NEW SIMPLE SYNTHESIS OF 2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE AND 2-ACETAMIDO-4-O-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-2-DEOXY-β-D-GLUCOPYRANOSYLAMINE AND PREPARATION OF THEIR N-ACYL DERIVATIVES

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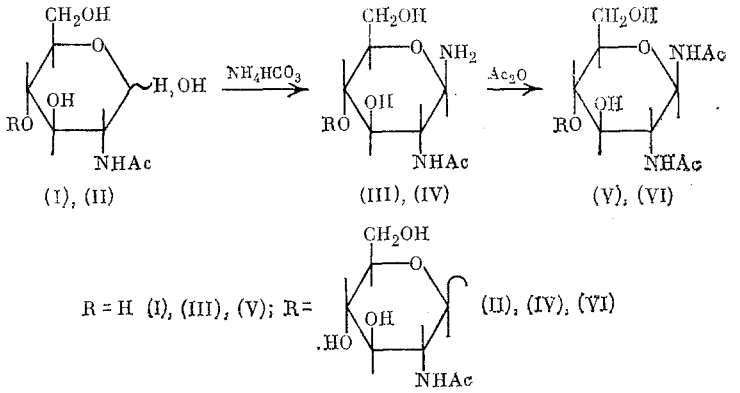
We have previously developed a method for cleavage of the N-glycosylamide carbohydrate–peptide bond in glycoproteins by the action of alkaline $LiBH_4$ that makes it possible to isolate the undergraded carbohydrate chains in up to 60% yields [1]. In order to perfect this method we decided to study the behavior of compounds that model the junction of the N-glycosylamide carbohydrate–peptide bond in glycoproteins. The synthesis of model compounds of this type is well known [2]. As a rule, β-D-glycosylamides of derivatives of L-aspartic acid (Asp) are obtained by acylation of per-O-acetylglycosylamines (less commonly the free glycosylamines [3]) with N-protected L-Asp α-esters by the carbodiimide method [4, 5]. A method for the synthesis of such compounds based on the reaction of per-O-acetylglycosyl isothiocyanates with 1-benzyl N-benzyloxycarbonyl-L-aspartate has also been proposed [6]. All of these methods are distinguished by their multistep character, chiefly in the preparation of the starting glycosylamine.

The present paper is devoted to a new simple method for the synthesis of 2-acetamido-2-deoxymono- (III) and 2-acetamido-2-deoxydi-β-D-hexopyranosylamine (IV) and their N-acyl derivatives. The chief difference in the developed method is direct condensation of 2-acetamido-2-deoxy-D-glucose (I) or 2-acetamido-4-O-(2-acetamido-3-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucose (II) with NA_4HCO_3 in water, which makes it possible to obtain glycosylamines III and IV in 65-80% yields.



Condensation with NH_4HCO_3 was monitored by means of paper chromatography (PC) (system C). In addition to starting I (R_f 0.43) or II (R_f 0.32) and glycosylamine III (R_f 0.32) or IV (R_f 0.23), minor products with R_f 0.22 (from I) and 0.14 (from II) were observed. The amounts of the latter increased when the reaction was carried out in MeOH. These products were probably bisglycosylamines, the formation of which (preferably in MeOH) was previously noted [7]. Minor products were virtually absent when an aqueous solution of NH_4HCO_3 was used at starting I or II concentrations below 0.3 M. The optimum reaction conditions were worked out in the case of I. When the reaction was carried out in saturated NH_4HCO_3 (~20°C, 1.5 months), the yield of glycosylamine III reached 80%; at 30°C the reaction was complete after 7 days, and the yield of glycosylamine III was 65%. It was necessary to

