Synthesis and membranoprotective properties of new disulfides with monoterpene and carbohydrate fragments*

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A cooxidation of carbohydrate and terpene thiols gives mixtures of disulfides containing 51—90% of the unsymmetric product. Membranoprotective and antioxidant properties of obtained unsymmetric and symmetric disulfides were evaluated based on their ability to inhibit the H_2O_2-induced hemolysis of erythrocytes, as well as the accumulation of secondary products of the peroxy oxidation of lipids and the oxidation of hemoglobin.

Key words: disulfides, monoterpenes, thiols, monosaccharides, membranoprotective properties, erythrocytes, oxidation hemolysis, antioxidants.

The synthesis of new efficient and low-toxic sulfur-containing antioxidants based on natural substrates is of significant interest for pharmacology, since the agents based on natural raw materials, as a rule, are less dangerous for living organisms and environment. Glutathione,[1,2] cystine,[3] and lypoic acid[4] are the commonly known endogenic sulfur-containing compounds with pronounced antioxidant activity. In cells, these compounds are in a labile equilibrium of the oxidized and the reduced forms (—SS—/—SH), with the reduced form only being active.[5] The transformation of the oxidized forms to the reduced ones takes place upon treatment with highly specific enzymes, for example, glutathione reductase and cystine reductase, due to which a constant redox potential of the cell is maintained.[6] At the same time, disulfides also can exhibit high antioxidant activity, in particular, such an activity was described for diallyl disulfide encountered with in plants of the genus *Allium* and formed from allicin thiosulfinate and/or its precursors.[7]

Disulfides can be involved in the oxidation reactions with many active oxidants due to the presence of two reaction centers capable of binding with four and more equivalents of an oxidant. An increase in the amount of the oxidant can lead to a stepwise formation of products with the growing oxidation state of sulfur: thiosulfinates—thiosulfonates—sulfinylsulfonates—disulfones, however, the instability of thiosulfinates formed in the first step interferes with further oxidation reactions.[8] It is known that thiosulfinates are prone to the disproportionation reactions, leading to the starting disulfide and the corresponding thiosulfonate, which can be subjected to oxidation, as well. The formation of the starting disulfides from thiosulfinates proceeds spontaneously without involvement of enzymes.[9]

The disulfides containing monosaccharide and monoterpenoid fragments seem promising for pharmacology, since they can possess not only an increased antioxidant activity due to the presence of a terpene fragment,[10] but also relatively high bioaccessibility and biocompatibility due to the presence of a carbohydrate fragment.[11]

The purpose of the present work is the synthesis and primary screening of biological activity of symmetric and unsymmetric disulfides containing terpene and/or carbohydrate fragments in different combinations. The studies also included evaluation of the contribution of each of the fragments to the antioxidant and membranoprotective activity of the conjugates obtained.

Results and Discussion

In the present work, the target disulfides were obtained from 6-thiodiisopropylidenegalactopyranose and 1-thiodiisopropylidenefructopyranose as the carbohydrate component and neomenthanethiol, isobornanethiol, *cis*-myrtanethiol, *trans*-verbenethiol, and myrtenethiol as the terpene component. The cooxidation of two different thiols with iodine proceeded nonselectively with the formation of two symmetric and one unsymmetric (containing a terpene and a carbohydrate fragment) disulfides.

* Dedicated to Academician of the Russian Academy of Sciences V. I. Minkin on the occasion of his 80th birthday.

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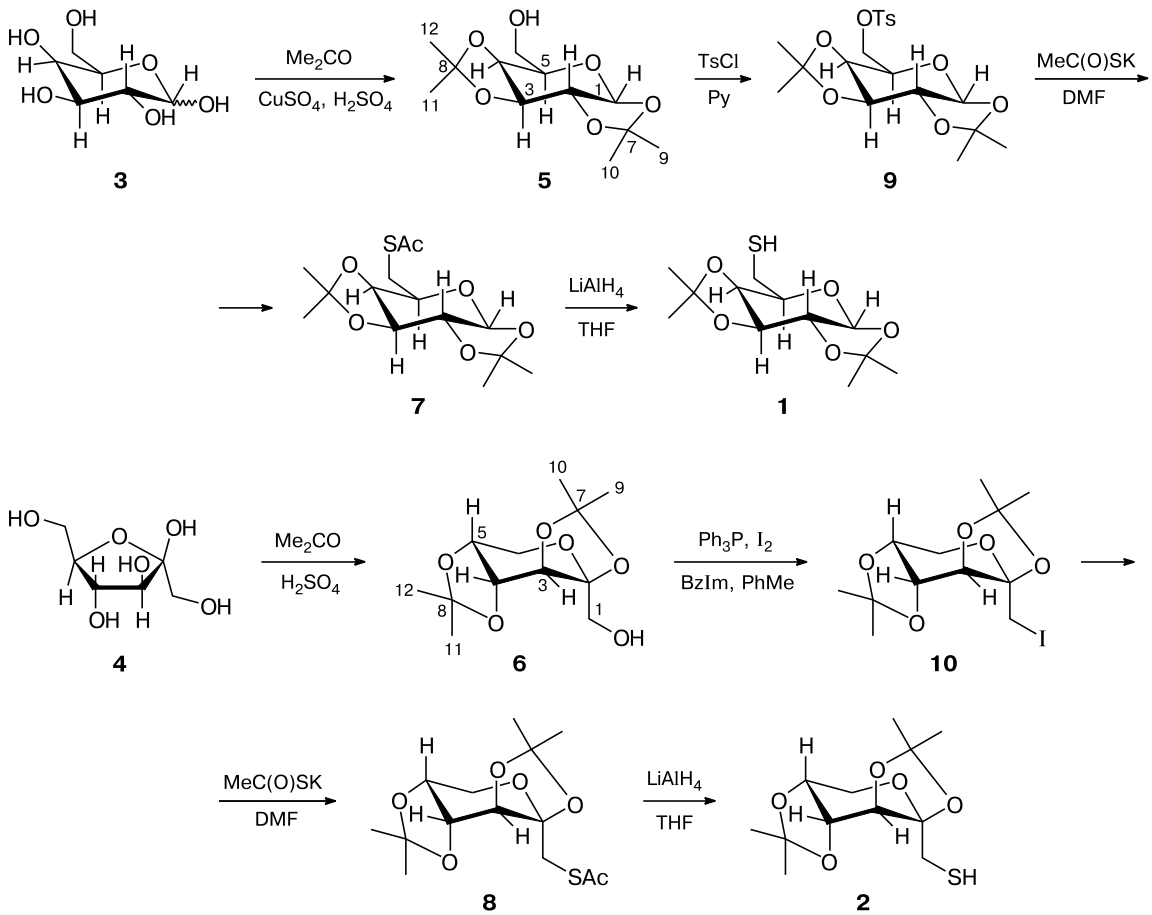
The starting 6-thiodiisopropylidenegalactopyranose (**1**) and 1-thiodiisopropylidenefructopyranose (**2**) were obtained from D-galactose **3** and D-fructose **4** (Scheme 1). Their reaction with acetone gave rise to the corresponding diisopropylidenepyranoses **5** and **6**; a preserved OH group at positions 6 and 1, respectively, was used for further transformations. The substitution for the OH group with a SH group was carried out in three stages through the step of the formation of thioacetates **7** and **8**. The thioacetates were obtained from the corresponding tosylate **9** and iodide **10** (see Scheme 1).

The terpene thiols were synthesized using known procedures based on the substitution for the *p*-toluenesulfonyl group in the corresponding tosylates with the thioacetic acid moiety[12] (thiols **11a**,**b**), or on the addition of thioacetic acid at the double bond of the monoterpenes[13] (thiol **11c**), or on the reaction of *N*-sulfinylbenzenesulfonamide with monoterpenes[14] (thiol **11d**) with subsequent reduction of the formed thioacetates and sulfinylsulfonamides. *trans*-Verbenethiol (**11e**) was obtained by the reduction of the corresponding thioacetate, in turn, synthesized by the reaction of thioacetic acid and *trans*-verbenol in the presence of a $ZnCl_2$ catalyst.[15]

The oxidation of a mixture of two different thiols with iodine (~20 °C, a complete conversion) is a nonselective process, which gives all the possible disulfides, however, the rate of their formation is not the same and is determined by steric effects, which facilitate or, *vice versa*, hinder the coupling of two sulfanyl fragments. Unsymmetric disulfides **12a**—**e**, **13a**—**e** were found to be the major products (Scheme 2). The largest amount of them was formed in the oxidation of a mixture of thiol **2** and isobornanethiol **11b** — up to 90% calculated on the total amount of products, whereas the smallest amount was formed in the case of thiol **2** and neomenthanethiol **11a** (51%). Symmetric disulfides **14a**—**e** based on terpene thiols were formed in lower amounts: from 7 to 41% calculated on the total amount of reaction products. In all the cases, the formation of symmetric disulfides **15** and **16** with monosaccharide fragments did not exceed 13% (Table 1).

