1.0 \times 10^{-8}$), respectively, but is inactive in the rat pressor assay. In order to determine whether the parallelism between similarly modified analogs of oxytocin and LVP would apply to the dibromotyrosine modification, the synthesis and pharmacological testing of [1-β-mercaptopropionic acid, 2-(3,5-dibromotyrosine)]-8-lysine-vasopressin ([β-Mpa[1], Dbt[2]]-LVP) was undertaken and is reported in this communication.

RESULTS AND DISCUSSION

This analog was prepared by "ring-plus-tail" condensation of [β-Mpa[1], Dbt[2]]-pressinoic acid with Pro–Lys(Boc)–Gly–NH_2. Preformed disulfide rings have been used in peptide fragment condensations previously by Hiskey and Smith (*9*), Kamber et al. (*10*), Mühlemann et al. (*11*), and Jones et al. (*12*). The key intermediate for the ring portion of the molecule, β-Mpa(Ec)–Dbt–Phe–Gln–Asn–Cys(Ec)–OMe, was prepared by a stepwise sequence from the C-terminus, using N^{α}-Boc protection throughout and the dicyclohexylcarbodiimide with 1-hydroxybenzotriazole preactivation procedure (*13*) as modified by Nestor et al. (*14*), for the incorporation of most amino acid residues. 3,5-Dibromotyrosine was incorporated as the 2,4,5-trichlorophenyl ester (*15*). The base-labile ethylcarbamoyl (*16*) protecting groups were used instead of the more commonly employed benzyl groups for the protection of the sulfur atoms of cysteine and β-mercaptopropionic acid to avoid the necessity of subjecting the halogen-containing peptide to Na in NH_3 to obtain the disulfhydryl compound. The Ec groups and the methyl ester were hydrolyzed simultaneously in 80% MeOH with NaOH (*16*), and the resulting disulfhydryl compound was oxidized to the disulfide with diiodoethane (*17*). The [β-Mpa[1], Dbt[2]]pressinoic acid thus obtained was purified by partition chromatography (*18*) in *n*-BuOH/C_6H_6/pyridine/HOAc/H_2O (750:250:100:1:900), followed by gel filtration (*19*) in 20% acetic acid and finally by partition chromatography in *n*-BuOH/pyridine/H_2O (6:1:7).

The second key intermediate, Z-Pro–Lys(Boc)–Gly–NH_2, was prepared by coupling Z-Pro–OH to Lys(Boc)–Gly-NH_2 (*12*) by DCC with 1-hydroxybenzotriazole, but

[3] The pA_2 values are defined by Schild (*25*) and are the negative logarithms to the base 10 of the average molar concentrations ($\bar{M}$) of antagonist which will reduce the appropriate biological response to $2x$ units of pharmacologically active compound (agonist) to the response to x units of the agonist. The standard deviation in $\bar{M}$ is represented by σ. Specific details of the antioxytocic and anti-AVD assay are described by Vavrek et al. (*26*), and the rat antipressor assay is described by Dyckes et al. (*27*).

without preactivation (*13*). The benzyloxycarbonyl protecting group was removed by catalytic hydrogenation and the resulting peptide condensed with [β-Mpa^1,Dbt^2]pressinoic acid, using DCC with 1-hydroxybenzotriazole but without preactivation. The remaining *t*-butyloxycarbonyl protecting group was removed by anhydrous trifluoroacetic acid. [β-Mpa^1,Dbt^2]-LVP was then purified by partition chromatography in *n*-BuOH/C_6H_6/HOAc/H_2O (8:3:2:8), followed by gel filtration in 0.2 *N* HOAc.

The highly purified analog was assayed[4] for oxytocic, avian vasodepressor (AVD), and rat pressor activities against the U.S.P. posterior pituitary standard. No measurable agonist activities were detected. When assayed for inhibition[3] of the oxytocic and AVD responses to synthetic oxytocin, [β-Mpa^1,Dbt^2]-LVP was found to inhibit both responses with pA_2 values of 6.29 ($\bar{M} = 5.1 \times 10^{-7}$, $\sigma = 1.4 \times 10^{-7}$) and 6.97 ($\bar{M} = 1.1 \times 10^{-7}$, $\sigma = 0.4 \times 10^{-7}$), respectively. When assayed as an inhibitor of the rat pressor response to synthetic LVP, no antagonist activity was found. In all of these biological assays [β-Mpa^1,Dbt^2]-LVP exhibits properties parallel to those of the corresponding oxytocin analog (*8*). It has approximately one-sixth of the antioxytocic and one-third of the anti-AVD potency of [β-Mpa^1,Dbt^2]oxytocin. Both analogs lack antipressor activity.

