1972

Synthesis of 3-deoxy-N-acetylglucosamine and Attempted Synthesis of 4-deoxy-N-acetylglucosamine as Possible Antibacterial Agents

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SYNTHESIS OF 3-DEOXY-N-ACETYLGLUCOSAMINE AND ATTEMPTED SYNTHESIS OF 4-DEOXY-N-ACETYLGLUCOSAMINE AS POSSIBLE ANTIBACTERIAL AGENTS

BY

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A thesis submitted
in partial fulfillment of the requirements for the
degree of Master of Science, Major in
Pharmaceutical Chemistry
South Dakota State University
1972

SYNTHESIS OF 3-DEOXY-N-ACETYLGLUCOSAMINE

AND ATTEMPTED SYNTHESIS OF 4-DEOXY-N-ACETYLGLUCOSAMINE

AS POSSIBLE ANTIBACTERIAL AGENTS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor Date

Head, Pharmaceutical Chemistry Department Date

ACKNOWLEDGEMENTS

I wish to express my gratitude to my advisor, Dr. Gary W. Omodt, Head of the Department of Pharmaceutical Chemistry for his guidance and encouragement in the development and completion of this thesis. It has been a privilege to work with him.

I also wish to thank South Dakota State University for the Graduate Assistantships for the academic years 1970-71, 1971-72.

GVR

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INTRODUCTION

An antibacterial agent is a substance which destroys or suppresses bacterial growth or reproduction. Gerhard Domagk's discovery of the antibacterial activity of the sulfonamides began one of the brightest eras in modern antibacterial chemotherapy. The sulfonamides are effective and are still used today but the introduction of the antibiotics has made them less popular.

Antibacterial agents cannot be classified by one chemical grouping or mechanism of action. The phenols and alcohols act by denaturing protein while others, such as arsenic and the heavy metals, exert their effect by combining with the sulfhydryl grouping present as an active site on certain enzymes. The sulfonamides exert their effect by interfering with the utilization of p-aminobenzoic acid in bacteria. The antibiotics including cycloserine, vancomycin, penicillin, and cephalothin inhibit normal build-up of the cell wall of the bacteria.

The proposed compounds would theoretically inhibit the normal cell wall synthesis of bacteria because these compounds would have the necessary binding sites needed for normal cell wall synthesis. However, the compounds synthesized should be bio-isosteric to the normal precursors to be accepted by the enzymes needed for biological synthesis.

RESEARCH OBJECTIVE

Bacteria are small microorganisms with rigid cell walls. This wall surrounds the protoplast of the cell and usually makes up about twenty per cent of the dry weight of the cell (1). The cell wall is actually a large sac-like, covalently linked molecule called a sacculus and is made up of a complex polysaccharide-polypeptide called a murein (2).

In 1951, Park (3) was able to isolate and characterize the uridine phosphate derivative of muramic acid from penicillin inhibited cultures of Staphylococcus aureus. Park and Strominger (4) related this accumulation of uridine phosphate derivative to the mechanism of the action of penicillin. They proposed that penicillin interfered with the utilization of muramic acid in the cell wall synthesis. Because gram positive bacteria have an osmotic pressure greater than the external environment, the weakened condition of the cell wall would allow the cell to lyse.

Work (5) was able to show that the cell wall of gram positive bacteria was made up of a hexosamine polymer comprised of muramic acid and glucosamine and sometimes galactosamine.

Ghuysen and Strominger (6) made studies on the cell wall structures of S. aureus. They hydrolyzed the insoluble portion of the cell wall with hydrochloric acid. The products were analyzed and indentified by using paper and ion exchange chromatography and were found to be glucosamine and muramic acid.

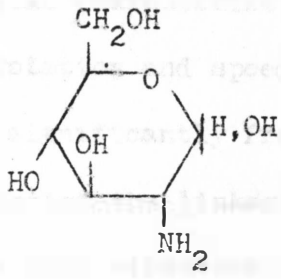


Fig. 1 Glucosamine

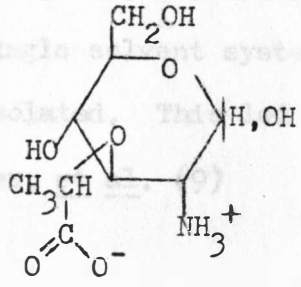
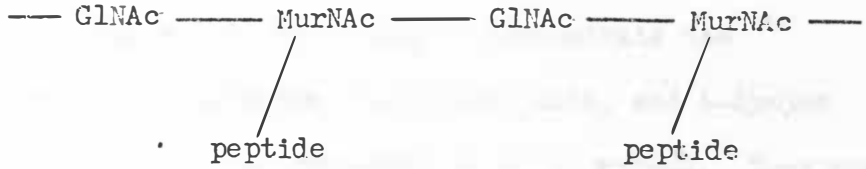


Fig. 2 Muramic Acid

Early work done by Salton and Ghuysen (7) led them to propose that glucosamine and muramic acid were linked together and that a peptide chain was attached to the carboxyl group of the muramic acid.



GlNAc = N-Acetylglucosamine
MurNAc = N-Acetylmuramic acid

Fig. 3 Backbone structure of cell wall

Jeanloz, et al. (8) in 1963, synthesized the disaccharide N-acetylglucosaminyl β (1→6) N-acetylmuramic acid and compared

it with the disaccharide extracted from Micrococcus lysodeiktius. The physical characteristics of the synthetic compound, especially the mutarotation and speed of migration in a single solvent system, differed significantly from those of the one isolated. This led to the assumption the linkage was β (1→4). Tipper, et al. (9) confirmed this structure using S. aureus.

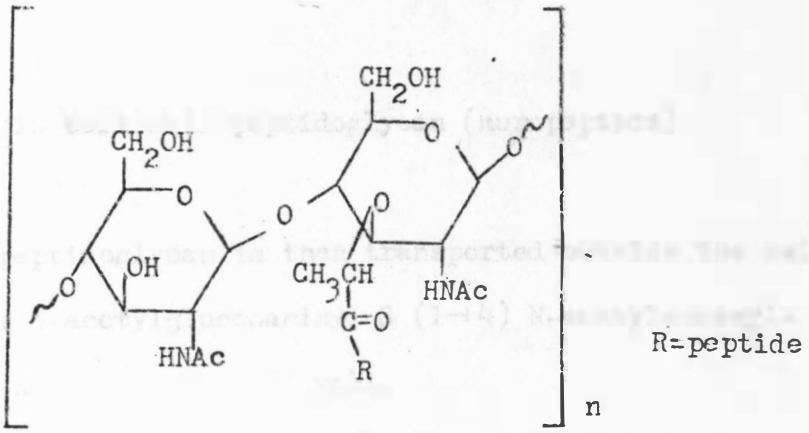


Fig. 4 Basic cell wall

Ito and Strominger (10) were able to demonstrate the sequential addition of L-alanine, D-glutamic acid, and L-lysine to UDP-muramic acid by enzyme preparations of S. aureus. They were also able to show the addition of D-alanyl-D-alanine to the lysine residue of the growing peptide.

Anderson, et al. (11) showed that N-acetylmuramyl-pentapeptide attached to a lipid receptor on the cell membrane. Anderson and Strominger (12) were able to show that N-acetyl-glucosamine from UDP-N-acetylglucosamine attaches irreversibly to the N-acetylmuramyl-pentapeptide (Fig. 5).

