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Glycosyl Esters of Amino Acids. VI*. Synthesis and Properties of Unprotected Glucosyl and Glucuronic Esters of Glycine and Alanine

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Treatment of 1-*O*-(*N*-*tert*-butyloxycarbonyl-glycyl- and -L--alanyl)-β-D-glucopyranoses with trifluoroacetic acid at —10 °C, led to a clean removal of the BOC-protecting group without affecting the C-1 ester linkage. The corresponding unprotected glucosyl esters I and II were isolated as trifluoroacetate salts and characterized by physical methods and by conversion into the known *N*-acetyl derivatives III and IV. In the glucuronic ester series, conditions for selective deprotection of the sugar moiety were examined with benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-acetyl-L-alanyl- and -1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranuronates (V and VI). Catalytic hydrogenation of the β anomers of V and VI gave the corresponding 1-*O*-acylaminoacyl-β-D-glucopyranuronic acids VII and VIII; for characterization purposes, VIII was converted into methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranuronate (IX-β), prepared also by an alternative route. Complete deprotection of VIII afforded 1-*O*-(L-alanyl)-β-D-glucopyranuronic acid trifluoroacetate salt (X) which was also obtained by catalytic hydrogenation of benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-L-alanyl)-β-D--glucopyranuronate (XI) in the presence of trifluoroacetic acid. Selective deprotection of the hydroxyl and carboxyl functions in the α anomer of VI by catalytic hydrogenation, led to a concomitant 1 → 2 acyl migration; the rearrangement product was characterized as methyl 1,3,4-tri-*O*-acetyl-2-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (XII), highly enriched in the α anomer.

INTRODUCTION

In previous reports from this laboratory[1,2], the synthesis of protected glucosyl and glucuronic esters of amino acids was described. The compounds were 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 acids in the presence of dicyclohexylcarbodi-imide (DCC). Selective removal of the benzyl blocking groups from the sugar moiety was studied in the glucosyl ester series, and a number of 1-*O*-acylaminoacyl-D--glucopyranoses was prepared and characterized[3]. In the case of the α anomers of 1-*O*-(*tert*-butyloxycarbonylaminoacyl)-D-glucopyranoses a subsequent 1 → 2 acyl migration, proceeding presumably *via* an ortho-ester intermediate, was

* Part V: Ref. 3.

established[3]. Selective removal of the benzyloxycarbonyl group from the aglycon amino function was examined in the glucuronic ester series, and the preparation of the crystalline mono-oxalate salt of methyl tri-*O*-methyl-1-*O*-glycyl-β-D-glucofuranuronate was described[4].

Studies on the simultaneous deprotection of amino and hydroxyl functions were initiated with the β anomer of tetra-*O*-benzyl-1-*O*-(1-benzyl *N*-benzyloxycarbonyl-L-aspart-4-oyl)-D-glucopyranose; it was presumed that the presence of a free carboxylic group and the remoteness of the amino group from the ester bond will add to the stability of the glucosidic ester linkage. In fact, catalytic hydrogenation of this compound afforded 1-*O*-(L-β-aspartyl)-β-D-glucopyranose which was fully characterized[3].

The present paper deals with an extension of this research to the synthesis of unprotected glucosyl and glucuronic esters containing a neutral α amino acid as the aglycon — the first model compounds of this type. In the glucuronic ester series we first had to examine whether selective deprotection of the hydroxyl and carboxyl functioons in the sugar moiety could be achieved without affecting the 1-ester linkage. The results obtained are reported and compared to those for the corresponding glucosyl esters.

RESULTS AND DISCUSSION

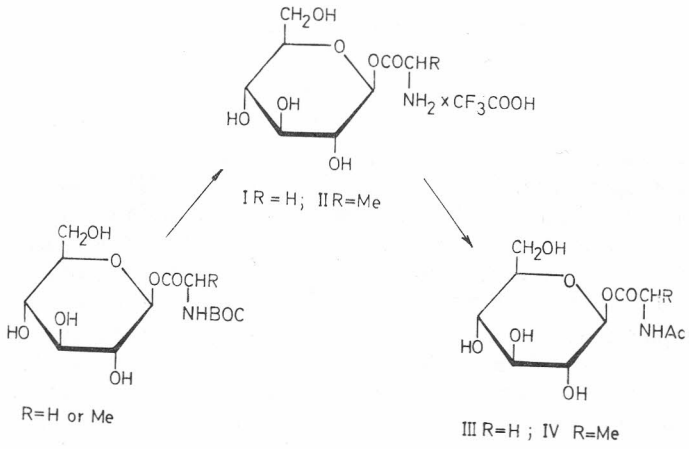
Conditions for the complete deprotection were first examined with the β anomers of fully benzylated D-glucosyl esters having the aglycon amino function blocked by the *tert*-butyloxycarbonyl (BOC) group. Recently the BOC nitrogen protective group has become widely used in peptide synthesis, mainly due to the relatively mild acidic conditions required for its removal[5], and it appeared that this property might also be advantageous for our objectives. However, numerous attempts to treat tetra-*O*-benzyl-1-*O*-(*N*-*tert*-butyloxycarbonylglycyl-β-D-glucopyranose[2] and tetra-*O*-benzyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranose[2], respectively, alone or in different solvent mixtures, with trifluoroacetic or formic acid have proved unsatisfactory. In all cases the cleavage of the BOC group was either uncomplete or accompanied by fission of the *O*-benzyl ether and C-1 ester bonds.

On the contrary, when the above compounds were first subjected to catalytic hydrogenation, and the resulting hydroxyl-free β-D-glucosyl esters dissolved in 98% trifluoroacetic acid at — 10 °C, a clean and rapid cleavage of the BOC group, without concomitant side-reactions, took place. Hence, under the conditions described, the known[3] 1-*O*-(*tert*-butyloxycarbonylglycyl)-β-D-glucopyranose and 1-*O*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranose afforded 1-*O*-glycyl-β-D-glucopyranose (I) and 1-*O*-(L-alanyl)-β-D-glucopyranose (II), respectively, as cristalline, highly hygroscopic trifluoroacetate salts.

