Doklady Chemistry, Vol. 383, Nos. 4–6, 2002, pp. 89–92. Translated from Doklady Akademii Nauk, Vol. 383, No. 4, 2002, pp. 500–503.

CHEMISTRY

New Efficient Synthesis of β-Glucosylamines of Mono- and Disaccharides with the Use of Ammonium Carbamate

L. M. Likhosherstov, O. S. Novikova, and V. N. Shibaev

Presented by Academician N. K. Kochetkov January 8, 2002

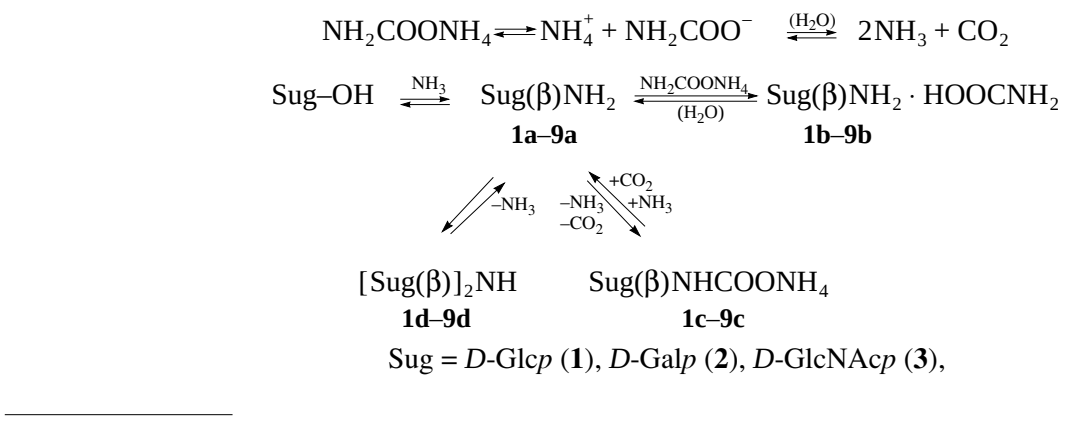
Received January 9, 2002

In recent years, strategies to synthesize different neoglucoconjugates used for studying the specificity of biological interactions with the participation of carbohydrate residues and for targeted transport of different pharmaceuticals in organisms have been extensively developed. Glucosylamines of mono- and oligosaccharides are promising starting compounds for the synthesis of similar compounds. However, the available methods for the synthesis of these compounds need improvement.

Classical methods based on the reaction of monosaccharides with NH_3 in a methanol solution (see reviews [1, 2]) require a very long time for the reaction to be completed. Poor solubilities of oligosaccharides in methanol hinder the application of this method, and attempts to obtain glucosylamine from 2-acetamido-2-deoxy-**D**-glucose have resulted in many side reactions [3]. In 1986, we put forward an efficient method for the synthesis of β-glucopyranosylamines from 2-acetamido-2-deoxyhexoses and their derivatives, which is based on the use of a saturated aqueous solution of NH_4HCO_3 [4]; the method was widely applied subsequently to the preparation of derivatives of hexoses, 6-deoxyhexoses, and oligosaccharides with different chain lengths [3, 5, 6]. The necessity of having a prolonged and labor-consuming procedure for the evaporation of aqueous solutions to remove considerable amounts of the volatile salt restricts, however, the application of the method in preparative syntheses. Moreover, glucosylamines are unstable in aqueous solutions and undergo fast hydrolysis at pH 1.5 to 9.0 and produce diglucosylamines in concentrated solutions. The rate of these reactions, which occur upon the isolation of glucosylamines, depends considerably on the nature of the sugar used, which strongly affects the yield and purity of the resulting glucosylamines.

Variation of the glucosylamine synthesis based on the use of a saturated aqueous solution of $(NH_4)_2CO_3$ [7], as well as aqueous [8] or methanolic solutions [9] of NH_3 in the presence of small amounts of $(NH_4)_2CO_3$, were proposed later. The two last modifications made it possible to reduce the reaction time; however, they could not serve as general methods for preparing these compounds.

