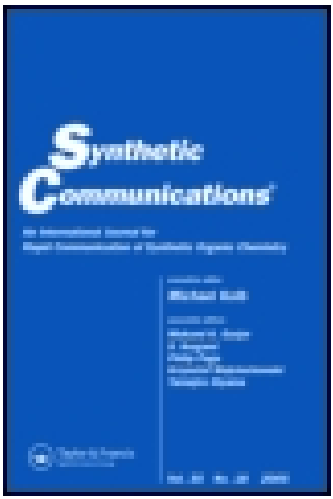

Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

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Published online: 15 Aug 2006.

To cite this article: Frank Navas III & Mark H. Norman (1996) The Design and Synthesis of a Hapten for 1192U90, a Potential Atypical Antipsychotic Agent, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 26:7, 1411-1421, DOI: 10.1080/00397919608003503

To link to this article: http://dx.doi.org/10.1080/00397919608003503

SYNTHETIC COMMUNICATIONS, 26(7), 1411-1421 (1996)

THE DESIGN AND SYNTHESIS OF A HAPTEN FOR 1192U90, A POTENTIAL ATYPICAL ANTIPSYCHOTIC AGENT

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Abstract: The design and synthesis of a hapten for a radioimmunoassay procedure for the potential atypical antipsychotic agent 1192U90 (**1**) is described. The hapten, a butyric acid derivative of *ortho*-amino benzamide 1192U90, was prepared in five steps starting from 5-hydroxy-2-nitrobenzaldehyde.

1192U90 (2-amino-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl) benzamide) (**1**) is currently in clinical trials as a potential atypical antipsychotic agent.[1] Due to the high potency of 1192U90, an analytical method capable of detecting the compound in biological fluids and tissues at concentrations in the ng/mL range or lower was needed for clinical pharmacokinetic studies. Since radioimmunoassay (RIA)[2] is a specific and sensitive analytical technique that permits the rapid processing of a large number of samples, we investigated the development of an RIA procedure for **1**. This analytical method requires the formation of an immunogen capable of generating antisera specific for the drug. Since small molecules are not usually immunogenic, the immunogen is prepared by coupling a hapten (the drug or a derivative of the drug) to a carrier protein

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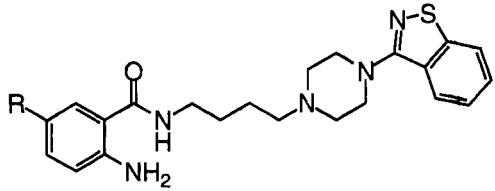
(e.g., bovine serum albumin). The resulting drug-protein conjugate is used in immunization procedures designed to generate antibodies that are specific to the small hapten molecule. To develop a suitable RIA for *ortho*-amino benzamide **1**, it was necessary to modify its structure to provide a hapten that could be coupled to a carrier protein. The design and synthesis of a hapten for 1192U90 (**1**) is discussed herein.

