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CO-OLIGOMERS OF GLYCINE AND GAMMA-METHYL-L-GLUTAMATE

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CO-OLIGOMERS OF GLYCINE AND γ-METHYL-L-GLUTAMATE

by

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ABSTRACT

A series of protected co-oligopeptides consisting of γ-methyl-L-glutamate and one glycine were synthesized from the di- through hepta-peptides. Glycine was placed at each position of the oligopeptide backbone except the carboxyl terminus. Determination of the effect of such a substitution on the conformation of the glutamate backbone was achieved via circular dichroism in both trifluoroethanol and hexafluoroisopropanol.

Synthesis was achieved via the mixed carbonic-carboxylic anhydride coupling procedure. Purity for all peptides was equal to or greater than 97% as determined by normal phase HPLC.

Glycine insertion at the amine terminus of the heptapeptide resulted in C.D. spectra indicative of a slight decrease of helicity as compared to the homo-heptamer in trifluoroethanol. Progressive movement of glycine to the interior positions of the heptapeptide resulted in a gradual loss of C.D. patterns associated with peptides in an α-helical array. Glycine positioned at the fourth position resulted in a complete loss of helicity. Further movement past this internal position toward the carboxyl terminus marked the return of C.D. patterns that indicated some helical character. Virtually all oligopeptides were disordered in trifluoroethanol and hexafluoro-isopropanol. Polarimetric analysis in trifluoroethanol and hexa-fluoroisopropanol supports the conclusions of the C.D. studies.

HPLC on normal phase silica suggests a dependence of mobility of the compounds on their individual conformational distribution.

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To my brother, Dave, and my sister-in-law, Marsh, who provided me with sound advice and many good meals.

To the Baffi Family who cared for me as one of their own sons.

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To Robert Angelo Baffi who taught me alot.

And, finally, to Mary who typed my thesis.

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INTRODUCTION

Proteins are among the most prevalent and important molecules in all organisms. Composed of long chains of amino acids (polypeptides) they function as enzymes catalyzing the anabolic and catabolic reactions in living tissue. Enzymes also carry out the complex reactions necessary for expression of the genetic code and the replication of both DNA and RNA. In addition proteins in conjunction with lipids maintain the structural integrity of all cell membranes. Many hormones are small proteins or peptides, and some of the molecules involved in nerve impulse transmission and immune response reactions are also peptides. The three dimensional structure of proteins and peptides is related to their biological activity, and is dependent upon their primary structure, their concentration, the solvent environment, and the temperature.

In order to elucidate conformational characteristics of proteins with respect to the above variables many oligopeptides have been synthesized[1] and studied as model compounds. The application of x-ray diffraction[2] (in the case of simple amides only) and infrared spectroscopy[3,4] in solid state studies, and I.R.,[3,4,5] optical rotation,[6,7,8,9,10] optical rotatory dispersion,[11] circular dichroism,[11,12,13,14,15,16,17,18,19] and nuclear magnetic resonance,[12, 20,22,23,24] in solution studies has successfully determined the secondary structure of many homo-oligopeptides and some co-oligopeptides. Such studies have revealed the dependence of secondary structure on chain length, solvent-chain interactions, concentration and temperature.

Of the many model compounds studied, among the first were homopolymers[25] and oligomers[26] of γ-methyl-L-glutamate. A great deal of

physico-chemical data on these compounds was made available. Later studies involved the analysis of co-oligopeptides in light of the fact that every biologically active peptide is composed of more than one type of amino acid. Co-oligomers composed of methionine and one valine or methionine and one glycine[18] were extensively analyzed via C.D. and optical rotation, and yielded much information regarding secondary structure in comparison to methionine homo-oligomers. To gain a better understanding of protein secondary structure it would now be useful to analyze a series of co-oligomers containing one glycine as a "guest" amino acid, and the well characterized γ-methyl-L-glutamate as the "host" or backbone residue. The goal of this research is the investigation of the effects on secondary structure of glycine insertion in protected γ-methyl-L-glutamate oligomers. Towards this end we will synthesize a series of compounds composed of γ-methyl-L-glutamate and glycine. All peptides, from dimer through heptamer will contain only one glycine at various positions in the chain with the remaining residues being γ-methyl-L-glutamate. Synthesis will be performed in a stepwise manner (backing off procedure), via a mixed carbonic-carboxylic anhydride coupling.[27] All peptides must be reasonably pure for meaningful spectral analysis (> 97%). Purity of peptides will be determined by normal phase high pressure liquid chromatography and thin layer chromatography on silica. In the presentation of chromatographic data as evidence of purity a discussion of the effects of positional isomerism with respect to HPLC mobility and resolvability will be offered.

Conformational analysis will be performed using C.D. Peptides will be examined in a structure supporting solvent (trifluoroethanol) and in

a non-structure supporting solvent (hexafluoroisopropanol). Conformational studies of co-oligopeptides will be compared to conformational data obtained for the γ-methyl-L-glutamate homo-oligomers. The results will serve to elucidate the effects on secondary structure of glycine insertion in a well studied model oligopeptide.

HISTORICAL

One of the first investigations involving proteins dates back to the experiments of Payer and Persoy in 1833.[29] These investigators demonstrated that an alcohol precipitate of malt extract contained a thermolabile substance which converted starch into sugar (amylase). Later in that century Emil Fischer demonstrated the close steric relationship between enzyme and substrate which led to the lock and key hypothesis.[30] It has been clearly proved in recent years that the activity of biologically active proteins is dependent upon their three dimensional conformation, which in turn is determined by the amino acid sequence of the molecule.[31] Although a protein can be visualized as existing in an infinite number of three dimensional structures there are discrete thermodynamically stable states which predominate.[32,33,34]

One of the first conformational studies performed on polypeptides was the x-ray diffraction analysis of fibrous proteins.[35] The results suggested a regular folding of the peptide backbone. Subsequent studies of α and β keratin reinforced the presence of a periodicity or regularity of folding.[36,37] Aided by the work of Huggins[38,39] which involved an attempt to determine the principle of protein folding, Pauling and coworkers made theoretical predictions as to the secondary structure of proteins.[2] In conjunction with x-ray studies of crystals of simple amide containing compounds (di and tripeptides), the peptide bond was determined to have a planar geometry. This geometry results from the partial double bond character of the amide bond which restricts rotation and shortens the C-N bond length.

X-ray analysis of α-keratin displayed a major periodicity of ~ 5.4 Å and a minor periodicity of 1.5 Å in the diffraction patterns. Pauling and Corey found that the simplest arrangement for the polypeptide which explained the above periodicity was a helical structure.[40,41] This secondary structure was termed the α-helix and contained a non-integral number of residues per helix turn (3.6) and thirteen atoms in a ring closed by a hydrogen bond. One complete turn of the helix was responsible for the periodicity of 5.4 Å and an axial translation of 1.5 Å per residue in the chain was responsible for the minor periodicity. Molecular models revealed an almost linear disposition of hydrogen bonds between the amide proton and the carbonyl oxygen in the peptide backbone. This H-bond has been implicated in stabilization of the helix.[41]

The existence of another secondary structure, the extended or β form, was first recognized by Meyer et al.[35] in silk and Astbury and Woods[37] in β-keratin. These investigators proposed the existence of interchain hydrogen bonds stabilizing the extended structure. Although the clearest evidence for the existence of the β-structure was from a study of polyamides (Kinoshita[42]), many investigators examining such polyamino acids as polyglycine,[43] poly-L-alanine,[44] poly β-n-propyl-L-aspartate,[45] and poly-S-carbobenzoxy-L-cysteine,[46] gave evidence for the antiparallel cross β-structure suggested by Pauling and Corey.[47] The existence of still another secondary structure, the ω-helix (a strained α-helix) has also been demonstrated in films of poly β-benzyl aspartate but is not common to most other polyamino acids.[48]

As stated previously the formation of intra- and intermolecular hydrogen bonds is an integral component of secondary structure. However,

early studies were performed in the solid state, while biologically active polypeptides function in solution. Physicochemical factors affecting secondary structure in the solid state such as van der Waals forces and electrostatic interactions may be significantly perturbed in solution. Furthermore, structure stabilizing hydrogen bonds are certainly affected by the dielectric constant of the solution medium. Hydrophobic interactions which can occur upon solubilization of peptides also greatly affect secondary structure.

Structural analysis by x-ray diffraction cannot be carried out in solution, however, infrared spectroscopy is capable of structural analysis of both polypeptide and peptide films and some dilute polypeptide and peptide solutions. This was an important instrumentational transition in that polypeptides and peptides previously characterized by x-ray crystallography could now be studied under biologically relevant conditions.

In 1950 Ambrose and Elliot[49] investigated the secondary structure of polypeptide films and dilute polypeptide organic solutions using infrared absorption techniques. The results of these studies indicated that oriented films of poly γ-methyl-L-glutamate and poly γ-benzyl-L-glutamate exhibit parallel dichroism for the NH and C=O stretching mode when cast from chloroform or m-cresol. These films exhibited characteristic absorption frequencies for the N-H stretching mode of 3305 cm^{-1} and 3070 cm^{-1}, and a frequency band of 1660 cm^{-1} for the C=O or Amide I stretching mode. Perpendicular dichroism was also observed for the N-H deformation mode at 1560 cm^{-1}, the Amide II band. These results were indicative of the α-helix or what was termed "α-structure". When films of the above polymers were cast from formic acid (a strong

hydrogen bonding solvent) frequency shifts were observed for the carbonyl, Amide I stretching modes, from 1660 cm^{-1} to 1640 cm^{-1}, and for the N-H in plane deformation mode from 1560 cm^{-1} to 1530 cm^{-1}. Perpendicular dichroism was now observed for the stretching modes and parallel dichroism was observed for the N-H deformation. These polarizations were opposite to those observed in α-structure and this structure was termed β. With knowledge of the above data the same investigators carried out dilute solution studies on poly γ-benzyl-L-glutamate in chloroform. The infrared spectra indicated that the N-H stretching frequency appeared at 3305 cm^{-1}; the identical frequency for the N-H stretching mode observed in the oriented film of the same polymer. These results indicated that all amide protons were hydrogen bonded, and that the characteristic absorption frequencies for the α-structure as a film exist for poly γ-benzyl-L-glutamate in the non-hydrogen bonding solvent chloroform. It was also recognized that polypeptide structural transitions occurred as a function of solvent.

Subsequent studies of films confirmed that α-helix, anti-parallel extended, and parallel chain extended conformation displayed either parallel or perpendicular dichroism in their Amide I and Amide II polarized infrared absorption bands.[50] These characteristic bands were explicable in terms of intra and interchain vibrational interactions.[50]

IR studies of lower molecular weight model oligopeptide films and solutions have provided a great deal of information on secondary structure with respect to critical chain size, protecting groups, side chain, solvents and temperature changes.[4,5,51] Toniolo and coworkers performed a series of experiments on protected oligomers with unbranched,

β-branched, γ-branched and sulfur containing hydrophobic side chains.[4] Specifically the protected oligomers of L-alanine, L-valine, L-leucine, L-isoleucine, L-norvaline, L-phenylalanine, L-methionine and L-methylcysteine were synthesized for IR conformational analysis. It was found that all of these peptides in the solid state exhibited one of two types of β-structure, and had a critical chain size of between five and seven residues. Higher oligomers of these amino acid residues maintained the extended structure, however, increase in temperature or dilutions of peptide solutions resulted in loss of any β-structure at any chain length.

When the solubilizing polymer poly(oxyethylene) was attached to L-Ala, L-Met, and L-Val oligomers, IR analysis in solution demonstrated the presence of substantial helical structure for the POE bound L-methionine decamer through tridecamer, and helical content to a lesser extent in POE bound L-alanine and L-valine tridecamers.[16] Infrared spectra studies of another type of oligopeptide, protected γ-ethyl glutamate, were performed by Goodman et al.[3] It was found that the solvent trimethylphosphate supported folded structures above the heptamer, while in chloroform the oligopeptides assumed β-structures above the pentamer.

In summary infrared spectroscopy was the first instrumental method to successfully distinguish the presence of α and β structures in polypeptides and oligopeptides in solution and proved to be an invaluable analytical tool. Solution studies however were limited to the use of solvents which were capable of dissolving polypeptides and peptides and at the same time maintaining transparency to IR radiation. In addition detailed analysis of globular proteins via IR was hindered

by overlap of critical absorption bands making resolution and quantification of specific 2° structures at best difficult.

Ultraviolet absorption spectrophotometry is better suited for the elucidation of peptide and protein secondary structure (as compared to IR) because electronic transitions of the amide chromophore occur in the far U.V., are discrete, are highly sensitive to changes in 2° structure and are readily quantified. U.V. absorption techniques also permit the use of aqueous solvent and a variety of organic solvents.

Polarized U.V. spectra of α-helical poly-L-lysine and poly-L-glutamic acid[52] revealed an unambiguous shoulder on the absorption bands in the 190-220 nm region. Other investigators demonstrated the same effect in poly γ-methyl-L-glutamate oriented films and in trifluoroethanol solutions of the same polypeptide.[53] These shoulders were a result of exciton splitting of $\pi \rightarrow \pi^*$ transitions in the α-helical structure. Other U.V. spectral phenomena are helpful in the analysis of protein structure. U.V. absorption spectra of α-helical poly-L-lysine in aqueous[52] solution revealed a hypochromic effect for the $\pi \rightarrow \pi^*$ absorption when compared to the spectral bands of the same polypeptide in a random coil or β-structure. This phenomenon is due to an interaction of the transition moment of the lowest energy band (the band of interest) with the transition moment from adjoining residue high energy bands. This phenomena is strictly associated with the α-helix, while a hyperchromic effect is seen at 194 nm in the U.V. absorption spectra of poly-L-lysine in the β-structure.[52]

U.V. absorption spectra exhibited observable differences between random coil, α-helix and extended forms, however partial overlap of peaks made it difficult to definitively assign the different secondary

structures. Later instrumental advances made available spectral methods capable of distinguishing between the three major types of secondary structure. One method was based on the optical activity of the amide chromophore in different secondary structures. The primary source of protein optical activity is the chiral α-carbon. This chiral center does not itself provide any transitions in accessible spectral ranges but its inherent asymmetry perturbs the amide group in proteins resulting in optical activity. The protein backbone upon taking on a regular folded, or extended structure itself becomes non-superimposable on its mirror image. This form of asymmetry also perturbs the amide chomophore. One of the first spectral methods utilized for measurement of the optical activity of peptides was polarimetry. Although this method does not usually use radiation in the far U.V. it can be readily applied to conformational analysis of oligopeptides.

Polarimetry at the sodium D line is a technique which has been employed as a sensitive criterion for the determination of the critical chain size for onset of secondary structure. Goodman et al.[54] performed optical rotation measurements of protected oligomers of γ-methyl-L-glutamate in various solvents. Plots of specific rotation versus residue number of oligomers dissolved in dichloroacetic acid yielded curves consistent with an increase in the number of chiral centers. Dissolution of oligomers in dioxane, a non-hydrogen bonding solvent, produced similar graphs of specific rotation vs. residue number up to and including the tetramer. From the pentamer through the undecamer a rather large deviation occurred for the dioxane-peptide solutions as compared to solutions in dichloroacetic acid. This

deviation has been shown to be a result of the contribution of the secondary structure to the rotation of polarized light.[55-61] More recent studies of oligopeptide conformation have used molar rotation values, instead of specific rotation, to generate nearly linear plots with respect to the number of residues when secondary structure is absent.[1]

The application of polarimetry as an indication of optical purity during synthesis of L-homo-oligopeptides[1] has also been possible when peptides are dissolved in solutions of strong hydrogen bonding solvent, such as hexafluoroacetone and hexafluoroisopropanol. However in optical rotation studies of co-oligomers positional isomers, values of molar rotations varied for a series of six hexamers containing one glycine and five L-methionines.[10] These deviations suggested that either there are internal interactions between adjacent chiral centers in the molecule or that there are conformational differences between the hexamers even in hexafluoroacetone.

As a result of a number of polarimetry studies of L-aspartate, L-methionine, γ-ethyl-L-glutamate, L-alanine, and γ-methyl-L-glutamate oligomers, Goodman[1] developed an equation describing the various elements that contribute to the total molar rotation of a peptide:

$$[\phi]_M = [\phi]_{end} + n'[\phi]_{internal} + [\phi]_{conformation}$$

where $[\phi]_M$ is the total molar rotation of the peptide, $[\phi]_{end}$ is the contribution from C and N terminal residues, which are chemically distinct from internal residues. $[\phi]_{internal}$ represents the optical activity from the internal residues and n' is the number of

internal residues. $[\phi]_{conformation}$ is the contribution from secondary structure. The form of the equation was shown to be linear for plots of $[\phi]_M$ vs. n' in the absence of secondary structure. Using this equation this investigator was able to predict the molar rotation of higher oligomers of a peptide series in various solvents based upon rotations of lower molecular weight model compounds of the same series.[1]

Another physical method that took advantage of the optical activity of peptides is optical rotatory dispersion, defined as the change in the magnitude of the rotation with wavelength. O.R.D. has been applied with limited success in the conformational analysis of proteins. Unfortunately contributions from chromophoric transitions many wave numbers away from the transition of interest can distort or cause overlap of peaks in critical regions of the dispersion spectrum.

In spectral regions where the protein or peptide molecules absorb light the related phenomenon of circular dichroism occurs. Circular dichroism has proven to be very sensitive to peptide secondary structure and provides characteristic spectral patterns which can be assigned to α-helical, β-sheet and random coil structures.[62] Due to the narrowness of the C.D. band it is superior to O.R.D. for conformational analysis of peptides and proteins. Circular dichroism was the experimental method used in this thesis for analysis of synthetic oligopeptides. C.D. can be explained as a difference in absorption of the left and right handed circularly polarized components of incident radiation by an optically active sample (plane polarized light can be visualized as the vector sum of two circularly polarized components). This preferential absorption results in the two vectors being of unequal size so that their vector sum traces out an ellipse. This ellipse is tilted relative to

the original plane of polarization. C.D. is directly recorded in terms of ellipticity, Θ, (Fig. 1). C.D. is also reported in terms of the difference in the molar extinction coefficients for left and right handed components: $\Delta\varepsilon = \varepsilon_L - \varepsilon_R$ defined as the circular dichroism.

$$[\theta] = 3300(\varepsilon_L - \varepsilon_R)$$

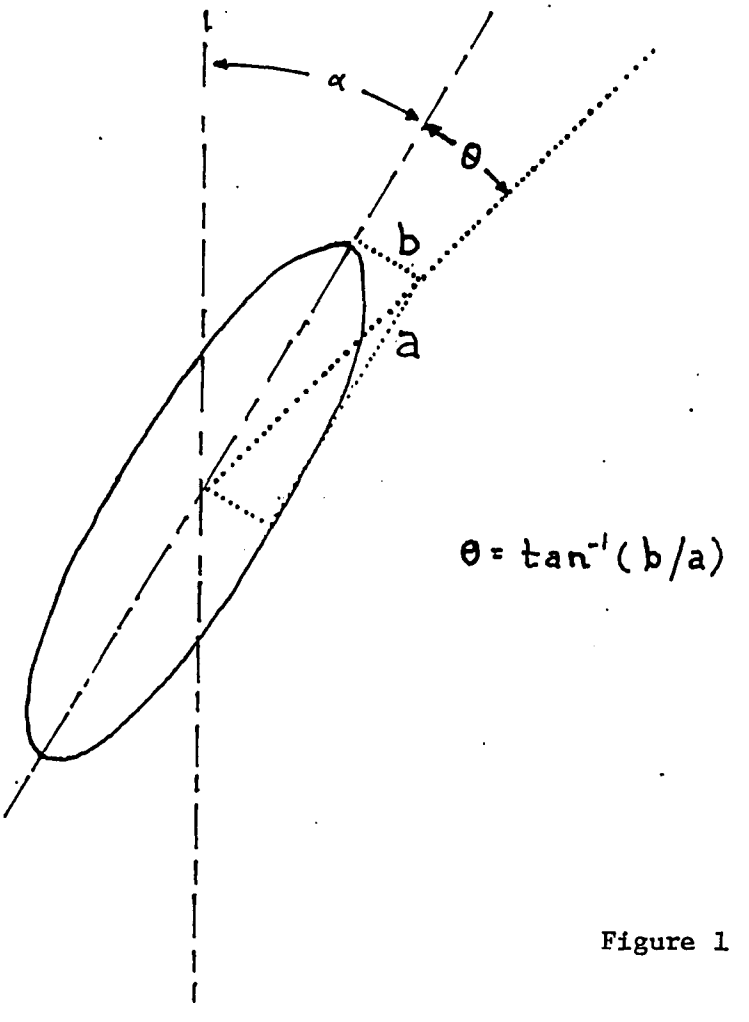


Figure 1

The characteristic differences in CD patterns for various secondary structures can be used in conformational analysis of oligo- and polypeptides, Fig. 1a. Holzwarth and Doty in 1965 performed extensive studies which correlated U.V. absorbtion data from poly γ-methyl-L-glutamate with C.D. spectra obtained from the same compound.[63] Three distinct Cotton

effects located between 185 nm and 245 nm were found to correspond to the U.V. absorbtion bands. The three rotatory bands were assigned to the $n \rightarrow \pi^*$ transition at 222 nm, the parallel polarized $\pi \rightarrow \pi^*$ exciton transition at 206 nm and the perpendicularly polarized $\pi \rightarrow \pi^*$ exciton transition at 190 nm. In the same study C.D. spectra obtained from polypeptides in random or disordered structure revealed a region of weak positive circular dichroism from 210 nm to 235 nm, and a strong negative dichroism centered near 200 nm. Goodman et al.[12] using C.D. analysis reported the appearance of α-helical structures beginning in the heptapeptide of protected γ-ethyl-L-glutamate oligopeptides. Extensive C.D. analysis of homooligomers containing aliphatic side chains was performed by Toniolo et al.[9,64,65] Protected homooligopeptides of the β-branched amino acids L-valine and L-isoleucine behaved similarly, both assuming β structure at the heptamer in TFE (trifluoroethanol). Analysis of the γ-branched oligomers of L-leucine exhibited a critical chain size for β structure of seven residues, occurring however in TFE:H_2O mixtures. In conjunction with the above studies Goodman and Naider found that protected alanine oligomers also formed β structures in TFE at the heptamer but while the above γ and β branched aliphatic oligomers became structureless upon dilution at the heptamer or larger peptides, alanine oligomers formed α helical structures between the heptamer and nonamer with a decrease in concentration.[1] These results indicate that formation of secondary structure is a function of hydrophobicity and chain branching or steric interactions.

In 1974 Naider and Becker performed C.D. analysis of L-methionine oligopeptides.[69] Protected homooligomers of L-methionine, tetramer

through nonamer were synthesized and conformationally characterized. For the tetramer, pentamer and hexamer in TFE they reported a long wavelength, weak positive band at 220 nm, and a short wavelength negative maxima at ~ 197. These bands were assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ amide chromophore transitions, respectively. These assignments were based upon comparisons with previous C.D. examinations of oligopeptides[12] and polypeptides.[63] For the heptamer in TFE a 4 nm red shift was reported for the $\pi \rightarrow \pi^*$ transition and a significant development of a negative ellipticity at 222.5 nm for the $n \rightarrow \pi^*$ transition. These spectral changes were magnified for the nonamer in the same solvent. The similarity between the C.D. patterns of $Boc(Met)_7OMe$ in hexafluoroacetone and those of the tetramer, pentamer, and hexamer in TFE supported the contention that the lower oligomers are disordered in the latter solvent and that L-methionine protected oligopeptides begin forming helices at the heptamer.

In light of the above and other previous studies regarding C.D. analysis of model homo-oligopeptides the next step was to insert a guest amino acid residue into various positions of a homooligopeptide backbone. Determination of the effect of amino acid replacement on secondary structure of a well characterized homooligomer would yield biologically relevant information because proteins are composed of more than one type of amino acid. Naider et al.[18] have determined the effects of homologous amino acid substitution on the conformation of oligopeptides composed of L-methionine and L-valine, and L-methionine and glycine. These experiments found that substitution of one L-valine at an internal position or at the N-terminal position in the L-methionine backbone of the heptamer did not affect the critical chain length compared to the

homo-methionine oligomers. In contrast to those results substitution of glycine in an internal position of the methionine heptamer backbone did prevent or destabilize the formation of the α-helix.

The previously mentioned L-methionine-glycine co-oligopeptides have also been utilized in elucidation of secondary structure by ^{1}H-NMR techniques on $CDCl_3$. This procedure is known as the "host-guest" technique and is used for assignment of the amide proton and α-CH resonances of each residue in the chain.[20] Assignments could be made provided that there was no change in secondary structure with the insertion of the guest amino acid residue. That this was the case was confirmed by the synthesis of a complete series of α-^{2}H methionine oligopeptides.[23] The assignments obtained for the selectively deuterated series were in complete agreement with those obtained for the host-guest series. Once assignments were made for the amide proton and α-CH resonances in the homo-oligomer, chemical shifts of specific resonances acted as sensitive probes responding to changes in secondary structure. ^{1}H-NMR proved to be successful in structural analysis of homo-oligopeptides of L-methionine and also of γ-ethyl-L-glutamate homo-oligomers.[12,23] In both cases it was possible to suggest model structures for the heptamers. Thus it was suggested that the heptamers of both series favor a structure with seven membered intramolecular H-bonded rings in $CDCl_3$ and α-helices in TFE.[23]

Over the past years a variety of physical methods have been made available for detailed conformational analysis of various model oligopeptides with emphasis on homooligomers. Up to this point no detailed study of secondary structural effects of glycine insertion on the well studied γ-methyl-L-glutamate oligomers have been performed via C.D. or

any other physical method. In light of scant structural knowledge on co-oligopeptides this thesis was designed to elucidate some of the structural aspects of glycine-γ-methyl-L-glutamate co-oligopeptides in the hope of providing more information relevant to biological macromolecules.

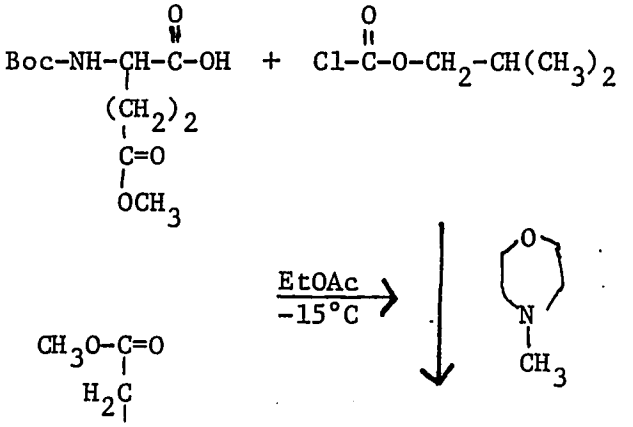
Circular dichroism data from γ-methyl-L-glutamate homo-oligomers and γ-methyl-L-glutamate-glycine co-oligomers was obtained in hexafluoro-isopropanol and trifluoroethanol. The data were interpreted and analyzed by comparison of the data to characteristic C.D. patterns from the literature.

All compounds were synthesized by the author and determined to be $\geq$ 97% pure via normal phase HPLC.

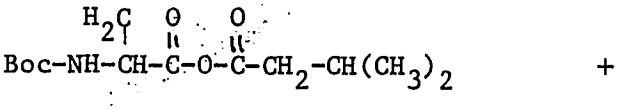
Synthetic Strategy

Synthesis of protected γ-methyl-L-glutamate homo-oligopeptides and protected γ-methyl-L-glutamate-glycine co-oligopeptides was achieved via the mixed carbonic-carboxylic anhydride coupling reaction of Anderson et al.[27] The synthetic strategy called for the stepwise addition of an amino acid residue (#1) N-protected with the tertiary-butoxycarbonyl group (Boc), to a second amino acid protected with a methyl ester on its carboxyl terminus (#3). This formed the completely protected dipeptide. The free carboxyl of the BocGlu(γOMe)OH was activated with isobutylchloroformate to form compound (#2), which greatly enhanced the reactivity to the nucleophile (#3). The γ-carboxyl groups were protected from reaction as the methyl ester.

