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(54) Title: OLIGOPEPTIDES FOR DRUG DELIVERY		
(57) Abstract <p>The present invention relates to oligopeptides and compositions prepared from them. Theses compositions comprising an oligopeptide, and an active agent are useful in the delivery of a cargo to a target, and particularly in the oral delivery of biologically or chemically active agents. Methods for the preparation and for the administration of such compositions are also disclosed.</p>		

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OLIGOPEPTIDES FOR DRUG DELIVERY

FIELD OF THE INVENTION

The present invention relates to compositions prepared from oligopeptides. These compositions are useful in the delivery of a cargo to a target, and particularly in the oral delivery of biologically or chemically active agents. Methods for the preparation and for the administration of such compositions are also disclosed.

BACKGROUND OF THE INVENTION

Conventional means for delivering active agents to their intended targets, such as human organs, tumor sites, etc., are often severely limited by biological, chemical, and physical barriers. Typically, these barriers are imposed by the environment through which delivery occurs, the environment of the target for delivery, or the target itself.

Biologically active agents are particularly vulnerable to such barriers. Oral delivery to the circulatory system would be the route of choice for administration of many active agents to animals if not for physical barriers such lipid bilayers, and various organ membranes that are relatively impermeable to certain biologically active agents, but which must be traversed before an agent delivered via the oral route can reach the circulatory system. Additionally, oral delivery is impeded by chemical barriers such as the varying pH of the gastro-intestinal (GI) tract and the presence of powerful digestive enzymes.

Earlier methods for orally administering vulnerable

pharmacological agents have relied on the co-administration of adjuvants (e.g., resorcinols and non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether) to increase artificially the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors (e.g., pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol) to inhibit enzymatic degradation.

Liposomes have also been described as drug delivery systems for insulin and heparin. See, for example, U.S. Patent No. 4,239,754; Patel et al. (1976), *FEBS Letters*, Vol. 62, pg. 60; and Hashimoto et al. (1979), *Endocrinology Japan*, Vol. 26, pg. 337.

However, broad spectrum use of such drug delivery systems is precluded because: (1) the systems require toxic amounts of adjuvants or inhibitors; (2) the systems are typically suitable only for low molecular weight cargos, (3) the systems exhibit poor stability and inadequate shelf life; (4) the systems are difficult to manufacture; (5) the systems fail to protect the active agent (cargo); (6) the systems adversely alter the active agent; or (7) the systems fail to allow or promote absorption of the active agent.

More recently, microspheres of artificial polymers of mixed amino acids (proteinoids) have been used to deliver pharmaceuticals. For example, U.S. Patent No. 4,925,673 describes drug-containing proteinoid microsphere carriers as well as methods for their preparation and use. These proteinoid microspheres are useful for the delivery of a number of active agents.

Further studies have demonstrated that cyclic peptides with an even number of alternating L- and D-amino acids were able to form organic nanotubes. (See, Whitesides et al., *Science* 1991, 254, 1312, 1319; Ghadiri, M.R. et al., *Nature* 1993, 366, 324-327.) Additionally, stabilized spherical micelles and tubular vesicles have been prepared from amphiphiles and bolamphiphiles. (See, Fuhrhop, J.H. et al., *J. Amer. Chem. Soc.*, 1991, 113, 7437, 7439; Frankel, D.A. et al. *J. Amer. Chem. Soc.*, 1991, 113, 7436,-7437; Fuhrhop, J.H. et al., *J. Amer. Chem. Soc.*, 1993, 115, 1600-1601.) L-Asp-diketopiperazines appended with amino acid subunits were found to

self assemble into microspheres by Bergeron et al., *J. Amer. Chem. Soc.* (1994) 116:8479-8484. This self assembly process was sensitive to solution pH and substrate concentration.

However, there is still a need in the art for simple, inexpensive delivery systems which are easily prepared and which can delivery a broad range of active agents.

SUMMARY OF THE INVENTION

The present invention provides structurally defined oligopeptides. Several oligopeptides have been synthesized. The oligopeptides are useful for the delivery of active agents. The oligopeptides have from 2 to about 11 amino acid residues. They can be linear (all α -bonding) or branched (α - and side chain bonding) peptides.

One type of oligopeptide useful in practicing the invention is a pyroglutamic acid initiated oligopeptide having the formula:



where each X is an amino acid residue and n is an integer from 1 to about 10.

Another type of oligopeptide useful in practicing the invention is a proline initiated oligopeptide having the formula:



where each X is an amino acid residue and n is an integer from 1 to about 10.

The oligopeptides of the present invention may be combined with active agent(s). Also contemplated are methods for administering compositions that includes an active agent.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a scheme illustrating the synthesis of branched tripeptides starting from pyroglutamic acid.

30 Figure 2 is a scheme illustrating the synthesis of branched tetrapeptides starting from pyroglutamic acid.

Figure 3 is a scheme illustrating the synthesis of tripeptides and tetrapeptides starting from L-Proline.

Figure 4 is a scheme illustrating the synthesis of tripeptides using a hydrobromide dipeptide and DPPA as a coupling agent.

Figure 5 is a ^1H NMR spectrum of DiBzOCOGLuTsOH.

Figure 6 is an IR spectrum of DiBzOCOGLuTsOH.

5 Figure 7 is a ^1H -NMR spectrum of TsOH-GluBz.

Figure 8 is a ^1H NMR spectrum of DiBzOCOAspTsOH.

Figure 9 is an IR spectrum of DiBzOCOAspTsOH.

Figure 10 is a ^1H NMR spectrum of BzOCOPheTsOH.

Figure 11 is an IR spectrum of BzOCOPheTsOH.

10 Figure 12 is a ^1H -NMR spectrum of TsOH-D-PheBz.

Figure 13 is a ^1H NMR spectrum of BzOCOTyrTsOH.

Figure 14 is an IR spectrum of BzOCOTyrTsOH.

Figure 15 is a ^1H -NMR spectrum of TsOH-AlaBz.

Figure 16 is a ^1H -NMR spectrum of TsOH-L-LeuBz.

15 Figure 17 is a ^1H -NMR spectrum of D-diBzOCOGLu-TsOH.

Figure 18 is a ^1H NMR spectrum of BzOCO-NHAsp- β Bz.

Figure 19 is an IR spectrum of BzOCO-NHAsp- β Bz.

Figure 20 is a ^1H NMR spectrum of BzOCO-NHAsp.

Figure 21 is a ^1H NMR spectrum of BzOCO-NHGlu.

20 Figure 22 is an IR spectrum of BzOCO-NHGlu.

Figure 23 is a ^1H NMR spectrum of BzOCO-NHPhe.

Figure 24 is an IR spectrum of BzOCONH-Phe.

Figure 25 is a ^1H -NMR spectrum of t-Boc-L-Glu.

Figure 26 is a ^1H -NMR spectrum of BzOCO-L-Proline.

25 Figure 27 is a ^1H NMR spectrum of PyGluGlu.

Figure 28 is a ^1H NMR spectrum of PyGluAsp.

Figure 29 is a ^1H NMR spectrum of PyGlu-Phe.

Figure 30 is an IR spectrum of PyGlu-Phe.

Figure 31 is a ^1H -NMR spectrum of PyGlu- α -Phe- α -Phe.

30 Figure 32 is an IR spectrum of PyGlu- α -Phe- α -Phe.

Figure 33 is a ^1H -NMR spectrum of PyGlu-Glu-(γ -Phe).

Figure 34 is a ^1H -NMR COSSY spectrum of PyGlu-Glu-(γ -Phe).

Figure 35 is an IR spectrum of PyGlu-Gly-(γ -Phe).

- Figure 36 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Phe).
- Figure 37 is an IR spectrum of PyGlu-Asp-(β -Tyr).
- Figure 38 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Tyr).
- Figure 39 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu- γ -Gly.
- 5 Figure 40 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu- γ -Ala.
- Figure 41 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu- γ -Leu.
- Figure 42 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp- β -Gly.
- Figure 43 is an IR spectrum of PyGlu-Asp- β -Gly.
- Figure 44 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp- β -Ala.
- 10 Figure 45 is an IR spectrum of PyGlu-Asp- β -Ala.
- Figure 46 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu- α -Phe- γ -Phe.
- Figure 47 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp- α -Gly- β -Gly.
- Figure 48 is an IR spectrum of PyGlu-Asp- α -Gly- β -Gly.
- Figure 49 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp- α -Ala- β -Ala.
- 15 Figure 50 is a $^1\text{H-NMR}$ spectrum of PyGlu- α -Phe- α -Phe- α -Tyr.
- Figure 51 is a $^1\text{H-NMR}$ spectrum of PyGlu-L-Phe-L-Phe-L-Glu.
- Figure 52 is an IR spectrum of PyGlu-L-Phe-L-Phe-L-Glu.
- Figure 53 is a $^1\text{H-NMR}$ spectrum of PyGlu-L-Phe-L-Phe-D-Glu.
- Figure 54 is a $^1\text{H-NMR}$ spectrum of PyGlu-Phe-Phe-Asp.
- 20 Figure 55 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Phe)- α -Phe.
- Figure 56 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Phe)- α -Phe.
- Figure 57 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Ala)- α -Ala.
- Figure 58 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Gly)- α -Gly.
- Figure 59 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Tyr)- α -Tyr.
- 25 Figure 60 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ ,D-Phe)- α ,D-Phe.
- Figure 61 is an IR spectrum of PyGlu-Glu-(γ ,D-Phe)- α ,D-Phe.
- Figure 62 is a $^1\text{H-NMR}$ spectrum of Py-L-Glu-D-Glu(γ -Phe)- α -Phe.
- Figure 63 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Phe)- α -Gly.
- Figure 64 is an IR spectrum of PyGlu-Glu-(γ -Phe)- α -Gly.
- 30 Figure 65 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Phe)- α -Ala.
- Figure 66 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Tyr)- α -Phe.
- Figure 67 is an IR spectrum of PyGlu-Glu-(γ -Phe)- α -Phe.
- Figure 68 is a $^1\text{H-NMR}$ spectrum of PyGlu-Glu-(γ -Phe)- α -Phe.

Figure 69 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Phe)- α -Phe.

Figure 70 is a $^1\text{H-NMR}$ spectrum of PyGlu-Asp-(β -Tyr-Phe)- α -Tyr-Phe.

Figure 71 is a $^1\text{H-NMR}$ COSSY spectrum of L-Pro-L-Glu- γ -Phe.

5

Figure 72 is a $^1\text{H-NMR}$ of L-Pro-L-Glu- γ -Phe.

Figure 73 is a $^1\text{H-NMR}$ of L-Pro-L-Asp- β -Phe.

Figure 74 is an IR spectrum of L-Pro-Glu- γ -Phe.

Figure 75 is a $^1\text{H-NMR}$ spectrum of L-Pro-Glu- γ -Phe.

Figure 76 is a $^1\text{H-NMR}$ spectrum of L-Pro-Glu.

10

Figure 77 is a $^1\text{H-NMR}$ spectrum of BzOCO-Pro-Asp.

Figure 78 is a $^1\text{H-NMR}$ spectrum of BzOCO-Pro-Glu.

Figure 79 is a $^1\text{H-NMR}$ spectrum of BzOCO-ProNHS.

Figure 80 is a $^1\text{H-NMR}$ spectrum of L-Pro-Glu- α -Phe- γ -Phe.

Figure 81 is an IR spectrum of L-Pro-Glu- α -Phe- γ -Phe.

15

Figure 82 is a $^1\text{H-NMR}$ spectrum of L-Pro-Glu- α -Phe- γ -Phe.

Figure 83 is a $^1\text{H NMR}$ COSSY spectrum of L-Pro-L-Asp- α -Gly- γ -

Gly.

Figure 84 is a $^1\text{H NMR}$ spectrum of L-Pro-L-Asp- α -Gly- β -Gly.

Figure 85 is a $^1\text{H-NMR}$ spectrum of L-Pro-L-Asp- α -Phe- β -Phe.

20

Figure 86 is a $^1\text{H-NMR}$ spectrum of L-Pro-L-Glu- α -Gly- γ -Gly.

Figure 87 is a $^1\text{H NMR}$ COSSY spectrum of L-Pro-L-Glu- α -Gly- γ -

Gly.

Figure 88 is a $^1\text{H-NMR}$ spectrum of L-Pro-L-Glu- α -Tyr- γ -Tyr.

Figure 89 is an IR spectrum of L-Pro-L-Glu- α -Tyr- γ -Tyr.

25

Figure 90 is a $^1\text{H-NMR}$ spectrum of L-Pro-L-Glu- α -Leu- γ -Leu.

Figure 91 is a $^1\text{H-NMR}$ spectrum of BzOCONPhe-PheBz.

Figure 92 is a $^1\text{H-NMR}$ spectrum of BrH_3N^+ -Phe-PheBz.

Figure 93 is a $^1\text{H-NMR}$ spectrum of (L-Asp- β -Glu).

Figure 94 is a $^1\text{H-NMR}$ spectrum of a mixture of (L-Glu- γ -Asp) and L-Glu- α -Phe- γ -Asp.

30

Figure 95 is a $^1\text{H NMR}$ COSSY spectrum of L-Glu- γ -Asp and L-Glu- α -Phe- γ -Asp.

Figure 96 is a $^1\text{H-NMR}$ spectrum of $\text{Br}^+\text{H}_3\text{N}^+$ -L-Glu- α -Phe- γ -Phe.

Figure 97 is a $^1\text{H-NMR}$ spectrum of $\text{Cl}^-\text{H}_3\text{N}^+-\text{Glu}-\alpha\text{-Phe-Bz}-\gamma\text{-Phe-Bz}$.

Figure 98 is a $^1\text{H-NMR}$ spectrum of $\text{Cl}^-\text{H}_3\text{N}^+-\text{L-Glu}-\alpha\text{-Phe}-\gamma\text{-Phe}$.

Figure 99 is a graphic illustration of the results of oral gavage testing in rats using heparin with PyGlu-Glu- γ -Phe carrier.

Figure 100 is a graphic illustration of Hydrophobicity (Partition Coefficient) of Oligopeptides.

Figure 101 is a graphic illustration of Binding Affinity of Oligopeptides to Heparin at $\text{pH} = 2.27$.

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DETAILED DESCRIPTION OF THE INVENTION

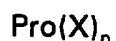
The present invention provides structurally defined oligopeptides. Several oligopeptides have been synthesized. The oligopeptides are useful for the delivery of active agents. The oligopeptides have from 2 to about 11 amino acid residues. They can be linear (all α -bonding) or branched (α - and side chain bonding) peptides.

One type of oligopeptide useful in practicing the invention is pyroglutamic acid initiated oligopeptides having the formula:



where each X is an amino acid residue and n is an integer from 1 to about 10.

Another type of oligopeptide useful in practicing the invention is proline initiated oligopeptides having the formula:



where each X is an amino acid residue and n is an integer from 1 to about 10.

Each X independently is an amino acid radical or a poly amino acid radicals.

In a preferred embodiment the oligopeptides can have from 4 to about 6 amino acid residues and n is from about 3 to about 5.

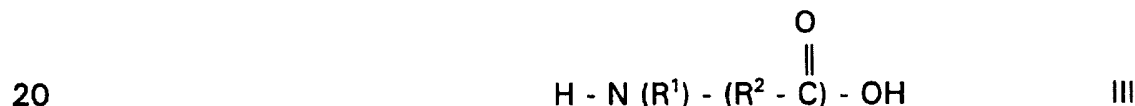
It has been found that, delivery of heparin, a hydrophilic drug, is enhanced when mixed with an oligopeptide having at least one hydrophobic and at least one hydrophilic amino acid. Thus, oral delivery can be effected.

Preferably, the hydrophobic amino acid has at least one aromatic group.

An amino acid is any carboxylic acid having at least one free amine group and includes naturally occurring and synthetic amino acids. An amino acid radical is an amino acid in which either one hydrogen atom of a free amine group or the hydroxyl from the carboxyl group has been removed such as by, for example, a condensation reaction in the formation of the oligopeptide.

Amino acid radicals are derived from naturally occurring or synthetic amino acids. Amino acid radicals are preferably derived from α -amino acids, and most preferably from naturally occurring α -amino acids. Many amino acids and amino acid esters are readily available from a number of commercial sources such as Aldrich Chemical Co. (Milwaukee, WI, USA); Sigma Chemical Co. (St. Louis, MO, USA); and Fluka Chemical Corp (Ronkonkoma, N.Y. USA).

Representative, but not limiting, amino acids from which amino acid radicals suitable for use in the present invention may be derived are generally of the formula



wherein: R^1 is hydrogen, C_1 - C_4 alkyl, or C_2 - C_4 alkenyl;

R^2 is C_1 - C_{24} alkyl, C_2 - C_{24} alkenyl, C_3 - C_{10} cycloalkyl, C_3 - C_{10} cycloalkenyl, phenyl, naphthyl, (C_1 - C_{10} alkyl) phenyl, (C_2 - C_{10} alkenyl) phenyl, (C_1 - C_{10} alkyl) naphthyl, (C_2 - C_{10} alkenyl) naphthyl, phenyl (C_1 - C_{10} alkyl), phenyl (C_2 - C_{10} alkenyl), naphthyl (C_1 - C_{10} alkyl), or naphthyl (C_2 - C_{10} alkenyl);

R^2 being optionally substituted with C_1 - C_4 alkyl, C_2 - C_4 alkenyl, C_1 - C_4 alkoxy, -OH, -SH, $-\text{CO}_2\text{R}^3$, C_3 - C_{10} cycloalkyl, C_3 - C_{10} cycloalkenyl, heterocycle having 3-10 ring atoms wherein the hetero atom is one or more of N, O, S, or any combination thereof, aryl, (C_1 - C_{10} alk)aryl, ar(C_1 - C_{10} alkyl) or any combination thereof;

R² being optionally interrupted by oxygen, nitrogen, sulfur, or any combination thereof; and

R³ is hydrogen, C₁-C₄ alkyl, or C₂-C₄ alkenyl.

The naturally occurring amino acids useful in practicing the invention are alanine, arginine, asparagine, aspartic acid, citrulline, cysteine, cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, proline, hydroxy proline, γ -carboxyglutamate, phenylglycine, or O-phosphoserine.

The non-naturally occurring amino acids useful in practicing the invention are β -alanine, α -amino butyric acid, γ -amino butyric acid, γ -(aminophenyl) butyric acid, α -amino isobutyric acid, citrulline, ϵ -amino caproic acid, 7-amino heptanoic acid, β -aspartic acid, aminobenzoic acid, aminophenyl acetic acid, aminophenyl butyric acid, γ -glutamic acid, cysteine (ACM), ϵ -lysine, ϵ -lysine (A-Fmoc), methionine sulfone, norleucine, norvaline, ornithine, d-ornithine, p-nitro-phenylalanine, hydroxy proline, 1,2,3,4,-tetrahydroisoquinoline-3-carboxylic acid, and thioproline.

The preferred amino acids are pyroglutamic, glutamic, aspartic, α -alanine, γ -alanine, γ -leucine, lysine, α -phenylalanine, β -phenylalanine, γ -phenylalanine, α -tyrosine, γ -tyrosine, tryptophan, proline, and γ -valine.

Poly amino acids can be used to form the oligopeptides. Typically, poly amino acids are either peptides or two or more amino acids linked by a bond formed by other groups which can be linked, *e.g.*, an ester, anhydride or an anhydride linkage. Poly amino acids can be homo- or hetero-poly amino acids, and can include natural amino acids, synthetic amino acids, or any combination thereof. Poly amino acids can be homo- or hetero- poly amino acids, and can include natural amino acids, synthetic amino acids, or any combination thereof. Poly amino acid radicals are poly amino acids in which at least one, and preferably one, hydrogen atom of a free amine group has been removed such as by, for example, a condensation reaction in the formation of the oligopeptide.

Peptides are two or more amino acids joined by a peptide bond. Peptides can vary in length from di-peptides with two amino acids to

polypeptides with several hundred amino acids. See, Walker, Chambers Biological Dictionary, Cambridge, England: Chambers Cambridge, 1989, page 215.

Active Agents

5 Active agents suitable for use in the present invention include biologically active agents and chemically active agents, including, but not limited to, fragrances, as well as other active agents such as, for example, cosmetics.

10 Biologically active agents include, but are not limited to, pesticides, pharmacological agents, and therapeutic agents. For example, biologically active agents suitable for use in the present invention include, but are not limited to, peptides, and particularly small peptides; hormones, and particularly hormones which by themselves do not or only pass slowly through the gastro-intestinal mucosa and/or are susceptible to chemical
15 cleavage by acids and enzymes in the gastro-intestinal tract; polysaccharides, and particularly mixtures of muco-polysaccharides; carbohydrates; lipids; or any combination thereof. Further examples include, but are not limited to, human growth hormones; bovine growth hormones; growth releasing hormones; interferons; interleukin-1; insulin; heparin, and particularly low
20 molecular weight heparin; calcitonin; erythropoietin; atrial natriuretic factor; antigens; monoclonal antibodies; somatostatin; adrenocorticotropin, gonadotropin releasing hormone; oxytocin; vasopressin; cromolyn sodium (sodium or disodium chromoglycate); vancomycin; desferrioxamine (DFO); anti-microbials, including, but not limited to anti-fungal agents; or any
25 combination thereof.

The methods and compositions of the present invention may combine one or more active agents.

30 In a typical liquid (solution) composition, the final solution can contain from about 10 mg to about 2000 mg of oligopeptide per ml of solution, preferably between about 20 to about 500 mg of oligopeptide per ml of solution, and most preferably from about 20 to about 200 mg per ml. Optionally, the mixture is heated to a temperature between about 20° C and about 60° C, preferably about 40° C, until the oligopeptide dissolves.

Particulates remaining in the solution may be filtered out by conventional means such as gravity filtration over filter paper.

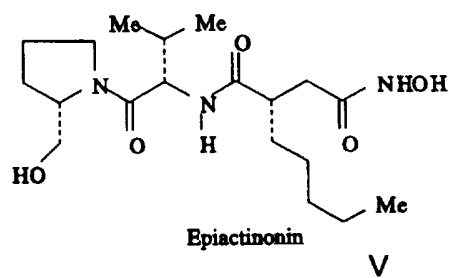
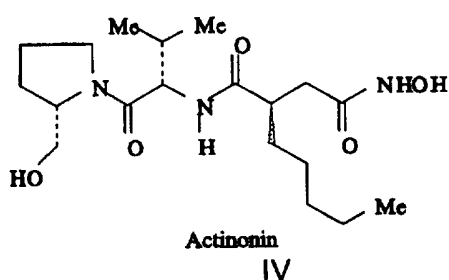
Additives

5 The compositions may optionally contain additives such as stabilizing additives. The presence of such additives promotes the stability and dispersability of any active agent in solution. The stabilizing additives may be employed at a concentration ranging between about 0.1 and 5% (w/v), preferably about 0.5% (w/v). Suitable, but non-limiting examples of
10 stabilizing additives include buffer salts, gum acacia, gelatin, methyl cellulose, polyethylene glycol, polypropylene glycol, and polylysine. The preferred stabilizing agents are gum acacia, gelatin, and methyl cellulose.

 The oligopeptides may be used directly as an active agent carrier by simply mixing one or more oligopeptides with the active agent(s) prior to
15 administration.

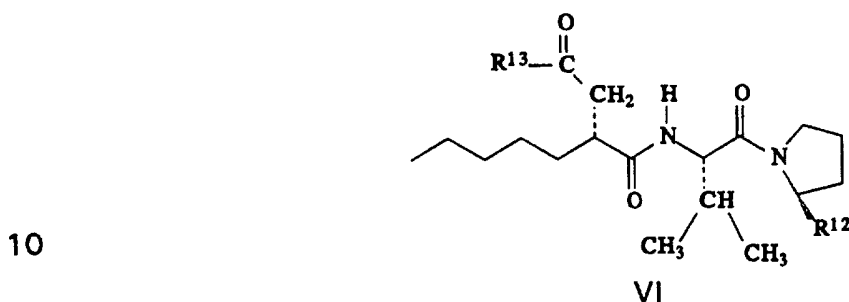
 The compositions of the present invention may be formulated into dosage units by the addition of one or more excipient(s), diluent(s), disintegrant(s), lubricant(s), plasticizer(s), colorant(s), or dosing vehicle(s). Preferred dosage unit forms are oral dosage unit forms. Most preferred
20 dosage unit forms include, but not limited to, tablets, capsules, or liquids. The dosage unit forms can include biologically, pharmacologically, therapeutically, or chemically effective amounts of the active agent or can include less than such an amount if multiple dosage unit forms are to be used to administer a total dosage of the active agent. Dosage unit forms are
25 prepared by methods conventional in the art.

 The compositions of the present invention may also include one or more enzyme inhibitors. Such enzyme inhibitors include, but are not limited to, compounds such as actinonin or epiactinonin and derivatives thereof. These compounds have the formulas below:
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Derivatives of these compounds are disclosed in U.S. Patent No. 5,206,384.

5 Actinonin derivatives have the formula:



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wherein R^{12} is sulfoxymethyl or carboxyl or a substituted carboxy group selected from carboxamide, hydroxylaminocarbonyl and alkoxy carbonyl groups; and R^{13} is hydroxyl, alkoxy, hydroxyamino or sulfoxyamino group. Other enzyme inhibitors include, but are not limited to, aprotinin (Trasylol) and Bowman-Birk inhibitor.

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Administration

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The compositions of the subject invention are useful for administering biologically active agents to any animals such as birds; mammals, such as primates and particularly humans; and insects. The system is particularly advantageous for delivering chemical or biologically active agents which would otherwise be destroyed or rendered less effective by conditions encountered before the composition reaches its target zone (i.e. the area in which the active agent of the delivery composition are to be released) and within the body of the animal to which they are administered. Particularly, the compositions of the present invention are useful in orally administering active agents, especially those which are not ordinarily orally

25

deliverable.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following examples illustrate the invention without
5 limitation. All reagents were purchased either from the Aldrich
Chemical Co. or the Sigma Chemical Co. and were used without further
purification. Silica gel 40 mm, obtained from J.T. Baker, was used for flash
column chromatography. ^1H NMR spectra were recorded at 300 MHz and
 ^{13}C NMR were recorded at 75 MHz. Chemical shifts are given in parts per
10 million downfield from an internal tetramethylsilane standard.

All amino acids used were in the L-configuration unless
otherwise indicated. All intermediates in the synthesis were measured by ^1H
NMR in D_6 -DMSO and the structures were confirmed. The final products were
confirmed by ^1H NMR, ^1H - ^{13}C Hetcor NMR, ES/MS and RHPLC.

15

Chromatography: Reversed-Phase HPLC

Reversed-phase HPLC (RHPLC) was carried out on a Waters-600
HPLC system (Delta-Pak C_{18} Column, 3.9 x 150 mm) by using the 50% (v/v)
 CH_3OH in deionized water plus 0.002% TFA (pH=2.30) as the mobile phase
20 at a flow rate of 0.5 mL/min and detection at $\lambda = 230$ nm. The following
standards were used to calibrate the partition coefficients of the
oligopeptides: pyridine, aniline, benzyl alcohol, benzoic acid, benzene. $K' =$
 $(V_R - V_0)/V_0$, $V_0 = 2.81$ mL (KI was used as a non-retained reference
compound). The linear equation to fit the standards is: $\text{Log } P_{\text{oct}} = 1.43 +$
25 $0.85 \text{ Log } K'$

Preparation of α -Amino Acid Benzyl Esters

EXAMPLE 1A:

Synthesis of L-Glutamic α,γ -Di(benzyl Ester) p-Toluensulfonate,
30 (DiBzGluTsOH)

L-Glutamic acid (150 mmol 22.07 g) and p-toluenesulfonic acid
(monohydrate, 32 g 162.8 mmol) were added to a mixture of freshly distilled
benzyl alcohol (220 ml) and toluene (100 ml) in a 500 ml round-bottom flask.

The mixture was heated to reflux (110-120°C) and the water formed in the reaction was trapped in a Dean-Stark receiver. When no more water appeared in the distillate (about 5 h) the mixture is allowed to cool to room
5 temperature and precipitated with ether (1000 ml). The crystalline p-toluenesulfonate of glutamic α,γ -di(benzyl ester) was collected on a filter, washed with 200 ml of ether and dried in air. After recrystallization from methanol-ether 67.37g (90%) of DiBzGluTsOH were obtained. M.P. 137°C.

The ^1H NMR spectrum is shown in Figure 5.

10 The IR spectrum is shown in Figure 6.

EXAMPLE 2A

Synthesis of Glycyl Benzyl Ester of p-Toluenesulfonate (TsOH-Gly-Bz)

Glycine (18.8 g, 250 mmol) and p-toluenesulfonic acid
15 (monohydrate; 48.5 g, 255 mmol) were added to a mixture of freshly distilled benzyl alcohol (100 mL) and benzene or toluene (50 mL) in a 500 mL round-bottom flask. The mixture was heated to reflux. The water formed was collected in a Dean-Stark receiver for about 5 hours. When no more water was formed in the distillate, the mixture was allowed to cool to room
20 temperature. The reaction mixture was then diluted with ether (500 mL) and refrigerated for 2 hours.

The crystalline product, the p-toluenesulfonate glycine benzyl ester, was filtered, washed with ether (200 mL), and air-dried. After recrystallization from methanol/ether, 30.4 g (yield 90%) was obtained. M.P.
25 132-134°C.

The ^1H NMR spectrum is shown in Figure 7.

EXAMPLE 3A

Synthesis of L-Aspartic α,β -Di(benzyl Ester) p-Toluenesulfonate,
30 (DiBzAsp,TsOH)

The product was obtained by following the procedure described in Example 1A, above (63.3 g, 87%). M.P. 154°C.

The ^1H NMR spectrum is shown in Figure 8.

The IR spectrum is shown in Figure 9.

EXAMPLE 4A

Synthesis of L-Phenylalanine Benzyl Ester p-Toluenesulfonate, (BzPhe,TsOH)

5 The product was obtained by following the procedure described in Example 1A, above, with 150 mmol of phenylalanine, 75 ml of benzyl alcohol, 75 ml of toluene and 32 g p-toluenesulfonate acid monohydrate (51.4 g, 80%). M.P. 159-160°C.

The ¹H NMR spectrum is shown in Figure 10.

10 The IR spectrum is shown in Figure 11.

Synthesis of D-Phenylalanine Benzyl Ester p-Toluenesulfonate, (Bz-D-Phe,TsOH)

15 The product was obtained by following the procedure described above. M.P. 157-158°C.

The ¹H NMR spectrum is shown in Figure 12.

EXAMPLE 5A

Synthesis of L-Tyrosine Benzyl Ester p-Toluenesulfonate, (BzTyr,TsOH)

20 The product was obtained by following the procedure described in Example 1A, above, with 150 mmol of tyrosine, 75 ml of benzyl alcohol, 75 ml toluene, and 32 g p-toluenesulfonate acid monohydrate (50 g, 75%). M.P. 166-168°C.

The ¹H NMR spectrum is shown in Figure 13.

25 The IR spectrum is shown in Figure 14.

EXAMPLE 6A

Synthesis of Alanine Benzyl Ester of p-Toluenesulfonate (TsOH-Ala-Bz)

30 The product was obtained by following the procedure as described in Example 1A, above (yield 88%). M.P. 110-111°C.

The ¹H NMR spectrum is shown in Figure 15.

EXAMPLE 7A**Synthesis of Leucyl Benzyl Ester of p-Toluenesulfonate (TsOH-L-Leu-Bz)**

The product was obtained starting with 19.67 g, 150 mmol, of leucine, by following the procedure in Example 1A, above (30.4 g, 90%
5 yield). M.P. 197-198°C.

The IR spectrum is shown in Figure 16.

EXAMPLE 8A**Synthesis of D-Glutamic Di(Benzyl Ester) of p-Toluenesulfonate (D-DiBz-Glu-
10 TsOH)**

The product was obtained by following the procedure as described in Example 1A, above. M.P. 130-131°C.

The ¹H NMR spectrum is shown in Figure 17.

15 A. Synthesis N-Benzyloxycarbonyl and N-tert-Butyloxycarbonyl Amino Acids**EXAMPLE 1B****Synthesis of N-Benzyloxycarbonyl L-Aspartic Acid β -Benzyl Ester (BzOCO-N-Asp- β Bz)**

20 L-Aspartic acid β -benzyl ester (150 mmol, 11.86 g) was dissolved in hot water (750 ml). The solution was allowed to cool to 60°C and then treated with NaHCO₃ (125 mmol, 10.5 g) and benzyl chlorocarbonate (60 mmol, 9 ml) and this solution was allowed to cool slowly to room temperature under vigorous stirring. Stirring was continued for
25 about 3 h. The solution was extracted with ether (2 X 250 ml) and acidified to Congo Blue with 5 N HCl. After several hours in the refrigerator the material was collected on a filter, washed with water, and dried in vacuo. N-Benzyloxycarbonyl β -benzyl L-aspartic acid was obtained after
recrystallization from CCl₄; (13.0 g, 73.6%). M.P. 107-108°C.

30 The ¹H NMR spectrum is shown in Figure 18.

The IR spectrum is shown in Figure 19.

EXAMPLE 2B**Synthesis of N-Benzyloxycarbonyl L-Aspartic Acid (BzOCO-N-Asp)**

A solution of L-aspartic acid (13.3 g 100 mmol) in 2N NaOH (150 ml) was cooled in an ice-water bath and stirred with a magnetic stirrer. 5 Benzyl chlorocarbonate (15.8 ml, 110 mmol) and 2N NaOH (250 ml) were added alternately in about ten portions each, (the reaction of the mixture was maintained distinctly alkaline). The temperature of the reaction mixture was maintained between 5 and 10°C for about 30 minutes, and controlled by the rate of addition of the reactants. The ice-water bath was replaced and 10 vigorous stirring was continued at room temperature for 45 min. The alkaline solution was extracted four times with ether (75 ml each). The aqueous layer was acidified to Congo Blue by the addition 5N HCl. An oil separated. After the removal of water by decantation. The product solidified in the refrigerator after about 6 hours (18.7 g, 70%). M.P. 114-115°C.

15 The ¹H NMR spectrum is shown in Figure 20.

EXAMPLE 3B**Synthesis of N-Benzyloxycarbonyl L-Glutamic Acid (BzOCO-N-Glu)**

The product was obtained by following the procedures in 20 Examples 2A and 2B. The amount of product obtained was 19 g, 70%. M.P. 116-118°C.

The ¹H NMR spectrum is shown in Figure 21.

The IR spectrum is shown in Figure 22.

25 EXAMPLE 4B**Synthesis of N-Benzyloxycarbonyl L-Phenylalanine (BzOCO-N-Phe)**

The product was obtained by following the procedures in Examples 2A and 2B (25 g, 83.3%). M.P. 86-88°C.

The ¹H NMR spectrum is shown in Figure 23.

30 The IR spectrum is shown in Figure 24.

EXAMPLE 5B**Synthesis of N-tert-Butyloxycarbonyl Glutamic Acid (t-Boc-Glu)**

A solution of 100 mmol of glutamic acid in a mixture of dioxane (200 mL), water (100 mL), and 2N NaOH (100 mL) was stirred and cooled in an ice-water bath. Di-tert-butylpyrocarbonate (24 g, 110 mmol) was added and stirring was continued at room temperature for ½ hour. The solution was concentrated *in vacuo* to about 100 mL, cooled in an ice-water bath, covered with a layer of ethyl acetate (300 mL) and acidified with a dilute solution of KHSO₄ to pH 2-3. The aqueous phase was extracted with ethyl acetate (150 mL), and the extraction was repeated. The ethyl acetate extracts were combined, washed with water (2 X 200 mL), and dried over anhydrous sodium sulfate. The t-Boc-Glu was recrystallized from ethyl acetate-hexane.

The ¹H NMR spectrum is shown in Figure 25.

15

EXAMPLE 6B**Synthesis of N-Benzyloxycarbonyl L-Proline (BzOCO-N-Pro)**

A solution of L-proline (11.5 g, 100 mmol) in 2N NaOH (50 mL) was cooled in an ice-water bath and stirred with a magnetic stirrer. Benzylchlorocarbonate (18.7 g, 110 mmol) and 2N NaOH (55 mL) were added in about 10 portions alternatively. The reaction mixture was maintained distinctly alkaline by addition of more of 2N NaOH, if necessary. The temperature of reaction mixture was kept between 5°C and 10°C by the rate of the reactants addition during 1 ½ hours. The ice-water bath was replaced and the stirring was continued for ½ hour at room temperature. The alkaline solution was extracted with ether (4 X 50 mL). The aqueous layer was removed and then acidified to pH 2-3 by the addition of 5N HCl. An oil was separated, washed with water, and dried *in vacuo*.

The ¹H NMR spectrum is shown in Figure 26

30

B. Preparation of Sequential Peptides Initiated with PyGlu

A typical synthesis procedure for the PyGlu initiated peptide is described as follows:

EXAMPLE 1C Synthesis of Pyroglutamyl Glutamic acid dipeptide (PyGluGlu):

To a stirred mixture of the PyGlu (0.65 g, 5.0 mmol) and DiBzGlu (2.5 g, 5 mmol) in dimethylformamide (DMF (10 mL) was added to
5 diphenylphosphoryl azide (DPPA) (1.41 mL, 6.5 mmol) in 5 mL of DMF at 0°C followed by the addition of triethylamine (TEA) (2.32 mL, 16.5 mmol) in 5 mL of DMF at 0°C during 5-10 min. period. The mixture was stirred at room temperature overnight. The mixture was then diluted with 300 mL of benzene/ethyl acetate (1:2 in volume). The solution was washed with 5% of
10 HCl aqueous solution (2 x 35 mL) aqueous solution, 5% NaHCO₃ solution (2 x 35 mL), water (35 mL), and finally a saturated NaCl solution (2 x 35 mL). After drying over Na₂SO₄ the solution was filtered, followed by removal of the solvent under reduced pressure. Yield 1.9 g (60.3%).

Removal of the ester protecting groups:

15 The benzyl ester groups were removed by hydrogenation: 1.8 g of the dipeptide in 200 mL of the methanol/tetrahydrofuran (THF) (1:1) was stirred at room temperature. To this solution was added 0.18 g. Pd/C (10 w/w%). Hydrogen gas was introduced into the system from a H₂ balloon. The mixture was stirred overnight. The catalyst was removed by
20 filtration. After removal of the solvent, the residue was recrystallized from chloroform to give 1.1 g (100%) of the unprotected dipeptide.

The ¹H NMR spectrum is shown in Figure 27.

EXAMPLE 2C. Synthesis of Pyroglutamyl Aspartic acid (PyGlu-Asp)

25

The product was obtained by following the procedure as described in Example 1C above.

The ¹H NMR spectrum is shown in Figure 28.

30 EXAMPLE 3C. Synthesis of Pyroglutamyl Phenylalanine (PyGlu-Phe)

The product was obtained by following the procedure as

described in Example 1C(a) above.

The ¹H NMR spectrum is shown in Figure 29.

The IR spectrum is shown in Figure 30.

5 EXAMPLE 4C. Synthesis of Pyroglutamyl Phenylalananyl
Phenylalanine (PyGlu-Phe-Phe):

(PyGlu-Phe-Phe) (mp 203-204) was obtained by
condensation of PyGlu-Phe with TsOH-PheBz at a molar ratio of 1:1 in the
presence of DPPA as a catalyst followed by the removal of the protecting
10 ester group by hydrogenation.

The ¹H NMR spectrum is shown in Figure 31.

The IR spectrum is shown in Figure 32.

15 EXAMPLE 5C. Synthesis of Pyroglutamyl Glutamyl γ -Phenylalanine
(PyGlu-Glu- γ -Phe):

Pyroglutamylglutamyl- γ -phenylalanine (PyGlu-Glu- γ -Phe)
was obtained by condensation of PyGlu-Glu and TsOH-Phe-Bz at a molar ratio
of 1:1 using DPPA as a catalyst, followed by the removal of the protecting
group by hydrogenation.

20 The ¹H NMR spectra are shown in Figure 33 and Figure 34.

The IR spectrum is shown in Figure 35.

 EXAMPLE 6C. Synthesis of Pyroglutamyl Aspartyl β -Phenylalanine
(PyGlu-Asp- β -Phe):

25 Pyroglutamylaspartyl β -phenylalanine (PyGlu-Asp- β -Phe)
was obtained following the procedure described above in Example 1C(b).

The ¹H NMR spectrum is shown in Figure 36.

The IR spectrum is shown in Figure 37.

30 EXAMPLE 7C. Synthesis of Pyroglutamyl Aspartyl β -Tyrosine
(PyGlu-Asp- β -Tyr):

Pyroglutamylaspartyl β -tyrosine (PyGlu-Asp- β -Tyr) was obtained
following the procedure described above in Example 1C. M.P. 208-210°C.

The ^1H NMR spectrum is shown in Figure 38.

Additional tripeptides were obtained according to the general route in the schemes disclosed in Figures 1 and 2. All tripeptides obtained by these routes were isolated after hydrogenation by freeze-dry technique.

EXAMPLE 8C. Synthesis of Pyroglutamyl Glutamyl Glycine (PyGlu-Glu- γ -Gly):

The trimer PyGlu-Glu- γ -Gly was synthesized following the schemes disclosed in Figures 1 and 2.

The ^1H NMR spectrum is shown in Figure 39.

EXAMPLE 9C. Synthesis of Pyroglutamyl Glutamyl Alanine (PyGlu-Glu- γ -Ala):

The trimer PyGlu-Glu- γ -Ala was synthesized following the schemes disclosed in Figures 1 and 2.

The ^1H NMR spectrum is shown in Figure 40.

EXAMPLE 10C. Synthesis of Pyroglutamyl Glutamyl Leucine (PyGlu-Glu- γ -Leu):

The trimer PyGlu-Glu- γ -Leu was synthesized following the schemes disclosed in Figures 1 and 2.

The ^1H NMR spectrum is shown in Figure 41.

EXAMPLE 11C. Synthesis of Pyroglutamyl Aspartyl Glycine (PyGlu-Asp- β -Gly):

The trimer, PyGlu-Asp- β -Gly, was synthesized following the schemes disclosed in Figures 1 and 2.

The ^1H NMR spectrum is shown in Figure 42.

The IR spectrum is shown in Figure 43.

EXAMPLE 12C. Synthesis of Pyroglutamyl Aspartyl Leucine (PyGlu-Asp- β -Ala):

The trimer, PyGlu-Asp- β -Ala were synthesized following the schemes disclosed in Figures 1 and 2.

5 The ^1H NMR spectrum is shown in Figure 44.

The IR spectrum is shown in Figure 45.

EXAMPLE 13C. Synthesis of Pyroglutamyl Glutamyl Phenylalanyl Phenylalanine (PyGlu-Glu- α -Phe- γ -Phe):

10 1 g (3.9 mmol) of the dipeptide PyGluGlu was dissolved in 10 mL of DMF followed by the addition of 3.32 g (7.8 mmol) of BzPhe. To this were added 2.19 mL of DPPA in 5 mL DMF and 3.59 mL of TEA in 5 mL of DMF at 0°C. The mixture was stirred at room temperature for 1 day. After completion, the reaction product was purified as described above. The
15 yield was 1.5 g (56.4%).

The ^1H NMR spectrum is shown in Figure 46.

EXAMPLE 14C. General Synthesis of Tetrapeptides:

20 Tetrapeptides may be classified as linear (all α -bonding) and branched (α - and side chain bonding) tetrapeptides. The structures are illustrated in Table 2.

Linear tetrapeptides (α) were obtained by sequentially repeating the condensation and deprotection procedures. The following linear α -tetrapeptides were prepared: pyroglutamylphenylalanyltyrosyl tyrosine (Py-
25 Glu- α -Phe- α -Phe- α -Tyr); pyroglutamylphenylalanylphenylalanyl glutamic acid (Py- α ,L-Glu- α ,L-Phe- α ,Phe- α ,L-Glu); Py- α ,L-Glu- α ,L-Phe- α ,L-Phe- α ,D-Glu, and PyGlu-Phe-Phe-Asp.

30 Tetrapeptides, such as α,γ and α,β , with identical amino acids were obtained by the condensation of PyGlu-Glu or PyGlu-Asp, respectively, with TsOH-Phe-Bz, TsOH-Ala-Bz, and TsOH-Glu-Bz at a molar ratio of 1:2.3. DPPA was used as a catalyst. The condensation was followed by the removal of the protecting group by hydrogenation. The following compounds were synthesized: PyGlu- α -Glu- α -Phe- γ -Phe; PyGlu-Asp- α -Phe- β -Phe; PyGlu-

Glu- α -Ala- γ -Ala; PyGlu- α -Glu- α -Gly- γ -Gly; PyGlu-Glu- α ,D-Phe- γ ,D-Phe, PyGlu- α ,D-Glu- α -Phe- γ -Phe, and PyGlu-Asp- α -Tyr- β -Tyr. Additional purification was used for PyGlu-Glu- α -Phe- γ -Phe and for PyGlu- α -Asp- α -Phe- β -Phe. The purification was carried out by suspending oligopeptides in acetone, stirring
5 for 30 min., and filtering.

Tetrapeptides, such as α,γ and α,β , with different amino acids in positions α,γ or α,β , were obtained from tripeptide and a benzyl ester tosylate of the corresponding amino acid. Tetrapeptide PyGlu-Glu- γ -Phe- α -Gly was synthesized by the condensation of PyGlu-Glu- γ -Phe-Bz with TsOH-GlyBz,
10 followed by hydrogenation. The similar procedure was used to obtain PyGlu- α -Glu- γ -Phe- α -Ala and PyGlu-Asp- β -Tyr- α -Phe.

Tetrapeptides, such as γ,γ and β,β , were obtained by condensation of PyGlu-Glu and PyGlu-Asp with Phenylalanyl phenylalanine benzyl ester hydrobromide at a molar ratio of 1:1, using DPPA as a catalyst.
15 The following oligopeptides were synthesized: PyGlu-Glu- γ -(Phe-Phe), and PyGlu-Asp- β -(Phe-Phe).

The tetrapeptides, PyGlu-Asp- α -Gly- β -Gly and PyGlu-Asp- α -Ala- β -Ala were prepared as described above.

The oligopeptide structures were determined using $^1\text{H-NMR}$ and
20 IR. The $^1\text{H NMR}$ and IR spectra are shown in Figures 47 to 69

The Hexapeptide PyGlu-Asp- β -(Tyr-Phe)- α -(Tyr-Phe) was obtained by the condensation of PyGlu-Asp- α -Tyr- β -Tyr with TsOH-PheBz, followed by the removal of the protecting groups.

25 The $^1\text{H NMR}$ is shown in Figures 70.

The oligopeptides prepared using PyGlu initiation according to the procedures described above are tabulated in Tables 1 and 2, below. The oligopeptides in table 1 have had molecular weights of the products
30 determined by GPC using a low molecular weight column (M_w range, 50-1500); Ultrastyrigel Column 100A $^\circ$ (Waters).

TABLE 1

Peptides Initiated With PyGlu

Sequence	Yield (%)	M(obs.)	M(calc.)
PyGlu-Glu	60.3	464	438
PyGlu-Asp	65.0	399	424
PyGlu-Glu- α -Phe- γ -Phe	56.4	803	432
PyGlu-Glu-Tyr- γ -Tyr	53.2	1006	764
PyGlu-Asp-Phe- β -Phe	60.3	718	718
PyGlu-Asp-Tyr- β -Tyr	55.1	873	750
PyGlu-Asp-Phe- β -Tyr	49.0	529	734
PyGlu-Glu-Phe- γ -Tyr	51.6	933	748

TABLE 2
OLIGOPEPTIDES INITIATED WITH PYROGLUTAMIC ACID

Dipeptides	Tripeptides	Linear Tetrapeptides	Branched Oligopeptides
Py-L-Glu-L-Glu	PyGlu-Glu- γ -Gly	PyGlu-Glu- α -Leu- γ -Leu	PyGlu- α -Glu- γ -(Phe- α -Phe)
Py-L-Glu-D-Glu	PyGlu-Glu- α -Ala	PyGlu-Asp- α -Gly- β -Gly	PyGlu- α -Asp- β -(Phe- α -Phe)
PyGlu-Asp	PyGlu-Glu- γ -Leu	PyGlu-Asp- α -Ala- β -Ala	PyGlu-Glu-(γ -Ala)- α -Ala
PyGlu-Phe	PyGlu-Asp- β -Gly	PyGlu- α -Phe- α -Phe- α -Tyr	PyGlu-Glu-(γ Phe)- α -Gly
	PyGlu-Asp- β -Ala	Py-L-Glu- α ,L-Phe- α ,L-Phe- α ,L-Glu	PyGlu-Glu-(γ Gly)- α -Gly
	Py-Glu-Glu- γ -Phe	Py-L-Glu- α ,L-Phe- α ,L-Phe- α ,D-Glu	PyGlu-Glu-(γ -Phe)- α -Phe
	PyGlu-Asp- β -Phe	Py-L-Glu- α ,L-Phe- α ,L-Phe- α ,L-Asp	PyGlu-Glu-(γ ,D-Phe)- α ,D-Phe
	PyGlu-Asp- β -Tyr	PyGlu-Glu- α -Phe- γ -Phe	Py-L-Glu-D-Glu(γ -Phe)- α -Phe
	PyGlu- α -Phe- α -Phe	PyGlu-Glu-Tyr- γ -Tyr	PyGlu-Asp-(β -Phe)- α -Phe
	PyGlu-Glu- γ -Ala	PyGlu-Asp-Phe- β -Phe	PyGlu-Asp-(β Tyr)- α -Tyr
	PyGluGlu- α -Phe	PyGlu-Asp-Tyr- β -Tyr	PyGlu-Glu-(γ -Phe)- α -Ala
	PyGluGlu- α -Tyr	PyGlu-Asp-Phe- β -Tyr	PyGlu-Asp-(β -Tyr)- α -Phe
	PyGluGlu- α -Val	PyGlu-Glu-Phe- γ -Tyr	PyGlu-Asp- α -(Tyr-Phe)- β -(Tyr-Phe)
	PyGluGlu- α -Leu	PyGlu-Glu-(α)Phe(γ)Tyr	
	PyGluGlu- γ -Tyr	PyGlu-Glu-(α)Phe(γ)Ala	
	PyGluGlu- γ -Ala	PyGlu-Glu-(α)Phe(γ)Val	
	GluGlu- γ -Val	PyGlu-Glu- γ -Phe- α -Gly	
	PyGluAsp- α -Tyr	PyGlu- α -Glu- γ -Phe- α -Ala	
		PyGlu-Asp- β -Tyr- α -Phe	
		PyGluGlu(α)Phe(γ)Leu	

5

10

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20

EXAMPLE D**Synthesis of Oligopeptides Initiated by L-Proline:****EXAMPLE 10 Synthesis of N-benzyloxycarbonyl-L-proline-N'-
5 hydroxysuccinimide ester:**

N-hydroxysuccinimide (4.2 g, 0.036 mol) was added to a solution of N-benzyloxycarbonyl-L-proline (9.15 g, 0.0366 mol, prepared in Example 5B) in THF. The mixture was cooled in an ice-water bath, and dicyclohexylcarbodiimide (7.6 g, 0.0368 mol) was added, with stirring for 24
10 hours. The separated N,N'-dicyclohexylurea was removed by filtration and the solvent was evaporated *in vacuo*. The crude product was recrystallized from isopropanol.

The ¹H NMR spectrum is shown in Figure 78.

**15 EXAMPLE 2D. Synthesis of BzOCO-Prolyl Glutamic acid with
z-proline-N-hydroxysuccinimide ester:**

L-Glutamic acid (0.0224 mol) was dissolved in aqueous, 5.7% NaHCO₃. The solution was treated with a solution of benzyloxy-carbonylproline (0.024 mol) in absolute methanol. The solution was stirred
20 for 1 hour at room temperature and acidified to pH 2 with 5N HCl. The separated solid was washed with water and dried *in vacuo*.

The ¹H NMR spectrum is shown in Figure 77.

**EXAMPLE 3D. Synthesis of BzOCO-Prolyl Aspartic acid with
25 z-proline-N-hydroxysuccinimide ester:**

Aspartic acid (0.0224 mol) was dissolved in aqueous, 5.7% NaHCO₃. The solution was treated with a solution of benzyloxy-carbonylproline (0.024 mol) in absolute methanol. The solution was stirred
30 for 1 hour at room temperature and acidified to pH 2 with 5N HCl. The separated solid was washed with water and dried *in vacuo*.

The ¹H NMR spectrum is shown in Figure 77.

EXAMPLE 4D. Synthesis of L-Prolyl glutamic acid (L-Pro-Glu)Dimer:

L-Prolyl glutamic acid (L-Pro-Glu) was obtained by condensation of z-Pro with diBz-Glu-TsOH using DPPA as a condensation agent, followed with reduction with H₂, Pd/C.

The ¹H NMR spectrum is shown in Figure 76.

