serum albumin was utilized as the standard.

The concentrations of the pyrazoloquinolines that inhibit specific [^{3}H]flunitrazepam binding by 50% (IC_{50}) were determined by log–probit analysis with four to six concentrations of the displacers, each performed in triplicate.

Registry No. **1a**, 97789-05-4; **1b**, 97993-50-5; **1c**, 97993-47-0; **1d**, 97993-49-2; **1e**, 97993-46-9; **2a**, 71814-52-3; **2b**, 98990-72-8; **2c**, 98990-73-9; **2d**, 98990-74-0; **2e**, 98990-75-1; **3a**, 98990-76-2; **3b**, 98990-77-3; **3c**, 98990-78-4; **3d**, 98990-79-5; **3e**, 98990-80-8; **4a**, 98990-81-9; **4b**, 98990-82-0; **4c**, 98990-83-1; **4d**, 98990-84-2; **4e**, 98990-85-3; **5a**, 98990-86-4; **5b**, 98990-87-5; **5c**, 98990-88-6; **5d**, 98990-89-7; **5e**, 98990-90-0; **6a**, 98990-91-1; **6b**, 98990-92-2; **6c**, 98990-93-3; **6d**, 98990-94-4; **6e**, 98990-95-5; **7a**, 98990-96-6; **7b**, 98990-97-7; **7c**, 98990-98-8; **7d**, 98990-99-9; **7e**, 98991-00-5; **8**, 37638-10-1.

Ketomethylene Pseudopeptide Analogues of Substance P: Synthesis and Biological Activity†

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Two pseudopeptide analogues [Bz-(*RS*)Phe$^8\psi$(COCH$_2$)Gly^9]SP$_{8-11}$ (**I**) and [pGlu^6,(*RS*)Phe$^8\psi$(COCH$_2$)Gly^9]SP$_{6-11}$ (II) of the substance P related C-terminal hexapeptide [pGlu^6]SP$_{6-11}$ were prepared as follows. The pseudodipeptidic unit H(*RS*)Pheψ(COCH$_2$)GlyOH was synthesized by using a modified Dakin–West reaction between Bz-Phe-OH and monomethyl succinoyl chloride. The N$^\alpha$-protected pseudopeptidic unit was then incorporated into the appropriate peptide by using various coupling methods. The two pseudopeptide analogues were purified, characterized, and tested for their biological activity and inhibitory effect on SP degrading enzymes. Analogue II was a full agonist contracting the isolated guinea pig ileum with a potency of 70% compared to the parent hexapeptide [pGlu^6]SP$_{6-11}$. It was also a potent inhibitor of SP degrading activity in rat diencephalon membranes with a K_i of 20 μM whereas analogue I was a weak inhibitor.

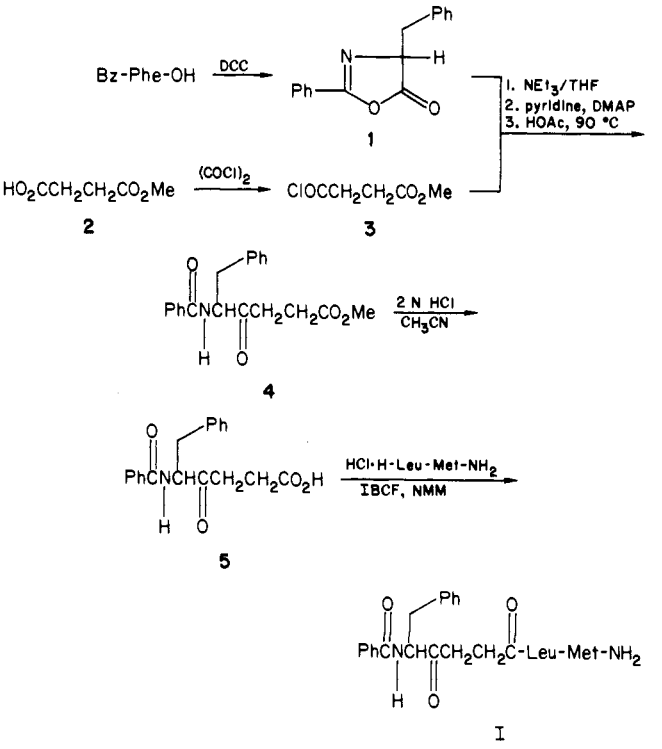
Substance P (SP), an undecapeptide with the sequence H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$, is a putative neurotransmitter or neuromodulator.[1] It has been shown that C-terminal hexapeptide fragments and analogues are equipotent to the parent compound in most bioassays. For example, the hexapeptide [pGlu^6]SP$_{6-11}$ is more potent than SP in contracting the guinea pig ileum[2] and in depolarizing spinal cord motoneurons;[3] it is equipotent to SP in inducing K^+ release from rat parotid slices.[4]

