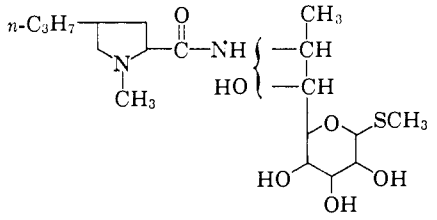
Lincomycin. III.[1a-e] The Structure and Stereochemistry of the Carbohydrate Moiety

W. Schroeder, B. Bannister, and H. Hoeksema[2]

Contribution from the Research Laboratories of the Upjohn Company, Kalamazoo, Michigan. *Received December 20, 1966*

Abstract: Cleavage of lincomycin at the amide bond into methyl thiolincosaminide and an amino acid was readily effected in refluxing hydrazine. The carbohydrate structure was determined by various physical and chemical procedures to be an unbranched 6-aminooctose. The stereochemistry of six of its seven asymmetric centers was established by isolation of D-galactose α-methylphenylhydrazone and N-2,4-dinitrophenyl-D-allothreonine, following oxidative cleavages of the appropriate derivatives. The configuration of the thiomethyl group was assigned from considerations of optical rotations.

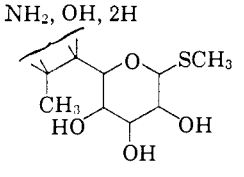
Partial structure **1** for lincomycin was derived in paper II[1c] of this series. The strenuous hydrolytic procedures employed therein freed the peripheral moieties, methyl mercaptan and a propylhygric acid, but failed to provide the intact carbohydrate. Hydrazine hydrate at reflux temperatures[3] efficiently cleaved the amide bond, liberating methyl thiolincosaminide (MTL) in high yield. MTL appeared to be the intact carbohydrate.



1

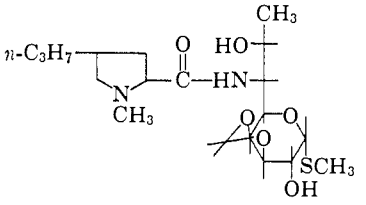
The amino acid fragment was isolated as the hydrazide which could be hydrolyzed in aqueous acid to the propylhygric acid described in the earlier work. To show that the vigorous reaction conditions had induced no rearrangements or racemizations in MTL, highly purified MTL was reacylated with the amino acid moiety. The reconstituted lincomycin so obtained was identical in every way with the antibiotic isolated from fermentations.

Consideration of the partial structure **1** led to working structure **2** for MTL.[4] Regardless of the ambiguity



2

of the C_6 and C_7 substituents, oxidative cleavage of the vicinal amino alcohol would provide an aldehyde capable of either oxidation or reduction to a known compound after removal of the thioaglycone. Thus, structural and stereochemical information concerning most of the MTL would be provided. Blocking of the ring hydroxyls of **2** to periodate cleavage would provide an intermediate suitable for cleavage at the desired position. Since an acetonide **3** of lincomycin itself formed



3

readily with ring hydroxyls as the most likely locations for the isopropylidene group, hydrazinolysis of this compound was expected to provide the desired intermediate (**7**). However, isopropylidenelincomycin proved completely resistant to hydrazinolysis and could be recovered unchanged following 140 hr of refluxing in hydrazine hydrate. Reasons for this are not completely clear. It may be due to the high degree of insolubility of the compound at reaction temperatures. Alternately, studies of models show that the back-side attack of the amide carbonyl by hydrazine is severely hindered when the conformation is such that the large acyl group is adjacent to the isopropylidene group. Hindrance to hydrazinolytic cleavage of amides has recently been reported.[5]

An alternate route to the desired intermediate was to convert MTL (**4**) to N-acetyl-MTL (**5**) with acetic anhydride in methanol. Subsequent treatment of **5** with acetone and sulfuric acid gave N-acetylisopropylidene-MTL (**6**) in good over-all yield. This compound underwent hydrazinolysis more readily, affording the key intermediate **7**. Consumption by **7** of 3 moles of periodate produced acetaldehyde and a compound considered to be aldehyde **8**.

Oxidation of **8** with nitric acid yielded a dibasic acid which was identified by infrared spectra and mixture melting points as mucic acid. Treatment of **8** with borohydride, followed by acid hydrolysis in the pres-

(1) (a) Preliminary accounts of this work appeared during the 148th National Meeting of the American Chemical Society, Chicago, Ill., Aug–Sept 1964; (b) part I: H. Hoeksema, B. Bannister, R. D. Birkenmeyer, F. Kagan, B. J. Magerlein, F. A. MacKellar, W. Schroeder, G. Slomp, and R. R. Herr, *J. Am. Chem. Soc.*, **86**, 4223 (1964); (c) part II: R. R. Herr and G. Slomp, *ibid.*, **89**, 2444 (1967); (d) part IV: G. Slomp and F. A. MacKellar, *ibid.*, **89**, 2454 (1967); (e) part V: B. J. Magerlein, R. D. Birkenmeyer, R. R. Herr, and F. Kagan, *ibid.*, **89**, 2459 (1967).

(2) To whom inquiries should be addressed.

