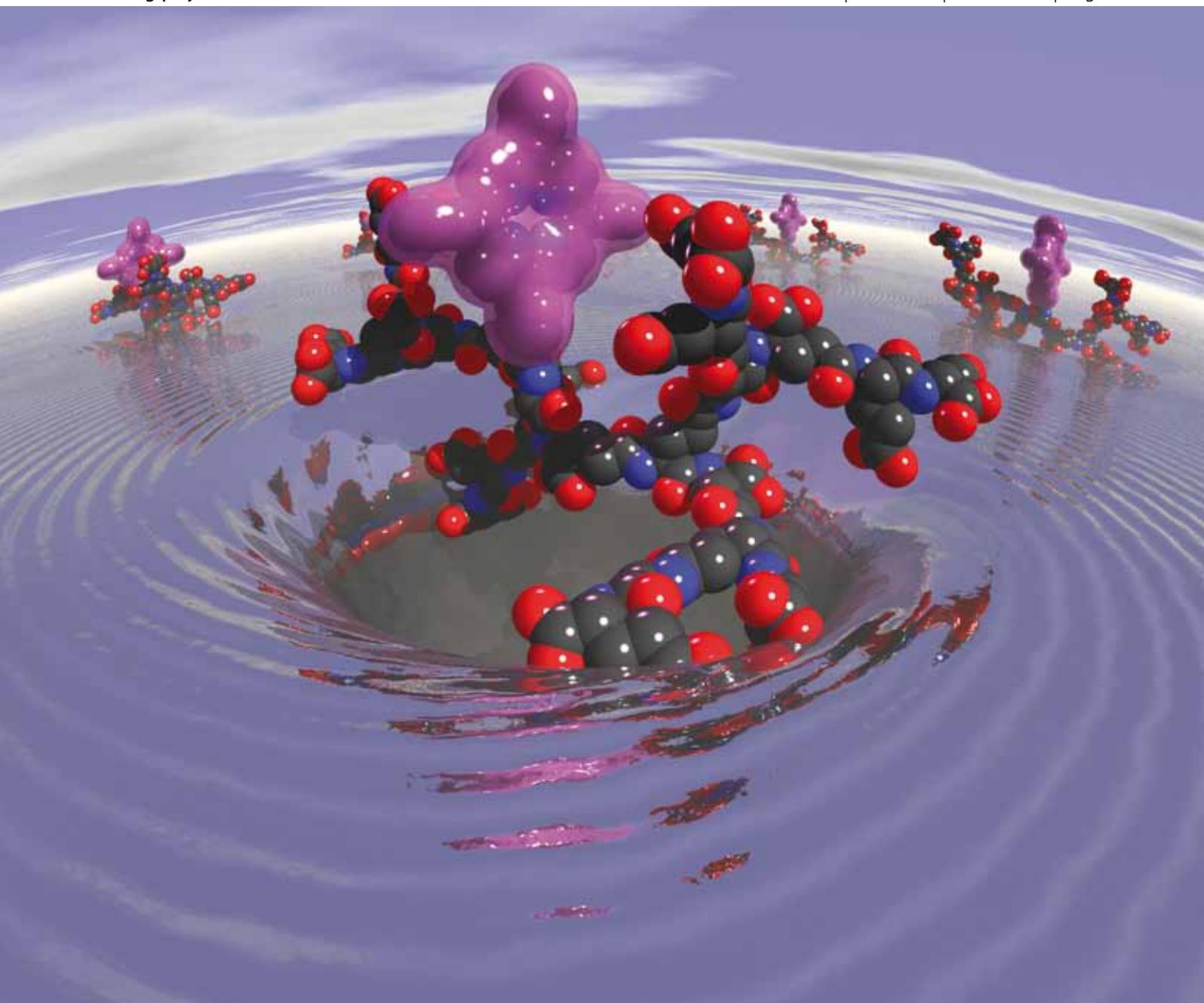


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COMMUNICATION

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Exponential growth of functional poly(glutamic acid) dendrimers with variable stereochemistry

REVIEW

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Thiol-ene "click" reactions and recent applications in polymer and materials synthesis

Exponential growth of functional poly(glutamic acid) dendrimers with variable stereochemistry†

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Polyglutamate dendrimers up to the fourth generation have been prepared via an accelerated iterative divergent/convergent binomial synthesis, which allows incorporation of either *all*-(*L*) or (*D-alt-L*) stereochemistry in the peptide backbone and enables versatile postfunctionalization of the dendritic core and periphery.

