Conjugates of polyhedral boron compounds with carbohydrates
8.* Synthesis and properties of *nido-ortho*-carborane glycoconjugates containing one to three β-lactosylamine residues**

L. M. Likhosherstov,[a] O. S. Novikova,[a] A. O. Chizhov,[a] I. B. Sivaev,[b] and V. I. Bregadze[b]*

[a]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499) 135 5328. E-mail: likhosherstov@mail.ru*
[b]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation*

β-Lactosylamine derivatives containing one to three disaccharide residues with the bromine atom at the terminal position of aglycon were synthesized. Conjugation of these derivatives with 1-mercapto-1,2-dicarba-*closo*-dodecaborane was carried out in DMSO, which was accompanied by deboronation to form *nido-ortho*-carborane (7,8-dicarba-*nido*-undecaborate) glycoconjugates in up to 70% yield. The hydrolytic stability of glycoconjugates and the binding efficiency of their triethylammonium salts to castor-bean galectin (*Ricinus communis* aggluinin, RCA_{120}) were estimated. Glycoconjugates are decomposed in an aqueous solution by ~15% at 37 °C in three days. The glycoconjugate salt with one β-lactosylamine residue binds to galectin analogously to lactose; after indroduction of the second β-lactosylamine residue into glycoconjugate, the binding efficiency increases ~6-fold due to the cluster effect.

Key words: glycoconjugates, β-lactosylamine, 1-mercapto-1,2-dicarba-*closo*-dodecaborane, *nido-ortho*-carborane, galectin RCA_{120}.

closo-ortho-Carborane (1,2-dicarba-*closo*-dodecarborane) is a polyhedral boron compound widely used for design of novel drugs, *viz.*, potential agents for cancer therapy and detection, including the conjugates containing carbohydrates.[2] Two approaches to the synthesis of *closo-ortho*-carborane glycoconjugates are known (see Ref. 3 and references cited therein). The first approach is based on the addition of decaborane to the triple bond being a part of the spacer of the *O*- and *C*-glycosides. The second approach is based on conjugation of different functional *closo-ortho*-carborane derivatives containing the hydroxyl, carboxyl, and amino groups with the corresponding mono- or disaccharide derivatives. We used such approach in the synthesis of glycoconjugates from *ortho*-carboranylacetic acid and derivatives of lactose linked through the *O*- or *N*-glycoside bond with aglycon containing the terminal amino group (see Refs 4 and 5 and references cited therein).

The *nido-ortho*-carborane conjugates are prepared from the *closo-ortho*-carborane conjugates by deboronation in the presence of bases.[2] These conjugates possess a higher water solubility and allow easy introduction of radioactive halogens or metals to the *nido-ortho*-carborane residue and preparation of the agents for radiologic diagnosis and cancer therapy in this way.[2] In the case of glycoconjugates, partial deboronation of the *closo-ortho*-carborane residue to form the *nido-ortho*-carborane glycoconjugate has been observed as a side process upon conjugation[6] and alkaline treatment for deacetylation[7], as well as in aqueous solutions of glycoconjugates.[1,8]

This work is aimed at the synthesis of novel glycoconjugates by *S*-alkylation of 1-mercapto-1,2-dicarba-*closo*-dodecaborate ($[1\text{-S-}1,2\text{-}C_2B_{10}H_{11}]^-PyH^+$ (**1**)[9a]) with the spacered derivatives of β-lactosylamine containing one to three disaccharide residues with the bromine atom in the terminal position of aglycon. The *S*-alkylation reaction has not been used for the synthesis of *closo*- и *nido*-carborane conjugates and was described only by the example of the 1-mercapto-*closo*-dodecaborate anion $[B_{12}H_{11}SH]^{2-}$ and peracetylated derivatives of glucose or glucuronic acid with the bromine or iodine atom in the positions 6 or 1 of monosaccharide (see Ref. 9b and references therein). The acetyl groups in glucoconjugates were not removed and an attempt to deacetylation (only one example desribed in the literature) afforded the glycoconjugate in a low yield. The use of the bromine derivatives of lactosylamine with the free hydrogyxl groups allowed us to not only reduce the number of synthetic steps, but also avoid complications noted upon deacetylation of the *closo-ortho*-carbo-

