GLYCOSYL ESTERS OF AMINO ACIDS

PART IV*. SYNTHESIS OF 1-*O*-(ACYLAMINOACYL)-2,3,4,6-TETRA-*O*-BENZYL-D-GLUCOPYRANOSES BY THE IMIDAZOLE-PROMOTED ACTIVE ESTER AND DICYCLOHEXYLCARBODIIMIDE METHODS

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ABSTRACT

1-*O*-(Acylaminoacyl)-2,3,4,6-tetra-*O*-benzyl-D-glucopyranoses were prepared in high yields by two routes involving direct participation of imidazole in the ester linkage formation; namely, (*a*) the accelerated active-ester method, and (*b*) the imidazole-promoted dicyclohexylcarbodiimide condensation. The compounds were synthesized from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and pentachlorophenyl esters of optically active benzyloxycarbonyl- and *tert*-butyloxycarbonyl-amino acids in method (*a*) or benzyloxycarbonyl- and acetyl-amino acids in method (*b*). By both methods, anomeric mixtures of D-glucosyl esters were obtained; they were resolved by column chromatography and the α and β anomers were fully characterized. The retention of configuration of the amino acid moiety was determined from optical rotations of acylamino acid methyl esters formed from D-glucosyl esters with methanolic sodium methoxide. With benzyloxycarbonyl and *tert*-butyloxycarbonyl protecting groups, a high degree of retention of optical activity was established in both methods–method (*a*) being slightly superior.

INTRODUCTION

Previous work in this laboratory[1,2] has been concerned with the synthesis of fully acetylated D-glucosyl esters containing an ester-bonded *N*-acylamino acid as the aglycon group; the esters were prepared by the silver salt and dicyclohexylcarbodiimide (DCC) method. In the search for more-effective synthetic routes which might also be of general applicability for the preparation of the hitherto unknown "ester type" glycopeptides, several methods involving activation of the acylamino acid carboxyl group and commonly used for the formation of peptide bonds, were investigated. In a preliminary communication[3], it was claimed that, under conventional conditions used in peptide-coupling procedures, the activated esters of acylamino acids do not react with HO-1 of an otherwise fully protected sugar moiety, whereas application of the "accelerated active ester" (AAE) method[4,5], involving direct

*Part III: Ref. 3.

TABLE I

1-*O*-ACYLAMINOACYL-2,3,4,6-TETRA-*O*-BENZYL-D-GLUCOPYRANOSES*

Starting amino acid component	D-*Glucosyl esters*													
	Compound No.	*Method of preparation*	*Yield (%)*	*Anomer*	*M.p. (degrees)*	$[\alpha]_D^{22}$ *(degrees)*[a]	*N.m.r. data*[b] $\tau(J_{1,2})$ *for H-1*	*Found (%)*			*Formula*	*Calc. (%)*		
								C	*H*	*N*		*C*	*H*	*N*
Z-DL-Ala-OPCP	1	AAE	75	β	80–83[c]	+10.9	4.34d (7)	72.32	6.44	2.04				
				α	oil	+48.6	3.58d (3)	72.20	6.50	1.95				
Z-L-Ala-OPCP	1	AAE	76	β	88–89[c]	+ 2.5	4.33d (7)	72.38	6.47	2.16				
				α	oil	+52.0	3.59d (3)	72.12	6.45	2.00				
Z-L-Ala-OH	1	DCC+Im.	60	β	88–89[c]	+ 3.5								
				α[d]										
Z-D-Ala-OPCP	1	AAE	74	β	83–84[c]	+19.5	4.31d (7.5)	72.49	6.48	2.14	$C_{45}H_{47}NO_9$	72.46	6.35	1.88
				α	oil	+50.6	3.62d (3)	72.53	6.38	2.09				
Z-β-Ala-OPCP	2	AAE	50	β	98–99[e]	+ 5.0	4.32d (7)	72.32	6.45	1.91				
				α	77–78[e]	+50.4	3.63d (3)	72.61	6.38	2.13				
Z-β-Ala-OH	2	DCC+Im.	30	β	98–99[e]	+ 5.5								
				α	76–78[e]	+48.5								
Z-L-Phe-OPCP	3	AAE	73	β	103–105[c]	+ 8.6	4.28d (7)	74.24	6.40	1.68	$C_{51}H_{51}NO_9$	74.52	6.26	1.70
				α	oil	+58.5	3.54d (3)	74.74	6.48	1.83				