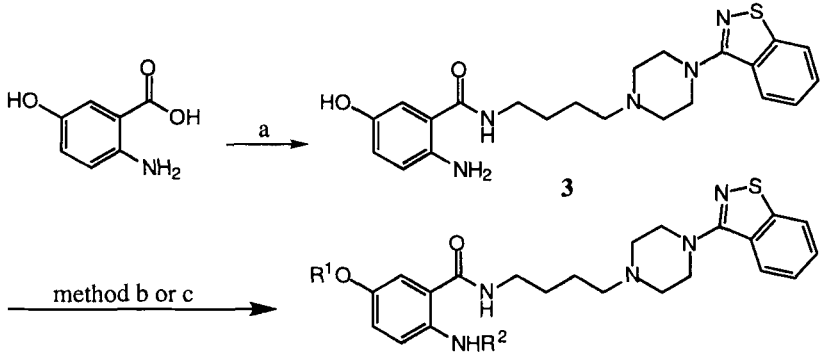


1: R = H
2: R = $O(CH_2)_3CO_2H$
3: R = OH

In considering the design of a hapten, it is important to address the need for a functional group that could be coupled to a carrier protein, and to recognize that antibody specificity is directed toward that part of the hapten molecule that is furthest from the site of attachment. A bridge is often inserted between the small molecule and the carrier protein so that important functional groups of the hapten may act as immunological discriminants and produce antisera specific for the drug. We felt that a molecule such as **2**, in which an alkyl bridge containing a terminal carboxyl group has been introduced to the benzamide ring of 1192U90, would fulfill the requirements of a hapten for RIA development. The alkyl chain of **2** would increase the distance between the carrier protein and important functional groups of 1192U90, and the carboxyl group could be attached to free amino groups of the carrier protein by either carbodiimide or mixed anhydride methodology.[2-4]

Initially, we envisioned that alkylation of 2-amino-N-4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl-5-hydroxybenzamide (**3**) with ethyl 4-bromobutyrate followed by ester hydrolysis would provide the desired hapten (**2**). This reaction sequence was investigated, since literature precedent existed for the alkylation of a phenolic oxygen in the presence of an unprotected aromatic amine. For example, Monkovic and coworkers alkylated 2-hydroxy-4-aminobenzamides in the presence of potassium carbonate to give aryl ethers,[5] while Musser *et al.* prepared napthol ethers from aminonapthols using sodium methoxide in N,N-dimethylformamide.[6] Benzamide **3** was readily prepared via the carbodiimide coupling of 5-hydroxyanthranilic acid with 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole[7] (Scheme I).

Scheme I



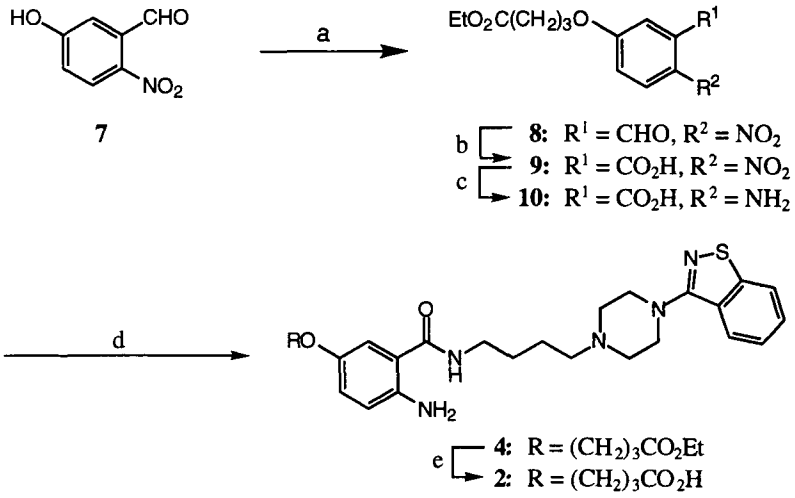
4: $R^1 = (CH_2)_3CO_2Et$; $R^2 = H$
5: $R^1 = H$, $R^2 = (CH_2)_3CO_2Et$
6: $R^1 = R^2 = (CH_2)_3CO_2Et$

(a) 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole,[7] dicyclohexylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF, 0 °C to room temperature. (b) *i.* NaOMe (1.0 eq), MeOH, room temperature, 1 h; *ii.* ethyl 4-bromobutyrate (1.0 eq), DMF, 0 °C to room temperature, 20 h; *iii.* NaI (0.25 eq), reflux, 20 h. (c) *i.* ethyl 4-bromobutyrate (1.0 eq), K_2CO_3 (2.0 eq), DMF, room temperature, 20 h; *ii.* NaI (0.24 eq), reflux, 20 h.

