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SYNTHESIS OF AMINOACYL DERIVATIVES OF SUGARS

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Abstract—A simple and convenient method for preparation of aminoacyl derivatives of simple sugars by the condensation of unprotected carbohydrate with N-carbobenzoxyaminoacid in the presence of carbodiimide has been developed. The condensation of glucose with N-carbobenzoxyaminoacid affords almost exclusively the 6-O (N-carbobenzoxyaminoacyl)-sugars. By this method 6-O (N-carbobenzoxyaminoacyl)-D-glucoses containing residues of glycine, D,L-alanine, D,L-valine, D,L-norleucine, β-alanine and ϵ-aminocaproic acid have been prepared.

The unprotected aminosugars condense smoothly with N-carbobenzoxyaminoacid in the presence of carbodiimide in aqueous pyridine to form N-(N'-carbobenzoxyaminoacyl)-hexosamine. The N-carbobenzoxy derivatives were converted to O- and N-aminoacyl derivatives with free amino groups by hydrogenolysis in aqueous methanol in the presence of oxalic acid. Corresponding O- and N-aminoacyl derivatives have been isolated as oxalates.

CARBOHYDRATE–protein compounds (mucopolysaccharides, mucoproteins, etc.) forming the group known as glycopeptides have recently attracted considerable attention but the main problem—the nature of the carbohydrate–peptide bond—still remains to be solved. Owing to difficulties connected with the natural glycopeptides, a more convenient approach to this problem would be the use of model compounds with a different type or the carbohydrate–aminoacid bond.

In contrast to the N-glycosides of amino acids the aminoacyl derivatives of sugars with ester or amide bonds are almost unknown and it is this type of bond that is most frequently suggested for natural glycopeptides.[1]

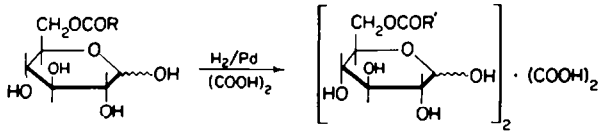
Synthesis of 6-aminoacyl derivatives of glucose

The few known[2,3] O-aminoacyl derivatives of carbohydrates were synthesized under drastic conditions using suitably protected carbohydrates. Recently Japanese workers have described the synthesis of 6-O-(N-carbobenzoxyglycyl)-glucose by the mixed anhydride[4] and the carbodiimide[5] methods, the sugars being protected.

A simple method has been developed for the condensation of unprotected carbohydrates and N-carbobenzoxyamino acids.[6] Both the primary and secondary hydroxyl groups are acylated but the reaction rate of the former is much greater and the glycoside hydroxyl probably undergoes no acylation at all. When excess monosaccharide is used the condensation reaction affords almost exclusively the 6-O-(N-carbobenzoxyaminoacyl)-sugars. By this method 6-O-(N-carbobenzoxyaminoacyl)-D-glucose (I)

[1] F. R. Bettelheim Jevons, *Adv. Prot. Chem.* **13**, 35 (1958).
[2] M. Bergmann, L. Zervas and J. Overhoff, *Z. physiol. Chem.* **224**, 52 (1934).
[3] Ling Yang, V. A. Derevitskaja and Z. A. Rogovin, *Vissokomolekularnye Soedinenya (USSR)* **1**, 157 (1959).
[4] S. Okui and S. Suzuki, *J. Pharm. Soc. Japan* **79**, 471 (1959).
[5] J. Ukita and S. Suzuki, *J. Pharm. Soc. Japan* **81**, 222 (1961).
[6] N. K. Kochetkov, V. A. Derevitskaja and L. M. Likhosherstov, *Chem. Ind.* 1532 (1960); *Zh. V. Kh. O. Im. Mendeleeva (USSR)* **6**, 228 (1961).

containing residues of glycine, D,L-alanine, D,L-valine, D,L-norleucine, β-alanine and ε-aminocaproic acid have been prepared. A variety of amino acids were selected with a view to establish the scope of the method and subsequently to obtain data on the effect of the amino acid moieties on the stability of the ester bond.



I VI

R = CbzNHCH$_2$–
R = CbzNHCHCH$_3$
R = CbzNHCH(CH$_2$)$_3$CH$_3$
R = CbzNHCH$_2$CH$_2$–
R = CbzNH(CH$_2$)$_5$–
R = CbzNHCHCH(CH$_3$)$_2$

R′ = NH$_2$CH$_2$–
R′ = NH$_2$CHCH$_3$
R′ = NH$_2$CH(CH$_2$)$_3$CH$_3$
R′ = NH$_2$CH$_2$CH$_2$CH$_2$–
R′ = NH$_2$(CH$_2$)$_5$–

Cbz = —COOCH$_2$C$_6$H$_5$

The compounds obtained gave positive aniline phthalate (glycoside hydroxyl) and fluorescein (carbobenzoxy group) reactions. The IR spectra exhibited absorption bands at 1740–1755 cm^{-1} (ester carbonyl) and 1675–1710 cm^{-1} (urethane carbonyl). The IR spectra of I (R = CBzNHCH$_2$—) are given in Fig. 1.