In this paper, we report on a new synthesis of β-glucosylamines which provides a rather fast preparation of derivatives of hexoses, pentoses, 2-acetamido-2-deoxyhexoses, and a number of disaccharides with high yields in preparative amounts. The synthesis is carried out with the use of mixtures of ammonium carbamate and an aqueous NH_3 solution and involves intermediate isolation of the salts of glucosylamines with carbamic acid, which are rather stable upon storage. Scheme 1 shows the reaction processes.



Sug = *D*-Glc*p* (**1**), *D*-Gal*p* (**2**), *D*-GlcNAc*p* (**3**),

Zelinskii Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii pr. 47, Moscow, 119991 Russia

0012-5008/02/0004-0089$27.00

D-Xyl*p* (**4**), **D**-Rib*p* (**5**), **D**-Gal*p*(β1–4)-**D**-Glc*p* (**6**),
D-Gal*p*(α1–6)-**D**-Glc*p* (**7**), **D**-Glc*p*(β1–4)-**D**-Glc*p* (**8**),
D-Glc*p*(β1–6)-**D**-Glc*p* (**9**)

Scheme 1.

Optimum conditions were found using the reaction with **D**-glucose as an example (Table 1). After keeping a solution of the monosaccharide in a methanol–NH_4OH mixture with 2 equiv. of ammonium carbamate (24 h at 37°C), the initial compound virtually completely transforms into glucosylamine **1a** according to electrophoresis data. Cooling of the reaction mixture resulted in the precipitation of salt **1b**, whose composition was confirmed by elemental analysis. The ^{1}H NMR spectrum of a DMSO-d_6 solution of the compound obtained showed only the signals characteristic for β-glucopyranosylamine. At the same time in the region of anomeric protons, the ^{1}H NMR spectrum of a solution of the same compound in D_2O (Table 2) showed a rather intense signal from H-1 of *N*-(β-glucosyl)carbamate **1c** (δ 4.68 ppm, cf. [6]) along with a signal from H-1 of **1a** (δ 4.08 ppm) and a weak signal corresponding to H-1 of di-β-glucosylamine **1d**. The formation of compound **1c** in an aqueous solution could be explained by the hydrolysis of the salt, the decarboxylation of the resulting carbamic acid, and the reaction of the liberated glucosylamine with CO_2 (Scheme 1). The conversion of **1b** into **1c** also occurs on adding water to a solution of **1b** in DMSO (see note to Table 2).

A facile transformation of salt **1b** into unstable glucosylcarbamate **1c** was used for regeneration of free glucosylamine **1a**, which was isolated in quantitative yield by concentrating a solution of the salt in methanol containing a small amount of water and *N*,*N*-diisopropylethylamine to prevent the hydrolysis of glucosylamine (method A).

Similar reaction conditions proved to be suitable for preparing glucosylammonium carbamates **2b**–**9b** derived from a series of mono- and disaccharides (Table 1). The ratio of methanol to concentrated NH_4OH in a reaction mixture and the reaction duration were selected in a series of preliminary experiments taking into account the solubility of initial sugars and reaction products. The formation of derivatives of pentoses **D**-xylose (**4b**) and **D**-ribose (**5b**) proceeds markedly faster than the synthesis of derivatives of hexoses **D**-glucose (**1b**) and **D**-galactose (**2b**). The derivatives of monosaccharides **2b**–**5b**, lactose **6b**, and gentiobiose **9b** were precipitated from the reaction mixture, while the glucosylammonium carbamates of melibiose **7b** and cellobiose **8b** were precipitated by adding 2-propanol.

By example of the preparation of 2-acetamido-2-deoxy-**D**-glucopyranosylammonium carbamate (**3b**), we demonstrated that the reaction could be carried out without addition of concentrated NH_4OH, although the molar ratio of ammonium carbamate to sugar and the reaction duration should be increased. Such a procedure may be very useful for the synthesis of glucosylamine derivatives from carbohydrates that are unstable in the presence of aqueous ammonia solutions.

