Conjugates of polyhedral boron compounds with carbohydrates. 5. Synthesis of glycoconjugates of *closo*-*ortho*-carborane and *N*-acyl-β-lactosylamines with various spacers*

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Conjugates of β-lactosylamine derivatives bearing terminal amino groups in aglycon with *ortho*-carboranylacetic acid were synthesized. Five glycoconjugates with spacers of different length (from 9 to 18 atoms) and hydrophilicity containing di-, tri-, and pentapeptide fragments constructed of glycine and serine residues and spacers incorporating ethylenediamine and tartaric acid or tertiary amine residues were prepared.

Key words: glycoconjugates, *ortho*-carboranylacetic acid, *N*-acyllactosylamines, aglycon spacer.

Hyperexpression of a number of lectins,[1] in particular, galectins, *i.e.*, proteins that bind specifically to β-galactose residues, is often observed on the tumor cell surface.[2] Drug conjugates with lactose containing a terminal β-galactose residue may prove suitable for drug targeting to the tumor cells. The possibility of selective delivery of these glycoconjugates, for example, to breast carcinoma cells, was successfully demonstrated.[3] It can be expected that conjugates of polyhedral boron compounds (PBC) with lactose would also be able to bind selectively to the surface of tumor cells, thus ensuring high boron concentration needed for successful boron neutron capture therapy (BNCT) of cancer. The efficiency of interaction of the glycoconjugate carbohydrate moiety with the cell surface lectin may be substantially affected by the chemical nature of the spacer connecting carbohydrate with the drug (see Ref. 4 and references cited therein).

Previously,[5] conjugates of PBC with lactose with a short (two atoms) spacer were synthesized by the addition of decaborane(14) to the triple bond in the aglycon of O- and C-lactosides. Recently, we have prepared the first examples of conjugates of PBC with lactose bearing six- and nine-atomic spacers.[6] The glycoconjugates were synthesized using amino-containing O- and N-lactosides (2-aminoethyl β-lactoside and *N*-glycyl-β-lactosylamine) and two carboxyl-containing PBC derivatives (*closo*-dodecaborate(12) and 1,2-dicarba-*closo*-dodecaborane(12) (*closo*-*ortho*-carborane)), which were coupled using *N*-methyl-*N*-(4,6-dimethoxy-1,3,5-triazin-2-yl)morpholinium chloride in aqueous MeOH (20 °C, 22—48 h). Under these conditions, *closo*-*ortho*-carborane glycoconjugates are formed together with by-products (up to 15%), *viz.*, *nido*-*ortho*-carborane glycoconjugates containing one boron atom less than the *closo*-analogs. It was shown[7] that in aqueous solutions of *closo*-conjugates at room temperature, deboration takes place to give boric acid and *nido*-*ortho*-carborane glycoconjugates; at 60 °C, this reaction may proceed almost to completion. The stability of such *closo*-conjugates against hydrolytic deboration was found to depend substantially on the nature of the spacer between the carborane and carbohydrate moieties.[8]

This work is devoted to further development of the methods of synthesis of PBC glycoconjugates exemplified by *ortho*-carboranylacetic acid (**1**)[9] and the previously obtained[4] set of *N*-lactosylamides with spacers of different lengths (from 7 to 16 atoms) and hydrophilicities containing terminal amino groups in aglycones. For the glycoconjugate synthesis, water-soluble lactosylamides **2a—5a** and **6a**·2HCl (Scheme 1) were used, as water solubility is a requirement to BNCT agents.

In view of the previously found instability of *closo*-*ortho*-carborane glycoconjugates in water, especially at elevated temperature, glycoconjugates were synthesized in anhydrous medium at reduced temperature. The con-