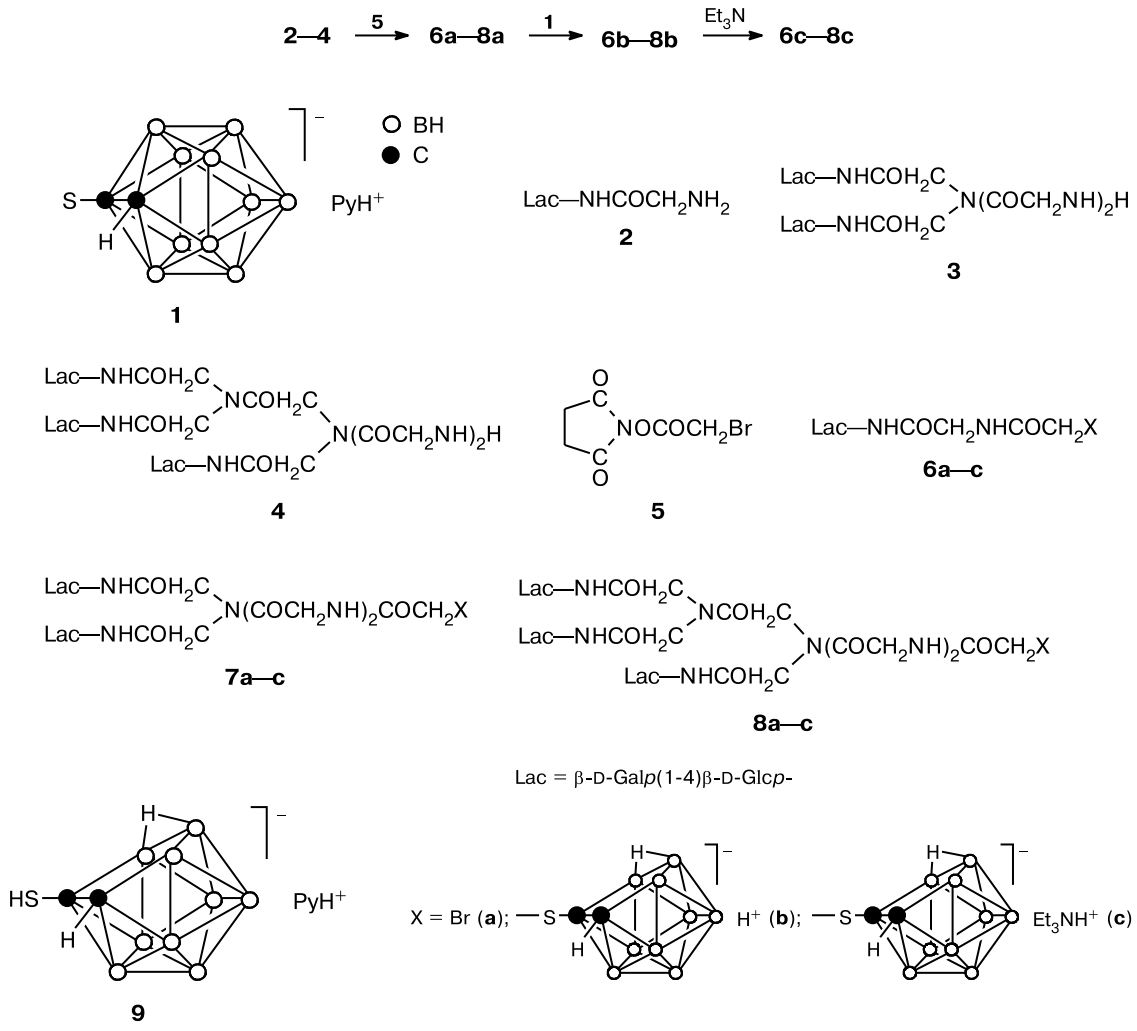
* For Part 7 see Ref. 1.
** Dedicated to the Academician of the Russian Academy of Sciences O. M. Nefedov on occasion of his 80th anniversary.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2312—2317, November, 2011.
1066-5285/11/6011-2359 © 2011 Springer Science+Business Media, Inc.

rane glycoconjugates.[7] Unlike the published works[9b] on the synthesis of glycoconjugates based on the $[B_{12}H_{11}SH]^{2-}$ anion without using the spacer, for conjugation with carboranylthiol **1** we used as before the lactosylamine derivatives with the spacer containing at least six atoms. It is known[10] that the pharmacophore fragment in a glucoconjugate containing a long spacer does not hinder the carbohydrate-protein interaction, which increases the transport efficiency of glycoconjugate to a target cell containing carbohydrate-specific receptors (proteins, so called lectins). The synthesized in the present work lactosylamine derivatives with two and three disaccharide residues allowed us to rely on increase in the galectin-binding efficiency of glycoconjugate due to the so called polyvalent or cluster effect.