Z-L-Asp(OBZL)-OPCP	4	AAE	85	β	59–61[e]	+ 6.5	4.34d (7)				$C_{53}H_{53}NO_{11}$	72.34	6.07	1.59
				α	oil	+51.0	3.61d (3)	72.19	6.09					
Z-L-Asp(OBZL)-OH	4	CDC+Im.	60	β	59–61[e]	+ 7.7		72.52	6.30	1.70				
				α	oil	+49.5		72.57	6.32	1.56				
BOC-Gly-OPCP	5	AAE	84	β	118–119[e]	+ 8.0	4.31d (7)	70.43	6.96	2.20	$C_{41}H_{47}NO_9$	70.57	6.79	2.00
				α	oil	+55.5	3.58d (3)	70.71	7.07	2.03				
BOC-L-Ala-OPCP	6	AAE	77	β	99–100[e]	+ 1.0	4.35d (7)	70.90	7.10	2.07	$C_{42}H_{49}NO_9$	70.86	6.94	1.97
				α	oil	+53.2	3.56d (3)	70.73	6.95	2.23				
BOC-L-Phe-OPCP	7	AAE	80	β	65–67[f]	+14.0	4.31d (7)	73.30	6.73	1.96	$C_{48}H_{53}NO_9$	73.17	6.78	1.77
				α[d]										
Ac-L-Ala-OH	8	DCC+Im.	62	β	130–133[g,h]	+ 2.6	4.32d (7)				$C_{39}H_{43}NO_8$	71.65	6.63	2.24
				α	45–47[e,h]	+62.5	3.61d (3)	71.71	6.94	2.07				
Ac-D-Ala-OH	8	DCC+Im.	66	β	136–137[g]	+15.0	4.31d (7)	71.52	6.46	2.23				
				α	oil	+60.0	3.61d (3)	71.73	6.89	2.35				
Ac-L-Phe-OH	9	DCC+Im.	61	β	135–137[g]	+11.9	4.30d (7)[i]				$C_{45}H_{47}NO_8$	74.05	6.49	1.92
				α	oil	+73.1	3.59d (3)	74.05	6.62	2.01				
Ac-L-Phe-OAg	9	Ag-salt	30	β	139–140[g]	+11.5	4.31d (7)[i]	74.12	6.45	2.03				
Ac-L-Met-OH	10	DCC+Im.		β	118–120	+13.7	4.30d (7)[i]	69.09	6.40	2.16	$C_{41}H_{47}NO_8S$	68.99	6.64	1.96
				α[d]										
Ac-L-Met-OAg	10	Ag-salt	20	β	124–125	+13.2	4.30d (7)[i]	68.76	6.52	2.28[j]				

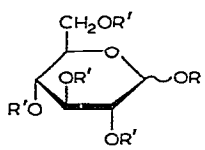
*Abbreviations: Z, benzyloxycarbonyl; PCP, pentachlorophenyl; BZL, benzyl; BOC, *tert*-butyloxycarbonyl; Im., imidazole.
[a]Determined in chloroform (*c* 1–2). [b]Measured in chloroform-*d*; coupling constant (*J*) in Hz. [c]From ethanol. [d]Not determined. [e]From ether–light petroleum. [f]From ethanol+some drops of water. [g]From ether–acetone (2:1) followed by light petroleum. [h]Prepared by silver salt method (Ref. 2): β anomer, m.p. 134–135°; α anomer, m.p. 45–46°. [i]After deuterium exchange of the NH signal. [j]Found: S, 4.21; calc. 4.48.

participation of imidazole in the transacylation reaction, leads to a rapid formation of the corresponding 1-ester. The catalytic activity of imidazole was also observed[3] in the DCC condensation of a sugar fully protected other than at position 1 and acylamino acid components. It has been suggested[3] 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 acyl group to the sugar component.

