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Synthesis of N-Acetyllincosamine[1]

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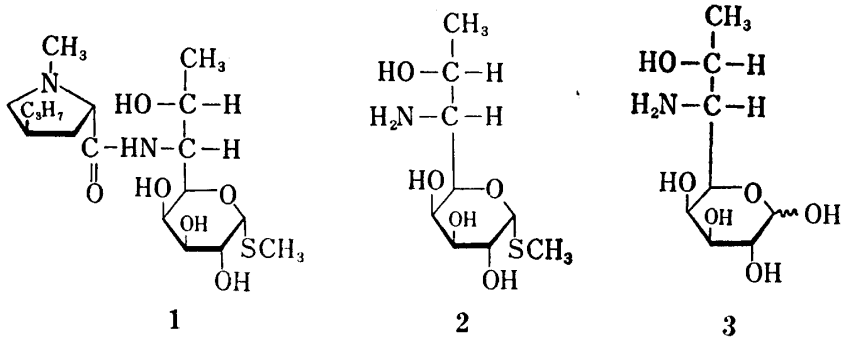
1,2:3,4-Di-O-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose (**4**) was treated with sodium cyanide in aqueous methanol and the resulting cyanohydrin mixture (**5a** and **6a**) was tosylated to give 1,2:3,4-di-O-isopropylidene-6-O-tosyl-L-*glycero*- (**5b**) and -D-*glycero*-α-D-*galacto*-heptopyranurononitrile (**6b**). Lithium aluminum hydride reduction of these tosylates **5b** and **6b**, followed by acetylation yielded D-*glycero*-N-acetylepimine (**7a**) and L-*glycero*-N-acetylepimine (**8**), respectively. The D-*glycero*-epimer (**7a**) thereby obtained was treated in warm acetic acid and gave a 6-acetamido-7-O-acetate (**17a**) exclusively, whose deacetylation with sodium methoxide yielded a 7-deacetyl derivative (**19**). Pfitzner–Moffatt oxidation of **19**, followed by treatment of the resulting 7-oxo derivative (**20**) with methylmagnesium bromide gave 6-acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-*threo*-α-D-*galacto*-octopyranose (**26**). The latter (**26**) was converted into its D-*erythro*-derivative (**24**) by oxidation with chromium trioxide in pyridine and successive reduction with sodium borohydride. Hydrolysis of **24** with aqueous acetic acid or Amberlite IR-120 (H^+) gave the N-acetate of lincosamine which constitutes a sugar component of antibacterial antibiotic, lincomycin.

Recently, interest in the synthesis of amino sugars has been very much stimulated by their widespread distribution as structural components of many antibiotics. Lincomycin, a fermentation product of *Streptomyces lincolnensis* var. *lincolnensis* n. sp.,[3] is one of such aminoglycoside antibiotics, which is orally effective in man for the clinical treatment of diseases caused by gram-positive bacteria.[4] Lincomycin (**1**) is a novel member of monosaccharide antibiotics characterized by an amino-octose derivative, methyl thiolincosaminide,



1 **2** **3**

Chart 1

1) Preliminary details of this work have been published: H. Saeki, T. Iwashige, and E. Ohki, Abstr. papers, 13th Symposium on the Chemistry of Natural Products, 1969, p. 1 (Sapporo, Hokkaido, September 25, 1969); H. Saeki and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1974 (1969); *idem*, *ibid.*, **18**, 412 (1970).

2) Location: *Hiromachi, Shinagawa-ku, Tokyo.*

3) D.J. Mason, A. Dietz, and C. DeBoer, *Antimicrobial Agents and Chemotherapy*, **1962**, 554; R.R. Herr and M.E. Bergy, *ibid.*, 560 (1962).

4) L.J. Hanka, D.J. Mason, M.R. Burch, and R.W. Treick, *Antimicrobial Agents and Chemotherapy*, **1962**, 565; C. Lewis, H.W. Clapp, and J.E. Grady, *ibid.*, **1962**, 570; W.J. Holloway, R.A. Kahlbaugh, and E.G. Scott, *ibid.*, **1963**, 200; J. Harnecker, J. Contreras, B. Gilabert, and V. Ubilla, *ibid.*, **1963**, 204; E.W. Walters, M.J. Romansky, and A.J. Johnson, *ibid.*, **1963**, 210; J.C. Trakas and H. Lind, *ibid.*, **1963**, 216.

joined to an amino acid, *trans*-N-methyl-4-propyl-L-proline, *via* an amide bond.[5] Further, methyl thiolincosaminide has been represented as methyl 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside (**2**) by the structural study of Schroeder, *et al.*[5c] 1-Demethylthio-1-hydroxyl derivative of **2**, 6-amino-6,8-dideoxy-D-*erythro*-D-*galacto*-octose (**3**), is called lincosamine.

Lincomycin (**1**) may be cleaved into the amino acid and methyl thiolincosaminide (**2**) by hydrolysis.[5a,c] Reproduction of the antibiotic from these components[5c] and preparation of the amino acid and its homologs have already been reported,[5e] while synthesis of lincosamine derivatives has not been achieved.[6] We wish to report herein the synthesis of N-acetyllincosamine and related octose derivatives by stepwise extension of the carbon chain from D-galactose through 6,7-epimino-6,7-dideoxy-D-*galacto*-heptose derivatives.

For the starting material of this synthesis, 1,2:3,4-di-O-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose[8,11,12] (**4**), which was easily prepared from D-galactose, was provided. Treatment of **4** with sodium cyanide in aqueous methanol afforded a mixture of 1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranurononitrile (**5a**) and its D-*glycero*-epimer (**6a**) in a good yield. This mixture was not fully separable into each epimer and only a small amount of the L-*glycero*-isomer (**5a**) was isolated as a pure crystalline substance. The relative ratio of **5a** and **6a** in the cyanohydrin mixture was determined by gas chromatographic analysis of its acetylated product. The ratio depended on the reaction temperature and varied between 1:1.8 and 1:2.5 with predominance of the D-*glycero*-isomer (**6a**). The cyanohydrin mixture was tosylated with *p*-toluenesulfonyl chloride in pyridine, yielding a crystalline mass which was successfully resolved into L-*glycero*-6-tosylate (**5a**) and D-*glycero*-6-tosylate (**6b**) by fractional recrystallization. In fact, the structure of these nitriles remained equivocal at this stage but was proved by determining the structures of 6,7-acylepimines (**7a**, **7b** and **8**) derived from these nitriles as will be described later. Infrared spectra of these crystalline nitriles (**5a**, **5b**, and **6b**) show very weak or no nitrile absorption band.[13]

Recently, Ichimura and Ohta[14] reported an ingenious method of preparing monosubstituted aziridine by treatment of α-halonitrile or α-sulfonyloxynitrile with lithium aluminum hydride. This reaction includes an initial reduction of nitrile into a primary amine and subsequent nucleophilic displacement of the β-substituent by the amine formed with a Walden inversion. Accordingly, we also attempted analogous reduction on these 6-*O*-tosylheptopyranurononitriles (**5b** and **6b**) in the following way. The L-*glycero*-tosylate (**5b**) easily furnished an epimine on its treatment with lithium aluminum hydride in ether, and the successive N-acetylation or N-benzoylation afforded 6,7-(acetylepimino)-6,7-dideoxy-1,2:3,4-di-*O*-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (**7a**) as a syrup or the 6,7-benzoylepimine (**7b**) as crystals, respectively. On the other hand, the reduction pattern of the D-*glycero*-tosylate

5) *a*) H. Hoeksema, B. Bannister, R.D. Birkenmeyer, F. Kagan, B.J. Magerlein, F.A. MacKeller, W. Schroeder, G. Slomp, and R.R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964); *b*) R.R. Herr and G. Slomp, *ibid.*, **89**, 2444 (1967); *c*) W. Schroeder, B. Bannister, and H. Hoeksema, *ibid.*, **89**, 2448 (1967); *d*) G. Slomp and F.A. MacKeller, *ibid.*, **89**, 2454 (1967); *e*) B.J. Magerlein, R.D. Birkenmeyer, R.R. Herr, and F. Kagan, *ibid.*, **89**, 2459 (1967).

6) There are several reports on the synthesis of D-*galacto*-octopyranose derivatives which include extension of the side chain of D-galactose by treatment of **4** with ethynyl-[7] or vinyl-magnesium bromide,[8,10] ethylidenetriphenylphosphorane,[9,10] or nitroethane.[8]

7) D. Horton, J.B. Hughes, and J.M.J. Tronchet, *Chem. Commun.*, **1965**, 481.

8) G.B. Howarth, D.G. Lance, W.A. Szarek, and J.K.N. Jones, *Can. J. Chem.*, **47**, 75 (1969).

9) D.G. Lance and W.A. Szarek, *Carbohydrate Res.*, **10**, 306 (1969).

10) D.G. Lance, .W.A. Szarek, J.K.N. Jones, and G.B. Howarth, *Can. J. Chem.*, **47**, 2871 (1969).

11) D. Horton, M. Nakadate, and J.M.J. Tronchet, *Carbohydrate Res.*, **7**, 56 (1968).

12) H. Saeki, T. Iwashige, E. Ohki, K. Furuya, and M. Shirasaka, *Ann. Sankyo Res. Lab.*, **19**, 137 (1967).

13) L.J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen & Co., Ltd., London, 1954, p. 266.

14) K. Ichimura and M. Ohta, *Bull. Chem. Soc. Japan*, **40**, 432 (1967); *idem*, *ibid.*, in press.

(**6b**) was a little more complicated than that of **5b**. Under a limited condition, **6b** mainly gave the corresponding epimine which yielded 6,7-(acetylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (**8**) as crystals on successive N-acetylation.

