Controlling Polymer Architecture in the Thermal Hyperbranched Polymerization of L-Lysine

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ABSTRACT: Because of the unequal reactivity of the two amine groups of L-lysine hydrochloride, thermal polymerization of this asymmetrical AB_2 monomer results in hyperbranched polymers, which contain ∼2.5 times more N^{ϵ}-linked linear compared to N^{α}-linked linear structural units. This report discusses the feasibility of three approaches to control polymer architecture during the thermal hyperbranched polymerization of L-lysine hydrochloride. The reactivity of the more reactive ϵ-NH_2 group was modulated by introducing temporary protective groups that preferentially block the ϵ-NH_2 position. This was achieved by (i) addition of *o*-vanillin to the polymerization, (ii) copolymerization of N^{ϵ}-benzylidene-L-lysine, and (iii) copolymerization of α-amino-ϵ-caprolactam. Analysis of the degree of branching (DB) and the average number of branches (ANB) of the obtained polymers did not provide evidence for any significant structural changes. Analysis of the distribution of structural units, in contrast, revealed major structural changes and indicated a rearrangement from N^{ϵ}-linked linear structural units to N^{α}-linked linear structural units upon addition of *o*-vanillin and N^{ϵ}-benzylidene-L-lysine. Unlike *o*-vanillin and N^{ϵ}-benzylidene-L-lysine, α-amino-ϵ-caprolactam was found to be rather ineffective in modulating polymer architecture.

Introduction

Dendrimers are perfectly monodisperse macromolecules with a regular and highly branched three-dimensional architecture that is essentially defect-free.[1,2] The synthesis of dendrimers typically involves iterative procedures, which require workup and purification after completion of each reaction cycle. Although they allow virtually perfect control over dendrimer molecular weight and architecture, it is obvious that such multistep protocols are not very attractive from an industrial point of view. Hyperbranched polymers, which can be obtained by polymerization of AB_n monomers ($n \geq 2$) or by copolymerization of A_2 and B_n ($n \geq 2$) monomers,[3−6] have emerged as an attractive alternative to perfect dendrimers. In contrast to dendrimers, hyperbranched polymers are synthesized in a one-step polymerization reaction and can be easily prepared in large quantities. The simplification of the synthesis, however, goes at the expense of control over molecular weight and structural integrity. Hyperbranched polymers are neither perfectly monodisperse nor free of structural defects. In spite of these limitations, however, the field of hyperbranched polymers has received increasing attention and has witnessed impressive advances over the past 10−15 years not least driven by an industrial interest to explore the potential of branched polymer architectures.

The structure of hyperbranched polymers is commonly discussed in terms of a characteristic parameter, referred to as the degree of branching (DB).[7] Under normal circumstances, the DB, i.e., the architecture, of a hyperbranched polymer prepared from an AB_n monomer depends on the reactivity of the functional groups A and B and the multiplicity of group B (i.e., the value of *n*). For a purely statistical polymerization of an AB_2 type monomer with B groups of equal reactivity, the most probable value for DB is 0.5.[8] A number of strategies have been developed to control, and in particular to increase, the DB of hyperbranched polymers. Two general strategies that have been successfully used to increase the DB are the addition of a multifunctional core molecule B_x ($x \geq 2$) to the reaction mixture and the "slow monomer addition".[4,5] In addition, several more specific strategies to control the DB of certain classes of hyperbranched polymers have been developed. The DB of hyperbranched polymers prepared via chain hyperbranched polymerization can be controlled using late-transition-metal catalysts.[9] Cobalt chain-transfer catalysts, for example, have been used to control the competition between chain propagation and chain transfer in the free radical polymerization of divinyl monomers. Along the same lines, Pd^{II}−α-bisimine catalysts have been used to control the architecture of polyethylene by regulation of the competition between propagation and chain walking. Very recently, an elegant approach to control the DB in the step hyperbranched copolymerization of 1-(2-aminoethyl)-piperazine (BB_2') and divinylsulfone (A_2) was reported by Zhu, Yan, and co-workers.[10] These authors found that 1-(2-aminoethyl)piperazine forms inclusion complexes with β-cyclodextrin and observed that the complexed monomer reacted as a B_2 rather than B_3 building block. This allowed to control the DB of hyperbranched polymers prepared from 1-(2-aminoethyl)piperazine and divinylsulfone from ∼0.4 to ∼0.02 by increasing the amount of β-cyclodextrin that was added to the reaction mixture.

The subject of this report is hyperbranched polymers based on L-lysine. These polymers may be interesting alternatives for perfect lysine dendrimers and of potential interest as nonviral vectors for gene delivery,[11−14] as drug carriers,[15] for the development of multiple antigen peptide systems,[16,17] and as magnetic resonance imaging agents,[18] among others. The first hyperbranched polypeptides already date back to the 1950s and

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1960s, where a number of authors investigated the thermal polymerization of AB_2-type amino acids such as L-aspartic acid, L-glutamic acid, and L-lysine.[19−26] Although it was speculated that the polymers had a high degree of branching and a three-dimensional structure, no detailed characterization data were provided. Via dinitrophenylation and subsequent hydrolysis, Heinrich, Rohlfing, and Bugna could show that polymers obtained by thermal polymerization of the free base of L-lysine at 195 °C were composed of N^{ϵ}-linked linear lysine units, dendritic lysine units, N^{α}-linked linear lysine units, and terminal lysine units in a ratio of 4:3:1:1.[21] Fox and Suzuki demonstrated that the ratio between N^{α}- and N^{ϵ}-linked linear lysine units could be changed by copolymerization of L-lysine with other amino acids, e.g., from 1:2.5 (Lys, Asp) to 1:0.8 (Lys, Glu).[23] Very recently, we have reinvestigated the thermal polymerization of L-lysine monohydrochloride and characterized the structure of the resulting hyperbranched polymers by means of ^{1}H NMR spectroscopy.[27] The DB of the hyperbranched polylysines obtained via thermal polymerization of L-lysine was typically 0.35−0.45.[27] This is smaller than 0.5, which is the maximum DB for a hyperbranched polymer prepared via a purely statistical AB_2 polymerization. The difference is due to the unequal reactivity of the two amine groups of the lysine monomer. The ϵ-NH_2 group has a higher reactivity than the α-NH_2 group, which under normal circumstances leads to preferred polymerization via the ϵ-NH_2 group.[27] Consequently, appropriate strategies to modulate the reactivity of the ϵ-NH_2 group may improve control over the polymer architecture in the thermal hyperbranched polymerization of L-lysine. The reactivity of the ϵ-NH_2 group of the L-lysine monomer during the polymerization may be modulated by introducing reversible or temporary protective groups.

In this contribution, the feasibility of three approaches to vary the DB during the thermal hyperbranched polymerization of L-lysine will be discussed: (i) the addition of *o*-vanillin to the thermal polymerization of L-lysine, (ii) the thermal copolymerization of *N*$^{\epsilon}$-benzylidene-L-lysine and L-lysine, and (iii) the thermal copolymerization of α-amino-ϵ-caprolactam and L-lysine. Each of these approaches will be discussed and compared in terms of kinetics of the polymerization and the structure of the resulting hyperbranched polymers.

Experimental Section

Materials. Unless stated otherwise, all reagents and solvents were commercial grade and used as received. L-Lysine HCl, α-amino-ϵ-caprolactam, and MeOH were obtained from BASF AG (Ludwigshafen, Germany). Deuterated solvents for NMR spectroscopy were acquired from Armar Chemicals (Döttigen, Switzerland). All other chemical and reagents were acquired from Sigma Aldrich (Buchs, Switzerland).

