GLYCOPEPTIDES

COMMUNICATION 9. SYNTHESIS OF O-(AMINO-ACYL) DERIVATIVES OF SOME MONOSACCHARIDES

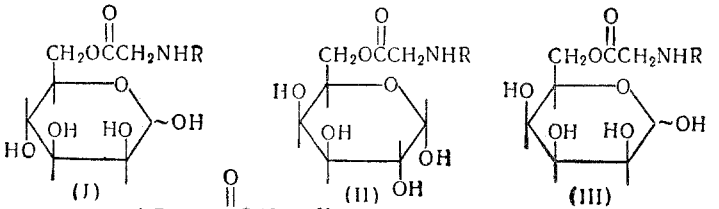
(UDC 542.91+547.455)

N. K. Kochetkov, V. A. Derevitskaya, and L. M. Likhosherstov

Institute for the Chemistry of Natural Products, Academy of Sciences, USSR
Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 6,
pp. 1045-1051, June, 1965
Original article submitted April 23, 1963

The previously developed carbodiimide method for the preparation of O-(amino acyl) and peptide derivatives of monosaccharides, which permits the preparation of simple models of natural glycopeptides, has been used by us up to now only for the preparation of glucose derivatives [1-4]. We now report results on the preparation of O-(amino-acyl) derivatives of mannose, galactose, talose, and ribose by this method. The preparation of these compounds was of interest in that it widened the possibilities of the proposed carbodiimide method for the synthesis of O-(amino-acyl) derivatives of carbohydrates and made it possible to establish the effect of the nature of the monosaccharide on this condensation. It is of extreme importance here that mannose, galactose, and ribose are components of natural mixed biopolymers, including glycopeptides. Finally, study of the chemical behavior of the compounds synthesized, in particular the stability of the ester link, will make it possible to get an idea of the effect of the nature of the carbohydrate residues on the properties of glycopeptides.

We have carried out the condensation of mannose, galactose, talose, and ribose with (benzyloxycarbonyl)glycine under the conditions used in the preparation of the corresponding glucose derivatives [1], i.e., in presence of dicyclohexylcarbodiimide in dry pyridine at 0°. However, because of the poor solubility of galactose the amount of pyridine was greatly increased. In all cases condensation went fairly smoothly and at approximately the same rate as in the case of glucose. As stated earlier [1, 2], the condensation of (benzyloxycarbonyl)glycine with glucose leads mainly to the formation of the 6-O-(amino-acyl) derivative; the acylation of secondary hydroxy groups goes only to a slight extent in spite of the fact that in the glucose molecule all the secondary hydroxy groups are in trans equatorial positions, which favor acylation. It might be expected that the amount of by-products formed by the acylation of secondary hydroxyls would be still less in other monosaccharides with a different disposition of hydroxyls. The correctness of this view was confirmed. For all the three hexoses taken the 6-O-[(benzyloxycarbonyl)glycyl] derivatives (Ia)-(IIIa) were the main reaction products.



a) R = —COCH$_2$C$_6$H$_5$
b) R = H

Chromatographic investigation of the reaction mixture showed that, apart from these by-products, very small amounts of other condensation products were formed, which were probably monoacyl derivatives, formed by reaction at one of the secondary hydroxyls, and diacyl derivatives. For galactose the amount of the by-product, which judging from its chromatographic behavior was a mono(amino-acyl) derivative formed by reaction with one of the secondary hydroxyls, corresponded to the amount of 3-O-[(benzyloxycarbonyl)glycyl]glucose formed in the analogous condensation [1, 2], but in the case of mannose only traces of such a by-product could be detected; the same applied to talose.

