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

Ali Ghadban, Luca Albertin *, Rédéo W. Moussavou Mounguengui, Alexandre Peruchon, Alain Heyraud

Centre de Recherches sur les Macromolécules Végétales (CERMAV-CNRS),† BP53, 38041 Grenoble cedex 9, France

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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.

* Corresponding author. Tel.: +33 04 76 03 76 25; fax: +33 04 76 54 72 03.
E-mail addresses: luca.albertin@cermav.cnrs.fr, luca_albertin@yahoo.com (L. Albertin).
† Affiliated with Université Joseph Fourier, and member of the Institut de Chimie Moléculaire de Grenoble.

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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]

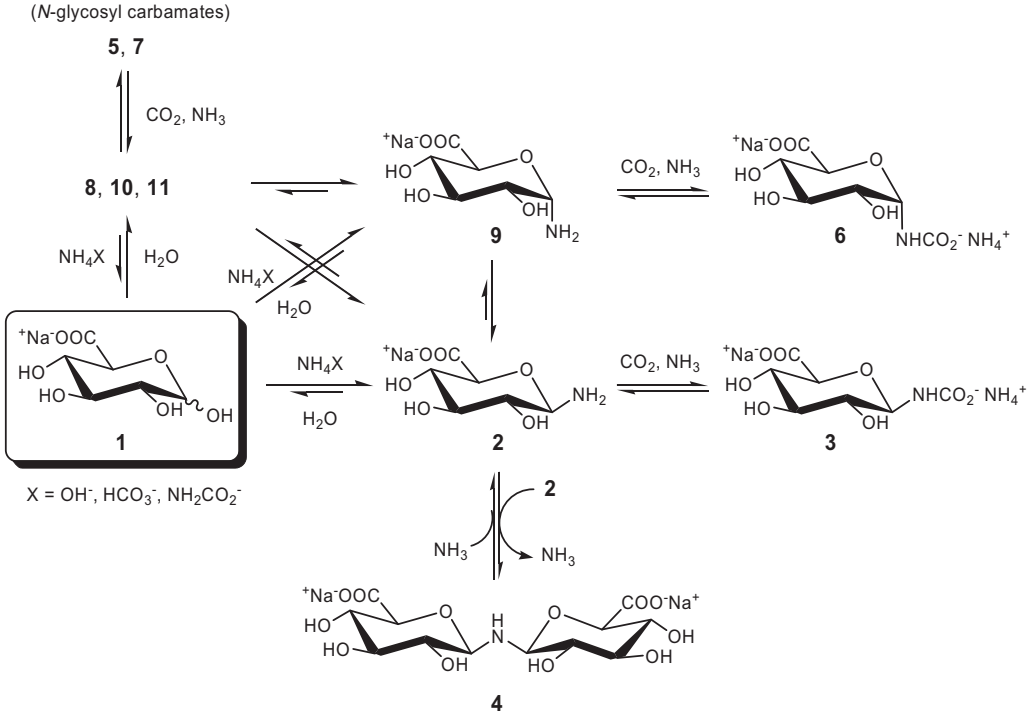
Whenever possible, the exact conditions used for uronic acids are listed.
[a] *Note:* $[s]_0$ indicates the initial concentration of species 's'.
[b] Solubility in water: NH_4HCO_3, 284 g/kg at 30 °C; $(NH_4)_2CO_3$, 320 g/L at 20 °C.
[c] Room temperature in the original paper.
[d] NH_3 15 M/CH_3OH 1:1.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

Table 2
Summary of the ammination experiments performed in this study[a]

Entry	Protocol[b]	Substrate	T (°C)	$[NH_3]_0$ (M)	Salt	$[salt]_0$ (M)	$[salt]_0/[subst.]_0$	Highest yield (%)		
								Time (h)	β-carbNHX[c]	By-products[d]
1	**A**.0.S	GlcA	30	—	NH_4HCO_3	Satd (~3.6)[e]	~18	24	75 (19)	11
2		Glc	30	—	NH_4HCO_3	Satd (~3.6)[e]	~18	168[f]	64 (14)	10
3		GlcA	40	—	NH_4HCO_3	Satd (>3.6)[e]	>18	7.5	78 (22)	9
4	**A**.1.06	GlcA	30	1	NH_4HCO_3	0.60	3	33[f]	67 (21)	21
5	**A**.5.02	GlcA	30	5	NH_4HCO_3	0.20	1	32.5[f]	80 (34)	15
6		GlcA	40	5	NH_4HCO_3	0.20	1	9.5	75 (22)	16
7	**A**.5.06	GlcA	30	5	NH_4HCO_3	0.60	3	33[f]	84 (17)	15
8	**A**.9.06	GlcA	30	8.8	NH_4HCO_3	0.60	3	33	86 (17)	13
9	**A**.14.02	GlcA	30	14.5	NH_4HCO_3	0.20	1	33[f]	84 (28)	13
10		Glc	30	14.5	NH_4HCO_3	0.20	1	168[f]	82 (22)	13
11	**B**.0.10	GlcA	~30[g]	—	$NH_2CO_2NH_4$	1.0	5	24	69 (47)	8
12	**B**.0.20	GlcA	~30[g]	—	$NH_2CO_2NH_4$	2.0	10	24	84 (51)	8
13	**B**.0.30	GlcA	~30[g]	—	$NH_2CO_2NH_4$	3.0	15	24	87 (45)	8
14	**B**.0.40	GlcA	~30[g]	—	$NH_2CO_2NH_4$	4.0	20	24	89 (47)	8
15	**B**.0.50	GlcA	~30[g]	—	$NH_2CO_2NH_4$	5.0	25	24	88 (48)	8
16	**B**.0.S	GlcA	30	—	$NH_2CO_2NH_4$	Satd (>5.4)[e]	>27	5.5	89 (23)	9
17		Glc	30	—	$NH_2CO_2NH_4$	Satd (>5.4)[e]	>27	96	87 (6)	13
18		GlcA	40	—	$NH_2CO_2NH_4$	Satd (>5.4)[e]	>27	1.5	87 (29)	11
19	**B**.1.06	GlcA	30	1	$NH_2CO_2NH_4$	0.60	3	33[f]	79 (22)	19
20	**B**.5.02	GlcA	30	5	$NH_2CO_2NH_4$	0.20	1	32.5[f]	80 (32)	15
21		GlcA	40	5	$NH_2CO_2NH_4$	0.20	1	24	75 (19)	13
22	**B**.5.06	GlcA	30	5	$NH_2CO_2NH_4$	0.60	3	33	84 (12)	14
23	**B**.9.06	GlcA	30	8.8	$NH_2CO_2NH_4$	0.60	3	33[f]	86 (17)	12
24	**B**.14.02	GlcA	30	14.5	$NH_2CO_2NH_4$	0.20	1	33[f]	86 (31)	12
25		Glc	30	14.5	$NH_2CO_2NH_4$	0.20	1	168[f]	81 (25)	14
26	**X**.14.00	GlcA	30	14.5	—	—	—	48[f]	82 (78)	16
27		Glc	30	14.5	—	—	—	48[f]	33 (32)	33

[a] *Note:* $[s]_0$ indicates the initial concentration of species 's'. Unless otherwise specified, $[carb]_0 = 0.20$ M.
[b] The numbering of each protocol reveals the experimental conditions used. Hence **A**.1.06 indicates that the salt used was NH_4HCO_3 ('**A**'), and that the initial concentration of ammonia and salt was 1 and 0.6 M, respectively. In this context '**B**' indicates $NH_2CO_2NH_4$, '**X**' indicates that no salt was added and 'S' stands for 'saturated'.
[c] Total glycosylamine plus *N*-glycosyl carbamate; the parenthetical value indicates the yield of free glycosylamine.
[d] Total by-products **4**–**11**.
[e] Solubility in water/NH_4HCO_3, 284 g/kg at 30 °C; $NH_2CO_2NH_4$, 423 g/L at 20 °C.
[f] End of reaction.
[g] Ambient temperature.

