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UNIVERSITÉ D'OTTAWA  
UNIVERSITY OF OTTAWA

To my family  
and  
Diane

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## Abstract

The new disaccharides, crystalline 6-deoxy- $\alpha$ -D-glucopyranosyl 6-deoxy- $\alpha$ -D-glucopyranoside (36) and the corresponding dicarboxylic acid (6-deoxy- $\alpha$ -D-glucopyranosyluronic acid) 6-deoxy- $\alpha$ -D-glucopyranosiduronic acid (5) were synthesized by chemical modification of  $\alpha$ ,  $\alpha$ -trehalose (1). Preparation of per-O-acetyl- $\alpha$ , $\alpha$ -trehalose 6,6'-ditriflate 15 from known 2,3,4,2',3',4'-hexa-O-acetyl- $\alpha$ , $\alpha$ -trehalose, followed by cyanide displacement in the former to generate the peracetylated dinitrile 16 and subsequent hydrolysis with alkaline hydrogen peroxide produced 5. The latter was acetylated to give the 2,3,4,2',3',4'-hexaacetate 12 of 5 which was subsequently reduced with borane-oxolane complex to furnish the disaccharide 36. This approach was also extended to synthesize the analogous 2,3,4,2',3',4'-hexabenzyl ether of the dinitrile 28 which was similarly hydrolysed, but only to the stage of the corresponding diamide. The peracetylated dicarboxylic acid 12 was converted to the dichloride and subsequently esterified with 1-octanol, to form the di-octyl ester. Similar esterification with  $C_{15}$ ,  $C_{16}$ ,  $C_{17}$  and  $C_{18}$  1-alkanols produced the analogous diesters. Removal of the acetyl groups was achieved by solvolysis using p-toluenesulfonic acid in the presence of the appropriate 1-alkanol at high temperature, but yields of the deacetylated diesters were low. A different route to these dialkyl bis(heptosiduronates) involved conversion of the dicarboxylic acid into its potassium salt 53 which reacted in acceptable yields with corresponding 1-alkanol mesylates. Synthesis of cord factor analog 55 via both approaches was attempted but difficulties were encountered that could not be overcome.

The very little-known mycolic acid chemistry field was expanded and as a result, three new mycolyl derivatives were obtained in high yields. 3-O-(2-Tetrahydropyranyl)mycolyl alcohol (**59**) was converted to its corresponding mesylate **63** which was subsequently solvolized to the mycolyl mesylate **64**.

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## **A. Introduction**

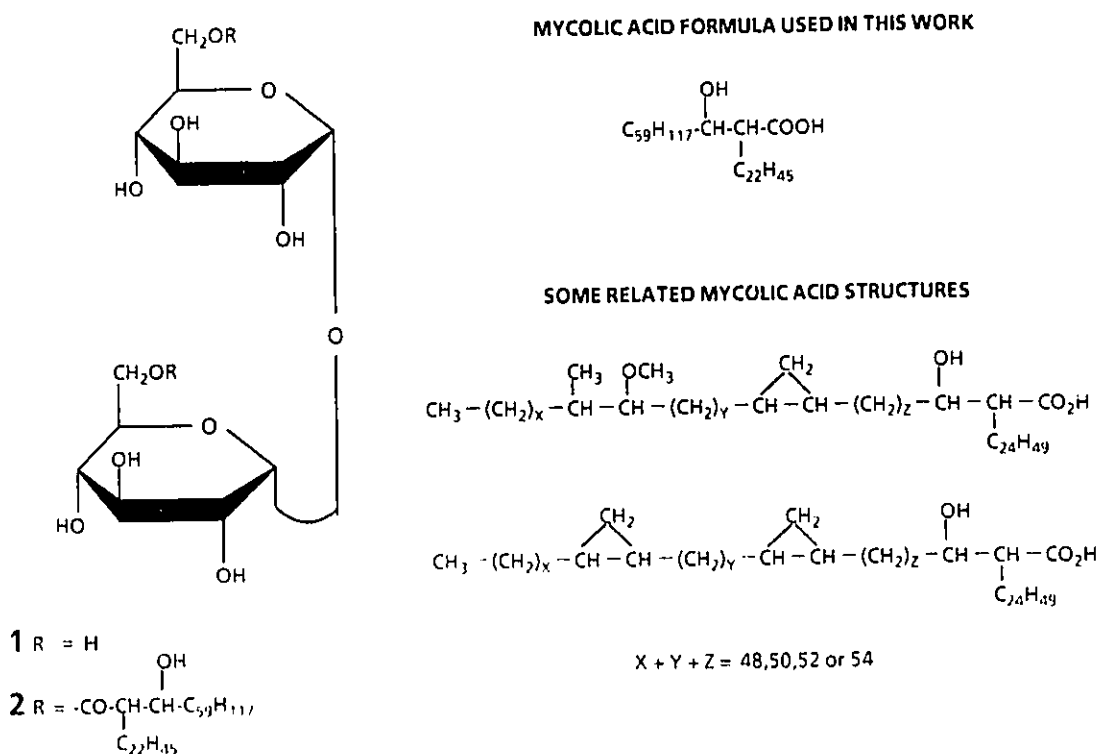
### **A.1. Some aspects of the problem of tuberculosis**

Tuberculosis is a chronic infectious disease in humans and animals caused by tubercle bacilli, *Mycobacterium tuberculosis*. 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<sup>1</sup>. 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 healthy individuals<sup>2</sup>.

It has been estimated that more than half of the human population is infected by *M. tuberculosis*. Most of the infected carry the bacilli throughout their entire lives, but only in a small proportion of people this infection progresses into the disease, tuberculosis, and these individuals become transmitters of tubercle bacilli to the non-infected. No means exist at present to eliminate the bacilli from the healthy infected individuals, and control of the spread of tuberculosis depends on early diagnosis and treatment of persons who are at high risk of developing the disease and thus becoming transmitters. Thus, contrary to a widespread popular belief, tuberculosis is still a serious health problem today.

## A.2. Background and objectives of this thesis

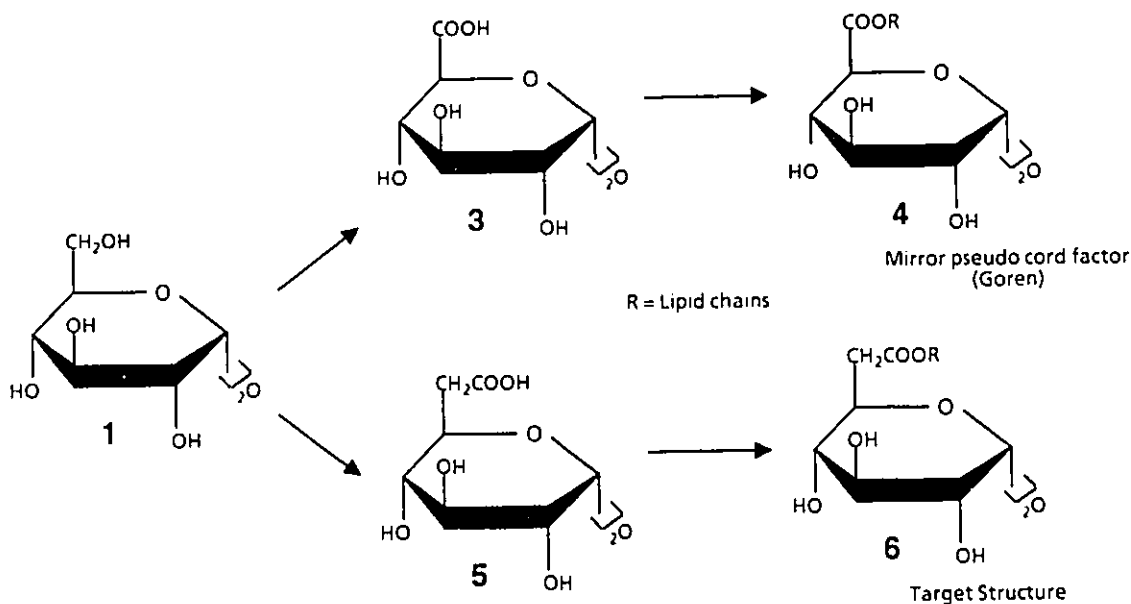
The objective of this project was to provide, by chemical synthesis, certain glycolipids which are expected to become useful tools for the study of biochemistry and immunochemistry of mycobacteria, in particular, *Mycobacterium tuberculosis*. A precise knowledge of the structures and biological functions 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 factor is the 6,6'-dimycolic acid ester 2 of  $\alpha,\alpha$ -trehalose 1 (Scheme 1) and is a characteristic lipid fraction extractable from mycobacteria, believed to be implicated in the morphology of virulent strains which grow in serpentine "cords".



Scheme 1

The mycolic acid used in this study (Scheme 1) is really a mixture of acids which contains approximately 84 carbon atoms and are high-molecular weight aliphatic  $\beta$ -hydroxy acids substituted at the  $\alpha$ -position with a long aliphatic chain and somewhere along the lengthier chain bears cyclopropane rings and a methoxy component<sup>34,37</sup>. Some related mycolic acid structures are also described.

Among the biological activities attributed to cord factor are: Inhibition of leucocyte migration<sup>3</sup>; toxic effects due to depression of NAD-dependent microsomal enzymes in various tissues<sup>4</sup>, apparently as a result of interaction of the cord factor with mitochondrial membranes<sup>5</sup>; effects on pyruvate metabolism and depression of muscle and liver glycogen synthesis<sup>6</sup>; granuloma formation<sup>7</sup>; immuno-stimulant properties and anti-tumor activity<sup>8,9</sup> through induction of the production of interleucin-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 relationships 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. Synthesis of cord factor analogs has been actively pursued in several laboratories<sup>10-12</sup>, with structural modifications 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<sup>13</sup>, of cord factor-like molecules in which the ester functionalities at C-6,6' of trehalose are regio-inverted, as shown in formula 4 (Scheme 2); such dialkyl (hexosyluronate hexosiduronate) structures have been termed "mirror pseudo cord factors". The purpose of the present study was to embark upon the synthesis of a novel type of "mirror" cord factor analogs 6. It is derived from a (hitherto unknown) dicarboxylic 5, namely bis-(6-deoxy- $\alpha$ -D)-gluco-heptosiduronic acid), a 2x7-carbon homolog of Goren's trehalosuronic acid 3.



**Scheme 2**

From a structural point of view, these 6-deoxyheptosyluronate 6-deoxyheptosiduronates **6** contain as the carbohydrate moiety, a 2x7-carbon framework, thus the latter would mimick the natural cord factor **2** more closely, yet possess the feature of a positionally inverted ester linkage.

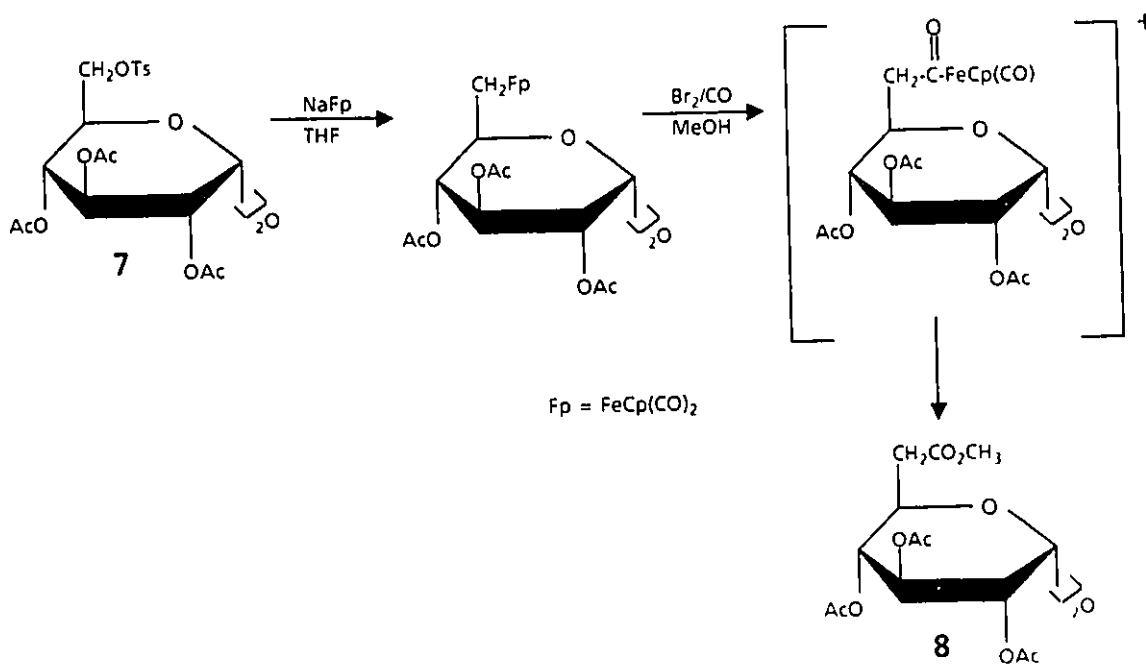
To this end, the bis-uronic acid **5** was first targeted. In our earlier attempts\*, two approaches starting from commercially available  $\alpha,\alpha$ -trehalose **1** were contemplated. The first was to make use of the methodology elaborated by Baer

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\* R.L. Breton, B.Sc. Thesis, University of Ottawa (1987).

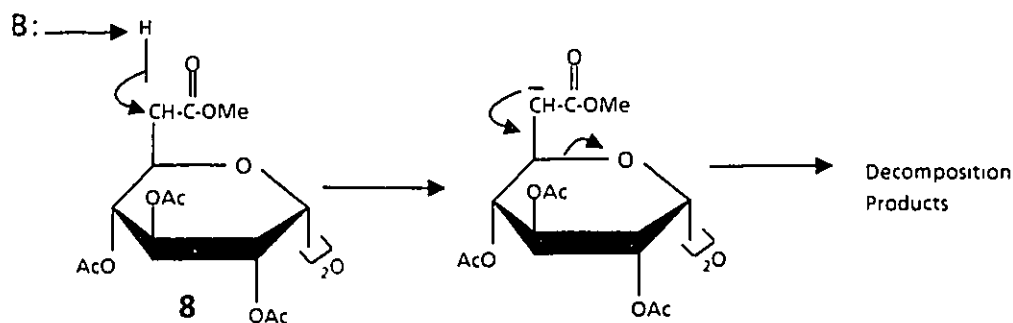
and Hanna<sup>14</sup> which involves chain elongation by ironcarbonyl chemistry, and the second approach employed an Arndt-Eistert<sup>15,16</sup> synthesis using the known trehalosuronic acid **3** as building block.

In the former route, hexa-*O*-acetyl- $\alpha,\alpha$ -trehalose 6,6'-ditosylate **7** was treated under strictly anhydrous conditions with sodium dicarbonyl- $\eta^5$ -cyclopentadienyliron (NaFp) obtained from dicarbonylcyclopentadienyliron dimer with sodium amalgam in tetrahydrofuran, which generated a sugar-iron compound (Scheme 3) that was not isolated but instead was treated in situ with bromine and methanol in the presence of carbon monoxide to effect carbonyl insertion and methanolysis. The dimethyl ester **8** was isolated crystalline in 42% yield.



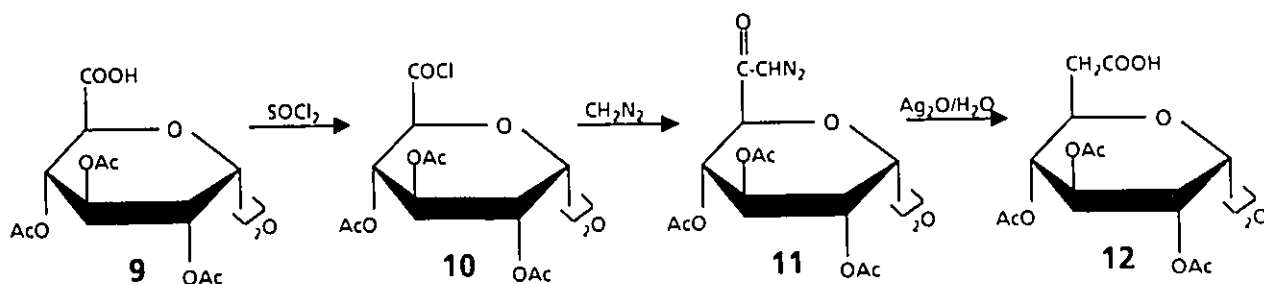
Scheme 3

In order to use **8** in synthesis of cord factor analogs, it must first be converted into the unprotected acid **5**. Conditions of acid hydrolysis of the methyl ester were not essayed because of the acid lability of the glycosidic bond. Several procedures for deprotection under basic conditions were therefore tried, including the standard Zemplén method of methoxide-catalyzed methanolysis which seldom entails any difficulties, and it was thought that **8** could be so deacetylated and thereby rendered water soluble; hydrolysis of the methyl esters could then be achieved by aqueous base. However, extensive degradation was observed and it had to be concluded that straightforward deacetylation of **8** was not feasible. A possible explanation for these difficulties may be that the hydrogen at C-6 in **8** is sufficiently activated by the neighbouring ester function to incur abstraction by the base, with degradation of the disaccharide by  $\beta$ -elimination ensuing (Scheme 4):



Scheme 4

The second approach investigated consisted of synthesizing the known trehalosuronic acid hexaacetate<sup>17</sup> **9**, and employing it for an Arndt- Eistert synthesis, also known as Wolff rearrangement (Scheme 5):



**Scheme 5**

The method consists of converting an acid into its next-higher homolog by insertion of a methylene group. The synthesis involves three operations: formation of an acid dichloride **10**, conversion of the latter into a diazoketone **11**, and rearrangement to the target molecule **12**. A yield of 65% of bis-diazoketone was obtained. The last step of the procedure, namely the molecular rearrangement of **11** to **12**, created a problem which could not be solved. Different catalysts and reaction conditions used in repeated trials afforded only large numbers of unidentified products.

Alternative routes to the bis-heptosiduronic acid **5** were therefore proposed. One of them, which was reasonably successful, was elaborated in parallel work in this laboratory\* and involved a modification of the aforementioned ironcarbonyl procedure: Use of water in place of methanol in the oxidative carbonyl insertion and solvolysis (**7**  $\rightarrow$  **8**) gave directly the acetylated dicarboxylic acid **12**, which could be

\*Doctoral studies by Y. Shen.

deacetylated to 5. A different route was successfully pursued in the present work, as will be described herein.

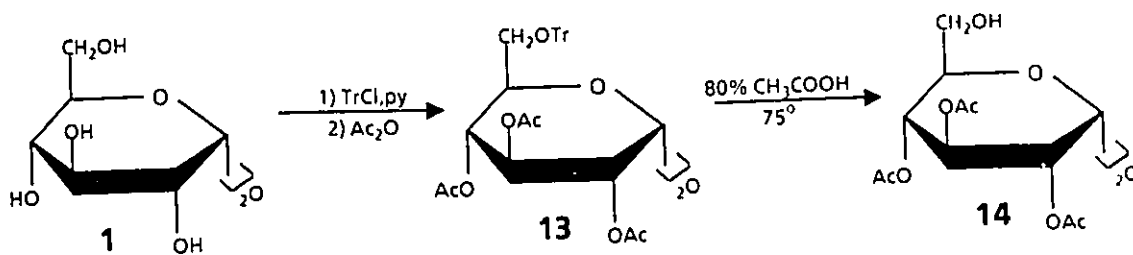
Once the dicarboxylic acid 5 and its hexaacetate 12 were in hand, the synthesis of long-chain fatty esters became the next goal of the project. Initially, simple alkanols of intermediate chain length ( $C_8$ - $C_{18}$ ) were to be attached before the more challenging task of attaching mycolic alcohol could be contemplated. The synthesis of such simple cord factor analogs as diesters containing, for example, hexadecyl or octadecyl residues was, however, to be considered as being more than a preliminary exercise in learning to master methodology. Relatively simple, trehalose-derived glycolipids do occur naturally in mycobacteria, and some exhibit interesting biological properties. Thus,  $\alpha,\alpha$ -trehalose 6,6'- dipalmitate has been isolated from tubercle bacilli and, like cord factor, shown to possess antitumor activity<sup>8,9</sup>. Recently, a 2,3- di-*O*-acyl- $\alpha,\alpha$ -trehalose 2'-sulfate containing palmitoyl and stearyl groups was isolated from *M.tuberculosis* in minute quantities and shown to be a highly specific antigen with great promise for the development of sensitive serodiagnostic assays<sup>2,18</sup>. Consequently, the preparation of "mirror" cord factor analogs with lipid chains of intermediate size appeared to be a worthwhile enterprise.

## B. Results and Discussion

### B.1. Synthesis of (6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronic acid) 6-deoxy- $\alpha$ -D-gluco-heptopyranosiduronic acid (5) and its precursors

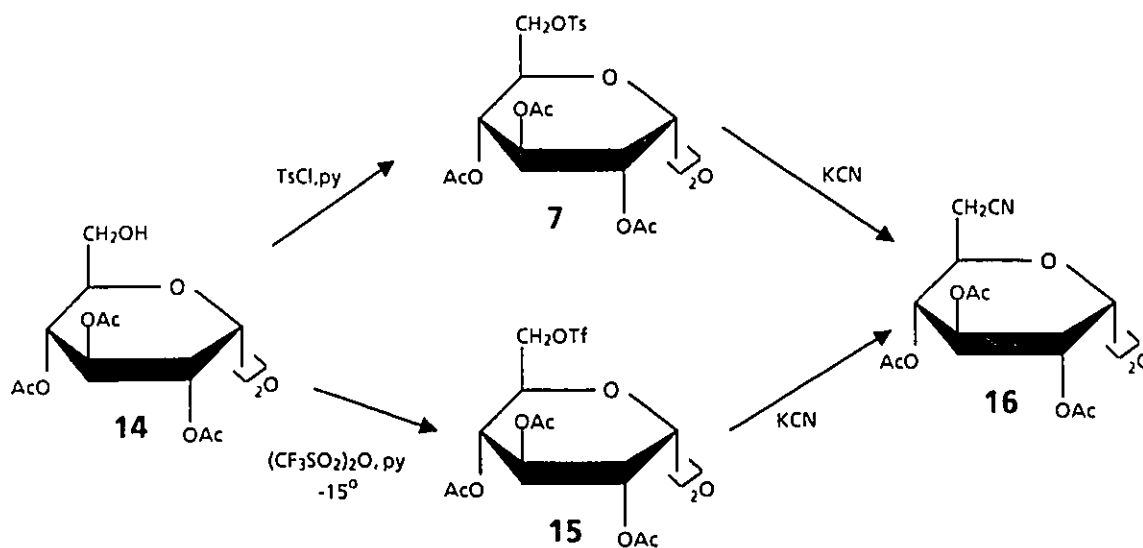
The study of the targeted cord factor analogs **6** required the initial synthesis of **5** due to the important C-6 deoxy position at both sugar residue of the latter which mimicks more closely the genuine cord factor than the "mirror" analogs **4** prepared by A. Liav and M.B. Goren<sup>13</sup>.