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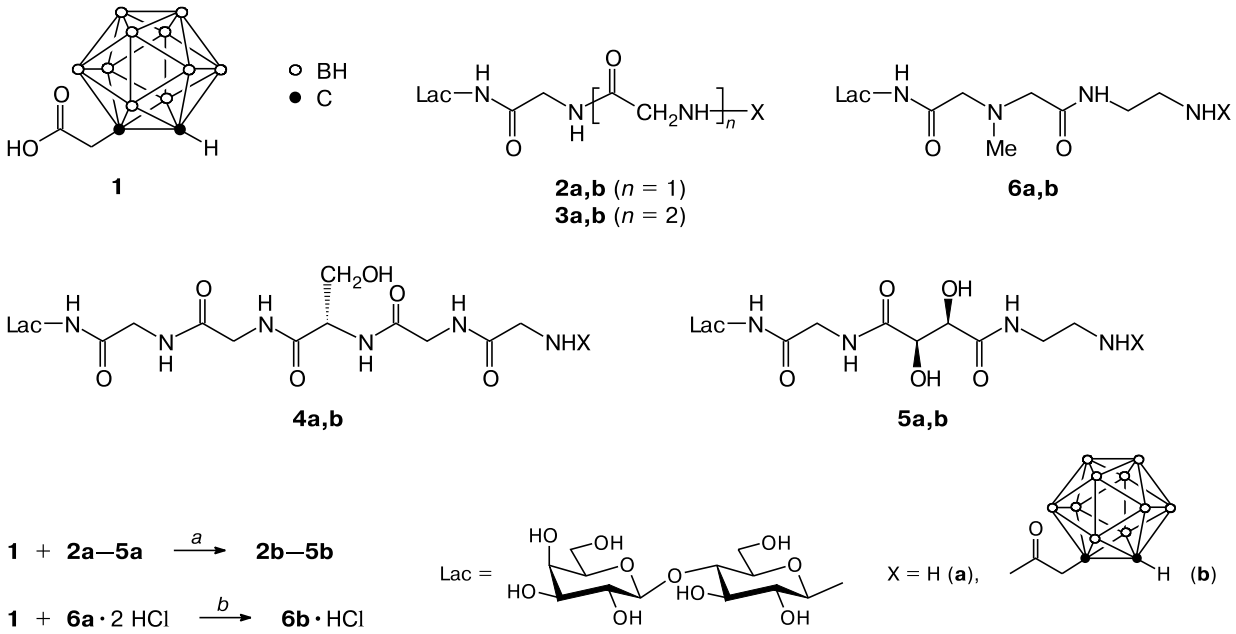
Scheme 1



Reagents and conditions: *a*. DCC, NHS, DMSO—DMF (4 : 1); *b*. DCC, NHS, Et_3N, DMSO—DMF (4 : 1).

densing agent used was DCC in the presence of NHS in a mixture of dry DMSO and DMF at 10 °C. The coupling of lactosylamides **2a—5a** with acid **1** was carried out for 24 h. Paper electrophoresis showed that the conversion of lactosylamides (positively charged compounds) over this period was ~95%, and the reaction gave the target neutral products (glycoconjugates of *closo*-carborane) and negatively charged by-products (glycoconjugates of *nido*-carborane, up to 3—5%). Glycoconjugates **2b—5b** were precipitated from reaction mixtures with ether, the precipitates were washed with ether and acetone and finally purified from the remainder of lactosylamides **2a—5a** and from by-products by treatment with a cation and anion exchange resins. The synthesis and isolation of glycoconjugate **6b** · HCl from lactosylamide **6a** · 2HCl and acid **1** were performed under the same conditions but in the presence of 0.5 equiv. Et_3N. Glycoconjugate **6b** · HCl was finally purified by gel chromatography on a column with Sephadex G-25 at 5 °C. After purification, aqueous solutions of glycoconjugates **2b—5b** and **6b** · HCl were immediately frozen and freeze-dried. The glycoconjugates obtained in 51 to 63% yields and containing 4 to 10% H_2O, were stored at 5 °C; in the case of long-term storage (more than a month), the temperature was decreased to −18 °C.