Figure 1 shows the evolution of the proton spectrum with time during the reaction of sodium D-glucuronate (**1**) with 5 M ammonia and 0.6 M ammonium carbamate, as well as the assignment of the peaks used for integration. From these spectra it is clear that, with the exception of **8** and **11**, at least one diagnostic peak per each species can be accurately integrated.

The mole fraction of each compound was then calculated from the proton spectra on the basis of the assignment previously reported.[27] To this end, we assumed that each signal in the ^{1}H NMR spectrum is due to a CH group, that is, that there is no rearrangement of C–H bonds during the reaction. This idea is supported by the absence of signals attributable to $R_2C{=}O$ or RCH_2–OH carbons in the ^{13}C and DEPT NMR spectra. A normalizing constant 'S' was then calculated according to the formula:

$$S = \sum_{i=1}^{12} \frac{A_\delta^i}{n_\delta^i} = A_{5.23}^{1\alpha} + A_{4.63}^{1\beta} + A_{3.18}^{2} + A_{4.70}^{3} + \frac{\left(A_{3.27} - A_{4.63}^{1\beta}\right)}{2} + A_{5.63}^{5} + A_{5.37}^{6} + A_{5.13}^{7} + A_{4.84}^{9} + + A_{4.58}^{10} + \frac{\left(A_{4.20-4.40} - A^4 - 2A_{5.63}^5 - 3A_{5.13}^7 - 3A_{4.58}^{10}\right)}{5} \quad (1)$$

where A_δ^i indicates the area of the methine signal of compound i having a chemical shift δ (ppm), and n_δ^i is the number such methine groups in species i (e.g., there are two equivalent H2 protons in diglycosylamine **4**). It should be noted that since the H2 signal of **4** superimposes with that of $\mathbf{1}_\beta$, the former was corrected by subtracting the integral of anomeric proton of $\mathbf{1}_\beta$ at 4.63 ppm. Also, the contribution of species **8** and **11** was combined in the last factor since neither can be quantified individually. The mole fraction x of each species (with the exception of **8** and **11**) is then calculated from:

$$x^i = \frac{A_\delta^i / n_\delta^i}{S} \quad (2)$$

Ammonium *N*-β-D-glucopyranuronosyl carbamate (**3**) forms from the reaction of **2** with the CO_2 liberated by the decomposition of bicarbonate or carbamate anions.[19,21] Since it decomposes fairly easily upon standing in aqueous solution and/or during repeated freeze drying cycles,[21] its proportion was included in the calculation of product yields in Table 2. Also, throughout this report compound **4** and intermediate species **5**–**11** will be all considered as by-products of the synthesis of β-D-glucopyranuronosylamine (**2**), unless otherwise specified.

As mentioned above, all methods reported in the literature for the synthesis of glycosylamines in aqueous or aqueous methanol solution (Table 1) involve the use of volatile ammonium salts that release CO_2 upon decomposition (carbonate, bicarbonate or carbamate) and trap the product as the more stable *N*-glycosyl carbamate.[28,29] The rationale behind this choice is that studies published in the period of 1950–1970 demonstrate that, upon standing in concentrated aqueous ammonia, carbohydrates degrade and epimerize to give a large number of compounds[30] of which the corresponding glycosylamines are sometimes a minor component. Since none of these reports focused on uronic acids though, we decided to react D-glucose and sodium D-glucuronate

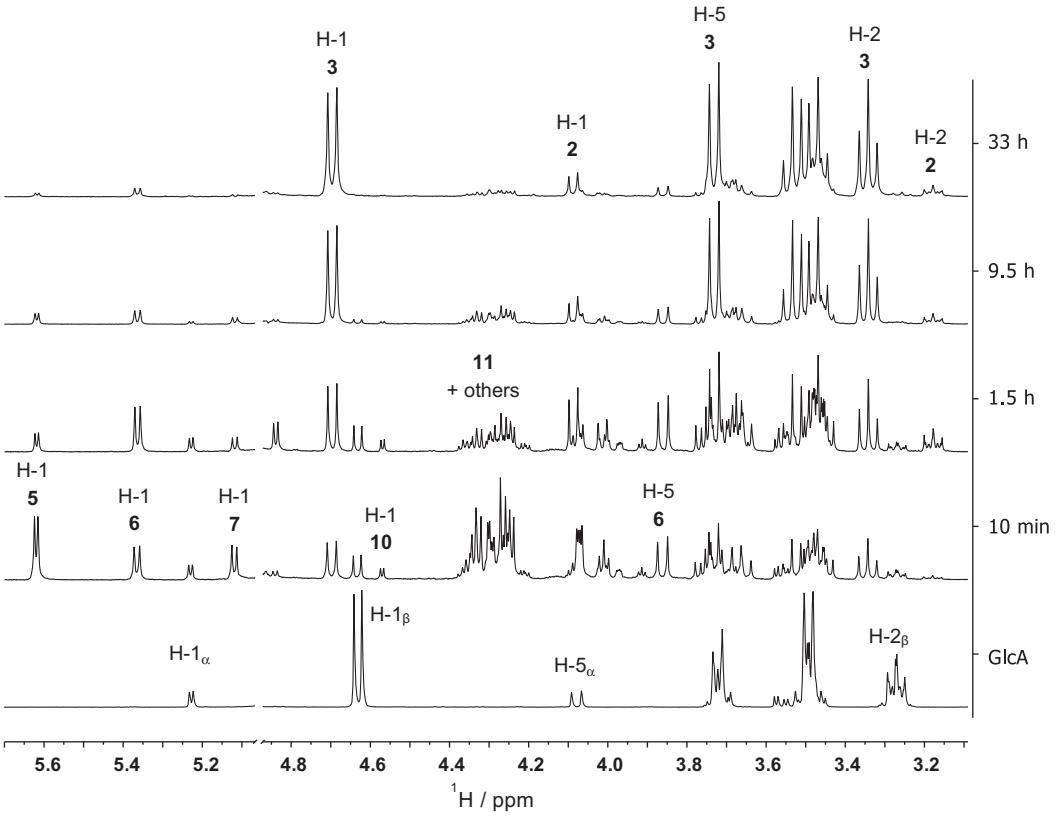


Figure 1. Evolution of the 1D ^{1}H spectrum (400.13 MHz) with time during the transformation of sodium D-glucuronate (**1**) into a mixture of **2** and **3** according to protocol **B**.5.06. The assignment of some peaks is also shown (D_2O, 278 K, δ (^{1}H)TSP = −0.017 ppm).

with commercial aqueous ammonia (~14.5 M) at 30 °C and to analyze the gross products by NMR spectroscopy (protocol **X**.14.00 in Table 2). As expected, in the case of glucose equal amounts of by-products and of glycosylamine/*N*-glycosyl carbamate formed: 21% and 33% after 25 and 48 h, respectively. By contrast, for the same reaction times sodium D-glucuronate afforded 70% and 82% of **2** + **3**, with only 20% and 16% of by-products, respectively. The latter result is rather positive, especially taking into account the simplicity of the procedure, and it will be used as benchmark for all other protocols tested.

