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# The Synthesis of *O*-Serine Glycosides*

Kichang Kum† and Saul Roseman‡

ABSTRACT: A general, three-step procedure was developed for the chemical synthesis of *O*-glycosylserine glycosides, starting with the acetobromoglycoses and *N*-carbobenzoxy-L-serine benzyl ester. The acetobromo derivatives of D-glucose, D-galactose, and D-xylose were used.

The synthesis involved Koenigs–Knorr condensation of the bromo sugar with the serine derivative, hydrogenolysis to remove the benzyl and carbobenzoxy groups, and ammonolysis to remove the acetyl groups. The acetyl derivatives and the final products were obtained as crystalline solids, and the over-all yields ranged between 24 and 40%. The optical rotations of the 3-*O*-(D-glycopyranosyl)-L-serine derivatives corresponded to those expected for the $\beta$ anomers.

Glycoproteins contain carbohydrate side chains linked to the polypeptide by glycosidic bonds. In some cases, as in ovalbumin and the blood glycoproteins, this bond involves the amide nitrogen atom of asparagine (Marshall and Neuberger, 1964) and the bond is stable to alkali. Another class of glycoproteins contains sugars linked to protein through alkali-sensitive bonds; this group includes the submaxillary mucins (Tanaka *et al.*, 1964), blood group substances (Schiffman *et al.*, 1964), and at least some mucopolysaccharide–protein complexes (Anderson *et al.*, 1965; Lindahl *et al.*, 1965). The sensitivity to alkali results from the fact that the sugar chains are *O*-glycosides of serine (and sometimes threonine), and are therefore susceptible to $\beta$ elimination in the presence of alkali. In the case of the mucopolysaccharides, such as the chondromucoprotein from cartilage, and a heparin–protein complex, the polysaccharide chains contain equimolar quantities of sulfated hexoamine and uronic acid, and are linked to the protein through xylose, and perhaps galactose. Thus, in studies concerned with the chemistry of these macromolecules, *O*-xylosylserine, and *O*-galactosylserine are of prime interest. In addition to its presence in the complex polymers, xylosylserine has been isolated from normal human urine; 1 mg/l. was obtained (Tominaga *et al.*, 1965), and the proposed structure was 3-*O*-($\beta$-D-xylosyl)serine.[1]

Our interest in the serine glycosides was stimulated by enzymatic studies on the biosynthesis of glycoproteins, including the mucins (Carlson *et al.*, 1964), and sphingoglycolipids such as the gangliosides and cerebrosides (Basu *et al.*, 1965). In the glycolipids, the

* From the Rackham Arthritis Research Unit and the Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan. *Received May 26, 1966.* The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of The University of Michigan. This work was supported by a grant from the National Institutes of Arthritis and Metabolic Diseases, The National Institutes of Health (A-512).

† Present address: The Robert W. Lovett Memorial Group for the Study of Diseases, Laboratory for Carbohydrate Research, Massachusetts General Hospital, Boston, Mass. 02114.

‡ Present address and to whom inquiries should be directed: McCollum–Pratt Institute and Department of Biology, The Johns Hopkins University, Baltimore, Md.

[1] Unless otherwise indicated, sugars are of the D configuration, glycosides are pyranosides, and serine and its derivatives are of the L configuration. The term carbobenzoxy signifies the benzyloxycarbonyl group. Acetobromoglycose signifies a fully *O*-acetylated 1-bromoglycopyranose, *e.g.*, 2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-glucopyranosyl bromide is described by its conventional name, acetobromoglucose. The *O*-glycosides of serine described in the literature are not named in a consistent manner. Some examples of these are as follows: 1-*O*-$\beta$-L-seryl-*N*-acetyl-D-glucosaminide (Jones *et al.*, 1961), *O*-$\beta$-D-(2,3,4,6-tetra-*O*-acetyl)-glucopyranoside of the methyl "ether" of *N*-carbobenzoxy-DL-serine (Derevitskaya *et al.*, 1964), *O*-$\beta$-D-xylopyranosyl-L-serine (Lindberg and Silvander, 1965), and 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-$\beta$-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-L-serine methyl ester (Vercellotti and Luetzow, 1966). While an abbreviated nomenclature is employed in some cases in the present paper, such as "serine glycosides," these terms are meant to describe only 3-*O*-($\beta$-D-glycopyranosyl)-L-serine and its derivatives. For future reference, we suggest that *O*-glycosides of serine be named in a manner similar to that employed for oligosaccharides, *viz.*, $\beta$-D-glycopyranosyl-(1→3)-L-serine.

