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Glucuronic Esters. VI*. Syntheses of Fully Protected 1-*O*-Acylaminoacyl-D-Glucuronic Acids by the Imidazole Promoted Active Ester and Dicyclohexylcarbodi-imide Methods

*G. Roglić** and D. Keglević*

Tracer Laboratory, Institute »Ruđer Bošković«, 41000 Zagreb, Croatia, Yugoslavia

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Fully protected D-glucuronic esters linked at C-1 to the carboxyl group of *N*-acyl-DL and L-amino acids were synthesized by two methods involving direct participation of imidazole in the ester linkage formation: (a) accelerated active ester method and (b) imidazole promoted DCC condensation. The synthesis was performed with fully-methylated and -benzylated C-1 free D-glucuronic acid as the sugar component and with pentachlorophenyl esters of benzyloxycarbonyl- and *tert*-butyloxycarbonyl- amino acids in method (a), or with benzyloxycarbonyl- and acetyl-amino acids in method (b). Fully methylated D-glucuronic acid I, prepared from D-glucurono-6,3-lactone and dimethyl sulphate, was used as the starting material for the synthesis of methylated sugar component II; GLC and chemical studies indicate the furanose structure for I. The corresponding glucuronic esters were obtained as anomeric mixtures which were resolved and characterized. Conditions for the cleavage of the amino acid protecting group were studied and the fully methylated 1-*O*-glycyl-β-D-glucuronate was obtained as the monooxalate salt in crystalline form.

INTRODUCTION

Previous work[1,2] in this laboratory has been concerned with the syntheses of fully protected D-glucuronic esters containing an ester bound aromatic or aliphatic acid as the aglycone; the compounds were prepared by the dicyclohexylcarbodi-imide (DCC), silver salt- or acid chloride-method. Recent interest in the nature of sugar-amino acid linkages in glycopeptides and glycoproteins prompted us to study[3,4] glucosyl esters of amino acids as possible model compounds of the hitherto unknown »ester« type linkage, involving the C-1 hydroxyl of the carbohydrate and the carboxyl group of the amino acid. However, the above synthetic methods proved to be of limited value when applied to various kinds of sugar and amino acid components.

As the next, reactions based on the use of acylamino acid active esters, commonly applied in peptide coupling procedures, were examined as potential routes to C-1 sugar-amino acid esters. However, under conditions normally used for the peptide bond formation, the activated esters of amino acids failed to react with the C-1 hydroxyl group of the fully protected sugar component.[5]

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On the other hand, the application of the »accelerated active ester« (AAE) method[6–8], involving participation of imidazole, led to a smooth formation of the corresponding C-1 ester derivatives[5]. In addition, it was observed that the synthesis of these compounds by the DCC method could be substantially promoted in the presence of imidazole[5]. The results obtained were rationalized by assuming that in both reactions the intermediate *N*-acylimidazole, formed by the nucleophilic attack of imidazole on the activated amino acid ester and the *O*-acylurea, respectively, participates directly in the transfer of the *N*-acylaminoacyl group to the sugar component.

In a recent paper[9] the two imidazole catalyzed reactions were studied in more detail, and as the result, two procedures to link the fully benzylated C-1 free D-glucopyranose to various *N*-acylamino acids *via* the ester bond were developed.

It seemed us of interest to extend the above procedures to the uronic acid series and to synthesize fully-methylated and -benzylated D-glucuronic esters of amino acids. In addition, we wanted to investigate conditions under which the amino acid protecting groups could be cleaved; owing to the stability of the *O*-methyl groups toward catalytic hydrogenation, the fully methylated C-1 free glucuronic acid was chosen as a suitable sugar component in the first studies. In the present paper the synthesis, anomeric resolution and characterization of a variety of fully protected 1-*O*-acylaminoacyl-D-glucuronates is reported, as well as preliminary results on the cleavage of the amino acid protecting group.

RESULTS AND DISCUSSION

Fully methylated D-glucuronic acid I, the immediate precursor of the C-1 free sugar component II, was obtained from D-glucurono-6,3-lactone in dimethylformamide with dimethyl sulphate as described by Kuhn and Trischmann[10]; the anomeric resolution was achieved on a silicagel column. The NMR spectrum of the slower moving component ($+ 110^0$) showed the anomeric proton (τ 5.06) with a coupling constant $J_{1,2}$ of 4 Hz and that of the faster moving component ($- 75^0$) revealed the signal ascribed to H-1 (τ 5.19) as a narrow doublet with the magnitude of splitting of 2 Hz. Methylation of D-glucuronic acid, performed in the same fashion, also gave I; physical data of the resolved anomers were indistinguishable from those of the samples obtained above.

Recently, Hashimoto *et al.*[11] prepared methyl (methyl 2,3,4-tri-*O*-methyl--D-glucopyranosid)uronate, highly enriched in the α-anomer, from D-glucurono--6,3-lactone; the procedure[12] involved treatment with methanolic hydrogen chloride followed by methylation of the intermediate methyl (methyl-D-glucopyranosid)uronate[12] with methyl iodide and silver oxide. The authors claimed to have ascertained the β-anomer in the reaction product, but no relevant data had been reported. We repeated the above procedure and compared the resolved anomers with those of the compound I. The two α-anomers revealed a high similarity in optical rotations and NMR spectra; however, the β-anomer of the fully methylated glucopyranosiduronic acid, isolated in a very low yield, gave in contrast to I-β the NMR spectrum consistent with the β-pyranose structure in the *C1*(D) conformation. Results obtained by GLC analysis suggested the furanose structure for I: the retention times of the anomers, relative

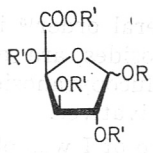
to each other were in agreement with the general order[13] in which anomeric methyl *O*-methylglyco-pyranosides and -furanosides are eluted, as well as with the observation[13] that fully methylated glucopyranosides have a higher mobility than the corresponding furanoside derivatives.

