New Synthesis of Meromycolic Acid

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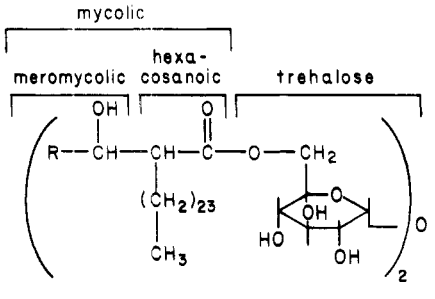
The meromycolic compound, *cis*-19,20:*cis*-35,36-bis(methylene)tetrapentacontanoic acid, has been prepared by a branching synthesis starting with 1-halo-16-(tetrahydropyranyloxy)-*cis*-8,9-methylenehexadecane, which was obtained in two ways. One sequence, starting with 1,14-dichloro-7-tetradecyne, proceeded by cyanide chain elongation and hydrolysis to 8-hexadecenedioic acid and methylenation of the dimethyl ester. Reduction of the cyclopropane diester product with lithium aluminum hydride afforded the glycol 1,16-dihydroxy-*cis*-8,9-methylenehexadecane which was then masked at one end by attaching the tetrahydropyranyl group. The remaining open hydroxyl was converted to bromide by tosylation and displacement of tosyloxy with bromide ion so as to arrive at the desired 1-bromo-16-(tetrahydropyranyloxy)-*cis*-8,9-methylenehexadecane. In a different approach, the corresponding chloro derivative compound was synthesized by starting with 1-hydroxy-3-nonyne. Base-catalyzed isomerization, by shifting the triple bond to the end of the chain, formed 1-hydroxy-8-nonyne. This was converted to its tetrahydropyranyl derivative and then alkylated with 1,7-dichloroheptane to give 1-chloro-16-(tetrahydropyranyloxy)-8-hexadecyne. Half-hydrogenation followed by methylenation with diethylzinc–methylene diiodide furnished the necessary intermediate, 1-chloro-16-(tetrahydropyranyloxy)-*cis*-8,9-methylenehexadecane. The synthesis continued by elaborating 1-halo-16-(tetrahydropyranyloxy)-*cis*-8,9-methylenehexadecane in two directions to obtain moieties that were eventually recombined. Along one branch, the Grignard reagent of the halo compound was allowed to react with methylcopper(I) followed by undecyl iodide to form 1-(tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane, which with triphenylphosphine dibromide gave 1-bromo-*cis*-8,9-methyleneheptacosane. Along the other branch, the same Grignard reagent after treatment with methylcopper(I) was allowed to couple with methyl 11-iodoundecanoate. The coupling product, methyl 27-(tetrahydropyranyloxy)-*cis*-19,20-methyleneheptacosanoate, was converted in two steps to methyl 27-iodo-*cis*-19,20-methyleneheptacosanoate. Finally, the two fragments were combined to form methyl meromycolate by forming the Grignard reagent of 1-bromo-*cis*-8,9-methyleneheptacosane, introducing methylcopper(I) to generate the disubstituted cuprate(I) complex, and by coupling with the iodo methyl ester. This synthesis, by furnishing product in an uncorrected overall yield of 6–8%, represents a practical source of meromycolic acid, which we need as a starting material for projected syntheses of cord factor. Hexacosanoic acid, also required for the further work, was conveniently reached by coupling pentadecylmagnesium bromide via its methylcopper complex with methyl 11-iodoundecanoate and saponifying the product.

Introduction

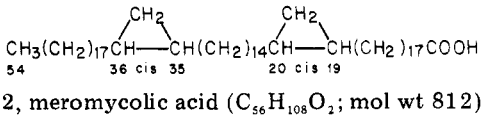
The work reported here is part of a program aimed at synthesizing 6,6′-dimycolyltrehaloses (**1**) or "cord factors",[1]



1
cord factor

which are high molecular weight glycolipids isolated from tubercle bacilli. These compounds have diverse physiological, biochemical, and immunological properties, including some of considerable promise for tumor control.[2] Formulation **1** is labeled so as to identify structural sections of the cord factor molecule. On this basis, cord factor is made up of trehalose, or 1α,(1α)′-diglucoside, which is esterified at both of its primary alcohol groups with hexacosanoic acid, the 26-carbon normal acid. In turn, the hexacosanoic acid is substituted at its α position with a large fragment derived from meromycolic acid, which constitutes the distal sections of the cord factor. Dissecting cord factor in this manner suggests ways of putting the molecule together, and, accordingly, the individual parts became of interest to us. While trehalose was readily available, the two acids were not and required synthesis.

Different meromycolic acid sections of cord factor (**1**) have been described. These parts of the molecule, providing most of the weight of the cord factors, carry a variety of functions attached to a long chain of carbon atoms—generally more than 50. Noting that most of the meromycolic acids incorporate one or more fused cyclopropane rings, we settled on meromycolic acid (**2**), having



2, meromycolic acid ($C_{56}H_{108}O_2$; mol wt 812)

two rings cis fused at the 19,20- and 35,36-positions of the 54-carbon normal acid, as our synthesis target.[3] Although

(1) Although of little significance other than historical, the name cord factor, first suggested by Bloch, is convenient and appears to be entrenched. Cf. H. Bloch, *J. Exp. Med.*, **91**, 197 (1950); H, Noll, H. Bloch, J. Asselineau, and E. Lederer, *Biochim. Biophys. Acta*, **20**, 299 (1956). Several reviews of cord factors are available: cf. J. Asselineau, "The Bacterial Lipids", Holden-Day, San Francisco, CA, 1966; M. B. Goren, *Tubercle*, **56**, 65 (1975); E. Lederer, *Chem. Phys. Lipids*, **16**, 91 (1976); *Med. Chem., Proc. Int. Symp., 5th* (1977); C. Asselineau and J. Asselineau, *Prog. Chem. Fats Other Lipids*, **16**, 59 (1978).

(2) A small sampling of the extensive literature is as follows: A. Bekierkunst, L. Wang, R. Toubiana, and E. Lederer, *Infect. Immun.*, **10**, 1044 (1974); J.-F. Petit and E. Lederer, *Symp. Soc. Gen. Microbiol.*, **38**, 177 (1978); R. Toubiana, E. Ribi, C. McLaughlin, and S. M. Strain, *Cancer Immunol. Immunother.*, **2**, 189 (1977); C. Leclerc, et al., *ibid.*, **1**, 227 (1976); M. Parant, et al., *J. Infect. Dis.*, **135**, 771 (1977), *Infect. Immun.*, **20**, 12 (1978). The citations in ref 1 are also relevant.

(3) The 17–14–17 pattern for the linear methylene groups has been found for natural mycobacterial cord factors: cf. A. H. Etemadi, F. Pinte, and J. Markovits, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **262**, 1343 (1966); G. Lamonica and A. G. Etemadi, *ibid.*, **264**, 1711 (1967); E. Lederer, *Pure Appl. Chem.*, **25**, 135 (1971). Also cf. E. Lederer, *Chem. Phys. Lipids*, 1, 294 (1967) and M. M. Lanéelle and G. Lanéelle, *Eur. J. Biochem.*, **12**, 296 (1970). Furthermore, meromycolic acid (**2**) is also representative, so that our approach could be adapted easily to the synthesis of related molecules. Note that continued refinement in separation and analytical techniques have produced results supporting the occurrence of meromycolic sections such as **2** although not necessarily with exactly the same number of methylene groups in the three parts: cf. N. Qureshi, K. Takayama, H. C. Jordi, and H. K. Schnoes, "Biological/Biomedical Applications of Liquid Chromatography", G. Hawk, Ed., Marcel Dekker, New York, in press; W. J. Gensler and J. P. Marshall, *Chem. Phys. Lipids*, **19**, 128 (1977).

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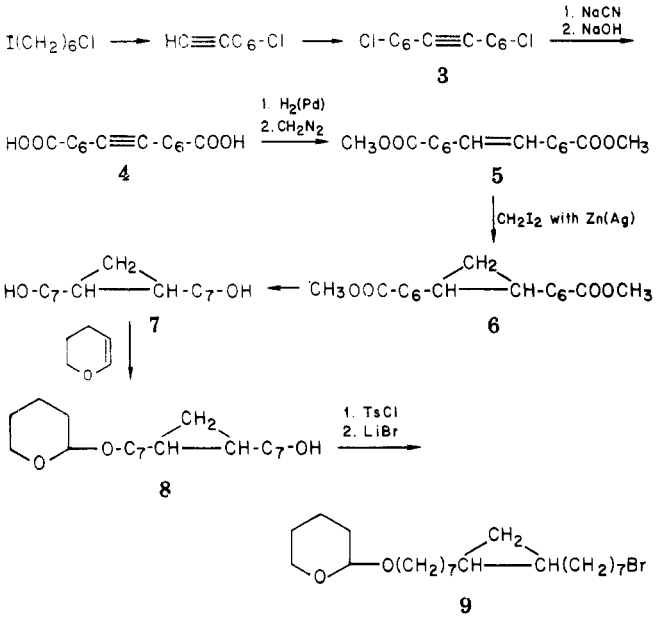
compound 2 had been obtained before as a synthetic material,[4] it had become clear that the earlier synthesis would furnish meromycolic acid in quantities sufficient for our purpose only with considerable effort. Accordingly, we worked out an alternate sequence. The present paper describes this short synthesis of meromycolic acid. It also describes a short, convenient synthesis of hexacosanoic acid.

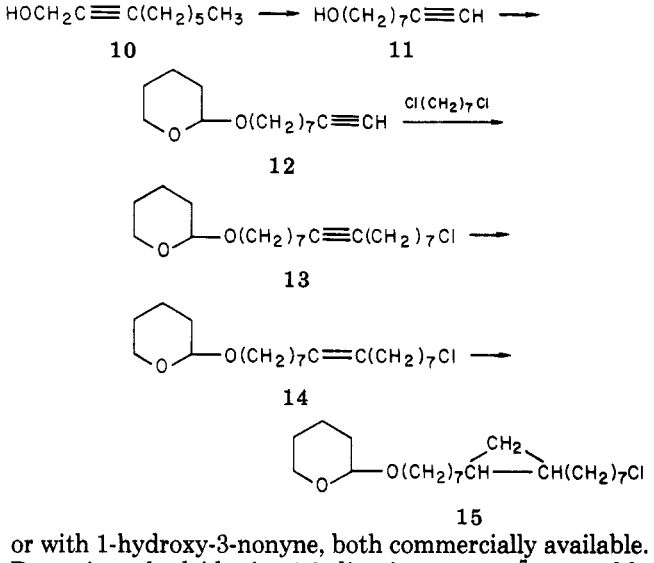
Synthesis of Meromycolic Acid (2). As will become evident, the plan for reaching meromycolic acid relied heavily on intermediate 9 (or 15), which, because of its



involvement at two places in the synthesis, provides 34 of the total of 56 carbon atoms in the finished molecule, including the two cyclopropane rings. Two pathways leading to this key intermediate were worked out.

