Dendritic−Graft Polypeptides

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ABSTRACT: A new synthetic strategy for the preparation of highly branched, or dendritic−graft, polypeptides is presented. The synthetic strategy is based on a repetitive sequence of ring-opening copolymerization and deprotection steps. Comonomers for the ring-opening polymerization are a set of orthogonally protected L-lysine *N*-carboxyanhydride derivatives. After each polymerization step, selective removal of the ϵ-NH_2 protecting groups of one of the monomers generates a number of new initiator sites that allow grafting of a successive generation of polypeptide branches. Following this strategy, dendritic−graft polypeptides containing up to ∼160 α-amino acids, corresponding to a number-average molecular weight of ∼40 kDa, could be obtained after only four ring-opening polymerization−deprotection cycles. In contrast to perfect dendrimers, the dendritic−graft polypeptides are neither structurally uniform nor monodisperse. Dendritic−graft polypeptides, however, are conveniently prepared in multigram quantities and do not require laborious purification procedures.

Introduction

Branched or dendritic polypeptides, in particular poly(L-lysine), have attracted interest for various medical applications, e.g., for the development of multiple antigen peptides (MAPs),[1] as nonviral transfection agents in gene therapy,[2] as therapeutics in boron neutron capture therapy,[3] or as contrasting agents for magnetic resonance imaging purposes.[4] Dendritic polypeptides are generally prepared in a stepwise fashion, either in homogeneous solution or via solid-phase synthesis.[1−5] Provided the synthesis and purification are performed carefully, this strategy affords structurally well-defined and perfectly monodisperse dendrimers/dendrons. Synthesis and purification, however, are laborious, and a large number of steps are required to obtain high molecular weight branched polypeptides.

In contrast to structurally defined and molecularly uniform dendrimers/dendrons, some publications have reported the use of poly(L-lysine) or copolymers of L-lysine and other α-amino acids as macroinitiators for the ring-opening polymerization of α-amino acid *N*-carboxyanhydrides (NCA's) to prepare branched or graft copolymers, which were termed multichain polypeptides.[6] The NCA ring-opening polymerization is very simple in terms of both synthesis and purification and allows rapid access to high molecular weight polypeptides.[7] The ease of synthesis, however, is at the expense of molecular uniformity; i.e., the resulting polypeptides are both polydisperse and contain structural defects. In principle, incorporation of suitable α-amino acids in the branches that are able to initiate a subsequent NCA ring-opening polymerization step, and repetition of this grafting cycle should result in highly branched polypeptides. Such repetitive graft-on-graft strategies have already been successfully used for the preparation of so-called dendritic−graft poly(ethylene imine),[8] poly(ethylene imine)−poly(2-ethyl-2-oxazoline) copolymers,[8] poly(styrene),[9] poly(butadiene),[10] and poly(styrene)−poly(*n*-butyl methacrylate) copolymers.[11] So far, no reports have been published that describe the use of such synthetic approaches for the preparation of highly branched polypeptides. In this contribution, we will demonstrate that high molecular weight branched or dendritic−graft polypeptides can indeed be prepared through a repetitive sequence of NCA ring-opening polymerization grafting steps.

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Experimental Part

Materials. *N*,*N*′-Dimethylformamide (DMF; Riedel-de-Haën, 99.5%) was distilled from CaH_2 (Fluka, >97,0%) under reduced pressure and subsequently stored over molecular sieves (4 Å, Acros Organics) under an argon atmosphere. *n*-Hexylamine (Aldrich, 99%) was distilled from CaH_2 and stored under an argon atmosphere. N^{ϵ}-(*tert*-Butoxycarbonyl)-L-lysine *N*-carboxyanhydride (BOC-lys NCA)[12] and N^{ϵ}-(benzyloxycarbonyl)-L-lysine *N*-carboxyanhydride (Z-lys NCA)[13] were prepared according to literature procedures. All other solvents and reagents were purchased from commercial suppliers and used as received.

