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Trifluoroalanine *N*-Carboxy Anhydride: A Reactive Intermediate for the Synthesis of Low Surface Energy Polypeptides

E. Dessipri and D. A. Tirrell*

Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

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ABSTRACT: D,L-Trifluoroalanine *N*-carboxy anhydride (D,L-TFANCA) was synthesized by phosgenation of 3,3,3-D,L-trifluoroalanine in tetrahydrofuran. ^{1}H 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. D,L-TFANCA was polymerized with aniline, thiophenol, or triethylamine as initiator. 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 L-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.

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 *p*-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, nonadhesiveness, 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.[7-12] In particular 3,3,3-D,L-trifluoroalanine has been studied as a suicide inhibitor of pyridoxal phosphate dependent enzymes.[13] 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 also been 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 the solvent exposure of specific functional groups and therefore conformations of fluorinated molecules.[7].

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.[3-6] 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.

Experimental Section

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 universal UV lamp Model 51402 operating at 254 nm, the ninhydrin test, and the Cl_2/toluidine test. Column chromatography was performed with the use of silica gel 60 (Merck mesh size 230–400).

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^{1}H NMR spectra were recorded on Bruker AC-200 (200 MHz ^{1}H), Varian XL-200 (200 MHz ^{1}H), and Varian XL-300 (300 MHz ^{1}H) 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 the 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 the X-ray source.

Time of flight matrix assisted laser desorption mass spectra were acquired at Millipore Corp. The spectrometer, custom built at Millipore Corp., 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 2 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 oligo(trifluoroalanine) 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 four measurements.

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 2 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**, bp 123–126 °C (lit.[15] 40–41 °C/21 mmHg). ^{1}H NMR ($CDCl_3$): δ 3.58 (s, 3H, OCH_3), 3.90 ppm (s, 3H, $COOCH_3$). ^{19}F NMR ($CDCl_3$): δ −11.3 (s, CF_3), 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 min. 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, bp 85–87 °C (lit.[15] 84–86 °C). ^{1}H NMR ($CDCl_3$): δ 4.99 (s, 1H), 4.97 ppm (s, 1H). ^{19}F NMR ($CDCl_3$): δ −5.0 (s, 1F), −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) was obtained. TLC analysis (ethyl acetate/hexane/acetic acid 9/1/.1) showed one only spot R_f = 0.73, mp 76–78 °C (lit.[14] 67 °C). ^{1}H NMR (acetone-d_6): δ = 1.40 (s, 9H, $OC(CH_3)_3$), 3.83 (s, 3H, OCH_3), 6.42 (s, broad, NH), 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, 0.098 mol) was dissolved in 350 mL of 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 one 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, bp 70 °C/0.6 Torr (lit.[14] 49 °C/0.2 Torr). ^{1}H NMR ($CDCl_3$): δ 1.61 (s, 10H, $OC(CH_3)_3$), 3.98 ppm (s, 3H, OCH_3). ^{19}F NMR ($CDCl_3$): δ 0 ppm.

Table 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 for 14 days. [b] Polymerizations in dioxane were run for 22 days. [c] Weight average degree of polymerization as determined by TOF-MALDMS.