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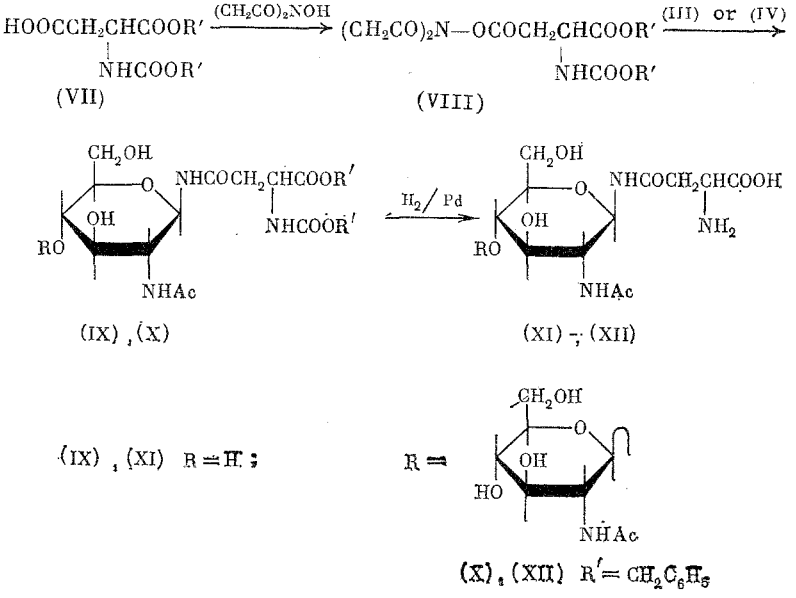
remove the liberated CO_2 from the reaction media, since the yields of glycosylamines did not exceed 30% in sealed ampuls.

Virtually no degradation and epimerization of I and II were observed under the adopted conditions; this followed from quantitative analysis of the reaction mixtures after hydrolysis with 2 M HCl; only 2-amino-2-deoxy-D-glucose (GlcN) was detected, and 2-amino-2-deoxy-D-mannose was virtually absent. The reaction mixtures after removal of NH_4HCO_3 in vacuo were separated on Amberlite 15 (H^+) into neutral (aqueous) fractions containing unchanged I or II and basic (ammonical) fractions containing glycosylamine III or IV. The glycosylamines obtained were quite stable in dry form during storage in a desiccator or in aqueous dilute (0.1 M or lower) solutions at 5°C. Glycosylamines III and IV gave positive reactions with ninhydrin, and the negative angles of optical rotation indicated a β configuration of the glycosylamine bond. The ^{13}C NMR data for glycosylamines III and IV confirmed their structures and indicated the absence of α anomers. The chemical shifts in the ^{13}C NMR spectrum of glycosylamine III coincided with the previously published values [7]. The assignment of the chemical shifts in the ^{13}C NMR spectrum of glycosylamine IV was made on the basis of a comparison with the ^{13}C NMR spectra of glycosylamine III and N,N'-diacetylchitobiose [8]. Acetylation of glycosylamines III and IV with Ac_2O in aqueous MeOH gave their 1-N-acetyl derivatives V and VI in 85% yields. The constants of diacetate V coincides with the previously described values [9], and the β configuration and structure of triacetate VI were confirmed by PMR spectral data.

Glycosylamines III and IV were subsequently used for the synthesis of model glycopeptides XI and XII with an N-glycosylamide carbohydrate-peptide bond via the scheme

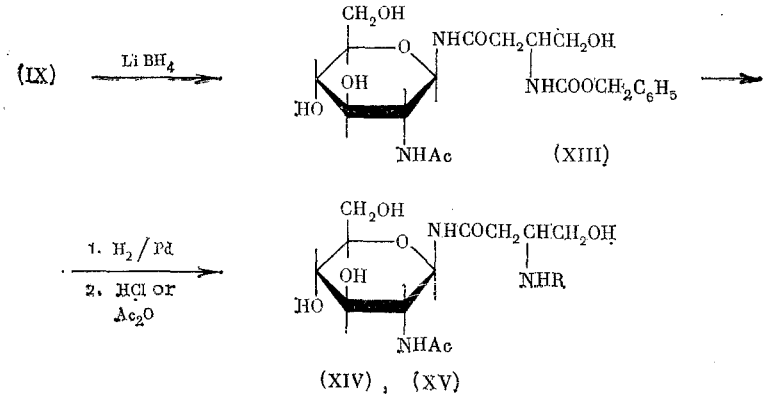


Activated ester VIII was obtained by condensation of 1-benzyl N-benzyloxycarbonyl-L-aspartate with N-hydroxysuccinimide in the presence of N,N'-dicyclohexylcarbodiimide [10]. The resulting 1-benzyl N-benzyloxycarbonyl-4-(N-succinimido)-L-aspartate (VIII) was used without additional purification for the acylation of glycosylamine III in 80% aqueous dimethylformamide (DMF) to give IX in 50% to 60% yield. Structure IX was confirmed by elementary analysis and quantitative determination of GlcN and Asp (in a ratio of 1:1).

