Comparison of columns 4 and 5 to $(k_3)_n$, which we assume defines the "normal" kinetic specificity of CT, suggests that in acylation with *p*-nitrophenylates the kinetic and/or binding partiality of CT for acetyl-L-tryptophan is lower than under "normal" circumstances.[73] To isolate the cause requires a separation of k_2 and K_s.[74]

The available stereochemical data (columns 7 and 8) provide stronger evidence for a diminished selectivity in acylation reactions with *p*-nitrophenyl esters.[75] If $(k_3)_{fast}/(k_3)_{slow}$ measures the kinetic stereoselectivity of CT, then $(k_c/K_m)_{fast}/(k_c/K_m)_{slow}$ should be much larger, since it reflects kinetic stereoselectivity plus the preferential productive binding of the more rapidly hydrolyzed isomer. The methyl esters fullfil this expectation but the *p*-nitrophenyl esters do not. With APME, for example, the predicted[76] total stereoselectivity is $\sim 2 \times 10^6$ while the observed figure is $>7 \times 10^5$. For APNPE, the prediction is the same and the discrepancy between prediction and observation is at least 10^3. It appears that this discrepancy arises from an unexpectedly high reactivity for D-APNPE with CT.

Among possible explanations for the NPE effect are the following two. First, methyl and *p*-nitrophenyl esters may have different hydrogen-bonding requirements.[23] The generality of this statement is limited by the observation that amides and methyl esters show the same structural selectivity[19] although their hydrogen-bond requirements should be quite dissimilar. Second, *p*-nitrophenyl esters may be capable of entering into multiple productive binding modes, contrary to the usual assumptions.[19,45,73]

(73) The small $(k_c/K_m)_nK_i$ for AcGlyCOME probably illustrates the relatively great tendency for it and $AcGlyCONH_2$ (but not AcGlyCONPE?) to enter into nonproductive binding. See the population analysis of ref 45.

(74) Hess and coworkers recently reported k_2 and K_s for L-AcTrpCOEE and L-AcPheCOME and estimated them for L-AcTrpCONPE. Their data accord with the analysis of Table II but do not permit resolution of the question we raise. See (a) K. G. Brandt, A. Himoe, and G. P. Hess, *J. Biol. Chem.*, **242**, 3973 (1967); (b) A. Himoe, K. G. Brandt, R. J. DeSa, and G. P. Hess, *ibid.*, **244**, 3483 (1969).

(75) Reference 53 reports stereoselectivity in acylation with the enantiomeric azlactones of N-benzoyltyrosine less than in deacylation of the resultant acylenzymes.

(76) Population analysis[45] shows productive binding for L-AcPheCONH$_2$ 300 times more favorable than for the D isomer. Couple 300 to the 8×10^4 of column 9 and 2×10^6 results.

The Synthesis and Proof of Structure of Perosamine (4-Amino-4,6-dideoxy-D-mannose) Derivatives[1]

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Contribution from the Department of Chemistry, Wayne State University, Detroit, Michigan 48202. *Received October 23, 1969*

Abstract: The synthesis and structure of proof of derivatives of 4-amino-4,6-dideoxy-D-mannose are described. These compounds are shown to be identical with the corresponding derivatives of perosamine, the carbohydrate moiety of the antibiotic perimycin. Most critical to the synthetic sequence was the ability to change the direction of the epoxide opening of methyl 3:4-anhydro-6-deoxy-α-D-talopyranoside (**3a**) by structure modification, so as to favor attack of azide ion at C-4 rather than at C-3. The ratio of C-3:C-4 attack on **3a** was ~1:3 but could be altered to a 4:1 ratio by benzoylating the C-2 hydroxyl prior to azide opening of the epoxide.

Efforts in this laboratory have led to recent disclosures of the synthesis and chemistry of six of a possible eight members of a new class of carbohydrates, the 4-amino-4,6-dideoxy-D-hexoses having the glucose,[2a] galactose,[2b] idose,[3] altrose,[3] gulose,[4] and talose[5] configurations. The importance of these new aminosugars is underscored by numerous reports of their occurrence and isolation from a variety of natural sources.[6a-i] As an extension of these efforts, routes to 4-amino-4,6-dideoxy-D-mannose (**1**) were investigated. As this work was nearing completion, Schaffner and Lee reported[6i] the isolation of a basic carbohydrate moiety, perosamine, from the acid hydrolysis of the

(1) Preliminary communication: C. L. Stevens, S. K. Gupta, R. P. Glinski, K. G. Taylor, P. Blumbergs, C. P. Schaffner, and C. H. Lee, *Carbohyd. Res.*, 7, 502 (1968). Since the appearance of this communication, an alternate synthesis of perosamine derivatives has appeared: J. S. Brimacombe, O. A. Ching, and M. Stacey, *ibid.*, 8, 498 (1968).

(2) (a) C. L. Stevens, P. Blumbergs, F. A. Daniher, D. H. Otterbach, and K. G. Taylor, *J. Org. Chem.*, 31, 2822 (1966); (b) C. L. Stevens, P. Blumbergs, and D. H. Otterbach, *ibid.*, **31**, 2817 (1966).

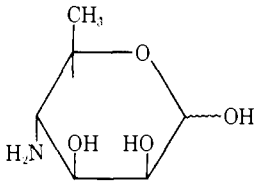
(3) C. L. Stevens, P. Blumbergs, J. P. Dickerson, and D. Chitharanjan, Abstracts, 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965, p 5C.

(4) C. L. Stevens, J. P. Dickerson, and K. G. Taylor, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, p 17C.

(5) (a) C. L. Stevens, R. P. Glinski, and K. G. Taylor, Abstracts, 152nd National Meeting of the American Chemical Society, New York, N. Y., Sept 1966, p 16D; *J. Org. Chem.*, 33, 1586 (1968); alternate syntheses have been reported since; (b) J. Jary, P. Novák, Z. Ksandr, and Z. Samek, *Chem. Ind.* (London), 1490 (1967); (c) J. Jarý and P. Novák, *Collect. Czech. Chem. Commun.*, **33**, 1744 (1968); (d) S. W. Gunner, W. G. Overend, and N. R. Williams, *Carbohyd. Res.*, 4, 498 (1967).

(6) (a) C. L. Stevens, K. Nagaranjan, and T. H. Haskell, *J. Org. Chem.*, 27, 2991 (1962); (b) C. L. Stevens and F. A. Daniher, *J. Amer. Chem. Soc.*, **84**, 1552 (1963); (c) R. W. Wheat, E. L. Rollins, and J. M. Leatherwood, *Biochem. Biophys. Res. Commun.*, **9**, 120 (1962); (d) C. L. Stevens, P. Blumbergs, F. A. Daniher, R. W. Wheat, A. Kiyomoto, and E. L. Rollins, *J. Amer. Chem. Soc.*, **85**, 3061 (1963); (e) C. L. Stevens, P. Blumbergs, D. H. Otterbach, J. L. Strominger, M. Matsuhashi, and D. N. Dietzler, *ibid.*, **86**, 2937 (1964); (f) C. L. Stevens, P. Blumbergs, F. A. Daniher, J. L. Strominger, M. Matsuhashi, and D. N. Dietzler, *ibid.*, **86**, 2939 (1964); (g) C. L. Stevens, G. E. Gutowski, K. G. Taylor, and C. P. Bryant, *Tetrahedron Lett.*, 649 (1967); (h) B. Jann and K. Jann, *Europ. J. Biochem.*, **2**, 26(1967); (i) C. H. Lee and C. P. Schaffner, *Tetrahedron Lett.*, 5837 (1966).

antibiotic perimycin. On the basis of the then available evidence, the tentative assignment of the 4-amino-4,6-dideoxy-D-mannose structure to perosamine was made.[6i] The synthesis and proof of structure of derivatives of 4-amino-4,6-dideoxy-D-mannose (**1**), as well as their



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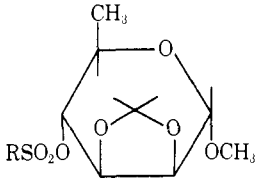
unequivocal identity with derivatives of natural perosamine form the subject matter of this paper.

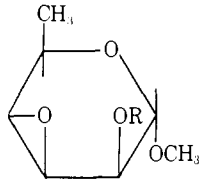
The first synthetic sequence which was undertaken involved an attempted double inversion at C-4 of methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-mesyl-α-D-mannopyranoside (**2**). The route consisted of four



2, R = CH_3

steps: (a) an SN2 displacement with acetate or benzoate anion; (b) saponification; (c) remesylation; (d) an SN2 displacement with azide ion. This procedure was used successfully in the synthesis of derivatives of 4-amino-4,6-dideoxy-D-glucose[2a] and -D-altrose;[3] the procedure was unsuccessful in the synthesis of the title compound. Step (a) of the double inversion sequence led to an unusual and unexpected ring-contraction rearrangement, giving furanoside products having the D-*talo* and L-*allo* configuration.[7]

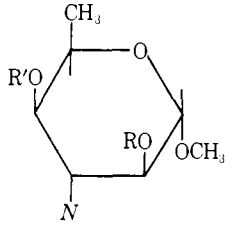
Alternately, an epoxide opening at C-4 of the methyl 3:4-anhydro-6-deoxy-α-D-talopyranoside (**3a**) with the



3a, R = H
3b, R = CC_6H_5 (=O)

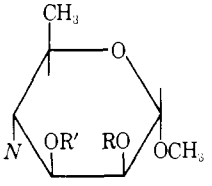
appropriate nitrogen-containing nucleophile seemed a reasonable approach. However, Jarý and coworkers[8] had noted that opening of the oxirane ring of the L enantiomer of **3a** with ammonia afforded products arising from exclusive attack at the C-3 position. Nevertheless, it was felt that with proper modifications, it might be possible to direct the attack of the nucleophile to the C-4 position to a considerably greater extent.

