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Conjugates of polyhedral boron compounds with carbohydrates. 2. Unexpected easy *closo*- to *nido*-transformation of a carborane–carbohydrate conjugate in neutral aqueous solution ☆

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Abstract

A novel 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c**, when dissolved in water or methanol, is subject to unexpected deboronation in *neutral* conditions leading to the formation of the corresponding *nido*-counterpart (**5**) as detected by ^{11}B NMR spectroscopy. After heating the aqueous solution of the conjugate **4c** at 60 °C for 17 h pure 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** was obtained.

Keywords: Boron neutron capture therapy; BNCT; 1,2-Dicarba-*closo*-dodecaborane–lactose conjugate; 1,2-Dicarba-*nido*-undecaborane–lactose conjugate; Deboronation; Neutral conditions

1. Introduction

We are developing a novel approach [1] for the preparation of carborane–carbohydrate conjugates [4–6] as BNCT agents that can possibly be used [1,2,4] for carbohydrate-mediated targeting [3] the tumor cells. We have recently synthesized [1a] carborane–carbohydrate conjugate **4a** (Scheme 1) with *O*-acetylated hydroxy groups of the lactose residue from amine **2a**. As a next step we attempted to remove protective groups and prepare the unprotected conjugate **4c**. In this communication we describe the results obtained along this line including an observation of unusually easy deboronation of *closo*-carborane to *nido*-carborane in neutral conditions.

2. Results and discussion

De-*O*-acetylation of the carborane–carbohydrate conjugate **4a** [1a] was performed with Et_3N–MeOH–H_2O (1:5:2) at room temperature (cf. [7]). ^{1}H and ^{13}C NMR analysis of the residue after removal of the volatiles from the reaction mixture revealed complete removal of acetates and confirmed that the structure of the lactose fragment remained intact. The ^{11}B NMR spectrum (CD_3OD) indicated the presence of a *closo*-carborane cage (δ_B −2.2, −5.1, −9.4 (br)) corresponding to the target structure **4c** (Scheme 1) contaminated with the *nido*-carborane **5** (ca. 16%, identified by characteristic signals at δ_B −32.3, −36.4) and boric acid (δ_B 18.9). The presence of

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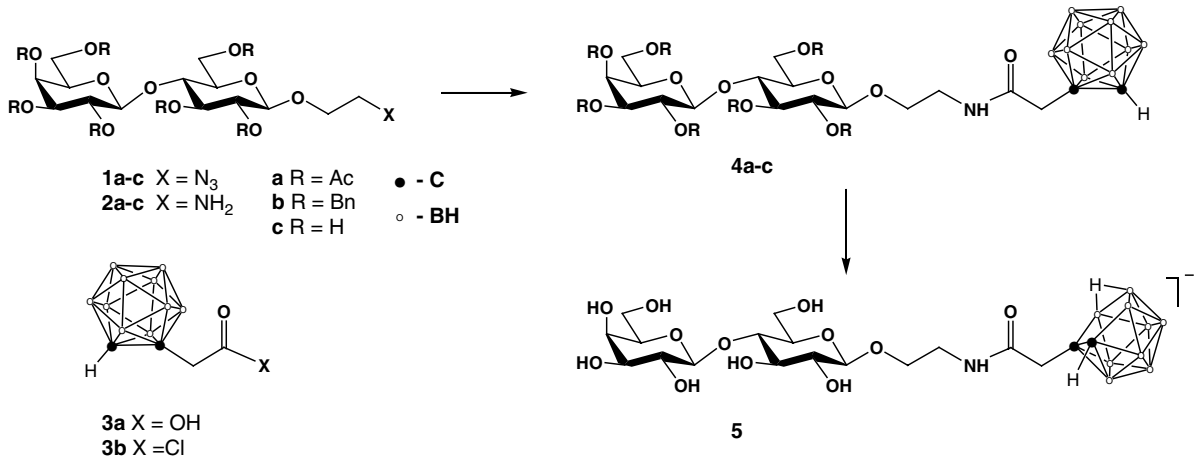

Scheme 1.

nido-carborane can apparently be attributed to basic conditions used for deprotection. No attempts to purify compound **4c** were made at this stage. Instead, we turned our attention to the preparation of carborane–carbohydrate conjugate **4b** with *O*-benzyl protective groups, which can be removed by catalytic hydrogenolysis at neutral conditions. Basing on a precedent [5], we hoped that it would be possible to suppress deboronation of *closo*-carborane **4c** by avoiding basic conditions.

