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Synthesis, Antibacterial Activity, and Cytotoxicity of Azido-Propargyloxy 1,3,5-Triazine Derivatives and Hyperbranched Polymers

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Abstract: A new method for the synthesis of azido-propargyloxy derivatives of 1,3,5-triazine has been developed utilizing the nitrosation of hydrazyno-1,3,5-triazines. New hydrazines (2-hydrazino-4,6-bis(propargyloxy)-1,3,5-triazine and 2,4-dihydrazino-6-propargyloxy-1,3,5-triazine) were synthesized and characterized via FTIR, NMR spectroscopy and elemental analysis. The hyperbranched polymers with azide (diazide monomer) and propargyloxy terminal groups were obtained via the azide-alkyne polycycloaddition reaction of diazide and monoazide AB_2-type monomers. The antibacterial activity against *Escherichia coli* bacteria of 2,4,6-trispropargyloxy-1,3,5-triazine, 2-azido-4,6-bispropargyloxy-1,3,5-triazine, and 2,4-diazido-6-propargyloxy-1,3,5-triazine and their hyperbranched polymers was studied. Only 2,4-diazido-6-propargyloxy-1,3,5-triazine has weak antibacterial activity in comparison with ampicillin. The cytotoxicity of these compounds against M-HeLa, FetMSC, and Vero cell lines was also studied. 2,4,6-trispropargyloxy-1,3,5-triazine does not show any cytotoxic effect ($IC_{50} \geq 280$ μM). It was shown that the presence of an azide group in the compound directly affects the cytotoxic effect. Hyperbranched polymers have a less cytotoxic effect against M-HeLa ($IC_{50} > 100$) in comparison with monomers (IC_{50} = 90–99 μM). This makes it possible to use these polymers as the basis for biocompatible materials in biomedical applications.

Keywords: 1,3,5-triazine; nucleophilic substitution; azido-acetylene cycloaddition; hyperbranched polymers; antibacterial activity; cytotoxicity; M-HeLa; Vero; FetMSC; *E. coli*

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1. Introduction

Nowadays, the search for new compounds for medico-biological applicants is an urgent task. Along with small molecules, polymer compounds are being actively studied as potential bioactive compounds [1–6].

Hyperbranched polymers (HBPs) are a class of compounds with globular and three-dimensional irregular macromolecular structures, which provide unique physical and chemical properties. The sizes of macromolecules vary from several to tens of nanometers and are characterized by a high density of functional groups, low viscosity, and high solubility compared to linear and branched polymers of a similar structure [7]. A large number of functional terminals allow them to modify HBPs to tune their physicochemical properties or construct complex molecular structures of the core–shell type [8–10]. Despite the similarity of the structure and properties of HBP with dendrimers, their synthesis is much simpler to scale [11].

There are several approaches to obtaining HBPs [12]. A widely used strategy for producing HBP is the polyaddition or polycondensation of AB_n-type monomers ($n \geq 2$) [13–16].

This approach makes it possible to obtain polymers with high molecular weights without the gelation process [17,18]. Interest in this approach has grown significantly with the development of click reactions with simplicity, reaction robustness, and a high atom economy [12]. Azide-alkyne cycloaddition is a typical click reaction that allows HBPs to be easily obtained using a thermally induced reaction (Huisgen Cycloaddition, AAC) [19] or a copper-catalytic reaction (CuAAC) [20].

Previously, we demonstrated the synthesis of triazine–triazole HBPs in bulk using a simple thermal azide-alkyne cycloaddition of 1,3,5-triazine monomers of the AB_2 type [21,22]. Despite the convenience and simplicity of obtaining HBPs via the polycycloaddition of AB_2-type monomers, it is necessary to develop effective methods for the preparation of monomers that are stable during synthesis and storage. The proposed technique should be simple, accessible, and provide a sufficiently high yield of the target products.

The unique structural properties and accessibility of HBPs explain the active investigations of their properties for use in biological and medical applications as fluorescent polymers [23], nanocontainers for drug delivery to cancer tumors [24], photosensitizers for photodynamic therapy [25], and nanomaterials for cancer diagnostics [26].

On the one hand, it has been shown that polymers based on 1,3,5-triazine have low cytotoxicity towards various cell lines with the standard drug doxorubicin [27–29] and might also prove to be useful for drug delivery, applied to gene delivery [30,31] and other bioapplications [32]. On the other hand, organic azido compounds are found to be efficient antibacterials, but their toxicity strongly limits their use as antimicrobial compounds [33]. However, there are some examples of the application of azido polymers as agents in medicinal chemistry [34–36].

