ELECTRONIC SUPPLEMENTARY INFORMATION

Lysine-based dendrimer with double arginine residues

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Synthesis of Lys-2Arg dendrimer

Materials. Amino acids (L-lysine, L-arginine, L-alanine) were obtained from "Iris Biotech GMBH" (Germany); trifluoromethanesulfonicacid (TFMSA), diisipropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), thioanisole, ethanedithiol and other reagents were purchased from Sigma–Aldrich (Germany) and used as received. Triethylamine, dichloromethane were purchased from Vecton Ltd. (Russia) and distilled prior to use. Dimethylformamide (DMF), also purchased from Vecton Ltd. (Russia), was dried under molecular sieves 4 Å and distilled under vacuum. Trifluoroacetic acid (TFA) purchased from Panreac (Spain) was distilled before application. All solvents were purified and distilled using standard procedures.

Instruments. Reaction products were analyzed by reversed-phase HPLC on a chromatograph Shimadzu LC-20 Prominence system (Japan) and the Luna C18(2), 4.6 × 150 mm column, 5μ mm, for analytical chromatography and Discovery C18 21.6 × 250 mm, 5μ for the preparative.

Synthesis and characterization. Lysine-based dendrimers were synthesized by standard solid phase peptide synthesis (SPPS), performed on a polymer support, p-methylbenzhydrylamine resin (Bachem Biochemica GmbH) using the BOC-strategy,

DIC/HOBt as a condensing mixture, and trifluoroacetic acid for deblocking at the acylation stage. To protect functional groups of amino acids, the tert-butylhydroxycarbonyl (Boc), and 2- chlorobensylhydroxycarbonyl groups were used. At the C-terminus of the dendrimers of this series, an alanine (Ala) residue was introduced so that the amino acid analysis of the synthesized dendrimers could reasonably monitor their structure. Nε,Nα-di-(tert-butylhydroxycarbonyl)lysine was introduced into the branching point and, subsequently, doubled amounts of derivative amino acids were added. The excess of the reagent introduced into the reaction was generally 3-4 equivalents per amino group based on the initial capacity of the polymeric carrier. The completeness of the acylation reaction was controlled by the presence of free amino groups using the Kaiser test (ninhydrin test). The protective BOC- group was removed during the synthesis by the action of trifluoroacetic acid (TFA). The final stage of the synthesis was the cleavage of the target dendrimer molecule from the polymeric carrier with the simultaneous complete deprotection of the trifluoromethanesulfonic acid/trifluoroacetic acid (TMSA/TFA) system in the presence of scavengers. Isolation and purification of the target molecules was carried out by chromatographic methods.

The following main stage was used to obtain 0.2 g of p-methylbenzhydrylamine resin (capacity 0.85 mmol/g): (1) deprotection, 50% TFA/CH2Cl2 (5ml), 20 min; (2) deprotonation, 10% Et3N/DMF (5ml x 2), 15 min; (3) coupling, 1.5 mM Boc-aminoacid, 15 mM DIC, 1.5 mM HOBt/DMF (5ml), 2 h; and a ninhydrin test. For this, the resin was washed with dimethylformamide and dichloromethane. In the case of the incomplete coupling (positive ninhydrin reaction) the protocol was repeated from stage (2).

Note that the problem of exhaustive acylation of the branched oligolysyl polymer appeared when passing from a dispherical to a trispherical construct in the process of dendrimers Lys-2Arg synthesis: two acylations with the activated derivative of N,N-di-(tert-butyloxycarbonyl)lysine and an increase in the acylation time were necessary to complete the reaction. And reaction times were prolonged for the amino acids after one branching unit (180 min), after two branching units (240 min), and three branching units

(360 min)); the equivalents of amino acid, DIC, HOBt were accordingly increased. The last stage of the growth of the dendrimer was necessary to add 4-N,N-dimethylaminopyridine (DMAP) to the reaction mixture as a catalyst for complete conversion.

