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SYNTHESIS AND CHARACTERIZATION OF FLUORINATED POLYPEPTIDES

A Dissertation Presented

by

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ABSTRACT

SYNTHESIS AND CHARACTERIZATION OF FLUORINATED POLYPEPTIDES

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Fluorinated polymers possess special properties, including stability at high temperatures, toughness and flexibility at very low temperatures, non-adhesiveness, insolubility, chemical resistance and, in some cases, biocompatibility. Incorporation of fluorinated amino acids has been proposed as a means to impart some of these properties to polypeptides.

Trifluoroalanine N-carboxy anhydride (TFANCA) was synthesized by phosgenation of 3,3,3-DL-trifluoroalanine in tetrahydrofuran and was polymerized with aniline, thiophenol, or triethylamine as initiators. Time of flight matrix assisted laser desorption mass spectrometry was used to determine the absolute molecular weights and molecular weight distributions of the resulting polypeptides. Knowledge of the exact molecular weight confirmed the structural integrity of the products and gave insight into the mechanism of polymerization. TFANCA was also copolymerized with γ-benzyl glutamate NCA. Incorporation of 20 mol-% trifluoroalanine into poly(γ-benzyl-L-glutamate) leads to a marked decrease in the surface energy of the polymer as determined by contact angle measurements.

Poly(α,L-glutamate)s carrying C_8, C_{10}, and C_{12} fluorinated side chains were synthesized by polymerization of the corresponding amino acid N-carboxyanhydrides. Contact angle measurements with water were used to assess the effects of fluorination on the surface energies of films of the resulting polypeptides. The wettability of the polymers was found to decrease with increasing fluorine content, as expected. A remarkably high contact angle (121°) was measured for the homopolymer carrying C_{12} fluorinated side chains, indicating a surface consisting of closely packed trifluoromethyl groups. Side chain crystallization, consistent with the formation of such a surface, was suggested by the results of x-ray scattering and calorimetric measurements.

Supercritical carbon dioxide was also used as a solvent for the polymerization of the above mentioned fluoroglutamate N-carboxyanhydrides. *In situ* IR monitoring verified that polymerization takes place in that solvent despite the necessary evolution of carbon dioxide during the reaction. The polymerization of the NCA with the C_{12} fluorinated side chain proceeded homogeneously. Spectral differences between the polypeptides prepared in tetrahydrofuran and in carbon dioxide are being investigated.

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CHAPTER 1

INTRODUCTION

1.1 Motivation

One of the goals in polymer science is the ability to design and synthesize materials with "tailor-made" properties. In that respect polypeptides are unique among different classes of polymers particularly because of the combination of two features. First, they can assume secondary and tertiary structures that depend on their primary sequence and second, monodisperse copolypeptides of controlled sequence and stereochemistry can be synthesized using genetic engineering methodology. However, the design of materials for specific applications may involve the presence of functional groups that are not found in any of the natural amino acids. This apparent limitation of the biological approach is being addressed by exploring methods for the incorporation of unnatural monomers into polypeptides prepared in bacterial hosts[1,2]. The new amino acids are designed to impart to polypeptides special properties.

Fluorinated analogs of amino acids may be particularly useful in such experiments, for two reasons. First, fluorine has steric requirements similar to those of hydrogen and therefore replacement of hydrogen by fluorine does not significantly perturb the molecular geometry. Second, fluorine-containing polymers possess special properties, including stability at high temperatures, toughness and flexibility at very low temperatures, non-adhesiveness, insolubility, chemical resistance and, in some cases, biocompatibility.

The main goal of the research described herein is the incorporation of fluorinated amino acids into polypeptides by polymerization of their N-carboxy anhydrides (NCAs) in order to determine the effectiveness of fluorination in decreasing the surface energy of polypeptides. Two classes of fluorinated amino acids have attracted our attention. First,

analogs of natural amino acids and in particular 3,3,3 trifluoroalanine and second, monomers derived from modification of natural amino acids such as esters of glutamic acid with fluorinated alcohols. The thesis is organized into four self-contained chapters. The introduction describes the motivation for the choice of the particular amino acids to study. A summary of the mechanisms of NCA polymerization is also provided. The synthesis and characterization of polymers containing trifluoroalanine is reported in Chapter 2. Chapter 3 describes the synthesis of fluorinated polyglutamates in organic solvents and their structural characterization while Chapter 4 deals with the synthesis of fluorinated glutamate homopolymers in supercritical carbon dioxide.

1.2 Fluorine Containing Molecules in Bioorganic Chemistry

Fluorinated analogs of biologically active compounds have been of increasing interest in biomedicine and biochemistry[3-6]. Being the second smallest substituent (the van der Waals' radii of F: 1.35 Å ; of H: 1.2 Å), but also the most electronegative element, fluorine closely mimics hydrogen with respect to steric requirements but has polar effects similar to those of oxygen. Introduction of fluorine provides a significant change in chemical character without perturbing the molecular geometry[7]. Substitution of hydrogen by fluorine increases the lipid solubility of a molecule. The CF_3 group is considered to be among the most lipophilic substituents and its incorporation into molecules has been used in drug design to ease transfer through lipophilic membranes. Selective fluorination has been used to modify biologically active compounds and facilitate the study of biosynthetic pathways. Replacing hydrogen by fluorine at or near the reactive site often causes inhibition of metabolism because of the exceptionally high C-F bond energy, which can result in irreversible binding. In particular, fluorinated amino acid analogs have been used to study decarboxylation because they act as specific inhibitors of the decarboxylation

enzymes[5]. Fluorinated pyrimidines have tumor inhibiting activity and their potential use in cancer chemotherapy is being studied[3]. Selective fluorination has been also used to probe biological compounds. Introduction of a fluorinated amino acid into a protein can make it detectable by ^{19}F NMR spectroscopy. In addition, the chemical shift in ^{19}F NMR spectroscopy is so sensitive to the chemical environment, that it can be used to detect exposure of a particular group to a solvent and therefore conformations of molecules[3].

It is therefore not surprising that fluorinated analogs of many amino acids have been synthesized and their biological activity has been tested[5,8,9]. 3,3,3-Trifluoroalanine is of particular interest because the trifluoromethyl group, being so close to the amino and carboxylic acid functions, greatly influences their activity. Indeed, the pKa value of the amino group of trifluoroalanine is 5.85, four units less than that of alanine, and as a result the fluorinated analog is not in a zwitterionic form in physiological solutions[10]. The function of trifluoroalanine as suicide inhibitor of pyridoxal phosphate dependent enzymes is well established[5,11-13]. Substitution of alanine by trifluoroalanine could lead to the synthesis of selectively fluorinated enzymes, hormones and other biologically active peptides. Synthetic fluoropeptides have been found to act as proteolytic enzyme inhibitors, while fluorinated hormone analogs can have increased or decreased reactivity compared to the parent hormone and their potential role in pharmacology is being investigated[4]. From the materials point of view, incorporation of trifluoroalanine into polypeptides is expected to decrease surface energy, increase water-repellant characteristics (and thereby their stability to hydrolysis), and perhaps decrease the surface friction coefficient.

1.3 Polyglutamates

Poly(α-L-glutamates) have attracted a lot of attention for their ability to exist in well defined α-helical conformations independently of the side chain substitution[14]. The latter

though can affect other properties including solubility in different solvents and thermal transitions. In particular poly(γ-alkyl-L-glutamates) (PALGs) have been studied for their ability to form lyotropic or thermotropic liquid crystals[15], as discussed in Chapter 3. They have also been found to be soluble in paraffinic solvents which indicates that the side chains control the solubility of the polymers[15]. We have been interested in the fluorinated analogs of these polypeptides (PFLGs) for their structural and surface properties but also for their solubility in supercritical carbon dioxide. CO_2 is a non-toxic, easily removable solvent, ideal for the synthesis of biomaterials. It is also a unique alternative to freons for solubilizing highly fluorinated polymers[16]. Furthermore, the polymerization of N-carboxy anhydrides depends on the carbon dioxide concentration in a way that is not fully understood (as discussed in the following section and in Chapter 4) and polymerization of fluorinated monomers in supercritical CO_2 could provide some insight into the mechanism of NCA polymerization.

1.4 α-Amino Acid N-Carboxyanhydrides

α-Amino acid N-carboxyanhydrides (α-NCAs) were discovered by Leuchs in 1906, who intended to use them as activated intermediates in peptide synthesis[17]. Their tendency to polymerize was their major shortcoming in stepwise preparations but rendered them valuable monomers for the synthesis of homopolypeptides and random copolypeptides. Historically it is interesting that the polymerization of NCAs was first reported when most chemists denied the existence of high molecular weight polymers. Investigation of the mechanism of polymerization started as early as 1920, but the course of the base initiated reaction has not yet been fully elucidated. Theoretical complexities and experimental difficulties summarized below are the reasons for ambiguities.

NCAs possess four reactive sites: two electrophilic carbonyl groups and the NH and α-CH groups that after deprotonation yield the nucleophilic amide anion and carbanion

respectively (Scheme 1.1). It is experimentally documented that the C-5 carbonyl is more electrophilic than the C-2 and therefore the first to be attacked by a nucleophile. Also the amide proton is far more acidic than the CH and so deprotonation of the methine is of importance only for N-protected NCAs. The insolubility of oligopeptides in organic solvents complicates kinetic studies of the polymerization. Precipitation of the oligopeptides formed and changes in conformation that accompany the increase in their molecular weight affect the rate of propagation[17]. The peculiarity in the behavior of some amino acid NCAs has also been the source of confusion in the literature. Generalization of the data taken for one particular amino acid NCA, although tempting, is not always correct. Finally, apparent contradiction in the literature has resulted from the use of different solvents, or reaction conditions (temperature, pressure, CO_2 concentration) that can affect the course of the polymerization.

Detailed discussions and reviews of the studies done to elucidate the mechanism of NCA polymerization can be found in the literature[17-20]. Following is a brief summary of the main considerations. Since initiation (by bases, nucleophiles, organometallic compounds, metal salts, or heat) influences the reaction pathways, mechanistic studies are subdivided according to the type of the initiation used.

Nucleophilic initiators, such as primary or less sterically hindered secondary amines, attack the C-5 carbonyl (Scheme 1.2)[18]. The carbamate anion, produced after opening of the NCA ring, is nucleophilic enough to attack another monomer and that pathway is described as the carbamate mechanism (Scheme 1.3). Alternatively, decarboxylation prior to propagation allows for the more nucleophilic amine to attack the next monomer, a mechanism called the amine or normal mechanism (Scheme 1.3). The relative contributions of these two mechanisms depend on the stability of the carbamate and the nucleophilicity of the carbamate versus the amine. Although carbamate anions are stable at temperatures below 50 °C, if they are not protonated, carbamate end groups have not been experimentally verified. Conditions that slow down decarboxylation have been

successfully used in stepwise synthesis to minimize polymerization, but contradictory results can be found in the literature on the effect of CO_2 pressure on the rate of polymerization[21,22]. To this point no analytical tool has allowed a reliable determination of the extent to which an individual polymerization involves an amine or carbamate mechanism[17].

The mechanism of the base initiated polymerization is still under investigation. It is reasonable that initiation by strong bases, like tertiary amines, first involves abstraction of the NH proton and subsequent attack of the amide anion on the C-5 carbonyl of another NCA (Scheme 1.4). Further propagation can occur according to the carbamate or amine mechanism. However, addition of N-acetyl glycine NCA as a coinitiator accelerates the polymerization and results in the formation of polypeptides containing one acetyl and one anhydride end group[23]. These data led to the formulation of an activated monomer mechanism, similar to the one proposed for the polymerization of lactams (Scheme 1.5). It should be pointed out, though, that there are significant differences between the lactams and the NCAs[20] and that unambiguous evidence for the formation of high molecular weight polypeptides via the activated monomer mechanism is still lacking[17].

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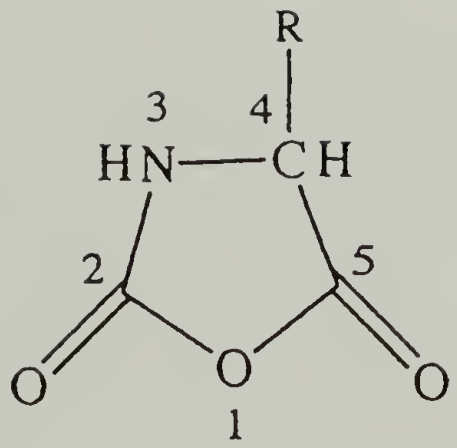
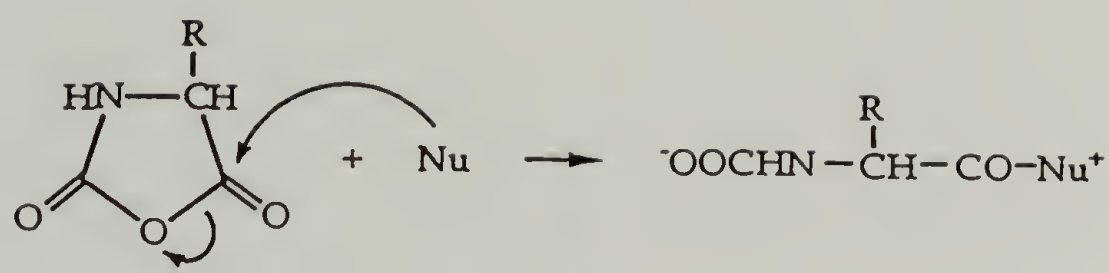
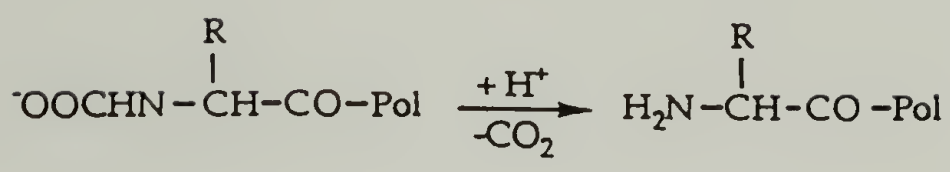


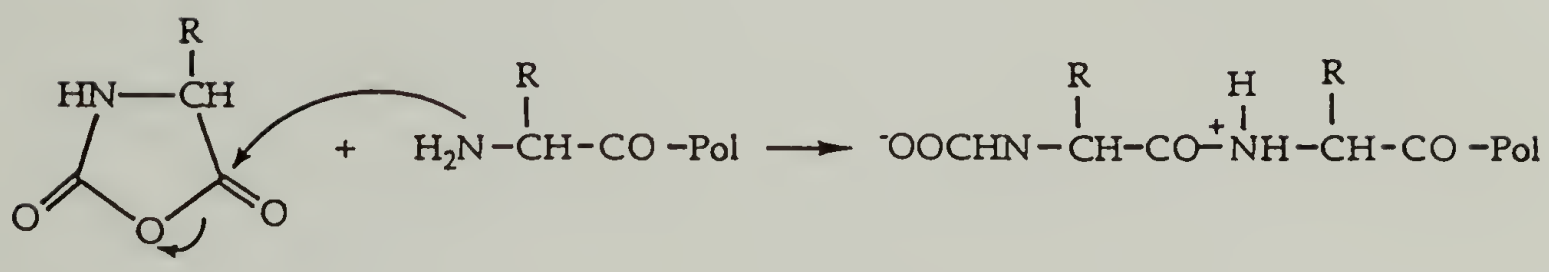
Scheme 1.1 α-Amino acid N-carboxy anhydride.



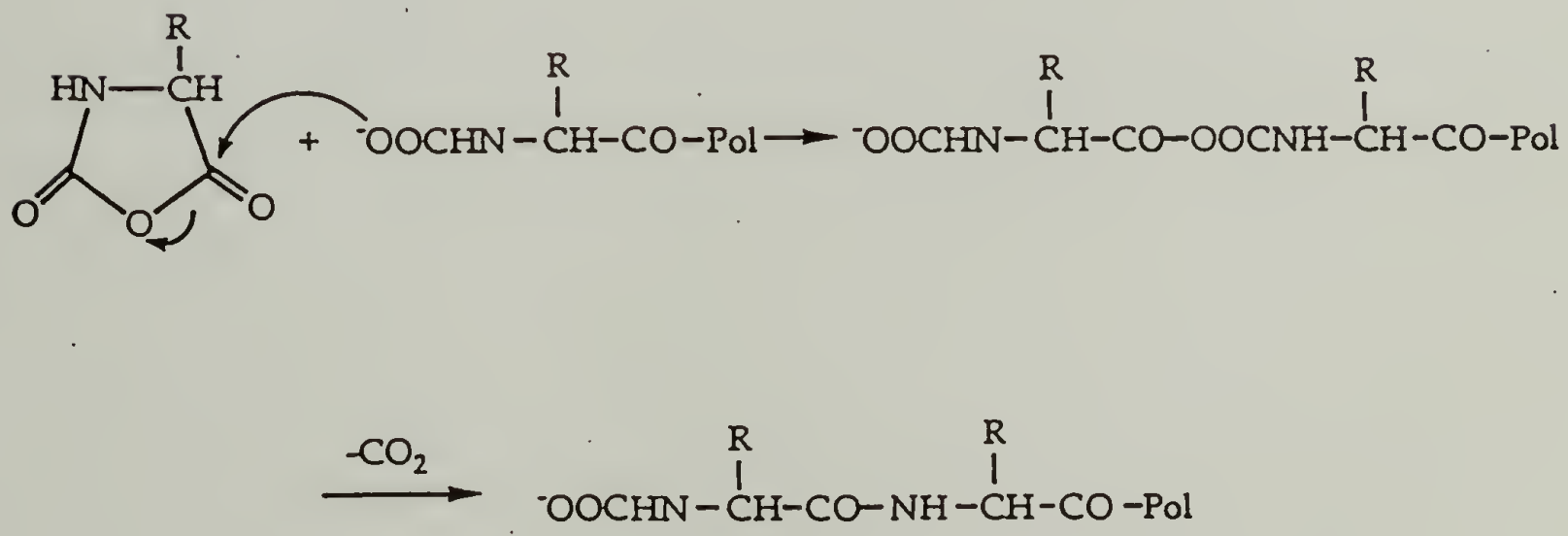
Scheme 1.2 Initiation of NCA polymerization by nucleophiles.

1. Amine Mechanism

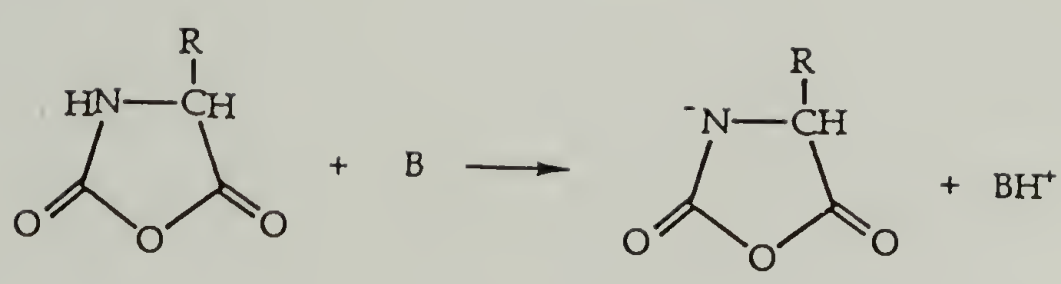


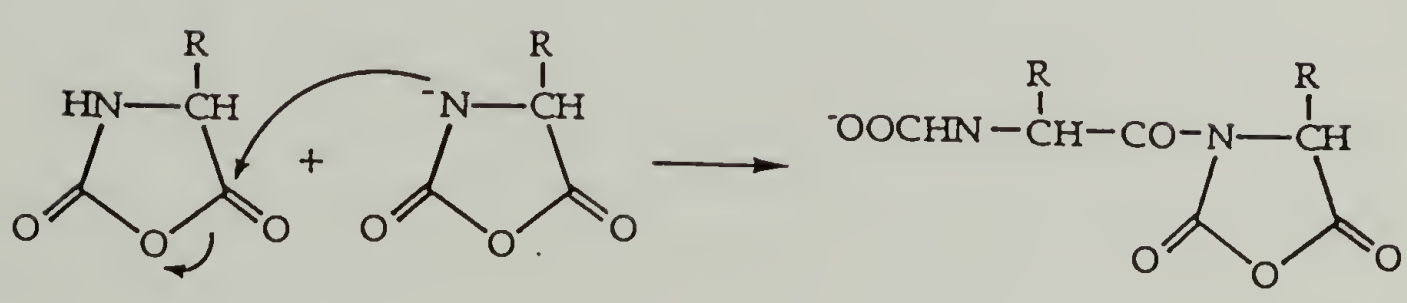


2. Carbamate mechanism

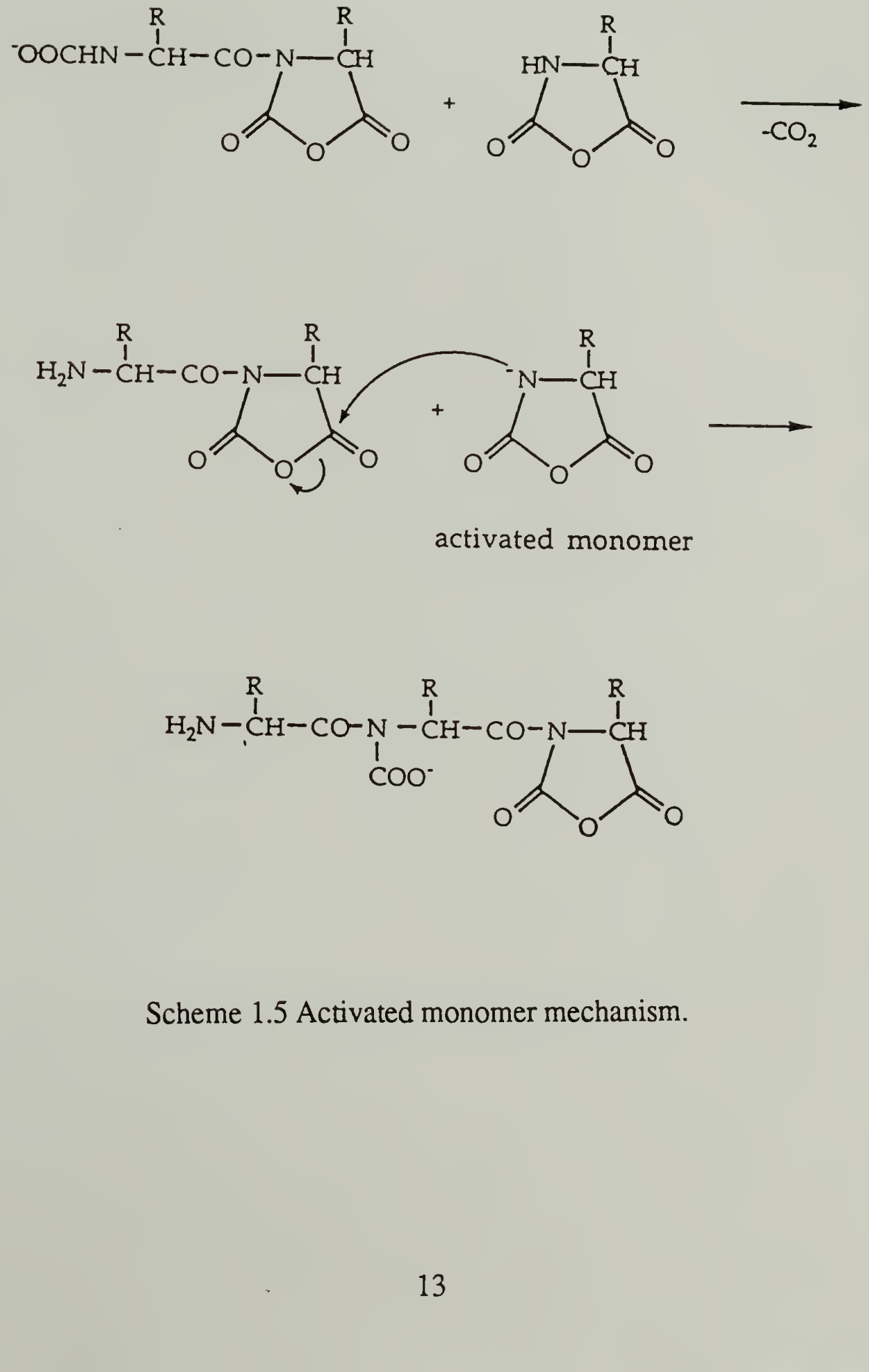


Scheme 1.3 Propagation via the amine or carbamate mechanisms.





Scheme 1.4 Initiation of NCA polymerization by bases.



Scheme 1.5 Activated monomer mechanism.

CHAPTER 2

SYNTHESIS OF TRIFLUOROALANINE N-CARBOXYANHYDRIDE AND POLYMERS AND COPOLYMERS THEREFROM

2.1 Introduction

Polypeptides are unique among polymeric materials. The high density of inter- and intramolecular hydrogen bonds formed by the backbone amide functions allows polypeptides to assume secondary and tertiary structures that depend in subtle ways on the primary chemical sequence. Furthermore, monodisperse copolypeptides of controlled sequence and stereochemistry can now be synthesized using genetic engineering methodology, making this class of materials unique in terms of uniformity of structure. The versatility of the biological approach to the synthesis of polypeptides will be determined in large part by the range of monomeric building blocks that can be utilized. In order to address this problem, we have begun a broadly based exploration of methods for the incorporation of unnatural monomers into polypeptides prepared in bacterial hosts. In our initial experiments, selenomethionine[1] and *para*-fluorophenylalanine[2] have been incorporated into repetitive polypeptides designed to adopt specific solid state structures.

Fluorinated analogs of amino acids may be particularly useful in such experiments, for two reasons. First, fluorine has steric requirements similar to those of hydrogen and therefore replacement of hydrogen by fluorine does not significantly perturb the molecular geometry. Second, fluorine-containing polymers possess special properties, including stability at high temperatures, toughness and flexibility at very low temperatures, non-adhesiveness, insolubility, chemical resistance and, in some cases, biocompatibility. Fluorinated polypeptides might be expected to have interesting surface properties, since

they combine the highly hydrophilic amide backbone with hydrophobic fluorocarbon side chains.

Fluorinated analogs of amino acids and other biologically active compounds are also of increasing interest in biochemistry and in medicine[3-8]. In particular 3,3,3-D, L-trifluoroalanine has been studied as a suicide inhibitor of pyridoxal phosphate dependent enzymes[9-11]. Substitution of alanine by trifluoroalanine can lead to selectively fluorinated enzymes, hormones and other biologically active peptides. Synthetic fluoropeptides have been found to act as proteolytic enzyme inhibitors, while fluorinated hormone analogs have altered reactivity compared to the parent hormones. The potential role of such compounds in pharmacology is being investigated. Selective fluorination has been also used to probe the structures of biological compounds. Because the chemical shift in ^{19}F NMR spectroscopy is sensitive to the chemical environment, the method can be used to detect solvent exposure of specific functional groups and therefore conformations of fluorinated molecules[3].

In the work described herein polypeptides containing trifluoroalanine were prepared by polymerization of D, L-trifluoroalanine NCA (D, L-TFANCA) and by copolymerization of D, L-TFANCA with γ-benzyl glutamate NCA. The effect of fluorination on the surface energy of polypeptide films was examined by means of contact angle measurements. The absolute molecular weights and molecular weight distributions of these novel polypeptides were determined by time of flight laser desorption matrix assisted mass spectrometry (TOF-MALDMS). Knowledge of the exact molecular weights proved to carry information concerning the mechanism of polymerization.

Although polymerization of α-amino acid N-carboxy anhydrides has been used for almost a century for the synthesis of high molecular weight polypeptides, the mechanism of the base initiated polymerization is still not fully elucidated[12-15]. The strongly electron withdrawing trifluoromethyl group in the α position of TFANCA results in reactivity significantly different from that of any other α amino acid NCA. Study of the mechanism

of polymerization of TFANCA should provide insights useful in understanding the mechanism of polymerization of natural amino acid NCAs.

2.2 Experimental Section

2.2.1 Materials and Methods

Methanol, petroleum ether, triethylamine, and aniline were dried by distillation from CaH_2. Tetrahydrofuran (THF) and dioxane used for the polymerizations were dried by distillation from sodium/ benzophenone. All other chemicals were purchased from Aldrich Chemical Co and used without further purification.

Thin layer chromatography (TLC) was effected with Silica Gel 60 F_{254} (Merck) on precoated aluminum plates, spots being visualized by means of a universal UV lamp model 51402 operating at 254 nm, the ninhydrin test and the Cl_2/ toluidine test. Column chromatography was performed with use of Silica Gel 60 (Merck mesh size 230-400).

^1H NMR spectra were recorded on Bruker AC-200 (200 MHz ^1H), Varian XL-200 (200 MHz ^1H) and Varian XL-300 (300 MHz ^1H) Spectrometers using deuterated chloroform, acetone, DMF and DMSO as solvents. ^{13}C NMR spectra were recorded on a Varian XL-300 (75.43 MHz ^{13}C) Spectrometer using deuterated DMSO as solvent. ^{19}F NMR spectra were recorded on a Varian XL-300 (282.28 MHz ^{19}F) Spectrometer using deuterated chloroform, acetone, and DMSO as solvents; trifluoroacetic acid in DMSO-d_6 was used as external reference, its chemical shift being assigned as 0 ppm. Melting point measurements were made on a Fisher-Johns melting point apparatus. Contact angle measurements were done using a Rame-Hart goniometer. X-ray photoelectron spectra were acquired with a Perkin Elmer 5100 instrument; Mg was used as X-ray source.

Time-of-flight matrix assisted laser desorption mass spectra were acquired at Millipore Corporation. The spectrometer, custom built at Millipore Corporation, has a 50

cm flight tube kept at a pressure of 10^{-6} Torr. Ions were formed by laser desorption at 337 nm (N_2 laser) and accelerated by a 30 kV potential. Negative ions were detected. Samples were prepared in a trihydroxyacetophenone matrix; insulin was used for calibration (m/z 5733).