The ^{1}H NMR spectra of glucosylammonium carbamates **2b**–**9b** in D_2O (Table 2) always show signals

Table 1. Conditions of the synthesis of glucosylammonium carbamates **1b**–**9b** and their transformation into free glucosylamines **1a**–**9a**

Glucosylammonium carbamate	Yield, %	Reaction conditions*			Method of preparation of amine from salt**
		Initial sugar concentration, M	Volume ratio MeOH/conc. NH_4OH	Reaction time, h*	
1b	89	0.4	19	24	A
2b	93	0.4	4	15	A
3b	83	0.4	59***	48	B
4b	93	0.8	19	5	C
5b	94	0.8	19	3	A
6b	85	0.4	4	20	C
7b	87	0.4	4	10	C
8b	82	0.4	1	25	C
9b	85	0.4	4	12	C

* All reactions were carried out at 37°C and at an ammonium carbamate/sugar ratio of 2.0.
** See Experimental.
*** Water was used instead of concentrated NH_4OH; the ammonium carbamate/sugar ratio was 4.0.

corresponding to β-glucopyranosylamines **2a**–**9a** and β-glucosylcarbamates **2c**–**9c** whose ratio depends on the sugar nature and sometimes signals indicating a slight admixture of diglucosylamines. As shown using compounds **3b** and **6b**, as examples, only signals corresponding to glucosylamines were observed in DMSO-d_6 solutions, while the addition of D_2O resulted in the emergence of signals indicating their partial transformation into glucosylcarbamates **3c** and **6c**.

Along with the above method A for the regeneration of glucosylamine from its carbamate, we developed two other simpler but not universal variants of the process. One of them (method B with **3b** as an example) is based on the evaporation of methanolic solutions without base added and is suitable for salts soluble in methanol. In the other variant (method C with **4b** and **6b**–**9b** as examples), aqueous salt solutions are treated with triethylamine, followed by precipitation of glucosylamines via addition of alcohols.

Thus, we developed a method for the synthesis of β-glucosylamines with the use of ammonium carbamate which provides an opportunity to obtain relatively stable salts of these compounds with carbamic acid on a preparative scale in a rather simple manner; the salts may be further converted into the corresponding glucosylamines. The suggested method seems to be very promising for the preparation of these important compounds.

Table 2. ^{1}H NMR data for solutions of carbamates **1b**–**9b** in D_2O

Carbamate	Ratio of a/c	Admixture of *d*, %	Signals of H-1 (δ, ppm)[a]		
			a	c	d
1b	2.0[b]	2	4.08	4.68	4.29
2b	2.0	3	4.02	4.65	4.23
3b	3.3[c]	not detected[d]	4.13	4.72	–
4b	1.3	3	4.02	4.61	4.16
5b	0.6	not detected	4.27	4.87	–
6b	2.0[e]	not detected	4.11	4.72	–
7b	1.1	5	4.14	4.74	4.31
8b	1.5	2	4.13	4.73	4.32
9b	2.0	not detected	4.10	4.70	–

[a] All signals are doublets with J = 8.6–9.0 Hz.

[b] Only **1a** in DMSO-d_6 (signal from H-1 at δ 3.77 ppm), a mixture of **1a** and **1c** (4 : 1, signals of H-1 at 3.76 and 4.41 ppm, respectively) in DMSO-d_6–D_2O (4 : 1).

[c] Only **3a** in DMSO-d_6 (signal from H-1 at δ 3.82 ppm), a mixture of **3a** and **3c** (4 : 1, signals of H-1 at 3.85 and 4.49 ppm, respectively) in DMSO-d_6–D_2O (6 : 1).

[d] Not detected.

[e] Only **6a** (signal from H-1 at δ 3.80 ppm) in DMSO-d_6, a mixture of **6a** and **6c** (6 : 1, signals of H-1 at 3.81 and 4.46 ppm, respectively) in DMSO-d_6–D_2O (4 : 1).

