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Synthesis and Properties of Dendritic Polymers Based on Natural Amino Acids

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Summary: Different approaches, including polycondensation, polymerization, polymer analog condensation, and ionic binding have been investigated for synthesis of amino acid-based dendritic polymers. It was shown that a growth of dendrons generation prevents obtaining of products with high polymerization degree in polycondensation and polymerization procedures. In polymer analog condensation a growth of dendrons generation leads to considerable decrease of polymer analog reaction rate as well as substitution degree. Degree of ionic binding depends on a strength of ionogenic groups and dendrns generation.

Keywords: α–amino acids, dendritic polymers, solubility, synthesis, viscosity

Introduction

Dendritic polymers are composed of linear macromolecular core to which dendrons have been attached in any way. They display a complex of unusual properties due to which they attract attention to their synthesis and study. First they were reported in 1987.[1] Their physical properties as well as conformational properties of their macromolecules are under thorough investigation.[2-4] The polymers consisting of dendritic macromolecules are considered as very promising objects, especially for biomedical purposes,[5] but complexity of their structure and difficulties and ambiguity of their synthesis as well as "inadequate analytical methodology precluded unequivocal characterization until now".[6] First dendritic lysine-based polypeptides were reported by Denkewalter in 1981.[7]

At present the most studied group of dendrimers are those, which have small molecule with a few functional groups as a core. Usually molecules of these dendrimers have a shape close to spherical. Replacement of the small molecule core by macromolecule leads to formation of new type of polymer molecules – dendritic macromolecules, whose shape is assumed to be

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close to cylindrical. The bigger and the more complex the core molecule, the more complex the final dendritic macromolecule is arranged.

Several parts of such molecule determine final properties of dendritic polymer. Firstly, it is the core chain, its rigidity, its skeletal chemical bonds and other structural features. Secondly, they are "anchor" groups, which join pendent dendrons to the core. Thirdly, they are the pendent dendrons proper, which constitute the main part of inner volume of the macromolecule. And finally, they are terminal groups of the dendrons located at periphery of the macromolecule. Each of these parts contributes to properties and behavior of the resulting dendritic polymer depending on their chemical nature and mass portion. Several teams successfully work in this area since early 90-th.[1-6]

Most of described denderitic polymers were prepared by chain polymerization, whereas information dealing with other routes is insufficient. Many approaches and their combination may be used for design of dendritic molecules.

In the present work we have investigated synthesis of amino acids based dendritic polymers with use of various approaches. The choice of trifunctional amino acids as monomers for design of pendent dendrons and sometimes core macromolecule was determined by their very attractive properties. These compounds are biocompatible, which makes them very convenient for synthesis of polymers for biomedical purposes. A presence of chiral carbon atom enables to use them as chirality carriers. Due to the progress in biosynthesis of α-amino acids they became accessible monomers at the moderate price. Besides, they may be easily transformed into polymers via polymerization as well as polycondensation procedures. Endo gave the detailed consideration of use of amino acids as monomers for polymer synthesis.[8]

The present paper gives a brief overview of our recent works in dendritic polymers.

Objectives of the Work

i. Development of synthetic procedures to preparing dendritic polymers based on amino acids
ii. Preparation of dendritic polymers samples for their detailed study
iii. Investigation of synthesis and properties of the polymers.

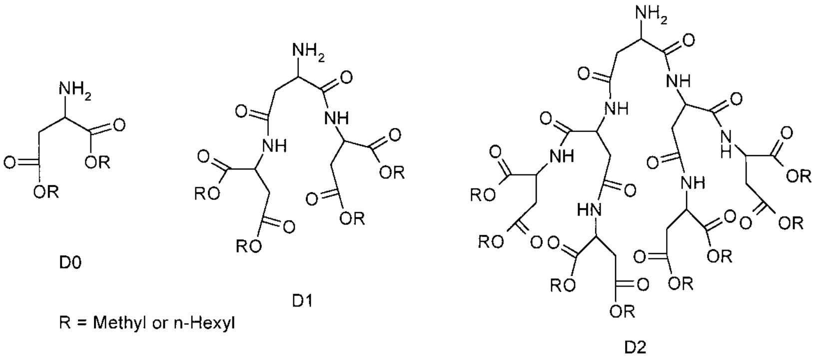
Approach: Gradual transition in the study of synthesis and properties of the polymers from relatively simple structures of their macromolecules (zero-generations of pendent dendrons) to more and more complicated ones.