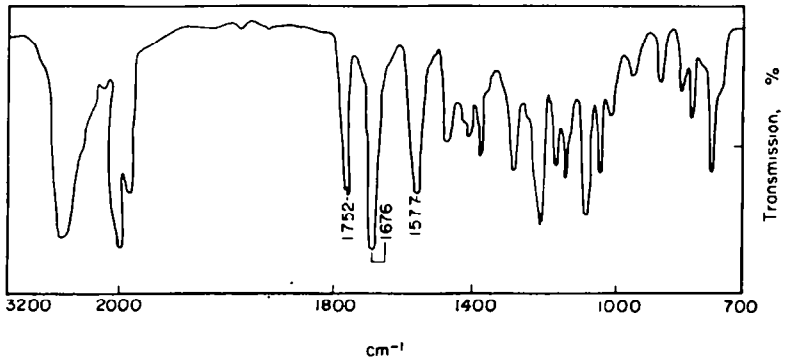
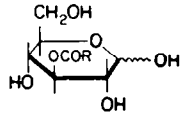


FIG. 1. IR-spectrum: 6-O-(N-carbobenzoxyglycyl)-glucose; vaseline oil.

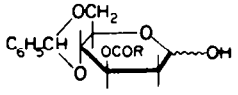
The structure of the 6-O-aminoacyl derivatives was confirmed by oxidation with periodic acid in acetate buffer at pH 4·3 (4 moles HIO_4 consumed and 4 moles HCO_2H produced). It is noteworthy that when the oxidation is carried out in the absence of buffer more oxidizing agent is used, evidently due to oxidation of glucose liberated as the result of hydrolysis of the ester bond.

As mentioned, aminocylation of glucose in the presence of carbodiimide also affects the secondary hydroxyl. Detailed investigation of the reaction with N-carbobenzoxyglycine shows that besides I (R = CBzNHCH$_2$—) small amounts of two other

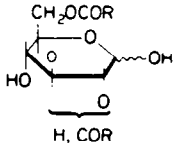
compounds are formed. One of these is the monoacyl derivative of II (R = $CBzNHCH_2$—)isomeric with I. This is confirmed by elementary analysis, IR spectra, positive gycoside-hydroxyl reactions and periodic acid oxidation (consumption of 3 moles HIO_4 and evolution of 2 moles HCO_2H). Reaction of II(R = $CBzNHCH_2$—) with benzaldehyde yielded the 4,6-benzylidene derivative (III; R = $CBzNHCH_2$—) as seen by formation of 4,6-benzylideneglucose on hydrolysis with aqueous methanolic ammonia. In the oxidation of III, 1 mole of HIO_4 is consumed and 1 mole HCO_2H formed and this is only possible in the case of 4,6-benzylidene-3-O-(N-carbobenzoxyglycyl)-glucose; hence II(R = $CBzNHCH_2$—)is 3-O-(N-carbobenzoxyglycyl)-glucose. This is supported by a comparison of III (R = $CBzNHCH_2$—) with the benzylidene derivative prepared by direct synthesis from diisopropylideneglucose (see below). The



II



III



IV

second minor compound isolated is the bis-acyl derivative (IV), based on elementary analysis, IR spectrum and chromatography. The structure has not yet been fully established. Periodate oxidation (2 moles HIO_4 consumed and 1 mole HCO_2H formed) and reactivity of the glucose hydroxyls suggest that IV is probably 3,6- or 2,6-bis-O,O′-(N-carbobenzoxyglycyl)-glucose.

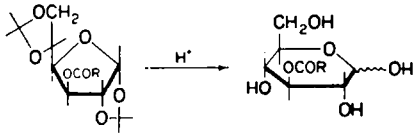
Formation of similar side products was observed in other cases of aminoacylation. For example, in the condensation of glucose with N-carbobenzoxynorleucine, II (R = $CBzNHCH(CH_2)_3CH_3$) was isolated. However, in the synthesis of 6-O-aminoacyl derivatives of glucose the side reactions (formation of 3-O- and bis-O,O′-aminoacyl derivatives) take place only to an insignificant extent, constituting not more than 10–15 per cent of the yield of I.

As varying reaction conditions such as temperature, concentration, etc. did not eliminate the side reactions so we elaborated a procedure for eliminating II and IV and obtaining pure 6-aminoacyl derivatives was elaborated. The bis-acyl derivative (IV) was extracted with ether. The 3-O-acyl derivative (II) was separated by treating the mixture with benzaldehyde in the presence of $ZnCl_2$, II quantitatively being converted to the 4,6 benzylidene derivative (III) which was then extracted with ether. In some cases this procedure was unnecessary since I could be purified by recrystallization.

In order to ascertain the effect of a free glycoside hydroxyl on the stability of the ester bond, the aminoacylation of α-methylglucopyranoside was also carried out.

Preliminary investigations show that this method may be applied to the synthesis of 6-O-aminoacyl derivatives of other monosaccharides, as well as of monosaccharide derivatives with the aminoacyl group at secondary carbon atoms. It is, therefore, a general method for the synthesis of O-aminoacyl derivatives of carbohydrates. Thus, for example, 3-O-(N-carbobenzoxyglycyl)-1,2:5,6-di-O-isopropylideneglucose (V) was prepared by condensation of 1,2:5,6-di-O-isopropylideneglucose with N-carbobenzoxyglycine in the presence of carbodiimide in anhydrous pyridine. Acid hydrolysis of V affords 3-O-(N-carbobenzoxyglycyl)glucose (II).