The transient nature of most in vitro and in vivo pharmacological effects stimulated by SP and [pGlu^6]-SP$_{6-11}$ is due to fast proteolytic degradation by various enzymes.[5] Lee and co-workers have reported the isolation of a membrane-bound neutral metalloendopeptidase from human brain, which cleaves SP between the residues Gln^6-Phe^7, Phe^7-Phe^8, and Phe^8-Gly^9.[5] We have recently reported the characterization of the degradation patterns of the hexapeptide [pGlu^6]SP$_{6-11}$ by rat parotid and hypothalamic slices.[6]

It has been reported that other membrane-bound brain metalloendopeptidases like enkephalinase and angiotensin converting enzyme (ACE) are capable of cleaving SP, yet it is not clear at present whether these peptidases also play significant roles in the inactivation of SP at the synaptic level.[7,8]

In order to extend the duration of substance P activity, degradation resistant analogues thereof have been designed and prepared. These were found to elicit more sustained biological responses.[9-11] An alternative approach to extend substance P action involves preparation of specific inhib-

Scheme I



† Abbreviations according to IUPAC–IUB Commission (1972), *Biochemistry*, *11*, 1726–1732, and Specialist Periodical Reports, "Amino Acids, Peptides and Proteins", Volume 11 (The Chemical Society, London, 1980, R. C. Sheppard, Ed.), are used throughout. The following special abbreviations are used for the ketomethylene peptides and fragments. The standard three-letter notation for amino acid residues preceded by the symbols ψ(COCH$_2$) represents the ketomethylene-modified residue of the pseudodipeptide.

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itors of the degrading enzymes. This approach had been successfully applied to prepare highly potent and specific inhibitors of enkephalinase and ACE.[12,13]

Since the proteases which are involved in the degradation of SP are metalloendopeptidases, the incorporation of mildly chelating isosteric moieties replacing a scissile amide bond in the substrate could furnish an inhibitor.

The recent reports by Almquist[14] and Ondetti[15] concerning the synthesis of highly potent inhibitors of ACE that include a ketomethylene group replacing a peptide bond in a substrate fragment of ACE prompted us to attempt the inclusion of such a backbone modification into the biologically active C-terminal hexapeptide analogue of SP.

In this paper we report the preparation, as well as the biological and inhibitory activities, of two pseudopeptide analogues of C-terminal sequences of substance P, containing a ketomethylene group replacing the Phe^8-Gly^9 peptide bond.

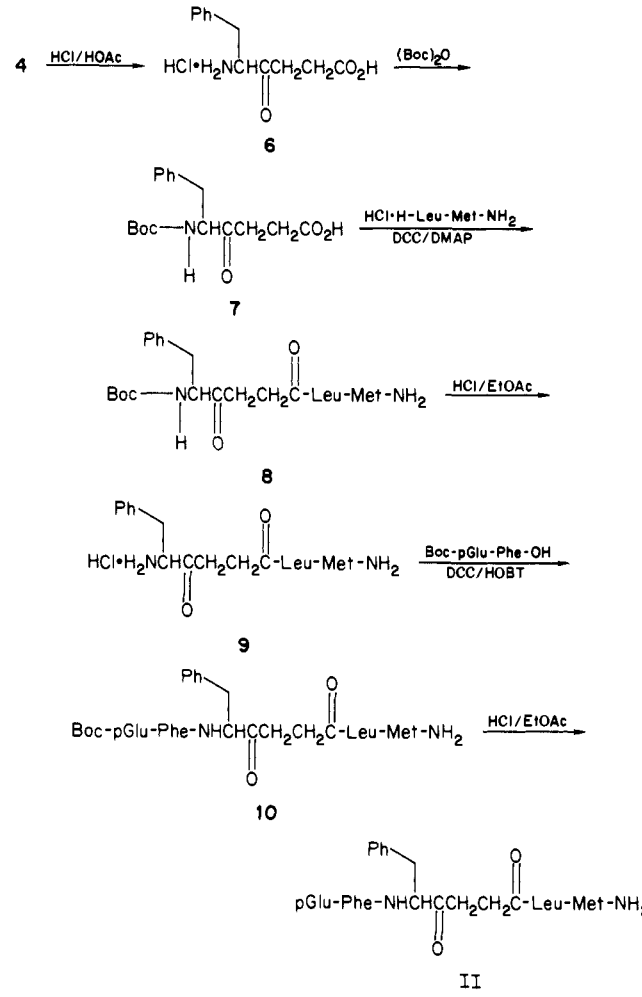
The two analogues prepared, [Bz(R,S)Phe$^8\psi$(COCH$_2$)-Gly^9]SP$_{8-11}$ (I) and [pGlu^6,(*RS*)Phe$^8\psi$(COCH$_2$)Gly^9]SP$_{6-11}$ (II), were aimed at probing the active site requirements of the enzymes that degrade SP. Analogue I lacks a dipeptidyl unit from the amino terminus, i.e., pGlu^6-Phe^5, which enabled us to evaluate the importance of these two residues for inhibitory activity.

