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O-PIVALOYL-D-GLUCOFURANURONO-6,3-LACTONES: USE OF THE PIVALOYL GROUP FOR POSITIONAL ASSIGNMENTS OF SUBSTITUENTS IN THE SUGAR RING*

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ABSTRACT

Selective acylation of D-glucofuranurono-6,3-lactone and its derivatives with pivaloyl chloride–pyridine gave products having the *O*-pivaloyl group at positions 5, 2,5, 1,2,5, 1, and 1,5. The relative reactivities (HO-5 > HO-2 > HO-1) of the hydroxyl groups are greatly influenced by the reaction conditions. The chemical shifts of the p.m.r. signals for the pivaloyl groups can be used for positional assignments of pivaloyl groups and, from their relative intensities, the $\alpha\beta$-ratios in anomeric mixtures can be determined. Evidence that *O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucofuranurono-6,3-lactone, a minor product[1] of the reaction of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranuronic acid with diazomethane, has the acyl residue at position 2 is provided by p.m.r. spectroscopy of its 1,5-dipivaloate derivative.

INTRODUCTION

In a prior study[1], we showed that esterification of α-D-glucopyranosyluronic esters of *N*-acylamino acids with ethereal diazomethane proceeds with concomitant 1→2 acyl migration to give, after acetylation, the corresponding 2-*O*-acyl *O*-acetyl methyl ester derivatives. For 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranuronic acid (**19**), the above treatment yielded, in addition to methyl 1,3,4-tri-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranuronate, di-*O*-acetyl-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucofuranurono-6,3-lactone as a minor product. P.m.r. data for the latter compound suggested that the acetyl groups were located at positions 1 and 5, indicating that the internal cyclisation and ring contraction had occurred after the acyl migration step. However, unequivocal structural assignment was not possible, because of the lack of adequate reference compounds and difficulties in synthesis by direct routes.

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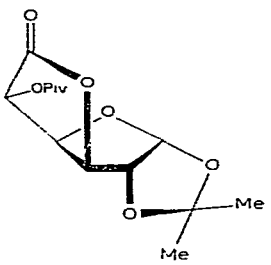
In order to elucidate the sequence of reactions leading to the lactone minor-product, we have examined the selectivity of pivaloyl chloride in the esterification of D-glucofuranurono-6,3-lactone and its derivatives. Preliminary experiments showed[2] the utility of the pivaloyl group for the synthesis of partially protected methyl α-D-glucopyranosides.

RESULTS AND DISCUSSION

O-Pivaloyl-D-glucofuranurono-6,3-lactones. — Treatment of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone in ether with pivaloyl chloride–pyridine gave (80%) the syrupy 5-pivaloate **1**. The close similarity of the coupling constants of the ring protons (see Experimental) in the p.m.r. spectrum (acetone-d_6) of **1** with those observed for 1,2-*O*-isopropylidene-5-*O*-toluene-*p*-sulphonyl-[3] and 5-*O*-acetyl- and -benzoyl-1,2-*O*-benzylidene-α-D-glucofuranurono-6,3-lactone[4] suggests[3] that **1** exists predominantly in the $^3T_2$ conformation. It is noteworthy that the low-field position of the H-5 doublet in the spectra of the cited compounds, which was ascribed[3,4] to the diamagnetic anisotropy of the lactone carbonyl group, has been observed in this work for each of the 5-*O*-acylated D-glucofuranurono-6,3-lactone derivatives. This suggests that the 5-ester carbonyl group also contributes to the increased paramagnetic shift of the H-5 signal.



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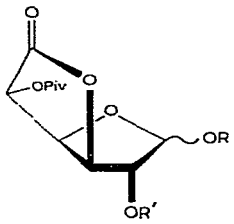
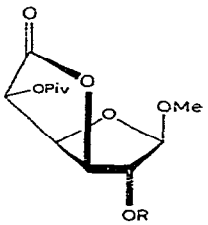
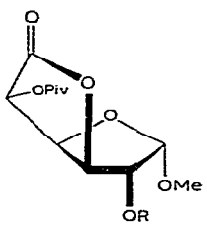


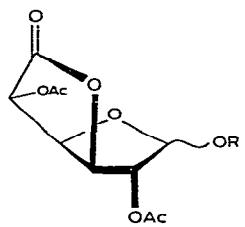
2 R = R′ = H
3 R = R′ = Ac
9 R = H, R′ = Piv
10 R = R′ = Piv
11 R = Ac, R′ = Piv
14 R = PhCH$_2$CHC(=O)NHBoc, R′ = Piv



4 R = H
5 R = Piv
6 R = Ac



7 R = H
8 R = Piv



12 R = Piv
13 R = PhCH$_2$CHC(=O)NHBoc

Piv = $Me_3CC(=O)$

Boc = $Me_3COC(=O)$

TABLE I

^{1}H-N.M.R. DATA (100 MHz) FOR *O*-PIVALOYL-D-GLUCOFURANURONO-6,3-LACTONE DERIVATIVES[a]