#1

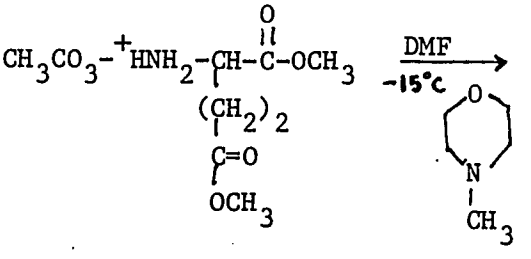


#2

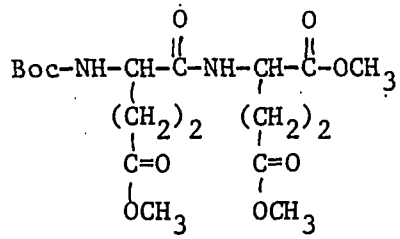


activated intermediate
mixed carbonic-carboxylic anhydride

#3



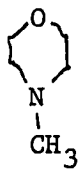
#4



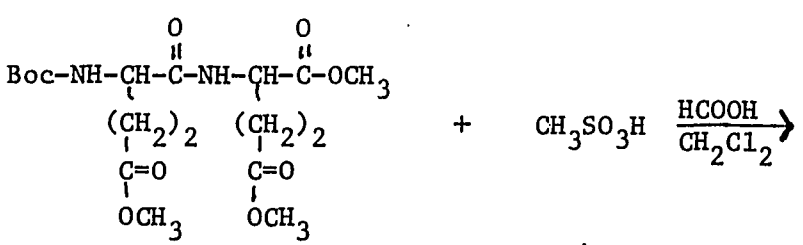
protected dipeptide

Boc = tertiarybutoxycarbonyl = $(CH_3)_3C-O-C-$ (O above C)

DMF = dimethyl formamide

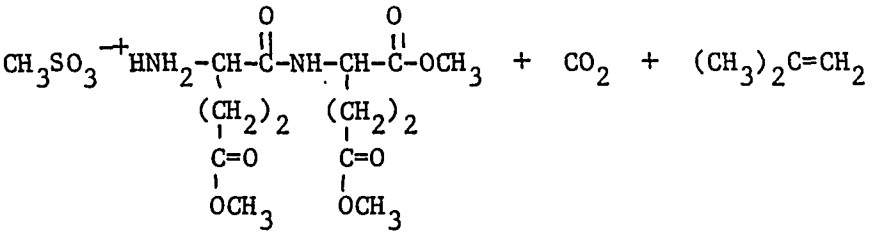
 = N-methyl morpholine

This coupling method maintains optical purity of the L-amino acids, and usually results in reasonable chemical purity and concommitant high yields (> 60%). The use of the Boc protecting group affords good solubility in the organic solvents utilized during synthesis and in helix supporting solvents employed for conformational analysis. Once the protected dipeptide product #4 is isolated the Boc group is rapidly and selectively removed via acidolysis using methanesulfonic acid under anhydrous conditions.[72] This reaction is complete within a few minutes and accomplished without cleavage of any of the methyl ester protecting groups. Specifically, the protected peptide was dissolved in equivalent volumes of anhydrous formic acid and methylene chloride, with the addition of one equivalent of methane sulfonic acid:



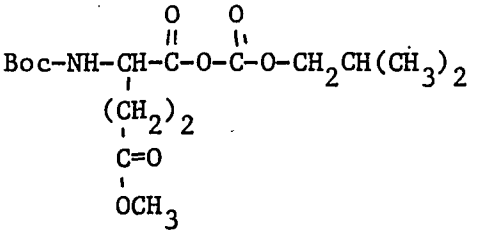
protected dipeptide

#5



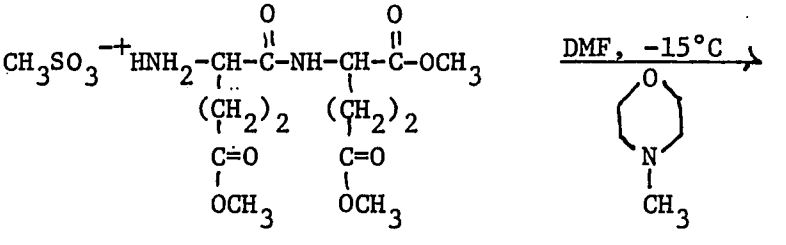
Removal of the Boc group frees the N-terminus of the dipeptide for reaction with the carboxyl activated N-blocked amino acid (#2).

#2



activated in situ

+



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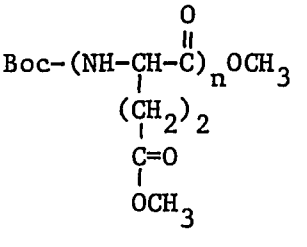


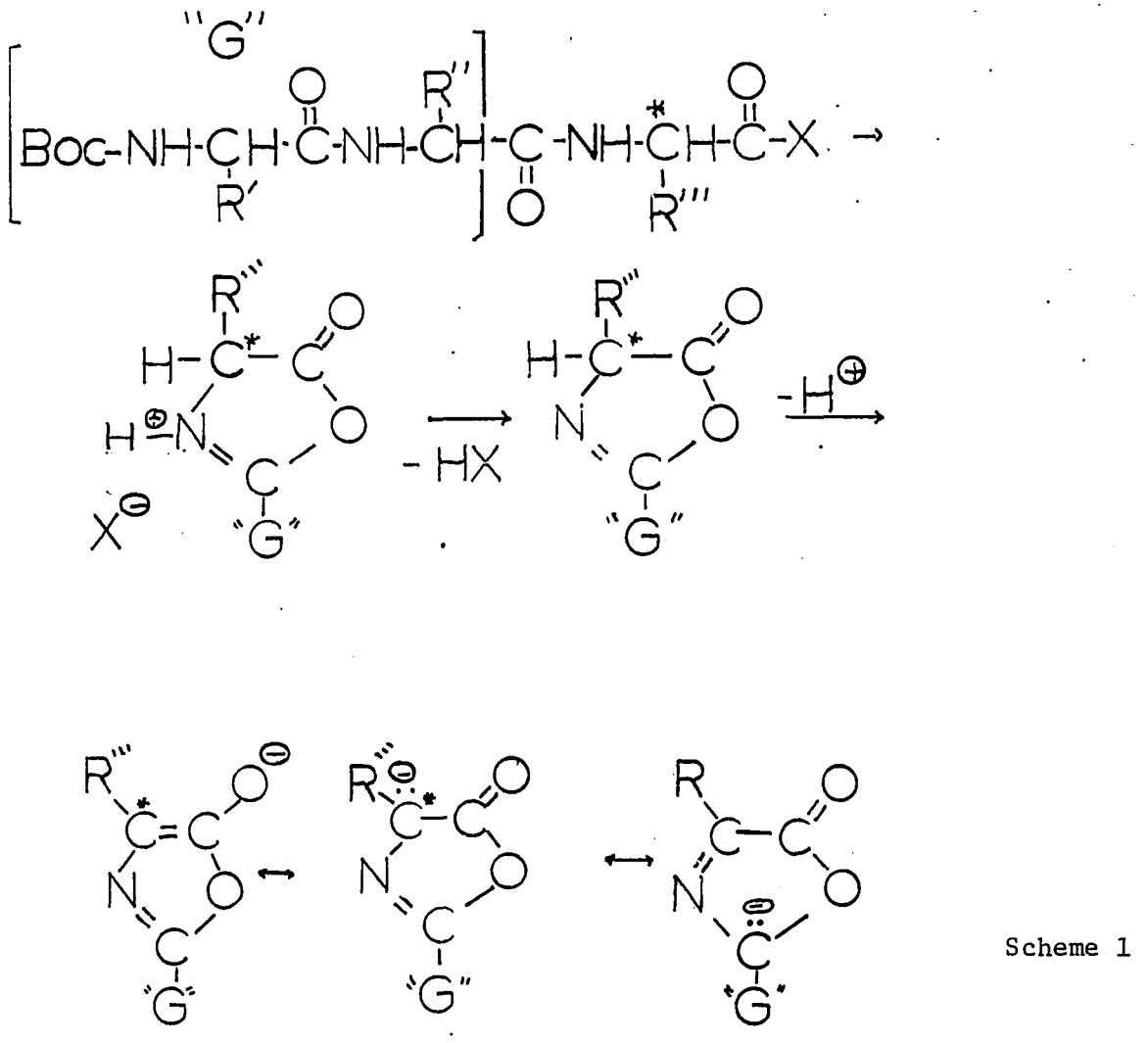
Yields from Boc cleavages were quantitative. The reaction was followed to completion by thin layer chromatography on silica plates. The resulting methanesulfonate peptide salt, #5, was easily precipitated from anhydrous diethyl ether after the removal of reaction solvent by roto-evaporation under reduced pressure.

All protected oligopeptides were required to be at least 97% pure as determined by high pressure liquid chromatography on normal phase silica. Higher molecular weight protected oligomers (n > 5) were rarely this pure after routine synthetic workup as described in the experimental section. Standard chemical purification of oligopeptides usually requires column chromatographic techniques to obtain highly pure compounds. Because of substantial losses that can be incurred during column purification, and decreased quantities of higher peptides, I employed a purification process which limited loss of product to less than 10% during purification. The process involved the suspension of contaminated product in 5% $NaHCO_3$ with overnight stirring. The product was filtered, rinsed with distilled water and rinsed with anhydrous ether a number of times. In the cases where the above procedure was not entirely successful, the protected oligopeptide was suspended in 5% citrate solution and stirred overnight. The product was filtered, rinsed with distilled water, and rinsed several times with anhydrous ether. This overall process, which was successful in all instances, probably removes the less soluble (with respect to aqueous washing solutions) acidic, and/or basic impurities via slow conversion to salts during overnight stirring.

Yields for most of the lower peptides (dimers through pentamers) were between 60% and 90%, excluding the series which contained glycine as the next to last residue. The comparatively low yields obtained for the $Boc(Glu)_n GlyGluOMe$ series (n = 0,1,2) were a result of the increased
OMe
solubility of the peptides in the aqueous wash solution. This was substantiated via thin layer chromatography on silica plates.

As chain length increased to the hexa- and heptapeptides yields in general were substantially reduced. This could have been due to the

reduced nucleophilicity of the nucleophile and certainly was due to the formation of emulsions during workup, as a result of decreased solubility of the higher molecular weight oligomers in the organic phase. Because the entire series of compounds were synthesized in a stepwise manner, the formation of emulsions and the resulting loss of product during workup at the hexamer level and above, presented a problem when attempting to secure reasonable amounts of the following heptamer of the series. This problem could be circumvented in solution phase peptide synthesis via an alternative coupling procedure. The fragment coupling method reacts two small fragments; $Boc(A.A.)_3OH$ + $H_2N(A.A.)_4OMe$, yielding in one step the heptamer and eliminating the loss of the precursors from previous stepwise addition reactions. There is however the possibility of racemization of the $Boc(A.A.)_3OH$ carboxyl terminal A.A. via oxazolone formation (Scheme 1). Loss of the proton at the α-carbon atom and subsequent recapture can result in inversion of configuration and thus conversion of the carboxyl terminal amino acid from the L-isomer to the corresponding D-isomer. In C.D. and polarimetric analysis of oligopeptide conformation optical purity must be maintained for results to be meaningful. With respect to the series of compounds synthesized for this study the above problem could be avoided by using glycine as the carboxyl terminal fragment and varying the number of glutamate residues in either or both fragments to obtain the desired glycine insertion. The above method was attempted, but the greatly reduced solubility of $Boc(Glu\gamma OMe)_nGlyOH$ fragments in organic solution made coupling via the mixed anhydride procedure impossible. An aqueous fragment coupling procedure was attempted using a water soluble carbodimide coupling procedure but the same fragments proved to be also insoluble in water, even with the addition of DMF.



Scheme 1

Despite the low yields of certain peptides the stepwise mixed anhydride procedure was used exclusively for the synthesis of all compounds due to the high solubility of the MSA·nucleophile in the DMF reaction mixture regardless of chain length. This procedure also yielded crude products rarely more impure than 15%.

EXPERIMENTAL

Synthetic

γ-Methyl-L-Glutamate·HCl (I)

Thionyl chloride (280 mmol; 20 ml) was added dropwise, over 15 minutes, to absolute methanol (140 ml) previously cooled to -15°C. L-Glutamic acid (200 mmol; 29.4 g) was added to the above solution and the entire reaction mixture shaken vigorously until most of the solid dissolved. The mixture was then allowed to stand at room temperature for 25 minutes. The product was precipitated by the addition of 400 ml of anhydrous ether. The crystals were collected by filtration and washed several times with anhydrous ether. The product was stored under vacuum overnight. Yield 60%, 23.5 g, m.p. 161°C, $[\alpha]_D^{25}$ = +25.2 (c = 0.22, CH_3OH),(lit. m.p. 166°C).[73]

L-Glutamic Acid Dimethyl Ester·HCl (II)

Thionylchloride (400 mmol; 28 ml) was added dropwise, with constant stirring, over 20 minutes, to absolute methanol (100 ml) previously cooled to -15°C. L-Glutamic acid (200 mmol; 29.4 g) was added to the above solution over a period of 45 minutes while the temperature of the reaction mixture was maintained at -15°C. When the addition was complete the reaction mixture was allowed to come to room temperature and after 2 hours the solution became clear and yellow. The reaction mixture was stirred overnight and then refluxed for 3 hours. The clear yellow liquid was concentrated on a roto-evaporator under reduced pressure. The resulting clear yellow syrup was crystallized by stirring overnight in the presence of 1 liter of anhydrous ether. The product, a white powder, was collected by filtration and washed several times with anhydrous ether. The product

was stored in a desiccator under vacuum overnight. Yield 95%, 39.9 g, m.p. 197°C, $[\alpha]_D^{25}$ = +15.3, (c = 0.17, H_2O).

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamic Acid (III)

Triethylamine (250 mmol; 34.8 ml) was added to γ-methyl-L-glutamate·HCl (100 mmol; 19.7 g) with continuous stirring. To this was added 60 ml of dioxane and 60 ml of distilled water. Upon dissolution of the amino acid 2-(tertiarybutoxycarbonyloxyimino)-2-phenylacetonitrile (100 mmol; 24.6 g) was added. The resulting solution was allowed to stir overnight and 100 ml of ethyl acetate and 100 ml of distilled water were added. The mixture was shaken and the organic layer discarded. The aqueous fraction was washed three times with 100 ml of diethyl ether. The aqueous fraction was acidified with 5% citric acid and extracted five times with 100 ml portions of ethyl acetate. The organic fractions were pooled, decolorized with Norit A and dried with anhydrous $MgSO_4$. The solution was concentrated on a roto-evaporator under reduced pressure to a thick, pale yellow syrup. The resulting syrup was triturated with petroleum ether until solidification of product occurred. The product, a white powder was used without further purification. Yield 79%, 20.6 g, m.p. 72-73°C, $[\alpha]_D^{25}$ = -7.8, (c = 0.18, CF_3CH_2OH). Rf = 0.57 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.49 in butanol:water:acetic acid, 4:5:1 upper phase.

N-t-Butoxycarbonyl Glycine (IV)[72]

The synthesis and workup of this compound was identical to that of Boc-γ-methyl-L-glutamic acid (compound III). The reaction was carried out on a 200 mmol scale. The product, a white powder, was

used without further purification. Yield 80%, 28.0 g, m.p. 87-88°C (lit. m.p. 87-88°C).[72]

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl Dimethyl L-Glutamate (V)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (10 mmol; 2.61 g), was added to 30 ml ethyl acetate. The solution was stirred and cooled to -15°C in a dry ice-acetone bath. N-methylmorpholine (10 mmol; 1.1 ml) and isobutylchloroformate (10 mmol; 1.37 ml) were added and stirring was continued for ten minutes at -15°C. During this time a white precipitate (N-methylmorpholine·HCl salt) was observed to form. To the above mixture was added dimethyl-L-glutamate·HCl (10 mmol; 2.1 g) and N-methylmorpholine (10 mmol; 1.1 ml) in dimethylformamide (10 ml). The resulting mixture was stirred at -15°C for ten minutes at which time the dry ice bath was removed and the reaction mixture stirred at room temperature for one hour. The reaction mixture was concentrated on a rotary evaporator under reduced pressure to near dryness. The above residue was dissolved in 300 ml ethyl acetate and the organic solution was washed three times with 100 ml portions of 5% citric acid, three times with 100 ml portions of 5% $NaHCO_3$ and three times with 100 ml portions saturated NaCl solution. The organic phase was dried with anhydrous $MgSO_4$, filtered, and concentrated on a rotary evaporator under reduced pressure to a volume of ~ 30 ml. The product was precipitated from petroleum ether as a white powder and was used without further purification. Yield 86%, 3.6 g, m.p. 87°C, $[\alpha]_D^{25}$ = 2.75 (c = 0.15, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.75 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.83 in butanol:water:acetic acid, 4:5:1 upper phase.

γ-Methyl-L-Glutamyl Dimethyl L-Glutamate·Methane Sulfonate (VI)

N-t-Butoxycarbonyl-L-methyl-glutamyl diethyl L-glutamate (40 mmol; 4.15 g) was dissolved in 12.5 ml of CH_2Cl_2. To this solution was added 12.5 ml anhydrous formic acid. Methane sulfonic acid (10 mmol; 0.64 ml) was then added and the solution swirled. Evolution of CO_2 was observed. The reaction was followed by thin layer chromatography on silica plates and usually reached completion within fifteen minutes. The solvents were removed on a rotary evaporator under reduced pressure and the N-terminal deprotected dipeptide·MSA salt precipitated with anhydrous diethyl ether as an oil. Yield was quantitative. The oil was homogeneous on thin layers of silica in butanol:water:acetic acid, 4:5:1. Rf = 0.45. It was used without further purification.

N-t-Butoxycarbonyl-di-(γ-Methyl-L-Glutamyl) Dimethyl L-Glutamate (VII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (10 mmol; 2.61 g) was reacted with γ-methyl-L-glutamyl dimethyl L-glutamate·MSA using the mixed anhydride coupling procedure described for the analogous dimer. The workup was identical to that for the analogous dimer. The product was a white powder. Yield = 90%, 5.0 g, m.p. 115°C, $[\alpha]_D^{25}$ = -4.41 (c = .14, CF_3CH_2OH). Purity via normal phase HPLC > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.71 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.77 in butanol: water:acetic acid, 4:5:1 upper phase.

Di-(γ-Methyl-L-Glutamyl) Dimethyl L-Glutamate·Methane Sulfonate (VIII)

N-t-Butoxycarbonyl-di-(γ-methyl-L-glutamyl)-dimethyl L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described for the analogous dimer. Workup was

identical as that for the analogous dimer. The N-deprotected tripeptide·MSA salt was recovered as a white powder. Yield was quantitative. m.p. 141-144°C. Rf = 0.36 on silica thin layers in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl L-Glutamate (IX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (5.4 mmol; 1.41 g) was reacted with di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (5.4 mmol; 3 g) using the mixed anhydride coupling procedure described for the analogous trimer. The workup was identical to that of the analogous trimer. The product was a white powder. Yield 72%, 2.73 g, m.p. 149-152°C, $[\alpha]_D^{25} = -44.0$ (c = 0.18, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.61 on thin layer silica plates in ethylacetate:methanol, 2:1. Rf = 0.64 in butanol:water:acetic acid, 4:5:1 upper phase.

Tri-(γ-Methyl-L-Glutamyl) Dimethyl-L-Glutamate·Methane Sulfonate (X)

N-t-Butoxycarbonyl-tri-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (2.4 mmol; 1.7 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl/formic acid as described for the analogous trimer. Workup was identical to that of the analogous trimer. The N-deprotected tetrapeptide·MSA salt was recovered as a white solid. Yield was quantitative. m.p. 168-169°C. Rf = 0.33 on thin layers of silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tetra-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XI)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (2.5 mmol; 0.65 g) was reacted with tri-(γ-methyl-L-glutamyl) dimethyl-L-glutamate·MSA (2.5 mmol; 1.75 g) using the mixed anhydride coupling procedure described

for the analogous tetramer. The workup was identical to that of the analogous tetramer. The product was a white powder. Yield 51%, 1.07 g, m.p. 191°C, $[\alpha]_D^{25}$ = -38.5 (c = .17, CF_3CH_2OH). Purity via HPLC > 97, in cyclohexane:isopropanol:methanol, 8:1:1. Rf - 0.74 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = .67 in butanol:water:acetic acid, 4:5:1 upper phase.

Tetra-(γ-Methyl-L-Glutamyl) Dimethyl L-Glutamate·Methane Sulfonate (XII)

N-t-Butoxycarbonyl-tetra-(γ-methyl-L-glutamyl) dimethyl L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/ formic acid as described for the analogous tetramer. The workup was identical to that of the analogous tetramer. The N-deprotected pentamer·MSA salt was recovered as a white solid. Yield was quantitative. m.p. > 195°C. Rf = 0.26 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-penta-(γ-Methyl-L-Glutamyl)-Dimethyl L-Glutamate (XIII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid 0.6 mmol; 0.16 g) was reacted with tetra-(γ-methyl-L-glutamyl)-dimethyl L-glutamate·MSA (.6 mmol; 0.51 g) using the mixed anhydride coupling procedure described for the previous protected compounds. The workup was identical to that of the lower oligomers, however HPLC analysis of this product revealed impurities greater than 10%. The white powder (product) 300 mg, was stirred overnight in 5% citric acid, filtered and rinsed with distilled water. The filtered product was then stirred overnight in 5% $NaHCO_3$, filtered and rinsed with distilled water and then rinsed extensively with anhydrous diethyl ether. The recovered product was dried and used

without further purification. Yield = 76%, 0.45 g, m.p. 228°C, $[\alpha]_D^{25}$ = -32.7 (c = 0.13, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.79 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.55 = 0.55 in butanol:water:acetic acid, 4:5:1 upper phase.

Penta-(γ-Methyl-L-Glutamyl) Dimethyl L-Glutamate·Methane Sulfonate (XIV)

N-t-Butoxycarbonyl-penta-(γ-methyl-L-glutamyl) dimethyl L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/ formic acid as described for the analogous deprotected peptides. Workup was identical to that of the analogous deprotected peptides. The N-deprotected hexapeptide·MSA salt was recovered as a white solid. m.p. > 193°C. Rf = 0.26 on thin layers of silica in butanol:water: acetic acid, 4:5:1.

N-t-Butoxycarbonyl-hexa-(γ-Methyl-L-Glutamyl)-Dimethyl L-Glutamate (XV)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.25 mmol; 0.07 g) was reacted with penta-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (using the mixed anhydride procedure described previously). The workup was identical to that of the analogous hexamer. The product was a white powder. Yield 55%, 0.16 g, m.p. 230-231°C, $[\alpha]_D^{25}$ = -27.4 (c = .16, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.37 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.44 in butanol:water:acetic acid, 4:5:1 upper phase.

N-t-Butoxycarbonyl-Glycyl-Dimethyl-L-Glutamate (XVI)

N-t-Butoxycarbonyl glycine (10 mmol; 1.75 g) was reacted with dimethyl L-glutamate·HCl (using the mixed anhydride coupling procedure previously described for the glutamate homo-oligomers). The workup was

identical to that of compound XI except for the presence of a major impurity in the reaction product. To remove this biproduct 10 mmoles of the product (which was an oil) was dissolved in 50 ml ethylacetate and stirred overnight in the presence of 50 ml 5% $NaHCO_3$. The organic layer was separated, dried with anhydrous $MgSO_4$ and filtered. The solvent was removed using a rotary evaporator under reduced pressure. The product was recovered as a clear oil and used without further purification. Yield 33%, 1.1 g, $[\alpha]_D^{25} = -8.1$, (c = 0.29, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = .68 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = .80 in butanol:water:acetic acid, 4:5:1.

Glycyl-Dimethyl-L-Glutamate·Methane Sulfonate (XVII)

N-t-Butoxycarbonyl-glycyl-dimethyl-L-glutamate (20 mmol; 6.6 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously for the glutamate homo-oligomers. Workup was identical to that of the glutamate homooligomers. The N-deprotected dipeptide·MSA salt was precipitated as an oil. Yield was quantitative. Rf = 0.30 on thin layer silica in butanol:water: acetic acid, 4:5:1. The product was used without further purification.

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl Glycyl Dimethyl L-Glutamate (XVIII)

N-t-Butoxy-γ-methyl-L-glutamic acid (33 mmol; 8.6 g) was reacted with glycyl-dimethyl L-glutamate·MSA (using the mixed anhydride coupling procedure). The workup was identical to that for compound XI. The product was recovered as an oil, and used without any further purification. Yield 47%, 7.3 g, $[\alpha]_D^{25} = -10.5$ (c = 0.4, CF_3CH_2OH). Purity via

HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.31 on thin layer silica plates in ethylacetate:methanol, 2:1. Rf = 0.63 in butanol:water:acetic acid, 4:5:1.

γ-Methyl-L-Glutamyl Glycyl Dimethyl L-Glutamate·Methane Sulfonate (XIX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamyl-glycyl-dimethyl-L-glutamate, (2.6 mmol; 1.23 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid. Workup was identical to that of the analogous dimer. The N-deprotected tripeptide·MSA salt was collected as an oil. Yield was quantitative. Rf = 0.31 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-di-(γ-Methyl-L-Glutamyl)-Glycyl Dimethyl L-Glutamate (XX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (15.4 mmol; 4.02 g) was reacted with γ-methyl-L-glutamyl-glycyl-dimethyl-L-glutamate·MSA (using the mixed anhydride coupling procedure mentioned previously). The workup was identical to that of compound XI. The product was recovered as a white powder. Yield 31%, 3.1 g, m.p. 108°C, $[\alpha]_D^{25}$ = -17.7 (c = 14, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane: isopropanol:methanol, 8:1:1. Rf = 0.70 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.68 in butanol:water:acetic acid, 4:5:1.

Di-(γ-Methyl-L-Glutamyl)-Glycyl Dimethyl L-Glutamate·Methane Sulfonate (XXI)

N-t-Butoxycarbonyl-di-(γ-methyl-L-glutamyl) glycyl dimethyl L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The N-deprotected

tetrapeptide·MSA salt was collected as an oil. Yield was quantitative. Rf = 0.34 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tri-(γ-Methyl-L-Glutamyl) Glycyl Dimethyl L-Glutamate (XXII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (5 mmol; 1.3 g) was reacted with di-(γ-methyl-L-glutamyl)-glycyl-dimethyl-L-glutamate·MSA (5 mmol; 3.06 g) using the mixed anhydride coupling procedure described previously. The workup was identical to that of compound XI. The product was recovered as a white powder. Yield 80%, 3.0 g, m.p. 166°C, $[\alpha]_D^{25} = -20.3$ (c = 0.18, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.69 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.54 in butanol:water:acetic acid, 4:5:1.

Tri-(γ-Methyl-L-Glutamyl)-Glycyl-Dimethyl-L-Glutamate·Methane Sulfonate (XXIII)

N-t-Butoxycarbonyl-tri-γ-methyl-L-glutamyl-glycyl-dimethyl-L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The N-deprotected pentapeptide·MSA salt was collected as an oil. Yield was quantitative. Rf = .30 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tetra-(γ-Methyl-L-Glutamyl) Glycyl Dimethyl L-Glutamate (XXIV)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (2.2 mmol; .57 g) was reacted with tri-(γ-methyl-L-glutamyl)glycyl dimethyl-L-glutamate·MSA (using the mixed anhydride coupling procedure described previously). The workup was identical to that for compound V. The product, which was

recovered as a white powder (~500 mg) was stirred overnight in 10 ml 5% $NaHCO_3$, filtered and washed with distilled water and anhydrous diethyl ether. The product was recovered as a white solid. Yield 25%, 0.5 g, m.p. 194°C, $[\alpha]_D^{25} = -18.0$ (c = 0.17, CF_3CH_2OH). Purity via HPLC > 97%, in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.39 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.46 in butanol:water:acetic acid, 4:5:1.

Tetra-(γ-Methyl-L-Glutamyl)-Glycyl-Dimethyl-L-Glutamate·Methane Sulfonate (XXV)

N-t-Butoxycarbonyl-tetra-(γ-methyl-L-glutamyl) glycyl dimethyl L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The workup was as described previously. The N-deprotected hexapeptide·MSA salt was collected as a white solid. Yield was quantitative. Compound decomposed above 182°C. Rf = 0.31 on thin layer silica in butanol: water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-penta-(γ-Methyl-L-Glutamyl) Glycyl Dimethyl L-Glutamate (XXVI)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.75 mmol; 2 g) was reacted with tetra-(γ-methyl-L-glutamyl) glycyl dimethyl L-glutamate (0.75 mmol; 0.67 g) using the mixed anhydride coupling procedure described previously. The workup was identical to that of compound XXIV. The compound was recovered as a white powder. Yield 56%, 0.44 g, m.p. 225-228°C, $[\alpha]_D^{25} = -21.6°$ (c = 0.16, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol: methanol, 8:1:1. Rf = 0.67 on thin layer silica plates in ethyl acetate: methanol, 2:1. Rf = 0.65 in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate (XXVII)

N-t-Butoxycarbonyl-glycine (5.8 mmol; 1.02 g) was reacted with γ-methyl-L-glutamyl-dimethyl L-glutamate·MSA (using the mixed anhydride procedure previously described). The workup was identical to that of compound V. The product was collected as a white powder. Yield 39%, 1.08 g, m.p. 68°C, $[\alpha]_D^{25}$ = -24.7 (c = 0.22, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane: isopropanol:Methanol, 8:1:1. Rf = 0.37 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.71 in butanol:water:acetic acid, 4:5:1.

Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate·Methane Sulfonate (XXVIII)

N-t-Butoxycarbonyl-glycyl-γ-methyl-L-glutamate-dimethyl-L-glutamate (0.32 mmol; 0.15 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The N-deprotected tripeptide·MSA salt was recovered as a white solid. Yield was quantitative. m.p. 141-143°C. Rf = 0.36 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl-Glycyl-γ-Methyl-L-Glutamyl Dimethyl-L-Glutamate (XXIX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.32 mmol; 0.08 g) was reacted with glycyl-di-(γ-methyl-L-glutamyl)dimethyl-L-glutamate·MSA (using the mixed anhydride procedure previously described). The workup was identical to that for compound V. The product was recovered as a white powder. Yield 75%, 0.16 g, m.p. 122-124°C, $[\alpha]_D^{25}$ = -30.4 (c = 0.17, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography

> 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf - 0.78 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.78 in butanol:water:acetic acid, 4:5:1.

γ-Methyl-L-Glutamyl-Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate· Methane Sulfonate (XXX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamyl-glycyl γ-methyl-L-glutamyl dimethyl-L-glutamate (2.5 mmol; 1.9 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl/formic acid as described previously. The deprotected tetrapeptide·MSA salt was recovered as an oil. Yield was quantitative. Rf - 0.20 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-di-(γ-Methyl-L-Glutamyl)-Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate (XXXI)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (2.5 mmol; 1.63 g) was reacted with γ-methyl-L-glutamyl glycyl γ-methyl-L-glutamyl dimethyl L-glutamate·MSA (using the mixed anhydride procedure described previously). The workup was identical to that of compound V. The product was collected as a white powder. Yield 60%, 1.14 g, m.p. 132-133°C, $[\alpha]_D^{25} = -19.7$ (c = 0.16, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.72 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.62 in butanol:water:acetic acid, 4:5:1.

Di-(γ-Methyl-L-Glutamyl)-Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate· Methane Sulfonate (XXXII)

N-t-Butoxycarbonyl-di-(γ-methyl-L-glutamyl) glycyl γ-methyl-L-glutamyl dimethyl L-glutamate (1.2 mmol; 0.9 g) was deprotected at the

N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as previously described. The N-deprotected pentapeptide was collected as a white solid. Yield was quantitative. m.p. 129-133°C. Rf = 0.25 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tri-(γ-Methyl-L-Glutamyl)-Glycyl-γ-Methyl-L-Glutamyl Dimethyl-L-Glutamate (XXXIII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (1.2 mmol; 0.31 g) was reacted with di-(γ-methyl-L-glutamyl)-glycyl-γ-methyl-L-glutamyl-dimethyl-L-glutamate·MSA (1.2 mmol; 0.9 g) using the mixed anhydride coupling procedure previously described. The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 71%, 0.77 g, m.p. 216-219°C, $[\alpha]_D^{25}$ = -23.0 (c = 0.17, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.71 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.45 in butanol:water:acetic acid, 4:5:1.

Tri-(γ-Methyl-L-Glutamyl)-Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate·Methane Sulfonate (XXXIV)

N-t-Butoxycarbonyl-tri-(γ-methyl-L-glutamyl)-glycyl-γ-methyl-L-glutamyl-dimethyl-L-glutamate (0.5 mmol; 0.45 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The N-deprotected hexamer·MSA salt was collected as a white solid. Yield was quantitative. m.p. 181-186°C. Rf = 0.33 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tetra-(γ-Methyl-L-Glutamyl)-Glycyl-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamate (XXXV)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.5 mmol; 0.13 g) was reacted with tri-(γ-methyl-L-glutamyl)-glycyl-γ-methyl-L-glutamyl-dimethyl-L-glutamate·MSA (0.5 mmol; 0.45 g) using the mixed anhydride coupling procedure described previously. The workup was identical to that of compound XIII. The product was recovered as a white powder and used without further purification. Yield 50%, 0.26 g, m.p. 228°C, $[\alpha]_D^{25}$ = -27.7 (c = 0.16, CF_3CH_2OH). Purity via normal phase HPLC > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.65 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.41 in butanol:water: acetic acid, 4:5:1.

N-t-Butoxycarbonyl-Glycyl-di-γ-Methyl-L-Glutamyl-Dimethyl-L-Glutamyl-Dimethyl-L-Glutamate (XXXVI)

N-t-Butoxycarbonyl glycine (4.4 mmol; 0.77 g) was reacted with di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (using the mixed anhydride procedure previously described). The workup was identical to that for compound V. The product was recovered as a white powder. Yield 78%, 2.1 g, m.p. 154°C, $[\alpha]_D^{25}$ = -33.6 (c = 0.14, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.74 on thin layer silica plates in ethyl acetate:methanol, 3:1. Rf = 0.73 in butanol:water: acetic acid, 4:5:1.

Glycyl-di-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XXXVII)

N-t-Butoxycarbonyl-glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (2.1 mmol; 1.3 g) was deprotected at the N-terminus using

methane sulfonic acid in CH_2Cl_2/formic acid as described previously for the protected glutamate dipeptide. The workup was identical to that of the protected glutamate dipeptide. The N-deprotected tetrapeptide·MSA salt was collected as a white solid. Yield was quantitative. m.p. 144-146°C. Rf = 0.22 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl-Glycyl-di-(γ-Methyl-L-glutamyl)-Dimethyl-L-Glutamate (XXXVIII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (2.1 mmol; 0.55 g) was reacted with glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (2.1 mmol; 1.3 g) using the mixed anhydride procedure previously described. The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 34%, 0.54 g, m.p. 162°C, $[\alpha]_D^{25} = -25.4$ (c = 0.14, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.69 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.54 in butanol:water:acetic acid, 4:5:1.

γ-Methyl-L-Glutamyl-Glycyl-di-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XXXIX)

N-t-Butoxycarbonyl-γ-methyl-L-glutamyl-glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate was deprotected using methane sulfonic acid in CH_2Cl_2/formic acid as described previously. The N-deprotected pentapeptide·MSA salt was collected as an oil. Yield was quantitative. Rf = 0.21 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-di-(γ-Methyl-L-Glutamyl)-Glycyl-di-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XL)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.5 mmol; 0.13 g) was reacted with γ-methyl-L-glutamyl-glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (0.5 mmol; 0.38 g) using the mixed anhydride procedure described previously. The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 48%, 0.22 g, m.p. 210-212°C, $[\alpha]_D^{25}$ = -31.3 (c = 0.15, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane: isopropanol:methanol, 8:1:1. Rf = 0.72 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.45 in butanol:water:acetic acid 4:5:1.

Di-(γ-Methyl-L-Glutamyl)-Glycyl-di-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XLI)

N-t-Butoxycarbonyl-di-(γ-methyl-L-glutamyl)-glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described for the protected glutamate dipeptide. The N-deprotected hexapeptide·MSA salt was collected as a white solid. Yield was quantitative. m.p. 148-150°C. Rf = 0.44 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-tri-(γ-Methyl-L-Glutamyl)-Glycyl-di-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XLII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.2 mmol; 0.05 g) was reacted with di-(γ-methyl-L-glutamyl)-glycyl-di-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (0.2 mmol; 0.18 g) using the mixed

anhydride coupling procedure described previously. The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 48%, 0.1 g, m.p. 217-220°C, $[\alpha]_D^{25}$ = -23.9 (c = 0.18, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.69 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.40 in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-Glycyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XLIII)

N-t-Butoxycarbonyl-glycine (2.0 mmol; 0.35 g) was reacted with tri-(γ-methyl-L-glutamyl) dimethyl-L-glutamate·MSA (using the mixed anhydride procedure described previously). The workup was identical to that of compound V. The product was recovered as a white powder. Yield 39%, 0.6 g, m.p. 165-169°C, $[\alpha]_D^{25}$ = -35.4 (c = 0.13, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.70 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.57 in butanol: water:acetic acid, 4:5:1.

Glycyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XLIV)

N-t-Butoxycarbonyl-glycyl-tri-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (0.8 mmol; 0.6 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously for the protected glutamate dipeptide. The N-deprotected tetrapeptide·MSA salt was collected as an oil. Yield was quantitative.

Rf = 0.20 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl-Glycyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XLV)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.8 mmol; 0.2 g) was reacted with glycyl-tri-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (using the mixed anhydride procedure described previously). The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 87%, 0.56 g, m.p. 210°C; $[\alpha]_D^{25} = -27.91$ (c = 0.17, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf - 0.70 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf - 0.45 in butanol:water:acetic acid, 4:5:1.

γ-Methyl-L-Glutamyl-Glycyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XLVI)

N-t-Butoxycarbonyl-γ-methyl-L-glutamyl-glycyl-tri-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (1.1 mmol; 1.0 g) was deprotected at the N-terminus using methane sulfonic in CH_2Cl_2/formic acid as described for the glutamate dipeptide. The N-deprotected hexapeptide·MSA salt was collected as a white powder. Yield was quantitative. m.p. 149-151°C. Rf = 0.31 on thin layer silica in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-di-(γ-Methyl-L-Glutamyl)-Glycyl-tri-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XLVII)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (1.1 mmol; 0.29 g) was reacted with γ-methyl-L-glutamyl-glycyl-tri-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate·MSA (using the mixed anhydride procedure previously described). The workup was identical to that for compound XIII. The

product was recovered as a white powder. Yield 55%, 0.63 g, m.p. 222-227, $[\alpha]_D^{25}$ = -28.0 (c = 0.14, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol: methanol, 8:1:1. Rf = 0.74 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf - 0.52 in butanol:water:acetic acid, 4:5:1.

N-t-Butoxycarbonyl-Glycyl-tetra-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (XLVIII)

N-t-Butoxycarbonyl-glycine (1.5 mmol; 0.26 g) was reacted with tetra-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate methane sulfonate (using the mixed anhydride procedure previously described). The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 40%, 0.6 g, m.p. 205-210°C, $[\alpha]_D^{25}$ = -35.4 (c = 0.13, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol: methanol, 8:1:1. Rf = 0.71 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.51 in butanol:water:acetic acid, 4:5:1.

Glycyl-tetra-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate·Methane Sulfonate (XLIX)

N-t-Butoxycarbonyl-glycyl-tetra-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate (0.6 mmol; 0.54 g) was deprotected at the N-terminus using methane sulfonic acid in CH_2Cl_2/formic acid as described previously for the glutamate dipeptide. The N-deprotected hexapeptide·MSA salt was collected as a white solid. Yield was quantitative. Compound decomposed above 202°C. Rf = 0.30 on thin layer silica in butanol:water: acetic acid, 4:5:1.

N-t-Butoxycarbonyl-γ-Methyl-L-Glutamyl-Glycyl-tetra-(γ-Methyl-L-Glutamyl)-Dimethyl-L-Glutamate (L)

N-t-Butoxycarbonyl-γ-methyl-L-glutamic acid (0.4 mmol; 0.1 g) was reacted with glycyl-tetra-(γ-methyl-L-glutamyl)-dimethyl-L-glutamate methane sulfonate (using the mixed anhydride procedure described previously). The workup was identical to that of compound XIII. The product was recovered as a white powder. Yield 35%, 0.15 g, m.p. 225-227°C, $[\alpha]_D^{25} = -19.4$ (c = 0.15, CF_3CH_2OH). Purity via normal phase high pressure liquid chromatography > 97% in cyclohexane:isopropanol:methanol, 8:1:1. Rf = 0.70 on thin layer silica plates in ethyl acetate:methanol, 2:1. Rf = 0.48 in butanol:water:acetic acid, 4:5:1.

High Performance Liquid Chromatography

Examination of oligopeptide conformation requires the synthesis of chemically and optically pure materials. High performance liquid chromatography (HPLC), on normal phase silica is well suited to study the homogeneity of the protected homo-oligopeptides of γ-methyl-L-glutamate and the co-oligopeptide of γ-methyl-L-glutamate and glycine.

HPLC analysis of the protected homo-oligomers was carried out in a solvent system consisting of cyclohexane:isopropanol:methanol, 8:1:1, using a flow rate of 2 ml/min. A few drops of trifluoroethanol was added to the heptamer solutions due to the decreased solubility of this homo-oligomer in methylene chloride. Retention times or K' values of homo-oligopeptide peaks increased with an increase in chain length.

$$K' = \frac{V_1 - V_o}{V_o},$$

where V_1 is the retention volume of the component of interest and V_o is the retention volume of the solvent front. Complete peak separation was achieved for all homo-oligomers using the above solvent system. Injection of a mixture of all homo-oligomers demonstrated the great resolving power of this system (Fig. 2).

The HPLC analysis of the co-oligopeptides was performed under the conditions identical to the above. During evaluation of chemical homogeneity several co-oligopeptides of the same molecular weight but different sequence displayed significant differences in retention times on μPorasil (normal phase silica). These results must be indicative of the high sensitivity of the system to slight changes in molecular structure. As seen in Table 1 and chromatograms (Figs. 2

through 15), the effect of glycine insertion on the tri-, tetra-, and pentapeptides results in stronger retention of the co-oligomers by the column as compared to the corresponding homo-oligomers. These observations are consistent with glycine behaving as a more polar residue than γ-methyl-L-glutamate and therefore interacting more strongly with the silica support. Two of the co-hexapeptides (Fig. 19, 20), and three of the co-heptapeptides (Fig. 24, 25, 26), however, have significantly higher mobility than the parent homo-oligopeptide. Thus in several instances glycine behaves as if it is a less polar species than γ-methyl-L-glutamate.

In experiments performed by Naider and Huchital,[28] comparison of the glutamate-glycine hexamers with L-methionine-glycine cohexamers revealed significant similarities. In both cohexamer series glycine in position 2 resulted in greatest retention, whereas glycine in position 3 and 4 gave the highest mobilities. These results suggest that both the glutamate and the methionine copeptides have similar conformational distributions and may interact in a similar manner with the HPLC support. These findings may be a consequence of a major perturbation to the three dimensional conformation of the peptide upon insertion of glycine. It is known that many homo-oligopeptides have critical chain lengths for onset of secondary structure of between six or seven residues.[1] In light of this the skewed chromatographic pattern of the homoheptamer (Fig. 22), and the greatly increased retention time (as compared to the lower homo-oligomers), may be a result of effects on mobility due to the onset of a unique secondary structure at this chain length. The insertion of glycine into the backbone may perturb this structure thus reducing glycine interaction with the column resulting in a reduced retention time. Unfortunately,

due to poor solubility of the higher peptides in the mobile phase employed it is extremely difficult to carry out C.D. measurements in this solvent.

With the proper choice of solvent system and flow rate, high performance liquid chromatography has proven to be a highly effective method for chemical purity analysis. HPLC might also serve as a system which could detect via retention times slight changes in molecular conformation as a result of differential interaction of the various co-oligomers with the silica support.

TABLE 1

Mobility of Glycine-γ-Methyl Glutamate

Co-oligopeptides on μPorasil

Compound	Mobile Phase (Cyclohexane-Isopropanol-Methanol)	K'[a]
Boc-[Glu(OMe)]$_3$OMe	92:5:2	3.40[b]
Boc-Gly[Glu(OMe)]$_2$OMe	92:6:2	4.54[b]
Boc-Glu(OMe)-Gly-Glu(OMe)OMe	92:6:2	6.01[b]
Boc-[Glu(OMe)]$_4$OMe	92:6:2	7.96[b]
Boc-Gly-[Glu(OMe)]$_3$OMe	92:6:2	7.98[b,c]
Boc-Glu(OMe)-Gly[Glu(OMe)]$_2$OMe	92:6:2	11.91[b]
Boc-[Glu(OMe)]$_2$-Gly-Glu(OMe)OMe	92:6:2	12.37[b]
Boc-[Glu(OMe)]$_3$OMe	80:10:10	1.08[b]
Boc-Gly-[Glu(OMe)]$_2$OMe	80:10:10	1.08[b]
Boc-Glu(OMe)-Gly-Glu(OMe)OMe	80:10:10	1.30[b]
Boc-[Glu(OMe)]$_4$OMe	80:10:10	1.58[b]
Boc-Gly-[Glu(OMe)]$_3$OMe	80:10:10	1.63[b]
Boc-Glu(OMe)-Gly-[Glu(OMe)]$_2$OMe	80:10:10	1.89[b]
Boc-[Glu(OMe)]$_2$-Gly-Glu(OMe)OMe	80:10:10	1.91[b]
Boc-[Glu(OMe)]$_5$-OMe	80:10:10	2.57[b]
Boc-Gly-[Glu(OMe)]$_4$OMe	80:10:10	2.90[b]
Boc-Glu(OMe)-Gly[Glu(OMe)]$_3$OMe	80:10:10	3.20[b]
Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_2$OMe	80:10:10	2.62[b]
Boc-[Glu(OMe)]$_3$-Gly-Glu(OMe)OMe	80:10:10	2.75[b]
Boc-[Glu(OMe)]$_6$OMe	80:10:10	4.26[d]
Boc-Gly-[Glu(OMe)]$_5$OMe	80:10:10	4.65[d]

TABLE 1 (Cont'd.)

Mobility of Glycine-γ-Methyl Glutamate Co-oligopeptides on μPorasil

Compound	Mobile Phase (Cyclohexane-Isopropanol-Methanol)	K'[a]
Boc-Glu(OMe)-Gly-[Glu(OMe)]$_4$OMe	80:10:10	4.85[d]
Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_3$OMe	80:10:10	4.19[d]
Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_2$OMe	80:10:10	3.89[d]
Boc-[Glu(OMe)]$_4$-Gly-Glu(OMe)OMe	80:10:10	4.37[d]
Boc-[Glu(OMe)]$_7$OMe	80:10:10	7.08[c,d]
Boc-Glu(OMe)-Gly-[Glu(OMe)]$_5$OMe	80:10:10	7.89[d]
Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_4$OMe	80:10:10	6.14[d]
Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_3$OMe	80:10:10	5.94[d]
Boc-[Glu(OMe)]$_4$-Gly-[Glu(OMe)]$_2$OMe	80:10:10	5.83[d]
Boc-[Glu(OMe)]$_5$-Gly-[Glu(OMe)]$_1$OMe	80:10:10	5.45[d]

[a] $K' = V_1 - V_0/V_0$, where V_1 is the retention volume of the component of interest and V_0 the dead volume; flow rate = 2.0 mL/min.

[b] Peptide injected in methylene chloride.

[c] Assymmetric-distorted peak shape.

[d] Peptide injected in methylene chloride/trifluoroethanol (10:1).