EXAMPLE 5D. Synthesis of L-Prolyl Glutamyl-γ-Phenylalanine acid (L-Pro-Glu-γ-Phe):

Pro-Glu-γ-Phe was synthesized using the ester activating procedure, DPPA and TEA, followed by removal of the protecting groups as depicted in the reaction scheme in Figure 4.

The ¹H NMR spectrum is shown in Figure 75.

The IR spectrum is shown in Figure 74.

EXAMPLE 6D. Synthesis of L-Prolyl Aspartyl-β-Phenylalanine acid (L-Pro-Asp-β-Phe):

Pro-Asp-β-Phe was synthesized using the ester activating procedure, DPPA and TEA, followed by removal of the protecting groups as depicted in the reaction scheme in Figure 4.

The ¹H NMR spectrum is shown in Figure 73.

EXAMPLE 7D. Syntheses of L-Prolyl L-Glutamyl γ-Phenylalanine acid (L-Pro-L-Glu-γ-Phe) Trimer:

L-Pro-L-Glu-γ-Phe was obtained by condensation of z-Pro with γ-phenylalanine benzyl ester of glutamic acid bromohydrate (HBr · NH₂-Glu-γ-Phe-Bz) using DPPA as a condensation agent followed by the removal of the protecting groups by hydrogenation (Pd/C) following the procedure in Figure 3.

The ¹H NMR spectra are shown in Figures 71 and 72.

Other oligopeptides initiated using proline were prepared following the procedures described herein. The oligopeptide structures were

determining using $^1\text{H-NMR}$ and IR.

The $^1\text{H NMR}$ and IR spectra are shown in Figures 74 to 90.

The oligopeptides prepared using Proline initiation according to the procedures described above. The results are tabulated in Table 3, below.

5

TABLE 3
Oligopeptides Initiated with Proline

Dipeptides	Tripeptides	Linear Tetrapeptides
Pro-Glu	Pro-Glu- γ -Phe	Pro-Glu- α -Phe- γ -Phe
Pro-Asp	Pro-Asp- γ Phe	Pro-Asp- α -Phe- β -Phe
	Pro-Glu- α -Phe	Pro-Glu- α -Gly- γ -Gly
		L-Pro-L-Glu- α -Leu- γ -Leu
		Pro-Glu- α -Tyr- γ Tyr
		Pro-Asp- α -Gly- β -Gly

15

EXAMPLE E **Other Oligopeptides**

EXAMPLE 1E(a). Synthesis of Phenylalanyl Phenylalanine Benzyl Ester Hydrobromide ($\text{Br-H}_3\text{N}^+\text{-Phe-Phe-Bz}$):

BzOCONPhe-PheBz was prepared by condensation of
 20 N-benzyloxycarbonyl L-phenylalanine with TsOH-PheBz in DMF and TEA with DPPA as a catalyst (yield 84%, M.P. 154.5-155°C) following the procedure illustrated in the scheme in Figure 2.

The $^1\text{H NMR}$ spectrum is shown in Figure 91.

25 EXAMPLE 1E(b). Selective Acidolysis:

$\text{Br-H}_3\text{N}^+\text{-Phe-Phe-Bz}$ was obtained by selective acidolysis of the N-benzyloxycarbonyl L-phenylalanyl phenylalanine benzyl ester (BzOCONPhe-PheBz).

A solution of HBr (30 wt.%) in acetic acid (30mL) was placed in
 30 a 250 mL round-bottom flask. N-benzyloxycarbonyl L-phenylalanyl

phenylalanine benzyl ester (BzOCONPhe-PheBz) (10 mmol) was added with stirring. The flask was closed with a cotton-filled drying tube. The reaction mixture was stirred to allow for a complete dissolution of the protected dipeptide, with a vigorous evolution of CO₂. When the gas evolution ceased
5 (after about 25 min.), dry ether (200 mL) was added with stirring. The reaction mixture was kept in a refrigerator for several hours. The precipitate formed was collected on a filter, washed with ether (50 mL), and dried over NaOH pellets *in vacuo*. The product phenylalanyl phenylalanine benzyl ester hydrobromide was recrystallized from methanol/ether.

10 The ¹H NMR spectrum is shown in Figure 92.

EXAMPLE 2E. Synthesis of L-Aspartyl β-Glutamic Acid (L-Asp-β-Glu):

L-Aspartyl β-Glutamic Acid (L-Asp-β-Glu) was obtained by
15 condensation of N-(carbobenzyloxy)-L-aspartic acid with DiBzOCO-Glu-TsOH with DPPA as a catalyst followed by the removal of the protecting groups by hydrogenation.

The ¹H NMR spectrum is shown in Figure 93.

20 EXAMPLE 3E. Synthesis of a mixture of β-Glutamyl-γ-Aspartic acid (L-Glu-γ-L-Asp) and L-Glutamyl-α-Phenylalanine-γ-L-Aspartic acid (L-Glu-α-Phe-γ-L-Asp) :

β-Glutamyl-γ-aspartic acid (L-Glu-γ-L-Asp) was obtained in a mixture with L-glutamyl-α-phenylalanine-γ-L-aspartic acid (L-Glu-α-Phe-γ-L-
25 Asp) by condensation of N-(carbobenzyloxy)-L-glutamic acid with DiBzOCO-Asp-TsOH with DiBzOCO-Glu-TsOH with DPPA as a catalyst. After purification the product was reacted by condensation with PheBzOCO-TsOH followed by the removal of the protecting groups by hydrogenation. The mixture of (L-Glu-γ-L-Asp) and (L-Glu-α-Phe-γ-L-Asp) was obtained at a molar
30 ratio of 80:20, determined by NMR.

The ¹H NMR spectrum is shown in Figure 94.

The ¹H NMR COSSY spectrum is shown in Figure 95.

EXAMPLE 4E. Synthesis of a mixture of L-Glutamyl- α -Phenylalanine- γ -Phenylalanine (L-Glu- α -Phe- γ -Phe):

L-Glutamyl- α -Phenylalanine- γ -Phenylalanine (L-Glu- α -Phe- γ -Phe) was obtained by two procedures.

- 5 (a) N-(carbobenzyloxy)-L-glutamic acid was reacted with PheBzOCO-TsOH using DPPA as a catalyst. The products BzOCO-Glu- α -Phe- γ -Phe-Bz and BzOCO-Glu- γ -Phe- α -Phe)-Bz were obtained. The protecting groups were removed by hydrogenation (Pd/C).

The ^1H NMR spectrum is shown in Figure 96.

- 10 (b) The protecting groups were removed selectively using HCl/dioxane (4 M; room temperature, 1 hour) from BzOCO-Glu- α -Phe-Bz- γ -Phe-Bz. The product was isolated by precipitation with ether. The structure was determined by NMR. The benzyl ester groups were removed by hydrogenation.

- 15 The ^1H NMR spectrum of the dibenzyl ester is shown in Figure 97.

The ^1H NMR spectrum of the free acid is shown in Figure 98.

The other oligopeptides prepared according to the procedures described above are tabulated in Tables 4, below.

20

TABLE 4
Other Oligopeptides

Dipeptides	Tripeptides
L-Asp- β -L-Glu	L-Glu- γ -Asp- α -Phe
L-Glu- γ -L-Asp	L-Glu- α -Phe- γ -Asp
Phe-Phe	L-Glu- α -Phe- γ -Phe
	L-Glu- γ -Asp- α -Phe

25

Example F Delivery of Heparin to rats**Example 1F Preparation of Dosing solutions.**

The oligopeptide, PyGlu-Glu- γ -Phe, was dissolved in distilled water and adjusted to pH 7.2-8.0. A solution containing heparin was prepared. Heparin was dissolved in a solution of 1.7 N citric acid and 0.5% gum arabic. The solutions were warmed to about 40°C and mixed. Two samples were prepared the first sample had a carrier concentration of 100 mg/mL. The heparin concentration was 33.3 mg/mL.

Following a similar procedure a second sample having a carrier concentration of 200 mg/mL and a heparin concentration of 33.3 mg/mL was prepared.

Example 2F HEPARIN *In Vivo* EXPERIMENTS IN RATS

For each sample a group of fasted rats were anesthetized. The rats were administered, by oral gavage, one of the heparin/carrier dosages prepared in Example 1F. In the first group each rat was administered a dosage of 100 mg/kg of heparin and 300 mg/kg of carrier. In the second group each rat was administered a dosage of 100 mg/kg of heparin and 600 mg/kg of carrier.

Blood samples were collected serially from the tail artery. Heparin activity was determined by utilizing the activated partial thromboplastin time (APTT) according to the method of Henry, J.B., Clinical Diagnosis and Management by Laboratory Methods; Philadelphia, PA; WB Saunders (1979).

The results of the test are illustrated in Figure 99.

It can be clearly seen from Example F that the oligopeptides of the invention are capable of delivering active agents to a target preferably through the GI tract.

Example G**HEPARIN BINDING AFFINITY EXPERIMENTS****Syntheses**

The following oligopeptide series were synthesized according to

the procedures described herein and the synthesis routes were optimized. (The numbers in the brackets represent the code number of the oligopeptides.) These groups of peptides were tested to determine their binding affinities with heparin.

5

	<u>Group 1</u>	<u>Group 2</u>	<u>Group 3</u>
	1) PyGluGlu- α -Phe (101)	PyGluGlu- γ -Phe (102)	PyGluGlu- α -Phe- γ -Phe (103)
	2) PyGluAsp- α -Phe (201)	PyGluAsp- β -Phe (202)	PyGluAsp- α -Phe- β -Phe (203)
	3) PyGluGlu- α -Tyr (301)	PyGluGlu- γ -Tyr (302)	PyGluGlu- α -Phe- γ -Tyr (303)
10	4) PyGluGlu- α -Ala (401)	PyGluGlu- γ -Ala (402)	PyGluGlu- α -Phe- γ -Ala (403)
	5) PyGluGlu- α -Val (501)	PyGluGlu- γ -Val (502)	PyGluGlu- α -Phe- γ -Val (503)
	6) PyGluGlu- α -Leu (601)	PyGluGlu- γ -Leu (602)	PyGluGlu- α -Phe- γ -Leu (603)
	7) ProGlu- α -Phe (701)	ProGlu- γ -Phe (702)	ProGlu- α -Phe- γ -Phe(703)

All amino acids used were in the L-configuration.

15

Since glutamic acid is hydrophilic, it is essential to have hydrophobic amino acids such as phenylalanine, tyrosine, alanine, valine, leucine as the terminal amino acid for the oligopeptides to balance the hydrophobicity in order to enhance delivery of the active agents. These oligopeptides were purified on a preparative reversed-phase HPLC column (Delta-Pak C₁₈, Waters). The relative purity of the oligopeptides was measured on an analytical reversed-phase HPLC column (Delta-Pak C₁₈, Waters).

25 Example 1G Circular Dichroism

Circular dichroism studies were carried out on a Jasco-600 for PyGluGlu- α -Phe- γ -Phe with the heparin fraction #1 which is the first fraction (high molecular weight) obtained by preparative Bio-Rad P-30 GPC chromatography. Samples were prepared in a phosphate buffer with variable concentrations of oligopeptide from 0.01 mg/mL to 0.05 mg/mL and 0.5 mg/mL of heparin at pH 7.0 and ultrasonicated for 20 minutes before scanning.

30

Example 2G Differential Scanning Calorimetry

Differential scanning calorimetry was carried out on a Microcal MC-2 scanning calorimeter. Samples were prepared in the same manner as above.

5 Circular Dichroism experiments did not provide any CD pattern changes at neutral pH even though the differential scanning calorimetry data indicated that the addition of PyGluGlu- α -Phe- γ -Phe, PyGluAsp- α -Phe- β -Phe or PyGluGlu- α -Phe- γ -Tyr eliminates the thermal transition peak for heparin. Possible explanations are that either at neutral pH, the oligopeptides do not
10 bind to heparin or heparin polymer conformation is not as sensitive to such weak binding.

Example 3G Equilibrium Dialysis

Equilibrium dialysis was carried out by using Spectra/Por CE
15 (cellulose ester) membrane -MWCO = 1000) to estimate the binding parameters. The heparin fraction #1 solution with constant concentration was contained inside the membrane, which is immersed into the oligopeptide solution at specific concentrations. The oligopeptide concentration was monitored by UV absorption ($\lambda = 257$ nm for PyGluGlu- γ -Phe and PyGluGlu- α -
20 Phe- γ -Phe; $\lambda = 274$ nm for PyGluGlu- α -Phe- γ -Tyr) until equilibrium was reached. The buffer composition was 100 mM NaCl in 25 mM phosphate plus 0.005% NaN₃ at pH=6.85 or 100 mM NaCl in 25 mM acetate buffer plus 0.005% NaN₃ at pH=2.27. By assuming n equivalent and independent binding sites between heparin and oligopeptides, the dissociation constants
25 and binding numbers were determined by the application of the following equation:

$$r / [P] = 1 / n + K_d / (n \times [P]), \quad r = [P]_{\text{bound}} / [D]_{\text{total}}$$

where [P] is the concentration of unbound or free oligopeptides,
[P]_{bound} is the difference of total oligopeptide concentration and
30 free oligopeptide concentration and [D]_{total} is the total heparin concentration. The binding number is n and K_d is the dissociation constant.

The partition coefficient P_{oct} between octanol and water is one of

the most effective physical parameters of bioactive compounds for predicting their biological activities in the study of quantitative structure-activity relations, K. Miyake et al., *Chem. Pharm. Bull.*, 1987, 35(1), 377-388. It was reported that P_{oct} has a linear relationship with reversed phase HPLC index K' . The hydrophobicity of these oligopeptides, evaluated by analytical RHPL, is shown in Figure 100. It was found that the tetramers have higher hydrophobicity than either the α -series trimers or the (β/γ)-series trimers. The hydrophobicity tendency for the tetramers is in agreement with the hydrophobicity of the amino acids themselves except that PyGluGlu- α -Phe- γ -Tyr has much lower hydrophobicity than PyGluGlu- α -Phe- γ -Phe even though Phe and Tyr have almost similar hydrophobicity characteristics.

Example 4G Heparin Affinity Chromatography

Heparin affinity chromatography was carried out on a SigmaChrom™ AF-Heparin affinity HPLC column. The mobile phase was 100 mM NaCl in 25 mM phosphate buffer plus 0.005% NaN₃ at pH = 6.85 or 100 mM NaCl in 25 mM acetate buffer plus 0.005% NaN₃ at pH = 2.27 with and without organic additives such as isopropanol or ethylene glycol. The flow rate was 1.0 mL/min. $K' = (V - V_0) / V_0$, $V_0 = 2.25$ (NaNO₂ was used as the non-retained reference).

It was found that the binding affinity of these oligopeptides to heparin increased with decreasing pH. Oligomers PyGluGlu- α -Phe- γ -Phe, PyGluAsp- α -Phe- β -Phe, PyGluGlu- α -Phe- γ -Tyr, and ProGlu- α -Phe- γ -Phe, which are tetramers and contain Phe or Tyr, bind to heparin better at low pH ~ 2.3 [Figure 101] than at higher pH ~ 6.8 (actually there was no apparent retention at neutral pH). The other oligopeptides do not bind to heparin at either the lower (~2) or the higher pH (~7). Upon addition of different concentrations of isopropanol (which was used to prevent hydrophobic interaction) to the mobile phase, the retention of oligopeptides to heparin is decreased. The addition of ethylene glycol (which was used to reduce the hydrogen-bonding) to the mobile phase also decreased the retention of oligopeptides on heparin. These data indicate that the binding affinities are due to hydrophobicity and hydrogen-binding. Correlation between heparin affinity and hydrophobicity

data indicates that the hydrophobic aromatic ring plays a role in the binding since PyGluGlu- α -Phe- γ -Leu, which has high hydrophobic character with leucine as the terminal groups, has very low retention on the heparin column.

The results are illustrated in Figure 101.

5

Correspondingly, the binding affinities of oligopeptides to heparin were also evaluated by equilibrium membrane dialysis to estimate the binding parameters. Both natural and commercially available heparin have a broad molecular weight distribution. Therefore, fractionation of commercial heparin was carried out on a Bio-Rad Gel SEC (size exclusion chromatography) column to obtain a high molecular weight fraction which was anticipated to have some degree of secondary structure.

10

Table 5 Affinity Parameters of Oligopeptides to Heparin Fraction #1

	PyGluGlu- γ -Phe	PyGluGlu- α -Phe- γ -Phe	PyGluGlu- α -Phe- γ -Tyr
n	5.7	5.5	87.0*
Kd	1.42×10^{-4}	1.46×10^{-4}	8.0×10^{-3} *

* pH = 2.27

20

The results, shown in Table 5 indicate that at neutral pH, the oligopeptides PyGluGlu- γ -Phe, PyGluGlu- α -Phe- γ -Phe, and PyGluGlu- α -Phe- γ -Tyr have a weaker binding affinity to the high molecular weight heparin fraction. The estimated binding number was 3-6 and the dissociation constant is 10^{-4} . At pH ~ 2, PyGluGlu- α -Phe- γ -Phe has a slightly weaker binding to heparin. However, a higher binding number (87) was found at this lower pH. These data indicate that the interactions are weak, which is not surprising since these oligopeptides do not contain a basic amino acid. However, it has been reported that basic amino acid containing oligopeptides such as KWK-COOH and RWR-COOH have similar order interaction with heparin ($K_a = 3.2$ or 4.5×10^{-3} respectively), D. P. Mascotti and T. M.

25

30

Lohman, *Biochemistry*, 1995, 34, 2908(2915).

All patents, patent applications, literature publications and test methods cited herein are hereby incorporated by reference.

Many variations of the present invention will suggest themselves to those skilled in the art in light of the above detailed disclosure. All such
5 modifications are within the full intended scope of the appended claims.

CLAIMS:

- 1 1. A composition comprising:
2 (a) an oligopeptide having the formula:
3 $Y(X)_n$
4 wherein Y is PyGlu or Pro,
5 each X is an independent amino acid residue,
6 and n is an integer from 1 to about 10; and
7 (b) an active agent.
- 1 2. A composition as defined in claim 1, wherein said
2 oligopeptide has the formula:
3 $PyGlu(X)_n$
4 wherein each X is an independent amino acid residue and
5 n is an integer from 1 to about 10.
- 1 3. A composition as defined in claim 1, wherein said
2 oligopeptide has the formula:
3 $Pro(X)_n$
4 wherein each X is an independent amino acid residue and
5 n is an integer from 1 to about 10.
- 1 4. A composition as defined in claim 1, wherein n is from
2 about 3 to about 5.
- 1 5. A composition as defined in claim 1, wherein each X is a
2 naturally occurring amino acid independently selected from the group
3 consisting of alanine, arginine, asparagine, aspartic acid, citrulline, cysteine,
4 cystine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine,
5 ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine,
6 valine, proline, hydroxy proline, γ -carboxyglutamate, phenylglycine, or
7 O-phosphoserine.

1 6. A composition as defined in claim 1, wherein each X is a
2 non-naturally occurring amino acid independently selected from the group
3 consisting of β -alanine, α -amino butyric acid, γ -amino butyric acid, γ -
4 (aminophenyl) butyric acid, α -amino isobutyric acid, citrulline, ϵ -amino caproic
5 acid, 7-amino heptanoic acid, β -aspartic acid, aminobenzoic acid,
6 aminophenyl acetic acid, aminophenyl butyric acid, γ -glutamic acid, cysteine
7 (ACM), ϵ -lysine, ϵ -lysine (A-Fmoc), methionine sulfone, norleucine, norvaline,
8 ornithine, d-ornithine, p-nitro-phenylalanine, hydroxy proline, 1,2,3,4,-
9 tetrahydroisoquinoline-3-carboxylic acid, and thioproline.

1 7. A composition as defined in claim 1, wherein each X is an
2 amino acid independently selected from the group consisting of pyroglutamic,
3 glutamic, aspartic, α -alanine, γ -alanine, γ -leucine, lysine, α -phenylalanine, β -
4 phenylalanine, γ -phenylalanine, α -tyrosine, γ -tyrosine, tryptophan, proline,
5 and γ -valine.

1 8. A composition as defined in claim 1, wherein said active
2 agent is selected from the group consisting of a biologically active agent, a
3 chemically active agent, or a combination thereof.

1 9. A composition as defined in claim 8, wherein said
2 biologically active agent comprises at least one peptide, mucopolysaccharide,
3 carbohydrate, or lipid.

1 10. A composition as defined in claim 8, wherein said
2 biologically active agent is selected from the group consisting of human
3 growth hormone, bovine growth hormone, growth hormone-releasing
4 hormone, an interferon, interleukin-1, insulin, heparin, low molecular weight
5 heparin, calcitonin, erythropoietin, atrial natriuretic factor, an antigen, a
6 monoclonal antibody, somatostatin, adrenocorticotropin, gonadotropin
7 releasing hormone, oxytocin, vasopressin, cromolyn sodium, vancomycin,
8 desferrioxamine (DFO), antimicrobials, anti-fungal agents, or any combination
9 thereof.

1 11. A composition as defined in claim 8, wherein said
2 biologically active agent comprises heparin.

1 12. A composition as defined in claim 8, wherein said
2 biologically active agent comprises low molecular weight heparin.

1 13. A composition as defined in claim 1, further comprising a
2 stabilizing additive.

1 14. A composition as defined in claim 13, wherein said
2 stabilizing additive is selected from the group consisting of buffer salts, gum
3 acacia, gelatin, methyl cellulose, polyethylene glycol, polypropylene glycol,
4 and polylysine.

1 15. A composition as defined in claim 14, wherein said
2 stabilizing additive is selected from the group consisting of gum acacia,
3 gelatin, and methyl cellulose.

- 1 16. A dosage unit form comprising
2 (A) a composition as defined in claim 1; and
3 (B) (a) an excipient
4 (b) a diluent,
5 (c) a disintegrant,
6 (d) a lubricant,
7 (e) a plasticizer,
8 (f) a colorant,
9 (g) a dosing vehicle, or
10 (h) any combination thereof.

1 17. A dosage unit form as defined in claim 16, comprising a
2 tablet, a capsule, or a liquid.

1 18. A composition as defined in claim 1, further comprising an

1 enzyme inhibitor.

2

1

19. A composition as defined in claim 18, wherein said enzyme inhibitor comprises actinonin or derivatives thereof, epiactinonin or derivatives thereof, aprotinin (TRASYLOL), or Bowman-Birk inhibitor.

2

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4

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20. A method for administering an active agent to an animal in need of said agent, said method comprising administering orally to said animal a composition as defined in claim 1.

2

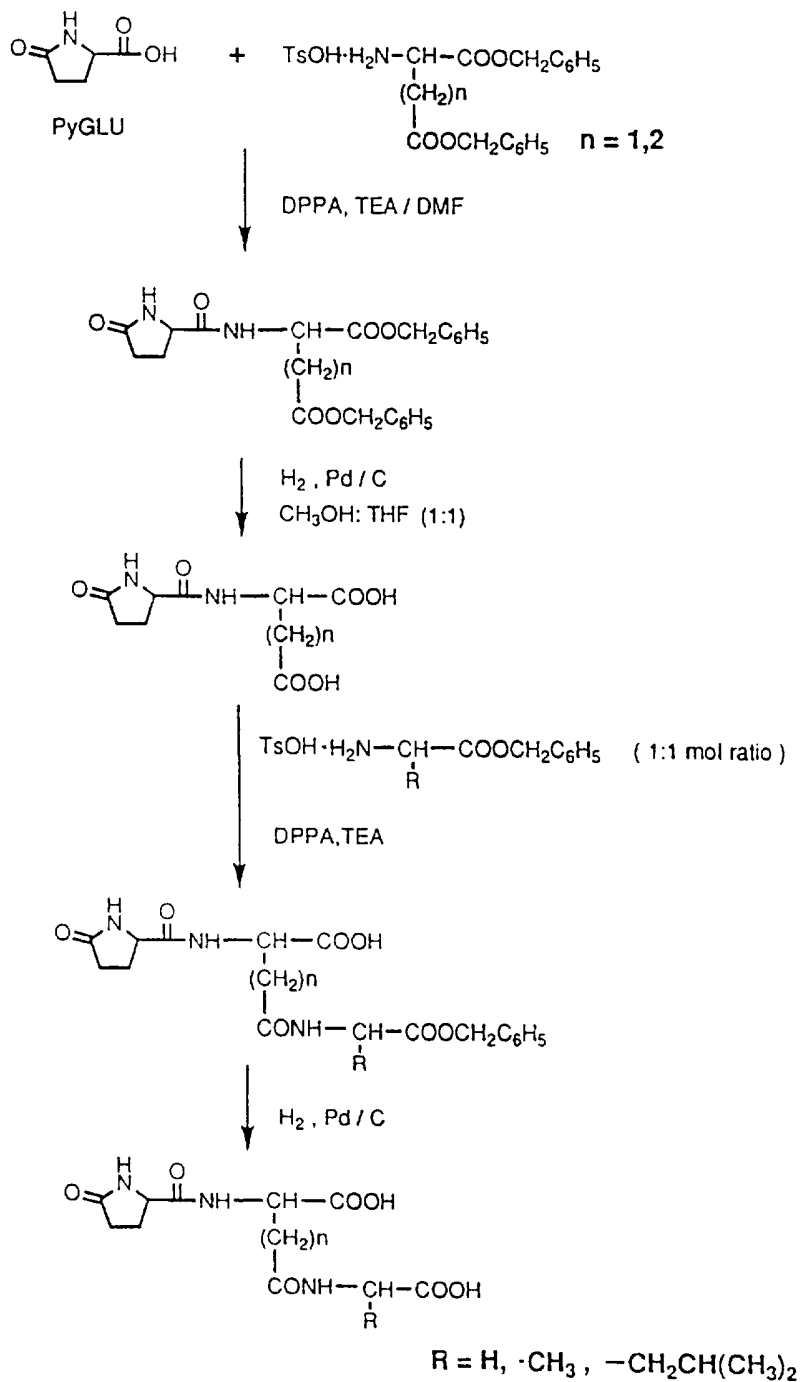
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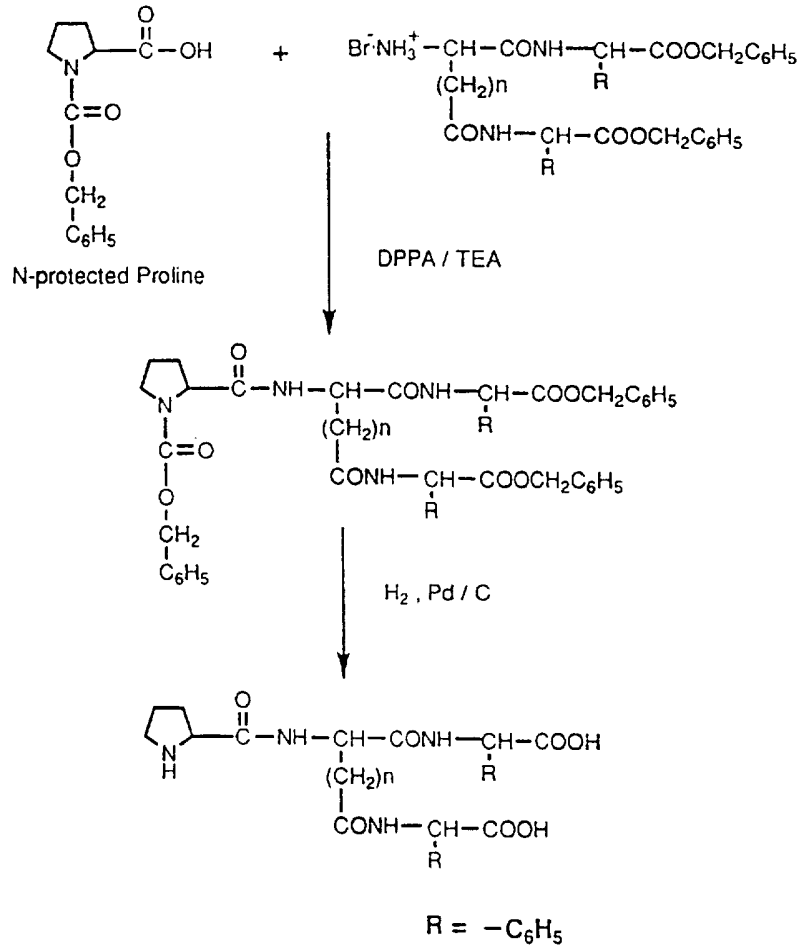
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1/101
Figure 1



4/10
 Figure 4



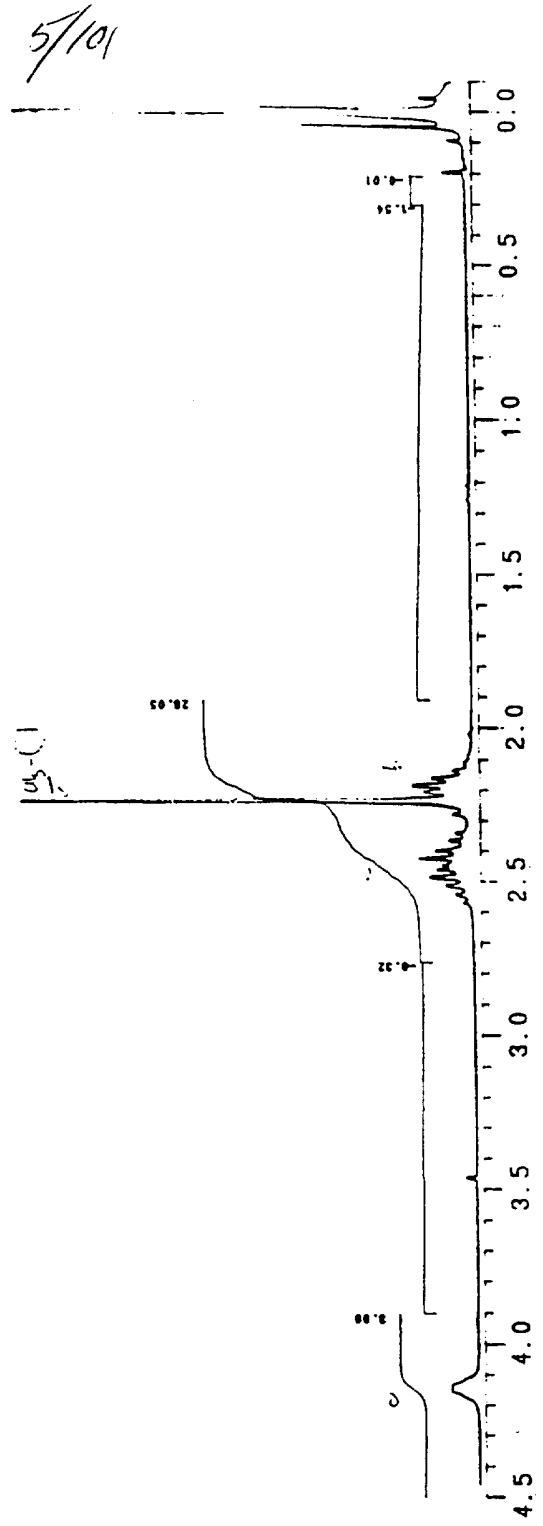
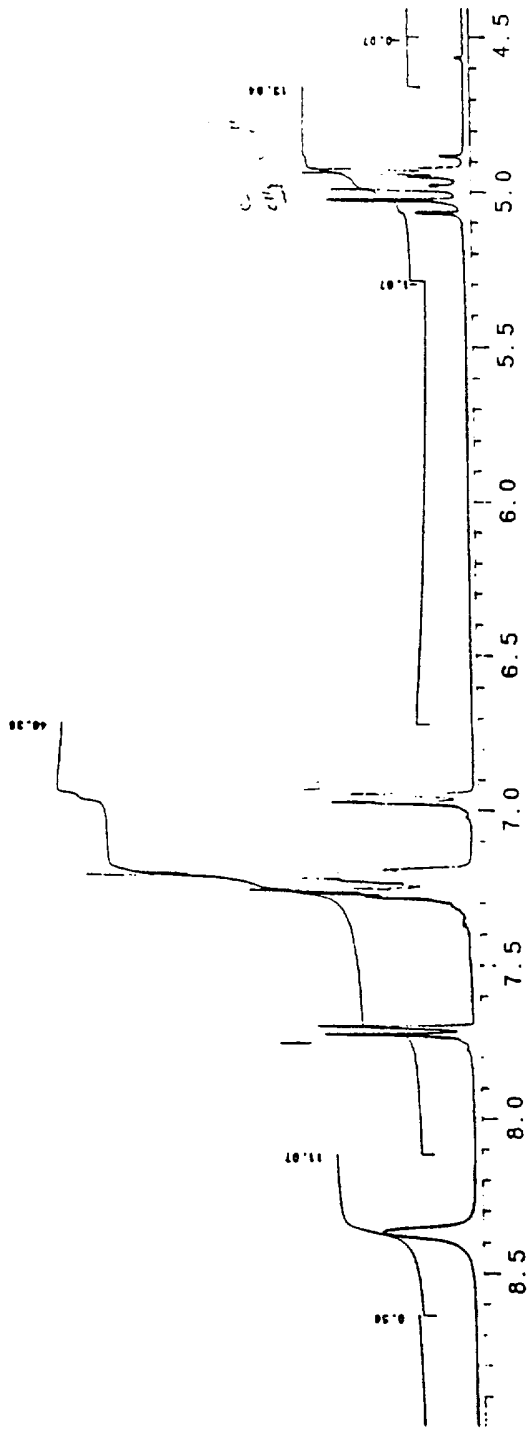


Fig 5 1H NMR dibzglutsoh

6/101

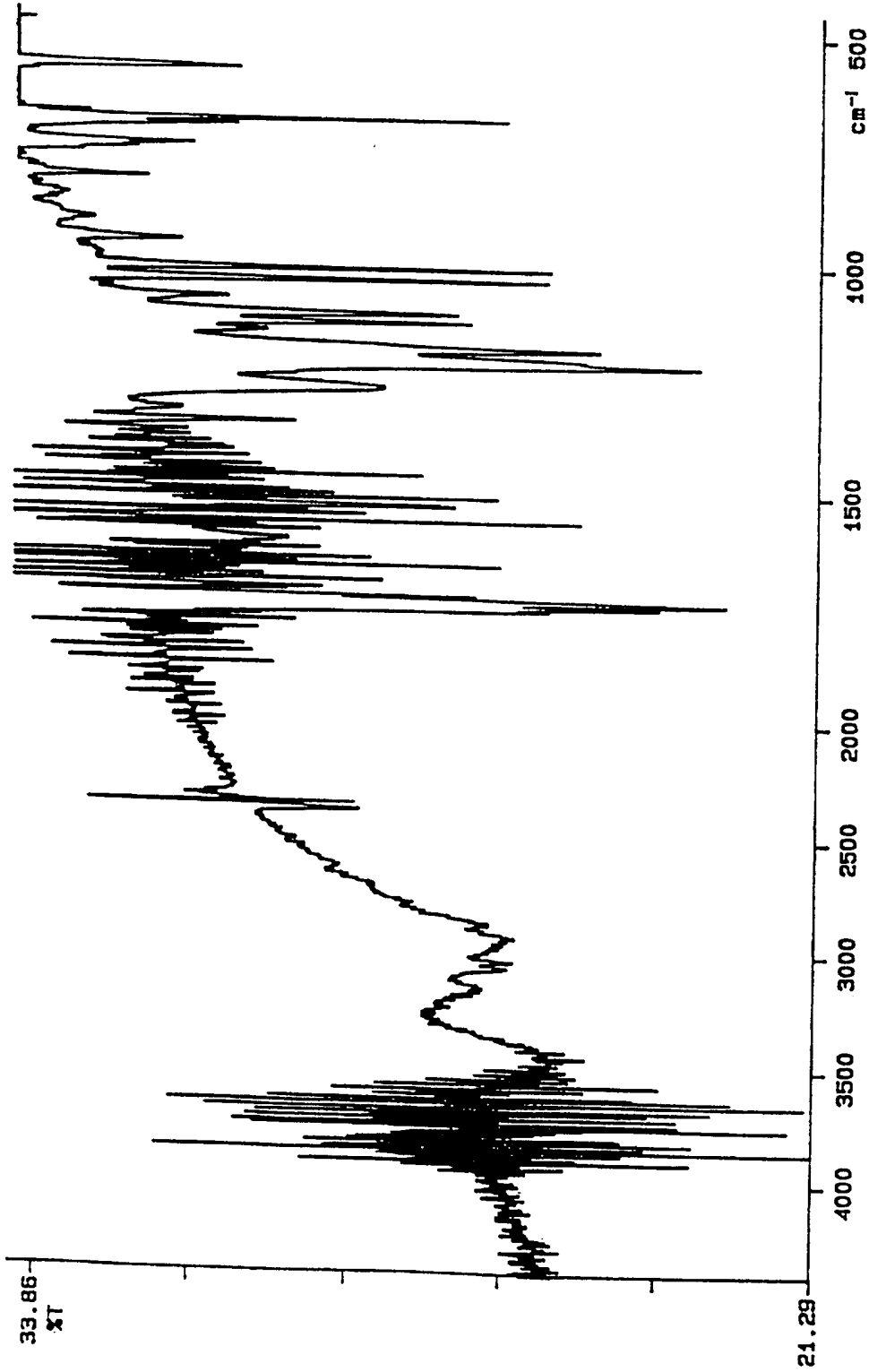


Fig 6 IR DIBzGLUTSOH

7/101

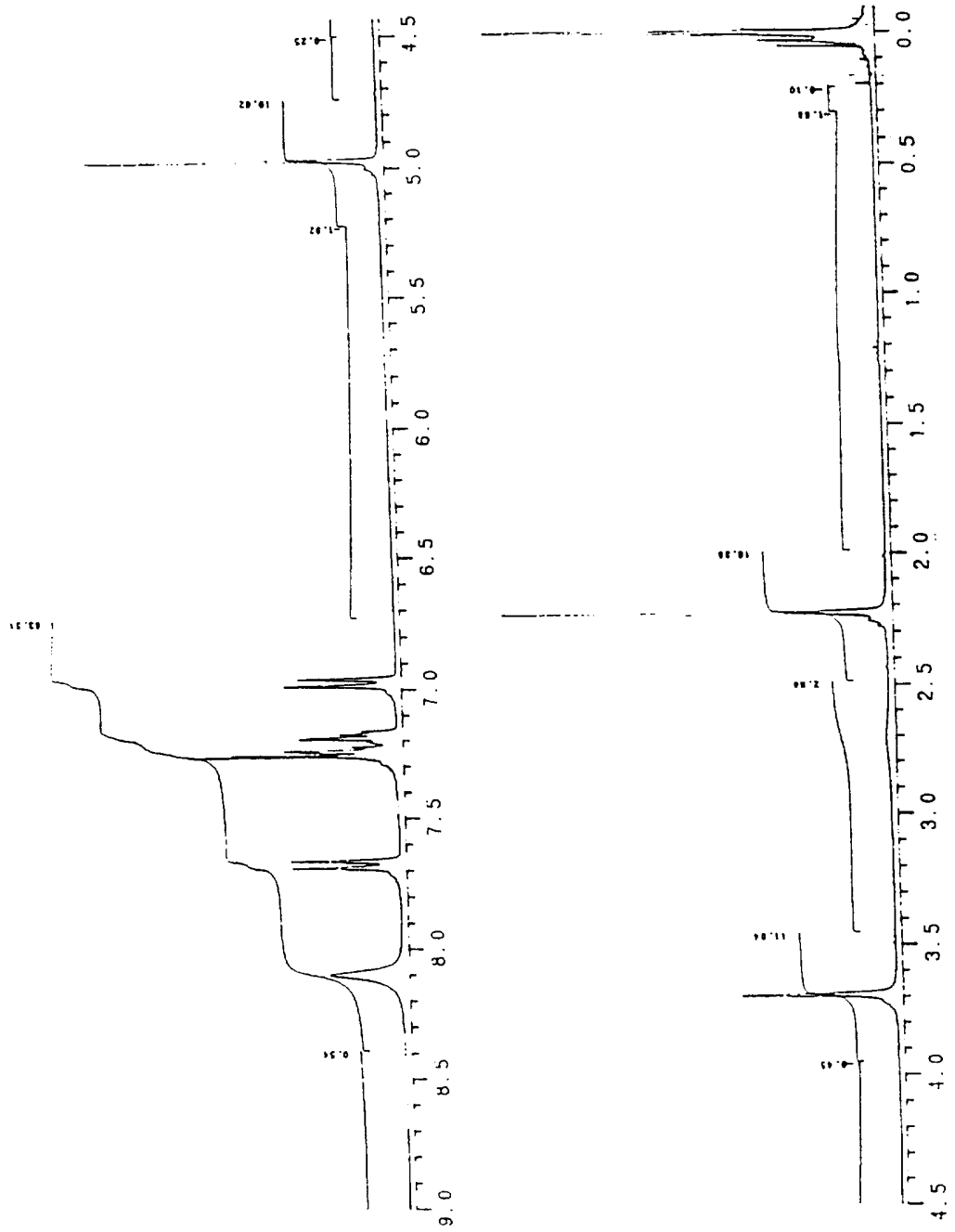


Figure 7 ¹H-NMR, TsOH-GluBz

8/101

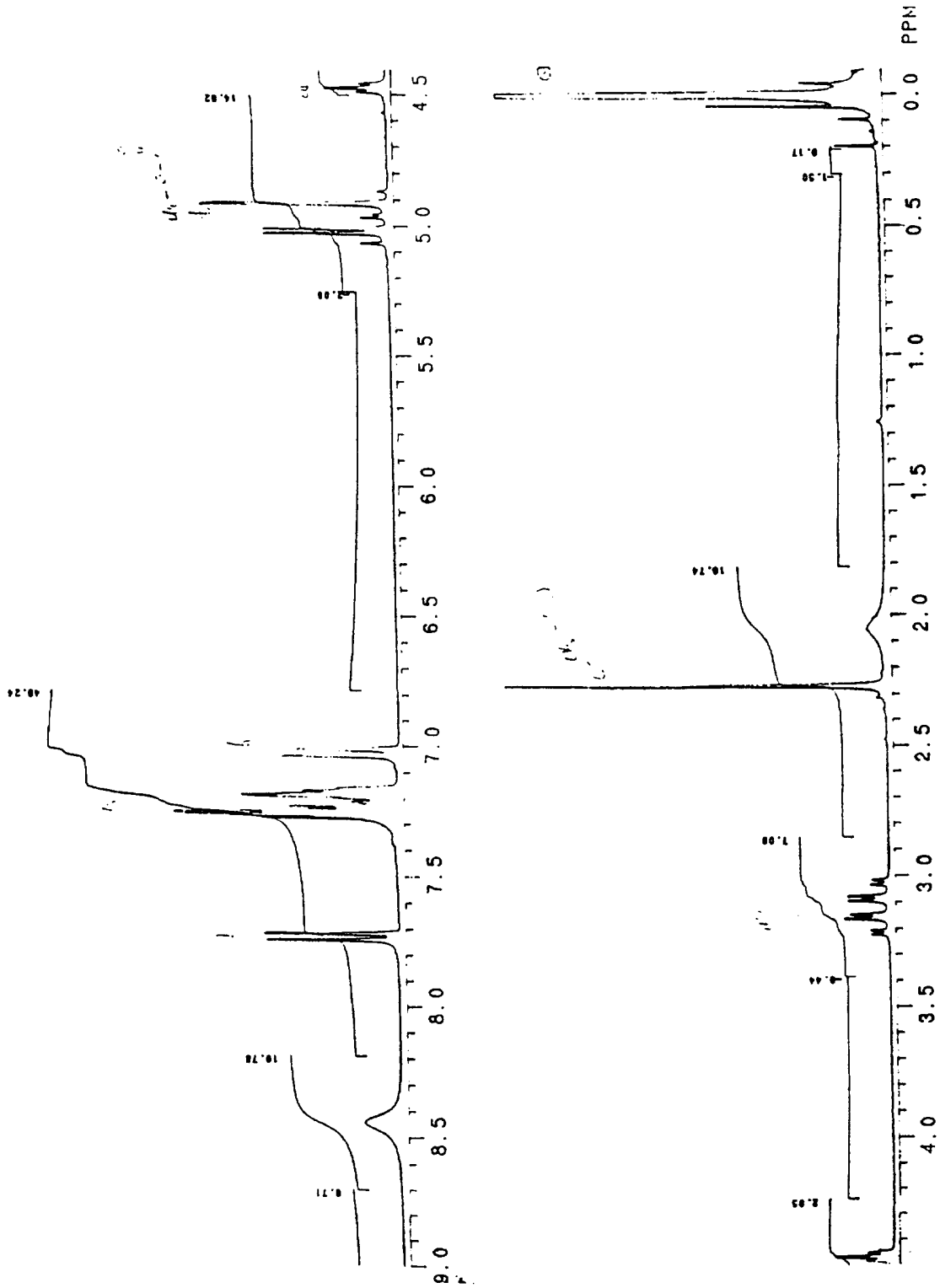


Fig 8 ¹H NMR dibzabptsoh

9/10/01

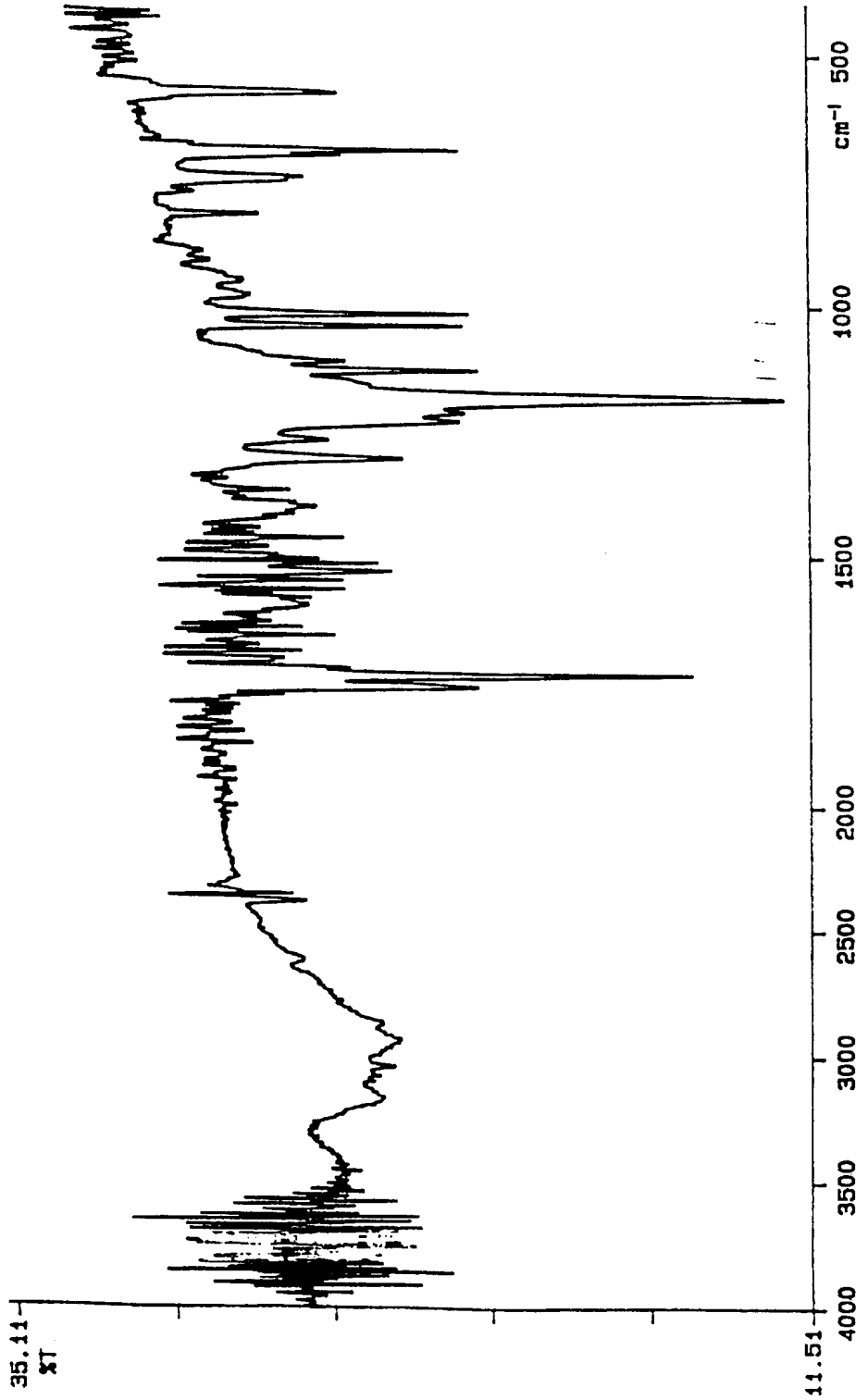


Fig 9 IR DIBZASPTSOH

10/10/01

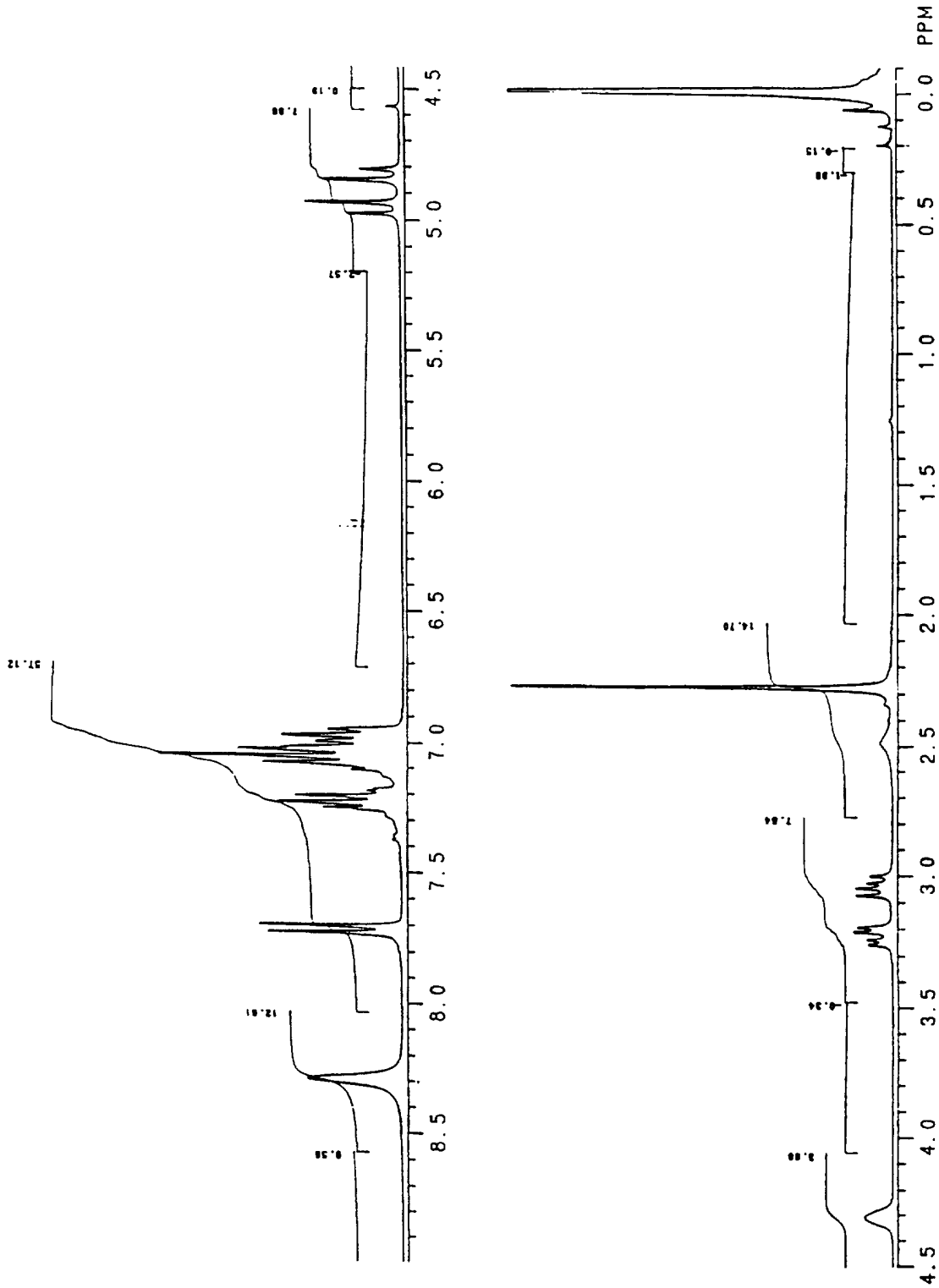


Fig 10 ¹H NMR BzPheTsoH

11/101

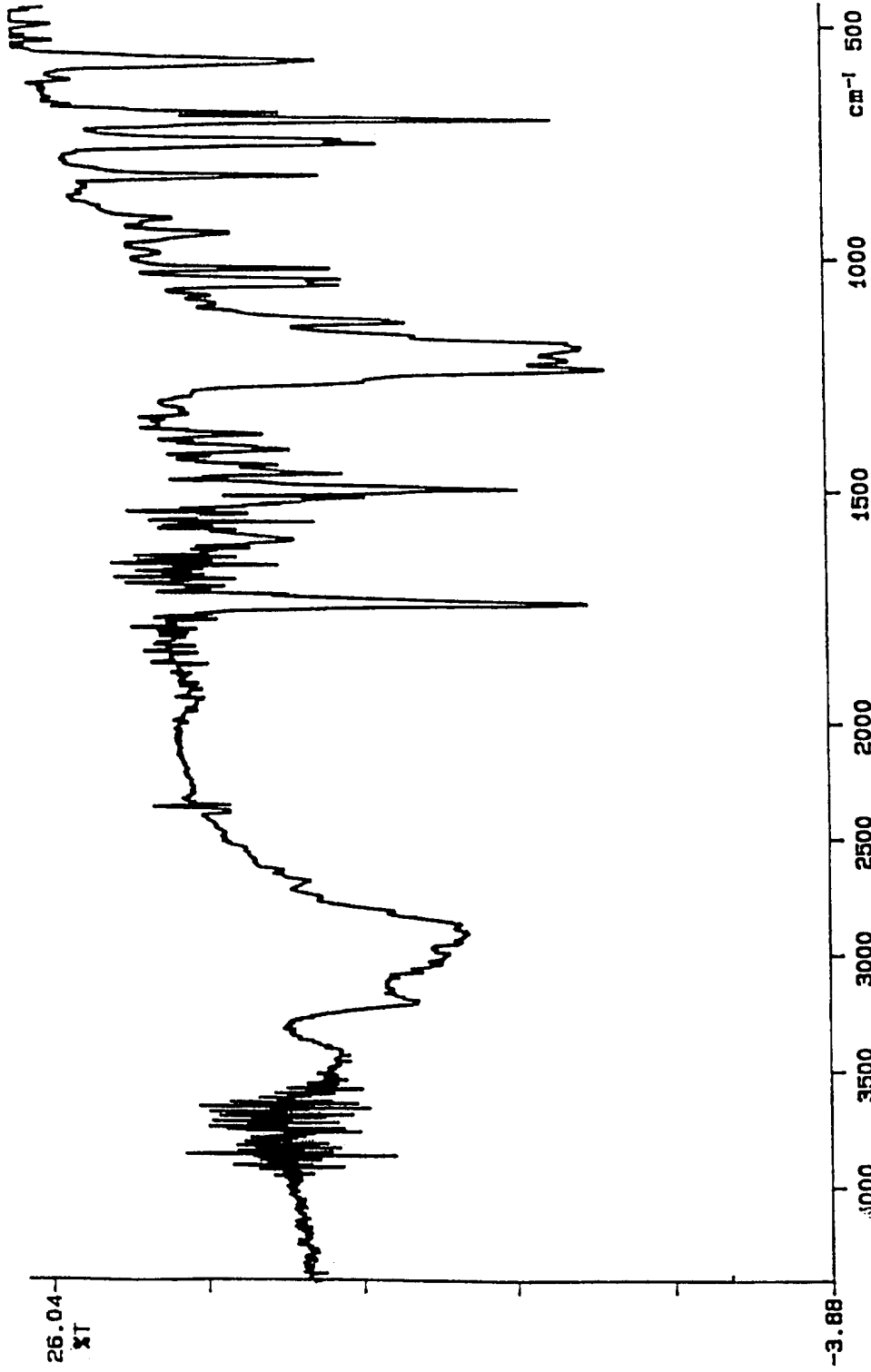
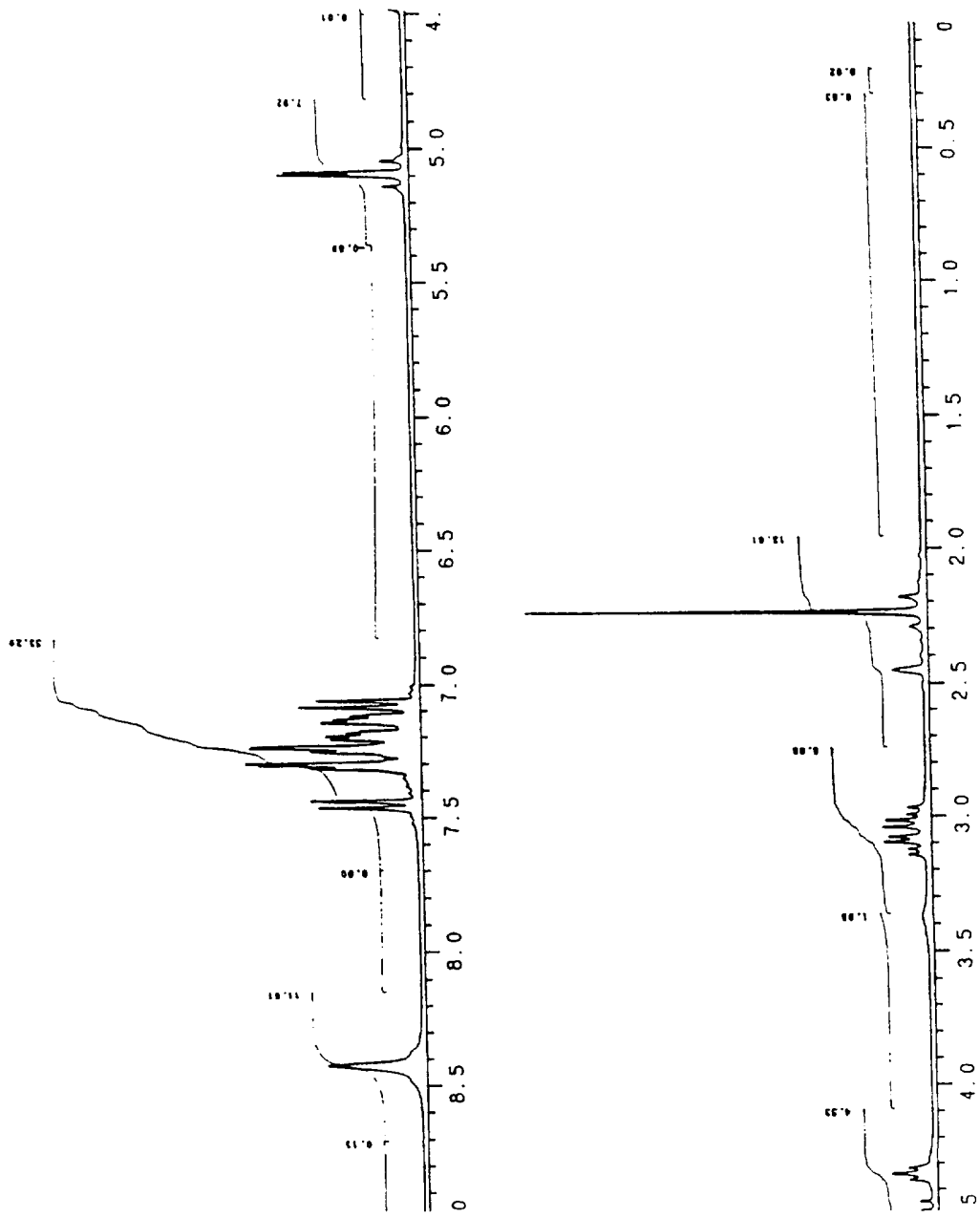


