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Synthesis and Chemical Behaviour of α- and β-D-Glucopyranosyl Esters of L-Serine and Their Derivatives*

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Simultaneous and stepwise deprotection of the fully benzylated α and β-D-glucopyranosyl esters of *N*-benzyloxycarbonyl- and *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine (I and V) was studied, and α and β anomers of 1-*O*-(L-seryl)-D-glucopyranose were isolated as hygroscopic trifluoroacetate salts II α and II β which were characterized by subsequent conversion into the corresponding *N*-acetyl- and -*O*-acetylated derivatives III and IV, respectively. Catalytic hydrogenation of V β afforded 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-β-D-glucopyranose (VI β), whereas the same treatment of the α anomer of V led to concomitant 1→2 acyl migration to give a mixture of VI α and its 2-*O*-isomer VII, highly enriched in the α anomer. Acetylation of the mixture yielded the peracetylated 1- and 2-*O*-acyl derivatives IX α and XII α, also prepared by definite methods. The ^{1}H NMR spectra of α-D-anomers of 2,3,4,6-tetra-*O*-acetyl-1-*O*- and 1,3,4,6-tetra-*O*-acetyl-2-O-[*N*-(*tert*-butoxycarbonyl)-*O*-(benzyl)-L-seryl] glucopyranoses (VIII α and X α) revealed that the seryl *O*-benzyl substituent causes strong shielding of the adjacent acetoxyl groups.

INTRODUCTION

In early studies concerned with structure elucidation of the *O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-L-serine linkage in submaxillary-gland glycoproteins, it has been suggested that the sugar moiety is bound to the amino acid residue by the glycosyl ester linkage[1,2]. Unambigous proof for the presence in glycoproteins of *O*-glycosylated L-serine and L-threonine residues was provided independently by several laboratories in 1964[1,2], and additional evidence was deduced from the chemical investigations of Fletcher and coworkers[3,4] who synthesized the α and β anomers of 2-acetamido-1-*O*-acetyl and benzoyl-2-deoxy-D-glucopyranoses and -D-galactopyranoses. These authors showed that the glycosidic ester linkage in the investigated compounds was highly unstable. To the best of our knowledge, the synthesis of a glycosyl ester derivative of serine has not been described so far.

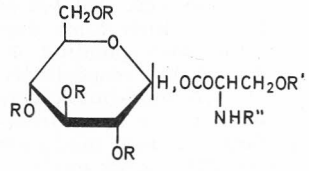
The protected glucosyl and glucosyluronic esters of amino acids can be obtained by the imidazole-promoted reactions of the fully benzylated, C-1 free sugar component with the activated esters of acylamino acids or with acylamino

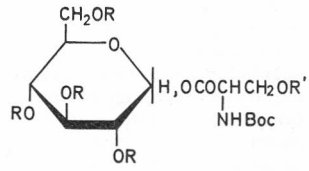
* Glycosyl Esters of Amino Acids: Part XI. For Part X, see Ref. 10.

acids in the presence of dicyclohexylcarbodi-imide (DCC)[5,6]. It has been shown[7,8], that the relative stabilities of the unprotected C-1 esters, available by simultaneous or stepwise removal of the protecting groups, are significantly different, depending on the aglycon structure and anomeric configuration of the sugar moiety. It seemed of interest to examine by the above-mentioned routes the preparation of D-glucopyranosyl esters of L-serine, and we now report on the synthesis of these compounds and comment on their reactivity.

RESULTS AND DISCUSSION

The fully protected D-glucopyranosyl esters I and V were obtained by the imidazole-promoted condensation[5,6] of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and the pentachlorophenyl ester of *N*-benzyloxycarbonyl-*O*-benzyl-L-serine and *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine, respectively. The products were obtained as anomeric mixtures which were resolved by repeated silica gel chromatography and fully characterized. The α, β ratio in I and V was ~ 2 : 1 and ~ 1 : 1, respectively, but, owing to extensive column fractionation, the oily α anomers were recovered in pure state in considerably lower yields than the corresponding crystalline β anomers.





I R = R' = CH_2Ph ; R" = Z
III R = R' = H ; R" = Ac
IV R = R' = R" = Ac

V R = R' = CH_2Ph
VI R = R' = H
VIII R = Ac ; R' = CH_2Ph
IX R = R' = Ac

Z = $PhCH_2OCO$; Boc = Me_3COCO

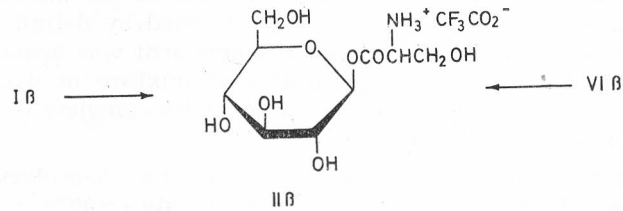
Hydrogenolysis of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[*N*-(benzyloxycarbonyl)-*O*-(benzyl)-L-seryl]-β-D-glucopyranose (Iβ) over palladium-on-charcoal in acetic acid-2-methoxyethanol (2 : 1) led to decomposition of the compound. TLC of the hydrogenation mixture revealed the presence of glucose and several iodine-positive spots, but not of serine, thus suggesting that β-elimination processes were operative under these conditions. The same procedure performed with the α anomer of I resulted in the transfer of the aglycon acyl group to the alcoholic solvent, to give L-serine 2-methoxyethyl ester and glucose as the principal products. The ester was isolated (70%) pure by column chromatography of the hydrogenation mixture on cellulose and characterized by comparison with an authentic sample. The latter was prepared by condensation of the adequately protected L-serine pentachlorophenyl ester with 2-methoxyethanol, followed by removal of the *N*- and *O*-protecting groups.

