274° (lit.[4] mp 272–274°); identical by ir, nmr, and tlc with a sample prepared by reduction of ethyl 5-benzyloxy-6-azaindole-2-carboxylate.[4]

Benzyl 5-Oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-2-carboxylate (39).—The 5-carbethoxylactam **38** (2 g) was dissolved in 20 ml of benzyl alcohol and 50 mg of sodium was added. The mixture was heated at 100° for 2 hr; 5 ml of the solvent was then distilled *in vacuo* at the same temperature. An equal volume of benzyl alcohol was added to the mixture and the heating was continued for an additional 4 hr. The benzyl alcohol was then evaporated to dryness *in vacuo* and the residue crystallized from a large volume of ethanol: 2.2 g (80%); mp 286–287°; ir 1690 (CO ester), 1667 (CO lactam), 695 cm^{-1} (C_6H_5).

Anal. Calcd for $C_{15}H_{14}O_3N_2$: C, 66.7; H, 5.2; N, 10.4. Found: C, 66.6; H, 5.2; N, 10.3.

5-Oxo-4,5,6,7-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-2-carboxylic Acid (40).—The benzyl ester lactam **39** (1 g) was dissolved in 50 ml of glacial acetic acid and hydrogenated at 50 psi for 2 hr over 300 mg of 10% palladium on charcoal. The catalyst was filtered, the solution evaporated to dryness *in vacuo* at 50°, and the residue crystallized by dissolving in a 1 *N* sodium hydroxide solution and precipitating with concentrated acetic acid: 530 mg (80%); mp dec above 300°; R_f 0.58 (tlc, on cellulose); uv_{max} 270 nm (ε 15,000).

Anal. Calcd for $C_8H_8O_3N_2$: C, 58.88; H, 4.44; N, 15.55. The product was identical by tlc, ir, and uv with a sample prepared by reduction of 5-oxo-5,6-dihydro-1*H*-pyrrolo[2,3-*c*]-pyridine-2-carboxylic acid.[4]

Diethyl 5-Oxo-3a,4,5,6-tetrahydro-1*H*-pyrrolo[2,3-*c*]pyridine-2,3-dicarboxylate (43).—The 6-azaindanone **28** (300 mg) was dissolved in 20 ml of ethanol and was reduced with hydrogen over 100 mg of 10% palladium on charcoal at 10 psi for 90 min. The catalyst was filtered, the solvent was evaporated to dryness *in vacuo*, and the residue was crystallized from ethanol: 130 mg (43%); mp 216–218°; ir 1635, 1610 cm^{-1} (CO lactam); nmr (TFA) δ 1.35 (t, 6, CH_3), 3.6 (m, 2, CH_2CO), 6.4 (q, 4, CH_2CH_3), 5.25 (m, 1, $CHCH_2CO$), 7.1 (s, 1, H_7); mass spectrum *m/e* rel intensity) 280 (M^+, 90), 235 (M − OC_2H_5, 20), 207 (M − $COOC_2H_5$, base peak), 163 (207 − OC_2H_4, 30), 135 (163 − CO, 90), 107 (135 − CO, 80).

Anal. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.7; H, 5.7; N, 10.0. Found: C, 55.6; H, 5.7; N, 10.2.

The same product was obtained by reducing diethyl 5-benzyloxy-6-azaindole-2,3-dicarboxylate **25** at 50 psi for 2 hr under the described conditions.

Registry No.—2 potassium salt, 38312-68-4; **4**, 38312-69-5; **5**, 38312-70-8; **6**, 38312-71-9; **7**, 38312-72-0; **8**, 38312-73-1; **9**, 38312-74-2; **10**, 38312-75-3; **11**, 38312-76-4; **12**, 38312-77-5; **13**, 38312-78-6; **14**, 38312-79-7; **15**, 38312-80-0; **16**, 38312-81-1; **17**, 38312-82-2; **18**, 38309-19-2; **19**, 38309-20-5; **21**, 38309-21-6; **22**, 38309-22-7; **23**, 38309-23-8; **24**, 38309-24-9; **25**, 38309-25-0; **26**, 38309-26-1; **27**, 38309-27-2; **28**, 38309-28-3; **29**, 38309-29-4; **30**, 38309-30-7; **31**, 33034-45-6; **32**, 38309-32-9; **33**, 32794-21-1; **34**, 38309-34-1; **35**, 32794-17-5; **36**, 38309-36-3; **38**, 22772-51-6; **39**, 38309-38-5; **40**, 32794-19-7; **43**, 38309-40-9; 2-chloro-4-methyl-5-nitropyridine, 23056-33-9.

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Synthesis of Oligosaccharides Containing 2-Acetamido-2-deoxyxylose by Chemical and Enzymic Methods[1a]

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O-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxy-D-xylopyranose (**1**) was prepared from the β(1→4)-linked *N*-acetylglucosamine dimer (**2**) by formation of the diethyl dithioacetal (**3**), glycol cleavage with periodate, reduction with ^3H-$NaBH_4$, and dithioacetal hydrolysis. **1** was isolated by charcoal–Celite column chromatography. A by-product, *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxy-L-arabinopyranose, was isolated as well. **1** was also isolated from the lysozyme-catalyzed reaction of the *N*-acetylglucosamine tetramer with 5-^3H-2-acetamido-2-deoxy-α-D-xylopyranose (**10**), demonstrating the structure of **1** and supporting a β(1→4) linkage for the higher oligomers containing *N*-acetylxylosamine and two or three *N*-acetylglucosamine residues, which were also produced in the enzymic reaction.

In the past six years, more and more evidence has accumulated for the fascinating, but by no means new,[2] theory that the structure of an enzyme active site is "designed" to fit a conformation of the substrate close to the reaction transition state better than it fits the substrate's ground-state conformation.[3] The synthesis of organic molecules designed to test this theory is a challenging task for the chemist.