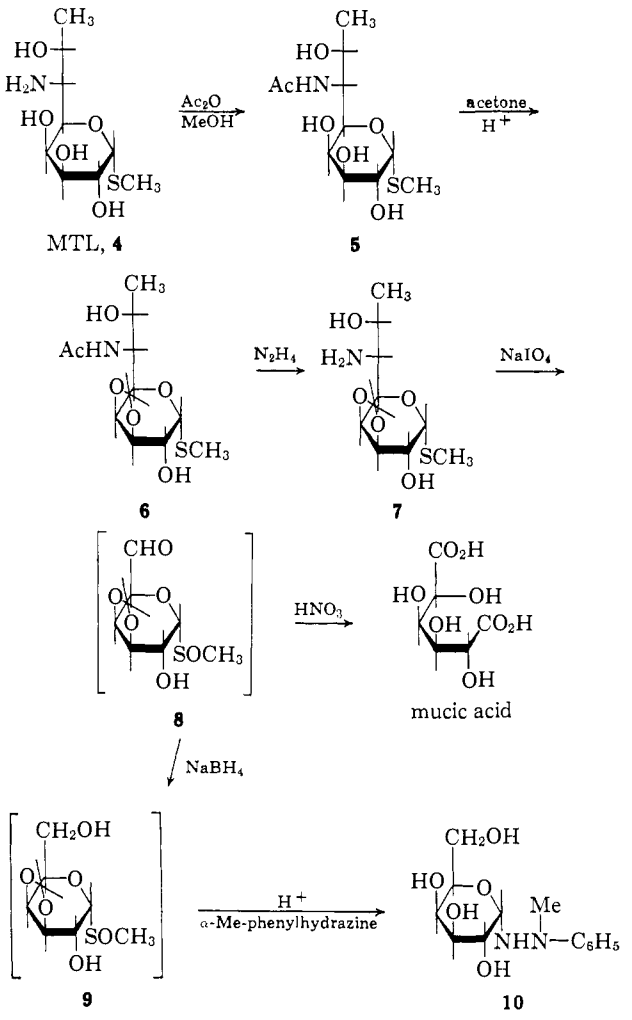
(3) M. L. Wolfrom and B. O. Julaiano, *J. Am. Chem. Soc.*, **82**, 2588 (1960).

(4) Hereafter, the complete structural formulas as ultimately determined will be employed, although some uncertainties, particularly that relating to substituents on C_6 and C_7 as illustrated in **2**, actually persisted to the very end of this investigation.

(5) M. Fujinaga and Y. Matsushima, *Bull. Chem. Soc. Japan*, **39**, 185 (1966).

ence of α-methylphenylhydrazine, afforded D-galactose α-methylphenylhydrazone, presumably *via* **9** which was not isolated. The isolation of the hydrazone in 58% (from **7**) yield confirmed stereochemistry of carbons 2–5, as well as establishing the D series (see Chart I).

Chart I



Relative locations of nitrogen and oxygen and the stereochemistry on carbons 6 and 7 remained to be elucidated. Although lincomycin and MTL (and subsequently compounds **11**, **15**, and **16**) failed to give an iodoform test, the nmr[6] spectrum of MTL favored assignment of nitrogen to C_6 and oxygen to C_7. This issue was further clouded by the positive iodoform test obtained following nitrous acid deamination of **4**. Efforts to resolve the impasse by periodate studies[7] in the open-chain series of compounds **11**–**16** (Chart II) were inconclusive.

The location of the groups was established by characterization of the ketone formed by oxidation of the hydroxyl in question. Thus, compound **6**, obtained from MTL, was desulfurized with Raney nickel, affording **17**, containing one ring hydroxyl which was inert to mild chromic acid oxidation and a side-chain hydroxyl. Such oxidation of **17** in either pyridine or glacial acetic acid yielded a crystalline ketone **18**, displaying carbonyl absorption at 1720 cm^{-1}, which now gave a positive iodoform test. Furthermore, nmr studies of **17** and **18** show that the terminal methyl doublet of **17** at 1.10 and 1.21 ppm had disappeared in the spectrum of **18**. Instead, a new singlet at 2.33 ppm was displayed. Thus, formulation A for the side chain cannot obtain, leaving the alternative B. Reduction of **18** with borohydride afforded a mixture from which iodoform again could not be obtained.

Confirmation of the above information as well as elucidation of the stereochemistry of carbons 6 and 7 were derived through the isolation of the terminal four carbons as an amino acid. Following numerous unsuccessful attempts to obtain such fragments from such intermediates as **14**, **15**, **16**, or N-benzoylated **13** and **15**, a single compound was finally isolated using **19**, the dinitrophenyl (DNP) derivative of **15**. After oxidation with periodate–permanganate mixture,[8] an amorphous product was purified by countercurrent distribution. The analytical data and paper chromatograms indicated an allothreonine-DNP, excluding the alternate choice, alanine-DNP, Rotational data, appearing in Table I, for the isomeric threonine and for L-allothreonine leave no doubt that the acid here isolated was D-allothreonine-DNP.

Table I. Comparison of Known 2,4-DNP Derivatives of the Isomeric Threonines with the 2,4-DNP Acid from Lincomycin

	[M]D, degrees			
Compd	D-Threonine	L-Threonine[a]	L-Allothreonine[a]	Unknown
HOAc	+141	−141	−84	+83.4
$NaHCO_3$	−305	+305	+305	−320[b]

[a] J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1961, p 1564. [b] The error in this value is considered to be due to the necessity for a vacuum filtration of the test solution through a Millipore filter, resulting in some evaporative concentration.

The assumption of stereochemical assignment at carbon 1 was based originally on considerations of the nmr spectrum of MTL.[1e] That the configuration of the methylthio group was α, however, was an important consideration in the process of factoring the entire spectrum and predicting galactose stereochemistry.

Proof of the axial configuration of the thioglycoside was obtained as follows. Acetylation of MTL in acetic anhydride–pyridine gave the pentaacetyl derivative **20**, mp 218–220°, $[\alpha]D$ +223° ($CHCl_3$). In ethanol-free chloroform solution, this pentaacetyl thioglycoside reacted rapidly with bromine[9] with the formation of the acetobromo derivative, considered to be the β anomer **21**, but which could not be isolated crystalline.

The acetobromo sugar reacted readily with thiourea[10] in acetone and the isothiouronium salt, which was not isolated, was hydrolyzed and the resulting mercaptan methylated with methyl iodide in aqueous carbonate.

(6) Nmr studies on MTL, which also led to the conclusion that the stereochemistry of C_2–C_5 was *galacto*, appear in ref 1d.