Methods: In the present work we used all available methods of polymer and peptide synthesis. The main method for characterization and investigation of the products was study of behavior of macromolecules in (dilute) solutions. Special attention in the present work was paid to solubility and viscosity behavior of the products.

Different approaches were used to prepare the polymers:

1. Polycondensation
2. Polymerization
3. Polymer analog condensation
4. Ionic binding of polyelectrolyte macromolecule with ionogenic focal groups of dendrons

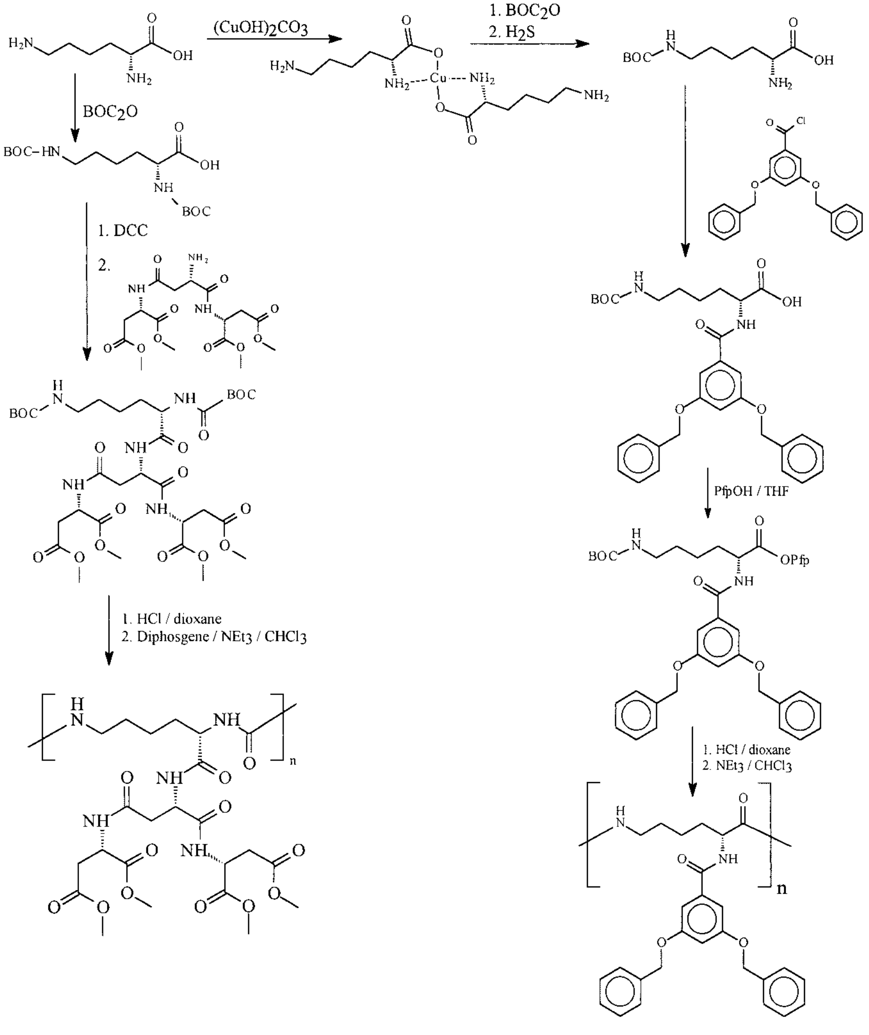
L-aspartic acid and L-glutamic acids were used for design of dendrons similar to those described in ref.[9]