EXPERIMENTAL SECTION

Melting points were determined in capillary tubes and are uncorrected. Specific rotations were determined on a Perkin–Elmer 141 polarimeter. Precoated plates of silica gel F-254 (0.25 mm, E. Merck) were used for thin layer chromatography (tlc) of 5–20-μg samples and were developed with the solvent systems: (A) $CHCl_3$/MeOH/HOAc (9:1:1); (B) $CHCl_3$/MeOH/HOAc (5:1:1); (C) $CHCl_3$/MeOH/HOAc (3:1:1); (D) BuOH/HOAc/H_2O (3:1:1); (E) BuOH/pyridine/H_2O (20:10:11); (F) BuOH/pyridine/HOAc/H_2O (15:10:3:12); and (G) BuOH/pyridine/HOAc/H_2O (6:10:6:3). Spots were visualized by uv and ninhydrin, where possible, and in all cases by chlorination followed by starch–KI spray. Where compounds are formulated as containing solvent, this does not necessarily imply that they are defined solvates; particularly in the case of amorphous products, solvent retention may be due to mild drying conditions (24 hr, 22°C, 0.005 Torr, over P_2O_5).

Boc protection was removed from intermediate peptides by treatment with saturated HCl in EtOAc or with anhydrous TFA as indicated. Deprotected peptide salts were precipitated and washed with ether, dried *in vacuo*, then dissolved in dimethylformamide (DMF) and neutralized with *N*-methylmorpholine. For most condensation reactions, the Boc amino acids (1.2 equiv) were dissolved in DMF at 0°C and preactivated with DCC (1.2 equiv) in the presence of 1-hydroxybenzotriazole (1.8 equiv) for 1 hr at 0°C and 1 hr at room temperature. This reaction mixture was then filtered directly into the

[4] Oxytocic assays were performed on uteri from rats in natural estrus according to the method of Holton (*20*), as modified by Munsick (*21*), with the use of Mg-free van Dyke–Hastings solution as the bathing fluid. Avian vasodepressor assays were performed on conscious chickens by the method of Coon (*22*) as modified by Munsick et al. (*23*). Pressor assays were carried out on anesthetized male rats (*24*).

DMF solution of deprotected peptide (1.0 equiv), and two washings of the precipitated DCU were added also. Where needed, additional aliquots of *N*-methylmorpholine were added to maintain the pH near neutrality. The progress of the coupling reactions was followed by the quantitative Kaiser test (*27*, *28*).

HCl·H-Cys(Ec)-OMe (**I**)

S-Ethylcarbamoylcysteine (*16*) (10.0 g, 52.0 mmol) was suspended in anhydrous MeOH (150 ml), and HCl (scrubbed through naphthalene and $CaCl_2$ towers) was bubbled into the suspension until all the material dissolved. The solution was cooled to 0°C, saturated with HCl, allowed to stand at room temperature for 4 hr, and evaporated to dryness under reduced pressure. The residue was twice dissolved in MeOH and evaporated to dryness *in vacuo*. The resulting white solid was recrystallized from MeOH/Et_2O (1:4): 11.38 g (90%); pure by tlc (C); mp 148–149°C; $[\alpha]_D^{21} - 17.1°$ (*c* 2, H_2O).

Anal. Calcd for $C_7H_{14}N_2O_3S \cdot HCl$: C, 34.64; H, 6.23; N, 11.54. Found: C, 34.56, H, 6.13; N, 11.40.

Boc-Asn–Cys(Ec)-OMe (**II**)

This compound was prepared on a 50 m*M* scale in 155 ml of DMF as described above, except that **I** was used in a 5% excess. After 24 hr, the reaction was complete and the solution was evaporated to dryness under reduced pressure to yield a viscous, yellow oil that solidified on trituration with H_2O. This solid was collected, washed with 5% $NaHCO_3$ (2 × 50 ml), H_2O (2 × 100 ml), 1 *N* HOAc (2 × 100 ml), and H_2O, dried *in vacuo*, and crystallized from 420 ml of EtOH/EtOAc (1:6): 15.3 g (73%); pure by tlc (A); mp 165–166°C; $[\alpha]_D^{21} - 26.2°$ (*c* 0.6, DMF).

Anal. Calcd for $C_{16}H_{28}N_4O_7S$: C, 45.69; H, 6.72; N, 13.32. Found: C, 45.86; H, 6.71; N, 13.03.

