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SYNTHESIS OF THE *N*-CARBOXY-α-AMINO ACID ANHYDRIDES OF SEVERAL *O*-ACETYLATED SERINE GLYCOSIDES

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ABSTRACT

The *O*-acetylated *O*-(β-glycopyranosyl)-L-serine derivatives of D-galactose, lactose, cellobiose, 2-acetamido-2-deoxy- and 2-deoxy-2-dodecamido-D-glucose, and *O*-acetylated *O*-(α-L-rhamnopyranosyl)-L-serine were synthesized by Koenigs–Knorr condensation of the corresponding acetobromo sugars with *N*-benzyloxycarbonyl-L-serine benzyl ester, followed by hydrogenolysis of the benzyloxycarbonyl and benzyl ester groups Treatment of the *O*-acetylated serine glycosides with phosgene gave the corresponding *N*-carboxy-α-amino acid anhydrides or Leuchs' anhydrides which are useful reagents to link sugars to linear and branched synthetic polypeptide antigens The free serine glycosides were obtained by treatment of the *O*-acetylated derivatives with methanolic ammonia

INTRODUCTION

Synthetic polypeptides or poly-α-amino acids have been used extensively during the past few years as model antigens in the study of the various physical and chemical parameters that are necessary to endow a macromolecule with the capacity to elicit antibodies in experimental animals[1] Since sugars are of great importance as determinants in many natural antigens we began to investigate their influence on the immunological properties of such synthetic antigens Of particular interest was the question whether sugar molecules when attached to a non-antigenic amino acid polymer would be able to convert it into an immunogen

For this study, a method was developed to link D-glucose to a branched synthetic polypeptide *via* the β-D-glucoside of serine[2,3] *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine was synthesized from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and *N*-benzyloxycarbonyl-L-serine benzyl ester by the Koenigs–Knorr procedure, followed by hydrogenolysis of the benzyloxycarbonyl and benzyl ester groups Treatment of the serine derivative with phosgene gave the *N*-carboxy anhydride (or Leuchs' anhydride) of *O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-L-serine. The polymerization reaction of this anhydride in aqueous medium, using as initiators the free α-amino terminals of the side chains of a branched synthetic polypeptide, led to elongation of the side chains with short peptides of *O*-acetylated

glucopyranosylserine units After removal of the *O*-acetyl groups, the resulting synthetic glycoprotein analog was used for immunological studies

It soon appeared to be of interest to extend these experiments to other monosaccharides and, if possible, to disaccharides In addition we wanted to determine if introduction of an internal adjuvant in the form of long-chain fatty acids attached to the amino group of D-glucosamine might increase the immunogenicity of sugar–polypeptide conjugates. Similar structures are present in the so-called lipid A fraction of bacterial endotoxins, and are possibly related to their adjuvant activity[4]

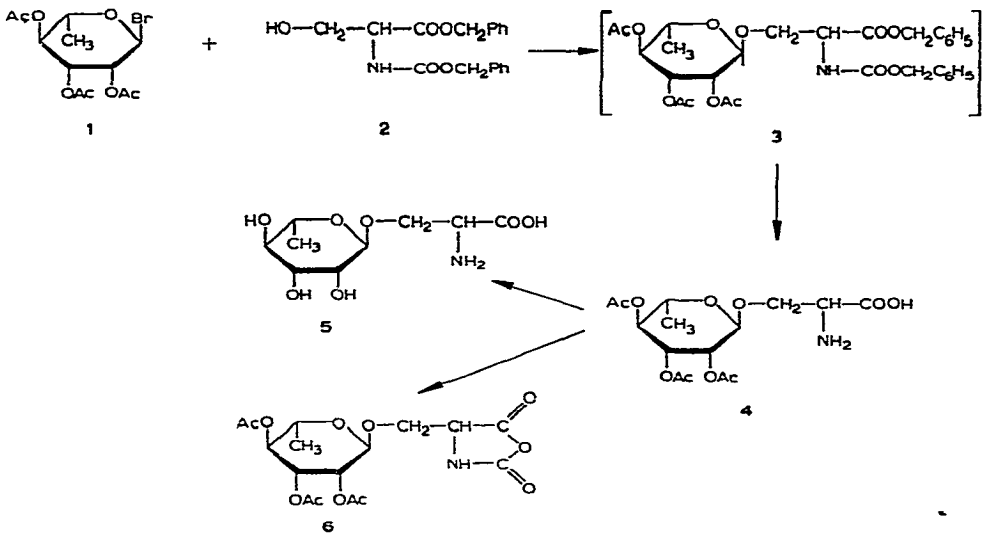
Few reports had appeared on the synthesis of serine glycosides[5,6] before interest in this type of compound was stimulated by the finding that glycosides of serine or threonine form the covalent linkage between peptide chains and carbohydrate moieties of several glycoproteins[7] In a preliminary communication Lindberg and Silvander[8] described the synthesis of β-D-xylopyranosylserine The relatively low yield of the product obtained was probably due to the difficulty of removal of the *N*-tosyl and methyl ester protecting groups of serine During the course of the present work, Brendel and Davidson[9] published the synthesis of α- and β-D-xylopyranosylserine, while Kum and Roseman[10] synthesized β-D-xylopyranosyl-, β-D-glucopyranosyl-, and β-D-galactopyranosyl-serine by essentially the same procedure as outlined above for the synthesis of the glucose derivative *O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(2,4-dinitrophenyl)-L-serine methyl ester was prepared by Vercellotti and Luetzow[11] as a model compound to study the alkali lability of serine glycosides More recently Derevitskaya *et al*[12] also described the synthesis of β-D-glucopyranosyl-and β-D-galactopyranosyl-serine by the Koenigs–Knorr procedure using *N*-benzyloxycarbonyl-L-serine methyl ester as starting material The same authors prepared β-D-galactofuranosylserine by a new method using *O*-acetylated 1,2-*O*-alkyl orthoesters of sugars as glycosylating agents

