904. *Nucleotides. Part XLV.*[1] *Derivatives of* β-2-*Amino*-2-*deoxy*-D-*glucose* (β-D-*Glucosamine*) 1-*Phosphate.*

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2-Acetamido-2-deoxy-β-D-glucose (β-*N*-acetylglucosamine) 1-phosphate has been prepared through derivatives of 2-deoxy-2-phthalimidoglucose. Attempts to convert this phosphate into the β-anomer of UDPAG, a natural coenzyme, failed, probably because the 1-pyrophosphate residue was displaced by the neighbouring 2-acetamido-group.

The structure (I) of " uridine diphosphate *N*-acetylglucosamine " (UDPAG, UDPX) was deduced by Leloir and his co-workers from the results of their degradative work,[2] and this substance was naturally included in our programme of synthesis in the field of uridine coenzymes.[3] The requisite 2-acetamido-2-deoxy-α-D-glucose 1-phosphate can be prepared[4] by a method of the kind usually successful for sugar 1-phosphates, but in this instance there is the complication of the very easy displacement of halide ion from the 1-halogeno-compound by the neighbouring acetamido-group. Thus the crystalline, water-soluble " acetobromoglucosamine " described by Moggridge and Neuberger[5] is actually 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucose hydrobromide,[6] and rapid manipulation of the 1-bromo-compound is necessary to forestall displacement of the halogen. Apparently the 1-chloro-compound is slightly more stable,[7] and it has been employed more successfully.[4] Although Dr. Leloir kindly informed us of his method before its publication, it seemed worthwhile investigating alternative routes in which this displacement would be precluded.

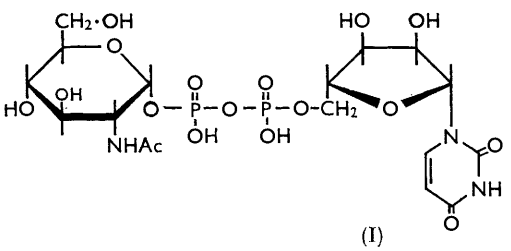
One possibility was to employ salts of the 2-amino-sugar, introducing the acetyl group only at the last stage, and for this purpose 3,4,6-tri-*O*-acetyl-2-amino-1-bromo-2-deoxy-α-D-glucose hydrobromide, which has long been known,[8] was an attractive starting material. It reacted smoothly with silver dibenzyl phosphate, but we were unable to remove the protecting groups cleanly from the product. However, after our work, Maley, Maley, and Lardy[9] described an efficient synthesis of 2-acetamido-2-deoxy-α-D-glucose 1-phosphate, beginning with reaction between the same 1-bromo-compound and triethylammonium diphenyl phosphate. Comparison of the optical rotations of the dibenzyl and the diphenyl phosphates suggests that our product belongs to the β-series (cf. discussion below), and the situation is reminiscent of the synthesis of the anomeric 1-phosphates of glucose and galactose.[10] There, too, β-compounds are derived from dibenzyl phosphate and α-compounds from diphenyl phosphate, but this rule does not extend to mannose and xylose, and the difference between silver and triethylammonium salts may be just as important.

An alternative way of precluding displacement, which also offered advantages in manipulation of the intermediates, was protection of the 2-amino-group with a residue which would not attack the neighbouring 1-position, and for this purpose the phthaloyl group seemed most suitable. Glucosamine was smoothly converted by phthalic anhydride in aqueous dioxan into its *o*-carboxybenzoyl derivative, and hence by acetic anhydride