Scheme 1

Acrylic chains were used as core macromolecules for polymerization and polymer analog condensation. Lysine was used as monomer for formation of the core macromolecule in polycondensation procedure. Usually, polycondensation is not applied for synthesis of poly-α-amino acids because of competitive cyclization reactions. Amino acids may be inserted in polymer chains by polycondensation in some specific routes.[8]

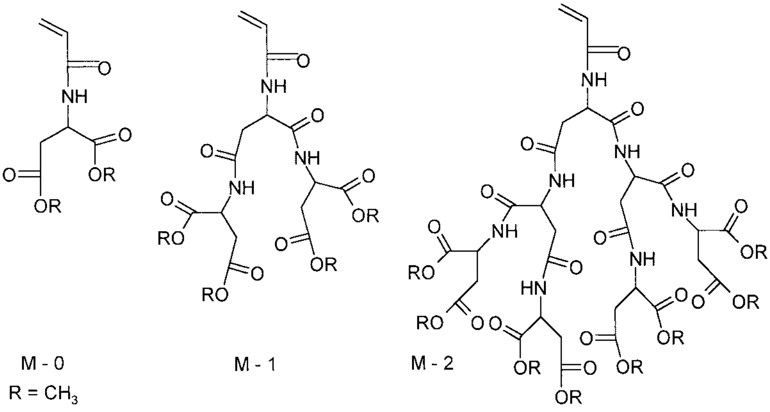
We performed polycondensation with use of lysine as a monomer for formation of a core macromolecule (see the scheme below). Aspartic dendrons bound to carboxylic group were used in one route, whereas zero-generation "Frechet-type" dendron was used in another route.



Scheme 2.

The resulting polymers were isolated as white powders, their structure was confirmed by ^{1}H NMR. Relatively low intrinsic viscosity (0.08 dl/g) may indicate rather low polymerization degree. At the same we showed that rather high molecular weight dendritic polymers might have low [η] values (see below).

Free-radical polymerization of acrylic monomers bearing aspartic and glutamic dendrons of different generations as well as conformational properties of the macromolecules are described in ref.[10,11] in detail. Structures of monomers used for the polymerization are given below:



Scheme 3.

The mostly interesting feature of the polymerization is high M_w of the polymers derived from M_0 and M_1 (about 10^6). Similar facts were mentioned for polymerization of N-acryloyl amino acids by Endo.[8] Polymerization of M_2 gave oligomers only. Some of the prepared polymers were selectively hydrolyzed at methoxy group carboxy-terminated dendritic polymers.

In order to perform polymer analog condensation of core macromolecule with dendrons we have synthesized activated polyacrylates p-nitrophenyl and N-hydroxysuccinimide esters of polyacrylic acid, which were introduced in a reaction with aspartic dendrons with focal amino group. The main series of the syntheses were carried out with use of poly-p-nitrophenyl acrylate (PNPA) with intrinsic viscosity 0.14 dl/g. In order to determine its polymerization degree we have transformed it to polyacrylamide, whose molecular weight was calculated by

Mark-Kuhn equation $[\eta] = 0.631*10^{-4}M_{\eta}^{0,8}$.[12] The polyacryamide had intrinsic viscosity 0.17 dl/g, which corresponds to molecular weight 19000 and polymerization degree about 270. Figure 1 shows that both reaction rate as well as substitution degree decrease considerably for higher generation dendrons. It should be noted that the content of p-nitrophenyl units in polyacrylic chains decreased to higher extent as compared with increase of the bound dendrons. We explain this fact by formation of succinimide and glutarimide cycles in which two carboxlic groups are bound with one focal group of dendrons. NMR spectra confirm a presence of such structures.

