for the *1:1 association* of acid (HA) and reference base (B). The logarithm of $K_{BHA}$ is regarded as the analog of the corresponding $pK_a$ value. In the past, a more usual practice has been to express comparative acidities as ratios, taking acetic acid or benzoic acid as the basis for comparison. (3) The reference base adopted, *1,3-diphenylguanidine*, is stronger than the reference bases ordinarily used. For example, it associates readily with carboxylic acids, commonly giving equilibrium constants in the range $10^4$–$10^7$. By comparison, the extent of other hydrogen-bonding reactions under the experimental conditions used is slight and can be neglected. (4) However, slightly more accurate association constants can be obtained by correcting for carboxylic acid present as the dimer, and in the process of correction, a *monomer–dimer equilibrium constant* for the carboxylic acid can be deduced. (Dimerization constants thus deduced compare favorably with results from other methods.) (5) The reference indicator acid, *tetrabromophenolphthalein ethyl ester* (*"bromophthalein magenta E"*), is well suited for determining the strengths of aliphatic and aromatic carboxylic acids in aprotic solvents because it matches them well in strength. (6) The results presented in this paper are in harmony with previous results, whose validity was indicated by clear-cut linear relationships of acidic strengths in benzene with those in water, alcohols, and partly aqueous solvents.[1,37] (7) The method is applicable to varied chemical types of acids.[38] Moreover, it is useful for acids which are difficultly soluble, scarce in amount, or unstable toward moisture. (8) The log $K_{BHA}$ scale is more suitable than the $pK_a$ scale for correlations of acid–base behavior in an aprotic solvent.

(37) It should also be noted that Hammett substituent constants calculated from the results for *meta*- and *para*-substituted acids agreed closely with constants derived from many other rate and equilibrium studies using other types of solvents, in particular, with "normal" substituent constants [R. W. Taft, Jr., *et al.*, *J. Am. Chem. Soc.*, **81**, 5343, 5352 (1959); J. E. Leffler and E. Grunwald, "Rates and Equilibria of Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1963, Chapter 7].

(38) Exploratory measurements have indicated that the reference acid–base system used here can probably be used in determining the strengths of saccharin, sulfonic acids, and some of the stronger phenols.

# Syntheses with Partially Benzylated Sugars. VI.[1] Some Solvolytic Reactions of 2-Acetamido-1-*O*-acyl-2-deoxy-D-glucopyranose and -D-galactopyranose Derivatives

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2-Acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoses and -D-galactopyranoses react readily with methanol to give methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside and -D-galactopyranoside. Since 2-acetamido-1-*O*-benzoyl-2-deoxy-β-D-galactopyranose behaves in a similar manner, the benzyl groups do not appear to be involved in the reaction. 2-Acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoses and -D-galactopyranoses yield no glycoside when heated with methanol, but undergo simple transesterification with the solvent. 2-Acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranoses and -D-galactopyranoses hydrolyze in aqueous dioxane at 50°, the β anomers cleaving more rapidly than the α anomers and β benzoates cleaving more rapidly than a β acetate. The mechanistic features of these reactions are discussed and their potential bearing on the origin of *O*-glycosides in mucoproteins is indicated.

The suggestion that some of the alkali-labile 2-acetamido-2-deoxy-D-galactopyranosyl moieties in ovine submaxillary gland mucoprotein (OSM) may be attached through C-1 with an ester linkage to the nonpeptide-bonded carboxyl groups of aspartic and glutamic acids[3] led Harrison and Fletcher[4] to develop a synthetic procedure for the preparation of C-1 esters of 2-acetamido-2-deoxy-D-glucose and of 2-acetamido-2-deoxy-D-galactose. This synthetic procedure has now been improved, several new substances needed for the present research have been prepared, and a study of the solvolytic reactions of some C-1 esters of substituted hexosamines has been carried out. The synthetic aspects of the present work will be discussed first.

(1) Paper V of this series: H. G. Fletcher, Jr., and H. W. Diehl, *J. Org. Chem.*, **30**, 2321 (1965).

(2) Fellow in the Visiting Program of the National Institutes of Health, 1964–1965.

(3) E. R. B. Graham, W. H. Murphy, and A. Gottschalk, *Biochem. Biophys. Acta*, **74**, 222 (1963).

(4) R. Harrison and H. G. Fletcher, Jr., *J. Org. Chem.*, **30**, 2317 (1965).