Fig 11 IR BzPheTsoH

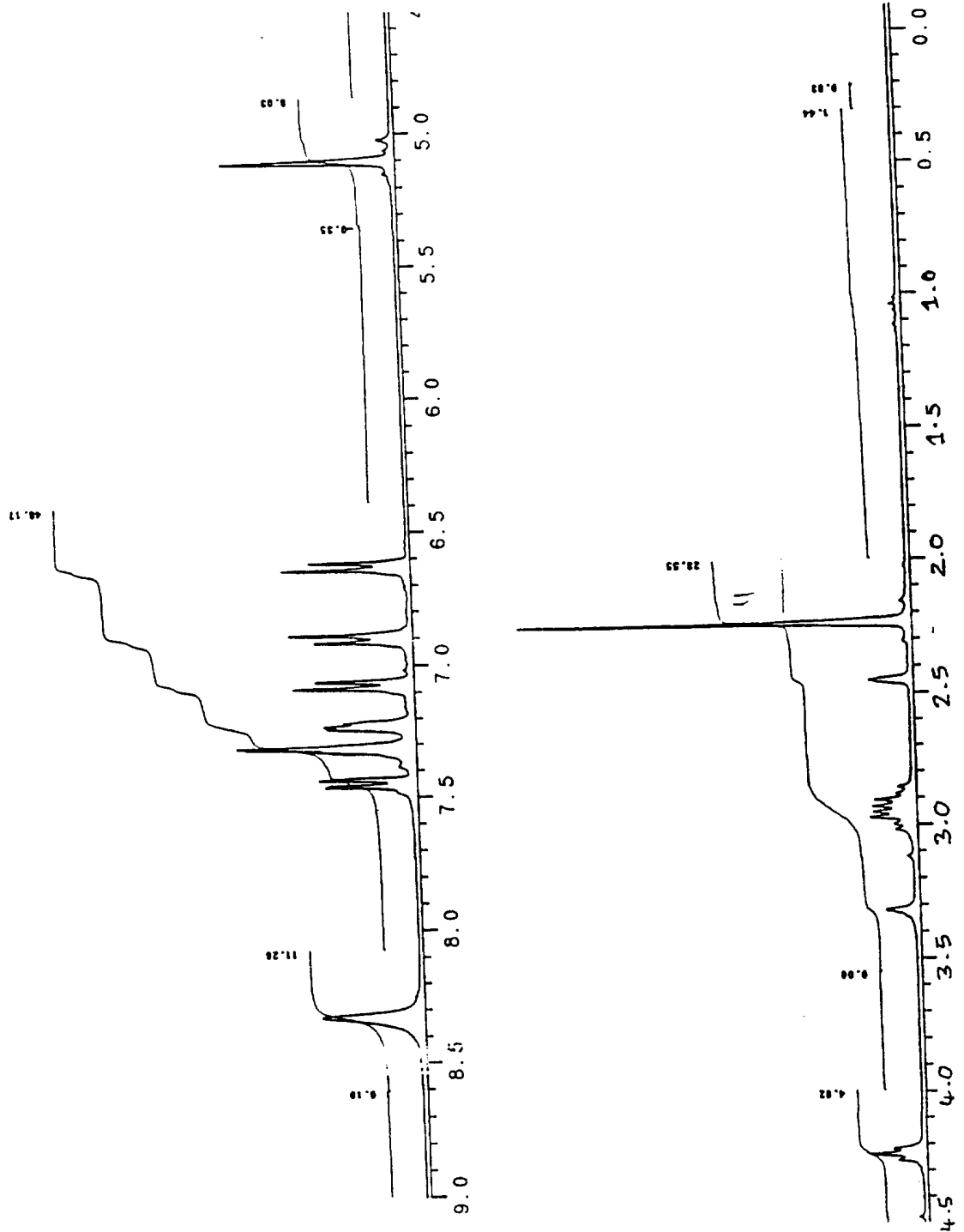
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Figure 12. ¹H-NMR, TsOH-D-PheBz



13/101

Fig 13 ¹H NMR BPTTTSOH



14/101

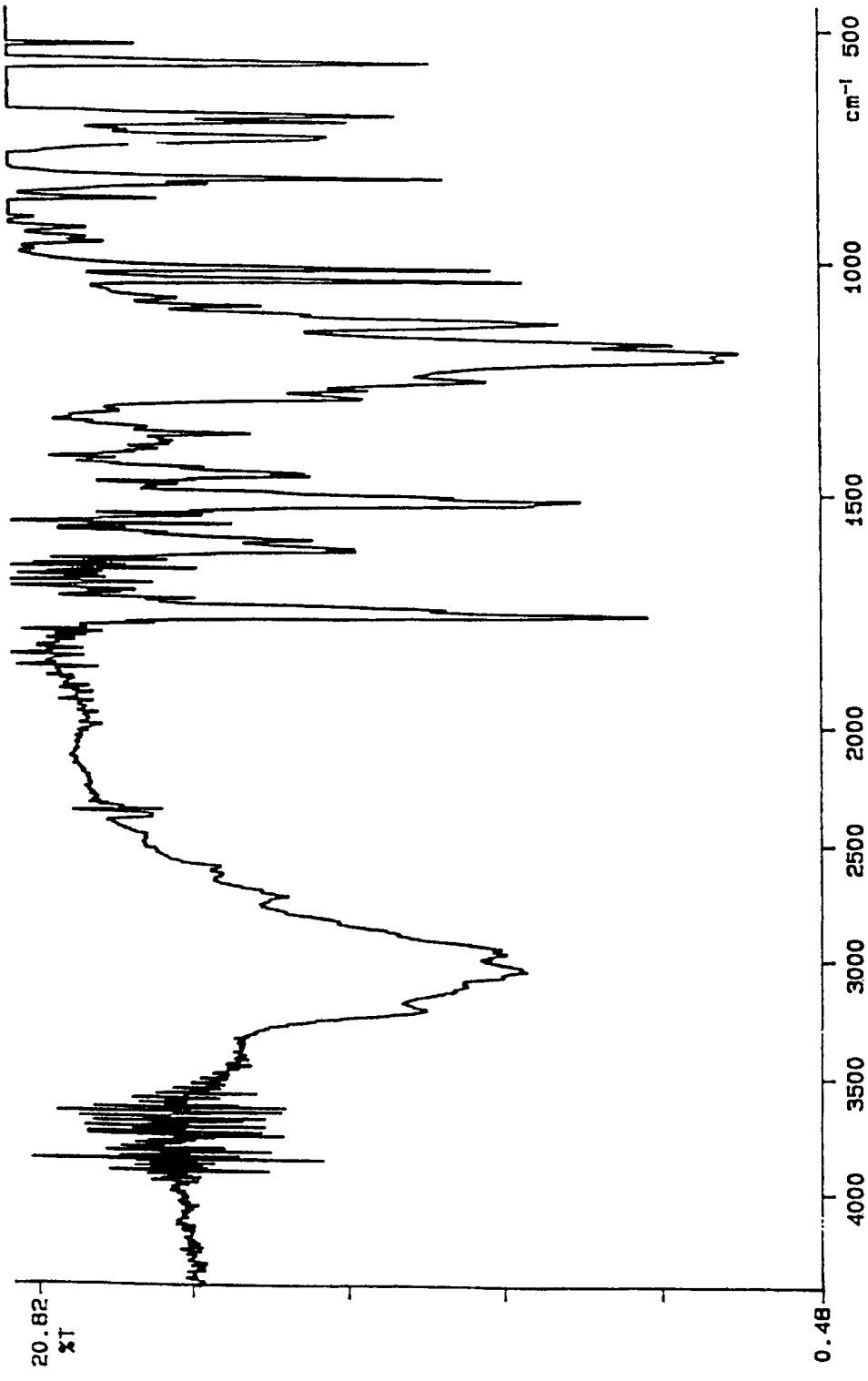


Fig 14 IR BzTYTsoH

15/101

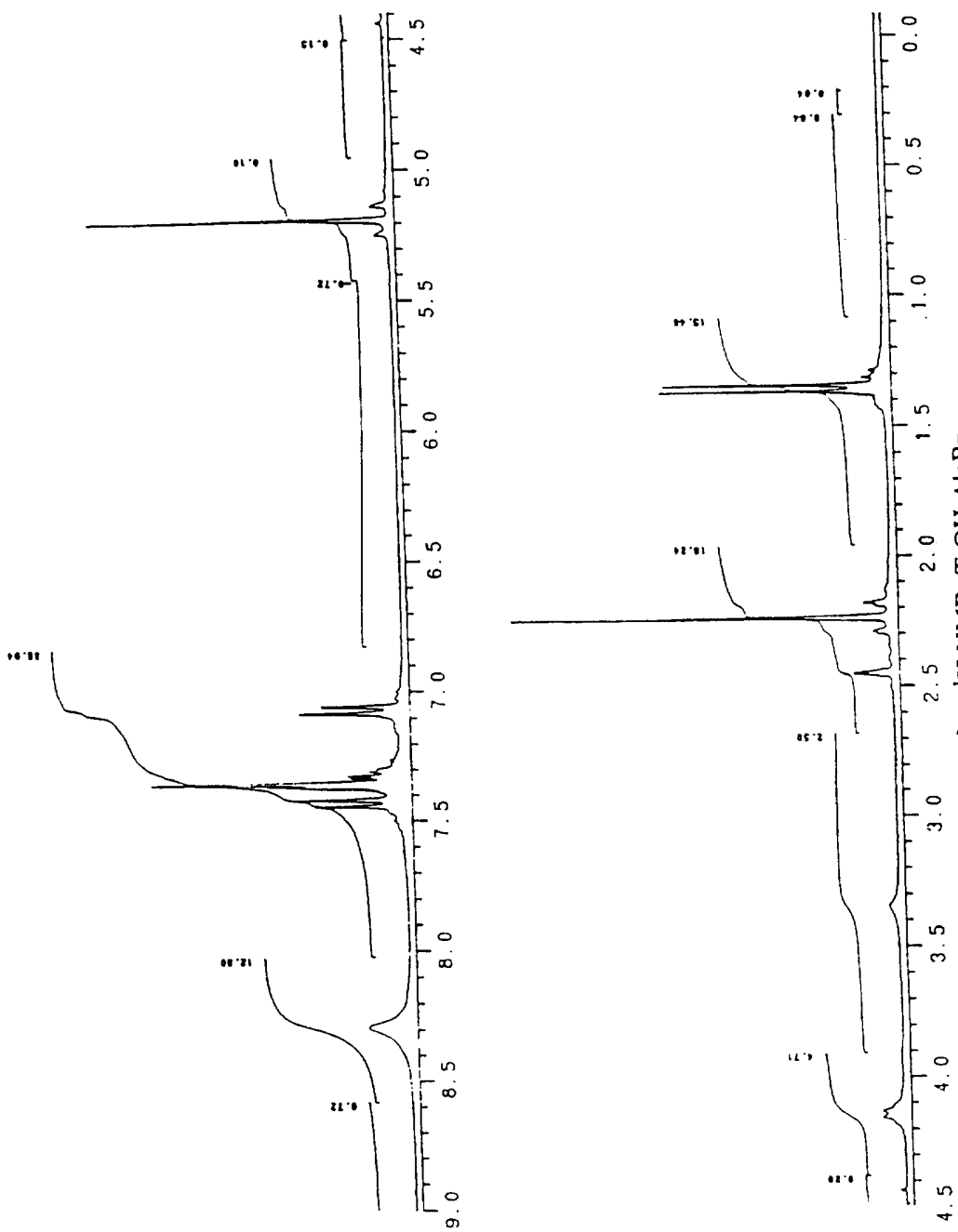


Figure 15 ¹H-NMR, TsOH-AlaBz

16/101

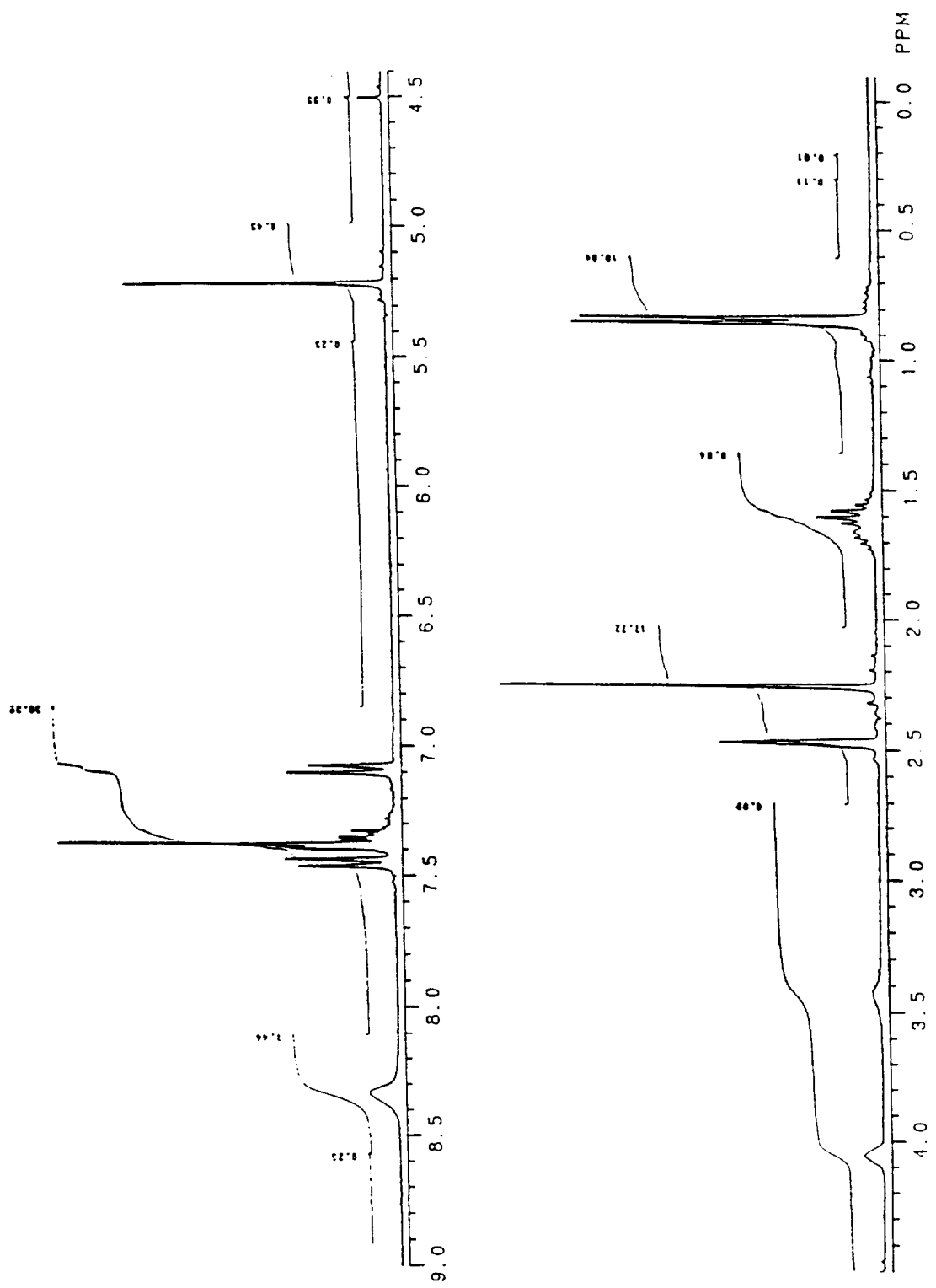


Figure 16 ¹H-NMR. L-leucine benzyl ester of p-toluenesulfonate (TsOH-L-LeuBz)

18/101

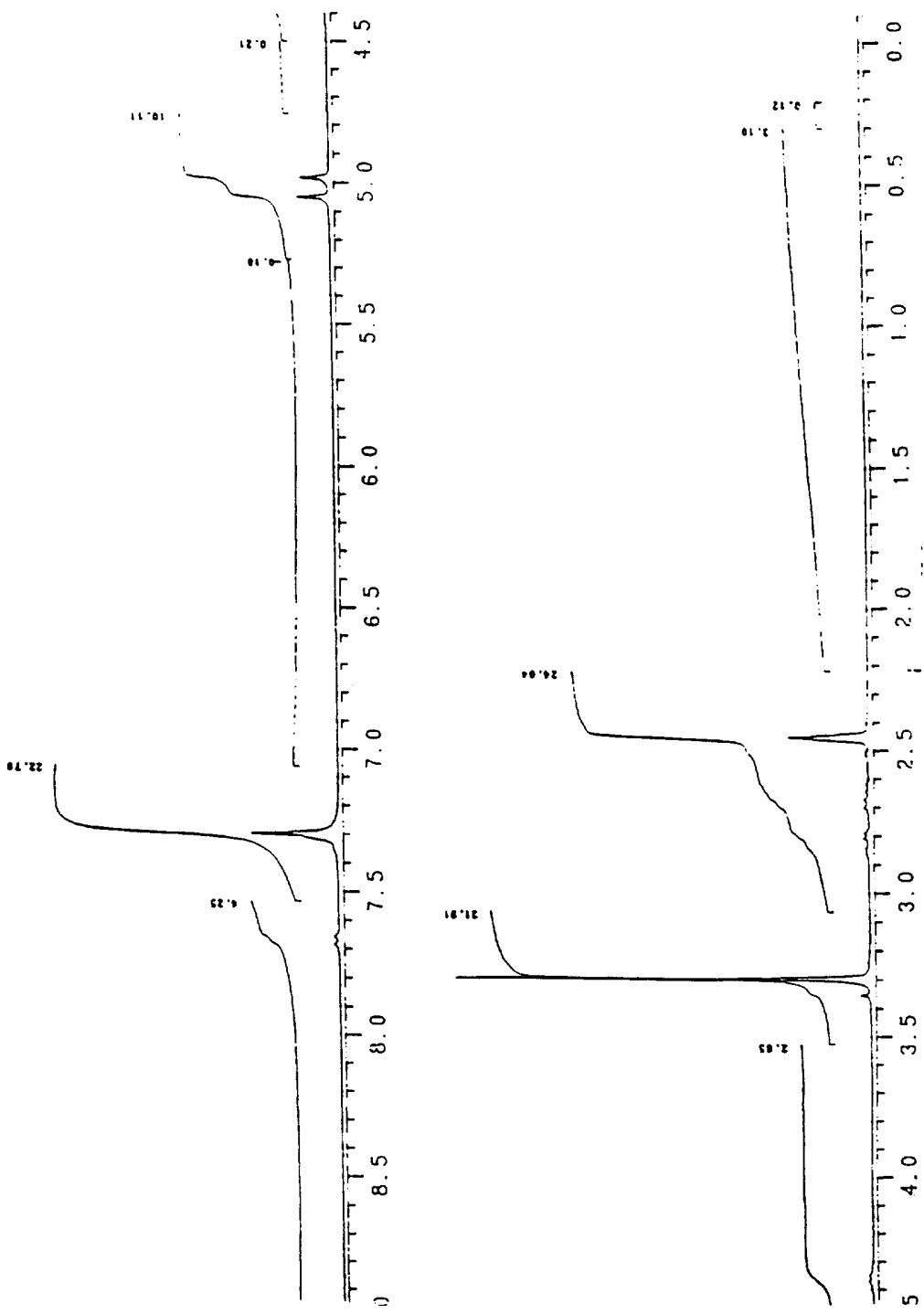


Fig 18 ¹H NMR BZCO-NHASP-β Bz

19/101

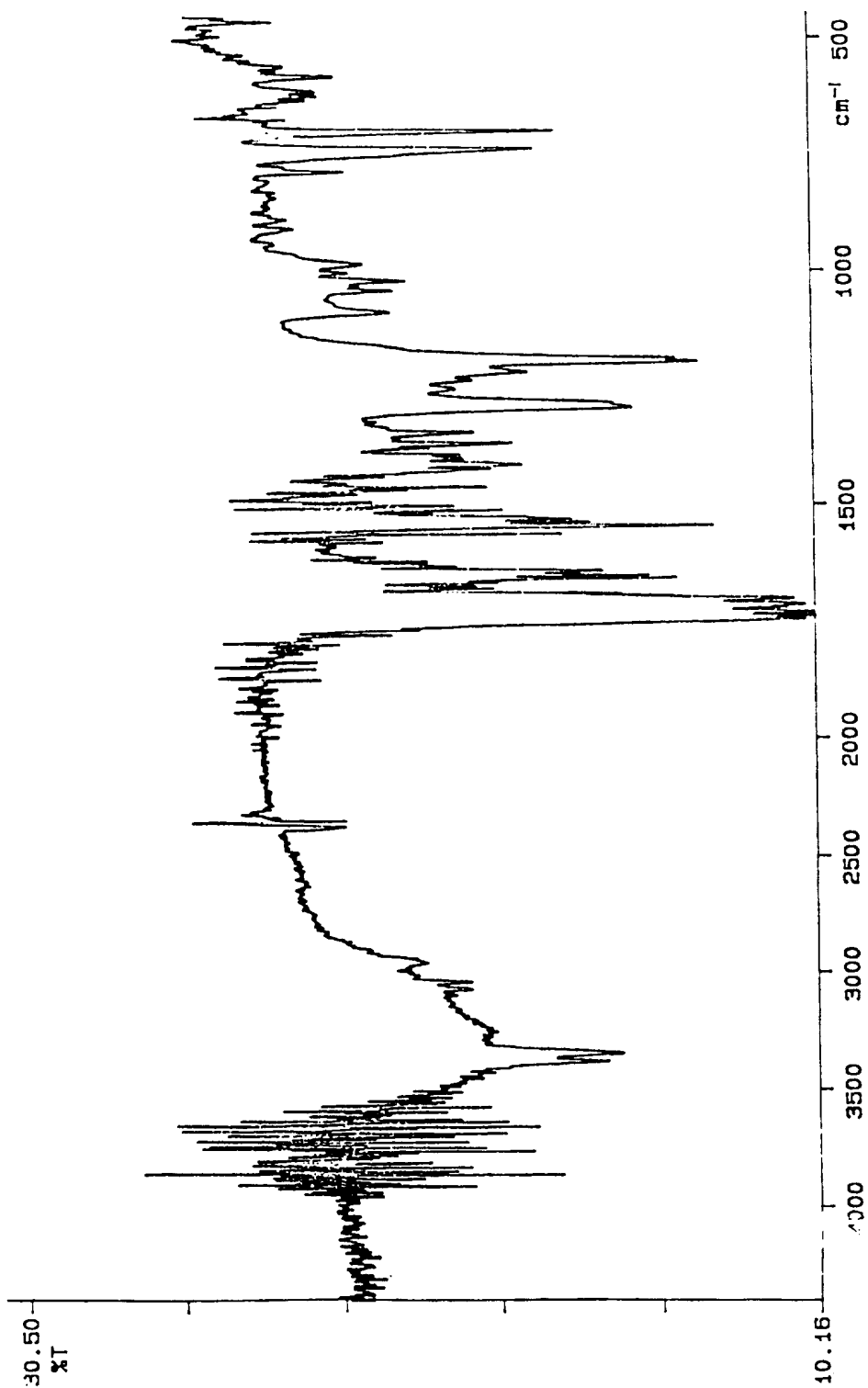


Fig 19 IR BzOCO-NHAsp-β Bz

20/101

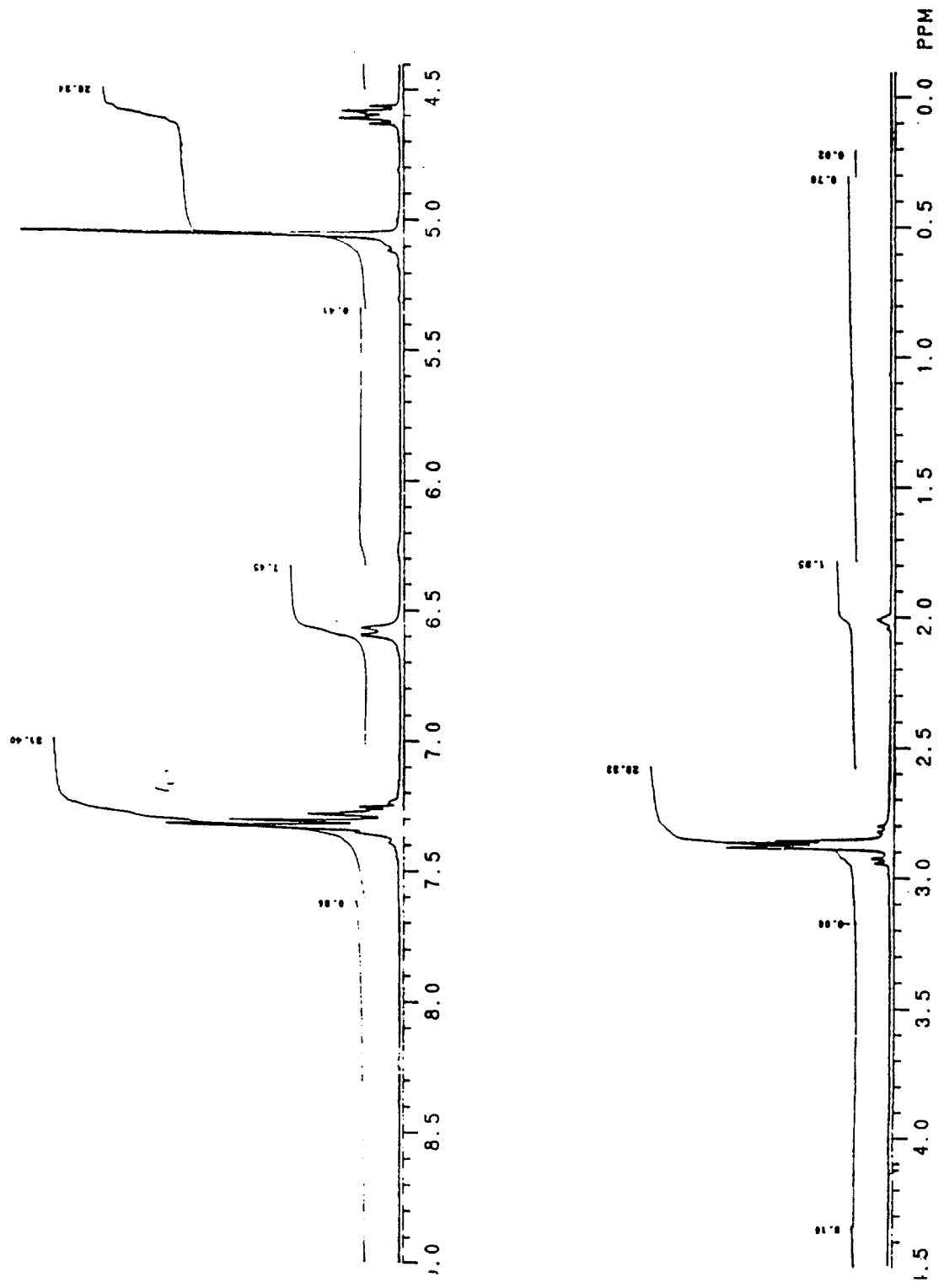


Fig 20 ¹H NMR BzOCO-NHASP

21/101

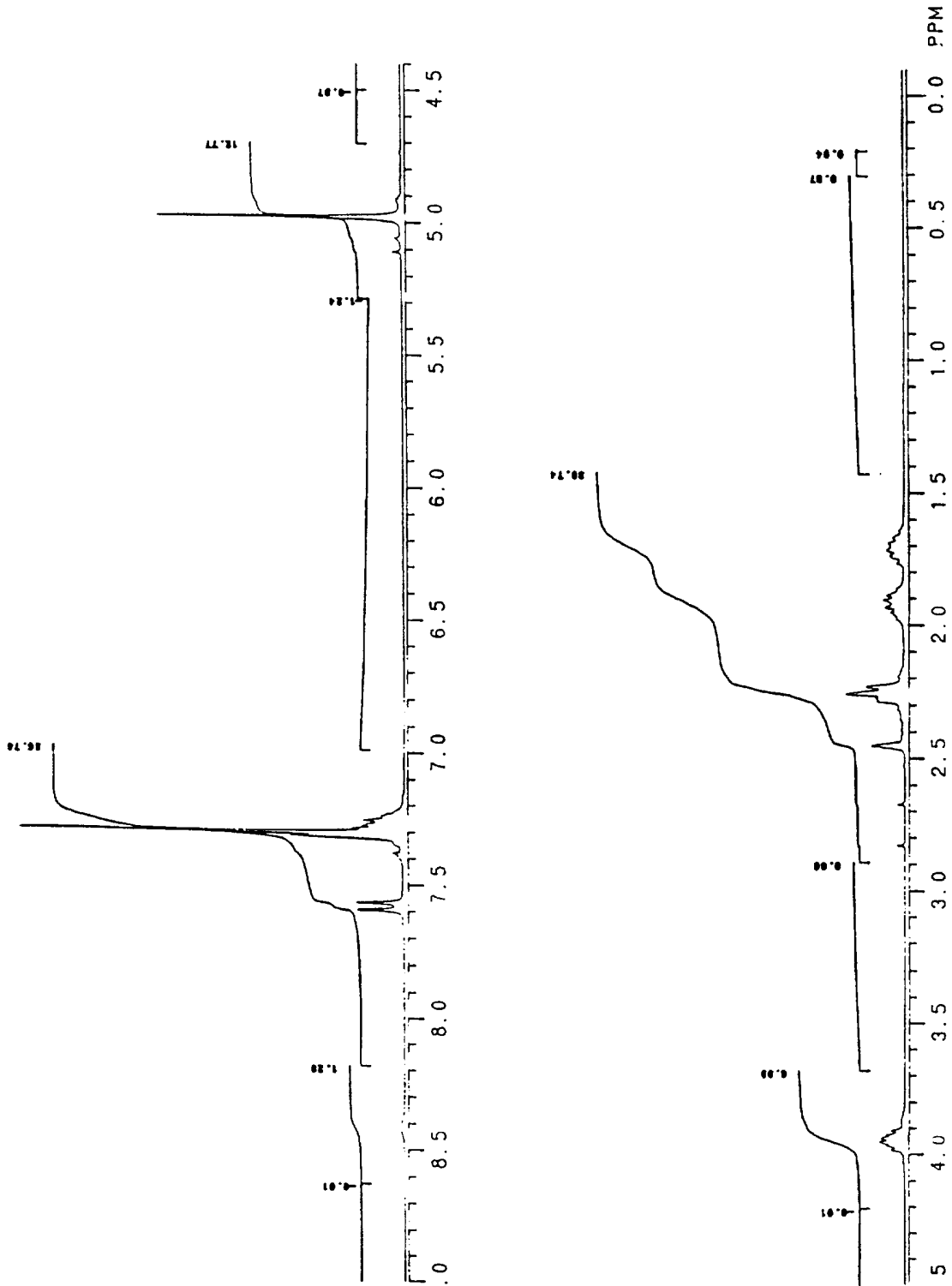


Fig 21 ¹H NMR BocO-NH-Glu

10/1/01

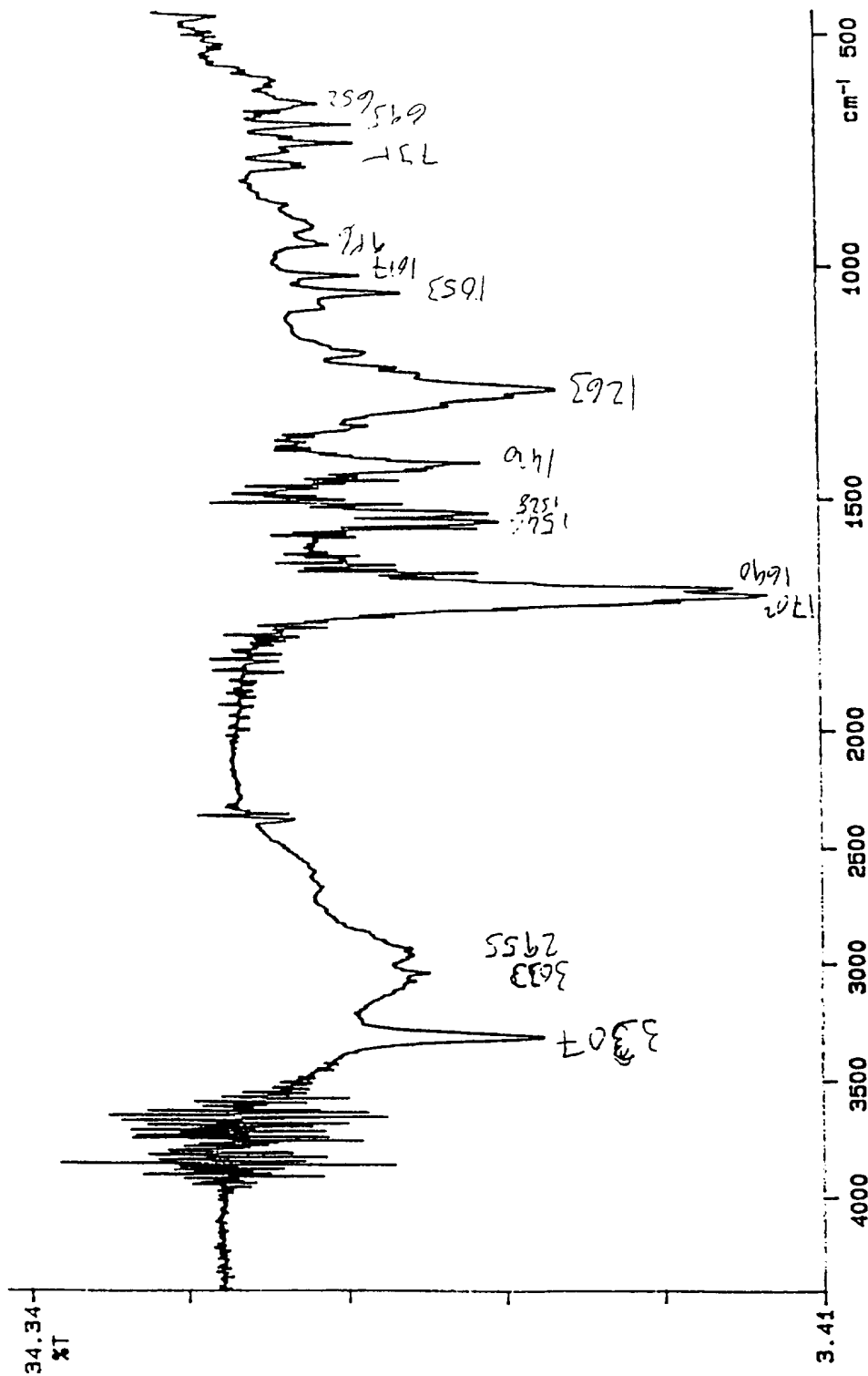


Fig 22 IR Bzoco-NHglu

24/101

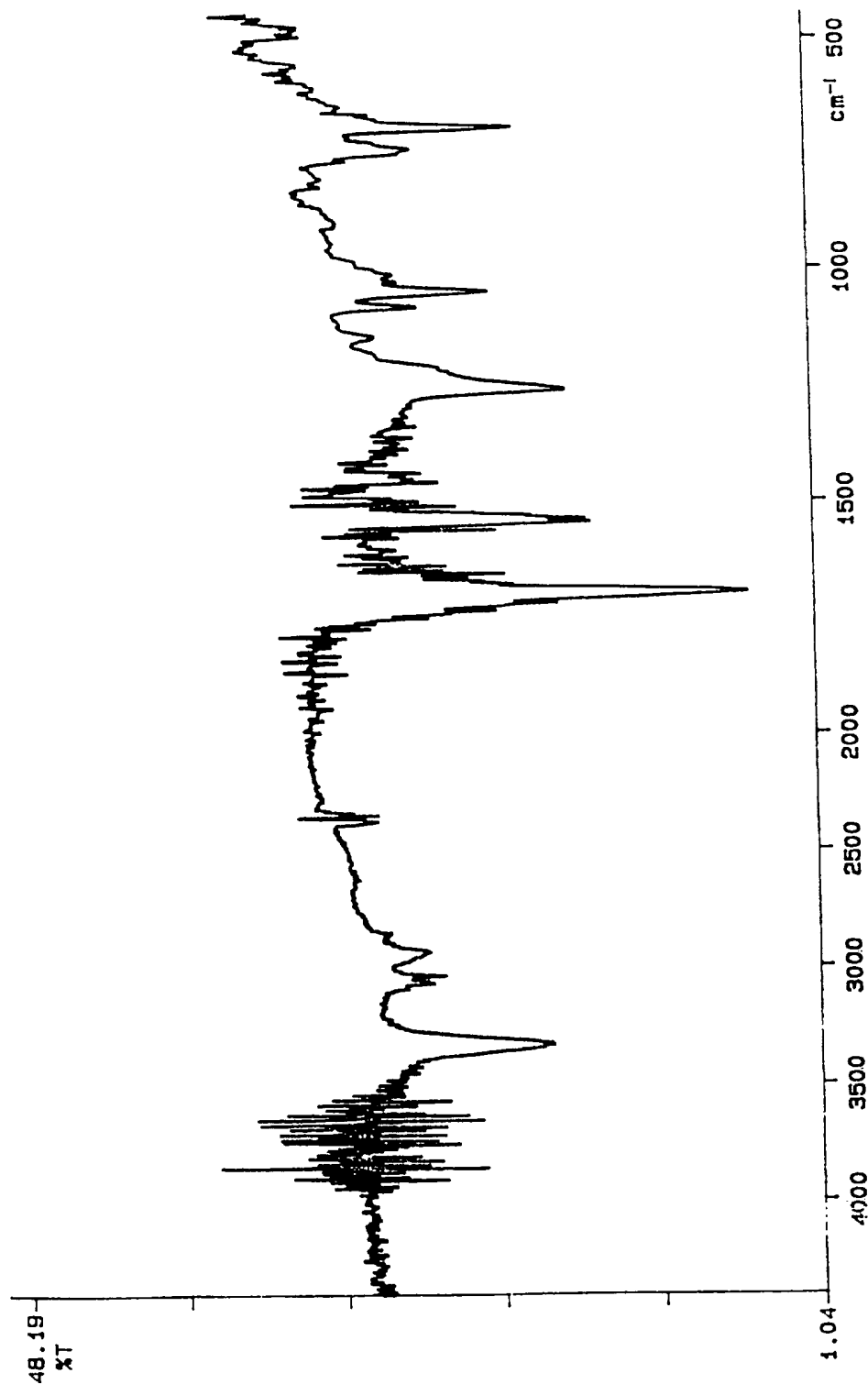


Fig 24 IR BZOCONH-Phe

25/10/

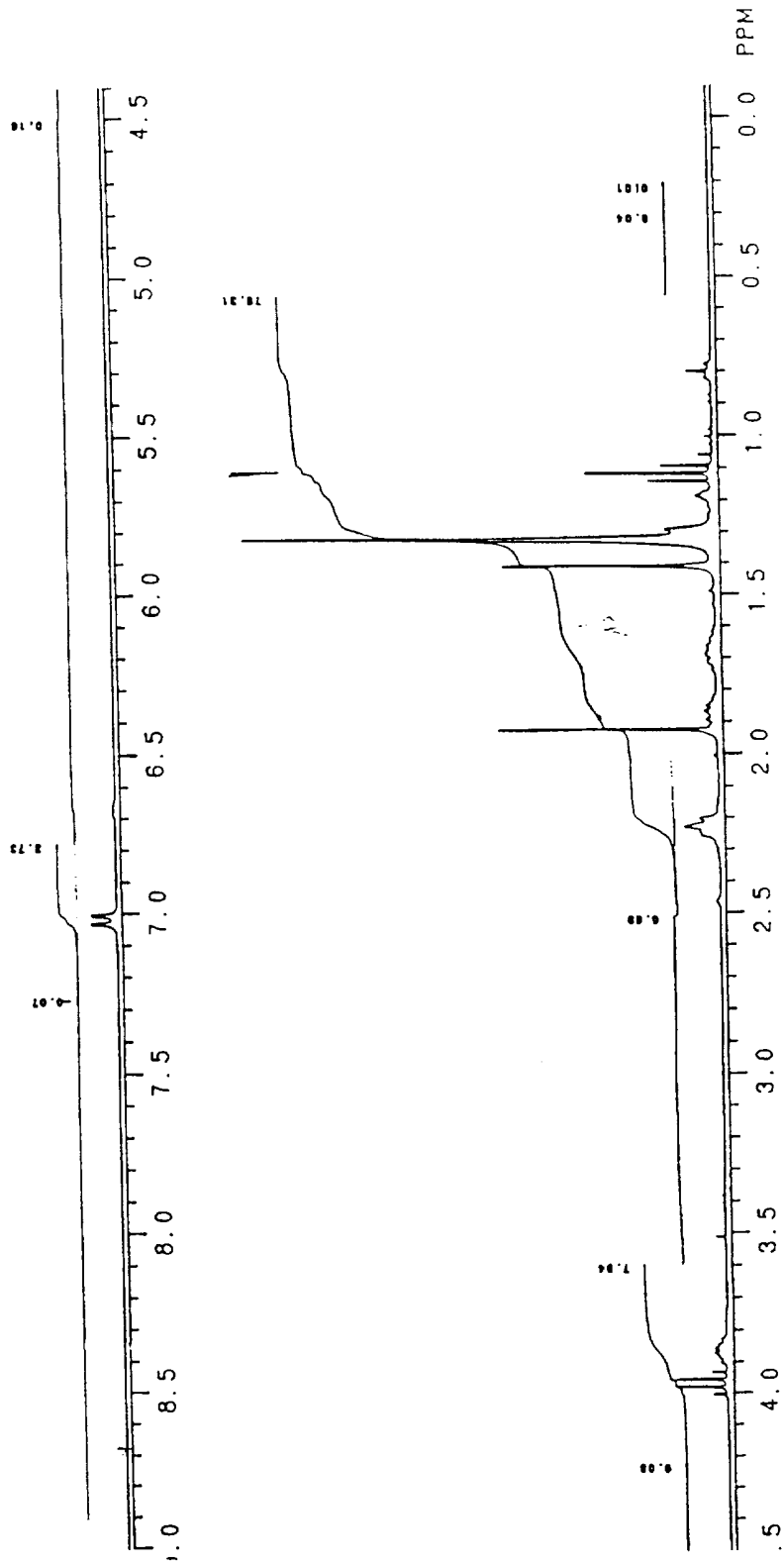


Figure 205 ¹H-NMR, N-tert-butyloxycarbonyl glutamic acid (t-Boc-L-Glu)