Table 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

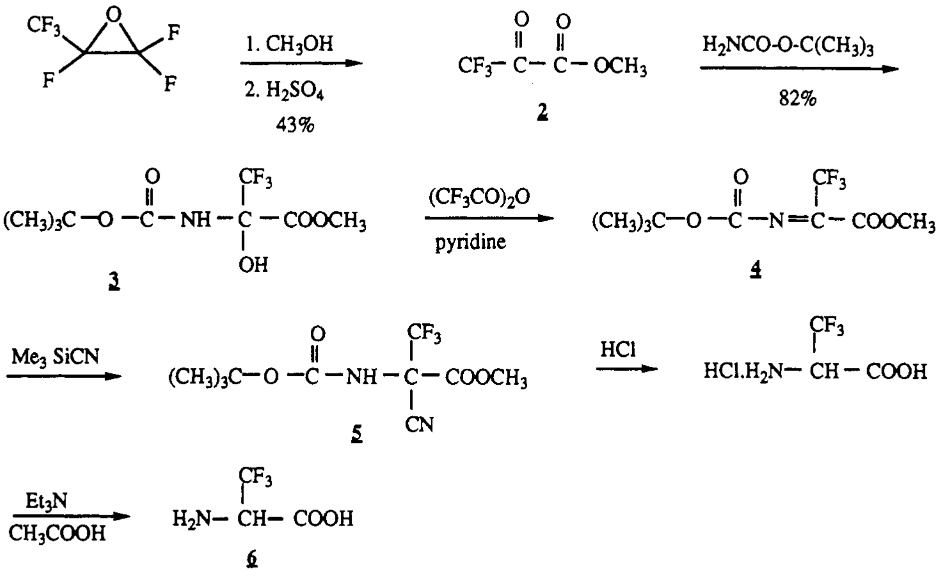
[a] Copolymerizations were run for 25 days. [b] Molar ratio of trifluoroalanine NCA:γ-benzyl glutamate NCA. [c] From elemental analysis. [d] Based on elemental analysis for % F. [e] Intrinsic viscosities (dL/g) in dichloroacetic acid at 25.2 °C. [f] Advancing water contact angles on films cast from DMSO.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

$(CH_3)_3COCONHC(CN)(CF_3)COOCH_3$ (5). Trimethylsilyl cyanide (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 h. Excess trimethylsilyl cyanide 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 of 0, 0.21, 0.33, and 0.39. Evaporation of chloroform gave 20 g (0.071 mol, 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 a 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 nondetectable by NMR. The solvents were evaporated to give **5** as an oily colorless product. ^{1}H NMR (acetone-d_6): δ 1.41 (s, 9H, $OC(CH_3)_3$), 3.98 (s, 3H, OCH_3), 8.46 ppm (s, broad, NH). ^{19}F NMR (acetone-d_6): δ +2.1 ppm.

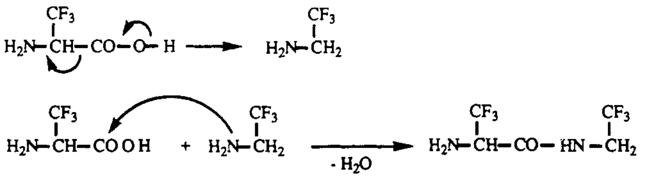
$H_2NCH(CH_3)COOH$ (6). Crude **5** (20 g, 0.071 mol) was heated to 80 °C for 8 h with 70 mL of concentrated HCl. After cooling to room temperature, the mixture was extracted with 200 mL of ether. The aqueous phase was evaporated to give 16 g of yellow solid. The solid was suspended in chloroform and 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, mp >210 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.1 (q, CH), 7.0 ppm (s, broad, NH_2 and COOH). ^{13}C NMR (DMSO-d_6): δ 55 (CH), 124 (CF_3), 165 ppm (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 (0.715 g, 5 mmol) was suspended in 5 mL of dry THF. Triphosgene (0.495 g, 5 mequiv) dissolved in 1 mL of 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 1 h 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 a reduced pressure. More dry THF was added and reevapo-

Scheme 1. Synthesis of 3,3,3-D,L-trifluoroalanine (Overall Yield of Last Four Reactions = 30%)



Scheme 2. Reaction Pathway That Can Account for the Decomposition of Trifluoroalanine Observed by ^{1}H NMR Spectroscopy (Figure 1)