The unprotected glucosyl esters I and II gave elemental analyses and spectral data fully consistent with the structures proposed. The i. r. spectra revealed the presence of absorptions characteristic of hydroxyl and ester functions and indicated an ionic carboxyl absorption (1640 cm^{-1}, shoulder) as well as two characteristic bands in the region of 1680—1520 cm^{-1} associated with the NH_3^+ deformations. The n. m. r. spectra in deuterium oxide showed H-1 signals at the position and with the $J_{1,2}$ value indicative of the β-D-configuration. When kept under anhydrous conditions at room temperature, the crystalline trifluoroacetate salts I and II were reasonably stable; a prominent decomposition into glucose and the parent amino acid could be

detected (TLC) after about three months of storage. However, in aqueous solutions the cleavage of the C-1 ester bond proceeded at a much faster rate (24—48 h), the glucosyl ester of alanine being definitely more susceptible to hydrolysis than the glucosyl ester of glycine.

For further characterization, compounds I and II were subjected to selective acetylation of the aglycon amino function with 2% acetic anhydride in aceton-water to give 1-*O*-(*N*-acetylglycyl)-β-D-glucopyranose (III) and 1-*O*-(*N*-acetyl-L-alanyl)-β-D-glycopyranose (IV), respectively. The pure products were obtained by chromatography on cellulose and their structures were assigned by comparison with III and IV prepared earlier[3] by catalytic hydrogenation of the tetra-*O*-benzyl-β-D-glucosyl esters of *N*-acetylglycine and *N*-acetyl-L-alanine, respectively. (Scheme 1).

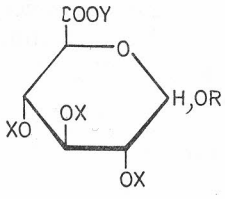


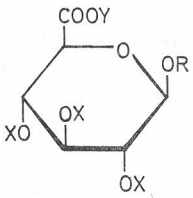
Scheme 1.

In order to check whether the same procedures could be applied in the glucuronic ester series, we have chosen for our deprotection experiments fully benzylated glucuronic esters of *N*-acetyl-, *N*-*tert*-butyloxycarbonyl- and *N*-benzyloxycarbonyl-L-alanine as the starting material.

Benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-acetyl-L-alanyl)-D-glucopyranuronate (V) and benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (VI) were synthesized by the imidazole-promoted condensations of the fully benzylated, C-1 free, glucuronic acid with *N*-acetyl-L-alanine in the presence of DCC and with *N*-*tert*-butyloxycarbonyl-L-alanine pentachlorophenyl ester, respectively. The products were obtained as anomeric mixtures which were resolved by silica gel chromatography and crystallisation. The β anomers of V and VI were crystalline and the corresponding α anomers were viscous oils; the proposed structures were in agreement with elemental analyses, optical rotatory data, i. r. spectra and n. m. r. evidence. (Scheme 2).

Selective deprotection of the sugar moiety in the β anomers of V and VI was performed by catalytic hydrogenation in methoxyethanol over palladium-on-charcoal in the presence of acetic acid. The products gave elemental analyses required for 1-*O*-(*N*-acetyl-L-alanyl)-β-D-glucopyranuronic acid (VII) and 1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranuronic acid (VIII),





V X=Y=CH_2Ph ; R=Ac-Ala-

VI X=Y=CH_2Ph ; R=BOC-Ala-

IX X=Ac ; Y=Me ; R=BOC-Ala-

VII X=Y=H ; R=Ac-Ala-

VIII X=Y=H ; R=BOC-Ala-

XI X=Y=CH_2Ph ; R=Z-Ala-

Scheme 2.

respectively; the former was obtained in crystalline form and the latter as a solid hygroscopic foam. The i. r. spectra of VII and VIII revealed the presence of hydroxyl absorption and the absence of bands associated with the aromatic ring, and the n. m. r spectra in deuterium oxide showed the anomeric proton as one doublet at position and with coupling constant indicative for the β-D-configuration and consistent with the observed optical rotation.

As compared to the analogous β-D-glucosyl esters, glucuronic esters VII and VIII showed to be less stable compounds. On the other hand, a remarkable difference in stability between VII and VIII, obviously due to the nature of the amino-protecting group, was observed. When kept dry under anhydrous conditions, the glucuronic ester VII, containing the *N*-acetylalanyl residue, revealed (TLC) the presence of glucuronic acid, glucurolactone and acetylalanine after about two months, while VIII, containing the BOC-alanyl residue, decomposed to about the same extent already within two weeks of storage.

For further characterization VIII was subjected to esterification with diazomethane, followed by acetylation with acetic anhydride in pyridine to give the crystalline methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L--alanyl)-β-D-glucopyranuronate (IX-β). The assignment of the structure to IX-β was definitely confirmed by comparison of its n. m. r. spectrum (Table I) with that of the β anomer of IX prepared by an independent route as described below. (Scheme 3).

The alternative synthesis of the anomeric mixture of methyl 2,3,4-tri-*O*--acetyl-1-*O*-(*N*-tert-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (IX) was achieved by the imidazole-promoted DCC condensation of methyl 2,3,4-tri-*O*--acetyl-D-glucopyranuronate[6] with *N*-*tert*-butyloxycarbonyl-L-alanine. The product, after column chromatography and fractional crystallisation, afforded the pure α anomer of IX and a fraction highly enriched in the β anomer which could not be completely resolved. The compounds were fully characterized and their n. m. r. parameters are given in Table I.

