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Primary Amine-Initiated Polymerizations of α–Amino Acid N-Thiocarbonic Acid Anhydrosulfide

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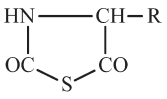
The N-thiocarbonic acid anhydrosulfides NTAs of D,L-leucine, D,L-phenylalanine and sarcosine were polymerized in dioxane by addition of n-hexylamine as initiator. Despite variation of the monomer-initiator ratio (M/I) only low yields of oligopeptides were obtained from D,L-Leu- and D,L-Phe-NTA. Both yields and molecular weights were almost twice as high for polymerizations of Sar-NTA. MALDI-TOF mass spectra confirmed that the isolated oligo- and polypeptides possess the expected structure with one reactive amino end group. Therefore, it is surprising that the polymerizations stopped at low conversions. Two hypotheses explaining this phenomenon are discussed.

Keywords: ring-opening polymerization; polypeptides; D,L-PHENYLALANINE; SARCOSINE; MALDI-TOF

1 Introduction

Polymerizations of α-amino acid N-carboxyanhydrides (NCAs) were described in hundreds of publications beginning with the work of Leuchs one hundred years ago (1–6). An advantage of NCAs is their high reactivity allowing for fast polymerizations and preparation of high molar mass polypeptides. A characteristic disadvantage is their high sensitivity to moisture or heating and their instability upon storage. Their thioanalogs the N-thiocarboxyanhydrosulfides (NTAs), **1**, are in principle, known for more than fifty years (7–9). Yet, most of the work on preparation and application of NTAs concerned stepwise syntheses of polypeptides (9–11). Modification of wool by grafting of oligopeptides by means of NTA was reported (12), but homopolymerizations initiated by a low molar mass initiator were not performed in previous publications (7–12). The only systematic studies in this direction were conducted by the first author who studied polymerizations of Gly-NTA (13), L-Phe-NTA (13), L-Leu-NTA (13, 14) and Sar NTA (15) and analogous polymerizations of the corresponding NCAs. It was found that regardless of monomers initiator and reaction conditions the molecular weights (i.e., the solution viscosities) of the polypeptides derived from Gly-NTAs were lower than those obtained from NCAs. Furthermore, yields and molecular weights of polysarcosine derived from the NTA were lower than those obtained from the NCA. Since in the years prior to 1971 neither high resolution ^{1}H-NMR nor ^{13}C-NMR spectroscopy nor FAB or MALDI-TOF mass spectrometry were available, it was difficult to identify endgroups and side reactions. In this context, it was the purpose of the present work to reinvestigate primary amine-initiated polymerizations of three NTAs, the polypeptides which are much more soluble in organic solvents than polyglycine or poly(L-Phe) and poly(L-Leu). It should be elucidated, if side reactions such as the formation of thiohydantoic acids (Scheme 1) stop the polymerization process, or if high molar mass polypeptides can be obtained.

Finally, it should be mentioned that greater interest in the chemistry of NTAs results from their potential role in molecular evolution (e.g. Scheme 2) (14). Anaerobic microorganisms working with a metabolism based on sulfur chemistry have been detected. Furthermore, heavy metal sulfides (e.g. FeS or NiS) were found to catalyze various reactions of sulfur compounds which may have played an important role in the molecular evolution (16–18).



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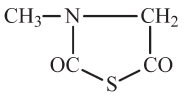

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2 Experimental

2.1 Materials

D,L-Leucine, D,L-phenylalanine, sarcosine, n-hexylamine, carbon disulfide, chloroacetic acid and phosphorous tribromide were all purchased from Acros Organics (Geel,