Results

Scheme I summarizes the synthetic pathway followed in the preparation of analogue I. The modified Dakin–West reaction was chosen as the most straightforward procedure to obtain the desired pseudodipeptide unit, albeit the fact that it affords an enantiomeric mixture.

Thus the reaction of the oxazolone of Bz-Phe-OH, 1, with monomethyl succinoyl chloride (3) in dry THF, in the presence of triethylamine, was followed by treatment of the crude product with pyridine and DMAP and then warmed with HOAc at 90 °C for a short period (30 min). The protected pseudodipeptidic product 4 was purified by recrystallization. For the preparation of pseudopeptide I, the methyl ester was hydrolyzed to yield 6-phenyl-5-(*N*-benzoylamino)-4-ketohexanoic acid (5). This was then coupled by the mixed anhydride method to H-Leu-Met-NH_2, yielding the pure N-blocked pseudotetrapeptide I as a diastereomeric mixture as shown by HPLC. The preparation of the pseudohexapeptide II is depicted in Scheme II. For this purpose the crude fully blocked pseudodipeptide unit was deprotected by acid hydrolysis under

Scheme II



reflux. The hydrochloride of 6-phenyl-5-amino-4-ketohexanoic acid (6) precipitated in practically pure form and reasonable yield from acetonitrile/ether after evaporation of solvents and reactants. Attempts to couple the *N*-(*tert*-butoxycarbonyl)-protected pseudodipeptidic unit to the C-terminal dipeptide carboxamide, namely H-Leu-Met-NH_2, via the mixed anhydride method failed. Since this coupling requires the activation of a pseudo-Gly residue, the use of DMAP involved no danger of racemization. Thus the use of DCC/DMAP as coupling reagents gave the desired product in a pure form. Deprotection of the Boc-protected pseudotetrapeptide was achieved by HCl/EtOAc to give the pseudotetrapeptide 9, which was coupled via DCC/HOBT to Boc-pGlu-Phe-OH. Finally the protected pseudohexapeptide 10 was in turn deprotected by HCl/EtOAc to give the desired analogue II. This was obtained in a pure form by recrystallization.

The purity of the pseudopeptides I and II as well as that of the pseudodipeptidic unit was assessed by HPLC and TLC. They were characterized by elemental analysis, amino acid analysis of their hydrolysates, and FAB-MS molecular ions and sequence matching. Pseudopeptides I and II were also characterized by ^{1}H NMR and the structure of Bz(*RS*)Pheψ(COCH$_2$)Gly-OMe (4) was also determined by single-crystal X-ray analysis. These results will be published elsewhere.

In both pseudopeptides, HPLC analysis gave two closely eluting peaks in a roughly 1:1 ratio (details given in the Experimental Section). We attributed these peaks to the two diastereomers and they were characterized as a mixture. For amino acid analysis, calibration runs were per-

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Table I. Relative Potencies of Several Pseudopeptidic Analogues of [pGlu]^{6}SP$_{6-11}$ Containing a Modified Phe^8-Gly^9 Bond[a]

analogue[b]	rel potency,[c] %
[pGlu^6,Phe$^8\psi$(COCH$_2$)Gly^9]SP$_{6-11}$	70
[**p**Glu^6,Phe$^8\psi$(CH=CH)Gly^9]SP$_{6-11}$	24[d]
[pGlu^6,Phe$^8\psi$(NH—CO)Gly^9]SP$_{6-11}$	22[e]
[pGlu^6,Phe$^8\psi$(CH$_2$-O)Gly^9]SP$_{6-11}$	25[f]
[pGlu^6,Sar^9]SP$_{6-11}$	65[g]

[a] As determined by the isolated guinea pig ileum assay. [b] The notation ψ(-)Xxx implies a modification of the peptide bond with the function that substitutes the amide bond between parentheses. [c] Relative to [pGlu^6]SP$_{6-11}$ = 100%, EC$_{50}$ = 2 × 10^{-9} M. [d] Reference 17. [e] Reference 9. [f] Reference 18. [g] Reference 19.

formed to determine the retention time of the deprotected pseudodipeptidic unit **6**. It elutes at the same retention time as His in the normal amino acid standard mixtures.