Chemical evidence for the furanose structure of I was obtained as follows. Ammonolysis of I-α failed to give a defined product while the parallel experiment with methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate led to the corresponding crystalline amide[12,14]. On the other hand, oxidation of I with nitric acid, followed by esterification gave the crystalline 2,3,5-tri-*O*--methyl-D-glucosaccharo-1,4-lactone 6-methyl ester.[15] Thus, the results suggest that under the conditions employed, methylation of D-glucofuranurono-6,3--lactone occurs before the opening of the lactone ring, and as the consequence methyl (methyl 2,3,5-tri-*O*-methyl-D-glucofuranosid)uronate (I) is formed as the final product. On the other hand, it has been shown[16] that under strong alkaline conditions the direct methylation of D-glucurono-6,3-lactone with dimethyl sulphate initially hydrolyses the lactone and converts the sugar to the pyranoid form, yielding methyl 2,3,4-tri-*O*-methyl-D-glucopyranosiduronic acid as the final product.

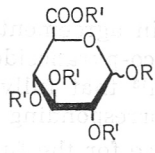
Hydrolysis of the glycosidic methyl group of I was smoothly effected by shaking an aqueous solution of I with the H^+ form of the cation-exchange resin at room temperature. After purification on a silicagel column, the oily product gave analytical data consistent with the fully methylated C-1 free glucuronic acid II: IR spectrum showed a band (3300 cm^{-1}) for the hydroxyl group and the NMR spectrum revealed one three-proton singlet ($\tau = 6.22$) and three three-proton singlets ($\tau = 6.52$, 6.56 and 6.58) assigned to the methyl ester and the methoxyl groups, respectively. Two resonances observed at τ 4.55 (doublet $\sim$ 2 Hz) and at τ 4.80 (singlet) integrated for one proton and were ascribed to H-1; the fact that the signal ascribed to the axial proton appeared as a singlet, suggests that in II the furanose structure has been retained. Hydrolysis of the pure α-anomer of I gave a product the NMR spectrum of which was indistinguishable from that of the anomeric mixture of II obtained above.

For further characterization, II was converted into the corresponding 1-*O*-acetyl-, 1-*O*-*p*-nitrobenzoyl- and 1-*O*-phthaloylglycyl- derivatives III, IV and V. Acetolysis of the glycoside group of I gave III as an anomeric mixture which was resolved by silicagel chromatography into two oily components revealing a high difference in the values (+ 72° and — 48°) for optical rotation. Acylation of II with *p*-nitrobenzoyl chloride in pyridine gave an anomeric mixture of IV from which the component with higher mobility on TLC was obtained in crystalline form. Reaction of II and phthalolglycyl chloride resulted in the sugar-amino acid ester V; upon anomeric resolution the faster moving component crystallised.

The structure of the above compounds was deduced from elemental analysis, IR spectra and NMR evidence. To the slower moving components of III, IV and V, the α-D-configuration was ascribed on the basis of high positive values for specific rotations and the $J_{1,2}$ values (4 Hz) observed in their NMR spectra. The assignment of the β-D-configuration to the faster moving components was consistent with their optical rotatory data and the positions of the



I–XIII R'=Me



XV–XVI R'=PhCH$_2$

No.	R	No.	R
I	Me	IX	L-PhCH$_2$CHCO | NHZ
II	OH	X	L-PhCH$_2$O$_2$CCH$_2$CHCO | NHZ
III	Ac	XI	L-MeCHCO | NHBOC
IV	*p*-NO$_2$C$_6$H$_4$CO	XII	CH$_2$CO | NHAc
V	CH$_2$CO | NPhth	XIII	L-MeCHCO | NHAc
VI	CH$_2$CO | NHZ	XV	DL-MeCHCO | NHZ
VII	DL-MeCHCO | NHZ	XVI	L-PhCH$_2$CHCO | NHZ
VIII	L-MeCHCO | NHZ		

Phth = $C_6H_4(CO)_2$; Z = $PhCH_2OCO$; BOC = Me_3COCO

H-1 signals at higher fields than those of the corresponding α-anomers; the $J_{1,2}$ values (~ 1 Hz for III and < 0.8 Hz for IV and V) were compatible with the furanose structure having a *trans* H-1, H-2 relationship.

The syntheses of fully methylated 1-*O*-acylaminoacyl-D-glucuronates by the (a) accelerated active ester and (b) imidazole promoted DCC methods were carried out following essentially the procedures elaborated[9] for the preparation of the corresponding fully benzylated glucosyl esters: in method (a) the protected sugar component was treated with a slight excess of the appropriate *N*-acyl-L- or DL-amino acid pentachlorophenyl ester in the presence of five equivalents of imidazole in dichloromethane, and in method (b) the condensation of II with the appropriate *N*-acylamino acid was carried out in the presence of two equivalents of imidazole. By these means, glucuronic esters VI—XIII were

TABLE I

Syntheses of Methyl 1-O-acylaminoacyl-2,3,5-tri-O-methyl-D-glucuronates

Compound No.	Method of preparation	Yield (%)
VI	AAE[a]	65
	DCC + Im[b]	45
VII	AAE	65
	DCC + Im	50
VIII	AAE	73
	DCC + Im	63
IX	AAE	70
X	AAE	65
XI	AAE	78
XII	DCC + Im	49
XIII	DCC + Im	48

[a]Accelerated active ester method.
[b]Imidazole promoted dicyclohexylcarbodi-imide method.

formed in good to excellent yields as anomeric mixtures in different proportions (Table I); method (a) proved to be superior to method (b).

The resolution of VI—XI into pure anomers was easily achieved by silica-gel column chromatography; on TLC in benzene-ethyl acetate (1 : 1), the relevant anomeric pairs appeared as two well-resolved homogeneous spots. On the other hand, attempts to obtain a clear anomeric separation of XII and XIII failed, due to the practically identical mobilities of the anomers in a number of solvents tried. With the exception of the laevorotatory anomer of the glucuronic ester XI, isolated from the reaction product of II and *tert*-butyloxycarbonyl-L-alanine pentachlorophenyl ester, both anomeric forms of the other glucuronic esters were oily products which could not be induced to crystallisation. The glucuronic ester VIII was prepared by both methods through benzyloxycarbonyl-L-alanine and its pentachlorophenyl ester, respectively: optical rotations of the resolved anomeric forms showed no differences with respect to the method of preparation.