In this first approach, the first step involved straightforward conversion of  $\alpha,\alpha$ -trehalose **1** to the hexaacetate ditrityl derivative **13** followed by selective removal of the trityl groups which delivered known<sup>17</sup> trehalose 2,3,4,2',3',4'-hexaacetate **14** (Scheme 6).



Scheme 6

The purpose of preparing compound **14** was to execute a cyanide displacement of a good leaving group at both ends of the sugar units, to produce a dinitrile which would then be subjected to aqueous base hydrolysis. The leaving groups chosen for this task were the tosyl and triflyl group (Scheme 7).



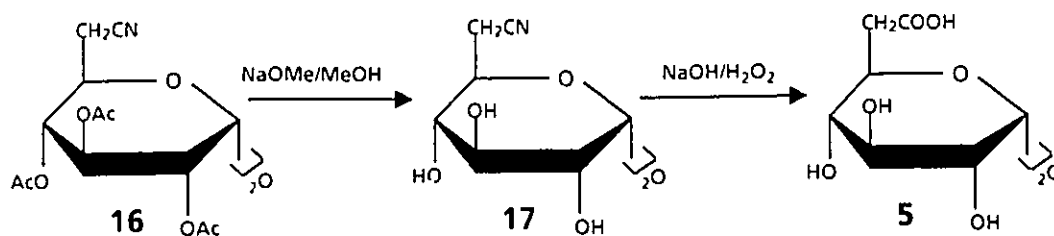
Scheme 7

Preliminary studies showed that the 6,6'-ditosylate\* 7, prepared from the sugar 14 by conventional tosylation, underwent reaction with potassium cyanide in a variety of solvent systems. However, mixtures of several products were always observed in t.l.c.. For this reason, 2,3,4,2',3',4'-hexa-O-acetyl-6,6'-di-O-(trifluoromethyl)sulfonyl-  $\alpha,\alpha$ -trehalose (15) was prepared as an alternative substrate for displacement. It was obtained in 79% yield by reaction of 14 with trifluoromethane sulfonic anhydride in the presence of pyridine during 15 min at -15°. The ditriflate demonstrated excellent cyanide displacement in 10% aqueous acetonitrile, to furnish the previously unknown dinitrile 16 in 81% yield after a

\*Prepared by co-worker.

reaction for 2 h at room temperature. Most importantly, the product showed a single spot in t.l.c. and could, after processing, be crystallized easily from methanol. Initial investigations employing many different solvent systems were carried out; facile displacement was observed also in dimethyl sulfoxide and N,N-dimethylformamide, but yields of **16** were erratic and generally lower than with aqueous acetonitrile. It was soon realized that very expeditious processing under mild conditions is essential to avoid decomposition of the sensitive **16**, and this was difficult with DMSO or DMF. It was feared at first that 10% aqueous acetonitrile used as the solvent for such a type of reaction might have an effect on the acetyl groups, because it is known that cyanide in the presence of water can be used for deacetylation purposes<sup>19</sup>. As it turned out, no deacetylation occurred within the reaction time required for complete detriflation of **15**, but after prolonged reaction times, t.l.c. indicated the presence of some slower-moving products suspected to originate from partial deacetylation. These small proportions of by-products were not further investigated.

Instead, much faster and cleaner deacetylation of **16** was achieved by use of a solution of methanolic methoxide, which rendered the material water-soluble by forming the corresponding hexaol **17** in quantitative yield. The latter was not characterized but used directly for the next step, namely, alkaline hydrolysis to the targeted diacid **5** (Scheme 8).

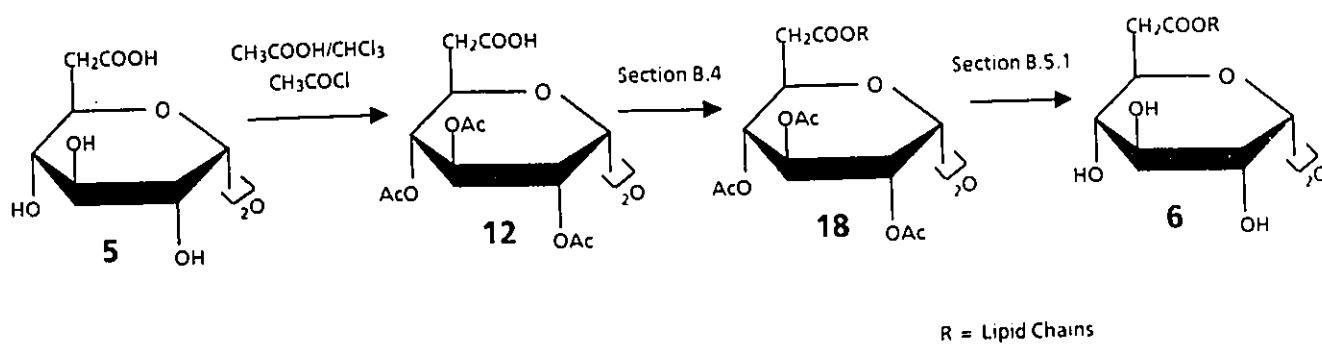


**Scheme 8**

The use of alkaline hydrogen peroxide was necessary in order to form compound **5** quantitatively. Straightforward base hydrolysis proceeded very poorly and showed extensive decomposition in t.l.c.. Thus, the addition of hydrogen peroxide<sup>20</sup> was compulsory. The new crystalline **5** was characterized by elemental and spectral analysis. 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 centered 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 centered 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. <sup>13</sup>C-n.m.r. and infrared spectroscopy afforded extra evidence (acid CO) which also confirmed the structure of the compound **5**.

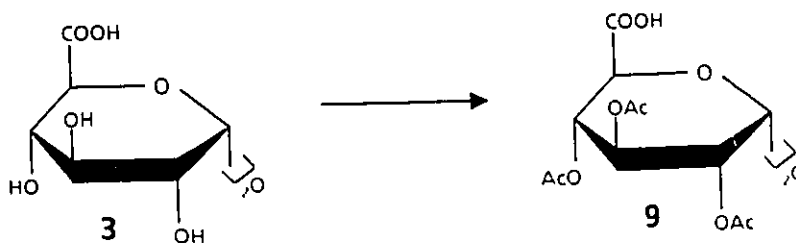
The attachment of lipid chains to the carbohydrate **5** by way of esterification of its carboxyl groups with alkanols required prior protection of the secondary alcohol functions. Acetyl groups<sup>13</sup> were chosen, and after esterification with various lipid alcohols, subsequent removal of the acetyl protecting groups would generate the desired cord factor analogs **6** (Scheme 9). Deacetylation with little or no loss of

lipid ester groups appeared to be a challenge, but should not present an insurmountable problem as such deacetylations had been accomplished in cord factor synthesis<sup>13</sup>.



Scheme 9

It was surprisingly difficult to acetylate compound 5. Attempts to peracetylate 5 with acetic anhydride-pyridine, or with acetic anhydride in the presence of sodium acetate invariably led to mixtures. The same problem had previously been encountered by M.B. Goren and K.S. Jiang<sup>21</sup> when they tried to acetylate their trehalosuronic acid 3 to give 9 (Scheme 10).



Scheme 10

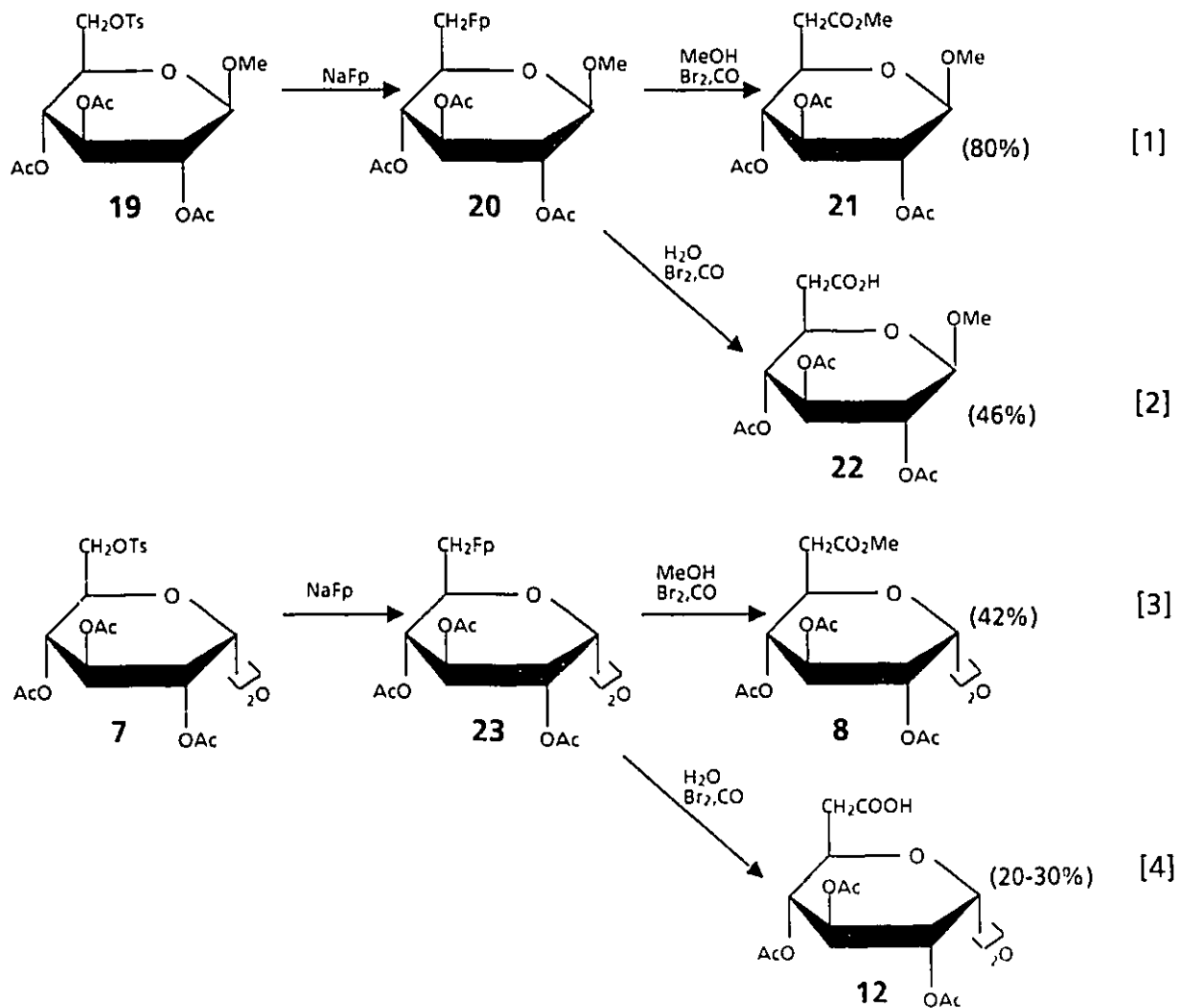
The authors explained that mixtures may have resulted from partial lactonization (i.r. evidence), and other components were probably acetylated, mixed anhydrides of **3** and acetic acid; some of the products may have had both features. In their case, rapid acetylation was achieved when the free acid **3** was dissolved (or suspended) in acetic anhydride and a trace of conc. sulfuric acid was added. The product **9** (64%) was generally obtained homogeneous in t.l.c. , but occasionally a (presumably) lactonized product(s) was obtained, as indicated by i.r. spectroscopy and by titration with alkali. However, this sulfuric acid procedure also failed to afford compound **12** in reasonable yield and again, a complex mixture of products was detected. The best results were obtained by dissolving **5** in a mixture of acetic acid/chloroform in the presence of acetyl chloride. After a 1-h reaction, the yield of **12** was 65% (Scheme 9). Chloroform was essential in order to prevent precipitation of not fully acetylated products, thus keeping the solution homogeneous.

Towards the synthesis of compound **12**, a second approach was elaborated, namely, chain elongation of the trehalose ditosylate **7** by the method using an iron carbonyl reagent, sodium dicarbonyl- $\eta^5$ -cyclopentadienyliron (NaFp)<sup>14</sup>. The method had first been elaborated<sup>14</sup> in various monosaccharides, e.g. **19** (Scheme 11, [1]) and had subsequently been shown\* to be applicable to the disaccharide derivative **7** (Scheme 11, [3]). Following these investigations, which had led to methyl esters (**21** and **8**, respectively), Y. Shen in this Laboratory examined the possibility of replacing methanol by water in the oxidative carbonyl insertion step. Model experiments using methyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl- $\beta$ -D-glucopyranoside (**19**) as the starting compound did furnish methyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\beta$ -D-*gluco*-heptopyranosiduronic acid (**22**) in a 46% yield. (Scheme 11, [2]). Applied to the

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\* R.L. Breton, B.Sc. Thesis, University of Ottawa (1987).

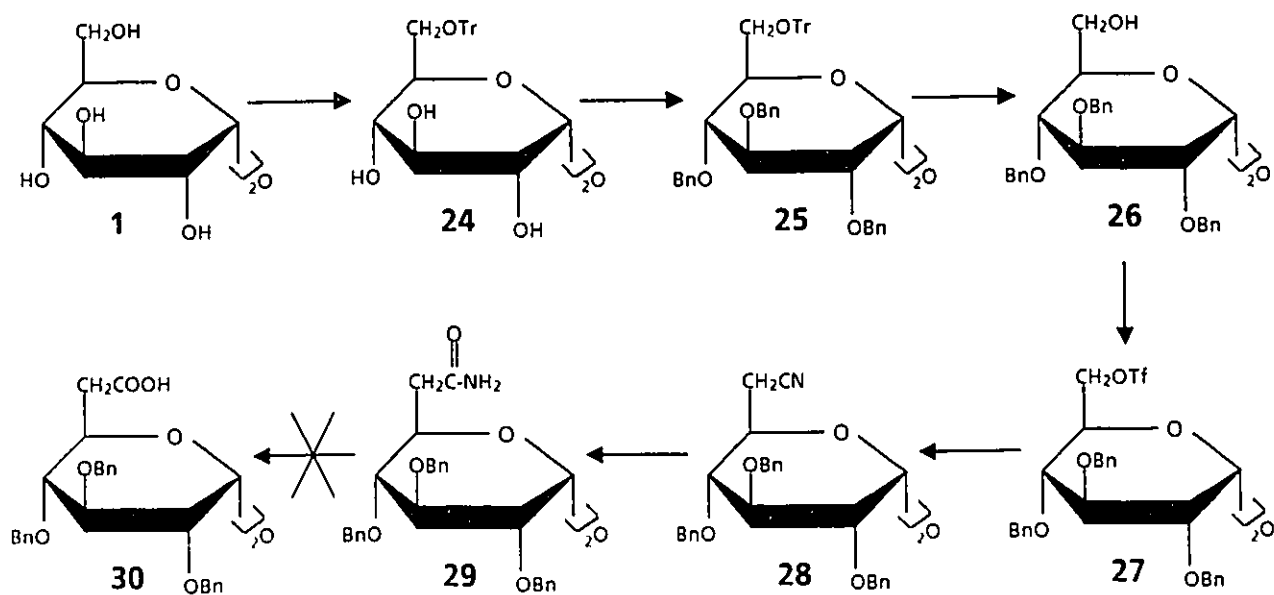
disaccharidic intermediate **23**, the procedure afforded crystalline **12** but in disappointing yields (20-30%). However, the n.m.r. and infrared data, optical rotation, and melting point agreed perfectly with the data for **12** from the previous procedure.



Scheme 11

## B.2. An approach to the synthesis of (2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosiduronic acid (30)

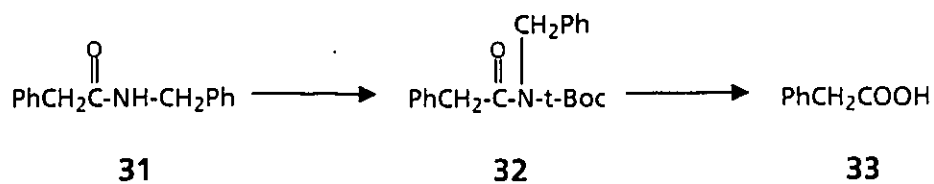
For future synthetic uses of 5, it appeared desirable to possess derivatives whose secondary hydroxyl groups are protected as benzyl ethers. Thus, if major difficulties would arise during the deacetylation process (Scheme 9) of 18, benzyl groups would be smoothly removable by catalytic hydrogenolysis in the presence of ester functions. Consequently, 2,3,4,2',3',4'-hexa-O-benzyl- $\alpha,\alpha$ -trehalose (26) was prepared from 1 via the trityl ethers 24 and 25, and the 6,6'-ditriflate 27 obtained from 26 was then converted, by displacement with potassium cyanide, into the dinitrile 28 (Scheme 12).



Scheme 12

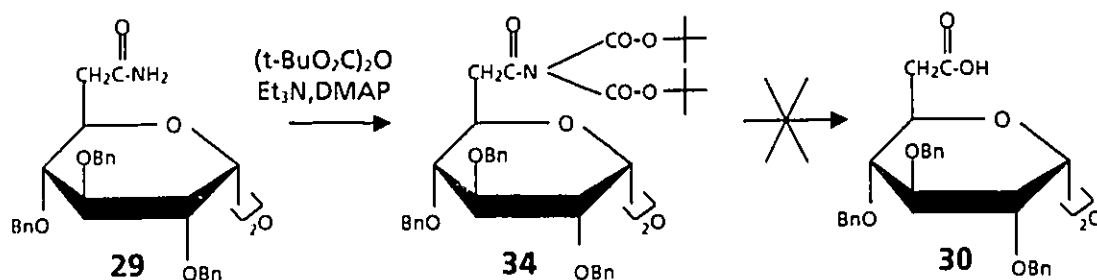
Hydrolysis of **28** with sodium hydroxide and hydrogen peroxide at room temperature proceeded readily but, in remarkable contrast to the aforementioned hydrolysis of the analog **16**, it stopped completely at the amide stage and the diamide **29** was isolated crystalline in 83% yield. Characteristic evidence for its structure was obtained by elemental analysis, infrared spectroscopy ( $\nu_{\text{max}}^{\text{Nujol}}$  1670 and 1610  $\text{cm}^{-1}$ , amide I and amide II bands) and the  $^1\text{H-n.m.r.}$  spectrum (which showed two broad, exchangeable singlets at 5.93 and 5.73 ppm for  $\text{NH}_2$ ). The diamide proved exceptionally resistant to further hydrolysis under a variety of alkaline conditions, and it also failed to undergo deamination with nitrous acid.

P.A. Grieco and co-workers reported<sup>23</sup> a mild, two-step hydrolysis / methanolysis of secondary amides in which N-tert-butoxycarbonyl derivatives of amides, prepared through the agency of di-tert-butyl dicarbonate, suffer regioselective hydrolysis or methanolysis, leading to the corresponding acids or esters respectively. For example, compound **31** can be converted [(t-BuO<sub>2</sub>C)<sub>2</sub>O, Et<sub>3</sub>N, DMAP, 25°, 6h] in 87% yield into the N-t-Boc derivative **32**, which upon treatment with 3 equiv. of lithium hydroxide in aqueous tetrahydrofuran at room temperature, provided 91% yield of carboxylic acid **33** (Scheme 13).



**Scheme 13**

Therefore, by applying Grieco's method to a primary amide, the following reaction sequence was investigated (Scheme 14).



**Scheme 14**

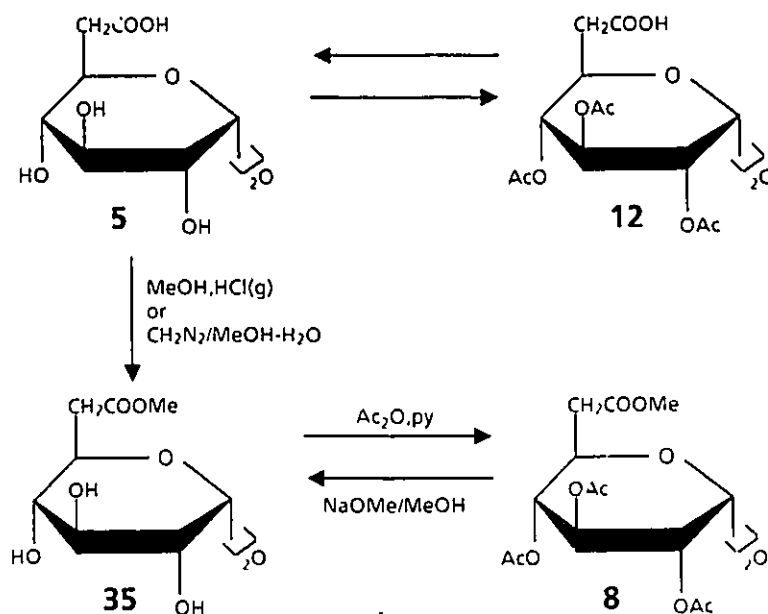
At this stage, this outlined strategy was desperately a shot in the dark, but had nothing to lose. The reaction  $29 \rightarrow 34$  was carried out with appropriately adjusted proportions of reagents, and after stirring the mixture overnight at room temperature, t.l.c. showed the formation of three faster-moving spots. Eventually, when no further change seemed to occur, a large excess of di-tert-butyl dicarbonate was added and the mixture was boiled under reflux for 10 h after which a single product appeared to be present. The  $^1\text{H}$ - and  $^{13}\text{C}$ -n.m.r. spectra suggested strongly that the product might have been **34**, although a couple of signals could not be identified in the  $^{13}\text{C}$  spectrum and were perhaps due to excess reagent still present. The latter could not be seen on t.l.c. and might have come off the silica gel column at the same time as **34**. Nevertheless, the presumed N-t-Boc derivative **34** was subjected to hydrolysis and methanolysis as proposed by Grieco, but the reactions afforded only starting material. Thus, this route was abandoned.

Reduction of nitriles with diisobutylaluminum hydride, followed by hydrolysis of the intermediate imine are known<sup>22</sup> to form aldehydes. Thus, the dinitrile **28** was

subjected to this treatment in the hope of obtaining the corresponding dialdehyde which could then be oxidized to **30**. However, the dinitrile did not seem to undergo reduction as only starting material was recovered. As a last attempt at synthesis of the target molecule **30**, the dinitrile **28** was dissolved in methanol, acidified with hydrochloric acid, and the solution was stirred overnight at room temperature (Pinner reaction<sup>24</sup>). Once again, a disappointing result was obtained, as only starting material was recovered.