Boc-Gln–Asn–Cys(Ec)-OMe (**III**)

This compound was prepared on an 11.9 m*M* scale by the standard procedure after deprotection of **II** in HCl/EtOAc for 0.5 hr. The reaction was carried out in 100 ml of DMF, and after 5 hr, tlc (B) showed no remaining dipeptide. The solution was evaporated to dryness *in vacuo* and the residue was triturated with EtOAc (100 ml). The resulting solid was collected, washed with EtOAc (4 × 50 ml), 95% EtOH (4 × 50 ml) and Et_2O (4 × 50 ml), and dried *in vacuo*: 6.15 g (94%). Tlc (B) showed that the product was contaminated by a trace of DCU. This material was crystallized from 250 ml of DMF/EtOAc (1:4): 5.50 g (89% recovery, 84% overall yield): pure by tlc (B); mp 189.5–190.5°C; $[\alpha]_D^{21} - 29.2°$ (*c* 0.4, DMF).

Anal. Calcd for $C_{21}H_{36}N_6O_5S$: C, 45.97; H, 6.63; N, 15.32. Found: C, 46.16; H, 6.66; N, 15.09.

Boc-Phe–Gln–Asn–Cys(Ec)-OMe (**IV**)

This compound was prepared on a 1.77 m*M* scale by the standard procedure after deprotection of **III** in HCl/EtOAc for 0.5 hr. The reaction was carried out in DMF (20 ml), and was complete after 3 hr. The solution was evaporated to dryness *in vacuo*,

and the residue was triturated with EtOAc (15 ml). The resulting solid was collected, washed with EtOAc (4 × 10 ml), 95% EtOH (4 × 10 ml), and Et_2O (5 × 20 ml), dried *in vacuo*, and precipitated from 25 ml of DMF/EtOAc (1:4): 1.14 g (93%); pure by tlc (B); mp 227–228°C; $[\alpha]_D^{21} - 16.2°$ (*c* 1, DMF).

Anal. Calcd for $C_{30}H_{45}N_7O_{10}S \cdot H_2O$: C, 50.83; H, 6.68; N, 13.83. Found: C, 51.16; H, 6.57; N, 13.79.

N-t-*Butyloxycarbonyl-3,5-Dibromotyrosine* (**V**)

Following the procedure of Schnabel (*29*), 3,5-dibromotyrosine·H_2O (3.57 g, 10.0 mmol) was suspended in H_2O (10 ml) and peroxide-free dioxane (10 ml). NaOH, 4 *N*, was added until all the material dissolved, and Boc-N_3 (1.53 ml, 11.0 mmol) was added. The reaction was carried out in a pH-Stat at pH 9.80, and after 24 hr the uptake of base had ceased. The solution was diluted with H_2O (100 ml) and extracted with Et_2O (3 × 50 ml). The aqueous layer was covered with EtOAc (100 ml) and acidified to pH 2 at 0°C with vigorous stirring. The layers were separated and the aqueous phase extracted with EtOAc (2 × 100 ml). The organic extractions were combined, dried over $MgSO_4$ and evaporated to dryness under reduced pressure to yield a white, crystalline solid. This material was recrystallized twice from EtOAc/hexane: 2.88 g (66%); pure by tlc (A); mp 151–151.5°C; $[\alpha]_D^{21} - 16.7°$ (*c* 1, DMF). Analysis by nmr spectroscopy showed some EtOAc present in the crystalline material. The amount of EtOAc was constant and independent of the nmr solvent used.

Anal. Calcd for $C_{14}H_{17}Br_2NO_5 \cdot 1/4EtOAc$: C, 39.06; H, 4.16; Br, 34.66; N, 3.04. Found: C, 39.19; H, 4.30; Br, 34.59; N, 3.17.

N-t-*Butyloxycarbonyl-3,5-dibromotyrosine 2,4,5-trichlorophenyl ester* (**VI**)

The Boc amino acid **V** (4.61 g, 10.0 mmol) and 2,4,5-trichlorophenol (*15*) (2.17 g, 11.0 mmol) were dissolved in EtOAc (33 ml). The solution was cooled to 0°C and DCC (2.06 g, 10.0 mmol) was added. The reaction was stirred at 0°C for 2 hr and then at room temperature overnight. The precipitated DCU was filtered off and washed with EtOAc, and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized twice from absolute EtOH: 3.90 g (63%); tlc (A) showed the compound to be contaminated by a trace of $HOPhCl_3$; mp 165–166°C; $[\alpha]_D^{21} - 41.6°$ (*c* 2, DMF).