The essential physical constants of VI—XIII are given in Table II. The IR spectra of all compounds showed absorptions characteristic of amide and ester carbonyl functions, and NMR spectra revealed the presence of methyl ester and methoxyl groups, superimposed on the non-anomeric C—H signals in the expected 1 : 3 ratio. In the NMR spectra of VI—XI, the broad NH signal appeared at τ 4.0—4.7 as an ill-defined doublet (J 7 Hz) at higher fields than the H-1 resonances, while in the spectra of XII and XIII the signal overlapped the region of the H-1 resonances, which could be observed after deuterium exchange of the NH proton. The relative positions of the H-1 signals and the specific rotation observed for the slower- and faster-moving anomeric forms of VI—XI are indicative for the α- and β-D-anomeric configuration, respectively. Assuming that the glucuronic acid moieties are in furanoid form, the observed $J_{1,2}$ values were in agreement with the above formulation.

TABLE II

Physical Constants and Analytical Data of Methyl 1-O-acylaminoacyl-2,3,5-tri-O-methyl-α- and β-D-glucuronates

Compound. No.	Acylaminoacyl[a]	Anomer[b]	$[\alpha]_D^{22}$ (°)[c]	NMR data[d], τ (J)			Found (%)			Formula	Calc'd. (%)		
				H-1	COOMe	3 OMe	C	H	N		C	H	N
VI	Z-Gly	α	+ 57.5	3.63 d (4)	6.23	6.50, 6.60, 6.63	54.13	6.27	3.20	$C_{20}H_{27}NO_{10}$	54.42	6.17	3.17
		β	— 40.0	3.90	6.29	6.62[e], 6.64	54.22	6.42	3.42				
VII	Z-DL-Ala	α	+ 50.3	3.64 d (4)	6.23	6.49, 6.58, 6.63	55.22	6.31	3.30	$C_{21}H_{29}NO_{10}$	55.38	6.42	3.07
		β	— 28.2	3.90	6.29	6.60[e], 6.64	55.11	6.13	3.28				
VIII	Z-L-Ala	α	+ 49.8	3.66 d (4)	6.24	6.50, 6.59, 6.66	55.06	6.52	3.01	$C_{21}H_{29}NO_{10}$	55.38	6.42	3.07
		β	— 32.8	3.90	6.27	6.59[e], 6.63	55.32	6.61	2.92				
IX	Z-L-Phe	α	+ 66.0	3.65 d (4)	6.22	6.48, 6.57, 6.62	60.86	6.33	2.77	$C_{27}H_{33}NO_{10}$	61.01	6.26	2.63
		β	— 12.0	3.87	6.27	6.63[f]	61.28	6.46	2.63				
X	Z-L-Asp(OBZL)	α	+ 46.7	3.67 d (4)	6.23	6.49, 6.60, 6.74	59.26	6.17	2.26	$C_{29}H_{35}NO_{12}$	59.08	5.98	2.37
		β	— 17.4	3.88	6.28	6.60[e], 6.66	58.92	5.86	2.36				
XI	BOC-L-Ala	α	+ 58.0	3.64 d (4)	6.25	6.48, 6.57, 6.62[g]	50.99	7.20	3.40	$C_{18}H_{31}NO_{10}$	51.30	7.41	3.32
		β	— 38.0	3.89	6.24	6.57[e,g]	51.40	7.40	3.44				
XII	Ac-Gly	α + β[h]	— 1.65	3.63 d (4) / 3.88 [i]	6.22	6.56[e], 6.60[j]	47.90	6.77	4.22	$C_{14}H_{23}NO_9$	48.13	6.64	4.01
XIII	Ac-L-Ala	α + β[h]	+ 7.25	3.65 d (4) / 3.90 [i]	6.22	6.56[e], 6.62[k]	49.30	7.15	4.14	$C_{15}H_{25}NO_9$	49.58	6.93	3.85

[a] Abbreviations: Z = benxyloxycarbonyl; BZL = Benzyl; BOC = *tert*-butyloxycarbonyl. [b] The α- and β-anomers of VI—XIII were oils except XI-β, m. p. 69—69.5° (ether-petroleum ether). [c] Determined in chloroform (c, 1—2). [d] Measured in chloroform-*d*; chemical shifts are in τ values and coupling constants (J) in Hz; multiplicity of signals: d = doublet; the absence of any indication implies that a singlet was observed. [e] Signal of intensity six. [f] Signal of intensity nine. [g] Me_3C, τ = 8.57, singlet, 9 H. [h] Unresolved. [i] Integrating for one proton. [j] N-Ac, τ = 7.94. [k] N-Ac, τ = 7.98; *Me*-CH(NH), τ = 8.55 d (7).

The susceptibility of the AAE and the imidazole promoted DCC methods to racemization of the amino acid moiety has been studied[9] in details on the fully benzylated D-glucosyl esters of various *N*-acylamino acids; in both methods, using benzyloxycarbonyl or *tert*-butyloxycarbonyl groups, a high degree of retention of the optical activity has been established. This has also been confirmed in the fully methylated glucuronic ester series: the treatment of the α- and β-anomers of VIII with one equivalent of sodium methoxide in methanol have yielded the optically pure benzyloxycarbonyl-L-alanine methyl ester.

Several attempts to obtain the fully methylated glucuronic esters containing an unprotected amino acid failed, mainly because of the instability of the products which decomposed during the isolation procedure. However, catalytic hydrogenation of the β-anomer of fully methylated 1-*O*-benzyloxycarbonylglycyl-D-glucuronate VI performed in methoxyethanol with an equimolar amount of oxalic acid, led to the crystalline monooxalate salt of the corresponding 1-*O*-glycyl-β-D-glucuronate XIV. The compound was characterized by elemental analysis, IR and NMR spectra; in water it showed a high negative value (— 68°) for specific rotation. The NMR spectrum of XIV revealed a one-proton singlet at τ 3.78 in deuterium oxide, and at τ 3.98 in dimethylsulphoxide-*d* which was ascribed to the anomeric proton. The monooxalate salt of XIV, in crystalline form, proved to be surprisingly stable, but in aqueous, neutral or slightly acidic solutions it underwent decomposition within several hours; in weak alkaline solutions the decomposition was almost instanteous.

For further characterization, XIV was converted into the corresponding *N*-acetyl derivative XII-β which was isolated as a chromatographically homogeneous syrup showing negative optical rotation in chloroform. In the NMR spectrum of XII-β, the one-proton singlet at τ 3.88, ascribed to the anomeric proton, was at the same position as the signal assigned to the β-anomeric proton in the NMR spectrum of XII obtained by the imidazole promoted DCC method; the signal assigned to the α-anomeric proton of XII was absent in the spectrum of XII-β.

