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An Expeditious Multigram-Scale Synthesis of Lysine Dendrigraft (DGL) Polymers by Aqueous *N*-Carboxyanhydride Polycondensation

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Abstract: The synthesis and characterisation of new arborescent architectures of poly(L-lysine), called lysine dendrigraft (DGL) polymers, are described. DGL polymers were prepared through a multiple-generation scheme (up to generation 5) in a weakly acidic aqueous medium by polycondensing N^{ε}-trifluoroacetyl-L-lysine-*N*-carboxyanhydride (Lys(Tfa)-NCA) onto the previous generation G(n−1) of DGL, which was used as a macroinitiator. The first generation employed spontaneous NCA polycondensation in water without a macroinitiator; this afforded low-molecular-weight, linear poly(L-lysine) G1 with a polymerisation degree of 8 and a polydispersity index of 1.2. The spontaneous precipitation of the growing N^{ε}-Tfa-protected polymer (GnP) ensures moderate control of the molecular weight (with unimodal distribution) and easy work-up. The subsequent alkaline removal of Tfa protecting groups afforded generation Gn of DGL as a free form (with 35–60% overall yield from NCA precursor, depending on the DGL generation) that was either used directly in the synthesis of the next generation (G(n+1)) or collected for other uses. Unprotected forms of DGL G1–G5 were characterised by size-exclusion chromatography, capillary electrophoresis and ^{1}H NMR spectroscopy. The latter technique allowed us to assess the branching density of DGL, the degree of which (ca. 25%) turned out to be intermediate between previously described dendritic graft poly(L-lysines) and lysine dendrimers. An optimised monomer (NCA) versus macroinitiator (DGL G-(n−1)) ratio allowed us to obtain unimodal molecular weight distributions with polydispersity indexes ranging from 1.3 to 1.5. Together with the possibility of reaching high molecular weights (with a polymerisation degree of ca. 1000 for G5) within a few synthetic steps, this synthetic route to DGL provides an easy, cost-efficient, multigram-scale access to dendritic polylysines with various potential applications in biology and in other domains.

Keywords: carboxyanhydrides · dendrimers · polylysine · polymerization · polymers

Introduction

Dendritic polymers have been recognised as the fourth major class of synthetic polymer architecture (after linear, cross-linked and branched),[1–3] characterised by a cascade-branching structure typically obtained from polyfunctional monomers under more or less strictly controlled polymerisation conditions. This class is itself commonly subdivided into three main subsets: dendrimers, hyperbranched polymers and dendrigraft (or dendritic graft) polymers.[3] The set

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of hyperbranched polymers represent the less controlled architecture, often obtained from one-pot self-condensation reactions of AB_n-type monomers. Conversely, dendrimers (historically the first subset reported in the literature), result from a generation-based scheme involving cycles of protection, condensation, and deprotection of AB_n-type building blocks.

Since their discovery, dendrimers have found numerous applications in areas such as nanomaterials, diagnostics, drug delivery,[4] biocides, gene transfer[5] and catalysts.[6–9] Among hundreds of dendrimer families that have already been synthesised over the last 25 years, those based on amide or peptide bonds (e.g., PAMAM or lysine dendrimers) have gained an increasing importance for in vivo applications because of their structural similarity with globular proteins and their biocompatibility/biodegradability properties.[6,10] Despite their macromolecular size, dendrimers are theoretically single-molecule compounds; from the polymerist's point of view, dendrimers exhibit remarkable monodispersity (e.g., PAMAM generations 1 to 9 have been obtained with polydispersity indexes M_w/M_n of between 1.000002 and 1.005[11]).

However, the possibility of defects during the synthesis, as a consequence of the difficulty in driving the reactions of a large set of identical groups to completion, commonly leads to mixtures resulting from side-reactions, rather than to single compounds; some commercial PAMAM samples have for example, been found to correspond to mixtures with polydispersity indexes (M_w/M_n) significantly higher than 1.[12] Moreover, such preparation routes involving numerous synthetic and purification steps when high molecular weight is desired, make dendrimers expensive products. Simplifying their synthetic preparation has been a major challenge to overcome for the commercial utilisation of these unique structures in industrial areas that require large quantities of inexpensive materials.[4] An alternative is to abandon the aim of obtaining single-molecule compounds and to concentrate on the properties required for the final material. Such a strategy might be further supported by, for example, the higher efficiency in gene delivery observed when using chemically degraded PAMAM dendrimers compared with intact molecules, which had been attributed to a higher flexibility.[13]

