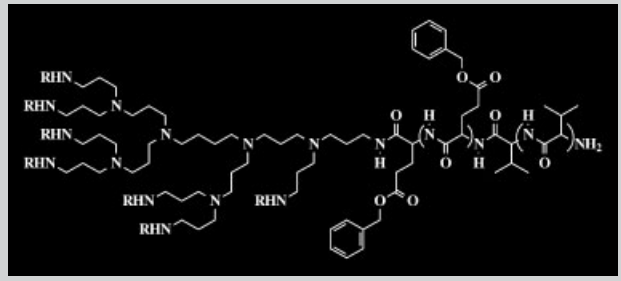
Summary: Polypeptide-shelled poly(propylene imine) dendrimers were realized by ring-opening polymerization of α-amino acid *N*-carboxyanhydrides, initiated by dendrimers as core molecules. Polypeptides with 2nd generation core were used as model compounds to investigate interior complexes between metal ion and surface-modified dendrimers. Microcalorimetric measurements outlined the formation of approximate 1:1 complexes between Cu^{II} and polypeptide-shelled dendrimers and the influence of polypeptide chain compositions on differential molar heats of complexation.



Composition of one of the polypeptides synthesized.

Polypeptide-Shelled Poly(propylene imine) Dendrimers and Their Complexing Properties towards Copper(II) Ions[a]

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Introduction

The ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCAs) reveals a well-established working tool to realize different polypeptide derivatives.[1] Thus, homopolymers, random, and block copolypeptides[2] were successfully obtained to investigate the chain conformation of the polypeptides as α helices (αh), *β* sheets (*β*s), or coil states. Their applications to various fields have been reported in the last few years.[3] The preparation of star-shaped polypeptides was also realized by NCA ring-opening polymerization. The outcome of this structural feature was the enhancement of the tertiary structure, especially in aqueous solution (e.g., switching between the αh and coil state of the chain conformation). This fundamental requirement is important to let such polypeptides act as artificial proteins or container molecules. Klok et al. investigated the switching of the conformational structure of star-shaped and water-soluble fluorescent polypeptides based on tetra-amino-functionalized perylene core.[4] Also Inoue et al. outlined the pH-dependent structures of several six-armed star poly(*γ*-benzyl-L-glutamate)s with a hexa-amino-substituted cyclotriphosphazene core.[5] Higashi et al. reported the first synthesis of a water-soluble poly(glutamic acid)-shelled dendrimer (PAMAM core with 32 amino surface groups) as a model protein.[6] The secondary structure of the helical poly(glutamic acid) chains of this polypeptide material allowed the enantioselective binding and stable encapsulation of α-amino acids in aqueous solutions. Such new polypeptides, with defined three-dimensional

[a] Supporting information for this article (NMR, SEC, and MALDI-TOF-MS data for **3** and **4** and ^{13}C NMR comparison between **3**, **4** and **5**) is available at the bottom of the article's abstract page, which can be accessed from the journal's homepage at http://www.mrc-journal.de, or from the author.

structure, might be very promising materials for new developments and improvement of functionalities (e.g., controlled release by conformational change of the helix segment caused by pH variation) based on amino acid derivatives.

Our concept for increased stability of interior metal complexes of polypeptide-shelled dendrimers is adopted from the aforementioned examples. The necessity of this synthetic approach is the requirement for increasingly stable metal complexes without additionally coupled complexing ligands in the interior of the commercially available PAMAM or PPI dendrimers. The coupled polypeptide chains on the dendrimer surface should act as an additional protection shell for complexed metal ions in the interior of the dendrimer, towards attacks from a biological environment, without significantly reducing the thermodynamic complex stability.

