Conjugates of polyhedral boron compounds with carbohydrates
1. New approach to the design of selective agents for boron neutron capture therapy of cancer

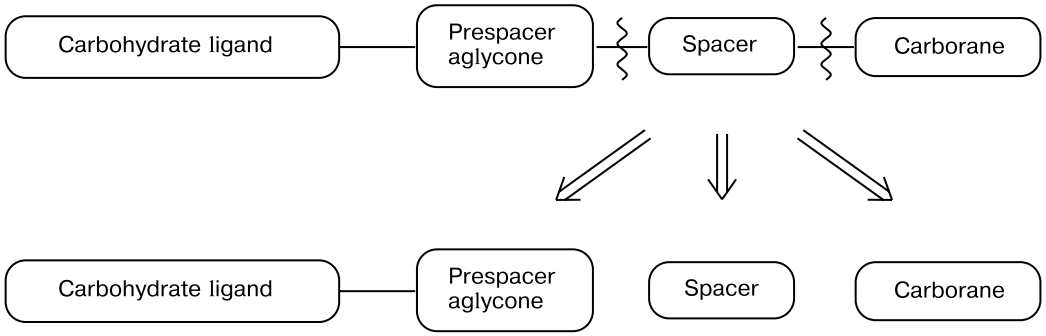
A. V. Orlova,[a] A. I. Zinin,[a] N. N. Malysheva,[a] L. O. Kononov,[a]★ I. B. Sivaev,[b] and V. I. Bregadze[b]

[a]N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (095) 135 5328. E-mail: kononov@ioc.ac.ru
[b]A. N. Nesmeyanov Institute of Organoelement Compounds of the Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation. Fax: +7 (095) 135 5085. E-mail: bre@ineos.ac.ru

Boron neutron capture therapy (BNCT) of cancer is a binary (chemo-radiotherapeutic) method for the treatment of cancer based on the introduction of the stable ^{10}B isotope into a tumor. Subsequent irradiation of the tumor by a flux of thermal neutrons gives rise to high-energy fission products with a path length comparable with cell dimensions, which allows selective destruction of the tumor cells without affecting the surrounding healthy tissue.[1] The second-generation BNCT agents used currently in clinical practice do not exhibit the required high selectivity of accumulation in the tumor. A way of increasing the selectivity of BNCT agents may be the use of targeted delivery of boron compounds to the tumor cells, which may be based, for example, on carbohydrate—protein interactions. Endogenous lectins (receptors of the protein nature) located on the surface of many normal and tumor cells function as specific receptors and are mediators in the carbohydrate-specific endocytosis of (neo)glycoconjugates.[2] Conjugates of polyhedral boron compounds with carbohydrates representing ligands of the lectins that are expressed on the surface of cancer cells can serve as promising agents for BNCT.[3a] A known approach[3] to the conjugation of carbohydrates with polyhedral boron compounds is based on the preparation of glycosides containing a triple bond in the aglycone and the subsequent addition of decaborane(14) to give ω-(*ortho*-carboranyl)alkylglycosides (derivatives of a number of monosaccharides and simple disaccharides including lactose have been described). The main drawback of this approach is the lack of flexibility and versatility for the preparation of a large set of neoglycoconjugates because of the neccesity of performing the non-trivial glycosylation and the addition of highly toxic decaborane(14) for each alkynol.

Here we report a new approach to the preparation of conjugates of *ortho*-carboranes with the carbohydrate ligands of lectins. It is based on the use of prespacer strategy,[4] which is highly flexible and efficient and allows reliable preparation of large sets (libraries) of neoglycoconjugates from only one (oligo)saccharide glycoside with a functional group in the terminal position of a rather simple (short) prespacer aglycone (Scheme 1). Then spacers of an appropriate structure are introduced into the aglycone (the introduction of a sufficiently long spacer between the carbohydrate part and the relatively bulky carborane cage is needed to ensure the accessibility of the carbohydrate fragment of the neoglycoconjugate for the interaction with lectin), and the resulting set of spacered