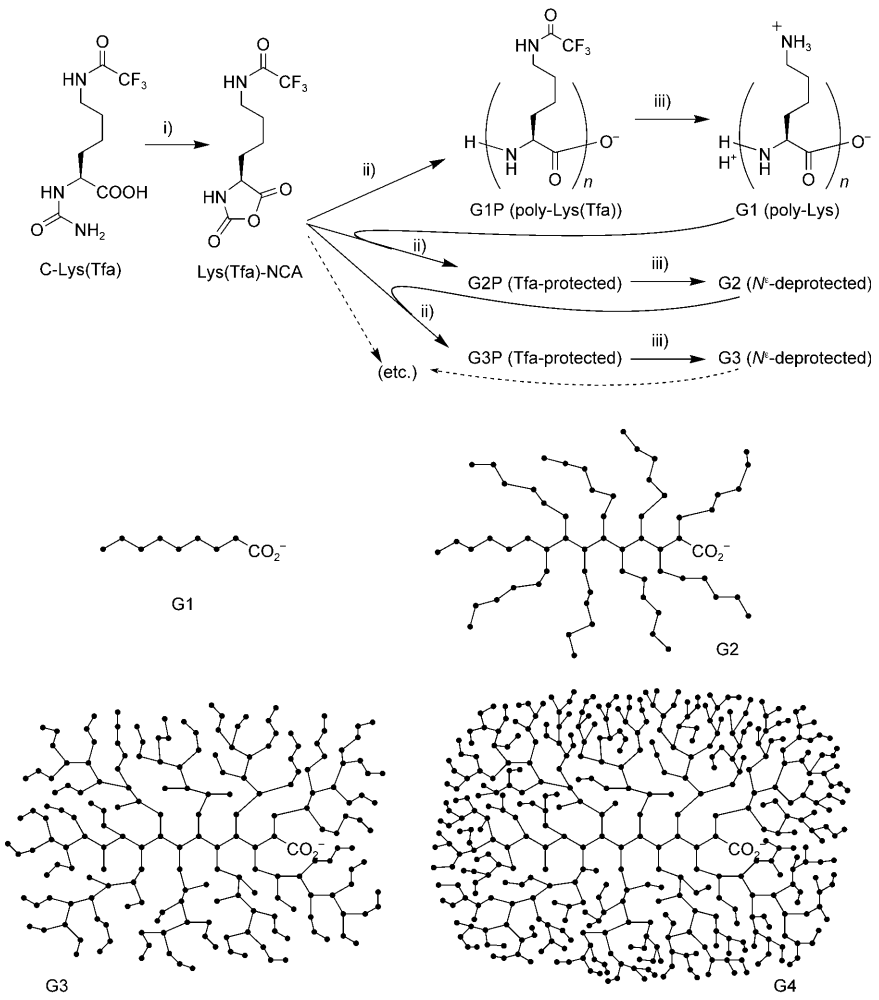
Dendrigraft (also called dendritic graft or arborescent) polymers are the third, most recently discovered subset of dendritic polymers. Like dendrimers, their preparation in a generation-based scheme involves protective group manipulation combined with polymerisation steps, that is, either by cycles of protection, monomer polymerisation, and deprotection (so-called "grafting from" method),[14] or by using preformed polymers instead of molecular compounds as AB_n building blocks in cycles of protection, condensation and deprotection (so-called "grafting onto" method).[3] Because they are no longer single-molecule compounds, dendrigraft polymers have a less controlled structure than dendrimers, but usually a much more regular architecture than hyperbranched polymers. Nevertheless, owing to their synthetic procedure, their molecular weight increases more rapidly with each generation than that of dendrimers. This dramatic molecular weight increase combined with the possibility of using relatively inexpensive monomers offers the potential to manufacture and develop dendrigraft polymers possessing dendrimer-like properties at a much lower cost.[15] As an example based on peptide bonds, Klok et al.[14,16] reported the synthesis of dendritic graft poly(L-lysine)s by iterative co-polycondensation of two differently N^{ε}-protected amino acid *N*-carboxyanhydrides (NCA) in dimethylformamide, followed by the selective removal of one of these sets of protecting groups; a final, complete deprotection step then afforded the desired dendritic graft poly(L-lysine).[14]

Our recent investigations on NCA chemistry in water (in connection with their possible key role in the emergence of life through the prebiotic chemistry of peptides),[17–19] gave us the opportunity to address the synthesis of lysine arborescent polymer in an original manner. Because of their high

