A Clean Conversion of D-Glucosamine Hydrochloride to a Pyrazine in the Presence of Phenylboronate or Borate

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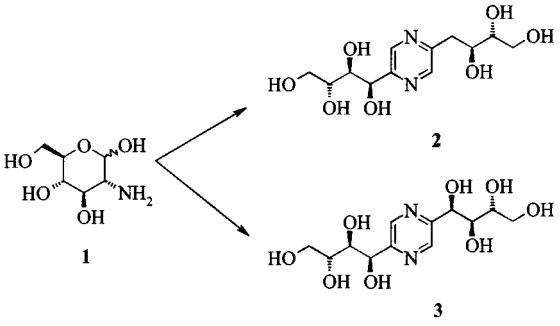
D-Glucosamine was found to undergo a condensation to give 2-(*arabo*-tetrahydroxybutyl)-5-(*erythro*-2,3,4-trihydroxybutyl)-pyrazine (**2**) as practically the sole product in the presence of phenylboronate or borate. The reaction proceeds in aqueous solutions at room temperature in 3 h in 58% isolated yield. In D_2O solutions, the incorporation of one deuterium into the methylene group of the trihydroxybutyl arm was found. The borate esters of the product were investigated by ^{11}B and ^{13}C NMR spectroscopy.

Introduction

Borate and phenylboronate are known to enhance the selectivity in a variety of reactions with carbohydrates, including isomerization,[1] esterification,[2] isopropylidation,[3] oxidation with bromine,[4] hydrogenation,[5] dehydration,[6] and alkaline oxidative degradation of aldohexoses by hydrogen peroxide.[7] Generally, the rationale for the selectivity enhancement is the formation of borate or boronate esters of the desired product. Moreover, these boron compounds exert a protecting role with respect to degradation reactions.

Aminosaccharides are able to undergo a variety of unique reactions, due to the presence of both amino and carbonyl groups, which may result in complex reaction mixtures due to, for example, Amadori rearrangements of initially formed Schiff bases, followed by further rearrangement and dehydration steps leading to browning.[8] The nature of the products formed is strongly dependent on the reaction conditions, particularly on the pH. It has been reported that D-glucosamine (**1**), in aqueous solutions, can be converted into complex mixtures of products, containing the pyrazine derivatives deoxyfructosazine [2-(*arabo*-tetrahydroxybutyl)-5-(*erythro*-2,3,4-trihydroxybutyl)-pyrazine] (**2**) and fructosazine [2,5-bis(*arabo*-tetrahydroxybutyl)pyrazine] (**3**) as major components (see Scheme 1).[9–11] Besides pyrazine derivatives, 5-hydroxymethyl-2-furaldehyde and products of its decomposition have also been observed. The starting amino sugar **1**, as well as products **2** and **3**, are of pharmaceutical interest, as they are reagents for DNA strand cleavage.[9]

The distribution of the reaction products can be influenced by the addition of amino acids (glycine, cysteine) and by the pH of the reaction mixture. The effect of the buffer used to maintain the pH has been studied: a mixture of pyrazine derivatives **2** and **3** is always formed, leading to the need for an HPLC separation. The use of a stoichiometric amount of cysteine can increase the yield of **2**.[9] Better yields of compound **2** have been obtained by heating D-glucosamine in acetic acid.[12] The disadvantage of this procedure is that the purification of compound **2** from the brown complex reaction mixture obtained is tedious.



Scheme 1. The main reaction pathways for the cyclocondensation of two molecules glucosamine

Here, we report a clean and selective conversion of glucosamine hydrochloride into compound **2** in the presence of phenylboronic acid or boric acid and NaOH. The borate esters of the product were investigated by ^{11}B and ^{13}C NMR spectroscopy.

Results and Discussion

The reaction of glucosamine hydrochloride in aqueous solution and in the presence of phenylboronic acid and NaOH gave compound **2** almost exclusively (96%) in 2 h. The reaction mixture was only slightly yellow and no elaborate separation steps were needed to isolate **2**. After removal of the phenylboronate by extraction with diethyl ether and recrystallization of the residue, pure **2** was obtained in 58% yield. Similar results were obtained with disodium tetraborate, sodium metaborate or a solution of boric acid in sodium hydroxide.