However, when catalytic hydrogenation of Iα and Iβ was performed in 2-methoxyethanol as the solvent and in the presence of trifluoroacetic acid[9] (98%, ~3 cm^3/mmol), the reaction proceeded without cleavage of the glycosyl ester bond, and the corresponding anomer of 1-*O*-(L-seryl)-D-glucopyranose was isolated as the trifluoroacetate salt IIα and IIβ, respectively,

in high yield. The structure assignments were based on analytical, spectral and optical rotation data, and for further characterization both anomers were sumbitted to selective *N*-acetylation, followed by conventional *O*-acetylation, to give the respective anomer of 1-*O*-(*N*-acetyl-L-seryl)-D-glucopyranose (III) and 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(acetyl)-*O*-(acetyl)-L-seryl]-D-glucopyranose (IV).

In dry state, the unprotected D-glucopyranosyl esters II α and II β were found to be fairly stable compounds; after one month of storage under anhydrous conditions, the estimated (TLC, solvent *C*) cleavage of the glycosyl ester bond was less than 20%. However, hydrolysis proceeded much faster in water (~50% within the first 24 hours) and was practically instantaneous in even very slightly alkaline aqueous media.

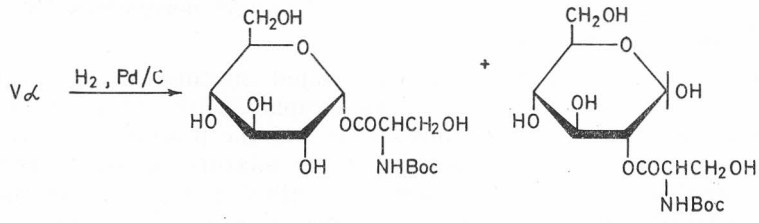
Catalytic hydrogenation of the fully benzylated β anomer of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(benzyl)-L-seryl]-D-glucopyranose (V β) in acetic acid-2-methoxyethanol (2 : 1) yielded 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-β-D-glucopyranose (VI β) which, upon treatment with trifluoroacetic acid at —10 °C, gave the product identical (analytical and spectral data) with the trifluoroacetate salt II β obtained by simultaneous deprotection of I β (Scheme 1.). Structural



Scheme 1.

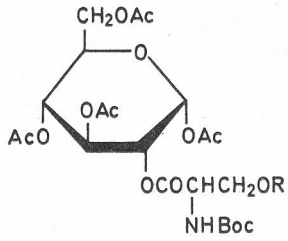
proof for VI β was provided by its conversion into the crystalline peracetylated derivative which showed physical properties identical to those of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-β-D-glucopyranose (IX β), prepared by definite route (see below).

Deprotection of the α anomer of V was accompanied by a partial 1 → 2 *O*-acyl migration to give a ~2 : 1 mixture of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-seryal]-α-D-glucopyranose (VI α) and its 2-*O*-acyl isomer VII (Scheme 2.).



Scheme 2. VI α VII α >> VII β

On TLC (solvent *B*), the product revealed two closely-migrating ninhydrin- and silver nitrate-positive spots, and ^{1}H NMR spectrum (D_2O) showed the anomeric proton as two doublets at τ 3.91 ($J_{1,2}$ 3 Hz) and 4.72 ($J_{1,2}$ 4 Hz) in a ~2 : 1 ratio. Conventional acetylation afforded a mixture of 2,3,4,6-tetra-*O*-acetyl--1- and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-



Xα R = CH_2Ph

XIα R = H

XIIα R = Ac

-α-D-glucopyranose (IX α and XII α), contaminated with the β anomer of XII. Assignments of the structures IX α and XII α to the major components were made on the basis of analytical and spectral data of the mixture, as well as by comparison with the authentic samples prepared by definite methods (see below). The structure XII β for the minor component was deduced, *inter alia*, from the NMR spectrum of the peracetylated mixture in which the signal assigned to the C-1 acetoxy group appeared as two singlets (τ 7.81 and 7.87) of different intensities (*ax* : *eq* ~ 5 : 1).

Compared to II β, an aqueous solution of the *N*-protected derivative VI β decomposed (TLC, solvent *B*) at a slower rate (~50% after 3 days) to give glucose and Boc-serine as the only detectable products. On the other hand, monitoring of an aqueous solution of the isomeric mixture, obtained by catalytic deprotection of V α, showed that 1 → 2 acyl migration and hydrolysis were operative under these conditions; after 24 h, TLC revealed the 2-*O*-acyl isomer VII as the major, and VI α, glucose and Boc-serine as the minor components of the mixture. Treatment of this material with acetic anhydride—pyridine resulted in a heterogeneous product which could be resolved by silica gel chromatography into the faster-eluting penta-*O*-acetyl-D--glucose and a mixture consisting of 1- and 2-*O*-acyl derivatives IX α, XII α and XII β (ratios ~0.5 : 1 : 1).