Analytical Methods. Polymer molecular weights were determined by gel permeation chromatography (GPC) using a Waters 150CV instrument modified for on-line differential viscosimetry. All analyses were carried out at 25 °C using two Shodex OHPak columns (SB-803HQ + SB-804HQ) in series using 0.1 M $NaHCO_3$ as eluent at a flow rate of 0.5 mL/min. Elution times were converted into absolute molecular weights using the "universal calibration" curve, which was constructed using poly(ethylene oxide) standards with a narrow molecular weight distribution. Sample elution was monitored with a UV detector at a wavelength of 230 nm. In addition, molecular weights of selected hyperbranched polylysine samples were measured by triple detection GPC (refractive index/viscometry/light scattering). The results indicated a good agreement between the data obtained using light scattering and the "universal calibration" method (see Supporting Information).

To monitor monomer conversion as a function of polymerization time, samples were analyzed by GPC using a Viscotek TDA Model 300 instrument. All analyses were carried out at 25 °C using two PSS PROTEEMA columns in series using a 0.2 M acetic acid/0.1 M sodium acetate buffer as eluent at a flow rate of 0.6 mL/min. Sample elution was monitored with a refractive index (RI) detector. The obtained chromatograms were exported to Origin. The area under the curves of the polymer samples as well as for a pure monomer sample was normalized to 1, and in the case of overlapping peaks they were deconvoluted. The deconvoluted peaks were integrated to determine monomer conversion and reaction kinetics. Further details are described elsewhere.[27]

^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance 400 spectrometer. CD_3OD with 0.03% (v/v) TMS was used as the solvent. Proton and carbon chemical shifts are reported using the TMS peak as the reference.

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded on a Nicolet Magna 560 FTIR spectrometer.

Melting points (mp) were measured on a Büchi Smp 20 apparatus. All melting points were measured in open capillaries and are reported uncorrected.

Procedures. Synthesis of Hyperbranched Polylysine. A mixture of L-lysine HCl (54.79 g, 300 mmol) and KOH (16.83 g, 300 mmol) was thoroughly mixed in a mortar until it had a pulplike consistence. The pulp was introduced into an open 1 L reactor equipped with a PTFE stir bar and subsequently heated to 150 °C. When the mixture was molten, $Zr(O^nBu)_4$ (7.2 mL, 3 mol % with respect to the L-lysine monomer) was added as catalyst. The water that formed during the polymerization was allowed to escape from the open reactor. After 2, 4, 6, 8, 10, 12, 24, 30, and 36 h samples were taken from the reaction mixture. After 48 h, the reaction was stopped by cooling the reactor to room temperature. The samples, which were taken during the polymerization, as well as the final, brownish reaction product, were taken up in methanol, and the precipitated KCl was filtered off. The solution was evaporated to dryness and the residue taken up in water. Zirconium dioxide, which precipitated from the aqueous solution, was removed via filtration. Finally, the aqueous solution was lyophilized to afford the polymer as a yellow-brown solid. All polymers have been characterized by NMR, IR, and aqueous GPC. IR: 3271 s, 3068 m, 2926 s, 2858 m, 1637 m, 1525 m, 1436 m, 1373 w, 1304 w, 1252 w, 941 w, 669 w. ^{1}H NMR (400 MHz, CD_3OD, 298 K): 4.24 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 4.02 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 3.33 (br, 1 H, COC*H*(R)N$^{\alpha}$H$_2$), 3.25 (br, 1 H, COC*H*(R)N$^{\alpha}$H$_2$), 3.18 (m, 2H, C*H*$_2$−N$^{\epsilon}$H), 2.74 (m, 2H, C*H*$_2$−N$^{\epsilon}$H$_2$), 1.92−1.30 (br m, 6 H, CH_2). ^{13}C NMR (100.6 MHz, CD_3OD, 298 K): 174.78 (*C*(O)−NH), 172.95 (*C*(O)−NH), 55.14 (CO*C*H(R)N$^{\alpha}$H), 53.62 (CO*C*H(R)N$^{\alpha}$H, CO*C*H(R)N$^{\alpha}$H$_2$), 40.37 (−*C*H$_2$−N$^{\epsilon}$H$_2$), 38.43 (−*C*H$_2$−N$^{\epsilon}$H), 34.71 (N$^{\alpha}$H−CH−*C*H$_2$), 30.19 (*C*H$_2$−CH$_2$−N$^{\epsilon}$H$_2$), 28.87 (*C*H$_2$−CH$_2$−N$^{\epsilon}$H$_2$), 22.69 (−CH$_2$−*C*H$_2$−CH$_2$).

Synthesis of Hyperbranched Polylysine with *o*-Vanillin as Additive. A mixture of L-lysine HCl (54.79 g, 300 mmol), KOH (16.83 g, 300 mmol), and the appropriate amount of *o*-vanillin was thoroughly mixed in a mortar until it had a pulplike consistence. The yellow pulp was introduced into an open 1 L reactor equipped with a PTFE stir bar and heated to 150 °C. When the mixture was molten, $Zr(O^nBu)_4$ (7.2 mL, 3 mol % with respect to L-lysine) was added as catalyst. The water that formed during the polymerization was allowed to escape from the open reactor. After 2, 4, 6, 8, 10, 12, 24, 30, and 36 h samples were taken from the reaction mixture. After 48 h, the reaction was stopped by cooling the reactor to room temperature. The samples, which were taken during the polymerization, as well as the final, brownish reaction product, were taken up in methanol and the precipitated KCl was filtered off. The solution was evaporated to dryness and the residue taken up in water. Zirconium dioxide and the yellow *o*-vanillin, which precipitated from the aqueous solution, were removed via filtration. Finally, the aqueous solution was lyophilized to afford the polymer as a yellow-brown solid in quantitative yield. All polymers have been characterized by NMR, IR, and aqueous GPC. IR: 3271 s, 3068 m, 2926 s, 2858 m, 1637 m, 1525 m, 1436 m, 1373 w, 1304 w, 1252 w, 941 w, 669 w. ^{1}H NMR (400 MHz, CD_3OD, 298 K):

4.24 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 4.02 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 3.33 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.25 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.18 (m, 2H, CH_2−N$^{\epsilon}$H), 2.74 (m, 2H, CH_2−N$^{\epsilon}H_2$), 1.92−1.30 (br m, 6 H, CH_2). ^{13}C NMR (100.6 MHz, CD_3OD, 298 K): 174.78 (*C*(O)−NH), 172.95 (*C*(O)−NH), 55.14 (COC*H*(R)N$^{\alpha}$H), 53.62 (COC*H*-(R)N$^{\alpha}$H, COC*H*(R)N$^{\alpha}H_2$), 40.37 (−CH_2−N$^{\epsilon}H_2$), 38.43 (−CH_2−N$^{\epsilon}$H), 34.71 (N$^{\alpha}$H−CH−CH_2), 30.19 (CH_2−CH_2−N$^{\epsilon}H_2$), 28.87 (CH_2−CH_2−N$^{\epsilon}H_2$), 22.69 (−CH_2−CH_2−CH_2).