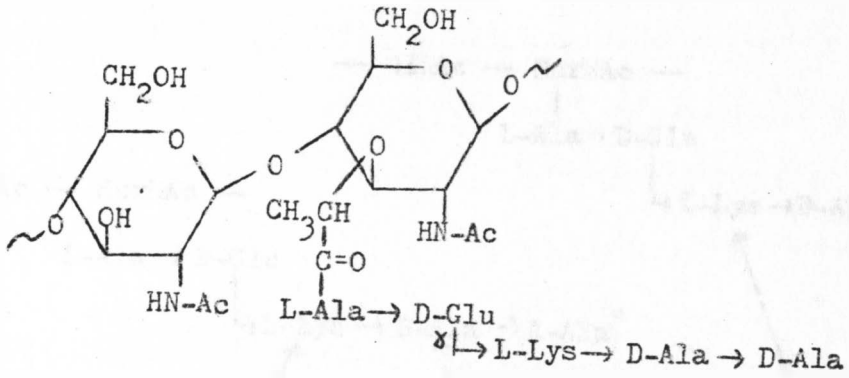


Fig. 5 Basic cell wall peptidoglycan (muropeptide)

The lipid peptidoglycan is then transported outside the cell membrane and the N-acetylglucosaminyl β (1→4) N-acetylmuramyl-pentapeptide is added to the cell wall.

The final step of the cell wall formation is the cross-linking reaction between the peptide moieties of separate peptidoglycans. In the case of S. aureus, a pentaglycine unit from transfer-RNA is added to the -amino group of L-lysine while the muropeptide is still attached to the lipid carrier. In the cross-linking reaction on the surface of the cell membrane, the N-terminal amino group of the pentaglycine moiety attacks the amide linkage of the D-alanyl-D-alanine residue of the closest murein strand (Fig. 6) (13).

Collins and Richmond (14) showed by wire models that N-acetylmuramic acid and the anion of benzyl penicillin were very similar in structure (Fig. 7).

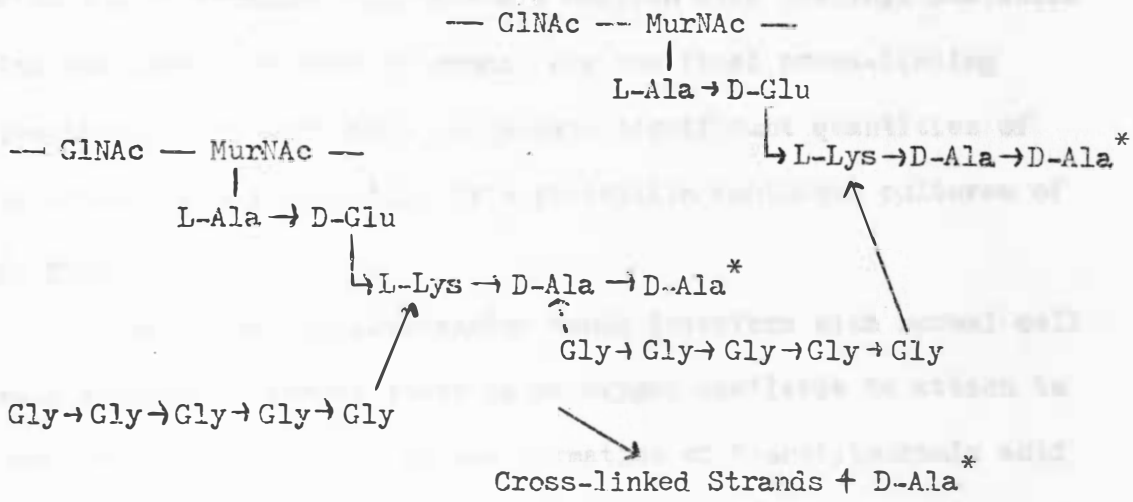


Fig. 6 Cross-linkage reaction in S. aureus

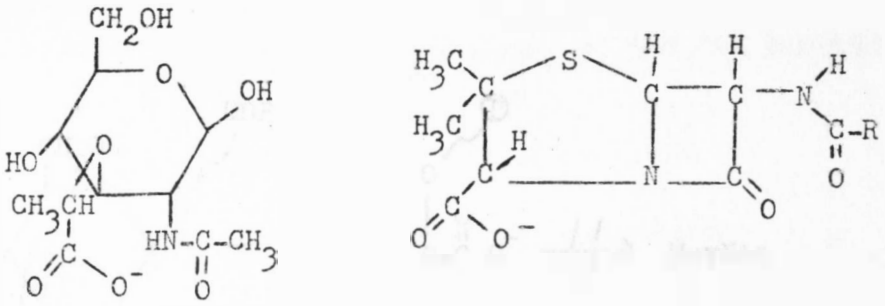


Fig. 7 Comparison of anions of N-acetylmuramic acid and penicillin

This led them to propose that penicillin is similar enough to N-acetylmuramic acid to competitively inhibit the enzyme necessary for the formation of the polysaccharide backbone.

However, Tipper and Strominger (15) and Wise and Park (16) presented much stronger evidence that penicillin did not compete

with N-acetylmuramic acid but did compete with D-alanyl-D-alanine for the transpeptidase necessary for the final cross-linking reaction. They were able to isolate significant quantities of uncross-linked muropeptide from penicillin inhibited cultures of S. aureus.

3-Decxy N-acetylglucosamine could interfere with normal cell wall synthesis because there is no oxygen available to attach to the lactic acid residue in the formation of N-acetylmuramic acid (Fig. 8). Richmond and Perkins (17) studied the biosynthesis of muramic acid in S. aureus and found that the lactic acid residue donor was probably pyruvate or phosphoenol pyruvate.

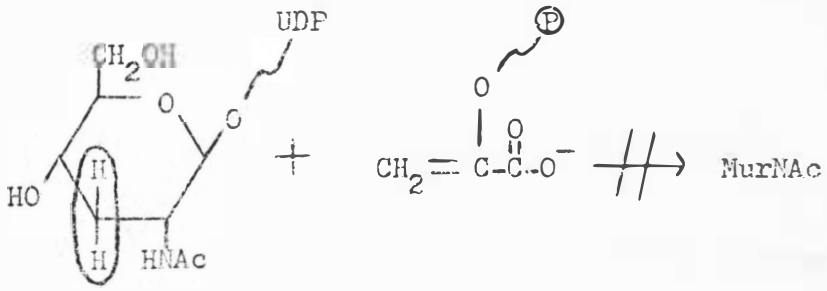


Fig. 8 Reaction blocked by 3-deoxy N-acetylglucosamine

Because of the lack of the 3-O-carboxyethyl group, there could be no addition of amino acids necessary for cross-linking and the cell wall would be incomplete (Fig. 9).

A 4-deoxy sugar could interfere in more than one reaction. The compound, 4-deoxy-N-acetylmuramyl-pentapeptide phospholipid,

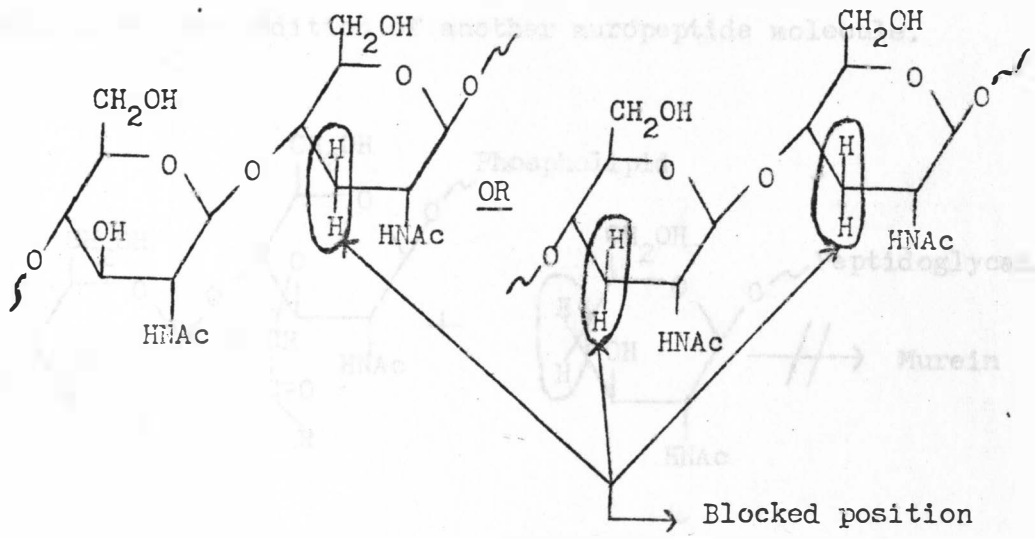


Fig. 9 Incomplete cell wall

would not be able to accept N-acetylglucosamine from UDP-N-acetyl-glucosamine because of the lack of an oxygen at the C-4 position.

