This is to certify that the

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Synthetic Approaches to the Structural Analogs of Lipid A and Total Syntheses of Structurally Non-related Lipid A Antagonists

presented by

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has been accepted towards fulfillment
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SYNTHETIC APPROACHES TO THE STRUCTURAL ANALOGS OF LIPID A AND TOTAL SYNTHESES OF STRUCTURALLY NON-RELATED LIPID A ANTAGONISTS

BY

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ABSTRACT

SYNTHETIC APPROACHES TO THE STRUCTURAL ANALOGS OF LIPID A AND TOTAL SYNTHESES OF STRUCTURALLY NON-RELATED LIPID A ANTAGONISTS

By

Kyung-Il Kim

The lipid A moiety of lipopolysaccharides (LPS) of gram-negative bacteria (endotoxin) have tremendous potential as therapeutic agents. They are usually diphosphorylated glucosamine disaccharides which are esterified and amidated with fatty acid chains. Despite their potential, the use of these molecules has been severely limited because preparations from bacterial sources are very variable, ill-defined, heterogeneous and contain contaminants which have deleterious effects. The optimum structure of the active components of these complex mixtures and their modes of action are still to be determined. Biological studies with well defined, pure lipid A species are necessary for obtaining structure activity relationship information. In order to test various theories on structure and how it determines reactivity, we designed two new lipid A analogs, **TM1** and **TM2**, in which phosphate groups are replaced by carboxy methyl groups and is glucosamine ring of lipid X moiety by a glucuronic acid ring in the case of **TM2**. This was done based on a combination of chemical, computational and biological

considerations. Analogs of the type described here are synthetically challenging and their synthesis unprecedented because they involve carbon-carbon connectivities which involve difficulty to achieve stereochemical and logistical constraints.

In this study, key intermediates in the synthesis of **TM2**, 1-O-allyl-4-O-methyl-α,β-glucuronic acid benzylester (**7**), 2-acetamido-1-C-ethoxycarbomethyl-1,2-dideoxy-3-O-trimethylacetyl-4,6-O-benzylidene-β-D-glucopyranose (**4**) and (R)-3-hydroxytetradecanoic acid (**8**) were synthesized. These molecules and the associated synthetic methodology developed here should make the synthesis of a variety of structural analogs of lipid A possible.

Structurally non-related lipid A antagonists **TM3** and **TM4** were also designed and synthesized to examine the roles of the charged head groups and lipid chains in the biological activities of lipid A at the molecular level, by considering these aspects separately without the saccharide components.

Dedicated to my Mom, Dad, Youngah, Youngho

and most importantly My Wife Mee-Sook

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LIST OF ABBREVIATIONS

4A	4 Angstrom
Ac	Acetyl
All	Allyl
n-BuLi	n-Butyl lithium
Bn	Benzyl
Bz	Benzoyl
^{13}C-NMR	Carbon (^{13}C) NMR
18-Crown-6	1,4,7,10,13,16-Hexaoxacyclooctadecane
d	Doublet
δ	Chemical shift
DMF	N,N-Dimethylformamide
DMSO	Dimethyl sulfoxide
2D-NMR	Two dimensional NMR
Et	Ethyl
ether	Diethyl ether
Hz	Hertz
Imid.	Imidazole
in vacuo	In vacuum oven
^{1}H-NMR	Proton NMR
LDA	Lithium diisopropylamide
LPS	Lipopolysaccharide
m	Multiplet

Me	Methyl
NMR	Nuclear Magnetic Resonance Spectroscopy
PDC	Pyridium dichromate
pet. ether	Petroleum ether
Ph	Phenyl
PMP	para-Methoxyphenyl
Pv	Pivaloyl (Trimethylacetyl)
Pyr.	Pyridine
q	Quartet
R_F	Rate of flow
s	Singlet
t	Triplet
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
Tf	Trifluoromethanesulfonyl
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TM1	The first target molecule designed
TM2	The second target molecule designed
TM3	The third target molecule designed
TM4	The forth target molecule designed
TMS	Tetramethylsilane
Tr	Trityl (Triphenylmethyl)
p-TsOH	para-Tolunesulfonic acid

INTRODUCTION

Gram-negative bacteria contain in their cell surface a toxic principle which causes fever and lethal shock in higher animals. This toxin is called endotoxin since it is firmly bound to the cells in living bacteria and released only after the cells are destroyed. Endotoxin exhibits not only undesired toxic activities (fever, lethal shock) but also beneficial ones such as stimulation of immune response (strong leukocytosis) and induction of a tumor-necrotizing factor (causing hemorrhage of tumors). These toxic and beneficial biological activities have been attributed to the lipopolysaccharide (LPS) moiety, which is a complex amphipathic molecule found in the outer membrane of gram negative bacterial cells.

In 1954, Westphal and Luderitz isolated the lipophilic part of LPS by mild acidic hydrolysis and termed it "Lipid A", whose structure is shown in **Figure 1.**[1,2] They observed that lipid A manifested most of the endotoxic activities of LPS. This extremely important discovery was neither fully nor unanimously accepted by endotoxin investigators at that time. Later this concept was unequivocally confirmed by Japanese colleagues, based on a total synthesis of *Escherichia coli* lipid A and a comparison of synthetic lipid A's biological activity with that of natural lipid A.[3] It is now generally accepted that the lipophilic terminal substructure of LPS (lipid A) is, in fact, responsible for its immunopharmacological activities and the full induction of endotoxicity. Since LPS (endotoxin) is the most powerful immunostimulant known to date, the full induction of endotoxicity is believed to be an over-reaction of the immune system, as manifested by changes of cardiovascular parameters and white blood cell count,

disseminated intravascular coagulation, and finally, multi-organ failure leading to irreversible shock and death.

In 1983, the structure of the lipid A component of *Escherichia coli* LPS was determined completely by means of chemical and 2D-NMR methods[4] to be a glucosamine β-(1'-6)-disaccharide 1,4'-diphosphate acylated at two hydroxyls (3 and 3') and the two amino groups, as shown in **Figure 1.** By referring to the determined structure, numerous studies were done to determine the structural features of lipid A associated with its biological activities.

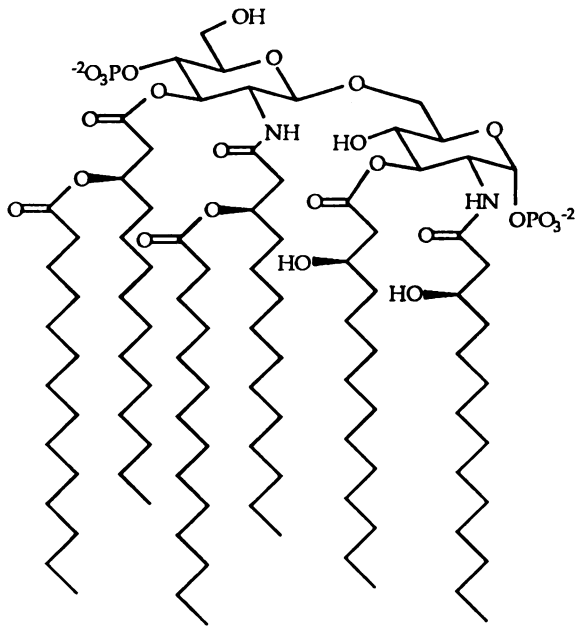


Figure 1

The structure of *Escherichia coli* lipid A

Generally, it was believed that the phosphate groups in lipid A were necessary for endotoxic activity, based on the fact that lipid A derivatives without phosphate groups in 1 and 4' positions were completely inactive, whereas a monophosphate at either 1 or 4' exhibited much weaker but still definite endotoxic activity.[5,6] This result doesn't mean necessarily that the phosphate group is the only charged group which can lead to the

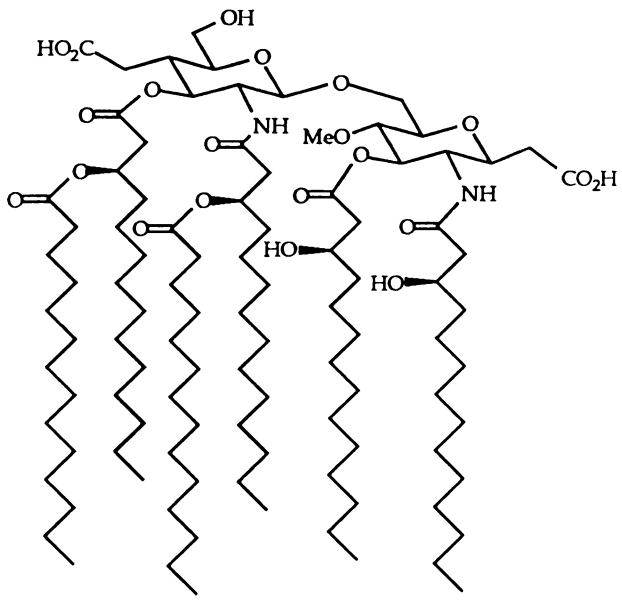
manifestation of the endotoxic properties of lipid A. We thought that phosphate groups funtion primarily to provide hydrophilic and anionic sites on the head group of lipid A, as well as contributing to the conformation of lipid A; but were not involved in chemical processes which are involved in the response to LPS. Recently published results[7] are very encouraging, since they indicate that the phosphate group can be substituted by a carboxymethyl group with retention of similar biological activity.

Besides efforts to determine the structural requirements for lipid A to exhibit endotoxic activity, numerous studies have addressed the possibility of dissociating detrimental endotoxic properties from beneficial immunostimulatory effects at the molecular level by chemical modification of lipid A or its simpler analogues. Efforts to date have concentrated on the total synthesis of lipid A[3,8] and its derivatives,[6,7,8] and on analogues representing both the nonreducing[9,10,11] and the reducing sugar moiety[1,12] such as lipid X and lipid Y.

The final goal of this study is the elucidation of the molecular basis of the biological properties of lipid A. As a first step to this goal, we designed the first target molecule **TM1**, as shown in **Figure 2**, primarily to investigate structural requirements (primarily phosphate substituents) of lipid A for endotoxicity. One model is that the phosphate group might be actively transfered as part of the mechanism. While the synthetic approach to **TM1** is to be discussed in a later chapter, the difficulty of synthesizing **TM1** forced us to design the second target molecule **TM2** as shown in **Figure 3**.

Besides the investigation of structural requirements of lipid A, structurally non-related lipid A antagonists, **TM3** and **TM4**, as shown in **Figure 4**, were also designed and synthesized; primarily based on the fact that the biological activity of lipid A is very sensitive to counter cationic species, since the aggregate structure of lipid A has been known to be more relevant to its biological activity than the actual head group structure. The mechanism by which **TM3** and **TM4** inhibit the endotoxicity of lipid A is

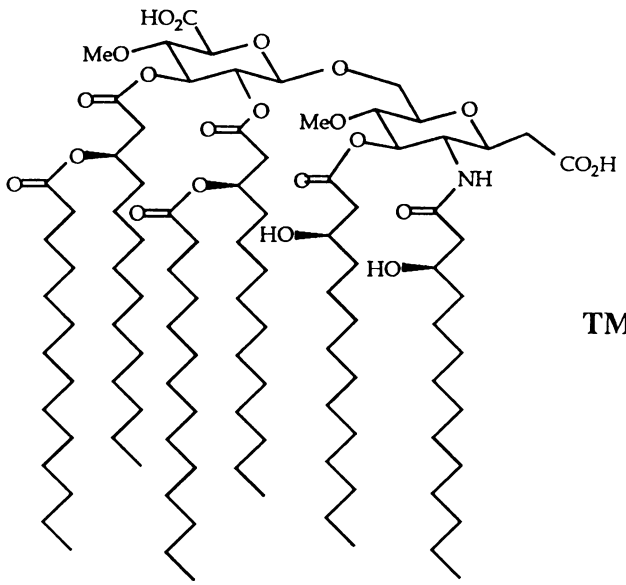
thought to be by membrane perturbation. Although the results of biological activity tests of **TM3** and **TM4** as endotoxin antagonists were more interesting[13] than expected, only the synthetic methods for obtaining them will be discussed here.



TM1

Figure 2

The structure of **TM1**



TM2

Figure 3

The structure of **TM2**

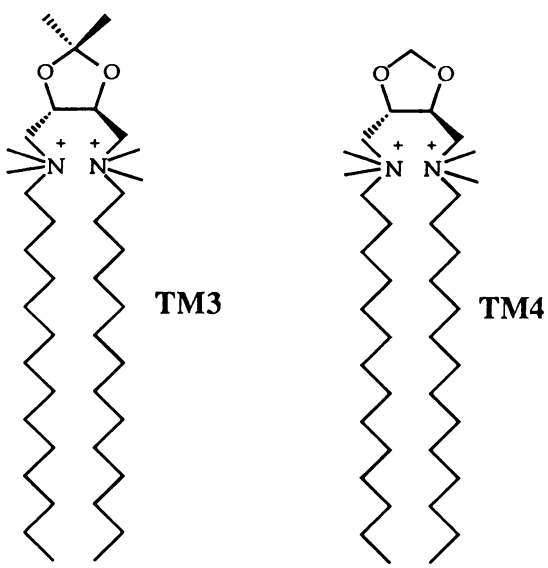


Figure 4

Structures of **TM3** and **TM4**

RETROSYNTHETIC ANALYSES

A retrosynthetic strategy for **TM1** is shown in **Scheme 1**. The basic strategy for the synthesis of **TM1** had to be quite different from that employed in the synthesis of *Escherichia coli* lipid A by Japanese colleagues,[3,6] because of the different substituents in 1 and 4' positions of lipid A. In the synthesis of *Escherichia coli* lipid A, phosphorylations of the 1 and 4' hydroxyl groups were performed after 3, 3' esterifications and 2, 2' amidations by fatty acids. In the synthesis of **TM1**, alkylations of the 1 and 4' carbons with the synthon of the carboxy methyl group were planned prior to esterifications or amidations of 3, 3' or 2, 2' by fatty acids, since C-alkylations at 1 and 4' were expected to be more difficult than O-alkylations of 1 and 4' hydroxyl groups.

The reactions planned for the transformation from **3** to **1** are selective deprotections of acetyl groups on the 2 nitrogen of **3** and the trimethylacetyl groups on the 3 oxygen of **3**, and mild coupling conditions with fatty acids for esterification and amidation. Selective deprotection of the N-acetyl group of **3** can be performed by a known method,[6] the treatment with Meerwein's reagent, triethyloxonium tetrafluoroborate, followed by acid hydrolysis of the resultant imidate. The free carboxylic acid groups on 1 and 4' positions of **TM1** should be developed at the last stage without perturbing other ester groups. Since benzyl esters can be selectively cleaved by catalytic hydrogenation, the transesterification of ethyl ester **3** to a benzyl ester, prior to coupling with fatty acid was necessary. Therefore the deprotection of the trimethylacetyl group was intended to be by treatment with NaOBn in BnOH to place a benzylester on the 4 position of **3**. In order to get **1** and **2**, obviously **3** and **4** are key

intermediates prior to coupling with the fatty acids. The synthetic approach to **3** and synthesis of **4** from **5** will be discussed in the next chapter.

The retrosynthetic strategy for **TM2** is different from the one for **TM1** only for the lipid X moiety as shown in **Scheme 2**. The transformation from **7** to **6** would be even easier than in the case of **3**. The known method[14] for the deprotection of allyl ethers using tristriphenylphosphinerhodium(I) chloride would be applied to the selective deprotection of allyl glycoside **6**. Since **7** is the key intermediate in **Scheme 2**, the synthesis of **7** from β-D-glucose will be discussed in the next chapter.