Methods. ^{1}H NMR experiments were performed on Bruker AMX500 and WS700 spectrometers. DMSO-d_6 or D_2O was used as solvent, and the residual proton signal was taken as internal standard. Gel permeation chromatography (GPC) was performed at 60 °C with a setup consisting of a Waters 510 pump and a series of three PSS SDV columns (bead size: 10 μm; $L \times D$: 300 × 8 mm) with pore sizes of 500, 10^4, and 10^6 Å. A 1 g/L solution of LiBr in DMF was used as the mobile phase at a flow rate of 1 mL/min. Sample elution was monitored with an ERC 7512 refractive index detector. Elution times were converted to molecular weights using a calibration curve constructed from narrow polydispersity poly(ethylene oxide) standards.

Procedures. Synthesis of the Poly([Z-L-lysine]-*co*-[BOC-L-lysine]) Core. A thoroughly dried Schlenk flask, which was purged with Ar and fitted with a drying tube, was charged with a mixture of Z-lys NCA (2.0 g, 6.5 mmol) and BOC-lys NCA (0.2 g, 0.73 mmol) in 12 mL of DMF. Then, *n*-hexylamine (48 μL, 0.36 mmol) was added. After 5 days, the reaction mixture was added to ∼200 mL of water to precipitate the polymer. Filtration and freeze-drying afforded 1.31 g (70%) of the desired polypeptide. ^{1}H NMR (500 MHz, DMSO-d_6, 333 K): δ = 7.4−7.1 (b, Ar*H* + N*H*, 5H × n_{Z-lys} + 1H × n), 4.9 (b, ArCH_2−, 2H × n_{Z-lys}), 4.3−3.7 (b, αCH, 1H × n), 2.9 (b, $-CH_2CH_2-NH(C{=}O)-$ + $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 2H × n + 2H), 2.0−1.1 (b, $-CH(CH_2)_3CH_2NH-$ + $-OC(CH_3)_3$ + $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 6H × n + 9H × $n_{BOC-lys}$ + 8H), 0.8 (t, $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 3H). GPC (DMF): M_w = 3880

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Table 1. Isolated Yields and Molecular Characteristics of Dendritic−Graft Poly(Z-L-lysine)s of Different Generations

		GPC[b]			^{1}H NMR[e]	
polymer	yield[a] (%)	M_w (g/mol)[c]	M_n (g/mol)[d]	M_w/M_n	DP_n[f]	M_n (g/mol)
core	70	3 880	3 300	1.18	14	3 760
G0	59	11 650	7 120	1.64	41	10 480
G1	63	32 450	22 730	1.43	93	23 180
G2	61	45 000	29 490	1.53	161	40 180

[a] Isolated yields. Determined gravimetrically after precipitation, filtration, and freeze-drying of the dendritic−graft poly(Z-L-lysine)s. [b] Gel permeation chromatography (GPC) in DMF relative to poly(ethylene oxide). [c] Weight-average molecular weight. [d] Number-average molecular weight. [e] 500 MHz ^{1}H NMR experiments in DMSO-d_6 at 60 °C. [f] Number-average degree of polymerization. DP_n was determined by comparison of the integral of the CH_3 group of the *n*-hexylamine initiator moiety at 0.8 ppm with that of the CH_2 group of the Z groups at 4.9 ppm and the broad signal between 2 and 1.1 ppm, which is due to the $-(CH_2)_3CH_2NH-$ methylene groups and the BOC groups of the L-lysine units and the $CH_3(CH_2)_4CH_2NH-$ methylene groups of the initiator.

g/mol, M_n = 3300 g/mol, M_w/M_n = 1.18. Note: n = number-average degree of polymerization, n_{Z-lys} = number of Z-lys repeat units, and $n_{BOC-lys}$ = number of BOC-lys repeat units ($n = n_{Z-lys} + n_{BOC-lys}$).