In order to convert the N-carbobenzoxy derivatives to compounds (IV) with free amino groups, hydrogenolysis of the carbobenzoxy grouping of I was investigated. The Japanese workers[4] were unable to obtain 6-O-glycyl-glucose by hydrogenolysis of the N-carbobenzoxy derivative; and only report degradation of the latter during the reaction.



V R = CbzNHCH$_2$–

The electrophoretic study of the reaction of 6-O-(N-carbobenzoxyglycyl)-glucose in presence Pd/$BaSO_4$ in aqueous or methanolic solutions shows rapid hydrolysis (or alcoholysis) of the resultant glycylglucose (VI, R = —CH_2NH_2) half of which is decomposed in 3–4 hours. The hydrogenolysis of the carbobenzoxy group in the presence of acids was carried out in order to obtain the more stable salts of the 6-O-aminoacyl derivatives of glucose.

Hydrogenolysis in methanol in the presence of HCl affords the hydrochloride (VI) as a hygroscopic oil, difficult to purify. Hydrogenolysis is best accomplished in 75 per cent aqueous methanol with Pd/$BaSO_4$ in the presence of oxalic acid. Under these conditions neutral oxalates of the 6-O-aminoacyl derivatives (VI) of glucose, in yields of about 70 per cent, are easily obtained in an analytically pure state, chromatographically and electrophoretically homogeneous. They give a positive reaction for a glycoside hydroxyl and with ninhydrin for a free amino group. The IR spectra display bands in the regions 1755 cm^{-1} (ester carbonyl) and 1610 cm^{-1} (ionized oxalic acid carboxyl). The IR spectrum of VI (R = —CH_2NH_2) is presented in Fig. 2.

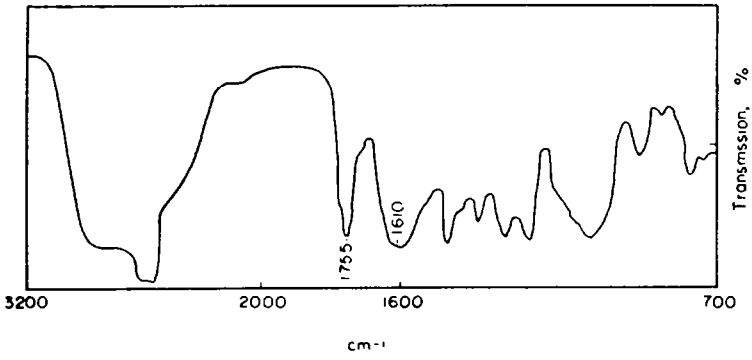


FIG. 2. IR-spectrum: 6-O-glycylglucose oxalate; vaseline oil.

Hydrogenolysis of N-carbobenzoxyaminoacyl derivatives in the presence of oxalic acid enabled for the first time, the isolation of O-aminoacyl derivatives of simple sugars. All compounds of this type, i.e. containing a free, non-protonized amino group, readily undergo solvolysis (hydrolysis, alcoholysis) in solutions of hydroxyl-containing solvents, being stable only within narrow pH ranges (about pH 4). The ester bond in VI is especially inclined to hydrolyse in the case of α-amino acid residues; the ε-aminocaproic acid derivative (VI, R′ = $NH_2(CH_2)_5$—) splits much more slowly,

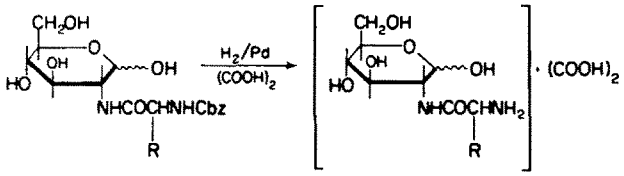
but the reaction itself is more complicated. The details of chemical behaviour, in particular the effect of pH and of the nature of the aminoacyl residue on the stability of the ester bond, which apparently has a direct bearing on the problem of the structure of natural glycopeptides will be communicated later.

Synthesis of N-*aminoacyl derivatives of aminosugars*

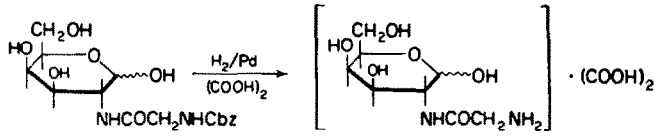
Bergmann and Zervas synthesized N-aminoacyl derivatives of glucosamine by acylation of the O-tetra-acetate of the aminosugar with N-carbobenzoxyamino acid chloride followed by hydrolysis of the acetyl groups.[7] Later additional compounds were obtained by analogous[8] or modified[9] methods but all comprised several stages.

Making use of the selectivity of amide bond formation in the presence of carbodiimide we were able to devise a simple method for the direct selective N-acylation of unprotected aminosugars.[10]

The aminosugar (glucosamine, galactosamine) condenses smoothly with N-carbobenzoxyamino acids (glycine, D,L-alanine) in the presence of dicyclohexylcarbodiimide in aqueous pyridine at room temperature to form N-(N′-carbobenzoxyaminoacyl)-glucosamine (VII) or, correspondingly, galactosamine (VIII).