Synthesis of *N*$^{\epsilon}$-Benzylidene-L-lysine.[28] L-Lysine HCl (27.3 g, 150 mmol) was dissolved in aqueous 2 M lithium hydroxide (75 mL, 150 mmol), and the mixture was cooled to 4 °C. Freshly distilled benzaldehyde (15.9 mL, 160 mmol) was added, and the solution was shaken vigorously. A white precipitate formed and the mixture set solid. The mixture was cooled to 4 °C. After 2 h, cold ethanol (100 mL) was added and the slurry filtered through sintered glass. The precipitate was resuspended in cold ethanol (2 × 30 mL) and refiltered. The product was dried in vacuo, yielding *N*$^{\epsilon}$-benzylidene-L-lysine as a fine white powder (30.1 g, 129 mmol, 86%); mp 187−189 °C.

To unequivocally demonstrate the N$^{\epsilon}$-derivatization of L-lysine HCl, the insoluble *N*$^{\epsilon}$-benzylidene-L-lysine (5.85 g, 25 mmol) was suspended in a mixture of 1 M sodium hydroxide (25 mL) and ethanol (25 mL), which had been precooled to −20 °C. Precooled solutions of benzyloxycarbonyl chloride (7.75 mL, 50 mmol) and 1 M sodium hydroxide (60 mL, 0.18 mol) in ethanol (50 mL) were added in portions of equivalent amounts over 30 min while the temperature of the reaction mixture was held at −20 °C. After that, the temperature was allowed to rise to −5 °C and precooled hydrochloric acid (25 mL) was added. The solution was stirred for additional 5 min, heated up to 50 °C for 5 min, and concentrated on a rotary evaporator until all the ethanol had been removed. The solution was adjusted to pH 6.2 with 1 M NaOH and left overnight at 4 °C. The white precipitate was collected by filtration to yield *N*$^{\alpha}$-benzyloxycarbonyl-L-lysine. ^{1}H NMR (400 MHz, D_2O, 298 K): 7.29−7.11 (m, 5H, Ar*H*), 4.58 (s, 2H, CH_2−Ar), 3.80−3.75 (t, J = 6.1 Hz, 1H, COC*H*(R)N$^{\alpha}$H), 2.86 (t, J = 7.6 Hz, 2H, COC*H*(R)N$^{\alpha}H_2$), 1.77−1.38 (br m, 6 H, CH_2). This derivatization reaction proves that the original reaction between L-lysine HCl and benzaldehyde exclusively resulted in the N$^{\epsilon}$-substituted product.

Synthesis of Hyperbranched Polylysine with *N*$^{\epsilon}$-Benzylidene-L-lysine as Additive. A mixture of L-lysine HCl (54.79 g, 300 mmol), KOH (16.83 g, 300 mmol), and the appropriate amount of *N*$^{\epsilon}$-benzylidene-L-lysine was thoroughly mixed in a mortar until it had a pulplike consistence. The white pulp was introduced into an open 1 L reactor equipped with a PTFE stir bar and heated to 150 °C. When the mixture was molten, Zr(O^{n}Bu)$_4$ (7.2 mL, 3 mol % with respect to L-lysine) was added as catalyst. The water that formed during the polymerization was allowed to escape from the open reactor. After 2, 4, 6, 8, 10, 12, 24, 30, and 36 h samples were taken from the reaction mixture. After 48 h, the reaction was stopped by cooling the reactor to room temperature. The samples, which were taken during the polymerization, as well as the final, brownish reaction product, were taken up in methanol, and the precipitated KCl was filtered off. The solution was evaporated to dryness, and the residue was taken up in water. Zirconium dioxide and benzaldehyde, which precipitated from the aqueous solution, were removed via filtration. Finally, the aqueous solution was lyophilized to afford the polymer as a yellow-brown solid in quantitative yield. All polymers have been characterized by NMR, IR, and aqueous GPC. IR: 3271 s, 3068 m, 2926 s, 2858 m, 1637 m, 1525 m, 1436 m, 1373 w, 1304 w, 1252 w, 941 w, 669 w. ^{1}H NMR (400 MHz, CD_3OD, 298 K): 4.24 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 4.02 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 3.33 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.25 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.18 (m, 2H, CH_2−N$^{\epsilon}$H), 2.74 (m, 2H, CH_2−N$^{\epsilon}H_2$), 1.92−1.30 (br m, 6 H, CH_2). ^{13}C NMR (100.6 MHz, CD_3OD, 298 K): 174.78 (*C*(O)−NH), 172.95 (*C*(O)−NH), 55.14 (COC*H*(R)N$^{\alpha}$H), 53.62 (COC*H*(R)N$^{\alpha}$H, COC*H*(R)N$^{\alpha}H_2$), 40.37 (−CH_2−N$^{\epsilon}H_2$), 38.43 (−CH_2−N$^{\epsilon}$H), 34.71 (N$^{\alpha}$H−CH−CH_2), 30.19 (CH_2−CH_2−N$^{\epsilon}H_2$), 28.87 (CH_2−CH_2−N$^{\epsilon}H_2$), 22.69 (−CH_2−CH_2−CH_2).

Synthesis of Hyperbranched Polylysine with α-Amino-ϵ-caprolactam as Additive. A mixture of L-lysine HCl (54.79 g, 300 mmol), KOH (16.83 g, 300 mmol), and the appropriate amount of α-amino-ϵ-caprolactam was thoroughly mixed in a mortar until it had a pulplike consistence. The white pulp was introduced into an open 1 L reactor equipped with a PTFE stir bar and heated to 150 °C. When the mixture was molten, Zr(O^{n}Bu)$_4$ (7.2 mL, 3 mol % with respect to L-lysine) was added as catalyst. The water that formed during the polymerization was allowed to escape from the open reactor. After 2, 4, 6, 8, 10, 12, 24, 30, and 36 h samples were taken from the reaction mixture. After 48 h, the reaction was stopped by cooling the reactor to room temperature. The samples, which were taken during the polymerization, as well as the final, brownish reaction product, were taken up in methanol, and the precipitated KCl was filtered off. The solution was evaporated to dryness, and the residue was taken up in water. Zirconium dioxide, which precipitated from the aqueous solution, was removed via filtration. Finally, the aqueous solution was lyophilized to afford the polymer as a yellow-brown solid. All polymers have been characterized by NMR, IR, and aqueous GPC. IR: 3271 s, 3068 m, 2926 s, 2858 m, 1637 m, 1525 m, 1436 m, 1373 w, 1304 w, 1252 w, 941 w, 669 w. ^{1}H NMR (400 MHz, CD_3OD, 298 K): 4.24 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 4.02 (br, 1 H, COC*H*(R)N$^{\alpha}$H), 3.33 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.25 (br, 1 H, COC*H*(R)N$^{\alpha}H_2$), 3.18 (m, 2H, CH_2−N$^{\epsilon}$H), 2.74 (m, 2H, CH_2−N$^{\epsilon}H_2$), 1.92−1.30 (br m, 6 H, CH_2). ^{13}C NMR (100.6 MHz, CD_3OD, 298 K): 174.78 (*C*(O)−NH), 172.95 (*C*(O)−NH), 55.14 (COC*H*(R)N$^{\alpha}$H), 53.62 (COC*H*(R)N$^{\alpha}$H, COC*H*(R)N$^{\alpha}H_2$), 40.37 (−CH_2−N$^{\epsilon}H_2$), 38.43 (−CH_2−N$^{\epsilon}$H), 34.71 (N$^{\alpha}$H−CH−CH_2), 30.19 (CH_2−CH_2−N$^{\epsilon}H_2$), 28.87 (CH_2−CH_2−N$^{\epsilon}H_2$), 22.69 (−CH_2−CH_2−CH_2).