2.1.1. Saturated salt and concentrated ammonia protocols

We began our study by comparing the kinetics obtained with a saturated solution of $NH_4^+HCO_3^-$, that is, the original protocol of Kochetkov and collaborators (**A**.0.S; entries 1 and 2 in Table 2),[14] with those obtained with a saturated solution of ammonium carbamate (**B**.0.S; entries 16 and 17 in Table 2), which is less stable and thus easier to eliminate by freeze-drying.[23] Surprisingly, when judged from the time required to obtain a 67% of **2** + **3**, the carbamate protocol was about nine times faster than the bicarbonate one (Fig. 2). Moreover, the equilibrium (plateau) fraction of **2** + **3** was 75% in the case of **A**.0.S and 89% in the case of **B**.0.S. Part of this difference can be explained by the higher solubility of $NH_4^+NH_2CO_2^-$ (>5.4 M vs ~3.6 M for $NH_4^+HCO_3^-$), and by the fact that each formula unit of carbamate decomposes to give 2 molecules of ammonia, but other factors must be at work. Similar behavior was observed with D-glucose (entries 2 and 17): to attain a 52% yield in glycosylamine/*N*-glycosyl carbamate it took protocol **B**.0.S one eighth of the time required using protocol **A**.0.S. What is more, the rate of formation of glycosylamine/*N*-glycosyl carbamate is ~4–5 times slower in the case of D-glucose than in the case of GlcA (**1**), although the same fraction of product is present at equilibrium.

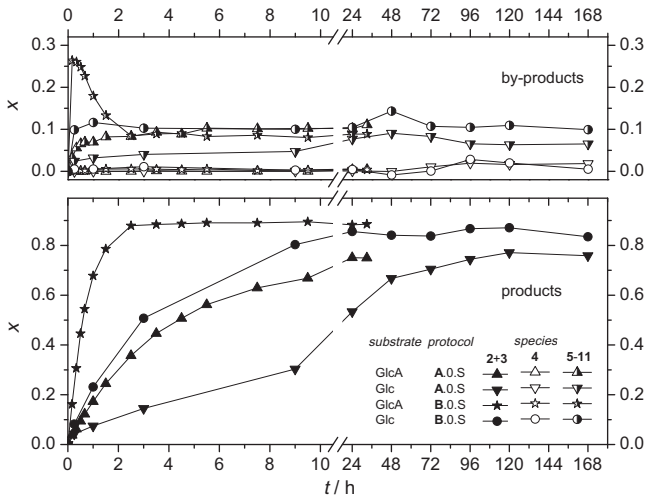


Figure 2. Evolution of the composition with time for the reaction of sodium D-glucuronate and D-glucose with saturated ammonium bicarbonate or ammonium carbamate solutions at 30 °C (see Table 2, entries 1, 2, 16, and 17). The mole fractions (x) of different species are shown.

Concerning the amount of by-products **5**–**11**, in case of GlcA protocol **B**.0.S led to an initial increase that was followed by a rapid stabilization around 8%, a value similar to that observed for **A**.0.S (11%). The salt effect was less pronounced in the case of Glc, with ~10% of by-products in the presence of ammonium carbamate against ~6% for ammonium bicarbonate. Finally, in all cases the amount of diglycosylamine remained close to zero during the first 33 h of reaction, and it only started to increase later on (the latter data is only available for Glc).

Although the high yield and the short reaction time obtained with protocol **B**.0.S are absolutely remarkable, the large amount of salt used could restrict its scope in synthesis. For instance, oligoglycuronans are not soluble at high ionic strength. Thus, we tested the effect of a decreasing amount of ammonium carbamate on the reaction outcome (entries 11–15 in Table 2). As it turned out, by using a 2 M initial concentration of $NH_4^+NH_2CO_2^-$, 84% of **2** + **3** is still obtained after 24 h of reaction.

For comparison, a number of protocols based on the combination of concentrated aqueous ammonia (14.5 M) and ammonium salts were investigated as well. Figure 3 shows the results obtained with Lubineau's protocol (**A**.14.02; entries 9 and 10 in Table 2)[17] as well as with a modification in which ammonium bicarbonate was replaced with the less stable ammonium carbamate (**B**.14.02; entries 24 and 25 in Table 2). It is evident that the same kinetic profiles were obtained with the two salts, that the conversion in glycosylamine is ~7–8 times faster for GlcA (**1**) than for Glc, and that the amount of diglycosylamine remains close to zero during the first 33 h of reaction and starts to increase later on (the latter data are only available for Glc). Also, when compared with the corresponding saturated salt protocols (Fig. 2), it is clear that a much larger fraction of by-products is present with concentrated ammonia during the first ~10 h of reaction, and that it takes ~24 h to attain a plateau value (~10% in all cases). This observation is consistent with intermediate species taking longer to transform into the final products **2** and **3**. Concerning the absolute rate of reaction, for both sugars it is comparable to that observed with protocol **A**.0.S (saturated ammonium bicarbonate) although somewhat higher yields of **2** + **3** are obtained for long reaction times. It is interesting to observe that, with respect to concentrated ammonia alone (**X**.14.00; entries 26 and 27 in Table 2), the addition of 0.2 M ammonium salt results in a moderate increase in the proportion of glycosylamine/*N*-glycosylcarbamate in the case of GlcA (82% after 24–25 h of reaction), and in a two-fold increase in the case of Glc (43%), both measured after 24–25 h of reaction.

2.1.2. Effect of a lower concentration of reagents

In order to optimize the synthesis of β-D-glucopyranuronosylamine, we looked into the possibility of reducing the amount of reagents used. At first, we examined the effect of a decreased concentration of ammonia for a given concentration of ammonium salt. Figure 4a shows that, in the presence of 0.6 M $NH_4^+HCO_3^-$ (entries 4, 7, and 8 in Table 2), the use of 9 M or 5 M ammonia results in almost identical kinetic profiles, whereas a further diminution to 1 M ammonia slows the reaction down significantly and results in ~20% less glycosylamine formed at equilibrium. The opposite can be said about the amount of by-products, which goes from 10% to 20% at equilibrium when the ammonia concentration decreases from 9 to 1 M. A qualitatively similar effect is observed with $NH_4^+NH_2CO_2^-$ (entries 19, 22, and 23 in Table 2), but the same is much less pronounced (Fig. 4b). The fraction of diglycosylamine **4** remained close to zero in all cases. Here it should be noted that after 33 h, the yield in **2** + **3** is virtually identical for protocols **A**.14.02 and **A**.5.06, meaning that a small increase in the amount of salt effectively compensates for a three-fold decrease in ammonia concentration. The same can be said for protocols **B**.14.02 and **B**.5.06.

