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Synthesis of β-D-glucopyranuronosylamine in aqueous solution: kinetic study and synthetic potential

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ABSTRACT

A systematic study of the synthesis of β-D-glucopyranuronosylamine in water is reported. When sodium D-glucuronate was reacted with ammonia and/or volatile ammonium salts in water a mixture of β-D-glucopyranuronosylamine and ammonium *N*-β-D-glucopyranuronosyl carbamate was obtained at a rate that strongly depended on the experimental conditions. In general higher ammonia and/or ammonium salt concentrations led to a faster conversion of the starting sugar into intermediate species and of the latter into the final products. Yet, some interesting trends and exceptions were observed. The use of saturated ammonium carbamate led to the fastest rates and the highest final yields of β-D-glucopyranuronosylamine/carbamate. With the exception of 1 M ammonia and 0.6 M ammonium salt, after 24 h of reaction all tested protocols led to higher yields of β-glycosylamine/carbamate than concentrated commercial ammonia alone. The mole fraction of α-D-glucopyranuronosylamine/carbamate at equilibrium was found to be 7–8% in water at 30 °C. Concerning bis(β-D-glucopyranuronosyl)amine, less than 3% of it is formed in all cases, with a minimum value of 0.5% in the case of saturated ammonium carbamate. Surprisingly, the reaction was consistently faster in the case of sodium D-glucuronate than in the case of D-glucose (4–8 times faster). Finally, the synthetic usefulness of our approach was demonstrated by the synthesis of three *N*-acyl-β-D-glucopyranuronosylamines and one *N*-alkylcarbamoyl-β-D-glucopyranuronosylamine directly in aqueous–organic solution without resorting to protective group chemistry.

1. Introduction

Uronic acids are monocarboxylic acids formally derived from aldoses by replacement of the hydroxymethyl group CH_2OH with a carboxy group.[1] In nature, they are found in polysaccharides fulfilling diverse biological and structural functions such as glycosaminoglycans (e.g., heparin, hyaluronan, and chondroitin), and homoglycuronans (e.g., alginates and pectins).[2] In order to incorporate uronic acids into glycoconjugates, it would be advantageous to selectively functionalize their reducing end without resorting to protective group chemistry, which tends to be rather cumbersome in the case of monosaccharides[3] and exceedingly time consuming in the case of oligoglycuronans.[4] A possible solution could be the transformation of unprotected uronic acids into the corresponding glycosylamines directly in water.

Glycosylamines have already been used as intermediates in the synthesis of a number of glycoconjugates,[5–7] such as glycopeptides,[8–14] surfactants,[11,15–17] glycopolymers,[15,18,19] and *N*-glycan probes.[20,21] Beginning in 1986 with the pioneering work of Kochetkov and collaborators,[14] four original protocols have been described for the synthesis of β-glycopyranosylamines in aqueous or aqueous methanol solutions (Table 1).[17,22–25] They are all based on the use of ammonia and/or volatile ammonium salts, and have found widespread application in the derivatization of hexoses, 6-deoxyhexoses, and oligosaccharides of different chain length.[13,18,19,21]

The main advantage of these aqueous-based methods resides in their applicability to unprotected and/or charged carbohydrates. Nevertheless, the considerable amount of salt used, the labor-consuming procedures needed to remove it, and the formation of diglycosylamine restricts their scope in preparative synthesis. Surprisingly, a detailed study on the formation of glycosylamines in aqueous solution is lacking[26] and only two papers claim the preparation of glycuronosylamines in aqueous[22] or aqueous methanolic solution while providing precious little details (only the salt with carbamic acid was isolated).

In order to palliate to this dearth of information, we have carried out a systematic study of the synthesis of glycuronosylamines in aqueous solution. In particular, we tried to verify whether such transformations could be conveniently performed, and to identify the experimental conditions leading to the maximum yield in the shortest reaction time, and with the smallest amount of reagents.

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Table 1
Experimental protocols reported in the literature for the synthesis of glycosylamines in aqueous solution[a]

Method	$[carb]_0$ (M)	$[NH_3]_0$ (M)	Salt	$[salt]_0$ (M)	T (°C)	React. time	Substrate	Yield (%)
Kochetkov[14]	⩽0.2	0	NH_4HCO_3	Satd (~3.6)[b]	30	6 d	GlcNAc	80
Lubineau[17,26]	0.2	~16 M	NH_4HCO_3	0.2	42	36 h	D-Glc	100
Gallop[22]	⩽0.06	0	$(NH_4)_2CO_3$	Satd (~3.3)[b]	~25[c]	5 d	D-GlcA	60
Likhosherstov[24,25]	0.8	~7.5 M[d]	$NH_2CO_2NH_4$	3.2	20	48 h	D-GlcA	81[e]