The complete deprotection and splitting off of the dendrimer from polymer was carried out using trifluoromethanesulfonic acid (1 ml) in TFA (10 ml) in the presence of thioanisole (1 ml) and ethanedithiol (0.5 ml) for 1 h at 0°C and then 1.5 h at room temperature. The mixture was diluted with ethyl ether (30 ml) and filtered. The dendrimer was dissolved in TFA (10 ml), filtered to remove the resin, and precipitated by dry ether (100 ml).

The crude Lys-2Arg dendrimers were isolated by gel-filtration on Sephadex G-50 column (2.5 x 50 cm) using 10% acetic acid as eluent and purified by RP-HPLC in the system "water–acetonitrile–0.1% trifluoroacetic acid" with the use of linear ascending acetonitrile gradient. The isolated fractions had 95% purity by counting from the basic line (the UV detection was performed at 230 nm). After lyophilization of the corresponding fractions, the purification degree of product was analyzed by RP-HPLC.

^{1}H and ^{13}C NMR spectroscopy analysis

The chemical structure of Lys-2Arg dendrimer is shown in Fig. 2. We presented the ^{1}H and ^{13}C NMR spectra of the dendrimer in Figures 3 and 4. We utilized the two-dimensional ^{1}H-^{13}C HSQC (Fig. S1) and HMBC (Fig. 7) spectra to analyze the Lys-2Arg dendrimer structure in detail.