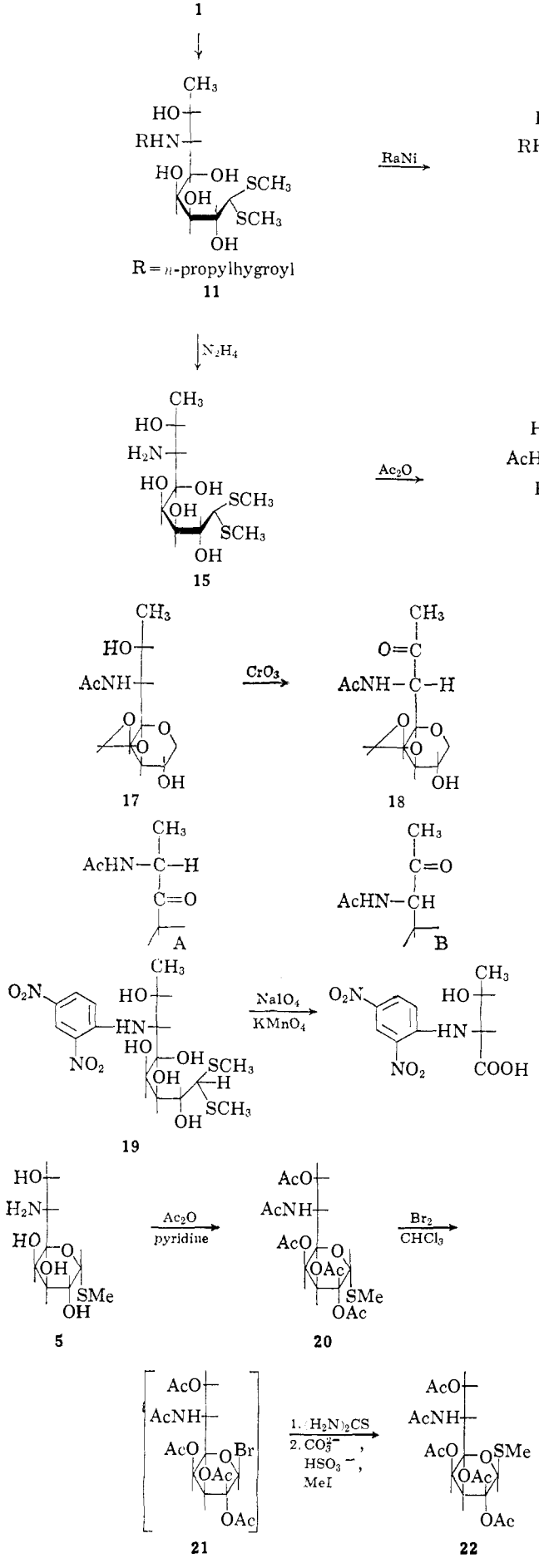
(7) Both the sulfur [see L. Hough and M. I. Taha, *J. Chem. Soc.*, 3994 (1957)] and propylhygroyl fragments can contribute to periodate complications.

(8) R. V. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1711 (1955).

(9) (a) W. A. Bonner, *J. Am. Chem. Soc.*, **70**, 770, 3491 (1948); (b) F. Weygand and H. Ziemann, *Ann.*, **657**, 179 (1962); (c) M. L. Wolfrom and W. Groebke, *J. Org. Chem.*, **28**, 2986 (1963).

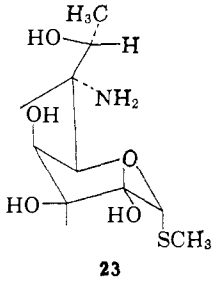
(10) M. Cerny, J. Vrkoc, and J. Stanek, *Collection Czech. Chem. Commun.*, **24**, 64 (1959); M. Cerny and J. Pacak, *ibid.*, **24**, 2566 (1959).

Chart II



A crystalline methyl pentaacetylthioglycoside, **22**, was isolated in excellent yield as the sole reaction product, mp 272–274°, $[\alpha]D$ +31° ($CHCl_3$). Since no opportunity exists in this sequence of reactions for a change in ring size to occur, stereospecific anomerization due to participation by the neighboring acetate group leads to the β anomer, showing a much lower positive rotation than pentaacetyl-MTL.

MTL, then, can best be represented by structure **23**, methyl 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside.



23

Experimental Section[11]

Hydrazinolysis of Lincomycin. Methyl Thiolincosaminide (4). A solution of 4.06 g (0.01 mole) of lincomycin base in 40 ml of hydrazine hydrate was refluxed for 21 hr. The excess hydrazine was then distilled off on the steam bath under reduced pressure (house vacuum). The residue set to semisolid mush of crystals when most of the hydrazine was gone. The residue was cooled and then stirred with acetonitrile until all of the lumps had broken up. The crystals were collected and washed with acetonitrile and then ether. After being dried *in vacuo* the crude product weighed 2.1 g (83%). This material was recrystallized by dissolving 1.5 g in a mixture of 5 ml of H_2O and 10 ml of methanol and adding 40 ml of 1-butanol. The resulting solution was filtered and the water and methanol were slowly evaporated *in vacuo*. The crystals which slowly separated from the butanol were collected, washed with butanol, and dried *in vacuo* to afford 700 mg of white crystals, mp 225–228°, $[\alpha]^{25}D$ +264° (*c* 0.718, H_2O). Material crystallized in this manner was

(11) Melting points were determined on a Kofler micro hot-stage apparatus. Infrared spectra were consistent with the proposed structures.

hygroscopic. The analytical sample contained 3.15% H_2O by Karl Fisher titration.

Anal. Calcd for $C_9H_{19}NO_5S$ + 3.15% H_2O: C, 41.6; H, 7.72; N, 5.42; S, 12.35; equiv wt (for hemihydrate), 262. Found: C, 41.86; H, 7.45; N, 5.63; S, 12.07; equiv wt (by titration), 257; $pK_a' = 7.5$.

Material subsequently recrystallized from ten volumes of dimethylformamide was found not to be hygroscopic.

Hydrazinolysis of Lincomycin. Propyl-L-hygric Acid Hydrazide Dipicrate. Evaporation to dryness of the acetonitrile washes obtained from procedures as described above yielded crude propylhygric acid hydrazide. A 5-g sample of crude 4-*n*-propylhygric acid hydrazide so obtained was dissolved in water and extracted with several equal volumes of chloroform. The chloroform extracts were then reextracted with one-fourth volume of water. This procedure served to remove hydrazine and methyl thiolincosaminide from the hydrazide. The chloroform solution was then dried over sodium sulfate and evaporated on a rotary evaporator at 50° to leave 2.5 g of a heavy oily residue. This residue of partially purified hydrazide crystallized on standing overnight, but the waxy, low-melting material was hygroscopic and resisted crystallization from a number of common solvents. An alcoholic solution when treated with picric acid, however, deposited a crystalline dipicrate which could be recrystallized from ethanol to afford an analytical sample.

