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Organic Chemistry

Synthesis and Biological Activity of some Derivatives of N-(4-Carboxyphenyl)-2,3,4,6-Tetra-O-Acetyl-β-D-Mannopyranosylamine

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Abstract. The N-β-glycosidic bond is widely represented in a large number of natural compounds, primarily in nucleic acids, glycoproteins, and some coenzymes, as well as synthetic analogues of nucleotides, the antimetabolites, which are used in the treatment of tumor diseases and viral infections. The development of efficient synthetic methodologies for various N-mannosides and their derivatives will be useful in designing and preparing synthetic analogues of natural product. The purpose of our work was the synthesis of glycoconjugates of N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine (III) and prediction of their biological activity. The N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine was synthesized by condensation of D-mannose with 4-aminobenzoic acid, followed by acetylation of synthesized N-mannopyranosylamine. By condensation of N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine with some L-amino acid ester dihydrochlorides, in the presence of N,N'- dicyclohexylcarbodiimide and trimethylamine, the corresponding glycoconjugantes were obtained. The structures of the obtained compounds were established by physico-chemical methods of analysis. Using the Pass Online program, based on the analysis of structure-activity relationships, we obtained a wide range of possible biological activities and toxic/side effects for the synthesized compounds.

Keywords: β-D-mannopyranosylamine, glycoconjugates, dicyclohexylcarbodiimide, biological activity