Benzylation of the known [9] 2-azidoethyl lactoside (**1c**) with BnCl in DMSO in the presence of NaOH afforded the target *O*-benzylated azide **1b** in 74% yield. Azide group in **1b** was reduced with Ph_3P–H_2O–NH_3 [14] in THF–EtOH mixture to give *O*-benzylated amine **2b** which was used for the preparation of carborane–carbohydrate conjugate **4b** without purification. The yields of **4b** depended on the condensing agent used for the reaction of **2b** with carboranylacetic acid (**3a**) [10] and were not very high (7% yield in the presence of 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) [11] in MeOH, 15% yield in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC)–*N*-hydroxysuccinimide [12] in THF). The best yield (53%) was obtained in the reaction of *O*-benzylated amine **2b** with carboranylacetic acid chloride **3b** [13] in the presence of $NaHCO_3$. The amide **4b** was purified by HPLC on a silica gel column. Data of ^1H, ^{13}C and ^{11}B NMR spectroscopy and mass-spectrometry were in full accord with the proposed structure of compound **4b**. It is important to note that only signals of the *closo*-carborane cage were present in the ^{11}B NMR spectrum ($CDCl_3$) of **4b** (δ_B −2.8, −5.6, −10.2 (br)).

Catalytic hydrogenolysis of **4b** in MeOH cleanly removed *O*-benzyl protective groups to afford the unprotected 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c** (84%), identical to that prepared from acetylated derivative **4a**, also contaminated with 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** (16%). The structure of compound **4c** was confirmed by an independent synthesis using the reaction of 2-aminoethyl lactoside (**2c**) [9] with carboranylacetic acid (**3a**) [10] in the presence of 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) [11] as a condensing agent. This route renders the removal of protective groups from the carborane–carbohydrate conjugate unnecessary. Again, the product (82% yield) was a mixture of **4c** and **5** in 9.8:1 molar ratio. This batch of compound **4c** was purified by reversed phase chromatography on a SepPak C18 cartridge (gradient elution from H_2O to MeOH) to give a sample containing *closo*-carborane **4c** (94%), *nido*-carborane **5** (5%) and boric acid (1%) (^{11}B NMR data). Data of ^1H, ^{13}C and ^{11}B NMR spectroscopy and mass-spectrometry of this sample were in full accord with the proposed structure of compound **4c**.

The amount of the *nido*-carborane **5** present in all samples of *closo*-carborane–lactose conjugate **4c** varied from batch to batch and method of preparation. We could not obtain a sample of **4c** free from **5** even when the former was prepared from **4b** in *neutral* conditions. More importantly, the amount of **5** gradually increased in time for all samples of **4c** dissolved in methanol or water. The deboronation proceeds in *neutral* conditions (pH not greater than 6 according to pH indicating paper). Apparently, the conversion of *closo*-carborane **4c** to the *nido*-carborane **5** is not related to the presence of basic impurities that might be present in some samples since *all* samples of the conjugate **4c** prepared by three *different* routes experience the same transformation.

This deboronation of the unprotected 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c** leading to the *nido*-carborane **5** can be accelerated at higher temperatures. Thus, an aqueous (D_2O) solution of a sample containing *closo*-carborane **4c**, which was obtained from benzylated conjugate **4b** by hydrogenolysis, was heated at 60 °C in a NMR tube with ^{11}B NMR monitoring (Fig. 1). The intensity of the signals of the *nido*-carborane **5** was gradually increasing with time. After 17 h of heating no signals of the *closo*-carborane **4c** could

