CHROM. 21 815

PREPARATIVE-SCALE SYNTHESIS AND REVERSED-PHASE PURIFICATION OF A GONADOTROPIN-RELEASING HORMONE ANTAGONIST

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SUMMARY

The preparation of "Nal–Glu" antagonist (Ac–D-Nal–D-Cpa–D-Pal–Ser–Arg–D-2-amino-5-oxo-5-(4-methoxyphenyl)pentanoic acid–Leu–Arg–Pro–D-Ala–NH$_2$) was accomplished in two steps: (i) preparation of [Ac–D-Nal^1, D-Cpa^2, D-Pal^3, Arg^5, D-Glu^6, D-Ala^{10}]-GnRH via standard solid-phase synthetic techniques and (ii) acylation of anisole (Friedel–Crafts) by the glutamic acid residue in position 6. The preparative-scale, reversed-phase high-performance liquid chromatographic (HPLC) purification of the crude "Nal–Glu" antagonist employs first a triethylammonium phosphate (TEAP) (pH 2.25)–acetonitrile solvent system, followed by an HPLC-based desalting procedure, yielding the acetate salt of the peptide. This repetitive process of purification in TEAP–acetonitrile, followed by counter-ion exchange with 0.5% acetic acid–acetonitrile is highly reproducible and allows large amounts of a given peptide to be purified efficiently in a batchwise fashion. The procedure described for the synthesis, purification and characterization of the "Nal–Glu" antagonist is presented as a model for the multi-gram synthesis and purification of peptides to be used in clinical investigations.

INTRODUCTION

The role assumed by high-performance liquid chromatography (HPLC) in the isolation and purification of biologically significant peptides and proteins is well documented[1]. As new peptides were isolated and characterized and the need for their duplication by total synthesis became imperative, the usefulness and power of preparative reversed-phase (RP) HPLC was recognized[2]. The technological advances that have arisen in the synthesis and chromatographic handling of peptides have led to a situation where it is now recognized that selected (10- to 40-residue) peptides synthesized in the solid phase pioneered by Merrifield[3] can be purified to the point where the most sophisticated analytical techniques cannot detect significant amounts of impurities. Work in this laboratory has demonstrated that such peptides [in our case, the releasing factors gonadotropin-releasing hormone (GnRH)[4], somatostatin (SS)[5], corticotropin-releasing factor (CRF)[6,7] and growth hormone-releasing factor (GRF)[8]] can be successfully and safely used in a clinical setting. Since large (multi-

gram) amounts of these peptides are needed for toxicological studies and clinical investigations *per se,* we have developed large-scale methodologies for both the synthesis and purification of these peptides.

One peptide of current clinical interest prepared in this laboratory is "Nal–Glu" antagonist: Ac–D-Nal–D-Cpa–D-Pal–Ser–Arg–D-2-amino-5-oxo-5-(4-methoxyphenyl)pentanoic acid–Leu–Arg–Pro–D-Ala–NH$_2$.[9] This peptide, a GnRH antagonist, is prepared via a hydrofluoric acid-mediated Friedel–Crafts acylation of the glutamic acid residue of [Ac-D-Nal[1], D-Cpa[2], D-Pal[3], Arg[5], D-Glu[6], D-Ala[10]]-GnRH by anisole. The purification of this compound employs a triethylammonium phosphate–acetonitrile solvent system, followed by an HPLC-based desalting procedure to obtain the acetate salt. We describe here the synthesis, purification and characterization of this unique GnRH antagonist, the procedure for which we offer as a model for the large-scale preparation of clinical peptides.

EXPERIMENTAL

Apparatus

The analytical chromatographic system consisted of a Perkin-Elmer Series 400 liquid chromatograph with quaternary gradient capabilities, a Houston Instruments Omniscribe strip-chart recorder, a Hewlett-Packard 3390A reporting integrator, a Rheodyne 7125 injector and a Kratos 757Z variable-wavelength UV detector.

The preparative chromatographic system consisted of a Waters Assoc. DeltaPrep Model 3000 instrument, a Houston Instruments Omniscribe strip-chart recorder and a Kratos Model 757 variable-wavelength UV detector.

Analytical columns

The analytical columns (25 × 0.46 cm I.D.) were packed with Vydac (5-μm, particle size and 300 Å pore size) C$_{18}$ silica[10], obtained from the Separations Group (Hesperia, CA, U.S.A.).

Preparative cartridges

Empty polyethylene cartridges and frits (part numbers 50411 and 50421) obtained from Waters Assoc. were dry-packed in our laboratory with Vydac C$_{18}$ derivatized silica (15–20-μm particle size and 300-Å pore size). For a discussion regarding packing materials for preparative chromatography, see ref. 10.