Dendrimers are discrete macromolecules with a well defined and perfectly branched structure, presenting attractive functional scaffolds for biomedical and pharmaceutical applications.^{1–3} In view of their high potential biocompatibility and biodegradability, particularly peptide dendrimers composed of branched AB₂-type‡ amino acids, *i.e.* lysine⁴ on the one hand or glutamic^{5–7} and aspartic acid⁸ on the other hand, are particularly interesting targets. Two of the main advantages of dendrimers are the possibility of precise attachment of many functional groups in close proximity (multivalency) and their adjustable size, both of which can be tuned by generation number. In the case of higher generation dendrimers these features enable not only the attachment of a large number of peripheral functionalities interacting with the environment but also other beneficial effects, such as enhanced circulation times *in vivo*.⁹ However, the eminent bottleneck in the use of dendrimers for any type of application requiring significant amounts of material is their demanding synthesis, limiting their size and availability.§ Nowadays, dendrimers are prepared *via* either divergent¹⁰ or convergent¹¹ syntheses, including double-stage convergent,¹² accelerated,¹³ or exponential growth¹⁴ approaches. Here, we report on the unprecedented accelerated synthesis of polyglutamate dendrimers up to the fourth generation *via* an iterative divergent/convergent exponential growth approach.^{14,15} Our synthesis provides exquisite control of backbone stereochemistry,¹⁶ *i.e.* either *all*-(*L*) or (*D-alt-L*), to tune structure and biodegradability,¹⁷ and allows for regioselective introduction of functional groups.

The incorporation of an alternating (*D,L*)-stereochemistry into a macromolecule can be conveniently accomplished by using a dimeric building block as repeat unit. To achieve rapid dendrimer growth, the exponential synthesis strategy is very attractive.¹⁴ As the dipeptide is extended at the side chain functionalities and not along the backbone, our synthesis requires orthogonal temporary protecting groups at the *N*-terminus and the carboxylate side chains while maintaining a permanent protecting group at the *C*-terminus. For this purpose, we chose the acid labile Boc-group as the *N*-terminal protecting group, while the carboxylic acid side chains were protected with benzyl esters, readily cleavable by hydrolysis. The

C-terminus was masked as methyl ester instead, allowing for cleavage *via* saponification at a later stage of the synthesis.

The dendrimer synthesis is based on the G1 dipeptide building blocks **1a** and **1b**, readily prepared from commercially available Boc-*L*-Glu(*Z*) *via* a 3-step route involving esterification of the *C*-terminus, Boc-cleavage, and subsequent coupling with either Boc-*L*-Glu(*Z*) or Boc-*D*-Glu(*Z*).† This synthesis route provided access to both monomers in almost quantitative yields and in excellent purity, which were then subjected to our exponential growth procedure to give both series of the corresponding G2 and G4 dendrons **4a/b** and **7a/b** (Scheme 1).