Abstract in French: *Nous décrivons la synthèse et la caractérisation de nouvelles architectures arborescentes de poly(*L*-lysine), nommées dendrimères greffés de lysine (DGL). La préparation des DGL repose sur un schéma multi-générations (jusqu'à 5) de polycondensation en milieu aqueux faiblement acide du* N*-carboxyanhydride de la* N*-epsilon-trifluoroacetyl-*L*-lysine (Lys(Tfa)-NCA) sur le DGL de la génération précédente G(*n*−1) pris comme macro-amorceur –- la première génération est obtenue par polycondensation spontanée du NCA dans l'eau en l'absence de macro-amorceur, donnant une poly(*L*-lysine) linéaire G1 de faible* M_w *(*DP_n*=8 pour un indice de polydispersité de 1.2). La précipitation spontanée du polymère en croissance (G*n*P) exerce un contrôle sur son poids moléculaire (assurant une distribution de* M_w *unimodale), et permet d'isoler aisément le produit. La suppression subséquente en milieu alcalin des groupes protecteurs Tfa donne le DGL G*n *sous sa forme libre (rendement depuis le précurseur du NCA: 50–60% selon la génération), qui est directement employé dans la synthèse de la génération suivante G(*n*+1), ou isolé pour d'aures fins. Les DGL G1–G5 déprotégés ont été caractérisés par chromatographie d'exclusion stérique, électrophorèse capillaire et RMN* ^1H*, cette dernière permettant d'évaluer la densité de ramifications du polymère, dont la valeur (ca. 25%) s'avère intermédiaire entre celle de polylysines (hyper)ramifiées précédemment décrites dans la littérature, et celle des dendrimères de lysine. L'optimisation du rapport monomère (NCA) sur macro-amorceur (DGL G(*n*−1)) a permis d'obtenir des distributions de masse unimodales pour des indices de polydispersité entre 1.3 et 1.5. Outre la possibilité d'atteindre en peu d'étapes de synthèse de hauts poids moléculaires (*DP *avoisinant 1000 pour G5), cette voie offre un accès facile et économique aux polylysines arborescentes, lesquelles présentent de nombreuses applications potentielles en biologie et dans d'autres domaines.*

sensitivity to hydrolysis, NCAs[20,21] are nowadays predominantly reacted in aprotic solvents under strictly anhydrous conditions, whereas earlier studies (in the 1950s) conducted in aqueous media[22–25] were later abandoned because simultaneous NCA hydrolysis was considered to be deleterious to the control of NCA polycondensation. Reinvestigating Bartlett's works, our group observed that NCA aminolysis occurs 100 times faster than NCA hydrolysis in weakly acidic aqueous media (pH 5–6.5).[26] These observations led us to re-investigate NCA aqueous polycondensation for preparative and applicative purposes by focusing on derivatives of non-polar amino acids. This latter feature results in the formation of water-insoluble polymers, thus greatly simplifying synthetic and purification procedures while exerting an unexpected control on the molecular weight and structure of resulting materials.

In this paper we describe a new synthetic route to highly branched dendrigraft poly(L-lysine)s (DGLs),[1] involving the iteration of the following sequence over five generations: 1) the polycondensation of lysine NCA bearing a single set of N^{ε}-protecting groups in water, 2) the collection of the precipitated polymeric material, followed by 3) the removal of N^{ε}-protective groups (Scheme 1). This straightforward synthetic procedure features most advantages of dendrigraft polymer synthesis (e.g., the fast molecular weight increase with generation), and as well as the formation of relatively short poly(L-lysine) arms, it allows the resulting DGL to display a high branching ratio, which is potentially useful for the replacement of lysine dendrimers by cheaper materials while increasing the scope of their potential applications.

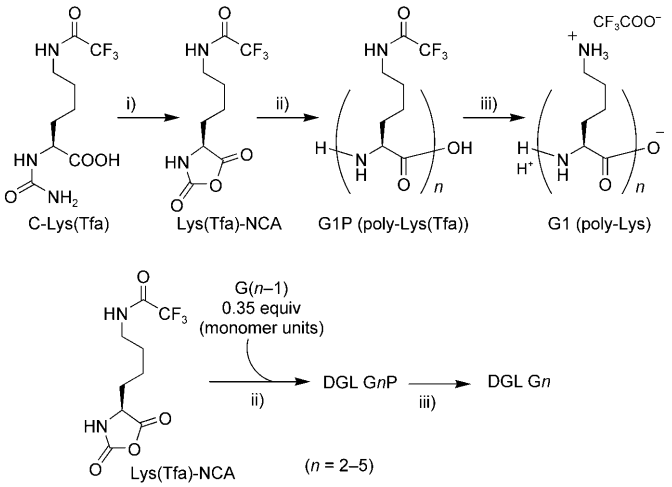


Scheme 1. Top: General scheme of the DGL synthesis. Conditions: i) NO/O_2 (4:1), MeCN, 0 °C, 1 h 45 min; ii) aqueous $NaHCO_3$ (0.2 N, pH 6.5), 0 °C, 15 h; iii) NH_3, H_2O/NeOH, 40 °C, 15 h. Bottom: Overview of DGL G1–G4 topology; each dot represents a Lys residue; pending free amino groups are not represented.