Compound	*Solvent*	*Me_3C-CO_2*[b]			*H-1* ($J_{1,2}$)[c]		*Others*
		1	*2*	*5*	*α*	*β*[d]	
1	$(CD_3)_2CO$			1.24	6.07 (3.91)		CMe_2 1.33, 1.47
2	$(CD_3)_2CO$[e]			1.24	5.40 (3.42)	5.02	
	C_5D_5N			1.26 1.29	6.10 (3.17)	6.02	
3	$CDCl_3$			1.27 1.25	6.53 (4.64)	6.24	2 OAc 2.07, 2.08; 2.13
	C_5D_5N			1.25 1.27	6.98 (4.88)	6.64	2 OAc 1.98, 2.00; 2.05, 2.10
3α	C_5D_5N			1.25	6.98 (4.88)		2 OAc 1.99; 2.07
4	$(CD_3)_2CO$			1.28		4.96	OMe 3.34
	C_5D_5N			1.33		5.42	OMe 3.48
5	$(CD_3)_2CO$		1.20	1.28		5.12	OMe 3.38
	C_5D_5N		1.17	1.33		5.54	OMe 3.45
6	$CDCl_3$			1.32		5.02	OMe 3.41 AcO-2 2.12
7	$(CD_3)_2CO$[e]			1.25	5.10 (4.39)		OMe 3.44
	C_5D_5N			1.31	[f]		OMe 3.44
8	$(CD_3)_2CO$[e]		1.21	1.25	5.28 (4.39)		OMe 3.35
	C_5D_5N		1.21	1.30	[f]		OMe 3.31
9	$(CD_3)_2CO$[e]		1.20	1.24	[f]	[f]	
	C_5D_5N		1.18	1.26	[f]	[f]	
10	$(CD_3)_2CO$	1.21 1.18	1.23	1.23	6.55 (4.64)	6.21	
	$CDCl_3$	1.20	1.23 1.22	1.27 1.25	6.53 (4.64)	6.24	
	C_5D_5N	1.22 1.29	1.19 1.13	1.23 1.31	6.98 (4.64)	6.71	
10β	$(CD_3)_2CO$	1.18	1.23	1.23		6.20	
	$CDCl_3$	1.20	1.22	1.25		6.22	
	C_5D_5N	1.29	1.13	1.31		6.71	
11	$CDCl_3$		1.23 1.22	1.27 1.25	6.54 (4.64)	6.21	AcO-1 2.06
11α	$CDCl_3$		1.23	1.27	6.54 (4.64)		AcO-1 2.06
	C_5D_5N		1.19	1.25	6.99 (4.88)		AcO-1 2.01
12	$(CD_3)_2CO$	1.20 1.17			6.56 (4.40)	6.24	2 OAc 2.10; 2.12
	$CDCl_3$	1.20			6.56 (4.88)	6.26	2 OAc 2.12, 2.15; 2.17, 2.24
	C_5D_5N	1.15 1.26			7.00 (4.88)	6.75	2 OAc 2.02; 2.07, 2.08
12α	$CDCl_3$	1.20			6.56 (4.88)		2 OAc 2.12; 2.24
	C_5D_5N	1.14			7.00 (4.88)		2 OAc 2.02; 2.08
12β	$CDCl_3$	1.20				6.26	2 OAc 2.16; 2.17
	C_5D_5N	1.26				6.75	2 OAc 2.02; 2.07
13β	$CDCl_3$					6.39	2 OAc 2.12; 2.15 *Boc*-PheO-1 1.38
14β	$(CD_3)_2CO$		1.21	1.24		6.37	*Boc*-PheO-1 1.32
	$CDCl_3$		1.22	1.22		6.34	*Boc*-PheO-1 1.37
	C_5D_5N		1.13	1.25		6.78	*Boc*-PheO-1 1.38

TABLE I *(continued)*

Compound	*Solvent*	*Me_3C-CO_2*[b]			*H-1* ($J_{1,2}$)[c]		*Others*
		1	*2*	*5*	*α*	*β*[d]	
15	$(CD_3)_2CO$			1.24	[f]	[f]	*Boc*-PheO-2 1.36
	C_5D_5N			1.25 1.27	[f]	[f]	*Boc*-PheO-2 1.42, 1.45
16β	$CDCl_3$			1.21		6.26	*Boc*-PheO-1 1.37; *Boc*-PheO-2 1.43
	C_5D_5N			1.24		6.82	*Boc*-PheO-1 1.40; *Boc*-PheO-2 1.44
17	$CDCl_3$			1.26 1.23	6.53 (4.64)	6.17	*Boc*-PheO-2 1.43, 1.40 AcO-1 2.04, 2.08
	C_5D_5N			1.24 1.26	7.14 (4.88)	6.64	*Boc*-PheO-2 1.40, 1.43 AcO-1 2.08, 2.16
18	C_5D_5N	1.23 1.26		1.23 1.27	6.97 (4.88)	6.75	*Boc*-PheO-2 1.44, 1.45
18β	$(CD_3)_2CO$	1.18		1.22		6.26	*Boc*-PheO-2 1.37
	$CDCl_3$	1.18		1.24		6.20	*Boc*-PheO-2 1.43
	C_5D_5N	1.26		1.28		6.75	*Boc*-PheO-2 1.43

[a]Internal Me_4Si; chemical shifts on δ scale and *J* in Hz. [b]All signals are singlets; in the case of two signals for one group-position, the upper value refers to the signal assigned to the α and the lower to the β anomer. [c]Only first-order values are given. [d]$J_{1,2} \leq 0.5$ Hz. [e]After deuteration. [f]Not amenable to first-order analysis.

Removal of the isopropylidene group in **1** by hot acetic acid proceeded without affecting the ester bond, to give the crystalline 5-pivaloate **2**, which was converted into the rather unstable 1,2-diacetate **3**, obtained as an anomeric mixture. The p.m.r. spectra (Table I) of **2** (in pyridine-d_5) and **3** (in chloroform-*d* and pyridine-d_5) contained two, closely spaced pivaloyl singlets that integrated for nine protons; on the basis of the intensities of the signals for H-1α and H-1β and comparison with the spectra of pure **3α**, isolated by silica gel chromatography, they were assigned to the 5-*O*-pivaloyl groups in the α and β anomers of **2** and **3**, respectively.