One approach made use of 1,14-dichloro-7-tetradecyne (3), obtained by a two-stage alkylation of acetylene with 1-chloro-6-iodohexane. 6-Chlorohexyl *p*-toluenesulfonate was also available for use in place of the chloroiodohexane. Replacing the two chloro groups of 1,14-dichloro-7-tetradecyne (3) with cyano groups gave the expected acetylenic dinitrile and lengthened the carbon chain by two. Saponification converted the dinitrile to diacid 4, which was then half-hydrogenated to *cis*-8-hexadecenedioic acid. The corresponding diester 5 on treatment with methylene diiodide plus zinc coated with silver[5] produced the cyclopropane derivative, dimethyl *cis*-8,9-methylenehexadecanedioate (6). The Zn–Ag modification of the Simmons–Smith cyclopropane synthesis gave better yields than those using the more familiar zinc–copper couple.[6] Lithium aluminum hydride reduced cyclopropane diester 6 to the corresponding diol 7, which with dihydropyran under controlled conditions furnished 16-(tetrahydropyranyloxy)-*cis*-8,9-methylene-1-hydroxyhexadecane (8). Taking into account the recovered unchanged diol 7 as well as its bis(tetrahydropyranyl) derivative allows the 57% direct yield to be corrected significantly upward. The same unsymmetrical derivative 8 was also obtained conveniently by an acid-catalyzed redistribution of tetrahydropyranyl groups between the diol and its bis(tetrahydropyranyl) derivative. For this equilibration process we prepared the bis derivative either from diol 7 or, alternatively, by going back several steps in the sequence to *cis*-8-hexadecenedioic acid and reducing it to 1,16-dihydroxy-*cis*-8-hexadecene. Then, after masking both hydroxy functions with tetrahydropyranyl groups, the desired symmetrical cyclopropane derivative was formed by methylenation at the double bond. With the unsymmetrical alcohol 8 available, tosylation followed by replacement of the tosyloxy group with bromide furnished the necessary intermediate 9.

A different series of reactions leading to the intermediate alkyl halide started either with 1-hydroxy-2-nonyne (10)

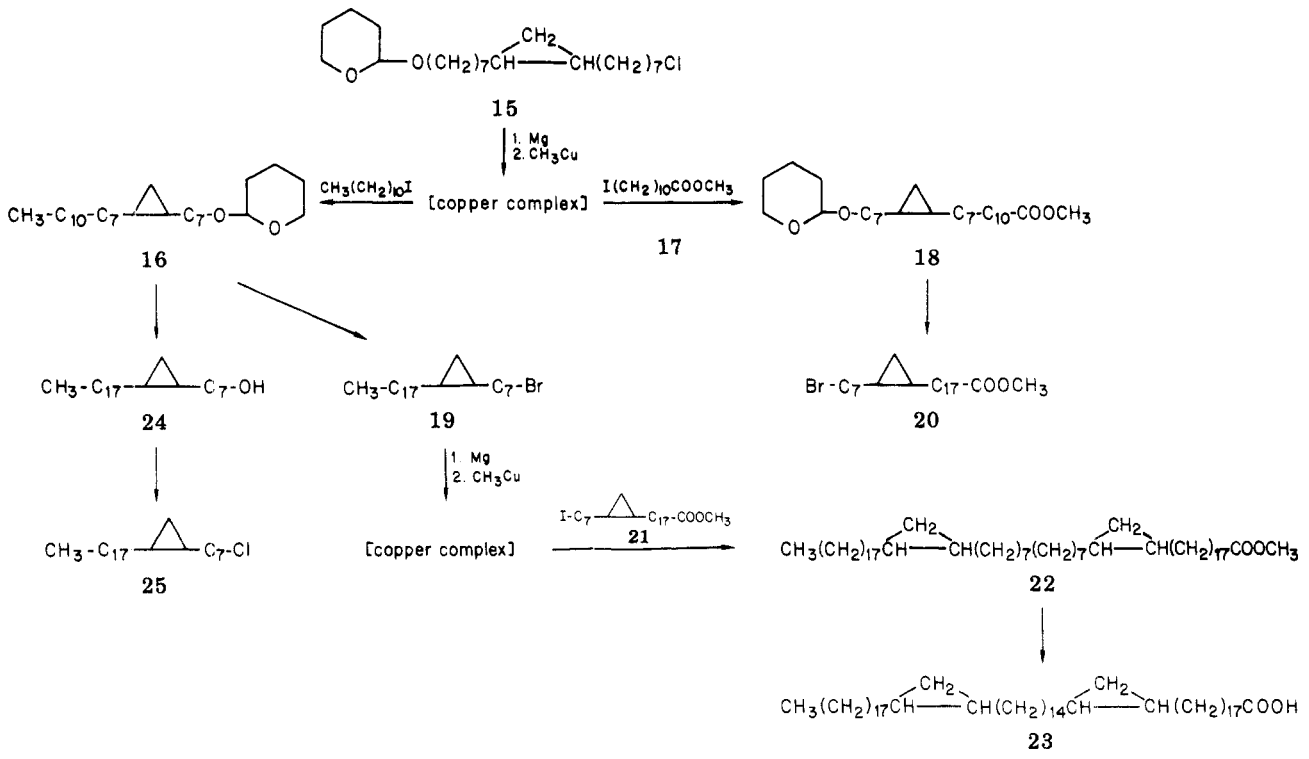


or with 1-hydroxy-3-nonyne, both commercially available. Potassium hydride in 1,3-diaminopropane[7] smoothly isomerized either compound to 1-hydroxy-8-nonyne (11). With the hydroxyl group masked with tetrahydropyranyl, as in 12, the terminal acetylenic group via its lithium acetylide derivative was alkylated with 1,7-dichloroheptane. 1-(Tetrahydropyranyloxy)-16-chloro-8-hexadecyne (13) was obtained in good yield together with minor amounts of the unwanted side product resulting from attachment of acetylides to both ends of the same dichloride. Acetylene 13 absorbed 1 mol of hydrogen over a Lindlar catalyst to yield 1-(tetrahydropyranyloxy)-16-chloro-*cis*-8-hexadecene (14). Concern about hydrogenolytic loss of chlorine proved groundless, excess hydrogen giving rise only to the saturated compound 1-(tetrahydropyranyloxy)-16-chlorohexadecane. Of the several methods tried for converting olefin 13 to the desired intermediate 15, the methylene diodide–diethylzinc process[8] for inserting methylene was clearly the best and was relied on.

When the two approaches were compared, the latter, with a 37% overall yield from 10 to 15, offered advantages and was preferred.

With the cyclopropane intermediate available, the synthesis continued in two directions, one branch leading to the hydrocarbon end of the meromycolate product and the second to the ester end. Both branches required chain elongation, and for both we made use of a procedure[9]

(4) W. J. Gensler, J. P. Marshall, J. J. Langone, and J. C. Chen, *J. Org. Chem.*, **42**, 118 (1977).
(5) J. M. Denis, C. Girard, and J. M. Conia, *Synthesis*, 549 (1972).
(6) Interestingly, a methylenation trial using the diacid precursor of diester 5 with the zinc–copper couple gave rise to a product (25%), which turned out to be the monomethyl ester corresponding to diester 6. Presumably the methyl ester is generated by zinc reduction of an intermediate iodomethyl ester.
(7) C. A. Brown and A. Yamashita, *J. Am. Chem. Soc.*, **97**, 891 (1975); *J. Chem. Soc., Chem. Commun.*, 959 (1976); C. A. Brown, *ibid.*, 222 (1975); J. C. Lindhoudt, G. L. van Mourik, and H. J. J. Pabon, *Tetrahedron Lett.*, 2565 (1976); E.-I. Negishi and A. Abramovitch, *Tetrahedron Lett.* 411 (1977).
(8) See J. Nishimura, J. Furakawa, N. Kawabata, and M. Kitayama, *Tetrahedron*, **27**, 1799 (1971).
(9) See D. E. Bergbreiter and G. M. Whitesides, *J. Org. Chem.*, **40**, 779 (1975); D. E. Bergbreiter and J. M. Killough, *ibid.*, **41**, 2750 (1976).

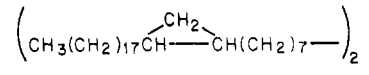


tantamount to a controlled Wurtz coupling of two different alkyl halides. The Grignard reagent from the key intermediate **15**, upon reaction with methylcopper(I), formed a complex, which for simplicity is described as an alkylmethylcopper(I) anion. Along one of the branches, coupling of this complex with undecyl iodide led to 1-(tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane (**16**). In the other direction, coupling of the same complex with methyl 11-iodoundecanoate (**17**) led to methyl 27-(tetrahydropyranyloxy)-*cis*-19,20-methyleneheptacosanoate (**18**). The parallel treatment of the two moieties continued for an additional step, in which triphenylphosphine dibromide transformed the tetrahydropyranyloxy groups directly to bromides.[10] In this way, **16** gave rise to 1-bromo-*cis*-8,9-methyleneheptacosane (**19**), and **18** gave rise to methyl 27-bromo-*cis*-19,20-methyleneheptacosanoate (**20**).

The iodo ester **21**, prepared without difficulty from the corresponding bromo ester **20**, served as one of the alkyl halides in a final coupling reaction. The other coupling partner, the one presented as the Grignard derivative, was either the bromide **19** or the analogous chloride **25**. The latter was prepared by removing the tetrahydropyranyl group of **16** to form alcohol **24** and then converting the alcohol to the chloride with triphenylphosphine in carbon tetrachloride.[11] The coupling, performed by a procedure similar to those used earlier in the sequence, joined the two halides **19** and **21** and so yielded the desired product, methyl meromycolate (**22**). Saponification gave meromycolic acid (**23**) and completed the synthesis.

With the exception of the last coupling step, the yields in all reactions were good to excellent. So far as the mixed couplings are concerned, connecting relatively small fragments (e.g., intermediate chloride **15** with undecyl iodide or with iodo ester **17**) gave products in yields at the 80% level. In sharp contrast, the last coupling produced methyl meromycolate (**22**) in only 28% yield calculated from the bromide **19** or in yields varying from 59 to 83% when based on nonrecovered iodide **21**.

Examination of the fractions obtained on processing this coupling reaction showed that although none of the alkyl bromide **19** survived, much iodo ester **21** could be recovered unchanged. High-resolution mass spectroscopic as well as other evidence was obtained for the side products formulated as in **24**–**30**.



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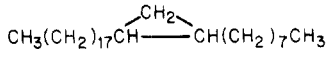

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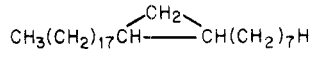

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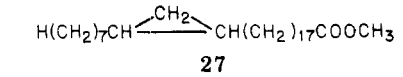

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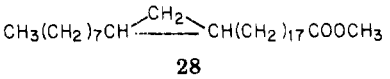

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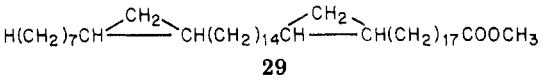

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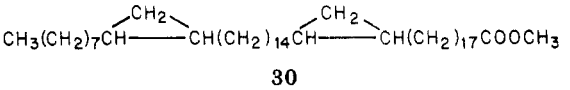

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A straightforward rationalization attributes these results to the observed limited solubility of one or both of the reactants, i.e., iodo ester **21** or the copper complex from the Grignard derivative of alkyl bromide **19**. The resulting heterogeneity would leave much of the iodo ester unchanged. The symmetrical hydrocarbon **24** could arise as a normal side product in the reaction of alkyl bromide **19** with magnesium, or, if some portions of the bromide **19**

(10) See P. E. Sonnet, *Synth. Commun.*, **6**, 21 (1976); *J. Chem. Soc., Chem. Commun.*, 337 (1977); M. Schwarz, J. E. Oliver, and P. E. Sonnet, *J. Org. Chem.*, **40**, 2410 (1975).

