Preparation of 2-Acetamido-2-deoxy-α-glycopyranosides[1]

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The α and the β anomer of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-D-galactopyranoside could be prepared by application of improved conditions developed for production of corresponding glycosides of glucosamine by fusion of glucosamine pentaacetate with phenol; the zinc chloride catalyzed condensation was studied in some detail. *o*-Nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside and its *p*-nitro isomer were prepared from the phenyl glycoside by nitration in acetic anhydride–acetic acid solution; orientation in analogous nitrations was studied briefly. The glycosides produced by O-deacetylation of the acetate esters mentioned were characterized. Some anomalous optical rotation values were observed and these are discussed.

The preparation of aryl β-glycosides of N-acetylglucosamine[2] and N-acetylgalactosamine[3] affords no problem, since these glycosides are available from the corresponding acetochlorohexosamines. On the other hand, synthesis of the corresponding α-glycosides, which were required as enzyme substrates,[4] has given difficulty. Phenyl 2-acetamido-3-4-6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside (1) has been prepared by fusion of phenol with the α- and β-glucosamine pentaacetates in presence of zinc chloride and has been deacetylated to produce phenyl 2-acetamido-2-deoxy-α-D-glucopyranoside (2).[5] Repetitions of this procedure gave pure **1** in 3–11% yield. To improve this preparation, a re-examination of the acid-catalyzed condensation[6] of phenol with the glucosamine pentaacetates[5a,7] was undertaken, with the additional intention of using the reaction as a model for similar glycoside syntheses. Conditions were found which permitted preparation of pure **1** and its β anomer in reasonable yield, and which could be extended readily to preparation of the phenyl glycosides of glactosamine. Attempts to produce *p*-nitrophenyl α-glycosides of hexosamines by this route were unfruitful. It proved possible, however, to prepare *o*- and *p*-nitrophenyl glycosides of glucosamine by nitration of phenyl glycosides.

The preparation[7] of phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (3), by reaction at 100° of phenol with α- and β-glucosamine pentaacetates in presence of *p*-toluenesulfonic acid, was readily improved (47% yield of pure product) by substitution of β-glucosamine pentaacetate and by application of forcing conditions, *i.e.*, vacuum distillation of acetic acid formed in the reaction.[8] Phenyl-2-acetamido-2-deoxy-β-D-glucopyranoside (4), also known previously, was available by O-deacetylation.

A study using vapor phase chromatography (vpc) was made of optimum conditions for preparation of phenyl α-glucosaminide tetraacetate (1) by anomerization of **3** or by condensation of α- and β-glucosamine pentaacetates with phenol in presence of zinc chloride. The results, summarized in part in Table I, did not regularly include analyses for the β-glycoside **3**, since this compound was labile on chromatography. In limited preparative experiments, starting either with **3** or with α- and β-glucosamine pentaacetates, considerable **3** was isolated after 7 hr at 125°, or after 15 min at 150°. After 1 hr at 150°, little **3** could be isolated, and the amount of chloroform-insoluble resinous material present was large. These as well as other results indicated that at high temperatures the anomeric equilibrium favored the α-glycoside **1**. Also, at high temperatures the reaction of the pentaacetates was complete, and β-glycoside was apparently destroyed preferentially. All of these circumstances facilitated isolation of the pure α-glycoside **1**, which could readily be produced preparatively in pure state from α- and β-glucosamine pentaacetates in 41% yield.

TABLE I

ZINC CHLORIDE CATALYZED ANOMERIZATION OF PHENYL 2-ACETAMIDO-3,4,6-TRI-O-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSIDE (**3**)[a]

Starting material	Temp, °C	Time, hr	Compn of mixture, %[b]: α-Glycoside (*1*)	Ac$_5$-α-GN	Ac$_5$-β-GN
β-Glycoside (**3**)	100[c]	4	4		
	100[c]	7	17		
	125[d]	1	36		
	125[d]	2	25		
	150[e]	0.5	48		
	150[e]	1	66		
	150[e]	2	57		
Ac$_5$ α,β-GN[f]	125[d]	1	28	27	5
	125[d]	4	35	12	4
	150[e]	2	56	0	1

[a] Starting material was heated at reduced pressure with phenol and zinc chloride. See text for details. [b] Expressed as mole per cent, based on starting material. [c] Distillation pressure maintained at 30 mm. [d] Pressure, 70 mm. [e] Pressure, 200 mm. [f] 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α,β-D-glucopyranosides, a crystalline mixture containing 72% of α anomer.