Scheme 1



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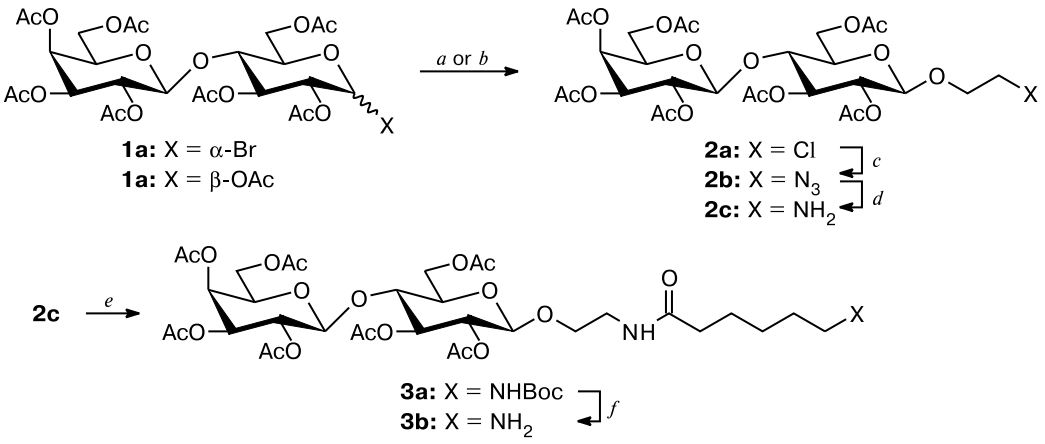
Scheme 2



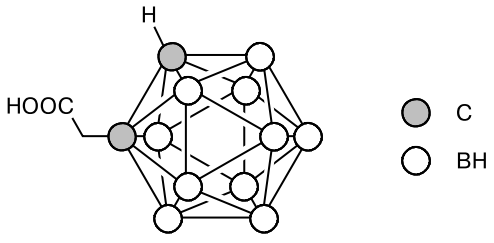
Reagents, conditions, and yields: *a*. $HOCH_2CH_2Cl$, $Hg(CN)_2$, refluxing, Ar, yield 59%; *b*. $HOCH_2CH_2Cl$, $SnCl_4$, MeCN, Ar, yield 50%; *c*. NaN_3, 18-crown-6, DMF, Δ, yield 95%; *d*. $NaBH_4$, H_3BO_3, $NiCl_2 \cdot 6H_2O$, EtOH, yield 71%; *e*. $BocNH(CH_2)_5COOH$, DCC, Et_3N, yield 55%; *f*. (1) TFA, (2) Et_3N, yield ~100%.

(oligo)saccharides is used for linking with the fragments to be conjugated (in this particular case, carboranes).

As a representative example, we chose the conjugates of 1,2-dicarba-*closo*-dodecaborane(12) (*ortho*-carborane) with the disaccharide lactose, which is the ligand of lectins that are expressed on the surface of melanoma cells.[5]

Lactose derivatives with a free amino group in the aglycone were synthesized from acetobromolactose (**1a**)[6] or from lactose octaacetate (**1b**);[7] the key steps of the synthesis included glycosylation[8a] of 2-chloroethanol and subsequent introduction[8] of the azido group into the aglycone of the lactoside **2a** (Scheme 2). The reduction of the azido function in lactoside **2b** by nickel boride generated *in situ* furnished the desired selectively protected 2-aminoethyl lactoside **2c**. Lactoside **3a** with an elongated spacer was prepared by condensation of amine **2c** with *N*-Boc-6-aminohexanoic acid in the presence of DCC (yield 55%). Removal of the Boc group in **3a** by treatment with TFA smoothly gave the target amine **3b**.

The condensation of amines **2c** and **3b** with an activated ester of carboranylacetic acid prepared *in situ* from acid **4** [9] (*N*-hydroxysuccinimide (NHS), DCC, THF) resulted in acetylated conjugates **5** and **6** in 19 and 32% yields, respectively (Scheme 3). The *O*-acetyl protective groups present in conjugates **5** and **6** can, apparently, be easily removed by treatment with MeONa in MeOH, as has been carried out previously[3] for other carborane—carbohydrate conjugates.



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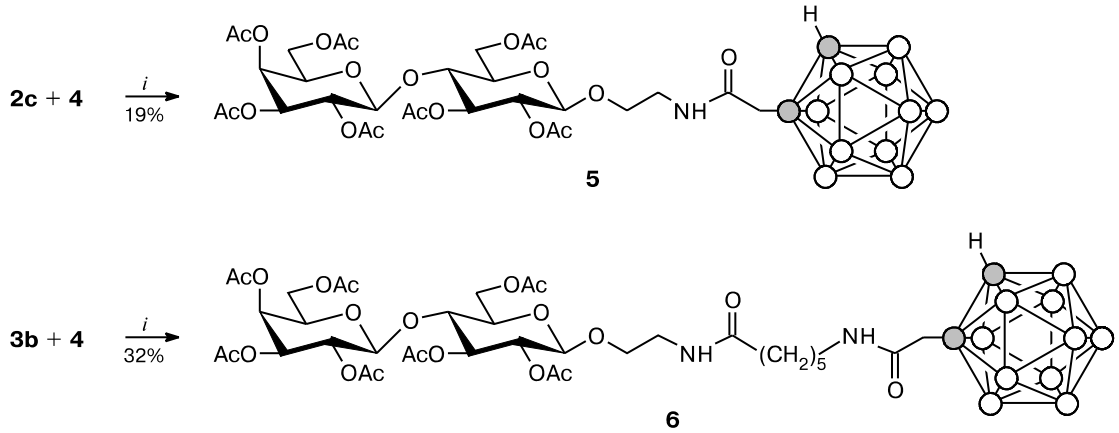
The synthesized compounds were isolated by chromatography on silica gel and characterized by ^1H, ^{13}C, and ^{11}B NMR spectroscopy (Bruker AC-200, $CDCl_3$), IR spectroscopy (Specord M-80, thin film from a solution in $CDCl_3$), and mass spectrometry (Finnigan MAT LCQ, electrospray (ESI)).