The present report deals with the synthesis of several *O*-acetylated and unesterified glycosides of serine by a procedure analogous to the one used previously for the preparation of the corresponding glucose derivative[3] The *O*-acetylated serine glycosides were treated with phosgene in order to prepare the corresponding *N*-carboxy anhydrides, which are useful reagents for the binding of sugars to synthetic polypeptides

RESULTS AND DISCUSSION

Serine glycosides of mono- and di-saccharides — The serine glycosides of L-rhamnose, D-galactose, lactose, and cellobiose were synthesized as exemplified for the rhamnose derivative in Scheme 1. The respective acetylglycosyl bromide derivative **1** was condensed with *N*-benzyloxycarbonyl-L-serine benzyl ester (**2**) in the presence of mercuric cyanide in nitromethane as solvent[13] Only the *O*-(hepta-*O*-acetyl-β-cellobiopyranosyl)-*N*-benzyloxycarbonyl-L-serine benzyl ester (analog of **3**) crystallized directly from the crude reaction product and was obtained in pure form Generally the reaction mixtures were complex, as shown by thin-layer chromatography, and the isolation of the fully substituted glycosides (**3** and analogs) would have been possible

only by chromatography on silica gel This operation, which is very inconvenient for large-scale preparations, was avoided by the following procedure: The crude reaction product was hydrogenated to remove the benzyloxycarbonyl and benzyl ester protecting groups The *O*-acetylated serine glycosides (**4** and analogs) were precipitated with ethyl acetate or ethyl acetate–ether, and most of the side products resulting from the decomposition or hydrogenation of unreacted acetylglycosyl bromides remained



Scheme 1

in the supernatant. The main impurities still present were serine and small amounts of unidentified ninhydrin-positive substances which on paper chromatograms migrated between serine and the *O*-acetylated serine glycosides (**4** and analogs) In the case of lactose, all these impurities could be removed by crystallization from water, but the L-rhamnose and D-galactose derivatives **4** were too soluble for application of the same procedure The latter derivatives were conveniently purified in relatively large amounts by partition chromatography on a column of moist Celite as stationary phase and water-saturated butyl alcohol as mobile phase[14]. The *O*-acetylated serine glycosides (**4** and analogs) were eluted first. They crystallized readily and were chromatographically pure A very low and broad second peak, which also contained a serine glycoside, appeared sometimes It was clearly separated from the first peak. This compound, possibly the anomer glycoside, had a slightly lower paper-chromatographic mobility than that of the main product. It could not be isolated in crystalline form or in sufficient amount for further characterization.

The serine glycosides (**5** and analogs) were prepared from the *O*-acetylated derivatives (**4** and analogs, **12** and **13**) by treatment with methanolic ammonia at 4°.