Without further modification, the procedure used for preparation of the β-glucosaminide tetraacetate **3** gave phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranoside (5), mp 186.5–187.5°, in 65% yield from β-galactosamine pentaacetate. A compound assigned this structure, but with mp 165°, was reported to result from action of sodium phenolate on acetochlorogalactosamine.[3] Repetition of this preparation and purification of the product gave material identical in properties with **5**, confirming the β-pyranoside structure assigned to this compound. The β-galactosaminide tetraacetate **5** was O-deacetylated to give phenyl 2-acetamido-2-deoxy-β-D-galactopyranoside (6). Anomerization of **5**, under conditions suggested by the reac-

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tion studies already described, readily produced phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranoside (**7**). This compound could also be prepared directly from β-galactosamine pentaacetate by fusion with phenol (zinc chloride catalysis, 43% yield of pure product). O-Deacetylation yielded phenyl 2-acetamido-2-deoxy-α-D-galactopyranoside (**8**). The α-pyranoside structures of **7** and **8** were assigned on the basis of analogy of their methods of preparation to those of the corresponding glucosamine derivatives **1** and **2** and on application of Hudson's rules to optical rotation data shown in Table II. The presumed anomeric relationship between the galactosaminide tetraacetates **7** and **5** and between the N-acetylgalactosaminides (**8** and **6**) is confirmed by agreement of the values of 2*A* for each of these pairs with the corresponding values for glucosamine and glucose derivatives. This is a finding to be expected[9] for compounds having the same substituent and configuration at carbon 2.

TABLE II

MOLAR ROTATION VALUES FOR SOME ARYL GLYCOSIDES[a]

Compound	—$[M]_D \times 10^{-3}$, deg— α anomer	β anomer	$[M]_D(\alpha) - [M]_D(\beta)$, deg ($2A \times 10^{-3}$)
2-Acetamido-2-deoxy-D-glucopyranosides[b]			
Phenyl tri-O-acetyl	+63.6	−5.1	+68.7
o-Nitrophenyl tri-O-acetyl	+13.2	+30.4	−17.2
p-Nitrophenyl tri-O-acetyl	+90.5	−12.3	+102.8
Phenyl	+62.1	−1.7	+63.8
o-Nitrophenyl	+75.0	−11.9	+86.9
p-Nitrophenyl	+93.5	−6.6	+100.1
2-Acetamido-2-deoxy-D-galactopyranasides[b]			
Phenyl tri-O-acetyl	+73.3	−1.0	+74.3
Phenyl	+76.8	+11.1	+65.7
D-Glucopyranosides			
Phenyl tetra-O-acetyl[c]	+71.2	−9.5	+80.7
o-Nitrophenyl tetra-O-acetyl[c]	+78.4	+21.1	+57.3
p-Nitrophenyl tetra-O-acetyl[c]	+93.9	−19.2	+113.1
Phenyl[d]	+46.2	−18.4	+64.6
o-Nitrophenyl[c]	+62.1	−25.0	+87.1
p-Nitrophenyl[c]	+64.8	−31.0	+95.8
D-Galactopyranosides			
o-Nitrophenyl tetra-O-acetyl	+88.2[e]	+32.8[f]	+55.4
p-Nitrophenyl tetra-O-acetyl	+100.7[e]	−3.9[g]	+104.6

[a] Values are calculated from optical rotation measurements at room temperature in this work and in the literature, as indicated. Chloroform was the solvent for acetate esters, water for the other glycosides. [b] This work. [c] E. M. Montgomery, N. K. Richtmyer, and C. S. Hudson, *J. Am. Chem. Soc.*, **64**, 690 (1942). [d] E. Fischer and L. von Mechel, *Ber.*, **49**, 2813 (1916). [e] B. Helferich and K. H. Jung, *Ann. Chem.*, **589**, 77 (1954); **595**, 242 (1955). [f] M. Seidman and K. P. Link, *J. Am. Chem. Soc.*, **72**, 4234 (1950). [g] W. F. Goebel and O. T. Avery, *J. Exptl. Med.*, **50**, 521 (1929).

Attempts to condense *p*-nitrophenol with glucosamine pentaacetate under conditions expected to yield an α-glycoside gave reaction mixtures from which no characterized product could be isolated. Nitrophenyl α-glycosides were regarded, however, as particularly desirable chromogenic enzyme substrates. After some trials, nitration of phenyl α-glucosaminide tetraacetate (**1**) was accomplished without hydrolysis by using acetic anhydride as a reaction solvent; addition of acetic acid as a diluent improved convenience without apparent effect on result.[10] The nitration gave the *o*- and *p*-nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosides (**9** and **10**), separated by fractional crystallization. These were O-deacetylated, yielding the desired *o*- and *p*-nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranosides (**11** and **12**). The *o*- and *p*-nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosides (**13** and **14**), resulting from nitration of **3**, were also separable by fractional crystallization. However, this nitration has limited preparative value, since these β-glycosides are more readily accessible through acetochloroglucosamine.[2] The nitration product from phenyl α-galactosaminide tetraacetate (**5**) was a syrup, so far unresolved; the mixture of nitrophenyl glycosides obtained on O-deacetylation was crystalline, but preparative separation has not been achieved.

