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Synthesis and Membrane Protective Activity of Bis-Sulfides Derived from Monoterpenoids and Monosaccharides

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Abstract—Hydroxyl- and chloroethyl derivatives of neomenthane- and isobornanethiol in yields of to 80% were synthesized. They served as the basis for the preparation of new bis-sulfides with diacetone-protected galacto- and fructopyranose fragments in yields up to 98%. The bis-sulfides synthesized were screened for membrane protective and antioxidant properties in a model cell system (in vitro) based on their ability to inhibit the H_2O_2-induced hemolysis of erythrocytes and retard the oxyhemoglobin oxidation.

Keywords: antioxidant, bis-sulfides, erythrocytes, membrane-protective activity, monosaccharide, oxidative hemolysis

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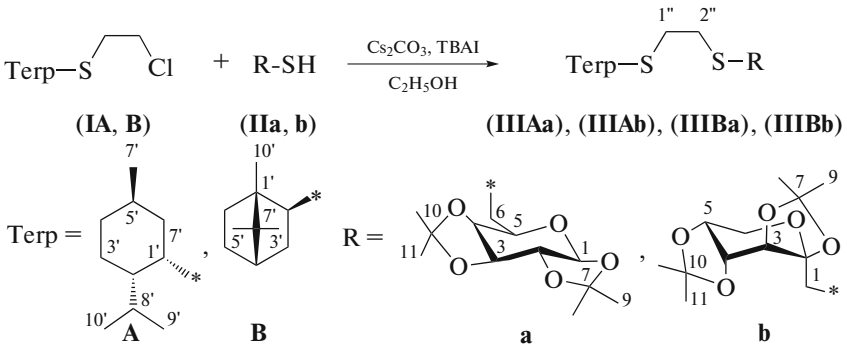
INTRODUCTION

A wide application of sulfur-containing compounds is based on their ability to stabilize polymeric materials by inhibition of oxidative processes. A considerable number of sulfur-containing inhibitors are represented by bis-sulfides containing two sulfur atoms separated by a hydrocarbon bridge [1]. The bis-sulfides bearing glycoside fragments are widely used in the organic synthesis of functionalities for the preparation of dendrimers as artificial glycoproteins [2], photosensitizers [3], as well as in the synthesis of the agents with antituberculous [4] and antidiabetic [5] activities. Two sulfur atoms, which can be involved in oxidation reactions, can also provide antioxidant properties stipulated by the antiperoxide activity of sulfur-containing groups [6, 7]. An essential factor supporting the use of bis-sulfides with glycoside fragments in pharmacology is their low toxicity. Due to the necessity to develop methods of preparation of biologically active compounds bearing natural nontoxic or low-toxic fragments we first synthesized bis-sulfides derived from monoterpenoids and monosaccharides.

RESULTS AND DICUSSION

Previously, we obtained sulfides, sulfoxides [8, 9], and disulfides [10] with various monoterpenoid and monosaccharide moieties. As a continuation of these studies, we synthesized nonsymmetrical bis-sulfides (**III**) with two sulfur atoms linked by an ethane bridge by the interaction of terpenyl sulfanyl ethyl chlorides (**IA**) or (**IB**) with monosaccharide thiols (**IIa**) or (**IIb**) in different combinations in the presence of a Cs_2CO_3/TBAI catalytic system (Scheme 1). The yields of bis-sulfides were maximal for neomenthane-bearing compounds (98%) and minimal in the case of isobornane- and diisopropylidenefructopyranose-derived compounds (63%) without regard to the monosaccharide component.



Scheme 1.

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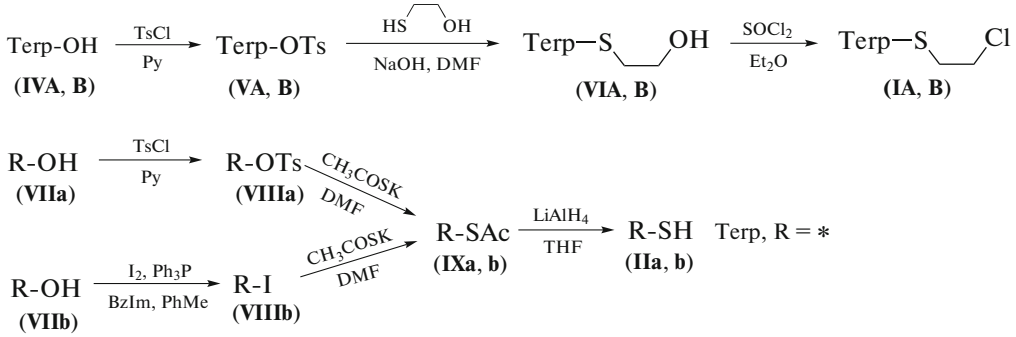
Abbreviations: AAPH, 2,2-azo-bis(amidinopropane) dihydrochloride; TBAI, tetrabutylammonium iodide.

The ^{1}H NMR spectra of bis-sulfides (**III**) contained proton resonances characteristic for the monosaccharide fragments protected by isopropylidene groups (**a**, **b**). If compared with starting chlorides (**IA, B**) the resonances of bis-sulfides (**III**) C2" protons (3.60–3.69 ppm) shifted upfield (2.60–2.94 ppm), whereas in ^{13}C NMR spectra the resonance of C2" atoms shifted upfield by ~10 ppm.

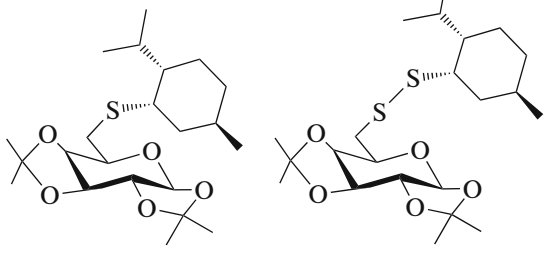
Terpenyl sulfanyl ethyl chlorides (**IA**) and (**IB**) were obtained from *L*-menthol (**IVA**) and borneol (**IVB**) via terpene tosylates (**VA, B**) and alcohols (**VIA, B**) (Scheme 2). Alcohols (**VI**) were synthesized by the reaction of tosylates with 2-mercaptoethanol in the presence of a base. The treatment of hydroxyethyl sulfides (**VIA, B**) with thionyl chloride in ether in the presence of a catalytic quantity of pyridine resulted in chlorides (**IA, B**). In their ^{13}C NMR spectra an upfield shift by ~17 ppm of the carbon resonance of the methylene group directly linked to the chlorine atom was observed if compared with the resonances of similar atoms in alcohols (**VIA, B**).