Complexation studies of unmodified poly(amidoamine) and poly(propylene imine) dendrimers were reported for a variety of metal ions.[7] However, the thermodynamic properties of these ion-dendrimer complexes are still partly unknown. Isothermal titration calorimetry (ITC) has become a standard method for the determination of complexation thermodynamics especially in macrocyclic chemistry[8] and biochemistry.[9] However, only very few applications of ITC in dendrimer chemistry were reported in the literature.[10]

Therefore, we have selected the 2nd generation poly(propylene imine) dendrimer for use as a first model substance for wrapping polypeptide chains on a dendrimer surface in order to increase the stability of metal-dendrimer complexes. In this paper, we present the synthesis and characterization (NMR, SEC, and IR) of polypeptide-shelled dendrimers realized by NCA ring-opening polymerization with 1st and 2nd generation poly(propylene imine) dendrimer as core molecules, and first investigations of Cu^{II}-complex formation with poly(γ-benzyl-L-glutamate-*co*-DL-valine)s with a 2nd generation dendrimer core in dimethylsulfoxide (DMSO) by micro-calorimetric titration measurements. The aim of the complexation studies is to prove and characterize defined complexes between Cu^{II} ions and the polypeptide-shelled dendrimer for tuning the next generation polypeptide-shelled dendrimers used in metal complexation.

Experimental Part

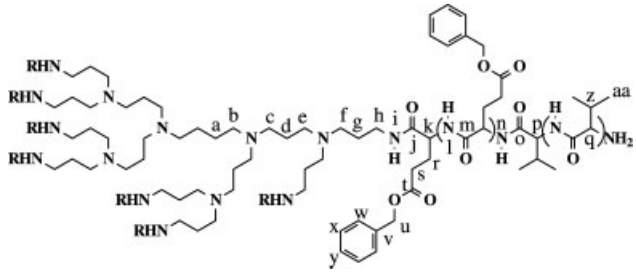
Chemicals

γ-Benzyl-L-glutamate *N*-carboxyanhydride (**1**) and DL-valine *N*-carboxyanhydride (**2**) were synthesized according to the method described by Daly and Poché.[11] All other solvents and reagents, including the 1st and 2nd generation poly(propylene imine) dendrimers, DAB-Am4 and DAB-Am8, and copper(II) trifluoromethanesulfonate (98%), used as the Cu^{II} source, were purchased from Fluka and Aldrich and were used as received. All solutions for titration microcalorimetry were prepared with dried DMSO.

Polymerization (General Procedure)

A round bottom flask was charged with a solution of the corresponding ratio of **1** and **2** in dry *N*,*N*-dimethylformamide ($0.08\ g \cdot mL^{-1}$) under argon atmosphere. Then, the corresponding initiator (hexylamine, DAB-Am4, or DAB-Am8) was added and the reaction mixture was stirred at room temperature. The used amount of the initiator was adjusted to the desired molecular weights of the polypeptide-shelled dendrimers controlled by the molar ratio of **1** and **2** and initiator (Table 1 for octa-armed polypeptides **5** and **6**, Table for linear- and tetra-armed polypeptide **3** and **4** in Supporting Information). After five days, the solution was slowly added to a 20-fold (v/v) excess of distilled water. The precipitated polypeptide was separated and dried in the vacuum.

*Poly(γ-benzyl-L-glutamate-co-DL-valine) with DAB-Am8 Core Based on a 2:1 Ratio for the Monomers **1** and 2-Polypeptide **5a***



^{1}H NMR (500 MHz; DMSO-d_6): $\delta = 0.7-0.9$ (z), 1.28 (a), 1.40 (d), 1.45 (g), 1.7–2.7 (b, c, e, f, q, r, and y), 2.85–3.15 (h), 3.8–4.4 (k and p), 5.0 (t), 7.15–7.4 (v–x), 7.7–8.5 ppm (i, l, and n). ^{1}H NMR spectra for polypeptide **5b–e** and **6** (without structure of DL-valine compared with **5b–e**) are similar to ^{1}H NMR spectrum of **5a**.

^{13}C NMR (125 MHz; DMSO-d_6): $\delta = 16.7-19.7$ (z), 24.0 (d), 24.5 (a), 24.9–25.5 (q), 26.7 (g), 26.9–27.8 (q), 29.1–31.5 (r and y), 37.1 (h), 51.1 (f), 51.3–52.1 (c, e, f, k, βs), 53.5 (b), 55.2–56.1 (k, αh), 57.0–60.0 (p), 65.5 (v), 127.05–128.5 (v–x), 136.2 (u), 170.5 (j), 170.6–171.1 (o), 171.5–173.0 (s, m, βs), 173.0–175.5 ppm (m, αh).

