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REACTIONS OF 2-(2′,4′-DINITROPHENYL)-AMINO-2-DEOXY-D-GLUCOSE, (DNP-D-GLUCOSAMINE), AND DERIVATIVES*

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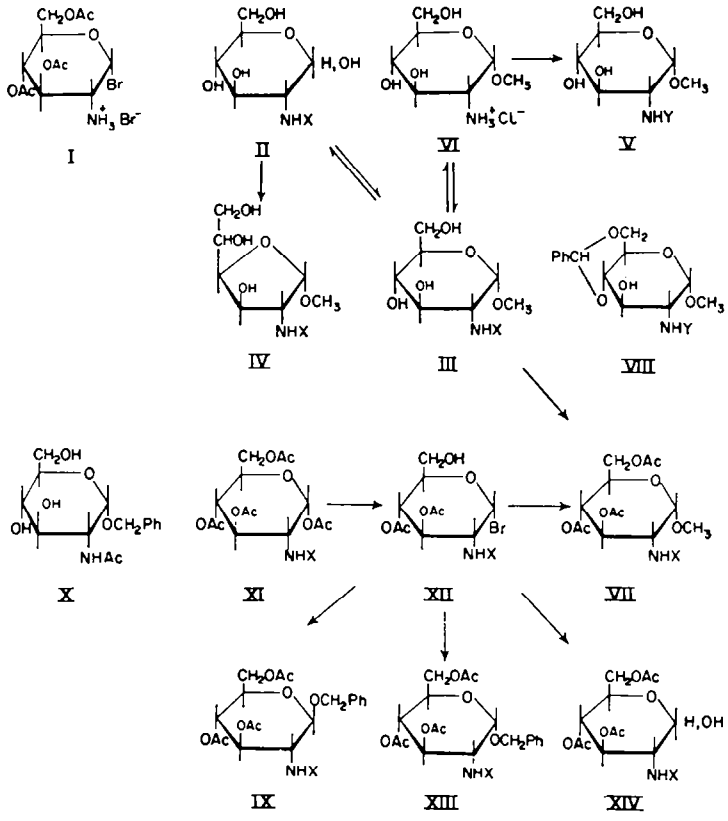
Abstract—3,4,6-Tri-O-acetyl-2-amino-1-bromo-2-deoxy-α-D-glucose hydrobromide is of limited value for the synthesis of glycosides. Treatment of DNP-D-glucosamine with methanolic hydrogen chloride yielded a mixture containing a pyranoside and a furanoside. The stability of the N-dinitrophenyl group under acidic and alkaline conditions has been investigated and a method for its removal utilizing an anionic ion-exchange resin is described. An acetobromo derivative of DNP-D-glucos amine was obtained which reacted with alcohols to yield glycopyranosides which, in some cases, were of the α-configuration.

THE biologically-important glycosides of the amino-sugars cannot be obtained directly by acid-catalysed condensation of the sugar with an alcohol, a method commonly used to prepare glycosides of non-nitrogenous sugars. However, Irvine *et al.* obtained an acetobromo derivative,[1,2] 3,4,6-tri-O-acetyl-2-amino-1-bromo-2-deoxy-α-D-glucose hydrobromide (I), which on treatment with alcohols and pyridine, morphine, or silver carbonate[3] yielded β-glycosides. When this method was examined by us mercuric acetate was found to be a more effective catalyst, but even when this salt was employed as condensation agent and the products were separated by chromatography, yields of pure (methyl) glycoside never exceeded 50 per cent and were often considerably lower than this. Attempts to obtain a disaccharide by interaction of the bromo compound I and 1,2,3,4-tetra-O-acetyl-α-D-glucose were unrewarding: chromatographic analysis of the acetylated products on alumina or silica gel furnished crystalline derivatives of the monosaccharide starting materials and a large number of smaller amorphous fractions. It seems likely that there occurred *inter alia* condensation of the compound I with free amino groups to form disaccharides and higher polymers linked through N-glycoside bridges.

When the amino group of a hexosamine is acylated the resultant derivative resembles the aldohexoses in many of its reactions. Thus 2-acetamido-2-deoxy-D-glucose forms glycosides directly with alcohols and acid,[4] and also it can be converted to an acetobromo compound[5] which may be condensed with alcohols to yield β-glycosides. Unfortunately the removal of an N-acetyl group from the resultant glycoside, as in the case of methyl 2-acetamido-2-deoxy-α-D-glycopyranoside,[6] is attended by scission of glycosidic linkages when acid hydrolysis is used, and although

* For a preliminary account of part of this work see *Chem. & Ind.* 917 (1955).