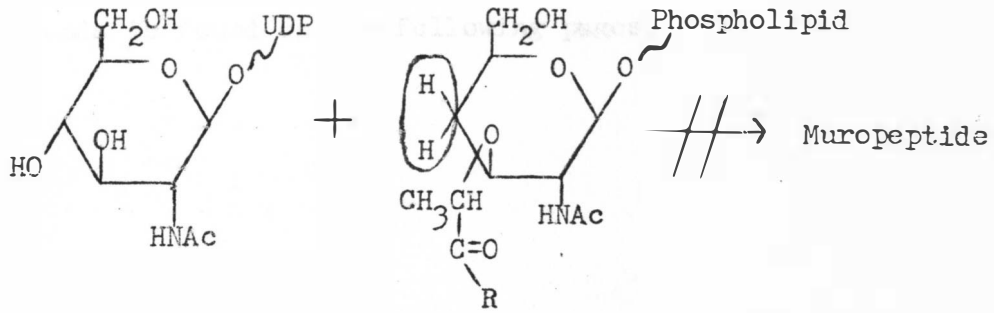


Fig. 10 Synthetic step inside cell membrane blocked by 4-deoxy N-acetylglucosamine

The 4-deoxy compound could also block cell wall formation outside the cell membrane. The absence of an oxygen at the C-4 position of a terminal N-acetylglucosamine of a growing cell wall

would block the addition of another muropeptide molecule.

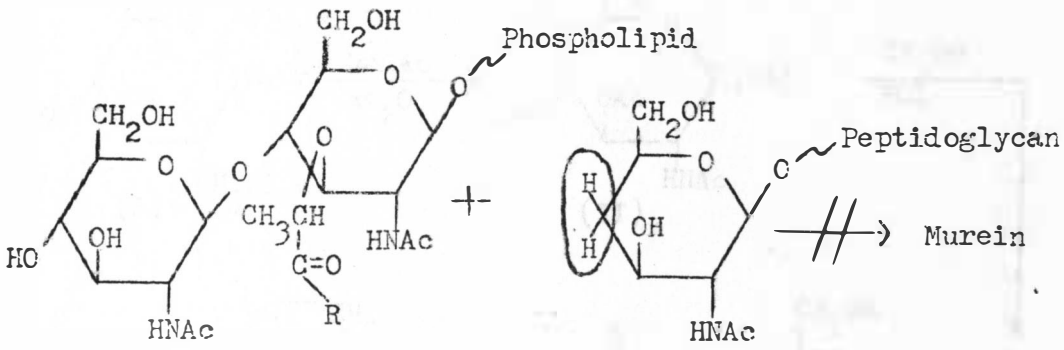
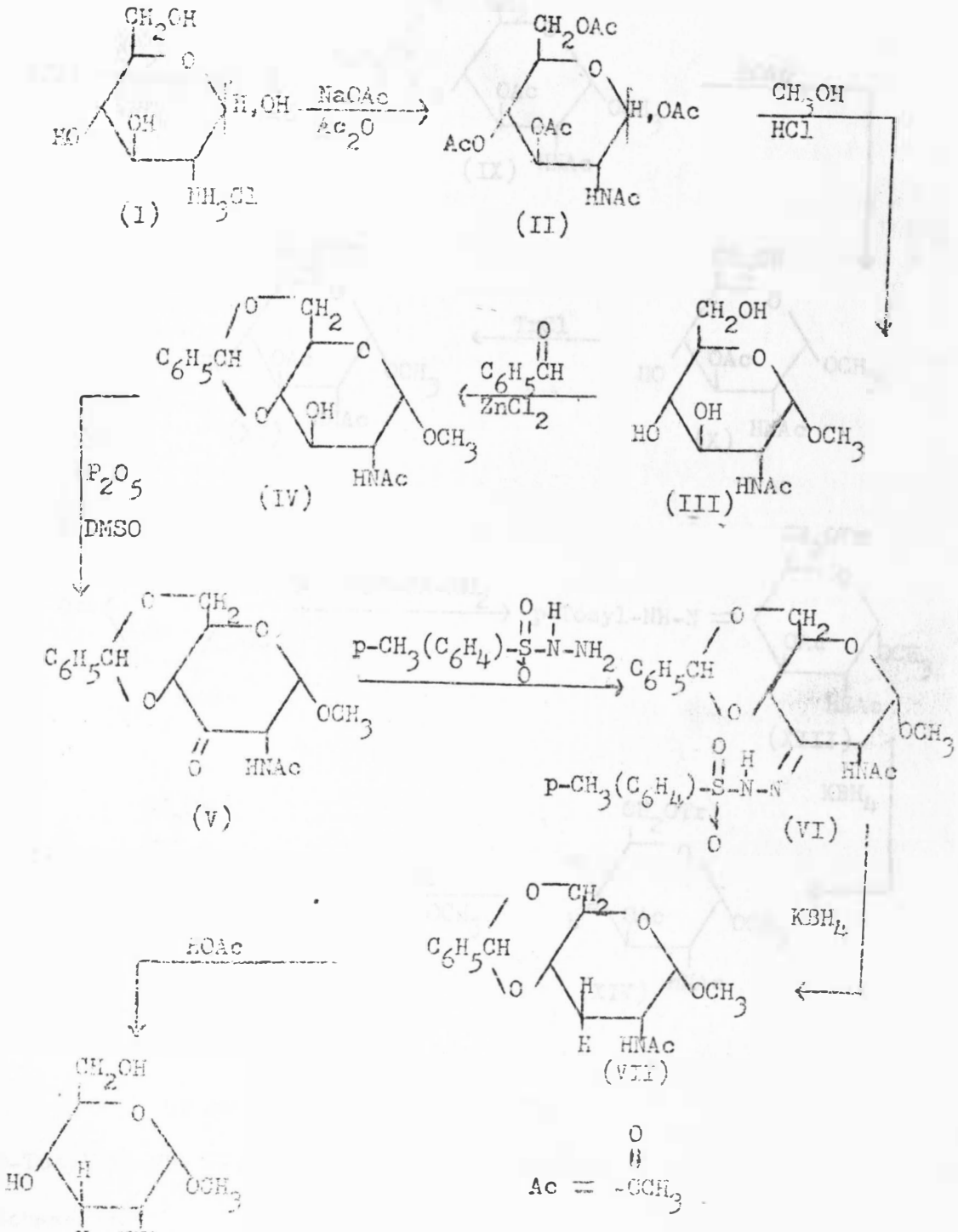