26/101

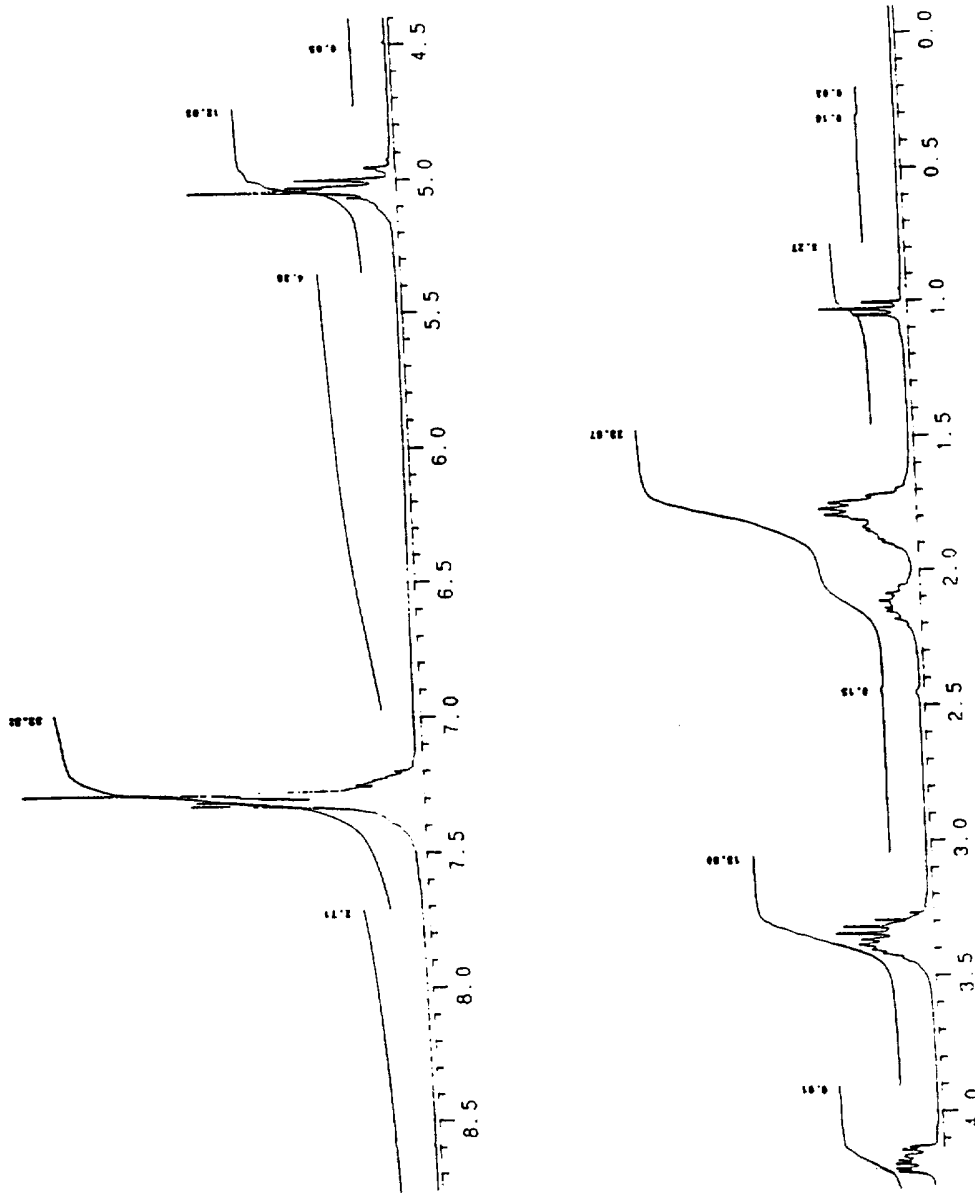


Figure 26. ¹H-NMR, β-L-proline,

27/10/

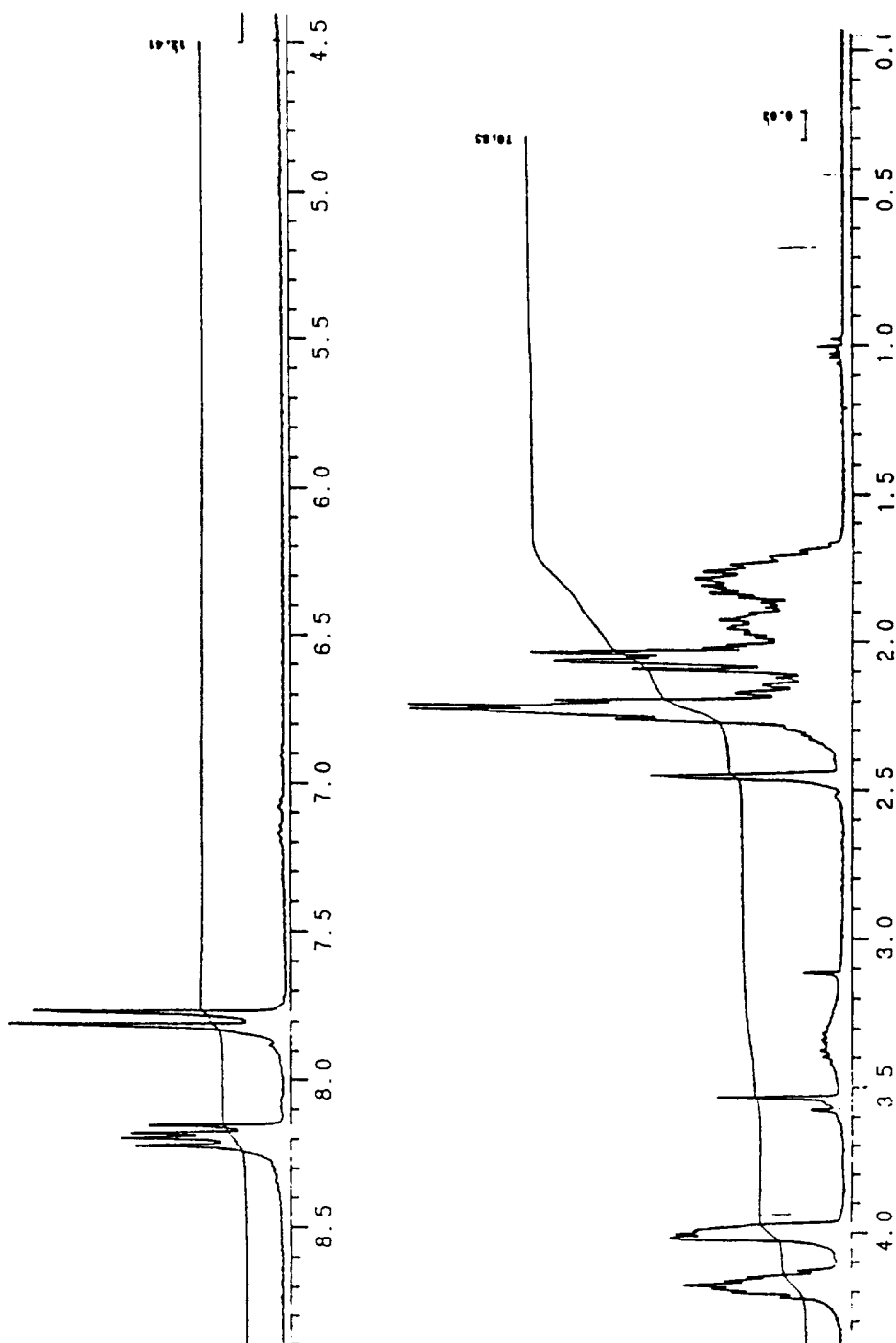


Fig. 27 ¹H NMR PyGluGlu

28/10/

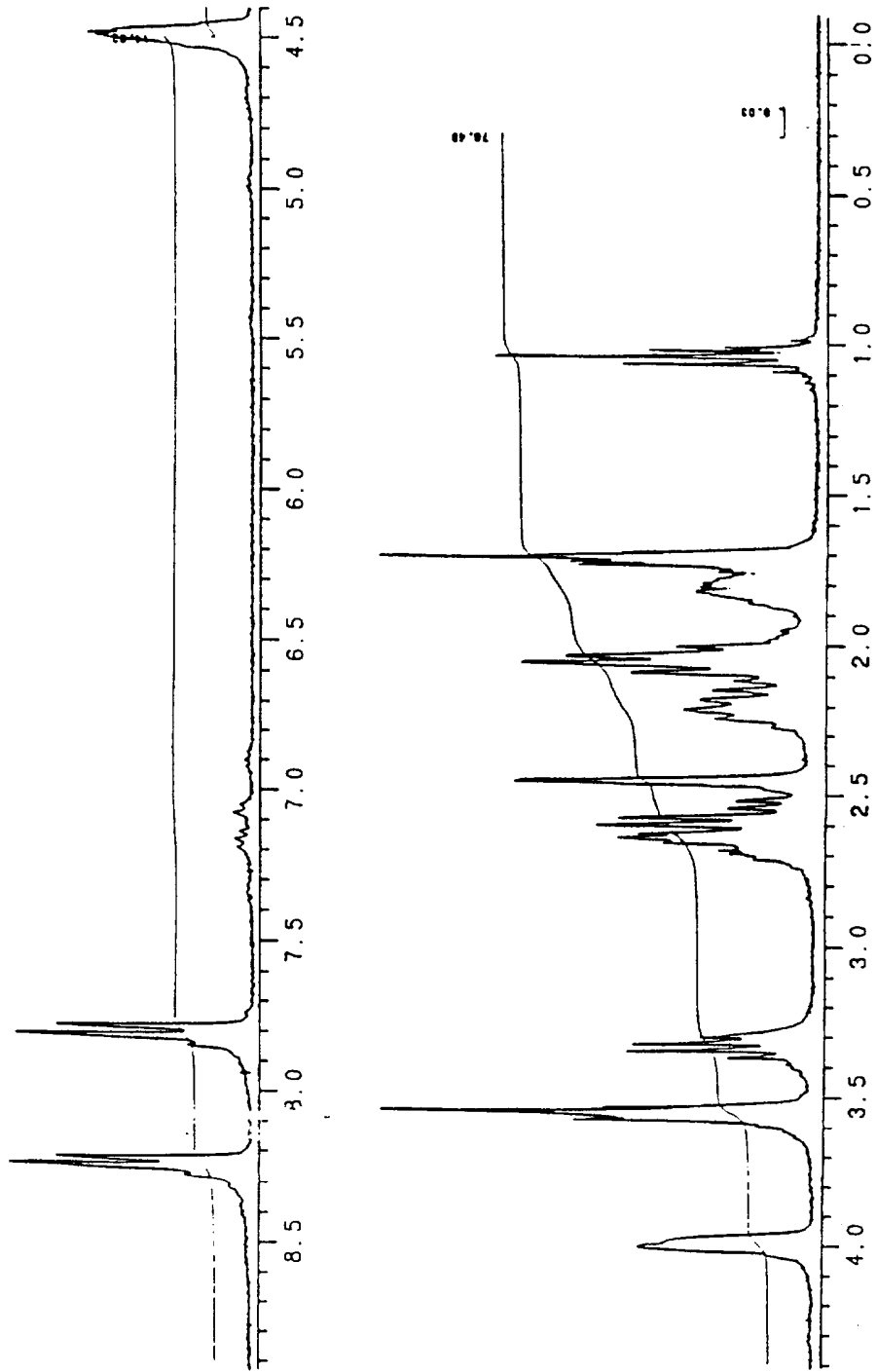
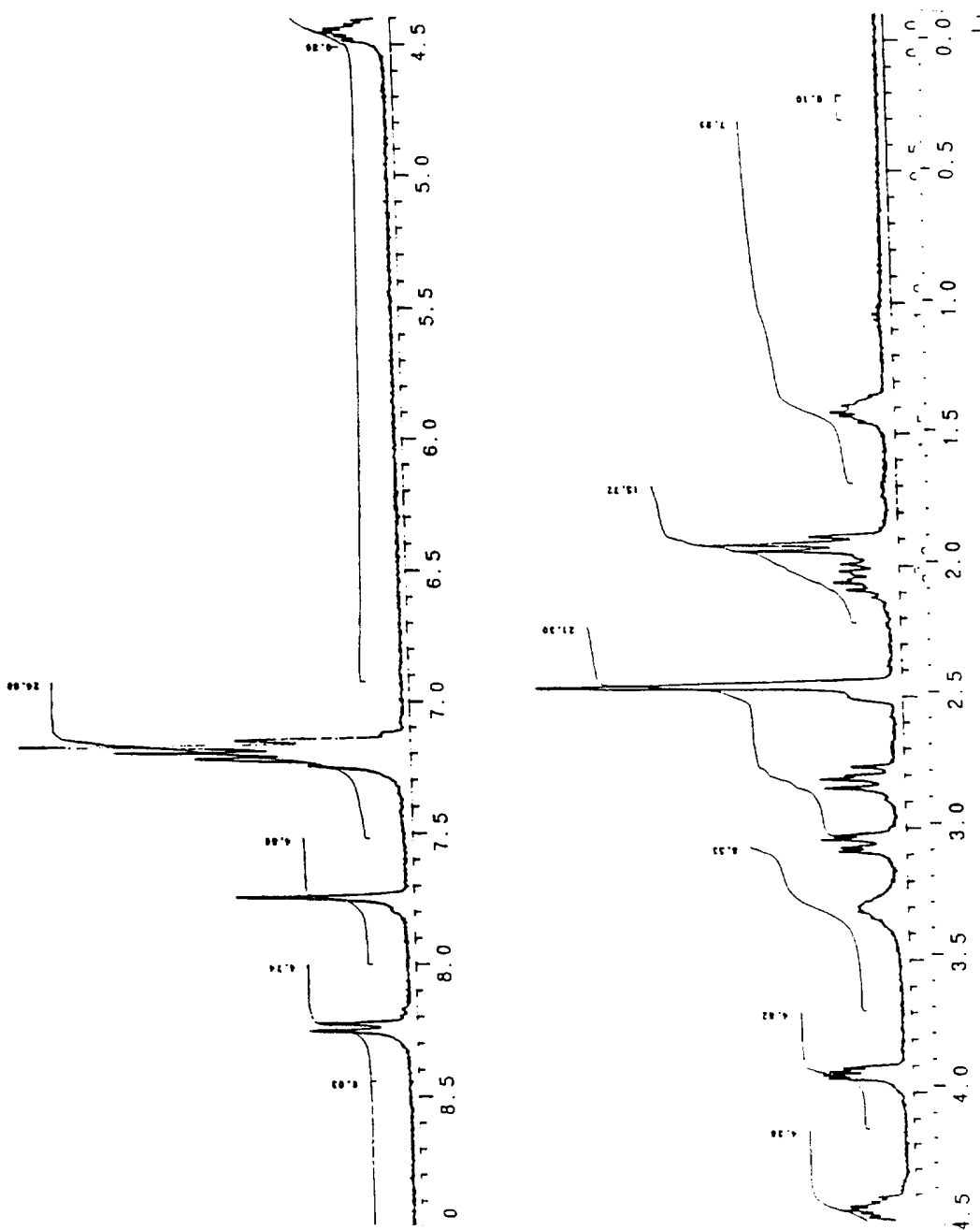


Fig. 28 1H NMR PyGluAsp

29/101

Figure 29 ¹H-NMR, PyGlu-Phe



30/10/

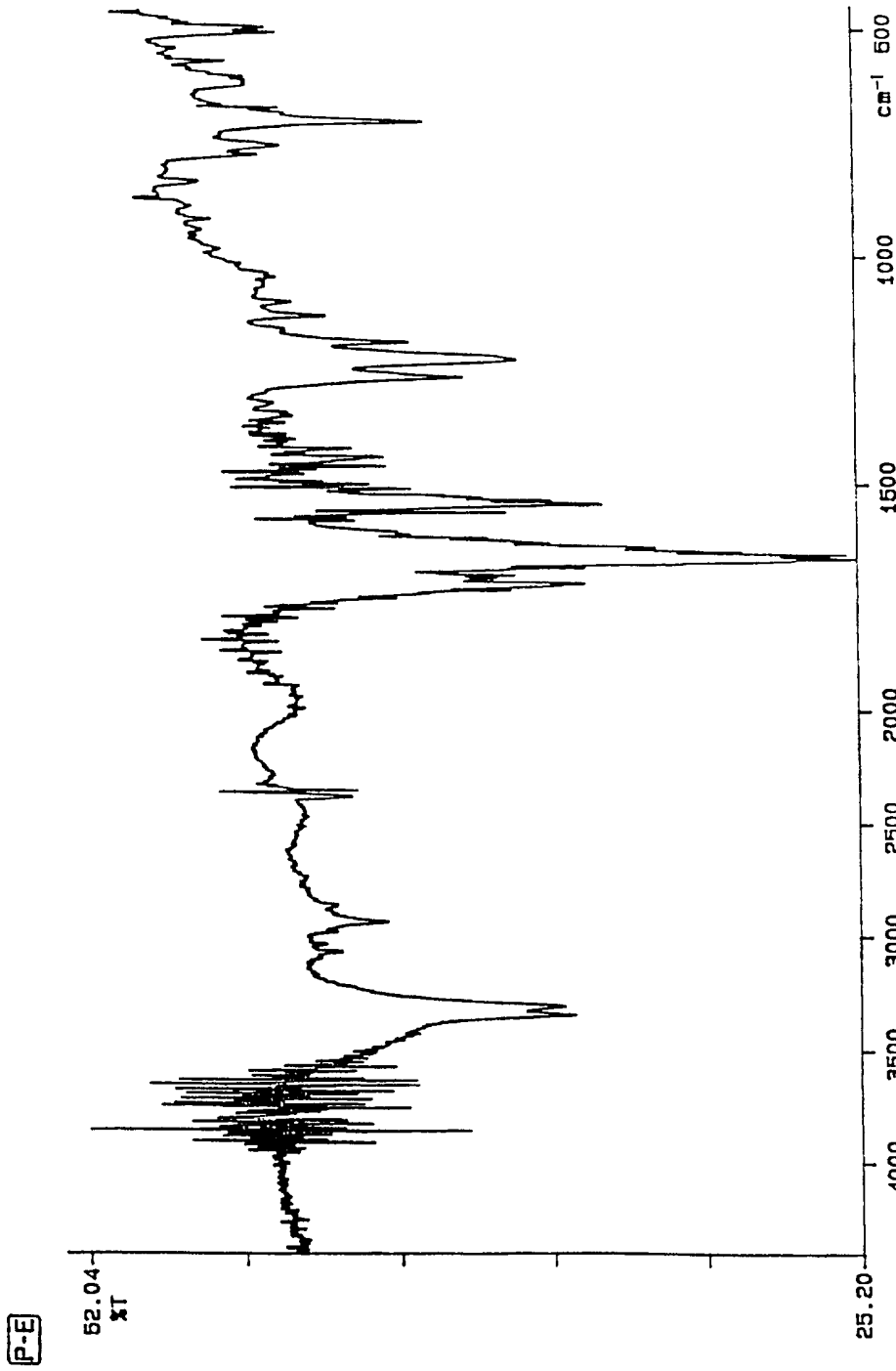


Figure 30 IR, PyGlu-Phe

3/1/01

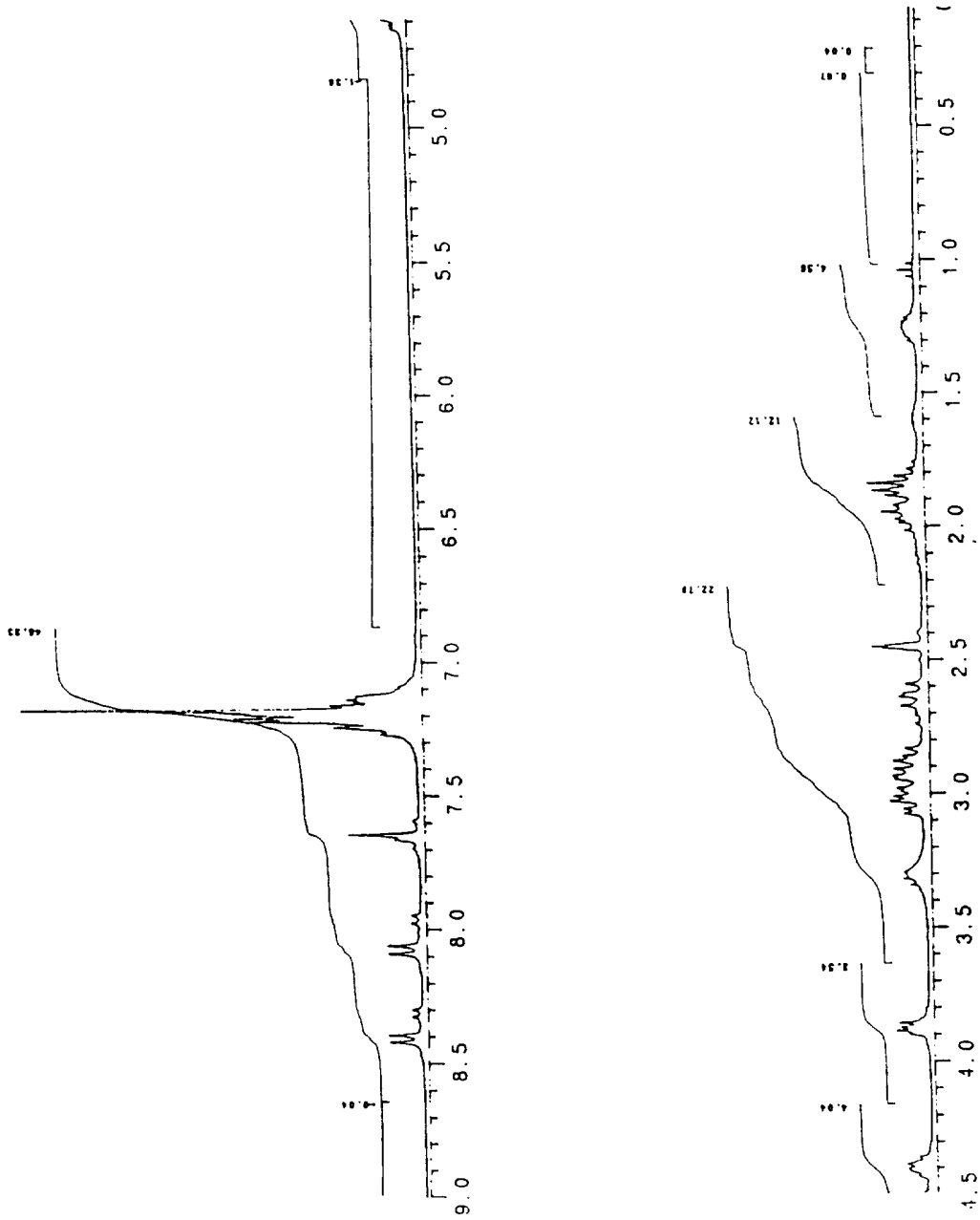


Fig 3| ¹H-NMR, PyGlu-α-Phe

3/2/01

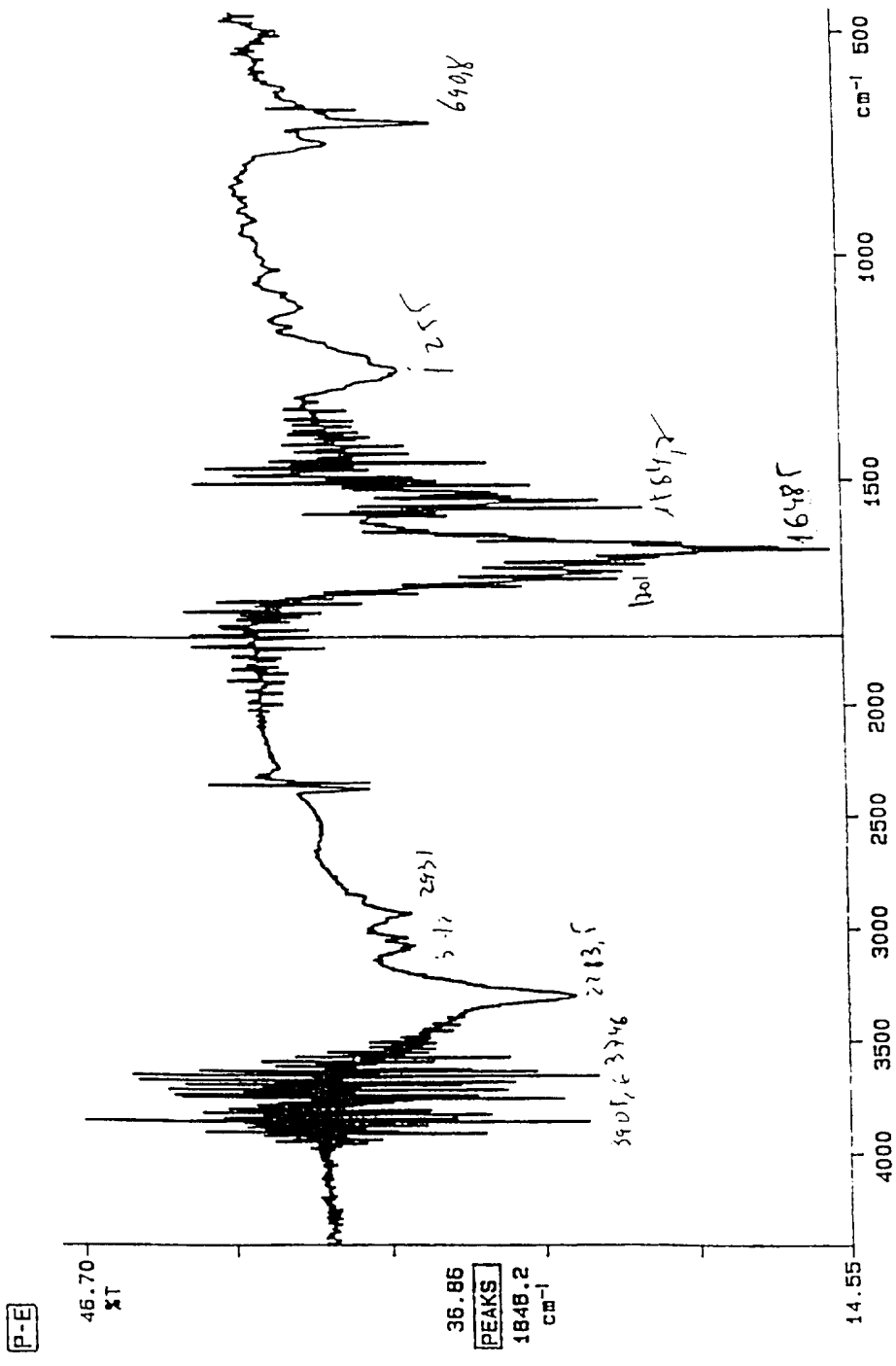
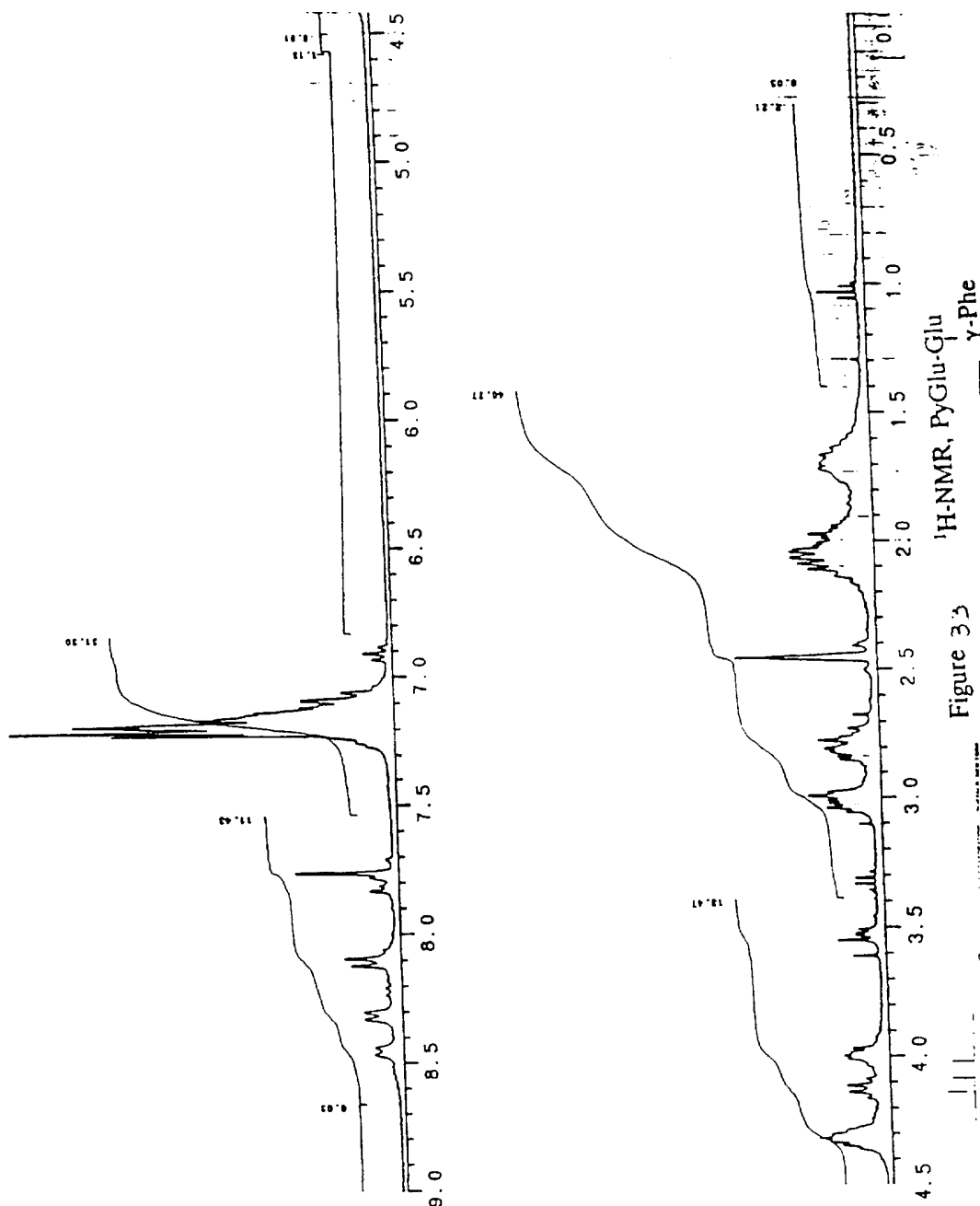


Figure 3λ IR, PyGlu-α-Phe-α-Phe

33/101



¹H-NMR, PyGlu-Glu
— γ -Phe
Figure 3b

34/101

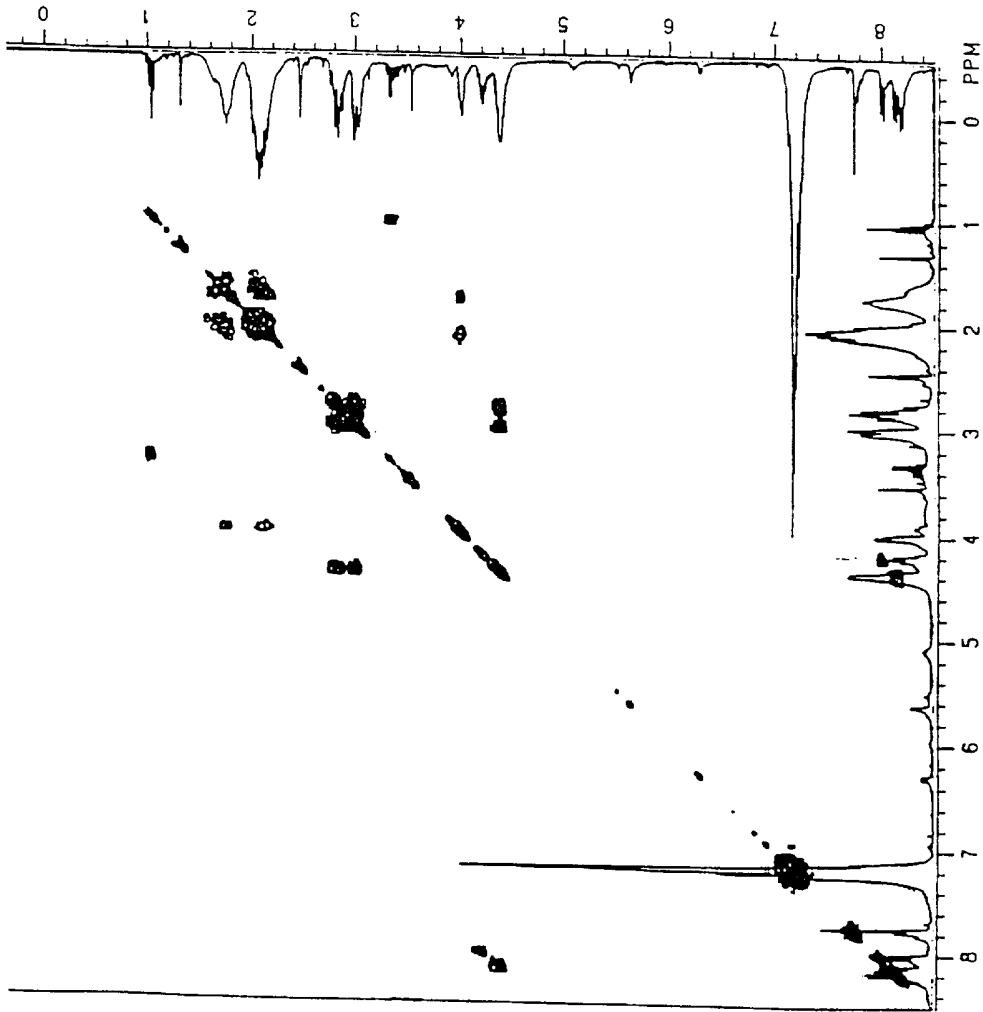


Fig. 34 COSY, PyGlu-Glu- γ -Phe

35/101

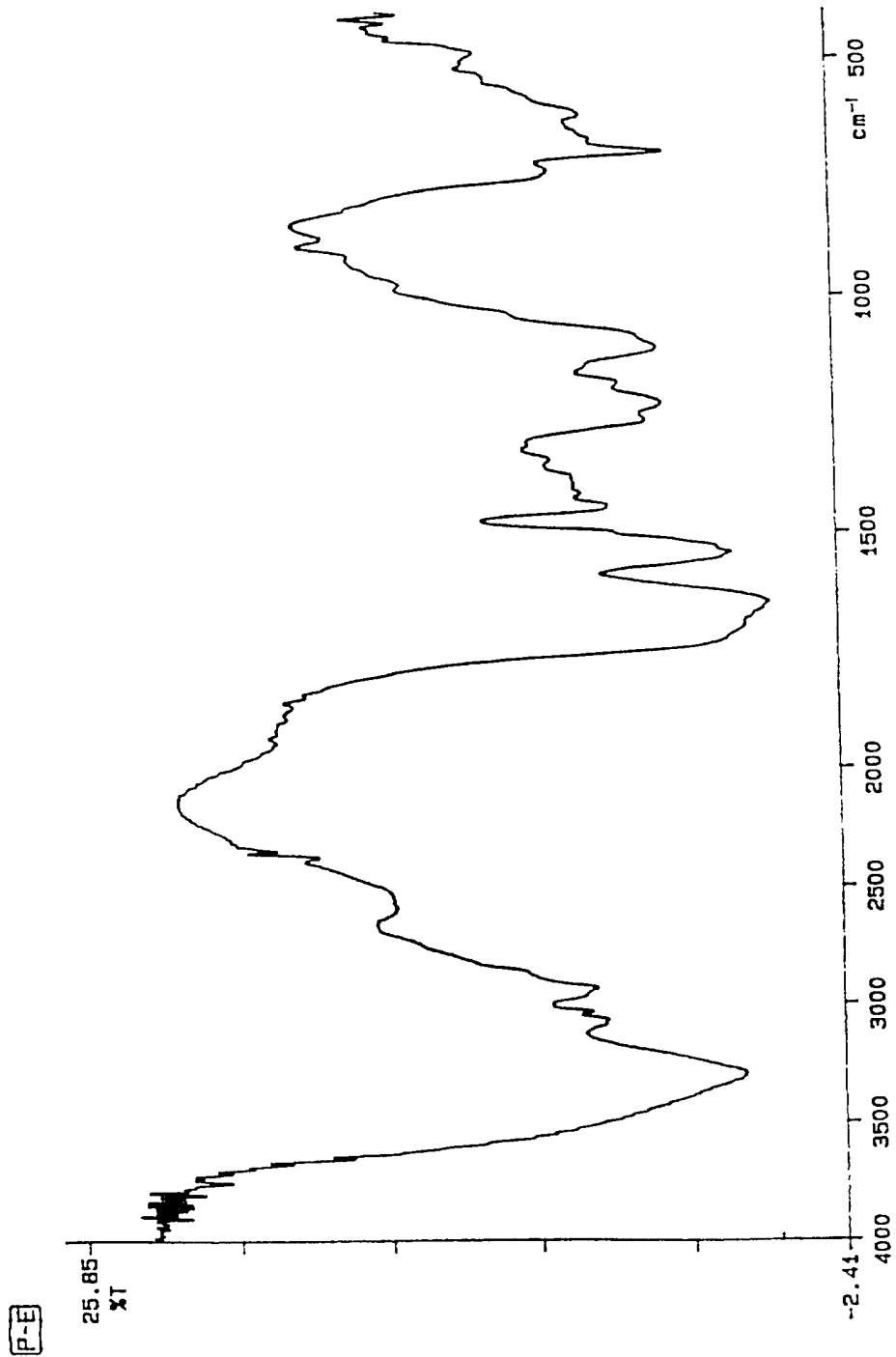


Fig. 35 IR, PyGlu-Glu- γ -Phe

36/101

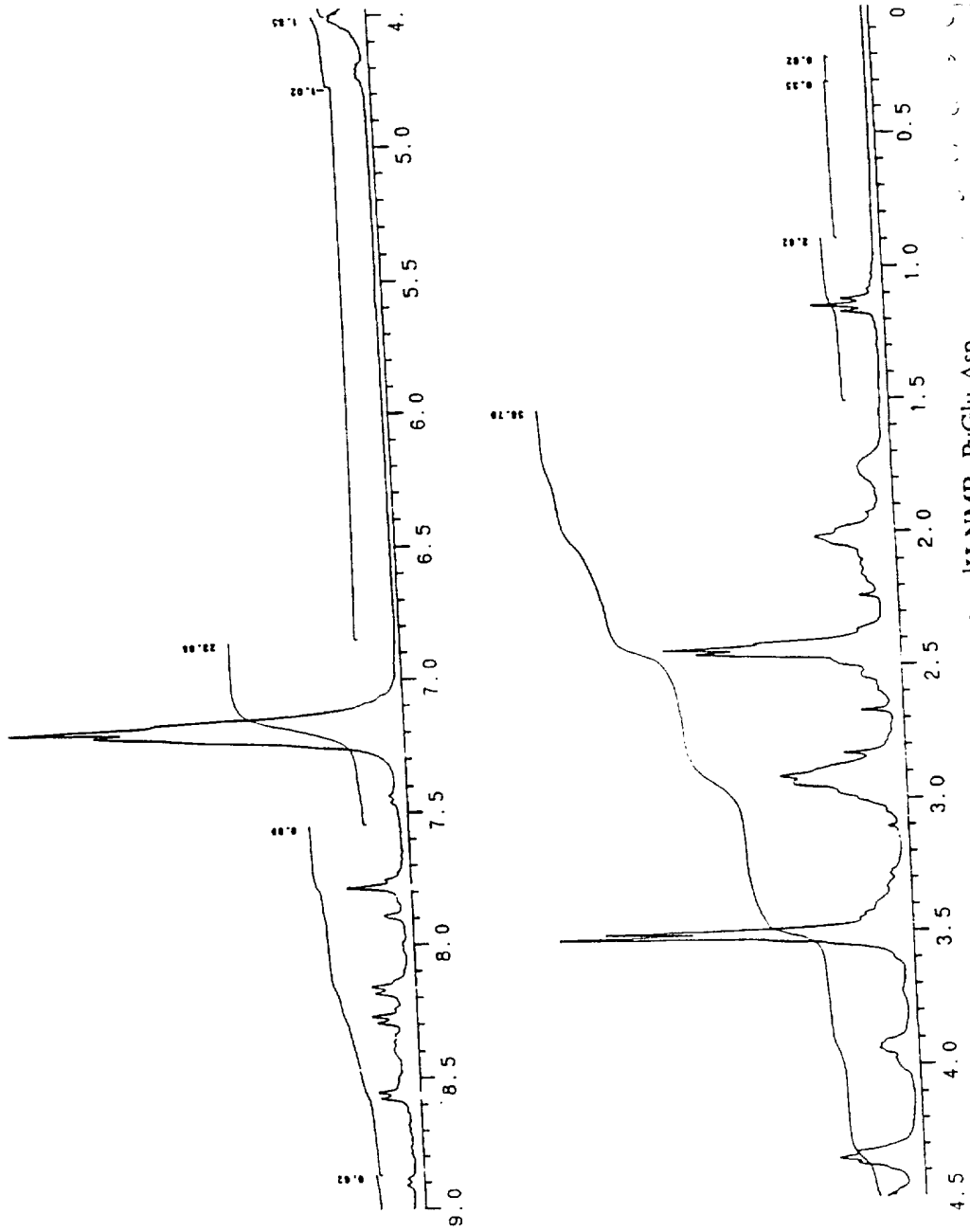


Figure 3-6 ¹H-NMR, PyGlu-Asp
β-Phe

37/101

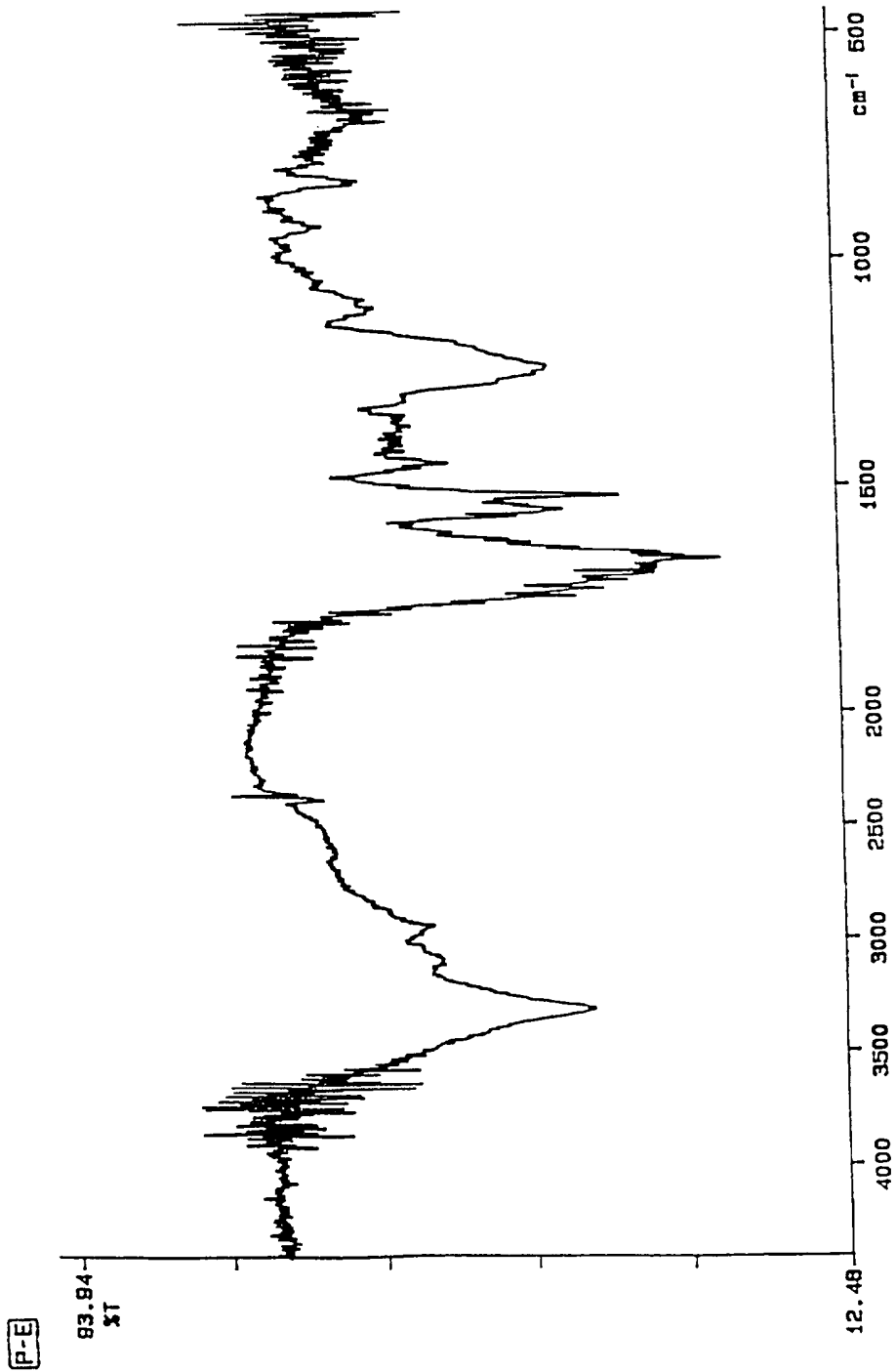


Figure 37 IR, PyGlu-Asp
β-Tyr

38/101

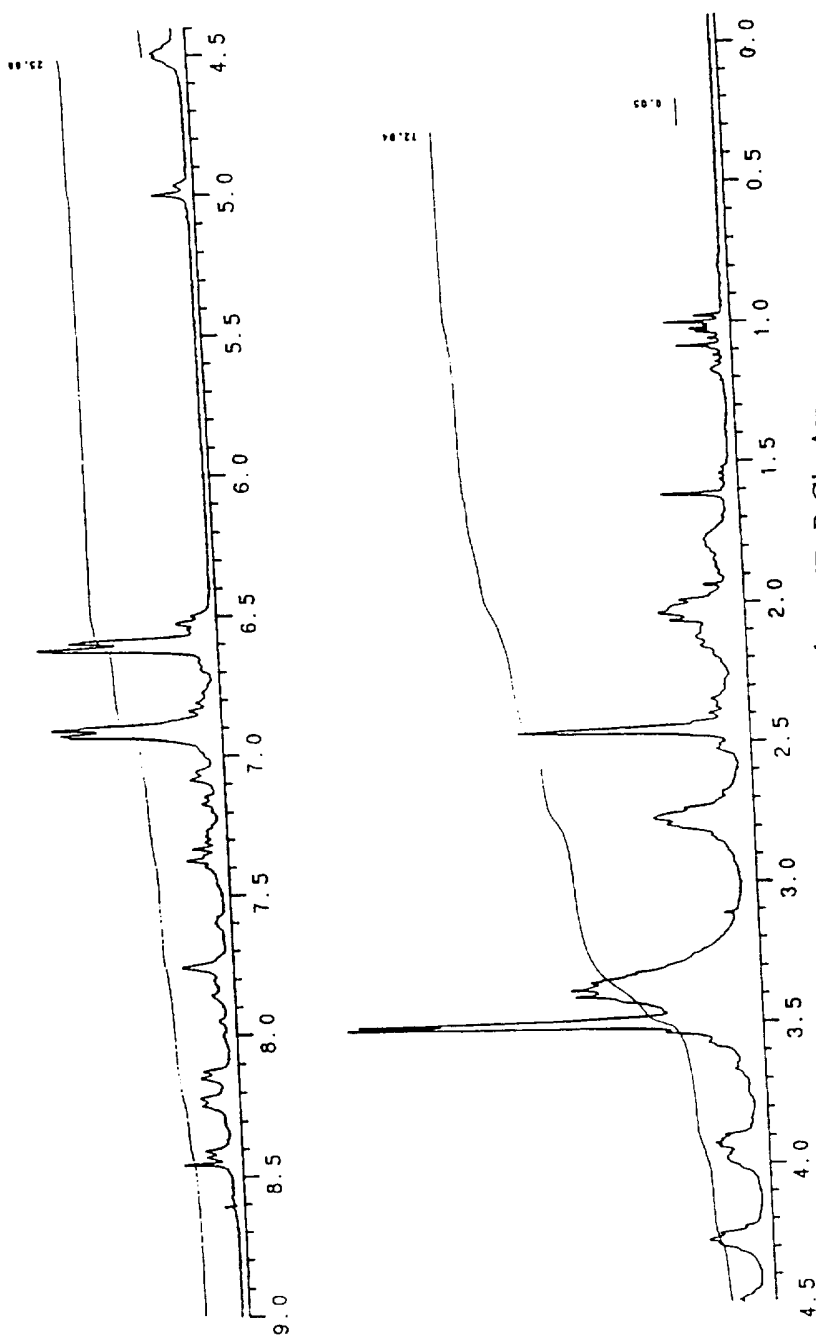


Figure 38 ¹H-NMR, PyGlu-Asp B-Tyr

39/101

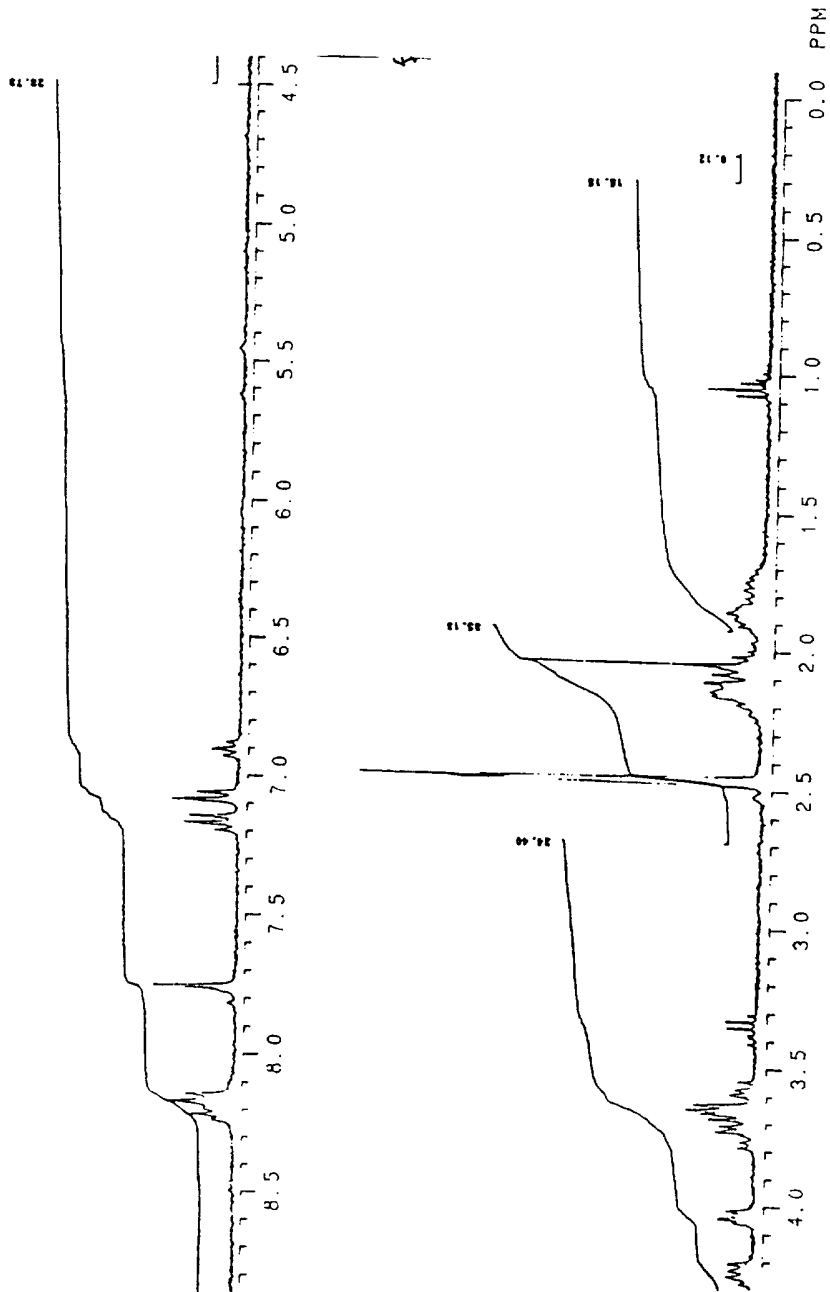


Figure 39 ¹H-NMR, PyGlu-Glu-γ-Gly

40/101

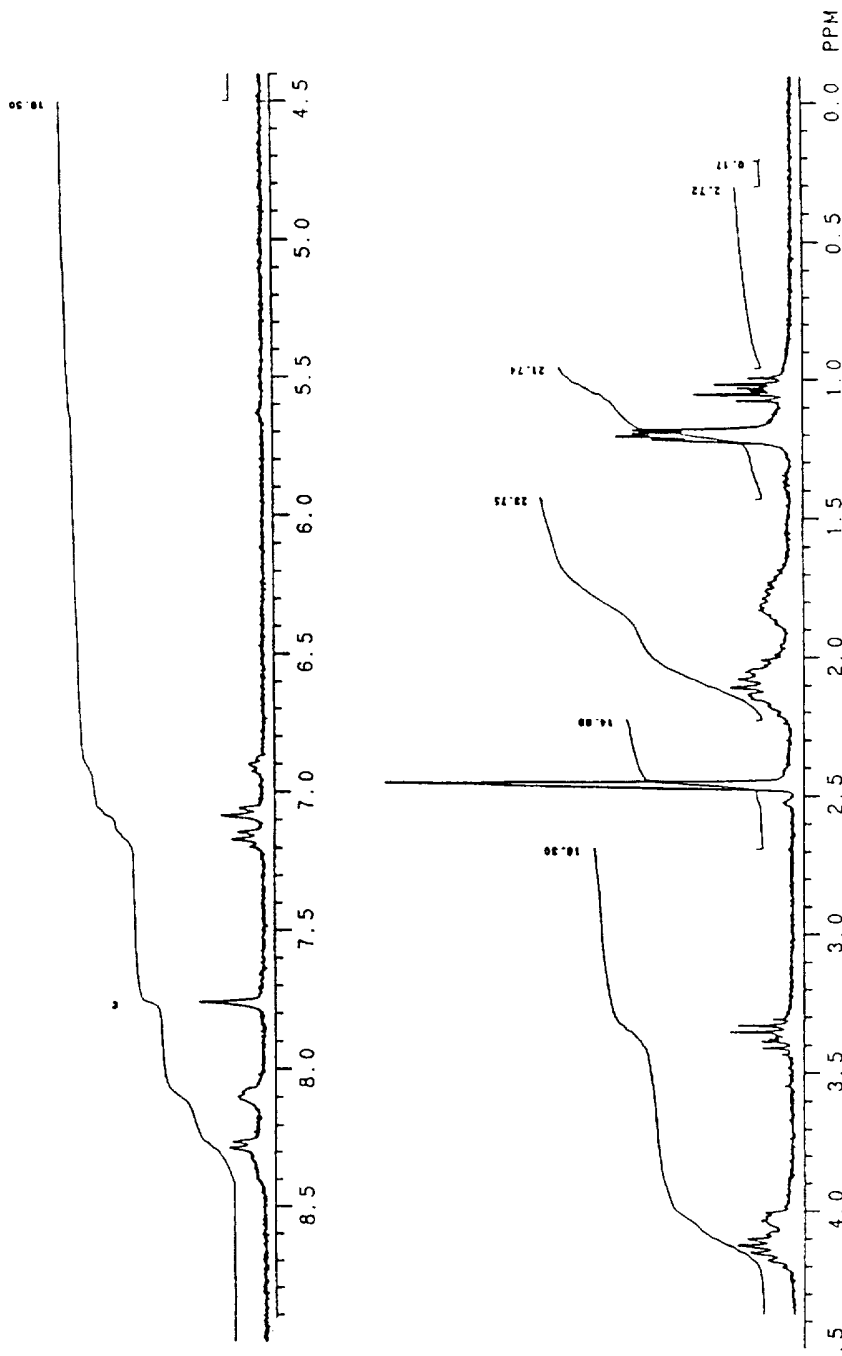


Figure 40 ¹H-NMR, PyGlu-Glu-γ-Ala

4/1/01

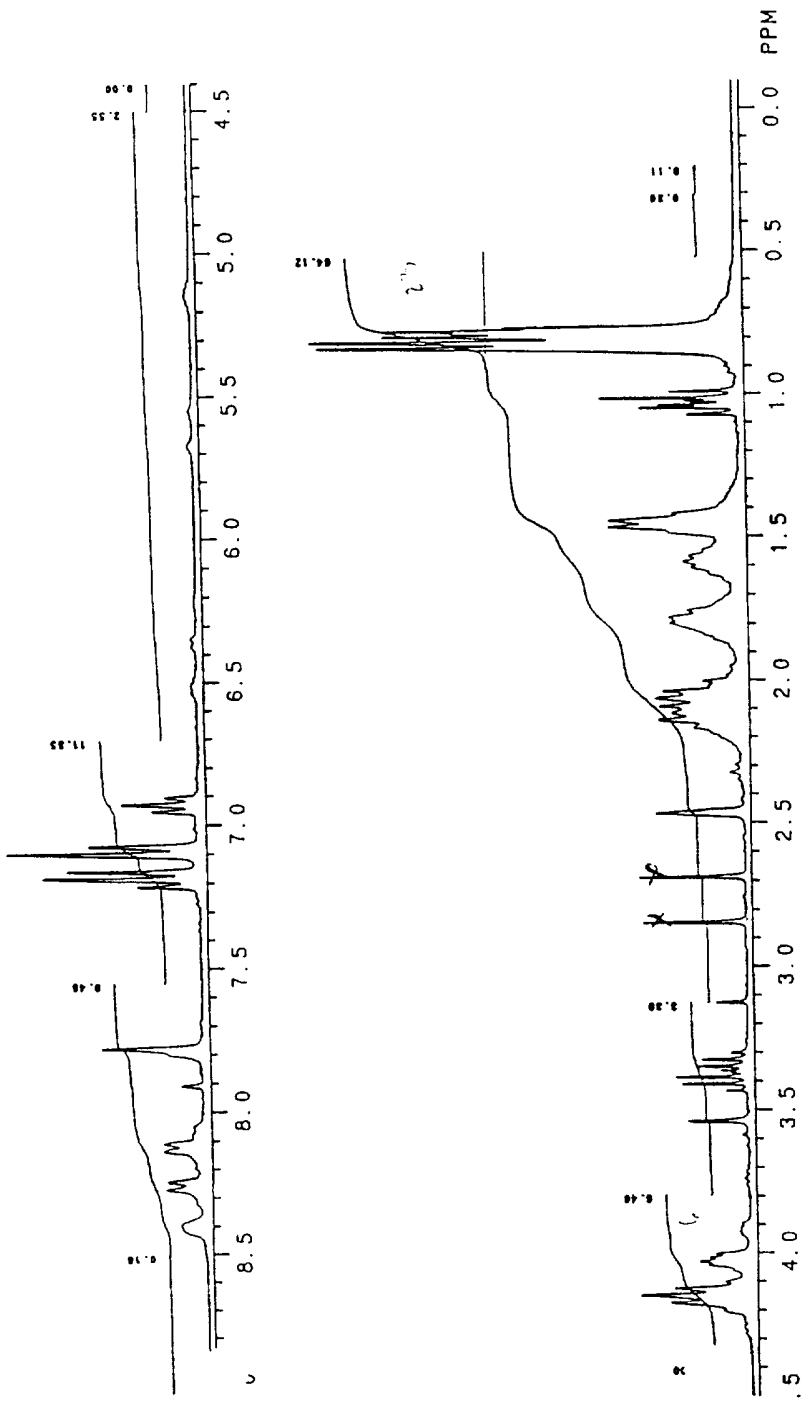
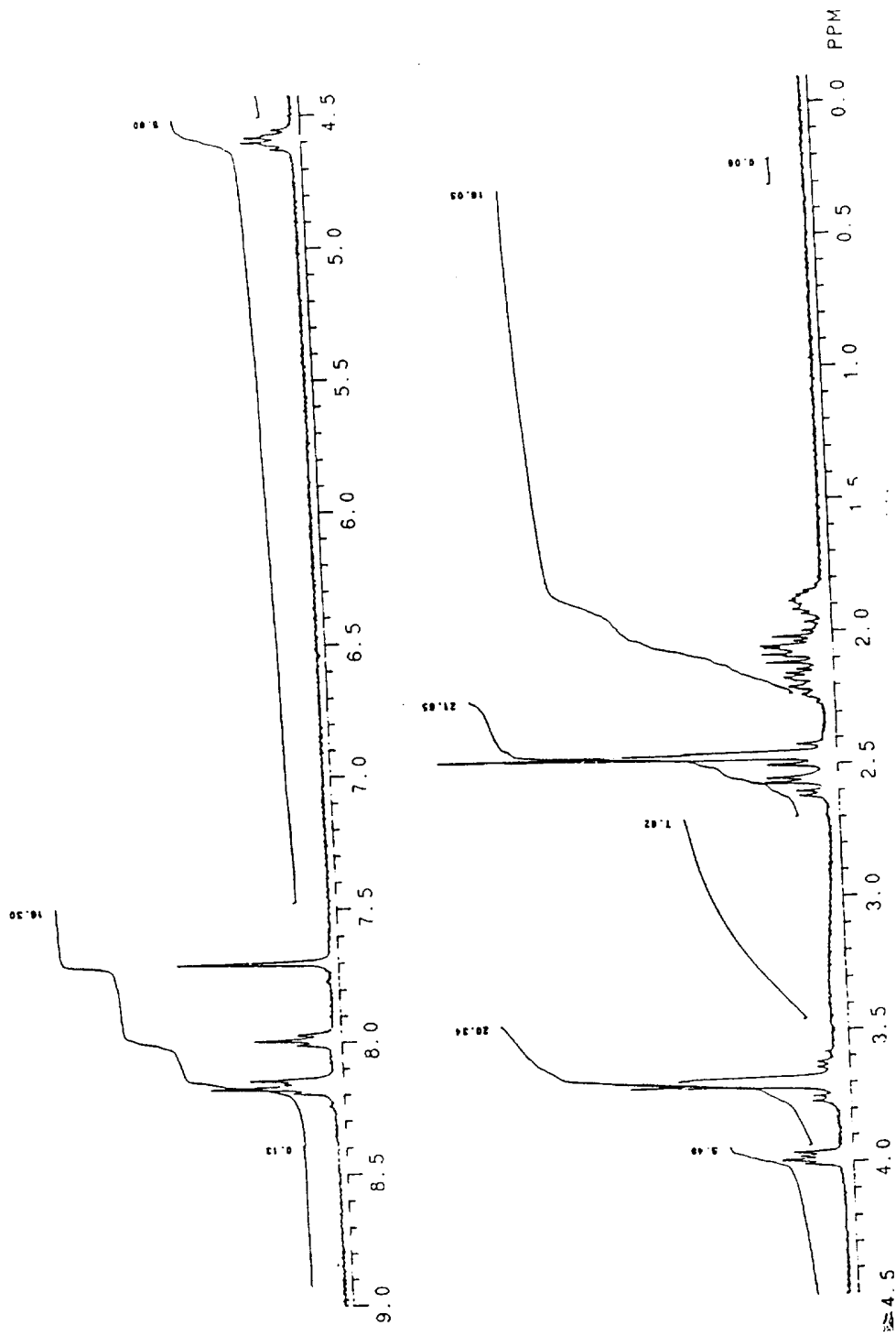