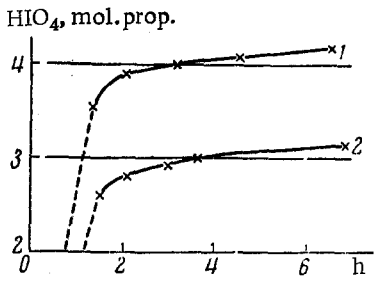
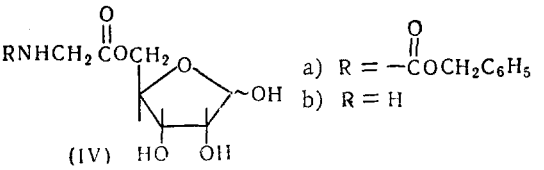


Fig. 1. Relation of the consumption of periodic acid to the duration of oxidation of 6-O-[(benzyloxycarbonyl)glycyl]-D-mannose (1) and 5-O-[(benzyloxycarbonyl)glycyl]-D-ribose (2).

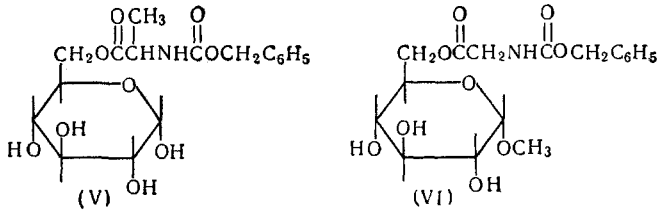
Special interest was presented by the amino-acylation of ribose, the first pentose to be used in this reaction, for there is no primary hydroxyl in the pyranose form of ribose. It was found that in this case ribose reacts in the furanose form with formation of the product of the acylation of the primary hydroxy group (IVa). Paper chromatography showed by-products of the acylation of ribose were almost completely absent.



This result lends particular emphasis to the predominant formation of amino-acyl derivatives at the primary hydroxy group under the action of carbodiimide, and it is of interest in this connection to study the analogous reaction of other pentoses. The formation of small amounts of by-products does not affect the preparative value of the method substantially, for the product of the main reaction is readily isolated in the pure state. The products of condensation with galactose (IIa) and talose (IIIa) were isolated in a crystalline form and obtained in about 25% yield in a chromatographically pure state. On dissolution of (IIa) in water its angle of rotation diminishes, which enables us to assign (IIa) to the α-galactose series. The product of condensation with mannose (Ia) did not crystallize, and the oily substance contained a very small amount of an isomeric impurity. The latter was readily separated by gradient partition chromatography on cellulose, after which (Ia) was obtained as an amorphous chromatographically homogeneous substance in 50% yield. The product of the condensation of ribose (IVa) was obtained directly from the reaction mixture in the chromatographically homogeneous amorphous state in about 50% yield.

The structure of the condensation products (Ia)-(IVa) was proved by periodate oxidation. As stated in [1], the oxidation of amino-acyl derivatives of glucose, particularly those containing glycine residues, in absence of a buffer leads to an abnormally high consumption of oxidant. In the case of (Ia)-(IVa) we were unable fully to avoid overoxidation by varying the oxidant (sodium metaperiodate, periodic acid), the pH of the acetate buffer, the concentration of the reagents, and the temperature. We therefore determined the change in the consumption of oxidant with time. The results are given in the table (see Experimental) and in Fig. 1, and they show that in the oxidation of (Ia)-(IIIa) about 4 molecular proportions of periodic acid are consumed rapidly and in the oxidation of (IVa) about 3 molecular proportions of periodic acid are consumed rapidly, after which the slow process of overoxidation occurs. This proves the structure of (Ia)-(IVa) unequivocally, for the isomers in which any one of the secondary hydroxyls is acylated should absorb not more than 3 molecular proportions of the oxidant in the case of hexoses and not more than 2 in the case of pentoses.

In our previous [1-3] syntheses of O-(amino-acyl) derivatives of glucose we used racemic amino acids. In a study of the stability of the ester link in O-(amino-acy) derivatives of glucose [5] it became necessary to check the results for derivatives of optically active acids, and we therefore carried out the condensation of glucose with (benzyloxycarbonyl)-L-alanine. The reaction, which was carried out under standard conditions [1], led in about 30% yield to crystalline 6-O-[N-(benzyloxycarbonyl)-L-alanine]-D-glucose (V), which on dissolution in water mutarotates with diminution in the angle of rotation, which permits us to assign (V) to the α-glucose series.