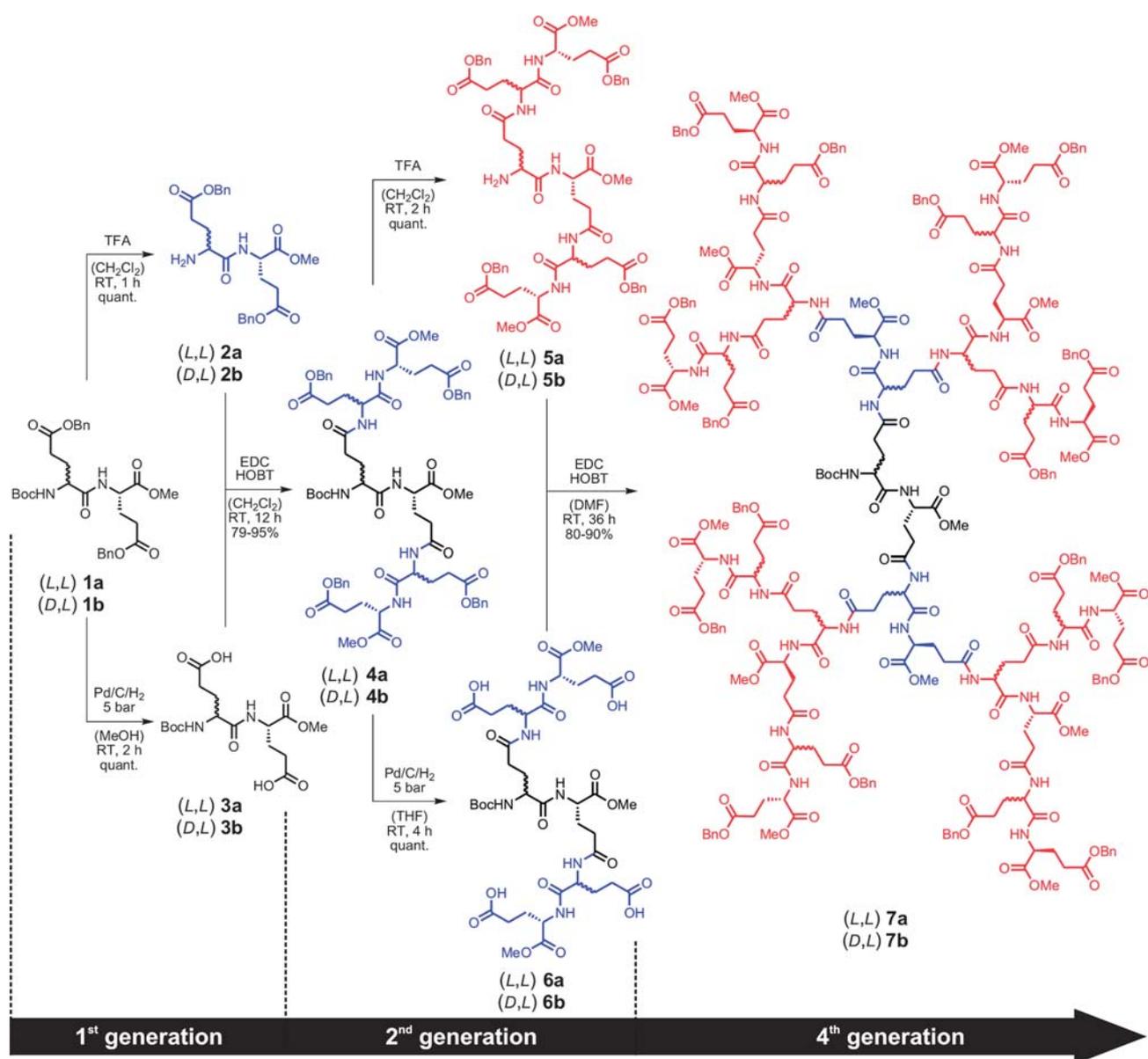
The dendron growth sequence starts with removal of the orthogonal Boc and *Z* protecting groups to yield the *N*- and *C*_γ-deprotected dipeptides **2a/b** and **3a/b**, respectively. All deprotection reactions proceeded smoothly and gave quantitative yields of the respective activated fragments, which were used without further purification. The subsequent coupling afforded both G2 dendrons **4a** and **4b** in high yields. Purification of **4a** and **4b** was readily achieved by column chromatography and repetitive precipitation in diethyl ether. Again, deprotection of the *N*-terminal Boc and *C*_γ-*Z* groups gave the activated dendrons **5a/b** and **6a/b**, respectively, in quantitative yields. Without further purification, the orthogonal fragments were coupled to yield the desired G4 dendrons **7a** and **7b** in excellent yields. Please note that purification of the G4 dendrons could easily be achieved by precipitation in diethyl ether and extensive washing of the solid residue with diethyl ether and MeOH, yielding the desired products as white powders of high purity.

