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Conjugates of polyhedral boron compounds with carbohydrates. 4. Hydrolytic stability of carborane–lactose conjugates depends on the structure of a spacer between the carborane cage and sugar moiety

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A novel 1,2-dicarba-*closo*-dodecaborane–lactose conjugate (4a) with an *N*-glycosidic linkage was synthesized. This conjugate was found to be much more stable against hydrolytic deboronation (*closo* to *nido* tranformation of the carborane cage) under neutral conditions than a related carborane–lactose conjugate (1a) with an *O*-glycosidic linkage. This result demonstrates that the hydrolytic stability of carborane–carbohydrate conjugates in neutral aqueous solutions may depend dramatically on the chemical nature of the spacer that links the carbohydrate moiety with the boron cage, the rate of hydrolysis varying by orders of magnitude. We relate a significant decrease in the deboronation rate to the formation of more strongly bound supramolecular aggregates, in which the boron cage is less accessible to nucleophilic attack by solvent molecules, in the solution of the carborane–*N*-lactoside conjugate 4a. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: boron neutron capture therapy, BNCT; 1,2-dicarba-*closo*-dodecaborane-lactose conjugate; 1,2-dicarba-*nido*-undecaborane-lactose conjugate; deboronation; spacer; hydrolytic stability

INTRODUCTION

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.[4] The second generation of BNCT agents (including polyhedral boron compounds) used currently in clinical practice does not exhibit the required high selectivity of accumulation in the tumor.[4] Targeted delivery of boron compounds to the tumor cells can be regarded as a way of increasing the selectivity of BNCT agents.

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.[5] Malignant transformation often results in the change in lectin composition of the cell surface and is usually accompanied by over-expression of these lectins.[6,7] Conjugates of polyhedral boron compounds with carbohydrates representing ligands of the lectins can serve as promising agents for BNCT.[5–8] The selection of an oligosaccharide 'vector' suitable for glycotargeting is based on the knowledge of the carbohydrate-binding specificity of the tissue in question, which depends on its lectin composition. We are developing a novel approach[1–3] for the preparation of carborane–carbohydrate conjugates[1–3,8–10] as BNCT agents that can possibly be used for carbohydrate-mediated targeting[5–7] of the tumor cells.

Chemical stability of carborane–carbohydrate conjugates under physiological conditions is an important issue from

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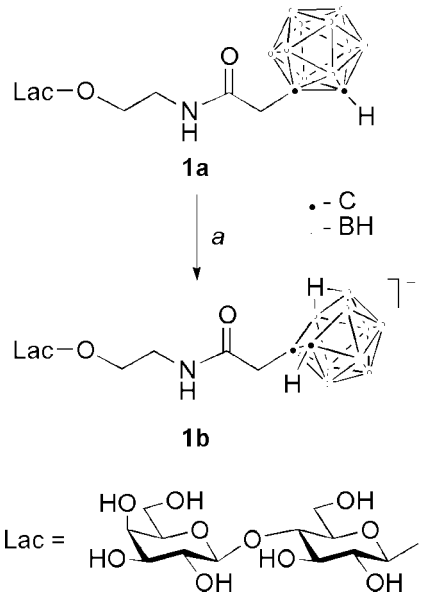

Figure 1. Reagents and conditions: *a*, D_2O, 60 °C, 17 h.

the viewpoint of further possible use of the conjugates in clinical practice. We have recently discovered that a novel 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **1a** (Fig. 1), when dissolved in water, is subject to unusual deboronation under *neutral* conditions leading to the formation of the corresponding *nido*-counterpart (**1b**).[2] In this communication we describe the synthesis and properties of a similar conjugate **4a** (Fig. 2), which is featured by an *N*-glycosidic linkage rather than the *O*-glycosidic bond present in the conjugate **1a**.