On TLC (solvent *A*, 2 : 1), the peracetylated mixture revealed 3 closely migrating spots, two of which co-chromatographed with authentic IX α and XII α, respectively. The relative concentration of the β anomer of XII could be determined from the NMR spectrum of the mixture in which the AcO-1 signal appeared as two singlets of almost identical intensities and the peak areas of *eq* AcO-1 : *ax* H-1 were in the expected 3 : 1 ratio. Thus, assuming that the rearrangement of VI α into VII proceeds via a cyclic ortho-ester intermediate, the above results indicate that the 1 → 2 acyl migration is followed by anomerization of the initial α form of the 2-isomer released[10].

The alternative synthesis of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-D-glucopyranose (IX) started from the adequately protected sugar- and amino acid-component which were condensed in the presence of imidazole to give 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-

-*O*-(benzyl)-L-seryl]-D-glucopyranose (VIII). The separation of the anomers by silica gel chromatography was rather inefficient, and only the α anomer of VIII could be obtained anomerically pure, by repeated column fractionation. The anomeric mixture of VIII was submitted to catalytic hydrogenation, by which the *O*-benzyl protective group of the seryl residue was removed, and the crude product was treated immediately with acetic anhydride—pyridine. The resulting compound IX was readily resolved into the α and β anomers which were fully characterized.

The preparation of 1,3,4,6-tetra-*O*-acetyl-2-*O*-[*N*-*tert*-butoxycarbonyl)-*O*--(acetyl)-L-seryl]-α-D-glucopyranose (XII α) involved three reaction steps: first, the condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and the adequately protected L-serine pentachlorophenyl ester to give 1,3,4,6-tetra-*O*-acetyl--2-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(benzyl)-L-seryl]-α-D-glucopyranose (X α), second, deprotection of the seryl hydroxyl group to give the 2-*O*-acyl derivative XI α, and, third, *O*-acetylation of this group to give XII α.

It is well known that aryl substituents in a peracetylated carbohydrate derivative cause specific shielding or deshielding of individual acetoxyl groups[11,12], and large upfield shifts of the AcO-2 group have been observed[13] in the ^{1}H NMR spectra of acetylated 1-*O*-arylacetyl-D-glucopyranoses. During the course of the present work, we observed that benzyl protection of the seryl hydroxyl group causes strong shielding of the adjacent acetoxyl groups in the α anomers of acetylated 1- and 2-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(benzyl)--L-seryl]-D-glucopyranoses VIII α and X α. Thus, in the spectrum (chloroform-*d*) of VIII α the signal assigned to AcO-2 appeared at τ 8.21; inspection of Dreiding models of α and β anomers of VIII revealed that spacial relationships in the former allow an interaction between the 2-acetoxyl-methyl protons and the *O*-benzyl aromatic ring. The finding that in the spectrum of the 2-*O*-acyl derivative X α, the *ax* AcO-1 and *eq* AcO-2 signals were shifted to higher fields (~ 0.05 and 0.18 ppm) relative to the same signals in the spectra of XI α and XII α, indicates that the orientation of the *O*-benzyl substituent in X α is such that both adjacent acetoxyl groups fall in the shielding region of the benzene nucleus.

EXPERIMENTAL

General

Concentrations were carried out at reduced pressure on a rotary evaporator at $< 35\,^{\circ}$C, if not stated otherwise, and solutions were dried with sodium sulphate. Column chromatography was performed on silica gel (Merck, 0.05—0.2 mm) or cellulose powder (Whatman, Standard grade), packed as a slurry by using a plunger, and TLC on Kiesel G (Merck). Solvent systems (by volume) used were: *A* benzene--ethyl acetate (proportions are given in the text); *B* 2-propanol-light petroleum--water (5 : 3 : 1), *C* acetonitril-2-propanol-water (2 : 2 : 1). Detection on TLC plates was effected by charring with sulphuric acid, with ninhydrin-reagent, or with alkaline silver nitrate. Optical rotations were determined for 1% solutions in chloroform, unless otherwise stated. IR spectra were recorded with a Perkin-Elmer Model 297 spectrometer, and ^{1}H NMR spectra with a Varian A-60A spectrometer for solutions in chloroform-*d* with tetramethylsilane as internal standard, if not stated otherwise.

Serine Derivatives

N-*tert*-Butoxycarbonyl-*O*-benzyl-L-serine pentachlorophenyl ester was obtained from *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine[14] and dicyclohexylcarbodi-imide-pen-

tachlorophenol complex (DDC × 3PCPOH) in ethyl acetate, as described in the general procedure of Kovacs et al.[15]. The product (45% yield) was recrystallised from ethyl acetate—light petroleum, m. p. 102—103 °C, $[\alpha]_D$ — 6.6° (DMF).