The explanation for the formation of **2** during the reaction is based on a previously published mechanism,[9,12] taking into account the formation of borate (or boronate) es-

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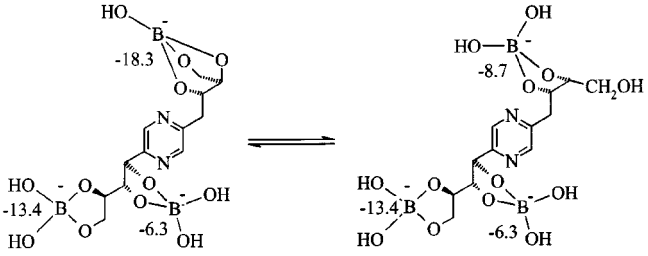
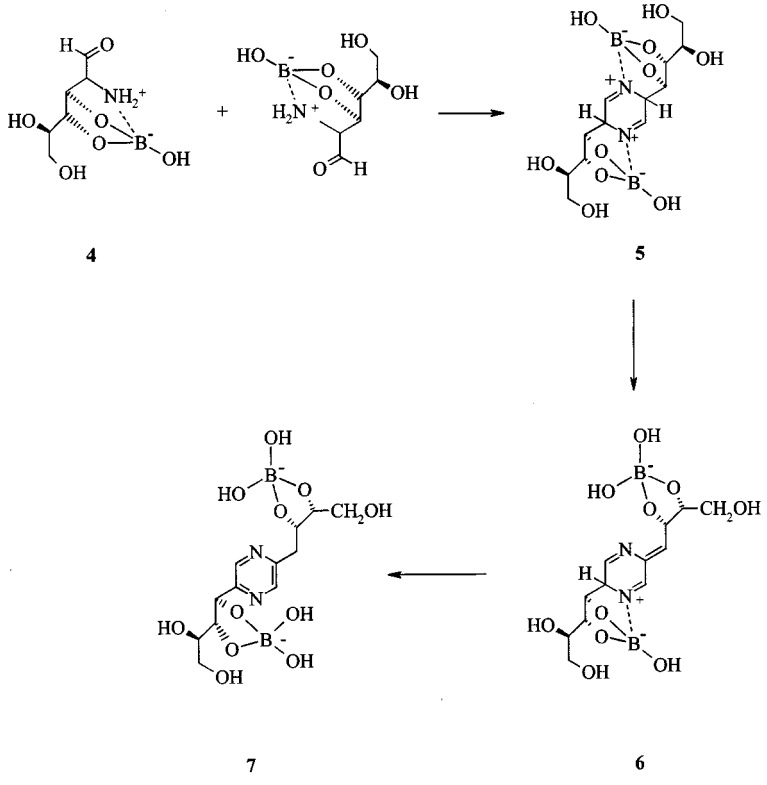
ters. The reaction in the presence of borate was too fast to follow by NMR spectroscopy, and only the borate esters of the reaction product **2** could be observed and identified (see below). However, from the high reactivity and selectivity of the reaction in the presence of borate, it can be concluded that borate esters play an important role in the mechanism. The starting compound glucosamine may occur in the pyranose, furanose and open forms. From previous studies regarding the stability of borate esters of diol groups in various configurations,[13,14] it is evident that the open form has the highest affinity for borate. A good model is the corresponding D-glucosamine oxime, for which the local association constants for borate ester formation at the *threo*-3,4-diol and at the *erythro*-4,5-diol moieties have been determined to be log$K$ = 2.6 and log$K$ = 1.5, respectively.[15] Here K is defined by $K = [B^-L]/[B^-][L]$, where B^-L denotes the borate ester, B^- free borate $[B(OH)_4^-]$ and L the free sugar. As a result of the high affinity of borate for the open form of glucosamine, the population of this form is relatively high in the mixture of borate esters. Condensation of two molecules of the borate esters of the open form of glucosamine (**4**) then gives the borate esters of the dihydropyrazine derivative **5**. The elimination of water then results in the borate esters of the corresponding pyrazine (**6**). This reaction step could be assisted by the borate ester moiety. The isomerisation of **6** by protonation/deprotonation gives the borate ester of **2** (**7**) (see Scheme 2). Under the conditions applied, a competitive oxidation of **5** to the borate ester of **3** does not proceed to a large extent (4%). The borate ester **7** is converted into free **2** by acidification with HCl.

To support the proposed reaction mechanism, we performed an experiment using D_2O and NaOD as the reaction medium. ^{13}C NMR measurements and an APT experiment[16] on the product showed no deuteration of the pyrazine ring carbons, while one deuterium was incorporated into the methylene group, -CHD-. This leads to the conclusion that the deuterium is incorporated into **2** during the protonation/deprotonation step of the intermediate **6** upon formation of the aromatic ring.

