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Models of Biopolymers by Ring-Opening Polymerization

Edited by
Stanislaw Penczek

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PREFACE

There are three major classes of biopolymers, namely, polypeptides (poly(α-amino acids)s), polysaccharides (polyacetals), and nucleic acids (polyesters of phosphoric acid). Similar backbones also have teichoic acids. There are also less prominent polymers of α-hydroxy acids, serving as the food reserve of some bacteria.

All of these polymers are produced in living organisms and play important structural and/or functional roles.

Evolution has chosen these particular chemical structures to perform highly specialized functions.

Long polymer chains are required because they can provide not only the unique chemical structures but also, due to the special interactions in solution and in the solid state, they provide several additional levels of hierarchical order.

Understanding the primary chemical structures of biopolymers as well as their secondary and tertiary structures is one of the major responsibilities of science. Models of biopolymers, sometimes simplified analogs of the native products, help to realize how the biopolymers are built and how they function.

Ring-opening polymerization has been used since the turn of the century to prepare some of these models. Synthesis of polypeptides in this way has the oldest history, going back to the work of Leuchs in 1906. Many years later came the method of the ring-opening polymerization of anhydro sugars, providing polysaccharides, including synthetic dextran.

The history of the polymerization of cyclic phosphorus containing monomers is the youngest one. The first high molecular weight poly(alkylene phosphate) was prepared by ring-opening polymerization in this author's laboratory in 1976.

The models of the above discussed biopolymers allowed us to understand a number of structural and functional features of biopolymers. On the other hand, some of the simplified models have been prepared in order to mimic functions, using only some of the structural elements of biopolymers. These simplified polymers, belonging rather to the group of bioanalogous polymers, have then been used in practice. Here belongs, for instance, artificial (synthetic) skin based on polypeptides, or poly(alkylene phosphate)s-specifically binding ions (by analogy with teichoic acids).

In this monograph, four chapters are written by authors active in the covered areas. Preparation (by ring-opening polymerization), properties, and some applications of the models of biopolymers are described. This is the first comprehensive treatment of the major classes of biopolymers in one monograph.

Ring-opening polymerization continues to be the most versatile method of synthesis of these four major groups of biopolymers, particularly when they are required in quantity. Some of these synthetic methods will certainly compete in the future with the new methods brought by biotechnology.

Stanislaw Penczek

THE EDITOR

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Professor St. Penczek graduated in 1963 from the Leningrad Institute of Technology, USSR, and presented habilitation (D.Sc.) at the Lodz Technical University in 1970. He is Chairman of the Section of Polymers, Polish Chemical Society, Member of the Editorial Boards of *Die Makromolekulare Chemie* (Basel), *Progress in Polymer Science* (Oxford), *British Polymer Journal*, and Member of the Polymer Division, A.C.S.

Professor St. Penczek served as Visiting Professor in CRM-CNRS in Strasburg, Universities in Mainz, Cleveland, Kyoto, and Ghent. He presented invited lectures in several IUPAC Congresses and Symposia and numerous guest lectures at Universities and Institutes in Europe, Japan, and North America. His current major research interests include kinetics, thermodynamics and mechanisms of the polymerization processes, and synthesis of bioanalogous polymers.

I wish to thank the Polish Academy of Sciences, where the new polyphosphate models of biopolymers have been developed (grant 01.13). I wish also to thank the CRC Staff for converting the original language of the chapters into better readable English.

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Chapter 1

POLYPEPTIDES

Hans R. Kricheldorf

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ABBREVIATIONS

Moc = Methoxycarbonyl
BPA = Bovin plasma albumin
DCA = Dichloroacetic acid
FA = Formic acid
MSA = Methanesulfonic acid
TFA = Trifluoroacetic acid
Bzl = Benzyl
DMF = Dimethylformamide
Et = Ethyl
HFI = Hexafluoroisopropanol
Hiba = α-Hydroxyisobutyric acid
Me = Methyl
PEO = Polyethylenoxide
THF = Tetrahydrofuran
Z = Benzyloxycarbonyl
CP/MAS = Crosspolarization/magic angle spinning
CD = Circular dichroism
$\overline{DP}$ = Average degree of polymerization
GPC = Gel permeation chromatography
M/I = Molar monomer/initiator ratio
$\overline{M}_n$ = Number average molecular weight
$\overline{M}_w$ = Weight average molecular weight
MWD = Molecular weight distribution

ORD = Optical rotation dispersion
VPO = Vapor pressure osmometry
α-Aibu = α-Aminoisobutyric acid
Ala = Alanine
Arg = Arginine
Asn = Asparagine
Asp = Aspartic acid
Cys = Cysteine
Gla = Glutamine
Glu = Glutamic acid
Gly = Glycine
His = Histidine
Ile = Isoleucine
Leu = Leucine
Lys = Lysine
Nva = Norvaline
Orn = Ornithine
Phe = Phenylalanine
Pro = Proline
Sar = Sarcosine
Ser = Serine
Thr = Threonine
Trp = Tryptophane
Tyr = Tyrosine
Val = Valine

I. INTRODUCTION

α-Amino acid *N*-carboxyanhydrides (α-NCAs) were first described in 1906 by Hermann Leuchs (Berlin). He obtained the NCAs in the course of the stepwise peptide syntheses when he tried to purify *N*-Methoxycarbonyl or *N*-ethoxycarbonyl amino acid chlorides by distillation. He also observed their polymerization and isolated the first synthetic polypeptides. However, in the years before World War I polymer science did not yet exist; most chemists did not accept the existence of covalently bound macromolecules. For instance, Emil Fischer, the Nobel Prize Laureate of 1902, predicted that polymers with molecular weights above 5000 cannot exist, because he was not able to synthesize polypeptides or polysaccharides with molecular weights above 4000. Therefore, Leuchs formulated his polypeptides as a kind of associates of α-lactams and not as a polymer chain consisting of covalent bonds.

Systematic studies of polymerizations of NCAs and the properties of the resulting polypeptides began with the work of Wessely's group in Vienna in the years around 1925. Since then, the interest of polymer chemists and biochemists in the polymerizations process itself and in the properties of polypeptides has steadily increased. Homo- and copolypeptides prepared from NCAs were used as models or substitutes of proteins in various ways. Most studies in this field concern conformational analyses. The modern characterization of polypeptides and proteins by means of ORD, CD, IR-, UV-, and NMR spectroscopy or X-ray diffraction are largely based on the experience polymer chemists, biochemists, and physicists gathered with synthetic polypeptides. Numerous homo- and copolypeptides were used for systematic studies of the genetic control of the immunogenicity of proteins. Polypeptides also have served as substrates of various enzymes, in particular proteases, and they were used as chiral components of catalysts as synthetic analogs of enzymes. Furthermore, textile fibers consisting of synthetic polypeptides can be used instead of silk or wool. Poly(γ-*O*-

Bzl-L-glutamate) was the first polymer, where a lyotropic, cholesteric liquid crystalline phase was detected.

Recently, the interest in biocompatible and biodegradable materials for various technical, pharmaceutical, and medical applications has been rapidly increasing, and this situation entails a permanent interest in polypeptides prepared from NCA. Although the polymerization of NCAs does not enable syntheses of special amino acid sequences, such as copies of enzymes, it still has the advantage that numerous high molecular weight homo- and copolypeptides are easily accessible without any significant risk of racemization.

II. SYNTHESIS AND CHARACTERIZATION OF α-AMINO ACID *N*-CARBOXY-ANHYDRIDES

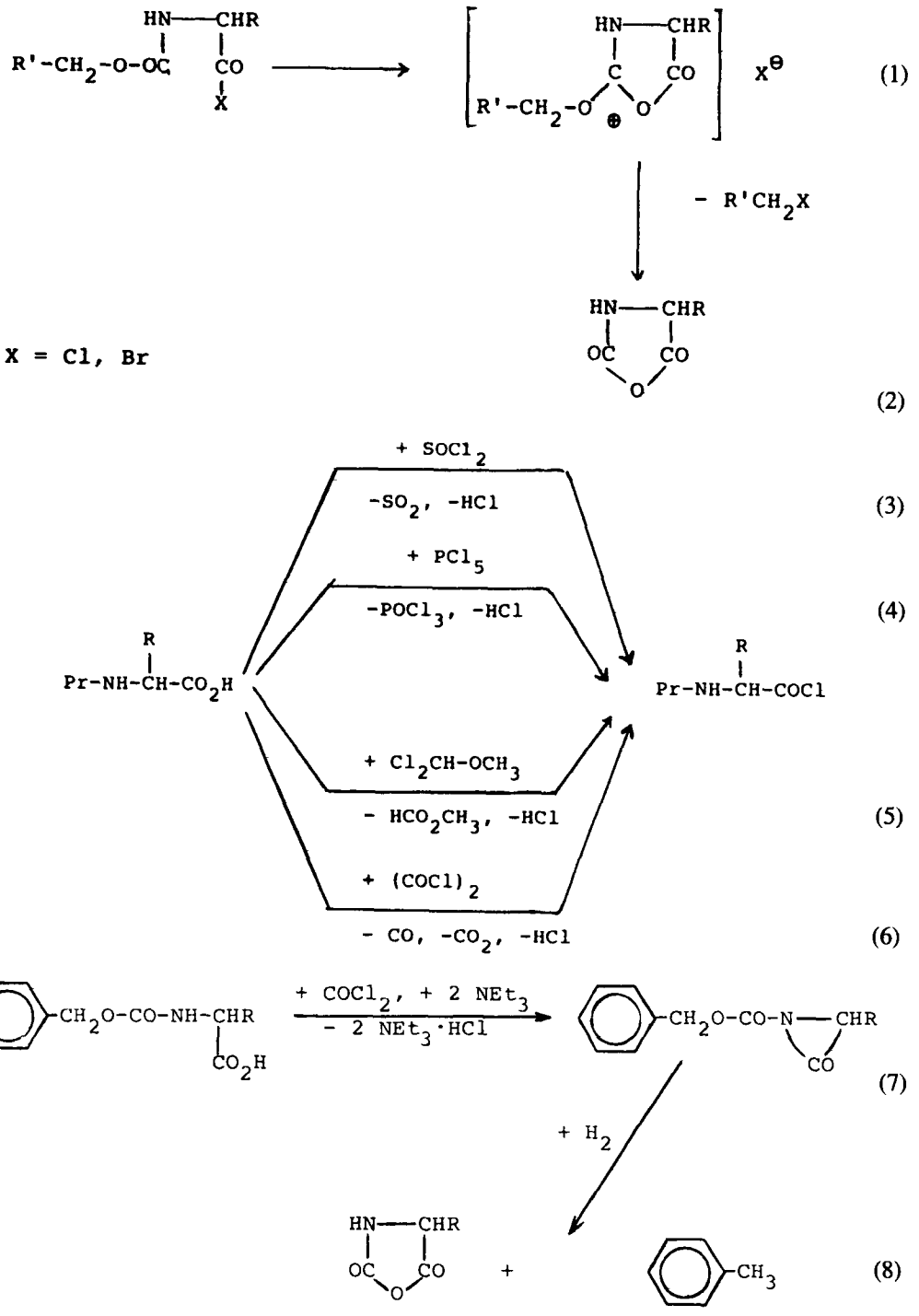
A. NCAs of *N*-Unsubstituted Amino Acids

Two basically different approaches exist for the synthesis of *N*-unsubstituted α-amino acid NCAs:

1. Cyclization of *N*-alkoxycarbonyl-amino acid halogenides, the so-called "Leuchs Method"
2. Phosgenation of free α-amino acids or suitable derivatives, the so-called "Fuchs-Farthing Method"

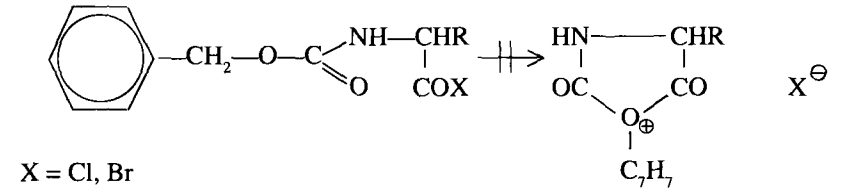
For the synthesis of *N*-unsubstituted β- and γ-amino acid NCAs, a third approach is known, namely the cyclization of β- and γ-isocyanatocarboxylic acids.

The original version of the Leuchs method, i.e., the cyclization of *N*-ethoxycarbonyl or *N*-methoxycarbonyl-amino acid chlorides (Equations 1 and 2) requires prolonged heating at temperatures in the range of 70 to 90°C.[1-3] At these reaction temperatures amino acid NCAs may begin to decompose and hydrogen chloride causes cleavage of the NCA ring. Therefore, several improvements of the Leuchs method were reported. Originally, thionyl chloride was used for the preparation of the acid chloride function.[1-3] This reagent has the advantage of gaseous by-products (Equation 4); yet it requires long reaction times at low temperatures or reaction temperatures >40°C. Phosphorus pentachloride is more reactive,[4] but the phosphorus oxide chloride formed as a by-product (Equation 4) may affect the crystallization of NCAs. Dichloromethyl methyl ether (Equation 5) was recommended[5] for the high purity of the resulting NCAs. However, this reagent was not commercially available in previous decades. The treatment of *N*-benzyloxycarbonyl-amino acid with phosgene yields at low temperature in the presence of triethylamine *N*-benzyloxycarbonyl-aziridine-2-ones which, upon catalytic hydrogenation, yields NCAs (Equations 7 and 8).[7,8] This method is not recommendable for preparative purposes because the phosgenation of the free amino acids gives NCAs in a simpler and more direct way. The most useful halogenating reagent is phosphorus tribromide.[9] The bromide ion is a better leaving group than chloride in the cyclization step (Equation 1), and it is a better nucleophile in the dealkylation step (Equation 2). Therefore, the bromination of *N*-alkoxycarbonyl-amino acids proceeds well even at temperatures below 25°C.

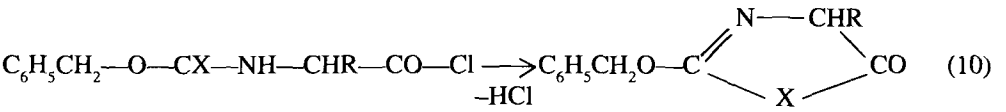


Leuchs had already observed that *N*-methoxycarbonyl-amino acid chlorides cyclize more readily than the ethoxycarbonyl derivatives. Other groups found that *N*-allyloxycarbonyl-[10] or *N*-benzyloxycarbonyl-amino acids cyclize still more rapidly. These findings suggest that the rate-determining step of this NCA synthesis is the alkylation of the halogenide ion (Equation 2), because benzyl derivatives are better alkylating reagents than methyl derivatives which, in turn, are more electrophilic than ethyl groups. However, this consideration also fits an alternative mechanism involving an oxonium ion (Equation 9) which was postulated

by several authors.[11,12] Nonetheless, the formation of such an oxonium ion is unlikely, because the positive charge cannot be stabilized by delocalization in contrast to the carbenium ion depicted in Equation 1. Furthermore, the carbonyl oxygen, which is acylated according to Equation 1, is considerably more nucleophilic than the "ether-oxygen" acylated as in Equation 9. A third mechanism proposed by Goodmann and Kenner[13] postulates the intermediate formation of an azalactone (Equation 10). However, the formation of such an intermediate is unlikely in the absence of a base for two reasons. First, enolization of urethane groups is more difficult to achieve than that of amide groups. Second, alkyloxycarbonyl derivatives of sarcosine and proline yield NCAs, even though they cannot enolize.[14] Additional evidence for the reaction mechanism of Equations 1 and 2 was obtained by cyclization of *N*-ethylmercaptocarbonyl-amino acid chlorides (Equation 11). In this case the stable carbenium chloride was isolated[15] demonstrating that not the nucleophilic sulfur but the carbonyl oxygen was acylated. Also, in agreement with the mechanism of Equations 1 and 2, a typical side reaction of NCAs with thiopyrimidine side chains was found.[16] The high alkylating power of the intermediately formed benzyloxy-carbenium ion results in benzylation of the thioxo group (Equation 12), although this group is not nucleophilic enough to react with free benzyl chloride.