We used amino compounds **2**—**4** (see Ref. 11) as the starting compounds for the synthesis of spacered bromine derivatives of lactosylamine containing one to three disaccharide resudes. *N*-Acylation of these derivatives was performed using the excess (1.5—2 mol) of *N*-bromoacetoxysuccinimide (**5**) in DMSO (Scheme 1). According to the electrophoretic data, at the end of the reaction, there were ~5—10% of the starting amino compound, ~80—90% of a neutral product, and ~5—10% a side positively charged product with a lower electrophorectic mobility than that of amino compounds **2**—**4** (there appears to be *N*-alkylated product, which is suggested by the presence of the bromide ion in the reaction mixture). After isolation and purification, the bromine compounds **6a**—**8a** were obtained in 70—88% yields, their structures being confirmed by the data of elemental analysis (for **6a**), mass spectrometry (for **7a**—**8a**), and ^{1}H NMR spectroscopy (for **6a**—**8a**). In the NMR spectra, the chemical shifts of the protons of the bromine compounds **6a**—**8a** and corresponding starting amino compounds **2**—**4** were almost identical, besides the chemical shifts of the protons of the CH_2NH_2 group, which were shifted downfield by δ_H ~0.6 after acylation.

Scheme 1



Conjugation of the bromine derivatives **6a**—**8a** with pyridinium 1,2-dicarbadodecaboran-1-ylthiolate **1** was performed in DMSO at ~22 °C for 24 h. It followed from the electrophoretic data that after this time, ~5—10% of the starting compounds **6a**—**8a** remained and the main reaction product is a negatively charged compound, *i.e.*, it seems that deboronation occured to form the *nido-ortho*-carborane glucoconjugates **6b**—**8b**, which was confirmed by subsequent investigation. ^{1}H NMR spectroscopy showed that *closo*-carborane **1** is deboronated rapidly in DMSO-d_6 to form *nido*-carborane **9**. After 5 min dissolution of carborane **1**, the spectrum exhibited the signal for HC_{carb} at δ_H 5.08 in the region typical of *closo*-carboranes and, after 2 h (no trasnformation dynamics was recorded), this signal was absent and a new signal appeared at δ_H 2.12 in the region typical of *nido*-carboranes.

Earlier,[12] such fast deboronation has been observed for the α-carbonyl-containing *closo-ortho*-carboranes in DMSO, while they were stable in dry DMSO and other solvents (MeOH, THF, acetone, acetonitrile, and dioxane containing up to 5% of H_2O). It should be noted that due to the solubility problems of the bromine compounds **6a**—**8a** and carborane **1**, for conjugation we could use only DMSO as a solvent. Since we used DMSO without drying (see Experimental) and the starting lactose derivatives **6a**—**8a** could contain a small amount of hard-to-remove water, apparently, some amount of water was present in the reaction mixture.

After isolation of glycoconjugates from the reaction mixtures and final chromatographic purification, the *nido-ortho*-carborane glycoconjugates **6b**—**8b** were obtained in yields of 61—70% and no corresponding *closo-ortho*-carborane glucoconjugates were found.

The structures of *nido-ortho*-carborane glycoconjugates **6b**—**8b** were confirmed by ^{1}H and ^{11}B NMR spectroscopy and mass spectrometry. The high-resolution negative-ion mass spectra of these glycoconjugates contained isotope clusters typical of carboranes; the *m/z* values of these components had a good agreement ($|\delta| < 5$ ppm) with the corresponding calculation data. The ^{1}H NMR spectra exhibited the signal of the HC_{carb} proton at δ_H ~2.2 typical of *nido-ortho*-carboranes and proton signals analogous to those of the corresponding bromine compounds **6a**—**8a**, besides the signals of the CH_2Br protons. These signals as part of the CH_2S group were shifted upfield by δ_H ~0.5. The ^{11}B NMR spectra of glucoconjugates **6b**—**8b** contained eight signals for the boron atom (one signal of double intensity) in the region of δ_B from −37 to −11 typical of *nido-ortho*-carboranes.