Selective pivaloylation was examined for both anomers of methyl D-glucofuranosidurono-6,3-lactone. Their reaction with acetic anhydride, acetyl chloride, or benzoyl chloride in the presence of pyridine showed[5] no selectivity, but with equimolar amounts of ethoxy- and benzyloxy-carbonyl chloride, there was[6] an appreciable selectivity for HO-5, particularly in the case of the β anomer. When the latter anomer was treated in ether with 2.4 mol. equiv. each of pivaloyl chloride and pyridine for 1 h at 4°, the 5-pivaloate **4**, characterised as the 2-acetate **6**, was obtained in 70% yield. When the reaction was conducted at room temperature for 48 h, the crystalline 2,5-dipivaloate **5** was practically the only product. By following the same procedures, methyl 5- (**7**) and 2,5-di-*O*-pivaloyl-α-D-glucofuranosidurono-6,3-lactone (**8**) were prepared in yields of 60 and 85%, respectively. In the p.m.r. spectrum (in acetone-d_6) of **4**, H-2 appeared as a doublet which, after deuteration, collapsed to a singlet, thus providing evidence that the pivaloyl substituent was at O-5.

Similarly, treatment of D-glucofuranurono-6,3-lactone in ether with 3.3 mol. equiv. each of pivaloyl chloride and pyridine for 1 h at 4° and 48 h at room temperature yielded a mixture of 2,5-di- (**9**) and 1,2,5-tri-*O*-pivaloyl-D-glucofuranurono-6,3-lactone (**10**) in the ratios ~5:1 and ~1:1.5, respectively. The products (>90%) were isolated by chromatography and both were obtained crystalline. Conventional acetylation of **9** gave an anomeric mixture of the 1-acetate **11** from which the α anomer was obtained crystalline. However, attempts to resolve (either by chromatography or crystallisation) the anomeric mixture (αβ ratio, ~1:1) of **10** were unsuccessful. Pivaloylation of 2,5-di-*O*-acetyl-D-glucofuranurono-6,3-lactone[7] in the above manner proceeded (t.l.c. monitoring) very slowly, even when a large excess (13 equiv. each) of pivaloyl chloride–pyridine was used; when ether was omitted from the reaction, the 1-pivaloate **12** was formed in 64% yield within 4 h, thus indicating the great influence of the solvent on the relative reactivity of HO-1.

Pivaloylation of D-glucofuranurono-6,3-lactone in (*a*) the absence of ether and (*b*) with pyridine as the solvent gave the tripivaloate **10** with αβ ratios of ~1:2 and ~1:7, respectively; from the latter preparation, the pure β anomer was obtained by crystallisation. When cold pyridine is used as solvent and reaction catalyst in acylations of free sugars, it is known[8] that the esterification reaction proceeds faster than mutarotation of the unreacted sugar molecule. Since crystalline D-glucofuranurono-6,3-lactone consists almost exclusively of the β-D form[9], the preponderant formation of **10β**, under the foregoing conditions, might be explained in this way.

These results indicate a high selectivity of the hydroxyl groups in the D-glucofuranurono-6,3-lactone series towards acylation with pivaloyl chloride. The order of reactivity HO-5 > HO-2 > HO-1 agrees[5] well with a higher degree of hydrogen bonding of HO-5 as compared to that of HO-2, and with a lower nucleophilicity of O-1 as compared to that of O-2. The β anomers were more stable than their α counterparts, which decomposed easily during chromatography on silica gel.

The chemical shifts of the pivaloyl groups in the p.m.r. spectra, measured in three solvents, of **1**–**12** are listed in Table I. Comparison of the spectra indicates that the chemical shift of a pivaloyl group depends upon its position in the molecule, the presence and orientation of the adjacent substituents, and the nature of the solvent used. Generally, the sequence of the chemical shifts of the pivaloyl-proton signals in acetone-d_6 and chloroform-*d* is very similar and, to some extent, different from that observed with pyridine-d_5 as the solvent, which gave the best resolution of the signals.

For all three solvents, the *tert*-butyl signal at the lowest field of the set of pivaloyl signals was assigned to the 5-pivaloyl group (**5**, **8**–**11**); only in the acetone-d_6 spectrum of **10β** did this signal coincide with that of the 2-pivaloyl group. There is a small but distinct difference between the chemical shifts of the 5-pivaloyl signals of the two anomeric forms: in acetone-d_6 (**4** and **7**, **5** and **8**) and chloroform-*d* (**3**, **10**, **11**), the 5-pivaloyl signal assigned to the α anomer resonates at a lower field (higher δ value) than that of its β counterpart, and the opposite is valid when pyridine-d_5 (**2**–**4** and **7**, **5** and **8**, **10**) is the solvent. The signal at second to lowest field for solutions

in acetone-d_6 and chloroform-*d* was assigned to the 2-pivaloyl group (**5**, **8**–**11**); in both solvents, the signals of the two anomeric forms are essentially coincident. By contrast, with pyridine-d_5 as the solvent, the 2-pivaloyl signal of the β anomer (**5**, **10**β) is shifted to higher field (0.04 and 0.06 p.p.m.) relative to that of its α counterpart (**8** and **10**α, respectively), thus indicating a strong solvent-effect upon the chemical shifts of individual pivaloyl-group resonances. For solutions in acetone-d_6 and chloroform-*d*, the signal observed at highest field of the set was assigned to the 1-pivaloyl group (**10**, **12**); the signals of the two anomeric forms are slightly separated in the former, and coincident in the latter, solvent. In pyridine-d_5, the 1-pivaloyl signals of **10**β and **12**β are shifted 0.07 and 0.11 p.p.m., respectively, downfield relative to those of their α counterparts; the order is opposite to that observed with acetone-d_6 as the solvent.

The foregoing data indicate the utility of the pivaloyl group for positional assignments of substituents in D-glucofuranurono-6,3-lactone derivatives: *e.g.*, the spectrum of the tripivaloate **10** in pyridine-d_5 exhibited six distinct singlets in the region 1.31–1.13 p.p.m., thus allowing identification of each pivaloyl group and, from the relative intensities of the signals, determination of the $\alpha\beta$-ratio.