Scheme 2. * For terpene and monosaccharide fragments see Scheme 1.

Carbohydrate thiols (**IIa**) and (**IIb**) were synthesized from isopropylidene-protected monosaccharides (**VIIa, b**) via intermediate tosylate/iodide (**VIIIa**)/(**VIIIb**) followed by the reduction of thioacetates (**IXa, b**) obtained from them.

The primary evaluation of the biological activity of the compounds under study was performed in vitro with blood erythrocytes of laboratory animals. The previously obtained sulfide (**A-S-a**) [8] and disulfide (**A-S-S-a**) [10] bearing neomenthane (**A**) and diisopropylidenegalactopyranose (**a**) moiety were used as comparatives:



A-S-a **A-S-S-a**

The goal of the comparison was the evaluation of the dependence of membrane-protective and antioxidant properties on the number and position of sulfur atoms.

Since the potential toxicity of the bis-sulfides and the comparatives could considerably limit their use, we first assessed their hemolytic activity.

Among the compounds tested, disulfide (**A-S-S-a**) demonstrated the highest toxicity (Fig. 1), which met the previously obtained data [10]. The presence of an ethane bridge separating sulfur atoms resulted in an essential decrease in the hemolytic activity of the compounds. It is noteworthy that, in general, the bis-sulfides containing a neomenthane fragment (**A**) were more toxic than similar compounds with an isobornane moiety (**B**) (Fig. 1).

The results of the study of membrane-protective activity under the conditions of oxidative stress due to the addition of hydrogen peroxide are shown in Figs. 2 and 3.

The analysis of the data in the Fig. 2 showed that bis-sulfides with a diisopropylidenegalactopyranose moiety (**IIIAa**) and (**IIIBa**) were somewhat more active than the similar compounds containing a diisopropylidenefructose fragment (**IIIAb**) and (**IIIBb**), although the difference was not always statistically significant. The same trend was also found for the membrane protective activity of the disulfides synthesized previously and lacking a spacer between the sulfur atoms [10].

The comparison of the compounds bearing both neomenthane and diisopropylidenegalactopyranose moieties demonstrated that the number of sulfur atoms and the presence of an ethane bridge between them impacted the membrane protective activity (Fig. 3). Bis-sulfide (**IIIAa**) with a dimethylene spacer between the sulfur atoms was the most active compound. Disulfide (**A-S-S-a**) lacking this bridge was less active, although the difference could be due to a considerably higher hemolytic activity of the latter.

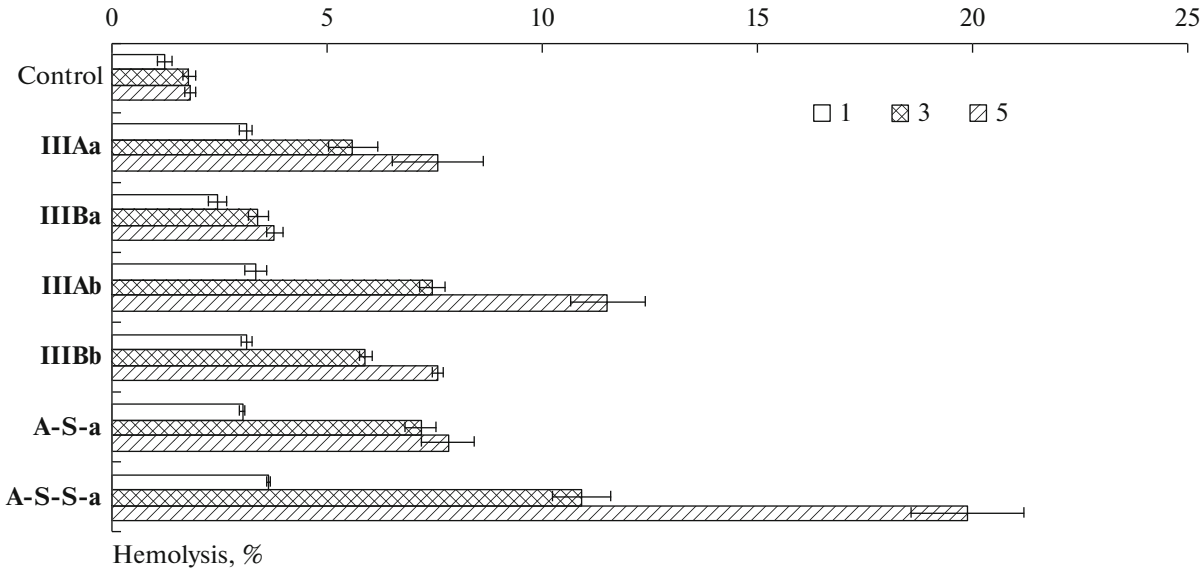


Fig. 1. Hemolytic activities of the compounds at a concentration of 10 μM (ethanol solutions; 1, 3, and 5 h incubation).

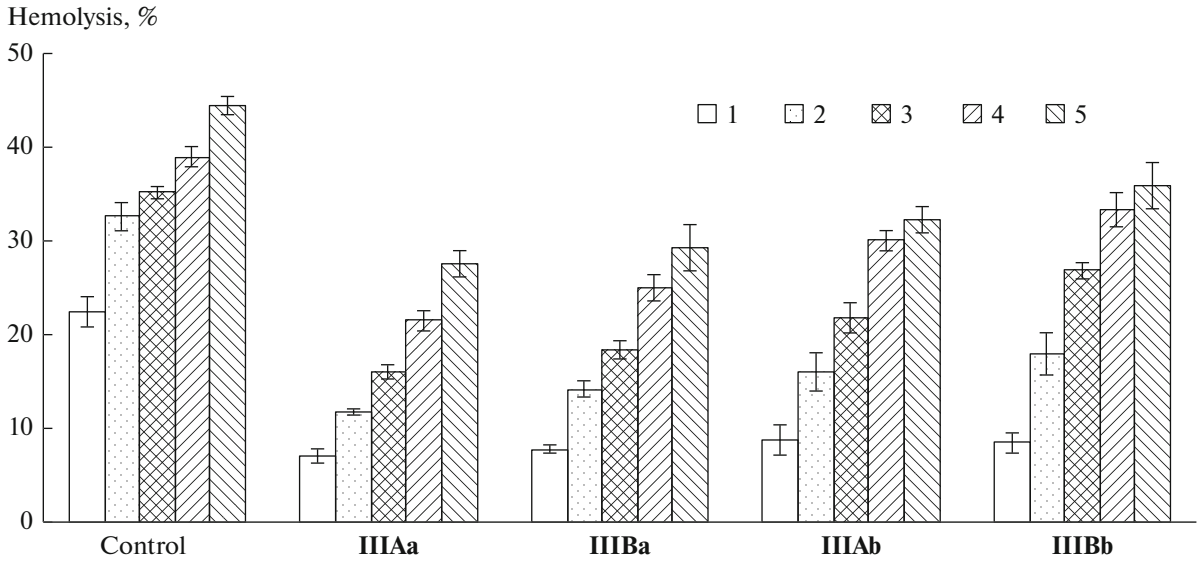


Fig. 2. The effect of bis-sulfides (**IIIAa**), (**IIIBa**), (**IIIAb**), and (**IIIBb**) at a concentration of 10 μM on the degree of H_2O_2-induced erythrocyte hemolysis after 1–5 h incubation.