Solvent systems

Distilled, deionized, sterile water was used in all purification steps. The triethylammonium phosphate (pH 2.25) (TEAP) and the trifluoroacetic acid (TFA) solvent systems have been described earlier[11,12]. The TEAP system was prepared from 0.9% (v/v) phosphoric acid and 0.9% (v/v) triethylamine and the pH was adjusted to 2.25 by addition of either phosphoric acid or triethylamine. Ammonium acetate (puriss. p.a.) was obtained from Fluka (Buchs, Switzerland). Solvent A was always the aqueous buffer and solvent B was acetonitrile–buffer A (60:40) unless indicated otherwise. Analytical and preparative flow-rates were 2 and 85–100 ml/min, respectively. The wavelength at which the eluent was monitored and the absorbance scale used are shown on the left-hand ordinate of the figures, the gradient shape is indicated as an

overlay, with percentage of acetonitrile values indicated on the right-hand ordinate, and the abscissa indicates the time of elution.

Peptides

[Ac–D-Nal^1, D-Cpa^2, D-Pal^3, Arg^5, D-Glu^6, D-Ala^{10}]-GnRH was synthesized manually by the solid-phase approach using classical solid-phase peptide synthesis techniques[13,14]. In brief, this peptide was assembled on 250 g of a methylbenzhydryl-amine resin, as the ultimate desired final product had a C-terminal carboxamide (the substitution level was 0.92 mequiv. NH$_2$/g). Hydrogen fluoride cleavage and depro-tection at 0°C for 1.5 h in the presence of anisole as a carbocation scavenger yielded, after extraction and lyophilization, the crude peptidic preparation, which was con-verted to "Nal–Glu" antagonist by subjecting this material in a batchwise fashion to an anhydrous hydrofluoric acid mediated Friedel–Crafts acylation reaction with ani-sole (see discussion below). After removal of the hydrofluoric acid and unreacted anisole under vacuum, the peptide was treated with anhydrous diethyl ether to re-move residual hydrofluoric acid and anisole. The material thus obtained was filtered, dissolved in acetonitrile–water (10:90) and lyophilized.

Sample preparation and loading

Approximately 1–3 g of the crude, lyophilized material obtained after the Frie-del–Crafts reaction was dissolved, just prior to chromatography, in a buffer (100–200 ml) whose concentration of organic modifier was equal to or below that used for the equilibration of the cartridge prior to the run and the pH of the solution was adjusted to *ca.* 7.0 with 6 *M* sodium-hydroxide. After stirring for 20–30 min the solution was acidified and filtered to remove particulates. This solution, often slightly opalescent, was then loaded onto the radially compressed column through the pumps and chro-matographed using the gradient conditions given in the individual figure legends.

Sample composition analysis

The composition of the final purified product was determined by a number of methods, including mass spectral analysis (City of Hope, Duarte, CA, U.S.A.); C,H,N and ash determination (Galbraith Labs., Knoxville, TN, U.S.A.), Karl Fisch-er water determination (Bachem, Torrance, CA, U.S.A.), and analysis of counter ions (see Table I). Amino acid composition was determined by hydrolysis with 4 *M* meth-anesulfonic acid (Pierce, Rockford, IL, U.S.A.) at 110°C for 24 h, followed by amino acid analysis.

RESULTS AND DISCUSSION

The structure of "Nal–Glu" antagonist is shown in Fig. 1. It contains six sub-stitutions of the native GnRH structure (pGlu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly–NH$_2$), five of which are D-amino acids. The unusual amino acid present in the 6-position, D-2-amino-5-oxo-5-(4-methoxyphenyl)pentanoic acid (I), is prepared via a hydrofluoric acid-mediated Friedel–Crafts acylation and modification of D-glutamic acid with anisole[15,16]. With the exception of this modified glutamic acid, the remaining amino acids are either commercially available or readily prepared in suffi-cient amounts for the synthesis of this peptide[9]. The keto amino acid I must be
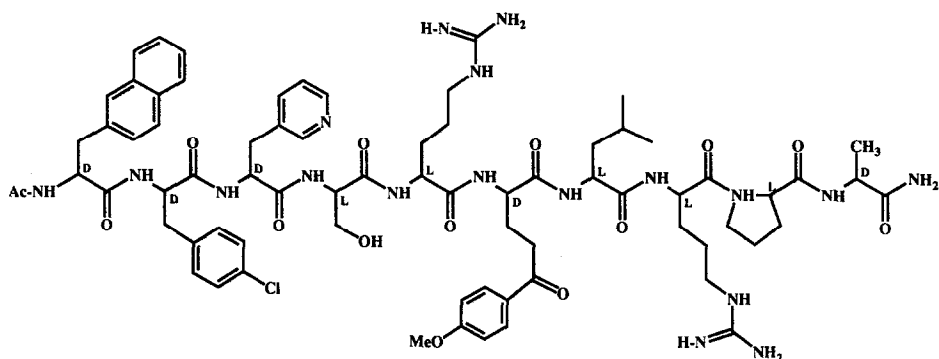
Fig. 1. Structure of "Nal–Glu" antagonist (Ac–D-Nal–D-Cpa–D-Pal–Ser–Arg–D-2-amino-5-oxo-5-(4-methoxyphenyl)pentanoic acid–Leu–Arg–Pro–D-Ala–NH$_2$). The chirality of the individual residues is represented by an appropriate stereochemical label on the C-α carbons.