However, when **6b** was reduced in higher concentration of the reagent and substrate, a primary amine was mainly obtained. This amine formed a crystalline acetamido derivative whose elementary analytical values corresponded to a monoacetamido-dideoxy-di-O-isopropylidene-heptose. The nuclear magnetic resonance (NMR) spectrum of the acetamido derivative exhibited the presence of 1,2:3,4-di-O-isopropylidene-α-D-*galacto*-pyranose moiety, but of no additional methyl group except that of the acetamido group. These facts suggest that the amine in question is a 7-amino derivative and would be designated as 7-amino-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-α-D-*galacto*-heptopyranose (**9**), which presumably was formed by removal of the 6-tosyloxy group without a 6,7-epimine formation during the reduction. On the other hand, similar formation of the primary amine (**9**) could not be observed in the case of the reduction of the L-*glycero*-epimer (**5b**) in various conditions examined. Such different behaviors of these epimeric α-tosyloxynitriles (**5b** and **6b**) to lithium aluminum hydride reduction is of interest to note.

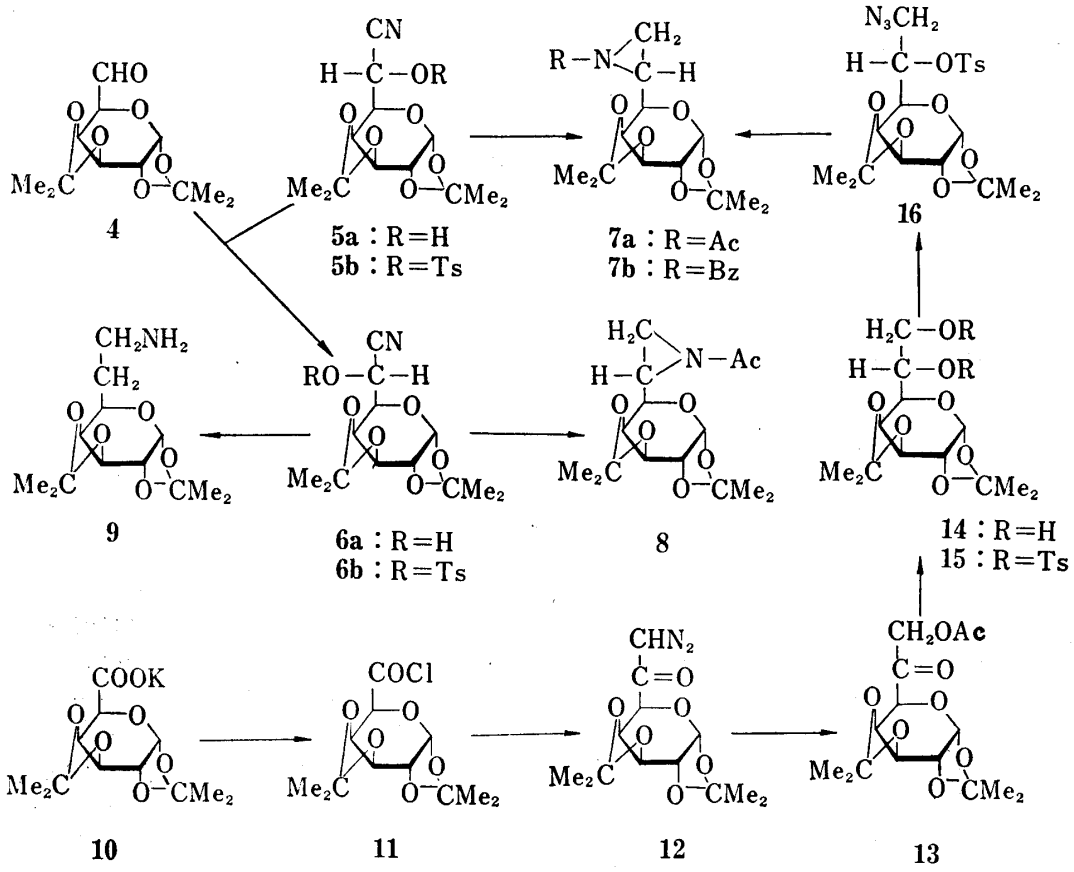


Chart 2

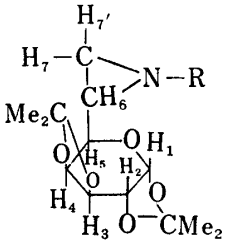
The presence of an epimine ring in **7a**, **7b**, and **8** was shown by a characteristic infrared absorption[15] of the acylepimine carbonyl group. In addition, NMR spectra of these acylepimines were completely assigned, reflecting these structures as illustrated in Table I. For determination of the configuration at the 6-position of these acylepimines, an unequivocal synthetic route toward **7a** and **7b** was examined. Following the method of David and Popot,[16]

15) H.L. Spell, *Anal. Chem.*, 39, 185 (1967).
16) S. David and M.O. Popot, *Carbohydrate Res.*, 5, 234 (1967).

Table I. NMR Data of 6,7-(Acetylepimino)- and 6,7-(Benzoylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (**7a** and **7b**) and 6,7-(Acetylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (**8**)

	Chemical shift (δ ppm)				Coupling constant (Hz)		
	7a	**8**	**7b**		**7a**	**8**	**7b**
H_1	5.47(d)	5.55(d)	5.53(d)				
				$J_{1,2}$	5	5.5	5
H_2	4.27(dd)	4.28(dd)	4.32(dd)				
				$J_{2,3}$	2	2.5	2
H_3	4.62(dd)	4.60(dd)	4.65(dd)				
				$J_{3,4}$	8	8	8
H_4	4.35(dd)	4.18(dd)	4.36(dd)				
				$J_{4,5}$	2	2	2
H_5	3.40(dd)	3.28(dd)	3.83(dd)				
				$J_{5,6}$	6.5	7	4.5
H_6	2.75(ddd)	2.75(ddd)	2.95(ddd)				
				$J_{6,7}$	6	6	6
				$J_{6,7'}$	3	3	4
H_7	2.43(d)	2.38(d)	2.57(d)				
$H_{7'}$	2.10(d)	2.10(d)	2.52(d)				
				$J_{7,7'}$	0	0	0
CH_3	1.45(6s)	1.48(3s)	1.47(6s)				
(Ip)	1.36(3s)	1.47(3s)	1.34(6s)				
	1.30(3s)	1.35(3s)					
		1.34(3s)					
Etc.	2.15(3s)	2.21(3s)	8.2–7.3(5m)				
	(CH_3CO–)	(CH_3CO–)	(C_6H_5CO–)				

*n*s: singlet,
*n*m: multiplet,
d: doublet
dd: doublet of doublets, *etc.*, where *n* is the number of protons.
Ip: isopropylidene



7a: R=$COCH_3$
7b: R=COC_6H_5
8: R=$COCH_3$

treatment of potassium 1,2:3,4-di-O-isopropylidene-α-D-galactopyranuronate[17] (**10**) with oxalyl chloride in ether gave a crystalline chloride[18] (**11**), which formed a diazoketone (**12**) on treatment with diazomethane in ether. The diazoketone (**12**) was converted into a crystalline acetoxyketone[18] (**13**) by solvolysis with acetic acid in the presence of cupric acetate. Lithium aluminum hydride reduction of **13** in ether predominantly gave 1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose[19] (**14**). Tosylation of **14** and successive treatment of the resultant syrupy 6,7-ditosylate (**15**) with sodium azide in dimethyl sulfoxide gave 7-azido-7-deoxy-6-tosylate (**16**). The latter compound (**16**) was reduced with lithium aluminum hydride in ether as described in our preceding papers on the synthesis of 5,6-epimino-hexofuranoses,[20] affording a 6,7-deoxy-6,7-epimino-heptose, whose N-acetyl and N-benzoyl derivatives were identified with **7a** and **7b**, respectively, by means of thin-layer chromatography, mixed melting point test, and infrared and NMR spectrometry. Since a Walden inversion at the 6-position would occur at the stage of the conversion of the L-*glycero*-7-azido-6-tosylate (**16**) into the

17) H.M. Sell and K.F. Link, *J. Am. Chem. Soc.*, **60**, 1813 (1938).
18) These compounds were described as syrups in the literature.[16]
19) S. David and M.O. Popot, *Carbohydrate Res.*, **8**, 350 (1968).
20) H. Saeki and E. Ohki, *Chem. Pharm. Bull.* (Tokyo), **16**, 2471, 2477 (1968).

epimine,[20] the acylepimines (**7a** and **7b**) would have a D-*glycero*-configuration at the 6-position; consequently, the original nitrile (**5b**), which gave **7a** or **7b**, is the L-*glycero*-epimer, because analogous inversion at the 6-position would occur in the formation of epimine ring.[14] As a result, structure of all the cyanohydrins and epimines prepared as above was clarified.