Acid hydrolysis of the ketal function of the known methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulfonyl-α-D-mannopyranoside[7] (**2**), followed by treatment of the 2,3-diol intermediate with sodium hydroxide, gave compound **3a**.[8] Reaction of **3a** with sodium azide in methyl Cellosolve containing ammonium chloride, followed by catalytic hydrogenation, afforded methyl 3-amino-3,6-dideoxy-α-D-idopyranoside (**4a**) in 45% overall yield for the two reactions. The physical constants of **4a** were in complete agreement with those of the L enantiomer reported earlier by Jarý, *et al.*[8] Reaction of **3a** with methanol–ammonia by a procedure previously successful in the L series,[8] improved the yield of the 3-aminosugar **4a** to 80%. Repeated fractional



(R = R′ = H unless otherwise stated)
4a, *N* = NH_2
4b, *N* = $N(CH_3)_2 \cdot HCl$
4c, *N* = N_3
4d, *N* = N_3; R = COC_6H_5
4e, *N* = NHAc
4f, *N* = NHAc; R = R′ = Ac
4g, *N* = $NHCOC_6H_5$; R = R′ = COC_6H_5

crystallizations of the mother liquors of this reaction yielded a small amount (less than 1%) of a new aminosugar, which was subsequently identified as the product of epoxide opening at C-4, methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**). Isolation of the



(R = R′ = H unless otherwise stated)
5a, *N* = NH_2
5b, *N* = NHAc
5c, *N* = NHAc; R = R′ = Ac
5d, *N* = $N(CH_3)_2$; R = R′ = Ac
5e, *N* = N_3
5f, *N* = N_3; R = R′ = COC_6H_5(H)
5g, *N* = N_3; R = R′ = H(COC_6H_5)
5h, *N* = $NH{-}C(=O){-}OCH_3$
5i, *N* = $NHCH_3 \cdot HCl$
5j, *N* = SO_2CH_3
5k, *N* = $N(CH_3)_2$
5l, *N* = NHAc·HBr
R = R′ = Ac

L enantiomer of **5a** had not been reported previously.[8] It was clear, however, that the low yield rendered this route inadequate for the synthesis of **5a**. For purposes of further characterization, **5a** was converted into the crystalline hydrochloride, *N*-acetyl (**5b**) and triacetyl (**5c**) derivatives.

Reaction of **3a** with aqueous dimethylamine produced a mixture of two *N*,*N*-dimethylaminosugars from opening of the epoxide at both C-3 and C-4. Under similar conditions in the L series, separation of the two *N*,*N*-dimethylaminosugars was achieved *via* an elaborate column chromatography yielding both isomers as

(7) C. L. Stevens, R. P. Glinski, K. G. Taylor, P. Blumbergs, and F. Sirokman, *J. Amer. Chem. Soc.*, 88, 2073 (1966).

(8) J. Jarý, K. Čapek, and J. Kovář, *Collect. Czech. Chem. Commun.*, 28, 2171 (1963).

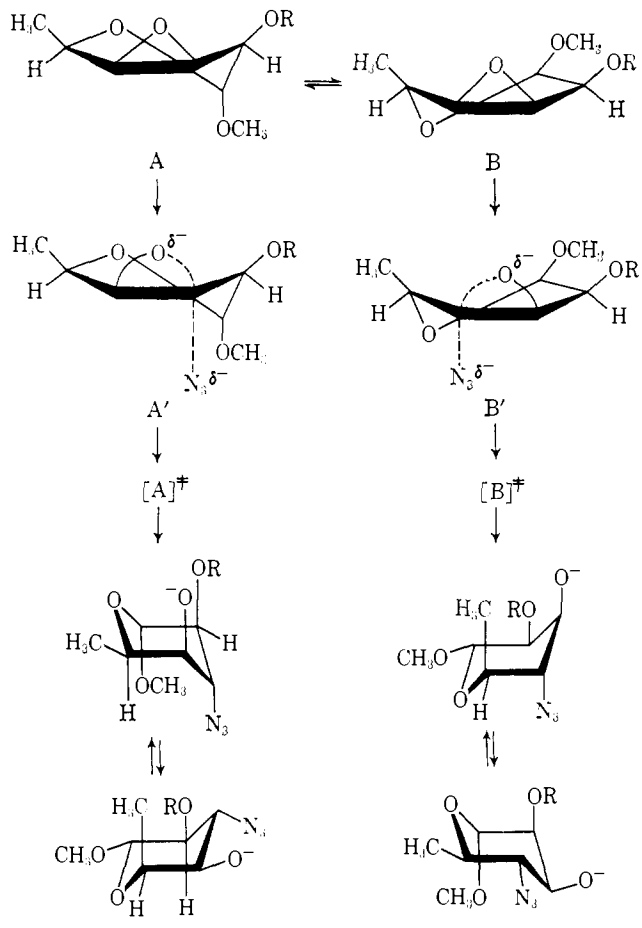
syrups.[8] More conveniently in our hands, the mixture was treated with an isopropyl alcohol–hydrogen chloride solution. Methyl 3,6-dideoxy-3-(*N*,*N*-dimethylamino)-α-D-idopyranoside hydrochloride (**4b**) crystallized in 70% overall yield. Acetylation of the mother liquors, followed by column chromatography over Woelm Grade 1 alumina, resulted in a 5.7% overall yield of crystalline methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*-dimethylamino)-α-D-mannopyranoside (**5d**).

Thus in summary, openings of the 3:4 epoxide function of **3a** exhibit a marked preference for attack at C-3.

Since the primary aim was to obtain the product resulting from opening at C-4, it was necessary to modify the structure of **3a** in an attempt to destabilize the transition state leading to attack at C-3 relative to a transition state where opening at C-4 might be preferred.

Because the conformation of compound **3a** is not stabilized in any way, it is considered to exist as an equilibrium mixture of the two half-chair conformations A and B (see Scheme I). Generally, *trans*-diaxial opening is preferred to *trans*-diequatorial opening.[9,10] However, a diaxial opening may give rise to two different products, depending upon which conformation the ring assumes during the attack. The major product of the azide reaction, **4c**, may be pictured as arising *via* the expected *trans*-diaxial attack at C-3 of conformation A in which R = H. It is likely that bond breaking will have progressed to a considerable extent in any of the possible transition states because of the strain inherent in the three-membered ring. Moreover, transformation from a half-chair to a chair conformation probably begins to occur almost simultaneously with relief of strain, so as to establish steric relationships closely resembling those expected in the normal chair conformation. Thus, the transition states are considered to resemble products and A′ and B′ are drawn with bent bonds to help illustrate these points.

Scheme I



When R = H, the transition state resulting from the attack of azide ion on C-3 in A is lower in energy than that resulting from attack on C-4 in B as is indicated by the product distribution. Increased steric bulk at C-2 was predicted to affect transition state $A^{\ddagger}$ (with its C-2 axial-OH) in an adverse manner to a greater extent than transition state $B^{\ddagger}$ (with its C-2 equatorial-OH). The overall effect might be a relative destabilization of transition states leading to opening at C-3, with respect to those fostering attack at C-4.

While it is true that increasing the effective size of the substituent at C-2 may serve to alter the conformational equilibrium, decreasing the proportion of A to B, the magnitude of such a change is not necessarily meaningful. Without information on the relative reaction rates from each conformer, the magnitude of such a change in the ground state does not necessarily reflect comparable changes in the corresponding activated complexes.

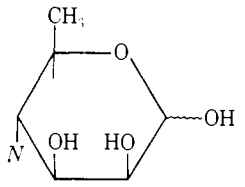
The benzoyl group was chosen to increase bulk about C-2 because of its ease of formation and removal. Thus, reaction of azide anion with methyl 3:4-anhydro-2-*O*-benzoyl-6-deoxy-α-D-talopyranoside (**3b**) under conditions identical with those utilized for compound **3a**, produced products resulting predominantly from attack at C-4 instead of C-3. Five azide-containing products were isolated and separated by preparative tlc on silica gel H: **5e**, mp 81.5–82.5° (20%); **5g**, an oil (4%); **5f**, mp 116–117° (8%); **4c**, mp 52–53° (3%); **4d**, mp 106–107° (11%). Compounds **5e**, **5f**, and **5g** arise from oxirane opening at C-4 and **4c** and **4d** arise from opening at C-3. When the reaction was performed using a sealed tube at 110°, only azides **5e** (43%), **4c** (9%), and **4d** (2%) could be isolated by preparative tlc. In order to demonstrate quantitively the influence of the benzoyl group at C-2, **3a** and **3b** were subjected to the reaction with azide anion and the products were analyzed as their trimethylsilyl derivatives by vpc.[11] The results of vpc analysis agreed closely with actual separations obtained on preparative tlc. The data are tabulated in the Experimental Section. Although significant debenzoylation occurred in the reaction mixture, close examination by tlc indicated that **3b** is not converted into **3a** since no **3a** could be detected as the reaction progressed. Thus, since debenzoylation occurred after epoxide opening, products **4c** and **4d** arose from **3b** and not **3a**, and the product ratio is truly a measure of the effect of the benzoyl group.