X = Cl, Br (9)



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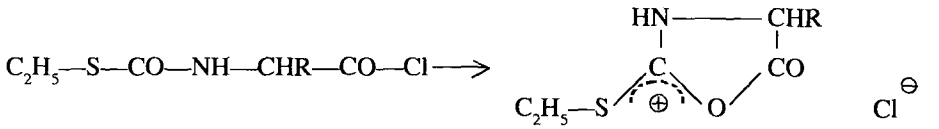
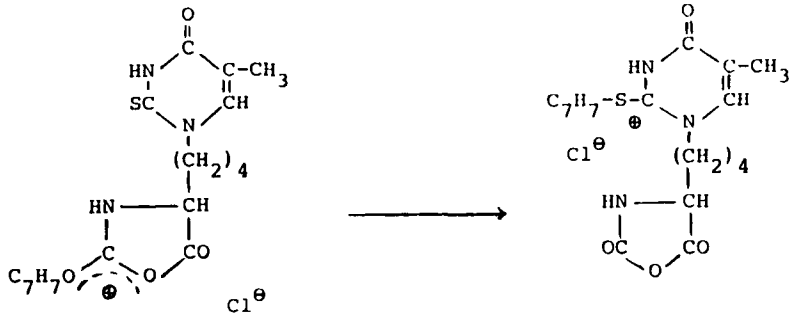


(11)

The "Leuchs method" is not only of historic interest, but it still plays a role for the preparation of special NCAs, as N^{ε}-benzyloxycarbonyl-lysine-NCA,[17,18] N^{δ}-benzyloxycarbonyl-ornithine,[19] arginine-NCA hydrobromide,[20] glutamine- and asparagine-NCA[20,21] or NCAs with nucleobases in the side chain.[16,22,23] Of particular interest is the preparation of N^{ε}-Z-lysine or N^{δ}-Z-ornithine-NCA from the free amino acids, because protection of the ω-amino groups and formation of the NCA ring is attainable in a "one-pot" procedure (Equation 15). This reaction sequence is also an attractive method for the preparation of selectively N^{ω}-protected lysine and ornithine (Equation 16). The preparation of glutamine-NCA with unprotected γ-amide group was reported;[18] however, a sophisticated purification procedure was required and the yield was low. Because the CO-amide groups of glutamine and asparagine react easily with dehydrating reagents to yield nitriles,[24] their protection prior to NCA synthesis is recommendable.[21] A useful variant of the Leuchs method is the conversion of *N*-benzyloxycarbonyl-amino acid trimethylsilyl esters with thionyl chloride.[25] The use of silyl esters increases the solubility of the starting materials in nonpolar solvents such as chloroform or carbon tetrachloride. It eases the reaction with thionyl chloride and prevents the formation of hydrogen chloride. This procedure is recommendable for the synthesis of Gly-NCA and α-Aibu-NCA, because in both cases the phosgenation of the free amino acids is difficult to achieve, in particular when quantities >0.1 mol are to be synthesized.



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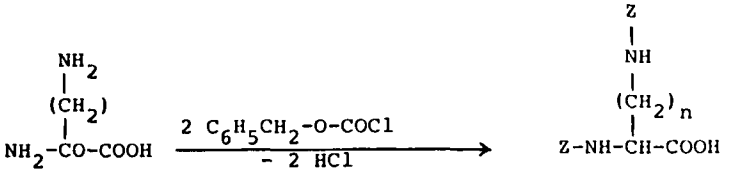

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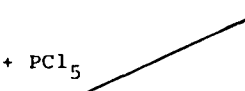

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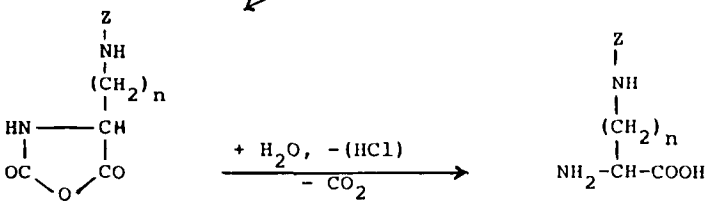

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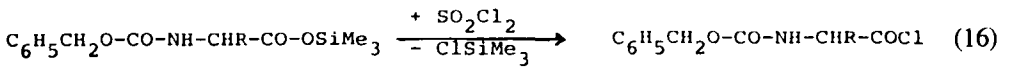
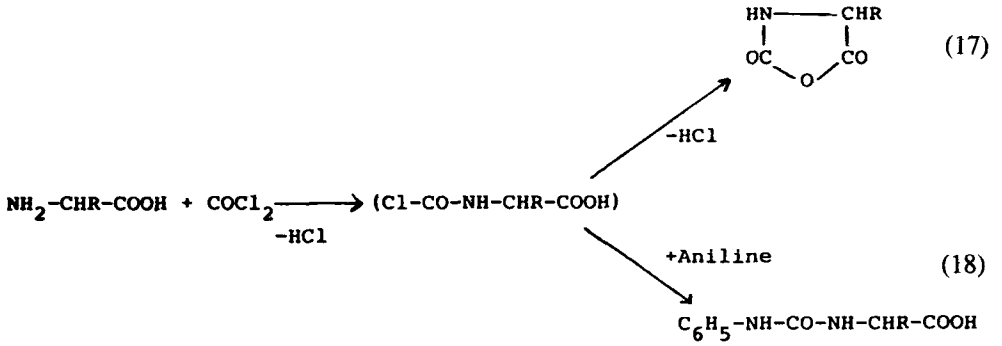
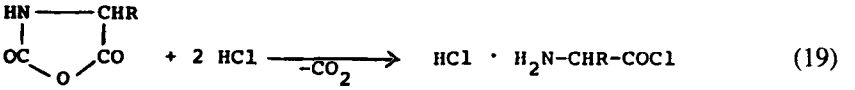


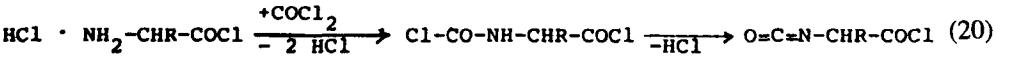
(16)

The most widely used method for the preparation of NCAs is the phosgenation of free amino acids. This method was first applied by Fuchs[26] for the preparation of *N*-phenyl-Gly-NCA and was later elaborated by Farthing et al.,[27-30] Coleman,[27,30,31] and Levy[32] for a broad variety of NCAs. The first step of the phosgenation seems to be the formation of an *N*-chloroformyl-amino acid (Equation 17), because addition of aniline yields 5-phenyl-hydantoic acids (Equation 18).[29,33] Tetrahydrofuran and 1,4-dioxane are the most widely used reaction media, although both solvents give side reactions when treated with hydrogen chloride for several hours. In principle, all solvents inert against phosgene may be used as reaction media; yet, in less-polar solvents such as chloroform, carbon tetrachloride, toluene, or ethyl acetate, the reaction time increases by a factor of 10 to 30. Acetonitrile is the only common highly polar solvent which can be used, and this solvent is recommendable for glycine and α-aminoisobutyric acid. Other polar solvents such as acetone, dimethylformide, or dimethylsulfoxide are not inert enough.



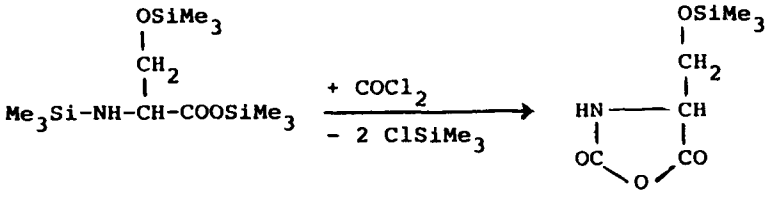
Nonetheless, cyclic ethers possess one shortcoming as reaction media, namely the good solubility of hydrogen chloride. High concentrations of hydrogen chloride, high temperatures, and long reaction times favor cleavage of NCAs according to Equation 19. The resulting amino acid chlorides hydrochlorides react with phosgene to carbamoylchlorides and finally yield α-isocyanatocarboxylic acid chlorides (Equation 20). Under suitable reaction conditions these isocyanates may become the main product.[34] These side reactions caused by hydrogen chloride may be a major problem when larger quantities of amino acids (≥0.5 mol) are to be phosgenated. According to the experience of the author, boiling mixtures of tetrahydrofuran and dichloromethane (approximately 1:1 v/v) are recommendable as reaction media in this case. The relatively poor solubility of hydrogen chloride in halogenated hydrocarbons and the working under reflux allows at least partial removal of the acid during phosgenation. Nonetheless, the reaction time increases when a part of the cyclic ethers is replaced by dichloromethane or chloroform, and it may be reasonable to stop the phosgenation immediately before completion. Since free amino acids are insoluble in all aprotic organic solvents, it is easier to separate NCAs from unreacted amino acids than from the acid chlorides depicted in Equation 20.





The preparation of NCAs from trifunctional amino acids may present difficulties depending on the nature of the third function. The imidazole ring of histidine and the ω-amino groups of ornithine and lysine need protection. When an acid-sensitive protecting group is used, an HCl acceptor is required. For this purpose, silver cyanide was used by two groups,[20,35] yet this expensive reagent is certainly not suited for the preparation of larger quantities of NCAs. A cheaper and generally applicable method which also avoids the formation of free hydrochloric acid is the phosgenation of silylated amino acids[36] (Equation 21). When the amino acids are silylated with trimethylchlorosilane/triethylamine,[37] distillation of the highly water-sensitive *N*-trimethylsilyl amino acid trimethylsilyl ester is not necessary. As demonstrated by the author[38] and later by Pfaender et al.,[39] the phosgenation of silylated tyrosine, serine, and threonine offers the advantage that the hydroxy group is protected by the easily removable trimethylsilyl group (Equation 22). Further, rarely used methods are the phosgenation of amino acid copper complexes[40] and the phosgenation of *N*-trimethylsiloxycarbonyl-amino acid trimethylsilyl esters (Equation 22).[38] When trichloromethyl chloroformate is available, it is more convenient to use this liquid reagent instead of the gaseous phosgene.[41] Finally, methods of mainly historical interest are to be mentioned. The first one is based

on the "dehydratization" of sodium amino acid carbonates by means of thionyl chloride.[29] This method requires first the preparation of purified, water-free amino acid carbonates from amino acids in sodium carbonate solution. Because of this stepwise procedure and the relatively low yields of NCAs, this approach is not attractive. A modern version of this approach, which also avoids the use of phosgene, is the conversion of *N*-trimethylsiloxycarbonyl-amino acid trimethylsilyl esters with thionyl chloride[39] (Equation 22). The main disadvantage of this version is the isolation of the highly water-sensitive starting materials.



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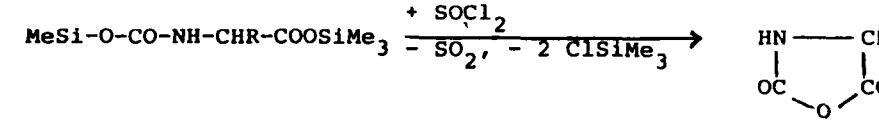


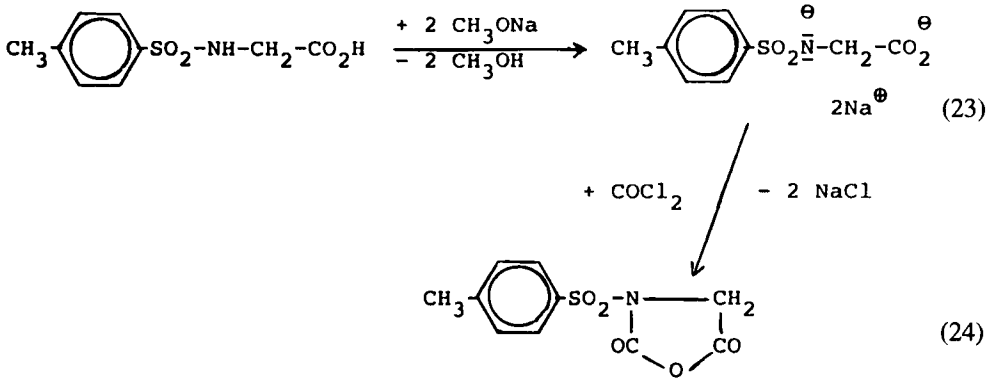
(22)

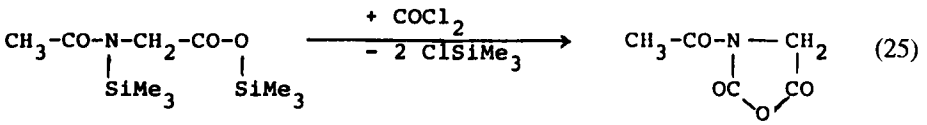
B. NCAs of *N*-Substituted Amino Acids

NCAs with alkyl or aryl groups attached to the nitrogen can easily be prepared by both the Leuchs and the Fuchs-Farthing method.[42-45] However, in the case of proline, spontaneous cyclization of the first-formed *N*-chloroformyl derivative does not take place[46] and addition of an HCl acceptor is necessary. For this purpose, silver oxide was recommended;[47] yet careful addition of equimolar amounts of triethylamine at 0°C obviously gives better results.[48,49]

NCAs with electron withdrawing *N*-substituent cannot be prepared by direct phosgenation of the free amino acid, because nitrogen is not nucleophilic enough to react with phosgene at moderate temperatures. Hence, either the nucleophilicity needs to be increased prior to phosgenation, or an alternative approach, e.g., electrophilic substitution of *N*-unsubstituted NCAs, is required. In the case of tosylamino acids, the high acidity of the sulfonamide group enables the preparation of metal salts by means of alkali metal alcoholates and their phosgenation yields the desired *N*-tosyl-NCAs (Equations 23 and 24).[50]

An analogous *N*-metallation of *N*-acyl-amino acids requires stronger bases than alcoholates, e.g., sodium amide or butyl lithium. However, such strong bases increase the risk of racemization or side reactions with functional groups in the side chains. A less rigorous method was developed by the author[51] for various *N*-acyl-glycine-NCAs. In the case of *N*-acetyl-, *N*-methoxycarbonyl-, and *N*-ethoxycarbonyl-glycine complete silylation with trimethylchlorosilane and triethylamine is feasible, and the *N*-silylated glycine derivatives react easily with phosgene (Equation 25). This synthesis of *N*-acetyl-glycine-NCA was reproduced by two other groups.[52,53] Unfortunately, complete silylation of other *N*-acyl-amino acids is not feasible because of steric hindrance.