Unfortunately, alkylation of **3** using either sodium methoxide or potassium carbonate as a base (Scheme I, methods b and c, respectively) resulted in incomplete reaction and a mixture of products. Products identified from the reaction included the desired O-alkylated product **4** as well as the N-alkylated and N,O-dialkylated products, **5** and **6**, respectively. The N- and O-alkylated products (**4-6**) were readily distinguished from one another by ^{1}H and ^{13}C NMR analysis since the methylene group attached to a phenolic oxygen was shifted downfield relative to the similarly substituted aromatic amine. For example, the -O-CH_2- group of phenolic ether **4** exhibited chemical shifts at 3.93 and 67.5 ppm in the ^{1}H and ^{13}C NMR spectra, respectively, while the corresponding chemical shifts of the methylene group in the N-alkylated product (**5**) were further upfield at 3.12 and 42.8 ppm. High performance liquid chromatography (HPLC) analysis of the crude reaction mixtures indicated that the desired product (**4**) was formed in less than 15% yield in each case. Our inability to selectively alkylate the phenolic oxygen of **3** in satisfactory yield led us to investigate an alternative approach.

We previously demonstrated that substituted anthranilic acids may be converted directly to their corresponding benzamides via carbodiimide couplings.[8] To prepare the desired hapten by this methodology, a substituted anthranilic acid with the butyrate side chain already in place was required (i.e., compound **10**). Venuti and coworkers described the synthesis of a similarly substituted 5-alkoxyanthranilic acid in their preparation of cilostamide and anagrelide analogues.[9] Appropriate modifications to the Venuti procedures led to the desired 5-alkoxyanthranilic acid **10** as outlined in Scheme II. Alkylation of 5-hydroxy-2-nitrobenzaldehyde (**7**) with ethyl 4-bromobutyrate in the presence of potassium carbonate gave ester-aldehyde **8** in 86% yield. The substituted benzoic acid **9** was obtained by oxidation of **8** with aqueous potassium permanganate.

Scheme II



(a) $Br(CH_2)_3CO_2Et$, K_2CO_3, DMF. (b) $KMnO_4$, H_2O. (c) 10% Pd-C, H_2, EtOH. (d) 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole,[7] 1,3-dicyclohexylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF. (e) KOH, EtOH, room temperature.

The nitro group of **9** was reduced by catalytic hydrogenation over 10% Pd/C to give the requisite 5-alkoxyanthranilic acid **10**. Since it was unnecessary to protect the weakly nucleophilic aromatic amino group, compound **10** was converted directly to the corresponding benzamide-ester **4** by a carbodiimide coupling with 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole.[7] The ethyl ester was hydrolyzed by treatment with ethanolic potassium hydroxide to give the desired hapten, 4-(4-amino-3-(N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl) carbamoyl)phenoxy)butyric acid (**2**).

In summary, a hapten for the potential antipsychotic agent 1192U90 has been synthesized. An alkyl bridge containing a terminal carboxyl group has been attached to the benzamide ring of 1192U90 *via* a five-step reaction sequence starting from 5-hydroxy-2-nitrobenzaldehyde. The hapten will be conjugated to a

carrier protein and the resulting immunogen will be used to generate antisera for a radioimmunoassay procedure. It is anticipated that insertion of a bridge between the carrier protein and 1192U90 at a site distal to important functional groups will result in the production of antibodies with both high affinity and specificity to 1192U90.

Experimental

General. Thin-layer chromatography (TLC) was performed on Analtech silica gel GF TLC plates (250 μ). Flash[10] and flush chromatography were performed with EM Science silica gel 60 (230-400-mesh ASTM). Anhydrous N,N-dimethylformamide (DMF) was obtained from Aldrich Chemical Co. in a Sure/Seal bottle. ^{1}H NMR spectra were determined with superconducting FT NMR spectrometers operating at 200, 300, and 400 MHz. ^{13}C NMR were measured at 75.43 or 100.58 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, and coupling constants in Hz. Mass spectra were obtained on a Fisons platform mass spectrometer operating in the positive ion atmospheric pressure chemical ionization (APCI) mode. Elemental analyses were performed by either Atlantic Microlab, Inc., Norcross, GA, or Galbraith Laboratories, Inc., Knoxville, TN. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Analytical high performance liquid chromatography (HPLC) was conducted on a Waters Millennium 2010 HPLC system with a photodiode array detector. A Delta-pak C-18 column was used with a 9:1 to 1:9 gradient of H_2O:CH_3CN (with 0.1% CF_3CO_2H as solvent modifier) as eluant.