Figure 41 ¹H-NMR, PyGlu-Glu-γ-Leu,

42/101



1H-NMR, PyGlu-Asp-beta-Gly

Figure 4a

43/101

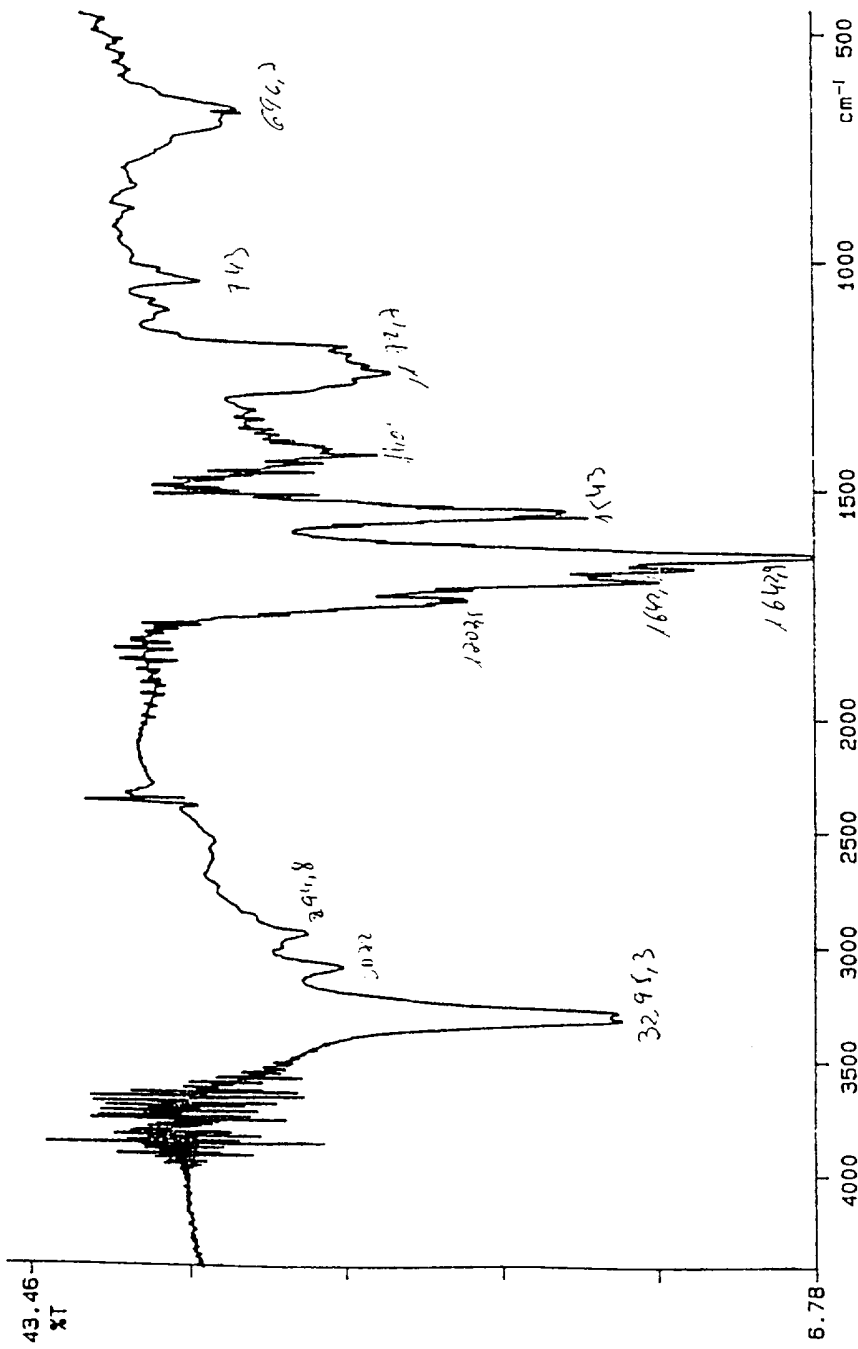


Figure 43. IR, PyGlu-Asp-β-Gly,

44/101

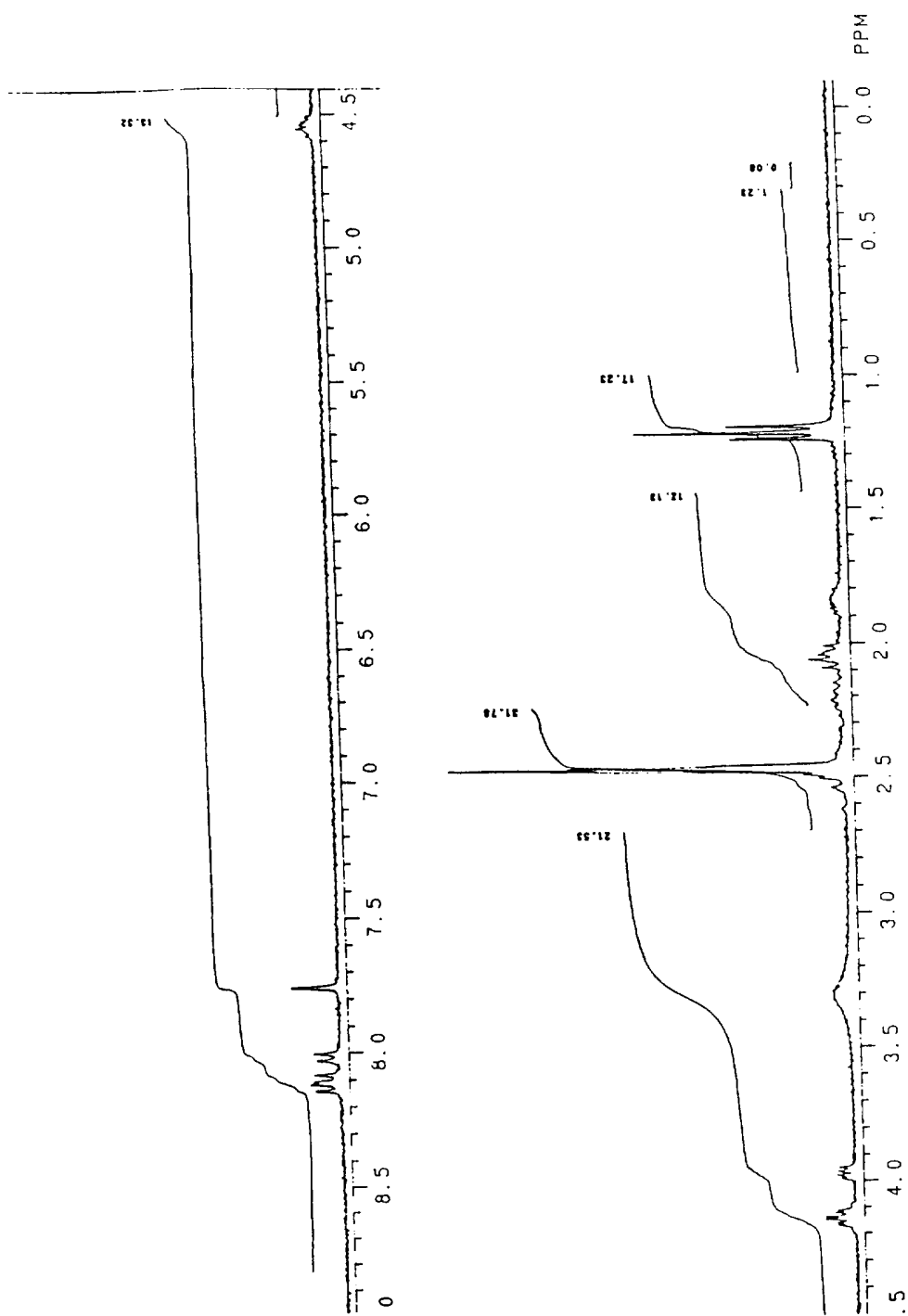


Figure 44 ¹H-NMR, PyGlu-Asp-β-Ala,

45/101

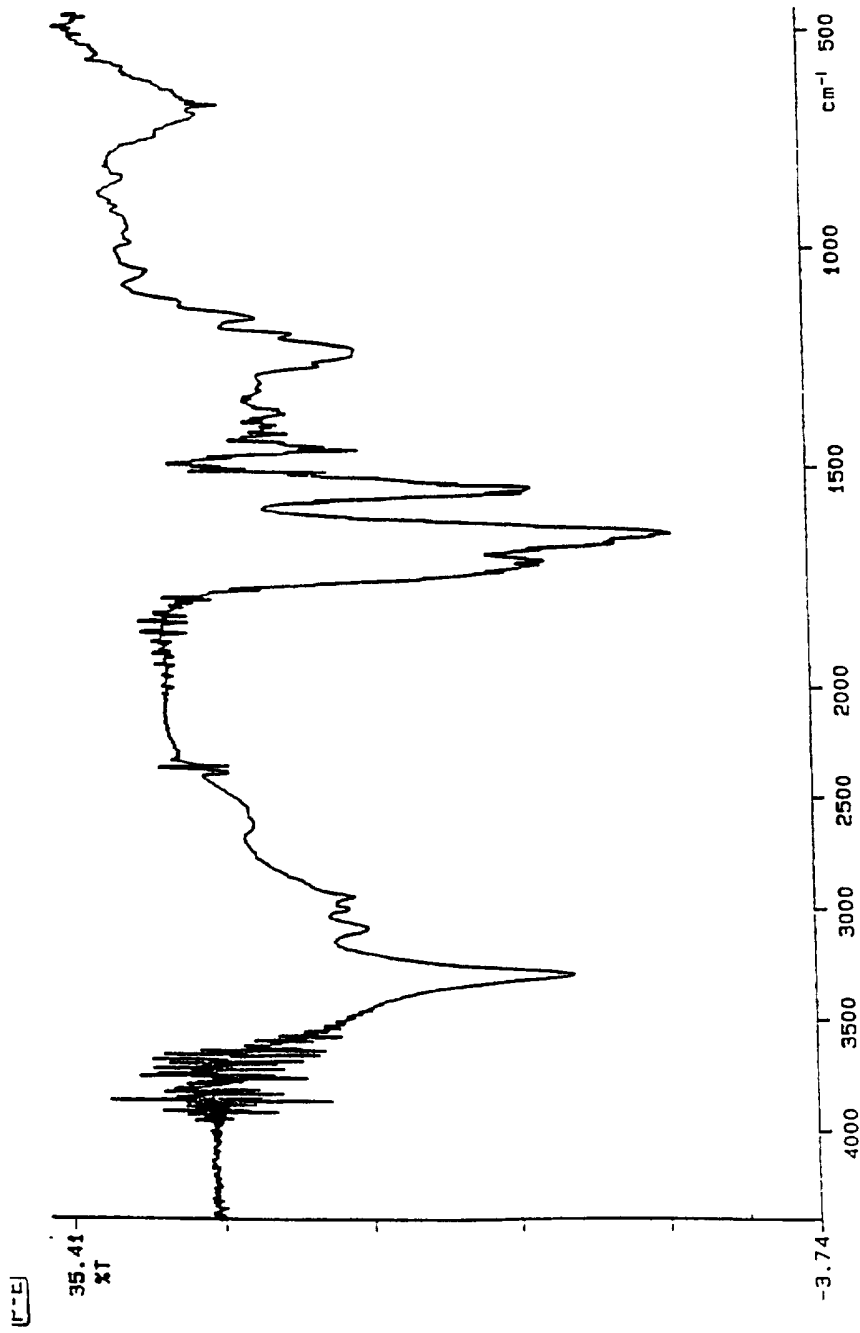


Figure 45 IR, PyGlu-Asp-β-Ala.

46/101

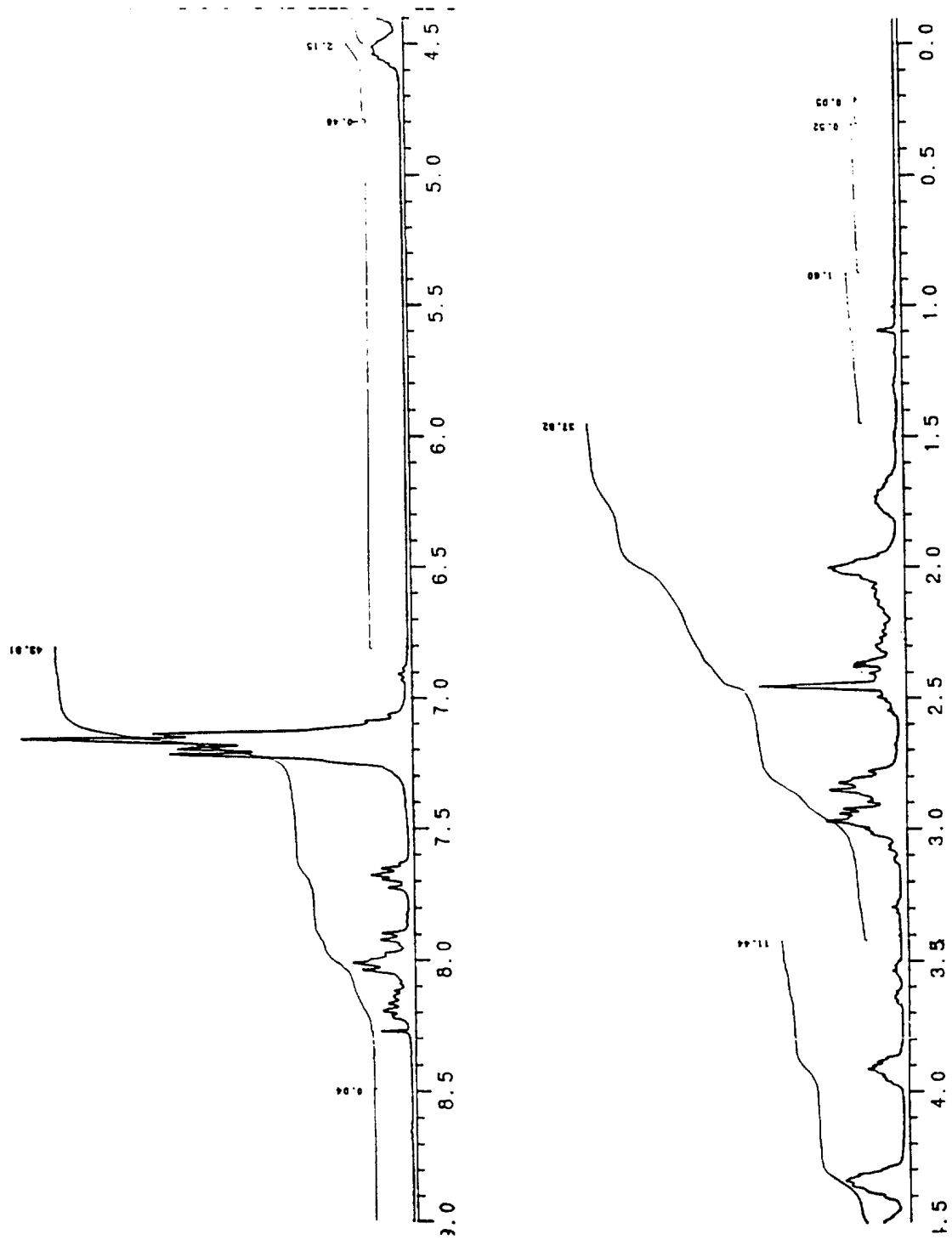


Fig. 4/6 ¹H NMR PyGluGlu α -Phe- γ -Phe

47/101

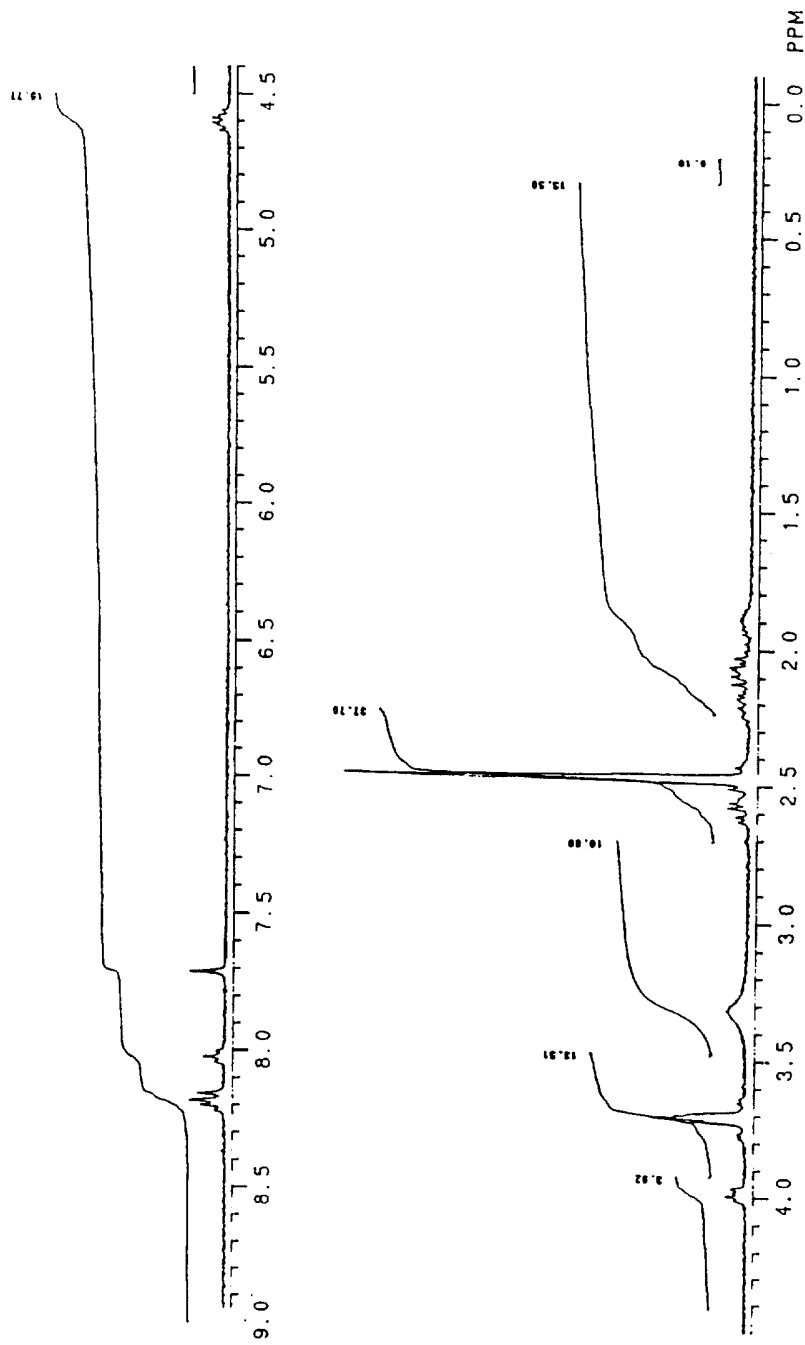


Figure 47 ¹H-NMR, PyGlu-Asp-α-Gly-β-Gly

48/01

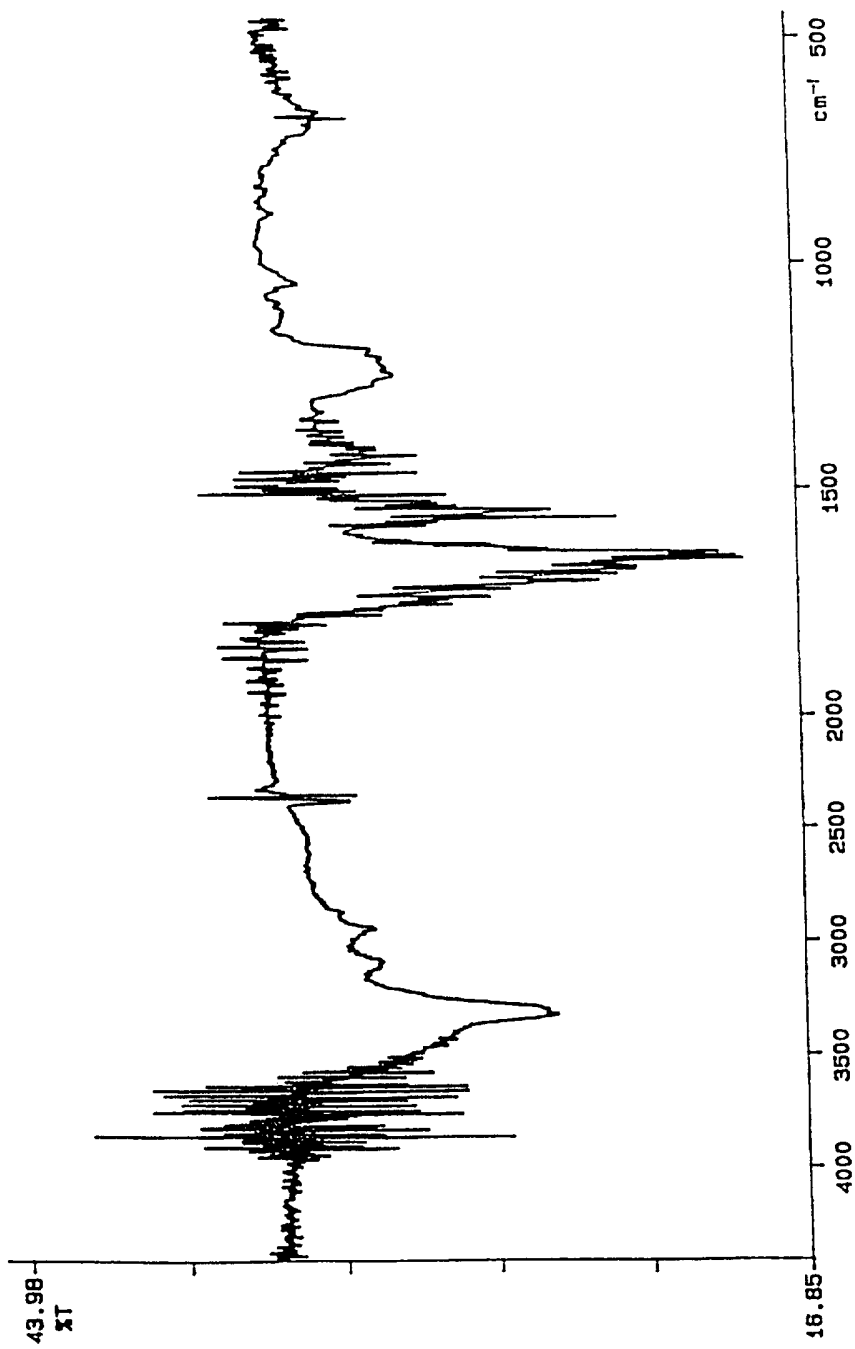


Figure 4/δ IR, PyGlu-Asp-α-Gly-β-Gly

49/101

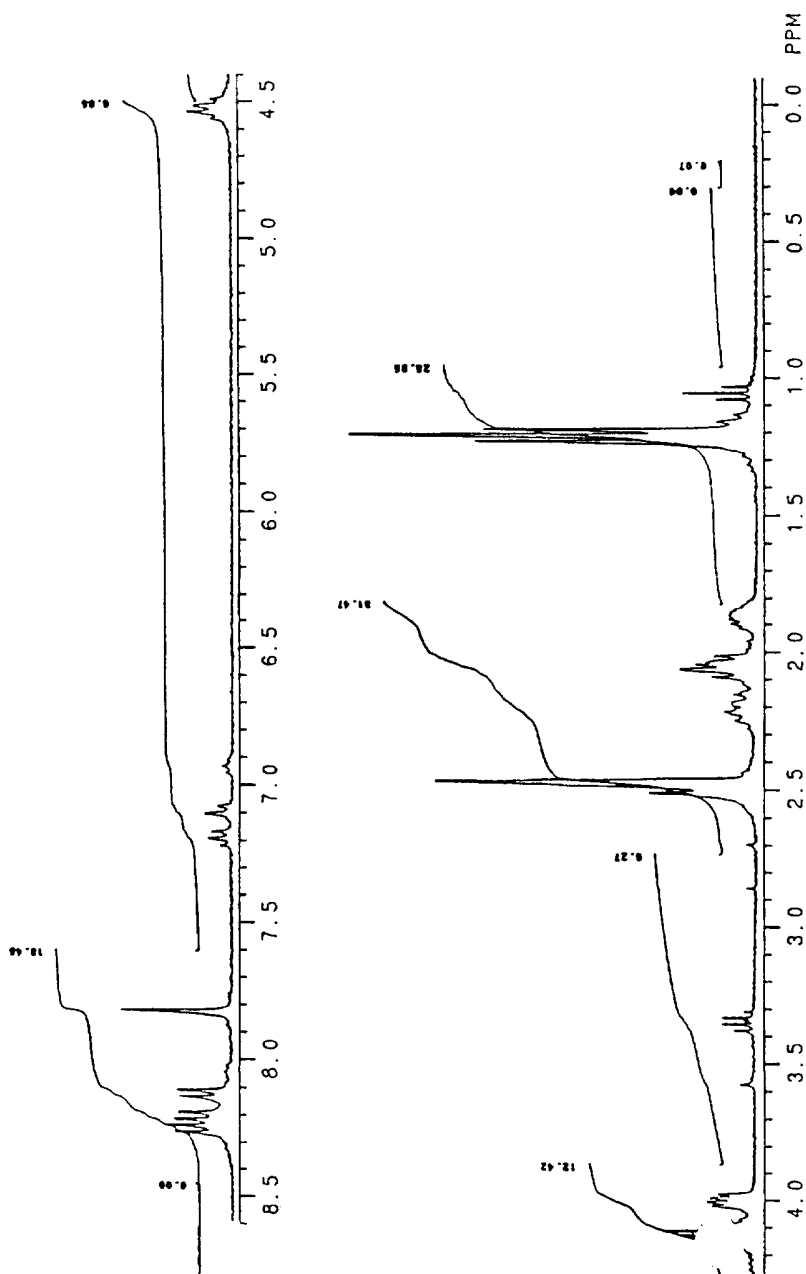


Figure 49 ¹H-NMR, PyGlu-Asp-α-Ala-β-Ala,

50/101

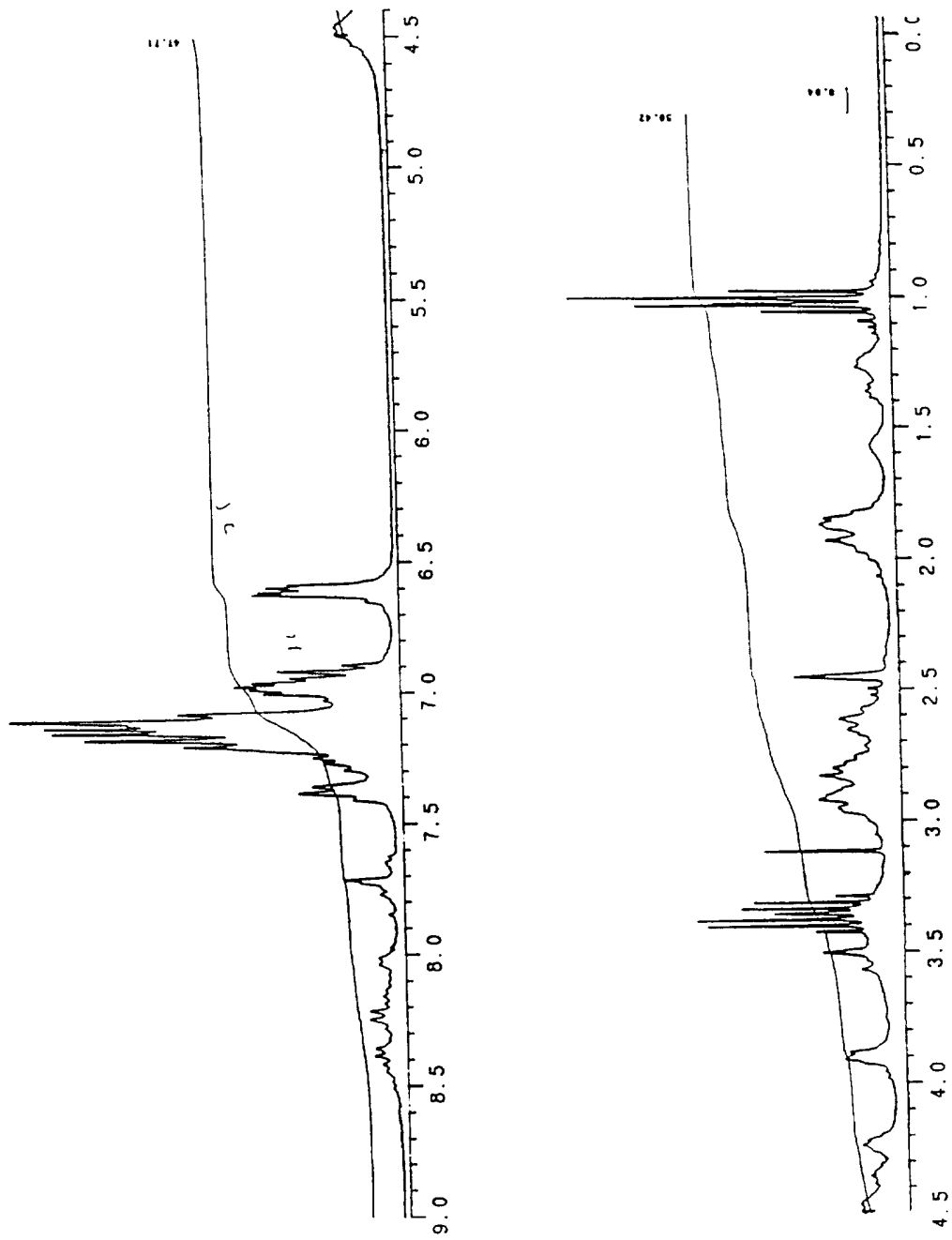


Figure 50 ¹H-NMR, PyGlu-α-Phe-α-Phe-α-Tyr

5/1/01

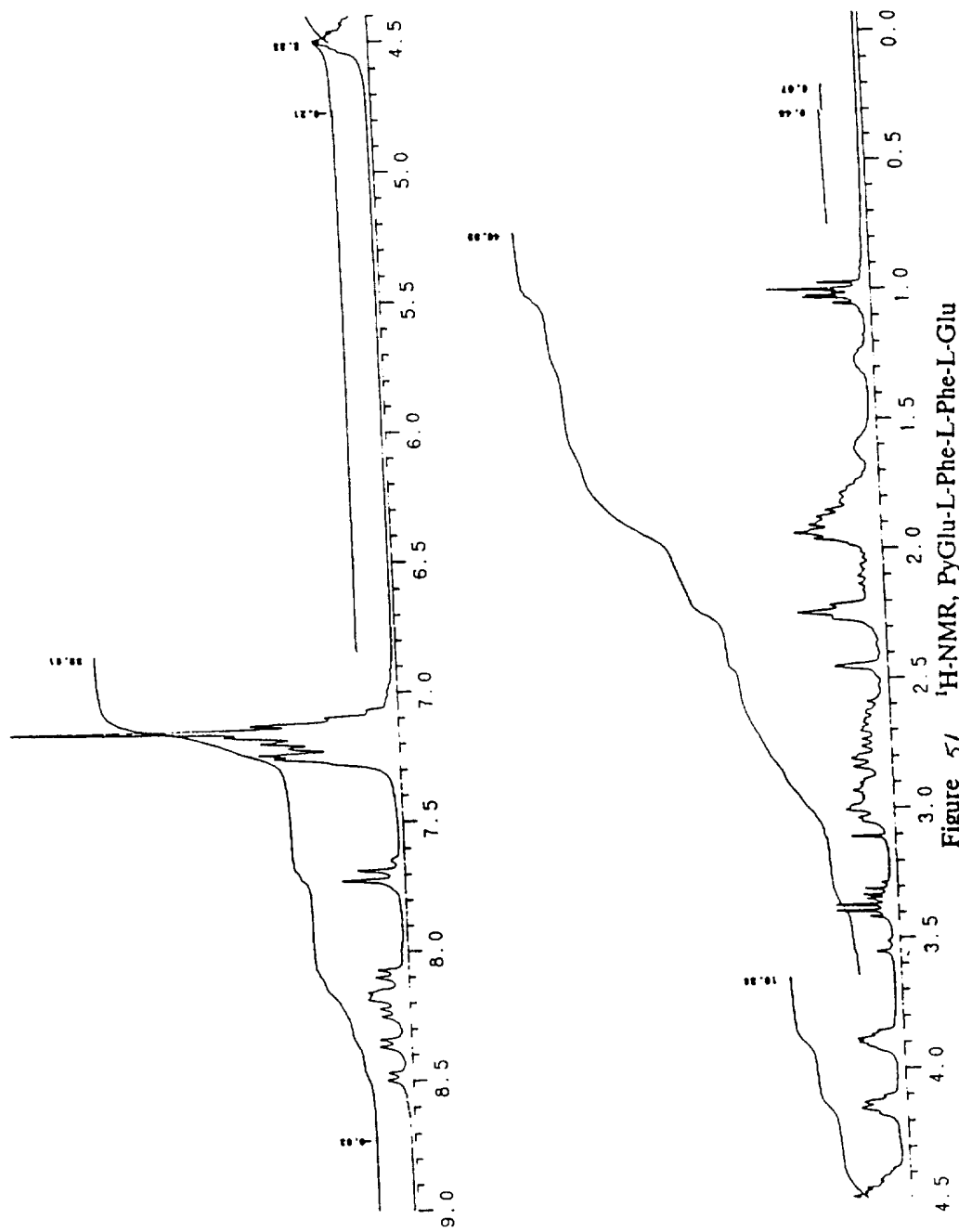
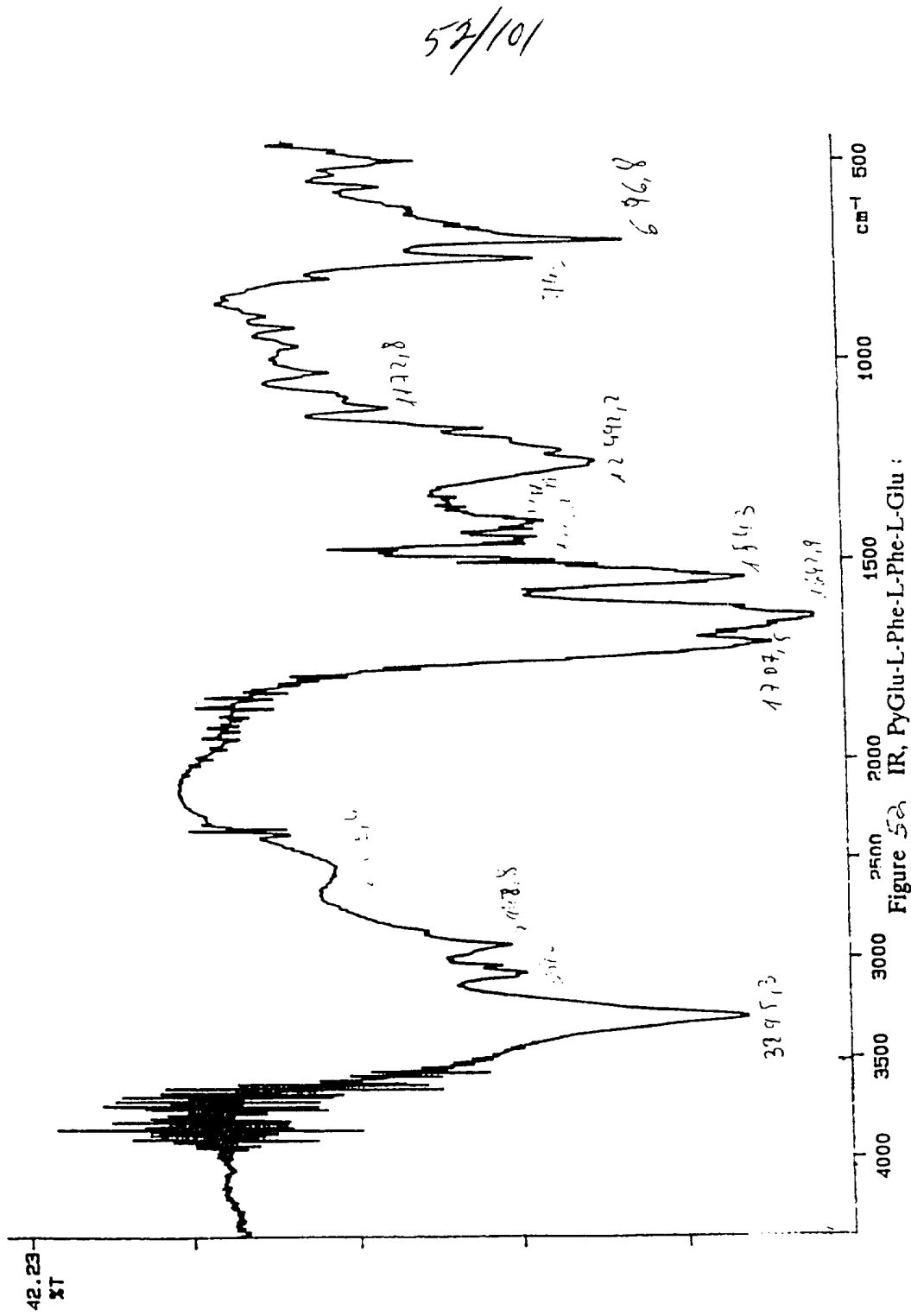
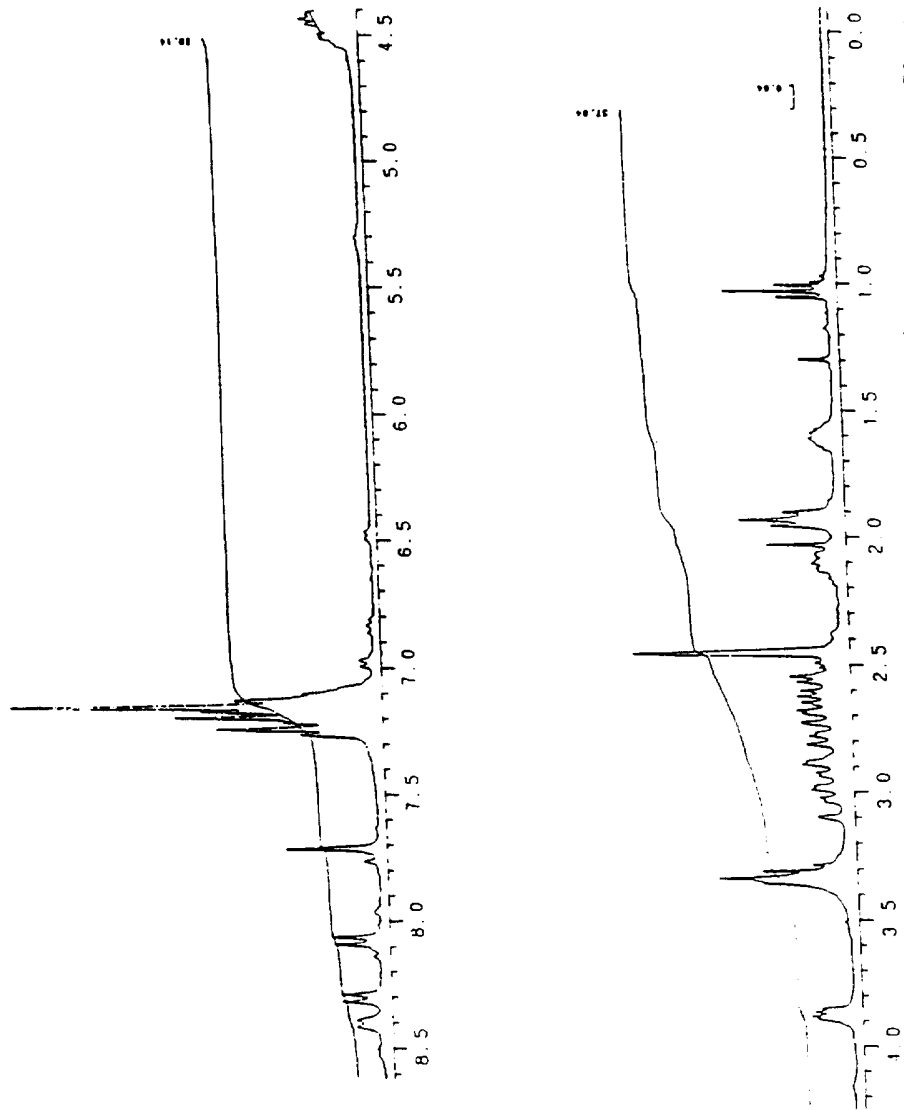