prepared post-synthetically, as the free amino acid is known to undergo cyclization to form the dehydroproline derivative **II** (eqn. 1)[16].

$$\text{(1)}$$
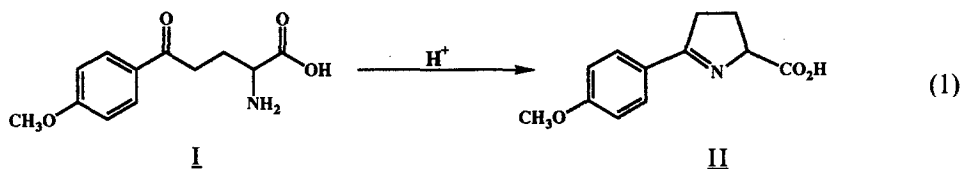
This reaction is also responsible for the inability of the modified Glu to be detected upon amino acid analysis of the hydrolyzed peptide. Although methods could undoubtedly be devised for the synthesis of **I**, we opted to prepare "Nal–Glu" antagonist via the Friedel–Crafts acylation reaction, as this reaction is generally a reliable method for its preparation.

Synthesis of "Nal–Glu" antagonist

    The general procedure for the synthesis of "Nal–Glu" antagonist has been reported elsewhere[9]; it is based on a side reaction of glutamic acid first elucidated in 1975[15,16]. The first step in this reaction involves the preparation of [Ac–D-Nal[1], D-Cpa[2], D-Pal[3], Arg[5], D-Glu[6], D-Ala[10]]-GnRH. This peptide is synthesized utilizing standard solid-phase peptide synthesis techniques[13,14]. Batchwise, concurrent deprotection and cleavage of the resin-bound peptide by hydrofluoric acid–anisole at 0°C provided, after extraction and lyophilization, 227 g of this crude "Nal–Glu" precursor. The analytical HPLC–UV trace of product run in 0.1% TFA–acetonitrile is presented in Fig. 2; very few impurities are seen in this crude peptide preparation. It has been determined in this laboratory that the crude [Ac–D-Nal[1], D-Cpa[2], D-Pal[3], Arg[5], D-Glu[6], D-Ala[10]]-GnRH is sufficiently pure to carry on to the Friedel-Crafts acylation step, as neither the impurities nor the salts and non-peptidic materials interfere to a significant extent with the subsequent conversion to and purification of crude "Nal–Glu" antagonist.
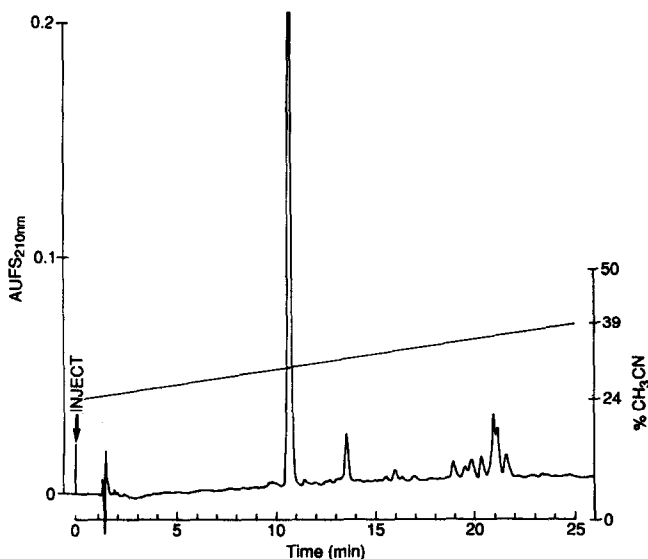
Fig. 2. Load: crude, lyophilized [Ac–D-Nal^1, D-Cpa^2, D-Pal^3, Arg^5, D-Glu^6, D-Ala^{10}]-GnRH from HF cleavage (1.5 μl, *ca.* 5 μg). Column: Vydac (5 μm) C$_{18}$, 25 × 0.46 cm I.D. Solvent: 0.1% TFA in water–acetonitrile. Gradient: 24–39% acetonitrile in 25 min. Flow-rate: 2.0 ml/min, 2000 p.s.i. back-pressure.

