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Synthesis and NMR properties of the first boron analogues of uracil

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ABSTRACT

Synthesis of 4-hydroxyborauracil and 4-hydroxy-3-methylborauracil, the first boron analogues of uracil, and comparison of their ^{1}H and ^{13}C NMR properties with those of uracil, are presented. The analyses of NMR-monitored boron compound–alcohol and boron compound–amine interactions pointed to the existence of sp^3-hybridized, B,B-bis-methoxyborauracils and pyridine-/*n*-butylamine-borauracils ate-complexes in solution.

1. Introduction

There are only few known boron analogues of nucleic acid bases, including benzoborauracils, and recently presented 5,6-dihydroborauracils and 5,6-dihydroborathymines [1–3]. It should be noted that borauracils, borathymines or boracytosines, and their derivatives, should be interesting compounds to be considered in search for new inhibitors of enzymes involved in nucleotide metabolism. Boron analogues of biologically active compounds show unique properties that may promote enzyme inhibition. Of particular interest is a capability to form tetrahedral sp^3-hybridized boron "ate" complexes following the nucleophilic attack of an enzyme onto the boron atom of borinic/boronic acids and their esters or amides. The "ate" complexes with enzymes are highly stable which results in effective inhibition [4–6]. Additionally, boron-containing compounds can be considered potentially active in boron-neutron capture therapy (BNCT) [7]. In this paper we present the synthesis of the first boron analogues of uracil (borauracil) along with NMR study of its structure and coordination properties.

2. Results and discussion

2.1. Boron analogues of 5,6-dihydrouracil and uracil

Only few heterocyclic compounds, containing covalently bound boron atom or atoms within ring structure, are known. The first benzoborauracils, and thiopheneuracil, containing boron at four-position of heterocyclic ring, were presented by Zhuo et al. [1]. Benzoborauracils were synthesized from *o*-aminebenzeneboronic acids that have a *cis* orientated 1,2-groups required for the ring closing reaction. The presence of the benzene moiety promotes formation of benzoborauracils, with benzene ring fused at the positions 5 and 6 of the heterocyclic moiety [1]. Unfortunately, compounds of this type have very limited applicability, since a considerable steric hindrance of aromatic ring makes them poor pyrimidine analogues. The closest uracil analogues so far are recently presented 5,6-saturated boron derivatives of uracil (5,6-dihydroborauracils) and 5,6-saturated borathymine (5,6-dihydroborathymines), containing boron atom at the position 4 of the heterocyclic ring [2,3].

The known boron analogues, compared to the parent uracil, show many structural and functional disadvantages structure. In particular, as pointed by the results of NMR analyses, 5,6-saturated borauracils differ structurally from the corresponding uracils by the lack of the ring planarity and ring current, characteristic for aromatic compounds. Benzoborauracils, in turn, shown to be aromatic compounds, contain additional 5,6-fused benzene ring, causing a considerable steric hindrance [1].

In contrast, judging based on various NMR data, the 5,6-unsaturated 4-hydroxyborauracils presented here show a marked structural and electronic similarity to 'natural' uracil, thus being good candidates for new building blocks of biologically active compounds, e.g. new enzyme inhibitors. Moreover, a unique boron property to form relatively stable "ate" complexes with various alcohols, amines, carboxylates, etc., allows to expect unusual inhibitory activities, not observed with carbon-based compounds [4–6].

2.2. Synthesis of 4-hydroxyborauracils

In our recent works two different routes of synthesis of 5,6-saturated borauracils and 5,6-saturated borathymines (4-hydroxy-5,6-dihydroborauracils and 4-hydroxy-5,6-dihydroborathymines respectively) have been presented. One included the hydroboration of N-vinylurea with dibromoborane [2], its major disadvantage being