RESULTS AND DISCUSSION

A relatively large set of carborane–carbohydrate conjugates needs to be prepared in order to ensure the success of the carbohydrate-mediated targeting. The synthesis of oligosaccharide glycosides with various aglycons (even if the sugar part is the same) requires separate optimization of glycosylation steps in each particular case (this is a characteristic feature of the current level of development of oligosaccharide synthesis).[11,12] For this reason, oligosaccharides with free reducing terminus isolated from natural sources have become popular for the synthesis of neoglycoconjugates.[12] The advantage of using neoglycoconjugates based on *N*-(aminoacetyl)glycosylamines[13] similar to the lactose derivative **2**[14] (Fig. 2) comes from the possibility of utilizing both synthetic carbohydrates[11] and oligosaccharides isolated[12] from natural sources for their preparation. Enhanced chemical stability of the glycosylamide linkage and altered hydrophilicity of the aglycon provide additional benefits.

As the first step along this line, we attempted the synthesis of a conjugate of carboranylacetic acid (**3**)[15] with the known

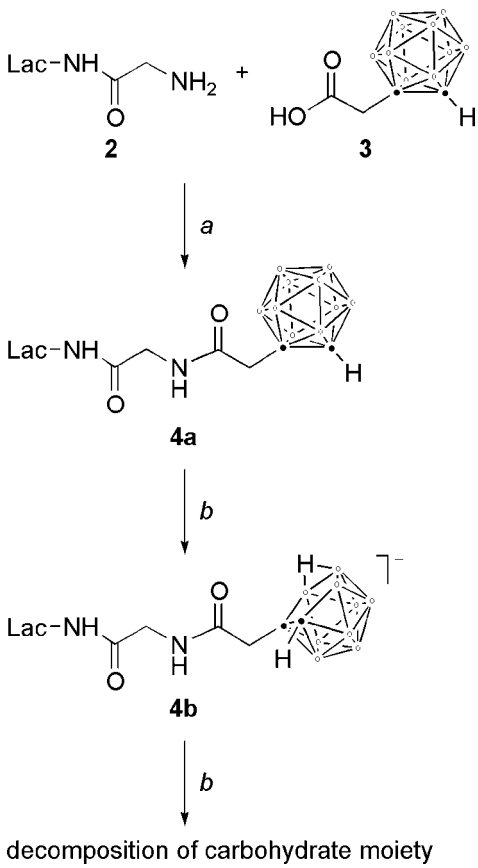

Figure 2. Reagents and conditions: *a*, DMT-MM, MeOH–H_2O; *b*, D_2O, 60 °C, 165 h.

N-(aminoacetyl)lactosylamine (**2**)[14] using the procedure developed earlier[2] for the synthesis of conjugate **1a** with the *O*-glycosidic linkage (Fig. 2). Condensation of the amine **2**[14] with the acid **3**[15] in the presence of 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[16] proceeded smoothly and afforded the target amide **4a** at 50% yield after purification by reversed phase chromatography. Data of ^1H, ^{13}C and ^{11}B NMR spectroscopy and mass spectrometry were in full accord with the proposed structure of compound **4a**.

The conjugate **4a** was found to be much more stable against hydrolytic deboronation (*closo* to *nido* tranformation of the carborane cage) under neutral conditions than a related carborane–lactose conjugate **1a**[2] with the *O*-glycosidic linkage. An ^{11}B NMR spectrum of an aqueous (D_2O) solution of *closo*-conjugate **4a** at ambient temperature contained no signals that could be assigned to the *nido*-conjugate **4b**. Since we knew from previous experience[2] that deboronation of the *closo*-conjugate **1a** is accelerated at higher temperatures, a sample of a solution of *closo*-conjugate **4a** was heated at 60 °C in a NMR tube with ^{11}B NMR monitoring. The intensity of the signals of the *nido*-carborane **4b** was gradually increasing with time. It is important to note that only after 165 h of heating could no signals of the *closo*-carborane **4a** be detected, the *nido*-carborane (δ_B −37.6, −33.5, −20.2,