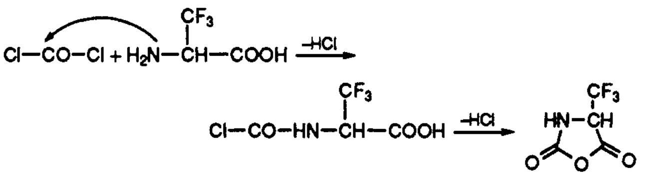
rated twice. The anhydride was very soluble in THF but partially crystallized in the form of white needles when most of the solvent was evaporated. After standing in the freezer overnight D,L-TFANCA (3.7 mmol, 75% yield) was obtained by filtration as a light yellow solid. ^{1}H NMR (DMSO-d_6): δ 5.6 (q, CH), 10.3 ppm (s, NH). ^{13}C NMR (DMSO-d_6): δ 58.7 (q, CH), 121.5 (q, CF$_3$), 151.4 (s, CO-5), 162.8 ppm (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 out, washed with water, and dried. The results are given in Table 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.

Results and Discussion

Synthesis and Chemistry of TFANCA. D,L-Trifluoroalanine was conveniently prepared on a 5-g scale via the route shown in Scheme 1.[14,15] Solutions of the amino acid proved to be labile; Figure 1 compares ^{1}H NMR spectra of DMSO solutions of D,L-trifluoroalanine taken immediately after preparation (Figure 1a) and after 50 days at room temperature (Figure 1b). Partial decarboxylation of trifluoroalanine followed by attack of the resulting trifluoroethylamine on the remaining amino acid (Scheme 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 ref 16 for natural amino acids was followed. The suspension of trifluoroalanine became clear within 15 min after addition of triphosgene at room temperature. We have performed the phosgenation of other amino acids and observed (in agreement with ref 16) 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:[3]



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 toward 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

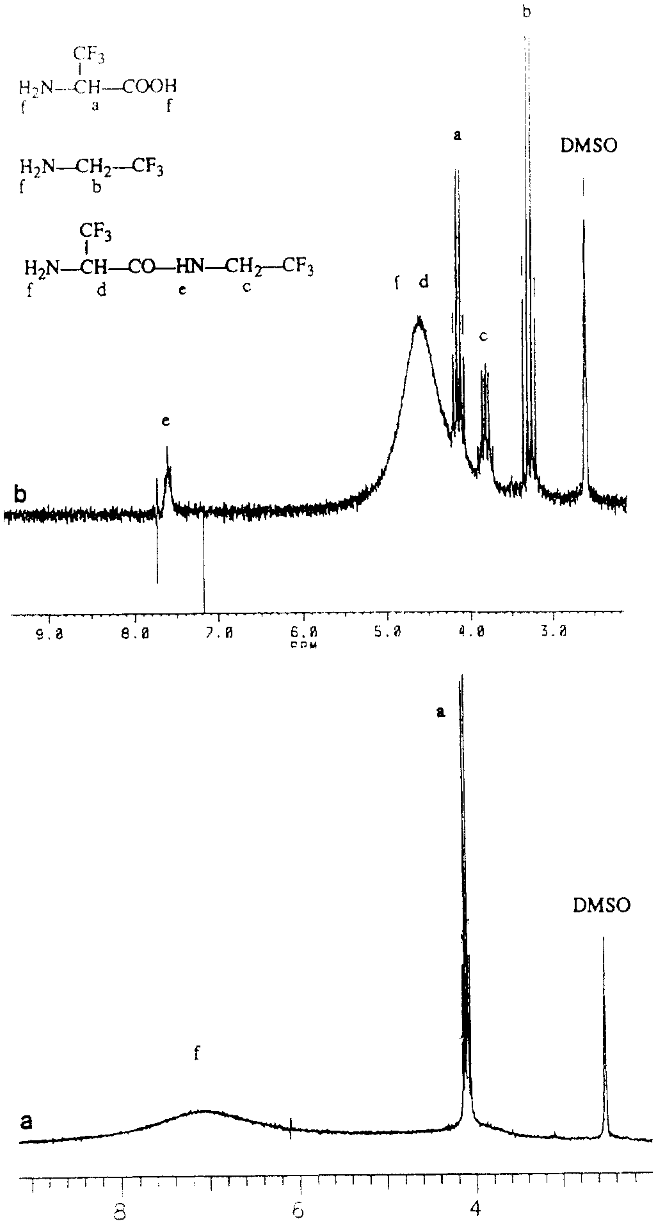


Figure 1. (a) 300-MHz ^{1}H NMR spectrum of trifluoroalanine in DMSO-d_6 immediately after the preparation of the solution. (b) 200-MHz ^{1}H NMR spectrum of the same sample after 50 days.