The next studies of antioxidant and membrane protective properties of the compounds synthesized were performed with AAPH as a source of radicals. It is known that AAPH can form peroxide radicals upon thermal degradation in an aqueous phase at physiological temperatures. Unlike hydrogen peroxide, it cannot penetrate cells and affect erythrocytes from the outer membrane surface [11]. The primary target of the H_2O_2 action in erythrocytes is intracellular hemoglobin, whereas in the case of AAPH the primary effect is the free radical-induced damage of plasmatic membrane.

According to the data in Fig. 4, all the four bis-sulfides (**III**) displayed a high membrane protective activity relative to a peroxide radical without regard to the structures of carbohydrate and terpene moieties.

At the same time, the number of sulfur atoms and the spacer between them had a noticeable impact on the biological activity of the compounds synthesized (Fig. 5). The analysis of the dynamics of the hemolytic process showed that bis-sulfide (**IIIAa**) containing two sulfur atoms separated by a methylene bridge was the most active, and compound (**A-S-a**) with one sulfur atom, the least active.

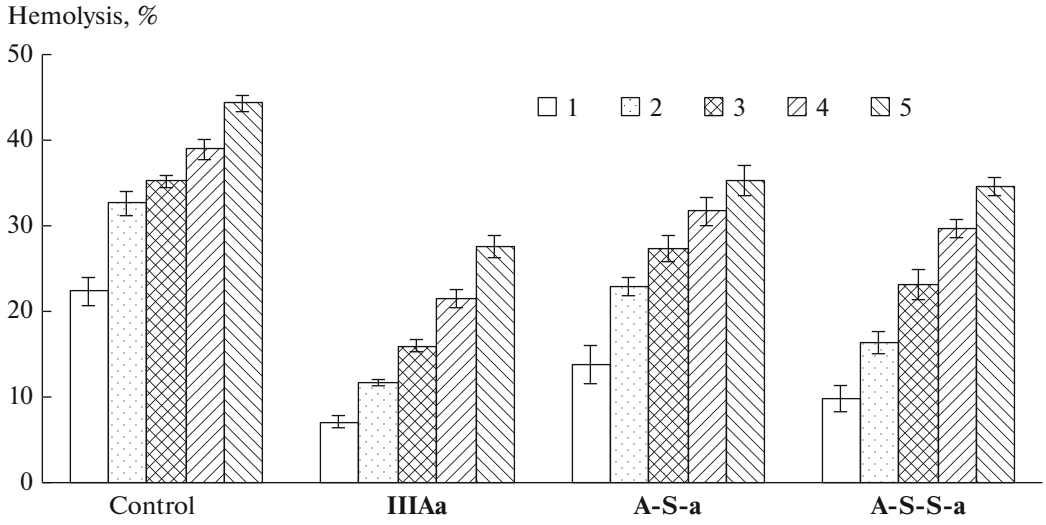


Fig. 3. The effect of **(IIIAa)**, **(A-S-a)**, and **(A-S-S-a)** at a concentration of 10 μM on the degree of H_2O_2-induced erythrocyte hemolysis after 1–5 h incubation.

The assessment of the cell death dynamics under conditions of acute oxidative stress allowed the conclusion that in general the compounds under study are only active at early stages and have low activity in the final stage of the process.

A low activity of the sulfide if compared with those of disulfide and bis-sulfide can be explained by the capacity of compounds with two sulfur atoms to display a higher antioxidant effect. At the same time, the bis-sulfide, in which two sulfur atoms are separated by a bridge, manifested a higher membrane protective activity if compared with the disulfide, because the effect of an electron acceptor group SO formed upon the oxidation of one of the sulfide groups of compound **(IIIAa)** on the other sulfur atom was lower than that in disulfide **(A-S-S-a)**.

The same regularity was observed upon the analysis of AOA evaluated by the ratio of the native (oxyhemoglobin (oxyHb)) and oxidized hemoglobin forms (methemoglobin (metHb) and ferryl hemoglobin (ferrylHb)) in erythrocyte hemolysates after induction of the AAPH-induced oxidative stress (Fig. 6). Bis-sulfides **(III)** bearing two sulfur atoms separated by a spacer were characterized by a higher AOA if compared with a similar sulfide **(A-S-a)**.

To summarize, we developed the methods of synthesis of bis-sulfides containing terpene and monosaccharide fragments as well as terpene alcohols and chlorides used for the preparation of these bis-sulfides. The composition and structures of terpene thioethanols, thioethyl chlorides, and bis-sulfides of various structures were confirmed by NMR and IR spectroscopy and element analysis.

The analysis of the results demonstrated the trend, according to which the compounds with a neomenthane fragment **(A)** were characterized by a higher hemolytic activity than structurally similar compounds with an isobornyl group. The introduction of a dimethylene bridge between the sulfur atoms resulted in a decreased toxicity of the bis-sulfides if compared with the corresponding disulfides lacking this fragment. Under the conditions of oxidative stress induced by a hydroxyl radical, bis-sulfides with a galactose moiety were distinguished by a higher membrane protective activity than the corresponding compounds with a fructose moiety. A similar dependency was also found upon the analysis of the membrane protective activity of the corresponding disulfides synthesized previously and lacking a spacer between the sulfur atoms.

We showed that the number of sulfur atoms and the presence of a spacer between them in the compounds containing neomenthane and galactose fragments, with all else being equal, had an impact on their membrane protective and antioxidant activities. Bis-sulfide **(IIIAa)** containing sulfur atoms separated by a dimethylene spacer was the most active compound in the model cell system of the acute oxidative stress induced by either hydroxyl or peroxide radicals.