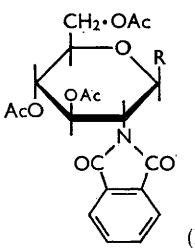
[1] Part XLIV, Griffin and Todd, *J.*, 1958, 1389.
[2] Paladini and Leloir, *Biochem. J.*, 1952, **51**, 426; Cabib, Leloir, and Cardini, *J. Biol. Chem.*, 1953, **203**, 1055.
[3] Michelson and Todd, *J.*, 1956, 3459, and earlier papers in this series.
[4] Leloir and Cardini, *Biochim. Biophys. Acta*, 1956, **20**, 33.
[5] Moggridge and Neuberger, *J.*, 1936, 745.
[6] Micheel, van de Kamp, and Wulff, *Chem. Ber.*, 1955, **88**, 2011; Inouye, Onodera, Kitaoka, and Ochiai, *J. Amer. Chem. Soc.*, 1957, **79**, 4218.
[7] Baker, Joseph, Schaub, and Williams, *J. Org. Chem.*, 1954, **19**, 1786.
[8] Irvine, McNicoll, and Hynd, *J.*, 1911, **99**, 250.
[9] Maley, Maley, and Lardy, *J. Amer. Chem. Soc.*, 1956, **78**, 5303.
[10] Leloir, *Fortschr. Chem. org. Naturstoffe*, 1951, **8**, 47; Foster and Overend, *Quart. Rev.*, 1957, **11**, 61.

into 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-α-D-glucose (II; R = OAc).* The action of hydrogen chloride on an ethereal suspension of this compound afforded the crystalline β-1-chloro-derivative (II; R = Cl), and, from the latter, crystalline dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose 1-phosphate [II; R = $O{\cdot}PO(O{\cdot}CH_2Ph)_2$] was obtained by reaction with silver dibenzyl phosphate. Triethylammonium dibenzyl phosphate, which is sometimes preferable to the silver salt in preparing phosphates,[12] did not appear to react in this instance. Similarly, triethylammonium diphenyl phosphate does not attack 2-acetamido-3,4,6-tri-*O*-acetyl-1-chloro-2-deoxy-α-D-glucose,[9] in contrast to the smooth reaction with the α-2-amino-1-bromo-derivative. All the protecting groups were removed cleanly from the phthalimido-phosphate by hydrogenolysis followed by hydrazinolysis, but *N*-acetylation of the crystalline product (III; R = H) was less satisfactory. Repeated treatment with acetic anhydride in aqueous triethylamine was required before a substantially homogeneous product was obtained; *N*-acetylation of the sodium salt, however, readily gave a crystalline product.

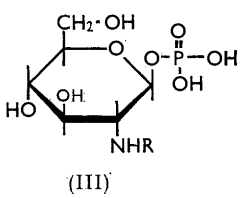
The optical rotation of our disodium salt of 2-acetamido-2-deoxy-D-glucose 1-phosphate (III; R = Ac) is considerably less negative than has been predicted [9] for the β-anomer by Maley, Maley, and Lardy, who have transferred the well-established data for the methyl glycosides [13] by simple application of Hudson's rules. In the meantime, however, it has



(I)



(II)



(III)

been shown [14] that 1-phosphates do not obey Hudson's rules, in that B values are greater than with methyl glycosides; probably a more elaborate calculation, like that developed for phenyl glycosides, glycosyl halides, etc.,[15] is required. The intermediates in our synthesis are unlikely to be mixtures of anomers, because they crystallise well, and their optical rotations are in the region for β-compounds and clearly different from those of the α-series; accordingly, we assign the β-configuration to these compounds. It may be that the bulky 2-phthalimido-group favours the β-configuration, but this is not apparent from molecular models.

Several unsuccessful attempts, which will not be described in detail, were made to convert 2-acetamido-2-deoxy-β-D-glucose 1-phosphate (III; R = Ac) into the uridine diphosphate derivative (anomer of UDPAG) by reaction between its tri-n-octylammonium salt and 2',3'-di-*O*-benzyluridine-5'-benzyl phosphorochloridate and subsequent hydrogenolysis.[3] The major components of the resulting mixture, resolved by anion-exchange chromatography, were uridine-5' pyrophosphate and its 2'(or 3')-*O*-monobenzyl derivative. Their presence indicated initial formation of the desired pyrophosphate, which presumably then decomposed through displacement of the pyrophosphate anion, like bromide ion, from the β-1-position by the neighbouring 2-acetamido-group. Probably the β-isomer is much

* Material, which has a higher melting point but does not yield a crystalline 1-chloro-derivative, has been prepared [7] from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-D-glucose.[11]

[11] Bergmann and Zervas, *Ber.*, 1931, **64**, 975.
[12] Wright and Khorana, *J. Amer. Chem. Soc.*, 1956, **78**, 811.
[13] Neuberger and Rivers, *J.*, 1939, 122.
[14] Putman and Hassid, *J. Amer. Chem. Soc.*, 1957, **79**, 5057.
[15] Korytnyk, *J.*, 1959, 650.

less stable than the natural coenzyme. Synthesis of the latter from 2-acetamido-2-deoxy-α-D-glucose 1-phosphate has not been attempted by us.