2-Chloroethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glycopyranoside (2a), 2-azidoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (2b), 2-aminoethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (2c). Characteristic signals in the ^{13}C NMR spectra, δ: 42.3 (**2a**, X = Cl), 50.1 (**2b**, X = N_3), 41.6 (**2c**, X = NH_2) ($OCH_2\underline{C}H_2X$); 100.9 and 100.7 (**2a**), 100.9 and 100.3 (**2b**), 101.0 and 100.7 (**2c**) (C(1), Glc and Gal, respectively). Mass spectrum of **2a**, *m/z* 721.0 [M + Na]. $C_{28}H_{39}ClNaO_{18}$. Calculated: *m/z* 721.2 [M + Na]. IR spectrum of **2b** (thin film from a solution in $CDCl_3$), ν/cm^{-1}: 1752 (CO), 2108 (N_3). Mass spectrum of **2b**, *m/z* 728.2 [M + Na]. $C_{28}H_{39}N_3NaO_{18}$. Calculated: *m/z* 728.2 [M+Na].

2-[6-(*tert*-Butoxycarbonylamino)hexanoylamino]ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (3a). ^{13}C NMR, δ: 20.4, 20.5, 20.7, 20.8 ($\underline{C}H_3CO$); 25.1 ($CH_2CH_2\underline{C}H_2CH_2CH_2$); 26.3 ($\underline{C}H_2CH_2CO$), 28.3 ($C\underline{Me}_3$); 29.7 ($\underline{C}H_2CH_2NH$); 36.2 ($CH_2CH_2\underline{C}H_2CO$); 39.0 ($\underline{C}H_2NH$); 40.3 ($(CH_2)_4\underline{C}H_2NH$); 60.6 (C(6), Gal); 61.7 (C(6), Glc); 66.5 (C(4), Gal); 69.0 (C(2), Gal); 69.3 ($O\underline{C}H_2CH_2NH$); 70.5 (C(5), Gal); 70.8 (C(3), Gal); 71.5 (C(2), Glc); 72.5 (C(5), Glc); 72.7 (C(3), Glc); 76.0 (C(4), Glc); 100.7 (C(1), Gal); 100.9 (C(1), Glc); 168.9, 169.6, 170.0, 170.3, 172.9 (C=O). MS, *m/z* 917.2 [M + Na]. $C_{39}H_{62}N_2NaO_{21}$. Calculated: *m/z* 917.4 [M + Na].

2-[(1,2-Dicarba-*closo*-dodecaboran(12)-1-yl)acetylamino]ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (5). ^{13}C NMR, δ: 20.5, 20.6, 20.8, 21.0 ($\underline{C}H_3CO$); 39.6 ($\underline{C}H_2NH$); 42.8

Scheme 3



Reagents and conditions: *i.* NHS, DCC, THF.

(($C_2HB_{10}H_{10}$)$\underline{C}H_2CO$); 58.7 (($\underline{C}HB_{10}H_{10}C$)); 60.7 (C(6), Gal); 61.2 (C(6), Glc); 66.5 (C(4), Gal); 69.1 ($O\underline{C}H_2CH_2NH$); 69.1 (C(2), Gal); 70.7 (C(5), Gal); 70.9 (C(3), Gal); 71.5 (C(2), Glc); 72.5 (C(3), Glc); 73.2 (C(5), Glc); 75.6 (C(4), Glc); 100.8 (C(1), Gal); 101.0 (C(1), Glc); 166.5, 170.1 (CO). $^{11}B\{^1H\}$ NMR, δ: −2.5 (1 B); −5.6 (1 B); −9.9 (8 B). IR, ν/cm^{-1}: 1752 (CO), 2592 (BH), 3380 (NH). MS, *m/z* 887.0 [M + Na]. $C_{32}H_{53}B_{10}NNaO_{19}$. Calculated: *m/z* 888.4 [M + Na].