Catalytic hydrogenation of the glucuronic ester VIII, performed under identical conditions as with VI, gave a product which could not be obtained in pure state. However, when the crude reaction product was subjected immediately to acetylation, a crystalline compound was isolated to which the structure of methyl 2,3,5-tri-*O*-methyl-1-*O*-(*N*-acetyl-L-alanyl)-β-D-glucuronate has been ascribed.

In order to check whether the AAE method could also be applied for the synthesis of the fully benzylated C-1 free glucuronic acids, benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate[17] was brought into reaction with pentachlorophenyl esters of benzyloxycarbonyl-DL-alanine and -L-phenylalanine, respectively, in the presence of five equivalents of imidazole. The corresponding glucuronic esters XV and XVI were obtained as anomeric mixtures in very good yields. The β-anomers were isolated as crystalline solids by direct crystallisation of the crude reaction products, and the α-anomers were obtained in pure form by submitting the relevant mother liquors to silicagel column chromatography. The products were characterized, and the NMR data of the resolved anomers were consistent with the pyranose structure in the *C1* (D) conformation.

EXPERIMENTAL

Melting points are uncorrected. Evaporations were performed in a rotatory evaporator *in vacuo* the bath temperature being kept below 50°, if not stated otherwise. Column chromatography was performed on Silicagel (Merck, 0.05—0.2 mm.); proportion of the substance to silicagel was 1 : 30—50, and the ratio of diameter to length of the column was 0.5—1.0 : 30—70 cm. Solvent systems used were: benzene--ethyl acetate 1 : 1, *A* and 1 : 2, *B*; ether-petroleum ether 1 : 1, *C*; butanol-acetic acid-water 60 : 15 : 25, *D*; ethyl acetate-methanol 9 : 1, *E*. Thin-layer chromatography (TLC) was carried out on chromatoplates of Silicagel G (Merck); spots were located with 10% sulphuric acid and heating, with iodine vapour or with ninhydrin reagent. Optical rotations were determined for 1% solutions at 20—24° in the solvent specified. IR spectra were recorded on a Perkin-Elmer 137 infracord spectrophotometer, NMR spectra on a Varian A-60A spectrometer with chloroform-*d* as solvent unless otherwise specified. Chemical shifts refer to an internal standard of tetramethylsilane (τ 10.00) and coupling constants (J) are measured in Hz. GLC was performed on a Hewlet-Packard 700—231 chromatograph; a 0.25 in. x 6 ft. stainless-steel column of 10% SE 52 on Chromosorb W was used with helium as carrier gas.

Methyl (methyl 2,3,5-tri-O-methyl-D-glucofuranosid)uronate (I)

(a) From D-glucurono-6,3-lactone. — D-glucurono-6,3-lactone (2.0 g., 11.4 mmoles) in dimethylformamide was methylated with dimethyl sulphate in the presence of barium oxide and barium hydroxide as described by Kuhn and Trischmann[10]. After working up, the crude anomeric product was chromatographed on a silicagel column (100 g.) with solvent *A* (5 ml. fractions, monitoring by TLC). Fractions (No 30—36) containing the faster moving β-anomer of I (820 mg) were collected apart from those (No 37—42) containing the anomeric mixture of I (420 mg.) and those (No 43—51) containing the slower moving α-anomer of I (500 mg; total yield on I: 60.5%).

I-β: syrup, $[\alpha]_D$ — 75.0° (MeOH). IR spectrum (neat): 2900s (Me); 1760s (C = O); 1120s (C—O—C) cm^{-1}. NMR spectrum: τ = 5.19 d (1H, $J_{1,2}$ 2 Hz, H-1); 6.23s (3 H, CO_2Me); 6.57—6.65 m (12 H, 4 OMe).

Anal. $C_{11}H_{20}O_7$ (264.28) calc'd.: C 49.99; H 7.63%
found: C 50.12; H 7.41%

I-α: oil, $[\alpha]_D$ + 110.0° (MeOH). IR spectrum (neat): 2900s (Me); 1750s (C = O); 1120s (C—O—C) cm^{-1}. NMR spectrum: τ = 5.06 d (H, $J_{1,2}$ 4 Hz, H-1); 6.23s (3 H, CO_2Me) and 6.56—6.62 m (12 H, 4 OMe).

Anal. $C_{11}H_{20}O_7$ (264.28) found: C 50.00; H 7.57%

(b) From D-glucuronic acid. — D-Glucuronic acid (2.2 g., 11.4 mmoles) was treated with dimethyl sulphate exactly as described for D-glucurono-6,3-lactone. After working up, the crude anomeric mixture of I (1.5 g.) was eluted from silicagel as described under (a). The resolved anomers revealed optical rotation, IR and NMR spectra identical with I-α and I-β, respectively, obtained under (a); total yield on I: 41%.

Structure Examination of I

A. — Methyl (methyl 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronate was prepared from D-glucurono-6,3-lactone (1.05 g.) as already described[11,12]. The faster moving β-anomer was separated from the preponderant α-anomer (500 mg., $[\alpha]_D$ + 115°, MeOH) by repeated column chromatography with solvent *A* and obtained as a chromatographically homogeneous oil (80 mg.) which gave satisfactory elemental analysis: $[\alpha]_D$ — 13.0° (MeOH). NMR spectrum: τ = 5.77d (1 H, $J_{1,2}$ 7 Hz, H-1); 6.20s (3 H, CO_2Me); 6.40, 6.47, 6.50 and 6.52s (12 H, OMe).

B. — *GLC analysis.* — Samples (50—150 μg.) of I and of methyl (methyl 2,3,4--tri-*O*-methyl-D-glucopyranosid)uronate in dioxane (2—7 μl.) were injected as the single anomers and as known mixtures into the gas chromatograph; column temp.: 160°, thermal conductivity detector: 300°, helium flow-rate: 34 ml./min. Retention times are based on the moment of elution of dioxane which was taken as zero.

