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To Chi and Kevin

PREFACE

This thesis consists of three self-contained, independent sections dealing with synthetic chemistry in the areas of trehalose-based glycolipids, cyclodextrin, and 3-deoxy-D-manno-2-octulosonic acid (KDO). It was in each of these diverse areas that procedures for chain elongation at the nonreducing terminal of sugar chains were to be studied, which constitutes a unifying concept. In each section a separate numbering system for compounds is used, and the same applies to reaction schemes and literature references.

ABSTRACT

SECTION I

The new disaccharide, crystalline (6-deoxy-α-D-gluco-heptopyranosyluronic acid) 6-deoxy-α-D-gluco-heptopyranosiduronic acid **5** was synthesized from α,α-trehalose **1**. Reaction of 2,3,4,2',3',4'-hexa-O-acetyl-6,6'-di-O-tosyl-α,α-trehalose **7** with sodium dicarbonylcyclopentadienyliron, followed by oxidative carbonyl insertion and hydrolysis with bromine and water, gave the 2,3,4,2',3',4'-hexa-acetate **16** of **5**, which was then O-deacetylated (Zemplén). The protected bis-heptosiduronic acid, (2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid **26**, was similarly synthesized by the ironcarbonyl method of chain elongation, starting from 2,3,4,2',3',4'-hexa-O-benzyl-6,6'-di-O-tosyl-α,α-trehalose **24**. In this case, oxidative carbonyl insertion was effected with iodine in the presence of methanol, and the dimethyl ester **25** obtained was subsequently saponified to **26**. The dimethyl ester **25** was also prepared by acid-catalyzed methanolysis of the corresponding diamide **29**, which can be obtained from the ditosylate **24** by cyanide displacement and alkaline hydrolysis of the resulting dinitrile **28**. Mitsunobu esterification of the diacid **26** with (racemic) diastereomeric 3-O-benzylcorynomycolyl alcohols, obtained by reduction of synthetic, 3-O-benzylated methyl C_{32}-corynomycolates with lithium aluminum hydride, furnished the corresponding diesters in high yields. Hydrogenolytic debenzylation of the

products led to "mirror" coryno-cord factors **47a** and **47b**.

SECTION II

Approaches to chain extension at the C-6 positions in cyclomaltoheptaose (**2**) were examined with the aim of producing novel beta-cyclodextrin analogs composed of heptose or hepturonic acid units. Ironcarbonyl-mediated methoxycarbonylation, and nucleophilic displacement by cyanide, in the fully acetylated heptakis(6-deoxy-6-iodo) and heptakis(6-O-mesyl) derivatives of **2** respectively were unsuccessful, as were similar reactions attempted with the newly synthesized, analogous allyl-protected derivatives of **2**. However, reaction of unprotected heptakis(6-deoxy-6-iodo)cyclomaltoheptaose with lithium cyanide in N,N-dimethylformamide afforded a high yield of the corresponding heptakis(6-cyano-6-deoxy) compound, i.e., cyclohepta-(1→4)-(6-deoxy-α-D-gluco-heptopyranosid)urononitrile **26**, catalytic hydrogenation of which gave the title compound, cyclo-(1→4)-(7-amino-6,7-dideoxy-α-D-gluco-heptopyrano)heptaose **6**, isolated as its peracetyl derivative **29**.

SECTION III

An approach to the synthesis of 3-deoxy-D-manno-2-octulosonic acid (KDO) **1** by the ironcarbonyl method of chain-elongation starting from methyl α-D-mannopyranoside **2** was attempted. Reaction of methyl 2,3,4-tri-O-acetyl-6-O-p-tolylsulfonyl-α-D-mannopyranoside **3** with sodium dicarbonylcyclopentadienyliron,

followed by oxidative carbonyl insertion and hydrolysis with bromine and water, gave the methyl 2,3,4-tri-O-acetyl-6-deoxy-α-D-manno-heptopyranosiduronic acid **5**. O-Deacetylation then afforded methyl 6-deoxy-α-D-manno-heptopyranosiduronic acid **6** in high yield. Compound **6** can also be obtained from methyl (methyl 2,3,4-tri-O-benzoyl-6-deoxy-α-D-manno-heptopyranosid)uronate **8** by O-debenzoylation and alkaline hydrolysis of the resulting methyl ester **9**. Acid hydrolysis of **6** gave the free hepturonic acid **10** and an unknown product. Treatment of this mixture with sodium borohydride, followed by acetylation, provided the corresponding penta-O-acetyl-2-deoxy-D-manno-heptonic acid **11**.

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LIST OF ABBREVIATIONS

Ac	acetyl
AcOH	acetic acid
ADEPT	auto DEPT
All	allyl
aq	aqueous
Ar	aryl
Bn	benzyl
Bz	benzoyl
br	broad
°C	degrees Celsius
c.i.	chemical ionization
d	doublet
DCC	N,N-dicyclohexylcarbodiimide
DEPT	distortionless enhanced polarization transfer
DIAD	diisopropyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulfoxide
equiv	equivalent
Et	ethyl

Et_2O	diethyl ether
EtOAc	ethyl acetate
f.a.b.	fast atom bombardment
NaFp	sodium dicarbonyl-η^5-cyclopentadienyliron
g	gram
h	hour(s)
Hz	Hertz
IR	infrared
L	liter
M	molar
m	multiplet
M^+	parent molecular ion
Me	methyl
min	minute(s)
mL	milliliter
mol	mole
m.p.	melting point
MS	mass spectrum
Ms	methylsulfonyl (mesyl)
m/z	mass to charge ratio
n	narrow
n.m.r.	nuclear magnetic resonance

OAc	acetate
OMe	methoxy
Ph	phenyl
ppm	parts per million
py	pyridine
q	quartet
R_F	retention factor
s	singlet
sept	septet
t	triplet
TBDMSCl	tert-butyldimethylsilyl chloride
Tf	trifluoromethylsulfonyl
t.l.c.	thin layer chromatography
Ts	p-tolylsulfonyl (tosyl)
TsOH	p-toluenesulfonic acid

SECTION I

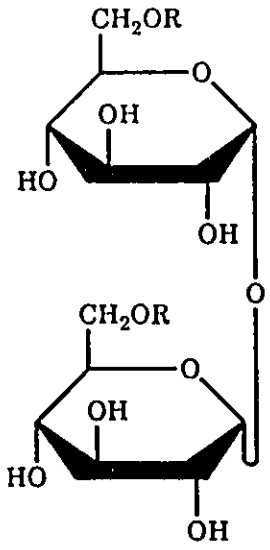
SYNTHESIS OF MIRROR CORD FACTORS

INTRODUCTION

The objective of this project was to provide, by chemical synthesis, certain glycolipids which are expected to become useful tools for the study of biochemistry and immunochemistry of mycobacteria. Tuberculosis is a chronic infectious disease in humans caused by tubercle bacilli, Mycobacterium tuberculosis. Similar diseases caused by related mycobacteria occur in animals, especially cattle and birds. For much of the world population and for most of recorded history, tuberculosis has been the most frequent cause of long-lasting sickness and the principal cause of death. As many as 10 million new cases occur in the world each year[1]. Although the situation is most worrisome in underdeveloped regions, even in Canada some 2000 new cases are reported annually, and it appears that worldwide the problem has been getting worse rather than better in recent years. Another threatening aspect of mycobacterial disease is that AIDS patients are prone to infection by Mycobacterium avium which does not normally pose a risk to a healthy individual[2].

A precise knowledge of the structures and biological functions of the glycolipids present in the bacteria and most importantly, the convenient procurement of such compounds in a state of high purity and in large quantities by chemical synthesis should be useful in developing sensitive and practical serodiagnostic methods for the early and reliable diagnosis of tuberculosis

infection. Cord factors 2 are 6,6'-diesters of α,α-trehalose 1 (Scheme 1) with long-



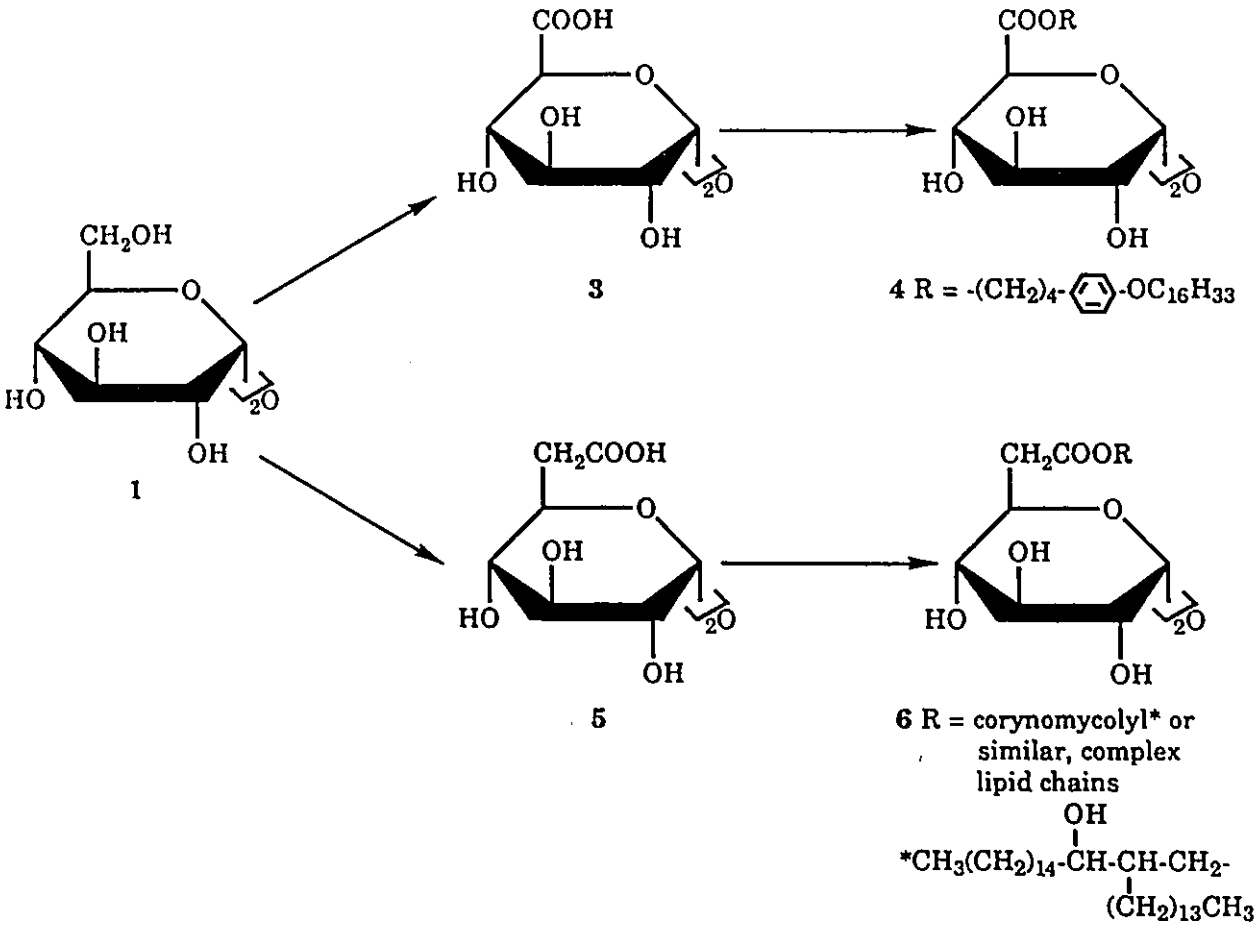
1 R = H

2 R = -CO-CH(C$_{22}$H$_{45}$)-CH(OH)-C$_{59}$H$_{117}$ and some related structures

Scheme 1

chain, 2-alkyl, 3-hydroxy fatty acids generically called mycolic acids. They are widely distributed in bacteria of the order Actinomycetales where they play important metabolic roles. Different bacterial genera are distinguished by different ranges of numbers of carbon atoms in the fatty acids. Thus, Mycobacteria produce, with some exceptions, cord factors containing C_{74}-C_{90} acids (mycolic acids proper), whereas Nocardiae and corynebacteria produce analogous esters of C_{32}-C_{56} (nocardomycolic) and C_{20}-C_{36} (corynomycolic) acids, respectively[3-5]. Among the biological activities attributed to cord factor are: Inhibition of leucocyte migration[6]; toxic effects due to depression of NAD-dependent microsomal enzymes in various tissues[7], apparently as a result of interaction of the cord factor with mitochondrial membranes[8]; effects on pyruvate metabolism and depression of muscle and liver

glycogen synthesis[9]; granuloma formation[10]; immuno-stimulant properties and anti-tumor activity[11,12] through induction of the production of interleukin-I and tumor necrosis factor in macrophages. In view of this impressive array of biological activities it is obvious that there exists great interest in studies aimed at unravelling structure-activity relationship in cord factor. In order to pinpoint functionally significant molecular features for the various and divergent activities, it is necessary to have in hand a collection of well-defined structural analogs. Syntheses of cord factor analogs have been actively pursued in several laboratories[13-15], with structural modification being introduced both with respect to the carbohydrate and to the lipid part of the molecule. One special objective in that direction was the synthesis, by Goren and Jiang[14b], of cord factor-like molecules which they termed "mirror" pseudo cord factors. An example is compound **4**, a diester of trehalose dicarboxylic acid **3** (trehalosuronic acid) with a long-chain lipid alcohol.



Scheme 2

The essential constitutional difference between **2** and **4** is a regioinverted ester functionality, apart from the nature of the lipid chains. Whereas only one example of a "mirror" ester of type **4** appears to have been described (with R as indicated), several analogous "mirror" amide pseudo cord factors were prepared[14b,16], having CONHR or $CONR_2$ groups in place of the ester functions, Among these were particularly interesting analogs wherein R represented spacer-borne corynomycolamido and mycolamido groups, and although details of

preparation and physical constants for these compounds were not published, they were reported[16] to show significantly lower toxicity in mice than true cord factors.

The purpose of the present study was to embark upon the synthesis of novel types of "mirror" cord factors, namely diesters **6** derived from the hitherto unknown dicarboxylic acid **5**, a bis-(6-deoxy-α-D-gluco-heptopyranosiduronic acid). This acid is a 2 x 7- carbon homolog of Goren's trehalosuronic acid **3**. Unlike Goren's "mirror" esters **4** derived therefrom, the natural cord factors of type **2** and our targeted "mirror" compounds of type **6** have their ester functionalities (acyloxy and alkoxycarbonyl groups, respectively) attached to the same position (C-6,6') of the trehalose carbon skeleton; synthetic **6** would be, in that structural regard, a closer analog to natural **2** than is synthetic **4**.

Lipid alcohols with which **5** was to be esterified would be mycolyl or corynomycolyl alcohols. These must first be prepared by reduction of the corresponding acids, which are not available commercially. Mycolic acids can only be obtained by extraction from mycobacteria and are in extremely short supply. Corynomycolic acids, on the other hand, are readily accessible by synthesis, in view of which we planned to use them in the present project.

Availability of these "mirror" pseudo cord factor analogs will make possible a comparison of the ultimate influence of the reversal of functionality at the ester

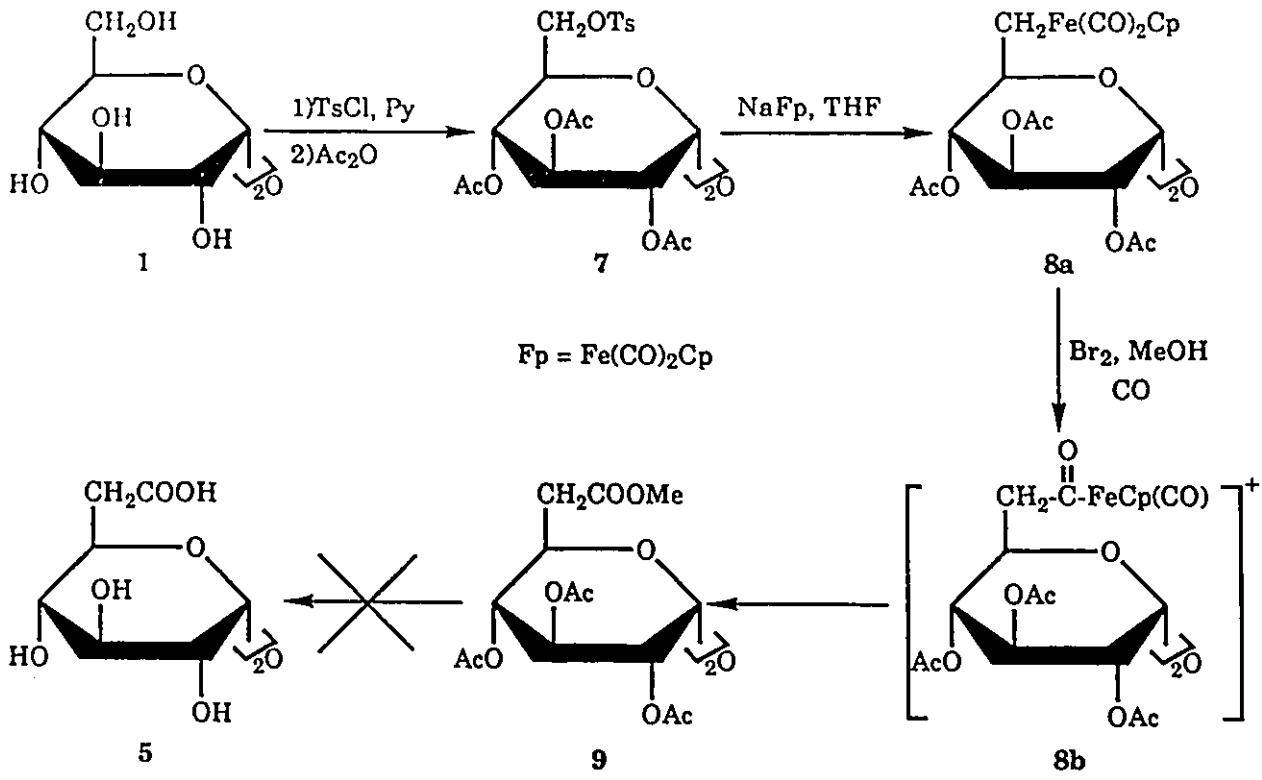
linkage upon the unusual biological activities exhibited by the natural cord factor. This is based upon the simplistic assumption that some of the biological activities may depend principally upon the amphipatic (and probably surface-active) properties possessed by a polar core moiety substituted by sufficiently large lipophilic functions.

RESULTS AND DISCUSSION

A. Synthesis of (6-deoxy-α-D-gluco-heptopyranosyluronic acid) 6-deoxy-α-D-gluco-heptopyranosiduronic acid (5) and its precursors

A prerequisite for the preparation of cord factor analogs of type **6** was the synthesis of the bis-heptosiduronic acid **5** and certain simple derivatives thereof. This task was initiated by R. L. Breton[17,18] in this laboratory, and the studies were expanded in the course of the present research. In the paragraphs that follow, Breton's results will first be delineated.

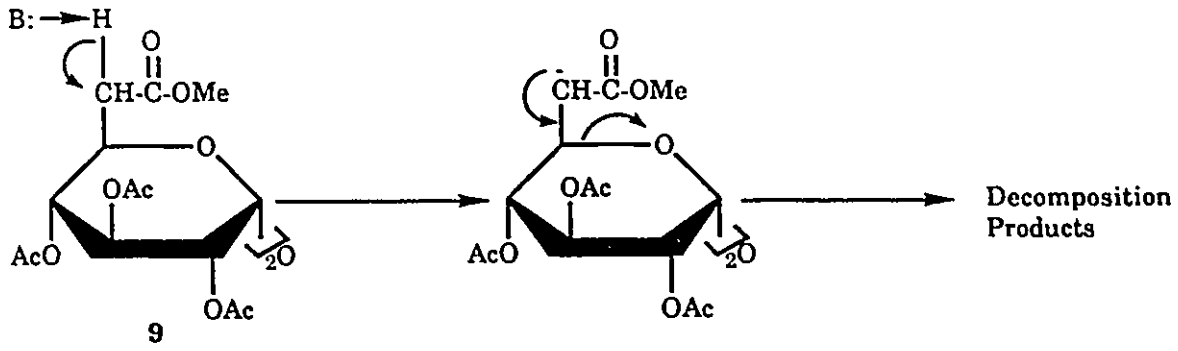
The first approach made use of the ironcarbonyl method of sugar chain elongation, elaborated earlier by Baer and Hanna[19] in monosaccharide chemistry. Thus, hexa-O-acetyl-α,α-trehalose 6,6'-ditosylate **7**, prepared from trehalose **1** by selective tosylation followed by acetylation, was treated under strictly anhydrous conditions with the powerfully nucleophilic reagent, sodium dicarbonyl-η^5-cyclopentadienyliron [$Na^+ \cdot Fe(CO)_2Cp$, often abbreviated NaFp]. Displacement occurred to generate the sugar-iron compound **8a** which was treated in situ with bromine and methanol under a carbon monoxide atmosphere. This effected oxidative carbonyl insertion, producing the unstable ionic complex **8b** that underwent immediate methanolysis to form the dimethyl ester **9**, isolated crystalline in 42% yield (Scheme 3).



Scheme 3

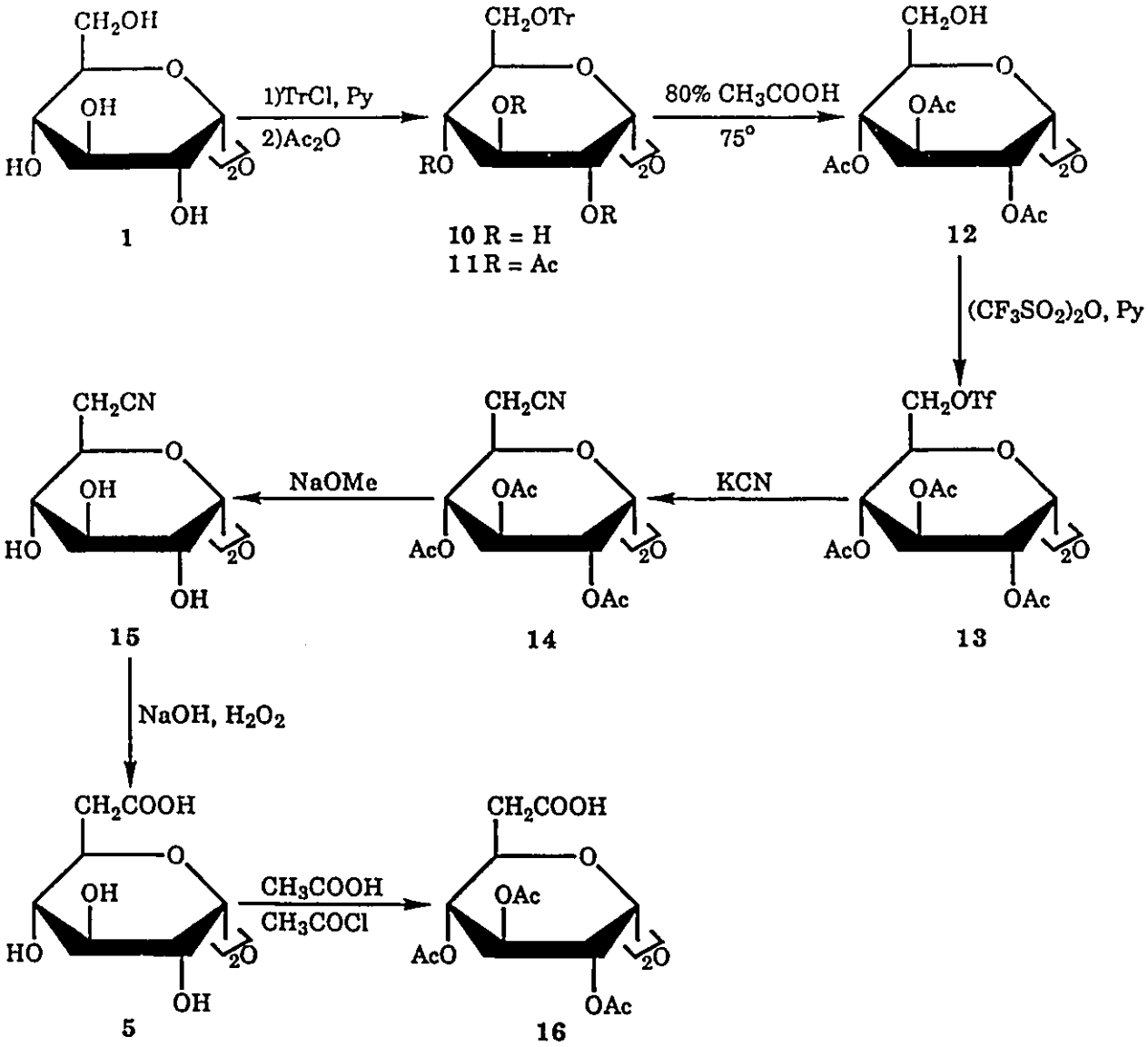
However, the seemingly trivial proposition of generating the free diacid **5** from the protected diester **9** proved problematical. Several procedures for deprotection under basic conditions were tried, including the standard Zemplén method of methoxide-catalyzed methanolysis which seldom entails any difficulties, and it was thought that **9** could be so deacetylated and thereby rendered water soluble; hydrolysis of the methyl esters should then be achievable by aqueous base. However, extensive degradation was observed and it had to be concluded that straightforward deacetylation of **9** was not feasible. A possible explanation for these difficulties may be that the hydrogen at C-6 in **9** is sufficiently activated

by the neighbouring ester function to incur abstraction by the base, with degradation of the disaccharide by β-elimination ensuing (Scheme 4):



Scheme 4

A second synthesis performed by Breton[18] is depicted in Scheme 5. 2,3,4,2',3',4'-Hexa-O-acetyl-α,α-trehalose **12**, prepared by established procedures from **1** via its 6,6'-dityityl ether **10** and the hexaacetate **11**, was trifluoromethane-sulfonated to give the 6,6'-ditriflate **13**. Displacement by potassium cyanide furnished the peracetylated bis-heptosidurono nitrile **14**, which on subsequent deacetylation to **15**, and alkaline hydrolysis of the nitrile groups in the presence of hydrogen peroxide provided the desired diacid **5**. Reacetylation of the alcoholic hydroxyls gave the hexaacetate **16** which was to be used in some of the studies planned in the laboratory.