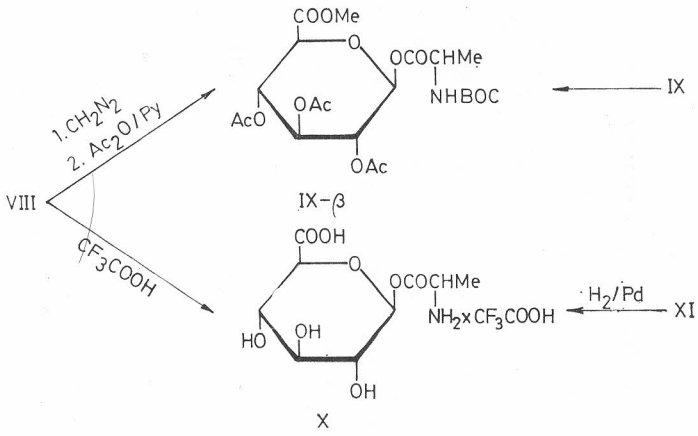
In principle, complete deprotection of a fully blocked glucuronic ester of amino acid can proceed, depending upon the nature of protecting groups, either

TABLE I

*N. m. r. Parameters of the Peracetylated Methyl 1- and 2-*O-(N-tert-*butyloxycarbonyl-*L-*alanyl)-*D-*glucopyranuronates*

Compounds and Anomeric Form	Solvent[a]	Chemical Shifts (τ values)[b] and Assignments				
		H-1 Doublet ($J_{1,2}$/Hz)	COOMe[c]	OAc[d]	Me_3C[e]	*Me*-CH[f]
Methylated and Peracetylated Product of VIII	A	4.21(7)	6.28	7.97, 7.99[2][g]	8.60	8.60[h]
IX-β[i]	A	4.20(7)	6.28	7.97, 7.99[2][g]	8.60	8.60[h]
IX-α	A	3.60(3)	6.28	7.98[2][g], 8.02	8.60	8.60[h]
	B	3.76(3)	6.38	8.03—8.07[3][g]	8.63	8.71[h]
Methylated and Peracetylated Hydrogenolysis product of VI-α (XII)	A	3.62(3) + 4.37(8.5)[j]	6.28	7.82 + 7.88[k], 7.98—8.00[2][g]	8.60	8.72
	B	3.81(3) + 3.99(8)[j]	6.38	7.84 + 7.95[k], 8.03[2][g]	8.68	8.84

[a] A = chloroform-*d*, B = methyl sulphoxide-d_6. [b] Data taken from spectra measured at 60 MHz. [c] Three-proton singlets. [d] Three-proton singlets unless otherwise indicated. [e] Nine-proton singlets. [f] Three-proton doublets, J=7 Hz. [g] Unresolved, the number in parentheses indicates the number of acetyl groups. [h] Overlapped by the Me_3C signal. [i] Not completely resolved from the α anomer. [j] Integrating for one proton, α : β ratio ~ 4 : 1. [k] Integrating for one OAc signal, relative intensity ~ 4 : 1.

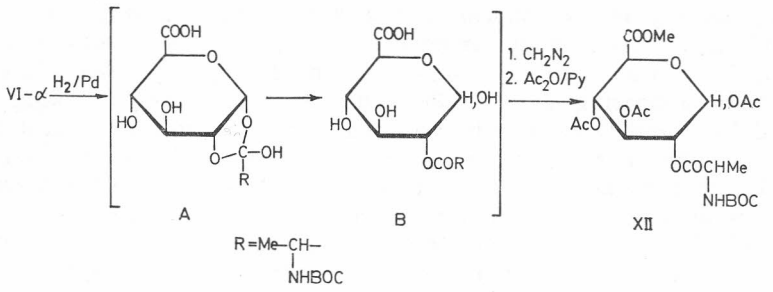


Scheme 3.

in a stepwise manner through different types of reactions, or simultaneously in one-type reaction. We have examined both approaches by employing 1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranuronate (VIII) and the known[1] benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-L-alanyl)-β-D-glucopyranuronate (XI) as the starting material.

Treatment of VIII with trifluoroacetic acid, under conditions used in preparation of unprotected glucosyl esters I and II, led to a rapid scission of the BOC protecting group. The product was obtained as a white hygroscopic solid with no definite melting point in 57.8% yield; on TLC it revealed one major ninhydrin- and silver nitrate-positive spot together with traces of glucuronic acid, glucurolactone and alanine. Several attempts of recrystallization resulted in extensive degradation, and consequently efforts to abtain an analytical sample of 1-*O*-(L-alanyl)-β-D-glucopyranuronic acid trifluoroacetate (X) failed. The n. m. r spectrum of the product in deuterium oxide revealed the anomeric proton as one doublet at $\tau = 4.33$ with the splitting of 7 Hz and the alanine methyl group as a doublet at $\tau = 8.41$ ($J = 7$ Hz); the observed optical rotation, $[\alpha]_D$ — 21.8° in water was also consistent with the β-D--configuration.

Hydrogenolysis of the fully benzylated glucuronic ester of *N*-benzyloxycarbonyl-L-alanine (XI) was performed in methoxyethanol with palladium-on--charcoal in the presence of an excess of trifluoroacetic acid. In order to prevent hydrolysis of the C-1 ester bond, the reaction was conducted in two successive steps: 1. removal of the *N*-benzyloxycarbonyl group by passing a stream of hydrogen through the reaction mixture and 2. removal of the benzyl ether and ester groups by shaking the mixture with hydrogen in a closed system. The isolated product showed on TLC one spot which was coincident with that of the major product formed from VIII with trifluoroacetic acid. In addition, the compound gave elemental analysis required for 1-*O*-(L-alanyl)--β-D-glucopyranuronic acid trifluoroacetate salt (X) and had $[\alpha]_D$ — 20.0° in water. The assignment of the anomeric structure to X was further supported by i. r. and n. m. r. spectra which were superimposable upon those of the product obtained from VIII.