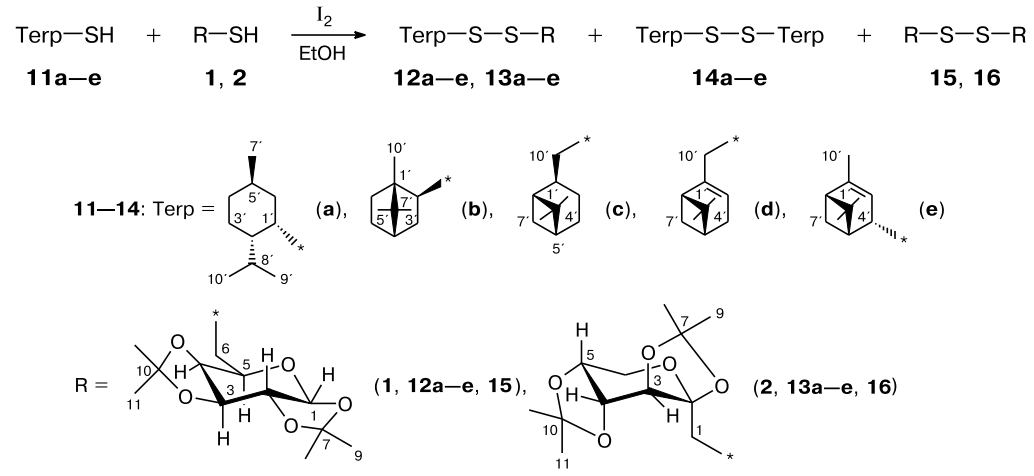
All the disulfides were isolated by column chromatography in the individual state, their structure was estab-

Scheme 1



BzIm is the benzimidazole. Py is the pyridine.

Scheme 2



lished by IR and NMR spectroscopy, as well as by elemental analysis.

The screening of biological activity of disulfides was carried out in the *in vitro* system using blood erythrocytes of laboratory animals.[16—21] Since the toxicity of disulfides can impose considerable limitations on their further use, the compounds were first evaluated on their hemolytic activity.

Among all the conjugates under study, the hemolytic activity was found only in unsymmetric disulfides containing neomenthyl (**12a**, **13a**) or isobornyl (**13b**) fragments. The addition of these compounds to a suspension of erythrocytes in the concentration of 10 μmol L^{-1} caused a 2.1—3.4 times excess of a spontaneous hemolysis level.

Most of the tested compounds showed an ability to inhibit the death of erythrocytes under the acute stress conditions induced by the addition of hydrogen peroxide. The membranoprotective activity of compounds with the galactopyranose fragment decreases in the following order (Fig. 1): **12d** = **12c** > **12a** = **12b** > **15** = standard (di-*n*-propyl disulfide) > **12e**. This means that the conjugates with *cis*-myrtanyl, myrtenyl, neomenthyl, and isobornyl moieties are the most active. The lowest activity was observed for the disulfide with *trans*-verbenyl fragment.

Table 1. Oxidation of mixtures of terpene and carbohydrate thiols

Mixture of thiols	Products	Proportion of corresponding disulfide* (%)
1 + **11a**	**12a** + **14a** + **15**	53 : 34 : 13
1 + **11b**	**12b** + **14b** + **15**	75 : 19 : 6
1 + **11c**	**12c** + **14c** + **15**	71 : 24 : 5
1 + **11d**	**12d** + **14d** + **15**	64 : 29 : 7
1 + **11e**	**12e** + **14e** + **15**	78 : 18 : 4
2 + **11a**	**13a** + **14a** + **16**	51 : 41 : 8
2 + **11b**	**13b** + **14b** + **16**	90 : 7 : 3
2 + **11c**	**13c** + **14c** + **16**	72 : 20 : 8
2 + **11d**	**13d** + **14d** + **16**	62 : 30 : 8
2 + **11e**	**13e** + **14e** + **16**	83 : 10 : 7

* Calculated from the ratio of integral intensities of nonoverlapping signals for the protons in the NMR spectra of the reaction mixture.

The structurally similar compounds **13a**—**e** containing a fructopyranose moiety exhibited a lower activity as compared to disulfides with galactopyranose fragment (Fig. 2). The calculations showed that the replacement of the galactopyranose fragment with a fructopyranose one led to a 1.2—1.6 times decrease in the membranoprotective activity for most compounds, except a low-active sulfide **12e** containing a *trans*-verbenyl fragment (see Figs 1 and 2). Thus, in the case when erythrocytes were protected by the conjugates with a galactopyranose fragment, the value of relative hemolysis was 44—69%, whereas when similar compounds with a fructopyranose fragment were used, it was 69—91%. However, among the disulfides with a fructopyranose moiety the compounds containing myrtenyl, *cis*-myrtanyl, or neomenthyl fragments were found to be the most active. The regressive analysis indicates the presence of a statistically significant close positive relationship between the activities of the corresponding galacto- and fructoso-containing disulfides with five different terpene fragments in the composition ($R_S = 0.9$, $p = 0.037$).

For the symmetric disulfides containing no sugar fragments (Fig. 3), the highest membranoprotective activity was found for compounds containing *cis*-myrtanyl (**14d**), myrtenyl (**14c**), and isobornyl (**14b)** moieties. The lowest activity was found in the disulfide with *trans*-verbenyl fragment (**14e**). For the symmetric neomenthane-derived disulfide (**14a**), a statistically significant membranoprotective activity was not found (no data reported).

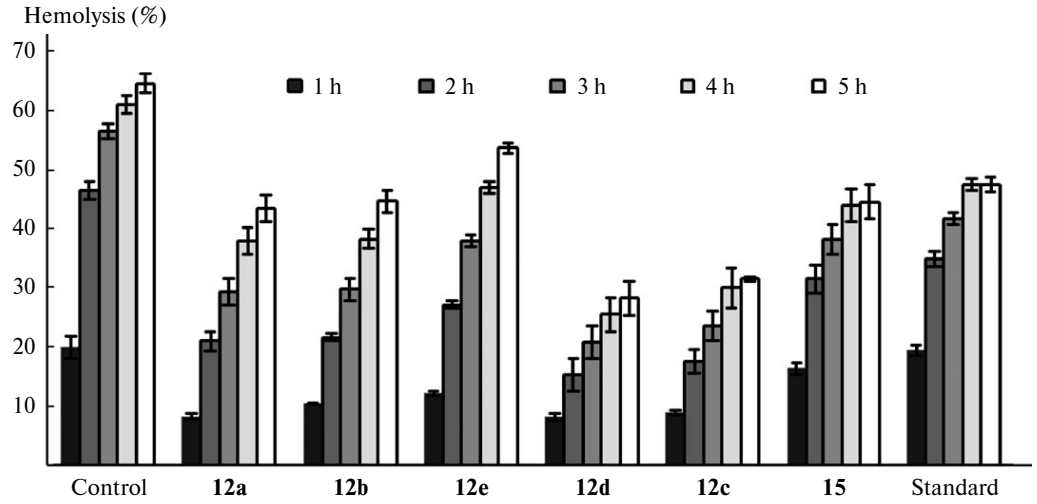


Fig. 1. Influence of compounds **12a**—**e**, **15** and $Pr^n_2S_2$ (a standard) in the concentration of 10 μmol L^{-1} on the degree of the H_2O_2-induced hemolysis of erythrocytes after 1—5 h of incubation.