Thus, it was shown for the first time that upon conjugation in wet DMSO, one can prepare the *nido-ortho*-carborane glucoconjugates from *closo-ortho*-carboranylthiol **1** in high yields. Our results and literature data[12] allow assumption of that *nido-ortho*-carborane glucoconjugates can be synthesized from other *closo-ortho*-carborane derivatives by pre-estimating their stabilities using ^{1}H NMR spectroscopy in DMSO-d_6.

Earlier,[1] we have reported that the *closo-ortho*-carborane glycoconjugates can be deboronated partially in aqueous solutions at 5, 20, 37, and 60 °C to form the *nido-ortho*-carborane glycoconjugates. It was noted also that degradation did not terminate at this point and continued slowly to form boric acid and other fragments, which were mainly unidentified. In this regard, by considering glycoconjugates **6b**—**8b** as potential agents for cancer therapy and diagnostics, aqueous solutions of glycoconjugates were kept at 37 °C for three days. The products of hydrolysis were separated by reversed-phase chromatography in water to give two fractions. As followed from the electrophoretic and ^{1}H NMR spectral data, the fraction eluted with delay on sorbent contained the starting glycoconjugate (~85%) and the fraction eluted without delay on sorbent contained unidentified fragments. The NMR spectrum of this fraction exhibited broadened and doubled signals for the protons of the carbohydrate part of the corresponding glycoconjugates and the signal for the HC_{carb} proton was absent. The comparison of the hydrolytic stabilities of the *closo*- and *nido-ortho*-carboranes (without considering the influence of the spacer structure) shows that in 0.05 *M* aqueous solutions at 37 °C for three days, the *closo-ortho*-carborane glycoconjugates remained unchanged by ~70% (see Ref. 1) and the *nido-ortho*-carborane glycoconjugates **6b**—**8b** remained unchaged by ~85%.

In Ref. 8, by hemagglutination we estimated the galectin-binding efficiency (RCA_{120}) of the *closo-ortho*-glycoconjugates containing one to three lactosylamine residues. We attempted to perform the analogous study for the *nido-ortho*-carborane glyconjugates. However, glycoconjugates **6b**—**8b** themselves induce hemagglutination. Assuming that this is due to the acid nature of the *nido-ortho*-carborane glycoconjugates, we prepared their triethylammonium salts **6c**—**8c**, which did not cause hemagglutination, and estimated the galectin-binding efficiency. As it follows from Table 1, lactose and salt **6c** containing one lactosylamine residue possess close inhibiting properties. Due to two- or three-pointing binding to lectin, the glycoconjugate salts **7c** and **8c** containing two or three lactosylamine residues possess a higher inhibiting ability than the glycoconjugate salt **6c** containing one lactosylamine resi-

Table 1. The minimum concentration (C_{min}) of the triethylammonium salts **6c**—**8c** and lactose inhibiting hemagglutination with galectin RCA_{120}

Inhibitor	$C_{min} \cdot 10$/mmol L^{-1}
6c	0.81
7c	0.14
8c	0.14
Lactose	0.92

due, *i.e.*, the so called polyvalent or cluster effect was observed for them. The minimum inhibiting concentration of salts **7c** and **8c** with two and three lactosylamine residues decreased ~6-fold as compared with that of salt **6c** with one lactosylamine residue. It should be noted that the presence of the third lactosylamine residue had no effect on the increase in the lectin-binding efficiency. Thus, introduction of two lactosylamine residues to glycoconjugate can be even sufficient for manifestation of the cluster effect.