Encouraged by the good results of protocols **A**/**B**.5.06, and following the same rationale of minimizing the amount of reagents used, we also tested the effect of a lesser amount of ammonium salt (entries 5 and 20 in Table 2). As shown in Figure 5, in the presence of 5 M ammonia a reduction from 0.6 to 0.2 M of the initial concentration of ammonium bicarbonate/carbamate only results in a slight decrease in the rate of formation of **2** + **3**, and in a ~5% decrease of their proportion at equilibrium. Concerning the

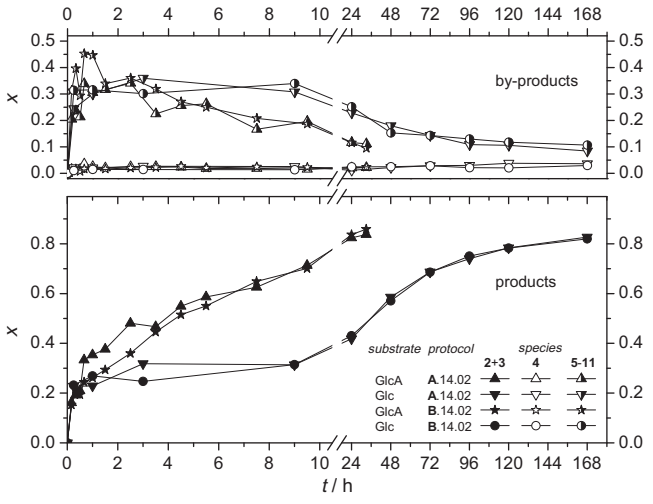


Figure 3. Evolution of the composition with time for the reaction of sodium D-glucuronate and D-glucose with concentrated ammonia (14.5 M) and one equivalent ammonium salt (0.2 M; entries 9, 10, 24, and 25 in Table 2). The mole fractions (x) of the different species are shown.

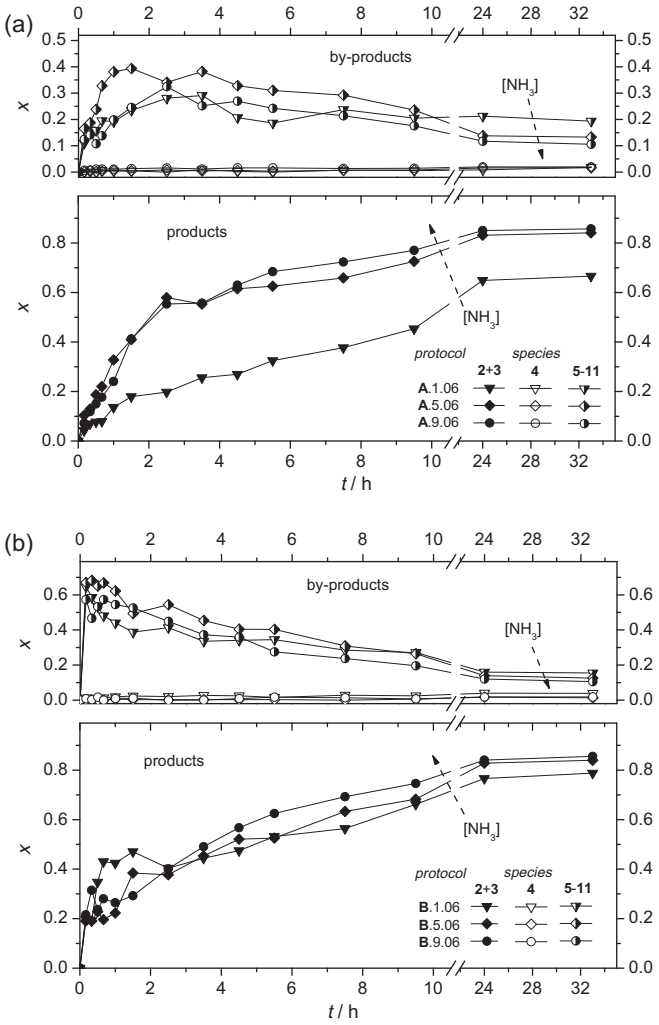


Figure 4. Evolution of the composition with time for the reaction of sodium D-glucuronate with different concentrations of ammonia and (a) 0.6 M ammonium bicarbonate (Table 2, entries 4, 7, and 8), or (b) 0.6 M ammonium carbamate (entries 19, 22, and 23). The mole fractions (x) of the different species are shown.

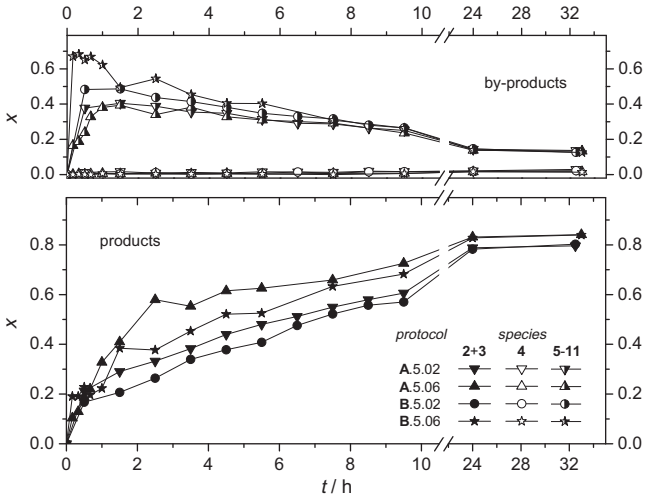


Figure 5. Effect of ammonium salt concentration (0.2 M or 0.6 M) on the evolution of the composition with time for the reaction of sodium D-glucuronate with 5 M ammonia (Table 2, entries 5, 7, 20, and 22). The mole fractions (x) of different species are shown.

amount of by-products **5**–**11**, there was no effect in the case of ammonium bicarbonate, while a higher concentration of ammonium carbamate led to a higher fraction of **5**–**11** being present during the first hours of reaction. This difference disappeared after ~7 h, though. The fraction of diglycosylamine **4** remained close to zero in all cases, and attained a maximum of ~3% after 33 h.

2.1.3. Effect of temperature

The last parameter to be tested was the reaction temperature, and in particular whether an increase in temperature would accelerate the rate of reaction without increasing the amount of by-products (Fig. 6). As it turned out, raising the temperature from 30 to 40 °C resulted in a four-fold increase in the rate of production of glycosylamine/*N*-glycosylcarbamate in the case of protocol **A**.0.S (entries 1 and 3 in Table 2), and in a three-fold increase in the case of **B**.0.S (entries 16 and 18), both measured at 70% conversion. All the same, the highest yield attained remains almost identical for the two temperatures, and for long reaction times, a decrease in the proportion of **2** + **3** is actually observed in the case of **B**.0.S: This certainly results from the poorer thermal stability of ammonium carbamate, which decomposes at T >30 °C.[23] As for the amount of by-products **5**–**11**, raising the temperature resulted in a marginal increase, whereas the fraction of diglycosylamine **4** remained close to zero in all cases.