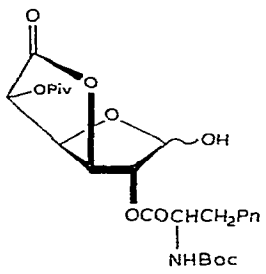
The chemical shifts and coupling constants of the anomeric protons (Table I) for **1**–**12** are fairly constant throughout the series and consistent with a *trans* and *cis* relationship, respectively, between H-1 and H-2. The spectra of the α-D derivatives revealed the anomeric proton doublet to lower field of the other ring protons, except for methyl 5- (**7**) and 2,5-di-*O*-pivaloyl-D-glucofuranosidurono-6,3-lactone (**8**), where the H-1 signal was strongly shifted to higher field and the H-5 doublet was the lowest-field signal. The spectra of **4** and **5** (β-D series) and **7**, **8**, **11**, and **12** (α-D series) showed (see Experimental) distinguishable signals for the other ring protons; signal assignments were verified, as necessary, by spin decoupling. In the β-D series, the observed coupling constants for H-2,3 were ~0 and those for H-3,4 and H-4,5 were ~6 and ~6.5 Hz, respectively. The spectra of α-D derivatives showed H-3 ($J_{2,3}$ ~1 Hz) as singlets, and H-2 ($J_{2,1}$ ~4.5 Hz) and H-5 ($J_{4,5}$ ~5 Hz) as well-resolved doublets. These results are in line with those reported[5,1c] for methyl β- and α-D-glucofuranosidurono-6,3-lactone, respectively, and suggest a predominance of the $^3T_2$ conformation in the foregoing compounds.

The sequences of the acetoxyl signals observed (Table I) in the spectra of **3**, **6**, **11**, and **12** are very similar to those found for the pivaloyl signals. However, a rather narrow separation of the peaks, which occur in a region not completely devoid of other resonances, and the lower intensity of the acetoxyl singlet, as compared to that exhibited by the pivaloyl group, make the identification of individual acetyl groups in the above compounds much more ambiguous.

Evidence for the structure of the minor product. — The reaction of 2,5-di-*O*-acetyl-D-glucofuranurono-6,3-lactone with *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester in the presence of imidazole gave (27%) the rather unstable 2,5-di-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucofuranurono-6,3-lactone (**13**β); its p.m.r. spectrum (Table I) was similar to, but not identical

with, that[1] of the acetylated derivative of the minor product formed from 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranuronic acid in the reaction with diazomethane. Analogous reaction of the 2,5-dipivaloate **9** with the amino acid active ester afforded (36%) 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-2,5-di-*O*-pivaloyl-β-D-glucofuranurono-6,3-lactone (**14β**). In both preparations, only the β anomers could be obtained chromatographically homogeneous, whereas the respective α anomers (t.l.c. and p.m.r. evidence) decomposed during isolation.

Condensation of the 5-pivaloate **2** with one and two equivalents of the amino acid component gave a mixture of 2-*O*- (**15**) and 1,2-di-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-5-*O*-pivaloyl-D-glucofuranurono-6,3-lactone (**16**) (ratios ~1 : 0.6 and 1 : 1.5, respectively) which was fractionated by chromatography, to give **15**, as an anomeric mixture, and anomerically pure **16β**. In the p.m.r. spectra (Table I) of the latter, the two *tert*-butoxycarbonyl (Boc) amino-protecting groups appeared as two, well-resolved singlets, one of which resonated at the same position as the Boc-group protons in the spectra of **13β** and **14β**. The spectrum of **15** in solution in pyridine-d_5 showed the Boc signal as two narrow-spaced singlets that integrated for nine protons: one (δ 1.45) coincided with the lower-field Boc signal in the spectrum of **16β**. Thus, the chemical shifts of Boc-group resonances are affected by the position of the Boc-aminoacyl residue in the lactone and by the anomeric configuration. Conventional acetylation of **15** gave the 1-acetate **17** as an anomeric mixture, and treatment of **15** with pivaloyl chloride–pyridine afforded 2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-1,5-di-*O*-pivaloyl-β-D-glucofuranurono-6,3-lactone (**18β**). The p.m.r. spectrum (in acetone-d_6 and pyridine-d_5) of **18β** was amenable to first-order analysis (see Experimental) and fully consistent with the structure proposed; the corresponding α anomer was insufficiently stable to be isolated pure.



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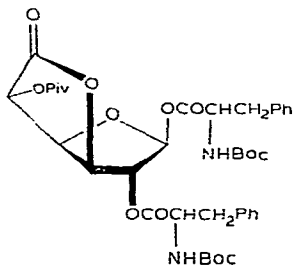

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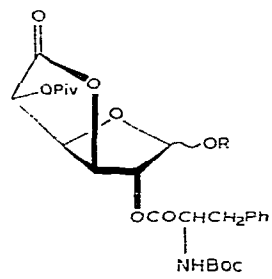

17 R = Ac
18 R = Piv

The resonance positions and order of individual pivaloyl signals in the p.m.r. spectra of the foregoing compounds are in excellent agreement with those observed for the model pivaloates. The spectra of **14β** and **18β** allow a ready differentiation of the two compounds on the basis of the chemical shifts of their pivaloyl- and Boc-group signals and thus permit the positional assignment of the Boc-aminoacyl residue in the sugar ring.