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded in D_2O at 24–30°C on a Bruker WM-250 spectrometer (operating at 250 MHz for ^{1}H and 75 MHz for ^{13}C) relative to acetone or residual protons of the solvent (as an internal standard). Electrophoresis was performed on Filtrak FN1 paper in a 6% HCOOH solution (12 V cm^{-1}, 1 h). The compounds were detected by ninhydrin staining and with the reagent sequence KIO_4–$AgNO_3$–KOH. The reactions were carried out in screw-capped tubes. Solutions were concentrated in vacuum on a water bath at <30°C.

β-D-Glucopyranosylammonium carbamates (general procedure). Methanol was added to a solution of hexose, pentose, or disaccharide and ammonium carbamate in concentrated NH_4OH, and the mixture was kept at 37°C (see Table 1). Carbamates **1b**, **2b**, **4b**–**6b**, and **9b** precipitated upon cooling the mixtures (5 to –10°C, 1–16 h). Carbamates **7b** and **8b** were precipitated from the reaction mixtures by 2-propanol (1.2 volumes), and the resulting oily sediment was allowed to stand for 3 h at –10°C and, then, was separated and triturated with a methanol–2-propanol mixture (1 : 1) until powder formation. The product was separated by filtration, washed with cold methanol and ether, and dried to give salts **1b**, **2b**, and **4b**–**9b** in a yield from 82 to 94% (Table 1).

2-Acetamido-2-deoxy-β-D-glucopyranosylammonium carbamate (3b). A mixture containing 2.21 g (10 mmol) of powdered 2-acetamido-2-deoxy-**D**-glucose and 3.12 g (40 mmol) of ammonium carbamate in 24.6 mL of methanol and 0.42 mL of water was kept for 48 h at 37°C and 16 h at 5°C. The resultant precipitate was separated by filtration, washed with methanol and ether, and dried to give 2.32 g (83%) of salt **3b**.

Transformation of Glucosylammonium Carbamates into Glucosylamines

Method A. An 8-mL volume of methanol and 0.022 mL of *N*,*N*-diisopropylethylamine were added to a solution of 50 mg of salt **1b**, **2b**, or **5b** in 0.2 mL of water. The solution was concentrated at 90 mmHg to 1 mL, and 3 mL of 2-propanol was added. The resulting mixture was evaporated to dryness at 12 mmHg. The residue was dried to produce glucosylamines **1a**, **2a**, and **5a** in ~100% yield.

Method B. A 7-mL volume of methanol was added to 50 mg of salt **3b**, and the salt was dissolved at 50°C. The solution was concentrated at 90 mmHg to 1 mL; then, 2 mL of methanol was added and the mixture was concentrated at 90 mmHg to 0.5 mL. Then, another 2 mL of methanol was added and the mixture was concentrated at 12 mmHg to dryness to leave 38.8 mg (99%) of amorphous glucosylamine **3a**.

Method C. (a) Triethylamine (0.04 mL), 0.25 mL of methanol, and 0.5 mL of absolute ethanol were added under stirring to a solution of 50 mg of salt **4b** in 0.1 mL

Table 3. Properties of glucosylammonium carbamates **1b**–**9b** and glucosylamines **1a**–**9a**

Carbamate	Molecular formula	Elemental analysis data, found/calculated, %			$[\alpha]_D$ (*c* 1, water)	Glucosylamine	$[\alpha]_D$ (c 1, water)	$[\alpha]_D$ (reference)
		C	H	N				
1b	$C_7H_{16}N_2O_7$	34.99/35.00	6.65/6.71	11.35/11.66	+5.3°	**1a**	+19.8°	+20.8° [10]
2b	$C_7H_{16}N_2O_7$	34.90/35.00	6.73/6.71	11.61/11.66	+28.6°	**2a**	+52.2°	+62.2° [10]
3b	$C_9H_{19}N_3O_7$	38.43/38.43	7.20/6.81	14.73/14.94	+1.3°	**3a**	–5.2°	–4.7°[4]
4b	$C_6H_{14}N_2O_6$	34.73/34.29	6.84/6.71	13.36/13.33	–12.1°	**4a**	–13.8°	–17.0°[9]
5b	$C_6H_{14}N_2O_6$	34.58/34.29	6.76/6.71	13.24/13.33	–18.3°	**5a**	–23.3°	–17.4° [11]
6b	$C_{13}H_{26}N_2O_{12}$	38.57/38.81	6.63/6.51	6.81/6.96	+27.4°	**6a**	+38.4°	+38.5° [12]
7b	$C_{13}H_{28}N_2O_{13}$*	36.67/37.14	6.78/6.72	6.88/6.66	+84.9°	**7a****	+100.1°	–
8b	$C_{13}H_{26}N_2O_{12}$	38.87/38.81	7.03/6.51	7.31/6.96	+12.0°	**8a**	+17.7°	+20.0° [12]
9b	$C_{13}H_{26}N_2O_{12}$	37.98/38.81	6.86/6.51	7.05/6.96	–13.5°	**9a**	–5.4°	–