Results and Discussion

Synthesis: The synthesis of the first generation of poly(L-lysine) was carried out by a three-step sequence (Scheme 2) by implementing the knowledge from our research group[17,28,29] and by starting from *N*-carbamoyl derivative C-Lys(Tfa), which was prepared by a straightforward *N*-carbamoylation of N^{ε}-trifluoroacetyl-L-lysine.[29] $NO+O_2$-promoted nitrosation[28] of C-Lys(Tfa) in acetonitrile gave monomer Lys(Tfa)-NCA, which was then reacted in aqueous sodium hydrogen carbonate (pH 6.5) to give N^{ε}-protected oligo(L-lysine) G1P. An important feature of this reaction sequence is that both nitrosation and polycondensation steps were carried out in one pot without isolating the NCA intermediate. Equally important is the fact that the resulting polymer, G1P, spontaneously precipitated from the reaction medium, which facilitated its isolation by filtration prior to deprotection of the ε-amino groups. The latter step was achieved by alkaline treatment of G1P with ammonia in metha-

[1] For clarity reasons, the term dendritic graft poly(L-lysine), introduced by Klok et al., is only used in this paper when referring to the materials prepared according to their methods.[14,27] We selected the term dendrigraft and the abbreviation DGL (which denotes both dendrigraft poly(L-lysine) and L-lysine dendrigraft polymer) to refer to the new materials described in this report.



Scheme 2. Synthesis of poly(L-lysine) G1 and DGL G2–G5. i) NO, O_2, MeCN, 0 °C, 1 h 45 min; ii) $NaHCO_3$ (0.2 N), 0 °C, 15 h; iii) NH_3, H_2O/MeOH, 40 °C, 15 h.

nol/water to give oligo(L-lysine) G1 with a DP_n of approximately 8 in 50–60 % overall yield from C-Lys(Tfa).

The original dendritic materials were then prepared by simply implementing the above-described reaction sequence in a multi-generation sequence (Scheme 2). Upon reacting crude Lys(Tfa)-NCA in aqueous $NaHCO_3$ in the presence of poly(L-lysine) G1 (30 % *w*/*w*; 0.36 equiv in monomer units), polymer G2P spontaneously precipitated from the reaction medium, which, after isolation, underwent alkaline N^{ε}-deprotection to afford poly(L-lysine) G2 with a DP_n of about 48 and 36 % overall yield from C-Lys(Tfa). The next generations of DGL G*n*P (protected) and then G*n* (deprotected) were similarly obtained through initiating the NCA polycondensation step by using 0.36 equivalents (monomer units) of the DGL G(*n*-1); G*n* was obtained in 50 to 60 % yield at each generation. In all generations, the growing polymer G*n*P spontaneously precipitated from the reaction medium, thus allowing easier isolation. DGLs G1–G5 are readily soluble in water up to at least 60 g L^{-1}.

Physical characterisation of DGL molecular weight and architecture: DGL physical characterisation data are summarised in Table 1. Poly(L-lysine) G1 (assumed to be linear) was characterised by capillary electrophoresis (CE), MALDI-TOF mass spectrometry and ^{1}H NMR spectroscopy; this showed a DP_n of 8 and a *PI* of 1.2, with fair agreement between various techniques. Further DGL generations were characterised by size-exclusion chromatography (SEC) coupled to refractive index and light scattering detection, and by ^{1}H NMR spectroscopy in D_2O. SEC analysis of DGL G2–G5 showed an exponential increase of molecular weight with generation (molecular weight geometric increment of 2.7 over the G2–G5 range with an $R^2 > 0.999$), together with polydispersity indexes ranging from 1.35 to 1.45 (Table 1). For generations above 3, additional, high-molecular-weight SEC peaks were observed, assigned to long-lived, multimolecular aggregates that form in aqueous solutions (molecular weights were therefore estimated on the basis of unimer peaks). Evaluation of polymerisation degrees DP_n from molecular weights measured by SEC (Figure 1), took into account the 51 % counterion condensation on polymer chains (according to Manning's condensation law[30]) in the molecular weight of monomer units. Additional physical data of DGL[31] are in agreement with a trifunctional dendritic topology: the almost linear correlation between DGL generation and hydrodynamic radius (measured by Taylor dispersion analysis); the bell-shaped variation of intrinsic viscosity versus generation with maximum for G3.

Table 1. Physical characterisations of DGL G1–G5, branching density and synthetic yield efficiencies.