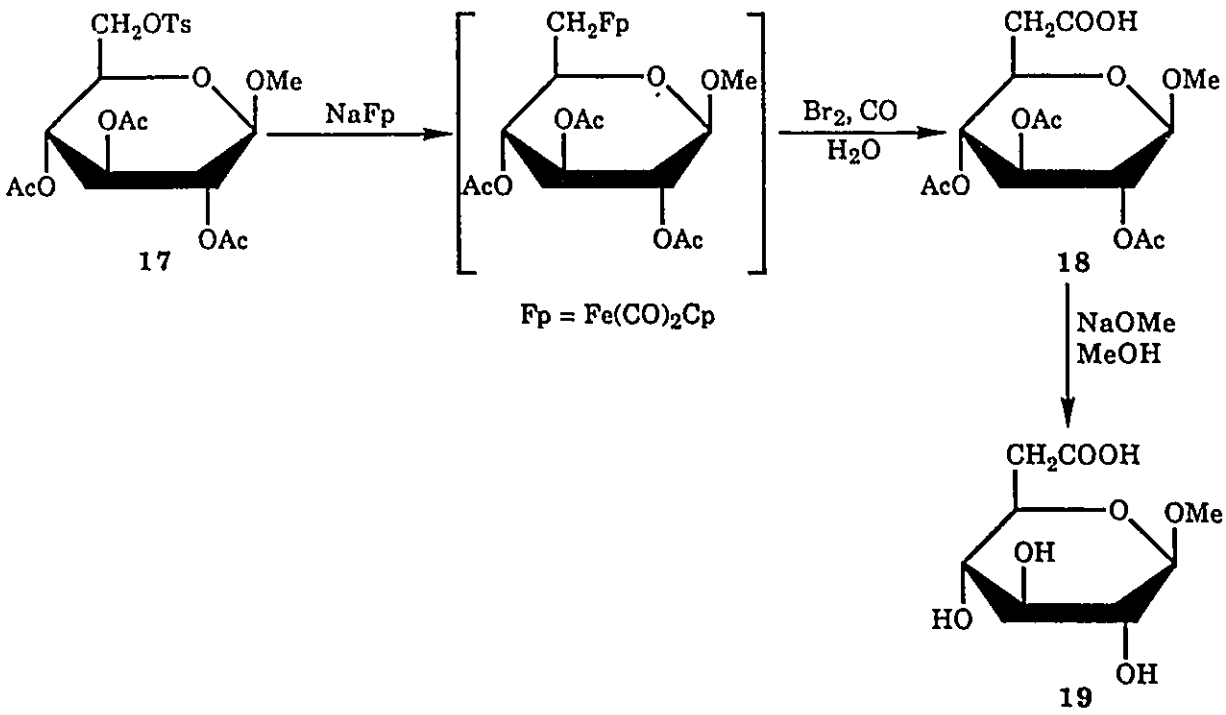


Scheme 5

Although the objective had thus been realized in principle, the results were not entirely satisfactory. The nitrile hydrolysis (**15**→**5**, Scheme 5) was difficult and proceeded rather inefficiently, and moreover, the classical approach of this second synthesis was lengthy (7 and 8 steps from **1** to **5** and **16**, respectively) compared to the more elegant ironcarbonyl approach (Scheme 3). As already mentioned, the

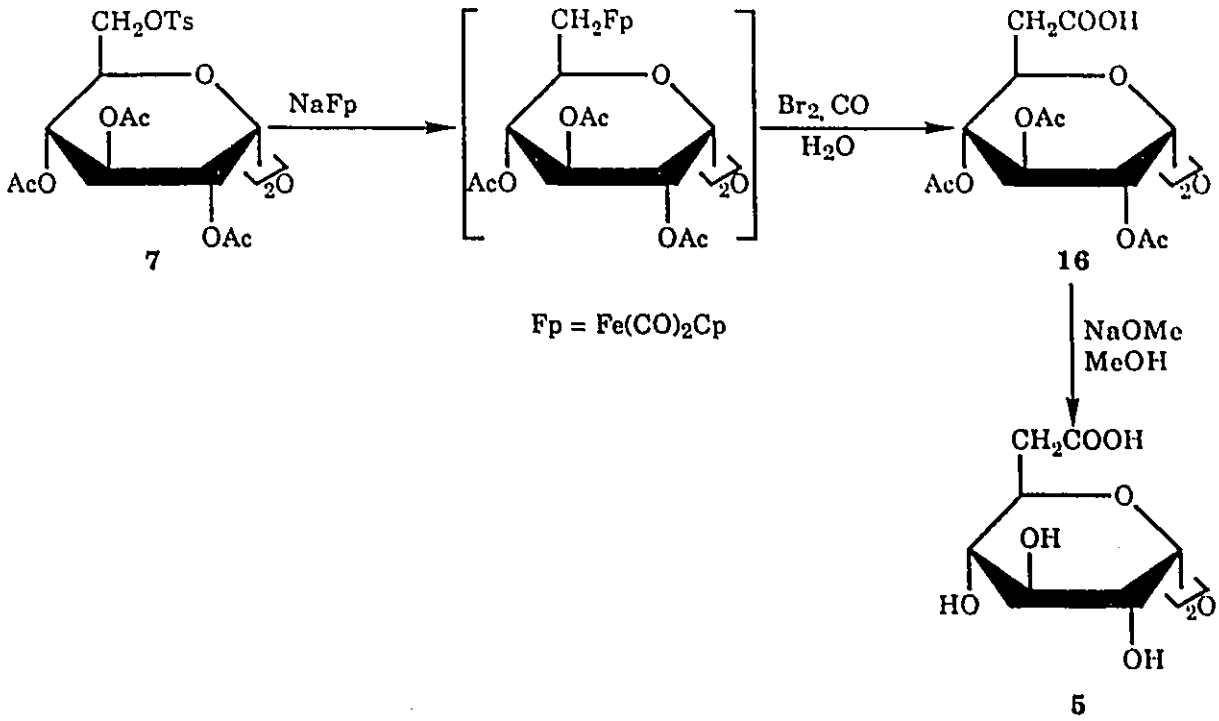
latter had been successful to the extent that chain elongation was accomplished, but had fallen short of its goal due to difficulties encountered in manipulating the product. The challenge remained to modify the procedure so as to deliver the required free acid **5** and (or) its hexaacetate **16**. This became part of the present thesis project and was achieved as follows.

The simple idea occurred to us that water could perhaps be substituted for methanol in the medium during the oxidative carbonyl insertion (compare **8a**→**8b** in Scheme 3), to cause hydrolysis rather than methanolysis of the ionic sugar-iron complex and so produce directly the free diacid **16** instead of its dimethyl esters **9**. We found no precedent in the review literature on this carbonylation method for such a variant and therefore decided to test the idea first with a monosaccharide model. Reaction of methyl 2,3,4-tri-O-acetyl-6-O-tosyl-β-D-glucopyranoside **17** with NaFp, followed by reaction with bromine in the presence of water, did indeed furnish methyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-gluco-heptopyranosiduronic acid **18**, although the yield of isolated, crystalline product (46%) did not match that (80%) achieved[19] in the synthesis of the corresponding methyl ester. However, methoxide-catalyzed deacetylation of **18** readily provided the crystalline heptopyranosiduronic acid **19** in 92% yield (Scheme 6). Both of these new compounds were fully characterized by physical data, elemental analysis, and n.m.r. data that were in accord with their structures (see Experimental).



Scheme 6

The same procedure was then applied (Scheme 7) to the peracetylated trehalose 6,6'-ditosylate **7**, and the desired diacid **16**, (2,3,4-tri-O-acetyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-acetyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid, was obtained after column chromatography as a yellow oil in high yield. The crude product crystallized after treatment with activated charcoal, but several recrystallizations were needed to obtain it in analytically pure form and yields of pure **16** were 20-30% in several experiments. Zemplén deacetylation of **16** gave crystalline (6-deoxy-α-D-gluco-heptopyranosyluronic acid) 6-deoxy-α-D-gluco-heptopyranosiduronic acid **5** in 84% yield.



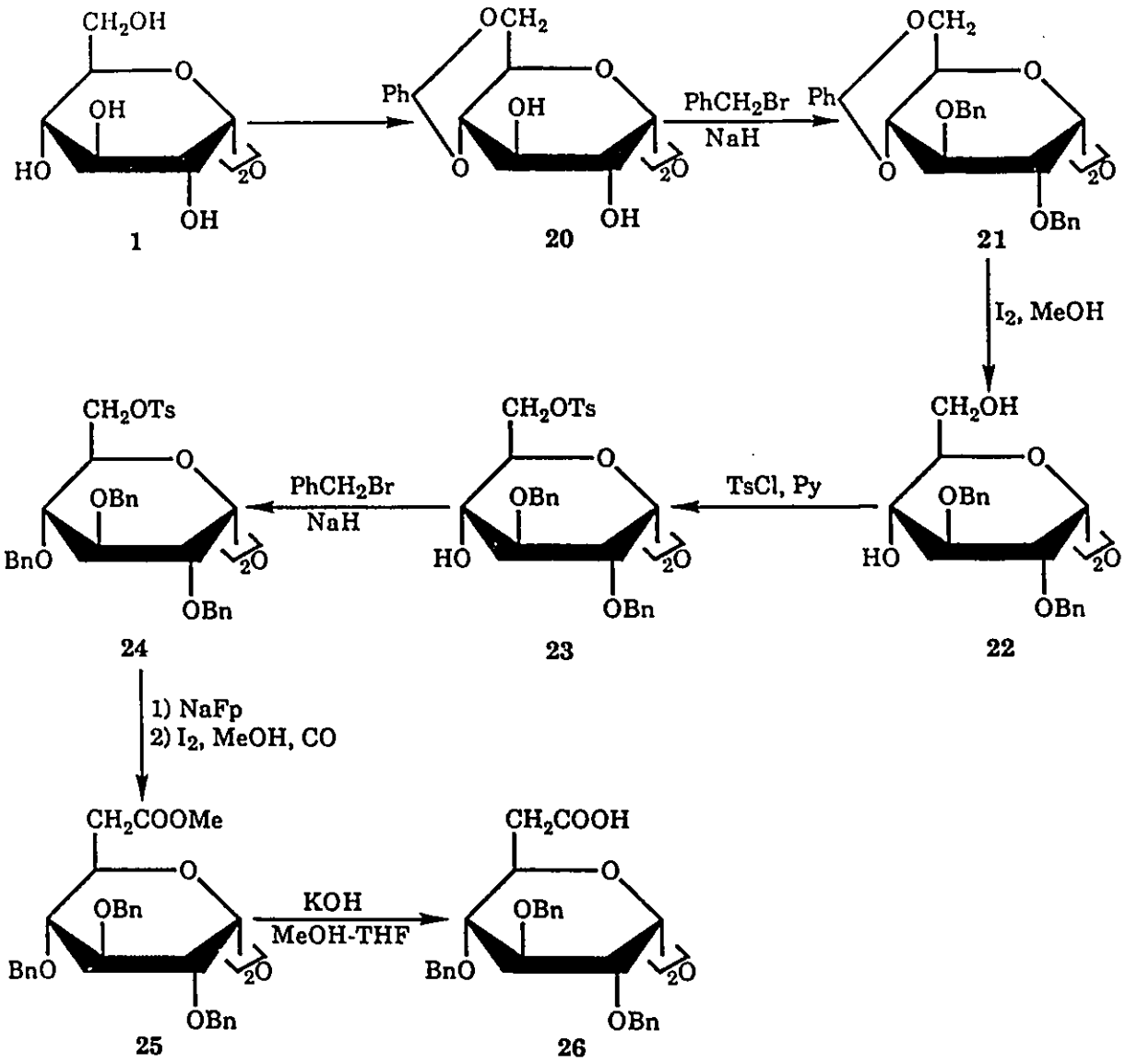
Scheme 7

Compounds **5** and **16** were characterized by elemental and spectral analysis. They both showed molecular ion peaks in their f.a.b. mass spectra, $[M+1]^+$ at m/z 399 and 651 respectively, as expected. In the ^{1}H-n.m.r. spectrum of **5**, the H-1 doublet appeared at 5.20 ppm with $J_{1,2}$ 3.85 Hz. The H-2 proton gave a doublet of doublets centred at 3.70 ppm having $J_{1,2}$ 3.9 Hz and $J_{2,3}$ 9.9 Hz. Doublets of doublets were also observed for both H-3 and H-4 at 3.88 ppm and 3.35 ppm, respectively. Coupling constants were found to be $J_{3,4}$ 9.0 Hz and $J_{4,5}$ 9.9 Hz. Proton H-5 was observed as a doublet of triplets centred at 4.16 ppm having $J_{5,6}$ 2.9 Hz and $J_{5,6'}$ 9.9 Hz. Two doublets of doublets were recorded at 2.97 and 2.52 ppm which belonged to H-6 and H-6', respectively. The latter showed $J_{6,6'}$ 15.8 Hz.

^{13}C-n.m.r. and infrared spectroscopy afforded extra evidence which also confirmed the structure of the compound **5**; in particular, a ^{13}C signal at δ 176.6 indicated carboxyl, and one at δ 37.4 represented the C-6 methylene groups. Similarly, the ^{1}H-n.m.r. spectrum of the acetylated derivative **16** displayed ring proton signals in accord with the α-pyranosidic structure: A doublet at δ 5.09 with $J_{1,2}$ 3.8 Hz for H-1, a doublet of doublets at δ 5.19 with $J_{1,2}$ 3.8 Hz and $J_{2,3}$ 10 Hz for H-2, and triplets or double doublets with J ~9.2-10 Hz for H-3 (δ 5.49) and H-4 (δ 4.86); the C-6 methylene group gave a 2-proton multiplet at δ 2.50, and three acetyl singlets were present at δ 2.03-1.99. The ^{13}C n.m.r. spectra of **16** exhibited signals for carboxyl CO (δ 176.1), ester CO (169.8-169.4), C-6 methylene (δ 36.2), and acetyl CH_3 (20.8-20.7). The spectra, optical rotations, and melting points of **5** and **16** agreed perfectly with the data from the aforementioned parallel work depicted in Scheme 5.

B. Synthesis of (2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid 26

For synthetic uses of the diacid **5**, it appeared desirable to possess derivatives whose secondary hydroxyl group are protected as benzyl ethers, as possible alternatives to the hexaacetate **16**. Thus, if major difficulties would arise during the deacetylation of lipid esters, benzyl groups would be smoothly removable by catalytic hydrogenolysis in the presence of ester functions. Therefore, the synthesis of the hexa-O-benzyl diacid **26** was undertaken by application of the ironcarbonyl method to 6,6'-di-O-tosyl-α,α-trehalose hexabenzyl ether **24** (Scheme 8). The known[14d] **24** was procured from trehalose essentially according to the published directions, with some procedural variations and improvements. Thus, the readily obtainable 4,6; 4',6'-di-O-benzylidene derivative[20] **20** of trehalose was benzylated with benzyl bromide and sodium hydride in dry N,N-dimethylformamide, and the product **21** deacetalated with iodine in methanol[21], to furnish 2,3,2',3'-tetra-O-benzyl-α,α-trehalose[20b,22] **22** in 83% yield. Selective tosylation of **22** with tosyl chloride (1.1 mol. equiv.) and pyridine yielded 85% of its crystalline 6,6'-ditosylate **23** (previously obtained[14c] as a syrup in 27% yield), which was benzylated to 2,3,4,2',3',4'-hexa-O-benzyl-6,6'-di-O-p-tolylsulfonyl-α,α-trehalose **24**. Treatment of **24** with sodium dicarbonylcyclopentadienyliron in oxolane effected displacement of tosyloxy by $Fe(CO)_2Cp$, and reaction of the resulting sugar-iron intermediate with iodine and methanol at room temperature



Scheme 8

under CO caused oxidative carbonyl insertion followed by methanolysis, to afford the dimethyl ester **25** in 63% yield. The key to success in this step was the use of iodine as oxidant. In previous instances (see ref. 19, and the reactions described in the preceding chapter) bromine was preferred as it reacts at a higher rate[19];

however, it appeared incompatible with benzyl groups present in the molecule, as was found out in unsuccessful pilot experiments. Saponification of **25** with aqueous potassium hydroxide in methanol and oxolane at room temperature for 8 hours then gave the crystalline diacid **26** in an acceptable yield* of 72%. The ^{1}H-n.m.r. spectrum completely supported its structure. The benzyl groups were observed as multiplets at 7.3-7.1 ppm and 4.98-4.52 ppm. The anomeric proton appeared as a doublet at 5.35 ppm having $J_{1,2}$ = 3.0 Hz. The H-2 proton gave a doublet of doublets centred at 3.58 ppm having $J_{1,2}$ = 3.1 Hz and $J_{2,3}$ = 9.6 Hz. Triplets with splittings of ~9.3 Hz were observed for both H-3 and H-4 at 4.13 ppm and 3.27 ppm, respectively. Proton H-5 was observed as a doublet of triplets centred at 4.30 ppm, having $J_{5,6}$ = 2 Hz and $J_{5,6'}$ = 10.4 Hz. Two doublets of doublets were recorded at 2.75 and 2.30 ppm which belonged to H-6 and H-6', respectively. The ^{13}C-n.m.r. data (see Experimental) also confirmed the structure of **26**, with signals occurring at 178.2 ppm (carboxyl), 138.7, 138.3, and 138.3 (C-1 of Ph) and 37.1 ppm (C-6). Spectral analysis of the precursor **25** revealed the same patterns for the benzylic and sugar-ring protons and ^{13}C atoms; the ester methyl group gave a signal at δ 3.59 (^{1}H) and a signal at δ 51.8 (^{13}C).

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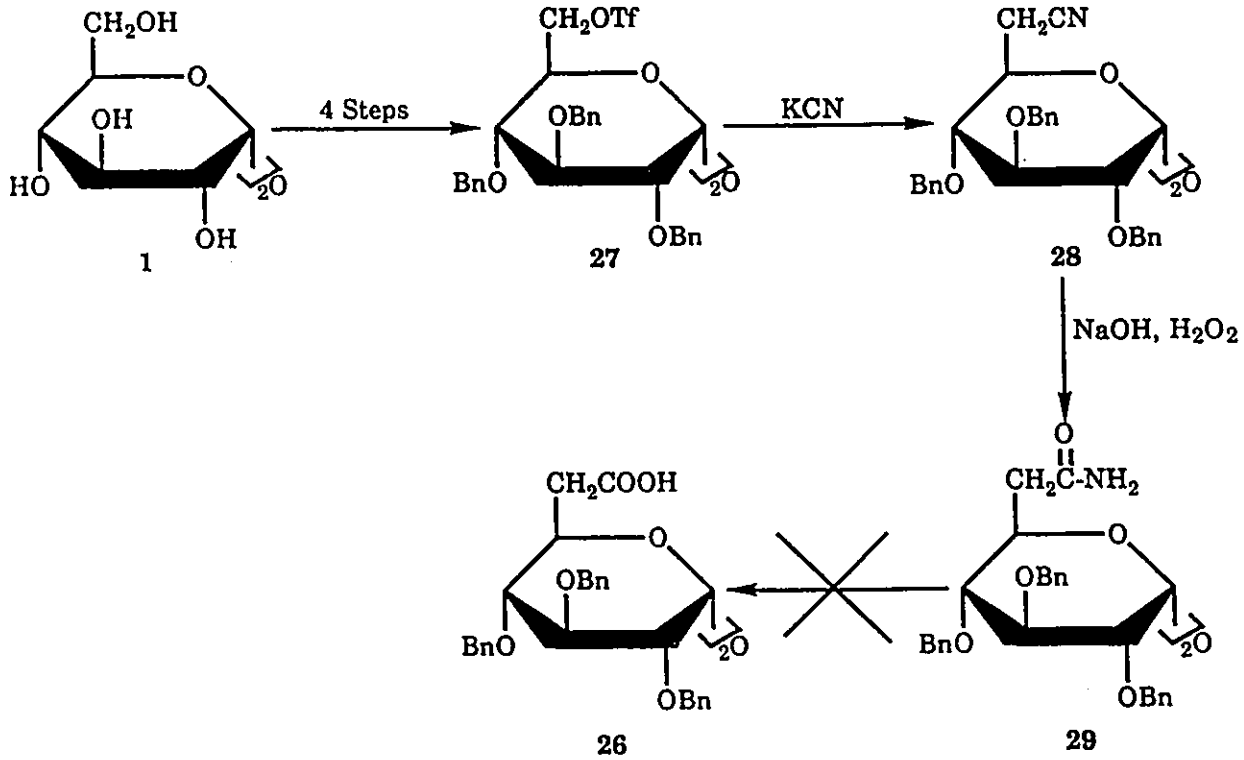
*A diminution in yield may have been due to some concomitant degradation of **25** through β-elimination of ring oxygen (compare Scheme 4). At any rate, in light of the previous experience that the analogous dimethyl ester **9** did not tolerate base treatment, the success of this alkaline hydrolysis appears noteworthy.

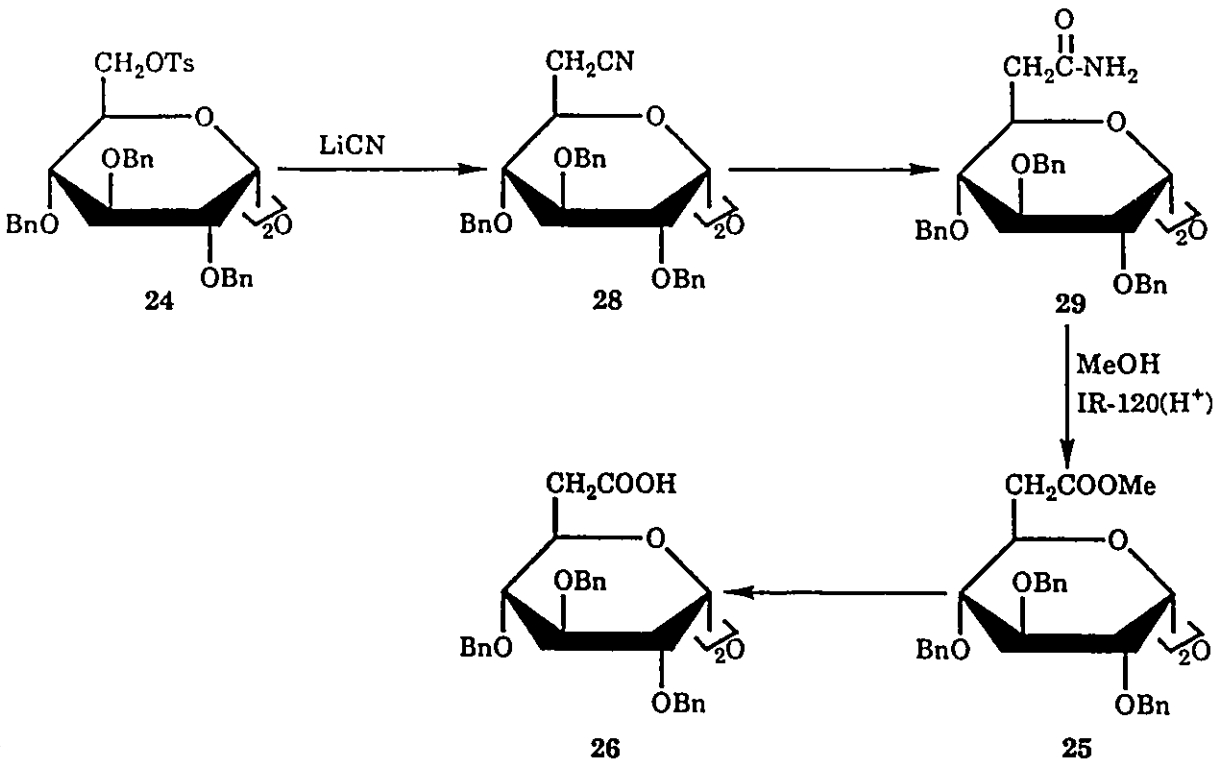
In concurrent studies performed in this laboratory, Breton[18] examined an alternative approach to the hexabenzyl diacid **26**, paralleling his synthesis of **5** involving cyanide displacement (see Scheme 5). He prepared 2,3,4,2',3',4'-hexa-O-benzyl-α,α-trehalose 6,6'-ditriflate **27** from trehalose **1** in four steps including tritylation (at O-6,6'), perbenzylation, detritylation, and finally trifluoromethanesulfonylation. The ditriflate **27** smoothly underwent displacement with potassium cyanide to give the dinitrile **28**. Hydrolysis of **28** with sodium hydroxide and hydrogen peroxide proceeded readily but, in remarkable contrast to the



Scheme 9

aforementioned hydrolysis of the analog **15**, it stopped at the amide stage and the diamide **29**, isolated crystalline in 83% yield, resisted all efforts at further alkaline hydrolysis to **26** (Scheme 9).

For the present thesis, further study seemed warranted to see if the problem of amide hydrolysis can be overcome and the benzylated diacid procurred. For these studies a fresh supply of dinitrile **28** had to be prepared, and it was examined whether the hexa-O-benzyl-α,α-trehalose 6,6'-ditosylate **24**, in hand from previous work (see Scheme 8), can be utilized for that purpose in place of the (more reactive) ditriflate **27**. It turned out that displacement with lithium cyanide in N,N-dimethylformamide during 20 hours at 25°C was in fact very effective, affording a 91% yield of **28** which was then converted into the diamide **29** as described before (Scheme 10).

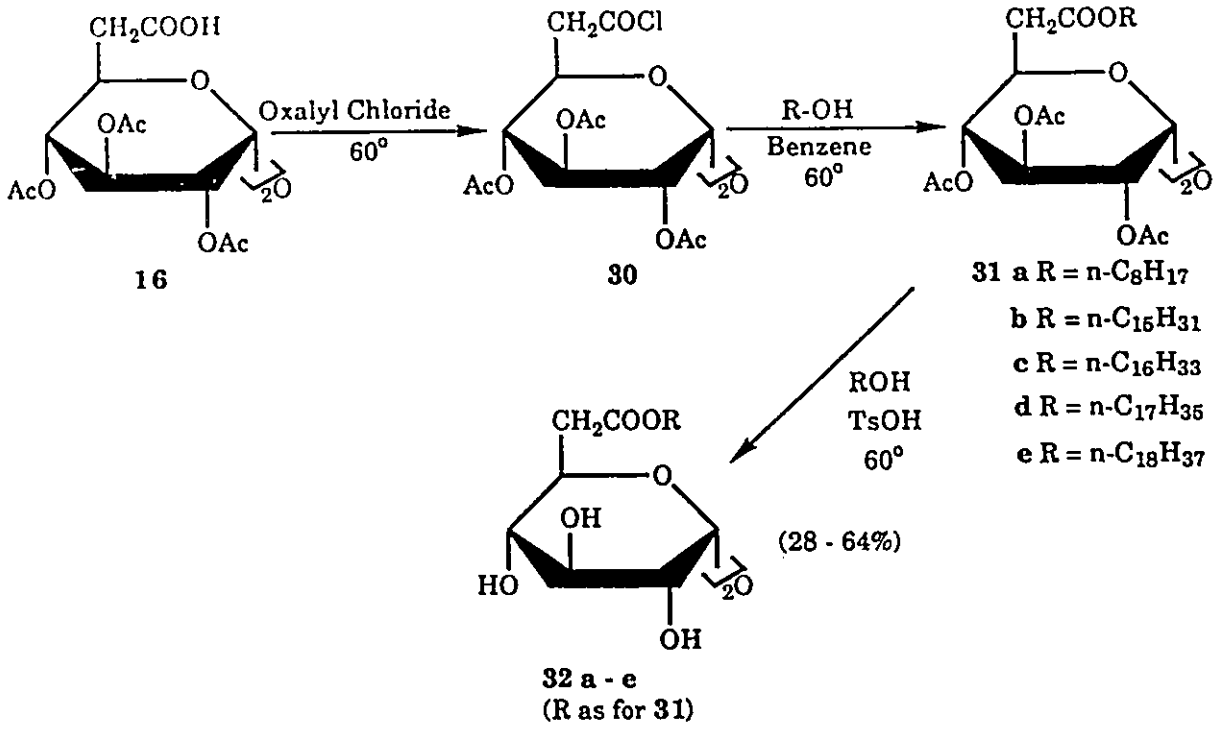


Scheme 10

Further attempts were then made to convert **29** into the diacid **26**. For example, **29** was transformed into its N,N-di-tert-butoxycarbonyl derivative which was subjected to hydrolysis or methanolysis as proposed by Grieco[23], but the reactions afforded only starting material. However, the problem was finally solved when we became aware of the excellent method of Greenlee and Thorseff[24] for conversion of amides into methyl esters, consisting simply of treatment with boiling methanol in the presence of a cation exchange resin. The diester **25** was thus obtained from **29** in 78% yield (Scheme 10). It is recalled that saponification of **25** to **26** had previously been accomplished (Scheme 8).

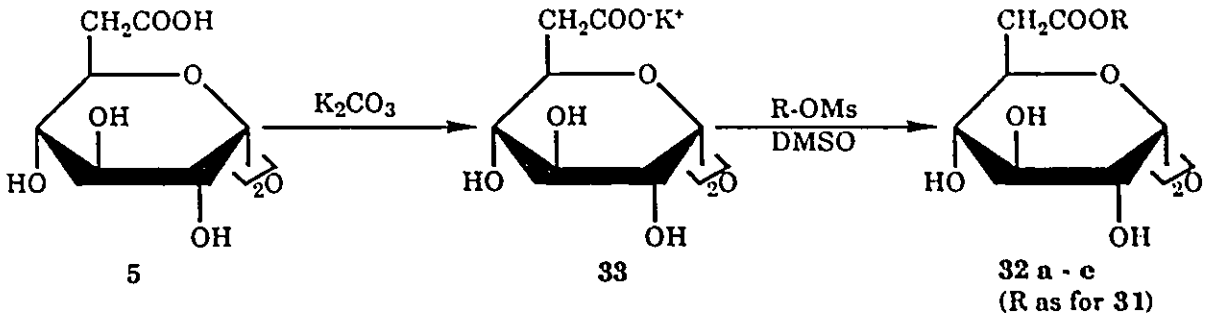
C. Synthesis of mirror coryno-cord factors 47a and 47b

Having compounds **5** and **16** in hand, we proceeded to examine their esterification with long-chain fatty alcohols, to generate novel "mirror" pseudo cord factors. Goren and Jiang[13c,14b] had prepared similar "mirror" pseudo cord factors derived from the trehalosuronic acid **3** which was activated by treatment with thionyl chloride and then coupled by nucleophilic attack with 1-alkanols. It was envisaged to apply the same experimental procedure to **16**. Breton in this laboratory carried out this approach[18,25]. In a model experiment, the acetylated acid **16** was converted by oxalyl chloride into its dichloride **30**, which was allowed to react with 1-octanol to form the di-octylester **31a** in 96% yield (Scheme 11). Similar esterification with C_{15} to C_{18} 1-alkanols by way of the same strategy produced the analogous diesters **31b-e**, all in high yields. Selective removal of the acetate groups in **31** in the presence of the lipid ester groups posed a problem. Deacetylation was achieved by transesterification using the same alcohol which constitutes the lipid chains of the molecule, at 60°, with benzene or 1,2-dichloroethane as solvent, in the presence of p-toluenesulfonic acid (Scheme 11). Yields of deacetylated products **32** were somewhat disappointing, they ranged from 28 to 64%. After the synthesis[25] of these simpler analogs, attempts were made by Breton[18] to esterify **30** with 3-O-(2-tetrahydropyranyl) mycolyl alcohol, but they failed to give the expected product.