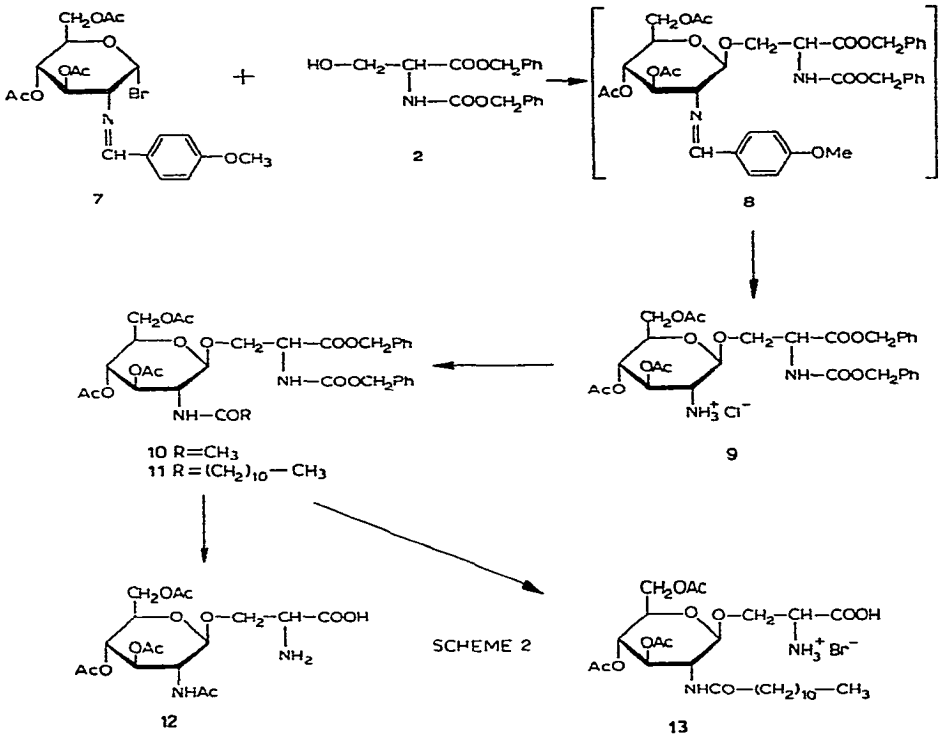
Several reports in the literature and our own experience in the synthesis of serine glucoside indicated that both mercuric cyanide and silver carbonate, when used as acid acceptors in the Koenigs–Knorr reaction, favor the formation of the same

anomeric glycoside[13,15]. This is the β-D-glycoside for most of the common sugars such as D-glucose, D-galactose, D-xylose, and lactose There are some cases, however, in which α-D-(1→2)- and α-D-(1→3)-linked disaccharides have been isolated from reactions with the 2,3,4,6-tetra-*O*-acetyl-α-D-glycosyl bromide derivatives of D-galactose, D-xylose, and L-fucose in the presence of mercuric cyanide[16–18]. On the other hand, 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide usually give α-D- and α-L-glycosides, respectively, under conditions similar to those of our condensation procedure[19] In these cases the configuration at the anomeric center is retained, probably owing to participation of the C-2 acetoxy group of D-mannose and L-rhamnose in the glycosidation reaction As shown in Table I, a comparison of the optical rotation of the synthetic serine glycosides with that of the corresponding α-D- and β-D-methyl glycosides indicates that the β-glycosides of D-galactose, lactose, cellobiose, and the α-glycoside of L-rhamnose have been obtained as the major products

Serine glycosides of 2-acylamido-2-deoxy-D-glucose — For the reasons just mentioned, we planned to link, in addition to L-rhamnose, D-galactose, lactose, and cellobiose, various 2-acylamido-2-deoxy-D-glucoses (acyl = acetyl, lauroyl, and eventually higher fatty acids) to synthetic polypeptides It was obvious to use also for this purpose the "serine glycoside" method

Several glycosyl halide derivatives of 2-amino-2-deoxy-D-glucose are known as glycosylating agents[20] They differ mainly in the manner in which the amino group is protected For our purpose, an amino-blocking group, which could be easily removed after glycoside synthesis and replaced by an acetyl or lauroyl group appeared to be advantageous We selected 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)amino-α-D-glucopyranosyl bromide (**7**) because the *p*-methoxybenzylidene group could be removed under mild acidic conditions[21], whereas blocking groups such as dinitrophenyl[22] or dichloroacetyl[20] require alkaline hydrolysis which might cause β-elimination of the serine glycoside Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)imino-α-D-glucopyranosyl bromide (**7**) with *N*-benzyloxycarbonyl-L-serine benzyl ester (**2**) was performed in the presence of silver carbonate (Scheme 2) In contrast to the experiments described in the preceding part, mercuric cyanide gave completely negative results A crystalline side product was separated from the reaction mixture in considerable quantity. The nature of this compound, probably a nonreducing disaccharide, is currently under investigation Apparently the same side product has been isolated by Hardy *et al*[23], but it was not studied in detail The crude reaction product containing the fully protected serine 2-amino-2-deoxy-D-glucopyranoside (**8**) was then treated with dilute acid and *O*-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-*N*-benzyloxycarbonyl-L-serine benzyl ester hydrochloride (**9**) was easily obtained in pure form The low yield of 15% was probably due in part to the use of impure starting material (**7**) (because of its instability it was not recrystallized before use), and to the formation of the side product just mentioned After acylation of the free amino group of **9** with acetic anhydride or dodecanoyl (lauroyl chloride), the benzyloxycarbonyl and benzyl ester groups of **10**

and **11** were removed by hydrogenolysis to give the *O*-(3,4,6-tri-*O*-acetyl-2-acylamido-2-deoxy-β-D-glucopyranosyl)-L-serines (**12** and **13**).