2-{6-[(1,2-Dicarba-*closo*-dodecaboran(12)-1-yl)acetylamino]hexanoylamino}ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranoside (6). ^{13}C NMR, δ: 20.5, 20.6, 20.8 ($\underline{C}H_3CO$); 23.8 ($CH_2CH_2\underline{C}H_2CH_2CH_2$); 25.7 (($CH_2)_3\underline{C}H_2CH_2CO$); 27.8 (($CH_2)_3\underline{C}H_2CH_2NH$); 35.5 (($CH_2)_4\underline{C}H_2CO$); 38.9 ($OCH_2\underline{C}H_2NH$); 39.3 (($CH_2)_4\underline{C}H_2NH$); 43.3 (($C_2HB_{10}H_{10}$)$\underline{C}H_2CO$); 58.7 ([$\underline{C}HB_{10}H_{10}C$]); 60.7 (C(6), Gal); 61.7 (C(6), Glc); 66.5 (C(4), Gal); 69.1 (C(2), Gal); 69.4 ($O\underline{C}H_2CH_2NH$); 70.7 (C(5), Gal); 70.9 (C(3), Gal); 71.6 (C(2), Glc); 72.5 (C(5), Glc); 72.9 (C(3), Glc); 76.0 (C(4), Glc); 100.8 (C(1), Gal); 101.0 (C(1), Glc); 170.3, 173.1 (CO). $^{11}B\{^1H\}$ NMR, δ: −2.6 (1 B), −5.5 (1 B), −10.0 (8 B). IR, ν/cm^{-1}: 1752 (CO), 2593 (BH), 3444 (NH). MS, *m/z* 1001.3 [M + Na]. $C_{38}H_{64}B_{10}N_2NaO_{20}$. Calculated: *m/z* 1001.5 [M + Na].

The approach we propose to the synthesis of carborane—carbohydrate conjugates can also be used successfully to prepare conjugates with polyhedral boron compounds of various structures, for example, *closo*-dodecaborates (see a review[10] dealing with their use in BNCT), which is impossible within the framework of the previously described approach[3] to the synthesis of carborane—carbohydrate conjugates based on the addition of decaborane(14) to acetylene glycosides.

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References

1. A. H. Soloway, W. Tyarks, B. A. Barnum, F.-G. Rong, R. F. Barth, I. M. Codogni, and J. G. Wilson, *Chem. Rev.*, 1998, **98**, 1515.
2. M. S. Wadhwa and K. G. Rice, *J. Drug Target.*, 1995, **3**, 111.
3. (a) G. B. Giovenzana, L. Lay, D. Monty, G. Palmisano, and L. Panza, *Tetrahedron*, 1999, **55**, 14123; (b) L. F. Tietze, U. Griebach, I. Schuberth, U. Bothe, A. Marra, and A. Dondoni, *Chem. Eur. J.*, 2003, **9**, 1296 and references cited therein.
4. G. Magnusson, A. Ya. Chernyak, J. Kihlberg, and L. O. Kononov, in *Neoglycoconjugates: Preparation and Application*, Eds. Y. C. Lee and R. T. Lee, Acad. Press, Inc., San Diego, California, 1994, p. 53.
5. B. Dean, H. Oguchi, S. Cai, E. Otsuji, K. Tashiro, S. Hakomori, and T. Toyokuni, *Carbohydr. Res.*, 1993, **245**, 175.
6. L. O. Kononov, A. V. Kornilov, A. A. Sherman, E. V. Zyryanov, G. V. Zatonskii, A. S. Shashkov, and N. E. Nifant´ev, *Bioorg. Khim.*, 1998, **24**, 608 [*Russ. J. Biorg. Chem.*, 1998, **24** (Engl. Transl.)] and references cited therein.
7. C. S. Hudson and J. M. Johnson, *J. Am. Chem. Soc.*, 1915, **37**, 1270.
8. (a) A. Ya. Chernyak, G. V. M. Sharma, L. O. Kononov, P. Radha Krishna, A. B. Levinsky, N. K. Kochetkov, and A. V. Rama Rao, *Carbohydr. Res.*, 1992, **223**, 303; (b) O. N. Yudina, A. A. Sherman, and N. E. Nifantiev, *Carbohydr. Res.*, 2001, **332**, 363.
9. L. I. Zakharkin, A. V. Grebennikov, L. E. Vinogradova, and L. A. Leites, *Zhurn. Obshch. Khim.*, 1968, **38**, 1048 [*Russ. J. Gen. Chem.*, 1968, **38** (Engl. Transl.)].
10. I. B. Sivaev, V. I. Bregadze, and N. T. Kuznetsov, *Izv. Akad. Nauk, Ser. Khim.*, 2002, 1256 [*Russ. Chem. Bull., Int. Ed.*, 2002, **51**, 1362].

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