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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Synthesis of a Methacrylic Monomer Containing an *L*-Lysine-Based Dendron

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Abstract—Procedures were developed for preparing a polylysine dendron of second generation, containing terminal Boc-protected amino groups and a free focal amino group; from this substance, a previously unknown methacrylic monomer linked to the *L*-lysine-based dendron was prepared.

Much attention is given today to development of hybrid materials combining dendritic and linear structural fragments [1]. The use of dendritic monomers as building blocks along with linear monomers substantially extends the possibilities of macromolecular design; it has led to preparation of polymers of unusual architecture [2]. By using various structural elements forming a polymeric framework, it is possible not only to modify the physical properties of polymers, but also to impart additional functions to the polymer skeleton, such as complexing power, response to certain physical actions, and possibilities of further transformations. Introduction of dendrimers combining a high functionality with a compact molecular structure opens wide prospects for application of such materials in biology, medicine, and medical diagnostics [3–7].

Dendrimers prepared from natural amino acids by peptide synthesis [8–10] contain on the periphery a controllable amount of reactive functional groups.

The interest in polymers containing branched polylysine [11, 12] is due to the possibility of preparing new amphiphilic materials to be used in molecular biology. Dendrimers derived from *L*-lysine and block copolymers containing dendritic poly-*L*-lysine exhibit a high compacting power toward DNA [13, 14].

Two types of denritic-linear polymers containing poly-*L*-lysine fragments are known: dumbbell-like compounds prepared by reactions of bifunctional linear compounds (low-molecular-weight or polymeric) with dendritic fragments [12] and compounds in which one end of a polymer chain is linked to the focal point of a dendron [11, 12].

Published data on synthesis of dendritic polymers and copolymers containing monomeric units with dendritic poly-*L*-lysine fragments are lacking. There are three fundamentally different routes to such substances: (1) synthesis of a linear polymer, followed by addition of separately prepared dendrons to the chain (convergent route); (2) synthesis of a polymer with active substituents, followed by stepwise build-up of a dendron at the polymer chain (divergent route); and (3) synthesis of macromonomers, i.e., dendrons containing a polymerizable functional group at the focal point, followed by polymerization [1, 15]. Use of such macromonomers containing terminal functional groups capable of further chemical transformations under relatively mild conditions may be promising for preparation of new polymeric materials and macroporous polymeric sorbents [16].

The goal of this study was to develop a procedure for preparing a methacrylic monomer containing a fragment of a poly-*L*-lysine dendron of second generation.

EXPERIMENTAL

We used the following chemicals: *L*-lysine hydrochloride (Reanal, Hungary), di-*tert*-butyl pyrocarbonate, dicyclohexylcarbodiimide (DCC), *N*-hydroxybenzotriazole (HOBT) (all Sigma–Aldrich, Germany), methacryloyl chloride, and thionyl chloride (both Reakhim, Russia).

Methylene chloride, ethyl acetate, and chloroform were dried over P_2O_5 and then distilled (bp 40, 77, and 62°C, respectively). Tetrahydrofuran and diethyl ether were refluxed over alkali and then distilled (bp 65 and 35°C, respectively). Acetone was refluxed

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over anhydrous K_2CO_3, distilled (bp 56°C), and stored over molecular sieves. Thionyl chloride was distilled (bp 79°C). Other solvents and solid chemicals were used without additional purification.

The ^1H and ^{13}C NMR spectra were recorded on Bruker AC 300 (300 MHz) and Bruker DRX 500 (500 MHz) spectrometers in DMSO-d_6 or acetone-d_6.

MALDI-TOF mass spectra were taken on a Bruker IV device. Samples were ionized with an N_2 laser from a matrix of 2,4-dihydroxybenzoic acid.

The IR spectra were recorded on a Bruker IFS 88 spectrophotometer.

