Polysiloxane based on hydroxyl-containing monomer. Preparation, properties and biomedical application

T. S. Lyubova,[a] N. S. Zakharycheva,[b] E. A. Zakharychev,[b] S. A. Lermontova,[a] E. Yu. Ladilina,[a]★ and L. G. Klapshina[a]

[a]*Razuvaev Institute of Organometallic Chemistry, Russian Academy of Sciences, 49 ul. Tropinina, 603950 Nizhni Novgorod, Russian Federation. Fax: +7 (831) 462 7497. E-mail: eladilina@gmail.com*
[b]*Lobachevsky State University of Nizhny Novgorod, 23 prosp. Gagarina, 603950 Nizhny Novgorod, Russian Federation. Fax: +7 (831) 462 3085*

A method for synthesis of hyperbranched polysiloxane based on *N*-methyl-*N*-(2,3,4,5,6-pentahydroxyhexyl)-*N*′-(3-triethoxysilylpropyl)urea is proposed. In water, the polymer forms nanoparticles capable of holding low soluble luminophores (tetracyanotetraarylporphyrazines) due to non-covalent interaction. Intensively luminescent stable aqueous suspensions based on non-toxic siloxane nanoparticles can be used in bioimaging.

Key words: dynamic light scattering, drug delivery, luminescence, nanoparticles, polysiloxanes.

Nanoparticles that are used as carriers of various drugs and diagnostic agents can be organic or inorganic in their nature.[1] Organic nanoparticles suitable for the delivery of water insoluble drugs can be formed by lipids,[2] biopolymers (such as albumin[3] and chitosan[4]) or synthetic polymers.[1] The essential advantages of the latter are high reproducibility and scalability of their syntheses and also their good safety profiles. The availability of different highly accessible functional groups necessary for the interaction with drugs to be delivered belong to the most important characteristics of the drug delivery systems along with their aqueous solubility and lack of toxicity.[5] Polyfunctional linear polymers are used most widely in biomedicine because of relative simplicity of their synthesis.[6]

An important issue in the development of polymeric nanomaterials for imaging systems is to achieve their solubility and stability.[7] The presence of a large number of amine[4], hydroxyl[4,8] or carboxyl groups[9,10] in the substituents of the main chain ensures good solubility of the polymers in water. The polymer *n*-donor groups such as ether, amine,[4] carbonyl, or amide groups[11] determine efficient interaction of the polymers with drugs. The polymers containing the listed groups are pH-sensitive[3,11,12] and can be used in the diagnosis and treatment of tumors since a more acidic environment is observed near cancer cells due to the specifics of their metabolism.[13]

The most attractive materials for the nanocarriers are organic-inorganic hybrid materials.[14] Nanoparticles with functionalized surfaces can be obtained on the basis of siloxanes of different structure by hydrolysis of trialkoxysilanes containing various organic substituents[11,15] or by their co-hydrolysis with tetraethoxysilane.[16] Previously, we synthesized new organotrialkoxysilanes, on the basis of which it was possible to obtain stable aqueous suspensions with different compounds and, in particular, with metal complexes, which are insoluble or poorly soluble in water.[17,18] It was established that interaction of siloxanes with metal complexes takes place due to the amide groups of the silicon non-hydrolyzable substituent and occurs with elimination of coordination water.

The present study is aimed at preparation and investigation of the structure and properties of siloxane formed upon hydrolysis of *N*-methyl-*N*-2,3,4,5,6-pentahydroxyhexyl-*N*′-(3-triethoxysilylpropyl)urea.

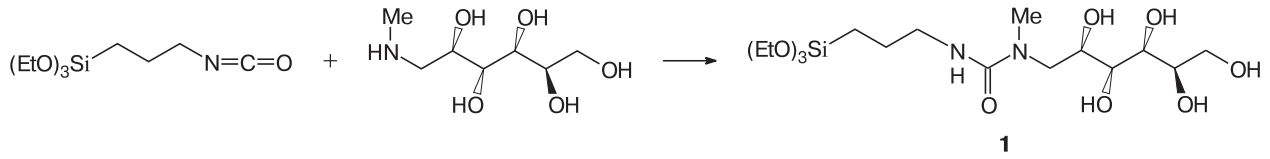
Results and Discussion

Polyfunctional trialkoxysilane containing five hydroxyl groups in the non-hydrolyzable silicon substituent was prepared from isocyanatopropyltriethoxysilane and *N*-methylglucamine (Scheme 1).