The compositions and structures of glycoconjugates **2b—5b** and **6b** · HCl were determined based on data from elemental analysis and ^1H, ^{13}C, and ^{11}B NMR spectroscopy. The ^1H NMR spectra contained, in addition to the proton signals that coincide with those of the starting lactosylamides,[4] signals for the protons of the CH_2C and CHB groups (δ 3.30 and 4.55, respectively), the *ortho*-carboranylacetic acid residue, and a very broad signal of the $B_{10}H_{10}$ group protons (δ 1.5—2.9) typical of carboranes. The ^{13}C NMR spectrum recorded for glycoconjugate **2b** exhibits, in addition to the carbon signals of the lactose residue, signals for three methylene groups of the spacer (δ_C 43.3, 43.6, 43.9) and carbon signals for the carborane CHB and CB groups (δ 62.2 and 70.6, respectively). The ^{11}B NMR spectra of glycoconjugates **2b—5b** and **6b** · HCl recorded immediately after dissolution in D_2O contained three broad signals for the *closo-ortho*-carborane boron atoms (δ −3.0, −10.1, −11.5). After 5 h at room temperature (~20 °C), low-intensity signals characteristic of the *nido*-anion (δ_B 33.1 and 37.4) and boric acid (δ_B 19.2) appeared. Note that during storage of solutions of glycoconjugates in D_2O, the intensity of the ^{11}B NMR signals typical of *nido*-carboranes and boric acid increased, while simultaneously the spot of the negatively charged compound in the electrophoregram increased, thus confirming that this spot corresponds to the glycoconjugate of *nido*-carborane. The presence of low-intensity spots of by-products with similar mobilities after the synthesis of glycoconjugates indicates that side deboration reaction, which is probably caused by the presence of traces of water in the starting lactosylamides and solvents, takes place to a minor extent.

Thus, we synthesized glycoconjugates of β-lactosyl amine derivatives under conditions that minimized side deboration. The products include glycoconjugates with

spacers of different lengths (9 to 18 atoms) and hydrophilicities containing di-, tri-, and pentapeptide fragments constructed of glycine and serine residues and spacers containing ethylenediamine and tartaric acid or tertiary amine residues. Using this set of glycoconjugates, potential BNCT agents, it would be possible in the future to choose the optimal spacers ensuring most selective binding of glycoconjugates to the surface of tumor cells, which is expected to increase the efficiency of cancer therapy.

Experimental

^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded in D_2O on a Bruker AC-200 spectrometer (operating at 200.13, 50.32, and 64.21 MHz, respectively) with respect to acetone, 1,4-dioxane, and $BF_3 \cdot Et_2O$ signals, respectively (external standards). Optical rotation was determined on a PU-07 polarimeter (Russia). Electrophoresis (30 V cm^{-1}, 1 h) was carried out on Filtrak FN1 paper in pyridinium acetate buffer (0.05 *M* for Py, pH 4.5). Compounds were detected with ninhydrin and the KIO_4—$AgNO_3$—KOH sequence of reagents (see Ref. 10). Water was determined by the Fisher method.

Condensation of lactosylamides 2a—5a with *ortho*-carboranylacetic acid 1 (general procedure). A solution of an amine component (lactosylamides **2a—5a**)[4] (0.2 mmol), *ortho*-carboranylacetic acid (**1**)[9] (40.4 mg, 0.2 mmol), and NHS (25.3 mg, 0.22 mmol) in dry DMSO (0.8 mL) and dry DMF (0.2 mL) (in the case of compound **2a**, in DMSO (1.2 mL) and DMF (0.3 mL)) was cooled to 10 °C, and DCC (49.5 mg, 0.24 mmol) was added. The reaction mixture was stirred for 1 h and kept for 23 h at 10 °C until lactosylamides **2a—5a** were converted almost completely (monitoring by electrophoresis). The precipitated *N*,*N*′-dicyclohexylurea was filtered off and washed with DMSO (0.2 mL). The filtrate was added with stirring to Et_2O (20 mL), and after clarification, the liquid was separated from the oily precipitate by decantation. The precipitate was triturated several times with Et_2O (5-mL portions) to obtain a thick mass, which was then triturated several times with acetone (2-mL portions) to remove the remaining NHS. The powder thus obtained was filtered off, washed with acetone and Et_2O, dried, and dissolved in water (10 mL). Dowex 50w×8 (H^+) cation exchange resin (40 mg) was added to the solution, the mixture was stirred for 10 min, Dowex 21K (OH^-) anion exchange resin (150 mg) was added, and the mixture was stirred for 30 min. The resin was filtered off and washed with water (15 mL), the filtrates were combined, immediately frozen, and freeze-dried. Products **2b—5b** were dried for 24 h *in vacuo* over $CaCl_2$ and stored at 5 °C.