4-(4-Amino-3-(N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl) carbamoyl)phenoxy)butyric acid hydrate (2). Ethyl 4-(4-amino-3-(N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)carbamoyl) phenoxy)butyrate (**4**) (1.68 g, 3.11 mmol) was dissolved in EtOH (40 mL) and a solution of 5 N potassium hydroxide (10 mL) was added dropwise. The reaction mixture was allowed to stir at room temperature for 1 h. The pH of the yellow-orange solution was adjusted to pH 6-7 (pH paper) by the addition of 1 N HCl. The EtOH was removed with a rotary evaporator and H_2O (50 mL) was added to the concentrated aqueous solution. The solution was extracted twice with CH_2Cl_2. The organic layers were combined and washed with H_2O (2x100 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give the desired compound as a pale beige amorphous solid. The solid was partially dissolved in Et_2O and the suspension was concentrated *in vacuo*. The solid was treated a second time with Et_2O and dried to give 0.618 g (39%) of the title compound as an off-white amorphous solid. ^1H NMR (DMSO-d_6; 300 MHz): δ 1.52 (m, 4), 1.87 (quin, 2, J = 6.8), 2.35 (t, 2, J = 7.4), 2.37 (m, 2), 2.58 (m, 4), 3.22 (m, 2), 3.42 (m, 4), 3.87 (t, 2, J = 6.3), 5.91 (br s, 1), 6.61 (d, 1, J = 8.9), 6.80 (dd, 1, J = 2.7, 8.9), 7.03 (d, 1, J = 2.7), 7.41 (ddd, 1, J = 1.2, 7.0, 8.1), 7.53 (ddd, 1, J = 1.2, 7.0, 8.1), 8.03 (dm, 2, J = 8.4), 8.21 (br t, 1, J = 5.5). ^{13}C NMR (DMSO-d_6; 75.43 MHz): δ 24.92, 25.56, 28.25, 31.33, 39.85, 50.77, 53.66, 58.72, 68.39, 114.26, 116.46, 118.67, 120.88, 122.21, 125.28, 125.53, 128.47, 128.98, 144.93, 149.59, 153.09, 164.67, 169.58, 175.35. APCI MS: 512(MH^+). Anal. Calcd for $C_{26}H_{33}N_5O_4S$•0.25 H_2O: C, 60.50; H, 6.54; N, 13.57; H_2O, 0.87. Found: C, 60.44; H, 6.59; N, 13.57; H_2O, 0.70.

Ethyl 4-(4-amino-3-(N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl) butyl)carbamoyl) phenoxy)butyrate hydrochloride (4). 2-Amino-5-(4-ethoxy-4-oxobutoxy)benzoic acid (**10**) (1.58 g, 5.91 mmol), 1-hydroxybenzotriazole