Determination of Structural Units and Branching Parameters. The mole fractions of N$^{\alpha}$-linked linear units (L_{α}), N$^{\epsilon}$-linked linear units (L_{ϵ}), dendritic (D) units, and terminal (T) structural units were estimated by ^{1}H NMR spectroscopy using the integrals of the α-CH signals corresponding to the different structural units.[27] The ^{1}H NMR signal of the terminal unit overlaps with that of the L-lysine monomer. To avoid an overestimation of the fraction of terminal units, the integral corresponding to this signal was corrected for the rest monomer content, which was determined by GPC.

From the mole fractions of the structural units, the degree of branching (*DB*) was calculated following the definition by Fréchet et al.:[7]

$$DB = \frac{D+T}{D+L+T} = \frac{D+T}{D+L_{\alpha}+L_{\epsilon}+T} \quad (1)$$

For this paper, the *DB* of the monomer was assumed to be 1. The average number of branches (*ANB*) was calculated as follows:[29]

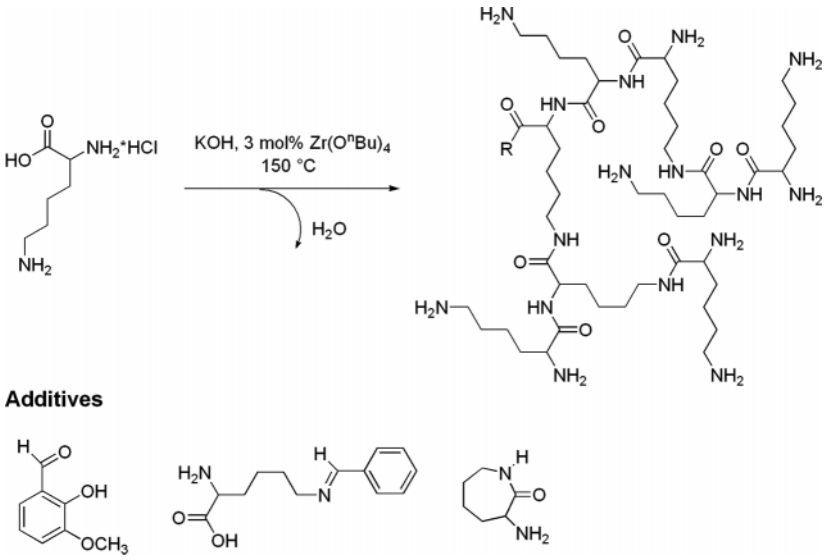
$$ANB = \frac{D}{D+L} \quad (2)$$

For this paper, the *ANB* of the monomer was assumed to be 0.

Results and Discussion

The thermal hyperbranched polymerization of L-lysine monohydrochloride is outlined in Scheme 1. When this monomer is mixed with 1 equiv of KOH and subsequently heated at 150 °C in the presence of 3 mol % Zr(O^{n}Bu)$_4$ as catalyst, a hyperbranched polymer is formed with a number-average molecular weight M_n = 6000 g/mol after 48 h.[27] ^{1}H NMR analysis revealed that this polymer consisted of ∼53 mol % N$^{\epsilon}$-linked linear structural units and ∼14 mol % N$^{\alpha}$-linked linear structural units and had a degree of branching (DB) of ∼0.35. The unequal amounts of N$^{\alpha}$ and N$^{\epsilon}$ linear structural units reflect the difference in reactivity between the two amine groups of the monomer. The higher reactivity of the ϵ-NH_2 group leads to nonstatistical chain growth and a DB which is smaller than the maximum value that can be obtained using an ideal AB_2

Scheme 1



monomer. In this contribution, we explore the feasibility of three different approaches to modulate the reactivity of the ϵ-NH_2 group and to control the polymer architecture during the thermal hyperbranched polymerization of L-lysine HCl. The first strategy involves the use of *o*-vanillin as an additive during the thermal polymerization of L-lysine HCl. Reaction of the aldehyde group of *o*-vanillin with an amine group of the L-lysine monomer or the polymer leads in situ to the formation of an imine. Since imines are hydrolytically labile and may be cleaved by the water that is generated during the polymerization, they may be regarded as temporary protective groups. Because of the higher reactivity of the ϵ-NH_2 group, it was anticipated that imine formation would preferably occur through the ϵ-NH_2 group and polymerization via the α-NH_2 group would be promoted. The rationale behind the second approach was very similar, with the difference that in this case a preformed imine (N^{ϵ}-benzylidene-L-lysine) instead of an aldehyde was used as additive. The third additive, which has been investigated, is α-amino-ϵ-caprolactam. In this case, the ϵ-amino group is protected in the form of an amide, which is liberated during polymerization upon opening of the lactam ring.

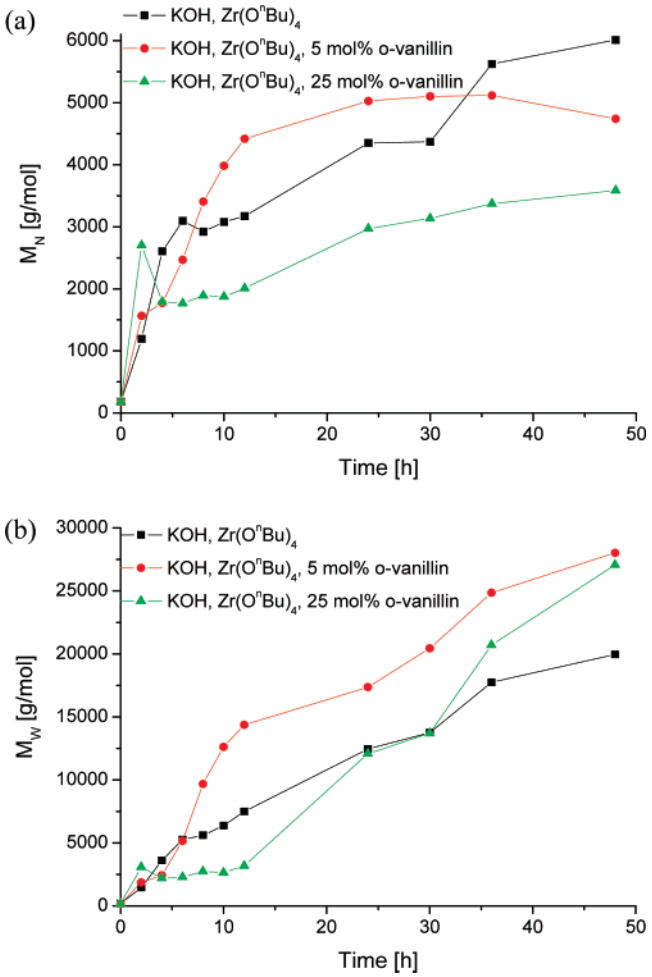


Figure 1. (a) Development of the number-average molecular weight and (b) the weight-average molecular weight as a function of time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of *o*-vanillin.