In the particular case of lysozyme, Phillips has proposed, on the basis of crystallographic studies of the hen egg white enzyme, that the catalytic region of the active site ("subsite D") can bind an *N*-acetylglucosamine residue in the "half chair" conformation, but cannot bind such a hexopyranose unit in its ground-state "chair" conformation because of steric hindrance to the hydroxymethyl group at C-5 in the latter conformation.[4] The preparation of substrate analogs containing *N*-acetylxylosamine (2-acetamido-2-deoxy-D-xylose), *i.e.*, in which a single C-5 hydroxymethyl group has been removed from an *N*-acetylglucosamine oligomer, would obviously be valuable in the further testing of Phillips' hypothesis. We have briefly reported elsewhere studies of such compounds which support this hypothesis.[5] In this paper we report the

(1) (a) Taken in part from the Ph.D. Thesis of P. v. E. (MIT, 1971), who thanks the A. D. Little Company for a fellowship; and the M.S. Thesis of W. A. W. (MIT, 1970), who thanks the NSF for a traineeship. Research support from the U. S. National Institutes of Health (Grant AM-13590) and the Merck Co. Foundation are gratefully acknowledged. (b) Department of Biology, University of the Negev, Beer Sheva, Israel.

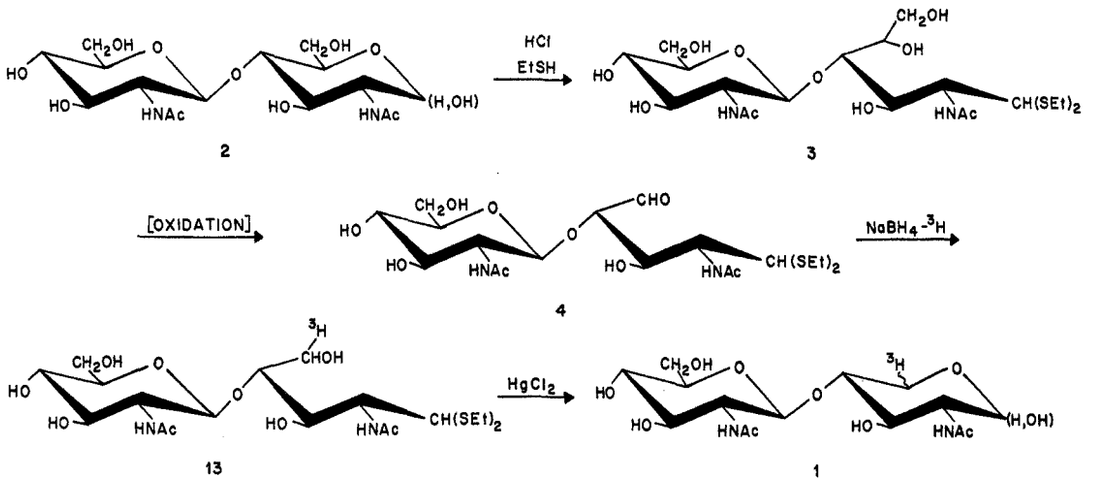
(2) J. H. Quastel, *Biochem. J.*, **20**, 166 (1926); L. Pauling, *Chem. Eng. News*, **24**, 1375 (1946).

(3) For recent discussions, see D. M. Blow and T. A. Steitz, *Ann. Rev. Biochem.*, **39**, 63 (1970); R. Wolfenden, *Accounts Chem. Res.*, **5**, 10 (1972).

(4) C. C. F. Blake, L. N. Johnson, G. A. Mair, A. C. T. North, D. C. Phillips, and V. R. Sarma, *Proc. Roy. Soc., Ser. B*, **167**, 378 (1967); L. N. Johnson, D. C. Phillips, and J. A. Rupley, *Brookhaven Symp. Biol.*, **21**, 120 (1968).

(5) P. van Eikeren and D. M. Chipman, *J. Amer. Chem. Soc.*, **94**, 4788 (1972).

SCHEME I



details of the synthesis of *N*-acetylglucosamine-β-(1→4)-*N*-acetylxylosamine (**1**, *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-xylopyranose), and some related compounds, by both chemical and enzymic techniques.

The standard technique for the synthesis of oligosaccharides is, of course, to form new glycosidic bonds between the monosaccharide moieties in question, usually by the Koenigs–Knorr condensation.[6] Since xylosamine itself apparently does not occur naturally, and is not readily available synthetically,[7] and since a facile route to a xylosamine derivative protected everywhere but O-4 is not obvious, such an approach appeared unattractive to us. We chose instead to attempt the synthesis of **1** from the readily available *N*-acetylglucosamine dimer (**2**) by the route outlined in Scheme I. Although degradative routes to oligosaccharides have been fairly widely used in the past to produce oligosaccharides with glycosidic linkages of known configuration,[8] to our knowledge this is the first example of such a degradation which does not involve loss of the reducing terminal carbon.

A third alternative for preparing the desired oligosaccharides is to use an enzyme to form one or more new glycosidic linkages between saccharides. Lysozyme is known to catalyze transglycosylation reactions of chitin oligomers[9] and bacterial cell wall oligosaccharides,[10] and to demonstrate considerable specificity toward acceptors,[11] but this specificity is not absolute. For instance, Sharon and Pollock showed that the products of the incubation of the bacterial cell wall tetrasaccharide and D-xylose with lysozyme included compounds with β(1→2), β(1→3), and β(1→4) linkages to xylose.[12] Use of the enzymic route with an acceptor other than *N*-acetylglucosamine or an oligomer thereof thus requires a proof of the structure of the new linkage. The synthesis of **1** by both the degradative chemical route and the enzymic route, and the demonstration of the identity of the two products, provides a rigorous proof of the structure of **1**, as well as information which is useful for the preparation of further compounds in the series.