Anal. Calcd for $C_{21}H_{25}N_9O_{15}$: C, 39.19; H, 3.92; N, 19.60. Found: C, 39.47; H, 3.71; N, 19.39.

Conversion of 4-*n*-Propylhygric Acid Hydrazide to 4-*n*-Propylhygric Acid Hydrochloride. A solution of 6 g of crude 4-*n*-propylhygric acid hydrazide in 20 ml of 6 *N* hydrochloric acid was refluxed 3.5 hr. The reaction mixture was evaporated to dryness *in vacuo* and the gummy residue was washed thoroughly with boiling *t*-butyl alcohol until it become granular. This was dissolved in 20 ml of 2-propanol, treated with activated carbon, then diluted with 150 ml of acetone and treated again with carbon at boiling temperatures. Finally, 50 ml of ether (and later a second 50-ml portion) brought about crystallization for a total yield of 2.96 g. Recrystallization of 0.5 g of this from 15 ml of acetonitrile yielded crystals identical by infrared with that obtained from acid hydrolysis,[1c] $[\alpha]_D$ −46.8° (*c* 3.42, H_2O).

Methyl N-Acetylthiolincosaminide (5). Methyl thiolincosaminide (**4**) (5 g) was suspended in 50 ml of methanol and treated under vigorous stirring with 3.75 ml of acetic anhydride at room temperature. Most of the solid dissolved within a few minutes, and then was replaced by a new precipitate. After stirring overnight at room temperature, the solid was removed at the pump, washed with methanol, and recrystallized from the same solvent, giving small colorless needles, mp 243–245°; $[\alpha]_D$ +248° (95% EtOH), +265° (H_2O).

Anal. Calcd for $C_{11}H_{21}O_6NS$: C, 44.72; H, 7.17; N, 4.74; S, 10.85. Found: C, 44.87; H, 7.10; N, 4.65; S, 10.99.

Resynthesis of Lincomycin. A suspension of finely divided *n*-propyl-N-methylproline hydrochloride (4.14 g, 0.02 mole) in 150 ml of dry acetone was treated with 11.2 ml (0.08 mole) of triethylamine. The acid hydrochloride dissolved rapidly and triethylamine hydrochloride precipitated. To this suspension was added 2.3 ml (slight excess of 0.02 mole) of *n*-propyl chloroformate and the mixture was stirred at room temperature for 1 hr. The resulting suspension was cooled in ice and filtered rapidly with suction into a cold (15°) solution of 5.06 g (0.02 mole) of methyl thiolincosaminide (**4**) in 150 ml of water. The mixture was stirred well for 15 min and then air sucked through the filter for 45 min to evaporate most of the acetone. The remainder of the acetone was removed *in vacuo* at 40°. The remaining aqueous phase was extracted with three 150-ml portions of methylene chloride and the extracts were washed with 30 ml of water and dried over $MgSO_4$. The methylene chloride was removed *in vacuo* to leave 2 g of residue. This residue was dissolved in 10 ml of 1 *N* HCl and 20 ml of acetone was added. A small amount of insolubles was removed by filtration and 180 ml more acetone was added to the filtrate. Crystals separated which were collected after standing for a while and washed with acetone and ether. After drying *in vacuo* at room temperature the yield of white crystals was 1.0 g. The substance was recrystallized from acetone–water and dried *in vacuo* at room temperature for analysis. Karl Fisher titration showed the presence of 0.615 mole of water; pK_a' in water = 7.55; $[\alpha]^{25}D$ +137° (*c* 1.044, H_2O). The infrared spectrum was identical with that of a lincomycin reference standard.

Anal. Calcd for $C_{18}H_{35}N_2O_6SCl \cdot 0.615H_2O$: C, 47.61; H, 8.05; N, 6.17; S, 7.05; Cl, 7.82. Found: C, 47.41; H, 8.24; N, 6.76; S, 6.92; Cl, 7.75.

3,4-O-Isopropylidenelincomycin (3). A solution of 9.8 g of lincomycin in 150 ml of acetone was added to a solution of 9.8 g of *p*-toluenesulfonic acid monohydrate in 100 ml of acetone with good stirring. The mixture was stirred at ambient temperature for 1 hr, after which 100 ml of anhydrous ether was added and stirring was continued in an ice bath for 0.5 hr. The crystals were filtered off and dried *in vacuo* at 50°; yield 13.35 g (85.5%) of 3,4-O-isopropylidenelincomycin *p*-toluenesulfonate. An additional 1.15 g (7.4%) could be recovered from the mother liquors by adding 350 ml of anhydrous ether and chilling the solution for 1 hr. Conversion to the free base was achieved by suspending the combined crops in 200 ml of ether and shaking vigorously with 125 ml of 5% potassium bicarbonate solution. The aqueous layer was back-extracted with two 100-ml portions of ether. The ether extracts were washed with 50 ml of saturated sodium chloride solution and filtered through anhydrous sodium sulfate, and the ether was evaporated *in vacuo*, leaving 7.9 g (73.1%) of 3,4-O-isopropylidenelincomycin, which was then dissolved in 25 ml of ethyl acetate. The solution was concentrated to about 10–15 ml and allowed to stand at room temperature for several hours. After refrigerating overnight, the crystals were filtered from the solution and washed sparingly with cold ethyl acetate; yield 4.55 g (42.2%) of 3,4-O-isopropylidenelincomycin having a melting point of 126–128°, $[\alpha]^{25}D$ 102° (*c* 1, methylene chloride).

Anal. Calcd for $C_{21}H_{38}N_2O_6S$: C, 56.48; H, 8.58; N, 6.27; S, 7.18. Found: C, 56.37; H, 8.55; N, 6.01; S, 7.24.