In the present work, the imidazole-promoted active ester and DCC methods were studied in more detail and successfully applied to the synthesis of a variety of 1-esters of fully benzylated D-glucopyranoses involving optically active *N*-acylamino acids. The extent of racemization of the amino acid moiety during ester bond-formation was investigated with respect to the amino acid protecting-group, the nature of the amino acid, and the coupling procedure employed.

RESULTS AND DISCUSSION

In the synthesis of amino acid D-glucosyl esters by the AAE method, pentachlorophenyl esters of benzyloxycarbonyl- and *tert*-butyloxycarbonyl-amino acids were used. Preliminary experiments with several active esters suggested that pentachlorophenyl esters were the most suitable activated carboxyl components, due to their high reactivity and the ease with which pentachlorophenol liberated in the coupling reaction could be removed from the reaction mixture. It has been found that use of four to six equivalents of imidazole is optimal. With lower concentrations of imidazole, the yields of D-glucosyl esters dropped substantially, even after prolonging the reaction time up to 24 h, while larger amounts of the base led to cleavage of the 1-ester linkage. Thus, the procedure finally adopted was to treat 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with a slight excess (10%) of the appropriate *N*-acyl-L-,



1-10 R' = $PhCH_2$ 16, 17 R' = Ac

1 R = MeCHCO (NHZ)
2 R = CH_2CH_2CO (NHZ)
3 R = $PhCH_2CHCO$ (NHZ)
4 R = $PhCH_2O_2CCH_2CHCO$ (NHZ)
5 R = CH_2CO (NHBOC)
6 R = MeCHCO (NHBOC)
7 R = $PhCH_2CHCO$ (NHBOC)
8 R = MeCHCO (NHAc)
9 R = $PhCH_2CHCO$ (NHAc)
10 R = $MeSCH_2CH_2CHCO$ (NHAc)
16 R = MeCHCO (NHAc)
17 R = $MeSCH_2CH_2CHCO$ (NHAc)

Z = $PhCH_2OCO$; BOC = Me_3COCO

D-, or DL-amino acid pentachlorophenyl ester in the presence of five equivalents of imidazole in dichloromethane for 5 h at room temperature; in this way, the corresponding D-glucosyl esters **1–7** were obtained as anomeric mixtures in excellent yields (Table I).

The condensation of tetra-*O*-benzyl-α-D-glucopyranose with benzyloxycarbonyl- and acetyl-L-, D-, or DL-amino acids by the DCC method in the presence of two equivalents of imidazole gave the anomeric mixtures of D-glucosyl esters **1**, **4**, and **8–10** in very good yields (Table I). When imidazole was omitted, only traces of the corresponding product could be detected by t.l.c. β-Alanine glucosyl ester **2** was obtained, by both procedures, in substantially lower yields; this could be ascribed to a lower degree of activation of the carbonyl group in the β-amino acid active ester.

The anomeric separation of compounds **1–10** was achieved by direct crystallisation and/or by column chromatography on silica gel. The β-D anomers were crystalline, and column chromatography on activated charcoal allowed the isolation of pure α-D anomers which were usually syrups. The α,β-ratio for both methods was ~1:1.5, but, due to extensive chromatography, pure α anomers were recovered in considerably lower yields than the corresponding β anomers.

The structures of compounds **1–10** were based on elemental analyses, and i.r. and n.m.r. spectral and optical rotatory data. The i.r. spectra of all products showed absorptions characteristic of amide and ester carbonyl functions. The n.m.r. spectra of **5–7** exhibited one singlet integrating for nine protons at τ 8.55–8.60 which demonstrated the presence of the *tert*-butyloxycarbonyl group, and the spectra of **8–10** contained one 3-proton singlet at τ 8.02–8.06 assigned to the *N*-acetyl group. The positions of the H-1 signals and the magnitude of the $J_{1,2}$ values were consistent with the observed optical rotations (see Table I), thus allowing assignment of anomeric configuration. In the case of β-D anomers of **9** and **10**, the H-1 resonances could be observed only after deuterium exchange of the broad N–H signal.