Catalytic hydrogenation of glycopeptide IX led to 2-acetamido-1-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (XI), which was previously described in [3, 4]; this was confirmed by the quantitative formation of GlcN and Asp in an equimolar ratio. 2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (XII) was synthesized similarly, except that intermediate X was not isolated in pure form because of its low solubility. Hydrolysis of glycopeptide XII with 4 M HCl led to GlcN and Asp in a ratio of 2:1.

The structure of XII was also confirmed by the PMR and ^{13}C NMR spectra, which have not been previously described. The assignment of the signals for XII was made on the basis of a comparison of its spectrum with the ^{13}C NMR spectra of N,N'-diacetylchitobiose and

2-acetamido-1-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine [11].



R = H·HCl (XIV), Ac (XV).

The next step was the synthesis of XIV and XV, which contained a hydroxymethyl group instead of a carboxy group. Similar compounds are formed in the reductive cleavage of the peptide bond of glycoproteins with alkaline $LiBH_4$, and they may prove to be useful in the further study of this process. Treatment of IX with $LiBH_4$ in 70% aqueous tert-BuOH at 0°C led to reduction of the ester grouping and the formation of XIII, in which the N-benzyloxycarbonyl grouping was retained. This followed from data from the IR spectrum of product XIII, in which absorption bands at 1650 and 1690 cm^{-1} (carbonyl groups of amide and urethane groupings, respectively) were present, and an absorption band at 1730 cm^{-1} of the carbonyl group of the ester grouping of starting IX was absent. An analysis of the products of acidic hydrolysis of XIII, after which Asp was not detected, also indicated reduction of the ester grouping.

Catalytic hydrogenation of XIII led to 2-acetamido-1-N-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine, which was isolated in the form of the hydrochloride (XIV) and the N-acetate (XV) in ~90% yields. To prove the structure, hydrochloride XIV or N-acetate XV was hydrolyzed with 4 M HCl; GlcN was formed in virtually quantitative yield. 3-Amino-4-hydroxybutanoic acid, which, as is well known, readily undergoes ring closure to a γ-lactone [12], is formed from the aglycone. The formation of this lactone was confirmed after its reduction with $LiBH_4$ to 2-aminobutane-1,4-diol in 86% yield.

EXPERIMENTAL

2-Amino-2-deoxyglycose (GlcN), aspartic acid (Asp), and the other amino derivatives were determined quantitatively after hydrolysis (with 2 M or 4 M HCl, 100°C, 16 h) with a Biotronik LC-4010 amino acid analyzer with an Aminex A-5 column (23 by 0.9 cm) and the use of standard Na–citrate–hydrochloric acid buffers [0.2 M Na^+, pH 3.25 (A); 0.35 M Na^+, pH 5.28 (B)] at 63°C and an elution rate of 80 ml/h.

The PMR and ^{13}C NMR spectra of solutions of the compounds in D_2O were recorded with a Bruker WM-250 spectrometer (250 and 62.89 MHz, respectively) with sodium 2,2-dimethyl-2-silapentane-5-sulfonate and methanol, respectively, as the internal standards; H' and C' are the hydrogen and carbon atoms of the 2-acetamido-2-deoxy-D-glucose residue with the O-glycoside bond, and H_α and H_β are the corresponding protons of the Asp residue.

The optical rotation was measured with a Perkin–Elmer 141 polarimeter. The melting points were determined with a Kofler stage. Paper chromatography (ascending) was carried out on Whatmann No. 1 paper with iso-BuOH–n-PrOH–water (1:2:1.25) as the solvent system (C). The substances were detected with ninhydrin, $AgNO_3$–KOH, and Cl_2–KI with starch. Thin-layer chromatography (TLC) was carried out on plates with a loose layer of LS 5/40 μm silica gel containing 13% gypsum in the following systems: chloroform–ethanol (19:0.8) (D) and ethyl acetate–MeOH (7:3) (E). The substances were detected by heating with H_2SO_4.

1,2-Dimethoxyethane was shaken with KOH until peroxides were absent, after which it was distilled over $LiAlH_4$. The DMF was distilled over ninhydrin in vacuo.

2-Acetamido-2-deoxy-β-D-glucopyranosylamine (III). A solution of 1 g of I and 7 g of NH_4HCO_3 in 15 ml of H_2O was maintained in a thin sealed flask at 30°C for 7 days with periodic

opening of the flask and stirring of the contents. The course of the reaction was monitored by paper chromatography. The mixture was evaporated with water (eight 25-ml portions), each time to ∿15 ml (at a bath temperature ≤30°C). The solution was diluted with water to 35 ml, the aqueous mixture was cooled with ice water, 8 ml of Amberlite 15 (H^+) was added, and the mixture was stirred for 15 min. The cation-exchange resin was removed by filtration and washed with 80 ml of cold water. The filtrate and the wash waters were combined and evaporated to dryness to give 0.23 g (23%) of unchanged I.