[1] J. C. Irvine, D. McNicoll and A. Hynd, *J. Chem. Soc.* **99**, 250 (1911); J. C. Irvine and A. Hynd, *Ibid.* 41 (1913).
[2] F. Micheel, F. P. Van de Kamp and H. Wulff, *Chem. Ber.* **88**, 2011 (1955).
[3] M. L. Hamlin, *J. Amer. Chem. Soc.* **33**, 766 (1911).
[4] F. Zilliken, C. S. Rose, G. A. Braun and P. Gyorgy, *Arch. Biochem. Biophys.* **54**, 392 (1955).
[5] R. Kuhn and W. Kirshenlohr, *Chem. Ber.* **86**, 1331 (1953).
[6] A. B. Foster, D. Horton and M. Stacey, *J. Chem. Soc.* 81 (1957).



elimination of the acyl group under alkaline conditions is feasible, drastic conditions are required.[7] 2-Benzyloxycarbonylamino-2-deoxy-D-glucose is now often used for glycoside preparation because in this case the "blocking group" can be removed catalytically under very mild conditions when required. Regrettably, when attempts were made to prepare an acetobromo derivative of this compound, the hydrobromide I was obtained.[8]

The present work, a study of 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose (DNP-D-glucosamine, II) and its derivatives, was undertaken with the object of devising new methods for the synthesis of glycosides and oligosaccharides derived from aminosugars containing free, as well as substituted, amino groups. Earlier workers[9,10,11] had shown that on account of their crystalline properties and distinctive colour 2′,4′-dinitrophenyl-(DNP-) derivatives of hexosamine were very suitable for characterization and separation. In order to assess its value as a "blocking group" in the synthetic studies envisaged, information concerning the stability of

[7] P. W. Kent and M. W. Whitehouse, *Biochemistry of the Aminosugars* p. 232. Butterworths, London (1955).
[8] E. L. May and E. Mosettig, *J. Org. Chem.* **15**, 890 (1950).
[9] P. W. Kent, *Research* **3**, 427 (1950); P. W. Kent, G. Lawson and A. Senior, *Science* **113**, 354 (1951).
[10] K. H. Meyer and D. E. Schwartz, *Helv. Chim. Acta* **33**, 1651 (1950).
[11] E. F. Annison, A. T. James and W. T. J. Morgan, *Biochem. J.* **48**, 477 (1951).

the N-DNP group in hexosamines was sought together with a method for its removal which was mild and would not affect glycosidic linkages.

DNP-D-glucosamine (II) was prepared by treating 2-amino-2-deoxy-D-glucose hydrochloride with 1-fluoro-2,4-dinitrobenzene (FDNB) and sodium bicarbonate. A high yield of crude material was obtained which invariably suffered much loss on subsequent purification. Although some variation in experimental procedure was made the yield of pure product did not exceed 35 per cent.

On treatment of DNP-D-glucosamine (II) with methanolic hydrogen chloride at 65°, a complex mixture was obtained. The presence of some unchanged II was demonstrated chromatographically and fractional crystallization of the product eventually furnished small amounts of methyl 2-(2′,4-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside (III), $[\alpha]_D + 12{\cdot}7°$ and methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucofuranoside (IV), $[\alpha]_D + 30{\cdot}6°$. The identity of the pyranoside III was established by an alternative synthesis from methyl 2-benzyloxycarbonyl-amino-2-deoxy-α-D-glucopyranoside (V)[12] of known structure. The latter was converted to methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride (VI) according to the directions of Neuberger and Rivers[12] and thence to III by condensation with FDNB. A crystalline derivative of II, methyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside (VII), was obtained on acetylation with pyridine and acetic anhydride.

From the value of its specific rotation it seemed unlikely that the glycoside IV was the anomeric form of the α-pyranoside III. Moreover the two glycosides were quite different in their susceptibilities to acid hydrolysis: whereas the substance IV was slowly hydrolysed to DNP-D-glucosamine (II) by 1N hydrochloric acid at room temperature, the α-pyranoside (III) was practically unaffected. The course of hydrolysis was followed polarimetrically and chromatographically. The substance IV was therefore tentatively assigned the furanoside structure indicated above since it is generally recognized that glycofuranosides are much more labile than glycopyranosides. Indeed the lability of the methyl 2-acetamido-2-deoxy-glucofuranoside obtained by Kent[9] established that the furanoside form of an N-substituted hexosamine displayed essentially the same ease of glycoside-breakdown as had been commonly observed with furanoside forms of non-nitrogeneous hexoses. The fact that the substance IV yielded 1 mole of formaldehyde on periodate oxidation gave support to the structure assigned. It should however be mentioned that in another experiment designed to determine the amount of periodate consumed, consistent results could not be obtained. Again, the isolation of a higher yield of IV (the only crystalline product isolated) when the acid-catalysed interaction of II and methanol was allowed to occur at room temperature was in accordance with the structure allocated to IV, corresponding as it did with the recognized tendency for furanoside structures to be formed under such mild conditions. From a consideration of the specific rotations of III and IV the latter is assumed to have the α-configuration. Compound IV formed a crystalline triacetate.