Figure 2. γ-Methyl Glutamate Homo-oligopeptides

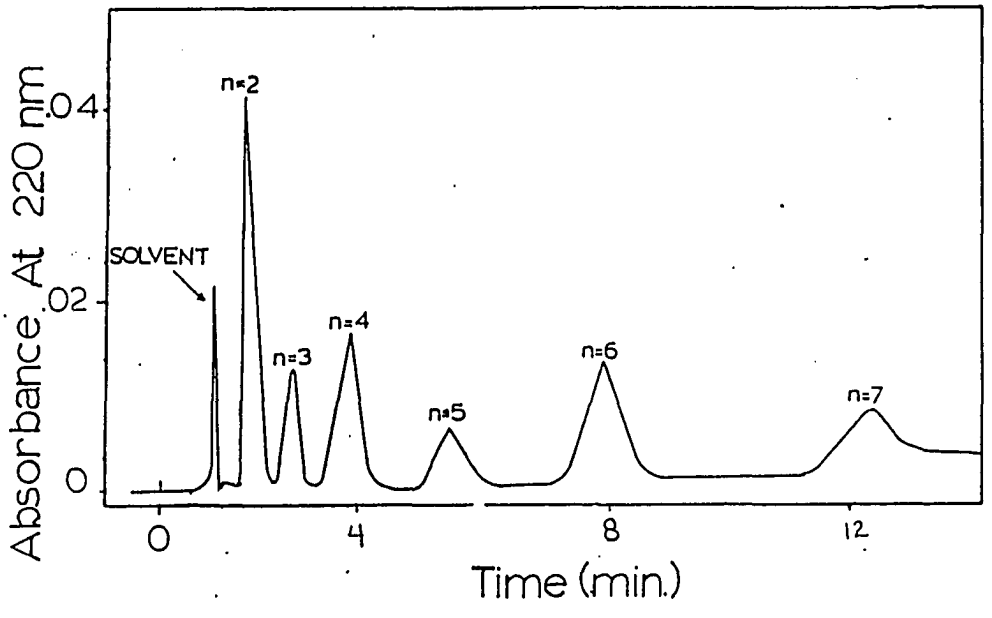


Figure 3. Boc-[Glu(OMe)]$_2$OMe

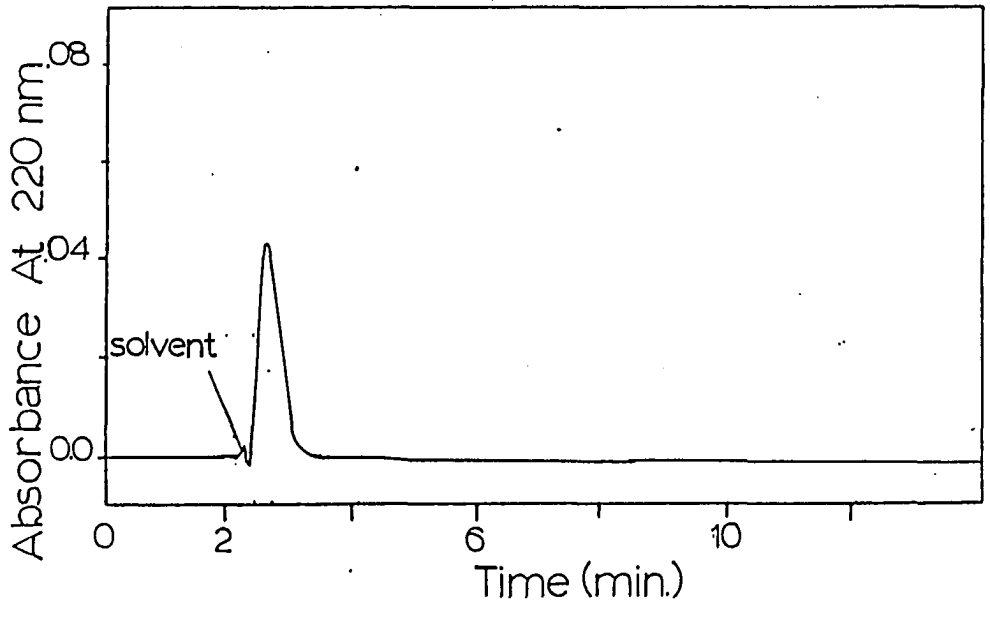


Figure 4. Boc-[Glu(OMe)]$_3$OMe

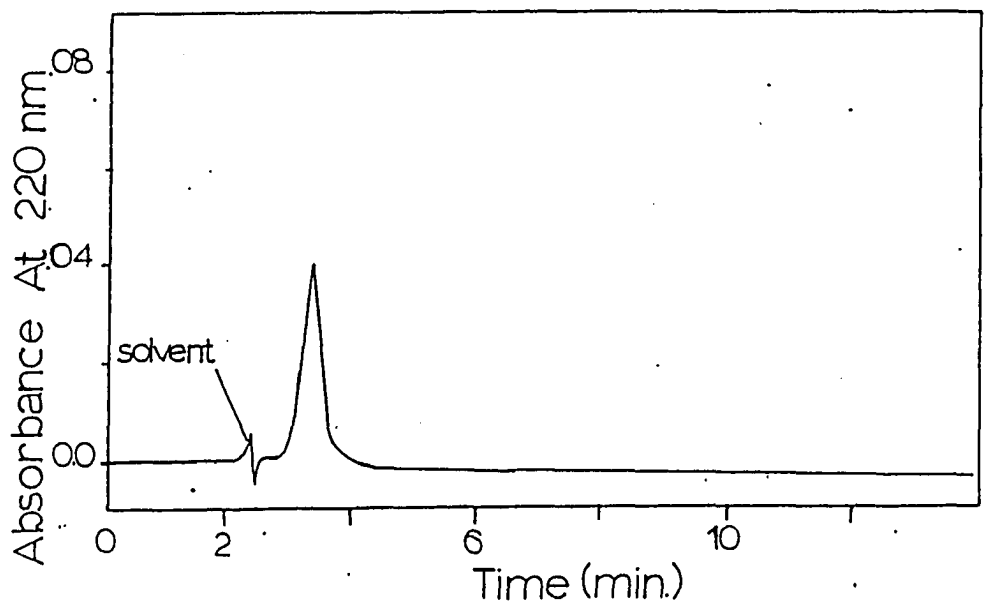


Figure 5. Boc-Gly[Glu(OMe)]$_2$OMe

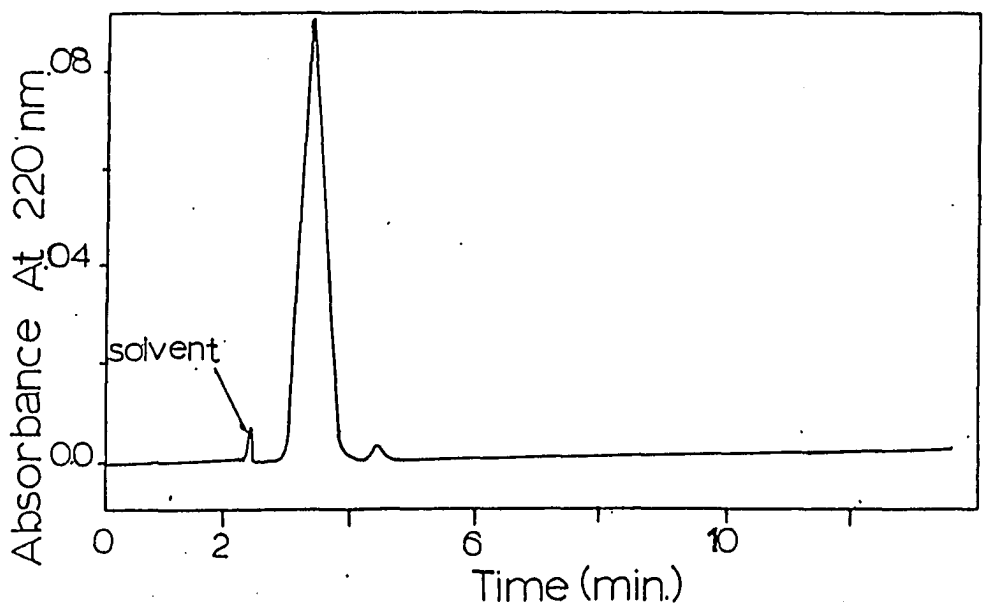


Figure 6. Boc-Glu(OMe)-Gly-Glu(OMe)OMe

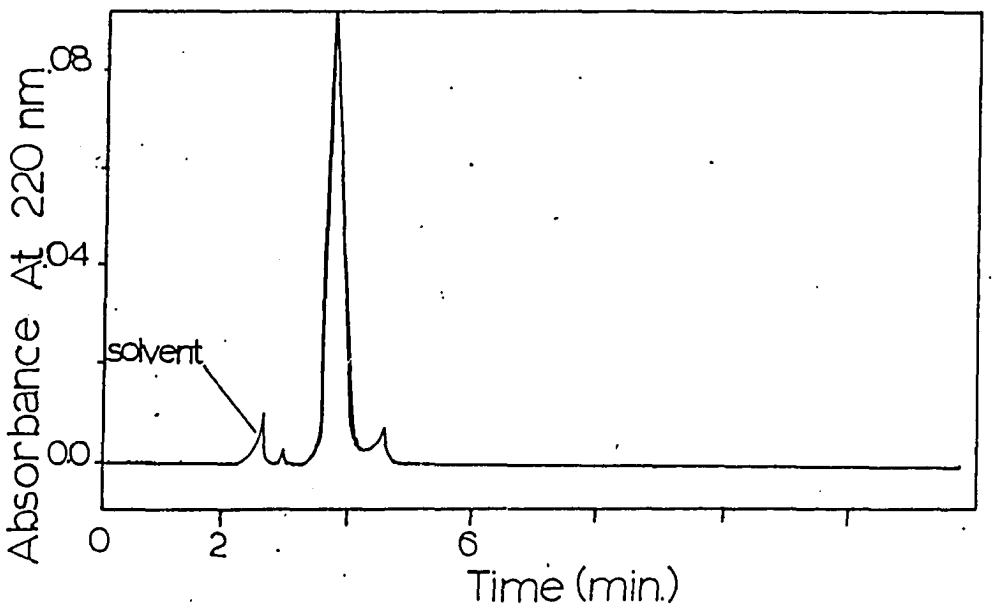


Figure 7. Boc-[Glu(OMe)]$_4$OMe

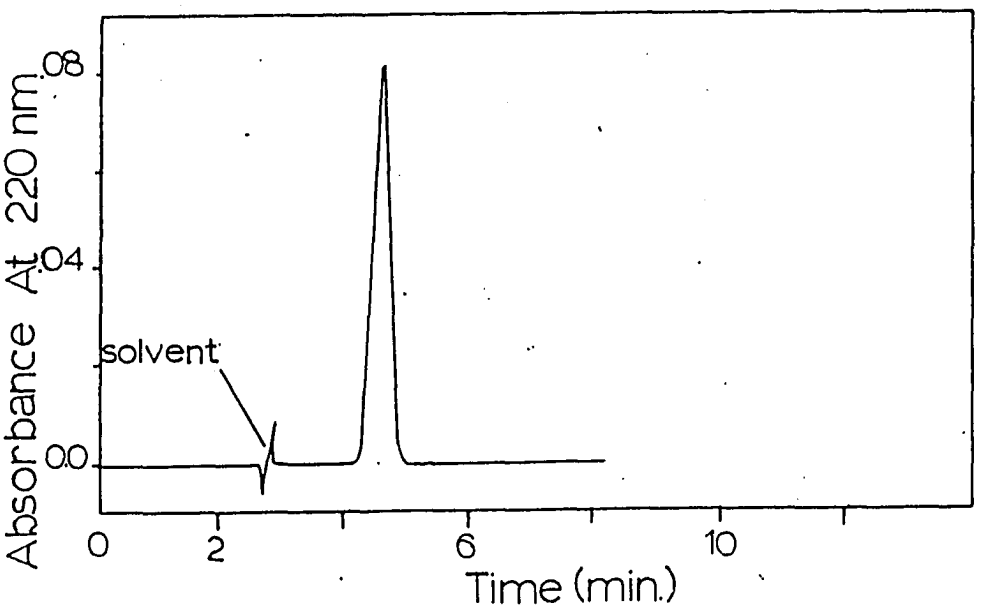


Figure 8. Boc-Gly-[Glu(OMe)]$_3$OMe

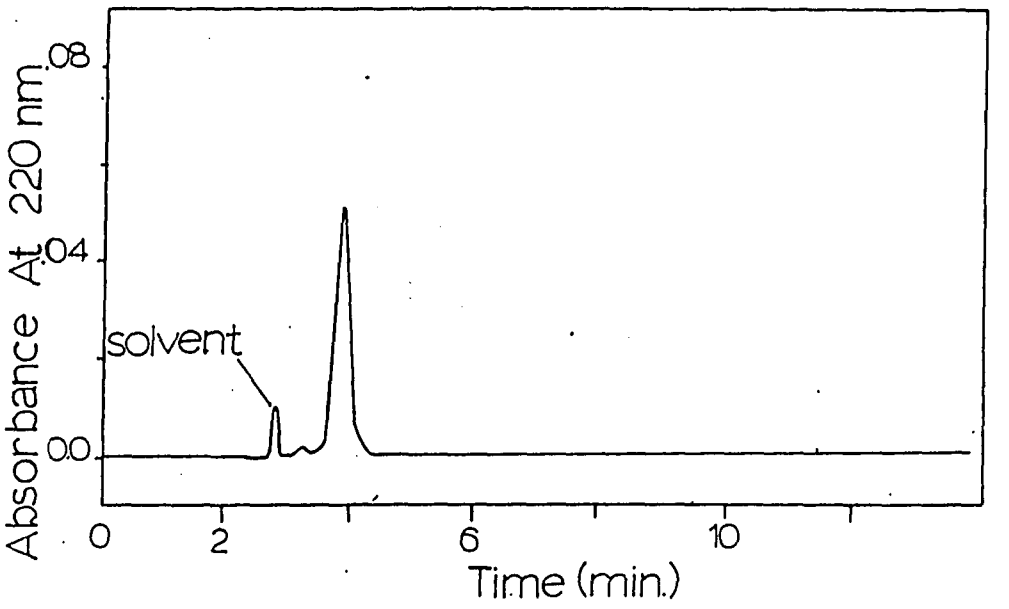


Figure 9. Boc-Glu(OMe)-Gly[Glu(OMe)]$_2$OMe

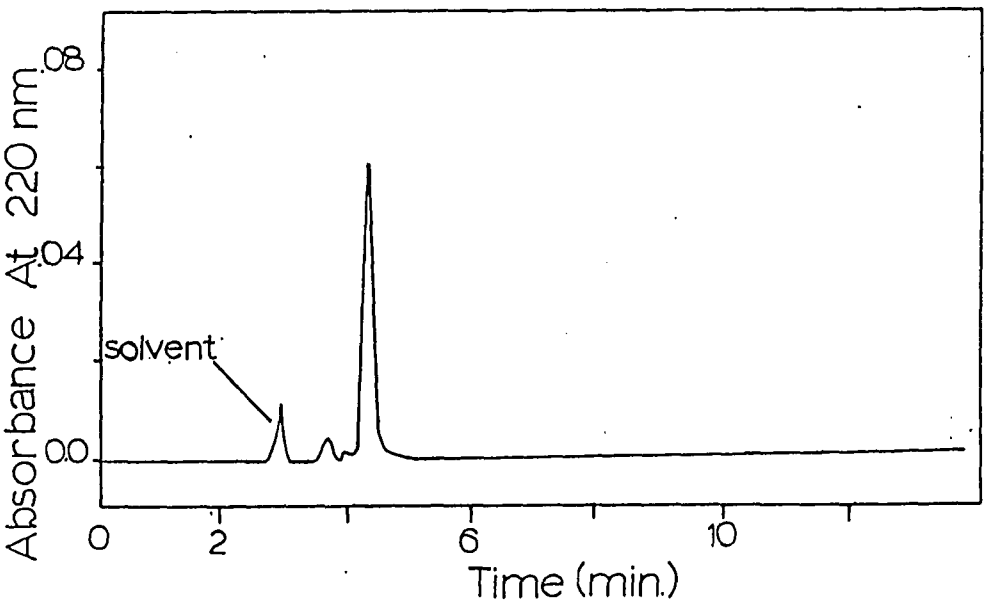


Figure 10. Boc-[Glu(OMe)]$_2$-Gly-Glu(OMe)OMe

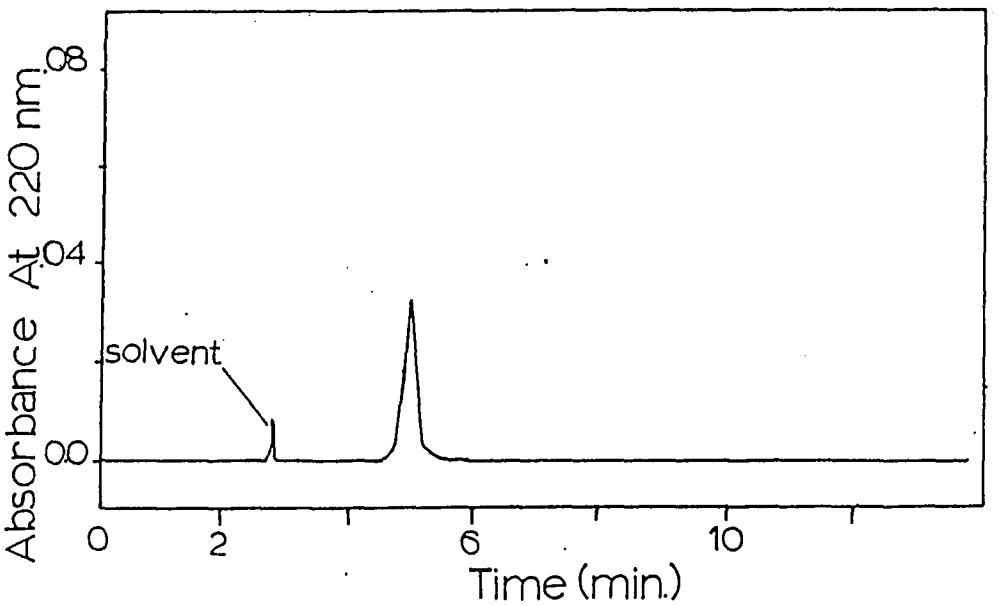


Figure 11. Boc-[Glu(OMe)]$_5$-OMe

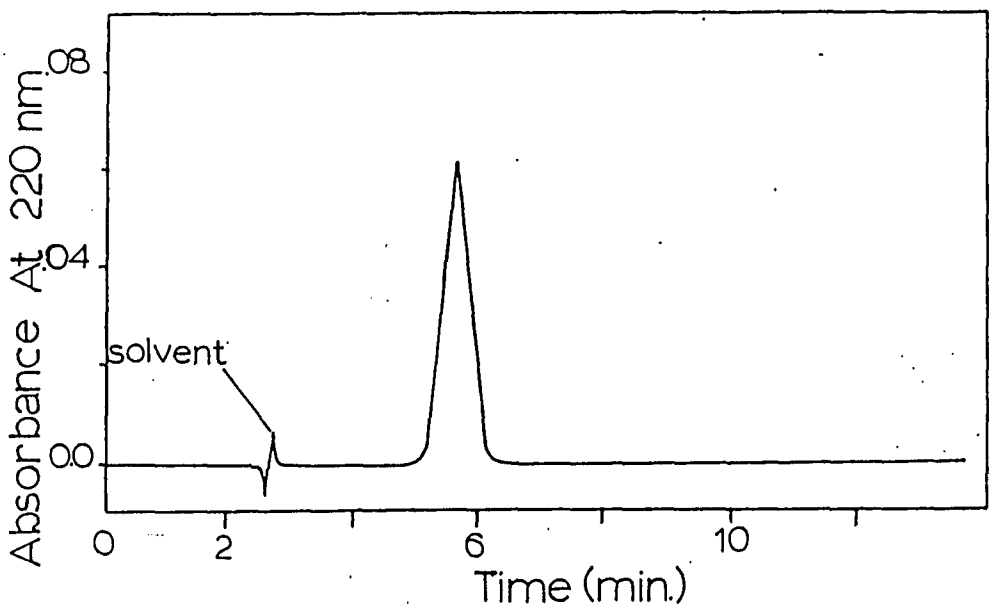


Figure 12. Boc-Glu(OMe)-Gly[Glu(OMe)]$_3$OMe

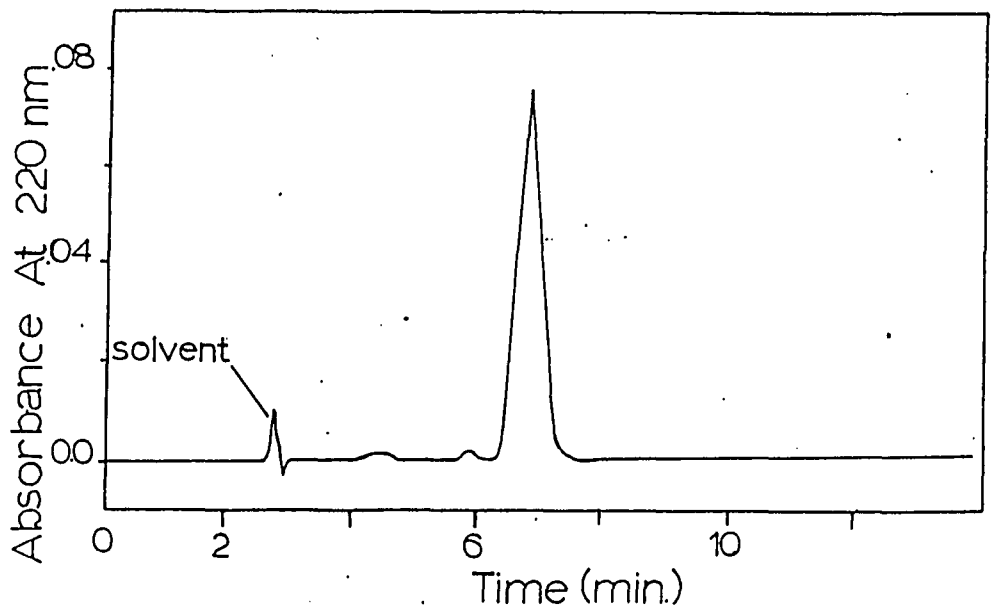


Figure 13. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_2$OMe

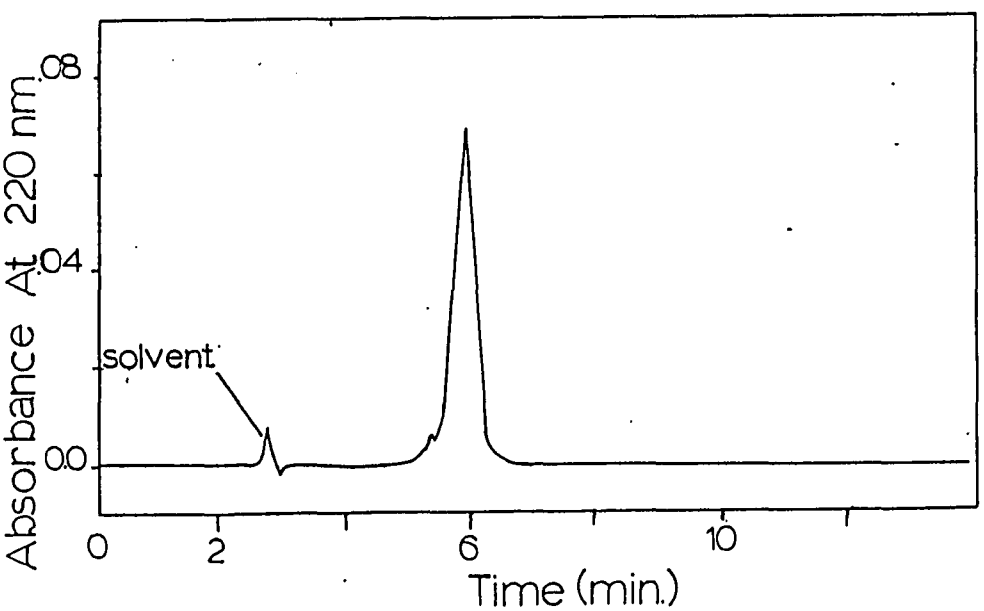


Figure 14. Boc-[Glu(OMe)]$_3$-Gly-Glu(OMe)OMe

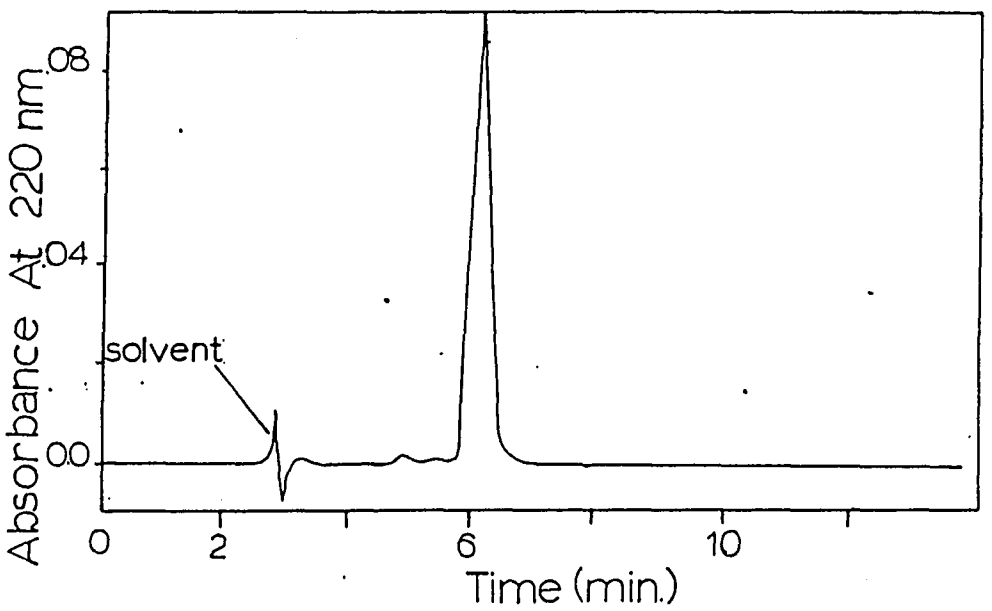


Figure 15. Boc-Gly-[Glu(OMe)]$_4$OMe

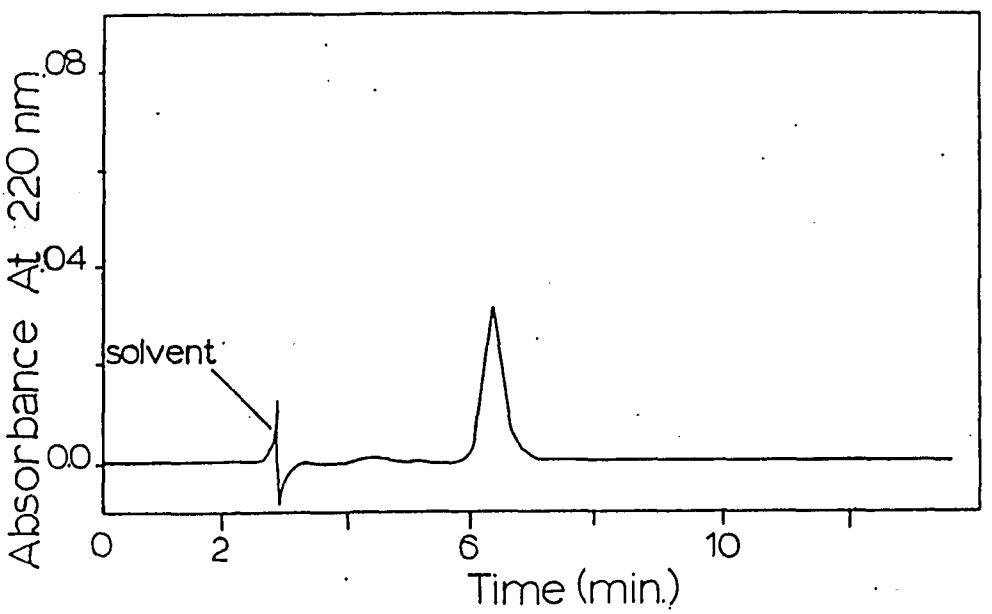


Figure 16. Boc-[Glu(OMe)]$_6$OMe

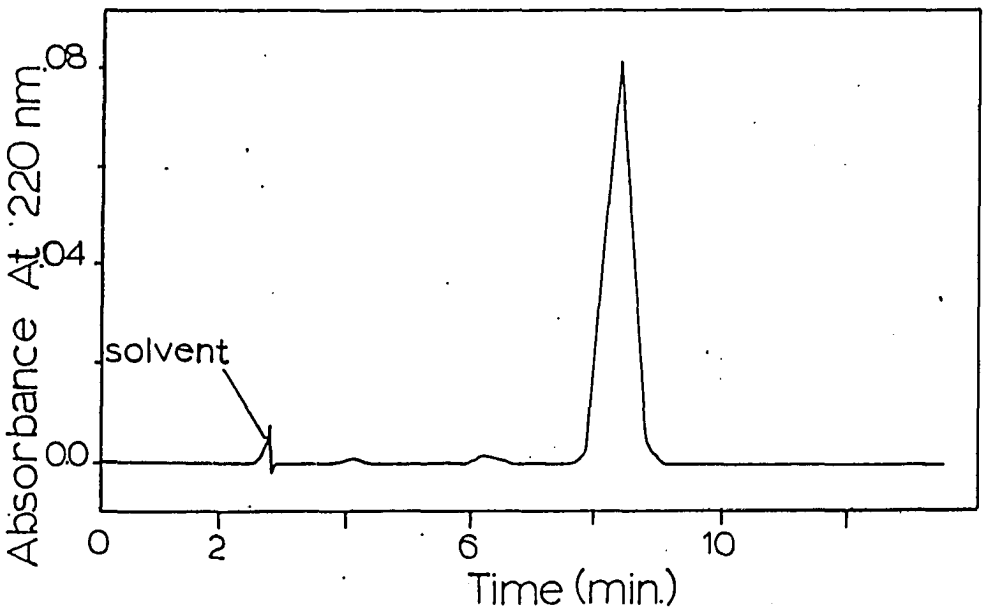


Figure 17. Boc-Gly-[Glu(OMe)]$_5$OMe

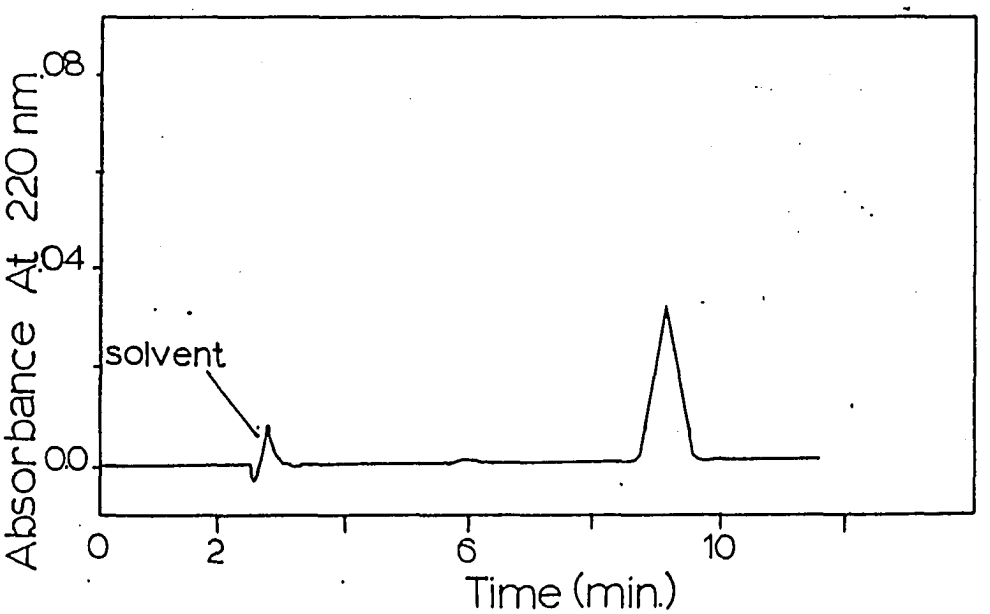


Figure 18. Boc-Glu(OMe)-Gly-[Glu(OMe)]$_4$OMe

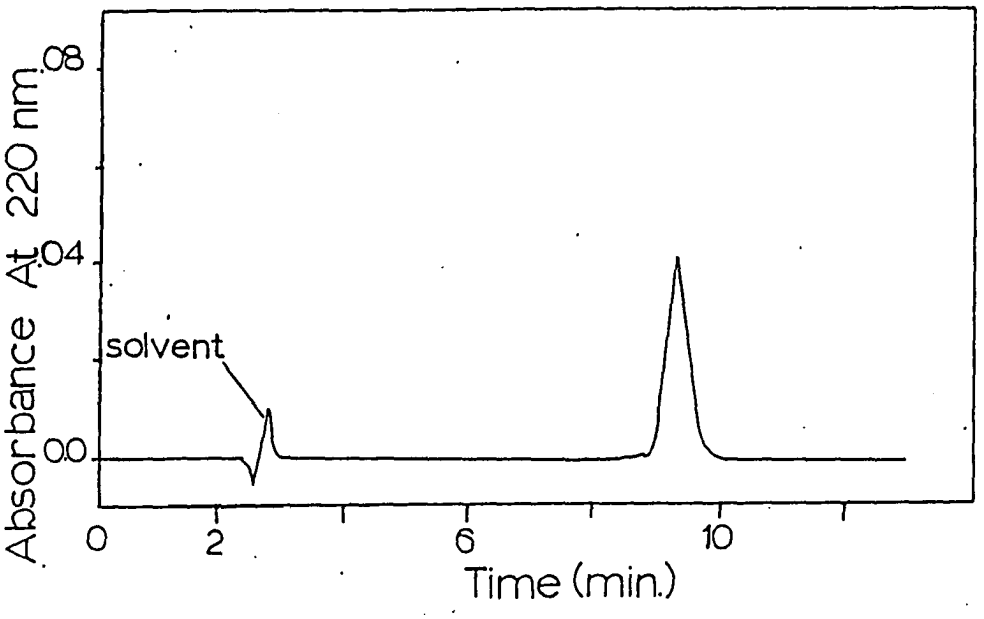


Figure 19. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_3$OMe

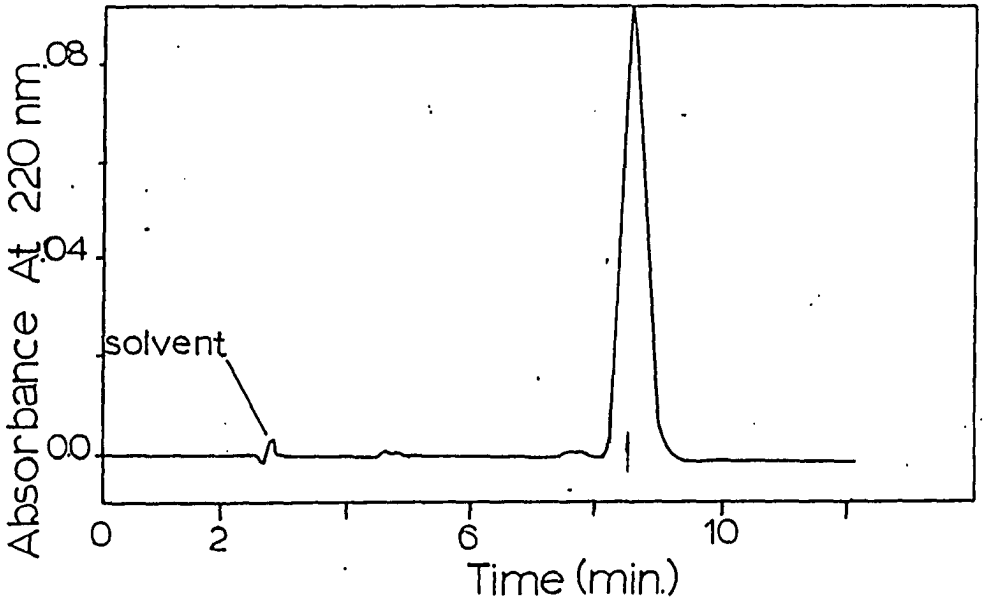


Figure 20. Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_2$OMe

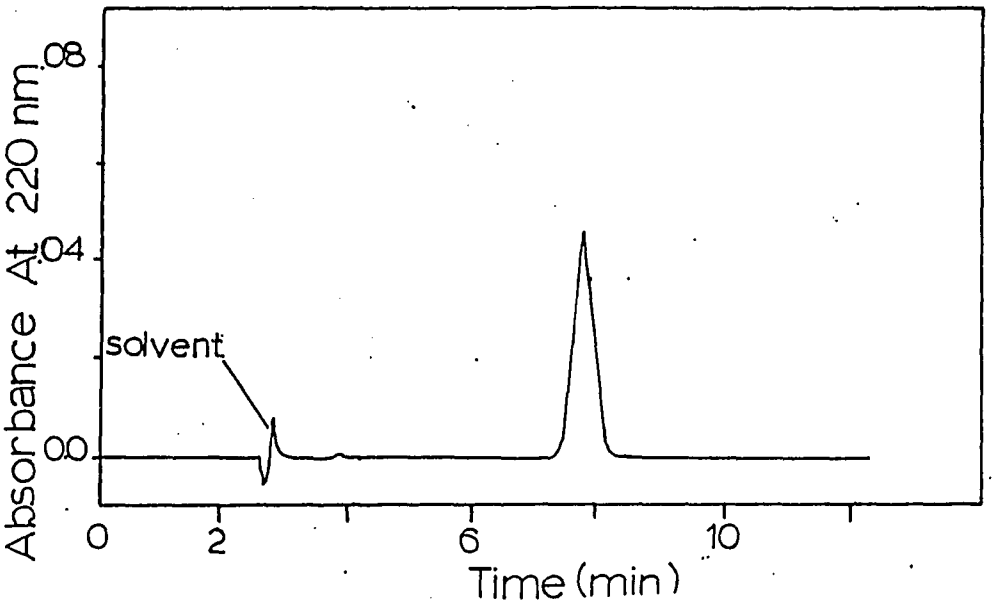


Figure 21. Boc-[Glu(OMe)]$_4$-Gly-Glu(OMe)OMe

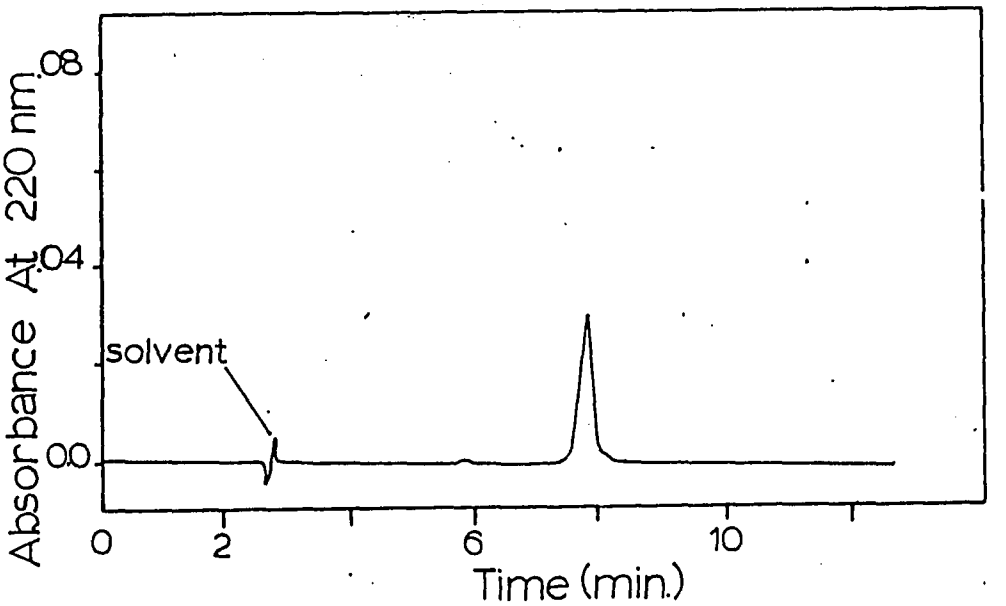


Figure 22. Boc-[Glu(OMe)]$_7$OMe

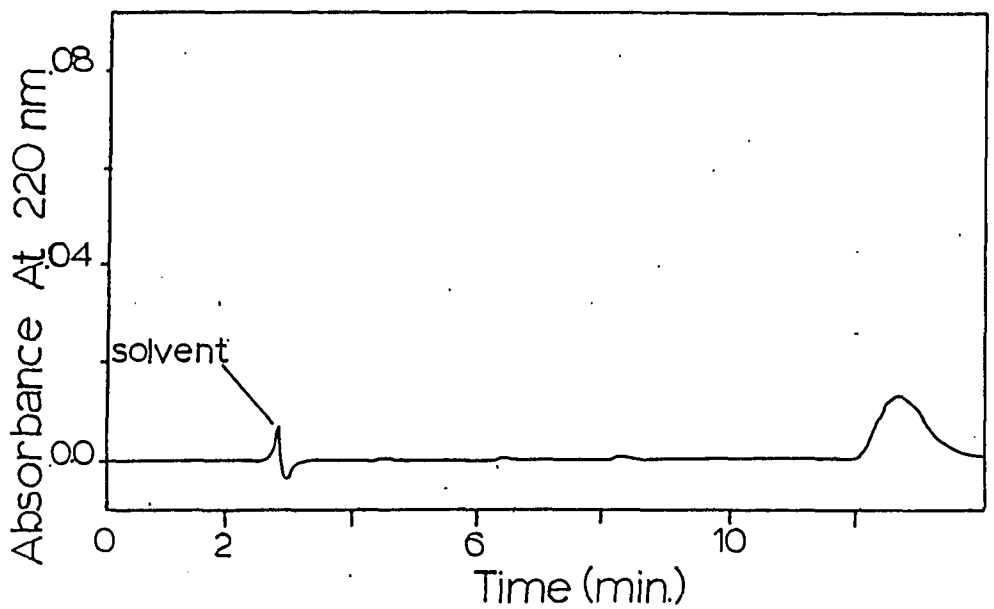


Figure 23. Boc-Glu(OMe)-Gly-[Glu(OMe)]$_5$OMe

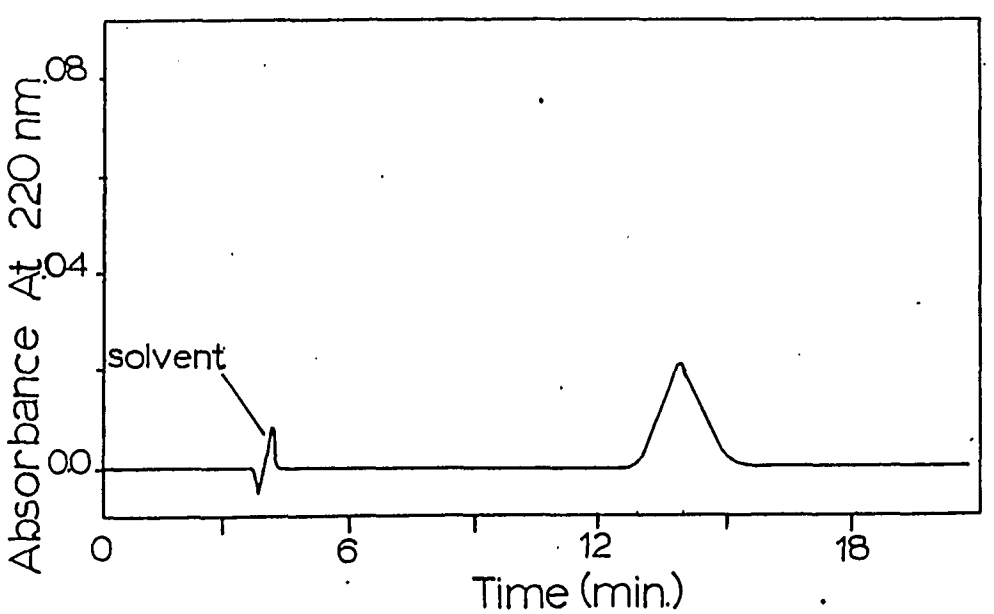


Figure 24. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_4$OMe

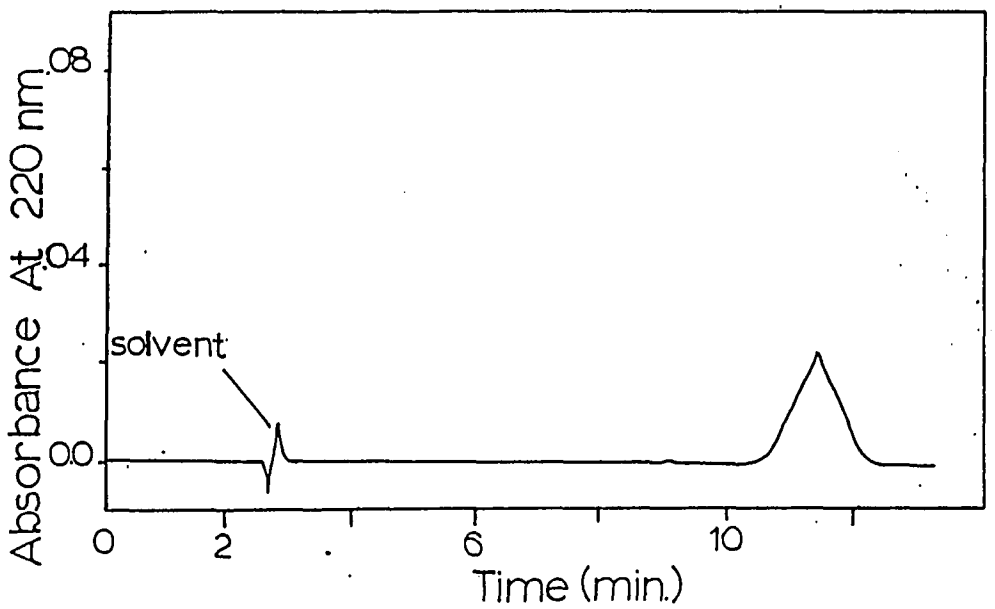


Figure 25. Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_3$OMe

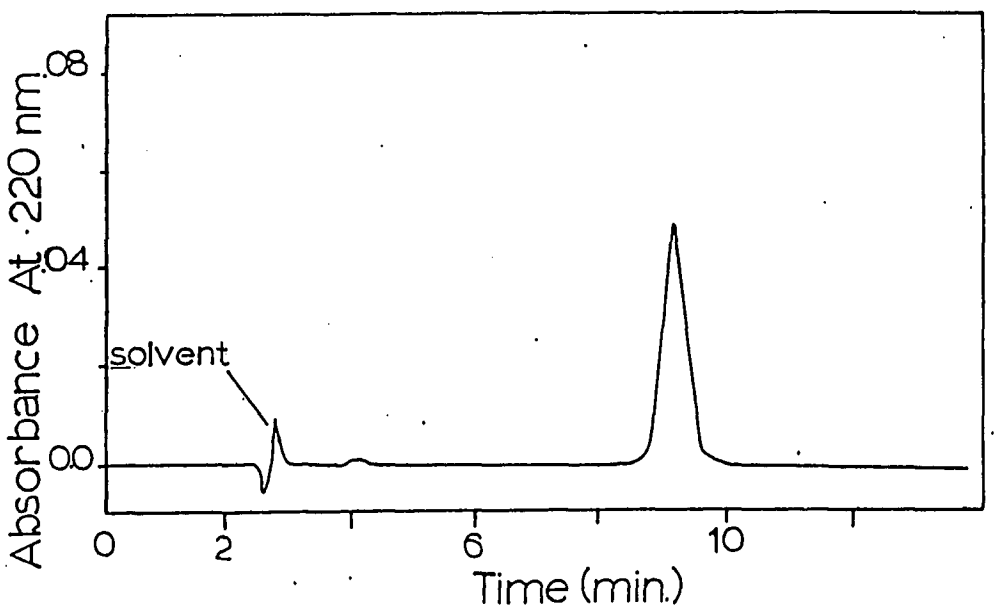


Figure 26. Boc-[Glu(OMe)]$_4$-Gly-[Glu(OMe)]$_2$OMe

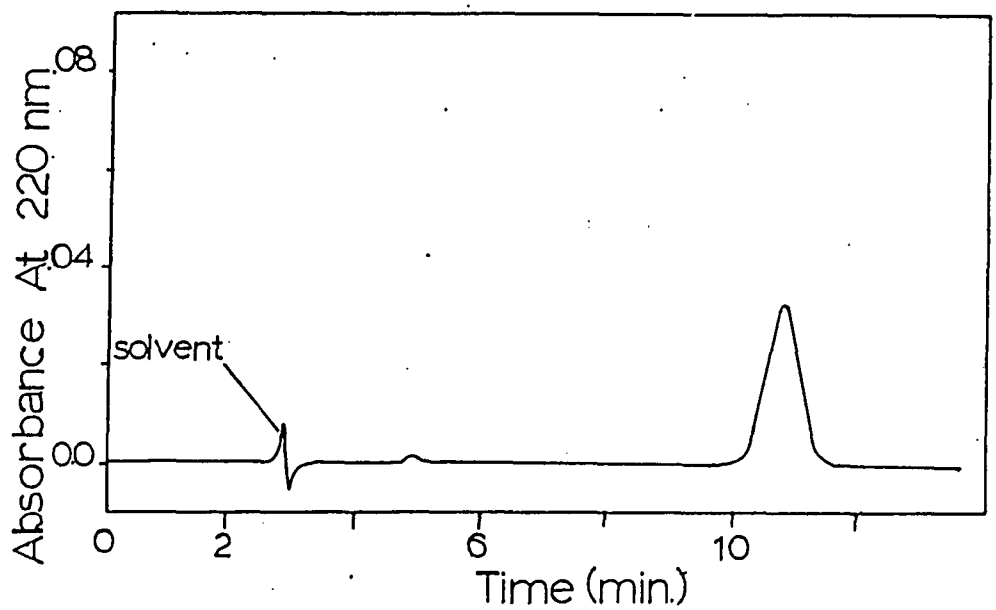
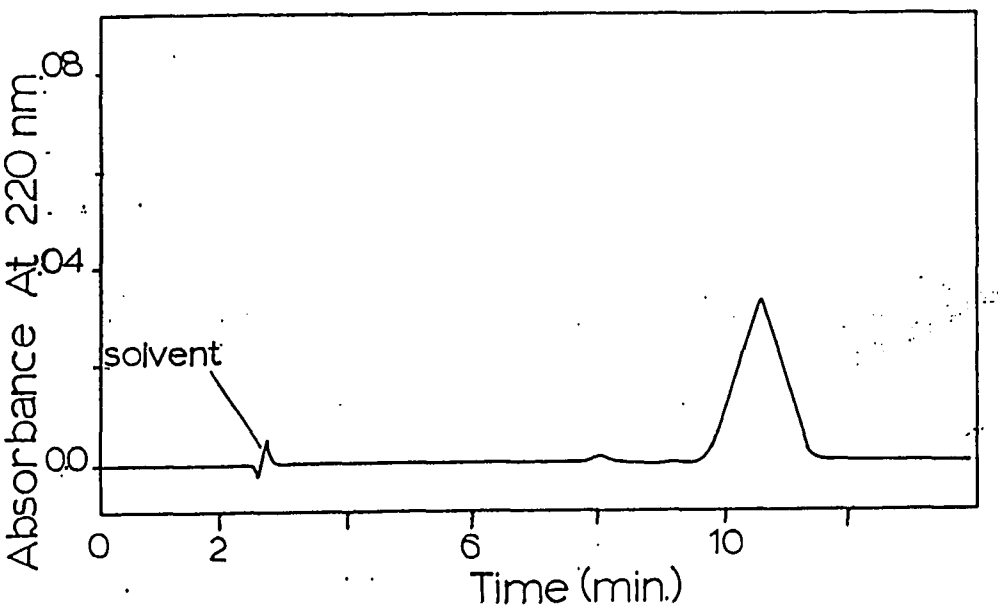


Figure 27. Boc-[Glu(OMe)]$_5$-Gly-[Glu(OMe)]$_1$OMe



Polarimetry

The optical activity of all protected oligomers was measured at the sodium D line, 589 nm, in both trifluoroethanol, a structure supporting solvent and hexafluoroisopropanol, a structure destabilizing solvent. The molar rotations were calculated according to the following equation:[67,68]

$$[\phi]_M = \frac{(\text{molecular weight}) \cdot ([\alpha]_D^{25})}{10{,}000}$$

$[\alpha]_D^{25}$ = specific rotation

A plot of the molar rotation of the homo-oligomer series versus the number of residues (n), in trifluoroethanol and hexafluoroisopropanol is presented in Figure 28. A linear relationship exists between the molar rotation and the chain length when these homo-oligomers are dissolved in hexafluoroisopropanol. These results are in close agreement with earlier studies.[6,67] Dissolution of the above series in trifluoroethanol yields values for molar rotation that are quite similar with those values in hexafluoroisopropanol for the dimer, trimer, and tetramer. At the pentapeptide a deviation from linearity occurs and continues through the heptamer for the peptides dissolved in trifluoroethanol. These results are also consistent with earlier studies of similar peptides. This deviation from linearity has been attributed to a change in secondary structure of the oligo-peptides.[55-61] Because hexafluoroisopropanol is a structure destabilizing solvent, it can be reasonably concluded that a transition of the peptide structure from statistical coil in HFIP to an ordered structure in TFE has occurred. These results establish a critical chain length for the onset of ordered structure of between five and six residues in the homo-oligomers.

A plot of the molar rotation values in hexafluoroisopropanol and trifluoroethanol vs. n, the number of glutamate residues is depicted in Fig. 29 for the co-oligopeptide series Boc-Gly-$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe. The co-oligopeptides dissolved in HFIP yielded $[\phi]_M$ values that are linear with respect to the number of residues. The slope of the curve is almost identical to the slope of the homo-oligomer series in HFIP. The slope as previously mentioned is equal to the contribution from $[\phi]_{\text{internal residue}}$. These results demonstrate the maintenance of optical purity during synthesis and the integrity of the instrumental analysis.

In Fig. 29 the BocGly$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe series dissolved in TFE shows a slight deviation from linearity at n = 4 and a substantial deviation at n = 5. This would indicate a contribution to total molar rotation from some form of secondary structure which initiates between four and five γ-methyl glutamate residues and is stabilized with increasing chain length up to the co-heptapeptide.

In Fig. 30 $[\phi]_M$ of the co-oligomer series BocGluGly$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe vs. n, in HFIP demonstrates again a linear relationship between $[\phi]_M$ and the number of glutamate residues in the peptide. In trifluoroethanol this copeptide displays a very slight deviation from linearity at n = 4, that is BocGluGly$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_4$OMe. The substantial deviation from the linearity of the HFIP curve occurs at n = 5 for this series in TFE.