Polymer Characterization

The NMR experiments were performed on a Bruker DRX 500 NMR spectrometer operating at 500.13 MHz for ^{1}H and at 125.75 MHz for ^{13}C, respectively. DMSO-d_6 was used as solvent. For internal calibration, the solvent peak of DMSO-d_6 was used: δ (^{13}C) = 39.6 ppm; δ (^{1}H) = 2.50 ppm. The signal assignment was done by both, 1D and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, and ^{1}H-^{13}C HMBC) NMR experiments using the standard pulse sequences provided by Bruker.

The IR investigations were carried out with a Bruker IFS66 spectrometer equipped with a heatable Golden Gate Diamond

Table 1. Polypeptide-shelled dendrimers **5** and **6** (synthetic conditions and structure parameters).

Sample	Ratio		Yield	$\bar{M}_n$ [a]	$\bar{M}_n$ [b]	$\bar{M}_w$ [b]	$\bar{M}_w/\bar{M}_n$ [b]	NMR[c]
	1/2	M/I	%	g · mol^{-1}	g · mol^{-1}	g · mol^{-1}		**1/2**
5a	2	36	55.4	7 220	7 300	8 300	1.13	2.1
5b[d]	10	26.4	59.4	6 270	10 300	13 600	1.32	9.7
5c[d]	10	44	76.0	9 940	10 000	12 200	1.22	10.5
5d[d]	10	70.4	72.5	15 440	13 900	19 200	1.38	10.5
5e[d]	10	88	66.4	19 110	15 600	21 100	1.35	9.25
6[d]	–	40	71.0	9 540	9 800	10 900	1.12	–

[a] Includes $\bar{M}_w$ of initiator.
[b] Determined by SEC.
[c] Determined by the ratio between benzylic CH_2 group (**1**) and CH_3 group (**2**).
[d] Used for complexation studies.

ATR-Unit (SPECAC). 100 scans for one spectrum were co-added at a spectral resolution of 4 cm^{-1}. The SEC measurements were performed with a modular chromatographic equipment, Agilent Series 1100, containing a HPLC-pump, refractive index detector, and autosampler (Agilent, Germany) at ambient temperature. The column set, containing PL Oligo-Pore (Polymer Laboratories, United Kingdom), was used. The injection volume was 20 μL. The sample concentration was $c = 3\ \mathrm{g \cdot L^{-1}}$. The flow rate was 1 mL · min^{-1}. The experiments were carried out with a mixture of dimethylacetamide/water (98v/2v/LiCl 3g/L) as eluent. The molar masses were calculated using poly(vinylpyridine) standards.

Isothermal Titration Calorimetry

All titration calorimetric measurements were carried out at 25 °C with a TAM titration microcalorimeter (Thermometric AB, Sweden), equipped with the high performance 4 mL calorimetric module 2201, a 2 mL titration micro-reaction ampoule 2251, and a Lund precision syringe pump 6120. The 2 mL reaction vessel was filled with approximately 1.2 mL of a $2–5 \times 10^{-4}$ mol · L^{-1} dendrimer solution, and the copper solution (2×10^{-2} mol · L^{-1}) was added in portions of 8 μL from a 250 μL Hamilton syringe with a gold capillary driven by the syringe pump. Titration experiments consisted of 30 injections with a time delay of 30 min between the injections, allowing the reaction mixtures to equilibrate. The complete titration experiment and data acquisition were controlled by the instrument software (Digitam 4.1). The heat associated with each injection was calculated by integration of the peaks in the heat flow versus time curve using the Digitam software. The calibration of the calorimeter was performed by electrical heat pulses after each titration run. Blank experiments were performed under identical experimental conditions by titrating the copper solution into pure DMSO. The blank effects were subtracted in order to correct for dilution, mixing, and injection effects.