As described earlier in our reports[20] 5,6-acetylepimino-hexofuranoses were extremely sensitive to acids and easily converted into 5-acetamido-6-O-acetyl-5-deoxy-hexofuranoses with opening of the epimine ring on treatment with acetic acid. These acylepimines (**7a**, **7b**, and **8**) were similarly labile to acids. Treatment of the D-*glycero*-acetylepimine (**7a**) and the benzoylepimine (**7b**) with warm acetic acid easily afforded 6-acetamido-7-O-acetyl-6-deoxy-1,2;3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (**17a**) and the 6-benzamido homolog (**17b**) as thick syrups, respectively. The L-*glycero*-acetylepimine (**8**) was much more easily converted into the corresponding L-*glycero*-6-acetamido-7-acetate (**18**) on treatment with acetic acid even at a lower temperature. Ring opening with terminal attack of an acetoxyl group was considered reasonable on the basis of the case of 5,6-acetylepimines.[20] Mass spectrometry of these heptopyranoses (**17a** and **18**) also supports this fact; mass spectra of both **17a** and **18** exhibit peaks at *m/e* 113, 100, and 85, which would originate from 1,2:3,4-di-O-isopropylidene-galactose moiety[21] as shown in Fig. 1 and 2. Further, they show additional common characteristic strong peaks assignable to the fragments illustrated in Chart 3. Peaks at *m/e* 144, 102, and 84 would be due to fragments A, B, and C arisen from a cleavage

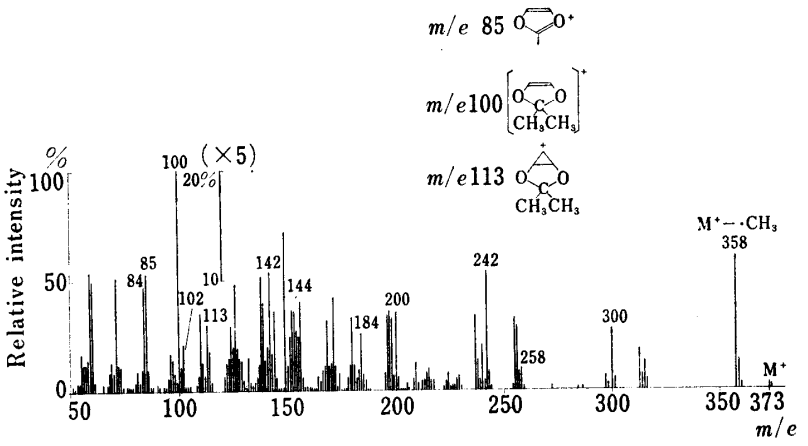


Fig. 1. Mass Spectrum of 6-Acetamido-7-O-acetyl-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (**17a**)

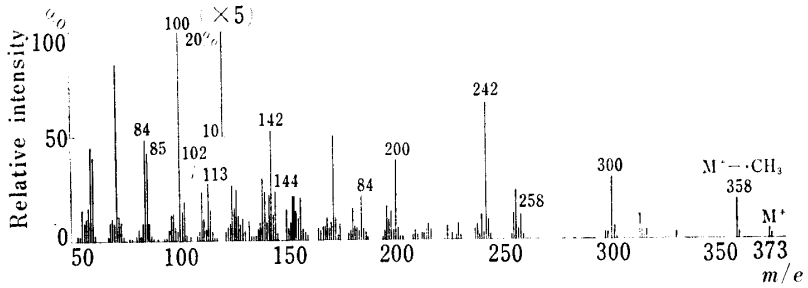
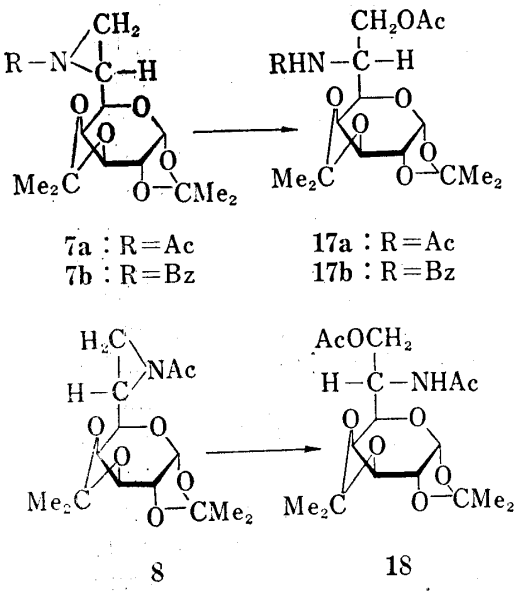


Fig. 2. Mass Spectrum of 6-Acetamido-7-O-acetyl-6-deoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (**18**)

of the carbons at the 5- and 6-position, and peaks at *m/e* 242, 200, 184, and 142 to fragments D, E, F, and G, arisen from removal of the terminal acetoxymethyl group and successive further

21) D.C. DeJongh and K. Biemann, *J. Am. Chem. Soc.*, **86**, 67 (1964).



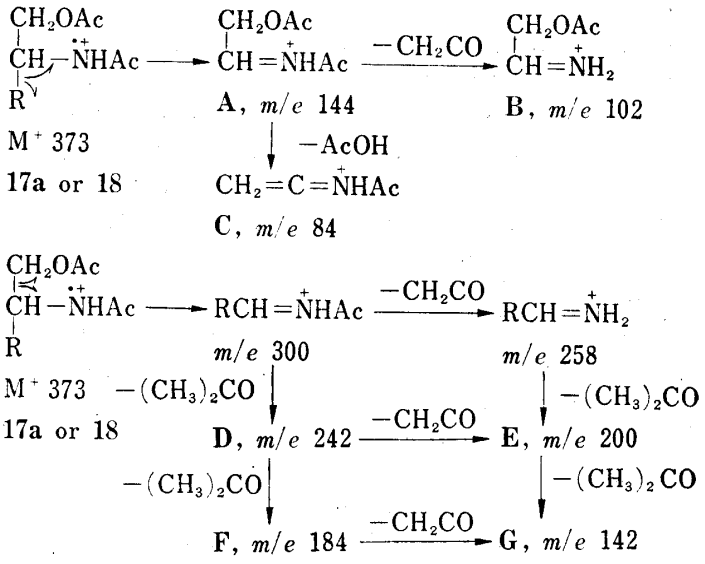


Chart 3

fragmentations. These data indicate the presence of an acetoxy group at the terminal position in these acetamidoacetates (**17a** and **18**).

The D-*glycero*-6-acetamido-7-O-acetate (**17a**) thus obtained was assumed to be a promising intermediate for the synthesis of lincosamine derivatives because **17a** has the same hexose sequence as lincosamine. Further extension of the side chain from **17a** was carried out as follows. Selective de-O-acetylation of **17a** was conducted with a catalytic amount of sodium methoxide in methanol, giving an acetamido–alcohol (**19**) as a thick syrup. Following the method of Pfitzner and Moffatt,[22] **19** was treated with dicyclohexylcarbodiimide in sulfoxide in the presence of phosphoric acid to give a syrupy aldehyde (**20**), which reduced the Fehling reagent and showed an infrared absorption at 1725 cm^{-1}; further, its NMR spectrum revealed an aldehyde proton. Preliminary test on reduction of the aldehyde (**20**) thus obtained with sodium borohydride exclusively yielded the parent alcohol (**19**) and this fact indicates that the aldehyde (**20**) kept the D-*glycero*-configuration at the 6-position during this oxidation. However, it was found that attempted chromatographic purification of the crude syrup of **20** caused partial isomerization at the 6-position. It was at first presumed that there is some doubt about the purity of the starting material (**19**), because **19** could not be obtained as crystals suitable for purification. Accordingly, the 6-acylamide (**17a**, **17b**, or **19**) was hydrolysed with aqueous barium hydroxide solution to give a crystalline amino–alcohol (**21**), which, after purification by repeated recrystallization, was *N*-acetylated and oxidized into the aldehyde (**20**). This aldehyde (**20**), which showed a single spot on thin-layer chromatogram, was charged on a silica gel column for two days and the column was washed with ethyl acetate-hexane (1:1,v/v). Thin-layer chromatography of the syrupy aldehyde fraction thereby obtained showed the presence of two components. Reduction of the aldehyde fraction with sodium borohydride, followed by acetylation in pyridine, gave a crystalline L-*glycero*-6-acetamido-7-acetate (**18**) and the D-*glycero*-epimer (**17a**) in approximate ratio of 1:1. Formation of the L-*glycero*-epimer (**18**) indicates that the absorption of the D-*glycero*-aldehyde (**20**) on silica gel induced an epimerization of its 6-position, giving the L-*glycero*-aldehyde (**22**), and the latter was converted into **18** *via* the alcohol (**23**). Further, selective de-O-acetylation of the L-*glycero*-6-acetamido-7-O-acetate (**18**) with sodium methoxide gave the L-*glycero*-acetamido-alcohol (**23**) as crystals which was oxidized with the Pfitzner-Moffatt reagent to give a syrupy L-*glycero*-aldehyde (**22**). These compounds were identified by thin-layer chromatography.

22) K.E. Pfitzner and J.G. Moffatt, *J. Am. Chem. Soc.*, **85**, 3027 (1963); *idem*, *ibid.*, **87**, 5661 (1965).

As the aldehyde (**20**) was found to be labile to ordinary purification, **20** was used as the material for the next Grignard reaction without purification or after a rapid chromatographic procedure which did not affect it. Moreover, in this oxidation reaction, use of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate in place of dicyclohexylcarbodiimide gave the aldehyde (**20**) without contamination of urea derivatives.