Experimental

^{1}H NMR spectra were recorded in D_2O on a Bruker AM-300 (300.13 MHz) spectrometer and ^{11}B NMR spectra were recorded on a Bruker DRX-500 spectrometer relative to the signals for acetone and $BF_3 \cdot Et_2O$, respectively (external standards). High-resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF II instrument by electrospray ionization (ESI).[13] The measurements were performed on positive and/or negative ions (capillary voltage is 4500 or 3200 V, respectively). The mass scanning range is m/z 50—3000 Da and calibration was external or internal (Electrospray Calibrant Solution, Fluka); the m/z values for experimental monoisotope clusters (for **7a** and **8a**) or the most intensive (for **6b**—**8b**) peaks of isotope clusters and their corresponding calculated m/z values are given. Syringe injection of a substance dissolved in aqueous methanol and diluted with MeCN or MeCN—H_2O (1 : 1) was used. The flow rate was 3 μL min^{-1}. The dispensing gas was nitrogen (4 L min^{-1}) and the interface temperature was 180 °C. Optical rotation was determined on a PU-07 polarimeter (Russia). Electrophoresis (30 V cm^{-1}, 1 h) was performed on a Filtrak FN1 paper in a pyridinium acetate buffer (0.025 mol L^{-1} based on Py, pH 4.5) on a EFA-1 instrument (USSR). Substances were determined using the reagent sequence KIO_4—$AgNO_3$—KOH (see Ref. 14). The reactions were carried out in the dark in the argon atmosphere and concentrating was performed under the pressure of ~10 Torr at the bath temperature of ~30 °C. DMSO (pure, Chemapol) recrystallized twice was used. The elution of substances upon column chromatography on Silica gel 100 C_{18}-Reversed phase (Fluka) was monitored by UV absorption at 206 nm.

***N*-Bromoacetoxysuccinimide (5).** To a ice-cooled solution of bromoacetic acid (2.82 g, 0.02 mol) and *N*-hydroxysuccinimide (2.45 g, 0.021 mol) in dry THF (20 mL), a cold solution of DCC (4.65 g, 0.0226 mol) in dry THF was added with stirring and the reaction mixture was kept at 5 °C for six days. The precipitate was filtered off and washed with THF and the solution was evaporated to dryness. The residue was washed with petroleum ether (b.p. 40—70 °C)—THF (2 : 1) (2 × 30 mL) and petroleum ehter to yield ester **5** (4.6 g, 97%). ^{1}H NMR, ($CDCl_3$), δ: 2.88 (s, 4 H, CH_2CH_2); 4.12 (s, 2 H, CH_2Br). The spectrum contained minor signals (δ_H ~1.0—2.0) for impurities, which did not prevent the use ester **5**.

***N*-[*N*-(Bromoacetyl)glycyl]-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosylamine (6a).** To a solution of 4-*O*-(β-D-galactopyranosyl)-*N*-glycyl-β-D-glucopyranosylamine (**2**)[11] (0.1 g, 0.25 mmol) in H_2O (0.35 mL), DMF (1.4 mL) was added and the mixture was stirred and ice-cooled. Ester **5** (0.075 g, 0.318 mmol) was added and the mixture was stirred until the ester was dissolved and kept at 0 °C for 3 h. The reaction temperature was increased to ~22 °C and DMF (1.5 mL) was added dropwise with stirring and the reaction mixture was kept at ~22 °C for 20 h. The precipitate was filtered off and washed with 90% aqueous DMF (0.5 mL), acetone, and Et_2O and dried to yield compound **6a** (0.05 g). The mother liquid was concentrated to remove volatile impurities (the residue is a solution in DMF) and Et_2O (30 mL) was added with stirring. After clarification, the liquid was decanted from the oily precipitate. The precipitate was tritirated several times with Et_2O (3 mL) and acetone (3 mL) until a powder was obtained. The powder was washed with dry EtOH (3×3 mL) and dried. The residue was dissolved in H_2O (1 mL), cationite KU-2 (H^+) (1 mL) was added, and the reaction mixture was stirred for 30 min. The resin was filtered off and washed with water (4×2.5 mL). The solution was concentrated to ~3 mL, filtered through a filter (0.45 μm), and concentrated to ~0.5 mL. MeOH was added with stirring until a precipitate formed. The precipitate was filtered off, washed with MeOH and Et_2O, and dried to yield additional portion of amorphous compound **6a** (0.042 g, the overall yield is 70%), $[\alpha]_D^{22}$ +3.2 (*c* 1, H_2O). Found (%): C, 37.11; H, 5.14; Br, 14.69; N, 5.34. $C_{16}H_{27}BrN_2O_{12}$. Calculated (%): C, 37.00; H, 5.24; Br, 15.39; N, 5.39. ^{1}H NMR, δ: 3.44—3.60 (m, 2 H); 3.63—3.87 (m, 8 H); 3.93 (br.s, 2 H); 4.02 (s, 2 H, CH_2); 4.05 (br.s, 2 H, CH_2); 4.45 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz).