Obtained *N*-methyl-*N*-2,3,4,5,6-pentahydroxyhexyl-*N*′-(3-triethoxysilylpropyl)urea (**1**) contains the easily hydrolyzable triethoxysilyl moiety and the carbamide group, which can efficiently solvate organic compounds and metal complexes. The linear substituent at the nitrogen atom with five hydroxyl groups provides the solubility in water of both the monomer itself and functionalized siloxane particles based on it and also can interact with organic compounds and metal complexes, which have low solubility in water. The dissolution of the monomer in water is accompanied by its triethoxysilyl moiety hydrolysis, which occurs slowly (for 5—7 days), but does not require the addition of a catalyst.

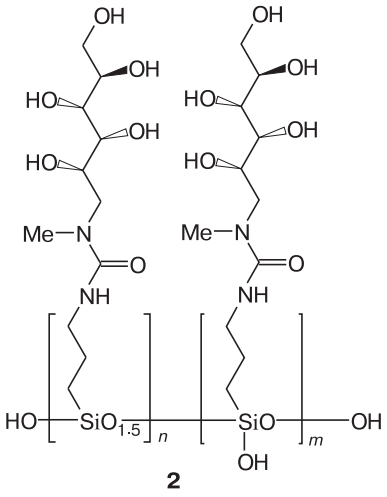
Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 1075—1080, May, 2019.
1066-5285/19/6805-1075

Scheme 1



1

Polymer **2**, which was formed upon hydrolysis, was isolated from the aqueous solution by drying. Obtained polysiloxane is amorphous. The only intensive maximum in its X-ray diffraction pattern lies in the 2θ region 15—25°. Polymer has no regular structure. There are two broad signals at δ 60—70 (Fig. 1) in the ^{29}Si NMR spectrum of polymer **2**. They belong to the T^2 (with the maximum at δ −58) and T^3 fragments (with the maxima at δ −66 and −68).[19,20] Since the signal corresponding to the T^3 fragment has two maxima, one may conclude that the polymeric macromolecule include cyclic and, in particular, cyclolinear and polyhedral[20—22] structures (T_8 and T_{10}). The integral intensity of the signal of the T^3 fragment is three times as large as that of the signals of the T^2 fragment. This fact implies that the ratio between these fragments in polymer **2** is $n : m = 3 : 1$.



2

$n : m = 3 : 1$

Because of insolubility of polysiloxane in THF, its hydroxyl groups were blocked by the interaction with phenyl isocyanate before determination of its molecular weight characteristics. The completeness of the reaction was controlled by the absence of signals of the OH group protons in the ^{1}H NMR spectra. The molecular mass of the obtained sample of the polymer, which does not contain hydroxyl groups, corresponds to 9—11 siloxane links. This leads to the estimated molecular mass of unmodified (initial) sample of 3100—3500 Da.

As can be seen from Fig. 2, the dynamic viscosity of aqueous solutions of polysiloxane varies little with increasing its concentration and is close to the water viscosity at low concentrations. The hyperbranched structure of the macromolecules can be a reason of this fact since it is known that hyperbranched polymers have well solubility, while their solutions have low viscosities.[23] The degree of branching determined for polymer **2** from the ^{29}Si NMR data[24] is 0.75, which is rather large for this type of polymers.[23]

The analysis of an aqueous solution of polymer **2** by the dynamic light scattering (DLS) showed that it contained nanoparticles, size distribution of which had bimodal character with a main maximum at 100 nm (Fig. 3). It can be assumed that these particles are micelles, the shell of which consists of polar groups (carbamide and pentahydroxyhexyl), and the core includes siloxane chains. The relative width of the distribution at half maximum is 78% (see Fig. 3). The contribution into the second mode is made by a small part of strongly scattering particles as

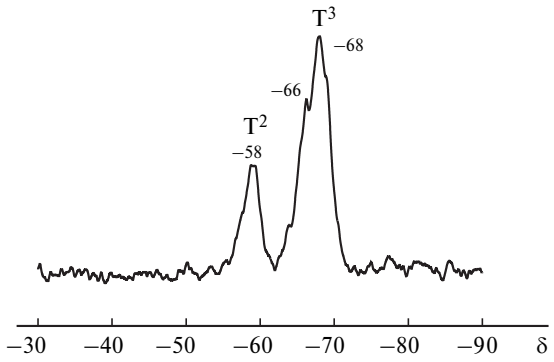


Fig. 1. ^{29}Si NMR spectrum of polysiloxane **2**.