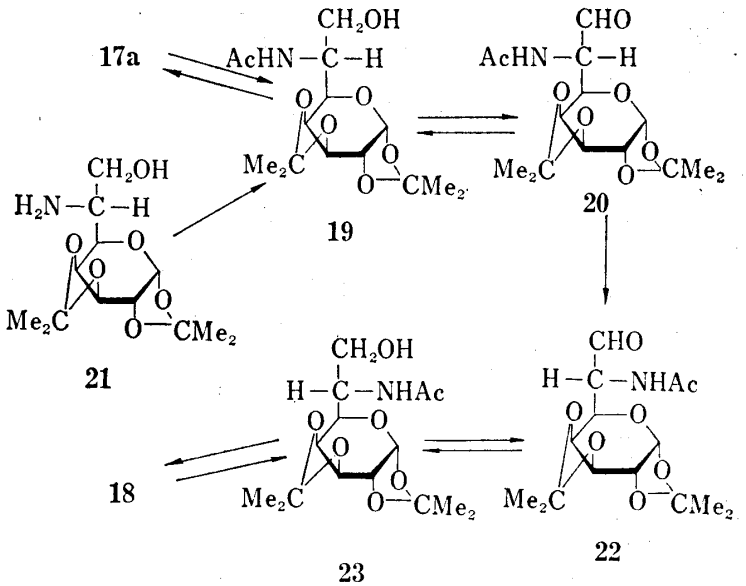


Chart 4

Extension of the carbon chain from the D-*glycero*-aldehyde (**20**) was carried out by the Grignard reaction using methylmagnesium bromide. Treatment of **20** with excess of the reagent in ether or tetrahydrofuran and succeeding column chromatography afforded only one crystalline product, whose NMR spectrum (100 MHz) indicated a new methyl absorption at 1.14 ppm as a doublet with J=6.5 Hz and whose infrared spectrum exhibited no carbonyl but hydroxyl absorption. Examination of the reaction mixture by thin-layer chromatography also did not reveal the presence of other possible isomer. In parallel with this experiment, N-acetyl-1,2:3,4-di-O-isopropylidenelincosamine (**24**) was prepared as follows: Hydrazinolysis[5c] of lincomycin[23] and successive N-acetylation gave methyl N-acetylthiolincosaminide which was treated with mercuric chloride in water,[24] giving N-acetyllincosamine (**25**) as crystals.[25] Treatment of **25** with acetone in the presence of zinc chloride and phosphoric acid gave its 1,2:3,4-di-O-isopropylidene derivative (**24**). However, the above-mentioned Grignard reaction product was different from **24** in thin-layer chromatography, melting point test, and infrared and NMR spectra. Therefore, the product was designated as 6-acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-*threo*-α-D-*galacto*-octopyranose (**26**) which is isomeric with **24** at the 7-position.

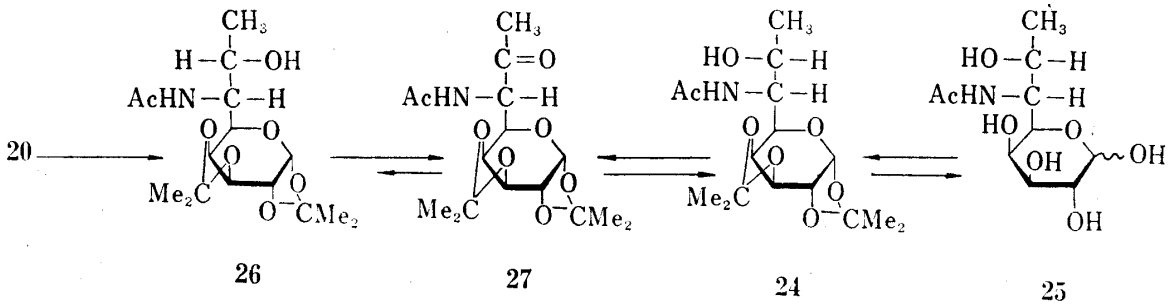


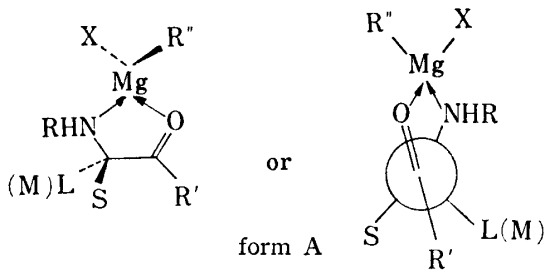
Chart 5

The L-*threo*-octose derivative (**26**) thus obtained was oxidized with chromium trioxide–pyridine complex,[26] yielding a 7-oxo derivative (**27**) in a good yield, whose infrared spectrum

23) Obtained from Lincocin capsule (Upjohn Co.).
24) French Patent 1451314 (1966) (Upjohn).
25) N-Acetyllincosamine was described as an amorphous powder and has not been characterized well.
26) G.I. Poos, G.E. Arth, R.E. Boeyler, and L.H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953); J.M. Sugihara and G.U. Yuen, *ibid.*, **79**, 5780 (1957).

contained a carbonyl absorption at 1725 cm^{-1}. Analogous oxidation of N-acetyl-di-O-isopropylidenelincosamine (**24**), gave the same octulose (**27**) in a good yield. This fact is also an evidence that the 6-acetamido-octose (**26**) synthesized from **20** has an L-*threo*-configuration isomeric with the lincosamine derivative (**24**) at the 7-position. Reduction of the octulose (**27**) with sodium borohydride in methanol mainly afforded the desired lincosamine derivative (**24**), along with its epimer (**26**). The relative ratio of **24** to **26** was determined as 2.2:1 by vapor–phase chromatographic analysis of the O-acetylated reduction product over 10% XE-60 on Chromosorb W at 210°. This result is quite consistent with the fact that sodium borohydride reduction of 2-deoxy-3,4-O-isopropylidene-7-oxolincomycin gave 70% of the corresponding D-*erythro*-derivative and 30% of the L-*threo*-derivative.[27] The N-acetyllincosamine derivative (**24**) synthesized from the octulose (**27**) was identified with the sample derived from lincomycin by mixed melting point test and a comparison of spectral data. Further, the synthesized **24** was hydrolysed with aqueous acetic acid or Amberlite IR-120 (H^+) in water, giving N-acetyllincosamine as crystals which was also indistinguishable from the authentic sample by thin–layer chromatography and infrared spectra.

Moreover, we wish to mention about the stereochemical course in the conversion of **20** into **26** and of **27** into **24**. The major diastereomer resulting from chemical addition to a carbonyl group directly attached to an asymmetric carbon atom can be generally predicted from the investigations of Cram and his co–workers.[28] They have formulated a principle that the favorable diastereoisomer will be that which results from the approach of the entering group from the side least hindered by the groups on the existing adjacent asymmetric center. When the asymmetric center carries oxygen or nitrogen which may form a complex with the reagent, the principle will be modified.[29] For example, reaction of the Grignard reagent and α-acylamino-ketone or aldehyde will result in an initial formation of a complex illustrated as form A. The entering group R″ will approach from the side where a smaller substituent (S) is oriented. In the case of metal hydride reduction or Meerwein–Ponndorf reduction, similar assumption will be applied, as shown in the reduction of dehydrochloramphenicol and related compounds.[30]



form A

Chart 6

The stereospecific formation of the L-*threo*-octose derivative (**26**) from the aldehyde (**20**) is in good accordance with the above–described principle. In the reaction of **20** and methylmagnesium bromide, one can postulate the initial formation of an intermediate, form B, with the participation of a nitrogen atom of the neighboring acetamido group. The Dreiding model of this intermediate indicates that di-O-isopropylidene-α-D-galactopyranose moiety is a very large substituent and, in particular, its 1,2-O-isopropylidene group overspreads the hindered (right) side, suggesting that the possible attack of the methyl anion from this side is extremely unlikely. Accordingly, the entering methyl anion approaches from the unhindered (left) side of this intermediate and the exclusive formation of the L-*threo*-octose (**26**), form C, will result. Predominant formation of the D-*erythro*-octose (lincosamine) derivative (**24**), form E, on the treatment of the octulose (**27**) with sodium borohydride would also be explained

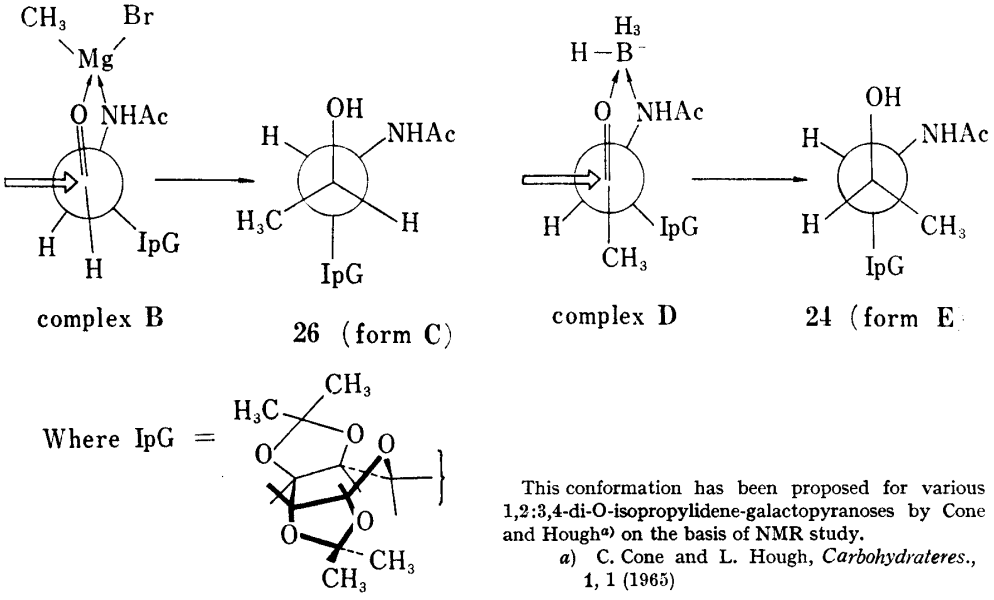
27) Private communication from Dr. H. Hoeksema, Upjohn Co., U.S.A., to whom we express our appreciation.

28) D.J. Cram and D.R. Wilson, *J. Am. Chem. Soc.*, **85**, 1245 (1963), and its preceding papers. An improved rationalization of Cram's rule was discussed. *cf.* G J. Karabatsos, *J. Am. Chem. Soc.*, **89**, 1367 (1967).