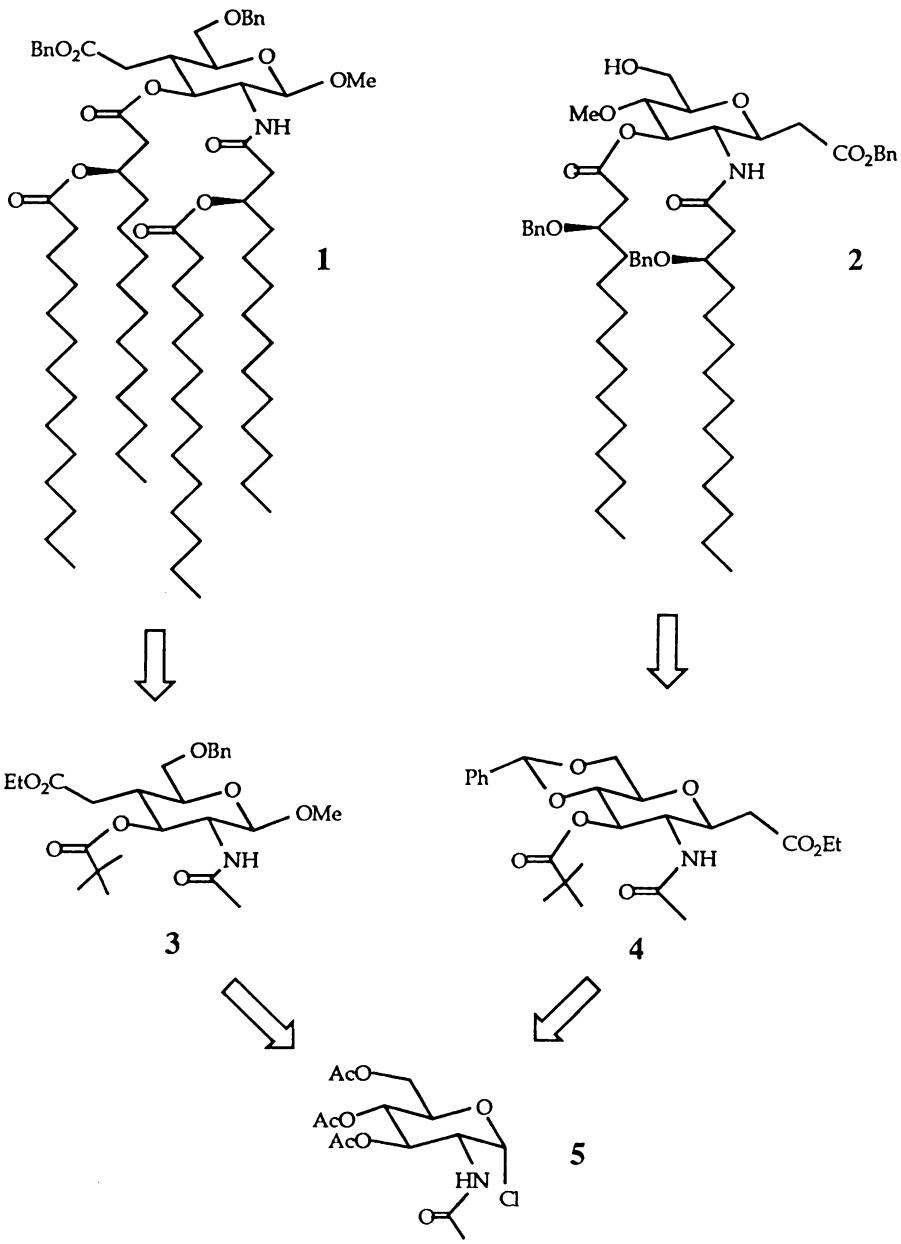
Another important key intermediate for **TM1** and **TM2** is optically pure (R)-3-hydroxytetradecanoic acid, **8**, which was chemically made by Yoshihara Izumi[15] in 1980. However, the best optical purity obtained by this method was 85% enantiomer excess. In order to get **8** in 100% enantiomer excess, the synthetic route from carbohydrate became inevitable. What was recently discovered in our group as shown in **Figure 5**, made this more than possible. Structure **41** was designed and synthesized as the key intermediate in the synthesis of **8** as shown in **Scheme 3**, based on the reaction mechanism in **Figure 5**. The synthesis of **8** from β-D-glucose will be discussed in the next chapter.

TM3 and **TM4** were made from L-tartaric acid as shown in **Scheme 4** because, in addition to endotoxin antagonist, the possibility of these molecules for chiral auxilliary was another concern in this research. At this point, the key intermediates for the synthesis of **TM1** and **TM2**, in addition to **TM3** and **TM4**, which will be discussed in **RESULTS AND DISCUSSION** chapter, are summarized in **Figure 6**.

Scheme 1

Retrosynthetic analysis of **TM1**

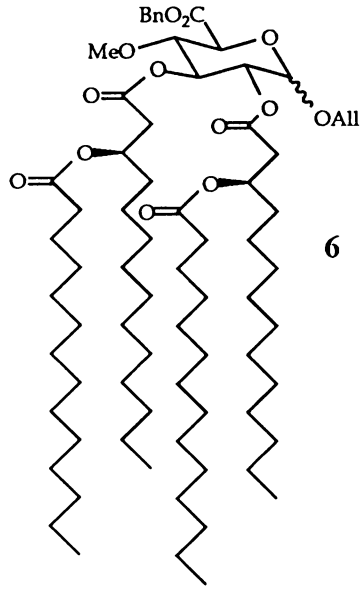
TM1


OBn
BnO2C
O
OMe
O
NH
O
O
O
O
1
HO
MeO
O
O
CO2Bn
NH
O
BnO
BnO
2
OBn
EtO2C
O
OMe
O
NH
O
3
Ph
O
O
O
O
CO2Et
NH
4
AcO
AcO
AcO
O
HN
Cl
O
5

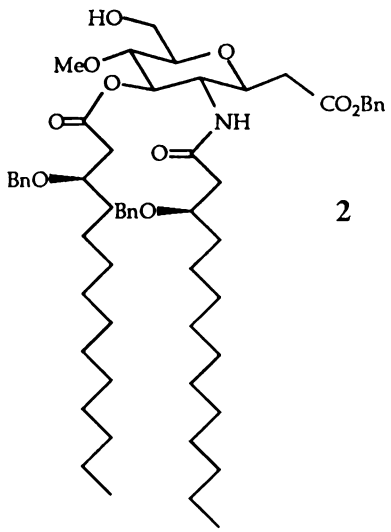
Scheme 2

Retrosynthetic analysis of **TM2**

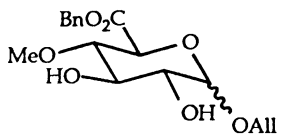
TM2



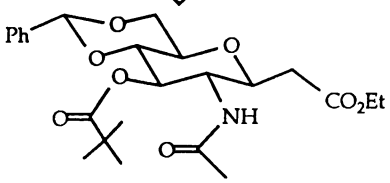
6



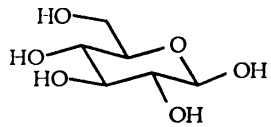
2



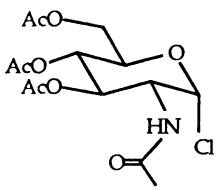
7



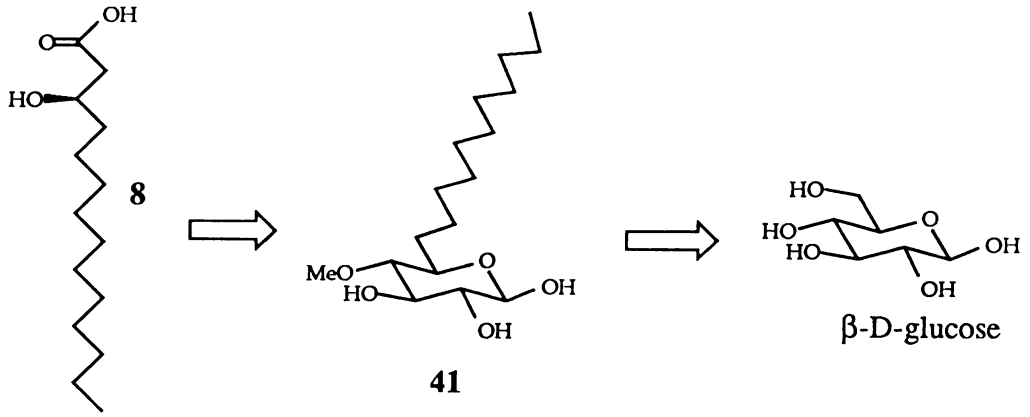
4



β-D-glucose

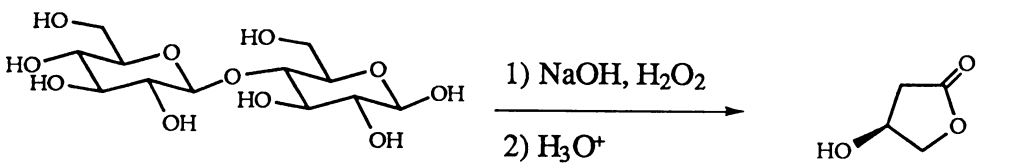


5



Scheme 3

Retrosynthetic analysis of **8**

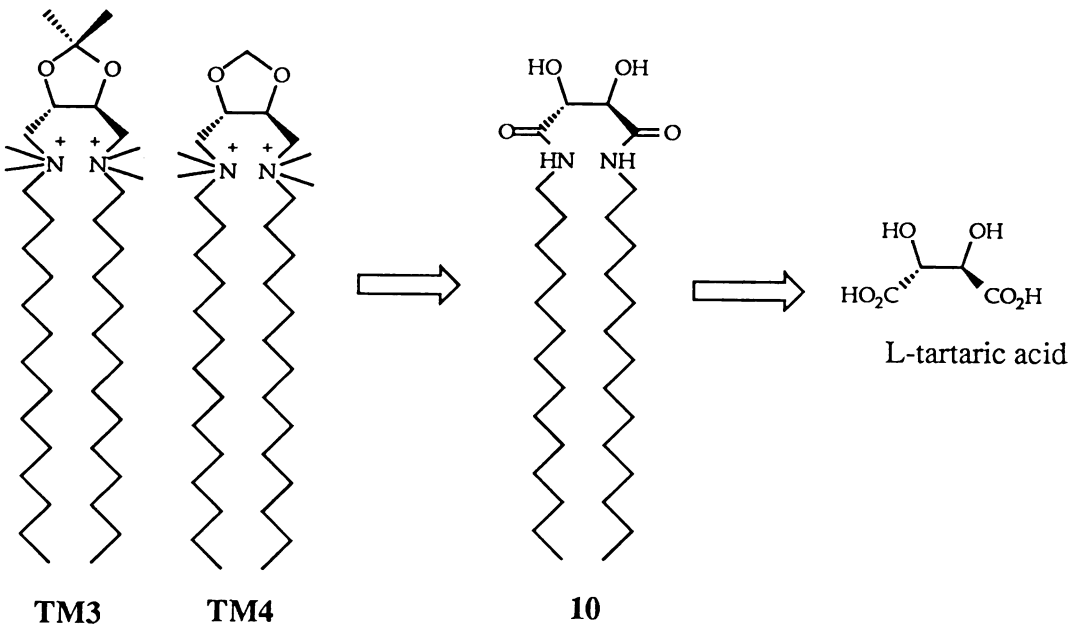


D-maltose

(S)-3-hydroxy-γ-butyrolactone

Figure 5

Reaction basis for the synthesis of **8**



Scheme 4

Retrosynthetic analysis of **TM3** and **TM4**

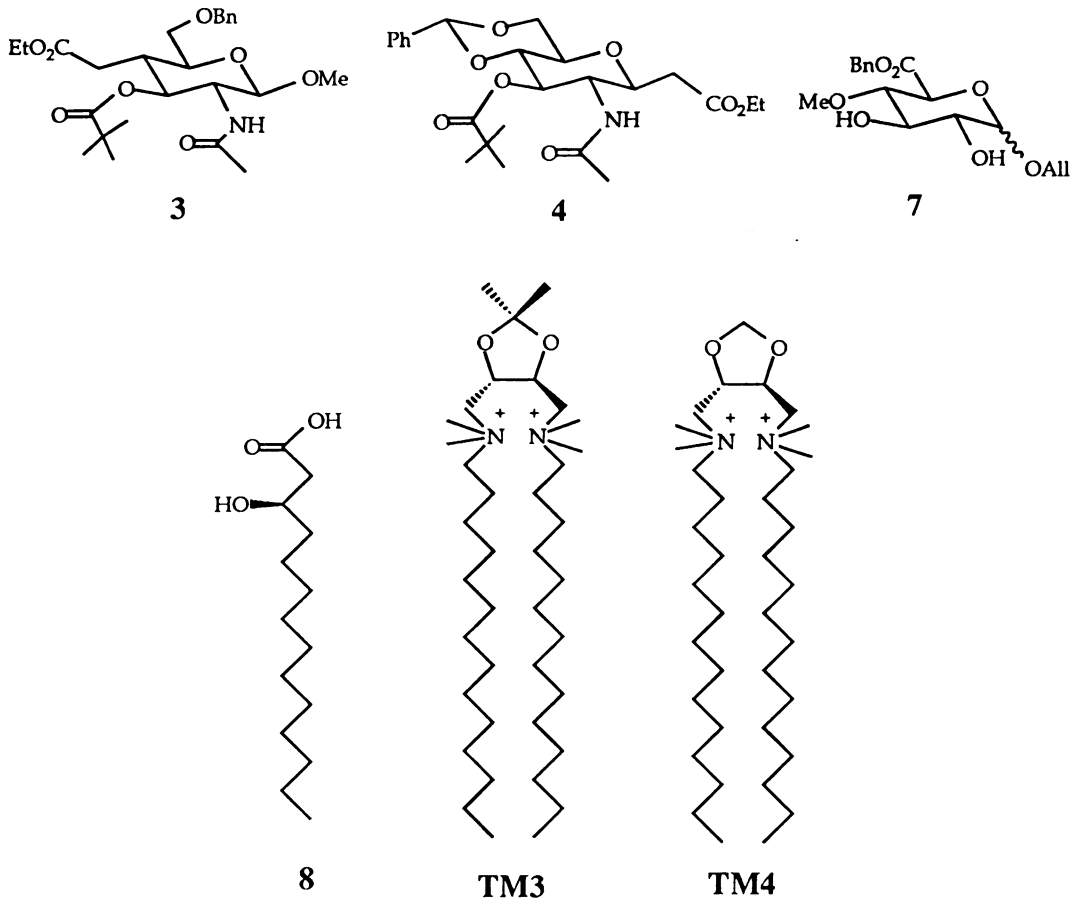


Figure 6

The list of molecules targeted in this research

RESULTS AND DISCUSSION

1. PREPARATION OF THE LEFT MOIETY OF TM1

The general strategy for obtaining the C-alkylated, protected amino-glycosyl intermediate for the left ring of the lipid A analogue **TM1** was to prepare a 4-keto derivative and achieve C-C bond formation via the Horner[21] or a related reaction. This strategy is detailed in **Scheme 5**. Intermediate **5** was prepared by the peracetylation of β-D-glucosamine hydrochloride, followed by chlorination of the anomeric position by treatment of the peracetate with gaseous HCl in acetic anhydride.[16] The methyl glycoside **11** was prepared from the 1-chloro by the Koenig-Knorr method[16], utilizing Ag_2O in methanol. Deacetylation of **11** was performed with MeOH and K_2CO_3, excess K_2CO_3 and insoluble byproduct were removed by filtration and the filtrate containing the crude product was concentrated and used for the preparation of **12**[17] without further purification. Trimethylacetylation of **12** was unexpectedly difficult when standard conditions (trimethylacetyl chloride, imidazole and pyridine) was used. The poor yield (32%) was attributed to further acylation of the amide nitrogen. This was evident from the TLC analysis of the reaction mixture. The acidic byproduct (pyridnium chloride) seemed to be responsible for promoting this N-acylation even under fairly vigorous condition (75°C, 16 hours). This problem was solved when NaH was used as base and trimethylacetyl chloride alone was employed thus maintaining the reaction mixture basic all the way and effecting complete deprotonation of the hydroxyl groups.

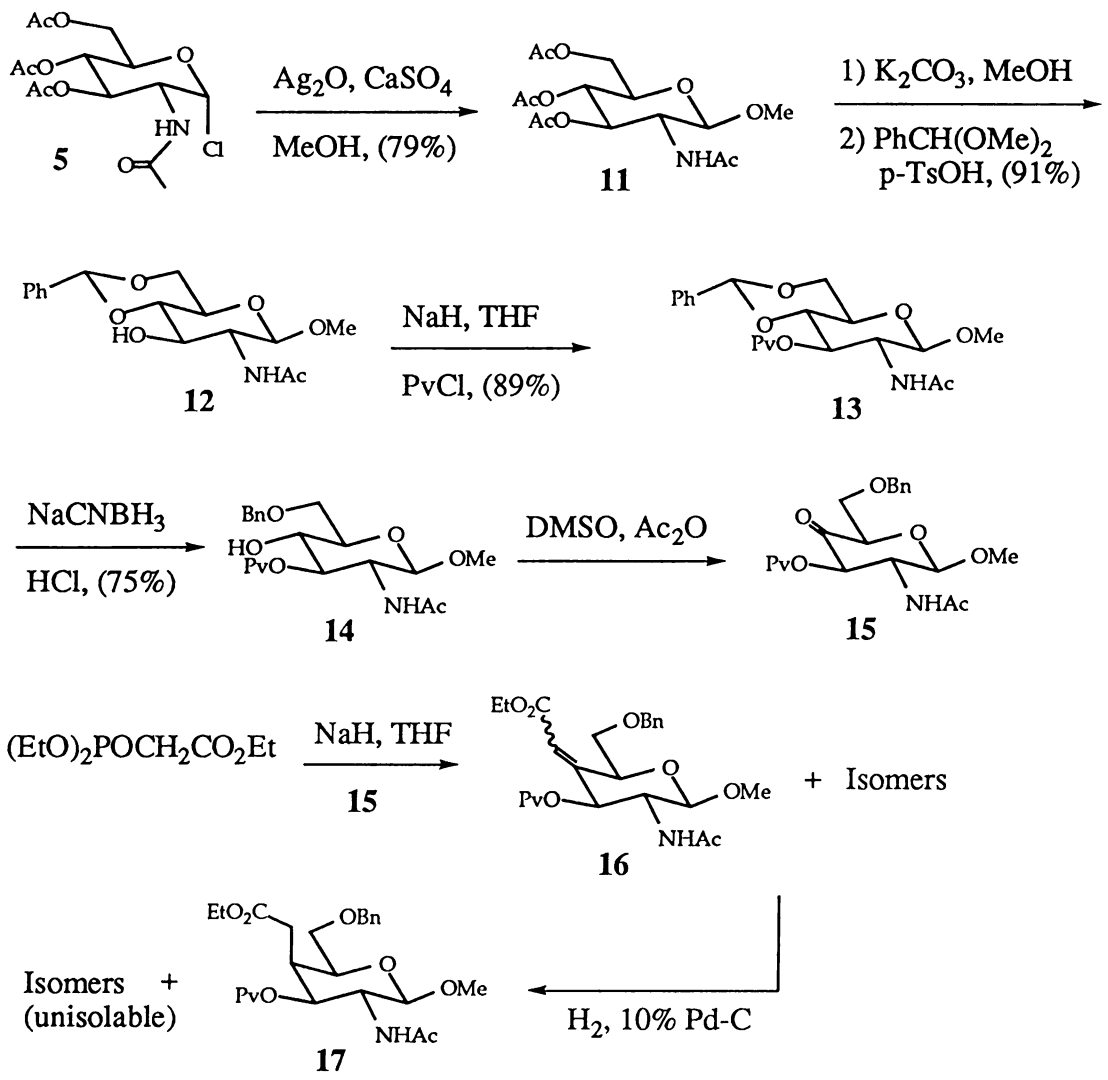
Regioselective benzylidene acetal ring cleavage of **13** was performed by employing the reductive ring opening method developed by Per J. Garegg[18] to give the

4-benzyl ether **14** in good yield. For the oxidation of **14**, Collins' oxidation[19] and the DMSO-acetic anhydride method[20] were both applied. The latter method turned out to be better for the preparation of the ketone **15**. Due to its poor stability during silica gel column chromatography, **15** prepared by Procedure A was used for the next reaction without further purification.