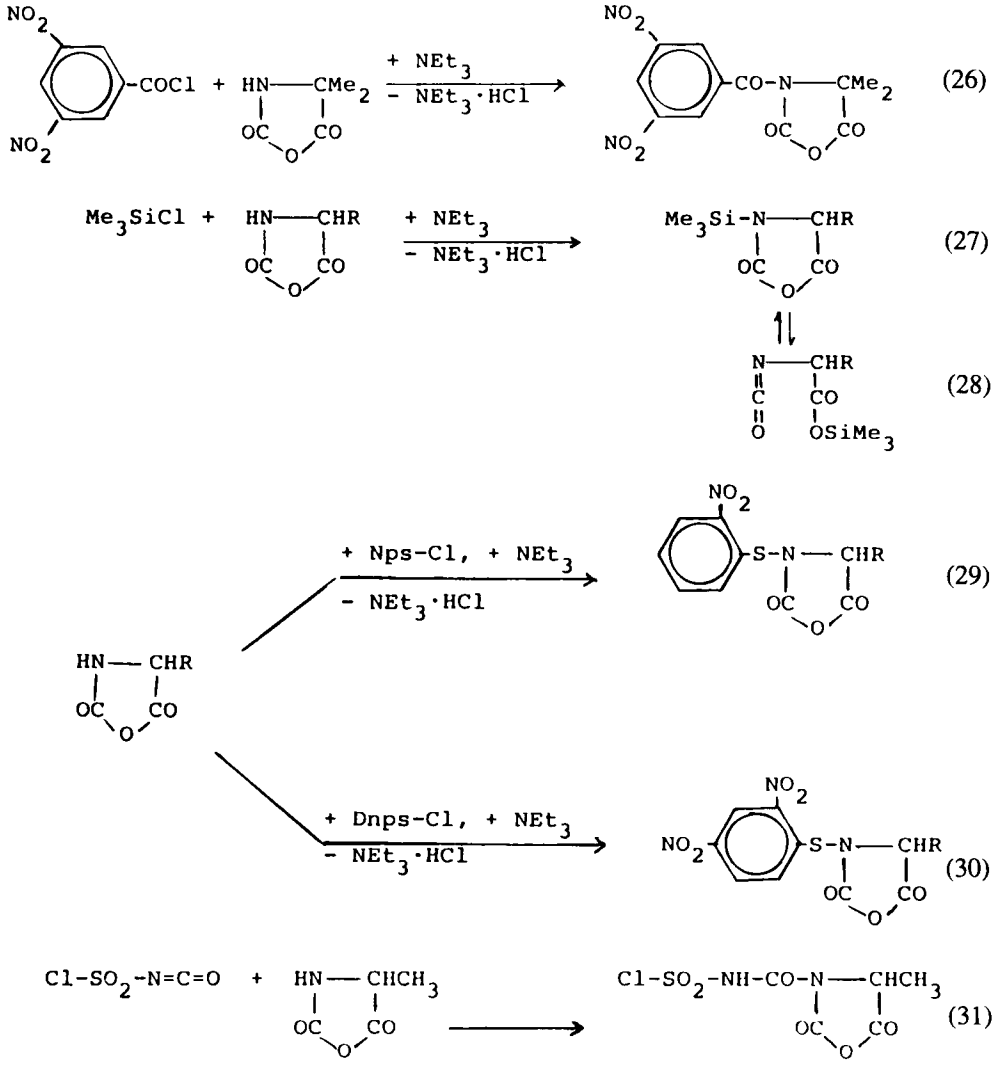


A quite different approach, namely the direct *N*-substitution of NCAs was investigated by the author using various acylating reagents. Since NCAs themselves are activated acylating reagents, the reaction partner must be a stronger electrophile, if polymerization is to be avoided. Aliphatic acid chlorides meet the requirement of a high electrophilicity, yet in the presence of a strong base, such as triethylamine, they yield ketones and other by-products, so that isolation of *N*-acyl-NCAs was never successful. Aromatic acid chlorides are less reactive; yet, when the relatively stable α-aminoisobutyric acid NCA was used, acylation with 3′,5′-dinitrobenzoylchloride was successful (Equation 26).[51] Quantitative *N*-substitution of all NCAs was easily obtained with trimethylchlorosilane (Equation 27).[54] Surprisingly, the *N*-silylated NCAs undergo a fast rearrangement to the corresponding α-isocyanatocarboxylic acid trimethylsilyl esters (Equation 28), and both components of this equilibrium react with each other yielding oligomerized isocyanates (nylon-1 backbone) with silylated carboxyl group in the side chain.[54]

More successful synthesis of stable *N*-substituted NCAs was based on the reaction with 2-nitrophenylsulfenyl chloride (Nps-Cl), (Equation 29).[55,56] Because aromatic sulfenyl chlorides are highly electrophilic, the substitution proceeds fast, even at 0°C, if an HCl acceptor is used which is basic enough to deprotonate the NCAs. The difficulty lies in the high sensitivity of Nps-NCAs to racemization. Even a 1% solution of triethylamine in 1,4-dioxane suffices to cause fast racemization at room temperature.

It is obvious that the risk of racemization decreases with decreasing basicity of the tertiary amine. Unfortunately, tertiary amines that are less basic than triethylamine do not yield a quantitative sulfenylation of the NCAs.[56] However, Katakai et al.[57,58] confirmed that careful dosage of triethylamine and reaction temperatures ≤0°C allow the synthesis of the optically pure Nps-NCAs. It is also necessary to isolate the Nps-NCAs as rapidly as possible from the reaction mixture. For this purpose it may be useful to synthesize the better crystallizing *N*-(2,4-dinitrophenylsulfenyl)-NCAs[56] (Equation 30). In this connection it is noteworthy that most functional groups in the side chains of the NCAs are not attacked by sulfenyl chlorides under the above-mentioned reaction conditions. In order to circumvent the problem of racemization, Halstrom and Kovacs[59] attempted to synthesize Nps-NCAs by phosgenation of Nps-amino acids. However, the yields were poor because the acidic by-products cleave the S-N bond. Finally, it is to be noted that the N-H group of NCAs adds to activated isocyanates, such as acyl- and sulfonylisocyanates.[51] Chlorosulfonylisocyanate reacts exothermically with NCAs or their thioanalogs and isolation of *N*-(chlorosulfonylcarbamoyl)-

alanine-NCA was reported[51] (Equation 31). However, isolation of other *N*-carbamoyl-α-amino acid NCAs failed, whereas addition of β-amino acid NCAs onto isocyanates was more successful.[60]



C. Purification and Titration of NCAs

The preparation of high molecular weight polypeptides demands for its success NCAs in a high state of purity. The method of synthesis of NCAs determines the contaminants to be expected. When the Leuchs method, using thionyl chloride, phosphorus pentachloride, or phosphorus tribromide is applied, ferric chloride, benzylhalogenides, phosphoroxy trihalogenides, and HCl are the usual contaminants. The Fuchs-Farthing method mainly yields *N*-chloroformyl-amino acid chlorides and α-isocyanato-acid chlorides as impurities. Because all of these impurities have an acidic or electrophilic character, they affect the polymerization of NCAs which is usually initiated by nucleophilic or basic reagents. Thus, these contaminants need to be completely removed before polymerization is initiated; however, it is worth mentioning that NCAs containing electrophilic impurities are more stable on storage, because initiation by traces of water is hindered. Extremely pure NCAs should be used within a few days after the last purification step. Since all aforementioned contaminants contain chlorine

or bromine; $AgNO_3$ enables their detection after dissolution of the NCA in concentrated nitric acid.

Most NCAs are crystalline in contrast to the usual contaminants, and thus, two to four recrystallizations from suitable solvents (and nonsolvents) is the standard purification procedure. Ethyl acetate and ligroine or cyclohexane, on the other hand, is the most widely used solvent mixture for the recrystallization of NCAs.[64] Gly-NCA, L-Ala-NCA and α-Aibu-NCA, which are nearly insoluble in ethyl acetate, are best dissolved in warm tetrahydrofuran or 1,4-dioxane and crystallized by portionwise addition of chloroform (for Gly-NCA) or carbon tetrachloride. These two nonsolvents are necessary because the contaminants of these three NCAs are insoluble in solvent mixtures containing ligroine or cyclohexane. Furthermore, it may be useful to treat solutions of crude NCAs with dry charcoal prior to crystallization. This procedure requires, of course, charcoal carefully dried over phosphorus pentoxide in vacuo. Several authors[43,62,63] purified NCAs by sublimation in a high vacuum. However, this method is limited to the purification of small quantities of thermally stable NCAs and does not reliably prevent contamination with high boiling acid chlorides.

The optical purity of NCAs is, of course, best checked by direct optical rotation measurements; yet, unfortunately only relatively few optical rotations were reported so far (cf. Tables 1 to 3 in Reference 64). Thus, hydrolysis of the NCA in dilute hydrochloric acid and comparison of the optical rotation with that of the original amino acid is the method of choice. NCAs may be quantitatively determined in nonacidic solutions, such as 1,4-dioxane, pyridine, dimethylformamide, or dimethylsulfoxide, by titration with sodium methoxide.[65] When thymol blue is used as indicator, the color changes sharply from yellow to blue upon addition of equimolar amounts of alcoholate. In analogy with the corresponding titration of normal acid anhydrides it is assumed that sodium carbamates of amino acid methyl esters are formed upon titration. Slow polymerization initiated by the titrant does not prevent a successful application of this titration procedure because sodium methoxide also reacts quantitatively with CO_2. In order to avoid the loss of CO_2 the titration should be conducted in dilute solution and at low temperatures. If this titration method is applied to determine residual NCA after incomplete polymerization, care must be taken to remove completely the CO_2 of the preceeding polymerization. A generally applicable and routine method which enables the determination of the chemical structure and the detection of impurities is high resolution ^1H NMR spectroscopy at frequencies $\geqslant$ 200 MHz.

D. IR and NMR Spectroscopy

IR spectra allow a rapid and reliable identification of individual NCAs, if the spectrum of an original sample is available for comparison. However, even if such a comparison is not feasible, the typical carbonyl bands of NCAs (Figure 1) allow at least a clear distinction from other amino acid derivatives. In addition to the frequencies (1855 $\pm$ 5 cm^{-1} and 1785 $\pm$ 5 cm^{-1}) the extinction coefficients are characteristic and allow an assignment of both stretching modes. Because in the case of the carbamoyl groups (C-2) not only a lone electron pair of the neighboring oxygen but also a lone pair of the nitrogen is delocalized, a reduced frequency along with a higher extinction coefficient is expected (Formula 32a).

However, it is noteworthy that symmetric cyclic anhydrides, such as succinic anhydride, exhibit a similar pattern of "carbonyl bands". In this case, the so-called "Fermi coupling" of the two originally identical CO frequencies is considered to explain the existence of two carbonyl bands of different extinction coefficients.[66] Nonetheless, there is an experimental evidence that the assignment of the vibration at 1785 cm^{-1} to the carbamoyl group is correct, namely a comparison with thiazolidine-2,5-diones and 2-thioxo-oxazolidine-5-ones (Formula 32b, c).[25,67,68] The latter class of cyclic anhydrides only exhibits the carbonyl band around 1850 cm^{-1}.[15,68]

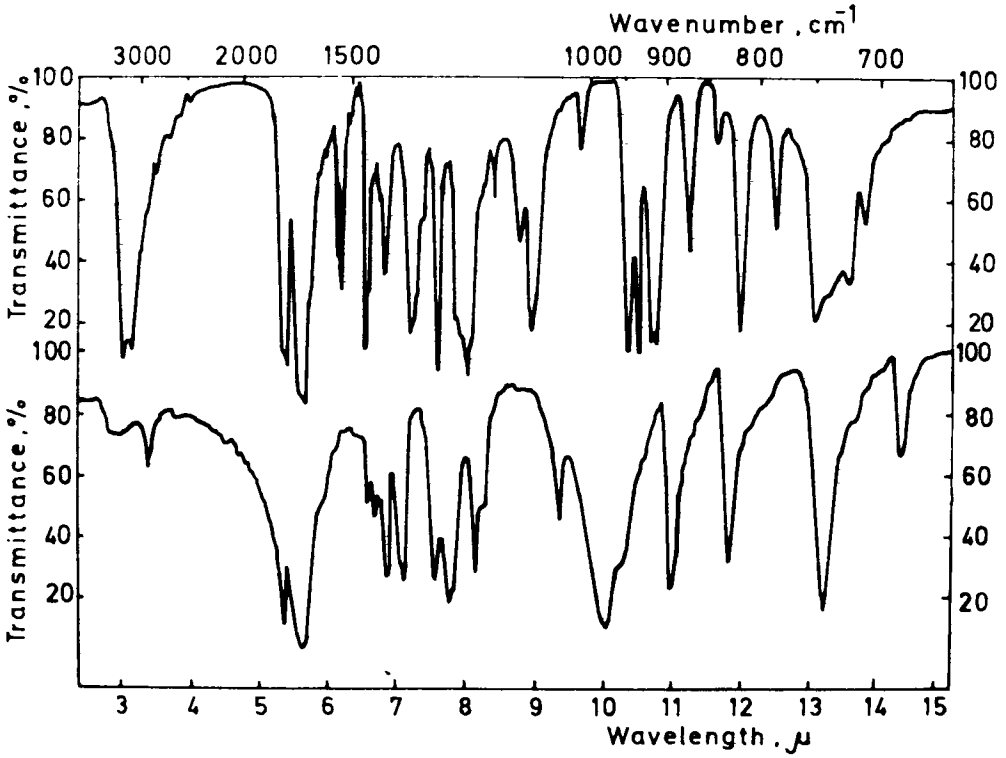
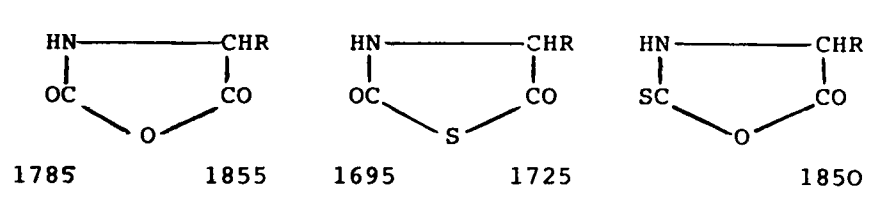


FIGURE 1. IR spectra (measured in KBr) of (A) L-Tyr-NCA and (B) Sar-NCA (Kricheldorf, unpublished results).



(32)

Wavenumbers (in cm^{-1}, variation ca. $\pm$ 5 cm^{-1}) of CO-bands

When NCAs contain additional carbonyl groups in the side chain, separate CO-bands are observable in the case of urethane (Z or Boc) groups and in the case of amide groups (e.g., glutamine-NCA). However, the CO-bands of ester groups largely overlap with the carbamoyl band of the NCA ring (Figure 1A). When the NCAs begin to polymerize during prolonged storage, the typical CO-bands of the NCA ring gradually disappear whereas the CO- and NH-bands of the peptide groups (approximately 1650 and 1530 cm^{-1}) gain in intensity (Figure 2A, B). Therefore, IR spectra allow an easy and rapid estimation whether, and to what extent, an NCA underwent polymerization upon storage.