Anal. $C_{21}H_{20}Cl_5NO_5$ (543.68) calc'd.: C 46.39; H 3.71; N 2.58%
found: C 46.53; H 3.94; N 2.33%

N-Benzyloxycarbonyl-*O*-benzyl-L-serine was prepared from the *N*-protected amino acid and benzyl bromide in the presence of 80% sodium hydride in dimethylformamide, as described[14] for the corresponding Boc-protected derivative; yield 73%, m. p. 95—97 °C (ethylacetate—light petroleum), $[\alpha]_D$ + 17.1° (EtOH). Lit.[16]: m. p. 98 °C, $[\alpha]_D$ + 17.3° (EtOH). Condensation of this compound with pentachlorophenol, under conditions cited[15] above, yielded *N*-benzyloxycarbonyl-*O*-benzyl-L-serine pentachlorophenyl ester (62.5% yield), m. p. 122—123 °C (ethyl acetate—light petroleum), $[\alpha]_D$ — 5° (benzene ,c 2), — 4° (DMF, c 2).

Anal. $C_{24}H_{18}Cl_5NO_5$ (577.69) calc'd.: C 49.90; H 3.14; N 2.43%
found: C 50.14; H 3.37; N 2.60%

N-Benzyloxycarbonyl-*O*-benzyl-L-serine 2-methoxyethyl ester was prepared from the corresponding pentachlorophenyl ester (577 mg) and 2-methoxyethanol (20 ml) in the presence of imidazole (340 mg). After 24 h at room temperature, the solvent was evaporated, the residue was extracted with ethyl acetate and worked-up in conventional way. The crude product was passed through silica gel (solvent *A*, 5 : 1), and fractions containing the homogenous title product (300 mg, 77%) were subjected to catalytic hydrogenation in acetic acid-2-methoxy-ethanol (2 : 1). After removal of the catalyst and solvent, the residue was dissolved in chloroform; subsequent addition of light petroleum precipitated pure L-serine 2-methoxyethyl ester (20 mg, 34%) as a hygroscopic solid mass, $[\alpha]_D$ — 4° (water, c 2). ν_{max}^{film} 3300 broad, vs (OH, NH), 1740 cm^{-1} (C=O).

Anal. $C_6H_{13}NO_4$ (163.17) calc'd.: C 44.16; H 8.03; N 8.58%
found: C 44.38; H 7.93; N 8.57%

2,3,4,6-Tetra-O-benzyl-1-O-[N-(benzyloxycarbonyl)-O-(benzyl)-L-seryl]-D-glucopyranose (I)

2,3,4,6-Tetra-*O*-benzyl-α D-glucopyranose (1.08 g, 2 mmol), *N*-benzyloxycarbonyl-*O*-benzy-L-serine pentachlorophenyl ester (1.5 g, 2 mmol) and imidazole (680 mg, 10 mmol) were dissolved in dichloromethane (20 cm^3) at 0 °C under stirring; the mixture was stirred at 0 °C for 1 h and then at room temperature for 24 h, whereupon pentachlorophenol was filtered off. The filtrate was washed with water, 10% citric acid, water, aqueous sodium hydrogen carbonate, and water, dried and concentrated. The residue was passed through a silica gel column (solvent *A*, 10 : 1) to give chromatographically homogenous I (1.47 g, 85%) as an anomeric mixture. Crystallisation from chloroform—light petroleum afforded the β anomer of I (756 mg), m. p. 60—62 °C, $[\alpha]_D$ + 6.8°. ν_{max}^{KBr} 3220 (NH), 1760, 1730, 1695 and 1685 (C=O), 1535 (amide II), 1080 cm^{-1} (C—O—C). NMR data: τ 2.66—3.00 (m, 30 H, 6 Ph), 4.20—4.52 (m, 2 H, H-1 + NH; deuteration led to one-proton doublet 4.40, $J_{1,2}$ 7 Hz), 4.80 (s, Ph CH_2OCO).

Anal. $C_{52}H_{53}NO_{10}$ (852.00) calc'd.: C 73.31; H 6.27; N 1.64%
found: C 73.50; H 6.27; N 1.69%

The mother liquor was evaporated to dryness, and the residue was submitted to silica gel chromatography (3 ×) with the same solvent. The pure α anomer was obtained as a viscous oil (450 mg), $[\alpha]_D$ + 26.2° (c 2). NMR data: τ 2.58—2.78 (m, 30 H, 6 Ph), 3.60 (d, $J_{1,2}$ 3 Hz, H-1), 4.30 (d, *J* 7.5 Hz, disappeared on deuteration, NH), 4.86 (s, Ph CH_2OCO).