Preparative experiments suggested a dependence of the yield of *o*- and *p*-nitro derivatives on the configuration of the sugar moiety. This dependence was investigated by hydrolysis of reaction mixtures obtained from nitrations in acetic anhydride–acetic acid, and estimation of the *o*- and *p*-nitrophenols liberated. For nitration of phenyl α-glucosaminide tetraacetate (**1**) the *ortho* to *para* ratio was 1.4; for its β anomer (**3**), 2.4. For phenyl α-galactosaminide tetraacetate (**7**) the ratio was 1.5; for its β anomer (**5**), 2.5. For phenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside the ratio was 1.5; for its β anomer, 2.1. Nitration in acetic anhydride of anisole, a noncarbohydrate analog, gave *o*- and *p*-nitroanisoles in ratio of 2.5.[11] It would appear that influence of the sugar moiety on the course of nitration is slight for phenyl β-glycosides. For their α anomers, however, steric factors markedly decrease the probability of *ortho* substitution. Speculation seems unfruitful for interpretation of these findings, in absence of a more perfect understanding of the reaction with anisole.[12]

Some interesting features are exhibited by the optical rotation constants for the aryl glycosides of the glucosamines series now known. These constants are summarized as molar rotation values in Table II. A striking anomaly is seen in the case of the *o*-nitrophenyl glucosaminide tetraacetates (**9** and **13**); the β anomer has a higher positive rotation than the α anomer. This phenomenon is undoubtedly related in part to observations made previously on the *o*-nitrophenyl 2,3,4,6-tetra-O-acetyl-D-glucopyranosides and the corresponding galactosides.[13] For each of these acetylated *o*-nitrophenyl glycosides (Table II), the α anomer had a molar rotation reasonably consistent with that for other acetylated aryl glycosides of the same series; the β anomer, however, had a positive rather than a negative value, in contrast to the other β-glycosides. This disparity largely disappeared at higher temperatures

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(9) W. Pigman, Ed., "The Carbohydrates," Academic Press Inc., New York, N. Y., 1957, p 70.

(10) *Cf.* B. Lindberg, *Acta Chem. Scand.*, **2**, 936 (1948).

(11) K. Halvorson and L. Melander, *Arkiv Kemi*, **11**, 77 (1957).

(12) For a review, see P. B. D. DeLaMare and J. H. Ridd, "Aromatic Substitution," Butterworth and Co. (Publishers) Ltd., London, 1959.

(13) (a) W. W. Pigman, *J. Res. Natl. Bur. Std.*, **33**, 129 (1944); (b) J. A. Snyder and K. P. Link, *J. Am. Chem. Soc.*, **75**, 1758 (1953); (c) N. Capon, W. G. Overend, and M. Sobell, *J. Chem. Soc.*, 5172 (1961).

and was abolished by removal of the ester groups. It was inferred that the observed phenomena were a manifestation of interaction between the *o*-nitro group and acetyl groups, in the form of weak bonds between them or of steric interference to free rotation of the phenyl group.[13a] Further investigation showed that the optical rotations of nonesterified *o*-nitrophenyl β-glycosides also had a large temperature coefficient;[13b] also, other acetylated aryl glycosides having bulky *ortho* substituents behave normally.[13c] The hypothesis has been advanced[13c] that in *o*-nitrophenyl β-glycosides, interactions occur between the strongly dipolar nitro group and the dipole of an acetyl or hydroxyl group at carbon 2; these interactions are thought to be in some way responsible for conformational differences reflected in the phenomena observed.

For the acetylated *o*-nitrophenyl glycosides of the glucosamine series, removal of O-acetyl groups also leads to normal rotations. What seems significant, however, is that in this case the polar substituent at carbon 2 remains unchanged. The circumstances suggest that the conformational changes presumed to be involved may be influenced by a steric effect of the O-acetyl groups at carbon atoms 3, 4, and 6, which is superimposed on the dipole–dipole interactions already implicated. Behavior of *o*-nitrophenyl α-glucosaminide tetraacetate (**9**) departs from parallelism with the glucose and galactose series, since the rotation of this α-glycoside is also highly abnormal. It may be hazarded, without attempting detailed rationalization, that the possibilities for strong hydrogen bonding between the nitro and amide groups place the presently reported anomeric pair in a special category. Other than those discussed, molar rotation values in the glucosamine series parallel those in the glucose series (Table II).