Scheme 11

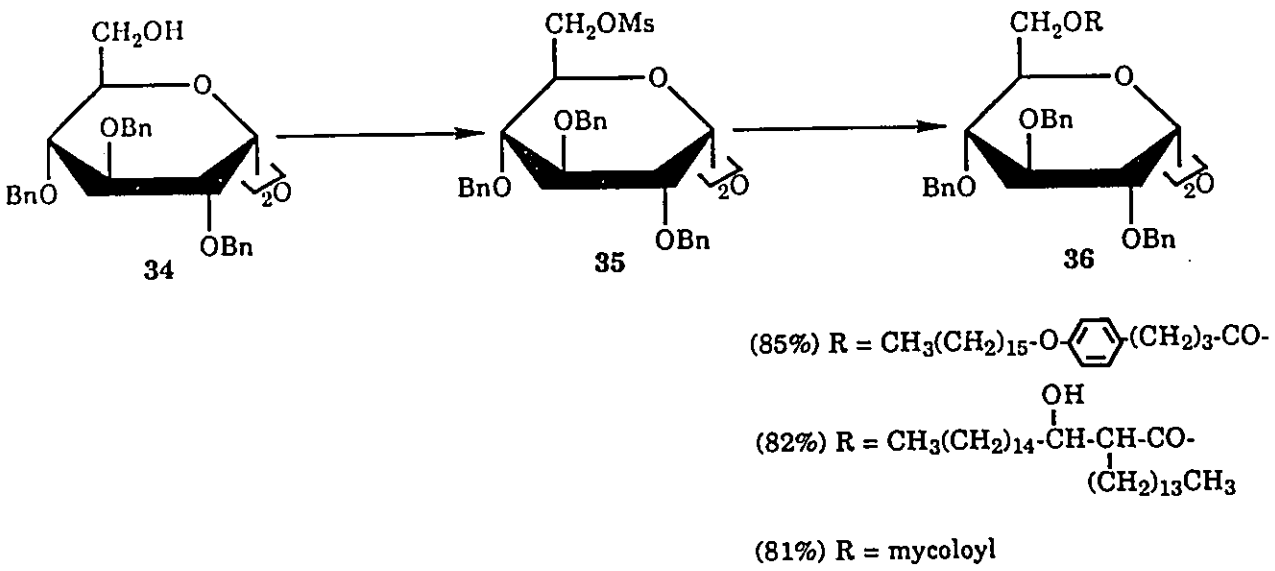
An alternative approach to compounds **32**, using the reaction of alkanol mesylates with the potassium salt of **5**, had also been elaborated[18,25]. The potassium salt **33** was generated by neutralizing **5** in water with potassium carbonate and lyophilizing the solution. The salt was then allowed to react with the alkyl mesylate in dimethyl sulfoxide solution at 75° for ~ 1.5 hours. The reaction mixture was processed by freeze-drying followed by chromatography, to yield 56-72% of the desired esters; no deprotection procedure was needed (Scheme 12). However, attempts to prepare the true analog of natural cord factor by this improved method again were unsuccessful[18].



5 33 32 a - c (R as for 31)

Scheme 12

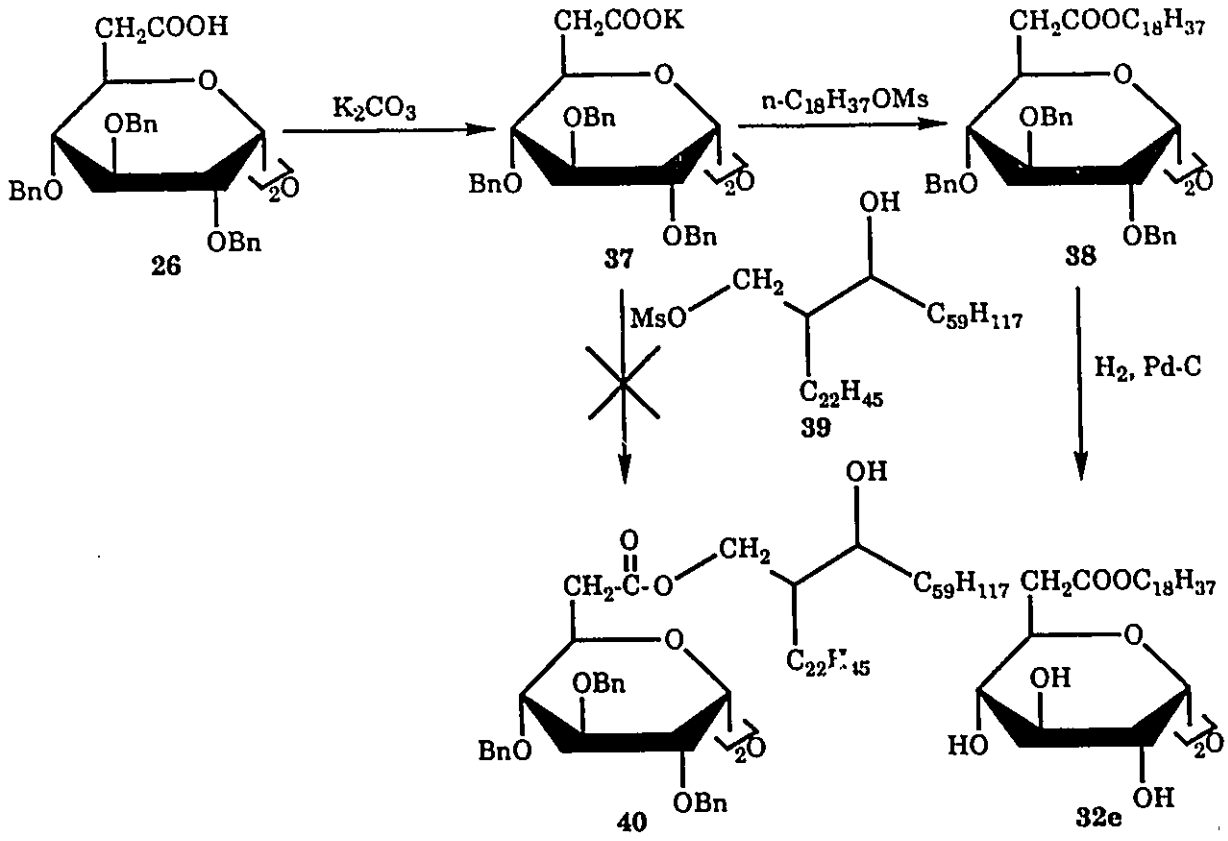
Goren and Liav advocated the use of benzyl-protected reactants and reported[13d,13h,14c] successful syntheses of cord factor and analogs by the strategy outlined in Scheme 13. Thus, 2,3,4,2',3',4'-hexa-O-benzyl-α,α-trehalose **34** was converted into the corresponding dimesylate **35** in quantitative yield by treatment with methanesulfonyl chloride in pyridine. When compound **35** was brought into reaction with the potassium salts of long-chain fatty acids in hexamethyl-phosphoric triamide (HMPA) at 95-100°, the 6,6'-diester derivatives **36** were obtained as the major products (Scheme 13). Catalytic hydrogenolysis then afforded the debenzylated compounds in good yields.



Scheme 13

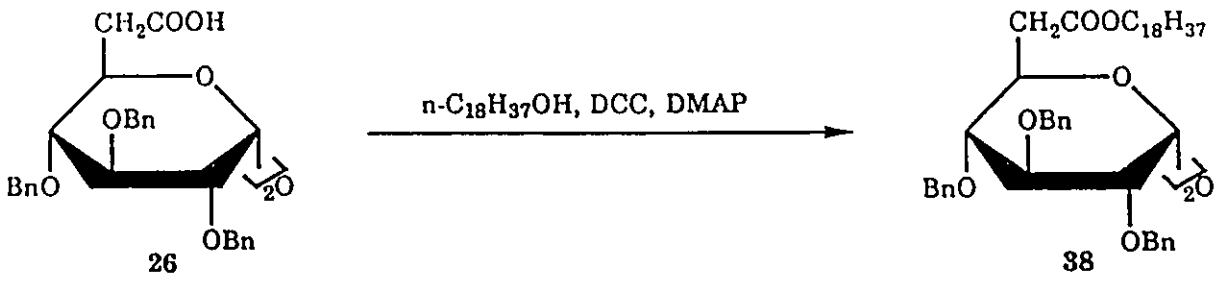
By analogy to this procedure, we first wished to establish, in a model experiment, the suitability of the new perbenzylated diacid **26** for condensation with fatty alcohols and elaborate appropriated condition for subsequent debenzylation. Reaction of the potassium salt **37** of the acid with octadecyl mesylate in dimethyl sulfoxide solution at 70° gave a 77% yield of the protected distearyl ester **38**, which by hydrogenolytic debenzylation over Pd-C gave **32e** (70%), identical with the product synthesized previously (Scheme 14). However, this mode of esterification when tried with mycolyl mesylate** **39** failed to give the "mirror" cord factor **40**, and we therefore turned to other methods.

The mesylate **39 was prepared in this laboratory[18] from mycolic acid (originating from natural cord factor "Peurois" and kindly donated by Dr. A. Liav), which was protected as 3-O-(2-tetrahydropyranyl) derivative[14c], reduced to the alcohol by $LiAlH_4$ or BH_3, conventionally mesylated, and deprotected.



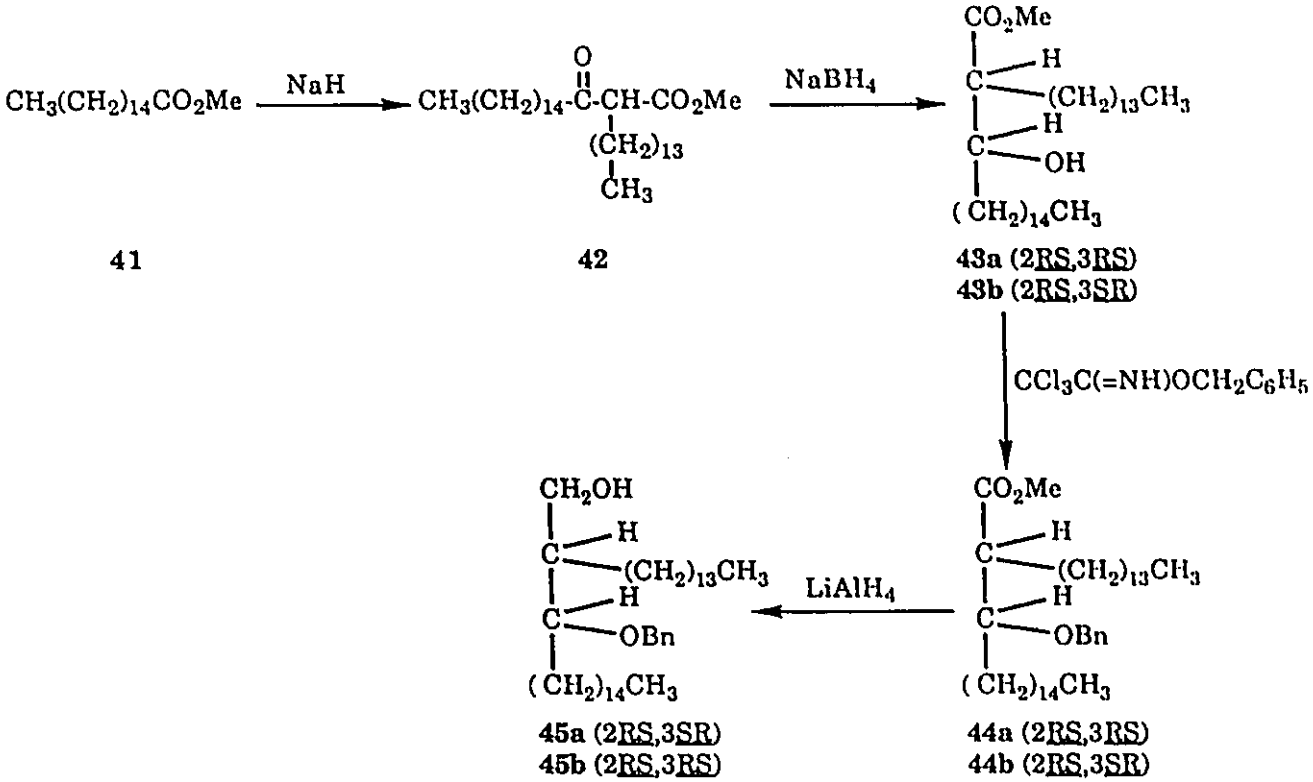
Scheme 14

N,N-Dicyclohexylcarbodiimide (DCC) has been used widely for the synthesis of peptides[26], depsides[27] and other types of products[28], and condensation products of alcohols and acids are usually obtained in good yields[29] . Having observed in t.l.c. that **38** is also formed on reaction of **26** with 1-octadecanol in the presence of DCC as the activating agent and 4-dimethylaminopyridine as catalyst (Scheme 15), it was decided to apply this method[29] to a 3-O-benzyl-corynomycolyl alcohol. To procure this, synthetic methyl C_{32}-corynomycolate[30] was prepared, following directions recently given by Datta et al.[131] Methyl palmitate **41** was subject to self-



Scheme 15

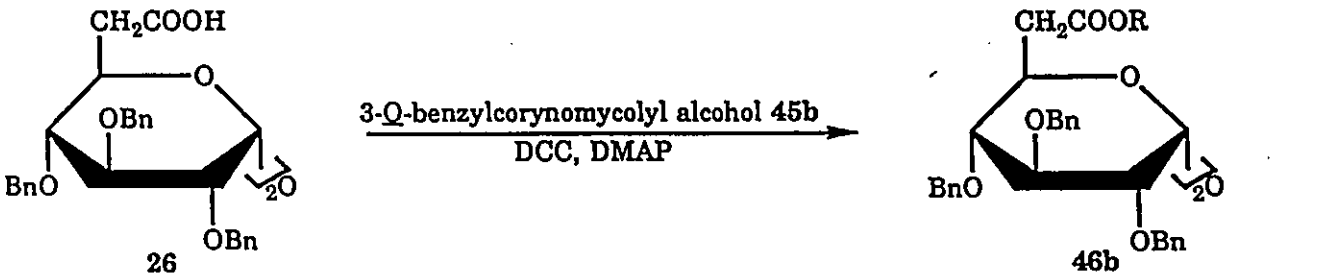
condensation (Claisen), and the resulting keto ester **42** was reduced with sodium borohydride to a ~2:3 mixture of diastereomeric hydroxyesters, separated by column chromatography as racemates **43a** (2RS,3RS configuration) and **43b** (2RS,3SR configuration) (Scheme 16).



Scheme 16[***]

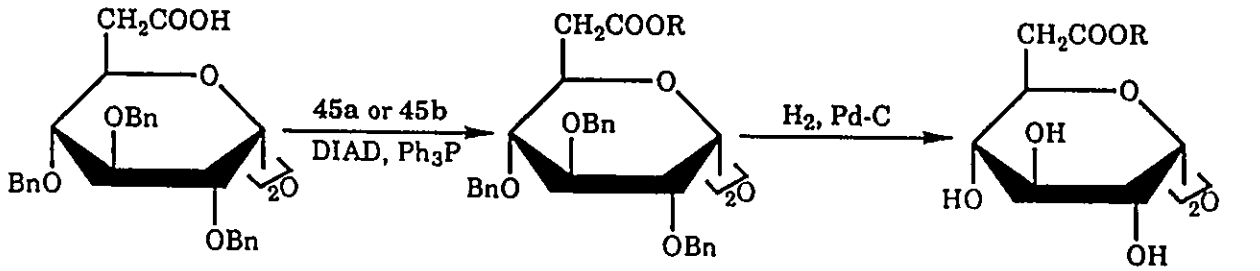
[***]Note that the reduction **44**→**45** entails a curious change in configurational designation. Due to the change in oxidation state of C-1, the priority sequence about C-2 becomes reversed, whereas that about C-3 remains unchanged. Hence one would logically designate **45a** as 2SR,3RS, and **45b** as 2SR,3SR. However, the IUPAC rule for racemates states: "If there is more than one chiral center, the first is labelled RS and the others are labelled RS or SR according to whether they are R or S when the first is R". Consequently, **45a** is labelled 2RS,3SR, and **45b** is 2RS,3RS.

For the protection of the 3-hydroxy function, necessary to prevent acylation during the coupling step, it seemed desirable to use a group that would be removed under the same conditions as the sugar-bound O-benzyl groups. Therefore, both **43a** and **43b** were benzylated by use of benzyl 2,2,2-trichloroacetimidate in the presence of trifluoromethanesulfonic acid[31], to give the protected esters **44a** and **44b**. These were reduced by lithium aluminum hydride to (2RS,3SR)-3-benzyloxy-2-tetradecyloctadecanol **45a** and (2RS,3RS)-3-benzyloxy-2-tetradecyloctadecanol **45b** (Scheme 16). The alcohol **45b** (obtained in higher yield) was then condensed with **26** by DCC in toluene during 5 hours at 70°, giving a 52% yield of the fully benzylated dicorynomycolyl ester **46b** after chromatographic separation from unreacted material and a by-product that appeared to be a monoester bearing on C-7' a DCC-related grouping. Extension of the reaction time afforded no improvement (Scheme 17).



Scheme 17

Better results were achieved by Mitsunobu esterification[32], a method previously employed to advantage in cord factor syntheses[14e]. Both alcohols **45a** and **45b** readily underwent condensation with the diacid **26**, mediated by diisopropyl azodicarboxylate (DIAD) and triphenylphosphine, to furnish high yields (~88%) of esters **46a** and **46b**. Hydrogenolytic debenzylation finally led to the target compounds (2RS,3SR)-3-hydroxy-2-tetradecyloctadecyl [((2RS,3SR)-3-hydroxy-2-tetradecyloctadecyl 6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-heptopyranosid] uronate **47a** and (2RS,3RS)-3-hydroxy-2-tetradecyloctadecyl [((2RS,3RS)-3-hydroxy-2-tetradecyloctadecyl 6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-heptopyranosid]uronate **47b** in yields of ~66% (Scheme 18). These synthetic "mirror" coryno-cord factors are, by necessity, diastereomeric mixtures as they were generated by combination of racemic alcohols with a chiral acid. This was reflected in certain features of the n.m.r.



Scheme 18

spectra of the precursors **46a** and **46b**. Thus, their pyranosidic H-1 signals appeared as narrow multiplets (instead of doublets), and the H-2, H-6, and H-6' signals were doublets of narrow multiplets (instead of doublets of doublets), doubtless because they represented superpositions of slightly shifted signals from diastereomers. Similarly, ^{13}C signal duplications were discernible for C-6 of the sugar and for the benzylic carbon atom of the ester group (See Experimental). Product **47a** must contain, as one of three components, the diester with two 2S,3R-corynomycolyl groups, stereochemically corresponding to the naturally occurring corynomycolic[33a] and mycolic[33b] acids. The latter possess 2R,3R configuration[33], as does one enantiomer[13l] in racemic **43a** and **44a**. Reduction of the methyl carboxylate to a primary carbinol function, however, entails a reversal of sequence rule priorities for the C-2 substituents as already mentioned (see footnote to Scheme 16), and hence requires the 2S,3R designation for the enantiomer in the resulting, racemic alcohol **45a**, even though bonding about the chiral centers remained the same as in **44a**.

Conclusion

The novel bis (6-deoxy-heptopyranosiduronic acid) **5** and its hexaacetate **16** were synthesized from 6,6'-di-O-tosyl-α,α-trehalose hexaacetate **7** by a modification of the ironcarbonyl method of chain extension. The protected hexa-O-benzyl diacid **26** was similarly synthesized from 6,6'-di-O-tosyl-α,α-trehalose hexabenzyl ether **24** by the ironcarbonyl method, or alternatively, by nucleophilic displacement with lithium cyanide, followed by elaboration of carboxyl groups from the generated nitrile functions.

Esterification of (2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid **26** with 3-O-benzylcorynomycolyl alcohol **45b** in the presence of dicyclohexylcarbodiimide and 4-dimethylaminopyridine gave the diester **46b** in good yield. An alternative and superior method for the synthesis of such esters involved Mitsunobu esterification of the diacid **26**, which furnished the benzyl-protected corynomycolyl diesters **46a** and **46b** in 88% yields. Debenzylation of the products by catalytic hydrogenolysis over Pd-C afforded the "mirror" coryno-cord factors **47a** and **47b** in yields of ~60%.

The corynomycolyl alcohols **45a** and **45b** used for this work were prepared for the first time, from synthetic corynomycolic acid.

Some of the "mirror" esters with lipid chains of intermediate length (**31b-e**) exhibited interesting antigenic properties in studies evaluating various synthetic cord factor analogs as immunoreactants for the serodiagnosis of tuberculosis[34,35], and they were found to inhibit the release of interleukin-6 and T-cell proliferation, induced in human blood mononuclear cells by mycobacterial antigens and bacterial endotoxins[36]. It should be interesting to test the activity of the new cord factor analogs in such biological systems.

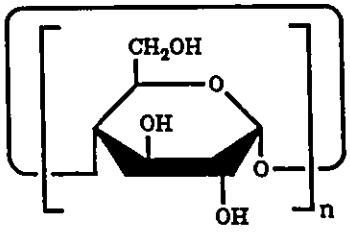
The contributions described in this chapter have been incorporated in two publications[38,39].

SECTION II

SYNTHESIS OF A CYCLOHEPTAOSE CONSISTING OF (1→4)-LINKED 7 AMINO-6,7-DIDEOXY-α-D-GLUCO-HEPTOPYRANOSYL UNITS: A NEW ANALOG OF CYCLOMALTOHEPTAOSE

1.Introduction

The cyclodextrins, sometimes called Schardinger dextrins, cycloamyloses, or cycloglucans, are a series of oligosaccharides produced by the action of the amylase of Bacillus macerans on starch and related compounds. Though cyclodextrins were discovered in 1891 by Villiers[1], the first detailed description of their preparation and isolation was made by Schardinger[2,3]. They are composed of a number of D-glucopyranose units connected by (1→4)-linkages. They are designated by a Greek letter to denote the number of glucose units: α- for 6, β- for 7, γ- for 8 and so on. Although cyclodextrins with up to 12 glucose residues are known[4], only the first three homologs have been studied extensively (Scheme 1).



1 n=6, α-cyclodextrin (cyclomaltohexaose)

2 n=7, β-cyclodextrin (cyclomaltoheptaose)

3 n=8, γ-cyclodextrin (cyclomaltoctaose)

Scheme 1

Cyclodextrins have doughnut-shapes with all the glucose units in substantially undistorted $^4C_1$ (D) chair conformations. The secondary hydroxyl groups (on the C-2 and C-3 atoms of the glucose units) are located on one side of the torus. The interior of the torus consists of a ring of C-H groups, a ring of the pyranoside ring oxygens and another ring of C-H groups.

Among the biochemical properties of cyclodextrins may be noted: They are metabolized by glucanotransferase from Klebsiella pneumoniae[5], cyclodextrinases in Taka diastase[6], and by amylases from Aspergillus niger and oryzae and Bacillus polymyxa[7]. They are largely resistant to the action of amylases from Bacillus subtilis and human saliva[7], and in general to the action of amylases which degrade linear polysaccharides from their terminus. Cyclodextrins also act as competitive inhibitors for phosphorylase[7,8] and β-amylase[7,9] from potatoes, for pancreatic amylase (which can simultaneously bind three β-cyclodextrin molecules in its active centre[10-12]), and for pullulanase from Aerobacter aerogenes[13].

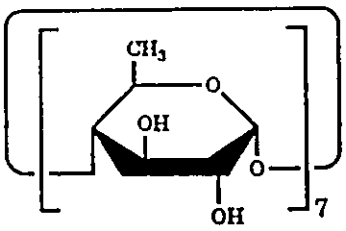
The most remarkable of the properties of cyclodextrins is their ability to form inclusion complexes with a variety of organic and inorganic compounds. These (crystalline) complexes are of interest for scientific research as, contrary to the classical clathrates, they exist in aqueous solution and can be used to study the hydrophobic interactions which are so important in biological systems. Cyclodextrin stabilizes a large number of different compounds in liquid and in

solid state. In some cases, the surounding hydroxyl groups and the apolar cavity will exert specific catalytic effects[14,15,16], and this makes cyclodextrins suitable for generating useful enzyme models[17]. The potential applications of cyclodextrins are wide. Micro-encapsulated active substances enclosed whithin them are protected from the effects of light and atmosphere and can be easily handled and stored in powder form. (For example, pyrethrins which are yellowish, light-sensitive oils useful as insecticides but of limited effectiveness due to their instability, are included in β-cyclodextrin to give a powder which is easy to handle, very stable, and toxic for insects long after its application [7,18]). Substances which are not very soluble in water become more soluble in the presence of cyclodextrins — creams and emulsions can be stabilized, and the growth and yield of grain harvests can be increased[19]. The easily modified hydroxyl groups make it suitable for polymerization to produce resins of specialized properties.

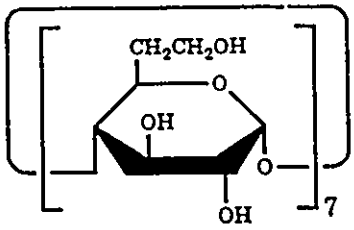
For example, polymer-linked cyclodextrins can be used as filling materials for chromatography (gel inclusion chromatography), with the advantage that they strongly retard molecules of an appropriate size, and molecules with similar molecular weights or even isomers can be separated. Separations of o- and p-nitrophenol[20], or benzoic and o-chlorobenzoic acid[21] are examples. Similar separations have been achieved with amino acids — particularly those with aromatic side groups[20,22].

In continuation of the work in our laboratory[23] on chemical modification of cyclomaltoheptaose (beta-cyclodextrin, **2**), which has provided new, convenient procedures for the preparation of the heptakis-6-deoxy derivative **4** (Scheme 2), it was decided to prepare a number of other derivatives specifically and uniformly modified at the 6-position. Targets of particular interest were chain-elongated analogs of **2** constituted of 6-deoxyheptopyranosyl units (e.g., **5** or **6**) and 6-deoxyheptopyranosyluronic acid units (e.g., **7**) (Scheme 2). It was to be examined whether the various chain extension procedures succesfully used[24] to modify α,α-trehalose (see Section I) can be applied to cyclomalto-oligosaccharides. Cyclo-oligosaccharides composed of heptoses appear to be unknown.

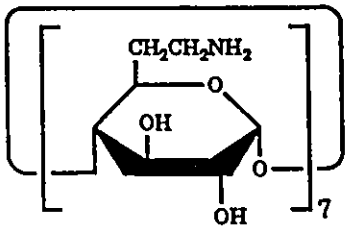
The vast literature[25] on chemical modification of cyclodextrins contains several references to the fact that regioselective and complete protection of all primary hydroxyl group by standard procedures is difficult to achieve. For example, full O-tritylation of **2** proved impossible[26], and although selective heptamolar 6-methanesulfonylation[26,27] and 6-p-toluenesulfonylation[26-28] were claimed, regioisomeric uniformity of the products has not been established and, at least for the case of tosylation, has been disputed[29,30]. Even with the more-selective mesitylsulfonyl chloride, the desired heptakis-6-sulfonate was not the sole product formed[29]. On the other hand, direct selective persubstitutions of the OH-6 group in **2** by azido[30], bromo[23,31], iodo[23], and phenylthio[23] substituents have been achieved with high yields. Another recent advance was primary-selective



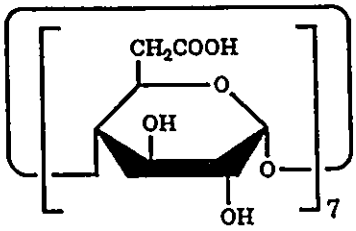
4 heptakis(6-deoxy)cyclomalto-heptaose



5 cyclo(1→4)-(6-deoxy-α-D-gluco-heptopyrano)heptaose



6 cyclo(1→4)-(7-amino-6,7-dideoxy-α-D-gluco-heptopyrano)heptaose



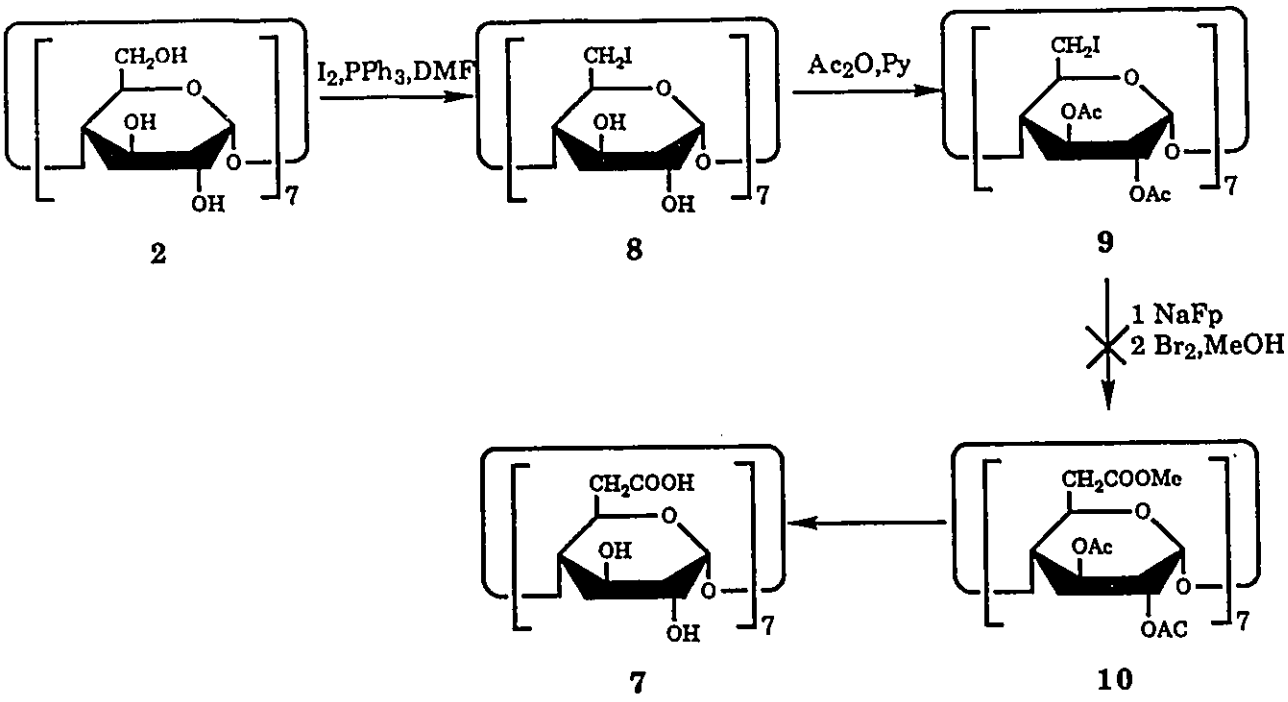
7 cyclohepta(1→4)-(6-deoxy-α-D-gluco-heptopyranosid)uronic acid

Scheme 2

tert-butyldimethylsilylation in 2, efficiently conducted either in N,N-dimethyl-formamide in the presence of imidazole[32], or better still, in pyridine[33]. (In the former system, secondary hydroxyl groups may also undergo silylation[34]).