Figure 5/ ¹H-NMR, PyGlu-L-Phe-L-Glu



54/101



70 FIG. 54 ¹H-NMR, PyGlu-Phe-Phe-Asp

55/101

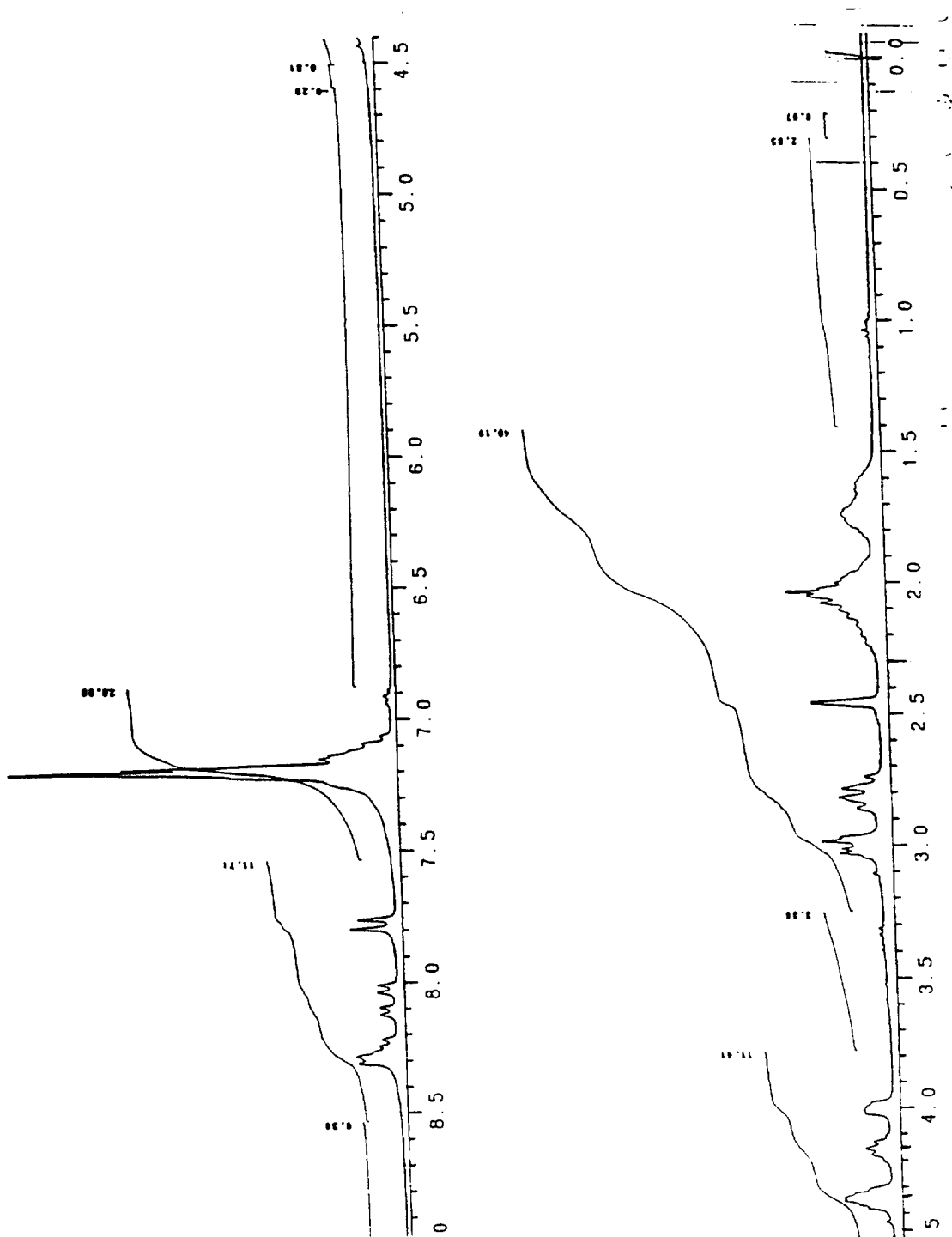


Figure 65 ¹H-NMR, PyGlu-Glu-α-Phe-γ-Phe

56/101

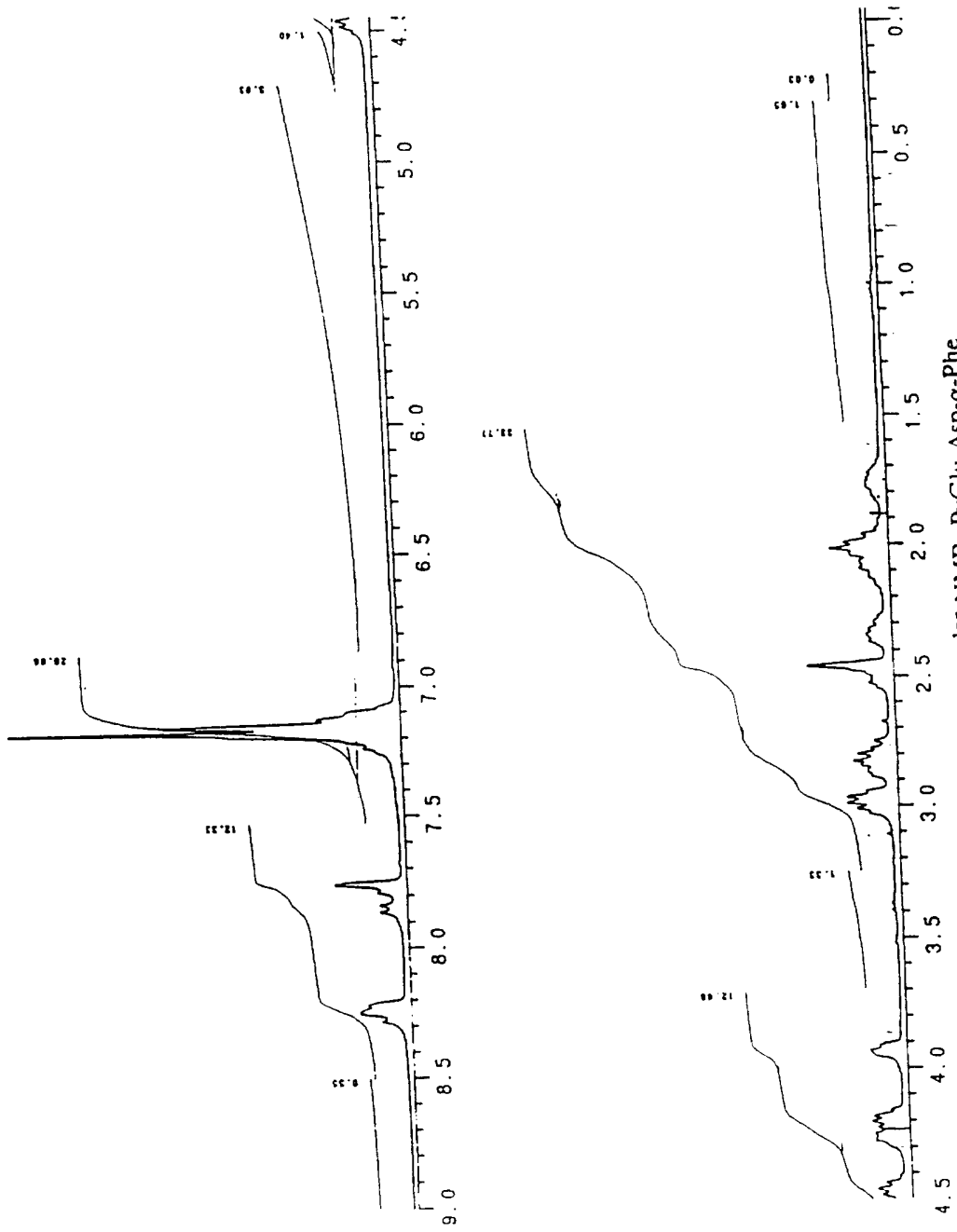


Figure 56 ¹H-NMR, PyGlu-Asp- α -Phe β -Phe

59/101

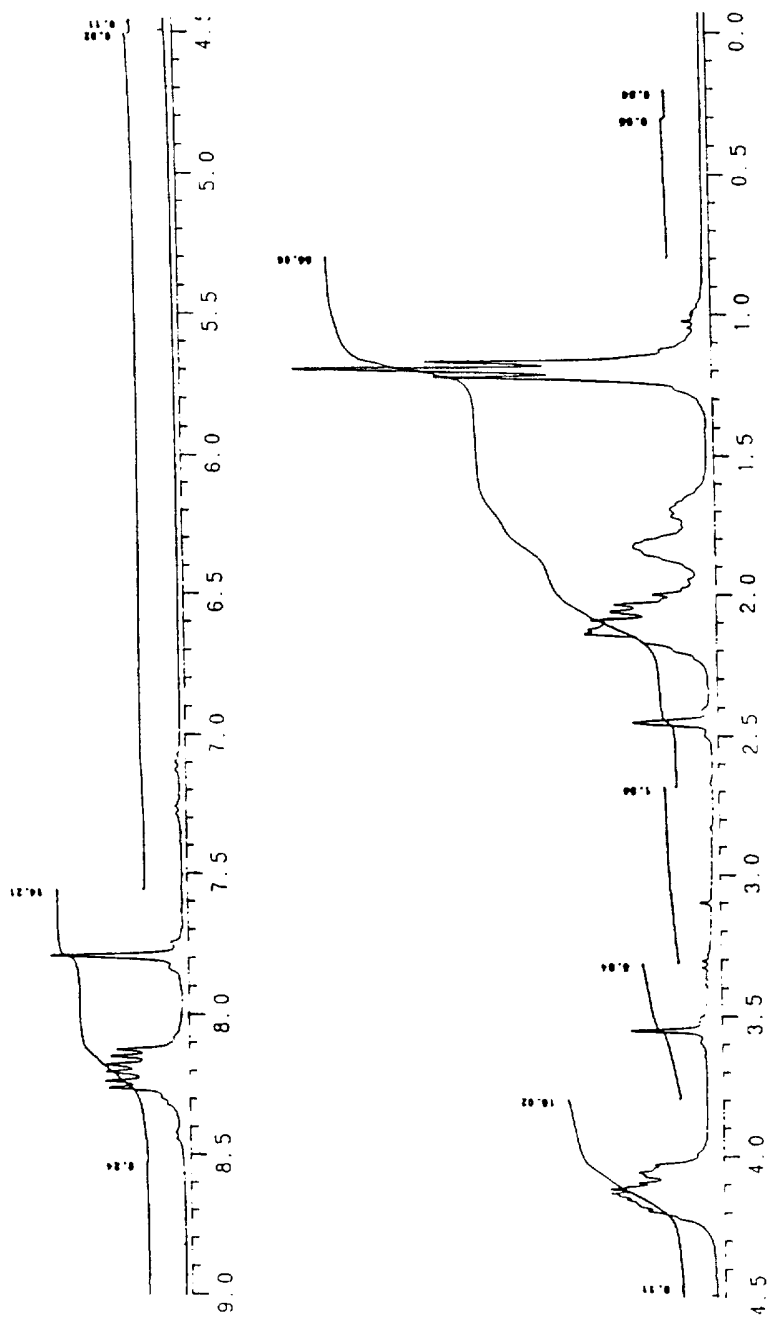


Figure 57 ¹H-NMR, PyGlu-Glu-α-Ala-γ-Ala

60/101

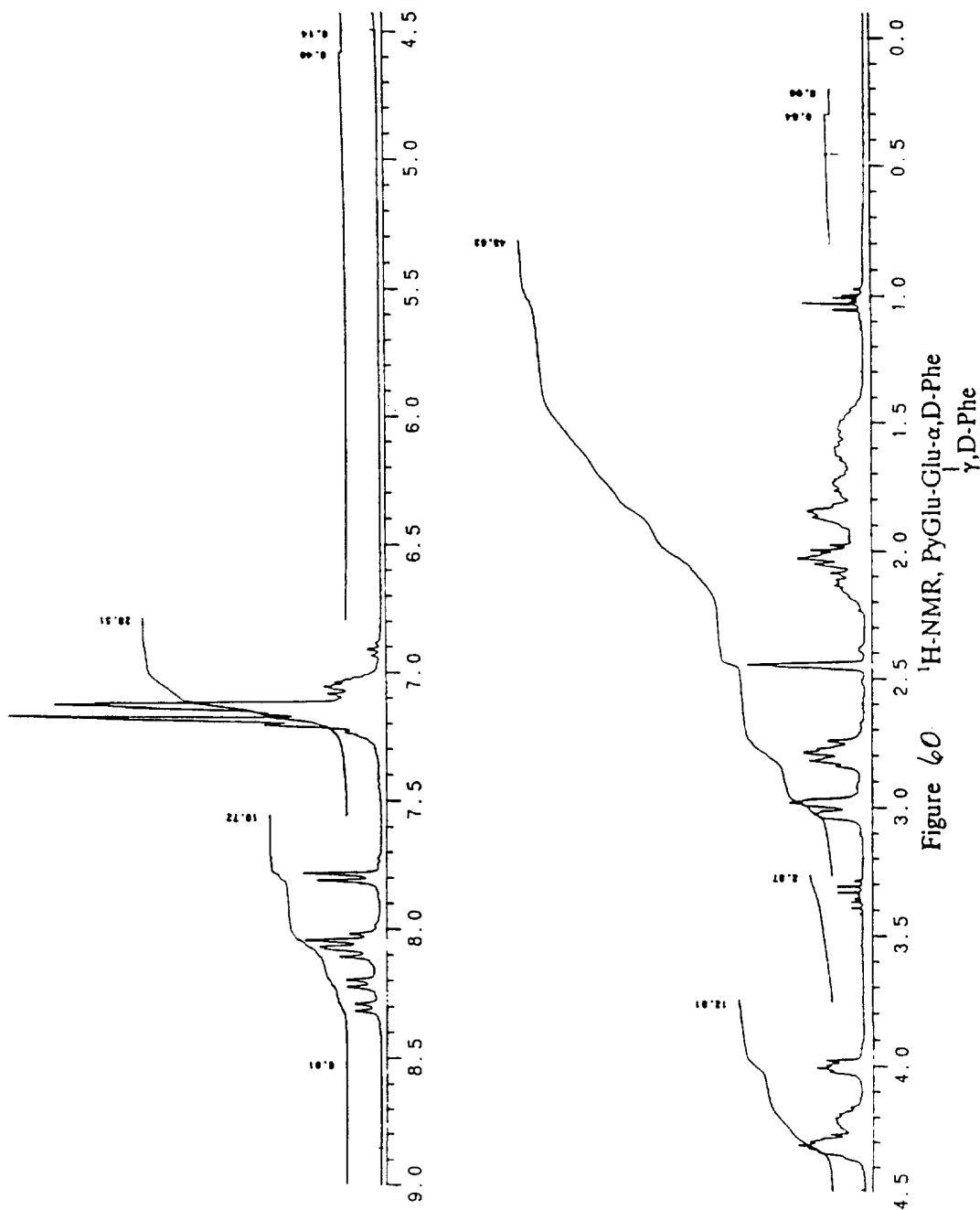


Figure 60. ¹H-NMR, PyGlu-Glu-α, D-Phe γ, D-Phe

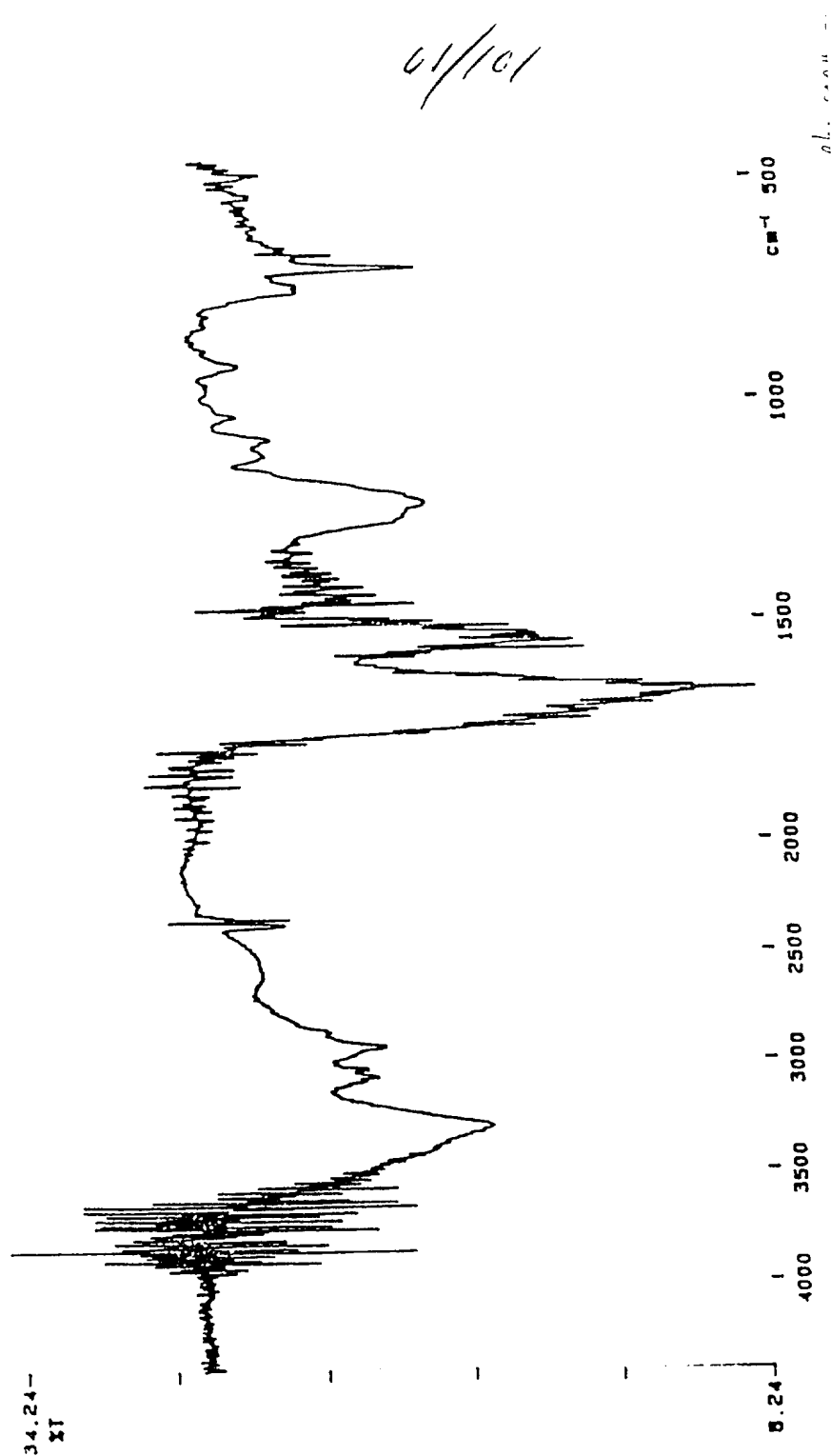


Fig. 6/ IR, PyGlu-Glu- α ,D-Phe
 γ ,D-Phe

10/1/80

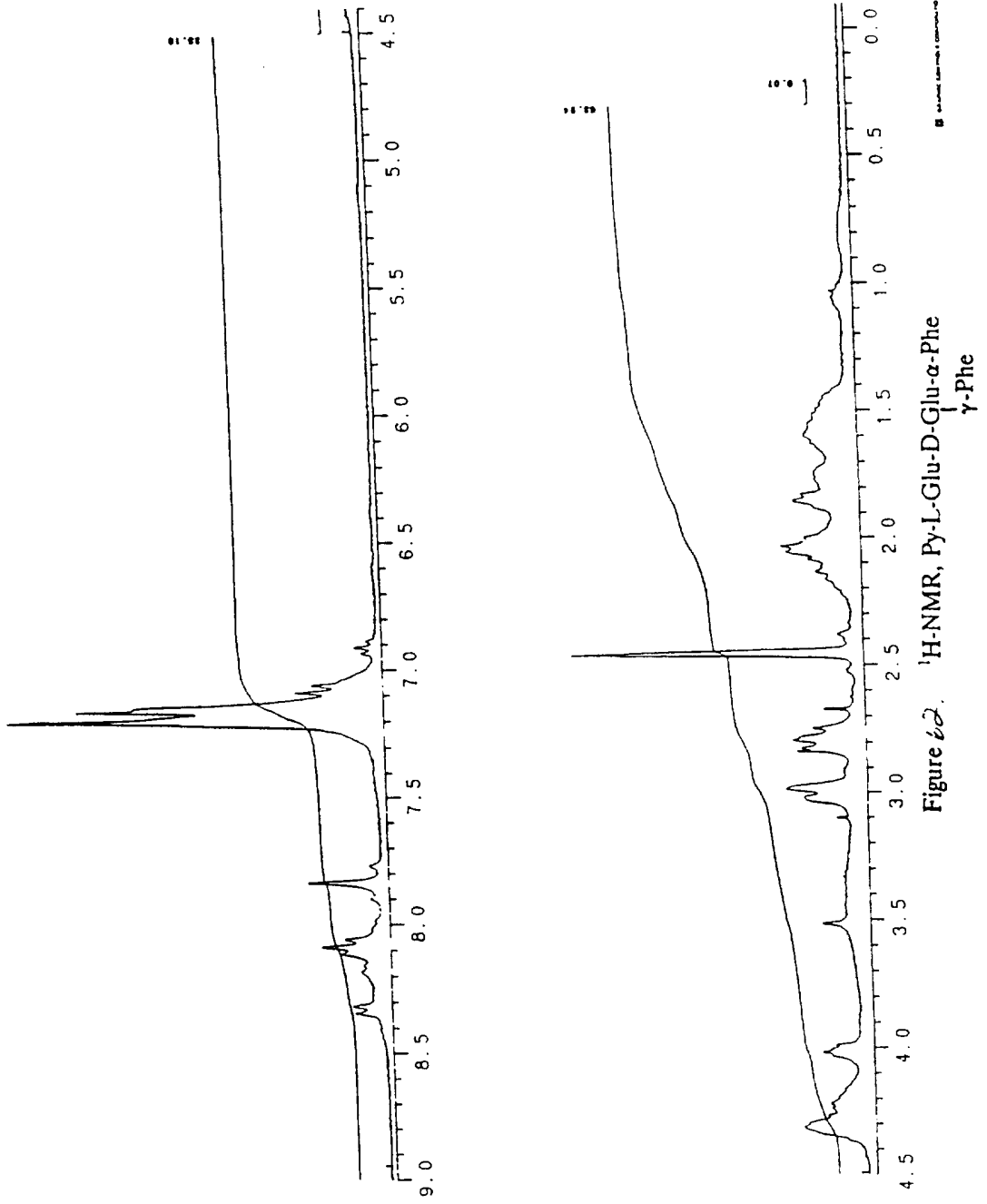


Figure 62. ¹H-NMR, Py-L-Glu-D-Glu-α-Phe-γ-Phe

63/101

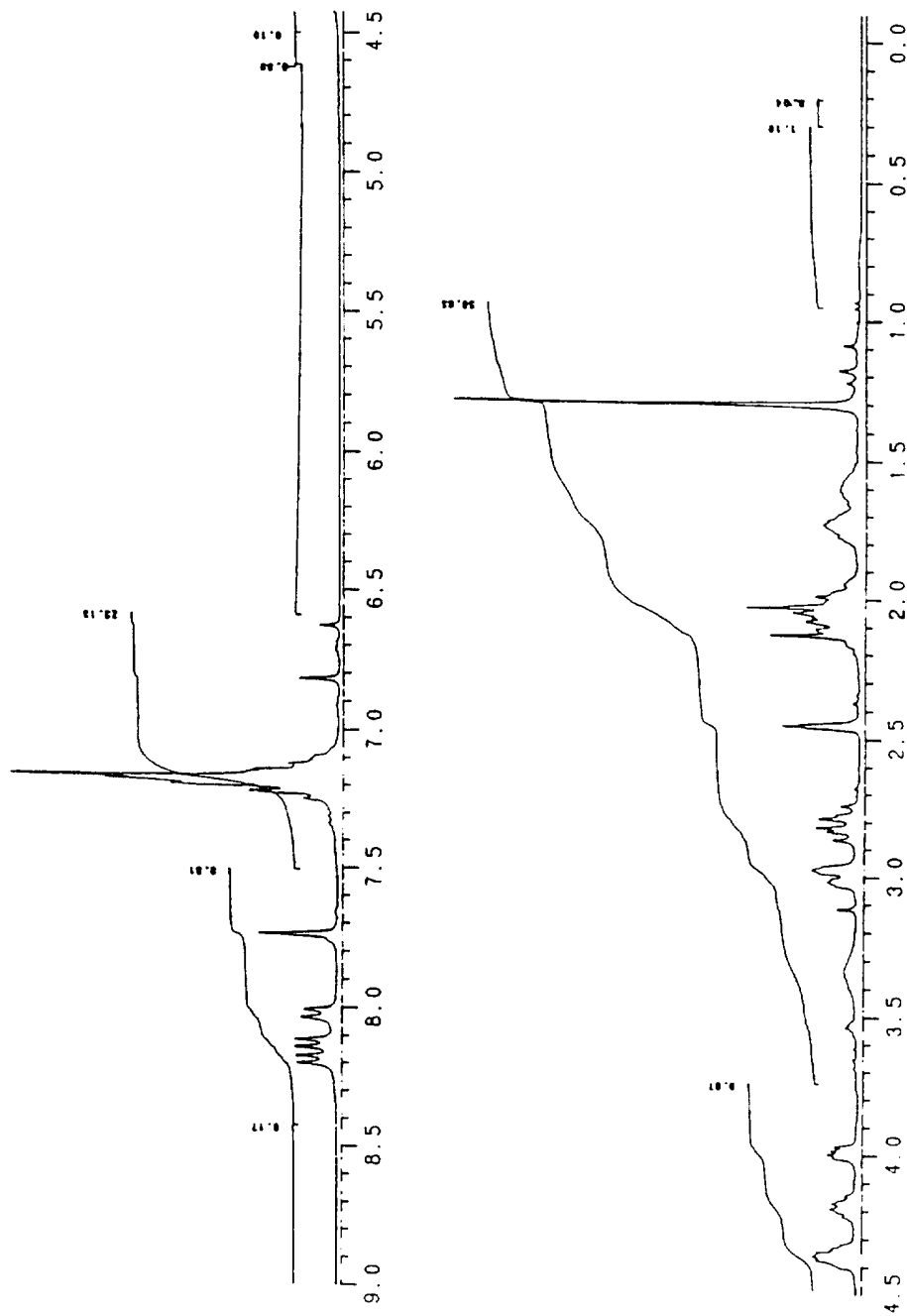


Figure 63 ¹H-NMR, PyGlu-Glu-α-Gly-γ-Phe

64/101

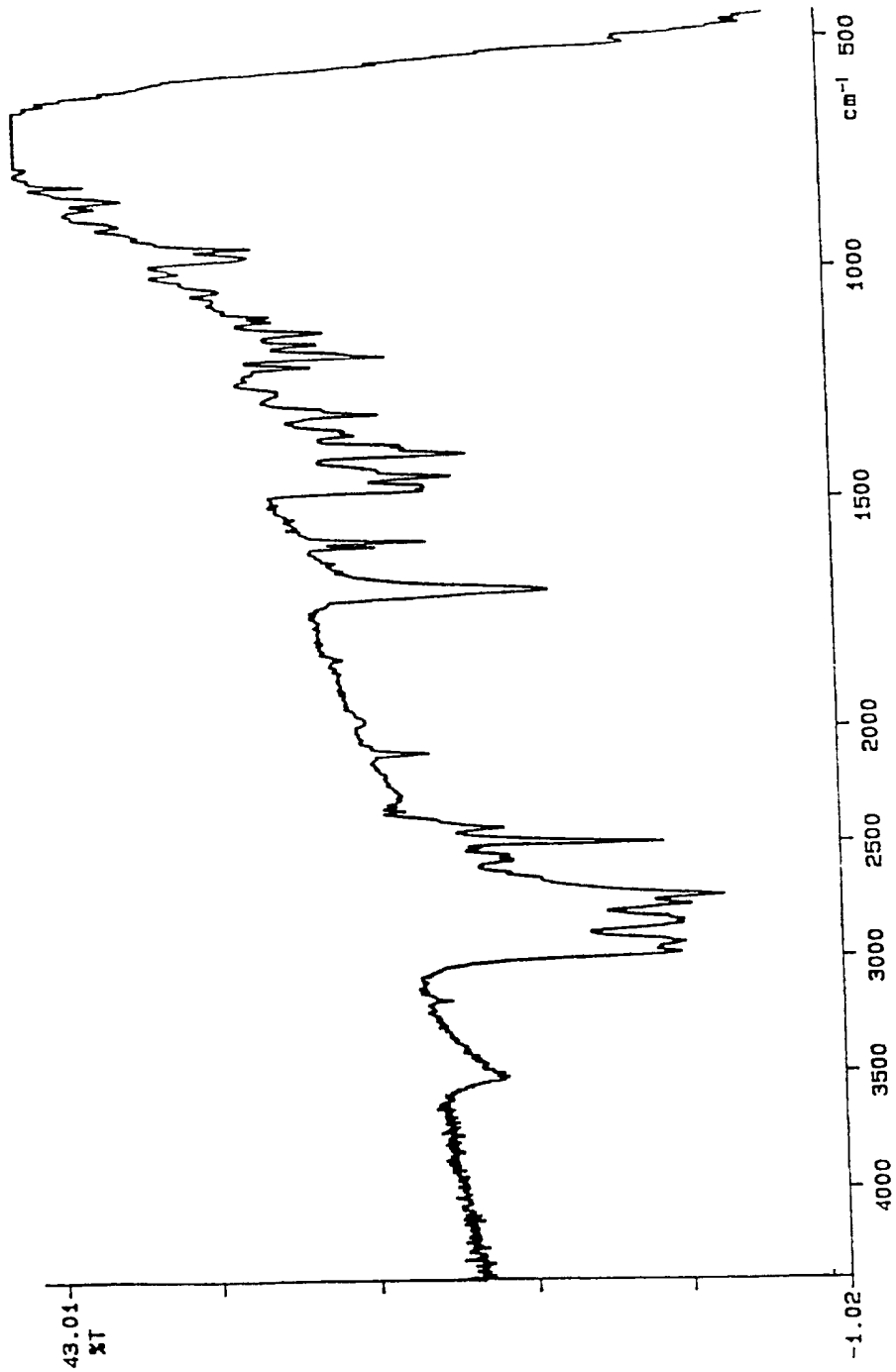


Figure 64 IR, PyGlu-Glu- α -Gly- γ -Phe

66/101

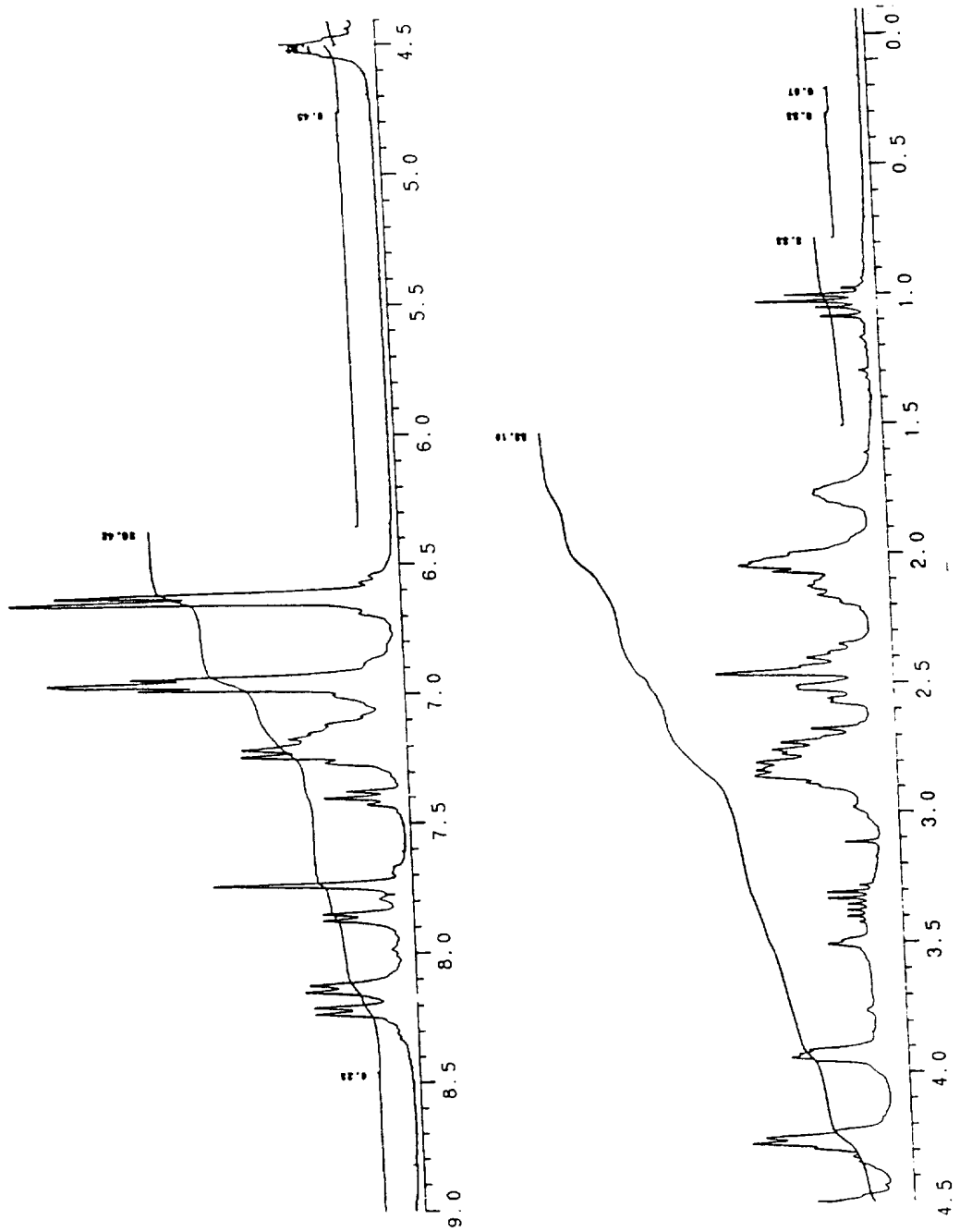


Figure 66 ¹H-NMR, PyGlu-Asp- α -Phe
 β -Tyr

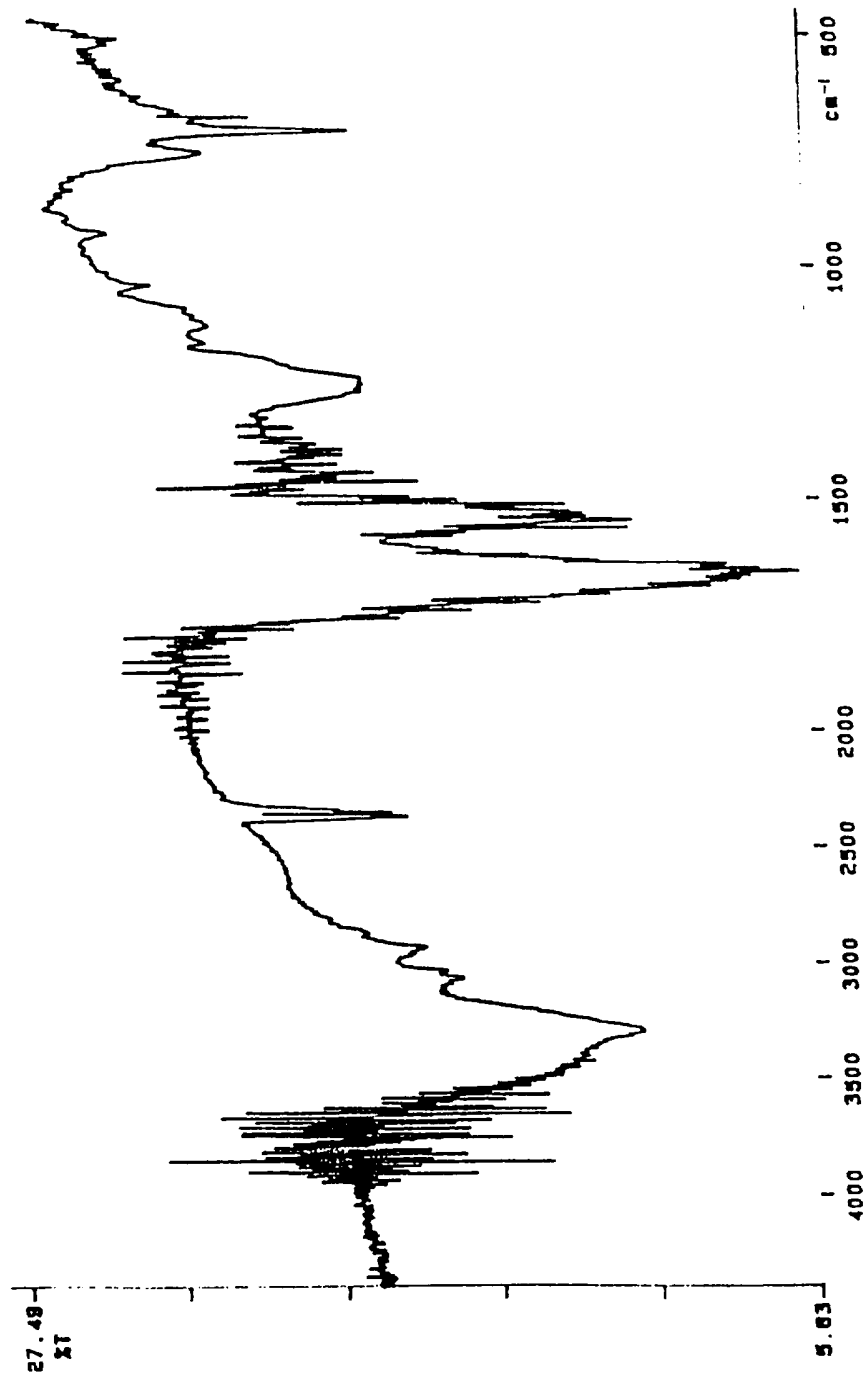


Figure 67 IR, PyGlu-Glu- α -Phe
 γ -Phe (Sp932)

10/1/97

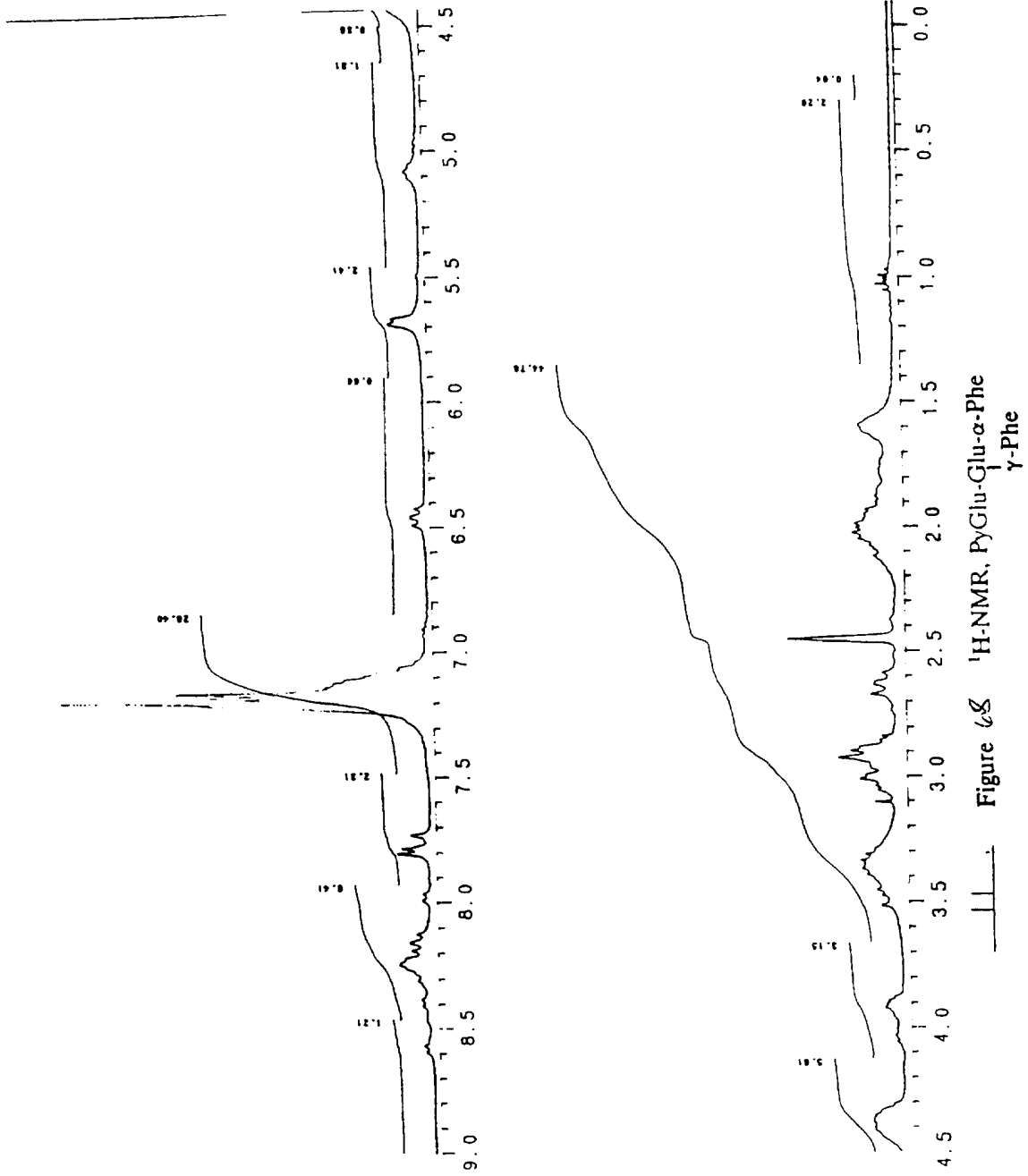


Figure 68 ¹H-NMR, PyGlu-Glu-α-Phe
γ-Phe

69/101

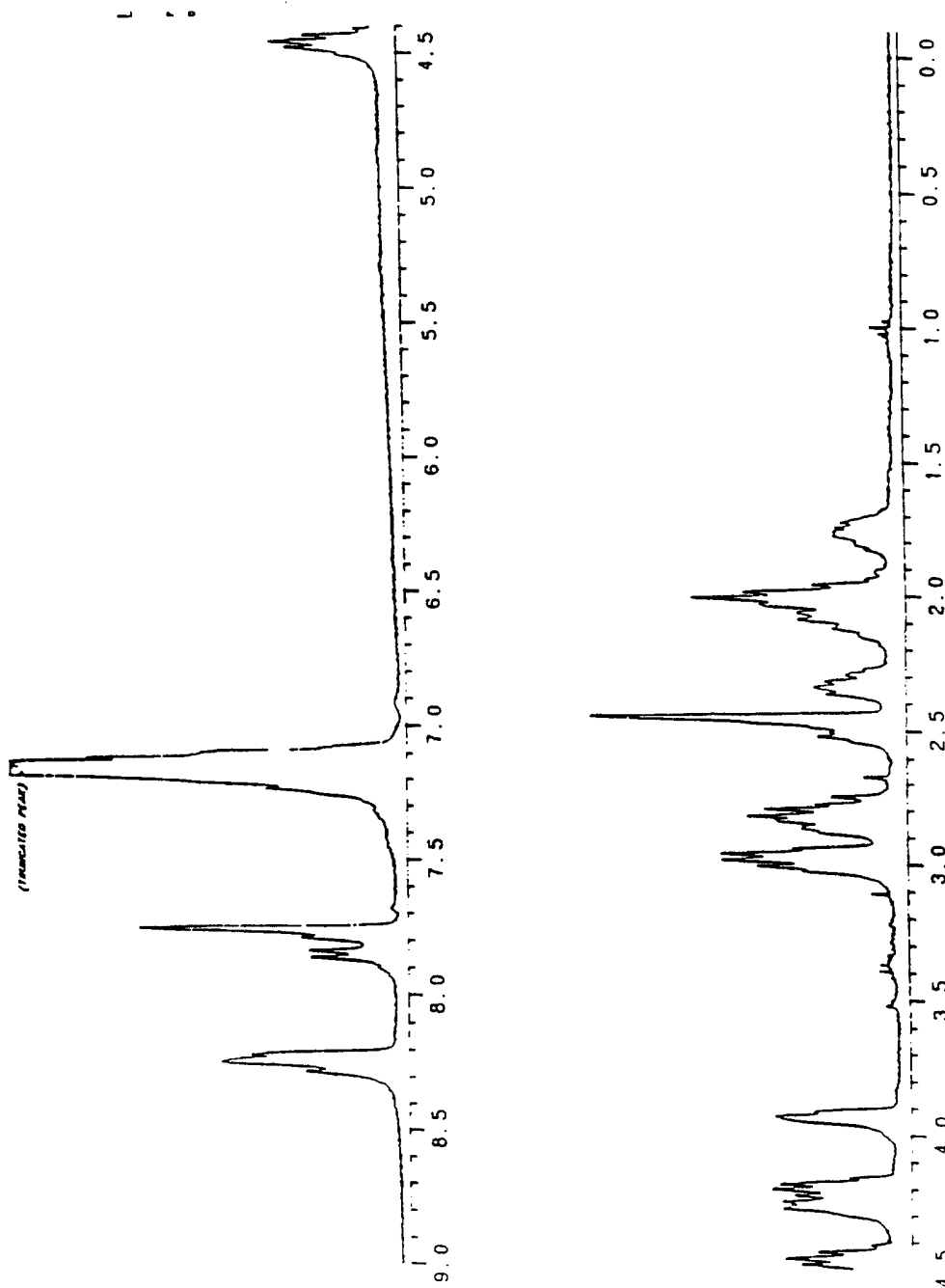
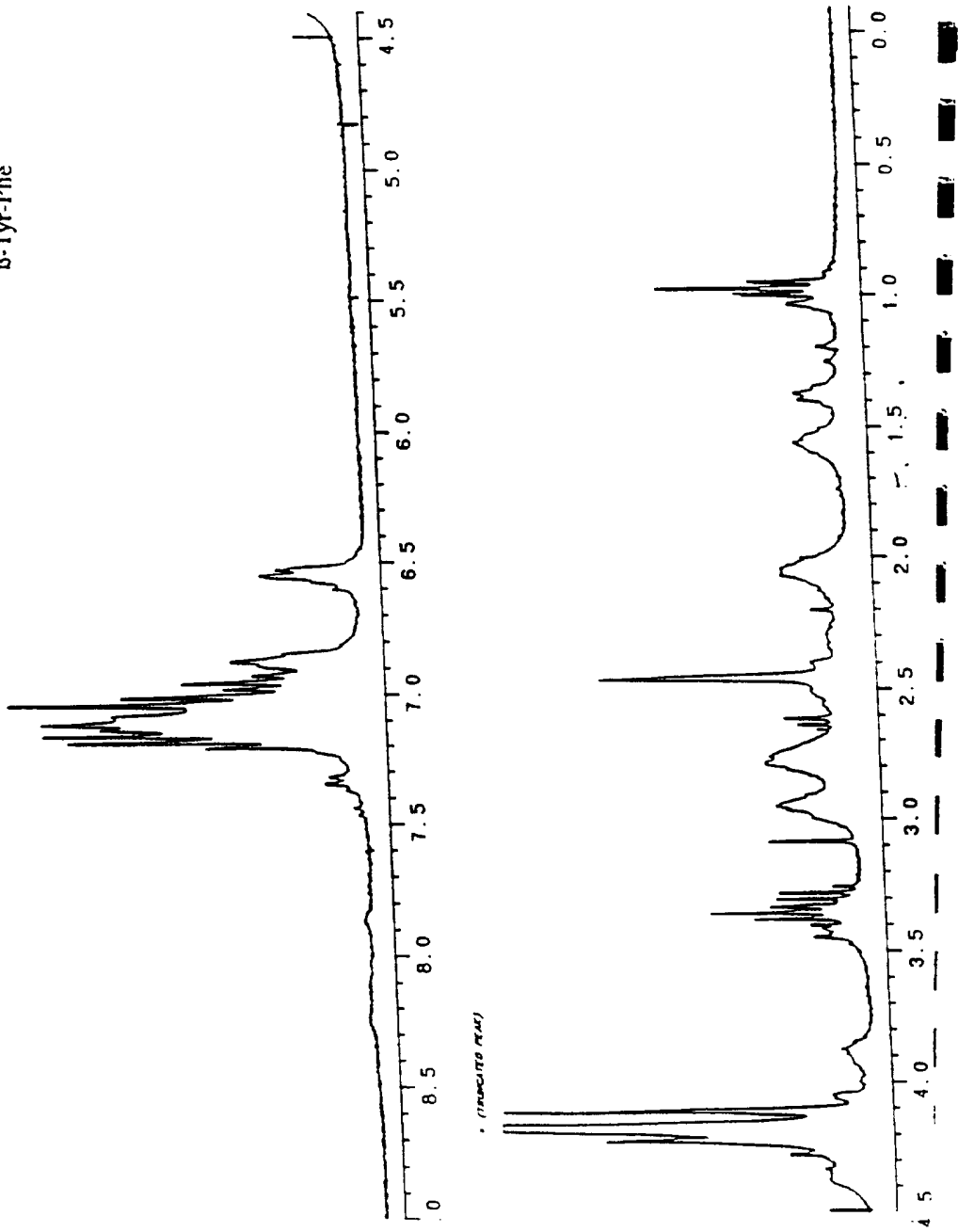


Figure 69 ¹H-NMR, PyGlu-Asp-α-Phe β-Phe

Figure 70 ¹H-NMR, PyGlu-Asp- α -Tyr-Phe
 β -Tyr-Phe



10/1/01

Figure 11
COSSY, L-Pro-L-Glu-γ-Phe,
C[C@@H](C(=O)O)[C@@H](C(=O)O)[C@@H](C(=O)O)C1=CC=CC=C1

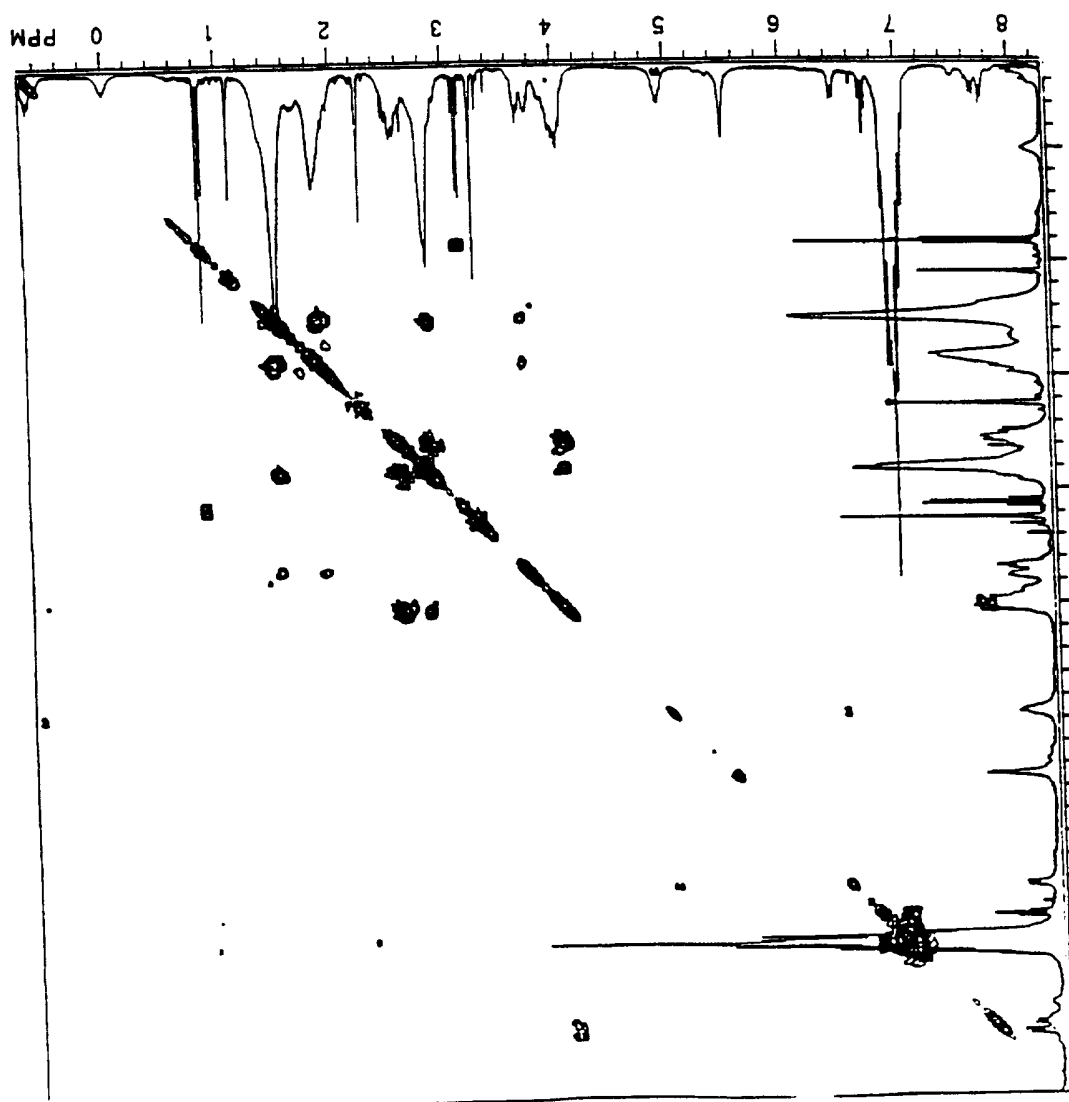
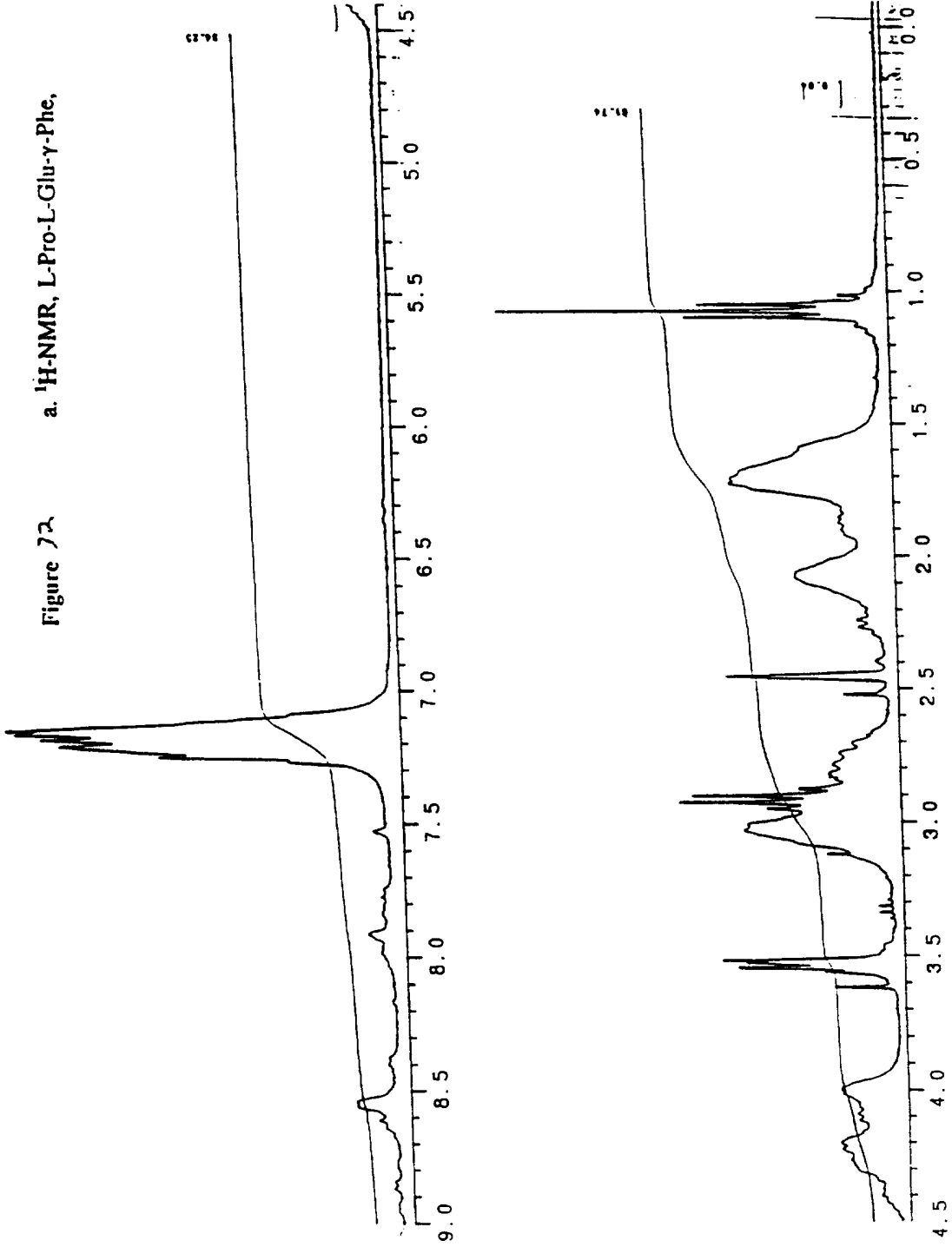
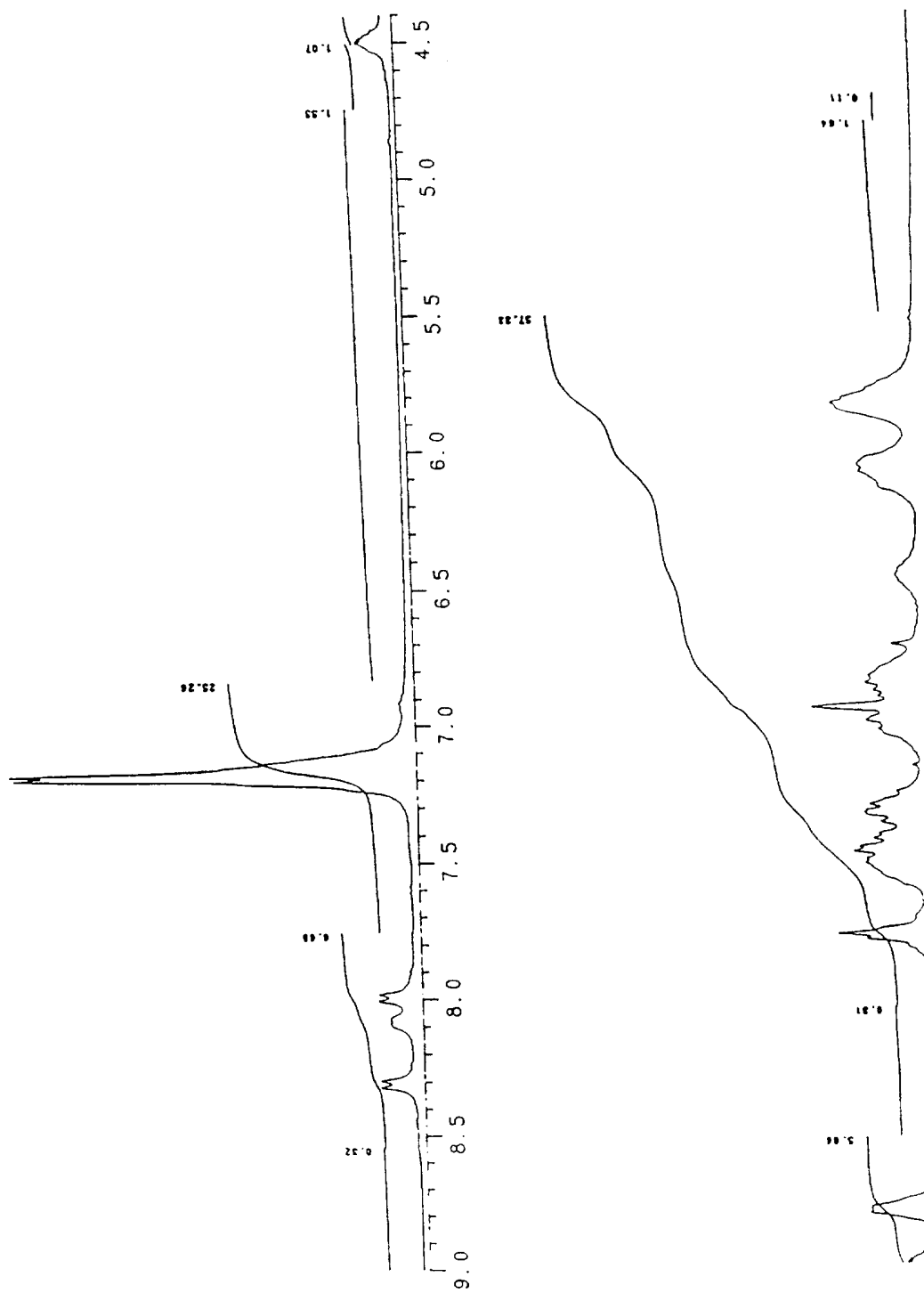


Figure 7a a. ¹H-NMR, L-Pro-L-Glu-γ-Phe,



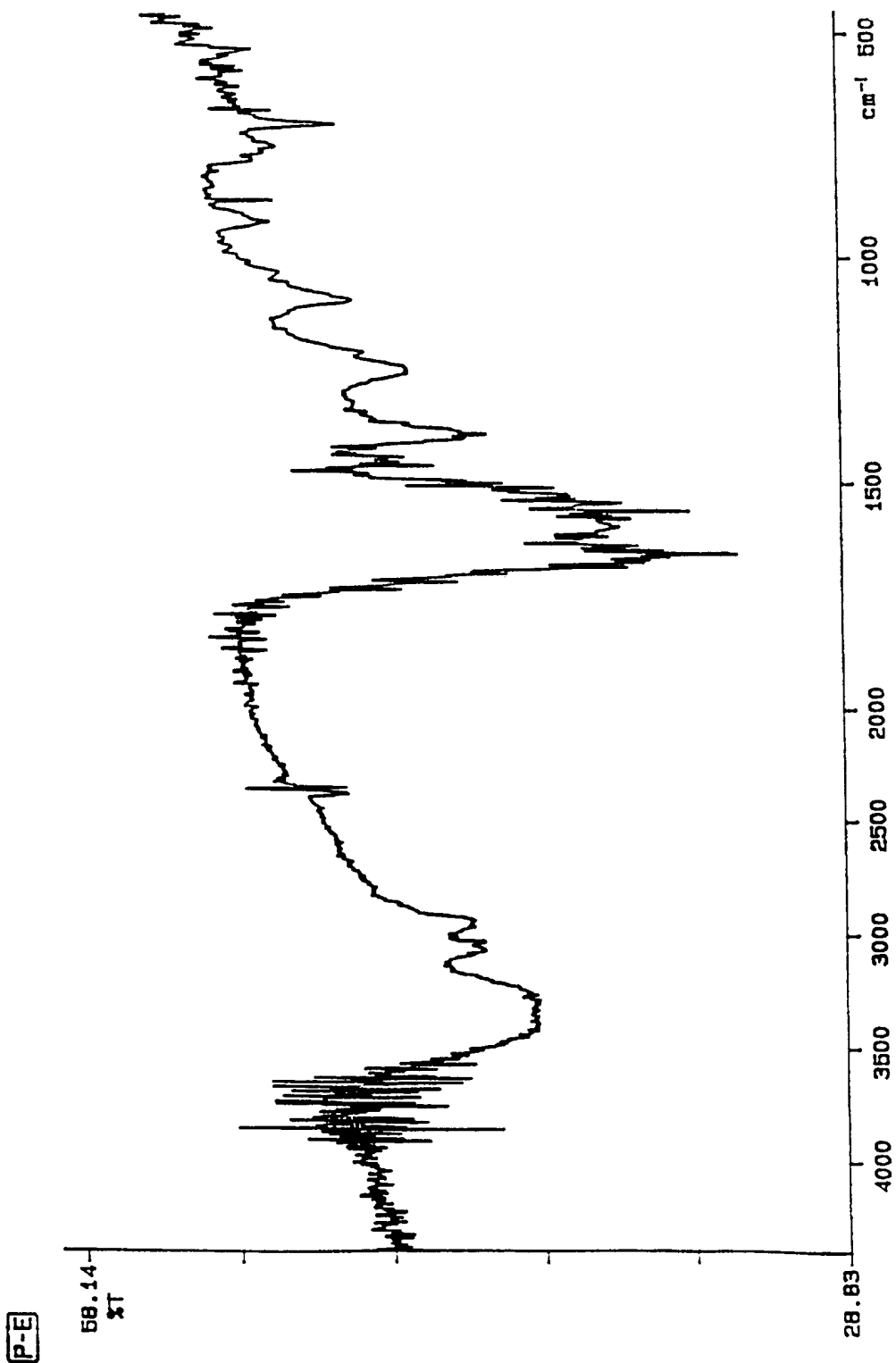
73/101



¹H-NMR, L-Pro-L-Asp-β-Phe,

Figure 73

10/1/96



IR, L-Pro-Glu- γ -Phe.