Catalytic hydrogenation of **5e** afforded methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**). The physical constants of **5a** were in agreement with those of methyl perosaminide, prepared by the methanolysis of perosamine. Acetylation of **5a** with acetic anhydride in methanol gave methyl 4-acetamido-4,6-dideoxy-α-D-mannopyranoside (**5b**). A mixture melting point of **5b** with methyl *N*-acetylperosaminide[12] prepared

(9) G. Charalambous and E. Percival, *J. Chem. Soc.*, 2443 (1954).
(10) R. E. Parker and N. S. Isaacs, *Chem. Rev.*, **59**, 737 (1959).
(11) C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, 85, 2497 (1963).

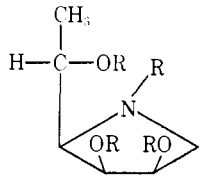
from the natural material was undepressed, and the infrared spectra (KBr) of the two compounds were identical. Derivatives **5c**, **5d**, **5h**, **5i**, and the hydrochloride salt of **5a** were prepared from **5a** by procedures used with other 4-amino-4,6-dideoxyhexoses.[1,2]

Thus far, attempts to isolate perosamine hydrochloride (**6a**) from the acid hydrolysis of **5a** or several derivatives of **5a** have been unsuccessful. Paper chromatography (pc) and tlc suggested that extensive decomposition had occurred. Acidic hydrolysis of compound **5e**, however, gave crystalline 4-azido-4,6-dideoxy-D-mannose (**6b**). Mutarotation studies ($[\alpha]^{27}D$



6a, $N = NH_2 \cdot HCl$
6b, $N = N_3$ (C_1–OH is β)
6c, $N = N(CH_3)_2 \cdot HCl$

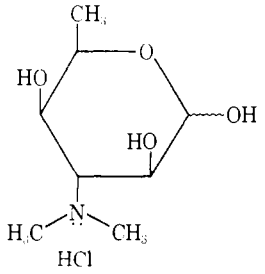
$+21.6° \rightarrow +57.4°$ in 0.5 hr (*c* 1.0, CH_3OH)) indicated that **6b** possessed the β configuration at C-1. Unfortunately, catalytic hydrogenation of **6b** in neutral or in acidic media failed to give the desired aminosugar **6a**. Instead, condensation of the C-4 amine with the C-1 aldehyde function, followed by reduction, afforded a pyrrole derivative (**7a**). The structure proof of **7a**



7a, R = H
7b, R = $COCH_3$

(and acetylated derivative **7b**) is summarized in the Experimental Section. The aldehyde group of **6b** could be selectively reduced in the presence of the azido group at C-4 with sodium borohydride to afford crystalline 4-azido-4,6-dideoxy-D-mannitol (**9**).

In contrast to the hydrolyses of aminosugars **4a** and **5a** (which led to extensive decomposition), the hydrolyses of the corresponding *N*,*N*-dimethylamino homologs proceeded smoothly and furnished crystalline-free sugar hydrochlorides. Thus, hydrolysis of **4b** gave **8**



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in 60% yield and similar conditions applied to **5d** produced **6c** in 50% yield.

(12) A sample of methyl *N*-acetylperosaminide was supplied for comparison by Professor C. P. Schaffner; see ref 6i.

Structure Proof

In addition to using the conventional methods of structure proof, a new method of degradation of some of these compounds was developed.

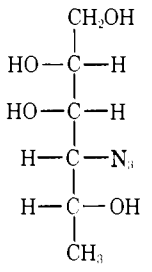
Spectroscopic Studies. The infrared spectra and the nmr spectra of the key compounds were found to be consistent with the structures proposed. The mass spectra of the *N*-acetyl and the triacetyl derivatives of **4a** and **5a** were also consistent with the structures. The mass fragmentation of **5b** and **5c** followed the same pattern as the derivatives of other methyl 4-amino-4,6-dideoxyhexoses.[3a,7]

Periodate Oxidation. The normal procedure was used for periodate oxidation of these sugars. An aqueous solution (0.05 *M*) of sodium metaperiodate was used in all the titrations. The periodate uptake was determined by iodometric titration with sodium arsenite.[13] As expected, **5a** consumed 2 mol of the reagent, whereas the *N*-acetyl derivative of **5a** (**5b**) consumed only 1 mol. Compound **4a** and the *N*-acetyl derivative of **4a** (**4e**) also gave the expected results, requiring 2 and 0 mol of periodate, respectively. Periodate oxidation of some azidosugars has been reported to give anomalous results.[14] However, the periodate oxidation of the azidosugars **4c**, **5e**, **6b**, and **9** proceeded according to theory, as can be seen from Table I.

Table I

Time (min)	Mol of periodate/mol of compound							
	4a	**4e**	**5a**	**5b**	**5e**	**4c**	**6b**	**9**
30		0.00	1.44		0.99	0.10	1.84	1.76
60	1.91	0.00	1.92	0.61	1.01		2.01	1.93
90	1.91	0.00	1.94	0.60	1.06	0.14	2.06	1.97
240	1.97	0.00	2.29	1.03	1.10	0.042	2.06	1.98
480					1.16	0.042	2.10	2.06
1440	2.01	0.00	2.13	1.05	1.44	0.042	2.19	2.19

Degradation Studies. The conventional degradation sequence of subjecting the compound in question to consecutive acid hydrolysis, periodate oxidation, bromine water oxidation, and, finally, acid hydrolysis to give the amino acid was attempted initially and failed



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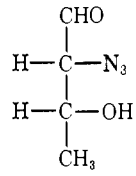
to give conclusive results. A successful new approach, however, was developed. The azidosugars **6b** and **9** were subjected to sodium metaperiodate oxidation. Both compounds led to the same product, *erythro*-2-azido-3-hydroxybutyraldehyde (**10**). Sodium borohydride reduction of **10** gave an oily amine which furnished a crystalline hydrogen oxalate salt (**11**) in 18% overall yield for the entire sequence.

(13) E. Müller and D. Friedberger, *Chem. Ber.*, 35, 2652 (1902).
(14) C. B. Varlow, R. D. Guthrie, and D. Murphy, *J. Chem. Soc.*, 3870 (1965).



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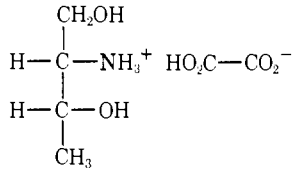

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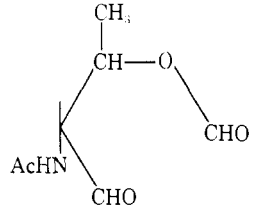
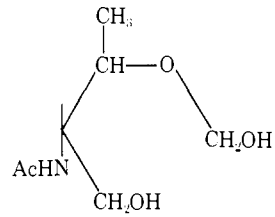
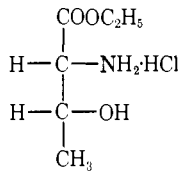
In order to establish conclusive identification of **11**, authentic samples of D- and L-allothreoninol, as well as D- and L-threoninol hydrogen oxalates were prepared.[15] The starting materials for these amino-alcohols were D- and L-threonine (obtained commercially) and D- and L-allothreonine (obtained by resolution[16] of commercial DL-allothreonine). Comparison of the product from degradation of **6b** and **9** with each of these four isomers revealed it to be identical with D-allothreoninol hydrogen oxalate **11**. The comparison was based on undepressed mixture melting points, comparable specific rotations, and superimposable infrared spectra (KBr). The infrared spectra (KBr) of D- and L-allothreoninol hydrogen oxalates were identical. Similarly, the infrared spectra (KBr) of D- or L-threoninol hydrogen oxalates were identical but different from the spectra of D- or L-allothreoninol salts. The main differences were in the regions of 3350, 1575, and 1050 cm^{-1}. Subsequently, a general method[17] was developed for degrading *N*-acetamido-4,6-dideoxyhexoses to the corresponding aminoalcohol hydrogen oxalates. Using this method, **5b** gave 20% overall yield of D-allothreoninol hydrogen oxalate **11**.