shifted by ca. 20 cm^{-1} to higher frequency (to 1875 and 1800 cm^{-1}) than in common α amino acid NCAs,[3] indicative of higher electrophilicity. Figure 2 shows the ^{1}H 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 h after the preparation of the solution in DMSO but is much slower in DMF. As shown in Figure 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).

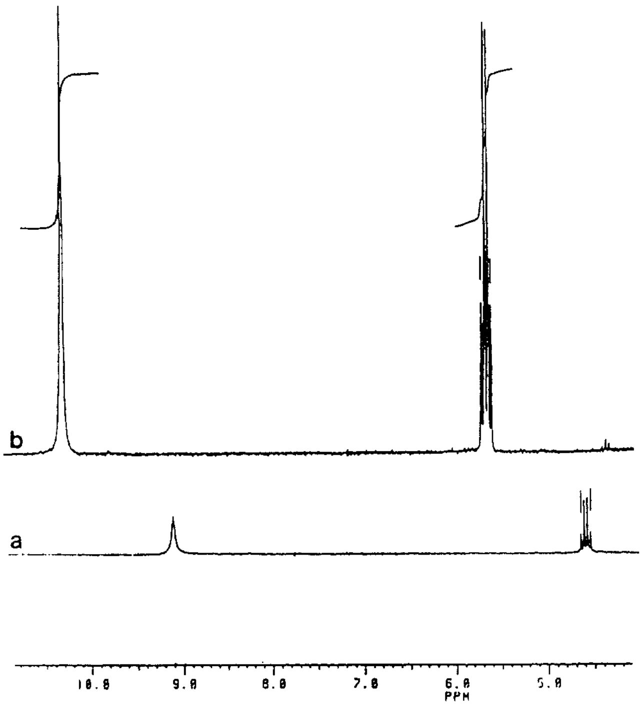
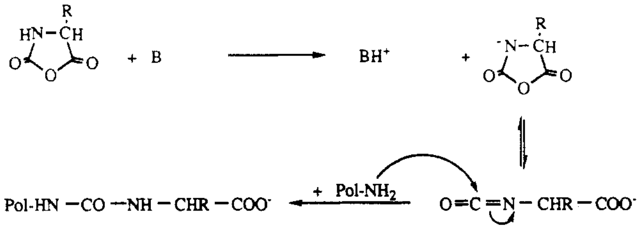


Figure 2. 200-MHz ^{1}H NMR spectra in DMSO-d_6 of (a) alanine NCA and (b) TFANCA.

Scheme 3. Termination via Hydantoic Acid Formation



Polymerization of D,L-TFANCA. Preliminary polymerization experiments with TFANCA were performed in dioxane using triethylamine, benzylamine, triethylaluminum, or sodium methoxide as initiator (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 3.[3] 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 the initiator. Thiophenol is a relatively good nucleophile (nucleophilic constant n_{CH_3I} = 5.70[18]) but is not basic. Polymerizations of D,L-TFANCA were also performed with aniline which has a similar nucleophilicity but a higher basicity (n_{CH_3I} = 5.70, pK_a of conjugate acid in methanol 4.58[18]), and with triethylamine, which is a good base (n_{CH_3I} = 6.66, pK_a of conjugate acid in methanol 10.7[18]). The polymerizations were carried out in dioxane and THF and were monitored by infrared