All the compounds that we report were synthesized with radiochemical labels. While the original reason for introducing tritium into these compounds was to make possible certain biochemical experiments, the labels turned out to be extremely useful for following the reactions and supporting the structures proposed for the products.

Results and Discussion

The diethyl dithioacetal **3** was produced from **2**[13] by the usual method with concentrated hydrochloric acid and ethanethiol.[14] No evidence for deacetylation was observed, and cleavage of the glycosidic bond occurred to a very small extent.

The crucial and most difficult step in the synthesis is the specific glycol cleavage of **3** to **4**, since **3** contains both acyclic and trans-diequatorial vicinal glycols, as well as the readily oxidizable sulfur atoms.[15] Following Wolfrom's synthesis of 2-amino-2-deoxyxylose,[7] we examined the reaction of **3** with lead tetraacetate. Immediate reduction, hydrolysis, trimethylsilylation, and vpc analysis of the reaction mixture indicated that apparently exclusive cleavage of the trans-diequatorial glycol had occurred. Examination of the reaction with periodate by the same method indicated that the glycol cleavage was exclusively at the open chain, but other evidence suggested that extensive oxidation of sulfur was taking place as well. Since no direct method (tlc, spectra) could be found for following the reaction, indirect methods were used to determine the optimum conditions for the oxidation. The disappearance of periodate was followed spectrophotometrically by the method of Dixon and Lipkin.[16] With a 50% excess of periodate at 0°, 1 mol of periodate per mole of **3** was consumed in about 2 min, and consumption of the remaining reagent occurred with a half-life of about 7

(6) W. Koenigs and E. Knorr, *Ber.*, **34**, 957 (1901).
(7) M. L. Wolfrom and M. W. Winkley, *J. Org. Chem.*, **31**, 1169 (1966).
(8) J. Staněk, M. Černý, and J. Pacák, "The Oligosaccharides," Academic Press, New York, N. Y., 1965, pp 95–100.
(9) N. A. Kravchenko and V. I. Maksimov, *Izv. Akad. Nauk SSR, Ser. Khim.*, 584 (1964).
(10) N. Sharon and S. Seifter, *J. Biol. Chem.*, **239**, 2398 (1964).
(11) J. J. Pollock, D. M. Chipman, and N. Sharon, *Biochem. Biophys. Res. Commun.*, **28**, 779 (1967); J. J. Pollock, Ph.D. Thesis, Weizmann Institute of Science, Rehovot, Israel, 1969.
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(13) J. A. Rupley, *Biochim. Biophys. Acta*, **83**, 245 (1964).
(14) J. Staněk, M. Černý, J. Kocourek, and J. Pacák, "The Monosaccharides," Academic Press, New York, N. Y., 1963, pp 589–605.
(15) D. Horton and D. M. Hutson, *Advan. Carbohyd. Chem.*, **18**, 123 (1963), contains a discussion of thioacetal sulfur oxidation.
(16) J. S. Dixon and D. Lipkin, *Anal. Chem.*, **26**, 1092 (1954).

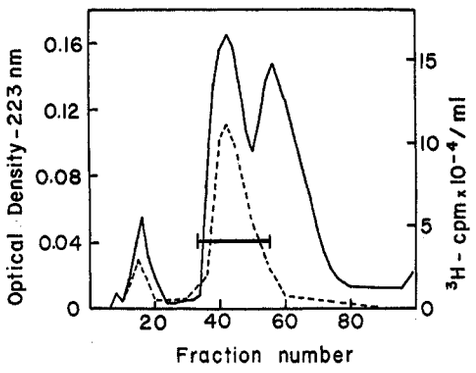


Figure 1.—First chromatography of crude 1 on charcoal–Celite column (1 × 43 cm, eluted with 0–20% ethanol gradient over 2 l.): solid line, optical density; broken line, radioactivity. Fractions marked by bar pooled for further resolution.

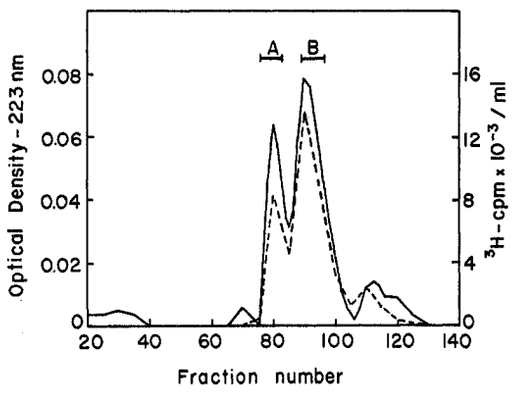


Figure 2.—Chromatographic resolution of 1 and 5 (1 × 58 cm charcoal–Celite column, eluted with 1–10% ethanol gradient over 2 l.): solid line, optical density; broken line, radioactivity. Fractions marked A pooled to isolate **5**, fractions B pooled to isolate **1**.

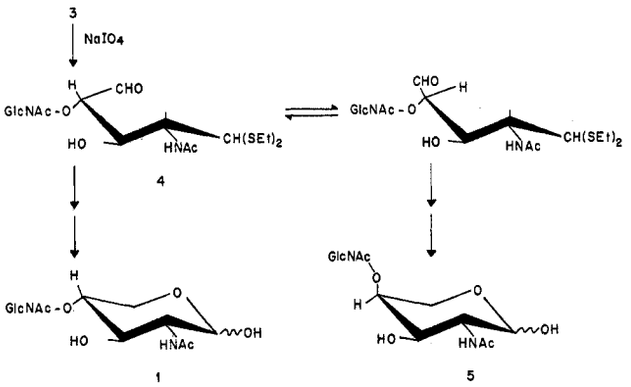
min. The incorporation of tritium into the sugar upon reduction with ^3H-borohydride was also examined. Comparison with a control reduction (*N*-acetyl-D-glucosamine) indicated that, under the final conditions chosen (30% mole excess of periodate, 5 min at 0°), oxidation of the glycol had proceeded to about 60% completion.