Films for contact angle measurements were cast on glass plates from solutions in DMSO (of concentration ca. 25 mg/ml). The glass substrate was cleaned by treatment with Nochromix® for two days, then washed with distilled water and dried in a vacuum oven. The dilute DMSO solutions were allowed to evaporate slowly and films were finally dried in a vacuum oven. The films were then peeled off the glass plate so that the contact angle measurement was done on the surface that was in contact with the glass. Thin films of oligotrifluoroalanine were cast from dilute DMSO solutions (ca. 10 mg/ml) on microscope slides. The slides were put in petri dishes and the solvent was allowed to evaporate very slowly. Films were finally dried under a nitrogen stream. The advancing contact angles reported in Table 2 are the average of at least 4 measurements.

2.2.2 Preparations

$CF_3CF(OCH_3)COOCH_3$ (1) : Gaseous hexafluoropropylene oxide (50 g, 0.30 mol) was bubbled through 260 mL of dry methanol for two days. Water (700 mL) was added to the alcoholic solution; the lower layer was separated, washed twice with water, dried over Na_2SO_4 and distilled to give 38.7 g (0.20 mol, 68% yield) of (1), b.p. 123°-126° C (lit.[15] 40 °-41 °C/ 21 mm Hg). ^1H NMR ($CDCl_3$) : δ=3.58 ppm (s, 3H, OCH_3); δ=3.90 ppm (s, 3H, $COOCH_3$). ^{19}F NMR ($CDCl_3$): δ=-11.3 ppm (s, CF_3) and δ=-64.8 ppm (s, CF).

$CF_3COCOOCH_3$ (2) : Concentrated sulfuric acid (48 mL), silica gel (4 g), and (1) (37.6 g, 0.19 mol) were placed in a flask fitted with a reflux condenser topped with a calcium chloride drying tube. The mixture was heated to 140 °C in an oil bath and allowed to stand at 135 °-140 °C for 25 minutes. The flask was cooled and connected to a distillation condenser, and the product was distilled at atmospheric pressure. Redistillation gave 18.6 g (0.12 mol, 63% yield) of methyl trifluoropyruvate, b.p. 85 °-87 °C (lit.[15] 84 °-86 °C). ^1H NMR ($CDCl_3$) : δ=4.99 ppm (s, 1H); δ=4.97 ppm (s, 1H). ^{19}F NMR ($CDCl_3$) : δ=-5.0 ppm (s, 1F) and δ=-5.7 ppm (s, 1F).

$(CH_3)_3COCONHC(CF_3)(OH)COOCH_3$ (3) : tert-Butyl carbamate (14.1 g, 0.12 mol) was dissolved in a minimum volume of methylene chloride (ca. 35 mL) and methyl trifluoropyruvate (2) (18.6 g, 0.12 mol). The solution was stirred at room temperature under nitrogen for three days. The precipitate formed was filtered and 26.84 g of (3) (0.098 mol, 82 % yield) were obtained. TLC analysis (ethyl acetate/hexane/acetic acid 9/1/.1) showed one only spot R_f=0.73, m.p. 76°-78° C (lit.[14] 67° C). ^1H NMR (acetone d_6) : δ=1.40 ppm (s, 9H, $OC(CH_3)_3$); δ=3.83 ppm (s, 3H, OCH_3); δ=6.42 ppm (s, broad, NH); δ=7.78 ppm (s, broad, OH). ^{19}F NMR (acetone d_6) : δ=-5.3 ppm.

$(CH_3)_3OCON=C(CF_3)COOCH_3$ (4) : Compound 3 (26.84 g, 0.098mol) was dissolved in 350 mL anhydrous ether. The solution was cooled to 0 °-1 °C. Trifluoroacetic acid anhydride (14 mL, 0.1 mol) and pyridine (16 mL, 0.2 mol) were added under vigorous stirring during a period of 90 min, and the solution was stirred at 0 °-2 °C for 1 more hour. The pyridinium trifluoroacetate was filtered and the solvent was evaporated in a rotary evaporator. The remaining oily product was treated with dry hexane and filtered again. The hexane was evaporated to give 20 g of the crude imine (4)

(0.078 mol, 80% yield), that can be distilled at reduced pressure, b.p. 70 °C/0.6 torr (lit.[14] 49 °C/0.2 torr). ^1H NMR ($CDCl_3$) : δ= 1.61 (s, 10H, $OC(CH_3)_3$), δ= 3.98 ppm (s, 3H, OCH_3) . ^{19}F NMR ($CDCl_3$) : δ=0 ppm.

$(CH_3)_3COCONHC(CN)(CF_3)COOCH_3$ (5) : Trimethylsilylcyanide (12 mL, 0.092 mol) was added dropwise to crude (4) (20 g, 0.078 mol) at 80 °C and the solution was left stirring at that temperature for 1 hour. Excess trimethylsilylcyanide was removed under vacuum. The remaining yellow oily product was dissolved in chloroform, filtered through Kieselgel and washed with chloroform. TLC analysis of the filtrate (hexane/ethyl acetate ; 5/1 ; v/v) showed four spots with R_f values 0, 0.21, 0.33 and 0.39. Evaporation of chloroform gave 20g (0.071mol, 90% yield) of yellow residue. Column chromatography was used in a smaller scale synthesis for the purification of (5). Silica gel was stirred in and the solvent was evaporated under nitrogen. The solid mixture was loaded onto the top of a 3×42 cm column packed with silica gel. The column was eluted with hexane/EtOAc 5/1 mixture. TLC of fractions containing the product (5) of R_f=0.21 revealed traces of impurities of R_f=0.33 non-detectable by NMR. The solvents were evaporated to give (5) as an oily colorless product. ^1H NMR (acetone d_6) : δ=1.41 ppm (s, 9H, $OC(CH_3)_3$), δ=3.98 ppm (s, 3H, OCH_3), δ=8.46 ppm (s, broad, NH). ^{19}F NMR (acetone d_6) : δ=+2.1 ppm.

$H_2NCH(CF_3)COOH$ (6) : Crude (5) (20g, 0.071 mol) was heated to 80 °C for 8 hours with 70 mL concentrated HCl. After cooling to room temperature the mixture was extracted with 200 mL ether. The aqueous phase was evaporated to give 16 g of yellow solid. The solid was suspended in chloroform and treated with excess triethylamine until most of the residue dissolved. The mixture was filtered and (6) was precipitated from the filtrate by addition of glacial acetic acid, collected by filtration, and dried to give 5.7 g

(0.04 mol, 56 % yield) of D, L-trifluoroalanine in the form of a white powder. Recrystallization from water/acetone gave 4.6 g (0.03 mol, 42% yield) of crystalline trifluoroalanine, m.p.>210 °C (d.). ^{1}H NMR (DMSO-d_6): δ=4.1ppm (q,CH), δ=7.0ppm (s, broad, NH_2 & COOH). ^{13}C NMR(DMSO-d_6): δ= 55ppm (CH), δ=124ppm(CF_3), δ= 165ppm (CO). Anal. calcd for $C_3H_4NF_3O_2$: C: 25.18%, H: 2.82%, N: 9.79%, F: 39.84%. Found: C: 24.92%, H: 3.00%, N: 9.79%, F: 39.61%.

TFANCA (7) : D, L-Trifluoroalanine (0.715g, 5 mmol) was suspended in 5 mL dry THF. Triphosgene (0.495g, 5 meq) dissolved in 1 mL dry THF was added dropwise. The mixture was purged with nitrogen periodically to remove excess HCl. The solution became clear after ca. 10 min but a slight cloudiness developed during the course of the reaction. After one hour the solution was filtered and cold dry petroleum ether was added to the filtrate. A small amount of precipitate was filtered off, the filtrate was purged with nitrogen, and most of the solvent was evaporated at reduced pressure. More dry THF was added and reevaporated twice. The anhydride was very soluble in THF but partially crystallized in the form of white needles when most of the solvent was evaporated. After standing in the freezer overnight D, L-TFANCA (3.7 mmol, 75% yield) was obtained by filtration as a light yellow solid. ^{1}H NMR (DMSO-d_6) : δ= 5.6 ppm (q, CH), δ=10.3 ppm (s, NH). ^{13}C NMR (DMSO-d_6): δ= 58.7ppm (q, CH), δ=121.5ppm (q, CF_3), δ=151.4ppm (s, CO-5), δ=162.8ppm (s,CO-2). Anal. calcd for $C_4H_2NF_3O_3$: C: 28.41%, H: 1.19%, N: 8.29%, F: 33.71%. Found: C: 28.39%, H: 1.23%, N: 8.10%, F: 33.6%.

Polymerization of TFANCA

A typical polymerization procedure was as follows. D, L-Trifluoroalanine NCA was dissolved in dry THF under nitrogen at room temperature. Triethylamine was added and

the solution was stirred at room temperature in a round bottom flask closed with a septum. The flask was purged with nitrogen periodically and the polymerization was monitored by infrared spectroscopy . When no more anhydride was detectable, water was added to the mixture. The precipitated product was filtered,washed with water and dried. The results are given in Table 2.1. The same procedure was used for the copolymerizations. D, L-TFANCA and γ-benzyl-L-glutamate NCA were charged to a round bottom flask and dissolved in THF. Thiophenol was added as initiator in an amount corresponding to 1 mol-% of the monomer. The results are given in Table 2.2.

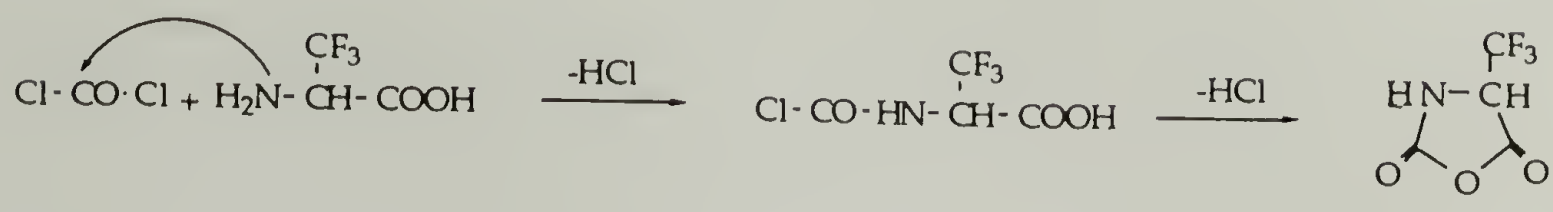
2.3 Results and Discussion

2.3.1 Synthesis and Chemistry of TFANCA

D, L-Trifluoroalanine was conveniently prepared on a 5 g scale via the route shown in Scheme 2.1[16-19]. Solutions of the amino acid proved to be labile; figure 2.1 compares ^1H NMR spectra of DMSO solutions of D, L-trifluoroalanine taken immediately after preparation (figure 2.1a) and after 50 days at room temperature (figure 2.1b). Partial decarboxylation of trifluoroalanine followed by attack of the resulting trifluoroethylamine on the remaining amino acid (Scheme 2.2) accounts completely for the observed spectral changes. D, L-Trifluoroalanine is stable upon storage in the solid state.

D, L-TFANCA was synthesized by phosgenation of the free amino acid in tetrahydrofuran (THF). Solid triphosgene was used as the phosgene source and the procedure described in reference 20 for natural amino acids was followed. The suspension of trifluoroalanine became clear within 15 minutes after addition of triphosgene at room temperature. We have performed the phosgenation of other amino acids and observed (in agreement with reference 20) that heating of the reaction mixture to 50 °C is necessary to drive the reaction to completion. The higher reactivity of

trifluoroalanine was not anticipated, since formation of the anhydride is believed to proceed via the following pathway[12]:



Because the nucleophilicity of the amino group of trifluoroalanine is reduced by the strongly electron withdrawing trifluoromethyl group in the α position, the first step in anhydride formation is expected to be retarded. Three possible explanations for the observed high rate of phosgenation can be provided. First is the possibility that the ring closure is rate determining; in such a case the nucleophilicity of the amine does not affect the overall rate. The second is that the hydrogen chloride produced as byproduct during the phosgenation reaction protonates the amino group of most natural amino acids, rendering them unreactive towards phosgene, while protonation of the less basic trifluoroalanine is reduced. Faster phosgenation would then result from the higher concentration of free amino groups in the case of trifluoroalanine. Daly et al. postulate that poor solubility accounts for the low yields characteristic of the phosgenation of alanine, valine, and leucine. The good solubility of the fluorinated anhydride in THF may also contribute to the faster phosgenation of trifluoroalanine.

The strongly electron withdrawing trifluoromethyl group in the α position was expected to have a significant effect on the reactivity of the functional groups of TFANCA. Indeed the stretching vibrations of the carbonyl groups in the infrared spectrum of TFANCA are shifted by ca. 20 cm^{-1} to higher frequency (to 1875 cm^{-1} and 1800 cm^{-1}) than in common α amino acid NCAs[12], indicative of higher electrophilicity. Figure 2.2 shows the ^1H NMR spectra of L-alanine NCA and D, L-TFANCA. The resonances of both the α-CH (5.7 ppm) and the NH (10.3 ppm) protons of the

fluorinated monomer are shifted more than 1 ppm downfield, owing to the electron withdrawing nature of the trifluoromethyl group.

D, L-TFANCA oligomerizes in DMSO or DMF even in the absence of added initiator. In dilute solutions, oligomerization is detectable 5 hours after the preparation of the solution in DMSO but is much slower in DMF. As shown in figure 2.3, signals due to oligotrifluoroalanine are detected on a time scale of days in solutions of TFANCA in DMF. Traces of water in the above solvents may be responsible for initiation of the polymerization; however, L-alanine NCA is much more stable in the same solvents. Oligomerization is believed to be initiated by deprotonation of D, L-TFANCA to produce activated monomer, which then acts as a nucleophile in attacking a second anhydride *(vide infra)*.

2.3.2 Polymerization of D, L-TFANCA

Preliminary polymerization experiments with TFANCA were performed in dioxane using triethylamine, benzyl amine, triethyl aluminum or sodium methoxide as initiators (initial molar ratios of monomer to initiator of 50-100; room temperature). In all cases the polymerizations were heterogeneous and were characterized by long times (more than 10 days) for complete consumption of the anhydride, as detected by infrared spectroscopy. The molecular weights of the resulting peptides were found to be low, with typical degrees of polymerization around 10 as estimated from ^{1}H NMR spectra and viscosity measurements.

A potential termination reaction limiting the chain lengths attainable in TFANCA polymerization is the formation of hydantoic acid end groups, as in Scheme 2.3 [12]. Since the amide proton of TFANCA is more acidic than that of other amino acid NCAs it is reasonable to postulate that termination reactions through this mechanism would be particularly prominent in the polymerization of the fluorinated anhydride.

In order to suppress the ionization of TFANCA, thiophenol was used as initiator. Thiophenol is a relatively good nucleophile (nucleophilic constant $n_{CH_3I} = 5.70$[21]) but is not basic. Polymerizations of D, L-TFANCA were also performed with aniline which has similar nucleophilicity but higher basicity ($n_{CH_3I} = 5.70$, pK_a of conjugate acid in methanol 4.58[21]), and with triethylamine, which is a good base ($n_{CH_3I} = 6.66$, pK_a of conjugate acid in methanol 10.7[21]). The polymerizations were carried out in dioxane and THF and were monitored by infrared spectroscopy. After complete consumption of the anhydride the products were precipitated in water, washed and dried. The results are shown in Table 2.1.

Time of flight matrix assisted laser desorption mass spectrometry allowed for the absolute determination of the molecular weights of the resulting polypeptides. Polymerizations in THF gave unimodal molecular weight distributions while those in dioxane gave multimodal distributions containing a particularly large fraction of heptamer. Bimodal and trimodal molecular weight distributions have been previously reported for polypeptides prepared by ring opening polymerization[12]. For example poly(L-lysine) obtained using n-butylamine as initiator in DMF showed a bimodal distribution with a first maximum at a degree of polymerization of 7. The use of thiophenol did not result in higher molecular weight products; however, knowledge of the exact molecular weights, as determined by the TOF-MALDMS technique, provided important insight into the nature of the polymerization.

The mass spectrum obtained from the polymer prepared using triethylamine as initiator (run 1, Table 2.1) is shown in figure 2.4. All of the fragments observed have masses that correspond to n trifluoroalanyl repeats (mass 125 per repeat) plus 18 mass units from the hydroxyl and proton chain ends. For example a fragment of mass 1519 corresponds to a dodecamer (12×125 + 18=1518) and the next fragment (mass 1643) is the corresponding tridecapeptide (13×125 + 18=1643). Termination via hydantoic acid formation would result in fragments that contain the elements of an extra CO_2 unit and

therefore have masses increased by 44. For example, a 13 repeat oligopeptide terminated via hydantoic acid formation would have a mass of 1687. Such species are not detected.

Figure 2.5 shows the mass spectrum obtained from the polymer prepared using thiophenol as initiator (run 3, Table 2.1). The large fraction with m/z = 890.5 corresponds to a heptamer (7×125 + 18 = 893). In the lower molecular weight region there are fragments of molar masses that could correspond to polypeptide chains terminated by hydantoic acids. For example the fragment of mass 811 corresponds to a hexamer terminated via hydantoic acid formation (6×125 + 18 + 44 = 812), and the fragment of molar mass 934.9 may be the corresponding heptamer. However all of the fragments in the higher molecular weight region correspond (as in the case of triethylamine as initiator) to n trifluoroalanyl repeats plus hydroxyl and proton chain ends. This observation was unanticipated in view of the fact that thiophenol is expected to initiate polymerization via nucleophilic attack at the C(5) carbonyl of the anhydride. Subsequent chain growth would normally occur via the carbamate or the amine mechanisms of NCA polymerization[12] and in either case thiophenol should be found at the chain end. The molar mass of thiophenol is 110, and chains with thiophenol end groups should therefore be readily distinguished from those with hydroxyl termini. For example, a dodecamer bearing thiophenol has a mass of 1610 (12×125 + 110); the hexadecamer, 2110 (16×125 + 110). Such species are not detected.

The absence of thiophenol end groups was surprising, especially because previous experiments had indicated that thiophenol does indeed attack TFANCA. Figure 2.6 shows ^{1}H NMR spectra of a). thiophenol, b). thiophenol and TFANCA a few minutes after the preparation of the solution and c). same sample after 18 hours. As thiophenol reacts with the NCA the aromatic proton resonances shift downfield from 7.1-7.5 ppm to 7.4-7.7 ppm. After 18 hours no free thiophenol remains. Where are the chains bearing thiophenol end groups? As mentioned above, after complete consumption of the anhydride, water was added to the polymerization mixture and the precipitated

polypeptide was filtered and dried. Figure 2.7 shows the ^{1}H NMR spectra of a). free thiophenol, b). the sample obtained from the supernatant after solvent evaporation and c). the precipitated polypeptide from run 3 (Table 2.1). These spectra show clearly that thiophenol is incorporated predominantly into the fragments that remain in the supernatant and are presumably of lower molecular weight. Integration of the ^{1}H NMR signals shows that the molar ratio of monomer units to bound thiophenol in the precipitate (85% of product) is 123:1, while in the supernatant (15%) this ratio is 14:1. The total ratio of monomeric units to bound thiophenol can be then calculated from the ^{1}H NMR spectra to be 58:1. Since the initial ratio of monomer to initiator was 53:1 (Table 1), there can be no appreciable amount of free thiophenol, either from unreacted initiator or from endgroup hydrolysis. The presence of thiophenol attached essentially only to the lower molecular weight fragments can be explained if the rate of propagation by the amine or carbamate mechanism is slow, such that there is an alternative faster mechanism of propagation. Slow propagation through the amine or carbamate mechanism is of course likely, since the trifluoromethyl group is expected to decrease the nucleophilicity of the amine. This suggestion is in accord with the results of Schierlinger *et al.*, who have reported that treatment of α-amino acid esters with α-trifluoromethylamino acid NCAs leads to high yields of dipeptides, without significant chain extension via homopolymerization of the fluorinated anhydrides[22]. Furthermore, the increased acidity of the amide proton of TFANCA should allow for the formation of activated monomer even in the presence of protic initiators. The reaction of TFANCA with thiophenol is shown in Scheme 2.4. The NMR data show that thiophenol adds to the NCA via nucleophilic attack, but the results of mass spectrometry demand that further propagation must occur predominantly through the activated monomer mechanism. We note that the preparation of high molecular weight polypeptides strictly via an activated monomer mechanism is debated for common NCAs; polycondensation reactions have been also postulated to be taking place in such systems[15,23]. A reduced

rate of polycondensation, owing to the reduced nucleophilicity of the amine, may account for the low molecular weights obtained in the polymerization of TFANCA. In agreement with this hypothesis polymerization of TFANCA in THF at higher temperature (40 °C) lead to an increase in the molecular weight of the product polytrifluoroalanine by a factor of 2 as estimated by ^{1}H NMR spectroscopy. Further increase in the temperature (refluxing THF, 66 °C) caused discoloration of the reaction mixture and no increase in the molecular weight of the product.

2.3.3 Copolymerization

D, L-TFANCA was copolymerized with γ-benzyl-L-glutamate NCA in THF using thiophenol as initiator to give polypeptides of molecular weight 25,000-50,000, as estimated from intrinsic viscosity measurements (Table 2.2) using the Mark-Houwink constants for PBLG[24]. The products are soluble in dichloroacetic acid (in contrast to oligotrifluoroalanine), which indicates that they are indeed copolymers. The content of trifluoroalanine in each copolymer, as estimated from elemental analysis and ^{19}F NMR spectroscopy, is close to that of the feed composition, as required by the high reaction conversion. The detailed structures of the copolypeptides, e.g. the degree of blockiness, are being investigated by solution ^{1}H and ^{13}C NMR spectroscopy.

2.3.4 Surface Structure and Properties

Thin films of the trifluoroalanine homopolymer on glass plates were made by slow evaporation of dilute solutions of the polymer in DMSO. The advancing contact angle of water on these films was 104°, indicating a striking reduction of the surface energy as a consequence of side chain fluorination. The advancing contact angle of water on films of the copolypeptides was also found to increase with increasing incorporation of the

fluorinated monomer (Table 2.2). Fluorination, even at relatively modest levels (ca. one monomer unit in four), is effective in decreasing the surface energy of the film. Two different films were made from the copolymer derived from run 4 to assure the reproducibility of the results, and all values reported in Table 2.2 are means of four measurements. The receding contact angles were found to vary with the time between advancing and receding contact angle measurement but a systematic study of the kinetics of spreading was not done.

X-ray photoelectron spectroscopy (XPS) was used to determine if the fluorinated monomer is preferentially localized at the surface of the copolymer. Films for XPS measurements were prepared by slow evaporation of dilute DMSO solutions on small glass plates (1×1 cm^2) that fit directly in the sample holder of the spectrometer. The atomic compositions of trifluoroalanine homopolymer determined at 15 ° and 75 ° take-off angles were identical and equal to that of the bulk, as expected. The results from the analysis of the copolymer prepared in run 4, Table 2.2 are shown in Table 2.3. The atomic ratio of fluorine to nitrogen at the 15° take-off angle (ca. 10 Å probe depth) is almost twice that of the bulk. This indicates that the surface of the copolymer is significantly enriched in the fluorinated amino acid. At 75 ° take-off angle (ca. 40 Å probe depth) the difference is considerably smaller.

2.3.5 Water absorption measurements

The % per weight of water retained at different humidities by polytrifluoroalanine and commercial polyalanine is plotted in Figure 2.8. Measurements were done similtaneously for the two materials. The samples were weighed in Eppendorf tubes, dried to constant weight and placed in a dessicator above a saturated aqueous solution of the appropriate inorganic salt for the desirable relative humidity[25]. The dessicator was

kept at room temperature. The samples were left to equilibrate to constant weight and then dried again. The cycle was repeated for different humidity. An empty tube was also weighted for reference. The error is of the order of ±1%. In all cases the water retained by the fluorinated material is less than by the non-fluorinated, as expected.

2.4 Conclusions

D,L-Trifluoroalanine N-carboxy anhydride (D,L-TFANCA) was synthesized by phosgenation of 3,3,3-DL-trifluoroalanine in tetrahydrofuran. ^1H Nuclear magnetic resonance and infrared spectra indicate that the electron withdrawing trifluoromethyl group in the α position increases the acidity of the amide proton and the electrophilicity of the carbonyl groups of the anhydride. TFANCA was polymerized with aniline, thiophenol, or triethylamine as initiators. Time of flight matrix assisted laser desorption mass spectrometry was used to determine the absolute molecular weights and molecular weight distributions of the resulting polypeptides. Knowledge of the exact molecular weight confirmed the structural integrity of the products and gave insight into the mechanism of polymerization. D,L-TFANCA was also copolymerized with γ-benzyl glutamate NCA. Incorporation of 20 mol-% D,L-trifluoroalanine into poly(γ-benzyl-L-glutamate) leads to a marked decrease in the surface energy of the polymer as determined by contact angle measurements.

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Table 2.1 Polymerization of TFANCA.

run	[TFANCA] (M)	initiator	solvent	M_0/I_0 (M)	yield %	DP_w[c]
1[a]	0.60	Et_3N	THF	48	70	17
2[a]	0.63	aniline	THF	93	85	19
3[b]	0.65	thiophenol	dioxane	53	85	16
4[b]	0.70	Et_3N	dioxane	56	60	15

[a] Polymerizations in THF were run at room temperature for 14 days. [b] Polymerizations in dioxane were run for 22 days. [c] Weight average degree of polymerization as determined by TOF-MALDS.

Table 2.2 Copolymerization of Trifluoroalanine NCA with γ-Benzyl Glutamate NCA[a].

run	feed ratio[b]	% F[c]	copolymer ratio[d]	yield (%)	[n][e]	contact angle[f] (deg)
1	1:18.4	1.15	1:22	75.5	0.37	64 ± 2
2	1:10.1	2.04	1:12.2	77.7	0.27	66 ± 3
3	1:4.7	4.48	1:5.2	75.1	0.19	72 ± 3
4	1:3.3	5.94	1:3.8	75.0	0.18	84 ± 2

[a] Copolymerizations were run at room temperature for 25 days. [b] Molar ratio of trifluoroalanine NCA:γ-benzyl glutamate NCA. [c] From elemental analysis. [d] Based on elemental analysis for % F. [e] Intrinsic viscosities (dL/g) in dichloroacetic acid at 25.2 °C. [f] Advancing water contact angles on films cast from DMSO. For comparison PBLG = 65 ° and poly(trifluoroalanine) (45.6% F) = 104 °.

Table 2.3 XPS data for the copolymer from run 4 (Table 2.2).

	normalized atomic ratios		
element	bulk[a]	40 Å[b]	10 Å[c]
C	10	9.9	11.7
O	2.7	2.6	2.2
F	0.6	0.85	1.1
N	1	1	1

[a] From elemental analysis. [b] 75° takeoff angle. [c] 15 ° takeoff angle.

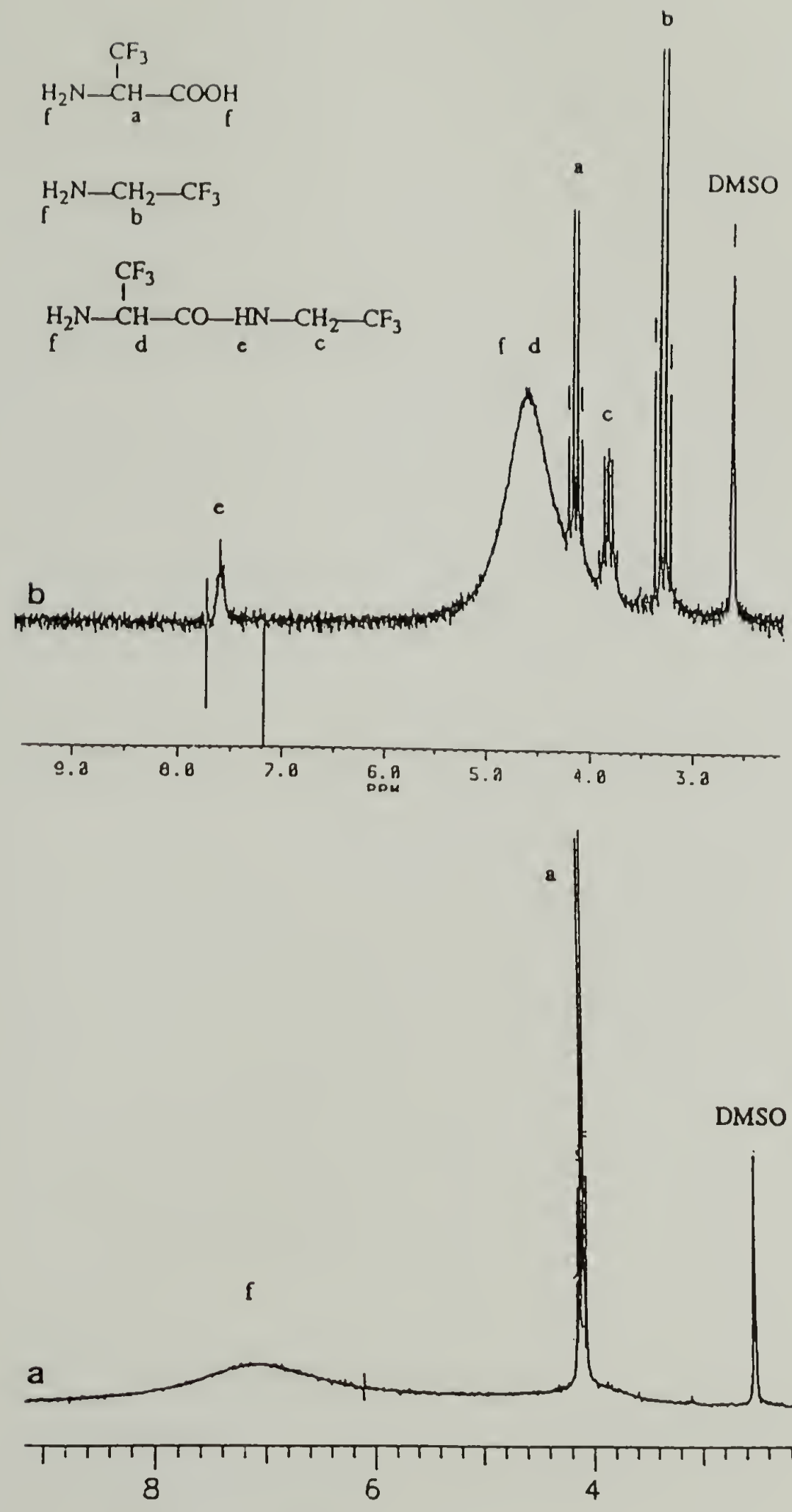


Figure 2.1 Decomposition of trifluoroalanine. (a) 300-MHz ^{1}H NMR spectrum of trifluoroalanine in DMSO-d_6 immediately after the preparation of the solution. (b) 200-MHz ^{1}H NMR spectrum of the same sample after 50 days.