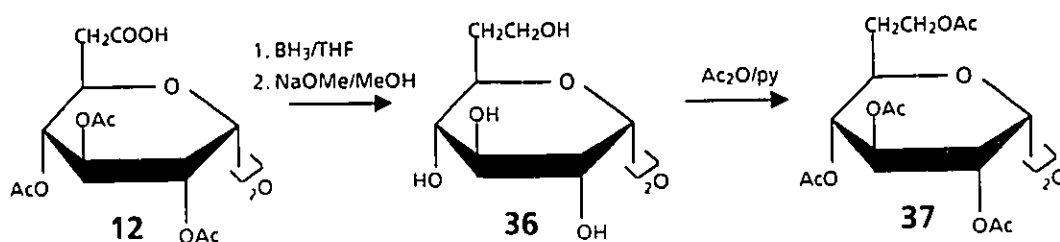
### B.3. Deacetylated and peracetylated dimethyl bis(heptosiduronates) **35** and **8** and the trehalose homolog **36**

Having compounds **12** and **5** in hand, we were able to undertake the synthesis of cord factor analogs (See Parts B.4, B.5.1 and B.5.2). However, before proceeding to the next section, some ancillary chemical transformations were performed (Scheme 15).



Both hexaacetates **12** and **8** were readily *O*-deacetylated (Zemplén) to free **5** (84%) and its dimethyl ester **35** (92%). As previously mentioned in the introduction, deacetylation of **8** to **35** had been unsuccessful in the early stages of this project. It was suggested that an aged sodium methoxide (solid) introduced directly into the methanolic solution of **8** might have somehow lost its activity. However, when a freshly prepared reagent (sodium metal dissolved in methanol) was used, **35** was obtained in a high yield. Mild esterification of **5** with methanol-hydrogen chloride, or with diazomethane, converted it into **35** in 87% and 99% yields, respectively. Acetylation of **35** employing the straightforward method of acetic anhydride pyridine afforded **8** in 64% yield; the melting point, infrared spectrum and <sup>1</sup>H-n.m.r. spectrum were all identical with those of **8** obtained by the iron carbonyl method (reaction [3], Scheme 11).

Treatment of the diacid hexaacetate **12** with borane in oxolane led to reduction of the carboxyl groups and partial deacetylation. Completion of the deacetylation (Zemplén) then furnished the hitherto unknown trehalose homolog, 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranoside (**36**), whereas reacetylation gave the octaacetate **37** of this new disaccharide (Scheme 16).



**Scheme 16**

The key features for the characterization of **36** were based on elemental analysis, the mass spectrum showing  $m/z$  371 (100%,  $[M + 1]^+$ ), the <sup>1</sup>H-n.m.r.

spectrum, and the  $^{13}\text{C}$ -n.m.r. spectrum which showed the expected number of seven signals. Compound 37 was identified by its  $^1\text{H}$ -n.m.r. spectrum which displayed four O-acetyl signals.

In summary, both compounds 5 and 12 were prepared in reasonable yields for use as starting materials in the synthesis of cord factor analogs as described in the next sections. Although the route in which the secondary hydroxyl groups were protected as benzyl ethers did not lead to its full potential, attempts to procure the diacid 30 are being continued. Furthermore, many new compounds were synthesized, all of which were fully characterized.

The successful studies reported in Sections B.1-B.3 have been published as an invited contribution to the Bicentenary Volume of *Carbohydrate Research*: H.H. Baer, R.L. Breton, and Y. Shen, *Carbohydr.Res.*200 (1990) 377-389.

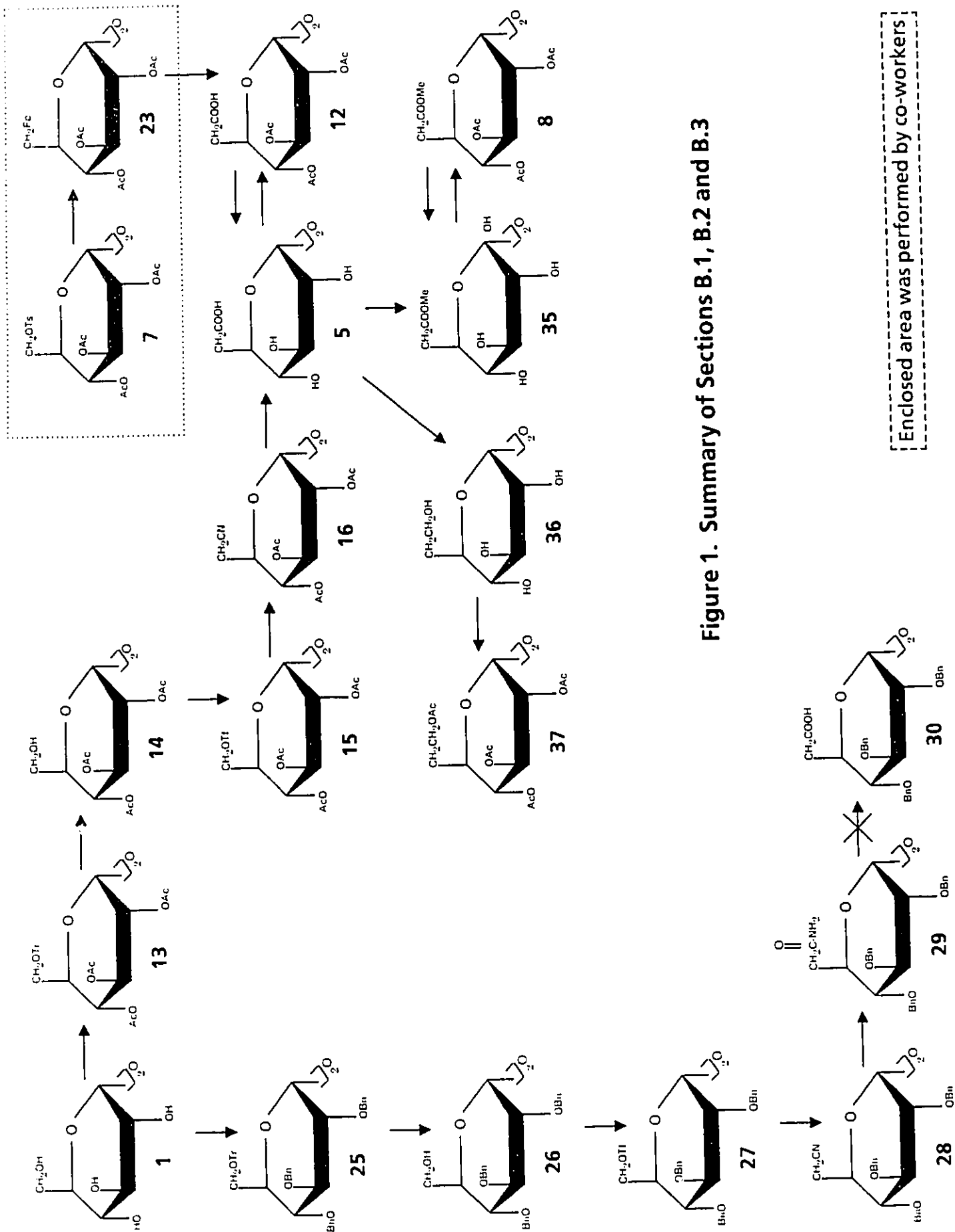
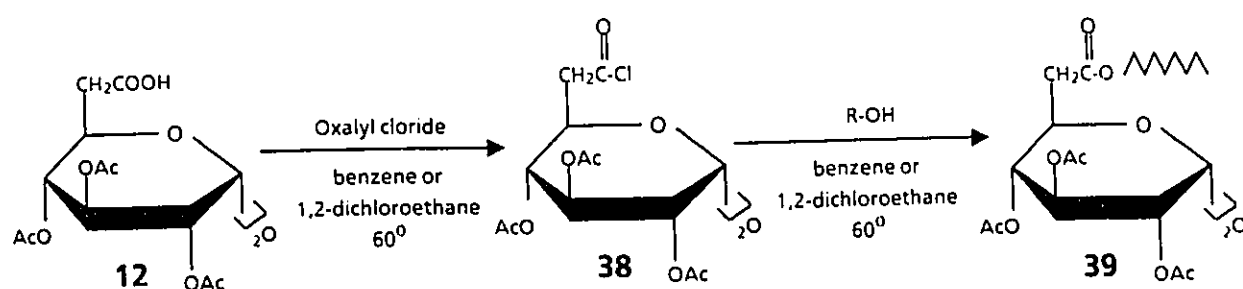


Figure 1. Summary of Sections B.1, B.2 and B.3

#### B.4. Synthesis of peracetylated dialkyl bis(heptosiduronates) 39-43

Having compound **12** in hand, we proceeded with the synthesis of higher esters of the type **8**. As a first model experiment, the acetylated acid **12** was converted into its dichloride **38**, which was allowed to react with 1-octanol, to form the di-octyl ester **39** (Scheme 17).



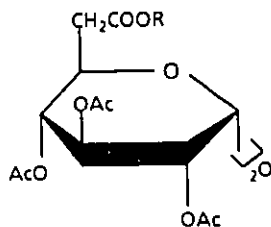
Scheme 17

M.B. Goren and K.S. Jiang<sup>13</sup> prepared similar "mirror pseudo cord factors" but used the trehalosuronic acid **3** as starting material and activated it by treatment with thionyl chloride, which was followed by nucleophilic attack of 1-alkanols. Thus, it was envisaged to employ the same experimental procedure to **12**. Although some target product of **39** was seen in t.l.c., the yields appeared very low and some trials gave little or no product. Because of this, processing of the reaction mixtures did not seem worthwhile and instead, a variety of other activating agents were investigated in order to obtain higher yields.

One method attempted was direct esterification analogous to that of Scheme 15 in which compound **5** was esterified to the methyl ester in high yield by methanol-hydrogen chloride. However, 1-octanol did not react at moderate

temperature, and decomposition occurred (t.l.c.) at elevated temperature. A milder method, investigated by several authors<sup>25</sup>, makes use of trifluoroacetic anhydride as a condensing agent. Although methanolysis seemed to give moderate yields (by t.l.c.) of dimethyl ester **8**, longer-chain alcohols showed no sign of reaction. N,N'-Dicyclohexylcarbodiimide (DCC) has been used widely for the synthesis of peptides<sup>26</sup>, depsides<sup>27</sup> and other types of products<sup>28</sup>, and condensation products of alcohols and acids are usually obtained in good yields. Consequently, 1-octanol and **12** were reacted overnight in the presence of DCC, which generated two products, namely the di- and mono-octyl esters, with the former obtained in lower yield. Even upon addition of more DCC and 1-octanol, the reaction did not progress satisfactorily. Both esters were separated and characterized by <sup>1</sup>H- n.m.r. spectroscopy. A useful catalyst, such as 4-pyrrolidinopyridine has been known<sup>29</sup> to increase yields of DCC condensation reactions, but this method furnished a third, slower moving, spot in t.l.c., and afforded no improvement. Nevertheless, the best results thus far obtained were those of the DCC reaction, and if it had not been for the next breakthrough, the mono-octyl ester might have been subjected to further condensation with DCC and 1-octanol in a separate reaction, in the hope of increasing the yield of diester **39**.

However, as previously mentioned, much more successful was esterification according to Scheme 17, but using oxalyl chloride as the chlorinating agent. After processing of compound **38**, 1-octanol was introduced in excess, and after heating the mixture at 75° for 2-h, processing afforded the dioctyl ester **39** in 96% yield. Similar esterification with C<sub>15</sub> to C<sub>18</sub> 1-alkanols by way of the same strategy produced the analogous diesters **40-43**, all in high yields (Scheme 18).



39 R = n-C<sub>8</sub>H<sub>17</sub>

40 R = n-C<sub>15</sub>H<sub>31</sub>

41 R = n-C<sub>16</sub>H<sub>33</sub>

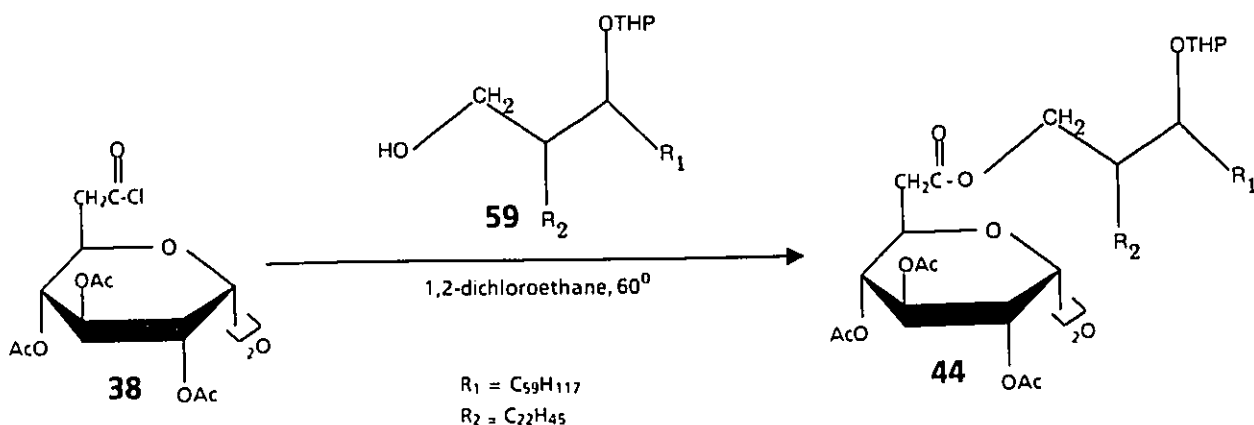
42 R = n-C<sub>17</sub>H<sub>35</sub>

43 R = n-C<sub>18</sub>H<sub>37</sub>

Scheme 18

As previously mentioned, deacetylation of **39-43** was next required in order to generate the targeted "mirror" molecules **45-49**. This is discussed in section B.5.1.

After the synthesis of these simpler analogs, attempts were made to prepare **44**, the acetylated, true analog of natural cord factor from **38** and the protected mycolic alcohol **59** (Scheme 19). The alcohol **59** was successfully prepared from natural mycolic acid (see chapter B.6), but unfortunately its condensation with **38** encountered major difficulties. After 45 min the reaction did not progress any further as judged by t.l.c., and **44** was formed in only ~5% yield (verified by <sup>1</sup>H-n.m.r.). Repeated reactions afforded no better result.



**Scheme 19**

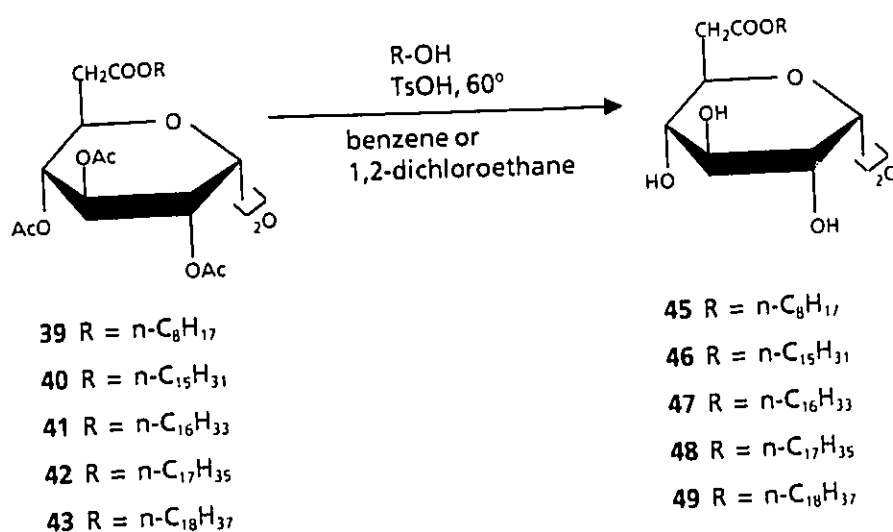
A different approach which affords directly the non-acetylated "mirror cord factor" analogs **45-49**, employing **5** as starting material, is described in section B.5.2.

## B.5. Synthesis of dialkyl bis(heptosiduronates) **45-49**.

### B.5.1. By deacetylation of peracetates **39-43**

Conversion of the hexa-*O*-acetyl diacid chloride **38** to the symmetrical diester hexaacetates **39-43** proceeded smoothly as just described; but deblocking of the carbohydrate core by selective removal of the acetyl groups proved more difficult. Deacetylation of these intermediates is necessary because acetylated cord factors are devoid of toxicity<sup>30</sup> and antitumor activity<sup>31</sup>. Similar deprotection was achieved by M.B. Goren and K.S. Jiang<sup>13</sup> who found a relatively simple solution: 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 and an excess of the appropriate alcohol, a slow progressive deacetylation occurred that was monitored by t.l.c. until no further change was evident. Following termination of the reaction, the pseudo cord factor was separated from the solvolytic alcohol, the alcohol acetate, the incompletely deacetylated material, and in part from a faster moving U.V.-active by-product by column chromatography, and the incompletely deacetylated products were recycled. This was done several times. When the last cycle was no longer worthwhile processing, the deacetylated concentrates were pooled and purification from hot methanol afforded crystalline products **45-49** (Scheme 20).



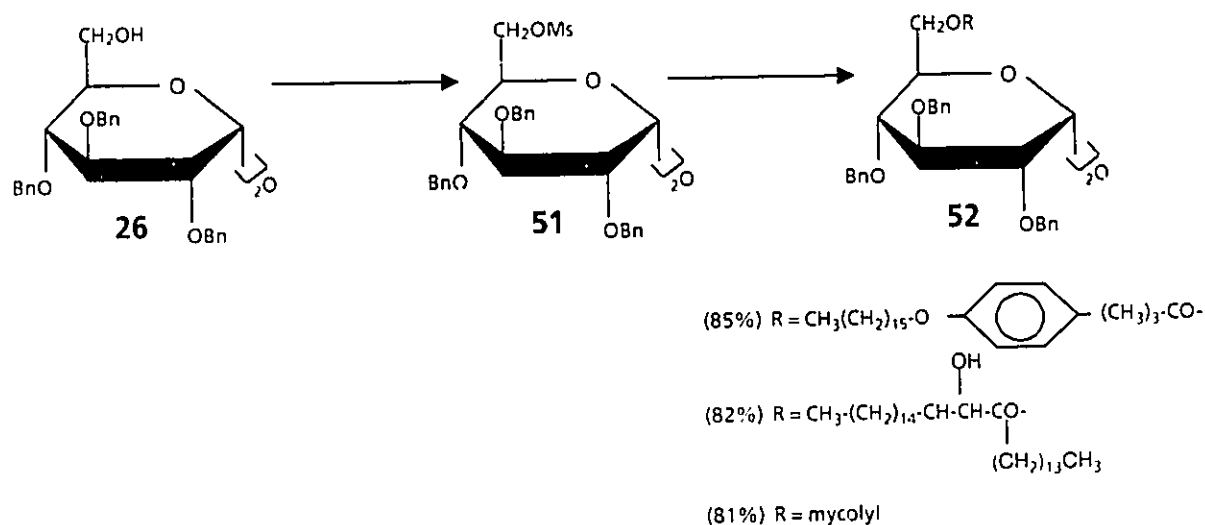
Scheme 20

Several recrystallizations were often necessary in order to remove the U.V.-active by-product which should have separated easily by column chromatography in view of its high R<sub>f</sub> value in t.l.c. but instead appeared to be migrating in part with the deacetylated products. The impurity exhibited aromatic proton resonances in its <sup>1</sup>H-n.m.r. spectrum and therefore probably originated from the sulfonic acid used as a catalyst, but its exact nature remained unclear. Yields of analytically pure

deacetylated products were somewhat disappointing, they ranged from 28 to 64%. Compounds such as 45-49 were targeted because they closely mirror trehalose 6,6'-dipalmitate, a mycobacterial glycolipid of considerable interest<sup>32</sup>, and in fact, 46 is isomeric with that dipalmitate.

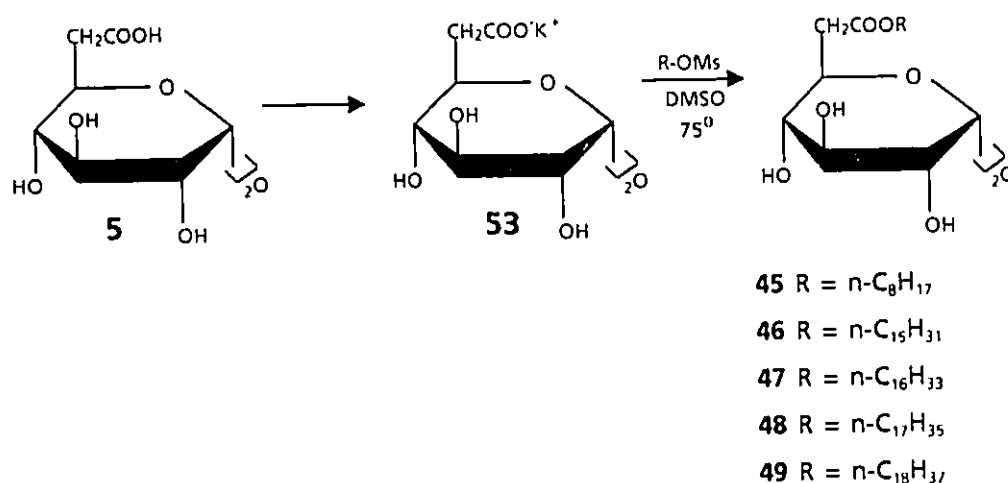
### B.5.2. Alternative synthesis of 45-49, by use of alkanol mesylates

M.B. Goren and A. Liav reported<sup>33</sup> successful syntheses of cord factor and analogs by the strategy outlined in Scheme 21. 2,3,4,2',3',4'-Hexa-O-benzyl- $\alpha,\alpha$ -trehalose (26) was converted into the corresponding dimesylate 51 in quantitative yield by treatment with methanesulphonyl chloride in pyridine. When compound 51 was brought into reaction with the potassium salts of long-chain fatty acids in hexamethylphosphoric triamide (HMPA) at 95-100°, the 6,6'-diester derivatives 52 were obtained as the major products. Catalytic hydrogenolysis then afforded the debenzylated compounds in good yields.



Scheme 21

By analogy to this procedure, C<sub>8</sub> and C<sub>15</sub> to C<sub>18</sub> 1-alkanols were mesylated as previously described. Triflation was also investigated and gave single spots in t.l.c., but the alkyl triflates readily decomposed during processing. The alkyl mesylates were then allowed to react with the potassium salt **53** of the unprotected diacid **5**, which produced the diesters **45-49** directly, and no deprotection procedure was needed (Scheme 22).