Partial Deprotection of the Poly([Z-L-lysine]-*co*-[BOC-L-lysine]) Core. BOC groups were removed by treating the poly([Z-L-lysine]-*co*-[BOC-L-lysine]) core (500 mg, ∼0.13 mmol, calculated using M_n obtained from ^{1}H NMR) with 15 mL of CF_3COOH for 60 min at room temperature. After that, the polymer was precipitated in water. After washing extensively with dilute NaOH(aq) and freeze-drying, the polymer was isolated in quantitative yield. ^{1}H NMR (500 MHz, DMSO-d_6, 333 K): δ = 7.4−7.1 (b, Ar*H* + N*H*, 5H × n_{Z-lys} + 1H × n), 4.9 (b, Ar−CH_2−, 2H × n_{Z-lys}), 4.3−3.7 (b, α-C*H*, 1H × n), 2.9 (b, $-CH_2CH_2NH(C{=}O)-$ + $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 2H × n + 2H), 2.0−1.1 (b, $-CH(CH_2)_3CH_2NH-$ + $CH_3(CH_2)_4CH_2NH-(C{=}O)-$, 6H × n + 8H), 0.8 (t, $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 3H). Note: n = number-average degree of polymerization, n_{Z-lys} = number of Z-lys repeat units, and $n_{BOC-lys}$ = number of BOC-lys repeat units ($n = n_{Z-lys} + n_{BOC-lys}$).

Total Deprotection of the Poly([Z-L-lysine]-*co*-[BOC-L-lysine]) Core. To a solution of the poly([Z-L-lysine]-*co*-[BOC-L-lysine]) core (0.1 g, ∼0.027 mmol, calculated using M_n obtained from ^{1}H NMR) in 3 mL of CF_3COOH was added a 4-fold molar excess with respect to the total number of L-lysine repeat units of a 33 wt % solution of HBr in CH_3COOH. After stirring for 1 h at room temperature, diethyl ether was added, and the precipitated polymer was isolated via filtration. After extensive washing with diethyl ether, the polymer was vacuum-dried and isolated in quantitative yield. ^{1}H NMR (700 MHz, D_2O, 298 K): δ = 4.45 (b, αC*H*, 1H × (n − 2)), 4.32 (b, C-terminal αC*H*, 1H), 4.18 (b, N-terminal αC*H*, 1H), 3.1 (b, $-CH_2CH_2NH_2$ + $CH_3(CH_2)_4CH_2NH(C{=}O)-$, 2H × n + 2H), 1.90−1.70 (b, $-CHCH_2CH_2CH_2CH_2NH_2$, 4H × n), 1.65−1.44 (b, $-CHCH_2CH_2CH_2CH_2NH_2$, 2H × n), 1.44−1.30 (b, $CH_3-(CH_2)_4CH_2NH(C{=}O)-$, 8H), 0.97 (t, $CH_3(CH_2)_5NH(C{=}O)-$, 3H). Note: n = number-average degree of polymerization.

Synthesis of Higher-Generation Dendritic−Graft Poly(L-lysine)s. The synthesis of higher-generation dendritic−graft poly(L-lysine)s starts with the ring-opening copolymerization of Z-lys NCA and BOC-lys NCA as described above. In this case, however, the partially deprotected poly([Z-L-lysine]-*co*-[BOC-L-lysine]) core instead of *n*-hexylamine is used as initiator. Monomer concentrations of ∼0.2 g/mL were used. For the calculation of the amount of NCA monomer, the M_n of the partially deprotected peptide of a preceding generation and the total number of grafting sites (BP_{tot}) were used, which were obtained from ^{1}H NMR experiments on the fully deprotected peptides. The monomer-to-initiator ratios, or the theoretically expected arm lengths ($DP_{th,arm}$), for each of the NCA ring-opening polymerization steps are listed in Table 2. Partial and full deprotection of the G0, G1, and G2 dendritic−graft poly(Z-L-lysine)s was performed according to the procedures outlined above.

Results and Discussion

Design and Synthesis. The synthesis of the dendritic−graft polypeptides is outlined in Scheme 1. In general terms, the synthetic strategy is based on the ring-opening copolymerization of two orthogonally N^ϵ-protected L-lysine NCA's.[7] One of the monomers contains a temporary protective group, which can be removed under relatively mild conditions. The ϵ-NH_2 group of the other L-lysine monomer is masked with a permanent protective group, which should be stable under the conditions applied for the removal of the temporary protective group. The permanent protective group may be removed in the very last step of the synthesis. In the first synthetic step, a primary amine is used to initiate the ring-opening copolymerization of the two NCA's to prepare the core of the targeted polypeptide. Removal of the temporary protective group generates a number of primary amine groups, which can act as an initiator to graft a first generation of peptide arms onto the core. Repetition of this NCA ring-opening polymerization/deprotection cycle yields highly branched, or dendritic−graft, polypeptides. Following commonly accepted conventions, a dendritic−graft polymer of generation n − 1 (G[n − 1]) is obtained after n deprotection/grafting cycles.[8,9]

