N-Protected Peptide Esters (Table I). The N-protected amino acid (0.01 mol) was dissolved in dry CH_2Cl_2 (50 mL) and a solution of amino acid ester *p*-tosylate (0.01 mol) in dry CH_2Cl_2 (25 mL) containing N-methylmorpholine (0.01 mol) was added. The solution was cooled to 0 °C and dicyclohexylcarbodiimide (DCC) (0.01 mol) was added. The solution was allowed to warm to room temperature, stirred overnight, and filtered, and the filtrate was washed with 20% citric acid solution (2 × 50 mL) and 3% sodium bicarbonate (3 × 50 mL). The organic phase was dried over $MgSO_4$, the solvent evaporated, and the residual oil crystallized from the appropriate solvent (Table I).

Removal of N-Protecting Groups (Table II). (a) The *N*-Cbz peptide *tert*-butyl ester (0.01 mol) was dissolved in MeOH (10 mL) and hydrogenated at ambient temperature and pressure over 10% Pd/C (500 mg). When the theoretical amount of hydrogen had been adsorbed, the solution was filtered through Kieselguhr and the solvent evaporated to leave the free amine.

(b) The *N*-Boc peptide ester (0.01 mol) in EtOH (50 mL) was cooled to 0 °C and treated dropwise with *p*-toluenesulfonic acid (0.01 mol) in EtOH (10 mL). The solution was allowed to warm to room temperature; then the solvent was evaporated and the residual oil triturated with ether to give the TosOH salt of the amine.

Introduction of *N*-Halogenoacetyl Groups (Table III). Iodo- or bromoacetic acid (0.01 mol) in dry CH_2Cl_2 (50 mL) containing *N*-methylmorpholine (0.01 mol) was cooled to −5 °C and isobutyl chloroformate (0.01 mol) was added over 15 min. A solution of the amine (0.01 mol) (or of the amine TosOH salt plus *N*-methylmorpholine) in CH_2Cl_2 was added portionwise at −5 °C and the mixture was stirred thus for 30 min, then allowed to warm to room temperature, and washed with 20% citric acid followed by 3% sodium bicarbonate solution. The organic phase was dried ($MgSO_4$) and evaporated to leave the desired amide.

Halogenoacetyl Peptides (Table IV). The *N*-halogenoacetyl peptide *tert*-butyl ester (0.01 mol) was stirred with trifluoroacetic acid (2 mL) at room temperature for 1 h. Dry ether (50 mL) was added, the suspension was stirred for a further hour, and the solid product was collected by filtration.

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Synthesis of β-Spiro[pyrrolidinoindolines], Their Binding to the Glycine Receptor, and in Vivo Biological Activity

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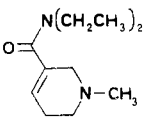
A series of β-spiro[pyrrolidinoindolines], **3a–d**, was prepared and evaluated for their ability to bind to the glycine receptor. These compounds were also tested in vivo to determine if they would produce convulsant or anxiolytic effects. The target indolines were chosen because they represent rings A, B, E, and a portion of ring C of strychnine. Results of this study indicate that, in this series, an acetylindoline in the endo configuration and a tertiary amine, such as that of the pyrrolidine ring nitrogen, are required for biological activity. In all of the cases studied, the activity was of a convulsant rather than a relaxant nature. Excellent correlation was found to exist between the binding affinities to the strychnine site of the glycine receptor and clonic convulsions (ED_{50}) and death (LD_{50}) in the mouse.

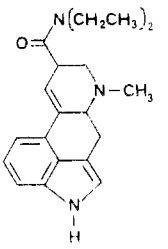
The excitatory actions of strychnine on the central nervous system (CNS) have been attributed to its ability to interact with postsynaptic receptors which are sensitive to glycine, thereby blocking the inhibitory effects of this amino acid neurotransmitter.[1] Snyder et al. have demonstrated that [^{3}H]strychnine binds to synaptosomal membrane fragments obtained from the rat brain stem and spinal cord[2] and that the regional location of this binding within the CNS correlates with endogenous glycine concentrations.[3]