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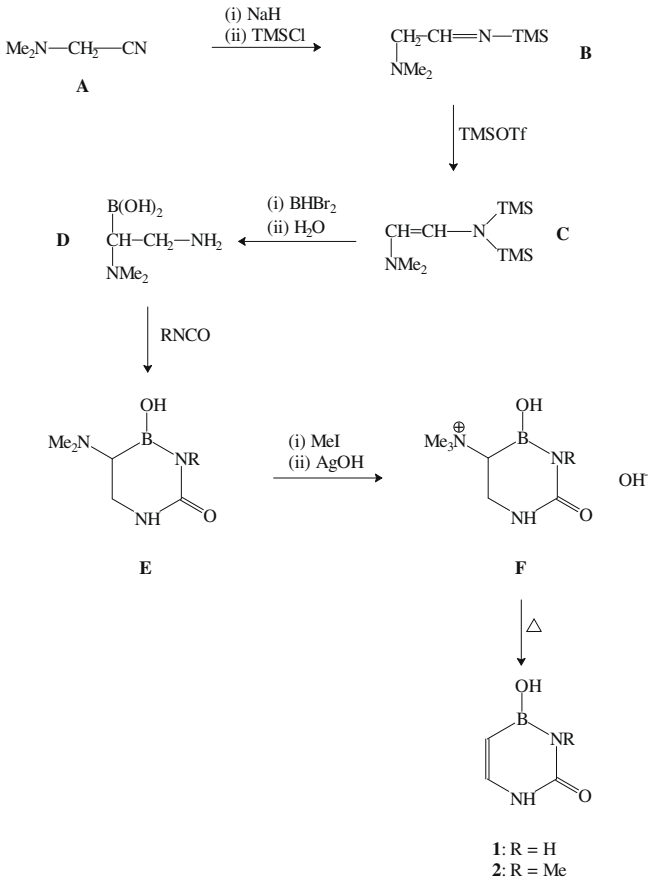

Fig. 1. Synthesis of 4-hydroxyborauracils.

a poor commercial availability of N-vinylurea, and especially its derivatives. The other, involving addition of hydrides and carboanions to the nitriles, followed by TMS protection steps, hydroboration with borane–dimethylsulfide and ring closing reaction [3], is time-consuming and uses highly toxic and pyrophoric reagents but allows to synthesize 5,6-dihydroborauracil derivatives with various substituents at the ring positions 3, 5 and 6 [3]. The compounds **1** and **2** were prepared using the latter method. Nitrile compound ((dimethylamino)acetonitrile, see Fig. 1) reaction with the hydride, followed by TMSCl protection step, gives the protected imine (Fig. 1B).

The N-trimethylsilylimino product (Fig. 1B) underwent reaction with trimethylsilyl triflate to give N,N-bis(trimethylsilyl)enamine (Fig. 1C), whose hydroboration with dibromoborane–dimethylsulfide complex, followed by hydrolysis, led to the formation of β-boronic amino acid (Fig. 1D). The cyclic product was obtained by the Zhuo method [1] with the use of two isocyanates, i.e. methyl isocyanate (R=Me, compound **2**) or isocyanic acid generated *in situ* in the reaction of potassium cyanate and acetic acid (R=H, compound **1**). The resulting cyclic products (5-N,N-dimethylamino-4-hydroxy-5,6-dihydroborauracils, Fig. 1E) were then used in a modified Hoffman elimination process, involving the reaction with methyl iodide, followed by ion exchange reaction with aqueous silver oxide suspension and thermal elimination step to give 5,6-unsaturated 4-hydroxyborauracil **1** and 4-hydroxy-3-methylborauracil **2**. It should be noted that 4-hydroxyborauracils, similarly to 5,6-dihydroborauracils are stable when stored in solid state, under oxygen-free conditions and at temperature below −10 °C.

2.3. NMR properties of borauracils

Boron compounds present very unique coordination behavior due to relatively easy change of boron hybridization state. Boronic and borinic acids, boranes and other boron compounds of sp^2 hybridization form stable sp^3-hybridized "ate" complexes upon coordination of an electron pair donor. The latter phenomenon may take place at an enzyme active site, resulting in the formation of sp^3 anionic form covalently bonded to enzyme protein [8,9].

The ^1H and ^{13}C NMR chemical shifts of compounds **1** and **2** are similar to those found for uracil. The ^{13}C chemical shift of carbonyl C(2) atoms of **1** and **2** are 153.1 and 153.2 ppm respectively, similar to that of 151.0 ppm, found for 'natural' uracil. In accord, ^1H chemical shift values of N(1)H (DMSO-d_6) for **1** and **2** are 10.41 and 10.32 ppm respectively, similar to the corresponding value of 10.9 ppm for uracil. The most interesting fragment, the 5,6-unsaturated region of 4-hydroxyborauracils, contains the C(5) carbon atom and its C(5)H hydrogen atom, showing chemical shift values of 95.0 and 5.18 ppm, respectively, comparable to the corresponding uracil values of 100.0 and 5.49 ppm, respectively. It should be noted that the $\delta_{C(6)}$ and $\delta_{C(6)H}$ values for the previously described 5,6-saturated 5,6-dihydroborauracils are located in entirely different regions of approx. 17–28 ppm and 1.3–1.5 ppm, respectively [2].