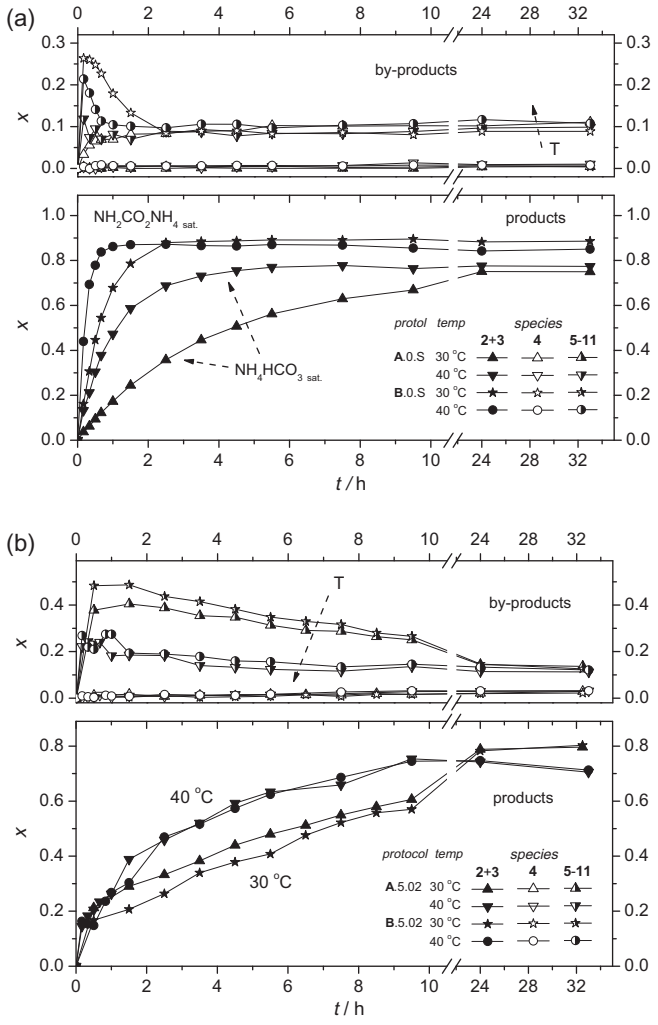


Figure 6. Effect of temperature on the evolution of the composition with time for the reaction of sodium D-glucuronate with (a) saturated ammonium salt (Table 2, entries 1, 3, 16, and 18) and (b) 5 M ammonia containing ammonium salt 0.2 M (entries 5, 6, 20, and 21). The mole fractions (x) of the different species are shown.

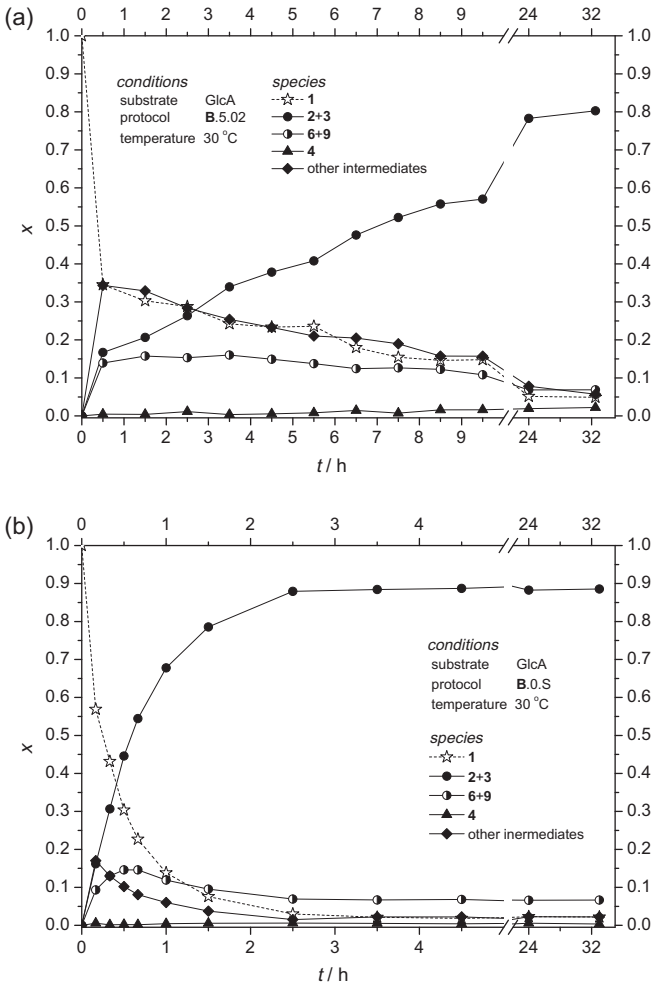
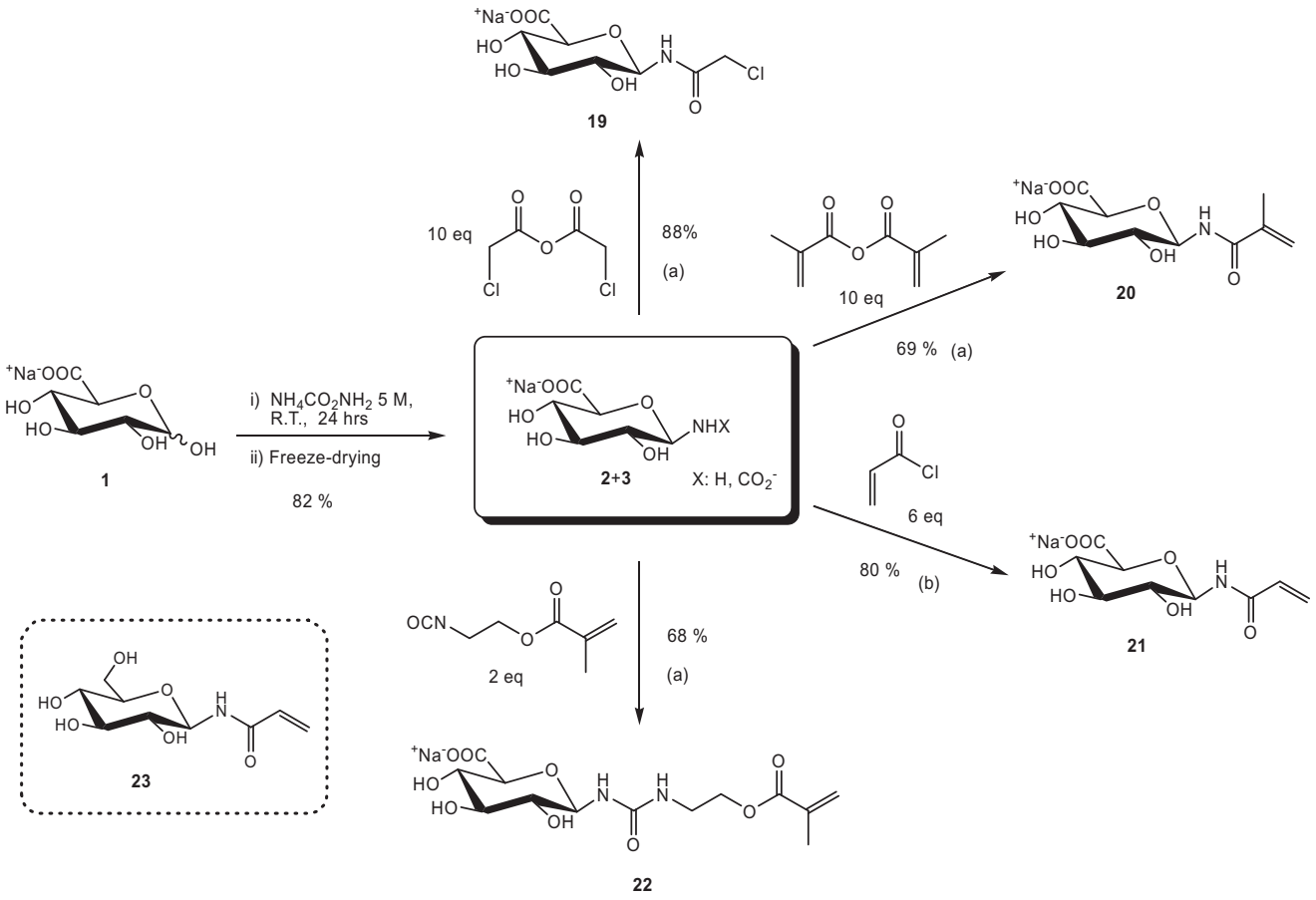


Figure 7. Comparison between the kinetics at 30 °C of (a) a 'slow' protocol (**B**.5.02) and (b) the fastest protocol tested (**B**.0.S; entries 16 and 20 in Table 2). The mole fractions (x) of different species are shown.