***N*-[*N*-(Bromoacetyl)diglycyl]-*N*-di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-gluclopyranosyl]carbamoylmethyl}amine (7a).** A suspension of *N*-di{*N*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]carbamoylmethyl}-*N*-(diglycyl)amine (**3**)[11] (0.22 g, 0.23 mmol) in H_2O (0.7 mL) was heated at 50 °C until dissolution, cooled to ~21 °C and a solution of ester **5** (0.111 g, 0.47 mmol) in DMF (2.8 mL) was added. The reaction mixture was kept at ~21 °C for 3 h and then H_2O was removed. Et_2O (30 mL) was added with stirring to the solution in DMF and the mixture was left at 5 °C for 20 h. The precipitate was filtered off, washed several times with Et_2O and hot acetone, and dried. The residue was dissolved in H_2O (1 mL) and MeOH was added with stirring until precipitation. The mixture was heated to ~50 °C and ketp at ~21 °C for 3 h and at 5 °C for 20 h. The precipitate that formed was filtered off, washed with 80% aqueous MeOH (2×3 mL), MeOH, and Et_2O, and dried to yield amorphous compound **7a** (0.18 g, 70%), $[\alpha]_D^{20}$ +3.8 (*c* 1, H_2O). ^{1}H NMR, δ: 3.43—3.60 (m, 4 H); 3.61—3.87 (m, 16 H); 3.88—3.98 (m, 4 H); 4.01 (s, 2 H, CH_2); 4.02 (s, 2 H, CH_2); 4.14 (br.s, 2 H, CH_2); 4.17 (br.s, 2 H, CH_2); 4.36 (br.s, 2 H, CH_2); 4.44 (br.d, 2 H, 2 H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz); 5.07 (d, 1 H, H(1) Glc, J = 9.0 Hz). MS (negative ions), m/z 1012.2374. Calculated for $C_{34}H_{56}BrN_5O_{25}$, $[M - H]^-$: m/z 1012.2375. MS (positive ions), m/z 1036.2342; 1052.2077. Calculated for $C_{34}H_{56}BrN_5O_{25}$, $[M + Na]^+$: m/z 1036.2340; $[M + K]^+$: m/z 1052.2078.

***N*-[*N*-(Bromoacetyl)diglycyl]-*N*-{*N*-[4-*O*-(β-D-galactopyrasonyl)-β-D-glucopyrasonyl]carbamoylmethyl}-*N*-(*N*-{*N*-bis[4-*O*-(β-D-galactopyrasonyl)-β-D-glucopyrasonyl]carbamoylmethyl}-carbamoylmethyl)amine (8a).** A suspension of *N*-{*N*-[4-*O*-(β-D-galactopyrasonyl)-β-D-glucopyrasonyl]carbamoylmethyl}-*N*-(*N*-{*N*-bis[4-*O*-(β-D-galactopyrasonyl)-β-D-glucopyrasonyl]-carbamoylmethyl}carbamoylmethyl)-*N*-(diglycyl)amine (**4**)[11] (0.19 g, 0.14 mmol) in DMSO (1.4 mL) was heated at 50 °C until dissolution, cooled to ~23 °C and ester **5** (0.066 g, 0.28 mmol) was added. The reaction mixture was stirred and kept at ~23 °C

for 6 h. Et_2O (15 mL) was added with stirring. The supernatant was decanted from the resulted oily product, which was triturated with Et_2O until a viscous mass was obtained. The mass was treated several times with hot acetone until a powder was obtained. The poweder was treated several times with MeOH, filtered off, and dried to yield amorphous compound **8a** (0.18 g, (88%), $[\alpha]_D^{20}$ +3.9 (*c* 1, H_2O). ^1H NMR, δ: 3.42—3.59 (m, 6 H); 3.61—3.86 (m, 24 H); 3.88—3.97 (m, 6 H); 3.98—4.58 (m, 17 H); 4.97—5.10 (m, 3 H, 3 H(1) Glc). MS (negative ions), *m/z:* 1450.3844; 724.6910. Calculated for $C_{50}H_{82}BrN_7O_{37}$, $[M - H]^-$: *m/z* 1450.3861; $[M - 2H]^{2-}$: *m/z* 724.6894. MS (positive ions), *m/z* 1474.3819. Calculated for $C_{50}H_{82}BrN_7O_{37}$, $[M + Na]^+$: *m/z* 1474.3826.