Experimental Section[14]

Anomerization in phenol solution and condensation with phenol were carried out in vacuum distillation equipment of appropriate size, arranged to have short, unconstricted vapor path; the distillation pressure was adjusted to ensure distillation of 10–20% of the phenol during the reaction interval.

For O-deacetylation,[15] the glycoside acetate ester (0.01 mole) was dissolved or suspended in 25 ml of chloroform, and the mixture was boiled in a flask on a hot plate until about 5 ml of solvent and any water present had been lost. Methanol (20 ml, magnesium dried) was added, and, when the mixture boiled again, 0.1 ml of 2 *M* sodium methoxide was added. After boiling for 3 min longer, the mixture was allowed to stand (stoppered) for several hours at room temperature. One drop of acetic acid was added, and the reaction mixture was evaporated. In some cases, the product had already crystallized, and evaporation could be omitted.

Chromatographic Methods.—The vpc of carbohydrate esters on columns of QF-1 (1%, coated on Gas-Chrom P, 120–140 mesh; supplied by Applied Science Laboratories, State College, Pa.), as described briefly by VandenHeuvel and Horning,[16] was extended. Glass columns, 150 × 0.5 cm, were operated at 195°, with argon as the carrier, at a flow rate of 200 ml/min. Retention times of the compounds of interest (2–10 μg) relative to hexaacetyl D-sorbitol (1.00, 6.7 min), follow: α-glucosamine pentaacetate, 3.19; β-glucosamine pentaacetate, 0.45; phenyl α-glucosaminide tetraacetate (**1**), 4.87. Phenyl β-glucosaminide tetraacetate (**3**) apparently decomposed on this column and was not recovered. All elution curves exhibited trailing. Quantitative analysis of mixtures was possible, however, by calibration of peak heights with mixtures of known concentrations, relative to the peak height for phenyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (relative retention time, 2.24); this compound was used throughout as an internal standard, at fixed concentration. The remarkable selectivity of this column, already indicated,[16] was further illustrated by some other relative retention times observed: phenyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside, 1.81; β-galactosamine pentaacetate, 0.47; β-mannosamine pentaacetate, 2.56; α-galactose pentaacetate, 0.88; β-galactose pentaacetate, 0.73; methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside, 1.43; methyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside, 1.91.

For estimation of glycosidically linked *o*-nitrophenol and *p*-nitrophenol, small samples of compounds or mixtures were hydrolyzed in 0.1 *M* hydrochloric acid, in sealed tubes heated at 150° for 2 hr. After addition of excess sodium carbonate solution, aliquots of the hydrolysates were chromatographed on Whatman No. 1 paper, using 1 *M* sodium bicarbonate as a developer (*o*-nitrophenol, R_F 0.63; *p*-nitrophenol, R_F 0.49). Spots were extracted from the paper with 0.1 *M* sodium bicarbonate, and the nitrophenols in the extracts were determined spectrophotometrically, using as standards known amounts of *o*- and *p*-nitrophenol, chromatographed identically. In the experiments on orientation in nitration of acetylated phenyl glycosides, reaction mixtures were treated with water and chloroform. After the chloroform extracts were washed with excess sodium carbonate solution and water, they were concentrated, and the residues were used for hydrolysis and chromatography.

α- and β-Glucosamine pentaacetates (2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α,β-D-glucopyranose) were obtained as a solid mixture by crystallization of the syrup[17] from chloroform–ether; the optical rotation of the solid preparation, $[\alpha]^{22}\mathrm{D}$ +63.6° (*c* 2, chloroform), indicated[18] a 72% content of the α anomer.

β-Galactosamine pentaacetate (2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-galactopyranose) crystallized when 10 g of β-galactosamine hydrochloride was stirred for 6 hr at 25° and for an additional 24 hr at 4° with a solution of 45 ml of acetic anhydride in 70 ml of dry pyridine. Crystallization was completed by dilution with water, and the solid was washed with water and ethanol. In three runs there were obtained 87–90% yields of pure β-pentaacetate,[19] $[\alpha]^{23}\mathrm{D}$ +10.0° (*c* 0.5, chloroform) [lit.[18] $[\alpha]^{20}\mathrm{D}$ +10.5° (chloroform)].