hydrate (0.846 g, 6.26 mmol, 1.06 eq), 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole[7] (1.71 g, 5.89 mmol, 1.0 eq), and anhydrous DMF (40 mL) were combined in a 250-mL, round-bottomed flask and cooled in an ice-water bath. A solution of 1,3-dicyclohexylcarbodiimide (1.41 g, 6.83 mmol, 1.16 eq) in anhydrous DMF (20 mL) was added dropwise to the stirred cooled reaction mixture. The ice-water bath was removed and the reaction mixture was allowed to stir at room temperature under N_2 for 24 h. The reaction mixture was filtered and concentrated *in vacuo*. The reaction mixture was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to give a dark orange-brown residue. The crude product was purified by flash chromatography with CH_2Cl_2:MeOH (93:7) as eluant to give 1.76 g of the free base as an orange oil. Impure fractions were combined and purified by flash chromatography with CH_2Cl_2:MeOH (99:1) followed by CH_2Cl_2:MeOH (97:3) as eluant to give another 0.707 g of the desired product for a total yield of 2.47 g (78%). A portion of the free base (0.41 g, 0.76 mmol) was dissolved in ethyl acetate and 0.76 mL of 1 N ethereal HCl (1.0 eq) was added. The salt was recrystallized from ethyl acetate/methanol to give 0.185 g (42%) of the title compound as a pale beige solid. Mp: 208-210 °C. ^{1}H NMR (DMSO-d_6; 300 MHz): δ 1.15 (t, 3, J = 7.2), 1.57 (m, 2), 1.80 (m, 2), 1.92 (quin, 2, J = 6.8), 2.43 (t, 2, J = 7.3), 3.17 (m, 2), 3.26 (m, 4), 3.54 (br s, 4), 3.94 (t, 2, J = 6.3), 4.03 (m, 2), 4.04 (q, 2, J = 7.2), 6.81 (d, 1, J = 8.8), 6.89 (dd, 1, J = 8.8, 2.9), 7.17 (d, 1, J = 2.9), 7.45 (ddd, 1, J = 1.1, 7.1, 8.1), 7.57 (ddd, 1, J = 1.1, 7.1, 8.1), 8.10 (t, 2, J = 8.3), 8.54 (br t, 1, J = 5.3). ^{13}C NMR (DMSO-d_6; 75.43 MHz): δ 14.46, 20.97, 24.69, 26.61, 30.49, 38.48, 46.72, 50.80, 55.48, 60.22, 67.54, 113.81, 119.75, 119.81, 119.82, 121.55, 124.35, 124.97, 127.30, 128.47, 150.74, 150.76, 152.45, 162.58, 168.31, 172.91. APCI

MS: 540(MH^+). Anal. Calcd for $C_{28}H_{37}N_5O_4S$•1.25 HCl: C, 57.46; H, 6.59; N, 11.97; Cl, 7.57. Found: C, 57.50; H, 6.60; N, 11.95; Cl, 7.72.

Ethyl-4-(3-formyl-4-nitrophenoxy)butyrate (8). This compound was prepared according to the method described by Venuti *et al.*[9] with modifications. Ethyl 4-bromobutyrate (4.61 g, 23.6 mmol), 5-hydroxy-2-nitrobenzaldehyde (**7**) (3.31 g, 19.8 mmol), potassium carbonate (3.11 g, 22.5 mol), and DMF (25 mL) were added to a 250-mL, round-bottomed flask and the reaction mixture was heated at 90-105 °C for 1.5 h under N_2. The reaction mixture was allowed to cool and was concentrated with a rotary evaporator. The residue was partitioned between saturated aqueous K_2CO_3 and EtOAc. The organic layer was separated and washed with saturated aqueous K_2CO_3 followed by brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 6.35 g of dark red-brown liquid. The crude product was purified by flush chromatography with hexanes:EtOAc (9:1) followed by hexanes:EtOAc (3:1) as eluant to give 4.78 g (86%) of the title compound as an orange liquid. ^1H NMR (DMSO-d_6; 300 MHz): δ 1.16 (t, 3, J = 7.1), 1.99 (quin, 2, J = 6.8), 2.46 (t, 2, J = 7.4), 4.05 (q, 2, J = 7.1), 4.17 (t, 2, J = 6.5), 7.21 (d, 1, J = 2.8), 7.32 (dd, 1, J = 2.8, 9.2), 8.15 (d, 1, J = 9.2), 10.26 (s, 1). ^{13}C NMR (DMSO-d_6; 75.43 MHz): δ 14.40, 24.21, 30.26, 60.25, 68.40, 114.35, 118.77, 127.59, 134.59, 142.09, 163.07, 172.70, 190.15. Anal. Calcd for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N,4.98. Found: C, 55.43; H, 5.42; N, 4.95.