Scheme 4.

Aqueous and particularly acetic acid solutions of X were highly labile and underwent complete hydrolysis within the first 24 h. However, in dry state under anhydrous conditions the compound decomposed to only about 50% after one month of storage.

Earlier results[3] have shown that the α anomers of D-glucopyranosyl esters, containing a BOC-protected amino acid as the aglycon, underwent the $1 \rightarrow 2$ acyl migration. In order to examine whether such a rearrangement also takes place in the α-D-glucopyranuronic ester series, we submitted benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-α-D-glucopyranuronate (VI-α) to catalytic hydrogenation under conditions used in debenzylation of VI-β. The product obtained revealed on TLC a major ninhydrin-and silver nitrate-positive component which underwent successive decomposition during chromatography on silica gel.

However, when the crude hydrogenolysis product of VI-α was immediately subjected to esterification followed by acetylation, the resulting mixture afforded, after purification by silica gel chromatography, a stable, crystalline product. Its elemental analysis was in agreement with the peracetylated methyl *tert*-butyloxycarbonylalanyl glucopyranuronate structure. On TLC the compound had practically identical mobility as methyl 2,3,4-tri-*O*-acetyl-1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (IX), but its n. m. r. spectrum in deuterated chloroform and dimenthylsulphoxide, respectively, differed from the spectra of IX-α and IX-β and was indicative for the methyl 1,3,4-tri-*O*-acetyl-2-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (XII) structure. The pertinent n. m. r signals in the spectra of the methylated and peracetylated hydrogenolysis product of VI-α, IX-α and IX-β are recorded in Table I.

The n. m. r. spectrum of the methylated and peracetylated derivative XII showed the anomeric proton as two separate doublets of different intensities. The signal assigned to the α anomer strongly predominated, the α : β ratio being about 4 : 1. In addition, one of the signals assigned to the acetyl methyl protons was shifted downfield, whereas in the spectra of IX-α as well as of IX-β the signals of all the three acetyl groups were concentrated in a narrow region at higher field. Following the well established principle[7] that in an anomeric pair of acetylated pyranoses the axial 1-*O*-acetyl signal resonates at lower field than the equatorial, the signal appearing in the spectrum of XII at $\tau = 7.82$ and 7.84, respectively, may be assigned to the

axial 1-acetoxyl group. Furthermore, the relative peak intensity of the axial 1-*O*-acetyl signal reflected the anomeric proportion of XII deduced from the mutual ratio of the doublets assigned to the H-1 proton. Accordingly, the weak singlet appearing at $\tau = 7.88$ and 7.95, respectively, lacking in the spectra of IX-α and IX-β, may be ascribed to the equatorial 1-*O*-acetyl group of XII. The other n. m. r signals as well as the optical rotatory data of the methylated and peracetylated hydrogenolysis product of VI-α were fully consistent with structure XII, highly enriched in the α anomer.

According to the generally accepted mechanism of acyl migration in partially acylated polyhydroxylic systems[8,9], the rearrangement of the unprotected α anomer of glucuronic ester of BOC-alanine should proceed *via* a cyclic ortho-ester intermediate *A* as depicted in the Scheme 4. The formation of a low amount of the β anomer of the peracetylated methyl derivative XII may be rationalized by assuming that the migration of the BOC-alanyl group is followed by subsequent anomerization of that form (presumably α) which was initially released from the cyclic intermediate *A*.

EXPERIMENTAL

Melting points are uncorrected. Evaporations were perfromed in a rotary evaporator *in vacuo* at bath temperature below 40 °C, if not stated otherwise. Specific rotations were measured at 20—23 °C, as 1% solutions in chloroform, if not stated otherwise. 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. TLC was conducted on plates coated with Kieselgel G (Merck), if not stated otherwise, followed by detection with 10% sulphuric acid and heating, with ninhydrin reagent or alkaline silver nitrate. Solvent systems (by volume): *A* chloroform—methanol—water (15 : 4 : 1); *B* acetonitrile—water (3 : 1); *C* benzene--ethyl acetate (proportions are given in the text); *D* *n*-butanol—acetic acid—water (12 : 3 : 5); *E* *iso*propanol—petroleum ether—water (5 : 3 : 1); *F* chloroform—methanol—acetic acid (15 : 4 : 1).

I. r. spectra were determined on a Perkin-Elmer Model 137 spectrometer. N. m. r spectra were recorded in solutions of chloroform-*d* unless otherwise stated, with tetramethylsilane as internal stanard, using a Varian A-60 A spectrometer.

1-O-Glycyl-β-D-glucopyranose trifluoroacetate salt (I)

To 1-*O*-(*tert*-butyloxycarbonylglycyl)-β-D-glucopyranose[1] (337 mg, 1 mmol), 98% trifluoroacetic acid (2 ml) was added at —10 °C, and the solution was kept at this temperature for 30 min (monitoring by TLC, solvent system *A*). After removal of trifluoroacetic acid, the residue was triturated with dry ether to give a solid which was recrystallized from *iso*propanol-dry ether. On cooling, pure I (318 mg, 90.5%) deposited as hygroscopic crystals with no definite melting point, $[\alpha]_D + 10.7^0$ (water). ν_{max}^{KBr} 3450 vs (OH and NH), 1760 vs (C=O), 1670 vs and 1525 (NH_3^+ deformations), 1640 sh (ionized trifluoroacetic acid carboxyl), 1070 cm^{-1} vs (C—O—C). N. m. r. data (D_2O): $\tau = 4.36$ (1-proton doublet, $J_{1,2} = 7$ Hz, H-1).