A comparison of the physical constants of **1–10** did not allow any conclusions about the retention of optical activity of the amino acid moiety. The β-D anomer of **1**, obtained through the benzyloxycarbonylalanine pentachlorophenyl ester, revealed some differences with respect to the initial configuration of the amino acid component. On the other hand, both the AAE and the imidazole-promoted DCC methods afforded identical preparations of **1**, **2** and **3**, respectively. In a previous paper[2], it was found that the amino acid moiety in fully acetylated 1-*O*-(acetylaminoacyl)-D-glucopyranoses, prepared by the DCC method in the presence of triethylamine, underwent complete racemization, whereas the synthesis by the silver salt method proceeded with 80–85% retention of configuration, regardless of the amino acid protecting-group. For comparison, the fully benzylated D-glucosyl esters **9** and **10**, containing the *N*-acetyl protecting group, were also prepared by the silver salt method; melting points of the products were higher than those of **9** and **10** obtained by the imidazole-promoted DCC condensation (Table I).

The susceptibility of the above methods to racemization was estimated from the optical rotations of acylamino acid methyl esters formed through transesterifica-

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TABLE II

DATA FOR *N*-ACYLAMINO ACID METHYL ESTERS FORMED FROM FULLY BENZYLATED 1-*O*-(2-ACYLAMINOACYL)-D-GLUCOPYRANOSES WITH METHANOLIC 0.1M SODIUM METHOXIDE

Configuration of the starting amino acid component	D-*Glucosyl ester*		*Ester isolated*			
	Compound and anomeric configuration	*Method of preparation*	*Compound name and No.*	*M.p. (degrees)*	$[\alpha]_D^{22}$ *(degrees c 1-2)*	*Retention of optical activity (%)*
L-	1β	AAE	Benzyloxycarbonylalanine methyl ester (**11**)	44–45	−36.0[a]	100 (±2)
		DCC+Im.		44–45	−33.8[a]	94 (±2)
L-	3β	AAE	Benzyloxycarbonylphenylalanine methyl ester (**12**)	oil	+52.4[b]	97 (±2)
L-	4α	AAE	Dimethyl benzyloxycarbonyl-aspartate (**14**)	oil	+21.5[b]	73 (±5)[c]
L-	6β	AAE	*tert*-Butyloxycarbonylalanine methyl ester (**15**)	oil	−41.0[a]	100 (±2)
L-	8β	DCC+Im.	*N*-Acetylalanine methyl ester[d]	oil	−68.0[e]	75 (±3)
D-				oil	+76.2[e]	84 (±3)
L-	9α+β	DCC+Im.	N-Acetylphenylalanine methyl ester[f]	85–86	+15.6[a]	82 (±5)
		Ag-salt		87–88	+17.0[a]	90 (±5)
L-	10β	DCC+Im.	*N*-Acetylmethionine methyl ester[g]	79–80	− 3.9[h]	19 (±5)
		Ag-salt		45–47	−20.5[h]	97 (±5)

[a]In methanol. [b]In chloroform. [c]Calculated on the basis of optical rotation of **14** obtained from 4-benzyl benzyloxycarbonylaspartic acid (96%). [d]Ref. 6, b.p. 76° (0.25 Torr), $[\alpha]_D$ −91.7° (water). [e]In water. [f]Ref. 7, m.p. 89–90°, $[\alpha]_D$ +19.0° (methanol). [g]Ref. 8, m.p. 43.5–44.5°, $[\alpha]_D$ −21.1° (ethanol). [h]In ethanol.

tion of the fully benzylated D-glucosyl esters with methanol in the presence of one equivalent of sodium methoxide. Control experiments revealed that racemization in this base-catalyzed reaction was within experimental error. As it can be seen from Table II, D-glucosyl esters having the amino acid aglycon group protected by a benzyloxycarbonyl or *tert*-butyloxycarbonyl group retained the configuration of the amino acid component to a high degree. By comparing the AAE and imidazole-promoted DCC method, conservation of optical purity seemed to be higher during the former coupling procedure. In the case of **4**, the benzyl ester group of the aspartyl residue also underwent an exchange reaction and dimethyl benzyloxycarbonyl-aspartate was formed. Control experiments (see Experimental) suggested that some loss of optical activity had occurred during this accompanying reaction.