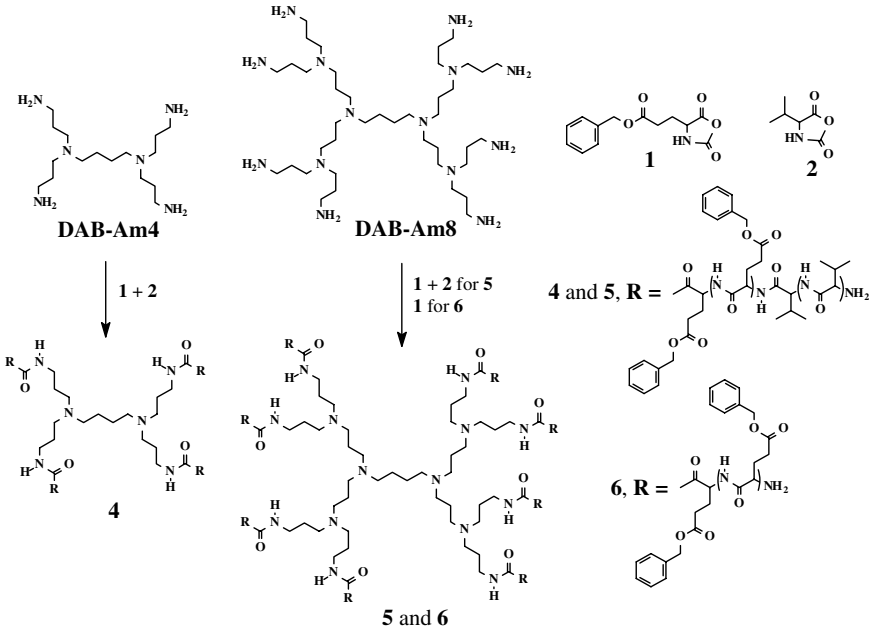
Results and Discussion

A well-defined protective shell, caused by the α helix or random-coil state of attached polypeptide chains on a dendrimer surface is one of the prerequisites for stable metal complexes to manifest their biological functions. Therefore, the poly(propylene imine) dendrimer DAB-Am8 was selected as core building block to establish the first polypeptide-shelled dendrimer with interior complexing properties, for example, for Cu^{II} as a model for defined metal-dendrimer complexes.

In order to optimize the synthesis and (structural) characterization of the desired polypeptide-shelled poly(propylene imine) dendrimer (**5**) (Scheme 1), first poly(γ-benzyl-L-glutamate-DL-valine) derivatives **3** and **4** were successfully synthesized by ring-opening copolymerization of γ-benzyl-L-glutamate *N*-carboxyanhydride (**1**) and DL-valine *N*-carboxyanhydride (**2**) with the initiators *n*-hexylamine and DAB-Am4, respectively. The characterization of the linear and tetra-armed polypeptides **3** and **4** is presented as Supporting Information.

The octa-armed poly(γ-benzyl-L-glutamate-*co*-DL-valine)s (**5**) with DAB-Am8 as core molecule (Scheme 1), were characterized by ^{1}H NMR, ^{13}C NMR, SEC, and IR. The molecular weight data and the **1/2** ratio of **5** are summarized in Table 1. The determined and theoretical number-average molecular weights are in good agreement. Also the molecular weight distributions are narrow for the random copolymerization of **1** and **2** initiated by DAB-Am8. The determined ratio of **1** and **2** by NMR is similar to the ratio used for initiating the NCA polymerization. The IR spectra of **5** revealed bands at 1 650/1 651 cm^{-1} (amide I) and 1 531/1 540 cm^{-1} (amide II). These results imply that the polypeptide chains are not organized as α helices in the solid state.[12,13] To better understand the complexing behavior of **5**, also the octa-armed poly(γ-benzyl-L-glutamate) (**6**) with DAB-Am8 as core molecule was realized and characterized as described for polypeptides **5**. As shown in Table 1, **6** possesses similar structure parameters as **5c**.