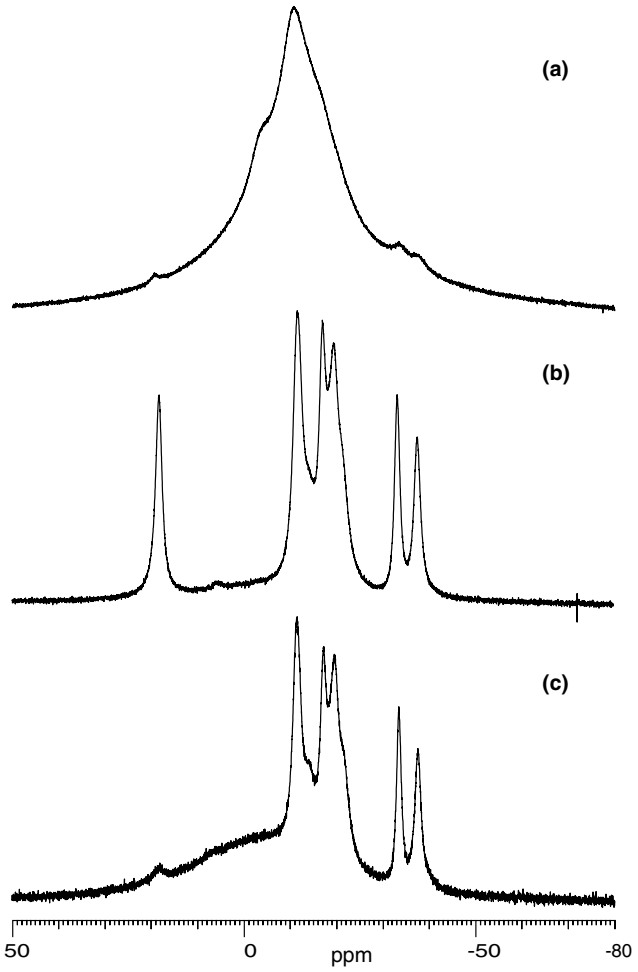

Fig. 1. ^{11}B {^{1}H} NMR spectra in D_2O of 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c** (a), 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** obtained by heating aqueous (D_2O) solution of **4c** at 60 °C for 17 h (*b*), the same as (b) after removal of H_3BO_3 (c).

be detected, the *nido*-carborane **5** and boric acid being the only components of the mixture (Fig. 1(b)). A sample of the 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** free from boric acid was obtained by repeated addition of MeOH and AcOH and evaporation of the volatiles (Fig. 1(c)). It is interesting to note that the value of optical rotation measured for the sample of 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** dissolved in water was negative while that for a sample of the parent *closo*-conjugate **4c** (and all its lactose precursors) was positive. This might indicate that deboronation proceeded stereoselectively to give rise to one diastereomer of **5** predominantly since the resulting *nido* cage is chiral.

Transformation of *closo*- to *nido*-carboranes in basic conditions [8a] and in the presence fluoride-ion [8b] is well known. Although the problem of removal of *O*-acetyl groups from carborane–carbohydrate conjugates is recognized [6], other reports [4,5] did not mention deboronation and the formation of the *nido*-carboranes during de-*O*-acetylation of *closo*-carborane–carbohydrate conjugate in basic conditions (MeONa in MeOH). In this communication we disclose our observation of facile conversion of *closo*- to *nido*-carborane–carbohydrate conjugate, in *neutral* aqueous or methanolic solutions. Recently, a report on instability of α-carbonyl substituted carboranes in essentially neutral conditions (in DMSO–H_2O or DMSO–MeOH mixtures) was published [8c] and electronic influence of α-carbonyl substituent was claimed to be responsible for the ease of the transformation. It was demonstrated that β-carbonyl substituted carboranes and, in particular, methyl ester of carboranylacetic acid are stable in these conditions [8c]. Self-degradation of racemic *o*-carboranylalanine to *nido*-carboranylalanine in buffered water–MeOH solutions was also described [8d]. The authors demonstrated that the reaction proceeds in an intramolecular fashion and that both the carboxylate ion and the ammonium ion in carboranylalanine are needed for an optimum reaction rate, which is maximum in the pH range 3–7, where the *zwitterionic* form predominates. It should be stressed that unprotected 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c**, described in this communication, experienced facile deboronation in neutral conditions *in the absence* of any *ionic* compounds. The reasons of hydrolytic instability of *closo*-carborane cage in **4c** are now under investigation in our laboratory.

The newly found easy *closo*- to *nido*-transformation of carborane–carbohydrate conjugate is important from practical point of view, especially if one considers their possible use as BNCT agents in vivo. The *nido*-conjugate **5** is characterized by much higher hydrophilicity as compared to the parent *closo*-carborane–carbohydrate conjugate **4c**, which is a typical surfactant that forms foaming solutions in water. The biological activity of *closo*- and *nido*-carborane–carbohydrate conjugates may be different.

3. Conclusions

In conclusion, we have synthesized a novel 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c** using three different routes. All samples of the conjugate **4c**, when dissolved in water or methanol, are subject to unexpected deboronation leading to the formation of the 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5**. This observation may have important consequences for their use in BNCT.