The results obtained with D-glucosyl esters of acetylamino acids, which were synthesized by the imidazole-promoted DCC method, revealed that the degree of racemization during 1-ester formation was dependent on the nature of the amino acid involved. Whereas the extent of racemization in **8** and **9** did not exceed 25%, the acetylmethionine component in **10** was almost completely racemized. On the contrary, the preparation of **9** and **10** by the silver salt method proceeded almost without racemization.

Evidence that the nature of the acetylated amino acid residues has a strong influence on the extent of racemization was also obtained in the fully acetylated D-glucosyl ester series. Condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose with *N*-acetyl-L-alanine and -L-methionine in the presence of DCC and imidazole afforded the corresponding esters **16** and **17** in 70 and 48% yield, respectively. An ester-exchange reaction of **16** and **17** with methanol resulted in 70% optically pure *N*-acetylalanine methyl ester and a completely racemized acetylmethionine methyl ester. The results could be rationalized by assuming a different rate of formation of the intermediate acylimidazoles: in the case of acetylmethionine, the formation of this intermediate is too slow to prevent (*a*) the $O \rightarrow N$ acyl rearrangement of the *O*-acylurea derivative, and (*b*) the formation of the corresponding oxazolone which is followed by immediate racemization.

In the n.m.r. spectra of fully acetylated and fully benzylated D-glucosyl esters containing acetylalanine as the aglycon group, a small change in chemical shift of the methyl resonances with respect to the configuration of the amino acid has been observed. The methyl doublet signal at τ 8.60 (J 7.5 Hz) in the D-glucosyl ester linked to *N*-acetyl-L-alanine appeared at a slightly lower field than the equivalent signal for the diastereoisomeric D-glucosyl ester containing an *N*-acetyl-D-alanyl residue; D-glucosyl esters with partially racemized *N*-acetylalanyl residues revealed two sets of doublets of different intensities. It is known[9,10] that methyl resonances in the n.m.r. spectra of *N*-protected peptide derivatives of alanine, particularly those containing an aromatic ring, reveal differences between LL (or DD) and DL (or LD) compounds sufficient to be used as a convenient tool for the analysis of racemization. The above observations suggest that this could also be applied in the field of sugar–amino acid compounds.

EXPERIMENTAL

General. — Melting points are uncorrected. Evaporations were performed in a rotatory evaporator *in vacuo*, the bath temperature being kept below 45°, if not stated otherwise. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm) or carbon–Celite (Charcoal activated, BDH; Kieselguhr, BDH), prepared as a 2:1 (w/w) intimate mixture. The proportion of the substance to silica gel and carbon–Celite was 1:30–50 and 1:50–70, respectively. Solvent systems: *A* 10:1 benzene–ethyl acetate; *B* 5:1 benzene–ethyl acetate; *C* 2:1 benzene–ethyl acetate; *D* 5:1:1 ether–acetone–light petroleum. T.l.c. was performed on Kieselgel G (Merck); spots were located with 10% sulphuric acid and heating or with chlorine–starch–iodide reagent for acylated amino acids. Optical rotations were determined for 1% solutions in chloroform unless otherwise stated. I.r. spectra were determined on a Perkin–Elmer Model 137 spectrometer. N.m.r. spectra were recorded on solutions in chloroform-*d* with tetramethylsilane as internal standard using a Varian A-60A spectrometer.