SCHEME 2

Scheme 2

N-Carboxy-α-amino acid anhydrides are usually prepared by treatment of amino acids with phosgene in inert solvents (Fuchs–Farthing method)[24] Using this procedure it was possible to synthesize the *N*-carboxy anhydrides of the *O*-acetylated serine glycosides (**6**) shown in Table IV All the anhydrides, except the lactose derivative, are crystalline solids which in the case of 2-acetamido-2-deoxy-D-glucose and L-rhamnose contain 1 molecule of dioxane Their equivalent weights were determined by titration with sodium methoxide in inert solvents[25] For the synthesis of long-chain, linear poly-α-amino acids by polymerization in organic solvents, *N*-carboxy-α-amino acid anhydrides of very high purity are required[24] If only short peptide chains are to be attached to the amino groups of a macromolecule by polymerization in aqueous solution, such highly purified anhydrides are not essential Nevertheless, the anhydrides of the serine glycosides were carefully purified by repeated crystallization or precipitation, and several of these anhydrides were used to attach serine glycosides to branched synthetic polypeptides

EXPERIMENTAL

General — Melting points were determined with a Tottoli melting-point

apparatus (Buchi, Flawil, Switzerland) and are uncorrected Optical rotations were measured with a Perkin–Elmer Model 141 polarimeter Descending paper chromatography was performed on Whatman No 1 paper with the following solvents (v/v) butyl alcohol–pyridine–water (6:4:3) (solvent A) and (6 5 5) (solvent B) The serine glycosides were detected by spraying with a 1% solution of ninhydrin in acetone and heating to 90° Thin-layer chromatography was performed on glass plates coated with Kieselgel G (E. Merck, Darmstadt, Germany). Depending on the type of com-

TABLE I

OPTICAL ROTATIONS OF SERINE GLYCOPYRANOSIDES AND OF THE CORRESPONDING METHYL α- AND β-D-GLYCOPYRANOSIDES

Compound	$[\alpha]_D$	*Solvent*	*Temp* (°C)	$[M]_D$
O-(α-L-Rhamnosyl)-L-serine[a]	− 41 3	water	22	−10377
Methyl α-L-rhamnoside[26]	− 62 5	water	20	−11137
Methyl β-L-rhamnoside[26]	+ 95 4	water	20	+17000
O-(β-D-Galactosyl)-L-serine[a]	− 1 6	water	22	−428
O-(β-D-Galactosyl)-L-serine[10]	− 2 1	water	24	−561
Methyl β-D-galactoside[26]	− 0 4	water	20	−78
Methyl α-D-galactoside[26]	+196 6	water	20	+38177
O-(β-Lactosyl)-L-serine[a]	− 0 6	water	22	−258
Methyl β-lactoside[27]	+ 5 6	water	26	+1995
Phenyl α-lactoside[28]	+ 88 7	water		+37113
O-(β-Cellobiosyl)-L-serine[a]	− 17 4	water	22	−7472
Methyl β-cellobioside[28]	− 19 1	water	20	−6806
Methyl α-cellobioside[28]	+ 96 8	water	20	+34493
O-(2-Acetamido-2-deoxy-β-D-glucosyl)-L-serine[a]	− 32 1	water	22	− 9896
O-(2-Acetamido-2-deoxy-β-D-glucosyl)-L-serine[6]	− 31 0	water	24	− 9557
O-(2-Deoxy-2-dodecanamido-β-D-glucosyl)-L-serine[a]	− 29 2	MeOH	22	−13098
Methyl 2-acetamido-2-deoxy-β-D-glucoside[29]	− 43 0	water		−10116
Methyl 2-acetamido-2-deoxy-α-D-glucoside[29]	+105 0	water		+24701

[a]This publication

pound the following solvents were used. chloroform–ethyl acetate (70 30, v/v), benzene–ethyl acetate (50 50, v/v), and ethyl acetate–methanol (20 80 v/v) The spots were detected by spraying with a 10% (v/v) solution of sulfuric acid in ethanol and subsequent heating, or with ninhydrin All compounds, except the Leuchs anhydrides, were routinely tested by thin-layer or paper chromatography, and after purification gave single spots Evaporations were performed with a flash evaporator at 40° bath-temperature and 12 mm Hg Solvents were purified and dried according to known procedures[30] Celite 535 was washed with conc. hydrochloric acid, then with distilled water until neutral, and dried The *N*-benzyloxycarbonyl-L-serine benzyl ester was prepared according to Baer and Maurukas from *N*-benzyloxycarbonyl-L-serine[31] Deacetylation of the *O*-acetylated serine glycosides (4 and analogs) was performed in all cases by treatment with methanolic ammonia as described below in detail for the rhamnose derivative The solvents used for recrystallization of the free serine