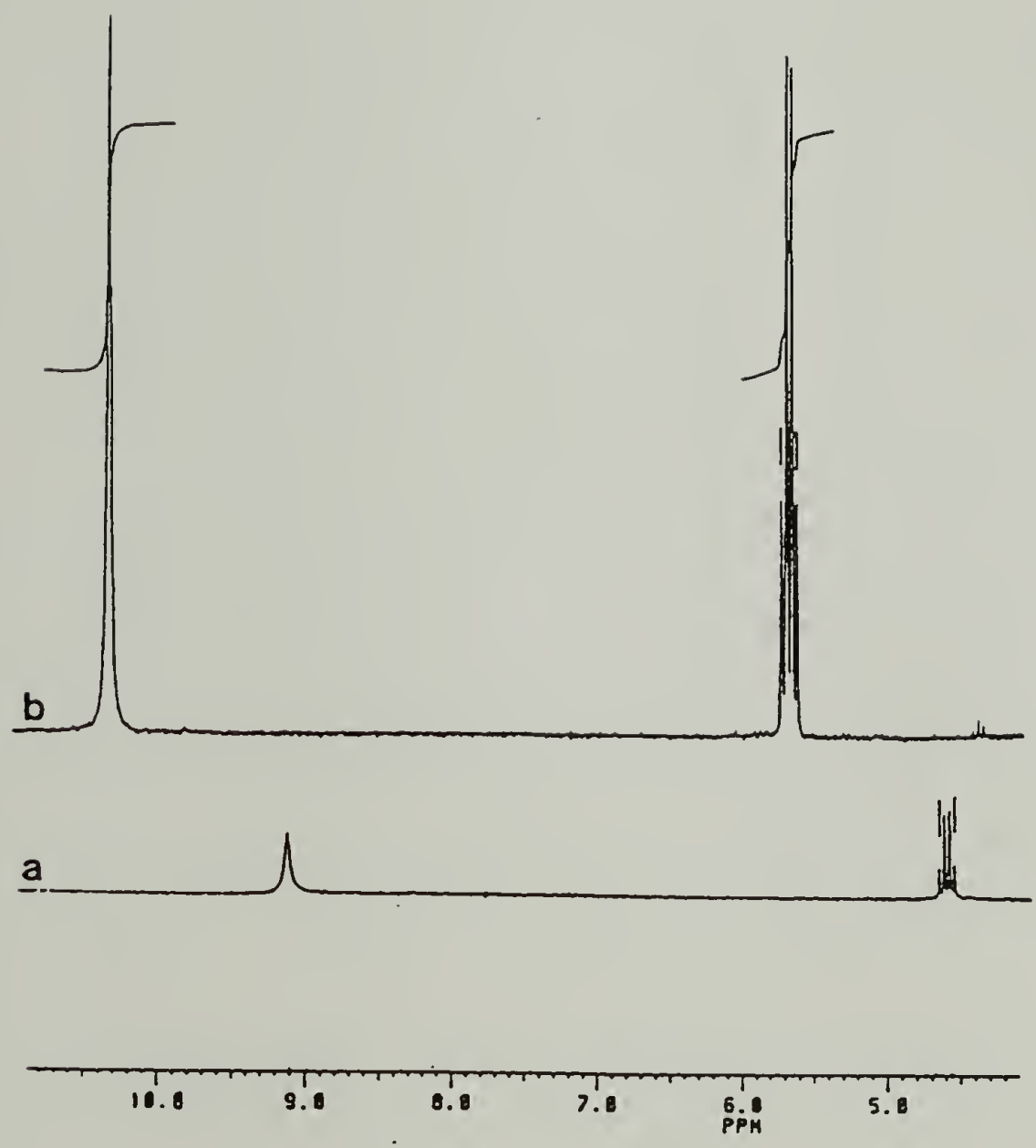


Figure 2.2 200-MHz ^{1}H NMR spectra in DMSO-d_6 of (a) alanine NCA and (b) TFANCA.

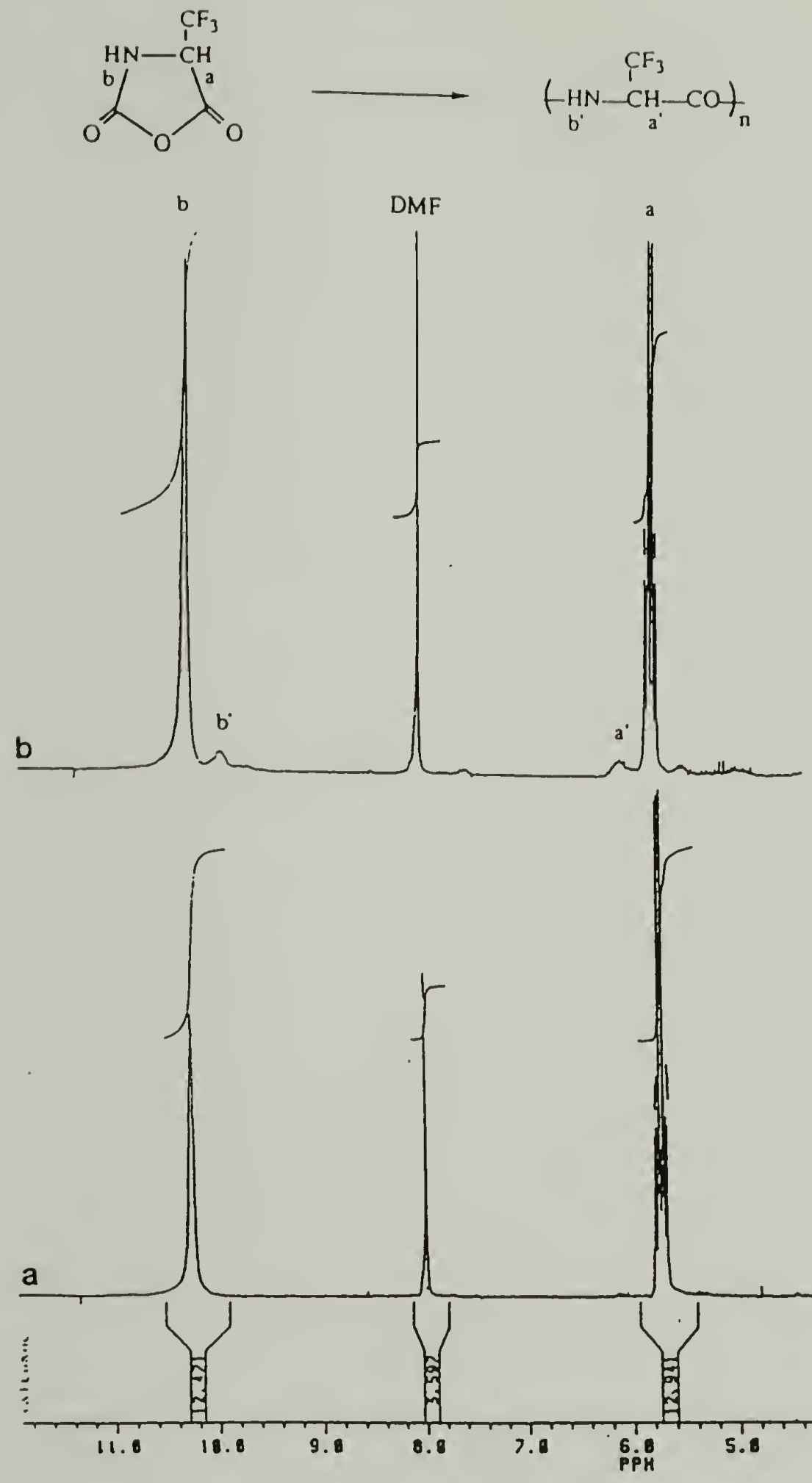


Figure 2.3 200-MHz ^{1}H NMR spetra of trifluoroalanine NCA in DMF-d_7 (a) immediately after the preparation of the solution and (b) 7 days later.

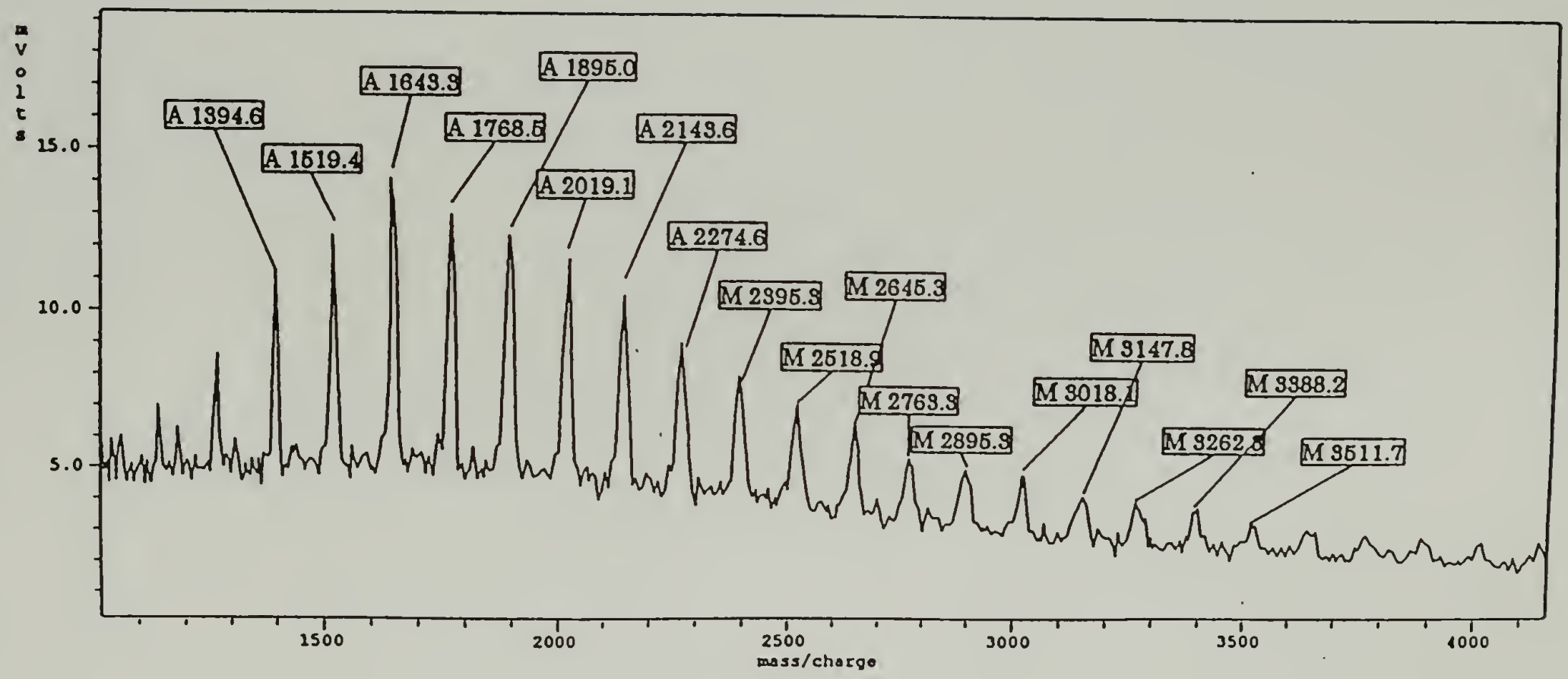


Figure 2.4 TOF-MALDMS of poly(trifluoroalanine) synthesized in THF with triethylamine as the initiator.

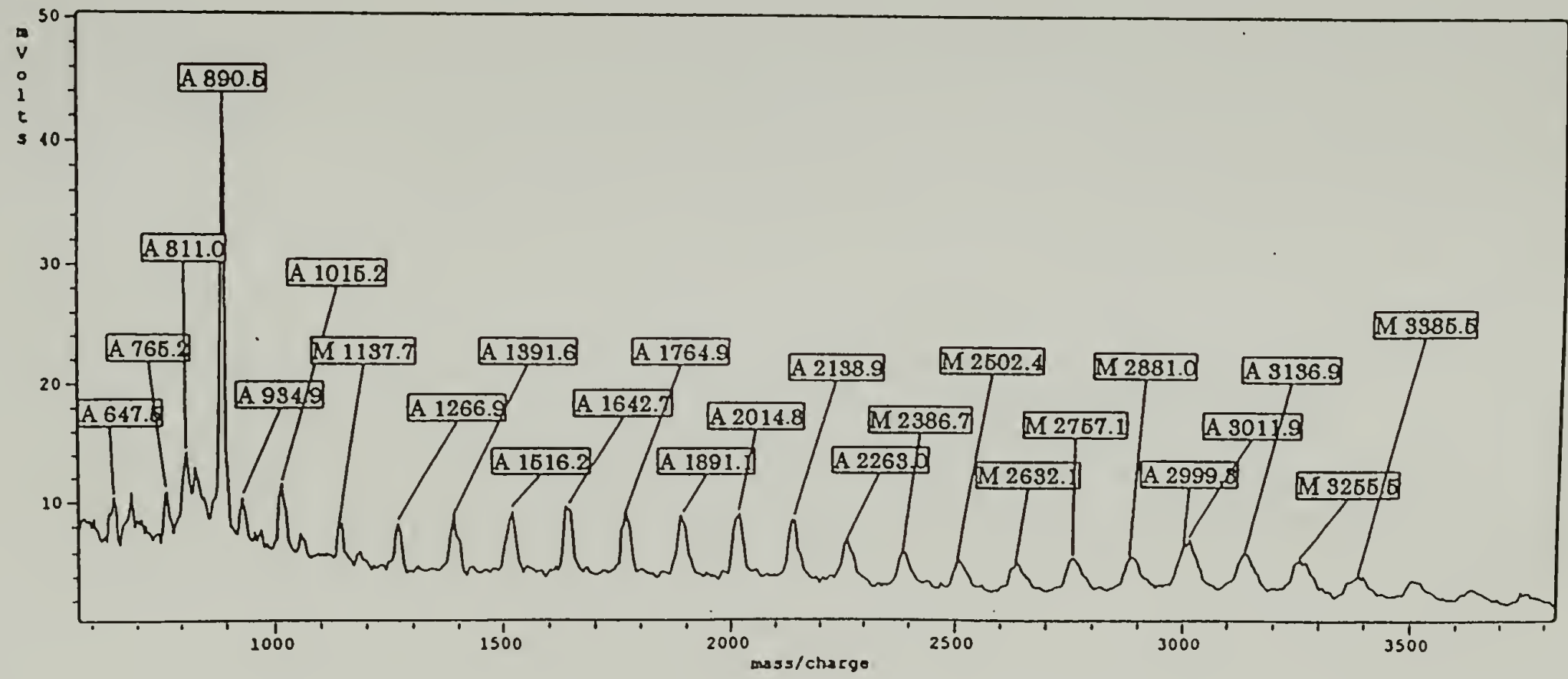


Figure 2.5 TOF-MALDMS of poly(trifluoroalanine) synthesized in dioxane with thiophenol as the initiator.

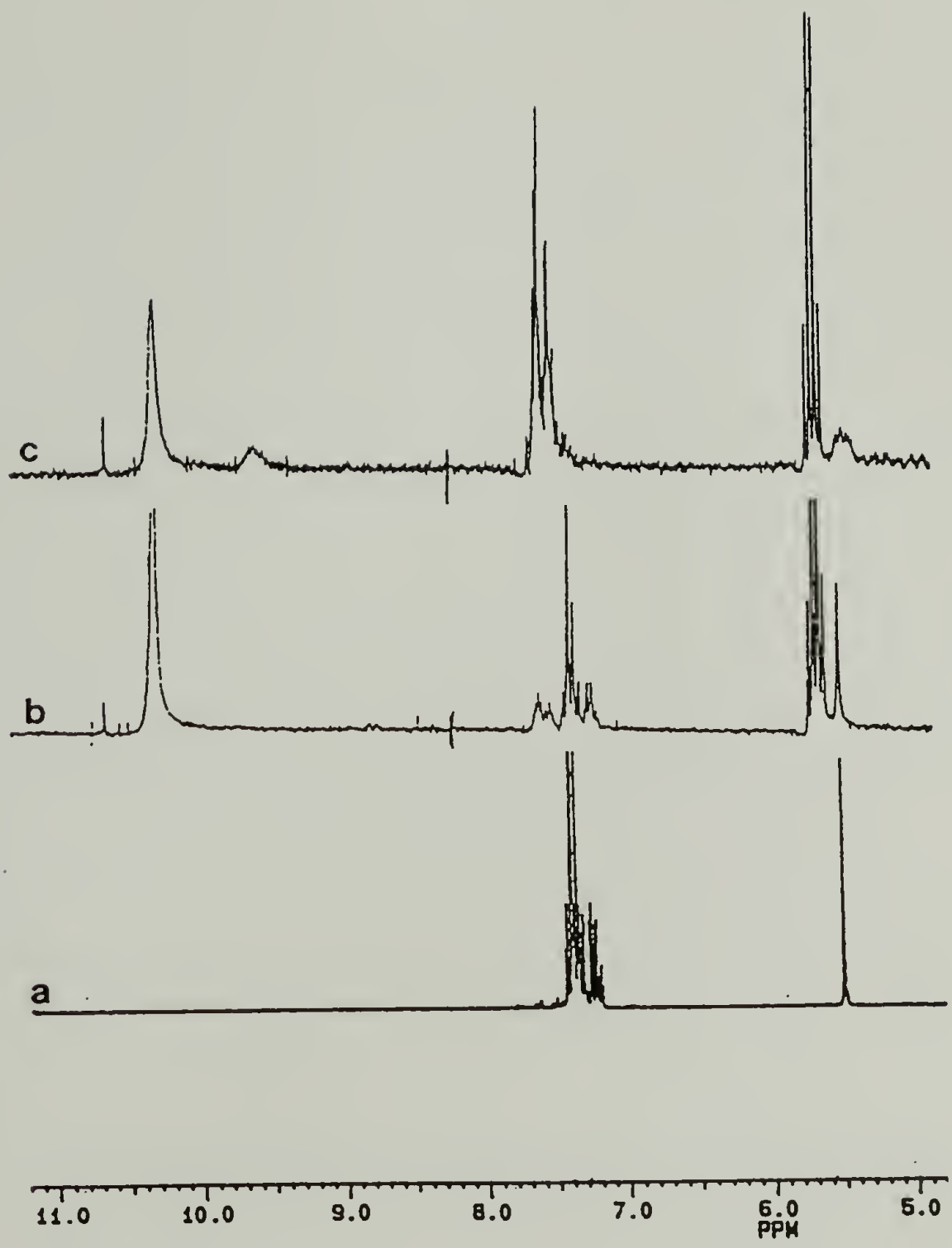


Figure 2.6 200-MHz ^{1}H NMR spetra in DMSO-d_6 of (a) thiophenol, (b) trifluoroalanine NCA and thiophenol a few minutes after the preparation of the sample, and (c) the same sample after 18 hours.

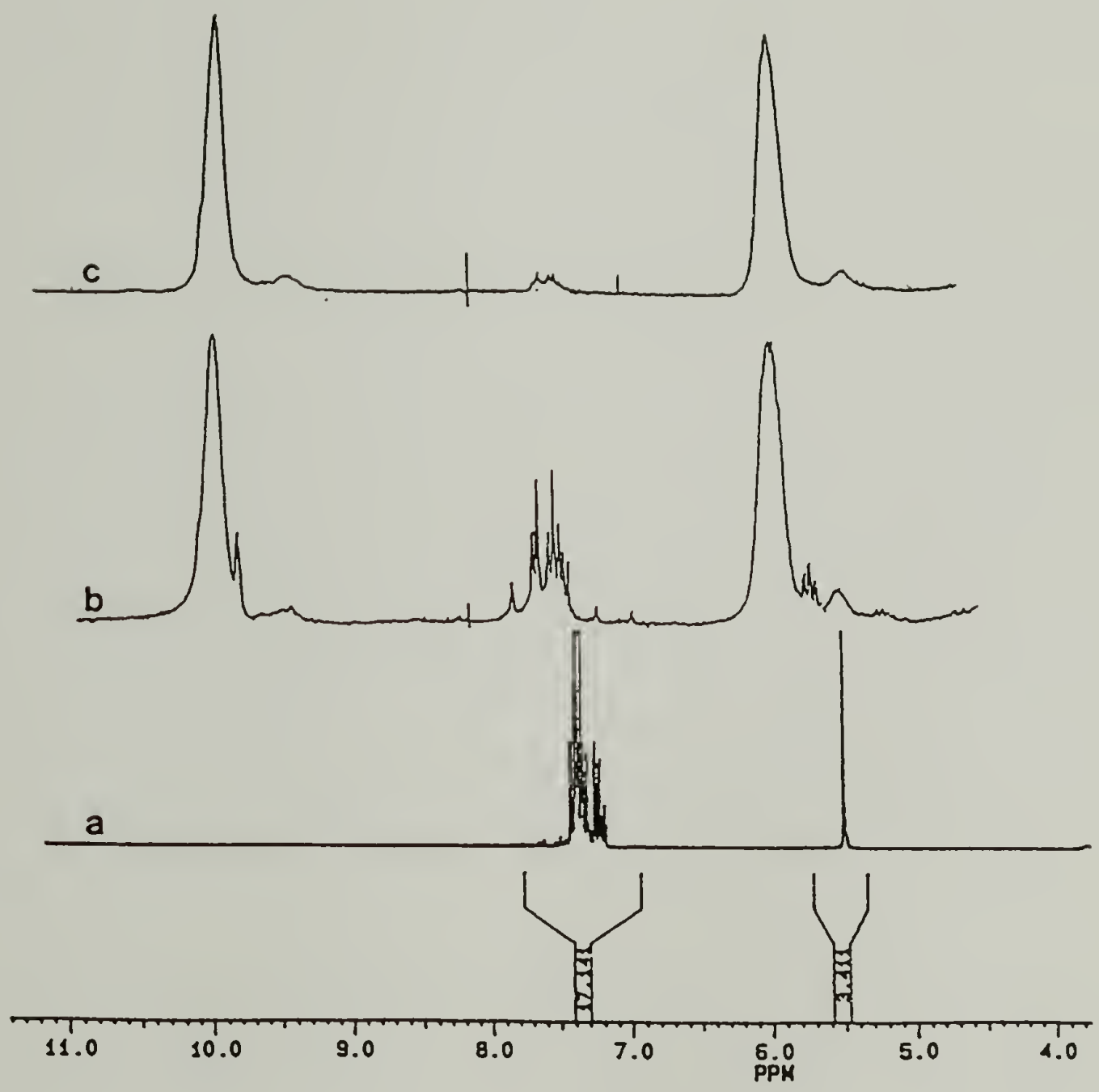


Figure 2.7 200-MHz ^1H NMR spetra in DMSO-d_6 of (a) free thiophenol, (b) the product remaining in the supernatant after precipitation with H_2O, and (c) the precipitated polypeptide.

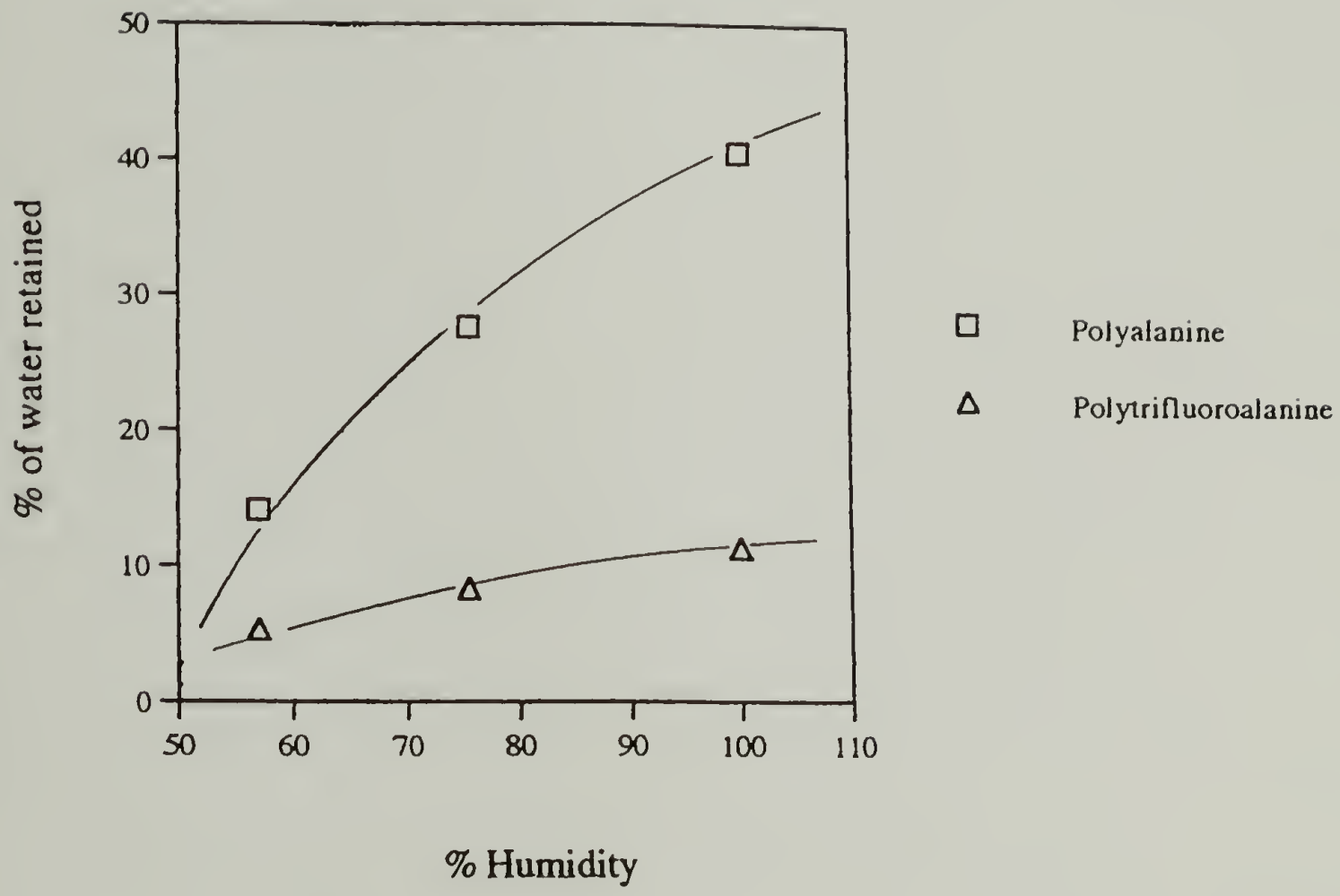
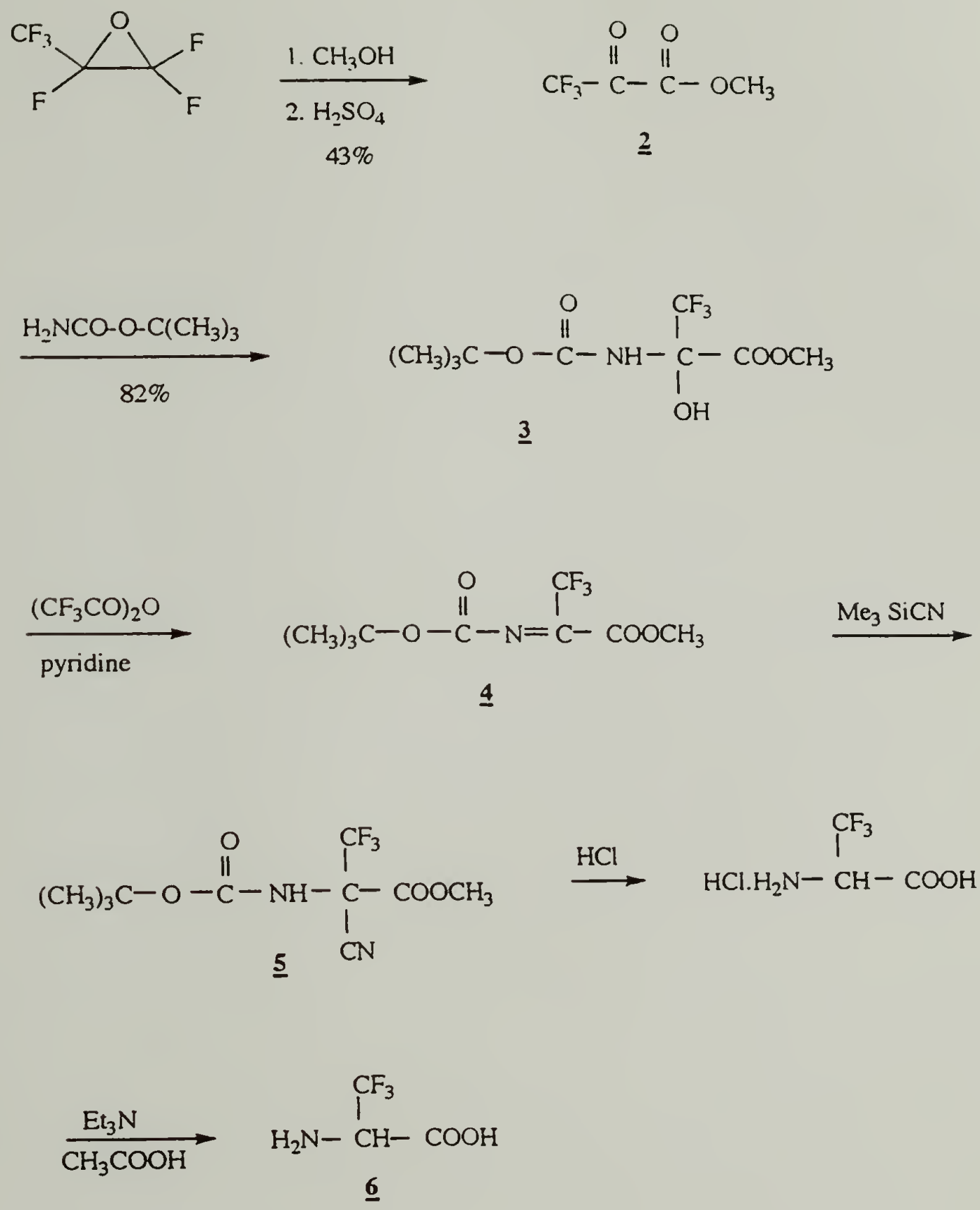
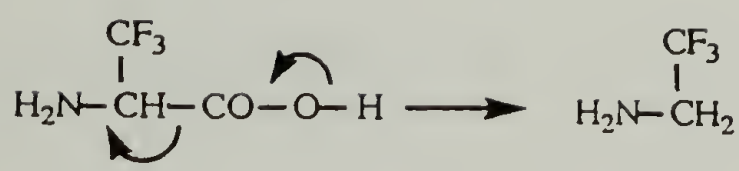


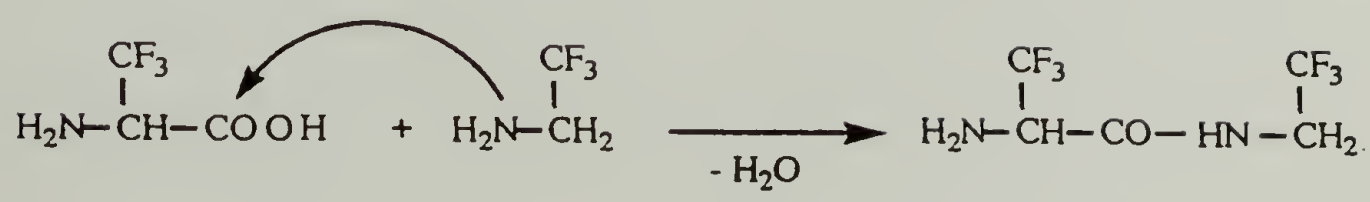
Figure 2.8 Comparison of the % of weight increase of polyalanine and alanine after equilibration at various relative humidities.