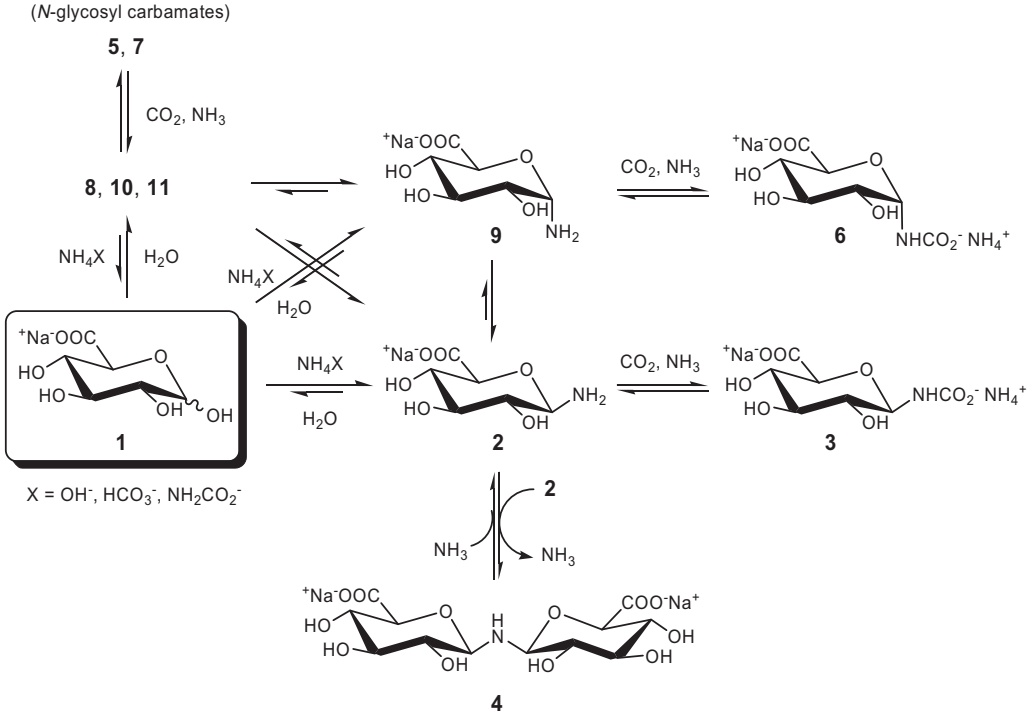
Whenever possible, the exact conditions used for uronic acids are listed.
[a] *Note:* $[s]_0$ indicates the initial concentration of species 's'.
[b] Solubility in water: NH_4HCO_3, 284 g/kg at 30 °C; $(NH_4)_2CO_3$, 320 g/L at 20 °C.
[c] Room temperature in the original paper.
[d] NH_3 15 M/CH_3OH 1:1.
[e] Only the salt with carbamic acid was isolated.

In a previous communication[27] we reported that the reaction of sodium D-glucuronate with ammonia and/or volatile ammonium salts in water yields the expected β-D-glucopyranuronosylamine and ammonium *N*-β-D-glucopyranuronosyl carbamate for long reaction times (~24 h), whereas intermediate samples contain a considerable amount of transient species (Scheme 1). From NMR and MS experiments we could establish that two such species (**6** and **9**) are the α anomer of the main products, whereas the others are precursors to the formation of α/β-D-glucopyranuronosylamine and ammonium *N*-α,β-D-glucopyranuronosyl carbamate. To date, the exact structures of species **5**, **7**, **8**, **10**, and **11** are unknown, other than the fact that **5** and **7** are *N*-glycosyl carbamates. Based on the ^1H NMR assignments made in that study, we developed a protocol for quantifying of the different compounds taking part to the reaction and used it to carry out a kinetic study of the same transformation under different experimental conditions. For comparison, some of experimental conditions tested were also applied to D-glucose. We now report the results of this quantitative study together with a few examples of the synthetic potential of our approach.

2. Results and discussion

2.1. Kinetic study

Sodium D-glucuronate (**1**) was reacted with ammonia and/or volatile ammonium salts (NH_4HCO_3 or $NH_2CO_2NH_4$) in water according to 18 different protocols at 30 and 40 °C (Scheme 1). For comparison, some protocols were also applied to D-glucose. The exact experimental conditions used and the final composition of the gross products are summarized in Table 2 (see also the Experimental part for the meaning of protocol numbering). Samples were drawn at preset reaction times, frozen in liquid nitrogen and freeze-dried overnight to eliminate water and most of the salts. No further purification was performed, and all reported analyses refer to the gross products obtained this way. In order to monitor the time course of the reaction, individual samples were redissolved in cold D_2O to afford clear solutions of pD ~9 that were immediately analyzed by ^1H NMR spectroscopy. Spectra were acquired at 278 K in order to inhibit hydrolysis of the product and to prevent the peak of residual HDO from interfering with integration.



Scheme 1. Reactions taking place during the synthesis of β-D-glucopyranuronosylamine **2** in aqueous solution.[27]