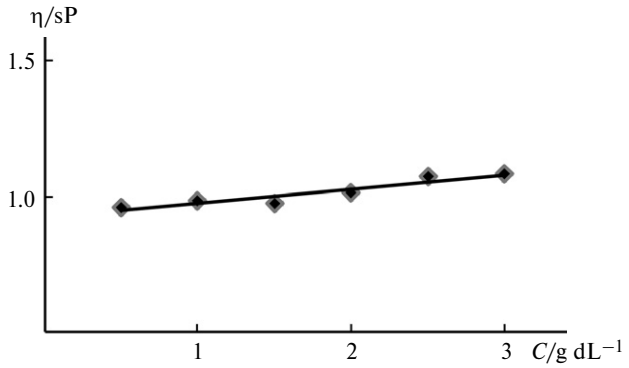


Fig. 2. Dependencies of dynamic viscosity of aqueous solutions of polysiloxane **2** on its concentration.

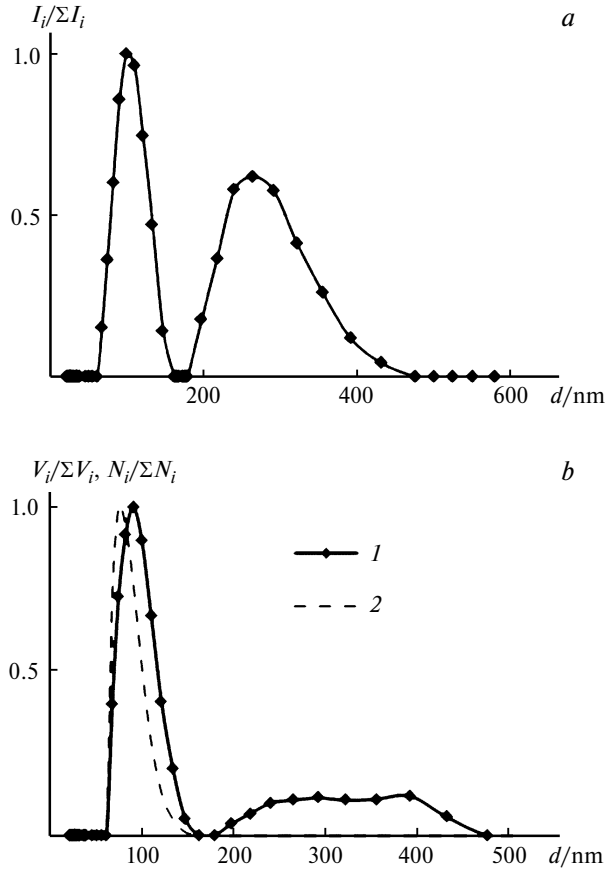
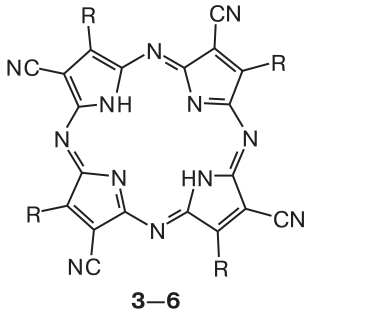


Fig. 3. Distributions by scattering intensity (*a*), by volume (*b,* curve *1*) and a number of particles (*b*, curve *2*), which were obtained from correlation function measurements for polysiloxane **2**.

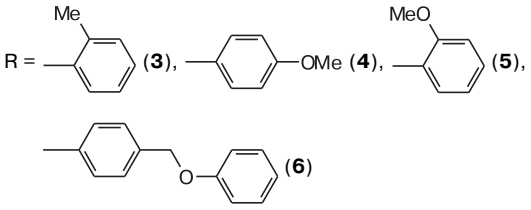
is demonstrated by the data shown in Fig. 3, *b*. The tendency, albeit not large, of polysiloxane particles towards association is confirmed by the boundary value of their zeta potential (–29 mV). However, formation of larger particles and sedimentation of polysiloxane over time were not observed. Its aqueous solutions can exist unchanged for several years.

We tested the applicability of obtained polymer **2** for drug delivery in the diagnosis and treatment of tumors using the photodynamic therapy (PDT). It is known that luminophores such as porphyrazines can be successfully used as photosensitizers in PDT.[25] Therefore we investigated interaction of nanoparticles of siloxane **2** with porphyrazines **3**—**6**.