Anal. $C_{52}H_{53}NO_{10}$ (852.00) found: C 73.42; H 6.24; N 1.61%

1-O-(L-Seryl)-β-D-glucopyranose Trifluoroacetate Salt (II β)

A solution of I β (600 mg, 0.7 mmol) in 2-methoxyethanol (15 cm^3) was hydrogenated at room temperature and pressure in the presence of 10% palladium-on-charcoal and trifluoroacetic acid (98%, 2 cm^3) until termination of hydrogen uptake (~ 16 h). The catalyst was centrifuged off, the supernatant was concentrated (0.1 Torr), anhydrous ether was added, and the precipitate was centrifuged off. Trituration of the residue with dry ether gave II β as a hygroscopic solid (218 mg, 81.3%), $[\alpha]_D$ 0° (water, c 2). ν_{max}^{KBr} 3360 broad, vs (OH, NH), 1770 (C=O), 1680 and 1520 (amide I and II), 1075 (C—O—C), 720 cm^{-1} (CF_3). NMR data (D_2O): τ 4.31 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. $C_{11}H_{18}F_3NO_{10}$ (380.92) calc'd.: C 34.68; H 4.76; N 3.68%
found: C 34.59; H 5.05; N 4.04%

A solution of II β (64 mg) in 10% acetic anhydride solution in acetone—water (1 : 1, 30 cm^3) was kept at room temperature for ~ 12 h (monitoring by TLC, solvent *C*). After removal of the solvent (0.1 Torr), the residue was dissolved in methanol, and the product was precipitated with dry ether. A second dissolution and precipitation afforded pure 1-*O*-(*N*-acetyl-L-seryl)-β-D-glucopyranose (III β, 36 mg, 70%) as a hygroscopic solid, $[\alpha]_D$ — 9.8° (water, c 2). NMR data (D_2O): τ 4.32 (d, $J_{1,2}$ 7 Hz, H-1), 7.90 (s, N*Ac*).

Anal. $C_{11}H_{19}NO_9 \times 2H_2O$ (327.27) calc'd.: C 40.36; H 6.46; N 4.27%
found: C 40.29; H 6.46; N 4.19%

A sample (54 mg) of III β was treated with acetic anhydride-pyridine (5 : 1, 8 cm^3) at room temperature for ~ 24 h (monitoring by TLC, ethyl acetate). After evaporation of the solvent and extraction of the residue with dry ether, the solution was concentrated to ~ 2 cm^3; subsequent addition of light petroleum precipitated crystals of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(acetyl)-*O*-(acetyl)-L-seryl]-β-D-glucopyranose (IV β), yield: 31 mg, 35%, m. p. 132—134 °C, $[\alpha]_D$ + 7.7°. ν_{max}^{KBr} 3380 (NH), 1750 (C=O), 1675 and 1535 cm^{-1} (amide I and II). NMR data: τ 4.32 (d, $J_{1,2}$ 7 Hz, H-1), 7.93—7.98 (18 H, 5 O*Ac* + N*Ac*).

Anal. $C_{21}H_{29}NO_{14}$ (519.45) calc'd.: C 48.55; H 5.62; N 2.69%
found: C 48.28; H 5.64; N 2.90%

Catalytic Hydrogenation of I α

(*a*) Without trifluoroacetic acid. — A solution of I α (407 mg, 0.477 mmol) in acetic acid-2-methoxyethanol (2 : 1, 15 cm^3) was shaken with hydrogen in the presence of 10% palladium-on-charcoal (300 mg) overnight. After removal of the catalyst and solvent, the residue was fractionated on a cellulose column (solvent *C*) to give a chromatographically homogeneous hygroscopic mass (54 mg, 70%) the TLC mobility, analytical and spectral data of which were indistinguishable from those of an authentic sample of L-serine 2-methoxyethyl ester.

(*b*) In the presence of trifluoroacetic acid. — Catalytic hydrogenation of I α (800 mg), performed as described for II β, afforded 1-*O*-(L-seryl)-α-D-glucopyranose trifluoroacetate salt (II α, 250 mg, 70%) as a hygroscopic solid, $[\alpha]_D$ + 47.9° (water, c 2), ν_{max}^{KBr} 3380 broad, vs (OH, NH), 1755 (C=O), 1678 and 1510 (amide I and II), 725 cm^{-1} (CF_3). NMR data (D_2O): τ 3.65 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. $C_{11}H_{18}F_3NO_{10}$ (380.92) found: C 34.77; H 5.11; N 4.06%

N-Acetylation of a sample (76 mg) of II α, performed as described for the β anomer, gave 1-*O*-(*N*-acetyl-L-seryl)-α-D-glucopyranose (III α) as a hygroscopic solid, $[\alpha]_D$ + 54.3° (water, c 2). NMR data (D_2O): τ 3.70 (d, $J_{1,2}$ 3 Hz, H-1), 7.85 (s, N*Ac*).

Anal. $C_{11}H_{19}NO_9 \times 2H_2O$ (327.27) found: C 40.19; H 6.41; N 4.29%

Conventional acetylation of III α (36 mg), performed as described for the β anomer, yielded, after extraction of the crude product with chloroform and precipitation of the solution with light petroleum, 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(acetyl)-*O*-

-(acetyl)-L-seryl]-α-D-glucopyranose (IV α) as a viscous oil (36 mg, 60%), $[\alpha]_D$ + 61.0°. NMR data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 7.97, 8.00, 8.03 (18 H, 5 *OAc* + N*Ac*).