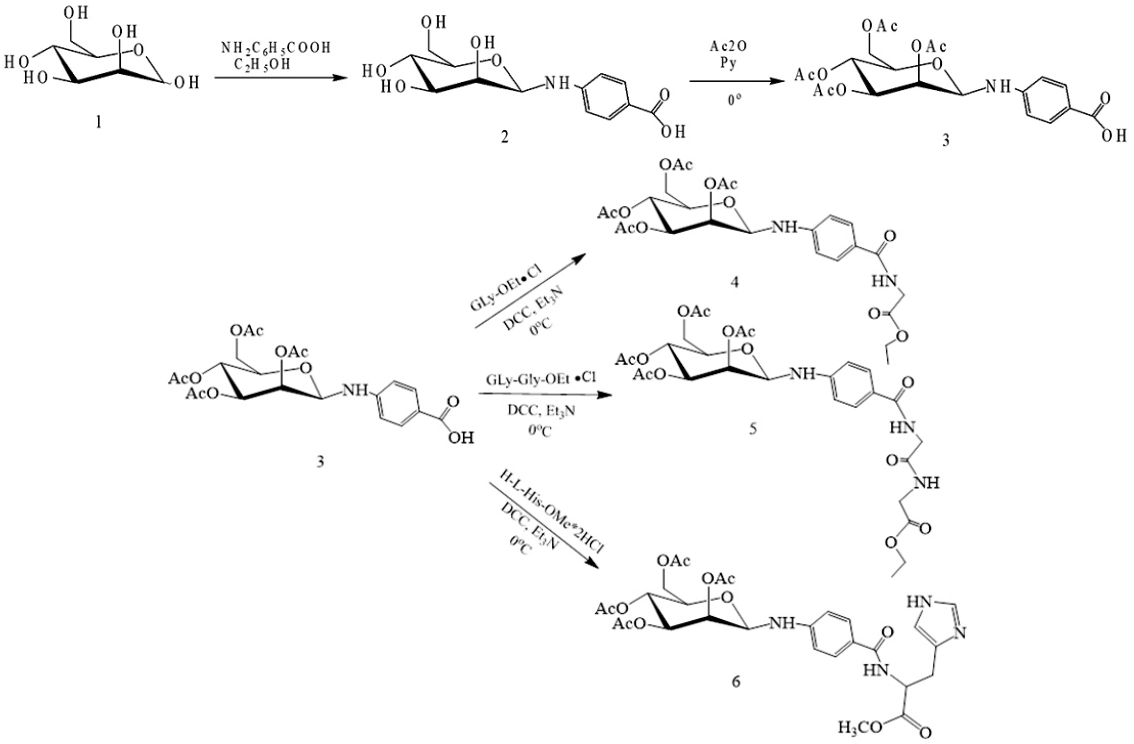
Introduction

Reactions between carbohydrates and amino compounds play an important but not yet fully understood role in biological systems. The N-glycolytic bond is quite common in both natural and synthetic products. N-glycosides are part of nucleic acids, which are responsible for the transfer and storage of genetic information, as well as other important processes in the body. Glycosylamines are important intermediates in the synthesis of nucleosides and chemotherapeutic drugs. N-glycosides are a number of natural nucleotides and nucleosides that are characterized by antiviral activity. N-glycosides are used as mediators and stimulants of the central nervous system. The pharmaceutical industry makes extensive use of the chemistry of glucosylamine derivatives, which are also used in agriculture against pests. Some N-

glycosides are characterized by antimicrobial activity. According to the researchers, the study of N-glycosylamines and their new derivatives will facilitate the understanding of the chemical and biological properties of these compounds as well as the mechanisms of their action in life processes (Ghoneim & El-Sherif, 2019; Kaletina, 1988; Metwally et al., 2010; Sidamonidze et al., 2007; 2023).

The goal of our work was to synthesize derivatives of N-(4-carboxyphenyl)-β-D-mannopyranosylamine and predict their potential biological activity.

In the first step, N-(4-carboxyphenyl)-β-D-mannopyranosylamine (2) was synthesized by condensation of D-mannose (1) with 4-aminobenzoic acid in a 95% ethanol solution, in the presence of the catalyst glacial acetic acid. To protect the hydroxyl groups, the acetylation reaction of compound (2) was performed with acetic anhydride in pyridine at 0°C. As a result, N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine (3) was synthesized. The final glycoconjugates N-[4-N'-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoic acid-L-glycine ethyl ester (4), N-[4-N'-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoic acid-L-glycine glycine ethyl ester (5) and N-[4-N'-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoyl-L-histidine methyl ester (6) were obtained by condensation of N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine (3) with the hydrochlorides of corresponding L-Glycine ethyl ester, L-histidine methyl ester and Glycyl-glycine ethyl ester in the presence of N,N'-dicyclohexylcarbodiimide and triethylamine at 0°C (Scheme).



Scheme. Synthesis of glycoconugates of N-[4-N'-(2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl)] aminobenzoic acid.

Reaction mechanism: Using the carbodiimide method to form amide bonds, N,N'-dicyclohexyl-carbodiimide (DCC) acts as the condensing agent. The basic process proceeds as follows:

$$R\text{-}COOH + NH_2\text{-}R^{I} + R^{II}\text{–}N{=}C{=}N\text{–}R^{II} \rightarrow R\text{-}CO\text{-}NH\text{-}R^{I} + R^{II}\text{–}NH\text{-}CO\text{-}NHR^{II}.$$

The hydrogen of carboxyl group interacts with double bond of the carbodiimide, which makes the carboxyl group ready to react. The reaction can then go two ways: a) the acyl group moves from oxygen to nitrogen (O→N acyl migration), creating N-acylurea; b) if it's acidic, another hydrogen is added, forming both symmetric anhydride and disubstituted N-acylurea. Finally, adding an amine to either the N-acylurea or the symmetric anhydride creates the amide bond (Schroeder & Lubke, 1967).

While direction (a) enables the target product to be obtained with a yield of 60-80%, direction (b) limits the greatest feasible yield to 50%. As a result, we employed direction (a).

The use of the carbodiimide method in amidic synthesis has certain advantages. For example, N,N'-dicyclohexylcarbodiimide is quite resistant to water, so moisture does not affect the condensation process. In contrast, other methods require specific precautions in this regard. Additionally, the *N,N'-dicyclohexylurea* by-product formed during the reaction is insoluble in most solvents, making it easy to remove from the reaction mixture by filtration.

The literature indicates that the carbodiimide technique of amidic synthesis works well at ambient temperature as well as at 0°C. The peptide is known to be produced at 0°C with a maximum yield of 74% (Grinstein & Vinits, 1965; Lagidze et al., 1983).