29) D.J. Cram and K.R. Kopecky, *J. Am. Chem. Soc.*, **81**, 2748 (1957).

30) J. Sicher, M. Svoboda, M. Hrda, J. Rudinger, and F. Šorm, *Collection Czech. Chem. Commun.*, **18**, 487 (1953).

by assuming that the reaction proceeds *via* an analogous intermediate, form D, with the attack of a hydride ion on the less hindered (left) side. Smaller stereospecificity of the reaction with formation of the minor epimer (**26**) would be ascribed to the small size of the entering anion such as the metal hydride.

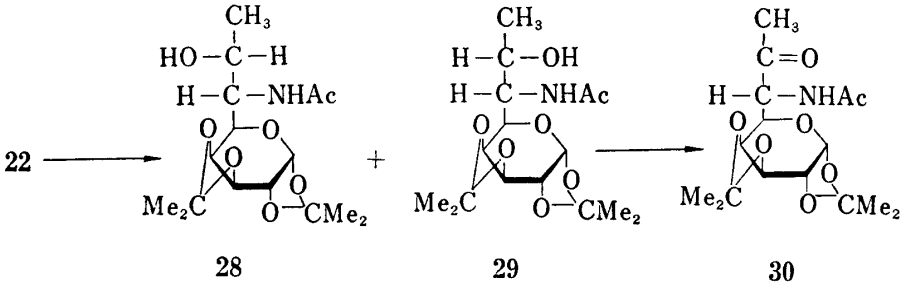


This conformation has been proposed for various 1,2:3,4-di-O-isopropylidene-galactopyranoses by Cone and Hough[a] on the basis of NMR study.

a) C. Cone and L. Hough, *Carbohydrateres.*, 1, 1 (1965)

Chart 7

Finally, we wish to mention an analogous sequence of reactions starting from the L-*glycero*-aldehyde (**22**). Somewhat different from the exclusive formation of the L-*threo*-octose derivative (**26**) on treatment of the D-*glycero*-aldehyde (**20**) with the Grignard reagent, the reaction of **22** and methylmagnesium bromide resulted in the formation of an epimeric octose mixture with predominant formation of one component. Thin-layer chromatogram of the product revealed two spots. The major component was isolated from the mixture as crystals after column chromatography which was different from the above-described L-*threo*-octose (**26**) or D-*erythro*-octose (lincosamine) derivative (**24**) by melting point test and by infrared and NMR spectrometry. The behavior of this isomer on thin-layer chromatogram quite resembled that of the L-*threo*-octose derivative (**26**). Further, the stereochemical process of the Grignard reaction as described above would be applicable to this reaction of the L-*glycero* series, suggesting that the predominant isomer is tentatively assignable as 6-acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-*threo*-α-D-*galacto*-octopyranose (**28**) and, consequently, the other minor isomer would be L-*erythro*-epimer (**29**). The latter could not be characterized well by the lack of an adequate supply of the sample. Oxidation of **28** either in pure state or in a mixed state



28 29 30

Chart 8

with the minor isomer (**29**) yielded an L-*glycero*-octulose derivative (**30**) as crystals which was distinguished from the D-*glycero*-octulose (**27**) by mixed melting point test and spectral data.

After this manuscript was finished, a short communication of Howarth, *et al.*[31] appeared in the publication received shortly afterwards describing the synthesis of *N*-acetyllincosamine by an entirely different route.

Experimental

Melting points are not corrected. Infrared spectra were determined on a Perkin–Elmer Model 221 or Perkin–Elmer Infracord, NMR spectra on a Varian A-60 or HA-100 spectrometer with tetramethylsilane as an internal standard, and mass spectra on a JEOL JMS-01SG spectrometer. The removal of solvent was accomplished *in vacuo* by a rotating flash evaporator at 20—30 mmHg and usually at 35—50°. Plates for thin-layer chromatography were prepared with Silica Gel G (E. Merck AG). Development of spots was effected by spraying a solution of NH_4VO_3 in 50% H_2SO_4, followed by heating. Column chromatography was carried out on a column packed with silica gel (Kanto Chemical Co., Tokyo). In the description of NMR spectra, the signals are expressed as *n*s (singlet), *n*d (doublet), *n*dd (doublet of doublets), *n*br. (broad absorption), where *n* is the number of protons indicated by integration, the δ values as ppm, and the coupling constants, J, as Hz, respectively.

1,2:3,4-Di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranurononitrile (5a)——To an ice-cold solution of 1.3 g of 1,2:3,4-di-O-isopropylidene-α-D-*galacto*-hexodialdo-1,5-pyranose[8,11,12] (**4**) in 15 ml of MeOH, a solution of 1 g of NaCN in 6 ml of H_2O was slowly added with stirring. After 1 hr's stirring, 100 ml of $CHCl_3$ and 50 ml of saturated aq. NaCl were added to this mixture. The $CHCl_3$ layer was separated after extraction procedure, dried over anhyd. Na_2SO_4, and 1.15 g of a syrup was obtained by evaporation of the solvent. To a solution of the syrup in a few ml of benzene, hexane was added to a slight turbidity and the resultant mixture was allowed to stand overnight in a refrigerator. Recrystallization of crystals (133 mg) thus obtained from benzene–ligroin afforded **5a** as needles, mp 145—149°, $[\alpha]_D^{19}$ −71.7° (c=3.9, $CHCl_3$). *Anal.* Calcd. for $C_{13}H_{19}O_6N$: C, 54.73; H, 6.71; N, 4.91. Found: C, 54.93; H, 6.69; N, 4.76.

1,2:3,4-Di-O-isopropylidene-6-O-tosyl-L-*glycero*-α-D-*galacto*-heptopyranurononitrile (5b) and its D-*glycero*-Epimer (6b)——A similar treatment of 18.3 g of **4** with 14.1 g of NaCN as described above gave a mixture (18.9 g) of **5a** and **6a** as a syrup. The syrup was tosylated with 6.96 g (1.1 mole) of TsCl in 46 ml of pyridine in a usual manner. After standing overnight at room temperature, the reaction mixture was diluted with 300 ml of $CHCl_3$ and the solution was washed successively with dil. HCl, aq. $NaHCO_3$, and H_2O dried over anhyd. $MgSO_4$, and evaporated, leaving 8 g of a syrup which crystallized on trituration with AcOEt–hexane. Fractional recrystallization from AcOEt–hexane easily gave the D-*glycero*-6-tosylate (**6b**) as prisms or rods of mp 145—145.5°, $[\alpha]_D^{21}$ −110.4° (c=2.3, $CHCl_3$). The L-*glycero*-epimer (**5b**) was also separated as needles of mp 152—154°, $[\alpha]_D^{19}$ −46.1° (c=5.5, $CHCl_3$), but with more difficulties. Infrared spectra of **5b** and **6b** did not show a nitrile absorption at 2260—2240 cm^{-1}. NMR (60 MHz) ($CDCl_3$) for **5b**: 5.38 (1d, $J_{1,2}$=5.0, H_1), 5.15 (1d, $J_{5,6}$=8.5, H_6), 4.65 (1dd, $J_{2,3}$=2.5, $J_{3,4}$=7.5, H_3), 4.37 (1dd, $J_{3,4}$=7.5, $J_{4,5}$=2.0, H_4), 4.30 (1dd, $J_{1,2}$=5.0, $J_{2,3}$=2.5, H_2), 4.02 (1dd, $J_{4,5}$=2.0, $J_{5,6}$=8.5, H_5), 2.45 (3s, CH_3 of tosyl), 1.66 (3s), 1.40 (3s) and 1.32 (6s) (CH_3 of isopropylidene); for **6b**: 5.49 (1d, $J_{1,2}$=5.0, H_1), 5.13 (1d, $J_{5,6}$=8.5, H_6), 4.58 (1dd, $J_{2,3}$=2.5, $J_{3,4}$=8.0, H_3), 4.31 (1dd, $J_{1,2}$=5.0, $J_{2,3}$=2.5, H_2), 4.11 (1dd, $J_{3,4}$=8.0, $J_{4,5}$=2.0, H_4), 4.05 (1dd, $J_{4,5}$=2.0, $J_{5,6}$=8.5, H_5), 2.45 (3s, CH_3 of tosyl), 1.52 (3s), 1.33 (6s), and 1.22 (3s) (CH_3 of isopropylidene). *Anal.* Calcd. for $C_{20}H_{24}O_8NS$: C, 54.78; H, 5.52; N, 3.19; S, 7.31. Found for **5b**: C, 54.36; H, 5.57; N, 3.12; S, 7.29; for **6b**: C, 54.68; H, 5.77; N, 3.03; S, 7.21.

The L-*glycero*-6-tosylate (**5b**) was also obtained from **5a** by tosylation of 17 mg of **5a** with 20 mg of TsCl in 0.1 ml of pyridine for 32 hr, followed by the usual procedure. Yield, 15 mg of **5b**.