To explore the feasibility of the concept outlined in Scheme 1, N^ϵ-(*tert*-butoxycarbonyl)-L-lysine NCA (BOC-lys NCA) and N^ϵ-(benzyloxycarbonyl)-L-lysine NCA (Z-lys NCA) were chosen as monomers. Both BOC-lys NCA[12] and Z-lys NCA[13] are easily prepared following published procedures. Whereas BOC groups are smoothly removed under moderately acidic conditions, Z-lys requires HBr/AcOH or hydrogenation for effective deprotection.[14] Thus, BOC-lys NCA appears to be a suitable temporary protected monomer, which after deprotection could act as a branching point and initiate the ring-opening copolymerization of a successive series of grafts. The advantage of using L-lysine-based NCA monomers bearing N^ϵ protective groups that differ in acid sensitivity is that after the last grafting step the temporary and permanent protective groups can be simultaneously removed. The use of acid stable permanent protective groups, which can be cleaved for example photolytically or under basic conditions, would require an additional reaction step to achieve complete deprotection of the dendritic−graft polypeptides. A disadvantage of using BOC and Z groups as temporary and permanent protective groups, respectively, is that during the course of the synthesis some Z groups may be cleaved upon prolonged exposure to acid.[15] Since the objective of the work described in this paper was to develop a convenient and fast route for the synthesis of highly branched poly(L-lysine), we decided to explore the BOC−Z protective group strategy, even if this could go at the expense of structural perfection.

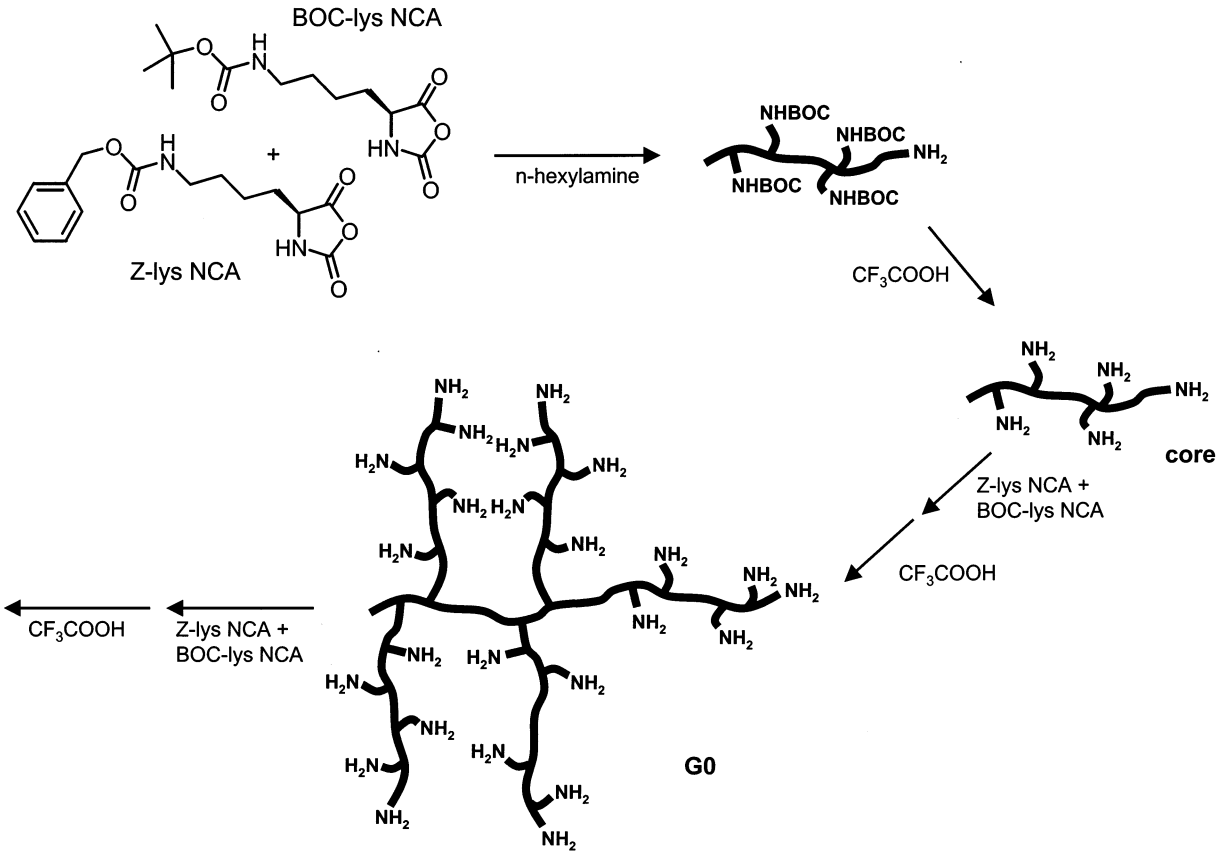
Branched polypeptide block copolymers topologically related to the dendritic−graft polypeptides shown in Scheme 1 have been reported previously by Birchall and North.[16] Their synthetic strategy involved quenching the NCA ring-opening polymerization by addition of N^α,N^ϵ-di(benzyloxycarbonyl)-L-lysine-(*p*-nitrophenyl ester), which after hydrogenation affords two new terminal amino groups that can act as initiator for a succes-