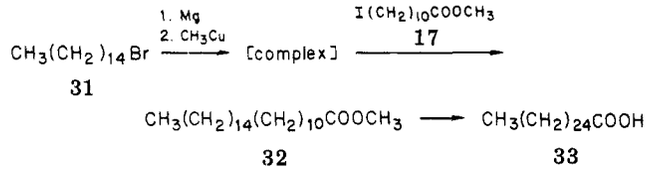
(11) J. B. Lee and T. J. Nolan, *Can. J. Chem.*, **44**, 1331 (1966); R. Appel, *Angew. Chem., Int. Ed. Engl.*, **14**, 801 (1975); C. Georgoulis and G. Ville, *Bull. Soc. Chim. Fr.*, 607 (1975).

escaped conversion to its Grignard derivative, this surviving bromide could react with the first-formed methylcopper(I) anion.[12] The result would be a complex in which *both* of the large alkyl groups came from **19**, and, therefore, one that would collapse to give the symmetrical hydrocarbon **24**. Hydrocarbon **26**, isolated as a mixture with **25**, would come from the insoluble alkylmethylcopper(I) anion when the reaction mixture was hydrolyzed. Actually, a very significant portion of the starting alkyl bromide **19**—as much as 40%—could be accounted for as the hydrocarbon mixture of **25** and **26**. Methylated compounds **25** and **28** are accounted for by reaction of the precursor halides **19** and **21** with residual methylcopper(I)[13] or by a methyl transfer in the last stages of the coupling procedure. Compound **27** can be explained by protonation of the final copper complex either inadvertently or on hydrolysis. Mass spectrometry showed the presence of esters **29** and **30**, the origins of which remain obscure.

We had little opportunity to test this interpretation for the low yield of meromycolate or to discover how to improve it. Nonetheless, despite the last coupling reaction, the new 13-step synthesis gives methyl meromycolate (**22**) in an overall uncorrected yield of 6–8% and so provides a practical source of the compound for continued work.[14] This may be compared to the earlier 27-step synthesis[4] giving methyl meromycolate (**22**) in 0.02–0.05% overall yield.

When chloride **25** was used in the last coupling in place of bromide **19**, methyl meromycolate was formed in poor yield, again accompanied by some of the same side products.

Synthesis of Hexacosanoic Acid (33). A convenient way to synthesize hexacosanoic acid was found in the mixed coupling of pentadecyl bromide (**31**) with methyl 11-iodoundecanoate (**17**). Saponification of the resulting methyl hexacosanoate (**32**) gave the desired acid (**33**).



Experimental Details

General Methods. Routine nuclear magnetic spectra were determined at 60 MHz, with chemical shifts referenced either to internal tetramethylsilane (δ 0.00) or to internal chloroform (δ 7.27). A few critical, more precise curves were obtained at 270.0146 or 250.0 MHz. High-resolution mass spectral data came from an AEI MS-902 instrument operating at 60 eV or from a CEC (Du Pont) 110B instrument. Thin-layer chromatography made use of Brinkmann E.M. brand plates precoated with 0.1 mm of silica gel on a 5 × 10 cm glass base. TLC spots were made visible by spraying the plate with 5% sulfuric acid and heating at 250–300 °C or by exposing the developed plate to iodine vapors; occasionally fluorescence under ultraviolet irradiation was helpful. For wet-column chromatography, we relied on E. Merck and Co. silica gel for chromatography, 0.06–0.20 mm, or on MC/B Florisil (60–100 mesh). The silica gel found effective for dry-column chromatography was purchased from ICN Pharmaceuticals, Inc., Life Sciences Group, Cleveland, OH, and was specified as having an average particle size of 0.05 mm, showing Brockmann Activity III and containing 0.5% of an inorganic fluorescent indicator. Nylon tubing with 0.04-mm wall thickness served to contain the column. Volatile solvents were routinely stripped from solution by distillation in a rotary still under water-pump pressures and with the flask in a bath maintained at room temperature or slightly above. The petroleum ether used here was specified with bp 35–60 °C. Analyses for elements were performed by Galbraith Laboratories, Knoxville, TN.

1,14-Dichloro-7-tetradecyne (3). Coupling sodium acetylide with 1-chloro-6-iodohexane essentially according to published direction[15] gave 8-chloro-1-octyne (**12**) in good yield. Gas–liquid chromatography showed only a single peak; NMR ($CDCl_3$) δ 3.4 (t, J = 12 Hz, 2, CH_2Cl), 2.3–1.4 (m, 11, remaining H's).

6-Chlorohexyl *p*-toluenesulfonate, an attractive equivalent to chloroiodohexane, was prepared[16] by stirring a solution of 6-chlorohexyl alcohol (22 g, 0.16 mol) and *p*-toluenesulfonyl chloride (49 g, 0.26 mol) in dry pyridine (250 mL) for 3.5 h at 10 °C. The mixture was poured into ice–water and extracted with ether. After washing the extract with water and drying, removing all volatiles left the desired product, 6-chlorohexyl *p*-toluenesulfonate, mp 37–37.5 °C, as a white powder (23 g, 56%): NMR ($CDCl_3$) δ 7.9–7.3 (2 d's, 4, aromatic H's), 4.0 (t, J = 12 Hz, 2, CH_2Cl), 3.4 (t, J = 15 Hz, 2, CH_2O), 2.5 (s, 3, $ArCH_3$), 2–1 (m, 8, remaining H's).

Combining the sodio derivative of 8-chloro-1-octyne (**12**) with 1-chloro-6-iodohexane in liquid ammonia afforded 1,14-dichloro-7-tetradecyne (**3**),[17] homogeneous according to GLC.

8-Hexadecynedioic Acid (4). When 3.2 g (12 mmol) of 1,14-dichloro-7-tetradecyne (**3**) was added to a stirred slurry of sodium cyanide (1.5 g, 30 mmol) in dimethyl sulfoxide[18] at 90 °C, the temperature rose rapidly to 150–160 °C, where it was maintained for 10 min. The cooled reaction mixture was poured into saturated salt solution, and the organic material was extracted with methylene chloride. The rinsed and dried extract, when stripped of volatiles, left a residue of dark-colored, crude 8-hexadecynedinitrile.

A small sample of the dinitrile, purified by silica gel column chromatography with chloroform as solvent, showed a single spot on a TLC plate (R_f 0.57 with $CHCl_3$): IR ($CHCl_3$) 2225 cm^{-1}; NMR ($CDCl_3$) δ 2.3 (m, 8, $CH_2C{\equiv}CCH_2$ and 2 CH_2CN), 1.5 (br s, 16, remaining CH_2's). Anal. Calcd for $C_{15}H_{24}N_2$: C, 78.68; H, 9.83; N, 11.47. Found: C, 78.65; H, 9.87; N, 11.40.

Hydrolysis was effected by refluxing the crude dinitrile in 75 mL of 2 N sodium hydroxide plus 75 mL of ethanol for 36 h or until no further ammonia was evolved. Chromatography of the dried, precipitated acid through a column of silica gel using methanol–chloroform (1:10) as solvent, with or without preliminary crystallization from benzene–petroleum ether (bp 40–50 °C), afforded 8-hexadecynedioic acid (**4**): mp 93–95 °C; NMR ($CDCl_3$) δ 11.5 (s, 2, 2 COOH), 2.51–2 (m, 8, $CH_2{\equiv}CCH_2$ and 2 CH_2COO), 1.6 (m, 16, other CH_2's). The 11.5-ppm signal disappeared on adding D_2O). Anal. Calcd for $C_{16}H_{26}O_4$: C, 68.03; H, 9.28. Found: C, 67.93; H, 9.07.

The yield of diacid from dichloride varied from 50 to 70%.

***cis*-8-Hexadecenedioic Acid.** Stirring a suspension of 5% Pd-on-$BaSO_4$ hydrogenation catalyst (30 mg) in a solution of 8-hexadecynedioic acid (**4**; 1.5 g, 5.4 mmol) in methanol (50 mL) containing five drops of quinoline under hydrogen at a slight positive pressure for 0.5 h resulted in absorption of very close to

(12) The alkyl bromide would serve as effectively as the alkyl iodide. Thus, in closely related processes, in which alkyllithium instead of Grignard reagents are used to form the intermediate disubstituted copper(I) anion, alkyl chlorides, bromides, and even tosylates react smoothly to give the expected coupling products [cf. G. H. Posner, *Org. React.*, **22**, 253 (1975); W. H. Mandeville and G. M. Whitesides, *J. Org. Chem.*, **39**, 400 (1974)].

(13) Examples of reactions with methylcopper are given by Posner (cf. citation in ref 12). Also cf. J. F. Normant, C. Cahiez, C. Chuit, A. Alexis, and J. Villiers, *J. Organomet. Chem.*, **40**, C49 (1972).

(14) As obtained here, synthetic meromycolate **2** undoubtedly is a mixture of two racemates, one of the four chiral forms of which correspond to the natural configuration. Unfortunately, aside from the cis arrangement of the ring substituents, nothing is known about the natural stereochemistry, so that which chiral form is correct cannot be stated. Our plans call for obtaining the individual optically active meromycolates and elaborating each separately to the corresponding mycolic acids and then on to the cord factors. We intend to reach the optically active meromycolates by separating the synthetic material **2** into the two racemates (recrystallization or chromatography) and then resolving them. Another way is to go through the entire synthesis using preresolved intermediates such as the enantiomers of **15** or of the smaller seven-carbon ring containing synthon of our first synthesis.[4] All four chiral forms of meromycolate **2** become accessible by introducing the two enantiomers in different combinations.

(15) W. J. Gensler and G. R. Thomas, *J. Am. Chem. Soc.*, **73**, 4601 (1951); R. A. Raphael and F. Sondheimer, *J. Chem. Soc.*, 2100 (1950).

(16) Unpublished work by Gayle Roset.

(17) W. J. Gensler and H. N. Schlein, *J. Am. Chem. Soc.*, **77**, 4846 (1955).

(18) R. A. Smiley and C. Arnold, *J. Org. Chem.*, **25**, 257 (1960).

the equimolar amount of gas. After the catalyst and solvent were removed, the residue in solution with chloroform was washed with dilute hydrochloric acid and water before drying. Chromatography (silica gel and $CHCl_3$) removed a trace of fast-running impurity and afforded 1.4 g (92%) of *cis*-8-hexadecenedioic acid, homogeneous according to TLC (R_f 0.5 with 1:9 CH_3OH–$CHCl_3$): mp 70–72 °C; NMR ($CDCl_3$) δ 11.4 (s, 2, 2 OH), 5.4 (t, J = 4 Hz, 2, CH=CH), 2.3 and 2.0 (br q and m, 8, 2 CH_2C=O and 2 CH_2C=C), 1.3 (s, 16, CH_2's). The 11.4-ppm singlet disappeared when deuterated water was added. Anal. Calcd for $C_{16}H_{28}O_4$: C, 67.60; H, 9.85. Found: C, 67.46; H, 10.06.