The C-alkylation of **15**, the key reaction in **Scheme 5**, was attempted using the Horner reaction[21]. However, the formation of side products which could not be separated from **16**, and apparently isomers of **16** formed by base catalyzed isomerization, drove us to try other way of performing the C-alkylation as described in **Scheme 6.** The strategy here was to prepare a triflate at the 4-position and displace it directly by appropriate two carbon unit.

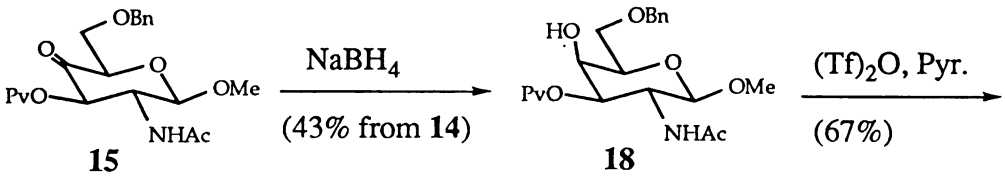
Axial triflate **19** was prepared according to the known procedure[22] utilizing $(Tf)_2O$ and pyridine, after inverting C-4 stereochemistry of **14** to give **18**. When **19** was treated with potassium diethylmalonate and 18-crown-6 in CH_2Cl_2 or DMSO, no reaction occurred even at higher temperature (100°C). Surprisingly **19** was not only insusceptible to attack by mild nucleophiles such as diethylmalonate anion, but also very stable to elimination under these reaction conditions. The treatment of **19** with stronger nucleophiles was attempted. The trimethylsilylacetate-TBAF method[23] and trimethylsilylacetate-LDA method[24] were both employed, but no desired product was formed even though all starting material was consumed in the reaction. Although the side products were not identified, it was obvious that the axial 4-triflate group survived these reactions.

A different route or approach was clearly required for the synthesis of **3**, but a biological precedent in Rhizobial lipid A structure combined with the synthetic difficulties in preparing the lipid X moiety of **TM1** drove us to modify the first designed analogue **TM1** to **TM2** in which the carboxylic acid funtion is placed on the 5-position. This is discussed later.

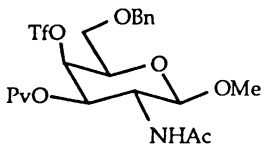


Scheme 5

Synthetic approach for the synthesis of **3**



15 **18**



19

$K^{+-}CH(CO_2Et)_2$, 18-Crown-6 → No reaction.

$(Me_3Si)CH_2CO_2Et$ —TBAF then **19**→ No **3** formed.

$(Me_3Si)CH_2CO_2Et$ —LDA then **19**→ No **3** formed.

Scheme 6

Reactions tried for the synthesis of **3**

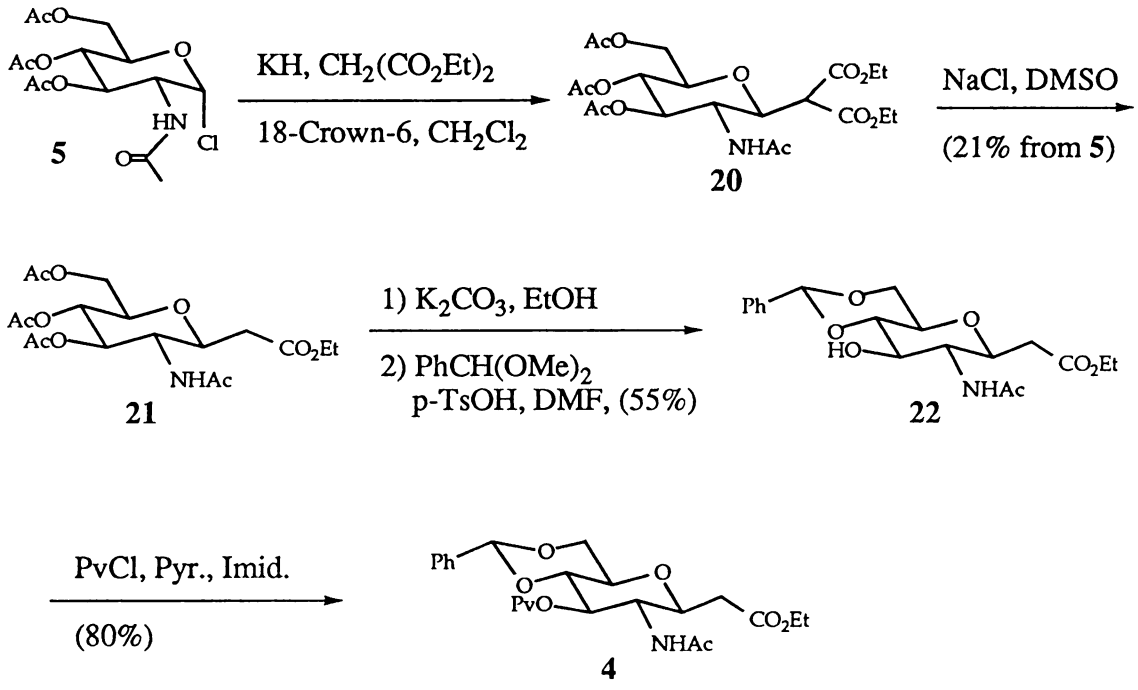
2. PREPARATION OF THE RIGHT MOIETY OF TM1

In the synthesis of **4**, obviously the C-alkylation of **5** to give **20** is the key reaction as shown in **Scheme 7**. Though syntheses of N-acetylglucosamine C-glycoside have been reported[25,26] in the preparation of lipid A analogues, their use has been limited because multistep routes are required for their preparations.

The C-alkylation of **5** by malonate anion in the presence of 18-crown-6 was very efficient and stereoselective compared to known methods. The only problem in this transformation to give **21** in two steps was the low yield (21% from **5**), caused by the formation of side products whose R_F values in the TLC were close to **20**. It has been reported[6] that the treatment of the bromo analogue of **5** (bearing Br instead of Cl) with pyridine at room temperature for 20 min. gave the oxazoline **5b** in high yield (90%). Although not all of side products were identified, the major one of them should probably be the oxazoline **5b** as shown in **Scheme 8**. Due to the competitive path to yield **5b**, it was necessary to maximize the effective concentration of the nucleophile (malonate anion) for the formation of **20** to dominate over that of **5b**. Moisture in the reaction mixture also seemed to accelerate the formation of **5b**. As a result, it was important to add the minimum amount of the solvent and keep the reaction mixture anhydrous. Even with the low yield, the efficiency and high stereoselectivity (uniquely β-isomer) made this reaction noteworthy.[27]

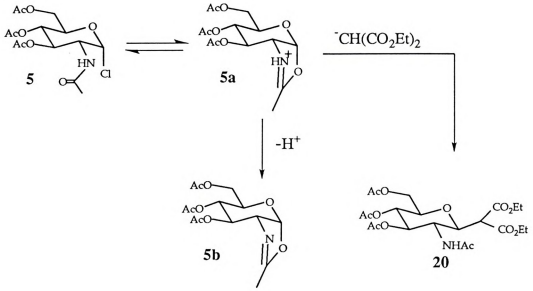
Reactions used for the transformation from **21** to **22** were identical to ones used for the synthesis of **12** in **Scheme 5**, except that EtOH instead of MeOH was used as solvent to reduce the possibility of exchange of the ethyl group of the ester function.

The trimethylacetylation of **22** was successful to give **4** in high yield (80%), when the standard method was applied unlike in the case of **12**. The difference in reactivities of **12** and **22** to this method (PvCl, Pyr. and Imid.) is difficult to explain.



Scheme 7

Synthetic scheme for the synthesis of **4**



Scheme 8

The reaction of **5** with malonate anion

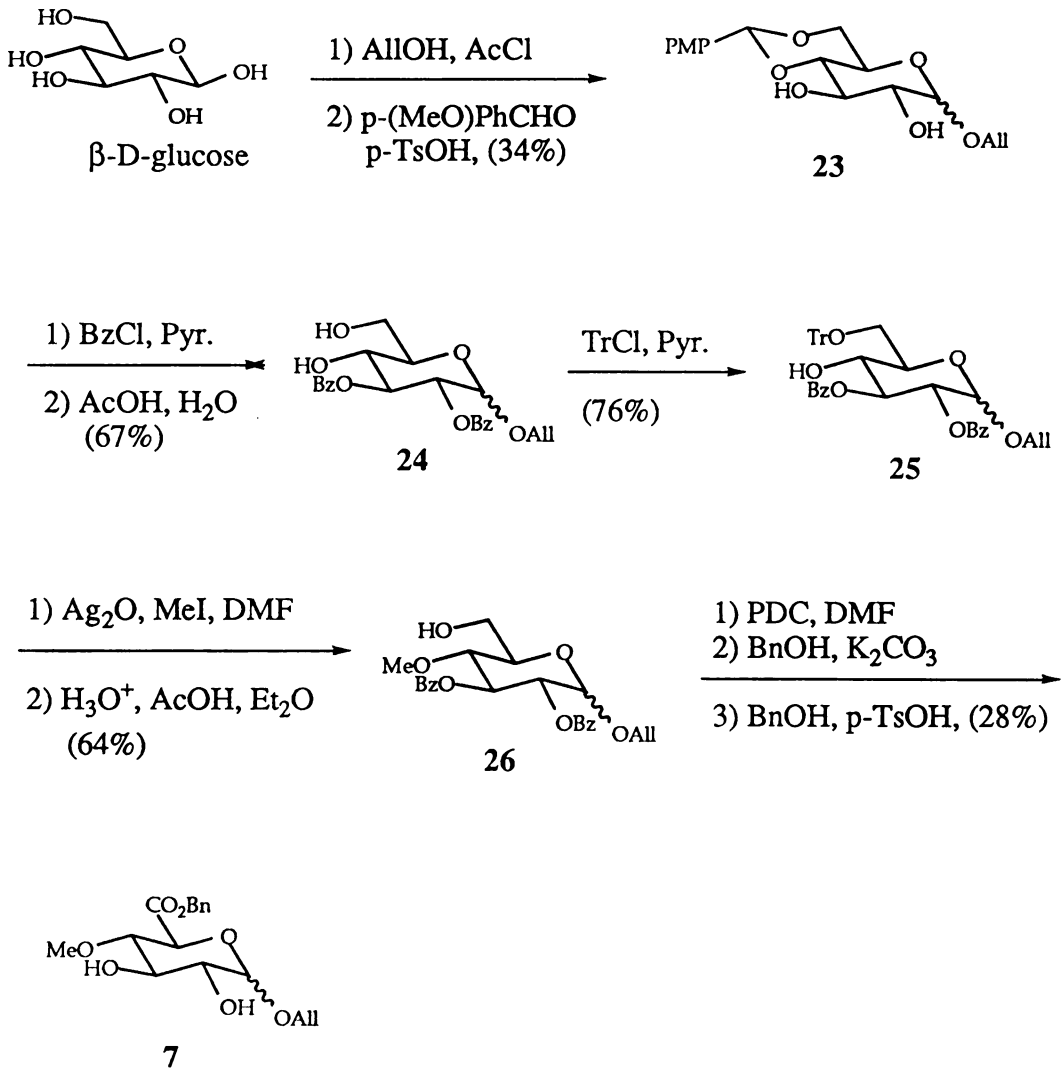
3. PREPARATION OF THE LEFT MOIETY OF TM2

The synthetic scheme for the synthesis of **7** is shown in **Scheme 9.** Intermediate **23** was prepared from β-D-glucose by adopting a p-methoxybenzylidene acetal group instead of a benzylidene acetal group, because the selective removal of the standard benzyl ether formed by reductive cleavage of a benzylidene acetal was anticipated to be very difficult without affecting the allyl ether group. After benzoylation of **23**, reductive cleavage was performed with $NaCNBH_3$ and p-TsOH. But the only product formed was completely deprotected diol **24** even in the presence of starting material. It seemed that the reduction of the p-methoxybenzyl ether reduced was even faster than that of the p-methoxybenzylidene acetal. Therefore the acidic hydrolysis of the p-methoxybenzylidene acetal to effect total deprotection was employed to give **24.** Subsequent protection of the 6-position by tritylation, methylation of the 4-position and detritylation gave **26.**

The pyridinium dichromate (PDC)[28] method was applied to the oxidation of **26** and the result was successful. However, the Jones reagent[29] didn't work for this case and seemed to cleave the benzoate groups. Subsequent debenzoylation and benzyl ester formation of the oxidized product of **26** gave **7.**

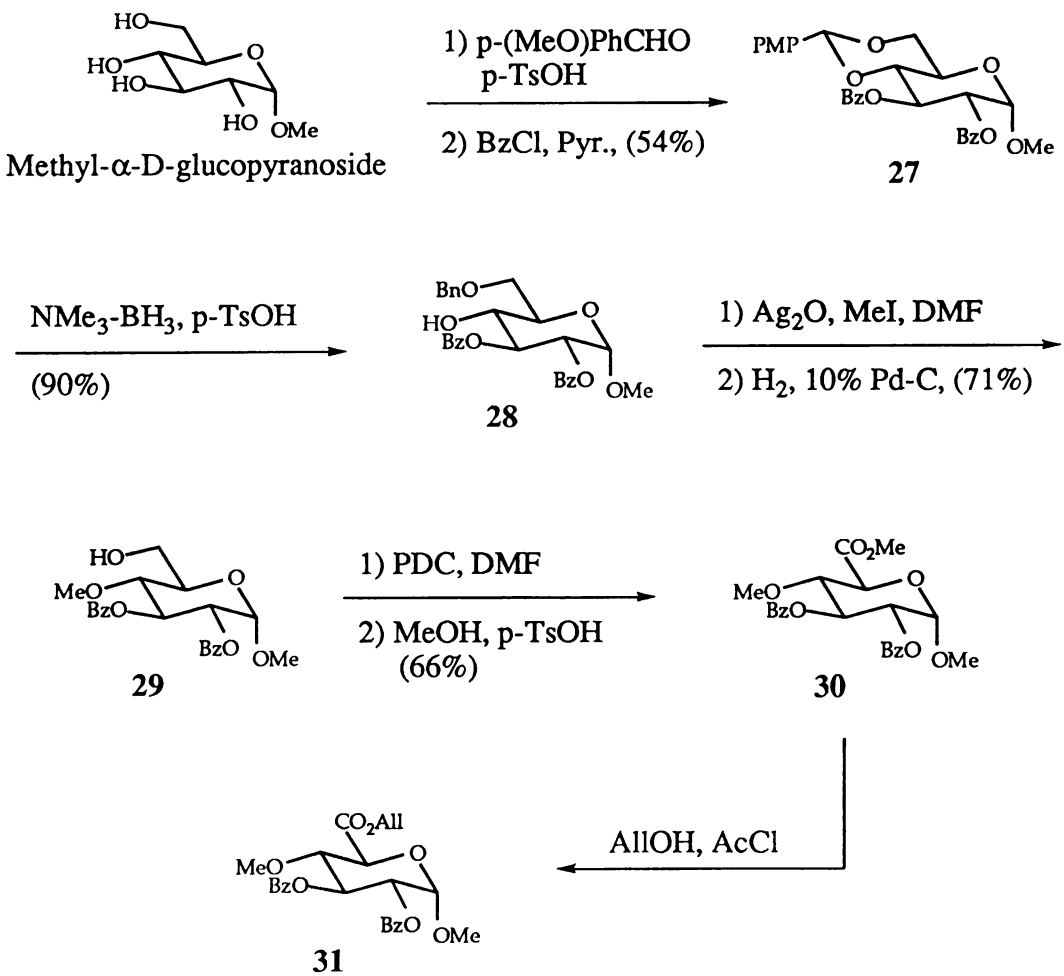
Another synthetic approach to **7** was attempted as shown in **Scheme 10.** The advantage of this approach was that we could work with only one isomer through most steps of the synthesis, rather than working on a mixture of α and β isomers for every step in **Scheme 9.** However, the glycosidic bond cleavage of **30** was unfortunately very difficult in a variety of conditions (70% aqueous HCO_2H, AllOH-BF_3, AllOH-(p-TsOH), HF, AllOH-AcCl). Although there was no conversion in most conditions, a couple of cases gave undesirable results. When HF was applied to **30** at 0°C and 25°C for one hour, the result was a mixture of products at 0°C and no

conversion at -25°C. When **30** was treated with AllOH and AcCl, intermediate **31** was the major product with no desired product observed by TLC or NMR. Based on the latter result, it seemed that carbonyl oxygen of the carboxylate retarded the glycosidic bond cleavage of **30** by reducing the basicity the acetal oxygen of glycoside **30**. Intermediate **7** was successfully prepared by **Scheme 9**.