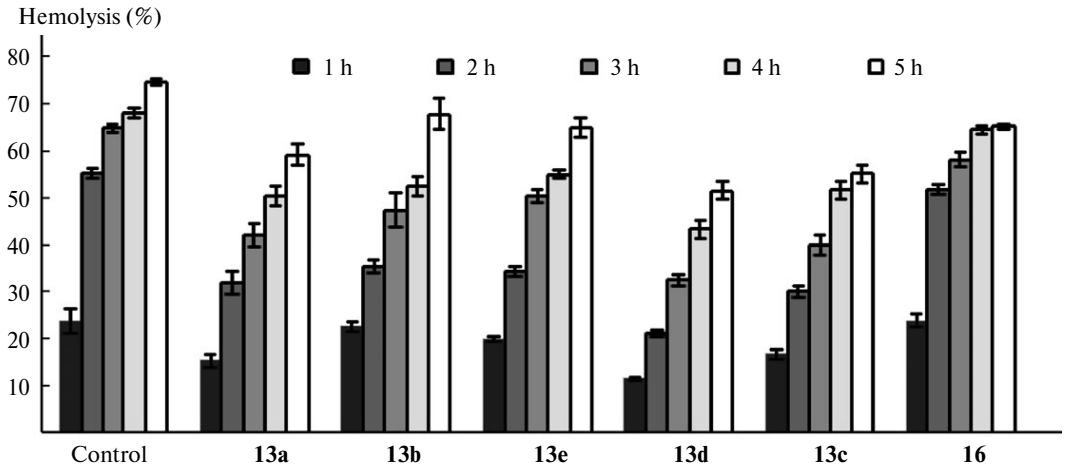


Fig. 2. Influence compounds **13a**—**e**, **16** in the concentration of 10 μmol L^{-1} on the degree of the H_2O_2-induced hemolysis of erythrocytes after 1—5 h of incubation.

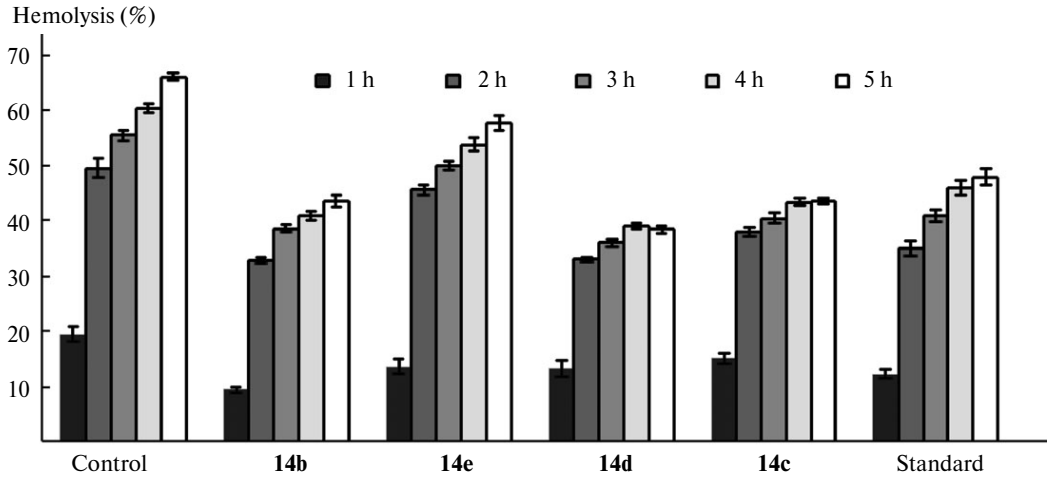


Fig. 3. Influence compounds **14b—e** and $Pr^n_2S_2$ (standard) in the concentration of 10 μmol L^{-1} on the degree of the H_2O_2-induced hemolysis of erythrocytes after 1—5 h of incubation.

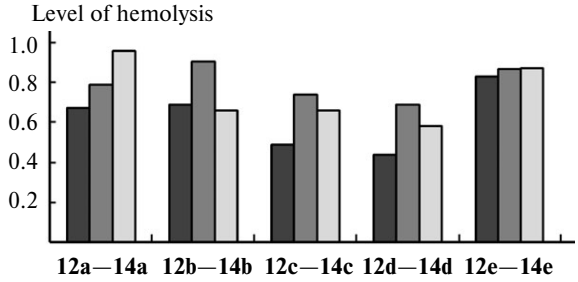


Fig. 4. Relative membranoprotective activity (the ratio of the hemolysis level in the tested and control samples 5 h after addition of H_2O_2) of compounds **12a—e—14a—e** in the concentration of 10 μmol L^{-1}.

A comparative evaluation of the membranoprotective activity showed (Fig. 4) that for most disulfides it depends on the structure of both the terpene and the carbohydrate fragments. Only for the conjugates with a *trans*-verbenyl moiety, the presence and the character of the carbohydrate part of the molecule have no influence on the biological activity of the compounds. Generally, it can be stated that the highest ability to protect cells from the influence of hydrogen peroxide is possessed by compounds containing *cis*-myrtanyl/myrtenyl and galactose fragments.

An ability of compounds to inhibit the process of peroxide oxidation of lipids (POL) in erythrocytes under conditions of the acute oxidation stress can be evaluated based on the data given in Fig. 5.

The disulfides with the *cis*-myrtanyl/myrtenyl fragments in a combintion with the galactose moiety inhibited accumulation of secondary products of POL most actively. A comparison reference and symmetric disulfides with two carbohydrate fragments showed relatively low activity in this model system, inhibiting POL to 72—80% from the control data.

All seventeen conjugates studied are characterized by the statistically significant positive dependence ($R_S = 0.56$, $p = 0.020$) between the ability to inhibit the accumulation

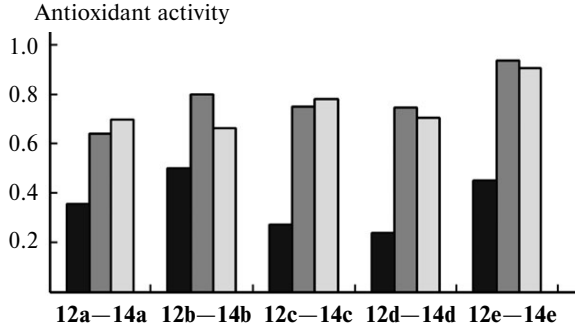


Fig. 5. Relative antioxidant activity (the ratio of the content of 2-thiobarbituric acid in in the tested and control samples 5 h after addition of H_2O_2) of compounds **12a—e—14a—e** in the concentration of 10 μmol L^{-1}.

of secondary products of POL and to protect cells from damage.

Finally, most disulfides obtained in this work (except those containing a *trans*-verbenyl fragment) exhibited the ability to inhibit transformation of oxyhemoglobin of erythrocytes to methemoglobin upon treatment with hydrogen peroxide, which also indicates the presence of a pronounced antioxidant activity. The highest activity was found in the conjugates with the myrtenyl/*cis*-myrtanyl fragments (independent of the structure of the second substituent). The preincubation of erythrocytes with these disulfides led to a 2—2.4 times decrease in the level of accumulation of methemoglobin as compared to the control samples (containing no compounds under study), whereas a comparison agent inhibited the formation of methemoglobin only by 1.4 times.

In conclusion, we for the first time synthesized symmetric and unsymmetric disulfides containing five terpene and two carbohydrate moieties in different combinations. A biological model system was used to show that the antioxidant and membranoprotective activity of the conjugates obtained depended on the structure of both substituents (the terpene and the carbohydrate fragments). The most active are disulfides, which have *cis*-myrtanyl, myrtenyl, and neomenthyl fragments in the composition, with the activity of compounds with a galactopyranose fragment being slightly higher than that of similar structures with a fructopyranose fragment. For symmetric disulfides containing no sugar moieties, the highest activity was found for compounds containing *cis*-myrtanyl, myrtenyl, and isobornyl fragments. A low activity in this model system was found for disulfides of different structure containing *trans*-verbenyl fragment.

The found relationships between the structure and the biological activity of disulfides with terpene and/or carbohydrate moieties in the composition allow us to carry out a purposeful synthesis of new biologically active compounds, promising for pharmacology and food production.

Experimental

IR spectra were recorded on a Shimadzu IR Prestige 21 Fourier-transform IR spectrometer for neat samples or in KBr pellets. Melting points were determined on a Gallencamp-Sanyo apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance-300 spectrometer (300.17 (^1H) and 75.48 MHz (^{13}C)) in solutions in $CDCl_3$, using residual signals of chloroform as a reference. The ^1H and ^{13}C signals were completely assigned using 2D homo- ($^1H—^1H$ COSY, $^1H—^1H$ NOESY) and heteronuclear experiments ($^1H—^{13}C$ HSQC, $^1H—^{13}C$ HMBC). Optical rotation angles were measured on a Kruss P3002RS automated digital polarimeter. Thin-layer chromatography was carried out on Sorbfil plates in the Et_2O—benzene, $CHCl_3—Et_2O$, and light petroleum—AcOEt solvent systems in different gradient ratio; solutions of phosphomolybdic acid and $KMnO_4$ were used for visualization. Elemental anlysis was carried out on an EA

1110 CHNS-O automated analyzer. All the reactions were carried out with freshly distilled solvents. Column chromatography was performed on Alfa Aesar silica gel (0.06—0.2 mm) in the same solvent systems as for TLC.