Treatment of the pyranoside III with 1N hydrochloric acid at 100° led to scission of the glycosidic linkage but the DNP group remained intact as judged from a chromatographic investigation of the hydrolysis: under alkaline conditions the DNP

[12] A. Neuberger and R. P. Rivers, *Biochem. J.* 23, 1581 (1939); A. B. Foster, D. Horton and M. Stacey, *Chem. Soc.* 81 (1957).

group was quickly eliminated with regeneration of the parent aminosugar glycoside. A convenient way of carrying out this alkaline hydrolysis consisted in treating the pyranoside III in aqueous–acetone with an anion-exchange resin (OH form). The resin not only effected the required de-dinitrophenylation but also retained the liberated 2,4-dinitrophenol. The product, methyl 2-amino-2-deoxy-α-D-glucopyranoside, which was obtained in excellent yield simply by filtration and evaporation, was characterized as the N-benzyloxycarbonyl-(V) and 4,6-O-benzylidene-N-benzyloxycarbonyl-(VIII) derivatives. This method of removal of the dinitrophenyl group was subsequently applied to other glycosides of II and in every case only the parent glucosamine derivative was isolated from the reaction products, there being no indication that scission of the (sugar) carbon-nitrogen linkage occurs to any significant extent.

The dinitrophenyl group was unaffected by treatment at room temperature with methanolic ammonia, a much-used reagent for de-O-acetylation. Thus benzyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)amino-2-deoxy-β-D-glucopyranoside (IX) the synthesis of which is described below, was smoothly deacetylated by this means. Subsequent alkaline (ion-exchange resin) hydrolysis of the deacetylation product furnished benzyl 2-amino-2-deoxy-β-D-glucopyranoside which was isolated as the N-acetyl derivative X by simultaneous O- and N-acetylation with acetic anhydride followed by de-O-acetylation with methanolic ammonia. The derivative X was identical with the benzyl 2-acetamido-2-deoxy β-D-glucopyranoside of known structure which was prepared from authentic benzyl 3,4,6-tri-O-acetyl-2-acetomida-2-deoxy-β-D-glucopyranoside[13] by de-O-acetylation.

The formation of an acetobromo derivative of DNP-D-glucosamine II was then attempted. 1,3,4,6-Tetra-O-acetyl-2-amino-2-deoxy-α-D-glucose hydrochloride[14] was dinitrophenylated with FDNB and sodium carbonate, the product 1,3,4,6-tetra-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucose (XI) being obtained in good yield. An alternative method of arylation with FDNB and silver carbonate in chloroform solution gave the same product in lower yield. The compound XI was converted to 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-1-bromo-2-deoxy-α-D-glucose (XII) in near quantitative yield by treatment with HBr in glacial acetic acid and chloroform. This acetobromo compound XII is highly crystalline and can be kept in a dry atmosphere for several months without decomposition.

Concurrently with a study of the reactions of the bromo compound XII the syntheses of reference β-glycosides of known structures were carried out. The acetobromoglucosamine hydrobromide I of Irvine *et al.*[1] reacted with benzyl alcohol in the presence of pyridine forming benzyl 3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucoside hydrobromide, which was converted to benzyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-β-D-glucoside (IX) by the usual method with FDNB. A parallel preparation of the corresponding β-methyl glycoside gave a syrupy product which, although analysing in the manner expected of methyl 3,4,6-O-acetyl-2-(2′, 4′-dinitrophenyl)-amino-2-deoxy-β-D-glucoside after chromatographic purification on alumina, could not be crystallized.