Scheme 9

Synthetic scheme for the synthesis of **7**



Scheme 10

The unsuccessful synthetic approach to **7**

4. THE SYNTHESIS OF (R)-3-Hydroxytetradecanoic acid

The successful scheme for the synthesis of **8** is shown in **Scheme 11**. The p-methoxybenzylidene acetal **33** was prepared from β-D-glucose by the same method as described for **23** except using BnOH instead of AllOH for preparing the glycoside. Dibenzylation of **33** was successfully performed by the reaction with NaH and BnBr to give **34** in high yield (95%). For the transformation of **34** to **37**, generally the same reactions were used as are described for the preparation of **26** in **Scheme 9**. One change was that NaH-MeI was used for the methylation of **36**. This method could not be applied for the methylation of **25** due to anticipated migration of the benzoyl group under the strongly basic conditions. Subsequent detritylation of the methylated product of **36** gave **37**.

In order to make **40** from **37**, two options were explored. One was a Wittig reaction between the ylide of bromide **43** and 1-decanal, the other was a Wittig reaction between the ylide of 1-iododecane and the aldehyde **38**. Originally the latter one was chosen because the isomerization at C-5 of aldehyde **38** was anticipated under the reaction condition for ylide formation. However, as shown in **Schemes 12** and **13**, even the preparation of the phosphonium bromide of **46** was either difficult or not successful due to poor conversion and/or difficulty in isolating the product. Since, in general, the amount of phosphonium halide required is in excess of the counterpart aldehyde, using the phosphonium halide of **46** prepared through multisteps synthesis in excess to commercially available 1-decanal turned out to be an extremely inefficient route, particularly with poor conversion of **46** to the phosphonium bromide. By the same reasoning, **Scheme 12** designed to work on single isomer all the way rather than working on a mixture (α and β anomers) as in **Scheme 11**, was discarded even though the isolated yield of **45** was higher (28%) than the case of **Scheme 13**. Fortunately,

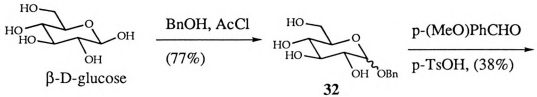
the anticipated isomerization of **38** prepared from **37** by the known method did not occur at all during the preparation of **40**. In addition to that, the preparation of the phosphonium iodide **39** was very successful giving almost quantititive yield (94%).

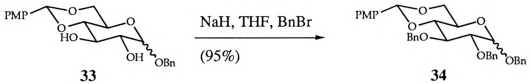
The reduction and debenzylation of **40** by palladium catalyzed hydrogenation was successfully done to give **41** in quantititive yield.

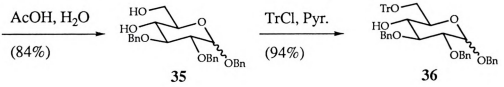
The degradation of **41** to give (R)-3-hydroxytetradecanoic acid **8**, the key reaction in **Scheme 11**, was performed in a mixture (1 : 1) of 0.2 M aqueous NaOH solution and 0.2 M aqueous H_2O_2 solution at 80°C for 2 days. Although TLC indicated that all starting material **41** was consumed after the first 10 hours, no desired product **8** was observed by ^{1}H-NMR analysis. The intermediates formed during the first 10 hours were not identified, but converted slowly to the desired product **8** without giving the α,β–unsaturated tetradecanoic acid. The successful result in this reaction introduces a very useful method for the syntheses of chiral compounds.

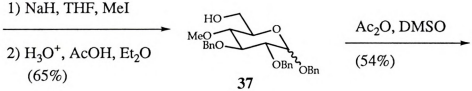
Scheme11

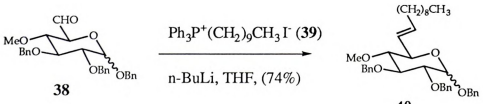
Synthetic scheme for the synthesis of **8**

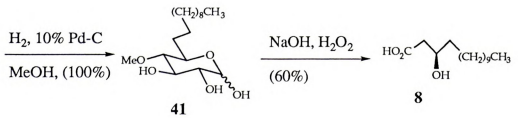

β-D-glucose
BnOH, AcCl
(77%)
32
p-(MeO)PhCHO
p-TsOH, (38%)

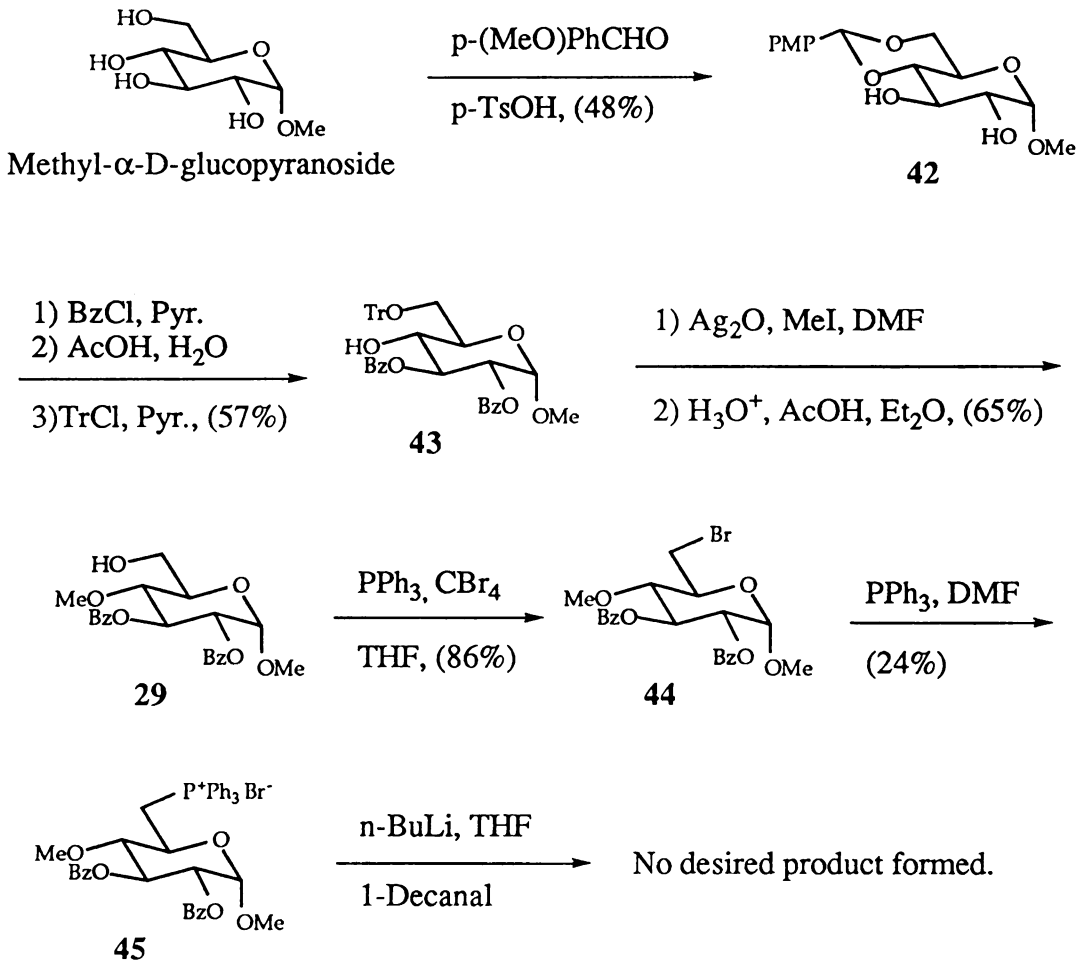

33
NaH, THF, BnBr
(95%)
34


AcOH, H2O
(84%)
35
TrCl, Pyr.
(94%)
36


1) NaH, THF, MeI
2) H3O+, AcOH, Et2O
(65%)
37
Ac2O, DMSO
(54%)

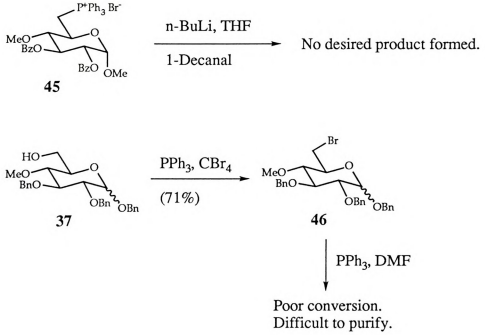

38
Ph3P+(CH2)9CH3 I- (39)
n-BuLi, THF, (74%)
40


H2, 10% Pd-C
MeOH, (100%)
41
NaOH, H2O2
(60%)
8



Scheme 12

Another synthetic approach for the synthesis of **8**



Scheme 13

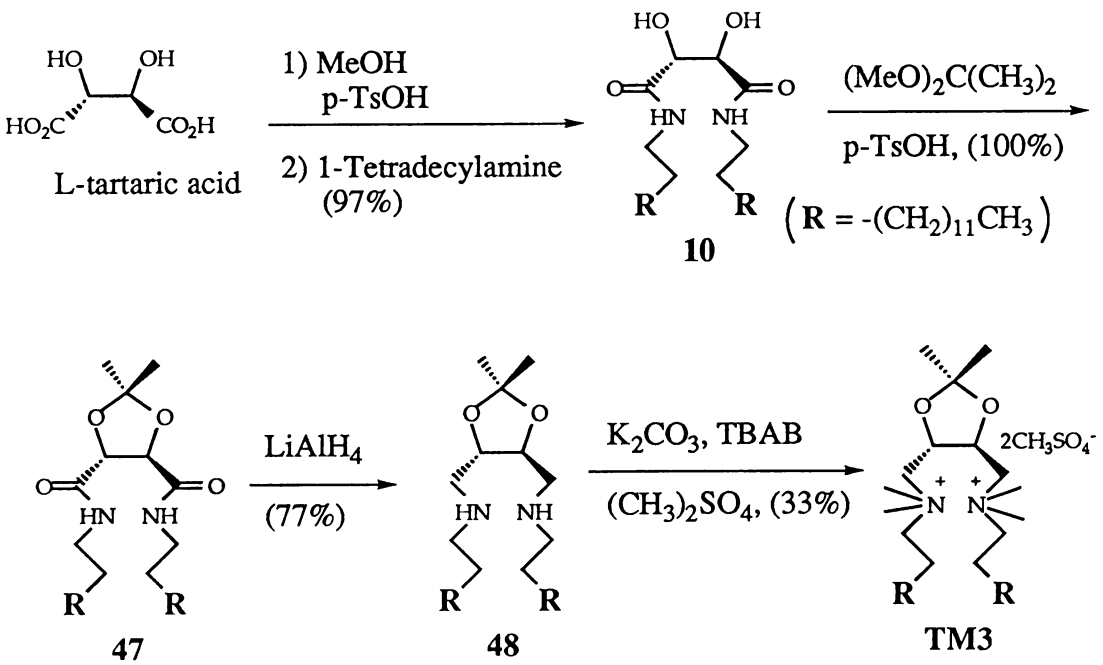
Alternative trial of the Wittig reaction of **37**

5. SYNTHESES OF TM3 AND TM4

TM3 was prepared from L-tartaric acid by **Scheme 14.** Tartaric acid diamide **10** was prepared in two steps through dimethyltartrate. Although the one step coupling of L-tartaric acid with 1-decylamine was attempted with DCC, ammonolysis of the diester turned out to be the best way because of the easy work. No further purification was needed. **47** was prepared in excellent yield and used in the next step without purification. Reduction of **47** was performed by lithium aluminium hydride to give **48.** Methylation of **48** was performed in heterogeneous solution to give **TM3** in poor yield (33%), but, because of the isolation procedure, the purity of the product was so high that no further isolation or purification step was needed after washing the reaction mixture once with water.

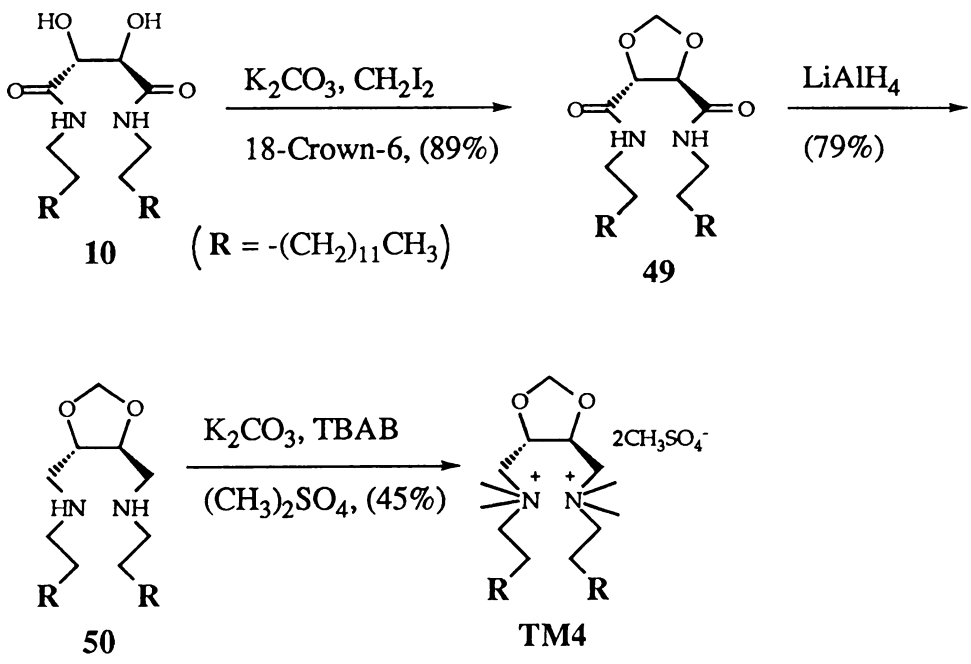
TM4 was prepared by the same reaction sequences as **TM3** except for one step as shown in **Scheme 15. 49** could not be made by any acid catalyzed acetalization (e.g. formalin-HCl, paraformaldehyde-HCl, $CH_2(OMe)_2$-(p-TsOH), $CH_2(OMe)_2$-BF_3, $CH_2(OMe)_2$-P_2O_5). In acid catalized reactions for the synthesis of **49,** many of side products were formed and some of them were identified as shown in **Figure 7** based on ^{1}HNMR spectra. Structures of **51, 52** and **53** gave rise to the quite interesting question of how can the small difference in similar reagents ($CH_2(OMe)_2$ and $Me_2C(OMe)_2$) made have such different effects on the results of the transacetalization of **10.** Based on the structures in **Figure 7,** the equilibrium between conformations of **10,** in addition to the difference in reagents, seemed to cause quite different results in the syntheses of **47** and **49.** In the reaction of **10** with $Me_2C(OMe)_2$ (bp 83°C), the byproduct MeOH (bp 64.6°C) was removable by fractional distillation to drive the equilibrium to the formation of the product. However, in the reaction of **10** with $CH_2(OMe)_2$, the higher boiling point (64.6°C) of MeOH compared to 42°C for the reagent $CH_2(OMe)_2$ made it impossible

for the equilibrium to be driven to the formation of the product **49** by this method. Another important factor should be considered in explaining the formation of the side products **51, 52** and **53**. The side product **51** was formed when the diol **10** was treated with the reagent paraformaldehyde. The other side products **52** and **53** were formed when the diol **10** was treated with $CH_2(OMe)_2$ in various acidic conditions. The structures of **51, 52** and **53** indicated that the intramolecular cyclization to form five-membered acetal ring was not favorable at all in these cases. The conformational analysis of the diol **10** seemed to provide a reasonable rationalization of these results. There are three possible conformers for the diol **10**. There are **CF1, CF2 and CF3** as shown in **Figure 8**. **CF1** seemed the most stable conformer compared to **CF2** or **CF3**, if one considers the H-bonding between hydroxyl and amide groups, and the lipophilic interaction between the C-14 alkyl chains. The two hydroxyl groups of the diol **10** were anti to each other in the conformer **CF1**, so that compound **51** with the seven-membered ring would have had to be formed when the diol **10** was treated with paraformaldehyde even with the removal of the byproduct (water). In the case of the reagent $CH_2(OMe)_2$, the presence of the byproduct MeOH in the reaction mixture, seemed to prevent the formation of the desired product **49** which would have been formed through the (unfavorable) conformer **CF2** or **CF3**. As soon as the desired product **49** was formed, the byproduct MeOH seemed to cleave **49** and drive the equilibrium to the favorable conformer **CF1**. Therefore, treatment with the reagent $CH_2(OMe)_2$ did not lead to intramolecular cyclization in acidic conditions. On the other hand, once the product **49** was formed, the reagent CH_2I_2 with a base K_2CO_3 gave no chance for the product **49** to be cleaved by the byproduct (potassium iodide), even though (also in the basic reaction condition) the product **49** had to be formed through either conformer of **CF2** or **CF3** . These results seemed to provide some structural information about how the long alkyl chains should be aligned in the molecule.