The conversion of this crude peptide to "Nal–Glu" antagonist was accomplished in a batchwise fashion as follows: 5.0–24.0 g (average 20 g) of [Ac–D-Nal^1, D-Cpa^2, D-Pal^3, Arg^5, D-Glu^6, D-Ala^{10}]-GnRH were placed in a graduated Kel-F HF cleavage vessel containing 20 ml of anisole and a magnetic stirring bar. After cooling the vessel for 5 min in a liquid nitrogen bath, it was evacuated and subsequently charged with 160–180 ml of anhydrous hydrofluoric acid. The mixture was then stirred in a hood at ambient temperature (22°C) overnight (16–18 h). This procedure, although potentially dangerous, is a convenient and facile method for the preparation of "Nal–Glu" antagonist. After the hydrofluoric acid treatment of all of the [Ac–D-Nal^1, D-Cpa^2, D-Pal^3, Arg^5, D-Glu^6, D-Ala^{10}]-GnRH analog, the weight of crude "Nal–Glu" antagonist obtained was 210 g. The absorbance profile obtained for this crude material is presented in Fig. 3.

Purification of "Nal–Glu" antagonist

The crude material obtained directly after the acylation reaction contains an approximately 1:1 mixture of the desired "Nal–Glu" antagonist (starred peak) and a closely associated hydrophilic impurity (Fig. 3), in addition to a number of other hydrophobic and hydrophilic impurities. When one examines the mechanism and reaction conditions employed in the synthesis of "Nal–Glu" antagonist, two interesting facets to the reaction become apparent. The first arises out of the mechanism of the Friedel–Crafts reaction. The presumed intermediate in the conversion of the glutamic acid to the keto amino acid I is the acylium ion; this reactive species can add not only to anisole but also to other nucleophilic species in the reaction medium, the most prevalent being the amide nitrogens in the peptide backbone[15,16]. The peptides aris-
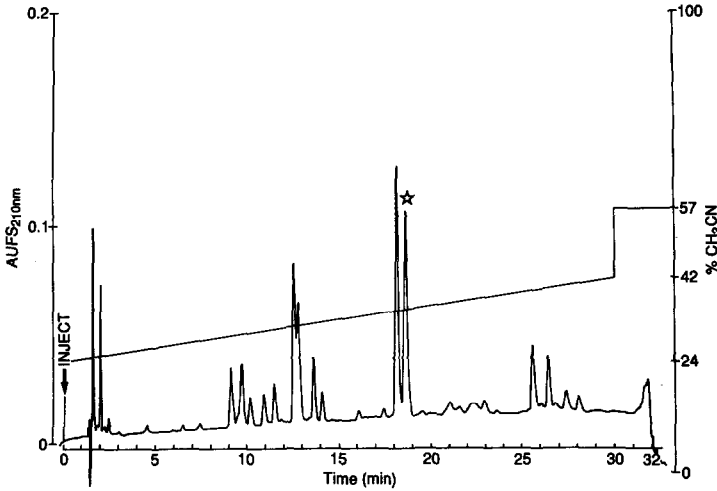
Fig. 3. Load: crude, lyophilized "Nal–Glu" antagonist (10 μl, 10 μg). Column: Vydac (5 μm) C_{18}, 25 × 0.46 cm I.D. Solvent: 0.1% TFA in water–acetonitrile. Gradient: 24–42% acetonitrile in 30 min. Flow-rate: 2.0 ml/min, 2000 p.s.i. back-pressure. The starred peak is the desired compound.