1,16-Dihydroxy-*cis*-8-hexadecene from *cis*-8-Hexadecenedioic Acid. A suspension of lithium aluminum hydride (0.41 g, 11 mmol) in tetrahydrofuran (110 mL) containing 0.70 g (2.5 mmol) of the diacid was refluxed for 5 h. The cooled mixture was treated with drops of water (2 mL) followed by 15% aqueous NaOH (2 mL) and then 6 mL of water. The resulting solids were separated by decantation and rinsed with ether. The combined tetrahydrofuran–ether solutions were shaken with water, dried, and freed of solvent. Chromatography (SiO_2 with 50:1 $CHCl_3$–CH_3OH) afforded the colorless, homogeneous diol (0.57 g or 90%): R_f 0.75 (1:30 CH_3OH–$CHCl_3$); NMR ($CDCl_3$) δ 5.4 (t, J = 4 Hz, 2, CH=CH), 3.6 (t, J = 4 Hz, 4, 2 CH_2OH), 2.45 (s, 2, 2 OH), 2.0 (m, 4, CH_2C=CCH_2), 1.3 (br s, 20, other CH_2's). Anal. Calcd for $C_{16}H_{32}O_2$: C, 75.00; H, 12.50. Found: C, 74.77; H, 12.34.

Dimethyl *cis*-8-Hexadecenedioate (5). A solution of the diacid (1.2 g, 4.2 mmol) in anhydrous ether (50 mL) was treated with an excess of distilled diazomethane in ether. After standing for 12 h, the solution was sparged with nitrogen and then stripped of all volatiles. Column chromatography (SiO_2 with $CHCl_3$) led to dimethyl *cis*-8-hexadecenedioate (5) as a colorless oil (1.3 g, 97%): one spot on TLC, R_f 0.75 with $CHCl_3$; NMR ($CDCl_3$) δ 5.4 (t, J = 4 Hz, 2, CH=CH), 3.7 (s, 6, 2 OCH_3), 2.5–1.8 (m, 8, CH_2C=CCH_2 and 2 CH_2C=O), 1.35 (s, 16, remaining H's). Anal. Calcd for $C_{18}H_{32}O_4$: C, 69.23; H, 10.25. Found: C, 69.07; H, 10.32.

***cis*-8,9-Methylene-1,16-hexadecanediol (7) by Methylenation and Reduction of Dimethyl *cis*-8-Hexadecenedioate (5).** Dimethyl *cis*-8-hexadecenedioate (5; 0.50 g, 1.6 mmol) in ether (100 mL) was stirred for 10 min in the presence of a zinc–silver couple[5] (17 g) plus powdered silver (1 g of 100 mesh). With the mixture at reflux, methylene diiodide (34 g, 0.13 mol) was added dropwise over a period of 1 h. Thereafter, stirring and refluxing were continued for 80 h.

Pyridine (12.6 g, 0.16 mol) was added by drops into the ice-cold reaction mixture. The solids were collected and washed on the funnel with ether, which was combined with the main solution. Additional small amounts of pyridine gave more precipitate. Finally, all volatiles were removed, leaving crude dimethyl 8,9-methylenehexadecanedioate (6) as the residue (0.5 g).

An ether solution (100 mL) of the diester (3.0 g) from several runs was refluxed with excess lithium aluminum hydride (2.4 g, 66 mmol) for 10 h. A small volume of water was added carefully followed by 3% aqueous NaOH. The ether layer was separated, washed with water, dried, and stripped of solvent to leave a waxy residue. When the wax was triturated with pentane, impurities were dissolved, and homogeneous, low-melting cis-8,9-methylene-1,16-hexadecanediol (7; 1.1 g, 42% from the olefinic diester) was obtained with R_f 0.75 (1:10 CH_3OH–$CHCl_3$). Chromatography through silica gel (1:50 CH_3OH–$CHCl_3$) afforded the diol with the following properties: NMR ($CDCl_3$) δ 3.62 (t, J = 4 Hz, 4, 2 CH_2OH), 2.3 (s, 2, 2 OH), 1.35 (s, 24, CH_2's), 0.6 (br s, 3, cyclopropane H's cis to each other), –0.3 (m, 1, cyclopropane H cis to alkyls). The δ 2.3 signal was removed in the presence of D_2O. Anal. Calcd for $C_{17}H_{34}O_2$: C, 75.55; H, 12.59. Found: C, 75.49; H, 12.67. In some runs, the appearance of an NMR signal at δ 5.4 in the crude reduction product indicated the presence of olefinic material (ca. 5–10%). This was removed by stirring the product in methylene dichloride with *m*-chloroperbenzoic acid for 3 h.

When copper-coated zinc[19] was used for methylenation instead of the silver–zinc combination and the diacid 15 was taken in place of the diester 16, unchanged diacid was recovered (25%) mixed with the oily monomethyl ester of *cis*-8,9-methylene-hexadecanedioic acid (25%): IR (neat) 1750, 1700 cm^{-1}; NMR ($CDCl_3$) δ 13.4 (br, 1, OH), 3.7 (s, 3, OCH_3), 2.3 (m, 4, 2 CH_2CO), 1.35 (s, ca. 20, 10 CH_2), 0.6 (s, 3, cyclopropane H's cis to each other), -0.3 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{18}H_{32}O_4$: C, 69.23; H, 10.25. Found: C, 68.56; H, 10.19.

Mono(tetrahydropyranyloxy) Derivative (8) of 8,9-Methylene-1,16-hexadecanediol. A mixture of diol 7 (0.25 g, 0.93 mmol) and dihydropyran (77 mg, 0.93 mmol) in dry methylene dichloride–tetrahydrofuran (50 and 12 mL, respectively) containing 5 mg of *p*-toluenesulfonic acid was stirred at –3 °C for 1 h and then at –10 °C for 3 h. Triethylamine (0.5 g) was added, and all volatile solvent was removed. Stirring the colorless residual oil with pentane (50 mL) dissolved all the monotetrahydropyran 8 and ditetrahydropyran derivatives plus a trace of diol 7. Most of the unchanged diol in the crude reaction mixture was recoverable as pentane-insoluble material.

The pentane solution was stripped of solvent, and the remaining oil was chromatographed through Florisil. The ditetrahydropyranyl compound was eluted with 1:1 $CHCl_3$–pentane. Continued elution with $CHCl_3$ alone gave the desired monotetrahydropyran derivative 8, and finally 1:15 CH_3OH–$CHCl_3$ gave the diol 7.

The total recovered diol 7 weighed 50 mg (20%) and showed one spot on TLC (R_f 0.37 with 1:30 CH_3OH–$CHCl_3$).

The ditetrahydropyran derivative (43 mg, 11%) was homogeneous according to TLC (R_f 0.74 with 1:30 CH_3OH–$CHCl_3$): IR ($CHCl_3$) no absorption maxima at 3500 cm^{-1}; NMR ($CDCl_3$) δ 4.5 (br s, 2, 2 OCHO), 3.5 (m, 8, 4 CH_2O), 1.55 (br s, 36, CH_2's), 0.55 (br s, 3, cyclopropane H's cis to each other), –0.35 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{27}H_{50}O_4$: C, 73.97; H, 11.41. Found: C, 74.07; H, 11.48.

16-(Tetrahydropyranyloxy)-8,9-methylene-1-hexadecanol (8), obtained as an oil (0.19 g, 57%), showed one TLC spot (R_f 0.59 with 1:30 CH_3OH–$CHCl_3$): IR 3500 cm^{-1}; NMR ($CDCl_3$) δ 4.55 (br s, 1, OCHO), 3.55 (m, 7, OH and 3 CH_2O), 1.45 (br s, 30, CH_2's), 0.55 (m, 3, cyclopropane H's), –0.3 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{22}H_{42}O_3$: C, 74.57; H, 11.86. Found: C, 74.47; H, 11.88.

1-(Tetrahydropyranyloxy)-8,9-methylene-16-bromohexadecane (9) from the Corresponding Alcohol (8). A solution of recrystallized *p*-toluenesulfonyl chloride (0.2 g, 1 mmol) and alcohol 8 (0.27 g, 0.77 mmol) in 35 mL of dry pyridine was kept at 1–3 °C for 20 h. Water (10 mL) was added, and the mixture was quickly extracted with cold ether. The extract was rinsed several times with cold water before drying and removing the solvent. The residue (0.35 g, 88%) was taken as the *p*-toluenesulfonate of alcohol 8.

Without further treatment, the *p*-toluenesulfonate was refluxed for 2 h with dry lithium bromide (0.30 g, 3.2 mmol) in dry acetone (75 mL). Aqueous bicarbonate was added, and the product was taken up in ethyl acetate. The extract was rinsed with water, dried, and stripped of solvent to leave the product (tetrahydropyranyloxy)hexadecyl bromide 9 (0.17 g, 74% from the tosylate). The NMR spectrum matched the assigned structure; no signals corresponding to the tosyl group were present.

1-Hydroxy-8-nonyne (11) from 1-Hydroxy-2-nonyne (10).[7] A portion of the thick paste that had settled out of a cream-colored 24% slurry of potassium hydride was removed, pressed between filter papers, weighed quickly (49 g, ca. 1.2 mol), and introduced without delay to a three-necked flask filled with argon. 1,3-Diaminopropane was distilled (bp 135–136 °C) from calcium hydride, 500 mL being collected directly onto the potassium hydride. Cooling maintained the temperature at 20 °C. After the mixture was stirred for 1 h, 1-hydroxy-2-nonyne (10; 29.8 g, 0.21 mol) was injected dropwise (10 min) at 10–15 °C to the stirred red-brown mixture, and the resulting slurry was stirred further under argon for 30 min.

With cooling, a large volume of water was added, cautiously at first, and the mixture was extracted thoroughly with ether. The extract was shaken with 1 N hydrochloric acid and with water before drying the product and removing the solvent. Fractional distillation of the viscous red-brown residue furnished 25.6 g (86%) of colorless 1-hydroxy-8-nonyne (11), bp 75–76 °C (1.1 mmHg), reported before [20,21] with bp 68–69 °C (0.5 mmHg). Prolonged

(19) E. LeGoff, *J. Org. Chem.*, **29**, 2048 (1964). See also R. D. Smith and H. E. Simmons, "Organic Synthesis", Collect. Vol. V, Wiley, New York, 1973, p 885; and H. E. Simmons, T. L. Cairns, S. A. Vladuchik, and C. M. Hoiness, *Org. React.*, **20**, 1 (1973).

distillation was avoided, since this led to serious decomposition. The product developed a single spot on TLC with R_f 0.31 (5:1 benzene–ethyl acetate) and gave a single peak on GLC: IR (neat) 3350, 3320, 2120 cm^{-1}; NMR (CCl_4) δ 3.7 (s, 1, OH), 3.5 (t, J = 6 Hz, 2 CH_2OH), 2.38–1.95 (m, 2, $CH_2C{\equiv}C$), 1.83 (t, J = 2.25 Hz, 1, C≡CH), 1.7–1.26 (m, 10, remaining H's). The δ 3.5 signal disappeared when D_2O was added.

Essentially the same results were obtained with 1-hydroxy-3-nonyne as starting material in place of 1-hydroxy-2-nonyne (**10**).