Experimental

[3,4,6-*Tri*-O-*acetyl*-2-*amino*-2-*deoxy*-β-D-*glucose* 1-(*Dibenzyl Phosphate*)] *Dibenzyl Phosphate*.—Silver dibenzyl phosphate (4·1 g.) was added to 3,4,6-tri-*O*-acetyl-2-amino-1-bromo-2-deoxy-α-D-glucose hydrobromide [8] (2 g.) in dry acetonitrile (50 ml.), and the mixture shaken for 52 hr. with exclusion of light and moisture. After removal of solvent, the residue was extracted with hot chloroform, and the extract filtered and evaporated under reduced pressure. Trituration of the residue with ethanol afforded [3,4,6-*tri*-O-*acetyl*-2-*amino*-2-*deoxy*-β-D-*glucose* 1-(*dibenzyl phosphate*)] *dibenzyl phosphate* (2·4 g., 64%), m. p. 146—147°, $[\alpha]_D^{19}$ +23·7° (*c* 0·9 in $CHCl_3$) (Found: C, 57·05; H, 5·4; N, 1·75. $C_{40}H_{47}NO_{15}P_2$ requires C, 56·9; H, 5·6; N, 1·7%).

2-O-*Carboxybenzamido*-2-*deoxy*-D-*glucose*.—To D-glucosamine hydrochloride (1 g.) and sodium hydrogen carbonate (0·78 g.) in water (20 ml.) was added phthalic anhydride (0·68 g.) in dioxan (15 ml.) during 15 min. and the solution set aside for 3 days at room temperature. Acidification with 2N-hydrochloric acid gave the *acid* (1·24 g., 82%) as colourless needles, m. p. 184°, $[\alpha]_D^{20}$ +67·3° (*c* 0·9 in H_2O) (Found: C, 51·1; H, 5·2; N, 4·3. $C_{14}H_{17}NO_8$ requires C, 51·4; H, 5·2; N, 4·3%).

1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucose*.—Anhydrous sodium acetate (3 g.), 2-*o*-carboxybenzamido-2-deoxy-D-glucose (3 g.), and acetic anhydride (18 ml.) were boiled under reflux for 5 min., cooled, and treated with water (30 ml.), and the solution taken to pH 7 with sodium hydrogen carbonate. Extraction with chloroform, followed by evaporation under reduced pressure of the washed and dried ($CaCl_2$) extracts, gave the *phthalimide* (3·9 g., 89%) which separated from tetrahydrofuran–cyclohexane as colourless prisms of indefinite m. p. $[\alpha]_D^{20}$ +59·4° (*c* 3·8 in C_6H_6) (Found: C, 54·8; H, 5·4; N, 2·7. $C_{22}H_{23}NO_{11}$ requires C, 55·3; H, 4·9; N, 2·9%). Recrystallisation from carbon tetrachloride gave needles, m. p. 81—83°, containing solvent of crystallisation which could not be completely removed. From ethyl acetate–light petroleum (b. p. 40—60°) the phthalimide separated in colourless prisms, m. p. 95—97°, which, after drying *in vacuo* at 65° overnight, sintered at 97° (change of crystal form?) and melted sharply at 146—148° (Found: C, 55·4; H, 5·0; N, 2·8%). Baker *et al.*[7] record m. p. 199—200°. Acetylation of the crude sodium salt of the carboxybenzamido-compound (obtained by removal of the aqueous dioxan under reduced pressure) gave a similar yield (68% overall) of the phthalimide.