Polymerization with *o*-Vanillin as Additive. The influence of *o*-vanillin on the thermal hyperbranched polymerization of L-lysine hydrochloride, and the structure of the resulting polymers was studied by carrying out a series of polymerization experiments in which 0, 5, 25, 50, and 100 mol % *o*-vanillin was added to the reaction mixture. Polymerizations were carried out by heating stoichiometric mixtures of L-lysine HCl and KOH for 48 h at 150 °C in the presence of 3 mol % $Zr(O^nBu)_4$ as the catalyst. Figure 1 shows the evolution of polymer molecular weight as a function of reaction time. Whereas the addition of more than 25 mol % *o*-vanillin did not result in the formation of polymer after 48 h, polymerization of L-lysine hydrochloride in the presence of 5 mol % *o*-vanillin afforded polymers with molecular weights, which were slightly reduced compared to those obtained in absence of the additive. Polymerization in the presence of 25 mol % *o*-vanillin resulted in number-average molecular weights of ~3600 g/mol after 48 h, compared to 6000 g/mol without the addition of *o*-vanillin. ^{1}H NMR spectra of the final product did not reveal any *o*-vanillin signals, demonstrating that the *o*-vanillin is not incorporated into the polymer by reacting with the carboxylic acid groups of the monomer or polymer (see Supporting Information).

Using aqueous GPC, monomer consumption during the polymerization was followed as a function of time. Figure 2 compares monomer conversion for a polymerization, which was carried out without *o*-vanillin, with that of two polymerizations, which were carried out using 5 and 25 mol % *o*-vanillin as an additive. The results clearly indicate that the polymerization is

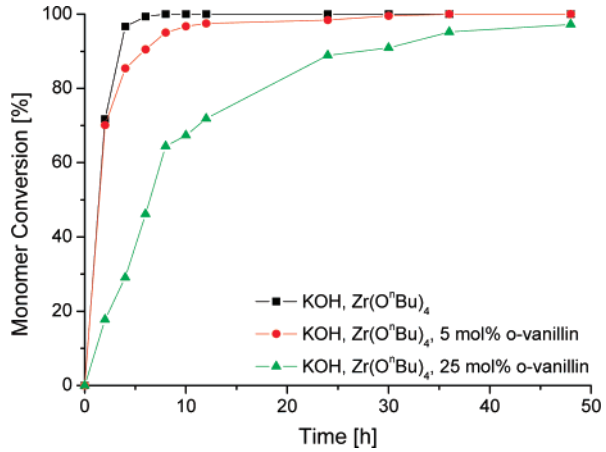


Figure 2. Monomer conversion as a function of time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of *o*-vanillin.

retarded in the presence of *o*-vanillin. In the absence of *o*-vanillin, full monomer conversion was reached after 12 h. In the presence of 5 or 25 mol % *o*-vanillin, however, the monomer conversion after 12 h decreased to 97% or 72%, respectively. The decreased polymerization rates in the presence of *o*-vanillin are in agreement with a temporary protection of the ϵ-amine groups and enhanced chain growth via the less reactive α-amine groups. After a reaction time of 48 h, full monomer conversion was also reached for the polymerization carried out in the presence of 25 mol % *o*-vanillin.

The hyperbranched polymers obtained via thermal polymerization of L-lysine HCl consist of four different structural units: (i) dendritic units (D), (ii) terminal units (T), (iii) N$^{\alpha}$-linked (L_{α}), and (iv) N$^{\epsilon}$-linked linear units (L_{ϵ}). The mole fractions of these different structural units can be determined using ^{1}H NMR spectroscopy, as described previously.[27] From the mole fractions of the structural units, the degree of branching (DB) and the average number of branches (ANB) can be calculated. Figure 3 shows the development of the mole fractions of the different structural units as a function of reaction time for polymerizations carried out in the absence of *o*-vanillin and in the presence of 5 and 25 mol % *o*-vanillin. The results clearly show that the addition of *o*-vanillin influences polymer structure. Upon increasing the amount of *o*-vanillin from 0 to 5 to 25 mol %, the mole fraction of L_{ϵ} units decreased from 0.53 to 0.47 to 0.39. The fraction of T units increased from 0.23 to 0.26 and 0.36 when respectively 5 and 25 mol % *o*-vanillin was added to the reaction mixture. The increase in the fraction of T units is also reflected in the decreased molecular weight of the polymers, which were prepared in the presence of 25 mol % *o*-vanillin (Figure 1). The addition of 5 and 25 mol % *o*-vanillin to the polymerization resulted in an increase in the L_{α} fraction from 0.14 to 0.16 and 0.19 and a decrease in the mole fraction of D structural units from 0.10 to 0.05, respectively. These observations indicate that the addition of small amounts (5 mol %) *o*-vanillin leads to a redistribution of linear structural units and results in polymers with an increased fraction of L_{α} linear structural units. This is in agreement with the proposed temporal, in-situ protection of the more reactive ϵ-NH_2 groups. The addition of larger amounts of *o*-vanillin (25 mol %) does not only lead to a decrease in the L_{ϵ} content and an increase in the L_{α} fraction but also results in an increased fraction of T structural units and a concomitant decrease in the polymer molecular weight, as well as a slight decrease in the mole fraction of dendritic structural units. This suggests that the addition of larger amounts (25 mol %) of *o*-vanillin does

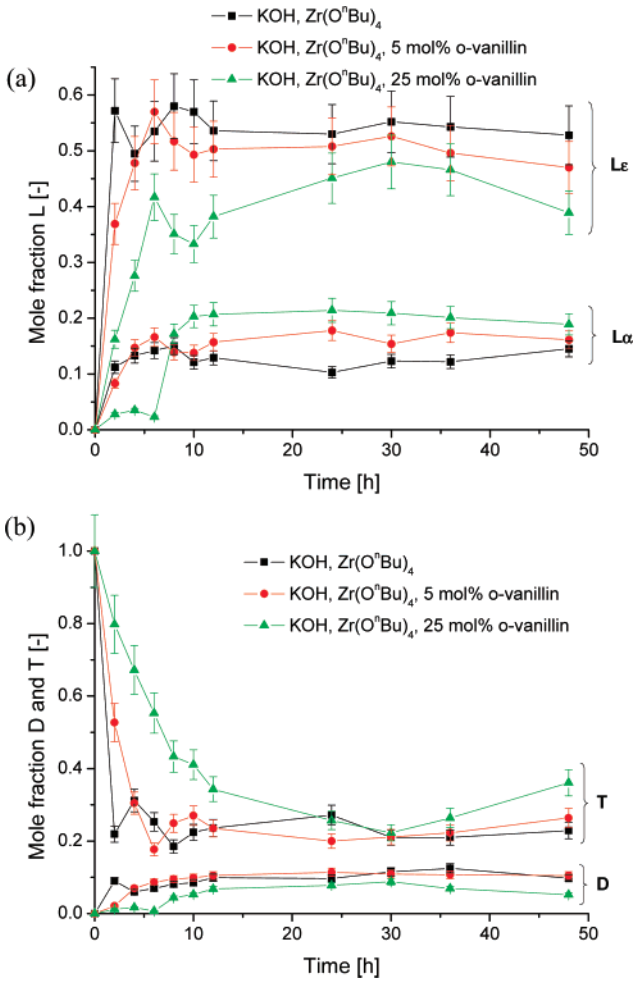


Figure 3. Development of the mole fraction of (a) L_{α} and L_{ϵ} and (b) T and D structural units as a function of polymerization time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of *o*-vanillin.

result not only in a redistribution of linear units from L_{ϵ} to L_{α} but also in polymers with a lower degree of polymerization and a more linear architecture.