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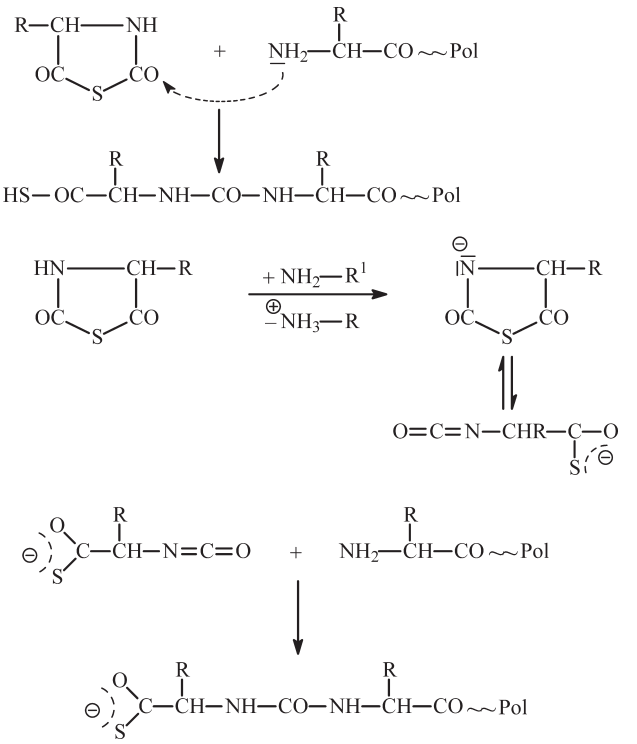
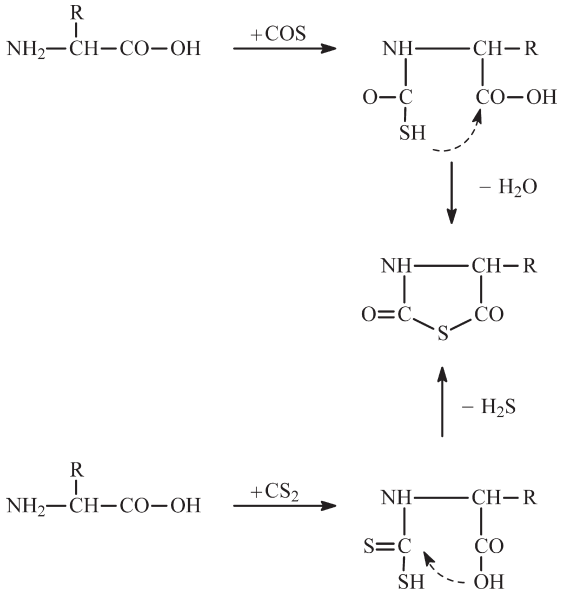


Sch. 1. Alternative routes of the potential formation of thiohydantoic acid endgroups.

Belgium) and used as received. Dioxane was distilled over sodium and DMF was distilled over P_4O_{10} *in vacuo*.

2.2 S-Ethoxythiocarbonyl Mercaptoacetic Acid (XAA)

The procedure described in Reference 19 was slightly modified to achieve higher purity. NaOH (1.0 mol) was dissolved in water (600 mL) and ethanol (3.4 mol) was added. Nitrogen was bubbled through this solution for 10 min and afterwards carbon disulfide (1.2 mol) was added rapidly but dropwise under cooling with ice. This reaction mixture was stirred for 2 h in a nitrogen atmosphere. Meanwhile, a solution of NaOH (1.0 mol) and chloroacetic acid (1.0 mol) in water (400 mL) was prepared under cooling with ice and then added rapidly to the xanthogenate solution. This mixture was stirred for 24 h at 20–22°C and acidified with conc. hydrochloric acid to pH 1. The product was extracted with three 300 mL portions of chloroform. The combined extracts were dried with Na_2SO_4 and concentrated *in vacuo*. The residual oil was dissolved in refluxing hexane by the dropwise addition of chloroform. When part of the product had crystallized at 20–22°C, the suspension was stored in a refrigerator for 20 h to complete the crystallization. The product was isolated by filtration and the filtrate was concentrated *in vacuo* and stored again in a refrigerator to obtain a small protion of a second crop. The combined crystalline products were recrystallized again. Yield, 33%, m.p. 57–57.5°C (m.p. 57–58°C in Reference (19)). ^{1}H-NMR ($CDCl_3$/TMS) δ: 1.43 (t, 3H), 3.98 (s, 2H), 4.66 (q, 2H).