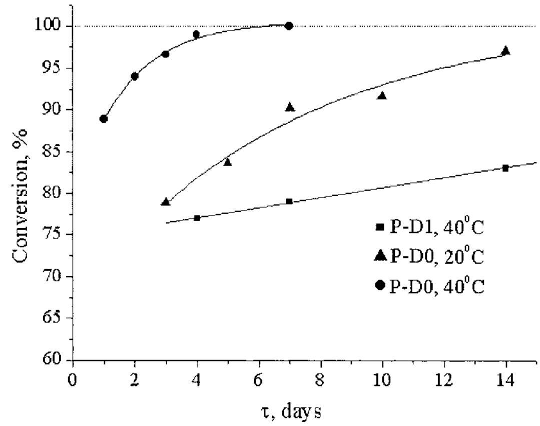


Fig. 1. Conversion degree of p-nitrophenyl groups determined by UV-spectrometry during reaction of PNPA with aspartic dendrons.

Assuming that polymerization degree doesn't change after polymer analog condensation molecular weight (M) of polymers with higher generations dendrons should increase. But we didn't observe increase in intrinsic viscosity of the products in chloroform at 30° (Table 1). Moreover, it decreased from 0.16 dl/g for P-D0 (acrylic polymer with attached D0; M = 53000) to 0.12 dl/g for P-D1 (acrylic polymer with attached D1; M = 115000) and to 0.11 dl/g for P-D2 (polymer with attached D2; M = 230000). The same tendency was observed for polymers prepared from N-oxysuccinimide ester of polyacrylic acid. More detailed characteristics of the condensation process and the products are given in Table 1.

Table 1. Characteristics of the process of polymer analog condensation of PNPA with aspartic dendrons of different generations.

Polymer	[η], dl/g	M calculated	Conversion of NP groups, %	Substitution degree, % (NMR)	Dendrons attached via imides, %
PNPA	0.14 (Ph-NO_2)	52000	-	-	-
P-D0	0.16 ($CHCl_3$)	53000	100	87	15
P-D1	0.12 ($CHCl_3$)	115000	83	~83	~2
P-D2	0.11 ($CHCl_3$)	230000	86	~86	0

Table 1 shows that complete conversions of p-nitrophenyl groups takes place in a reaction with dimethyl ester of aspartic acid only. Some of dendrons were bound by formation of not only amide bond, but by imide bond too. Growth of the generation number of dendron doesn‘t lead to decrease of substitution degree with respect to attached dendrons, but decreases a number of imide cycles formed, which may be explained by steric factors.

One of the most important directions of the area is self-assembling of complex macromolecular structures based on non-covalent interactions.[5] Hydrogen bonding of dendrons bearing terminal amino groups with poly(vinylpyridine) is described in.[13] We have studied an opportunity to form proper dendritic macromolecules due to self-assembly based on ionic binding of polyelectrolyte macromolecules with dendrons bearing oppositely charged groups at their focal point.

Aspartic dendrons have basic amino group at their focal point thus they are able to form ionic bond with polyanionites. We have studied ionic interaction of these dendrons with macromolecules of weak and strong polyacids. Polyacrylic and polystyrene sulfonic (PSS) acids were used as those. Ionic binding of weakly basic focal amino group of dendrons with weakly acidic carboxylic group of polyacrylic acid proceeds with binding degree not above 60% not depending on dendron generation. As to strongly acidic polystyrene sulfonic acid the binding of dendrons proceeds with binding degree close to 100%, which was confirmed by IR, NMR and elemental analysis data (N/S ratio).

Solubility and Viscosity Properties

We have studied properties of the synthesized dendritic polymers with use of different methods. Conformational properties of macromolecules of the polymers were investigated by flow and electrical birefringence as well as by diffusion and sedimentation.[10] In the present publication we will briefly discuss unusual solubility and viscosity behavior of some polymers.