6,7-(Acetylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (7a)——To a stirred solution of 2.50 g of **5b** in 300 ml of dry ether was added 420 mg of $LiAlH_4$ in one portion at room temperature. After having been stirred for 1 hr at room temperature, the mixture was diluted with hydrous ether slowly to decompose the excess reagent and filtered. The filtrate was dried over anhyd. Na_2SO_4 and evaporated to leave 1.44 g (93.2%) of a colorless syrup. This syrup was dissolved in 5 ml of MeOH and treated with 0.2 ml of Ac_2O for 5 min, the reaction mixture was neutralized with saturated aq. $NaHCO_3$, and extracted twice with 25 ml of $CHCl_3$. The combined extract was washed with H_2O, dried over anhyd. $MgSO_4$, and evaporated below 40° (bath temp.) to leave a colorless syrup (1.69 g). This syrup was absorbed on a silica gel column (25 g packed in hexane) and the column was washed with AcOEt–hexane (1:4, v/v). Evaporation of the solvent from the eluate gave 1.825 g (71.7% **5b**) of **7a** as a colorless syrup of $[\alpha]_D^{20}$ −50.9° (c=2.5, $CHCl_3$). IR $\nu_{max}^{liq.}$: 1710 cm^{-1} (amide), no N–H absorption. *Anal.* Calcd. for $C_{15}H_{23}O_6N$: C, 57.49; H, 7.40; N, 4.47. Found: C, 57.49; H, 7.49; N, 4.25.

31) G.B. Haworth, W.A. Szarek, and J.K.N. Jones, *Chem. Commun.*, **1969**, 1339.

6,7-(Benzoylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (7b)——Analogously, 500 mg of **5b** was treated with 84 mg of $LiAlH_4$ and the resulting epimine was dissolved in 2 ml of MeOH. To the stirred solution was added 260 mg of benzoic anhydride and the mixture was allowed to stand for 1 hr at room temperature. The mixture was neutralized by slow addition of a cold saturated aq. $NaHCO_3$, the crystals thereby formed were collected, washed with H_2O, and dried to 357 mg (81.5% from **5b**) of crude **7b**. Recrystallization from iso-PrOH–hexane gave **7b** as needles of mp 107—108° $[\alpha]_D^{22}$ −7.4° (c=3.1, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1680 (amide), 1605, 1585 (aromatic). *Anal.* Calcd. for $C_{20}H_{25}O_6N$: C, 63.98; H, 6.71; N, 3.73. Found: C, 64.13; H, 6.45; N, 3.68.

6,7-(Acetylepimino)-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose(8)——A solution of 3.0 g of **6b** in 450 ml of dry ether was treated with 0.5 g of $LiAlH_4$ for 1 hr. The reaction mixture was worked up as described for the preparation of **7a**, and yielded 2.21 g of crude **8** as a colorless syrup which crystallized by the addition of 1 ml of iso-PrOH. After further addition of 5 ml of hexane, the crystals were collected to 1.76 g of needles. Recrystallization from hexane containing a small amount of iso-PrOH afforded **8** as needles of mp 95—96°, $[\alpha]_D^{21}$ −121.0° (c=0.7, $CHCl_3$). IR ν_{max}^{Nujol} 1700 cm^{-1} (amide). *Anal.* Calcd. for $C_{15}H_{23}O_6N$: C, 57.49; H, 7.40; N, 4.47. Found: C, 57.76; H, 7.75; N, 4.34.

7-Acetamido-6,7-dideoxy-1,2:3,4-di-O-isopropylidene-α-D-*galacto*-heptopyranose (N-Acetate of 9)——To a stirred solution of 500 mg of **6b** in 17 ml of dry ether was added 88 mg of $LiAlH_4$ in one portion. After being stirred for 1.5 hr, the reaction mixture was treated as described for the preparation of **7a**, and afforded an amine as a colorless syrup which was N-acetylated with 0.5 ml of Ac_2O in 2 ml of MeOH. The resultant syrup (353 mg) was worked up in the usual manner and allowed to stand for crystallization. The crystals were collected and recrystallized from AcOEt–hexane to 264 mg of the N-acetate of **9**, mp 116—117°. IR ν_{max}^{Nujol} cm^{-1} 1660, 1540 (amide). NMR (60 MHz) ($CDCl_3$): 6.5—5.8 (1br. –NHCO–), no CH_3 signal in 1.7—0.5. *Anal.* Calcd. for $C_{15}H_{25}O_6N$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.19; H, 7.96; N, 4.03.

The syrup (46 mg) left by evaporation of the recrystallization mother liquor was found not to contain **7a**, as a result of infrared spectrometry.

1,2:3,4-Di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (14)——Following the procedure of David and Popot,[16] potassium 1,2:3,4-di-O-isopropylidene-α-D-galactopyranuronate[17] **(10)** was treated with oxalyl chloride in dry ether in place of dry benzene reported by them[16] and afforded the crude chloride as a syrup which crystallized on trituration with hexane. Recrystallization from benzene–hexane gave 1,2:3,4-di-O-isopropylidene-α-D-galactopyranuronyl chloride (**11**) as needles of mp 65—66°. Treatment of **11** with CH_2N_2 in ether yielded the diazoketone (**12**) as needles of mp 118—128°. Treatment of **12** with AcOH in the presence of $Cu(OAc)_2$ at 100—110° for 3.5 hr yielded the crude **13** as a syrup which crystallized on trituration with hexane. Recrystallization from AcOEt–hexane gave the acetoxyketone (**13**) as prisms of mp 90—92°. Following the procedure of David and Popot,[19] reduction of **13** with $LiAlH_4$ afforded **14** as a powder (from iso-Pr_2O) of mp 98—101°.

1,2:3,4-Di-O-isopropylidene-6,7-di-O-tosyl-L-*glycero*-α-D-*galacto*-heptopyranose (15)——A mixture of 328 mg of **14**, 500 mg of TsCl, and 4 ml of pyridine was allowed to stand for 5 days at room temperature. Then the mixture was diluted with ice water and extracted twice with 30 ml of $CHCl_3$. The combined extract was washed successively with dil. HCl, H_2O, saturated aq. $NaHCO_3$, dried over anhyd. $MgSO_4$, and evaporated, leaving a syrup which was absorbed on a column of silica gel (10 g packed in benzene). The column was washed with 50 ml of benzene and then eluted with 150 ml of AcOEt–benzene (3:97, v/v). Evaporation of the solvent from the eluate gave **15** as a colorless powder (579 mg), $[\alpha]_D^{21}$ −63.9° (c=2.4, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1600, 1190, (tosyl), no OH absorption.

7-Azido-7-deoxy-1,2:3,4-di-O-isopropylidene-6-O-tosyl-L-*glycero*-α-D-*galacto*-heptopyranose (16)——A mixture of 376 mg of **15**, 45 mg of NaN_3, and 3 ml of Me_2SO was heated at 90—100° for 1.5 hr and then was diluted with $CHCl_3$. After the solution was shaken with saturated aq. NaCl, the $CHCl_3$ layer was separated, dried over anhyd. $MgSO_4$, and evaporated. The syrup thus obtained was purified by chromatography on a column of silica gel (7 g packed in benzene) using AcOEt–benzene (1:49, v/v) for elution, affording 234 mg of **16** as a syrup, $[\alpha]_D^{21}$ −82.7° (c=1.7 $CHCl_3$). IR $\nu_{max}^{liq.}$ cm^{-1}: 2100 (azide), 1600, 1190 (tosyl). *Anal.* Calcd. for $C_{20}H_{27}O_8N_3S$: C, 51.16; H, 5.81; N, 8.95; S, 6.83. Found: C, 51.34; H, 5.80; N, 8.90; S, 6.63.

Conversion of 16 into the Acylepimines (7a and 7b)——To an ice-cold solution of 225 mg of **16** in 5 ml dry ether was added 37 mg of $LiAlH_4$ with stirring. After being stirred at 0° for 5 min and further at room temperature for 45 min, the reaction mixture was treated in a usual manner, affording the crude epimine. The epimine thus obtained was N-acetylated and purified by column chromatography, yielding 75 mg of **7a**, which was identified with the sample obtained from **5b** by thin-layer chromatography and NMR spectrometer. Alternately, the free epimine was *N*-benzoylated in the same way as described earlier and gave the N-benzoylepimine (**7b**) which was identified with the sample obtained earlier, by mixed melting point test and infrared spectrometry.

6-Acetamido-7-O-acetyl-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (17a)——A solution of 1.44 g of **7a** in 8 ml of AcOH was heated at 50—55° for 1 hr. The solution was neutralized with solid $NaHCO_3$, diluted with ice water, and extracted with three 20 ml portions of $CHCl_3$. The combined extract was washed successively with saturated aq. $NaHCO_3$ and H_2O, dried over anhyd. $MgSO_4$, and evaporated, leaving 1.47 g of a thick syrup. Its column chromatography on silica gel (30 g) using AcOEt contain-

ing a gradient amount of MeOH (0—5%, v/v) for elution, gave **17a** as a colorless syrup or powder, $[\alpha]_D^{21}$ −47.9° (c=3.4, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1750 (acetate), 1665 and 1550 (acetamide). NMR (60 MHz) ($CDCl_3$): 2.02 (3s, CH_3 of acetate), 1.95 (3s, CH_3 of acetamide), *ca.* 6.2 (1br., –NH–CO–), *Anal.* Calcd. for $C_{13}H_{23}O_6N$: C, 53.96; H, 8.01; N, 4.84. Found: C, 53.90; H, 8.09; N, 4.89.

7-O-Acetyl-6-benzamido-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (17b)——A solution of 350 mg of **7b** in 5 ml of AcOH was warmed at 50—55° for 45 min. Evaporation of AcOH from the mixture under a diminished pressure gave 390 mg of **17b** as an amorphous powder. IR ν_{max}^{Nujol} cm^{-1}: 1750 (acetate), 1650, 1610 and 1585 (benzamide).