Anal. $C_{10}H_{16}F_3NO_9$ (351.24) calc'd.: C 34.19; H 4.59; N 3.99%
found: C 34.12; H 4.82; N 4.08%

N-Acetylation of compound I (222 mg, 0.63 mmol) in water (50 ml) was performed with a 2% solution of acetic anhydride in acetone (50 ml) at room temperature. After completion of the reaction (~ 3 h, monitoring by TLC in solvent system *B*), the solvent was evaporated (0.1 Torr, bath 30 °C), traces of the anhydride were removed by repeated addition and distillation of ethanol, and the oily residue was eluted from a column (60 × 1.2 cm) of cellulose (30 g) with solvent system *B*. Chromatographically homogeneous fractions were pooled and evaporated to dryness to give 1-*O*-(*N*-acetylglycyl)-β-D-glucopyranose (III, 120 mg, 69%) as a solid foam, $[\alpha]_D - 8.8^0$ (water).

Anal. $C_{10}H_{17}NO_8$ (279.24) calc'd.: C 43.01; H 6.15; N 5.01%
found: C 42.95; H 6 03; N 5.11%

The i. r. and n. m. r. spectra of III were superimposable upon those of the product obtained[3] by catalytic hydrogenation of 1-*O*-(*N*-acetylglycyl)-2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranose.

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

Deprotection of 1-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranose[2] (351 mg, 1 mmol) with trifluoroacetic acid was conducted as described above; the product was crystallised from *iso*propanol-dry ether to yield II (261 mg, 71.5%) as hygroscopic crystals with no definite melting point, $[\alpha]_D + 6.9^0$ (water). ν_{max}^{KBr} 3450 vs (OH and NH), 1770 s (C=O), 1680 vs and 1520 w (NH_3^+ deformations), 1640 sh (ionized trifluoroacetic acid carboxyl), 1070 cm^{-1} vs (C—O—C). N. m. r. data (D_2O): τ 4.27 (1-proton doublet, $J_{1,2} = 7$ Hz, H-1), 8.42 (3-proton doublet, $J = 8$ Hz, Me—CH).

Anal. $C_{11}H_{18}F_3NO_9$ (365.26) calc'd.: C 36.17; H 4.97; N 3.83%
found: C 36.00; H 4.92; N 3.96%

N-Acetylation of compound II (160 mg, 0.44 mmol) was performed as described for III; the crude product was eluted from a cellulose column with solvent system *B* to give 1-*O*-(*N*-acetyl-L-alanyl)-β-D-glucopyranose (IV, 54 mg, 51%) as a solid foam, $[\alpha]_D - 47.0^0$ (water).

Anal. $C_{11}H_{19}NO_8$ (293.28) calc'd.: C 45.05; H 6.53; N 4.77%
found: C 45.29; H 6.67; N 4.48%

The i. r. and n. m. r. spectra of IV were superimposable upon those of the product obtained[3] by catalytic hydrogenation of 1-*O*-(*N*-acetyl-L-alanyl)-2,3,4,6-tetra-*O*--benzyl-β-D-glucopyranose.

Benzyl 1-O-(N-acetyl-L-alanyl)-2,3,4-tri-O-benzyl-D-glucopyranuronate (V)

To a solution of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[10] (555 mg, 1 mmol), *N*-acetyl-L-alanine (144 mg, 1.1 mmol) and imidazole (136 mg, 2 mmol) in dichloromethane-dimetylformamide (4 : 1, 10 ml), dicyclohexylcarbodi-imide (DCC, 206 mg, 1 mmol) in dichloromethane (5 ml) was added at 0 °C under shaking. After 1 h, more *N*-acetyl-L-alanine (13 mg) was added, and the reaction mixture was left at room temperature for additional 20 h. Dicyclohexylurea was filtered off, washed with dichloromethane, and the combined filtrate and washings were poured onto ice; the organic layer was washed with water, 1.5% sulphuric acid, water, aqueous sodium hydrogen carbonate and water, and dried (sodium sulphate). After evaporation of the solvent the residue was dissolved in a minimal amount of solvent system *C* (2 : 1), the remaining dicyclohexylurea was filtered off, and the filtrate was passed through a silica gel column (40 × 0.8 cm) with the same solvent. Combination and concentration of the appropriate fractions (monitoring by TLC) gave the chromatographically homogeneous anomeric mixture of V (259 mg, 38.7%).

The β-D-anomer of V was obtained by dissolution of the anomeric mixture in benzene and subsequent precipitation with petroleum ether; yield: 126 mg. A second crystallization afforded pure V-β, m. p. 139—140 °C, $[\alpha]_D - 18.2^0$. ν_{max}^{KBr} 3400 vs (NH), 1750 vs and 1775 vs (C=O), 1650 vs (amide I), 1570 m (amide II), 1100 vs (C—O—C), 735 m and 698 cm^{-1} vs (aromatic CH). N. m. r. data: τ 2.59—2.82 (multiplet, 20 H, 4 × Ph), 3.98 (1-proton doublet, $J = 7$ Hz, NH), 4.26 (1-proton doublet, $J_{1,2} = 7$ Hz, H-1), 8.04 (3-proton, singlet, N—Ac), 8.61 (3-proton doublet, $J = 7$ Hz, Me—CH).

Anal. $C_{39}H_{41}NO_9$ (667.73) calc'd.: C 70.15; H 6.19; N 2.10%
found: C 70.01; H 6.43; N 2.06%

The mother liquors were evaporated to dryness, and the oily residue was re-chromatographed on silica gel as described above. The fractions containing the slightly faster moving α-D-anomer of V were pooled and evaporated to dryness:

oil, $[\alpha]_D$ + 40.5°. N. m. r. data: τ 2.59—2.82 (multiplet, 20 H, 4 × Ph), 3.60 (1-proton doublet, $J_{1,2}$ = 3 Hz, H-1), 3.92 (1-proton doublet, J = 7 Hz, NH), 8.00 (3-proton singlet, N—Ac), 8.60 (3-proton doublet, J = 7 Hz, Me—CH).