Being inspired by these findings from the literature, we hypothesize that the HBP obtained via the cycloaddition reaction of azido-acetylene 1,3,5-triazine monomers of the AB_2 type have a low cytotoxic effect and are able to be used for drug delivery. Moreover, the azido derivatives of 1,3,5-triazine and HBP with azido terminal groups have antibacterial activity.

This work aimed to optimize the method of the synthesis of the azido-propargyloxy-substituted 1,3,5-triazines, the synthesis of HBPs, and the study of the cytotoxicity and antibacterial activity of obtained compounds to assess their applicability in biomedical applications.

2. Materials and Methods

2.1. General Information

Commercially available reagents (cyanurchloride, 2-propynol, hydrazine hydrate, NaN_3, $NaNO_2$, $NaHCO_3$, NaOH, HCl) and solvents (acetone, isopropanol, DMSO, CH_2Cl_2) (Sigma Aldrich, Burlington, MA, USA; Acros organics, Morris Plains, NJ, USA; Fluka Chemie AG, Buchs, Switzerland) were used without preliminary purification, Na_2SO_4 was calcined, and THF was dried over KOH and distilled over CaH_2. The melting point was measured on a Boetius-type heating table with a heating rate of 4 °C·min^{-1} at the melting point. IR spectra were recorded on an Alpha Bruker FT-IR spectrometer; solid samples were prepared in the form of KBr tablets; the IR spectra of solutions were recorded in dismountable CaF_2 cuvettes. The NMR spectra were recorded on a superconducting pulsed broadband two-channel NMR spectrometer AVANCE III 500 MHz Bruker BioSpin for liquid-phase samples at 500 MHz (^{1}H spectra) and 126 MHz (^{13}C spectra) for samples in DMSO-d^6 and $CDCl_3$ with 1% tetramethylsilane (TMS). Elemental analysis was performed on a CHNS/O elemental analyzer "Vario Micro cube" Elementar GmbH. The optical density of MTT-formazan dissolved in DMSO was measured on an EFOS 9305 immunoenzyme tablet photometer (JSC "MZ "Sapphire", Moscow, Russia) at a wavelength of 570 nm. Nonspecific sorption was measured at a wavelength of 650 nm.

*2.2. Synthesis of 2,4,6-Tripropargyloxy-1,3,5-triazine (**3**)*

In a 250 mL three-necked flask, 10.00 g (54 mmol) of cyanuric chloride and 10.12 g (180 mmol) of 2-propynol (molar ratio of 2-propynol: cyanuric chloride = 1:3.33) were mixed in 130 mL of acetone at 20 °C. After complete dissolution, the mixture was cooled to 0–2 °C. A 5% NaOH solution was added to the mixture, adjusting its supply so that the temperature of the mixture did not rise above 5 °C. The completion of the reaction was checked using Belstein's test (the absence of chlorine in the sample of the substance). After the reaction was complete, the solvent was removed under reduced pressure, and the resulting white precipitate was washed with water and dried on air. Yield 8.90 g (69%). Colorless crystals, mp. 79–80 °C (*i*-PrOH). IR spectrum (KBr tablet), ν, cm^{-1}: 3267 (≡CH), 2965, 2938 (stretching vibrations of CH_2), 2135 (-C≡CH), 1571 (stretching vibrations of C-N triazine), 1452 (deformation CH_2 vibrations), 1135, 1332 (stretching vibrations of C-O-C), 816 (bending vibrations of C-N triazine). ^{1}H NMR spectrum (500 MHz, DMSO-d^6), δ, ppm. (J, Hz): 3.64 (3H, t, J = 2.4, CH); 5.06 (6H, d, J = 2.4, CH_2). ^{13}C NMR (126 MHz, $CDCl_3$), δ, ppm: 172.46; 77.16; 75.95; 55.94. Found, %: C 59.08; H 3.86; N 17.02. $C_{12}H_9N_3O_3$ calculated, %: C 59.26; H 3.73; N 17.28.