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−16.7, −11.5) and boric acid (δ_B18.9) being the only boron-containing components of the mixture according to the data of ^{11}B NMR spectroscopy. ^{13}C NMR spectroscopy clearly indicated significant decomposition of carbohydrate moiety under these conditions of prolonged heating since many signals were present in the anomeric region of the ^{13}C NMR spectrum of the reaction mixture rather than two signals of the anomeric carbons expected for compound **4b**. These signals may belong to glycosidically linked saccharides in pyranose (δ_C 101.7, 102.5, 103.5, 103.8, 104.6, 104.9) and furanose forms (δ_C 106.9, 109.1) as well as to reducing sugars (δ_C 93.0, 96.6, 97.5, 98.2).[17] According to the data of mass-spectrometry, the *nido*-conjugate **4b** (m/z 597.4 $[M + Na + H]^+$; m/z 573.5 $[M]^-$) was indeed present in the reaction mixture along with the *nido*-carborane derivatives corresponding to the sequential cleavage of one (m/z 411.3 $[M - Gal + H]^-$) and two (m/z 249.2 $[M - Lac + H]^-$) monosaccharide residues from **4b**.

This significant difference in the rate of hydrolytic deboronation of conjugates **1a** and **4a** (complete conversion of the starting material after 17 and 165 h, respectively) under identical conditions requires special comment. The difference in structures of conjugates **1a** and **4a** seems to be minimal (Fig. 3). Moreover, the different fragments (marked with dashed boxes in Fig. 3) are *remote* from the carborane cage, which is the actual reaction site (shown with arrows in Fig. 3). For this reason, it is rather difficult to explain such a dramatic difference in the reactivities of conjugates **1a** and **4a** upon change in the spacer. We believe that the key issue is the presence of the second amide bond in the conjugate **4a**, which is an additional site for intermolecular hydrogen bonding, while only one site of this kind is present in the conjugate **1a**. This extra hydrogen bond might cross-link molecules of **4a** and *additionally* stabilize micelle-like aggregates apparently formed in aqueous solutions of these typical surfactants, which form foaming solutions in water.[2] The existence of apparently stronger intermolecular hydrogen bond network in solutions of the conjugate **4a** might lead to the formation of supramolecular aggregates of the amide **4a**, in which the accessibility of the carborane cage for nucleophylic attack by water molecules is reduced in comparison with that in aggregates formed by the conjugate **1a**. This would decrease the rate of deboronation of the carborane cage in solutions of the conjugate **4a** with respect to that in solutions of the conjugate **1a**.

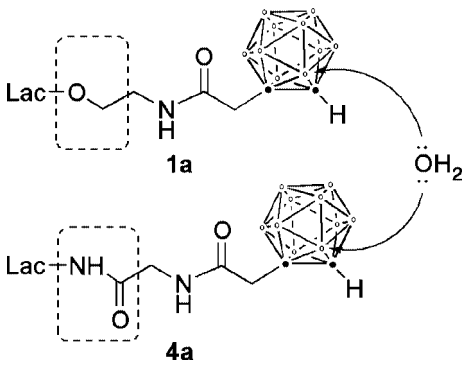

Figure 3. Different fragments are remote from the site of nucleophilic attack.

The presence of more *strongly bound* supramolecular aggregates in solutions of the conjugate **4a** in comparison to those formed in solutions of the conjugate **1a** is indirectly corroborated by mass spectrometry data. An ESI mass spectrum of solution of the *di*amide **4a** contains a peak of a dimer (m/z 1188.3 $[M_2 + Na]$) as the most abundant component (100%) along with the peaks of the molecular ion (33%) and a trimer (16%). It is important to note that the peak of a dimer (m/z 1165.4 $[M_2 + Na]$) in the mass spectrum of the *mono*amide **1a** was less abundant (19%) than the peak of the molecular ion [m/z 594.4 $[M + Na]$ (30%)].[2]