Treatment of the DNP acetobromo compound XII with benzyl alcohol in the presence of silver carbonate yielded the β-glucoside IX as expected, but a different

[13] W. O. Cutler and S. Peat, *J. Chem. Soc.* 274 (1939).
[14] L. Bergmann and M. Zervas, *Ber. Dtsch. Chem. Ges.* **64**, 975 (1931).

product XIII was obtained when the reaction was carried out in acetone solution with pyridine as condensation agent. From the fact that the new compound was non-reducing and in accordance with analytical and optical rotational data it was tentatively designated benzyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside. Under the conditions that appeared to favour the formation of the α-glycoside, a synthesis of methyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside (VII) was attempted by condensation of the bromo compound XII with methanol. On this occasion the product obtained was clearly of the α-configuration since it was identical with the authentic α-glycoside VII prepared as described above.

The yields of glycosides isolated after condensation of XII and an alcohol were not high and repeated recrystallization of the crude glycoside was necessary during the purification process. This was thought to be due, in part at least, to the presence in the reaction products of 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose (XIV); this compound was actually isolated during one experiment. Its structure was inferred from its reducing character and elemental analysis data.

It seems clear from these investigations that DNP-D-glucosamine and derivatives are potentially of considerable value for glycoside formation and other synthetic operations in the glucosamine field. A detailed consideration of the reactions of the bromo compound XII will be presented in a subsequent communication.

EXPERIMENTAL

Paper chromatography. The upper phase of a water : 1-butanol : ethanol (5 : 4 : 1 by volume) mixture was used as mobile phase for paper chromatography and all R_F values given below refer to this solvent system.

In all reactions involving bromo compounds dry reactants were used and rigorously anhydrous conditions were observed.

2-(2′,4′-Dinitrophenyl)-amino-2-deoxy-D-glucose

2-Deoxy-2-amino-D-glucose hydrochloride (2·00 g), sodium carbonate (0·98 g) and 1-fluoro-2,4-dinitrobenzene in acetone : water (50 ml, 1 : 4 v/v) were shaken overnight. The acetone was removed by evaporation under reduced pressure and the residue washed with ice-water (45 ml) and dried. The crude product (80% yield) was dissolved in hot methanol and 4–5 volumes of benzene and sufficient light petroleum (b.p. 60–80) to produce turbidity added. On standing the product (1·12 g, 35% separated as orange needles m.p. 194–196°, $[\alpha]_D^{18}$ 4 + 1·2° (*c.* 1·0 in ethanol) + 63·7 (*c.* 0·8 in 80% (v/v) ethanol, R_F 0·82. Kent[9] reports m.p. 167–169, $[\alpha]_D^{22}$ + 50° (ethanol), Meyer and Schwartz,[10] m.p. 196 ± 2° and Annison *et al.*[11] give m.p. 202–204, $[\alpha]_{5461}$ + 65 ± 3° (80% (v/v) ethanol).

Reaction of 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose with methanolic hydrogen chloride

(a) *At* 65°. A solution of 2-(2′,4′-dinitrophenyl)-2-amino-2-deoxy-D-glucose (5·0 g) in dry methanol (250 ml) containing 2·1% hydrogen chloride was heated under reflux until the optical rotation became constant, $[\alpha]_D^{20}$ + 48·0° (5 min) → + 55·0° (80 min). The solution was neutralized with silver oxide, filtered, treated with hydrogen sulphide, filtered and evaporated to a yellow syrup. This was dissolved in acetone and 4 volumes of ether and light petroleum (b.p. 80–100°) added until the solution was turbid. On standing, yellow prisms (Fraction A, 1·15 g), m.p. 201–204°, were deposited. From the mother liquors, after evaporation, dissolution in ethanol and addition of water at 0° a second crystalline fraction (Fraction B, 1·07 g), m.p. 95° unsharply, was obtained. Evaporation of the mother liquors and tritration with ethanol : light petroleum furnished a third crystalline fraction (Fraction C, 0·42 g) m.p. 134–136°.

Repeated recrystallization of fraction A from ethanol : light petroleum and from aqueous acetone afforded yellow prisms of *methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside* (0·21 g),

m.p. 216–218°, $[\alpha]_D^{19}$ + 12·7° (*c* 1·0 in acetone), R_F 0·88 (Found: C, 43·5, H, 4·9; N, 11·3. $C_{18}H_{17}O_9N_3$ required: C, 43·7; H, 4·8, N, 11·7; OMe, 8·6%).

After many recrystallizations of fraction B from ethanol : light petroleum, yellow prisms of *methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucofuranoside* (0·31 g) were obtained. This compound had m.p. 166–167°, $[\alpha]_D^{18}$ + 30·6° (*c* 1·0 in acetone) R_F 0·88 and was generally more soluble in water and organic solvents than the corresponding pyranoside. (Found: C, 43·8; H, 4·7; N, 11·9, mol. wt. (Rast) 350. $C_{18}H_{17}O_9N_3$ requires: C, 43·7; H, 4·8; N, 11·7%; mol. wt. 359).