Compound	Retention time
Methyl (methyl 2,3,5-tri-*O*-methyl-α-D-glucofuranosid)uronate	8.4 min.
Methyl (methyl 2,3,5-tri-*O*-methyl-β-D-glucofuranosid)uronate	7.8 „
Methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate	7.9 „
Methyl (methyl 2,3,4-tri-*O*-methyl-β-D-glucopyranosid)uronate	6.8 „

C. — Ammonolysis. — Samples (190 mg.) of I-α and methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate were dissolved in methanolic ammonia (2% w/w, 100 ml.) and kept at 0° for 2 days. After removal of the solvent, the residue was crystallised from acetone-petroleum ether; only in the case of the pyranose derivative a solid (30 mg., m. p. 169—170°) deposited. A second crystallisation afforded analytically pure amide of methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronic acid, m. p. 182—183°, $[\alpha]_D$ + 153.0° (c (0.59, water). Lit.[14]: m. p. 183°, $[\alpha]_D$ + 137.5°.

D. — Oxidation of I-β *with nitric acid.* — A sample of I-β (500 mg., 1.9 mmoles) was treated with nitric acid (d 1.39, 8 ml.) as described by Smith[15]; after work-up the residue was dissolved in methanol, a solution of diazomethane in ether (50 ml.) was added, and the mixture was left at 0° for 3 h. The solvent was evaporated, and the remaining oil was purified on a silicagel column with solvent *A*. 2,3,5-Tri-*O*-methyl-D-glucosaccharo-1,4-lactone 6-methyl ester was obtained as a chromatographically homogeneous semi-crystalline mass (150 mg.); after two recrystallisations from ether, shiny crystals with m. p. 76—77°, $[\alpha]_D$ — 12.0° (c, 0.5, water) were obtained. Lit.[15]: m. p. 77—78°, $[\alpha]_D$ — 10° (water). IR spectrum (KBr): 3000m (Me); 1770vs and 1800vs (C = O); 1060vs (C—O—C).

Anal: $C_{10}H_{16}O_7$ (248.24) calc'd.: C 48.39; H 6.49%
found: C 48.56; H 6.69%.

*Methyl 2,3,5-tri-*O*-methyl-*D*-glucuronate* (II)

Compound I (1.0 g., 3.8 mmoles) in water (40 ml.) was shaken with Dowex 50 W X4 exchange resin (H^+ form, 30 ml.) at room temperature for 24 h. The resin was filtered off, washed with water, the combined filtrates were evaporated to dryness, and the remaining oily product was passed through silicagel (60 g.) with solvent *B*. Chromatographically homogeneous fractions were pooled and evaporated to dryness leaving pure II (600 mg., 60%) as a colourless oil, $[\alpha]_D$ — 16.0° ($CHCl_3$). IR spectrum (neat): 3300m (OH); 2850m (Me); 1725s (C = O); 1115s (C—O—C) cm^{-1}. NMR spectrum: τ = 4.55d ($J_{1,2}$ 2 Hz) and 4.80 s (integrating for 1 H, H-1); 6.22s (3 H, CO_2Me); 6.52, 6.56 and 6.58s (9 H, OMe).

Anal. $C_{10}H_{18}O_7$ (250.25) calc'd.: C 48.00; H 7.23%
found: C 48.27; H 7.65%

*Methyl 1-*O*-acetyl-2,3,5-tri-*O*-methyl-*D*-glucuronate* (III)

Compound I (1.7 g., 6.5 mmoles) was dissolved in a mixture of acetic acid (37 ml.) and acetic acid anhydride (6 ml.) whereupon 10% sulphuric acid in acetic acid (4 ml.) was added under shaking at 0°. After standing for 5 h. at room temperature, the reaction mixture was poured on to ice, extracted with chloroform, and the combined extracts were washed with water, aqueous $NaHCO_3$ and water and dried over sodium sulphate. After removal of the solvent the remaining oil was chromatographed on a silicagel (30 g.) column with solvent *A* (fractions 2—3 ml., monitoring by TLC). The first fractions enriched in the β-anomer of III (182 mg., $[\alpha]_D$ — 27.5°, MeOH) were set apart from those containing the anomeric mixture of III (1223 mg.) and those containing predominantly the slower moving α-anomer of III (110 mg., $[\alpha]_D$ + 45.5°, MeOH; total yield on III: 80.5%).

Rechromatography (solvent *A*) of the first fractions on a silicagel column afforded the pure β-anomer of III: oil, $[\alpha]_D$ — 48.0° (MeOH). IR spectrum (neat):

2900 m (Me); 1750s (C = O); 1120s (C—O—C) cm^{-1}. NMR spectrum: $\tau = 3.95$d (1 H, $J_{1,2}$ 1 Hz, H-1); 6.23s (3 H, CO_2Me); 6.56s (9 H, OMe); 7.90s (3 H, OAc).

Anal. $C_{12}H_{20}O_8$ (292.29) calc'd.: C 49.31; H 6.89%
found: C 49.16; H 6.77%

Rechromatography of the final fractions, performed in the same manner as with III-β, gave the pure α-anomer of III: oil, $[\alpha]_D$ + 72.0° (MeOH). IR spectrum (neat): 2900m (Me); 1750s (C = O); 1120s (C—O—C) cm^{-1}. NMR spectrum: $\tau = 3.67$d (1 H, $J_{1,2}$ 4 Hz, H-1); 6.22s (3 H, CO_2Me); 6.48s (3 H, OMe) and 6.57s (6 H, 2 OMe); 7.90s (3 H, OAc).

Anal. $C_{12}H_{20}O_8$ (292.29) found: C 49.01; H 6.77%.

Methyl 2,3,5-tri-O-*methyl-1*-O-(*p-nitrobenzoyl*)-D-*glucuronate* (IV)

To a solution of II (400 mg., 1.6 mmoles) in pyridine (2 ml.) a solution of *p*-nitrobenzoylchloride (569 mg., 3.2 mmoles) in pyridine (6 ml.), prepared immediately prior to the reaction, was dropped under shaking at 0°. The reaction mixture was left at room temperature for about 40 min. (monitoring by TLC in solvent *A*) whereupon it was diluted with chloroform (20 ml.), and the organic layer was washed with cold water, 3% sulphuric acid (3x) and water, dried (sodium sulphate) and evaporated. The remaining semicrystalline mass was dissolved in hot ethanol (5 ml.), and to the cooled solution water was added until turbidity persisted; after standing overnight at 0°, crystals of the β-anomer of IV (120 mg., 19%) separated. A second crystallisation afforded pure IV-β: m. p. 102—103°, $[\alpha]_D$ — 42.5° ($CHCl_3$). IR spectrum (KBr): 2870m (Me); 1750s and 1725s (C = O); 1525s and 1350s (NO_2); 725s (aromatic CH) cm^{-1}. NMR spectrum: $\tau = 1.70$s (4 H, aromatic); 3.65s (1 H, H-1); 6.28s (3 H, CO_2Me); 6.50s (6 H, 2 OMe) and 6.58s (3 H, OMe).