EXPERIMENTAL

IR spectra were recorded on an IR-Fourier spectrometer in a thin film or KBr tablets. Melting points were measured on a Gallencamp-Sanyo device. ^1H and ^{13}C NMR spectra (δ, ppm; *J*, Hz) were recorded on a Bruker Avance-300 spectrometer (300.17 MHz for ^1H and 75.48 MHz for ^{13}C) in $CDCl_3$; chloroform resonances as an internal standard. The complete assignment of ^1H and ^{13}C resonances was conducted using dimeric homo-(^1H–^1H COSY, ^1H–^1H NOESY) and heteronuclear (^1H–^{13}C HSQC, ^1H–^{13}C HMBC) experiments. Optical rotation was measured

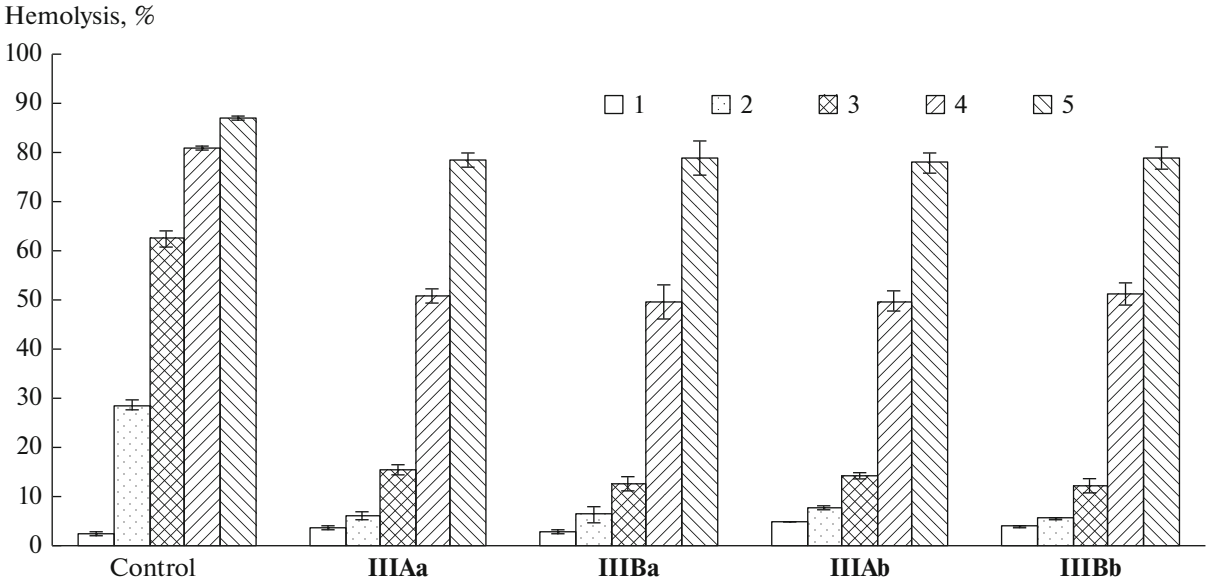


Fig. 4. The effect of bis-sulfides **(IIIAa)**, **(IIIBa)**, **(IIIAb)**, and **(IIIBb)** at a concentration of 10 μM on the degree of AAPH-induced erythrocyte hemolysis after 1–5 h incubation.

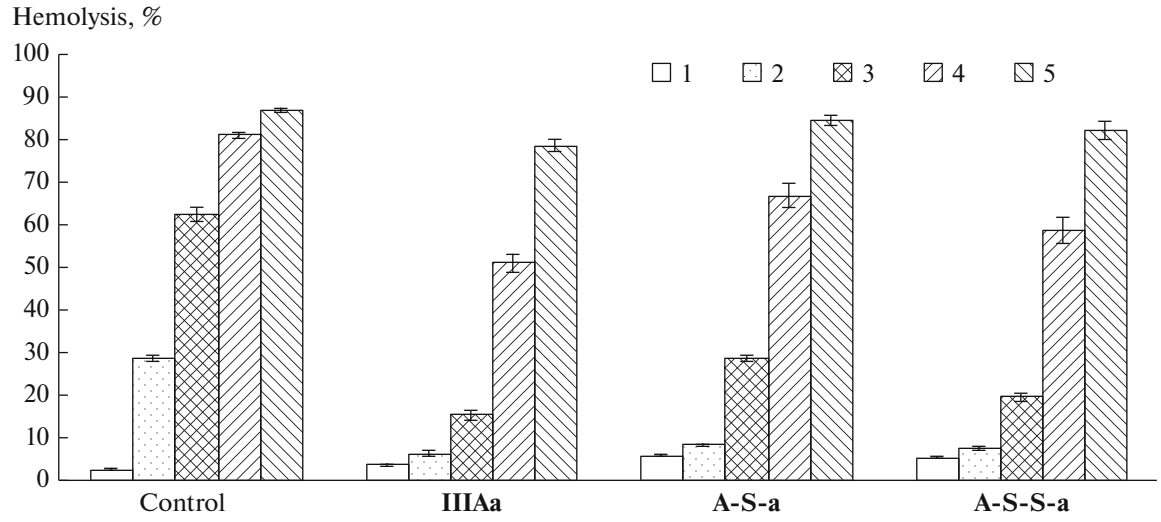


Fig. 5. The effect of **(IIIAa)**, **(A-S-a)**, and **(A-S-S-a)** at a concentration of 10 μM on the degree of AAPH-induced erythrocyte hemolysis after 1–5 h incubation.

on an automatic digital P3002RS polarimeter (Kruss). TLC was performed on Sorbfil plates eluting with a $CHCl_3$–Et_2O mixture at various ratios; the compounds were developed using phosphormolybdic acid and $KMnO_4$. Element analysis was carried out on an automatic EA 1110 CHNS-O device. All the reactions were performed in freshly distilled solvents. Column chromatography was carried out on Alfa Aesar silica gel (0.06–0.2 mm) in the same solvent systems as for TLC.

L-menthol *p*-toluenesulfonate (VA) and borneol *p*-toluenesulfonate (VB) were obtained as described in [12].

2-(((1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl)-thio)ethanol (VIA) and **2-(((1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)thio)ethanol (VIB)** were synthesized by the reaction of 2-mercaptoethanol with *p*-toluenesulfonates (**V**) in the presence of NaOH [13].