The effect of pseudopeptides I and II as inhibitors of SP degrading peptidase activity in rat diencephalon membranes was determined by using the radiolabeled substrate N^{α}([^{125}I]desaminotyrosyl)SP$_{6-11}$ according to the procedure described earlier.[18]

Pseudopeptide II was a potent inhibitor of the degradation with a K_i of 20 μM whereas pseudopeptide I did not show a strong inhibitory effect ($K_i \simeq 200$ μM). The inhibitory effects of pseudopeptide II were compared to the parent peptide [pGlu^6]SP$_{6-11}$ ($K_i \simeq 200$ μM) and those of pseudopeptide I to Boc-SP$_{8-11}$ ($K_i >> 200$ μM). The relative spasmogenic activity of peptide II on the isolated guinea pig ileum is shown in Table I. This analogue had 70% of the potency of [pGlu^6]SP$_{6-11}$.

Discussion

As part of the screening process for the design and synthesis of potential inhibitors of SP degrading activities, the ketomethylene analogues presented a relatively simply achieved modification of the peptide backbone with the advantageous features of mild active-site metal chelation combined with the required side-chain configuration for maximum enzymatic recognition. As an agonist, the smaller rigidity of the pseudo peptide's ketone methylene function, having a free rotation around the ketomethylene bond compared with that of the parent compound amide bond, could in principle afford a more suitable range of conformations facilitating peptide–receptor interactions.

The synthetic approach we adopted, although simple in principle, provided some unexpected stumbling blocks concerning mainly the incorporation of the ketomethylene unit into the peptide chain. Whereas the coupling of the protected *N*-benzoyl-γ-keto free acid to H-Leu-Met-NH$_2$ via the mixed anhydride method took place in a smooth and quantitative manner, this coupling performed on the *N*-Boc-protected pseudodipeptide free acid by the same method gave a mixture of unwanted products. We succeeded in obtaining the *N*-Boc-protected pseudotetrapeptide by using DCC/DMAP as coupling reagents. Although the cause of this is not yet fully understood, a partial explanation of this result could lie in the higher electron withdrawing tendency of the alkylurethane protecting group, which would render the ketone carbonyl more prone to nucleophilic attack by amines than in the case of the *N*-benzoyl-protected unit. This could then be offset by a higher acylation rate obtained by the use of DMAP as catalyst, which was indeed observed.

The fact that no parallel phenomenon was observed while coupling the pseudopeptide's free amino group to the carbonyl of an amino acid residue without DMAP as catalyst could serve as further indication to this phenomenon. (In this case lower electron withdrawing effects of the ketone carbonyl due to adjacent free amine would make nucleophilic attack less favorable.)

A fragment condensation strategy allowed us to obtain acceptable yields, based on the observation that in all coupling steps involving the ketomethylene unit, reaction yields were smaller than those normally obtained in "conventional" peptide couplings.

Analogue II was intended to contain all residues present in the parent hexapeptide, thus ensuring maximum recognition by SP degrading peptidases. The low inhibitory activity shown by analogue I indicated the possibility that at least two consecutive hydrophobic residues such as Phe-Phe are necessary on the C side of the cleaved bond in order to achieve active-site recognition. This was clearly borne out by the fact that analogue II showed distinct inhibitory potency. Subsequent experiments with other compounds[16] further confirmed this observation.

The replacement of the Phe^8-Gly^9 peptide bond by its ketomethylene isostere did not affect the biological activity, as measured by the contraction of the guinea pig ileum. Pseudopeptide II was a full agonist with 70% of the biological activity of the parent compound [pGlu^6]SP$_{6-11}$.

The activity of this analogue can be compared to that of several other pseudopeptide analogues of [pGlu^6]SP$_{6-11}$ in which the Phe^8-Gly^9 bond has been replaced by a nonpeptide function (see Table I). Thus the peptide bond between Phe^8-Gly^9 in [pGlu^6]SP$_{6-11}$ tolerates a certain degree of chemical modification without seriously hampering the biological activity.