12



13



14

Experimental Section

All melting points were taken on a Thomas Hoover melting point apparatus and are uncorrected. Tlc was performed using silica gel H from Brinkman Instruments on 5 × 15 cm glass plates. Preparative tlc was carried out on 20 × 40 cm glass plates coated with a 1-mm thickness of silica gel H. The compounds were detected with 6 *N* sulfuric acid spray followed by baking at 110° for 10 min–0.5 hr. The bands on preparative plates were detected by spraying a 1-in. perpendicular band on both sides of the plates with sulfuric acid and baking. pK_a's were determined in aqueous 50% methanol. Vpc analyses were performed on an F & M Scientific Corporation instrument (Model 810) fitted with a flame-ionization detector. Microanalyses were performed by Midwest Microlab Inc., Indianapolis, Ind.

Methyl 6-Deoxy-4-*O*-methanesulfonyl-α-D-mannopyranoside (5j). Methyl 6-deoxy-2,3-isopropylidene-4-*O*-methanesulfonyl-α-D-mannopyranoside (**2**) was prepared according to the literature procedure.[7] A slurry of 40 g of **2** in 600 ml of 20% acetic acid was refluxed with vigorous stirring for 0.5 hr, until the solution became homogeneous. The solution was evaporated to dryness *in vacuo* and the last traces of acetic acid were removed by azeotropic distillation with toluene. The resulting colorless oil was crystallized from ethanol–pentane to yield 28 g (81%) of methyl 6-deoxy-4-*O*-methanesulfonyl-α-D-mannopyranoside (**5j**) with mp 81–84°. A sample was recrystallized from ethanol–*n*-pentane for analysis: mp 85–86.5°; $[\alpha]^{25}D$ +80.30 (*c* 1.05, $CHCl_3$).

Anal. Calcd for $C_8H_{16}O_7S$: C, 37.50; H, 6.25; S, 12.50. Found: C, 37.45; H, 6.00; S, 12.38.

Methyl 3,4-Anhydro-6-deoxy-α-D-talopyranoside (3a). A solution of 28 g of **5j** in 60 ml of ethanol was titrated with 2 *N* aqueous sodium hydroxide at 75° until the solution was permanently pink to phenolphthalein. Sodium methanesulfonate was filtered off and the solution evaporated to dryness. Extraction of the residue with dry ethyl acetate at room temperature, removal of the solvent, and recrystallization of the residue from warm light petroleum ether (bp 40–60°) gave crystalline methyl 3,4-anhydro-6-deoxy-α-D-mannopyranoside (**3a**): 14.1 g (80%); mp 64–66°. One more recrystallization of a sample gave colorless needles: mp 65–66°; $[\alpha]^{23.5}D$ +114.37 (*c* 1.1, H_2O) [lit.[8] mp 65°; $[\alpha]^{18}D$ −116° (*c* 1.20, H_2O) for the L enantiomer of **3a**].

Methyl 3-Amino-3,6-dideoxy-α-D-idopyranoside (4a) and Hydrochloride Salt. a. A solution of 1 g of **3a**, 80 mg of ammonium chloride, and 2.5 g of sodium azide in 75 ml of dry methyl Cellosolve was refluxed with stirring for 4 hr. The hot reaction mixture was diluted with excess anhydrous ether (200–300 ml) and the salts were removed by filtration using a Celite bed. The filtrate was evaporated to dryness. The residue was washed with anhydrous ether and filtered through Celite. This process was repeated until all inorganic materials were removed and a slightly yellow oil (0.95 g, 75%) was obtained. The product was pure enough for further reactions as indicated by tlc and infrared analysis. The yellow oil was dissolved in 30 ml of methanol and hydrogenated in the presence of 10% Pd–C (100 mg) at atmospheric pressure and room temperature for 2 hr. The reaction mixture was filtered through Celite and the filtrate was evaporated to dryness *in vacuo* to give a slightly yellow oil. The oil gave 510 mg (45% overall yield for two steps) of colorless crystals of methyl 3-amino-3,6-dideoxy-α-D-idopyranoside (**4a**) with mp 165–167°. A pure sample was obtained by one recrystallization from ethanol–ether: mp 166–167.5°; $[\alpha]^{28}D$ +91.1 (*c* 1.0, H_2O) [lit.[8] mp 168–169°; $[\alpha]^{20}D$ −93.83 (*c* 1.04, H_2O) for methyl 3-amino-3,6-dideoxy-α-L-idopyranoside].

Anal. Calcd for $C_7H_{15}NO_4$: C, 47.46; H, 8.47; N, 7.90, Found: C, 47.52; H, 8.66; N, 7.77.

b. A solution of 2 g of **3a** in 60 ml of methanol saturated with ammonia at 0° was heated in an autoclave for 30 hr at 120–130°. The brown reaction mixture was evaporated to dryness *in vacuo* and decolorized with activated charcoal in ethanol. The product was recrystallized from ethanol–ether to give 1.7 g (76.8%) of methyl 3-amino-3,6-dideoxy-α-D-idopyranoside (**4a**) with mp 164–166°.

On repeated fractional crystallizations from ethanol–ether, 3.5 mg of crystals (mp 152–154°) was obtained from the mother liquors. These crystals were subsequently identified as methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**).

For the preparation of the hydrochloride, 100 mg of the crystalline **4a** was dissolved in 1 ml of anhydrous isopropyl alcohol and a slight excess (by pH paper) of isopropyl alcohol–hydrogen chloride was added. On cooling, the hydrochloride salt of **4a** crystallized. The solvent was decanted and the crystals washed several times with anhydrous ether to remove any excess hydrogen chloride to afford 95 mg (79%) with mp 140–142° dec. A sample of the hydrochloride salt of **4a** was recrystallized once from ethanol–ether for analysis: mp 141–143°; $[\alpha]^{28}D$ +89.4 (*c* 1.0, MeOH).

Anal. Calcd for $C_7H_{16}NO_4Cl$: C, 39.35; H, 7.96; N, 6.55; Cl, 16.61. Found: C, 39.52; H, 7.72; N, 6.77; Cl, 16.87.

Methyl 3-Acetamido-2,4-di-*O*-acetyl-3,6-dideoxy-α-D-idopyranoside (4f). Compound **4a** (1 g) was acetylated using excess acetic

(15) T. Inui and T. Kaneko, *J. Chem. Soc., Jap.*, **82**, 1078 (1961).
(16) H. Seki, K. Koga, H. Matsuo, S. Ohki, I. Matsuo, and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **13**, 995 (1965).
(17) C. L. Stevens, S. K. Gupta, R. P. Glinski, G. E. Gutowski, and C. P. Bryant, *Tetrahedron Lett.*, 1817 (1968).

anhydride in pyridine at room temperature overnight. Standard work-up gave 1.5 g (87.6%) of crystalline triacetyl derivative (**4f**) with mp 133.5°. A pure sample was obtained by one recrystallization from chloroform–light petroleum ether (bp 40–60°): mp 134.5°; $[\alpha]^{28}D$ +61.5 (*c* 1.0, $CHCl_3$) [lit.[8] mp 143.5–144.5°; $[\alpha]^{21}D$ −61.25° (*c* 0.72, $CHCl_3$) for methyl 3-acetamido-2,4-di-*O*-acetyl-3,6-dideoxy-α-L-idopyranoside].

Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.35; H, 7.19; N, 4.43.

Methyl 3-Acetamido-3,6-dideoxy-α-D-idopyranoside (4e). A solution of 200 mg of **4a** in 20 ml of methanol was cooled (0°) and a twofold excess of acetic anhydride was added dropwise over a period of 30 min with stirring. The solution was allowed to stand at room temperature for 30 min. The solvent was removed *in vacuo* and the last traces of acetic anhydride were removed by azeotropic distillation with toluene. The residue crystallized to give 180 mg (72.7%, mp 163–164°) of methyl 3-acetamido-3,6-dideoxy-α-D-idopyranoside (**4e**). A pure sample was obtained by recrystallization from chloroform–petroleum ether (bp 40–60°): mp 164–165°; $[\alpha]^{25.8}D$ +86.7 (*c* 1.0, H_2O) [lit.[8] mp 164.5–165.5°; $[\alpha]^{23}D$ −83.95 (*c* 0.75, H_2O) for methyl 3-acetamido-3,6-dideoxy-α-L-idopyranoside].

Anal. Calcd for $C_9H_{17}NO_5$: C, 49.77; H, 7.76; N, 6.30. Found: C, 49.61; H, 7.96; N, 6.60.

Methyl 3-Benzamido-2,4-di-*O*-benzoyl-3,6-dideoxy-α-D-idopyranoside (4g). A solution of 350 mg of **4a** in 10 ml of dry pyridine was cooled (0°) and 4.5 mol/mol of distilled benzoyl chloride was added while stirring over a period of 0.5 hr. The solution was allowed to stand at room temperature for an additional hour and was poured over ice and stirred overnight. White crystals of the tribenzoyl derivative (**4g**) separated. Filtration yielded 794 mg (82%) of crystals with mp 127–129°. A sample was recrystallized from ether–*n*-pentane for analysis: mp 128–130°; $[\alpha]^{25.5}D$ +68.43 (*c* 0.96, $CHCl_3$).

Anal. Calcd for $C_{28}H_{27}NO_7$: C, 68.70; H, 5.56; N, 2.86. Found: C, 68.61; H, 5.46; N, 2.93.