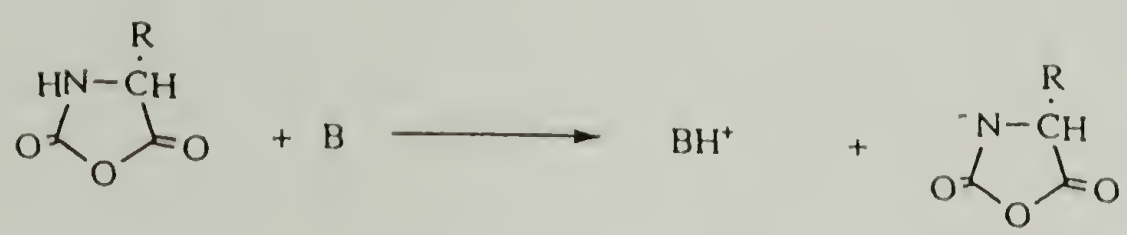
Scheme 2.1 Synthesis of 3,3,3 D,L-trifluoroalanine.

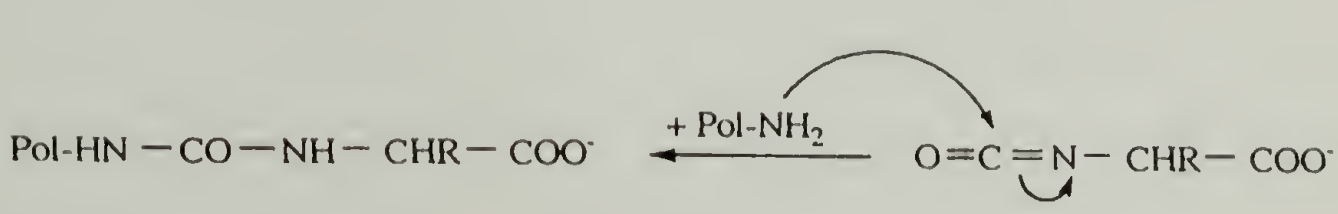
(overall yield of last four reactions 30%)



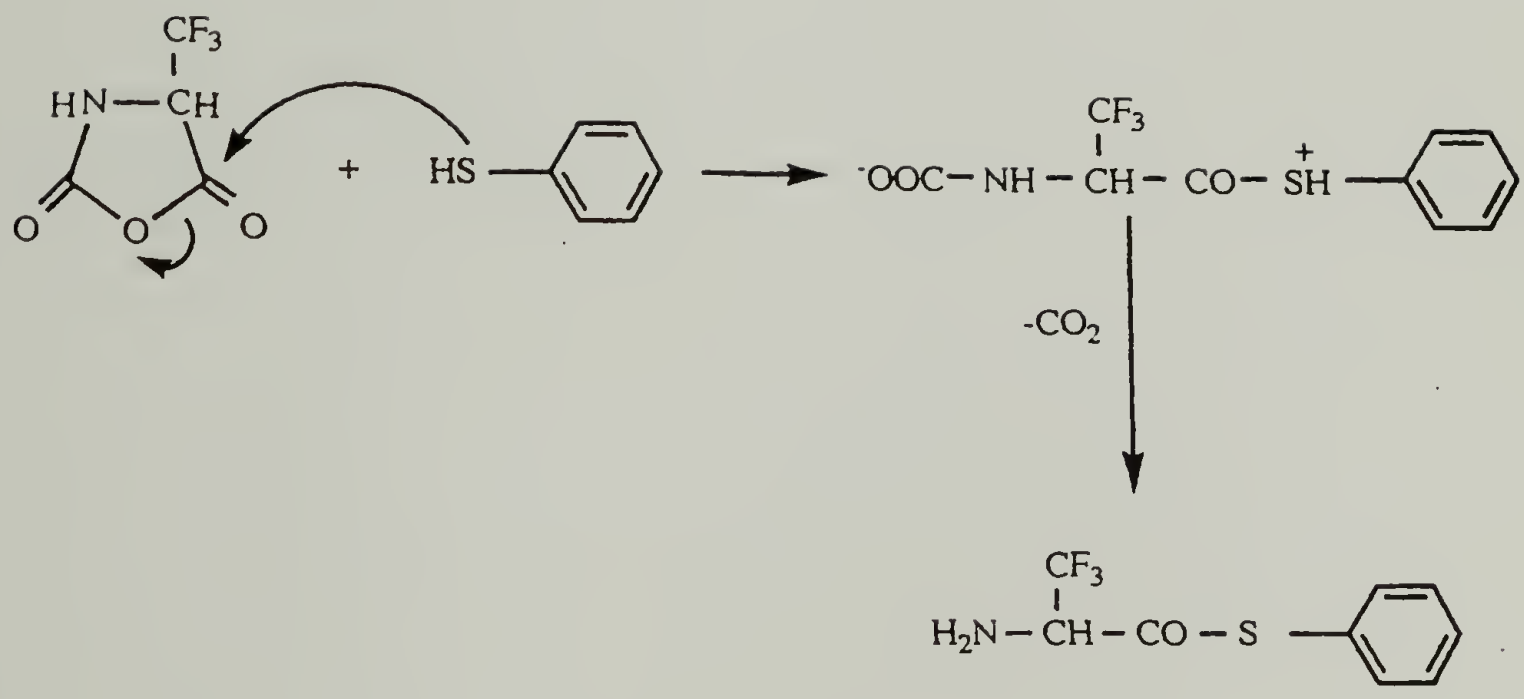


Scheme 2.2 Reaction pathway that can account for the decomposition of trifluoroalanine observed by ^{1}H NMR spectroscopy (Figure 2.1).





Scheme 2.3 Termination via hydantoic acid formation.



Scheme 2.4 Reaction of TFANCA with thiophenol.

CHAPTER 3

FLUORINATED POLYGLUTAMATES

3.1 Introduction

Fluorinated polymers exhibit special properties, including stability at high temperatures, toughness and flexibility at very low temperatures, non-adhesiveness, insolubility, chemical inertness and, in some cases, biocompatibility[1]. Incorporation of fluorinated amino acids has been proposed as a means to impart some of these properties to polypeptides.

The synthesis of polypeptides containing D,L-trifluoroalanine (TFA) has been reported in the previous chapter, and we have found that the surface energy of films of copolypeptides of TFA and γ-benzyl-L-glutamate (BLG) is significantly reduced compared to that of the PBLG homopolymer only when the fluorinated monomer is present at a significant level (> 20%)[2]. In some circumstances, it would be desirable to use fluorinated comonomers that would alter polymer properties even when present in very small amounts. Möller and coworkers have shown that copolymers of styrene with monomers carrying C_4F_9 and C_8F_{17} substituents have very low surface energies, even when only a few percent of the fluorinated comonomer is incorporated[3]. An extremely low critical surface tension of 6 mN/m was found for the homopolymer with the perfluorooctyl side chain. This value is as low as that reported for monolayers of perfluorocarbon acids or thiols[4] and is indicative of a surface composed of closely packed trifluoromethyl groups. Differential scanning calorimetry on the *p*-perfluorodecyl-ethyleneoxymethyl styrene homopolymer showed a first order phase transition at 82°C which was attributed to melting of side chain crystals. It seems likely that in such systems, surface energy is influenced by side chain crystallization, which leads to highly

ordered fluorocarbon surfaces. Similar very low surface energies have been reported for acrylate and methacrylate polymers[4-6], and for polysiloxanes with long fluorinated side chains[7,8]. These results prompted us to undertake the synthesis of polypeptides prepared from γ-esters of glutamic acid with long fluorinated alcohols.

Poly(α,L-glutamate)s have attracted attention both as model polymers for theoretical studies and as products for potential commercial applications[9]. Their most interesting feature is their ability to exist in well defined α-helical conformations independent of the side chain substitution, and to maintain that structure in solution. PBLG is the most thoroughly studied of such polymers, but various poly(γ-alkyl-L-glutamate)s with long paraffinic chains (PALGs) have been of increasing interest, owing to their ability to form thermotropic liquid crystals and ordered Langmuir-Blodgett films[10-14]. The liquid crystalline properties of such polymers are a result of the combination of the rigid rod character imparted by the α-helical structure, and the flexible aliphatic side chains that melt and act as a solvent for the backbone. PALGs with side chains varying from 1 to 18 carbon atoms have been synthesized and their thermal and structural properties have been reported[10]. The α-helical conformation is not affected by the side chains even when the latter are long enough to crystallize. In that case the spatial arrangement of the helices is dictated by the packing of the side chains and as a result the typical hexagonal lattice is replaced by a layered structure[12].

Generally PALGs can be prepared either by polymerization of the corresponding amino acid N-carboxyanhydrides (NCAs) or by ester interchange reactions with PBLG or PMLG[9-10]. The NCA route is more painstaking but it is preferred for the synthesis of homopolymers that are difficult to produce by ester interchange without breaking the polypeptide backbone. Polyglutamates containing fluorinated side chains have been synthesized from PMLG by ester interchange reactions with fluorinated alcohols[15]. The N_2 and O_2 permeabilities of films of these compounds have been measured. We report herein the synthesis of γ-esters of glutamic acid with alcohols of the general formula

$F(CF_2)_n(CH_2)_2OH$, with n=6, 8, and 10, conversion to the corresponding NCAs, polymerization of the NCAs, and copolymerization with γ-benzyl α,L-glutamate NCA. The NCA of the fluorinated ester with n=8 has been reported previously but its characterization has not been described[16]. The effects of side chain length on the structure, crystallinity, and surface properties of the polypeptides. Side chain crystallization, which has been observed for aliphatic side chains longer than 10 carbons, was very pronounced for the fluorinated ester with n=10 as indicated by the x-ray diffraction pattern. The high degree of crystallinity was accompanied by a decrease in the surface energy.

3.2 Experimental Section

3.2.1 Materials

All reagents and solvents used are reported below. The materials were used as received unless otherwise indicated. Letter codes are used to indicate the source of the material.

1H, 1H, 2H, 2H, Perfluorodecanol	(P)
1H, 1H, 2H, 2H, Perfluorododecanol	(P)
1H, 1H, 2H, 2H, Perfluorooctanol	(P)
Chloroform -d_1	(A)
Dichloroacetic acid	(A)
Dimethyl sulfoxide -d_6 gold label	(A)
Ethyl ether	(F)
γ-Benzyl L-glutamate	(F)
Methanol	(F)

Sulfuric acid, 96%	(F)
Tetrahydrofuran, distilled (bp 65-67 °C) over Na/benzophenone	(F)
Triethylamine, distilled (bp 89 °C) over calcium hydride	(A)
Triphosgene	(A)

Aldrich=A; Fisher=F; PCR Incorporated=P

3.2.2 Methods

Proton magnetic resonance spectra were recorded on a Bruker AC-200 (200 MHz ^{1}H) Spectrometer. Chemical shifts are reported as parts per million downfield from tetramethylsilane (TMS). ^{19}F NMR spectra were recorded on a Bruker AC-200 (188.19 MHz ^{19}F) Spectrometer. Chemical shifts are reported as parts per million downfield from trifluoroacetic acid. Melting point measurements were made on a Fisher-Johns melting point apparatus and are reported uncorrected. Films for contact angle measurements were molded on a Model C Carver Laboratory press. The samples were kept at 130 °C (115 °C for C_{12}-FGlu polymers) for 1 minute and then compressed at 1500 psi for an additional minute while at the same temperature. Contact angle measurements with double distilled water were done using a Ramé-Hart goniometer. Five measurements were made on each different film at different spots. Differential scanning calorimetric measurements were performed on Perkin Elmer DSC 7 analyzer. Samples were in powder form. After the first run (25 °C to 200 °C) the samples were quenched to 25°C. The second and third runs (25°C to 250 °C, 40 °C/min) were followed by slow cooling (40 °C/min) to 25 °C. Infrared spectra were obtained on a Perkin Elmer 1600 Fourier Transform Spectrophotometer. Samples were either in the form of film prepared by evaporation of solution on a NaCl plate or in the form of a KBr pellet. Wide angle X-ray diffraction

patterns were obtained on an evacuated flat plate Statton x-ray camera with a Cu-K_α (1.54Å) Ni filtered radiation from a sealed tube anode on film at a camera length of 53 mm. Small angle X-ray patterns were obtained on a Rigaku Denki x-ray camera using a Cu-K_α Ni filtered radiation at a camera length of 230 mm. Circular dichroism spectra were obtained with an Aviv Model 62DS Circular Dichroism Spectrometer in the wavelength range 205 to 240 nm. 1H, 1H, 2H, 2H-Perfluorooctanol was used as solvent. Intrinsic viscosity measurements of solutions of polymers in dichloroacetic acid were performed with a CANNON® 100 Ubbelohde viscometer. A water bath with a Lauda temperature controller was used to regulate the temperature to within 0.1 °C.

Elemental analysis measurements were performed by the Microanalytical Laboratory, Office of Research Services, University of Massachusetts, Amherst, MA 01003.

3.2.3 Preparations

3.2.3.1 Esterification of L-Glutamic acid

A typical esterification procedure has as follows: A 250-mL 3-neck round bottom flask equipped with an addition funnel and a reflux condenser charged with molecular sieves, was charged with L-glutamic acid (13.23 g, 0.09 mol), *tert*-butanol (100 mL), concentrated sulfuric acid (7.8 mL, 0.14 mol), and benzene (18 mL). The mixture was stirred and heated to ca. 70 °C until complete dissolution occurred (typically 20 to 30 minutes). 1H, 1H, 2H, 2H-Perfluorooctanol (39 mL, 0.18 mol) was added dropwise and the solution was stirred and maintained at 65-70 °C for one day. The initially clear solution became cloudy within an hour after the addition of the fluorinated alcohol. The final mixture, which looked like an emulsion, was evaporated to dryness. *tert*-Butanol (50 mL) was added and the product was stirred until completely dissolved. Triethylamine

(7.2 mL, 0.05 mol) was added dropwise to neutralize the excess sulfuric acid, and was followed by the addition of water (22.5 mL) and 95% ethanol (315 mL). Additional triethylamine (27.0 mL, 0.19 mol) was added dropwise. The mixture was stirred for 30 minutes to allow complete precipitation of the ester and then centrifuged until the supernatant became clear. The recovered precipitate was slurried for 20 minutes at 65 °C with 240 mL of water and recentrifuged hot. The supernatant was deccanted and the precipitate was washed with 60 mL each of methanol and 60 ml of ethyl ether, and then dried in a vacuum oven (50 °C) overnight. The crude ester (**I**), obtained in the form of a white powder (24.2 g, 54.4% yield), was recrystallized from an isopropanol/water (2/1) mixture (80°C), washed again with methanol and ether, and dried to give 15.1 g (0.031 mol, 34% yield) of the pure ester. ^{1}H NMR (DMSO-d_6/TFA): δ=2.13ppm (m, 2H, β-CH_2), δ=2.65ppm (m, 4H, γ-CH_2 and -CH_2-CF_2-), δ=4.03ppm (m, 1H, α-CH), δ=4.42ppm (triplet, 2H, O-CH_2), δ=8.33ppm (s, 3H, NH_3^+). Esters (**II**) and (**III**) were obtained at 44% and 32% yields respectively and afforded ^{1}H NMR spectra identical to that of (**I**).

3.2.3.2 Synthesis of N-carboxyanhydrides

A typical procedure for the synthesis of the N-carboxyanhydrides of the fluorinated esters was as follows. A 100 mL 3-neck round bottom flask equipped with a reflux condenser and a dropping funnel was charged with (**I**) (3.93 g, 8 mmol) and dry THF (30 mL). The suspension was stirred and heated to 50 °C. A solution of triphosgene (0.87 g, 8.8 meq) in 10 mL THF was added dropwise and the mixture was stirred under nitrogen for ca. 3 hours. During that time the initial suspension became almost completely clear. The small amount of the remaining precipitate was removed by centrifugation. The supernatant was transferred to a 100 mL round bottom flask, purged with nitrogen and concentrated to ca. half the initial volume. Hexane (ca. 50 mL) was added and the mixture

was placed in the freezer overnight to allow complete precipitation of the anhydride. The product was filtered under nitrogen, washed with hexane and dried to give 3.50 g (84% yield) of crude anhydride in the form of a white powder. Recrystallization from THF/hexane 1/3 mixture gave 2.97 g of (**IV**) (5.7 mmol, 71% yield). ^{1}H NMR (DMSO-d_6): δ=2.15ppm (m, 2H, β-CH_2), δ=2.6-2.9ppm (m, 4H, γ-CH_2 and -CH_2-CF_2-), δ=4.52ppm (triplet, 2H, -OCH_2-), δ=4.67ppm (triplet, 1H, α-CH), δ=9.20ppm (singlet, 1H, NH). Anal. calcd for $C_{14}H_{10}NO_5F_{13}$: C: 32.4%, H: 1.94%, N: 2.70%, F: 47.6%. Found: C: 32.4%, H: 1.73%, N: 2.86%, F: 47.8%.

The N-carboxy anhydrides of (**II**) and (**III**) were prepared according to the same procedure. Their ^{1}H NMR spectra are identical to that of (**IV**). For (**V**): yield 63%, anal. calcd for $C_{16}H_{10}NO_5F_{17}$: C: 31.0%, H: 1.63%, N: 2.26%, F: 52.2%. Found: C: 30.8%, H: 1.35%, N: 2.42%, F: 51.9%. For (**VI**): yield 56% anal. calcd for $C_{18}H_{10}NO_5F_{21}$: C: 30.05%, H: 1.40%, N: 1.95%, F: 55.5%. Found: C: 29.8%, H: 1.14%, N: 2.06%, F: 55.1%.

3.2.3.3 Polymerizations

A typical polymerization procedure was as follows. A 25 mL round bottom flask equipped with a drying tube was charged with (**IV**) (1.036 g, 2 mmol). THF (5 mL) was added via cannula and after dissolution of the anhydride, triethyl amine (2.8 µL, 0.02 mmol) was added. The polymerization was monitored by infrared spectroscopy until complete disappearance of the bands corresponding to the anhydride carbonyl stretching vibrations (1780 and 1855 cm^{-1}). Methanol (ca. 20 mL) was added to the reaction mixture and the precipitate was filtered, washed with methanol and ether, and dried in a vacuum oven (50 °C) overnight. In the case of copolymerizations, both monomers were charged at the onset and the same procedure was followed.

3.3 Results and Discussion

3.3.1 Esterification of L-glutamic acid

γ-Esters of glutamic acid with long fluorinated alcohols were prepared by esterification of glutamic acid under acidic conditions[17] (Scheme 1). Three fluorinated alcohols were used: 1H, 1H, 2H, 2H perfluorooctanol (n=6), 1H, 1H, 2H, 2H perfluorodecanol (n=8), and 1H, 1H, 2H, 2H perfluorododecanol (n=10).

The esterification was initially performed as described for aliphatic alcohols[17]. Free glutamic acid was suspended in tert-butanol, sulfuric acid was added and the suspension was heated to 65°C until complete dissolution of the amino acid. A four-fold excess of the alcohol was added to the clear solution. Although all of the fluorinated alcohols are soluble in tert-butanol, each of the reaction mixtures became heterogeneous, with emulsions appearing ca. 20 minutes after addition of the alcohol. Termination of the reaction after one hour lead to no detectable product and even when the reaction was terminated after one day the yields of fluorinated esters were lower than 10%. Phase separation, which is not observed in esterifications with aliphatic alcohols, may contribute to the low yields. The use of hexafluoroisopropanol as a substitute for tert-butanol did not alleviate the problem.

Since esterification is an equilibrium reaction, the low yields of the esters may reflect the unfavorable equilibrium for reaction of the fluorinated alcohol. Removal of water should shift the equilibrium toward ester formation. In order to remove water efficiently, the reaction flask was equipped with a condenser charged with molecular sieves. The temperature of the reaction was kept at 65-70°C, and a small amount of benzene was added. (tert-Butanol, water and benzene form an azeotrope with a boiling point of 67.3°C). In this way we achieved yields up to 35% after recrystallization even using

stoichiometric amounts of the fluorinated alcohols. The main product of the esterification is the γ-ester, but small amounts (5 to 10 %) of α-ester are also observed by ^{1}H NMR spectroscopy. Recrystallization from isopropanol/water (2:1) results in enrichment in the γ-ester; after two recrystallizations no α-ester was anymore detectable.

The reaction was also performed at higher temperatures and in the absence of tert-butanol. In both cases we found 50-60 % of α-ester and diester byproducts as estimated by ^{1}H NMR spectroscopy. Reaction times longer than one day did not increase the reaction yield.

Evaporation of the solvent before termination by addition of triethylamine appears to be necessary to allow subsequent precipitation of the ester. After the reaction mixture was evaporated to dryness, addition of tert-butanol lead to clear solutions. The excess sulfuric acid was neutralized by addition of triethylamine, and water and ethanol were added. Excess triethylamine was then added to precipitate the free glutamate ester. The precipitate was collected by centrifugation, slurried with water at 65°C for 20 minutes, and recentrifuged hot. This procedure was necessary to remove free glutamic acid. Further washings with methanol and ethyl ether removed any residual alcohol.

3.3.2 Synthesis of N-carboxy anhydrides

Synthesis of the N-carboxy anhydrides (NCAs) of the fluorinated esters was performed in tetrahydrofuran with use of triphosgene[18] (Scheme 2). The amino acid esters are insoluble in THF, but their NCAs are soluble. Solutions of triphosgene in THF were added to suspensions of the amino acids in THF at 50 °C. Phosgenation was most efficient when the amino acid suspensions were heated to 50 °C before addition of triphosgene. (Gelation and formation of an unidentified by-product as the major (80%) reaction product was observed upon addition of triphosgene to the suspension of the amino acid at room temperature and subsequent heating.) The initial suspension becomes

almost clear ca. 2.5 to 3.5 hours after the addition of triphosgene. The NCAs are obtained in high yield (56-71%) after precipitation with hexane and recrysttallization. The phosgenation reaction can be monitored by infrared spectroscopy; as the reaction proceeds the bands at 1780 and 1855 cm^{-1} (which correspond to the anhydride) increase in intensity relative to the ester absorption at 1730 cm^{-1}. Purging with N_2 during the phosgenation is generally recommended for the removal of the excess of HCl[18] but it was avoided in this case because of the surface activity of the fluorinated NCAs, which caused foaming.

3.3.3 Polymerization

Homopolymers and statistical copolymers of **I, II,** and **III** with γ-benzyl L-glutamate were prepared by polymerization of the respective NCAs in THF using triethylamine as initiator (Tables 1-3). All of the polymerizations were monitored by infrared spectroscopy and were terminated (after complete conversion of the NCAs) by addition of methanol. The reaction mixtures became inhomogeneous shortly after addition of the initiator. Infrared spectra taken during the course of the polymerization indicated the initial formation of polypeptides in a ß-sheet conformation. Figure 3.1 shows characteristic infrared spectra recorded during the copolymerization of (**V**) and (**VII**) (run 3). As the copolymerization proceeds, the absorptions at 1852 cm^{-1} and 1783 cm^{-1} assigned to the carbonyl groups of the anhydrides decrease in intensity. Two amide I vibrations of the polypeptide are seen, one at 1624 cm^{-1} assigned to a ß-sheet conformation[19] and one at 1651 cm^{-1} assigned to the α-helical conformation[19] . The percentage of α-helical content increases with time in agreement with the literature[20]. Solid state spectra of the precipitated polypeptide, recorded subsequently, show considerable enrichment in α-helical content as shown in Figure 3.2. Figure 3.3 shows the circular dichroism spectrum of the homopolymer of 1 in 1H, 1H, 2H, 2H

perfluorooctanol (1.2 mg/ml) at 25°C. Secondary structure analysis based on a set of four spectra (100% of alpha helix, beta sheet, beta turn, and random coil respectively) indicated the existance of 29% of α-helical conformation.

Copolymers were prepared to allow determination of the dependence of the surface energy on percent fluorination; thus conversions were high and feed ratios were chosen to give a useful range of fluorine contents. Elemental analysis of the copolypeptides showed that the copolymer compositions were close to the feed compositions (as expected at high conversion) favoring slightly the fluorinated amino acid. On the other hand, since the recovered yields were not 100%, the appearance of enhanced reactivity of (**IV-VI**) may have resulted from preferential precipitation of chains enriched in fluorinated units. The solubility of these copolypeptides in different organic solvents varies with fluorine content. Polymerization products with 5 mole percent of any of the fluorinated comonomers are soluble in the solvents that solubilize PBLG, e.g., THF, DMF and chloroform. This indicates that they are indeed copolymers, since the homopolymers of the fluorinated esters are not soluble in these solvents. The fluorinated homopolymers are soluble in trifluoroacetic acid and hexafluoroisopropanol but not in dichloroacetic acid; the latter is a solvent for the copolymers.

3.3.4 Contact angle measurements

Films for contact angle measurements were prepared by pressing powder samples between Kapton® films at 130 °C (115 °C in the case of C_{12}-FGlu polymers) in a melt press. The samples were kept at 130 °C for 1 minute and then compressed at 1500 psi for an additional minute while at the same temperature. This method proved convenient and effective in the preparation of films with smooth surfaces.

Tables 4-6 list the water advancing and receding contact angles of the various homopolymers and copolymers. The values reported are averages of 5 measurements. All

films show hysteresis comparable but higher than that reported for the water contact angles on a copolymer of tetrafluoroethylene and perfluoropropylene (ca. 25 °)[21]. In general differences between advancing and receding contact angles can be result of surface roughness, heterogeneity, and deformability[21]. Figure 4 shows the dependence of the advancing contact angle on the total fluorine content. Results obtained previously on the polymers of trifluoroalanine are included. In each case, the contact angle increases significantly between 0 and 10% fluorine and levels off. The surface energy correlates better with the total fluorine content than with the molar percent of fluorinated monomer. The C_{12}-FGlu homopolypeptide shows an exceptionally high contact angle. The result was verified by five measurements in two different films. Zisman has demonstrated that the surface energy depends on both the constitution and structure of the surface and that the lowest surface energy is that of closely packed trifluoromethyl groups[4,22]. Indicative of the effect of packing in lowering the surface energy is the fact that the water contact angle on Teflon is 108°[8] while on self-assembled perfluorinated alkanethiol monolayers is 118°[23] and on poly(1H, 1H-pentadecafluorooctyl methacrylate) 120°[24]. The contact angle for the C_{12}-FGlu homopolymer indicates a highly ordered fluorocarbon surface that can result from side chain crystallization. Side chain crystallization has been further verified by X-ray scattering and differential scanning calorimetry as discussed below. It is interesting that Möller and coworkers also find very low surface energy for the fluorinated homopolymer when side chain crystallization takes place. However, in their work γ_s^D varies linearly with fluorine content and is not further affected by side chain crystallization as shown in Figure 3.5[3].