The glycol cleavage product **4** was reduced with tritiated sodium borohydride, and the dithioacetal was hydrolyzed with mercuric chloride and lead carbonate without intermediate work-up. The nmr spectrum of the crude product indicated that one quarter of the ethyl groups were not removed even after prolonged treatment; presumably oxidation at sulfur rendered the dithioacetal refractive to hydrolysis. The extent of glycol cleavage and sulfur oxidation seem to be in accord with the notion that the open-chain glycol is only a factor of two–fivefold more reactive toward periodate than is the dithioacetal sulfur, as indicated by the studies of periodate disappearance.

The product was isolated and purified by column chromatography on charcoal–Celite columns[13,17] in two stages. These columns proved to be extremely powerful separation tools when used with sufficiently gradual gradients of aqueous ethanol. The first column (Figure 1) served to separate the tritiated disaccharides from fragmented and deacetylated impurities, the bulk of the recovered **2**, and unhydrolyzed dithioacetals (not eluted with 20% ethanol). The second column (Figure 2) resolved two tritiated disaccharides, **1** and *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-L-arabinopyranose (**5**). Epimerization of the D-xylo residue to the L-arabino configuration presumably occurred at the aldehyde stage (Scheme II).

1 was obtained in 17% yield from **3**, or 14% overall from the *N*-acetylglucosamine dimer **2**. The product was shown to be a radiochemically pure compound on several chromatographic systems. Upon hydrolysis, D-glucosamine and D-xylosamine were obtained in equimolar quantities; only the latter was radiochemically labeled, demonstrating that the sequence of reactions had occurred as outlined in Scheme I. Hydrolysis of **5** yielded D-glucosamine and a tritium-labeled amino sugar which was assumed to be L-arabinosamine on the basis of chromatographic properties[18] and the method of preparation.

SCHEME II



A sequence of reactions identical with those used to synthesize **1** was carried out on the *N*-acetylglucosamine trimer (**6**) in an attempt to produce the next homolog of **1**, the trisaccharide *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-xylopyranose (**7**). The dithioacetal **8** was produced in 73% yield from **6**, and its oxidation, reduction, and dithioacetal hydrolysis were followed with results similar to those reported for the disaccharide insofar as could be determined by tritium incorporation, nmr, etc. However, all attempts to resolve the expected tritiated D-xylosamine- and L-arabinosamine-containing trisaccharides failed, even though the behavior of the product in binding experiments with lysozyme showed clearly that it was a mixture of at least two labeled compounds.

In order to further support the assigned structure of **1**, and to produce the homologous trisaccharide **7** and tetrasaccharide **9**, enzymic preparation of these *N*-acetylxylosamine-containing saccharides was also carried out. Tritium-labeled 2-acetamido-2-deoxy-α-D-xylopyranose (**10**) was prepared by the sequence of Scheme III. The ethylthiofuranoside of *N*-acetylxylosamine (**11**) was produced by the method of Wolfrom and Winkley,[7] with the introduction of tritium at C-5 from labeled borohydride, and hydrolyzed to **10** by a variation of Wolfrom and Anno's procedure.[19]

(17) S. A. Barker, A. B. Foster, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 2218 (1958).

(18) R. Kuhn and G. Baschang, *Justus Liebigs Ann. Chem.*, **628**, 193 (1959).

(19) M. L. Wolfrom and K. Anno, *J. Amer. Chem. Soc.*, **75**, 1038 (1953).

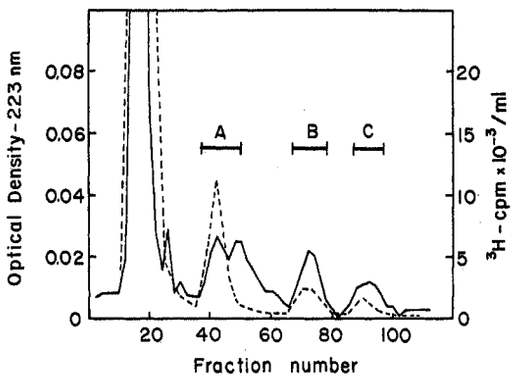
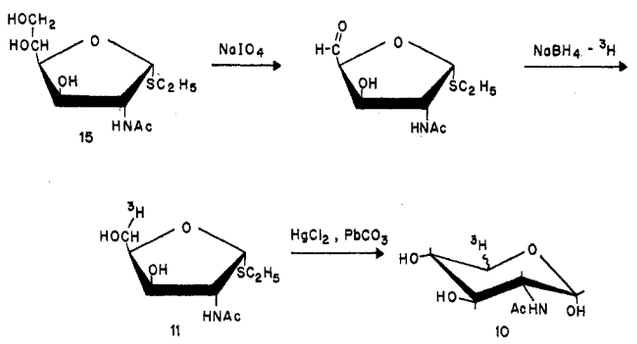


Figure 3.—Chromatographic separation of lysozyme-catalyzed reaction of *N*-acetylglucosamine tetramer (**12**) with labeled *N*-acetylxylosamine (**10**) (1 × 30 cm charcoal–Celite column, eluted with 0–40% ethanol gradient over 2 l.): solid line, optical density; broken line, radioactivity. Fractions A, B, and C pooled to isolate **1**, **7**, and **9**, respectively.