Various non-covalent interactions between the drug and carrier can occur in the drug delivery systems.[26,27] The interaction of nanoparticles of polysiloxane **2** with porphyrazines, apparently, takes place due to formation of numerous hydrogen bonds between polymer functional groups and luminophore. As is known,[17,28] the interaction of the compounds of this type with polymers in water results in the increase in their luminescence intensity. In our case, a considerable increase in the intensity of luminescence of porphyrazines **3**—**6** was also observed on



3—**6**



going from their aqueous solutions to nanoparticle suspensions (Table. 1). This increase depended on the nature and position of the substituent in the benzene ring. The increase in the luminescence intensity was stronger for porphyrazine **4** with the methoxy substituent in *para*-position than for compound **5** with the same substituent in *ortho*-position. It can be connected with formation of hydrogen bonds by amide and hydroxyl groups of polymer not only with the nitrile groups and central nitrogen atoms of macrocycles, but also with oxygen containing substituents of porphyrazines, with the latter interaction being most effective with substituents located in *para*-position. The π-system of the aryl substituent is involved in the non-covalent interaction too[29] since a significant luminescence increase was also observed for porphyrazine **3**, which has no oxygen containing moiety. The maximum luminescence increase was detected for porphyrazine **6** (see Table 1). This indicates the largest interaction of this compound with the functionalized surfaces of nanoparticles of polysiloxane **2** in water due to the presence in its structure of the largest number of aromatic rings in addition to *n*-donor oxygen atoms.

The dependence of the luminescence intensity on the concentration of polysiloxane **2** was studied for lumino-

Table 1. Luminescence intensity changes for porphyrazines **3**—**6** in suspensions (I_s) in comparison with their aqueous solutions (I_w) (λ_{ex} = 590 nm)

Porphyrazine	$C \cdot 10^6$ /mol L^{-1}	λ_{max} /nm	I_s	I_w	I_s/I_w
			(arb. unit)		
3	7.7	637	160	45	3.6
4	7.1	646	69	12	5.8
5	7.9	648	65	27	2.4
6	8.7	658	122	5	24.4

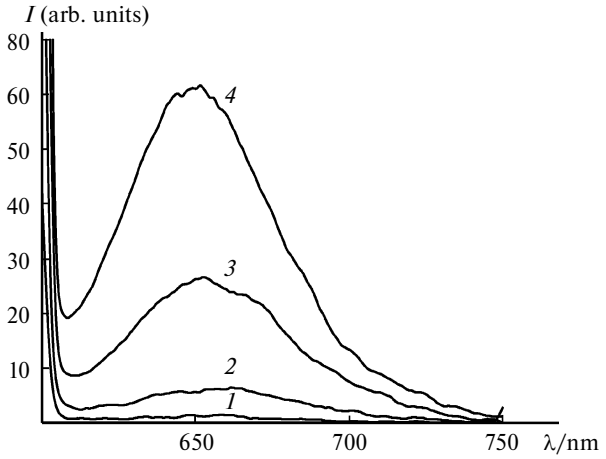


Fig. 4. Luminescence spectra of porphyrazine **6** in water (*1*) and in solutions of polysiloxane **2** with the concentrations of the latter of 0.1 (*2*), 0.5 (*3*) and 1% (*4*).

phore **6**. As is seen from the spectra shown in Fig. 4, it increases with increasing the polymer content in the solution. It is known that for the compounds of the considered type, the luminescence intensity can depend on the medium viscosity. At the same time, the viscosity of polymer **2** solutions only slightly differs from the water viscosity and insignificantly changes with the increase in its concentration (see Fig. 2). Therefore, the observed substantial luminescence intensification can only be due to an interaction of luminophore **6** with the polymer, *i.e.*, due to the local viscosity increase inside nanoparticles containing porphyrazine.

The luminescence intensity enchancement was also observed when the content of the luminophore relative to that of polysiloxane was increased. It was established for compound **4** that this takes place up to a ratio of $3 \cdot 10^{-6}$ mmol of luminophore **4** to 14.5 mg of polymer **2**. At this ratio, the complete saturation of siloxane particles with luminophore is achieved, in which no interaction of luminophore molecules with each other occurs. Further addition of porphyrazine to the polysiloxane solution results in a decrease in its luminescence intensity, apparently, due to concentration quenching.

The fact of participation of peripheral functional groups of polysiloxane in the interaction with porphyrazine was also confirmed by the DLS study of the polymer nanoparticles doped with luminophore **6** in water. As can be seen from the particle distributions by the scattering intensity and also by their volumes and numbers, which are shown in Fig. 5, the propensity of the particles of the doped polymer to formation of associates remains, however the sizes of both particles themselves (71 *vs.* 99 nm) and their aggregates (175 *vs.* 264 nm) are smaller than for non-doped polysiloxane. At the same time, the contribution of associates to light scattering for the doped polymer occurs to a much greater degree in spite of that the portion of