1-(Tetrahydropyranyloxy)-8-nonyne (12). A solution of 1-hydroxy-8-nonyne (6.5 g, 0.046 mol), dihydropyran (6 g, 0.07 mol), and 4 mg of dry *p*-toluenesulfonic acid in ether was stirred for 8 h at 15 °C. After a small volume of aqueous ammonia was added to neutralize the acid, more ether was added, and the solution was rinsed with several portions of water, dried, and stripped of volatiles. Fractional distillation of the residue gave 10.2 g (98%) of 1-(tetrahydropyranyloxy)-8-nonyne (**12**), bp 73–74 °C (0.3 mmHg). The colorless product [lit.[20] bp 98–101 °C (0.05 mmHg)] was homogeneous according to TLC (R_f 0.78 with 5:1 benzene–ethyl acetate) and according to GLC: IR (neat) 3310, 2120 cm^{-1}; NMR (CCl_4) δ 4.48 (poor t, J = 3 Hz, 1, OHCO), 4–3.05 (m, 4, 2 OCH_2), 2.15 (d and m, J = 3 Hz, 2, $CH_2C{\equiv}C$), 1.79 (t, J = 2.25 Hz, 1, C≡CH), 1.7–1.1 (m, 16, other H's).

1-(Tetrahydropyranyloxy)-16-chloro-8-hexadecyne (13). Under a slow current of pure argon, a 2.2 M solution of butyllithium in hexane (30.4 mL, 0.067 mol) was injected (10 min) into a cold (−58 °C), stirred solution of 1-(tetrapyranyloxy)-8-nonyne (**23**; 13.6 g, 0.061 mol) in tetrahydrofuran (50 mL) that had been freshly distilled from lithium aluminum hydride. With the temperature brought to 10 °C, hexamethylphosphoramide[22] (30 mL) distilled from calcium hydride was added, and the cold solution was stirred for 1 h. The color darkened gradually from pale yellow to dark red. 1,7-Dichloroheptane[23] (15 g, 0.091 mol) in hexamethylphosphoramide (30 mL) was added quickly, and the mixture, in which the color faded slowly, was stirred overnight at 10–15 °C.

After a large volume of ice–water was added, the reaction mixture was extracted with ether, which was then shaken with several portions of water, dried, and evaporated at reduced pressures. Distillation of the residue through a short-path still allowed most of the excess 1,7-dichloroheptane to be recovered with bp 96–97 °C (9.1 mmHg). After a short exposure to temperatures at 160 °C (10^{-5} mmHg), the material remaining in the distillation flask was chromatographed through Florisil with 3:1 hexane–benzene as solvent. Two materials could be separated.

The main product, the tetrahydropyranyl derivative (**13**) of 1-hydroxy-16-chloro-8-hexadecyne, was obtained as a colorless liquid (14.3 g, 66%): R_f 0.59 (1:10 ethyl acetate–benzene, very faint extra spots appeared); IR (neat) 3320, 2120 cm^{-1}; NMR (CCl_4) δ 4.45 (poor t, 1, OCHO), 3.95–3.0 (m, 6, 2 OCH_2 and CH_2Cl), 2.3–1.9 and 1.9–1.0 (2 m's, 30, $CH_2C{\equiv}CCH_2$ at ca. 2.03 and remaining H's). Anal. Calcd for $C_{21}H_{37}ClO_2$: C, 70.68; H, 10.37; Cl, 9.95. Found: C, 70.83; H, 10.53; Cl, 9.93.

Another homogeneous material was obtained from the chromatography whose properties corresponded to those expected for the bis condensation product, the ditetrahydropyranyl derivative of 1,25-dihydroxy-8,17-pentacosadiyne. This side product (1.7 g) accounted for 10% of the starting terminal acetylene **12**; it showed one spot on a TLC plate with R_f 0.41 (1:10 ethyl acetate–benzene): NMR (CCl_4 with $CHCl_3$ as internal reference) δ 4.55 (poor t, J = 3 Hz, 2 OCHO), 4.05–3.1 (m, 8, 4, CH_2)), 2.4–2 and 2–1.2 (2 m's, 50, 4 $CH_2C{\equiv}C$ and all other protons).

The yield of product **13** was not improved by increasing the proportion of 1,7-dichloroheptane to acetylene **23** from 1.5:1 to 2:1 (70% after 2.5 h of reaction time), nor was there any advantage to using tetrahydrofuran as solvent[24] at temperatures ranging from −70 to 70 °C, hexamethylphosphoramide alone[25] at temperatures of −5 to +20 °C, or sodium in liquid ammonia.[17] Replacing the dichloroheptane with 1-iodo-7-chloroheptane[23,26] also failed to give better results.

1-(Tetrahydropyranyloxy)-16-chloro-*cis*-8-hexadecene (14). A mixture of 1-(tetrahydropyranyloxy)-16-chloro-8-hexadecyne (**13**; 14.2 g, 39.8 mmol) and 0.7 g of Pd-on-$CaCO_3$ (Pb) Lindlar catalyst in reagent hexanes (140 mL) was stirred under an atmosphere of hydrogen for 3 h or until the calculated equimolar amount of hydrogen was absorbed. Removing the catalyst and solvent afforded 14.1 g (99%) of product **14**, showing only one TLC spot either on a standard silica gel plate or on one impregnated with silver nitrate.

A small sample processed further either by chromatography or by crystallization in hexanes at −65 °C showed R_f 0.61 on silica gel or 0.44 on silica gel containing silver nitrate (ca. 5–7%), both with 1:20 ethyl acetate–benzene as solvent: NMR (CCl_4) δ 5.3 (t, $J \approx$ 4.5–5 Hz, 2, CH=CH), 4.50 (ill-defined t, 1, OCHO), 3.98–3.03 (m, 6, 2 CH_2O and CH_2Cl), 2.4–1.1 (m, 30, remaining H's). Anal. Calcd for $C_{21}H_{39}ClO_2$: C, 70.29; H, 10.88; Cl, 9.90. Found: C, 70.60; H, 11.02; Cl, 9.74.

Allowing the hydrogenation to continue past the 1-mol point to saturation produced 1-(tetrahydropyranyloxy)-16-chlorohexadecane as a low-melting solid: R_f 0.57 (1:10 ethyl acetate–benzene). Anal. Calcd for $C_{21}H_{41}ClO_2$: C, 69.90; H, 11.37; Cl, 9.85. Found: C, 70.11; H, 11.43; Cl, 9.52.

Silica gel TLC plates dipped into aqueous silver nitrate and dried in the dark at 110–120 °C cleanly separated the unsaturated molecule **25** from the saturated compound; the R_f values with 1:20 ethyl acetate–benzene were respectively 0.44 and 0.61.

1-(Tetrahydropyranyloxy)-16-chloro-*cis*-8,9-methylenehexadecane (15). Scrupulously dried glassware was used here, with dry argon blanketing the reagents and the reaction mixture throughout the procedure. Commercial diethylzinc[8] (0.91 mL, 8.9 mmol) was added dropwise (10 min) from a calibrated dropping funnel to a stirred solution of cis olefin **14** (2.13 g, 5.95 mmol) in 10 mL of benzene that had been freshly distilled from calcium hydride. The flask was held in a bath at 10–15 °C. The olefin was followed by methylene diiodide (0.96 mL or 12 mmol) injected over 5 min. Stirring was continued at 20–25 °C for 30–45 min. Occasionally, the temperature rose spontaneously, in which case better results were obtained when no attempt was made to cool the flask. The argon flow was then replaced with a bubbler through which a slow stream of dry oxygen[27] was passed into the reaction overnight. Too rapid a development of turbidity was avoided by adjusting the rate of oxygen flow.

After addition of 150 mL of saturated aqueous ammonium chloride to the stirred, ice-cold mixture, it was extracted thoroughly with ether. The extract was shaken with several portions of water, dried, and then stripped of solvent. As judged from its nuclear magnetic resonance spectrum, the residue consisted of cyclopropane product **15** plus olefinic starting material **14** in a 4:1 ratio.

Several experiments were performed in an effort to improve the yield by methylenating the mixture a second and even a third time. However, the development of additional impurities complicated purification, so that there was no worthwhile increase in yield. Recovery of unchanged olefin by shaking a carbon tetrachloride solution of the crude product with methanolic silver nitrate[28] failed. Attempts at separation by column chromatography with silver nitrate impregnated silica gel[29] led to considerable decomposition and low recovery.

The persistent olefin was removed eventually by stirring an acetone (60 mL) solution of the crude methylenation product with a solution of sodium periodate (5.6 g) and potassium permanganate (0.2 g) in 60 mL of phosphate buffer at pH 7–7.4[30] overnight at

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(22) See M. Schwarz and R. M. Water, *Synth. Commun.*, 567 (1972).
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(25) D. M. Brattesani and C. H. Heathcock, *Synth. Commun.*, **3**, 245 (1973).
(26) W. F. Huber, *J. Am. Chem. Soc.*, **73**, 2730 (1951).
(27) See M. Sotaro and H. Hashimoto, *Bull. Soc. Chem. Jpn.*, **46**, 892 (1973).
(28) P. L. Nichols, *J. Am. Chem. Soc.*, **74**, 1091 (1952).
(29) A. T. James and L. J. Morris, Eds., "New Biochemical Separations", Van Nostrand, New York, 1964, p 295.

room temperature. Solids were removed and washed thoroughly on the funnel with chloroform. The filtrate plus washings were diluted with water before extracting with ether. The extract, after being rinsed and dried, furnished a solvent-free residue (1.8 g), which was free of olefin as shown by the absence of a signal at δ 5.25 and the absence of an olefin spot (R_f 0.57 with 10:1 benzene–ethyl acetate) on a silica gel TLC plate carrying 8–10% silver nitrate. Column chromatography through silica gel using benzene–hexanes (1:3) as the eluting solvent furnished 1.5 g (67%) of 1-(tetrahydropyranyloxy)-16-chloro-*cis*-8,9-methylenehexadecane (**15**), which from the NMR spectrum and the appearance of a TLC plate could contain no more than a trace of impurities.

A small sample of **15** further purified by silica gel preparative plate chromatography with 10:1 benzene–ethyl acetate as solvent showed the following properties: one spot on TLC with R_f 0.58 (10:1 benzene–ethyl acetate) or 0.44 (20:1 benzene–ethyl acetate); NMR (CCl_4 with $CHCl_3$ as reference) δ 4.53 (t, 1, OCHO), 4.06–3.06 (m, 6, 2 CH_2 and CH_2Cl), 2.06–1.06 (m, 30, 15 CH_2), 0.68 (br s, 3, cyclopropane cis H's), –0.14 to –0.34 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{22}H_{41}ClO_2$: C, 70.87; H, 11.01; Cl, 9.53. Found: C, 70.54; H, 11.21; Cl, 9.29.

Scaling the procedure up to the 5–6-g level of starting olefin **14** consistently gave the same results. Larger runs led to somewhat lower yields. Other methods of oxidatively removing unchanged olefin from the cyclopropanation product, for example, epoxidation[31] or low-temperature ozonolysis,[32] gave no better results than the periodate–permanganate procedure.

When the Simmons–Smith process was carried out with either Zn(Cu)[19,33] or Zn(Ag),[5] the maximum yield of cyclopropane product **26** was 40%. The products, even after multiple exposure to the reagents, always contained unchanged olefin in appreciable proportions.