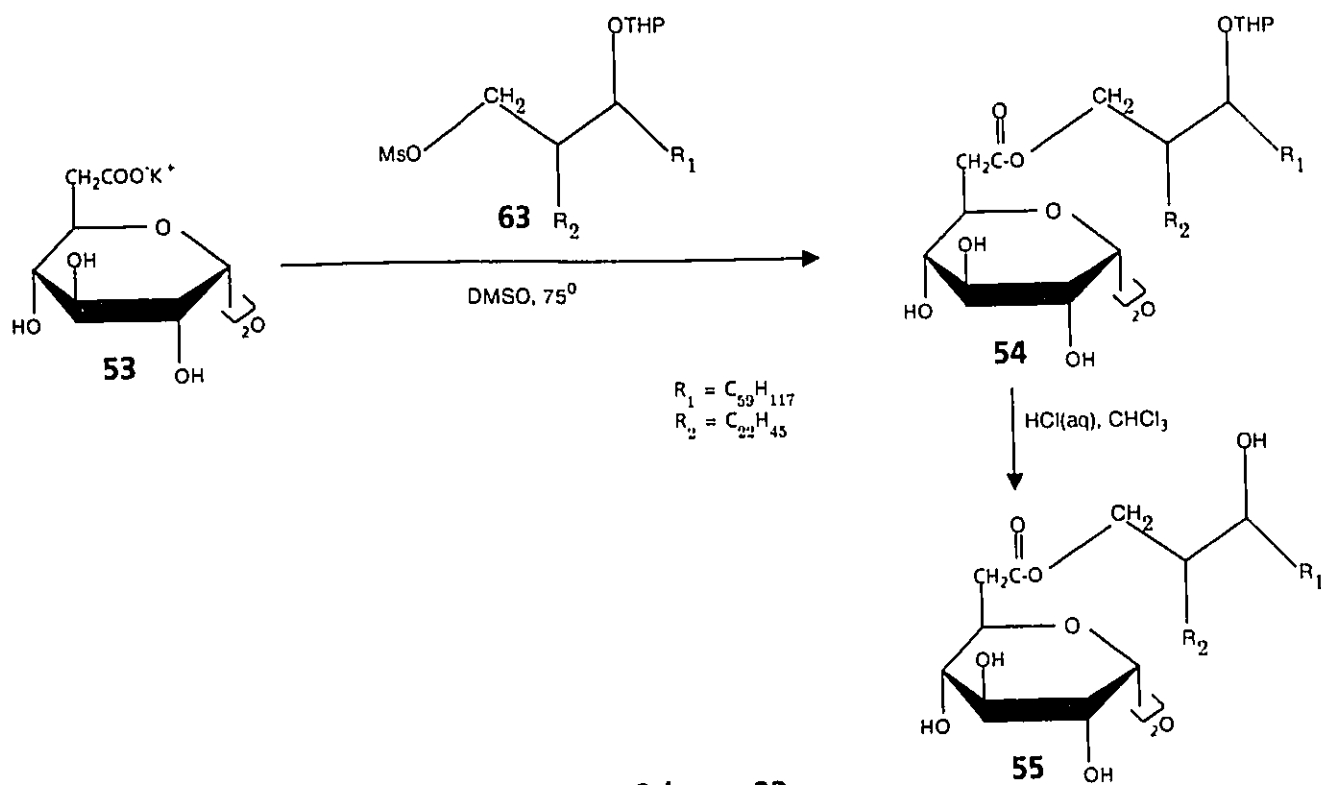
Scheme 22

The potassium salt **53** 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 h. The reaction mixture was processed by freeze-drying followed by chromatography, to yield 56-72% of the desired esters. In one experiment the cesium salt of **5** was used, but no increase in yield was noticed. The cord factor analogs were characterized by elemental analysis and all displayed practically identical infrared, <sup>1</sup>H-, and <sup>13</sup>C-n.m.r. spectra. In their mass spectra (+ve f.a.b.), two of them (**45** and **46**) showed the protonated molecular ion, *m/z* 623 (4%) and 819 (8%), respectively. All of them

gave peaks at  $m/z$   $[0.5 (M - 16)^+]$ , which represented the glycosyl ion resulting from cleavage of the disaccharidic bond.

Based on starting material **5**, the overall yields in the synthesis of **45** to **49** via acetylation of **5**, esterification with the alcohols, and solvolysis of the acetyl groups (section B.4 and B.5.1) were 23-61%. Compared to this, the present method is much simpler and less time-consuming, and typically affords higher yields (56-72%) so that it constitutes a significant improvement.

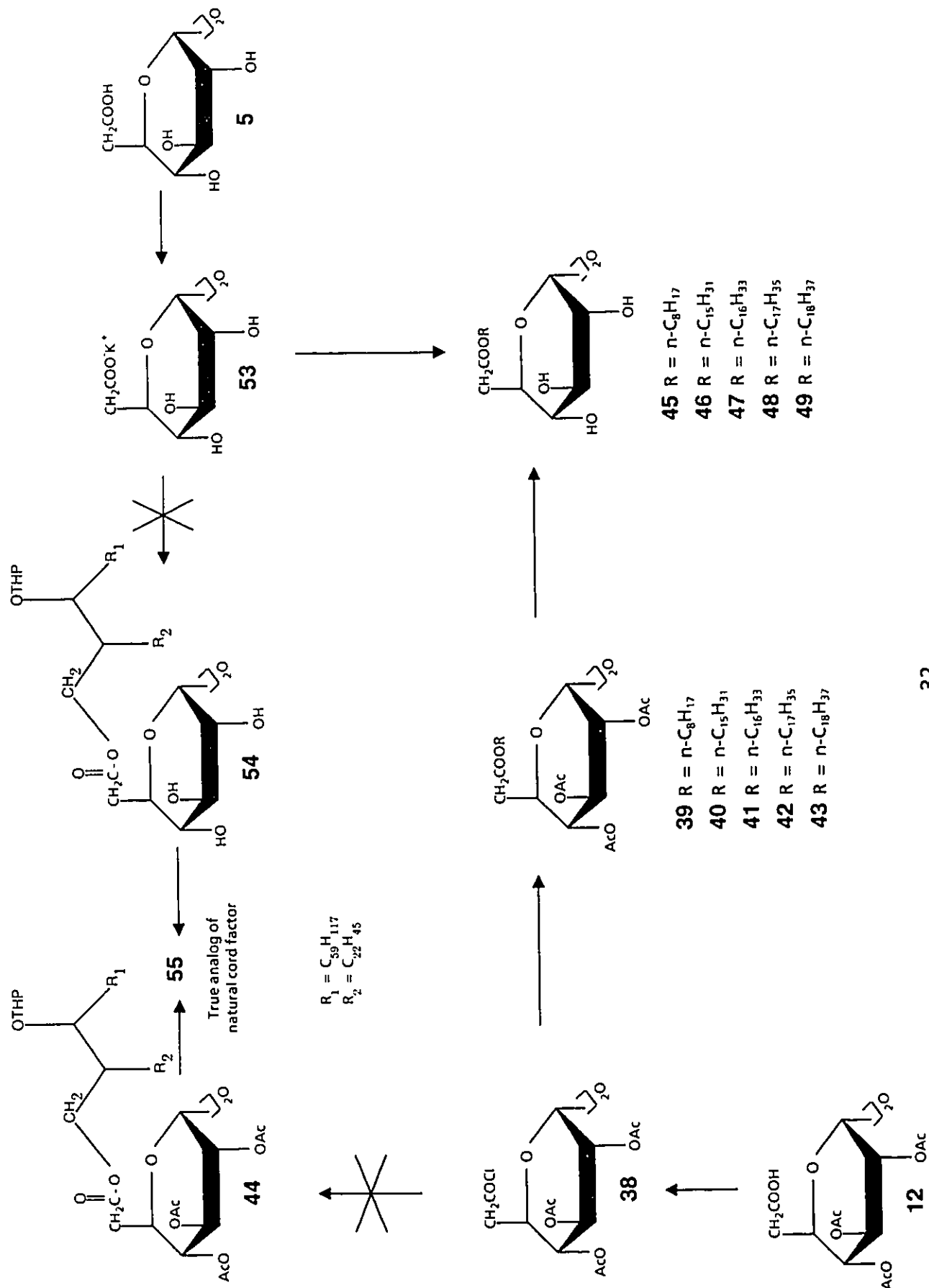
This new and improved method prompted us to reexamine the possibility of synthesizing the blocked mycolyl ester **54** which should be convertible by mild acid hydrolysis<sup>34</sup> into the cord factor analog **55** (Scheme 23). Therefore, the previously mentioned (Scheme 19) mycolyl alcohol **59** was mesylated using the same procedure as for the shorter-chain 1-alkanols, to give the mycolyl mesylate **63** (see section B.6 for more details). Much to our disappointment, the reaction of **63** with **53** did not produce any desired **54**; only starting materials (acid **5** and mycolic mesylate **63**) along with some deprotected mycolic mesylate **64** (having lost the tetrahydropyranyl group) were isolated after processing and chromatography.



**Scheme 23**

As a last attempt, phase transfer methodology was briefly examined. In two parallel but separate reactions, tetrahexylammonium hydrogen sulfate was introduced into an aqueous solution of **53** that was stirred with a dichloromethane phase containing, in one experiment, 1-heptadecylmesylate, and in the other, the mycolic mesylate **63**. Both reactions were performed at room temperature and then at the reflux temperature for several hours, but no product formation was observed in t.l.c. in either case. Addition of 18-crown-6 did not cause any change. Present work is still underway in the hope of obtaining the interesting and important cord factor analog **55**.

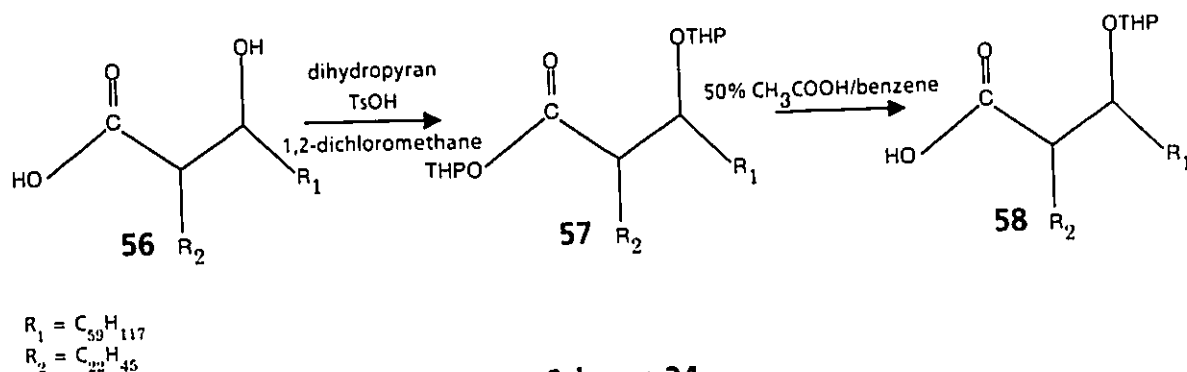
Figure 2. Summary of Sections B.4 and B.5



## B.6. Mycolyl lipids reactions

### B.6.1. The synthesis of 3-O-(2-tetrahydropyranyl) mycolyl alcohol (59) and 3-O-(2-tetrahydropyranyl) mycolyl mesylate (63) and their precursors

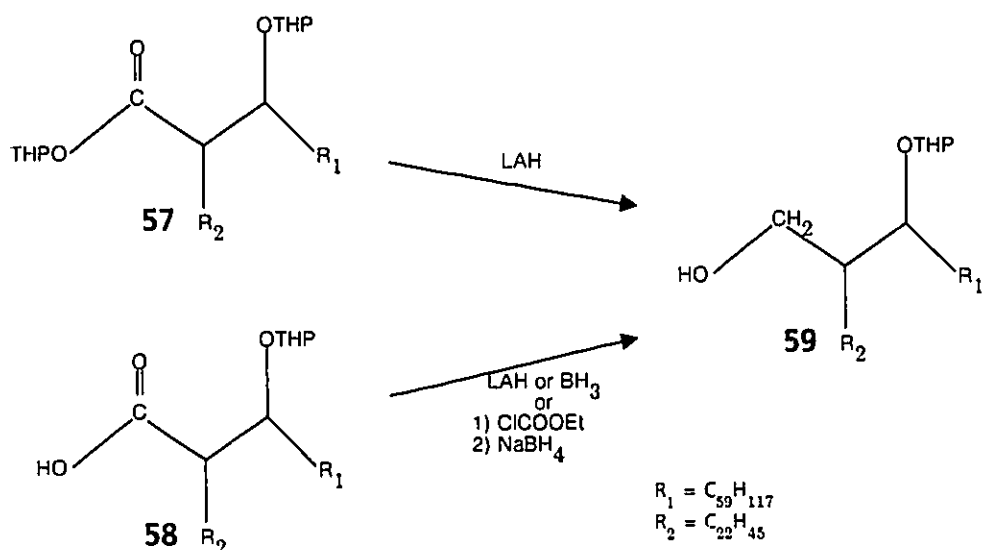
Chemical modifications of mycolic acid **56** have been studied before by I.D. Jenkins and M.B. Goren<sup>34</sup>, and of particular interest were analogues in which the mycolyl residues were significantly altered in the vicinity of the  $\beta$ -position. Cord factors were synthesized in very satisfactory manner, and it was hoped to gain information about whether the free  $\beta$ -hydroxyl group of the mycolic acids is necessary for expression of biological activities, such as antitumor activity<sup>8,9</sup>, and whether activities are altered by blocking of this hydroxyl. To our knowledge, this is the first and only work in the literature that dealt with chemical transformations of mycolic acid. In order to prepare the hitherto unknown mycolyl alcohol and some derivatives thereof, we performed a number of reactions as described in the paragraphs that follow. First, mycolic acid **56** was protected with tetrahydropyranyl groups as already reported<sup>34</sup> (Scheme 24).



Scheme 24

Treatment of mycolic acid **56** with dihydropyran in dichloromethane in the presence of a catalytic amount of *p*-toluenesulfonic acid gave the bis-

tetrahydropyranyl derivative **57**. The latter was selectively deprotected by overnight treatment with acetic acid in benzene at room temperature. Chromatography of the product mixture gave the acid **58** in 35% overall yield from **56**. A large amount of mycolic acid (44%) was regenerated and recovered. In a separate experiment using the same procedure, compound **57** was isolated chromatographically in 81% yield and not subjected to ester hydrolysis. The first desired and new target compound, 3-O-(2-tetrahydropyranyl) mycolyl alcohol (**59**) was then prepared in 86% yield by reduction of **57** using lithium aluminum hydride (LAH) in ether at the reflux temperature (Scheme 25).

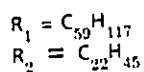
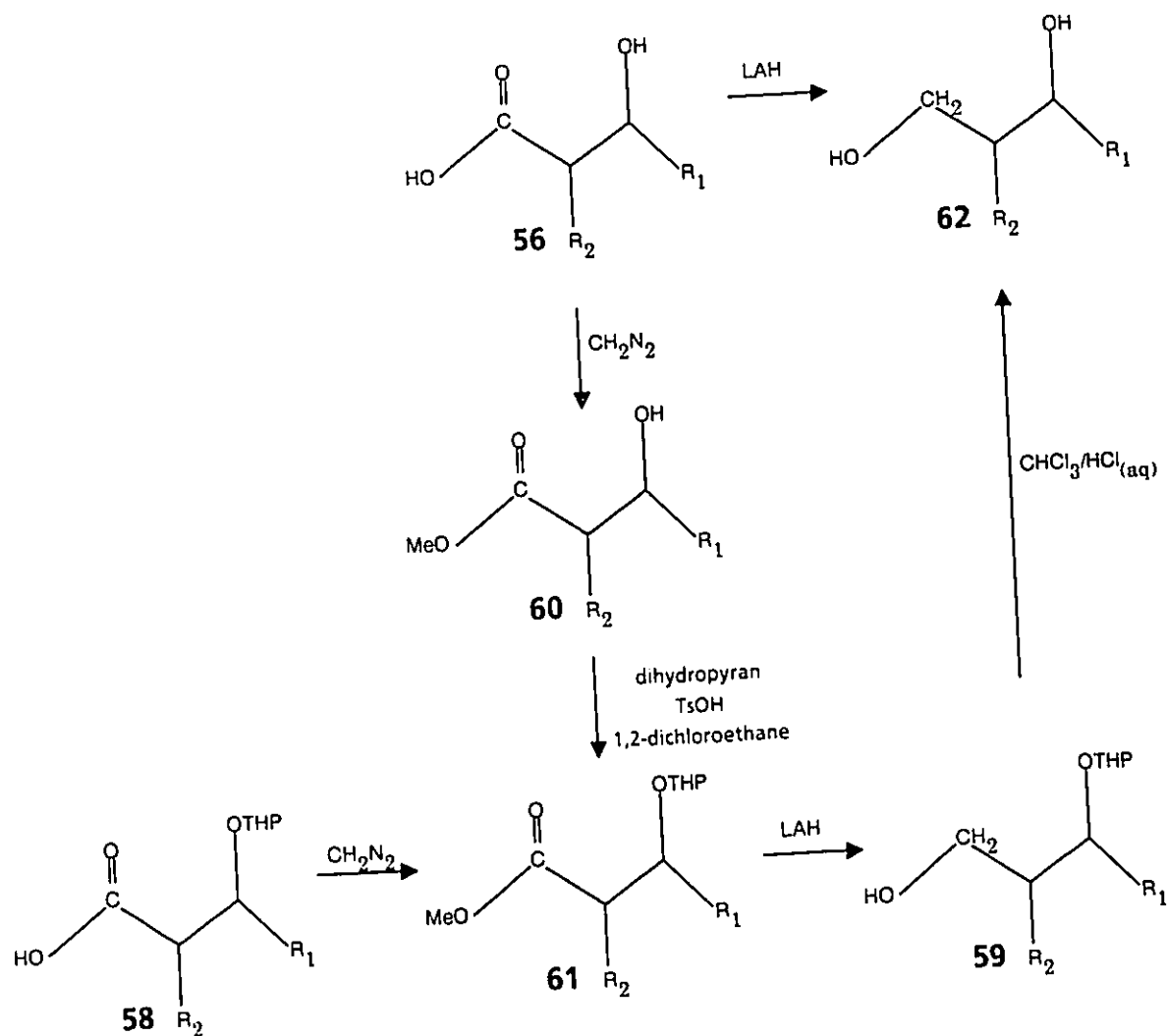


Scheme 25

T.l.c. indicated two distinct spots for **59** which were attributed to diastereoisomeric tetrahydropyranyl acetals. Irrigation of the latter seven times in a less polar solvent system resolved the spots into four, and it appeared reasonable to

assume the sample of mycolic acid used was not constitutionally homogeneous. This assumption has been previously supported<sup>34,35</sup> as mycolic acid(s) are a mixture of different components varying in type and in homology, the empirical formula is representative only. However, elemental analyses of mycolic acid derivatives prepared in the present study are in accord with this formula. Compound 59 was also elaborated from the acid 58 by the action of LAH (74%), or borane in oxolane (83%), and by a two-step procedure that involved sodium borohydride reduction of a mixed anhydride generated from 58 with ethyl chloroformate (yield, 88%). Infrared spectra of the different preparations were all identical except for a very small, broad signal at  $1730\text{ cm}^{-1}$  that could not be explained in the borane reaction. Elemental analysis of 59 was correct, and two extra  $\text{CH}_2$  signals appearing in the carbon-13 spectrum between 60-70 ppm indicated the conversion of the carboxyl into a primary alcohol group. Compound 59 was used in the attempted synthesis of the acetylated cord factor as previously mentioned in section B.4.

Definitive characterization of such high molecular weight compounds is difficult; evidence provided by IR and n.m.r. is often inconclusive. For this reason, a number of small-scale "test reactions" were run (1-10 mg scale), in support of the lipid structures dealt with in this section. These test reactions, outlined in Scheme 26, provided chromatographic and spectroscopic evidence for the structures concerned.



**Scheme 26**

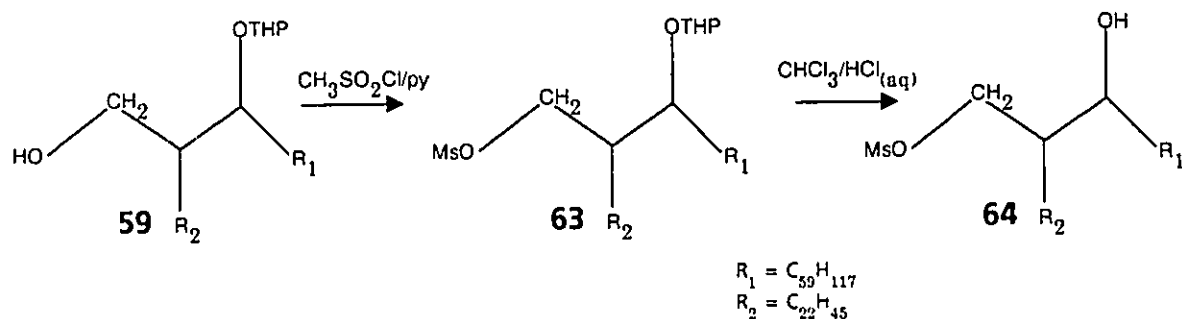
Thus, mycolic acid **56** was treated with an ether solution of diazomethane, to produce its methyl ester **60**. The infrared spectrum showed a carbonyl band shifting from  $1680\text{ cm}^{-1}$  (acid) to  $1725\text{ cm}^{-1}$  (ester).  $^1\text{H-n.m.r.}$  indicated a singlet at 3.68 p.p.m. ( $\text{COOCH}_3$ ). Protection of the secondary hydroxyl group using dihydropyran and a catalytic amount of p-toluenesulfonic acid in 1,2-dichloroethane converted **60** into the acetal **61**, which was isolated pure by means of chromatography. The hydroxyl

absorption in the IR spectrum disappeared, and <sup>1</sup>H-n.m.r. showed two singlets, at 3.63 and 3.65 ppm. (COOCH<sub>3</sub>), one for each diastereoisomers. In a parallel manner, treatment of compound **58** with a diazomethane-ether solution afforded a major product which by its R<sub>f</sub>-value, IR spectrum and <sup>1</sup>H-n.m.r. spectrum was revealed to be **61**, identical with the product obtained from **60**.

For curiosity's sake, product **61** was subjected to reduction with LAH, and formation of compound **59** was observed in t.l.c.. The IR spectrum of the product was identical with that of **59** obtained from the procedure described in Scheme 20; the <sup>1</sup>H-n.m.r. spectrum indicated absence of methoxyl resonances.