Esterification of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranuronic acid (**19**) with ethereal diazomethane, followed by treatment of the crude product with pivaloyl chloride–pyridine, gave a heterogeneous product from which an anomeric mixture of **18**, contaminated with some decomposition products, was isolated in 20% yield; further purification on silica gel afforded pure **18β**, indistinguishable (t.l.c., i.r., p.m.r. data) from the sample prepared by direct synthesis. This result implies that the diazomethane-catalysed 1→2 acyl migration occurred before the lactonisation step. It may be presumed that the Boc-aminoacyl residue at C-2 enhances the nucleophilicity of HO-3 and thus facilitates its attack on the carbonyl carbon of the C-5 carboxylate; the resulting 2-*O*-acyl-D-glucopyranurono-6,3-lactone then rearranges into the thermodynamically more stable[5] D-glucofuranurono-6,3-lactone derivative.

EXPERIMENTAL

General. — Melting points are uncorrected. Optical rotations were determined for 1% solutions in methanol, unless otherwise stated. Concentrations were performed at diminished pressure on a rotary evaporator at <35°, if not stated otherwise, and solutions were dried with anhydrous sodium sulphate. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm), and t.l.c. on Kieselgel G (Merck); the solvents employed were: *A*, benzene–ether (proportions are given in the text); *B*, benzene–methanol (5:1); *C*, chloroform–ethyl acetate–benzene (5:3:2); *D*, chloroform–ethyl acetate (5:3); and *E*, acetonitrile–water (5:1). Detection on t.l.c. plates was effected by charring with sulphuric acid, or with alkaline silver nitrate, the ninhydrin reagent, or the chlorine–starch–iodine reagent for peptides. I.r. spectra were recorded with a Perkin–Elmer 297 spectrometer, and p.m.r. spectra (100 MHz, internal Me_4Si) with a Jeol JNM FX-100 Fourier-transform NMR spectrometer. The amount of diazomethane in ethereal solutions (redistilled before use) was determined by titration of an aliquot portion with benzoic acid, and the concentration of the reagent was adjusted to ~0.2 mmol/ml).

1,2-O-Isopropylidene-5-O-pivaloyl-α-D-glucofuranurono-6,3-lactone (**1**). — To a stirred suspension of 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone[11] (2.16 g) in dry ether (50 ml) was added, at room temperature, pyridine (1 ml) followed by pivaloyl chloride (1.45 g). The mixture was stirred for 6 h, and then poured onto ice and extracted with chloroform. The combined extracts were washed with 10% aqueous citric acid, water, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. The residual oil was eluted from silica gel with solvent *A* (5:1), to give chromatographically homogeneous (R_F 0.48) **1** as a glass (2.4 g, 80%), $[\alpha]_D$ +90°; ν_{max}^{film} 1810 (C=O, γ lactone) and 1740 cm^{-1} (ester). P.m.r. data (acetone-d_6): δ 6.07 (d, $J_{1,2}$ 3.9 Hz, H-1), 5.75 (d, $J_{4,5}$ 4.1 Hz, H-5), 5.09 (dd, 1 H, H-4), 5.05 (s, $J_{2,3}$ <0.5 Hz, H-3), and 4.90 (d, $J_{2,1}$ 3.9 Hz, collapsed on irradiation of H-1 into a singlet, H-2); also see Table I.

Anal. Calc. for $C_{14}H_{20}O_7$: C, 55.99; H, 6.71. Found: C, 55.86; H, 6.74.

5-O-Pivaloyl-D-glucofuranurono-6,3-lactone (**2**). — A solution of **1** (100 mg) in acetic acid (8 ml) [containing a small amount of Dowex 50 (H^+) resin] was heated to 100°, water (2 ml) was added, and the mixture was kept at 100° for 4 h [t.l.c. (solvent *B*) then indicated the absence of **1** and the presence of two, closely migrating spots, R_F 0.33 (major) and 0.28 (trace)]. After removal of the solvent, the residue was dissolved in hot propan-2-ol; addition of light petroleum precipitated **2** (61 mg, 70%), m.p. 155–157°, $[\alpha]_D$ +92→+48° (upon addition of two drops of pyridine, after 16 h); ν_{max}^{KBr} 3520 (broad, OH), 1800 (C=O, γ lactone), and 1715 cm^{-1} (ester).

Anal. Calc. for $C_{11}H_{16}O_7$: C, 50.77; H, 6.20. Found: C, 50.96; H, 6.13.

Treatment of **2** (320 mg) with acetic anhydride–pyridine (1:1, 8 ml) for 3 h at 4°, followed by concentration (0.1 Torr) of the solution and fast elution of the residue from silica gel with solvent *A* (10:3), gave syrupy 1,2-di-*O*-acetyl-5-*O*-pivaloyl-D-glucofuranurono-6,3-lactone (**3**; 254 mg, 60%) as an anomeric mixture [R_F 0.30 (major) and 0.34 (minor)], $[\alpha]_D$ +128°.

Anal. Calc. for $C_{15}H_{20}O_9$: C, 52.32; H, 5.86. Found: C, 52.63; H, 5.82.

Rechromatography (2×) of the above material on silica gel, with the same solvent, was accompanied by decomposition, to give only a few fractions containing pure (t.l.c. and p.m.r. evidence) α anomer (8 mg, R_F 0.30).