*2.3. Synthesis of 2-Hydrazino-4,6-dipropargyloxy-1,3,5-triazine (**4**)*

In a 100 mL flask, 10.0 g (41 mmol) of compound **3** was dissolved in 37 mL of *i*-PrOH and 16 mL of THF, and 2.83 mL (57 mmol) of hydrazine hydrate was added. The mixture was stirred for 2 h at room temperature. The resulting mixture was filtered, and the precipitate was washed with 12 mL of *i*-PrOH and dried on air. Yield 7.75 g (86%). White crystals, mp. 141–143 °C. IR spectrum (KBr tablet), ν, cm^{-1}: 3351, 3222, 3181, 3116 (-$NHNH_2$), 3294 (≡CH), 2135, 2122 (stretching vibrations -C≡CH), 1673, 1608 (bending vibrations of $NHNH_2$), 1583, 1544 (stretching vibrations -C=N of triazine), 1461 (bending vibrations of CH_2), 1332, 1112 (stretching vibrations of C-O-C), 813 (bending vibrations -C=N of triazine). ^{1}H NMR spectrum (500 MHz, DMSO-d^6), δ, ppm: 3.56 (1H, t, J = 1.9, CH); 3.58 (1H, t, J = 1.9, CH); 4.44 (2H, s, NH_2); 4.94 (2H, d, J = 1.9, CH_2); 5.02 (2H, d, J = 1.9, CH_2); 9.15 (1H, m, NH). ^{13}C NMR spectrum (126 MHz, DMSO-d^6), δ, ppm: 170.66; 168.96; 78.83; 78.06; 54.52. Found, %: C 48.79; H 4.28; N 31.60. $C_9H_9N_5O_2$. Calculated, %: C 49.31; H 4.14; N 31.95.

*2.4. Synthesis of 2,4-Dihydrazino-6-propargyloxy-1,3,5-triazine (**5**)*

In a 100 mL flask, 10.0 g (41 mmol) of compound **3** was dissolved in 74 mL of *i*-PrOH, and 5.65 mL (114 mmol) of hydrazine hydrate was added. The mixture was refluxed for 3.5 h. The resulting mixture was filtered, and the precipitate was dried on air. Yield 7.84 g (98%). White crystals, mp. 188–189 °C. IR spectrum (KBr tablet), ν, cm^{-1}: 3324 (≡CH), 3341 (NH), 3236, 3169 (NH_2), 2121 (-C≡CH), 1667, 1598 (bending vibrations of $NHNH_2$), 1584, 1541 (stretching vibrations of -C=N triazine), 1456 (bending vibrations of CH_2), 1328, 1106, (stretching vibrations of C-O-C), 805 (bending vibrations of -C=N triazine). ^{1}H NMR spectrum (500 MHz, DMSO-d^6), δ, ppm: 3.49 (1H, s, CH); 4.08–4.50 (4H, m, NH_2); 4.78–5.12 (2H, m, CH_2); 8.20–8.62 (2H, m, NH). ^{13}C NMR spectrum (126 MHz, DMSO-d^6), δ, ppm: 168.43; 79.81; 77.80; 53.83. Found (%): C 36.56; H 4.93; N 50.75. $C_6H_9N_7O$. Calculated (%): C 36.92; H 4.65; N 50.23.

*2.5. Synthesis of 2-Azido-4,6-dipropargyloxy-1,3,5-triazine (**1**)*

The solution of 10.0 mmol of concentrated HCl in 5 mL of water was added to a suspension of 1.53 g (7.0 mmol) of hydrazine **4** in 10 mL of water with cooling (using a bath of ice and salt), and the mixture was stirred until the solid was dissolved. Then, 10 mL of CH_2Cl_2 was added, and a solution of 0.48 g (7.0 mmol) $NaNO_2$ in 2–3 mL of water was dropwise-added. The reaction mixture was stirred for 10 min while cooling, and then $NaHCO_3$ was added until pH = 7.0. The organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 25 mL) and dried over calcined Na_2SO_4. A solution of

the substance in CH_2Cl_2 was passed through a thin layer of silica gel, and the solvent was distilled on a rotary evaporator.

Yield 1.29 g (80%). White crystals, mp. 40–41 °C (CH_2Cl_2) (mp 40–41 °C [15]). IR spectrum (KBr tablet), ν, cm^{-1}: 3278 (≡CH), 2159, 2132, 1332 (stretching vibrations of N_3), 1583, 1548 (stretching vibrations -C=N of triazine), 1489 (bending vibrations of CH_2), 1381, 1054, (stretching vibrations of C-O-C), 814 (bending vibrations -C=N of triazine). ^{1}H NMR spectrum (500 MHz, DMSO-d^6), δ, ppm. (J, Hz): 3.66 (2H, t, J = 2.3, CH), 5.06 (4H, d, J = 2.3, CH_2). ^{13}C NMR spectrum (126 MHz, DMSO-d^6), δ, ppm: 171.84; 171.60; 78.73; 77.69; 55.96. Found, %: C 46.81; H 2.83; N 37.18. $C_9H_6N_6O_2$. Calculated, %: C 46.96; H 2.63; N 36.51.