The last test reactions involved the formation of **62** via **56** and **59**. Treatment **56** with LAH in anhydrous ether gave rise to a single spot in t.l.c. having the same R<sub>f</sub> value as that of the hydrolysis product obtained when **59** was treated with moist chloroform containing a small amount of hydrochloric acid. Both diastereoisomers of **59** converged to a single spot.

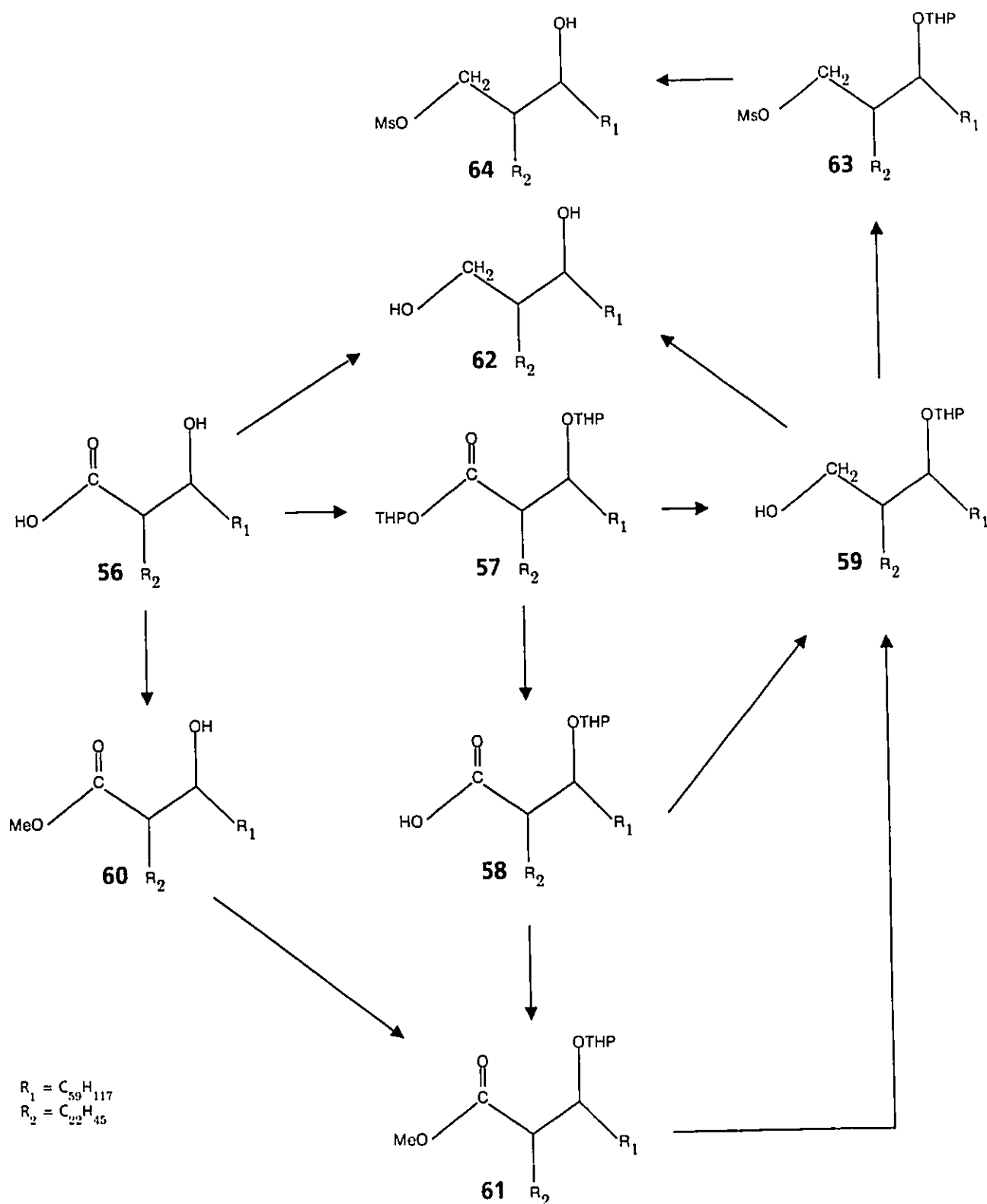
3-O-(2-Tetrahydropyranyl) mycolyl mesylate (**63**) was the last objective of these studies as this compound was used in several attempts to synthesize cord factor (see chapters B.4 and B.5.2.). Mesylation of **59** readily occurred within one hour when the compound was treated with methanesulfonyl chloride (1.2 molar eq.) in pyridine at room temperature. T.l.c. indicated a single spot, and after processing and chromatography the desired material **63** was isolated in 95% yield (Scheme 27).



**Scheme 27**

The mesylate was characterized by means of its IR spectrum which showed specific signals at 1360 and 1175  $\text{cm}^{-1}$  (S–O);  $^1\text{H}$ -n.m.r. exposed a singlet around 3 ppm ( $\text{SO}_2\text{CH}_3$ ) whereas two extra signals (diastereoisomers) appeared in the  $^{13}\text{C}$ -n.m.r. spectrum (APT) between 30-40 ppm and were assigned as  $\text{SO}_2\text{CH}_3$  signals. Because of the instability of the tetrahydropyranyl group in **63**, which was partly cleaved off during storage of the compound for a couple of days at room temperature (maybe by traces of acidic impurities present), the waxy product was unsuitable for submission to elemental analysis. Therefore, the THP group was deliberately removed by treatment of **63** with hydrochloric acid in moist chloroform, which afforded a quantitative yield of product **64**, homogeneous in t.l.c.. This mesylate showed a hydroxyl absorption at 3380  $\text{cm}^{-1}$  while the characteristic bands at 1340 and 1175  $\text{cm}^{-1}$  (S = O) still remained in the IR spectrum; a 3-proton singlet around 3 ppm in the  $^1\text{H}$ -n.m.r. spectrum was assigned to the mesyl group.

Figure 3. Summary of mycolyl lipid reactions B.6



Test Reactions were investigated in the formation of compound 60, 61, 62: Reactions were run on 1-10 mg scale. They provided further evidence in the characterization of compounds 57, 58 and 59 by their  $R_f$  values and/or  $^1H$ -n.m.r. and/or  $^{13}C$ -n.m.r. and/or IR parameters.

## C. Conclusion

The desired bis (6-deoxyheptopyranosiduronic acid) **5** used as starting material in the preparation of cord factor analogs was achieved in excellent overall yield when the secondary hydroxyl groups in the core of the sugar were protected with acetyl groups. However, when the latter groups were substituted with benzyl groups, the alkaline hydrolysis step going from the dinitrile **28** to the corresponding diacid **30** stopped at the diamide **29** stage, and various strategies tried to overcome this problem were not successful. Nevertheless, successful synthesis of **5** and its corresponding hexaacetate **12** for use in the next steps of this project was achieved.

Reaction of (2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronic acid) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosiduronic acid (**12**) with oxalyl chloride produced the dichloride **38** which subsequently reacted with 1-alkanols ( $C_8$ ,  $C_{15}$  to  $C_{18}$ ) yielding the analogous diesters **39-43** in yields of 81-96%. Removal of the acetyl groups with little or no loss of the lipid ester groups was accomplished by the method proposed by Goren and Jiang<sup>13</sup>. Transesterification by use of *p*-toluenesulfonic acid in the presence of an excess amount of the alcohol converted the hexaacetates into the "mirror" cord factor analogs **45-49** in fair to moderate yields (28 to 64%). The synthesis of acetylated dimycolyl ester **44** was studied using this method, but presented problems not yet overcome.

An alternative and superior method for the synthesis of **45-49** involved displacement of a mesylate group on the lipid chains by the potassium salt **53** of **5**, which delivered good yields and was less time-consuming than the first approach. The overall yields via acetylation of **5**, and esterification with the alcohols followed by solvolysis of the acetyl groups were 23-61% compared to 56-72% for the second

approach. However, this improved method was not successful in the preparation of the cord factor analog **55**.

Treatment of diacid hexaacetate **12** with borane in oxolane followed by complete deacetylation (Zemplén) furnished the hitherto unknown trehalose homolog, 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranoside (**36**).

Furthermore, the field of mycolic acid chemistry was expanded specifically by chemical transformation at the  $\beta$ -position and at carbon-1. As a result, three new compounds were isolated; mycolyl alcohol **59** was obtained by reduction of two previously known mycolyl derivatives, **57** and **58**, and the other two new lipids were mycolyl mesylates **63** and **64**.

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. The ester **45-49** were tested by Dr. A. J. Ulmer, Division of Cellular Immunology, Forschungs institut Borstel, Germany, for possible immunostimulant properties. It was found that they all were capable of inducing the production of interleukin-1, interleukin-6, and tumor necrosis factor in human monocytes. Serological assays performed by Dr. A. Laszlo, Laboratory Center for Disease Control, Health and Welfare Canada indicated that **46-49** (but not **45**) possess considerable affinity for antibodies present in sera of tuberculosis patients.

## D. Experimental

**General Methods.** – Column chromatography was performed with silica gel Merck 9385; 20-45  $\mu\text{m}$ , or BDH 7734; 70-230 mesh or BDH 9385; 230-400 mesh, the latter being indicated in experimental procedures where employed. Solvent systems were ethyl acetate-hexane *A*, 3:1; *B*, 2:1; *C*, 3:2; *D*, 1:1; *E*, 2:3; *F*, 1:2; *G*, 1:3; *H*, 1:4; *I*, 1:6; *J*, 1:8; and *K*, 1:18; water-methanol-ethyl acetate *L*, 4:5:10; *M*, 4:5:15; *N*, 4:5:20; and *O*, 4:5:30; methanol-ethyl acetate *P*, 1:19; methanol-chloroform *Q*, 1:19; and ether-hexane *R*, 3:1; *S*, 2:1; *T*, 1:1; *U*, 1:2; *V*, 1:3; and *W*, 1:5. Thin layer chromatography (t.l.c.) was done on Merck 60 F-254 silica gel plates: spots were visualized by spraying with 5% sulfuric acid in ethanol and heating. Melting points were measured in capillaries in a Gallenkamp apparatus and are uncorrected. Optical rotations were determined at  $\sim 25^\circ$  using an electronic Perkin Elmer polarimeter, model 241. Infrared data  $\nu_{\text{max}}$  were recorded with a Perkin Elmer 783 infrared spectrophotometer for all compounds described and were consistent with the assigned structures; only significant bands are reported. Mass spectra were obtained with a VG organic mass spectrometer 7070E by the c.i. mode (ether or isobutane), and some by the f.a.b. mode using glycerol as the matrix. The  $^1\text{H}$ - and  $^{13}\text{C}$ -n.m.r. spectra were recorded for solutions in  $\text{CDCl}_3$ , unless otherwise stated; data recorded with a Varian Gemini 200 instrument are denoted as 200 and 50.29 MHz, respectively, whereas data without special notation refer to spectra at 300 and 75.43 MHz obtained with a Varian XL 300 instrument. Assignments of  $^{13}\text{C}$  peaks were aided by ADEPT or APT experiments. Signals refer to carbon atoms shown in Roman type; locant atoms or groups attached thereto are underlined. The symmetrical disaccharide derivatives gave only one set of signals which, for convenience, are recorded with reference to one moiety.

## D.1. (6-Deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronic acid) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosiduronic acid (5) and its precursors

(6-Deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronic acid) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosiduronic acid (5). – A. From its hexa-acetate 12. Compound 12 (85 mg) was deacetylated at room temperature in methanol (4 mL) made alkaline (pH 9) with sodium methoxide. Deionization with Amberlite IR-120(H<sup>+</sup>) resin after 30 min and concentration of the solution gave colorless 5,  $R_f$  0.2 (t.l.c., solvent L) which crystallized on trituration with a little methanol and ethyl acetate. The white, somewhat hygroscopic powder (44mg, 84%) had no distinct melting point but began to sinter near 60° and turned gradually into a glassy foam on heating above 90° ;  $[\alpha]_D + 144^\circ$  (c 1.6, water);  $\nu_{\max}^{\text{Nujol}}$  3400-3100 (OH), 2600, 1710  $\text{cm}^{-1}$  (acid CO). Mass spectrum:  $m/z$  (+ve f.a.b.) 399 (7%, [M + 1]<sup>+</sup>);  $m/z$  (-ve f.a.b.) 397 (44%, [M - 1]<sup>-</sup>);  $m/z$  (c.i.) 381, 363, and 345 (9, 2, and 4%, [M + 1 - 1, 2, and 3H<sub>2</sub>O]<sup>+</sup>), 265 (23%), 191 (100%, 0.5 [M - 16]<sup>+</sup>), 173 (56%, [0.5(M - 16) - H<sub>2</sub>O]<sup>+</sup>), 155 (22%, [0.5 (M - 16) - H<sub>2</sub>O]<sup>+</sup>); N.m.r. data (D<sub>2</sub>O): <sup>1</sup>H,  $\delta$  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,  $J_{5,6'}$  9.9,  $J_{6,6'}$  15.8 Hz, H-6'); <sup>13</sup>C (50.29 MHz),  $\delta$  176.6 (CO<sub>2</sub>H), 93.6 (C-1), 74.0, 73.4, 72.1, 69.7 (C-2,3,4,5), 37.6 (C-6).

B. From the dinitrile 16. A solution of 16 (1.145 g) in methanol (20 mL) was made alkaline (pH 9) by dropwise addition of methanolic sodium methoxide, to effect O-deacetylation which was complete after 20 min, as indicated by the appearance of a single new spot ( $R_f$  0.73) in t.l.c. (solvent L). The solution was concentrated to a syrup which, together with powdered NaOH (340 mg), was dissolved in aqueous 25% H<sub>2</sub>O<sub>2</sub> (12 mL). The mixture was stored at room

temperature without agitation. Effervescence was observed, and when it ceased, additional 25% H<sub>2</sub>O<sub>2</sub> (5 mL) and NaOH (135 mg) were introduced. This was repeated several times in the course of 4 days. Monitoring of the reaction by t.l.c. (solvent L) revealed a slow formation of slow-moving 5 together with products of intermediate mobilities, which gradually disappeared again; eventually a single spot of 5 remained, R<sub>f</sub> 0.4 (triple irrigation). When the reaction mixture was stirred, the rate decreased and completion was then attained only after 1.5-2 weeks. The solution was deionized with Amberlite IR-120(H<sup>+</sup>) resin, filtered, boiled for 3 h in order to decompose H<sub>2</sub>O<sub>2</sub>, and concentrated to dryness, to give 5 as a white, hygroscopic powder (723 mg, 97%). Crystallization from methanol-ethyl acetate furnished 5 (666 mg, 89.5%), [α]<sub>D</sub> + 130° (c 0.7, water), whose melting-behavior, i.r. spectrum, and n.m.r. data proved its identity with the product obtained in Procedure A.

*Anal.* Calc. for C<sub>14</sub>H<sub>22</sub>O<sub>3</sub> (398.3): C, 42.21; H, 5.57. Found: C, 41.89; H, 5.65.

(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 (12). —To a solution of 5 (125 mg) in glacial acetic acid (3 mL) and CHCl<sub>3</sub> (1 mL) containing a catalytic amount of 4-dimethylaminopyridine was added acetyl chloride (1 mL). Formation of 12 (R<sub>f</sub> 0.6) was complete after 1 h (t.l.c. with solvent M). A minor precipitate was removed, and the filtrate was concentrated with addition of toluene and then ethanol. A solution of the light-brown residue in CHCl<sub>3</sub> was washed twice with water, dried (MgSO<sub>4</sub>), and concentrated. Flash chromatography (CHCl<sub>3</sub>, then solvent Q) of the foamy residue 12 (177 mg, 86%) on silica gel (4g) and crystallization from ethyl acetate-hexane gave 12 (134 mg, 65%), which sintered at ~50° and melted indistinctly at 75-95°, [α]<sub>D</sub> + 113° (c 0.7, CHCl<sub>3</sub>). Mass spectrum (+ve f.a.b.): m/z 651 (6%, [M + 1]<sup>+</sup>), 317 (51%, 0.5 [M - 16]<sup>+</sup>). N.m.r. data: <sup>1</sup>H, δ 5.49 (dd, J<sub>2,3</sub> 10, J<sub>3,4</sub> 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,  $J_{3,4} \sim J_{4,5} \sim 10$  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);  $^{13}\text{C}$ ,  $\delta$  176.1 (CO<sub>2</sub>H), 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<sub>36</sub>H<sub>34</sub>O<sub>19</sub> (650.5): C, 48.00; H, 5.27. Found: C, 48.19; H, 5.43.

Acetylation of 5 with acetic anhydride in the presence of conc. H<sub>2</sub>SO<sub>4</sub> gave crystalline 12 in low yield only (28%).

2,3,4,2',3',4'-Hexa-O-acetyl-6,6'-di-O-trityl- $\alpha,\alpha$ -trehalose (13). – Prepared according to Liav and Goren<sup>17</sup>, 13 had m.p. 241°, [ $\alpha$ ]<sub>D</sub> + 110° (c 0.6, CHCl<sub>3</sub>); lit.<sup>17</sup> m.p. 238-241°, [ $\alpha$ ]<sub>D</sub> + 112.3°. <sup>1</sup>H-N.m.r. data:  $\delta$  7.38 and 7.24 (2m, Ph), 5.44 (d,  $J_{1,2}$  3.7 Hz, H-1), 5.43 (dd,  $J_{3,4}$  9.3,  $J_{2,3}$  10.4 Hz, H-3), 5.17 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  10.3 Hz, H-2), 5.12 (dd,  $J_{3,4}$  9.3,  $J_{4,5}$  10.5 Hz, H-4), 4.10 (dt,  $J_{5,6} = J_{5,6'}$  = 3.5 Hz,  $J_{4,5}$  10.3 Hz, H-5), 3.08 (d, 2 H,  $J$  3.5 Hz, H-6,6'), 1.97, 1.87, 1.73 (3s, 3 H each, 3 OAc).

2,3,4,2',3',4'-Hexa-O-acetyl- $\alpha,\alpha$ -trehalose (14). – Compound 13 (36 g) was detritylated with aqueous 80% acetic acid (600 mL) by stirring the suspension for 1.5 h at 75°, as described<sup>17</sup>. The mixture was concentrated to half its volume, filtered from precipitated triphenylcarbinol, and brought to dryness by concentration with added toluene followed by ethanol. Crystallization of the residue from oxolane-hexane gave 14 (13.8 g) and flash chromatography (solvent B) of the material in the mother liquor on silica gel (190 g) gave more 14 (1.1 g, total yield, 75%), m.p. 84°; lit.<sup>15</sup> m.p. 82-86°. <sup>1</sup>H-N.m.r. data:  $\delta$  5.51 (dd,  $J_{3,4}$  9.4,  $J_{2,3}$  10.3 Hz, H-3), 5.28 (d,  $J_{1,2}$  3.9 Hz, H-1), 4.99 (dd,  $J_{3,4}$  9.4,  $J_{4,5}$  10.4 Hz, H-4), 4.96 (dd,  $J_{1,2}$  4,  $J_{2,3}$  10.3 Hz, H-2), 3.93 (m, H-5), 3.62 (m, H-6,6'), 2.07, 2.06, 2.02 (3s, 3 H each, 3 OAc).

2,3,4,2',3',4'-Hexa-O-acetyl-6,6'-di-O-(trifluoromethyl) sulfonyl- $\alpha,\alpha$ -trehalose (15). — To a mixture of dry 1,2-dichloroethane (200 mL, freshly distilled from  $P_2O_5$ ) and dry pyridine (18.6 mL) at  $-15^\circ$  was added, dropwise, trifluoromethanesulfonic anhydride (15.5 mL), under  $N_2$ . The mixture was stirred for 10 min and a solution of 14 (13.63 g) in dry 1,2-dichloroethane (30 mL) was introduced dropwise. The conversion of 14 ( $R_f$  0.3) into 15 ( $R_f$  0.65) was complete after 15 min (t.l.c. with solvent A). The mixture was extracted twice with aqueous 5% HCl, washed with aqueous  $NaHCO_3$  followed by water, dried ( $MgSO_4$ ), and concentrated. Trituration of the oil with anhydrous ether gave 15 as a white solid (15.59 g, 79%) which decomposed without melting;  $[\alpha]_D + 104^\circ$  (c 0.6,  $CHCl_3$ ). Mass spectrum (c.i.):  $m/z$  799 (2.4%,  $[M + 1 - AcOH]^+$ ), 709 (2.1%,  $[M + 1 - TfOH]^+$ ), 421 (96%,  $0.5[M - 16]^+$ ). N.m.r. data:  $^1H$ ,  $\delta$  5.47 (dd,  $J_{3,4}$  9.25,  $J_{2,3}$  10.35 Hz, H-3), 5.28 (d,  $J_{1,2}$  3.85 Hz, H-1), 5.09 (dd,  $J_{1,2}$  3.85,  $J_{2,3}$  10.3 Hz, H-2), 4.98 (dd,  $J_{3,4}$  9.2,  $J_{4,5}$  10.4 Hz, H-4), 4.50 (dd,  $J_{5,6}$  6.5,  $J_{6,6'}$  11.5 Hz, H-6), 4.39 (dd,  $J_{5,6'}$  2.5,  $J_{6,6'}$  11.5 Hz, H-6'), 4.24 (m, H-5), 2.08, 2.06, 2.02 (3s, 3 H each, 3 OAc);  $^{13}C$ ,  $\delta$  169.7, 169.3, 169.0 (MeCO), 93.3 (C-1), 73.3 (C-6), 69.5, 69.2, 68.3, 68.1 (C-2,3,4,5), 20.7, 20.6, 20.5 (COMe).

*Anal.* Calc. for  $C_{26}H_{32}F_6O_{21}S_2$  (858.6): C, 36.37; H, 3.76; S, 7.47. Found: C, 36.24; H, 3.92; S, 7.68.

2,3,4-Tri-O-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosylurononitrile 2,3,4-tri-O-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosidurononitrile (16). — A mixture of ditriflate 15 (15.5 g), acetonitrile (180 mL), water (20 mL), and KCN (3.1 g) was stirred at  $25^\circ$  for 2 h. The formation of 16 was indicated in t.l.c. (solvent A) by a spot having an  $R_f$  value (0.62) marginally lower than that of 15. After concentration, the oily residue was partitioned between  $CH_2Cl_2$  and water. The organic phase was washed twice with water, dried ( $MgSO_4$ ), and concentrated, to give crude 16 as a brown syrup.