Methyl 5-O- and 2,5-di-O-pivaloyl-β-D-glucofuranosidurono-6,3-lactone (**4** *and* **5**). — (*a*) A suspension of methyl β-D-glucofuranosidurono-6,3-lactone[12] (190 mg) in dry ether (15 ml) was treated at 4° with pyridine (0.19 ml) and pivaloyl chloride (0.29 g) for 1 h. The mixture was then poured onto ice, extracted with ethyl acetate, and worked-up as described for **1**. Fast elution of the product from silica gel (solvent *C*) afforded **4** (190 mg, 70%) as a chromatographically homogeneous (R_F 0.24) glass, $[\alpha]_D$ +44°; ν_{max}^{film} 3430 (broad, OH), 1800 (C=O, γ lactone), and 1740 cm^{-1} (ester). P.m.r. data (acetone-d_6): δ 5.54 (d, $J_{4,5}$ 6.4 Hz, H-5), 5.14 (dd, 1 H, collapsed on irradiation of H-5 into a doublet, $J_{4,3}$ 4.6 Hz, H-4), 4.99 (d, $J_{3,4}$ 6 Hz, H-3), 4.89 (d, $J_{HO\text{-}2,H\text{-}2}$ 4.4 Hz, disappeared on deuteration, HO-2), 4.96 (s, H-1), 4.27 (d, $J_{2,HO\text{-}2}$ 4.4 Hz, changed to a singlet on deuteration, H-2); pyridine-d_5: δ 6.06 (d, $J_{4,5}$ 6.6 Hz, H-5), 5.58 (dd, *J* 5 and 2 Hz, collapsed on irradiation of H-5 into a doublet, H-4), 5.42 (s, H-1), 5.34 (d, $J_{3,4}$ 5.9 Hz, H-3), and 4.84 (s, H-2); also see Table I.

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.62. Found: C, 52.35; H, 6.85.

Acetylation of **4** (50 mg), as described for **3**, gave, after chromatography (solvent *A*, 10:3), methyl 2-*O*-acetyl-5-*O*-pivaloyl-β-D-glucofuranosidurono-6,3-lactone (**6**; 52 mg, 90%), which crystallised from light petroleum; m.p. 80–82°, $[\alpha]_D$ +40°.

Anal. Calc. for $C_{14}H_{20}O_8$: C, 53.16; H, 6.37. Found: C, 52.15; H, 6.13.

(*b*) When the reaction in (*a*) was carried out at room temperature for 48 h, chromatography (solvent *C*) of the crude product afforded **5** (R_F 0.9; 300 mg, 84%), which crystallised spontaneously upon removal of the solvent; m.p. 76–77°, $[\alpha]_D$

+64°. P.m.r. data (pyridine-d_5): δ 6.05 (d, $J_{4,5}$ 6.6 Hz, H-5), 5.58–5.46 (m, 1 H, H-4), 5.54 (s, H-1), 5.31 (d, $J_{3,4}$ 7 Hz, H-3), and 5.07 (s, H-2); also see Table I.

Anal. Calc. for $C_{17}H_{26}O_8$: C, 56.97; H, 7.31. Found: C, 56.70; H, 7.24.

Methyl 5-O- and 2,5-di-O-pivaloyl-α-D-glucofuranosidurono-6,3-lactone (**7** *and* **8**). — (*a*) Acylation of methyl α-D-glucofuranosidurono-6,3-lactone[13] (95 mg), as described for the β anomer under (*a*), gave the monopivaloate **7** (82 mg, 60%) as a glass, $[\alpha]_D$ +165°, R_F 0.42 (solvent *C*). P.m.r. data (acetone-d_6 +D_2O): δ 5.62 (d, $J_{4,5}$ 5.37 Hz, H-5), 5.10 (d, $J_{1,2}$ 4.39 Hz, H-1), 4.99 (s, H-3), 5.07–4.95 (m, collapsed on irradiation of H-5 into a doublet, H-4), and 4.30 (d, $J_{2,1}$ 3.91 Hz, H-2); also see Table I.

Anal. Calc. for $C_{12}H_{18}O_7$: C, 52.55; H, 6.62. Found: C, 52.48; H, 6.76.

(*b*) When the above reaction was conducted at room temperature for 48 h, chromatography of the product afforded the dipivaloate **8** (150 mg, 85%), m.p. 84° (from light petroleum), $[\alpha]_D$ +176°, R_F 0.86 (solvent *C*). P.m.r. data (acetone-d_6): δ 5.64 (d, $J_{4,5}$ 5.37 Hz, H-5), 5.28 (d, $J_{1,2}$ 4.39 Hz, H-1), 5.21 (dd, $J_{2,1}$ 4.39, $J_{2,3}$ ~1 Hz, H-2), 5.03 (s, H-3), and 5.03–4.97 (m, collapsed on irradiation of H-5 into a doublet, H-4).

Anal. Calc. for $C_{17}H_{26}O_8$: C, 56.97; H, 7.31. Found: C, 57.00; H, 7.27.

2,5-Di- and 1,2,5-tri-O-pivaloyl-D-glucofuranurono-6,3-lactone (**9** *and* **10**). — (*a*) A suspension of D-glucofuranurono-6,3-lactone (176 mg) in dry ether (20 ml) was treated with pyridine (0.26 ml) and pivaloyl chloride (400 mg) for 2 h at 4°, and the mixture was further processed as described for **4**. Elution (solvent *A*, 5:1) of the product from silica gel gave chromatographically homogeneous **9** (R_F 0.28; 260 mg, 75.6%) as a solid that crystallised from propan-2-ol; m.p. 169–170°, $[\alpha]_D$ +102→ +45° (upon addition of two drops of pyridine after 16 h); ν_{max}^{KBr} 3440 (broad, OH), 1820 (C=O, γ lactone), 1730, and 1715 cm^{-1} (ester).

Anal. Calc. for $C_{16}H_{24}O_8$: C, 55.81; H, 7.03. Found: C, 55.51; H, 6.76.

Concentration of the faster-moving fractions afforded the tripivaloate **10** (αβ ratio, ~1:1; p.m.r. evidence) (64 mg, 15%), R_F 0.65, m.p. 90–92° (from light petroleum), $[\alpha]_D$ +94°.

Anal. Calc. for $C_{21}H_{32}O_9$: C, 58.87; H, 7.53. Found: C, 58.77; H, 7.39.

Conventional acetylation of **9**, as described for **3**, gave, after chromatography (solvent *A*, 10:3), 1-*O*-acetyl-2,5-di-*O*-pivaloyl-D-glucofuranurono-6,3-lactone (**11**; 50 mg, 89%) as an anomeric mixture [R_F 0.59 (major) and 0.63 (minor)], m.p. 122–123°, $[\alpha]_D$ +153°.