It is interesting to compare the influence of the addition of *o*-vanillin on the fractions of the different structural units with the effect of *o*-vanillin on the DB and ANB, which can be calculated from the mole fractions of the different structural units. Figure 4 shows the development of DB and ANB with reaction time for polymerizations carried out in the presence of 5 or 25 mol % *o*-vanillin. The DB increased slightly from 0.33 to 0.37 and 0.41 upon the addition of 5 and 25 mol % *o*-vanillin, respectively. The ANB, in contrast, remained virtually unchanged when the polymerization was carried out in the presence of 5 mol % *o*-vanillin but decreased to 0.08 upon the addition of 25 mol % *o*-vanillin. The changes in the ANB are in good agreement with the changes in the polymer architecture that were proposed on the basis of results shown in Figure 3. With the addition of increasing amounts of *o*-vanillin, the fraction of D structural units decreases from 0.15 to 0.05, while the sum of the mole fractions of L units ($L_{\alpha} + L_{\epsilon}$) remains relatively constant (0.62 and 058 for polymerization without *o*-vanillin and in the presence of 25 mol % *o*-vanillin, respectively), which leads to the observed decrease in ANB. The slight increase in DB seems contradictory but can be understood considering the data shown in Figure 1. DB increases with increasing amounts of *o*-vanillin since (i) the decrease in D structural units is less than the increase in T structural units (see numerator of eq 1

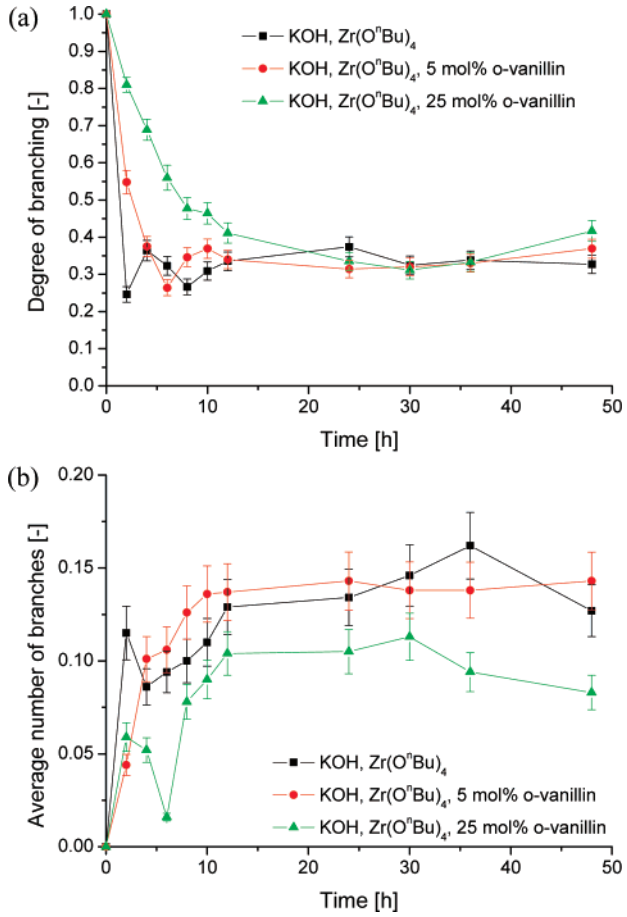


Figure 4. (a) Degree of branching and (b) average number of branches as a function of polymerization time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of *o*-vanillin.

and Figure 3) and (ii) the decrease in the sum of the mole fractions of D, L_α, and L_ϵ structural units is approximately equal to the increase in T structural units (see denominator of eq 1 and Figure 3). Figure 1 shows that the addition of *o*-vanillin to the polymerization results in a decrease in the number-average molecular weight and consequently in an increase in the fraction of T structural units. As a result, the observed increase in DB does not reflect changes in the polymer architecture but is related to the decrease in polymer molecular weight.

The preceding discussion also indicates that while DB and ANB are widely used to describe the structure of hyperbranched polymers, they are not necessarily the most informative characteristic parameters for hyperbranched polymers prepared from asymmetric monomers such as L-lysine hydrochloride. In the present study, most of the insights into the structural changes that occurred upon addition of *o*-vanillin to the reaction mixture were obtained by considering the distribution of different structural units, rather than DB and ANB.

It would have been interesting to study the kinetics of imine formation and hydrolysis during the polymerization. In principle, this can be accomplished using UV−vis spectroscopy.[30,31] The polymerization conditions used in this paper (bulk, 150 °C), however, do not allow the use of routine UV−vis spectroscopic methods. Detailed studies using in-situ techniques are planned and will hopefully allow a comparison of the polymerization kinetics with the kinetics of the imine formation and hydrolysis.

Polymerization with N^ϵ-Benzylidene-L-lysine as Additive. The Zr(O^{n}Bu)$_4$-catalyzed hyperbranched polymerization of L-lysine monohydrochloride was carried out in the presence of

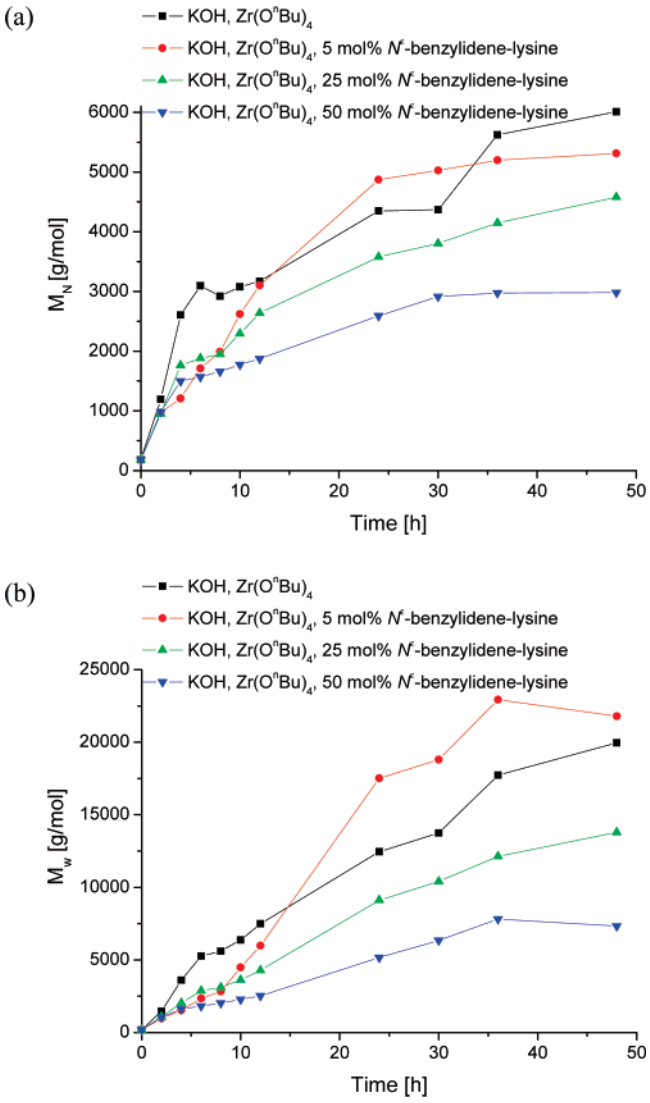


Figure 5. Development of (a) the number-average molecular weight and (b) the weight-average molecular weight as a function of time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of N^ϵ-benzylidene-L-lysine.