Anal. $C_{17}H_{21}NO_{10}$ (399.37) calc'd.: C 51.13; H 5.30; N 3.51%
found: C 51.28; H 5.38; N 3.38%

The mother liquor was diluted with water whereupon a second crop of IV (150 mg.) precipitated as a semicrystalline mass; TLC (solvent *A*) revealed the presence of both anomers in about 1 : 1 ratio. The filtrate was evaporated to dryness and the remaining syrup (120 mg., total yield on IV 61%), highly enriched in the α-anomer of IV, was eluted from silicagel with solvent *A*. Chromatographically homogeneous fractions were pooled and evaporated to dryness yielding pure α-anomer of IV as a colourless syrup, $[\alpha]_D$ + 71.0° ($CHCl_3$) IR spectrum (neat): 2900m (Me); 1740s (C = O); 1530s and 1350s (NO_2); 725s (aromatic CH). NMR spectrum: $\tau = 1.72$s (4 H, aromatic); 3.38d (1 H, $J_{1,2}$ 4 Hz, H-1); 6.20s (3 H, CO_2Me); 6.45, 6.51 and 6.56s (9 H, OMe).

Anal. $C_{17}H_{21}NO_{10}$ (399.37) found: C 51.20; H 5.07; N 3.33%

Methyl 2,3,5-tri-O-*methyl-1*-O-*phthaloyglycyl*-D-*glucuronate* (V)

To a solution of II (377 mg., 1.5 mmoles) in pyridine (2 ml.) a freshly prepared solution of phthaloylglycyl chloride (669 mg., 3.0 mmoles) in dichloromethane (3 ml.) was dropped at 0°. The reaction mixture was left at room temperature for 1 h. whereupon it was diluted with dichloromethane and worked up as described for IV. After removal of the solvent the remaining semicrystalline mass was passed through a column of silicagel (50 g.) with solvent *A* (fractions 3—5 ml., monitoring by TLC). The first fractions enriched in the β-anomer of V were pooled, evaporated to dryness, and the residue (253 mg.) was triturated with ether; on standing white crystals, m. p. 96—100°, precipitated. Crystallisation from chloroform-ether-petroleum ether afforded pure V-β: m. p. 99—100°, $[\alpha]_D$ — 26.5° ($CHCl_3$). IR spectrum (KBr): 2900m (Me); 1780—1720s (C = O); 720s (out of plane, aromatic CH) cm^{-1}. NMR spectrum: $\tau = 1.97$—2.33 m (4 H, aromatic); 3.68s (1 H, H-1); 5.50s (2 H, CH_2—NPhth); 6.20s (3 H, CO_2Me); 6.58s (3 H, OMe) and 6.69s (6 H, 2 OMe).

Anal. $C_{20}H_{23}NO_{10}$ (437.41 calc'd.: C 54.92; H 5.30; N 3.20%
found: C 54.93; H 5.59; N 3.30%

Subsequent fractions containing V highly enriched in the slower moving α-anomer were pooled, evaporated to dryness, and the residue (167 mg.; total yield on V: 64%) was fractionated on a second silicagel column with solvent *A*. Pure V-α was obtained as a colourless oil, $[\alpha]_D$ + 55.0° ($CHCl_3$). IR spectrum (neat): 2900m (Me); 1780—1720s (C = O); 720s (aromatic CH) cm^{-1}. NMR spectrum: τ = 1.99—2.34m (4 H, aromatic); 3.62d (1 H, $J_{1,2}$ 4 Hz, H-1); 5.50s (2 H, CH_2—NPhth); 6.24s (3 H, CO_2Me); 6.50s (3 H, OMe) and 6.62s (6 H, 2 OMe).

Anal. $C_{20}H_{23}NO_{10}$ (437.41) found: C 54.76; H 5.10; N 3.45%

Preparation of Methyl 1-O-acylaminoacyl-2,3,5-tri-O-methyl-D-glucuronates — General Procedure

(a) Accelerated active ester (AAE) method. — Methyl 2,3,5-tri-O-methyl-D--glucuronate (II, 250 mg., 1 mmole), the corresponding *N*-acylamino acid pentachlorophenyl ester (1 mmole) and imidazole (340 mg., 5 mmoles) were subsequently dissolved in dry dichloromethane (10 ml.) at room temperature. The reaction was monitored by TLC in solvent *A* and after 1—2 h. an additional amount (10—15% excess) of the amino acid component was added under shaking. After 5 h. the precipitated pentachlorophenol was filtered off, washed with dichloromethane, and the combined filtrates were poured on to ice and washed with water, 3% sulphuric acid or 10% citric acid (in reactions with compounds containing *tert*-butyloxycarbonyl group), water, aqueous $NaHCO_3$ and water. After drying (sodium sulphate) and evaporation, the residue was submitted to silicagel column chromatography in solvent system *A;* the β-anomers of the products migrated faster than the corresponding α-anomers. Fractions were combined according to their composition as revealed by TLC and those enriched in α- and β-anomer, respectively, were pooled, evaporated and re-chromatographed in the same solvent system. Yields, physical constants and analytical data of the α- and β-anomers of compounds VI—XIII are given in Tables I and II.

(b) Carbodi-imide + imidazole (DCC + Im) method. — To methyl 2,3,5-tri-O--methyl-D-glucuronate (II, 250 mg., 1 mmole), the appropriate acylamino acid (1 mmole) and imidazole (136 mg., 2 mmoles) dissolved in dichloromethane (10 ml.) or, in 5 : 1 dichloromethane-dimethylformamide (10 ml.), a solution of DCC (206 mg., 1 mmole) in dichloromethane (5 ml) was added under cooling. The progress of the reaction was monitored by TLC, and after 2—4 h., an additional amount (10—15% excess) of the corresponding acylamino acid was added. The total reaction time was 8—24 h., whereupon dicyclohexylurea was filtered off, the filtrates were poured on to ice and treated further as described under (a). In reactions with acetylamino acids traces of dimethylformamide were removed (bath 40°/0.1 mm. Hg) before submitting the residues to silicagel column chromatography; elution was performed with ethyl acetate followed by solvent *E*. Yields and physical constants of compounds VI—VIII, XII and XIII are given in Tables I and II.