Experimental Section

Melting points were measured on a Thomas-Hoover capillary melting point apparatus and are uncorrected. HPLC analysis was performed on a Spectra Physics 8000 liquid chromatograph, using a Whatman Partisil ODS column (10-μm mean particle size; 0.4 cm × 25 cm) or a Merck Hibar Lichrospher RP 8 column (5-μm particle mean size; 0.4 cm × 10 cm). TLC was done on precoated silica gel plastic plates Polygram Sil NH-8/UV 254 from Macherey Nagel Co. The solvent systems used were (A) methanol–methylene chloride, 1:9, (B) methanol–methylene chloride, 1:1, (C) methanol/methylene chloride, 1:4, (D) butanol–pyridine–0.1% HOAc, 5:3:11. The plates were developed with use of the following reagent sprays: (I) ninhydrin, 0.1% (Merck), (II) fluorescamine (Fluram, Hoffman La Roche & Co), (III) iodine vapors, (IV) *o*-dianisidine saturated solution in HOAc (glacial). Amino acid analysis was performed on an LKB 4400 amino acid analyzer, equipped with a Spectra Physics 4100 printer plotter computing integrator, using four component sodium buffer systems, and a standard 54-min program. Hydrolysis of samples for amino acid analysis was carried out on 1-mg samples with constant-boiling HCl (0.5 mL), which was degassed, sealed at 0.1 mmHg, and heated at 110 °C for 20 h. The hydrolysate was dried under vacuum over KOH pellets and diluted with sodium citrate buffer (0.2 mL), pH 2.2. Elementary microchemical analysis was carried out at the Microanalytical Laboratory of the Organic Chemistry Department. Where elemental analyses are indicated only by symbols of the elements, analytical results were within ±0.4% of the theoretical values. Mass spectrometry was performed by K. Eckart at the laboratory of Prof. H. Schwarz, The Technische Universitat, Department of Chemistry, Berlin, using a fast atom bombardment (FAB) ion source.

Solvent and Reagent Purification. EtOAc and CH_2Cl_2 were distilled over P_2O_5; DMF was distilled under reduced pressure from Al_2O_3; *N*-methylmorpholine (NMM) was distilled from KOH. Other solvents were distilled before use.

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General Procedures. General Coupling Procedure. (A) In a three-necked flask equipped with nitrogen inlet, mechanical stirrer and subzero thermometer, Boc-amino acid (1.4 equiv) in DMF (0.4–0.7 M) and 1 equiv of NMM were cooled to −20 °C. To the well-stirred solution was added isobutyl chloroformate (1.4 equiv). After 2–10 min at −15 °C a precooled solution of the amine hydrochloride component (1 equiv) in DMF was added followed by NMM (1 equiv). After 5 min the reaction mixture was tested for basicity by introduction of a wet universal pH paper into the vapor phase. If the universal pH paper did not indicate basicity, more NMM (in 0.1-equiv portions) was added. Completion of reaction was monitored by negative fluorescamine test. After 2 h at −15 °C a solution of $KHCO_3$ (2 M, 3 equiv) was added and the mixture stirred vigorously at 0 °C for 0.5 h. Then brine was added (4 times the volume of DMF). After 1 h at 0 °C the precipitate was collected by filtration and washed thoroughly with cold water (to negative Cl^- as checked by $AgNO_3$ reagent). The combined filtrates were kept in the cold until the yield was determined. The precipitate was dried under vacuum. If the yield was lower than 75%, the combined filtrates were extracted with EtOAc which was washed with brine, $KHCO_3$ (2M), and brine, dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure.

(B) In an ice-cooled reaction flask, under argon, the amine hydrochloride (1 equiv), the Boc-protected amino acid (1 equiv), and HOBt (2 equiv) or DMAP (0.2 equiv) were stirred in EtOAc or DMF. DCC (1 equiv) dissolved in the appropriate solvent was dropped in during 15 min concurrently with NMM (1 equiv). After addition, the mixture was allowed to warm up to room temperature and stirred for 1 h. DCU was then filtered off. When EtOAc was the reaction solvent, the mixture was then extracted twice with saturated $NaHCO_3$ solution and then with 0.2 M $KHSO_4$ solution and brine, dried over $MgSO_4$, and evaporated in vacuo. The remaining residue was purified by recrystallization. When DMF was the reaction solvent, after the removal of DCU a saturated solution of $NaHCO_3$ was added (4–6 times the total volume of DMF) with cooling. The precipitate obtained was collected, washed with water, and dried in a dessicator over P_2O_5 at high vacuum. It was then recrystallized, if not sufficiently pure.