The inhibition of [^{3}H]strychnine binding to synaptosomal preparations has thus far been reported for glycine and β-alanine,[2] a group of strychnine alkaloids,[4] and a series of 1,4-benzodiazepine derivatives.[5] The convulsant activities of the series of strychnos alkaloids studied were found to be highly correlated with their respective binding affinities. Likewise, Snyder was able to show that the rank order of potency of a series of 21 1,4-benzodiazepines in a variety of animal and human pharmacological and behavioral tests correlated with the ability of these compounds to displace bound [^{3}H]strychnine. This observation led to the hypothesis that the 1,4-benzodiazepines exert their anxiolytic, muscle-relaxant, and anticonvulsant effects by mimicing glycine at its receptor site.[5]

The purpose of this study was twofold. First, we were interested in determining what structural features within the strychnine molecule were responsible for the high binding affinity to the glycine receptor and, secondly, to establish if a portion of the strychnine skeleton would bind to this receptor and elicit anxiolytic, rather than convulsant, effects. Although the likelihood of finding a strychnine antagonist (glycine agonist) within the framework of the strychnine molecule might seem remote, such an approach has been successful elsewhere. The tetrahydropyridine, **1**, which constitutes a portion of the LSD molecule, **2**, was recently reported to bind with high affinity to the LSD receptor and, more importantly, was found to antagonize the behavioral effects of LSD.[6]



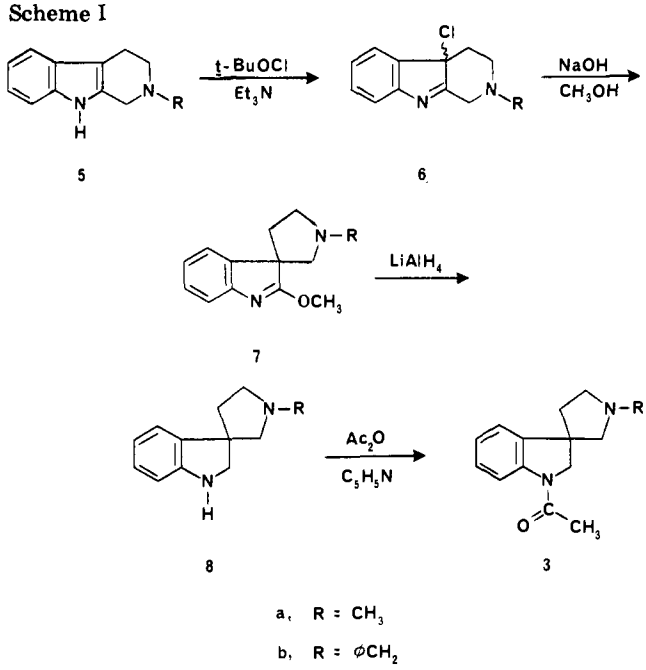
1



2

Furthermore, Rees and Smith[7] reported that certain seco derivatives of strychnine, strychnidine, and brucidine,

Scheme I

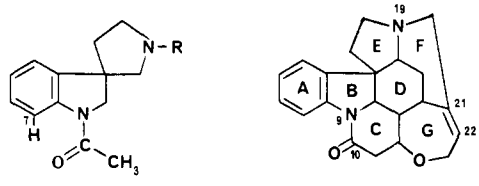


a, R = CH_3

b, R = ϕCH_2

formed by reductive fission and saturation of the 21,22 double bond, were antidepressants (potentiation of amphetamine) at 5 mg/kg and possessed tranquilizing activity at 25 mg/kg. The compounds did not exhibit strychnine-like convulsant activity at these doses.[7]

The target molecules for this study, **3a–d**, constitute rings A, B, E, and a portion of ring C of strychnine, **4**. The olefinic carbons of the allyl group in **3c** correspond to carbons 21 and 22 of strychnine.



a, R = CH_3

b, R = ϕCH_2

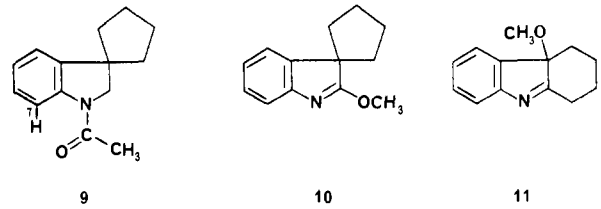
c, R = CH_2=$CHCH_2$

d, R = H

Chemistry. The synthesis of **3a** and **3b** proceeded from the corresponding 2-substituted 1,2,3,4-tetrahydro-β-carbolines, **5a** and **5b**, as shown in Scheme I. This approach was patterned after the sequence recently used by Owellen in the preparation of the spiro[cyclopentylindoline], **9**.[8]