0, 5, 25, and 50 mol % N^ϵ-benzylidene-L-lysine as an additive. It should be kept in mind that each mole of N^ϵ-benzylidene-L-lysine introduces 1 mol of benzaldehyde and 1 mol of L-lysine into the reaction system. Consequently, the addition of 5, 25, and 50 mol % N^ϵ-benzylidene-L-lysine effectively corresponds to the addition of 4.8, 20, and 33 mol % benzaldehyde. Figure 5 shows the development of the polymer molecular weight with reaction time for the different polymerizations. Whereas the addition of 5 mol % N^ϵ-benzylidene-L-lysine did not significantly affect the molecular weight, a strong reduction in molecular weight was observed when 50 mol % N^ϵ-benzylidene-L-lysine was added. In the ^{1}H NMR spectra of the final hyperbranched polylysines, no aromatic signals that would indicate the presence of residual benzylidene imine groups could be detected (see Supporting Information). Attempts to homopolymerize N^ϵ-benzylidene-L-lysine were not successful. We attribute this to the absence of neutralization water and the water insolubility of N^ϵ-benzylidene-L-lysine: In contrast to L-lysine HCl, N^ϵ-benzylidene-L-lysine was used as the free base, and no KOH was added to the polymerization to neutralize the HCl salt. We hypothesize that the water, which is formed upon neutralization of the HCl salt, initially solubilizes the reaction mixture and facilitates polymerization.

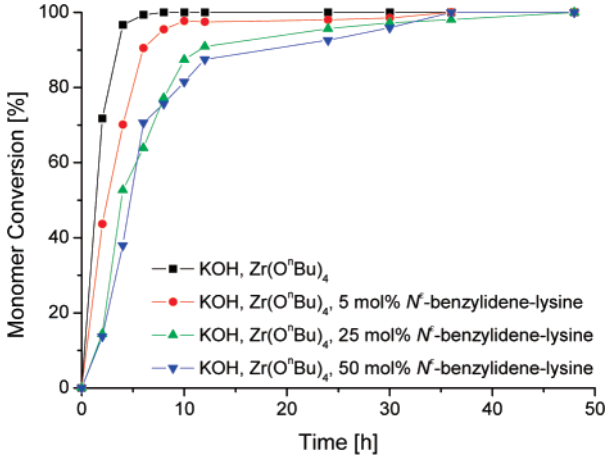


Figure 6. Monomer conversion as a function of time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of N^ϵ-benzylidene-L-lysine.

Figure 6 illustrates monomer conversion vs time for L-lysine monohydrochloride polymerizations carried out with 0, 5, 25, and 50 mol % N^ϵ-benzylidene-L-lysine. The data clearly show that the polymerization is retarded upon the addition of N^ϵ-benzylidene-L-lysine, which is in agreement with enhanced chain growth through the less reactive α-NH_2 group. Even in the presence of 50 mol % N^ϵ-benzylidene-L-lysine, however, full monomer conversion was reached after 36 h. Since the monomer conversion was quantitative in all cases, all of the L-lysine that was introduced in the reaction system in the form of N^ϵ-benzylidene-L-lysine has been incorporated in the hyperbranched polylysines (see also Supporting Information).

The mole fractions of the different structural units in the polymer were determined with ^{1}H NMR spectroscopy and are plotted as a function of polymerization time in Figure 7 for the different reaction conditions. Figure 7 shows that increasing the amount of N^ϵ-benzylidene-L-lysine from 0 to 50 mol % resulted in a decrease in the fraction of L_ϵ units from 0.53 to 0.38 and an increase in the fraction of L_α units from 0.14 to 0.28. The fractions of D and T structural units, in contrast, were virtually unaffected by the addition of N^ϵ-benzylidene-L-lysine. Only for the polymerization carried out with 25 mol % N^ϵ-benzylidene-L-lysine was the fraction of T units slightly enhanced. These observations indicate that the decrease in the fraction of L_ϵ structural units is essentially compensated for by the increase in the fraction of L_α units and that the addition of N^ϵ-benzylidene-L-lysine induces a redistribution ("isomerization") of linear units from L_ϵ to L_α. This is in agreement with a temporary protection of the ϵ-NH_2 groups and enhanced chain growth through the less reactive α-NH_2 group. Since D and T are not influenced and the decrease in L_ϵ units is compensated by the increase in L_α units, DB and ANB are not changed by the addition of N^ϵ-benzylidene-L-lysine to the reaction mixture (Figure 8). Only for the hyperbranched polymerization, which was carried out with 25 mol % N^ϵ-benzylidene-L-lysine, was a slight increase in DB observed, which may be related to the small increase in T structural units (Figure 7). These results, however, underline again the limited use of DB and ANB to characterize the structure of hyperbranched polymers prepared from asymmetric AB_2 monomers and emphasize the importance of studying the distribution of structural units in these polymers.

Polymerization with α-Amino-ϵ-caprolactam as Additive. The feasibility of α-amino-ϵ-caprolactam (ACL) as an additive to control polymer architecture was investigated in a series of

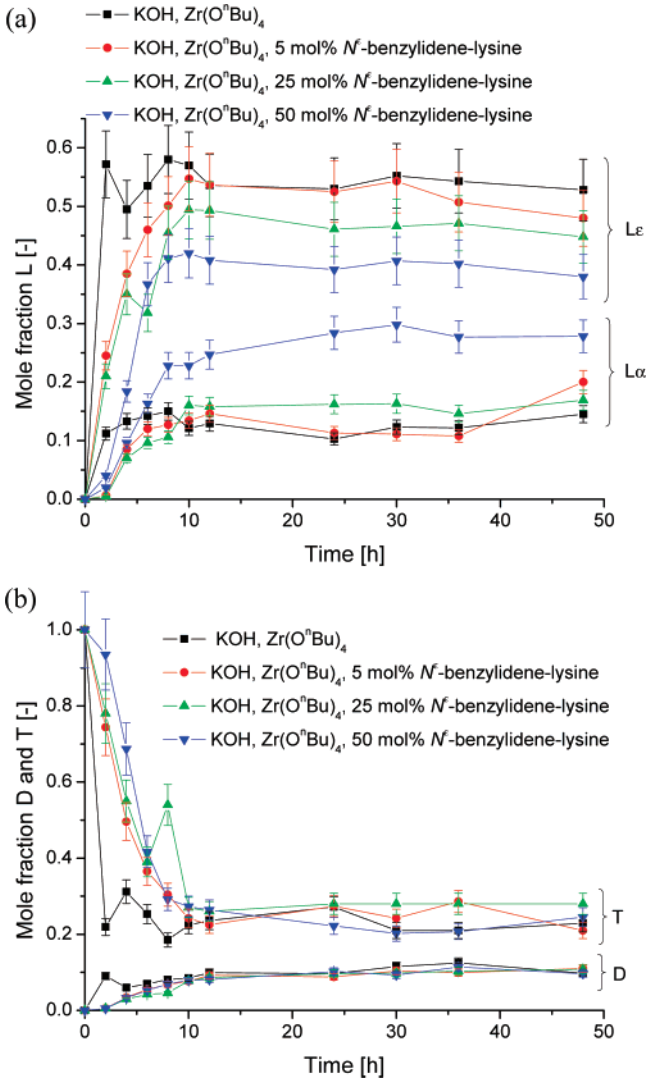


Figure 7. Development of the mole fraction of (a) L_α and L_ϵ and (b) T and D structural units as a function of polymerization time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of N^ϵ-benzylidene-L-lysine.