Solubility of the polymers with acrylic chain and methoxycarbonyl terminal groups decreases in common organic solvents with growth of generation number due to increased contribution of intramolecular hydrogen bonding. These polymers after selective hydrolysis of methyl ester group display pronounced polyelectrolyte effect.[11] The most interesting solubility and viscometry data were obtained for ionic complexes of polystyrene sulfonic acid and aspartic denrons bearing methoxycarbonyl and n-hexyloxycarbonyl terminal groups. Solubility of these complexes is influenced by a number of factors. Hydrophobicity and high flexibility of a core polystyrene macromolecule should promote solubility in organic solvents with moderate polarity. Anchor sulfoammonium salt groups should promote solubility in highly polar water and methanol. Dendritic part of a macromolecule should form a network of predominantly intramolecular hydrogen bonds, which should prevent solubility in aprotic organic solvents. And, finally terminal alkoxycarbonyl groups should promote solubility of the products in organic solvents with low polarity. Table 2 displays solubility of ionic complexes of PSS and aspartic dendrons bearing two (C^M-1 and C^H-1); four (C^M-2 and C^H-2) and eight (C^M-3 and C^H-3) methoxy and hexyloxy terminal groups respectively (dendrons D0; D1 and D2).

Table 2. Solubility of ionic complexes of PSS and aspartic dendrons.

	Hexane	Toluene	$CHCl_3$	MeOH	Dioxane	DMSO	Water
C^M-1	-	-	+	+	-	+	-
C^M-2	-	-	-	+ *	-	+	-
C^M-3	-	-	-	+ *	-	+	-
C^H-1	-	-	+	+	-	+	-
C^H-2	+ *	+	+	+	+	+	-
C^H-3	+ *	+*	+	+	+	+	-

* - At heating above 40°C

These data allows evaluating relative contributions of each component of ionically-assembled dendritic macromolecule to its solubility. Remarkable fact is the solubility of PSS polysalts in solvents with very low polarity.

Solubility of the complexes in a wide range of solvents made it possible to determine intrinsic viscosity values in different solvents. Viscometry data were used by some researchers for evaluating conformational properties of dendritic macromolecules.[14]

We have measured viscosity of ionic complexes of PSS and aspartic dendrons in different solvents. Some of the results are given at Figure 2 for hexyloxy-terminated dendritic polymers. The broad range of solvents allows drawing some preliminary conclusion about behavior of the macromolecules in these solvents. C^H-1 and C^H-2 display pronounced polyelectrolyte effect in polar methanol. At that reduced viscosity values for C^H-2 are lower than those for C^H-1, although molecular weight of the latter is much lower (165000 for C^H-1 and 300000 for C^H-2). In non-polar solvent such as toluene and chloroform the complexes do not display polyelectrolyte effect, but their intrinsic viscosity values look to be very small for the complexes of rather high molecular weights ([η] = 0.11 dl/g in chloroform for C^H-3 with M = 570000).

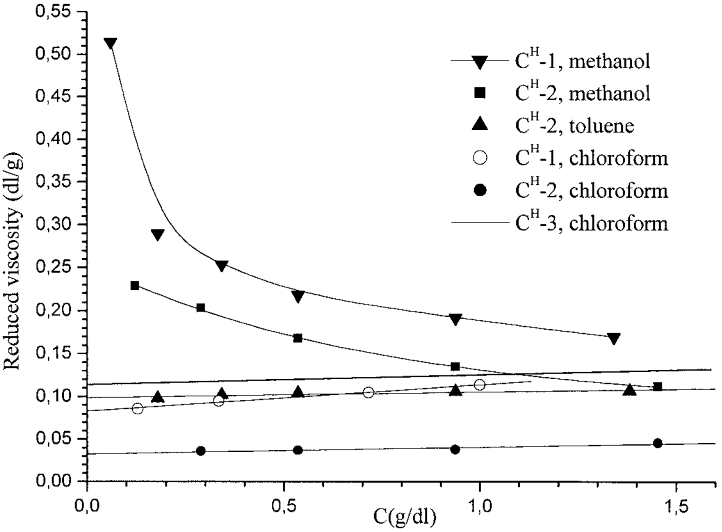


Fig. 2. Dependencies of reduced viscosity for the hexyloxy-terminated complexes on concentration in different solvents at 30° C.