Anal. $C_{21}H_{29}NO_{14}$ (519.45) found: C 48.54; H 5.68; N 2.63%

2,3,4,6-Tetra-O-benzyl-1-O-[N-(tert-butoxycarbonyl)-O-(benzyl)-L-seryl]-D--glucopyranose (V)

The compound was prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (2.7 g, 5 mmol) and *N-tert*-butoxycarbonyl-*O*-benzyl-L-serine pentachlorophenyl ester (2.71 g, 5 mmol), in the presence of imidazole (1.7 g, 25 mmol), as described for I. The crude product was passed through silica gel (solvent *A*, 10 : 2) to give chromatographically pure V (2.04 g, 50%) which, after crystallisation (3 ×) from ether—light petroleum, yielded crystalline β anomer, m. p. 88.—89 °C, $[\alpha]_D$ + 10°. ν_{max}^{KBr} 3445 (NH), 1765 (C=O), 1725 and 1500 (amide I and II), 1360 (Me_3C), 750, 725, 700 cm^{-1} (aromatic CH). NMR data: τ 2.67—2.82 (m, 5 Ph), 4.30 (d, $J_{1,2}$ 7 Hz, H-1), 4.68 (d, *J* 8 Hz, NH), 8.57 (s, Me_3C).

Anal. $C_{49}H_{55}NO_{10}$ (817.98) calc'd.: C 71.95; H 6.78; N 1.71%
found: C 71.90; H 6.89; N 1.89%

The residue, left after concentration of the mother liquor, was subjected to silica gel chromatography (3 ×) with the same solvent to give pure, slightly faster-moving, α anomer of V as a viscous oil (350 mg), $[\alpha]_D$ + 45°. NMR data: τ 2.68—2.82 (m, 5 Ph), 3.62 (d, $J_{1,2}$ 3 Hz, H-1), 4.63 (d, *J* 8 Hz, NH), 8.58 (s, Me_3C).

Anal. $C_{49}H_{55}NO_{10}$ (817.98) found: C 71.99; H 7.02; N 1.74%

1-O-[N-(tert-Butoxycarbonyl)-L-seryl]-β-D-glucopyranose (VI β)

Catalytic hydrogenation of V β (410 mg, 0.5 mmol) was performed in acetic acid-2-methoxyethanol (2 : 1, 15 cm^3) in the presence of 10% palladium-on-charcoal (400 mg) for ~ 20 h (monitoring by TLC, solvent *B*). After removal of the catalyst and solvent (0.1 Torr), the residue was triturated with dry ether, the precipitated material centrifuged off and dissolved in methanol; subsequent addition of dry ether at 0 °C deposited VI β (110 mg, 60%) as a white hygroscopic solid, $[\alpha]_D$ — 12.5° (water, *c* 2.5). ν_{max}^{KBr} 3380 broad, vs (OH, NH), 1760 (C=O), 1690 and 1520 (amide I and II), 1395 and 1370 cm^{-1} (Me_3C). NMR data (D_2O): τ 4.57 (d, $J_{1,2}$ 7 Hz, H-1), 8.65 (s, Me_3C).

Anal. $C_{14}H_{25}NO_{10}$ (367.36) calc'd.: C 45.77; H 6.85; N 3.77%
found: C 45.65; H 6.98; N 3.77%

A sample (87 mg) of VI β was dissolved in trifluoroacetic acid (98%, 5 cm^3) at 0 °C, and the solution was kept at room temperature for ~ 1 h (monitoring by TLC, solvent *C*) whereupon it was concentrated (0.1 Torr), and the residue was dissolved in methanol. Subsequent addition of dry ether precipitated a white hygroscopic solid (48 mg, 55%) which TLC mobility (R_F ~ 0.8, solvent *C*), IR and NMR spectra were superimposable to those of 1-*O*-(L-seryl)-β-D-glucopyranose trifluoroacetate salt (II *β*) obtained by simultaneous deprotection of I β.

Anal. $C_{11}H_{18}F_3NO_{10}$ (380.92) found: C 34.94; H 4.98%

Treatment of a sample (75 mg) of VI β (75 mg, 0.2 mmol) with acetic anhydride--pyridine (5 : 1, 6 cm^3) overnight at room temperature, followed by concentration (0.1 Torr) of the solvent, gave a solid foam (90.4 mg, 77%) which subsequently crystallised (m. p. 64—66 °C) and was indistinguishable (mixed m. p., IR and NMR spectra) from an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*--acetyl-L-seryl]-β-D-glucopyranose (IX β) prepared by unambigous route.

Anal. $C_{24}H_{35}NO_{15}$ (577.55) calc'd.: C 49.91; H 6.11; N 2.42%
found: C 49.74; H 6.13; N 2.21%

Catalytic Hydrogenation of V α

Catalytic debenzylation of V α (540 mg, 0.66 mmol) was performed as described for VI β to give a ~ 2 : 1 mixture of 1-*O*-[*N*-*tert*-butoxycarbonyl)-L-seryl]-α-D-glucopyranose (VI α) and 2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-seryl]-α-D-glucopyranose (VII α) as a white solid (137 mg, 56%), $[\alpha]_D$ + 34.9° (water). TLC (solvent *B*), R_F 0.73 and 0.66. NMR data (D_2O): τ [3.91 + 4.72 (d, ~ 0.6 H, $J_{1,2}$ 3 Hz) + (d, ~ 0.3 H, $J_{1,2}$ 4 Hz), H-1 of VI α and VII α], 8.60 (s, Me_3C).