experiments in which L-lysine monohydrochloride was copolymerized with 5, 25, or 900 mol % ACL. In addition, several unsuccessful attempts to homopolymerize ACL were made. Heating ACL to 150 °C as the neat monomer, with addition of 3 mol % Zr(O^{n}Bu)$_4$ as well as in the presence of 20 mol % water, solely resulted in the formation of a colorless liquid, which turned brown with time, but did not afford any polymer after 48 h. The evolution of polymer molecular weight and monomer conversion during the copolymerization of L-lysine monohydrochloride with ACL was monitored with GPC (see Supporting Information). Since ACL and L-lysine hydrochloride eluted differently from the GPC column, ACL consumption (i.e., ACL ring opening) could be followed during the polymerization. Polymer yields were quantitative in all cases, meaning that all of the added ACL was incorporated in the final polymer. It was found that the addition of 5 and 25 mol % ACL did not significantly affect the number-average molecular weight of the polymer. The number-average molecular weight, however, significantly decreased when the hyperbranched polymerization of L-lysine HCl was carried out in the presence of a large excess ACL (900 mol %). ^{1}H NMR analysis of the resulting hyperbranched polylysines indicated that the mole fractions of the different structural units, and consequently DB and ANB, were

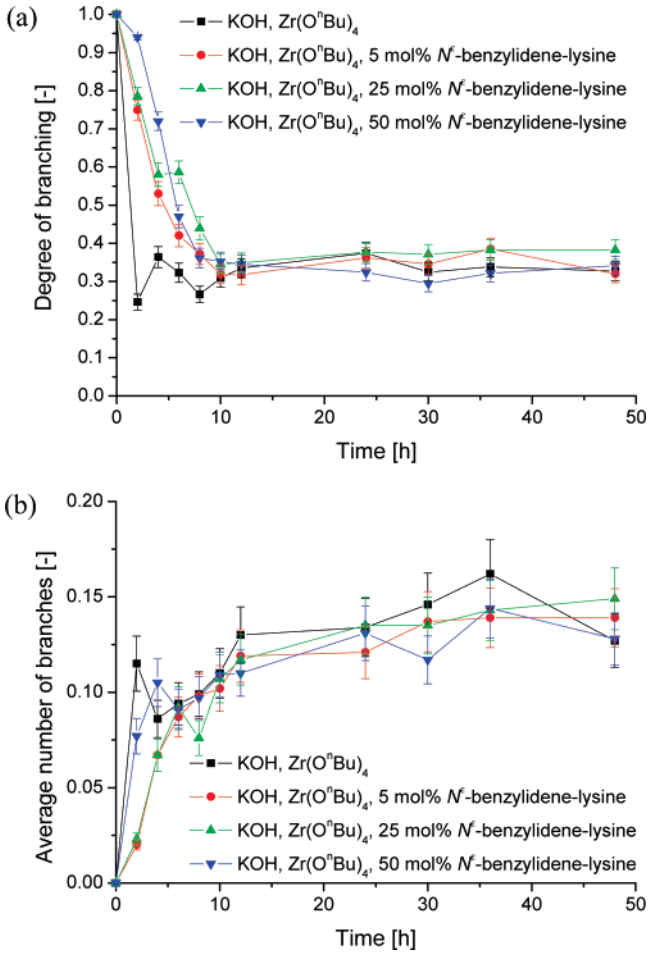


Figure 8. (a) Degree of branching and (b) average number of branches as a function of polymerization time during the thermal hyperbranched polymerization of L-lysine in the presence of different amounts of N^ϵ-benzylidene-L-lysine.

not significantly changed upon the addition of ACL (see Supporting Information).

Conclusions

Thermal polymerization of L-lysine hydrochloride in the presence of 1 equiv of KOH and 3 mol % Zr(O^{n}Bu)$_4$ as the catalyst at 150 °C affords hyperbranched polymers with a DB of 0.35−0.45 and an ANB of 0.15−0.25. In these polymers, the mole fraction of N$^\epsilon$-linked linear structural units is ∼2.5 times larger than the fraction of N$^\alpha$-linked linear structural units. In this paper, we have investigated the feasibility of three approaches to control the architecture of these hyperbranched polylysines. The rationale behind these three approaches was to modulate the reactivity of the more reactive ϵ-NH$_2$ group of the L-lysine HCl monomer and promote polymerization through the less reactive α-NH$_2$ group. The reactivity of the ϵ-NH$_2$ group was controlled by the introduction of temporary or reversible protection groups. This was attempted in three ways: (i) by addition of *o*-vanillin to the polymerization, (ii) by copolymerization of N^ϵ-benzylidene-L-lysine and L-lysine HCl, and (iii) copolymerization of α-amino-ϵ-caprolactam and L-lysine HCl.

The addition of 5 mol % *o*-vanillin to the polymerization did not significantly affect polymer molecular weight and the polymerization kinetics. The polymer molecular weight, however, was reduced when 25 mol % *o*-vanillin was added. Analysis of the DB and ANB of the polymers obtained in the presence of different amounts of *o*-vanillin did not suggest major structural changes. Only in the presence of 25 mol % *o*-vanillin was the ANB found to decrease from 0.13 to 0.08. Careful analysis of the development of the mole fractions of the different structural units, however, provided evidence for significant structural changes. Upon increasing the amount of *o*-vanillin from 0 to 25 mol %, the mole fraction of L$_\epsilon$ units decreased from 0.53 to 0.39 and the mole fraction of L$_\alpha$ units increased 0.14 to 0.19, indicating a rearrangement of linear structural units.

Copolymerization of L-lysine hydrochloride with 5 mol % N^ϵ-benzylidene-L-lysine did not affect polymer molecular weight or polymerization kinetics. Polymer molecular weights decreased and monomer conversion was retarded upon copolymerization of 25 or 50 mol % N^ϵ-benzylidene-L-lysine. The DB and ANB of the resulting hyperbranched polymers were barely influenced by the addition of N^ϵ-benzylidene-L-lysine to the reaction mixture. ^{1}H NMR analysis of the mole fractions of the different structural units, however, demonstrated that the addition of N^ϵ-benzylidene-L-lysine resulted in major structural changes. Upon increasing the amount of N^ϵ-benzylidene-L-lysine from 0 to 50 mol %, the mole fraction of L$_\epsilon$ was found to decrease from 0.53 to 0.38 with a concomitant increase in the mole fraction of L$_\alpha$ from 0.14 to 0.28. This suggests that the addition of N^ϵ-benzylidene-L-lysine essentially induced a redistribution ("isomerization") of linear structural units from L$_\epsilon$ to L$_\alpha$. These experiments also illustrate the limitations of DB and ANB to discuss the structure of hyperbranched polymers based on asymmetric AB$_2$ monomers and underline the importance of analyzing the relative amounts of different structural units.

In contrast to *o*-vanillin and N^ϵ-benzylidene-L-lysine, α-amino-ϵ-caprolactam was rather ineffective to modulate polymer architecture. The mole fractions of the different structural units and the DB and ANB were not significantly changed upon addition of 5 or 25 mol % α-amino-ϵ-caprolactam to the polymerization. Only the addition of a large excess (900 mol %) of α-amino-ϵ-caprolactam resulted in a slight decrease in the mole fraction of D structural units and the ANB.

Acknowledgment. This work was financially supported by BASF AG. Philippe Charpilloz is acknowledged for GPC measurements.

Supporting Information Available: Comparison of universal calibration and triple detection GPC, additional GPC and ^{1}H NMR data, and results of the polymerizations with α-amino-ϵ-caprolactam as an additive. This material is available free of charge via the Internet at http://pubs.acs.org.

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