Methyl 3,6-Dideoxy-3-(*N*,*N*-dimethylamino)-α-D-iodopyranoside Hydrochloride (4b). **a.** A solution of 100 mg of **4a** in 0.7 ml of 86% formic acid and 0.2 ml of 40% formalin solution was heated under reflux on a steam bath for 20 hr. The solvent was removed *in vacuo*. The residual dark brown oil was dissolved in 2 ml of methanol. The solution was cooled to 0° and 3 drops of acetic anhydride was added over a period of 30 min. The reaction mixture was applied to a Dowex 50-X2 (H^+) column. The column was washed with methanol to remove the neutral components. The basic material was eluted with 3% aqueous ammonium hydroxide in methanol. The resulting dark brown oil (70 mg) was evaporatively distilled *in vacuo* (0.01 mm/135–140° bath temperature) to yield a colorless oil. The oil was dissolved in isopropyl alcohol and treated with a slight excess (by pH paper) of hydrogen chloride in isopropyl alcohol. On cooling, crystals of the hydrochloride salt of **4b** formed. The solvent was decanted. The crystals were washed with anhydrous ether to remove excess hydrogen chloride to give 35 mg (26%) of methyl 3,6-dideoxy-3-(*N*,*N*-dimethylamino)-α-D-idopyranoside hydrochloride (**4b**) with mp 176–178°.

b. A solution of 3 g of compound **3a** in 50 ml of 25% aqueous dimethylamine was heated in an autoclave at 120–125° for 30 hr. Evaporation of the solvent *in vacuo* gave 3.9 g of a brown syrup which was decolorized by treatment with activated charcoal in ether solution. The resulting slightly yellow syrup was dissolved in isopropyl alcohol and a slight excess of isopropyl alcohol–hydrogen chloride solution was added. On cooling, crystals of methyl 3,6-dideoxy-3-(*N*,*N*-dimethylamino)-α-D-idopyranoside hydrochloride salt (**4b**) formed. Work-up as in method a gave 3 g (71%) of crystals with mp 186–188°. An analytically pure sample was prepared by recrystallizing a small sample from ethanol–ether: mp 187–188°; $[\alpha]^{25.8}D$ +65.43 (*c* 0.975, H_2O).

Anal. Calcd for $C_9H_{20}NO_4Cl$: C, 44.73; H, 8.34; N, 5.79; Cl, 14.67. Found: C, 44.91; H, 8.24; N, 6.32; Cl, 14.80.

The free base of **4b** was prepared by passing the hydrochloride salt in methanol through column of Dowex 1-X2 (OH^-). A colorless syrup was obtained: $[\alpha]^{24.8}D$ +83.9 (*c* 1.05, H_2O) [lit.[8] $[\alpha]^{25}D$ −82.29 (*c* 0.51, H_2O) for methyl 3,6-dideoxy-3-(*N*,*N*-dimethylamino)-α-L-idopyranoside].

Methyl 2,3-Di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*-dimethylamino-α-D-mannopyranoside (5d). The mother liquor (600 mg) from the previous experiment (method b) was concentrated *in vacuo*. The residue was dissolved in methanol and applied to a column of Dowex 1 × 2 (OH^-). Elution of the column with methanol gave 520 mg of a dark brown oil (free base) which was acetylated in pyridine using excess acetic anhydride at 0° for 2 hr and at room temperature overnight. Standard work-up gave 510 mg of light brown oil which was purified by chromatography using Woelm grade 1 alumina. Elution of the column with ether–*n*-pentane (4:1) afforded 310 mg (5.7% based on compound **3a**) of white crystals (mp 86–87°) of methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*-dimethylamino)-α-D-mannopyranoside (**5d**). A sample was recrystallized from ether–*n*-pentane for analysis: mp 87–88°; $[\alpha]^{25}D$ +64.51 (*c* 1.1, MeOH); nmr ($CDCl_3$) δ 1.27 (d, 3, $J_{5,6}$ = 5.5 Hz, C-5 CH_3), 2.01 (s, 3, C-3 C(O)CH_3), 2.09 (s, 3, C-2 C(O)CH_3), 2.32 (s, 6, C-4 N(CH_3)$_2$), 2.6 (t, 1, $J_{4,5}$ = $J_{3,4}$ = 8.2 Hz, C-4 *H*), 3.28 (s, 3, C-1 OCH_3), 3.69 (m, 1, C-5 *H*), 4.4 (d, 1, $J_{1,2}$ = 2.1 Hz, C-1 H), 5.1 (m, 2, C-2 *H*, C-3 *H*).

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.94; H, 8.02; N, 4.84. Found: C, 54.15; H, 8.16; N, 5.03.

3,6-Dideoxy-3-(*N*,*N*-dimethylamino)-D-idose Hydrochloride (8). A solution of 200 mg of **4b** in 200 ml of 1.5 *N* hydrochloric acid was heated on a steam bath for 6 hr. The colorless solution was carefully evaporated to dryness at 30° (0.1 mm). The residue colorless foam was washed several times with anhydrous ether to remove excess hydrogen chloride. The foam was crystallized from ethanol–ether to give 98 mg (52.5%) of compound **8** with mp 147–149°. Recrystallization of **8** from ethanol–ether gave an analytically pure sample: mp 149–150°; $[\alpha]^{25.8}D$ +30.94 (*c* 0.85, H_2O).

Anal. Calcd for $C_8H_{16}NO_4Cl$: C, 42.20; H, 7.96; N, 6.15. Found: C, 42.37; H, 8.14; N, 6.08.

Methyl 3,4-Anhydro-2-*O*-benzoyl-6-deoxy-α-D-talopyranoside (3b). A solution of 2 g of **3a** in 15 ml of pyridine was cooled to 0° and 1.5 equiv/equiv of benzoyl chloride was added dropwise over a period of 1 hr with vigorous magnetic stirring. The mixture was stirred for an additional hr at 0°. The reaction mixture became pink during the course of reaction. The reaction mixture was poured over ice with vigorous stirring. After some time a white solid separated. The solid was removed by filtration and washed with cold water. Crystallization of the solid from ether–pentane gave 2.65 g (80.3%) of colorless crystals, with mp 71–73°, of methyl 3,4-anhydro-2-*O*-benzoyl-6-deoxy-α-D-talopyranoside (**3b**). One more recrystallization from ether–*n*-pentane gave an analytically pure sample: mp 72.5–73.5°; $[\alpha]^{25.8}D$ +139.38 (*c* 0.98, $CHCl_3$).

Anal. Calcd for $C_{14}H_{16}O_5$: C, 63.63; H, 6.12; O, 30.30. Found: C, 63.69; H, 6.22; O, 30.11.

Reaction of 3b with Lithium Azide. **a.** A mixture of 1 g of **3b**, 2 g of lithium azide, 2.5 g of ammonium chloride, and 50 ml of methyl Cellosolve was refluxed for 36 hr with stirring. The reaction mixture was diluted with an excess of anhydrous ether (400–500 ml) while hot and the precipitated inorganic salts were removed by filtration using a Celite bed. The filtrate was evaporated to dryness, diluted again with ether, and filtered to remove the salts. This process was repeated until all the inorganic salts were removed. The resulting yellow oil (650 mg) was found to be a mixture of five components by tlc using ether–*n*-pentane (5:4) as the developing solvent (R_f's 0.13, 0.21, 0.33, 0.45, and 0.67). The five components were separated by preparative tlc using ether–*n*-pentane (5:4) as the developing solvent. Anhydrous ether was used to extract individual fractions from the silica gel. Filtration and concentration of the ether extracts *in vacuo* gave the following five azides.

(i) **Methyl 4-Azido-4,6-dideoxy-α-D-mannopyranoside (5e).** Fraction 1 (R_f 0.13) gave 157 mg (20.3%) of colorless crystals of **5e** with mp 81–83°. A sample was recrystallized once from ether–*n*-pentane for analysis: mp 81.5–82.5°; $[\alpha]^{27}D$ +126.9 (*c* 1.0, MeOH).

Anal. Calcd for $C_7H_{13}N_3O_4$: C, 41.38; H, 6.45; N, 20.68. Found: C, 41.13; H, 6.46; N, 20.59.

(ii) **Methyl 3-Azido-3,6-dideoxy-α-D-idopyranoside (4c).** Fraction 2 (R_f 0.21) gave 23 mg (3%) of colorless crystals of **4c** with mp 50–52°. Recrystallization from ether–*n*-pentane gave an analytically pure sample: mp 52–53°; $[\alpha]^{26}D$ +110.76 (*c* 1.05, MeOH).

Anal. Calcd for $C_7H_{13}N_3O_4$: C, 41.38; H, 6.45; N, 20.68. Found: C, 41.51; H, 6.64; N, 20.96.

The structure of **4c** was proved by hydrogenation of a solution of 100 mg in 5 ml of ethanol in the presence of 50 mg of 10% Pd-C for 2 hr. The catalyst was removed by filtration and the filtrate was concentrated *in vacuo* to give 70 mg (80%) of colorless crystals of methyl 3-amino-3,6-dideoxy-α-D-idopyranoside (**4a**) with mp 165–167°.