Anal. $C_{39}H_{41}NO_9$ (667.73) found: C 69.94; H 6.12; N 2.16%.

Benzyl 2,3,4-tri-O-benzyl-1-O-(N-tert-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (VI)

Benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (555 mg, 1 mmol), *N-tert*-butyloxycarbonyl-L-alanine pentachlorophenyl ester (482 mg, 1.1 mmol) and imidazole (340 mg, 5 mmol) were subsequently dissolved in dry dichloromethane (10 ml) at room temperature. The progress of the reaction was monitored by TLC in solvent *C* (10 : 1) and after about 1 h an additional amount of the amino acid component (44 mg) was added under shaking. After 24 h, the precipitated pentachlorophenol was filtered off, washed with dichloromethane, and the combined filtrate and washings were poured onto ice. The organic layer was worked up as described for V, except that washing with sulphuric acid was replaced with 10% citric acid. After removal of the solvent, the crude product was submitted to silica gel column chromatography in solvent system *C* (10 : 1) to give VI (418 mg, 57.6%) as a chromatographically homogeneous anomeric mixture.

The β-D-anomer of VI was obtained by crystallisation of the anomeric mixture from ethanol; yield: 221 mg, m. p. 129—130 °C, $[\alpha]_D$ — 6.0°. ν_{max}^{KBr} 3440 m (NH), 1780 s and 1740 s (C=O), 1520 s (amide II), 1360 m (Me_3C), 1100 vs (C—O—C), 755 s and 699 cm^{-1} s (aromatic CH) N. m. r. data: τ 2.59—2.82 (multiplet, 20 H, 4 × Ph), 4.24 (1-proton doublet, $J_{1,2}$ = 7 Hz, H-1), 8.50—8.74 (singlet, 12 protons, Me_3C + Me—CH).

Anal. $C_{42}H_{47}NO_{10}$ (725.80) calc'd.: C 69.50; H 6.53; N 1.93%
found: C 69.40; H 6.46; N 1.86%

The mother liquor was evaporated to dryness, and the residue was re-chromatographed on a silica gel column with solvent system *C* (10 : 1). Elution afforded the slightly faster moving α anomer of VI as a viscous oil, $[\alpha]_D$ + 40.0°. ν_{max}^{film} 3450 m (NH), 1760 vs and 1730 vs (C=O), 1510 s (amide II), 1365 s (Me_3C), 736 s and 699 cm^{-1} s (aromatic CH). N. m. r. data: τ 2.59—2.82 (multiplet, 20 H, 4 × Ph), 3.60 (1-proton doublet, $J_{1,2}$ = 3 Hz, H-1), 8.55—8.75 (singlet, 12 protons, Me_3C + Me—CH).

Anal. $C_{42}H_{47}NO_{10}$ (725.80) found: C 69.61; H 6.59; N 1.85%

1-O-(N-Acetyl-L-alanyl)-β-D-glucopyranuronic Acid (VII)

The β anomer of V (231 mg, 0.346 mmol) was dissolved in methoxyethanol (12 ml) and to the solution 10% Pd/C (Fluka, *puriss.*, 231 mg) and acetic acid (0.3 ml) were added; the mixture was shaken with hydrogen at room temperature and pressure until the uptake of hydrogen was complete (monitoring by TLC in solvent system *D*). The catalyst was centrifuged off, the supernatant was evaporated to dryness (0.1 Torr, bath 30 °C), and the residual syrup was crystallized from *iso*propanol-ether to afford analytically pure VII. Yield: 58.5 mg, 55.2%, m. p. 135—136 °C, $[\alpha]_D$ — 59.0° (water). ν_{max}^{KBr} 3440 vs, broad (OH, NH), 1770 s and 1720 s (C=O), 1600 s (amide I), 1550 vs (amide II), 1080 (C—O—C) cm^{-1} vs. N. m. r. data (D_2O): τ 4.39 (1-proton doublet, $J_{1,2}$ = 7 Hz, H-1), 8.00 (3-proton singlet, N—Ac), 8.58 (3-proton doublet, J = 7 Hz, Me—CH).

Anal. $C_{11}H_{17}NO_9$ (307.25) calc'd.: C 42.96; H 5.58; N 4.56%
found: C 42.99; H 5.78; N 4.81%

1-O-(N-tert-butyloxycarbonyl-L-alanyl)-β-D-glucopyranuronic Acid (VIII)

The β anomer of VI (170 mg, 0.377 mmol) was catalytically hydrogenated in methoxyethanol (7 ml) in the presence of 10% Pd/C (100 mg) and acetic acid (0.11 ml) as described for VII; the progress of the reaction was monitored by TLC in the solvent system *E*. After removal of the catalyst and the solvent (0.1 Torr, bath 30 °C),

the residue was dissolved in dry ethanol. Subsequent addition of dry ether at 0 °C led to precipitation of contaminant glucuronic acid and alanine (monitoring by TLC in solvent systems *E* and *F*). After centrifugation, the supernatant was evaporated to dryness to give pure VIII as a hydroscopic solid foam. Yield: 90 mg, 65.8%, $[\alpha]_D$ — 40.0° (*c* 0.5, MeOH). ν_{max}^{KBr} 3440 vs, broad (OH, NH), 1750 vs (C=O), 1530 vs (amide II), 1370 cm^{-1} s (Me_3C). N. m. r data (D_2O): τ 4.29 (1-proton doublet, $J_{1,2}$ = = 7 Hz, H-1), 8.56 (9-proton singlet, Me_3C), 8.58 (3-proton doublet, J = 7 Hz, partly masked by Me_3C signal, Me—CH).