Scheme 14

Synthetic scheme for the synthesis of **TM3**



Scheme 15

Synthetic scheme for the synthesis of **TM4**

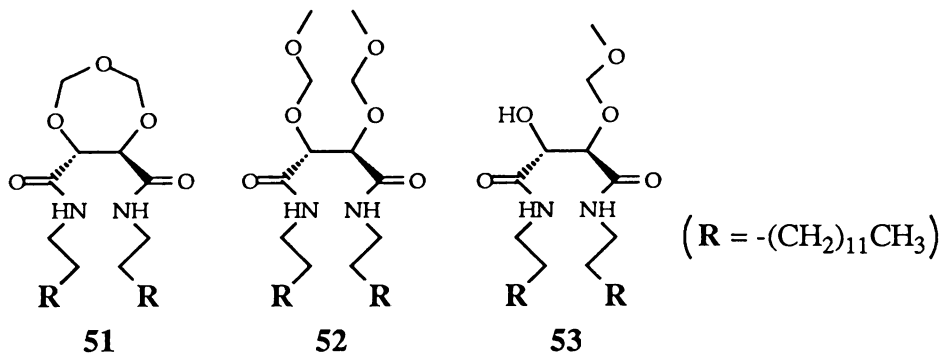
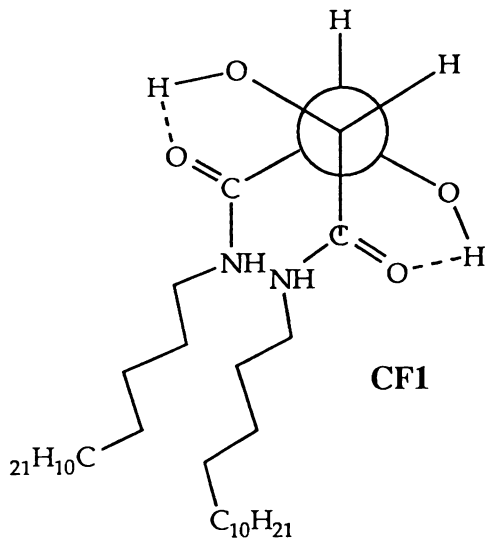


Figure 7

Side products formed in the reaction of **10** with $(HCHO)_n$ or $CH_2(OMe)_2$

Figure 8

Conformational analysis for the acid catalyzed acetalization of the diol **10**

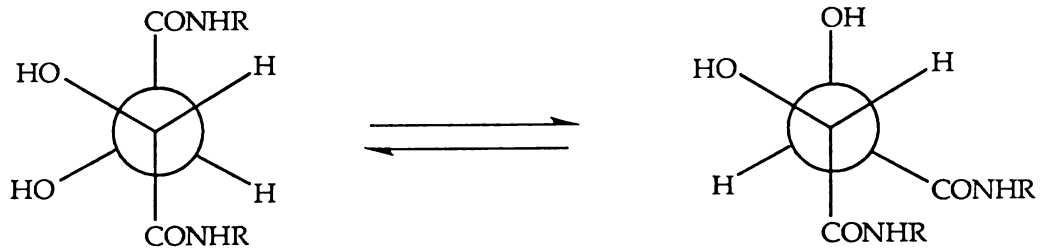


CF1

CF1; H-bondings and lipophilic interaction.

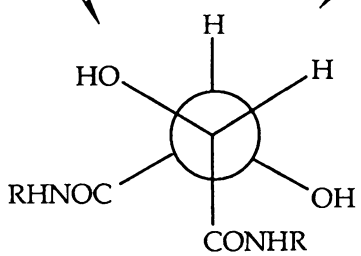
CF2; H-bondings only.

CF3; lipophilic interaction only.



CF2 **CF3**

R = $-(CH_2)_{13}CH_3$



CF1

CONCLUSIONS

The direct chemical synthesis of the lipid A region of lipopolysaccharides is a formidable synthetic challenge which has only very recently been realized and only by one group.[3,6] There are no published studies of lipid A analogs of similar structural complexity. One of the main problems in the design and execution of directed syntheses of lipid A molecules and related structures is the high density of functional groups. This requires elaborate protection and deprotection schemes and the development of several logistical strategies and methods. The most difficult aspect of synthetic organic chemistry is still the stereospecific and regiospecific formation of carbon-carbon bonds. This problem, coupled to the usual constraints of carbohydrate synthesis has made the preparation of lipid A analogs containing carboxylate fundtions instead of phophate groups one in which no successes have been recorded.

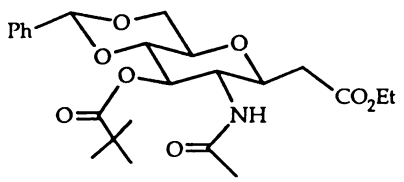
In this study, lipid X and lipid Y moieties (**4** and **7**) were synthesized for such an analog (**TM2**) as shown in **Figure 9**. The protected saccharide units **7** and **4** each has its own potential for use in the synthesis of structural analogs of lipid A. Another major step was the development of a synthetic scheme for the preparation of optically pure (R)-3-hydroxytetradecanoic acids. Synthetic strategies towards another related analog (**TM1**) are also presented and discussed.

The synthesis of (R)-3-hydroxytetradecanoic acid (**8**) introduces a new potential for the synthesis of other optically pure and related compounds. As shown in **Scheme 16**, in principle, any (R)-3-hydroxycarboxylic acid can be made by simply changing **R** groups. In so far as **R** group can be conserved under the reaction condition (0.1 M

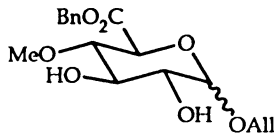
NaOH and H_2O_2 at 80°C for 48 hours), the variety of biologically related molecules which can be made is impressive.

The future aspects of the key intermediates (**4**, **7** and **8**) prepared in this study are summarized in **Scheme 17** and **Scheme 18**. The properly protected fatty acids (**54** and **55**) are intended to be prepared by developing a dianion of **8** followed by quenching with BnBr or tetradecanoyl chloride. The lipid X moiety **56** will possibly be made by coupling **55** with **7** followed by a sequence of reactions (selective cleavage of the allyl ether[14], and an activation of 1-OH group to X). Differently from the lipid X moiety **56**, the lipid Y moiety should be made by stepwise acylation as shown in **Scheme 18**. The monoacylated lipid Y moiety **57** will be possibly made by selective cleavage of 2-acetamide group[6] followed by a sequence of reactions (acylation of 2-amino group with **54**, cleavage of benzylidene acetal, tritylation of 6-OH, and methylation of 4-OH). The diacylated lipid Y moiety **2** will be made from **57** by the cleavage of 3-O-pivaloyl group with NaOBn/BnOH followed by a sequence of reactions (acylation of 3-OH group with **54**, and detritylation to give 6-OH). The coupling of **2** with **56** followed by catalytic hydrogenation will give **TM2** as shown in **Scheme 18**.

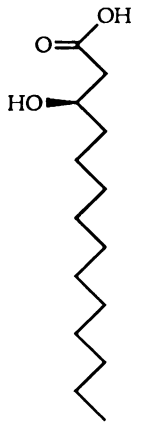
While the syntheses of the lipid X moiety **7**, lipid Y moiety **4** and (R)-3-hydroxytetradecanoic acid (**8**) are expected to make a big contribution to studies investigating the structural requirements for endotoxicity, the structurally non-related lipid A antagonists **TM3** and **TM4** containing no sugar ring would contribute to the biomechanism study of lipid A at the molecular level by examining the roles of the charged head groups and lipid chains which are very important components of lipid A.



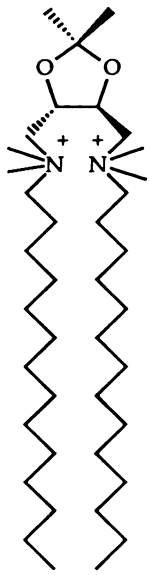
4



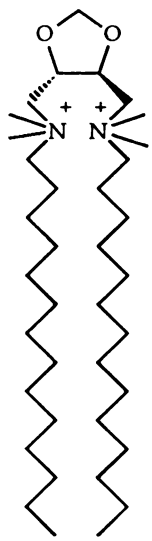
7



8



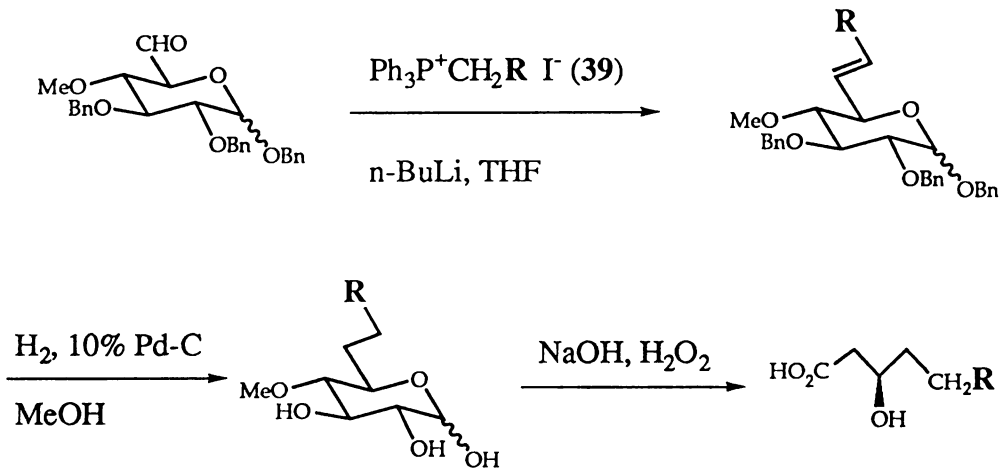
TM3



TM4

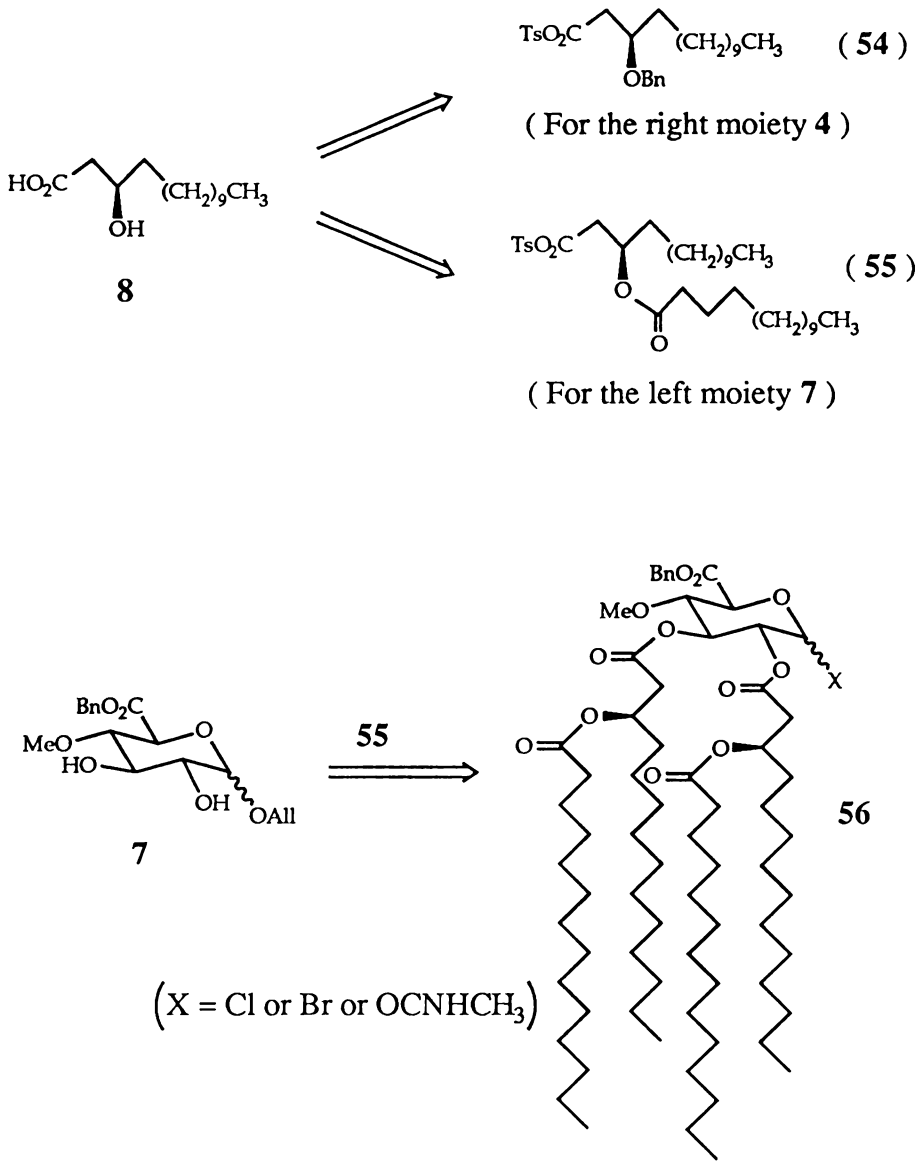
Figure 9

The list of molecules synthesized in this study



Scheme 16

The generalized scheme for the syntheses of chiral compounds from β-D-glucose

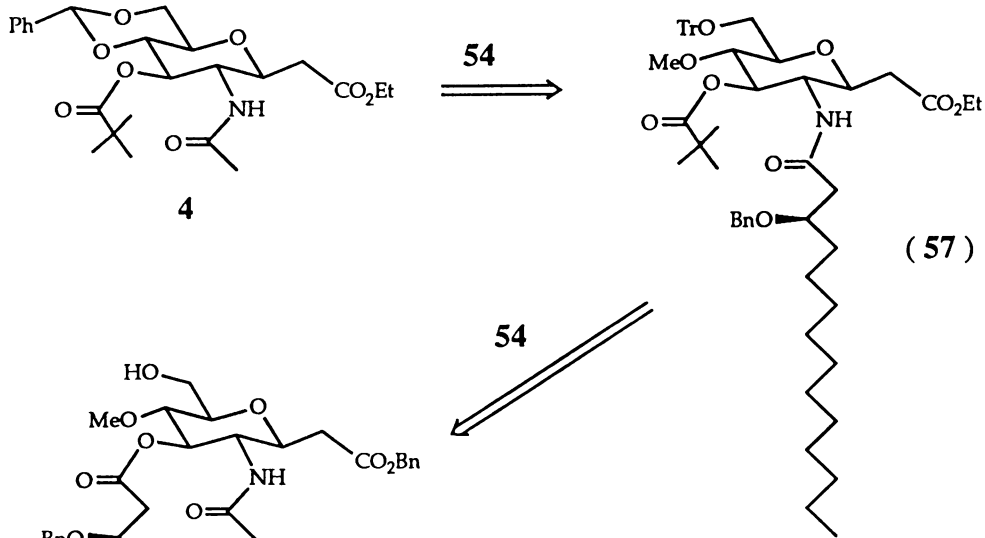


Scheme 17

The future synthetic scheme intended to be done for the left moiety of **TM2**

Scheme 18

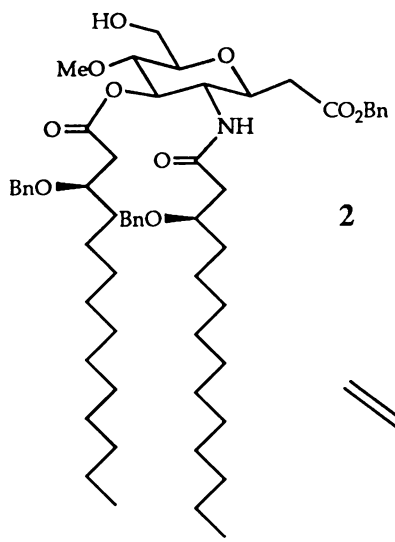
The future synthetic scheme intended to be done for the right moiety of **TM2** and the coupling of the left moiety with the right moiety to give **TM2**


Ph
O
O
O
O
O
NH
O
CO2Et
54
TrO
MeO
O
O
NH
CO2Et
O
BnO

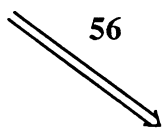
4

(57)

54


HO
MeO
O
O
NH
CO2Bn
O
BnO
BnO

2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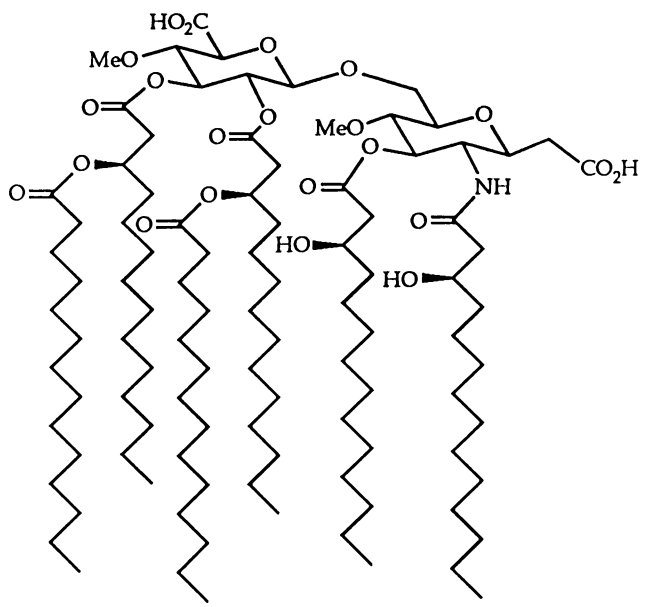
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MeO
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NH
CO2H
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HO
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TM2

EXPERIMENTAL

^{1}H-NMR and ^{13}C-NMR spectra were measured on a Varian Gemini-300 spectrometer (300 MHz) for chloroform-*d* solutions unless noted otherwise. The chemical shifts are given in δ values with TMS as the internal standard or relative to the chloroform line at 7.24 ppm for proton or 77.4 ppm for ^{13}C spectra. Silica gel flash column chromatography was carried out on silica using Kieselgel 60 (Merck), 0.040-0.063 mm. Precoated Kieselgel F_{254} plates (1mm thickness ; Uniplate) were used for preparative TLC. The intermediates **5** and **11** in **Scheme 5** were prepared by an earlier developed method[13] from β-D-glucosamine hydrochloride.

Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (12)

Compound **11** (30g, 82.9 mmol) was treated with potassium carbonate (0.5g, 3.6 mmol) and methanol (200 ml) at room temperature for 16 hours. After neutralization with 35% aqueous hydrochloric acid (0.5g, 5 mmol), insoluble materials were filtered off. The filtrate solution was concentrated under reduced pressure and the residue was dried *in vacuo*. This residue was dissolved in dry DMF (100 ml) and treated with benzaldehyde dimethylacetal (18.9g, 124 mmol) and p-TsOH (0.2g, 1 mmol) at room temperature for 2 hours. After evaporating at reduced pressure, potassium carbonate (0.3g, 2.2 mmol) was added. After additional stirring for 0.5 hour, insoluble materials were filtered off and washed with DMF (20 ml). The filtrate was combined with the washings and evaporated at reduced pressure. The residue was then treated with ether (150 ml) to precipitate the product. The product was crystalized

from ether, collected by filtration, washed with distilled water (50 ml) and ether (50 ml) and then dried *in vacuo*.

Yield ; 24.4g (91%). ^{1}H-NMR (300 MHz, $CDCl_3$-CD_3OD) δ 2.02(3H, s, CH_3), 3.51(3H, s, CH_3), 3.40-3.55(2H, m, H-5 and H-6ax), 3.62(1H, dd, J = 9 and 10 Hz, H-4), 3.77(1H, dd, J = 10 and 10 Hz, H-3), 3.89(1H, dd, J = 9 and 10 Hz, H-2), 4.35(1H, dd, J = 10 and 6 Hz, H-6eq), 4.54(1H, d, J = 9 Hz, H-1), 5.57(1H, s, H-benzyl), 7.34-7.53(5H, m, H-aromatic).

Methyl 2-acetamido-3-O-trimethylacetyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (13)

To a solution of **12** (19.2g, 59.4 mmol) in dry THF (300 ml) was added 60% NaH in mineral oil (2.7g, 67.5 mmol) at room temperature. After stirring the THF solution at room temperature for 18 hours, it was cooled to 0°C and trimethylacetyl chloride (7.2g, 59.4 mmol) was added keeping the solution at 0°C. The reaction mixture was allowed to be warmed up to room temperature and stirred for another 4 hours. The reaction mixture was then concentrated under reduced pressure. Dichloromethane (300 ml) and distilled water (100 ml) were added to the residue. The organic layer was separated in a separatory funnel, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The crude oily product was purified by flash column chromatography (CH_2Cl_2-ether : 1-1)

Yield ; 21.5g (89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.18(9H, s, $(CH_3)_3$), 1.93(3H, s, CH_3), 3.25(3H, s, OCH_3), 3.57(1H, m, H-5), 3.70(1H, dd, J = 10 and 10 Hz, H-6ax), 3.74(1H, dd, J = 10 and 10 Hz, H-4), 4.15-4.30(3H, m, H-1 H-2 and H-6eq), 5.28(1H, dd, J = 10 and 10 Hz, H-3), 5.50(1H, s, H-benzyl), 7.29-7.40(5H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 101.0(C-1), 170.5(C-carbonyl), 180.0(C-carbonyl).

Methyl 2-acetamido-3-O-trimethylacetyl-6-O-benzyl-2-deoxy-β-D-glucopyranoside (14)

A solution of the benzylidene acetal **13** (0.7g, 1.7 mmol) and sodium cyanoborohydride (0.9g, 14.0 mmol) in dry THF (20 ml) containing 4A-molecular sieves was cooled to 0°C. Anhydrous hydrogen chloride was bubbled into the reaction mixture until no gas evolution. After 10 min at 0°C, when TLC indicated complete reaction, the mixture was poured into ice water, and the product was extracted with dichloromethane (50 ml). The extract was washed with saturated aqueous sodium hydrogencarbonate, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was subjected to flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 0.53g (75%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.19(9H, s, $(CH_3)_3$), 1.92(3H, s, CH_3), 3.47(3H, s, OCH_3), 3.57(1H, m, H-5), 3.73(1H, dd, J = 10 and 10 Hz, H-4), 3.78(2H, d, J = 5 Hz, H-6), 4.03(1H, dd, J = 10 and 8 Hz, H-2), 4.38(1H, d, J = 8 Hz, H-1), 4.56(1H, d, J = 12 Hz, H-benzyl), 4.62(1H, d, J = 12 Hz, H-benzyl), 5.05(1H, dd, J = 10 and 9 Hz, H-3), 7.33(5H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 102.0(C-1), 170.4(C-carbonyl), 180.0(C-carbonyl).

Methyl 2-acetamido-3-O-trimethylacetyl-4-oxo-6-O-benzyl-2-deoxy-β-D-glucopyranoside (15)

To the solution of anhydrous DMSO (500 ml) and acetic anhydride (25 ml) was added **14** (2.5g, 6.1 mmole). The reaction mixture was stirred at 30°C for two days and concentrated by vacuum distillation at 30°C. The oily residue was used for the next reaction without further purification.

Methyl 2-acetamido-3-O-trimethylacetyl-cis,trans-4-ethoxycarbomethylene-6-O-benzyl-2,4-dideoxy-β-D-glucopyranoside (16)

To the suspension of 60% NaH in mineral oil (264 mg, 6.6 mmole) and dry THF (10 ml) was added ethyl diethylphophonoacetate (1.30g, 6.1 mmole) at 0°C and the reaction mixture was stirred at room temperature for 3 hours. The ketone **15** was added to the reaction mixture which was stirred at room temperature for additional 16 hours. After checking the completion of the reaction by TLC, the reaction mixture was concentrated and the residue was treated with dichloromethane (50 ml) and distilled water (20 ml). The organic layer was separated in a separatory funnel, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. Reaction products were subjected to flash column chromatography and analyzed by ^{1}H-NMR.

Methyl 2-acetamido-3-O-trimethylacetyl-6-O-benzyl-2-deoxy-β-D-galactopyranoside (18)

The crude oxidized product **15** from **14** (2.0g, 4.89 mmole) was treated with $NaBH_4$ (0.5g, 13.2 mmole) and TBAB (2 mg) in dichloromethane (15 ml) and distilled water (5 ml). After stirring for 16 hours, the completion of the reaction was checked by TLC and the organic layer was separated, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The oily residue was subjected to flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 850 mg (43%). TLC ; R_F = 0.25 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.21(9H, s, $(CH_3)_3$), 2.03(3H, s, CH_3), 3.52(3H, s, OCH_3), 3.48-3.60(2H, m, H-6), 3.74-3.85(2H, m, H-2 and H-5), 3.91(1H, dd, J = 11 and 3 Hz, H-3), 4.41(1H, d, J = 10 Hz, H-1), 4.47(1H, d, J = 12 Hz, H-benzyl), 4.53(1H, d, J = 12 Hz, H-benzyl), 5.33(1H, dd, J = 3 and 1 Hz, H-4), 7.25-7.36(5H,

m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 101.5(C-1), 173.5(C-carbonyl), 178.0(C-carbonyl).

Methyl 2-acetamido-3-O-trimethylacetyl-4-O-trifluoromethanesulfonyl-6-O-benzyl-2-deoxy-β-D-galactopyranoside (19)

To the solution of **18** (90 mg, 0.22 mmole) in dry pyridine (2 ml) was added trifluoromethanesulfonic anhydride (100 mg, 35 mmole) at -15ºC. The reaction mixture was warmed up to room temperature and stirred for 2 hours. It was then treated with 4% aqueous HCl solution (35 ml) and dichloromethane (20 ml). The organic layer was separated and washed with a 5% aqueous solution of $NaHCO_3$ (10 ml). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The oily residue was subjected to flash column chromatography (CH_2Cl_2-MeOH : 50-1).

Yield ; 80 mg (67%). TLC ; R_F = 0.30 (CH_2Cl_2-MeOH : 50-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.17(9H, s, $(CH_3)_3$), 2.12(3H, s, CH_3), 3.53(3H, s, OCH_3), 3.45-3.58(2H, m, H-6), 3.71(1H, ddd, J = 10, 10 and 4 Hz, H-2), 4.10(1H, ddd, J = 10, 10 and 2 Hz, H-5), 4.43(1H, d, J = 12 Hz, H-benzyl), 4.51(1H, d, J = 12 Hz, H-benzyl), 4.56(1H, d, J = 10 Hz, H-1),5.01(1H, dd, J = 4 and 2 Hz, H-4), 5.13(1H, dd, J = 4 and 4 Hz, H-3), 5.67(1H, d, J = 10 Hz, NH), 7.24-7.36(5H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 99.5(C-1), 119.4(q, J = 320 Hz, CF_3), 168.7(C-carbonyl), 176.5(C-carbonyl).

Ethyl 2-acetamido-1,2-dideoxy-3,4,6-tri-O-acetyl-β-D-glucopyranosylacetate (21)

To a 60% mineral oil dispersion of KH (2.67g, 40 mmole), was added anhydrous dichloromethane (20 ml). Diethyl malonate (6.4g, 40 mmole) and

18-crown-6 (5.3g, 20 mmole) were then added to the suspension while maintaining it at 0°C. After additional stirring at 0°C for 15 min, α-D-acetochloroglucosamine **5** (3.66g, 10 mmole) was added in one portion. After additional stirring at room temperature for 20 min, acetic acid (2.4g) was added to quench the reaction. The solution was washed with 5% aqueous sodium hydrogencarbonate (100 ml), dried over anhydrous magnesium sulfate, filtered and concentrated. The product was purified by flash column cchromatography (CH_2Cl_2-MeOH : 30-1). The first purified oily product **20** was dissolved in DMSO (24 ml) containing NaCl (2.0g, 34 mmole). After refluxing for 16 hours, DMSO was removed by vacuum distillation and the residue was treated with distilled water (50 ml) and dichloromethane (100 ml). The organic layer was isolated, dried over anhydrous magnesium sulfate, filtered and concentrated. The product was purified by flash column chromatography (CH_2Cl_2-MeOH : 30-1).

Yield ; 0.88g (21%). TLC ; R_F = 0.35 (CH_2Cl_2-MeOH : 20-1). $[\alpha]_D^{20}$; -1.94 (c 0.09, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.23(3H, t, J=7 Hz, CH_3), 1.90(3H, s, CH_3), 2.00(3H, s, CH_3), 2.01(3H, s, CH_3), 2.04(3H, s, CH_3), 2.58(2H, d, J=6.0 Hz, CH_2), 3.62(1H, m, H-5), 3.79(1H, dt, J=10 and 6 Hz, H-1), 4.01-4.22(4H, m, H-2, H-6 and O$\underline{CH_2}CH_3$), 4.98(1H, dd, J=10 and 10 Hz, H-4), 5.06(1H, dd, J=10 and 10 Hz, H-3). ^{13}C-NMR (75.5 MHZ, $CDCl_3$) δ 37.7(C-2). MS (Neg. FAB, matrix: NBA) 416.2(M-1).

2-Acetamido-1-C-ethoxycarbomethyl-1,2-dideoxy-4,6-O-benzylidene-β-D-glucopyranose (22)

The procedure for the preparation of **22** was the same as that employed for the preparation of **12**, except that EtOH was used instead of MeOH.

Yield ; 55% . TLC ; R_F = 0.20 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.25(3H, t, J=7 Hz, CH_3), 1.94(3H, s, CH_3), 2.55(2H, m, CH_2), 3.36-3.92(5H, m, H-2, H-3, H-4, H-5 and H-6),

4.14(2H, q, J = 7 Hz, OCH_2), 4.26(1H, dd, J = 10 and 4 Hz, H-6), 5.47(1H, s, H-benzyl), 7.33-7.48(5H, m, H-aromatic).

2-Acetamido-1-C-ethoxycarbomethyl-1,2-dideoxy-3-O-trimethylacetyl-4,6-O-benzylidene-β-D-glucopyranose (4)

To the solution of imidazole (0.6g, 8.8 mmole) in dry pyridine (12 ml) in an ice bath, was added trimethylacetyl chloride (1.0g, 8.3 mmole). After removing ice bath, the solution was stirred at room temperature for 0.5 hour. Compound **22** (1.8g, 3.9 mmole) was added to the solution and it was stirred at 80°C for 16 hours. After evaporating pyridine under reduced pressure, the residue was treated with dichloromethane (50 ml) and 5% aqueous HCl solution (20 ml). The organic layer was isolated and washed with 5% aqueous NaOH solution (10 ml). The organic layer was collected, dried over anhydrous magnesium sulfate and concentrated. The product was purified by flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 1.7g (80%). TLC ; R_F = 0.30 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.19(9H, s, $(CH_3)_3$), 1.25(3H, t, J=7 Hz, CH_3), 2.51(1H, dd, J = 18 and 10 Hz, CHH'), 2.62(1H, dd, J = 18 and 5 Hz, CHH'), 3.49(1H, m, H-1), 3.69(2H, m, H-5 and H-6), 3.86(1H, m, H-2), 4.12(1H, dd, J = 10 and 10 Hz, H-2), 4.15(2H, q, J = 7 Hz, OCH_2), 4.24(1H, dd, J = 10 and 5 Hz, H-6), 5.17(1H, dd, J = 10 and 10 Hz, H-3), 5.52(1H, s, H-benzyl), 7.33-7.43(5H, m, H-aromatic).

Allyl 4,6-(4-methoxy)benzylidene-α,β-D-glucopyranoside (23, α/β=3/2)

To a solution of β-D-glucose (60g, 0.33 mole) and allyl alcohol (300 ml) was added acetyl chloride (0.5g, 6.4 mmole). The reaction mixture was refluxed for 18 hours and concentrated under reduced pressure after checking for completion by periodically obtaining ^{1}H-NMR spectra in D_2O. Dry DMF (100 ml), p-TsOH (0.5g)

and p-anisaldehyde (45.3g, 0.33 mole) were then added to the residue. After stirring the reaction mixture at room temperature for 2 hours, toluene (200 ml) was added and it was then evaporated under reduced pressure. This addition-evaporation step with toluene was repeated at least three times. Anhydrous potassium carbonate (1.0g) was then added to the reaction mixture and it was stirred for 0.5 hour at room temperature. It was then concentrated *in vacuo* and the residue treated with ether (250 ml) The ethereal was allowed to stand in the refrigerator (-20°C) for 18 hours. The precipitate which formed was collected by filtration and the filter cake was washed with a 1:1 mixture (200 ml) of pet. ether and ether. The filter cake was redissolved and the product isolated by flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 38g (34% from D-glucose). TLC ; R_F = 0.33 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, DMSO-d^6) δ 3.12(1H, ddd, J = 10, 10 and 7 Hz, H(β)-6), 3.32-3.40(1H, m, H(α)-6), 3.58-3.69(2H, m, H-3 and H-4), 3.73(3H, s, OCH_3), 3.93-4.27(3H, m, H-5 and H(α)-allyl), 4.37(1H, d, J = 10 Hz, H(β)-1), 4.78(1H, d, J = 4 Hz, H(α)-1), 4.99(2H, d, J = 8 Hz, H(β)-allyl), 5.13-5.19(2H, m, H-2 and H(β)-vinyl), 5.27-5.39(2H, m, H(α)-vinyl), 5.50(1H, s, H-benzyl), 5.83-6.00(1H, m, H-vinyl), 6.90(2H, d, J = 10 Hz, H-aromatic), 7.36(2H, d, J = 10 Hz, H-aromatic).