*2.6. Synthesis of 2,4-Diazido-6-propargyloxy-1,3,5-triazine (**2**)*

A solution of 20.0 mmol of concentrated HCl in 5 mL of water was added to a suspension of 1.37 g (7.0 mmol) of dihydrazine **5** in 10 mL of water with cooling (using a bath of ice and salt), and the mixture was stirred until it dissolved. A total of 10 mL of CH_2Cl_2 was added, and a solution of 0.99 g (14.4 mmol) $NaNO_2$ in 2–3 mL of water was dropwise-added. The reaction mixture was stirred for 15 min while cooling before $NaHCO_3$ was added until neutral. The organic layer was separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL), and the organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 25 mL) and dried over calcined Na_2SO_4. A solution of the substance in CH_2Cl_2 was passed through a thin layer of silica gel, and the solvent was distilled on a rotary evaporator.

Yield 1.17 (77%). White crystals, mp. 57–58 °C (mp. 57–58 [14]). IR spectrum (KBr tablet), ν, cm^{-1}: sq. 3277 (≡CH), 2180, 2140, 1352 (stretching vibrations of N_3), 1594, 1547 (stretching vibrations -C=N of triazine), 1451 (bending vibrations of CH_2), 1360, 1076, (stretching vibrations of C-O-C), 809 (bending vibrations -C=N of triazine). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm. (J, Hz): 2.62 (1H, t, J = 2.4, CH); 5.07 (2H, d, J = 2.4, CH_2). ^{13}C NMR spectrum (126 MHz, $CDCl_3$), δ, ppm: 172.15; 171.53; 77.51; 77.25; 56.20. Found, %: C 33.41, H 1.89, N 57.20. $C_6H_3N_9O$. Calculated, %: C 33.19, H 1.39, N 58.05.

2.7. Synthesis of Hyperbranched Polymers in Bulk

The thermally initiated polycycloaddition reaction of monomers **1** and **2** was carried out via stepwise heating as follows: 2 h at a temperature of 60 °C, 2 h at 90 °C, and 10 h at 120 °C.

2.8. Determination of Compound's Solubility in Nutrient Media

A portion of the compound under study was dissolved in DMSO to obtain a solution with an initial concentration of the compound at 10 wt.%. Then, this solution was added dropwise to the minimal essential medium (MEM) and nutrient medium at such an amount that [DMSO] ≈ 0.1 vol.%. If the solution was colloid (the cone of light from the laser beam became noticeable), then the solution of the test compound in DMSO was diluted, and the entire procedure was repeated until the solution became optically clear in a mixture of 99.9 vol.% nutrient medium/0.1 vol.% DMSO.

2.9. Antibacterial Activity of Polymer Films

The volumetric samples of HBPs (10–15 mg) were placed on the surface of an agar inoculated with *E. coli* bacteria to qualitatively assess the antibacterial activity of polymers. After this, the cups were kept in a thermostat at a temperature of 37 °C for 24 h. Then, the area of the microbial growth inhibition zone was determined.

2.10. Antibacterial Activity by the Disk Diffusion Method (DDM)

The determination of the antibacterial activity of samples was carried out following the methodology [37]. A total of 20 mL of melted nutrient agar was poured into sterile Petri dishes with a diameter of 10 cm. A solution of the test *E. coli* culture at a concentration of 10^7 pcs/mL was evenly distributed over the surface of the dish; excess liquid was removed

with a Pasteur pipette, and the agar was dried on the worktable with the dish closed. A total of 5 mL of the solutions of the studied low-molecular substances in various concentrations of acetone were dosed onto filter paper disks with a diameter of 8 mm.

For the polymers, a series of disks impregnated with solutions of monomers in acetone were prepared for the quantitative assessment of the antibacterial activity of HBPs (Table 1). The disks were dried from the solvent, and the reaction of polycycloaddition was carried out at 80 °C for 24 h. Disks with polymers were evenly laid out on the surface of the seeded agar at a distance of 2 cm from the edge of the dish. The cups were kept in a thermostat at 37 °C for 24 h.

Table 1. Concentrations of the HBPs in the DDM method.

№	Sample	Mass of Sample at the Filter, mg
1	HBP-2	2.5
2	HBP-2	1.5
3	HBP-2	0.9
4	HBP-2	0.3
5	HBP-1	1.7

To take into account the results, we determined the width of the microbial growth inhibition zone around the discs using a caliper. The absence of a zone of microbial growth inhibition indicated the resistance of the studied bacteria to this substance. The zones whose diameter did not exceed 15 mm (when using discs with a diameter of 8 mm) indicated poor sensitivity. Zones from 15 to 25 mm were found in sensitive microbes. Highly sensitive microbes were characterized by zones with a diameter greater than 25 mm.