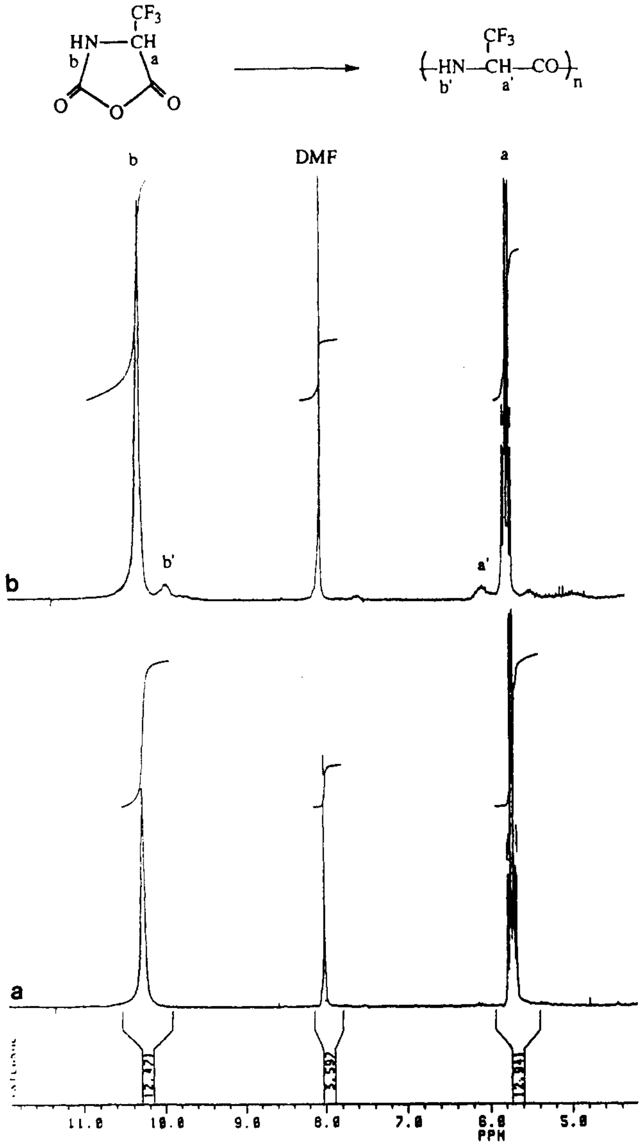


Figure 3. 200-MHz ^{1}H NMR spectra of trifluoroalanine NCA in DMF-d_7 (a) immediately after the preparation of the solution and (b) 7 days later.

spectroscopy. After complete consumption of the anhydride the products were precipitated in water, washed, and dried. The results are shown in Table 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.[3] For example poly(L-lysine) obtained using *n*-butylamine as the 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 1) is shown in Figure 4. All of the oligomers 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 the signal at *m*/*z* 1519 corresponds to a dodecamer (12 × 125 + 18 = 1518) and the next (*m*/*z* 1643) is the corresponding tridecapeptide (13 × 125 + 18 = 1643). Termination via hydantoic acid formation would result in chains 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 5 shows the mass spectrum obtained from the polymer prepared using thiophenol as the initiator (run 3, Table 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 signal at *m*/*z* 811 corresponds to a hexamer terminated via hydantoic acid formation (6 × 125 + 18 + 44 = 812), and that at *m*/*z* 934.9 may be the corresponding heptamer. However, all of the signals 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