4-*O*-(β-D-Galactopyranosyl)-*N*-{*N*-[(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)acetyl]diglycyl}-β-D-glucopyranosylamine (2b), yield 58%, amorphous compound, $[\alpha]_D^{25}$ +1.8 (*c* 0.5, H_2O). Found (%): C, 36.13; H, 6.55; B, 15.50; N, 6.28; H_2O, 3.62. $C_{20}H_{41}B_{10}N_3O_{13} \cdot 1.5\ H_2O$. Calculated (%): C, 36.03; H, 6.65; B, 16.21; N, 6.30; H_2O, 4.05. ^{1}H NMR, δ: 3.30 (s, 2 H, CH_2C); 3.47—3.60 (m, 2 H); 3.63—3.84 (m, 8 H); 3.93 (br.s, 2 H); 3.98 (br.s, 2 H, CH_2NH); 4.00—4.05 (m, 2 H); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.53 (br.s, 1 H, CHB); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz). ^{13}C NMR, δ: 43.3 (CH_2); 43.6 (CH_2); 43.9 (CH_2); 60.8 (C(6), Glc); 61.8 (C(6), Gal); 62.2 (CHB); 69.4 (C(4), Gal); 70.6 (CB); 71.8 (C(2), Gal); 72.3 (C(2), Glc); 73.4 (C(3), Gal); 75.8 (C(3)Glc); 76.2 (C(5)Gal); 77.3 (C(5), Glc); 78.8 (C(4), Glc); 80.0 (C(1), Glc); 103.8 (C(1), Gal); 170.1 (CO); 172.3 (CO); 173.2 (CO). ^{11}B NMR, δ: 1.0 to −5.0 (2 B); −5.0 to −20.0 (8 B).

4-*O*-(β-D-Galactopyranosyl)-*N*-{*N*-[(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)acetyl]triglycyl}-β-D-glucopyranosylamine (3b), yield 51%, amorphous compound, $[\alpha]_D^{25}$ +4.0 (*c* 0.5, H_2O). Found (%): C, 35.69; H, 6.65; B, 14.54; N, 7.62; H_2O, 4.43. $C_{22}H_{44}B_{10}N_4O_{14} \cdot 2\ H_2O$. Calculated (%): C, 36.06; H, 6.60; B, 14.75; N, 7.65; H_2O, 4.92. ^{1}H NMR, δ: 3.30 (br.s, 2 H, CH_2C); 3.45—3.61 (m, 2 H); 3.62—3.87 (m, 8 H); 3.88—3.95 (m, 2 H); 3.98 (br.s, 2 H, CH_2NH); 4.00 (br.s, 2 H, CH_2NH); 4.01 (br.s, 2 H, CH_2NH); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.53 (br.s, 1 H, CHB); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz). ^{11}B NMR, δ: −1.0 to −3.5 (1 B); −3.5 to −7.5 (1 B); −7.5 to −11.0 (3 B); −11.0 to −18.0 (5 B).