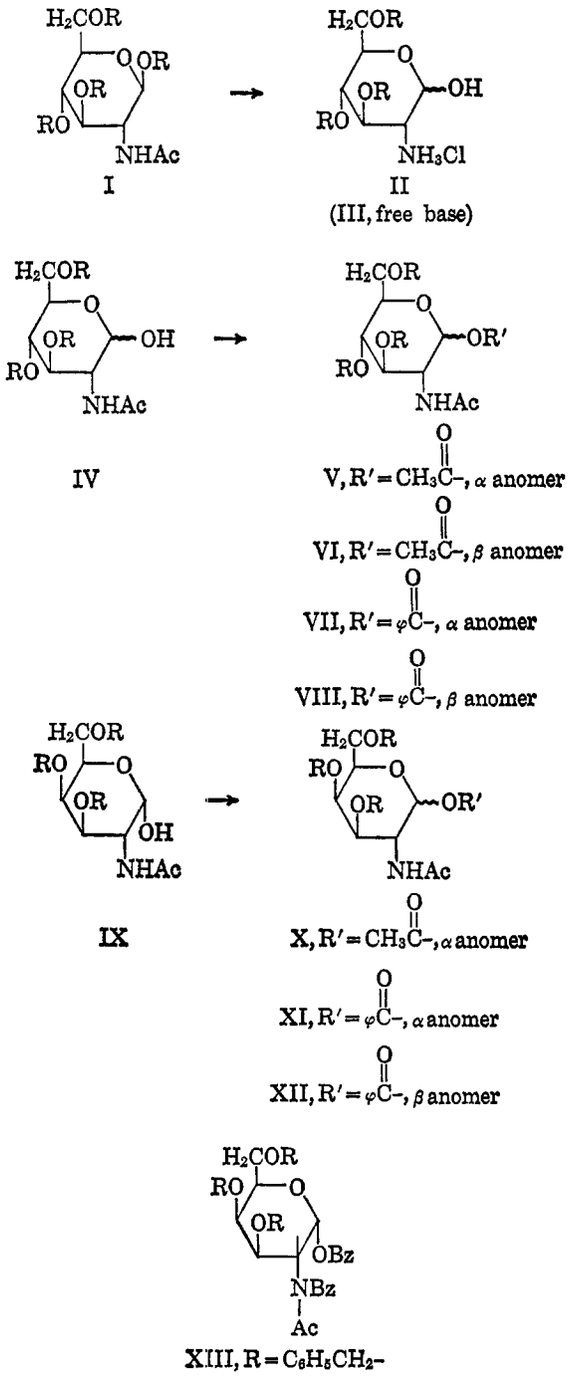
## Preparation of Substrates

The acetylation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (IV) (Scheme I) with acetic anhydride in pyridine affords 2-acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (V) in 78% yield.[4] In order to obtain the corresponding β 1-*O*-acetyl derivative (VI), IV was acetylated by a variety of procedures which have been reported as suitable for the preparation of β 1-*O*-acyl derivatives of 2-acetamido-2-deoxyhexoses;[5] none of these proved satisfactory. Anderson and Percival[6] found that tritylation of 2-acetamido-2-deoxy-α-D-glucopyranose with trityl chloride in pyridine at 100°, followed by acetylation with acetic anhydride, yielded a substantial proportion of the β anomer of 2-acetamido-1,3,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-D-glucopyranose, a fact that suggests that warm pyridine and the pres-

(5) C. S. Hudson and J. K. Dale, *J. Am. Chem. Soc.*, **38**, 1431 (1916); B. R. Baker, J. J. Joseph, R. E. Schaub, and J. H. Williams, *J. Org. Chem.*, **25**, 1786 (1954); J. K. N. Jones, M. B. Perry, B. Shelton, and D. J. Walton, *Can. J. Chem.*, **39**, 1005 (1961); M. Stacey, *J. Chem. Soc.*, 272 (1944).

(6) J. M. Anderson and E. Percival, *ibid.*, 814 (1956).

SCHEME I




ence of pyridine hydrochloride may favor the formation of a β anomer. 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (IV) was, therefore, heated in pyridine solution with pyridine hydrochloride prior to acetylation; the desired β 1-*O*-acetyl derivative (VI) was then isolated in 60% yield.

In the earlier study[4] of the synthesis of 2-acetamido-1-*O*-acyl-2-deoxyhexopyranoses, benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxyhexopyranosides were subjected to acidic hydrolysis. In addition to removal of the aglycon, this process resulted in partial loss of the *N*-acetyl group; to obtain the desired 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxyhexopyranose, the immediate product of the hydrolysis was acetylated and *O*-deacetylated. In the course of the present work, isolation of crystalline 2-amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose hydrochloride (II) directly from the hydrolysis of I was found practicable; the substance was also obtained as the free base, III, simply by chromatography of II on silica gel. *N*-Acetylation of II with 1 mole of acetic anhydride in pyridine at room temperature, followed by *O*-acetylation at an elevated temperature, yielded *ca.* equal portions of V and VI.

Harrison and Fletcher[4] prepared the β 1-*O*-benzoyl derivative of IV (VIII) in low yield through the condensation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranosyl bromide with silver benzoate; we have now found that VIII is more readily accessible through the benzoylation of IV after equilibration in pyridine or in pyridine containing pyridine hydrochloride. It is essential that the excess of benzoyl chloride be minimized in such acylations for, as shown later in this paper (and elaborated on in the following paper[7]), this reagent attacks the acetamido group to give a di-*N*-acyl derivative.

Several new C-1 esters in the *N*-acetyl-D-galactosamine series were synthesized. Acetylation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose[4] (IX) in normal fashion afforded its α 1-*O*-acetyl derivative (X). Benzoylation of IX with slightly more than 1 mole of benzoyl chloride gave a mixture from which the α 1-*O*-benzoyl derivative (XI) was isolated in 31% yield and its β anomer, XII, in 10% yield.[8] In addition to XI and XII, the benzoylation of IX yielded 2-(*N*-acetylbenzamido)-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (XIII) in crystalline form; further details regarding *N*,*N*-diacyl derivatives related to XIII are described in the following paper.[7]