(iii) **Methyl 4-Azido-2(3)-*O*-benzoyl-4,6-dideoxy-α-D-mannopyranoside (5f).** Fraction 3 (R_f 0.33) gave a slightly yellow oil (**5f**,

Table II

	Reaction mixture a		Reaction mixture b	
	Amount indicated by vpc analysis	Amount isolated by tlc	Amount indicated by vpc analysis	Amount isolated by tlc
Azide **5e**	55.3 mg (17.4%)	38.5 mg (12.1%) mp 81–83°	131.3 mg (33.9%)	107 mg (27%) mp 81–83°
Azide **4c**	145 mg (45.5%)	99 mg (31.2%) mp 51–53°	72 mg (18.6%)	43 mg (11%) mp 50–52°

48 mg, 4%) which was decolorized by treatment with activated charcoal. The oil was homogeneous by tlc: $[\alpha]^{25}D$ −15.2 (*c* 1.18, $CHCl_3$).

A solution of 20 mg of the oil **5f** in 5 ml of ethanol was treated with a catalytic amount of sodium ethoxide at room temperature for 12 hr. The reaction mixture was neutralized with excess solid carbon dioxide. Removal of the solvent *in vacuo* and extraction of the residue with dry ethyl ether gave 10.2 mg (75%) of methyl 4-azido-4,6-dideoxy-α-D-mannopyranoside (**5e**) with mp 79–81°.

(iv) **Methyl 3-Azido-2-*O*-benzoyl-3,6-dideoxy-α-D-idopyranoside (4d).** Fraction 4 (R_f 0.45) gave 128 mg (11%) of colorless crystals of **4d** with mp 105–107°. The analytically pure sample was obtained by one more recrystallization from ether–*n*-pentane: mp 106–107°; $[\alpha]^{27}D$ +18.6 (*c* 1.0, MeOH).

Anal. Calcd for $C_{13}H_{13}N_3O_5$: C, 54.72; H, 5.58; N, 13.67. Found: C, 54.98; H, 5.67; N, 13.67.

For structure proof, a solution of 50 mg of **4d** in 10 ml of ethanol was hydrogenated in the presence of 40 mg of 10% Pd–C for 4 hr. Standard work-up gave 39 mg (85%) of an oil. The oil was dissolved in 3 ml of ethanol saturated with ammonia at 0°. The solution was heated in a sealed tube at 80° for 8 hr. The solvent was removed *in vacuo*. The resulting residue was dissolved in methanol and the solution was applied to a Dowex 50-X2 (H^+) column. The column was eluted with 3% methanol–ammonium hydroxide to give 17.5 mg of colorless crystals of methyl 3-amino-3,6-dideoxy-α-D-idopyranoside (**4a**) with mp 164–166°.

(v) **Methyl 4-Azido-3(2)-*O*-benzoyl-4,6-dideoxy-α-D-mannopyranoside (5g).** a. Fraction 5 (R_f 0.67) gave 92 mg (8%) of crystalline **5g** with mp 115–117°. A sample was recrystallized once from ether–pentane for analysis: mp 116–117°; $[\alpha]^{25}D$ −48.3 (*c* 1.04, $CHCl_3$).

Anal. Calcd for $C_{13}H_{17}N_3O_5$: C, 54.72; H, 5.58; N, 13.67 Found: C, 55.02; H, 5.60; N, 13.51.

The structure of **5g** was proved by subjecting a 30-mg sample to the same sequence of reactions as was applied to **4d** and by isolating 10 mg (65%) of colorless crystals with mp 150–152° of methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**).

b. A mixture of 5 g of **3b**, 10 g of lithium azide, 12.5 g of ammonium chloride, and 140 mg of methyl Cellosolve was heated in a sealed tube at 110° for 18 hr. Processing as in method a and subsequent preparative tlc furnished the following compounds containing an azide group: **5e**, 1.65 g (43%), mp 81–83°; **4c**, 350 mg (9%), mp 50–52°; **4d**, 125 mg (2%), mp 104–106°.

Comparison of the Reaction of Lithium Azide with 3a and 3b. a. A mixture of 250 mg of **3a**, 500 mg of lithium azide, and 625 mg of ammonium chloride in 12.5 ml of methyl Cellosolve was heated under reflux for 6 hr. Processing as in method a of the previous experiment gave 230 mg of a yellow oil.

b. A mixture of 500 mg of **3b**, 1 g of lithium azide, and 1.25 g of ammonium chloride in 25 ml of methyl Cellosolve was heated under reflux for 36 hr. The usual isolation procedure gave 420 mg of a yellow oil which was saponified by a catalytic amount of sodium ethoxide at room temperature for 24 hr. The reaction mixture was neutralized by the addition of excess solid carbon dioxide and stirring for 2 hr. The solvent was removed *in vacuo* and extraction of the resulting residue with ethyl ether gave 340 mg of a yellow oil.

Vpc Analyses of the Products from a and b. *Ca.* 10 mg of each of the products from reactions a and b were converted into the trimethylsilyl (TMS) derivatives according to the literature procedure.[11] Vpc analyses were performed on (6 ft × 1/4 in. and 8 ft × 1/4 in., 15%) ethylene glycol succinate columns at 160°, using ether solutions of the TMS derivatives. The data are given in Table II.

Tlc Analyses of a and b. The azides from reaction mixtures a and b were separated by preparative tlc. The data are given in Table II.

Methyl 4-Amino-4,6-dideoxy-α-D-mannopyranoside (5a) and Hydrochloride. A solution of 500 mg of **5e** in 30 ml of methanol was hydrogenated in the presence of 250 mg of 5% Pd–C at room temperature for 3 hr. The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to yield 375 mg (85%) of colorless crystals of methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**) with mp 151–153°. One recrystallization from ethanol–ether gave an analytically pure sample: mp 152–153°; $[\alpha]^{27}D$ +82.5 (*c* 1.0, MeOH) [lit.[1,6i] mp 150–151°; $[\alpha]^{23}D$ +80 (*c* 1.0, MeOH) for methyl perosaminide].

Anal. Calcd for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 47.65; H, 8.78; N, 7.64.

For the preparation of the hydrochloride salt of **5a**, the same procedure was used as described for the preparation of the hydrochloride salt of **4a**. Colorless crystals of the hydrochloride salt of **5a** were obtained in 88% yield with mp 179–181° dec. A sample was recrystallized from ethanol–ether for analysis: mp 188–189° dec; $[\alpha]^{27}D$ +57.6 (*c* 1.0, MeOH).

Anal. Calcd for $C_7H_{16}NO_4Cl$: C, 39.35; H, 7.96; N, 6.55. Found: C, 39.31; H, 7.86; N, 6.83.

Methyl 4-Acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside (5c). Compound **5a** was acetylated using excess acetic anhydride in pyridine at room temperature overnight. Isolation in the usual manner gave crystalline **5c** with mp 156–158° in 90% yield. One recrystallization from ether–*n*-pentane gave an analytically pure sample: mp 159–160°; $[\alpha]^{25}D$ +95.61 (*c* 0.98, MeOH).

Anal. Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.32; H, 6.97; N, 4.90.

Methyl 4-Acetamido-4,6-dideoxy-α-D-mannopyranoside (5b). Acetylation of **5a** (free base) in methanol and acetic anhydride at 0°, using a method similar to that described for the preparation of **4c**, gave **5b** with mp 184–186° in 95% yield. A sample was recrystallized from ethanol–ethyl ether for analysis: mp 185–186°; $[\alpha]^{26}D$ +85.2 (c 1.0, MeOH). A mixture melting point of **5b** with methyl *N*-acetylperosaminide[12] prepared from perosamine was undepressed, and the infrared spectra (KBr) of the two compounds were identical.

Anal. Calcd for $C_9H_{17}NO_5$: C, 49.77; H, 7.76; N, 6.30. Found: C, 49.76; H, 7.77; N, 6.30.

Alkaline Hydrolysis of Methyl 4-Acetamido-4,6-dideoxy-α-D-mannopyranoside (5b). A solution of 20 mg of **5b** and 100 mg of barium hydroxide in 3 ml of water was heated on a steam bath for 4 days. The reaction mixture was neutralized with 1 *N* sulfuric acid to pH 7.0 (pH paper). The precipitate of barium sulfate was removed by filtration. The filtrate applied to a column of Dowex 1-X2 (OH^-). The column was eluted with methanol. The effluent was concentrated *in vacuo* to give a gum. The gum was dissolved in methanol and the solution was applied to a Dowex 50-X2 (H^+) column. The column was eluted with methanol. The effluent was concentrated *in vacuo* to give 5 mg of **5b**. Subsequent elution of the column with 3% methanol–ammonium hydroxide gave 5 mg of methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**5a**) with mp 151–152°.