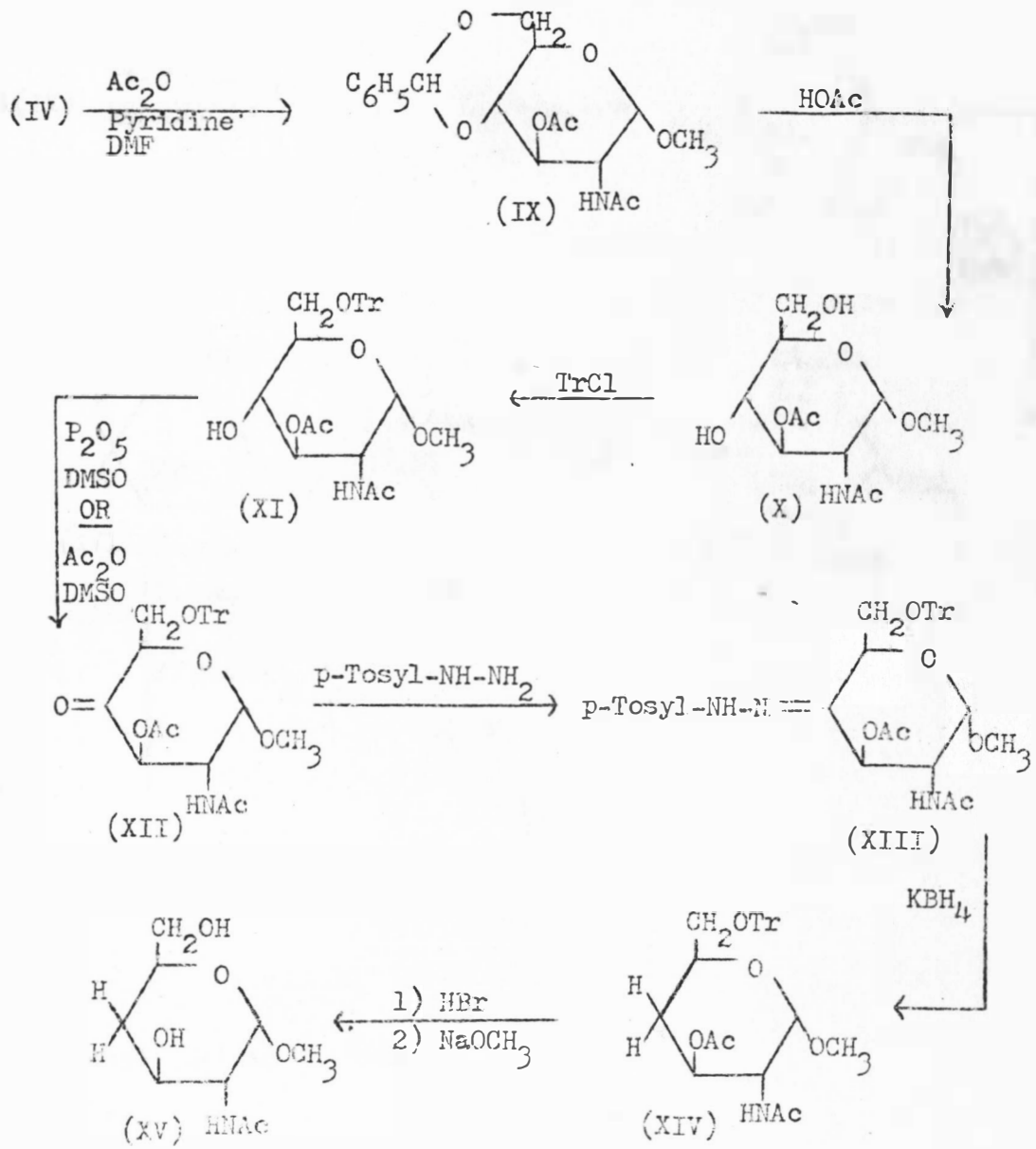
Fig. 11 Synthetic step blocked outside cell membrane

Both the 3-deoxy and 4-deoxy N-acetylglucosamines should be bio-isosteric to the parent compound to be accepted by the enzymes necessary for biological synthesis.

A theoretical scheme for the synthesis of both of these compounds is found on the following pages.


NaOAc
Ac2O
CH3OH
HCl
P2O5
DMSO
ZnCl2
HOAc
KBH4
(I)
(II)
(III)
(IV)
(V)
(VI)
(VII)
(VIII)
Ac = -C(=O)CH3

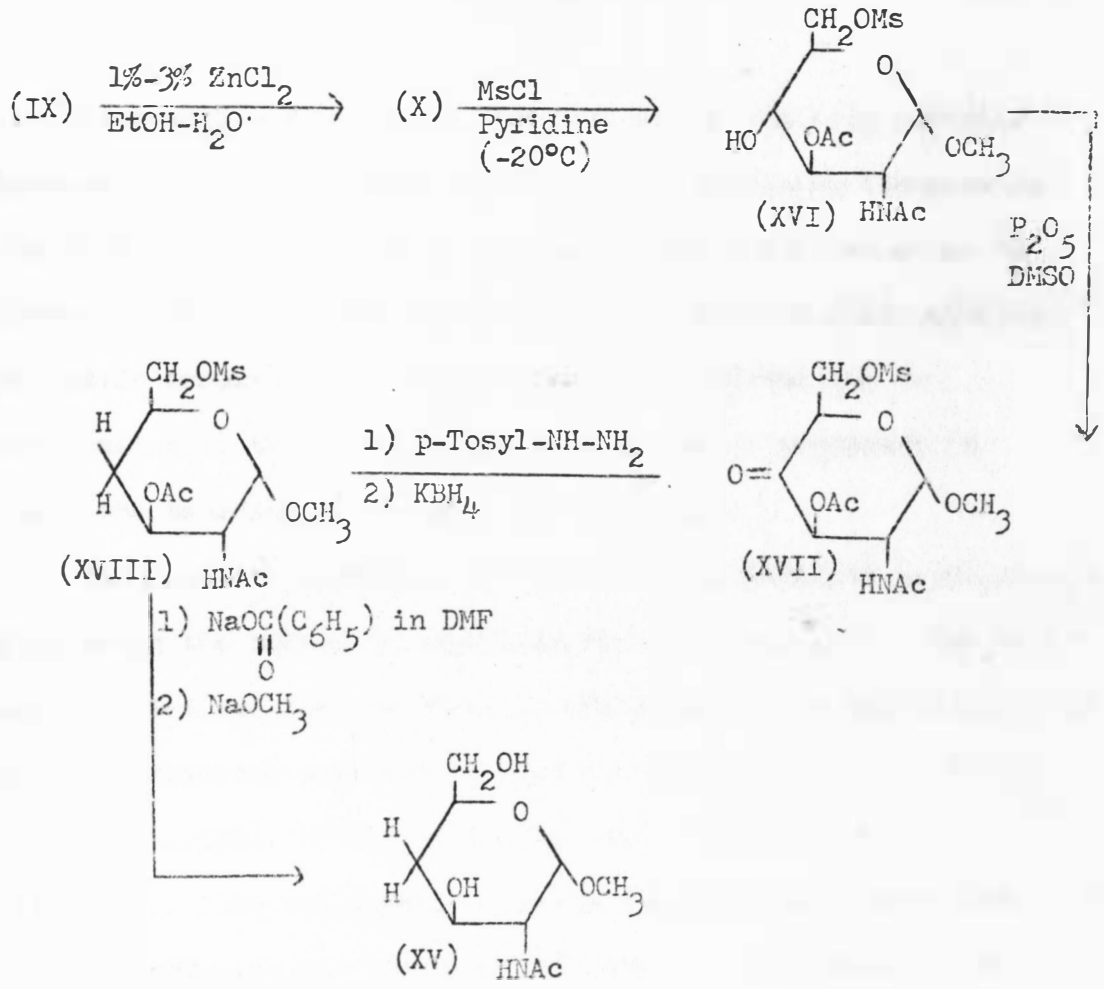
Schere I



Tr = Trityl (Triphenylmethyl)

p-Tosyl-NH-NH_2 = p-Toluenesulfonylhydrazine

Scheme II



Ms = Mesyl (Methanesulfonyl)

Scheme III

DISCUSSION

Glucosamine HCl (I) was selected as the starting material because it is commercially available and relatively inexpensive. The first step was complete acetylation using the procedure of Lobrey De Bruyn and Van Eckenstein (18). Because acid catalyzed glycoside formation of 2-amino sugars is hindered due to protonation of the amino group, acetylation is necessary to lower the basicity of the amino groups (19).