The DDM test was carried out five times for each compound. Data from independent experiments were presented as the range of values of diameters for the growth inhibition zone.

2.11. Cytotoxicity Test by the MTT Test

Investigations were carried out on M-HeLa (subclone M, epithelioid carcinoma of the human cervix), FetMSC (mesenchymal stem cells from the bone marrow of a 5–6 weeks embryo), and Vero (African green monkey kidney cells) cell lines. These cell lines were obtained from the Institute of Cytology RAS (St. Petersburg, Russia). The M-HeLa cells were grown in an EMEM medium (PanEco, Moscow, Russia), FetMSC cells were grown in a DMEM/F12 medium (PanEco), and Vero cells were grown in a DMEM medium (PanEco). Cells were cultured in media containing 10% fetal calf serum (Biowest, France), penicillin (50 U/mL), and streptomycin (50 μg/mL) in an atmosphere of 5% CO2 at 37 °C.

The determination of the cytotoxicity of the test samples with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was carried out following the described method [38].

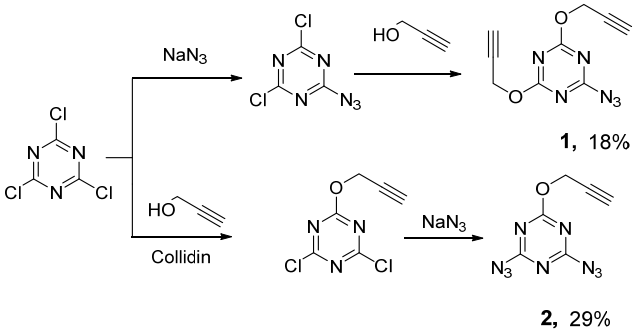
M-HeLa and Vero cells were seeded into 96-well plates at a rate of 5×10^3 cells per well. FetMSC cells were seeded at 10×10^3 cells per well. The test substances were added to the culture medium 24 h after seeding in the form of a solution in a mixture of the nutrient medium/DMSO (0.1%) in various concentrations. MTT was added to a final concentration of 0.5 mg/mL 72 h after adding the test compounds to the culture medium, and the cells were incubated in an atmosphere of 5% CO_2 at 37 °C for 3 h. The incubation medium and the formed crystals of MTT-formazan were removed from the wells after MTT staining. The color intensity was determined photometrically at a wavelength of 570 nm and a reference wavelength of 620 nm. Cell viability was assessed via MTT staining. The intensity of MTT staining in the control samples was taken as 100%. IC_{50} doses were calculated using the median effect analysis based on dose–response data [39].

The MTT test was carried out three times for each compound. Data from independent experiments are presented as the mean ± standard deviation.

3. Results and Discussion

3.1. Optimization of the Method for the Synthesis of Azido-Propargyloxy Monomers

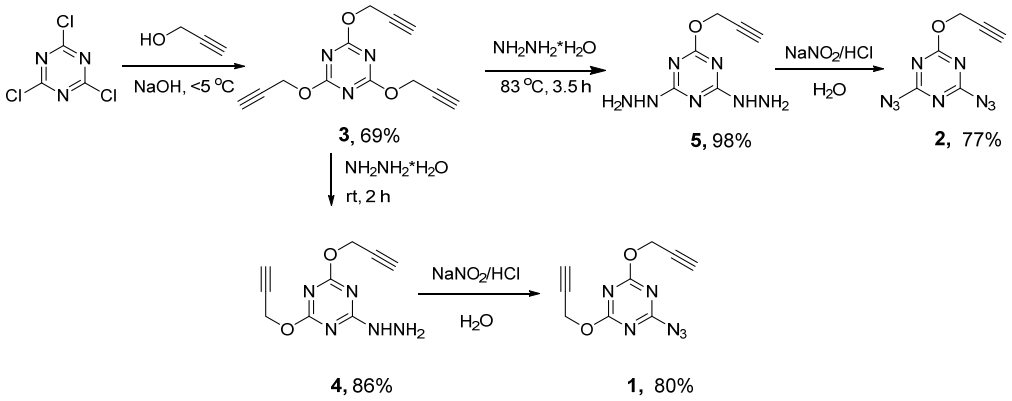
Despite the fact that the studied monomers were obtained and described earlier, the methods of their synthesis only provided low yields (Scheme 1) [22,40].



Scheme 1. The original methods for monomer synthesis [22,40].