The identity of the specific rotation of the oily reaction product obtained initially with that of purified (V) shows that in the condensation of glucose with (benzyloxycarbonyl)-L-alanine racemization, if it occurs at all, occurs only to a very slight extent. This is fully in accord with known data on the smallness of the extent of racemization of L amino acids under the conditions of the carbodiimide synthesis of esters of peptides. For the purposes stated above

we also synthesized methyl 6-O-[N-(benzyloxycarbonyl)glycyl]-α-D-glucopyranoside (VI). The transition from the N-(benzyloxycarbonyl) derivatives (Ia)-(IVa) to substances with a free amino group was effected by hydrogenolysis over palladium on barium sulfate in 50% methanol in presence of oxalic acid. In this way we obtained high yields of the oxalates of 6-O-glycylmannose (Ib), 6-O-glycylgalactose (IIb), and 5-O-glycylribose (IVb). The hydrogenolysis of the benzyloxycarbonyl derivatives (Ia)-(IVa) in water or methanol without the addition of oxalic acid led, as in the case of glucose derivatives [1-3], to unstable, readily decomposable bases. Preliminary experiments show that the amino-acyl derivatives of galactose, mannose, talose, and ribose (Ib)-(IVb) have somewhat lower hydrolytic stabilities than the corresponding glucose derivatives. Data on the stability of these compounds at various pH values will be published later.

EXPERIMENTAL

All the substances obtained were checked for their chromatographic or electrophoretic homogeneity. Chromatography and electrophoresis were conducted on "M" chromatographic paper from the Leningrad factory No. 2. Ascending chromatograms were prepared. The mixtures of solvents used in the paper chromatography were: isobutyl alcohol saturated with water (System No. 1), 4:1:5 butyl alcohol-acetic acid-water, upper layer (System No. 2). Electrophoresis was conducted in a pH 4.3-4.5 buffer containing pyridine (2 ml), acetic acid (4 ml), and water (to 1 liter). The detection of spots on the chromatograms and electrophoregrams was carried out with silver nitrate, aniline phthalate, copper-potassium periodate complex, ninhydrin, and fluorescein.

6-O-[N-(Benzyloxycarbonyl)glycyl]-D-mannose (Ia). 3.6 g of mannose and 2.1 g of (benzyloxycarbonyl)-glycine were dissolved in 60 ml of dry pyridine, the solution was cooled to 0°, and 2.5 g of dicyclohexylcarbodiimide was added; the mixture was left for 48 h at 0°. Pyridine was vacuum-distilled off, the residue was treated with a 1 : 1 mixture of water and ether, crystals of 1,3-dicyclohexylurea were filtered off, the ether layer was separated, and the aqueous layer was extracted six times with ether. A drop of acetic acid was added to the aqueous solution, which was then extracted seven times with butyl alcohol. The extract was vacuum-evaporated, and we obtained 2.1 g of an oily substance, which according to paper chromatography in System No. 1 contained 6-O-[(benzyloxycarbonyl)glycyl]mannose, R_f 0.60 (large intense spot), an isomeric substance, R_f 0.70 (traces), and mannose (a small spot). This mixture was subjected to gradient partition chromatography on 600 g of cellulose. For this purpose a solution of the substance in 7 ml of isobutyl alcohol saturated with water was applied to a column of cellulose in the same solvent, and 500 ml of isobutyl alcohol was added. When 50-70 ml of solvent had left the column we added 150 ml of isobutyl alcohol saturated with water, and then with the aid of communicating vessel isobutyl alcohol saturated with water was added continuously as solvent flowed out. The first fractions, containing a substance with R_f 0.70, were rejected, and subsequent fractions, containing only a substance with R_f 0.60, were evaporated. We obtained 1.84 g [50%, based on the (benzyloxycarbonyl)glycine] of (Ia) as a colorless amorphous chromatographically homogeneous hygroscopic powder, $[\alpha]_D^{20}$ +11.6° (with 0.5; water). Found: C 51.85; 51.98; H 5.78; 5.84%. $C_{16}H_{21}O_9N$. Calculated: C 51.75; H 5.70%.