Anal. Calcd for $C_{20}H_{18}Br_2Cl_3NO_5$: C, 38.84; H, 2.93; N, 2.26. Found: C, 38.94; H, 3.26; N, 2.24.

Boc-Dbt-Phe–Gln–Asn–Cys(Ec)-OMe (**VII**)

The tetrapeptide **IV** (1.50 g, 2.16 mmol) was treated with anhydrous TFA for 15 min. The deprotected peptide was precipitated with Et_2O, collected, washed with Et_2O and dried *in vacuo*. This material was dissolved in DMF (20 ml) and neutralized with 0.27 ml (2.41 mmol) of *N*-methylmorpholine. The active ester **VI** was added and additional base was supplied during the course of the reaction to keep the pH near neutrality. After 48 hr the solution was evaporated to dryness under reduced pressure and the residue triturated with EtOAc (50 ml). The precipitate was isolated by centrifugation,

washed by centrifugation with EtOAc (3 × 50 ml), 95% EtOH (3 × 50 ml) and Et_2O (5 × 50 ml), and dried *in vacuo*: 1.98 g (90%); pure by tlc (B); mp 231–234°C; $[\alpha]_D^{21}$ – 13.3° (*c* 0.5, DMF).

Anal. Calcd for $C_{39}H_{52}Br_2N_8O_{12}S$: C, 46.06; H, 5.16; N, 11.02; Br, 15.72. Found: C, 45.99; H, 5.18; N, 10.73; Br, 16.08.

[β-Mpa¹, Dbt²]pressinoic acid (VIII)

After deprotection of 0.394 mmol of **VII** in TFA, Mpa(Ec)–OH was coupled by the standard procedure. The reaction was carried out in 11 ml of DMF. The reaction stopped after 5 hr at 70% completion, and another 40% of preactivation solution was added. After 22 hr, the reaction was complete, and the solution was evaporated to dryness under reduced pressure. The yellow residue was triturated with EtOAc (25 ml), isolated by centrifugation, washed with EtOAc (4 × 15 ml), 95% EtOH (4 × 15 ml) and Et_2O, dried *in vacuo*: 404 mg (96%). The material had a minor impurity, as shown by tlc (B), but was used satisfactorily in the next step.

This protected intermediate, β-Mpa(Ec)-Dbt-Phe–Gln–Asn–Cys(Ec)-OMe, (161 mg, 0.150 mmol) was suspended in 80% MeOH (15 ml). The suspension was deoxygenated with helium, cooled to 0°C, and 1 *N* NaOH (0.60 ml, 0.60 mmol) was added. The reaction was allowed to proceed under He for 1 hr, and another 0.15 ml of 1 *N* NaOH was added. After another 2 hr the solution was diluted with 400 ml of MeOH/H_2O (1:1) and the disulfhydryl peptide thus obtained was oxidized to the disulfide with 63.5 mg (0.225 mmol) of diiodoethane (*17*). After 15 min the Ellman test (*30*) indicated that the oxidation was complete. The solution was evaporated to dryness under reduced pressure and the residue lyophilized from glacial HOAc. This material was subjected to partition chromatography (*18*) on Sephadex G-25 (100–200 mesh, 2.8 × 70 cm) in the system *n*-BuOH/C_6H_6/pyridine/HOAc/H_2O (750:250:100:1:900) at a flow rate of 18.4 ml/hr. Peptide materials, detected by the Folin–Lowry method (*31*), emerged in two major peaks at R_f 0.90 and R_f 0.23. The materials isolated from the two peaks were tentatively identified as [β-Mpa¹, Dbt²]pressinoic acid methyl ester and [β-Mpa¹, Dbt²]pressinoic acid, respectively. The acid was subjected to gel filtration (*19*) on Sephadex G-25 (200–270 mesh, 2.8 × 68 cm) in 20% HOAc at a flow rate of 24 ml/hr. A single sharp peak emerged at 116% of the column volume. The material isolated from this peak was further purified by partition chromatography on Sephadex G-25 (100–200 mesh, 2.8 × 70 cm) in *n*-BuOH/pyridine/H_2O (6:1:7) at a flow rate of 17.6 ml/hr. A single peak emerged at R_f 0.32 containing 37 mg (27% overall yield) of product: pure by tlc (D, E, F); $[\alpha]_D^{21}$ – 28.9° (*c* 0.13, 1 *N* HOAc).

Anal. Calcd for $C_{33}H_{39}Br_2N_7O_{10}S_2 \cdot CH_3CO_2H \cdot H_2O$: C, 42.22; H, 4.56; Br, 16.05; N, 9.85. Found: C, 42.25; H, 4.53; Br, 16.10; N, 10.03.