SCHEME III



Incubation of **10** and the *N*-acetylglucosamine tetramer **12** with lysozyme produced a mixture of *N*-acetylglucosamine oligomers and compounds containing tritium-labeled *N*-acetylxylosamine, which could be at least partly resolved on a charcoal–Celite column (Figure 3).

On the basis of the known transglycosylation reactions of lysozyme,[20] all the tritium-labeled oligomers must contain *N*-acetylxylosamine at their reducing termini. Although the linkage to *N*-acetylxylosamine is very probably β, there is no *a priori* reason to believe that these linkages are exclusively to O-4.[12] The identity of the *N*-acetylxylosamine-containing disaccharide produced synthetically and enzymically was demonstrated by perfect cochromatography on systems including a charcoal–Celite column. The fact that binding constants with lysozyme determined by the dialysis equilibrium technique were identical for the two products and independent of saccharide and protein concentrations[5] further demonstrates both the identity and purity of the samples. On the basis of the two alternative methods of preparation and the analysis of synthetic product, the structure of the compound obtained can only be the desired *N*-acetylglucosamine-β(1→4)-*N*-acetylxylosamine (**1**). Given that the disaccharide formed by lysozyme-catalyzed transglycosylation is exclusively the β(1→4) linked compound, it is reasonable to assume that the higher oligosaccharides **7** and **9** so produced have analogous structures.[21] They are also expected to be radiochemically pure, although neither **7** nor **9** could be freed of the respective nonradioactive *N*-acetylglucosamine oligomer of similar size.

The syntheses described here provide important compounds for testing hypotheses concerning the mechanism of lysozyme action, and starting materials and references for the preparation of more complex compounds for the further study of enzyme mechanisms. It is also likely that the synthetic scheme used here can be extended to the modification of other oligosaccharides. The introduction of a radiochemical label in the course of the synthesis turned out to be invaluable for following reactions, for verifying the course of the synthesis, and for analysis of the product. We feel that this technique will turn out to be generally useful for a wide variety of synthetic manipulations.

Experimental Section

General.—Tritiated sodium borohydride was obtained from New England Nuclear Corp. Rexyn 300, a mixed bed ion exchange resin which could be used to remove free reducing sugars[23] as well as ions in product work-ups, was obtained from Fisher Scientific Co. Unlabeled 2-amino-2-deoxy-D-xylose was prepared by the method of Wolfrom and Winkley.[7]

Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter in 10-cm microcells of 1-ml capacity.

Charcoal–Celite columns were prepared by the method of Rupley[13] from equal weights of Darco G-60 and Celite 535, and used only once before discarding. Linear gradients of increasing ethanol concentration were used for elution. Acetamido sugars were detected in the effluent by their end absorption in the uv (223 nm was generally used).

Several analytical chromatographic systems were used: system I, "Baker-flex" silica gel IB plates developed with *n*-butyl alcohol–ethanol–water (5:3:3 v/v) and visualized with a spray of 0.5 g of $KMnO_4$ and 40 g of NaOH in 100 ml of water; system II, Analtech "Avicell" cellulose glass plates developed with *n*-butyl alcohol–acetic acid–water (4:2:3 v/v) and visualized with silver nitrate–base[24] or the chlorine–starch iodide method of Powning and Irzkiewiecz;[25] system III, Analtech cellulose 300 MN glass plates developed with *n*-butyl alcohol–ethanol–water (4:1:2 v/v) and visualized as for II; system IV, Cellulose 300 MN developed with pyridine–ethyl acetate–acetic acid–water (5:5:1:3 v/v) and visualized with 0.2% ninhydrin in ethanol; and system V, descending paper chromatography on Whatman No. 1 paper with the eluent as in IV in a chamber saturated with pyridine–ethyl-acetate–water (11:40:60 v/v), developed with ninhydrin. Radiochromatograms were analyzed by scraping or slicing sections into scintillation vials, adding 1 ml of water, and allowing the vials to stand overnight. "Aquasol" liquid scintillation fluid (New England Nuclear) was then added and the vials were counted in a Packard 3375 counter.

Amino Sugar Analysis.—Samples of 1–2 mg of saccharide were hydrolyzed by heating on a steam bath for 2 hr in 2 ml of 6 *N* HCl in a sealed tube. The solvent was removed *in vacuo* and the residue was dried by repeated evaporations with absolute ethanol. For qualitative analysis, chromatography in systems IV and V was used.

For quantitative analysis, the sample dissolved in 0.5 ml of 0.3 *N* HCl was chromatographed on a 1 × 20 cm column of Dowex 50-x8 resin (Cl^- form, 200–400 mesh) equilibrated and eluted with 0.3 *N* HCl, and 1-ml fractions were collected.[26]

(20) D. M. Chipman, J. J. Pollock, and N. Sharon, *J. Biol. Chem.*, **243**, 487 (1968); D. M. Chipman and N. Sharon, *Science*, **165**, 454 (1969).

(21) The disaccharide itself is undoubtedly produced by fragmentation of larger *N*-acetylglucosamine-containing saccharides, rather than direct transfer of an *N*-acetylglucosaminyl residue to *N*-acetylxylosamine.[22] The specificity of *N*-acetylxylosamine as an acceptor as compared to D-xylose[12] is not surprising after the fact, in view of the apparent presence of a strong binding site for the acetamido group in the lysozyme acceptor subsite *E*.[4]

(22) D. M. Chipman, *Biochemistry*, **10**, 1714 (1971).

(23) S. Roseman, R. H. Abeles, and A. Dorfman, *Arch. Biochem. Biophys.*, **36**, 232 (1952).

(24) J. Trevelyan, *Nature (London)*, **166**, 444 (1950).

(25) R. F. Powning and H. Irzkiewiecz, *J. Chromatogr.*, **17**, 621 (1965).