hydroxyl group at C-1 of *N*-acylsphingosine is linked to either D-glucose or D-galactose. Earlier *in vivo* isotope experiments had shown that C-1, C-2, and the amino group of sphingosine are derived from C-3, and C-2, and the amino group of serine, respectively (Sprinson and Coulon, 1954). *In vitro* studies suggested that L-serine was condensed with palmitaldehyde, with the concomitant loss of the carboxyl group, to yield sphingosine (Brady and Koval, 1958). However, it appeared possible that an alternate pathway existed, namely the condensation of *O*-(glycosyl)-L-serine with palmitaldehyde to give psychosine.[2] An examination of this hypothesis required substrate quantities of the glycosylserine derivatives.

Apparently, the first reported synthesis of an *O*-(glycosyl)serine was that of Jones *et al.* (1961), who coupled 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride with *N*-carbobenzoxy-L-serine methyl ester by the Koenigs–Knorr procedure. The protecting groups were removed in the following sequence: deacetylation with methanol containing barium methoxide, hydrogenolysis of the carbobenzoxy group, and saponification of the methyl ester. The four steps gave an over-all yield approximating 2%.

More recently, Vercellotti and Luetzow (1966), employing methods similar to those used by Jones *et al.* (1961), synthesized 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-2,4-dinitrophenyl-L-serine methyl ester. These authors noted that the derivative was markedly sensitive to alkali, and that β elimination proceeded more rapidly than did hydrolysis of the *O*-acetyl groups.

During the course of the synthetic studies reported below, preliminary communications appeared on the synthesis of glucopyranosylserine methyl ester (Derevitskaya *et al.*, 1964), and of xylopyranosylserine (Lindberg and Silvander, 1965). The glucose derivatives were prepared by condensing 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with *N*-carbobenzoxyserine methyl ester (DL and L), followed by deacetylation, and hydrogenolysis; the over-all yields of the glucoside methyl esters approximated 31%. The xylose derivative was prepared similarly, except that the serine derivative used in the condensation reaction was *N*-tosyl-L-serine methyl ester, and after alkaline hydrolysis of the product, the tosyl group was removed by reductive cleavage; here, the yield of 3-*O*-(β-D-xylopyranosyl)-L-serine was 1.2%, and the authors suggest that the alkaline conditions used to remove the protecting groups resulted in large losses, presumably through the β-elimination reaction.

The present studies involved the synthesis of 3-*O*-(β-D-glycopyranosyl)-L-serine, where the glycose units were xylose, glucose, and galactose. The procedure consisted of the following steps. (a) *N*-Carbobenzoxy-L-serine benzyl ester was condensed with the acetobromoglycose in the presence of silver carbonate, catalytic quantities of silver perchlorate (Wolfrom *et al.*, 1961), and a drying agent. After purification by column chromatography, the products were obtained in 40–59% yields. (b) Hydrogenolysis of the syrups removed both the benzyl- and carbobenzoxy-protecting groups, and the resulting serine derivatives were isolated as crystalline solids in 85–90% yields. (c) After deacetylation with methanolic ammonia, the desired 3-*O*-(D-glycopyranosyl)-L-serine derivatives were obtained as crystalline solids in 70–75% yields. The over-all yields therefore ranged between 24 and 40%.