Methyl N-Acetylisopropylidenethiolincosaminide (6). A suspension of 5.3 g of finely powdered methyl N-acetylthiolincosaminide (**5**) was stirred for 60 hr at room temperature with 500 ml of acetone and 0.5 ml of concentrated H_2SO_4. Most of the starting material was still insoluble at this time. An additional 5 ml of H_2SO_4 was added and the insoluble material dissolved rather rapidly. Stirring was continued for 30 min at room temperature. The solution was then added to a suspension of 150 g of barium carbonate in 100 ml of water and the mixture stirred until neutral. The barium salts were filtered off and washed with acetone. The filtrate was evaporated to dryness *in vacuo* at 50°. The residue was taken up in acetone–ether (10:1) and the insolubles were filtered off. The filtrate was evaporated to dryness and the residue was taken up in 100 ml of ethanol, which was then removed *in vacuo* to leave a yellow gum. The gum was dissolved in 20 ml of warm water containing a small amount of sodium carbonate, some insolubles were filtered off, and the filtrate was cooled. The crystals which formed were collected after standing in the refrigerator for 4 hr and washed with cold water. After drying *in vacuo* they weighed 2.0 g. A portion was recrystallized from water for analysis, mp 174–175°; $[\alpha]^{25}D$ +189° (*c* 0.4136, H_2O).

Anal. Calcd for $C_{14}H_{25}NO_6S$: C, 50.12; H, 7.52; N, 4.17; S, 9.55. Found: C, 49.87; H, 7.46; N, 4.11; S, 9.56; H_2O, none by Karl Fisher.

Methyl Isopropylidenethiolincosaminide (7). A solution of 1 g of methyl N-acetyl-3,4-O-isopropylidenethiolincosaminide (**6**) in 10 ml of hydrazine hydrate was refluxed for 24 hr. The excess hydrazine was distilled off in a nitrogen stream on the steam bath. The crystalline residue was taken up in 5 ml of water and allowed to recrystallize. The crystals were collected and washed with cold water. After drying *in vacuo* the crystals weighed 450 mg. Recrystallization from ethanol afforded the analytical sample, mp 177–178°; $[\alpha]^{25}D$ +186° (*c* 0.689, H_2O).

Anal. Calcd for $C_{12}H_{23}NO_5S$: C, 49.12; H, 7.90; N, 4.78; S, 10.91. Found: C, 49.27; H, 8.0; N, 5.01; S, 10.92.

Larger Scale Preparation of Methyl 3,4-O-Isopropylidenethiolincosaminide (7). A suspension of 20 g of MTL (**4**) in 400 ml of methanol was stirred and 20 ml of acetic anhydride added. All of the solid dissolved in a few minutes, and then crystals of the N-acetyl derivative started to separate. After cooling in the refrigerator 1 hr the crystals were collected and washed with methanol. Drying *in vacuo* overnight afforded 19.3 g of N-acetate. This material was well powdered and suspended in 1500 ml of acetone. While stirring, 15 ml of concentrated H_2SO_4 was added. After stirring for 1 hr at room temperature, all was in solution. After cooling in the refrigerator for an additional hour a slight excess (Hydrion paper) of ammonia gas was passed in to neutralize the sulfuric acid. The ammonium sulfate was filtered off and washed with acetone. The colorless filtrate was evaporated *in vacuo* at 50° to a frothy gum. The yield of crude N-acetylisopropylidene derivative was 24.8 g. This crude material was dissolved in 210 ml of hydrazine hydrate. Crystals started to form, but they redissolved on heating. The

resulting solution was refluxed for 23 hr. The excess hydrazine was removed *in vacuo* on the steam bath. The residue was taken up in ethanol, some insolubles were filtered off, and the ethanol was then removed *in vacuo*. The crystalline residue was slurried with 50 ml of acetonitrile to break up lumps and the crystals were collected, washed with acetonitrile and ether, and dried *in vacuo*. The yield of first crop was 12.35 g. The filtrate upon evaporation afforded another 1.0 g of product. The total yield of material identical with that prepared previously was 13.35 g (57.5%).

Mucic Acid from 7. A solution of 3.2 g (slight excess of 3 equiv) of sodium periodate in 25 ml of warm water was cooled to room temperature and 1.5 g of methyl 3,4-O-isopropylidenethiolincosaminide was added simultaneously with good stirring. The MTL derivative dissolved rapidly and some salt precipitated (probably $NaIO_3$). The temperature was maintained at room temperature by occasional cooling. The odor of acetaldehyde was strong. The solution was placed in a flask fitted with a sparger, N_2 gas was passed through, and the acetaldehyde was converted to its 2,4-DNP derivative with Brady's reagent. After 3 hr the formation of 2,4-DNP had practically ceased. The yield of acetaldehyde 2,4-DNP was 750 mg (65%). The original reaction mixture was filtered from precipitated sodium iodate and a slight excess of barium acetate was added to the filtrate to remove the remainder of the iodate. The salts were removed and washed with water. The filtrate (50 ml) was treated with 15 ml of concentrated HNO_3 and heated in an open beaker on the steam bath for 3 hr. By this time the solution had gone to dryness. The residue was dissolved in 15 ml of water and the resulting solution was permitted to stand overnight. After cooling in the refrigerator for 1 hr, the crystals which had separated were collected and washed with water, ethanol, and ether. After being dried *in vacuo* they weighed 250 mg and were identified by solubility, infrared spectrum, and direct comparison with mucic acid; $[\alpha]D$ 0° (*c* 0.5, NaOH). An additional 205 mg of mucic acid separated from the filtrate on standing in the refrigerator for several days. The total yield was therefore 455 mg (41%).