5-O-[N-(Benzyloxycarbonyl)glycyl]-D-ribose (IVa). 3 g of ribose and 2.1 g of (benzyloxycarbonyl)glycine were dissolved in 40 ml of dry pyridine, the solution was cooled to 0°, 2.5 g of dicyclohexylcarbodiimide was added, and the mixture was left for 48 h at 0°. Pyridine was vacuum-distilled off, and the residue was treated with a 1 : 1 mixture of water and ether. Crystals of 1,3-dicyclohexylurea were filtered off, the ether layer was separated, and the aqueous layer was extracted six times with ether. The ether extracts were washed five times with water, which after two extractions with ether was combined with the main aqueous solution. One drop of acetic acid was added to the aqueous solution, which was extracted six times with butyl alcohol. The extract was washed ten times with water, and the wash waters were extracted three times with butyl alcohol, which after five washes with water was combined with the main solution. The butyl alcohol solution was vacuum-evaporated, and we obtained 1.86 g [54.5%, based on the (benzyloxycarbonyl)glycine] of a colorless hygroscopic chromatographically homogeneous amorphous powder with $[\alpha]_D^{20}$ −14.7° (with 0.36; water); R_f 0.73 (System No. 1). Found: C 53.09; 53.11; H 5.91; 5.93%. $C_{15}H_{19}O_8N$. Calculated: C 52.79; H 5.61%.

6-O-[N-(Benzyloxycarbonyl)glycyl]-D-galactose (IIa). This was prepared by the procedure described for 5-O-[(benzyloxycarbonyl)glycyl]ribose from 3.6 g of finely ground galactose, 2.1 g of (benzyloxycarbonyl)glycine, and 2.5 g of dicyclohexylcarbodiimide in 450 ml of dry pyridine with the difference that in the course of the removal of butyl alcohol by distillation 6-O-[(benzyloxycarbonyl)glycyl]galactose crystallized out and was filtered off and washed with ether and ethyl acetate. After recrystallization from alcohol we obtained 1.04 g [28%, based on the (benzyloxycarbonyl)glycine] of colorless crystals, m. p. 171-172° (decomp.); $[\alpha]_D^{20}$ +52.5° (after 15 min), +42.7°

(constant) (c 0.15; water); R_f 0.45 (System No. 1). Found: C 51.81; 51.90; H 5.73; 5.71%. $C_{16}H_{21}O_9N$. Calculated: C 51.75; H 5.70%.

6-O-[N-(Benzyloxycarbonyl)glycyl]-D-talose (IIIa). This was prepared by the procedure described for 5-O-[(benzyloxycarbonyl)glycyl]ribose from 3.6 g of talose, 2.1 g of (benzyloxycarbonyl)glycine, and 2.5 g of dicyclohexylcarbodiimide with the difference that after the removal of butyl alcohol by distillation the residue was dissolved in a little alcohol, the solution was left in the cold, and the crystals which precipitated with recrystallized from a 1 : 1 mixture of alcohol and ethyl acetate. Yield 0.9 g [24%, based on the (benzyloxycarbonyl)glycine] of colorless crystals of m. p. 124.5-125°; $[\alpha]_D^{20}$ +11.0° (c 1.0; water); R_f 0.67 (System No. 1). Found: C 52.01; 52.06; H 5.80; 5.83%. $C_{16}H_{21}O_9N$. Calculated: C 51.75; H 5.70%.