6-Acetamido-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (19)——To a solution of 2.90 g of **17a** in 50 ml of MeOH was added 1 ml of methanolic 1N $NaOCH_3$ and the mixture was allowed to stand overnight in a refrigerator or for 2 hr at room temperature. The mixture was diluted with 500 ml of $CHCl_3$ and 100 ml of saturated aq. NaCl. The $CHCl_3$ layer separated after vigorous shaking, dried over anhyd. $MgSO_4$, and evaporated to dryness, leaving **19** as an amorphous powder which was pure on thin-layer chromatogram. Analytical sample was obtained by chromatography of the amorphous powder (860 mg) over 20 g of silica gel and elution with hexane containing a gradient amount of AcOEt (50—100%), which gave 796 mg of **19** as a powder, $[\alpha]_D^{20}$ −41.5° (c=2.3, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300—3500 (OH, NH), 1660 and 1550 (acetamide), no acetoxyl band at 1750. NMR (60 MHz) ($CDCl_3$): 3.2—2.8 (1br., –OH), 1.95 (3s, CH_3 of acetamide). *Anal.* Calcd. for $C_{15}H_{25}O_7N$: C, 54.37; H, 7.61; N, 4.23. Found: C, 53.99; H, 7.54; N, 4.32.

6-Amino-6-deoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-heptopyranose (21)——A mixture of 503 mg of **19**, 3 ml of acetone, and 15 ml of saturated aq. $Ba(OH)_2$ was refluxed on a steam bath for 7 hr. The mixture was saturated with CO_2 and filtered with activated carbon. The filtrate was concentrated *in vacuo* to 1 ml, basified with 2 ml of conc. NH_4OH, and extracted with 30 ml of $CHCl_3$. The extract was washed with H_2O, dried over anhyd. $MgSO_4$, and evaporated. A syrup thus obtained crystallized on addition of a small amount of ether–hexane, yielding 269 mg of **21** as needles of mp 113—116°. Recrystallization from AcOEt gave the analytical sample as needles of mp 118—120°, $[\alpha]_D^{20}$ −57.5° (c=3.3, $CHCl_3$). Ninhydrin test, positive. IR ν_{max}^{Nujol} cm^{-1}: 3400, 3200 (OH, NH), 1585 (NH_2). *Anal.* Calcd. for $C_{13}H_{23}O_6N$: C, 53.96; H, 8.01; N, 4.84. Found: C, 53.90; H, 8.09; N, 4.89.

Alternately, a mixture of 780 mg of **17a**, 3 ml of acetone, and 20 ml of saturated aq. $Ba(OH)_2$ was refluxed on a steam bath for 9 hr. Similar treatment as described above gave 290 mg of **21**. The analogous treatment of **17b** (436 mg) in 5 ml of EtOH, with aq. $Ba(OH)_2$, yielded 291 mg of **21**.

6-Acetamido-7-O-acetyl-6-deoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (18)——A solution of 2.0 g of **8** in 10 ml of AcOH was allowed to stand for 1—2 hr at room temperature or for 1—5 min at 50—60°. The mixture was diluted with 5 ml of H_2O and partly neutralized with solid $NaHCO_3$ with cooling. The precipitate which appeared by further dilution with H_2O was collected and dried over KOH pellets, giving 2.1 g of **18** which, on recrystallization from AcOEt, afforded prisms of mp 137—140°, $[\alpha]_D^{21}$ −46.4° (c=3.8, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3350, 3250—3200, 3100 (NH, H_2O of crystallization), 1720 (acetate), 1660, 1580 (amide). NMR (60 MHz)($CDCl_3$), 5.91 (1br. d, –NH–CO–), 2.03 (3s, CH_3 of acetate), 1.96 (3s, CH_3 of acetamide). *Anal.* Calcd. for $C_{17}H_{27}O_8N \cdot \frac{1}{2}H_2O$: C, 53.39; H, 7.38; N, 3.66. Found: C, 53.67; H, 7.53; N, 3.60.

These prisms gave another crystalline form when dried over P_2O_5 at 100—110° and at 5 mmHg for 3 hr, but did not become completely anhydrous. IR ν_{max}^{Nujol} cm^{-1}: 3350 (NH), 1750 (acetoxy), 1655, 1540 (amide). *Anal.* Found: C, 53.92; H, 7.23; N, 3.72. *cf.* Calcd. for $C_{17}H_{27}O_8N$ (anhydrous): C, 54.68; H, 7.29; N, 3.75.

6-Acetamido-6-deoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-heptopyranose (23)——To a solution of 2.1 g of **18** in 15 ml of MeOH was added 0.5 ml of methanolic 1N $NaOCH_3$. After standing at room temperature for 15 min, the solution was treated with Amberlite IR-120 (H^+) to remove $NaOCH_3$ and evaporated to leave a crystalline mass. Recrystallization from AcOEt–hexane gave 1.6 g of **23** as needles, mp 129—133°, $[\alpha]_D^{21}$ −43.5° (c=3.6, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3250 (OH, NH), 1655, 1550, 1530 (acetamide), NMR (60 MHz) ($CDCl_3$): 1.98 (3s, CH_3 of acetamide). *Anal.* Calcd. for $C_{15}H_{25}O_7N$: C, 54.37; H, 7.61; N, 4.23. Found: C, 54.30; H, 7.76; N, 4.20.

6-Acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-*threo*-α-D-*galacto*-octopyranose (N-Acetyl-1,2:3,4-di-O-isopropylidene-*epi*-lincosamine) (26)——To a solution of 1.008 g of **19** and 1.9 g of dicyclohexylcarbodiimide in 15 ml of Me_2SO was added 1.5 ml of 1M H_3PO_4 in Me_2SO and the resulting mixture was allowed to stand for 3.5 hr at room temperature. MeOH solution of oxalic acid was slowly added to the mixture until evolution of CO_2 gas ceased. After addition of saturated aq. NaCl, the mixture was filtered, the filtrate was diluted with saturated aq. $NaHCO_3$, and extracted with two 70 ml portions of $CHCl_3$ and further with 30 ml of $CHCl_3$. The combined extract was washed with aq. NaCl, dried over anhyd. $MgSO_4$, and evaporated. The residue was dissolved in cold acetone and undissolved material (dicyclohexylurea) was removed by filtration. Evaporation of the solvent afforded a syrup which was treated several times by the same procedure for removal of the urea derivative, yielding 936 mg of crude aldehyde (**20**) as a syrup. IR $\nu_{max}^{liq.}$: 1725 cm^{-1} (aldehyde).

To the solution of this crude aldehyde (**20**) in 6 ml of tetrahydrofuran was added dropwise a mixture of 9 ml of 3M MeMgBr in Bu_2O (Tokyo Kasei Kogyo Co., Ltd.) and 4 ml of tetrahydrofuran with stirring and cooling. After stirring was continued for 1 hr at room temperature, the reaction mixture was diluted with saturated aq. NH_4Cl to dissolve the precipitates and extracted with three 30 ml portions of $CHCl_3$. The combined extract was washed with H_2O, dried over anhyd. $MgSO_4$, and evaporated, giving 735 mg of a powder. This powder was dissolved in a small amount of benzene and absorbed on a silica gel column (12 g packed in hexane). The column was first washed with AcOEt–hexane (1:1, v/v) to remove the contaminated urea derivative and then with AcOEt. Further elution with MeOH–AcOEt (1:9, v/v) and evaporation of the solvent from the eluate gave 431 mg of crude **26** as a syrup which revealed one spot on thin-layer chromatogram. The syrup crystallized on trituration with AcOEt–hexane and recrystallization of the collected crystals from AcOEt–hexane gave **26** as needles of mp 143.5—145°, $[\alpha]_D^{21}$ −33.1° (c=2.3, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3600, 3275, 3100 (OH, NH), 1640, 1570 (acetamide), no aldehyde absorption at 1720—1750 cm^{-1}. NMR (100 MHz) ($CDCl_3$): 2.95 (1d, J=3, -O<u>H</u>), 2.02 (3s, C<u>H</u>$_3$ of acetamide), 1.14 (3d, J=6.5, -C<u>H</u>-CH$_3$); (60 MHz): 2.84 (1d, J=3, -O<u>H</u>), 2.02 (3s, C<u>H</u>$_3$ of acetamide), 1.14 (3d, J=6.5, -C<u>H</u>-CH$_3$). *Anal.* Calcd. for $C_{16}H_{27}O_7N$: C, 55.64; H, 7.88; N, 4.06. Found: C, 55.35; H, 7.78; N, 4.14.

6-Acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-*erythro*-α-D-*galacto*-octopyranose (N-Acetyl-1,2:3,4-di-O-isopropylidenelincosamine) (24)——To a suspension of 3.5 g of N-acetyllincosamine[24,25] (**25**) in 165 ml of dry acetone was added 0.4 g of 100% H_3PO_4 with vigorous stirring at room temperature. After having been stirred for 2 hr, the mixture was allowed to stand overnight at room temperature. Then the mixture was neutralized with aq. NaOH, filtered, and the filtrate was extracted with $CHCl_3$. The extract was washed with H_2O, dried over anhyd. $MgSO_4$ and evaporated. The residue crystallized on standing or on treatment with a small amount of AcOEt. Recrystallization from AcOEt gave 1.75 g of **24** as leaflets or platelets of mp 165.5—166.5°, $[\alpha]_D^{22}$ −53.8° [32] (c=3.2, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3300, 3200 (shoulder), 3100 (OH, NH), 1650, 1570 (acetamide). NMR (60 MHz) ($CDCl_3$): 3.24 (1d, J=5.0, -O<u>H</u>), 1.98 (3s, C<u>H</u>$_3$ of acetamide), 1.23 (3d, J=6.0, -C<u>H</u>-CH$_3$); (100 MHz): 3.24 (1d, J=5.0, -O<u>H</u>), 1.96 (3s, C<u>H</u>$_3$ of acetamide), 1.22 (3d, J=6.0, -C<u>H</u>-CH$_3$). *Anal.* Calcd. for $C_{16}H_{27}O_7N$: C, 55.64; H, 7.88; N, 4.06. Found: C, 55.57; H, 7.71; N, 4.08.