Sch. 2. Speculative reaction pathways for the formation of NTAs in prebiotic molecular evolution under anaerobic conditions.

2.3 D,L-Phe-NTA

Sodium hydroxide (0.5 mol) was dissolved in water (450 mL), D,L-phenylalanine (0.2 mol) were then dissolved in the aqueous NaOH and XAA (0.2 mol) were added. This reaction mixture was stirred for 3 days at 20–25°C and then acidified with conc. HCl. The precipitated N-ethoxythiocarbonyl D,L-Phe was extracted with two 500 mL portions of chloroform. The combined extracts were washed with aqueous citric acid (5 wt%) dried with Na_2SO_4 and concentrated *in vacuo*.

The remaining product was dissolved in 300 mL of chloroform and cooled with ice. PBr_3 (0.11 mol) was then added dropwise with stirring in an atmosphere of dry nitrogen. After complete addition, the reaction mixture was stirred for 10 min with cooling and 60 min without cooling. Afterwards, this chloroform solution was washed with a saturated $NaHCO_3$ solution (200 mL) and three times with water (200 mL portions). The remaining chloroform solution was dried with $MgSO_4$ and concentrated *in vacuo*. The crude NTA was recrystallized from a 1:1 ethylacetate/ligroin mixture. Yield, 41%, m.p. 109–111°C. Anal. calcd. for $C_{10}H_2CO_2$ (193.25), C 62.31, H 4.64, S 16.55, found: C 62.09, H 4.71, S 16,36%. ^{1}H-NMR ($CDCl_3$/TMS), δ: 2.90 (dd, 1H), 3.35 (dd, 1H), 4.50 (dd, 1H) 7.20–7.48 (m, 5H) ppm.

D,L-Leu-NTA was prepared analogously: m.p. 80–82°C (m.p. 81–82°C in Reference (20)).

2.4 Sarcosine-NTA (15)

NaOH (0.6 mol) was dissolved in water (400 mL) and sarcosine (0.3 mol) and XAA (0.3 mol) were added with stirring.

Table 1. n-Hexylamine-initiated polymerizations of D,L-Leu-NTA

Expt. no.	Reaction medium	Temp. (°C)	NTA/Init.	Yield (%)	η^{a}_{inh} (dL/g)
1	Dioxane	20	20	30	0.08
2	Dioxane	20	40	27	0.09
3	Dioxane	20	60	0	—
4	Dioxane	20	100	0	—
5	DMF	60	20	47	0.08
6	DMF	60	40	64	0.09

[a]Measured at 20°C with c = 2 g/L in dichloroacetic acid.

After 3 days, the reaction mixture was acidified with conc. HCl and twice extracted with 400 mL portions of chloroform. The combined extracts were washed with aqueous citric acid (200 mL) and dried with Na_2SO_4 and concentrated *in vacuo*.

The remaining N-ethoxythiocarbonyl sarcosine was dissolved in dry chloroform (350 mL) and PBr_3 (0.17 mol) was added dropwise with stirring and under cooling with ice. After complete addition, the reaction mixture was stirred for 10 more minutes with cooling and for 1 h without cooling. Afterwards, it was washed with a concentrated $NaHCO_3$ solution (200 mL) and twice with water (200 mL portions). The chloroform solution of the NTA was then dried with MgSO4 and concentrated *in vacuo*, so that the temperature did not exceed 50°C. Finally, the crude product was distilled in a vacuum of 10^{-3} mbar over a short-path apparatus (bath temp. up to 100°C). Yield, 31%, ^{1}H-NMR in $CDCL_3$/TMS, $\delta = 3.11$ (3H, s) and 4.21 (2H, s) ppm.