* For monohydrate. Content of H_2O: found (%): 3.56, calculated (%): 4.28.
** The substance contains 5% of **7d** according to the NMR spectrum in D_2O.

of water, and the resulting mixture was kept for 16 h at 5°C. The resulting precipitate was separated by filtration, washed with a methanol–absolute ethanol (1 : 1) mixture and ether, and dried to yield 29.2 mg (94%) of glucosylamine **4a**.

(b) Triethylamine (0.042 ml) and 0.6 mL of methanol were added under stirring to a solution of 80 mg of salts **6b**–**9b** in 0.1 mL of water, and absolute ethanol was added under stirring until the appearance of intense turbidity. The mixture was kept for 16 h at 5°C. The resulting precipitate was separated by filtration, washed with absolute ethanol and ether, and dried to produce 60–65 mg (88–95%) of glucosylamines **6a**–**9a**.

The data of ^{1}H NMR spectra for the compounds obtained are presented in Table 2, and elemental analyses and optical rotation data are listed in Table 3.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 01–03–33267.

REFERENCES

1. Monsigny, M., Quetard, C., Bourgerie, S., Delay, D., Pichon, C., Midoux, P., Mayer, R., and Roche, A.C., *Biochimie*, 1998, vol. 80, pp. 99–108.
2. Isbell, H.S. and Frush, H.L., *Methods in Carbohydrate Chemistry*, New York: Academic, 1980, vol. 8, pp. 255–259.
3. Manger, I.D., Rademacher, T.W., and Dwek, R.A., *Biochemistry*, 1992, vol. 31, pp. 10724–10732.
4. Likhosherstov, L.M., Novikova, O.S., Derevitskaya, V.A., and Kochetkov, N.K., *Carbohydr. Res.*, 1986, vol. 146, pp. C1–C5.
5. Urge, L., Otvos, L., Lang, E., Wroblewski, K., Laczko, I., and Hollosi, M., *Carbohydr. Res.*, 1992, vol. 235, pp. 83–93.
6. Kallin, E., Lonn, H., Norberg, T., and Elofsson, M., *J. Carbohydr. Chem.*, 1989, vol. 8, no. 4, pp. 597–611.
7. Vetter, D. and Gallop, M.A., *Bioconjugate Chem.*, 1995, vol. 6, pp. 316–318.
8. Lubineau, A., Auge, J., and Drouillat, B., *Carbohydr. Res.*, 1995, vol. 266, no. 2, pp. 211–219.
9. Likhosherstov, L.M., Novikova, O.S., Shibaev, V.N., and Kochetkov, N.K., *Izv. Akad. Nauk, Ser. Khim.*, 1996, no. 7, pp. 1848–1851.
10. Isbell, H.S. and Frush, H.L., *J. Org. Chem.*, 1958, vol. 23, no. 9, pp. 1309–1319.
11. Tipson, R.S., *J. Org. Chem.*, 1961, vol. 26, no. 7, pp. 2462–2465.
12. Micheel, F., Frier, R., Plate, E., and Hiller, A., *Chem. Ber.*, 1952, vol. 85, no. 11, pp. 1092–1096.