N-alkyl or *N*-aryl substituents do not significantly influence the CO-vibrations; yet all bands resulting from N-H vibrations (e.g., 3200 cm^{-1}) are, of course, lacking. Electron-withdrawing substituents, such as acyl, carbamoyl, or sulfenyl groups shift both carbonyl bands to higher frequencies (1880 and 1810 cm^{-1}, respectively). In the case of *N*-acetyl-glycine-NCA, three CO-bands are clearly observable[51] because the acetyl group absorbs at a lower frequency than the NCA ring. The typical high frequencies of *N*-acyl-NCA end

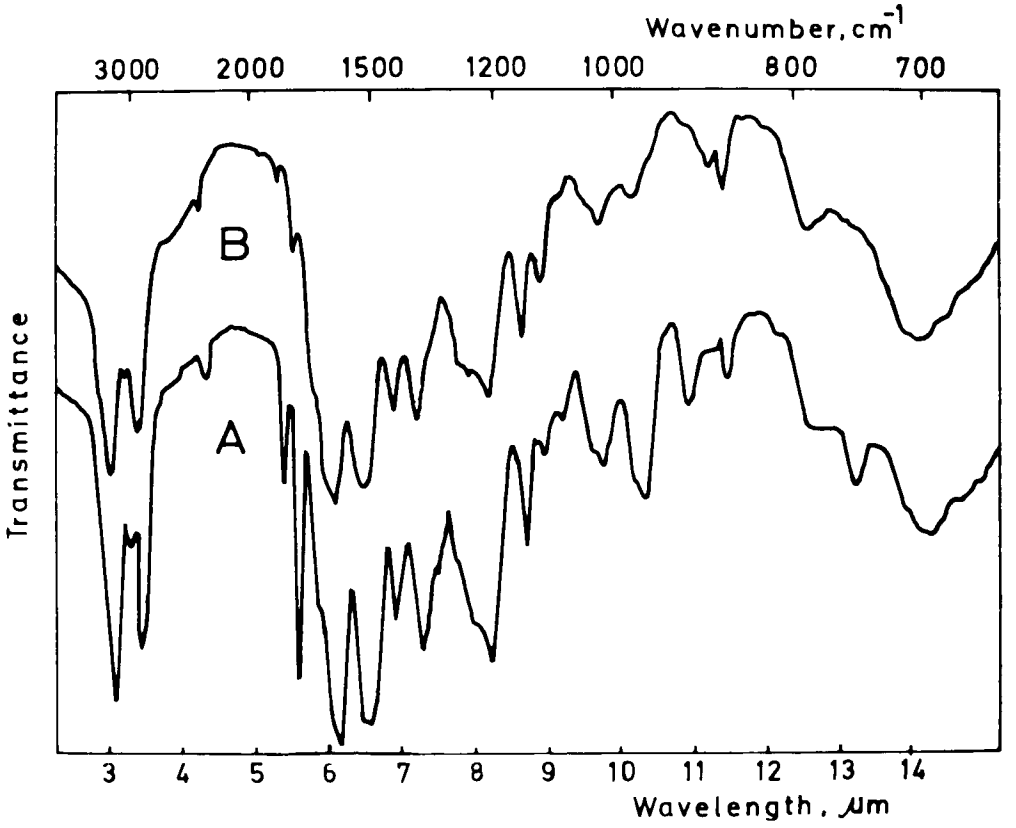


FIGURE 2. IR spectra (measured in KBr) of poly(D,L-Ile) initiated with *N*-acetyl-Gly-NCA and triethylamine. (A) After 14% conversion; (B) after 39% conversion. (Adapted from Reference 51.)

groups, which are worthwhile indicators of the so-called "activated monomer mechanism" (cf. Section III), are easily detectable in the spectra of oligopeptides (Figure 2A, B). However, when a normal grating IR spectrometer is used, the observation of *N*-acyl-NCA end groups is limited to a $\overline{DP}_n$ of approximately 20. Measurements of subtraction spectra by means of FT-IR spectrometer will certainly enhance the sensitivity of this analytical method.

In the past 2 decades NMR spectroscopy has become the most powerful analytical tool for the characterization of chemical structures and conformations in organic chemistry; yet only few NMR data were reported on NCAs.[25,51] This discrepancy certainly results from the fact that comparison of melting points, optical rotations, and IR spectra allow sufficient characterization and identification of NCAs. Furthermore, the NCA ring is a stiff system and not promising for conformational studies. Nonetheless, the detection of NCAs or low molecular weight by-products in reaction mixtures (usually after filtration from precipitated oligo- and polypeptides) and the identification of end groups of oligo- and polypeptides are problems that can be better solved by NMR spectroscopy. Unfortunately, the detection of NCAs or by-products in reaction mixtures requires the (expensive) use of deuterated solvents. ^{15}N NMR measurements are not affected by most solvents used for polymerization of NCAs; yet, its sensitivity is at least 10^4 times lower than that of ^1H NMR spectroscopy. Thus, the ideal method for the detection and identification of low concentrations of NCAs in reaction mixtures is still lacking.

^1H NMR spectra of NCAs do not display unusual features and resemble those of *N*-acylated amino acid derivatives (Table 1). Nonetheless, it is to be mentioned that the neighborhood of a chiral center (C-α) render the two chemically equal methyl groups of

Table 1
90 MHz ^{1}H NMR SPECTRA OF VARIOUS OXAZOLIDINE-2,5-DIONES (α-NCAs)[a]

Monomer	Solvent	Chemical shifts δ(ppm)[a] and coupling constants J (Hz)
Gly-NCA	TFA-d	4.46 (s)
	Acetone-d_6	4.37 (s)
N-*t*-butyl-Gly-NCA	TFA-d	4.47 (s); 1.56 (s)
N-acetyl-Gly-NCA	TFA-d	4.74 (s); 2.75 (s)
N-methoxycarbonyl-Gly-NCA	TFA-d	4.74 (s); 4.05 (s)
L-Ala-NCA	TFA-d	4.66(q; 1.77(d,J = 6.5 Hz); 7.54 (s)
	$CDCl_3$	4.48 (q); 1.57(d,J = 6.5 Hz)
L-Leu-NCA	$CDCl_3$	4.39 (dd); 1 77 (m); 1.00; (1:3:6)
L-Phe-NCA	TFA-d	4.84 (dd); 3.29 (dd); 7.33 (s, broad)
D-Phe-NCA	TFA-d	5.56 (s); 7.45; 7.42 (s, broad)

[a] Relative to internal TMS.

Adapted from References 51 and 72 and from unpublished results of the author.

valine and leucine NCA magnetically unequal. The resolution of both methyl signals depends, of course, on solvent and magnetic field strength. For reasons of chirality, the two β-protons of amino acids, such as leucine, phenylalanine, serine, or glutamic acid, are also magnetically nonequivalent. However, resolution of separate signals, including the geminal coupling, normally requires frequencies above 100 MHz. The nonequivalence of the β-protons has consequences for the signal pattern of the α-proton. Whereas at low frequencies a "triplet pattern" is observed, a doublet of doublets is resolved at frequencies above 100 MHz.

α-Amino acid NCAs differ from most other α-amino acid derivatives by a greater downfield shift of the C_α-proton (0.2 to 0.5 ppm depending on the amino acid derivative). The chemical shift of the C_α-proton depends on the nature of the side chain, whereas alkyl or aryl groups, attached to the nitrogen, do not have a significant influence. However, electron withdrawing substituents, such as acyl or sulfonyl groups, cause an additional downfield shift of approximately 0.3 to 0.4 ppm (Table 1). This shift effect is of great analytical interest for the elucidation of the polymerization mechanisms initiated by aprotic bases. As discussed in more detail in Section III.C and D, the polymerization mechanism proposed by Bamford et al.[69,71] postulates *N*-acyl-NCA end groups as active chain ends. In addition to IR spectroscopy, ^{1}H NMR spectroscopy was successfully used to prove the formation of *N*-acyl-NCA end groups under certain circumstances.[51,72] The downfield shift of the C_α-protons enabled the detection of the *N*-acyl-NCA end group despite the presence of the polyglycine chain (Figure 3). In addition to the NCA end group, the glycyl unit directly attached to the nitrogen of the NCA ring could be identified (Figure 3). Again, the C_α-protons of this glycyl unit absorb downfield of the main chain units because the NCA ring itself is a strongly electron-withdrawing substituent for other groups. Unfortunately, the ^{1}H NMR spectroscopic identification of *N*-acyl-NCA end groups cannot be easily applied to other polypeptides. Due to the relatively small shift differences of end groups and main chain, low molecular weight *N*-acyl-NCAs are required as spectroscopic models. In the case of polyglycine *N*-acetyl- and *N*-methoxycarbonyl-Gly-NCA were available. Furthermore, the glycine system is advantageous, because the C_α-protons give a singlet signal; whereas the signal patterns of the C_α-protons of almost all amino acid units and NCAs are doublets, triplets, or doublets of doublets. Hence, end group analyses require frequencies above 200 MHz, and suitable model compounds such as *N*-acetyl-NCAs are indispensable. Since syntheses of *N*-acyl-NCAs other than *N*-acyl-Gly-NCA were not yet successful, the ^{1}H NMR spec-

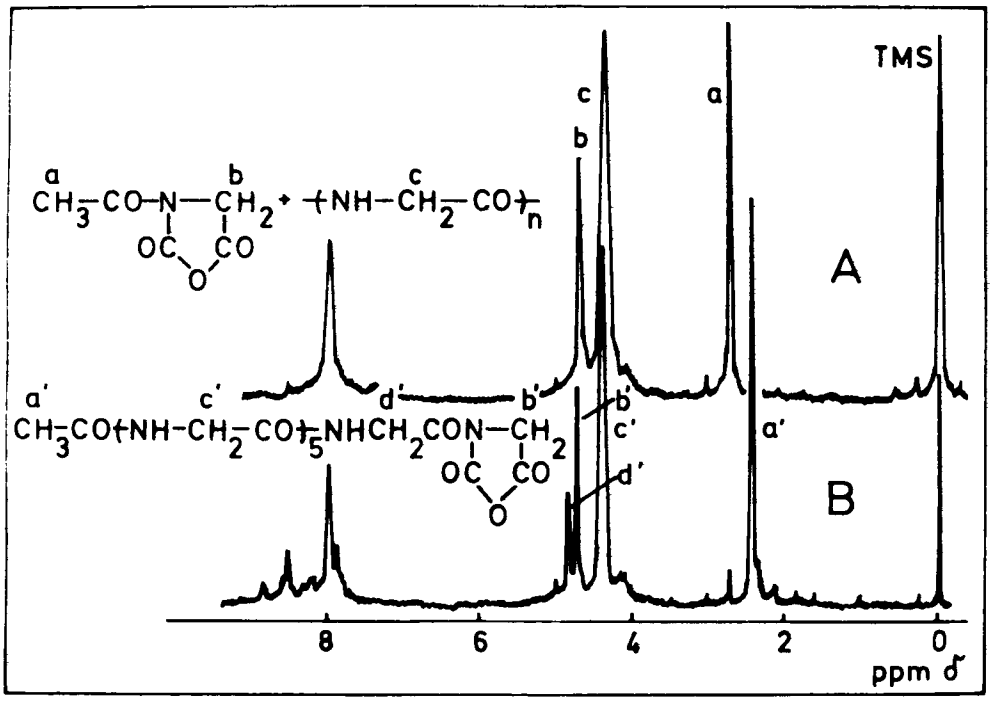


FIGURE 3. 220 MHz ^{1}H NMR spectra (measured in TFA) of (A) mixture of *N*-acetyl-Gly-NCA and poly(Gly); (B) oligo (Gly) initiated with *N*-acetyl-Gly-NCA and triethylamine. (Adapted from Reference 51.)

troscopic identification of *N*-acyl-NCA end groups is currently limited to the glycine system. However, ^{1}H NMR spectroscopy proved to be very useful for the detection and quantification of various dead chain ends (cf. Section V).

In addition to ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy is the most useful tool for identification of end groups and (isolated) side products. Again, only few data were hitherto published in the literature. The chemical shifts of C_α and of the side chain carbon obey not exactly, but rather closely, the substitution (or increment) rules developed by Paul and Grant[73,74] for other aliphatic compounds. For instance, a carbon directly attached to C_α (i.e., C_β) causes a downfield shift of approximately 8 ppm relative to Gly-NCA. A carbon in β-position to C_α (i.e., C_γ) causes a downfield shift of approximately 5 ppm; whereas a carbon in γ-position (i.e., C_δ) causes an upfield shift of approximately 1 ppm. These shift effects are nearly additive. The chemical shifts of the two carbonyl groups differ by approximately 15 ppm. The assignments are based on comparison with other amino acid and carbamic acid derivatives. It is a general tendency in ^{13}C NMR spectroscopy of amino acids and peptides that the CO signals of carbamic acid derivatives absorb in the range of 150 to 160 ppm, whereas the CO signals of amide and ester groups show up between 168 and 180 ppm. Since the signal of CO-5 falls into the shift range characteristic of amide and ester groups, it is the signal of the carbonyl group C-2 which enables the detection of NCAs and *N*-acyl-NCA end groups in reaction mixtures containing higher concentrations of peptide and ester groups. Finally, it is noteworthy that a more comprehensive discussion of NMR spectra of NCAs, including ^{13}C and ^{15}N NMR data was published in a recent monograph.[64] The same monograph contains tables with melting points and optical rotations of all α- and ω-amino acid NCAs published so far.

E. Syntheses of NCAs: Exemplary Procedures

1. Glycine-NCA

Z-glycine (105 g, 0.5 mol) and hexamethyldisilazane (63.5 g, 0.3 mol) are refluxed in

500 ml of dry xylene until the evolution of ammonia ceases. The xylene and excess hexamethyldisilazane are removed in vacuo and the residual Z-Gly trimethylsilylester is diluted with 400 ml of dry chloroform. Distilled thionyl chloride (72g, 0.6 mol) is added, and the reaction mixture is refluxed until the evolution of SO_2 ceases. After cooling with ice, the crystallized Gly-NCA is isolated by filtration and washed with dry chloroform. The crude Gly-NCA is dissolved in the smallest possible quantity of boiling dry THF and upon cooling, 400 ml of dry toluene is portionwise added. The resulting suspension is concentrated in vacuo to a volume of approximately 300 ml, and the crystallized NCA is isolated by filtration. The procedure is repeated until the NCA is free of chloride.