General Deprotection Procedure. Boc-protected peptides were dissolved in 4 N HCl in glacial acetic acid or in saturated HCl in EtOAc in the presence of 1% (v/v) thioanisole. The solution was kept 30 min at room temperature protected with $CaCl_2$ tube. Solvent was removed under reduced pressure and the residue was triturated with dry ether and after decanting the supernatant was kept overnight under vacuum over KOH pellets. The solid was recrystallized from MeOH–ether.

Materials. Phosphoramidon was purchased from the Peptide Research Institute (Osaka, Japan), captopril was graciously provided to us by Dr. M. A. Ondetti (Squibb Institute, Princeton, NJ), and [pGlu^6]substance P (6–11), and N^{α}[^{125}I](desamino-iodotyrosyl)substance P (6–11) were prepared according to previously published procedures.[20]

Methyl 6-Phenyl-5-(benzoylamino)-4-oxohexanoate (4). A magnetically stirred and cooled solution of Bz-Phe-OH (20 mmol, 5.39 g) in dry THF (40 mL) was treated with cooling with DCC (22 mmol, 4.54 g). After 1 h the solution was allowed to warm up to room temperature and left stirring for a further 24 h. Removal of DCU by filtration was followed by cooling of the filtrate and treatment with triethylamine (24 mmol, 3.3 mL) and then by the dropwise addition of a solution of monomethyl succinoyl chloride (22 mmol, 3 g). This was stirred during 2 h, and triethylamine hydrochloride was filtered off and the solvent evaporated. The residue was taken up in 10 mL of pyridine with the addition of DMAP (0.032 mmol, 40 mg). Stirring of this for 1 h at 25 °C was followed by warming up in an oil bath to 90 °C and by the addition of HOAc (10 mL). After 1 h, the mixture was cooled and ethyl acetate was added (150 mL) and this was washed consecutively with water, 1 N HCl, water, and a saturated $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$ (anhydrous) and evaporated to dryness. The residue was taken up in methanol and upon the addition of a few milliliters of petroleum-ether a precipitate was obtained which was then collected by filtration and dried at high vacuum: yield 5.42 g (80%); mp 101–103 °C (lit.[21] mp 102–104 °C); TLC R_f (A) 0.63, (D) 0.76; HPLC, k'(MeOH %/H_2O %) 5.57 (55/45), 2.25 (65/35); FAB mass spectrum, m/e 339 [M + H$^+$]. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

6-Phenyl-5-(benzoylamino)-4-oxohexanoic Acid (5). Ester 4 (3 mmol, 1 g) was stirred at 40 °C in 2 N HCl (50 mL) and acetonitrile (25 mL) with reaction followup by TLC. After 24 h no starting material was present. Acetonitrile and HCl were evaporated in vacuo, and the solid residue was collected. After recrystallization from MeOH and drying of the recovered material, the yield was 0.8 g (82%): mp 180 °C [lit.[21] mp 182–184 °C); TLC R_f (A) 0.24; HPLC, k'(MeOH %/H_2O %) 4.42 (65/35); FAB MS, m/e 326 [M + H$^+$]. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

***N*-Benzoyl(*RS*)Pheψ(COCH$_2$)Gly-Leu-Met-NH$_2$ (I).** Coupling was performed following method A. Compound 5 (0.6 mmol, 0.2 g) in dry DMF (2 mL) was reacted with IBCF (0.6 mmol, 71 μL) in the presence of NMM (0.6 mmol, 67 μL). HCl·H-Leu-Met-NH_2 (0.5 mmol, 0.15 g) in DMF (1 mL) was added gradually into the reaction mixture at the same time as a solution of NMM (0.51 mmol, 57 μL) in DMF (0.5 mL) was dropped in. The crude product was recrystallized from hot methanol, affording 0.1 g (18%) of material: mp 212–218 °C; TLC R_f (B) 0.83, (D) 0.86; HPLC, k'(MeOH %/H_2O %) (a) 2.5, (b) 2.8 (70/30) [(a) and (b) refer to the fast and slow peaks, respectively]; FAB MS, m/e 569 [M + H$^+$]. Amino acid analysis: leucine: 1.00; methionine, 1.00; X: 1.00 (X, the pseudo ketomethylene unit is detected at a t_R similar to that of His, established by an independent control run performed with a buffered sample of 6. Anal. ($C_{30}H_{40}N_4O_5S$) C, H, N.