Phenyl 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside (1). A. Reaction Studies.—A small vacuum distillation apparatus was charged with 1.00 g of phenol, 0.13 g of zinc chloride (in solution), and 0.50 g of phenyl β-glucosaminide tetraacetate (**3**) or of α- and β-glucosamine pentaacetates. After heating in an oil bath under reduced pressure with frequent shaking, the reaction mixture was dissolved in chloroform. The solution was washed with 10% sodium sulfate, cold 2 *M* sodium hydroxide, and sodium sulfate solution. The washed extract was diluted to measured volume and dried, and an aliquot was analyzed by vpc. Selected reaction conditions and results are shown in Table I.

B. Preparative Experiment.—A mixture of 260 g of molten phenol, 130 g of α- and β-glucosamine pentaacetates, and 32.5 g of zinc chloride (in solution) contained in a vacuum distillation apparatus, was heated by an oil bath. While heating for 1 hr at 125° and 70 mm with frequent shaking, considerable distilla-

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(14) Melting points are corrected. Optical rotation was measured in a 0.35-dm tube with an EPL-NPL automatic polarimeter (Bendix Corp., Cincinnati, Ohio), using sucrose for calibration; the estimated precision corresponded to $[\alpha]\mathrm{D}$ ±0.5°. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Nitric acid (90%, fuming) was catalog item 2713 of Mallinckrodt Chemical Works. Phenol was redistilled before use. Pyridine was dried over barium oxide. In all cases where zinc chloride was used, it was added as a 25% solution in acetic acid containing 5% of acetic anhydride. Preparation by Dr. David Dennen of β-galactosamine hydrochloride from purified bovine cartilage chondroitin sulfate, instruction by Mr. William Lehnhardt in use of gas chromatography equipment, and assistance of Miss Dorotea Friederici in some of the enzyme experiments are gratefully acknowledged.

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(18) C. S. Hudson and J. K. Dale, *J. Am. Chem. Soc.*, **38**, 1431 (1916).

(19) *Cf.*, however, M. Stacey, *J. Chem. Soc.*, 222 (1944); P. J. Stoffyn and R. W. Jeanloz, *J. Am. Chem. Soc.*, **76**, 561 (1954); Z. Tarasiejka and R. W. Jeanloz, *ibid.*, **80**, 6325 (1958). These investigators reported varying proportions of α- and β-pentaacetates from seemingly similar acetylations. Consistently used in present experiments was the β-hydrochloride, *e.g.*, $[\alpha]^{23}\mathrm{D}$ +45.5 → +95.7° (*c* 0.5, water).

tion occurred; heating at 125° was continued for an additional hour at 90 mm. The bath temperature was raised rapidly and the mixture was heated for 1 hr at 150° and 240 mm. After cooling, the dark residue (351 g) was dissolved in 2 l. of chloroform and washed with 3 l. of 10% sodium sulfate solution; much insoluble, resinous material separated, but did not hinder the operation. The extract was washed with excess cold 2 *M* sodium hydroxide and with sodium sulfate solution, and it was dried. Passage through a pad of chromatographic silicic acid removed the turbidity present, as well as some color. Removal of chloroform at reduced pressure was repeated after addition of 2-propanol to the residue. Crystallization from 2-propanol gave 28 g of solid, mp 119–120°, and a second crop (after addition of pentane), 50 g, mp 114–116°. Two recrystallizations from 2-propanol gave 58 g (41%) of the tetraacetyl α-glycoside **1**, mp 119.5–120°, $[\alpha]^{23}$D +149.9° (*c* 0.68, chloroform), unchanged by successive recrystallizations from 2-propanol, benzene, and ethyl acetate–cyclohexane [lit.[5a] mp 122–123°, $[\alpha]^{25}$D +138° (chloroform)].

O-Deacetylation of the tetraacetate **1** and recrystallization of the product from methanol gave the α-glycoside **2** in 67% yield, mp 248° dec, $[\alpha]^{23}$D +208.6° (*c* 0.51 water), unchanged by three recrystallizations from methanol [lit. $[\alpha]$D +203° (water),[5b] +213° (water),[5a] +210° (water)[20]].

Phenyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (4).—A mixture of 40 g of molten phenol, 0.6 g of *p*-toluenesulfonic acid monohydrate, and 30 g of β-glucosamine pentaacetate[21] was heated at 100° for 40 min with frequent shaking in a vacuum distillation apparatus maintained at a pressure of 25 mm. The reaction mixture was dissolved in chloroform, washed with excess cold 2 *M* sodium hydroxide and with water, and dried. The turbid extract was clarified with silicic acid. Evaporation left a crystalline residue. Two recrystallizations from 2-propanol gave the β-glycoside tetraacetate **3**, 15.5 g (47%), mp 204.5–205°, transition at 187°, $[\alpha]^{23}$D −12.0° (*c* 0.70, chloroform), −15.8° (*c* 0.58, acetone), unchanged by successive recrystallizations from 2-propanol, benzene, and ethyl acetate [lit. mp 201.5–205°, transition at 185°, $[\alpha]^{18}$D −10° (chloroform);[7] mp 204–205°, $[\alpha]^{25}$D − 21.0° (chloroform);[5a] mp 204°, $[\alpha]^{18}$D −14.5° (acetone)[2]]. Substitution of α- and β-glucosamine pentaacetates in the procedure as described decreased the yield to 21%.