VII R=H, CH_3– IX



VIII X

The exclusive formation of N-substituted derivatives under the chosen conditions is due to the specific action of carbodiimide and the instability of O-acyl derivatives in aqueous pyridine.

The N-(N′-carbobenzoxyaminoacyl) derivatives of the aminosugars (VII and VIII) may be easily isolated in analytically pure state. Their individuality has been confirmed by paper chromatography in several solvent systems. They give a positive aniline phthalate test (glycoside hydroxyl) and positive test with fluorescein (carbobenzoxy grouping) but no colouration with ninhydrin. All this is in complete accord with the structure of N-aminoacyl derivatives. The IR spectra display absorption bands in the region of 1700 cm^{-1} (carbonyl group of the urethane system) and in the region of 1650 and 1550 cm^{-1} (amide carbonyl). The IR spectrum of N-(N′-carbobenzoxy-D,L-α-alanyl)-glucosamine (VII, R = CH_3—) is presented in Fig. 3.

[7] M. Bergmann and L. Zervas, *Ber. Dtsch. Chem. Ges.* **65**, 1201 (1932).
[8] D. G. Doherty, E. A. Popenoe and K. P. Link, *J. Amer. Chem. Soc.* **75**, 3466 (1953).
[9] A. Bertho and J. Maier, *Liebigs Ann.* **495**, 113 (1932); *Ibid.* **498**, 50 (1932).
[10] N. K. Kochetkov, V. A. Derevitskaja and N. V. Molodtsov, *Chem. Ind.* 1159 (1961).

The specific N-acylation of unprotected aminosugars in the presence of carbodiimide of a more general character is a convenient method for the preparation of other N-acyl derivatives. By this method N-hippuroylglucosamine and N-acetylglucosamine were obtained in good yields.

The carbobenzoxy derivatives (VII and VIII) may be converted to N-aminoacyl derivatives IX and X containing a free amino group by hydrogenolysis in aqueous methanol. In order to prevent the formation of N-glycosides[10] the hydrogenolysis was carried out in the presence of acids, and here also oxalic acid proved to be the most convenient. The resultant powdery acid oxalates are easily isolated and purified. Hydrochloric and hydrobromic acids are less suitable because the hydrohalides of aminoacylderivatives are much more difficult to purify.

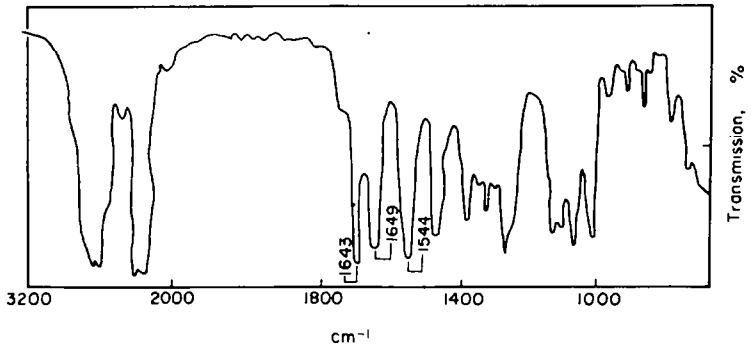


FIG. 3. IR-spectrum: N-(N′-carbobenzoxy-D,L-alanyl)-glucosamine; vaseline oil.

The acid oxalates IX and X are chromatographically and electrophoretically homogeneous. They give positive reactions for a glycoside hydroxyl and a free amino group. The IR spectra display absorption bands in the region 1637 cm^{-1} (amide carbonyl) and 1617 cm^{-1} (weakly ionized carboxyl of oxalic acid). The N-aminoacyl derivatives of the aminosugars IX and X are more stable towards hydrolysis than the O-aminoacyl derivatives of sugars. A more detailed study of this question will be described in a later report.

It thus can be seen that the carbodiimide method of synthesizing aminoacyl derivatives of carbohydrates opens a simple route to the two main types of compounds serving as models of the structural units of natural glycopeptides, namely O-aminoacyl derivatives of ordinary monosaccharides and N-aminoacyl derivatives of aminosugars. Available data show that the method can be used also in solving more complicated synthetical problems in this field.

EXPERIMENTAL

Paper chromatography and electrophoresis were carried out on paper "M" (Leningrad No. 2 Factory). The chromatograms were ascending; the mobile phase: water–saturated isobutanol (system No. 1); upper layer of n-butanol, acetic acid, water 4:1:5 (system No. 2).

Electrophoresis was carried out in the buffer system: pyridine (2 ml)–acetic acid (4 ml)–water (to 1 liter); pH 4·2–4·5 and applied voltage 900V. Spots were detected with the aid of aniline phthalate, silver nitrate, potassium copper periodate complex, ninhydrin and fluorescein. IR spectra were taken in vaseline oil.