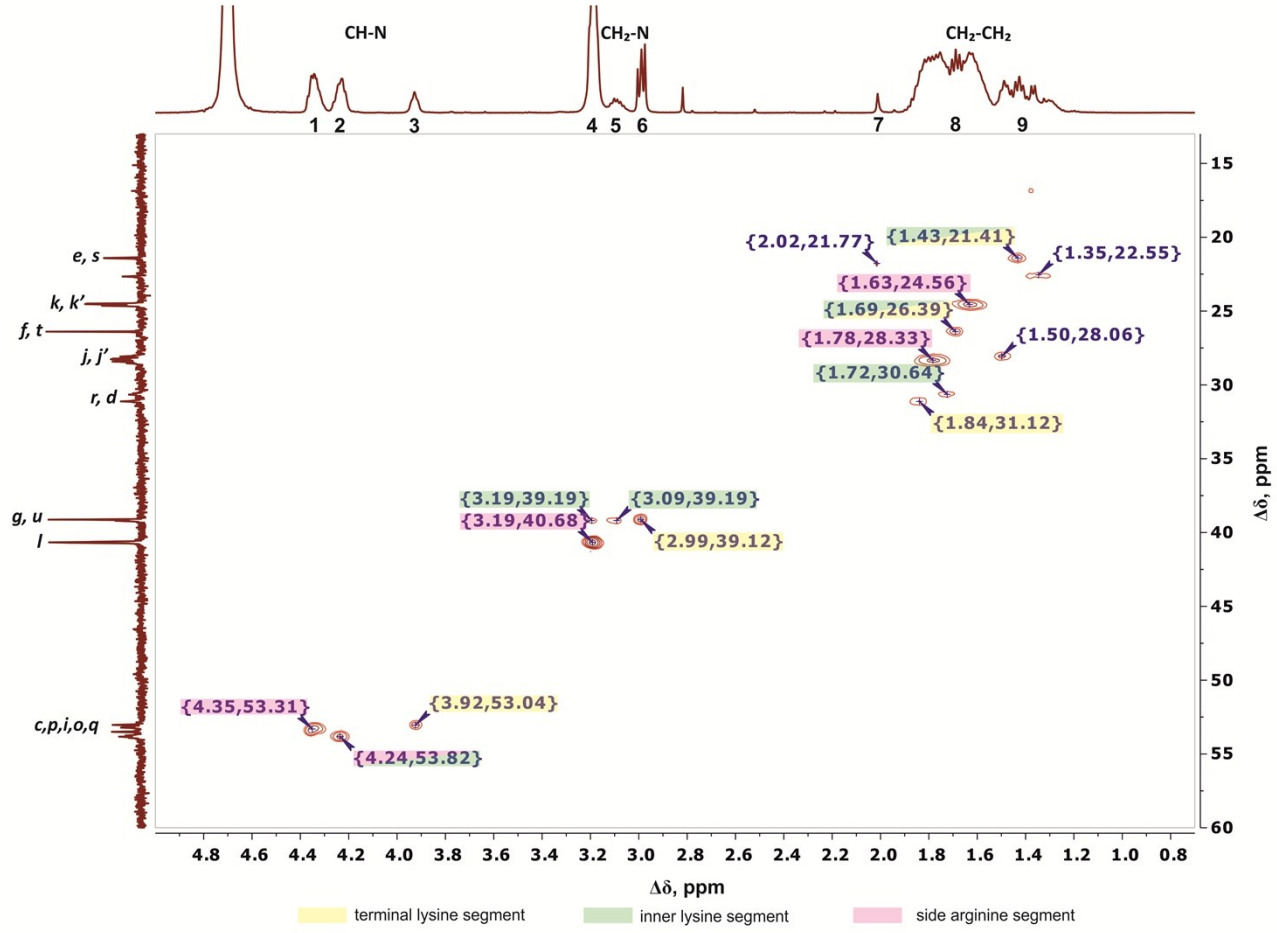

Figure S1. *^{1}H-^{13}C HSQC spectrum of Lys-2Arg dendrimer in the range of 5.0-0.7 ppm at 298K. The letter symbols correspond to the designations of the groups in Fig. 1.*

Cross peaks (3.19; 40.68) (Fig. S1), (3.19; 24.58) and (3.19; 28.26) (Fig. 7) give us information about chemical shifts of carbons in side segments 40.68 ppm, 24.58 and 28.26 ppm (symbols *l, k, j* in Fig. 1 and 3).

The chemical shifts of the carbons in carboxyl groups are given by the following groups of the cross peaks (3.08; 173.06), (3.20; 173.06) and (4.23; 173.06) (Fig. 7). We have defined that the chemical shifts of the protons in the inner groups are 3.09 and 3.19 ppm. Therefore, the cross-peaks (3.08; 173.06) and (3.20; 173.06) indicate a connection between the protons in the inner groups and carboxyl carbons. According to the cross-peak (4.23; 173.06), the protons of the CH groups are connected with this carbons (173.06 ppm). The seven protons in CH groups (symbol *i* in Fig. 2) have a chemical shift equal to 4.23 ppm correspond to this condition. It means that the chemical shift of carbon in the carboxyl group (symbol *h* in Fig. 1 and 3) is 173.06 ppm.

The cross-peaks (4.36; 24.49) and (4.36; 28.08) in Fig. 7 suggest that protons in CH groups (peak 1 in Fig. 3) are connected only with carbons in Arg residues. According to the chemical structure, these protons are in CH groups of the side segments (Fig. 2, blue open circles).

Thus, we conclude that 28 protons in CH groups of the side segments are distributed between peaks 1 and 2 as follows: seven protons (symbol *i* in Fig. 2) give a signal at peak 2 (4.23 ppm); and all other protons (symbol *o* in Fig. 2) contribute to peak 1 (4.34 ppm).

The cross peak (2.99; 39.12) (Fig. S1) indicates chemical shifts of hydrogen and carbon atoms located in the CH_2-(N) groups in the terminal segments (symbol *u* in Fig. 2 and 4). Therefore, the cross-peaks (2.99; 26.43) and (2.98; 21.39) in Fig. 7 give us the chemical shifts of two more carbons in the terminal segments of 26.43 and 21.39 ppm (symbols *t* and *s* in Fig. 2 and 4).