O-Deacetylation and crystallization from methanol gave the β-glycoside **4**, mp 250° dec, $[\alpha]^{23}$D −5.5° (*c* 0.50, water), unchanged by two recrystallizations from methanol [lit. $[\alpha]$D −5.4° (water),[7] −10.3° (water),[5a] −8.4° (water),[2] −3° (water)[20]].

Phenyl 2-Acetamido-2-deoxy-β-D-galactopyranoside (6).—Phenol (15 g), *p*-toluenesulfonic acid monohydrate (0.25 g), and β-galactosamine pentaacetate (10 g) were allowed to react for 40 min at 100° and 25 mm, and the reaction mixture was worked up as described for the preceeding preparation. Evaporation of the chloroform extract left a syrup. This crystallized readily from ethyl acetate, yielding the β-glycoside tetraacetate **5**, 6.1 g, mp 186.5–187.5°, $[\alpha]^{20}$D −2.3° (*c* 4, chloroform), unchanged on recrystallization from 2-propanol. Concentration of the filtrate and addition of isopropyl ether gave additional material, pure after recrystallization (0.9 g, total yield 65%).

Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.7; H, 5.95; N, 3.31. Found: C, 56.4; H, 6.05; N, 3.26.

A compound of structure **5**, but with mp 165° (no optical rotation or analysis given), has been reported[8] to result from reaction of acetochlorogalactosamine with sodium phenolate. Repetition of the reported procedure, and purification of the product gave a preparation of **5** (25% yield) identical (melting point and mixture melting point) with the one now described.

On O-deacetylation of **5**, the product crystallized in pure state from the hot reaction mixture. After cooling and acidification of the mixture, the solid was collected and washed with chloroform–methanol, ethanol, and ether. The β-glycoside **6** (87% yield) had mp 237° dec, $[\alpha]^{23}$D +37.3° (*c* 0.57, water), unchanged on recrystallization from methanol.

Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.44; N, 4.71. Found: C, 56.6; H, 6.39; N, 4.85.

Phenyl 2-Acetamido-2-deoxy-α-D-galactopyranoside (8). A. From 5.—A mixture of 6.5 g of phenyl β-galactosaminide tetraacetate (**5**), 13 g of phenol, and 1.6 g of zinc chloride (in solution) was heated at 150° for 1 hr with frequent shaking in a distillation apparatus maintained at a pressure of 240 mm. Considerable resinous material separated when a chloroform solution of the melt was shaken with 10% sodium sulfate solution. Washing of the extract with cold 2 *M* sodium hydroxide and sodium sulfate solution, drying, clarification with silicic acid, and evaporation gave an amber syrup. This crystallized readily from ethyl acetate–isopropyl ether–pentane. Recrystallization from 2-propanol gave the α-glycoside tetraacetate **7**, 3.0 g (47%), mp 130–131°, $[\alpha]^{23}$D +173.1° (*c* 0.50, chloroform), unchanged on two recrystallizations from ethyl acetate–isopropyl ether.

Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.7; H, 5.95; N, 3.31. Found: C, 56.9; H, 5.99; N, 3.22.

On cooling of the reaction mixture following deacetylation of **7**, the product crystallized in part. Crystallization was completed by addition of 1 drop of acetic acid and dilution with ether. The solid was collected, washed with absolute ethanol and ether, and recrystallized from absolute ethanol giving the α-glycoside **8** (85% yield), mp 248° dec, $[\alpha]^{23}$D +258.3° (*c* 0.41, water), unchanged on recrystallization from absolute ethanol.

Anal. Calcd for $C_{14}H_{19}NO_6$: C, 56.6; H, 6.44; N, 4.71. Found: C, 56.7; H, 6.43; N, 4.59.

B. From β-Galactosamine Pentaacetate.—Reaction of 16.7 g of β-galactosamine pentaacetate, 32 g of phenol, and 4 g of zinc chloride (in solution) for 2 hr at 125° and 70 mm, and for 1 hr at 150° and 240 mm, followed by work-up as before, gave a syrup. This was crystallized from ethyl acetate–cyclohexane. Pure α-glycoside tetraacetate (**7**), 7.4 g (43%), identical with that already described (melting point, mixture melting point, and rotation), was obtained by recrystallization from 2-propanol.