4. Experimental

4.1. General

The reactions were performed in an argon atmosphere with the use of anhydrous (where appropriate)

solvents purified according to standard procedures and commercial reagents (Aldrich and Fluka). Column chromatography was performed on silica gel L (40–100 μm, Chemapol) and Silasorb 600 (7 μm, Chemapol). 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). Compound containing NH fragment (amides, amines) were detected by treatment with chlorine gas followed by 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, ^{11}B, and ^{31}P NMR spectra were recorded on Bruker AC-200 instrument (200.13, 50.32, 64.21, and 81.02 MHz, respectively). The ^1H NMR chemical shifts are referred to the residual signal of $CHCl_3$ (δ_H 7.27), the ^{13}C NMR- to the $CDCl_3$ signal (δ_C 77.0), ^{11}B NMR- to $BF_3 \cdot Et_2O$ (δ_B 0.0, external standard), ^{31}P NMR- to 75% H_3PO_4 in D_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 abundance (I_{rel} (%)) for monoisotopic peaks are quoted. The observed isotopic patterns in mass spectra of compounds **4b**,**c** and **5** fit well the expected ones for boron-containing compounds with the respective structures. The optical rotation was measured on a JASCO DIP-360 polarimeter at 20–25 °C.

4.2. Azidoethyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (*1b*)

To a stirred suspension of finely ground NaOH (1.031 g, 25.8 mmol) in DMSO (3 ml) a solution of 2-azidoethyl lactoside (**1c**) [9] (498 mg, 1.2 mmol) in DMSO (4 ml) was added. A solution of BnCl (1.96 ml, 17.0 mmol) in DMSO (2 ml) was then added dropwise and the resulting mixture was stirred at 18 °C for 18 h. The reaction was quenched by addition of MeOH (6 ml, 150 mmol). After 1 h the reaction mixture was diluted with water (10 ml) and extracted with Et_2O (2×30 ml). Combined extracts were filtered through a cotton wool plug and concentrated. The residue was purified by chromatography on a silica gel column (170×40 mm, Silicagel L, 40–100 μm) with gradient elution (hexanes → hexanes–AcOEt, 8:2) to give pure **1b** (928.4 mg, 73%), R_f 0.73 (hexanes–AcOEt, 6:4), $[\alpha]_D^{20} + 14.3$ (*c* 10.0, CH_2Cl_2).

^{13}C NMR ($CDCl_3$): δ 51.0 (CH_2N); 68.1, 68.2 (C(6), C(6′)); 68.1 (OCH_2); 72.5, 73.1, 73.4, 74.7, 75.0, 75.2, 75.3 (OCH_2Ph); 73.0, 73.5, 75.1, 76.6, 79.9, 81.7, 82.5, 82.8 (C(2), C(3), C(4), C(5), C(2′), C(3′), C(4′), C(5′)); 102.8, 103.6 (C(1), C(1′)); 127.0, 127.3, 127.4, 127.6, 127.7, 127.9, 128.0, 128.1, 128.2 (Ph); 137.9, 138.2, 138.4, 138.5, 138.6, 139.0 (2C) (quat. Ph).

MS, *m/z* (I_{rel} (%)) 1064.3 [M + Na] (100). $C_{63}H_{67}N_3NaO_{11}$. Calc.: *m/z* 1064.5 [M + Na].

4.3. Synthesis of carborane–carbohydrate conjugate with O-benzyl protective groups

4.3.1. Aminoethyl 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (*2b*)

To a solution of azide **1b** (394 mg, 0.378 mmol) in THF (10 ml) 25% aqueous NH_3 (2 ml) and 96% EtOH (1 ml) were added. To the resulting homogeneous solution Ph_3P (150 mg, 0.572 mmol) was added and the reaction mixture was stirred at room temperature for 2 days. Volatiles were removed on a rotary evaporator and the residue was dried in vacuo to give crude amine **2b** (R_f 0.54, EtOH–*n*-BuOH–Py–AcOH–H_2O (100:10:10:10:3)), which was used in the next step without any purification. Triphenylphosphine oxide (^{31}P NMR ($CDCl_3$): δ_P 29.5) present in this sample is compatible with the conditions of the amidation step, Ph_3PO being easily removed at the next step.