Cbz-Pro–Lys(Boc)–Gly-NH_2 (IX)

Lys(Boc)–Gly-NH_2 (*32*) (302 mg, 1.00 mmol), 1-hydroxybenzotriazole (243 mg, 1.80 mmol) and Cbz-Pro-OH (300 mg, 1.20 mmol) were dissolved in DMF (4 ml) and the solution was cooled to 0°C. DCC (247 mg, 1.20 mmol) was added and the reaction was stirred at 0°C for 1 hr and overnight at room temperature. The solution was cooled

to 0°C and the DCU was filtered off and washed with DMF (2 × 2 ml). The combined filtrates were evaporated *in vacuo* to a yellow oil. The oil was triturated with H_2O to yield a crystalline solid which was collected, washed with H_2O, 5% $NaHCO_3$ (3 × 5 ml), H_2O, 1 *N* HOAc (3 × 5 ml) and H_2O, dried *in vacuo* and recrystallized from EtOH/H_2O (1:4): 330 mg (63%). Analysis by tlc (C) showed the product contaminated by a trace of DCU which could not be removed by further recrystallization; mp 149°–150°C; $[\alpha]_D^{21} - 31.7°$ (*c* 1, DMF).

Anal. Calcd for $C_{26}H_{39}N_5O_7$: C, 58.52; H, 7.37; N, 13.12. Found: C, 58.87; H, 7.49; N, 12.80.

[*β-Mpa*^{1}, *Dbt*^{2}]-*8-lysine-vasopressin* (X)

The tripeptide **IX** (200 mg, 0.376 mmol) was dissolved in absolute EtOH (20 ml). The solution was deoxygenated with N_2, then 50 mg of 10% palladium on charcoal was added, and the compound was hydrogenated at atmospheric pressure for 90 min. The catalyst was filtered off through Celite and the filtrate evaporated to dryness to yield a colorless oil. This oil was dissolved in 3.65 ml of DMF to give a concentration of 37 mg/ml of Pro–Lys(Boc)–Gly-NH_2. Into 0.5 ml of this solution (18.5 mg, 66 μmol of the free tripeptide) was dissolved 30.2 mg (33 μmol) of **VIII** and 8.96 mg (66 μmol) of 1-hydroxybenzotriazole. The solution was cooled to 0°C and 8.16 mg (40 μmol) of DCC was added. The reaction was allowed to proceed for 2 hr at 0°C and for 24 hr at room temperature. The precipitated DCU was filtered off, washed on the filter with DMF (4 × 1 ml), and the combined filtrates were evaporated to dryness under reduced pressure. The residue was treated with anhydrous TFA for 15 min, then diluted with 40 ml of H_2O and evaporated (below 30°C) to about 5 ml. This solution was again diluted with 40 ml of H_2O, evaporated to dryness under reduced pressure, and the residue lyophilized from glacial HOAc. The crude product was subjected to partition chromatography on Sephadex G-25 (100–200 mesh, 2.8 cm × 70 cm) in *n*-BuOH/C_6H_6/HOAc/H_2O (8:3:2:8) at a flow rate of 20 ml/hr. The products were detected by the Folin–Lowry method and emerged as a single peak at R_f 0.16. The material from this peak was subjected to gel filtration on Sephadex G-25 (200–270 mesh, 2.8 cm × 68 cm) in 0.2 *N* HOAc at a flow rate of 22 ml/hr. A single sharp peak emerged at 137% of the column volume. The fractions comprising this peak were pooled and lyophilized: 26 mg (66%); pure by tlc (F, G); $[\alpha]_D^{21} - 72.2°$ (*c* 0.3, 1 *N* HOAc). Amino acid analysis (*33*) of a sample hydrolyzed in 6 *N* HCl at 110°C for 24 hr was performed on a Beckman Model 116 analyzer using a single column system (*34*). Dibromotyrosine emerged between Lys and NH_3. The following molar ratios were obtained: Asp, 0.98; Glu, 1.03; Pro, 1.00; Gly, 0.96; ½Cys, 0.46; mixed disulfide of Cys and β-Mpa, 0.61; Phe, 0.98; Lys, 0.96; Dbt, 0.91; NH_3, 3.09.

Anal. Calcd for $C_{46}H_{62}Br_2N_{12}O_{12}S_2 \cdot CH_3CO_2H \cdot 4H_2O$: C, 43.31; H, 5.68; Br, 12.01; N, 12.63. Found: C, 43.62; H, 5.53; Br, 12.02; N, 12.49.

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