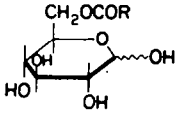
I. *Synthesis of* 6-O-(N-*carbobenzoxyaminoacyl*) *glucose*

(a) N,N′-dicyclohexylcarbodiimide (0·012 mole) was added to a solution of anhydrous glucose (0·02 mole) and N-carbobenzoxyamino acid (0·01 mole) in dry pyridine (80 ml) which had been cooled to 5° and the mixture maintained at that temp for 35–40 hr. Pyridine was distilled off *in vacuo*, the residue treated with water–ether mixture, the precipitate of dicyclohexylurea filtered off, and the aqueous layer separated and extracted several times with ether. The ether extracts in the case of D,L-valine, D,L-norleucine and ε-aminocaproic acid were well washed with water acidified with acetic acid and the washings added to the main aqueous solution. The aqueous solution, slightly acidified with acetic acid, was repeatedly extracted with butanol and the butanol extract evaporated. The oily residue partially crystallized. The non-crystallized portion was subjected either to partition chromatography on a cellulose column with water saturated isobutanol as mobile phase (in the case of glucine) or to treatment according to method B (in the case of ε-aminocaproic acid). The additional amounts of crystalline substance were added to the earlier obtained crystals and the whole was recrystallized from a mixture of ethyl acetate and small amounts of methanol.

(b) The synthesis was carried out as before but the residue after distillation of the butanol was subjected to chromatography on cellulose (100 g) with water-saturated butanol as mobile phase. A syrupy substance was dissolved in 15 ml freshly distilled benzaldehyde, 0·5 g freshly fused $ZnCl_2$ added and the mixture shaken at room temp for 3–4 hr. The excess benzaldehyde was removed by repeated treatment with pet ether. The undissolved residue was treated with a mixture of ether and 3% aqueous $(NH_4)_2SO_4$ solution, filtered and the ether layer separated. The aqueous layer was extracted several times with ether and the ether extracts in the case of D,L-valine, D,L-norleucine and ε-aminocaproic acid thoroughly washed with water acidified with acetic acid and the washings added to the main aqueous solution. When necessary the benzylidene derivatives of 3-O-(N-carbobenzoxyaminoacyl) glucose could be isolated from the ether extracts (see below).

The aqueous solution was extracted several times with butanol. The butanol extracts were washed 3 times with a small amount of water and the butanol evaporated. The residue in the case of β-alanine and ε-aminocaproic acid was recrystallized from a mixture of ethyl acetate with a small amount of methanol. In the case of D,L-α-alanine, D,L-valine and D,L-norleucine the residue was dissolved in alcohol, the solution decolourized with charcoal, the alcohol evaporated, ether added and 6-O-(N-carbobenzoxyaminoacyl) glucose obtained as a colourless, amorphous, chromatographically homogeneous powder.

The constants of 6-O-(N-carbobenzoxyaminoacyl)-glucose prepared according to method (a) and (b) are presented in Table 1.



II. *Isolation of* 3-O-(N-*carbobenzoxyglycyl*)-D-*glucose*

The fractions obtained in the chromatographic separation of the non-crystalline residue in the synthesis of 6-O-(N-carbobenzoxyglycyl)-D-glucose (see method Ia) and which gave a single spot on the paper chromatograms with R_f 0·65 (system 1), were decolorized with charcoal and a hygroscopic amorphous powder obtained in a yield of 0·3 g (8%).$[\alpha]_D^{20}$ +22·9°(*c*, 0·96, methanol) after 20 hr. (Found C, 51·95; 52·11; H, 5·78; 6·00; N, 3·85; 3·59. Calc. for $C_{16}H_{21}O_9N$: C, 51·75; H, 5·70; N, 3·77%).

III. *Isolation of* 3,6-(*or* 2,6-)-O,O′-*bis*-(N-*carbobenzoxyglycyl*)-D-*glucose*

3,6-(or 2,6-)-O,O′-bis-(N-carbobenzoxyglycyl-)-D-glucose was isolated from the ether extracts, obtained by treatment of the reaction mixture in the synthesis of 6-O-(N-carbobenzoxyglycyl-) glucose (see method Ia). This was achieved by repeated extraction of the ether solution with 5% aqueous acetic acid and the latter extracted with butanol. The residue after the removal of butanol crystallized and was purified from ethanol. M.p. 180°(with decomp). $[\alpha]_D^{20}$ +50·0° (*c*, 0·6, methanol) in 6 hr. R_f 0·81 (system 1). (Found C, 55·77; 55·79; H, 5·47; 5·48; Calc. for $C_{26}H_{30}O_{12}N_2$: C, 55·51; H, 5·38%) IR-spectrum: 1535 cm^{-1}, 1708 cm^{-1} (urethane carbonyl); 1756 cm^{-1} (ester carbonyl).