Table 2. Results of the ^{1}H NMR Characterization of the Dendritic−Graft Poly(L-lysine)s

	^{1}H NMR integrals[a]										
polymer	$I(H_a)$	$I(H_b)$	$I(H_c)$	$I(H_d)$	$[I(H_a) + I(H_b) + I(H_c) + I(H_d)]/I(H_c)$	DP_n[b]	M_n[c] (g/mol)	DP_{arm}[d]	BP_{tot}[e]	BOC-lys[f] (mol %)	$DP_{th,arm}$[g]
core	18		1	1	20	20	2750	20	3	11	20
G0	50	4	3		19	57	7390	11	6	9	25
G1	75	6	6		15	87	11250	5	14	25	36
G2	157	18	14		14	189	24270	7			30

[a] Integrals are reported relative to that of the signal of the methyl group of the initiator moiety at 0.97 ppm, which was set equal to 3. [b] Total number-average degree of polymerization of the poly(L-lysine)s. [c] Total number-average molecular weight of the poly(L-lysine)s. [d] Number-average degree of polymerization of the arms that were added during the last grafting step. [e] Total number of grafting sites present in the molecule [f] BOC-lys content of the arms added during the last grafting step. [g] Theoretically expected number-average arm length. This number is calculated from the amount of α-amino acid *N*-carboxyanhydride that was used during the last polymerization step and the number of grafting sites based on the amount of BOC-lys repeat units that were deprotected prior to the last polymerization step.

Scheme 1



sive NCA ring-opening polymerization step. The highly branched block copolymers prepared in this way, however, were exclusively composed of water-insoluble α-amino acids and, presumably due to solubility problems, were only of moderate molecular weight (<∼9 kDa).[16]

Dendritic−graft poly(Z-L-lysine)s of different generations were prepared in *N*,*N*′-dimethylformamide (DMF), using a monomer mixture containing 10 mol % BOC-lys NCA throughout all polymerization steps. After each ring-opening polymerization step, the polypeptides were treated with trifluoroacetic acid (TFA) to remove the BOC groups and generate a number of new initiator sites. The ring-opening polymerization−deprotection cycle was repeated four times to ultimately afford a second-generation (G2) dendritic−graft poly(Z-L-lysine). Treatment of the dendritic-graft poly(Z-L-lysine)s with HBr/AcOH resulted in removal of the carbamate permanent protective groups and afforded the corresponding water-soluble dendritic−graft poly(L-lysine)s.