In Fig. 31 glycine is shifted stepwise and closer to the carboxyl terminus in the copeptide series Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_2Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe; molar rotation values for

these peptides in HFIP fall on a curve which displays a relationship between $[\phi]_M$ and n not quite as linear as the previously mentioned curves, however the absolute value of $[\phi]_M$ continues to increase as n increases. Deviation from linearity for the same series dissolved in TFE are not as pronounced as seen for the homo-oligomers, BocGly$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe and BocGluGly$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe in TFE. This may be explained by the inhibition of onset of structure due to the fact that the glycyl residue is at the interior of the oligopeptide. Certainly in the Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_3Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe series the deviation from linearity in TFE does not exist (Fig. 32).

It is suggested that the presence of glycine in the third or fourth positions in the tri- through hepta- or tetra- through hepta- co-oligopeptides respectively, may disrupt the formation or onset of ordered structure in TFE. This assertion can be substantiated by the absence of deviation from linearity in graphs of $[\phi]_M$ vs. n for these co-oligomers in TFE. Further evidence implicates a glycyl residue as a disruptor of structure in the Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_2Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe and Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_3Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe series when a comparison of the slopes (within a series) of the HFIP vs. TFE plots are made. Specifically, in both the Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_2Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe series and the Boc$(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_3Gly(\underset{\underset{\text{OMe}}{|}}{\text{Glu}})_n$OMe series the slopes of $[\phi]_M$ vs. n in HFIP as compared to TFE do not vary substantially. This slope is equal to the contribution to rotation or rotatory power, of the internal residues, $[\phi]_{\text{internal residue}}$. The rotatory power in turn is a function of the electric and magnetic field environment

surrounding the peptide chromophore. These fields can vary from solvent to solvent, as a function of primary structure (that is field effects from adjacent residues), and as a function of secondary structure.

Comparison of the slopes of $[\phi]_M$ vs. n plots in HFIP for the homo-oligomer series ($n < 5$), BocGly(Glu)$_n$OMe series ($n < 5$), and
OMe

BocGluGly(Glu)OMe series ($n < 5$), reveals very little if any difference
OMe OMe

in slope. All of these plots also maintain their linearity regardless of glycine position. It is therefore concluded that the deviations from linearity for these series in TFE is primarily a result of change in secondary structure of the glycine substituted glutamate backbone.

The ability to analyze the type of secondary structure assumed by a peptide is not possible via polarimetry. Polarimetry does however provide a method by which one is able to monitor the onset and/or presence of secondary structure in an oligopeptide series when compared to the same oligomers in a disordered or statistical coil conformation. Polarimetry also serves as a criteria for the determination of optical purity. The maintenance of optical purity is evident for all the above compounds from the linearity of plots of all peptides in HFIP.

TABLE 2

Polarimetry Results

Compound	$[\alpha]_D^{25}$ TFE	$[\alpha]_D^{25}$ HFIP	$[\phi]_M$ TFE	$[\phi]_M$ HFIP
Boc(Glu)$_2$OMe γ-OMe	-27.50 c = 0.15	-28.58 c = 0.22	-1.14	-1.19
Boc(Glu)$_3$OMe γ-OMe	-44.10 c = 0.14	-44.49 c = 0.16	-2.47	-2.49
Boc(Glu)$_4$OMe γ-OMe	-44.00 c = 0.18	-47.02 c = 0.19	-3.10	-3.31
Boc(Glu)$_5$OMe γ-OMe	-38.46 c = 0.17	-49.12 c = 0.16	-3.26	-4.16
Boc(Glu)$_6$OMe γ-OMe	-32.70 c = 0.13	-53.00 c = 0.17	-3.23	-5.20
Boc(Glu)$_7$OMe γ-OMe	-27.42 c = 0.16	-52.00 c = 0.17	-3.11	-5.90
BocGlyGluOMe γ-OMe	-8.10 c = 0.29	-6.00 c = 0.26	-.27	-.20
BocGly(Glu)$_2$OMe γ-OMe	-24.66 c = 0.22	-28.20 c = 0.18	-1.17	-1.20
BocGly(Glu)$_3$OMe γ-OMe	-33.58 c = 0.14	-35.40 c = 0.18	-2.08	-2.22
BocGly(Glu)$_4$OMe γ-OMe	-35.41 c = 0.13	-42.80 c = 0.17	-2.70	-3.30
BocGly(Glu)$_5$OMe γ-OMe	-35.44 c = 0.13	-52.60 c = 0.18	-3.21	-4.60
BocGly(Glu)$_6$OMe γ-OMe	-35.40 c = 0.13	-43.99 c = 0.13	-2.31	-4.61
BocGluGlyGluOMe γ-OMe γ-OMe	-10.50 c = 0.40	-12.29 c = 0.32	-0.50	-0.59
BocGluGly(Glu)$_2$OMe γ-OMe γ-OMe	-30.43 c = 0.17	-35.38 c = 0.21	-1.88	-2.19

Polarimetry Results (Cont'd.)

Compound	$[\alpha]_D^{25}$ TFE	$[\alpha]_D^{25}$ HFIP	$[\phi]_M$ TFE	$[\phi]_M$ HFIP
Boc(Glu(γ-OMe)Gly(Glu(γ-OMe))$_3$OMe	-25.41 c = 0.27	-40.20 c = 0.19	-1.94	-3.06
Boc(Glu(γ-OMe)Gly(Glu(γ-OMe))$_4$OMe	-27.91 c = 0.17	-45.20 c = 0.17	-2.53	-4.11
Boc(Glu(γ-OMe)Gly(Glu(γ-OMe))$_5$OMe	-19.39 c = 0.15	-49.50 c = 0.19	-2.03	-4.60
Boc(Glu(γ-OMe))$_2$GlyGlu(γ-OMe)OMe	-17.70 c = 0.27	-24.74 c = 0.16	-1.10	-1.53
Boc(Glu(γ-OMe))$_2$Gly(Glu(γ-OMe))$_2$OMe	-19.66 c = 0.16	-40.55 c = 0.18	-1.48	-3.90
Boc(Glu(γ-OMe))$_2$Gly(Glu(γ-OMe))$_3$OMe	-31.32 c = 0.15	-47.09 c = 0.18	-2.83	-4.26
Boc(Glu(γ-OMe))$_2$Gly(Glu(γ-OMe))$_4$OMe	-29.01 c = 0.14	-49.48 c = 0.16	-2.81	-5.19
Boc(Glu(γ-OMe))$_3$GlyGlu(γ-OMe)OMe	-20.27 c = 0.18	-35.80 c = 0.19	-1.54	-2.73
Boc(Glu(γ-OMe))$_3$Gly(Glu(γ-OMe))$_2$OMe	-23.02 c = 0.16	-44.44 c = 0.15	-2.07	-4.02
Boc(Glu(γ-OMe))$_3$Gly(Glu(γ-OMe))$_3$OMe	-23.89 c = 0.18	-47.92 c = 0.16	-2.50	-5.02