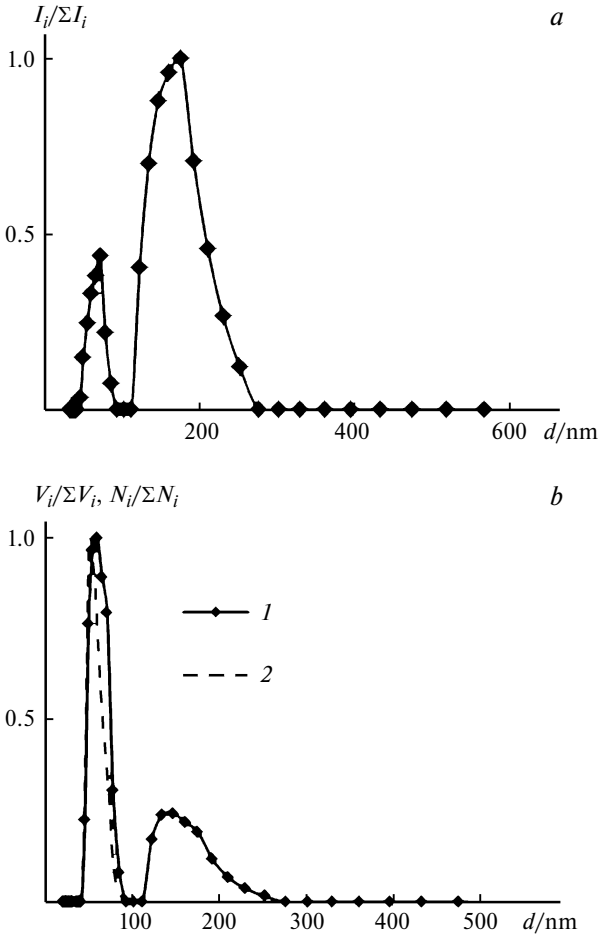


Fig. 5. Distributions by scattering intensity (*a*), by volume (*b,* curve *1*) and a number of particles (*b*, curve *2*), which were obtained from correlation function measurements for polysiloxane **2** doped with porphyrazine **6**.

aggregated particles is small in general, and these particles are completely absent in the numerical distribution.

In the course of the study of the luminescence of polymer particles doped with porphyrazine **4** in the presence of blood serum or serum albumin, it was found that the increase in the luminescence intensity is stronger in the second case. It indicates the efficient interaction of polymer particles loaded with luminophore with proteins due to the formation of strong hydrogen bonds between the latter and amide and hydroxyl groups of the side substituents in polysiloxane **2**. We already observed a similar effect for another water-soluble polysiloxane.[17]

A common method of assessing the cytotoxicity of a drug is to measure its concentration causing a decrease in cell growth (or cell death) by 50% (IC_{50}). As studies on cells of human epidermoid carcinoma A431 show, there is no significant reduction in cell growth when applying polysiloxane **2** to its concentration of 5 mg mL^{-1}. Therefore, the polymer in this concentration is not cytotoxic and can be used as a carrier for delivery of diagnostic agents

and drugs and, in particular, luminophores for bioimaging and PDT. The absence of toxicity is determined by the chemical inertness of polysiloxane, as well as by the specifics of the method for its synthesis,[1] which does not involve the use of any organic solvents and, therefore, makes it possible to use the polymer aqueous solutions without the separation stage or an additional purification.

Thus, on the basis of a monomer containing five hydroxyl groups, we obtained a hyperbranched water-soluble non-toxic polysiloxane in a single-step "green" method. In water, its particles, which are smaller than 100 nm in size, are capable to retain luminophores due to non-covalent interactions. Therefore, it is possible to prepare intensively luminescent aqueous suspensions based on it. Despite the absence of covalent binding with a dye, the polymer can be used as a carrier for luminescent compounds in bioimaging.

Experimental

IR spectra were recorded using a Fourier IR spectrometer FSM-1201 (Monitoring Ltd., Russian Federation) in vaseline oil between KBr plates for samples in the form of suspensions or as films on ZnSe plates for samples of polymers. A Bruker Avance DPX-200 spectrometer (200 MHz for ^{1}H) and a Bruker Avance III spectrometer (79.5 MHz) were used to obtain ^{29}Si NMR spectra, respectively. The spectra were recorded at 25 °C; chromium(III) acetylacetonate was added to samples to reduce their relaxation times; Me_4Si was used as an internal standard. Hydrodynamic diameters of nanoparticles in water were measured by DLS using multimodal analysis of the correlation function with the use of a NanoBrook Omni analyzer (Brookhaven Instruments). The particle sizes were measured in polystyrene cuvettes (1 cm) at an angle of 90° at 25 °C. Zeta potential was obtained by phase analysis light scattering (PALS). The Hückel model was used to convert the electrophoretic mobility into the zeta potential. A Perkin-Elmer LS-55 fluorimeter was used in the studies of solution luminescence. The X-ray phase analysis was carried out using the filtered CuKα radiation (λ = 1.54178 Å) with a Shimadzu XRD-6000 X-ray diffractometer. The molecular weight characteristics of the polymer were measured using a Knauer Smartline chromatograph equipped with Phenogel Phenomenex column (300×7.8 mm, 5 μm) and UV detector (λ = 254 nm). Calibration was carried out using polystyrene standards with molecular masses in the range from 3420 to 2570000, THF was used as a mobile phase with flow rate of 2 mL min^{-1}. Viscosities of polymer aqueous solutions were measured by an Ostwald viscometer at 25 °C, the characteristic viscosity was obtained by the dilution procedure. Densities of polysiloxane solutions were measured using a pycnometer of 0.35 mL in volume.