The GPC-traces of the peptide dendrons (Fig. 1, bottom) nicely illustrate the rapid growth of the hydrodynamic volume as a function of generation. Interestingly, the corresponding molecular masses determined by GPC, calibrated with polystyrene standards, correlate reasonably well with the real molecular masses of the molecules. As expected for discrete dendritic macromolecules, polydispersities determined by GPC were close to unity (PDI ≤ 1.04). Assuming well solvated peptide repeat units in DMF, the increase in hydrodynamic volume can qualitatively be visualized by molecular models of each generation (Fig. 1, top). The absence of structural defects, potentially arising from incomplete deprotection or coupling steps, was verified by both mass spectrometry and ¹H NMR spectroscopy.†

Unfortunately, further growth of the G8 dendrons by another divergent/convergent cycle failed. Initial Boc-deprotection proceeded smoothly yielding the G4 dendron with a single free amine functionality at the focal point and also *Z*-deprotection gave the G4 dendron with 16 terminal free carboxylic acid functionalities. However, subsequent coupling of both fragments did not lead to formation of the desired G8 dendron and only starting material was isolated. Most likely, the focal amine functionality of the G4 dendron was sterically too shielded and hence not accessible for the activated terminal acid

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† Electronic supplementary information (ESI) available: Synthesis details and characterization data. See DOI: 10.1039/b9py00217k



Scheme 1 Synthesis of the (L,L)- and (D,L)-G4 dendrons **7a** and **7b** starting from the (L,L)- and (D,L)-G1 dipeptide monomers **1a** and **1b**, respectively, via an accelerated exponential growth approach.

groups. Such inhibition of further dendritic growth in the iterative divergent/convergent binomial synthesis has been observed by Moore and coworkers in the case of *meta*-phenylene ethynylene dendrons.¹⁴

The peptide dendrons serve as versatile scaffolds, allowing for solubilization of various functional moieties in water. These functional entities could be attached either covalently to the focal amine group (or non-covalently in the dendritic interior) while terminal deprotection of the dendrons would furnish dendritic polyelectrolytes with many solubilizing carboxylate groups. Therefore, for example water-insoluble dyes could be encapsulated within a given dendritic shell¹⁸ and thus be transferred into a physiological environment for various biomedical and biosensing purposes. In this context, (metallo)porphyrins constitute a very attractive class of dyes with unique photophysical and photochemical features, in particular with regard to their singlet oxygen generation ability⁶ as an important prerequisite for photodynamic therapy.¹⁹ In order to covalently attach

a tetraphenylporphyrin (TPP) core to the dendritic interior, *i.e.* the focal amine functionality, 5-(4'-carboxyphenyl)-10,15,20-triphenylporphyrin²⁰ was prepared and successfully coupled to the *D,L*-G4 dendrimer **7b**. The fully protected G4 dendron carrying a porphyrin focal point **8** is insoluble in water and remains in the organic layer, whereas after saponification **9** is readily water-soluble (Fig. 2).

In summary, polyglutamate dendrons up to the fourth generation have been synthesized using an exponential growth approach and were readily functionalized both at their focal point as well as periphery. Due to the precise control over dimension, core and exterior functionality²¹ as well as backbone stereochemistry, these dendrons and derived dendrimers constitute a versatile platform for the design of functional macromolecules. Ongoing work in our laboratories is concerned with exploiting this dendrimer family as structure-directing building blocks in polyelectrolyte complexes and as scaffold in bio-applications.