Preparation of glycoconjugates 6b—8b (general procedure). To a solution of bromine compounds **6a—8a** (0.1 mmol) in DMSO (0.5 mL), pyridinium 1,2-dicarba-*closo*-dodecaboran-1-yl thiolate (**1**)[9] (28 mg, 0.11 mmol) was added and the mixture was kept at ~22 °C for 24 h. Et_2O (10 mL) was added to the reaction mixture. The supernatant was decanted from the precipitated oily product, which was triturated with Et_2O until a viscous mass was obtained.

Glycoconjugate 6b. The viscous mass was dissolved in H_2O (10 mL) and extracted with CH_2Cl_2 (3×4 mL). The aqueous solution was diluted with H_2O (40 mL) and concentrated until a syrup was obtained. The syrup was dissolved in H_2O (3 mL) and applied on a column with C_{18} silica gel (30 mL). The column was washed with H_2O (~200 mL) before the UV absorption started to grow and then with 10% aqueous MeOH (~150 mL). The water-methanol solution containing the product was concentrated to 2 mL, diluted with H_2O (50 mL), concentrated to 3 mL to remove pyridine, and chromatographed again under the same conditions. The water-methanol solution was concentrated to 1 mL and lyophilized to yield amorphous compound **6b** (40 mg, 66%), $[\alpha]_D^{25}$ +3.8 (*c* 1, H_2O). ^1H NMR, δ: 2.19 (br.s, 1 H, HC_{carb}); 3.36, 3.58 (both d, 1 H each, CH_2S, J = 14.5 Hz); 3.42—3.56 (m, 2 H); 3.61—3.83 (m, 8 H); 3.88—3.95 (m, 2 H); 4.00, 4.10, and 4.04, 4.12 (both AB systems, 1 H each, CH_2, J = 25.5 Hz, J = 9.5 Hz); 4.44 (d, 1 H, H(1) Gal, J = 7.5 Hz); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz). ^{11}B NMR, δ: −37.25 (1 B); −32.94 (1 B); −21.27 (2 B); −18.68 (1 B); −16.76 (1 B); −14.50 (1 B); −11.15 (1 B); −9.91 (1 B). MS (negative ions), *m/z* 604.3050. Calculated for $C_{18}H_{39}B_9N_2O_{12}S$, $[M - H]^-$: *m/z* 604.3039.

Glycoconjugate 7b. The viscous mass was triturated several times with acetone until a powder was obtained. The powder was triturated in H_2O (50 mL), the resulted mixture was evaporated to dryness, and this procedure was repeated until pyridine disappeared completely (UV control at 260 nm). The residue was chromatographed as described for compound **6b** to yield amorphous compound **7b** (77 mg, 70%), $[\alpha]_D^{25}$ +3.0 (*c* 1, H_2O). ^1H NMR, δ: 2.18 (s, 1 H, HC_{carb}); 3.37, 3.59 (both d, 1 H each, CH_2S, J = 14.5 Hz); 3.42—3.57 (m, 4 H); 3.60—3.87 (m, 16 H); 3.88—3.96 (m, 4 H); 4.02, 4.10 (AB system, 2 H, CH_2, J = 15.0 Hz); 4.17 (m, 4 H, 2 CH_2); 4.37 (br.s, 2 H, CH_2); 4.44 (br.d, 2 H, 2 H(1) Gal, J = 7.5 Hz); 5.00 (d, 1 H, H(1) Glc, J = 9.0 Hz); 5.05 (d, 1 H, H(1) Glc, J = 9.0 Hz). ^{11}B NMR, δ: −37.26 (1 B); −32.93 (1 B); −21.23 (2 B); −18.64 (1 B); −16.78 (1 B); −14.53 (1 B); −10.99 (1 B); −9.94 (1 B). MS (negative ions), *m/z* 1099.4770. Calculated for $C_{36}H_{68}B_9N_5O_{25}S$, $[M - H]^-$: *m/z* 1099.4752.