D-Galactose α-Methylphenylhydrazone (10) from 7. A solution of 6.4 g of sodium periodate in 75 ml of water was cooled and 3.0 g of isopropylidene-MTL (**7**) added all at once. The mixture was stirred and cooled to moderate the reaction. After standing in the refrigerator overnight, the salts were filtered off and barium acetate was added in slight excess to remove the remainder of the iodate. The salts were again removed and the filtrate was treated with 400 mg of sodium borohydride. After standing at room temperature for 1 hr, an additional 400 mg of borohydride was added. After 30 min, acetic acid was added dropwise to pH 6 to destroy excess borohydride. Some insoluble material was filtered off and about one-fifth volume of IR 120-H^+ resin added along with a few milligrams of sodium bisulfite to reduce some iodine liberated on acidification. The resulting suspension was heated on the steam bath for 4.5 hr, at which time a test for reducing sugar (Benedict) was at its maximum. Some Darco G-60 was added and the mixture was filtered. The filtrate was then reduced in volume to about 70 ml *in vacuo* at 50°. To this solution was added 2 g of sodium acetate, 2 g of sodium bisulfite, 1.5 g of α-methylphenylhydrazine, and 1 ml of acetic acid. Crystals separated on standing. After overnight in the refrigerator, the crystals were collected, washed with water, and dried *in vacuo*. The slightly orange crystals weighed 900 mg, mp 187–189°. After standing an additional day in the refrigerator, the filtrate had deposited another 800 mg of product; total yield 1700 mg (58.5%). Recrystallization from hot water afforded colorless crystals of D-galactose α-methylphenylhydrazone; $[\alpha]^{26}D$ +12.4° (*c* 2.06, DMSO). An authentic sample of D-galactose α-methylphenylhydrazone had $[\alpha]^{26}D$ +12.2° (*c* 2.0, DMSO) and was identical in melting point and infrared spectrum with the material derived from isopropylidene-MTL.

N-(4-Propyl-L-hygroyl)lincosamine Dimethyl Mercaptal (11). In a three-necked flask equipped with a Dry Ice condenser, 150 ml of concentrated hydrochloric acid and 50 ml of methanethiol were chilled to 0°. After rapid addition of 15 g of lincomycin hydrochloride the mixture was vigorously stirred for 5 hr, then diluted with one volume of ice water and extracted with two 100-ml portions of pentane (or Skellysolve B). The extract was discarded. The 6 *N* hydrochloric acid solution was next partially neutralized by addition of a subequivalent amount (100 g) of potassium hydroxide pellets at 20–30° (Dry Ice–acetone cooling permitted rapid addition). The potassium chloride was removed by filtration. Next, 200 ml of chloroform was added to the filtrate which was then brought to pH 10 with sodium hydroxide. The chloroform layer was removed and a second extract was made with chloroform. The combined extracts were washed with three 50-ml portions of water (emulsions), and the chloroform was evaporated *in vacuo* in the presence of water, transferring the product to the aqueous phase which was then freeze dried.

The freeze-dried product was crystallized from 75 ml of boiling acetone to give 7.5 g, mp 134–140°. A second crystallization gave material, mp 146–148°; $[\alpha]D$ −33° (*c* 1, methylene chloride).

Anal. Calcd for $C_{19}H_{36}N_2O_6S_2$: C, 50.19; H, 8.42; N, 6.16; S, 14.10. Found: C, 50.15; H, 8.20; N, 5.94; S, 14.31.

N-(4 Propyl-L-hygroyl)-1-deoxylincosaminol (12). A suspension of 100 g of W-3 Raney nickel (washed to pH 7 with water, then washed three times with absolute ethanol) was stirred and heated to reflux in 1 l. of absolute ethanol with 15 g of the mercaptal (**11**). After 4 hr the hot reaction mixture was filtered and the nickel was washed with 600 ml of hot ethanol. The combined filtrate and washings were evaporated to dryness. A five-tube countercurrent distribution at 60 ml/phase was run in the system 1-butanol–water, moving the upper phase. The contents of tubes 4 and 5 were evaporated to dryness (7 g) and crystallized from 100 ml of ethyl acetate (5.7 g), mp 105–108°.

Anal. Calcd for $C_{17}H_{34}N_2O_6$: C, 56.33; H, 9.46; N, 7.73. Found: C, 56.23, 56.29; H, 9.16, 9.30; N, 7.68.

1-Deoxylincosaminol (13) by Acid Hydrolysis. A solution of 1 g of N-(4-propyl-L-hygroyl)-1-deoxylincosaminol (**12**) was heated under reflux in 50 ml of 4 *N* sulfuric acid for 4 hr. The solution was cooled, brought to pH 8 with saturated barium hydroxide solution, and filtered. The filtrate was passed through a 0.75-in. column containing 10 g of Dowex 2 in the hydroxyl cycle, and the effluent was freeze dried, yielding 200 mg of white solid. A 120-mg aliquot was crystallized from absolute ethanol to yield 40 mg of product, mp 177–185°.

Anal. Calcd for $C_8H_{19}NO_5$: C, 45.92; H, 9.15; N, 6.69; equiv wt, 209.24. Found: C, 46.29; H, 9.24; N, 6.40; equiv wt, 207; titration showed 1 basic function, $pK_a' = 8.5$.

1-Deoxylincosaminol (13) by Hydrazinolysis. A 5-g quantity of compound **12** was heated under reflux for 6 days in 100 ml of hydrazine hydrate. The hydrazine was evaporated *in vacuo* and the residue was crystallized in two crops from 2 ml of water in 75 ml of absolute ethanol; yield 60%.

N-Benzoyl-1-deoxylincosaminol. To 700 mg of 1-deoxylincosaminol (**13**) in 50 ml of absolute ethanol was added 1 g of benzoic anhydride. This was stirred until all dissolved and until the new precipitate was completely formed. After overnight standing at room temperature 730 mg (70% yield) of white crystals was obtained, mp 188–190°.

Anal. Calcd for $C_{15}H_{23}NO_6$: C, 57.49; H, 7.40; N, 4.47; Found: C, 57.33; H, 7.45; N, 4.22.

N-Acetyl-1-deoxylincosaminol (14). To 1 g (0.0048 mole) of 1-deoxylincosaminol (**13**) in 100 ml of methanol was added 1 g (0.01 mole) of acetic anhydride at 5°. This mixture was stirred until everything dissolved, and then refrigerated. After 20 hr, 300 ml of ether was added, and the precipitate was filtered. The 800 mg of dried white solid was recrystallized from 30 ml of absolute ethanol, yielding 630 mg of white crystals, mp 174–176°.