RESULTS AND DISCUSSION

Targeting first the homologous cycloheptaose **7**, which consists of 6-deoxy-α-D-gluco-heptopyranosyluronic acid residues, we attempted to apply the ironcarbonyl method[24,35] of chain extension to heptakis(2,3-di-O-acetyl-6-deoxy-6-iodo)-cyclomaltoheptaose[23] **9** which was obtained by reaction of **2** with iodine and triphenylphosphine in N,N-dimethylformamide (to give **8**), followed by acetylation with acetic anhydride in the presence of pyridine. The procedure involves reaction of a terminal halide or tosylate with sodium dicarbonylcyclopentadienyliron, followed by oxidative carbonyl insertion in, and methanolysis of, a sugar-iron complex engendered, with methyl uronate being the expected product. Product yields had been high (70-90%) with monosaccharides[35], and lower but still adequate (42%) with trehalose[24] as substrates, but the method failed completely when applied to **9** (Scheme 3). Although this compound reacted rapidly with the reagent under the same conditions, as indicated by t.l.c., no methyl ester **10** or any other identifiable product could be isolated. Probably the steric requirements of the $Fe(CO)_2Cp^-$ anion prevented complete and effective substitution around the cyclodextrin torus (Scheme 3).



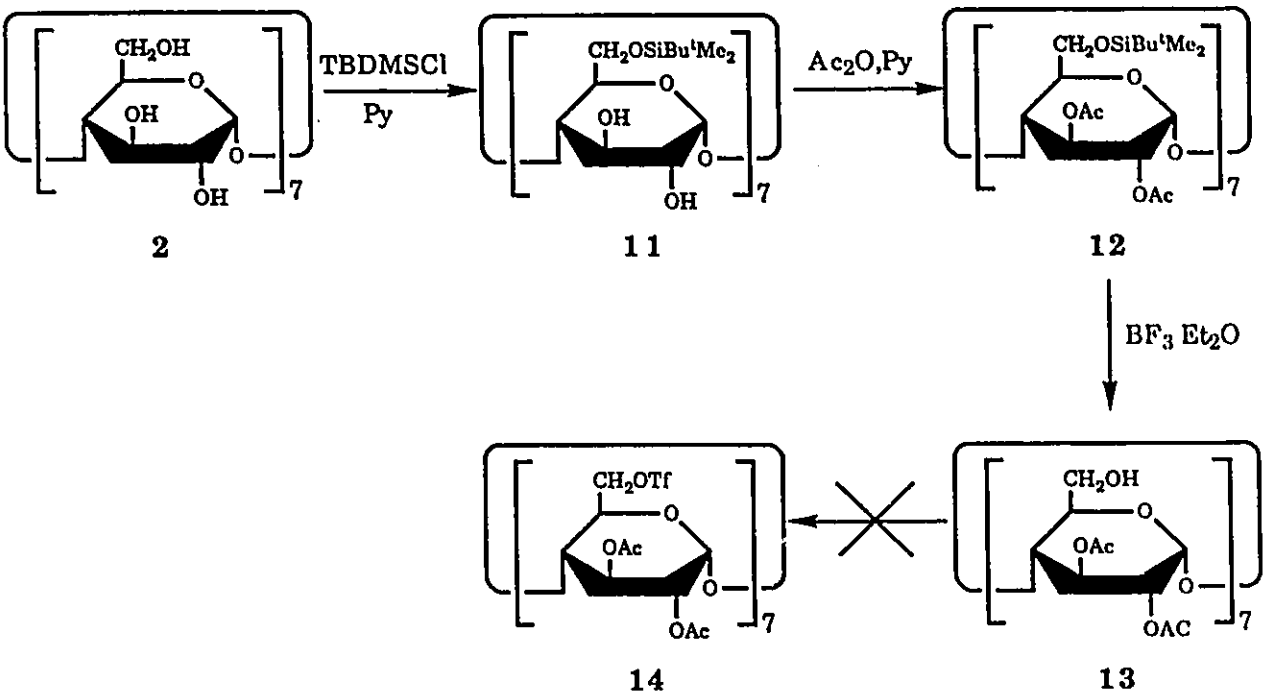
2 8 9

7 10

Scheme 3

We then turned to the second method which had proved successful[24] in the case of trehalose, namely, nucleophilic substitution of terminal sulfonic ester groups by cyanide ion, followed by elaboration of carboxyl groups from the generated nitrile functions. This approach led to nitriles after some initial difficulties were overcome. First, we intended to employ the hitherto unknown heptakis-trifluoromethanesulfonate **14** of heptakis(2,3-di-O-acetyl)cyclomalto-heptaose **13**, as the 6,6'-ditriflates of acetyl- and benzyl-protected trehalose had been amenable to facile cyanide displacement under mild conditions. Compound **13** is readily obtained by tert-butyldimethylsilylation of **2** as already mentioned,

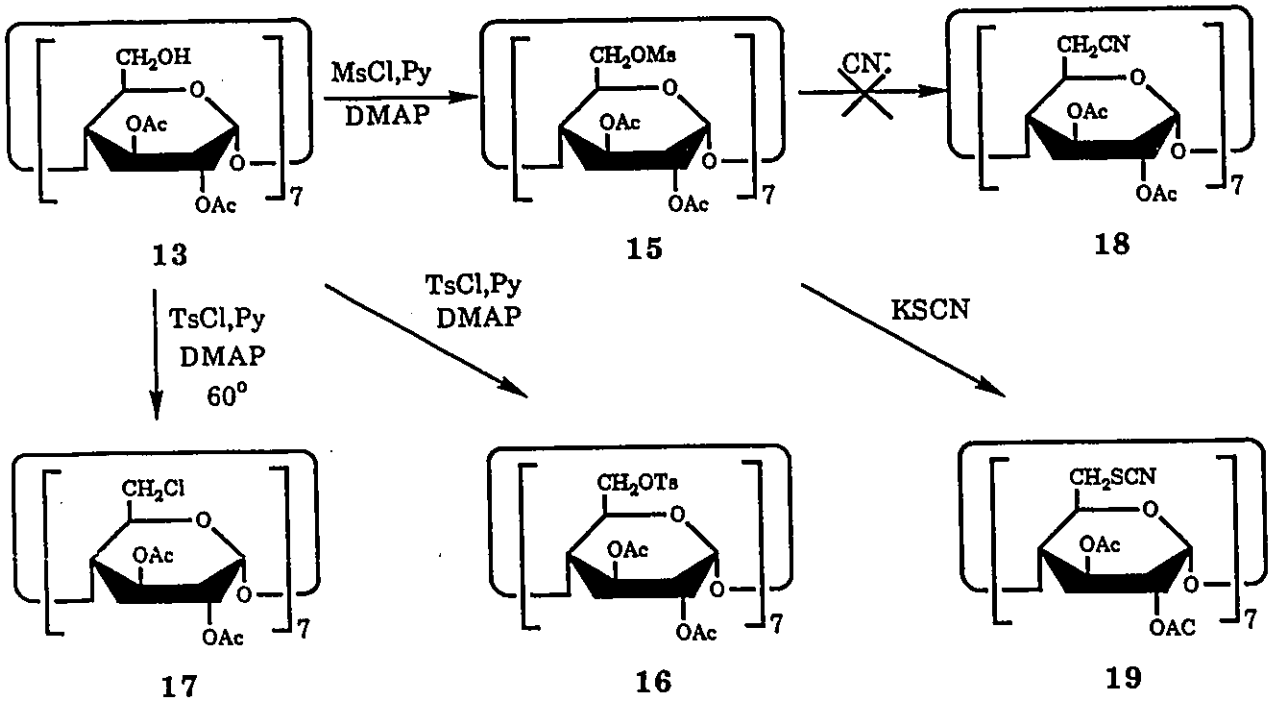
followed by peracetylation[32b,33c] of the silyl ether **11** (to give heptakis(2,3-di-O-acetyl-6-O-tert-butyldimethylsilyl)cyclomaltoheptaose **12**) and subsequent desilylation[32b] with boron trifluoride or toluenesulfonic acid, with each of these steps affording yields in the 80-90% range (Scheme 4). Unfortunately, several attempts at triflation of **13** failed to produce the desired **14** in pure, isolable form; the product appeared rather unstable and prone to decomposition during



Scheme 4

processing. We therefore considered the known, crystalline heptakis(2,3-di-O-acetyl-6-O-methylsulfonyl)cyclomaltoheptaose[26,27] **15** or the corresponding heptatosylate **16** (if obtainable) as alternative, though intrinsically less-reactive,

candidates for cyanide displacement. Whereas **15** could be prepared without problem by treatment of **13** with methanesulfonyl chloride and pyridine for 2 hours at 0^0 and 20 hours at room temperature (yield, 91%), the analogous tosylation to produce **16** was not straightforward (Scheme 5). When **13** was treated with a large excess of tosyl chloride in pyridine (containing dimethyl-



Scheme 5

aminopyridine as a catalyst) during three days at room temperature, a product showing a single spot in t.l.c. was obtained, but its ^{1}H- and ^{13}C-n.m.r. spectra and elemental analysis gave evidence of incomplete tosylation. Although ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$) did not show a signal for CH_2OH (expected near δ 61.0 ppm),

there were six signals in the δ 70.2-68.8 ppm range (C-2,3,5) instead of the three expected for a uniformly substituted product, and the C-1 signal (δ 96.2 ppm) also had a small companion (δ 96.3 ppm). The remaining resonances were at δ 170.4 and 169.4 ppm (2 CO), 145.2, 132.5, 130.0, and 128.1 ppm (arom.), 75.3 ppm (C-4), 21.4 ppm (Me of Ts), and 20.5 ppm (2 MeCO) (Figure 1). The ^{1}H-n.m.r. spectrum showed resonances for the tosyl group (δ 7.75, 7.30, and 2.40 ppm) which integrated to slightly less than 7 H as measured against the 6-proton integral for the signals (δ 1.98 and 1.95 ppm) of the two OAc groups per glucose unit. Moreover, the tosyl CH_3 signal was not a clean singlet, and the main resonances for the sugar ring proton (δ 4.15-3.55 ppm) were accompanied by some minor signals (Figure 2). Microanalytical data for C and S (found: C, 50.86; H, 5.23; S, 8.01) lay between those calculated for the heptakis-tosylate **16** (C, 50.99; H, 5.04; S. 8.01) and a hexakis-tosylate (C, 50.79; H, 5.10; S, 7.26), indicating that the compound was not uniformly substituted. A separate experiment had indicated no change to occur after prolonged storage.

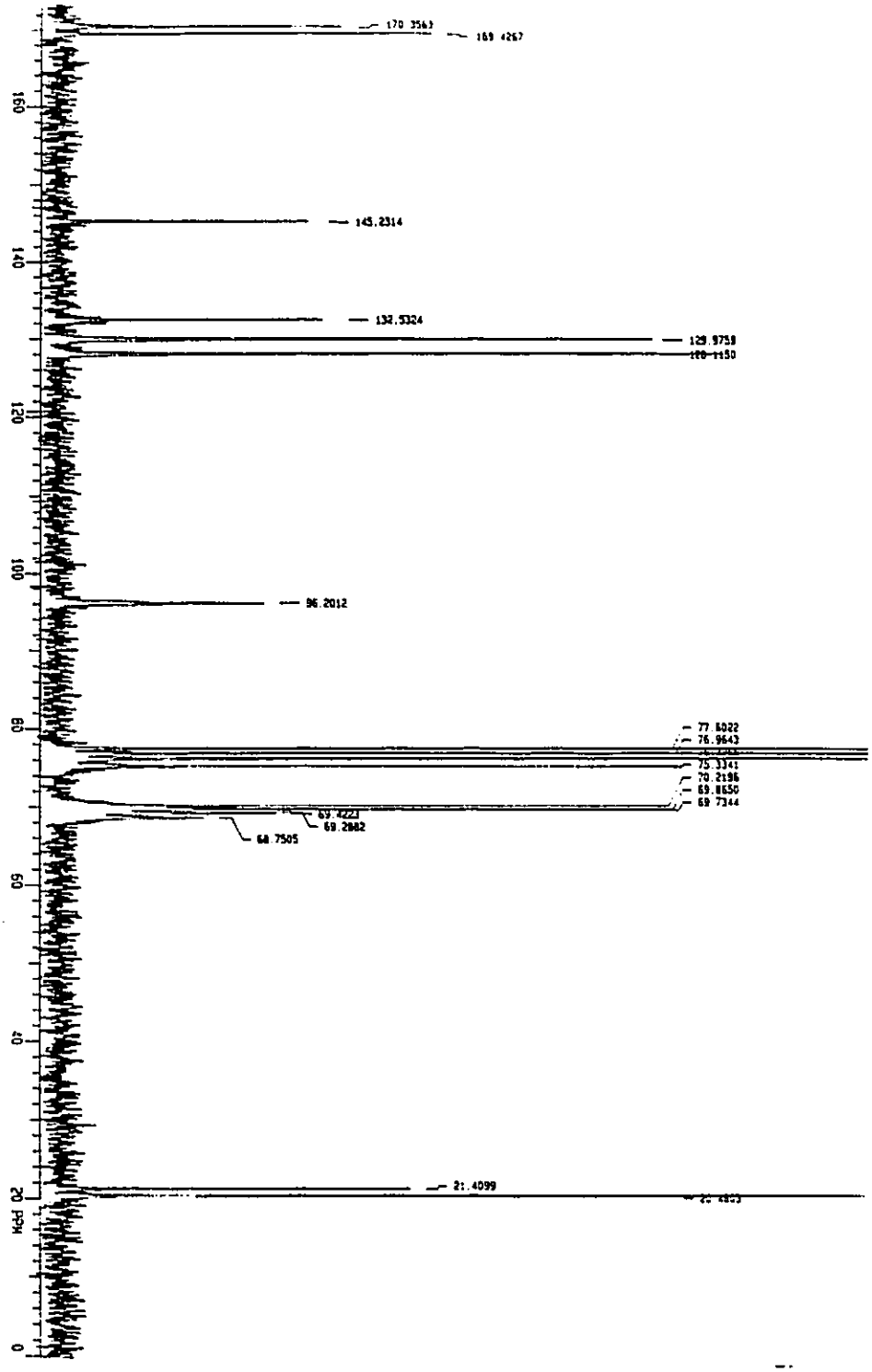


Figure 1. ^{13}C NMR spectrum of compound **16**

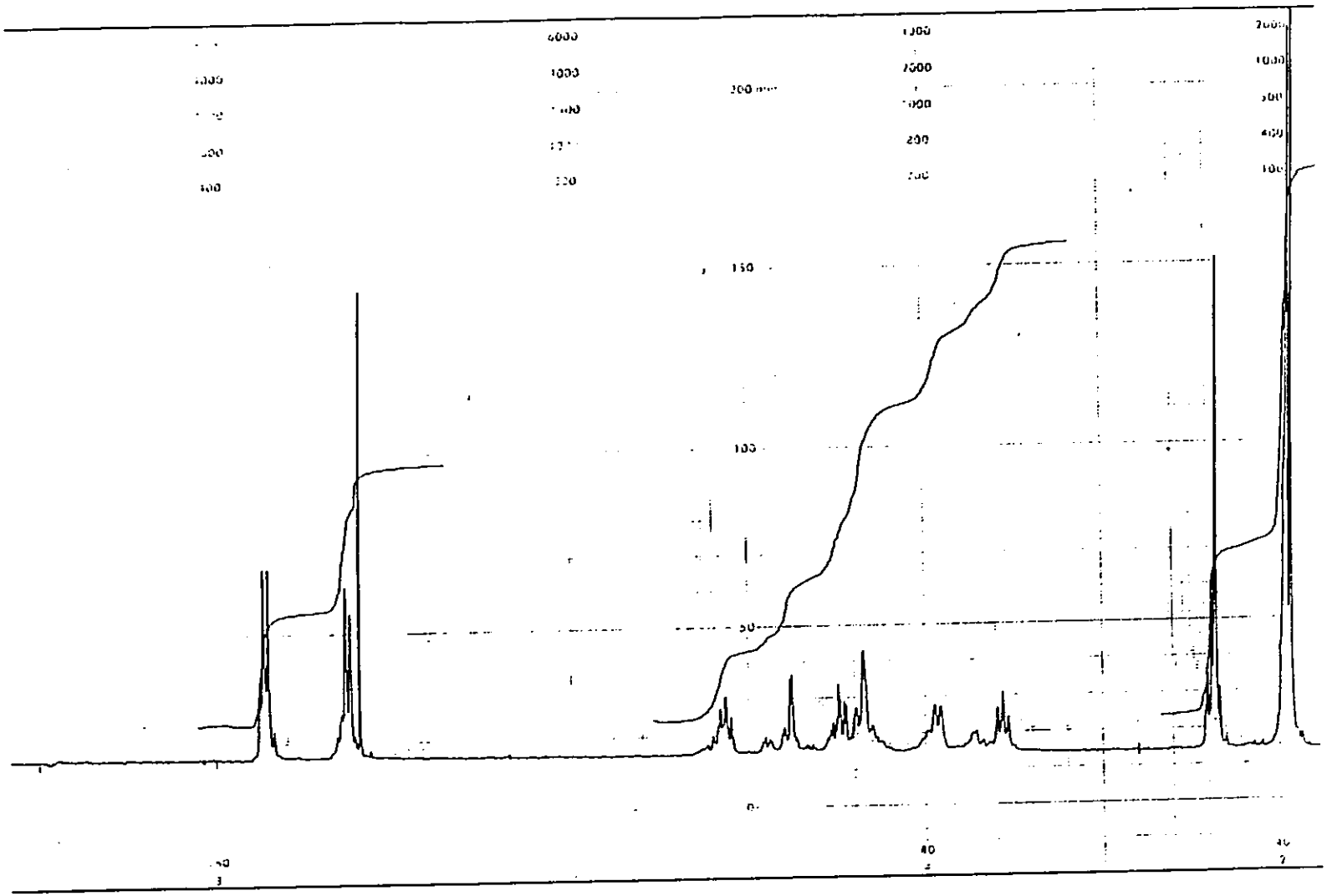
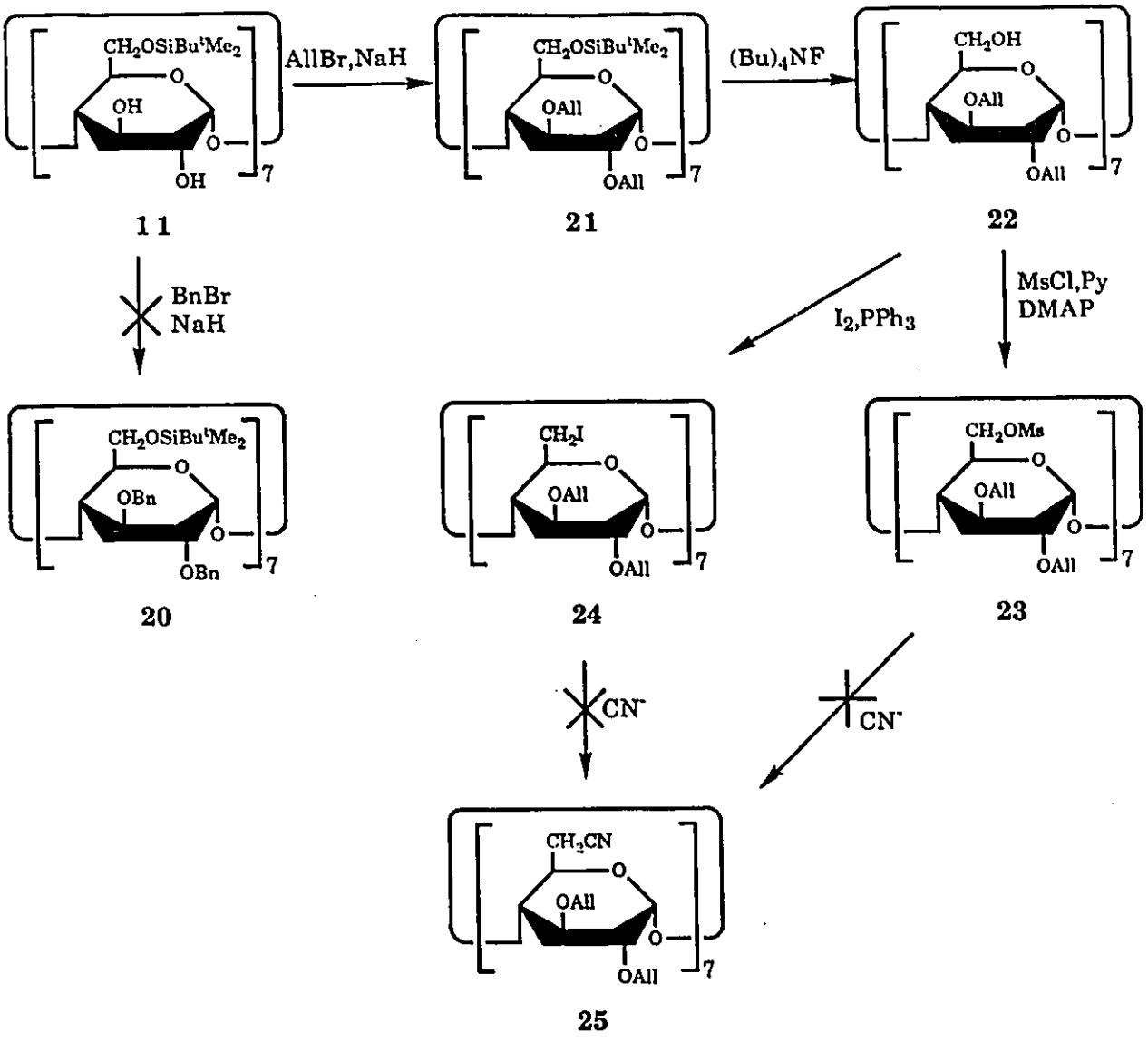


Figure 2. ^{1}H NMR spectrum of Compound **16**

When the reaction of **13** with tosyl chloride was performed at 60° for 40 hours, the product isolated in 92% yield was the known heptakis(2,3-di-O-acetyl-6-chloro-6-deoxy)cyclomaltoheptaose **17** (Scheme 5), previously obtained[32b] from the mesylate **15** by displacement with lithium chloride. Direct replacement of a hydroxyl group by a chloro substituent under similar conditions of tosylation has been observed before[36].

In the event, we tried mesyloxy displacement in **15** by potassium cyanide and found that it occurred only under forcing conditions (in N,N-dimethylformamide solution at 60°), under which far-reaching decomposition took place, probably as a consequence of simultaneous O-deacetylation induced by the cyanide. In aqueous acetonitrile solution at room temperature, where O-deacetylation should not be significant[24], cyanide was ineffective for displacement. By contrast, treatment of **15** with potassium thiocyanate in N,N-dimethyldormamide for 12 hours at 100° gave an 85% yield of crystalline heptakis(2,3-di-O-acetyl-6-deoxy-6-thiocyanato)-cyclomaltoheptaose **19**. Thiocyanate ion is a considerably weaker base than cyanide ion, and this result complements earlier observations[26,27,32b] that high-yielding displacements in **15** with such weaker basic nucleophiles as chloride, bromide, iodide, and azide ions can be achieved without compromising the O-acetyl protecting groups.



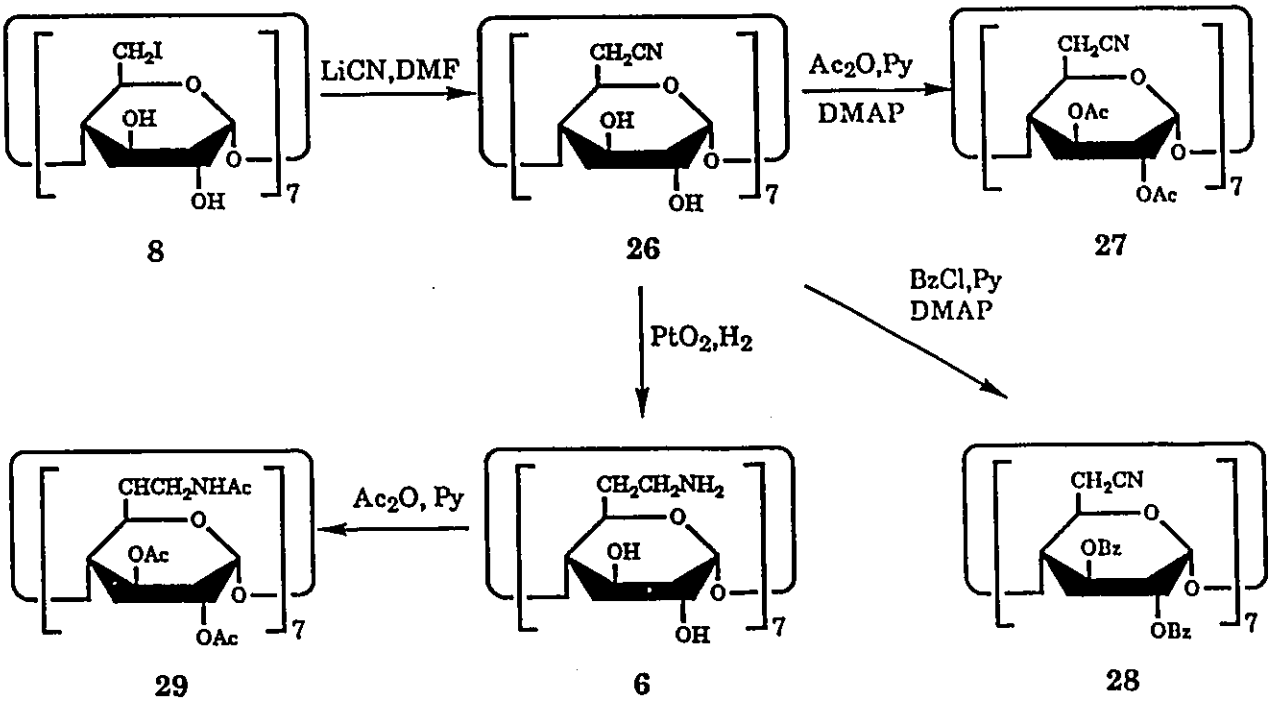
Scheme 6

It was hoped that the difficulties just mentioned might be circumvented by using a substrate bearing resistant protecting groups for cyanide displacement. First, we tried to achieve benzyl protection, which had been successful in the case

of analogous hexakis(6-O-tert-butyldimethylsilyl)cyclomaltohexaose[32a]. However, various attempts to benzylate **11** with sodium hydride and benzyl bromide failed to give heptakis(2,3-di-O-benzyl-6-O-tert-butyldimethylsilyl)cyclomaltoheptaose **20**; mixtures of several products were always observed in t.l.c.. Attention was then turned to allylation as an alternative method of protection. This was successful. Thus, the silyl ether **11** was treated with allyl bromide and sodium hydride to provide the tetradeca-O-allyl derivative **21**, desilylation of which with tetrabutylammonium fluoride in refluxing oxolane gave the crystalline heptakis(2,3-di-O-allyl)cyclomaltoheptaose **22**. Finally, mesylation of **22** furnished the heptamesylate **23** in 60% overall yield from **11** (Scheme 6). In addition, the corresponding perallylated heptakis-6-deoxy-6-iodo compound **24** was synthesized as an alternative substrate, by applying the previously elaborated[23], Vilsmeier-type iodination to **22**. Several attempts were then made to achieve displacement in **23** and **24**, using potassium, sodium, or lithium cyanide under various conditions, but again, none was successful and the nitrile **25** could not be obtained. However, it was then discovered (ironically, after all these labors) that the readily available[23], unprotected heptakis(6-deoxy-6-iodo)cyclomaltoheptaose **8** reacts well with lithium cyanide in N,N-dimethylformamide, to furnish the desired cyclohepta(6-deoxy-α-D-gluco-heptopyranosid)urononitrile **26** in high yields (74% after 26 hours at 45^0, 87% after 72 hours at about 30^0). The crystalline nitrile, which was converted into its per-O-acetyl and per-O-benzoyl derivatives **27** and **28** by standard procedures (Scheme 7), constitutes the first example of a

cyclooligosaccharide composed of a hepturonic acid derivative.

