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Synthesis of a $(B_{12}H_{11}S)^{2-}$ containing glucuronoside as potential prodrug for BNCT

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Abstract

A $B_{12}H_{11}SH^{2-}$ containing glycoside of glucuronic acid has been prepared, for possible use as prodrug in BNCT. The synthesis was carried out by the Koenigs–Knorr reaction of the acetylated glucopyranosyluronate bromide with the nucleophile cyanoethylthioundecahydro-*closo*-dodecaborate(2−). After removal of the cyanoethyl-group, deacetylation and saponification of the reaction product tris(tetramethylammonium)-[S-(β-D-glucuronate)-thio] undecahydro-*closo*-dodecaborate(3−) could be prepared.

Keywords: BNCT; Prodrug; BSH; Glucuronic acid

1. Introduction

One of the major limitations of boron neutron capture therapy (BNCT) often is the insufficient tumor-selectivity of the anticancer agent used. To solve the same problem in conventional chemotherapy the prodrug concept was developed. A nontoxic prodrug circulates through the body until it is converted, at the tumor site, into an active anticancer agent. To achieve tumor selectivity enzymes overexpressed or overactive in tumor tissue can be used.

Human β-glucuronidase catalyzes the cleavage of β-D-glucuronosides. Mürdter et al. [1] could show that the level of the active anthracycline anticancer agent doxorubicin in tumor tissue after using the nontoxic glucuronide prodrug HMR 1826 was 7-fold higher than after perfusion with doxorubicin itself. The extracellular pH is lower in tumors than in healthy lung tissue (6.46 ± 0.35 versus 7.30 ± 0.33). Activity of β-glucuronidase is higher at an acidic pH than at a neutral pH. At pH of the tumor there is a 4-fold higher enzyme activity than in healthy tissue. Therefore the low tumor pH promotes prodrug activation by β-D-glucuronidase [2].

Our aim was the synthesis of a $B_{12}H_{11}S^{2-}$-containing glycoside of glucuronic acid. The uptake of BSH ($B_{12}H_{11}SH^{2-}$) in tumor could perhaps be increased if the β-glucuronoside of BSH is cleaved by this enzyme. BSH is being used clinically for the treatment of brain tumors [3]. Its tumor specificity is not very expressed [4] despite the fact that a substantial dose differential between tumor tissue and surrounding healthy tissue can be achieved.

The stereoselective synthesis of glycosides of BSH has been already successfully carried out with several sugars, using the Koenigs–Knorr reaction [5]. We used the cyanoethyl group both as intermediate protecting group and to steer the BSH-cluster in β-position.

2. Results and discussion

Acetylated glucopyranosyluronate bromide (**1**) reacts successfully with the nucleophile cyanoethyl-derivative

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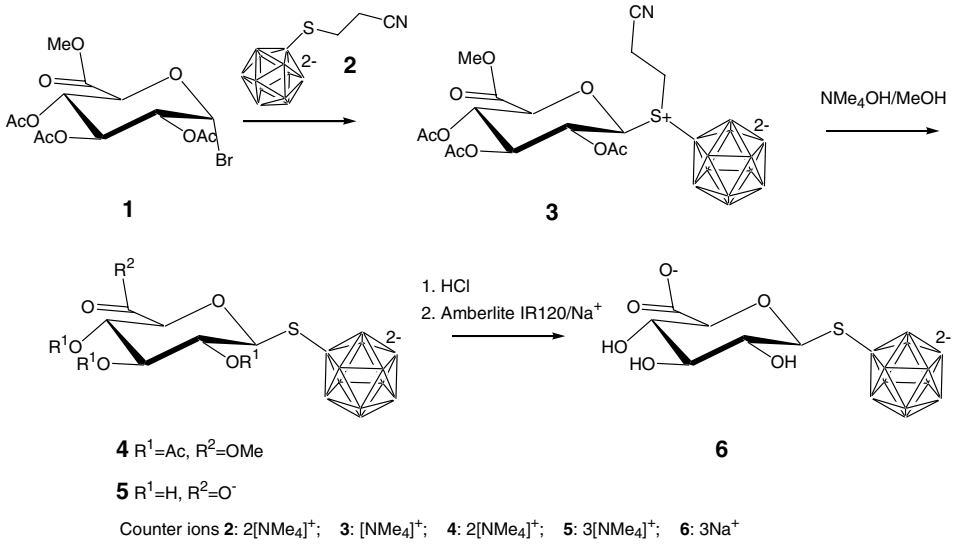