5-(4-Ethoxy-4-oxobutoxy)-2-nitrobenzoic acid (9). Distilled water (28 mL) and ethyl-4-(3-formyl-4-nitrophenoxy)butyrate (**8**) (2.0 g, 7.14 mmol) were combined in a 250-mL round-bottomed flask and the mixture was heated at 70 °C. A solution of potassium permanganate (1.61 g, 10.2 mmol, 1.43 eq) in H_2O (60 mL) was added dropwise and the reaction mixture was heated at

70-90 °C for 1 h. The reaction mixture was allowed to cool to room temperature and saturated aqueous $NaHCO_3$ (10 mL) was added. The mixture was filtered and the filtrate was acidified to pH 2 (pH paper) with 10% aqueous HCl. The acidic aqueous solution was extracted three times with CH_2Cl_2. The organic layers were combined and washed with saturated aqueous $NaHCO_3$. The basic aqueous solution was acidified to pH 1 (pH paper) with concentrated HCl and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$, filtered, and concentrated to give 1.0 g (47%) of the title compound as a pale yellow oil, which solidified to form an off-white solid. Mp: 77.5-79.5 °C. ^1H NMR ($CDCl_3$; 300 MHz): δ 1.27 (t, 3, J = 7.2), 2.17 (quin, 2, J = 6.6), 2.54 (t, 2, J = 7.1), 4.15 (t, 2, J = 6.2), 4.17 (q, 2, J = 7.2), 7.05 (dd, 1, J = 2.9, 9.0), 7.17 (d, 1, J = 2.9), 8.01 (d, 1, J = 9.0), 9.13 (br s, 1); ^{13}C NMR ($CDCl_3$; 75.43 MHz): δ 14.09, 24.10, 30.33, 60.71, 67.89, 114.79, 116.52, 126.57, 129.77, 140.14, 162.37, 170.09, 173.03. Anal. Calcd for $C_{13}H_{15}NO_7$: C, 52.53; H, 5.09; N, 4.71. Found: C, 52.54; H, 5.12; N, 4.70.

2-Amino-5-(4-ethoxy-4-oxobutoxy)benzoic acid (10). 5-(4-Ethoxy-4-oxobutoxy)-2-nitrobenzoic acid (**9**) (13.46 g, 45.3 mmol) was dissolved in EtOH (150 mL) and hydrogenated over 10% Pd-C (1.26 g) at 53 psi overnight. The reaction mixture was filtered through a pad of celite and the filtrate was concentrated to give 8.68 g (72%) of the title compound as an olive-green solid. Mp: 122.5-123 °C. ^1H NMR (DMSO-d_6 ; 200 MHz): δ 1.19 (t, 3, J = 7.1), 1.93 (quin, 2, J = 6.8), 2.48 (t, 2, J = 7.3), 3.87 (t, 2, J = 6.3), 4.08 (q, 2, J = 7.1), 6.71 (d, 1, J = 9.0) 6.95 (dd, 1, J = 3.0, 9.0), 7.19 (d, 1, J = 3.0), 8.45 (br s, 2). ^{13}C NMR (DMSO-d_6; 75.43 MHz): δ 14.42, 24.71, 30.51, 60.16, 67.39, 109.74, 114.44, 118.14, 123.68, 146.63, 148.36, 169.60, 172.90. Anal. Calcd for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24. Found: C, 58.30; H, 6.48; N, 5.18.

Acknowledgments

We thank Dr. John Findlay and Mr. Jeff Sailstad for their helpful discussions regarding radioimmunoassays, Ms. Ann Davis for her assistance with NMR analysis, and Ms. Laura Cotterman for proofreading this manuscript.

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(Received in the USA 24 September 1995)