Hydrogenation of the nitrile functions was difficult, as was first realized when Prof. F. Santoyo González* and his coworkers attempted reduction by two different reductants recommended in the literature[37,38] for the conversion of nitriles into amines. Thus, reduction of **26** with lithium borohydride in the



Scheme 7

*Co-investigator with H. H. Baer on a joint project supported by a NATO grant for international collaboration.

presence of trimethoxyborane[37], attempted in pyridine solution because of insolubility of **26** in the preferred solvents (ether or oxolane)[37], led to complex mixtures of products, as did reduction of **28** with sodium trifluoroacetoxy-borohydride[38] in oxolane. More successful was catalytic hydrogenation of **26** over platinum. The reaction was slow (10 days at room temperature and 4.5 atm H_2 pressure) and it also gave a mixture of products, but the desired amine **6** was evidently among them. Peracetylation and chromatography of the mixture afforded a modest yield (23%) of crystalline cyclo-(1→4)-(7-acetamido-2,3-di-O-acetyl-6,7-dideoxy-α-D-gluco-heptopyrano)heptaose **29**, the first cyclodextrin analog composed of heptose units. The crystals melted at 183-186°C and had $[\alpha]_D$= +38° (c 1, $CHCl_3$). Spectroscopic methods completely supported the structure of **29**. The NH signal was observed as a broadened singlet at 7.80 ppm. A doublet of doublets at 5.14 ppm was assigned to H-3 and had $J_{2,3}$=$J_{3,4}$=7.8 Hz while another doublet of doublets, representing H-2 was noticed at 4.75 ppm and had $J_{1,2}$=3.7 Hz and $J_{2,3}$=8.8 Hz. A triplet for H-5 was centred at 3.94 ppm having $J_{4,5}$≈$J_{5,6}$≈8 Hz and $J_{5,6'}$=1 Hz. The H-4 proton resonated at 3.38 Hz and appeared as a doublet of doublets having $J_{3,4}$≈$J_{4,5}$≈7.8 Hz. The H-6, H-6' and H-7, H-7' protons gave multiplets at 3.30, 1.75 ppm and 3.60, 3.09 ppm, respectively. The anomeric proton H-1 appeared as a doublet at 5.00 ppm ($J_{1,2}$=3.7 ppm). The acetyls were observed as singlets at 2.06, 2.00 and 1.97 ppm. ^{13}C-n.m.r. afforded extra evidence which also confirmed the structure of the compound **29**. The carbonyl carbons of the acetyl groups were found at 171.8, 170.7, and 169.4 ppm. The anomeric C-1

signal appeared at 97.4 and C-4 at 81.5 ppm. Signals at 70.6 and 69.9 (double intensity) ppm were assigned to C-2, 3, and 5. The C-7 signal was observed at 36.5 ppm and the C-6 signal was noticed at 31.5 ppm. The methyl carbon signals for the acetyl and acetamido groups appeared at 22.82 and 22.80 ppm.

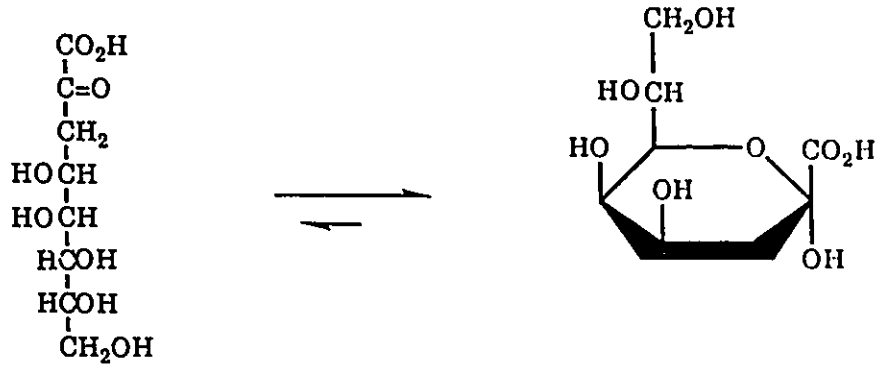
In summary, cyclomaltoheptaose **2** was converted into the corresponding per(6-deoxy-6-iodo) derivative **8** which reacted with lithium cyanide in N,N-dimethyl-formamide to give a high yield of the corresponding cyclohepta(1→4)-(6-deoxy-α-D-gluco-heptopyranosid)urononitrile **26**, catalytic hydrogenation of which gave cyclo(1→4)-(7-amino-6,7-dideoxy-α-D-gluco-heptopyrano)heptaose **6**, isolated as its peracetyl derivative **29**.

SECTION III

AN APPROACH TO THE SYNTHESIS OF 3-DEOXY-D-MANNO-2-OCTULOSONIC ACID (KDO)

INTRODUCTION

3-Deoxy-D-manno-2-octulosonic acid (**1**, "ketodeoxyoctonic acid", KDO)[1] is a higher monosaccharide that occurs as a ketosidic component in lipopolysaccharides (LPS) of Gram-negative bacteria, and it has also been identified in several acidic exopolysaccharides (K-antigens). The location of LPS and of K-antigens (capsular materials) at the cell surface of Gram-negative bacteria is shown diagrammatically[1] in Figure 1. KDO was first detected by Levin and Racker[2] through the formation of a characteristic, purple chromophore from its 8-phosphate by the action of periodate-thiobarbituric acid. The 8-phosphate is the product of the condensation of D-arabinose 5-phosphate with enolpyruvate phosphate, catalyzed by 3-deoxy-8-O-phosphonooctulosonate synthetase[2].



Keto form α-pyranose form

KDO (1)

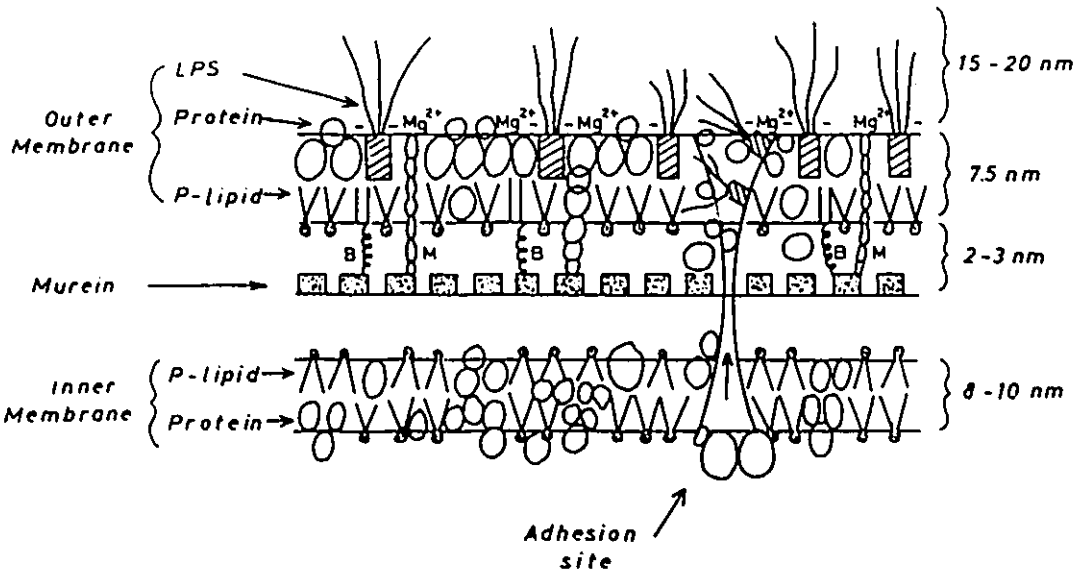


Figure 1. Schematic Representation of the Cell Envelope of Gram-Negative Bacteria. [The envelope is seen to consist of an inner (cytoplasmic) membrane, a murein (peptidoglycan) layer, and an outer membrane. The region between the inner and outer membranes is referred to as the periplasmic space. The outer membrane consists of phospholipid, protein, and lipopolysaccharide (LPS).The polysaccharide chains of LPS are seen to extend into the surrounding medium].

KDO appears to be unique to Gram-negative bacteria. In the LPS that have been studied, KDO residues are situated at the reducing ends of the polysaccharide domains, linking them, by ketosidic bonds, to the fatty acid-substituted 2-amino-2-deoxy-D-glucosyl disaccharides referred to as lipid A.

The incorporation of KDO appears to be a vital step in LPS biosynthesis[3] and, indeed, in the growth of the Gram-negative bacteria. The rate-limiting enzyme for the incorporation of KDO into these LPS is 3-deoxy-D-manno-octulosonate cytidylyl transferase (CMP-KDO synthetase)[4], and the preparation

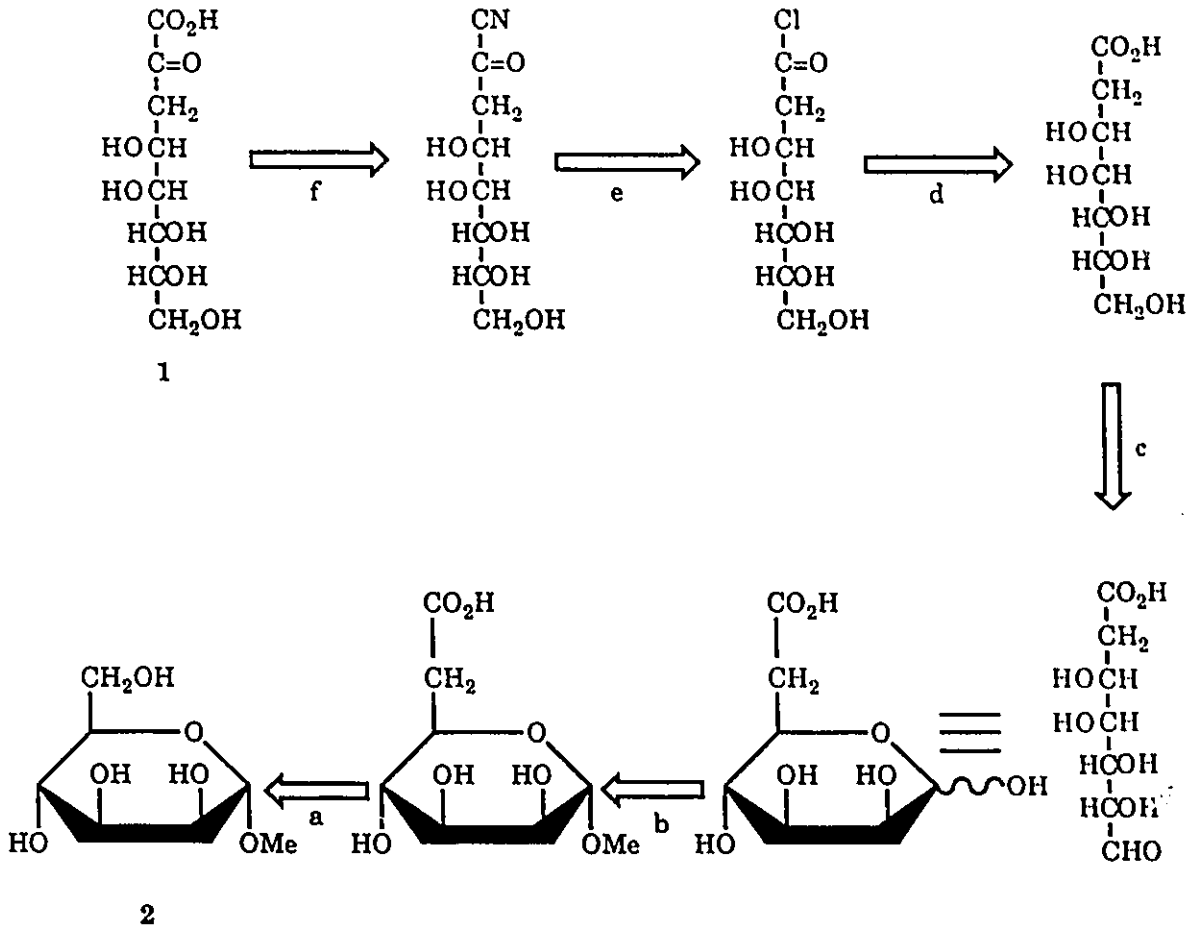
of analogues of 1 as potential inhibitors of this enzyme emerged as an attractive strategy for the discovery of novel antibiotics[5]. These investigation lead to the development of several effective antibacterial agents derived from 2-deoxy-KDO that specifically inhibit LPS biosynthesis.

The biological importance of KDO (1) has also provided an impetus for a number of efforts directed toward its total synthesis[6]. An often used approach is the synthesis of KDO from D-mannose in combination with a C_2 building unit, which avoids the possible formation of the D-gluco isomer. The chain elongation of aldehydo-D-mannose by Wittig- or aldol-type reactions has been reported in many variations[1,6e,l,j,o]. In most of these procedures, several additional steps are required to liberate the dormant 2-keto group. An interesting variation is the Horner-Emmons reaction of aldehydo-mannose with Horner's phosphonate that leads in only two steps to the KDO-1,4-lactone[6f]. Paquet and Sinay[6a] suggested the use of a phosphonate of a functionalized acceptor sugar, followed by mercuration-demercuration, thus providing directly a KDO-containing disaccharide in good yield. Shiba et al.[6b] reported the preparation of KDO by nucleophilic substitution of a triflate group at C-1 of a D-mannitol derivative with the anion of methyl glyoxylate dithioacetal. An interesting variation of this approach, using the 1,4-cyclic sulfate of D-mannitol, was reported by van Boom et al.[6g]. All of these strategies have one feature in common: for chain extension, C-1 of the precursor sugars is used. Recently, a synthetic method was reported employing C-6 of D-

mannose for chain extension. Branchaud et al.[6h] described the use of cobaltoxime-mediated radical alkyl-alkenyl cross coupling to construct the key C-C bond. Similarly, chain elongation of aldehydo-D-arabinose by three carbon atoms with Wittig- or aldol-type reactions is another common strategic device[1,6i,k,m,p]. Furthermore, de novo syntheses of KDO have been worked out[6d,n,q].

Ongoing research in this laboratory focused on the structural modification of monosaccharides and oligosaccharides by use of various procedures for chain elongation at the nonreducing terminal[7,8]. Examples were delineated in sections I and II of the thesis. Consideration of these experiences led to the formulation of an approach to KDO that involved chain elongation by ironcarbonyl chemistry. This approach is depicted in a retrosynthetic scheme that suggests the production of **1** from readily available methyl α-D-mannopyranoside **2** (Scheme 1). The salient features of the design are:

Chain extension at C-6 in **2** by the iron carbonyl method, to give a methyl 6-deoxyheptosiduronic acid (step **a**); hydrolysis of the methyl glycoside to afford the corresponding, reducing alduronic acid (step **b**); borohydride reduction of the alduronic acid to furnish a 2-deoxyheptonic acid (step **c**); sequential conversion of the latter into its acyl chloride (step **d**) and acyl cyanide (step **e**), and finally, transformation of the acyl cyanide (which is the nitrile of KDO) into the target acid **1**. It goes without saying that the scheme illustrates only the principle of the



Scheme 1

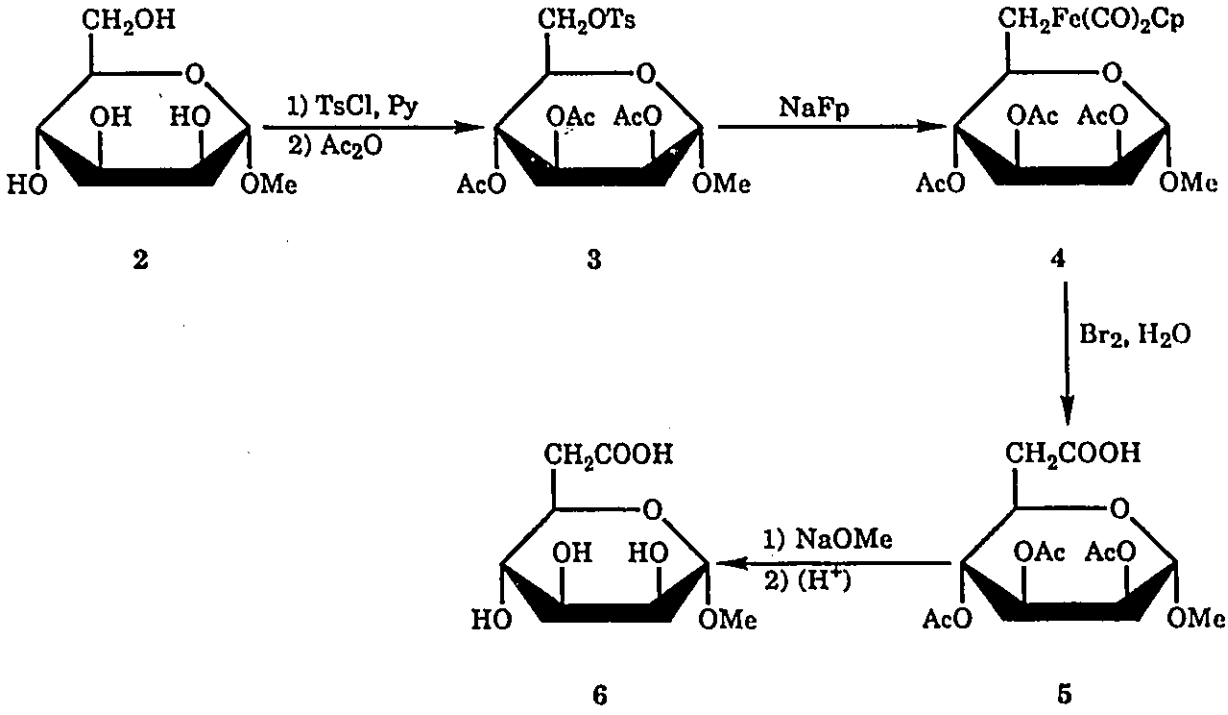
strategy; suitably derivatized and protected intermediates will have to be employed in its practical realization.

Results and Discussion

As just indicated (Scheme 1), the first key step in the proposed synthesis was to consist of an application, to D-mannose, of the synthesis of 6-deoxy-heptosiduronic acids elaborated in this laboratory[7]. In that work, several variously protected methyl D-glucopyranoside 6-tosylates and 6-deoxy-6-halo derivatives were allowed to react with sodium dicabonyl-η^5-cyclopentadienyliron (NaFp) in oxolane, to give by nucleophilic substitution a sugar-iron compound which, on treatment with an oxidant (Br_2, I_2, $CuSO_4$, or $FeCl_3$) and methanol underwent carbonyl insertion and methanolysis, to furnish methyl glycosides of 6-deoxy-gluco-heptosiduronic acid methyl esters in isolated yields of 70-95%. A single example of reaction of a substrate having the D-manno configuration, namely, methyl 2,3,4-tri-O-benzoyl-6-deoxy-6-iodo-α-D-mannopyranoside, was included in the original work; the yield of methyl uronate was 75% in that particular instance[7]. Early attempts to generate free uronic acids from such protected methyl esters by alkaline solvolysis had met with certain difficulties (since overcome), which had prompted the studies reported in Section I of this thesis, namely, the use of water instead of methanol for solvolysis of the sugar-iron intermediate, producing a free uronic acid directly. This strategy[8] was successful with a methyl β-D-glucopyranoside and with α,α-trehalose as substrates, and although yields were not as high as in the analogous methyl ester syntheses, the present project provided an opportunity to study the method further, by extending it to an

example of the D-manno-series.

Methyl 2,3,4-tri-O-acetyl-6-O-p-tolylsulfonyl-α-D-mannopyranoside **3**, prepared[9] from methyl α-D-mannopyranoside **2** by selective tosylation followed by acetylation, was treated under strictly anhydrous conditions with NaFp obtained from dicarbonylcyclopentadienyliron dimer with sodium amalgam (Scheme 2).



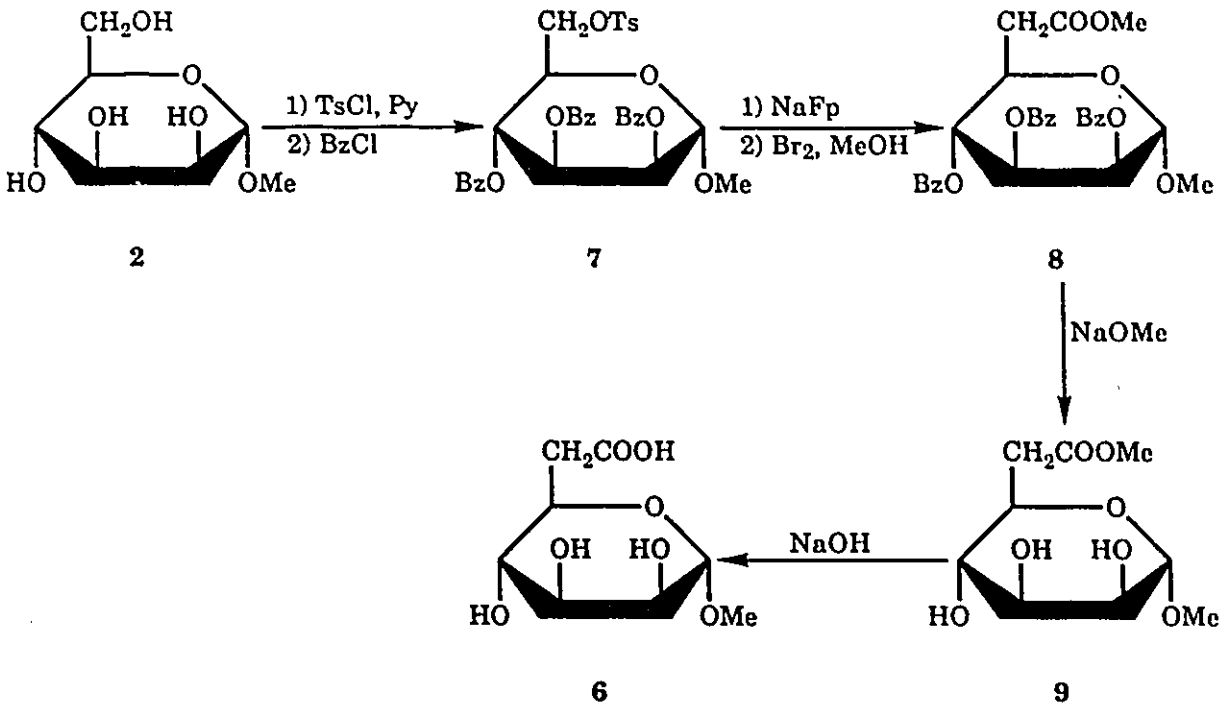
2 **3** **4** **5** **6**

Scheme 2

The sugar-iron compound **4** generated was not isolated; instead, it was treated directly with bromine and water, thereby furnishing methyl 2,3,4-tri-O-acetyl-6-deoxy-α-D-manno-heptopyranosiduronic acid **5** in 36% yield and thus supporting

the generality of this variant of the ironcarbonyl chain extension. Methoxide-catalyzed methanolysis (Zemplén deacetylation) of **5** provided the heptopyranosiduronic acid **6** in 88% yield.

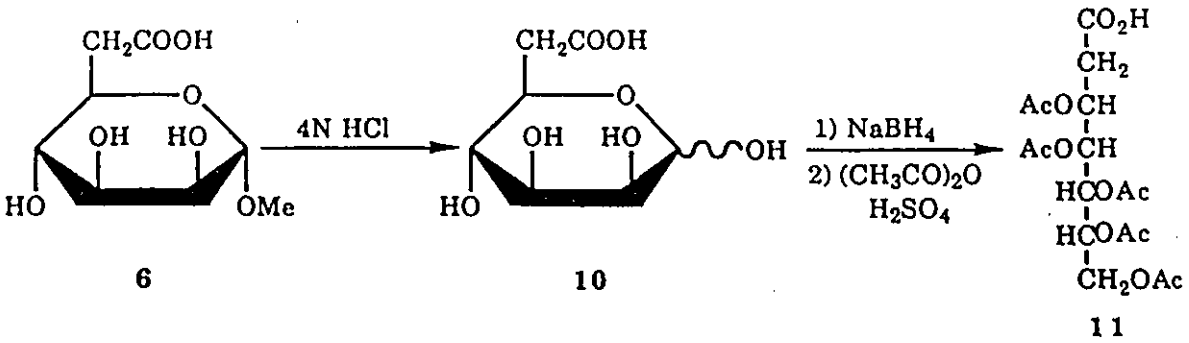
However, since the yield of **5** was modest and did not match that (75%) achieved[7] in the aforementioned methyl ester synthesis, it was decided to re-examine the possibility of methyl ester saponification. Thus, the methyl ester **8** was synthesized as described by Baer and Hanna[7], except that methyl 2,3,4-tri-O-benzoyl-6-O-p-tolylsulfonyl-α-D-mannopyranoside[10] **7** was used instead of the corresponding 6-iodo derivative used previously. The yield of **8** was similar (in fact, somewhat better: 80%), reinforcing previous observations[7,8] that tosylates and halides are practically interchangeable as reactants in the ironcarbonyl procedure. Debenzoylation of **8** with a catalytic amount of sodium methoxide in methanol (1 hour at 25°) gave a 95% yield of the methyl (methyl 6-deoxy-α-D-manno-hepto-pyranosid)uronate **9**. Saponification of the methyl ester **9** was then achieved by 1.66% sodium hydroxide in aqueous methanal (1 hour at 98°), affording the acid **6** in 90% yield (Scheme 3).



2 7 8 9 6

Scheme 3

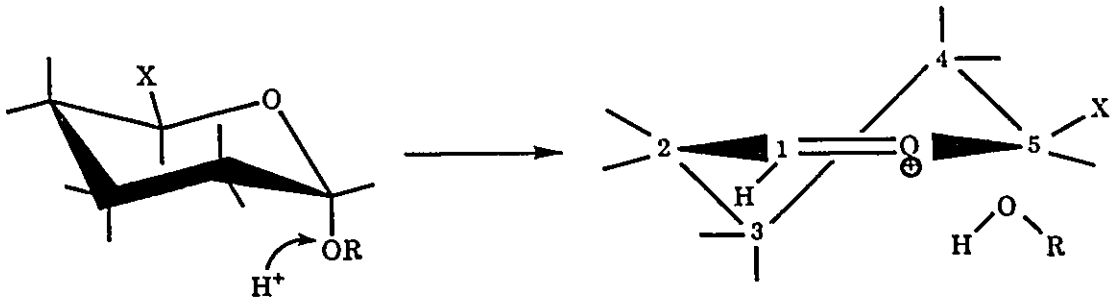
The next requirement for the synthesis (step **b** in Scheme 1) was the conversion of the glycosiduronic acid **6** into the glycuronic acid **10**, involving liberation of the anomeric center (Scheme 4). Acid-catalyzed hydrolysis of glycosides is one of the most common operations in carbohydrate chemistry and seldom entails difficulties[11,12]. There was some concern in the present case, however. Glycosides of alduronic acids derived from hexoses differ from ordinary glycosides in that they are normally more resistant to hydrolysis[13]. The problem is of particular practical relevance in polysaccharide chemistry where yields of monomeric uronic acids from hydrolysis of polymers are often low because the glycosidic linkages of uronic acids are more stable than those of neutral sugar



Scheme 4

constituents, and when liberated under forcing conditions the free uronic acids tend to be degraded more readily than neutral sugars[14]. The degradations in question involve decarboxylation and dehydration[15]; in fact, procedures for the quantitative estimation of uronic acids are based on decarboxylation effected by strong mineral acids[16]. Partial acid hydrolysis of heteropolysaccharides that contain both uronic acids and neutral sugar (such as hemicelluloses, plant gums, pneumococcal polysaccharides, heparin, hyaluronic acids, etc.) normally leads to an accumulation of aldobiouronic acids, i.e., disaccharidic fragments in which the nonreducing unit is a uronic acid glycosidically linked to a neutral, reducing sugar unit[14]; prior reduction of the carboxyl groups facilitates total hydrolysis[13,14,17a]. The kinetics and mechanism of uronoside hydrolysis have been the subjects of many investigations, which in part gave contradictory results and led to some controversy in interpretation. Polar and steric factors have been invoked to explain the enhanced stability of hexuronosides versus hexopyranosides; the most widely held view is that in the former the CO_2H substituent at C-5 of the

pyranoside ring offers greater steric hindrance than the CH_2OH substituent in the latter, to the adoption of a half-chair conformation required for a C-1 glycosyl carboxonium ion in the hydrolytic transition state[17b].



X = CO_2H or CH_2OH

However, all the previous studies concerned hexuronosides, and it was not known how the new class of 6-deoxyhepturonosides (wherein X in the above illustration is CH_2CO_2H) will behave in acid hydrolysis.

As it turned out, the hydrolysis of **6** proved difficult. Several trials were undertaken. Thus, refluxing of **6** in 80% acetic acid for 24 hours was without effect. Treatment with 30% trifluoroacetic acid under reflux for 24 hours gave two products as signalled by two overlapped spots in t.l.c.. Decrease of trifluoroacetic acid concentration led to similar results with longer reaction time.