2-(((1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl)-thio)ethanol (VIA). The yield 78%. Viscous yellow liquid, $[\alpha]_D^{20}$ + 91.1 (*c* 1.30; $CHCl_3$), R_f 0.41 ($CHCl_3$–Et_2O, 10 : 1). IR, ν, cm^{-1}: 690 (C–S), 1047 (CH_2OH), 1452, 2918, 3369 (OH). ^1H NMR: 0.84–0.94 (m, 1H, H4'*e*), 0.91 (d 3H, Me(7'), *J* 6.0), 0.93 (d, 3H,

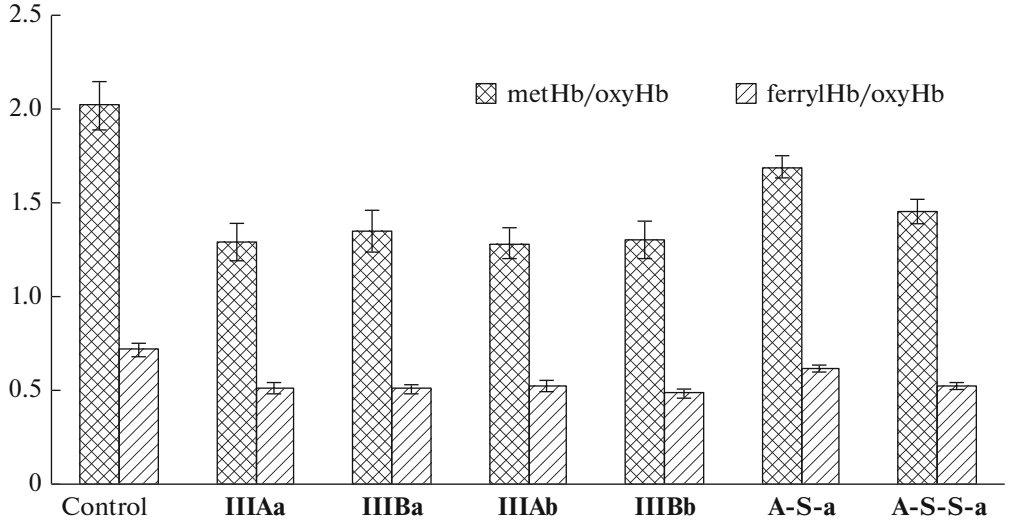


Fig. 6. The effect of (**IIIAa**), (**IIIBa**), (**IIIAb**), (**IIIBb**), (**A-S-a**), and (**A-S-S-a**) at a concentration of 10 μM on the ratio of native and oxidized hemoglobin forms in erythrocyte hemolysates under conditions of the oxidative stress in the presence of AAPH.

Me(10'), *J* 6.0), 0.97 (d, 3H, Me(9'), *J* 6.4), 1.08–1.37 (m, 3H, H2', H3'*e*, H6'*e*), 1.64–1.85 (m, 3H, H8', H3'*a*, H4'*a*), 1.87–2.06 (m, 2H, H5', H6'*a*), 2.37 (s, 1H, OH), 2.79 (t, 2H, H1'', *J* 5.9), 3.14–3.26 (m, 1H, H1'), 3.73 (t, 2H, H2'', *J* 5.9). ^{13}C NMR: 20.80 (C10'), 21.02 (C9'), 22.17 (C7'), 25.86 (C3'), 26.41 (C5'), 29.94 (C8'), 34.99 (C1''), 35.34 (C4'), 40.99 (C6'), 46.50 (C1'), 48.90 (C2'), 60.46 (C2''). Found, %: C, 66.35; H, 11.75; S, 14.83. $C_{12}H_{24}OS$. Calculated, %: C, 66.61; H, 11.18; S, 14.82.

2-(((1*S*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)thio)ethanol (VIB). The yield 75%. Viscous yellow liquid, $[\alpha]_D^{20}$ + 248.0 (*c* 0.30; $CDCl_3$), R_f 0.29 ($CHCl_3$–Et_2O, 10 : 1). IR, ν, cm^{-1}: 677 (C–S), 1049 (CH_2OH), 1456, 2951, 3385 (OH). ^{1}H NMR: 0.86 (s, 3H, Me(9'), 1.00 (s, 3H, Me(8'), 1.03 (s, 3H, Me(10'), 1.13–1.25 (m, 2H, H5'*en*, H6'*en*)), 1.67–1.82 (m, 3H, H5'*ex*, H6'*ex*, H4'), 1.86–1.97 (m 2H, H3), 2.24 (s, 1H, OH), 2.60–2.69 (m, 1H, H2'), 2.72–2.82 (m, 2H, H1''), 3.73 (t, 2H, H2'', *J* 5.9). ^{13}C NMR: 14.01 (C10'), 20.17 (C8'), 20.44 (C9'), 27.33 (C5'), 38.00 (C6'), 38.52 (C3'), 41.23 (C1''), 45.90 (C4'), 47.32 (C1'), 49.46 (C7'), 54.39 (C2'), 60.32 (C2''). Found, %: C, 67.26; H, 10.39; S, 14.85. $C_{12}H_{22}OS$. Calculated, %: C, 67.23; H, 10.34; S, 14.96.

(2-Chloroethyl)-((1*S*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl)sulfide (IA) and **(2-chloroethyl)-((1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)-sulfide (IB)** were obtained by the reaction of alcohols with thionyl chloride used for the synthesis of chlorides [14].

(2-Chloroethyl)-((1*S*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl)sulfide (IA). The yield 78%. Viscous yellow liquid, $[\alpha]_D^{20}$ +19.0 (*c* 0.70; $CDCl_3$), R_f 0.80 ($CHCl_3$). IR, ν, cm^{-1}: 700 (C–Cl), 1450, 2916. ^{1}H NMR: 0.82–0.95 (m, 1H, H4'*e*), 0.91 (d, 3H, Me(7'), *J* 6.3), 0.93 (d, 3H, Me(10'), *J* 6.6), 0.97 (d, 3H, Me(9'), *J* 6.6), 1.05–1.35 (m, 3H, H2', H3'*e*, H6'*e*), 1.58–1.81 (m, 3H, H8', H3'*a*, H4'*a*), 1.87–2.05 (m, 2H, H5', H6'*a*), 2.80–2.96 (m, 2H, H1''), 3.15–3.30 (m, 1H, H1'), 3.60–3.69 (m, 2H, H2''). ^{13}C NMR: 20.77 (C10'), 21.02 (C9'), 22.14 (C7'), 25.81 (C3'), 26.32 C(5'), 29.92 C(8'), 33.75 C(1''), 35.31 C(4'), 41.03 C(6'), 43.24 C(2''), 47.24 C(1'), 48.88 C(2'). Found, %: C, 61.42; H, 9.91; S, 13.76. $C_{12}H_{23}SCl$. Calculated, %: C, 61.38; H, 9.87; S, 13.65.