This result demonstrates for the first time that the hydrolytic stability of carborane–carbohydrate conjugates in neutral aqueous solutions may depend dramatically on the chemical nature of the spacer that links the carbohydrate moiety with the boron cage, the rate of hydrolysis varying by orders of magnitude. By careful selection of the spacer, one can hopefully modulate the stability of the carborane cage in aqueous solutions. At present, a large set of carborane–carbohydrate conjugates with different spacers is being synthesized in our laboratory. The results of the ongoing study of their stability with respect to deboronation will be published elsewhere.

CONCLUSIONS

In conclusion, we have synthesized a novel carborane–lactose conjugate **4a** with the *N*-glycosidic linkage and shown it to be more stable than a related carborane–lactose conjugate **1a** with the *O*-glycosidic linkage. This observation may have important consequences for their use in BNCT.

EXPERIMENTAL

The reactions were performed with the use of commercial reagents (Aldrich and Fluka) and solvents purified according to standard procedures. For reversed-phase chromatography a Superclean LC_{18} cartridge (Supelco) was used. Thin-layer chromatography was carried out on plates with silica gel 60 on aluminum foil (Merck). Spots of compounds containing carbohydrates were visualized with a solution of 85% H_3PO_4 in 96% EtOH (1 : 10) with subsequent heating (150 °C). Amines were detected with 5% ninhydrin in acetone with subsequent heating (80 °C). Compounds containing NH-fragment (amides, amines) were detected by treatment with

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chlorine gas followed by treatment with a solution of *o*-tolidine (160 mg) in AcOH (30 ml) and H_2O (500 ml). Spots of compounds containing boron hydride fragments were visualized with solution of $PdCl_2$ (1.256 g) in 10% aqueous HCl (25 ml) and MeOH (250 ml). The ^1H, ^{13}C, and ^{11}B NMR spectra were recorded on Bruker AC-200 instrument (200.13, 50.32 and 64.21 IHz, respectively). The ^1H NMR chemical shifts are referred to the residual signal of H_2O (δ_H 4.8), the ^{13}C NMR to the 1,4-dioxane (δ_C 67.4, external standard), and ^{11}B NMR to $BF_3 \cdot Et_2O$ (δ_B 0.0, external standard). The assignment of the signals in the ^{13}C NMR spectra was made based on the DEPT-135 experiments. Mass spectra (electrospray ionization, ESI) were recorded on a Finnigan LCQ mass spectrometer for 2×10^{-5} M solutions in MeOH in positive ions detection mode unless otherwise stated; m/z values and relative abundances [I_{rel} (%)] for monoisotopic peaks are quoted. The observed isotopic patterns in mass spectra fit well the expected ones for boron-containing compounds with the respective structures. In the description of mass spectra of the negatively charged *nido*-carborane derivatives, M denotes the exact mass of the anion. The optical rotation was measured on a Jasco DIP-360 polarimeter at 20–25 °C.

N-[(1,2-dicarba-*closo*-dodecaborane(12)-1-yl) acetyl]aminoacetyl-4-*O*-(β-D-galacto-pyranosyl)-β-D-glucopyranosylamine (4a)

To a stirred solution of carboranylacetic acid **3**[15] (44.4 mg, 0.23 mmol) and *N*-glycyl-β-lactosylamide[14] (**2**) (92 mg, 0.23 mmol) in MeOH–H_2O mixture (2 : 1, 1.5 ml), 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[16] (70.3 mg, 0.25 mmol) was added. After 45 h of stirring at room temperature volatiles were evaporated. The residue was purified by reversed phase chromatography on a Superclean LC_{18} cartridge (gradient elution from H_2O to MeOH) to give pure amide **4a** (67.4 mg, 50%), R_f 0.58 (EtOH–*n*-BuOH–Py–AcOH–H_2O, 100 : 10:10 : 10:3).

$$[\alpha]_D^{20} + 2.3 \ (c\ 4.3, H_2O).$$

^1H NMR (characteristic signals, D_2O, δ, J/Hz): 2.91 [s, 2I, $COC\underline{H}_2C(B_{10}H_{10})CH$], 4.44 (d, 1H, H-1 Gal, $J = 7.1$), 4.45 (br., 1H, N$\underline{H}$), 5.00 (d, 1H, H-1 Glc, $J = 9.3$).