Glycoconjugate 8b was prepared analogously to compound **7b** in a yield of 94 mg (61%), $[\alpha]_D^{25}$ +3.2 (*c* 1, H_2O). ^1H NMR, δ: 2.18 (br.s, 1 H, HC_{carb}); 3.31—3.57 (m, 8 H); 3.61—3.86 (m, 24 H); 3.88—3.97 (m, 6 H); 3.98—4.57 (m, 15 H); 4.97—5.10 (m, 3 H, 3 H(1) Glc). ^{11}B NMR, δ: −37.19 (1 B); −32.97 (1 B); −21.16 (2 B); −18.53 (1 B); −16.84 (1 B); −14.44 (1 B); −10.78 (1 B); −9.88 (1 B). MS, (negative ions), *m/z* 1537.6212; 768.3068. Calculated for $C_{52}H_{94}B_9N_7O_{37}S$, $[M - H]^-$: *m/z* 1537.6247; *m/z* $[M - 2H]^{2-}$: *m/z* 768.3087.

Hydrolytic stabilities of glycoconjugates 6b—8b. A solution of glycoconjugate **6b** (31 mg, 0.05 mmol) in water (1 mL) was heated at 37 °C for three days. The solution was diluted with water (2 mL), applied on a column with C_{18} silica gel (18 mL) in water, and eluted with water (~250 mL). The fraction eluting together with solvent front were combined, concentrated to ~0.2 mL, and lyophilized to yield a substance (4.5 mg), which was analyzed by electrophoresis and NMR spectroscopy. The fractions containing a homogeneous negatively charged substance (electrophoresis) eluted with delay on sorbent were combined, concentrated to ~0.5 mL, lyophilized, and dried to yield the starting glycoconjugate **6b** (26 mg, 85%).

The hydrolytic stabilities of glycoconjugates **7b** and **8b** were estimated analogously. The starting glycoconjugates **7b** and **8b** were isolated in amount of ~85%.

Triethylammonium salts of glycoconjugates (6c—8c). Solutions (1 mL each) containing glycoconjugates **6b—8b** (0.01 mmol) in D_2O were ice-cooled, a solution of Et_3N in D_2O (0.5 mL, 0.025 mmol L^{-1}) was added, and the mixtures were lyophilized. The residues were dissolved in D_2O (0.5 mL) and the ^1H NMR spectra were recorded. The solutions were lyophilized to yield salts **6c—8c**. The ^1H NMR spectra of the salts contained the proton signals for the Et_3ND^+ group in addition to the proton signals analogous to the corresponding starting glycoconjugates **6b—8b**, δ: 1.25 (t, 9 H, 3 CH_3, J = 6.0 Hz); 3.17 (q, 6 H, 3 CH_2, J = 6.0 Hz).

Inhibition of hemagglutination was performed according to the standard procedure.[15] A suspension of galectin RCA_{120} (2 mg) in 0.9% aqueous NaCl (1.5 mL) was stirred at room temperature for 1.5 h. The undissolved protein was separated by centrifugation and the supernatant was stored at 5 °C for no more than 3 days. A portion of the galectin solution was diluted with 0.9% NaCl (~10—20-fold) and the lectin concentration that upon fourfold dilution of the galecting solution did not cause agglutination of a 2% suspension of red cells of human blood group O was determined.

Solutions of salts **6c—8c** (4 mmol L^{-1}) in 0.9% NaCl were used for the analysis.

Solutions of NaCl (0.9%, 25 μL each) were placed to round-bottom wells of the enzyme immunoassay plate (96 wells) and a solution of the glycoconjugate salt (25 μL) was added to the first well and stirred. The resulted solution (25 μL) was taken and placed to the second well and, analogously, the solution from the second well (25 μL) was taken and placed to the third well and so on until the tenth well. Galectin (25 μL) was added to each well and the mixture was stirred and kept at ~20 °C for 1 h. A 2% suspension of red blood cells in a NaCl solution (25 μL each) was added and stirred and, after 3—5 h, emergence or absence of agglutination was observed.

The authors are grateful to V. A. Lapuk (IOC RAS) for the provided galectin RCA_{120}. This work was financially supported by the Russian Foundation for Basic Research (Project No. 07-03-00120).

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Received March 28, 2011