	G1[a]	G2	G3	G4	G5
dn/dC [mL g^{-1}]	n.m.[b]	0.127	0.134	0.138	0.136
R_h[c] [nm]	1.03(3 %)	1.96(1 %)	3.06(2 %)	3.69(5 %)	6.39(2 %)
M_w[d] [g mol^{-1}]	–	11 800	32 100	88 800	249 800
M_n[d] [g mol^{-1}]	–	8600	22 000	65 300	172 300
PI[d]	1.20	1.38	1.46	1.36	1.46
DP_w[d,e]	9.6	66	179	496	1396
DP_n[d,e]	8.0	48	123	365	963
DP_n geom. incr. G*n*/G($n-1$)[e]	n.a.[f]	6.0	2.56	2.97	2.64
BR %[g]	0	12.3	24.0	24.7	24.4
BP[h]	0	5.9	29.5	90	235
macroinitiator	–	G1	G2	G3	G4
global G*n* yield[i] [%]	61.2	36.5	60.9	67.9	69.6
yield eff. vs. I[j] [%]	n.a.[f]	23.3	91.1	87.6	101.1[k]
yield eff. vs. M[l] [%]	61.2	41.2	50.3	60.9	58.5

[a] The molecular-weight distribution (DP_n, DP_w, *PI*) of G1 was directly measured by using CE instead of SEC. [b] Not measured. [c] Hydrodynamic radii, R_h, were calculated from the diffusion coefficients determined by TDA in NaH_2PO_4 (50 g L^{-1}, pH 4.5, $I = 0.61$ M, viscosity 1.01×10^{-3} Pa s at 25 °C); values in brackets are RSD σ over five experiments; data reprinted from ref. [31]. [d] Determined by SEC coupled to static light scattering and refractive index detection for G2–G5 eluted in NaH_2PO_4 (50 g L^{-1}). [e] DP_w and DP_n calculated from M_w^{SEC} and M_n^{SEC}, respectively, assuming 51 % of Lys residues to be condensed with phosphate counterions (from SEC eluent) according to Manning's condensation theory,[30] to give an average residue molecular weight of 179 g mol^{-1} instead of 128 (bare Lys). [f] Not applicable. [g] Branching ratio of G*n* assessed from H^{ε} resonances of the ^{1}H NMR spectra (400 MHz) in acidic medium (D_2O with added TFA), see the Supporting Information. [h] Number of branching points (*BP*) of G*n* estimated by using the relationship $BP = DP_n^{SEC} \times BR\,\%^{NMR}$. [i] Global yield based on combined monomer units of both macroinitiator G($n-1$) and NCA monomer. [j] Yield efficiency based on monomer units from macroinitiator G($n-1$) only (I basis). [k] Within acceptable experimental error range of DP_n measurement by SEC. [l] Yield efficiency based on monomer units from (C-Lys(Tfa)/Lys(Tfa)-NCA) monomer only (M basis).

Branching ratio of DGL: The branching density of DGL was assessed by ^{1}H NMR spectroscopy in D_2O and was found to be significantly higher than that of dendritic graft poly(L-lysine)s previously described by Klok et al.[14] (cf. Supporting Information) with about 25 % of branched Lys

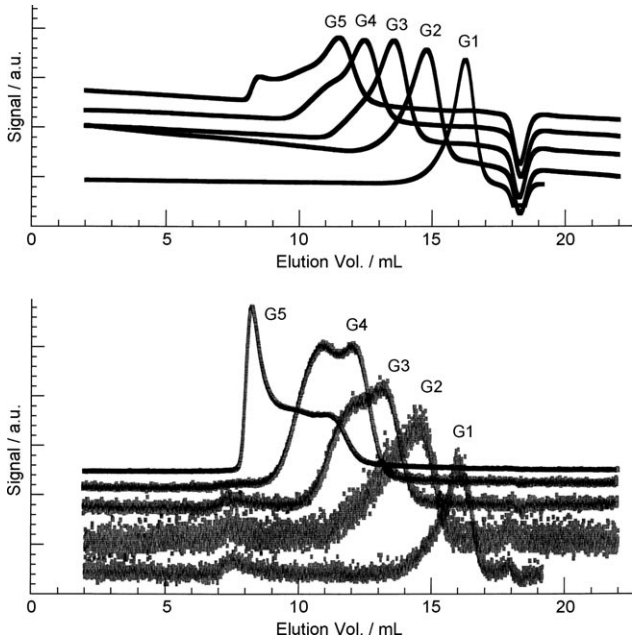


Figure 1. SEC analysis of DGL G1–G5 (phosphate eluent). Top: Refractive index (RI) signal. Bottom: Right-angle light scattering (RALS) signal.

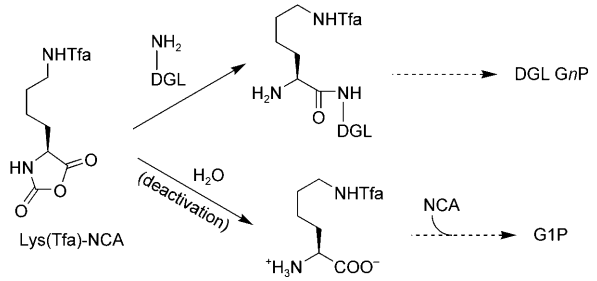
residues in DGL of generations >3, and G1 was confirmed to have a linear topology.