***L*-Lysine methyl ester dihydrochloride I.** Thionyl chloride (8 ml) was added dropwise with stirring to 60 ml of methanol cooled to –5°C with an ice–salt mixture, after which *L*-lysine hydrochloride (9.13 g, 0.005 mol) was added in portions with cooling. The mixture was stirred for 40 min at room temperature and then refluxed for 80 min. After cooling, the mixture was left overnight. The precipitate was filtered off and washed with diethyl ether (1 : 1). The precipitate was dried in air and then in a desiccator over NaOH. Yield 10.17 g (87.2%); white crystals.

^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.39 m (2H, CH_2), 1.59 m (2H, CH_2), 1.83 m (2H, CH_2), 2.74 m (2H, CH_2), 3.75 s (3H, OCH_3), 3.96 t (1H, CH), 8.16 br.s (NH_3^+), 8.72 (NH_3^+).

Di(*tert*-butoxycarbonylamino)-*L*-lysine II. To a solution of 3.2 g (0.08 mol) of NaOH in 30 ml of H_2O, we added with stirring *L*-lysine hydrochloride (7.306 g, 0.04 mol) and then sodium hydrocarbonate (1.68 g, 0.02 mol). A solution of 18.2 g (0.088 mol) of *tert*-butyl pyrocarbonate in 40 ml of ethanol was added dropwise with stirring to the solution of the amino acid, cooled with ice and water. The mixture was stirred for a day at room temperature, after which 50 ml of H_2O was added to dissolve the precipitate. Excess *tert*-butyl pyrocarbonate was extracted with petroleum ether. The aqueous phase was acidified with 1 M $NaHSO_4$. The product was extracted with ethyl acetate. The organic layer was washed with a saturated NaCl solution, dried over anhydrous Na_2SO_4, and evaporated; the residue was dried in a vacuum. Yield 10.98 g (85.4%); white crystals.

^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.2–1.4 m (20H, *t*-C_4H_9, CH_2), 1.53 m (2H, CH_2), 1.61 m (2H, CH_2), 2.88 m (2H, CH_2NHBoc), 3.82 m (1H, OCCH), 6.72 t (1H, NH), 6.96 t (1H, NH), 12.35 s (1H, COOH).

Found, %: C 55.42, H 8.81, N 8.16.

$C_{16}H_{30}N_2O_6$.

Calculated, %: C 55.47, H 8.73, N 8.09.

CH_3OLys(LysBoc$_2$)$_2$ III. To a solution of 3.244 g (0.014 mol) of *L*-lysine methyl ester dihydrochloride in 20 ml of DMF, we added 4.6 ml (0.033 mol) of triethylamine to obtain mixture A. To a solution of 10.5 g (0.042 mol) of di(*tert*-butoxycarbonylamino)-*L*-lysine in 40 ml of DMF, we added 3.97 g (0.033 mol) of HOBT and 6.84 g (0.033 mol) of DCC. After standing for 40 min, the solution of the activated ester was filtered through a glass frit and added to mixture A. The mixture was stirred for a day, and the precipitate was separated. The filtrate was evaporated in a vacuum to 1/3 of the initial volume. The resulting solution was diluted with 100 ml of ethyl acetate and washed successively with saturated NaCl solution (40 ml), 2% $NaHSO_4$ solution (2 × 20 ml), 5% $NaHCO_3$ solution (2 × 20 ml), and H_2O (2 × 20 ml). The organic layer was dried over Na_2SO_4 and evaporated; the yellow oily residue was purified by preparative adsorption chromatography on a column packed with silica gel (40–60 μm). The major fraction was additionally purified by repeated chromatography on a similar column. A mixture of chloroform with methanol (ratio from 10 : 1 to 5 : 1) was used as eluent. A white crystalline substance was obtained; yield 9.84 g (86%).