The method we previously developed for the synthesis of monomer **1** consisted of obtaining 2-azido-4,6-dichloro-1,3,5-triazine and the subsequent replacement of chlorine atoms with propargyloxy groups [22]. This approach allowed us to obtain monomer **1** in two stages but gave a low total yield (18%). In addition, it was necessary to use column chromatography to isolate the final product **1**. For monomer **2**, a two-step preparation method was also previously used, including the synthesis of 2-propargyloxy-4,6-dichloro-1,3,5-triazine from cyanuric chloride and propargyl alcohol using collidine as a base and the further interaction of the resulting dichloride with sodium azide [15]. Attempts to improve this method (replacing collidine with sodium carbonate and high-boiling toluene with methylene chloride) only reduced the synthesis time and simplified the isolation of product **2** but did not increase the total yield (29%). The main reason for the relatively low total yield of monomers was the non-selectivity of the substitution of chlorine atoms in cyanuric chloride and 2-azido-4,6-dichloro-1,3,5-triazine with propargyloxy groups (in general, with alkoxy groups). As a result, mixtures of products require distillation under reduced pressure or column chromatography for the separation of target compounds. It is known that the replacement of chlorine in cyanuric chloride with an alkoxy group(s) only slightly reduces the ability of the remaining chlorine atoms to undergo nucleophilic substitution, which leads to a sharp decrease in the mobility of the remaining chlorine atoms. Because of this fact, the selective mono-, di- or tri-replacement of chlorine atoms in cyanuric chloride with amino groups is possible [41,42].

In this work, a new general procedure for the synthesis of monomers **1** and **2** was developed and significantly increased the yield of target products, making it easier for their isolation. At the first stage, trisubstituted 1,3,5-triazine **3** was synthesized from cyanuric chloride according to the method reported by [43]. Then, one or two propargyloxy groups were replaced by hydrazino groups. In the third step, the reaction of nitrosation of hydrazino-1,3,5-triazines **4** and **5** was carried out according to the method [44] with sodium nitrite in hydrochloric acid. (Scheme 2). As a result, target products were obtained with a significantly higher overall yield (48% for **1** and 52% for **2**) than those previously obtained (18% and 29%, respectively) from the starting cyanuric chloride. Azidoacetylene monomers **1** and **2** are colorless crystalline substances with T_m = 41–42 °C and T_m = 57–58 °C, respectively, and are relatively stable at room temperature. The IR, ^{1}H NMR, and ^{13}C NMR spectra of the obtained monomers match well with the spectra of the corresponding compounds obtained [22,40].

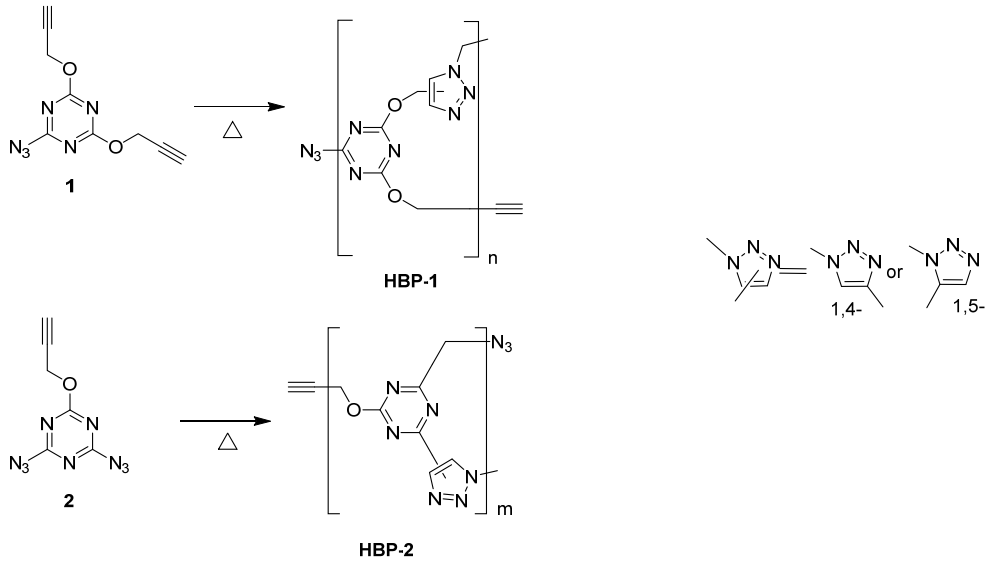


Scheme 2. The new methods of monomer synthesis.

As a result, a new general method for the simple synthesis of monomers **1** and **2** was developed, which made it possible to significantly increase the yield of the target products.