Characterization. The dendritic−graft poly(Z-L-lysine)s were analyzed by gel permeation chromatography (GPC) and ^{1}H NMR spectroscopy. GPC chromatograms of dendritic−graft poly(Z-L-lysine)s of different generations are shown in Figure 1. The GPC traces illustrate the rapid increase in molecular weight of the dendritic−graft polymers with increasing generation. The average molecular weights calculated from the GPC traces are listed in Table 1 together with the results obtained from the ^{1}H NMR experiments. Since elution times were converted to molecular weights using a calibration curve constructed from linear poly(ethylene oxide) standards, the GPC experiments should not provide useful quantitative molecular weight information. Nevertheless, the number-average molecular weights (M_n) determined by GPC and ^{1}H NMR agree fairly well. Generally, the M_n values obtained from GPC are slightly lower, which may be attributed to the relatively compact three-dimensional structure of the highly branched dendritic−graft polypeptides compared

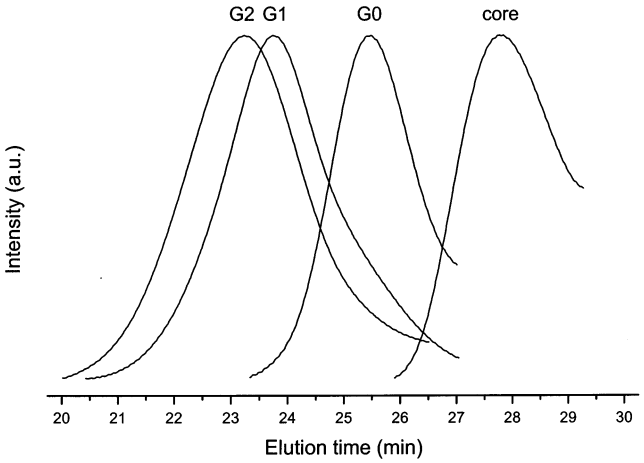


Figure 1. GPC chromatograms (refractive index detector signal) of dendritic−graft poly(Z-L-lysine)s of different generations.

to the linear calibration standards. The GPC and ^{1}H NMR data summarized in Table 1 demonstrate the concept is feasible and show that polypeptides containing up to ∼160 α-amino acids (M_n ∼ 40 kDa) can be prepared in only eight synthetic steps.

Although ^{1}H NMR experiments on the dendritic−graft poly(Z-L-lysine)s were useful to obtain absolute molecular weight information, the spectra did not provide any structural information. ^{1}H NMR spectra of the corresponding dendritic−graft poly(L-lysine)s recorded in D_2O, in contrast, were sufficiently resolved to provide both molecular weight information and also allow insight into the topology of the polypeptides. Figure 2 shows an expansion of the methine region of the ^{1}H NMR spectrum of different dendritic−graft poly(L-lysine)s.[17] The peaks labeled H_b, H_c, and H_d in Figure 2 can be assigned to the α-CH protons of L-lysine residues that are located at the C- and N-terminus of a peptide chain. The large signal labeled H_a is due to all other α-CH protons. Table 2 lists the ^{1}H NMR integrals determined for each of the signals H_a−H_d ($I(H_a) - I(H_d)$), relative to that of the resonance of the $-CH_3$ group of the hexylamine initiator species at ∼0.97 ppm, which was set at a value of 3. The sum of the integrals H_a−H_d yields the total number-average degree of polymerization (DP_n) of the dendritic−graft poly(L-lysine)s. These values, together with the corresponding number-average molecular weights, are also listed in Table 2. The number-average degrees of polymerization of the dendritic−graft poly(L-lysine)s agree reasonably well with those obtained from the ^{1}H NMR experiments on the corresponding poly(Z-L-lysine)s (see Table 1).