The α-methyl glycoside (III) was formed from the penta-acetate (II) using the method of Moggridge and Neuberger (20). The only modification was the use of a calculated amount of acetyl chloride in cold anhydrous methanol to form the required 2.2 per cent, weight in weight, hydrogen chloride in methanol needed for catalysis. This modification was much easier than generating hydrogen chloride and trapping it in anhydrous methanol. The methyl group was chosen as the masking group of the C-1 hydroxyl group because the methyl glycoside had been previously synthesized and its melting point is known.

In order to preferentially oxidize the C-3 hydroxyl group, the C-4 and C-6 hydroxyls were blocked with a benzylidene group following the method of Neuberger (21). The C-3 hydroxyl was oxidized to a carbonyl by the procedure of Onodera and Kashimura (22). The modification used by Baker and Buss (23) of shaking at room temperature in place of heating was used in this step as a precaution against decomposition of products.

The tosylhydrazone (VI) was formed according to the method of Helferick and Schirp (24). The reaction was run in benzene in place of absolute ethanol. The rate of reaction could be followed as the starting compound (V) is insoluble in benzene and the tosylhydrazone is scluble in benzene.

Grasseli and Caglioti (25) found that the tosylhydrazone of steroids could be reduced to methylene groups when subjected to reduction with sodium borohydride in dioxane. This procedure was used on the tosylhydrazone (VI) in the formation of (VII) except potassium borohydride was used in place of sodium borohydride.

The procedure of Richardson (26) was used for the removal of the benzylidene group from the 3-deoxy compound (VII) formed by the borohydride reduction. The resulting syrup was treated with ethanol and ether in an attempt to crystallize the final product (VIII). Dilutions of the ethanolic layer and the syrup were subjected to thin layer chromatography according to the method of Haer (27). In order to facilitate purification, an alumina column was used to remove impurities interfering with crystallization. This was done according to the procedure of Fieser (28).

Since the synthesis of the 3-deoxy compound was successful up to the failure to crystalize the final product, the same procedures should be adaptable to the synthesis of the 4-deoxy compound.

Starting with the benzylidene compound (IV) (Scheme I), it

is first necessary to block the hydroxyl group at C-3 with a group that can be removed easily in succeeding steps. The acetyl group was used as it can be removed selectively under basic conditions with retention of configuration and retention of the acetamide group (29). The benzylidene derivative (IV) was acetylated according to the method of Richardson (26).

In order to preferentially oxidize the hydroxyl at C-4, it must be freed from the benzylidene group and the C-6 hydroxyl blocked preferentially. The procedure of Richardson (26) was used to remove the benzylidene group. The trityl (triphenylmethyl) group was selected as the blocking group for the C-6 hydroxyl as it is preferential for primary hydroxyl groups under selected conditions (30). The method of Foster, et al. (31) was used. It was modified by extending the reaction time to forty-eight hours for completeness of reaction.

The trityl derivative (XI) (Scheme II) was subjected to oxidation by the procedure of Onodera and Kashimura (22). The resulting product did not give a positive test for ketones using 2,4-dinitrophenylhydrazine.

Albright and Goldman (32) reported the use of acetic anhydride and dimethyl sulfoxide (DMSO) for oxidizing sterically hindered secondary hydroxyl groups. Inouye, et al. (33) also reported the use of acetic anhydride and DMSO for the oxidation of primary hydroxyl groups, the two procedures only differing in the amount of reagents used.

Because of the size of the trityl group, it may sterically hinder the attack by the dimethyl sulfonium ion on the secondary hydroxyl group.. Removal of the trityl group prior to reacting with p-toluenesulfonylhydrazine may have favored reaction. Trityl groups can be specifically removed usi g hydrobromic acid in acetic acid without removing acetyl groups or breaking glycosidic linkages (34).

Another approach would be to use a smaller blocking group at C-6. Cramer (35) reported a method of selective mono-mesylation of the primary hydroxyl group. The mesyl (methanesulfonyl) group is preferred over the tosyl group because the mesyl group is much smaller and should not sterically hinder further reactions of the C-4 hydroxyl group. Care must be taken in removing the mesyl group in the presence of a 3-O-acetyl group as this may result in the formation of a 3,6-anhydro sugar (36).

Fig. 12 Formation of 3,6-anhydro sugar

However, a procedure using sodium benzoate in

dimethylformamide (DMF) has been published and could be used in this case (37).

The product (XVII) (Scheme III) was oxidized by the method of Onodera (22) and this ketone subjected to tosylhydrazine in benzene. No product was isolated.

EXPERIMENTAL

All melting points recorded in this work were taken using a capillary tube melting point apparatus (Tottoli) and all are uncorrected. All infrared data were taken using KBr discs in a Beckman IR-33 using a KBr blank. The elemental analyses, carbon and hydrogen, were performed by the Department of Chemistry, South Dakota State University.

SYNTHESIS OF 3-DEOXY-N-ACETYLGLUCOSAMINE

PREPARATION OF 2-ACETAMIDO-2-DEOXY-1,3,4,6-TETRA-O-ACETYL-α/β-D-GLUCOPYRANOSIDE (18):

Anhydrous sodium acetate (133 g., 1.62 mole) was added to boiling acetic anhydride (670 ml., 744 g., 7.29 mole) in a covered 2 l. Erlenmeyer flask equipped with a magnetic stirring bar and allowed to boil for fifteen minutes. To this was added glucosamine HCl (100 g., 0.463 mole) and allowed to boil for three minutes. The whole mixture was poured into a 4 l. beaker containing 1000 g. ice and 1000 ml. cold water with mechanical stirring and neutralized with sodium carbonate.

After neutralization, 1000 ml. of chloroform was added with vigorous stirring. The resulting mixture was siphoned into two 2 l. separatory funnels, the layers were seperated, and the aqueous

layers were extracted three times with 215 ml. portions of chloroform. The chloroform was removed at 60° in vacuo. When a thick syrup had formed, the temperature was raised to 90°. The syrup was stored at room temperature in a vacuum oven. The yield was 143.66 g. (79.560 %) based on glucosamine HCl.

PREPARATION OF METHYL 2-ACETAMIDO-2-DEOXY-α-D-GLUCOPYRANOSIDE (20):

Impure 2-acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-α/β-D-glucopyranoside (syrup from above) (143.66 g., 0.36897 mole) was refluced in 1553 ml. of anhydrous methanol containing 2.2 % w/w HCl (made by adding 53.1 ml., 58.7 g. of acetyl chloride to 1500 ml., 1180 g. of cold anhydrous methanol with stirring) for two hours. The dark solution was then added to 290 g. lead carbonate with stirring and stirred fifteen minutes. The suspension was suction filtered through a Celite-prepared Buchner funnel. The residue was washed with five 100 ml. portions of methanol. The methanol-methyl acetate solvent was removed in vacuo at 60-75°. The residue was recrystallized from 95 % ethanol. The yield was 53.2 g., (60.9 %), m. p. 185-187° (reported 188°), IR Data: 3400 cm^{-1} (OH); 3295 cm^{-1} (NH); 1645 cm^{-1}, 1545 cm^{-1} (CONH); 890 cm^{-1} (OCH_3).