^{13}C NMR (D_2O): δ 43.5 ($\underline{C}H_2N$); 44.1 ([$C_2HB_{10}H_{10}$]$\underline{C}H_2CO$); 60.7 (C-6 Gal); 61.9 (C-6 Glc); 69.4 (C-4 Gal); 70.6 (OCH_2); 71.8 ([$CHB_{10}H_{10}\underline{C}$]); 72.4 (C-2 Gal); 73.4 (C-3 Gal); 75.9 (C-2 Glc); 76.2 (2C, C-5, C-3 Glc); 77.3 (C-5 Gal); 78.6 (C-4 Glc); 80.1 (C-1 Gal); 103.8 (C-1 Glc); 169.7, 172.5 (CO).

^{11}B NMR (D_2O) : δ−2.3(br., 1 B),

−5.4(br., 1 B), −9.5(br., 8 B).

MS: m/z [I_{rel} (%)] 605.4 [M + Na] (33). $C_{18}H_{38}B_{10}N_2NaO_{12}$. Calculated: m/z 605.3 [M + Na]; m/z [I_{rel} (%)] 1188.3 [M_2 + Na] (100). $C_{36}H_{76}B_{20}N_4NaO_{24}$. Calculated: m/z 1187.7 [M_2 + Na]; m/z [I_{rel} (%)] 1770.1 [M_3 + Na] (16). $C_{54}H_{114}B_{30}N_6NaO_{36}$. Calculated: m/z 1770.0 [M_3 + Na].

Hydrolysis of amide 4a

A solution of a sample (40 mg) containing *closo*-carborane **4a** in D_2O (0.5 ml) was heated at 60 °C in a NMR tube, the course of the reaction being controlled by ^{11}B NMR monitoring. After 165 h of heating no signals of the *closo*-carborane **4a** could be detected. Given below are the data for this crude reaction mixture.

^{13}C NMR (D_2O, signals of anomeric region): δ 93.0, 96.6, 97.5, 98.2, 101.7, 102.5, 103.5, 103.8, 104.6, 104.9, 106.9, 109.1.

$^{11}B\{^1H\}$ NMR (D_2O): δ −37.6 (1 B), −33.5 (1 B), −20.2 (3 B), −16.7 (1 B), −11.5 (4 B).

Additional signal in the $^{11}B\{^1H\}$ NMR spectrum (D_2O): δ 18.9 (H_3BO_3).

MS, m/z [I_{rel} (%)]597.4 [M + Na + H] (32). $C_{18}H_{39}B_9NNa_2O_{12}$. Calculated: m/z 597.3 [M + Na + H].

MS (detection of negative ions), m/z [I_{rel} (%)] 249.3 [M − Lac + H] (14). $C_8H_{18}B_9N_2O_2$. Calculated: m/z 249.2 [M − Lac + H]; m/z [I_{rel} (%)] 411.4 [M − Gal + H] (23). $C_{12}H_{28}B_9N_2O_7$. Calculated: m/z 411.3 [M − Gal + H]; m/z [I_{rel} (%)] 573.5 [M] (100). $C_{18}H_{38}B_9N_2O_{12}$. Calculated: m/z 573.3 [M]; m/z [I_{rel} (%)] 845.5 [(M − Gal + H)$_2$ + Na] (4). $C_{24}H_{57}B_{18}N_4NaO_{14}$. Calculated: m/z 845.5 [(M − Gal + H)$_2$ + Na].

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