The tendency of lowering intrinsic viscosity with growth of molecular weight (dendrons generation numbers at the same polymerization degree of a core macromolecule) seems to be common for the polymers under investigation (see above). It may indicate significant compactization of macromolecules in some solvent. At present we study conformational properties of dendritic molecules in different solvents by methods hydrodynamics, electrooptics, diffusion and sedimentation.

Experimental Part

All solvents were purified by distillation. BOC-amino acids and hydrochlorides of diesters of aspartic and glutamic acids were prepared according to standard methods. NMR spectra were recorded by "Bruker DPX-300" instrument in $CDCl_3$ or DMSO-D_6. IR spectra were recorded by "Specord IR-75" spectrometer.

Experimental details of polymerization and polymer analog condensation procedures are given in [11] and [15] respectively. Dendritic derivative of di-BOC-lysine was synthesized by reaction of di-BOC-lysine with aspartic dendron D-1 with use of dicyclohexyl carbodiimide. NMR, elemental analysis and thin-layer chromatography confirmed structure, composition, and purity of all derivatives.

Typical procedure for synthesis of condensation polymer with pendent aspartic dendronG-1 is given below:

2.5 ml of 3 M HCl solution in dry dioxane was added to 400 mg of di-BOC-lysine dendronized derivative. After a few minutes colorless oil of lysine dendronized derivative dihydrochloride was precipitated. Dioxane and excess of HCl were removed in vacuum and resulting oil was dissolved in 2 ml of chloroform containing 400 mg of triethylamine. 0.1 ml of diphosgene in 3 ml of chloroform was added dropwise to this mixture for 15 min. Reaction mixture was poured in 50 ml of hexane, the precipitate was filtered off, washed with water, dissolved in chloroform and poured into hexane to give 120 mg of white solid. Intrinsic viscosity in chloroform at 30°C was 0.08 dl/g. NMR spectra and elemental analysis confirm its composition and structure.

Polystyrene sulfonic acid was obtained from its sodium salt (Aldrich, $M_W = 70000$) using ion exchange chromatography column filled with Dowex 50x8.

Dendrons were synthesized by method similar to those described in,[11] but hexan-1-ol was used instead of methanol.

Ionic complexes were obtained via mixing equimolar amounts of dendrons and polyacids in methanol solution followed by precipitation in ethylacetate and drying in a vacuum. The composition of complexes was confirmed by elemental analysis (see Table 3), NMR and IR spectroscopy. The presence of absorption bands located near 1600 cm^{-1} and 1500-1520 cm^{-1} are assigned to H_3N^+ and shows the formation of ionic bonds. It should be noted that the first band could be seen for C^M-1 and C^H-1 only. In all other complexes it is overlapped by broad and intensive band of amide groups.

Table 3. Elemental analysis data for ionic complexes of polystyrene sulfonic acid and aspartic dendrons esters.

Ionic Complex	Elemental analysis (%)								N/S Ratio	
	Found				Calculated					
	C	H	N	S	C	H	N	S	Found	Calculated
C^M-1	47.9	5.5	3.6	8.4	48.7	5.5	4.1	9.3	0.4	0.4
C^M-2	46.9	5.4	6.5	5.1	47.8	5.5	7.0	5.3	1.3	1.3
C^M-3	46.0	5.7	8.3	2.9	47.2	5.5	8.8	2.9	2.9	3.0
C^H-1	58.5	7.5	2.5	6.3	59.4	8.0	2.9	6.6	0.4	0.4
C^H-2	59.8	8.3	4.8	3.6	59.8	8.3	4.8	3.6	1.3	1.3
C^H-3	58.9	8.1	5.7	2.0	60.0	8.4	5.8	1.9	2.9	3.0

Conclusion

A set of dendritic polymers with aspartic dendrons and various core macromolecules was synthesized by polycondensation, polymerization, polymer analog condensation and ionic binding methods. The polymers were investigated in dilute solutions and some unusual features of their solubility and viscosity behavior were found.

Acknowledgement

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