2.5 Polymerizations

An α-amino acid NTA (10 mmol) was dissolved in dry dioxane (20 mL) and n-hexylamine (0.5 mmol) was added in the form of a 2M solution in dry dioxane under an atmosphere of argon. The reaction vessel was closed with a glass-stopper and steel spring. After storage for 48 h at 20–22°C, the reaction mixture was precipitated into diethyl ether (200 mL). The precipitated polypeptide was isolated by filtration and dried at 50°C *in vacuo*.

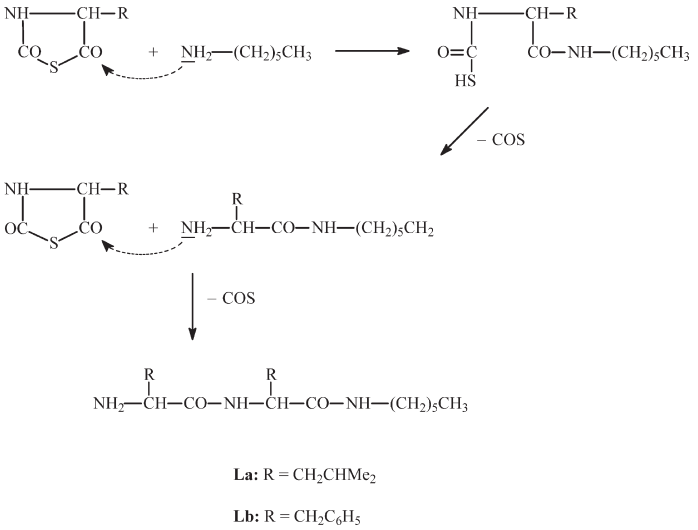
2.6 Measurements

The intrinsic viscosities were measured in dichloroacetic acid using an automated Ubbelohde viscometer thermostated at 20°C. The 400 MHz ^{1}H-NMR spectra were recorded on a Bruker "Avance 400" FT spectrometer in 5 mm o.d. sample tubes. The MALDI-TOF (MT) mass spectra were measured on a Bruker "Biflex III" mass spectrometer equipped with a nitrogen laser ($\lambda = 337$ nm). All mass spectra were recorded in the reflection mode with an acceleration voltage of 20 kV. The irradiation targets were prepared from peptide solutions in trifluoroacetic acid with dithranol as matrix and potassium trifluoroacetate as dopant.

3 Results and Discussion

For several reasons, most polymerizations conducted in this work were carried out in dioxane as the reaction medium. First, it is easy to dry and it is a standard reaction medium for polymerizations of NCAs. Second, it does not react with NCAs and NTAs, whereas polar solvents such as DMF, DMSO or N-methylpyrrolidone were found to initiate a Zwitterionic polymerization of NCAs (20). n-Hexylamine was selected as an initiator, because it is more nucleophilic than the amino end group of the peptide chains, so that this initiation should be faster than the growing steps. Such a kinetic scenario allows for a control of the molecular weight via the monomer-initiator ratio (M/I). For this reason, n-hexylamine was frequently used as initiator for NCAs (5).

Four polymerizations of D,L-Leu-NTA performed in dioxane with a variation of M/I were listed in Table 1 (Nos. 1–4). Low yields and low inherent viscosities were found for the first two experiments and no conversion was found for M/Is ≥ 60.



Sch. 3. Mechanism of primary amine-initiated polymerizations of D,L-Leu-NTA and D,L-Phe-NTA.

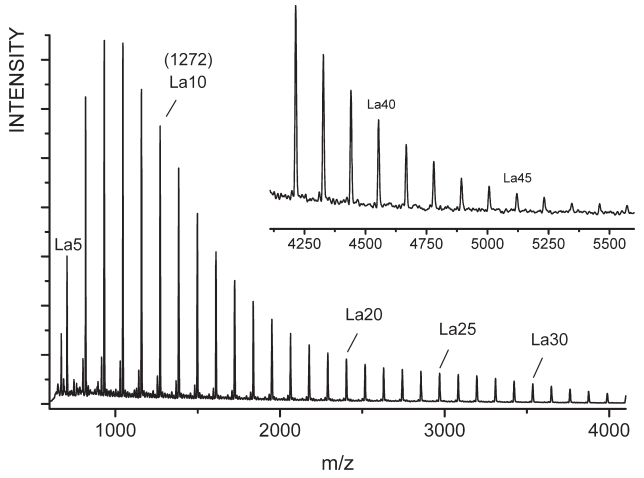


Fig. 1. MALDI-TOF mass spectrum of a poly(D,L-Leu) prepared by n-hexylamine-initiated polymerization in dioxane at 20°C (No. 1, Table 1). The weak peaks result from $Na^{\oplus}$ doping.