((2-Chloroethyl)-((1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)sulfide (IB). The yield 80%. Viscous yellow liquid, $[\alpha]_D^{20}$ +21.1 (*c* 0.70; $CDCl_3$), R_f 0.65 ($CHCl_3$). IR, ν, cm^{-1}: 702 (C–Cl), 1453, 2920. ^{1}H NMR: 0.86 (s, 3H, Me(9')), 0.97 (s, 3H, Me(8')), 1.03 (s, 3H, Me(10')), 1.14–1.23 (m, 2H, H5'*en*, H6'*en*), 1.68–1.79 (m, 3H, H5'*ex*), H6'*ex*, H4'), 1.88–1.98 (m, 2H, H3'), 2.62–2.70 (m, 1H, H2'), 2.84–2.94 (m, 2H, H1''), 3.64 (t, 2H, H2'', *J* 8.1). ^{13}C NMR: 14.04 C(10'), 20.15 C(8'), 20.41 C(9'), 27.34 C(5'), 36.90 C(6'), 38.52 C(3'), 41.37 C(1''), 43.17 C(2''), 45.89 C(4'), 47.32 C(1'), 49.57 C(7'), 55.45 C(2'). Found, %: C, 63.26; H, 8.65; S, 13.11. $C_{12}H_{21}SCl$. Calculated, %: C, 61.91; H, 9.09; S, 13.77.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-α-D-galatopyranose (VIIIa) was synthesized as described in [15]. The yield 98%; $[\alpha]_D^{22}$ – 44.6 (*c* 0.30, $CHCl_3$); ref. [15]: the yield 87%, $[\alpha]_D^{22}$ – 44.0 (*c* 0.28, $CHCl_3$).

6-Deoxy-6-thioacetyl-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (IXa) was obtained as described

in [16]. The yield 77%, $[\alpha]_D^{22}$ − 15.6 (*c* 0.30, $CHCl_3$); ref. [16]: yield 99%, $[\alpha]_D^{22}$ − 15.0 (*c* 1.80, $CHCl_3$).

1-Deoxy-1-iodo-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (VIIIb) was obtained as described in [17]. The yield 64%, $[\alpha]_D^{22}$ − 47.6 (*c* 0.88, $CHCl_3$).

1-Deoxy-1-thioacetyl-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (IXb) was synthesized as described in [18]. The yield 72%, $[\alpha]_D^{22}$ −3.1 (*c* 0.95, $(CH_3)_2CO$); ref. [18]: the yield 76%, $[\alpha]_D^{22}$ −2.9 (*c* 1.00, $CHCl_3$).

6-Deoxy-6-thio-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (IIa) was obtained as described in [19]. Viscous yellow liquid. The yield 91%, $[\alpha]_D^{22}$ − 70.0 (*c* 0.47, $CHCl_3$), ref. [19]: the yield 70%, − 88.7 (concentration not given, $CHCl_3$).

1-Deoxy-1-thio-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranose (IIb) was obtained as described in [20]. White powder. The yield 94%, $[\alpha]_D^{22}$ − 44.0 (*c* 0.40, $CHCl_3$); ref. [20]: the yield 65%, not shown.

Monoterpene- and monosaccharide derived bis-sulfides. A carbohydrate-containing thiol (0.359 g, 1.3 mmol), cesium carbonate (0.424 g, 1.3 mmol), and tetrabutylammonium iodide (0.480 g, 1.3 mmol) were dissolved in EtOH (3 mL) under stirring in an argon atmosphere. Terpenyl sulfanyl ethyl chlorides (**IA**) or (**IB**) (1.0 mmol) were added in 5 min and the mixture was refluxed for 6 h. The solvent was removed in vacuum and the product was isolated by column chromatography on silica gel eluting with 60 : 1 $CHCl_3$–Et_2O.

1-((1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexylsulfanyl)-2-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl-6-sulfanyl)ethane (IIIAa). The yield 98%. Viscous yellow liquid, $[\alpha]_D^{20}$ − 8.0 (*c* 0.80; $CHCl_3$), R_f 0.37 ($CHCl_3$–Et_2O, 60 : 1). IR, ν, cm^{-1}: 646 (C–S), 999, 1071(C–O), 1169 (O–C–O), 1211, 2916. ^1H NMR: 0.83–0.94 (m, 1H, H4'*e*), 0.90 (d, 3H, Me(7'), *J* 6.6), 0.92 (d, 3H, Me(10'), *J* 6.6), 0.97 (d, 3H, Me(9'), *J* 6.6), 1.07–1.32 (m, 3H, H2', H6'*e*, H3'*e*), 1.36 (c, 3H, Me(8)), 1.38 (s, 3H, Me(12)), 1.48 (s, 3H, Me(11)), 1.57 (s, 3H, Me(9)), 1.66–1.80 (m, 3H, H4'*a*, H8', H3'*a*), 1.87–2.10 (m, 1H, H5'), 1.88–2.03 (m, 1H, H6'*a*), 2.67–2.89 (m, 6H, H6, H1'', H2''), 3.14–3.24 (m, 1H, H1'), 3.84–3.95 (m, 1H, H5), 4.31–4.42 (m, 2H, H2, H3), 4.65 (dd, 1H, H4, *J* 8.0, 2.2), 5.56 (d, 1H, H1, *J* 5.0). ^{13}C NMR: 20.83 C(10'), 21.05 C(9'), 22.18 C(7'), 24.48 C(12), 24.92 C(8), 25.91 C(3'), 25.98 C(11), 25.99 C(5'), 26.36 C(9), 29.94 C(8'), 31.68 C(1''), 31.85 C(2''), 33.01 C(6), 35.37 C(4'), 40.95 C(6'), 46.82 C(1'), 48.92 C(2'), 68.00 C(5), 70.54 C(2), 70.91 C(4), 71.56 C(3), 96.64 C(1), 108.60 C(7), 109.28 C(10). Found, %: C, 62.35; H, 8.80; S, 13.72. $C_{24}H_{42}S_2O_5$. Calculated, %: C, 60.72; H, 8.92; S, 13.51.