6-Phenyl-5-amino-5-oxohexanoic Acid Hydrochloride (6). Compound 4 (8.5 mmol, 2.7 g) was dissolved in 75 mL of HOAc (glacial) and stirred under argon with HCl (37% 135 mL) and water (45 mL). The mixture was refluxed in an oil bath for 20 h (at 120 °C). Cooling and evaporation afforded a residue, which was dissolved in acetonitrile. Stirring with ether produced an off-white solid, which was collected and reprecipitated from acetonitrile–ether. Drying of this water-soluble material afforded 1.59 g (72.5%) of product: mp 130–135 °C (lit.[14] mp 130–132 °C); TLC R_f (A) 0.02, (B) 0.26; HPLC, k'(MeOH %/H_2O %) 6.81 (60/40). Anal. ($C_{12}H_{15}NO_3$·HCl·$^1/_2H_2O$) C, H, N.

6-Phenyl-5-[(*tert*-butoxycarbonyl)amino]-4-oxohexanoic Acid (7). The hydrochloride salt 6 (5.8 mmol, 15 g) was dissolved in a 2:1 solution of dioxane and water (30 mL). To this mixture was added 1 N NaOH (10 mL) with stirring and cooling (ice bath), followed by $(Boc)_2O$ (11 mmol, 2.45 g). After 1 h at 0 °C and an additional hour at room temperature, completion of reaction was determined by a negative fluorescamine test. Solvents were evaporated partially, and the aqueous residue was extracted once with ethyl acetate, acidified to pH 2 with saturated $KHSO_4$, and extracted three times with ethyl acetate. The combined organic extracts were washed once with brine and then dried over $MgSO_4$. Solvent evaporation and recrystallization of crude product from ethyl acetate/petroleum-ether afforded 1.29 g (69.3%) of pure (7): mp 126–128 °C; TLC R_f (A) 0.38, (B) 0.68; HPLC, k'(MeOH %/H_2O %) 2.25 (70/30); FAB MS, m/e 322 [M + H$^+$]. Anal. ($C_{17}H_{23}NO_5$) C, H, N.

Boc-(*RS*)Pheψ(COCH$_2$)Gly-Leu-Met-NH$_2$ (8). The N-protected pseudodipeptide 7 (0.62 mmol, 0.29 g) and HCl·H-Leu-Met-NH_2 (0.62 mmol, 0.18 g) were coupled in ethyl acetate (15 mL) according to method B using DCC (0.62 mmol, 0.13 g), DMAP (0.012 mmol, 18 mg), and NMM (0.62 mmol, 0.07 mL). Recrystallization of product from ethyl acetate/petroleum-ether afforded 0.13 g (37%) of 9: mp 158–163 °C; TLC R_f (A) 0.46, (B) 0.76, (D) 0.77; HPLC, k'(MeOH %/H_2O %) 3.33 (85/15), 4.57 (65/35). Anal. ($C_{28}H_{44}N_4O_6S$) C, H, N, S.

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HCl·H-(*RS*)Pheψ($COCH_2$)Gly-Leu-Met-NH_2 (9). The N-protected pseudopentapeptide **8** (0.39 mmol, 0.22 g) was deprotected according to the standard deprotection procedure given above. The precipitate obtained was dried in vacuo: yield 0.14 g (73.8%); mp 138–141 °C; TLC, R_f (B) 0.70, (D) 0.57; HPLC, k'(MeOH %/H_2O %) 7.5 (40/60). Anal. ($C_{23}H_{36}N_4O_4S$·HCl·H_2O) C, H, N.

Boc-pGlu-Phe-(*RS*)Pheψ($COCH_2$)Gly-Leu-Met-NH_2 (10). Amine component **9** (0.28 mmol, 0.14 g) was coupled to Boc-pGlu-Phe-OH (0.28 mmol, 0.10 g) following method B, in 2 mL of DMF, using DCC (0.28 mmol, 0.057 g), HOBt (0.56 mmol, 0.076 g), and NMM (0.28 mmol, 0.031 mL): yield 0.14 g (60.8%); mp 105–108 °C dec; TLC R_f (B) 0.92, (D) 0.73; HPLC, k' (MeOH %/H_2O %) (a) 5.41, (b) 7.0 (60/40) [(a) and (b) refer to fast and slow peaks, respectively]; FAB MS, m/e 823 [M + H^+]. Anal. ($C_{42}H_{58}N_6O_9S$) C, H, N.