Scheme 2. Synthesis of *N*-acyl-β-D-glucopyranuronosylamines **19**–**21** and of *N*-alkylcarbamoyl-β-D-glucopyranuronosylamine (**22**). Conditions: (a) Na_2CO_3, H_2O/DMSO 8:2, 0 °C to rt, 25 h; (b) Na_2CO_3, H_2O/CH_3OH 1:1, 0 °C, 0.5 h.

The same type of experiment was carried out with protocols **A**/**B**.5.02 (Fig. 6b; entries 5, 6, 20, and 21 in Table 2). In this case, raising the temperature from 30 to 40 °C resulted in a two-fold increase in the rate of formation of **2** + **3** (measured at 60% yield), but the evaporation of ammonia was rapid enough to lead to ~5% lower yields after 24 h. Accordingly, at 40 °C the proportion of **5**–**11** was significantly lower during the first ~10 h of reaction and bottomed out around 13% for longer reaction times, that is, the same value observed at 30 °C. Finally, for both temperatures tested the amount of diglycosylamine **4** remained close to zero during the first ~6 h and increased to ~3% by the end of the reaction.

2.1.4. Fate of intermediate species

Finally, it is worth looking more closely at the results obtained at 30 °C for a 'slow' protocol (**B**.5.02; entry 20 in Table 2) and for the fastest protocol tested (**B**.0.S; entry 16). Figure 7 shows that after 30 min of reaction between 65% (**B**.5.02) and 70% (**B**.0.S) of the initial sugar had already been consumed. In the case of **B**.5.02 though, only half of it was transformed into α,β-D-glucopyranuronosylamine and ammonium *N*-α,β-D-glucopyranuronosyl carbamate, whereas the other half yielded intermediates **5**, **7**, **8**, **10**, and **11**. It then took 32.5 h for the concentration of intermediates to reach its lowest value of 6% and for the yield in **2** + **3** to attain a maximum value of 80%. By contrast, in the case of **B**.0.S the fraction of intermediates present after 30 min was only 10%, and their concentration reached a minimum of ~2% in just 2.5 h. Consequently, at the same reaction time the system already contained ~60% of α,β-D-glucopyranuronosylamine and ammonium *N*-α,β-D-glucopyranuronosyl carbamate, and it reached a maximum yield of 89% in barely 5.5 h. At the end of the reaction, the proportion of residual GlcA **1** was 5% in the case of **B**.5.02 and 2% in case of **B**.0.S, and both systems contained the same amount (7%) of the α anomers **6** + **9**. We checked the final amount of **6** + **9** for three other protocols and found that nearly the same value was obtained with **A**.5.06 (8%), **A**.14.02 (8%), and **B**.9.06 (7%). This suggests that 7–8% is about the equilibrium fraction of α-D-glucopyranuronosylamine/carbamate in water at 30 °C.

2.2. Synthetic potential

Glucuronidation is a major detoxification pathway in all vertebrates. The reaction is catalyzed by UDP-glucuronosyltransferases (EC 2.4.1.17) and involves the transfer of a glucuronic acid residue from UDP-glucuronic acid to countless substances possessing hydroxyl, amino, carboxyl, or thiol groups, thus converting them to water-soluble β-D-glucopyranuronosyl derivatives.[31] What is more, pharmacologists have conjugated a number of drugs to glucuronic acid to allow for more effective delivery, while the toxicity of some substances is effectively reduced by glucuronidation.[32] In the field of drug delivery, Oku and Namba[33] have demonstrated that liposomes carrying β-D-glucopyranuronosyl residues at their surface bind to a lower extent to macrophage-like cells, have prolonged circulation, and passively accumulate in tumor tissue, thus reducing the toxic side effects of the therapeutic agents.

In this context, the possibility to transform D-glucuronic acid into β-D-glucopyranuronosyl derivatives without resorting to protective group chemistry would be highly advantageous. To probe the synthetic potential of our approach, four test reactions were carried out in which the title compound was reacted with various

Table 3
Summary of the characterization of compounds $\mathbf{1}_{\beta}$–**3** and **19**–**23**

Compound[a]	δ ^{1}H/ppm (m, *J*/Hz, assignment)	δ ^{13}C/ppm (assignment)	*m*/*z* (structure, monoisotopic ion mass, molecular ion)
$\mathbf{1}_{\beta}$	4.63 (d, 8.0, H1), 3.27 (m, H2), 3.50 (H3), 3.51 (m, H4), 3.72 (H5)	98.57 (C1), 76.70 (C2), 78.25 (C3), 74.53 (C4), 78.93 (C5), 178.64 (C6)	193 (1_H, 193.0, $[M-H]^-$); 217 (1_H, 217.0, $[M+Na]^+$); 239 (1_{Na}, 239.0, $[M+Na]^+$); 387 ($(1_H)_2$, 387.1, $[M-H]^-$); 455 ($(1_{Na})_2$, 455.0, $[M+Na]^+$)
2	4.09 (d, 8.8, H1), 3.18 (dd, 9.0, H2), 3.48 (H3), 3.48 (d, 9.5, H4), 3.69 (m, H5)	87.68 (C1), 76.76 (C2), 79.03 (C3),74.71 (C4), 79.92 (C5), 179.14 (C6)	192 (2_H, 192.1, $[M-H]^-$); 194 (2_H, 194.1, $[M+H]^+$); 216 (2_{Na}, 216.0, $[M+H]^+$); 238 (2_{Na}, 238.0, $[M+Na]^+$); 385 ($(2_H)_2$, 385.1, $[M-H]^-$)
3	4.70 (d, 9.1, H1), 3.34 (t, 9.0, H2), 3.52 (H3), 3.48 (H4), 3.73 (d, 9.4, H5), 6.25 (d, 9.8, $NHCO_2$)	85.68 (C1), 74.64 (C2), 79.02 (C3), 74.55 (C4), 79.87 (C5), 178.88 (C6), 165.86 ($NHCO_2$)	238 ($3_{H,\ H}$, 238.0, $[M+H]^+$); 260 ($3_{H,\ H}$, 260.0, $[M+Na]^+$)
19	5.03 (d, 9.1, H1), 4.20 (s, CH_2Cl)	82.16 (C1), 78.84, 78.60, 73.90, 73.73, 44.96 (CH_2Cl), 173.10 (amide)	268, 270 (22_H, 268.0, 270.0, $[M-H]^-$); (22_{Na}, 290.0, 292.0, $[M-H]^-$); (22_H, 292.0, 294.0, $[M+Na]^+$); 314, 316 (22_{Na}, 314.0, 316.0, $[M+Na]^+$)
20	5.06 (d, 8.6, H1), 3.80 (d, 9.5, H5)	82.12 (C1), 80.97, 78.95, 74.44, 175.54 (amide)	260 (19_H, 260.1, $[M-H]^-$); 262 (19_H, 262.1, $[M+H]^+$); 306 (19_{Na}, 306.1, $[M+Na]^+$)
21	5.04 (d, 9.0, H1), 3.81 (d, 9.6, H5)	81.95 (C1), 80.65, 78.89, 74.37, 132.08 (CH_2=CH), 172.04 (amide)	246 (20_H, 246.1, $[M-H]^-$); 268 (20_{Na}, 268.0, $[M-H]^-$); 292 (20_{Na}, 292.0, $[M+Na]^+$)
22	4.82 (d, 9.1, H1), 3.74 (d, 9.5, H5)	83.69 (C1), 80.62, 138.49 (CH_2=*C*), 129.67 (*CH_2*=C), 162.33 (urea)	347 (21_H, 347.1, $[M-H]^-$); 393 (21_{Na}, 393.1, $[M+Na]^+$); 717 ($(21_H)21_{Na}$, 717.2, $[M-H]^-$)
23[b]	5.05 (d, 9.2, H1); 3.87 (dd, 12.4, 2.1, H6); 3.72 (dd, 12.4, 5.4, H6); 3.54 (m, H3 and H5); 3.44 (m, H2 and H4); 6.32 (m, *CH_2*=CH); 5.87 (dd, 6.6, 4.9, CH_2=*CH*)	82.12 (C1), 80.30 (C5), 79.12 (C3), 74.48 (C2), 71.92 (C4), 63.26 (C6), 132.09, 132.11 (CH_2=CH), 172.02 (amide)	232 (23 232.1; $[M-H]^-$); 256 (23 256.1, $[M+Na]^+$); 489 ($(23)_2$, 489.2, $[M+Na]^+$)