Scheme 1.

of the BSH-cluster $(Me_4N)_2[B_{12}H_{11}S(C_2H_4CN)]$ **2** in a Koenigs–Knorr reaction (Scheme 1). As expected, only the β anomer of the glucuronyl sulfonium undecahydro-*closo*-dodecaborate(2−) **3** is formed.

Removal of the cyanoethyl group is achieved with an equimolar amount of tetramethylammonium hydroxid (TMAOH) in a methanol/acetone mixture. For this still acetylated glucuronyl thio undecahydro-*closo*-dodecaborate(2−) **4** the stereochemistry of the ring and its substituents could be determined from NMR spectroscopy, as the vicinal H,H coupling constants in the pyrane ring were between 7 and 12 Hz, indicating a dihedral angle of 180°.

With an excess of TMAOH in methanol the hydroxy functions of the peracetylated glucuronyl sulfonium undecahydro-*closo*-dodecaborate(2−) **3** are deacetylated, the methyl ester function is saponified, and the cyanoethyl group is removed, to give the tetramethylammonium salt of glucuronyl-thio-undecahydro-*closo*-dodecaborate(3−) **5**. Attempts to deprotect the hydroxyl groups with $NaOCH_3$ in CH_3OH led to degradation products.

To use this compound in future biological studies we exchanged the toxic TMA^+ ions to Na^+ cations in **6**.

3. Experimental

Bis (tetramethylammonium)-(2-cyanoethyl)-thio-undecahydro-*closo*-dodecaborate (2−) $(Me_4N)_2[B_{12}H_{11}S(CH_2)_2CN]$ **2** was prepared as described in the literature [6]. Acetylated glucopyranosyluronate bromide **1** was prepared as described [7]. The ^1H NMR spectra were collected using Bruker Avance DPX-200 spectrometer and referenced to Me_4Si. IR spectra were obtained on a Bio-Rad FTS 155 spectrometer. For mass spectrometry Esquire-LC 00061 spectrometer was used. Electrospray ionization (ESI) was used for ion formation. Ions with B_{12}-boron pattern are indicated with a star (*); the peak with highest intensity is given.

3.1. Tetramethylammonium-{S-[methyl-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-3-cyanoethyl-sulfonio}-undecahydro-closo-dodecaborate(1−) $(Me_4N)[B_{12}H_{11}S(C_2H_4CN)(C_{13}H_{17}O_9)]$ (3)

916 mg (2.31 mmol) **1** and 220 mg (588 μmol) **2** was stirred in 53 mL dry acetonitrile at 60 °C for 3 h under nitrogen. The precipitate of tetramethylammonium bromide was filtered off. The solvent of the filtrate was distilled off in vacuo. The crude product was dissolved in 7 mL acetone, freed from the rest of tetramethylammonium bromide by filtration and precipitated with a tenfold volume of diethyl ether. After an additional precipitation with diethyl ether 199 mg (55%) of slightly brown crystalline product was obtained.