By analogy with the terminal CH_2-(N) groups, we consider that the protons at peak 3 (Fig. 3) with the smallest chemical shift of 3.94 ppm belong to CH groups located in the terminal segments (Fig. 2, red open circles). Then, the cross peaks (4.25; 30.67) and (3.94; 30.95) (Fig. 7) give us chemical shifts of carbon in the inner and terminal segments (symbols *d* and *r* in Figs. 2 and 4), respectively.

The cross peak (4.25; 30.67) (Fig. 7) allow us to conclude that the protons of CH groups at Lys branching points between inner segments (Fig. 2, green open circles) contribute to integral of peak 2 (Fig. 3).

Then, according to ^{1}H-^{13}C HSQC data, the difference between the chemical shifts of the carbons in the inner and terminal CH_2-(N) groups is less 0.1 ppm. We assume that the carbons in CH_2 groups with the symbols *f* and *t* (Fig. 2) have the same chemical shift equal to 26.40 ppm (Fig. 4). Also CH_2 groups with the symbols *e* and *s* (Fig. 2) have the chemical shift equal to 21.40 ppm (Fig. 4).

Also, we should note that in the side segments there are two pairs of CH_2 groups with similar chemical shifts: i) CH_2 groups denoted as *j* and *j'* (Fig. 2); ii) CH_2 groups denoted as *k* and *k'* (Fig. 2). The protons (symbol *o* in Fig. 2) have the cross-peaks (4.36; 24.49) and (4.36; 28.08) (Fig. 7) with the carbons *k'* and *j'*, respectively.

In the schemes below we collected all information about cross-peaks from ^{1}H-^{13}C HMBC and HSQC spectra for the inner segments containing the ε- or α-part of Lys residues (Fig. S2 and S3) and terminal segments (Fig. S4).