The structure of the obtained compounds 4-6 was proved by physico-chemical methods of analysis.

Probable biological activity of synthesized substances. Study of the spectrum activity. For prediction of biological activity spectra of the synthesised compounds, based on the analysis of structure-activity relationship (SAR) the PASS program was used (Czerepak & Ryser, 2008; Poroikov et al., 2019; Ruddigkeit et al., 2013). The pass prediction tool gives the biological activities (including pharmaco-therapeutic as well as toxic effects) of the input 2D structure as a result of prediction in the descending order of their probability ratios Pa>Pi (probability to be active:probability to be inactive), hence giving the output list in the order of their most useful activities. We performed calculations on deacetylated products.

The data on the prediction of the biological activity of compounds 4-6 show that they possess potentially high antihemorrhagic, antiviral, antidiabetic, antimicrobial, and other types of biological activities.

Materials and Methods

Reagents. Analytical-grade D-mannose, 4-aminobenzoic acid, chloroform, triethylamine, hydrochloride of H-Gly-Gly ethyl ester, L-histidine methyl ester hydrochloride and H-L-Gly ethyl ester hydrochloride were used. The purity of the products and Rf values were determined on Silufol UV-254 using the solvent system: (a) dioxane:benzene (1:4); (b) benzene:ethanol (9:2); (c) benzene:ethanol (9:1). IR spectra were recorded using a "NICOLETis5" spectrometer. ^{13}CNMR spectra were registered in $CDCI_3$ on a "Ascend TM400".

Synthesis: N-(4-Carboxyphenyl)-β-D-mannopyranosylamine (2) 3.4 g (0.036 mol) of 4-aminobenzoic acid, 3.6 g (0.035 mol) of D-mannose, and 0.5 mL of glacial acetic acid were added to 50 mL of 96% ethanol. The reaction mixture was heated at 80°C for 40 minutes under constant stirring. The mixture cooled up to room temperature 50 ml of diethyl ether was added and after blending left for the night at room temperature. The precipitate was filtered, washed three times with a mixture of ethanol and ether (1:1), and dried. Recrystallization was carried out from ethanol. The obtained white crystalline substance had a yield of 5.1 g (88%). Melting point: 165°C, Rf= 0.25 (a) Literature data (Cherepanov & Belkov, 2022).

N-(4-Carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine (3) 1.4 g (0.005 mol) of N-(4-carboxyphenyl)-β-D-mannopyranosylamine was dissolved in 5 mL of pyridine under stirring and cooled in

an ice bath to 0°C. Then, freshly distillated 5 mL of acetic anhydride was added. After 2 hours of mechanical stirring, the reaction mixture was left in the refrigerator for 24 hours. The resulting mixture was then poured into 150 mL of ice-cold water. The precipitated crystals were filtered, washed first with cold distilled water (until the pyridine odor was removed) and then with a mixture of ether and ethanol (1:1). Recrystallization was carried out from ethanol. The obtained white precipitate was dried over anhydrous $CaCl_2$. Yield: 1.33 g (62%). Melting point: 100–105°C, M = 467, Rf=0,67, (b).

IR spectrum (ν, cm⁻¹): 3323.88 cm^{-1} ν(NH); 2929.15-2851.10 cm^{-1} ν(C-$H_{alifat.}$); 1744.29 cm^{-1} ν(C=O_{ester}.); 1696.4 cm^{-1} ν(C=O_{carbox}.); 1605.78-1580.16 cm^{-1} (C=C_{arom}.); 1527 cm^{-1} δ(N-H); 1499.72 cm^{-1} (C_1-$N_{-glicos.}$); 1330 cm^{-1} (C-$N_{secendary\ arom\ amin.}$); 1215.59-1164.00 cm^{-1} (C-O-C); 1030-1086 cm^{-1} δ(pyranose ring); 892.33 cm^{-1} (C_1-H); 640.18 cm^{-1} δ (C_1-$N_{-glicos.}$);