## Solvolytic Studies

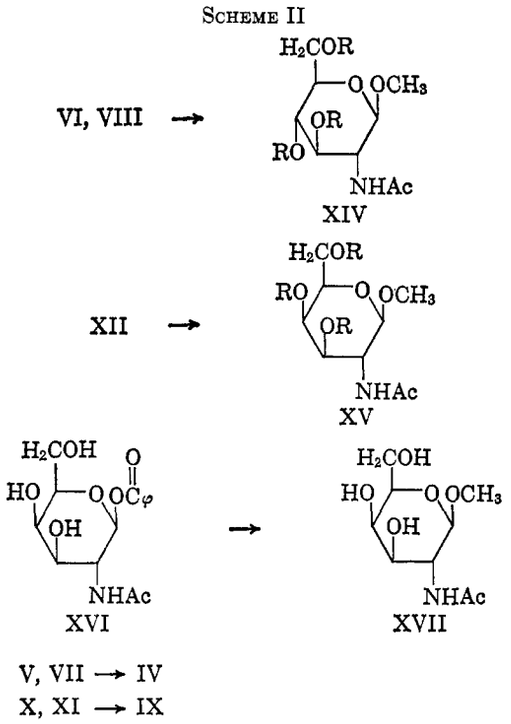
The instability of the 2-acetamido-1-*O*-acyl-2-deoxyhexopyranoses in methanol solution has been noted previously[4] and we, therefore, turned our attention to the problem of the methanolysis of a variety of 2-acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzylhexopyranoses. On boiling in methanolic solution, both 2-acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranose (VI) and 2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranose (VIII) lost their *O*-acyl groups and were converted to methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XIV) (Scheme II), the acidity of the reaction mixture indicating that the acyl group had appeared as the free acid. Similarly, 2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranose (XII) gave methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranoside (XV) and 2-acetamido-1-*O*-benzoyl-2-deoxy-β-D-galactopyranose[4] (XVI) was converted into methyl 2-acetamido-2-deoxy-β-D-galactopyranoside (XVII). The latter case is significant in showing that benzyl masking is not involved in the glycoside formation.

In all of the four cases described above, the ester function at C-1 is *trans* to the acetamido group at C-2; the behavior of the corresponding anomeric *cis* esters proved to be qualitatively different. Thus, 2-acet-

(7) T. D. Inch and H. G. Fletcher, Jr., *J. Org. Chem.*, **30**, 1815 (1965).

(8) In the earlier study[4] benzoylation of IX gave XII in 62% yield, none of XI being isolated. All attempts to repeat this result gave a preponderance of XI as described here.

SCHEME II

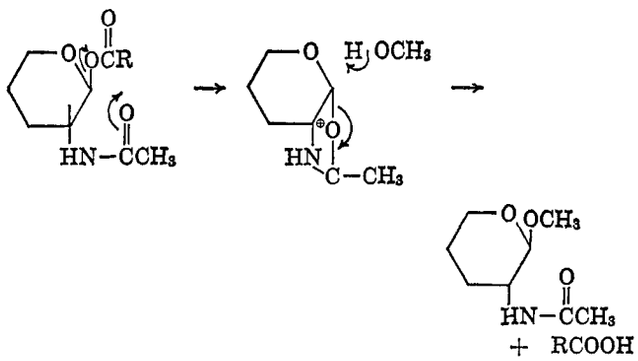



amido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (V) and the corresponding benzoate (VII) gave 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (IV) while the corresponding *cis* esters of the D-galactosamine series, X and XI, similarly gave 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-galactopyranose (IX). The odor of methyl benzoate was clearly evident in the methanolysis of the *cis* benzoates.

Gottschalk and his co-workers[3] measured the rate of cleavage of protein-bound hexosamine from OSM by 0.1 *N* sodium hydroxide at 100° and observed a rapid as well as a slower reaction. Attempts were made in the course of the present work to duplicate these measurements using the synthetic 1-*O*-acyl derivatives of the *N*-acetylhexosamines, but in all cases the rates of cleavage were too high under Gottschalk's conditions to permit kinetic study. Indeed aqueous dioxane alone at 50° was found to hydrolyze the 2-acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-2-deoxy-D-hexopyranoses at a convenient rate. The rates of hydrolysis of six such substances under these conditions are shown in Figure 1.

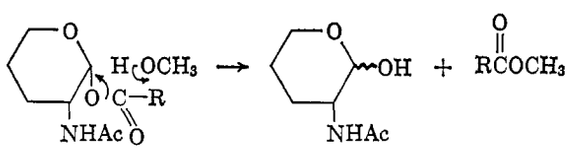
## Discussion

Normal 1-*O*-acylaldoses having a *trans* relationship between the ester group at C-1 and the hydroxyl group are comparatively stable substances while the corresponding *cis* esters readily undergo rearrangement, the acyl group migrating to C-2.[9] These properties contrast markedly with those of the 2-acetamido-1-*O*-acyl-2-deoxyaldose derivatives described here. The fact that the *trans* esters react with methanol in a stereospecific fashion to yield *trans* glycosides suggests the mechanism which is probably involved. The acetamido group may be viewed as providing anchimeric assistance in the departure of the acyloxy group from C-1, giving an intermediate ion which controls the direction



of attack of the methanol. As far as we are aware, this type of reaction has not hitherto been observed.[10]

Neighboring-group participation being sterically unlikely in the solvolysis of the *cis* esters, their cleavage may be regarded as a process of transesterification.