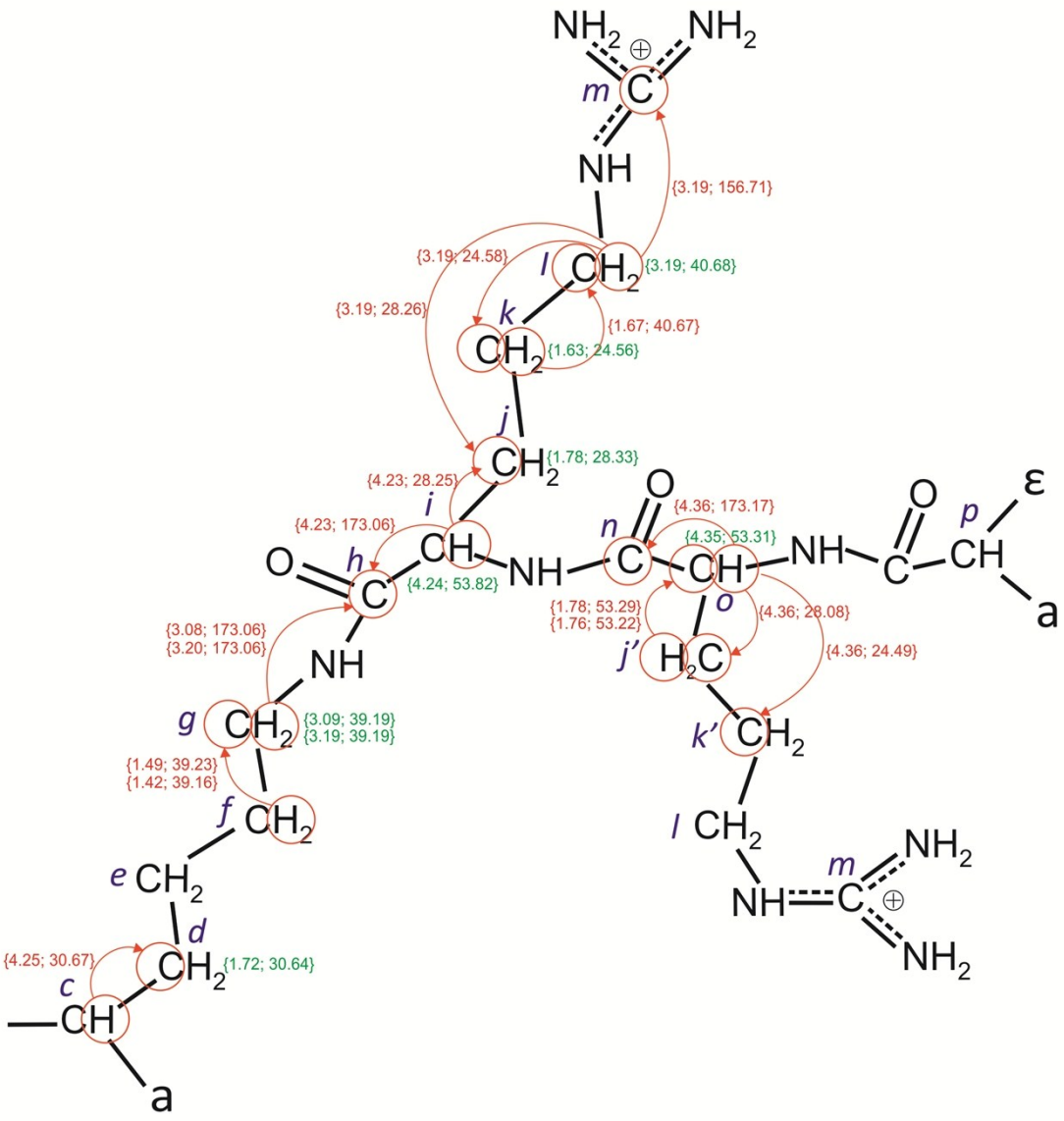

Figure S2. *The map of cross-peaks for the inner segment containing the ε-part of Lys residue. The green and red colors are the cross-peaks from the two-dimensional ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra, respectively. The red arrows show the interaction between hydrogen and carbon nuclei according to the ^{1}H-^{13}C HMBC spectrum.*