N-[4-N'-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoyl-L-glycine ethyl ester (4) A solution of 0.5 g (0.001 mol) of N-(4-carboxyphenyl)-2,3,4,6-tetra-O-acetyl-β-D-mannopyranosylamine in 10 mL of chloroform was mixed with 0.196 g (0.001 mol) of L-glycine ethyl ester hydrochloride, 0.2 g of N,N'-dicyclohexylcarbodiimide solution in 5 mL of chloroform, and 0.2 mL of triethylamine. The reaction mixture was stirred at 0°C for 2 hours and then placed in a refrigerator for 24 hours. The resulting precipitate was filtered, and the filtrate was treated with water, 5% citric acid solution, and water again. The organic phase was dried over Na_2SO_4. After filtration, the solution was concentrated and treated with petroleum ether until a precipitate formed. The precipitate was filtered and recrystallized from ethanol. Yield: 0.39 g (71%); Melting point: 116°C; Rf=0,66 (c).

IR spectrum (ν, cm⁻¹): 3317.09 cm^{-1} ν(NH); 2928.92-2851.02 cm^{-1} ν(C-$H_{alifat.}$); 1739.65 cm^{-1}, 1694.47 cm^{-1} ν(C=O_{carbo}.); 1636.82 cm^{-1} ν(CO-NH); 1606.71 cm^{-1} (C=C_{arom}.); 1526.39 cm^{-1} δ(N-H); 1366-1310 cm^{-1} (C-$N_{secendary\ arom\ amin}$); 1218.86-1163.89 cm^{-1} (C-O-C); 1099-986.89 cm^{-1} δ(pyranose ring); 834.72 cm^{-1}
(C_1-H); 640.50 cm^{-1} δ (C_1-$N_{-glicos.}$);

^{13}C NMR (δ, ppm): 81.12 (C-1); 73.35 (C-2); 71.80 (C-3); 65.77 (C-4); 71.70 (C-5); 62.52 (C-6); 49.20 (C-N); 20,57-22.98 (CO-CH_3); Aromatic ring: 146.78 (C-1); 113.78 (C-2); 129.14 (C-3); 128.09 (C-4); 129.14 (C-5); 113.78 (C-6); 170.95-169.65 (C=O_{ester}); 151 (CO-NH);

N-[4-N'-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoyl-L-glycylglycine ethyl ester (5) was synthesized under similar conditions.Yield: 62,6% (0,385g); Melting point**:** 109–112°C; Rf=0,62 (c).

IR spectrum (ν, cm⁻¹): 3321.71 cm^{-1} ν(NH); 2928.82-2849.88 cm^{-1} ν(C-$H_{alifat.}$); 1737.75 cm^{-1} ν(C=O_{carbo}.); 1642.74 cm^{-1}, 1624.71 cm^{-1} ν(CO-NH); 1607.68 cm^{-1} (C=C_{arom}.); 1511.17 cm^{-1} δ(N-H); 1367.43-1310.30 cm^{-1} (C-$N_{secendary\ arom\ amin}$); 1213.48 cm^{-1} (C-O-C); 1087.61-1030.93 cm^{-1} δ(pyranose ring); 840.69 cm^{-1} (C_1-H); 641.03 cm^{-1} δ (C_1-$N_{-glicos.}$);

^{13}C NMR (δ, ppm): 91.29 (C-1); 71.71 (C-2); 71.80 (C-3); 68.58 (C-4); 73.33 (C-5); 62.51 (C-6); 49.27 (C-N); 20,56-20.98 (CO-CH_3); Aromatic ring: 149.49 (C-1); 113.77 (C-2); 129.16 (C-3); 121 (C-4); 129.16 (C-5); 113.77 (C-6); 170.97-169.67 (C=O_{ester}); 1620 (CO-NH).

N-[4-N'-(2,3,4,6-Tetra-O-acetyl-β-D-mannopyranosyl)]aminobenzoyl-L-histidine methyl ester (6) Yield: 0.48 g (72%); Melting point (T_m): 135-137°C; Rf=0,72 (c).