3.2. Preparation of HBPs

HBPs were prepared via the thermal azide-alkyne cycloaddition reaction (Huisgen cycloaddition) (Scheme 3) [22]. The resulting polymer compounds **HBP-1** and **HBP-2** were yellow-brown glassy substances, completely soluble only in highly polar aprotic solvents, such as N,N-dimethylformamide (DMF), 1,3-dimethylamylamine (DMAA), and dimethyl sulfoxide (DMSO).



Scheme 3. The thermal reaction of obtaining hyperbranched polymers. 1 and 2 are the azide-alkyne monomers AB_2 type.

The goal of this work is the qualitative study of the biological activity of polymers to assess the prospects of their use in bioapplications. Because of this, the molecular mass and structure properties of obtaining HBPs were not considered.

3.3. Preparation Compounds for the Biological Tests

In the first step, the solubility in nutrient media of test compounds was determined. The monomers and HBPs used were hydrophobic; therefore, in biological tests, we used concentrated solutions of these compounds in the experimental mixture (99.9 vol.% nutrient medium/0.1 vol.% DMSO): compound **1** ~4 wt.%, compound **HBP-1** ~0.75 wt.%, compound **2** ~4 wt.%, compound **HBP-2** ~0.75 wt.%, compound **3** ~4 wt.%. Due to the low solubility

of the test compounds, along with the experiments using these solutions, experiments were carried out with solutions of nutrient media containing 1 vol.% DMSO.

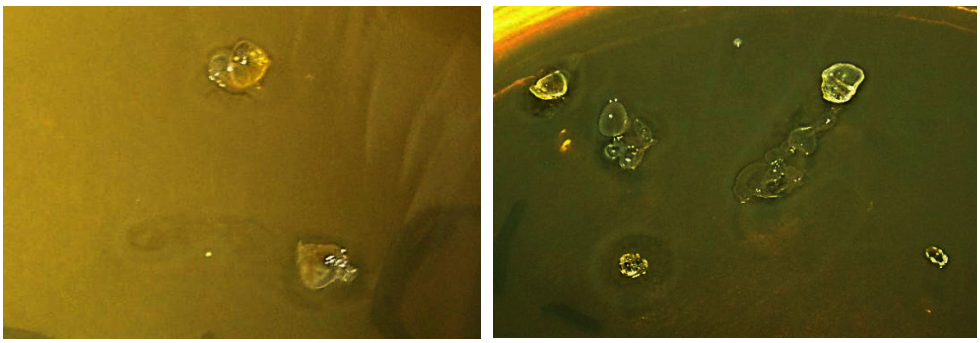
Antibacterial Activity

The antibacterial activity of the compounds was observed in the bacteria of the species *E. coli* using the disk diffusion method (DDM). As a comparison, common broad-spectrum penicillin antibiotic, ampicillin ((2S,5R,6R)-6-[(R)-2-amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3,2,0]-heptane-2-carboxylic acid) was chosen. Based on the results of the study (Table 2), trispropargyloxy-substituted 1,3,5-triazine **3** and monoazide monomer **1** did not have antibacterial activity against *E. coli*. Diazide monomer **2** in the concentration range from 0.2 to 2.5 mg/filter inhibited the growth of bacteria (*E. coli* growth inhibition zone width was 10–12 mm). Comparing the diameters of the zones of inhibition of *E. coli* growth for ampicillin and monomer **2**, we concluded that diazide monomer 2 is characterized by weak antibacterial activity.

Table 2. The results of the DDM method.

Sample	Mass of Sample at the Filter, mg	Diameter of Growth Inhibition Zone, mm
ampicillin	1×10^{-2}	20–22
	5×10^{-3}	14–17
	2.5×10^{-3}	12–15
	1.25×10^{-3}	13–15
1	1.6	<1
2	2.5	10–12
	1.6	9–11
	0.6	9–11
	0.2	8–10
3	1.6	<1

The qualitative test on volumetric samples of **HBP-1** and **HBP-2** showed that polymers have weak antibacterial activity; they reduce the density of the bacterial lawn but do not cause the appearance of a bacteria-free zone (Figure 1).



(**a**) (**b**)

Figure 1. Qualitative test of the antibacterial activity of bulk samples **HBP-1** (**a**) and **HBP-2** (**b**).

An analysis of the antibacterial activity of polymer films using the DDM method showed that these polymers do not exhibit activity against *E. coli* (where the diameter of the growth inhibition zone is less than 10 mm) (data are not presented). However, initial experiments on volumetric samples showed that there was still some activity. Thus, we can conclude that initial polymer samples have a very low concentration of active low-molecular compounds that are capable of diffusing into the aquatic environment in polymer samples.