Several features of the hydrolysis of these C-1 esters deserve special comment. First, it should be noted (Figure 1) that the *cis* esters (V, VII, and X) hydrolyze at a much slower rate than the *trans* esters (VI, VIII, and XII), indicating the anchimeric assistance provided by the acetamido group in the latter three cases. Second, the two *trans* benzoates VIII and XII are seen to hydrolyze at a more rapid rate than the *trans* acetate VI; this observation may be regarded as supporting the proposed mechanism inasmuch as benzoate is a more effective leaving group than acetate.

In view of the ease with which the *trans* esters described here undergo conversion to methyl β-glycosides, it is natural to inquire whether fully acylated *trans* esters of 2-acetamido-2-deoxy-D-glucose may react similarly with methanol. For this reason, 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose and the two anomeric forms of 2-benzamido-1,3,4,6-tetra-*O*-benzoyl-2-deoxy-D-glucopyranose were boiled in methanol solution for 24 hr; thin film chromatography showed that none of the three substances was significantly altered by this treatment and, indeed, each was recovered in high yield. It has recently been shown[11] that the replacement of benzyl groups by *p*-nitrobenzoyl groups at C-3 and C-5 in a pentofuranosyl chloride markedly reduces the rate of solvolysis of the chloride. It is possible that the acyl groups in the hexosamine esters exert a similar long-range effect in preventing the glycoside formation which is so facile when carbons 3, 4, and 6 bear unsubstituted hydroxyl groups or benzyloxy groups.

Whether or not ester-linked 2-acetamido-2-deoxy-β-D-galactopyranosyl moieties occur in glycoproteins to a significant extent, it is at least chemically possible

(9) S. Tejima and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 2999 (1963).

(10) In the course of the present research, a variety of *trans*-2-acetamidocyclohexanol esters was heated in dilute methanolic solution. In each case *trans*-2-acetamidocyclohexanol, together with a methyl ester, was formed. In view of the special character of C-1 in aldopyranose derivatives, it is not surprising to find that alicyclic esters do not undergo displacements analogous to those shown by the *trans*-2-acetamido-1-*O*-acyl-2-deoxyaldoses.

(11) C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **87**, 4636 (1965).

that the glycosidically attached moieties which have been found there[12] arose through a carboxyl–hydroxyl transfer reaction analogous to the methanolysis of *trans* esters reported here.

The generalization that axial acetoxy groups give an nmr signal at lower field than equatorial acetoxy groups[13] has been used by various groups of workers[14] to determine molecular structure. Although this generalization appears to hold for the majority of acetylated pyranoses, we wish to point out that the two anomeric 2-acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranoses (V and VI) appear to constitute an exception; the signals for the acetoxy protons of both these compounds appear at $\tau$ 7.94. While this anomaly may arise from the influence of the aromatic residues which are present, it in no way interferes with the assignment of anomeric configuration since the chemical shifts and coupling constants of $H_1$ in these structures suffice unequivocally for this purpose.

## Experimental Section[15]

**2-Amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose Hydrochloride (II).**[16]—Benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-$\beta$-D-glucopyranoside[4] (I, 10 g) was dissolved in tetrahydrofuran (900 ml) and the solution, diluted with 3 *N* hydrochloric acid (450 ml), boiled under reflux for 48 hr. The cooled reaction mixture was concentrated *in vacuo* at 32° (bath temperature) until the bulk of the tetrahydrofuran was removed and the resulting crystalline magma was stored at +5° overnight. The solid mass was then removed by filtration, washed with cold 1 *N* aqueous hydrochloric acid, and dried in the air: 8.8 g (calcd 8.4 g), mp 169–172° dec, $[\alpha]^{20}D$ +54.4° (*c* 0.70, 2-methoxyethanol). Recrystallization from dioxane partially saturated with hydrogen chloride afforded a product of mp 184–185° and $[\alpha]^{20}D$ +55.3° (*c* 2.1, 2-methoxyethanol).

*Anal.* Calcd for $C_{27}H_{32}ClNO_5$ (486.02): C, 66.72; H, 6.64; Cl, 7.30; N, 2.88. Found: C, 66.47; H, 6.44; Cl, 6.47; N, 2.70.

The low chlorine content of the material could not be raised by further crystallization from solvents containing hydrogen chloride. On thin film chromatography (benzene–methanol, 3:1, v/v) the product was homogeneous and indistinguishable from the free base described below. Evidently, dissociation of the salt was complete under the conditions of the chromatography, the hydrochloride migrating as the free base. It seems probable that the hydrochloride obtained is contaminated with the free base.

A sample (0.5 g) of the material was warmed to 50° for 1 hr in pyridine solution containing 1.1 molar equiv of benzoyl chloride. The solution was then poured into ice water and the precipitated product was removed by filtration. After drying, it was recrystallized from ethanol: 0.38 g (67%), mp 228–229°, $[\alpha]^{20}D$ +95° (*c* 0.5, pyridine). Harrison and Fletcher[4] reported mp 221–222° and $[\alpha]^{20}D$ +89° (pyridine) for 2-benzamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose; a mixture of their product with that described above showed mp 227–228°.