Methyl 2,3-Di-*O*-acetyl-4,6-dideoxy-4-(*N*,*N*-dimethylamino)-α-D-mannopyranoside (5d). A solution of 200 mg of **5a** in 10 ml of methanol was hydrogenated in the presence of 0.3 ml of 37% formaldehyde and 100 mg of 5% Pd–C at room temperature for 12 hr. The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to give 150 mg (77.7%) of methyl 4,6-dideoxy-4-(*N*,*N*-dimethylamino)-α-D-mannopyranoside (**5k**) as a colorless oil. The oil was acetylated at room temperature overnight using excess acetic anhydride in pyridine. Isolation in the usual manner gave colorless crystals of **5d** (70% overall) with mp 87–88°.

Methyl 4-Carbomethoxyamino-4,6-dideoxy-α-D-mannopyranoside (5b). To an ice-cold solution of 200 mg of **5a** in 35 ml of methanol was added 200 mg of anhydrous sodium bicarbonate and 0.6 ml of methyl chloroformate, dropwise, with stirring. The mixture was stirred for 2 hr at 0° and at room temperature for 8 hr. The reaction mixture was evaporated to dryness *in vacuo*. The resulting

white solid was extracted with chloroform. The chloroform extract was concentrated *in vacuo* to give a colorless oil. The oil was dissolved in methanol. The solution was applied to a column of Dowex 50-X2 (H^+) and the column was eluted with methanol to remove any basic impurities. The effluent was concentrated *in vacuo* to give a colorless oil which crystallized from ether to afford 185 mg (70%) of **5h** with mp 144–145°. A sample was recrystallized from ether for analysis: mp 145–146°; $[\alpha]^{27}D$ +71.1 (*c* 1.16, H_2O).

Anal. Calcd for $C_9H_{17}NO_6$: C, 45.95; H, 7.29; N, 5.95. Found: C, 46.23; H, 7.43; N, 6.14.

Methyl 4,6-Dideoxy-4-(*N*-methylamino)-α-D-mannopyranoside and Hydrochloride Salt (5i). A solution of 75 mg of **5h** in 20 ml of ether was heated with 100 mg of lithium aluminum hydride under reflux for 6 hr. The excess hydride was decomposed by the addition of ethyl acetate. Water was added with stirring until the precipitate coagulated. The ether was decanted and the aluminum hydroxide washed several times with hot ether. The combined ether extracts were evaporated *in vacuo* to yield 35 mg (60%) of a colorless oil. The oil was dissolved in ether and isopropyl alcohol–hydrogen chloride was added until the solution became acidic to pH paper. The resulting white precipitate was removed by filtration and crystallized from ethanol–ether to yield **5i** (40 mg) with mp 140–142° dec. An analytically pure sample was obtained by one recrystallization from ethanol–ether: mp 142–143° dec; $[\alpha]^{27}D$ +60.7 (*c* 0.84, MeOH).

Anal. Calcd for $C_8H_{18}NO_4Cl$: C, 42.20; H, 7.96; N, 6.15. Found: C, 41.94; H, 8.04; N, 6.26.

4,6-Dideoxy-4-(*N*,*N*-dimethylamino)-D-mannose Hydrochloride (6c). Acid Hydrolysis of 5d. A solution of 500 mg of **5d** in 4 ml of 1 *N* hydrochloric acid was heated in an oil bath at 80° for 24 hr. Isolation procedures similar to those described for the preparation of **8** yielded a colorless oil which crystallized from ethanol–ethyl ether to give 25.9 mg (65%) of 4-(*N*,*N*-dimethylamino)-4,6-dideoxy-D-mannose hydrochloride (**6c**) with mp 178–180°. A sample was recrystallized from ethanol–ethyl ether for analysis: mp 181.5–182.5° dec; $[\alpha]^{28}D$ (initial) +13.82; (18 hr) +10.27 (*c* 1.0, MeOH).

Anal. Calcd for $C_8H_{18}O_3NCl$: C, 42.20; H, 7.96; N, 6.15. Found: C, 42.20; H, 8.06; N, 6.42.

4-Azido-4,6-dideoxy-β-D-mannose (6b). A solution of 400 mg of **5e** in 50 ml of 1.5 *N* hydrochloric acid was heated in an oil bath at 70° for 4 days. The yellow solution was evaporated *in vacuo* and the last traces of hydrogen chloride were removed by azeotropic distillation with a 50:50 (v/v) mixture of ethanol–toluene. The resulting yellow foam was extracted with anhydrous diethyl ether and was treated with activated charcoal. Removal of the solvent left a colorless oil which crystallized from ethanol–*n*-pentane to give 210 mg (56%) of **6b** with mp 116–118°. A sample was recrystallized for analysis: mp 118–118.5°; $[\alpha]^{27}D$ (initial) +21.6; (30 min) +57.4 (*c* 1.0, MeOH).

Anal. Calcd for $C_6H_{11}N_3O_4$: C, 38.10; H, 5.86; N, 22.21. Found: C, 38.36; H, 5.99; N, 21.98.

4-Azido-4,6-dideoxy-D-mannitol (9). A solution of 100 mg of **6b** in 4 ml of water was cooled in an ice bath. A suspension of 80 mg (4 mol/mol) of sodium borohydride in 4 ml of water was added with stirring over a period of 0.5 hr. The mixture was stirred at 0° for 2 hr. The sodium borohydride complex of the product was decomposed by the addition of Dowex 50-X2 (H^+) and stirring at room temperature for *ca.* 1 hr. The reaction mixture was passed through a column of Dowex 50-X2 (H^+). The column was eluted with methanol. The effluent was concentrated *in vacuo* to yield a white solid. The solid was azeotroped with ethanol–toluene (1:1) several times and was extracted with anhydrous diethyl ether. Evaporation of the solvent yielded a white solid which crystallized from ether–*n*-pentane to give 64.5 mg (64%) of **9** with mp 70–75°. An analytically pure sample of **9** was obtained by one recrystallization from ether–*n*-pentane: mp 72–81°; $[\alpha]^{27}D$ −5° (*c* 0.9, MeOH).

Anal. Calcd for $C_6H_{13}N_3O_4$: C, 37.69; H, 6.85; N, 21.98. Found: C, 37.92; H, 7.01; N, 22.18.

Methyl 4-Acetamido-2,3-di-*O*-acetyl-4,6-dideoxy-α-D-mannopyranoside Hydrobromide (51). To a 50-ml round-bottom flask containing 50 mg of **5b** was added 1 ml of acetyl bromide. The mixture was stirred for 5 min; colorless crystals of **51** formed. The reaction mixture was diluted with dry petroleum ether (bp 60–70°) to complete the crystallization of **51**. The solvent was decanted and the crystals were washed with dry diethyl ether several times, until all the acetyl bromide had been removed. Recrystallization from chloroform–ether gave 60 mg (73%) of **51** with mp 154–155° dec. Compound **51** was also obtained in good yield (80%) by allowing **5c** to react with diethyl ether–hydrogen bromide. One further recrystallization gave an analytically pure sample: mp 155–156° dec; $[\alpha]^{26}D$ +75.4 (*c* 0.78, $CHCl_3$).

Anal. Calcd for $C_{13}H_{22}NO_7Br$: C, 40.66; H, 5.77; N, 3.64. Found: C, 40.62; H, 5.88; N, 3.67.

For structure proof, a solution of 10 mg of **51** in chloroform was washed with dilute aqueous sodium bicarbonate. The chloroform was dried ($MgSO_4$) and concentrated *in vacuo* to give 5 mg of **5c** with mp 158–159°. A solution of 20 mg in **51** in methanol was applied to a column of Dowex 50-X2 (H^+). The column was eluted with methanol. The effluent was concentrated *in vacuo* to afford 10 mg of **5b** with mp 184–186°.

Degradation of 9 and 6b. Sodium metaperiodate oxidation of compound **9** or the free sugar **6b** gave *erythro*-2-azido-3-hydroxy-D-butyraldehyde (**10**). An aqueous solution of 100 mg of **6b** in 50 ml of 0.1 *N* sodium metaperiodate was kept in the dark at room temperature for 4 hr. The solution was evaporated to dryness *in vacuo*. The resulting white solid was extracted several times with chloroform. Removal of the solvent *in vacuo* afforded 55 mg (81%) of **10** as a yellow oil, which was homogeneous by tlc. The infrared spectrum ($CHCl_3$) of **10** had strong bands (cm^{-1}) at 1725, 2100, and 3450.

A solution of 55 mg of **10** in 4 ml of water was cooled in an ice bath. A suspension of sodium borohydride (10 mol/mol) in 4 ml of water was slowly added with stirring. The mixture was stirred at 0° for 2 hr and at room temperature overnight. The sodium borohydride complex was decomposed by the addition of Dowex 50-X2 (H^+) and stirring for *ca.* 1 hr. The reaction mixture was percolated through a column of Dowex 50-X2 (H^+). The column was washed with methanol to remove any neutral compounds. Final elution of the column with 5% methanol–ammonium hydroxide and removal of the solvent *in vacuo* gave 22 mg of basic material as a colorless oil, which was homogenous by pc. The oil was dissolved in ethanol and a solution of oxalic acid in ethanol was added dropwise until the solution was slightly acidic. A white solid precipitated. Filtration, washing of the solid with ether, and crystallization from methanol furnished 14 mg (18% overall yield for the three steps) of colorless crystals with mp 174–175° dec. The crystals were identified as D-allothreoninol hydrogen oxalate (**11**) by comparison with an authentic sample. A sample was recrystallized from methanol for analysis: mp 174–175° dec; $[\alpha]^{26}D$ −28 (*c* 0.93, H_2O).