^{13}C NMR ($CDCl_3$): δ 41.8 (CH_2N); 67.9, 68.0 (C(6), C(6′)); 72.0 (OCH_2); 72.3, 72.9, 73.2, 74.5, 74.9, 75.1, 75.2 (OCH_2Ph); 72.8, 73.4, 74.8, 76.5, 79.8, 81.6, 82.3, 82.8 (C(2), C(3), C(4), C(5), C(2′), C(3′), C(4′), C(5′)); 102.6, 103.5 (C(1), C(1′)); 127.0, 127.2, 127.4, 127.5, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.5 (Ph); 137.9, 138.2, 138.1, 138.5, 138.6, 138.9, 139.0 (quat. Ph).

MS, *m/z* (I_{rel} (%)) 1038.7 [M + Na](100). $C_{63}H_{69}NNaO_{11}$. Calc.: *m/z* 1038.5 [M + Na].

4.3.2. {2-[(1,2-Dicarba-closo-dodecaborane(12)-1-yl)-acetylamino]ethyl} 2,3,6-tri-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (*4b*)

(A) To the solution of crude amine **2b** (0.100 mmol, calculated with respect to azide **1b** taken in the previous step), *N*-hydroxysuccinimide (13.9 mg, 0.121 mmol) and carboranylacetic acid **3a** [10] (19.7 mg, 0.102 mmol) in anhydrous THF (2 ml) *N*,*N*′-dicyclohexylcarbodiimide (DCC) (50.5 mg, 0.222 mmol) was added. The reaction mixture was stirred at room temperature for 2 days. Then the reaction mixture was filtered and the filtrate was washed successively with 2 M H_2SO_4 (20 ml), saturated aqueous $NaHCO_3$ (20 ml), and brine (30 ml), filtered through a cotton wool plug and concentrated. The residue was purified by HPLC on a silica gel column (250×15 mm, Silasorb 600, 7 μm) with gradient elution (hexanes → hexanes–AcOEt, 7:3) to give pure amide **4b** (18.6 mg, 15%), R_f 0.32 (hexanes–AcOEt, 7:3).

(B) To the solution of crude amine **2b** (0.087 mmol, calculated with respect to azide **1b** taken in the previous step), carboranylacetic acid **3a** [10] (17.0 mg, 0.087 mmol) in MeOH (1.5 ml) 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (26.5 mg, 0.096 mmol) was added. The reaction mixture was stirred at room temperature for 6 days. Volatiles were removed on a rotary evaporator and the residue was dissolved in Et_2O. This solution was washed successively with 2 M H_2SO_4 (20 ml), saturated aqueous $NaHCO_3$ (20 ml), and brine (30 ml), filtered through a cotton wool plug and concentrated. The residue was purified by HPLC on a silica gel column (250 × 15 mm, Silasorb 600, 7 μm) with gradient elution (hexanes → hexanes–AcOEt, 7:3) to give pure amide **4b** (7.3 mg, 7%), R_f 0.32 (hexanes–AcOEt, 7:3).

(C) To the solution of crude amine **2b** (0.087 mmol, calculated with respect to azide **1b** taken in the previous step) in CH_2Cl_2 (0.8 ml) saturated aqueous $NaHCO_3$ (0.8 ml) was added. To the resulting vigorously stirred two-phase mixture a suspension of carboranylacetic acid chloride **3b** [13] (55.6 mg, 0.267 mmol) in CH_2Cl_2 (1.2 ml) was added dropwise and the reaction mixture was stirred for 1h at room temperature. Organic layer was washed successively with 2 M H_2SO_4 (20 ml), saturated aqueous $NaHCO_3$ (20 ml), and brine (30 ml), filtered through a cotton wool plug and concentrated. The residue was purified by HPLC on a silica gel column (250 × 15 mm, Silasorb 600, 7 μm) with gradient elution (hexanes → hexanes–AcOEt, 7:3) to give pure amide **4b** (55.4 mg, 53%), R_f 0.32 (hexanes–AcOEt, 7:3), $[\alpha]_D^{20}$ + 4.8 (*c* 0.84, $CHCl_3$).