Oxidation of the furanoside with unbuffered aqueous 0·3 M sodium metaperiodate for 1½ hr followed by distillation[16] and treatment of the distillate with dimedone gave a precipitate (m.p. 188–189°) which corresponded to the formation of 0·96 mole formaldehyde.

The melting point of fraction C had not become constant after six recrystallizations. The product (0·030 g) then had m.p. 198–206°, $[\alpha]_D^{17}$ + 7·8 (*c* 0·5 in acetone), R_F 0·89.

Chromatographic examination of the mother liquors indicated that some unchanged starting material was present.

(b) *At room temperature.* A solution of 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose (0·500 g) in dry methanol (25 ml) containing 2·1% hydrogen chloride underwent a slow change in optical rotation which became constant after 14 days. $[\alpha]_D^{19}$ + 48·0 (zero time) → $[\alpha]_D^{18}$ 58·5° (14 days). The solution was neutralized with silver oxide, filtered and evaporated to dryness. The residue on dissolution in ethanol and addition of petroleum ether (b.p. 80–100°) furnished some crystalline material. Repeated recrystallization of the latter yielded methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucofuranoside (0·082 g) m.p. 166–167°.

Methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside

Methyl 2-amino-2-deoxy-α-D-glucopyranoside hydrochloride[13] (0·41 g), 1-fluoro-2,4-dinitro benzene (0·50 g) and sodium carbonate (0·19 g) in acetone : water (10 ml, 4 : 1 v/v) were shaken for 48 hr. After removal of the acetone by evaporation the residue was washed with ice-water and recrystallized from ethanol. The product (0·41 g, 65%) was identical with the product derived from fraction A (above). (Found: C, 43·9; H, 4·9; N, 11·7; oMe, 9·6%).

Acid hydrolysis of methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucosides

(a) *At room temperature.* Each glycoside (0·050 g) in acetone (4·0 ml) was treated with 5N hydrochloric acid (1·0 ml) and kept at room temp. The reaction was followed polarimetrically and also by chromatographic examination of aliquots at suitable intervals. The furanoside (R_F 0·89) was slowly hydrolysed and after 50 days 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose, but only a trace of the original furanoside, was detected chromatographically. The optical rotation had become constant. $[\alpha]_D^{30}$ + 63·7 → + 47·4°. Evaporation and recrystallization gave 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose.

The pyranoside remained unhydrolysed and was recovered unchanged (91%) after 50 days. 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose when subjected to the above acid treatment underwent no change, polarimetrically or chromatographically.

(b) *At* 100°. The hydrolysis of methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucofuranoside by 1·0N hydrochloric acid was followed chromatographically. After 2 hr, from the intensities of the spots about 30% of the glycoside had been hydrolysed. After 7 hr an intense spot corresponding to 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-D-glucose was seen and only a trace of the starting material. After development of the chromatogram with silver nitrate : sodium hydroxide sprays, no further spots appeared.

Methyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside

Methyl 2-(2′4′-dinitrophenyl)-amino-2-deoxy-α-D-glucopyranoside (0·103 g) was dissolved in acetic anhydride (1·0 ml) and pyridine (1·0 ml). After standing for 3 days at room temp the mixture was poured into a large volume of water and the yellow precipitate filtered and recrystallized from ethanol. The *tri-acetyl derivative* (0·109 g, 817%), needles, had m.p. 206°, $[\alpha]_D^{18}$ + 21·3° (*c* 0·5 in chloroform), $[\alpha]_D^{18}$ + 71·6 (*c* 0·5 in acetone) (Found: C, 46·9; H, 5·1; N, 8·7; $C_{18}H_{23}O_{12}N_3$ requires: C, 47·0; H, 4·8; N, 8·7%).

Methyl 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-amino-2-*deoxy*-α-D-glucofuranoside

This compound (79% yield) was obtained by acetylation of methyl 2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucofuranoside by the method described above. Recrystallized from ethanol *the product* had m.p. 95–97°, $[\alpha]_D^{22}$ +99·7 (*c* 0·7 in chloroform), R_F 0·95. (Found: C, 47·3; H, 4·6; N, 8·9. $C_{19}H_{22}O_{12}N_4$ requires: C, 47·0; H, 4·8; N, 8·7%).