Allyl 2,3-di-O-benzoyl-α,β-D-glucopyranoside (24, α/β=3/2)

To a cooled solution of **23** (18g, 53 mmole) in dry pyridine (50 ml) in an ice bath was added benzoyl chloride (15g, 106 mmole). The reaction mixture was then stirred at room temperature for 18 hours and concentrated under reduced pressure. Dichloromethane (100 ml) was added to the residue and the solution was washed with a 4% aqueous HCl solution (100 ml) followed by a 5% aqueous $NaHCO_3$ solution (50 ml). The organic layer was isolated, dried over anhydrous magnesium sulfate, filtered and concentrated under reduced pressure. The residue was treated with an

AcOH-H_2O (5 : 1) mixture (100 ml) at room temperature for 16 hours. The reaction mixture was then concentrated under reduced pressure until 80% of the solvent was evaporated. A 5% aqueous $NaHCO_3$ solution was added to the concentrated reaction mixture until no further gas evolution. The product was then extracted from the aqueous suspension by washing twice with dichloromethane (100 ml). The combined organic solution was dried over anhydrous magnesium sulfate, filtered and concentrated. The product was purified by flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 15.3g (67% from **23**). TLC ; R_F = 0.30 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.56(1H, m, H(β)-5), 3.85-4.02(3H, m, H-4, H(α)-5 and H-6), 3.98-4.36(2H, m, H-vinyl), 4.76(1H, d, J = 8 Hz, H(β)-1), 5.08-5.30(4H, m, H(α)-1, H-2 and H(α)-allyl), 5.38-5.47(2H, m, H(β)-allyl), 5.69-5.89(2H, m, H-3 and H-vinyl), 7.30-7.96(10H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 95.5 (C_α-1), 100.0(C_β-1), 118.0(C-allyl).

Allyl 2,3-di-O-benzoyl-6-O-triphenylmethyl-α,β-D-glucopyranoside (25, α/β=3/2)

To the solution of **24** (8.7g, 20.3 mmole) in dry pyridine (20 ml) was added triphenylmethyl chloride (6.3g, 22.3 mmole). The reaction mixture was stirred at room temperature for 16 hours. Dry ether (150 ml) was then added and stirring was continued for a further 4 hours. The reaction mixture was then washed with distilled water (50 ml) and 4% aqueous HCl solution (200 ml). The organic layer was collected and washed with 5% aqueous $NaHCO_3$ solution (50 ml). After drying it over anhydrous magnesium sulfate, the organic layer was concentrated under reduced pressure. The residue was subjected to flash column chromatography (pet. ether-CH_2Cl_2 : 1-1).

Yield ; 10.4g (76%). TLC ; R_F = 0.27 (pet. ether-CH_2Cl_2 : 1-2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.45-3.68(2H, m, H-5 and H-6), 3.90-4.44(4H, m, H-4, H-6 and H-vinyl), 4.27(1H, d, J = 10 Hz, H(β)-1), 5.15-5.50(3H, m, H(α)-1,

H-2 and H-allyl), 5.78-5.96(2H, m, H-3 and H-vinyl), 7.25-8.04(25H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 117.8(C-allyl).

Allyl 2,3-di-O-benzoyl-4-O-methyl-α,β-D-glucopyranoside (26, α/β=3/2)

To the solution of **25** (4.3g, 6.4 mmole) containing Ag_2O (2.97g, 12.8 mmole) in dry DMF (14 ml) at room temperature was added methyl iodide (1.82g, 12.8 mmole). The reaction mixture was stirred at room temperature for 18 hours and then filtered through celite after the addition of ether (100 ml). The filtrate was washed with distilled water (100 ml) and the organic layer was isolated and dried over anhydrous magnesium sulfate. The drying agent was removed ty filtration and the filtrate was concentrated and the residue was subjected to flash column chromatography (pet. ether-CH_2Cl_2 : 1-2). The purified product was treated with ether (20 ml), acetic acid (10 ml) and 10% HCl aqueous solution (5 ml). After stirring the reaction mixture at room temperature for 18 hours, only the ether was removed from the reaction mixture by evaporation under reduced pressure. After adding dichloromethane (120 ml) to the concentrated mixture, 5% aqueous $NaHCO_3$ solution was added until no further gas evolution. The organic layer was separated, dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was subjected to flash column chromatography (CH_2Cl_2-MeOH : 20-1).

Yield ; 1.7g (64%). TLC ; R_F = 0.40 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.45(3H, S, OCH_3(α or β)), 3.47(3H, S, OCH_3 (α or β)), 3.51-3.57(1H, m, H-5), 3.68(1H, dd, J = 10 and 10 Hz, H-4), 3.82-4.38(4H, m, H-vinyl and H-6), 4.77(1H, d, J = 9 Hz, H(β)-1), 5.10-5.32(4H, m, H(α)-1, H(α)-2 and H-allyl), 5.36(1H, dd, J = 10 and 9 Hz, H(β)-2), 5.65(1H, dd, J = 10 and 10 Hz, H(β)-3), 5.71-5.89(1H, m, H-vinyl), 6.00(1H, dd, J = 10 and 10 Hz, H(α)-3), 7.34-8.05(10H, m, H-aromatic).

1-O-Allyl-4-O-methyl-α,β-D-glucuronic acid benzylester (7, α/β=3/2)

Pyridinium dichromate (5.0g, 13.3 mmole) was added to a solution of **26** (1.7g, 3.8 mmole) in dry DMF (10 ml) at room temperature. After stirring at room temperature for 18 hours, distilled water (100 ml) and dichloromethane were added to the reaction mixture. The organic layer was separated, dried over anhydrous magnesium sulfate, filtered and concentrated. The concentrate was subjected to flash column chromatography (CH_2Cl_2-MeOH : 10-1). The product was collected and treated with benzyl alcohol (30 ml) and potassium carbonate (2.0g). After stirring at room temperature for 18 hours, the reaction mixture was filtered and p-TsOH was added to the filtrate until the solution was strongly acidic (pH < 2 on pH-paper). The solution was then stirred at room temperature for 18 hours and coevaporated with toluene (100 ml) under reduced pressure. Coevaporation with toluene was repeated three times, and the solution containing benzyl alcohol was treated with sodium bicarbonate (2g). After stirring for half an hour, it was filtered and subjected to flash column chromatography (CH_2Cl_2) to remove most of the benzyl alcohol in the fore-run. The product which was still adsorbed on the silica gel on the column was then eluted sith a more polar solvent system (CH_2Cl_2-MeOH : 20-1).

Yield ; 0.37g (28% from **26**). TLC ; R_F = 0.33 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.33(3H, S, OCH_3(α or β)), 3.35(1H, dd, J = 10 and 10 Hz, H(α)-3), 3.36(3H, S, OCH_3(α or β)), 3.45(1H, dd, J = 10 and 9 Hz, H(β)-2), 3.56(1H, dd, J = 10 and 4 Hz, H(α)-2), 3.62(1H, dd, J = 10 and 10 Hz, H(β)-3), 3.80(1H, d, J = 10 Hz, H(β)-5), 3.82(1H, dd, J = 10 and 10 Hz, H-4), 4.00-4.40(2H, m, H-vinyl), 4.14(1H, d, J = 10 Hz, H(α)-5), 4.32(1H, d, J = 10 Hz, H(β)-1), 4.95(1H, d, J = 4 Hz, H(α)-1), 5.19-5.30(4H, m, H-allyl and H-benzyl), 5.80-5.95(1H, m, H-vinyl), 7.32-7.38(5H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 80.6($C_{\alpha \text{ or } \beta}$-benzyl), 80.9($C_{\alpha \text{ or } \beta}$-benzyl), 97.4($C_\alpha$-1), 101.6($C_\beta$-1), 117.4($C_{\alpha \text{ or } \beta}$-allyl), 117.6($C_{\alpha \text{ or } \beta}$-allyl), 169.4(C-carbonyl).

Methyl 2,3-di-O-benzoyl-4,6-O-benzylidene-α-D-glucopyranoside (27)

To a suspension of methyl-α-D-glucopyranoside (29g, 0.15 mole) in dry DMF (60 ml) were added α,α-dimethoxytoluene (30g, 0.20 mole) and dry p-TsOH (200 mg) at room temperature. After stirring at room temperature for 18 hours, MeOH produced in the reaction mixture was removed by evaporation at reduced pressure. Anhydrous potassium carbonate (0.5g) was added to neutralize the reaction mixture. After filtering the precipitate from the reaction mixture, the filtrate was concentrated by vacuum distillation at 40°C. Ether (20 ml) was poured into the residue and the reaction mixture was stood in the refrigerator (0°C) over night. The product was precipitated further by adding pet. ether (200 ml) to the ethereal solution. The precipitate was collected by filtration, dried *in vacuo* and weighed. The yield was 35g. To a solution of the product (35g, 0.12 mole) in dry pyridine (120 ml) at 0°C was added benzoyl chloride (37.8g, 0.26 mole). The mixture was kept at 0°C for 0.5 hour. It was then stirred at room temperature for 4 hours and then poured into a mixture of distilled water (200 ml) and ether (300 ml). After stirring for additional 0.5 hour, the organic layer was separated and concentrated under reduced pressure. The residue was precipitated by a mixture of pet. ether (100 ml) and ether (50 ml). The precipitate was collected by filtration and the filter cake was dried *in vacuo* and weighed (33.4g). Additional product (6.7g) was isolated from the filtrate by flash column chromatography.

Yield ; 40.1g (54%). TLC ; R_F = 0.50 (CH_2Cl_2-ether : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.42(3H, S, OCH_3), 3.85(1H, dd, J = 10 and 10 Hz, H-6(ax)), 3.90(1H, dd, J = 10 and 10 Hz, H-4), 4.08(1H, ddd, J = 10, 10 and 5 Hz, H-5), 4.37(1H, dd, J = 10 and 5 Hz, H-6(eq)), 5.17(1H, d, J = 3 Hz, H-1), 5.25(1H, dd, J = 10 and 3 Hz, H-2), 5.57(1H, s, H-benzyl), 6.07(1H, dd, J = 10 and 10 Hz, H-3), 7.29-8.01(15H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 97.8(C-1), 101.6(C-benzyl), 165.5(C-carbonyl), 166.0(C-carbonyl).

Methyl 2,3-di-O-benzoyl-6-O-benzyl-α-D-glucopyranoside (28)

To a room temperature solution of **27** (33.4g, 67.6 mmole) and borane-trimethylamine complex (14.8g, 203 mmole) in dry toluene (1.2 l) was added, dropwise anhydrous p-TsOH (35g, 203 mmole) at room temperature over 0.5 hour. After stirring at room temperature for 18 hours, the reaction mixture was concentrated under reduced pressure. Ether (300 ml) and pet. ether (300 ml) were added to the residue and the precipitate formed was filtered off. The filtrate was concentrated and the product was purified by flash column chromatography (pet. ether-ether : 1-2).

Yield ; 30g (90%). TLC ; R_F = 0.45 (pet. ether-ether : 20-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.41(3H, s, OCH_3), 3.78-3.88(2H, m, H-6), 3.95(1H, m, H-5), 3.98(1H, dd, J = 10 and 10 Hz, H-4), 4.60(1H, d, J = 12 Hz, H-benzyl), 4.67(1H, d, J = 12 Hz, H-benzyl), 5.12(1H, d, J = 3 Hz, H-1), 5.25(1H, dd, J = 10 and 3 Hz, H-2), 5.75(1H, dd, J = 10 and 10 Hz, H-3), 7.27-7.98(15H, m, H-aromatic).

Methyl 2,3-di-O-benzoyl-4-O-methyl-α-D-glucopyranoside (29)

To a solution of **28** (30.0g, 60.5 mmole) in dry DMF (30 ml) were added Ag_2O (20.0g, 86.3 mmole), anhydrous Ca_2SO_4 (20g) and iodomethane (68.4g, 0.48 mmole) at room temperature. After stirring at room temperature for a further 18 hours, the reaction mixture was poured into ether (300 ml). The precipitate was removed by filtering through celite and the filtrate was concentrated under reduced pressure and the residual DMF was removed by vacuum distillation. The product was further purified by passage through a silica gel column with 1 liter of a mixture of pet. ether-ether (2-1). After concentrating the filtrate, ethyl alcohol (120 ml) and 10% palladium on activated carbon (10g) were added. The reaction mixture was hydrogenated by attaching a hydrogen balloon to the reaction flask. After stirring the reaction mixture for 2 days, the reaction was checked for completion by TLC. The

precipitate in the reaction mixture was removed by filtration through celite and the filtrate was concentrated. The product was purified by flash column chromatography.

Yield ; 18.0g (71%). TLC ; R_F = 0.23 (pet. ether-ether : 1-2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.38(3H, s, OCH_3), 3.45(3H, s, OCH_3), 3.64(1H, dd, J = 10 and 10 Hz, H-4), 3.77-3.94(3H, m, H-5 and H-6), 5.06(1H, dd, J = 10 and 3 Hz, H-2), 5.10(1H, d, J = 3 Hz, H-1), 5.94(1H, dd, J = 10 and 10 Hz, H-3), 7.32-8.03(10H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 97.0(C-1), 165.6(C-carbonyl), 166.0(C-carbonyl).

The alternative way for the preparation of **29** from **43** in **Scheme 12** was the same as that employed for the preparation of **26** (Yield ; 65%).

1-O-Methyl-2,3-di-O-benzoyl-4-O-methyl-α-D-glucuronic acid methylester (30)

To a room temperature solution of **29** (17g, 40.5 mmole) in dry DMF (90 ml) was added pyridinium dichromate (91.4g, 0.24 mole). The reaction mixture was stirred at room temperature for 18 hours after which it was treated with distilled water (800 ml) and ethyl acetate (500 ml) and stirred for 0.5 hour. The organic layer was isolated and washed with a aqueous 4% HCl solution (300 ml). The ethyl acetate layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure, followed by further concentration under high vacuum. To the residue were added MeOH (600 ml), anhydrous magnesium sulfate (20g) and p-TsOH (1.0g). The reaction mixture was stirred at room temperature for 18 hours after which it was treated with sodium bicarbonate (1.5g) for 0.5 hour and concentrated under reduced pressure. The residue was treated with dichloromethane (400 ml) for 0.5 hour and the precipitate was removed by filtration. The filtrate was concentrated and the residue was purified by flash column chromatography.

Yield ; 12.0g (66%). TLC ; R_F = 0.55 (CH_2Cl_2-ether : 20-1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.34(3H, s, OCH_3), 3.36(3H, s, OCH_3), 3.77(3H, s, OCH_3), 3.79(1H, dd, J = 10 and 10 Hz, H-4), 4.23(1H, d, J = 10 Hz, H-5), 5.07(1H, dd, J = 10 and 3 Hz, H-2), 5.11(1H, d, J = 3 Hz, H-1), 5.87(1H, dd, J = 10 and 10 Hz, H-3), 7.25-7.95(10H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 97.4(C-1), 165.4(C-carbonyl), 165.8(C-carbonyl), 169.4(C-carbonyl).

Benzyl-α,β-D-glucopyranoside (32, α/β=1/1)

To a suspension of β-D-glucose (60g, 0.33 mole) in benzyl alcohol (500 ml, 4.83 mole) and benzene (150 ml) was added acetyl chloride (2.0 ml). The reaction mixture was refluxed with a Dean-Stark apparatus for 18 hours to remove the water that was formed. After checking for completion of the reaction by ^{1}H-NMR spectroscopy, the reaction mixture was treated with anhydrous potassium carbonate (4.0g) at room temperature for 1 hour. Benzene in the reaction mixture was evaporated at reduced pressure, then the reaction flask was allowed to stand until all of the precipitate settled. The supernatant was carefully decanted into ether (200 ml). This ethereal solution was stirred with distilled water (500 ml) for 1 hour and the aqueous layer was isolated. The aqueous layer was washed again with ether (150 ml) and then concentrated under reduced pressure and dried further under high vacuum. The residue was used for the next reaction without further purification.

Yield ; 69g (77%). ^{1}H-NMR (300 MHz, D_2O) δ 3.10-3.78(4H, m, H(β)-2, H-3, H-4, H-5 and H-6), 3.39(1H, dd, J = 9 and 3 Hz, H(α)-2), 4.34(1H, d, J = 9 Hz, H(β)-1), 4.42-4.78(2H, m, H=benzyl), 4.85(1H, d, J = 3 Hz, H(α)-1), 7.20-7.31(5H, m, H-aromatic).

Benzyl 4,6-O-(4-methoxy)benzylidene-α,β-D-glucopyranoside (33, α/β=1/1)

The procedure for the preparation of **33** was the same as that employed for the preparation of **42**.

Yield ; 37.7g (38%). TLC ; R_F = 0.36 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR (300 MHz, DMSO-d^6) δ 3.15-4.20(6H, m, H-2, H-3, H-4, H-5 and H-6), 3.73(3H, s, OCH_3), 4.46(1H, d, J = 9 Hz, H(β)-1), 4.50(1H, d, J = 12 Hz, H(α)-benzyl), 4.58(1H, d, J = 12 Hz, H(β)-benzyl), 4.70(1H, d, J = 12 Hz, H(α)-benzyl), 4.78(1H, d, J = 12 Hz, H(β)-benzyl), 4.85(1H, d, J = 3 Hz, H(α)-1), 5.50(1H, s, H(α)-benzylidene), 5.51(1H, s, H(β)-benzylidene), 6.89-7.41(9H, m, H-aromatic).

Benzyl 2,3-di-O-benzyl-4,6-O-(p-methoxy)benzylidene-α,β-D-glucopyranoside (34, α/β=1/1)

To a room temperature solution of **33** (16g, 41.2 mmole) in dry THF (200 ml) was added (3.3g, 82.5 mmole) of a 60% dispersion of NaH in mineral oil and the mixture kept stirring at room temperature for 0.5 hour. Benzyl bromide (14.1g, 82.5 mmole) was then added and stirring maintained for an additional 18 hours after which the reaction mixture was concentrated and the residue was treated with distilled water (100 ml) and dichloromethane (200 ml). The organic layer was isolated and dried over anhydrous magnesium sulfate. The drying agent was removed by filtration and the filtrate was concentrated and the concentrate was used for the next reaction without further purification.