The cation-exchange resin was washed successively with cold MeOH (20 ml) and a cold 0.5 M solution of NH_3 in MeOH (10 12-ml portions, 45 min), and the eluate was evaporated in vacuo to ∿2 ml. Ether was added, and the resulting amorphous precipitate was separated by decantation, washed twice with ether, and dried in a vacuum desiccator over KOH to give 0.66 g (66%) of chromatographically homogeneous (system C) glycosylamine III with $[\alpha]_D^{20}$–4.7° (C 1.7, water) (see [3, 7]). ^{13}C NMR spectrum (δ, ppm): 175.4 ($NHCO\underline{C}H_3$), 84.9 (C^1), 77.5 (C^5), 75.3 (C^3), 70.8 (C^4), 61.6 (C^6), 57.1 (C^2), and 23.0 ($NHCO\underline{C}H_3$).

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (IV). A solution of 0.4 g of II and 3 g of NH_4HCO_3 in 6 ml of water was maintained at 30°C for 7 days. The course of the reaction and the isolation of IV were monitored as in the case of III. The yield of chromatographically homogeneous (system C) amorphous IV, with $[\alpha]_D^{21}$–10.5° (C 1.0, water), was 0.28 g (65%); dissolving of this product in methanol and evaporation of the solution in vacuo to 5 ml gave 0.2 g of crystalline IV with mp 221-222°C (dec.) and $[\alpha]_D^{20}$–11.0° (C 1.1, water). ^{13}C NMR spectrum (δ, ppm): 175.6 ($NH\underline{C}OCH_3$), 102.5 ($C^{1'}$), 85.2 (C^1), 81.0 (C^4), 77.0 ($C^{5'}$), 76.4 (C^5), 74.6 ($C^{3'}$), 74.2 (C^3), 70.9 ($C^{4'}$), 61.7 ($C^{6'}$), 61.4 (C^6), 56.9 and 56.7 ($C^{2'}$ and C^2), and 23.3 and 23.2 ($NHCO\underline{C}H_3$).

2-Acetamido-1-N-acetyl-2-deoxy-β-D-glucopyranosylamine (V). A 2-ml sample of MeOH and 1 ml of Ac_2O were added to a solution of 0.36 g of III in 8 ml of water, and the mixture was maintained at 20°C for 16 h. It was then diluted with MeOH and evaporated with toluene in vacuo to dryness. The residue was crystallized from ethanol to give 0.35 g (80%) of V with mp 264-265°C and $[\alpha]_D^{19}$ +25° (C 1.0, water) (see [9]).

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-N-acetyl-2-deoxy-β-D-glucopyranosylamine (VI). Compound VI, with mp 303-304°C (dec., from MeOH) and $[\alpha]_D^{20}$ +2.5° (C 1.0, water), was similarly obtained in 85% yield. PMR spectrum (δ, ppm; J, Hz): 5.08 (1H, H^1; $J_{1,2}$ = 9.2), 4.65 d (1H, $H^{1'}$; $J_{1,2}$ = 8.3), and 2.1 s (3H) and 2.04 s (6H) (9H, $NHCOC\underline{H}_3$). Found: C 46.21; H 6.82; N 8.85%. $C_{18}H_{31}N_3O_{11}$. Calculated: C 46.44; H 6.71; N 9.02%.

1-Benzyl N-Benzyloxycarbonyl-4-(N-succinimido)-L-aspartate (VIII). Solutions of 0.4 g (1.13 mmole) of 1-benzyl N-benzyloxycarbonyl-L-aspartate (VII) [5, 13] and 0.13 g (1.13 mmole) of N-hydroxysuccinimide in 1 ml of 1,2-dimethoxyethane and 0.26 g (1.26 mmole) of N,N'-dicyclohexylcarbodiimide in 2 ml of 1,2-dimethoxyethane were mixed at −10°C, and the mixture was maintained at −10°C for 1 h and at −5°C for 18 h. The resulting precipitate (0.24 g) was removed by filtration, and the solution was evaporated in vacuo to dryness to give activated ester VIII, with R_f 0.34 (system D), in the form of a viscous syrup, which, without additional purification, was subjected to reaction with glycosylamine III or IV.