Eventually, a hydrolysis was preformed in refluxing 4M hydrochloric acid during 5 hours. The glycoside was completely consumed, but a mixture of products

was formed, giving in t.l.c. two ill-separated spots with a visually estimated intensity ratio of 1:1. Column chromatography produced a small proportion of one of the components in pure form, which proved to be the desired acid **10** as a 3:2 mixture of α- and β-anomers (yield, 9%), whereas a major fraction (62%) consisted of **10** and an unidentified by-product of similar mobility. The structure of **10** was indicated by its well-resolved ^{1}H-n.m.r. spectrum which showed two sets of separated signals in a 3:2 intensity ratio for H-1 (δ 5.13 and 4.91, doublets), H-3 (δ 3.85 and 3.67, doublets of doublets), H-4 (δ 3.55 and 3.48, triplets), H-5 (δ 4.18 and 3.71, triplets of doublets), and H-6' (δ 2.53 and 2.52, doublets of doublets) for protons of the α- and β- forms, respectively; the H-2 protons of both anomers had essentially identical chemical shifts (δ 3.96, narrow multiplet), as did the H-6 protons (δ 2.95, doublet of narrow multiplets). The coupling constants listed in the Experimental were in accord with the geometry of the D-manno configuration in the $^4C_1$ chair conformation. The ^{13}C-n.m.r. spectrum afforded confirmatory evidence for the structure of **10**. The carboxyl carbons of the two anomers resonated at δ 178.73 and 178.68, C-1 at δ 96.5 and 96.1, C-2 to C-5 at δ 75.1-71.4 (six signals, two of which had double intensity), and C-6 of both anomers resonated at δ 39.4.

The isolation and spectroscopic characterization of **10** demonstrated that acid hydrolysis of **6** is possible in principle. However, further efforts will be necessary to optimize the reaction conditions, with a view to suppressing as far

as possible the formation of the unidentified by-product*.

The mixture of **10** and by-product was used for a small-scale pilot experiment to examine the feasibility of reduction of **10** to the corresponding aldonic acid (step **c** in Scheme 1). It is known that uronic acids can be so reduced, as salts, by the action of sodium borohydride[18]. Consequently, the material was treated with an excess of that reductant, after which the crude product was peracetylated with acetic anhydride in the presence of sulfuric acid, and processed by chromatography. There was obtained a syrupy product whose ^{1}H-n.m.r. spectrum was compatible with that expected for 2-deoxy-D-manno-heptonic acid pentaacetate **11** (Scheme 4). The spectrum exhibited five 3-proton acetyl singlets (δ 2.07-2.01), a 2-proton multiplet at high field (δ 2.58) assignable to the deoxy function (H-2,2'), two mutually coupled (J_{gem} 12.5 Hz) 1-proton doublets of doublets (δ 4.20 and 4.06) attributable to H-7,7', and overlapping multiplets (5.35-5.05) totalling 4 protons for H-2,3,4,5.

Unfortunately, lack of material and time precluded a more thorough characterization of the presumed aldonic acid **11**, its preparation on a larger scale, and its subsequent use as contemplated in Scheme **1**. The remaining steps toward

*The ^{1}H n.m.r. spectrum of the mixture ruled out the possibility that this was a lactone of **10** because there appeared no peaks in the 2 - 3 ppm region where H-6,6' (the protons in α-position to the lactone carbonyl) should be expected to resonate.

the goal of KDO would include conversion of **11** into its acid chloride (step **d**) by means of thionyl or oxalyl chloride; conversion of the chloride into the corresponding acyl cyanide (step **e**) by action[19,20] of trimethylsilyl cyanide; and finally, hydrolysis of the nitrile to furnish the α-oxo acid (step **f**).

The last-mentioned proposition requires comment. The reaction of acyl cyanides with water is strongly pH-dependent. In the range of pH 12 - 5 a fast bimolecular reaction with hydroxyl ion takes place, resulting in the displacement of the cyano group. This cleavage is inhibited by acids and becomes slow at pH values below 5. When the concentration of strong acid is increased, acid-catalyzed hydrolysis of the nitrile occurs, to give α-oxo acids[20,21]. This reaction is strongly catalyzed by bromide ions, and it can also be conducted in methanolic medium, in which case α-oxo methyl esters result[20,22]. High yields have been reported for simple alkyl and aryl cyanides, and it should be a challenging task to elaborate appropriate conditions for application with a more complex, protected polyhydroxyalkyl cyanide such as that derived from **11**.

EXPERIMENTAL

GENERAL TECHNIQUES

Solvents. -- Solvents were reagent grade and were used without further purification, except where indicated otherwise. Petroleum ether normally refers to the fraction boiling at 30-60°. The solvent systems used for chromatography are listed at the beginning of each section. Solvent evaporation during processing of reaction mixtures and chromatographic fractions was performed in a rotary evaporator, normally at 35-40° (bath temperature).

Handing of moisture- and air-sensitive materials. -- Glassware used in the experiments with moisture- and air-sensitive materials was dried by heating it overnight in an oven at ca. 120° and was then purged, while still hot, with a stream of dry nitrogen. An atmosphere of dry nitrogen (or carbon monoxide, as required) under slightly positive pressure was maintained in the apparatus during reactions. Reactant solutions were transferred, and test samples withdrawn, by means of syringes, rubber septa, and Teflon tubing.

The oxolane (tetrahydrofuran) employed as reaction medium was freshly dried immediately before each experiment by refluxing it over, and distilling it from, sodium metal in the presence of benzophenone radical anion.

Chromatography. -- Analytical thin layer chromatography (t.l.c.) was routinely employed to monitor reactions and to check the purity of products. It was

performed on precoated silica gel plates Merck 60 F-254. Spots were made visible by UV light and (or) by spraying the plates with 5% sulfuric acid in ethanol and heating them briefly on a hot-plate. Column chromatography was performed on silica gel Merck 9385; 20-45 m, or BDH 9385; 230-400 mesh.

N.M.R. spectroscopy. -- Most n.m.r. data were obtained by use of a Varian Gemini 200 instrument, operating at 200 (^{1}H) and 50.3 (^{13}C) MHz; ^{1}H data referring to 300-MHz spectra (Varian XL-300 instrument) are so denoted. Assignments of ^{13}C peaks were aided by ADEPT or APT experiments. Unless otherwise indicated, samples were dissolved in $CDCl_3$, and δ values are given with reference to the internal chloroform signal at δ 7.24 ppm. The symmetrical disaccharide and oligosaccharide derivatives gave only one set of signals which, for convenience, are recorded with reference to one moiety.

Other spectroscopic techniques. -- Infrared data (υ_{max}) were recorded on a Bomen MB-100 instrument; normally, only bands of particular structural importance are listed. Mass spectra were obtained with VG-Analytical-7070E or KRATOS-Concept-IIH mass spectrometers by the chemical ionization (c.i.) mode using ether as the ionizing gas, and some by the fast atom bombardment (f.a.b.) mode using glycerol as the matrix.

Optical rotations. -- Optical rotations ([α] values) were determined at ambient

temperature (usually about 25°) in a Perkin Elmer polarimeter, model 241, and refer to chloroform solutions unless otherwise stated.

Melting points were determined by use of capillaries in an electrically-heated aluminum block apparatus (Gallenkamp) and are uncorrected.

Microanalyses were performed by M-H-W Laboratories, Phoenix, Arizona.

SECTION I

Experimental. Section I

Unless otherwise noted, the following solvent combinations (v/v) were used for thin-layer and column chromatography on silica gel: EtOAc-hexanes, (A) 1:100, (B) 1:50, (C) 1:30, (D) 1:20, (E) 1:10, (F) 1:6, (G) 1:4, (H) 1:3, (I) 1:2, and (J) 1:1; (K) 1:5 MeOH-EtOAc; (L) 1:10 MeOH-CH_2Cl_2; (M) 1:5 MeOH-$CHCl_3$; and H_2O-MeOH-EtOAc, (N) 4:5:10, and (O) 4:5:30.

Methyl 2,3,4-tri-O-acetyl-6-deoxy-β-D-gluco-heptopyranosiduronic acid **18**.

Methyl 2,3,4-tri-O-acetyl-6-O-(p-tolylsulfonyl)-β-D-glucopyranoside[19] **17** (1.42 g, 3 mmol) was treated with NaFp (4 mmol) in oxolane (40 mL), as described[19] for the preparation of the methyl ester of **18**, except that water (30 mL) and Br_2 (1.4 mL) were used in the step of oxidative carbonyl insertion. The latter step was complete after a few minutes (t.l.c., solvent J). In the work-up procedure[19], washing of the ethyl acetate solution of the crude products with aqueous $NaHCO_3$ was omitted and flash chromatography was performed with solvent I. Compound **18** (R_F 0.45, solvent O) was obtained as a syrup that crystallized from ethyl acetate-hexane to give 483 mg (46.3%) of pure material, m.p. 167-168°, $[\alpha]_D$ - 12° (c 0.7, chloroform). Mass spectrum (c.i.): m/z 317 (98%, $[M - OMe]^+$), 289 (22%, $[M - OAc]^+$), 257 (21%, $[M - OMe - AcOH]^+$), 229 (7.5%, $[M + 1 - 2\ AcOH]^+$). N.m.r. data: ^1H, δ 5.18 (~t, $J_{2,3} \approx J_{3,4} \approx 9.5$ Hz, H-3), 4.94 (dd, $J_{1,2}$ 7.9, $J_{2,3}$ 9.7 Hz, H-2),

4.90 (dd, $J_{3,4}$ 9.5, $J_{4,5}$ 10 Hz, H-4), 4.33 (d, $J_{1,2}$ 7.9 Hz, H-1), 3.93 (ddd, $J_{5,6}$ 4.8, $J_{5,6'}$ 7.7, $J_{4,5}$ 10 Hz, H-5), 3.45 (s, 3 H, OMe), 2.59 (m, 2 H, H-6,6'), 2.03, 2.01, and 1.98 (3 s, 3 H each, 3 OAc); ^{13}C, δ 175.3 (CO_2H), 170.1, 169.5, 169.3 (MeCO), 101.5 (C-1), 72.8, 71.5, 71.4, 70.0 (C-2,3,4,5), 57.0 (OMe), 36.7 (C-6), 20.8, 20.7, 20.7 (COMe).

Anal. Calc. for $C_{14}H_{20}O_{10}$ (348.3): C, 48.27; H, 5.79. Found: C, 48.05; H, 5.87.

Methyl 6-deoxy-β-D-gluco-heptopyranosiduronic acid **19**.

The triacetate **18** (100 mg) was treated with methanolic 0.05 M sodium methoxide (10 mL) during 1 hour. Deionization with Amberlite IR-120 (H^+) resin and concentration of the solution gave a colourless syrup that crystallized from methanol-ethyl acetate to afford **19** (59 mg, 92.5%), m.p. 172-173°, $[\alpha]_D$ - 20° (c 0.45, methanol). N.m.r. data (D_2O): ^1H (200 MHz), δ 4.42 (d, $J_{1,2}$ 8 Hz, H-1), 3.83 (dt, $J_{5,6}$ 3.1, $J_{4,5} = J_{5,6'} = 9.6$ Hz, H-5), 3.56 (s, 3 H, OMe), 3.5-3.3 (m, 3 H, H-2,3,4), 2.99 (dd, $J_{5,6}$ 3.1, $J_{6,6'}$ 15.9 Hz, H-6), 2.55 (dd, $J_{5,6'}$ 9.7, $J_{6,6'}$ 15.9 Hz, H-6'); ^{13}C (50.29 MHz), δ 178.6 (CO_2H), 106.3 (C-1), 78.6, 76.2, 75.9, 75.3 (C-2,3,4,5), 60.1 (OMe), and 39.7 (C-6).

Anal. Calc. for $C_8H_{14}O_7$ (222.2): C, 43.24; H, 6.35. Found: C, 43.16; H, 6.38.

(2,3,4-Tri-O-acetyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4,-tri-O-acetyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid **16**.

2,3,4,2',3',4'-Hexa-O-acetyl-6,6'-di-O-p-tolylsulfonyl-α,α-trehalose[37] **7** (1.62g, 1.8 mmol) was reacted with a 3.3 mol excess of NaFp [from 2.13 g of $Fe(CO)_2Cp$ dimer, 12 mmol] in oxolane (60 mL) during 70 min. T.l.c. (solvent J) revealed no **7** but a faster-moving, yellow spot (visible without spraying) of the sugar-iron intermediate. A stream of CO was passed through the solution at 0°, and water (60 mL) followed by Br_2 (2 mL) was added. After 10 min, the solution was concentrated to a small volume and extracted repeatedly with ethyl acetate, and the combined extracts were washed with water, dried ($MgSO_4$), and concentrated. Column chromatography ($CHCl_3$, then with added methanol up to 2%) of the dark residue gave **16**, isolated as a yellow oil (1.35 g). Crystallization from ethyl acetate - light petroleum, after treatment with activated charcoal, and 3 recrystallizations from ethyl acetate-hexane gave colourless **16** (304 mg, 26%), which sintered at ~ 50° and melted indistinctly at 75-95°, $[\alpha]_D$ +112° (c 0.5, chloroform). Mass spectrum (+f.a.b.): m/z 651 (6%, $[M + 1]^+$), 317 (51%, $0.5[M - 16]^+$). N.m.r. data: ^1H, δ 5.49 (dd, $J_{2,3}$ 10, $J_{3,4}$ 9.2 Hz, H-3), 5.19 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 10 Hz, H-2), 5.09 (d, $J_{1,2}$ 3.8 Hz, H-1), 4.86 (t, $J \approx J_{4,5} \approx 10$ Hz, H-4), 4.33 (dt, H-5), 2.50 (m, 2 H, H-6,6'), 2.03, 2.01, and 1.99 (3s, 3 H each, 3 OAc); ^{13}C, δ 176.1 (CO_2H), 169.8, 169.7, 169.4 (MeCO), 90.6 (C-1), 71.5, 70.3, 69.0, 65.8 (C-2,3,4,5), 36.2 (C-6), 20.8, 20.8, 20.7 (COMe).

Anal. Calc. for $C_{36}H_{34}O_{19}$ (650.5): C, 48.00; H, 5.27. Found: C, 48.19; H, 5.43.

(6-Deoxy-α-D-gluco-heptopyranosyluronic acid)6-deoxy-α-D-gluco-heptopyranosid-uronic acid **5**.

Compound **16** (85 mg) was deacetylated at room temperature in methanol (4 mL) made alkaline (pH 9) with sodium methoxide. Deionization with Amberlite IR-120 (H^+) resin after 30 min and concentration of the solution gave colourless **5**, R_F 0.4 (t.l.c., solvent N), which crystallized on trituration with a little methanol and ethyl acetate. The white, somewhat hygroscopic powder (44 mg, 84%) had no distinct melting point but began to sinter near 60° and turned gradually into a glassy foam on heating above 90°; $[\alpha]_D$ +144° (c 1.6, water); υ_{max}^{Nujol} 3400-3100, 2600, 1710 cm^{-1}. Mass spectrum (+f.a.b.): m/z 399 (7%, $[M + 1]^+$); m/z (-f.a.b.) 397 (44%, $[M - 1]^-$; m/z (c.i.) 381, 363, and 345 (9, 2, and 4%, $[M + 1 - 1, 2, \text{and } 2H_2O]^+$), 265 (23%), 191 (100%, $0.5[M - 16) - 2H_2O]^+$). N.m.r. data (D_2O): ^1H, δ 5.20 (d, $J_{1,2}$ 3.85 Hz, H-1), 4.16 (dt, $J_{5,6}$ 2.9, $J_{4,5} = J_{5,6'}$ = 9.9 Hz, H-5), 3.88 (dd, $J_{3,4}$ 9.0, $J_{2,3}$ 9.9 Hz, H-3), 3.70 (dd, $J_{1,2}$ 3.9, $J_{2,3}$ 9.9 Hz, H-2), 3.35 (dd, $J_{3,4}$ 9, $J_{4,5}$ 9.9 Hz, H-4), 2.97 (dd, $J_{5,6}$ 2.9, $J_{6,6'}$ 15.8 Hz, H-6), 2.52 (dd, $J_{5,6'}$ 15.8 Hz, H-6'); ^{13}C (50.29 MHz), δ 176.6 (CO_2H), 93.6 (C-1), 74.0, 73.4, 72.1, 69.7 (C-2,3,4,5), 37.6 (C-6).

Anal. Calc. for $C_{14}H_{22}O_{13}$ (398.3): C, 42.21; H, 5.57. Found: C, 41.89; H, 5.65.

2,3,2',3'-Tetra-O-benzyl-4,6;4'6'-di-O-benzylidene-α,α-trehalose **21.**

The bisacetal[20c] **20** (7.7 g) and NaH (4.65 g of a 61% suspension in mineral oil, rinsed with toluene and added at 0°) in dry N,N-dimethylformamide (100 mL) were stirred for 3 hours at 25°. Benzyl bromide (14 mL) was then added and stirring continued overnight. Some conc., aqueous NH_3 was added to decompose residual NaH, and after 1 hours the mixture was diluted with water (500 mL) and extracted several times with toluene. The extract was washed with saturated, aqueous $NaHCO_3$ and water, dried, and evaporated. The residue crystallized from ether-hexane, giving **21** (10.95 g, 89%), m.p. 151-152°, $[\alpha]_D$ +51.3° (c 1, chloroform); lit.[20b] viscous syrup. ^{1}H-n.m.r.: δ 7.5-7.2 (m, Ph), 5.55 (s, PhCH), 5.11 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.99-4.69 (6 equally spaced lines for 2 partially overlapping AB-q, 4 H, 2 $PhCH_2$), 4.30-4.08 (m, 3H) and 3.71-3.55 (m, 3 H) for H-2,3,4,5,6,6'; ^{13}C-n.m.r.: δ 138.8, 138.1, 137.6 (C-1 of 3 Ph), 128.9-126.2 (multiple peaks, Ph), 101.3 (PhCH), 95.0 (C-1), 82.4 (C-4), 78.8, 78.7 (C-2,3), 75.4, 73.8 (2 $PhCH_2$), 69.0 (C-6), and 63.0 (C-5).

2,3,2',3'-Tetra-O-benzyl-α,α-trehalose **22**.

Compound **21** (10.7 g) was dissolved in MeOH (200 mL) containing I_2 (2 g), with addition of Me_2CO (20 mL) for complete dissolution. The mixture was boiled under reflux for 1 hour, cooled, and stirred with solid $Na_2S_2O_3$ (4 g) until it became colourless (~10 min). The residue obtained upon solvent evaporation was dissolved in CH_2Cl_2 and washed with aqueous $NaHCO_3$ solution and water. Evaporation of the dried solution gave **22** (7.13 g, 83.3%), crystallized from EtOAc-hexane; m.p. 199-200°, $[\alpha]_D$ +121° (c 0.6, chloroform); lit.[22] m.p. 186-188°, $[\alpha]_D$ +120°; lit.[20b] m.p. 186-189°, $[\alpha]_D$ +124°.

2,3,2',3'-Tetra-O-benzyl-6,6'-di-O-p-tolylsulfonyl-α,α-trehalose **23**.

Tosyl chloride (420 mg, 2.2 mmol) was added portionwise at 0° to a solution of **22** (750 mg, 1.0 mmol) in pyridine (5 mL, dried over CaH_2). After it was stirred overnight at room temperature, the mixture showed a single spot for **23** (R_F 0.6) in t.l.c. (solvent H). It was poured into ice water, and the product was extracted with CH_2Cl_2. The washed (M HCl followed by water), dried, and concentrated extract gave **23** (869 mg, 85%) upon crystallization by the addition of hexane; m.p. 146-147°, $[\alpha]_D$ +83.7° (c 0.7, chloroform), lit.[13k] $[\alpha]_D$ +70° for a syrupy product; ^{1}H-n.m.r.: δ 7.68 (d, J 8.4 Hz) and 7.32-7.22 (m) for aryl, 5.02 (d, $J_{1,2}$ 3.5 Hz, H-1), 4.85 (center of AB-q, 2 H, J 11.4 Hz, $PhCH_2$), 4.495 (center of AB-q, 2 H, J 12.1

Hz, $PhCH_2$), ~4.0, 3.76, and 3.46 (3 m, 2 H each, H-2,3,4,5,6,6'), 2.42 (s, 3 H, CH_3 of Ts), and 2.27 (d, $J_{4,OH}$ 3.2 Hz, OH-4); ^{13}C-n.m.r.: δ 144.8 (C-1 of Ts), 138.3 and 137.7 (C-1 of Ph), 132.7 (C-4 of Ts), 129.8-127.3 (multiple peaks, aryl), 94.2 (C-1), 80.0 and 78.8 (C-2,3), 75.3 and 72.7 (2 $PhCH_2$), 69.6 and 69.2 (C-4,5), 68.3 (C-6), and 21.7 (CH_3 of Ts).

Anal. Calc. for $C_{54}H_{58}O_{15}S_2$ (1011.1): C, 64.14; H, 5.78; S, 6.32. Found: C, 64.08; H, 5.88; S, 6.07.

2,3,4,2',3',4'-Hexa-O-benzyl-6,6'-di-O-p-tolylsulfonyl-α,α-trehalose **24**.

Compound **23** (5.0 g) was benzylated with benzyl bromide (2.4 mL) and NaH (780 mg of a 61% suspension in mineral oil) essentially as described for the benzylation of **20**, but in refluxing oxolane (200 mL) during 24 hours. Complete consumption of **23** (R_F 0.3) and formation of **24** (R_F 0.5) was seen by t.l.c. (solvent I). Decomposition of remnant NaH with some added MeOH, evaporation of the solvent, washing of a CH_2Cl_2 solution of the product with aqueous $NaHCO_3$ followed by water, and finally, column chromatography (solvent H) of the recovered crude product gave pure **24** as a colourless syrup (4.84 g, 82%), $[\alpha]_D$ +67.3° (c 0.5, chloroform), lit.[14d] $[\alpha]_D$ +73°; ^{1}H-n.m.r.: δ 7.67 (d, J 8.4 Hz) and 7.28-7.06 (m) for aryl, 4.96 (d, $J_{1,2}$ 3.4 Hz, H-1), 4.87, 4.58 and 4.56 (centers of 3 AB-q, 2 H each, J 10.9, 11.9, and 10.8 Hz, 3 $PhCH_2$), 4.1-3.75 (m, 4 H) and 3.5-3.4 (m, 2 H) for H-2,3,4,5,6,6' and 2.37 (s, 3 H, CH_3 of Ts); ^{13}C-n.m.r.: δ 145.0 (C-1 of Ts),

138.6, 137.9, 137.8 (C-1 of 3 Ph), 132.7 (C-4 of Ts), 129.9-127.4 (multiple peaks, aryl), 94.2 (C-1), 81.5 and 79.2 (C-2,3), 76.9 (C-4), 75.6, 75.2, and 73.0 (3 $PhCH_2$, 69.0 (C-5), 68.3 (C-6), and 21.7 (CH_3 of Ts).

Anal. Calc. for $C_{68}H_{70}O_{15}S_2$ (1191.4): C, 68.55; H, 5.92; S, 5.38. Found: C, 68.87; H, 5.97; S, 5.26.

Methyl [(methyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronate) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosid]uronate **25**.

(a) From **24**.-- Rigorously dried apparatus[19] and reagents, and careful exclusion of atmospheric moisture are crucial in this operation. The hexabenzyl ether **24** (3 g) was allowed to react under N_2 with sodium dicarbonyl-η^5-cyclopentadienylirone (NaFp) prepared from 2.67 g of $Fe(CO)_2Cp$ dimer (Aldrich Chemical Co.) in dry[19] oxolane (150 mL), exactly as detailed for the corresponding hexa-O-acetyl derivative, except that a longer reaction time (20 hours) was required for complete conversion of **24** (R_F 0.5) into the sugar-iron intermediate (R_F 0.7, yellow spot visible prior to spraying; t.l.c. with solvent I). A stream of CO was then bubbled through the solution, a slurry of I_2 (10 g) in MeOH (100 mL) was added portionwise at room temperature, and the mixture was stirred under CO for 20 hours. In t.l.c. the aforementioned yellow spot disappeared and a spot for **25** (R_F 0.55 visible upon spraying with 5% H_2SO_4 in EtOH, and heating) was seen. The solvent was evaporated and a solution of the residue in EtOAc was washed with

saturated aqueous $Na_2S_2O_3$ to remove I_2, followed by aqueous $NaHCO_3$ and water, dried, and concentrated. The material was chromatographed on SiO_2 (300 g) by use of solvent G to yield syrupy **25** (1.53 g, 62.7%), $[\alpha]_D$ +90.3° (c 0.8, chloroform), υ_{max}^{film} 1738 cm^{-1} (ester CO); ^{1}H-n.m.r.: δ 7.4-7.15 (m, Ph), 5.39 (d, $J_{1,2}$ 3.4 Hz, H-1), 5.00-4.55 (3 AB-q, 6 H, 3 $PhCH_2$), 4.345 (dt, H-5), 4.14 (t, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 3.59 (s, 3 H, OMe, partly overlapping dd for H-2), 3.31 (t, $J_{3,4} \approx J_{4,5} \approx$ 9.4 Hz, H-4), 2.70 (dd, $J_{5,6}$ 3, $J_{6,6'}$ 15.6 Hz, H-6), 2.36 (dd, $J_{5,6'}$ 9.3, $J_{6,6'}$ 15.6 Hz, H-6'); ^{13}C-n.m.r.: δ 138.9, 138.5, 138.5 (C-1 of Ph), 128.5-127.5 (multiple peaks, Ph), 90.6 (C-1), 81.4, 81.3 and 80.1 (C-2,3,4), 75.6, 74.9, and 73.0 (3 $PhCH_2$), 67.7 (C-5), 51.8 (OMe), and 37.1 (C-6).

Anal. Calc. for $C_{58}H_{62}O_{13}$ (967.1): C, 72.03; H, 6.46. Found: C, 71.90; H, 6.42.

(b) From **29**.-- A solution of diamide **29** (1.10 g of trihydrate, prepared[18] from dinitrile **28**) in MeOH (80 mL) was magnetically stirred with Amberlite IR-120(H^+) resin (30 g, washed with MeOH and dried prior to use), and gently boiled under reflux (oil bath, 80°) for 4 days. The resin was filtered off and washed successively with MeOH and EtOAc, the filtrate concentrated, and applied to a column (SiO_2). Elution with solvent G gave pure **25** (838 mg, 78%), identical with **25** obtained from **24** (^{1}H- and ^{13}C-n.m.r.).

(2,3,4-Tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosiduronic acid **26**.

A solution of diester **25** (1.00 g) in oxolane (20 mL), MeOH (10 mL), and aqueous, 20% KOH (10 mL) was kept at room temperature for 8 hours and then concentrated at reduced pressure (bath temperature, 40°) to remove organic solvent. The remaining, largely aqueous solution was carefully acidified to pH 2 (indicator paper) at 0° with 5% HCl and extracted with CH_2Cl_2. The extract was washed three times with water, dried, and evaporated, and the crude product purified by column chromatography (solvent L), to give syrupy **26** (960 mg) which from CH_2Cl_2 and hexane gave crystalline **26** (697 mg, 71.8%); m.p. 142-143°, $[\alpha]_D$ +96.7° (c 1, chloroform), υ_{max}^{KBr} 1713 cm^{-1} (CO); ^{1}H-n.m.r.: δ 7.3-7.1 (m, Ph), 5.35 (d, $J_{1,2}$ 3 Hz, H-1), 4.98-4.52 (m, 6 H, 3 $PhCH_2$), 4.30 (dt, H-5), 4.13 (t, $J_{2,3} \approx J_{3,4} \approx 9.3$ Hz, H-3), 3.58 (dd, $J_{1,2}$ 3.1, $J_{2,3}$ 9.6 Hz, H-2), 3.27 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 2.75 (dd, $J_{5,6} \approx 2$, $J_{6,6'} \approx 15$ Hz, H-6), 2.30 (dd, $J_{5,6'}$ 10.4, $J_{6,6'}$ 15.3 Hz, H-6'); ^{13}C-n.m.r.: δ 178.2 (CO), 138.7, 138.3, 138.3 (C-1 of Ph), 128.3-127.3 (multiple peaks, Ph), 90.1 (C-1), 81.1, 81.05, and 80.1 (C-2,3,4), 75.5, 74.8, and 72.9 (3 $PhCH_2$), 67.0 (C-5), and 37.1 (C-6).

Anal. Calc. for $C_{56}H_{58}O_{13}$ (939.1): C, 71.63; H, 6.23. Found: C, 71.69; H, 6.23.

2,3,4-Tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosylurononitrile 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosiduronononitrile **28** from **24**.

A solution of ditosylate **24** (4.0 g) in 0.5 M LiCN in N,N-dimethylformamide (20 mL; Aldrich Chemical Co.) was kept under N_2 for 20 hours at 85°, cooled, diluted with water (100 mL), and extracted with 1:1 EtOAc - Et_2O (3 x 75 mL). The extract was washed with water (2 x 25 mL), dried, concentrated, and subjected to column chromatography (solvent G), affording syrupy **28** (2.73 g, 91%), $[\alpha]_D$ +135.6° (c 1, chloroform), lit.[6] +132°. The ^1H- and ^{13}C-n.m.r. data were identical with those reported[18].

Octadecyl [(octadecyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronate) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosid]uronate **38**.