3.3.5 X-Ray Diffraction

Figure 5 shows X-ray diffraction patterns for the C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu homopolymers. Two points are obvious from the patterns and the spacings listed in Table

7. First, the low angle reflections can be attributed to the packing array of the α-helices and increase with increasing side chain length as expected. Second, in the wide angle region all three homopolymers show an intense diffraction ring that corresponds to ca. 5 Å spacing. There is a dramatic increase in the sharpness of that reflection with increase in the side chain length. Two more rings are observed at higher diffraction angles in the case of the C_{12}-FGlu homopolymer. The three signals index on a hexagonal lattice of side $a = 5.7$ Å and are believed to arise from packing of the fluorinated segments of the side chains. Polytetrafluoroethylene can crystallize in a hexagonal lattice of side $a = 5.66$ Å[25-27]. The sharpnening of the wide angle diffraction rings that is indicative of good packing of the side chains in the case of homopolymer 3 is coincident with the appearence of (*h*00) reflections in the low angle region that are characteristic of a layered structure. The same behavior has been reported by Watanabe and coworkers for PALGs with side chains of 14 methylene units or longer and the model shown in Figure 6 was proposed to explain both the development of good side chain crystals and the layered arrangement of the helices inferred from the low angle pattern[12]. For PALGs with side chains shorter than 14 methylene units the helices pack on hexagonal lattices but with increase in side chain length the overall structure is dictated by the crystallization of the aliphatic chains. As shown in Figure 6 the side chains are interdigitated. This is not directly observed but it is proposed because the distance between neighboring helices (spacing that corresponds to the 100 reflection) is shorter than two fully extended side chains. In the case of homopolymer 3 that spacing is 43 Å and can be reasonably assigned to the distance between two layers of helices if the side chains are fully extended and not interdigitated as shown in Figure 7. The diameter of poly (γ-methyl-L-glutamate) is considered about 14 Å[14]. Our structure has an additional methylene group (1.25 Å)[28] and 10 perfluorinated side chain carbon atoms (10×1.3 Å)[25]. So the diameter of helices bearing fully extended chains should be ca. 42.5 Å. The lack of interdigitation of the

fluorinated side chains could be due to their increased bulkiness, compared to the hydrocarbon chains, that could prevent them from interdigitating.

For homopolymer 2 the distance between neighboring helices is 36.4 Å. The difference between the interlayer distances of homopolymers 2 and 3 is 6.6 Å, a little bit larger than the difference corresponding to 4 CF_2 units ($4 \times 1.3 = 5.2$). This is expected since from the wide angle reflections the side chains in homopolymer 2 seem to have packed more loosely and are probably not fully extended.

The low angle reflections in homopolymer 1 can be indexed on a hexagonal lattice with $a = 18.9$ Å. The hydrogenated polyglutamate with 8 methylene units long side chain crystallizes in a hexagonal lattice with $a = 17.1$ Å. The slight increase in distance can be explained as a result of the increased bulkiness of the fluorocarbon chain. The side chains cannot form extended crystals and therefore the wide angle reflection is much broader.

The spacings from the X-ray diffraction patterns of the copolymers from runs 3 in Tables 1 and 2 (roughly 50/50) are listed in Table 8. The reflections can be indexed on tetragonal unit cells. Tetragonal packing has been observed for copolymers of γ-hexyl and γ-methyl glutamate of 30-70% constitution[11] although the corresponding homopolymers pack in a hexagonal configuration.

3.3.6 Thermal Analysis

Figure 3.9 shows the DSC heating traces of C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu homopolymers. C_{10}-FGlu and C_{12}-FGlu homopolymers show a first order transitions at 96.3 °C and 137.4 °C, respectively; no corresponding transition is observed in C_8-FGlu homopolymer. These transitions are assigned to melting of side chain crystals, consistent with the X-ray data on the C_{10}-FGlu and C_{12}-FGlu homopolymers. Upon cooling (40 °C/min) a crystallization exotherm is observed at 74 °C and 123 °C respectively. The transition enthalpy of the C_{12}-FGlu and C_{10}-FGlu homopolymers are 6.75 kJ/mol and

1.50 kJ/mol (3rd run). Möller et al. report the melting of pure $C_{12}F_{26}$ at 72 °C associated with a heat of melting of 21 kJ/mol that corresponds to 1.75 kJ/CF_2[29]. Adopting this value the number of 'crystalline' CF_2 units in the case of the C_{12}-FGlu homopolymer can be calculated to be 3.9 (6.75/1.75) and in the case of C_{10}-FGlu homopolymer 0.9. The corresponding values for the hydrogenated analogs have been calculated by Watanabe et al. to be 0.4, 1.4, 3.0, 5.9, and 7.7 for the 10, 12, 14, 16, and 18 methylene unit long side chains respectively[12].

3.4 Conclusions

Polyglutamates carrying long fluorinated side chains have been synthesized by polymerization of the corresponding amino acid N-carboxy anhydride. The surface energies of the fluorinated polypeptides were found to vary with total fluorine content, independent of the length of the side chain. The very high contact angle (121 °) measured for the C_{12}-FGlu homopolymer was attributed to the formation of a surface of closely packed trifluoromethyl groups. This explanation is consistent with X-ray scattering results, which indicate well developed side chain crystallinity. Crystallization of the fluorinated segments controls the spatial arrangement of the helices, which organize into layered arrays.

3.5 References

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Table 3.1 Copolymerization of C_8-FGlu NCA (**IV**) and BzGlu NCA (**VII**).

Run	**IV** (g)	**VII** (g)	C_8-FGlu Mol % (feed)	C_8-FGlu Mol % (polymer)[a]	[n] (dl/g)	% Yield
1	1.036	0	100	100[b]		87.6
2	0.509	0.258	50.0	52.4[c]	0.07	85.2
3	0.271	0.528	20.6	21.9[d]	0.08	81.4
4	0.260	0.921	12.5	13.4[e]	0.10	60.7
5	0.138	1.263	5.3	5.7[f]	0.17	84.8

[a] Copolymer compositions were estimated on the basis of elemental analysis. Fluorine contents were determined by Schöniger flask decomposition of the compound in an oxygen atmosphere. The resulting F^- was analyzed using Ion Selective Electrode techniques. [b] Anal calcd C: 32.8%, H: 2.12%, N: 2.95%, F: 52.0%. Found: C: 32.0%, H: 2.20%, N: 2.98%, F: 51.9%. [c] Anal calcd C: 42.6%, H: 3.26%, N: 3.96%, F: 36.6%. Found: C: 42.0%, H: 3.39%, N: 3.76%, F: 36.5%. [d] Anal calcd C: 53.3%, H: 4.52%, N: 5.09%, F: 19.6%. Found: C: 52.9%, H: 4.44%, N: 5.02%, F: 19.4%. [e] Anal calcd C: 57.5%, H: 5.01%, N: 5.52%, F: 13.1%. Found: C: 57.1%, H: 5.03%, N: 5.41%, F: 12.1%. [f] Anal calcd C: 61.9%, H: 5.53%, N: 5.99%, F: 6.0%. Found: C: 61.9%, H: 5.57%, N: 6.93%, F: 5.5%.

Table 3.2 Copolymerization of C_{10}-FGlu NCA(**V**) and BzGlu NCA (**VII**).

Run	**V** (g)	**VII** (g)	C_{10}-FGlu Mol % (feed)	C_{10}-FGlu Mol % (polymer)[a]	[n] (dl/g)	% Yield
1	0.423	0	100	100[b]		68.7
2	0.615	0.277	48.6	54.5[c]		62.3
3	0.314	0.556	19.4	19.5[d]	0.09	76.8
4	0.315	1.170	10.3	10.3[e]	0.13	86.8
5	0.155	1.250	5.0	5.9[f]	0.15	84.5

[a] Copolymer compositions were estimated on the basis of elemental analysis. Fluorine contents were determined by Schöniger flask decomposition of the compound in an oxygen atmosphere. The resulting F^- was analyzed using Ion Selective Electrode techniques. [b] Anal calcd C: 31.3%, H: 1.75%, N: 2.44%, F: 56.1%. Found: C: 30.4%, H: 1.72%, N: 2.61%, F: 52.2%. [c] Anal calcd C: 39.6%, H: 2.77%, N: 3.39%, F: 42.6%. Found: C: 39.6%, H: 2.73%, N: 3.20%, F: 41.5%. [d] Anal calcd C: 52.4%, H: 4.34%, N: 4.85%, F: 21.8%. Found: C: 51.8%, H: 4.10%, N: 4.56%, F: 21.0%. [e] Anal calcd C: 57.8%, H: 5.00%, N: 5.48%, F: 13.0%. Found: C: 57.3%, H: 5.08%, N: 5.51%, F: 12.7%. [f] Anal calcd C: 60.9%, H: 5.38%, N: 5.83%, F: 8.0%. Found: C: 60.9%, H: 5.46%, N: 5.79%, F: 7.5%.

Table 3.3 Copolymerization of C_{12}-FGlu NCA (**VI**) and BzGlu NCA(**VII**).

Run	**VI** (g)	**VII** (g)	C_{12}-FGlu Mol % (feed)	C_{12}-FGlu Mol % (polymer)[a]	[n] (dl/g)	% Yield
1	0.423	0	100	100[b]		81.4
2	0.196	0.754	8.7	8.7[c]	0.14	83.8

[a] Copolymer composition was estimated on the basis of elemental analysis. Fluorine contents were determined by Schöniger flask decomposition of the compound in an oxygen atmosphere. The resulting F^- was analyzed using Ion Selective Electrode techniques. [b] Anal calcd C: 30.4%, H: 1.49%, N: 2.07%, F: 59.1%. Found: C: 29.5%, H: 1.41%, N: 2.42%, F: 58.2%. [c] Anal calcd C: 57.4%, H: 4.92%, N: 5.37%, F: 13.9%. Found: C: 57.0%, H: 4.77%, N: 5.37%, F: 12.9%.

Table 3.4 Contact angles of water on films of copolymers of C_8-FGlu and BzGlu[a].

Run	C_8-FGlu Mol %	% F	Contact angle (°) advancing	receding
1	100	51.9	107±2	90±3
2	52	36.5	101±10	66±9
3	22	19.4	88±3	54±7
4	13	12.1	90±5	56±7
5	6	5.5	73±7	55±5

[a] Contact angle on PBLG θ_{adv}= 71±4, θ_{rec}= 56±4.

Table 3.5 Contact angles of water on films of copolymers of C_{10}-FGlu and BzGlu[a].

Run	C_{10}-FGlu Mol %	% F	Contact angle (°) advancing	receding
1	100	52.2	107±4	72±10
2	55	41.5	101±2	72±4
3	19	21.0	89±2	64±10
4	10	12.7	90±4	60±7
5	6	7.5	91±2	56±3

[a] Contact angle on PBLG θ_{adv}= 71±4, θ_{rec}= 56±4.

Table 3.6 Contact angles of water on films of copolymers of C_{12}-FGlu and BzGlu[a].

Run	C_{12}-FGlu Mol %	% F	Contact angle (°) advancing	receding
1	100	58.2	121±3	89±2
2	9	12.9	87±6	58±6

[a] Contact angle on PBLG θ_{adv}= 71±4, θ_{rec}= 56±4.

Table 3.7 X-ray spacings for C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu homopolymers.

C_8-FGlu homopolymer	C_{10}-FGlu homopolymer	C_{12}-FGlu homopolymer
	low-angle region	
16.4 s (10)	36.4 s (100)	43.0 vs (100)
9.3 m (11)	18.0 s (200)	21.5 s (200)
	12.2 s (300)	14.3 s (300)
	9.0 vw (400)	10.8 vw (400)
	7.2 w (500)	8.5 w (500)
		7.2 w (600)
	wide-angle region	
5.1 s, broad	5.1 s	4.9 vs (10)
		2.8 w (11)
		2.5 w (20)

Table 3.8 X-ray spacings for copolymers of roughly 50/50 composition.

C_8-FGlu Copolymer (run 2, Table 1)	C_{10}-FGlu Copolymer (run 2, Table 2)
17.2 s (100)	18.6 s (100)
12.4 m (110)	13.3 m (110)
8.6 w (200)	10.2 vw (200)

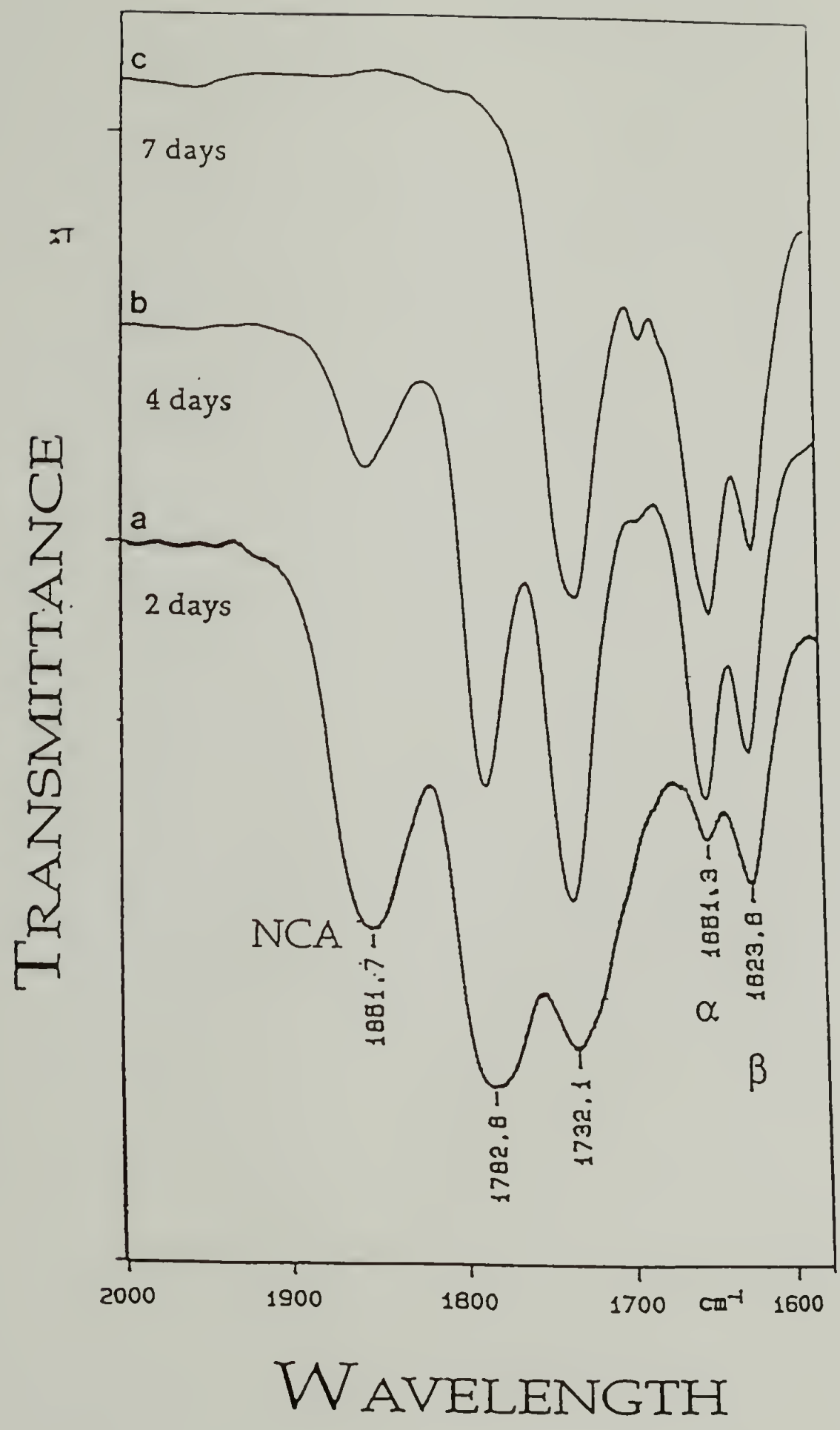


Figure 3.1 IR spectra recorded during the copolymerization of C_{10}-FGlu NCA with BzGlu NCA a. 2 days, b. 4 days, and c. 7 days after the addition of initiator. Samples were in the form of film prepared by evaporation of a reaction aliquot on a NaCl plate.

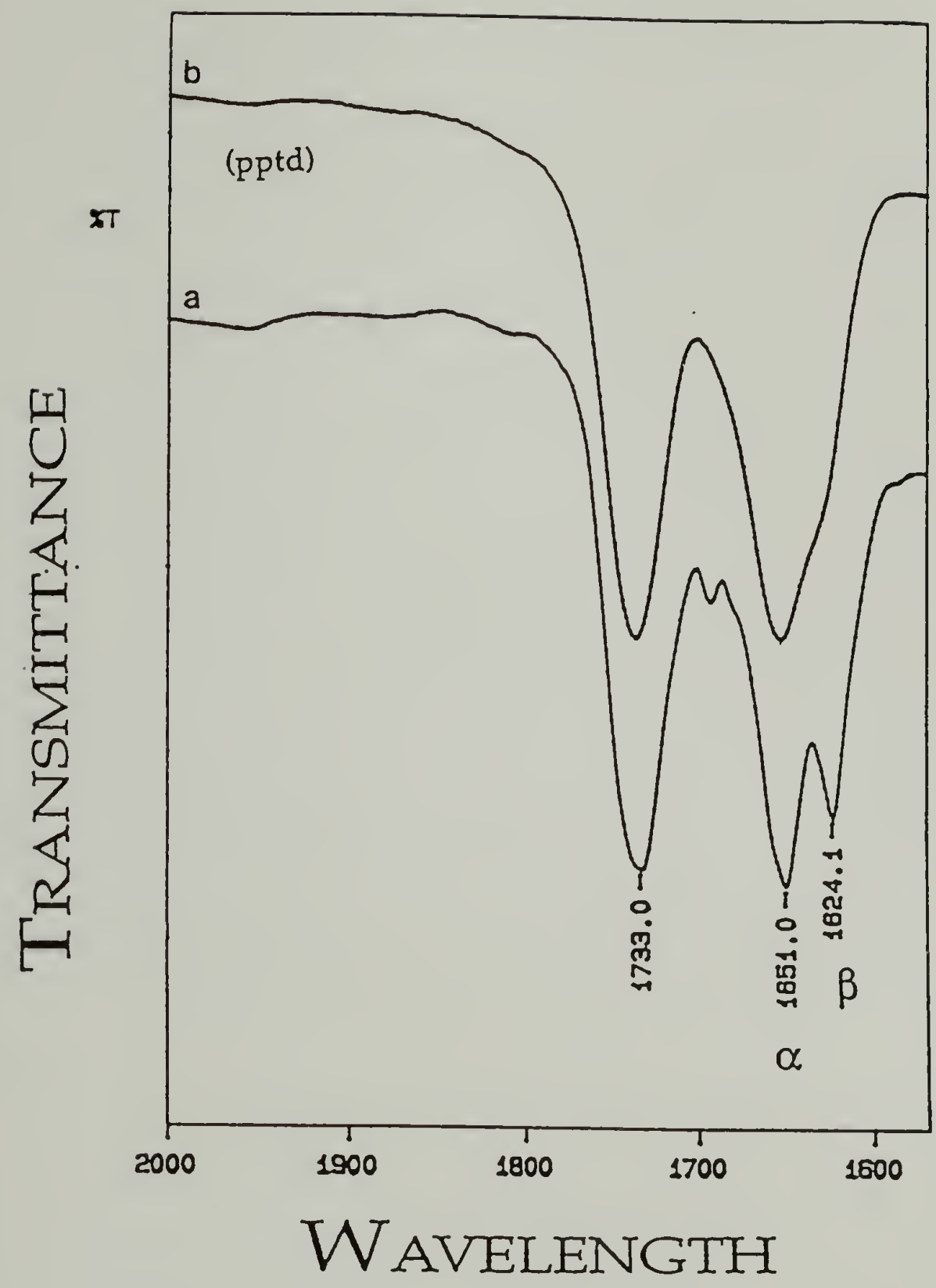


Figure 3.2 IR spectra recorded (a) during the copolymerization of C_{10}-FGlu NCA with BzGlu NCA, 7 days after the addition of initiator (film on NaCl plate), and (b) after precipitation of the copolypeptide (KBr pellet).

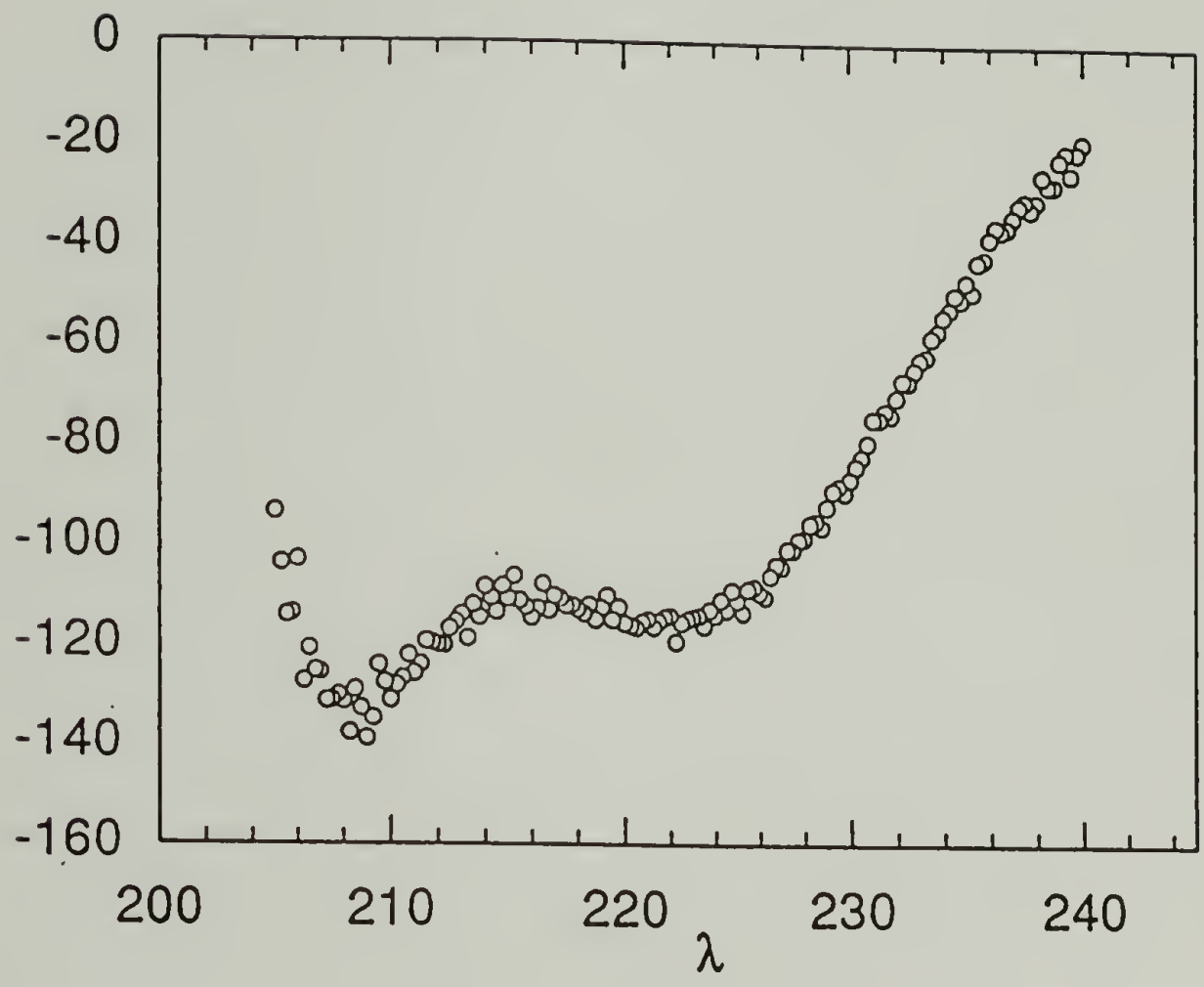


Figure 3.3 Circular dichroism spectrum of C_8-FGlu homopolymer in 1H, 1H, 2H, 2H, perfluorooctanol (1.2 mg/mL) at 25 °C.

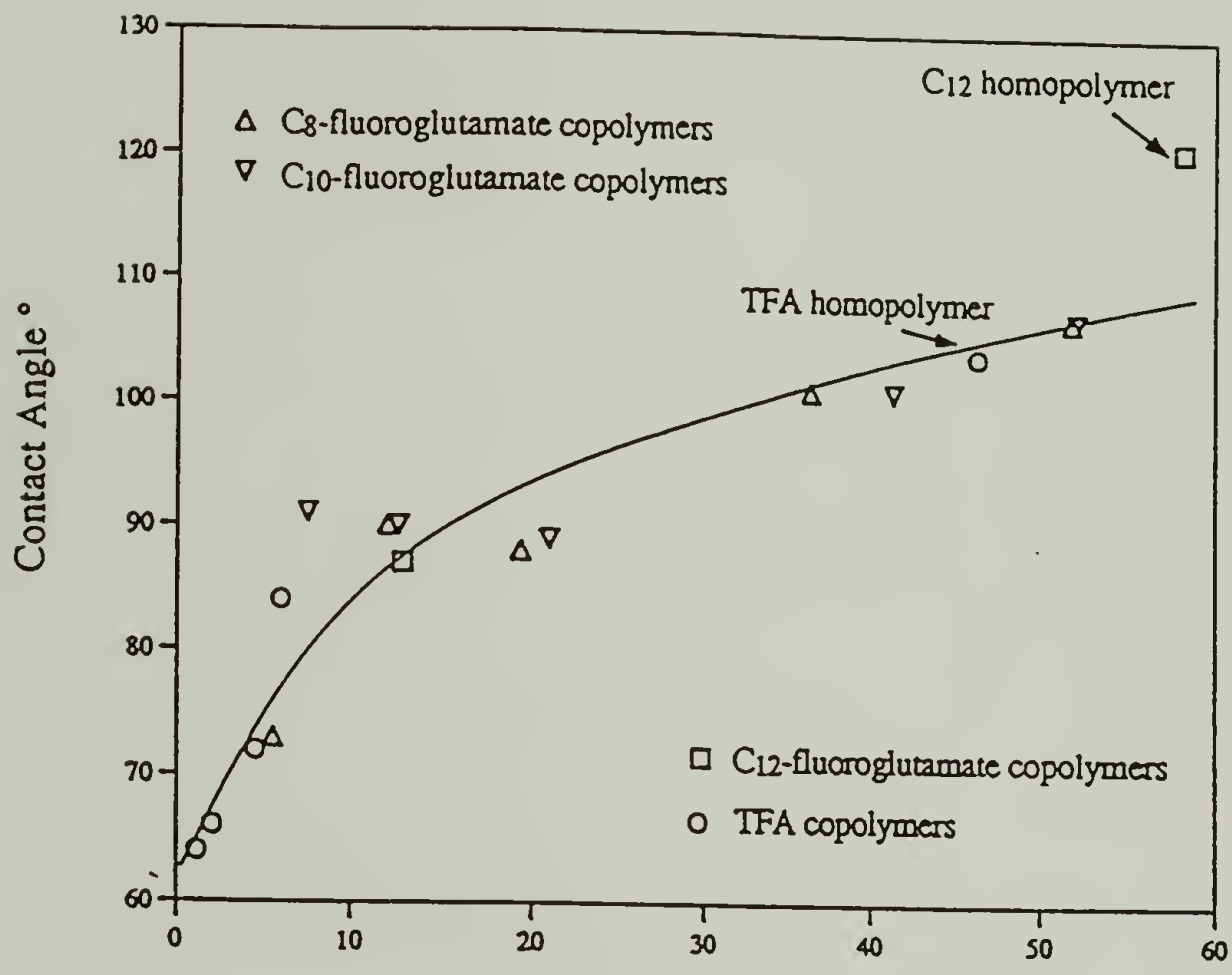


Figure 3.4 Advancing water contact angles vs fluorine content for various fluorinated polypeptides.

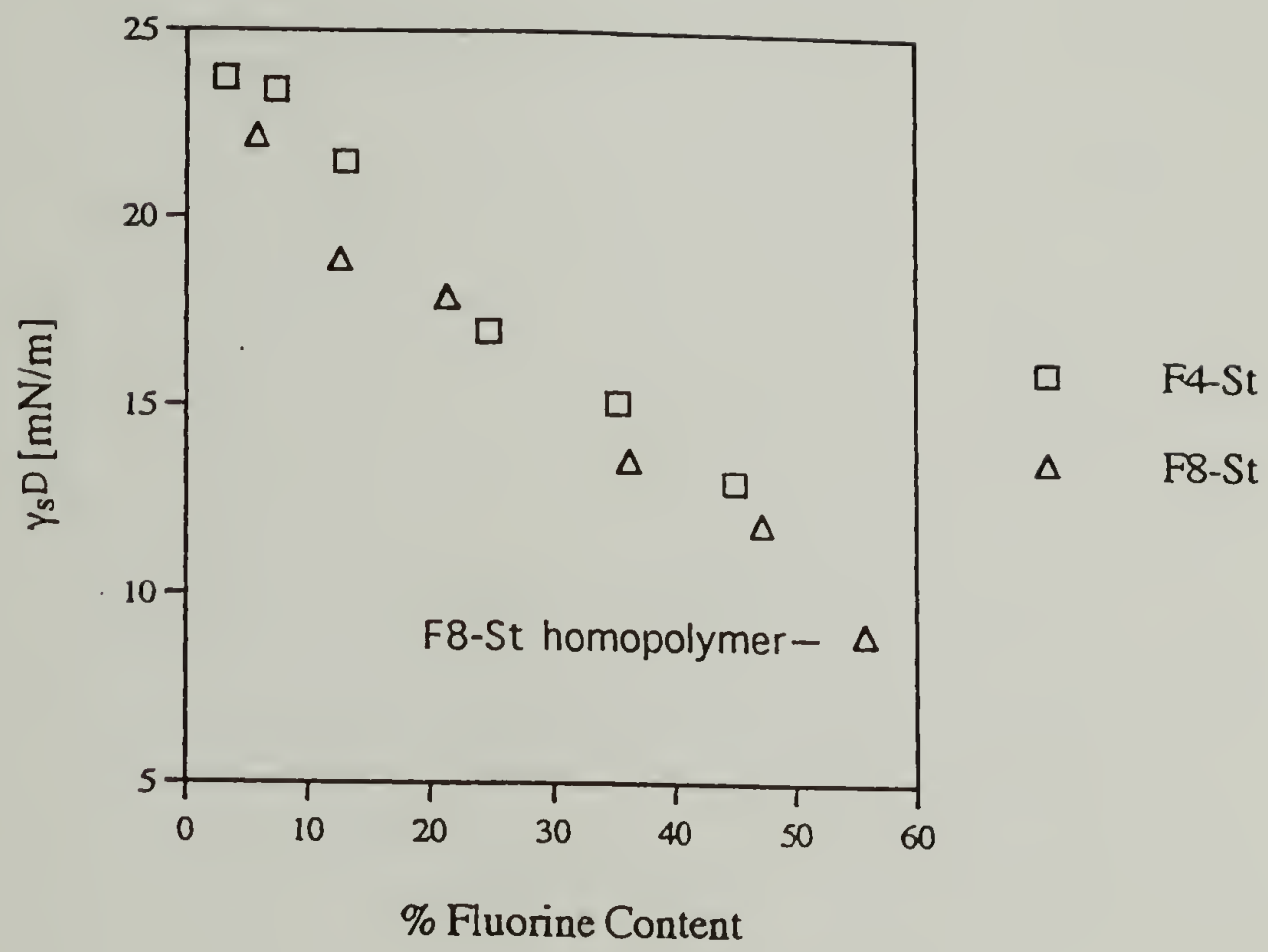


Figure 3.5 Plot of the dispersion force contribution to the surface energy γ_s^D versus the fluorine content of copolymers[3].