(1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexanethiol (neomenthanethiol (11a)) was obtained according to the procedure[22] from L-(−)-menthol (99%) made by Alfa Aesar ($[\alpha]_D^{25}$ −50.0, *c* 0.1, EtOH). A colorless liquid. The yield was 58%, $[\alpha]_D^{22}$ +53.2 (*c* 0.83, EtOH) (*cf.* Ref. 22: the yield 60%, $[\alpha]_D^{22}$ 53.0 (*c* 0.8, EtOH)).

(1*S*,2*S*,4*S*)-1,7,7-Trimethylbicyclo[2.2.1]heptane-2-thiol (isobornanethiol (11b)) was synthesized according to the procedure[23] from (−)-borneol (98%) made by Alfa Aesar ($[\alpha]_D^{22}$ −36.0, *c* 5.0, EtOH). A white powder. The yield was 83%, $[\alpha]_D^{22}$ +46.5 (*c* 0.1, EtOH) (*cf.* Ref. 23: the yield 85%, $[\alpha]_D^{22}$ +47.0 (*c* 0.1, EtOH)).

(1*R*,2*S*,5*R*)-(6,6-Dimethylbicyclo[3.1.1]hept-2-yl)methanethiol (*cis*-myrtanethiol (11c)) was obtained through thioacetate by a direct addition of thioacetic acid to (−)-β-pinene according to the described procedure.[24] A colorless liquid. The yield was 71%, $[\alpha]_D^{22}$ −45.6 (*c* 0.35, $CHCl_3$) (*cf.* Ref. 24: the yield 37%, $[\alpha]_D^{22}$ −75.9 (*c* 5.18, $CHCl_3$)).

(1*S*,5*R*)-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methanethiol (myrtenethiol (11d)) was synthesized according to the procedure[14] from (−)-β-pinene (99%) made by Alfa Aesar ($[\alpha]_D^{25}$ −21.0, without solvent). A colorless liquid. The yield was 51%, $[\alpha]_D^{22}$ −46.5 (*c* 0.1, EtOH) (*cf.* Ref. 14: the yield 84%, $[\alpha]_D^{22}$ −42.6 (*c* 0.4, $CHCl_3$)).

2,6,6-Trimethylbicyclo[3.1.1]hept-3-ene-2-thiol (*trans*-verbenethiol (11e)). The compounds MeC(O)SH (0.076 g, 1 mmol) and $ZnCl_2$ (0.014 g, 0.1 mmol) were sequentially added to *trans*-verbenol[25] (0.152 g, 1 mmol) ($[\alpha]_D^{25}$ +1.0, without solvent) in CH_2Cl_2 (5 mL) with stirring. The mixture was stirred for 10—15 min, treated with water (20 mL), and extracted with chloroform (3×10 mL). The combined organic layers were dried with $CaCl_2$, the solvent was evaporated *in vacuo*. The residue was dissolved in Et_2O (20 mL) and $LiAlH_4$ (0.019 g, 0.5 mmol) was added under argon. After 15 min, water (10 mL) was added slowly, extracted with light petroleum (3×15 mL), dried with $CaCl_2$, the solvent was evaporated *in vacuo*. The residue was separated by column chromatography (eluent light petroleum, visualization with $KMnO_4$). A colorless liquid. The yield was 90%. Found (%): C, 71.42; H, 9.48; S, 19.10. $C_{10}H_{16}S$. Calculated (%): C, 71.37; H, 9.58; S, 19.05. IR, ν/cm^{-1}: 675 (C—S), 820, 1372, 1645 (C=C), 2924. ^{1}H NMR ($CDCl_3$), δ: 0.90 (s, 3 H, 9′-Me); 1.35 (s, 3 H, 10′-Me); 1.31—1.43 (m, 1 H, H_A(7′)); 1.63 (d, 1 H, SH, *J* = 8.5 Hz); 1.72 (s, 3 H, 8′-Me); 2.03 (t, 1 H, H(5′), *J* = 5.4 Hz); 2.17—2.25 (m, 1 H, H(1′)); 2.35 (dt, 1 H, H_B(7′), *J* = 9.1 Hz, *J* = 5.6 Hz); 3.65—3.75 (m, 1 H, H(4′)); 5.26—5.32 (m, 1 H, H(3′)). ^{13}C NMR, δ: 20.37 (C(9′)), 22.63 (C(8′)), 26.52 (C(10′)), 28.29 (C(7′)), 40.81 (C(1′)), 42.82 (C(6′)), 47.35 (C(5′)), 49.54 (C(4′)), 119.82 (C(3′)), 145.69 (C(2′)).

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (5) was obtained according to the procedure.[26] The yield was 85%, $[\alpha]_D^{22}$ −45.6 (*c* 0.35, Me_2CO) (*cf.* Ref. 26: the yield 72—90%, $[\alpha]_D^{22}$ −46.0 (*c* 0.36, Me_2CO)).

1,2:3,4-Di-*O*-isopropylidene-6-*O*-(*p*-toluenesulfonyl)-α-D-galactopyranose (9) was synthesized according to the procedure.[27] The yield was 98%, $[\alpha]_D^{22}$ −44.6 (*c* 0.30, $CHCl_3$) (*cf.* Ref. 27: the yield 87%, $[\alpha]_D^{22}$ −44.0 (*c* 0.28, $CHCl_3$)).

1,2:3,4-Di-*O*-isopropylidene-6-thioacetyl-6-deoxy-α-D-galactopyranose (7) was obtained according to the procedure.[28] The yield was 77%, $[\alpha]_D^{22}$ −15.6 (*c* 0.30, $CHCl_3$) (*cf.* Ref. 28: the yield 99%, $[\alpha]_D^{22}$ −15.0 (*c* 1.8, $CHCl_3$)).

2,3:4,5-Di-*O*-isopropylidene-β-D-fructopyranose (6) was synthesized according to the procedure.[26] The yield was 85%, $[\alpha]_D^{22}$ −45.6 (*c* 0.35, Me_2CO) (*cf.* Ref. 26: the yield 90%, $[\alpha]_D^{22}$ −46.0 (*c* 0.36, Me_2CO)).

2,3:4,5-Di-*O*-isopropylidene-1-iodo-1-deoxy-β-D-fructopyranose (10) was obtained according to the procedure.[29] The yield was 64%, $[\alpha]_D^{22}$ −47.6 (*c* 0.88, $CHCl_3$).

2,3:4,5-Di-*O*-isopropylidene-1-thioacetyl-1-deoxy-β-D-fructopyranose (8) was synthesized according to the procedure.[30] The yield was 72%, $[\alpha]_D^{22}$ −3.1 (*c* 0.95, Me_2CO) (*cf.* Ref. 30: the yield 76%, $[\alpha]_D^{22}$ −2.9 (*c* 1.0, $CHCl_3$)).

1,2:3,4-Di-*O*-isopropylidene-6-mercapto-6-deoxy-α-D-galactopyranose (1) was obtained according to the procedure.[31] A dense yellow liquid. The yield was 91% (benzene—Et_2O, 10 : 1), $[\alpha]_D^{22}$ −70 (*c* 0.47, $CHCl_3$) (*cf.* Ref. 31: the yield 70%, $[\alpha]_D^{22}$ −88.7 ($CHCl_3$)).

2,3:4,5-Di-*O*-isopropylidene-1-mercapto-1-deoxy-β-D-fructopyranose (2) was obtained according to the procedure.[32] A white powder. The yield was 94% (benzene—Et_2O, 10 : 1), $[\alpha]_D^{22}$ −44.0 (*c* 0.40, $CHCl_3$) (*cf.* Ref. 32: the yield 65%, angle of rotation not reported).