TABLE 1. SYNTHESIS OF 6-O-(N-CARBOBENZOXYAMINOACYL)-D-GLUCOSE

No.	R	Method	Yield (%)	m.p.	$[\alpha]_D^{27}$	R_f Syst. 1	Analysis					
							% C		% H		% N	
							Found	Calc.	Found	Calc.	Found	Calc.
1	$CbzNHCH_2$—[a]	a	46	159–160	+30·4 water c 0·5	0·54	51·67 51·57	51·75	5·73 5·62	5·70	3·84 3·91	3·77
2	D,L—$CbzNHCHCH_3$ |	c	39	amorph.	+33·0[b] methanol c 0·45	0·62	52·90 53·13	52·98	6·08 6·03	6·02	3·60 3·55	3·63
3	$CbzNHCH_2CH_2$—	c	31	127–128	+31·5[c] water c 0·5	0·60	52·97 53·23	52·98	5·94 5·97	6·02	3·88 3·99	3·63
4	D,L—$CbzNHCHCH(CH_3)_2$ |	c	35	amorph.	+28·4 methanol c 1·0	0·82	54·99 55·07	55·20	6·82 6·89	6·58	3·47 3·55	3·39
5	D,L—$CbzNHCH(CH_2)_3CH_3$ |	c	26	amorph.	+42·8[d] methanol c 0·9	—	56·27 56·20	56·20	6·95 6·95	6·84	3·35 3·38	3·28
6	$CbzNH(CH_2)_5$—	a	42	119·5–120	+33·9 methanol c 1·0	—	56·21 56·00	56·20	6·61 6·95	6·84	3·19 3·23	3·28

[a] (5) m.p. 153°; $[\alpha]_D^{18}$ +30.0; [b] at 20; [c] at 24°; [d] at 20°.

IV. 4,6-*benzylidene*-3-O-(N-*carbobenzoxyglycyl*)-D-*glucose*

Solution of 200 mg 3-O-(N-carbobenzoxyglycyl)-D-glucose, isolated by method II, in 2 ml freshly distilled benzaldehyde was treated with 85 mg of freshly fused $ZnCl_2$ and shaken for 3 hr at room temp. Excess benzaldehyde was removed by extraction of the reaction mixture with light petroleum. The undissolved solid was treated with a mixture of ether and 3% aqueous $(NH_4)_2SO_4$. Crystalline 4,6-benzylidene-3-O-(N-carbobenzoxyglycyl-)-D-glucose was filtered off, the ether layer washed with water and ether removed *in vacuo*. The residue was dissolved in ethanol, the crystalline product filtered off and added to the first portion. Yield 150 mg (60·5%), m.p. 164–165° (recrystallized from ethanol), needles. $[\alpha]_D^{20}$ +10·4 (*c*, 0·47, methanol) in 6·5 hr. (Found: C, 60·33; 60·56; H, 5·74; 5·75; Calc. for $C_{23}H_{25}O_9N$: C, 60·13; H, 5·48%) IR-spectrum: 1549 cm^{-1}, 1700 cm^{-1} (urethane carbonyl); 1740 cm^{-1}) (ester carbonyl).

1 mg of the product was dissolved in 0·75 ml methanol, treated with 0·4 ml conc ammonia and left for 4 hr. Paper chromatography in various solvent systems, two of which were 1 and 2, indicated the presence of a single substance with R_f value characteristic of 4,6-benzylideneglucose.

V. *Isolation of* 4,6-*benzylidene*-3-O-(N-*carbobenzoxy*-D,L-*norleucyl*)-D-*glucose*

This substance was isolated from the reaction mixture, obtained in the synthesis of 6-O-(N-carbobenzoxy-D,L-norleucyl-)-D-glucose by method (b) after treatment with benzaldehyde, light petroleum and solution of the residue in a mixture of ether with 3% aqueous $(NH_4)_2SO_4$. Crystalline 4,6-benzylidene-3-O-(N-carbobenzoxy-D,L-norleucl-)-D-glucose was filtered off, the ether layer washed with water, ether removed *in vacuo*, the residue crystallized from ethanol and added to the first portion, m.p. 193–194°.

(Found: C, 62·53; 62·68; H, 6·41; 6·23; N, 2·73; 2·62; Calc. for $C_{27}H_{33}O_9N$: C, 62·90; H, 6·45; N, 2·71%).

VI. *Synthesis of* 6-O-(N-*carbobenzoxy*-D,L-α-*alanyl*-)-α-*methyl*-D-*glucopyranoside*

Synthesis was performed by method (b) but with extraction of the aqueous layer with isoamyl alcohol instead of butanol. The product was obtained as an amorphous powder in a yield of 31·5% $[\alpha]_D^{20}$ +74·5 (*c*, 1·1 methanol).

(Found: C, 53·93; 53·92; H, 6·40; 6·31; N, 3·51; 3·48; CH_3O-, 7·72; 7·64; Calc. for $C_{18}H_{25}O_9N$: C, 54·13; H, 6·31; N, 3·51; CH_3O-, 7·77%).