A mixture of diacid **26** (200 mg) in acetone (2 mL) and K_2CO_3 (32.4 mg) in water (1 mL) was evaporated to dryness at 40°, and the resulting salt was dried overnight in a high vacuum and then dissolved in dry Me_2SO (10 mL) together with octadecyl methanesulfonate[25] (221.4 mg). The mixture was heated under N_2 for 3 hours at 70°, then cooled, diluted with water, and extracted with ether (3 x 50 mL). The extract was washed with water, dried, concentrated, and purified by column chromatography (solvent E), to give **38** (236 mg, 76.7%) as a colourless syrup, $[\alpha]_D$ +62.5° (c 0.6, chloroform), υ_{max}^{film} 1736 cm^{-1} (ester CO); ^1H-n.m.r. (300

MHz, assignments confirmed by COSY): δ 7.4-7.15 (m, Ph), 5.43 (d, $J_{1,2}$ 3.4 Hz, H-1), 5.00-4.55 (3 AB-q, 6 H, 3 $PhCH_2$), 4.34 (dt, H-5), 4.13 (t, $J_{2,3} + J_{3,4}$ = 18.5 Hz, H-3), 3.99 (t, J 6.9 Hz, 2 H, H-1,1' of alkyl), 3.62 (dd, $J_{1,2}$ 3.4, $J_{2,3}$ 9.5 Hz, H-2), 3.31 (t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 2.69 (dd, $J_{5,6}$ 3.0, $J_{6,6'}$ 15.6 Hz, H-6), 2.37 (dd, $J_{5,6'}$ 9.3, $J_{6,6'}$ 15.9 Hz, H-6'), 1.54 (m, 2 H, H-2,2' of alkyl), 1.25 (m, large peak, internal CH_2 of alkyl), and 0.86 (t, terminal CH_3); ^{13}C-n.m.r.: δ 171.1 (CO), 138.8, 138.5, and 138.5 (C-1 of Ph), 128.3-127.4 (multiple peaks, Ph), 90.5 (C-1), 81.3, 81.25, and 80.0 (C-2,3,4), 75.5, 74.8, and 72.8 (3 $PhCH_2$), 67.5 (C-5), 64.8 (C-1 of alkyl), 37.0 (C-6), 32.0, 22.8, and 14.2 (terminal $CH_2CH_2CH_3$ of alkyl), 29.8-28.6 and 25.9 (internal CH_2 of alkyl).

Anal. Calc. for $C_{92}H_{130}O_{13}$ (1444.0): C, 76.52; H, 9.07. Found: C, 76.47; H, 8.96.

Octadecyl [(octadecyl 6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-heptopyranosid]uronate **32e**.

The benzyl derivative **38** (100 mg) dissolved in 1:1 EtOAc - EtOH (12 mL) was hydrogenated over 10% Pd-C (100 mg) during 10 hours at 3.5 kPa H_2 pressure and room temperature. The catalyst was filtered off and washed with oxolane, and the filtrate was evaporated. The product showed one major spot (R_F 0.45) in t.l.c. (solvent O), identical with that of a co-chromatographed authentic sample[25] of **38**, and faster-moving trace contaminants. The latter were removed by column

chromatography (solvent K), which gave solid **38** (44 mg, 70%); $[\alpha]_D$ +62.7° (c 1, chloroform), lit.[25] +61°; i.r. spectrum identical with that of authentic **38**.

Methyl (2RS,3RS)- and (2RS,3SR)-3-hydroxy-2-tetradecyloctadecanoates **43a** and **43b**.

Claisen condensation of methyl palmitate **41** was performed as described[131]. The resulting keto ester **42** showed the following n.m.r. data: ^{1}H, δ 3.69 (s, 3 H, OMe), 3.41 (t, J 7.4 Hz, H-2) 2.47 (m, 2 H) and 2.30 (m, 2 H) for H-4,4' of main chain and H-1.1' of 2-alkyl, 1.80 (m, 2 H), 1.60 (m, 2 H) and 1.22 (m, large peak) for internal CH_2 groups, and 0.85 (t, 2 terminal CH_3); ^{13}C: δ 205.5 (keto CO), 170.4 (ester CO), 59.0 (C-2), 52.2 (OMe), 41.9 (C-4), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 29.7-27.5 (multiple peaks), 24.7, and 23.5 (internal CH_2).

Borohydride reduction of the keto ester **42** and chromatographic separation of the diastereomeric hydroxy esters[131] furnished **43a** (R_F 0.4) and **43b** (R_F 0.55, t.l.c. with $CHCl_3$) in yields and with physical constants and ^{1}H-n.m.r. data in agreement with those reported; ^{13}C-n.m.r.: for **43a**, δ 176.2 (CO), 72.3 (C-3), 51.5 (OMe), 50.9 (C-2), 35.7 (C-4), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 29.7-29.4, 27.4, and 25.7 (internal CH_2); for **43b**, δ 176.1 (CO), 72.1 (C-3), 51.6 (OMe), 51.0 (C-2), 34.3 (C-4), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 29.7-29.4, 27.8, 26.9, and 25.9 (internal CH_2).

Methyl (2RS,3RS)-3-benzyloxy-2-tetradecyloctadecanoate **44a**.

To a stirred solution of **43a** (510 mg) in CH_2Cl_2 (5 mL) and cyclohexane (30 mL) was added benzyl trichloroacetimidate[31] (600 mg), followed by trifluoromethanesulfonic acid (0.2 mL). Upon overnight storage of the mixture, t.l.c. (solvent F) indicated complete replacement of **43a** (R_F 0.4) by **44a** (R_F 0.67). The reaction was quenched by addition of pyridine (1 mL), and the solution washed with water, dried, and evaporated to a syrup. This was triturated with hexane, whereby a white solid was formed. The filtrate therefrom was evaporated to dryness with repeated additions of water followed by acetone. Purification of the crude product by column chromatography (solvents A - E in sequence) furnished syrupy **44a** (550 mg, 92%) which, although contaminated by a small proportion of dibenzyl ether (^{1}H-n.m.r.: δ 4.55, s, $PhCH_2$), was sufficiently pure for further use. An analytical sample was freed from the Bn_2O impurity by repeated chromatography. ^{1}H-n.m.r.: δ 7.30-7.26 (m, Ph), 4.47 (AB-q, 2 H, J 11.4 Hz, $PhCH_2O$-3), 3.64 (s, 3 H, OMe), 3.61 (m, 1 H, H-3), 2.65 (quintet, 1 H, H-2), ~1.5(m) and 1.24 (large peak) for internal CH_2, and 0.86 (t, 6 H, J 6.3 Hz, 2 terminal CH_3); ^{13}C-n.m.r.: δ 175.3 (CO), 138.6 (C-1 of Ph), 128.4-127.5 (multiple peaks, Ph), 80.5 (C-3), 72.1 ($PhCH_2$), 51.4 (OMe), 49.9 (C-2), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 30.9, 29.7-29.4, 27.9, 27.7, and 24.5 (internal CH_2).

Anal. Calc. for $C_{40}H_{72}O_3$ (601.0): C, 79.94; H, 12.08. Found: C, 79.63; H, 11.92.

Methyl (2RS, 3SR)-3-benzyloxy-2-tetradecyloctadecanoate **44b**.

Prepared from **43b** in 93% yield as just described for **44a**, **44b** gave an ^{1}H-n.m.r. spectrum very similar to that of its diastereomer; ^{13}C-n.m.r.: δ 174.9 (CO), 138.4 (C-1 of Ph), 128.3, 127.8, and 127.6 (Ph), 80.0 m(C-3), 72.0 ($PhCH_2$), 51.4 (OMe), 49.7 (C-2), 31.9, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 32.1, 29.7-29.4, 28.5, 27.9, and 25.3 (internal CH_2).

Anal. Calc. for $C_{40}H_{72}O_3$ (601.0): C, 79.94; H, 12.08. Found: C, 80.07; H, 11.89.

(2RS,3SR)-3-Benzyloxy-2-tetradecyloctadecanol **45a**.

Compound **44a** (300 mg) and $LiAlH_4$ (80 mg) were boiled for 4 hours in refluxing oxolane (10 mL). To the cooled mixture was added EtOAc to decompose excess reductant, and the solution was filtered through Celite and evaporated to give a residue that was triturated with hexane. Insoluble parts were filtered off and the filtrate was washed with water, dried, and concentrated for column chromatography, performed by sequential use of solvents B - E, which gave oily **45a** (240 mg, 84%); ^{1}H-n.m.r.: δ 7.33-7.28 (Ph), 4.51 (AB-q, 2 H, J 11.3 Hz, $PhCH_2$), 3.83 and 3.55 (2 dd, 1 H each, H-1,1'), 3.51 (m, H-3), 2.81 (br. s, OH), 1.89 (m, H-2), 1.62 (m), 1.25 (large peak), and 1.15 (m) for internal CH_2, and 0.87 (t, 6 H, J 6.1 Hz, 2 terminal CH_3); ^{13}C-n.m.r.: δ 138.3 (C-1 of Ph), 128.4, 127.8, and

127.7 (Ph), 83.3 (C-3), 72.2 ($PhCH_2$), 63.3 (C-1), 42.6 (C-2), 32.0, 22.7, and 14.1 (terminal $CH_2CH_2CH_3$), 31.3, 29.9-29.4, 28.7, 27.4, and 25.2 (internal CH_2).

Anal. Calc. for $C_{39}H_{72}O_2$ (573.0): C, 81.75; H, 12.67. Found: C, 81.63, H, 12.55.

(2RS,3RS)-3-Benzyloxy-2-tetradecyloctadecanol **45b**.

Compound **45b** was prepared in 87% yield from **44b** as just described for **45a**; ^{1}H-n.m.r. (300 MHz): δ 7.34-7.24 (Ph), 4.55 (AB-q, 2 H, J 11.4 Hz, H-1'), 3.51 (m, H-3), 2.96 (br., OH), 2.00 (m- H-2), 1.58 (m), 1.44 (m), 1.24 (large peak), and 1.15 (m) for internal CH_2, and 0.86 (t, 6 H, J 6.6 Hz, 2 terminal CH_3); ^{13}C-n.m.r.: δ 138.2 (C-1 of Ph), 128.4, 128.0, and 127.8 (Ph), 82.8 (C-3), 71.8 $PhCH_2$, 64.3 (C-1), 41.2 (C-2), 32.0, 22.8, and 14.2 (terminal $CH_2CH_2CH_3$), 29.9-29.4, 27.8, 27.1, and 26.4 (internal CH_2).

Anal. Calc. for $C_{39}H_{72}O_2$ (573.0): C, 81.75; H, 12.67. Found: C, 81.88; H, 11.66.

(2RS,3SR)-3-Benzyloxy-2-tetradecyloctadecyl[((2RS,3SR)-3-benzyloxy-2-tetradecyloctadecyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronate) 2,3,4,-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosid]uronate **46a**.

A reaction flask was charged with diacid **26** (100 mg, 0.107 mmol), alcohol **45a** (147 mg, 0.256 mmol), and Ph_3P (112 mg, 0.43 mmol). The contents were thoroughly dried in high vacuum and then dissolved in toluene (5 mL, dried over molecular sieves). Diisopropylazodicarboxylate (87 mg, 0.085 mL, 0.43 mmol) was added by syringe at 0°. After overnight storage of the mixture under N_2 at room temperature, a strong spot for **46a** (R_F 0.70) and a weak spot for unreacted **45a** (R_F 0.60) were seen in t.l.c. (solvent G). The residue obtained upon solvent evaporation was triturated with hexane (30 mL); insoluble material was filtered off, washed with hexane (50 mL), and discarded. The hexane filtrates were evaporated and the residue was subjected to column chromatography. Initial elution with solvent B removed unreacted **45a**, and continued elution with solvent D gave **46a** as a homogeneous oil (193 mg, 88.5%), $[\alpha]_D$ +45.1° (c 0.7, chloroform), υ_{max}^{film} 1735 cm^{-1} (ester CO), 1458, 1325, 1175, 1095, 1070, and 1002, and bands typical for Bn at 735 and 696 cm^{-1}; ^{1}H-n.m.r. (300 MHz): δ 7.4-7.1 (Ph), 5.48 (nm, H-1), 5.0-4.4 (4 partially overlapping AB-q, 8 H, 4 $PhCH_2$), 4.35 (dt, H-5), 4.13 (t, $J_{2,3} = J_{3,4} = 9.0$ Hz, H-3), 4.05 (m, 2 H, H-1,1' of octadecyl), 3.61 (dnm, H-2), 3.34-3.27 (m, 2H, H-4 of sugar and H-3 of octadecyl), 2.65 (dnm, H-6), 2.35 (dnm, H-6'), 1.82 (m, H-2 of octadecyl), and 1.6-0.8 region (same pattern as in **45a**); ^{13}C-n.m.r.:

δ 171.0 (CO), 138.9-138.5 (C-1 of PH), 128.3-127.3 (Ph), 90.4 (C-1), 81.3 (C-3 of octadecyl), 81.1, 80.0, and 79.2 (C-2,3,4), 75.5, 74.7, and 72.8 (3 $PhCH_2$ on sugar), 71.8, 71.7 ($PhCH_2$ on alkyl), 67.3 (C-5), 64.9 and 40.3 (C-1 and C-2 of octadecyl), 36.75, 36.7 (C-6), 32.0, 22.8, and 14.2 (terminal $CH_2CH_2CH_3$), 30.6, 29.8-29.4, 27.5, and 25.7 (internal CH_2).

Anal. Calc. for $C_{134}H_{198}O_{15}$ (2049.0): C, 78.55; H, 9.74. Found: C, 78.54; H, 9.77.

(2RS,3RS)-3-Benzyloxy-2-tetradecyloctadecyl[(2RS,3RS)-3-benzyloxy-2-tetra-decyl-octadecyl 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosyluronate) 2,3,4-tri-O-benzyl-6-deoxy-α-D-gluco-heptopyranosid]uronate **46b**.

(a) By the DIAD method.

Condensation of **26** (50 mg) with **45b** (73 mg) by the method just described for **46a** gave **46b** as an oil (96 mg, 88%), [α] +39.1° (c 0.8, chloroform), υ_{max}^{film} 1736 (ester CO), 1460, 1324, 1175, 1095, 1070, 734, and 697 cm^{-1}. The ^{1}C-n.m.r. spectrum (300 MHz) was very similar to that of **46a**, although there were slight differences in the patterns of the benzylic resonances (δ 5.05-4.45), the H, H' protons of octadecyl (δ 4.0-3.9) and the sugar H-6 and H-6' protons (δ 2.65 and 2.35). ^{13}C-n.m.r.: δ 171.1 (CO), 138.9-138.6 (C-1 of Ph), 90.4 (C-1), 81.3, 80.1, and 79.2 (C-2,3,4), 78.8 (unassigned), 75.5, 75.7, and 72.8 (3 $PhCH_2$ on sugar), 71.8,

71.6 ($PhCH_2$ on alkyl), 67.3 (C-5), 65.0 (C-1 of octadecyl), 40.1, 40.0 (C-2 of octadecyl), 36.8 (C-6), 32.0, 22.8, and 14.2 (terminal $CH_2CH_2CH_3$), 30.7, 30.5, 30.0, 29.8-29.4, 27.8, and 27.0 (internal CH_2).

Anal. Calc. for $C_{134}H_{198}O_{15}$(2049.0): C, 78.55; H, 9.74. Found: C, 78.48; H, 9.71.

(b) By the DCC method.

Thoroughly vacuum-dried **26** (150 mg, 0.16 mmol), **45b** (220 mg, 0.38 mmol), dicyclohexylcarbodiimide (78 mg, 0.38 mmol), and 4-dimethylamino-pyridine (10 mg) were dissolved at 0° in dry toluene (10 mL), under N_2. The mixture was kept for 4 hours at room temperature and then for 5 hours at 70°. After cooling, filtration, and evaporation of the solution a residue was obtained which showed **46b** (R_F 0.75) and two less-mobile components (R_F 0.55-0.5) in t.l.c. (solvent G). The products were separated by column chromatography. Initial elution with solvent D curiously produced first the component of intermediate t.l.c. mobility, which proved to be unreacted **45b** (43 mg), followed by a small amount of a mixture of **45b** and **46b**. Continued elution with solvent E gave **46b** as an oil (171 mg, 52%), followed eventually by the unidentified, slow-moving by-product (96 mg). The ^{1}H-n.m.r. spectrum of **46b** was identical with that of **46b** from section (a). The ^{1}H- and ^{13}C-n.m.r. spectra of the by-product were exceedingly complex, suggestive of an unequally substituted disaccharide derivative, and

exhibited features tentatively attributable to cyclohexyl groups.

(2RS,3SR)-3-Hydroxy-2-tetradecyloctadecyl [((2RS,3SR)-3-hydroxy-2-tetra-decyl-octadecyl-6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-hepto-pyranosid]uronate **47a**.

Compound **46a** (80 mg) dissolved in EtOAc (6 mL) and EtOH (6 mL) was hydrogenated over 10% Pd-C (100 mg) during 24 hours at 3.5 kPa H_2 pressure and room temperature. A very strong spot for **47a** (R_F 0.4) appeared in t.l.c. (solvent M), along with faster moving trace spots of incompletely debenzylated products. The catalyst was removed and washed well with $CHCl_3$, and the solution evaporated. Column chromatography of the amorphous residue was started by elution with $CHCl_3$, which removed the minor impurities, and continued by elution with solvent M, which produced pure **47a** as an amorphous solid (34 mg, 65%), $[\alpha]_D$ +42.6° (c 0.4, chloroform); υ_{max}^{KBr} 3348 (OH), 1728 (ester CO), 1461, 1149, 1074, 1044, and 989 cm^{-1}. (Benzyl bands at 735-696 cm^{-1} were absent.). No well-resolved n.m.r. spectra could be obtained.

Anal. Calc. for $C_{78}H_{150}O_{15}$ (1328.0): C, 70.55; H, 11.38. Found: C, 70.76; H, 11.21.

(2RS,3RS)-3-Hydroxy-2-tetradecyloctadecyl [((2RS,3RS)-3-hydroxy-2-tetradecyloctadecyl 6-deoxy-α-D-gluco-heptopyranosyluronate) 6-deoxy-α-D-gluco-heptopyranosid]uronate **47b**.

Compound **46b** (45 mg) was hydrogenated as just described for **46a**, to give **47b** as an amorphous solid (19.5 mg, 67%) after chromatographic purification; R_F 0.45 (solvent L), $[\alpha]_D$ +39.8° (c 0.8, chloroform); υ_{max}^{KBr} 3353 (OH), 1722 (ester CO), 1462, 1148, 1074, 1044, and 991 cm^{-1}. (Benzyl bands at 735-696 cm^{-1} were absent.) No well-resolved n.m.r. spectra could be obtained.

Anal. Calc. for $C_{78}H_{150}O_{15}$ (1328.0): C, 70.55; H, 11.38. Found: C, 70.37; H, 11.42.

SECTION II

Experimental. Section II

Unless otherwise noted, the following solvent combinations (v/v) were used for thin-layer and column chromatography on silica gel: (A) 30:5:4 EtOAC-MeOH-H_2O; EtOAc-hexanes, (B) 20:1, (C) 10:1, (D) 1:1, and (E) 1:20; (F) 1:9 EtOH-benzene; MeOH-$CHCl_3$, (G) 1:5, (H) 1:7, (I) 1:9, and (J) 1:99.

Heptakis(2,3-di-O-acetyl)cyclomaltoheptaose **13**.

Beta-cyclodextrin **2** was dried to constant weight at 110° over P_4O_{10} in vacuo, and tert-butyldimethylsilylated in dry pyridine (freshly distilled from CaH_2) essentially according to Fügedi[33c], except that 8.5 instead of 7.7 molar equivs. of the trialkylchlorosilane was employed. The chromatographically purified silyl ether **11** (yields, 80-85%) had m.p. 314-318° (dec.), $[\alpha]_D$ +107° (c 1, $CHCl_3$); lit.[33c] m.p. 314-318° (dec.), $[\alpha]_D$ +105° and[32b] m.p. 299-302°, $[\alpha]_D$ +113°. For complete acetylation of **11**, treatment[33c] with Ac_2O and pyridine for 1 day at 60° was not entirely sufficient (t.l.c.) in our hands, but subsequent heating for 4 hours at [32b] 100° led to completion, and amorphous peracetate **12**, purified by chromatography, was obtained in yields of 90% in several experiments; $[\alpha]_D$ +81.5° (c 1.2, $CHCl_3$), lit.[32b] +82° and[33c] +83°.

Compound **12** (1.60 g) was dissolved in CH_2Cl_2 (20 mL) and desilylated at

room temperature by addition of BF_3-Et_2O (2 mL) following the procedure of Takeo et al.[32b]. Complete replacement of the fast-moving **12** by **13** (R_f 0.3) was noted after 4 hours (t.l.c. with solvent A). The mixture was diluted with CH_2Cl_2 (80 mL) and washed sequentially with ice water, saturated aq. $NaHCO_3$, and water (50 mL of each). The dried (Na_2SO_4) organic phase was evaporated and the residue subjected to flash chromatography on SiO_2 with solvent G, to give crystalline **13** (0.92 g, 81%), m.p. 208-212° , $[\alpha]_D$ +114° (c 0.6, $CHCl_3$), lit.[32b] m.p. 184-186°, $[\alpha]_D$ +115°; ^{1}H-n.m.r. (300 MHz, $CDCl_3$ at 55°): δ 5.33 (dd, $J_{3,4}$ 8.4, $J_{2,3}$ 9.7 Hz, H-3), 5.11 (d, $J_{1,2}$ 3.9 Hz, H-1), 4.78 (dd, $J_{1,2}$ 3.9, $J_{2,3}$ 9.7 Hz, H-2), 4.24 (br, OH), 4.1-3.8 (m, 3 H, H-5,6,6'), 3.75 (~ t, $J_{3,4}$ + $J_{4,5}$ = 17.8 Hz, H-4), 205 and 2.03 (2 s, 3 H each, 2 OAc); ^{13}C-n.m.r. (75.43 MHz, $CDCl_3$): δ 170.4 and 169.1 (2 CO), 96.5 (C-1), 76.2 (C-4), 72.4, 71.2, 70.7 (C-2,3,5), 61.4 (C-6), 20.7 and 20.6 (2 MeCO). The ^{13}C values were slightly higher, by 0.1-1.5 (average, 0.6) p.p.m. than those obtained[32b] at 22.6 MHz from a $(CD_3)_2SO$ solution.

Anal. Calc. for $C_{70}H_{98}O_{49}$ (1723.5): C, 48.78; H, 5.73. Found: C, 48.64; H, 5.84.

Heptakis(2,3-di-O-acetyl-6-O-methylsulfonyl)cyclomaltoheptaose **15**.

Compound **13** was mesylated essentially as described[32b], with minor procedural modifications. Methanesulfonyl chloride (1 mL) was added dropwise, at 0°, to a solution of **13** (0.50 g) in dry pyridine (15 mL). The mixture was kept for 2 hours at 0° and 20 hours at room temperature, whereafter a single product spot (R_F 0.4) was seen in t.l.c. (solvent F). Water (1 mL) was then added, and after 15 min the mixture was poured into ice water. The precipitate was washed with water and dissolved in $CHCl_3$ (50 mL), and the solution was washed with 5% HCl (100 mL), aq. $NaHCO_3$ (100 mL), and water, dried, and evaporated. The crude product was passed through a short column of SiO_2 with solvent B, to give crystalline **15** (0.60 g, 91%), m.p. 210-213°, $[\alpha]_D$ +110.3° (c 0.6, $CHCl_3$); lit.[4] m.p. 165°, $[\alpha]_D$ +114°, and [32b] amorphous, $[\alpha]_D$ +108°. ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 5.34 (dd, $J_{3,4}$ 8.5, $J_{2,3}$ 9.8 Hz, H-3), 5.12 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.78 (dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.9 Hz, H-2), 4.60 and 4.15 (2 m, 2 and 1 H, H-5,6,6'), 3.78 (dd, $J_{3,4}$ 8.5, $J_{4,5}$ 9.3 Hz, H-4), 3.09 (s, 3 H, $MeSO_3$), 2.05 and 2.03 (2 s, 3 H each, 2 OAc), in reasonable agreement with reported[32b] 90-MHz data; ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 170.6 and 169.5 (2 CO), 96.6 (C-1), 75.9 (C-4), 70.1, 70.1, 69.4 (C-2,3,5), 68.3 (C-6), 37.1 ($MeSO_3$), and 20.5 (2 MeCO), in good accord with reported[32b] 22.6-MHz data.

Anal. Calc. for $C_{77}H_{112}S_7O_{63}$ (2270.2): C, 40.74; H, 4.97; S, 9.89. Found: C, 40.64; H, 5.02: S, 10.06.

Attempted heptamolar tosylation of **13**.

(a) Reaction at room temperature. To a solution of **13** (200 mg) in dry pyridine (15 ml) containing a catalytic amount of 4-dimethylaminopyridine was added a large excess of p-toluenesulfonyl chloride (930 mg, 6 mol. equivs.). The mixture was kept for 3 days at room temperature, whereafter a single product spot (R_F 0.45) was seen in t.l.c. (solvent F, double irrigation). A separate experiment had indicated no change to occur after prolonged storage. Conventional processing, and purification of the product by passage through a short column of SiO_2 by means of solvent B, gave a crystalline tosylate (245 mg) whose microanalytical data for C and S (found: C, 50.86; H, 5.23; S, 7.76) lay between those calculated for a heptakis-tosylate (C, 50.99; H, 5.04; S, 8.01) and hexakistosylate (C, 50.79; H, 5.10; S, 7.26). Although ^{13}C-n.m.r.(50.3 MHz, $CDCl_3$) did not show a signal for CH_2OH (expected near δ 61.0), there were six signals in the δ 70.2-68.8 range (C-2,3,5) instead of the three expected for uniformly substituted product, and the C-1 signal (δ 96.2) also had a small companion (δ ~ 96.3). The remaining resonances were at δ 170.4 and 169.4 (2 CO), 145.2, 132.5 130.0 and 128.1 (arom.), 75.3 (C-4), 21.4 (Me of Ts), and 20.5 (2 MeCO). The ^1H-n.m.r. spectrum showed resonances for the tosyl group (δ 7.75, 7.30, and 2.40) which integrated to slightly less than 7 H as measured against the 6-proton integral for the signals (δ 1.98 and 1.95) of the two OAc groups per glucose unit. Moreover, the tosyl CH_3 signal was not a clean singlet, and main resonances for

sugar ring protons (δ 4.15-3.55) were accompanied by some minor signals, indicating that the compound was not uniformly substituted.

(b) Formation of heptakis(2,3-di-O-acetyl-6-chloro-6-deoxy)cyclomaltoheptaose **17**.

When **13** (50 mg) and TsCl (230 mg) in dry pyridine (5 mL) containing a catalytic amount of 4-dimethylaminopyridine were heated for 40 hours at 60° and reaction mixture was conventionally worked up, the crude product was homogeneous in t.l.c. (solvent F). After purification on a short column of SiO_2 using solvent C, crystalline **17** (50 mg, 92%) was obtained, m.p. 180-182°, $[\alpha]_D$ +104° (c 1, $CHCl_3$); lit. [32b] m.p. 179-180° $[\alpha]_D$ +102°; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 5.31 (t, $J_{2,3} + J_{3,4}$ = 17.8 Hz, H-3), 5.17 (d, $J_{1,2}$ 3.5 Hz, H-1), 4.79 (dd, J1,2 3.5, $J_{2,3}$ 9.9 Hz, H-2), 4.2-3.75 (m, 4 H, H-4,5,6,6'), 2.08 and 2.06 (2s, 3 H each, 2 OAc); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 170.6 and 169.5 (2 CO), 96.4 (C-1), 77.0 (C-4), 70.7, 70.3, 70.1 (C-2,3,5), 44.3 (C-6), and 20.5 (2 MeCO).

Heptakis(2,3-di-O-acetyl-6-deoxy-6-thiocyanato)cyclomaltoheptaose **19**.

A solution of **15** (60 mg) and KSCN (200 mg) in dry N,N-dimethylformamide (3 mL) was heated for 12 hours at 90-100°. The cooled mixture was diluted with water (25 mL) and extracted with 1:1 toluene-EtOAc (50 mL). The extract was washed with water (2 x 25 mL), dried (Na_2SO_4),and evaporated to a solid that was passed through a short column of SiO_2 with EtOAc as the eluent, to afford crystalline **19** (45 mg, 85%), m.p. 194-196°, $[\alpha]_D$ +116° (c 0.65, $CHCl_3$); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 5.27 (dd, $J_{3,4}$ 7.7, $J_{2,3}$ 9.3Hz, H-3), 5.10 (d, $J_{1,2}$ 3.8 Hz, H-1), 4.86 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 9.4 Hz, H-2), 4.28 (m- H-5), 3.77 (dd, $J_{5,6}$ 2.8, $J_{6,6'}$ 13.0 Hz, H-6), 3.63 (dd, $J_{3,4}$ 7.6, $J_{4,5}$ 9.1 Hz, H-4), 3.22 (dd, $J_{5,6'}$ 8.9, $J_{6,6'}$ 13.0 Hz, H-6'), 2.10 and 2.04 (2 s, 3H each, 2 OAc); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 170.6 and 169.4 (2 CO), 112.3 (-S-CN; by contrast, -N=C=S resonates[39] near 131), 97.2 (C-1), 79.9 (C-4), 70.5, 69.8, 69.6 (C-2,3,5), 35.7 (C-6), and 20.5 (2 MeCO).

Anal Calc for $C_{77}H_{91}N_7O_{42}S_7$ (2011.1): C, 45.99; H, 4.56; N, 4.88; S, 11.16. Found: C, 46.09; H, 4.54; N, 5.00; S, 10.97.

Heptakis(2,3-di-O-allyl-6-O-tert-butyldimethylsilyl)cyclomaltoheptaose **21**.