Anal. Calcd for $C_{10}H_{21}NO_6$: C, 47.39; H, 8.43; N, 5.58. Found: C, 48.09; H, 8.27; N, 5.43.

Lincosamine Dimethyl Mercaptal (15). A 10-g quantity of compound **11** in 100 g of hydrazine hydrate was heated under reflux for 6 days. The hydrazine hydrate was evaporated *in vacuo* using a nitrogen ebullator. The residue was crystallized from hot acetonitrile (100 ml) in two crops, for a total of 4.3 g (64% yield). The analytical sample was further crystallized from absolute ethanol yielding a sample, mp 142–144°; $pK_a' = 7.95$ in water.

Anal. Calcd for $C_{10}H_{23}NO_5S_2$: C, 39.84; H, 7.69; N, 4.65; S, 21.27; equiv wt, 301.41. Found: C, 39.74; H, 7.09; N, 4.87; S, 20.54; equiv wt, 320.

N-Benzoyllincosamine Dimethyl Mercaptal. A solution of 1.8 g (0.006 mole) of **15** in 100 ml of ethanol was heated under reflux for 1 hr with 3.6 g (0.016 mole) of benzoic anhydride. The mixture was evaporated to dryness and the residue washed with acetone. Recrystallization from acetone gave 300 mg, mp 182–184°.

Anal. Calcd for $C_{17}H_{27}NO_6S_2$: C, 50.35; H, 6.71; N, 3.45; S, 15.81. Found: C, 50.34; H, 6.81; N, 3.67; S, 15.96.

N-Acetyllincosamine Dimethyl Mercaptal (16). A 1-g (0.0033 mole) quantity of compound **15** suspended in 30 ml of methanol was stirred with 1 ml (0.011 mole) of acetic anhydride at room temperature for 2 hr. During this time, the starting material went into solution. Upon dilution with 20 ml of ether crystallization occurred, yielding 660 mg (59%) of dried, ether-washed, white solid, mp 177–179°. Recrystallization from 15 ml of absolute ethanol gave 0.5 g mp 179–179.5°.

Anal. Calcd for $C_{12}H_{25}NO_6S_2$: C, 41.96; H, 7.34; S, 18.67. Found: C, 41.92; H, 7.18; S, 19.15.

N-Acetyl-1-deoxylincosaminol (14) by Desulfurization. From 2.49 g (0.007 mole) of **16** and *ca.* 50 g of nickel catalyst, refluxed 10 hr in 200 ml of absolute ethanol, was obtained 1 g (0.004 mole) of N-acetyl-1-deoxylincosaminol, identical with that above.

N-Acetyl-3,4-O-isopropylidene-1-deoxylincosamine (17). A 15-g quantity of methyl N-acetyl-3,4-O-isopropylidenethiolincosaminide (**6**) was heated under reflux for 7 hr with 100 ml, loosely packed, of Raney nickel in 500 ml of ethanol. The mixture was filtered and the catalyst was washed with a total of 1 l. of boiling ethanol. The filtrate and washings were combined and evaporated to dryness, leaving a partially crystalline residue. This residue was purified by countercurrent distribution, 200 transfers, in the system 1-butanol–water. A major fraction, $K = 0.48$, was isolated by evaporation to give a white crystalline solid, mp 220–235° dec, $[\alpha]D$ +70° (*c* 1, 50% ethanol). This material gave a negative iodoform test.

Anal. Calcd for $C_{13}H_{23}NO_6$: C, 53.96; H, 8.09; N, 4.84. Found: C, 53.87; H, 8.23; N, 5.67, 4.92.

N-Acetyl-3,4-O-isopropylidene-7-dehydro-1-deoxylincosamine (18). A. Oxidation in Pyridine. To a solution of 1 g (0.0034 mole) of compound **17** in 10 ml of pyridine and 1 ml of water was added 1 g (0.0066 mole) of chromic oxide in 7 ml of pyridine and 1 ml of water. The mixture was stirred overnight, then added to a solution of 125 ml of ether and 125 ml of ethyl acetate. After standing 3 hr, this mixture was filtered and the filtrate was evaporated to dryness. The residual crystalline material was triturated with acetone and dried, yielding 225 mg of crystalline material. A second crop of 50 mg was recovered from the filtrate by countercurrent distribution in 1-butanol–water, $K = 0.78$. On recrystallization from acetone, a total of 185 mg of material was recovered which melted at 205–210°. This material showed new infrared absorption at 1730 cm^{-1}, and the nmr spectrum showed a new signal at 2.35 ppm. This product gave a positive iodoform test. Upon treatment of the product with sodium borohydride in methanol, the iodoform test again was negative. *Anal.* Calcd for $C_{13}H_{21}NO_6$: C, 54.34; H, 7.37; N, 4.88. Found: C, 54.39; H, 7.59; N, 5.06.

B. Oxidation in Glacial Acetic Acid. To a solution of 1 g (0.0034 mole) of compound **17** in 10 ml of glacial acetic acid was added 1 g (0.0066 mole) of chromic oxide in 10 ml of acetic acid. The mixture, which warmed immediately, was stirred overnight. A 250-ml solution of ethyl acetate and ether (1:1) was added and the mixture was filtered, then evaporated to dryness. Trituration of the residue with acetone produced 40 mg of white crystals identical with those obtained from the pyridine oxidation.

N-(2,4-Dinitrophenyl)lincosamine Dimethyl Mercaptal (19). To 1 g (0.0033 mole) of lincosamine dimethyl mercaptal in 20 ml of 50% ethanol was added 0.5 g of sodium bicarbonate and 1 g (0.0054 mole) of 1-fluoro-2,4-dinitrobenzene, the latter in 10 ml of ethanol. After 2 hr of stirring, the mixture was evaporated to dryness and the residue was leached with 50 ml of benzene, which was then discarded. Upon washing the residue twice with 25 ml of water, 1.2 g of yellow solid remained. This was recrystallized from boiling acetone to give a total of 820 mg of yellow crystals, mp 138–140 dec.

Anal. Calcd for $C_{16}H_{25}N_3O_9S_2$: C, 41.10; H, 5.39; N, 8.99; S, 13.72. Found: C, 41.12; H, 5.38; N, 8.69; S, 13.33.