Treatment of either **5a** or **5b** with freshly prepared *t*-BuOCl in the presence of Et_3N using a methylene chloride–carbon tetrachloride solvent system afforded the intermediate chloroindolenines, **6a** and **6b**, respectively. The progress of this reaction was monitored by TLC and found to be complete within 1–2 h at 5 °C. No attempt was made to isolate these intermediates or to determine the stereochemistry of the chlorine substituent. Instead, solutions of the intermediate chloroindolenines were poured into a refluxing solution of NaOH in methanol. Owellen found that these conditions gave the rearranged β-spiro[cyclopentylindolenine], **10**, rather than the isomeric 4a-methoxytetrahydrocarbazoleindolenine, **11**.[8]

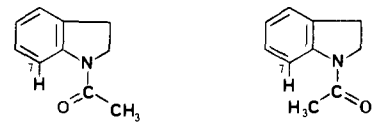
The results obtained here are in agreement with those of Owellen, in that only the β-spiro[pyrrolidinoindolenines], **7a** and **7b**, were obtained in overall yields of 90 and 95%, respectively. Examination of the methanolic reaction mixtures failed to show the presence of the unrearranged, isomeric indolenines corresponding to **11**.



Reduction of **7a** and **7b** with $LiAlH_4$ provided **8a** and **8b**, and acetylation of the resultant indolines furnished the desired acetylated products, **3a** and **3b**. **3a** was obtained as a solid, and **3b** was isolated as an oil, purified by bulb-to-bulb distillation and then converted to a stable, crystalline oxalate salt. Attempts to prepare the hydrochloride or maleate of **3b** resulted in unstable, hygroscopic salts.

The *N*-allyl derivative, **3c**, was prepared from **3b** by employing two additional steps. Removal of the *N*-benzyl group in **3b** by hydrogenolysis using palladium catalyst furnished the secondary amine, **3d**, which upon reaction with allyl bromide in DMF in the presence of K_2CO_3 afforded **3c**. Once again the free base, isolated as an oil, was converted to a stable, crystalline oxalate salt.

The selection of the *N*-acetyl moiety to simulate the C-10 carbonyl (lactam carbonyl) of strychnine was based on the realization that the amide group of 1-acetylindoline, **12**, exists in the "endo" rather than the "exo" configuration.



endo exo

This structural assignment for **12** has been based on dipole moment measurements,[9] ^1H NMR,[10] nuclear Overhauser effects in the ^1H NMR,[11] and, more recently, ^{13}C NMR.[12] We observed that the chemical shifts for the C-7 proton in compounds **3a–c**, **9**, and **12** were δ 8.14–8.22 appearing as broad doublets (J = 7 Hz). It should be noted, however, that this configurational preference is only true when the adjacent C-2 position of the indoline is unsubstituted. 1-Acetyl-2,3,3-trimethylindoline, **13**, exists as a mixture of endo and exo rotamers, as evidenced by its ^1H NMR spectrum.[10] Thus, by having the carbonyl group in the endo configuration, a similar spatial relationship exists between the amide atoms and pyrrolidine nitrogen in **3a–c** and the lactam (N-9, C-10) and tertiary amine (N-19) of strychnine. Although these similarities in spatial arrangements exist in the ground-state solution conformations, conformational changes may be induced during the binding of these substrates to the receptor.

Results and Discussion

The data obtained from the binding affinity determinations, the ED_{50} values for clonic and tonic convulsions, and the LD_{50} values are presented in Table I. Correlations exist between the rankings for the affinities of these compounds for the binding sites in the synaptic membrane preparation and the dosages required to produce clonic convulsions (r_s = 0.99) and death (r_s = 0.90) in the mouse. With one compound, **3b**, the clonic convulsions were not followed by tonic convulsions. This alters the rankings of

Table I. Correlation between Displacement of [^{3}H]Strychnine Binding in Vitro and Convulsant Activity and Death in the Mouse[a]

Compd	Binding		Clonic convulsions		Tonic convulsions		Death	
	IC_{50}, μM	Rank	ED_{50}, μmol/kg	Rank	ED_{50}, μmol/kg	Rank	LD_{50}, μmol/kg	Rank
3a	23.6	1	79.6 ± 3.5	1	83.9 ± 4.3	1	94.8 ± 6.1	1
3b	31.0	2	252 ± 45	2	>1010	6.5	505 ± 83	4
3c	67.9	3	300 ± 32	3	434 ± 29	3	396 ± 29	2
3d	295	5	542 ± 49	5	746 ± 92	4	687 ± 77	5
8a	282	4	365 ± 19	4	412 ± 28	2	412 ± 18	3
9	>1000	7	1646 ± 84	6	>7422	6.5	6884 ± 367	8
12	>1000	7	>2482	7	>2484	6.5	1404 ± 87	6
13	>1000	7	<1970		>3941	6.5	2832 ± 217	7