Oxidation of thiols with iodine (general procedure). A solution of I_2 (0.127 g, 0.5 mmol) in EtOH (6 mL) was added slowly to a mixture containing a terpene thiol (1 mmol) and a thiol carbohydrate (0.276 g, 1 mmol) in ethanol (4 mL) with vigorous stirring until a yellow color was persistent. The mixture was stirred for 2 h, the solvent was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ (30 mL), and washed with saturated solution of $Na_2S_2O_3$ (2 mL). The organic layer was separated, dried with anhydrous $CaCl_2$, the solvent was evaporated *in vacuo*, the residue was separated by column chromatography (eluent $CHCl_3$—Et_2O, 5 : 1; visualization with $KMnO_4$).

6-{[(1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl]disulfanyl}-1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-D-galactopyranose (12a). A dense yellow liquid. The yield was 53% (0.125 g), $[\alpha]_D^{22}$ +33.0 (*c* 0.71, $CHCl_3$). Found (%): C, 59.02; H, 8.66; S, 14.24. $C_{22}H_{38}O_5S_2$. Calculated (%): C, 59.16; H, 8.58; S, 14.36. IR, ν/cm^{-1}: 646 (C—S), 999, 1071 (C—O), 1169 (O—C—O), 1211, 2918. ^{1}H NMR ($CDCl_3$), δ: 0.86—0.92 (m, 1 H, H_e(4′)); 0.91 (d, 3 H, 7′-Me, *J* = 6.6 Hz); 0.93 (d, 3 H, 10′-Me, *J* = 6.6 Hz); 1.01 (d, 3 H, 9′-Me, *J* = 6.6 Hz; 1.10—1.33 (m, 3 H, H(2′), H_e(6′), H_e(3′)); 1.37 (s, 3 H, 8-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 11-Me); 1.62 (s, 3 H, 9-Me); 1.58—1.81 (m, 3 H, H_e(4′), H(8′), H_a(3′)); 1.81—2.00 (m, 1 H, H(5′)); 2.25—2.35 (m, 1 H, H_a(6′)); 2.90 (dd, 2 H, H(6), *J* = 6.7 Hz, *J* = 2.1 Hz); 3.36—3.43 (m, 1 H, H(1)); 4.11—4.18 (m, 1 H, H(5)); 4.30—4.39 (m, 2 H, H(2), H(3)); 4.67 (dd, 1 H, H(4), *J* = 8.0 Hz, *J* = 2.2 Hz); 5.57 (d, 1 H, H(1), *J* = 5.0 Hz). ^{13}C NMR, δ: 20.74 (C(10′)), 21.20 (C(9′)), 22.11 (C(7′)), 24.47 (C(12)), 25.04 (C(8)), 25.91 (C(3′)), 25.98 (C(11), C(5′)), 26.25 (C(9)), 29.99 (C(8′)), 35.38 (C(4′)), 38.30 (C(6)), 40.12 (C(6′)), 48.80 (C(2′)), 53.70 (C(1′)), 66.67 (C(5)), 70.64 (C(2)), 70.98 (C(4)), 71.70 (C(3)), 96.67 (C(1)), 108.76 (C(7)), 109.30 (C(10)).

1-{[(1*S*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl]disulfanyl}-2,3;4,5-di-*O*-isopropylidene-1-deoxy-β-D-fructopyranose (13a). A yellow powder. M.p. 53—54 °C. The yield was 51% (0.120 g), $[\alpha]_D^{22}$ +99.0 (*c* 0.58, $CHCl_3$). Found (%): C, 59.08; H, 8.49;

S, 14.30. $C_{22}H_{38}O_5S_2$. Calculated (%): C, 59.16; H, 8.58; S, 14.36. IR, ν/cm^{-1}: 511 (S—S), 642 (C—S), 988, 1067 (C—O), 1165 (O—C—O), 2918. ^{1}H NMR ($CDCl_3$), δ: 0.89 (d, 3 H, 7′-Me, J = 6.3 Hz); 0.91 (d, 3 H, 9′-Me, J = 6.3 Hz); 0.85—0.95 (m, 1 H, H_e(4′)); 0.99 (d, 3 H, 10′-Me, J = 6.6 Hz); 1.06—1.30 (m, 3 H, H(2′), H_e(6′), H_e(3′)); 1.36 (s, 3 H, 12-Me); 1.48 (s, 3 H, 8-Me); 1.50 (s, 3 H, 11-Me); 1.55 (s, 3 H, 9-Me); 1.55—1.80 (m, 3 H, H_a(3′), H_a(4′), H(8′)); 1.80—1.98 (m, 1 H, H(5′)); 2.24—2.36 (m, 1 H, H_a(6′)); 3.10 (d, 1 H, H_A(1), J = 13.8 Hz); 3.31—3.42 (m, 2 H, H_B(1), H(1′)); 3.76 (d, 1 H, H_A(6), J = 12.9 Hz); 3.93 (d, 1 H, H_B(6), J = 12.7 Hz); 4.24 (d, 1 H, H(5), J = 7.7 Hz); 4.39 (d, 1 H, H(3), J = 2.5 Hz); 4.63 (dd, 1 H, H(4), J = 8.0 Hz, J = 2.5 Hz). ^{13}C NMR, δ: 20.84 (C(10′)), 21.18 (C(9′)), 22.15 (C(7′)), 24.10 (C(12)), 25.76 (C(8)), 25.87 (C(3′)), 25.93 (C(11)), 26.18 (C(5′)), 26.64 (C(9)), 26.89 (C(8′)), 35.41 (C(4′)), 39.91 (C(6′)), 48.84 (C(2′)), 49.06 (C(1)), 54.16 (C(1′)), 61.68 (C(6)), 70.39 (C(4)), 70.82 (C(5)), 71.96 (C(3)), 102.93 (C(2)), 108.66 (C(7)), 109.08 (C(10)).

1,2:3,4-Di-*O*-isopropylidene-6-{[(1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl]disulfanyl}-6-deoxy-α-D-galactopyranose (12b). A dense colorless liquid. The yield was 73% (0.243 g), $[\alpha]_D^{22}$ −106.0 (*c* 0.22, $CHCl_3$). Found (%): C, 59.49; H, 8.22; S, 14.49. $C_{22}H_{36}O_5S_2$. Calculated (%): C, 59.43; H, 8.16; S, 14.42. IR, ν/cm^{-1}: 511 (S—S), 646 (C—S), 1071 (C—O), 1140, 1169 (O—C—O), 2953. ^{1}H NMR ($CDCl_3$), δ: 0.84 (s, 3 H, 8′-Me); 0.92 (s, 3 H, 9′-Me); 1.05 (s, 3 H, 10′-Me); 1.10—1.33 (m, 2 H, H_{en}(5′), H_{en}(6′)); 1.37 (s, 3 H, 8-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 11-Me); 1.61 (s, 3 H, 9-Me); 1.64—1.81 (m, 3 H, H_{ex}(5′), H_{ex}(6′), H(4′)); 1.83—2.08 (m, 2 H, H_{en}(3′), H_{ex}(3′)); 2.85—3.08 (m, 3 H, H(2′), H(6)); 4.10—4.19 (m, 1 H, H(5)); 4.35 (q, 1 H, H(2), J = 4.9 Hz, J = 2.6 Hz); 4.40 (dd, 1 H, H(3), J = 7.8 Hz, J = 1.8 Hz); 4.66 (dd, 1 H, H(4), J = 7.8 Hz, J = 2.3 Hz); 5.57 (d, 1 H, H(1), J = 5.0 Hz). ^{13}C NMR, δ: 14.02 (C(10′)), 20.00 (C(8′)), 20.48 (C(9′)), 24.46 (C(12)), 25.03 (C(8)), 25.99 (C(11)), 26.08 (C(9)), 27.31 (C(5′)), 38.25 (C(6′)), 38.33 (C(6)), 40.51 (C(3′)), 45.93 (C(4′)), 47.16 (C(1′)), 49.89 (C(7′)), 63.93 (C(2′)), 66.44 (C(5)), 70.66 (C(2)), 70.93 (C(4)), 71.30 (C(3)), 96.72 (C(1)), 108.76 (C(7)), 109.26 (C(10)).