2. Sarcosine-NCA

N-methoxycarbonylsarcosine (147 g, 1 mol) and chlorotrimethylsilane (109 g, 1 mol) are dissolved in 1.2 l of warm, dry toluene. Upon heating, 1 mol of triethylamine is added dropwise under stirring. The reaction mixture is refluxed for 30 min, cooled with ice and filtered in dry conditions. The filtrate is concentrated in vacuo and the residual *N*-methoxycarbonylsarcosine trimethylsilylester is diluted with approximately 800 ml of dry carbon tetrachloride. Phosphorus tribromide (135 g, 0.5 mol) is added to this solution which is refluxed for 2 h under stirring. After cooling with ice, the crystalline Sar-NCA is filtered off under exclusion of moisture. The crude NCA is dissolved in warm THF and stirred with charcoal (dried over phosphorus pentoxide in vacuo!) for approximately 10 min. The charcoal is filtered off, the filtrate concentrated in vacuo, and the NCA is crystallized by portionwise addition of carbon tetrachloride under stirring or shaking. This procedure is repeated until the product is free of halogen.

3. Phenylalanine-NCA

L-phenylalanine (16.5 g, 0.1 mol) is suspended in a mixture of 200 ml of dry 1,4-dioxane and 200 ml of dry dichloromethane, and a moderate stream of phosgene is introduced under stirring. After approximately 5 min the reaction mixture is heated to reflux, and these reaction conditions are maintained until most of the L-Phe crystals have disappeared. Then the introduction of phosgene is stopped, but stirring is continued until a clear solution is obtained (approximately 10 to 30 min). The excess phosgene is removed by a stream of carbon dioxide, and the reaction mixture is concentrated in vacuo. The crystallization of Phe-NCA is completed by portionwise addition of carbon tetrachloride and cooling with ice. The further purification may be conducted as described for Sar-NCA.

4. Proline-NCA

L-proline (11.5 g, 0.1 mol) is phosgenated in a 1:1 v/v mixture of dry THF and dichloromethane. The clear reaction mixture is concentrated in vacuo and the residual carbamoyl chloride is dissolved in 200 ml of dry ice cold ethyl acetate. A solution of 10.1 g (0.1 mol) of triethylamine in 50 mol of ice cold ethyl acetate is added dropwise under stirring and 10 min after complete addition the precipitated triethylamine hydrochloride is removed by filtration. The filtrate is treated with dry charcoal and concentrated in vacuo. Crystallization of Pro-NCA is achieved by the gradual addition of diethyl ether and ligroine under cooling with ice. The same treatment may be used for recrystallization.

III. POLYMERIZATION OF α-AMINO ACID *N*-CARBOXY-ANHYDRIDES

A. Initiation with Water or Alcohols

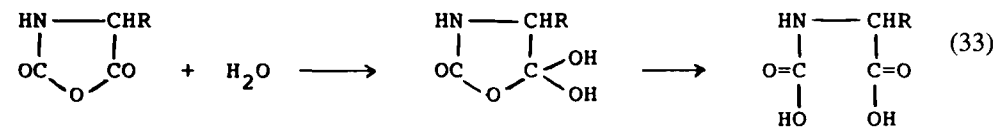
α-Amino acid NCAs have four reactive sites, namely, two electrophilic groups, CO-2 and CO-5, and two nucleophilic groups, NH and C-H after deprotonation. These inherent structural properties of NCAs are responsible for the complex chemistry resulting in the

long-lasting discussion of their polymerization mechanisms. Free radical initiators and acidic catalysts (with the exception of pure H_2F_2[75]) may decompose NCAs but do not initiate true polymerizations. Therefore, true initiators may be subdivided into two classes: protic nucleophiles and bases on the one hand and aprotic nucleophiles or bases on the other hand. Furthermore, it is useful to classify all initiators according to the following criteria:

1. Chemical course of the initiation, or in other words the reaction site of the NCA attacked by the initiator
2. Reactivity of the initiator relative to that of the active chain end
3. Incorporation of initiators into the peptide chain forming a dead chain end via a covalent bond

With regard to point 1, water, alcohols, and primary amines behave identically because they all exclusively attack the carbonyl group C-5 of the NCAs, at least when monomer/initiator (M/I) ratios >1 are used. However, in the case of secondary amines, tertiary amines, and anions, the course of initiation depends on the nucleophilicity/basicity (nuc/bas) ratio. With regard to point 2, water, alcohols, and aromatic amines are all less nucleophilic than the active chain ends (amine or carbamate groups). Consequently, the initiation step is slower than propapagation. With respect to point 3, water, alcohols, and primary and most secondary amines have in common that they can form dead chain ends via covalent bonds.

The hydrolysis of NCAs was the first reaction carefully investigated by several authors[1-3,42,62,76-80] beginning with Leuchs et al.[1-3] They found that the reaction of NCAs with water may take two extreme courses. High NCA/H_2O ratios (>10) result in the formation of polypeptides, whereas low NCA/H_2O ratios (<10^3) result in complete hydrolysis of the NCAs. Intermediate NCA/H_2O ratios favor the formation of oligopeptides. Gly-NCA and various other NCAs dissolve in large quantities of cold water with slow evolution of CO_2. Acidification accelerates the evolution of CO_2 and favors a clean hydrolysis, whereas increasing pH favors polymerization. When *N*-substituted Gly-NCAs are hydrolyzed at pH 7, the $\overline{DP}_n$ of the resulting oligopeptides decreases considerably with increasing bulkiness of the *N*-substituent. From these observations and from careful kinetic studies of Bartlett et al.,[79,80] the following hydrolytic mechanism was derived (Equations 33 to 35).



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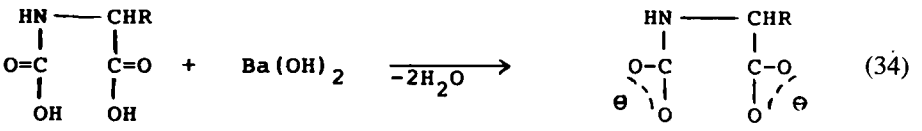

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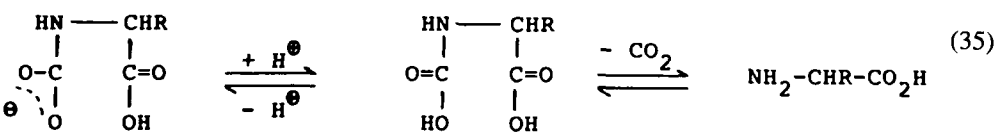

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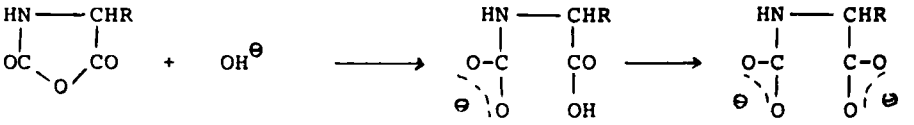
In neutral and acidic water it is mainly the H_2O molecule itself which attacks the carbonyl group C-5 of the NCA ring. The resulting carbamic acid derivative (Equation 33) is detectable

by the change of pH when the hydrolysis is conducted in neutral water. Furthermore, the addition of calcium or barium hydroxide to the ice-cold solutions of NCAs yields the so-called Siegfried salts (Equation 34). In contrast to the carbamate anions, free carbamic acids are not stable above 0°C, so that the first product of the hydrolysis is finally converted into the free amino acid (Equation 35). The decarboxylation rate depends on the equilibrium between anion and acid form and thus depends on the nature of the amino acid and on the pH. Complete hydrolysis of NCAs to amino acids without the formation of side products is best achieved in acidic water solution, because protonation of the amino group prevents oligomerization. Such an acidic hydrolysis is, for instance, the last step in a reaction sequence which enables the synthesis of N^{δ}-Z-L-ornithine,[18] N^{ε}-Z-L-lysine[81] (Equations 13 to 15), N^{g}-(*p*-nitrobenzyloxycarbonyl)-arginine,[81] or N^{β}-Z-α,β-diaminopropionic acid.[82] Nonetheless, the hydrolysis of the NCA ring itself, i.e., the ring opening step (Equation 33), is not catalyzed by protons[79,80] because acidic water solutions are not able to protonate the NCA ring. The stability of NCAs in water-free TFA is a clear evidence for the low basicity of NCAs.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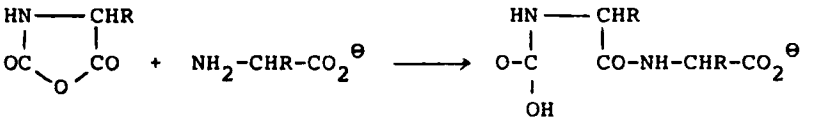
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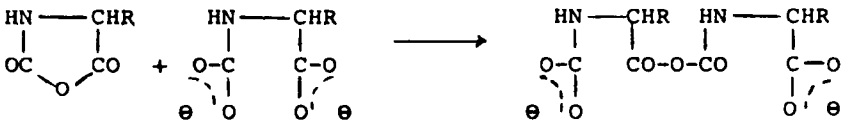
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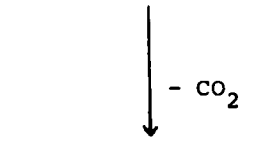
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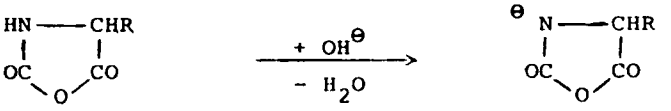
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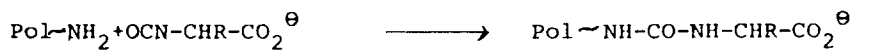
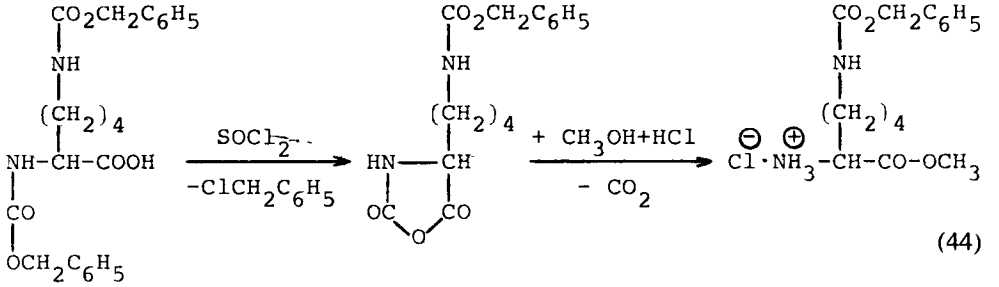

(42)

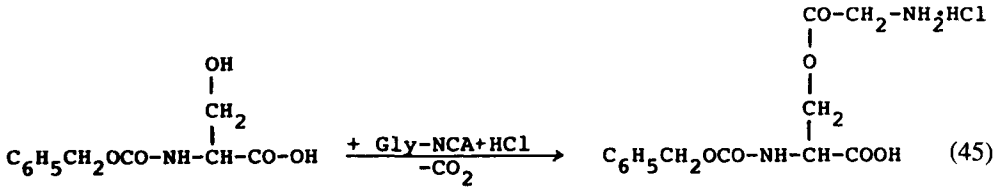
In contrast to protons, hydroxyl ions catalyze the hydrolysis of NCAs because they are considerably more nucleophilic than H_2O itself. Yet, pH above 7 does not only accelerate the hydrolysis but also favors the formation of oligopeptides (Equations 36 to 39). One reason is that the amino groups are not protonated, and due to their high nucleophilicity they can compete with H_2O and $OH^{\ominus}$ in attacking the unreacted NCAs. Another reason is the stabilization of the carbamate groups because the equilibrium (Equation 35) is shifted to the left side. The carbamate ions are more nucleophilic than H_2O, and thus their reaction with NCAs (Equations 38 and 39) can compete with hydrolysis. Hence, it is obvious that increasing pH favors the formation of oligopeptides at the expense of free amino acids. However, it must be emphasized that treatment of NCAs with alkaline water is not an optimum method for the preparation of high molecular weight polypeptides. Increasing pH favors the deprotonation of NCAs, the resulting NCA anions rearrange to α-isocyanatocarboxylates which cause termination steps by addition to amino end groups (Equations 40 to 42). Characteristic for this reactions sequence is the isolation of hydantoic acids from stepwise peptide syntheses in alkaline water.[83] For the preparation of polypeptides initiation with neutral water in water miscible solvents such as tetrahydrofuran, 1,4-dioxane or dimethylformamide is the method of choice. Nevertheless, water was only seldom used for preparative purposes on the following grounds. First, water-initiated polymerizations of NCAs are relatively slow. Second, high molecular weights (e.g., $\overline{DP}_n > 100$) are difficult to achieve. Third, the $\overline{DP}_n$ cannot be varied at will. Because propagation is faster than initiation, Equation 43 is not valid.

$$\overline{DP}_n = \frac{M}{I} \cdot \frac{\%\ \text{conversion}}{100} \tag{43}$$

The alcoholysis of NCAs resembles in many aspects their hydrolysis. Small amounts of alcohols initiate the polymerization of NCAs; yet initiation is considerably slower than propagation. With decreasing NCA/alcohol ratio the oligomer fraction increases at the expense of the polymer fraction. However, even a large excess of alcohol does not result in high yields of free amino acid esters because the nucleophilicity of primary amino groups is considerably higher than that of alcohol groups. Hence, the preparation of amino acid esters in high yields requires the presence of at least an equimolar amount of a strong acid, which prevents the formation of free amino groups. This way of preparing amino acid ester salts has become the standard method for the synthesis of N^{ω}-protected, α,ω-diamino acid esters. The preparation of N^{ϵ}-benzyloxycarbonyl-lysine methyl ester hydrochloride[81] is outlined in Equation 44. Ornithine[18] and *p*-aminophenylalanine[84] were modified in the same way. Furthermore, glycine units can be introduced in the side chain of serine[85] (Equation 45). Thus, it is obvious that the alcoholysis of NCAs under neutral or acidic conditions is useful mainly for the synthesis of amino acid esters or oligopeptides with ester end groups. The preparation of high molecular weight polypeptides is feasible by means of alkali alcoholates as discussed in Section III.E below.



(44)



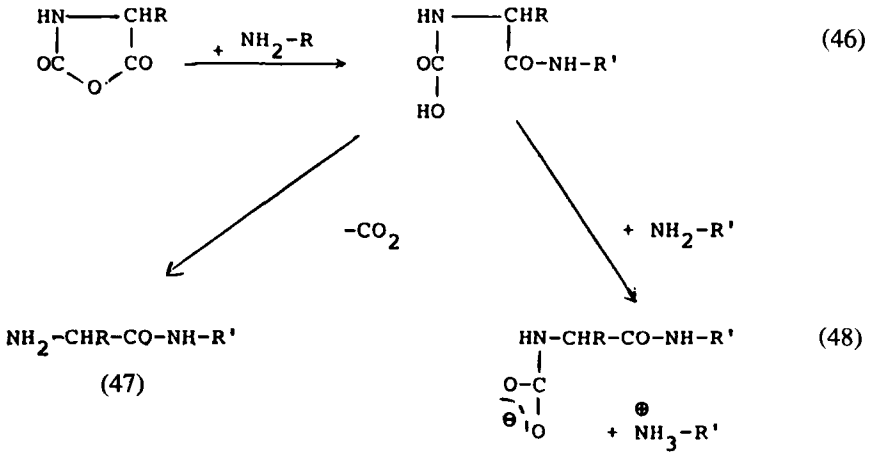
(45)

B. Initiation with Primary Amines

Primary amines can be subdivided into three categories with respect to their reactions with NCAs;

1. Aromatic amines, hydrazines, and hydroxylamines
2. α-Amino acid derivatives
3. Aliphatic amines

The first group of amines is characterized by lower basicity and lower nucleophilicity compared to the amino end group of peptide chains. Hence, reactions of NCAs with such primary amines lead in a homogeneous system to the formation of oligo- and polypeptides, even when equimolar amounts of reactants are used. In this case the initiation step (Equation 46) is slower than the most probable propagation step (Equation 49), in terms of rate constants: $k_{I1} < k_{p1}$ (Equations 50 and 51). A chain growth via carbamate chain ends (Equations 52 to 54) is less likely because the weakly basic amines cannot stabilize the carbamic acid by deprotonation (Equations 47 and 48). The reaction of D,L-Phe-NCA with excess aniline (or *N*-methylaniline) is characteristic for this reaction sequence.[62] In analogy to initiation with alcohol, polymerizations initiated with amines of group 1 are slow, and the $\overline{DP}$ is difficult to predict because Equation 43 is not valid.