6-O-[N-(Benzyloxycarbonyl)-L-alanyl]-D-glucose (V). 3.6 g of anhydrous glucose and 2.1 g (benzyloxycarbonyl)-L-alanine were dissolved in 80 ml of dry pyridine, the solution was cooled to 0°, 2.5 g of dicyclohexylcarbodiimide was added, and the mixture was left for 48 h at the same temperature. Pyridine was vacuum-distilled off, the residue was treated with a 1:1 mixture of water and ether, crystals of 1,3-dicyclohexylurea were filtered off, the ether layer was separated, and the aqueous layer was extracted five times with ether. One drop of acetic acid was added to the aqueous solution, which was extracted seven times with butyl alcohol. The extract was evaporated, and the oily residue was purified from glucose impurity by partition chromatography on a little cellulose (100 g) with a mobile phase of isobutyl alcohol saturated with water. We obtained 2.3 g of an oily substance, which in paper chromatography in System No. 1 gave two spots: (V) with R_f 0.62 (very intense spot) and probably 3-O-[(benzyloxycarbonyl)alanyl]glucose with R_f 0.72 (small spot).

The oily reaction product was dissolved in 15 ml of freshly distilled benzaldehyde, 0.5 g of freshly fused zinc chloride was added, and the mixture was shaken for 4 h at room temperature. The reaction mixture was treated 5-6 times with a large amount of petroleum ether, which was decanted each time from the oil formed. The residue was treated with a mixture of ether and 3% ammonium sulfate solution, the mixture was filtered, the ether layer was separated, and the aqueous layer was extracted again with ether four times and with butyl alcohol five times. The butyl alcohol solution was washed four times with water until a test for sulfate ions was negative, and was then evaporated. The oily residue crystallized on addition of ethyl acetate and was recrystallized from a 3:1 mixture of ethyl acetate and methanol. Yield 1.1 g [28.5%, based on the (benzyloxycarbonyl)alanine] of colorless crystals, m. p. 112-113°; $[\alpha]_D^{20}$ +21.1° (after 5 min) → +2.0° (constant) (c 0.5; water). Found: C 52.91; 52.69; H 5.93; 5.79%. $C_{17}H_{23}O_9N$. Calculated: C 52.98; H 6.02%.

Methyl 6-O-[N-(Benzyloxycarbonyl)glycyl]-α-D-glucopyranoside (VI). 3.9 g of methyl α-glycopyranoside and 2.1 g of (benzyloxycarbonyl)glycine were dissolved in 50 ml of dry pyridine, the solution was cooled to 0°, 2.5 g of dicyclohexylcarbodiimide was added, and the mixture was left for 48° at 0°. Pyridine was vacuum-distilled off, the residue was treated with a 1:1 mixture of water and ether, crystals of 1,3-dicyclohexylurea were filtered off, the ether layer was separated, and the aqueous layer was extracted five times with ether. One drop of acetic acid was added to the aqueous solution, which was extracted seven times with isopentyl alcohol. The extracts were washed twice with a little water and vacuum-evaporated. The residue was subjected to partition chromatography on cellulose (400 g) with a mobile phase of isobutyl alcohol saturated with water. Fractions containing a substance with R 0.68 (System No. 1) were evaporated. The residue crystallized on addition of ethyl acetate and was recrystallized from alcohol. Yield 1 g [26%, based on the (benzyloxycarbonyl)glycine] of colorless crystals, m. p. 136.5-137.5°; $[\alpha]_D^{20}$ +89.5° (c 1.0; methanol). Found: C 53.11; 53.17; H 6.10; 6.08%. $C_{17}H_{23}O_9N$. Calculated: C 52.98; H 6.02%.