Treatment of Methyl 1-O-(benzyloxycarbonyl-L-alanyl)-2,3,5-tri-O-methyl-α- and β-D-glucuronate with Methanolic Sodium Methoxide

To a solution of the β-anomer of VIII (140 mg., 0.31 mmole) in anhydrous methanol (5 ml.), 0.1 *M* methanolic sodium methoxide (3.1 ml.) was added at room temperature; the progress of the reaction was monitored by TLC in solvents *A* and *C*. After 1 h. methanol was removed *in vacuo,* the residue was suspended in water (10 ml.) and extracted with ether (3x). The combined extracts were dried (sodium sulphate), the solvent was evaporated, and the remaining syrup was passed through a silicagel column (11 g.) with solvent *C* (fractions 2 ml.). Elution afforded a chromatographically homogeneous syrup which after crystallisation from petroleum ether yielded optically pure benzyloxycarbonyl-L-alanine methyl ester (55 mg., 75%) with m. p. 44—45°, $[\alpha]_D$ —35.0° (MeOH); lit.[9]: m. p. 45—46°, $[\alpha]_D$ —36.0° (MeOH).

Identical treatment of VIII-α (120 mg., 0.26 mmole) with one equivalent of methanolic sodium methoxide gave 52 mg. (71%) of benzyloxycarbonyl-L-alanine methyl ester with m. p. 43.5—45° and $[\alpha]_D$ —35.2° (MeOH).

Methyl 1-O-glycyl-2,3,5-tri-O-methyl-β-D-glucuronate, monooxalate salt (XIV)

To a solution of VI-β (263 mg., 0.6 mmole) in methoxyethanol (10 ml.), 10% palladium on charcoal (30 mg.) and oxalic acid dihydrate (75 mg., 0.6 mmole) were

added, and a stream of hydrogen was bubbled through the system at room temperature. Effluent gases were tested for carbon dioxide, and after completion of the reaction (1 h., monitoring by TLC in solvents *A* and *D)* the catalyst was removed by filtration, the filtrate was evaporated at 40° (bath)/0.1 mm. Hg, and the oily residue was thoroughly dried over sulphuric acid in a vacuum dessicator. The product solidified upon trituration with dry ether to a lightly brown powder; after two recrystallisations from methoxyethanol-ether, analytically pure monooxalate-salt of XIV (100 mg., 41.5%) was obtained as a white powder: m. p. 131—132° (decomp.), $[\alpha]_D$ —68.0° (H_2O). IR spectrum (KBr): 3400m (OH); 2950m (Me); 1750vs (C=O); 1100vs (C—O—C) cm^{-1}. NMR spectrum (D_2O): τ = 3.78s (1H, H-1); 5.96s (2H, CH_2—N); 6.17s (3H, CO_2Me); 6.50, 6.53 and 6.56s (9 H, 3 OMe). NMR spectrum (dimethylsulphoxide-*d*): τ = 3.98s (1 H, H-1); 6.33s (3 H, CO_2Me); 6.63s (6 H, 2 OMe) and 6.70s (3H, OMe). The compound is very soluble in water and methoxyethanol, sparingly soluble in methanol and ethanol and insoluble in ethyl acetate, chloroform, ether and benzene. In crystalline state, the compound can be stored in a vacuum dessicator for many months with no appreciable change (monitoring by TLC and m. p.).

Anal. $C_{14}H_{23}NO_{12}$ (397.34) calc'd.: C 42.32; H 5.83; N 3.62%
found: C 42.03; H 5.88; N 3.77%

*Methyl 1-*O*-(*N*-acetylglycyl)-2,3,5-tri-*O*-methyl-*β*-*D*-glucuronate* (XII-β) — *Acetylation of* XIV

Monooxalate-salt XIV (130 mg., 0.33 mmoles) was dissolved in 2 ml. of a 5 : 1 mixture of pyridine-acetic anhydride at 0°, and the mixture was kept at 0° for 3 h. (monitoring by TLC in solvent *E*) whereupon chloroform (20 ml.) was added, and the solution was poured on to ice. The organic layer was washed with water, 3% sulphuric acid, water, aqueous $NaHCO_3$ and water, dried (sodium sulphate), and the solvent was removed *in vacuo*. The residual oil was put on a silicagel column (12 g., fractions 2 ml.) and eluted with ethyl acetate, followed by solvent *E*; the second solvent eluted XII-β as a chromatographically homogeneous syrup (110 mg., 96%), $[\alpha]_D$ —25.5° ($CHCl_3$). IR spectrum (neat): 3300m (NH); 2950m (Me); 1740vs (C = O); 1650s and 1525s (amide I and II); 1100s (C—O—C) cm^{-1}. NMR spectrum: τ = 3.88s (1 H, H-1); 6.24s (3H, CO_2Me); 6.57s (9H, 3 OMe); 7.95s (3H, NAc).

Anal. $C_{14}H_{23}NO_9$ (349.34) calc'd.: C 48.13; H 6.64; N 4.01%
found: C 48.35; H 6.82; N 4.04%

*Methyl 1-*O*-(*N*-acetyl-*L*-alanyl)-2,3,5-tri-*O*-methyl-*β*-*D*-glucuronate* (XIII-β) via *the Hydrogenation Product of* VIII-β

The β-anomer of VIII (455 mg., 1 mmole) in methoxyethanol (15 ml.) was catalytically hydrogenated in the presence of oxalic acid dihydrate (126 mg., 1 mmole) and 10% Pd/C (50 mg.) as described for VI-β; after 1 h. the reaction was complete. The crude hydrogenolysis product was evaporated to dryness, and the residual brown oil was immediately acetylated with 5 : 1 pyridine-acetic anhydride (4 ml.) as described in the preparation of XII-β. After workup, the crude product was passed through a silicagel column with ethyl acetate, followed by solvent *E;* the second solvent eluted XIII-β (100 mg., 27.5% calcd. on VIII-β as a colourless chromatographically homogeneous glass which crystallised upon trituration with ether-petroleum ether: m. p. 86—87.5°, $[\alpha]_D$ —47.0° ($CHCl_3$). IR spectrum (KBr): 3350m (NH); 2950m (Me); 1730vs (C=O); 1650s and 1525s (amide I and II); 1100vs (C—O—C) cm^{-1}. NMR spectrum: τ = 3.87s (1H, H-1); 6.23s (3H, CO_2Me); 6.56s (6H, 2 OMe) and 6.58s (3H, OMe); 7.97s (3H, NAc); 8.55d (3H, J 7 Hz, MeC).