VII. *Synthesis of* 3-O-(N-*carbobenzoxyglycyl*-)-D-*glucose by condensation of* 1,2:5,6-*di*-O-*isopropylidene glucose with* N-*carbobenzoxyglycine*

(a) 3-O-(N-*carbobenzoxyglycyl*)- 1,2:5,6- *di*-O-*isopropylideneglucose*. To a solution of 5·2 g (0·02 mole) 1,2:5,6-di-O-isopropylidene glucose and 4·2 g (0·02 mole) carbobenzoxyglycine in 50 ml dry pyridine 5 g (0·024 mole) of dicyclohexylcarbodiimide in 15 ml dry pyridine were added and the mixture left at room temp for 48 hr. Pyridine was distilled off *in vacuo* the residue treated with a large volume of ether, dicyclohexylurea filtered off, ether solution shaken with 5% acetic acid for 0·5 hr and dicyclohexylurea again filtered off. The ethereal solution was washed with water, 2% aqueous $NaHCO_3$ and again with water. The solution was then evaporated to dryness after addition of benzol, the residue dissolved in ether, the solution filtered and ether removed *in vacuo*. The resulting oil was dissolved in ether, hexane added to the solution in an open vessel. Crystalline 3-O-(N-carbobenzoxyglycyl)-isopropylideneglucose precipitated out. This was recrystallized from mixture CCl_4–C_6H_{14} (1:1). Yield 4·5 g (51%). M.p. 79–80°, $[\alpha]_D^{23}$ −14·4° (*c*, 1·0, methanol).

(Found: C, 58·48; 58·76; H, 6·45; 6·65. Calc. for $C_{22}H_{29}O_9N$: C, 58·53; H, 6·47%).

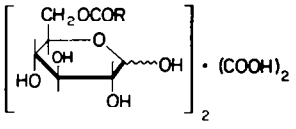
(b) 3-O-(N-*carbobenzoxyglycyl*-)-D-*glucose*. Heated to 45° a solution of 3·8 g 3-O-(N-carbobenzoxyglycyl-)-1,2:5,6-diisopropylidene-D-glucose in 25 ml acetone was treated with a mixture of 30 ml water with 2·12 ml conc hydrochloric acid and the mixture maintained at 45° for 3·5 hr. After removal of acetone *in vacuo* 60 ml water and 40 ml ether were added. On the solution of the solid, the ether layer was separated and the aqueous phase extracted 7 times with ether, brought to neutral pH with Ag_2CO_3, filtered, treated with several drops of acetic acid and extracted 5 times with butanol. The butanol was washed with very dil acetic acid, and the washings extracted with butanol, and added to the main portion. Butanol was removed *in vacuo*, the residue dissolved in ethanol, decolorized with charcoal, alcohol distilled off, the residue dissolved in a small volume ethyl acetate and left to crystallize

yield 750 mg (24%). m.p. 132–133° after recrystallization from ethyl acetate. $[\alpha]_D^{21}$ +38·0° (*c*, 0·5, water). (Found: C, 51·78; 51·64; H, 5·76; 5·79; Calc. for $C_{16}H_{21}O_9N$: C, 51·75; H, 5·70%).

The product was converted to 4,6-benzylidene-3-O-(N-carbobenzoxyglycyl-)-D-glucose by analogy with method above for comparison. The crystals m.p. 162–163° gave no depression with the product, obtained by method described above.

VIII. *Synthesis of* 6-O-*aminoacyl*-D-*glucose oxalates*

6-O-(N-Carbobenzoxyaminoacyl-)-D-glucose (0·001 mole) was dissolved in 75% methanol (8 ml), oxalic acid (0·0006 mole), 5% Pd/$BaSO_4$ (200 mg) added and the mixture subjected to hydrogenation for 1·5 hr. The catalyst was removed by centrifugation, the supernatant diluted with a large volume of ether, the appearing oil separated by centrifugation, dissolved in a small volume methanol, precipitated with ether, redissolved in methanol and precipitated with acetone. The solid obtained settled by centrifugation, chilled with dry acetone, acetone decanted and remaining acetone removed *in vacuo*. Amorphous hygroscopic colourless powders were obtained in yields of ca, 70%.



The constants of these substances are listed in Table 2.

IX. 6-O-(D,L-α-*alanyl*)-α-*methyl*-D-*glucopyranoside oxalate*

Obtained by analogy with above method (VIII) in yield of 70% as an amorphous colourless powder. $[\alpha]_D^{20}$ +86·0 (*c*, 0·6, water). (Found: C, 42·51; 42·57; H, 6·75; 6·75; N, 4·44; 4·32; Calc. for $(C_{10}H_{19}O_7N)_2.(COOH)_2$: C, 42·58; H, 6·50; N, 4·51.

X. *Synthesis of* N-(N′-*carbobenzoxyaminoacyl*)-*hexosamines*

Solution of hexosamine hydrochloride (0·01 mole) in water (5 ml) was treated at 0° with stirring with 2N alkali (5 ml; 0·01 mole) and in 15–20 min with N-carbobenzoxyglycine (0·01 mole) or D,L-α-alanine in 7·5 ml freshly distilled pyridine. Dicyclohexylcarbodiimide (0·015 mole) in pyridine (20 ml)

TABLE 2. SYNTHESIS OF 6-O-AMINOACYL-GLUCOSE

No.	R	$[\alpha]_D^{20}$	Analysis % C		% H		% N	
			Found	Calc.	Found	Calc.	Found	Calc.
1	NH_2CH_2—	+ 36·2 water *c*, 1·3 (in 4 hr)	38·16 37·90	38·30	5·85 5·79	5·71	4·67 4·52	4·96
2	D,L-NH_2CHCH_3	+ 31·5 water *c*, 0·4 (in 4 hr)	40·35 40·33	40·54	6·29 6·36	6·12	4·77 4·61	4·73
3	$NH_2CH_2CH_2$—	+ 32·1[a] water *c*, 0·5	40·20 40·13	40·54	5·93 6·07	6·12	4·54 4·43	4·73
4	D,L-$NH_2CH(CH_2)_3CH_3$	+ 36·2[b] water *c*, 0·5	—	—	—	—	4·11 4·23	4·14
5	$NH_2(CH_2)_5$—	+ 30·4[a] water *c*, 1·0	46·07 46·14	46·15	7·21 7·39	7·15	—	—