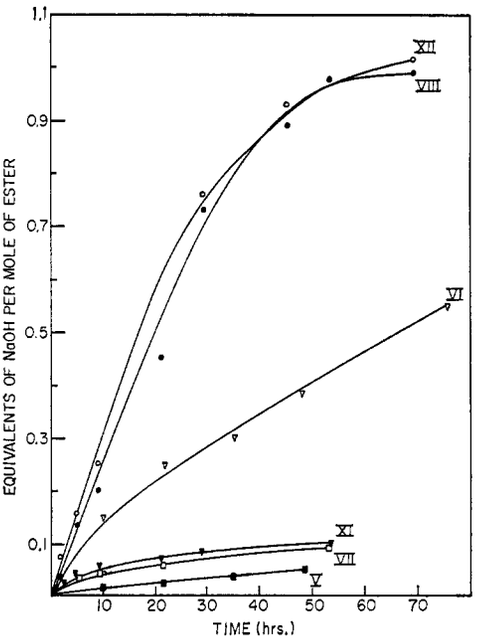



Figure 1.—Hydrolysis of 2-acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-2-deoxyhexopyranoses in aqueous dioxane at 50°. The acyl groups and hexoses are as follows: ○ benzoyl, $\beta$-D-galactose; ● benzoyl, $\beta$-D-glucose; ▽ acetyl, $\beta$-D-glucose; ▼ benzoyl, $\alpha$-D-galactose; □ benzoyl, $\alpha$-D-glucose; ■ acetyl, $\alpha$-D-glucose.

**2-Amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (III).**—A solution of the hydrochloride (2.76 g) in benzene–methanol (1:1, v/v) was poured on a column (3 × 28 cm) of silica gel and the column eluted with benzene–methanol (6:1, v/v). 2-Amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (2.1 g, 82%) was isolated from the first 500 ml of eluent; recrystallized from 2-propanol, it had mp 114–115° and $[\alpha]^{20}D$ +76° (*c* 1.1, chloroform). On standing at room temperature for several weeks, the substance decomposes.

*Anal.* Calcd for $C_{27}H_{31}NO_5$ (449.56): C, 72.14; H, 6.95; N, 3.12. Found: C, 72.41; H, 6.76; N, 3.04.

**2-Acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-$\beta$-D-glucopyranose (VI). A. From 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (IV).**—A solution of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (1.5 g) in pyridine (15 ml) to which pyridine hydrochloride (0.5 g) had been added was heated at 100° for 1 hr. Acetic anhydride (0.33 ml) was added to the hot solution which was then allowed to cool slowly (overnight) to room temperature. The solution was poured into ice water and the crystalline product was removed by filtration. Thin layer chromatography (benzene–ether–methanol, 14:14:1, v/v) indicated the product to be a mixture of two compounds, presumably V and VI. A solution of the mixture in chloroform was poured on a column (4 × 50 cm) of silica gel and the products eluted with benzene–ether–methanol (14:14:1, v/v), 15-ml fractions being collected.

Fractions 50–67 contained the pure $\beta$ anomer (VI) which was crystallized from ethyl acetate: 0.98 g (60%), mp 168–169°, $[\alpha]^{20}D$ +31° (*c* 0.9, $CHCl_3$).

*Anal.* Calcd for $C_{31}H_{35}NO_7$ (533.63): C, 69.77; H, 6.61; N, 2.62. Found: C 70.10; H, 6.53; N, 2.62.

Fractions 68–72 contained a mixture of $\alpha$ and $\beta$ anomers; subsequent fractions contained the pure $\alpha$ anomer which was crystallized from ethyl acetate–heptane: 0.15 g (9%), mp 146–147°, undepressed on admixture with 2-acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-$\alpha$-D-glucopyranose.[4]

The two anomers are clearly distinguished by their nmr spectra: $\alpha$ anomer, $\tau$ 3.85 ($H_1$, $J$ = 3.5 cps), 7.94 (OAc), 8.20 (NAc); $\beta$ anomer, $\tau$ 4.3 ($H_1$, $J$ = 8 cps), 7.94 (OAc), 8.20 (NAc).

**B. From 2-Amino-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose Hydrochloride (II).**—A solution of acetic anhydride (0.63 ml, 1 molar equiv) in pyridine (20 ml) was added dropwise to a solution of II (3 g) in pyridine (100 ml) at room temperature. After the addition was complete, the solution was stirred at

(12) V. P. Bhavanandan, E. Buddecke, R. Carubelli, and A. Gottschalk, *Biochem. Biophys. Res. Commun.*, **16**, 353 (1964); K. Tanaka, M. Bertolini, and W. Pigman, *ibid.*, **16**, 404 (1964); S. Harbon, G. Herman, B. Rossignol, P. Jollès, and H. Clauser, *ibid.*, **17**, 57 (1964); B. Anderson, N. Seno, P. Sampson, J. Riley, P. Hoffman, and K. Meyer, *J. Biol. Chem.*, **239**, 2716 (1964); R. Carubelli, V. P. Bhavanandan, and A. Gottschalk, *Biochim. Biophys. Acta*, **101**, 67 (1965); K. Tanaka and W. Pigman, *J. Biol. Chem.*, **240**, PC 1487 (1965).

(13) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Am. Chem. Soc.*, **80**, 6098 (1958).

(14) L. D. Hall, *Advan. Carbohydrate Chem.*, **19**, 51 (1964).