The most significant difference between NMR spectra of the analogue **1** and parent uracil, concerning the 5,6-unsaturated region, is apparent for the C(6) and C(6)H atoms, with the $\delta_{C(6)}$ values of 132.1 and 147.2 ppm, and $\delta_{C(6)H}$ values of 7.15 and 7.44 ppm, observed for **1** and uracil, respectively. The latter is apparently due to the lack in **1** of the mesomeric effect of the strongly electron-withdrawing pyrimidine C(4) carbonyl group, potentially resulting in a partial positive charge at the pyrimidine C(6). Comparison of the $\delta_{C(6)H}$ values suggests the above mentioned effect to be much weaker in the case of borauracils. The C(6) and C(6)H chemical shifts for **2** were 134.9 and 7.11 ppm, respectively, thus very similar to those found for **1**. Of note is that the $\delta_{C(6)}$ and $\delta_{C(6)H}$ values for 5,6-dihydroborauracils were found to be in the 34–43 ppm and 3.4–4.3 ppm ranges, respectively [2].

It is well known that the hybridization state of boron may be effectively analyzed with the use of ^{11}B NMR [2,3]. It was previously shown for a series of 4-hydroxy-5,6-dihydroborauracils with ^{11}B chemical shifts in the 24.6–29.5 ppm range in non-coordinating solvent. Interaction with large excess of boron-coordinating solvent (CD_3OD) resulted in a significant *upfield* shift, up to the −1.1–1.4 ppm range, with the formation of bis-methoxy species containing sp^3-hybridized, negatively charged boron atoms. Similar experiments done with compounds **1** and **2** showed the ^{11}B chemical shift in non-coordinating solvent (DMSO-d_6) of 32.1 and 32.0 ppm, respectively (Table 1), in a good agreement with earlier published data concerning similar compounds [1–3]. With both compounds studied in a coordinating solvent, the corresponding ^{11}B chemical shift values for (CD_3OD) were equal, 6.4 ppm and much lower (Table 1).

The average chemical shift difference value with **1** and **2** (25.7 ppm) is close to that found for 5,6-dihydroborauracils and 5,6-dihydroborathymines (23.0–24.4 ppm), pointing to the formation of analogical sp^3-hybridized bis-methoxy adducts (**1**·**(CD$_3$O)$_2$** and **2**·**(CD$_3$O)$_2$**, see Fig. 2) [3]. In accord with the previous reports, the solution of bis-methoxy adducts may contain the B–O form in equilibrium with the B–N form. The removal of methanol gives starting B–N form, as indicated by the ^{11}B NMR spectra.

In order to test potential interactions of the compounds **1** and **2** with nitrogen atoms of protein side chains, NMR-controlled experiments were done. As previously shown, those interactions can be studied using pyridine, mimicking tryptophan, or histidine and *n*-butylamine, mimicking lysine and proline side chains [3]. Table 1 presents the ^{11}B chemical shifts in non-coordinating solvent (DMSO-d_6, column 2), containing approx. 36 mM DMSO-d_6 solution of pyridine (column 5) or *n*-butylamine (column 6). The ^{11}B chemical shift values for complexes of **1** and **2** with pyridine were −4.1 and −4.4 ppm, respectively, significantly *upfield* shifted,

Table 1
^{11}B NMR chemical shifts of compounds **1** and **2** in DMSO-d_6, methanol-*d4* and in amine solutions.

Compound	δ ^{11}B in DMSO-d_6 (ppm)	δ ^{11}B in CD_3OD (ppm)	$\Delta\delta$ (ppm)[a]	δ ^{11}B in DMSO-d_6/py (ppm)[b]	δ ^{11}B in DMSO-d_6/$BuNH_2$ (ppm)[c]
1	32.1	6.4	25.7	−4.1	−3.3
2	32.0	6.4	25.6	−4.4	−3.6

[a] Difference of chemical shift between δ ^{11}B in DMSO-d_6 and δ ^{11}B in CD_3OD (ppm).
[b] 1:1 4-Hydroxyborauracils to pyridine molar ratios.
[c] 1:1 4-Hydroxyborauracils to *n*-butylamine molar ratios.