Methyl 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-amino-2-*deoxy*-β-D-*glucopyranoside*

Methyl 3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide (0·50 g) prepared according to the method of Irvine *et al.*,[1] 1-fluoro-2,4-dinitrobenzene (0·35 g), sodium carbonate (0·133 g), acetone (8 ml) and water (2 ml) were shaken together for 24 hr. The acetone was removed by distillation and the residue extracted with ether. After washing with dil hydrochloric acid and water the ethereal solution was evaporated to a bright yellow syrup, (0·749 g) which gave a single discrete spot, R_f 0·95, on the paper chromatogram. This could not be crystallized and charring occurred when distillation in a high vacuum was attempted on part of the product. It was purified by chromatography on an alumia column eluting successively with benzene : light petroleum (b.p 60–80°) (1 : 1 v/v), other mixtures of benzene and light petroleum containing higher proportions of benzene, benzene : chloroform (1 : 1 v/v) and benzene : chloroform (1 : 2 v/v). *Methyl* 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-β-D-*glucopyranoside* (80% yield) was obtained as a light yellow syrup $[\alpha]_D^{22}$ +58·1 (*c*, 0·9 in acetone). (Found: C, 47·5; H, 5·1; N, 8·8. $C_{19}H_{22}O_{12}N_4$ requires: C, 47·0; H, 4·8; N, 8·7%).

Benzyl 3,4,6-*tri*-O-*acetyl*-2-*amino*-2-*deoxy*-β-D-*glucopyranoside hydrobromide*

This compound was prepared by a modification of the method of Cutler and Peat,[13] 3, 4, 6-Tri-O-acetyl-2-amino-1-bromo-2-deoxy-α-D-glucose hydrobromide (1·00 g) was dissolved in benzyl alcohol (10 ml) and containing 2% anhydrous pyridine. After standing at room temp overnight, light petroleum (b.p. 80–100°): ether (80 ml, 3 : 1 v/v) was added and the gelatinous precipitate filtered off and suspended in ethyl acetate (25 ml). After filtering and washing with ether, recrystallization of the solid from ethanol : light petroleum afforded fine needles of the *benzyl glycoside* (0·443 g, 42%), which decomposed without melting at 237–241°, $[\alpha]_D^{23}$ +19·5° (*c* 0·5 in chloroform) (Found: C, 47·9; H, 5·4; N, 2·9. Calc. for $C_{19}H_{26}O_8NBr$: C, 47·9, H, 5·5; N, 3·0%). Cutler and Peat[13] give decomp 237–240°, $[\alpha]_D^{14}$ +24·2 (*c* 1.0 in chloroform).

Benzyl 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-β-D-*glucopyranoside*

The benzyl glycoside prepared above (0·15 g) was shaken with 1-fluoro-2,4-dinitrobenzene (0·088 g) and sodium bicarbonate (0·056 g) in 80% (v/v) aqueous acetone (5 ml) for 14 hr. Evaporation, crystallization by trituration with ethanol and recrystallization from the same solvent furnished the *dinitrophenylated compound*, large yellow prisms, (0·15 g, 85%), m.p. 160°, $[\alpha]_D^{23}$ +44·1° (*c* 1·0 in chloroform). (Found: C, 53·4; H, 4.7; N, 7·8 ($C_{25}H_{27}O_{12}N_3$ requires: C, 53·5; H, 4·9; N, 7·5%).

Benzyl 2-*acetamido*-2-*deoxy*-β-D-*glucopyranoside*

Benzyl 3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrobromide (0·055 g) was dissolved in pyridine (1·2 ml) and acetic anhydride (1·0 ml). After 3½ hr the mixture was poured into ice-water and the precipitate recrystallized from ethanol to give benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (0·035 g, 70%), m.p. 163°, $[\alpha]_D^{18}$ —42·3 (*c* 0·3 in chloroform). Kuhn *et al.*[5] give m.p. 165–167°, $[\alpha]_D^{20}$ —43·4 (in methanol) for this compound.

On de-O-acetylation with methanolic ammonia the tri-O-acetyl-derivative was converted to benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside (70% yield) m.p. 204–206° $[\alpha]_D^{19}$ —45·2 (*c* 0·4 in water). Kuhn *et al.*[5] give m.p. 205–206°, $[\alpha]_D$ —48° (in water).

Benzyl 2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-β-D-*glucopyranoside*

The tri-O-acetyl-derivative of this compound (0·200 g) was deacetylated by dissolving in dry methanol (20 ml) which had been saturated with ammonia at 0° and acetone (7 ml) and keeping

at room temp overnight. The solvents were removed by evaporation and the residue recrystallized from ethanol : light petroleum (b.p. 80–100°). The *product* of deacetylation (0·072, 50%) had m.p. 198°. (Found: C, 52·7; H, 4·7; N, 9·4. $C_{19}H_{21}O_9N_3$ requires: C, 52·4; H, 4·9; N, 9·6%).