Nitration of Phenyl α-D-Glucosaminide Tetraacetate (1).—A nitrating mixture was prepared by slow addition of 5 ml of 90% nitric acid to 16 ml of acetic anhydride with stirring and ice cooling. To the mixture was added at one time a solution of 21.2 g of the glycoside tetraacetate in 50 ml of acetic acid. The resulting solution was stored at 38° for 2 hr, then poured on ice (100 g). Sodium carbonate (2 *M*, 25 ml) was added. The oil which separated initially crystallized after stirring for several hours. After further dilution with water, chilling, and stirring, the solid (21 g, mp 155–172°) was collected. When a solution of the material in 240 ml of warm ethyl acetate–isopropyl ether (1:1) was allowed to cool undisturbed, 5.9 g of needles, mp 170–173°, separated. The filtrate from collection of this fraction, in the course of 2 days, deposited 3.8 g of hard, compact, crystalline aggregates, mp 186–195°. Systematic fractional crystallization from ethyl acetate–isopropyl ether and 2-propanol of these two materials and of materials recovered from the mother liquors eventually yielded two pure compounds.

The lower melting compound, which was rather soluble, but which crystallized rapidly, was shown by hydrolysis and paper chromatography to be a glycoside of *o*-nitrophenol. The total yield of pure *o*-nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside (**9**) was 8.6 g (36%). The compound had mp 173.5–174.5°, $[\alpha]^{23}$D +28.2° (*c* 0.49, chloroform), unchanged on recrystallization from ethyl acetate–isopropyl ether.

Anal. Calcd for $C_{20}H_{24}N_2O_{11}$: C, 51.3; H, 5.16; N, 5.98. Found: C, 51.3; H, 5.35; N, 5.97.

On hydrolysis of **9** with 1 *M* hydrochloric acid at 100°, crystals of *o*-nitrophenol, mp and mmp 45–45.5°, sublimed from the reaction mixture.

The higher melting component of the nitration product, which was less soluble than its isomer, but which crystallized slowly, was shown by hydrolysis and paper chromatography to be a glycoside of *p*-nitrophenol. The total yield of pure *p*-nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranoside (**10**) was 5.8 g (25%). The compound had mp 197.5–198.5°, $[\alpha]^{23}$D +192.8° (*c* 0.64, chloroform), unchanged on recrystallization from ethyl acetate–isopropyl ether.

Anal. Calcd for $C_{20}H_{24}N_2O_{11}$: C, 51.3; H, 5.16; N, 5.98. Found: C, 51.0; H, 5.19; N, 6.09.

After hydrolysis of **10** for 6 hr with 1 *M* hydrochloric acid at 100°, *p*-nitrophenol, mp and mmp 113–114°, was isolated from the reaction mixture by extraction with benzene and crystallization from benzene–cyclohexane.

***o*-Nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside (11)** was produced from its acetate ester (**9**). After recrystallizations from methanol and from ethanol the product was pure (70% yield). It had mp 208–208.5°, $[\alpha]^{23}$D +219.0° (*c* 0.48, water), unchanged by two recrystallizations from ethanol.

Anal. Calcd for $C_{14}H_{18}N_2O_8$: C, 49.1; H, 5.30; N, 8.19. Found: C, 48.9; H, 5.39; N, 8.07.

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***p*-Nitrophenyl 2-acetamido-2-deoxy-α-D-glucopyranoside (12)** crystallized in part (2.7 g) in pure state during O-deacetylation of 5.2 g of its acetate ester (**10**). Recrystallization from methanol of material recovered from the filtrate yielded additional pure α-glycoside (0.8 g, total yield 91%). The compound had mp 274° dec, $[\alpha]^{23}_{\mathrm{D}}$ +273.4° (*c* 0.67, water), unchanged by recrystallization from methanol.

Anal. Calcd for $C_{14}H_{18}N_2O_8$: C, 49.1; H, 5.30; N, 8.19. Found: C, 49.4; H, 5.25; N, 8.15.