Figure 74

75/101

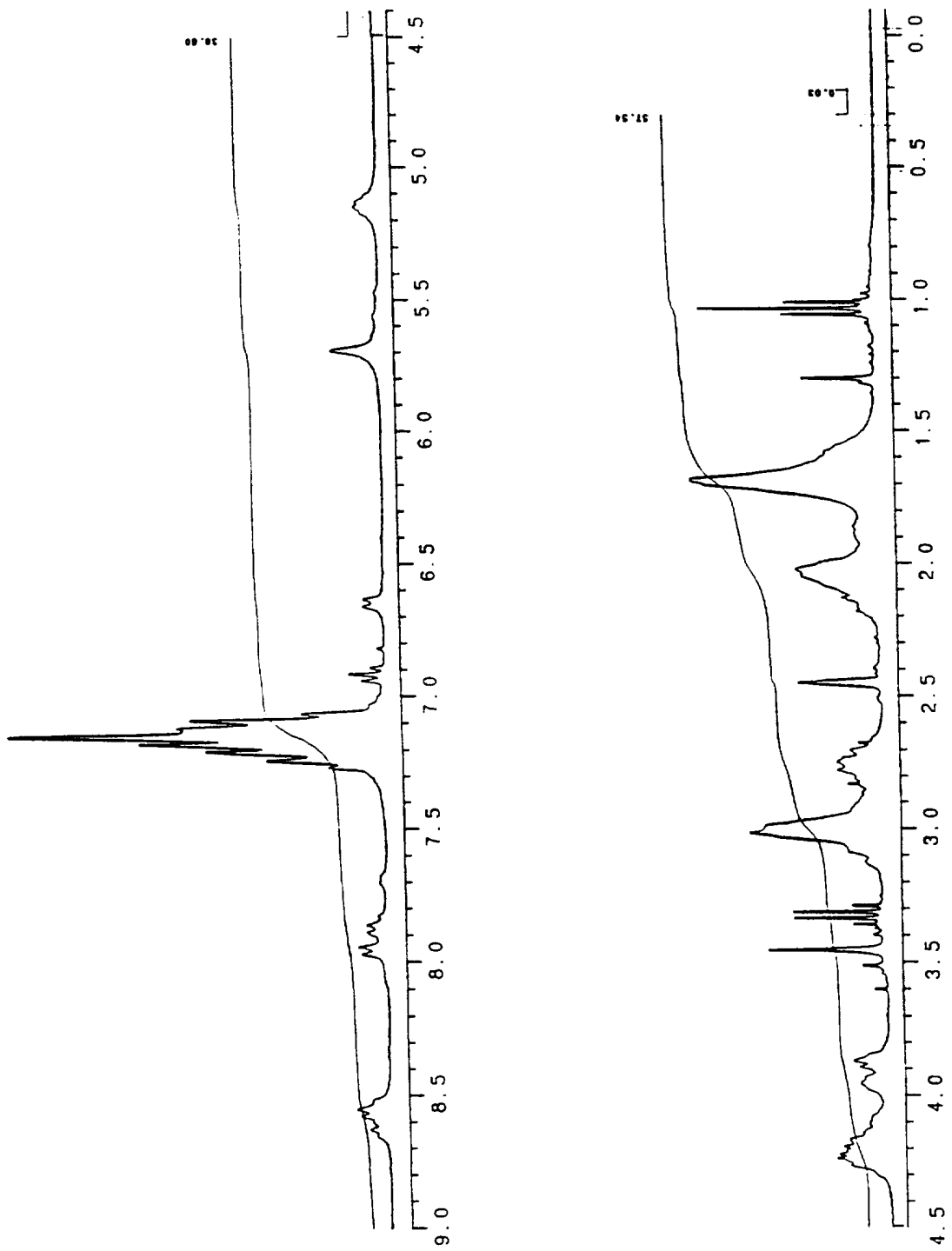


Figure 75 ¹H-NMR, L-Pro-Glu-γ-Phe,

76/101

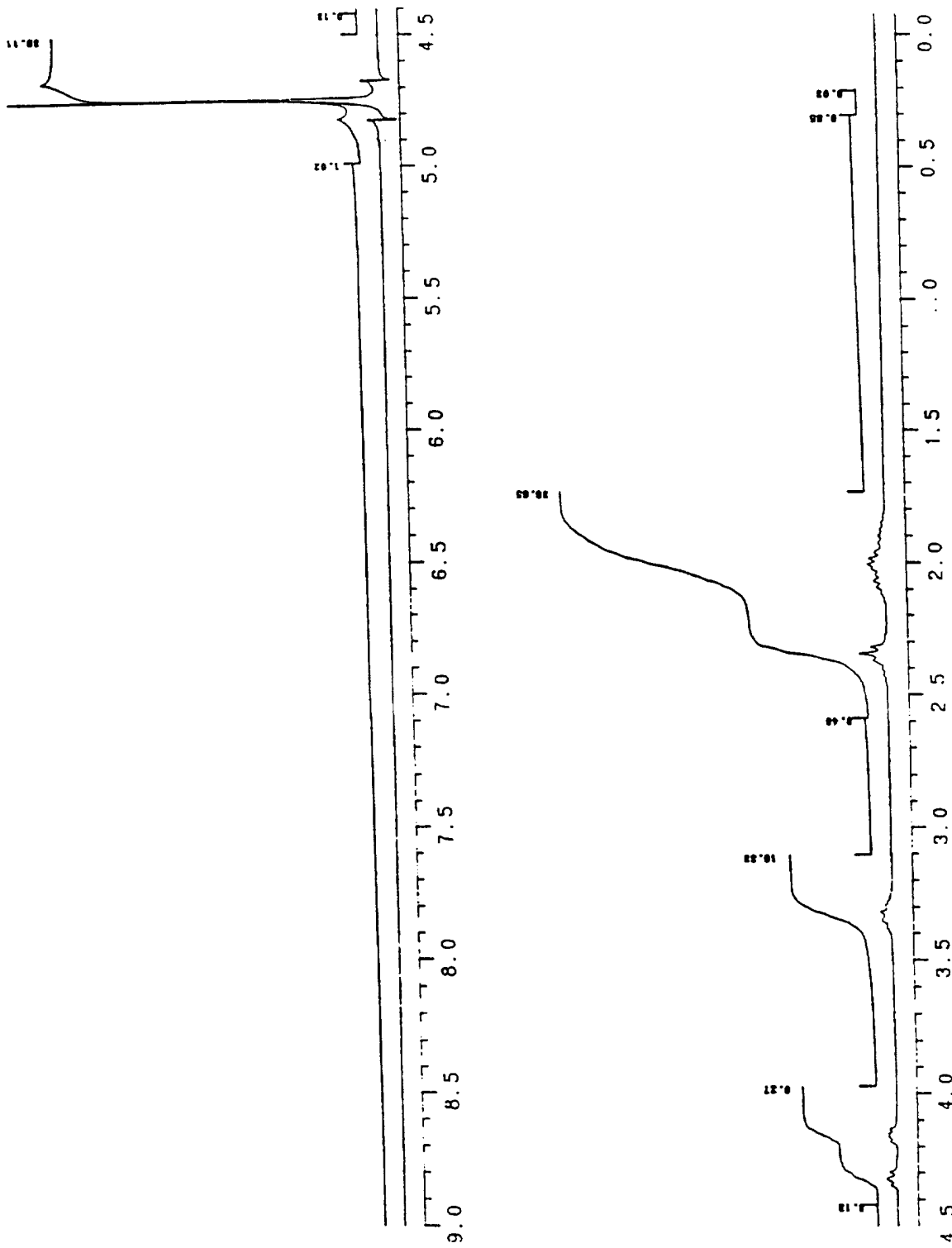


Figure 7c ¹H-NMR, L-Pro-Glu,

99/101

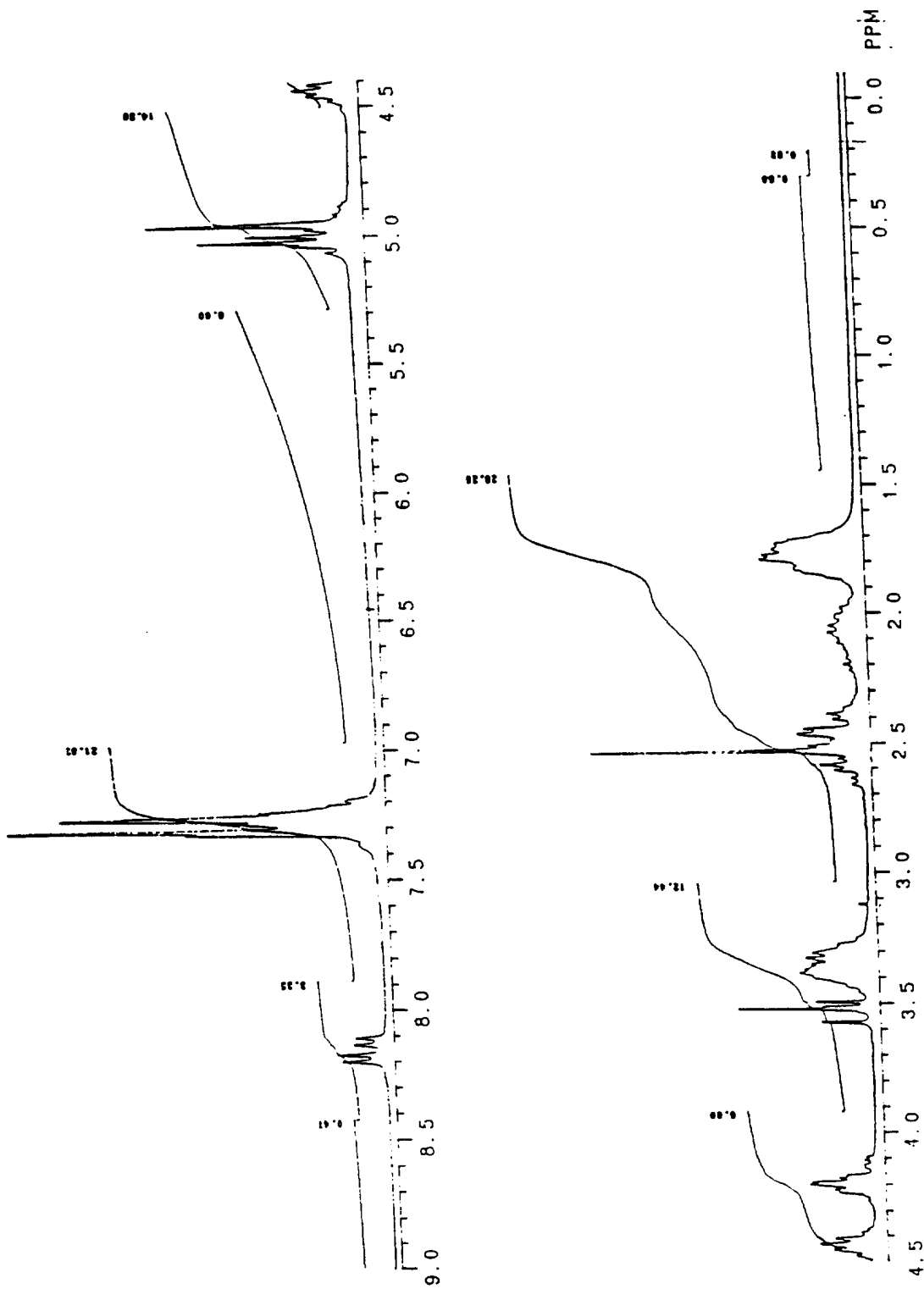


Figure 77 ¹H-NMR L-Pro-Asp.

10/1/86

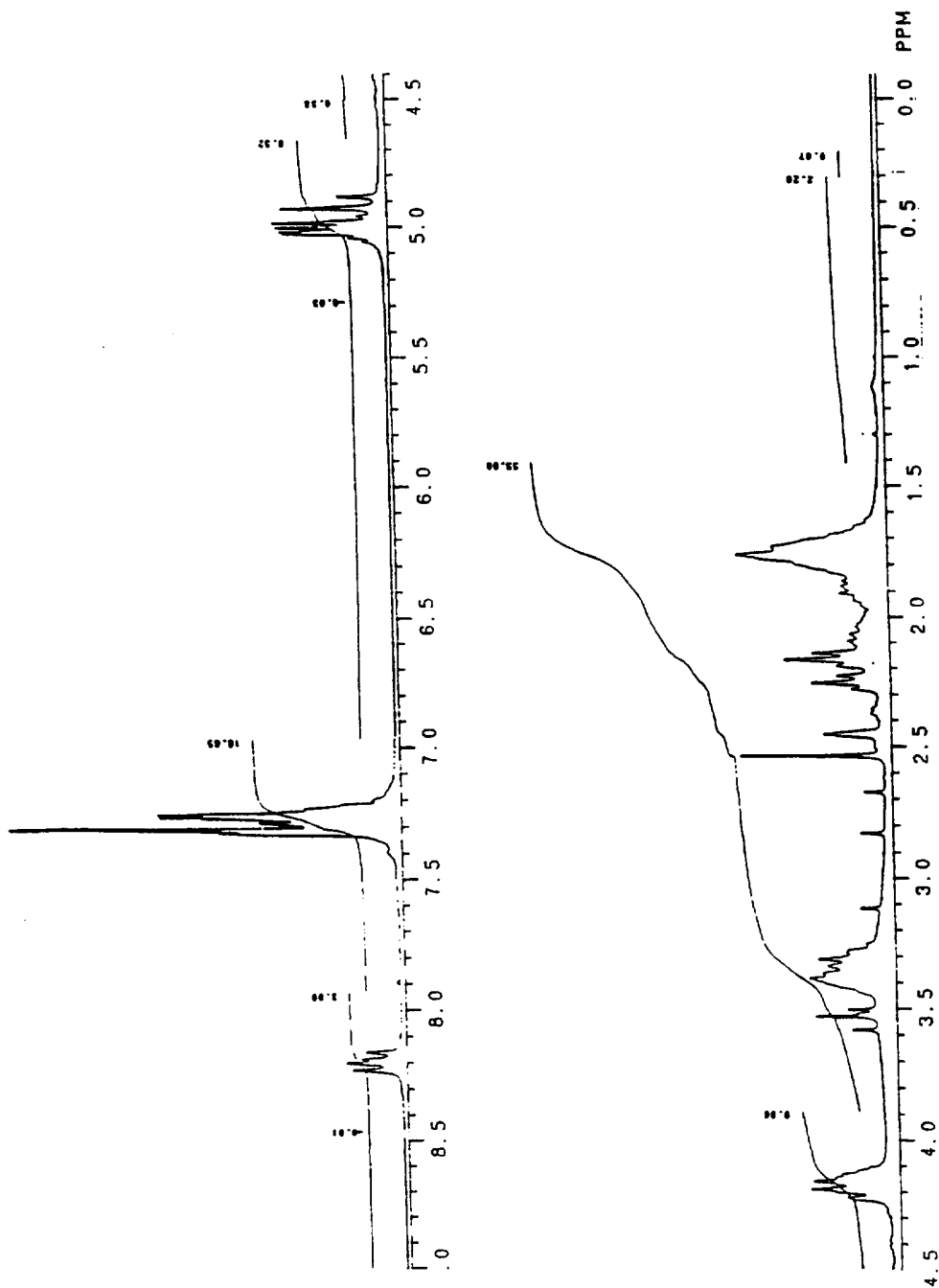


Figure 78 ¹H-NMR of L-Pro-Glu.

7/9/01

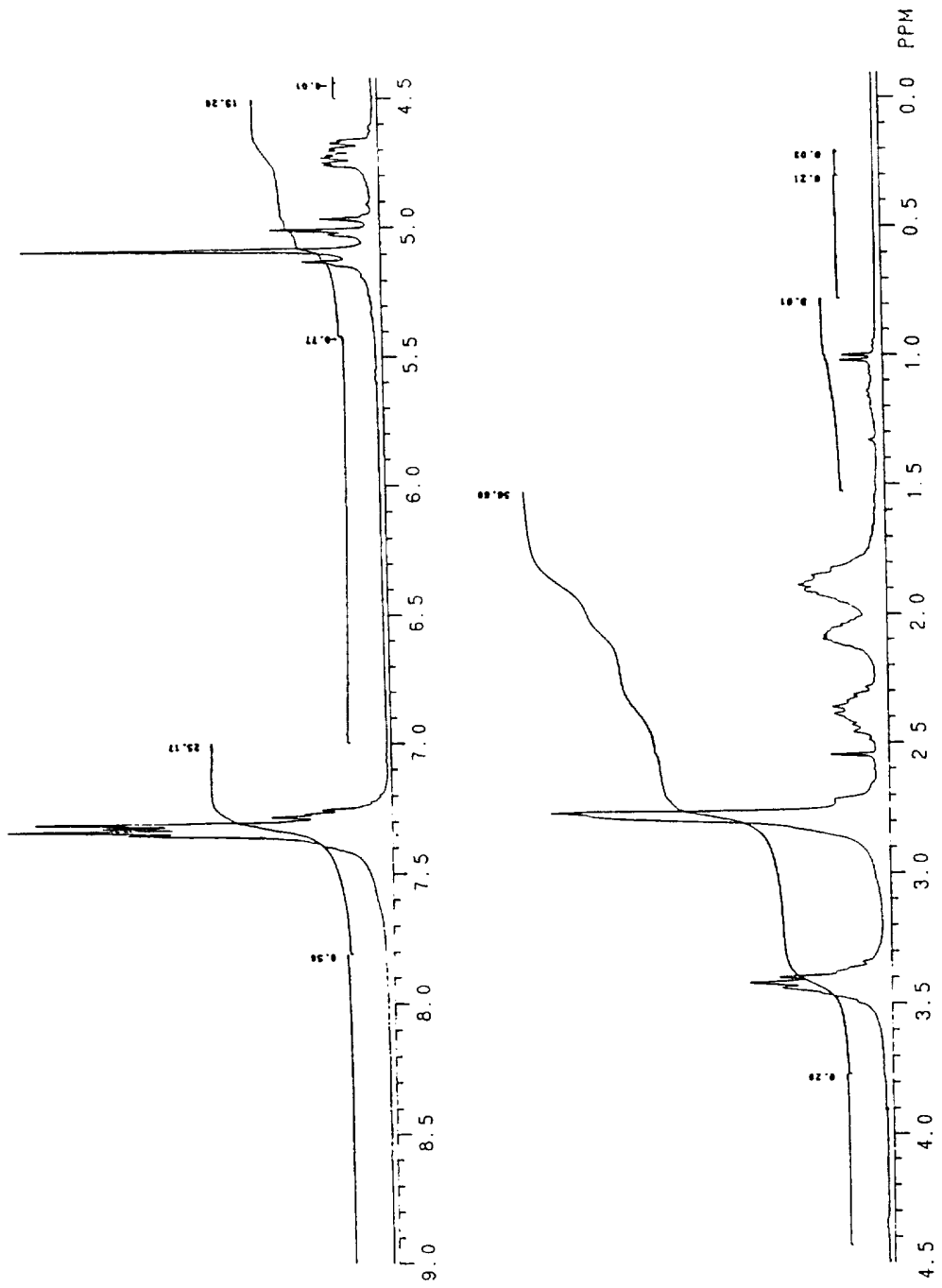


Figure 79 ¹H-NMR, β z-ProNHS,

10/108

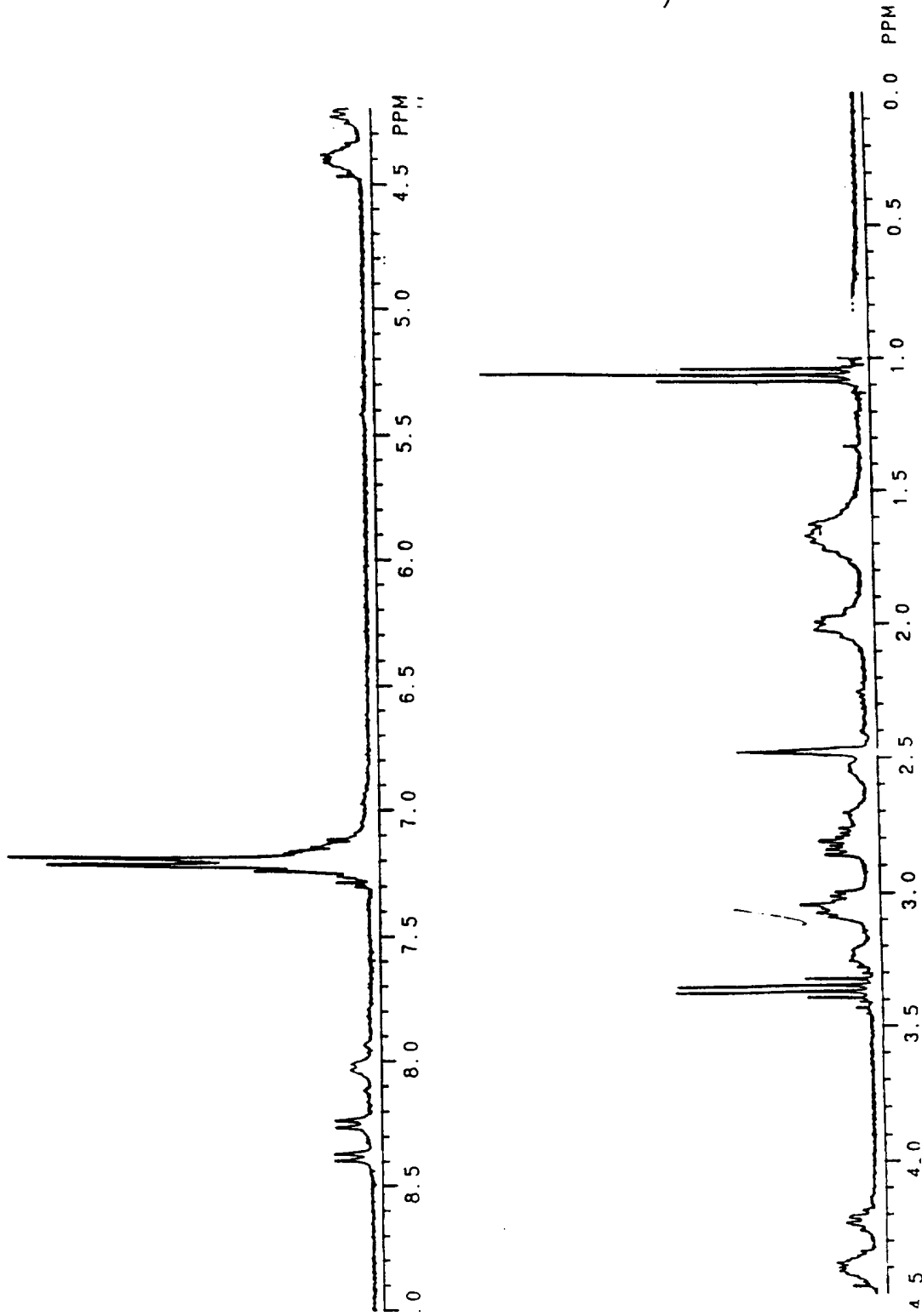


Figure 80 ¹H-NMR, L-Pro-Glu-α-Phe-γ-Phe,

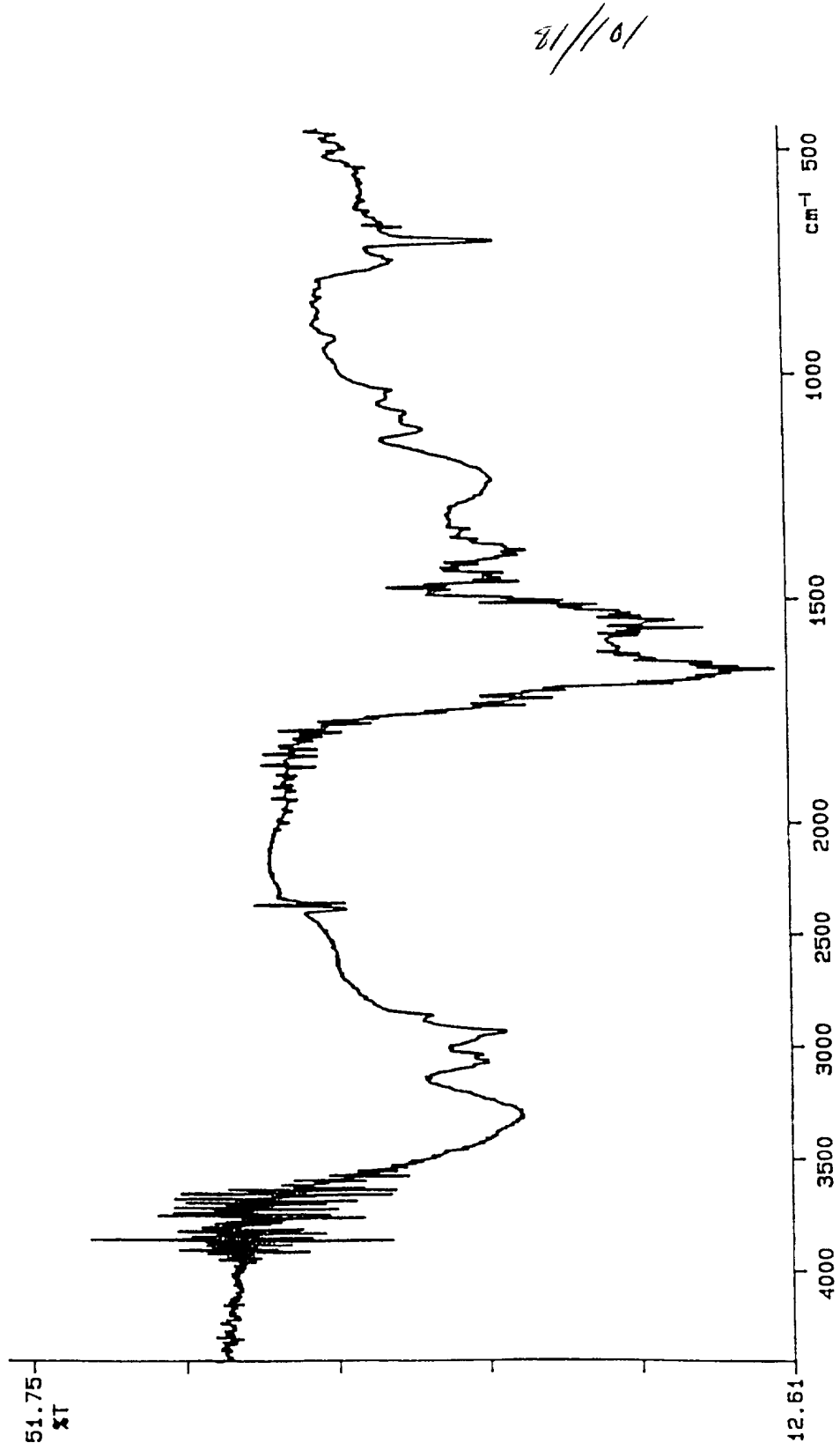
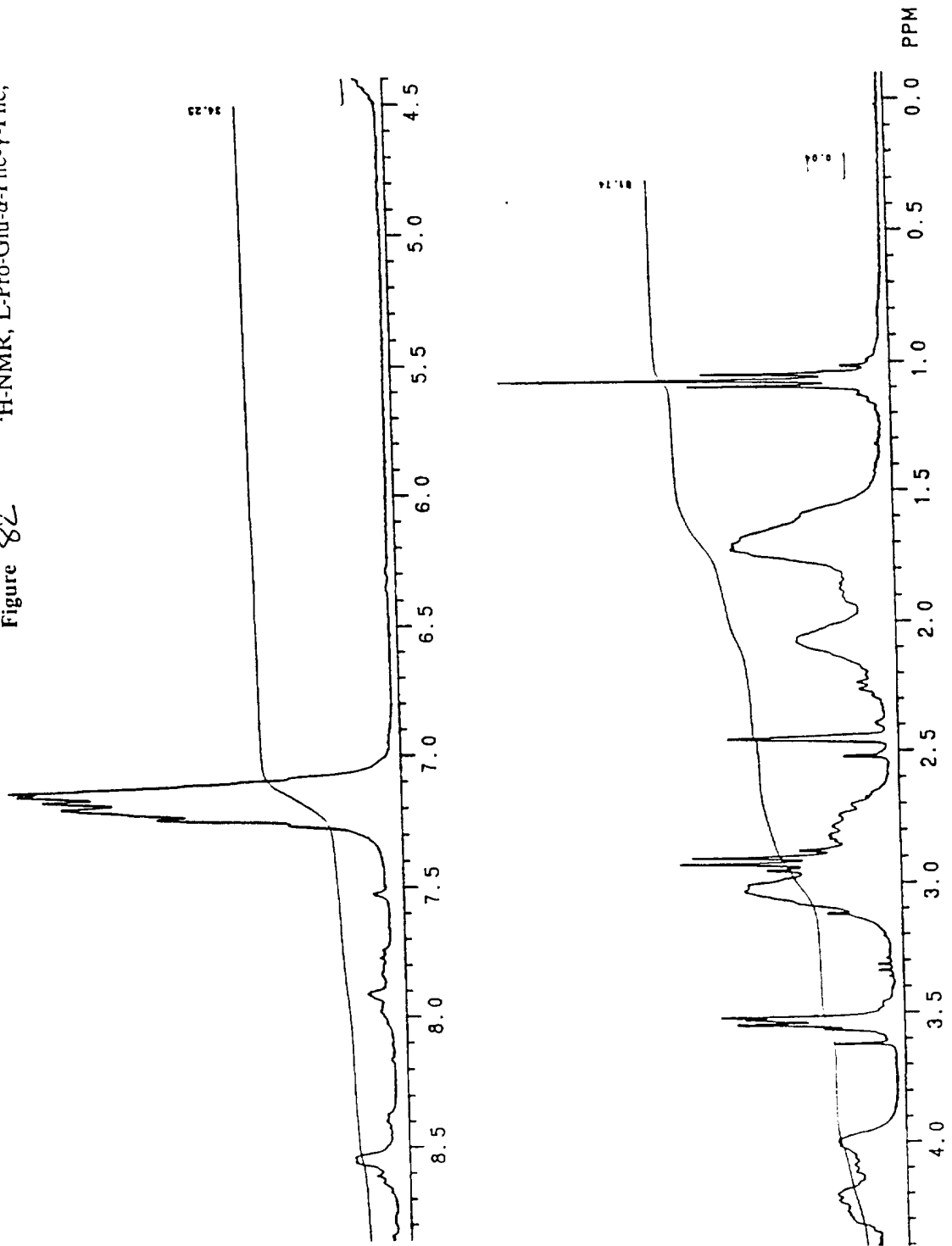


Figure 8) IR, L-Pro-Glu- α -Phe- γ -Phe,

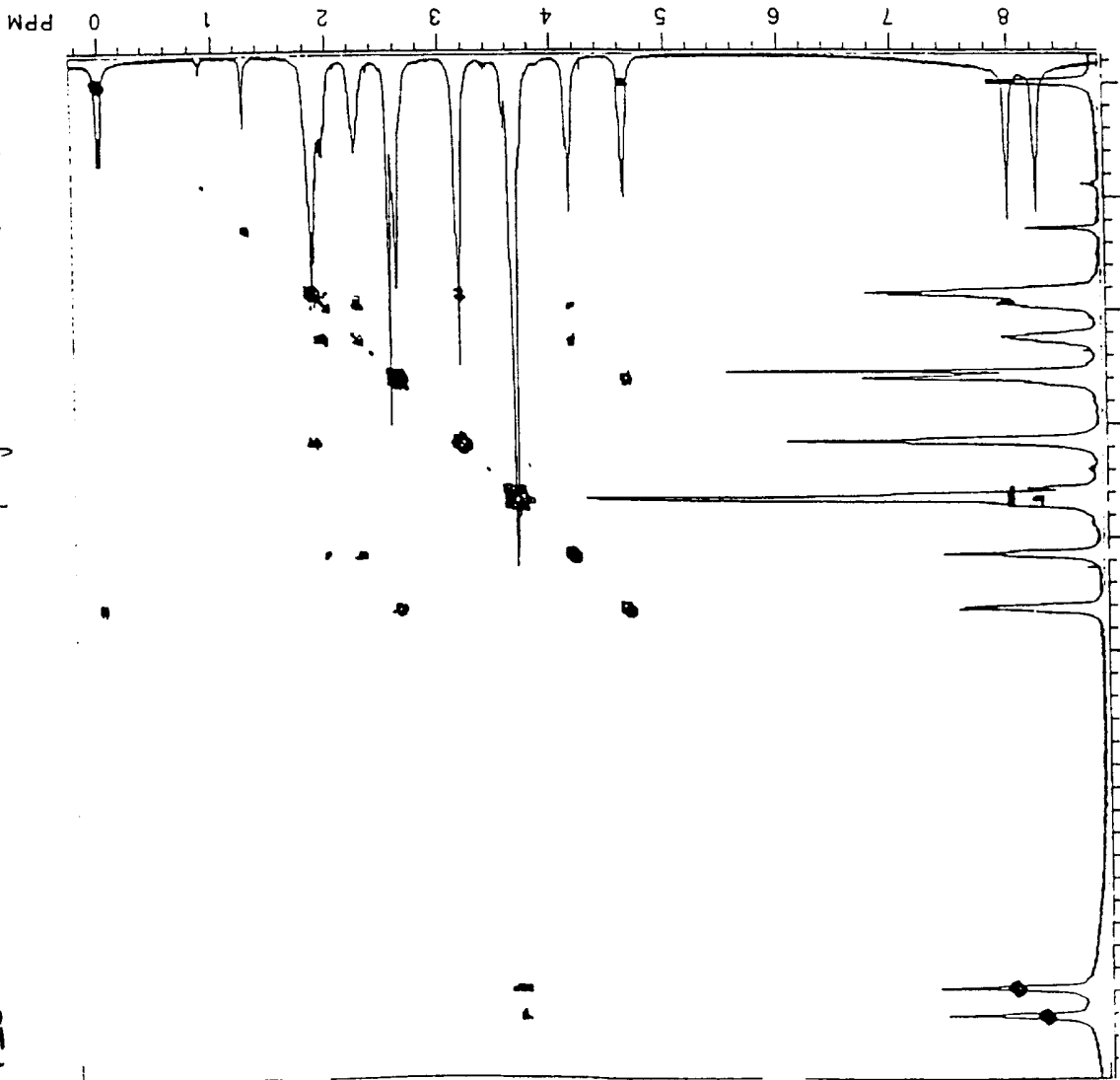
82/101

Figure 82 ¹H-NMR, L-Pro-Glu- α -Phe- γ -Phe,



83/101

FIGURE 83. ¹H NMR Cossy of a: L-Pro-L-Asp-α-Gly-β-Gly,



84/01

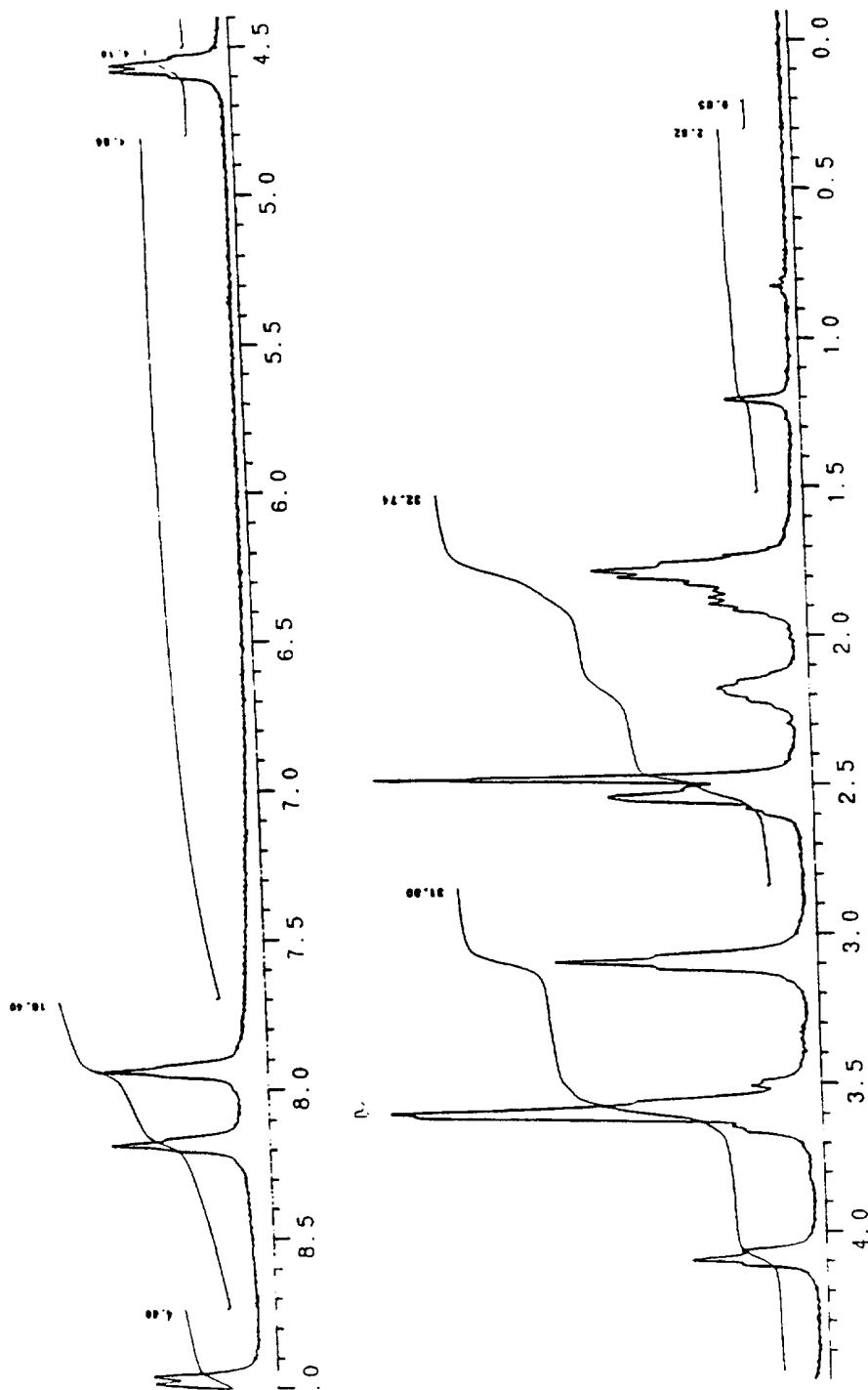


Figure 84 b. ¹H NMR of L-Pro-L-Asp-α-Gly-B-Gly.