PREPARATION OF METHYL 2-ACETAMIDO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-GLUCOPYRANSIDE (21):

Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (53.0 g., 0.226 mole), anhydrous zinc chloride (53.0 g., 0.388 mole) and freshly distilled benzaldehyde (136.8 ml., 142.5 g., 1.342 mole) were placed in a 500 ml. glass-stoppered Erlenmyer flask and shaken at room temperature for fifty-eight hours. The thick, syrupy liquid was poured into 2 l. of mechanically stirred cold water. Precipitation of product ensued immediately. Skelly C (350 ml.) was added with stirring to take up excess benzaldehyde. The precipitate was suction filtered with difficulty as the precipitate tends to enmesh water. The cake was pressed and allowed to air dry. The air-dried material was dissolved in 1.66 l. methanol and treated with 0.5 g. Darco charcoal to remove impurities. The product crystallized from methanol. The yield was 46.0 g. (61.5 %), m. p. 252-258° dec. (reported m. p. 255°), IR Data: 3420 cm^{-1} (OH); 3295 cm^{-1} (NH); 1645 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl).

PREPARATION OF METHYL 2-ACETAMIDO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-RIBO-HEXOPYRANOSIDE-3-ULOSE (22, 23):

Anhydrous phosphorous pentoxide (5.2 g., 0.037 mole) was added to freshly distilled dimethyl sulfoxide (410.0 ml., 451.0 g., 5.772 mole) in a 500 ml. glass-stoppered Erlenmeyer flask and shaken until dissolved. To this was added methyl 2-acetamido-4, 6-O-benzylidene-2-deoxy-α-D-glucopyranoside (20.0 g., 0.0617 mole). The mixture was shaken at room temperature for one hundred and

sixty-eight hours. At the end of this time the light yellow solution was poured into a mechanically stirred mixture of cold five per cent sodium bicarbonate (1225 ml.) and cold chloroform (2050 ml.) and stirred for thirty minutes. The layers were separated and the aqueous layer extracted three times with 200 ml. portions of cold chloroform. All chloroform layers were combined and filtered through anhydrous sodium sulfate. The chloroform was evaporated *in vacuo* at 90° and 15 mm. Hg. The remaining residue was recrystallized from 100 times its weight of acetone. The yield was 13. g. (65.8 %), m. p. 231.5-232.5° dec. (reported 227-228° dec. (23)), IR Data: 3290 cm^{-1} (NH); 1645 cm^{-1}, 1545 cm^{-1} (CONH); 1730 cm^{-1} (C=O); 690 cm^{-1}, 740 cm^{-1} (phenyl); 890 cm^{-1} (OCH_3). Loss of absorption at 3400 cm^{-1} (No OH).

PREPARATION OF METHYL 2-ACETAMIDO-4,6-O-BENZYLIDENE-2-DEOXY-α-D-*RIBO*-HEXOPYRANOSIDE-3-ULOSE TOSYLHYDRAZONE (24):

To a mechanically stirred solution of *p*-toluenesulfonylhydrazine (6.35 g., 0.341 mole) in benzene (1100 ml.) was added methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside-3-ulose (11.0 g., 0.0341 mole). A Dean-Stark trap and condenser were attached and the mixture stirred and refluxed until all material was in solution. This took approximately eighteen hours. The benzene was removed *in vacuo*. The remaining residue was refluxed in 570 ml. methanol until dissolved. At the boiling

point of the solvent 230 ml. of water were added and solution allowed to cool. Crystallization was immediate upon cooling. The yield was 6.7 g. (40 %), m. p. 191-192° dec. Anal.--Calcd. for $C_{23}H_{27}O_7N_3S$: C, 56.43; H, 5.56. Found: C, 56.31; H, 5.56. IR Data: 3280 cm^{-1}, 3400 cm^{-1} (NH); 1670 cm^{-1} (C=N); 1649 cm^{-1}, 1525 cm^{-1} (CONH); 1335 cm^{-1}, 1155 cm^{-1} (SO_2N); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl).

PREPARATION OF METHYL 2-ACETAMIDO-4,6-O-BENZYLIDENE-2,3-DIDEOXY-α-D-RIBO-HEXOPYRANOSIDE (25):

To a solution of the tosylhydrazone derivative (6.7 g., 0.014 mole) in anhydrous dioxane (335 ml.) in a one l. 3-necked, round-bottom flask equipped with a condenser and mechanical stirrrer, was added potassium borohydride (13.4 g., 0.248 mole). The suspension was refluxed and stirred for sixteen hours. The dioxane was removed in vacuo. The residue was refluxed with 250 ml. of chloroform. The chloroform layer was washed successively with 75 ml. water, 100 ml. five per cent sodium bicarbonate solution, and after standing twenty-four hours, 75 ml. water. The chloroform layer was filtered through anhydrous sodium sulfate and the chloroform removed in vacuo. The residue was dissolved in 250 ml. methanol, and the product was forced out with small volume of water. The yield was 2.5 g. (58 %), m. p. 231.5-232° dec. Anal.--Calcd. for $C_{16}H_{21}O_5N$: C, 62.52; H, 6.88. Found: C, 62.35; H, 6.27. IR Data: 3290 cm^{-1}

(NH); 1649 cm^{-1}, 1525 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl). Loss of peak at 1335 cm^{-1} (SO_2NH).

PREPARATION OF METHYL 2-ACETAMIDO-2,3-DIDEOXY-α-RIBO-HEXOPYRANOSIDE (26, 27, 28):

Methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-ribo-hexopyranoside (2.0 g., 0.0065 mole) was heated in 100 ml. of fifty per cent aqueous acetic acid with stirring for thirty minutes. The solvents were evaporated in vacuo. To the remaining brown syrup, water and toluene were added and evaporated between each addition. The syrup was taken up in methanol and filtered through a Celite-charcoal prepared Buchner funnel with suction. The solvent was evaporated in vacuo and the syrup was taken up in absolute ethanol. Ether and petroleum ether (b. p. 30-60°) were added to turbidity and allowed to stand in the refrigerator. No crystalline product was formed but an oil separated upon standing.

The ethanol-ether layer was separated from the oil and evaporated in vacuo to a syrup. Two mg./ml. dilutions in methanol of the oil and the syrup were chromatographed on silicic acid-impregnated glass fiber which had been impregnated with 0.1 M potassium dihydrogen phosphate and activated one hour at 110°. A solvent system of chloroform-glacial acetic acid-water (50:35:5) was used. The developed chromatograms were sprayed with a 0.5 per cent potassium permanganate in 1 N sodium hydroxide and heated

at 100° for two minutes. Sugars appear as pale yellow spots on a purple background. Two areas, one each from the syrup and the oil dilutions, were detected and Rf values of .75 were calculated for each spot.

The syrup and the oil were dissolved in small amounts of methanol and placed on separate alumina columns (25 g. alumina activated at 200° for three hours and stored in a dessicator). The columns were eluted with 50 ml. portions of petroleum ether (b. p. 30-60°), benzene, ether, ether-methanol (4:1; 1:1; 1:4), and methanol. Twenty-five milliliter samples of each were collected in tared flasks and evaporated to dryness on a steam bath. The dried, colorless fractions from the petroleum ether (b. p. 30-60°), benzene, ether, and methanol-ether elutions were dissolved in small amounts of 95 % ethanol and evaporated in vacuo. No products were isolated.