Optical Rotations of Nitrophenyl β-Glucosaminides.—These compounds, prepared as described by Leaback and Walker,[2] were purified with some care. *o*-Nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**13**) had mp 191–191.5°, $[\alpha]^{23}_{\mathrm{D}}$ +64.8° (*c* 0.55, chloroform), +2.8° (*c* 0.65, acetone), unchanged by successive recrystallizations from 2-propanol, benzene, and ethyl acetate [lit.[2] mp 196–197°, $[\alpha]^{23}_{\mathrm{D}}$ +3.4° (acetone)]. *o*-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside had mp 189–190° dec, $[\alpha]^{23}_{\mathrm{D}}$ −34.7° (*c* 0.52, water), unchanged by two recrystallizations from methanol [lit.[2] mp 192–194°, $[\alpha]^{20}_{\mathrm{D}}$ −33.1° (water)]. *p*-Nitrophenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (**14**) had mp 241° dec, $[\alpha]^{23}_{\mathrm{D}}$ −26.3° (*c* 0.55, chloroform), −44.2° (*c* 0.53, pyridine), −23.6° (*c* 0.57, acetone), unchanged by three recrystallizations from chloroform–methanol [lit. $[\alpha]^{18}_{\mathrm{D}}$ −46.8° (pyridine),[15] $[\alpha]^{18}_{\mathrm{D}}$ −46.2° (acetone),[2] $[\alpha]^{20}_{\mathrm{D}}$ −40° (acetone)[20]]. *p*-Nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside had mp 215° dec, $[\alpha]^{23}_{\mathrm{D}}$ −19.4° (*c* 0.31, water), unchanged by recrystallization from methanol [lit. $[\alpha]^{20}_{\mathrm{D}}$ −18.6° (water),[2] −15° (water)[22]].

Anomeric Purity of α-Glycosides.—To test for possible contamination with their β anomers of the α-glycosides prepared in this work, these were subjected to an enzymic test. Solutions of the glycosides in 0.05 *M* sodium citrate buffer of pH 4.3 were incubated for 1 hr at 37° with purified liver β-N-acetylglucosaminidase, present in sufficient quantity per milliliter of digest to liberate 1000 μg of *p*-nitrophenol from *p*-nitrophenyl β-N-acetylglucosaminide.[23] No liberation of aglycon above nonenzymic control levels was detectible with phenyl α-N-acetylglucosaminide (**2**) at 10 m*M* concentration; phenyl α-N-acetylgalactosaminide (**4**), 10 m*M*; *o*-nitrophenyl α-N-acetylglucosaminide (**11**), 5 m*M*; *p*-nitrophenyl α-N-acetylglucosaminide (**12**), 1 m*M*. When each digest was supplemented with an amount of the corresponding β-glycoside equivalent to 0.5% of the α-glycoside present, enzymic liberation of aglycon was demonstrable.

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The Stereospecific Synthesis of *cis* and *trans* Isomers of Glycidic Esters and Products of the Darzens Synthesis

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The synthesis of *cis* and *trans* isomers of ethyl 2,3-diphenylglycidate and of ethyl 2-methyl-3-phenylglycidate, and *trans* isomers of ethyl and *t*-butyl phenylglycidates by direct epoxidation of the corresponding α,β-unsaturated esters with *m*-chloroperbenzoic acid is described. By comparison of the nmr spectra of the pure isomers with the spectra of products of the Darzens synthesis, the Darzens method is shown to produce both isomers. It is shown also that the Darzens synthesis employing potassium *t*-butoxide in *t*-butyl alcohol results in considerable transesterification.

We have been interested in the question of whether the Darzens synthesis of glycidic esters[1] does not in fact yield both geometrical isomers when their existence is possible.[2] One aspect of the problem involved the synthesis of *cis* and *trans* isomers of several glycidic esters as reference compounds, and we have thus been searching for facile procedures for their preparation.

Various conditions for carrying out epoxidations are described in the literature. Epoxidation of alkenes bearing carbonyl substituents is generally regarded as more difficult than epoxidation of isolated ethylenic functions,[3] but examples of direct epoxidation of α,β-unsaturated carbonyl compounds have appeared.[3–6] Additionally, a method for interconverting geometrical isomers of glycidic esters has been described.[7] Epoxidation with organic peracids is evidently stereospecific.[6]

We have found that direct epoxidation of α,β-unsaturated esters with *m*-chloroperbenzoic acid[8] is satisfactory. By refluxing a solution of an unsaturated ester in methylene chloride with a small excess of the peracid for varying lengths of time, good yields of six glycidic esters (compounds **1–5** and **7**) have been obtained. That all of these compounds are pure stereoisomers is confirmed by their proton magnetic resonance spectra. Table I shows the important features of the nmr spectra of seven glycidic esters and of six corresponding cinnamates.

The spectra were readily correlated with structures on the basis of the known configurations[9] of the ethyl α,β-diphenylglycidates, **1** and **2**, and by noting that chemical shifts of certain proton resonances in the several isomers agree with predictions.[10] All of the compounds prepared carry β-phenyl groups. The effect of this structural feature is to shift the resonance frequency of the ethoxyl protons toward lower values of δ (parts per million) when the phenyl group is moved from a *trans* to a *cis* position relative to the ester group. Models show that the ethoxyl protons are located, part

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