1-[((1*S*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]-hept2-yl)sulfanyl]-2-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranosyl-6-sulfanyl)ethane (IIIBa). The yield 80%. Viscous yellow liquid, $[\alpha]_D^{20}$ −28.5 (*c* 0.50; $CHCl_3$), R_f 0.51 ($CHCl_3$–Et_2O, 60 : 1). IR, ν, cm^{-1}: 646 (C–S), 1071 (C–O), 1169 (O–C–O), 2951. ^1H NMR: 0.84 (s, 3H, Me(9')), 0.97 (s, 3H, Me(8')), 1.01 (s, 3H, Me(10')), 1.10–1.23 (m, 2H, H5'*en*, H6'*en*), 1.36 (s, 3H, Me(8)), 1.38 (s, 3H, Me(12)), 1.48 (s, 3H, Me(11)), 1.57 (s, 3H, Me(9)), 1.66–1.78 (m, 3H, H5'*ex*, H6'*ex*, H4'), 1.84–1.99 (m, 2H, H3'), 2.62–2.90 (m, 7H, H2', H2'', H1'', H6), 3.84–3.94 (m, 1H, H5), 4.27–4,38 (m, 2H, H2, H3), 4.65 (dd, 1H, H4, *J* 7.8, 2.3), 5.55 (d, 1H, H1, *J* 5.0). ^{13}C NMR: 14.01 C(10'), 20.20 C(8'), 20.42 C(9'), 24.47 C(12), 24.92 C(8), 26.00 C(11), 26.16C(9), 27.36 C(5'), 31.82 C(2''), 32.92 C(6), 34.76 C(1''), 38.53 C(6'), 41.19 C(3'), 45.90 C(4'), 47.33 C(1'), 49.48 C(7'), 54.93 C(2'), 68.02 C(5), 70.54 C(2), 70.90 C(4), 71.60 C(3), 96.64 C(1), 108.60 C(7), 109.28 C(10). Found, %: C, 61.27; H, 8.55; S, 13.54. $C_{24}H_{40}S_2O_5$. Calculated, %: C, 60.98; H, 8.53; S, 13.57.

1-((1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexylsulfanyl)-2-(1-deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranosyl-1-sulfanyl)ethane (IIIAb). The yield 98%. Viscous yellow liquid, $[\alpha]_D^{20}$ +10.6 (*c* 1.00; $CHCl_3$), R_f 0.42 ($CHCl_3$–Et_2O, 60 : 1). IR, ν, cm^{-1}: 640 (C–S), 1063 (C–O), 1167 (O–C–O), 2918. ^1H NMR: 0.89 (d, 3H, Me(7'), *J* 6.3), 0.92 (d, 3H, Me(9'), *J* 6.3), 0.84–0.94 (m, 1H, H4'*e*), 0.96 (d, 3H, Me(10'), *J* 6.6), 1.05–1.25 (d, 3H, H2', H3'*e*, H6'*e*), 1.37 (s, 3H, Me(12)), 1.48 (s, 3H, Me(8)), 1.50 (s, 3H, Me(11)), 1.56 (s, 3H, Me(9)), 1.59–1.80 (m, 3H, H8', H3'*a*, H4*a*'), 1.85–2.06 (m, 2H, H5', H6'*a*), 2.68–2.94 (m, 4H, H2'', H1''), 2.84 (d, 1H, $H1^A$, *J* 13.8), 3.10 (d, 1H, $H1^B$, *J* 13.8), 3.14–3.20 (m, 1H, H1'), 4.30 (d, 1H, $H6^A$, *J* 13.0), 4.63 (d, 1H, $H6^B$, *J* 12.6), 4.26 (d, 1H, H5, *J* 7.7), 4.34 (d, 1H, H3, *J* 2.5), 4.63 (dd, 1H, H4, *J* 8.0, 2.5). ^{13}C NMR: 20.80 C(10'), 21.06 C(9'), 22.18 C(7'), 24.07 C(12), 25.49 C(10), 25.93 C(3'), 25.98 C(5'), 26.37 C(11), 26.63 C(9), 29.94 C(8'), 31.50 C(2''), 24.16 C(1''), 35.37 C(4'), 40.14 C(6'), 40.95 C(1), 46.94 C(1'), 48.94 C(2'), 61.68 C(6), 70.39 C(4), 70.89 C(5), 72.25 C(3), 103.64 C(2), 108.55 C(7), 109.06 C(10). Found, %: C, 63.10; H, 8.77; S, 13.42. $C_{24}H_{42}S_2O_5$. Calculated, %: C, 60.72; H, 8.92; S, 13.51.

1-[((1*S*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]hept-2-yl)sulfanyl]-2-(1-deoxy-2,3:4,5-di-*O*-isopropylidene-β-D-fructopyranosyl-1-sulfanyl)ethane (IIIBb). The

yield 63%. Viscous yellow liquid, $[\alpha]_D^{20}$ −12.4 (*c* 0.34; $CHCl_3$), R_f 0.36 ($CHCl_3$–Et_2O, 60 : 1). IR, ν, cm^{-1}: 579 (C–S), 1063 (C–O), 1132, 1167 (O–C–O), 2941. ^1H NMR: 0.84 (s, 3H, Me(8')), 0.96 (s, 3H, Me(9')), 1.01 (s, 3H, Me(10')), 1.10–1.22 (m, 2H, H5'*en*, H6'*en*), 1.37 (s, 3H, Me(12)), 1.48 (s, 3H, Me(8)), 1.50 (s, 3H, Me(11)), 1.56 (s, 3H, Me(9)), 1.64–1.76 (m, 3H, H5'*ex*, H6'*ex*, H4'), 1.83–1.94 (m, 2H, H3'), 2.61–2.91 (m, 6H, H2', H1'', $H1^A$, H2''), 3.09 (d, 1H, $H1^B$, *J* 13.7), 3.79 (d, 1H, $H6^A$, *J* 13.2), 3.94 (dd, 1H, $H6^B$, *J* 12.9, 1.4), 4.25 (d, 1H, H5 *J* 7.7), 4.33 (d, 1H, H3, *J* 2.5), 4.63 (dd, 1H, H4, *J* 7.8, 2.6). ^{13}C NMR: 14.00 (C10'), 20.19 C(8'), 20.41 C(9'), 24.05 C(12), 25.50 C(8), 25.97 C(11), 26.63 C(9), 26.36 C(5'), 34.10 C(2''), 34.53 C(1''), 38.51 C(6'), 39.98 C(1), 41.23 C(3'), 45.90 C(4'), 47.29 C(1'), 49.45 C(7'), 55.02 C(2'), 61.68 C(6), 70.38 C(4), 70.88 C(5), 72.21 (C3), 103.65 (C2), 108.54 C(7), 109.06 C(10). Found, %: C, 61.78; H, 8.40; S, 13.28. $C_{24}H_{40}O_5S_2$. Calculated, %: C, 60.98; H, 8.53; S, 13.57.