Flash chromatography (190 g of silica gel; solvent *D*) and crystallization from methanol gave **16** as a colorless solid (8.95 g, 81%) which sintered at 85° and melted indistinctly at 95-110°;  $[\alpha]_D + 129^\circ$  (c 0.5, CHCl<sub>3</sub>);  $\nu_{\max}^{\text{Nujol}}$  2250 (weak but sharp, CN), 1750 cm<sup>-1</sup> (ester CO). Mass spectra: *m/z* (+ve f.a.b.) 613 (1%, [M + 1]<sup>+</sup>), 298 (49%, 0.5[M - 16]<sup>+</sup>); *m/z* (c.i.) 612 (1.4%, M<sup>+</sup>), 553 (1.2%, [M + 1 - AcOH]<sup>+</sup>), 298 (100%, 0.5 [M - 16]<sup>+</sup>). N.m.r. data: <sup>1</sup>H,  $\delta$  5.46 (dd, *J*<sub>3,4</sub> 9.3, *J*<sub>2,3</sub> 10.2 Hz, H-3), 5.31 (d, *J*<sub>1,2</sub> 3.9 Hz, H-1), 5.09 (dd, *J*<sub>1,2</sub> 3.9, *J*<sub>2,3</sub> 10.25 Hz, H-2), 4.93 (dd, *J*<sub>3,4</sub> 9.3, *J*<sub>4,5</sub> 9.9 Hz, H-4), 4.16 (dt, *J*<sub>5,6</sub> = *J*<sub>5,6'</sub> = 6.1, *J*<sub>4,5</sub> 10 Hz, H-5), 2.55 (d, 2 H, H-6,6'), 2.14, 2.10, 2.02 (3s, 3 H each, 3 OAc); <sup>13</sup>C,  $\delta$  169.7, 169.6, 169.3 (MeCO), 115.6 (CN), 93.1 (C-1), 71.6, 69.5, 69.4, 66.3 (C-2,3,4,5), 21.0 (C-6), 20.8, 20.75, 20.7 (COMe).

*Anal.* Calc. for C<sub>26</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> (612.5): C, 50.98; H, 5.27; N, 4.57. Found: C, 50.93; H, 5.49; N, 4.49.

## D.2. An approach to the synthesis of (2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronic acid) 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosiduronic acid (30)

2,3,4,2',3',4'-Hexa-O-benzyl-6,6'-di-O-trityl- $\alpha,\alpha$ -trehalose (25). – A mixture of 6,6'-di-O-trityl- $\alpha,\alpha$ -trehalose<sup>36</sup> (24, 5.08 g), benzyl bromide (4.8 mL), NaH (2.2 g), and *N,N*-dimethylformamide (120 mL), prepared at 0°, was stirred at room temperature for 2 days. After the careful addition of water, the mixture was concentrated at 65° under reduced pressure with repeated additions of water, and the remaining syrup was then partitioned between ether and water. The dried (Na<sub>2</sub>SO<sub>4</sub>) organic phase was concentrated to give a yellow material that was passed through a bed of silica gel with solvent *W*. Concentration of the filtrate gave amorphous 25 (7.73 g, 92%), R<sub>f</sub> 0.65 (t.l.c., solvent *U*). By precipitation from acetone-acetic acid solution by careful addition of water, an analytical sample was obtained as a white solid that sintered and melted indistinctly above 60°. The <sup>1</sup>H-n.m.r. spectrum indicated a 5:1 ratio of phenylic to benzylic protons. <sup>13</sup>C-N.m.r. data:  $\delta$  143.7 (C-1 in CPh<sub>3</sub>), 138.7, 138.1, 137.9 (C-1 in CH<sub>2</sub>Ph), 128.8-126.7 (multiple peaks, Ph), 94.8 (C-1), 86.2 (Ph<sub>3</sub>C), 81.8, 80.3, 77.9, 70.7 (C-2,3,4,5), 75.9, 75.5, 72.5 (PhCH<sub>2</sub>), 61.5 (C-6).

*Anal.* Calc. for C<sub>92</sub>H<sub>86</sub>O<sub>11</sub> (1367.6): C, 80.79; H, 6.34. Found: C, 80.68; H, 6.45.

2,3,4,2',3',4'-Hexa-O-benzyl- $\alpha,\alpha$ -trehalose (26). – Compound 21 (6.90 g) was detritylated with aqueous 80% acetic acid (150 mL) during 9 h at 85°, essentially as described for the crude<sup>36</sup> trityl ether. Chromatography<sup>36</sup> of the product furnished pure 26 (3.475 g, 78%), [ $\alpha$ ]<sub>D</sub> + 104° (c 1.6, CHCl<sub>3</sub>); lit.<sup>36</sup> [ $\alpha$ ]<sub>D</sub> + 99°. The <sup>1</sup>H-n.m.r. data agreed with those reported<sup>36</sup>. <sup>13</sup>C-N.m.r. data (50.29 MHz):  $\delta$  138.9, 138.4, 138.2 (C-

1 in CH<sub>2</sub>Ph), 128.5-127.6 (multiple peaks, Ph), 93.9 (C-1), 81.6, 79.6, 77.5, 71.5 (C-2,3,4,5), 75.6, 75.0, 73.0 (PhCH<sub>2</sub>), 61.4 (C-6).

2,3,4,2',3',4'-Hexa-O-benzyl-6,6'-di-O-(trifluoromethyl)sulfonyl- $\alpha,\alpha$ -trehalose (27). – Compound 26 (1.98 g) was triflated as detailed for 14, with appropriately adjusted proportions of reagents, to give 27, R<sub>f</sub> 0.65 (t.l.c., solvent G), which, after processing was obtained as a pale yellow foam (2.42 g, 94%), [ $\alpha$ ]<sub>D</sub> + 98° (c 1.2, CHCl<sub>3</sub>). A sample (170 mg) was purified on a small column (3 g of silica gel) by elution with 1:6 ethyl acetate-hexane, to give colorless 27 (127 mg), m.p. 106-107°. N.m.r. data: <sup>1</sup>H (200 MHz),  $\delta$  7.3 (m, 15 H, Ph), 5.13 (d, J<sub>1,2</sub> 3.5 Hz, H-1), 5.06-4.50 (m, 6 H, 3 PhCH<sub>2</sub>), 4.18-3.95 (m, 4 H, H-3,5,6,6'), 3.55 (dd, J<sub>1,2</sub> 3.5, J<sub>2,3</sub> 9.7 Hz, H-2), 3.47 (dd, J 9.3 and 9.9 Hz, H-4); <sup>13</sup>C (50.29 MHz)  $\delta$  138.3, 137.8, 137.6 (C-1 in CH<sub>2</sub>Ph), 128.6-127.7 (multiple peaks, Ph), 94.5 (C-1), 81.5, 79.3, 76.3, 68.6 (C-2,3,4,5), 75.5, 75.2, 74.2, 73.2 (PhCH<sub>2</sub> and C-6).

*Anal.* Calc. for C<sub>56</sub>H<sub>56</sub>F<sub>6</sub>O<sub>15</sub>S<sub>2</sub> (1147.1): C, 58.63; H, 4.92; F, 9.94; S, 5.59. Found: C, 59.76; H, 5.25; F, 8.94; S, 5.07. The values found fit a composition C<sub>55.8</sub>H<sub>56.2</sub>F<sub>5.4</sub>O<sub>14.4</sub>S<sub>1.8</sub>, representing a product having lost 10% of its triflyl groups; apparently, the analytical sample had deteriorated in storage and transit.

2,3,4-Tri-O-benzyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosylurononitrile 2,3,4-tri-O-benzyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosidurononitrile (28). – Crude 27 (2.25 g) was treated with KCN as described for 15, with appropriately adjusted proportions of reagents. Conversion of 27 (R<sub>f</sub> 0.65) into 28 (R<sub>f</sub> 0.25) was complete after 8 h (t.l.c., solvent G). The crude product, isolated as for 16, was subjected to flash chromatography (38 g of silica gel, solvent G), to give a pure syrupy 28 (1.21g) and 28 (0.57g) containing a trace impurity (t.l.c.). Pure 28 had [ $\alpha$ ]<sub>D</sub> + 132° (c 1.5, CHCl<sub>3</sub>);  $\nu_{\max}^{\text{Nujol}}$  2250 cm<sup>-1</sup> (CN). N.m.r. data: <sup>1</sup>H (200 MHz),  $\delta$  7.3 (m, 15 H, 3 Ph), 5.20 (d, J<sub>1,2</sub> 3.5

Hz, H-1), 5.09-4.60 (m, 6 H, 3 PhCH<sub>2</sub>), 4.15 (qn, *W* 19.2 Hz, H-5), 4.04 (t, *J*<sub>2,3</sub> ~ *J*<sub>3,4</sub> ~ 9.25 Hz, H-3), 3.64 (dd, *J*<sub>1,2</sub> 3.5, *J*<sub>2,3</sub> 9.5 Hz, H-2), 3.42 (t, *J*<sub>3,4</sub> ~ *J*<sub>4,5</sub> ~ 9.35 Hz, H-4), 2.33 (m, 2 H, H-6,6'); <sup>13</sup>C (50.29 MHz), δ 138.7, 138.1, 138.0 (C-1 in CH<sub>2</sub>Ph), 128-127.5 (multiple peaks, Ph), 117.2 (CN), 94.0 (C-1), 81.4, 80.3, 79.6, 66.9 (C-2,3,4,5), 75.6, 75.4, 73.2 (PhCH<sub>2</sub>), 20.3 (C-6).

*Anal.* Calc. for C<sub>56</sub>H<sub>56</sub>N<sub>2</sub>O<sub>4</sub> (901.1): C, 74.65; H, 6.26; N, 3.11. Found: C, 74.56; H, 6.48; N, 3.04.

2,3,4-Tri-*O*-benzyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronamide 2,3,4-tri-*O*-benzyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosiduronamide (29). —To a solution of 28 (947 mg) in acetone (30 mL) was added solid NaOH (535 mg) and aqueous 30% H<sub>2</sub>O<sub>2</sub> (10 mL). The mixture was stirred at room temperature for 20 h, during which time 28 (*R*<sub>f</sub> 1.0) was converted into one major product (*R*<sub>f</sub> 0.6) accompanied by traces of more-polar and less-polar by-products (t.l.c., solvent O). No visible changes occurred on prolonged treatment of the product under these conditions, or at elevated temperatures. The mixture was concentrated to a small volume, the residue was partitioned between ether and water, and the dried (MgSO<sub>4</sub>) ether phase was concentrated to an oily residue that was applied to a chromatographic column (25 g of silica gel; 99:1 ethyl acetate-methanol). Virtually pure 29 (syrup) yielded a crystalline trihydrate (852 mg, 82.5%) from ether (plus 2% of ethyl acetate) and hexane; m.p. 151-154°, [ $\alpha$ ]<sub>D</sub> + 77° (*c* 1, CHCl<sub>3</sub>);  $\nu_{\max}^{\text{Nujol}}$  3400, 3320, 3180 (NH, OH), 1680 (amide I), 1610 (amide II) cm<sup>-1</sup>. N.m.r. data: <sup>1</sup>H (200 MHz), δ 7.3 (m, 15 H, 3 Ph), 5.93 and 5.73 (2bs, 1 H each, exchangeable, NH<sub>2</sub>), 5.18 (d, *J*<sub>1,2</sub> 3.5 Hz, H-1), 4.95-4.55 (m, 6 H, 3 PhCH<sub>2</sub>), 4.23 (m, H-5), 4.07 (t, *J*<sub>2,3</sub> ~ *J*<sub>3,4</sub> ~ 10 Hz, H-3), 3.58 (dd, *J*<sub>1,2</sub> 3.5, *J*<sub>2,3</sub> 10 Hz, H-2), 3.28 (t, *J*<sub>3,4</sub> ~ *J*<sub>4,5</sub> ~ 10 Hz, H-4), 2.55 (dd, *J*<sub>5,6</sub> 2.5, *J*<sub>6,6'</sub> 15 Hz, H-6), 2.20 (dd, *J*<sub>5,6'</sub> 10, *J*<sub>6,6'</sub> 15 Hz, H-6'); <sup>13</sup>C (50.29 MHz), δ 174.1 (CO), 138.5, 138.1, 138.1 (C-1 in CH<sub>2</sub>Ph),

128.6-127.8 (multiple peaks, Ph), 92.3 (C-1), 81.2, 81.0, 79.4, 68.0 (C-2,3,4,5), 75.5, 74.9, 73.2 (PhCH<sub>2</sub>), 38.0 (C-6). There was an unexplained signal (secondary or quaternary C) at  $\delta$  109.6.

*Anal.* Calc. for C<sub>56</sub>H<sub>60</sub>N<sub>2</sub>O<sub>11</sub>·3H<sub>2</sub>O (991.1): C, 67.86; H, 6.71; N, 2.83. Found: C, 67.94; H, 6.60; N, 2.87.

### D.3. Deacetylated and peracetylated dimethyl bis(heptosiduronates) 35 and 8 and the trehalose homolog 36

Methyl [(methyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (**8**). – A solution of compound **35** (35 mg) in pyridine (4 mL) was treated overnight at 25° with acetic anhydride (1 mL) and 4-dimethylaminopyridine (3 mg). Formation of **8** ( $R_f$  0.6) was indicated by t.l.c. (solvent *B*). Concentration of the mixture followed by dissolution of the residue in  $\text{CHCl}_3$  and conventional processing gave **8** (31.5 mg, 64%) crystallized from methanol, m.p. 134-135°,  $[\alpha]_D + 144^\circ$  (c 0.5,  $\text{CHCl}_3$ );  $\nu_{\text{max}}^{\text{Nujol}} 1750 \text{ cm}^{-1}$  (ester CO). Mass spectrum (c.i.):  $m/z$  331 (100%, (0.5 [M – 16]<sup>+</sup>), 271 (10%, [0.5(M – 16) – AcOH]<sup>+</sup>), 211 (25%, [0.5 (M – 16) – 2AcOH]<sup>+</sup>). N.m.r data : <sup>1</sup>H,  $\delta$  5.50 (dd,  $J_{3,4}$  9.2,  $J_{2,3}$  10.0 Hz, H-3), 5.22 (d,  $J_{1,2}$  3.8 Hz, H-1), 5.17 (dd,  $J_{1,2}$  3.7,  $J_{3,3}$  10.2 Hz, H-2), 4.88 (dd,  $J_{3,4}$  9.2,  $J_{4,5}$  10.2 Hz, H-4), 4.34 (m, H-5), 3.62 (s, 3 H, OMe), 2.45 (m, 2 H, H-6,6'), 2.11, 2.02, and 2.00 (3s, 3 H each, 3 OAc); <sup>13</sup>C,  $\delta$  170.1, 169.9, 169.7, 169.7 (CO), 90.8 (C-1), 71.8, 70.2, 68.9, 66.3 (C-2,3,4,5), 52.0 (OMe), 36.6 (C-6), and 20.6 (COMe).

*Anal.* Calc. for  $\text{C}_{28}\text{H}_{38}\text{O}_{19}$  (678.6): C, 49.56; H, 5.64. Found: C, 49.36; H, 5.54.

Methyl [(methyl 5-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (**35**). – **A.** From hexa-acetate **8**. To a solution of **8** (50 mg) in methanol (5 mL) was added 1 drop of methanolic sodium methoxide at room temperature. Deacetylation was complete after 15 min as indicated by t.l.c. ( $R_f$  of **35**: 0.7 and 0.4 with solvents *L* and *N*, respectively). Deionization and concentration of the solution, followed by trituration of the residue with methanol-ether, gave **35** (29 mg, 92%) as a white solid that sintered near 90° and gradually turned into a viscous foam above 100°,  $[\alpha]_D + 138^\circ$  (c 1.1, methanol). Mass spectrum (c.i.):  $m/z$  205

(100%, 0.5 [M – 16]<sup>+</sup>), 187 (56%, [0.5 (M – 16) – H<sub>2</sub>O]<sup>+</sup>), 169 (34%, [0.5 (M – 16) – 2H<sub>2</sub>O]<sup>+</sup>). N.m.r. data (D<sub>2</sub>O): <sup>1</sup>H, δ 5.14 (d, J<sub>1,2</sub> 3.85 Hz, H-1), 4.17 (dt, J<sub>5,6</sub> 3, J<sub>4,5</sub> = J<sub>5,6</sub> = 9.9 Hz, H-5), 3.86 (t, J<sub>2,3</sub> ~ J<sub>3,4</sub> ~ 9.5 Hz, H-3), 3.74 (s, 3 H, OMe), 3.70 (dd, J<sub>1,2</sub> 3.85, J<sub>2,3</sub> 9.9 Hz, H-2), 3.36 (t, J<sub>3,4</sub> ~ J<sub>4,5</sub> ~ 9.5 Hz, H-4), 2.98 (dd, J<sub>5,6</sub> 3, J<sub>6,6'</sub> 15.9 Hz, H-6), 2.58 (dd, J<sub>5,6</sub> 9.8, J<sub>6,6'</sub> 15.9 Hz, H-6'); <sup>13</sup>C (50.29 MHz), δ 177.2 (CO), 95.8 (C-1), 76.0, 75.3, 74.1, 71.8 (C-2,3,4,5), 55.5 (OMe), 39.5 (C-6).

*Anal. Calc.* for C<sub>16</sub>H<sub>26</sub>O<sub>13</sub> (426.4): C, 45.07; H, 6.15. Found: C, 45.18; H, 6.22.

**B.** From dicarboxylic acid **5**. Under careful exclusion of moisture, a stream of HCl gas was passed for a few seconds into a solution of **5** (74 mg) in methanol (15 mL). The solution was kept overnight at 23°, then concentrated with several additions of fresh methanol, and the residue (R<sub>f</sub> 0.4, solvent *M*) was crystallized from methanol-ether to give **35** (60 mg, 87%). Alternatively, a solution of **5** (30 mg) in aqueous methanol was treated with an ethereal solution of diazomethane until the yellow color persisted. Evaporation of the solvents and trituration of the residue with methanol-ether gave solid **35** (31.8 mg, 99%).

**6-Deoxy-α-D-gluco-heptopyranosyl 6-deoxy-α-D-gluco-heptopyranoside (36).**  
– A solution of **12** (54 mg, 0.083 mmol) in oxolane (1.8 mL) containing 0.8 mmol of BH<sub>3</sub> was stirred under N<sub>2</sub> for 2 h at room temperature and then for 40 min at the reflux temperature. T.l.c (solvent *M*) revealed several spots attributable to partially deacetylated reduction products. The excess of reductant was decomposed by continued heating (10 min) with added methanol, the solvents were evaporated, and the residue was treated with sodium methoxide in methanol at pH 9 and for 15 min at ~23°, which effected complete deacetylation. T.l.c. (solvent *M*) then showed a single spot (R<sub>f</sub> 0.3). Upon deionization with Amberlite IR-120 (H<sup>+</sup>) resin and

concentration of the solution, **36** was obtained as a colorless syrup (29 mg, 94%), the  $^{13}\text{C}$ -n.m.r. spectrum of which agreed with the structure. Crystallization from methanol-acetone gave **36** with m.p.  $201^\circ$ ,  $[\alpha]_D + 183^\circ$  (c 1.2, water). Mass spectrum (c.i.):  $m/z$  371 (100%,  $[\text{M} + 1]^+$ ), 177 (27%,  $0.5[\text{M} - 16]^+$ ), 159 (83%,  $[0.5(\text{M} - 16) - \text{H}_2\text{O}]^+$ ). N.m.r. data ( $\text{D}_2\text{O}$ ):  $^1\text{H}$  (200 MHz),  $\delta$  5.10 ( $J_{1,2}$  3.8 Hz, H-1), 3.78-3.71 (m, 4 H, unresolved), 3.64 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.9, H-2), 3.27 (dd,  $J$  9.0 and 9.7 Hz), 2.09 and 1.69 (2m, 1 H each, H-6,6');  $^{13}\text{C}$  (50.29 MHz),  $\delta$  95.85 (C-1), 76.0, 75.1, 73.6, 71.3 (C-2,3,4,5), 60.75 (C-7), 35.6 (C-6).

*Anal.* Calc. for  $\text{C}_{14}\text{H}_{26}\text{O}_{11}$  (370.4): C, 45.40; H, 7.08. Found: C, 45.37; H, 7.05.

A solution of syrupy **36** (19 mg) in a few drops of N,N-dimethylformamide was acetylated overnight at  $\sim 23^\circ$  with 1:1 acetic anhydride-pyridine (1 mL). Removal of the reagent by co-evaporation with excess of toluene gave the syrupy octa-acetate **37**. N.m.r. data:  $^1\text{H}$  (200 MHz),  $\delta$  5.42, (dd,  $J$  9.3 and 10 Hz, H-3 or H-4), 5.21 (d,  $J_{1,2}$  3.95 Hz, H-1), 4.97 (dd,  $J_{1,2}$  3.85,  $J_{2,3}$  10.1 Hz, H-2), 4.88 (dd,  $J$  9.3 and 10 Hz, H-4 or H-3), 4.08 (octet, 2H,  $W$  44 Hz, H-7,7'), 3.82 (octet,  $J$  5,7, and 10 Hz, H-5), 2.10, 2.01, 2.00, 1.99 (4s, 3 H each, 4 AcO), 1.75 (m, 2H, H-6,6');  $^{13}\text{C}$  (75.43 MHz),  $\delta$  170.7, 169.8, 169.6, 169.5 (MeCO), 90.8 (C-1), 72.0, 70.2, 70.1, 66.9(C-2,3,4,5), 60.2 (C-7), 30.4 (C-4), 20.9, 29.75, 20.75, 20.7 (COMe).

#### D.4. Synthesis of peracetylated dialkyl bis(heptosiduronates) 39-43.

General procedure, illustrated on the preparation of octyl [(octyl 2,3,4-tri-O-acetyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronate) 2,3,4-tri-O-acetyl-6-deoxy- $\alpha$ -D-gluco-heptopyranosid]uronate (39). – To the peracetylated diacid 12 (70 mg, 0.1 mmol) dissolved in dry 1,2-dichloroethane or benzene (2 mL) was added a large excess of oxalyl chloride (0.5 mL) in an oven-dried (or flame-dried) round-bottom flask. A nitrogen atmosphere was provided, and the mixture was heated at 60° for 95 minutes and then evaporated to dryness under reduced pressure, with a drying tube being inserted between the rotary evaporator and the aspirator. Dichloroethane was added to, and evaporated from, the solid residue which was then dissolved in fresh 1,2-dichloroethane (2 mL) and treated with anhydrous 1-octanol (0.085 mL; 0.54 mmol) by stirring for 2 h at 75°. The light-brown reaction mixture, which showed a major spot ( $R_f$  0.30, solvent *T*) in t.l.c., was concentrated to an oil that was chromatographed on a column of silica gel (4 g, 230-400 mesh). Solvent *V* eluted the fast-moving, excess 1-octanol, and solvent *U* subsequently delivered the desired diester 39 as an oil (90.6 mg, 96%);  $[\alpha]_D + 108^\circ$  (c 0.87,  $\text{CHCl}_3$ );  $\nu_{\text{max}}^{\text{film}} 1745 \text{ cm}^{-1}$  (ester CO). Mass spectrum (c.i.):  $m/z$  429 (100%, 0.5 [M – 16]<sup>+</sup>). N.m.r. data:  $^1\text{H}$ ,  $\delta$  5.50 (dd,  $J_{2,3}$  9.5,  $J_{3,4}$  9.4 Hz, H-3), 5.20 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.8 Hz, H-2), 5.16 (d,  $J_{1,2}$  3.7 Hz, H-1), 4.89 (dd,  $J_{3,4}$  9.2,  $J_{4,5}$  10.3 Hz, H-4), 4.36 (m, H-5), 4.01 (m,  $\text{COOCH}_2$ ), 2.42 (m, H-6,6'), 2.10, 2.02, 2.00 (3s, 3 H each, 3 OAc), 1.55, 1.27, 0.86 (m for  $\text{C}_7\text{H}_{15}$  residue);  $^{13}\text{C}$ ,  $\delta$  169.8, 169.7, 169.6, 169.6 (CO), 90.9 (C-1), 71.8, 70.4, 69.0, 66.3 (C-2,3,4,5), 65.1 ( $\text{COOCH}_2$ ), 36.6 ( $\text{OCOCH}_2$ ), 20.8, 20.7, 20.7 ( $\text{OCOCH}_3$ ).