[a] The Spearman coefficients of rank correlation[16] are binding vs. clonic convulsions, $r_s = 0.992$, $p < 0.02$; binding vs. LD_{50}, $r_s = 0.905$, $p < 0.01$; binding vs. tonic convulsions, $r = 0.690$, not significant. Values are means ± SEM.

compounds with regard to their ability to cause tonic convulsions and, as a result, there does not appear to be a statistically significant correlation between binding affinity and tonic convulsions (ED_{50}).

In examining these data with regard to the initial purposes of this study, several observations can be made. The in vivo biological effects were, in all cases, of a convulsant rather than relaxant nature. All convulsant activity and fatalities occurred within the first 3-h period following administration of the test compounds. The dose–response curves for all active compounds were very steep, with all of the activity occurring between one-half and two times the ED_{50}. From this, one must conclude that the compounds are strychnine-like and are acting at the receptor site as glycine antagonists rather than glycine agonists. The high correlation between binding affinity and clonic convulsant dose and death is in keeping with observations of a similar correlation in the strychnine alkaloid study.[4]

The structure–activity relationships of this series using binding affinities, clonic convulsant activity, and death as the biological parameters under consideration yield the following conclusions. The nitrogen atom of the β-spiropyrrolidino ring is required for activity. All of the β-spiropyrrolidino compounds were active to some extent, while the spirocyclopentyl analogue, **9**, and the two simple indolines, **12** and **17**, were virtually inactive.

Within the group of β-spiropyrrolidino indolines, those that possessed an acetyl group on the indoline ring nitrogen were most active. In comparing **3a** with **8a**, there is a 12-fold difference in binding affinity and a fourfold difference in in vivo effects.

Finally, the nature of the substitutent on the pyrrolidine ring nitrogen has some effect, although of a minor nature. All three tertiary amines studied, **3a**, **3b**, and **3c**, had comparable binding affinities, while the secondary amine, **3d**, had about one-tenth the binding affinity of **3a**. The presence of the allyl substituent in **3c** did not result in an increase in the binding affinity or produce any differences in the in vivo biological effects.

From these observations, it appears that in this series the presence of an acetylindoline moiety which is suitably removed from a tertiary amine, such as an N-substituted pyrrolidine, is an absolute requirement for binding to the strychnine site of the glycine receptor and for strychnine-like in vivo responses. This conclusion is in agreement with earlier studies which have examined the structure–activity relationships of the strychnos alkaloids.[4,13,14]

Experimental Section

Melting points were taken on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. Ultraviolet spectra were recorded in methanolic solutions on a Beckman DK-2A spectrometer and are reported in nanometers (nm); infrared spectra were determined in $CHCl_3$ solutions on a Beckman IR-12 spectrometer; ^{1}H NMR spectra were obtained on a Varian Associates A-60 spectrometer, from $CDCl_3$ solutions unless otherwise stated, using tetramethylsilane as an internal standard. Chemical shifts are expressed in δ in parts per million (ppm). Microanalyses were performed by the Searle Laboratories Microanalytical Department and unless indicated are ±0.4% of the calculated values.

TLC for use in monitoring reactions employed 1 × 3 in. microscope slides coated with silica gel containing a fluorescent material (Camag, Inc., New Berlin, Wis.). A solution of 90% $CHCl_3$ and 10% CH_3OH was used to develop the plates and spots were visualized with short-wave UV or ethanolic phosphomolybdic acid and heat.

Organic solutions of products were dried over anhydrous sodium sulfate, and evaporations to dryness were carried out in vacuo using a Buchler rotary evaporator.