We studied the borate esters of **2** in solution by ^{11}B and ^{13}C NMR spectroscopy in the system disodium tetraborate (excess)/glucosamine hydrochloride. From integration of the spectra it can be deduced that compound **2** is bound to three borate moieties (see Scheme 3). The formation of bo-



Scheme 3. Schematic representation of the borate esters of compound **2**; the B atoms are labeled with the ^{11}B chemical shifts of the species



Scheme 2. Proposed mechanism for the cyclocondensation of two molecules of glucosamine in the presence of borate

rate esters starts at pH ≈ 6.5; this relatively low pH value (in comparison to glucose, for example) can be explained by the stabilization of the ester by the pyrazine nitrogen atom. A similar stabilization of the borate esters of aminodiols has been described for the system boric acid/3-dimethylamino-1,2-propandiol.[17] The effect of the pyrazine nitrogen atoms may be responsible for the formation of **2**.

The type of borate esters (mode of borate coordination) was established using known characteristic values of ^{11}B NMR chemical shifts for various types of borate esters.[14,17] The results are summarized in the Table 1.

Table 1. Comparison of the ^{11}B chemical shifts of compound **2** with literature values

Type of borate coordination in the ester	^{11}B chemical shift typical range	observed
1,2-Bidentate	−12.6 to −14.9[a]	−13.4
Tridentate	−18.1 to −19.4[a]	−18.3
1,2-Bidentate with interaction to nitrogen	−10.5 to −12.0[b]	−8.7; −6.3

[a] Ref.[7] – [b] Ref.[17] converted into the H_3BO_3 chemical-shift scale. For interaction of boronate with an aliphatic amino group.

Thus, the ^{11}B NMR spectrum reveals the presence of 1,2-bidentate and tridentate coordinated borate anions and two non-equivalent borate anions having an interaction with nitrogen. At the same time, the sum of the intensities of the signals at δ = −18.3 and −8.7 is approximately equal to the intensity of the signals at δ = −13.3 or δ = −6.3, probably due to an equilibrium between these forms. The structures of the borate esters in solution are depicted schematically in Scheme 3.

Experimental Section

NMR Experiments: NMR experiments were performed on a Varian Unity-Inova 300 spectrometer in D_2O as solvent at 25 °C. For the ^1H and ^{13}C NMR measurements *tert*-butyl alcohol was used as an internal standard with the methyl signal calibrated at δ = 1.2 and 31.2, repsectively. The ^{11}B chemical shifts are reported with respect to 0.1 M H_3BO_3 in D_2O as external standard (substitution method).

Deoxyfructosazine (2): Phenylboronic acid (3.05 g, 25 mmol) was added to a solution of NaOH (1.00 g, 25 mmol) in 60 mL of water. The resulting suspension was stirred until a clear solution was formed. D-Glucosamine hydrochloride (2.16 g, 10 mmol) was then added portionwise during 5 min. and the mixture obtained was stirred at room temperature for 3 h. During this time the color of the reaction mixture turned to light yellow. The pH was decreased to 2−3 by dropwise addition of 10% HCl. Phenylboronic acid precipitated and was quantitatively recovered by extraction with diethyl ether (5 × 40 mL) followed by evaporation of the solvent. The light-orange aqueous phase was evaporated to dryness in vacuo, with the bath temperature kept below 40 °C. The semi-solid residue was extracted with 15 mL of methanol/ethanol (2:1 v/v) and then with pure ethanol. The extract was evaporated to dryness and the procedure was repeated in order to remove the last portion of NaCl. The residue obtained was triturated in 5 mL of MeOH with heating to the boiling point. Dry ethanol (15 mL) was then added dropwise with stirring, after which the mixture was allowed to cool to room temperature. The light grey product was filtered off, washed with ethanol and dried at 80 °C. Recrystallization from MeOH/EtOH afforded 0.88 g (58%) of analytically pure **2**; m.p. 157−158 °C (ref.[12] 161−162 °C); $[\alpha]_D^{21}$ = −78.9 (*c* = 0.033 M) (ref.[12] −78). − ^1H NMR (399.9 MHz, D_2O): δ = 2.93 (m, 1 H), 3.17 (m, 1 H), 3.64 (m, 3 H), 3.77 (d, 1 H), 3.81 (m, 3 H), 3.98 (m, 1 H), 5.13 (d, 1 H), 8.50 (d, 1 H), 8.65 (d, 1 H) − ^{13}C NMR (100.6 MHz, D_2O): δ = 39.15 (CH_2), 64.08, 64.56, 72.64, 72.91, 72.96, 75.02, 76.05, (7 C, *C*HOH), 143.82 and 145.72 (2 C arom. *C*H), 154.84 and 155.64 (2 C, arom. C).

Acknowledgments

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