N-Methyl-D-glucamine (99%, Acros Organics), 3-triethoxysilylpropyl isocyanate (95%, Aldrich), and phenylisocyanate (≥99%, Sigma-Aldrich) were used without additional purification. Porphyrazines **3**[30], **4**, **5**,[31] and **6**[32] were synthesized according to the described methods.

Cell culture viability was assessed using the MTT assay.[33] The cells were placed in a 96-well plate by four thousand per a well and incubated for 12 h. Then the nutrient medium was replaced with 100 μL of a medium containing the polymer in a certain concentration, the cells were incubated for 4 h, and the medium in the wells was replaced with a fresh nutrient medium. In 24 h after incubation with polymer, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT, Alfa Aesar, GB) was added to the medium in the wells to get its final concentration of 0.5 mg mL^{-1}, and the cells were further incubated for 4 h. Then the medium was removed, whereas the crystals of formed colored MTT-formazan were dissolved in 100 μL of dimethyl sulfoxide. The optical density of the content of each well was measured using a Synergy MX microplate reader (BioTek, USA) at the wavelength of 570 nm. The cell viability was assessed from the ratios of the optical densities of the formazan solution from each well and a control sample.

***N*-Methyl-*N*-(2,3,4,5,6-pentahydroxyhexyl)-*N*′-(3-triethoxysilylpropyl)urea (1).** A solution of 2.00 g (8.09 mmol) of 3-triethoxysilylpropyl isocyanate in 25 mL of THF was added dropwise to a suspension of 1.59 g (8.09 mmol) of *N*-methylglucamine in 25 mL of THF for 30 min under stirring. The reaction mixture was stirred for 2 h under warming (80—90 °C), then THF was removed *in vacuo*. The residue was washed with hexane. This afforded 3.45 g (99%) of white powdered compound **1**. Found (%): C, 42.11; H, 8.15; Si, 5.14. $C_{17}H_{38}N_2O_9Si$. Calculated (%): C, 46.14; H, 8.65; Si, 6.35. IR, ν/cm^{-1}: 3330, 3271 (NH, OH); 2977, 2933, 2926 (CH); 1700, 1608, 1545 (C=O, NH); 1104 (C—O—H); 1080 (Si—O—C); 953, 775, 723 (Si—O—Et). ^{1}H NMR ($(CD_3)_2CO$), δ: 0.61—0.58 (m, 2 H, C$\underline{H}_2$Si); 1.18 (t, 9 H, C$\underline{H}_3$CH$_2$, $^3J_{H,H}$ = = 7.0 Hz); 1.61—1.56 (m, 2 H, CH$_2$C$\underline{H}_2$CH$_2$); 2.96 (s, 3 H, MeN); 3.15—3.12 (m, 2 H, NHC$\underline{H}_2$); 3.45—3.42 (m, 2 H, MeNC$\underline{H}_2$); 3.61—3.59 (m, 2 H, C$\underline{H}_2$OH); 3.73—3.69 (m, 4 H, C$\underline{H}$(OH)); 3.80 (q, 6 H CH$_3$C$\underline{H}_2$, $^3J_{H,H}$ = 7.0 Hz); 3.92—3.90 (m, 2 H, CH(O$\underline{H}$)); 4.09—4.07 (m, 1 H, CH(O$\underline{H}$)); 4.20—4.18 (m, 1 H, CH(O$\underline{H}$)); 4.86 (br.d, 1 H, CH$_2$O$\underline{H}$); 5.98 (br.t, 1 H, $\underline{H}$NCH$_2$). ^{29}Si NMR (DMSO-d$_6$), δ: −45.