Indeed, the ε-protons of DGL show two different (broad) resonances depending on whether the vicinal ε-nitrogen atom is under an amine or amide form (i.e. connected to a Lys residue), which gives simple access to the branching ratio (*BR*%; defined as the ratio between the number of ε-branched Lys residues to the total DP_n; cf. Table 1). Comparatively, α-proton resonances (up to five signals) show higher sensitivity to the topological environment within the DGL, as already noted by van Dijk-Wolthuis[27] and Klok;[14] this allows the current peptide residues, branching points and N termini of peptide arms to be distinguished and quantified. The assignment of ^{1}H NMR spectroscopy resonances taken from papers by van Dijk-Wolthuis and Klok were completed and confirmed by comparison to commercially available oligo- and poly(L-lysine)s. A calculation of DGL branching ratios based on either ε- or α-resonances also showed reasonable agreement with each other.

For comparison, the *BR*% parameter for other arborescent materials described in the literature was estimated to be 5–10% for the dendritic graft poly(L-lysine)s described by Klok;[14] whereas the *BR*% is expected to be 50% at any generation of genuine Lys dendrimers as those described by Denkewalter.[32]

Stereochemical integrity: Lys residues of DGL are not likely to undergo epimerisation to a significant extent, considering the synthetic pathway used in this work. Indeed, N-unprotected NCAs are commonly considered to be quite insensitive to racemisation under neutral conditions, and the NCA preparation method promoted by nitrosation was shown to be nonracemising.[28] Similarly, the conditions used for either NCA polycondensation or DGL deprotection steps are close to those used in classical operations of peptide chemistry, in which they are not known to give rise to significant loss of chiral integrity in peptides. For instance, according to Denkewalter et al.,[33] the condensation of tyrosine in aqueous solution at pH 10 proceeds with less than 0.004% racemisation of Tyr residues. The racemisation reaction is likely to be even less important at pH 6.5.

Insights into the conditions of DGL synthesis: The synthetic protocol to afford DGL described here turns out to be remarkably reproducible and robust, and allows the DGL preparation to be carried out in batches of between 1 and 100 g. The polycondensation temperature of 0°C was chosen to limit known side-reactions of NCA.[34] A pH value close to 6.5 was chosen for the reaction to maximise the ratio of NCA aminolysis (polycondensation) versus NCA hydrolysis.[26] Nevertheless, we set the NCA concentration as high as possible (and thus probably above the saturation threshold) to limit the extent of hydrolysis and to favour the precipitation of DGL G*n*P in the highest possible yield (Scheme 3). Similarly, the pH value was easy to maintain constantly at 6.5 because of the formation of CO_2 over the whole NCA polymerisation reaction.



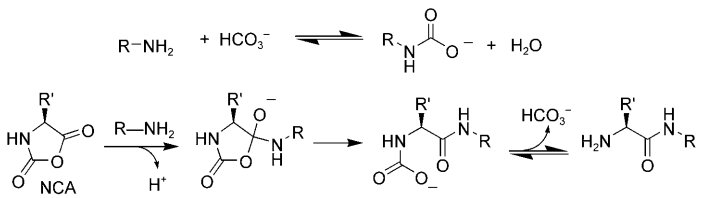
Scheme 3. NCA condensation in water: Competition between DGL macroinitiation/elongation (upper pathway) and monomer deactivation (lower pathway), the latter gives rise to formation of short, linear oligomers.

A key factor of this DGL synthetic route turns out to be the spontaneous precipitation of the growing G*n*P polymer from the aqueous NCA polycondensation medium, which is obviously primarily due to the hydrophobicity of N^{ε}-Tfa-protected side-chains. These decrease the solubility of G*n*P macromolecules as their molecular weight increases.

At the first generation, the free amino acid resulting from NCA hydrolysis initiates the polycondensation. At higher generations, the G(n−1) macroinitiator is added to replace the free amino acid; it is, therefore, essential to limit the NCA/G(n−1) ratio, so that the small Lys(Tfa) oligomers do not grow until reaching the precipitation threshold (this issue is critical for G2, cf. Supporting Information). This allowed us to isolate the G*n*P polymer with an unimodal, rather monodisperse molecular weight distribution.

In the first generation it is very likely that the precipitation of growing polymer also controls the molecular weight of G1P by preventing (or at least considerably retarding) the elongation of peptide chains, though there are indications in the literature that NCA polymerisation can take place in the solid phase or at the solid–liquid interface.[35] As a matter of fact, adding a preformed precipitate (composed of G1P material after completion of polycondensation) to initiate a further NCA polycondensation batch did not result in augmented DP_n; the precipitated material was identical to G1P. Upon precipitating, G1P probably aggregates as β sheets (a hypothesis consistent with literature observations on hydrophobic poly(amino acids),[36] and supported by the presence of an absorption band at 1630 cm^{-1} in the IR spectrum of crude, solid G1P), which is indeed likely to hinder the N termini of peptide chains,[21] thus slowing their reaction with NCA. Such a relationship between peptide insolubility and weak reactivity has also been identified in stepwise peptide synthesis (either by solid or solution-phase methods) with the so-called "difficult sequences".[37] In our case, faster parallel reactions that consume the NCA reactant still progress until the monomer in the medium is completely depleted, for example, the elongation of shorter peptide chains, or the continuous hydrolysis of NCA (the latter initiating the formation of new peptide chains). This accounts for the relative independence of the molecular weight of G1P from either the reaction time or NCA refeeding. Furthermore, G1P indeed meets the minimum DP of >7–10 required for peptides to ensure sufficient thermodynamic stability of β-sheet aggregates in neutral aqueous medium.[20]