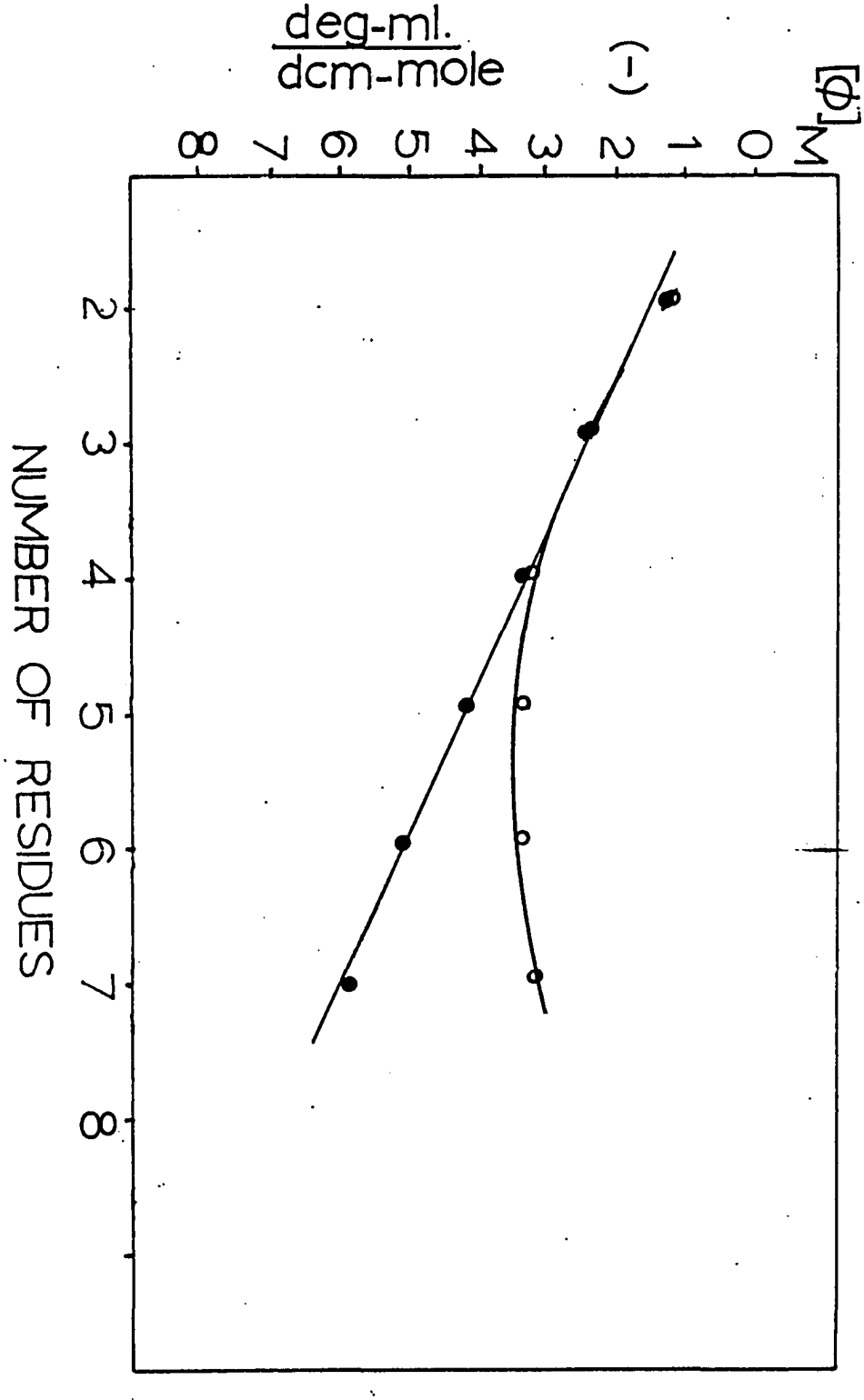


Figure 28. Boc-[Glu(OMe)]$_n$-OMe ○ TFE, ● HFIP

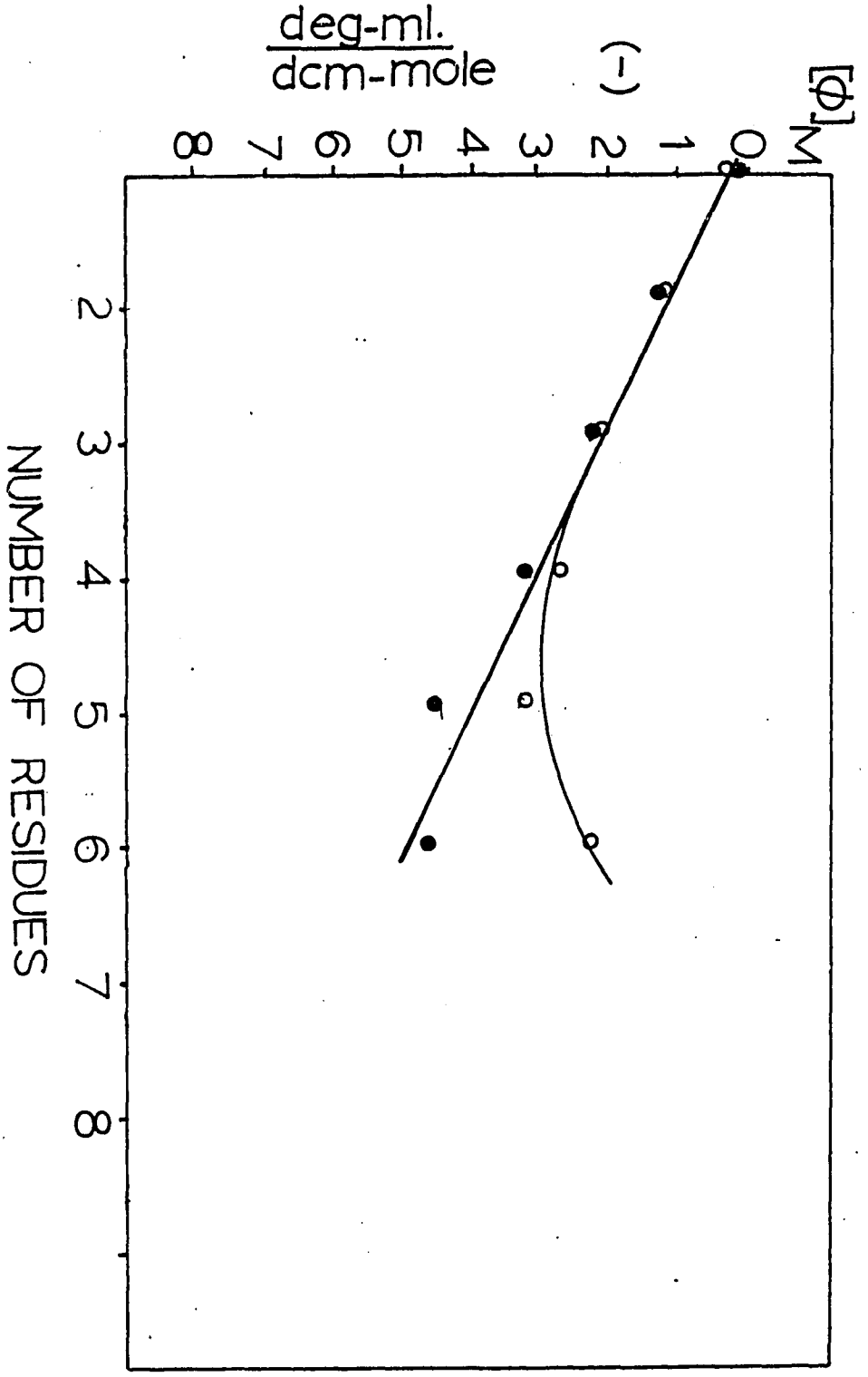


Figure 29. Boc-Gly-[Glu(OMe)]$_n$-OMe ○ TFE, ● HFIP

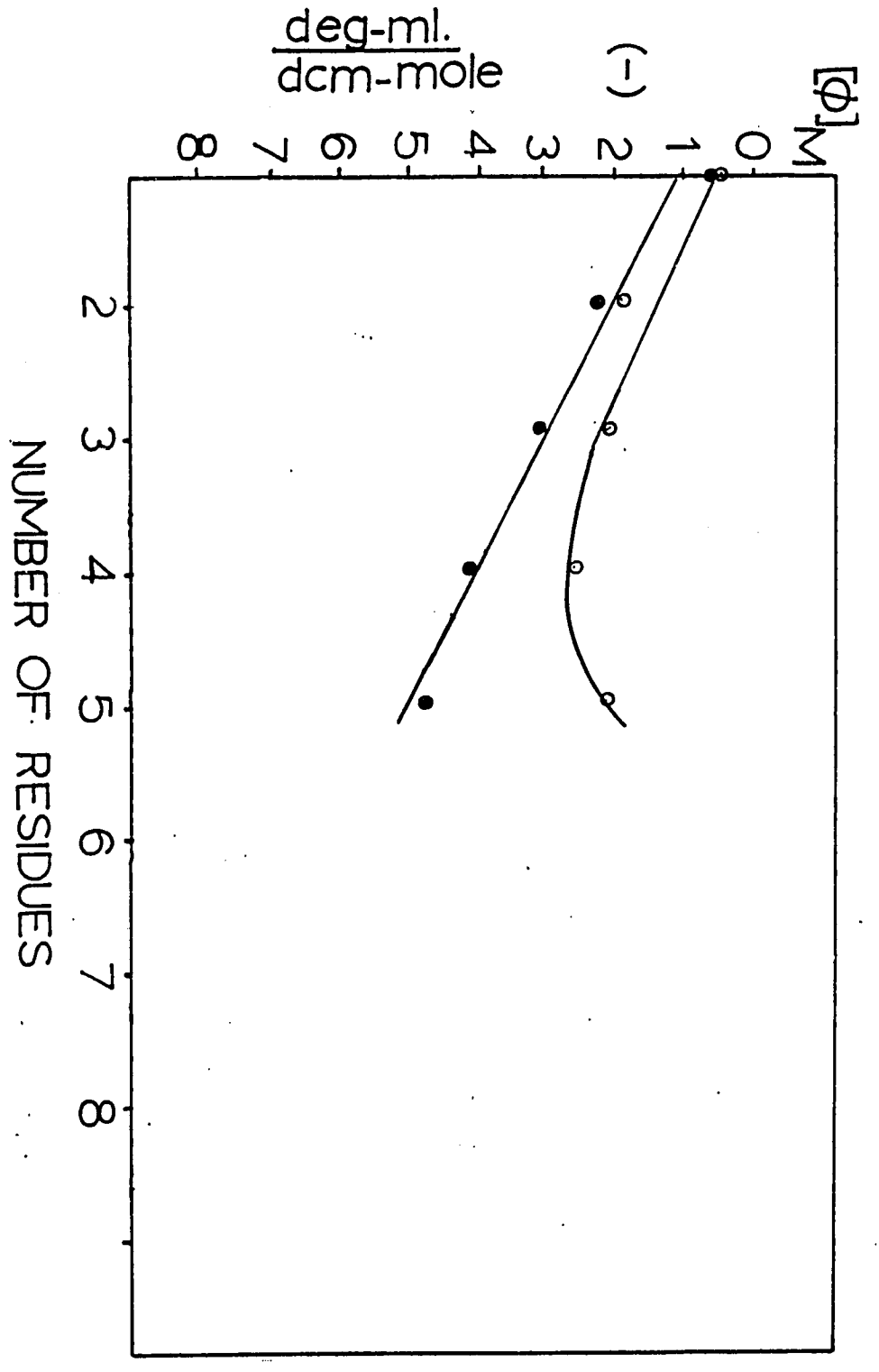


Figure 30. Boc-[Glu(OMe)]-Gly-[Glu(OMe)$_n$]-OMe ○ TFE, ● HFIP

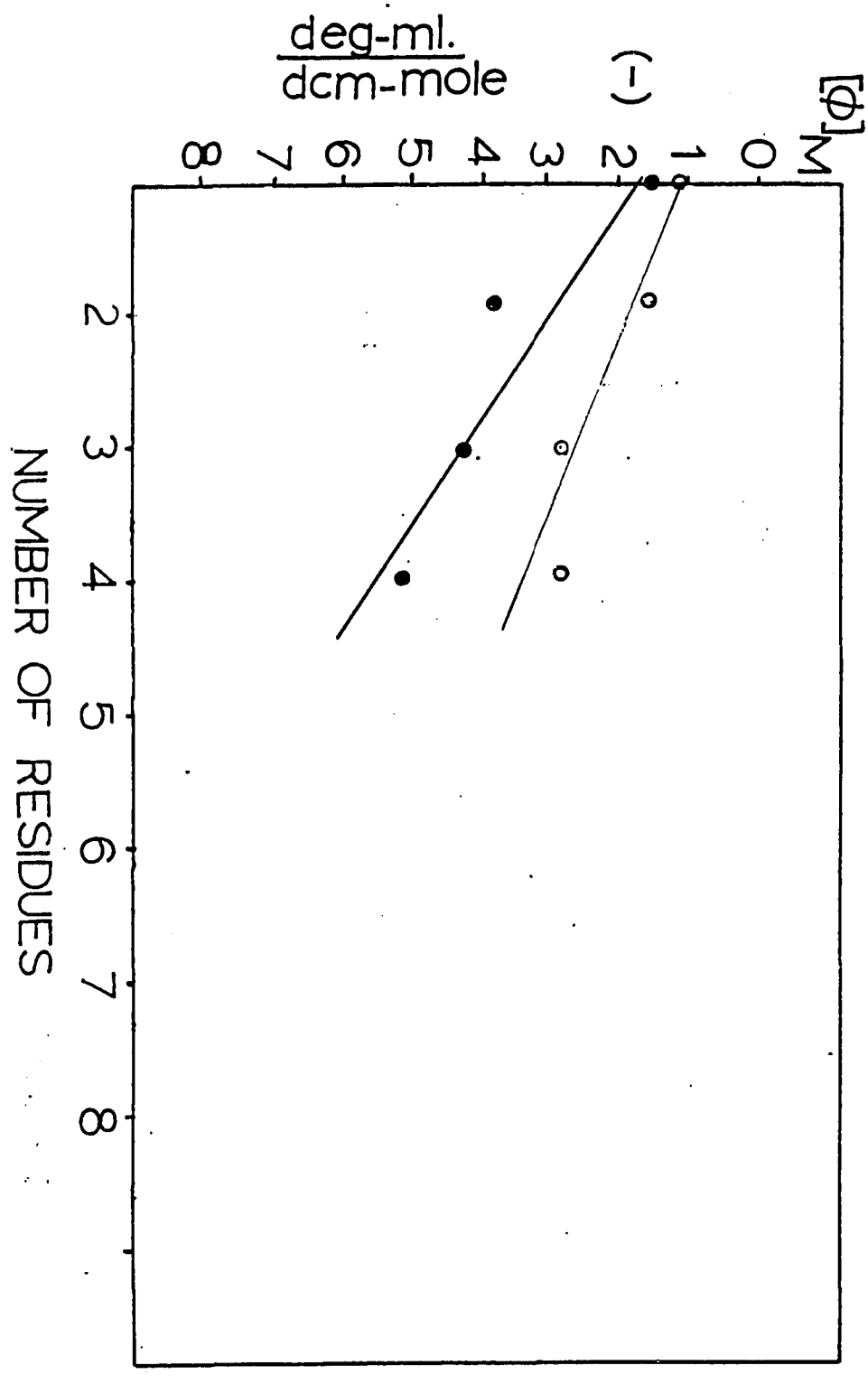


Figure 31. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_n$-OMe o TFE, ● HFIP

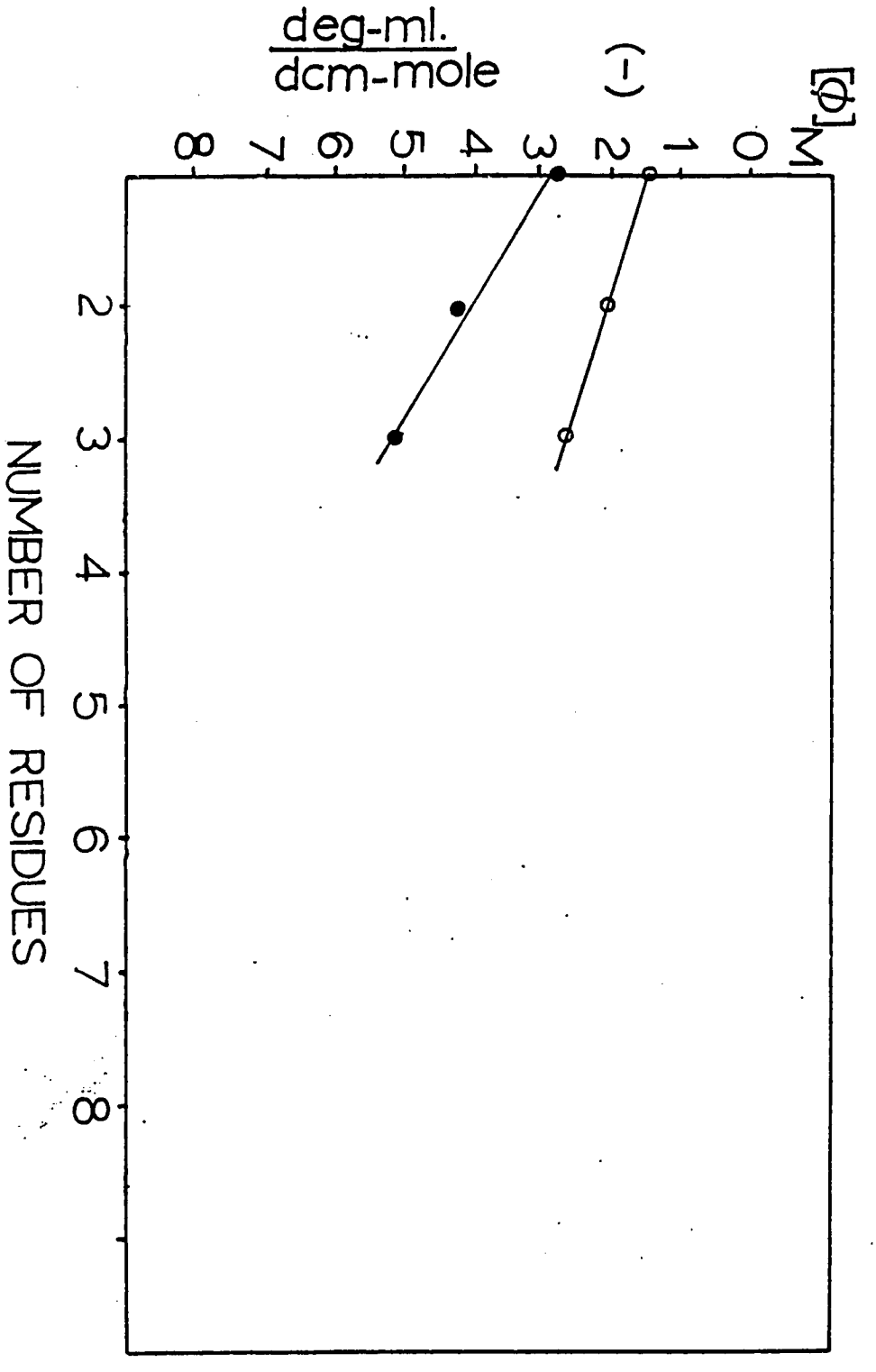


Figure 32. Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_n$-OMe ○ TFE, ● HFIP

Circular Dichroism

The circular dichroism of all peptides was measured from 250 nm to 190 nm in trifluoroethanol (TFE), a structure supporting solvent[1] and hexafluoroisopropanol (HFIP)[1] a structure destabilizing solvent for oligopeptides. The homooligomer series displayed similar C.D. spectra, with respect to the position of Cotton effects, when compared to previous studies of similar compounds.[70] The characteristic red shift of the λ_{max} for the amide $\pi \rightarrow \pi^*$ transition from ~ 192 nm for the tripeptide to 204 nm for the heptapeptide is present as seen in Fig. 33. The accompanying development of a trough at 222 nm for the heptamer in TFE is clearly evident and is consistent (with respect to wavelength) with the $n \rightarrow \pi^*$ transition observed in C.D. spectra of α-helical synthetic and natural polypeptides.[11] In addition to the above physical characteristics a crossover begins developing for the pentamer at 191 nm and is well established in the heptamer at 196 nm. This crossover has been shown to be due to exciton resonances as a result of coupling of electronic transitions of the amide chromophore in an α-helical array.[11,61,63]

The molar ellipticities of the two negative Cotton effects observed for the heptamer in TFE (one at 204 nm and the other at 222 nm), are not in close agreement with the C.D. spectra of partially α-helical γ-ethyl-L-glutamate[12] and partially α-helical γ-methyl-L-glutamates homooligomers in TFE.[70] The magnitude of ellipticities reported in the literature for γ-methyl-L-glutamates and γ-ethyl-L-glutamates are also not in close agreement.

Comparison of the C.D. spectra of the lower peptides n = 2 through n = 6 in TFE to the identical compounds measured in HFIP reveal

significant similarities (Fig. 34). Specifically, a weak positive band between 210 nm and 220 nm is present for the dipeptide, tripeptide, tetrapeptide and pentapeptide in TFE. The position of this positive band for the above oligomers is the same in HFIP and is certainly more pronounced. In TFE the hexapeptide does not exhibit any positive crossover or Cotton effect between 210 nm and 220 nm compared to the substantial positive Cotton effect centered at approximately 215 nm for the hexamer in HFIP. This may reflect a difference in structural distribution for the hexamer versus the lower peptides. In addition to the above spectral characteristics a trough was observed from 225 nm to 240 nm in the spectra of n = 3 through n = 6 of the homooligomers in HFIP. These results are consistent with the findings of Holtzworth and Doty[53] for spectra of random or disordered peptides.

The presence of a well developed negative Cotton effect centered at ~ 198 nm for the heptamer in HFIP and the absence of the weak positive band between 210 nm and 220 nm (present in the lower oligomers in HFIP) may be indicative of increased structural stability inherent in the homo-heptamer even in a highly destabilizing solvent. However the $n \rightarrow \pi^*$ shoulder at 222 nm is not well developed in HFIP, and the characteristic negative Cotton effect assigned to the parallel $\pi \rightarrow \pi^*$ exciton resonance is positioned at 198 nm as compared to 204 nm for the heptamer in TFE. These results suggest a lower percentage, if any, of helix-like structure for the heptamer in HFIP.

Examination of the C.D. spectra for the BocGly[Glu(OMe)]$_n$OMe series in TFE (Fig. 35), reveals a gradual red shift for the negative Cotton effect due to the $\pi \rightarrow \pi^*$ transition which appears at 192 nm for the co-tripeptide and 202 nm in the co-heptapeptide. Also present in

the co-heptapeptide spectra at ~ 222 nm is a well developed shoulder which can be assigned to the $n \rightarrow \pi^*$ transition of a peptide in a partially helical structure. The magnitude of ellipticity of the negative Cotton effect assigned to the $\pi \rightarrow \pi^*$ transition is in close agreement with the same transition in the homo-heptamer in TFE and the position of this transition is 202 nm as compared to 204 nm in the homo-heptamer. The ellipticity of the $n \rightarrow \pi^*$ band is reduced by approximately 30% in the co-heptamer as compared to the same transition for the homo-heptamer in the same solvent.

Examination of the spectra of the same compounds in HFIP (Fig. 36), reveals great differences in the position and sign of Cotton effects vs. those in TFE. There is no negative ellipticity between 226 nm and 207 nm in HFIP and a rather pronounced positive Cotton effect centered at 215 nm is observed from dimer to heptamer. These spectral characteristics are consistent with peptides in random structure as described in the literature.[53] It should also be emphasized that although BocGly[Glu(OMe)]$_6$OMe displays spectral characteristics in TFE quite similar to those of Boc[Glu(OMe)]$_7$OMe in TFE, its spectra in HFIP is very unlike that of the homo-heptamer in the same solvent. It is concluded that the stability of the secondary structure assumed by BocGly[Glu(OMe)]$_6$OMe is less than that of the homo-heptamer in the same solvent.

Examination of the C.D. spectra of the Boc[Glu(OMe)]-Gly-[Glu(OMe)]$_n$OMe series in TFE (Fig. 37), as compared to HFIP (Fig. 38), reveals many similarities for the peptides n = 1 through n = 4. In both solvents there appears in the spectra of these compounds a well developed positive Cotton effect between 205 nm and 225 nm. There is virtually no negative

ellipticity for these oligomers above 205 nm and the troughs below 205 nm are not highly developed. Thus for the above series with n = 1-4 there are no spectral characteristics associable with α-helical structures. It is concluded that all members of this series, save Boc[Glu(OMe)]-Gly[Glu(OMe)]$_5$OMe, display spectra associated with random structures regardless of the solvent.

Examination of the spectra of Boc[Glu(OMe)]-Gly[Glu(OMe)]$_5$OMe in HFIP reveals ellipticity patterns very similar to those of the lower oligomers in the same solvent. Spectra obtained for Boc[Glu(OMe)]-Gly[Glu(OMe)]$_5$OMe in TFE suggest the presence of some α-helical content in this molecule based upon the presence of a trough located between 220 nm and 235 nm, the absence of a positive crossover above 200 nm, a well developed negative Cotton effect centered at 202 nm, and a crossover at 196 nm.

When glycine is placed in position three of the peptide backbone, the Boc[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_n$-OMe series, the C.D. spectra of all oligomers (with the exception of Boc[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_4$OMe (Fig. 39and Fig.40), exhibit spectra lacking any Cotton effect characteristic of α-helical structure in both HFIP and TFE. When n = 1-3 the peptides display a crossover from negative ellipticity to positive ellipticity at ~ 225 nm. Only positive ellipticity is observed at least through 205 nm. These spectral patterns were obtained in both TFE and HFIP. Measurement of the C.D. spectra of Boc[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_4$-OMe in TFE reveals a substantial trough located close to the $n \rightarrow \pi^*$ transition at 222 nm that is characteristic of helical peptides. Absent is the crossover to positive ellipticity above 200 nm commonly associated with peptides in a random structure. Present is a

large negative Cotton effect centered at ~ 200 nm. It is concluded that this compound may possess some α-helical character.

The C.D. measurements of the Boc[Glu(OMe)]$_3$-Gly[Glu(OMe)]$_n$OMe series in TFE (Fig. 41) and HFIP (Fig. 42) are consistent with the previously discussed characteristics of random structure. Spectra of all compounds exhibit a weak trough at between 215 nm and 235 nm, a positive band centered at ~ 210 nm, and a negative Cotton effect below 200 nm. The co-heptamer in TFE displayed spectral patterns consistent with random structure in contrast to all the previously examined co-heptamers.

The Boc[Glu(OMe)]$_4$-Gly-[Glu(OMe)]$_n$-OMe series consists of two members, the co-hexamer and co-heptamer, which display very similar spectral patterns with respect to one another in TFE and HFIP. The C.D. spectra in HFIP of these two compounds (Fig. 44), exhibit characteristic patterns of peptides assuming a random structural distribution. Present in the HFIP spectra is the negative trough centered at 230 nm and a large positive Cotton effect at ~ 210 nm.

For the co-hexamer and co-heptamer in TFE there is present a negative trough at 230 nm accompanied by a substantial negative Cotton effect at ~ 201 nm. The absence of positive ellipticity above 205 nm suggest the presence of some ordered structure however the magnitude of ellipticity of the $\pi \rightarrow \pi^*$ transitions for both molecules in TFE argues against this conclusion. There may be present partial helicity in these oligopeptides in equilibrium with random conformations, and certainly these peptides do possess different structural distributions in TFE as compared to HFIP. Whether or not these conformers are of a helical nature is difficult to substantiate.

Boc[Glu(OMe)]$_5$-Gly[Glu(OMe)]-OMe dissolved in HFIP (Fig. 45) exhibits a spectrum typical of a peptide in a random or disordered structure. The C.D. spectrum of this peptide measured in TFE (Fig. 45), displays quite a different pattern. There appears a substantial negative Cotton effect at 200 nm and a steady, well developed shoulder centered at ~ 222 nm. There is absent a positive crossover between 225 nm and 205 nm which is present in the HFIP spectra. It is concluded that Boc[Glu(OMe)]$_5$-Gly[Glu(OMe)]-OMe definitely possess some α-helical character in TFE based upon the positions and magnitudes of the negative Cotton effects and the marked difference between CD patterns in TFE and HFIP.

C.D. spectra of the homo-oligomers in TFE displayed spectral patterns similar to those previously reported in the literature with respect to position of the Cotton effect assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The molar ellipticities for the two major Cotton effects in the homo-heptamer in TFE were half those reported for the N-carbobenzoxy-γ-ethyl-L-glutamate heptamer C.D. spectra in TFE.[13] The difference when compared to Z-[Glu(OMe)]$_7$-OMe in TFE was an order of magnitude.[70]

The CD investigation of the glycine-glutamate co-peptides has resulted in the following interpretations. Glycine in position 1 of a co-heptamer in TFE results in an approximately 30% reduction in molar ellipticity of the negative Cotton effect at 222 nm as compared to the homo-heptamer. There is no shift however in the position of the $n \rightarrow \pi^*$ transition. There is approximately a 5% reduction in ellipticity for the negative Cotton effect associated with the $\pi \rightarrow \pi^*$ transition and an accompanying shift from 204 nm in the homo-heptamer to 202 nm in the

co-heptamer. It is concluded that insertion of glycine in position 1 in the co-heptamer results in spectra (in TFE) representative of chromophores in a partially α-helical array. The extent of helicity in this co-heptamer is probably less than that of the homo-heptamer in the same solvent.

Movement of glycine to position 2 in the co-heptamer results in ~ 25% loss in ellipticity in the major Cotton effect assigned to the $\pi \rightarrow \pi^*$ transition when compared to the homo-heptamer. There is also about a 50% reduction in ellipticity in the $n \rightarrow \pi^*$ transition at 222 nm for this co-heptamer as compared to the homo-heptamer in TFE (Fig. 46). As glycine is moved to position 3 the similarity of the CD spectrum in TFE with that of an α-helical oligopeptide decreases further and finally when glycine is in position 4 all resemblence to an α-helical CD pattern disappears (Fig. 46).

The C.D. spectra in TFE of the co-heptamer having glycine in position 5 is difficult to interpret. Based upon the differences in the spectra of this peptide in HFIP vs. TFE it can however be concluded that there are structural differences. The spectra in HFIP reflects typical random or statistical coil conformation, while the spectra in TFE reflects at least a perturbation in magnetic and electric field environments for the identical chromophores.

The C.D. spectra of the co-heptamer in TFE possessing glycine in the 6th position clearly reflects α-helical content as it displays many of the characteristics previously discussed and recognized as those representative of partially helical structures.