Methyl 27-(Tetrahydropyranyloxy)-*cis*-19,20-methyleneheptacosanoate (18). Considerable care had to be taken with this and similar preparations to eliminate moisture and to use pure solvents and reagents. The scrupulously clean glass apparatus was routinely dried at 160 °C for 12 h. Solvents were redistilled just before use. Tetrahydrofuran was taken as the distillate from a mixture of sodium (10 g), benzophenone (20 g), and tetrahydrofuran (700 mL) that had been refluxed under nitrogen for 1 day. Pure argon was passed through a column of Drierite before entering the reaction vessels to provide an inert atmosphere that was maintained throughout the procedure. The magnesium powder (50 mesh; Alfa), or turnings, was dried overnight at 160 °C. Commercial copper(I) iodide, used as obtained, was specified as anhydrous, 98% pure material and was stored desiccated in a brown bottle. Methyllithium in ether was assayed at least monthly by a double-titration method.[34] Reactants were introduced by syringe injection through septum caps.

A small portion (ca. 1 ml) of a solution of 1-tetrahydropyranyl-16-chloro-*cis*-8,9-methylenehexadecane (**15**; 4.62 g, 12.5 mmol) in tetrahydrofuran (total volume 10 mL) was injected over magnesium powder (0.532 g, 22 mmol), and the mixture was stirred until reflux was evident. If there was a problem in starting the reaction, a catalytic amount of methyl iodide was added. The bulk of the tetrahydrofuran solution was introduced at a moderate rate, with the temperature maintained at 60 °C. An additional 10 mL of solvent washed in the last portions of reactant. The refluxing mixture was then stirred for 24 h.

Methylcopper was formed by injecting ethereal methyllithium (9.55 mL of a 1.304 M solution, 12.7 mmol) into a stirred mixture of copper(I) iodide (2.45 g, 12.9 mmol) and tetrahydrofuran (14 mL), taking care to keep the temperature at –60 to –65 °C. After 1 h, the pale yellow suspension was slowly (45 min) brought to 0 °C, whereupon the color changed to bright yellow. Without delay, the temperature was returned to –60 °C.

The Grignard solution was injected (10 min) into the stirred methylcopper suspension at –65 to –55 °C, the transfer being completed with the help of 10 mL of solvent. (The recovered magnesium weighed 0.232 g, so that the amount of magnesium consumed was 12.4 mmol.) The copper complex was formed by stirring the mixture at –60 °C for 1 h, warming it slowly to 0 °C, and, as soon as a clear green-purple solution developed, dropping the temperature back to –60 °C. Finally, after injection of methyl 11-iodoundecanoate[9] (4.64 g, 14.2 mmol) in 6 mL of tetrahydrofuran (10 min) followed by 5 mL of wash solvent, stirring was continued first for 1 h at –60 °C and then for 3 h at 25 °C.

The coupling reaction was quenched at 0 °C with saturated aqueous ammonium chloride and then extracted with ether. The extract was washed with portions of water and brine, dried, and freed of solvent. Chromatography of the residue on a 100-g column of Florisil made use of the following developing solvents: petroleum ether (200 mL), 4:1 petroleum ether–benzene (200 ml), 1:1 petroleum ether–benzene (300 mL), benzene (750 mL), benzene and chloroform (300 mL). The desired product appeared in the last liter of eluate and was obtained in pure form by stripping away all solvent. The earlier fractions (ca. 0.8 g) contained mainly unchanged iodo ester.

The colorless methyl 27-(tetrapyranyloxy)-*cis*-19,20-methyleneheptacosanoate (**18**), mp 35–36 °C, weighed 5.4 g (80% from the chloride **26**) and showed one TLC spot with R_f 0.36 (11:1 benzene–ethyl acetate); NMR (CCl_4 with $CHCl_3$ as internal reference) δ 4.51 (poor t, 1, OCHO), 3.58 and 3.93–3.03 (s bounded by br m, 7, OCH_3 and 2 OCH_2), 2.43–2.08 (br t, 2, CH_2CO), 1.47 (br s, ca. 50, CH_2's), 0.78–0.43 (br s, 3 cyclopropane cis H's), –0.17 to –0.47 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{34}H_{64}O_4$: C, 76.12; H, 11.94. Found: C, 75.93; H, 11.87.

When essentially the same procedure was doubled in scale, the percentage yields were about the same; here, ordinary magnesium turnings could be used without difficulty, and no methyl iodide starter was necessary.

Substituting 1-(tetrahydropyranyloxy)-*cis*-8,9-methylene-16-bromohexadecane (**9**) for the corresponding chloro compound (**15**) gave the same product (**18**) but in lower yields.

Methyl 27-Bromo-*cis*-19,20-methyleneheptacosanoate (20) from Methyl 27-Tetrahydropyranyl-*cis*-19,20-methyleneheptacosanoate (18). The tetrahydropyranyl starting material (**18**; 5.38 g, 10.0 mmol) was stirred at room temperature for 2 h under nitrogen with a pale yellow solution of triphenylphosphine dibromide[10] (5.3 g, 13 mmol) in 40 mL of methylene dichloride. Then anhydrous methanol (5 mL) was injected, and stirring was continued for another 0.5 h. Water was added, and the product was taken up in ether, which was washed with water and with brine, dried, and then stripped of volatiles. Most of the triphenylphosphine oxide was removed as the solid that formed on diluting a solution of the residue in 5 mL of benzene with 15 mL of petroleum ether. Further purification was effected by chromatography on Florisil (30 g) with 200 mL of 1:1 petroleum ether–benzene as eluant.

Methyl 27-bromo-*cis*-19,20-methyleneheptacosanoate (**20**) was obtained in this way (3.81 g, 74%): mp 38–39 °C; one spot on TLC with R_f 0.56 (11:1 benzene–ethyl acetate); NMR (CCl_4 with $CHCl_3$ reference) δ 3.61 (s, 3, OCH_3), 3.35 (t, J = 7 Hz, 2, CH_2Br), 2.25 (br t, J = 7 Hz, CH_2CO), 1.32 (s, CH_2's), 0.8–0.55 (br s, cyclopropane cis H's), –0.18 to –0.45 (m, 1, cyclopropane H cis to alkyls). The integration at δ 2.25 corresponded approximately to 2 H's, at δ 1.32 to 44 H's, and at δ 0.8–0.55 to 3 H's. Anal. Calcd for $C_{29}H_{55}BrO_2$: C, 67.57; H, 10.68; Br, 15.53. Found: C, 67.68; H, 10.44; Br, 15.71.

Methyl 27-Iodo-*cis*-19,20-methyleneheptacosanoate (21). A solution of the corresponding bromo ester **20** (3.81 g, 7.40 mmol) and sodium iodide (2.3 g, 15 mmol) in acetone (30 mL) was stirred and refluxed under a blanket of nitrogen for 2 h. After removal of the acetone, benzene was added to the residue, and insoluble solids were separated by filtration. The filtrate was passed through a short column of Florisil before it was stripped of all volatiles.

The residual methyl 27-iodo-*cis*-19,20-methyleneheptacosanoate (**21**) appeared as a solid (4.0 g, 96%) with mp 39–40 °C and with its other properties very similar to those of the precursor bromo compound **20**. The product was homogeneous according to TLC (R_f 0.56 with 11:1 benzene–ethyl acetate): NMR (CCl_4 with $CHCl_3$ internal reference) δ 3.66 (s, 3, OCH_3), 3.20 (t, J = 7 Hz, 2, ICH_2), 2.31 (br t, J = 7 Hz, ca. 2, CH_2CO), 1.39 (s, ca. 44, CH_2's), 0.72 (s, ca. 3, cyclopropane H's cis to each other), –0.11 to –0.41 (m,

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1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{29}H_{55}IO_2$: C, 61.92; H, 9.78; I, 22.60. Found: C, 61.80; H, 9.64; I, 22.50.

1-(Tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane (16). The procedure here was analogous to the one used to obtain ester **18**. The starting materials were 1-tetrahydropyranyl-16-chloro-*cis*-8,9-methylenehexadecane (**15**; 2.45 g, 6.57 mmol) with 10 mL of tetrahydrofuran followed by 5 mL of tetrahydrofuran for rinsing and later with another 5 mL for complete transfer of the Grignard reagent, magnesium powder (0.203 g, 8.35 mmol; 6.05 mmol was consumed; ordinary turnings were also used successfully), copper(I) iodide (6.99 mmol) with 10 mL of tetrahydrofuran, ethereal methyllithium (5.4 mL of a 1.24 M solution, 6.70 mmol), and undecyl iodide (2.30 g, 8.16 mmol) with 5 mL (plus 5 mL for rinsing) of tetrahydrofuran. The chromatographic separation made use of an 18-cm column of Florisil starting with hexane solvent, gradually introducing benzene (9:1, 4:1, 1:1), and ending with benzene alone.

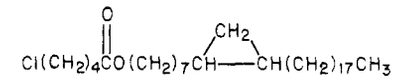
1-(Tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane (**16**) was isolated as a colorless, low-melting solid (2.6 g, 81% based on the starting chlorine or 87% based on magnesium): homogeneous according to TLC (R_f 0.40 with 11:1 benzene–ethyl acetate; R_f 0.58 with benzene); NMR ($CDCl_3$ with $CHCl_3$ as reference) δ 4.75–4.5 (t, 1, OCHO), 4.1–3.2 (br m, 4, 2 OCH_2), 2.1–1.2, 1.2–0.9, and 0.9–0.6 (m, s, and distorted t, 58, CH_2's, CH_3, cyclopropane cis H's), –0.05 to –0.35 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{33}H_{64}O_2$: C, 80.49; H, 13.01. Found: C, 80.39; H, 12.95.

1-Bromo-*cis*-8,9-methyleneheptacosane (19). The corresponding 1-tetrahydropyranyl derivative **16** was converted to its bromo analogue **19** essentially the way tetrahydropyranyl derivative **18** was converted to its bromo analogue **20**. The amounts used were as follows: 1-(tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane, 2.31 g (4.69 mmol); triphenylphosphine dibromide, 3.0 g (7.0 mmol); methylene dichloride (solvent), 13 mL. Column chromatography was performed with 9:1 petroleum ether–benzene as the developing solvent. The low-melting, colorless 1-bromo-*cis*-8,9-methyleneheptacosane (**19**; 2.05 g, 93%) showed a single spot on TLC, R_f 0.67 (11:1 benzene–ethyl acetate): NMR (CCl_4 with $CHCl_3$ reference) δ 3.43 (t, J = 6 Hz, 2, $BrCH_2$), 1.45 (s, ca. 46, CH_2's), 1.05 (distorted t, ca. 3, CH_3), 0.75 (s, ca. 3, cyclopropane cis H's), –0.05 to –0.35 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{28}H_{55}Br$: C, 71.34; H, 11.68; Br, 16.98. Found: C, 71.49; H, 11.77; Br, 17.07.