TABLE II

PROPERTIES AND ANALYSES OF SERINE GLYCOSIDES

O-*Glycopyranosyl*-L-*serine*	*Solvent of crystallization*	*Yield*[a] %	*M p* (°C) (*dec*)	$[\alpha]_D^{22}$ (*deg*)	*Solvent* (c, *l*)	R_{Ser}[b]	*Formula*	*Calc*			*Found*		
								C	*H*	*N*	*C*	*H*	*N*
α-L-Rhamnosyl-	H_2O-MeOH	61	173–175	−41.3	water	0 81	$C_9H_{17}NO_7\ H_2O$	40 15	7 11	5 20	40 32	7 24	5 11
β-D Galactosyl-	H_2O-MeOH	84	140–142	− 1.6	water	0 56	$C_9H_{17}NO_8$	40 45	6 41	5 24	40 59	6 62	5 37
β-Lactosyl-	H_2O-MeOH	70	200–206	− 0 6	water	0 34	$C_{15}H_{27}NO_{13}$ H_2O	40 27	6 53	3 13	40 40	6.68	3.10
β-Cellobiosyl-	H_2O-MeOH	73	211–228	−17.4	water	0 42	$C_{15}H_{27}NO_{13}$	41 96	6 34	3 26	42 00	6 28	3 17
2-Acetamido-2-deoxy-β-D-glucosyl-	H_2O-MeOH	77	200–216	−32 1	water	0 88	$C_{11}H_{20}N_2O_8$	42 85	6.54	9 09	42 79	6 60	8 96
2-Deoxy-2-dodecamido-β-D glucosyl-	MeOH-H_2O	85	174–178	−29 2	MeOH	4 3	$C_{21}H_{40}N_2O_8$	56 23	8 99	6 25	56 10	9 12	6 21

[a]The yields given in this Table refer to the deacetylation reaction

[b]Mobility on paper chromatograms relative to serine in solvent B

glycosides (**5** and analogs) and the yields are given in Table II The *N*-carboxy-α-amino acid anhydrides of the various serine glycosides (**6** and analogs) were prepared by treatment of the *O*-acetylated serine glycosides (**5** and analogs) with phosgene, essentially according to the procedure given for the rhamnose derivative The properties of these compounds are listed in Table IV Their equivalent weights were determined as described by Berger *et al*[25]. The anhydride (4-10 mg) was dissolved in anhydrous dioxane (about 0 7 ml) and titrated in the presence of thymol blue as indicator with 0 1M sodium methoxide delivered from an Agla micrometer syringe (Burroughs Wellcome and Co , London)

O-(*2,3,4-Tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-L-*serine* (**4**) — 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[32] (**1**) (3 53 g, 10 mmoles) and *N*-benzyloxycarbonyl-L-serine benzyl ester (**2**) (3 3 g, 10 mmoles) were dissolved in anhydrous nitromethane and powdered mercuric cyanide (2 8 g, 11 mmoles) was added After being stirred for 48 h at 40° with exclusion of moisture, the mixture was diluted with benzene, and the mercuric salts were filtered off The filtrate was extracted 3 times with M aqueous potassium bromide, 3 times with a saturated sodium bicarbonate solution, and finally with water The organic phase was dried with anhydrous sodium sulfate and evaporated to a colorless syrup which was dissolved in ethanol Water was added dropwise until a point was reached just before a slight uniform turbidity appeared The solution was hydrogenated at room temperature and atmospheric pressure in the presence of 5% palladium-on-charcoal The uptake of hydrogen was essentially complete after 3 h. After filtering off the catalyst, the solution was evaporated, and the remaining syrup was repeatedly dissolved in ethyl acetate and evaporated again to remove water Finally the syrup was dissolved in ethyl acetate, and ether was slowly added until precipitation was complete After centrifugation the supernatant was discarded, and the sediment was dissolved in a minimal amount of methanol. Part of the product crystallized Crystallization was completed by addition of ethyl acetate and ether The yield of crude acetylated *O*-(α-L-rhamnopyranosyl)-L-serine (**4**) was 2 5 g (66%)