^1H NMR spectrum (DMSO-d_6), δ, ppm: 1.10–1.75 m (54H; *t*-$C_4\underline{H}_9$, CH_2), 2.88 m (4H, $C\underline{H}_2$), 2.95–3.1 m (2H), 3.61 m (3H, $OC\underline{H}_3$), 3.80 m (1H, CH), 3.91 m (1H, CH), 4.20 m (1H, CH), 6.66 d (1H, NH), 6.71 m (3H, NH), 7.71 t (1H, NH), 8.05 t (1H, NH).

^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 22.51, 22.72, 22.83, 24.48, 25.37, 28.22, 28.31, 28.61, 29.24, 29.26, 30.58, 31.64, 31.83, 33.38, 38.15, 39.72, 40.83, 47.55, 51.75, 54.03, 54.37, 77.34, 77.94, 155.28 (CO), 156.59 (CO), 171.95 (CO), 172.41 (CO), 172.53 (CO).

Found, %: C 57.41, H 8.95, N 10.40.

$C_{39}H_{72}N_6O_{12}$.

Calculated, %: C 57.33, H 8.88, N 10.29.

MALDI, *m*/*z*: 818.1 $[M + H]^+$, 840.1 $[M + Na]^+$, 856.1 $[M + K]^+$. IR spectrum, *ν*, cm^{-1}: 3150–3500 (NH), 2978 (*t*-C_4H_9), 1693 (C=O, amide **I**), 1525 (amide **II**), 1170 (CO).

$NH_2CH_2CH_2NH$Lys(LysBoc$_2$)$_2$ IV. A solution of 1.58 g (1.93 mmol) of dendron **III** in 15 ml of absolute methanol was added dropwise with stirring to 15 ml of ethylenediamine, cooled with ice. The mixture was purged with nitrogen and left in the dark at room temperature for 3 days. After that, the solvent and excess ethylenediamine were distilled off in a vacuum. The product was purified by preparative adsorption chromatography on a column packed with

silica gel (40–60 μm), with chloroform–methanol (5 : 1) as eluent. A white crystalline product was obtained; yield 1.38 g (83.8%).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.10–1.75 m (56H), 2.54 m (2H, –CH_2–NH_2), 2.87 m (4H, CH_2), 2.95–3.08 m (4H, CH_2), 3.81 m (1H, CH), 3.85 m (1H, CH), 4.17 m (1H, CH), 6.66 d (1H, NH), 7.62 m (2H, NH), 6.86 d (1H, NH), 7.68 m (2H, NH), 7.79 t (1H, NH).

^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 22.52, 22.83, 28.22, 28.32, 28.79, 29.24, 31.44, 31.89, 31.96, 38.41, 39.69, 41.25, 42.29, 52.38, 54.33, 54.54, 77.34, 77.93, 78.15, 155.29 (CO), 155.46 (CO), 155.61 (CO), 171.46 (CO), 171.90 (CO), 171.96 (CO).

Found, %: C 56.93, H 9.35, N 13.21.

$C_{40}H_{76}N_8O_{11}$.

Calculated, %: C 56.85, H 9.06, N 13.26.

MALDI, *m*/*z*: 846 $[M + H]^+$, 868 $[M + Na]^+$, 884 $[M + K]^+$. IR spectrum, ν, cm^{-1}: 3150–3500 (NH), 2978 (*t*-C_4H_9), 1695 (C=O, amide **I**), 1525 (amide **II**), 1169 (CO).