Biological Methods. The preparation of tissues and the methods of determination of the binding affinities for the compounds in this study are the same as those described previously.[4] Convulsant activity (ED_{50}) for both clonic and tonic convulsions and LC_{50} values were determined in male mice of the COBS–CDI strain (Charles River Breeding Labs, Inc., Wilmington, Mass.). Groups of ten mice were used to evaluate each dose, and following ip injection of the compound, the animals were observed for 3 h and again after 24 h. Compounds were administered as solutions or suspensions in physiological saline containing 1% (v/v) Tween 80 and 1% (v/v) propylene glycol. The vehicle alone did not produce any observable effects and concentrations were adjusted so that the mice received 10 mL/kg. ED_{50} and LD_{50} values were calculated graphically on log probit paper.[15]

2-Methoxy-1′-methylspiro[3*H*-indole-3,3′-pyrrolidine] (7a). Tetrahydro-β-carboline,[17] **5** (9.3 g, 0.050 mol), was dissolved in Spectrograde CH_2Cl_2 (150 mL) containing Et_3N (5.55 g, 0.055 mol). The resultant solution was stirred and treated at 5 °C, in the dark under a nitrogen atmosphere, with a solution of freshly prepared *tert*-butyl hypochlorite[18] (6.1 g, 0.056 mol) in Spectrograde CCl_4 (50 mL). The addition was made in dropwise portions over a 1-h period, followed by an additional hour of stirring at 5 °C. TLC indicated that all of **5** had been consumed and a new spot (R_f 0.66) was present. Without attempting to isolate **6a**, this reaction mixture was added in a steady stream to a refluxing solution of NaOH (20 g) in methanol (750 mL). The reaction mixture was heated to reflux overnight, and the resultant orange mixture was evaporated to dryness. The residue was then suspended in water (200 mL) and extracted with ether (3 × 150 mL). The combined extract was dried and evaporated to dryness. **7a** was obtained as an orange oil: 9.70 g (90%); bp 60–65 °C (0.5 Torr); UV 255 nm (ε 5500); IR 1620 cm^{-1} (C=N); NMR 1.85–3.10 (m, 6 aliphatic H), 2.43 (s, CH_3N), 4.08 (s, CH_3O), 6.90–7.50 (m, 4 aromatic H). Anal. ($C_{13}H_{16}N_2O$) C, H, N.

2-Methoxyphenylmethylspiro[3*H*-indole-3,3′-pyrrolidine] (7b). Using the same procedures described for the preparation of **7a**, **5b** was converted into **7b** in 95% yield. **7b** was purified by bulb-to-bulb distillation [135 °C oven temperature (0.15 Torr)]: UV 252 nm (ε 6500), 257 (6500); IR 1620 cm^{-1} (C=N); NMR 2.05–3.30 (m, 6 aliphatic H), 3.75 (s, CH_2N), 4.10 (s, CH_3O),

6.90–7.55 (m, 9 aromatic H). Anal. ($C_{19}H_{20}N_2O$) H, N; C: calcd, 78.05; found, 77.40.

1,2-Dihydro-1′-methylspiro[3*H*-indole-3,3′-pyrrolidine] Oxalate (8a) and 1-Acetyl-1,2-dihydro-1′-methylspiro[3*H*-indole-3,3′-pyrrolidine] (3a). A solution of 7a (7.25 g, 0.034 mol) in anhydrous ether (25 mL) was added to a magnetically stirred suspension of $LiAlH_4$ powder (4.0 g) in anhydrous ether (100 mL). The mixture was stirred and heated to reflux for 2 h and then cooled to 5 °C and the excess $LiAlH_4$ was cautiously decomposed by the dropwise addition of water (5 mL), 15% NaOH solution (5 mL), and water (15 mL). The resultant suspension was filtered through a cake of Celite and $MgSO_4$ and the filtrate was diluted with anhydrous ether to a 250-mL vol in a volumetric flask.

This solution (50 mL) was treated with a solution of oxalic acid (1 g) in ether (250 mL). **8a** was obtained as a yellow solid: 1.26 g (67%); mp 191–193 °C dec; NMR (Me_2SO-d_6–D_2O) 2.20 (t, J = 7 Hz, CH_2), 2.90 (s, CH_3N), 3.25–3.70 (m, 6 aliphatic H), 6.50–7.40 (m, 4 aromatic H). Anal. ($C_{12}H_{16}N_2 \cdot 1.5C_2H_2O_4$) C, H, N. Recrystallization from methanol did not change the microanalytical results.