The main impurity still present, L-serine, was removed by partition chromatography[14]. Dry Celite (165 g) was thoroughly mixed with water saturated with butyl alcohol (150 ml of lower phase) and suspended in butyl alcohol saturated with water (upper phase) This mixture was poured onto a column (3.5 × 71 cm) The solution of the crude glycoside (**4**, 2 5 g) in butyl alcohol (8 ml) was applied to the column, and elution was performed at a rate of about 60 ml/h with butyl alcolol saturated with water. Fractions of 15 ml were collected Samples of each fraction were deposited on paper and stained with ninhydrin. Most of compound **4** was eluted in fractions 50–84, however, an additional small amount was eluted in fractions 85–100 Fractions 50–84 were pooled and evaporated. The chromatographed material was completely free from serine, and contained only trace amounts of a substance of slightly lower chromatographic mobility. This substance was removed by two recrystallizations from methanol–ether (yield 1 9 g)

Fractions 85–100 together with the combined mother-liquors were rechromato-

graphed on a column prepared from 60 g of Celite, and 5-ml fractions were collected Fractions 55–88 again contained 0 34 g of pure **4**, and fractions 128–190 contained a small amount of another rhamnosylserine which could not be crystallized The paper chromatographic mobility of this compound was slightly lower than that of the main product **4**. The total yield of pure **4** was 2 2 g (58%) (Table III)

O-(α-L-*Rhamnopyranosyl*)-L-*serine* (**5**) — *O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-serine (**4**, 0 46 g), dried *in vacuo*, was dissolved in anhydrous methanol (30 ml) The solution was cooled with ice, and then saturated with dry ammonia with exclusion of moisture The mixture was kept for 3 days at 4° and was then evaporated. The residue, after removal of acetamide by sublimation for 2 h at 60° and 0 2 mm Hg, was recrystallized twice from water–methanol The yield of *O*-(α-L-rhamnopyranosyl)-L-serine (**5**) was 0 20 g (61%) (Tables I and II)

O-(*2,3,4-Tri*-O-*acetyl*-α-L-*rhamnopyranosyl*)-N-*carboxy*-L-*serine anhydride* (**6**) — *O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-L-serine (**4**) (0 51 g), dried *in vacuo* over phosphorus pentaoxide, was suspended in anhydrous dioxane (40 ml) Dry phosgene was passed through the suspension for 1 h at 50° Within a few minutes the glycoside dissolved completely. The solution was evaporated to remove the excess phosgene The residue could not be crystallized from ethyl acetate–petroleum ether, but crystallized from dioxane–petroleum ether For recrystallization a small amount of dry charcoal was added, this effectively adsorbed trace amounts of chlorine-containing impurities As far as possible all operations were performed with exclusion of moisture and with anhydrous solvents The 3 times recrystallized anhydride **6** (0 56 g, 85%) began to melt at 65° and decomposed at 76° It contained one molecule of dioxane which was slowly released at 0 2 mm Hg by gradually raising the temperature from 60 to 80° (loss of weight 16 8%, calc for 1 molecule of dioxane 17 9%) The dioxane-free anhydride slowly decomposed from 80° to 115° (Table IV)

O-(*2,3,4,6-Tetra*-O-*acetyl*-β-D-*galactopyranosyl*)-L-*serine* — This compound was prepared essentially according to Kum and Roseman[10], but isolation and purification were performed as described for the rhamnose derivative The chromatographically pure β-D-galactoside was obtained in 25% yield

O-(*Hepta*-O-*acetyl*-β-*lactopyranosyl*)-L-*serine* — The condensation reaction of hepta-*O*-acetyl-α-lactopyranosyl bromide[33] (15 4 g, 22 mmoles) with *N*-benzyloxycarbonyl-L-serine benzyl ester (7 3 g, 22 mmoles), followed by hydrogenation was performed as described The crude reaction product was dissolved in a minimal amount of hot water The lactoside crystallized upon cooling with ice It was washed with ice water, redissolved in a small amount of ethanol, precipitated with ethyl acetate and ether, and again recrystallized from water The yield of chromatographically pure product, which contained 3 molecules of water, was 6.7 g (39%) (Table III)

O-(*Hepta*-O-*acetyl*-β-*cellobiopyranosyl*)-N-*benzyloxycarbonyl*-L-*serine benzyl ester* — Hepta-*O*-acetyl-α-cellobiopyranosyl bromide[33] (7 0 g, 10 mmoles) was treated with *N*-benzyloxycarbonyl-L-serine benzyl ester (3 3 g, 10 mmoles) in the presence of mercuric cyanide (2 8 g, 11 mmoles) as described for the rhamnose derivative The crude reaction product crystallized from methanol After several recrystal-