pGlu-Phe-(*RS*)Pheψ($COCH_2$)Gly-Leu-Met-NH_2 (II). The Boc-protected pseudohexapeptide **10** (0.16 mmol, 0.13 g) was deprotected by the method described above. The residue obtained was twice reprecipitated from absolute methanol, centrifuged at 2500 rpm, collected, and dried in vacuo, yielding 0.1 g (86.5%) of product: mp 262–265 °C dec; TLC R_f (B) 0.88, (D) 0.86; HPLC, k'(MeOH %/H_2O %) (a) 2.9, (b) 3.45 (60/40) [(a) and (b) refer to fast and slow peaks, respectively]; FAB MS, m/e 723 [M + H^+]. Amino acid analysis: Glu: 1.00; Phe: 1.00; Leu: 1.00; Met: 1.00; X = 1.00 (X, the pseudo ketomethylene unit is detected at a t_R similar to that of His, established by an independent control run performed with a buffered sample of **6**). Anal. ($C_{37}H_{50}N_6O_7S$) C, H, N.

Biological Assays. Isolated Guinea Pig Ileum Assay. This was done as previously described.[6]

Rat Diencephalon Membrane System. Diencephalons from seven male albino rats were homogenized in 25 mL of ice-cold Hepes buffer (50 mM, pH 7.4), with 10 strokes of a Teflon-glass homogenizer at 800 rpm, and centrifuged at 1000*g* for 10 min. The supernatant was recentrifuged at 17000*g* for a further 10 min. The resulting pellet was suspended with a Dounce homogenizer in cold buffer and centrifuged again at 17000*g* for 10 min. The final pellet was washed with cold buffer and resuspended in 5 mL of buffer (1–3 mg/mL of protein).

Assay of Peptidase Acting on the C-Terminal Sequence of Substance P. The degradation of N^{α}([^{125}I]-desaminoiodotyrosyl)SP_{6-11} and its inhibition by the above-mentioned compounds by rat diencephalon membrane preparation was assayed as previously described.[16] High concentrations (10^{-6} M each) of phosphoramidon and captopril, potent inhibitors displaying affinities in the nanomolar range toward angiotensin converting enzyme and enkephalinase, respectively,[12,13] were included in the assay in order to detect SP degrading activities distinct from these two enzymes. The inhibition constants, K_i, of peptides I and II were determined as described before.[20]

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Resolution of Flecainide Acetate, *N*-(2-Piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide Acetate, and Antiarrhythmic Properties of the Enantiomers

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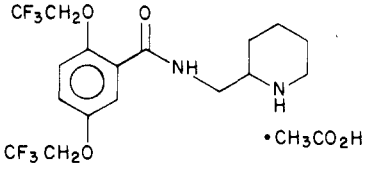
The antiarrhythmic agent flecainide acetate was resolved by fractional crystallization of its diastereomeric α-bromocamphor-π-sulfonate salts. Optical purity of the two enantiomers was shown to be >99% by an NMR technique using the chiral shift reagent Eu(hfbc)$_3$. Antiarrhythmic effects of flecainide and its enantiomers were assessed in two different animal models, chloroform-induced ventricular fibrillation in mice and ouabain-induced ventricular tachycardia in dogs. The two enantiomers were highly effective in suppressing both of these experimental arrhythmias and appeared to be essentially equipotent. No significant differences were found either between the two enantiomers or between the enantiomers and racemic flecainide.

Flecainide acetate (Tambocor, I), *N*-(2-piperidylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide acetate, is a clinically effective new agent used in the treatment of cardiac arrhythmias. The synthesis,[1] animal pharmacology,[2,3] metabolism,[4] and clinical properties[5] of racemic flecainide acetate have been previously described. In this paper we report a resolution of flecainide and an evaluation of the antiarrhythmic properties of both enantiomers compared to racemic flecainide acetate.



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Resolution. Racemic flecainide acetate was converted to its free base and resolved by fractional crystallization of the diastereomeric salts formed by addition of 1 equiv of ammonium (+)-α-bromocamphor-π-sulfonate to a solution of flecainide in methanol. Repeated crystallizations, first from isopropyl alcohol and then ethyl acetate, yielded a single diastereomeric salt, $[\alpha]^{26}_D$ +43.5°. Decomposition

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