^{13}C NMR ($CDCl_3$): δ 40.7 (CH_2N); 41.6 ([$C_2HB_{10}H_{10}$]CH_2CO); 58.4 ([$CHB_{10}H_{10}C$]); 68.1, 69.3, 69.4 (C(6), C(6′), OCH_2); 70.9 ([$CHB_{10}H_{10}C$]); 72.5, 73.4 (2C), 74.7, 75.2 (2C), 75.4 (OCH_2Ph); 73.2, 73.3, 74.5, 77.8, 79.8, 81.8, 82.6, 82.8 (C(2), C(3), C(4), C(5), C(2′), C(3′), C(4′), C(5′)); 103.3, 104.3 (C(1), C(1′)); 127.5, 127.5, 127.6, 127.8, 127.9, 128.0, 128.2, 128.4, 128.5 (Ph); 137.3, 138.1, 138.4 (2C), 138.8, 139.0 (2C) (quat. Ph); 166.5 (CO).

$^{11}B\{^1H\}$ NMR ($CDCl_3$): δ −2.8 (1B), −5.6 (1B), −10.2 (br, 8B).

MS, *m/z* (I_{rel} (%)) 1224.8 [M + Na] (82). $C_{67}H_{81}B_{10}NNaO_{12}$. Calc.: *m/z* 1224.66 [M + Na].

4.4. Synthesis of carborane–carbohydrate conjugate *4c* without protective groups

4.4.1. Synthesis of *4c* from *O*-benzylated conjugate *4b*

A degassed solution of *O*-benzyl ether **4b** (55.4 mg, 0.046 mmol) in MeOH (2 ml) containing a catalyst (10% Pd/C, 10 mg) was stirred in a hydrogen atmosphere (1 bar) for 18 h at room temperature. The solids were filtered off on a Celite pad and the filtrate was concentrated to give crude product (26.0 mg), which, according to ^{11}B NMR analysis, contained 84% of the target 1,2-dicarba-*closo*-dodecaborane–lactose conjugate **4c** contaminated with 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5**.

4.4.2. Synthesis of *4c* from 2-aminoethyl lactoside (*2c*)

4.4.2.1. Aminoethyl 4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (2c). A degassed solution of 2-azidoethyl lactoside (**1c**) [9] (340 mg, 0.83 mmol) in H_2O (20 ml) containing a catalyst (10% Pd/C, 10 mg) was stirred in a hydrogen atmosphere (1 bar) for 18 h at room temperature. The solids were filtered off on a Celite pad and the filtrate was concentrated to give crude amine **2c** (292 mg, 91%), which was used in the next step without any purification. R_f 0.11, EtOH–*n*-BuOH–Py–AcOH–H_2O (100:10:10:10:3); $[\alpha]_D^{20}$ + 2.5 (*c* 8.5, H_2O).

^{13}C NMR (D_2O): δ 40.9 (CH_2N); 60.9 (C(6′)); 61.9 (C(6)); 69.8 (OCH_2); 69.4 (C(4′)); 71.8 (C(2′)); 73.4 (C(3′)); 73.7 (C(2)); 75.1 (C(5)); 75.6 (C(3)); 76.2 (C(5′)); 79.2 (C(4)); 103.0 (C(1′)); 103.8 (C(1)).

4.4.2.2. {2-[(1,2-dicarba-closo-dodecaborane(12)-1-yl)-acetylamino]ethyl} 4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (4c). To a stirred solution of carboranylacetic acid **3a** [10] (71.3 mg, 0.370 mmol) and 2-aminoethyl lactoside (**2c**) (141.8 mg, 0.368 mmol) in 3 ml of mixture MeOH–H_2O (2:1) mixture 4-(4,6-dimethoxy[1.3.5]triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) [11] (111.9 mg, 0.405 mmol) was added. After 22 h of stirring at room temperature volatiles were evaporated and the residue was dissolved in H_2O and purified by reversed phase chromatography on a SepPak C18 cartridge (gradient elution from H_2O to MeOH) to give two fractions. Fraction **A** (127.5 mg), eluted first, was a mixture of *closo*-carborane **4c** (65%), *nido*-carborane **5** (8%) and boric acid (27%) while the second, more pure, fraction **B** (48.8 mg) contained *closo*-carborane **4c** (94%), *nido*-carborane **5** (5%) and boric acid (1%) (composition of each fraction (molar %) was determined by integration of the respective signals in the ^{11}B NMR spectra). Taking into account the molecular masses of **4c** and **5** (569.61 and 558.80, respectively) total yields of *closo*-carborane **4c** and *nido*-carborane **5** could be calculated based on the percentages shown. This procedure gave 74.2% and 7.6% yields for **4c** and **5**, respectively.

Data for fraction **B**:

R_f 0.38 ($CHCl_3$–MeOH–H_2O, 65:25:4); $[\alpha]_D^{20}$ + 7.0 (*c* 2.4, H_2O).