An important point of the structural characterization of the polypeptide-shelled dendrimers **4** and **5**, also for **6**, is to prove that all amino end groups of the dendritic initiators DAB-Am4 and DAB-Am8, respectively, participated in the initiation of the desired NCA polymerization, and were



Scheme 1. Synthesis of polypeptides **4**–**6** based on the conversion of DAB-Am4 or DAB-Am8 with α-amino acid *N*-carboxyanhydrides **1** and **2**.

converted into starting amide groups of the polypeptide chains. The ^{13}C NMR signal of the methylene group (C) in α position to the formed amide group can be well observed at about 37.2 ppm for both polypeptide-shelled dendrimers, **4** and **5** [Figure 1(a) and (b)]. As known from the spectra of DAB-Am4 and DAB-Am8, the carbon signal of residual CH_2-NH_2 groups (A) should appear at about 40.2 ppm. Unfortunately, the detection of a possibly small signal would be hampered due to overlapping signal of the solvent DMSO-d_6. However, this signal overlap does not occur in a two-dimensional ^1H-^{13}C HMQC spectrum, and one takes advantage of the higher sensitivity because of proton detection. Figure 1(c) exemplifies this for **5b**. Besides the intense cross-peak of group (C), only the signal of DMSO-d_6 appeared in the region of interest but no signal at 2.52/40.2 ppm could be observed for residual group (A). In such a way and within the sensitivity of the method, a complete conversion of initiator amino groups and so the formation of completely polypeptide-shelled dendrimers could be stated.

Copoly(peptide)-shelled dendrimers **5b**–**e** with six interior tertiary amino groups and the homopoly(peptide)-shelled dendrimer (**6**) were used for first complexation studies with Cu^{II} by isothermal titration microcalorimetry.[14] The results were compared with the complexation behavior of the unmodified 1st and 2nd generation poly(propylene imine) dendrimers DAB-Am4 and DAB-Am8. The calorimetric results are summarized in Figure 2 and Table 2. The results show that the core dendrimers DAB-Am4 and DAB-Am8, as well as the polypeptide-shelled dendrimers **5b**–**e** and **6**, are able to form complexes with Cu^{II}. Except for the 2nd generation poly(propylene imine) core dendrimer

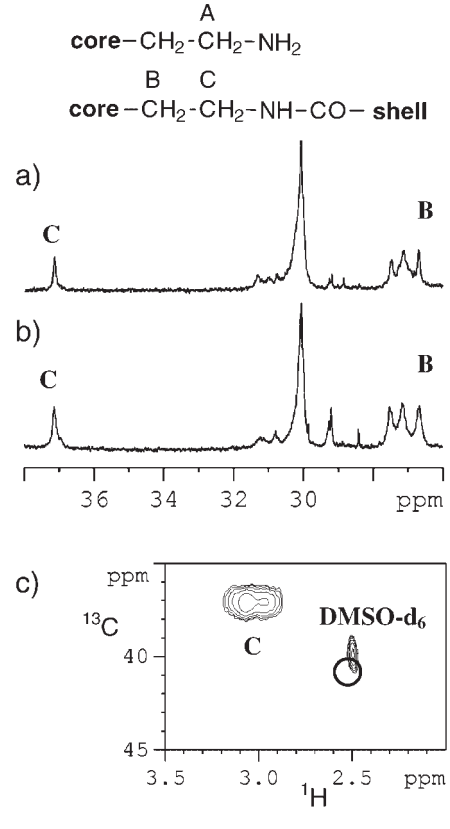


Figure 1. Regions of the ^{13}C NMR spectra of **4b** (a) and **5a** (b) showing signals B and C assigned to the connecting group between the dendritic core and polypeptide shell. The region of the ^1H-^{13}C HMQC spectrum of **5a** (c) demonstrates the absence of the signal for the CH_2-NH_2 group (A) (expected in the encircled region).

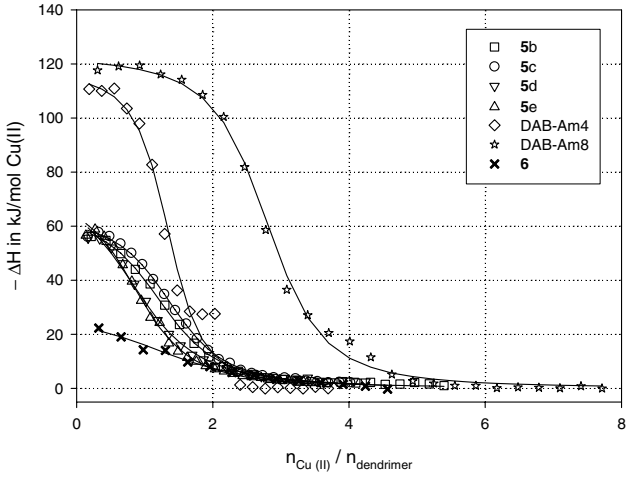


Figure 2. Experimental (symbols) and fitted (lines) differential molar heats of complexation as a function of the molar Cu^{II}/dendrimer ratio in DMSO solution at 298 K.