Benzyloxycarbonyl-DL-alanine pentachlorophenyl ester[11], benzyloxycarbonyl-L-alanine pentachlorophenyl ester[12], benzyloxycarbonyl-D-alanine pentachlorophenyl ester, benzyloxycarbonyl-L-phenylalanine pentachlorophenyl ester[12], benzyloxycarbonyl-β-alanine pentachlorophenyl ester[13], and 1-pentachlorophenyl 4-benzyl benzyloxycarbonylaspartate[14] were prepared by the procedure of Kovacs *et al.*[12]. Pentachlorophenyl esters of *tert*-butyloxycarbonyl-glycine, -L-alanine, and -L-phenylalanine were prepared by the method of Johnson and Trask[15].

Benzyloxycarbonyl-D-alanine pentachlorophenyl ester (yield 75%) had m.p. 172–173° (from methanol), and $[\alpha]_D^{25}$ +14.4° (*c* 2.21, chloroform).

Anal. Calc. for $C_{17}H_{12}Cl_5NO_4$: C, 43.30; H, 2.57: Cl, 37.59. Found: C, 43.52; H, 2.48; Cl, 37.36.

*Preparation of 1-*O*-(*N*-acylaminoacyl)-2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranoses* (**1–10**). — (*a*) *Accelerated active ester* (*AAE*) *method.* 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (540 mg, 1 mmole), the appropriate *N*-acylamino acid pentachlorophenyl ester (1 mmole), and imidazole (340 mg, 5 mmoles) were dissolved in dry dichloromethane (10 ml) at room temperature. The progress of the reaction was monitored by t.l.c. (solvent *A*), and after 1–2 h an additional amount (10% excess) of the amino acid component was added with shaking. After a total reaction time of 5 h, the precipitated pentachlorophenol was filtered off and washed with dichloromethane, and the combined filtrates were poured on to ice and washed with water, 1.5% sulphuric acid or 10% citric acid (in reactions with compounds containing *tert*-butyloxycarbonyl group), water, aqueous sodium hydrogen carbonate and water. After drying (sodium sulphate) and evaporation, the residue was treated in one of the following ways. The β anomers of **1** (formed in the reaction with benzyloxycarbonyl-L-alanine pentachlorophenyl ester) and of **3** were obtained by direct crystallisation of the residue from ethanol. Purification and anomeric separation of other D-glucosyl esters was effected on silica gel columns with solvent *A*; the α anomers of the products migrated slightly faster than the corresponding β anomers. Fractions were analysed

by t.l.c. and those enriched in β anomers were combined and evaporated and the residues were crystallised from the solvents indicated in Table I.

Fractions containing preponderantly the α anomers of the products were combined and evaporated, and the oily residues were rechromatographed on silica gel and then on carbon–Celite columns with solvent *A*. The anomeric purity of the fractions was monitored by optical rotation and n.m.r. spectroscopy. Physical constants, yields, and analytical data of the α and β anomers of compounds **1–7** are given in Table I.

(*b*) *Carbodiimide+imidazole* (*DCC+Im.*) *method.* To 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (1 mmole), the appropriate acylamino acid (1 mmole) and imidazole (136 mg, 2 mmoles) dissolved in dichloromethane (10 ml) [or, in reactions with acetylamino acids, in 5:1 dichloromethane–*N*,*N*-dimethylformamide (10 ml)], a solution of DCC (206 mg, 1 mmole) in dichloromethane (5 ml) was added at 0°. The progress of the reaction was monitored by t.l.c., and after 1–3 h an additional amount (10–15% excess) of the corresponding acylamino acid was added. The total reaction time was 5–6 h, whereupon *N*,*N*′-dicyclohexylurea was filtered off, and the filtrates were poured on to ice and treated further as described under (*a*). In reactions with acetylamino acids, traces of *N*,*N*-dimethylformamide were removed (bath 40°/0.12 Torr) before submitting the residues to column chromatography with solvent *C*. Physical constants of the products are given in Table I.