6-O-Glycyl-D-mannose (Oxalate). 185 mg of 6-O-[(benzyloxycarbonyl)glycyl]mannose was dissolved in 6 ml of 50% methanol, 38 mg of oxalic acid dihydrate and 100 mg of 5% palladium on barium sulfate were added, and hydrogenation was conducted for 1 h. Catalyst was separated by centrifugation, washed with 50% methanol, and again centrifuged. The solution obtained was filtered and diluted with a large amount of acetone. The precipitate that formed was centrifuged off, dissolved in the least possible amount of water, and precipitated with acetone; this reprecipitation treatment was repeated. The precipitate was ground in dry acetone and centrifuged; acetone was decanted, and residual acetone was removed in a vacuum. We obtained 97 mg (69%) of an electrophoretically and chromatographically (System No. 2) homogeneous colorless amorphous hygroscopic powder, $[\alpha]_D^{20}$ +11.3° (c 1.5; water). Found: C 38.44; 38.11; H 5.51; 5.56%. $(C_8H_{15}O_7N)_2 \cdot (COOH)_2$. Calculated: C 38.30; H 5.71%.

6-O-Glycyl-D-galactose (Oxalate). This was prepared like 6-O-glycylmannose, but as the 6-O-[(benzyloxycarbonyl)glycyl]galactose does not dissolve in 6 ml of 50% methanol a suspension of the substance was hydrogenated for 90 min. Yield 74% of a colorless amorphous hygroscopic powder with $[\alpha]_D^{20}$ +48.7° (c 1.5; water). Found: C 38.13; 38.08; H 6.04; 6.10%. $(C_8H_{15}O_7N)_2 \cdot (COOH)_2$. Calculated: C 38.30; H 5.71%.

Substance	HIO_4, mol. prop.		
	1.5 h	3 h	6.5 h
6-O-[(Benzyloxycarbonyl)glycyl]-D-mannose	3.59	3.96	4.18
6-O-[(Benzyloxycarbonyl)glycyl]-D-galactose	3.67	4.02	4.20
6-O-[(Benzyloxycarbonyl)glycyl]-D-talose	3.48	4.01	4.19
5-O-[(Benzyloxycarbonyl)glycyl]-D-ribose	2.6	2.93	3.15

5-O-Glycyl-D-ribose (Oxalate). This was prepared like 6-O-glycylmannose from 170 mg of 5-O-[(benzyloxycarbonyl)glycyl]ribose and 38 mg of oxalic acid dihydrate. Yield 84 mg (67%) of a colorless amorphous hygroscopic powder with $[\alpha]_D^{20}$ −16.5 (c 1.0; water). Found: C 38.24; 38.44; H 5.90; 5.74%. $(C_7H_{13}O_6N)_2 \cdot (COOH)_2$. Calculated: C 38.10; H 5.60%.

Oxidation with Periodic Acid. 20-22 mg of 6-O-[(benzyloxycarbonyl)glycyl]mannose, 6-O-[(benzyloxycarbonyl)glycyl]galactose, 6-O-[(benzyloxycarbonyl)glycyl]talose, or 5-O-[(benzyloxycarbonyl)glycyl]ribose was dissolved in 35 ml of acetate buffer of pH 5.3 at 15°, 10 ml of periodic solution (5.4 g/liter) was added at the same temperature, and water was added to make 50 ml. The solution was left at 15° in the dark, and samples of 5-10 ml were taken periodically; these were neutralized with alkali, 5-10 ml of 4% borax solution was added and then 0.5 g of KI, and the mixture was titrated with sodium arsenite (0.0282 N). The results of the oxidation are given in the table and in Fig. 1.

SUMMARY

1. By the previously developed carbodiimide method 6-O-[(benzyloxycarbonyl)glycyl] derivatives of hexoses (mannose, galactose, talose, glucose) and the 5-O-[(benzyloxycarbonyl)glycyl] derivative of a pentose (ribose) were synthesized.

2. By the hydrogenolysis of O-[(benzyloxycarbonylamino)-acyl] derivatives of monosaccharides in presence of oxalic acid, oxalates of O-(amino-acyl) monosaccharides were prepared.

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