Under the conditions used for the condensation reaction, essentially the Koenigs–Knorr reaction, the glycosylserine derivatives were expected to be the β anomers (Pigman, 1957). As shown in Table I, a comparison of the optical rotations of the synthetic compounds with those of the corresponding methyl α- and β-glycosides indicates that the crystalline products were, in fact, the β-glycosides.

## Materials and Methods[2]

All melting points are corrected. Optical rotations were measured with a Rudolph Model 80 polarimeter. Benzyl group determinations were performed by measuring the absorbancies of the compounds at 257 mμ, using 4% chloroform in ethanol as the solvent, and *N*-carbobenzoxy-L-serine benzyl ester as the reference compound. The anthrone method of Carroll (1960) was used for determining sugar concentrations. A commercial grade of activated silicic acid, 200–325 mesh, called Unisil (Clarkson Chemical Co.) was employed, without pretreatment, for column chromatography.

Thin layer chromatography was conducted with 4% methanol in benzene (v/v) on silica gel G (E. Merck AG., Darmstadt, Germany). Esters were detected on the chromatograms with the spray reagents of Tate and Bishop (1962); the esters gave orange, or grey-violet spots on a pale yellow background. Microanalyses were performed by the Spang Microanalytical Laboratories, Ann Arbor, Mich.

The solvents used for the Koenigs–Knorr condensation reactions were carefully purified by standard procedures and dried before use. Silver carbonate and silver perchlorate were prepared as described (Wolfrom *et al.*, 1961).

The following compounds were prepared by the described procedures, or by minor modifications of these procedures. (a) *N*-Carbobenzoxy-L-serine, by the method of Moore *et al.* (1954), was obtained in 78% yield and showed the expected elemental analyses mp 118–119°, $[\alpha]_D^{22}$ +5.83° (*c* 6, acetic acid). These values conform to those reported in the literature (Moore *et al.*, 1954; Baer and Maurukas, 1955; Fruton, 1942). (b) *N*-Carbobenzoxy-L-serine benzyl ester, by the method of Ben-Ishai and Berger (1952), was obtained in 75% yield and showed the expected elemental analyses mp 84–85°, $[\alpha]_D^{22}$ +6.1° (*c* 4, chloroform). These values agreed with those reported by Baer and Maurukas (1955). (c) The bromo sugars used in the

[2] This concept is discussed in a review concerned with the biosynthesis of gangliosides (Kaufman *et al.*, 1966).

TABLE I: Comparison of Molar Rotations of Known Glycosides and Synthetic Serine Glycopyranosides.[a]

| Compound | $[\alpha]_D^{24}$ (deg) | Mol Wt | [M]D (deg) |
|---|---|---|---|
| 3-*O*-(D-Glucosyl)-L-serine | −23.0 | 267.2 | −6,146 |
| Methyl β-D-glucopyranoside | | | −6,640 |
| Methyl α-D-glucopyranoside | | | +30,860 |
| 3-*O*-(D-Galactosyl)-L-serine | −2.1 | 267.2 | −561 |
| Methyl β-D-galactopyranoside | | | 0 |
| Methyl α-D-galactopyranoside | | | +38,050 |
| 3-*O*-(D-Xylosyl)-L-serine | −47.4 | 237.2 | −11,243 |
| Methyl β-D-xylopyranoside | | | −10,750 |
| Methyl α-D-xylopyranoside | | | +25,260 |

[a] The molar rotations of the glycosylserines were not corrected for the contribution by the L-serine ([M]D = −717° in water); the effect of pH on the rotations was not studied. The methyl glycoside rotations are literature values (Bates *et al.*, 1942).

condensation procedures were prepared by a standard method (Barczai-Martos and Korosy, 1950): acetobromoglucose, mp 88–89°; acetobromogalactose, mp 84–86°; acetobromoxylose, mp 102°. (d) 2,3,4,6-Tetra-*O*-acetyl-β-D-glucose (tetracetylglucose), by the method of McCloskey (1944), was obtained in 78% yield, gave the expected elemental analyses, and showed a mp 137–140°. The literature values reported most recently for this compound range between 132 and 138° (McCloskey, 1944; Georg, 1932; Allen, 1962). (e) Octa-*O*-acetyl-β-D-glucopyranosyl-β-D-glucopyranoside (trehalose octaacetate), by the method of McCloskey (1944), was obtained in 30% yield and gave the expected elemental analyses mp 181–183°, $[\alpha]_D^{25}$ −17.3° (*c* 3, chloroform). These values agreed with those reported in the literature (Allen, 1962; McCloskey, 1944).