Anal. Calc. for $C_{18}H_{26}O_9$: C, 55.95; H, 6.78. Found: C, 55.78; H, 6.95.

Recrystallisation (3×) of the above product from light petroleum afforded **11α**, m.p. 119–120°, $[\alpha]_D$ +162°. P.m.r. data (pyridine-d_5): δ 6.99 (d, $J_{1,2}$ 4.88 Hz, H-1), 6.26 (d, $J_{4,5}$ 5.37 Hz, H-5), 5.77 (d, $J_{2,1}$ 4.88 Hz, H-2), 5.62 (near s, H-3), and 5.62–5.58 (m, H-4); also see Table I.

(*b*) When the reaction in (*a*) was conducted at room temperature for 30 h, **9** and **10** were obtained in yields of 35 and 58%, respectively.

(*c*) To D-glucofuranurono-6,3-lactone (176 mg) in pyridine (0.26 ml) was

added pivaloyl chloride (400 mg) at 4°, and the thick paste was stirred at 4° for 2 h, iced water was then added, and the mixture was worked-up as described for **4**. Chromatography of the residue, as described above, gave **9** and **10** ($\alpha\beta$ ratio, ~ 1 : 2) in yields of 45 and 40%, respectively.

(*d*) When the reaction was performed as in (*c*), but using pyridine (15 ml) as the solvent, **9** and **10** ($\alpha\beta$ ratio, ~ 1 : 7) were obtained in yields of 34 and 48%, respectively. Crystallisation of the latter from light petroleum afforded **10β**, m.p. 119–121°, $[\alpha]_D$ +64.5° (Found: C, 59.00; H, 7.42).

*2,5-Di-*O*-acetyl-1-*O*-pivaloyl-*D*-glucofuranurono-6,3-lactone* (**12**). — (*a*) Treatment of 2,5-di-*O*-acetyl-D-glucofuranurono-6,3-lactone[7] (55 mg) in dry ether (5 ml) with pyridine (0.5 ml) and pivaloyl chloride (330 mg) for 48 h, with further processing of the reaction mixture as described for **1**, gave a residue that was eluted from silica gel with solvent *D*. Eluted first was an anomeric mixture ($\alpha > \beta$) of **12** (17 mg, 23%) as an oil.

Anal. Calc. for $C_{15}H_{20}O_9$: C, 52.32; H, 5.85. Found: C, 52.21; H, 5.92.

Eluted second was **12β** (p.m.r. evidence) (29 mg, 40%): glass, $[\alpha]_D$ +86° (*c* 1, chloroform) (Found: C, 52.55; H, 5.95).

(*b*) When the reaction was performed as in (*a*), but in the absence of ether for 4 h, **12** was obtained in 64% yield. Elution (2×) from silica gel with solvent *D* led to partial separation of the pure α anomer (21 mg); glass, $[\alpha]_D$ +157° (chloroform). P.m.r. data (pyridine-d_5): δ 7.00 (d, $J_{1,2}$ 4.88 Hz, H-1), 6.33 (d, $J_{4,5}$ 4.88 Hz, H-5), 5.75, 5.70 (dd, $J_{2,3}$ ~1, $J_{2,1}$ 4.88 Hz, collapsed on irradiation of H-1 into a broad singlet, H-2), 6.00 (near s, H-3), and 5.63–5.51 (near q, 1 H, H-4).

*2,5-Di-*O*-acetyl-1-*O*-[*N*-(tert-butoxycarbonyl)-*L*-phenylalanyl]-*D*-glucofuranurono-6,3-lactone* (**13**). — The condensation of 2,5-di-*O*-acetyl-D-glucofuranurono-6,3-lactone (520 mg) and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester (1.13 g) was performed in the presence of imidazole (680 mg) in dichloromethane (10 ml) at room temperature for 2.5 h; within this period, the reaction mixture turned red-brown. After removal of pentachlorophenol and work-up, as described for **1**, a solution of the residue in chloroform was filtered through a short column of silica gel which retained most of the coloured contaminants. After evaporation of the eluate, the residue was crystallised (2×) from chloroform–light petroleum, to give **13β** (274 mg, 27%), m.p. 139–141°; ν_{max}^{KBr} 3360 (NH), 1810 (C=O, γ lactone), 1755 (ester), 1700 and 1525 (Amide I and II), and 1375 cm^{-1} (Me_3C).

Anal. Calc. for $C_{24}H_{29}NO_{11}$: C, 56.79; H, 5.76; N, 2.76. Found: C, 56.53; H, 5.98; N, 2.52.

*1-*O*-[*N*-(tert-Butoxycarbonyl)-*L*-phenylalanyl]-2,5-di-*O*-pivaloyl-*D*-glucofuranurono-6,3-lactone* (**14**). — To a solution of **9** (134 mg) in dichloromethane (15 ml) was added *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester (240 mg) and imidazole (131 mg), and the reaction mixture was kept at room temperature for 20 h. After removal of pentachlorophenol and work-up, the residue was subjected to a fast elution from silica gel with solvent *A* (10 : 3), to give **14β** (83 mg, 35.9%) as a glass, $[\alpha]_D$ +49° (chloroform).

Anal. Calc. for $C_{30}H_{41}NO_{11}$: C, 60.90; H, 6.99; N, 2.37. Found: C, 60.75; H, 7.21; N, 2.54.