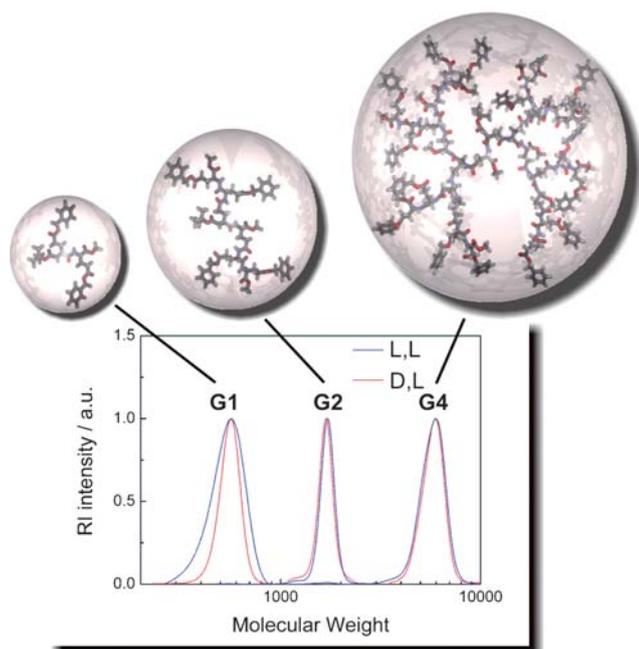


Fig. 1 Overlay of the GPC-traces of the (*D*)-*alt*-(*L*) G1, G2, and G4 dendrons **1b**, **4b**, and **7b** (red traces) including schematic representations of the three-dimensional shape of each generation. For comparison, the *all*-(*L*) series (**1a**, **4a**, and **7a**) is shown in blue (GPC in DMF at 70 °C, calibrated with narrow polystyrene standards).

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Notes and references

‡ In polymer chemistry, A and B groups are commonly used to depict chemical functionalities of orthogonal, *i.e.* nucleophilic and electrophilic, reactivity.

§ Only few dendrimer types, such as PPI or PAMAM dendrimers, are commercially available thus far.

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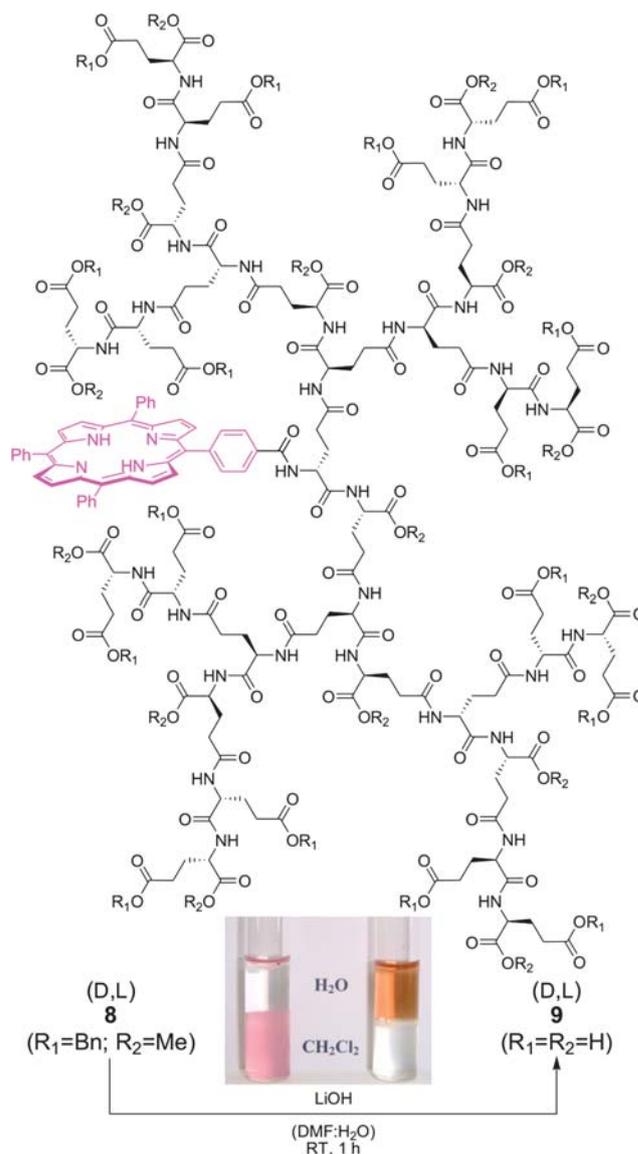


Fig. 2 The G4 dendron, carrying a porphyrin focal point, is insoluble in water in its completely protected form **8** (left) while saponification provides the fully deprotected derivative **9** with good water solubility (right).

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