Figure 85 ¹H-NMR, L-Pro-L-Asp- α -Phe- β -Phe,

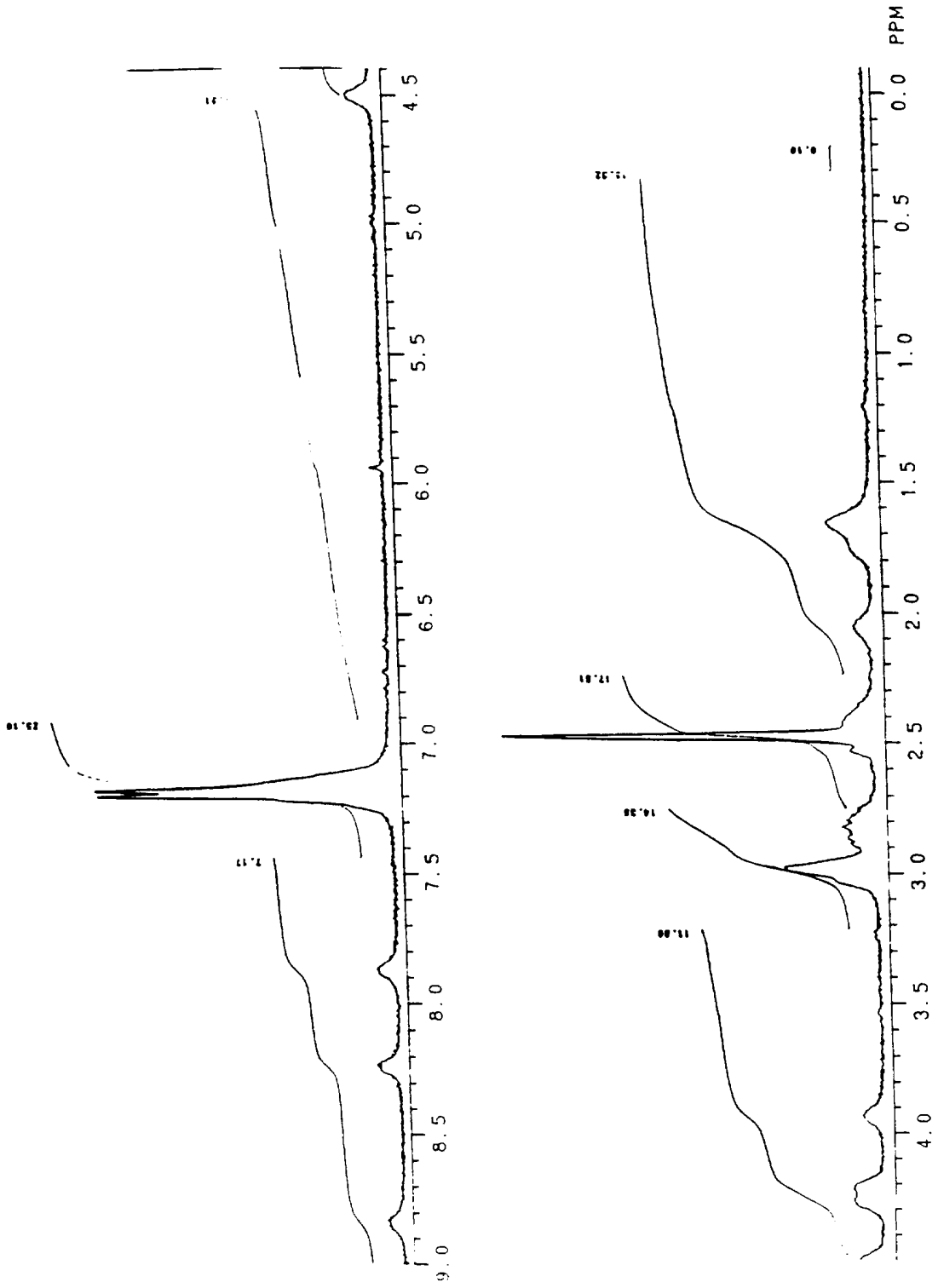
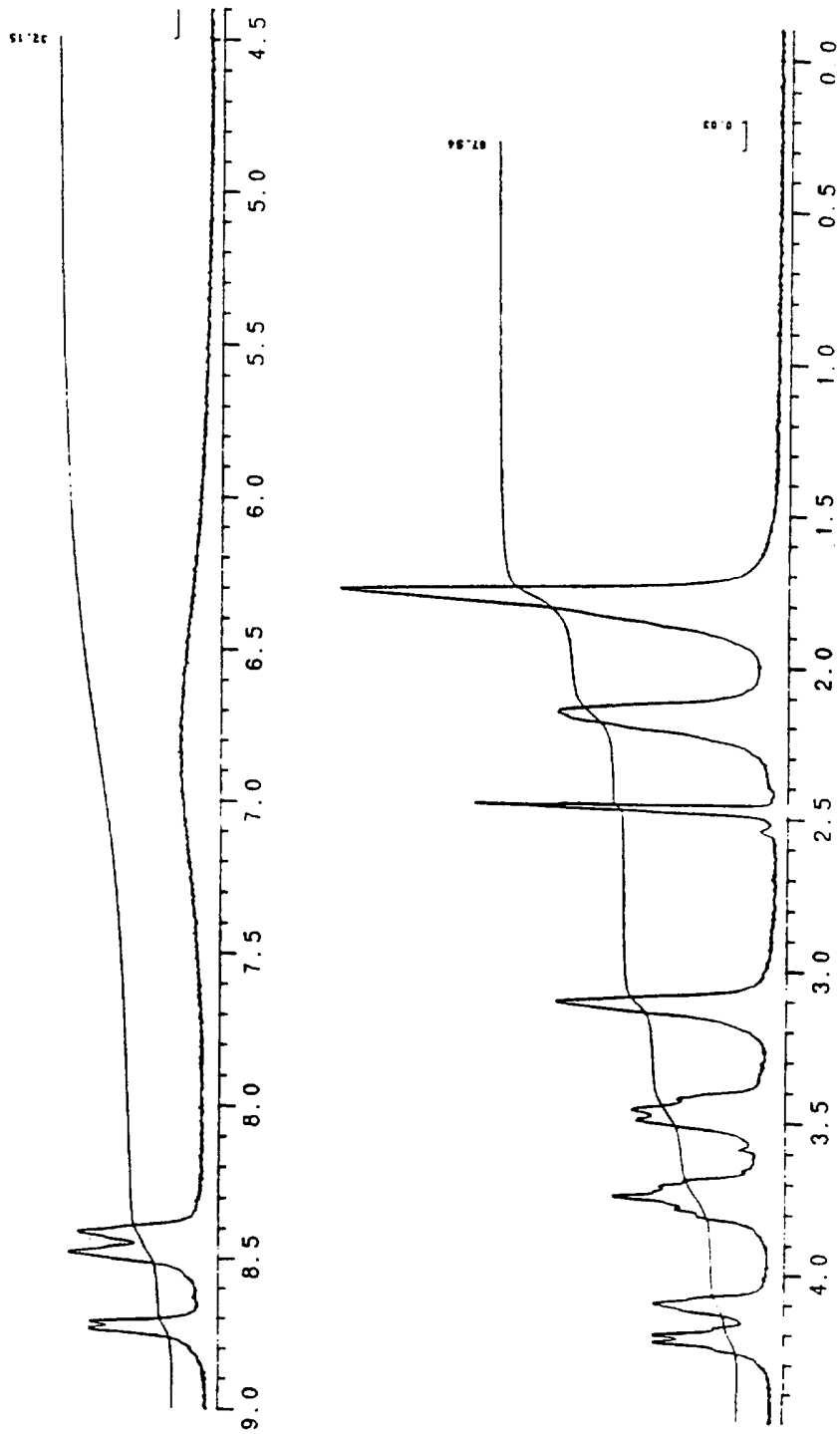


Figure 86 a. ¹H-NMR, L-Pro-L-Glu-α-Gly-γ-Gly,



89/101

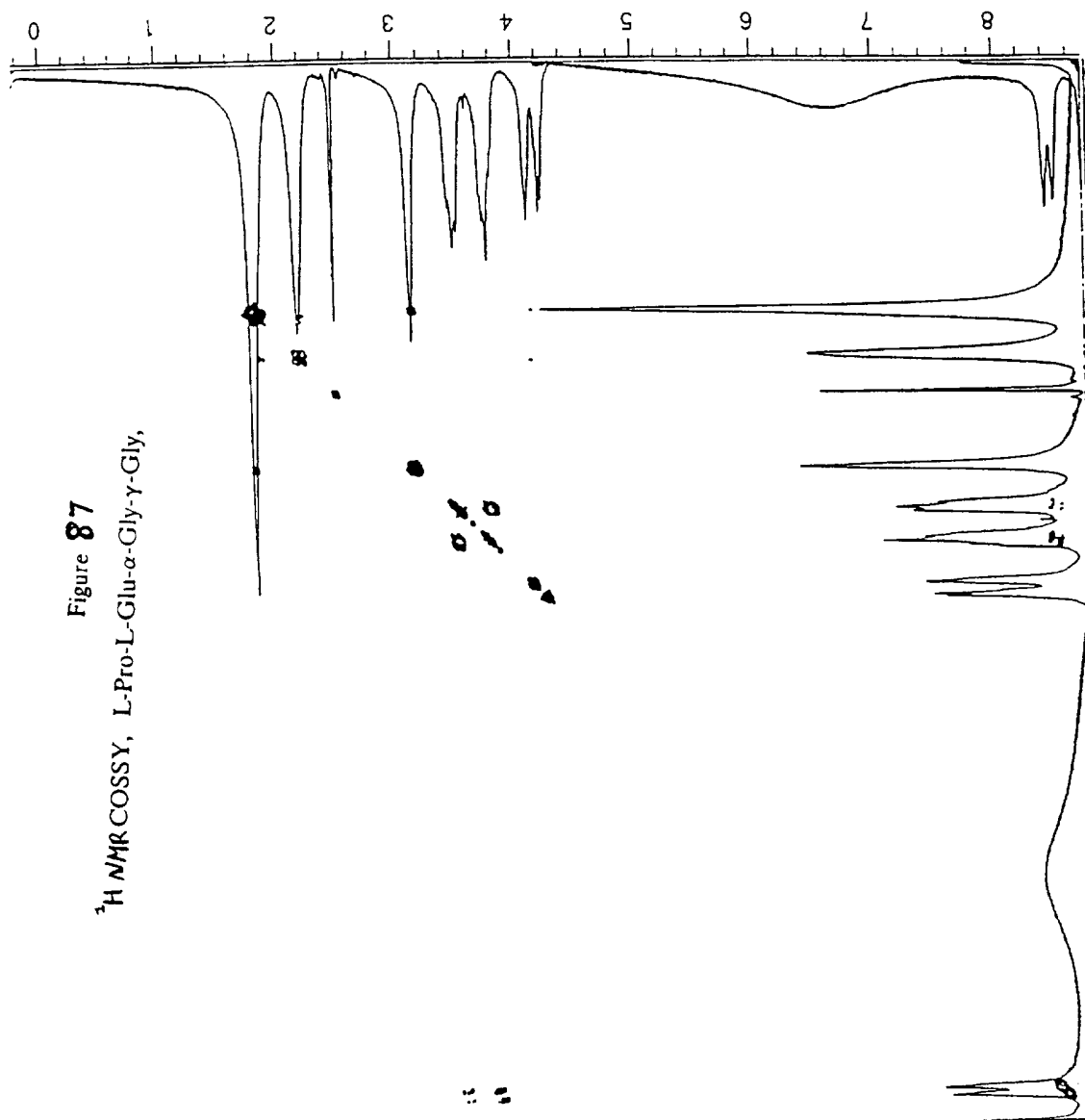
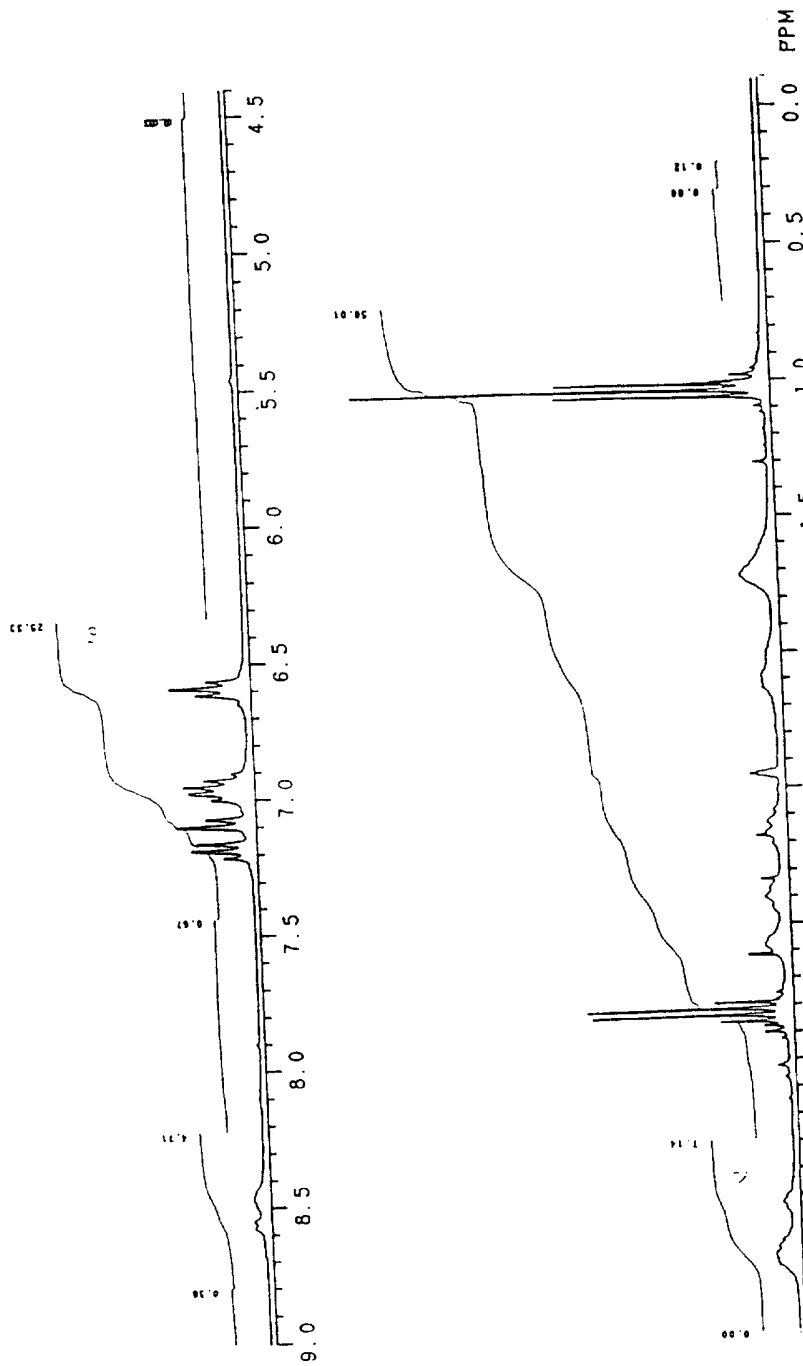


Figure 87
¹H NMR COSY, L-Pro-L-Glu-α-Gly-γ-Gly,

Figure 88 ¹H-NMR, L-Pro-L-Glu-α-Tyr-γ-Tyr,



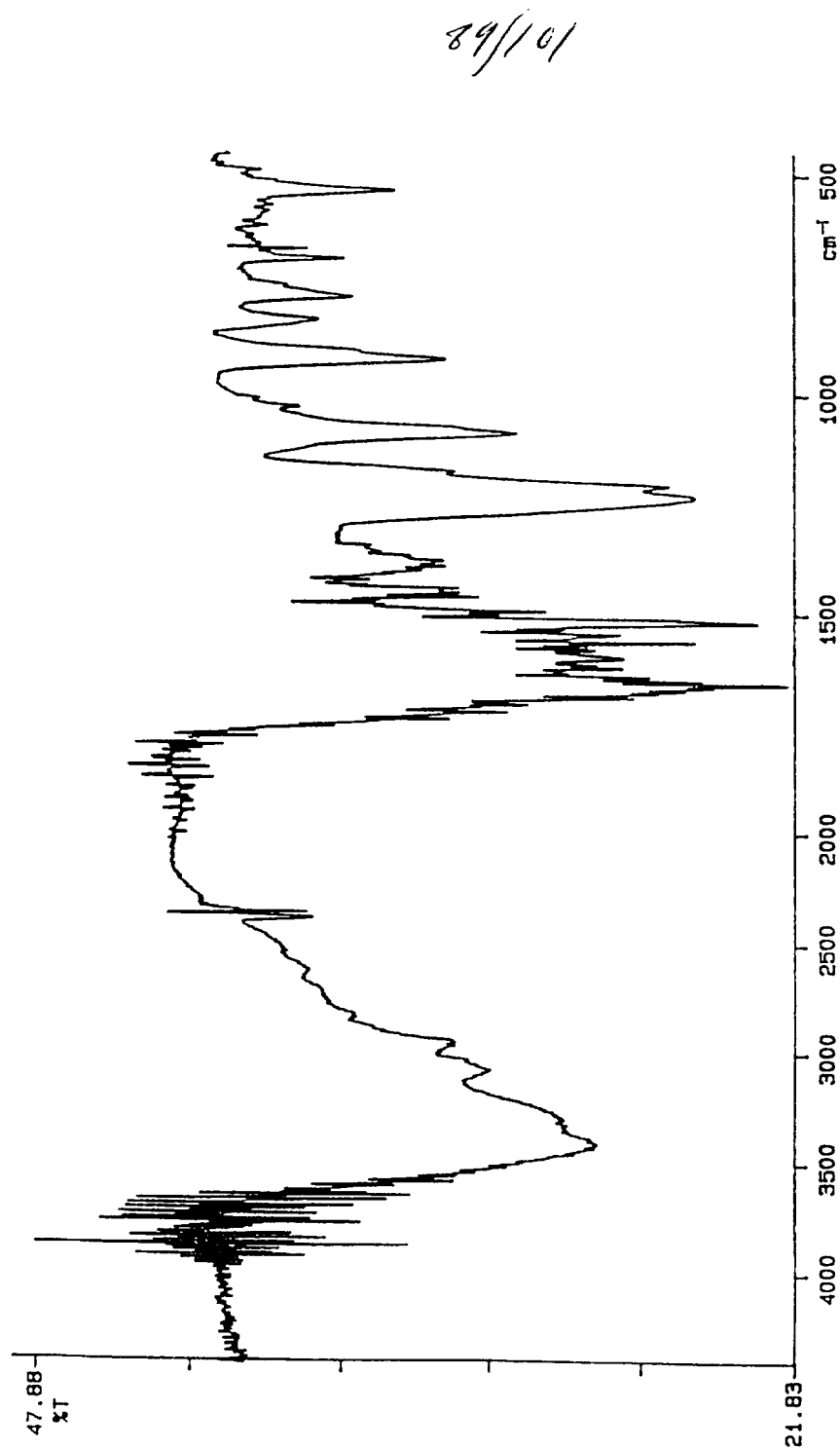
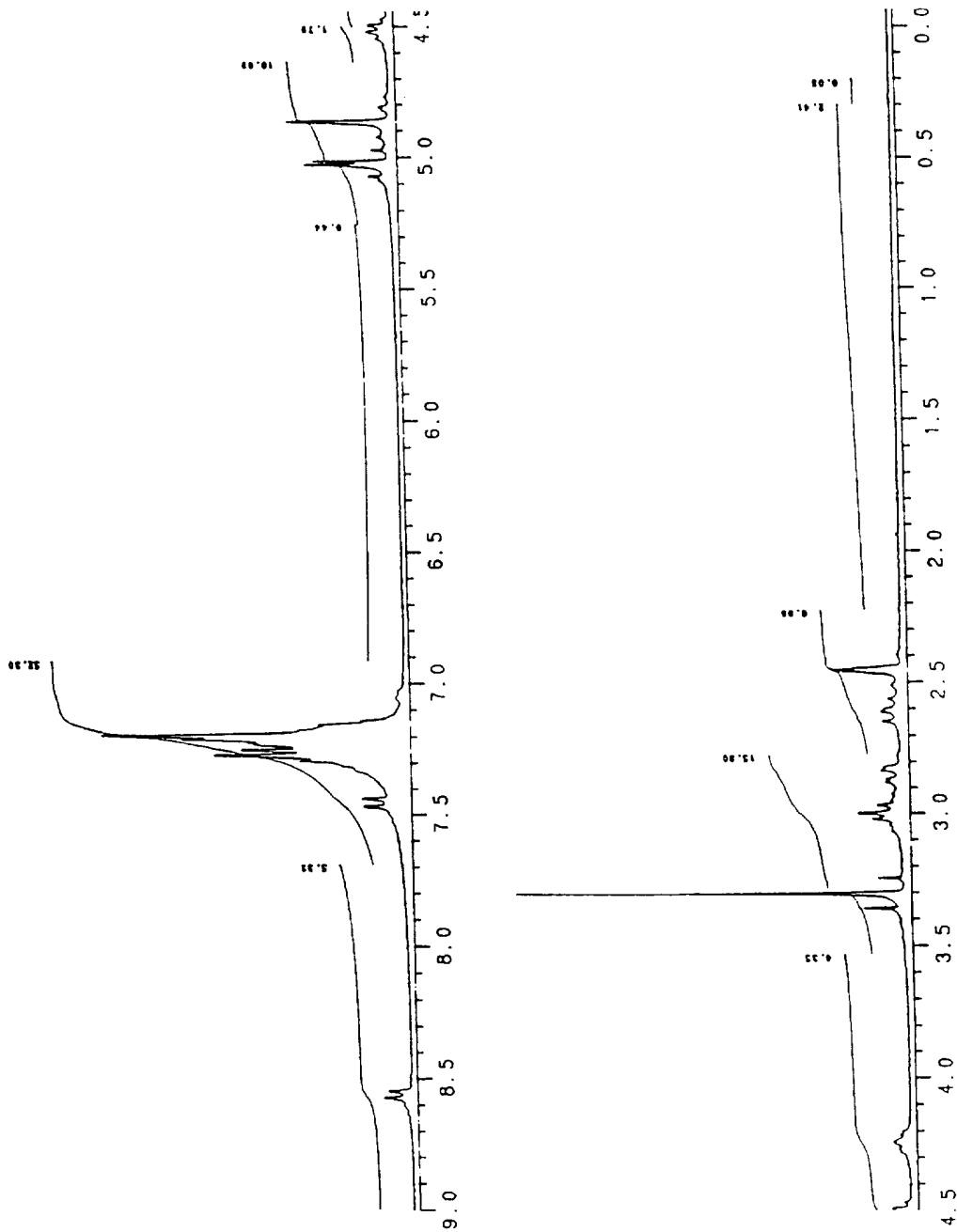


Figure 89 IR, L-Pro-L-Glu- α -Tyr- γ -Tyr,

Figure 90 ¹H-NMR, L-Pro-L-Glu- α -Leu- γ -Leu,



Figure 91 ¹H-NMR, BzOCOPhe-PheBz



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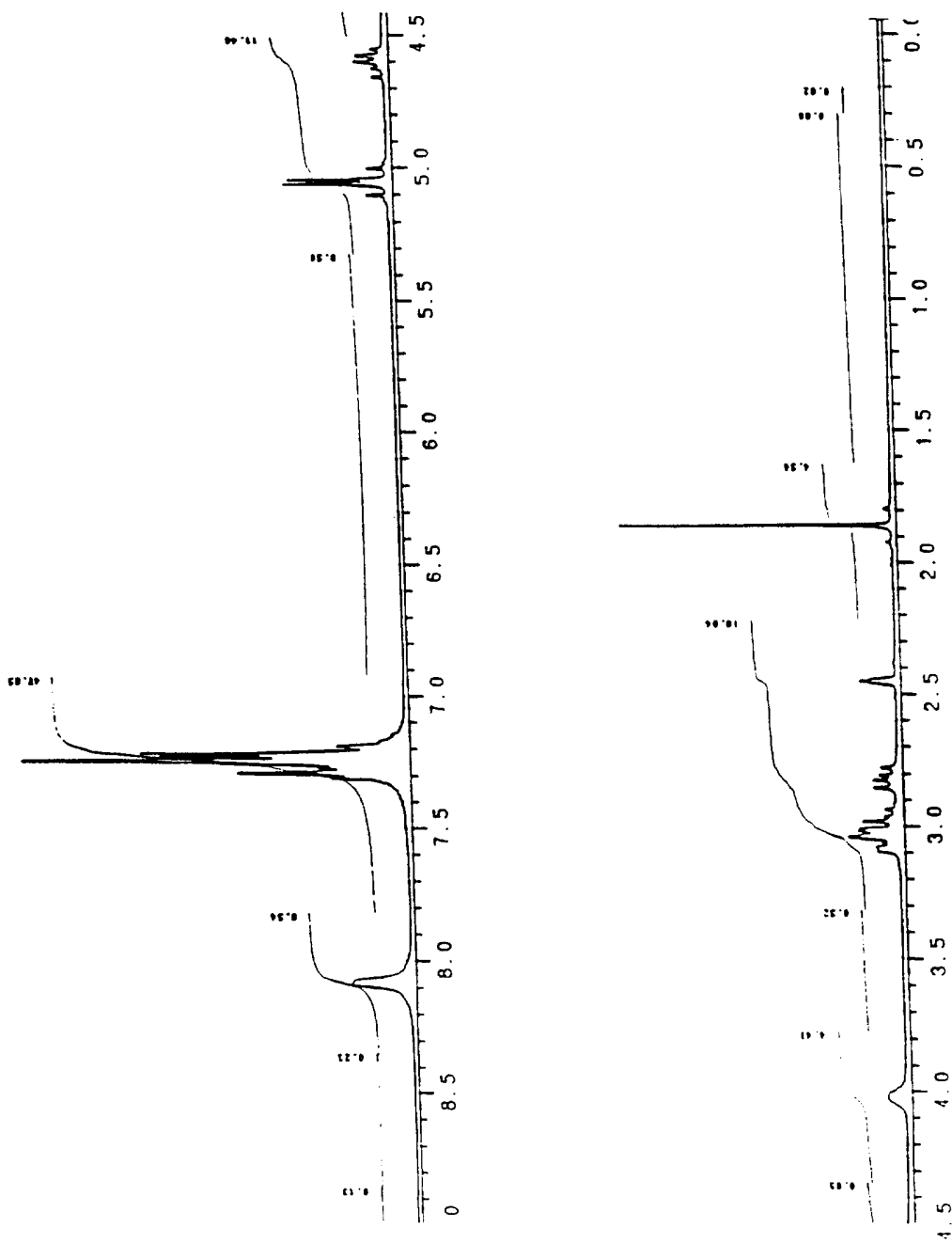


Figure 92 ¹H-NMR, Br·H₃N⁺-Phe-PheBz

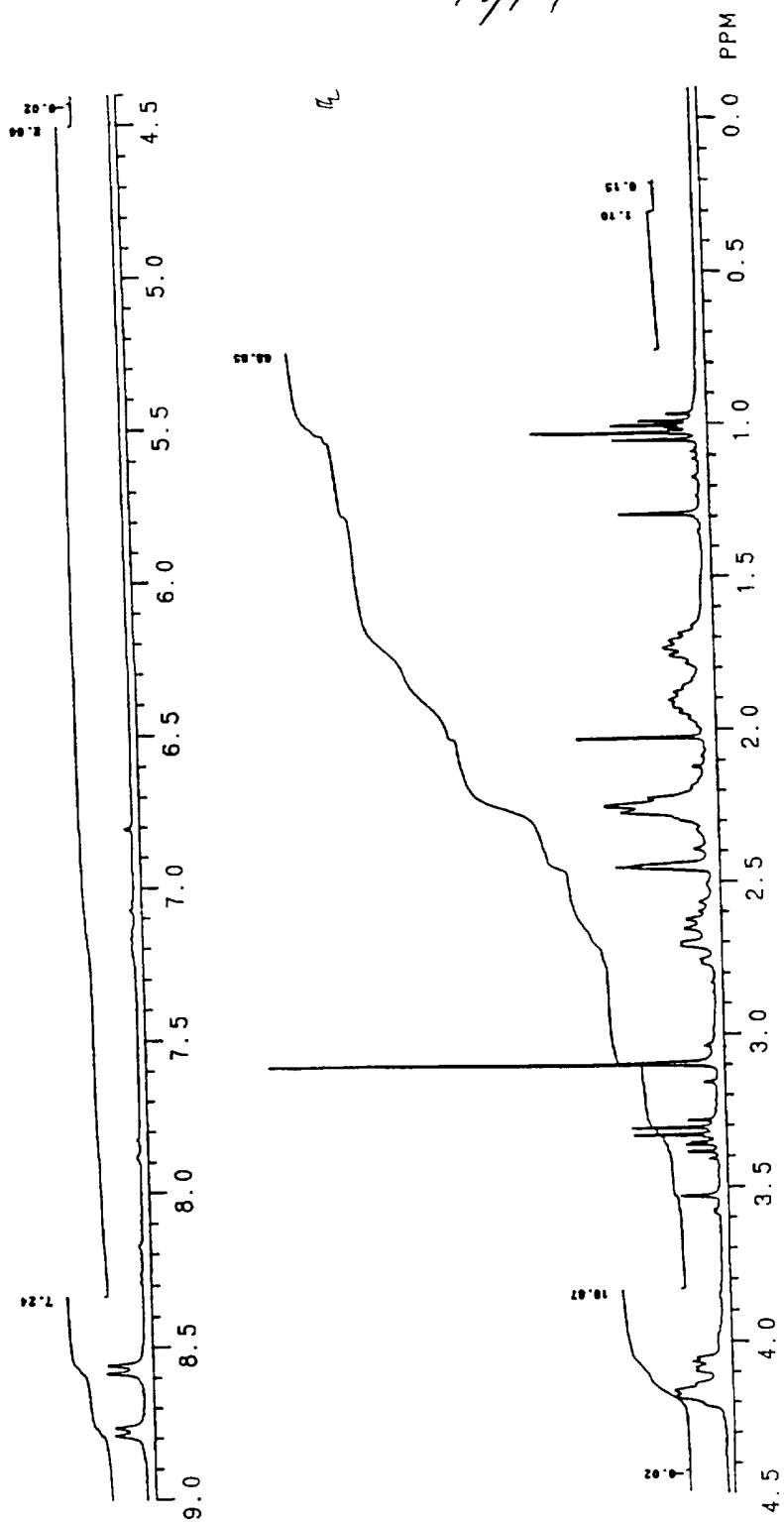


Figure 93 ¹H-NMR, L-aspartyl β-glutamic acid (L-Asp-β-Glu),

94/101

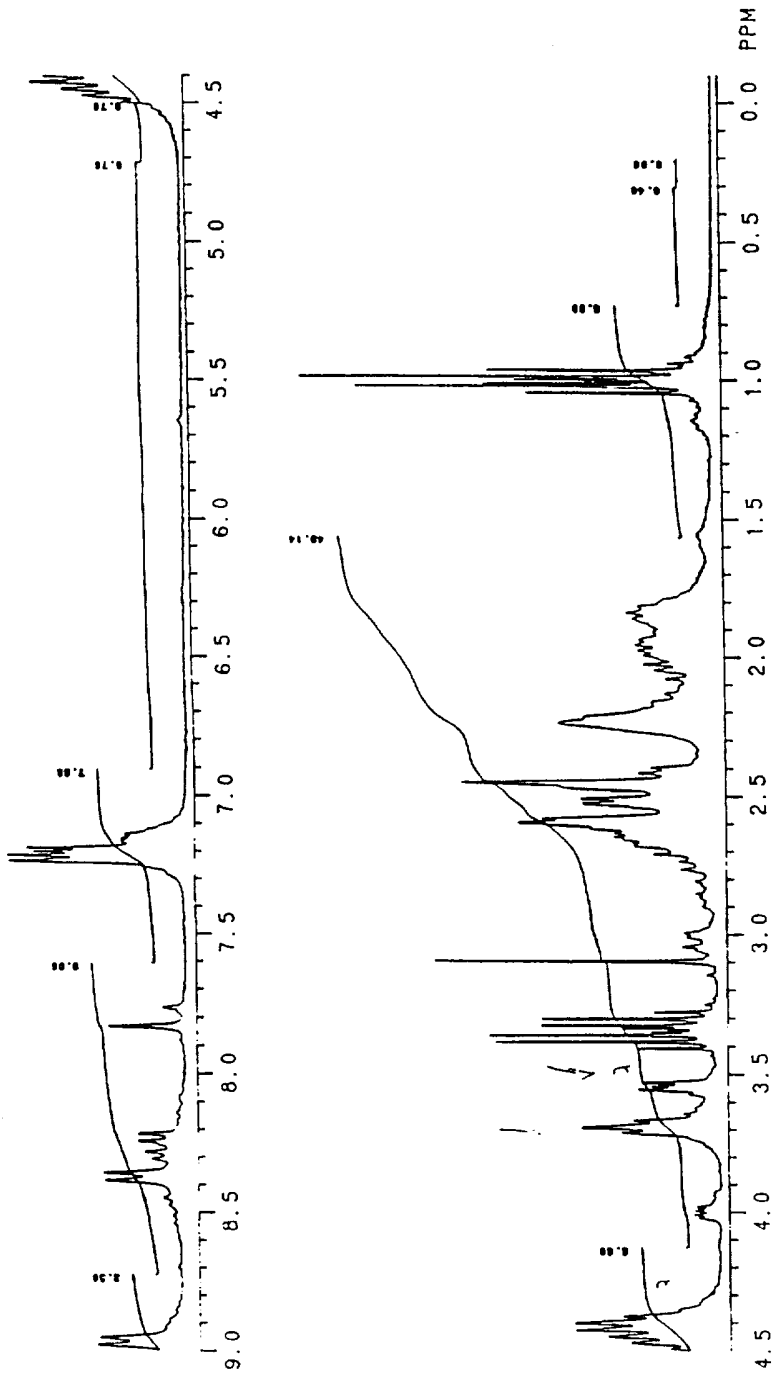


Figure 94 ¹H-NMR, L-glutamic β-aspartic acid (L-Glu-γ-Asp) AND L-
Glu-α-Phe-γ-Asp mixture.

95/101

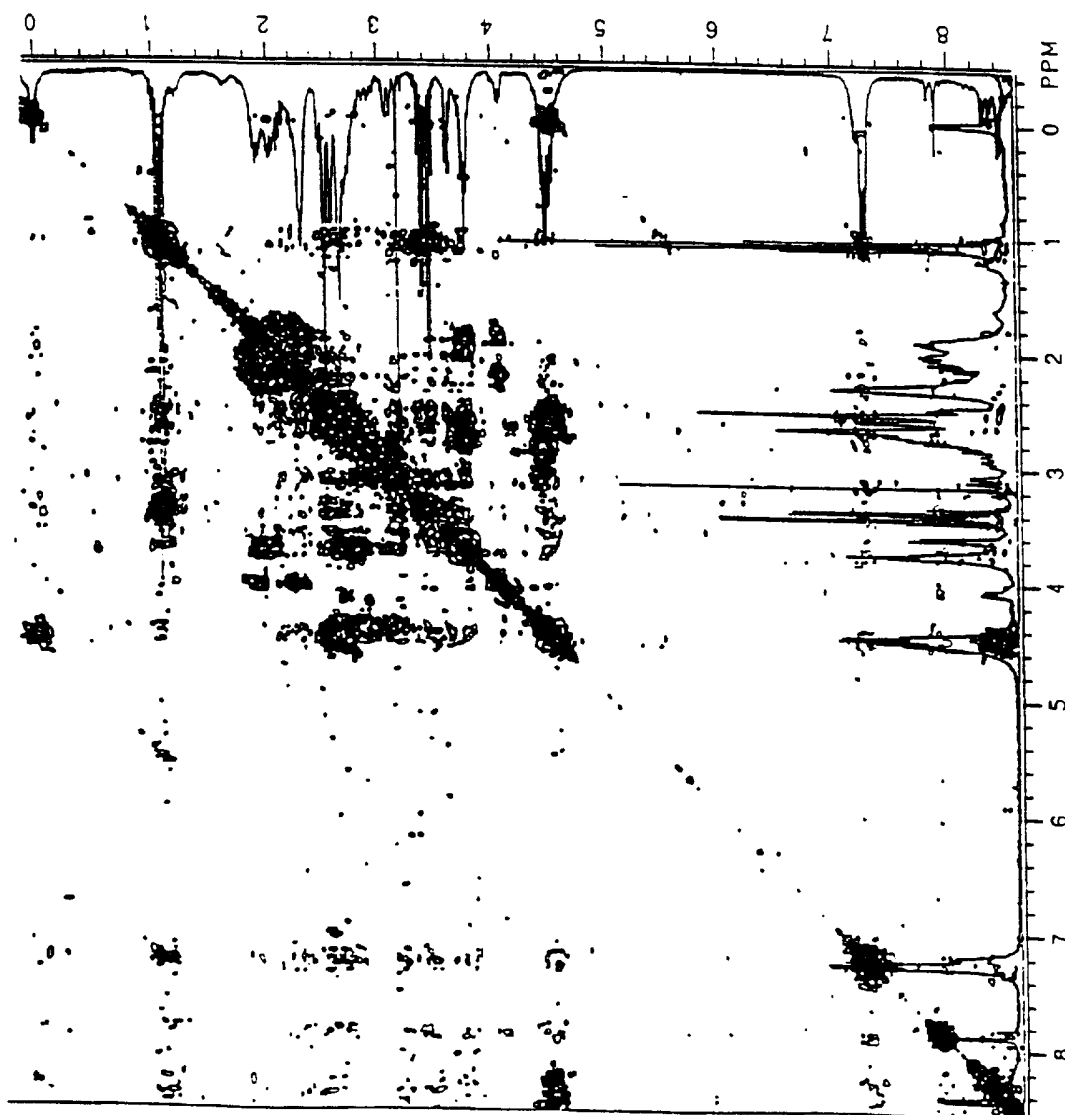


Figure 95 ¹H NMR COSY, L-Glu- γ -Asp and L-Glu- α -Phe- γ -Asp.

96/101

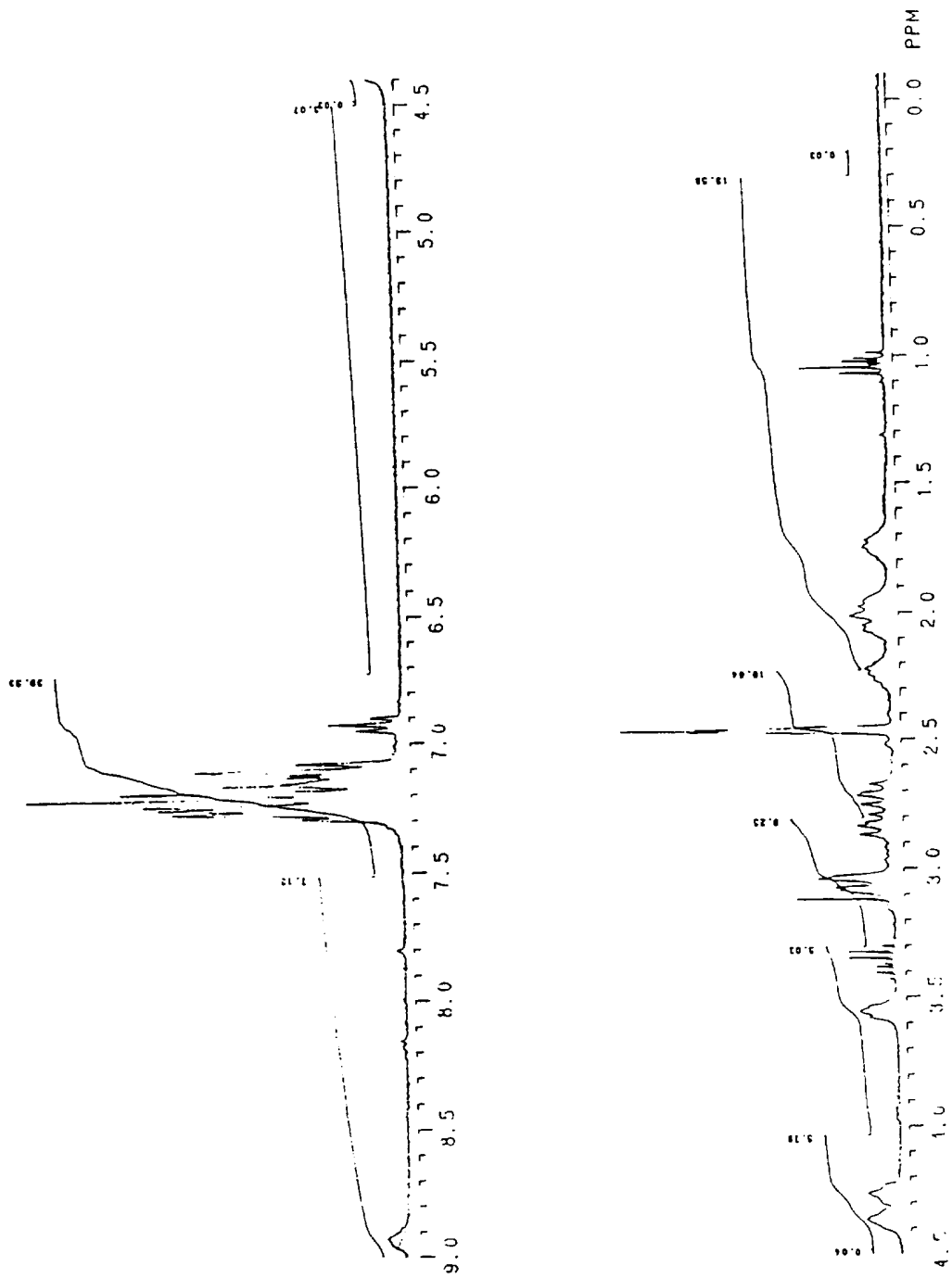


Figure 96 ¹H-NMR, BrH₃N⁺-L-Glu-α-Phe-γ-Phe,

10/1/96

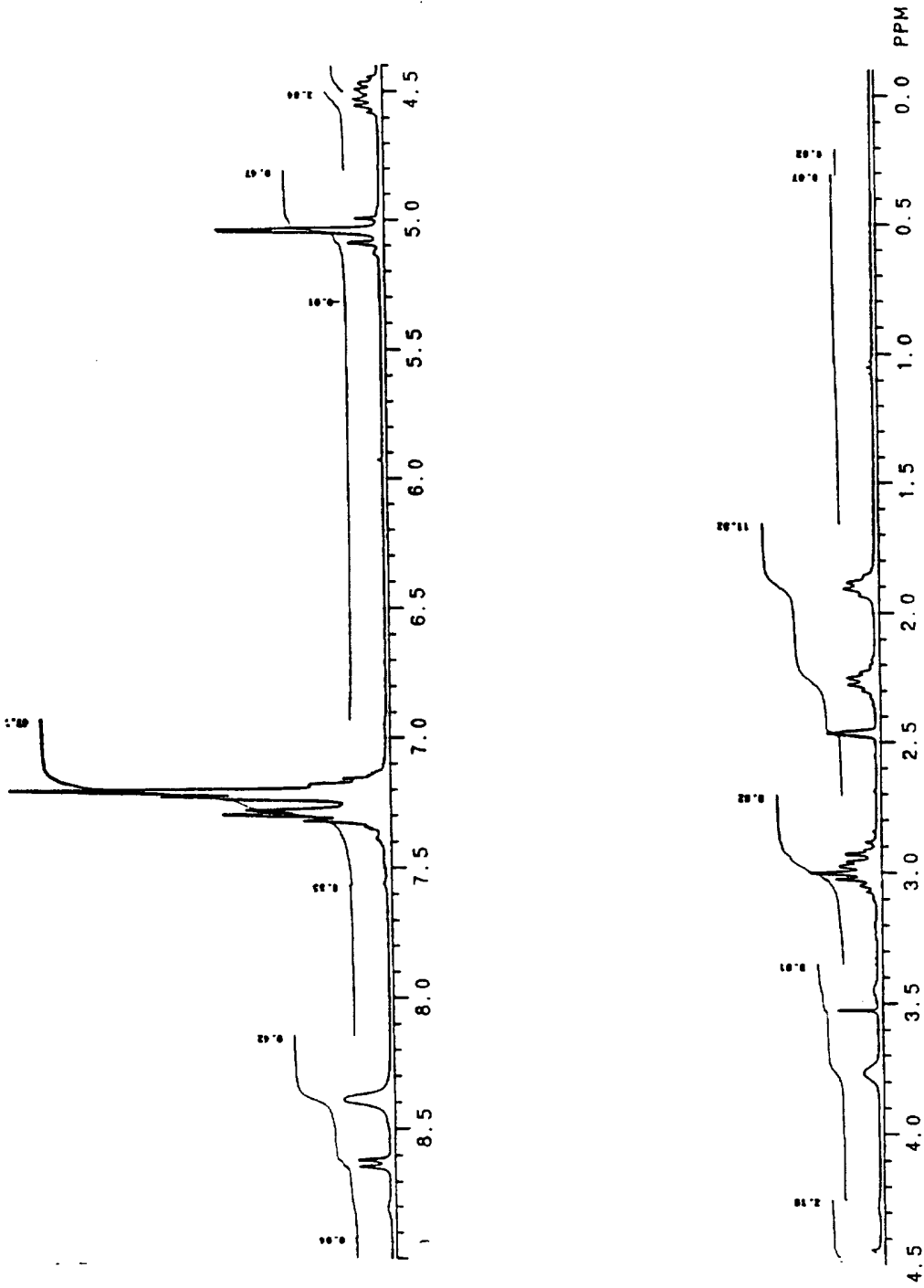


Figure 97 ¹H-NMR, C₁₇H₂₁N⁺Glu-α-Phe-Bz-γ-Phe-Bz,

98/101

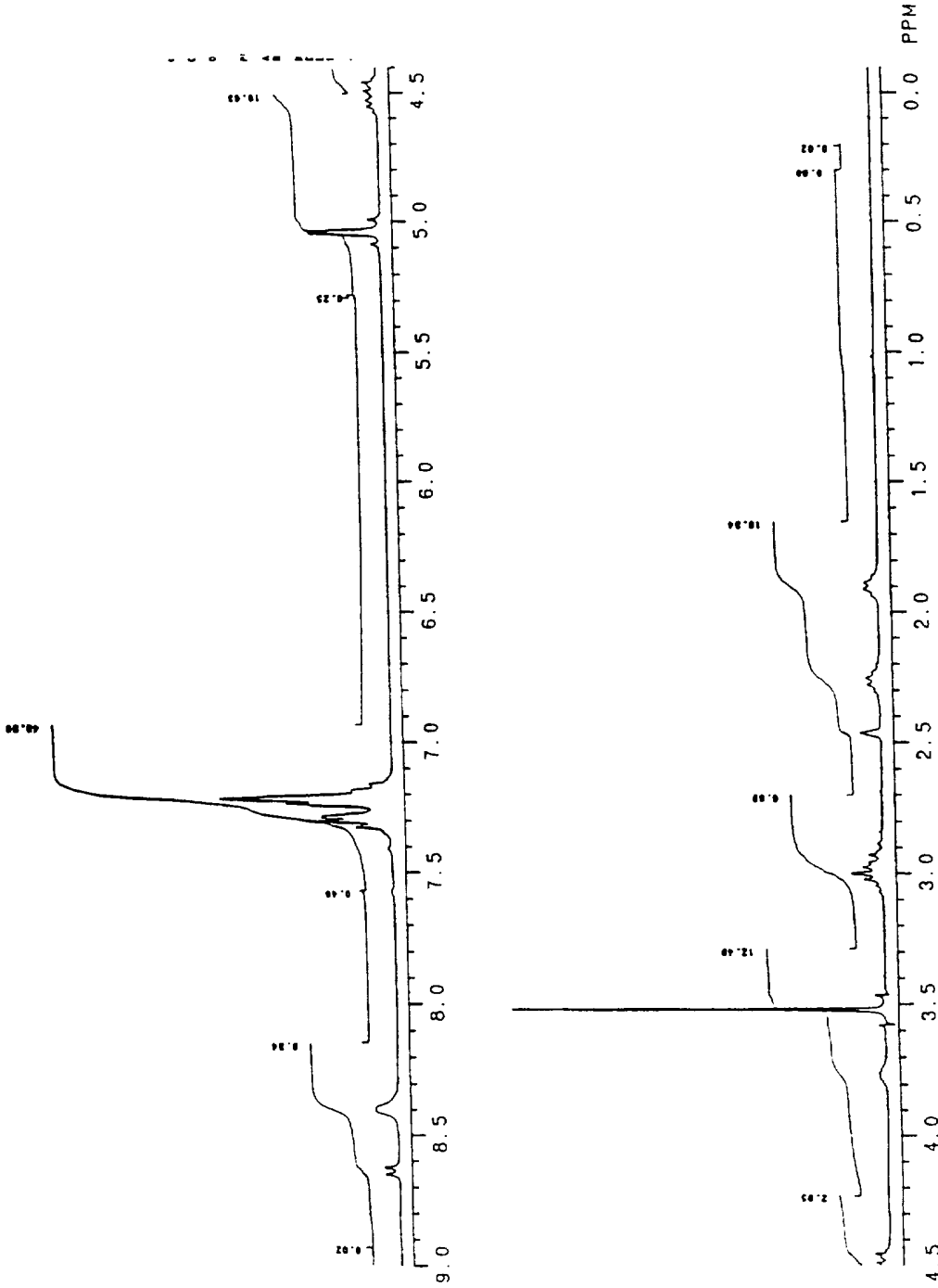
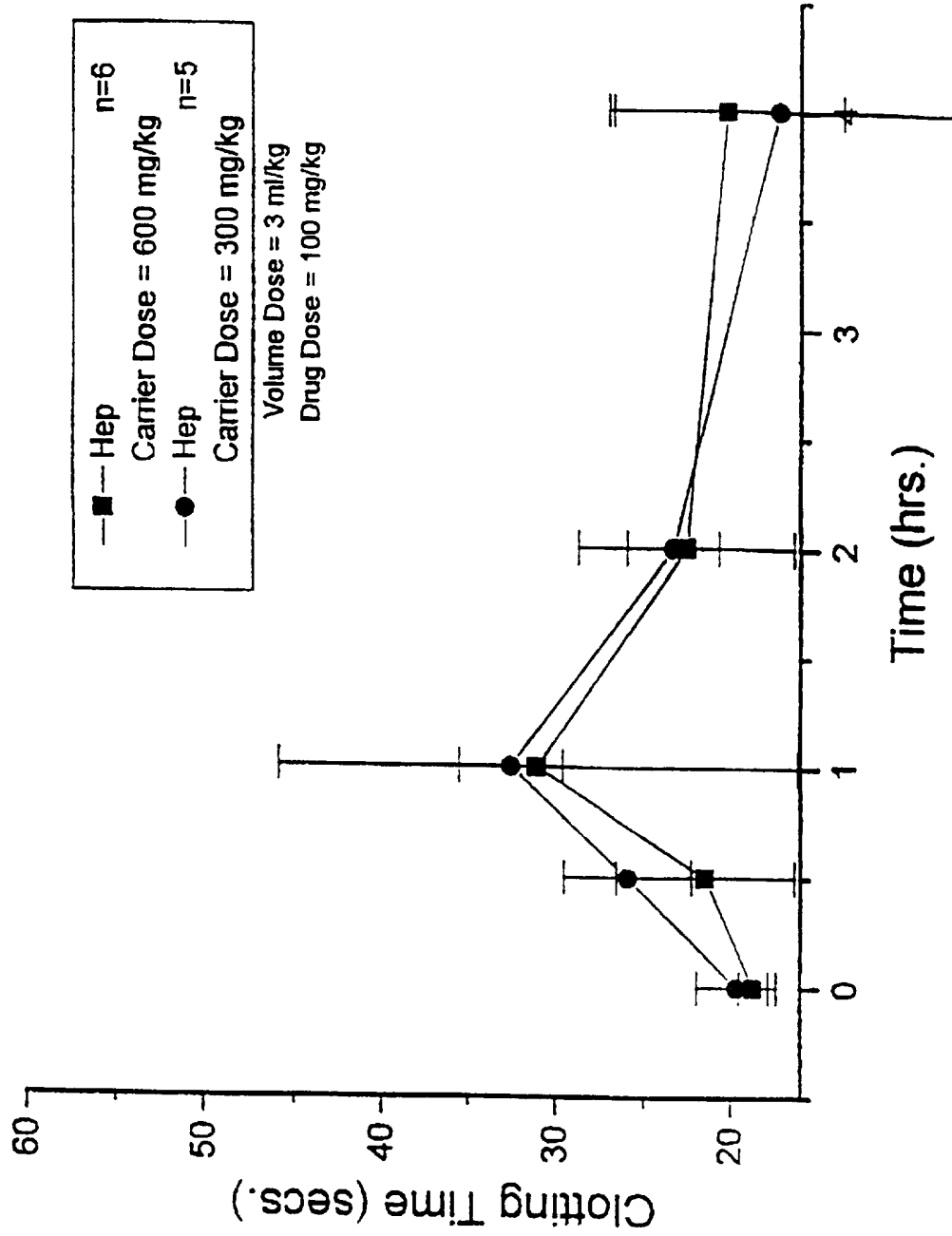


Figure 98 ¹H-NMR, C₁₁H₁₃N⁺-L-Glu-α-Phe-γ-Phe,

FIGURE 71
Heparin and p-Glu-Glu-Phe



100/101

Figure 100 Hydrophobicity (Partition Coefficient) of Oligopeptides

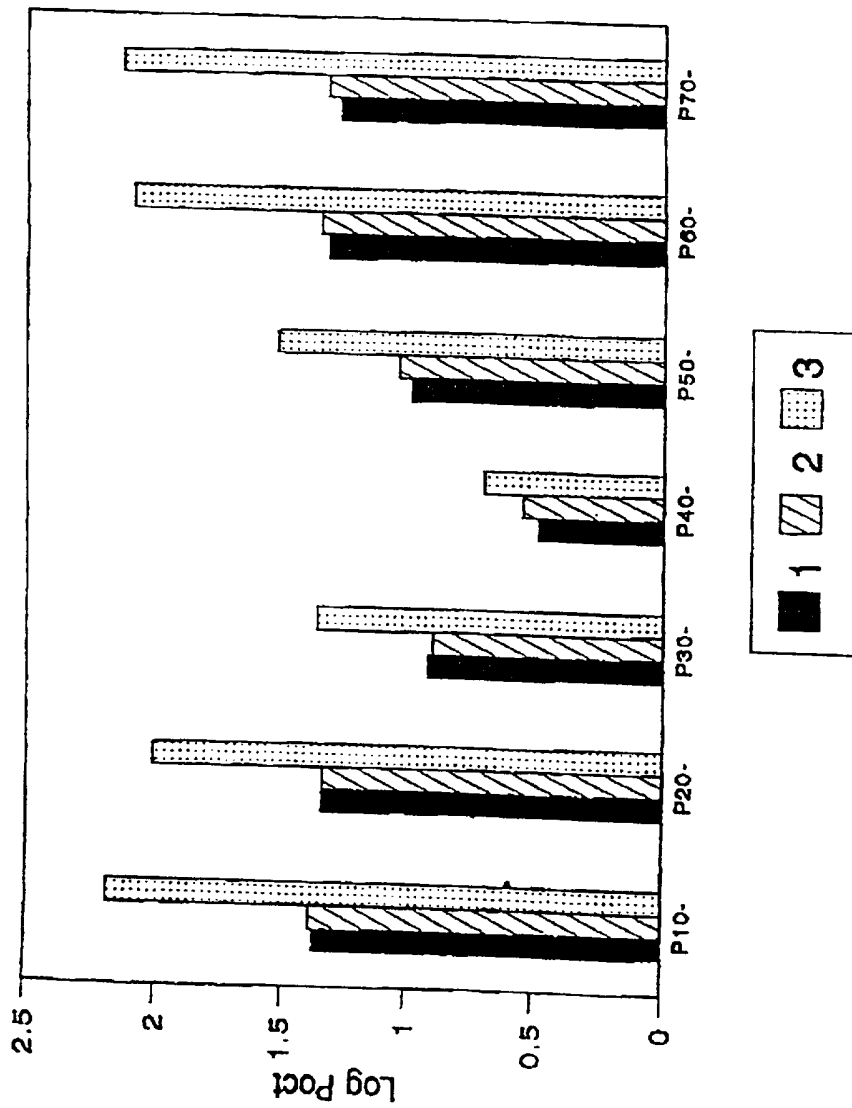
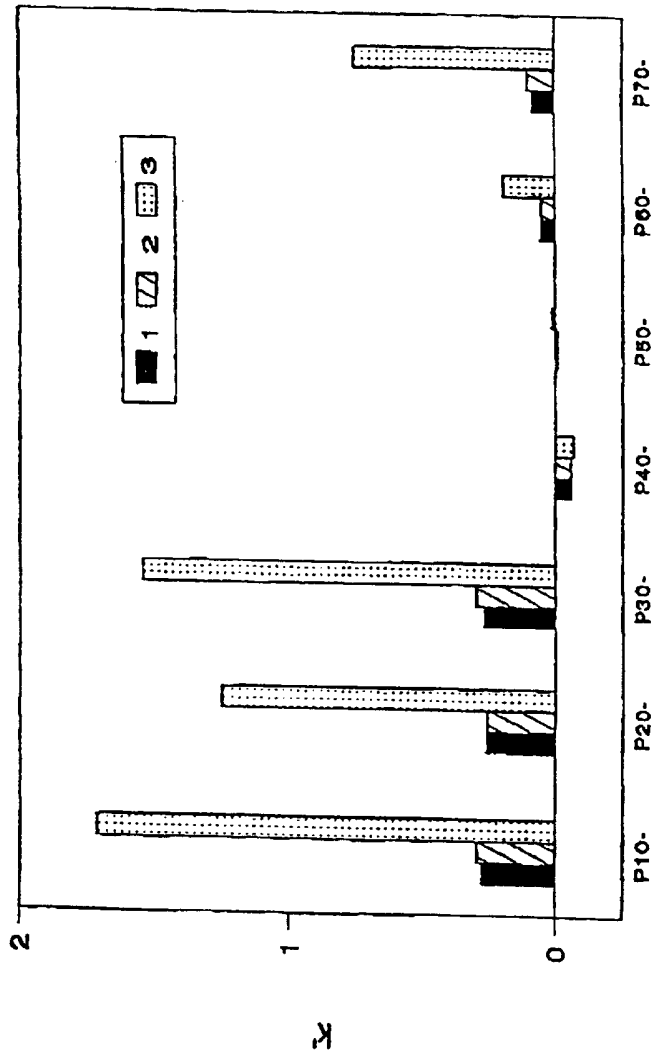


Figure 101 Binding Affinity of Oligopeptides to Heparin at pH=2.27



Chromatographic Conditions: Sigma-Aldrich AF-Heparin Affinity HPLC column (2.5 mL) at flow rate 1.0 mL/min; the mobile phase is 100 mM NaCl in 25 mM acetate buffer plus 0.005% Na₃N at pH=2.27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04051**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :C07K 5/00, 7/00; A61K 38/00

US CL :530/ 327, 328, 329, 330, 331; 514/15, 16, 17, 18, 19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/ 327, 328, 329, 330, 331; 514/15, 16, 17, 18, 19

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
none

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, APS

oligopeptide, active agent, delivery, pyroglutamyl, insulin, heparin, pyglu

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PASTORES, G. et al. The Use Of A Non-Porous Reversed Phase Column For Resolution Of Porcine Insulin From Low Molecular Weight Amides In The Same Matrix. Journal of Liquid Chromatography. 1995, Volume 18, No. 15, pages 3049-3059.	1-20
Y	JP 06-107682 A2 (NAMIKATA et al.) 19 April 1994 (19.04.94), entire document.	1-20
A	DE-2343073 A (KOENIG et al.) 06 March 1975(06.03.75), entire document.	1-20
A	SINHA et al. Functional Characterization of Human Blood Coagulation Factor XIa Using Hybridoma Antibodies. Journal of Biological Chemistry. 05 September 1985, Volume 260, No. 19, pages 10714-10719.	1-20

 Further documents are listed in the continuation of Box C.
 See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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