^{1}H-NMR spectra of the isolated poly(D,L-leucine)s indicated the covalent incorporation of hexylamine and the MT mass spectra confirmed the structure **La** (Scheme 3) as it was expected on the basis of a normal primary amine-initiated polymerization. Figure 1 exemplarily illustrates that most, if not all, poly(D,L-Leu) chains possess structure **La** and termination reactions such as that formulated in Scheme 1 were not detectable. Therefore, these findings present the unusual situation that the conversions were far from complete, although almost all oligo- or polypeptide chains possessed active amino endgroups.

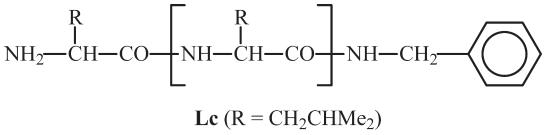
Analogous polymerizations of D,L-Phe-NTA in dioxane gave similar results (experiments Nos. 1–4, Table 2). The MT mass spectra confirmed again that the poly(D,L-Phe) chains of structure **Lb** were formed, and thiohydantoic acid endgroups (Scheme 1) were not observed in the MT mass spectra. For both monomers D,L-Leu-NTA and D,L-Phe-NTA, the ^{1}H-NMR spectra confirmed the covalent incorporation of n-hexylamine and the ^{13}C-NMR spectra did not give any indication for the presence of urea (hydantoic acid) groups. These results raise the question, how an early termination of the polymerizations process can be explained despite the presence of active amino endgroups. One potential explanation starts out from the fact that NTAs are less reactive than NCAs, so that the chain growth is slower under identical conditions. This point is particularly important, because the oligopeptides of D,L-Leu, D,L-Phe and most other α-aminoacids can associate via H-bonds forming soluble aggregates with hair-pin conformations or precipitate in the form of (distorted) β-sheet lamellae. It is well known from polymerizations of NCAs (Reference (5), Chapter 3) that such aggregation may cause a "physical death" of the polymerization, because the access of the monomers to the amino endgroup is sterically hindered. If this phenomenon was responsible for the low conversions and molecular weights in experiments Nos. 1–4, Tables 1 and 2, higher yields and molecular weights should be obtainable by: 1) polymerizations at higher temperatures, 2) polymerizations in polar solvents solvating H-bonds, 3) polymerization of Sar-NTA, because oligosarcosines cannot associate via H-bonds.

Table 2. n-Hexylamine-initiated polymerizations of D,L-Phe-NTA in dioxane

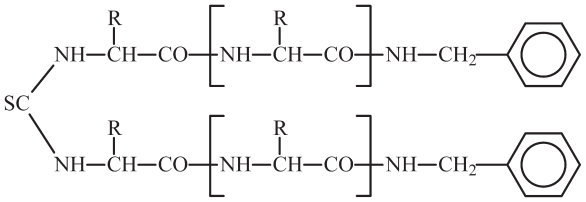
Expt. no.	NTA/Init.	Temp. (°C)	Yield (%)	η^a_{inh} (dL/g)
1	20	20	11	0.09
2	40	20	9	0.10
3	60	20	5	—
4	100	20	1	—
5	40	60	46	0.11

[a]Measured at 20°C with c = 2 g/L in dichloroacetic acid.