ing from this reaction account for the early-eluting hydrophilic impurities; the amounts of these materials formed can be minimized by optimization of concentrations of anisole (10%) and peptide–substrate (10 g of peptide per 100 ml of anhydrous hydrofluoric acid–anisole). Most important, however, is the observation that peptides containing serine, when treated under acidic conditions at elevated temperatures for
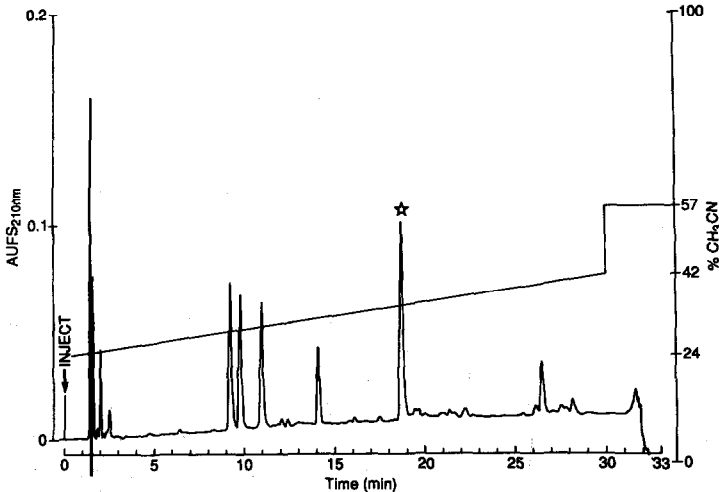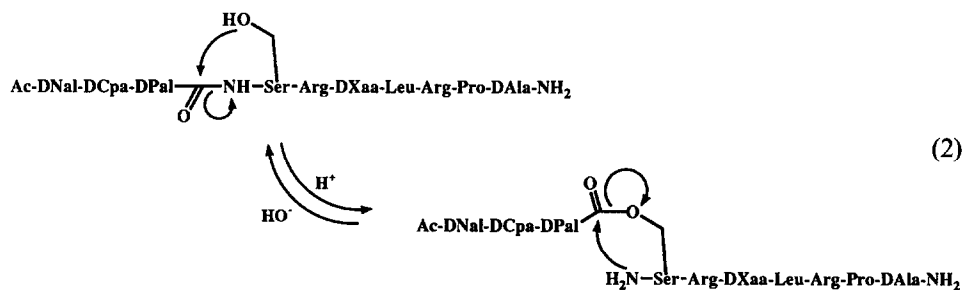

Fig. 4. Load: crude, lyophilized "Nal–Glu" antagonist (10 μl, 10 μg) after stirring at pH 6.5 for 30 min. Column: Vydac (5 μm) C_{18}, 25 × 0.46 cm I.D. Solvent: 0.1% TFA in water–acetonitrile. Gradient: 24–42% acetonitrile in 30 min. Flow-rate: 2.0 ml/min, 2000 p.s.i. back-pressure. The starred peak is the desired compound.

prolonged periods, can undergo an N-to-O acyl shift[17]. This side-reaction is not a serious problem, as adjustment of the pH of a solution of the crude peptide to 6.0–7.5 will result in a rapid reversal of this reaction (O-to-N acyl shift). In this instance, the closely associated hydrophilic "impurity" arises from just such an N-to-O acyl shift between the D-Pal[3] carboxamide nitrogen and the Ser[4] side-chain hydroxyl group (eqn. 2); stirring crude "Nal–Glu" antagonist at pH > 6 for 30 min reverses this shift and simplifies the analytical HPLC–UV trace considerably, as shown in Fig. 4 (compare with Figure 3; the starred peak is "Nal–Glu" antagonist). Thus, an understanding of the chemistry aids in the simplification of the purification of the desired peptide from the crude material and in an increase in yield.

HO.

Ac-DNal-DCpa-DPal—$\overset{\text{O}}{\Vert}$—NH—Ser-Arg-DXaa-Leu-Arg-Pro-DAla-NH$_2$

(2)

H^+

HO$^-$

Ac-DNal-DCpa-DPal

H_2N—Ser-Arg-DXaa-Leu-Arg-Pro-DAla-NH$_2$

Purification of "Nal–Glu" antagonist from the crude material was accomplished through HPLC procedures as described elsewhere[18,19]. A typical HPLC profile of the preparative-scale purification is shown in Fig. 5. Analytical isocratic conditions that will yield maximum information on the composition of the mixture around the desired product were first determined; in general this is obtained when the desired product elutes with a solute capacity factor (k') between 4 and 8; in the present instance, the ideal conditions employ a flow-rate of 2 ml/min with an isocratic solvent composition of 36% acetonitrile in water + 0.1% TFA.