^{13}C NMR (CD_3OD): **4c**,δ 40.7 (CH_2N); 44.0 ([$C_2HB_{10}H_{10}$]CH_2CO); 61.6 ([$CHB_{10}H_{10}C$]); 61.8 (C(6′)); 62.4 (C(6)); 69.3 (OCH_2); 70.2 (C(4′)); 71.6 ([$CHB_{10}H_{10}C$]); 72.4 (C(2′)); 74.7 (2C, C(3′)), (C(2)); 76.2 (C(5)); 76.4 (C(3)); 77.0 (C(5′)); 80.6 (C(4)); 104.2 (C(1′)); 105.0 (C(1)); 168.6 (CO).

$^{11}B\{^1H\}$ NMR (CD_3OD): **4c**, δ −2.3 (1B), −5.4 (1B), −9.5 (br, 8B).

$^{11}B\{^1H\}$ NMR (D_2O): **4c**, δ −5.1 (shoulder), −10.7 (br). See also Fig. 1(a).

Additional minor signals in the $^{11}B\{^1H\}$ NMR spectrum (CD_3OD): δ 18.9 (H_3BO_3); −32.8, −36.9 (*nido*-carborane **5**). Additional minor signals in the $^{11}B\{^1H\}$ NMR spectrum (D_2O): δ 19.1 (H_3BO_3); −33.3, −37.4 (*nido*-carborane **5**). See also Fig. 1(a).

MS, *m/z* (I_{rel} (%)) 594.4 [M + Na] (30). $C_{18}H_{39}B_{10}NNaO_{12}$. Calc.: *m/z* 594.33 [M + Na].

MS, *m/z* (I_{rel} (%)) 1165.4 [M_2 + Na] (19). $C_{36}H_{78}B_{20}N_2NaO_{24}$. Calc.: *m/z* 1165.67 [$M_2$ + Na].

4.5. {2-[(1,2-Dicarba-nido-undecaborane(12)-1-yl)-acetylamino]ethyl} 4-O-(β-D-galactopyranosyl)-β-D-glucopyranoside (5)

A solution of a sample (30 mg) containing *closo*-carborane **4c**, which was obtained from benzylated conjugate **4b** by hydrogenolysis, 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 (Fig. 1). After 17 h of heating no signals of the *closo*-carborane **4c** could be detected, the *nido*-carborane **5** (δ_B −11.7, −17.1, −19.5, −33.2, −37.6) and boric acid (δ_B 18.2) being the only components of the mixture (Fig. 1(b)). A sample of the 1,2-dicarba-*nido*-undecaborane–lactose conjugate **5** free from boric acid was obtained by repeated addition of MeOH and AcOH and evaporation of the volatiles (Fig. 1(c)).

R_f 0.0 ($CHCl_3$–MeOH–H_2O, 65:25:4); $[\alpha]_D^{24}$ − 15.4 (*c* 0.2, H_2O).

^{13}C NMR (D_2O): **5**, δ 40.1 (CH_2N); 45.8 ([$C_2HB_9H_{10}$]CH_2CO); 61.0 (C(6′)); 61.8 (2C, C(6)), ([$CHB_9H_{10}C$]); 69.4 (2C, C(4′), OCH_2); 70.5 ([$CHB_9H_{10}C$]); 71.8 (C(2′)); 73.4 (C(3′)); 73.7 (C(2)); 75.1 (C(5)); 75.6 (C(3)); 76.2 (C(5′)); 79.3 (C(4)); 103.1 (C(1′)); 103.8 (C(1)); 176.1 (CO).

$^{11}B\{^1H\}$ NMR (D_2O): **5**, δ −11.5 (2B), −17.2 (3B), −19.6 (2B), −33.4 (1B), −37.5 (1B).

Minor signal in the $^{11}B\{^1H\}$ NMR spectrum (D_2O): δ 18.1 (H_3BO_3). See also Fig. 1(c).

MS (detection of positive ions), *m/z* (I_{rel} (%)) 606.4 [M + 2Na] (58). $C_{18}H_{39}B_9NNaO_{12}$. Calc.: *m/z* 606.3 [M + 2Na].

MS (detection of negative ions), *m/z* (I_{rel} (%)) 560.5 [M] (64). $C_{18}H_{39}B_9NO_{12}$. Calc.: *m/z* 560.3 [M].

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