Since they are sufficiently resolved in the methine region to allow identification of the different end groups, the ^{1}H NMR spectra shown in Figure 2 can also provide structural information. This becomes evident upon comparison of the integral of the signal of the methine protons labeled H_c, which is a measure for the number of end groups present in the molecule, with that of the sum of the integrals of all methine protons (H_a−H_d in Figure 2). For a linear polypeptide, which only possesses a single end group (i.e., $I(H_c) = 1$), $[I(H_a) + I(H_b) + I(H_c) + I(H_d)]/I(H_c)$ would be equal to the number-average degree of polymerization. Accordingly, if the polypeptides prepared according to Scheme 1 are linear rather than branched, then the ratio $[I(H_a) + I(H_b) + I(H_c) + I(H_d)]/I(H_c)$ should increase from ∼20 (core) to ∼189 (G2) based on the degrees of polymerization that were previously determined. Table 2, however, shows that, except for the core, the values for $[I(H_a) + I(H_b) + I(H_c) + I(H_d)]/I(H_c)$ are much lower and slowly decrease from ∼19 (G0) to ∼14 (G2). This reflects the large number of end groups present in the dendritic−graft polypeptides and is indicative for their highly branched topology.

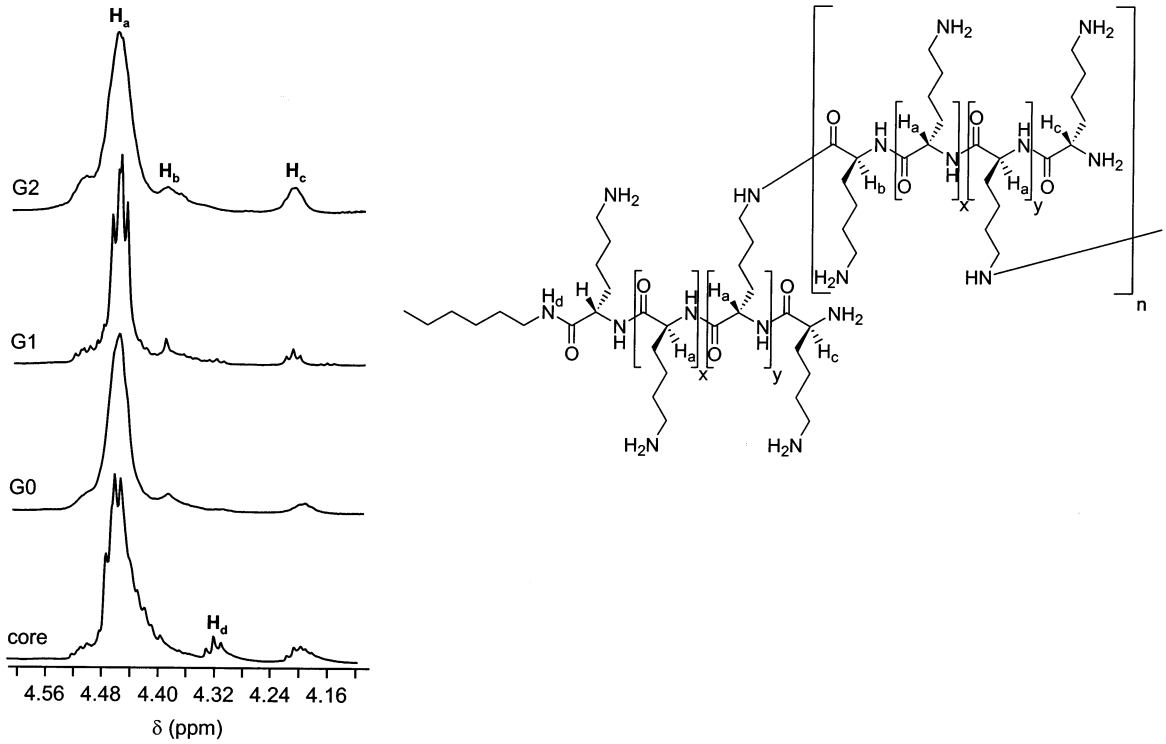


Figure 2. Part of the ^{1}H NMR spectra of a series of dendritic−graft poly(L-lysine)s. The spectra (700 MHz) were recorded in D_2O at 298 K; core: $n = 0$, G0: $n = 1$, G1: $n = 2$, G2: $n = 3$.