The analytical HPLC screening of various fractions obtained from the preparative HPLC run (depicted in Fig. 5) is presented in Fig. 6. A solvent system consisting of 0.1% TFA–acetonitrile is particularly convenient as the UV transparency of this solvent system at 210 nm allows for high sensitivity, in addition to providing an extended column life span, and reproducibly good separations, extremely important points when faced with purifications on the scale reported here. Determination of the isocratic analytical conditions allows the successive and rapid assessment of the identity and purity of the fractions obtained from the individual preparative HPLC purification runs. As we have found that the TEAP–acetonitrile solvent system generally gives higher resolution and different selectivity than the corresponding TFA system, preparative-scale purification is run first employing TEAP at pH 2.25 and acetonitrile, applying a gradient selected on the basis of the analytical chromatogram obtained for the crude material (see Figs. 3 and 4). Owing to the strong elutropic characteristics of the TEAP buffer, preparative gradient conditions are generally started at about 10% lower acetonitrile concentration than the isocratic analytical conditions in TFA with a slope of 1% increase in acetonitrile per 300 ml of solvent that is eluted.
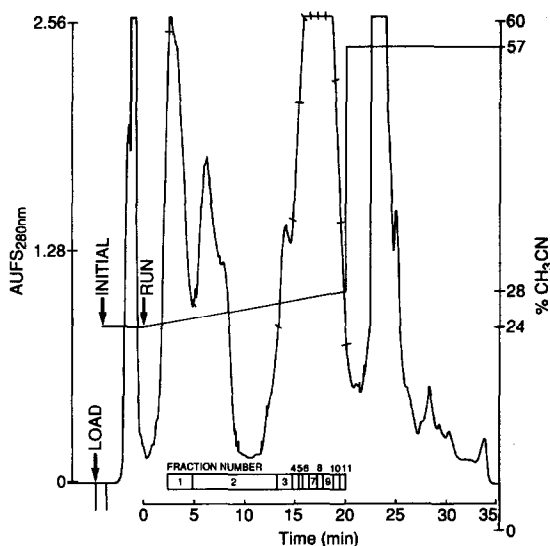
Fig. 5. Load: purified crude, lyophilized "Nal–Glu" antagonist after stirring at pH 6.5 for 30 min (3.0 g in 150 ml). Cartridge: 30 × 5 cm I.D., packed with Vydac (15–20 μm) C_{18}. Solvent: TEAP (pH 2.25)–acetonitrile. Gradient: 24–28% acetonitrile in 20 min. Flow-rate: 90 ml/min, 500 p.s.i. column back-pressure. Fractions taken are indicated by slash marks on the trace and boxes under the absorbing peak.

The fractions obtained from this purification step are screened analytically and pooled based on the composition as determined from analytical HPLC; in this fashion three pools are obtained: "good" (total impurities <1%) and "philic" and "phobic" (fractions containing hydrophilic or hydrophobic impurities, respectively,
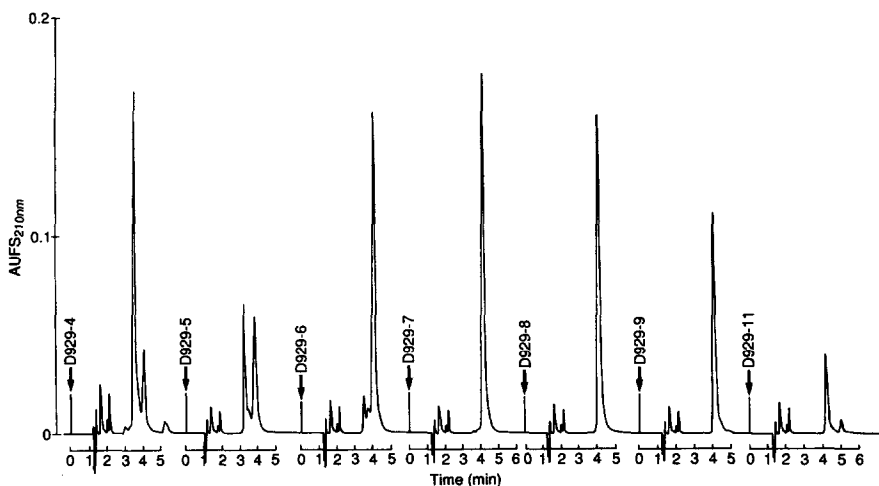

Fig. 6. Analytical screening of fractions 4–9 + 11 obtained from the TEAP purification shown in Fig. 5 (*ca.* 10 μl, 10 μg). Column: Vydac (5 μm), C_{18}, 25 × 0.46 cm I.D. Solvent: 0.1% TFA in water–acetonitrile; isocratic at 36% acetonitrile. Flow-rate: 2.0 ml/min, 2000 p.s.i. back-pressure.

of 1–30%). From the preparative run depicted in Fig. 5, the relative fates of the fractions obtained are as follows: 1–5, waste; 6, "philic"; 9–11, "phobic"; and 7–8, "good". The "phobic" and "philic" pools are then concentrated and separately re-purified to obtain additional pure material. This repurification, in the case of "Nal–Glu" antagonist, was accomplished using either a TEAP–acetonitrile solvent system and a different gradient or 0.5% acetic acid–acetonitrile. Although a high degree of purity can be achieved with the TEAP system, a desalting step utilizing 0.5% acetic acid–acetonitrile is required to free the peptide from any TEAP salt. Purification in two systems (TEAP followed by acetic acid) and analysis in another (TFA) was found in general to minimize the probability of missing any impurities and to maximize the probability of obtaining a pure final product while offering good recovery.