2,3;4,5-Di-*O*-isopropylidene-1-[(1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yldisulfanyl]-1-deoxy-β-D-fructopyranose (13b). A dense yellow liquid. The yield was 86% (0.342 g), $[\alpha]_D^{22}$ −24.0 (*c* 0.15, $CHCl_3$). Found (%): C, 59.49; H, 8.22; S, 14.49. $C_{22}H_{36}O_5S_2$. Calculated (%): C, 59.43; H, 8.16; S, 14.42. IR, ν/cm^{-1}: 520 (S—S), 579 (C—S), 1069 (C—O), 1130, 1165 (O—C—O), 2949. ^{1}H NMR ($CDCl_3$), δ: 0.84 (s, 3 H, 8′-Me); 0.91 (s, 3 H, 9′-Me); 1.06 (s, 3 H, 10′-Me); 1.09—1.32 (m, 2 H, H_{en}(5′), H_{en}(6′)); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 8-Me); 1.53 (s, 3 H, 11-Me); 1.57 (s, 3 H, 9-Me); 1.61—1.81 (m, 3 H, H_{ex}(5′), H_{ex}(6′), H(4′)); 1.80—2.07 (m, 2 H, H_{en}(3′), H_{ex}(3′)); 3.06 (dd, 1 H, H(2′), J = 9.4 Hz, J = 6.1 Hz); 3.13 (d, 1 H, H_A(1), J = 13.8 Hz); 3.44 (d, 1 H, H_B(1), J = 13.8 Hz); 3.79 (d, 1 H, H_A(6), J = 13.2 Hz); 3.94 (dd, 1 H, H_B(6), J = 12.9 Hz, J = 1.4 Hz); 4.26 (d, 1 H, H(5), J = 7.7 Hz); 4.36 (d, 1 H, H(3), J = 2.5 Hz); 4.64 (dd, 1 H, H(3), J = 7.8 Hz, J = 2.6 Hz). ^{13}C NMR, δ: 14.07 (C(10′)), 20.00 (C(8′)), 20.48 (C(9′)), 24.12 (C(12)), 25.29 (C(8)), 25.69 (C(11)), 25.99 (C(9)), 27.38 (C(5′)), 38.30 (C(6′)), 40.35 (C(3′)), 45.88 (C(4′)), 47.16 (C(1′)), 49.78 (C(7′), C(1)), 61.59 (C(6)), 63.96 (C(2′)), 70.40 (C(4)), 70.81 (C(5)), 72.11 (C(3)), 102.79 (C(2)), 108.59 (C(7)), 109.10 (C(10)).

6-[(*cis*)-(1*R*,2*R*,5*R*)-6,6-Dimethylbicyclo[3.1.1]hept-2-ylmethyldisulfanyl]-1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-D-galactopyranose (12c). A dense yellow liquid. The yield was 62% (0.195 g), $[\alpha]_D^{22}$ −113.0 (*c* 0.64, $CHCl_3$). Found (%): C, 59.51; H, 8.24; S, 14.31. $C_{22}H_{36}O_5S_2$. Calculated (%): C, 59.43; H, 8.16; S, 14.42. IR, ν/cm^{-1}: 511 (S—S), 646 (C—S), 1071 (C—O), 1169 (O—C—O), 2913. ^{1}H NMR ($CDCl_3$), δ: 0.83—1.04 (m, 1 H, H_A(6′)); 1.02 (s, 3 H, 9′-Me); 1.22 (s, 3 H, 8′-Me); 1.37 (s, 3 H, 8-Me); 1.38 (s, 3 H, 12-Me); 1.48 (s, 3 H, 11-Me); 1.48—1.64 (m, 1 H, H_A(6′)); 1.61 (s, 3 H, 9-Me); 1.75—2.16 (m, 3 H, H(1′), H(2′), H_B(4′)); 2.27—2.53 (m, 2 H, H(5′), H_B(6′)); 2.64—3.02 (m, 6 H, H(3′), H(10′), H(6)); 4.08—4.18 (m, 1 H, H(5)); 4.32—4.45 (m, 2 H, H(2), H(3)); 4.67 (dd, 1 H, H(4), J = 8.0 Hz, J = 2.2 Hz); 5.57 (d, 1 H, H(1), J = 5.0 Hz). ^{13}C NMR, δ: 21.81 (C(4′)), 23.31 (C(9′)), 24.48 (C(12)), 25.03 (C(8)), 26.00 (C(11)), 26.08 (C(9)), 27.93 (C(8′)), 33.25 (C(6′)), 38.04 (C(6)), 38.69 (C(7′)), 40.21 (C(5′)), 41.25 (C(1′)), 45.44 (C(3′)), 45.92 (C(10′)), 66.57 (C(5)), 70.61 (C(2)), 70.94 (C(4)), 71.50 (C(3)), 96.71 (C(1)), 108.79 (C(7)), 109.32 (C(10)).

1-[(*cis*)-(1*R*,2*R*,5*R*)-6,6-Dimethylbicyclo[3.1.1]hept-2-ylmethyldisulfanyl]-2,3;4,5-di-*O*-isopropylidene-1-deoxy-β-D-fructopyranose (13c). A dense yellow liquid. The yield was 60% (0.192 g), $[\alpha]_D^{22}$ −47.0 (*c* 0.32, $CHCl_3$). Found (%): C, 59.60; H, 8.09; S, 14.51. $C_{22}H_{36}O_5S_2$. Calculated (%): C, 59.43; H, 8.16; S, 14.42. IR, ν/cm^{-1}: 516 (S—S), 578 (C—S), 1065 (C—O), 1165 (O—C—O), 2913. ^{1}H NMR ($CDCl_3$), δ: 0.84—1.07 (m, 1 H, H_A(7′)); 1.01 (s, 3 H, 9′-Me); 1.21 (s, 3 H, 8′-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 8-Me); 1.53 (s, 3 H, 11-Me); 1.56 (s, 3 H, 9-Me); 1.45—1.62 (m, 1 H, H_A(4′)); 1.67—2.17 (m, 5 H, H(1′), H(2′), H_B(4′), H(3′)); 2.27—2.52 (m, 2 H, H(5′), H_B(7′)); 2.79—2.95 (m, 2 H, H(10′)); 3.09 (d, 1 H, H_A(1), J = 13.8 Hz); 3.36—3.45 (m, 3 H, H_B(1)); 3.79 (d, 1 H, H_A(6), J = 13.0 Hz); 3.94 (dd, 1 H, H_B(6), J = 12.9 Hz, J = 1.4 Hz); 4.26 (d, 1 H, H(5), J = 8.0 Hz); 4.33 (d, 1 H, H(3), J = 2.5 Hz); 4.63 (dd, 1 H, H(4), J = 8.0 Hz, J = 2.5 Hz)). ^{13}C NMR, δ: 20.17 (C(4′)), 21.83 (C(9′)), 24.13 (C(12)), 25.58 (C(8)), 25.94 (C(11)), 26.59 (C(9)), 26.00 (C(11)), 26.08 (C(9)), 26.10 (C(3′)), 27.94 (C(8′)), 33.25 (C(7′)), 38.69 (C(6′)), 40.12 (C(5′)), 41.26 (C(1′)), 45.58 (C(2′)), 46.37 (C(10′)), 49.69 (C(1)), 61.62 (C(6)), 70.41 (C(4)), 70.80 (C(5)), 72.24 (C(3)), 102.66 (C(2)), 108.60 (C(7)), 109.13 (C(10)).

1,2:3,4-Di-*O*-isopropylidene-6-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-ylmethyldisulfanyl]-6-deoxy-α-D-galactopyranose (12d). A dense yellow liquid. The yield was 58% (0.164 g), $[\alpha]_D^{22}$ −105.0 (*c* 0.94, $CHCl_3$). Found (%): C, 59.63; H, 7.51; S, 14.43. $C_{22}H_{34}O_5S_2$. Calculated (%): C, 59.70; H, 7.74; S, 14.49. IR, ν/cm^{-1}: 511 (S—S), 679 (C—S), 1071 (C—O), 1169 (O—C—O), 1645 (C=C), 2918. ^{1}H NMR ($CDCl_3$), δ: 0.85 (s, 3 H, 9′-Me); 1.16—1.31 (m, 1 H, H_A(4′)); 1.31 (s, 3 H, 8′-Me); 1.37 (s, 3 H, 8-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 11-Me); 1.61 (s, 3 H, 9-Me); 2.05—2.15 (m, 3 H, H(5′)); 2.21 (t, 1 H, H(1′), J = 5.2 Hz); 2.25—2.33 (m, 2 H, H(7′)); 2.38—2.48 (m, 1 H, H_B(4′)); 2.85—3.00 (m, 2 H, H(6)); 3.29—3.41 (m, 2 H, H(10′)); 4.12 (t, 1 H, H(5), J = 6.6 Hz); 4.35 (dd, 1 H, H(2), J = 5.0 Hz, J = 2.5 Hz); 4.41 (dd, 1 H, H(3), J = 8.0 Hz, J = 1.1 Hz); 4.66 (dd, 1 H, H(4), J = 7.8 Hz, J = 2.3 Hz); 5.50 (s, 1 H, H(3′)); 5.57 (d, 1 H, H(1), J = 5.0 Hz). ^{13}C NMR, δ: 21.29 (C(9′)), 24.47 (C(12)), 25.01 (C(8)), 25.98 (C(11)), 26.08 (C(9)), 26.16 (C(8′)), 31.43 (C(4′)), 31.74 (C(7′)), 37.95 (C(6)), 38.15 (C(6′)), 40.45 (C(5′)), 45.26 (C(1′)), 45.73 (C(10′)), 66.65 (C(5)), 70.63 (C(2)), 70.92 (C(4)), 71.36 (C(3)), 96.71 (C(1)), 108.74 (C(7)), 109.28 (C(10)), 121.63 (C(3′)), 142.83 (C(2′)).