The ether solution (200 mL), containing the free base of **8a**, was treated with acetic anhydride (15 mL) and pyridine (5 mL), and the mixture was set aside at room temperature in a stoppered flask for 2 days. The mixture was then washed with a 20% Na_2CO_3 solution (3 × 150 mL) and water (200 mL), dried, and evaporated to dryness. The resultant orange oil crystallized on standing. Recrystallization from hexane and decolorization with Darco furnished **3a** as a colorless solid: 2.85 g (45%); mp 85–87 °C; UV 253 nm (ε 15 000), 280 (3700), 289 (3000); IR 1665 cm^{-1} (amide C=O); NMR 1.85–3.10 (m, 6 aliphatic H), 2.22 (s, CH_3CO), 2.38 (s, CH_3N), 3.87 and 4.12 (AB q, J = 10.5 Hz, CH_2), 6.85–7.45 (m, 3 aromatic H), 8.20 (br d, J = 7 Hz, 1 aromatic H). Anal. ($C_{14}H_{18}N_2O$) C, H, N.

1-Acetyl-1,2-dihydro-1′-phenylmethylspiro[3*H*-indole-3,3′-pyrrolidine] Oxalate (3b). Following the procedures described for the synthesis of **8a**, **7b** (4.47 g, 0.015 mol) was reduced with $LiAlH_4$ (6.9 g) in anhydrous ether (320 mL). The ether solution containing **8b** was, without isolation of **8b**, acetylated using conditions reported for the preparation of **3a**. One additional procedure was added to the work-up and this involved the washing of the ether solution containing the acetylated product with a 0.1% cupric nitrate solution (100 mL). This washing removed the last traces of pyridine. The free base of **3b** was obtained as a yellow oil: 2.77 g (60%), bulb-to-bulb distillation [190 °C oven temperature (0.15 Torr)]; IR 1660 cm^{-1} (amide C=O); NMR 1.95–3.00 (m, 6 aliphatic H), 2.22 (s, CH_3CO), 3.68 (s, CH_2N), 3.86 and 4.08 (AB q, J = 11 Hz, CH_2), 6.95–7.50 (m, 8 aromatic H), 8.22 (br d, J = 7 Hz, 1 aromatic H). Anal. ($C_{20}H_{22}N_2O$) C, H, N. This free base was converted to its oxalate salt, **3b**, mp 221–222 °C (recrystallized from MeOH). Anal. ($C_{20}H_{22}N_2O \cdot C_2H_2O_4$) C, H, N.

1-Acetyl-1,2-dihydrospiro[3*H*-indole-3,3′-pyrrolidine] Oxalate (3d). A solution of the free base of **3b** (6.0 g, 0.0196 mol) in ethanol (150 mL) was hydrogenated on a Parr shaker apparatus for 4 h at 60 °C and 60 psi. Palladium on carbon (10%, 1.2 g) was used as the catalyst. The resultant mixture was filtered and evaporated to dryness leaving a yellow oil (4.0 g). NMR of this oil indicated a disappearance of the benzylic methylene protons as well as a diminution of the integration of the aromatic signals. A portion of this oil (1.0 g) was dissolved in ether and converted to an oxalate salt. This salt was recrystallized from methanol–ether and dried in vacuo at 60 °C. **3d** was obtained as a colorless solid: 0.70 g (50%); mp 188.5–189.5 °C. Anal. ($C_{13}H_{16}N_2O \cdot 0.75C_2H_2O_4$) C, H, N.

1-Acetyl-1,2-dihydro-1′-(2-propenyl)spiro[3*H*-indole-3,3′-pyrrolidine] Oxalate (3c). A solution of the free base of **3d** (1.8 g, 0.008 mol) in dry DMF (35 mL) containing pulverized K_2CO_3 (1.97 g, 0.014 mol) was stirred at room temperature and treated, in dropwise portions over a 10-min period, with a solution of allyl bromide (1.01 g, 0.008 mol) in dry DMF (15 mL). The mixture was stirred overnight at 25 °C and then poured into water (200 mL), and the resultant mixture was extracted with ethyl acetate (3 × 150 mL). The combined extract was washed with water (3 × 175 mL), dried, and evaporated to dryness leaving a yellow oil: 1.17 g (57%); IR 1660 cm^{-1} (amide C=O); NMR 1.90–3.25 (m, 8 aliphatic H), 2.22 (s, CH_3CO), 3.87 and 4.12 (AB q, J = 10.5 Hz, CH_2), 4.95–5.45 (m, 3 olefinic H), 6.90–7.45 (m, 3 aromatic H), 8.22 (br d, J = 7 Hz, 1 aromatic H). This oil was converted to an oxalate salt, **3c**, which was isolated as a yellow solid, mp 189–190 °C. Anal. ($C_{16}H_{20}N_2O \cdot C_2H_2O_4$) C, H, N.

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