Figure 33. Boc-[Glu(OMe)]$_n$-OMe in TFE

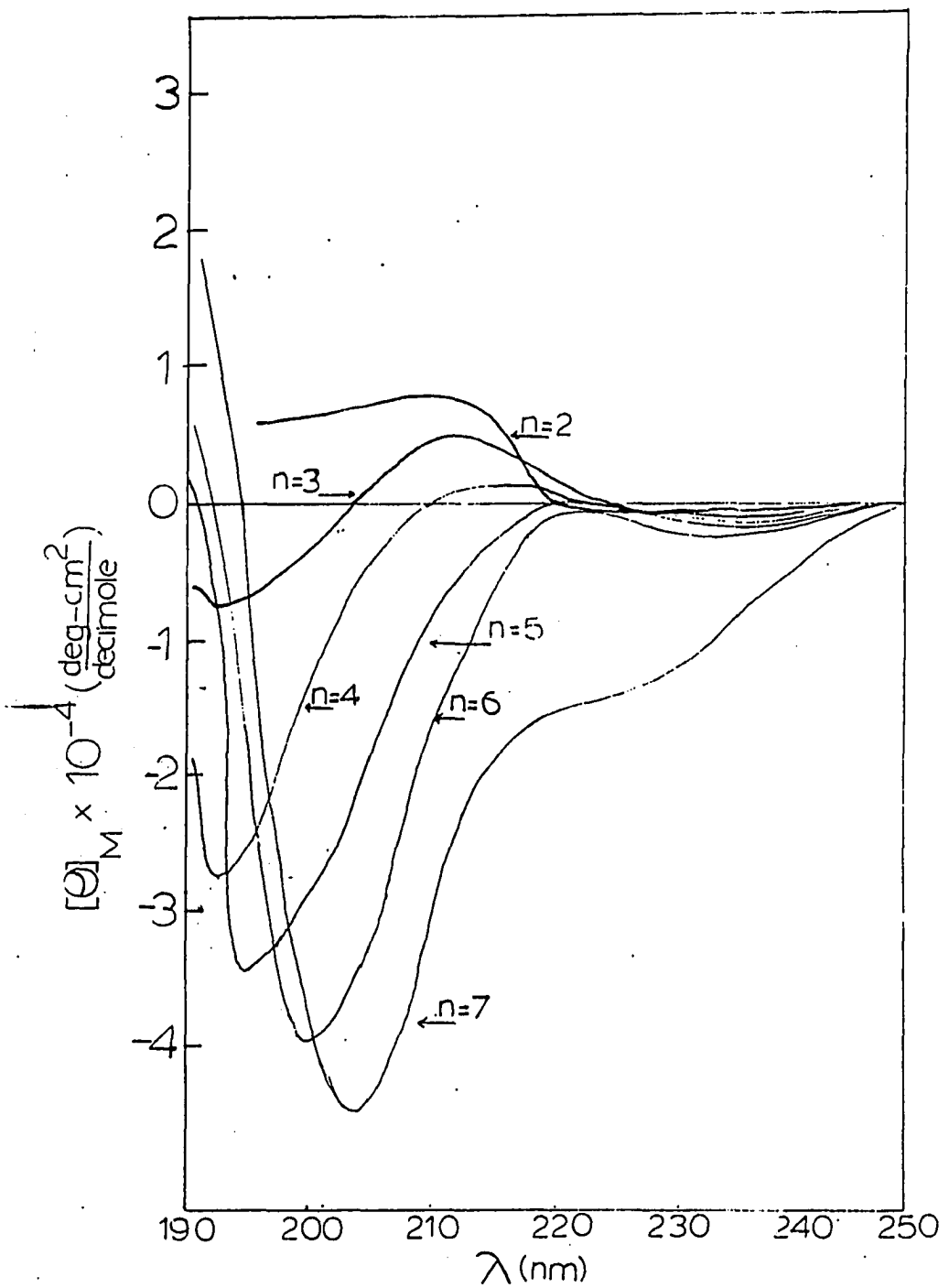


Figure 34. Boc-[Glu(OMe)]$_n$-OMe in HFIP

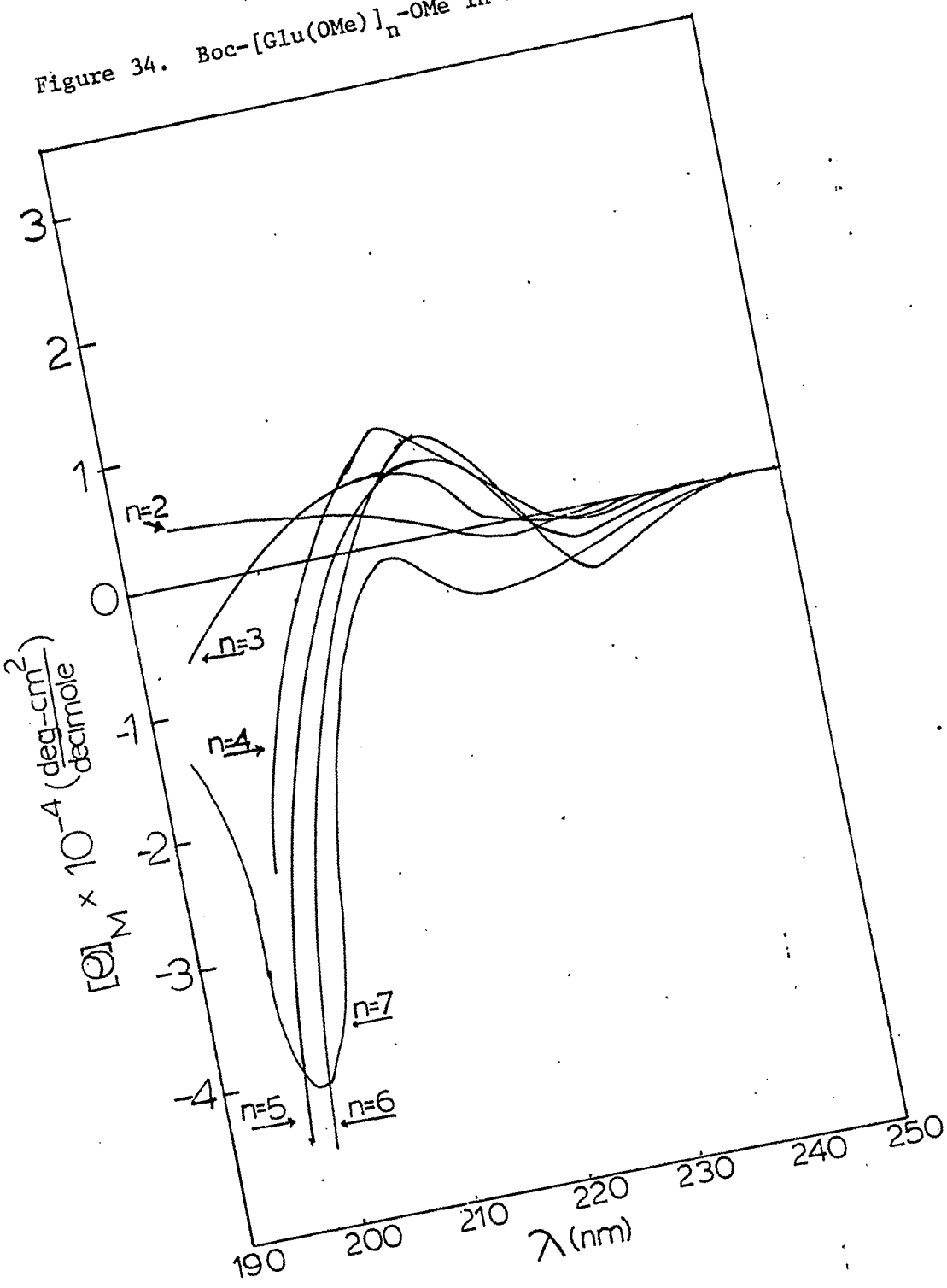


Figure 35. Boc-Gly-[Glu(OMe)]$_n$-OMe in TFE

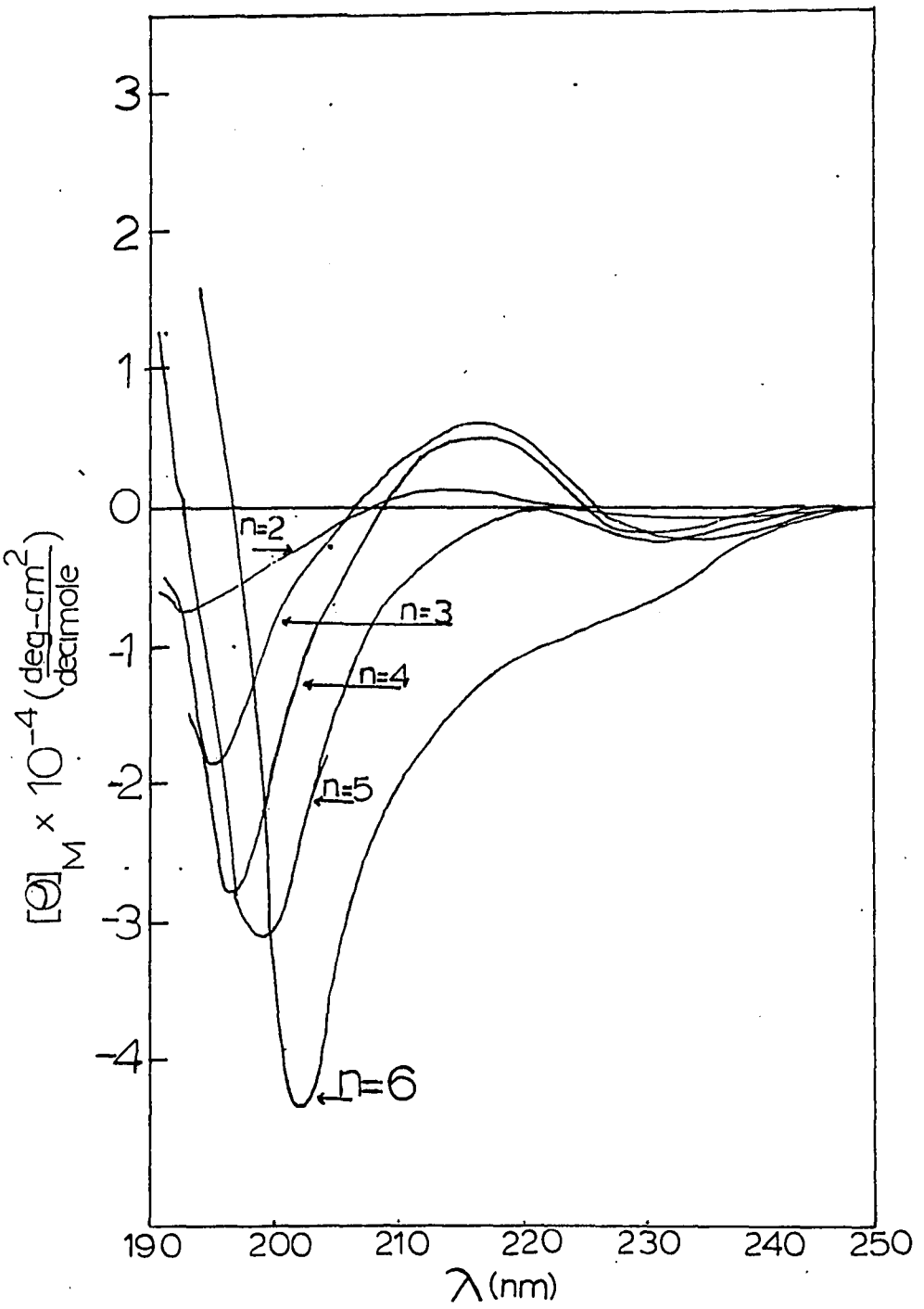


Figure 36. Boc-Gly-[Glu(OMe)$_n$]-OMe in HFIP

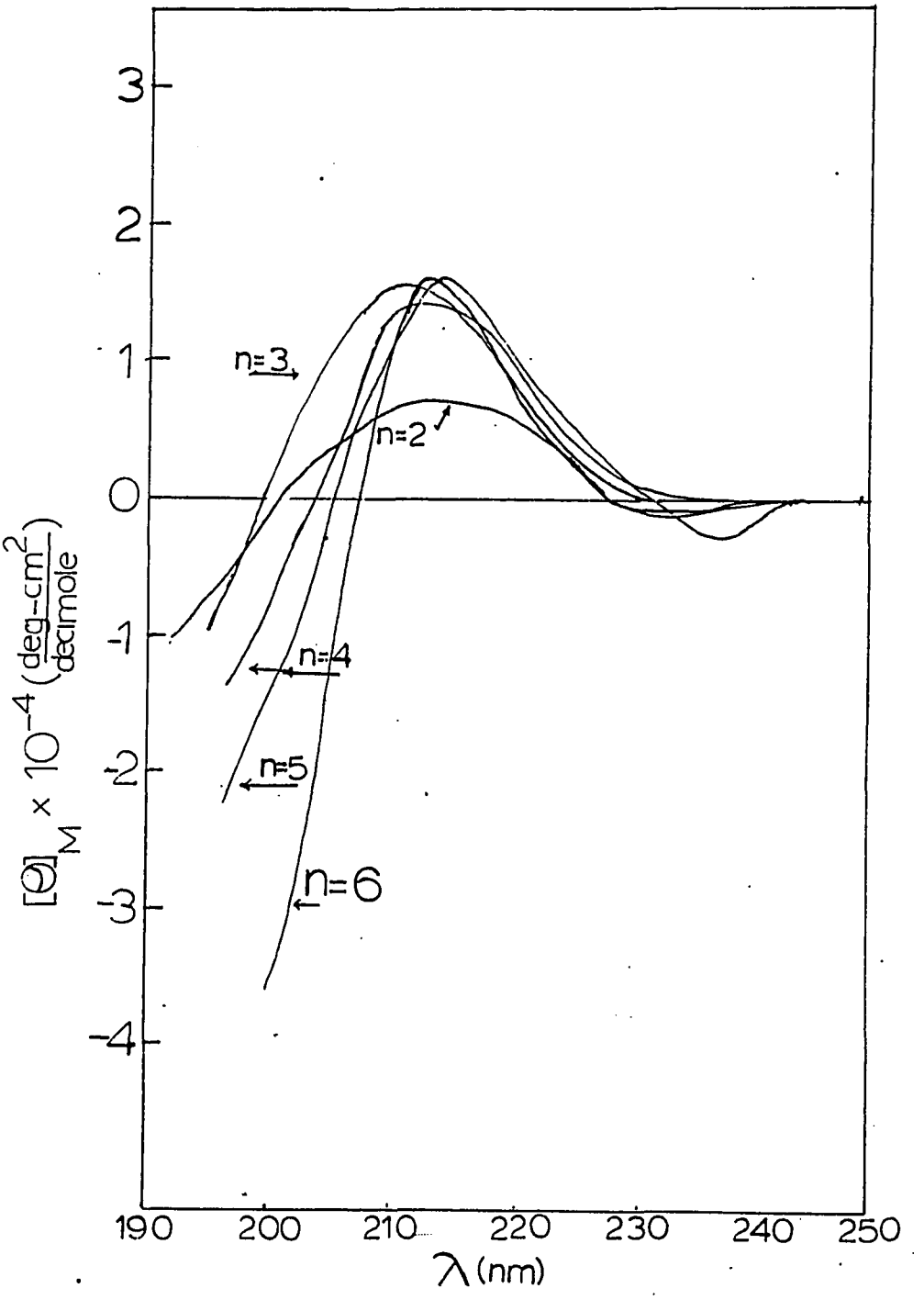


Figure 37. Boc-[Glu(OMe)]$_1$-Gly-[Glu(OMe)]$_n$-OMe in TFE

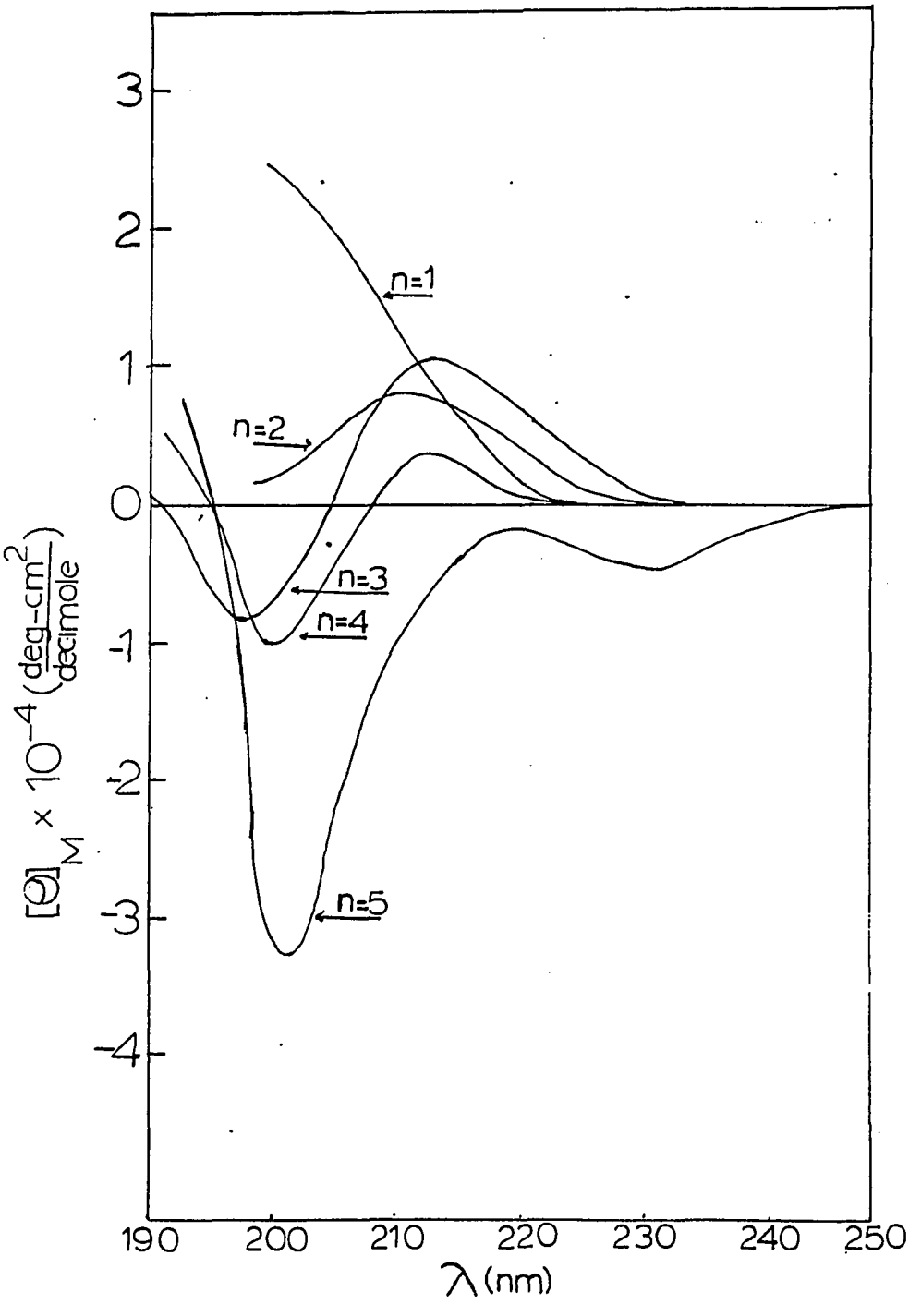


Figure 38. Boc-[Glu(OMe)]$_1$-Gly-[Glu(OMe)]$_n$-OMe in HFIP

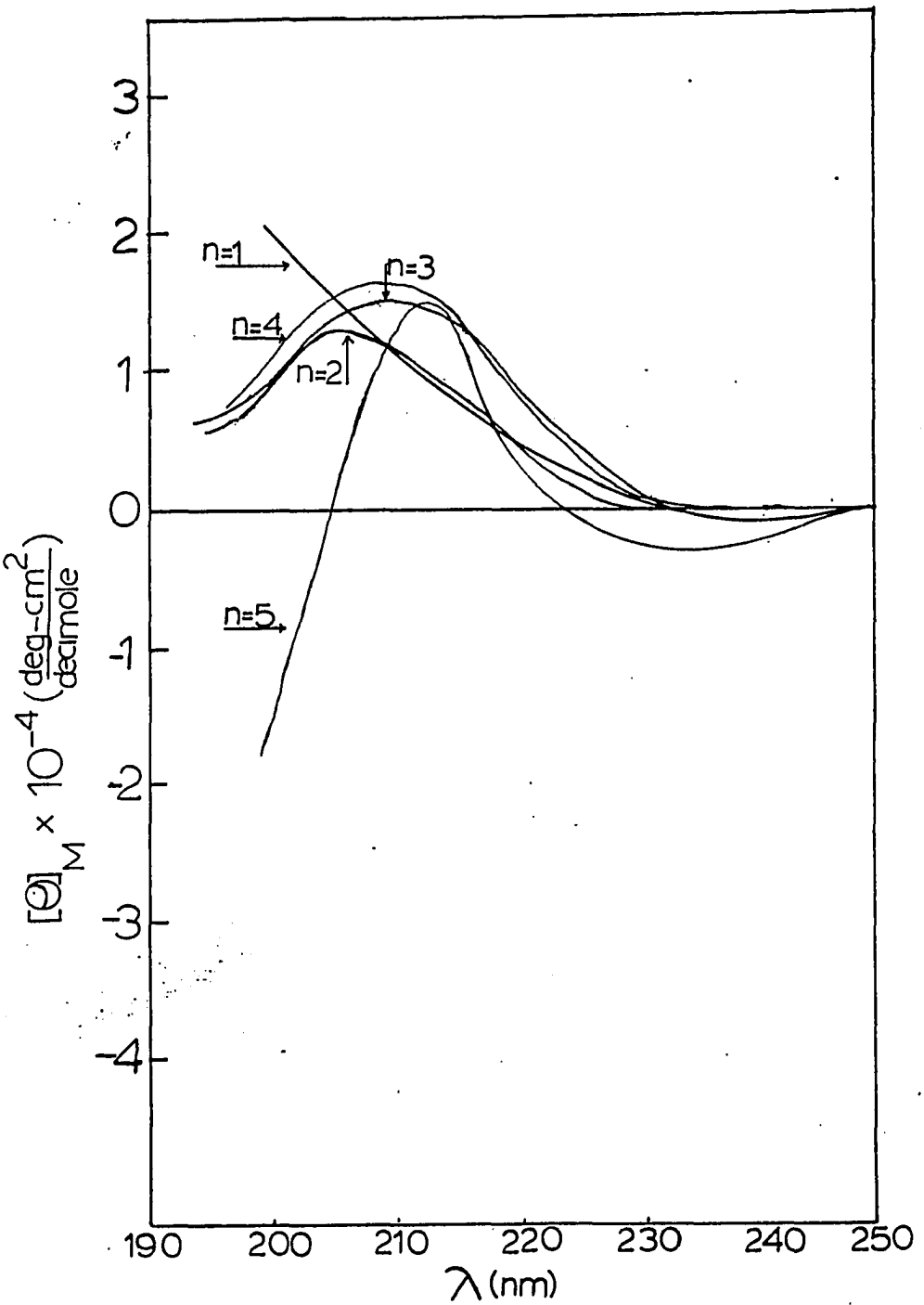


Figure 39. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_n$-OMe in TFE

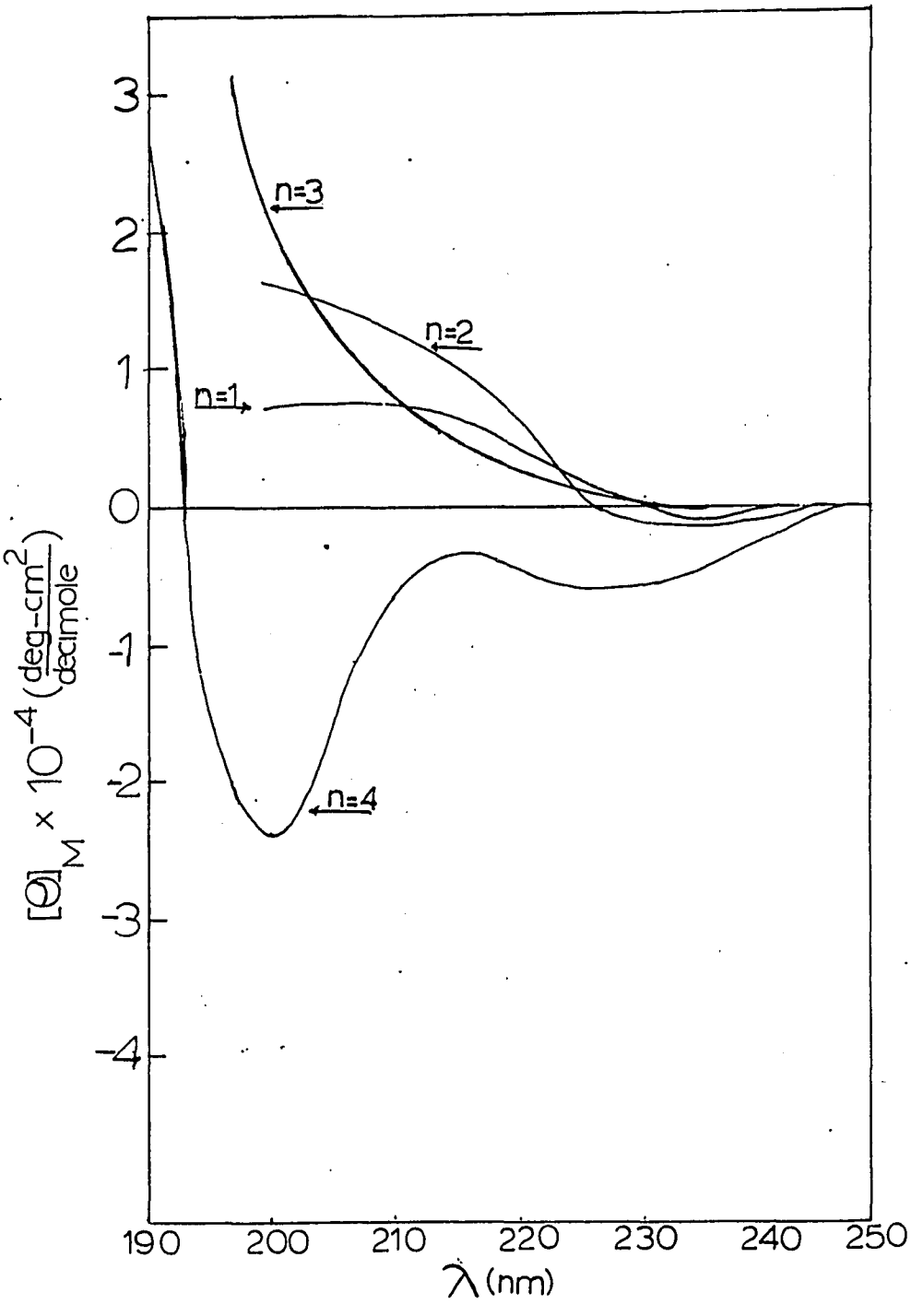


Figure 40. Boc-[Glu(OMe)]$_2$-Gly-[Glu(OMe)]$_n$-OMe in HFIP

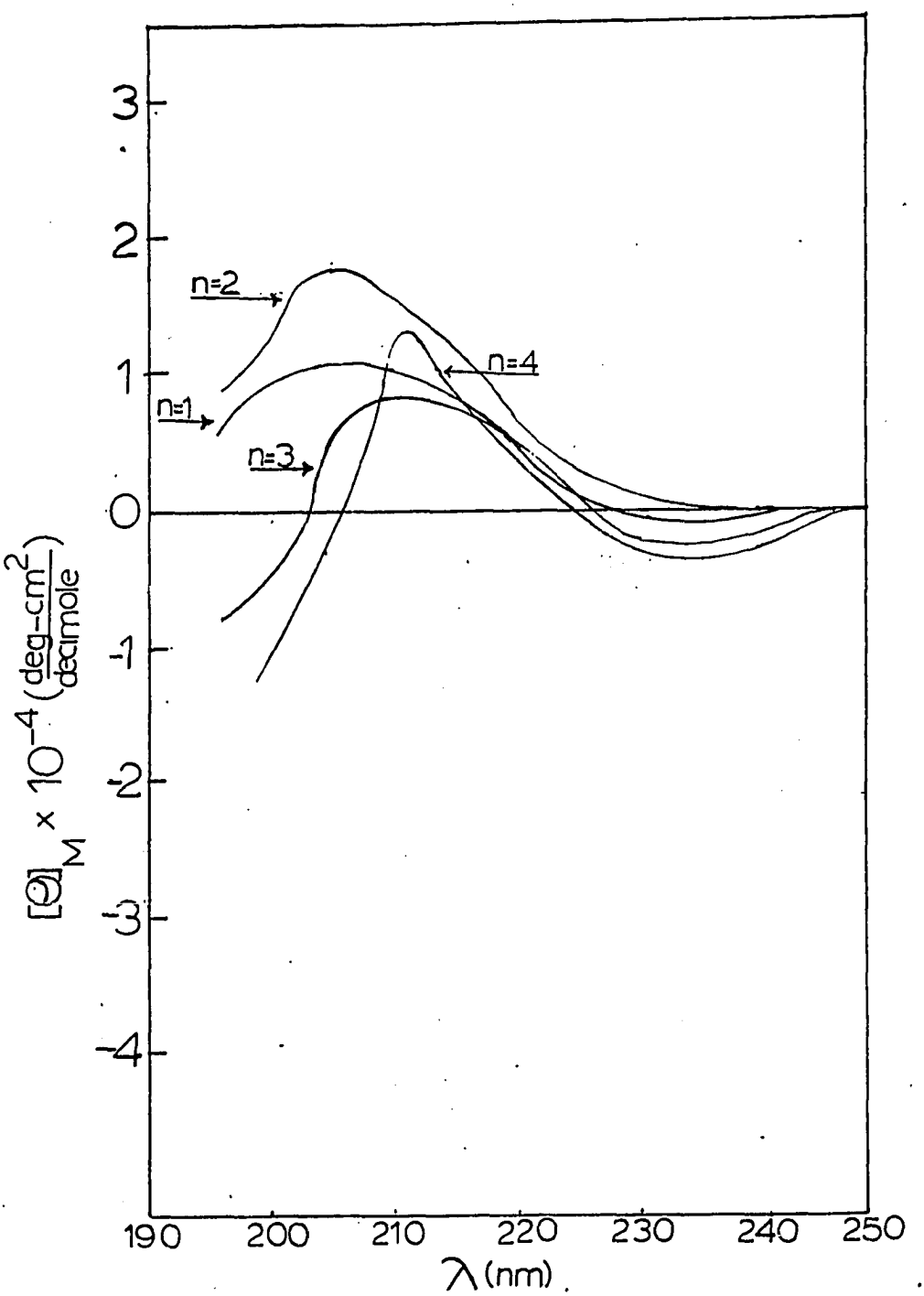


Figure 41. Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_n$-OMe in TFE

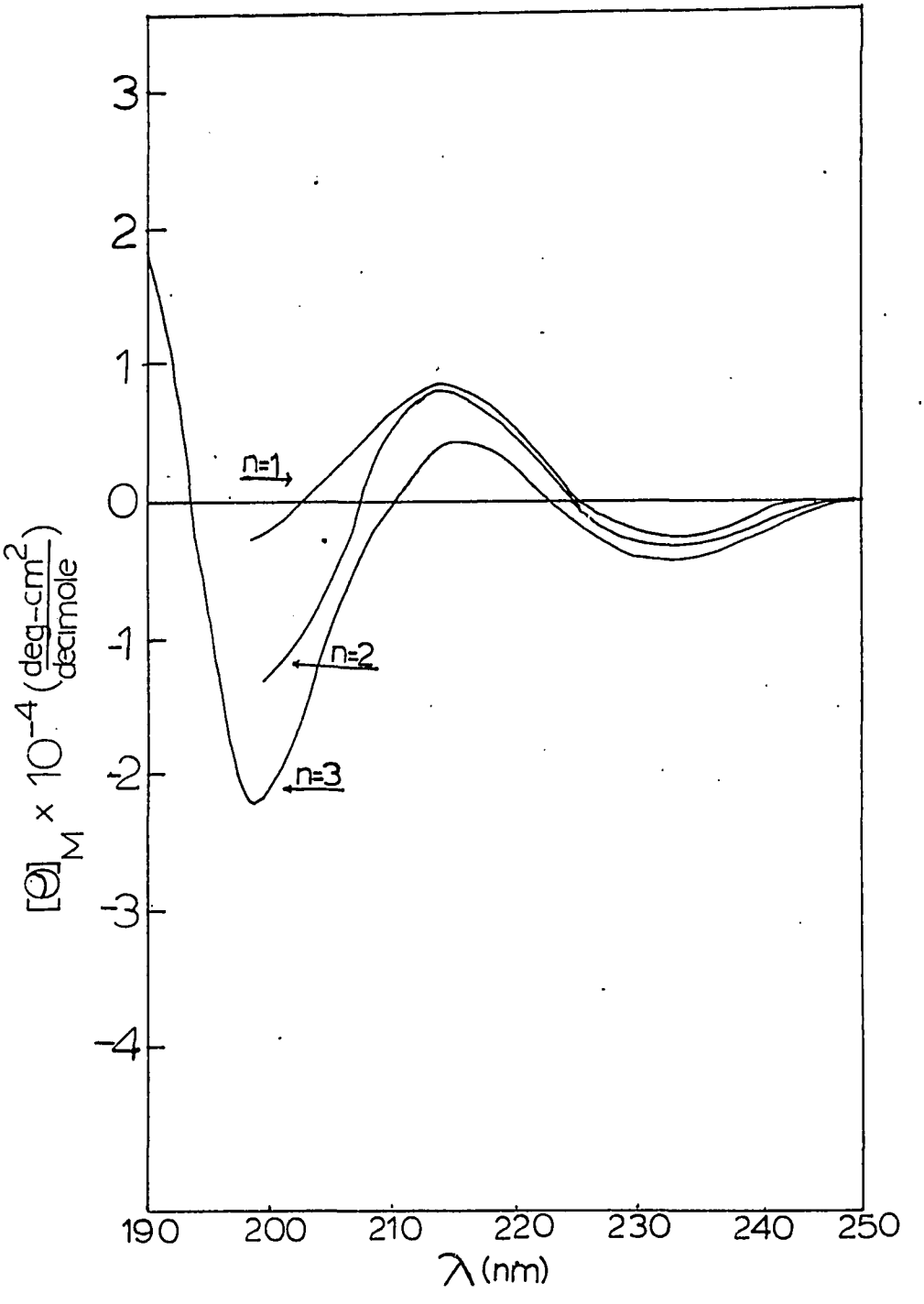


Figure 42. Boc-[Glu(OMe)]$_3$-Gly-[Glu(OMe)]$_n$-OMe in HFIP

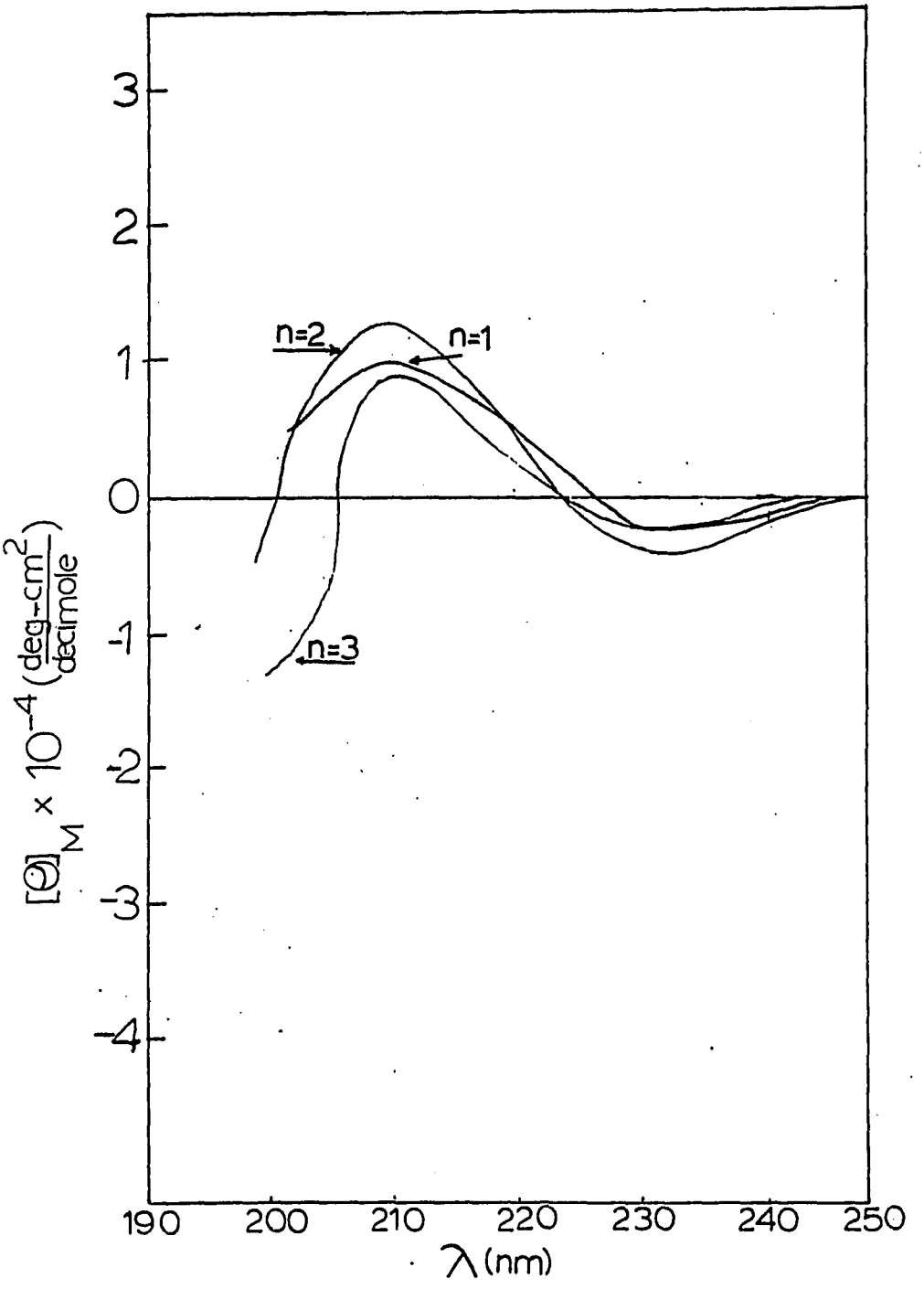


Figure 43. Boc-[Glu(OMe)]$_4$-Gly-[Glu(OMe)]$_n$-OMe in TFE

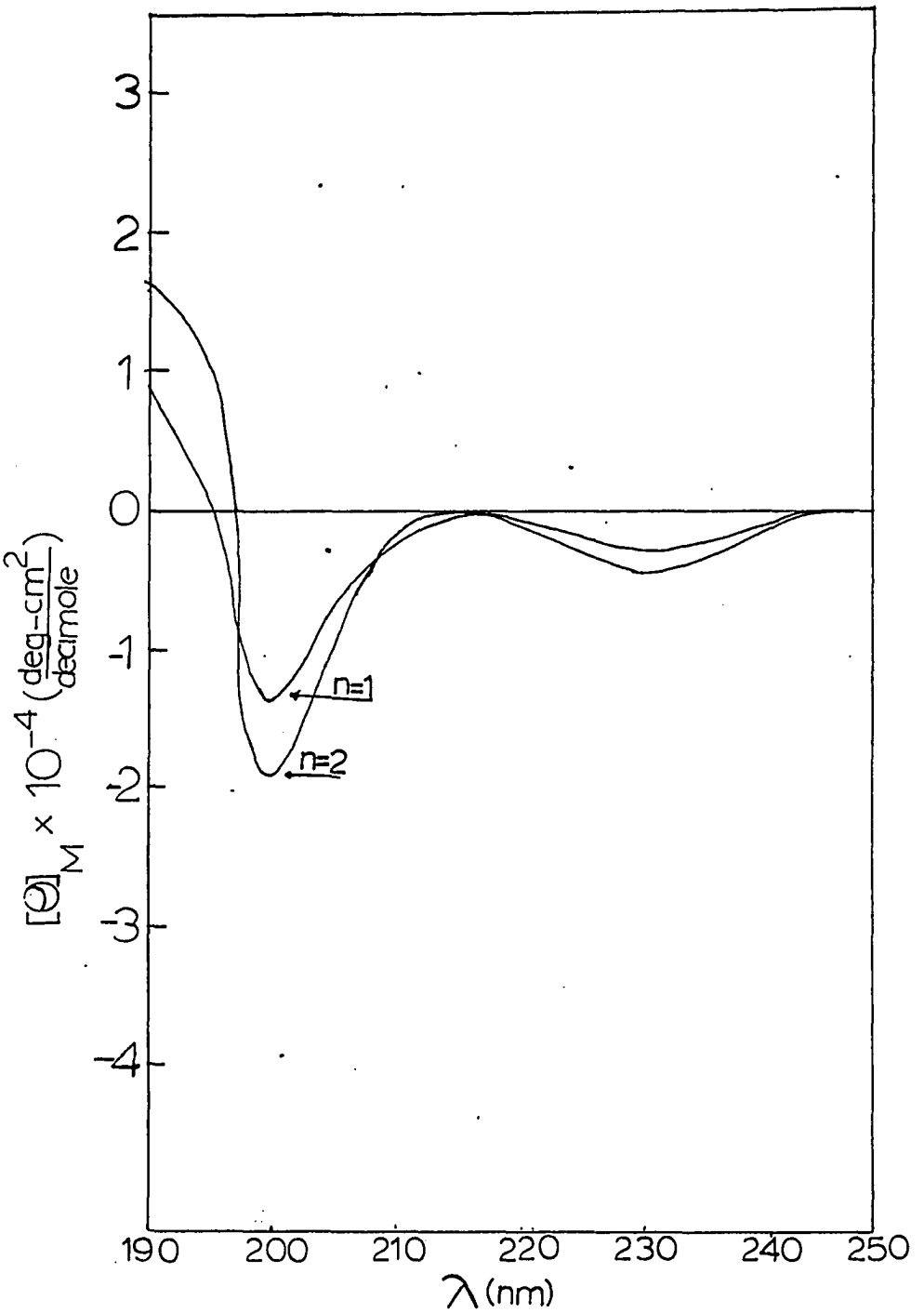


Figure 44. Boc-[Glu(OMe)]$_4$-Gly-[Glu(OMe)]$_n$-OMe in HFIP

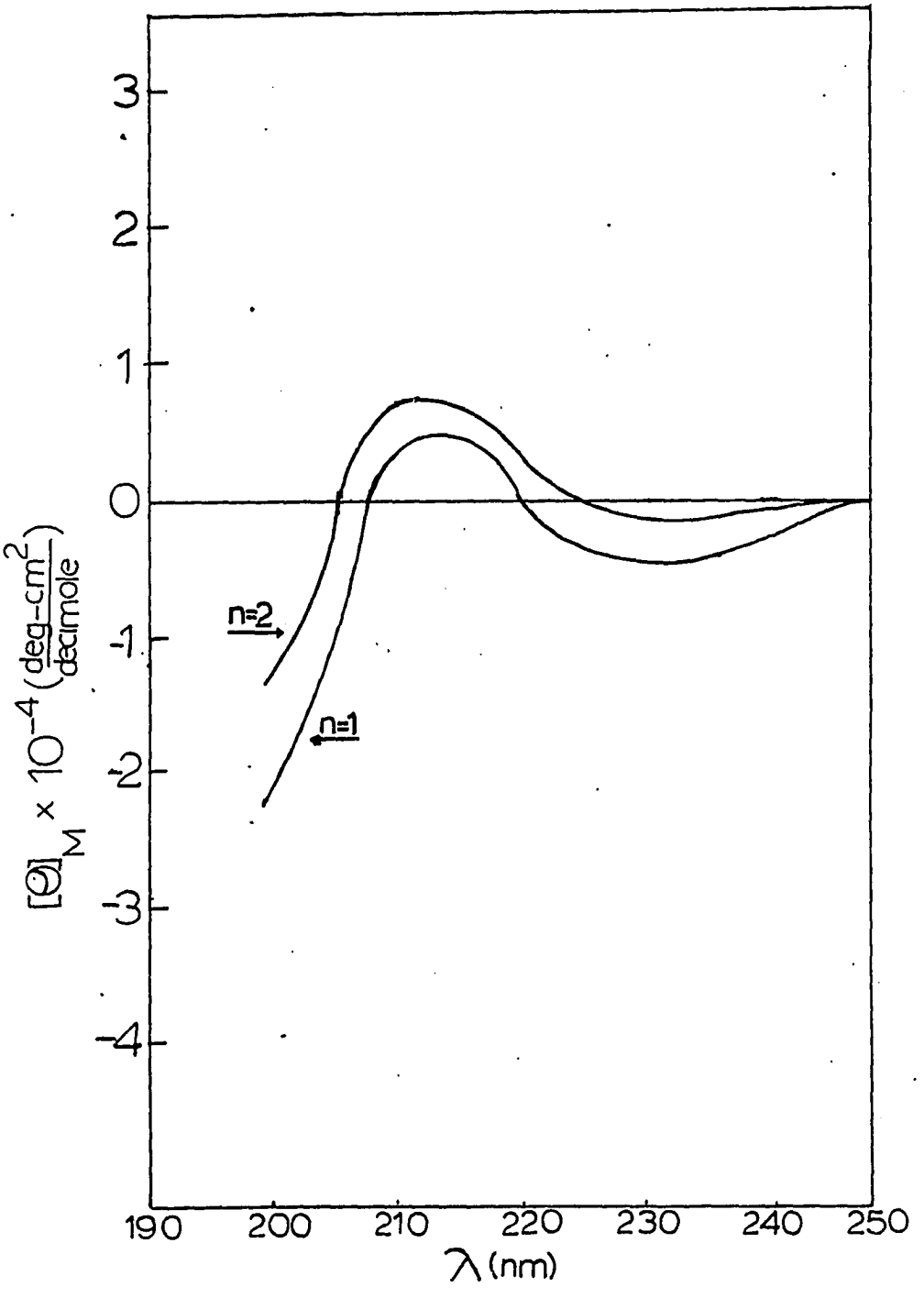


Figure 45. Boc-[Glu(OMe)]$_5$-Gly-[Glu(OMe)]$_1$-OMe in HFIP and TFE

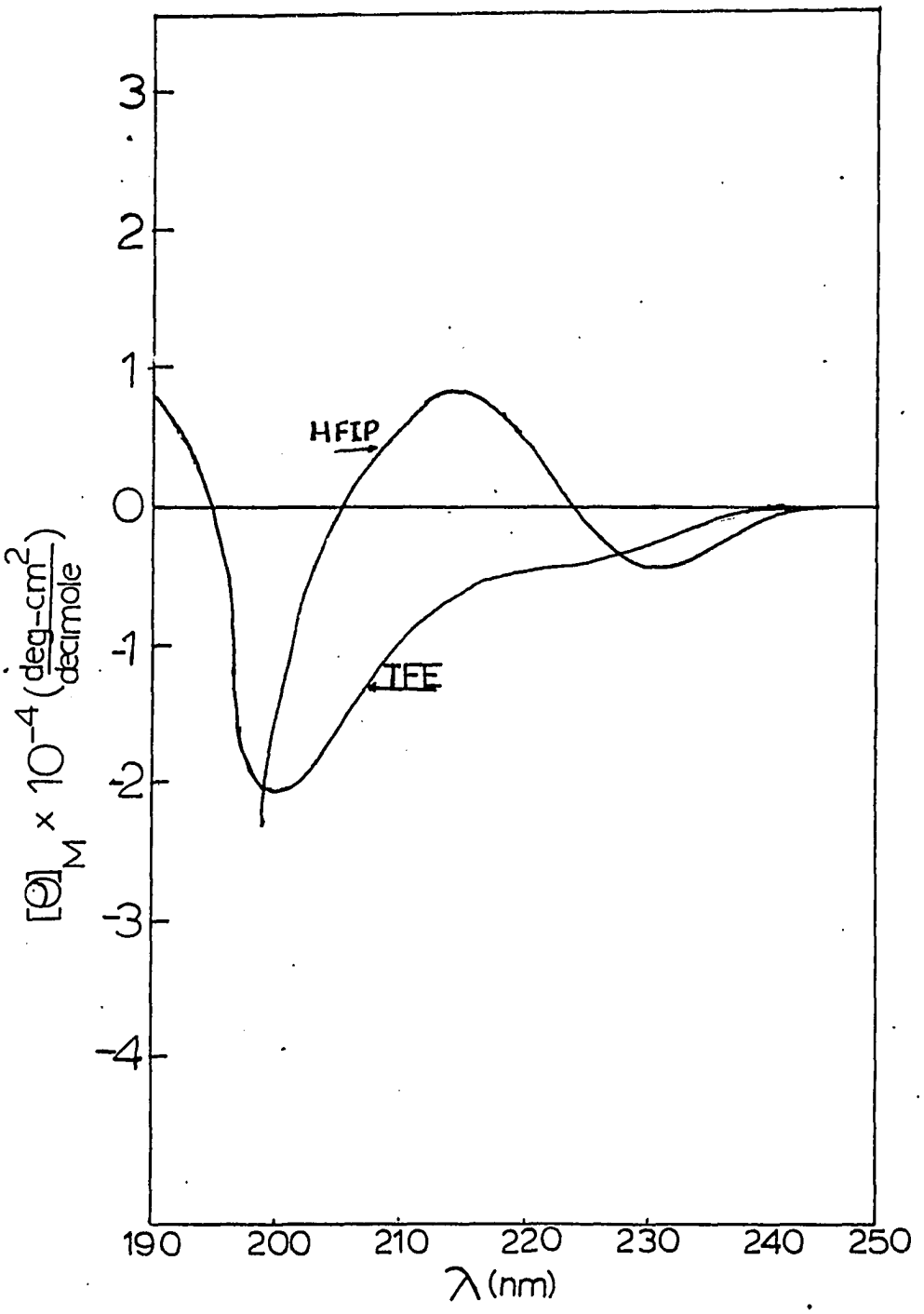
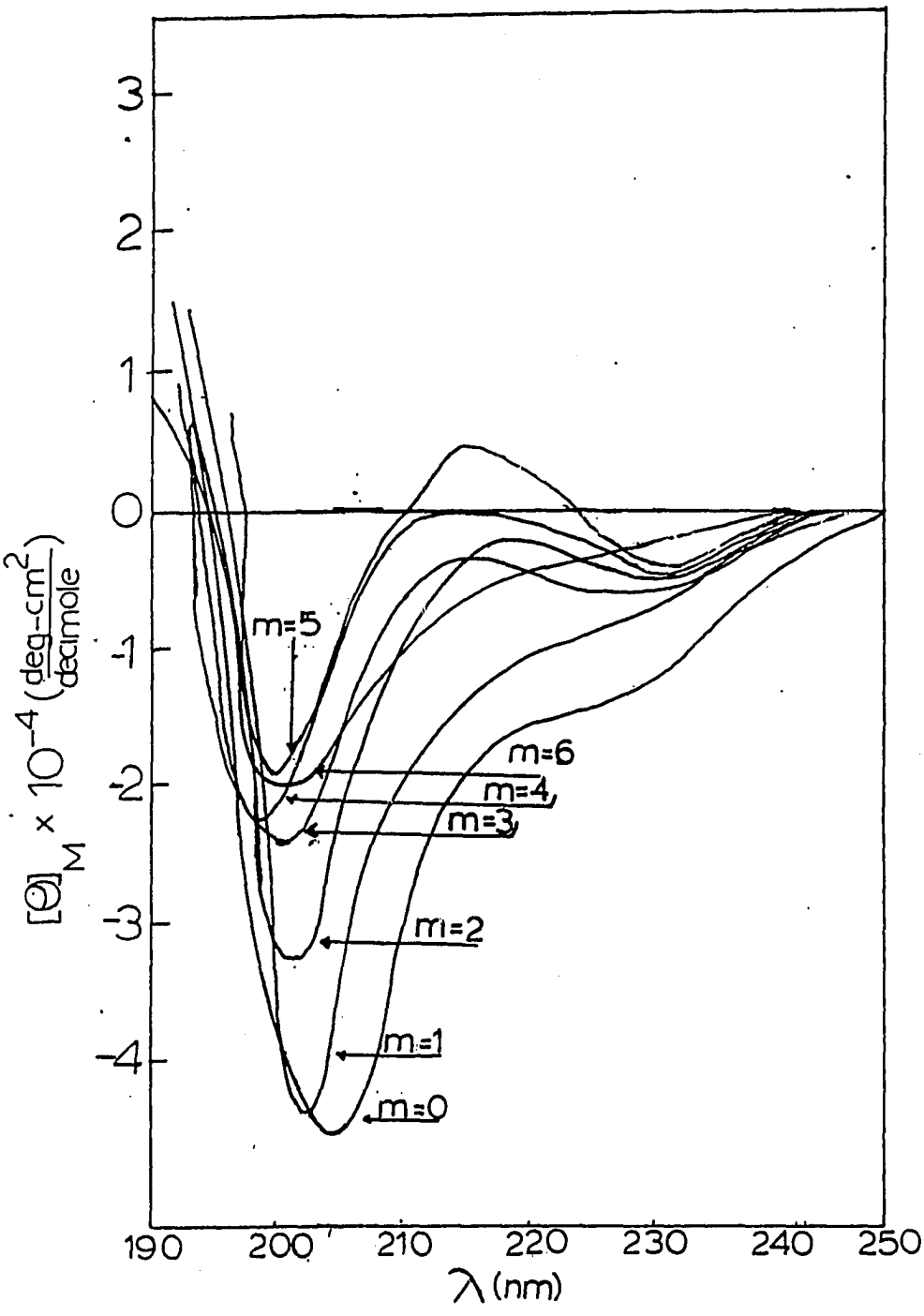


Figure 46. Co-heptamers in TFE



MATERIALS AND METHODS

L-Glutamic acid and glycine were purchased from Sigma Chemical Co., St. Louis, Missouri. N-methyl morpholine, 2-(tertiarybutoxycarbonyl-oxyimino)-2-phenylacetonitrile, isobutylchloroformate, methane sulfonic acid, trifluoroacetic acid and hexafluoroisopropanol were purchased from Aldrich Chemical Co., New Jersey. All other reagents and solvents were of the highest purity available.

The optical rotation measurements were carried out on a Perkin-Elmer Model 141 polarimeter.

The HPLC apparatus used was a Waters chromatograph (Waters Assoc., Milford, Mass.) equipped with a model 6000M solvent delivery system, a U6K injector, and a model 450 variable-wavelength uv detector operating at 220 nm. A 30 cm x 3.9 mm i.d. μPorasil silica column, also supplied by Waters Assoc., was used for all experiments.

Generally, the amount of sample injected was 5-20 μg dissolved in 5-25 μL dichloromethane. For some of the larger peptides a small amount of trifluoroethanol was required to attain dissolution. All samples were eluted isocratically with cyclohexane-isopropanol-methanol.

Solvents were spectral grade and were purchased from Fisher Scientific (Springfield, N.J.).

C.D. spectra were recorded on a Cary 60 spectropolarimeter using a .05 cm cell path length.

CONCLUSION

The major goal of this thesis was the determination of the effects on the secondary structure of non-homologous amino acid substitution at various positions in protected γ-methyl-L-glutamate homo-oligomers. To this end the solution synthesis of homo- and co-oligopeptides was required. A final purity of $\bar{>}$ 97% was deemed necessary for meaningful spectral analysis. Purity determination via normal phase HPLC was the criteria for purity.

The synthetic procedure employed was highly successful. All compounds synthesized were of a final purity of 97% or greater. The significance of this success lies in the ability to obtain for conformational comparison a pure series of compounds with identical amino acid content varying only slightly in sequence. In contrast to the solution phase synthesis, the more rapid solid phase method could not guarantee correct peptide sequence. When employing the solid phase method it is necessary to drive every reaction to 100% completion to insure homogeneity of the final product. Solution phase synthesis allows the facile removal at every step of any impurities resulting from unreacted starting materials.

The ability to purify all peptides without the use of column-liquid chromatography was an additional accomplishment of this thesis. The use of a simple and inexpensive washing procedure allowed recovery of virtually pure product. Losses were rarely greater than 10% using this procedure.

The purity determination of HPLC portion of this thesis yielded serendipitous discoveries. During the time at which these purity determinations were carried out analytical HPLC of protected oligopeptides