The conversion of the peptide from its TEAP salt to the corresponding acetate form is accomplished through HPLC procedures utilizing a 0.5% acetic acid–aceto-nitrile solvent system. This method of preparing acetate salts is based in part on an earlier procedure[19]. Briefly, this material was desalted and converted to the acetate salt form in the following manner: a pool of "acceptable" fractions containing ap-proximately 3–6 g of peptide (as determined by analytical HPLC) was diluted with an equal volume of 0.07 M ammonium acetate (pH 4.5). This solution was then applied to the same preparative HPLC cartridge as used in the purification above. After loading the peptide, the cartridge was washed with 1.5 l of 6% acetonitrile in water containing 0.5% acetic acid, after which the peptide was eluted from the cartridge by
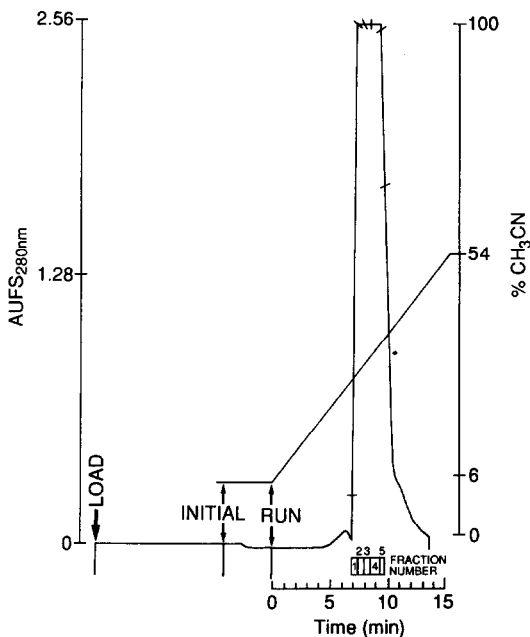

Fig. 7. Load: concentrated/desalted "good" "Nal–Glu" antagonist pool (ca. 3 g in 4 l). Cartridge: 30 × 5 cm I.D., packed with Vydac (15–20 μm) C_{18}. Solvent: 0.5% acetic acid–acetonitrile. Gradient: 6–54% acetonitrile in 15 min. Flow-rate: 95 ml/min, 500 p.s.i. column back-pressure. Fractions taken are indicated by slash marks on the trace and boxes under the absorbing peak.

application of a gradient running from 6 to 54% acetonitrile in water containing 0.5% acetic acid over 15 min at a flow-rate of 95 ml/min. The HPLC trace of this process is given in Fig. 7. This batch-wise procedure was repeated until all of the peptide had been converted to the corresponding acetate.

The fractions of the desired product that were deemed acceptable by analytical HPLC screening (data not shown) of the fractions obtained from these runs were collected and pooled for lyophilization. Each batch of lyophilized powder was re-lyophilized from 2.5 l of 0.5% acetic acid to ensure the complete removal of aceto-nitrile from the peptide. To ensure homogeneity of "Nal–Glu" antagonist, a final single-batch lyophilization was carried out. The purified peptide was dissolved in 2 l of deionized, distilled water and stirred until a clear solution was obtained (15 min). The solution was then filtered into clean, acid-washed lyophilizer bottles so that the resulting solution prior to lyophilization contained no visible particulate matter. After lyophilization at ambient temperature (20–22°C), 36.2 g of the "Nal–Glu" antag-onist were obtained as a fluffy white powder. We find this result, and also the method, to be both general and reliable for a wide range of peptides, and an alternative to the pyridine–acetic acid–2-propanol–water method of Gabriel[20] or the use of classical anion-exchange supports based on cellulose, Sephadex or polystyrene[21].