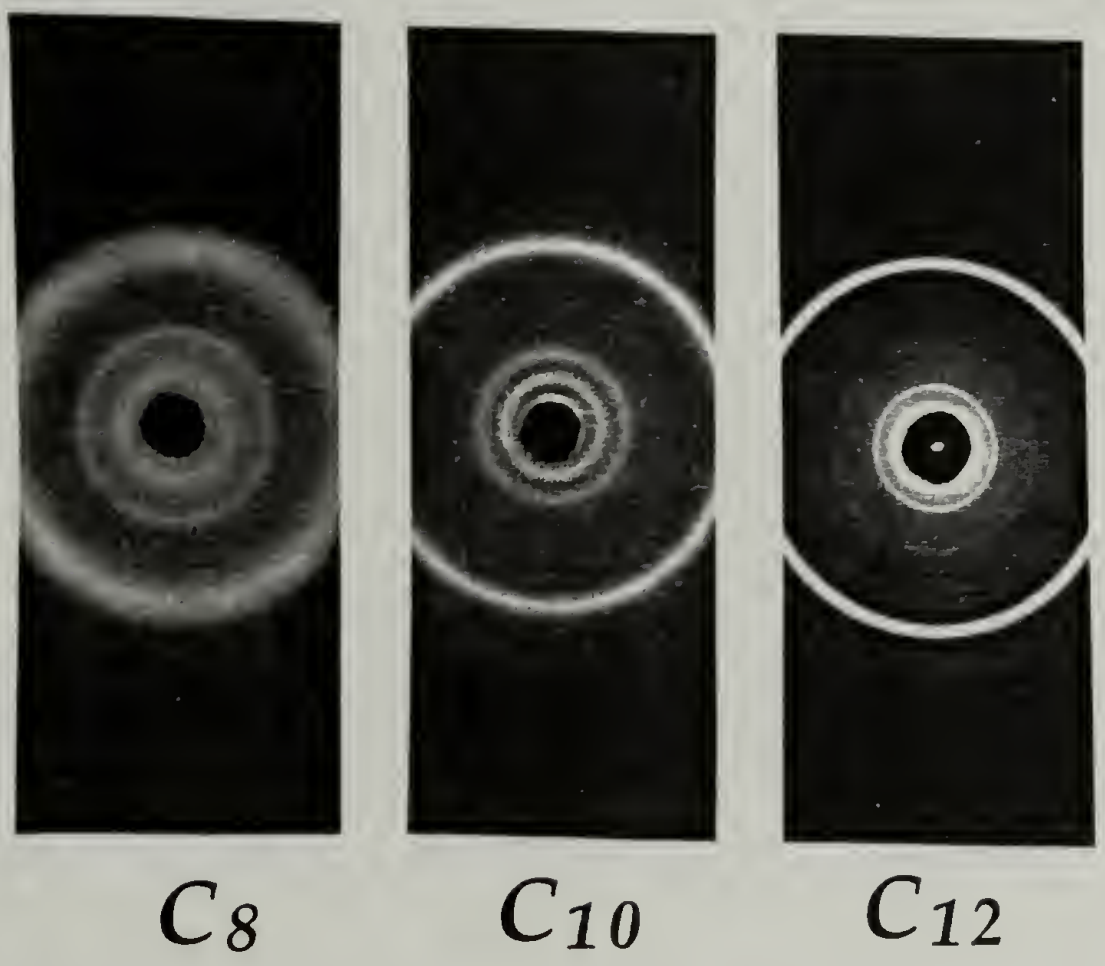


Figure 3.6 X-ray diffraction patterns of C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu homopolymers.

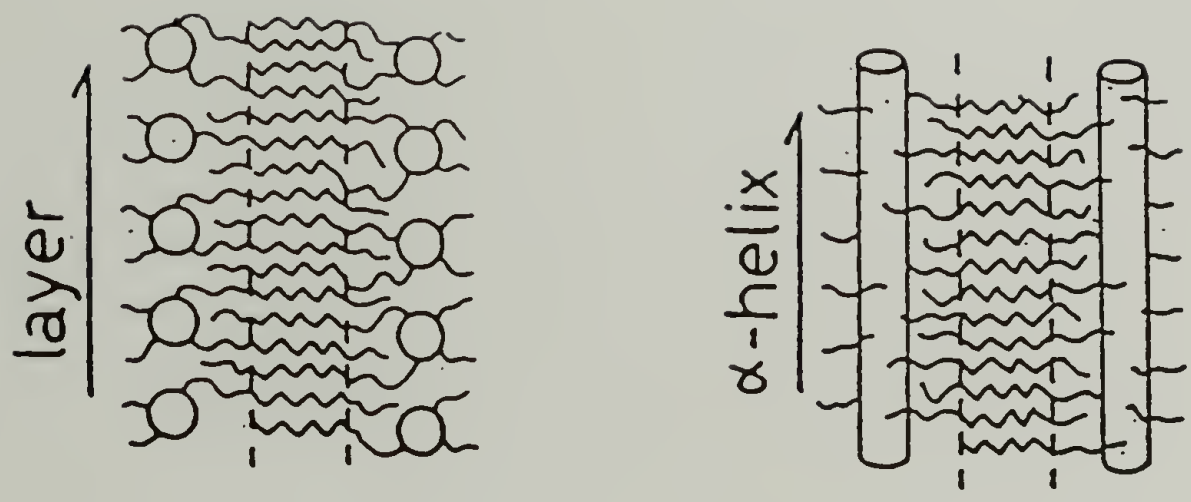


Figure 3.7 Model proposed by Watanabe et al. for the packing of PALG.

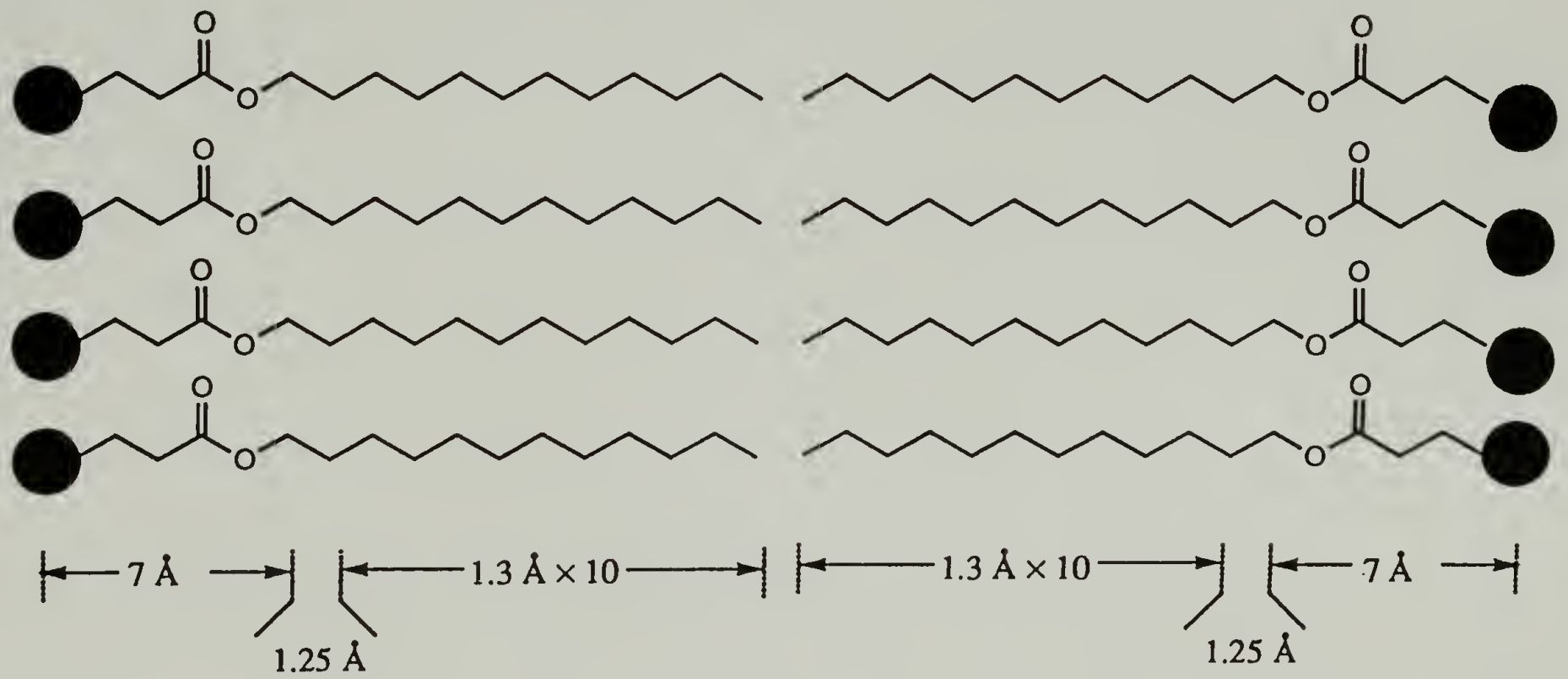


Figure 3.8 Interhelical distance for C_{12}-FGlu homopolymer with fully extended side chains.

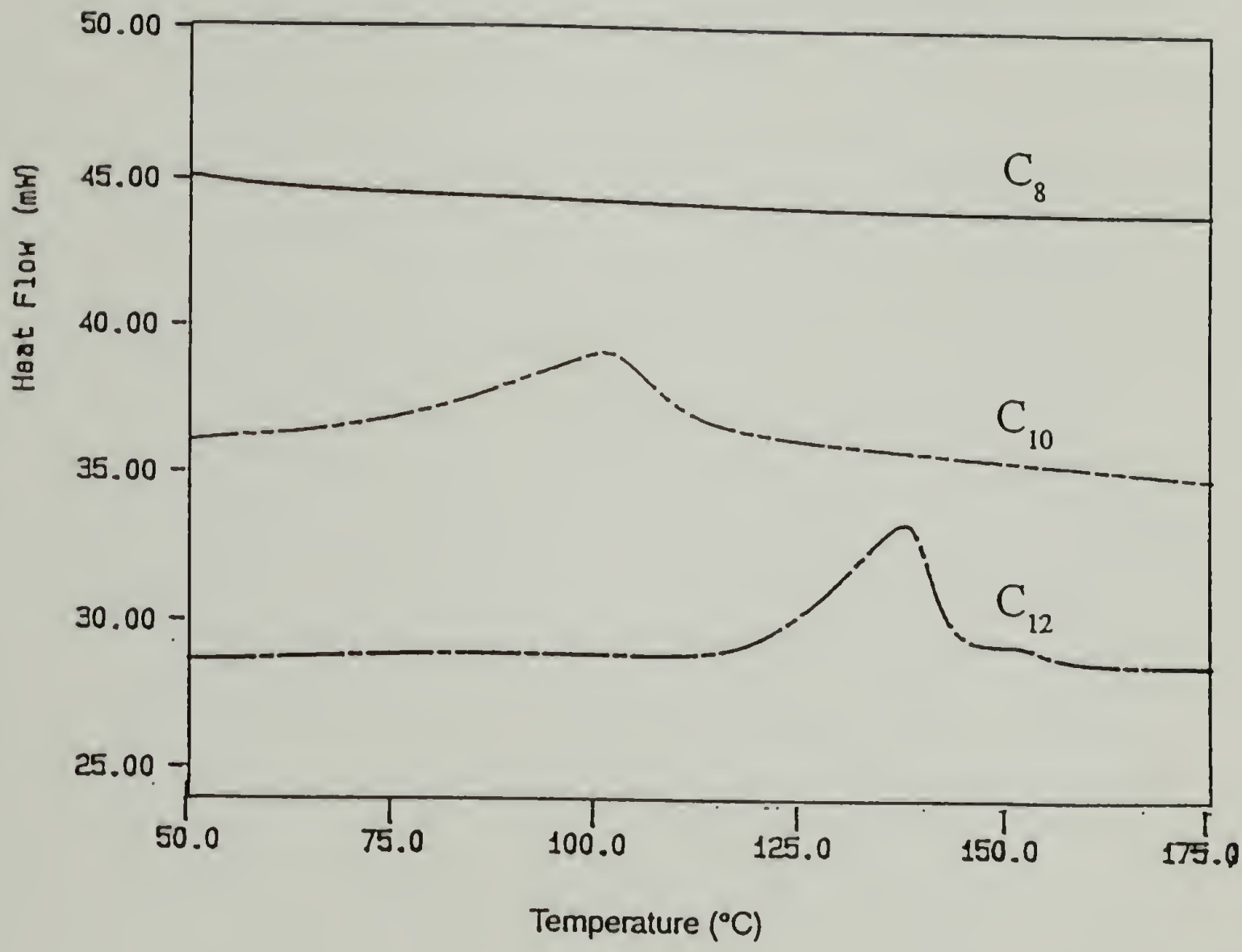
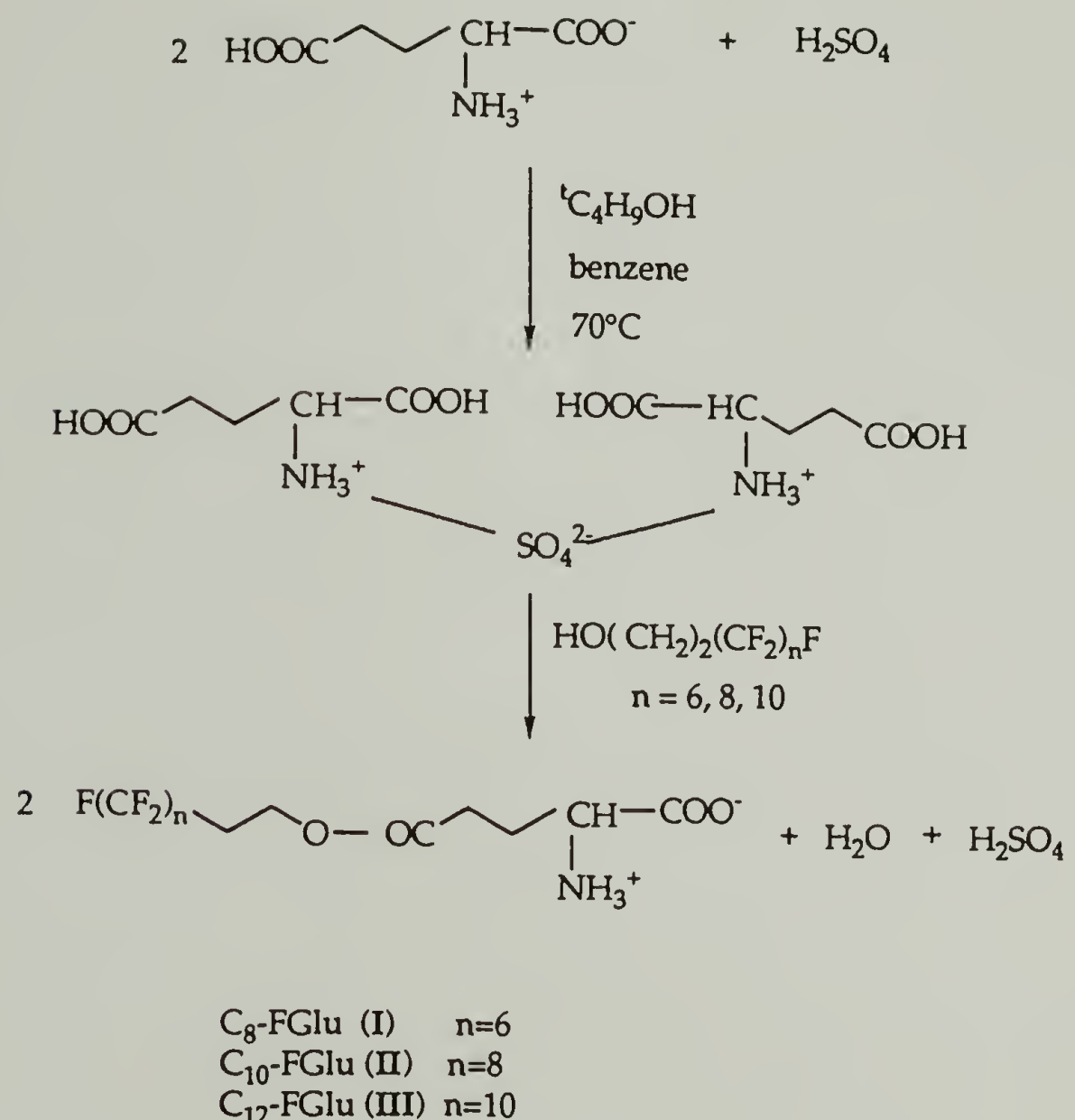
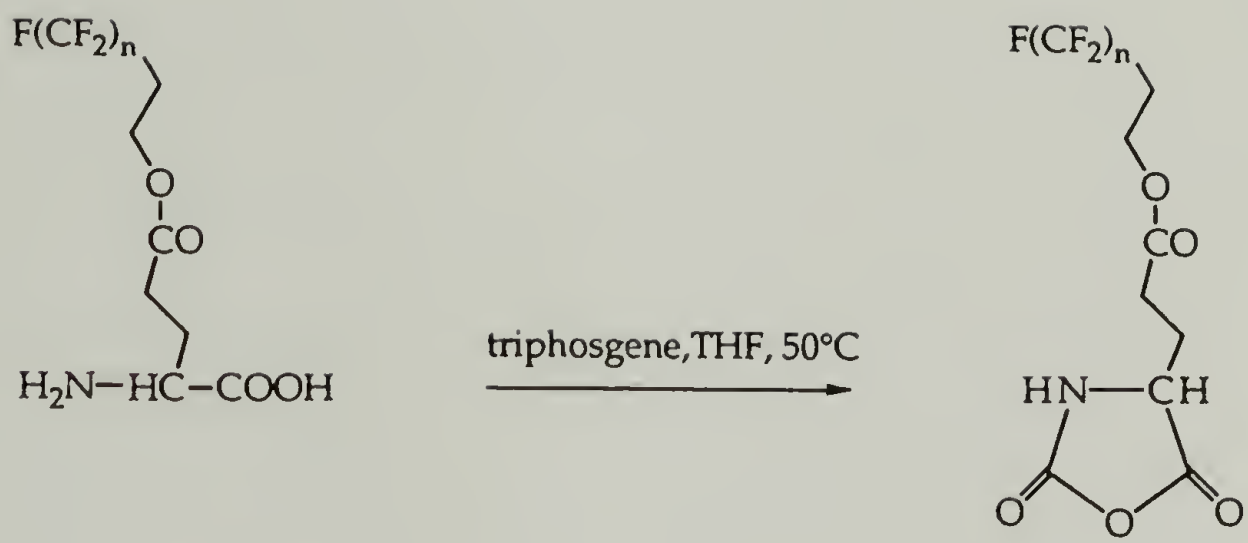


Figure 3.9 DSC heating traces of C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu homopolymers.



C_8-FGlu (I) n=6
C_{10}-FGlu (II) n=8
C_{12}-FGlu (III) n=10

Scheme 3.1 Synthesis of fluoroglutamate esters.



C_8-FGlu NCA (IV) n=6
C_{10}-FGlu NCA (V) n=8
C_{12}-FGlu NCA (VI) n=10

Scheme 3.2 Synthesis of N-Carboxyanhydrides.

CHAPTER 4

POLYMERIZATION OF FLUORINATED AMINO ACID N-CARBOXY ANHYDRIDES IN SUPERCRITICAL CARBON DIOXIDE

4.1 Introduction

The solubility of N-carboxy anhydrides (NCAs) of esters of glutamic acid with alcohols of the general formula {$F(CF_2)_n(CH_2)_2OH$, n=6, 8, and 10} (Scheme 3.2) in supercritical carbon dioxide and the possibility of their polymerization in that medium is of our interest. Natural amino acids and proteins are insoluble in carbon dioxide[1,2]. The reduction in polarity by tert-butoxy carbonyl (^{t}Boc) protection of the amino group of glycine (Gly) increases its solubility 5 orders of magnitude (from 10^{-5} wt % for glycine to 1wt % for ^{t}Boc-Gly)[3]. Long fluorinated side chains have been proposed to increase the solubility of NCAs and their corresponding polypeptides. In general carbon dioxide is a good solvent for highly fluorinated compounds[4]. Acrylates with long fluorinated chains and their polymers have been found to be soluble in supercritical CO_2[5]. Trifluoroalanine NCA and γ-benzyl L-glutamate NCA were found to be practically insoluble in CO_2[6].

Our interest in using supercritical carbon dioxide as the polymerization solvent has multiple origins. CO_2 has relatively modest critical temperature and pressure (31.1 °C and 72.8 atm)[4]. It is non-toxic and therefore an ideal solvent for the synthesis of biomaterials, and it serves as an alternative to freons in solubilizing fluorinated polymers[5]. The utility of supercritical CO_2 in polymer chemistry will depend in part on the variety of polymerizations that can be performed in CO_2 and the number of polymers that are soluble and can be processed in it. Typical anionic polymerizations are performed in CO_2-free environments[7] because anions react with carbon dioxide resulting in chain termination. The polymerization of α-amino acid N-carboxy anhydrides occurs via a ring

opening reaction that proceeds with evolution of carbon dioxide[8]. Initiation by nucleophiles proceeds via attack to the C-5 carbonyl and ring opening (Scheme 1.2). Decarboxylation is believed to occur either before or after the propagation step, giving rise to the amine or carbamate mechanisms, respectively (Scheme 1.3). No direct analytical method exists to distinguish between these two mechanisms. The extent of propagation via the amine or carbamate mechanism will depend on the stability of the carbamate and the relative nucleophilicity of the amine versus the carbamate. When basic initiators are used initiation is believed to occur through proton abstraction (Scheme 1.4). In that case propagation could also happen via the activated monomer mechanism (Scheme 1.5). Sekiguchi has proposed that in an actual polymerization all three mechanisms are operative to different extents[9].

Since the polymerization of NCAs involves evolution of carbon dioxide, the viability of such polymerizations under high CO_2 pressures is questionable. The effect of carbon dioxide concentration on the rates of polymerization of α-amino acid NCAs is not at all clear. Results from kinetic studies indicate that increasing carbon dioxide concentrations can lead to increased, decreased, or unaltered rates of NCA polymerization, depending on the solvent, amino acid, and initiator used[10-12]. For instance, the rate of polymerization of γ-benzyl L-glutamate NCA in dioxane using hexylamine as initiator is higher when CO_2 is removed by blowing N_2 over (slow removal) rather than through (fast removal) the reaction mixture[10]. The exact opposite is observed by the same authors for the copolymerization of equimolar amounts of γ-benzyl L-glutamate NCA and β-benzyl L-aspartate NCA[10]. In both cases the polymerizations follow 2 stage pseudo first order kinetics. Furthermore, polymerizations of L-leucine NCA were conducted using 1, 6 hexanediamine as initiator in dioxane or dimethylformamide (DMF)[12]. The reactor was initially filled with N_2 or CO_2. Two propagation stages were observed again. In DMF the kinetics were independent of the initial filling of the polymerization reactor with either carbon dioxide or nitrogen. In dioxane the first propagation rate constant is ca. 55 % of

that in DMF, when the reactor is initially filled with N_2 and further decreased by ca. 28 % when the reactor is initially filled with CO_2. The second propagation stage is not that significantly influenced by the initial carbon dioxide concentration. The results are interpreted by assuming propagation via the amine mechanism in DMF and in the initial stages of dioxane/N_2 and propagation via the carbamate mechanism in dioxane/CO_2. The kinetics are not influenced by the onset of precipitation. The transition from β-sheet conformation to α-helical is proposed as responsible for the two stage kinetics.

When supercritical carbon dioxide is used as the polymerization solvent the rate of carbamate decarboxylation would be expected to be considerably decreased. Therefore, if the carbamate mechanism is operative at all, it might be anticipated that it would predominate under these conditions. Propagation via the carbamate mechanism would result in the formation of mixed anhydrides that would subsequently lose carbon dioxide to give the polyamides (Scheme 1.3). However Kricheldorf suggests that the decarboxylation of linear mixed anhydrides can in general lead to urea type linkages[8] referencing older reports from reactions of aromatic isocyanates with carboxylic acids[13,14] as well as his own experiments[15]; this is used as an argument against any significant contribution of the carbamate mechanism to the NCA polymerization[8]. Specifically Kricheldorf has studied isocyanato carboxylic acids formed by hydrolysis of their trimethylsilyl esters. He found that aliphatic α- and β- substituted acids give spontaneously cyclic NCAs whereas higher homologues undergo polyaddition to form poly-N-carboxyanhydrides that decarboxylate to polyamides. The loss of carbon dioxide is catalyzed by bases. No reference to urea byproducts is done in this paper but in a later paper by the same author the reaction of thiocarboxylic acids with isocyanates or thioisocyanates is studied. Diarylureas are byproducts when aromatic isocyanates are used[16].

We report herein the polymerization of fluorinated amino acid N-carboxy anhydrides in supercritical carbon dioxide. ^{13}C NMR spectroscopy was used to determine the existence of urea linkages in the product polypeptides.

4.2 Experimental Section

4.2.1 Materials and Methods

Hexafluoroisopropanol (HFIP) was purchased from Aldrich Chemical Co. HFIP-d_2 was purchased from Cambridge Isotope Labs. Carbon dioxide (research grade) was purchased from Air Products and transferred to the high pressure cells by using an Isco Model No. 260D automatic syringe pump. Proton magnetic resonance spectra were recorded on a Bruker AC-200 (200 MHz ^1H) Spectrometer. Chemical shifts are reported as parts per million downfield from tetramethylsilane (TMS). ^{13}C NMR spectra were recorded on a Varian XL-300 (75.43 MHz ^{13}C) Spectrometer and on a Bruker AC-500 (125.72 MHz ^{13}C) using deuterated hexafluoroisopropanol as solvent. Solid state infrared spectra were obtained on a Perkin Elmer 1600 Fourier Transform Infrared Spectrophotometer. Samples were in the form of KBr pellets. Infrared spectra in supercritical carbon dioxide were recorded on a Biorad FTS 7 Spectrophotometer. A fixed volume (1 mL) high-pressure spectroscopic cell fitted with CaF_2 windows was used for all IR experiments. Solubility tests and polymerizations in supercritical carbon dioxide were conducted in a 2.5 mL fixed volume, high-pressure, view cell fitted with sapphire windows. Syntheses of C_8-FGlu NCA, C_{10}-FGlu NCA, and C_{12}-FGlu NCA have been described in Chapter 3. The anhydrides were recrystallized from THF/hexane before use. The synthesis of homopolymers of C_8-FGlu, C_{10}-FGlu, and C_{12}-FGlu in THF have been described in the previous chapter. Inherent viscosity measurements of

solutions of polymers in hexafluoroisopropanol were performed with a CANNON® 50 Ubbelohde viscometer. A water bath with a Lauda temperature controller was used to regulate the temperature within 0.1 °C. Elemental analysis measurements were performed by the Microanalytical Laboratory, Office of Research Services, University of Massachusetts, Amherst, MA 01003.

4.2.2 Solubility tests

The possibility of solubilizing the fluorinated amino acid NCAs and their homopolymers in supercritical CO_2 was tested in a high pressure fixed volume (2.5 mL) view cell. The cell was charged with the anhydride (40 to 60 mg) and purged with argon for 5 minutes. The temperature was raised to ca. 50 °C and carbon dioxide was introduced to the cell until the pressure reached 2500 psi. Clear solutions were observed for all three anhydrides, and all solutions became cloudy upon slow venting of the carbon dioxide. The same procedure was followed to test the solubility of the fluoropolypeptides but in this case smaller amounts (10 to 15 mg) were tested. C_8-FGlu and C_{10}-FGlu polypeptides remained practically insoluble whereas more than 90 % of the C_{12}-FGlu homopolymer (13.5 mg tested) dissolved. Upon slow venting of the carbon dioxide the solution became cloudy.

4.2.3 Polymerizations

Polymerization of C_8-FGlu NCA (**IV**) in IR cell: C_8-FGlu NCA (12.6 mg, 24 μmol) and triethyl amine (3 μL 1% solution in THF, 0.22 μmol) were charged to the IR cell. The cell was sealed, purged with argon for ca. 5 minutes, and placed in the spectrophotometer. The temperature was raised to ca. 50 °C and carbon dioxide was added so that the pressure reached ca. 3000 psi. Spectra (16 scans) were obtained every 1

hour. The same procedure was used for monitoring the polymerization of C_{12}-FGlu NCA (**VI**) in supercritical CO_2. 6.8 mg (9.5 μmol) of (**VI**) were polymerized using 1.4 mL triethyl amine solution (1% in THF, 0.10 μmol). In the later case the rection seemed homogeneous.