For the evaluation of toxicity and antioxidant and membrane protective activities of the compounds under study we used 0.5% (v/v) suspension of mouse erythrocytes in a phosphate buffer (PBS, pH 7.4). Ethanol was used as a solvent. The in vitro toxicity of the compounds under study was assessed by their capacity to induce hemolysis. The ethanol solutions of the compounds synthesized were added to an erythrocyte suspension and the mixture was incubated at 37°C for 5 h in a temperature-controlled Biosan ES-20 shaker (Latvia). The control samples contained the same volume of ethanol (0.1%). The membrane protective and antioxidant activities were evaluated by the inhibition degree of the induced hemolysis, inhibition of accumulation of secondary lipid peroxide products, and oxyhemoglobin oxidation in erythrocytes. To this end, the hemolysis was initiated 30 min after the addition of the tested solution into erythrocyte suspension using 0.006% hydrogen peroxide or 5 mM AAPH. The reaction mixture was incubated at 37°C for 5 h in a temperature-controlled shaker under slow stirring.

An aliquot was taken from the incubation mixture each hour and centrifuged for 5 min at 1600 g. The degree of hemolysis was assessed by the hemoglobin content in the supernatant on a ThermoSpectromic Genesys 20 spectrophotometer (United States) at λ 524 nm [21]. The hemolysis percentage was calculated relative to the total hemolysis of the sample. For the evaluation of accumulation of hemoglobin oxidation products after the incubation completion (5 h), an aliquot of erythrocyte suspension was subjected to the total hemolysis, centrifuged to precipitate erythrocyte ghosts, and an absorption spectrum was analyzed using a Fluorat-02-Panorama spectrofluorimeter (St. Petersburg). The contents of different hemoglobin forms (oxyHb, metHb, and ferrylHb) were calculated based on the proper absorption coefficients [22]. Each experiment was performed with five or six repeats. Statistical treatment and diagram plotting were carried out with Microsoft Office Excel 2007 software.

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REFERENCES

1. Balsells, J., Moyano, A., Pericas, M.A., and Riera, A., *Tetrahedron: Asymmetry*, 1997, vol. 8, pp. 1575–1580.
2. Ghirardello, M., Oberg, K., Staderini, S., Renaudet, O., Berthet, N., Dumy, P., Hed, Y., Marra, A., Malkoch, M., and Dondoni, A., *J. Polym. Sci., Part A: Polym. Chem.*, 2014, vol. 52, pp. 2422–2433
3. Titov, D.V., Gening, M.L., Tsvetkov, Yu.E., and Nifant'ev, N.E., *Usp. Khim.*, 2014, vol. 83, pp. 523–554.
4. Tiwari, V.K., Singh, B.K., Chaturvedi, V., Manju, Y.K., Srivatava, A.K., Sinha, S., Gaikwad, A., and Tripathi, R.P., *Indian J. Org. Chem.*, 2007, vol. 3, pp. 84–96.
5. Hinou, H., Sun, X.-L., and Ito, Y., *Org. Chem.*, 2003, vol. 68, pp. 5602–5613.
6. Ovchinnikova, L.P., Rotskaya, U.N., Vasyunina, E.A., Sinitsina, O.I., Kandalintseva, N.V., Prosenko, A.E., and Nevinskii, G.A., *Russ. J. Bioorg. Chem.*, 2009, vol. 35, pp. 379–384.
7. Terakh, E.I., Kandalintseva, N.V., Nikulina, V.V., Pinko, P.I., and Prosenko, A.E., *Neftekhimiya*¸ 2004, vol. 44, pp. 237–240.
8. Pestova, S.V., Sudarikov, D.V., Rubtsova, S.A., and Kutchin, A.V., *Russ. J. Org. Chem.*, 2013, vol. 49, pp. 366–373.
9. Pestova, S.V., Izmest'ev, E.S., Rubtsova, S.A., and Kuchin, A.V., *Russ. J. Org. Chem.*, 2014, vol. 50, pp. 670–677.

10. Pestova, S.V., Izmest'ev, E.S., Shevchenko, O.G., Rubtsova, S.A., and Kuchin, A.V., *Rus. Chem. Bull.*, 2015, vol. 64, no. 3, pp. 723–731.
11. Miki, M., Tamai, H., Mino, M., Yamamoto, Y., and Niki, E., *Arch. Biochem. Biophys.*, 1987, vol. 258, pp. 373–380.
12. Snow, A.W. and Foos, E.E., *Synthesis*, 2003, vol. 4, pp. 509–512.
13. Demakova, M.Ya., Sudarikov, D.V., Rubtsova, S.A., Slepukhin, P.A., and Kuchin, A.V., *Chem. Nat. Compd.*, 2012, vol. 47, pp. 899–902.
14. Volynskii, N.P., Perepelitchenko, L.I., and Zegel'man, L.A., *Izv. AN SSSR. Ser. Khim.*, 1987, vol. 11, pp. 2506–2508.
15. Lunau, N., Seelhorst, K., Kahl, S., Tscherch, K., Stacke, C., Rohn, S., Thiem, J., Hahn, U., and Meier, C., *Chem.-Eur. J.*, 2013, vol. 19, pp. 17379–17390.
16. Daniellou, R. and Le Narvor, C., *Adv. Synth. Catal.*, 2005, vol. 347, pp. 1863–1868.
17. Garegg, P.J. and Samuelsson, B., *J. Chem. Soc., Perkin Trans.*, 1980, vol. 1, pp. 2866–2869.
18. Goodwin, C.J., *Carbohydr. Res.*, 1989, vol. 195, pp. 150–152.
19. Martins, A.M.A., D'Accorso, N.B., and Thiel, I.M.E., *J. Heterocycl. Chem.*, 1996, vol. 33, pp. 1339–1343.
20. Carchon, G., Chre'tien, F., and Chapleur, Y., *Tetrahedron Lett.*, 2003, vol. 44, pp. 5715–5718.
21. Takebayashi, J., Chen, J., and Tai, A., Advanced Protocols in Oxidative Stress II, in *Methods in Molecular Biology, Armstrong*, D., Ed., New York: Humana Press, 2010, vol. 594, pp. 287–296.
22. Berg, J.M., Kamp, J.A.F., Lubin, B.H., Roelofsen, B., and Kuypers, F.A., *Free Radical Biol. Med.*, 1992, vol. 12, pp. 487–498.

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