Hydrolysis of 1. Compound **1** (0.81 g, 1.83 · 10^{-3} mol) was added to 81.84 g of water under stirring. The solution was kept for one weak. A transparent solid film of polysiloxane **2** was obtained by drying. The prepared polymer is soluble in water and methanol (ethanol) containing water. Polysiloxane **2** was obtained as white powder by mechanical removal of its film from the plate where it was produced. IR, ν/cm^{-1}: 3358 (NH, OH); 2937, 2878 (CH); 1604, 1547 (C=O, NH); 1398 (CH); 1084 (COH); 1057 (SiOSi). ^{1}H NMR (D$_2$O), δ: 0.59—0.57 (m, 2 H, C$\underline{H}_2$Si); 1.51—1.49 (m, 2 H, CH$_2$C$\underline{H}_2$CH$_2$); 2.85 (br.s, 4 H, C$\underline{H}$(OH)); 3.08—3.06 (m, 2 H, NHC$\underline{H}_2$); 3.67—3.63 (m, 5 H, $\underline{Me}$NC$\underline{H}_2$); 3.91—3.89 (m, 2 H, C$\underline{H}_2$OH); 4.57 (br.s, 1 H, $\underline{H}$NCH$_2$); 4.81 (br.s, 5 H, OH). ^{29}Si NMR (DMSO-d$_6$), δ: −58 (T^2);−66, −68 (T^3).

Blocking of hydroxyl groups in polysiloxane 2. Polysiloxane **2** (0.083 g, 2.5 · 10^{-4} mol) was dissolved in 2.8 g of DMSO and added to 0.304 g (2.5 · 10^{-3} mol) of phenylisocyanate under stirring. The reaction mixture was stirred for 24 h. DMSO was removed from the mixture *in vacuo*. The molecular mass of the obtained polymer without hydroxyl groups was $M_w : M_n$ = = 9900 : 8700, polydispersity index was 1.14. It corresponds to 9—11 siloxane links and to molecular mass of starting non-modified polysiloxane of $M_w : M_n$ = 3100 : 3500.

Preparation of luminescent aqueous suspensions. A sample (0.005—0.002 g) of porphyrazine or a metal complex was dissolved in small amount of ethanol. A 1% aqueous solution of polymer **2** was added to the prepared ethanol solution. The obtained suspensions were diluted with distilled water if necessary and used in measurements of luminescence spectra.

The work was performed within the framework of the state task of the Razuvaev Institute of Organometallic Chemistry of RAS (IOMC RAS) with the use of equipment of the centers of collective usage "Analytical centre of IOMC RAS" and "New Materials and Resource-saving Technologies" of the Research Institute for Chemistry of Lobachevsky State University of Nizhni Novgorod.

This work in part was financially supported by the Russian Science Foundation (Project No. 18-73-00194; porphyrazine syntheses).