Polymerization of C_8-FGlu NCA in view cell: C_8-FGlu NCA (58.1 mg, 0.112 mmol) and pyridine (10 μL 1% solution in THF, 1.2 μmol) were charged to the view cell. The cell was sealed and purged with argon for ca. 5 minutes before being filled with carbon dioxide. The polymerization was run at ca. 50 °C and 3500 psi. The initially clear solution became cloudy 20 minutes after the addition of the carbon dioxide and more precipitate formed during the following 2 hours. After 24 hours the carbon dioxide was vented slowly and the cell was opened. The polymer product (25 mg, 0.053 mmol, 47% yield) was obtained in the form of a white powder. $\eta_{inh(2.0g/dl)}$ = 0.12 dl/g. Anal. Calcd for $C_{13}H_{10}O_3NF_{13}$: C, 32.8%; H, 2.12%; N, 2.95%; F, 52.0%. Found: C, 32.6%; H, 1.78%; N, 3.28%; F, 49.2%.

The same procedure was followed using triethylamine as initiator, with shorter reaction time (3.5 hours) and slightly lower carbon dioxide pressure (3,000 psi). The product precipitated on the window and was obtained in the form of a white solid (40 mg, 0.084 mmol, 73.5% yield) . Anal. Calcd for $C_{13}H_{10}O_3NF_{13}$: C, 32.8%; H, 2.12%; N, 2.95%; F, 52.0%. Found: C, 33.0%; H, 2.26%; N, 3.14%; F, 50.9%.

4.3 Results and Discussion

4.3.1 Solubilities

The NCAs of the esters of glutamic acid with fluorinated alcohols (**IV-VI**) were found to be soluble in supercritical CO_2, even at moderate temperatures and pressures (50

°C, 1500 psi), in contrast to trifluoroalanine NCA and γ-benzyl L-glutamate NCA. From the homopolypeptides only the C_{12}-FGlu was found to be soluble. It is interesting that an increase in the fluorine content by only 3% (from 56% for poly C_{10}-FGlu to 59% for poly C_{12}-FGlu) has such an effect on the solubility.

Figure 4.1 shows the solid state IR spectrum and the IR spectrum in supercritical CO_2 of C_8-FGlu NCA. The stretching vibrations of the C-2 carbonyl is shifted by ca. 30 cm^{-1} to higher frequency in CO_2 compared to the solid state spectrum. Hyatt has compared solvent effects on the frequencies of acetone (C=O stetch), cyclohexanone (C=O stretch), and pyrrole (N-H stretch) already existing in the literature with the effect of subcritical and supercritical carbon dioxide[17]. The solvent effect is expressed as $10^3(\Delta\nu/\nu_o)$ where ν_o is the gas phase vibrational frequency in cm^{-1} and $\Delta\nu=\nu_{solvent}-\nu_o$. CO_2 behaves similarly to nonpolar hydrocarbon solvents (i. e. hexane) for a carbonyl ketone; $10^3(\Delta\nu/\nu_o) \approx 10$. However for the N-H of pyrrole, CO_2 behaves like a solvent in the ether to ethyl acetate range; $10^3(\Delta\nu/\nu_o) \approx 37$. The difference is attributed to hydrogen bonding between N-H and CO_2.

4.3.2 Polymerizations in IR cell

Figure 4.2 shows IR spectra aquired during the polymerization of C_8-FGlu NCA performed in an IR cell. Triethyl amine was used as initiator in a ratio of ca. 1/100 to the monomer. The polymerization was heterogeneous but could be monitored directly by IR spectroscopy. As the bands corresponding to the NCA disappeared a new band at 1607 cm^{-1} appeared. This band can be assigned to the amide I stretching vibration and is shifted by ca. 40 cm^{-1} to lower frequency compared to the solid state spectrum. The assignment was verified by the IR spectrum in CO_2 of C_{12}-FGlu homopolymer (synthesized previously in THF) shown in Figure 4.3 together with the solid state spectrum of that polymer. The experiment shows clearly that the presence of excess of

carbon dioxide does not prevent the polymerization of the N-carboxy anhydride. The same procedure was followed for the polymerization of C_{12}-FGlu NCA. In that case the reaction seemed to be homogeneous. Figure 4.4 shows an IR spectrum obtained 10.5 hours after the initiation of that polymerization.

The % conversion versus time for the C_8-FGlu NCA and the C_{12}-FGlu NCA polymerization was calculated based on the absorbance at 1875 cm^{-1} (peak height) and is shown in Figure 4.5. Assuming that the absorbance at 1875 cm^{-1} varies linearly with the anhydride concetration the % conversion was calculated from the formula $\%\ \text{conversion} = (A_0 - A_t)/A_0 \times 100$ where A_0 is the absorbance at t=0 and A_t is the absorbance at time t. The value for A_0 was estimated by extrapolation since IR measurements were started at least 0.5 hours after the addition of CO_2. In both cases the polymerization seems to follow two stage first order kinetics (Figure 4.6). Two stage kinetics has been reported in the literature for the polymerization of NCAs in various solvents[8,12,18,19]; some of these experiments are described in the introduction. In all cases the second stage is faster than the first, which is exactly opposite to what we observe in supercritical CO_2. The onset of the second stage occurs at conversion that depends on the initial monomer to initiator ratios as shown in Figure 4.7 for the polymerization of L-phenylalanine NCA (100 mmol/L) with N-methylbenzylamine as initiator in DMF at 25 °C[20]. The origin of the two stage kinetics has been attributed to a β-sheet to α-helix transition[20]. Although it is well established (by IR and x-ray studies of oligo and polypeptides) that helicogenic amino acids can form peptides of the two conformations depending on the degree of polymerization (DP>10 required for helices) the connection between the conformational transition and the onset of the second stage is not without dispute[8]. The strongest arguments come from the observation of two stage kinetics in the polymerizations of non-helicogenic amino acid NCAs such as glycine NCA[21], sarcosine NCA, and proline NCA[22,23]. The hypothesis that association of the NCAs to the growing oligopeptide chains increases the local NCA concentration and

leads to the faster second stage propagation has been adopted by Kricheldorf as the most plausible explanation[8]. It originated from the observation that preformed oligoglutamates can initiate the polymerization of L-γBzl-Glu NCA at rates dependent on their DP (initiation by oligoglutamate with DP=15 was found three times faster than when DP=3)[22]. That observation seems to be compatible with the helical growth hypothesis too but it also explains why the two stage kinetics is more pronounced in dioxane than in DMF while both solvents are helicogenic. Finally the carbon dioxide concentration has definitely an effect on the rate of the polymerization as discussed in the introduction and the transformation of carbamate end groups to amino end groups (or vice versa), supported by a conformational change, has been considered responsible for the two stage kinetics[10]. Since the presence of acidic impurities (that speed up the decarboxylation of the carbamate groups) results in a decreased rate of polymerization and only one stage kinetics[18], Kricheldorf concluded that in order to explain the two stage kinetics by change in the propagating mechanism one has to assume that higher concentrations of carbamates cause higher rates of polymerization. This does not necessarily need to be true since acidic impurities can also affect other factors apart from the rate of carbamate decarboxylation. The problem is that the carbon dioxide concentration has different effect on the polymerization of different amino acids as discussed in the introduction.

4.3.3 Polymerizations in view cell

Polymerization of C_8-FGlu NCA was also performed in a 2.5 mL stainless steel view cell using pyridine as initiator. The initial solution of the anhydride and initiator was clear. Cloudiness appeared ca. 10 minutes after dissolution in supercritical carbon dioxide. More precipitate forms with time for the first two hours but no visual change is observed after that. The reaction was run for 24 hours. The carbon dioxide was vented slowly and the polymeric product was obtained in the form of a fine powder. The yield reported

(47%) corresponds to the amount of polymer that was mechanically removed from the cell and does not represent the actual yield of the polymerization reaction. Since the product was not further purified the yield should have been 100%. The inherent viscosity of the product at a concentration of 2.0 g/dl in hexafluoroisopropanol (η = 0.12 dl/g) compares favorably to that measured for C_8-FGlu homopolymer synthesized in THF at room temperature, using triethylamine as initiator (η = 0.04 dl/g) (Table 3.1). As discussed later there are also structural differences between the two products.

The same procedure was repeated with a shorter reaction time (3.5 hours) since no change was visually observed after the first two hours. Triethyl amine was used as initiator in this case; most of the product formed precipitated on the window of the view cell and was easier to recover (yield 75%).

4.3.4 Spectroscopic characterization

Figure 4.8 shows the solid state IR spectra (carbonyl stretching region) of the C_8-FGlu polypeptides obtained in CO_2 (P=3,000 psi, T=50 °C, 42 hours, triethylamine (TEA) as initiator) and in THF (TEA as initiator). The polypeptide prepared in carbon dioxide seems to have a higher α-helical to β-sheet content as judged by the ratio of the amide I vibrations at 1656 and 1635 cm^{-1}. Formation of β-sheets is observed at the initial stages of NCA polymerization or when low monomer to initiator ratios are used[24]. Oligopeptides of helicogenic amino acids prepared by stepwise methods assume a β-sheet conformation. At higher degrees of polymerization the α-helical conformation is more stable. NCA polymerization can give a mixture of β-sheets and α-helices. Fractions containing β-sheets have been extracted with formic acid[25]. Figure 4.9 shows the solid state IR spectra of the polymers synthesized in CO_2 with the two different initiators. The polypeptide synthesized with triethylamine as initiator has a higher percentage of α-helical to β-sheet content.

Figure 4.10 shows the ^{13}C NMR spectra of the C_8-FGlu polypeptides synthesized in THF or in carbon dioxide using pyridine or triethyl amine as initiators. A peak at ca. 161 ppm is present only in the polymer synthesized in CO_2 using pyridine as initiator. This frequency is characteristic of urea carbonyl resonances[26,27]. Urea linkages are postulated byproducts of the decarboxylation of the linear α-amino acid N-carboxy anhydrides that are intermediates in the carbamate mechanism for NCA polymerization. This is because linear mixed anhydrides, formed by the addition of carboxylic acids into aromatic isocyanates, are known to yield symmetric anhydrides along with ureas[8,13-15,27] (Scheme 4.1). This has been an argument against the carbamate mechanism since no appreciable levels of urea linkages have been detected in polypeptides synthesized in conventional solvents. Urea type linkages could also form from termination reactions that lead to hydantoic acid end groups. Possible pathways for these reactions involving the intermediate formation of linear anhydrides are shown in Scheme 4.2[8,27-29]. It should be mentioned here that there are other reaction pathways that can lead to hydantoic acid end groups that do not involve the carbamate mechanism as discussed in Chapter 2 (Scheme 2.3). Finally the frequency at 161 ppm cannot be assigned to the aromatic carbons of pyridine or pyridinium salts (δ_{C1} = 150 ppm). If pyridine were present additional spurious peaks would have been observed at 136 ppm and 124 ppm (or at 142 ppm and 128 ppm for pyridinium salts).

The difference between the two polymers obtained in carbon dioxide may be the result of the different initiators, reaction times, or carbon dioxide pressure. Further experiments are necessary in order to explore these possibilities.

4.4 Conclusions

The addition of fluorocarbon side chains (6, 8, or 10 carbons long) to α-amino acid N-carboxy anhydrides proved efficient in rendering them soluble in supercritical carbon

dioxide at concentrations of at least 2.5 g/mL at moderate temperatures (50 °C) and pressures (1500 psi). *In situ* IR monitoring of the polymerization reaction proved unequivocally the consumption of the anhydrides and formation of polypeptides in the presence of the large excess of carbon dioxide. Only the polymerization of C_{12}-FGlu NCA seemed to be homogeneous in supercritical CO_2. To the best of our knowledge this is the only solvent for the homogeneous polymerization of the above anhydride. ^{13}C NMR spectroscopy suggests the possible presence of urea type linkages in the polymer obtained in carbon dioxide when pyridine was used as initiator but not when triethyl amine was used as initiator. Shorter reaction time and slightly lower carbon dioxide pressure were applied in the second case.

4.5 References

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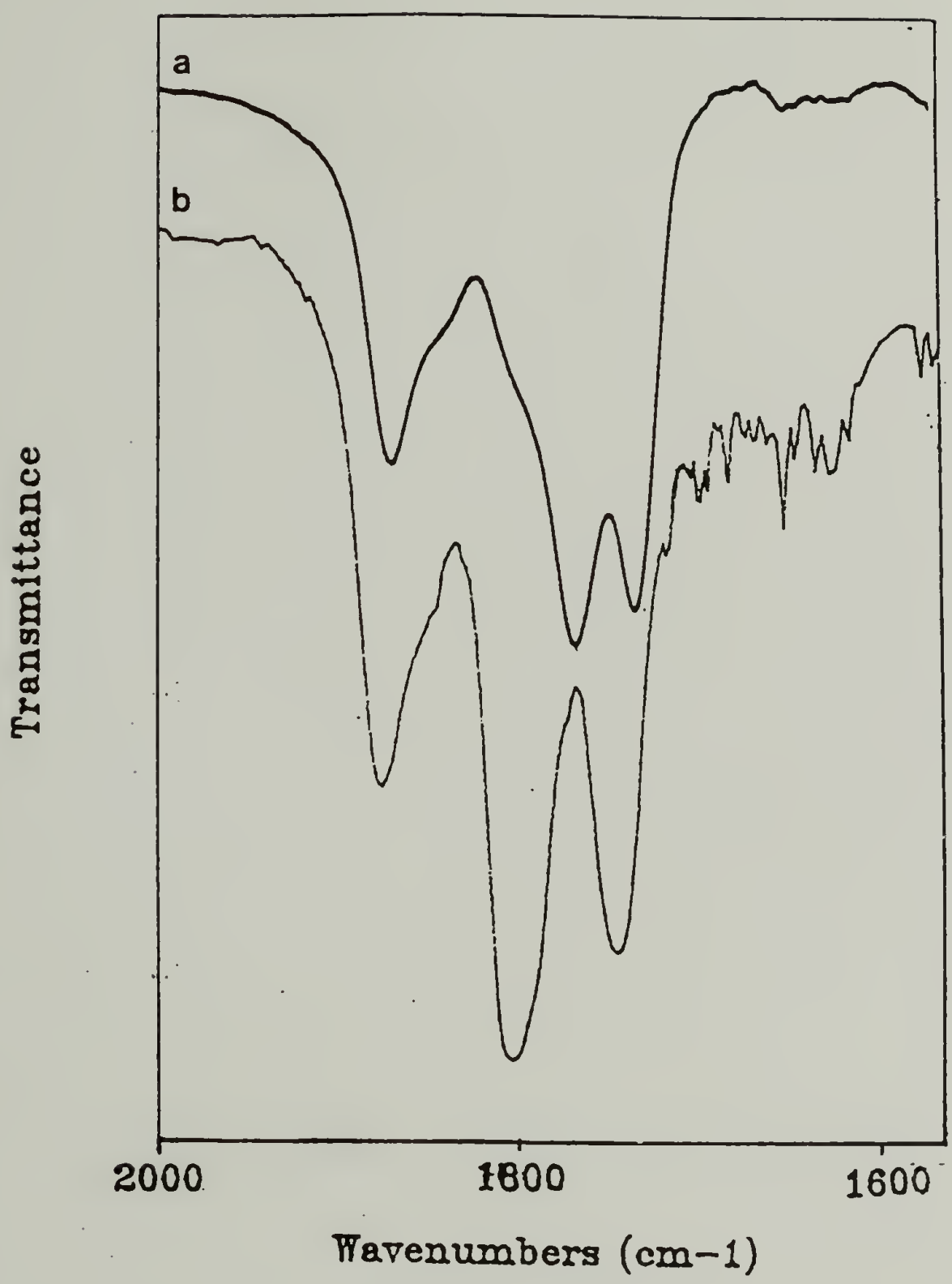


Figure 4.1 Comparison of the IR spectra of C_8-FGlu NCA (a) in the solid state (KBr pellet) and (b) in supercritical carbon dioxide (3000 psi, 50 °C).

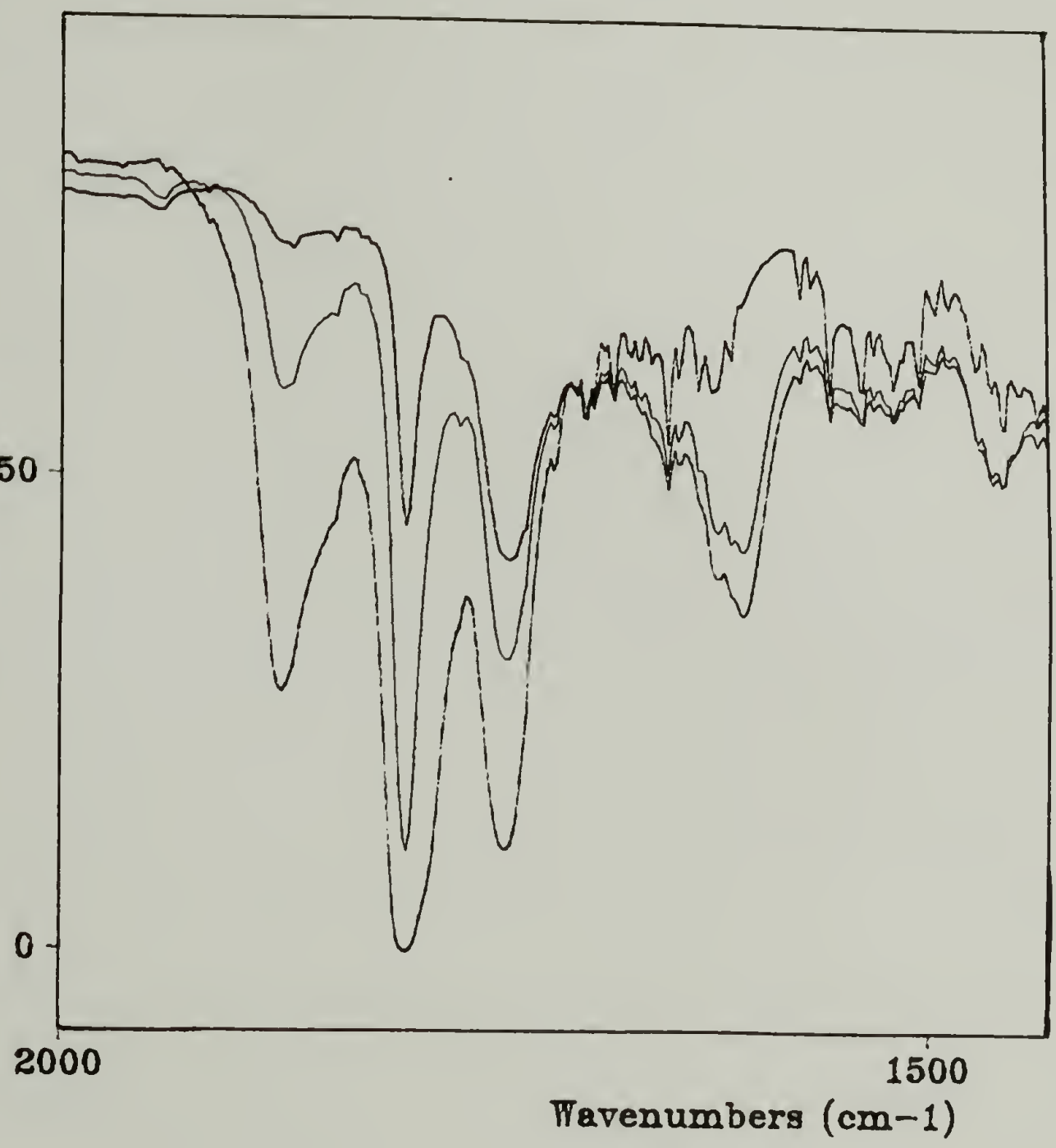


Figure 4.2 IR spectra during the polymerization of C_8-FGlu NCA (24 mmol/L) in supercritical carbon dioxide (3000 psi, 50 °C). The bands at 1875 and 1805 cm^{-1} decrease in intensity as a new band at 1607 cm^{-1} forms. The spectra are taken 1, 18, and 42 hours after the addition of triethylamine (0.22 mmol/L).

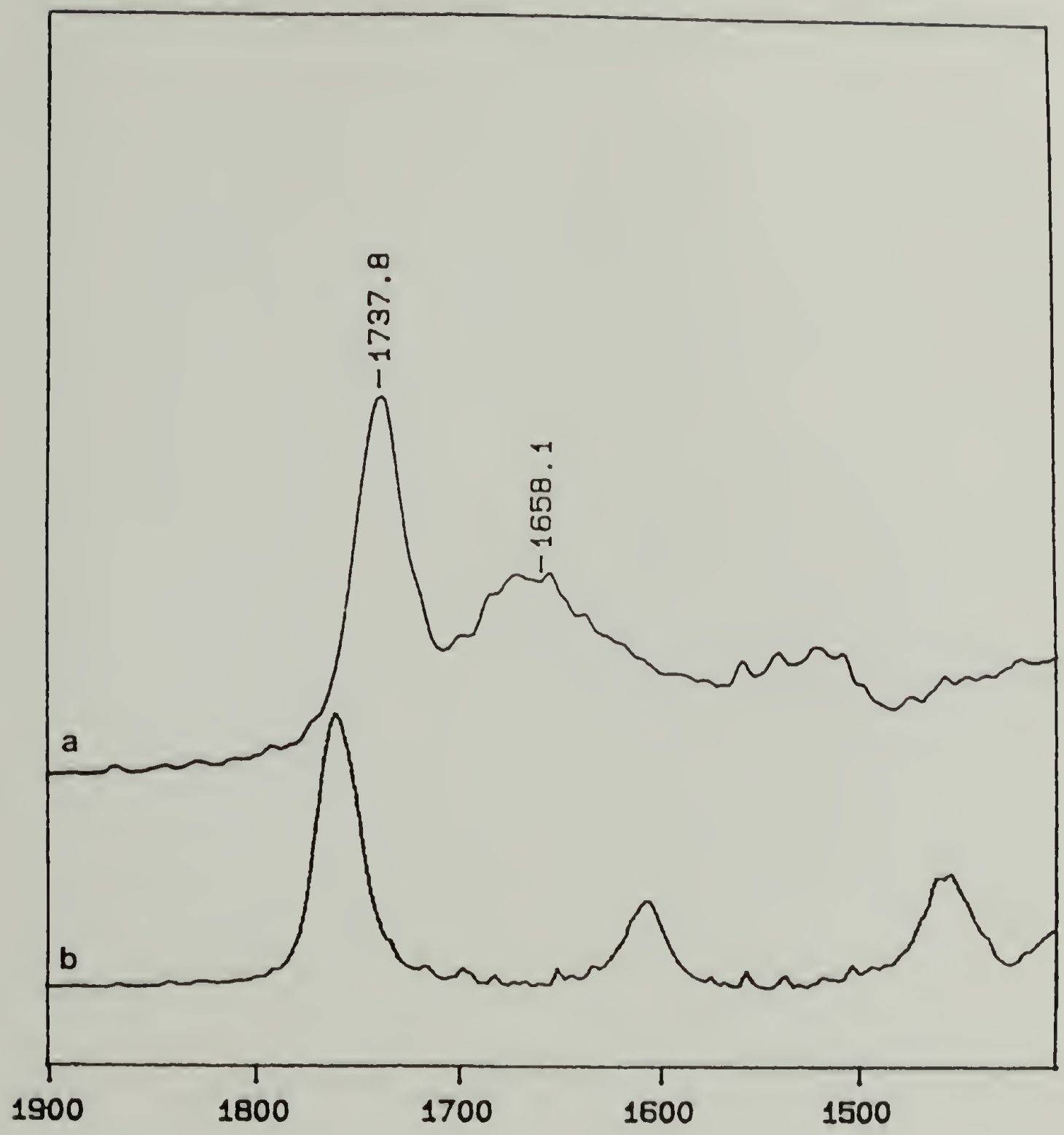


Figure 4.3 Comparison of the IR spectra of poly C_{12}-FGlu (synthesized in THF using triethylamine as initiator) (a) in the solid state (KBr pellet) and (b) in supercritical carbon dioxide.

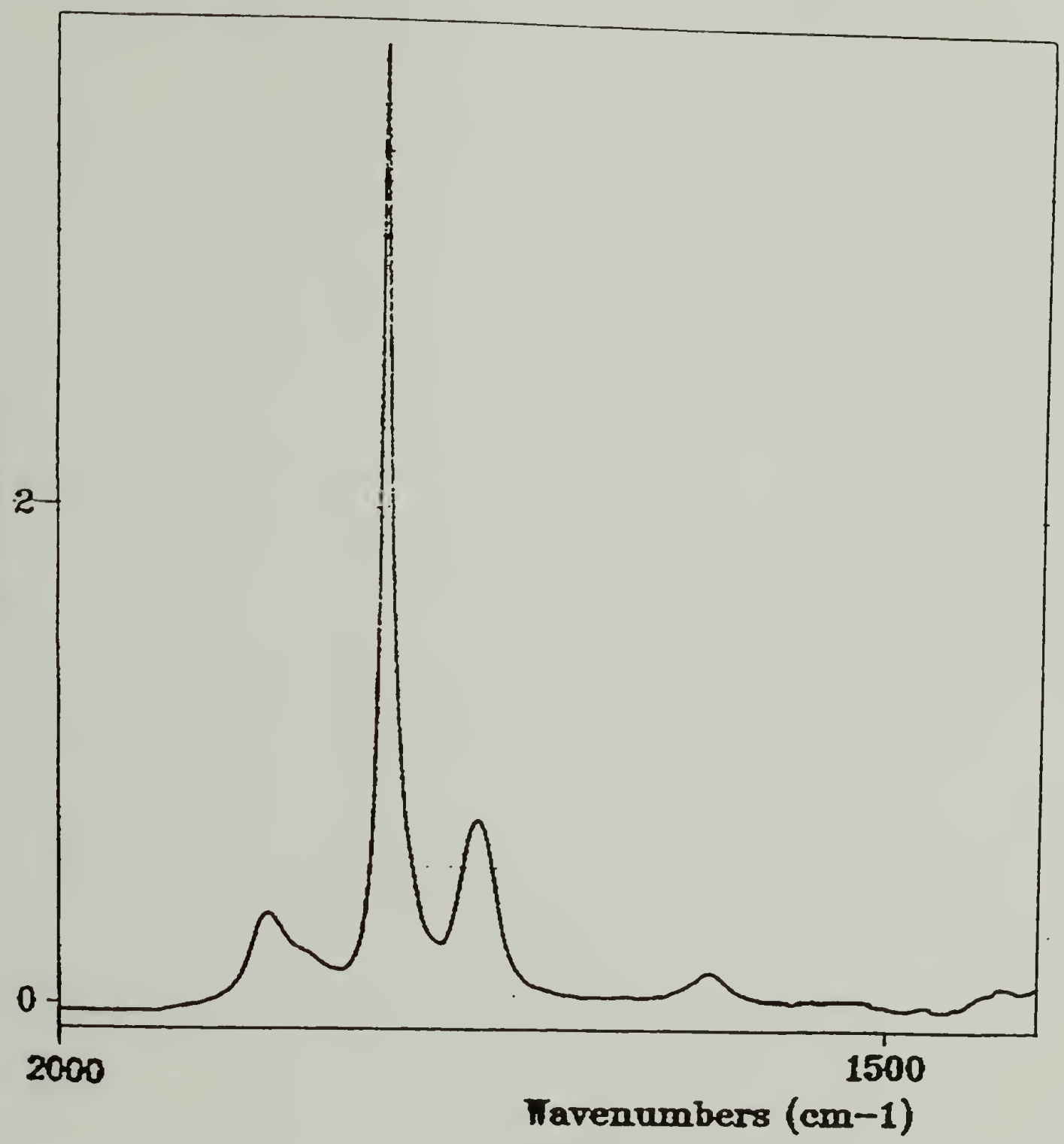


Figure 4.4 IR spectrum during the polymerization of C_{12}-FGlu NCA (9.5 mmol/L) in supercritical CO_2 (3200 psi, 50 °C). The spectrum is taken 10.5 hours after the addition of triethylamine (0.1 mmol/L).