TABLE III

PROPERTIES AND ANALYSES OF *O* ACETYLATED SERINE GLYCOSIDES

O-(*Per*-O-*acetyl-glyco-pyranosyl*)-L-*serine*	*M p* (°C)	$[\alpha]_D^{23}$ (*deg*)	*Solvent* (*c*, *l*)	R^a_{Ser}	R^a_F	*Formula*	*Calc*				*Found*			
							C	*H*	*N*	*Br*	*C*	*H*	*N*	*Br*
2,3,4-Tri-*O*-acetyl-α-L-rhamnosyl	169–171 (dec)	−65 2	water	4.3	0 56	$C_{15}H_{23}NO_{10}$	47 75	6 14	3 71		47 46	6 25	3 84	
2,3,4,6-Tetra-*O*-acetyl-β-D-galactosyl-	141–145 (dec)	−10 0	water	4 4	0 57	$C_{17}H_{25}NO_{12}$	46 89	5 79	3 22		46 98	5.87	3.26	
Hepta-*O*-acetyl-β-lactosyl-	168–170	−16.2	water	4 8	0 62	$C_{29}H_{41}NO_{20}$ $3H_2O$	44 79	6 09	1 80		44.93	6.24	1 90	
Hepta-*O*-acetyl-β-cellobiosyl-	165–177 (dec.)	−13 5	acetic acid	4 5	0 58	$C_{29}H_{41}NO_{20}$	48 13	5 71	1 94		48.07	5 66	1 85	
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucosyl- (12)	160–162 (dec.)	−47 3	methanol	3 5	0 48	$C_{17}H_{26}N_2O_{11}$	47.00	6.03	6 45		46 99	6 01	6.48	
3,4,6 Tri-*O*-acetyl-2-deoxy-2-dodecamido β-D glucosyl-hydrobromide (13)	147–151 (dec)	−11 3	methanol	6 0	0 83	$C_{27}H_{47}N_2O_{11}Br$	49 47	7 23	4.27	12 19	49 00	7.41	4 11	12.08

[a]Mobility on paper chromatograms relative to serine (R_{Ser}) and to the solvent (R_F) in solvent A

TABLE IV

PROPERTIES AND ANALYSES OF THE *N*-CARBOXY ANHYDRIDES OF *O*-ACETYLATED SERINE GLYCOSIDES

O-(*Per*-O-*acetyl-glycopyranosyl*)-N-*carboxy*-L-*serine anhydride*	*Solvent of crystallization*	*Yield* %	*M p* (°C) (*dec*)	*Equiv wt*		*Formula*	*Calc*			*Found*		
				Calc	*Found*		*C*	*H*	*N*	*C*	*H*	*N*
2,3,4-Tri-*O* acetyl α-L-rhamnosyl-	dioxane–petroleum ether	85	65– 76	491 5	494	$C_{16}H_{21}NO_{11}$ $C_4H_8O_2$[a]	48 88	5 95	2 85	48 98	5 93	2 96
2,3,4,6-Tetra-*O*-acetyl-β-D-galactosyl-	ethyl acetate–petroleum ether	52	90– 98	461 4	469	$C_{18}H_{23}NO_{13}$	46 86	5 02	3 04	46 67	5 21	2 99
Hepta-*O*-acetyl-β-lactosyl-	precipit from dioxane-petroleum ether, lyophilized from dioxane	90		749 7	753	$C_{30}H_{39}NO_{21}$	48 07	5 24	1 87	47 62	5 38	1 78
Hepta-*O*-acetyl-β-cellobiosyl-	ethyl acetate–petroleum ether	81	199–204	749 7	750	$C_{30}H_{39}NO_{21}$	48 07	5 24	1 87	47 78	5 37	1 75
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucosyl-	dioxane–petroleum ether	63	192–193	548 5	558	$C_{18}H_{24}N_2O_{12}$ $C_4H_8O_2$[b]	48 17	5 88	5 11	47.83	5 89	5 18
				460 4		$C_{18}H_{24}N_2O_{12}$	46 96	5 25	6 09	46 73	5.45	5.89
3,4,6-Tri-*O*-acetyl-2-deoxy-2-dodecamido-β D-glucosyl-	ethyl acetate–petroleum ether	66	169–173	600 7	590	$C_{28}H_{44}N_2O_{12}$	55 99	7 38	4 66	56 03	7 65	4 59

[a]The crystalline compound contains 1 molecule of dioxane which is slowly released by gradually raising the temperature from 60 to 80° at 0 2 mm Hg Loss of weight 16 8%, calc 17 8%

[b]The crystalline compound contains 1 molecule of dioxane which is released at 70° and 0 2 mm Hg Loss of weight 16 19%, calc. 16 06%

lizations from methanol, the yield was 4 5 g (47.5%), m p 145–148°, $[\alpha]_D^{23}$ −16 4° (*c* 1, ethyl acetate)

Anal Calc for $C_{44}H_{53}NO_{22}$· C, 55 75; H, 5.64, N, 1 48 Found C, 55.66, H, 5.61, N, 1.64.