2-O- and 1,2-di-O-[N-(tert-Butoxycarbonyl)-L-phenylalanyl]-5-O-pivaloyl-D-glucofuranurono-6,3-lactone (**15** *and* **16**). — (*a*) The 5-pivaloate **2** (90 mg) was treated with two equivalents of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester (365 mg) in the presence of imidazole (117 mg) in dichloromethane (10 ml) at room temperature for 4 h; t.l.c. (solvent *B*) monitoring revealed the appearance of a spot (R_F 0.46) whose intensity gradually decreased on account of a faster-moving spot (R_F 0.51). The mixture was processed, as described above, to give a residue that was eluted from silica gel with solvent *A* (10:3). The first component to be eluted was the β anomer of **16** (60 mg, 23%); glass, $[\alpha]_D$ +36° (chloroform). P.m.r. data (pyridine-d_5): δ 6.82 (s, H-1), 6.09 (d, $J_{4,5}$ 6.35 Hz, H-5), 5.79 (near s, H-2), 5.44 (t, $J_{4,3}$ 4.6, $J_{4,5}$ 6.4 Hz, H-4), and 5.26 (d, $J_{3,4}$ 4.7 Hz, H-3); also see Table I.

Anal. Calc. for $C_{39}H_{50}N_2O_{13}$: C, 62.06; H, 6.68; N, 3.71. Found: C, 61.78; H, 6.70; N, 3.69.

Eluted second was **15** (24 mg, 13.4%); solid foam, $[\alpha]_D$ +65° (methanol); ν_{max}^{KBr} 3380 (broad, OH, NH), 1810 (C=O, γ lactone), 1745 (ester), 1700 and 1500 (Amide I and II), 1370 (Me_3C), 700 and 755 cm^{-1} (aromatic).

Anal. Calc. for $C_{25}H_{33}NO_{10}$: C, 59.16; H, 6.55; N, 2.76. Found: C, 59.24; H, 6.84; N, 2.72.

(*b*) When the above reaction was performed with one equivalent of the amino acid component for 5 h, **15** and **16**β were obtained in yields of 30 and 18%, respectively.

Conventional acetylation of **15** (120 mg) for 2 h, followed by concentration (0.1 Torr), and fast elution of the residue from silica gel (solvent *A*, 10:3) afforded 1-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-5-*O*-pivaloyl-D-glucofuranurono-6,3-lactone (**17**; 68 mg, 53.5%) as an anomeric mixture ($\alpha\beta$ ratio, ~1:1.5), R_F 0.49 (major), 0.55 (minor).

Anal. Calc. for $C_{27}H_{35}NO_{11}$: C, 59.01; H, 6.42; N, 2.55. Found: C, 59.05; H, 6.35; N, 2.54.

2-O-[N-(tert-Butoxycarbonyl)-L-phenylalanyl]-1,5-di-O-pivaloyl-D-glucofuranurono-6,3-lactone (**18**). — Compound **15** (62 mg) in pyridine (0.45 ml) was treated with pivaloyl chloride (700 mg) at room temperature for 5 h and the mixture was then processed as described for **1**, to give a residue that was passed (2×) through silica gel with solvent *A* (10:3). Eluted first was an anomeric mixture ($\alpha < \beta$, R_F 0.53 < 0.50) of **18** (20 mg, 31%); oil, $[\alpha]_D$ +84° (chloroform).

Anal. Calc. for $C_{30}H_{41}NO_{11}$: C, 60.90; H, 6.99; N, 2.37. Found: C, 60.76; H, 7.11; N, 2.48.

Eluted second was the β anomer of **18** (12 mg, 18.5%), $[\alpha]_D$ +50° (chloroform), as a glass. P.m.r. data (acetone-d_6): δ 6.26 (s, H-1), 5.53 (d, $J_{4,5}$ 6.34 Hz, H-5), 5.29 (s, H-2), 5.12 (dd, *J* 4.9 and 4.4 Hz, H-4), 5.02 (d, $J_{3,4}$ 4.4 Hz, H-3), and 4.44 (q, 1 H, $PhCH_2CH$); (pyridine-d_5): δ 6.74 (s, H-1), 6.02 (d, $J_{4,5}$ 6.15 Hz, H-5), 5.77 (s, H-2), 5.41 (t, $J_{4,3}$ 4.98, $J_{4,5}$ 6.15 Hz, H-4), and 5.19 (d, $J_{3,4}$ 4.98 Hz, H-3) (Found: C, 60.82; H, 7.13; N, 2.18).

A sample of **18** was kept in solvent *A* (10:3) with silica gel overnight; t.l.c. monitoring showed complete disappearance of the spot having R_F 0.53 (α anomer), and the presence of Boc-phenylalanine and several silver-nitrate positive spots.

Evidence for the 2-O-[N-(tert-butoxycarbonyl)-L-phenylalanyl]-D-glucofuranurono-6,3-lactone structure. — A solution of[1] **19** (116 mg) in *N,N*-dimethylformamide (2 ml) was treated with ethereal diazomethane (0.16 mmol/ml, 2 ml; 1.2 equiv.) at 4° for 1 h and, after evaporation (0.1 Torr) of the solvent, the residue was dissolved in pyridine (1 ml) and treated with pivaloyl chloride (1.50 g) at 4° for 4 h. The mixture was poured onto ice and extracted with chloroform, and, after work-up as described for **1**, the crude product was eluted from silica gel with solvent *A* (10:3). The faster-moving fractions consisted of several components having glucopyranuronate (t.l.c. and p.m.r. evidence) structures and were not further investigated. The residue (31 mg, 20%) from the slower-moving fractions was a mixture of two, closely migrating components (t.l.c. mobilities identical with those of **18**α and β) and several minor contaminants. Re-chromatography (2×) of this material afforded the slower-moving component as a chromatographically homogeneous glass (12 mg, 8%) whose chromatographic mobility and i.r. and p.m.r. spectra (in three solvents) were indistinguishable from those of 2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-1,5-di-*O*-pivaloyl-β-D-glucofuranurono-6,3-lactone (**18**β).

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