2-Acetamido-1-N-(1-benzyloxy-N-benzyloxycarbonyl-4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (IX). Solutions of 0.5 g (2.27 mmole) of glycosylamine III in 2 ml of water and 1.02 g (2.25 mmole) of activated ester VIII in 7.5 ml of DMF were mixed at 0°C, and the resulting solution was maintained at 20°C for 16 h, after which it was diluted with toluene and evaporated in vacuo. The dry residue was washed repeatedly on the filter with ether and ice water. The product was dried in vacuo over P_2O_5, dissolved in MeOH, and precipitated with acetone. The amorphous residue was removed by filtration, washed with acetone and ether, and dried in vacuo to give 0.65 g (52%) of IX with $[\alpha]_D^{21}$+ 9.8° (C 1.0, MeOH). Found: C 57.58; H 6.01; N 7.43%. $C_{27}H_{33}N_3O_{10}$. Calculated: C 57.95; H 5.94; N 7.50%.

A 1.03-mg (1.84 μmole) sample of IX in 1 ml of 4 M HCl was hydrolyzed at 100°C for 16 h after which 1.78 μmole of Asp (peak at the 29th minute) and 1.7 μmole of GlcN (Peak at the 59th minute) were determined with the amino acid analyzer in buffers A (15 min) and B (50 min).

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-1-N-(1-benzyloxy-N-benzyloxycarbonyl-4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (X). Solutions of 0.65 g (1.54 mmole) of glycosylamine IV in 2.5 ml of water and 0.7 g (1.54 mmole) of ester VIII in 7.5 ml of DMF

were mixed at 0°C, and the mixture was maintained at 20°C for 16 h. The solvent was evaporated in vacuo, acetone was added, and the product was removed by filtration, washed with acetone, ice water, and MeOH, and dried to give 0.45 g (37%) of amorphous X.

The hydrolysis of X was carried out as in the case of IX; 1.18 μmole of Asp and 2.8 μmole of Glc were determined in 1 mg (1.31 μmole) of X.

2-Acetamido-1-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (XI). A solution of 0.8 g of IX in 20 ml of 70% aqueous MeOH was hydrogenated for 5 h over 0.4 g of 10% Pd/C at 20°C, after which the catalyst was removed by filtration and washed with aqueous MeOH, and the solution was evaporated to dryness. The residue was crystallized from aqueous ethanol to give 0.38 g (81%) of XI with mp 217-219°C (dec.) and $[\alpha]_D^{20}$ +25° (C 1.0, water) (see [3, 4]).

2-Acetamido-4-O-(2-acetamido-2-deoxy-β-B-glucopyranosyl)-1-N-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (XII). A suspension of 0.69 g of X in 50 ml of 70% MeOH was hydrogenated as in the case of IX. Two crystallizations from aqueous ethanol gave 0.31 g (64%) of XII with mp 255-257°C (dec.) and $[\alpha]_D$ +5.1° (C 1.0, water) (see [5]). PMR spectrum (δ, ppm; J, Hz): 5.12 d (1H, H^1; $J_{1,2}$ = 9.25), 4.65 d (1H, $H^{1'}$; $J_{1,2}$ = 8.25), 4.03 dd (H_α; $J_{\alpha,\beta}$ = 6.8; $J_{\alpha,\beta'}$ = 4.5), 3.0 dd ($H_{\beta'}$; $J_{\alpha,\beta'}$ = 4.5, $J_{\beta,\beta'}$ = −17.8), 2.9 dd (H_β; $J_{\alpha,\beta}$ = 6.8, $J_{\beta,\beta'}$ = −17.8). ^{13}C NMR spectrum (δ, ppm): 176.4, 176.1 ($NH\underline{C}OCH_3$); 174.3, 174.1 (COOH, $\underline{N}HCO$); 102.97 ($C^{1'}$), 80.6 (C^4), 79.7 (C^1), 77.8 ($C^{5'}$), 77.5 (C^5), 75.1 ($C^{3'}$), 74.4 (C^3), 71.3 ($C^{4'}$), 62.2 ($C^{6'}$), 61.6 (C^6), 57.2 ($C^{2'}$), 55.2 (C^2), 52.5 ($CH_2\underline{C}H$), 36.6 ($CO\underline{C}H_2$), 23.7 ($NHCO\underline{C}H_3$).