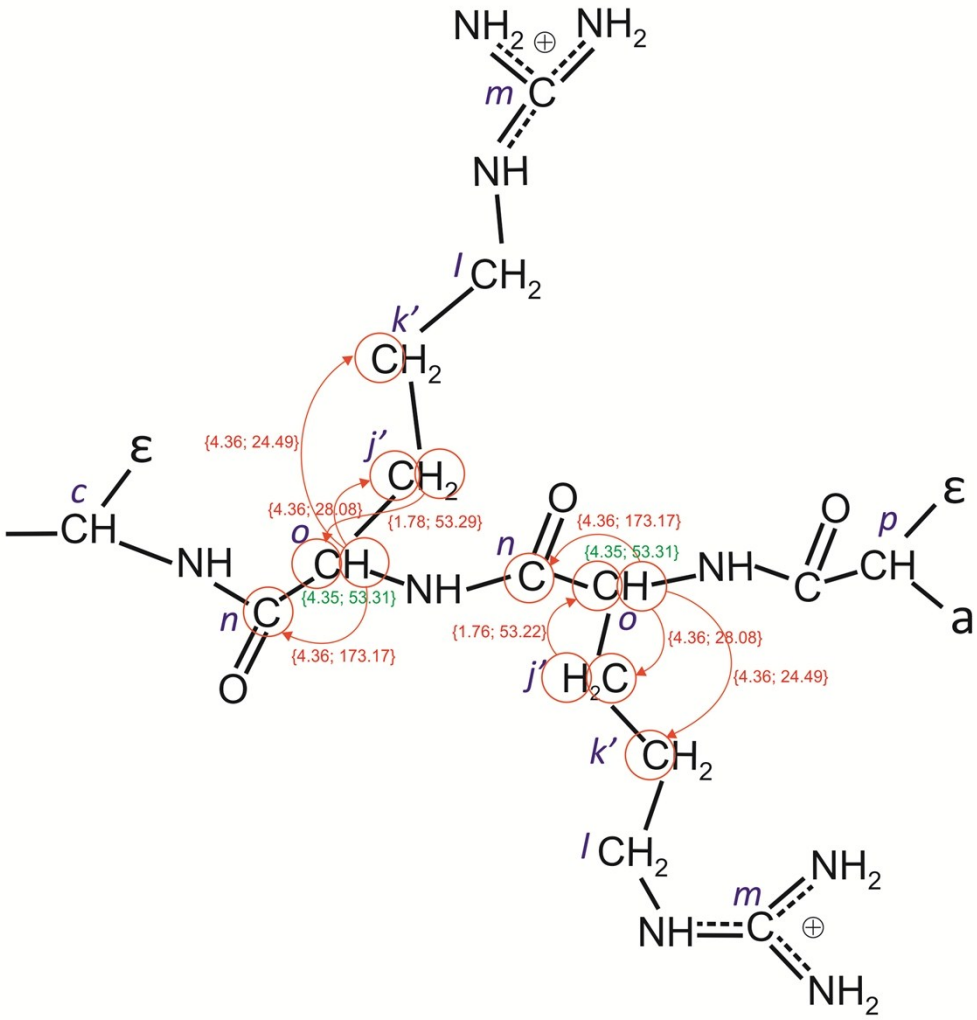

Figure S3. *The map of cross-peaks for the inner segment containing the α-part of Lys residue. The green and red colors are the cross-peaks from the two-dimensional ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra, respectively. The red arrows show the interaction between hydrogen and carbon nuclei according to the ^{1}H-^{13}C HMBC spectrum.*

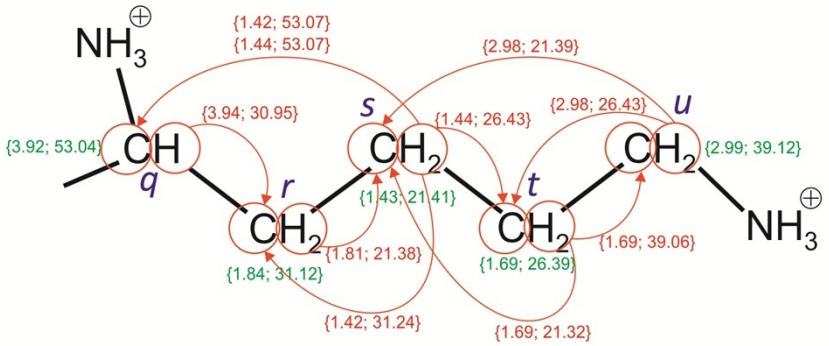

Figure S4. *The map of cross-peaks for the terminal segment. The green and red colors are the cross-peaks from the two-dimensional ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra, respectively. The red arrows show the interaction between hydrogen and carbon nuclei according to the ^{1}H-^{13}C HMBC spectrum.*

Diffusion

The diffusion coefficient was measured for each separate peak in the ^{1}H spectrum (see Fig. 3). The values of diffusion coefficients, *D*, for peaks 1-9 are collected in Tab. S1. To calculate the diffusion coefficient of the dendrimer, we averaged the data in the Tab. S1 taking into account the integral intensities of the peaks which are shown in Tab. 1 in the main text of the article.

Table S1. The diffusion coefficient values of peaks 1-9 in Lys-2Arg dendrimer. The attribution of these peaks is shown in Fig. 3.

Peak name	**Δδ, ppm**	***D**10^{10}, m^2/s**
1	4.34	1.01
2	4.23	1.04
3	3.92	1.03
4	3.19	0.99
5	3.09	1.03
6	2.99	1.06
7	2.01	1.07
8	1.69	0.99
9	1.40	0.97