4-*O*-(β-D-Galactopyranosyl)-*N*-{*N*-[(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)acetyl]diglycyl-L-seryldiglycyl}-β-D-glucopyranosylamine (4b), yield 62%, amorphous compound, $[\alpha]_D^{25}$ −4.8 (*c* 0.5, H_2O). Found (%): C, 35.40; H, 6.59; B, 11.85; N, 9.22; H_2O, 9.27. $C_{27}H_{52}B_{10}N_6O_{16} \cdot 5\ H_2O$. Calculated (%): C, 35.44; H, 6.83; B, 11.82; N, 9.19; H_2O, 9.85. ^{1}H NMR, δ: 3.30 (br.s, 2 H, CH_2C); 3.47—3.59 (m, 2 H); 3.63—3.84 (m, 8 H); 3.86—3.96 (m, 4 H); 3.99 (br.s, 2 H, CH_2NH); 4.00—4.06 (m, 6 H, 3 CH_2NH); 4.46 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.51 (t, 1 H, CHN, *J* = 4.5 Hz); 4.54 (br.s, 1 H, CHB); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz). ^{11}B NMR, δ: −1.0 to −3.5 (1 B); −3.5 to −7.5 (1 B); −7.5 to −11.0 (3 B); −11.0 to −18.0 (5 B).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-{(*R,R*)-2,3-dihydroxy-3-[*N*-(2-{(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)acetylamino}ethyl)carbamoyl]propionyl}glycyl)-β-D-glucopyranosylamine (5b), yield 60%, amorphous compound, $[\alpha]_D^{25}$ +43.4 (*c* 0.5, H_2O). Found (%): C, 36.38; H, 6.77; B, 13.23; N, 7.20; H_2O, 5.06. $C_{24}H_{48}B_{10}N_4O_{16} \cdot 2\ H_2O$. Calculated (%): C, 36.36; H, 6.61; B, 13.64; N, 7.07; H_2O, 4.54. ^{1}H NMR, δ: 3.19 (s, 2 H, CH_2); 3.38 (br.s, 4 H, 2 CH_2); 3.45—3.58 (m, 2 H); 3.62—3.84 (m, 8 H); 3.88—3.94 (m, 2 H); 4.04, 4.10 (AB system, 2 H, $COCH_2NH$, *J* = 17.0 Hz); 4.45 (d, 1 H, H(1) Gal, *J* = 7.5 Hz); 4.48 (br.s, 1 H, CHB); 4.57, 4.61 (both br.s, 1 H each, CHCH); 5.02 (d, 1 H, H(1) Glc, *J* = 9.0 Hz). ^{11}B NMR, δ: −1.0 to −3.5 (1 B); −3.5 to −7.5 (1 B); −7.5 to −11.0 (3 B); −11.0 to −18.0 (5 B).

4-*O*-(β-D-Galactopyranosyl)-*N*-(*N*-methyl-*N*-[*N*-(2-{(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)acetylamino}ethyl)carbamoylmethyl]glycyl)-β-D-glucopyranosylamine hydrochloride (6b · HCl) was prepared according to the general procedure from 4-*O*-(β-D-galactopyranosyl)-*N*-{*N*-methyl-*N*-[*N*-(2-aminoethyl)carbamoylmethyl]glycyl}-β-D-glucopyranosyl amine trihydrate dihydrochloride (**6a** · 2HCl)[4] (128 mg, 0.2 mmol) in dry DMSO (1.6 mL) and dry DMF (0.4 mL) in the presence of Et_3N (0.014 mL, 0.1 mmol). Instead of ion exchange resins, gel chromatography on a column (2.5×90 cm) with Sephadex G-25 in 0.01 *M* AcOH at 5 °C was used for purification. The course of elution was monitored by following UV absorption at 206 nm. The fractions containing the target product were combined, concentrated *in vacuo* at 10 Torr to 10 mL, and freeze-dried. The residue was dried for 24 h *in vacuo* over KOH to give 102 mg (63%) of amorphous compound **6b**·HCl, $[\alpha]_D^{25}$ +4.4 (*c* 0.5, H_2O). Found (%): C, 33.96; H, 6.75; B, 13.37; N, 6.65;

H_2O, 8.61. $C_{23}H_{48}B_{10}N_4O_{13}\cdot HCl\cdot 4\ H_2O$. Calculated (%): C, 34.30; H, 7.13; B, 13.42; N, 6.96; H_2O, 8.95. ^1H NMR, δ: 2.67 (s, 3 H, Me); 3.15 (s, 2 H, CH_2); 3.36 (br.s, 4 H, 2 CH_2); 3.42—3.59 (m, 2 H); 3.59—3.87 (m, 12 H); 3.87—3.97 (m, 2 H); 4.44 (d, 1 H, H(1) Gal, J = 7.5 Hz); 4.48 (br.s, 1 H, CHB); 5.02 (d, 1 H, H(1) Glc, J = 9.0 Hz). ^{11}B NMR, δ: 1.0 to −5.0 (2 B); −5.0 to −20.0 (8 B).

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