Anal. $C_{14}H_{23}NO_{10}$ (365.33) calc'd.: C 46.02; H 6.35; N 3.83%
found: C 45.86; H 6.34; N 3.63%

*Conversion of VIII into Methyl 2,34-tri-O-acetyl-1-O-(*N*-tert-butyloxycarbonyl--L-alanyl)-β-D-glucopyranuronate (IX-β)*

A sample of VI-β (375 mg, 0.517 mmol) was catalytically debenzylated as described above, and the crude VIII (236 mg) was immediately treated with diazomethane in ether (40 ml) at 0 °C. After about 1.5 h the solvent was removed, and to the oily residue a precooled mixture of acetic anhydride-dry pyridine (1 : 5, 6 ml) was added at 0 °C. After standing at 0 °C for about 16 h (monitoring by TLC in the solvent system *C*, 2 : 1), the mixture was evaporated to dryness (bath < 30 °C, 0.1 Torr), traces of pyridine were removed by repeated addition and evaporation of water, and the residue was dissolved in dry ether. Addition of few drops of petroleum ether at 0 °C deposited some impurities which were centrifuged off; addition of more petroleum ether deposited chromatographically homogeneous IX-β (110 mg, 42.2%, calc'd. on VI-β). After a second crystallization from the same solvent the compound was analytically pure: m. p. 62—64 °C, $[\alpha]_D$ — 5.13°. ν_{max}^{KBr} 3485 (NH), 1770 and 1720 s (C=O), 1520 m (amide II), 1375 cm^{-1} s (Me_3C). N. m. r. data are given in Table I.

Anal. $C_{21}H_{31}NO_{13}$ (505.47) calc'd.: C 49.90; H 6.18; N 2.77%
found: C 49.91; H 6.14; N 2.81%

*Methyl 2,3,4-tri-O-acetyl-1-O-(*N*-tert-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (IX)*

To a solution of methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate[6] (1670 mg, 5 mmol), *N-tert*-butyloxycarbonyl-L-alanine (946 mg, 5 mmol) and imidazole (681 mg, 10 mmol) in dichloromethane (15 ml), a solution of DCC (1030 mg, 5 mmol) in dichloromethane (5 ml) was added at 0 °C under shaking. After standing for 24 h at room temperature, the precipitated dicyclohexylurea was filtered off, and the filtrate was worked up as described for V, except that washing with sulphuric acid was replaced with 10% citric acid. After removal of the solvent, the oily residue was passed through a silica gel column with solvent system *C* (2 : 1) to give IX (767 mg, 30.4%) as a chromatographically homogeneous anomeric mixture. Dissolution in ethyl acetate followed by subsequent addition of petroleum ether at 0 °C afforded crystals consisting predominantly of the α anomer of IX; after 3 subsequent recrystallizations the compound had m. p. 133—134 °C and $[\alpha]_D$ + 86.0°. ν_{max}^{KBr}: 3460 m (NH), 1750 vs and 1720 s (C=O), 1520 m (amide II), 1370 cm^{-1} m (Me_3C). N.m.r. data: τ 3.60 (1-proton doublet, $J_{1,2}$ = 3 Hz, H-1), 6.28 (3-proton singlet, OMe), 8.03—8.07 (singlet, 9 H, 3 × OAc), 8.63 (9-proton singlet, Me_3C), 8.71 (3-proton doublet, J = 7 Hz, partly masked by Me_3C signal, Me—CH).

Anal. $C_{21}H_{31}NO_{13}$ (505.47) calc'd.: C 49.90; H 6.18; N 2.77%
found: C 49.68; H 6.30; N 2.75%

The mother liquors were evaporated to dryness, and the residue was submitted to a second silica gel chromatography with solvent *C* (2 : 1); fractions with the lowest optical rotation were pooled, evaporated to dryness, and the residue was crystallized from ethyl acetate-petroleum ether. Crystals with m. p. 58—60 °C, $[\alpha]_D$ + 9.3° containing preponderantly the β anomer of IX deposited. ν_{max}^{KBr} 3450 m

(NH), 1760 vs and 1710 s (C=O), 1520 m (amide II), 1370 cm^{-1} s (Me_3C). N. m. r data: τ 3.58 and 4.20 (doublets, 1 H, $J_{1,2} = 3$ Hz and $J_{1,2} = 7$ Hz, H-1), 6.27 (3-proton singlet, OMe) 8.00—8.05 (singlet, 9 H, 3 × OAc), 8.60 (singlet, 12 H, Me_3C + *Me*—CH overlapped).

Anal. $C_{21}H_{31}NO_{13}$ (505.47) found: C 49.93; H 5.96; N 2.74%

1-O-(L-Alanyl)-β-D-glucopyranuronic Acid Trifluoroacetate Salt (X)

(a) From 1-*O*-(*N*-*tert*-butyloxycarbonyl-L-alanyl)-β-D-glucopyranuronic acid (VIII). — A sample of pure VIII (80 mg, 0.219 mmol) was dissolved in 98%-trifluoroacetic acid (1.2 ml) at —10 °C, and the solution was kept at —10 °C for 45 min (monitoring by TLC, cellulose plates, solvent system *B*) whereupon the solvent was removed and the oily residue was triturated with dry ether. To the semi-solid mass warm *iso*propanol was added; on cooling and scratching X deposited as a white solid (37 mg) which was centrifuged off. Addition of dry ether to the supernatant precipitated a second crop of X (11 mg, total yield 57.8%). $[\alpha]_D$ —21.8° (*c* 0.55, water). ν_{max}^{KBr}: 3430 vs, broad (OH), 1795 vs (C=O), 1600 cm^{-1} vs (NH_3^+ deformation and ionized trifluoroacetic acid carboxyl). N. m. r. data (D_2O): τ 4.33 (1-proton doublet, $J_{1,2} = 7$ Hz, H-1), 8.41 (3-proton doublet, $J = 7$ Hz, Me—CH)

(b) From benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-L-alanyl)-β-D-glucopyranuronate[1] (XI). — To a suspension of XI (730 mg, 0.963 mmol) in methoxyethanol (25 ml) were added 10% Pd/C (70 mg) and trifluoroacetic acid (2.2 ml). Hydrogen was passed through the stirred suspension until evolution of carbon dioxyde [$Ba(OH)_2$ solution] ceased (~ 4 h). The reaction was discontinued, additional catalyst (660 mg) was added, and the mixture was shaken with hydrogen at atmospheric pressure and room temperature until termination of hydrogen uptake (monitoring by TLC, cellulose plates, solvent system *B*).