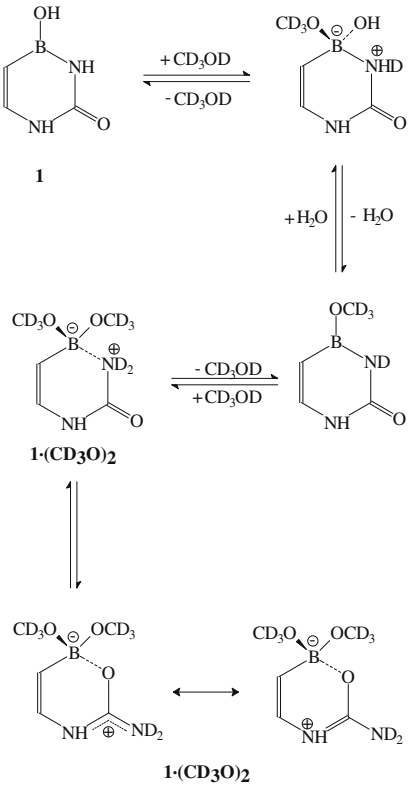

Fig. 2. Methanolysis equilibrium for **1**.

compared to 5,6-dihydroborauracils and 5,6-dihydroborathymines studied under identical conditions [3]. In similar, chemical shifts of complexes with *n*-$BuNH_2$ were −3.3 and −3.6 ppm for **1·BuNH$_2$** and **2·BuNH$_2$**, respectively. The observed chemical shift differences are in accord with the previously published data [1–3,10–18]. The presented values of ^{11}B chemical shifts prove the formation of boron sp^3-hybridized ate complexes.

3. Conclusions

The synthesis, reactivity and spectroscopic properties of first borauracils, 4-hydroxyborauracils and 4-hydroxy-3-methylborauracil, are presented. The synthetic route started with the reaction of nitrile with sodium hydride, followed by the trimethylsilyl chloride protection step. N-trimethylsilylimino product that formed, underwent the reaction with trimethylsilyltriflate to yield the enamine product. The following hydroboration produced boronic amino acid which was cyclised with the use of various isocyanates. A modified Hoffman elimination resulted in the formation of the unsaturated borauracil. The NMR data showed relatively high similarity of the chemical shifts of new compounds to those of uracil. The ^{11}B NMR chemical shifts of borauracils in methanol-*d4* are 6.4 ppm, pointing to the formation of B,B-bis-methoxy derivatives of analyzed compounds. Pyridine, mimicking interactions of tryptophan, or histidine and *n*-butylamine, mimicking interactions of lysine or proline side chains, were used in NMR-monitored experiments, demonstrating the formation of the "ate" complexes of borauracils containing additional nitrogen donors.

4. Experimental

4.1. Materials and methods

^{1}H NMR spectra were obtained with Bruker Avance II spectrometer, operating in the quadrature mode at 500 MHz. All ^{11}B spectra were performed using 5 mm pure quartz NMR tube. The residual peaks of deuterated solvents were used as internal standards. Elemental analysis was performed using Carlo Erba Elemental Analyser EA 1108. GC–MS analysis was carried out on Agilent Technologies 6890 N apparatus with 5973-Network mass detector. FTIR spectra were recorded on Perkin Elmer Paragon 1000 apparatus. All other reagents and deuterated solvents of the highest commercially available grade were purchased from Aldrich and used without further purification (with exception for DMSO-d_6 which was dried with anhydrous sodium sulfate). Rubber septa joints were purchased from Aldrich. All procedures, including preparation of samples for the NMR measurements, were carried out under nitrogen. The sodium hydride reactions steps, as well as all operations performed with the reagents, were performed in airbag filled with dry nitrogen.

4.2. Preparation of the compound *1* (4-hydroxyborauracil)

To the solution (−20 °C) of anhydrous (dimethylamino)acetonitrile (0.17 ml, 1.7 mmol) in tetrahydrofuran (1 ml) in two-neck round bottom flask filled with dry nitrogen, the sodium hydride suspension (38 mg, 1.6 mmol in 1 ml of dry THF) was added over the period of 1 h. The reaction mixture was a stirred for another 1 h, followed by addition of trimethylchlorosilane (0.2 ml; 1.6 mmol) and further 4 h stirring. The reaction mixture was then filtered under nitrogen atmosphere, filtrate dried under high vacuum and used in the next reaction step without further purification. Thus obtained imine derivative (0.22 g, 1.4 mmol) was inserted into a three-neck reaction flask containing triethylamine (0.4 ml, 2.9 mmol) and toluene (2 ml). Trimethylsilyltriflate (0.26 ml, 1.4 mmol) was then added to the reaction mixture over a period of 1 h, followed by 4 h stirring of the mixture at room temperature. The resulting enamine derivative was purified by vacuum distillation. Enamine (0.25 g, 1.1 mmol, Fig. 1C) was then dissolved in dichloromethane (0.5 ml) and added slowly with a syringe into a three-neck round bottom flask containing dibromoborane–dimethylsulfide complex dichloromethane solution (1.1 ml of 1 M dibromoborane–dimethylsulfide solution at 0 °C). After 24 h of stirring (final temperature ~20 °C), a solution of deoxygenated water (0.041 ml, 2.3 mmol) in THF (0.5 ml) was added and the mixture stirred for another 2 h. Solvents were then removed from the reaction mixture under high vacuum and the reaction product (0.13 g of solid product) used in the next synthetic step. To 63 mg (0.48 mmol) of thus obtained boronic amino acid (Fig. 1,