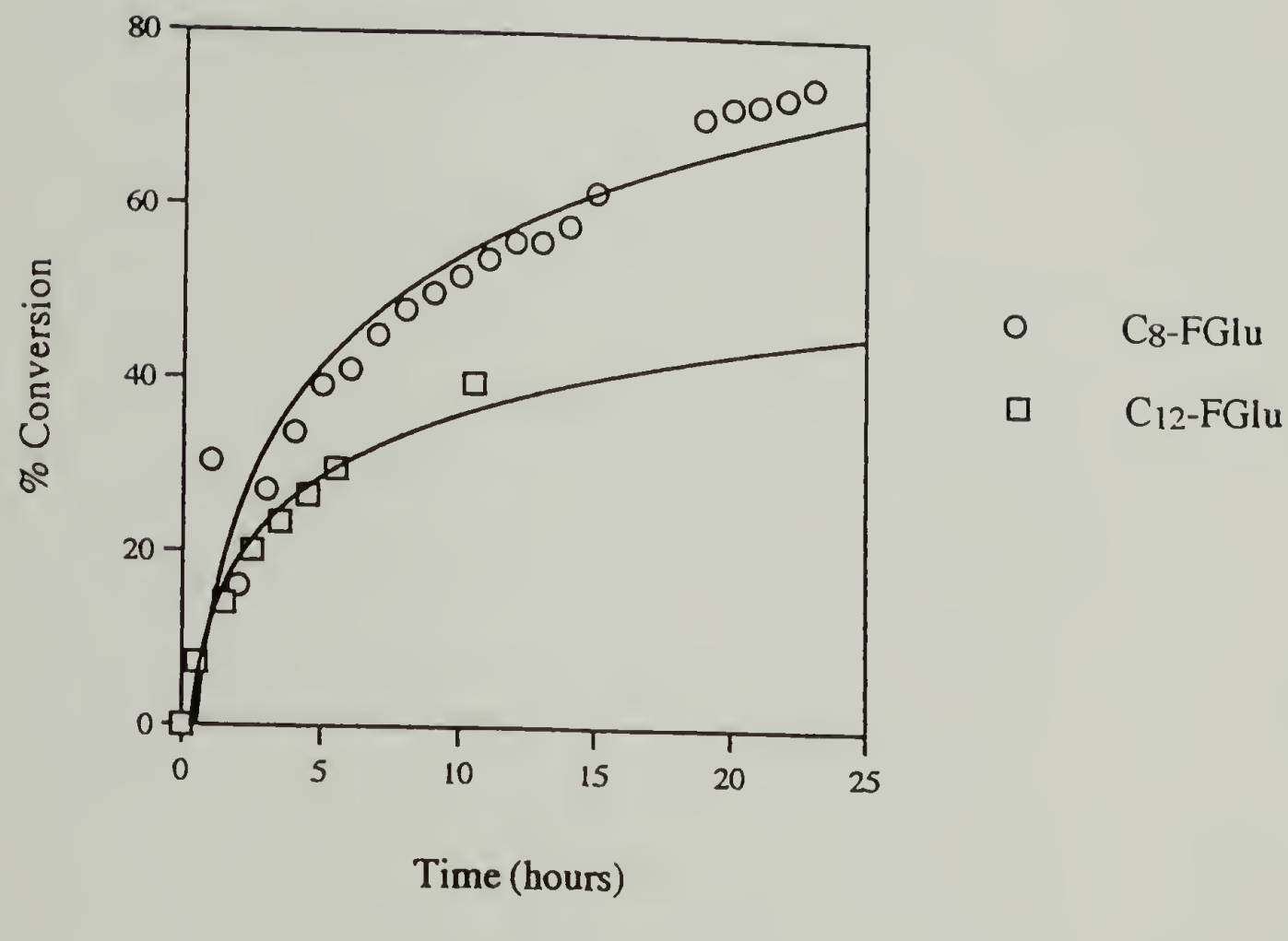
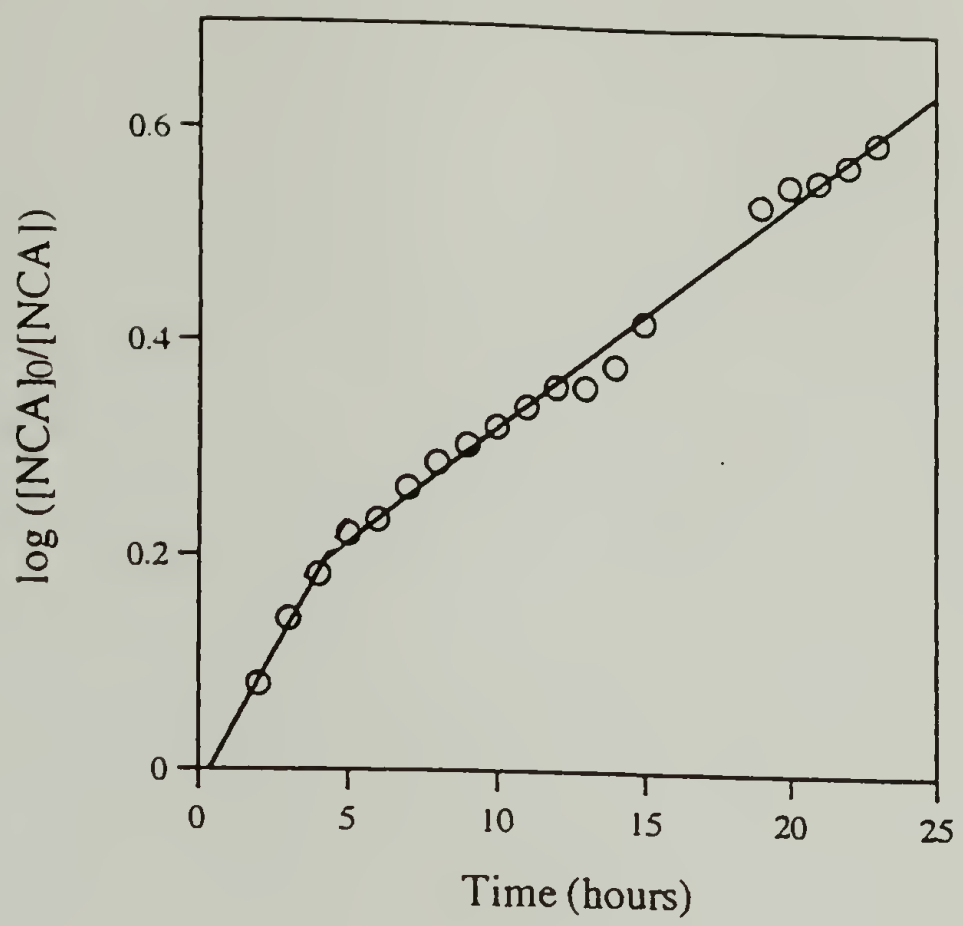


Figure 4.5 % Conversion versus time during the polymerization of C_8-FGlu NCA (0.024 mol/L) and C_{12}-FGlu NCA (0.009 mol/L) in supercritical CO_2.



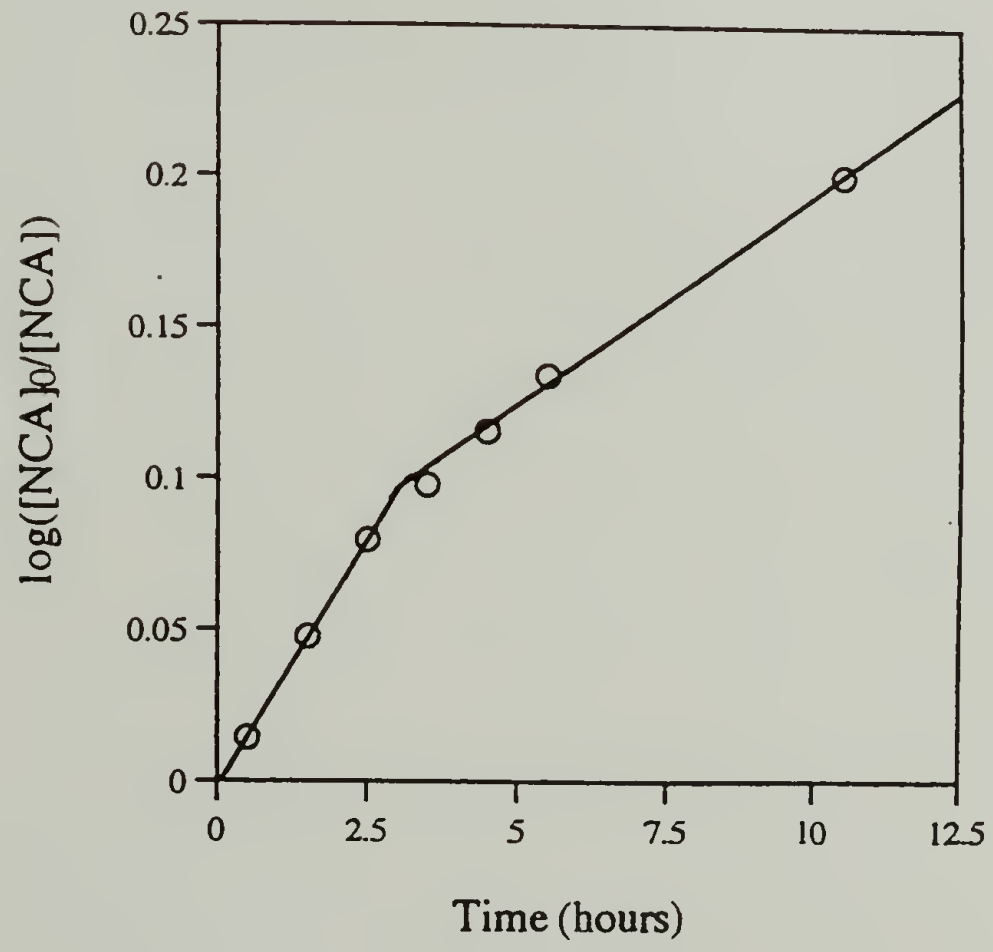


Figure 4.6 Triethyl amine initiated polymerization of a. C_8-FGlu NCA (0.024 mol/L) and b. C_{12}-FGlu NCA (0.009 mol/L) in supercritical CO_2.

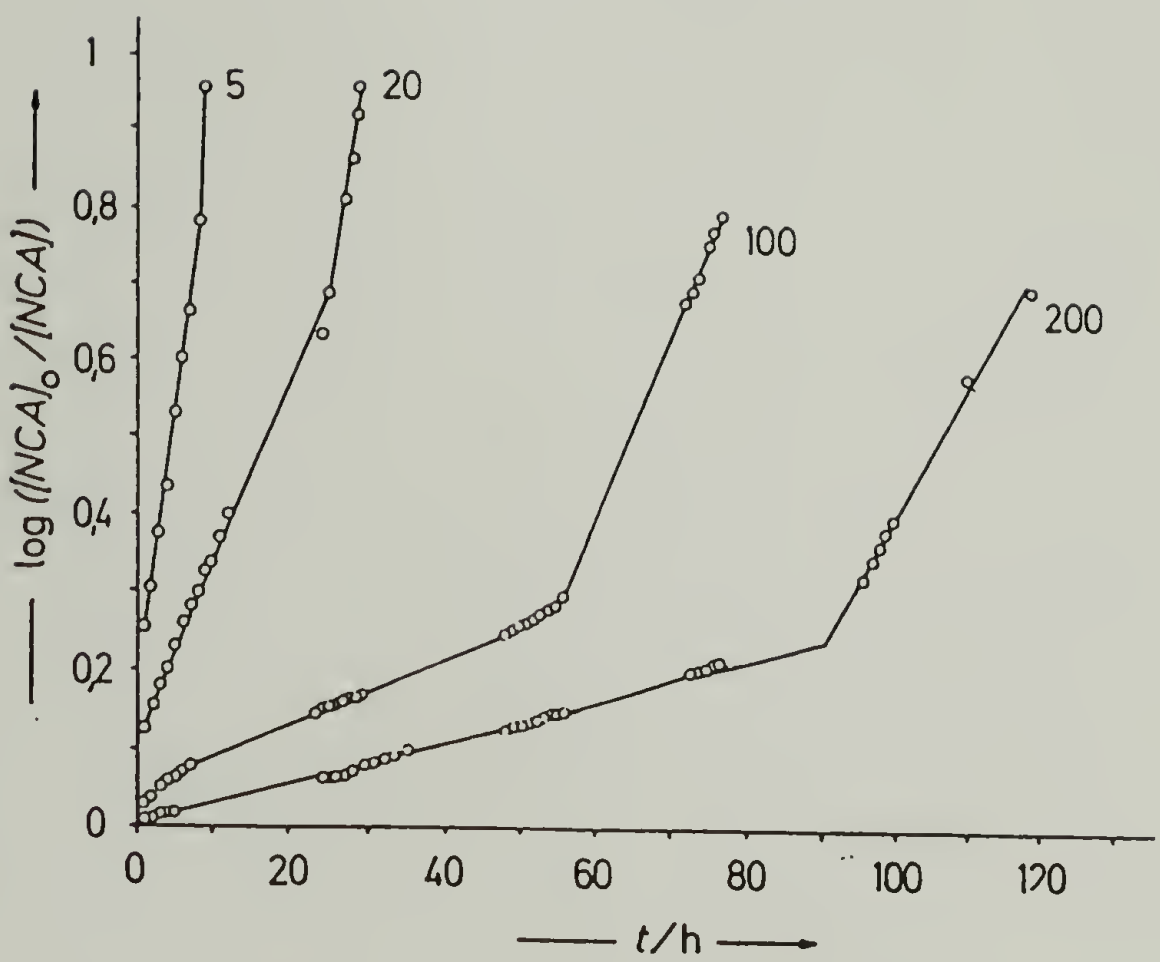


Figure 4.7 Pseudo-first order rate plots for the polymerization of L-phenylalanine NCA (100mmol/L) with N-methylbenzylamine as initiator in N, N-diethylformamide at 25 °C. numbers indicate the initial monomer/initiator ratios[20].

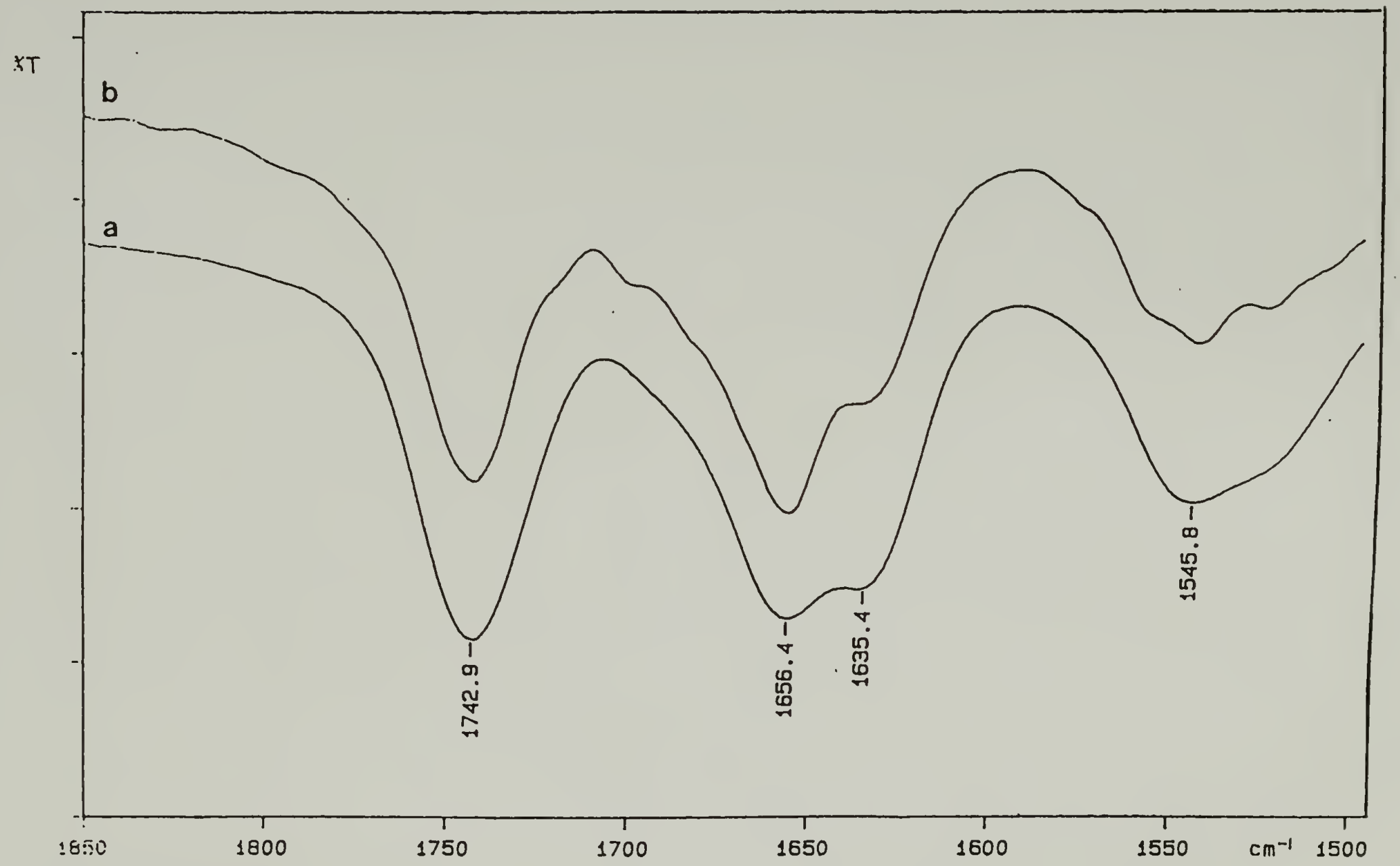


Figure 4.8 Solid state IR spectra (KBr) of C_8-FGlu polypeptides synthesized in a. THF and b. supercritical carbon dioxide.

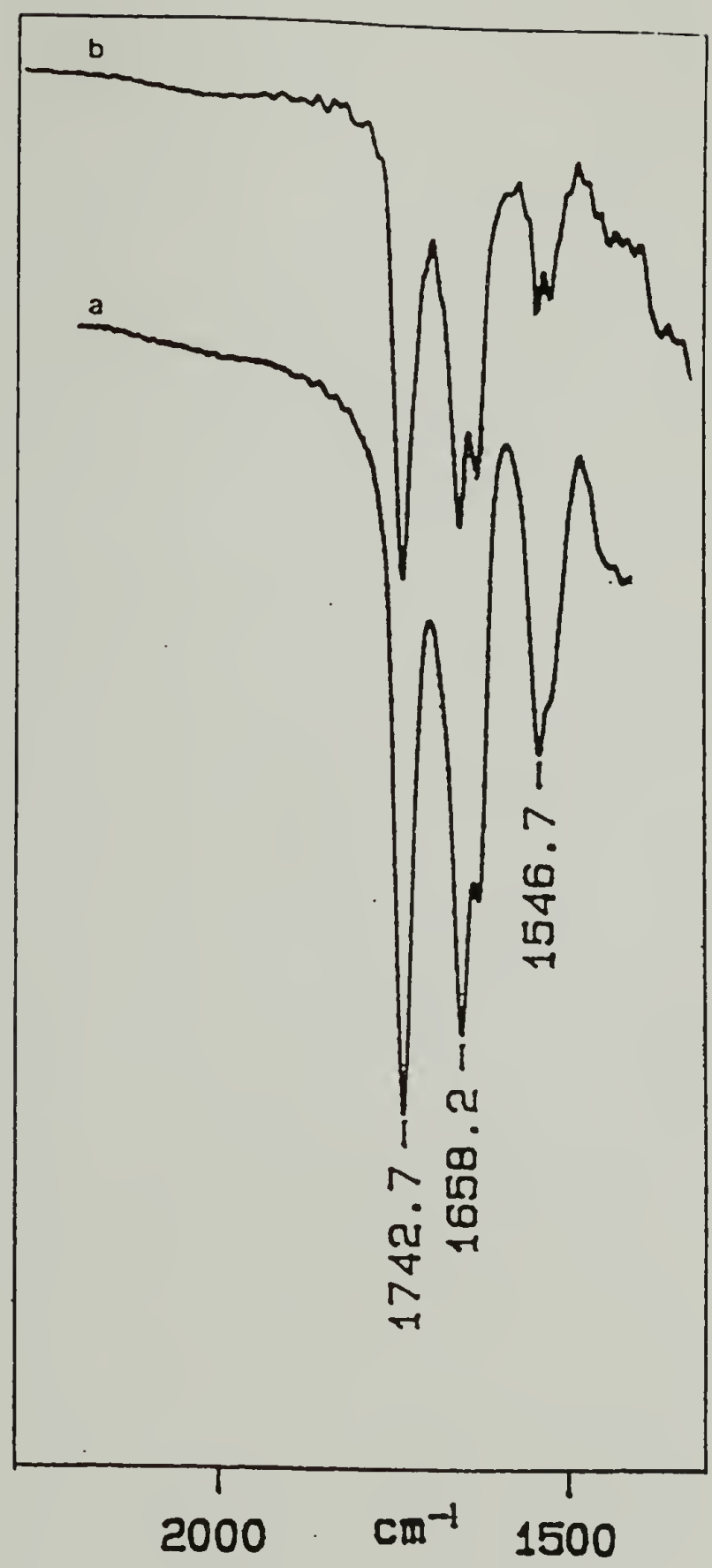


Figure 4.9 Solid state IR spectra (KBr) of C_8-FGlu polypeptides synthesized in supercritical carbon dioxide using a. triethyl amine (TEA) and b. pyridine as initiators.

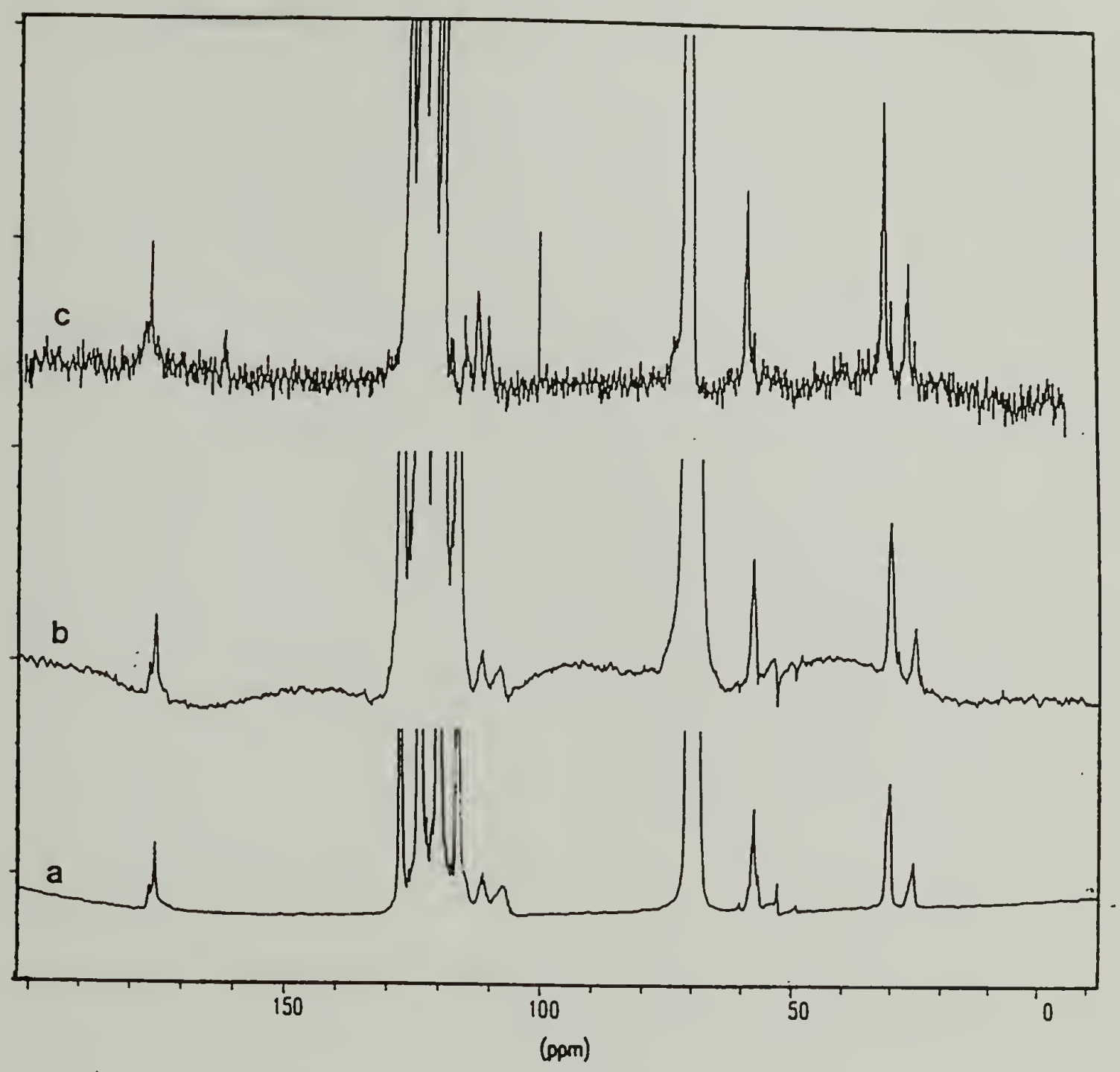
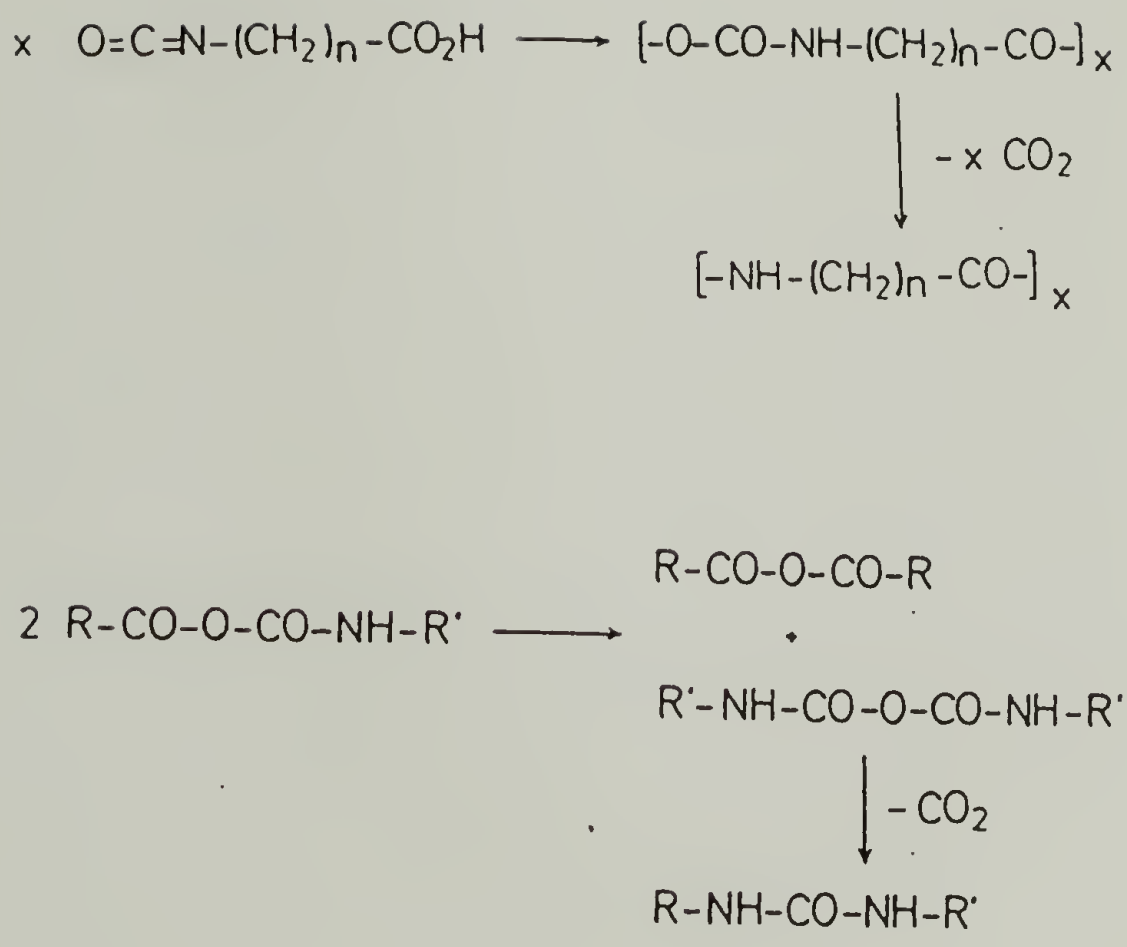
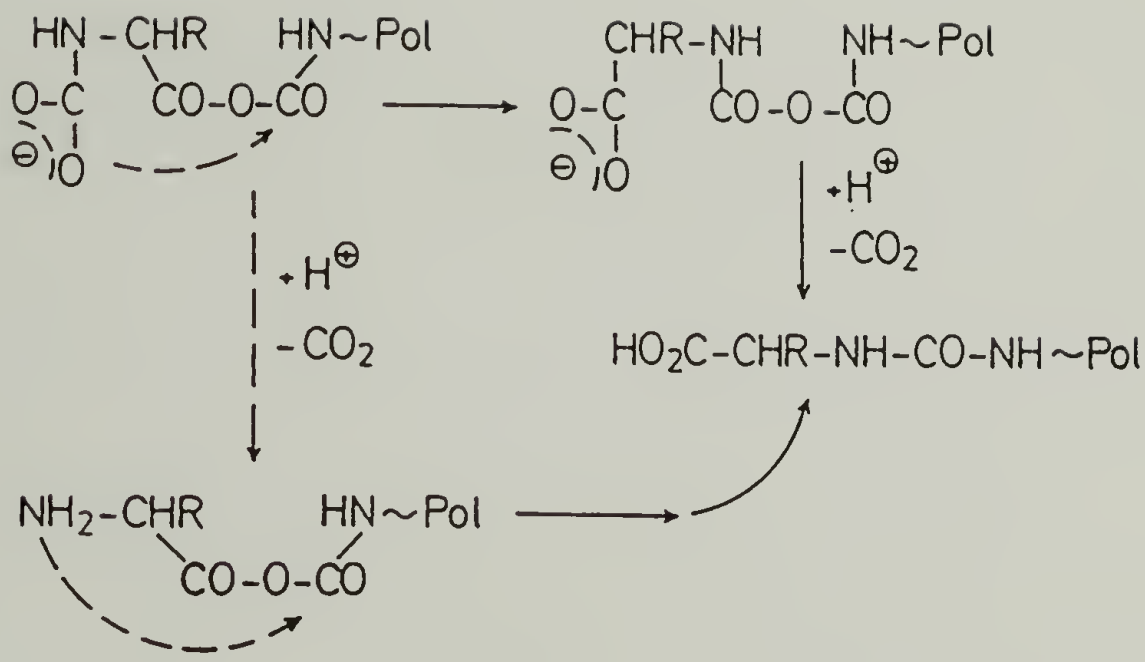


Figure 4.10 ^{13}C NMR spectra of C_8-FGlu polypeptides synthesized in a. THF using triethyl amine as initiator (75.43 MHz ^{13}C), b. supercritical carbon dioxide using triethyl amine as initiator (75.43 MHz ^{13}C), and c. supercritical carbon dioxide using pyridine as initiator (125.72 MHz ^{13}C).



Scheme 4.1 Linear anhydrides forming during the addition of carboxylic acids to isocyanates decarboxylate giving polypeptides but also ureas and symmetric anhydrides[8].



Scheme 4.2 Termination of carbamate propagation by the formation of hydantoic acid end groups[8].

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