Anal. $C_{15}H_{25}NO_9$ (363.37) calc'd.: C 49.58; H 6.93; N 3.85%
found: C 49.66; H 6.91; N 3.89%

*Benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-*DL*-alanyl)-α- and β-*D*-glucuronate* (XV)

The condensation of benzyl 2,3,4-tri-*O*-benzyl-D-glucopyranuronate (554 mg., 1 mmole) with benzyloxycarbonyl-DL-alanine pentachlorophenyl ester (1 mmole + 15% excess) in the presence of imidazole by the AAE method was performed as described

under General Procedure. The progress of the reaction was monitored by TLC in benzene-ethyl acetate (10 : 1), and after workup, the crude oily product was dissolved in hot ethanol; on cooling white crystals of the β-anomer of XV (209 mg., 28%) separaed. A second crystallisation afforded pure XV-β: m. p. 144—145°, [α] D —12.8° ($CHCl_3$). NMR spectrum: τ = 4.23d (1H, $J_{1,2}$ 7.5 Hz, H-1); 8.61d (3H, J 7 Hz, MeC).

Anal. $C_{45}H_{45}NO_{10}$ (759.82) calc'd.: C 71.13; H 5.97; N 1.84%
found: C 71.27; H 6.25; N 1.86%

The mother liquor was evaporated to dryness, and the remaining oil was passed through a silicagel column with benzene-ethyl acetate (10 : 1). The first fractions enriched in the α-anomer of XV (96 mg.) were set apart from those containing an approximately 1 : 1 anomeric mixture (120 mg.) and those in which the slower moving β-anomer of XV (65 mg.; total yield on XV: 65%) strongly prevailed. Rechromatography of the first fractions on silicagel in the same solvent afforded the pure α-anomer of XV: oil, [α] D + 32.0° ($CHCl_3$). NMR spectrum: τ = 3.59d (1H, $J_{1,2}$ 3.5 Hz, H-1); 8.57d (3H, J 7 Hz, MeC).

Anal. $C_{45}H_{45}NO_{10}$ (759.82) found: C 71.12; H 5.87; N 1.76%

*Benzyl 2,3,4-tri-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-*L*-phenylalanyl)-α- and β-*D*-glucuronate* (XVI)

The synthesis of XVI was performed with benzyl 2,3,4-tri-O-benzyl-D-glucopyranuronate and benzyloxycarbonyl-L-phenylalanine pentachlorophenyl ester by the AAE method as described for XV. The β-anomer was obtained in a 31% yield by direct crystallisation of the crude anomeric product from ethanol: m. p. 128—129°, [α] D —7.5° ($CHCl_3$). NMR spectrum: τ = 4.23d (1H, $J_{1,2}$ 7 Hz, H-1).

Anal. $C_{51}H_{49}NO_{10}$ (835.91) calc'd.: C 73.28; H 5.91; N 1.67%
found: C 73.04; H 6.14; N 1.69%

The mother liquor was subjected to silicagel column chromatography in benzene-ethylacetate (total yield on XVI: 71%), the fractions enriched in the faster moving α-anomer of XVI were pooled, and the remaining oil was subjected to two further fractionations on silicagel. Finally, pure α-anomer of XVI (55 mg.) was obtained as a colourless syrup, [α] D + 42.9° ($CHCl_3$). NMR spectrum: τ = 3.57d (1H, $J_{1,2}$ 3Hz, H-1).

Anal. $C_{51}H_{49}NO_{10}$ (835.91) found: C 73.13; H 6.16; N 1.87%

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IZVOD

Glukuronski esteri. VI. Sinteze potpuno zaštićenih 1-*O*-acilaminoacil-D-glukuronskih kiselina metodom aktivnih estera i dicikloheksilkarbidi-imid metodom, u prisustvu imidazola kao katalizatora

G. Roglić i D. Keglević

Potpuno zaštićeni D-glukuronski esteri, vezani preko C-1 atoma šećerne komponente s karboksilnom grupom *N*-acil-DL- i -L-aminokiselina, sintetizirani su dvjema metodama; u obadvije reakcije imidazol sudjeluje direktno kod stvaranja esterske veze šećer-aminokiselina. Kao aminokiselinska komponenta služili su u metodi (a) pentaklorofenil esteri benziloksikarbonil- i *tert*-butiloksikarbonil-aminokiselina, a u metodi (b) benziloksikarbonil- i acetil-aminokiseline; kao šećerna komponenta uzimana je potpuno metilirana, ili potpuno benzilirana, C-1 slobodna D-glukuronska kiselina.

Potpuno metilirana D-glukuronska kiselina I priređena je iz D-glukurono-6,3-laktona i dimetilsulfata; izneseni su dokazi da spoj I ima furanoidnu strukturu. Iz I, hidrolizom glikozidne metilne grupe, dobiven je metil 2,3,5-tri-*O*-metil-D-glukofuranuronat (II) koji je karakteriziran i uziman kao šećerna komponenta u sintezama glukuronskih estera.

Odgovarajući 1-*O*-acilaminoacil-D-glukuronati dobiveni su kao anomerne smjese; nakon kromatografije na silikagel kolonama, anomeri su odijeljeni i karakterizirani.

Studirani su uvjeti pod kojima dolazi do skidanja protektivnih grupa s aminokiselinskog dijela molekule, i priređen je potpuno metilirani 1-*O*-glicil-β-D-glukofuranuronat u obliku kristaliničnog monooksalata.

INSTITUT »RUĐER BOŠKOVIĆ«
TRACER LABORATORIJ
41000 ZAGREB

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