References

1. G. Chen, I. Roy, C. Yang, P. N. Prasad, *Chem. Rev.*, 2016, **116**, 2826.
2. W. Al-Jamal, K. Kostarelos, *Nanomedicine*, 2007, **2**, 85.
3. N. Sh. Lebedeva, E. S. Yurina, Y. A. Gubarev, O. I. Koifman, *Mendeleev Commun.*, 2017, **27**, 47.
4. N. E. Sedyakina, N. B. Feldman, S. V. Lutsenko, G. V. Avramenko, *Mendeleev Commun.*, 2018, **28**, 598.
5. T. M. Allen, P. R. Cullis, *Science*, 2004, **303**, 1818.
6. N. Kamaly, Z. Xiao, P. M. Valencia, A. F. Radovic-Moreno, O. C. Farokhzad, *Chem. Soc. Rev.*, 2012, **41**, 2971.
7. R. Srikar, A. Upendran, R. Kannan, *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, 2014, **6**, 245.
8. O. V. Nazarova, Yu. I. Zolotova, A. V. Dobrodumov, I. I. Malakhova, E. N. Vlasova, E. F. Panarin, *Russ. J. Appl. Chem.*, 2009, **82**, 1600].
9. O. V. Nazarova, M. L. Levit, T. N. Nekrasova, N. G. Bel´nikevich, A. V. Dobrodumov, E. F. Panarin, *Polym. Sci., Ser. B* (*Engl. Transl.*), 2009, **51**, 327 [*Vysokomolekulyar. Soedineniya. Ser. B*, 2009, **51**, 1671].
10. A. V. Yakimansky, T. K. Meleshko, D. M. Ilgach, M. A. Bauman, T. D. Anan´eva, L. G. Klapshina, S. A. Lermontova, I. V. Balalaeva, *J. Polym. Sci., Part A: Polymer Chemistry*, 2013, **51**, 4267.
11. G. A. Sarychev, M. S. Mironova, U. A. Budanova, Y. L. Sebyakin, *Mendeleev Commun.*, 2017, **27**, 155.
12. X. Hu, Y. Zhang, Z. Xie, X. Jing, A. Bellotti, Z. Gu, *Biomacromolecules*, 2017, **18**, 649.
13. B. Faubert, K. Y. Li, L. Cai, C. T. Hensley, J. Kim, L. G. Zacharias, C. Yang, Q. N. Do, S. Doucette, D. Burguete, H. Li, G. Huet, Q. Yuan, T. Wigal, Y. Butt, M. Ni, J. Torrealba, D. Oliver, R. E. Lenkinski, C. R. Malloy, J. W. Wachsmann, J. D. Young, K. Kernstine, R. J. De Berardinis, *Cell*, 2017, **171**, 358.
14. J. A. O. Junior, M. P. Abuçafy, E. B. Manaia, B. Lallo da Silva, B. G. Chiari-Andréo, L. A. Chiavacci, *Polymers*, 2016, **8**, 91.
15. L. John, M. Malik, M. Janeta, S. Szafert, *RSC Adv.*, 2017, **7**, 8394.
16. C. Sanchez, P. Belleville, M. Popalld, L. Nicole, *Chem. Soc. Rev.*, 2011, **40**, 696.
17. E. Yu. Ladilina, T. S. Lyubova, Yu. P. Klapshin, M. A. Lopatin, V. V. Semenov, G. A. Domrachev, *Dokl. Phys. Chem.* (*Engl. Transl.*), 2013, **453**, 282 [*Dokl. AN*, 2013, **453**, 634].
18. E. Yu. Ladilina, S. A. Lermontova, L. G. Klapshina, N. S. Zakharycheva, Yu. P. Klapshin, G. A. Domrachev, *Dokl. Phys. Chem.* (*Engl. Transl.*), 2018, **478**, 1 [*Dokl. AN*, 2018, **478**, 48].
19. S. Chen, D. Zhuo, J. Hu, *Industrial & Engineering Chemistry Research*, 2018, **57**, 10372.
20. S.-S. Choi, A. S. Lee, H. S. Lee, K.-Y. Baek, D. H. Choi, S. S. Hwang, *Macromol. Research*, 2011, **19**, 261.
21. S. Chimjarn, R. Kunthom, P. Chancharone, R. Sodkhomkhum, P. Sangtrirutnugula, V. Ervithayasuporn, *Dalton Trans.*, 2015, **44**, 916.
22. M. Janeta, Ł. John, J. Ejfler, T. Lis, S. Szafert, *Dalton Trans.*, 2016, **45**, 12312.
23. Y. Zheng, S. Li, Z. Wenga, C. Gao, *Chem. Soc. Rev.*, 2015, **44**, 4091.
24. M. Jikei, M.-A. Kakimoto, *Prog. Polym. Sci.*, 2001, **26**, 1233.
25. S. A. Lermontova, I. S. Grigor´ev, E. Yu. Ladilina, I. V. Balalaeva, N. Yu. Shilyagina, L. G. Klapshina, *Russ. J. Coord. Chem.*, 2018, **44**, 301.
26. K. Ulbrich, K. Holá, V. Šubr, A. Bakandritsos, J. Tuček, R. Zbořil, *Chem. Rev.*, 2016, **116**, 5338.
27. J. Ding, L. Chen, C. Xiao, L. Chen, X. Zhuanga, X. Chen, *Chem. Commun.*, 2014, **50**, 11274.
28. L. G. Klapshina, W. E. Douglas, I. S. Grigoryev, E. Yu. Ladilina, M. V. Shirmanova, S. A. Mysyagin, I. V. Balalaeva, E. V. Zagaynova, *Chem. Commun.*, 2010, **46**, 8398.
29. Q.-S. Du, Q.-Y. Wang, L.-Q. Du, D. Chen, R.-B. Huang, *Chem. Central. J.*, 2013, **7**, 2.
30. S. A. Lermontova, N. Y. Shilyagina, N. N. Peskova, I.V. Balalaeva, I. S. Grigoryev, L. G. Klapshina, M. V. Shirmanova, *Russ. J. Gen. Chem.* (*Engl. Transl.*), 2016, **86**, 1330.
31. RF Pat. 2665471 C1, 2017.
32. S. A. Lermontova, I. S. Grigoryev, N. N. Peskova, E. Yu. Ladilina, I. V. Balalaeva, L. G. Klapshina, V. P. Boyarskii, *Russ. J. Gen. Chem.*, 2017, **87**, 479.
33. R. J. Freshney, *Culture of Animal Cells: A Manual of Basic Technique*, Wiley-Liss, 1987, 397 pp.

Received December 29, 2018;
in revised form February 21, 2019;
accepted March 20, 2019