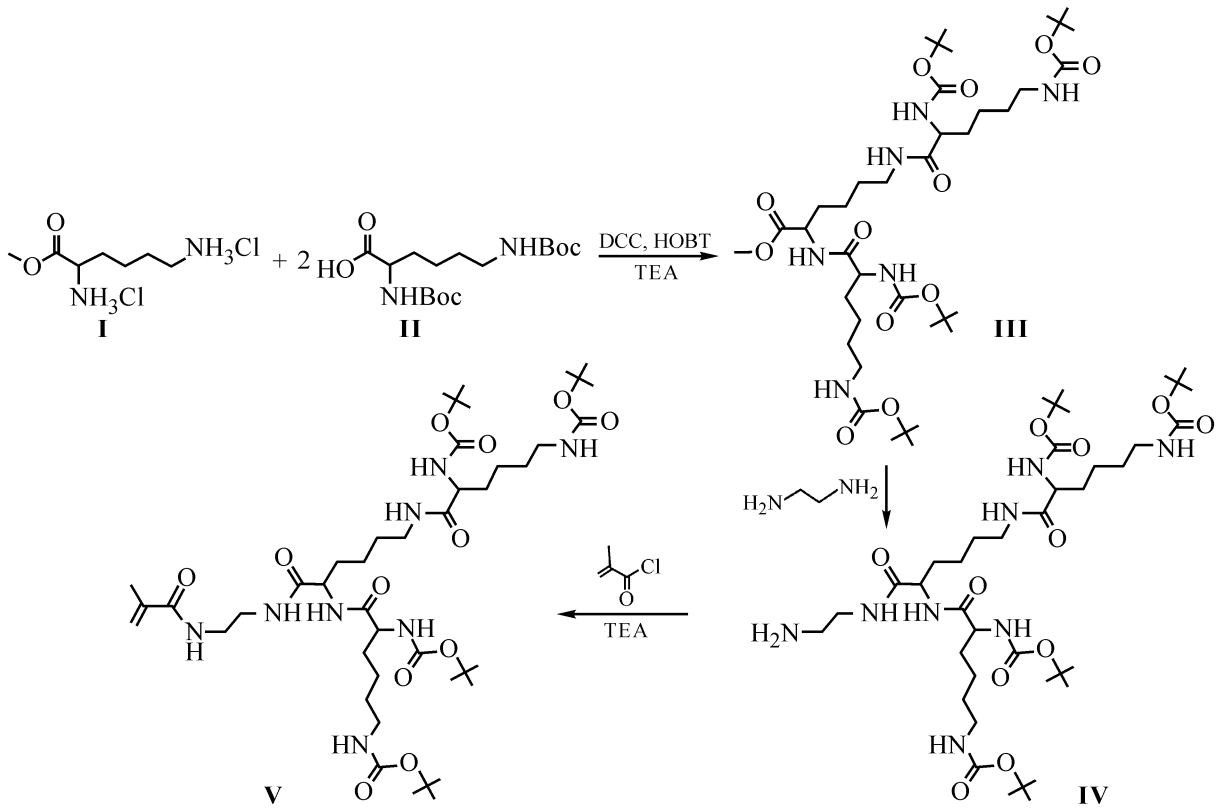
Methacrylic monomer V. To a solution of 0.97 g (1.45 mmol) of dendron **IV** in 12 ml of dry THF, cooled to –5°C, we added 0.223 ml (1.60 mmol) of triethylamine. Then we slowly added a solution of 1.167 g (1.6 mmol) of methacryloyl chloride in 2 ml of THF. The mixture was stirred for 3 h and left overnight. On the next day, the solvent was distilled off in a vacuum. The residue was dissolved in dichloromethane and washed with water, dilute Na_2CO_3, and again water. The organic layer was dried over Na_2SO_4 and concentrated. A white crystalline substance was obtained; yield 1.1 g (83.3%).

^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.1–1.75 m (52H), 1.98 s (3H, C=CCH_3), 2.87 m (4H, CH_2NHCO), 3.03 m (2H), 3.15 m (4H), 3.82 m (2H), 4.18 m (1H, CH), 5.28 (1H), 5.62 (1H), 5.95–6.9 (a series of broad NH signals).

^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 19.39, 21.55, 23.31, 23.66, 25.30, 25.97, 29.01, 29.10, 29.63, 30.06, 32.25, 32.71, 34.20, 39.81, 46.43, 48.37, 53.15, 55.15, 55.33, 55.70, 60.57, 67.86, 78.13, 78.72, 78.94, 119.93, 140.66, 156.09 (CO), 156.27 (CO), 156.40 (CO), 157.49 (CO), 168.37 (CO), 171.11 (CO), 172.62 (CO), 172.64 (CO).