*Anal.* Calc. for  $\text{C}_{42}\text{H}_{66}\text{O}_{19}$  (874.97): C, 57.65; H, 7.60. Found: C, 57.79; H, 7.70.

The diesters 40-43 were prepared by the same general procedure. The proportions of starting materials and the yields obtained are given in Table 1.

Pentadecyl [(pentadecyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyl-uronate) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (40).—

Compound 40 showed:  $R_f$  0.33 (solvent S),  $[\alpha]_D + 85^\circ$  (c 0.52,  $\text{CHCl}_3$ ),  $\nu_{\text{max}}^{\text{film}} 1745 \text{ cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  1071 (2%,  $[\text{M} + 1]^+$ ), 527 (48%, 0.5  $[\text{M} - 16]^+$ ). N.m.r. data:  $^1\text{H}$ ,  $\delta$  5.50 (dd,  $J_{2,3} 9.6$ ,  $J_{3,4} 9.2$  Hz, H-3) 5.20 (dd,  $J_{1,2} 3.7$ ,  $J_{2,3} 9.7$  Hz, H-2), 5.16 (d,  $J_{1,2} 3.7$  Hz, H-1), 4.89 (dd,  $J_{3,4} 9.2$ ,  $J_{4,5} 10.2$  Hz, H-4), 4.36 (m, H-5), 4.00 (m,  $\text{COOCH}_2$ ), 2.42 (m, H-6,6'), 2.10, 2.02, 2.00 (3s, 3H each, 3 OAc), 1.55, 1.25, 0.86 (m, for  $\text{C}_{14}\text{H}_{29}$  residue);  $^{13}\text{C}$ ,  $\delta$  169.7, 169.7, 169.6, 169.6 (CO), 90.9 (C-1), 71.8, 70.4, 69.0, 66.3 (C-2,3,4,5), 65.1 ( $\text{COOCH}_2$ ), 36.6 ( $\text{OCOCH}_2$ ), 20.8, 20.7, 20.7 ( $\text{OCOCH}_3$ ).

*Anal. Calc.* for  $\text{C}_{56}\text{H}_{94}\text{O}_{19}$  (1071.35): C, 62.78; H, 8.84. Found: C, 62.63; H, 8.96.

Hexadecyl [(hexadecyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyl-uronate) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (41).—

Compound 41 showed:  $R_f$  0.35 (solvent S),  $[\alpha]_D + 85^\circ$  (c 0.79,  $\text{CHCl}_3$ );  $\nu_{\text{max}}^{\text{film}} 1750 \text{ cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  541 (21%, 0.5  $[\text{M} - 16]^+$ ). N.m.r. data:  $^1\text{H}$ ,  $\delta$  5.50 (dd,  $J_{2,3} 9.5$ ,  $J_{3,4} 9.3$  Hz, H-3), 5.20 (dd,  $J_{1,2} 3.7$ ,  $J_{2,3} 9.7$  Hz, H-2), 5.16 (d,  $J_{1,2} 3.7$  Hz, H-1), 4.89 (dd,  $J_{3,4} 9.3$ ,  $J_{4,5} 10.2$  Hz, H-4), 4.36 (m, H-5), 4.00 (m,  $\text{COOCH}_2$ ), 2.42 (m, H-6,6'), 2.10, 2.02, 2.00 (3s, 3H each, 3 OAc), 1.53, 1.24, 0.86 (m, for  $\text{C}_{15}\text{H}_{31}$  residue);  $^{13}\text{C}$ ,  $\delta$  169.7, 169.7, 169.6, 169.6 (CO), 90.9 (C-1), 71.8, 70.4, 69.0, 66.3 (C-2,3,4,5), 65.1 ( $\text{COOCH}_2$ ), 36.6 ( $\text{OCOCH}_2$ ), 20.7, 20.7, 20.7 ( $\text{OCOCH}_3$ ).

*Anal. Calc.* for  $\text{C}_{58}\text{H}_{98}\text{O}_{19}$  (1099.40): C, 63.36; H, 8.98. Found: C, 63.57; H, 8.88.

Heptadecyl [(heptadecyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptapyranosyluronate) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptapyranosid]uronate (42).—

Compound 42 showed:  $R_f$  0.38 (solvent S),  $[\alpha]_D + 80^\circ$  (c 0.84,  $\text{CHCl}_3$ );  $\nu_{\text{max}}^{\text{film}}$  1750  $\text{cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  1127 (50%,  $[\text{M} + 1]^+$ ), 555 (25%, 0.5  $[\text{M} - 16]^+$ ). N.m.r. data:  $^1\text{H}$ ,  $\delta$  5.50 (dd,  $J_{2,3}$  9.5,  $J_{3,4}$  9.4 Hz, H-3), 5.20 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.7 Hz, H-2), 5.16 (d,  $J_{1,2}$  3.9 Hz, H-1), 4.89 (d,  $J_{3,4}$  9.2,  $J_{4,5}$  10.2 Hz, H-4), 4.36 (m, H-5), 4.00 (m,  $\text{COOCH}_2$ ), 2.42 (m, H-6, 6'), 2.10, 2.02, 2.00 (3s, 3 H each, 3 OAc), 1.53, 1.25, 0.86 (m, for  $\text{C}_{16}\text{H}_{33}$  residue);  $^{13}\text{C}$ ,  $\delta$  169.7, 169.7, 169.6, 169.6 (CO), 90.9 (C-1), 71.8, 70.4, 69.0, 66.3 (C-2,3,4,5), 65.1 ( $\text{COOCH}_2$ ), 36.6 ( $\text{OCOCH}_2$ ), 20.8, 20.7, 20.7 ( $\text{OCOCH}_3$ ).

Anal. Calc. for  $\text{C}_{60}\text{H}_{102}\text{O}_{19}$  (1127.45): C, 63.92; H, 9.12. Found: C, 63.77; H, 9.02.

Octadecyl [(octadecyl 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 2,3,4-tri-*O*-acetyl-6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (43).—

Compound 43 showed:  $R_f$  0.32 (solvent T),  $[\alpha]_D + 80^\circ$  (c 0.63,  $\text{CHCl}_3$ );  $\nu_{\text{max}}^{\text{film}}$  1750  $\text{cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  569 (100%, 0.5  $[\text{M} - 16]^+$ ). N.m.r. data:  $^1\text{H}$ ,  $\delta$  5.50 (dd,  $J_{2,3}$  9.5,  $J_{3,4}$  9.5 Hz, H-3), 5.20 (dd,  $J_{1,2}$  3.9,  $J_{2,3}$  9.8 Hz, H-2), 5.16 (d,  $J_{1,2}$  3.9 Hz, H-1), 4.89 (dd,  $J_{3,4}$  9.2,  $J_{4,5}$  10.2 Hz, H-4), 4.37 (m, H-5), 4.00 (m,  $\text{COOCH}_2$ ), 2.42 (m, H-6, 6'), 2.10, 2.02, 2.00 (3s, 3 H each, 3 OAc), 1.54, 1.26, 0.86 (m, for  $\text{C}_{17}\text{H}_{35}$  residue);  $^{13}\text{C}$ ,  $\delta$  169.7, 169.7, 169.6, 169.5 (CO), 90.9 (C-1), 71.8, 70.4, 69.0, 66.3 (C-2,3,4,5), 65.1 ( $\text{COOCH}_2$ ), 36.6 ( $\text{OCOCH}_2$ ), 20.8, 20.7, 20.7 ( $\text{OCOCH}_3$ ).

Anal. Calc. for  $\text{C}_{62}\text{H}_{106}\text{O}_{19}$  (1155.51): C, 64.45; H, 9.25. Found: C, 64.58; H, 9.38.

**Table 1. Preparation of peracetylated dialkyl bis(heptosiduronates) 39 - 43**

Compound	Starting materials		Yield of diester	
	Acid	Alcohol		
	mg (mmol)	mg (mmol)	mg (%)	
39	70 (0.108)	1-octanol 70 (0.54)	91 (96)	
40	78 (0.120)	1-pentadecanol 137 (0.60)	110 (86)	
41	70 (0.108)	1-hexadecanol 130 (0.54)	101 (86)	
42	64 (0.099)	1-heptadecanol 126 (0.49)	90 (81)	
43	74 (0.114)	1-octadecanol 155 (0.57)	106 <sup>a</sup> (81)	

<sup>a</sup>After crystallization from methanol, 80mg (61%) of crystalline **43** m.p. 37.5 - 39° was obtained; the remaining mother liquor material (26mg) was homogeneous in t.l.c..

## D.5. Synthesis of dialkyl bis(heptosiduronates) 45-49

### D.5.1. By deacetylation of peracetates 39-43

General procedures, illustrated on the preparation of octadecyl [(octadecyl 6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-gluco-heptopyranosid] uronate (49). – Compound **43** (80 mg, 0.069 mmol), 1-octadecanol (93 mg, 0.34 mmol), and a few crystals of p-toluenesulfonic acid were dissolved in dry 1,2-dichloroethane or benzene (2 mL). The solution was heated at 60°, and the reaction was monitored by t.l.c. using solvents *T* and *O*. After 2 days, the t.l.c. pattern ceased to change. Solvent *T* revealed some unreacted **43** ( $R_f$  0.32), some slower-moving products, and an immobile product giving a strong spot. Solvent *O* indicated the fully deacetylated diester **49** ( $R_f$  0.46) and several spots of higher  $R_f$  values, attributable to partially deacetylated products, 1-octadecanol, and its acetate. The least-polar product present gave a u.v.-active spot. Additional 1-octadecanol (90 mg) and a few more crystals of p-toluenesulfonic acid were introduced, and heating (60°) of the reaction mixture was continued for 2 days, after which all **43** had disappeared (solvent *T*) and the spot for **49** (solvent *O*) had increased in strength. However, incompletely deacetylated products were still present. The mixture was processed by column chromatography on silica gel (70-230 mesh), using sequentially hexane, 1:4 ethyl acetate-hexane, 1% methanol in ethyl acetate, and 2% methanol in ethyl acetate as the eluants. The less polar eluants eluted 1-octadecanol and its acetate, the incompletely deacetylated esters, and part of the u.v.-active material, whereas the last-mentioned eluant produced the desired diester **49**, contaminated with some of the u.v.-active compound. The fractions containing incompletely deacetylated diester were pooled and subjected again to solvolysis for 1 day as described (in the presence of 50 mg of added 1-octadecanol). Chromatography then

furnished additional **49**, but some incompletely solvolyzed material still remained. [In the preparation of the diesters **45-48**, such fractions were sometimes recycled once more when it was deemed worthwhile]. The combined fractions of **49** were freed from the u.v.-active contaminant by several recrystallizations from hot methanol, and eventually, 18 mg (28%) of pure **49** was obtained. [In the preparation of **45-48**, fewer recrystallizations were needed to remove the contaminant, and thus, higher yields of pure products were achieved]. Compound **49** sintered at 87° and melted at 114-116°;  $[\alpha]_D + 61^\circ$  (c 1, CHCl<sub>3</sub>);  $\nu_{\max}^{\text{film}} 3300 \text{ cm}^{-1}$  (OH),  $1730 \text{ cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  443 (7%, 0.5 [M-16]<sup>+</sup>), 425 (17%, [0.5 (M-16)-H<sub>2</sub>O]<sup>+</sup>), 407 (4%, [0.5 (M-16)-2H<sub>2</sub>O]<sup>+</sup>). N.m.r. data (CD<sub>3</sub>OD): <sup>1</sup>H,  $\delta$  5.09 (d,  $J_{1,2}$  3.8 Hz, H-1), 4.26 (m, 3H, H-5 and COOCH<sub>2</sub>), 3.75 (dd,  $J_{2,3}$  9.4,  $J_{3,4}$  9.2 Hz, H-3), 3.50 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.6 Hz, H-2), 3.15 (dd,  $J_{3,4}$  8.9,  $J_{4,5}$  9.9 Hz, H-4), 2.84 (dd,  $J_{5,6}$  2.8,  $J_{6,6'}$  15.6 Hz, H-6), 2.38 (dd,  $J_{5,6'}$  9.7,  $J_{6,6'}$  15.7 Hz, H-6'), 1.63, 1.30, 0.9 (m, for C<sub>17</sub>H<sub>35</sub> residue); <sup>13</sup>C,  $\delta$  173.2 (CO), 94.3 (C-1), 74.9, 74.5, 73.2, 69.7 (C-2,3,4,5), 65.6 (COOCH<sub>2</sub>), 37.9 (OCOCH<sub>2</sub>).

Anal. Calc. for C<sub>50</sub>H<sub>94</sub>O<sub>12</sub> (903.28): C, 66.48; H, 10.49. Found: C, 66.68; H, 10.43.

The deacetylations of **39-43** to give the diesters **45-49** were performed in the same manner. The proportions of reagents and yields of products are listed in Table 2.

Octyl [(octyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (**45**). — Compound **45** showed: m.p. 131 - 134°;  $[\alpha]_D + 63^\circ$  (c 0.58, CHCl<sub>3</sub>);  $\nu_{\max}^{\text{film}} 3300 \text{ cm}^{-1}$  (OH),  $1730 \text{ cm}^{-1}$  (ester CO). Mass spectrum (+ve f.a.b.):  $m/z$  623 (4%, [M+1]<sup>+</sup>), 605 (13%, [M+1-H<sub>2</sub>O]<sup>+</sup>), 587 (2%, [M+1-2H<sub>2</sub>O]<sup>+</sup>), 569 (4%, [M+1-3H<sub>2</sub>O]<sup>+</sup>), 303 (100%, 0.5 [M-16]<sup>+</sup>), 285 (100%, [0.5 (M-

16)  $-H_2O]^+$ ), 267 (47%,  $[0.5 (M - 16) - 2H_2O]^+$ ). N.m.r. data ( $CD_3OD$ ):  $^1H$ ,  $\delta$  5.09 (d,  $J_{1,2}$  3.8 Hz, H-1), 4.09 (m, 3H, H-5 and  $\underline{COOCH_2}$ ), 3.78 (dd,  $J_{2,3}$  9.3,  $J_{3,4}$  9.2 Hz, H-3), 3.49 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.6 Hz, H-2), 3.13 (dd,  $J_{3,4}$  9.0,  $J_{4,5}$  9.8 Hz, H-4), 2.85 (dd,  $J_{5,6}$  2.7,  $J_{6,6'}$  15.8 Hz, H-6), 2.36 (dd,  $J_{5,6'}$  9.9,  $J_{6,6'}$  15.8 Hz, H-6'), 1.62, 1.31, 0.90 (m, for  $C_7H_{15}$  residue);  $^{13}C$ ,  $\delta$  173.1 (CO), 94.4 (C-1), 75.0, 74.6, 73.3, 69.8 (C-2,3,4,5), 65.7 ( $\underline{COOCH_2}$ ), 38.1 ( $\underline{OCOCH_2}$ ).

Anal. Calc. for  $C_{30}H_{54}O_{13}$  (622.75): C, 57.86; H, 8.74. Found: C, 57.80; H, 8.70.

Pentadecyl [(pentadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (46). — Compound 46 showed: m.p. 122 - 124° (with sintering at 80°),  $[\alpha]_D + 62^\circ$  (c 0.79,  $CHCl_3$ );  $\nu_{max}^{film}$  3300  $cm^{-1}$  (OH), 1730  $cm^{-1}$  (ester CO). Mass spectrum (+ve .f.a.b):  $m/z$  819 (8%,  $[M + 1]^+$ ), 801 (4%,  $[M + 1 - H_2O]^+$ ), 401 (100%,  $0.5 [M - 16]^+$ ), 383 (99%,  $[0.5 (M - 16) - H_2O]^+$ ), 365 (22%,  $[0.5 (M - 16) - 2H_2O]^+$ ). N.m.r. data ( $CD_3OD$ ):  $^1H$ ,  $\delta$  5.09 (d,  $J_{1,2}$  3.8 Hz, H-1), 4.10 (m, 3H, H-5 and  $\underline{COOCH_2}$ ), 3.76 (dd,  $J_{2,3}$  9.3,  $J_{3,4}$  9.1 Hz, H-3), 3.49 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.6 Hz, H-2), 3.15 (dd,  $J_{3,4}$  9.0,  $J_{4,5}$  9.8 Hz, H-4), 2.84 (dd,  $J_{5,6}$  2.9,  $J_{6,6'}$  15.7 Hz, H-6), 2.38 (dd,  $J_{5,6'}$  9.6,  $J_{6,6'}$  15.7 Hz, H-6'), 1.63, 1.31, 0.89 (m, for  $C_{14}H_{29}$  residue);  $^{13}C$ ,  $\delta$  173.1 (CO), 94.3 (C-1), 75.0, 74.6, 73.3, 69.8 (C-2,3,4,5), 65.1 ( $\underline{COOCH_2}$ ), 38.1 ( $\underline{OCOCH_2}$ ).

Anal. Calc. for  $C_{44}H_{82}O_{13}$  (819.12): C, 64.52; H, 10.09. Found: C, 64.45; H, 10.12.

Hexadecyl [(hexadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (47). — Compound 47 showed: m.p. 122 - 123° (sintering at 82°),  $[\alpha]_D + 61^\circ$  (c 0.54,  $CHCl_3$ );  $\nu_{max}^{film}$  3300  $cm^{-1}$  (OH), 1730  $cm^{-1}$  (ester CO). Mass spectrum (+ve .f.a.b):  $m/z$  415 (12%,  $0.5 [M - 16]^+$ ), 397 (12%,  $[0.5 (M -$

16)  $-H_2O]^+$ ). N.m.r. data ( $CD_3OD$ ):  $^1H$ ,  $\delta$  5.09 (dd,  $J_{1,2}$  3.9 Hz, H-1), 4.09 (m, 3H, H-5 and  $COOCH_2$ ), 3.77 (dd,  $J_{2,3}$  9.6,  $J_{3,4}$  9.0 Hz, H-3), 3.48 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.7 Hz, H-2), 3.12 (dd,  $J_{3,4}$  9.0,  $J_{4,5}$  9.9 Hz, H-4), 2.84 (dd,  $J_{5,6}$  2.6,  $J_{6,6'}$  15.6 Hz, H-6), 2.37 (dd,  $J_{5,6'}$  10.2,  $J_{6,6'}$  15.7, H-6'), 1.62, 1.32, 0.92 (m, for  $C_{15}H_{31}$  residue);  $^{13}C$ ,  $\delta$  173.4 (CO), 94.4 (C-1), 75.2, 74.7, 73.5, 70.0 (C-2,3,4,5), 65.9 ( $COOCH_2$ ), 38.3 ( $OCOCH_2$ ).

Anal. Calc. for  $C_{46}H_{86}O_{13}$  (847.18): C, 65.22; H, 10.23. Found: C, 64.96; H, 10.14.

Heptadecyl [(heptadecyl6-deoxy- $\alpha$ -D-gluco-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-gluco-heptopyranosid]uronate (**48**). — Compound **48** showed: m.p. 119.5 - 122° (sintering at 82°),  $[\alpha]_D + 62^\circ$  (c 0.58,  $CHCl_3$ );  $\nu_{max}^{film}$  3300  $cm^{-1}$  (OH), 1730  $cm^{-1}$  (ester CO). Mass spectrum (+ve f.a.b):  $m/z$  429 (7%, 0.5  $[M - 16]^+$ ), 411 (3%, [0.5 (M - 16) -  $H_2O]^+$ ). N.m.r. data ( $CD_3OD$ ):  $^1H$ ,  $\delta$  5.09 (d,  $J_{1,2}$  3.8 Hz, H-1), 4.10 (m, 3H, H-5 and  $COOCH_2$ ), 3.76 (dd,  $J_{2,3}$  9.3,  $J_{3,4}$  9.1 Hz, H-3), 3.49 (dd,  $J_{1,2}$  3.8,  $J_{2,3}$  9.6 Hz, H-2), 3.15 (dd,  $J_{3,4}$  8.9,  $J_{4,5}$  9.9 Hz, H-4), 2.84 (dd,  $J_{5,6}$  2.9,  $J_{6,6'}$  15.7 Hz, H-6), 2.38 (dd,  $J_{5,6'}$  9.7,  $J_{6,6'}$  15.6 Hz, H-6'), 1.61, 1.29, 0.89 (m, for  $C_{16}H_{33}$  residue);  $^{13}C$ ,  $\delta$  173.1 (CO), 94.3 (C-1), 75.0, 74.6, 73.3, 69.8 (C-2,3,4,5), 65.7 ( $COOCH_2$ ), 38.1 ( $OCOCH_2$ ).

Anal. Calc. for  $C_{48}H_{90}O_{13}$  (875.23): C, 65.87; H, 10.36. Found: C, 65.76; H, 10.23.

**Table 2. Deacetylation of 39 - 43 to give dialkyl bis(heptosiduronates) 45 - 49**

Starting peracetate	Alcohol	Reaction time at 60°	Product <sup>a</sup>	Yield after recrystallization
mg (mmol)	mg (mmol)	days		mg (%)
39, 76 (0.087)	1-octanol 116 (0.89)	4	45	29 (54)
40, 65 (0.061)	1-pentadecanol 140 (0.61)	4 <sup>b</sup>	46	18 (36)
41, 66 (0.060)	1-hexadecanol 145 (0.25)	4	47	16 (31)
42, 76 (0.067)	1-heptadecanol 170 (0.67)	4 <sup>b</sup>	48	18 (30)
43, 80 (0.069)	1-octadecanol 183 (0.68)	4 <sup>b</sup>	49	18 (28)

<sup>a</sup>Compound 45 had R<sub>f</sub> 0.41, compounds 46 - 49 had R<sub>f</sub> 0.46 (solvent O). <sup>b</sup>Approximately half of the alcohol was introduced and reacted for 2 days after which, the remainder was added and the reaction continued for a total of 4 days.