The catalyst was filtered off, the filtrate was concentrated (0.1 Torr, bath 30 °C) to about 3 ml, and on addition of dry ether an oil precipitated which was centrifuged off. Treatment of the residue with *iso*propanol as described under (a) afforded pure X (total yield 130 mg, 34.5%), $[\alpha]_D$ —20.0° (water). The i. r. and n. m. r. spectra were superimposable upon those of the product obtained by (a).

Anal. $C_{11}H_{16}F_3NO_{10}$ (379.25) calc'd.: C 34.84; H 4.25; N 3.69%
found: C 34.95; H 4.39; N 3.94%

Catalytic Hydrogenation of VI-α and Direct Conversion of the Product into Methyl 1,3,4-tri-O-acetyl-2-O-(N-tert-butyloxycarbonyl-L-alanyl)-D-glucopyranuronate (XII)

The α anomer of VI (150 mg, 0.207 mmol) was catalytically hydrogenated as described for VIII. After removal of the catalyst and evaporation of the solvent, the crude product revealed (TLC, cellulose plates, solvent system *E*) one major (R_f ~ 0.5) silver nitrate- and ninhydrin-positive spot and traces of two compounds coincident with glucuronic acid and *tert*-butyloxycarbonylalanine, respectively.

The crude product (59 mg) was immediately treated with an etheral solution of diazomethane and then with acetic anhydride-pyridine as described for IX-β. After removal of pyridine-acetic anhydride, the residue was chromatographed on a silica gel column with solvent *C* (2 : 1). Chromatographically homogeneous fractions were pooled and evaporated to dryness to give 34 mg (42% calc'd. on VI-α) of a white solid. After one crystallization from ether-petroleum ether, the compound melted at 63—65 °C, partly resolidified, and then melted at 100—103 °C, $[\alpha]_D$ + 54.0°. ν_{max}^{KBr} 3480 m (NH), 1770 vs and 1720 s (C=O), 1525 m (amide II), 1380 cm^{-1} s (Me_3C). N. m. r. data are given in Table I.

Anal. $C_{21}H_{31}NO_{13}$ (505.47) calc'd.: C 49.90; H 6.18; N 2.77%
found: C 49.64; H 6.15; N 2.78%

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SAŽETAK

Glikozil-esteri aminokiselina. VI. Sinteza i karakteristike nezaštićenih glukozil- i glukuronskih estera glicina i alanina

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Deprotekcija amino funkcije u 1-*O*-(*N*-*tert*-butiloksikarbonil-glicil- i -L-alanil)-β-D-glukopiranozama sa trifluoroctenom kiselinom, pod uvjetima koji ne dovode do cijepanja C-1 esterske veze, rezultirala je u nezaštićenim D-glukozil esterima glicina i L-alanina. Produkti su izolirani kao trifluoroacetat soli i karakterizirani prevođenjem u odgovarajuće *N*-acetil derivate.

U redu D-glukuronskih estera aminokiselina uvjeti za selektivnu deprotekciju šećernog dijela molekule ispitivani su na benzil 2,3,4-tri-*O*-benzil-1-*O*-(*N*-acetil-L-alanil)- i -1-*O*-(*N*-*tert*-butiloksikarbonil-L-alanil)-D-glukopiranuronatima. Katalitičkim hidriranjem β anomera tih spojeva dobivene su odgovarajuće 1-*O*-acilaminoacil-β-D-glukopiranuronske kiseline; BOC-alanil derivat preveden je u metil 2,3,4-tri-*O*-acetil-1-*O*-(*N*-*tert*-butiloksikarbonil-L-alanil)-β-D-glukopiranuronat koji je prirađen i drugim sintetskim putem. Katalitičko hidriranje α anomera potpuno benziliranog D-glukuronskog estera BOC-alanina rezultiralo je u deprotekciji šećernog dijela molekule uz paralelnu 1 → 2 acil migraciju. Neposredna esterifikacija i acetilacija produkta hidriranja dala je metil 1,3,4-tri-*O*-acetil-2-*O*-(*N*-*tert*-butiloksikarbonil-L-alanil)-D-glukopiranuronat, pretežno α anomerne konfiguracije; struktura i konfiguracija tog spoja izvedena je na bazi uspoređivanja n. m. r. spektara paracetiliranih metil 1- i 2-*O*-acil-D-glukopiranuronata.

Sinteza 1-*O*-(L-alanil)-β-D-glukopiranuronske kiseline kao trifluoroacetat soli, provedena je na dva načina: (a) putem postepene deprotekcije, polazeći od benzil 2,3,4-tri-*O*-benzil-1-*O*-(*N*-*tert*-butiloksikarbonil-L-alanil)-β-D-glukopiranuronata preko 1-*O*-(*N*-tert-butiloksikarbonil-L-alanil)-β-D-glukopiranuronske kiseline, i (b) u jednom tipu reakcije, katalitičkim hidriranjem benzil 2,3,4-tri-*O*-benzil-1-*O*-(*N*-benziloksikarbonil-L-alanil)-β-D-glukopiranuronata.

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