Treatment of D-*glucosyl esters with methanolic sodium methoxide.* — To a suspension of the respective D-glucosyl ester (0.5 mmole) in anhydrous methanol (5 ml), 0.1M methanolic sodium methoxide (5 ml) was added, and the mixture was left with occasional shaking for 1 h at room temperature; the progress of the reaction was followed by t.l.c. in solvents *A*, *C*, or *D*. Precipitated tetra-*O*-benzyl-α-D-glucopyranose was filtered off and washed with cold methanol, and the combined filtrate and washings were passed through a column of Amberlite IR-120(H^+) resin (10 ml) prewashed with methanol. The effluent was evaporated to dryness, and the residue was eluted through a column of silica gel with solvent *A* (in reactions with D-glucosyl esters **1**, **3**, **4**, and **6**), *B* (in reactions with **8–10**), or *C* (in reactions with **16–17**). Elution afforded the corresponding acylamino acid methyl esters (yields 60–80%) and, in reactions with fully benzylated glucosyl esters, also an additional crop of tetra-*O*-benzyl-α-D-glucopyranose (total yields 80–90%). The isolated methyl esters were further purified by crystallisation or re-chromatography to constant optical rotation.

Benzyloxycarbonyl-L-*alanine methyl ester* (**11**). — To benzyloxycarbonyl-L-alanine (2 mmoles) in anhydrous methanol (5 ml), M thionyl chloride (freshly redistilled) in anhydrous methanol (5 ml) was added at 0°, and the reaction mixture was shaken at room temperature for 2 h. The solvent was removed, the residue taken up in chloroform, and the extract was washed with water, sodium hydrogen carbonate, and water, and dried (sodium sulphate). After evaporation of the solvent, the residue was crystallised from light petroleum. Yield: 85%; m.p. 45–46°, $[\alpha]_D$ −36.0° (methanol). I.r. data: ν_{max}^{KBr} 3400 (NH), 1760 (C=O), 1680 and 1525 (amide I and II), 755 and 700 cm^{-1} (CH aromatic).

Anal. Calc. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.84; H, 6.53; N, 6.11.

Benzyloxycarbonyl-L-phenylalanine methyl ester (**12**). — Benzyloxycarbonyl-L-phenylalanine was treated as described for **11**, and, after working up, the product was eluted from a silica gel column with solvent *A*. Yield: 81%; oil, $[\alpha]_D$ +53.9°. Hydrolysis of a sample with 6M HCl at 100° gave optically pure phenylalanine, $[\alpha]_D$ −36.0° (water).

Anal. Calc. for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47. Found: C, 69.09; H, 6.14; N, 5.58.

Dimethyl benzyloxycarbonyl-L-aspartate (**14**). — (*a*) Benzyloxycarbonyl-L-aspartic acid (2 mmoles) was added in small portions with shaking to a solution of diazomethane (tenfold excess) in ether (100 ml) at 0°. After standing at 0° for 3 h, ether was removed, and the oily residue was eluted from a silica gel column with solvent *A*. Yield: 58%, oil, $[\alpha]_D$ +29.4°.

Anal. Calc. for $C_{14}H_{17}NO_6$: C, 56.94; H, 5.80; N, 4.74. Found: C, 56.84; H, 5.79; N, 4.57.

(*b*) 4-Benzyl benzyloxycarbonyl-L-aspartic acid was treated with thionyl chloride–methanol as described for **11**, and, after working up, the product was eluted from a silica gel column with solvent *A*. Separate fractions containing 4-benzyl 1-methyl benzyloxycarbonylaspartate (**13**) and the slower-moving dimethyl ester **14** were obtained.

Compound **13** (50%) was isolated as an oil, $[\alpha]_D$ +21.7°.

Anal. Calc. for $C_{20}H_{21}NO_6$: C, 64.68; H, 5.70; N, 3.77. Found: C, 64.66; H, 5.67; N, 3.95.

Compound **14** (31%), also an oil, had $[\alpha]_D$ +22.4°. N.m.r. data: τ 2.72 (singlet, 5 protons, aromatic), 6.30, 6.37 (singlets, 2 × 3 protons, OMe).