As a result, it can be concluded that the azide group in the compound affects the antibacterial activity, as well as the diffusion of the low molecular weight fraction of the substance in the aqueous environment.

3.4. Cytotoxic Effect of Compounds

A cytotoxicity test was carried out with solutions of the test substances. Experiments to determine the cytotoxicity of these solutions were carried out using the MTT test. Based on the results of the study, dose–effect curves were constructed (Figure 2), and the concentrations of the half-maximal inhibition IC_{50} of all studied samples were calculated (Table 3).

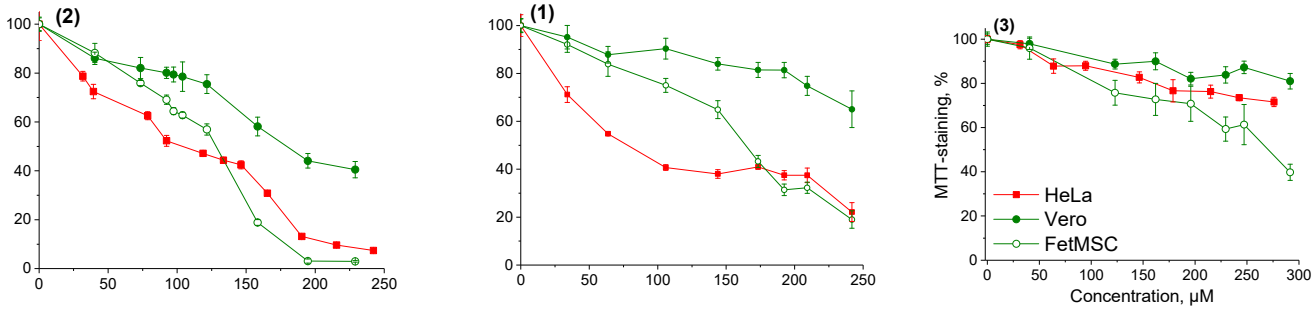


Figure 2. Changes in MTT staining of M-HeLa, Vero, and FetMSC cells after 72 h of incubation in the presence of compounds **3** (**right**), **1** (**center**), and **2** (**left**).

Table 3. IC_{50} values for the studied monomer and polymer samples.

Compound	IC_{50}, μM		
	M-HeLa	**Vero**	**FetMSC**
1	99 ± 18	>250	150 ± 36
2	90 ± 23	164 ± 48	83 ± 15
3	>280	>280	280 ± 21
HBP-1	>145	>145	>145
HBP-2	108 ± 20	>126	>125

Tripropargyloxy-substituted 1,3,5-triazine **3** showed no cytotoxic effect on M-HeLa, Vero, and FetMSC cells. Diazide compound **2** had the greatest toxic effect on M-HeLa, Vero, and FetMSC cells. Monazide compound **1** showed cytotoxicity to M-HeLa cells compared to Vero and FetMSC cells. HBPs had a less cytotoxic effect on M-HeLa cells, which can be explained by the spatial hindrances resulting from polycycloaddition for the interaction of functional groups located inside the macromolecule with the cell (its enzymes or receptors). In addition, polymeric compounds **HBP-1** and **HBP-2** have a low cytotoxic effect against normal cell lines (FetMSC and Vero) in comparison with monomers **1** and **2**. HBPs are little toxic compounds according to the classification of Halle and Göres [45]. This fact indicates the safety of using HBPs for bioapplications.

4. Conclusions

New methods for the synthesis of 2-azido-4,6-bispropynyloxy-1,3,5-triazine and 2,4-diazido-6-propynyloxy-1,3,5-triazine were developed, providing the yields of the target product of 48% and 52%, respectively. The hyperbranched polymers based on the developed compounds were obtained. Antibacterial activity against *E. coli* bacteria and cytotoxicity against M-HeLa, FetMSC, and Vero cell lines were studied. It was shown that with an increase in the content of terminal azide groups in compounds based on 1,3,5-triazine, antibacterial activity appeared. The cytotoxicity of these compounds against the M-HeLa, FetMSC, and Vero cell lines was also studied. 2,4,6-tripropargyloxy-1,3,5-triazine does not show any cytotoxic effect ($IC_{50} \geq 280$ μM). It was shown that the presence of an azide

group in the compound directly affects the cytotoxic effect. The hyperbranched polymers are little toxic compounds that have a less cytotoxic effect against M-HeLa (IC_{50} > 100) in comparison to monomers (IC_{50} = 90–99 μM). It was shown that HBPs, based on azido-acetylene AB_2 monomers and containing 1,3,5-triazine, do not exhibit significant toxicity. This makes it possible to use these polymers as the basis of biocompatible materials in biomedical applications.

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