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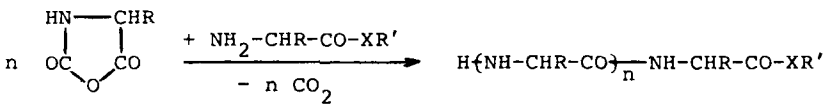
(47)

(48)

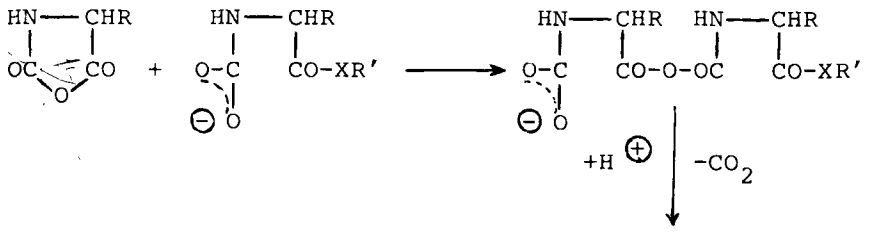


(49)

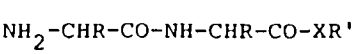
$$V_{I1} = k_{I1} [Am] [NCA] \quad (50)$$

$$V_{p1} = k_{p1} [Pol\text{-}NH_2] [NCA] \quad (51)$$

$$V_{p2} = k_{p2} [Pol\text{-}NH\text{-}CO_2^{\ominus}] [NCA] \quad (52)$$

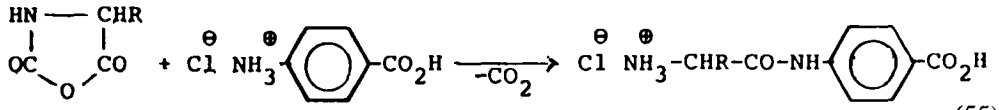


(53)



(54)

Different results were obtained, when *N*-phenylglycine-NCA was reacted with excess ammonia, aniline (or *N*-methylaniline). In this case, *N*-phenylglycine amides were the main products because the phenyl groups reduce the nucleophilicity of the nitrogen to such an extent that initiation is faster than propagation. However, other NCAs also yield mainly amino acid amides when the reaction with a less basic amine is conducted in water at the acidic pH. The salts of aromatic amines are hydrolyzed so that part of the amine is free. The free amino groups react with the NCA, and because the amino group generated from the NCA is more basic, it is preferentially protonated, and thus protected against oligomerization. In this way, α-amino acid derivatives of aromatic amino acids were prepared[86] (Equation 55).



(55)

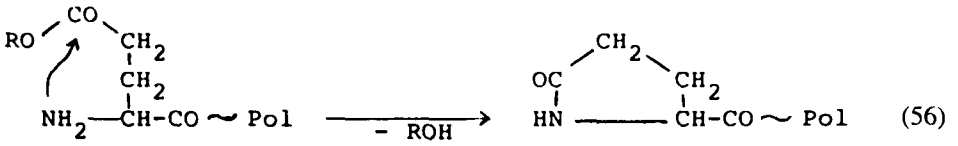
Amino acid esters or amides possess, of course, reactivities similar to those of amino end groups. Hence, their reactions with NCAs yield oligo- and polypeptides. At higher temperatures, 2,5-dioxopiperazines are the predominant by-products as demonstrated for the reaction of Gly-NCA with glycine ethyl ester.[87,88] Because free amino acid amides are difficult to isolate and amino esters are unstable on storage, such amines were only rarely used as initiators. More important is the reaction of amino acids or peptides with NCAs under conditions that allow stepwise syntheses of well-defined amino acid sequences. As summarized in several reviews,[64,89,90] various procedures were developed by numerous research groups. The most successful one consists of the conversion of NCAs with buffered water solutions of amino acids or peptides. In this way, various penicilline derivatives were prepared, and even the synthesis of the enzyme ribonuclease-*S* was successful.

Primary alkylamines (group 3), and in particular *n*-butyl-amine and *n*-hexylamine, are the most widely used initiators of all protic nucleophiles. Due to their higher nucleophilicity compared to the active chain end, initiation is more rapid than propagation ($k_{i1} > k_{p1}$ and $k_{i1} > k_{p2}$ in Equations 50 to 52). In analogy to the anionic "living polymerization" of vinyl monomers primary alkylamine-initiated polymerizations of NCAs obey Equation 47. Hence, the $\overline{DP}$ can be varied at will via the M/I ratio at least of M/I ratios <150, and when a nearly quantitative conversion is attainable. However, as discussed in Section IV, primary (or secondary) amine-initiated polymerization of NCAs do not result in narrow molecular weight distributions.

Because aliphatic primary amines are basic enough to stabilize the initially formed carbamic acid by deprotonation (Equations 46 and 48) the chain growth can proceed in two ways. First, the active chain end is an amino group (Equation 51): a mechanistic pathway which is called "amine mechanism" or "normal propagation". This mechanism was first investigated and formulated by Wessely et al.[42,76-78,91,92] and Watson et al.[43] Second, the active chain end can be a carbamate ion (Equations 53 and 54): the "carbamate mechanism",

as first formulated by Idelson and Blout.[93] Because both protonation of carbamate ions and decarboxylation of carbamic acids are reversible reactions, it will largely depend on the reaction conditions, in particular on temperature, solvent, CO_2 pressure,[63,94-97] and concentration of protons, to what extent an individual polymerization proceeds via amino or carbamate end groups. At the present time, no analytical tool exists which allows a reliable and at least semiquantitative determination to what extent an individual polymerization involves the amine mechanism or the carbamate mechanism. Nonetheless, two observations suggest that the carbamate mechanism plays only a minor role. First, the isolation of amino acid carbamates in the course of stepwise peptide syntheses[96,97] indicates that carbamates with primary or secondary ammonium counterions are less nucleophilic than amino end groups. Second, the rapid consumption of primary aliphatic amines at low conversion prevents the formation of ion pairs according to Equation 48 over the whole course of the polymerization. At any rate, the extent of the carbamate mechanism does not play any role for the preparative application of primary amine-initiated polymerizations.

A further problem of the "amine mechanism" is the nature and frequency of the termination steps. Despite the seemingly "living character" of polymerizations initiated by protic nucleophiles, high molecular weights ($\overline{DP}_n > 150$) are in general not accessible in this way (for MWD see Section V). In the case of polypeptides that cannot form α-helices, such as polyglycine, polyvaline, polyisoleucine, etc., the β-sheet structure may be responsible for a "physical death" of the chain growth (cf. Section V.D). However this argument is valid for all kinds of initiation, and high molecular weight polypeptides with β-sheet structure have indeed not yet been reported so far. In the case of polyglutamates, cyclization of the ultimate monomeric unit is likely to be a termination step (Equation 56).



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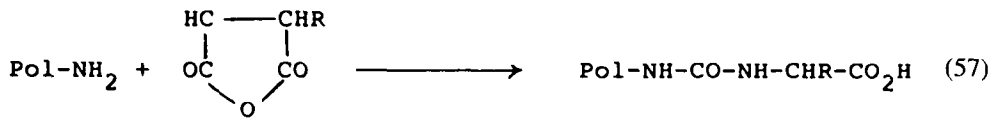

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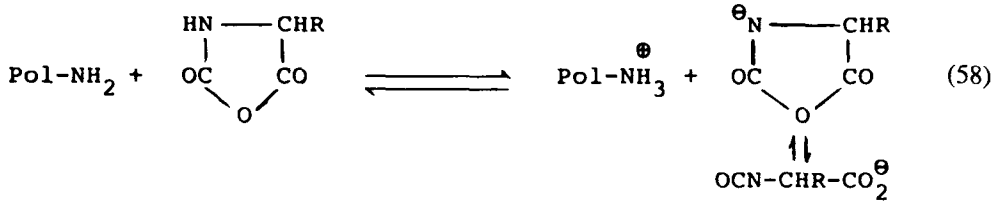

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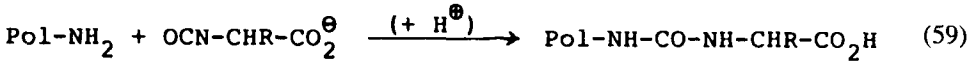


(59)

Sela and Berger,[101] who carefully investigated the end groups of various polypeptides, found hydantoic acid units in poly(D,L-alanine), poly(L-lysine), or poly(D,L-phenylalanine) initiated by water, diethylamine, or L-tyrosine ethyl ester. They proposed the termination step of Equation 57, which has been cited since then in numerous other papers and reviews. However, the formation of hydantoic acid units can also be explained by an alternative

Table 2
YIELD (IN %) OF 5-SUBSTITUTED HYDANTOIC ACIDS IN THE REACTION OF AMINES WITH VARIOUS AMINO ACID NCAs[a]

Amine	Gly-NCA	Sar-NCA	Phe-NCA
Diethylamine	100	—	80
Dimethylamine	Low	Low	25
t-Butyl	90	0	60
i-Propyl	45	0	35
Ethyl	55	—	10
Phenyl	—	—	0

[a] 0.5 g of NCA in 10 ml of 1:1 dichloromethane-amine at 0°C.

Adapted from Reference 102.

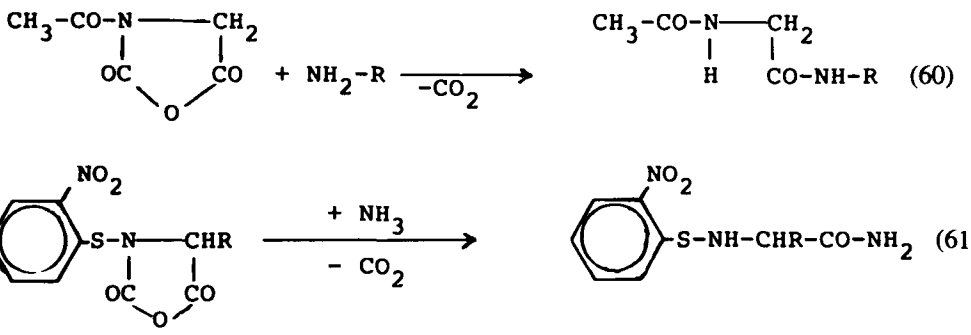
Table 3
CONVERSION OF α-AMINO ACID NCAs WITH STOICHIOMETRIC AMOUNTS OF SECONDARY AMINES IN 1,4-DIOXANE

No. of experiment	NCA	Secondary amine	Mole ratio NCA/amine	Yield of polypeptide in %	Yield of subst. hydantoic acid in %	Reaction[a] temp. in °C
1	D,L-phenyl-alanine	Diethylamine	1:1	0	32.0	25—40
2			1:3	0	75.0	25—40
3			1:6	0	86.0	25—40
4		Diisopropylamine	1:1	97.0	0	25—30
5		Dicyclohexylamine	1:1	98 0	0	25—30
6	Glycine	Morpholine	1:2	0	38.5[b]	25—45
7		Diethylamine	1:2	1 0	75.5[b]	25—45
8		Diisopropylamine	1:2	95.5	0	25—45
9		Dicyclohexylamine	1:2	97.0	0	25—45

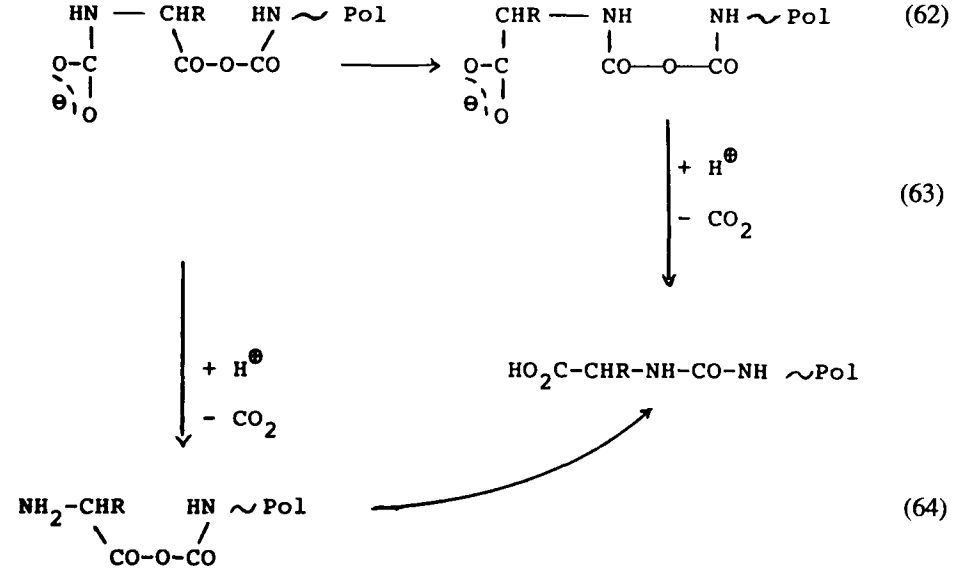
[a] The temperature rises during the exothermic reaction.
[b] Products soluble in alkaline water.

Adapted from Reference 105.

mechanism involving α-isocyanatocarboxylate ions (Equations 58 and 59). Whereas any experimental evidence for Sela and Berger's termination step is lacking, all model reactions favor the reaction mechanism of Equations 58 and 59. Such model reactions were first conducted by Kopple[102,103] and later by Seeney and Harwood[104] and by this author.[105] As demonstrated by the results summarized in Tables 2 and 3, increasing concentration and basicity of primary and secondary amines favor the formation of hydantoic acid unless sarcosine-NCA is used. Further evidence against the termination step of Equation 57 results from reactions of *N*-acetyl-Gly-NCA and Nps-Val-NCA with primary amines or ammonia[43,51,106] (Equations 60 and 61). Although the electron-withdrawing *N*-substituent favors a nucleophilic attack at C-2, hydantoic acids were never found.