(26) S. Gardell, *Acta Chem. Scand.*, **7**, 207 (1953).

Under these conditions, glucosamine was eluted in an effluent volume of about 76 ml and xylosamine in 100 ml. The fractions were analyzed by the Elson–Morgan color test for amino sugars[27] as extended by Crumpton.[28] Standard xylosamine produced 1.21 times the absorption at 530 nm produced by an equal weight of glucosamine.

***O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose** (2) and higher chitin oligosaccharides were prepared by the method of Rupley,[18] except that a longer charcoal–Celite column (4.5 × 60 cm) eluted with a very slow gradient (water to 20% ethanol over 4 l.) was used to obtain complete separation. From 13 g of chitin, 1.3 g of the dimer 1, $[\alpha]^{27}D$ +15.8° (lit.[18] $[\alpha]^{30}D$ +16°), 1.1 g of trimer 6, $[\alpha]^{27}D$ +3.25° (lit.[13] $[\alpha]^{30}D$ +2.5°), and 0.67 g of tetramer 12, $[\alpha]^{27}D$ −1.4° (lit.[13] $[\alpha]^{30}D$ −2.9°) (all rotations *c* 2, water, final), were obtained.

***O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose Diethyl Dithioacetal** (3).—2 (850 mg) was dissolved in 13 ml of concentrated hydrochloric acid at 0°, ethanethiol (22 ml) was added, and the mixture was stirred with a 5000-rpm mechanical stirrer for 12 hr at 0–3°. This time sufficed for all 2 to disappear (tlc, system I). The ethanethiol was decanted and the aqueous layer, which contained all of the desired product, was diluted to 125 ml and neutralized with basic lead carbonate with cooling. The suspension was filtered and the filtrate was passed through a 1.5 × 7.0 cm column of Rexyn 300 mixed bed resin.[28] The effluent (250 ml) was lyophilized, dissolved in ethanol, and filtered, and the filtrate was evaporated to yield 930 mg (82%) of white powder, which on tlc (system I) showed a major spot of R_f 0.64 and a trace of *N*-acetylglucosamine dithioacetal. Crystallization from water afforded pure **3**: mp 152–159°; $[\alpha]^{23}D$ −13.4° (*c* 0.82, ethanol); nmr (D_2O) δ 1.75 (t, 6, *J* = 8 Hz, CH_3CH_2S), 2.6 (s, 6, acetamido CH_3), and 3.2 (q, 4, *J* = 8 Hz, CH_3CH_2S); ir (KBr) 1670 and 1630 (amide I), 1572 and 1532 cm^{-1} (amide II).

Anal. Calcd for $C_{20}H_{38}N_2O_{10}S_2 \cdot H_2O$: C, 43.78; H, 7.35; N, 5.11; S, 11.69. Found: C, 43.56; H, 7.59; N, 4.85; S, 11.59.

***O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxy-D-xylopyranose** (1).—3 (500 mg, 0.905 mmol) was dissolved in 30 ml of water. The solution was cooled to 0° and to it was added a solution of 251 mg (1.18 mmol) of sodium periodate (1:1.3 molar ratio) in 30 ml of water cooled to 0°. The mixture was shaken vigorously, and then stirred at 0° for 5 min, after which 100 mg (0.58 mmol) of barium hydroxide in water was added. The reaction mixture was filtered and immediately lyophilized. The resulting white residue (presumably **4**) was dissolved in 100 ml of ethanol, and the solution was filtered through Celite and concentrated under vacuum to 40 ml.

A 60-ml portion of a 0.1 *M* solution of ^3H-$NaBH_4$ (1.7 mCi/mmol) in isopropyl alcohol was added immediately to the above solution, and the mixture was stirred for 3 hr at room temperature. The solvent was removed by evaporation, and the residue was dissolved in 100 ml of water and neutralized to pH 6 with 1 *N* HCl. The solution was stirred with 5 g of Rexyn 300 and filtered, and the resin was washed with water. Lyophilization of the combined filtrate and washings yielded 500 mg of white powder, presumably **13**.

The powder was dissolved in 30 ml of water, and 3 g of lead carbonate and 1.05 g of mercuric chloride were added. The reaction was stirred for 9 hr at room temperature and filtered. Then 3 ml of pyridine and 400 mg of lead carbonate were added to the filtrate, which was allowed to stand in an ice bath for 30 min. The resulting suspension was filtered several times and lyophilized to yield 525 mg of crude **1**. The nmr (D_2O) showed acetamido:ethylthio methyl groups in a 4.3:1 ratio.

Vpc Analysis of Oxidation Course.—After oxidation of 50 mg of **3** with periodate as described above, or oxidation of a similar quantity with 1 equiv of lead tetraacetate in 2 ml of pyridine, the samples were treated with Rexyn 300, dried, and reduced with a 10–20-fold excess of cold $NaBH_4$ in ethanol. After repeated addition of methanol and evaporation, the residue was hydrolyzed in 3 ml of 6 *N* HCl at 95° for 1 hr and dried *in vacuo*. The residue was then trimethylsilylated by the method of Sweeley, *et al.*,[29] using "Tri-Sil" reagent (Pierce Chemical Co.), and the pyridine was removed. The samples were taken up in hexane and analyzed by vpc on a 5 ft × 0.125 in. column of 5% QF-1 on 60/80 Chromosorb W, using flame ionization detection. The sample from lead tetraacetate oxidation showed peaks due to the TMS derivatives of glucosamine and glycerol, as identified by retention times and coinjection of authentic mixtures, but little or no material identifiable as xylosamine derivatives. The periodate oxidation product showed peaks due to the α and β derivatives of glucosamine, and xylosamine in about 1/3 the theoretical amount, as well as two unidentified peaks (arabinosamine?).