Isolation of D-Allothreonine. Oxidative Cleavage of N-2,4-Dinitrophenyllincosamine Dimethyl Mercaptal. A suspension of 4.3 g (0.0092 mole) of N-2,4-dinitrophenyllincosamine dimethyl mercaptal (**19**), 30 g of sodium metaperiodate, 3 g of sodium carbonate, and 650 mg of potassium permanganate in 1900 ml of water was stirred 6 hr. The mixture was extracted with 700 ml of ether, then acidified and further extracted with 200 ml of ether. The combined extracts, washed with 100 ml of water, were then dried over sodium sulfate and finally evaporated to dryness. The residue was taken into 50 ml of ether and washed with 50 ml of 5% bicarbonate, then with water. After acidification and reextraction of the alkaline layer, the dried (magnesium sulfate) ether solution was evaporated to dryness and the residue was subjected to countercurrent distribution in the system ethyl acetate–cyclohexane–95% ethanol–water (1:1:1:1, v/v). The contents of tubes 270–360 ($K = 1.28$) were pooled and isolated by evaporation to an aqueous phase and freeze drying to yield 175 mg of yellow amorphous solid, which failed to crystallize; $[M]D$ −320° (4% sodium bicarbonate solution), +83° (glacial acetic acid). Paper chromatographic comparison with DL-allothreonine-DNP and DL-threonine-DNP in the system 1-butanol–water–ethanol (4:5:1, v/v, use upper phase) showed similar R_f values for DL-allothreonine and the unknown compound (0.64), while that of threonine was lower (0.61).

Anal. Calcd for $C_{10}H_{11}N_3O_7$: C, 42.11; H, 3.89; N, 14.73. Found: C, 42.12; H, 4.19; N, 13.93.

Iodoform from Nitrous Acid Treated Methyl Thiolincosaminide. A solution of 100 mg of MTL in 1 ml of water and five drops of acetic acid was treated with 100 mg of sodium nitrite in portions over a 2-min period at room temperature. Gas evolution was vigorous and the solution turned pink. After standing at room temperature for 2 hr, 5 ml of 2 *N* sodium hydroxide and seven 1-ml portions of I_2–KI solution were added. Iodoform started to precipitate immediately. After cooling in the refrigerator for 2 hr, the precipitate was collected and dried in air. The yield of iodoform melting at 121–122° (Kofler) was 60 mg (39%).

The control experiment which contained no sodium nitrite gave no evidence of iodoform production.

Pentaacetyl-MTL (22) (Methyl 6-N-Acetyl-2,3,4,7-tetra-O-acetyl-1-thio-α-lincosaminide). Methyl 1-thio-α-lincosaminide (3.0 g) was acetylated by allowing a solution in pyridine (50 ml) and acetic anhydride (25 ml) to stand overnight at room temperature. Solvent was removed on the rotating evaporator at 40° (15 mm), and the syrupy residue was distributed between water and methylene chloride. The organic extract, after washing with dilute hydrochloric acid (1 *N*), water, and saturated aqueous sodium bicarbonate and drying over anhydrous sodium sulfate, was taken to dryness *in vacuo*, giving a colorless crystalline residue which was recrystallized from ethyl acetate–Skellysolve B to give rods, showing a double melting point of 210–212 and 218–200°; $[\alpha]D$ +223° (*c* 0.906, $CHCl_3$).

Anal. Calcd for $C_{19}H_{29}NO_{10}S$: C, 49.22; H, 6.31; N, 3.02; S, 6.92. Found: C, 49.10; H, 6.57; N, 3.23; S, 6.91.

Pentaacetyl-α-MTL (22) (Methyl 6-N-Acetyl-2,3,4,7-tetra-O-acetyl-1-thio-α-lincosaminide). The above α-pentaacetate (2.0 g, 4.3 mmoles), dissolved in ethanol-free chloroform (50 ml), was stirred magnetically and a solution of bromine (1.11 g, 0.36 ml, 6.9 mmoles) in the same solvent (20 ml) was added over the course of 10 min (Drierite tube). After stirring for an additional 15 min, no trace of starting material could be detected by thin layer chromatography (SiO_2, ethyl acetate–Skellysolve B, 1:1). Solvent was removed on a rotating evaporator at 40° (7 mm), giving a yellow-orange syrup which was redissolved in chloroform, the solvent removed as above, and the process repeated twice more, giving a colorless syrup which could not be induced to crystallize. This procedure removes the methylsulfinyl bromide.

A solution of this syrup in acetone (analytical reagent, 25 ml) was heated under reflux (Drierite tube) with thiourea (656 mg, 8.6 mmoles) for 1 hr. After cooling, potassium carbonate (680 mg), sodium bisulfite (860 mg), water (10 ml) and methyl iodide (3.06, g, 1.34 ml, 21.6 mmoles) were added, and the mixture was stirred vigorously for 3 hr at room temperature.

The acetone was removed *in vacuo*, and the residue was distributed between water and chloroform. The aqueous layer was extracted thoroughly with chloroform, and the combined extracts were washed twice with water and dried over anhydrous sodium sulfate. Removal of the solvent on a rotating evaporator at 40° (7 mm) gave a colorless solid (1.48 g) showing only one spot on thin layer chromatography (SiO_2, ethyl acetate–Skellysolve B, 1:1). The solid crystallized from ethyl acetate–Skellysolve B in colorless platelets, mp 272–274° (1.12 g, 56%), unchanged by further crystallization. Countercurrent distribution of the crude product in the system water–acetone–methyl ethyl ketone–cyclohexane (3:5:4:4, v/v) showed only one material, of $K = 1.14$, to be present; $[\alpha]D$ +31° (*c* 0.680, $CHCl_3$).

Anal. Calcd for $C_{19}H_{29}NO_{10}S$: C, 49.22; H, 6.31; N, 3.02; S, 6.92. Found: C, 49.15; H, 6.23, N, 3.00; S, 6.75.

Acknowledgment. The authors wish to thank Mr. James Shiley for valuable technical assistance and Mr. Raymond Anderson and his associates for the analytical results.