product D), dissolved in 50% acetic acid (1 ml), a solution of potassium isocyanate (82 mg, 1 mmol) in water (0.2 ml) was added, and the resulting mixture stirred for 48 h at RT. The solvents were then removed from the reaction mixture under high vacuum, and acetone (3 × 0.5 ml) was used to wash out the cyclic product (see Fig. 1E, where R=H), with 39 mg yield. To the methanol solution of the cyclic product (39 mg, 0.25 mmol in 5 ml of methanol) methyl iodide (0.017 ml, 0.27 mmol) was added dropwise over a 15-min period at RT. The reaction flask was then cooled in an ice bath (2 h) and stirred for another 24 h at RT. Product was vacuum dried, and washed three times with ethyl ether (3 × 0.3 ml) and again dried. Thus obtained iodide was then dissolved in water (1 ml) and reacted with silver oxide (*ca.* 53 mg) over a period of 12 h (RT), then filtered and liquid vacuum dried. Solid product was then carefully heated under vacuum on an oil bath to approx. 90–95 °C (1–1.5 h) following by vacuum drying. Yield 12 mg. Analytical data for **1**: MS (electrospray ionization, *m/z*): 112 (100%). Elemental analysis: calculated for $C_3H_5BN_2O_2$: C 32.20%, H 4.50%, N 25.04%; found C 31.86, H 4.62, N 24.95. ^{1}H NMR (DMSO-d_6, ppm): 10.41 (br, N(1)H, 1H); 8.92 (br, OH, 1H); 8.05 (br, N(3)H, 1H); 7.15 (m, C(6)H, 1H); 5.18 (m, C(5)H, 1H). ^{13}C NMR (DMSO-d_6, ppm): 153.1 (C=O); 132.1 (C(6)); 95.0 (br, C(5)). ^{11}B NMR (DMSO-d_6, ppm): 32.1. ^{11}B NMR (methanol-*d4*, ppm): 6.4.

4.3. Preparation of the compound **2** (4-hydroxy-3-methylborauracil)

It was obtained by the reaction of boronic amino acid (63 mg, Fig. 1, product D; see Section 4.2) with methyl isocyanate (60 μl, 1 mmol) in tetrahydrofuran solution (0.4 ml) at RT (48 h). The product was then vacuum dried. To the methanol solution of cyclic product (35 mg, 0.20 mmol in 1 ml of methanol) methyl iodide (14 μl, 0.22 mmol) was added dropwise over a 15-min period at RT. The reaction flask was then cooled in an ice bath (2 h) and stirred for another 24 h period at RT. The product was vacuum dried, later washed three times with ethyl ether (3 × 0.2 ml) and dried. Thus obtained iodide was then dissolved in water (1 ml) and reacted with silver oxide (44 mg, 0.19 mmol) over a period of 12 h (RT), then filtered and vacuum dried. Solid product was then carefully heated under vacuum in an oil bath to approx. 90–95 °C (1–1.5 h), followed by vacuum drying. Yield 11 mg. Analytical data for **2**: MS (electrospray ionization, *m/z*): 126 (100%). Elemental analysis: calculated for $C_4H_7BN_2O_2$: C 38.15%, H 5.60%, N 22.25%; found C 38.04, H 5.71, N 22.17. ^{1}H NMR (DMSO-d_6, ppm): 10.32 (br, N(1)H, 1H); 8.95 (br, OH, 1H); 7.11 (m, C(6)H, 1H); 5.20 (m, C(5)H, 1H); 2.91 (s, Me, 3H). ^{13}C NMR (DMSO-d_6, ppm): 153.2 (C=O); 134.9 (C(6)); 96.2 (br, C(5)); 28.9 (Me). ^{11}B NMR (DMSO-d_6, ppm): 32.0. ^{11}B NMR (methanol-*d4*, ppm): 6.4.

4.4. Interactions of **1** and **2** with pyridine and n-butylamine

Approx. 2 mg of a given cyclic compound was dissolved in 0.5 ml of DMSO-d_6, containing pyridine or *n*-butylamine (1:1 pyridine or *n*-butylamine to borauracil molar ratios). The NMR results are presented in Table 1.

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