1-Hydroxy-*cis*-8,9-methyleneheptacosane (24). 1-(Tetrahydropyranyloxy)-*cis*-8,9-methyleneheptacosane (**16**; 0.94 g, 1.9 mmol) in 15 mL of methylene dichloride containing 1 mL of ethanol and 6 mg of *p*-toluenesulfonic acid was stirred at 20 °C for 1 h. After some aqueous ammonia was added, the mixture was extracted thoroughly with ether. The extract was washed free of ammonia with water, dried, and then stripped of all solvent. Crystallization of the residue from hexane containing benzene, chloroform, or ether furnished 1-hydroxy-*cis*-8,9-methyleneheptacosane (**24**) as shiny crystals (0.70 g, 90%): mp 70–71 °C; single spot on TLC with R_f 0.17 (20:1 benzene–ethyl acetate) (R_f for the starting material 0.49); IR ($CDCl_3$) 3620 cm^{-1}; NMR ($CDCl_3$ with $CHCl_3$ as reference) δ 3.72 (t, J = 6 Hz, 2, CH_2OH), 1.85 (hump that disappears on adding D_2O, OH), 1.77–1.22 (m with s at 1.42, CH_2's), 1.06 (t, J = 4.5 Hz, CH_3), 0.92–0.62 (m, cyclopropane cis H's), 0.0 to –0.33 (m, 1, cyclopropane H cis to alkyls). Integration of all the signals from δ 1.85 to 0.62 showed the presence of 53 H's as required. Anal. Calcd for $C_{28}H_{56}O$: C, 82.35; H, 13.73. Found: C, 82.42; H, 13.63.

1-Chloro-*cis*-8,9-methyleneheptacosane (25). A solution of 1-hydroxy-*cis*-8,9-methyleneheptacosane (0.66 g, 1.6 mmol) in carbon tetrachloride (25 mL) containing triphenylphosphine (1.7 g, 6.5 mmol) was refluxed for 39 h. Solvent was removed, and the residue was passed through a small silica gel column using hexanes as the carrier solvent. The desired 1-chloro-*cis*-8,9-methyleneheptacosane (**25**) was obtained by stripping all volatiles from the appropriate fractions. The product proved to be a solid (0.67 g, 96%), melting at 78–79 °C and homogeneous according to TLC (R_f 4.9 with hexanes as solvent; R_f 0 for the starting alcohol **31**). Further treatment by preparative plate chromatography (1:10 benzene–hexanes) afforded analytically pure material: IR ($CDCl_3$) no maximum at 3620 cm^{-1}; NMR ($CDCl_3$ with $CHCl_3$ as reference) δ 3.6 (t, J = 6 Hz, 2, CH_2Cl), 2.2–1.2 (m surrounding an s at 1.42, CH_2's), 1.02 (t, CH_3), 0.90–0.57 (m, cyclopropane H's cis to each other), –0.08 to –0.33 (m, 1, cyclopropane H cis to alkyls). The combined integration of the signals from 2.2 to 0.57 ppm corresponded to 52 H's. Anal. Calcd for $C_{28}H_{55}Cl$: C, 78.78; H, 12.90; Cl, 8.32. Found: C, 78.94; H, 13.00; Cl, 8.22.

When triphenylphosphine dichloride[35] was allowed to react with 0.1 g of the tetrahydropyranyl derivative **16**, the corresponding chloride **25** was obtained in 65% yield. However, when 1 g of **16** was used, the yield of chloride dropped to an unsatisfactory 36%. Starting material (35%) could be recovered, and, in addition, a side product was obtained to which the following structure was tentatively assigned:



This material showed infrared absorption maxima at 1750 and 1737 cm^{-1} but none in the hydroxyl region; NMR ($CDCl_3$) δ 4.35 (t, J = 6 Hz, OCH_2), 3.67 (t, J = 6 Hz, $ClCH_2$), 2.87–1.17 (m), 1.17–0.87 (t, CH_3), 0.87–0.52 (m), –0.11 to –0.33 (m).

Methyl Meromycolate (22) by Coupling Bromide 19 with Iodo Ester 21. The procedure was similar to the one described before for the synthesis of ester **18**. The reactant 1-bromo-*cis*-8,9-methyleneheptacosane (**19**) (1.39 g, 3.00 mmol) in tetrahydrofuran (10 mL) was converted to its Grignard reagent by stirring with 168 mg (6.89 mmol) of magnesium powder. When the reaction was performed with double these quantities, ordinary magnesium turnings served satisfactorily, and no problem requiring the use of methyl iodide primer was encountered. To form the alkylmethylcopper(I) intermediate, we injected the Grignard solution into the yellow slurry of methylcopper(I), which was prepared as before from copper(I) iodide (0.702 g, 3.69 mmol) under tetrahydrofuran (6 mL) and 2.6 mL (3.0 mmol) of ethereal methyllithium. If the heavy slurry became too difficult to stir mechanically, it was shaken intermittently by hand. The coupling partner, methyl 27-iodo-*cis*-19,20-methyleneheptacosanoate (**21**; 1.86 g, 3.30 mmol) in tetrahydrofuran (14 mL), was introduced, and the mixture was treated as in the analogous preparations. At the last stages, quenching with saturated aqueous ammonium chloride, extraction, etc. afforded 2.34 g of solvent-free crude solid.

Crystallization first from chloroform or hexane and then from benzene furnished 0.69 g of methyl meromycolate (**22**): mp 72–78 °C; single spot on TLC with R_f 0.52 (85:5 hexane–ethyl acetate); ^1H NMR (250.0 MHz; $CDCl_3$ with $CHCl_3$ as internal reference at δ 7.26) δ 3.66 (s, 2.88, CH_3), 2.30 (t, J = 7.5 Hz, 1.95 in the ratio 1.1:1.9:1.1, CH_2COO), 1.61–1.55 (br t and sharp s, ca. 8.4, CH_2's at positions 18, 21, 34, 37), 1.43–1.07 with intense s at 1.27 (CH_2's), 0.90–0.85 (distorted t, CH_3–C), 0.68–0.55 (m, 6.2, cyclopropane H's cis to each other), –0.31 to –0.35 (t with some sign of q, J = 4.1 Hz, 1.8, cyclopropane H's cis to alkyls). An imprecise integration in the 1.43–0.085-ppm range corresponded approximately to 112 H's (97 required). A check determination at 270.0146 MHz produced integration values that corresponded to 2.9 for CH_3O, 2.1 for CH_2COO, 2.0 for cyclopropane H's cis to alkyls, and 103 for the remaining protons; proton decoupled ^{13}C NMR (62.9 MHz; $CDCl_3$ as solvent with δ 76.95 referenced to CS_2 at 192.90 ppm) δ 51.30 ($COOCH_3$), 34.14 (CH_2COO), 32.03, 30.30, 29.79, and 28.84 (CH_2's), 25.04 (CH_2CH_2COO), 22.76 (CH_3CH_2), 15.87 (cyclopropane methines), 14.13 (CH_3CH_2), 11.01 (cyclopropane methylenes). These ^{13}C chemical shifts agree well with those reported for related compounds.[36] Anal. Calcd for $C_{57}H_{110}O_2$: C, 82.81; H, 13.32. Found: C, 82.91; H, 13.41.

The high-resolution fragmentation pattern was determined for this methyl meromycolate (**22**) from m/e 70 to 850. The probe

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inlet was set at the relatively low temperature of 160–170 °C so as to promote survival of larger molecular pieces. The molecular mass (calcd for $C_{57}H_{110}O_2$, 826.8506; found, 826.8393 and 826.8446) appeared with the unusually high intensity of 44% of the base peak. Although the data amassed for this methyl meromycolate supported the product as an essentially homogeneous material, probing by sensitive mass spectroscopy revealed the presence of trace amounts of extraneous substances. Thus, minor blip peaks were noted at m/e 686 for $C_{47}H_{90}O_2$ (**30**) and 672 for $C_{46}H_{88}O_2$ (**29**); the same was true for the peaks at m/e 436 and 450 corresponding to $C_{29}H_{56}O_2$ (**27**) and $C_{30}H_{58}O_2$ (**28**), respectively, as well as for m/e 392 corresponding to the hydrocarbon $C_{28}H_{56}$ (**26**). A peak at m/e 782, attributed to the hydrocarbon $C_{56}H_{110}$ (**24**), appeared in an isolated scan with an intensity of 9%; however, the other data again sharply limited the amount that could be present to trace levels. Finally, some very low-intensity signals suggested the possible presence of phthalate esters (?), presumably as inadvertently introduced contaminants. In other preparations, high-resolution mass spectral signals obtained with less purified samples (inlet at 200 °C) indicated the presence of esters, **29**, **30**, **28** (the molecular mass for **28** was observed at m/e 450 as well as one at m/e 419 corresponding to the loss of CH_3OH), and **27** (the peak at m/e 404 corresponded to the molecular mass minus CH_3OH).

Aside from sketchy mass spectral data, the literature[3] offers no constants suitable for making comparisons between the degradation meromycolate and the synthetic material.

The solvent-free residue (1.59 g) in the mother liquor remaining after removing the methyl meromycolate was subjected to dry-column chromatography using 45 g of silica gel with 1:1 hexanes–benzene. A fast-running, nonpolar fraction was isolated (0.48 g, corresponding to 41% of the starting bromide **19**), which proved to be a mixture of 8,9-methyleneheptacosane (**26**) and 9,10-methyleneoctacosane (**25**). After crystallization from hexane, this product melted at 73–74 °C and gave a single well-defined spot on TLC with R_f 0.69–0.70 (11:1 benzene–ethyl acetate); NMR (CCl_4 with $CHCl_3$ as reference) δ 1.515 and 1.14 (s and t, 52–54, CH_2's and CH_3's), 0.84 (s, 3, cyclopropane H's cis to each other), 0.04 to −0.26 (m, 1, cyclopropane H cis to alkyls). Anal. Calcd for $C_{28}H_{56}$ or $C_{29}H_{58}$: C, 85.71; H, 14.28. Found: C, 85.68; H, 14.09.

High-resolution mass spectroscopy (70 eV; inlet probe at 200 °C) confirmed the identity [calcd for $C_{28}H_{56}$ and $C_{29}H_{58}$, m/e 392.4382 and 406.4539, respectively); found, respectively, for the molecular mass peaks, m/e 392.4362 (72% of base peak intensity) and 404.4545 (26% of the base peak)]. Repeated low-resolution scans of the same sample while the inlet temperature was gradually raised, starting with 100 °C, showed signs of fractionation, with more of the lower molecular weight material appearing at the lower temperatures. The intensity ratios of the m/e 392–406 peaks ranged from 4:1 to 2:1 and suggested an average close to 3:1.

The dry-column chromatography procedure also permitted separation of unchanged iodo ester **21** (1.05 g) as a slower moving fraction, which was homogeneous according to TLC (R_f 0.55 with 11:1 benzene–ethyl acetate). The yield of methyl meromycolate (**22**) calculated from the starting 1-bromo-*cis*-8,9-methyleneheptacosane (**19**) used was 28%; the yields calculated from the magnesium and the iodo ester consumed were 40 and 59%, respectively. In other preparatory runs, much more of the iodo ester **21** was recovered, so that the yield based on iodo ester consumed ranged as high as 80%.

Coupling reactions with 1-chloro-*cis*-8,9-methyleneheptacosane (**25**) used in place of the bromo analogue **19** were unsatisfactory. Either the chloride refused to react with magnesium or, when small amounts of ethylene dibromide were introduced as an entrainment agent, the methyl meromycolate product was obtained in only 10% yield. In the ethylene dibromide runs, most of the iodo ester **21** was recovered unchanged, the major product proving to be a mixture of hydrocarbons **25** and **26**. In addition, mass spectral data revealed the presence of compounds **27**–**30**.