[a] Solvents used for NMR analyses: D_2O (**19**, **21**, **23**); 8:2 D_2O–DMSO-d_6 (**20**) or 5:4 D_2O–DMSO-d_6 (**22**).
[b] Assignments obtained by HMQC.

acylating agents and with an isocyanate (Scheme 2). In particular, a mixture of **2** + **3** was prepared according to protocol **B**.0.50 and purified by two freeze-drying cycles. This procedure slightly reduces the global yield (82% vs 88% for a single cycle) but effectively removes all ammonium salt. The gross product was then reacted with chloroacetic anhydride, methacrylic anhydride, acryloyl chloride, and 2-isocyanoethyl methacrylate in cold aqueous/organic solutions. This way, *N*-acyl-β-D-glucopyranuronosylamines **19**–**21** and *N*-alkylcarbamoyl-β-D-glucopyranuronosylamine **22** where obtained in 68–88% yield. The compounds were not isolated, but their formation was confirmed by MS and NMR analysis of the crude products, as summarized in Table 3. In particular, we found that for compounds **19**–**21** the chemical shift and coupling constant of C1 (82 ppm) and H1 (5.05 ppm, doublet, 9 Hz) are almost identical to those of *N*-(prop-2-enoyl)-β-D-glucopyranosylamine (**23**), a pure sample of which was prepared in our laboratory. The same can be said for compound **22**, whose diagnostic peaks match those reported in the literature for several *N*-arylcarbamoyl-β-D-glucopyranosylamines.[34] Finally, it is interesting to note that the yields of **19** and **21** are comparable to those reported by Manger et al.[21] and Kallin et al.[19] for the analogous derivatives of *N*-acetylglucosamine (95%) and lactose (88%), respectively; whereas the yield of **22** matches those obtained by Somsák et al.[35] for the synthesis of *N*-arylcarbamoyl-β-D-glucopyranosylamines in pyridine (45–76%). Here it should be noted that compound **19** represents a classic starting point for the synthesis of 1-*N*-glycyl-β-D-glycosyl derivatives,[18,21] while compounds **20**–**22** can be either functionalized by thiol-ene chemistry[35] or used as glycomonomers for radical polymerization.[36,37]

3. Conclusions

When sodium D-glucuronate (**1**) was reacted with ammonia and/or volatile ammonium salts in water, the rate of formation of β-D-glucopyranuronosylamine (**2**) and ammonium *N*-(β-D-glucopyranuronosyl carbamate (**3**) 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 **5**–**11**, and of the latter into the final products **2** and **3**. Yet, some interesting trends and exceptions were observed:

i. **B**.0.S (saturated ammonium carbamate) was both the fastest protocol tested and the one leading to the highest final yields of **2** + **3** (89% in 5.5 h at 30 °C; 87% in 1.5 h at 40 °C). In particular, at 30 °C it was nine times faster than Kochetkov's and Lubineau's methods. A qualitatively similar result was obtained with D-glucose, and we postulate that this may be a general finding.
ii. Whenever a lesser amount of salt (or a lower ionic strength) is needed, protocols **B**.0.20/30 and **B**.5.06 should be preferred to **A**/**B**.9.06 and **A**/**B**.14.02, since they lead to the same yield in **2** + **3** (84–86% after 24–33 h) while requiring much less reagent.
iii. With the sole exception of **A**/**B**.1.06, after 24 h of reaction all tested protocols led to higher yields of **2** + **3** than concentrated commercial ammonia alone (**X**.14.00).
iv. The equilibrium fraction of α anomers **6** + **9** was found to be about 7–8% in water at 30 °C.
v. Concerning bis(β-D-glucopyranuronosyl)amine (**4**), less than 3% formed in all cases, with a minimum value of 0.5% in the case of **B**.0.S (after 24 h).
vi. The formation of β-glycopyranosylamine/*N*-β-glycopyranosyl carbamate is 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 transforming a mixture of β-D-glucopyranuronosylamine (**2**) and *N*-(β-D-glucopyranosyluronic acid) carbamate (**3**) into *N*-acyl-β-D-glucopyranuronosylamines **19**–**21** (69–88% yield) and *N*-alkylcarbamoyl-β-D-glucopyranuronosylamine **22** (68% yield) directly in aqueous/organic solution without resorting to protective group chemistry. The findings from this study are now being applied to the regioselective functionalization of oligoglycuronans directly in aqueous solution.

4. Experimental

4.1. Materials and methods

Unless otherwise specified, all chemicals were reagent grade and used as received. Ammonium bicarbonate (⩾99.0%), ammonium carbamate (⩾99.5%), D-glucose (⩾99.0%), and D-glucuronic acid sodium salt monohydrate (99%) were from Fluka Chemical Co. Ammonia (28% w/w, Carlo Erba), D_2O (99.9%-D, Euriso-top), 2-isocyanatoethyl methacrylate (>98.0%, TCI Europe), methacrylic anhydride (94%, Sigma–Aldrich), SiO_2 (15–40 μm, 60 Å, E. Merck), sodium carbonate monohydrate (⩾99.5%, Sigma–Aldrich), and TLC plates (SiO_2 F254, 15 μm, 60 Å, E. Merck) were obtained from the indicated suppliers. Acryloyl chloride (⩾96.0%, Fluka) and methacryloyl chloride (⩾97.0%, Fluka) were distilled under reduced pressure. Distilled or deionized water was used in all experiments.

4.2. Protocol numbering

Sodium D-glucuronate (**1**) (Scheme 1) and D-glucose were reacted at 30 and 40 °C with ammonia and/or volatile ammonium salts (NH_4HCO_3 or $NH_2CO_2NH_4$) in water according to different protocols. The numbering of each protocol reveals the exact experimental conditions used:

- The letter indicates the type of solid salt added (**A**, NH_4HCO_3; **B**, $NH_2CO_2NH_4$, **X**, no salt).
- The middle number indicates the initial formal concentration of liquid ammonia in mol L^{-1}.
- The last number indicates the initial formal concentration of salt in 10^{-1} mol L^{-1}.

For example, **A**.1.06 indicates that the salt used was NH_4HCO_3, and that the initial concentration of ammonia and salt were 1 and 0.6 M, respectively.