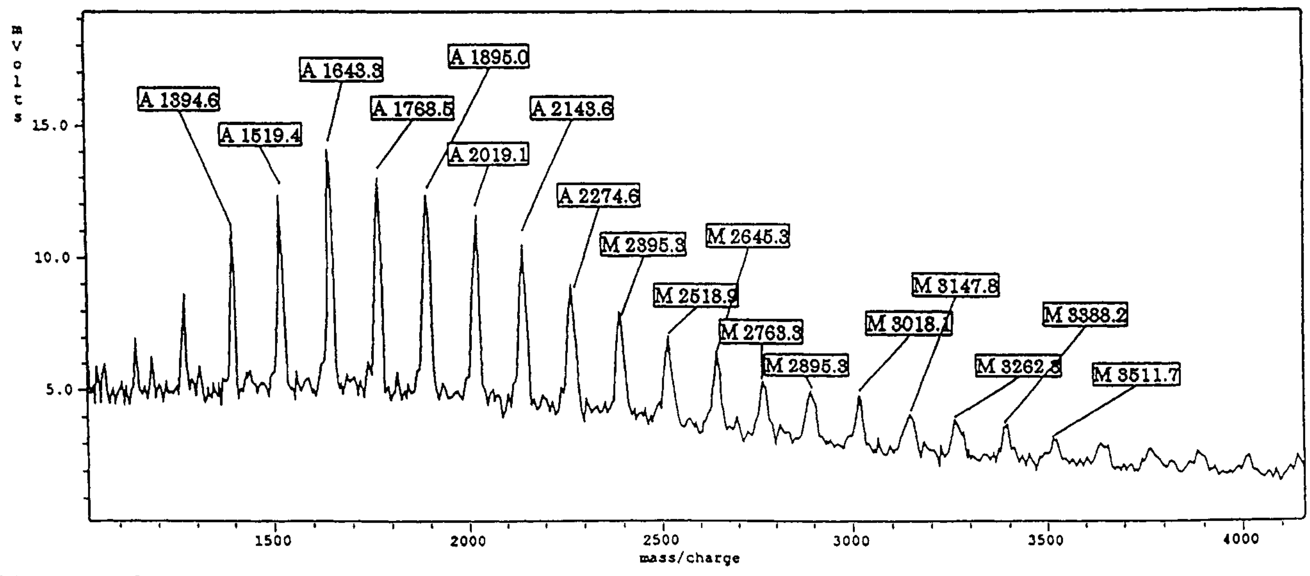


Figure 4. TOF-MALDMS of poly(trifluoroalanine) synthesized in THF with triethylamine as the initiator.