(15) Melting points are corrected. Thin layer chromatography was conducted on silica gel G (E. Merck A.-G., Darmstadt) in the solvent systems specified, development being by exposure to iodine vapor. All column chromatography was conducted on silica gel (0.05–0.20 mm) of E. Merck A.-G., this product proving markedly superior to other commercially available preparations. Nmr spectra were measured in $CDCl_3$ solution using a Varian A-60 spectrometer. Infrared absorption spectra were obtained using a Perkin-Elmer Model 521 spectrophotometer and 5 × $10^{-3}$ *M* solutions in dry $CCl_4$ or as mulls in Nujol, using a Perkin-Elmer Model 21 spectrometer.

(16) This substance was first isolated by Mr. Harry W. Diehl of this laboratory.

room temperature for 1 hr and then for a further hour at 95–100°. Acetic anhydride (1 ml) was added to the hot solution which was then cooled gradually to room temperature over the course of 5 hr. The reaction mixture was poured into ice-cold water and the product was separated by filtration and dried. It was then chromatographed on a column of silica gel as described under A above. Three major fractions were obtained: (1) VI, 1.1 g (33%); (2) V + VI, 0.6 g; (3) V, 1 g (30%).

**Benzoylation of 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (IV). Method A.**—Benzoyl chloride (0.5 ml, 1.1 molar equiv) was added to a cooled solution (ice bath) of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (2 g) in pyridine (20 ml) and the solution was then stored overnight at room temperature. It was poured into ice water and the crude product was extracted with dichloromethane. The extract was washed succesively with dilute hydrochloric acid, aqueous sodium bicarbonate solution, and water. Moisture was removed with magnesium sulfate and the solution was concentrated to a syrup which was dissolved in 3:1 benzene–ether and adsorbed on a column of silica gel (3.2 × 50 cm). Elution was carried out with the same solvent mixture, 10-ml fractions being collected. Early fractions contained 0.5 g of a yellow syrup, presumably 2-(*N*-acetylbenzamido)-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose.[7] Fractions 145–164 contained essentially pure **2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranose (VIII)** which was crystallized from methanol: 0.1 g (4.1%), mp 148–149° either alone or in admixture with material produced earlier;[4] nmr data, $\tau$ $H_1$ 4.09 ($J \sim 7$ cps), N-Ac 8.27; infrared spectrum, $\nu_{max}^{CCl_4}$, $cm^{-1}$, 1737.5 (CO), 1698 (NHAc).

Fractions 164–184 contained a mixture of α and β anomers. Fractions 185–247 contained essentially pure **2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (VII)** which was crystallized from isopropyl ether: 0.9 g (37%), mp 118–119° undepressed on admixture with material prepared earlier;[4] nmr data, $\tau$ $H_1$ 3.6 ($J$ = 3 cps), N Ac $\tau$ 8.29; infrared spectrum, $\nu_{max}^{CCl_4}$, $cm^{-1}$, 3449 and 3438 (NH), 1741 (CO), 1696 (NHAc). Some unreacted starting material was detected in later fractions by thin layer chromatography.

**Method B.**—A solution of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose (1.3 g) in pyridine (20 ml) containing pyridine hydrochloride (0.5 g) was heated at 90° for 1 hr, cooled to 20°, and treated with 1.1 molar equiv of benzoyl chloride. The solution was stored at room temperature for 1 hr, heated at 50° for 1 hr, and then worked up as described under method A. Chromatography on a column of silica gel (3.8 × 35 cm) was carried out as described under method A, a clean separation being obtained. The β anomer (VIII) was crystallized from methanol: 0.47 g (30%), mp 148–149°. The α anomer (VII) was crystallized from isopropyl ether: 0.39 g (25%), mp 116–117°.

**2-*O*-Acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (X).**—A solution of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose[4] (IX, 1 g) in a mixture of acetic anhydride (0.21 ml) and pyridine (10 ml) was stored overnight at room temperature and then poured into ice–water. The resulting crystalline precipitate was removed by filtration, dried, and recrystallized from isopropyl ether to give the pure ester X: 0.5 g (46%), mp 149–150°, $[\alpha]^{20}D$ +100° (*c* 1.06, $CHCl_3$); nmr data, $\tau$ 3.78 ($J$ = 3.5 cps) ($H_1$), 8.00 (OAc), 8.15 (NAc).

*Anal.* Calcd for $C_{31}H_{35}NO_7$ (533.63): C, 69.77; H, 6.61; N, 2.62. Found: C, 69.99; H, 6.56; N, 2.92.

**Benzoylation of 2-Acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranose (IX).**—Benzoyl chloride (0.4 ml, 1.1 molar equiv) was added to a cooled solution (ice bath) of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (IX, 1.6 g) in pyridine (15 ml) and the reaction mixture was stored at room temperature overnight. The solution was then poured into ice water and the product extracted with dichloromethane. After being washed successively with dilute hydrochloric acid, aqueous sodium bicarbonate solution, and water, the extract was dried with magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel (3 × 55 cm) using benzene–ether (5:2) as eluent. The first 600 ml of eluent contained a small quantity of yellow syrup, chromatographically indistinguishable from 2-(*N*-acetylbenzamido)-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (see below). Thereafter, eluent was collected in 15-ml portions. Fractions 19–43 contained essentially pure **2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranose (XII)** which was crystallized from methanol: 0.2 g (10%); mp 148–149°, undepressed on admixture with the product obtained by Harrison and Fletcher;[4] infrared spectrum, $\nu_{max}^{CCl_4}$, $cm^{-1}$, 1742 (CO), 1700 with shoulder at 1695 (NHAc).