was not a routine procedure. Initial attempts at analysis using a conventional bi-component (cyclohexane:isopropanol) solvent system, on silica, proved quite ineffective in resolving any of the peptides above the trimer. Application of reverse phase HPLC was also without success. This researcher devised a tri-component solvent system consisting of cyclohexane:isopropanol:methanol. All peptides synthesized for this thesis were highly resolvable in the new system. Most impressive from the HPLC analysis was the ability of the system to resolve oligomers of the identical molecular weight that differed only in the position of glycine insertion in the glutamate backbone. Another remarkable finding was the relationship of column mobility of the peptide to position of glycine insertion in the peptide. The most interior glycine positions in the hexamer and heptamers resulted in reduced retention time when compared to other heptamers and hexamers. This is in direct contrast to increased retention time (as compared to the homo-oligomers) with insertion of glycine in any of the lower oligomers. These results could be attributable to a conformational perturbation imposed by glycine insertion at the internal positions and subsequent differences in the glycyl interaction with the silica support.

The results of circular dichroism studies indicate that the homo-glutamates form helices at the heptapeptide. The spectra of $(BocGly(Glu)_6OMe)$ (with γOMe) in TFE was quite similar to the spectra of $Boc(Glu)_7\text{-}OMe$ (with OMe) in TFE. In a heptamer, four or at most five residues can exist in a helical structure because end residues solvate differently and are often unable to take part in the one or two

intramolecular stabilizing hydrogen bonds. It is plausible that a glycyl residue at the N-terminal position of a heptamer, otherwise containing only γ-methyl-L-glutamate, is not involved in hydrogen bonding and thus not involved in the stabilization of helical structure. Glycine in the second position must solvate differently than a glutamate residue and may therefore effect its H-bonding abilities, and certainly possess different ϕ,ψ angle distributions. These differences are reflected in the substantial loss of helical character from the C.D. patterns. This disappearance of α-helical structure becomes more pronounced in the C.D. of the co-heptamer $Boc(\underset{\gamma OMe}{Glu})_2Gly(\underset{\gamma OMe}{Glu})_4OMe$. The loss of all helical character is apparent in $Boc(\underset{\gamma OMe}{Glu})_3Gly(\underset{\gamma OMe}{Glu})_3OMe$ based upon its representative spectra. The fourth position is the position where a glycyl residue appears to be most effective in destabilizing structure. This may be a result of having the lowest number of consecutive glutamates in this molecule and therefore destroying the possibility of any H-bonding. Glycine may behave or function as a highly flexible hinge in this position. These findings are consistent with the results of Chou and Fasman[71] with respect to glycine's helix breaking ability in globular proteins in general. A glycyl residue in the fifth position displays a spectra in TFE which is difficult to interpret with respect to the presence of any definite helical structure. Finally the movement of the glycyl residue to the sixth position marks the return of some helical character to the C.D. (in TFE) of that coheptamer. The results of the above are consistent with those of polarimetry for the $Boc(\underset{\gamma OMe}{Glu})_nOMe$,

$Boc\underset{\gamma OMe}{Gly(Glu)}_n OMe$, $Boc\underset{\gamma OMe}{Glu}Gly\underset{\gamma OMe}{(Glu)}_n OMe$, $Boc\underset{\gamma OMe}{(Glu)}_2 Gly\underset{\gamma OMe}{(Glu)}_n OMe$, and $Boc\underset{\gamma OMe}{(Glu)}_3 Gly\underset{\gamma OMe}{(Glu)}_n OMe$ with respect to deviation from linearity in $[\phi]_M$ vs. n plots in TFE as a function of the onset of secondary structure. In the case of the remaining series graphic analysis of polarimetric results was not appropriate.

These circular dichroism studies demonstrate the ability of this spectral technique to distinguish between structures of linear oligopeptides varying only in the position of one non-homologous peptide in a homologous backbone. The ability to distinguish between small changes in peptide sequence via C.D. can be of major importance for the study of peptide hormones and drug design when such drugs and hormones are dependent on their primary and secondary conformations for biological activity.

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