Anal. $C_{14}H_{25}NO_{10}$ (367.36) found: C 45.47; H 7.07; N 3.72%

A sample (35 mg) of the above mixture was acetylated as described for the conversion of VI β into IX β, and the crude product was passed through silica gel (solvent *A*, 1 : 2) to give a solid foam (35 mg, 60.5%), identified as a ~ 2 : 1 mixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-α-D-glucopyranose (IX α) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-α-D-glucopyranose (XII α), contaminated with the β anomer of XII. TLC (solvent *A*, 2 : 1): R_F ~ 0.72, 0.66 (major spots) and 0.76 (traces). NMR data: τ [3.68 + + 3.73 (triplet made up of two doublets, ~ 0.9 H, $J_{1,2}$ 3 Hz and 3.5 Hz), H-1], [7.81 + + 7.87 (~ 0.4 × 3 H, *ax* : *eq* AcO-1 ~ 5 : 1), 7.92, 7.96, 8.00 (~ 14 H), 5 O*Ac*], 8.54 (s, Me_3C).

Anal. $C_{24}H_{35}NO_{15}$ (577.55) found: C 49.89; H 6.25; N 2.34%

A sample (75 mg) of the freshly prepared hydrogenolysis product (VI α + VII α) of V α was dissolved in water, and the solution was kept at room temperature for 2 days whereupon it was concentrated, and the residue was submitted to acetylation as described above. Fractionation of the crude product on silica gel (solvent *A*, 2 : 1) separated penta-*O*-acetyl-D-glucopyranose (25 mg) from a ~ 0.5 : 1 : 1 mixture of IX α, XII α and XII β, TLC: R_F ~ 0.66, 0.76 and 0.72, respectively. NMR data: τ [3.68 + 3.72 (triplet made up of two doublets, ~ 0.6 H, $J_{1,2}$ 3 Hz and 3.5 Hz) + 4.53 (d, ~ 0.4 H, $J_{1,2}$ 7 Hz), H-1], [7.81 + 7.87 (~ 0.8 × 3 H, *ax* : *eq* AcO-1 ~ 1 : 1), 7.92, 7.97, 8.00 (~ 13 H), 5 O*Ac*], 8.54 (s, Me_3C).

Anal. $C_{24}H_{35}NO_{15}$ (577.55) found: C 50.21; H 6.07; N 2.26%

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-[*N*-(tert-butoxycarbonyl)-*O*-(benzyl)-L-seryl]-D-glucopyranose (VIII)*

By using 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (630 mg, 1.82 mmol), *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine pentachlorophenyl ester (1.02 g, 1.82 mmol) and imidazole (615 mg, 9.1 mmol), the reaction was performed as described for I. After work-up, the crude product was passed through silica gel (solvent *A*, 1 : 2) to give VIII (1.14 g, 59.7%) as an anomeric mixture. The faster-moving fractions, enriched in the β anomer, were re-chromatographed (2 ×) on silica gel with solvent *A* (4 : 1) to give VIII β, still containing traces of the α anomer; solid foam, $[\alpha]_D$ + 3° (c 3). NMR data: τ 2.74 (Ph), 4.24 (d, $J_{1,2}$ 7 Hz, H-1), 7.99—8.01 4 × O*Ac*, 8.21 (< 1 H, s, AcO-2 of VIII α), 8.56 (s, Me_3C).

Anal. $C_{29}H_{39}NO_{14}$ (625.64) calc'd.: C 55.67; H 6.28; N 2.24%
found: C 55.90; H 6.30; N 2.35%

The residue left in slower-moving fractions was re-chromatographed (2 ×) as just described to give pure α anomer of VIII as a glass, $[\alpha]_D$ + 57,3°. NMR data: τ 2.73 (Ph), 3.63 (d, $J_{1,2}$ 3 Hz, H-1), 7.97, 7.99, 8.00, 8.21 (s, 4 × O*Ac*), 8.56 (s, Me_3C).

Anal. $C_{29}H_{39}NO_{14}$ (625.64) found: C 55.84; H 6.25; N 2.33%

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-[*N*-(tert-butoxycarbonyl)-*O*-(acetyl)-L-seryl]-D-glucopyranose (IX)*

To a solution of VIII (α > β, 450 mg, 0.7 mmol) in 2-methoxyethanol (20 cm^3), 10% palladium-on-charcoal (50 mg) and a few drops (~ 0.3 cm^3) of acetic acid were

added, and the mixture was shaken in an atmosphere of hydrogen ~ 20 h (monitoring by TLC, solvent *A*, 2 : 1). After removal of the catalyst and solvent, the residual viscous oil was dissolved in acetic anhydride-pyridine (1 : 5, 8 cm^3) at 0 °C, and the solution was left overnight at 0 °C whereupon it was concentrated (0.1 Torr), and the residue was passed through silica gel (solvent *A*, 2 : 1) to give chromatographically homogenous IX (272 mg, 67% calc'd. on VIII). The fractions containing the faster-moving β anomer of IX were combined and concentrated; crystallisation of the residue from di-isopropyl ether afforded pure IX β, m. p. 66—68 °C, $[\alpha]_D$ + 7.2°. NMR data: τ 4.26 (d, $J_{1,2}$ 7 Hz, H-1), 7.96, 8.00 (5 × O*Ac*), 8.56 (s, Me_3C).