DAB-Am8, the experimental titration data can be well-described by applying a simple 1:1 complexation model. DAB-Am8 is obviously able to bind more than one Cu^{II} and, consequently, the fit of the calorimetric results using a model based on the assumption of n independent equivalent binding sites,[15] gives a satisfactory approximation with $n = 3$. This result implies that three binding sites for Cu^{II} complexation exist in one DAB-Am8 molecule, which is also consistent with spectroscopic titration results of copper complexation in poly(propylene imine) dendrimers.[16] The complexation of Cu^{II} with DAB-Am8 and DAB-Am4 can be assigned to the interaction of Cu^{II} with the bis(3-aminopropyl)amine end groups.[16] The Cu^{II} ion complexed in **5** and **6** is assumed to be coordinated by the tertiary amino groups in the interior of the core dendrimer DAB-Am8. This assumption is supported by NMR studies of the Zn^{II} complexation by poly(propylene imine) dendrimers,[17] showing that interior tertiary amino groups are able to act as ligands for the metal ion inside the dendrimer.

Thermodynamic data, derived from the fit of the calorimetric results, are summarized in Table 2. Generally, the copper complexation is characterized by a relatively strong binding expressed by highly exothermic binding enthalpies and a large entropy loss, resulting in a considerable enthalpy-entropy-compensation effect.[18] Although, the complexation enthalpy of the unmodified dendrimers are approximately twice of the value of the copoly(peptide)-modified dendrimers, the stability constants are nearly identical also indicating the strong compensation of the enthalpy gain by a corresponding entropy penalty. The complexation properties of the different copoly(peptide)-modified dendrimer samples are very similar and no significant influence of the peptide chain length could be observed. A much smaller binding energy compared with the copoly(peptide)-shelled dendrimers **5** was found for the homopoly(peptide)-shelled dendrimer **6**. This can be understood by taking into account the reduced flexibility of the modified dendrimer due to the rigid poly(glutamate) chains. Therefore, the dendrimer is prevented from adopting the optimal conformation for complexation.

Table 2. Thermodynamic data of Cu^{II} complexation by dendrimers in DMSO at 298 K.

Dendrimer	ΔH	lg K	ΔS	Stoichiometry dendrimer: Cu^{II}
	$kJ \cdot mol^{-1}$		$J \cdot K^{-1} \cdot mol^{-1}$	
DAB-Am4	−117.7	4.72	−304	1:1
DAB-Am8	−123.5	4.82	−322	1:3
5b	−60.1	4.43	−117	1:1
5c	−58.3	4.43	−111	1:1
5d	−61.3	4.38	−122	1:1
5e	−64.2	4.22	−135	1:1
6	−28.1	4.08	−16	1:1

Conclusion

The calorimetric results support the concept of tailoring polypeptide-shelled dendrimers with interior complexing properties. The poly(propylene imine) dendrimer DAB-Am8 is suited to act as a complexing core in **5** and **6**. Attaching of polypeptide chains to the core dendrimer favors the formation of 1:1 complexes without significant loss of complex stability compared with the unmodified dendrimer DAB-Am8. This result is an essential prerequisite for tailoring the biological functionality and targeting properties of these promising new compounds. Additional synthetic approaches and complexation investigations are in progress to better understand the formation and properties of the Cu^{II} complexes with polypeptide-shelled dendrimers **5**.

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[14] Amine-initiated NCA polymerizations possess chain transfer and termination reactions. No termination reactions could be proven. However, a very low amount of linear homo/copolypeptides in **5** and **6** could be present (<1%). Such a low linear polymer content does not significantly influence the metal complexation studies of **5** and **6** by ITC.

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