Alkaline (resin) hydrolysis of derivatives of 2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-D-*glucose*

(a). *Methyl* 2-(2′4′-*dinitrophenyl*)-*amino*-2-*deoxy*-α-D-*glucopyranoside.* When this compound (0·200 g) was dissolved in acetone : water (10 ml, 2 : 1 v/v) and Amberlite "IRA-400-OH" (9 g) added and the mixture shaken rapid decolorization occurred. After shaking overnight the filtered solution was evaporated *in vacuo* to syrupy methyl 2-amino-2-deoxy-D-glucopyranoside (0·120 g). This was characterized by formation of the benzyloxycarbonyl- and benzylidene-benzyloxycarbonyl-derivatives as follows.

The syrupy product (0·120 g) was dissolved in water (1·2 ml) and sodium bicarbonate (0·094 g) added. Benzyloxycarbonyl chloride (0·152 g) was added dropwise over a period of 1 hr with vigorous shaking during the whole period. After 70 min the mixture was cooled to 0° for 2 hr and the crystalline methyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (0·091 g, 50%) separated. This had m.p. 160–161°, $[\alpha]_D$ +96·4° (*c* 1·0 in pyridine). The melting point was not depressed on admixture with an authentic specimen.[13] (Found C, 54·5; H, 6·5; N, 4·3. Calc. for $C_{15}H_{21}O_7N$: C, 55·0; H, 6·5, N, 4·3%).

The benzyloxycarbonyl derivative[15] (0·016 g) was stirred with freshly distilled benzaldehyde (0·13 ml) and zinc chloride (fused) (0·020 g) until solution was complete. After standing overnight three vol of ethanol were added. The precipitate was recrystallized from acetone which afforded five needles of *methyl* 4,6-0-*benzylidene*-2-*benzyloxycarbonylamine*-2-*deoxy*-α-D-*glucopyranoside* (0·014 g, 69%), m.p. 212–213° (Found: C, 64·1; H, 6·4; $C_{22}H_{25}O_7N$ requires: C, 63·6; H, 6·1%).

(b). *Benzyl* 2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-β-D-*glucopyranoside.* This compound (0·20 g) was dedinitrophenylated with "Amberlite IRA 400-04" by the method described above. The product, a syrup, (0·058 g, 47%) was N-acetylated by treatment with dry methanol : acetic anhydride (1·5 ml, 8 : 1 v/v) at room temp overnight. On evaporation and recrystallization of the residue, white needles of benzyl 2-acetamido-2-deoxy-β-D-glucoside (0·026 g, 43%) were obtained identical with an authentic sample, the preparation of which is described above.

1,3,4,6-*Tetra*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-α-D-*glucose*

(a). *Reaction in aqueous acetone solution.* 1,3,4,6-Tetra-O-acetyl-2-amino-2-deoxy-2-D-glucose hydrochloride (1·00 g) m.p. 230° (decomp), $[\alpha]_D^{23}$ +29·9 (*c* 1·2 in water), which had been prepared by the method of Bergmann and Zervas[14] was shaken with 1-fluoro-2,4-dinitrobenzene (0·49 g) and sodium bicarbonate (0·22 g) in acetone (16 ml) and water (4 ml) for 14 hr at room temp. The solution was concentrated to a small volume and the solid that separated recrystallized from ethanol yielding lemon needles of 1,3,4,6-*tetra*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-α-D-*glucose* (1·12 g, 84%), m.p. 166–167°, $[\alpha]_D^{19}$ +47·9 (*c* 1·0 in chloroform), R_F 0·95 (Found: C, 46·7; H, 4·2; N, 8·2; $C_{20}H_{23}O_{13}N_3$ requires: C, 46·8; H, 4·5; N, 8·2%).

(b). *Reaction in chloroform solution.* A solution of 1,3,4,6-tetra-O-acetyl-2-amino-2-deoxy-α-D-glucose (1·00 g), m.p. 141°[14], and 1-fluoro-2,4-dinitribenzene (0·51 g) in dry chloroform (20 ml) was shaken with dry silver carbonate (2.15 g) for 24 hr. The solution was filtered, evaporated, and the residue on recrystallization furnished the same product as obtained in (a). Yield 36%.