A third reaction mechanism which can explain the formation of hydantoic acid end groups is based on the carbamate mechanism. If propagation is faster than decarboxylation of the intermediately formed mixed anhydride (Equations 53 and 54), isomerization according to Equations 62 to 64 may occur. This mechanism has the advantage that neither a nucleophilic attack at CO-2 of an NCA nor deprotonation of NCAs by amino end groups needs to be postulated. Yet, any experimental evidence in favor or against this mechanism is still lacking. At any rate, whichever mechanism is responsible for the formation of the acidic end groups, it is obvious that water, alcohol, or primary-initiated polymerizations of NCAs do not have a true "living character". In this connection it should be noted that Woodward and Schramm[107] reported the synthesis of high molecular weight polypeptides ($\overline{DP}_n > 10^4$) by means of water in benzene. However, it was later demonstrated that these $\overline{DP}_n$ were by far overestimated (Section V.B), owing to association of the peptide chains.

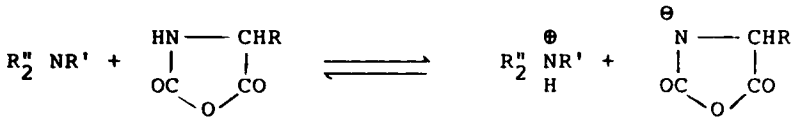


C. Initiation with Secondary Amines

Secondary amines can react with NCAs in two ways, namely as nucleophiles, attacking CO-5 in analogy to primary amines (Equations 46 and 49), or as bases, attacking the *N*-proton, in analogy to tertiary amines (Equation 65, R′ = H). The course of initiation depends on the nucleophilicity/basicity ratio (nuc/bas), and on the reaction conditions. Although a quantitative determination of the nuc/bas ratio is not feasible, numerous experimental results obtained from reactions with NCAs allow a subdivision of secondary amines into the following two classes:

- Class I with low nuc/bas ratio: To this class belong all aliphatic secondary amines with substituents bulkier than ethyl groups, e.g., di-*n*-propylamine, *N*-methyl-α-methylbenzylamine, *N*-methyl-alanine amides or dicyclohexylamine.
- Class II with high nuc/bas ratio: To this class belong also aromatic amines due to their low basicity, and dimethylamine, *N*-methyl-ω-amino acid amides, morpholine, piperidine, diethylamine, *N*-methylaniline.

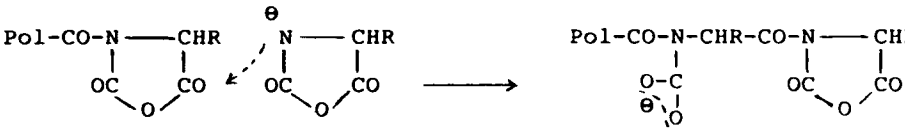
It must be emphasized that amines such as diethylamine, α-methylpiperidine, or *N*-methyl-α-methylbenzylamine represent the borderline between both classes, which means they can react more as a nucleophile or as a base, depending on the reaction conditions.



(65)

R, R' = H, Alkyl, R" = Alkyl,

(66)



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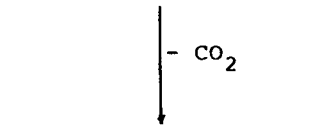


(68)

The experimental results on which this classification is based were obtained by means of the following methods: molecular weight measurements by physical methods, end group analyses, kinetic measurements, CO_2 evolution, and the kinetic effect of electrophilic additives. Blout and Karlson,[99] Bamford and Block,[98] and Goodman and Peggion[108] compared the $\overline{DP}$ of diethylamine or diisopropylamine-initiated polymerizations of γ-*O*-Bzl-Glu-NCA with those calculated according to Equation 43. From the much higher experimental $\overline{DP}$ they concluded that these secondary amines almost exclusively initiate the activated monomer mechanism (Equations 65 to 68). $\overline{DP}$ were calculated from viscosity measurements, and thus were close to the weight average values, which are useless for the desired purpose, because the $\overline{DP}_w/\overline{DP}_n$ ratios of primary or secondary amine-initiated poly(γ-*O*-Bzl-glutamate)s may be as high as 10 (Section V). Furthermore, it is noteworthy that the precipitation of the poly(γ-*O*-Bzl-glutamate)s was not described. However, oligo(γ-*O*-Bzl-glutamate) are soluble in most organic solvents, and fractionation may well affect the $\overline{DP}$ measurements, when M/I ratios <100 were used for the polymerizations. This critical comment is necessary

Table 4
RESULTS OF POLYMERIZATION OF THE NCA OF γ-*O*-BENZYL-L GLUTAMATE USING ^{14}C-LABELED CARBON

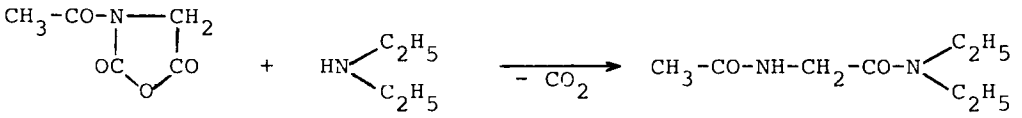
Initiator	A/I	$\overline{DP}_w$	Radioactivity in the polymer (%)	Initiator	A/I	$\overline{DP}_w$	Radioactivity in the polymer (%)
n-Hexylamine[a]	40	38	50.6	Diisopropylamine[b]	24	1210	10.9
Benzylamine[a]	50	79	72.2	Triethylamine	50	89	0.17
Isopropylamine[a]	16	242	100.0	Methyl diisopropylamine[b]	12	859	0
Diethylamine[a]	50	273	9.2				
Diisopropylamine[b]	15	693	11.4	Methyl diisopropylamine[b]	100	1722	0.49
				Na methylate		244	1.3

[a] Unpublished data of Goodman and Hutchison.
[b] Unpublished data of Scoffone et al.

Adapted from Reference 108.

because Shalitin and Katchalski[109] reported that their diethylamine-initiated polymerizations of γ-*O*-Bzl-Glu-NCA fit well to Equation 43.

More information is obtained from end group analyses. Using ^{14}C-labeled diethylamine, di-*n*-butylamine, and diisopropylamine, Goodman, Peggion, et al. could demonstrate[108-114] that di-*n*-butylamine and diisopropylamine exclusively deprotonate NCAs (Equation 65), whereas diethylamine reacts as a base and as a nucleophile (Table 4). These conclusions were confirmed by kinetic and spectroscopic studies of the author.[115] *N*-acetyl-Gly-NCA was added to morpholine, diethylamine, diisopropylamine, and dicyclohexylamine-initiated polymerizations of Gly-NCA. Since *N*-acetyl-Gly-NCA is a stronger electrophile than Gly-NCA, it acylates the secondary amine before it can initiate polymerization if the amine reacts as a nucleophile (Equation 69). Such a reaction sequence was found for morpholine and diethylamine[115] (Table 5). However, when diisopropylamine and dicyclohexylamine were used as initiators, *N*-acetyl-Gly-NCA reacted as cocatalyst and accelerated the polymerization. This acceleration effect results from the replacement of the slow initiation of Equation 66 by the faster initiation of Equations 70 and 71. Furthermore, IR and ^{1}H NMR spectra revealed the incorporation of *N*-acetyl-Gly-NCA, and thus, clearly prove that diisopropylamine and dicyclohexylamine initiate the activated monomer mechanism (AMM) (Table 5).



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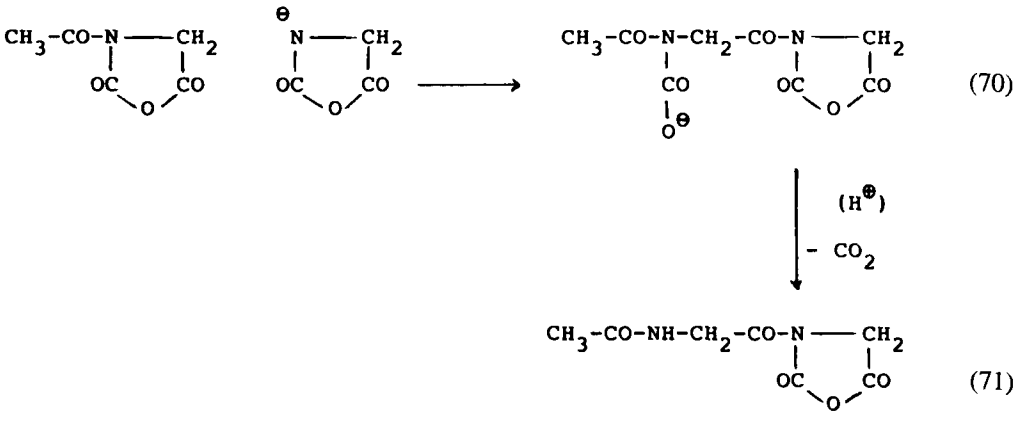


(70)

(71)

Table 5
CONDITIONS AND RESULTS OF THE POLYMERIZATION OF GLYCINE-NCA INITIATED BY VARIOUS SECONDARY AMINES IN THE PRESENCE OF *N*-ACETYL-GLYCINE-NCA. MOLE RATIO GLYCINE NCA/*N*-ACETYLGLYCINE-NCA = 4:1

Exp. no.	Catalyst	Mole ratio NCA/catalyst	Temp. (°C)	Time (h)	Yield[a] (%)	$\overline{DP}_n$[b]	Polyglycine	
							IR	*N*-acyl-NCA end groups by ^{1}H NMR
1	Dicyclohexylamine	250:1	30	1/12	65	8	+	+
2	Dicyclohexylamine	100:1	30	1/12	91	8	+	+
3	Diisopropylamine	250:1	30	1/12	70	8—9	+	+
4	Diisopropylamine	25:1	30	1/12	ca. 100	7	–	+
5	Diethylamine	250:1	25	12	0	—	–	–
6	Diethylamine	25:1	25	12	0	—	–	–
7	Diethylamine	4:1	25	12	<5	—	–	–
8	Morpholine	250:1	25	12	0	—	–	–
9	Morpholine	25:1	25	12	0	—	–	–
10	Morpholine	4:1	25	12	<5	—	–	–

[a] Related to CH_3-CO-(NH-CH_2-CO-)$_n$...

[b] The intensities of the acetyl signal (δ = 2.39) and of the CH_2-signal (δ = 4.32) in the ^{1}H NMR spectrum (CF_3COOH/TMS) were compared.

Adapted from Reference 115.

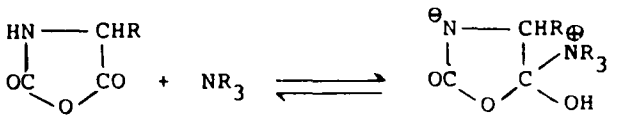
This clear-cut evidence of the AMM needs to be emphasized because Harwood et al.[104,116] doubted its existence until recently. Seeney and Harwood[104] reacted L-Phe-NCA with diethylamine at mole ratios <1 and seemingly found the diethylammonium carbamate of phenylalanine diethylamide (cf. Equations 46 and 48) as the sole reaction product. However, ^{1}H NMR spectra of the reaction mixture were their only evidence; they never isolated and

analyzed the carbamates. A reinvestigation of these reactions by the author[105] revealed that the conversion of diethylamine with L-Phe-NCA or Gly-NCA at M/I ratios <1 yields *N,N*-diethylhydantoic acids, in addition to the amino acid diethylamides. The yields of hydantoic acids increased with increasing concentrations of diethylamine (Table 3) in analogy with the results of Kopple.[102,103] Obviously, part of the diethylamine deprotonates the NCAs and reacts with the resulting isocyanatocarboxylates (cf. Equations 58 and 59). Under the same conditions morpholine gives low yields of hydantoic acids indicating that its nuc/bas ratio is higher than that of diethylamine. In contrast, diisopropylamine and dicyclohexylamine exclusively yield polypeptides because these amines are not nucleophilic enough to react with the isocyanatocarboxylates. In other words, the results of Harwood et al.[104] do not disprove initiation involving the activated monomer mechanism.

The classification of secondary amines is also based on block-copolymerizations and kinetic studies of Watson et al.,[4,117,118] Bamford et al.,[69,98,119] and Imanishi et al.[53,120-122] These authors describe in several papers the polymerization of D,L-Phe-NCA initiated by sarcosine dimethylamide and oligo(sarcosine dimethylamide)s. The isolation and characterization of block copolypeptides proves the nucleophilic attack of the sarcosyl unit on the Phe-NCA ring. Analogous investigations with oligomeric *N*-ethyl-, *N*-propyl-, and *N*-butylglycines are not conclusive because the CO_2 measurements were not combined with the isolation and characterization of the assumed block copolymers. Furthermore, the polymerization rates of L-NCAs and D,L-NCAs were compared upon initiation with various primary and secondary amines. The evaluation of the kinetic data was based on the hypothesis that (1) a stereospecific polymerization of D,L-NCAs is faster than a nonstereospecific one, and (2) only the activated monomer mechanism involves a high degree of stereospecificity. Although this hypothesis is not quite correct (cf. Section IV), the classification of secondary amines resulting from the kinetic data is reasonable. Sarcosine and *N*-methyl-ω-amino acid amides, piperidine and β- or γ-substituted piperidines, and *N*-methylbenzylamines predominantly react as nucleophiles (at M/I ratios >1), whereas *N*-methyl-D,L-alanine diethylamide and di-*n*-butylamine react as bases. However, it should always be kept in mind that in the case of secondary amines with high or moderate nuc/bas ratio the reaction conditions, in particular the M/I ratio, may influence the extent of nucleophilic and basic attack.

D. Initiation with Tertiary Amines

In the case of tertiary amines it is reasonable to differentiate between trialkylamines and pyridines because of the largely differing nuc/bas ratios. Trialkylamines have pK_s values around 11, whereas those of pyridines are of the order of 4 to 7 depending on the substituents. On the other hand, pyridines are more nucleophilic than triethylamine or higher trialkyl amines because the steric hindrance is lower if C-2 and C-6 are unsubstituted. Furthermore, the high polarizability of the aromatic π-electrons favors a nucleophilic attack. Tertiary amines are of particular interest as initiators for two reasons. First, as demonstrated for the first time by Blout and Karlson,[99] trialkylamines may yield with γ-*O*-Bzl-Glu-NCA the highest molecular weights of α-helix-forming polypeptides when compared to other initiators under identical conditions. For NCAs of *N*-substituted amino acids, pyridine seems to be the optimum reaction medium and initiator.[67] Second, when Bamford et al.[69-71,99] investigated the mechanism of trialkylamine and lithium chloride-initiated polymerizations, they developed a new mechanistic concept, the activated monomer mechanism (AMM). The AMM has since been the object of numerous investigations and controversial discussions.