### D.5.2. Alternative synthesis of 45-49 by use of alkanol mesylates

General procedure, illustrated on the preparation of hexadecyl [ (hexadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptapyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (47). – A solution of diacid 5 (14.2 mg, 0.036 mmol) and potassium carbonate (5.5 mg, 0.04 mmol) in water (1 mL) was freeze-dried using a Virtis freezemobile 24. To the residue 53 was added 1-hexadecyl mesylate (56 mg, 0.18 mmol) and dimethyl sulfoxide (0.8 mL), and the cloudy mixture was stirred in a stoppered vessel at 75° for ~1.5 h, during which it became homogeneous. The product had  $R_f$  0.46 (t.l.c. with solvent O) and was accompanied by faster- and slower-moving by-products. The reaction mixture was freeze-dried and then chromatographed on 3g of silica gel (70-230 mesh) by sequential use of 1:3 ethyl acetate-hexane, pure ethyl acetate, and 1:19 methanol-ethyl acetate as eluants, to yield 18.8 mg (62%) of the desired diester 47. Recrystallization from hot methanol gave analytically pure 47 (16 mg), m.p. 122-123.8° (with sintering at 82°).

Octyl [ (octyl 6-deoxy- $\alpha$ -D-*gluco*-heptapyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (45). – To the potassium salt prepared from diacid 5 (11.1 mg, 0.028 mmol) and potassium carbonate (5 mg, 0.036 mmol) was added 1-octyl mesylate (30 mg, 0.14 mmol) dissolved in ether (1 mL), under a nitrogen atmosphere. The ether was allowed to evaporate, and the stoppered vessel was then heated at 75° for 1.5 h. Processing and chromatography as described for 47 gave 45 (12.5 mg, 72%). Crystallized from methanol, the product (11 mg) showed  $R_f$  0.46 (solvent O), m.p. 132-134.5°.

Pentadecyl [ (pentadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptapyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (46). – Potassium salt prepared from 5 (22.3 mg,

0.056 mmol) and potassium carbonate (8.5 mg, 0.062 mmol) was allowed to react with 1-pentadecyl mesylate (85 mg, 0.28 mmol) to give **46** (29 mg, 63%) after chromatographic purification. Crystallized and recrystallized from methanol, the product (22 mg) had  $R_f$  0.46 (solvent O), m.p. 122.5-124.7° (with sintering at 78°).

Heptadecyl [ (heptadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (**48**). –The cesium salt prepared from **5** (5.4 mg, 0.013 mmol) and cesium carbonate (4.8 mg, 0.015 mmol) was allowed to react with 1-heptadecyl mesylate (22 mg, 0.066 mmol), to give **48** (6.4 mg, 54%),  $R_f$  0.46 (solvent O). Recrystallized from methanol, the product (5.5 mg, 47%) sintered at 87° and melted at 119-122°.

Use of the potassium salt of **5** gave **48** (m.p. 119-121.5°, with sintering at 87°) in similar yield (56% after chromatography; 44% after crystallization).

Octadecyl [ (octadecyl 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosyluronate) 6-deoxy- $\alpha$ -D-*gluco*-heptopyranosid]uronate (**49**). –To the potassium salt prepared from diacid **5** (8.1 mg, 0.02 mmol) and potassium carbonate (3.5 mg, 0.025 mmol) was added 1-octadecyl mesylate (36 mg, 0.1 mmol) which gave **49** (12.5 mg, 68%) after chromatographic purification; after crystallization from methanol, the product (10 mg, 54%) showed  $R_f$  0.46 (solvent O) and m.p. 114-116° (with sintering at 84°).

## D.6. Preparation of alkanol mesylates for the synthesis of 45-49

1-Octanol, 1-pentadecanol, 1-hexadecanol, 1-heptadecanol, and 1-octadecanol were mesylated by the following general procedure. — A solution of the alcohol (~150 mg) and methanesulfonyl chloride (1.2 mol. equiv) in pyridine (1-2 mL) was stirred for 1 h at room temperature. T.l.c. (solvent *D*) then indicated a single spot for the product. Cold water was added, and after 5 min the mixture was extracted with ether. The phases were separated and the aqueous layer was washed twice more with ether. The combined organic layers were washed sequentially with 5% HCl, saturated aqueous sodium bicarbonate and water, and dried with magnesium sulfate. After filtration, the ether was evaporated and the residue dissolved in acetone and stored in the freezer for several hours. The fine white needles of the mesylate were isolated in high yields. The compounds showed:  $\nu_{\text{max}}^{\text{film}}$  1360 and 1175  $\text{cm}^{-1}$  (S = O), 945  $\text{cm}^{-1}$  (S – O), 1465  $\text{cm}^{-1}$  ( $\text{CH}_2$ ). N.m.r. data:  $^1\text{H}$ ,  $\delta$  ~3.0 (s,  $\text{SO}_2\text{CH}_3$ );  $^{13}\text{C}$ ,  $\delta$  ~39 ( $\text{SO}_2\text{CH}_3$ ).

## D.7. Mycolyl lipids reactions

### D.7.1. The synthesis of 3-O-(2-tetrahydropyranyl) mycolyl alcohol (59) and 3-O-(2-tetrahydropyranyl) mycolyl mesylate (63) and their precursors

3-O-(2-tetrahydropyranyl) mycolic acid (58) and its 2-tetrahydropyranyl ester (57). —Mycolic acid\* (56,506 mg) was suspended in 1,2-dichloroethane (distilled over  $P_2O_5$ ). Dihydropyran (0.5 mL) and a few crystals of *p*-toluenesulfonic acid were added and the mixture was stirred overnight at room temperature. The light purple solution showed an intense spot having  $R_f$  0.9 in t.l.c. (solvent *H*) as described<sup>34</sup>. Hexane (30 mL) was added and the solution washed with saturated aqueous sodium bicarbonate and water. The organic phase was dried over magnesium sulfate and evaporated to a pale yellow oil (57) which was dried in vacuo;  $\nu_{\text{max}}^{\text{film}}$  3060  $\text{cm}^{-1}$  (cyclopropane), 1740  $\text{cm}^{-1}$  (ester CO), 1115, 1020  $\text{cm}^{-1}$  (C—O); no OH or COOH absorption.

The crude 57 was dissolved in dry benzene (5 mL), glacial acetic acid (5 mL) was added and the solution stirred overnight at room temperature. T.l.c. (solvent *H*) showed two spots, one having  $R_f$  0.36 (compound 58), the other  $R_f$  0.1 (regenerated 56). The benzene was evaporated at room temperature under reduced pressure, and hexane (50 mL) added to the residue. The hexane layer was washed three times with water (~30 mL each), dried with magnesium sulfate, and evaporated. The oily product was applied to a column of silica gel (50 g, 70-230 mesh) and eluted with 1:19 ethyl acetate-hexane which delivered compound 58 (187 mg, 35%). Some

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\*Kindly donated by Dr. A. Liav, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado.

mycolic acid (222 mg) was recovered by continued elution with solvent of increased polarity. Compound **58** showed  $\nu_{\text{max}}^{\text{film}}$  1705  $\text{cm}^{-1}$  (acid CO), as reported<sup>34</sup>; the hydroxyl absorption present in mycolic acid at 3350  $\text{cm}^{-1}$  was absent. T.l.c. of **58** (triple irrigation with solvent *I*) showed two distinct spots which were assumed to represent diastereoisomers.

In a separate experiment, starting with 240 mg of **56** and using the same procedure, compound **57** was isolated and not subjected to ester hydrolysis. The crude oily **57** ( $R_f$  0.9, solvent *H*) was applied to a silica gel column (25 g, 70-230 mesh) that was eluted with 1:19 ethyl acetate-hexane. Compound **57** came out in fractions 4 to 7 (30-mL fractions), which after concentration afforded 221 mg (81%) of the desired material identified by its IR spectrum.

3-O-(2-tetrahydropyranyl) mycolyl alcohol (**59**). – A. From **57** by lithium aluminum hydride reduction: Compound **57** (215 mg) was dissolved in ether, an excess of lithium aluminum hydride (LAH, 40 mg) was introduced, and the solution was boiled under reflux for 1 h. The reaction mixture was introduced into a separatory funnel containing hexane (30 mL) and dilute aqueous sodium hydroxide (10%, 10 mL). After the excess LAH was destroyed, the layers were separated and the organic phase was washed twice with water and dried over magnesium sulfate. T.l.c. (solvent *H*) indicated two distinct spots for **59** ( $R_f$  0.64, and 0.58; diastereoisomers), migrating more slowly than **57** and faster than the diol **62** ( $R_f$  0.38, see Test Reaction 1, p. 72). Irrigating **59** seven times in solvent *J* afforded four spots\*. After concentrating the solution, the resulting oil was applied to a silica gel

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\*See discussion

column (22g, 70-230 mesh) and was eluted with 1:19 ethyl acetate-hexane. By collecting 30-mL fractions, the two diastereoisomers were partly separated. The faster moving one ( $R_f$  0.64) was recovered from fractions 5-7 (58 mg), mixtures of both were found in fractions 8 and 9 (61 mg) while the slower-moving isomer was obtained from fractions 10-16 (54 mg), for a total yield of 86%. The mixture fractions were used in Test Reaction 1 for t.l.c. comparison with diol 62. Both diastereoisomers showed:  $\nu_{\text{max}}^{\text{film}}$  3500  $\text{cm}^{-1}$  (OH), 3060  $\text{cm}^{-1}$  (cyclopropane); carbonyl absorption was absent:  $^{13}\text{C}$ -n.m.r.: appearance of two new  $\text{CH}_2$  signals between  $\delta$  60-70 (diastereoisomers).

Anal. Calc. for  $\text{C}_{89}\text{H}_{176}\text{O}_3$  (1294.37): C, 82.59; H, 13.70. Found: C, 82.49; H, 13.48.

B.1 From 58 by lithium aluminum hydride reduction: Partly decomposed 58 (90 mg) was dissolved in anhydrous ether, LAH (20 mg) was introduced, and the solution was boiled under reflux for 1 hour. Work-up, t.l.c. conditions and results were identical with those of the previous procedure A except that a slower-moving spot was also observed in t.l.c., probably due to impure starting material. The weight of the combined diastereoisomers (66 mg) after column chromatography corresponded to a yield of 74%. The IR spectrum of the product was identical with that from procedure A.

B.2 From 58 by borane reduction: Compound 58 (66 mg) was dissolved in tetrahydrofuran (1 mL) and a 1M solution of borane in THF (1 mL) was added. The solution was stirred for 30 min at room temperature and then under reflux for 15 min. T.l.c. (solvent H) indicated the same two spots for the desired compound 59, accompanied by traces of slower-moving by-products and an intense, faster moving

spot ( $R_f$  0.95). Methanol was added to the reaction mixture and when effervescence had stopped, the solution was evaporated under reduced pressure and co-evaporated twice with methanol and three times with chloroform. At this stage, the fast-moving product ( $R_f$  0.95) had disappeared in t.l.c.; it was believed to be a borane complex destroyed by the methanol. The resulting oil was applied to a silica gel column (5 g, 70-230 mesh) and, after elution with 1:19 ethyl acetate-hexane, a total of 54 mg (83%) of **59** was obtained. The IR spectrum was the same as that of **59** from procedure **A**, except that a very small, broad signal was found at  $1730\text{ cm}^{-1}$  and could not be explained.

**B.3 From 58 by sodium borohydride reduction:** Compound **58** (112 mg) was dissolved in THF (10 mL, stored over 4A molecular sieves) and cooled to  $0^\circ$ . Ethyl chloroformate (5 drops) and triethylamine (7 drops) were introduced which formed immediately a white precipitate. The mixture was stirred at  $0^\circ$  for 15 min and then allowed to warm to room temperature at which the reaction was continued for an extra 5 min. Sodium borohydride (60 mg) followed by ethanol (~3 mL) were added and the solution was stirred for 25 min at room temperature. An intense spot ( $R_f$  0.8) was observed in t.l.c. (solvent *I*) which was believed to be a borane complex. Water was introduced, and once all excess sodium borohydride was destroyed, the THF was evaporated under reduced pressure at ambient temperature. Extraction of the aqueous, cloudy residue with 10:1 hexane-ethyl acetate and concentration of the dried ( $\text{MgSO}_4$ ) organic phase gave 97 mg (88%) of compound **59** (oil). T.l.c. revealed the same two spots ( $R_f$  0.64 and 0.58) with solvent *H* as seen in procedure **A**. No by-products were visible and column-chromatographic purification was therefore deemed unnecessary.

C. From 61 by lithium aluminum hydride reduction: see Test Reaction 3 (T.R.3)

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3-O-(2-Tetrahydropyranyl) mycolyl mesylate (**63**). -- A solution of **59** (130 mg) and methanesulfonyl chloride (0.12 mL) in pyridine (1 mL) was stirred at room temperature. After 1 h, t.l.c. showed that all starting material had disappeared and a single spot ( $R_f$  0.56, Solvent *H*) was present. Ice water was added and the aqueous phase was extracted with ether (3 x 15 mL). The combined organic phase was washed with 5% HCl, saturated aqueous sodium bicarbonate, water, dried with magnesium sulfate and concentrated to an oil. The latter was chromatographed on a silica gel column (13 g, 70-230 mesh) using 1:19 ethyl acetate-hexane as eluant, to give **63** (131 mg, 95%);  $\nu_{\max}^{\text{film}}$  3060  $\text{cm}^{-1}$  (cyclopropane), 1360 and 1175  $\text{cm}^{-1}$  (S=O), 945  $\text{cm}^{-1}$  (S-O). N.m.r. data:  $^1\text{H}$ ,  $\delta \sim 3$  ( $\text{SO}_2\text{CH}_3$ );  $^{13}\text{C}$ ,  $\delta \sim 30-40$  (appearance of two signals, ( $\text{SO}_2\text{CH}_3$  diastereoisomers).

Mycolyl mesylate (**64**). -- One small drop of concentrated hydrochloric acid was added to a solution of compound **63** (12 mg) in chloroform (1 mL), which was stirred for 30 min at room temperature. Solvent *H* revealed a single spot ( $R_f$  0.3). Sodium bicarbonate was introduced and the mixture was stirred for 10 min. After filtering the solution through a Celite plug, it was concentrated to an oil affording a quantitative yield (~12 mg);  $\nu_{\max}^{\text{film}}$  3380  $\text{cm}^{-1}$  (OH), 3060  $\text{cm}^{-1}$  (cyclopropane), 1340 and 1175  $\text{cm}^{-1}$  (S=O), 845  $\text{cm}^{-1}$  (S-O);  $^1\text{H}$ -n.m.r.:  $\delta \sim 3$  ( $\text{SO}_2\text{CH}_3$ ).

Anal. Calc. for  $\text{C}_{85}\text{H}_{170}\text{O}_4\text{S}$  (1288.34): C, 79.24; H, 13.30. Found: C, 78.86; H, 12.64.

### D.7.2. Test Reactions (T.R.)

T.R.1. Formation of mycolyl alcohol (62). – A. From 56. A solution of mycolic acid **56** (~5 mg) and LAH (~5 mg) in anhydrous ether (2 mL) was boiled under reflux for 1 h. The starting material was all reduced to the target diol **62** having  $R_f$  0.37 (solvent *H*). No work-up was carried out since this reaction was used only for spot checking.

B. From 59. In parallel reactions, each diastereoisomer of **59** was solvolyzed by dissolving 1-2 mg of sample in chloroform (~1 mL), adding 1 drop of concentrated hydrochloric acid and stirring at room temperature. After 30 min, both diastereoisomers had disappeared and generated the same, single spot ( $R_f$  0.38, solvent *H*), which proved identical with that seen in **A**.

T.R.2. Formation of methyl 3-O-(2-tetrahydropyranyl) mycolate (61). – A. From 56. Mycolic acid **56** (10 mg) was dissolved in a freshly prepared solution of diazomethane in ether. Nitrogen evolved immediately and after 5 min, t.l.c. showed a faster-moving, major spot for **60** ( $R_f$  0.35, solvent *K*) accompanied by traces of slower-moving by-products;  $\nu_{\max}^{\text{film}}$  3500  $\text{cm}^{-1}$  (OH), 3060  $\text{cm}^{-1}$  (cyclopropane), 1725  $\text{cm}^{-1}$  (ester CO), 1215  $\text{cm}^{-1}$  (C–O),  $^1\text{H-n.m.r.}$ :  $\delta$  3.68 ( $\text{OCH}_3$ ).

The crude **60** was dissolved in dichloromethane (2 mL), a catalytic amount of *p*-toluenesulfonic acid and dihydropyran (2 drops) were added, and the solution was stirred for 5 h at room temperature. The dark green reaction mixture was then stored overnight in a freezer. T.l.c. indicated an intense spot for **61** ( $R_f$  0.60, solvent

K) along with slower-moving contaminants. Compound **61** was isolated pure by means of a silica gel column (5 g, 70-230 mesh) using 1:40 ethyl acetate-hexane as eluant;  $\nu_{\max}^{\text{film}}$  OH was absent,  $1735\text{ cm}^{-1}$  (ester CO);  $^1\text{H-n.m.r.}$ :  $\delta$  3.63, 3.65 ( $\text{OCH}_3$  diastereoisomers).

**B. From 58.** Compound **58** (~5 mg) was dissolved in a freshly prepared solution of diazomethane in ether. After the evolution of nitrogen had ceased, the yellow solution was left standing at room temperature for 5 min. A major spot having a  $R_f$  value (0.60) identical to that seen in **A** was observed by t.l.c. (solvent K). The solution was concentrated to an oil, which was chromatographed on silica gel (3g, 70-230 mesh) using 1:40 ethyl acetate-hexane as eluant, to give pure **61**. Both  $^1\text{H-n.m.r.}$  and IR spectra of **61** obtained in procedures **A** and **B** were identical.

**T.R.3. Formation of 59. — From 61.** Compound **61** (~5 mg) and LAH (~5 mg) in anhydrous ether (2 mL) were heated for 1.5 h at reflux. The reaction mixture was diluted with hexane (~5 mL), washed with 2N NaOH (effervescence) and water, and dried with magnesium sulfate. The same two spots ( $R_f$  0.65 and 0.59, solvent *H*) as those generated in procedure **A** (p.68) were seen in t.l.c.. The IR spectrum of the product was identical with that of **59** from procedure **A**; the  $^1\text{H-n.m.r.}$  spectrum indicated absence of ester methoxyl.

**Figure 4. <sup>1</sup>H-NMR DATA (CDCl<sub>3</sub>)**

Peracetylated bis(6-deoxy- $\alpha$ -D-gluco-heptosiduronic acid) (**12**)\*

Chemical shifts ( $\delta$ )		Coupling constants (Hz)									
Compound	H-1	H-2	H-3	H-4	H-5	H-6,6'	OAC	J <sub>1,2</sub>	J <sub>2,3</sub>	J <sub>3,4</sub>	J <sub>4,5</sub>
<b>12</b>	5.09d	5.19dd	5.49dd	4.86t	4.33dt	2.50m	2.03 2.01 1.99	3.8 (H-1) 3.8 (H-2)	10.0 (H-2) 10.0 (H-3)	9.2 (H-3) 10.0(H-4)	10.0 (H-4)

\* Starting material for the synthesis of peracetylated cord factor analogs **39-43** (Section B.4).

**Figure 5. <sup>1</sup>H-NMR DATA (D<sub>2</sub>O)**

Bis(6-deoxy- $\alpha$ -D-gluco-heptosiduronic acid) (**5**)\*

Chemical shifts ( $\delta$ )		Coupling constants (Hz)												
Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-6'	J <sub>1,2</sub>	J <sub>2,3</sub>	J <sub>3,4</sub>	J <sub>4,5</sub>	J <sub>5,6</sub>	J <sub>5,6'</sub>	J <sub>6,6'</sub>
<b>5</b>	5.20d	3.70dd	3.88dd	3.35dd	4.16dt	2.97dd	2.52dd	3.85 (H-1) 3.9 (H-2)	9.9 (H-2) 9.9 (H-3)	9.0 (H-3) 9.0(H-4)	9.9 (H-5) 9.9 (H-5)	2.9 (H-5) 2.9 (H-6)	9.9 (H-5) 9.9 (H-6')	15.8 (H-6) 15.8 (H-6')

\* Starting material for the synthesis of cord factor analogs **45-49** (Section B.5.2).

Figure 6. <sup>1</sup>H-NMR DATA (CDCl<sub>3</sub>)

Peracetylated dioctyl bis(heptosiduronate) (39)\*

Chemical shifts (δ)

Coupling constants (Hz)

Compound	H-1	H-2	H-3	H-4	H-5	H-6,6'	<u>COOCH</u> <sub>2</sub>	OAC	J <sub>1,2</sub>	J <sub>2,3</sub>	J <sub>3,4</sub>	J <sub>4,5</sub>
39	5.16d	5.20dd	5.50ddd	4.89dd	4.36m	2.42m	4.01m	2.10 2.02 2.00	3.7 (H-1) 3.8 (H-2)	9.8 (H-2) 9.5 (H-3)	9.4 (H-3) 9.2 (H-4)	10.3 (H-4)

\*Other peracetylated dialkyl bis(heptosiduronates) 40-43 have practically identical n.m.r. values (see experimental section for exact signals).

Figure 7. <sup>1</sup>H-NMR DATA (CD<sub>3</sub>OD at 50-55°)

dioctadecyl bis(heptosiduronate) (49)\*

Chemical shifts (δ)

Coupling constants (Hz)

Compound	H-1	H-2	H-3	H-4	H-5 and <u>COOCH</u> <sub>2</sub>	H-6	H-6'	J <sub>1,2</sub>	J <sub>2,3</sub>	J <sub>3,4</sub>	J <sub>4,5</sub>	J <sub>5,6</sub>	J <sub>5,6'</sub>	J <sub>6,6'</sub>
49	5.09d	3.50dd	3.75ddd	3.15ddd	4.12m	2.84dd	2.38dd	3.8 (H-1) 3.8 (H-2)	9.6 (H-2) 9.4 (H-3)	9.2 (H-3) 8.9(H-4)	9.9 (H-4)	2.8 (H-6)	9.7 (H-6')	15.6 (H-6) 15.7 (H-6')

\*Other dialkyl bis(heptosiduronates) 45-48 have practically identical n.m.r. values (see experimental section for exact signals).

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