To a chilled (0°) solution of silyl ether **11** (2.30 g; see under preparation of **13**) in dry N,N-dimethylformamide (70 mL) was added NaH (2.5 g of a 60% oil immersion, rinsed with hexane), and the mixture was stirred under exclusion of moisture for 2 hours at 0° and overnight at room temperature. Allyl bromide (13 mL) was then added, and stirring was continued for 2 days. The excess of NaH was decomposed by addition of a small amount of MeOH, and the mixture was concentrated to dryness at 60° (bath temperature) under reduced pressure. A solution of the residue in $CHCl_3$ (100 mL) was washed successively with water, aq. $NaHCO_3$, and water, then dried, and evaporated. Column chromatography of the crude product, using solvent E as the eluent, yielded syrupy **21** that was dried in vacuo to a brittle glass (2.50 g, 82%), $[\alpha]_D$ +76.4° (c 1, $CHCl_3$); ^{1}H-n.m.r. (300 MHz, $CDCl_3$, assignments aided by COSY): δ 6.06-5.85 (2 m, partially overlapping, 1 H each, 2 -CH=), 5.28-5.21 (narrow m and dd, 1 H each, H-3 of 2 allyl groups), 5.17 (d, $J_{1,2}$ 3.6 z, H-1), 5.08 (center of 2 dd, 1 H each, H-3' of 2 allyl groups), 4.49 and 4.23 (2 dd, 1 H each, O-CH_2- of 1 allyl group), 4.16-4.12 (m, 3 H, H-6 and O-CH_2 of 1 allyl group), 3.80 (t, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), 3.70 (t, $J_{2,3} + J_{3,4}$ = 18.2 Hz, H-3), 3.56 (center of 4 lines space ~10 Hz, 2 H, H-5,6'), 3.20 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 9.5 Hz, H-2), 0.85 (s, Me_3C), –0.01 and –0.013 (2 s, $SiMe_2$). ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 136.4 and 135.4 (2 -CH=), 116.6 and 115.9 (2 H_2C=), 98.2 (C-1), 80.1, 79.3 77.8 (C-2,3,4), 74.6 and 72.0 (2 allylic O-CH_2), 72.1 (C-5), 62.2 (C-6), 25.7 ($\underline{Me}_3C$), 18.0

($Me_3\underline{C}Si$), -5.1 and -5.5 (Me_2Si).

Anal. Calc. for $C_{126}H_{224}O_{35}Si_7$ (2495.7): C, 60.63; H, 9.05. Found: C, 60.66; H, 8.97.

Heptakis(2,3-di-O-allyl)cyclomaltoheptaose **22**.

A mixture of **21** (1.10 g) in oxolane (30 mL) and 1M Bu_4NF in oxolane (5 mL) was boiled under reflux for 1.5 hours, after which a single product spot (R_F 0.4) was seen in t.l.c. (solvent H, triple irrigation). The solvent was evaporated and the residue taken up in $CHCl_3$ (100 mL). Evaporation of the dried (Na_2SO_4) solution and column chromatography of the residue with $CHCl_3$ followed by solvent I as eluents gave syrupy **22** (726 mg, dried in a high vacuum), $[\alpha]_D$ +116° (c 0.8, $CHCl_3$). An analytical sample was solidified by trituration with ether, and dried for 8 hours at 110°; it then analyzed as a trihydrate, and on this basis the yield of **22** was ~94%. ^{1}H-n.m.r. data (300 MHz, $CDCl_3$, assignments aided by COSY): δ 6.00-5.82 (complex m, 2H, 2 -CH=), 5.29-5.08 (2 m, 4 H, 2 H_2C=), 5.05 (d, H-1), 4.7 (broad s, exchangeable, OH), 4.31 (center of 2 dd, 1 H each, J 5.6 and 12.5 Hz, O-CH_2 of 1 allyl group), 4.12 narrow m, 2 H, O-CH_2 of 1 allyl group), 3.93 (narrow m, H-6), 3.77 (narrow m, 2 H, H-5,6'), 3.68 (t, $J_{2,3} + J_{3,4}$ = 17.3 Hz, H-3), 3.54 (t, $J_{3,4} + J_{4,5}$ = 16.9 Hz, H-4), and 3.33 (dd, $J_{1,2}$ 3.7, $J_{2,3}$ 9.2 Hz, H-2); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): 136.1 and 135.2 (2 -CH=), 116.8 and 115.8 (2 H_2C=), 98.5 (C-1), 79.7, 79.6, 78.8 (C-2,3,4), 74.2 and 72.0 (2 allylic O-CH_2), 72.4 (C-5), and 61.35

(C-6).

Anal. Calc. for $C_{84}H_{126}O_{35}$• 3 H_2O (1749.9):C, 57.65; H, 7.60. Found: C, 57.62; H. 7.42.

Heptakis(2,3-di-O-allyl-6-O-methylsulfonyl)cyclomaltoheptaose **23**.

Methanesulfonyl chloride (0.6 mL) was added at -10° to a solution of **22** (300 mg) in dry pyridine (15 mL), which was then kept overnight at +5°, diluted with $CHCl_3$, washed sequentially with 5% HCl, aq. $NaHCO_3$, and water, and evaporated to dryness with addition of several portions of toluene. The crude product was purified by column chromatography (solvent J), to give crystalline **23** (300 mg, 79%), dec.p. 186-188°, $[\alpha]_D$ +93° (c 0.5 $CHCl_3$); ^{1}H-n.m.r. (300 MHz, $CDCl_3$, assignments aided by COSY): δ 6.02-5.82 (complex m, 2 H, 2 -CH=), 5.31-5.07 (complex m, 5 H, 2 CH_2= , and H-1 at δ 5.11), 4.54 (~s, 2 H, H-6,6'), 4.44 and 4.23 (2 dd, J 5.5 and 12.2 Hz, 1allylic O-CH_2), 4.15 (narrow m, 2 H, 1 allylic O-CH_2), 3.90 (d with broadened lines, $J_{4,5}$ 9.1 Hz, H-5, weakly coupled with H-6,6'), 3.67 (sept, 2 H, H-3,4), 3.33 (dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.3 Hz, H-2), 3.05 (s, 3 H, $MeSO_3$); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 135.9 and 134.0 (2 -CH=), 117.3 and 116.0 (2 CH_2=), 98.9 (C-1), 79.2, 79.0, 78.6 (C-2,3,4), 74.4 and 72.3 (2 allylic O-CH_2), 69.4 (C-5,6), and 36.8 ($MeSO_3$).

Anal. Calc. for $C_{91}H_{140}O_{49}S_7$ • 10 H_2O (2422.6): C, 45.11; H, 6.66; S, 9.26. Found: C, 45.11; H, 6.65; S, 9.23.

Heptakis(2,3-di-O-allyl-6-deoxy-6-iodo)cyclomaltoheptaose 24.

To a stirred solution of PPh_3 (7.06 g, dried over P_4O_{10}) in dry N,N-dimethylformamide (40 mL) was added I_2 (6.83 g) in small portions, followed after 1 hour by 22 (2.4 g). The mixture was stirred overnight under N_2 at 60-70°, after which it showed a major (R_F 0.31) and minor (R_F 0.23) spot in t.l.c. (CH_2Cl_2). After concentration in vacuo to a small volume, the mixture was diluted with CH_2Cl_2, washed successively with aq. $Na_2S_2O_3$ solution, aq. $NaHCO_3$, and water, dried and evaporated . Flash chromatography (with CH_2Cl_2) of the residue gave amorphous 24 (1.96 g, 56%), $[\alpha]_D$ +76.3° (c 1, $CHCl_3$); ^{1}H-n.m.r. (200 MHz, $CDCl_3$, assignments aided by COSY): δ 5.90 (12-line m, 2H, 2 -C=), 5.31-5.07 (m, 5 H; 2 H_2C=, and d for H-1 at δ 5.17), 4.45 and 4.20 (2 dd, 1 H each, J 5.4 and 12.1 Hz, 1 allylic O-CH_2), 4.16 (narrow m, 2 H, 1 allylic O-CH_2), 3.80-3.50 (m, 5 H, H-3,4,5,6,6'), and 3.33 (dd, $J_{1,2}$ 3.6, $J_{2,3}$ 9.6 Hz, H-2); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 135.6 and 134.8 (2 -CH=), 117.2 and 115.9 (2 H_2C=), 98.2 (C-1), 83.0 (C-4), 79.1, 78.7 (C-2,3), 74.4 and 72.2 (2 allylic O-CH_2), 70.1 (C-5), and 10.2 (C-6).

The compound was stable only under refrigeration; it developed a brown discoloration on storage at room temperature.

Cyclohepta(1→4)-(6-deoxy-α-D-gluco-heptopyranosid)urononitrile [**26**, heptakis(6-cyano-6-deoxy)cyclomaltoheptaose].

A solution of heptakis(6-deoxy-6-iodo)cyclomaltoheptaose[23] (**8**, 2.0 g) in 0.5 M LiCN in N,N-dimethylformamide (40 mL; Aldrich Chemical Co.) was kept for 1 day at 45°, then poured into water (850 mL). The fine suspension of the precipitate formed was allowed to settle overnight, and then filtered to give solid **26** that was washed with water and dried over P_4O_{10} in vacuo; yield, 930 mg (74%). Another experiment, using the same amounts of reagents, was conducted for 3 days at room temperature (28-30°) and gave 1.10g (87%) of crystalline **26**, dec.p. 276-278°, $[\alpha]_D$ +138° (c 0.7, DMF); i.r.: υ_{max}^{KBr} 3369 (OH), 2247 (CN) cm^{-1} ; ^{13}C-n.m.r. (75.4 MHz, DMSO-d_6): δ 118.0 (CN), 102.0 (C-1), 85.2 (C-4), 72.1, 71.7, 66.9 (C-2,3,5), and 20.5 (C-6). However, the crude **26** so obtained contained some inorganic impurity, presumably Li_2CO_3, as suggested by microanalytical data. A sample was purified by reprecipitation (DMF-water) and dried at room temperature in a high vacuum over P_4O_{10}; it then analyzed correctly as a tetrahydrate inclusion complex, showed an unchanged decomposition point, and $[\alpha]_D$ +169° (c 0.7, DMF).

Anal. Calc. for $C_{49}H_{63}N_7O_{28} \cdot 4\ H_2O$ (1270.1): C, 46.33; H, 5.63; N, 7.72. Found: C, 46.18; H, 5.37; N, 7.99.

In later experiments, **26** was obtained free from ash by pouring the reaction mixture into dilute HCl instead of water.

Cyclohepta(1→4)-(2,3-di-O-acetyl-6-deoxy-α-D-gluco-heptopyranosid)urononitrile **27**.

Crude nitrile **26** obtained from 1.00 g of **8** was treated with Ac_2O (5 mL), pyridine (5 mL), and catalytic amount of 4-dimethylaminopyridine during 30 hours at room temperature. The mixture was concentrated with additions of MeOH, taken up in $CHCl_3$, washed successively with 5% HCl, aq. $NaHCO_3$, and water, dried (Na_2SO_4), and brought to dryness. The crude product was crystallized from EtOH to give pure **27** (660 mg, 70.4% based on **8**), m.p. 200-205°, $[\alpha]_D$ +117° (c 1, $CHCl_3$); i.r.:υ_{max}^{KBr} 3481 and 1634 (weak) for H_2O, 2257 (CN), and 1750 (ester CO) cm^{-1}; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 5.26 (dd, $J_{3,4}$ 8.0, $J_{2,3}$ 9.6 Hz, H-3), 5.11 (d, $J_{1,2}$ 3.8 Hz, H-1), 4.90 (dd, $J_{1,2}$ 3.8, $J_{2,3}$ 9.6 Hz, H-2), 4.20 (m, H-5), 3.70 (~t, $J_{3,4} \approx J_{4,5} \approx$ 8.5 Hz, H-4), 3.00 (m, 2H, H-6,6'), 2.05 and 2.03 (2 s, 3H each, 2 OAc); ^{13}C-n.m.r. (75.4 MHz, $CDCl_3$): δ 170.3 and 169.6 (2 CO), 117.3 (CN), 96.7 (C-1), 79.5 (C-4), 70.0, 69.6, 67.6 (C-2,3,5), 22.2 (C-6), 20.8 and 20.7 (2 MeCO). The analytical sample was dried in vacuo over P_4O_{10} at room temperature but still retained water as inclusion complex.

Anal. Calc. for $C_{77}H_{91}N_7O_{42} \cdot 5\ H_2O$ (1876.6): C, 49.28; H, 5.43; N, 5.22. Found: C, 49.03; H, 5.00; N, 4.98.

Cyclohepta(1→4)-(2,3-di-O-benzoyl-6-deoxy-α-D-gluco-heptopyranosid)urononitrile 28.

Crude nitrile **26** obtained from 180 mg of **8** was dissolved in dry pyridine (5 mL) containing a catalytic amount of 4-dimethylaminopyridine, and treated overnight at room temperature with benzoyl chloride (0.8 mL). The mixture was poured into ice water, the product extracted with CH_2Cl_2, and the extract was washed with aq. $NaHCO_3$, 5% HCl, aq. $NaHCO_3$ again, and water, dried, and evaporated. Column chromatography (solvent D) of the crude product yielded pure **28** (194 mg, 77.3%), m.p. 220-225°, $[\alpha]_D$ +80° (c 1, $CHCl_3$); ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 7.5-7.0 (10 H, arom.), 5.92 (dd, $J_{3,4}$ 8.8, $J_{2,3}$ 10.1 Hz, H-3), 5.52 (d, $J_{1,2}$ 4.0 Hz, H-1), 5.12 (dd, $J_{1,2}$ 3.9, $J_{2,3}$ 10.2 Hz, H-2), 4.61 (m, H-5), 4.00 (~t, $J_{3,4} \approx J_{4,5}$ = 9 Hz, H-4), 3.31 and 3.20 (centers of 2 dd, AB system, $J_{5,6}$ 3.2, $J_{5,6'}$ 7.2, $J_{6,6'}$ 17.3 Hz, H-6,6'); ^{13}C-n.m.r. (75.4 MHz, $CDCl_3$): δ165.8 and 164.6 (2 CO), 134-128 region (Ph), 117.4 (CN), 97.3 (C-1), 80.1 (C-4), 71.0, 70.7, 68.1 (C-2,3,5), and 22.7 (C-6).

Anal. Calc. for $C_{147}H_{119}O_{42}N_7$ (2655.5): C, 66.48; H, 4.52; N, 3.69. Found: C, 65.98; H, 4.58; N, 3.67.

Cyclo(1→4)-(7-acetamido-2,3-di-O-acetyl-6,7-dideoxy-α-D-gluco-heptopyrano)-heptaose **29**.

A suspension of PtO_2 (140 mg) in water (50 mL) and 1M HCl (20 mL) was prehydrogenated, compound **26** (200 mg) was added, and hydrogenation was continued for 10 days at 4.5 atm H_2 pressure. The catalyst was filtered off over Celite and washed well with water, and the combined filtrate and washings were concentrated to give a residue (containing the amine **6**) which was treated with Ac_2O (10 mL) and pyridine (5 mL) for 2 days at room temperature. Concentration of the mixture and column chromatography (solvent G) of the crude product gave fractions (R_F 0.8 and 0.7, t.l.c. with solvent G) containing mixtures of unidentified products, followed by a fraction (R_F 0.6) of **29**, isolated as a white, crystalline solid (88 mg, 23%), m.p. 183-186°, $[\alpha]_D$ +38° (c 1, $CHCl_3$); υ_{max}^{Nujol} 3400-3150 (NH, H_2O), 1752 (ester CO), 1647 and 1559 (AMIDE I and II) cm^{-1}; ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ7.80 (bs, NH), 5.14 (dd, $J_{2,3}$ 8.8 $J_{3,4}$ 7.8 Hz, H-3), 5.00 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.75 (dd, $J_{1,2}$ 3.7, $J_{2,3}$ 8.8 Hz, H-2), 3.94 (broadened t, $J_{4,5} \approx J_{5,6} \approx 8$, $J_{5,6'} \approx 1$ Hz, H-5), 3.60 (m, 1H, H-7), 3.38 (dd, $J_{3,4} \approx J_{4,5} \approx 7.8$ Hz, H-4), 3.09 (m, 1H, H-7'), 2.30 (m, 1H, H-6), 2.06, 2.00, and 1.97 (3s, 3 H each, 3 Ac), and 1.75 (m, 1H, H-6'); ^{13}C-n.m.r. (75.4 MHz, $CDCl_3$); δ171.8, 170.7, and 169.4 (3 CO), 97.4 (C-1), 81.4 (C-4), 70.6, 69.9, 69.9 (C-2,3,5), 36.5 (C-7), 31.5 (C-6), 22.82 (MeCOO) and 22.80 (MeCONH).

Anal. Calc. for $C_{91}H_{133}N_7O_{49} \cdot 6\ H_2O$ (2217.1): C, 49.29; H, 6.59. Found: C, 49.46; H, 6.66.

SECTION III

Experimental Section III

Unless otherwise noted, the following solvent combinations (v/v) were used for thin-layer and column chromatography on silica gel: EtOAc-hexanes, (A) 1:2 and (B) 1:1; MeOH-$CHCl_3$, (C) 5:95, (D) 1:9, and (E) 1:5; and H_2O-MeOH-EtOAc, (F) 4:5:30, and (G) 4:5:15.

Methyl 2,3,4-tri-O-acetyl-6-deoxy-α-D-manno-heptopyranosiduronic acid **5**.

Methyl 2,3,4-tri-O-acetyl-6-O-(p-tolylsulfonyl)-α-D-mannopyranoside[10] **3** (0.71g, 1.5 mmol) was treated with NaFp (2 mmol) in oxolane (30 mL), as described[7] for the preparation of the methyl ester of **8**, except that water (15 mL) and Br_2 (0.7 mL) were used in the step of oxidative carbonyl insertion. The latter step was complete after a few minutes (t.l.c. solvent B). In the work up-procedure[7], washing of the ethyl acetate solution of the crude products with aqueous $NaHCO_3$ was omitted, and flash chromatography (solvent A) of the dark residue gave **5** as a syrup (0.21 g, 36%). Mass spectrum (c.i): m/z 349 (5.1%, $[M + 1]^+$), 317 (71%, $[M - OMe]^+$), 289 (7.8%, $[M - OAc]^+$), 257 (14%, $[M - OMe - AcOH]^+$), 229 (3%, $[M + 1 - 2AcOH]^+$). N.m.r. data ($CDCl_3$): ^1H (200 MHz), δ 5.29 (dd, $J_{2,3}$ 3.3, $J_{3,4}$ 10 Hz, H-3), 5.19 (dd, $J_{1,2}$ 1.6, $J_{2,3}$ 3.3 Hz, H-2), 5.09 (t, $J_{3,4} + J_{4,5}$ = 19.9 Hz, H-4), 4.60 (d, $J_{1,2}$ 1.2 Hz, H-1), 4.22 (ddd, $J_{4,5}$ 9.7, $J_{5,6}$ 7.6, $J_{5,6'}$ 5.25 Hz, H-5), 3.38 (s, 3 H, OMe), 2.58 (m, 2 H, H-6,6'), 2.11, 2.01, and 1.95 (3 s, 3 H each, 3 OAc); ^{13}C (50.3 MHz), δ

176.4 (COOH), 170.2, 170.2, 170.1 (MeCO), 98.2 (C-1), 69.4, 68.8, 68.75, 66.4 (C-2,3,4,5), 55.2 (OMe), 36.5 (C-6), 20.6, 20.5, 20.4 (COMe).

Methyl 6-deoxy-α-D-manno-heptopyranosiduronic acid **6**.

(a) From **5**. -- The triacetate **5** (100 mg) was treated with methanolic 0.05M sodium methoxide (10 mL) during 1 hour. Deionization with Amberlite IR-120 (H^+) resin and concentration of the solution gave **6** as a colourless, amorphous solid (56 mg, 88.3%), $[\alpha]_D$ +71.6° (c 0.5, H_2O). Mass spectrum (c.i.): m/z 223 (0.7%, $[M + 1]^+$), 191 (18%, $[M - OMe]^+$), 173 (5.4%, $[M - OMe - H_2O]^+$). N.m.r. data (D_2O): ^1H (300 MHz), δ 4.71 (d, $J_{1,2}$ 1.6 Hz, H-1), 3.98 (td, $J_{5,6}$ 2.8, $J_{4,5} \approx J_{5,6'} \approx$ 10.2 Hz, H-5), 3.95 (dd, $J_{1,2}$ 1.8, $J_{2,3}$ 3.5 Hz, H-2), 3.78 (dd, $J_{2,3}$ 3.5, $J_{3,4}$ 9.7 Hz, H-3), 3.54 (t, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 3.43 (s, 3 H, OMe), 2.94 (dd, $J_{5,6}$ 2.8, $J_{6,6'}$ 15.4 Hz, H-6), 2.46 (dd, $J_{5,6'}$ 10.3 Hz, $J_{6,6'}$ 15.4 Hz, H-6'); ^{13}C (50.3 MHz, referenced to internal acetone signal at δ 29.8), δ 175.4 (COOH), 100.6 (C-1), 70.0, 69.6, 69.4, 68.9 (C-2,3,4,5), 54.3 (OMe), and 36.3 (C-6).

(b) From **9** -- A solution of methyl ester **9** (800 mg) in methanol (25 mL) and 10% NaOH (5 mL) was heated in a steam bath for one hour, after which a single product spot (R_f 0.3) was seen in t.l.c (solvent G). The solvent was evaporated and the residue was dissolved in water (20 mL). Deionization with Amberlite IR-120 (H^+) resin, treatment with activated charcoal, and evaporation of the solution gave

6 as a colourless amorphous solid (681 mg, 90.5%), identical with the product obtained from **5** according to n.m.r. spectra.

Methyl (methyl 2,3,4-tri-O-benzoyl-6-deoxy-α-D-manno-heptopyranosid)uronate **8**.

Compound **8** was prepared as described by Baer and Hanna[7], except by use of methyl 2,3,4-tri-O-benzoyl-6-O-p-tolylsulfonyl-α-D-mannopyranoside[10] **7** instead of the corresponding 6-iodo derivative as the starting material. It was obtained in 80% yield. Crystallized from ether-petroleum ether, it showed m.p. 135-137°, $[\alpha]_D$ -136° (c 1, chloroform); reported[7], m.p. 136.5-138°, $[\alpha]_D$ -135.6°.

Methyl (methyl 6-deoxy-α-D-manno-heptopyranosid)uronate **9**.

The tribenzoate **8** (3.70 g) in absolute methanol (100 mL) was treated with a catalytic amount of sodium methoxide (1 hour, 25°), after which it was seen by t.l.c. (solvent D) that **8** (R_F 0.9) was completely debenzoylated to **9** (R_F 0.25). Deionization with Amberlite IR-120(H^+) resin and concentration of the solution gave a crude product, which was passed through a short column of SiO_2 with solvent C, to give syrupy **9** (1.51 g, 94.7%), $[\alpha]_D$ +84.6° (c 2.6, chloroform). Mass spectrum (c.i.): m/z 237 (20%, $[M + 1]^+$), 205 (100%, $[M + 1 - MeOH]^+$). ^{1}H-n.m.r. (300 MHz, $CDCl_3$): δ 4.62 (d, $J_{1,2}$ 1.0, H-1), 4.16 (bs, 3 H, 3 OH), 3.96 (td, $J_{5,6}$ 2.9, $J_{4,5} \approx J_{5,6'} \approx$ 9.5 Hz, H-5), 3.90 (dd, $J_{1,2}$ 1.3, $J_{2,3}$ 3.2 Hz, H-2), 3.75 (dd, $J_{2,3}$ 3.1, $J_{3,4}$

9.4 Hz, H-3), 3.68 (s, 3 H, COOMe), 3.55 (t, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 3.36 (s, 3 H, OMe), 2.91 (dd, $J_{5,6}$ 2.9, $J_{6,6'}$ 15.7 Hz, H-6), 2.54 (dd, $J_{5,6'}$ 9.5, $J_{6,6'}$ 15.8 Hz, H-6'); ^{13}C-n.m.r. (50.3 MHz, $CDCl_3$): δ 172.6 (CO), 100.8 (C-1), 71.5, 70.6, 70.4, 68.6 (C-2,3,4,5), 54.8 (COOMe), 51.7 (OMe), 36.5 (C-6).

6-Deoxy-α,β-D-manno-heptopyranosiduronic acid **10**.

A solution of compound **6** (100 mg) in 4 M hydrochloric acid (10 mL) was boiled under reflux for 5 hours. Thereafter, t.l.c (solvent G) indicated complete consumption of **6** (R_F 0.3) but two ill-separated spots (R_F 0.2-0.23, ~1:1 ratio) representing **10** and an unknown product were seen. The acid was removed by coevaporation with added water and the residue was passed through a column of silica gel with solvent E, giving **10** (9 mg, syrup), along with 62 mg of a mixture of **10** and the unknown product. The pure **10** showed $[\alpha]_D = +8.1°$ (c 0.7, H_2O). N.m.r. data (D_2O): ^{1}H (300 MHz), δ 5.13 (d, 0.6 H, $J_{1,2}$ 1.7 Hz, H-1α), 4.91 (d, 0.4 H, $J_{1,2}$ 1.0 Hz, H-1β), 4.18 (td, 0.6 H, $J_{4,5} = J_{5,6'} = 9.8$, $J_{5,6}$ 3.0 Hz, H-5α), 3.96 (m, 1 H, H-2α,β), 3.85 (dd, 0.6 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.6 Hz, H-3α), 3.71 (td, 0.4 H, $J_{4,5} = H_{5,6'} = 9.5$, $J_{5,6'}$ 3.0 Hz, H-5β), 3.67 (dd, 0.4 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.6 Hz, H-3β), 3.55 (t, 0.6 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4α), 3.48 (t, 0.4 H, $J_{3,4} = J_{4,5} = 9.65$ Hz, H-4β), 2.95 (d of narrow t, 1 H, $J_{6,6'}$ 16 Hz, H-6α,β), 2.53 (dd, 0.4 H, $J_{5,6'}$ 9.5, $J_{6,6'}$ 16 Hz, H-6'β), and 2.52 (dd, 0.6 H, $J_{5,6'}$ 9.8, $J_{6,6'}$ 15.6 Hz, H-6'α). ^{13}C-n.m.r (50.3 MHz); δ 178.73 and 178.68 (COOH), 96.5 and 96.1 (C-1), 75.1, 75.0, 73.5, 73.0, 73.0, 72.0, 71.4, 71.4

(C-2,3,4,5), 39.4 (C-6).

Penta-O-acetyl-2-deoxy-D-manno-heptonic acid **11.**

A solution of 50 mg of the mixture of **10** with the unknown product in 5 mL water was cooled to 0°, and 50 mg of sodium borohydride was added in small portion with stirring. The reaction mixture was kept at room temperature for one hour and then deionized with Amberlite IR-120(H^+) until pH 2 was reached. After filtration, the solution was evaporated under reduced pressure to a thin syrup from which added methanol was repeatedly evaporated in order to remove the boric acid as volatile methyl borate. The resulting residue was stirred overnight at room temperature with acetic anhydride (3 mL) containing 2 drops of concentrated sulfuric acid. The mixture was diluted with water and extracted with chloroform. The extract was washed with water, dried, concentrated, and purified by column chromatography (solvent A), to give **11** (6 mg) as a colourless syrup (first fraction, R_F 0.4, t.l.c. with solvent F) ; ^{1}H-n.m.r.(200 MHz, $CDCl_3$) δ 5.35 (m, 3 H), 5.05 (m, 1 H), 4.20 (dd, $J_{6,7}$ 2.9, $J_{7,7'}$ 12.5 Hz, H-7), 4.06 (dd, $J_{6,7'}$ 5.1, $J_{7,7'}$ 12.5 Hz, H-7'), 2.58 (m, 2 H, H-2,2'), 2.07, 2.05, 2.03, 2.02 and 2.01 (s, 3 H each, 5 OAc).

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CLAIMS TO ORIGINAL RESEARCH

1. The novel bis (6-deoxy-heptopyranosiduronic acid **5** and its hexaacetate **16** were synthesized from 6,6'-di-O-tosyl-α,α-trehalose hexaacetate by a modification of the ironcarbonyl method of chain extension.

2. The protected hexa-O-benzyl diacid **26** was synthesized from 6,6'-di-O-tosyl-α,α-trehalose hexabenzyl ether by the ironcarbonyl method, or alternatively, by nucleophilic displacement with lithium cyanide, followed by elaboration of carboxyl groups from the generated nitrile functions.

3. The new "mirror" cord factors **47a** and **47b** were synthesized by esterification of the protected hexa-O-benzyl diacid with 3-O-benzylcorynomycolyl alcohol in the presence of dicyclohexylcarbodiimide, or alternatively, by the superior Mitsunobu method of esterification of the diacid, followed by debenzylation.

4. The corynomycolyl alcohols **45a** and **45b** were prepared for the first time, from synthetic corynomycolic acid.

5. The first cyclodextrin analog composed of heptose units, cyclo-(1→4)-(7-amino-6,7-dideoxy-α-D-gluco-heptopyrano)heptaose **6** (Section II), was synthesized by chain extension at the C-6 positions of β-cyclodextrin. Several new derivatives of

β-cyclodextrin were prepared in the course of this work.

6. A new approach to the synthesis of 3-deoxy-D-manno-2-octulosonic acid **1** (SECTION III), by the ironcarbonyl method of chain-elongation starting from methyl α-D-mannopyranoside was attempted, leading to penta-O-acetyl-2-deoxy-D-manno-heptonic acid **11**, a potential precursor for KDO.