O-(*Hepta-O-acetyl-β-cellobiopyranosyl*)-L-*serine* — *O*-(Hepta-*O*-acetyl-β-cellobiopyranosyl)-*N*-benzyloxycarbonyl-L-serine benzyl ester (9 48 g, 10 mmoles) was dissolved in methanol–ethanol (1:1, 350 ml) and a small amount of water was added. The solution was hydrogenated in the presence of (5%) palladium-on-charcoal (0 5 g) at atmospheric pressure, and the reaction product was crystallized from water–ethanol to give 6 67 g (92%) (Table III)

O-(*3,4,6-Tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl*)-N-*benzyloxycarbonyl-L-serine benzyl ester hydrochloride* (**9**) — 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylidene)-amino-α-D-glucopyranosyl bromide (24 3 g, 50 mmoles), prepared according to Zervas and Konstas[21] and used without recrystallization, and *N*-benzyloxycarbonyl-L-serine benzyl ester (**2**, 16.5 g, 50 mmoles) were dissolved in anhydrous 1,2-dichloromethane (100 ml) Silver carbonate (13 8 g, 50 mmoles) and anhydrous calcium sulfate (40 g) were added, and the mixture was shaken for 2 days in the dark Calcium sulfate and silver salts were filtered off, washed with 1,2-dichloromethane, and the filtrate was evaporated Upon redissolving the oily residue in acetone (90 ml) and cooling, a crystalline compound (2 4 g, m p 243–245°) was obtained, probably a nonreducing disaccharide

Conc hydrochloric acid (3 ml) was added to the mother liquor, and the solution was boiled for 1 min and evaporated *in vacuo* The residue was dissolved in ether (100 ml) and water (35 ml) Upon shaking or stirring for a few minutes, compound 9 crystallized, mainly at the interphase of ether and water It was filtered off and recrystallized from methanol–water and from methanol–ether to give 4 9 g (15%) of pure compound **9**, m p 213–218° (dec), $[\alpha]_D^{25}$ −4° (*c* 1 234, methanol)

Anal. Calc. for $C_{30}H_{37}ClN_2O_{12}$ C, 55 17, H. 5 71, Cl, 5 43; N, 4 29 Found C, 55 04, H, 5 62, Cl, 5 56, N, 4 33

O-(*2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl*)-N-*benzyloxycarbonyl-L-serine benzyl ester* (**10**) —Compound **9** (3 27 g, 5 mmoles) was dissolved in anhydrous pyridine (25 ml), and acetic anhydride (2 ml) was added with shaking The solution was kept in a stoppered flask for 1 day at room temperature, and was then poured into ice water A precipitate which formed was filtered off, washed with ice water, dried and recrystallized several times from ethyl acetate–petroleum ether and from methanol–water, yield 3 12 g (94 5%), m p 160–161°, $[\alpha]_D^{23}$ −13 9° (*c* 1, acetic acid).

Anal Calc for $C_{32}H_{38}N_2O_{13}$ C, 58 35, H, 5 81, N, 4 25 Found C, 58.46, H, 5 90, N, 4.18

O-(*2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl*)-L-*serine* (**12**) — Compound **10** (3 3 g, 5 mmoles) dissolved in ethanol (250 ml) was hydrogenated in the presence of palladium-on-charcoal in the usual way Crystallization from ethanol gave 2 0 g (92%) (Table III)

O-(3,4,6-Tri-O-acetyl-2-deoxy-2-dodecamido-β-D-glucopyranosyl)-N-benzyloxycarbonyl-L-serine benzyl ester (**11**) — Compound **9** (6 53 g, 10 mmoles) was partially dissolved in anhydrous pyridine (15 ml) and dodecanoyl chloride (lauroyl chloride) (2 18 g, 10 mmoles) was added with shaking After being kept for 1 day at room temperature, the solution was poured into ice water The precipitate which formed was washed with ice water, dried, and recrystallized 3 times from ethyl acetate–petroleum ether to give 6 55 g (82%), m p. 146–148°, $[\alpha]_D^{23}$ −5.8° (*c* 1, ethyl acetate)

Anal Calc for $C_{42}H_{58}N_2O_{13}$ C, 63 14; H, 7 32, N, 3 51 Found: C, 63 04, H, 7 40, N, 3 53

O-(3,4,6-Tri-O-acetyl-2-deoxy-2-dodecamido-β-D-glucopyranosyl)-L-serine (**13**) — Compound **11** (4 0 g, 5 mmoles) dissolved in 1:1 ethyl acetate–methanol (270 ml) was hydrogenated in the usual way Crystallization from methanol–water was very slow and a gel formed easily. Therefore, the hydrobromide was prepared by adding a few drops of 48% (w/w) aqueous hydrobromic acid until the solution of **13** in a small amount of methanol became acidic. Upon addition of ether, fine needles were obtained which were recrystallized from methanol–ether (2 7 g, 82%) (Table III)

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