Characterization of "Nal–Glu" antagonist

As the end usage of this preparation of "Nal–Glu" antagonist is for human clinical use and toxicological studies, it was subjected to a complete chemical and biological analysis. Table I gives a summary of some of the data obtained for this preparation of "Nal–Glu" antagonist. The homogeneity of this peptide in two analyt-ical HPLC solvent systems (TEAP–acetonitrile and 0.1% TFA–acetonitrile) is given in Fig. 8a and b. Finally, this compound was shown to co-elute with authentic "Nal–Glu" antagonist and the analytical HPLC for this experiment is shown in Fig. 8c. Homogeneity in at least two solvent systems and coelution with a standard (if avail-able) is a routine procedure in our laboratory for large batches of peptides; although it is not a final criterion of purity, it is always an important indicator. The amino acid analysis gave (with expected values in parentheses) Ser, 0.79 (1.00); Leu, 1.00 (1.00); Pro, 0.99 (1.00); Ala, 0.98 (1.00); Cpa, 1.03 (1.00); Nal, 1.02 (1.00); and Arg, 2.07 (2.00); neither the modified glutamic acid nor the pyridylalanine residues can be detected using currently available protocols; however, the presence of these two ami-

TABLE I

CHARACTERIZATION OF "NAL–GLU" ANTAGONIST

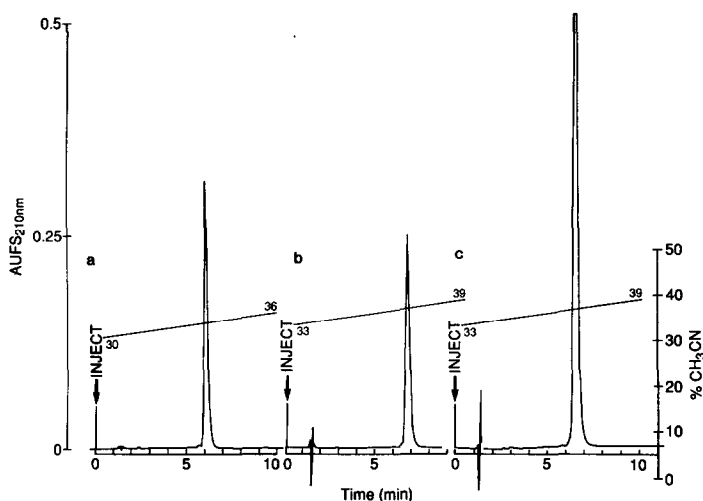| Parameter	Value
Mass spectrum	$MH^+ = 1485.52$ (calc., $MH^+ = 1485.72$)
C,H,N analysis	Found: C 54.96; H 6.88; N 15.11%
	Calc.: C 54.81; H 6.77; N 15.07%
Ash	<0.16%
Acetate content	$6.33 \pm 0.26\%$ CH_3COOH
Water content	$5.43\% \pm 0.13\%$ H_2O
Optical rotation	$-35.7°$ ($c = 1.00$; 50% CH_3COOH in H_2O)
Composition	$C_{73}H_{97}O_{18}Cl \cdot 1.67\ H_2O \cdot 4.7\ CH_3COOH$

Fig. 8. Analytical HPLC traces of purified "Nal–Glu" antagonist, including coelution experiment. For all three runs the following parameters apply: column, Vydac (5 μm) C_{18}, 25 × 0.46 cm I.D.; flow-rate, 2.0 ml/min, 2000 p.s.i. back-pressure. (a) Load: purified "Nal–Glu" antagonist (5 μl, 5 μg). Solvent: TEAP–acetonitrile. Gradient: 30–36% acetonitrile in 10 min. (b) Load: purified "Nal–Glu" antagonist (5 μl, 5 μg). Solvent: 0.1% TFA in water–acetonitrile. Gradient: 33–39% acetonitrile in 10 min. (c) Load: co-injection of purified "Nal–Glu" antagonist (4 μl, 4 μg) with "Nal–Glu" antagonist standard (4 μl, 4 μg). Solvent: 0.1% TFA in water–acetonitrile. Gradient: 33–39% acetonitrile in 10 min.

no acids is confirmed by both mass spectral and C,H,N analysis (see Table I). For completeness, the water and acetate counter-ion contents of the peptide were also determined; based on these data, and the peptide composition as determined by amino acid analysis, this preparation of peptide is found to be *ca.* 90% peptide, with the remainder attributable to acetate counter ions and water of hydration.

CONCLUSION

Over the past few years, the methodology and technology for preparing and purifying synthetic peptides on a scale sufficiently large to allow toxicological and clinical studies to be carried out has progressed considerably. We have shown previously that clinical batches of peptides can be synthesized and obtained in a highly purified form and that this can be accomplished in the setting of an academic laboratory. This is satisfying in that it is seldom possible (or plausible) for an academic research laboratory to have the opportunity to undertake and complete such a task. We have demonstrated that we can make this extension in the case of "Nal–Glu" antagonist and hopefully have convinced the reader that, at least in some instances, an academic laboratory can successfully accomplish industrial-type development.

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