2,3;4,5-Di-*O*-isopropylidene-1-[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-ylmethyldisulfanyl]-1-deoxy-β-D-fructopyr-

anose (13d). A dense yellow liquid. The yield was 52% (0.142 g), $[\alpha]_D^{22}$ –75.0 (*c* 0.51, $CHCl_3$). Found (%): C, 59.60; H, 7.58; S, 14.47. $C_{22}H_{34}O_5S_2$. Calculated (%): C, 59.70; H, 7.74; S, 14.49. IR, ν/cm^{-1}: 519 (S—S), 580 (C—S), 988, 1061 (C—O), 1165 (O—C—O), 1211, 1645 (C=C), 2931. ^{1}H NMR ($CDCl_3$), δ: 0.86 (s, 3 H, 9′-Me); 1.16—1.28 (m, 1 H, H_A(4′)); 1.31 (s, 3 H, 8′-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 8-Me); 1.54 (s, 3 H, 11-Me); 1.57 (s, 3 H, 9-Me); 2.06—2.16 (m, 1 H, H(5′)); 2.22 (t, 1 H, H(1′), *J* = 5.1 Hz); 2.25—2.34 (m, 2 H, H(7′)); 2.42 (dt, 1 H, H_B(4′), *J* = 8.6 Hz, *J* = 5.6 Hz); 3.12 (d, 1 H, H_A(1), *J* = 14.0 Hz); 3.35—3.46 (m, 3 H, H_B(1), H(10′)); 3.80 (d, 1 H, H_A(6), *J* = 12.9 Hz); 3.94 (dd, 1 H, H_B(6), *J* = 12.9 Hz, *J* = 1.4 Hz); 4.26 (d, 1 H, H(5), *J* = 8.0 Hz); 4.35 (d, 1 H, H(3), *J* = 2.5 Hz); 4.64 (dd, 1 H, H(4), *J* = 7.8 Hz, *J* = 2.6 Hz); 5.50 (s, 1 H, H(3′)). ^{13}C NMR, δ: 21.32 (C(9′)), 24.16 (C(12)), 25.59 (C(8)), 25.95 (C(11)), 26.17 (C(8′)), 26.59 (C(9)), 31.43 (C(4′)), 31.73 (C(7′)), 38.16 (C(6′)), 40.48 (C(5′)), 45.35 (C(1′)), 45.85 (C(10′)), 49.53 (C(1)), 61.60 (C(6)), 70.44 (C(4)), 70.82 (C(5)), 72.29 (C(3)), 102.63 (C(2)), 108.59 (C(7)), 109.15 (C(10)), 121.64 (C(3′)), 142.80 (C(2′)).

1,2:3,4-Di-*O*-isopropylidene-6-[(*trans*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-yldisulfanyl]-6-deoxy-α-D-galactopyranose (12e). A dense yellow liquid. The yield was 78% (0.269 g), $[\alpha]_D^{22}$ –84.0 (*c* 0.91, $CHCl_3$). Found (%): C, 59.57; H, 7.79; S, 14.42. $C_{22}H_{34}O_5S_2$. Calculated (%): C, 59.70; H, 7.74; S, 14.49. IR, ν/cm^{-1}: 511 (S—S), 646 (C—S), 1071 (C—O), 1169 (O—C—O), 1645 (C=C), 2930. ^{1}H NMR ($CDCl_3$), δ: 0.91 (s, 3 H, 9′-Me); 1.37 (s, 6 H, 10′-Me, 12-Me); 1.38 (s, 3 H, 8-Me); 1.38—1.48 (m, 1 H, H_A(7′)); 1.37 (s, 3 H, 8-Me); 1.38 (s, 3 H, 12-Me); 1.47 (s, 3 H, 11-Me); 1.61 (s, 3 H, 9-Me); 1.64—1.81 (m, 3 H, H_A(7′)); 1.48 (s, 3 H, 9-Me); 1.62 (s, 3 H, 11-Me); 1.73 (s, 3 H, 8′-Me); 2.06 (t, 1 H, H(5′), *J* = 5.3 Hz); 2.24—2.34 (m, 1 H, H_B(7′)); 2.40—2.48 (m, 1 H, H(1′)); 2.85—3.02 (m, 2 H, H(6)); 3.72—3.81 (m, 1 H, H(4′)); 4.16 (t, 1 H, H(5), *J* = 6.6 Hz); 4.36 (dd, 1 H, H(2), *J* = 5.0 Hz, *J* = 2.2 Hz); 4.37—4.43 (m, 1 H, H(3)); 4.67 (dd, 1 H, H(4), *J* = 8.0 Hz, *J* = 2.2 Hz); 5.27 (s, 1 H, H(3′)); 5.57 (d, 1 H, H(1), *J* = 5.0 Hz). ^{13}C NMR, δ: 20.60 (C(9′)), 22.98 (C(8′)), 24.49 (C(12)), 25.04 (C(8)), 26.00 (C(11)), 26.11 (C(9)), 26.50 (C(10′)), 28.58 (C(7′)), 38.87 (C(6)), 42.72 (C(6′)), 45.26 (C(1′)), 47.70 (C(5′)), 54.41 (C(4′)), 66.58 (C(5)), 70.64 (C(2)), 70.95 (C(4)), 71.58 (C(3)), 96.73 (C(1)), 108.81 (C(7)), 109.29 (C(10)), 115.65 (C(3′)), 149.22 (C(2′)).

2,3:4,5-Di-*O*-isopropylidene-1-[(*trans*)-2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-yldisulfanyl]-1-deoxy-β-D-fructopyranose (13e). A dense yellow liquid. The yield was 83% (0.305 g), $[\alpha]_D^{22}$ +6.0 (*c* 1.2, $CHCl_3$). Found (%): C, 59.41; H, 7.64; S, 14.59. $C_{22}H_{34}O_5S_2$. Calculated (%): C, 59.70; H, 7.74; S, 14.49. IR, ν/cm^{-1}: 517 (S—S), 573 (C—S), 1067 (C—O), 1165 (O—C—O), 1645 (C=C), 2932. ^{1}H NMR ($CDCl_3$), δ: 0.90 (s, 3 H, 9′-Me); 1.36 (s, 3 H, 10′-Me); 1.37 (s, 3 H, 12-Me); 1.34—1.50 (m, 1 H, H_A(7′)); 1.48 (s, 3 H, 8-Me); 1.52 (s, 3 H, 11-Me); 1.56 (s, 3 H, 9-Me); 1.72 (s, 3 H, 8′-Me); 2.05 (t, 1 H, H(5′), *J* = 5.4 Hz); 2.28 (dt, 1 H, H_B(7′), *J* = 9.1 Hz, *J* = 5.6 Hz); 2.41—2.53 (m, 1 H, H(1′)); 3.07 (d, 1 H, H_A(1), *J* = 13.8 Hz); 3.49 (d, 1 H, H_B(1), *J* = 13.8 Hz); 3.73—3.98 (m, 3 H, H(4′), H(6)); 4.96 (d, 1 H, H(5), *J* = 8.0 Hz); 4.34 (d, 1 H, H(3), *J* = 2.5 Hz); 4.63 (dd, 1 H, H(4), *J* = 7.7 Hz, *J* = 2.2 Hz); 5.28 (s, 1 H, H(3′)). ^{13}C NMR, δ: 20.58 (C(9′)), 22.94 (C(8′)), 24.10 (C(12)), 25.60 (C(8)), 25.97 (C(11)), 26.49 (C(9)), 26.60 (C(10′)), 28.55 (C(7′)), 42.68 (C(6′)), 45.21 (C(1′)), 47.71 (C(5′)), 50.75 (C(1)), 55.20 (C(4′)), 61.59 (C(6)), 70.40 (C(4)), 70.80 (C(5)), 72.23 (C(3)), 102.71 (C(2)), 108.57 (C(7)), 109.09 (C(10)), 115.72 (C(3′)), 149.04 (C(2′)).