In further generations (e.g., ≥ 2), the formation of well-defined β-sheet aggregates from G*n*P is impossible because of the branched structure of the polymers. Therefore, the origin of molecular weight control for G2 to G5 is likely to be directly related to the hydrophobic character of the protected DGL structure. Additional physicochemical factors might be involved in decreasing the solubility of the material, such as the possible formation of anionic carbamate groups by the (reversible) reaction of free amino groups in the aqueous CO_2/HCO_3^- buffer (Scheme 4), which is likely to reduce the effective charge of GnP.



Scheme 4. Amine–carbamate equilibrium and implication in NCA polycondensation.

The branching density of DGL is a result of the competition between the α- and ε-amino groups of macroinitiator G($n-1$) towards NCA; the reaction of α-amino groups result in the simple elongation of existing arms, whereas the reaction of ε-amino groups result in the formation of new arms (the ratio of α- to ε-amino groups in a DGL is roughly equal to its BR%). Indeed, at a given DGL generation some of the free (either α- or ε-) amino groups of G($n-1$) (theoretically in number equal to its DP_n) do not react with an NCA, which is consistent with both ^{1}H NMR spectroscopy data and the molecular-weight geometric increment. In this case, the precipitation of the product does not require a complete substitution of initially available ε-amino groups.

Conclusions

Applications and biological properties—comparison with other poly(L-lysine)s: The topology of DGL (Scheme 1) is characterised by significantly higher branching ratios than other dendritic polylysines described in the literature. Comparatively, the dendritic graft polylysines described by Klok et al.[14] exhibit a much less branched topology and lower DP_n after the same number of synthetic steps. Compared with Lys dendrimers, such as those described by Denkewalter et al.,[32] the DP_n geometric increment of approximately 2.7 also gives access to higher DP_n in much fewer synthetic steps. Finally, although overall yields of DGL synthesis are moderate, this must be compared with the relative inexpensiveness of the starting materials and simplicity of the synthetic methods.

Besides being characterised by the absence of "xenobiotic" moieties attached to the macromolecule (DGLs are made of Lys units only), DGLs present original biological properties that are likely related to their flexible structure, which is a result of a branching density intermediate between genuine Lys dendrimers[32] and otherwise-described Lys dendritic graft polymers.[14] Such structural flexibility (critical in some biological applications)[13] might be the origin of promising immunochemical properties of DGLs that are currently under investigation (to be published separately), as suggested by recent Raman optical activity measurements on DGLs.[38] Other biological applications are expected from the polycationic nature of DGL, which is susceptible to interactions with anionic materials (cell membrane, nucleic acids, nucleotides, etc.), as illustrated by, for example, a recent investigation on interactive transport properties of DGL (either native or chemically modified) through liposomal and cellular membranes.[39]

Experimental Section

Materials: N^{α}-Carbamoyl,N^{ε}-trifluoroacetyl-L-lysine, C-Lys(Tfa), was prepared and purified according to Taillades et al.[29] from N^{ε}-trifluoroacetyl-L-lysine Lys(Tfa), which was generously supplied by Degussa (purity 99% by HPLC), and used without further purification. Acetonitrile (MeCN; Baeckeroot Labo, Jacou, France) was stored over 4 Å molecular sieves. Nitric oxide and oxygen were obtained from L'Air Liquide (Le Pontet, France); Pure H_2O (18 MΩ cm^{-1}) was obtained by using a Milli-Q apparatus (Millipore). MeOH, NH_3 (28% *w*/*v*) and $NaHCO_3$ were ob-

tained from Aldrich. Reagents were used as received unless otherwise specified. The poly(N^{ε}-lysine) samples (DP_n 30, purity > 90%) used for ^{1}H NMR spectra were obtained from Chisso Corporation, Fine Chemicals Division (Chuo-ku, Japan).

Caution! Hazardous compound: Nitric oxide is a skin and mucous irritant. All operations involving the handling of this compound were carried out in a fume hood whilst wearing appropriate protective clothing. Effluents were destroyed by treatment with concd aq NaOH.