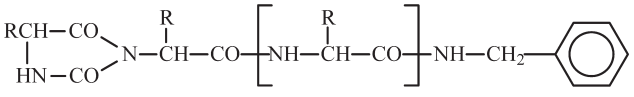
For these reasons, two benzylamine-initiated polymerizations of D,L-Leu-NTA were performed in DMF at 60°C (Nos. 5 + 6, Table 1). The yields were indeed significantly higher, but the viscosity values were still low and the conversion was still far from complete. The MT mass spectra showed that the expected linear chains having one benzylamide endgroup (**Lc** in Scheme 4) were the prevailing reaction product (Figure 2). Yet, surprisingly, four more



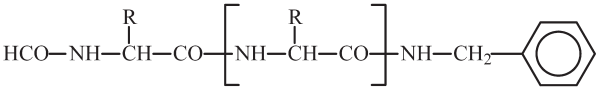
Lc (R = CH_2CHMe_2)



Ld (R = CH_2CHMe_2)



Le (R = CH_2CHMe_2)



Lf (R = CH_2CHMe_2)

Sch. 4. Potential reaction products of the benzylamine-initiated polymerizations of D,L-Leu-NTA in DMF at 60°C (Nos. 5 + 6, Table 1).

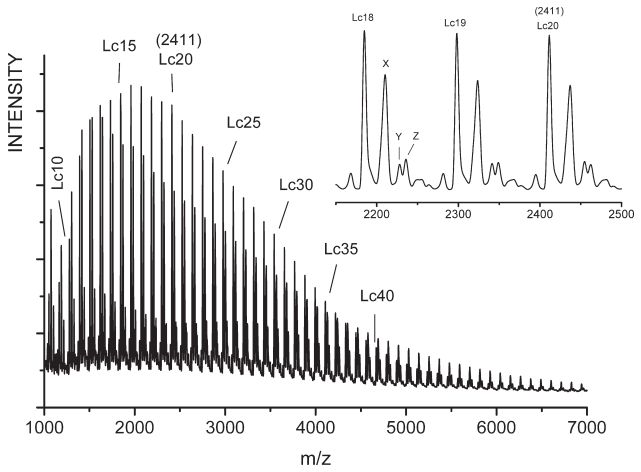


Fig. 2. MALDI-TOF mass spectrum of a poly(D,L-Leu) prepared by benzylamine-initiated polymerization in DMF at 60°C (No. 5, Table 1).

species were also found with additional masses around 21 Da, 24–25 Da (signal x), 41–42 Da (signal y) and 51–52 Da (signal z) relative to the **Lc** chains. The +21 Da peak fits in with structure **Ld** (Scheme 4), but a mechanistic explanation for the formation of this structure is hard to find. The +41 Da peak most likely results from structure **Le**, whereas no interpretation can be offered for the +25 and +52 Da peaks. Chains terminated by formyl groups (**Lf** resulting from the reaction of amino endgroups with DMF) were observed in primary amine-initiated polymerizations of NCA (21, 22). Yet, these chains have an additional mass of 28 Da which is too high for the +25 Da peak. A polymerization of D,L-Phe-NTA at 60°C in dioxane (Table 2) also proved that the peptide chains having one amide and one amino endgroup were the main reaction products. By-products having additional masses of +21–22 and +41–42 Da had lower concentrations than in the case poly(D,L-Leu). In summary, the polymerizations conducted at higher temperatures had in common to give higher yields, but the conversions stopped again below 100% despite the presence of NH_2-terminated polypeptide chains and intensive side reactions occurred.

Finally, four n-hexylamine-initiated polymerizations were performed with Sar-NTA (Table 3). In this series, the yields were considerable higher than those of poly(D,L-Leu) or poly(D,L-Phe) and polymerizations proceeded at all M/Is. Higher inherent viscosities were also obtained. However, complete conversion was only observed at M/I = 20, the conversions decreased with higher M/I ratios and were again far below 100% for M/I = 60 or 100. Assuming that the yields closely parallel the conversions multiplication of yields with M/I suggest that the molecular weights of experiments Nos. 2–4 were almost the same and the viscosity data agree with this calculation. The MT mass spectra of the samples Nos. 1–4 exclusively exhibited the peaks of the expected **Lg** chains. The mass spectra of samples Nos. 2–4

Table 3. n-Hexylamine-initiated polymerizations of Sar-NTA in dioxane

Expt. no.	NTA/Init.	Yield (%)	η_{inh}[a] (dL/g)
1	20	99	0.13
2	40	79	0.15
3	60	48	0.16
4	100	37	0.17

[a]Measured at 20°C with c = 2 g/L in dichloroacetic acid.