Fractions 43–51 contained a mixture of α and β anomers. Fractions 52–96 contained **2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (XI)** which was crystallized from methanol: 0.6 g (31%); mp 136–138°; $[\alpha]^{20}D$ +149° (*c* 0.67, $CHCl_3$); infrared spectrum, $\nu_{max}^{CCl_4}$, $cm^{-1}$, 1742.5 (CO), 1698 (NHAc); nmr data (XI), $\tau$ 3.53 ($H_1$, $J_{1,2}$ ~3 cps), 8.24 (NAc). XII: $\tau$ 3.94 ($H_1$, $J_{1,2}$ = 8 cps), 8.20 (NAc).

*Anal.* Calcd for $C_{36}H_{37}NO_7$ (595.70): C, 72.58; H, 6.26; N, 2.35. Found: C, 72.67; H, 6.39; N, 2.38.

**2-(*N*-Acetylbenzamido)-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (XIII).**—The products from several benzoylations of IX were pooled and chromatographed on silica gel using benzene–ether (1:1, v/v). A chromatographically pure compound which traveled essentially with the solvent front was obtained after removal of the solvent. On trituration with heptane, it crystallized; recrystallization from ether afforded a pure product: mp 130–131°; $[\alpha]^{20}D$ +97.5° (*c* 1.25, $CHCl_3$); infrared spectrum, $\nu_{max}^{Nujol}$, $cm^{-1}$ 1720 (s), 1710 (sh), 1665 (RCO), and 1600 (vw) ($C_6H_5$); nmr spectrum, $\tau$ 8.22 (NAc), 3.35 ($H_1$, 3.5 cps).

*Anal.* Calcd for $C_{43}H_{41}NO_8$ (699.81): C, 73.80; H, 5.91; N, 2.00. Found: C, 74.03; H, 5.66; N, 2.20.

**Methanolysis of 2-Acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranose (VI).**—A solution of VI (0.2 g) in methanol (10 ml) was boiled under reflux for 48 hr, cooled, and concentrated to dryness, the odor of acetic acid then being clearly noticeable. The residue was crystallized and recrystallized from methanol to yield **methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (XIV)**: 0.11 g (58%); mp 169–171°; $[\alpha]^{20}D$ +17° (*c* 1.2 in $CHCl_3$); nmr data, $\tau$ 4.25 ($H_1$, $J$ = 8 cps), 8.15 (NAc).

*Anal.* Calcd for $C_{30}H_{35}NO_6$ (505.62): C, 71.26; H, 6.98; N, 2.77. Found: C, 71.41; H, 6.82; N, 2.70.

**Methanolysis of 2-Acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranose (VIII).**—A solution of VIII (0.2 g) in methanol (5 ml) was boiled under reflux for 16 hr at which time thin layer chromatography (ether–benzene, 2:1, v/v) showed the presence of but a trace of starting material along with a slower moving major component. The solution was concentrated and the residue, dissolved in chloroform, adsorbed on a column of silica gel (2 × 15 cm). Unchanged starting material was eluted with ether–benzene (2:1) and the product was then eluted with ether–methanol (1:1); recrystallization from methanol afforded methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside (0.12 g, 71%) which melted at 169–171° either alone or in admixture with the product obtained by the methanolysis of VI.

**Methanolysis of 2-Acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (V).**—A solution of V (0.1 g) in methanol (3 ml) was boiled under reflux and examined at intervals by thin layer chromatography (benzene–ether–methanol, 14:14:1). After 3 hr, no starting material could be detected and the solution was cooled. 2-Acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose crystallized out directly: 0.08 g (87%), mp 221–222°. A mixture melting point with authentic material was undepressed.

**Methanolysis of 2-Acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranose (VII).**—A solution of VII (0.2 g) in methanol (5 ml) was boiled under reflux and examined at intervals by thin layer chromatography using benzene–ether (1:1). After 7 hr only a trace of starting material remained; the solution was concentrated to a residue which was dissolved in chloroform and freed of methyl benzoate by chromatography on silica gel using ether as eluent. The product was crystallized from methanol to yield pure 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose: 0.13 g (79%), mp 218–219°, undepressed on admixture with authentic material.

**Methanolysis of 2-Acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranose (XII).**—A solution of XII (0.2 g) in methanol (4 ml) was boiled under reflux for 5.25 hr. Thin layer chromatography (benzene–methanol, 7:1) showed some starting material to be left; the reaction mixture was left overnight at room temperature, no starting material then being detectable. The methyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-galactopyranoside (XV) which separated from the cooled solution was removed and recrystallized from methanol: 0.1 g, (59%), mp 200–201°, $[\alpha]^{20}D$ +14° (*c* 0.3, $CHCl_3$).

*Anal.* Calcd for $C_{30}H_{35}NO_6$ (505.62): C, 71.26; H, 6.98; N, 2.77. Found: C, 71.07; H, 7.15; N, 2.53.

**Methanolysis of 2-Acetamido-1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (X).**—The ester X (0.17 g) was dissolved in methanol (5 ml) and the solution boiled under reflux for 10 hr. The crystalline product which separated on cooling the solution was recrystallized from methanol to give essentially pure 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (IX): 0.08 g (51%), mp 189–190° alone or in admixture with authentic material.