Treatment of a sample of **13** with methanolic sodium methoxide, as described in the transesterification of D-glucosyl esters, gave the dimethyl ester **14** with $[\alpha]_D$ +15.0°.

tert-*Butyloxycarbonyl-L-alanine methyl ester* (**15**). — *tert*-Butyloxycarbonyl-L-alanine was treated with diazomethane as described for **14**, and the reaction product was purified on a silica gel column with solvent *A*. Yield: 72%; oil, $[\alpha]_D$ −40.0° (methanol). Treatment of a sample with one equivalent of 0.5M NaOH in acetone (1:1) gave *tert*-butyloxycarbonyl-L-alanine, m.p. 83–84°, $[\alpha]_D$ −23.0°; lit[16] m.p. 83–84°, $[\alpha]_D$ −22.4°.

Anal. Calc. for $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.40; H, 8.14; N, 6.66.

2,3,4,6-Tetra-O-acetyl-1-O-(acetylalanyl)-D-glucopyranose (**16**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (1.740 g, 5 mmoles), *N*-acetyl-L-alanine (660 mg, 5 mmoles), and imidazole (655 mg, 10 mmoles) in dichloromethane–*N*, *N*-dimethylformamide (10:1, 11 ml), a solution of DCC (1.030 g, 5 mmoles) in dichloromethane (5 ml) was added, and the reaction mixture was treated further as described for preparation of fully benzylated D-glucosyl esters. After working up, the

product was eluted from a column of silica gel with solvent *C*; crystallisation of the chromatographically pure anomeric mixture (1.60 g, 69.5%) from dry ether–acetone (2:1) by the addition of light petroleum afforded the β anomer of **16** (735 mg), m.p. 135–136°, $[\alpha]_D$ +4.9° (lit.[2] data for **16** by the silver salt method, m.p. 138–140°, $[\alpha]_D$ −5.0°).

The mother liquor was evaporated to dryness, and the residue was submitted to silica gel and carbon–Celite column chromatography to give the α anomer of **16** as a colourless syrup, $[\alpha]_D$ +82.5°.

Anal. Calc. for $C_{19}H_{27}NO_{12}$: C, 49.46; H, 5.90; N, 3.04. Found: C, 49.36; H, 5.88; N, 3.27.

2,3,4,6-Tetra-O-*acetyl-1*-O-(*acetylmethionyl*)-D-*glucopyranose* (**17**). — The reaction was performed with *N*-acetyl-L-methionine (5 mmoles) as the amino acid component, exactly as described for **16**, to give chromatographically homogenous **17** (1.230 g, 47.2%), Recrystallisation from ether–acetone–light petroleum afforded the β anomer of **17** (720 mg), m.p. 124–127°, $[\alpha]_D$ +4.8° (lit.[2] data for **17** by the silver salt method, m.p. 140–141°, $[\alpha]_D$ +3.8°).

The mother liquor was evaporated to dryness, and the oily residue was submitted to silica gel and carbon–Celite chromatography to give the α anomer of **17** as a syrup, $[\alpha]_D$ +71.5°. N.m.r. data: τ 3.18 (doublet, J 8.5 Hz, NH), 3.60 (doublet, $J_{1,2}$ 3 Hz, H-1), 7.85 (3-proton singlet, SMe), and 7.91, 7.96–7.99 (3 singlets, 12 H, 4 OAc).

Anal. Calc. for $C_{21}H_{31}NO_{12}S$: C, 48.36; H, 5.99; N, 2.69. Found: C, 48.51; H, 6.16; N, 2.78.

1-O-(*Acetyl*-L-*phenylalanyl*)-*and 1*-O-(*acetyl*-L-*methionyl*)-*2,3,4,6-tetra*-O-*benzyl*-β-D-*glucopyranoses* (**9** *and* **10**) *by the silver salt method.* — These compounds were prepared from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride[2,17] and the silver salt of *N*-acetyl-L-alanine and *N*-acetyl-L-methionine, respectively, by refluxing in dry benzene as described[2] for the alanine homologue. The products were purified on a column of silica gel with solvent *B*; the physical constants are given in Table I.

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