3,4,6-*Tri*-O-*acetyl*-1-*bromo*-2-*deoxy*-2-*phthalimido*-β-D-*glucose*.—To a 50% w/v solution (10 ml.) of hydrogen bromide in glacial acetic acid was added with stirring 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose (3·9 g.). After 3 hr. at room temperature the solution was diluted with chloroform (30 ml.) and poured into saturated ice-cold aqueous potassium hydrogen carbonate. The pH was adjusted to 6 with solid potassium hydrogen carbonate, the chloroform layer separated, and the aqueous layer extracted with further chloroform. Evaporation under reduced pressure of the combined washed and dried ($CaCl_2$) extracts, followed by trituration with dry ether, gave the 1-*bromo-compound* (2·3 g., 50%) in needles, m. p. 130°, $[\alpha]_D^{20}$ +18·1° (*c* 3·0 in C_6H_6) (Found: C, 48·5; H, 3·95; N, 2·9; Br, 16·1. $C_{20}H_{20}BrNO_9$ requires C, 48·2; H, 4·0; N, 2·8; Br, 16·1%).

3,4,6-*Tri*-O-*acetyl*-1-*chloro*-2-*deoxy*-2-*phthalimido*-β-D-*glucose*.—A solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose (4 g.) in dry ether (185 ml.) was saturated at 0° with dry hydrogen chloride and kept at 0° for 4 days. After removal of solvent under reduced pressure, dry benzene (20 ml.) was added and similarly removed. Trituration with dry ether gave the 1-*chloro-compound* (3·3 g., 86%) in prisms, m. p. 154°, $[\alpha]_D^{19}$ +27·5° (*c* 3·2 in C_6H_6) (Found: C, 53·0; H, 4·8; N, 3·4; Cl, 8·0. $C_{20}H_{20}ClNO_9$ requires C, 53·0; H, 4·45; N, 3·1; Cl, 7·8%).

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucose* 1-(*Dibenzyl Phosphate*).—(*a*) A mixture of silver dibenzyl phosphate (1·15 g.) and 3,4,6-tri-*O*-acetyl-1-bromo-2-deoxy-2-phthalimido-β-D-glucose (1 g.) in dry benzene (10 ml.) was shaken in the dark for 48 hr., filtered, and evaporated to dryness. The *dibenzyl phosphate* (0·9 g., 65%) separated from tetrahydrofuran–cyclohexane or from methanol in needles, m. p. 138—139·5°, $[\alpha]_D^{19\cdot5}$ +11·1° (*c* 3·6 in C_6H_6) (Found: C, 59·1; H, 5·25; N, 1·95. $C_{34}H_{34}NO_{13}P$ requires C, 58·7; H, 4·9; N, 2·0%).

(*b*) A mixture of silver dibenzyl phosphate (3 g.) and 3,4,6-tri-*O*-acetyl-1-chloro-2-deoxy-2-phthalimido-β-D-glucose (2·75 g.) in dry benzene (60 ml.) was boiled under reflux for 90 min. with exclusion of light, and the product (3·4 g., 81%) worked up as in (*a*).

(*c*) The use of triethylammonium dibenzyl phosphate in place of the silver salt in (*b*) led to recovery of the starting materials.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucose* 1-(*Cyclohexylammonium Benzyl Phosphate*).—A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose 1-(dibenzyl phosphate) (0·4 g.) and cyclohexylammonium thiocyanate (0·1 g.) in dry ethyl methyl ketone (4 ml.) was boiled under reflux for 30 min., cooled, and filtered. The *cyclohexylammonium salt* (0·33 g., 83%), when washed with ethyl methyl ketone, formed colourless needles, m. p. 226° (decomp.), unchanged by recrystallisation from ethanol, $[\alpha]_D^{19}$ +23·1° (*c* 2·2 in $CHCl_3$) (Found: C, 56·3; H, 6·3; N, 4·0. $C_{33}H_{41}N_2O_{13}P$ requires C, 56·25; H, 5·9; N, 4·0%).