[a] at 25°; [b] at 28°

TABLE 3. SYNTHESIS OF N-(N′-CARBOBENZOXYAMINOACYL)-HEXOSAMINES

No.	Substance	Yield (%)	m.p.	$[\alpha]_D^{20}$	R_f		Analysis			
							% C		% H	
					System 1	System 2	Found	Calc.	Found	Calc.
1	N-(N′-carbobenzoxyglycyl)-glucosamine	75–80	178–180°[a]	+ 77·5 pyridine *c*, 1·4	0·63	0·69	51·88 51·73	51·89	5·87 5·98	5·99
2	N-(N′-carbobenzoxy-D,L-alanyl)-glucosamine	63	+220° dec.[b]	+71·9 pyridine *c*, 1·0	0·77	0·74	53·08 53·11	53·12	6·24 6·17	6·29
3	N-(N′-carbobenzoxyglycyl)-galactosamine	64·5	178–179°	+ 64·0[c] pyridine *c*, 1·25	0·62	0·68	51·85 51·86	51·89	6·00 6·09	5·99

[a] m.p. 180°; [b] m.p. 230°; [c] at 25°.

were slowly dropped into the resulting solution. The mixture was left for a day at room temp diluted to 100–200 ml. with water and precipitated dicyclohexylurea was filtered off. The filtrate was extracted with ether (2 × 50 ml) and aqueous layer evaporated *in vacuo*, bath temp not exceeding 40–50°. The solid residue was extracted with hot ethanol (3 × 50 ml), and the latter removed *in vacuo*. The crystalline residue was recrystallized from either methanol or ethanol.

The constants of N-(N′-carbobenzoxyaminoacyl)-hexosamines are presented in Table 3.

XI. *Synthesis of* N-*hippuroylglucosamine*

This was obtained by analogy with above method (X) from glucosamine hydrochloride and hippuric acid. Colourless crystals, m.p. 211–212° (from ethyl acetate or aqueous ethanol). Yield 60%. $[\alpha]_D^{20}$ +38·3° (*c*, 1·2, pyridine) in 30 min; *R*, 0·44 (System 1) and 0·61 (System 2) at 25°. (m.p. 210·5–212°, $[\alpha]_D^{20}$ +43·4° (*c*, 1·3, pyridine) in 45 min[8]) IR-spectrum: 1640–1667 cm^{-1} and 1548^{-1} (amide bond carbonyl).

TABLE 4. SYNTHESIS OF N-AMINOACYLGLUCOSAMINES

No.	Substance	Yield (%)	$[\alpha]_D^{25}$	R_f	Analysis % C		% H	
				Syst. 2	Found	Calc.	Found	Calc.
1	N-glycylglucosamine oxalate	80	+ 24·4 water *c*, 1·5	0·15	36·76 36·69	36·81	5·66 5·64	5·58
2	N-glycylgalactosamine oxalate	68	+ 63·4 water *c*, 1·0	0·15	36·98 37·15	36·81	6·02 6·33	5·58
3	N-D,L-alanylglucosamine oxalate	50	+ 16·0 water *c*, 1·0	0·18	39·10 39·11	38·82	6·14 6·16	5·92

XII. *Synthesis of* N-*acetylglucosamine*

This was obtained by analogy with above method (X) yield after crystallization from ethanol 48%, m.p. 195–200°; $[\alpha]_D^{20} = +55{\cdot}0°$ (*c*, 1·6, water, in 0·5 hr); *R*, 0·13 (System 1) and 0·31 (System 2) at 25°.[1] ($[\alpha]_D^{20}$ +55·6°; m.p. 190°[11]).

IR-spectrum: 1629 cm^{-1} and 1548 cm^{-1} (amide bond carbonyl).

XIII. *Synthesis of* N-(*aminoacyl*)-*hexosamines*

Solution of N-(N′-carbobenzoxyaminoacyl)-hexosamine (0·00215 mole) in 50% in aqueous methanol (40 ml), containing oxalic acid (0·0032 mole) was treated with 5% Pd/$BaSO_4$ (0·5 g) and the mixture hydrogenized at normal press till the starting carbobenzoxy derivative dissappeared, which was indicated by paper electrophoresis (this takes ca. 2 hr). The catalyst was filtered off, the solution was evaporated to a small volume *in vacuo* (bath temp 30°), and acid N-(aminoacyl)-hexosamine oxalate precipitated with abs ethanol or acetone. The precipitate was centrifugated, washed several times with abs ethanol or acetone and dried *in vacuo*.

The constants of the acid N-(aminoacyl)-hexosamine oxalates obtained are listed in Table 4.

[11] A. Bertho, F. Holder, W. Meiser and F. Huther, *Liebigs Ann.* **465**, 127 (1931).