2-Acetamido-1-N-(3-benzyloxycarbonylamido-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine (XIII). A 0.55-g sample of $LiBH_4$ was added at 0°C to a solution of 0.6 g of IX in 25 ml of 70% tert-BuOH, and the mixture was maintained at 0°C for 2 h. The excess $LiBH_4$ was decomposed with 50 ml of water and 10 ml of AcOH, and the tert-BuOH was removed in vacuo. The solution was diluted with water to 100 ml, 85 ml of KU-2 (H^+) cation-exchange resin was added, and the mixture was stirred for 1 h. The cation-exchange resin was removed by filtration and washed with 30% MeOH. The filtrate and wash waters were evaporated, and the H_3BO_3 was removed by repeated evaporation with MeOH. The residue was washed with MeOH and ether and dried to give 0.22 g (45%) of amorphous XIII, with R_f 0.52 (system E), which was hydrogenated without additional purification.

2-Acetamido-1-N-(3-amino-4-hydroxybutyryl)-2-deoxy-β-D-glucopyranosylamine Hydrochloride (XIV) and 2-Acetamido-1-N-(3-acetamido-4-hydroxybutyryl)-2-deoxy-β-D glucopyranosylamine (XV). A solution of 0.4 g of XIII in 20 ml of 50% MeOH was hydrogenated for 3 h over 0.2 g of 10% Pd/C at 20°C, after which the catalyst was removed by filtration and washed with 20% MeOH. The solution was evaporated in vacuo, the residue was dissolved in 10 ml of water, and the solution was divided into two parts. One half of the solution was acidified to pH 2-3 with 0.2 M HCl and evaporated to dryness in vacuo. The residue was dissolved by heating in MeOH, ether was added until the solution became turbid, and the mixture was allowed to stand at 20°C. The crystalline product was removed by filtration, washed with MeOH and ether, and dried in vacuo to give 0.13 g (83%) of hydrochloride XIV with mp 235-236°C (dec.) and $[\alpha]_D$ +15° (C 1.4, water). Found: C 39.96; H 6.87; N 11.25%. $C_{12}H_{24}N_3ClO_7$. Calculated: C 40.28; H 6.76; N 11.74%.

The other half of the solution was treated with 1 ml of MeOH and 0.5 ml of Ac_2O, and the mixture was maintained at 20°C for 16 h. The solution was diluted with MeOH, toluene was added, and the mixture was evaporated to dryness. The residue was dissolved in the minimum amount of water, ethanol was added until the solution became turbid, and the mixture was allowed to stand at 20°C for 16 h. The crystals were removed by filtration, washed with ethanol and ether, and dried to give 0.12 g (76%) of acetate XV with mp 281-283°C (dec.) and $[\alpha]_D^{21}$ +24.0 (C 1.2, water). Found: C 45.84; H 7.03; N 11.41%. $C_{14}H_{25}N_3O_8$. Calculated: C 46.27; H 6.93; N 11.56%.

Determination of 2-Aminobutane-1,4-diol. A 1.25-mg sample of hydrochloride XIV was hydrolyzed in 2 ml of 4 M HCl at 100°C for 16 h, after which the mixture was evaporated to dryness. Water (1 ml) was added to the residue, and 0.25 ml of the solution was withdrawn and evaporated repeatedly in vacuo with water until it was neutral. The dry residue was treated with 0.25 ml of water, 0.7 ml of tert-BuOH, and 0.05 ml of 1 M LiOH, and the resulting solution was cooled with ice and treated with 22 mg of $LiBH_4$. The mixture was maintained at 40-45°C for 1 h, after which it was diluted with 3 ml of water, and the aqueous mixture was

acidified to pH ∿ 2 with 1 M HCl. The solution was evaporated to dryness, and the residue was analyzed in buffer B, as a result of which 0.75 μmole (86%) of 2-aminobutane-1,4-diol (peak at the 78th minute) and 0.82 μmole (90%) of 2-amino-2-deoxysorbitol (peak at the 53rd minute) were determined. Starting hydrochloride XIV in buffer B gave a peak at the 39th minute.

CONCLUSIONS

1. A new method for the preparation of 2-acetamido-2-deoxy-β-D-glucopyranosylamine and 2-acetamido-4-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine, based on the condensation of the corresponding N-acetamido sugars with NH_4HCO_3 in a saturated aqueous solution, was developed.

2. A number of model glycopeptides with an N-glycosylamide carbohydrate–peptide bond were synthesized.

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