MS: acetone/isopropanol (1:10), 2 μL/min; negative: 544* A^{-1}, positive: 74 TMA^+; ^1H-NMR (DMSO, ppm): −0.14–1.90 (11H, br, $B_{12}H_{11}$), 1.96–2.07 (9H, cm, OAc), 3.08 (12H, s, Me_4N^+) 3.27–3.50 (4H, m, $(CH_2)_2CN$), 3.62 (3H, s, MeO–), 4.57 (1H, d, 10.3 Hz, H-5), 5.15–5.27 (2H, cm, H-1, H-4), 5.46 (1H, t, 9.54 Hz, H-2), 5.67 (1H, t, 9.29 Hz, H-3); IR (KBr): 2503(s, B–H), 1754(s, C=O), 1486(w), 1375(w), 1242, 1221 (s, C–O), 1044 (m), 949(w).

3.2. Bis(tetramethylammonium)-{S-[methyl-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)uronate]-thio}-undecahydro-closo-dodecaborate(2−) ($Me_4N)_2[B_{12}H_{11}S(C_{13}H_{17}O_9)]$ (**4**)

134 mg (217 μmol) **3** was dissolved in 5 mL acetone. 91 μL (217 μmol) of a 25% solution of $(CH_3)_4NOH$ in methanol was added dropwise. After 1 h the precipitate was filtered and washed with a small amount of acetone to give 92 mg (67%) of slightly yellow crystals.

^{1}H-NMR (DMSO, ppm): 0.19–1.79 (11H, br, $B_{12}H_{11}$), 1.89–2.08 (9H, cm, AcO), 3.07 (24H, s, Me_4N^+), 3.61 (3H, s, MeO), 3.92 (1H, d, 9.78 Hz, H-5), 4.54 (1H, t, 9.54 Hz, H-2), 4.71 (1H, d, 10.3 Hz, H-1), 4.82 (1H, t, 10.2 Hz, H-3), 5.07 (1H, t, 9.29 Hz, H-4).

3.3. Tris(tetramethylammonium)-[S-(β-D-glucuronate)-thio]-undecahydro-closo-dodecaborate(3−) ($Me_4N)_3[B_{12}H_{11}S(C_6H_8O_6)]$ (**5**)

199 mg (322 μmol) sulfonium glucopyranosyluronate **3** was dissolved in 5 mL acetone. 380 μL (902 μmol) of a 25% solution of $(CH_3)_4NOH$ in methanol was added dropwise. After 1 h the residue was filtered and washed with a small amount of acetone. The crude product was digested in acetonitrile to give 90 mg (49%) slightly brown crystals.

MS: H_2O/methanol (1:1), 2 μL/min; negative: 141* $(B_{12}H_{11})^-$, 424* $(A^{-3} + Me_4N^+ + H^+)^-$, 446* $(A^{3-} + Me_4N^+ + Na^+)^-$, 497* $(A^{-3} + 2Me_4N^+)^-$, positive: 74 TMA^+ ; ^{1}H NMR (D_2O, ppm): 0.2–1.8 (11H, br, $B_{12}H_{11}$); 3.03 (36H, s, Me_4N^+), 3.23–3.43 (3H, cm, H-2, H-3, H-4); 3.47 (1H, d, 9.78 Hz, H-5), 4.37 (1H, d, 9.78 Hz, H-1).

3.4. Trissodium [S-(β-D-glucuronate)-thio]undecahydro-closo-dodecaborate(3−) $Na_3[B_{12}H_{11}S(C_6H_8O_6)]$ (**6**)

89.8 mg (157 μmol) **5** was dissolved in a small amount of water. After neutralization with 1N HCl, TMA^+-ions were exchanged to Na^+-ions by an Amberlite IR 120 ion exchange column. Bidestilled water was used as eluent. The water was removed in vacuo to give 52 mg (79%) slightly yellow crystals.

MS: H_2O/methanol (1:1), 2 μL/min; negative: 141* $(B_{12}H_{11})^-$, 186* $(A^{3-} + Na^+)^{2-}$, 395* $(A^{3-} + 2Na^+)^-$, 452* $(A^{3-} + 2K^+)^-$.

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