Anal. Calcd for $C_5H_{12}NO_4$: C, 39.96; H, 8.00; N, 9.33. Found: C, 39.87; H, 8.20; N, 9.49.

Degradation of 5b. An aqueous solution of 75 mg of the *N*-acetyl derivative (**5b**) in 25 ml of 0.1 *N* sodium metaperiodate was kept in the dark at room temperature for 6 hr. The solution was evaporated to dryness *in vacuo*. The residue was dried by azeotropic distillation with ethanol and was extracted with chloroform several times. Removal of the solvent *in vacuo* afforded 55 mg (72%) of **12** as a colorless oil, which was homogeneous by tlc. The infrared spectrum ($CHCl_3$) of **12** had strong bands (cm^{-1}) at 3450, 1750, 1650, and 1075.

A solution of 55 mg of **12** (from above) in 3 ml of water was cooled in an ice bath and 200 mg of sodium borohydride was added in small portions over a period of 0.5 hr. The mixture was stirred at 0° for 2 hr and at room temperature overnight. The resulting sodium borohydride complex was decomposed by the addition of Dowex 50-X2 (H^+) and stirring for *ca.* 1 hr. The solution was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was azeotroped with ethanol–benzene (1:1) several times and extracted with chloroform. Concentration of the chloroform extract *in vacuo* yielded 35 mg (64%) of **13** as a colorless oil. The infrared spectrum ($CHCl_3$) of **13** had strong bands (cm^{-1}) at 3450, 1650, and 1075.

A solution of 35 mg of **13** (from above) in 3 ml of 1.5 *N* hydrochloric acid was heated under reflux on a steam bath for 12 hr. The solution was evaporated to dryness *in vacuo*. The residue was dissolved in methanol. The solution was applied to a Dowex 50-X2 (H^+) column. The column was eluted with methanol to remove the neutrals and then eluted with 5% methanol–ammonium hydroxide. Removal of solvent gave 13 mg of a light brown oil. The oil was dissolved in ethanol and converted into the oxalate salt in the usual manner to give 16 mg of a brown solid. The solid was treated with activated charcoal and crystallized from methanol to afford 10 mg of colorless crystals with mp 174–175° dec. The crystals were identified as D-allothreoninol hydrogen oxalate (**11**) by mixture melting point determinations and infrared spectral comparisons with an authentic sample.

D-Allothreonine Ethyl Ester Hydrochloride. DL-Allothreonin was resolved into the D and L enantiomers according to the procedure of Inui and Kaneko.[15] To an ice-cold suspension of 350 mg of D-allothreonine in 12 ml of absolute ethanol was added 0.6 ml of thionyl chloride dropwise. The solution was refluxed for 4 hr and evaporated to dryness. The last traces of the acid were removed by azeotroping with ethanol–toluene (1:1) several times. The colorless, solid residue was crystallized from ethanol–ether to give 480 mg (80%) of D-allothreonine ethyl ester hydrochloride (**14**) with mp 153–154°. A sample was recrystallized from ethanol–ether for analysis: mp 155°; $[\alpha]^{25}D$ −12.44 (*c* 1.19, H_2O).

Anal. Calcd for $C_6H_{14}NO_3Cl$: C, 39.25; H, 7.68; N, 7.63. Found: C, 39.53; H, 7.77; N, 7.90.

L-Allothreonine Ethyl Ester Hydrochloride. L-Allothreonine ethyl ester hydrochloride was prepared in the same manner as the D enantiomer: mp 151–152°; $[\alpha]^{25}D$ −12.72 (*c* 1.05, H_2O).

D-Allothreoninol Hydrogen Oxalate (11). To an ice-cold solution of 400 mg of **14** in 10 ml of water 1 g of sodium borohydride was added in small portions with stirring. The mixture was stirred at 0° for 2 hr and at room temperature overnight. The resulting sodium borohydride complex was decomposed by the addition of Dowex 50-X2 (H^+). Isolation in a manner similar to that described for the degradation of **9** and **6b** furnished 160 mg (70%) of a colorless oil which was subsequently converted into 175 mg (76%) of D-allothreoninol hydrogen oxalate: mp 168–170° dec. One recrystallization from ethanol–water gave an analytically pure sample: mp 175–176° dec; $[\alpha]^{25}D$ −27 (*c* 0.93, H_2O).

L-Allothreoninol Hydrogen Oxalate. L-Allothreoninol hydrogen oxalate was prepared similar to the D enantiomer: mp 175–176° dec; $[\alpha]^{27}D$ +27.26 (*c* 0.73, H_2O). The infrared spectra (KBr) of the D and L enantiomers were superimposable and had strong bands (cm^{-1}) at 3375, 1575, 1300, and 1050.

D- and L-Threoninol Hydrogen Oxalates. D- and L-threoninol hydrogen oxalates were prepared starting from D- and L-threoninol using the same procedure as mentioned above. Their physical constants are given below: (a) D-threoninol hydrogen oxalate, mp 187–188°; $[\alpha]^{25}D$ +7.15 (*c* 0.7, H_2O) [lit.[15] mp 188–189° dec; $[\alpha]^{22.5}D$ +3.2 (*c* 1.078, H_2O)]; (b) L-threoninol hydrogen oxalate, mp 188.5–189° dec; $[\alpha]^{25}D$ −7.84 (*c* 0.9, H_2O).

2(*S*)-α(*R*)-Hydroxyethyl-3(*S*),4(*R*)-dihydroxypyrrolidine (7a). A solution of 100 mg of **6b** in 10 ml of water containing 0.2 ml of 2 *N* hydrochloric acid was hydrogenated in the presence of 50 mg of 5% Pd–C for 6 hr. The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to give a brown oil. The oil afforded 53 mg (54%) of colorless crystals of **7a** with mp 182–184°. Two more recrystallizations from ethanol–ether gave an analytically pure sample: mp 184–185°; pK_a = 8.15; $[\alpha]^{27}D$ −21.5 (*c* 0.39, MeOH).

Anal. Calcd for $C_6H_{14}NO_3Cl$: C, 39.25; H, 7.68; N, 7.63. Found: C, 39.51; H, 7.67; N, 7.62.

N-Acetyl-2(*S*)-α(*R*)-acetoxyethyl-3(*S*),4(*R*)-diacetoxypyrrolidine (7b). A solution of 60 mg of **6b** in 8 ml of methanol was hydrogenated in the presence of 30 mg of 10% Pd–C for 24 hr. The catalyst was removed by filtration. The filtrate was concentrated *in vacuo* to give a colorless gum. The gum was acetylated using excess acetic anhydride in pyridine at room temperature overnight. Isolation in the usual manner gave 35 mg (35% overall yield for the two steps) of **7b** with mp 159–161°. Recrystallization from ethanol–ether afforded an analytically pure sample: mp 163–164°; $[\alpha]^{27}D$ +29.03 (*c* 1.04, $CHCl_3$).

Anal. Calcd for $C_{14}H_{21}NO_7$: C, 53.36; H, 6.72; N, 4.44. Found: C, 53.65; H, 6.94; N, 4.51.

For structure proof, **7a** was acetylated in the presence of excess acetic anhydride in pyridine to give the tetraacetyl derivative (**7b**). The infrared spectrum of **7b** was devoid of NH absorptions and had strong bands (cm^{-1}) at 1750, 1645, 1375, 1300, and 1100. On oxidation with sodium metaperiodate, **7a** required 4 mol of the reagent for complete oxidation. The mass spectrum and the nmr spectrum of **7b** were also consistent with the proposed structure.

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Volume Changes Accompanying the Titration of Some Chemically Modified Ribonuclease Preparations[1]

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Abstract: The volume changes produced by the reaction of hydroxide ions with the basic groups of proteins are abnormally low in comparison with those observed with similar functional groups in small model compounds; approximately 16 ml/mol as compared with 25 ml/mol. An explanation of this phenomenon based on dilatometric measurements with highly purified preparations of ribonuclease and two denatured preparations of ribonuclease (reduced-carboxymethylated and oxidized) is offered. The reaction of tyrosine residues was shown to account for the abnormality in the pH range where the lysine residues titrate. Unfolding ribonuclease was found to have only a small, but measurable, effect on the volume change (and therefore the solvation) of the histidine residues and no apparent effect on the lysine residues. Evidence is presented to show that the presence of approximately one phosphate ion per molecule of native ribonuclease could produce the abnormality in the region of the p*K* of histidine. Finally polyvalyl ribonuclease was used to provide a measure of the solvation at particular sites on the protein surface.

Measurements of the volume changes produced by the reactions of the functional groups of proteins can yield information regarding subtle changes in the immediate environment of these groups. Of particular interest is the reaction of amino groups with base. Rasper and Kauzmann[2] made an extensive dilatometric

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(2) J. Rasper and W. Kauzmann, *J. Amer. Chem. Soc.*, 84, 1771 (1962).