Bis(1,2;3,4-di-*O*-isopropylidene-6-deoxy-α-D-galactopyranos-6-yl) disulfide (15). A dense yellow liquid. The yield was 13—20%, $[\alpha]_D^{22}$ –87.0 (*c* 1.70, $CHCl_3$) (*cf.* Ref. 33: $[\alpha]_D^{22}$ –75.3 (*c* 0.36, $CHCl_3$)).

Bis(2,3:4,5-di-*O*-isopropylidene-1-deoxy-β-D-fructopyranos-1-yl) disulfide (16). A dense yellow liquid. The yield was 15—18%, $[\alpha]_D^{22}$ –55.0 (*c* 0.67, $CHCl_3$) (*cf.* Ref. 30: $[\alpha]_D^{22}$ –54.1 (*c* 1.00, $CHCl_3$)).

Bis[(1*S*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl] disulfide (14a). White crystals. M.p. 52—53 °C. The yield was 30—35%, $[\alpha]_D^{22}$ +353.0 (*c* 1.1, Me_2CO) (*cf.* Ref. 34: $[\alpha]_D^{22}$ +350.0 (*c* 1.0, Me_2CO)).

Bis[(1*S*,2*S*,4*S*)-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl] disulfide (14b). A white powder. M.p. 215—216 °C. The yield was 20—22%, $[\alpha]_D^{22}$ –83.0 (*c* 0.90, $CHCl_3$) (*cf.* Ref. 35: $[\alpha]_D^{22}$ –81.7 (*c* 0.88, $CHCl_3$)).

Bis[*trans*-(1*S*,2*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-ylmethyl] disulfide (14c). A yellow liquid. The yield was 25—30%, $[\alpha]_D^{22}$ –73.8 (*c* 0.60, $CHCl_3$). Found (%): C, 70.98; H, 10.17; S, 18.85. $C_{20}H_{34}S_2$. Calculated (%): C, 70.94; H, 10.12; S, 18.94. IR, ν/cm^{-1}: 540 (S—S), 615 (C—S), 874 (C—S), 937, 2910. ^{1}H NMR ($CDCl_3$), δ: 0.84—1.02 (m, 1 H, H_A(7′)); 1.02 (s, 3 H, 8′-Me); 1.23 (s, 3 H, 9′-Me); 1.44—1.66 (m, 1 H, H_A(3′)); 1.67—2.17 (m, 5 H, H(1′), H_B(3′), H(4′), H(5′)); 2.26—2.50 (m, 2 H, H(2′), H_B(7′)); 2.70—2.87 (m, 2 H, H(10′)). ^{13}C NMR, δ: 21.91 (C(3′)), 23.30 (C(8′)), 26.14 (C(4′)), 27.97 (C(9′)), 33.31 (C(7′)), 38.70 (C(6′)), 40.32 (C(2′)), 41.31 (C(5′)), 45.42 (C(1′)), 46.19 (C(10′)).

Bis[(1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-ylmethyl] disulfide (14d). A dense yellow liquid. The yield was 26—30%, $[\alpha]_D^{22}$ –36.8 (*c* 0.52, $CHCl_3$). Found (%): C, 71.83; H, 9.15; S, 19.02. $C_{20}H_{30}S_2$. Calculated (%): C, 71.80; H, 9.04; S, 19.17. IR, ν/cm^{-1}: 542 (S—S), 599 (C—S), 1645 (C=C), 2918. ^{1}H NMR ($CDCl_3$), δ: 0.87 (s, 3 H, 9′-Me); 1.17—1.36 (m, 1 H, H_A(4′)); 1.33 (s, 3 H, 8′-Me); 2.07—2.18 (m, 1 H, H(5′)); 2.23 (t, 1 H, H(1′), *J* = 5.0 Hz); 2.26—2.36 (m, 2 H, H(7′)); 2.44 (td, 1 H, H_B(4′), *J* = 8.6 Hz, *J* = 5.6 Hz); 3.25—3.39 (m, 2 H, H(10′)); 5.49 (s, 1 H, H(3′)). ^{13}C NMR, δ: 21.34 (C(9′)), 26.21 (C(8′)), 31.44 (C(4′)), 31.78 (C(7′)), 38.19 (C(6′)), 40.50 (C(5′)), 45.36 (C(1′)), 45.74 (C(10′)), 121.32 (C(3′)), 143.02 (C(2′)).

Bis[2,6,6-trimethylbicyclo[3.1.1]hept-2-en-4-yl] disulfide (14e). A colorless liquid. The yield was 24—26%. Found (%): C, 71.70; H, 9.14; S, 19.16. $C_{20}H_{30}S_2$. Calculated (%): C, 71.80; H, 9.04; S, 19.17. IR, ν/cm^{-1}: 567 (S—S), 611 (C—S), 1645 (C=C), 2924. ^{1}H NMR ($CDCl_3$), δ: 0.92 (s, 3 H, 9′-Me); 1.38 (s, 3 H, 10′-Me); 1.40—1.50 (m, 1 H, H_A(6′)); 1.75 (s, 3 H, 8′-Me); 2.06 (t, 1 H, H(5′), *J* = 5.4 Hz); 2.23—2.35 (m, 1 H, H_B(6′)); 2.38—2.49 (m, 1 H, H(1′)); 3.68 (s, 1 H, H(4′)); 5.33 (s, 1 H, H(3′)). ^{13}C NMR, δ: 20.62 (C(9′)), 22.97 (C(8′)), 26.50 (C(10′)), 28.65 (C(6′)), 42.77 (C(7′)), 45.36 (C(1′)), 47.69 (C(5′)), 55.08 (C(4′)), 116.05 (C(3′)), 148.74 (C(2′)).

Evaluation of toxicity, antioxidant, and membranoprotective activity of obtained compounds. For *in vitro* evaluation of toxicity, antioxidant and membranoprotective activity, a 0.5% (v/v) suspension of laboratory mice erythrocytes in the phosphate-buffered saline (PBS, pH 7.4) was used. Ethanol was used as a solvent, while acetone was used for symmetric disulfides with two terpene fragments **14a—e**, which are poorly soluble in ethanol. Di-*n*-propyl disulfide with known antioxidant properties was chosen as a standard.[36]

Toxicity of compounds was evaluated based on their ability to induce hemolysis. Solutions of compounds were added to a suspension of erythrocytes and incubated for 5 h at 37 °C in a Biosan ES-20 temperature-controlled shaker (Latvia). Control samples contained a corresponding solvent (0.1% of a total volume of the incubation mixture). Membranoprotective and antioxidant activity of disulfides were determined based on their inhibition degree of the induced hemolysis, the inhibition of the accumulation of secondary products POL and the oxidation of oxyhemoglobin in erythrocytes. To accomplish this, hemolysis was initiated with an aqueous solution of hydrogen peroxide (0.006%) 30 min after the addition of solutions of compounds under study to the suspension of erythrocytes. Then, the reaction mixture was incubated for 5 h at 37 °C in a temperature-controlled shaker with a slow stirring.

An aliquot was collected from the incubation media, centrifuged for 5 min (1600 g), hemolysis degree was determined based on the content hemoglobin in the supernatant on a ThermoSpectronic Genesys 20 spectrophotometer (USA) at λ = 524 nm.[19] Completely hemolyzed sample served as a reference to calculate percentage of hemolysis.[18] The content of secondary products POL reacting with 2-thiobarbituric acid was determined spectrophotometrically.[37] To evaluate accumulation of the oxidation products of hemoglobin, the absorption spectrum of hemolyzate was analyzed, using a Lyumeks Flyuorat-02-Panorama spectrofluorimeter. The contents of oxyhemoglobin (oxyHb), methemoglobin (metHb), and ferrylhemoglobin (ferrylHb) were calculated with allowance for the corresponding molar absorption coefficients.[38] Each experiment was carried out 4—5 times. Statistical processing of data and building of diagrams were accomplished using the Microsoft Office Excel 2007 software package.

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