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucose* 1-*Phosphate*.—3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose 1-(dibenzyl phosphate) (1·5 g.) and palladised charcoal (0·3 g.) in pure dioxan (30 ml.) were shaken in hydrogen. The theoretical amount was rapidly absorbed. The solution was filtered, evaporated under reduced pressure, and twice evaporated with dry benzene (10 ml.) and the residue was triturated with dry hexane. The *phosphate* (1·06 g., 100%) was obtained as a microcrystalline powder which decomposed when heated (Found: C, 46·8; H, 4·4; N, 2·9. $C_{20}H_{22}NO_{13}P$ requires C, 46·6; H, 4·3; N, 2·7%).

2-*Amino*-2-*deoxy*-β-D-*glucose* 1-*Phosphate*.—A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucose 1-phosphate (0·27 g.) in redistilled methoxyethanol (1·5 ml.) was treated with 100% hydrazine hydrate (0·10 ml.) and heated at 100° with stirring for 8—9 min. After 2—3 min. a gum normally separated. Solvent was removed under reduced pressure, and water (5 ml.) was added, followed by glacial acetic acid to pH 5. After being kept at 0° for 30 min., the solution was filtered from phthalhydrazide and diluted with ethanol (5 ml.). When crystallisation had set in, further ethanol (15—20 ml.) was added, and the solution kept at 0° overnight and then centrifuged. After being washed with ethanol (thrice) and acetone (thrice) and dried *in vacuo* over phosphorus pentoxide 2-*amino*-2-*deoxy*-β-D-*glucose* 1-*phosphate* was obtained as a microcrystalline powder, m. p. 178—179° (decomp.) (Found: C, 27·7; H, 5·8; N, 5·3. $C_6H_{14}NO_8P$ requires C, 27·8; H, 5·4; N, 5·4%).

The *disodium salt* was obtained as a microcrystalline powder by neutralisation of the acid with the theoretical quantity of N-sodium hydroxide, followed by precipitation with ethanol (Found: C, 20·8; H, 4·5; N, 3·95. $C_6H_{12}NNa_2O_8P,2H_2O$ requires C, 21·2; H, 4·7; N, 4·1%).

2-*Acetamido*-2-*deoxy*-β-D-*glucose* 1-*Phosphate*.—(*a*) 2-Amino-2-deoxy-β-D-glucose 1-(sodium phosphate) (0·38 g.) in water (1·8 ml.) was treated with acetic anhydride (0·6 ml.) and shaken vigorously for 3 min. Further acetic anhydride (0·4 ml.) was then added and shaking continued for a further 3 min. Addition of ethanol (5 vol.) precipitated 2-*acetamido*-2-*deoxy*-β-D-*glucose* 1-(*disodium phosphate*) (0·26 g., 60%) as a microcrystalline powder, m. p. 170—171° (decomp.), $[\alpha]_D^{19}$ −1·6° (*c* 2·5 in H_2O) (Found: C, 28·05; H, 4·5; N, 4·05. $C_8H_{14}NNa_2O_9P$ requires C, 27·8; H, 4·1; N, 4·05%).

(*b*) 2-Amino-2-deoxy-β-D-glucose 1-phosphate (518 mg.) in water (2 ml.) was shaken with triethylamine (1·1 ml.) and acetic anhydride (0·37 ml.) for 5 min. with occasional cooling in ice. Further triethylamine (0·28 ml.) and acetic anhydride (0·18 ml.) were added, the mixture again shaken for 5 min., and the process repeated once more. Ethanol (20 ml.) was then added, and the mixture evaporated to dryness under reduced pressure and kept at 30°/0·001 mm. for 1 hr. The residual gum solidified at 0° in dry acetone (5 ml.), but was extremely hygroscopic and no analytical data were obtained.

The following R_F values (ascending) were observed:

	A	B	C
Glucosamine 1-phosphate	0·26	0·15	0·09
N-Acetylglucosamine 1-phosphate	0·49	0·22	0·19

System A: propan-2-ol–1% ammonium sulphate, on paper previously soaked in 1% aqueous ammonium sulphate and dried. System B: propan-1-ol–ammonia–water (6 : 3 : 1 v/v). System C: 95% ethanol–M-ammonium acetate (75 : 30).

This work was carried out during the tenure of an I.C.I. Fellowship (by B. H. C.) and a Fellowship of the Consejo Superior de Investigaciones Cientificas, Madrid (by G. B.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, June 1st,* 1960.]