**Methanolysis of 2-Acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (XI).**—A solution of XI (0.08 g) in methanol (5 ml) was boiled under reflux for 48 hr. Thin layer chromatography, using ether–benzene (2:1), then showed the presence of three components: methyl benzoate, starting material, and 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-galactopyranose (IX). The solution was concentrated, the residue was dissolved in chloroform and the solution was poured on a column of silica gel (2 × 15 cm). Elution with ether–benzene (2:1) gave, first, methyl benzoate and then 2-acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (0.022 g). Elution with ether–methanol (1:1) afforded 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose (IX): 0.035 g (73%, corrected for starting material recovered), mp 185–188° alone or in admixture with authentic IX.

**Methanolysis of 2-Acetamido-1-*O*-benzoyl-2-deoxy-β-D-galactopyranose (XVI).**—A solution of XVI[4] (100 mg) in methanol (10 ml) was boiled under reflux for 30 hr and then concentrated to a volume of *ca.* 2 ml to give a crystalline product (0.03 g) which was acetylated with acetic anhydride and pyridine in the usual manner to yield, from methanol, **methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranoside**: 20 mg (28%), mp 217–218°, $[\alpha]^{20}D$ −16.4° (*c* 0.75, $CHCl_3$). Tarasiejska and Jeanloz[17] reported mp 216–217° and $[\alpha]^{20}D$ −17° ($CHCl_3$) for this substance.

**Hydrolysis of 2-Acetamido-1-*O*-acyl-3,4,6-tri-*O*-benzyl-D-hexopyranoses.**—Each substance ($10^{-5}$ mole) was dissolved in purified dioxane (30 ml), previously heated to 50°, and water (20 ml), also preheated to 50°, was added. The reaction mixture was held at 49.5 ± 0.5° while, at intervals, 5-ml aliquots were removed, diluted with 20 ml of cold water, and titrated with 0.01 *N* sodium hydroxide, using phenolphthalein as an indicator. The titer of sodium hydroxide as a function of time is plotted in Figure 1; it will be noted that 1.00 ml of 0.01 *N* sodium hydroxide is equivalent to 1 molar equiv of benzoic acid liberated.

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# *N*-Acyl Derivatives of 2-Acylamino-2-deoxy-D-glucopyranose[1]

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*N*-Acetylbenzamidocyclohexane, (±)-*trans*-2-(*N*-acetylbenzamido)cyclohexyl benzoate, and (±)-*trans*-2-(*N*-acetylacetamido)cyclohexyl acetate have been prepared and the behavior of the first two has been studied with a variety of reagents. Such diacylamines are readily attacked by nucleophilic agents, losing a single acyl group; where two dissimilar acyl groups are attached to nitrogen a mixture of products often (if not always) results. Replacement of the nitrogen-attached proton in 2-acylamino-2-deoxy-D-glucopyranose derivatives by a second acyl group is comparatively easily accomplished and a variety of *N*-acylacylamino-2-deoxy-D-glucopyranose derivatives have been prepared. Like the cyclohexane derivatives mentioned above, these readily lose one of the nitrogen-attached acyl groups. Isopropenyl acetate in the presence of a trace of *p*-toluenesulfonic acid is shown to be an effective reagent both for *O*-acetylation and for the acetylation of monoacylamines.

In the course of a recent study[3] of the benzoylation of 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-D-glucopyranose and -D-galactopyranose, we have observed that, in addition to normal *O*-acylation, *N*-acylation may take place and we have described the preparation of 2-(*N*-acetylbenzamido)-1-*O*-benzoyl-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranose. Whereas some cyclic *N*-acylacylamino derivatives of the aminosugars such as the phthalimido[4] and succinimido[5] sugars have been investigated, comparatively little attention has been directed toward the acyclic analogs of these substances. Thus, Ohle and Lichtenstein[6] found that benzoylation of 6-amino-6-deoxy-D-glucose afforded a hexabenzoyl derivative while Druey and Huber[7] were able to obtain a hexaacetyl derivative of 1-amino-1-deoxy-D-fructose. More recently, Coxon and Fletcher[8,9] have reported several *N*-acylacylamino derivatives of 1-amino-2,6-anhydro-1-deoxyheptitols. It should be noted that the nitrogen atom in all these examples is attached to an exocyclic carbon atom while the attachment in the hexosamines is to a ring carbon atom. The investigation which we will report here was primarily designed to study the preparation and some of the properties of 2-(*N*-acylacylamino)-2-deoxy-D-glucopyranose derivatives.

Since the pioneering work of Titherley,[10] the problem of the acylation of amides has received only sporadic attention although Rothman and his

(1) Compounds of the type R-N(COR')$_2$ have been variously called diacylamines, acyclic imides, diacylimides, diacylamides, etc.; of these terms, we believe that diacylamine is least confusing. We have chosen to use the prefix *N*-acylacylamino when describing individual compounds since this is in accord with accepted practice although, from the viewpoint of clarity, prefixes such as *N*-acetyl-*N*-benzoylamino might be preferable since nomenclature of this type, although more cumbersome, emphasizes the fact that such compounds are indeed *N*-diacyl derivatives of primary amines. These compounds, incidentally, should not be confused with the *N,N'*-diacyl derivatives produced in the Wohl degradation of acylated nitriles. These substances, containing the -CH(NHCOCH$_3$)$_2$ group, have been widely termed diacetamides.

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