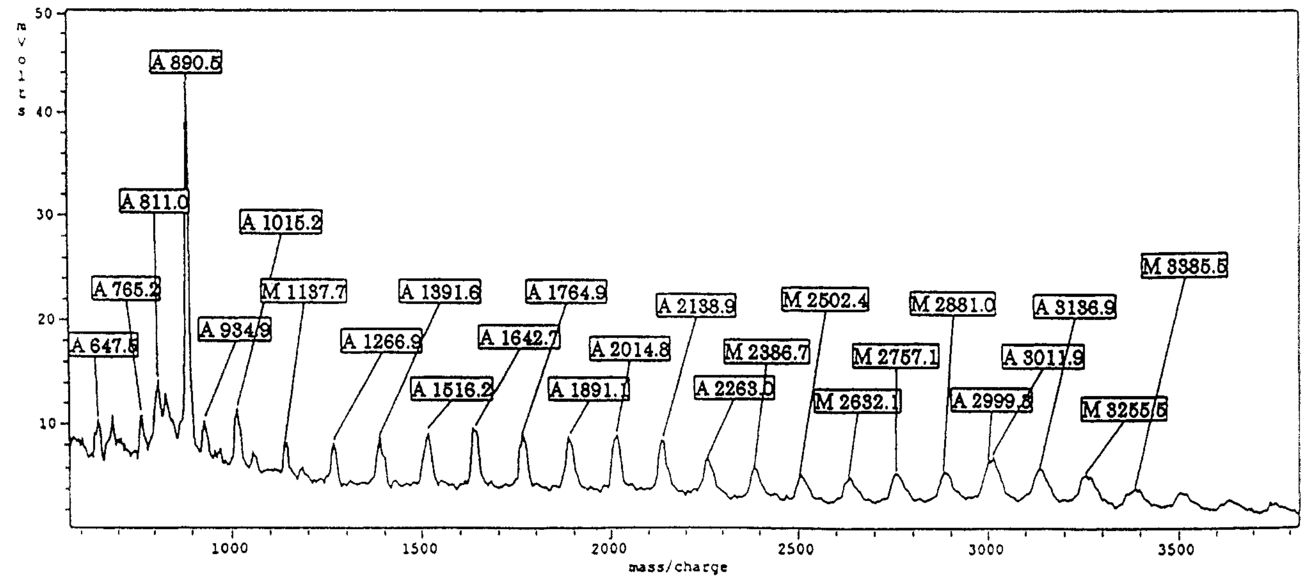


Figure 5. TOF-MALDMS of poly(trifluoroalanine) synthesized in dioxane with thiophenol as the initiator.

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,[3] 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.

Figure 6. 200-MHz ^{1}H NMR spectra 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 h.

The absence of thiophenol end groups was surprising, especially because previous experiments had indicated that thiophenol does indeed attack TFANCA. Figure 6 shows ^{1}H NMR spectra of (a) thiophenol, (b) thiophenol and TFANCA a few minutes after the preparation of the solution, and (c) the same sample after 18 h. As thiophenol reacts with the NCA, the aromatic proton resonances shift downfield from 7.1–7.5 to 7.4–7.7 ppm. After 18 h 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 out and dried. Figure 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 1). These spectra show clearly that thiophenol is incorporated predominantly into the chains that remain in the supernatant and are presumably of a 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 end group hydrolysis. The presence of thiophenol attached essentially only to the lower molecular weight species 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-(trifluoromethyl)amino acid NCAs leads to high yields of dipeptides, without significant chain extension via homopolymerization of the fluorinated anhydrides.[20] 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 4. The NMR data