4.3. Kinetic study

In a typical experiment (AG09-30_P2, protocol **B**.5.06), D-glucuronic acid sodium salt monohydrate (0.468 g, 2.0 mmol) and ammonium carbamate (0.468 g, 6.0 mmol) were weighed in a 25-mL round-bottom flask. A magnetic bar was added together with 10 mL of aqueous ammonia (5 M), the flask was sealed with a rubber septum, and a disposable needle (21 G) was passed through the septum to prevent pressure build-up. The flask was plunged in an oil bath pre-heated at 30 °C and stirred at 200 rpm. At pre-set intervals, ~250 μL samples were drawn with a syringe, transferred to a glass vial, diluted with 2 volumes of water, and frozen in liquid nitrogen. All samples were freeze-dried overnight and stored in a freezer (−18 °C) until needed. The mole fraction of each compound was then calculated by ^{1}H NMR spectroscopy according to (Eq 2). For protocols **A**/**B**.0.S, saturation was ensured by the constant presence of solid salt at the bottom of the flask.

4.4. Instrumental analyses

The composition of samples from the kinetic study was determined by NMR spectroscopy on a Bruker DPX400 spectrometer equipped with a Variable Temperature (VT) module (resonance frequency of 400.13 and 100.62 MHz for ^{1}H and ^{13}C nuclei, respectively). Two different 5-mm detection probes were used: QNP (direct) and BBIZ (inverse). Unless otherwise specified, 90° pulses and pulse sequence recycle times of 3 s were used. The probe temperature was calibrated in the range 275–300 K using neat methanol. The NMR probe was pre-equilibrated at 278 K, individual samples were redissolved in D_2O (~3% w/w), transferred to a NMR tube, and immediately lowered into the instrument magnet for analysis. 1D ^{1}H spectra were obtained with 32 scans and 32 K data points, and were re-processed using MestReNova software (v5.1). Sodium 3-(trimethylsilyl)propanoate (TSP) or sodium 3-(trimethylsilyl) propane-1-sulfonate (DSS) were used as an internal reference. Chemical shifts (in ppm) for ^{1}H and ^{13}C nuclei were referenced to $\delta_{TSP} = -0.017$ ppm (^{1}H) and $\delta_{TSP} = -0.149$ ppm (^{13}C), or to $\delta_{DSS} = 0.000$ ppm (^{1}H and ^{13}C). Mass spectrometry analyses were performed with a Waters ZQ (Altrincham, GB) single quadrupole atmospheric pressure ionization mass spectrometer fitted with a Z electrospray interface (ESI). The instrument was calibrated with mass spectra generated by electrospray ionization of a 0.1 mol L^{-1} solution of sodium iodide in aqueous acetonitrile (50%, v/v) in the mass range of 23–1972 amu. Nitrogen was used as the drying and nebulizing gas. Samples (~1 mg mL^{-1}) were dissolved in deionized water or water/MeOH mixtures and infused to the ESI interface at constant flow rate (50 μL min^{-1}).

4.5. Synthesis of β-D-glucopyranuronosylamine (2)

D-Glucuronic acid sodium salt monohydrate (1.00 g, 4.27 mmol) and ammonium carbamate (8.32 g, 0.106 mol) were weighed in a 50-mL round-bottom flask and dissolved in 21.3 mL of de-ionized water (experiment AP10-12, protocol **B**.0.50). A magnetic bar was added, the flask was sealed with a rubber septum, a disposable needle (21 G) was passed through the septum to prevent pressure build-up, and the mixture was stirred at ambient temperature (~25 °C) and 300 rpm. After 24 h of reaction, the content of the flask was frozen in liquid nitrogen and freeze dried overnight. The resulting powder was redissolved in deionized water (80 mL) and submitted to a second cycle of freeze-drying. The obtained white fluffy solid (1.13 g) had the following molar composition (as determined by ^{1}H NMR): **1** (10%), **2** (65%), **3** (17%), **4** (1%), **6** + **9** (4%), **5** + **7** + **8** + **10** + **11** (3%). The sample was then sealed in a round-bottom flask and stored in a freezer (−18 °C) until needed. The increase in the mass of the sample is mostly due to the presence of *N*-glycosylcarbamates **3**, **5**, and **6**, whose molar mass is larger than that of the starting sugar **1** and of glycosylamine **2**.

4.6. Synthesis of *N*-acyl-β-D-glucopyranuronosylamines (19–21) and of *N*-{[2-((2-methylprop-2-enoyl)oxy)ethyl]carbamoyl}-β-D-glucopyranuronosylamine (22)

In a typical experiment, β-D-glucopyranuronosylamine (100 mg of gross product) was dissolved in (a) 8:2 1 M Na_2CO_3–DMSO (3.9 mL) or (b) 1:1 1 M Na_2CO_3–DMSO (4.4 mL) at 0 °C under stirring. A calculated volume of acylating agent was added dropwise either as neat liquid (a) or as 1.5 M solution in THF (b). The resulting mixture was then left stirring on ice for 60 min (a) or 30 min (b), and at ambient temperature for another 24 h (a). MeOH was eliminated by rotary evaporation (b) and any unreacted acylating agent was removed by solvent extraction (EtOAc, 2 × 5 mL). Following further rotary evaporation at ambient temperature, the remaining solution was diluted with 2 volumes of water and freeze-dried overnight (experiments WM_03, WM_06, AP10-11, AP10-16 and AP10-17). Yields were calculated from the ^{1}H NMR spectra of the gross products by integrating to one the anomeric protons signals.

4.7. Synthesis of *N*-(prop-2-enoyl)-β-D-glucopyranosylamine (23)

D-Glucose (2.00 g, 11.1 mmol) and ammonium bicarbonate (2.60 g, 32.8 mmol) were weighed in a 250-mL round-bottom flask and dissolved in 55 mL of 5 M ammonia (experiment WM_04, protocol **A**.5.06). A magnetic bar was added, the flask was sealed with a rubber septum, a disposable needle (21 G) was passed through the septum to prevent pressure build-up, and the mixture was stirred at 35 °C and 300 rpm. After 30 h the flask was fitted to a rotary evaporator, and the reaction mixture was concentrated to ∼½ of the initial volume (p = 35 mbar, T_{bath} = 25 °C). More water was added (∼30 mL), and the process was repeated once. The resulting solution was freeze-dried overnight. A white fluffy solid (2.15 g) containing β-D-glucopyranosylamime was obtained that was mixed with $Na_2CO_3{\cdot}H_2O$ (6.13 g, 49.5 mmol) and redissolved in 108 mL of 1:1 CH_3OH–H_2O under stirring (WM_06). The resulting solution was cooled in an ice bath and acryloyl chloride (4.10 g, 45.2 mmol, diluted in 31 mL of anhyd THF) was added over a period of 5 min under vigorous stirring. After 30 min the reaction flask was fitted to a rotary evaporator, and the volatiles were eliminated at ambient temperature. The reaction mixture was then diluted with water (60 mL) and 2-PrOH (50 mL) and 63 g of chromatographic grade SiO_2 were added. In order to adsorb the gross product on silica, the flask was re-fitted to the rotary evaporator and water was eliminated as a binary azeotrope with 2-PrOH (T_b = 80 °C, 88% w/w alcohol) before evaporating the resulting slurry to dryness (p = 45 mbar, T_{bath} = 40 °C). The dry silica gel was then charged on the top of a prepacked column and eluted with 9:1 CH_3CN–H_2O. Fractions containing the product (R_f 0.34, 8:2 CH_3CN–H_2O) were pooled, stabilized with a few grains of BHT, concentrated at the rotary evaporator, and freeze-dried overnight. Isolated yield: 1.45 g (52%) of white fluffy powder. The sample was stored in a freezer (−18 °C) until needed. See Table 3 for spectroscopic characterization.

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