Anal. $C_{24}H_{35}NO_{15}$ (577.55) calc'd.: C 49.91; H 5.91; N 2.25%
found: C 49.66; H 5.91; N 2.30%

The residue from the slower-moving fractions was subjected to a second silica gel chromatography to give the α anomer of IX as an oil, $[\alpha]_D$ + 68°. NMR data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 7.92, 7.96, 8.00 (5 × O*Ac*), 8.54 (s, Me_3C).

Anal. $C_{24}H_{35}NO_{15}$ (577.55) found: C 49.72; H 6.04; N 2.67%

1,3,4,6-Tetra-O-*acetyl-2*-O-[N-(tert-*butoxycarbonyl*)-O-*(benzyl)*-L-*seryl*]-α-D-*glucopyranose* (*X α*)

The reaction of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (696 mg, 2 mmol) and *N-tert*-butoxycarbonyl-*O*-benzyl-L-serine pentachlorophenyl ester (1.09 g, 2 mmol) in the presence of imidazole (680 mg, 10 mmol)was performed as described for I to give, after work-up and silica gel chromatography (solvent *A*, 2 : 1) of the crude product, the title compound (747 mg, 59.7%) as a solid foam, $[\alpha]_D$ + 70.5°. NMR data: τ 2.79 (Ph), 3.76 (d, $J_{1,2}$ 3 Hz, H-1), 7.86, 7.93, 7.97, 8.18 (4 × O*Ac*), 8.56 (s, Me_3C).

Anal. $C_{29}H_{39}NO_{14}$ (625.64) calc'd.: C 55.67; H 6.28; N 2.24%
found: C 55.56; H 6.35; N 2.10%

1,3,4,6-Tetra-O-*acetyl-2*-O-[N-(tert-*butoxycarbonyl*)-L-*seryl*]-α-D-*glucopyranose (XI α)*

Catalytic hydrogenation of X α (470 mg, 0.75 mmol), performed as described for deprotection of VIII, gave the title compound (374 mg, 93%) as a solid foam. For analysis, a sample was passed through silica gel with solvent *A* (1 : 2); $[\alpha]_D$ + 67°. NMR data: τ 3.73 (d, $J_{1,2}$ 3 Hz, H-1), 7.81, 7.93, 7.98 (4 × O*Ac*), 8.56 (s, Me_3C).

Anal. $C_{22}H_{33}NO_{14}$ (535.51) calc'd.: C 49.34; H 6.21; N 2.62%
found: C 49.53; H 6.46; N 2.74%

1,3,4,6-Tetra-O-*acetyl-2*-O-[N-(tert-*butoxycarbonyl)*-O-*(acetyl)*-L-*seryl*]-α-D-*glucopyranose (XII α)*

Treatment of XI α (242 mg, 0.45 mmol) with acetic anhydride-pyridine (1 : 5, 6 cm^3) at 0 °C overnight, followed by concentration (0.1 Torr) of the reaction mixture and trituration of the residue with light petroleum, gave a solid mass which was dissolved in dry ether; addition of a few drops of light petroleum to the solution, deposited crystals (118 mg, 47.3%) of pure XII α, m. p. 123—125 °C, $[\alpha]_D$ + 80°. NMR data: τ 3.73 (d, $J_{1,2}$ 3.5 Hz, H-1), 7.81, 7.92, 7.97, 8.00 (5 × O*Ac*), 8.54 (s, Me_3C).

Anal. $C_{24}H_{35}NO_{15}$ (577.55) calc'd.: C 49.91; H 6.11; N 2.42%
found: C 50.05; H 6.14; N 2.26%

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SAŽETAK

Sinteza i kemijska svojstva α- i β-D-glukopiranozil estera L-serina i njihovih derivata

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Sintetizirani su potpuno benzilirani α i β anomeri D-glukopiranozil estera *N*-benziloksikarbonil- i *N*-*tert*-butoksikarbonil-*O*-benzil-L-serina (I i V), te su proučavane mogućnosti njihove simultane i postepene deprotekcije u slobodne glikozil estere. 1-*O*-(L-Seril)-α- i β-D-glukopiranoza (II α i II β) izolirane su kao trifluoroacetat soli i karakterizirane postepenim prevođenjem u odgovarajuće *N*-acetil- i per-*O*-acetil derivate III i IV. Katalitičko hidriranje α anomera spoja V rezultiralo je u djelomičnoj 1→2 acil migraciji i kao produkti su identificirani 1-*O*-(*N*-*tert*-butoksikarbonil-L-seril)-α-D-glukopiranoza (VI α) i njen 2-*O*-acil izomer VII (α ≫ β) koji su karakterizirani kao odgovarajući 1- i 2-*O*-acil peracetilirani derivati IX i X, pripravljeni također direktnom sintezom.

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