IR Spectrum (ν, cm^{-1}): 3323.17 cm^{-1} ν(NH); 2927.66-2851.17 cm^{-1} ν(C-H$_{alifat.}$); 1745.23 cm^{-1} ν(C=O$_{carbo.}$); 1637.96 cm^{-1} ν(CO-NH); 1626.35 cm^{-1} (C=N); 1606.52-1575.92 cm^{-1} (C=C$_{arom.}$); 1526.33 cm^{-1} δ(N-H); 1366.29-1310.71 cm^{-1} (C-N$_{secendary\ arom\ amin}$); 1218.31 cm^{-1} (C-O-C); 1088.22-1044.60 cm^{-1} δ(pyranose ring); 837.51 cm^{-1} (C$_1$-H); 641.20 cm^{-1} δ (C$_1$-N$_{-glycos.}$);

^{13}C NMR (δ, ppm): 81.64 (C-1); 73.79 (C-2); 71.35 (C-3); 68.6 (C-4); 71.35 (C-5); 62.32 (C-6); 54.15 (C-N); 20,80-22.95 (CO-CH$_3$); Aromatic ring: 154.37 (C-1); 112.94 (C-2); 129.11 (C-3); 127.37 (C-4); 129.11 (C-5); 112.94 (C-6); Histidine ring: 24.92 (CH$_3$); 49.61 (CH$_2$); 54.15 (CH); 113.59 (C-4); 117.48 (C-5); 132.56 (C-2); 170.45-168.69 (C=O$_{ester}$); 157.48 (C=N); 155.08 (CO-NH).

ორგანული ქიმია

N-(4-კარბოქსიფენილ)-2,3,4,6-ტეტრა-O-აცეტილ-β-D-მანოპირანოზილამინის წარმოებულების სინთეზი და ბიოლოგიური აქტიურობის პროგნოზი

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N-გლიკოზიდური ბმა ფართოდ არის გავრცელებული სხვადასხვა ტიპის ბუნებრივ ნაერთებში, ძირითადად ნუკლეინის მჟავასა და გლიკოპროტეინებში, ზოგიერთ კოენზიმში, ასევე ნუკლეოტიდების სინთეზურ ანალოგებსა და ანტიმეტაბოლიტებში, რომლებიც გამოიყენება სიმსივნური დაავადებებისა და ვირუსული ინფექციების სამკურნალოდ. სხვადასხვა ტიპის N-მანოზიდებისა და მათი წარმოებულების სინთეზის ეფექტური მეთოდების შექმნა სასარგებლო ბუნებრივი პროდუქტების სინთეზური ანალოგების სტრუქტურის დადგენისა და სინთეზისათვის. წინამდებარე სამუშაოს მიზანს წარმოადგენდა N-(4-კარბოქსიფენილ)-2,3,4,6-ტეტრა-O-აცეტილ-β-D-მანოპირანოზილამინის წარმოებულების, კერძოდ, L-ამინმჟავების ესტერებთან სინთეზი და მათი შესაძლო ბიოლოგიური აქტიურობის პროგნოზი. N-(4-კარბოქსიფენილ)-2,3,4,6-ტეტრა-O-აცეტილ-β-D-მანოპირანოზილამინის სინთეზი ჩატარდა D-მანოზის 4-ამინობენზოის მჟავასთან კონდენსაციით და მიღებული პროდუქტების შემდგომი აცეტილირებით. N-(4-კარბოქსიფენილ)-2,3,4,6-ტეტრა-O-აცეტილ-β-D-მანოპირანოზილამინის კონდენსაციით ზოგიერთი L-ამინმჟავას ესტერის ჰიდროქლორიდთან N,N'-დიციკლოჰექსილკარბოდიიმიდის და ტრიეთილამინის თანაობისას მიღებულ იქნა შესაბამისი ახალი ტიპის წარმოებულები. სინთეზირებულ ნაერთთა აღნაგობა დადგინდა კვლევის ფიზიკურ–ქიმიური მეთოდებით. კომპიუტერული პროგრამა Pass Online-ის მეშვეობით,

ნივთიერებების სტრუქტურა ბიოაქტიურობის ანალიზის საფუძველზე სინთეზირებული ნაერთებისათვის პროგნოზირებულ იქნა ბიოლოგიური აქტიურობის სპექტრი.

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