The data summarized in Tables 1 and 2 prove that the concept outlined in Scheme 1 is feasible and demonstrate that highly branched polypeptides with relatively high molecular weights can be prepared in a small number of simple synthetic steps. Table 2, however, also indicates that the synthesis is not completely free of side reactions. This is illustrated for example by the increasing difference between the theoretical and experimentally determined length of the peptide branches with increasing generation (see Table 2). This difference may be attributed to partial cleavage of benzyloxycarbonyl (Z) protective groups during the course of the preparation of the dendritic−graft poly(Z-L-lysine)s.[15,18] In a model experiment, in which a linear poly(Z-L-lysine) sample with a number-average degree of polymerization of 10 was treated with CF_3COOH under conditions identical to those applied for removal of the BOC protective groups, ∼40% of the Z groups were cleaved after 4 h. This reaction time is identical to the total time the Z groups are exposed to CF_3COOH for the synthesis of a G2 dendritic−graft poly(Z-L-lysine). Consequently, it seems likely that part of the Z groups are also cleaved during the synthesis of the dendritic−graft poly(Z-L-lysine)s. This results in a higher number of primary amine grafting sites than expected on the basis of the selective removal of the BOC groups and may account for the observed decrease in arm length with increasing generation. Currently, we are exploring different orthogonal protective group schemes in order to improve the control over peptide arm length.

At this point, it is interesting to compare the structure of the dendritic−graft polypeptides with that of dendrimers prepared in a divergent fashion.[19] Divergent synthesis starts from a central core and involves a repetitive sequence of high yielding reactions in which one branch after another is attached. The purity of dendrimers can be discussed in terms of polydispersity (M_w/M_n, which is a measure for chain length heterogeneity) and in terms of dendritic purity, which has been defined as the number of defect-free dendrimers divided by the total number of dendrimers multiplied with 100%.[20] Since they are prepared from low molecular weight building blocks in high yield (98−99%) reactions, divergent dendrimers can have polydispersities as low as 1.002.[20] However, since every new generation can hardly be purified, defects, which are the result of the very high yield but not quantitative reactions, propagate and accumulate statistically during synthesis. Since the fully converted and perfect product is the dominant species, it has been argued that the purity of dendrimers should be discussed in terms of dendritic purity.[20]

The dendritic−graft polypeptides discussed in this contribution are also prepared following a divergent strategy. In this case, however, a new generation of branches is not attached by coupling low molecular weight building blocks in high yielding reactions, but in a grafting step involving NCA ring-opening polymerization. Since the primary amine-initiated NCA ring-opening polymerization is hampered by various chain-transfer and chain-breaking reactions,[7] each grafting step produces a generation of branches that are heterogeneous with respect to chain length. The polydispersity of the polypeptide branches adds to further defects for example from incomplete conversion of grafting sites and results in a very heterogeneous product. This is reflected in the polydispersities (M_w/M_n) of the dendritic−graft poly(Z-L-lysine)s listed in Table 1. Taking into account the type of polymerization, the GPC values are probably an underestimate of the real polydispersity, but the reported values agree fairly well with results of earlier GPC experiments on a variety of Z-L-lysine-based polypeptides.[21]

Conclusions

In summary, we have introduced and demonstrated the feasibility of a new synthetic strategy for the preparation of highly branched, dendritic−graft, polypeptides. The method involves a repetitive sequence of NCA ring-opening polymerization and deprotection steps and uses appropriately N^{ϵ}-protected L-lysine derivatives as branching points. In contrast to the stepwise coupling and deprotection strategy used for the synthesis of peptide dendrimers, the graft-on-graft methodology introduced here neither affords structurally uniform nor produces perfectly monodisperse materials. However, it allows the preparation of high molecular weight branched polypeptides in a small number of simple reactions without the need for extensive isolation and purification steps. In addition, although not shown in the present contribution, the repetitive grafting strategy is very versatile with respect to tailoring, e.g., the branching density and chemical composition of the dendritic−graft polypeptides. The molecular weights of the reported dendritic−graft polypeptides are much higher than those of topologically related branched block copolypeptides, which have been reported before,[16] and are also not restricted by solubility problems. The reported dendritic−graft polypeptides are of potential interest as carriers for gene delivery and for the development of synthetic vaccines. As a consequence, future work will not only concentrate on expanding the synthetic concept (i.e., exploring different orthogonal protective group schemes and other α-amino acid comonomers) but will also address the practical utility of these new polypeptides.

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