6-Acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-*glycero*-α-D-*galacto*-octopyranos-7-ulose (27)——To a complex prepared from 300 mg of CrO_3 and 4 ml of pyridine was added 137 mg of **26** with stirring and the stirring was continued for 18 hr at room temperature. The mixture was diluted with 50 ml of AcOEt and filtered. The filtrate was evaporated to dryness and the solid residue was extracted with AcOEt. The extract was treated with activated carbon and evaporated, giving 135 mg of a crystalline mass which was recrystallized from AcOEt–hexane to afford **27** as silky needles, mp 174°. Further recrystallization from the same solvent gave needles of mp 181—183°, $[\alpha]_D^{21}$ −65.9° (c=4.2, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3210 (NH), 1725 (ketone), 1665, 1568 (acetamide). NMR (100 MHz) ($CDCl_3$): 4.83 (1dd, $J_{5,6}$=6.5 $J_{6,NH}$=8.0, H$_6$), 2.26 (3s, C<u>H</u>$_3$CO–), 2.00 (3s, C<u>H</u>$_3$ of acetamide): (60 MHz): 4.83 (1dd, H$_6$), 2.30 (3s, C<u>H</u>$_3$CO–), 2.02 (3s, C<u>H</u>$_3$ of acetamide). *Anal.* Calcd. for $C_{16}H_{25}O_7N$: C, 55.96; H, 7.34; N, 4.08. Found: C, 56.09; H, 7.56; N, 4.08.

In an analogous manner, 134 mg of **24** was oxidized with CrO_3–pyridine complex to give 122 mg of **27**, which was identical with the sample obtained as above by mixed melting point test and infrared and NMR spectrometry.

Reduction of 27 into N-Acetyllincosamine Derivative (24) and Its Epimer (26)——To a solution of 485 mg of **27** in 15 ml of MeOH was added 300 mg of $NaBH_4$ with stirring at room temperature. The mixture was stirred for 10 min, the excess reagent was decomposed with AcOH, and the solution was diluted with 150 ml of $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over anhyd. $MgSO_4$, and evaporated to give 480 mg of a syrup which crystallized on trituration with AcOEt–hexane. The crystalline mass exhibited two spots corresponding to **24** and **26** on thin-layer chromatogram. Column chromatography and recrystallization of the products afforded 171 mg of **24** and 55 mg of **26**. These samples were identified respectively with the authentic samples. The remaining mixture of **24** and **26** was difficult to be resolved to each epimer.

The relative ratio of **24** and **26** in the reaction mixture was determined as follows. The reduction mixture was fully acetylated with Ac_2O and pyridine, and the product obtained in a usual manner was analysed by gas-liquid chromatography on a glass tube (3 mm × 1 m) packed with 10% XE-60 on Chromosorb W (60/80) at 210°. The ratio of **24** and **26** thus determined was 2.2:1. Alternately, a comparison of OH proton signals in the NMR spectrum of the reaction mixture was carried out and the data thereby obtained supported the result from the gas chromatographic analysis.

6-Acetamido-6,8-dideoxy-D-*erythro*-D-*galacto*-octose (N-Acetyllincosamine) (25)——A solution of 400 mg of **24** in 50 ml of 50% aq. AcOH was refluxed for 30 min and the mixture was evaporated. The residue crystallized on trituration with EtOH and was stored in a refrigerator overnight. Recrystallization of the crystals (128 mg) from EtOH gave **25** as hygroscopic fine crystals, mp 130—141°, $[\alpha]_D^{25}$ +62.9° (5 min)→

32) The value was incorrectly listed in the preceding communication.[1]

+55.9° (20 hr, final) (c=3.0, H_2O). *Anal.* Calcd. for $C_{10}H_{19}O_7N$: C, 45.28; H, 7.22; N, 5.28. Found: C, 45.34, 45.58; H, 7.75, 7.53; N, 5.02.

The sample of **25** was identified with the authentic one by thin-layer chromatography and infrared spectrometry.

Analogous Conversion of the L-*glycero*-Epimer (23) into the Isomeric Octoses——To a solution of 1.5 g of **23** and 2.9 g of dicyclohexylcarbodimide in 23 ml of Me_2SO was added 2.23 ml of 1M H_3PO_4 in Me_2SO and the resulting mixture was allowed to stand overnight at room temperature. MeOH solution of oxalic acid was slowly added to the mixture until evolution of CO_2 gas ceased. After addition of 30 ml of ice water, the mixture was filtered. The filtrate was diluted with saturated aq. NaCl and extracted with three 30 ml portions of $CHCl_3$. The combined extract was washed with saturated aq. $NaHCO_3$ and H_2O, dried over anhyd. $MgSO_4$, and evaporated. The residue was dissolved in cold acetone and insoluble dicyclohexylurea was removed by filtration. After evaporation of the solvent, the same procedure was repeated several times, giving 1.321 g of crude **22** as a thick yellow oil. IR $\nu_{max}^{liq.}$: 1725 cm^{-1} (aldehyde).

Alternately, a solution of 713 mg of **23**, 2.74 g (3 mole) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate in 8 ml of Me_2SO and 1.8 ml of 1M H_3PO_4 in Me_2SO was allowed to stand for 2 hr at room temperature. The mixture was diluted with $CHCl_3$, filtered, and the filtrate was washed with saturated aq. NaCl. The $CHCl_3$ layer was dried over anhyd. $MgSO_4$ and evaporated to afford 712 mg of crude **22** as a syrup.

To a solution of 1.3 g of the crude **22** thus obtained in 10 ml of tetrahydrofuran was added dropwise a mixture of 12 ml of 3M MeMgBr in Bu_2O and 4 ml of tetrahydrofuran with stirring and cooling. After having been stirred for 1 hr at room temperature, the reaction mixture was treated in the same way as described earlier, affording a crystalline mass. On trituration with AcOEt–hexane, the product gave 600 mg of prisms, mp 154—161°, and 462 mg of a syrup. The latter was absorbed on a column of silica gel (8 g packed in hexane) and the column was eluted with AcOEt–hexane (1:1, v/v) (100 ml), AcOEt (100 ml), and MeOH–AcOEt (1:9, v/v) (100 ml). The fractions were monitored by thin-layer chromatography and the fractions of octose derivatives were collected and evaporated, giving 40 mg of further crop of crystals from the fast moving fractions, and 73 mg of a semi-crystalline oil from the slower moving fractions. The latter syrup revealed two spots corresponding to an epimeric mixture of octoses (**28** and **29**) on thin-layer chromatogram. All crystals were collected and recrystallized from AcOEt–hexane to give one epimer, which was tentatively designated as 6-acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-D-*threo*-α-D-*galacto*-octopyranose (**28**), as prisms, mp 163—165°, $[\alpha]_D^{21}$ −59.0° (c=5.3, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3350, 3100 (OH, NH), 1645, 1565 (acetamide). NMR (100 MHz) ($CDCl_3$): 3.74 (1ddd, H_6), 2.03 (3s, CH_3 of acetamide), 1.18 (3d, J=6.2, –CH–CH_3); (60 MHz): 3.73 (1ddd, $J_{5,6}$=6.5, $J_{6,7}$=2.0, $J_{6,NH}$=7.5), 2.05 (3s, CH_3 of acetamide), 1.19 (3d, J=6.2, –CH–CH_3). *Anal.* Calcd. for $C_{16}H_{27}O_7N$: C, 55.64; H, 7.88; N, 4.06. Found: C, 55.54; H, 7.94; N, 3.88.

6-Acetamido-6,8-dideoxy-1,2:3,4-di-O-isopropylidene-L-*glycero*-α-D-*galacto*-octopyranos-7-ulose (30)——To a complex prepared from 0.25 g of CrO_3 and 2 ml of pyridine was added a solution of 100 mg of **28** in 1.5 ml of pyridine with stirring at room temperature. After having been stirred overnight, the mixture was diluted with 10 ml of AcOEt and treated in the same way as described for **27**, giving 72 mg of crystals. Recrystallization from AcOEt–hexane gave **30** as platelets, mp 149—145°. IR ν_{max}^{Nujol} cm^{-1}: 3375 (NH), 1720 (ketone), 1660, 1525 (acetamide). NMR (60 MHz) ($CDCl_3$): 2.20 (3s, –CO–CH_3), 1.97 (3s, CH_3 of acetamide). *Anal.* Calcd. for $C_{16}H_{25}O_7N$: C, 55.96; H, 7.34; N, 4.08. Found: C, 55.75; H, 7.28; N, 4.08.

The epimeric mixture described above was also treated with CrO_3-pyridine complex to give **30** in a good yield.

Epimerization of the D-*glycero*-Aldehyde (20) on Silica Gel Column——N-Acetylation of the crystalline **21** with 0.2 ml of Ac_2O in 1 ml of MeOH, followed by oxidation with Pfitzner–Moffatt reagent as described above gave crude **20** which revealed one spot on thin-layer chromatogram. The syrup was absorbed on a column of silica gel (6 g packed in hexane) and developed with AcOEt–hexane (1:1, v/v). After standing for two days, the column was washed with hexane containing a gradient amount of AcOEt (50 to 100%) to give 146 mg of an aldehyde mixture which revealed the presence of two components on thin-layer chromatogram. The NMR spectrum (100 MHz) of this mixture also showed two aldehyde protons at 9.91 and 9.76 ppm. Reduction of this mixture with $NaBH_4$ in MeOH, followed by acetylation in pyridine gave a crystalline **18** (50 mg) and a syrupy **17a** (57 mg).

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