ATTEMPTED SYNTHESIS OF

4-DEOXY N-ACETYLGLUCOSAMINE

PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-4,6-O-BENZYLIDENE-2-DEOXY-α-D-GLUCOPYRANOSIDE (26):

To anhydrous pyridine (200 ml.) was added acetic anhydride (200.0 ml., 217.4 g., 2131 mole) and dimethylformamide (20.0 ml.,

18.9 g., 0.259 mole). To this mixture was added methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (36.0 g., 0.111 mole) and refluxed for thirty minutes at 100°. The solution was allowed to stand overnight at room temperature. Crushed ice (900 g.) was added and crystallization took place immediately. The precipitate was filtered with suction and recrystallized from one hundred times its weight of absolute ethanol. Two products were recovered upon recrystallization. The first crop of product consisted of long, needle-like crystals. The yield was 13.0 g. (34.4 %), m. p. 302-304° dec. (reported m. p. 203-205° (38)), Anal.--Calcd. for $C_{18}H_{23}O_7N$: C, 59.17; H, 6.34. Found: C, 59.36; H, 6.09. IR Data: 3290 cm^{-1} (NH); 1745 cm^{-1} (acetyl); 1650 cm^{-1}, 1550 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl). Loss of absorption at 3420 cm^{-1} (No OH).(1)

The mother liquor yielded spherical, cottony masses of crystalline material. The yield was 18.0 g. (44.4 %), m. p. 211-212° dec. Anal.--Calcd. for $C_{18}H_{23}O_7N$: C, 59.17; H, 6.34. Found: C, 59.36; H, 6.09. IR Data: 3290 cm^{-1} (NH); 1745 cm^{-1} (acetyl); 1650 cm^{-1}, 1550 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl). Loss of absorption at 3420 cm^{-1} (No OH).(1)

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(1). The isolation of two products with such widely differing melting points cannot readily be explained. One reason could be the formation of a 3,6-O-benzylidene derivative. Another reason could be a change in the configuration about the benzylidene anomeric carbon atom. However, ther is no precedent established in the literature to confirm either reason.

PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-α-D GLUCOPYRANOSIDE (26):

Methyl 2-acetamido-3-O-acetyl-4,6-benzylidene-2-deoxy-α-D glucopyranoside (m. p. 302-304°) (15.0 g., 0.041 mole) in fifty per cent aqueous acetic acid (172.5 ml.) was refluxed with stirring for thirty minutes at 100°. After standing two hours, the solvents were evaporated *in vacuo*. A distinct odor of acetic acid and benzaldehyde was present at this time. Absolute ethanol and benzene were added with each being evaporated *in vacuo* between each addition to remove last traces of benzaldehyde and acetic acid. The remaining product was dissolved in absolute ethanol and ether and petroleum ether (b. p. 30-60°) added to turbidity. The product crystallized upon standing in the freezer for two hours. The yield was 5.4 g. (47 %), m. p. 177-179°. IR Data: 3420 cm^{-1} (OH); 3240 cm^{-1} (NH); 1710 cm^{-1} (acetyl); 660 cm^{-1}, 1570 cm^{-1} (CONH); 890 cm^{-1} (OCH_3). Loss of phenyl absorption peaks at 690 cm^{-1} and 740 cm^{-1}.

The 3-O-acetyl derivative (m. p. 211-212°) (5.0 g., 0.014 mole) in fifty per cent aqueous acetic acid (57.5 ml.) was refluxed for thirty minutes at 100°. The solvents were evaporated *in vacuo* to a syrup which was treated as above. The syrup was treated with various solvents and combinations of solvents, including benzene, toluene, ether, Skelly C, methanol ethanol, and ethyl acetate, in an attempt to induce crystallization. No crystalline product was

isolated. The yield of the syrup was 3.7 g. (95 %).

PREPARTION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-TRITYL-α-D-GLUCOPYRANOSIDE (31):

To anhydrous pyridine (85 ml.) was added methyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (4.5 g., 0.016 mole) and triphenylchloromethane (4.4 g., 0.016 mole). This mixture was allowed to stir at room temperature for forty-eight hours. The reaction mixture was poured onto ice chips (250 g.) and chloroform (150 ml.) was added. This mixture was allowed to stir until all ice had melted. The layers were separated and the aqueous layer extracted four times with 50 ml. portions of chloroform. All chloroform layers were combined and washed with four 20 ml. portions of a saturated solution of sodium bicarbonate. The chloroform layer was filtered through anhydrous sodium sulfate and evaporated in vacuo. The remaining residue was washed with toluene to remove last traces of pyridine. The white residue was dissolved in absolute ethanol and upon gentle concentration with low heat the product crystallized. The yield was 5.2 g. (62 %), m. p. 233-235° dec. Anal.--Calcd. for $C_{30}H_{33}O_7N$: C, 69.35; H, 6.34. Found: C, 69.36; H, 7.0. IR Data: 3400 cm^{-1} (OH); 3230 cm^{-1} (NH); 1740 cm^{-1} (acetyl); 1650 cm^{-1}, 1550 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1} (phenyl).

The syrup from prededing preparation and triphenylchloromethane

(3.7 g., 0.014 mole) were dissolved in anhydrous pyridine (75 ml.) and stirred for ninety-six hours. The reaction mixture was worked up as described above. However, no products could be crystallized from absolute ethanol. The syrup foamed and the foam was dired, digested with ether, and filtered with suction. The yield was 1.84 g. (25.4 %), m. p. 222-224° dec. IR Data: 3400 cm^{-1} (OH); 3340 cm^{-1} (NH); 1730 cm^{-1} (acetyl); 1650 cm^{-1}, 1540 cm^{-1} (CONH); 890 cm^{-1} (OCH_3); 690 cm^{-1}, 740 cm^{-1}, 760 cm^{-1} (phenyl).

ATTEMPTED PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-TRITYL-α-D-RIBO-HEXOPYRANOSIDE-4-ULOSE USING PHOSPHOROUS PENTOXIDE (22,23):

Anhydrous phosphorous pentoxide (0.168 g., 0.00118 mole) was dissolved in anhydrous dimethyl sulfoxide (13.0 ml., 14.3 g., 0.183 mole). To this solution was added methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (1.0 g., 0.0019 mole) and the reaction mixture shaken at room temperature for seven days. The reaction mixture was poured into a mechanically stirred mixture of cold chloroform (65 ml.) and five per cent sodium bicarbonate solution (40 ml.) and stirred for thirty minutes. The layers were separated, the aqueous layer extracted three times with 30 ml. portions of cold chloroform, and all chloroform layers combined and evaporated *in vacuo*. The remaining syrup would not crystllize so ether (50 ml.) was added and allowed to stand overnight. The ether

was evaporated *in vacuo* leaving a white solid. The solid was suspended in ether (25 ml.) and Skelly C (25 ml.) and filtered with suction. The melting point and IR spectra were identical with that of the starting material.

ATTEMPTED PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-TRITYL-α-D-*RIBO*-HEXOPYRANOSIDE-4-ULOSE USING ACETIC ANHYDRIDE (32, 33):

Acetic anhydride (1.2 ml., 1.3 g., 0.013 mole) was added to anhydrous dimethyl sulfoxide (8.2 ml., 9.0 g., 0.12 mole) and methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (1.0 g., 0.0019 mole) was dissolved in this mixture. The reaction mixture was shaken at room temperature for three days, then worked up as previously described. The white solid from the ether digestion was recrystallized from ethanol upon the addition of a small amount of water. The yield was 0.300 g. (30.5 % based on oxidized product), m. p. 218-220° dec. *Anal.*--Calcd. for $C_{30}H_{31}O_7N$: C, 69.62; H, 6.04. Found: C, 67.43; H, 6.26. IR Data: Showed hydroxyl group absorption at 3400 cm^{-1}.

The procedure was run again using acetic anhydride (4.0 ml., 4.3 g., 0.043 mole), DMSO (6.0 ml., 6.6 g., 0.084 mole) and methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (1.0 g., 0.0019 mole). At the end of twenty-nine hours the reaction mixture was worked up as before. The yield from the

ether digestion was 0.100 g. (10.2 % based on oxidized product), m. p. 221-223°. IR Data: Retained hydroxyl absorption at 3400 cm^{-1}.

The methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (solidified foam, m. p. 222-224°) was divided into two samples of 0.900 g. each. The first sample was subjected to oxidation with acetic anhydride (1.1 ml., 1.2 g., 0.012 mole) and DMSO (7.4 ml., 8.1 g., 0.10 mole). The reaction mixture was shaken for eight days at room temperature and worked up as before. No product was isolated.

The second sample was subjected to oxidation with phosphorous pentoxide (0.16 g., 0.00011 mole) and DMSO (12.9 g., 0.165 mole). The reaction mixture was shaken at room temperature for four days and then worked up as before. No crystalline product was isolated. However, when two drops of an alcoholic solution of the syrupy residue was added to 3 ml. of 2,4-dinitrophenylhydrazine test solution a cloudy solution formed upon cooling. The cloudiness disappeared upon heating and returned upon cooling. The yield of the syrupy residue was 0.600 g. (66.9 % based on oxidized products).

ATTEMPTED PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-TRITYL-α-D-RIBO-HEXOPYRANOSIDE-4-ULOSE TOSYLHYDRAZONE (24):

Methyl 2-acetamido-3-O-acetyl-3-deoxy-6-O-trityl-α-D-ribo-hexopyranoside-4-ulose (0.600 g. syrupy residue, 0.00115 mole)

and p-toluenesulfonylhydrazine (0.216 g., 0.00115 mole) were suspended in benzene (60.0 ml.). The flask was equipped with a Dean-Stark apparatus and condenser and the reaction mixture was refluxed for seventeen hours. The benzene was evaporated in vacuo to a syrupy residue which crystallized after addition of water and methanol and evaporation of both at 40°. However, no prodcut was obtained upon recrystallization from methanol-water.

PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-α-D-GLUCOPYRANOSIDE USING A ZINC CHLORIDE CATALYST:

Methyl 2-acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (m. p. 211-212°) (5.0 g., 0.014 mole) was dissolved in 50 ml. of absolute ethanol. This solution was added to 200 ml. of water containing 3.0 g. of zinc chloride. This mixture was refluxed for four hours. At this time, 50 ml. of distillate was removed and material precipitated in the reaction flask. Two grams of zinc chloride were added and the solution refluxed an additional forty-five minutes. At this time, 75 ml. of distillate was removed and no precipitation took place in the reaction flask. The distillate had a very strong odor of benzaldehyde. The reaction mixture was allowed to cool to room temperature and was then treated with 78 g. of Rexyn I-300 (H-OH) resin (Fischer Chemical Company). The resin suspension was stirred until all resin turned from blue to light tan. The resin was filtered off and the solvents evaporated

in vacuo. The remaining syrup was washed with benzene, the benzene evaporated *in vacuo*, and dissolved in acetone at room temperature. The acetone was evaporated *in vacuo* at room temperature and the syrup foamed and hardened. The yield was 3.7 g. (95 %). The melting point was very indefinite (78-92°). IR Data: 3350 cm^{-1} (OH); 3290 cm^{-1} (NH); 1735 cm^{-1} (acetyl); 1650 cm^{-1}, 1540 cm^{-1} (CONH); 890 cm^{-1} (OCH_3). Loss of absorption at 690 cm^{-1} and 740 cm^{-1}. (No phenyl group present).

PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-MESYL-α-D-GLUCOPYRANOSIDE (35):

Methyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (dried from preceding reaction) (2.4 g., 0.0087 mole) was dissolved in anhydrous pyridine and cooled to -20° using a calcium chloride-crushed ice bath. Methanesulfonyl chloride (0.74 ml., 1.1 g., 0.0096 mole) was added dropwise with stirring. The reaction mixture was stored at 0° for twenty-four hours, then at 22° for twenty-four hours. At the end of this time the reaction mixture was broken down by the dropwise addition of water. The reaction mixture was then poured onto ice chips and 60 ml. of chloroform added with stirring. The layers were separated and the aqueous layer extracted with a 30 ml. portion of chloroform. All chloroform layers were combined and evaporated *in vacuo*. Various solvents (benzene, toluene, acetone, methanol, ethanol) were used in an attempt to

crystallize the resulting syrup. After the evaporation of acetone *in vacuo* at room temperature, the syrup foamed and hardened. The yield was 0.700 g. (23.3 %). The melting point was very indefinite (85-92°). IR Data: 3360 cm^{-1} (OH); 3290 cm^{-1} (NH); 1740 cm^{-1} (acetyl); 1640 cm^{-1}, 1530 cm^{-1} (CONH); 1165 cm^{-1}, 1345 cm^{-1} (sulfonate); 890 cm^{-1} (OCH_3).

ATTEMPTED PREPARATION OF METHYL 2-ACETAMIDO-3-O-ACETYL-2-DEOXY-6-O-MESYL-α-D-*RIBO*-HEXOPYRANOSIDE-4-ULOSE (22, 23):

Anhydrous phosphorous pentoxide (0.170 g., 0.00119 mole) was dissolved in DMSO (13.0 ml., 14.4 g., 0.183 mole) and this solution was added to the methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-mesyl-α-D-glucopyranoside (0.700 g., 0.00197 mole) and shaken until dissolved. The mixture was stirred at room temperature for seven days and then worked up as described for the other oxidation reactions. No product was obtained.

RESULTS AND CONCLUSIONS

In the first synthetic scheme, two new compounds were isolated, methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-ribo-hexopyranoside tosylhydrazone (VI) and methyl 2-acetamido-4,6-O-benzylidene-2,3-dideoxy-α-D-ribo-hexopyranoside (VII). In the crystallization of the tosylhydrazone, it was found that care must be taken not to heat the methanolic-water solution to boiling. Heating to the boiling point of the solvent caused decomposition of the product to the tosylhydrazone of benzaldehyde. This was proven by synthesis of the tosylhydrazone of benzaldehyde and comparing the melting points of the decomposition product and the synthesized product. The melting points were found to be identical.

Thin layer chromatography of the oil and syrup isolated from the acid hydrolysis of the benzylidene group from the 2,3-dideoxy compound (VII) did give identical Rf values for each phase. This is a good indication that each phase contains the same product. However, attempts to crystallize the colorless fractions collected from elution of the alumina columns did not yield a product that could be analyzed. Crystallization was probably hindered by the presence of too many impurities.

In the second synthetic scheme, two new compounds were isolated, methyl 2-acetamido-3-O-acetyl-2-deoxy-α-D-glucopyranoside (X) and methyl 2-acetamido-3-O-acetyl-2-deoxy-6-O-trityl-α-D-glucopyranoside (XI). The attempted oxidations of the 6-O-trityl compounds were

possibly blocked due to steric hindrance by the large bulky trityl groups. The retention of hydroxyl group absorption at 3400 cm^{-1} is a fairly good indication of no reaction at the C-4 hydroxyl group.

The oxidation procedure using DMSO and acetic anhydride may lead to acetylation of the free hydroxyl group. The carbon-hydrogen analysis of the compound (XII) isolated from the acetic anhydride-DMSO reaction mixture compares more closely to the theoretical carbon-hydrogen of the acetylated compound, (Calcd. for $C_{32}H_{35}O_8N$: C, 68.43; H, 6.28), than it does to the theoretical carbon-hydrogen of the oxidized compound.

It was found that zinc chloride could be used to remove the benzylidene group. However, it was difficult to isolate the product from the reaction mixture.

Theoretically, mono-mesylation of the primary hydroxyl would be ideal as the mesyl group is relatively small and should allow oxidation of the C-4 hydroxyl group. However, the conditions of the reaction are rather extreme and are hard to control. Also the amounts of reactants used in this procedure were relatively small and may have led to the difficulty in isolating the desired product.

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