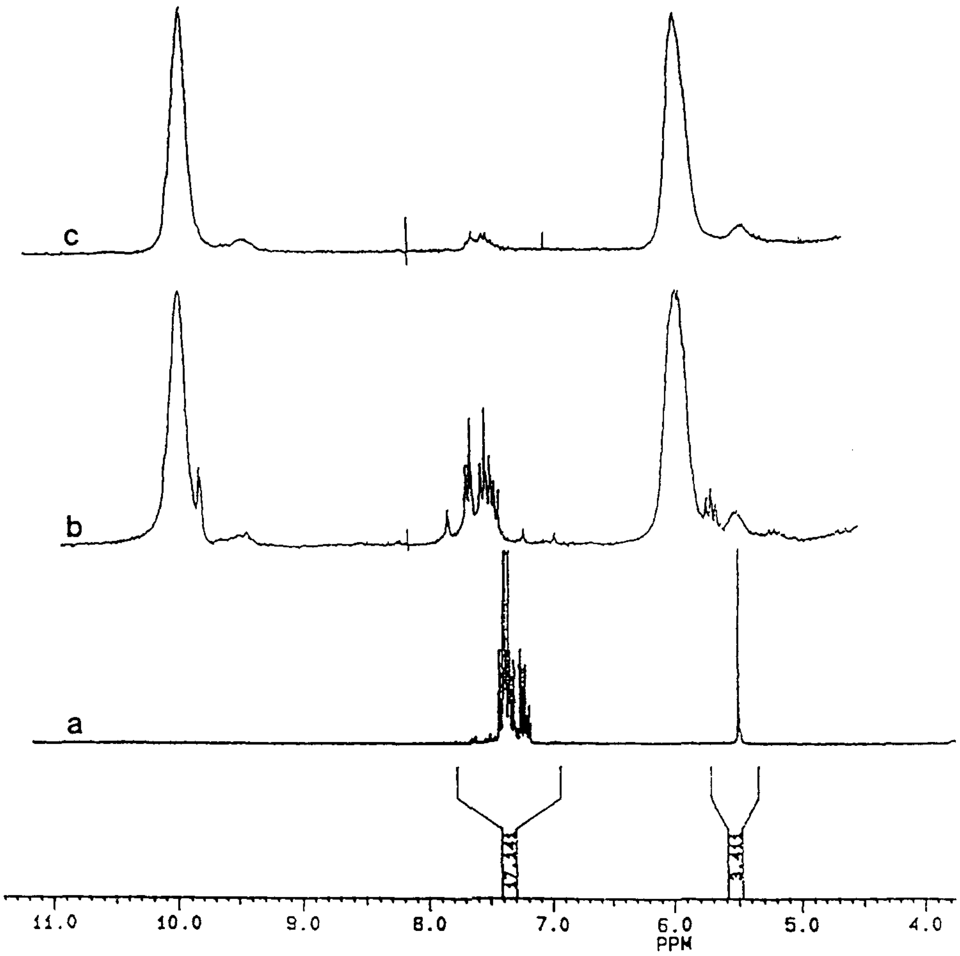
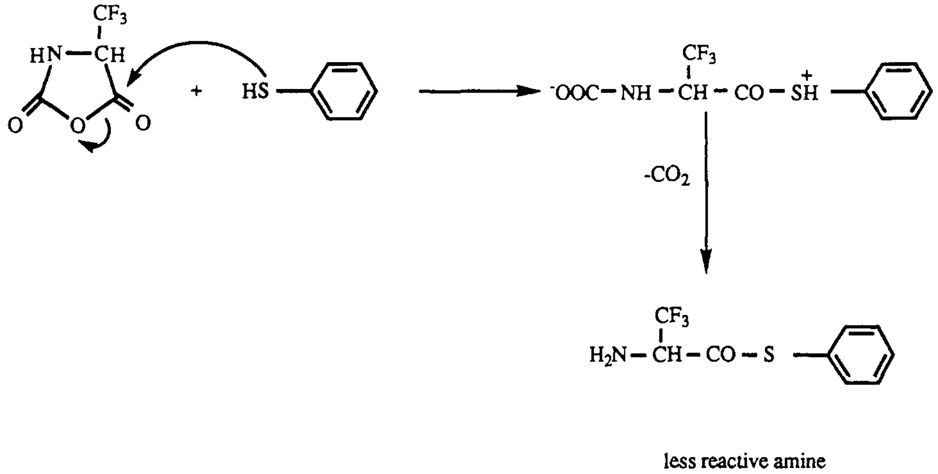


Figure 7. 200-MHz ^{1}H NMR spectra in DMSO-d_6 of (a) free thiophenol, (b) the product remaining in the supernatant after precipitation with H_2O, and (c) the precipitated polypeptide.

Scheme 4. Reaction of TFANCA with Thiophenol



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 also been postulated to be taking place in such systems.[6,17] 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.

Copolymerization. D,L-TFANCA was copolymerized with γ-benzyl L-glutamate NCA in THF using thiophenol as initiator to give polypeptides of molecular weights 25 000–50 000, as estimated from intrinsic viscosity measurements using the Mark-Houwink constants for PBLG.[19] The products are soluble in dichloroacetic acid (in contrast to oligo(trifluoroalanine)), 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.

Surface Structure and Properties. Thin films of the trifluoroalanine homopolymer on glass plates were made by slow evaporation of dilute solutions of the polymer

Table 3. XPS Data for the Copolymer from Run 4 (Table 2)

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

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

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 incoroporation of the fluorinated monomer (Table 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 are means of four measurements. The receding contact angles were found to vary with the time between advancing and receding contact angle measurements, 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 the trifluoroalanine homopolymer determined at 15 and 75° takeoff 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, are shown in Table 3. The atomic ratio of fluorine to nitrogen at the 15° takeoff 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° takeoff angle (ca. 40-Å probe depth) the difference is considerably smaller.

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