Meromycolic Acid (23). A solution of recrystallized methyl meromycolate (**22**; 0.861 g, 1.06 mmol) and potassium hydroxide (5 g) in dioxane (25 mL), methanol (22 mL), and water (3 mL) was refluxed for 3 h. After the cooled mixture had been acidified with 20% hydrochloric acid, it was diluted with 100 mL of water and left to stand overnight at 0 °C. The precipitate was collected, washed with cold water, and dried, after which it was triturated with cold chloroform to remove some soluble impurities. The dried, white meromycolic acid (**23**) weighed 0.76 g (98%), showed mp 90–95 °C, and produced a streaked TLC spot (R_f ca. 0.50 with 6:3:1 chloroform–hexane–methanol) whose density distribution and symmetry was consistent with homogeneity. Other than showing no signals at δ 5.3 (no CH_3O), the 60-MHz NMR spectrum was not very informative. Anal. Calcd for $C_{56}H_{108}O_2$: C, 82.76; H, 13.40. Found: C, 82.57; H, 13.30.

The high-resolution mass spectrum of this acid (inlet temperature approximately 160 °C) showed the molecular mass with 1.5% of the base peak intensity (calcd for $C_{56}H_{108}O_2$, m/e 812.835; found m/e 812.852). No signals were seen corresponding to ester **29** (or to its acid $C_{45}H_{86}O_2$ with m/e 658) or to ester **30** (or to its acid $C_{46}H_{88}O_2$ with m/e 672). The acid $C_{28}H_{54}O_2$ (m/e 422), which matched ester **27**, and the acid $C_{29}H_{56}O_2$ (m/e 436), which matched ester **28**, could be detected as minor peaks but only by making many successive scans on the same sample, during which fractionation and concentration were undoubtedly occurring. In one of these late, repeated scans, a peak for hydrocarbon **24** was evident at a significant level of intensity. Finally, some signals appearing at small m/e values (e.g., 149 for $C_8H_5O_3$, 167 for $C_8H_7O_4$, and 279 for $C_{16}H_{23}O_4$) could be indicative of the presence of trace amounts of phthalate ester (?) impurities.

Methyl Hexacosanoate (33). The procedure applied here is similar to the one used to obtain ester **18**. A mixture of pentadecyl bromide (8.4 g, 29 mmol) and magnesium turnings (0.85 g, 35 mmol) with 30 mL of anhydrous tetrahydrofuran was stirred and refluxed for 4 h to give a gray, turbid solution. (The appearance of a white precipitate at this point signaled a poor final yield.) The Grignard preparation at room temperature was added (ca. 15 min) by drops to a methylcopper suspension at −70 °C, which had been prepared from copper(I) iodide (5.6 g, 30 mmol) under tetrahydrofuran (30 mL) and 24.6 mL of 1.26 M ethereal methyllithium (30.9 mmol). At the stage at which the Grignard–methylcopper complex was brought to 0–10 °C, instead of the expected purple, an orange or pale pink developed. After the mixture was returned to −70 °C, methyl 11-iodoundecanoate[9] (10.6 g, 32.6 mmol) in 20 mL of tetrahydrofuran was introduced, and the reaction was allowed to proceed and was processed as before.

The crude, solvent-free product was chromatographed through a column of silica gel (210 g) with hexane and then hexane–benzene mixtures as developing solvents. Methyl hexacosanoate (**32**) emerged with the hexane–benzene and was obtained as a white solid (7.4 g, 62% from the alkyl bromide): mp 62–63 °C (lit.[37] mp 61.5–62 °C); single spot on TLC with R_f 0.59 (benzene); IR ($CHCl_3$) 1730 cm^{-1}; NMR ($CDCl_3$) δ 3.60 (s, 3, OCH_3), 2.45–2.15 (t, J = 7 Hz, 2, CH_2CO), 1.3 and 1.1–0.8 (s and t, 49, remaining protons). Anal. Calcd for $C_{27}H_{54}O_2$: C, 79.02; H, 13.17. Found: C, 79.13; H, 13.02.

The methyl hexacosanoate could also be purified by vacuum distillation or by crystallization from either ethanol or acetone.

Hexacosanoic Acid (33). A heterogeneous mixture of methyl hexacosanoate (2.6 g, 6.3 mmol), sodium hydroxide (2.1 g, 52 mmol), methanol (19 mL), dioxane (30 mL), and water (1 mL) was stirred and refluxed for 6 h. The cooled, still heterogeneous mixture was stirred for 30 min with excess cold 25% hydrochloric acid before diluting with ice-cold water and filtering. Crystallization of the solids from chloroform afforded 2.4 g (96%) of hexacosanoic acid: mp 87–89 °C (lit.[38] mp 86.3 °C); NMR ($CDCl_3$) δ 9.5–9.1 (br, 1, COOH), 2.5–2.2 (t, J = 7 Hz, 2, CH_2CO), 1.28–0.75 (s and t, 49, remaining H's). Addition of deuterated water caused the δ 9.5–9.1 peak to disappear in favor of an HOD peak at δ 4.6.

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Registry No. **3**, 71041-98-0; **4**, 71041-99-1; **5**, 63318-26-3; **6**, 71042-00-7; **7**, 71042-01-8; **7** bis(tetrahydropyran) derivative, 71042-28-9; **8**, 71042-02-9; **8** tosylate, 71042-03-0; **9**, 71042-04-1; **10**, 5921-73-3; **11**, 10160-28-8; **12**, 10160-26-6; **13**, 71042-05-2; **14**, 71042-06-3; **15**, 71042-07-4; **16**, 71042-08-5; **17**, 929-33-9; **18**, 71042-09-6; **19**, 71042-10-9; **20**, 71042-11-0; **21**, 71042-12-1; **22**, 59014-67-4; **23**, 71042-13-2; **24**, 71042-14-3; **25**, 71042-15-4; **26**, 71042-16-5; **27**, 71042-17-6; **28**, 71042-18-7; **29**, 71042-19-8; **30**, 71042-20-1; **31**, 629-72-1; **32**, 5802-82-4; **33**, 506-46-7; sodium acetylide, 1066-26-8; 1-chloro-6-iodohexane, 34683-73-3; 8-chloro-1-octyne, 24088-97-9; 6-chlorohexyl *p*-toluenesulfonate, 71042-21-2; 6-chlorohexyl alcohol, 2009-83-8; 8-hexadecynedinitrile, 71042-22-3; *cis*-8-hexadecenedioic acid, 71042-23-4; 1,16-dihydroxy-*cis*-8-hexadecene, 71042-24-5; methylene diiodide, 75-11-6; *cis*-8,9-methylenehexadecanedioic acid monomethyl ester, 71042-25-6; 1-hydroxy-8-nonyne, 10160-28-8; 1,7-dichloroheptane, 821-76-1; 1,25-bis(tetrahydropyranyloxy)-8,17-pentacosadiyne, 71042-26-7; undecyl iodide, 4282-44-4; *cis*-8,9-methyleneheptacosyl 4-chloropentanoate, 71042-27-8; 1-(tetrahydropyranyloxy)-16-chlorohexadecane, 71042-29-0; 9,10-methyleneoctacosane, 71042-30-3.

Oxidation of N^6,N^6-Dialkyl-2′,3′,5′-tri-*O*-acyladenosines with Ruthenium Tetroxide and a Novel Selective N-Monodealkylation Sequence

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Oxidation of 2′,3′,5′-tri-*O*-acetyl (benzoyl) derivatives of N^6,N^6-dimethyladenosine (**1d** and **1e**), N^6,N^6-diethyladenosine (**1f** and **1g**), N^6,N^6-dibenzyladenosine (**1h**), 6-(*N*-pyrrolidino)nebularine (**1i**), and 6-(*N*-piperidino)nebularine (**1j**) with RuO_4 in CCl_4 gave the corresponding monoamido derivatives **2a–g**. The reaction is selective—no formation of diamido (imido) compounds was observed—and in conjunction with deacylation effected by alkaline hydrolysis in the case of **2a–e** it represents a monodealkylation sequence of N^6,N^6-dialkyladenosines. A formal similarity of this transformation to the N-monodemethylation step in the metabolism of antibiotic puromycin is discussed. Selectivity of oxidation of the title compounds with RuO_4 can be explained in terms of electronic (inductive and/or resonance) effects of N^6-alkyl groups, the pyrimidine portion of the purine ring, and the carbonyl function in the reaction products.

Nucleosides derived from N^6,N^6-dimethyladenosine play an important role in biological processes. Thus, the antibiotic puromycin (**1a**, Scheme I) is a powerful inhibitor of protein synthesis, exhibiting distinct antibacterial and antitumor activity.[1] A related nucleoside—N^6,N^6-dimethyladenosine (**1b**)—occurs as a part of 16S and 18S ribosomal RNA[2] which is believed to be responsible for the binding of antibiotic kasugamycin.[3] Little is known of the metabolism of the N^6,N^6-dimethyl derivatives **1a** and **1b**. Puromycin (**1a**) is converted in vivo to the highly nephrotoxic aminonucleoside **1c** which is then selectively monodemethylated and phosphorylated to the corresponding 5′-phosphate[4] (Scheme II). The mechanism of demethylation has not yet been elucidated, although it is possible that as in cases of certain xenobiotics[5] the process involves an oxidation of an N^6-methyl group catalyzed by microsomal oxydase followed by cleavage (hydrolysis) of the resultant carbinolamine intermediate **4** to N^6-methylnucleoside **5**. No chemical (nonenzymic) model of this selective N-monodealkylation has been reported to date.

We wish to report on the results of oxidation of a series of N^6,N^6-dialkyl-2′,3′,5′-tri-*O*-acyladenosines, **1d–j**, with ruthenium tetroxide in a nonpolar solvent (CCl_4) to the corresponding monoamido derivatives **2a–g**. In the case of N^6-formyl, -acetyl, or -benzoyl derivatives **2a–e** a simple deacylation with ammonia in methanol afforded the N^6-monoalkylated nucleosides, thus accomplishing a selective removal of a single alkyl group from the starting N^6,N^6-dialkylnucleoside.

Results and Discussion

The results of oxidation of the title series of nucleosides **1d–j** are summarized in Table I. N^6,N^6-Dimethyl-2′,3′,5′-tri-*O*-acetyladenosine (**1d**) was readily oxidized with RuO_4 to give the N^6-formyl-N^6-methyl derivative **2a** in 72% yield along with a small amount (4%) of N^6-methyl-2′,3′,5′-tri-*O*-acetyladenosine (**1m**). Confirmation of the structure of **2a** came from the NMR spectrum which inter alia showed only one three-proton singlet for NCH_3 (δ 3.59) and another low-field one-proton singlet (δ 10.42) for the *N*-formyl group. The electron-impact mass spectrum (MS) indicated, in addition to the corresponding molecular peak (M^+, m/e 435), an ion of m/e 407 derived by decarbonylation of M^+. Compound **2a** on further oxidation with RuO_4 did not afford compound **1m**, which indicated that the formation of the latter did not result from an oxidative removal of the *N*-formyl group. Rather, it was formed in some preceding step, possibly decom-

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