Synthesis of DGL of generations $n=1$ to 5

Lys(Tfa)-NCA: This procedure was adapted from that of Collet et al.[28] C-Lys(Tfa) (100 g, 350 mmol) was placed in a 20 L, 3-necked flask fitted with a magnetic stirrer, a 2 L dropping funnel, and gas/vacuum connections, all with Teflon Rotaflo stops, and thermostated to 0°C. The apparatus was evacuated, filled with N_2, re-evacuated, then filled with gaseous NO to 90% of atmospheric pressure (total 20 L, 890 mmol). MeCN (400 mL; previously stored over molecular sieves and deaerated by N_2 flushing) was injected into the apparatus with a dropping funnel and the mixture was stirred. O_2 (3800 mL, 160 mmol at 1 atm) was injected in 10 fractions of decreasing size over 1 h, and the mixture was stirred at 0°C for 45 min. After in vacuo removal of the excess nitrogen oxides and solvent (followed by purging the reactor with N_2), the crude Lys(Tfa)-NCA was obtained in high yield as a white solid and was immediately used without further purification in the same vessel in the following step.

NCA condensation: The flask containing the crude, freshly prepared Lys(Tfa)-NCA (ca. 350 mmol), was thermostated to 0°C. Cold (0°C) aqueous $NaHCO_3$ (0.2 M, 2000 mL) and DGL G($n-1$) polymer (30.0 g, 124 mmol of monomer units, 2.83 equiv; when $n=1$ no DGL polymer was added) were added to the stirred mixture, and the reaction was then kept closed (with a controlled leak so that exuded CO_2 kept the pressure to 1.0–1.5 atm and thus the pH at ca. 6.5) and stirred for 15 h (overnight) at 0°C. The white, flocculent precipitate was separated by centrifuging three times, the solid residue was resuspended in aqueous $NaHCO_3$ (0.1 M, 500 mL), and the mixed supernatants were filtered. Exception: G1P was collected by filtration and then washing with H_2O (3×500 mL). In vacuo drying of combined precipitates afforded the G*n*P polymer as a white solid. Although Lys(Tfa)-NCA consumption was complete within 5 h according to CO_2 outgassing measurements, the recovery of precipitated G*n*P was easier after overnight stirring, possibly because of tighter aggregation of the polymers.

Deprotection: The above-described combined precipitates (G*n*P) were suspended in a mixture of H_2O (400 mL), NH_3 (10 N, 700 mL; 28% *w/v*) and MeOH (1200 mL) in a 5 L flask, and the mixture was stirred at 40°C for 15 h (overnight). The reaction was monitored by ^{19}F NMR spectroscopy analysis of aliquots by checking for the disappearance of CF_3CONH signal ($\delta_F=-74$ ppm, s) and the increase in the $CF_3CO_2^-$ signal ($\delta_F=-76$ ppm, s). After completion of the reaction, the mixture was concentrated in vacuo to 1/10 of its initial volume to remove MeOH and NH_3, then freeze-dried to afford a white solid (polymer G*n*, fully water-soluble), which was identified as poly(L-lysine), trifluoroacetate salt (overall yields from 100 g of C-Lys(Tfa): 52, 42, 70, 78 and 80 g for G1 to G5, respectively).

Analytical methods

NMR spectroscopy: ^{1}H NMR spectroscopic analyses of G1–G5 polymers were carried out by using a Bruker AM 400 apparatus (400 MHz) on polymer samples (25 mg) dissolved in D_2O (0.6 mL; Sigma). ^{19}F NMR spectroscopic monitoring was carried out by using a Bruker AC-250 apparatus. Chemical shift internal standards for NMR analyses: ^{1}H: H_2O signal at 4.72 ppm (D_2O) or $CHCl_3$ signal at 7.27 ppm ($CDCl_3$); ^{19}F: TFA signal at 76 ppm.

Size-exclusion chromatography (SEC): SEC analyses were carried out by using a chromatograph that included a Waters 515 HPLC pump, a 100 μL injection loop, a 300×10 mm agarose column (Superose 12 HR 10/30, Millipore), an Optilab DSP refraction index detector (Wyatt Technology, Santa Barbara, CA), and a Dawn DSP multiple-angle laser light scattering (MALLS) detector (Wyatt Technology) working at a wavelength of $\lambda=633$ nm. Data were collected and processed by using the ASTRA and EASI software (Wyatt Technology). The eluent was a solution of Na_2HPO_4 (50 g L^{-1}, Sigma Aldrich) in H_2O (pH 4.5), which was filtered on 0.45 μm and then 0.1 μm Millipore filters prior to use. Elution was carried out at 0.4 mL min^{-1} flow rate. The whole system (column and detectors) were thermostated to 35°C. Polymer samples (2 g L^{-1}) were dissolved in the eluent and then filtered on Millipore 0.45 μm filters prior to injection. Refractive-index increments *dn*/*dc* were measured at 35°C by using an Optilab DSP refractive index detector.

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