Chromatographic Purification of 1.—Crude **1** (250 mg) was applied to a 1 × 43 cm charcoal–Celite column and eluted with a gradient of water to 20% ethanol over 2 l. Fractions of 15 ml were collected at a flow rate of 0.3 ml/min. After analysis (Figure 1) fractions 33–55 were pooled and lyophilized to yield 91 mg of a white powder. A portion of this material (65 mg) was rechromatographed on a 1 × 58 cm charcoal–Celite column eluted with a gradient from water to 10% ethanol over 2 l. Fractions of 15 ml were collected at a flow rate of 0.75 ml/min. After analysis (Figure 2) fractions 89–102 (B) were pooled and lyophilized to yield 24.0 mg of **1** (calcd overall yield 17% from **3**). Rechromatography on a similar column and rejection of the front of the peak yielded material of specific activity 1.38 × 10^6 dpm/mg (0.29 mCi/mmol), $[\alpha]^{27}D$ −24.0° (*c* 2, water, final). Acid hydrolysis and analysis on chromatographic systems IV or V showed spots equivalent to authentic glucosamine and xylosamine (R_f glucosamine 1.3 in system V), only the latter of which contained radioactive label.

Anal. Calcd for $C_{15}H_{26}O_{10}N_2 \cdot 3H_2O$: C, 40.18; H, 7.18; N, 6.25. Found: C, 39.69; H, 6.65; N, 6.24.

***O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-deoxy-L-arabinopyranose** (5).—Fractions 76–83 from the second chromatography of crude **1** (A, Figure 2) were pooled and lyophilized to yield 15.3 mg (calcd 10% from **3**) of **5**. Rechromatography on a similar column and rejection of the tail of the peak yielded material of specific activity 1.27 × 10^6 dpm/mg (0.27 mCi/mmol), $[\alpha]^{27}D$ −13.5° (*c* 0.63, water, final). Amino sugar analysis with system V revealed a spot equivalent to authentic glucosamine and a spot of R_f glucosamine 1.18, slower than xylosamine (lit.[18] R_f glucosamine 1.1 for D-arabinosamine). Only the latter spot was radioactively labeled.

Anal. Calcd for $C_{15}H_{26}O_{10}N_2 \cdot 3H_2O$: C, 40.18; H, 7.18; N, 6.25. Found: C, 39.70; H, 6.59; N, 6.09.

***O*-2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucose Diethyl Dithioacetal** (8).—The trimer **6** (1.0 g) was treated with ethanethiol and hydrochloric acid under conditions identical with those used for synthesis of the dimer dithioacetal (**3**) and worked up similarly to yield 850 mg (72%) of a white product. On tlc (system I) the product had R_f 0.54, and contained traces of monomer and dimer dithioacetals as contaminants: nmr (D_2O) δ 1.75 (t, 6, *J* = 8 Hz, CH_3CH_2S), 2.55 (s, 9, acetamido CH_3), 3.25 (q, 4, *J* = 8 Hz, CH_3CH_2S).

Anal. Calcd for $C_{28}H_{51}N_3O_{15}S_2 \cdot 3H_2O$: C, 43.68; H, 7.20; N, 5.46; S, 8.33. Found: C, 43.36; H, 6.96; N, 5.44; S, 7.59.

Ethyl-5-^3H-2-acetamido-2-deoxy-1-thio-α-D-xylofuranoside (11).—Ethyl 2-acetamido-3,5,6-tri-*O*-acetyl-2-deoxy-1-thio-α-D-glucofuranoside (**15**), mp 124–125°, $[\alpha]^{27}D$ +133° (lit.[7] mp 122–123°, $[\alpha]^{20}D$ +139 ± 3°), was prepared by the method of Wolfrom and Winkley.[7] The *O*-deacetylated compound **14** was prepared from 1.95 g (5.0 mmol) of **15** and oxidized with sodium periodate, as described.[7] After the addition of barium chloride and removal of precipitated barium iodate, the solution was immediately lyophilized. The resulting residue was dissolved in 50 ml of absolute methanol, and 10 ml of a 0.05 *M* solution of ^3H-sodium borohydride in isopropyl alcohol (14 mCi/mmol) was added. After 30 min of stirring at room temperature, a further 226 mg (5.0 mmol) of sodium borohydride in 10 ml of methanol was added, and the mixture was stirred for 30 min more. The solvent was removed under reduced pressure, and the residue was taken up in water, neutralized to pH 7, and passed down a 15 × 2.5 cm column of Rexyn 300. The effluent (200 ml) was evaporated under high vacuum, and the solid was recrystallized from ethanol to yield two crops of solid **11**, 94 mg, mp 155–156°, and 110 mg, mp 152–154° (total yield 19%) (lit. mp 153–155°,[7] 157–158°[19] for unlabeled compound).

5-^3H-2-Acetamido-2-deoxy-α-D-xylose (10).—**11** (200 mg) was dissolved in 4 ml of water and a suspension of 1.4 g of lead carbonate and 500 mg of mercuric chloride in 12 ml of water was added. The mixture was stirred at room temperature for 4 hr and filtered. After addition of 2 ml of pyridine the solution was allowed to stand at 4° overnight. The precipitated pyridine

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complex of mercury was filtered off, and the filtrate was lyophilized. The solid was repeatedly dissolved in methanol, the solvent was evaporated, and the white powder resulting was finally recrystallized from methanol–acetone–ether to afford 107 mg (66%) of white needles, mp 180–182° dec, $[\alpha]^{27}D$ +8.4° (*c* 0.74, water, final) [lit.[19] mp 184–187° dec, $[\alpha]^{29}D$ +8° (*c* 1, water, final) for unlabeled compound]. This material was identical in several tlc systems with an authentic sample of *N*-acetyl-α-D-xylosamine provided by the late Professor Wolfrom. It was recrystallized to a constant specific activity of 3.10×10^6 dpm/mg (0.29 mCi/mmol).