MALDI-TOF, *m*/*z*: 935.7 $[M + Na]^+$, 951.7 $[M + K]^+$. IR spectrum, ν, cm^{-1}: 3150–3500 (NH), 3085 (=C–H), 2978 (*t*-C_4H_9), 1694 (C=O, amide **I**), 1642 (C=C), 1526 (amide **II**), 1169 (CO).

The poly-*L*-lysine dendritic macromonomer is prepared in several steps:



MALDI-TOF data

Dendron	Formula	Molar weight	MALDI-TOF data		
			$[M + H]^+$	$[M + Na]^+$	$[M + K]^+$
III	$C_{39}H_{72}N_6O_{12}$	817.02	818.02*	840.02	856.02
			818.1**	840.1	856.1
IV	$C_{41}H_{78}N_8O_{11}$	845.08	846.08	868.08	884.08
			846.1	868.1	884.1
V	$C_{44}H_80N_8O_{12}$	913.15	–	935.7	951.7
			–	935.7	951.7

* Found.

** Calculated.

First, we prepared by known procedures *L*-lysine methyl ester dihydrochloride and di-Boc-*L*-lysine. The carboxy group was protected by esterification with methanol in the presence of $SOCl_2$. The Boc protection of the amino groups of *L*-lysine was performed by a standard procedure with *tert*-butyl pyrocarbonate (Boc_2O) [17].

From *L*-lysine methyl ester hydrochloride **I** and di(*tert*-butyloxycarbonyl)-*L*-lysine **II**, we prepared a dendron of second generation, $CH_3OLys(LysBoc_2)_2$ **III**, containing four Boc-protected amino groups. For this reaction to be successful, the carboxy group should be activated. Various activating agents are used in the synthesis of dendritic poly-*L*-lysines. Choi *et al*. [12] used, when preparing a hybrid copolymer of poly(ethylene glycol) and polylysine dendron by a divergent procedure, *N*-α-*N*-ε-di-Fmoc-*L*-lysine (Fmoc = 9-fluorenylmethoxycarbonyl) and HOBT in the presence of diisopropylamine. Chapman et al. [11] used as reagent pentafluorophenyl ester of *N*-α-*N*-ε-di-Boc-*L*-lysine in the presence of diisopropylethylamine (DPEA).

In the synthesis of *L*-lysine dendron **III**, we activated the carboxy group with dicyclohexylcarbodiimide and *N*-hydroxybenzotriazole (DCC/HOBT). The reaction occurs within 3 days at room temperature. The product was purified by column chromatography on silica gel, with chloroform–methanol as eluent; yield 86%.

Compound **III** contains two types of protecting groups: Boc groups on the periphery and methyl group in the focal point. By saponification of the carboxy group, the dendron with the reactive carboxy group can be obtained; it can be used for preparing dendrons of further generations.

Schlüter *et al*. reported on preparation of dendron-containing monomers by a procedure involving addition of a spacer to an acrylic or methacrylic monomer, followed by its modification with a dendron containing an appropriate functional group [1, 18]. In this study, we modified dendron **III** with ethylenediamine to obtain $NH_2CH_2CH_2NHLys(LysBoc_2)_2$ **IV**. This compound contains a focal amino group. In the subsequent step, it is brought into condensation with methacryloyl chloride, i.e., the spacer is added directly to the dendritic fragment.

The structures of dendrons **III** and **IV** and of macromonomer **V** were confirmed by IR and ^1H and ^{13}C NMR spectroscopy, and also by MALDI-TOF spectrometry (see table).

CONCLUSIONS

(1) A poly-*L*-lysine dendron of second generation, containing a focal amino group and terminal Boc-protected amino groups, was prepared.

(2) A procedure was developed for preparing a methacrylic monomer linked to the poly-*L*-lysine dendron of second generation through a spacer.

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