3,4,6-*Tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-1-*bromo*-2-*deoxy*-α-D-*glucose*

1,3,4,6-Tetra-O-acetyl-2-(2′,4′-dinitrophenyl)-2-deoxy-α-D-glucose (2·90 g) was dissolved in a mixture of glacial acetic acid (45 ml) which had been saturated with dry HBr at 0° and chloroform (25 ml), and dry HBr was passed through the solution for 30 min. After standing for 2 hr at room temp the mixture was poured into ice-water and extracted with chloroform. The chloroform extract was washed with ice-cold sodium bicarbonate solution until free from acid, then washed with water, dried ($MgSO_4$) and evaporated under diminished pressure. The yellow residue, which crystallized spontaneously was the *bromo compound* (2·90 g, 96%); it was of a high degree of purity, m.p. 160–162°, $[\alpha]_D^{19}$ +45·6 (*c* 0·8 in chloroform) (Found: Br 14·4%). It was usually recrystallized from dry acetone–light petroleum (b.p. 100–120°) before further use when it (2·38 g) had m.p. 162–164°

[15] Z. Y. Kyi, Ph.D. Thesis, Birmingham (1951).

$[\alpha]_D^{19}$ + 46·0. It could also be recrystallized from glacial acetic acid or benzene, and was soluble in chloroform, acetone, ether and alcohols and insoluble in light petroleum and water. Normally, the compound was stored in a desiccator but a sample exposed to the atmosphere for several days suffered no decomposition. (Found: C, 40·6; H, 3·8; N, 8·1; Br, 14·8. $C_{18}H_{20}O_{11}N_3Br$ requires: C, 40·5; H, 3·8; N, 7·9; Br 14·8%).

Reaction of 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-1-*bromo*-2-*deoxy*-α-D-*glucose with alcohols*

(a) *With benzyl alcohol in the presence of silver carbonate.* A solution of the bromo compound (0·50 g) and benzyl alcohol (1·0 ml) in chloroform (10 ml) was stirred with silver carbonate (2·6 g) and anhydrous calcium sulphate (1·0 g) for 45 hr in the dark. The solution was filtered through a charcoal mat and evaporated to dryness. Trituration with ethanol caused crystallization and recrystallization from the same solvent gave yellow prisms of benzyl 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-β-D-*glucoside* (0·22 g, 41%), m.p. 160° unchanged on admixture with authentic material. (Found: C, 53·4; H, 4·9; N, 7·4%).

Evaporation of the mother liquors from the above recrystallization yielded a solid m.p. 125–130°. Repeated recrystallization from light petroleum (b.p. 60–80°) : ethyl acetate and from ethanol gave yellow needles of 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-D-*glucose* (0·028 g), m.p. 150–152·5 depressed to 128–136° when mixed with the β benzyl glycoside (above). It reduced ammoniacal silver nitrate. (Found: C, 45·6; H, 4·6; N, 8·4. $C_{18}H_{21}O_{13}N_3$ requires: C, 45·9; H, 4·5; N, 9·0%).

(b) *With benzyl alcohol in the presence of pyridine.* The bromo compound (0·072 g) was mixed with benzylalcohol (2·0 ml) containing 1% pyridine, acetone (1·0 ml) added to effect complete solution, and the reaction followed polarimetrically $[\alpha]_D^{20}$ + 100° (40 min) → − 69·2° (2 hr) → + 9·2 (14 hr) (constant). After 20 hr the acetone was removed by distillation and the remaining solution poured into light petroleum (40 ml, b.p. 40–60°) containing 4% ethanol. The precipitate which separated, on recrystallization from ethanol yielded *benzyl* 3,4,6-*tri*-O-*acetyl*-2-(2′,4′-*dinitrophenyl*)-*amino*-2-*deoxy*-α-D-*glucoside* (0·023 g, 30%), yellow needles, m.p. 198–200° (sintering at 180°), $[\alpha]_D^{19}$ + 9·0° (*c* 0·3 in chloroform). (Found: C, 53·0; H, 4·8; N, 7·9. $C_{25}H_{27}O_{13}N_3$ requires: C, 53·5; H, 4·8; N, 7·5%).

(c) *With methanol in the presence of pyridine.* Condensation of the bromo compound (0·12 g) with methanol (1·8 ml) by the method described in (b) above furnished methyl 3,4,6-tri-O-acetyl-2-(2′,4′-dinitrophenyl)-amino-2-deoxy-α-D-glucoside (0·045 g 45%), m.p. 206°, alone and when mixed with authentic material, R_F 0·95.

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[16] P. Karrer and K. Pfaehler, *Helv. Chim. Acta* 17, 766 (1934).