## Experimental Section

*Koenigs–Knorr Condensation.* The condensation reaction between the bromo sugars and *N*-carbobenzoxy-L-serine benzyl ester was studied in a number of solvents and with several catalytic reagents. The optimum yields were obtained with a mixture of silver carbonate and silver perchlorate, the latter being used to increase the rate of the reaction (Wolfrom *et al.*, 1961). The rates of the reactions and the yields also varied with the solvent used, and the most efficient solvent is indicated for each reaction.

The general procedure for the condensation was as follows. *N*-Carbobenzoxy-L-serine benzyl ester (0.02 mole) was dissolved in the solvent, and stirred for 10 min with 0.033 mole of anhydrous silver carbonate and 0.15 mole of Drierite (anhydrous calcium sulfate). The bromo sugar (0.02 mole) and 0.2 g of silver perchlorate were then added, and the mixture was stirred in the dark, with the exclusion of moisture, for 48 hr. The solvents used were as follows: 120 ml of nitromethane with acetobromoglucose, 200 ml of ether with acetobromogalactose, and 100 ml of benzene with acetobromoxylose. Aliquots of the reaction mixtures were filtered and examined by thin layer chromatography, as described above, to follow the course of the reaction. Finally, the reaction mixtures were filtered, the filtrates were diluted with benzene, washed with approximately 100 ml of cold saturated $NaHCO_3$ solution, at least three times with water, dried over anhydrous sodium sulfate, and concentrated to syrups under vacuum. Examination of the syrup obtained from the acetobromoglucose reaction mixture by thin layer chromatography showed the following components ($R_F$): acetobromoglucose, 0.81 (acetobromogalactose, 0.79; acetobromoxylose, 0.84); trehalose octaacetate, 0.34; *N*-carbobenzoxy-L-serine benzyl ester, 0.33; tetraacetylglucose, 0.18. By-products with $R_F$ values similar to those of trehalose octaacetate and tetraacetylglucose were observed in the galactose and xylose reaction mixtures. The desired condensation products, characterized by the presence of both aromatic and ester groups, migrated between the bromo sugars and the unreacted *N*-carbobenzoxy-L-serine benzyl ester. They showed the following $R_F$ values: product from glucose, 0.70; from galactose, 0.68; and from xylose, 0.72.

Isolation of the desired products was achieved by chromatography on silicic acid columns as follows. Columns (3.5 × 85 cm) were prepared containing 120 g of Unisil, which was slurried in benzene containing methanol (*c* 4, v/v), and packed into the columns by gravity filtration. After the columns were washed with the solvent, the syrups were dissolved in the same solvent, applied to the columns, and eluted with the benzene–methanol solvent system. Fractions, 600 ml each, were collected and examined by thin layer chromatography. The following substances were detected in the indicated fractions: *N*-carbobenzoxy-L-serine benzyl ester, fractions 5 and 6; the desired glycosylated

TABLE II: Properties and Analyses of Acetylated Serine Glycopyranosides.

| 3-*O*-Serine Glycoside | Mp (°C) | $[\alpha]_D^{24}$ (deg) | Solvent (concn 2%) | Calcd[a] | | | Found | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | C | H | N |
| 2,3,4,6-Tetra-*O*-acetyl-glucose | 155–157 | −23.8 | Methanol | 46.90 | 5.79 | 3.22 | 46.81 | 5.84 | 3.23 |
| 2,3,4,6-Tetra-*O*-acetyl-galactose | 151–153 | −11.2 | Water | 46.90 | 5.79 | 3.22 | 46.87 | 5.92 | 3.21 |
| 2,3,4-Tri-*O*-acetylxylose | 189–191 | −68.0 | Water | 46.28 | 5.83 | 3.86 | 46.32 | 5.71 | 3.96 |

[a] The calculated values are based on $C_{17}H_{25}NO_{12}$ for the hexosides and $C_{14}H_{21}NO_{10}$ for the xyloside.