Enzymic Synthesis of Oligosaccharides Containing Xylosamine.—In a typical experiment, 17 mg (0.020 mmol) of the β(1→4)-linked tetramer of 2-acetamido-2-deoxy-D-glucose (**12**) and 17 mg (0.085 mmol) of 5-^3H-2-acetamido-2-deoxy-α-D-xylopyranose (**10**, 3.10×10^6 dpm/mg) were incubated with 2 mg of lysozyme (Worthington LYSF, three times recrystallized salt free) in 2 ml of 0.1 *M* sodium acetate–acetic acid buffer, pH 5.2, at 39.5° for 25 hr, and the mixture was applied to a 1 × 30 cm charcoal–Celite column. The column was eluted with a gradient from water to 40% ethanol over 2 l. Fractions of 10 ml were collected at a rate of 1.5 ml/min and analyzed (Figure 3). Fractions 37–50 (A) were pooled and lyophilized to yield 4.5 mg of material which was rechromatographed on a 1 × 30 cm column with a 2-l. 0–20% ethanol gradient. The major peak was collected and lyophilized to yield pure *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxy-D-xylopyranose (**1**). This material was shown to be identical with that produced synthetically, by tlc (systems I, II, III) and cochromatography on a 1 × 30 cm charcoal–Celite column with a 2-l. 0–15% ethanol gradient.

Fractions 67–78 from the initial chromatography (B, Figure 3) contained *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxy-D-xylopyranose (**7**), together with the β(1→4)-linked trimer of 2-acetamido-2-deoxy-D-glucose (**6**), and fractions 87–97 (C) contained *O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-*O*-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-5-^3H-2-acetamido-2-deoxyxylopyranose (**9**), together with the β(1→4) tetramer of 2-acetamido-2-deoxy-D-glucose (**12**). Each of these peaks was pooled and rechromatographed twice, with the front of the peak being collected each time. Analysis of known weights of the final products for ^3H content revealed that the trisaccharide mixture contained 22 mol % of the xylosamine-containing compound **7** and that the tetrasaccharide mixture contained 16 mole % of the xylosamine-containing compound **9**.

Registry No.—**1**, 38864-17-4; **2**, 35061-50-8; **3**, 38864-18-5; **4**, 38864-19-6; **5**, 38864-20-9; **6**, 38864-21-0; **8**, 38864-22-1; **10**, 38864-23-2; **11**, 38864-24-3; **13**, 38864-25-4; **14**, 38859-04-0; **15**, 7115-40-4.

C-Glycosyl Nucleosides. II.[1] A Facile Synthesis of Derivatives of 2,5-Anhydro-D-allose

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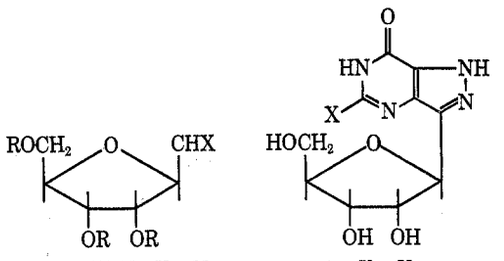
A very facile synthetic route to 3,4,6-substituted derivatives of 2,5-anhydro-D-allose is described. Reductive hydrolysis of 2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl cyanide (**3**) with Raney nickel and sodium hypophosphite in aqueous pyridine–acetic acid is accompanied by extensive elimination of benzoate to give furfural derivatives. In the presence of *N*,*N*′-diphenylethylenediamine (**6**), however, the initial aldehyde is trapped as a crystalline 1,3-diphenylimidazolidine derivative (**7**) which is obtained in 74% yield. In a similar way 5-*O*-benzoyl-2,3-*O*-isopropylidene-β-D-ribofuranosyl cyanide is converted into the corresponding imidazolidine derivative (**12**). Alkaline hydrolysis of (**7**) gives 1,3-diphenyl-2-(β-D-ribofuranosyl)imidazolidine (**8**) which can be converted into the tri-*O*-benzyl ether **9a** or the tri-*O*-acetate **9b**. Regeneration of the free 3,4,6-trisubstituted 2,5-anhydro-D-alloses from the imidazolidine derivatives can be achieved by mild acidic treatment.

In recent years a considerable number of *C*-glycosyl nucleosides have been isolated from natural sources.[2] The frequently interesting biological properties of these substances have made them interesting targets for chemical synthesis, but as yet this has proved to be a more formidable task than the preparation of conventional *N*-glycosyl nucleosides. Thus, while the preparation of 5-(β-D-ribofuranosyl)uracil (pseudouridine) has been achieved through carbon–carbon bond formation between a 5-lithiopyrimidine and a suitable derivative of ribose,[1,3] and this method has also been extended to other 5-glycosyluracils,[4] this route has not yet been readily adapted for use with other heterocycles.

A more versatile route would appear to involve the preparation of an appropriately C_1-functionalized derivative of 2,5-anhydro-D-allose or 2,5-anhydro-D-allitol (**1**), a compound already containing the desired elusive carbon–carbon bond, from which C_1 can be elaborated into a variety of heterocycles. One such derivative is the diazo compound **1a** which has been



1a, R = CH_2Ph; X = N_2
b, R = CH_2Ph; X = O
c, R = Bz; X = O

2a, X = H
b, X = OH

ingeniously converted into formycin B (**2a**)[5] and oxoformycin (**2b**)[6] *via* initial cycloaddition to dimethyl acetylenedicarboxylate.

In a related way the furanosyl keto ester (**1**, R = Ac; CHX = $COCO_2Me$) has been transformed into the

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