(Table 3) were almost identical with a maximum of the frequency distribution around 1 650 Da (Figure 3B), whereas a somewhat lower maximum (around 1 300 Da) was found for No. 1 (Figure 3A) in agreement with the lower viscosity value. In other words in experiments Nos. 2–4 (Table 3) the chain-growth stopped at nearly the same chain-length, although all chains had reactive amino endgroups. Incomplete conversions were also reported previously (15) for tert.-butyl amine-initiated polymerizations of Sar NTA in dioxane at 50°C (15).

This unexpected result is difficult to explain, since a "physical death" due to association via H-bonds cannot occur in the case of poly(Sar). The only hypothesis which

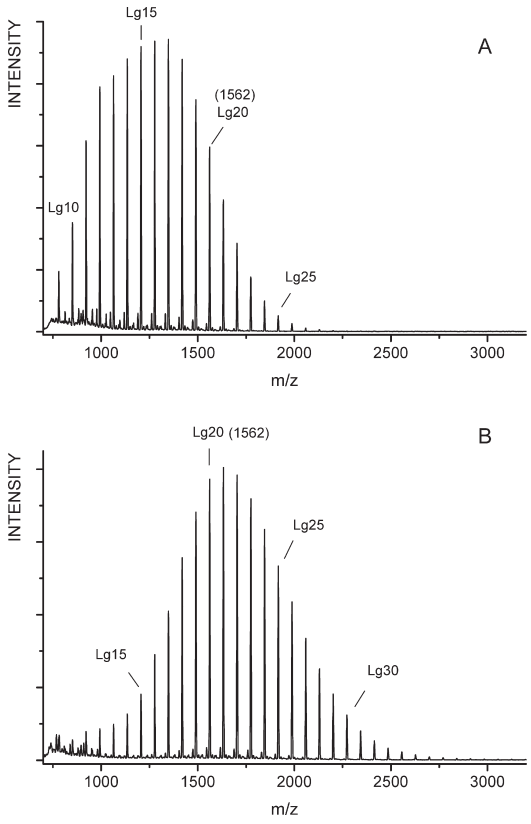


Fig. 3. MALDI-TOF mass spectra of n-hexylamine-initiated poly(Sar)s: A) M/I = 20/1 (No. 1, Table 3), B) M/I = 100/1 (No. 4, Table 3).

can be offered at this time is stabilization of thiocarbonate groups (formula **3**) by solvation with neighboring polySar chain segments. The polySar chain is far more polar than dioxane and comparable to N-methylpyrrolidone, therefore it can solvate and stabilize ion pairs more than dioxane. The thiocarbonate groups will decompose during the preparation of irradiation targets for MT mass spectrometry, and thus, are not detectable by this method. However, this hypothesis is mere speculation and a satisfactory explanation of the low conversions of Sar/NTA cannot be forwarded at this time.

4 Conclusions

The results of this work demonstrate, on the one hand, that primary amine-initiated polymerizations of NTAs proceed in the expected way, so that polypeptides having one amide and one amino endgroup are formed (Scheme 3). On the other hand, it is obvious that the conversion of almost all polymerizations stops far below 100%, despite the presence of peptide chains having a reactive endgroup. Association of peptide chains via H-bond causing a steric hindrance of the reaction between NTAs and endgroups partially explain this unexpected phenomenon. However, a convincing explanation for all aspects of these polymerizations was not found, and at the current state of affairs, NTAs are not promising candidates for the preparation of high molar mass polypeptides.

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