TABLE III: Properties and Analyses of Serine Glycopyranosides.

| 3-*O*-Serine Glycoside | Mp (°C) | $[\alpha]_D^{24}$ (deg) | Solvent (concn %) | Calcd[a] | | | Found | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N | C | H | N |
| D-Glucose | 223–225 dec | −23.0 | Water (2) | 40.45 | 6.41 | 5.24 | 40.20 | 6.31 | 5.21 |
| D-Galactose | 138–140 | −2.1 | Water (3) | 40.45 | 6.41 | 5.24 | 40.39 | 6.27 | 5.17 |
| D-Xylose | 224–227 dec | −47.4 | Water (2.2) | 40.50 | 6.37 | 5.91 | 40.65 | 6.29 | 5.94 |

[a] The calculated values are based on $C_9H_{15}NO_8$ for the hexosides and $C_8H_{15}NO_7$ for the xyloside.

serine derivatives, fractions 3–5; the bromo sugars, fractions 2 and 3; the by-products, in fractions 5–7. To obtain chromatographically pure material, the fractions containing the desired products were combined, concentrated under vacuum, and again subjected to column chromatography. This procedure was repeated until the syrups appeared homogeneous on thin layer chromatography. Column chromatography was repeated three times with the glucose derivative, four times with the galactose derivative, and four times with the xylose derivative.

Although the final products appeared to be homogeneous, they could not be crystallized. Analysis by the anthrone method using the corresponding sugars as standards, and for aromatic groups using *N*-carbobenzoxy-L-serine benzyl ester as the standard, showed that each of the products contained 1 mole of glycose/two benzyl residues (*i.e.*, one serine residue).

*3-O-(Tetraacetylglucosyl)-L-serine, 3-O-(Tetraacetylgalactosyl)-L-serine, and 3-O-(Triacetylxylosyl)-L-serine.* The glycosylated *N*-carbobenzoxy-L-serine benzyl esters described above (1.5–3.0 g) were dissolved in a mixture containing 80 ml of purified dioxane and 20 ml of water, and reduced with hydrogen at atmospheric pressure in the presence of a 10% Pd-on-charcoal catalyst (0.2 g). The absorption of $H_2$ essentially ceased after about 3–4 hr. Following filtration to remove the catalyst, the solutions were concentrated, giving syrups that crystallized spontaneously. The crude crystalline products were obtained in 90–95% yields. Recrystallization in the following solvent systems gave the purified materials in the indicated yields: tetraacetylgalactosyl-L-serine from methanol, 90%; triacetylxylosyl-L-serine from water-ethanol, 88%; tetraacetylglucosyl-L-serine from methanol–ethanol–petroleum ether (bp 30–60°), 85%. The analyses and physical constants of the products are shown in Table II.

*3-O-(β-D-Glycopyranosyl)-L-serine.* The acetylated glycosylserine derivatives were deacetylated with anhydrous ammoniacal methanol. From 0.3 to 0.8 g of the acetyl derivative was suspended in 50 ml of anhydrous methanol, cooled to 0°, and mixed with 50 ml of methanol saturated with ammonia at 0°. The mixture was slowly brought to room temperature, while moisture was excluded, and was maintained at room temperature for 5 hr. The colorless solutions were concentrated in a vacuum, at 30–35°, whereupon the residues were spontaneously crystallized. Recrystallization of the glucose and xylose derivatives were effected from a minimum of water and ethanol, while a water–methanol mixture was used for the galactose derivative. The final products were obtained in 70–75% yields, and their analytical

values and physical properties are given in Table III.

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