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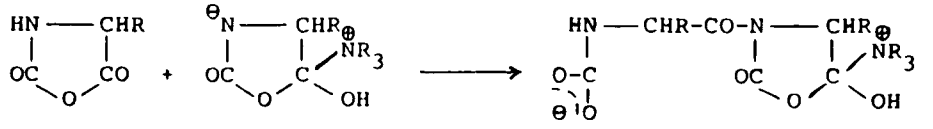

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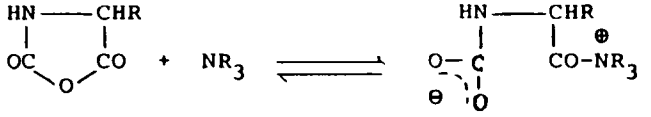

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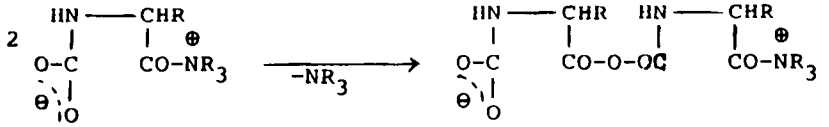

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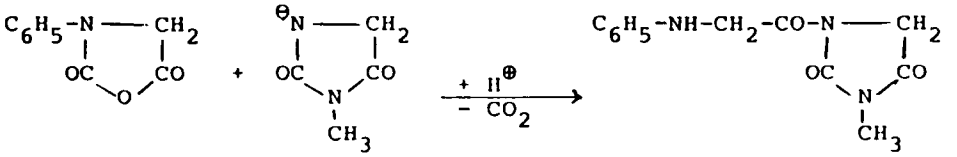

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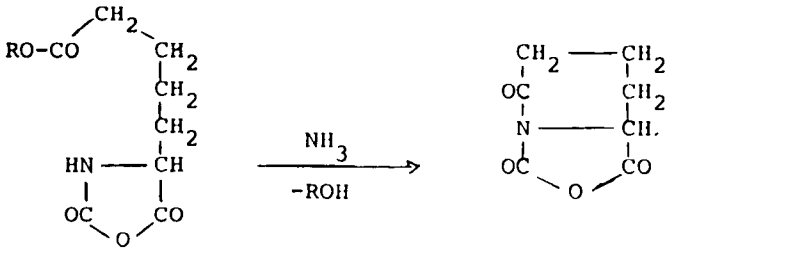


(77)

Details of the first mechanistic concept (Equations 72 and 73) published by Bamford et al.[69,123-127] are highly unlikely and were later abandoned in favor of the mechanistic scheme presented by Equations 65 to 68.[99] Both the first concept of the AMM and the "zwitterion mechanism" of Wieland[129] (Equations 74 and 75) postulate a nucleophilic attack of the tertiary amine at CO-5 of the NCA ring. For steric reasons, such a nucleophilic attack is unfavorable when trialkylamines (with the exception of 1,4-diazabicyclo[2.2.2]octane) are used, and deprotonation (Equation 65) is by far more likely. Any experimental evidence in favor of such a nucleophilic attack of trialkylamines is lacking, whereas numerous experiments demonstrate the existence of deprotonation. The first clue in this direction came from the reaction of 3-methylhydantoin (a nonpolymerizable model of Gly-NCA) with *N*-phenyl-Gly-NCA in the presence of triethylamine[71] (Equation 76). Similar reactions were conducted by Imanishi and Hashimoto[130] 2 decades later. In all cases 1-acylated 3-methylhydantoins were isolated. The intermediate formation of an NCA anion was for the first time demonstrated by Goodman and Choi who found that the NCA of δ-*O*-Bzl-α-aminoadipate forms a lactam ring of treatment with triethylamine (Equation 77). Finally, the author demonstrated[15,16,54-56] that numerous NCAs can be acylated, silylated, and sulfenylated in the presence of trialkylamines.

It is obvious that base-initiated polymerizations of *N*-substituted NCAs such as Sar- or Pro-NCA cannot obey the AMM. Bamford et al.[70,128] reported that Sar- and Pro-NCA polymerize very slowly with tri-*n*-butylamine in the absence of protic impurities, although γ-*O*-Bzl-Glu-NCA polymerizes rapidly under identical conditions (Figure 4). However, when

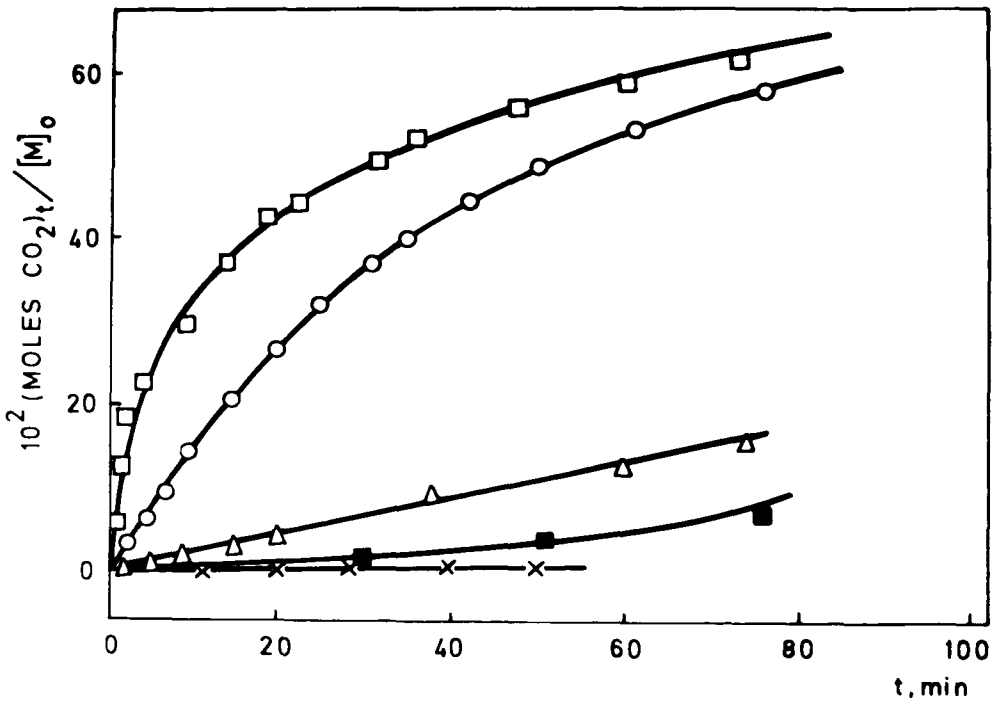
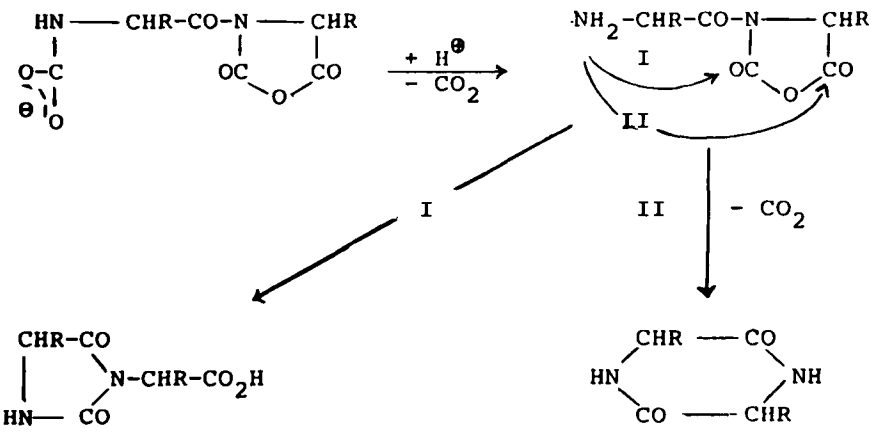


FIGURE 4. Conversion-time curves for tributylamine (B) initiated polymerizations of NCAs in DMF at 25°C (Adapted from Reference 20.) (□) γ-*O*-Bzl-Glu-NCA = 0.1 mol/l, B = 0.0126 mol/l (○) Sar-NCA = 0.332 mol/l, B = 0.042 mol/l, 3-methylhydantoine = 0.178 mol/l (△) L-Pro-NCA = 0.291 mol/l, B = 0.13 mol/l (■) Sar-NCA = 0.289 mol/l, B = 0.262 mol/l (×) Sar-NCA = 0.323 mol/l, 3-methylhydantoine = 0.175 mol/l.

protic cocatalysts were added, such as 3-methylhydantoin, a fast polymerization via nucleophilic chain ends was initiated (Figure 4). Thus, Bamford et al. concluded that trialkylamines do not directly interact with *N*-substituted NCAs, and acidic cocatalysts are necessary for a successful initiation. Nonetheless, the rapid racemization of Pro-NCA in the presence of sodium methoxide[131,132] and the racemization of Nps-NCAs in the presence of tertiary amines[56] suggest that deprotonation of C_α (Equation 78) might be an initiation reaction when more acidic cocatalysts are absent.

(78)

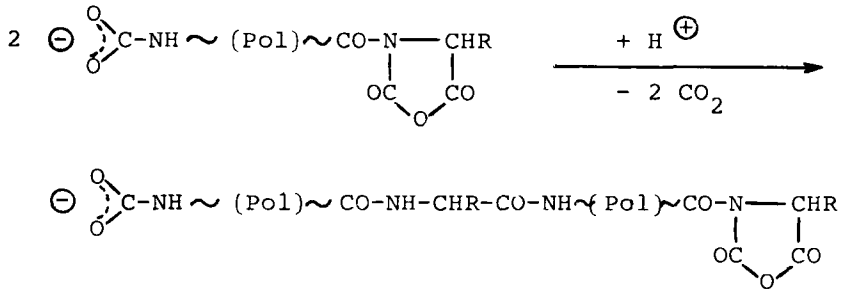
The initiation step of the AMM leads to a dimer which may possess a carbamate or an amino chain end (Equations 66 and 79). Seeney and Harwood doubted the existence of the AMM with the argument that the amino-terminated dimer would rather cyclize (Equation 80) than propagate. This argument is partially correct because cyclization of the amino group-terminated dimer is highly probable, and 2,5-dioxopiperazines and hydantoin-3-carboxylic acids are indeed by-products of base-initiated polymerizations. However, there is no evidence that the decarboxylation (Equation 79) is faster than propagation of the carbamate-terminated dimer, and cyclization of the latter is much less likely owing to seven- and nine-membered cyclic transitions states.



(79)

(80)

Since the formation of difunctional peptide chains having one *N*-acyl-NCA and one nucleophilic chain end is a basic requirement of the AMM, several authors attempted to prove or disprove their existence. Idelson and Blout[93] reported for sodium methoxide/methanol-initiated polymerizations of γ-*O*-Bzl-Glu-NCA in 1,4-dioxane that the $\overline{M}_w$ are constant after approximately 25% conversion for all M/I ratios. Peggion et al.,[133] on the other hand, conducted diisopropylamine-initiated polymerizations of the same NCA in DMF; they found increasing molecular weights upon prolonged storage and concluded that condensation of difunctional peptide chains takes place (Equation 81). The seeming contradiction between the results of both groups is possibly a consequence of the different reaction conditions. Peggion et al.[133] used DMF, a solvent in which poly(γ-*O*-Bzl-glutamate) is only weakly associated, whereas strong association occurs in 1,4-dioxane used by Idelson and Blout.[93,100] Polycondensation requires a high mobility of the peptide chain, and since most polypeptides strongly associate and even precipitate from the reaction mixture, polycondensation cannot play an important role for the course of the AMM.[134]



(81)

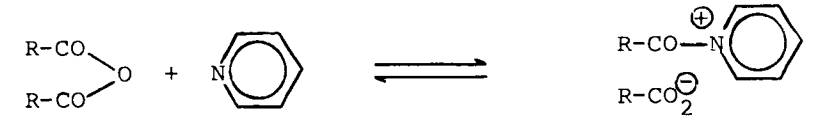
Szwarc,[134] like Sela and Katchalski,[135] emphasized in their comprehensive reviews on polymerization of NCAs that clear-cut proof of the AMM requires an unambiguous identification on *N*-acyl-NCA end groups. Shalitin,[136] on the other hand, proposed to study the cocatalytic effect of *N*-acyl-NCAs in analogy to acceleration of *N*-acyllactams on the anionic polymerization of lactams. An acceleration of the overall reaction rate is expected when the slow initiation of the original AMM (Equation 66) is replaced by the faster reaction of the *N*-acyl-NCA (Equation 70). Both problems were solved by the present author,[51] who investigated pyridine and triethylamine-initiated polymerizations of Gly-NCA, L-Ala-NCA, and L-Ile-NCA in the presence of *N*-acetyl-Gly-NCA. He could demonstrate that the addition of *N*-acetyl-Gly-NCA:

1. Accelerates the polymerizations
2. Reduces the $\overline{DP}$
3. Yields polypeptides possessing one *N*-acetylglycyl and one *N*-acyl-NCA chain end (Figures 2 and 3)

These results were later confirmed by Hashimoto and Imanishi[137] and Sekiguchi et al.[138] in similar experiments so that the existence of the AMM is unequivocally established.

Detailed information on the reactivity of potential cocatalysts was obtained by the present author[72] by studying the influence of various amides, imides, anhydrides, and isocyanates on base-initiated polymerizations of L-Ala-NCA, Gly-NCA, and Sar-NCA. Furthermore, Sekiguchi and Froyer conducted kinetic studies with α-Aibu-NCA as monomer and *N*-methylacetamide, 2-pyrrolidone, 2-oxazolidone, 3-methylhydantoin, 1-acetyl-2-pyrrolidone, 1-acetyl-2-oxazolidone and 1-acetyl-3-methylhydantoin as potential cocatalysts. A weak acceleration of the CO_2 evolution was found in most cases. Although all experiments with effective and ineffective cocatalysts clearly provide evidence for the existence of the AMM, they do not prove that this mechanism is predominant in all base-initiated polymerizations conducted without electrophilic cocatalysts. The cocatalyst prevents the formation of dimers with nucleophilic end groups. The question is, what happens when in the absence of a cocatalyst, difunctional oligomers are formed, and the *N*-acyl-NCA end groups are consumed by side reactions. A termination which completely stops the propagation of a difunctional peptide chain is their cyclization. Since macrocyclic and linear polypeptides have nearly identical properties and are insoluble in most organic solvents, the qualitative determination of cyclic peptides is in most cases nearly impossible. Nonetheless, in the case of Gly-NCA, the formation of several cycloglycines, in particular that of cyclohexaglycine, was observed.[123,141,142] Other terminations steps, with exception of reactions with nucleophilic impurities, are hypotheses without sufficient experimental evidence.[51]

The relatively high nuc/bas ratio of pyridine compared to trialkylamines is documented by rapid reactions with alkyl-halogenides (quaternization) and by the finding of Gold and Jefferson[143] that pyridine catalyzes the hydrolysis of carboxylic acid anhydrides by intermediate formation of *N*-acyl-pyridinium ions (Equation 82). These results suggest that pyridine, in contrast to trialkylamines, can attack CO-5 of NCAs (Equation 83). The alternative initiation mechanism is, of course, the N-H deprotonation of *N*-unsubstituted NCAs as formulated for trialkylamines in Equation 40. Bamford and Block[71] were the first who became aware that initiation by pyridines may take two different mechanistic courses. In order to differentiate between a nucleophilic and a basic attack on the NCA, γ-*O*-Bzl-L-Glu-NCA was polymerized in DMF with pyridine, picoline, and 2,6-lutidine as initiators. Whereas the nucleophilicity of pyridines depends largely on position and bulkiness of the substituents[98] the basicity is far less sensitive to the steric demands of substituents, because protons are the smallest possible reaction partners. Thus, the basicity slightly increases in the series pyridine <2-picoline <2,6-lutidine whereas the nucleophilicity strongly decreases. The polymerizations conducted by Bamford and Block[71] demonstrate that the rate of initiation depends on the basicity of the initiators and not on their nucleophilicity.



(82)

(83)