Yield ; 22.3g (95%). TLC ; R_F = 0.30 (CH_2Cl_2). ^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 3.53(1H, dd, J = 10 and 4 Hz, H-2), 3.58(1H, dd, J = 10 and 10 Hz, H-4), 3.66(1H, dd, J = 10 and 10 Hz, H-6), 3.80(3H, s, OCH_3), 3.88(1H, ddd, J = 10, 10 and 5 Hz, H-5), 4.09(1H, dd, J = 10 and 10 Hz,

H(α)-3), 4.17(1H, dd, J = 10 and 5 Hz, H-6), 4.55-4.93(6H, m, H-benzyl), 4.80(1H, d, J = 4 Hz, H-1), 5.50(1H, s, H-benzylidene), 6.89(19H, m, H-aromatic).

Benzyl 2,3-di-O-benzyl-α,β-D-glucopyranoside (35, α/β=1/1)

Compound **34** (22.3g, 39.3 mmole) was stirred with a mixture of acetic acid (100 ml) and distilled water (50 ml) at room temperature for 6 hours. The reaction mixture and additional distilled water (200 ml) were transfered to a 1 liter beaker, and sodium bicarbonate was added to the beaker until no further gas evolution. The product was extracted twice with dichloromethane (200 ml) from the aqueous solution. The combined organic layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated and the product was purified by flash column chromatography.

Yield ; 17.6g (84%). TLC ; R_F = 0.32 (CH_2Cl_2-MeOH : 20-1). ^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 3.48(1H, dd, J = 10 and 4 Hz, H-2), 3.55(1H, dd, J = 10 and 10 Hz, H-4), 3.68(1H, dd, J = 10 and 3 Hz, H-5), 3.72(2H, d, J = 3 Hz, H-6), 3.87(1H, dd, J = 10 and 10 Hz, H-3), 4.50-5.05(6H, m, H-benzyl), 7.28-7.42(15H, m, H-aromatic). ^{13}C-NMR of α-isomer (75.5 MHz, $CDCl_3$) δ 62.2, 69.1, 70.3, 71.0, 72.7, 75.3, 79.7, 81.3, 95.5, 127.8-128.5(C-aromatic), 137.0, 137.9, 138.7.

Benzyl 2,3-di-O-benzyl-6-O-triphenylmethyl-α,β-D-glucopyranoside (36, α/β=1/1)

To a solution of **35** (13.6g, 30.2 mmole) in dry pyridine (50 ml) was added triphenylmethyl chloride (9.3g, 33.2 mmole). The reaction mixture was stirred at 70°C for 18 hours. When the reaction was complete, the reaction mixture was treated with dichloromethane (200 ml) and distilled water (150 ml). The organic layer was isolated and dried over anhydrous magnesium sulfate. After filtering, the filtrate was concentrated

under reduced pressure until most of the pyridine was removed. The residue was subjected to flash column chromatography.

Yield ; 19.7g (94%). TLC ; R_F = 0.28 (CH_2Cl_2).

^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 3.19(1H, dd, J = 12 and 6 Hz, H-6), 3.24(1H, dd, J = 12 and 4 Hz, H-6), 3.46(1H, dd, J = 10 and 4 Hz, H-2), 3.50(1H, dd, J = 10 and 10 Hz, H-4),3.69-3.76(1H, m, H-5), 3.77(1H, dd, J = 10 and 10 Hz, H-3), 4.81(1H, d, J = 4 Hz, H-1), 7.12-7.42(30H, m, H-aromatic). ^{13}CNMR (75.5 MHz, $CDCl_3$) δ 63.7, 68.7, 70.3, 71.4, 72.7, 75.6, 79.6, 81.7, 86.7, 94.9, 127.0-128.7(C-aromatic), 137.1, 138.1, 138.8, 143.9.

Benzyl 2,3-di-O-benzyl-4-O-methyl-α,β-D-glucopyranoside (37, α/β=1/1)

To a room temperature solution of **36** (27.5g, 40.0 mmole) in dry THF (180 ml) was added 60% NaH in mineral oil (1.75g, 44.0 mmole). After stirring the suspension at room temperature for 1 hour, iodomethane (9.4g, 66.3 mmole) was added at room temperature over a period of 0.5 hour. The reaction mixture was stirred at room temperature for a further 18 hours and then concentrated. Dichloromethane (300 ml) and distilled water (100 ml) were added to the residue, and the organic layer was isolated and passed through a short column of silica gel. The filtrate was concentrated and the residue was treated with a mixture of 4% aqueous HCl solution (20 ml), acetic acid (40 ml) and dichloromethane (100 ml) for 18 hours. The reaction mixture was then poured slowly into 20% K_2CO_3 aqueous solution (250 ml) with vigorous stirring. The organic layer was isolated and dried over anhydrous magnesium sulfate. After filtation, the filtrate was concentrated and the product was isolated by flash column chromatography.

Yield ; 12.0g (65%). TLC ; R_F = 0.50 (CH_2Cl_2-MeOH : 20-1).

^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 3.18(1H, dd, J = 10 and 10 Hz, H-4), 3.37(1H, dd, J = 10 and 4 Hz, H-2), 3.49(3H, s, OCH_3), 3.55(1H, m, H-5), 3.61(1H,

dd, J = 12 and 4 Hz, H-6), 3.68(1H, dd, J = 12 and 3 Hz, H-6), 3.88(1H, dd, J = 10 and 10 Hz, H-3), 4.45-4.91(6H, m, H-benzyl), 4.72(1H, d, J = 4 Hz, H-1), 7.19-7.35(15H, m, H-aromatic). ^{13}C-NMR of α-isomer (75.5 MHz, $CDCl_3$) δ 60.8, 61.9, 69.2, 71.0, 73.1, 75.6, 79.7, 81.8, 95.6, 127.6-128.4(C-aromatic), 137.1, 138.1, 138.8.

1,2,3-Tri-O-benzyl-4-O-methyl-α,β-D-xylo-hex-6-al (38, α/β=1/1)

To the solution of acetic anhydride (90 ml) in anhydrous DMSO (1600 ml) was added **37** (9.0g, 19.4 mmole). The reaction mixture was stirred at 30°C for 48 hours and then concentrated by vacuum distillation below 30°C. The product was isolated from the oily residue by flash column chromatography.

Yield ; 4.8g (54%). TLC ; R_F = 0.23 (pet. ether-ether : 3-2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.28(1H, dd, J = 10 and 9 Hz, H(α)-4), 3.40-3.51(2H, m, H-2 and H(β)-4), 3.52(3H, s, OCH_3), 3.54(3H, s, OCH_3), 3.59(1H, d, J = 6 Hz, H(β)-5), 3.63(1H, dd, J = 9 and 9 Hz, H(α)-3), 3.78(1H, dd, J = 10 and 1 Hz, H(α)-5), 4.03(1H, dd, J = 9 and 9 Hz, H(β)-3), 4.11(1H, d, J = 10 Hz, H(β)-1), 4.50-4.99(7H, m, H-benzyl and H(α)-1), 7.25-7.40(15H, m, H-aromatic), 9.67(1H, s, H(β)-aldehyde), 9.72(1H, d, J = 1 Hz, H(α)-aldehyde).

(1-Decyl)triphenylphosphonium iodide (39)

To the solution of triphenylphosphine (3.15g, 12 mmole) in dry THF (10 ml) was added 1-iododecane (2.7g, 10 mmole). This reaction mixture was stirred at 70°C for 18 hours. When the reaction was complete, the reaction mixture was concentrated under reduced pressure and the residue was treated with ether (30 ml). The ethereal solution was stood in the refrigerator (-20°C) for 18 hours. The resultant precipitate was collected by filtration and dried *in vacuo* at 30°C.

Yield ; 5.0g (94%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.80(3H, t, J = 8 Hz, CH_3), 1.12-1.22(16H, m, CH_2), 1.57(2H, m, CH_2), 3.57(2H, m, PCH_2), 7.65-7.82(15H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.1, 22.2, 23.2, 29.0. 29.2, 30.1, 30.3, 31.5, 117.7(d, J = 83 Hz, PCH_2), 128.0-135.0(C-aromatic).

Benzyl 2,3-di-O-benzyl-4-O-methyl-5-trans(1-undecene-1-yl)-α,β-D-xylopyranoside (40, α/β=1/1)

To the solution of phosphonium iodide **39** (390 mg, 0.75 mmole) in dry THF (12 ml) was added 2.06M n-BuLi in hexane (0.37 ml, 0.76 mmole) at -78°C. This solution was stirred at 0°C for 1 hour and cooled again to -78°C. Intermediate **38** (160 mg, 0.35 mmole) dissolved in dry THF (3 ml) was then added to the reaction mixture, which was then warmed up to room temperature and stirred for 18 hours after which time it was complete. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in dichloromethane (20 ml). The dichloromethane solution was washed with distilled water (10 ml) and dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated and the product was purified by flash column chromatography.

Yield ; 150 mg (74%). TLC ; R_F = 0.37(α), 0.43(β) (CH_2Cl_2).
^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 0.85(3H, t, J = 7 Hz, CH_3), 1.23-1.40(14H, m, CH_2), 2.12(2H, m, CH_2), 3.01(1H, dd, J = 10 and 10 Hz, H-4), 3.48(1H, dd, J = 10 and 3 Hz, H-2), 3.49(3H, s, OCH_3), 3.95(1H, dd, J = 10 and 10 Hz, H-3), 4.43(1H, dd, J = 10 and 10 Hz, H-5), 4.51-4.95(6H, m, H-benzyl), 4.77(1H, d, J = 3 Hz, H-1), 5.35(1H, ddt, J = 10, 10 and 1Hz, H-6(vinyl)), 5.71(1H, dt, J = 10 and 7 Hz, H-7(vinyl)), 7.25-7.41(15H, m, H-aromatic).
^{1}H-NMR of β-isomer (300 MHz, $CDCl_3$) δ 0.85(3H, t, J = 7 Hz, CH_3), 1.23-1.40(14H, m, CH_2), 2.15(2H, m, CH_2), 3.08(1H, dd, J = 10 and 10 Hz, H-4),

3.45(1H, dd, J = 10 and 10 Hz, H-2 or H-3), 3.49(3H, s, OCH_3), 3.53(1H, dd, J = 10 and 10 Hz, H-2 or H-3), 4.00(1H, dd, J = 10 and 10 Hz, H-5), 4.52(1H, d, J = 10 Hz, H-1), 4.57-4.96(6H, m, H-benzyl), 5.44(1H, ddt, J = 10, 10 and 1Hz, H-6(vinyl)), 5.73(1H, dt, J = 10 and 8 Hz, H-7(vinyl)), 7.25-7.40(15H, m, H-aromatic).

^{13}C-NMR of α-isomer (75.5 MHz, $CDCl_3$) δ 14.0, 22.5, 28.3, 29.5, 29.7, 32.0, 61.0, 66.5, 68.7, 73.2, 75.7, 79.5, 81.6, 84.6, 95.2, 126.5-139.0(C-aromatic and C-vinyl).

4-O-methyl-6-undecyl-α,β-D-xylose (41, α/β=1/1)

To the solution of **40** (120 mg, 0.20 mole) in MeOH (5 ml) was added 10% palladium in activated charcoal (250 mg, 0.24 mole). This suspension was stirred under hydrogen using hydrogen filled balloon attached to the reaction flask, at room temperature for 18 hours. When the reaction was complete, the reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was used for the subsequent reaction without further purification.

Yield ; 66 mg (100%). TLC ; R_F = 0.35 (CH_2Cl_2-MeOH : 10-1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88(3H, t, J = 6 Hz, CH_3), 1.25-1.80(20H, m, CH_2), 2.83(1H, dd, J = 9 and 9 Hz, H(α or β)-4), 2.89(1H, dd, J = 9 and 9 Hz, H(α or β)-4), 3.21(1H, m, H(β)-5), 3.32(1H, dd, J = 9 and 9 Hz, H(β)-2), 3.50-3.62(2H, m, H(α)-2 and H(b)-3), 3.58(3H, s, OCH_3), 3.72(1H, m, H(α)-5), 3.81(1H, dd, J = 9 and 9 Hz, H(α)-3), 4.52(1H, d, J = 9 Hz, H(β)-1), 5.20(1H, d, J = 3 Hz, H(α)-1).

(R)-3-Hydroxytetradecanoic acid (8)

To the suspension of triol **41** (66 mg, 0.21 mmole) in 0.1 M aqueous NaOH (4 ml, 0.4 mmole)solution was added 30% aqueous H_2O_2 solution (45 mg, 0.4 mmole). The reaction mixture was then stirred at 80°C for 48 hours. After cooling to room temperature, dichloromethane (10 ml) was added to the reaction mixture. 4% aqueous HCl solution was added to the bilayer mixture with vigorous stirring until the pH

of the aqueous layer was strongly acidic (pH < 2 on pH-paper). The organic layer was then isolated and concentrated. The product in the concentrate was identified by ^{1}H-NMR analysis.

Yield ; 60% (based on ^{1}H-NMR analysis).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.87(3H, t, J = 7 Hz, CH_3), 1.20-1.60(20H, m, CH_2), 2.47(1H, dd, J = 17 and 9 Hz, $COCH_2$), 2.58(1H, dd, J = 17 and 3 Hz, $COCH_2$), 4.04(1H, m, CHOH).

Methyl 4,6-O-(4-methoxy)benzylidene-α-D-glucopyranoside (42)

To a suspension of methyl-α-D-glucopyranose (60g, 0.31 mole) in dry DMF (100 ml) were added p-methoxybenzaldehyde (44.4g, 0.33 mole) and anhydrous p-TsOH (0.5g). After stirring at room temperature for 2 hours, toluene (200 ml) was added to the reaction mixture and evaporated at reduced pressure. This evaporation step with toluene was repeated three times. Anhydrous potassium carbonate (1.5g) was added to the reaction mixture which was subsequently concentrated by vacuum distillation. The residue was treated with a mixture (500 ml) of pet. ether and ether (1 : 1), and the precipitate formed was collected by filtration. The product was extracted from the filter cake with 1.2 liter of 5% methanolic dichloromethane. The extract was passed through a short silica gel (20g) column and concentrated.

Yield ; 46g (48%). TLC ; R_F = 0.30 (CH_2Cl_2-MeOH : 20-1).

^{1}H-NMR (300 MHz, DMSO-d^6) δ 3.30(3H, s, OCH_3), 3.30-3.37(2H, m, H-3 and H-6), 3.52-3.60(2H, m, H-2 and H-5), 3.65(1H, dd, J = 10 and 10 Hz, H-4), 3.74(3H, s, OCH_3), 4.12(1H, dd, J = 10 and 4 Hz, H-6), 5.13(1H, d, J = 4 Hz, H-1), 5.49(1H, s, H-benzyl), 6.90(2H, d, J = 10 Hz, H-aromatic), 7.35(2H, d, J = 10 Hz, H-aromatic).

Methyl 2,3-di-O-benzoyl-6-O-triphenylmethyl-α-D-glucopyranoside (43)

The procedure for the preparation of **43** was the same as that employed for the preparation of **25** from **23**.

Yield ; 57%. TLC ; R_F = 0.27 (CH_2Cl_2-pet. ether : 2-1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.42(3H, s, OCH3), 3.43-3.54(2H, m, H-4 and H-5), 3.85-3.94(2H, m, H-6), 5.13(1H, d, J = 3 Hz, H-1), 5.23(1H, dd, J = 10 and 3 Hz, H-2), 5.75(1H, dd, J = 10 and 10 Hz, H-3), 7.21-7.99(35H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 87.2(C-trityl), 96.9(C-1), 166.0(C-carbonyl), 167.0(C-carbonyl).

2,3-Di-O-benzoyl-1,4-di-O-methyl-6-bromo-6-deoxy-α-D-glucopyranoside (44)

To a solution of **29** (6.8g, 16.3 mmole) in dry THF (50 ml) was added tetrabromomethane (10.84g, 32.7 mmole) and triphenylphosphine (8.6g, 32.8 mmole). After stirring at room temperature for 16 hours, THF was evaporated under reduced pressure. The residue was treated with ether (250 ml) and the precipitate formed was filtered through celite. The filtrate was concentrated and the product was purified by flash column chromatography.

Yield ; 6.7g (86%). TLC ; R_F = 0.45 (CH_2Cl_2).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.41(3H, s, OCH_3), 3.48(3H, s, OCH_3), 3.58(1H, dd, J = 10 and 10 Hz, H-4), 3.68-3.77(2H, m, H-6), 3.91-3.98(1H, m, H-5), 5.11(1H, dd, J = 10 and 3 Hz, H-2), 5.13(1H, d, J = 3 Hz, H-1), 5.94(1H, m, H-3), 7.32-8.02(10H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 79.8(C-6), 97.0(C-1), 165.6(C-carbonyl), 165.9(C-carbonyl).

2,3-Di-O-benzoyl-6-deoxy-1,4-di-O-methyl-6-triphenylphosphino-α-D-glucopyranosyl bromide (45)

Compound **44** (6.8g, 14.2 mmole) and triphenylphosphine (3.72g, 14.2 mmole) were dissolved in dry DMF (30 ml). The solution was then stirred at 100°C for 2 days. After concentrating the reaction mixture by vacuum distillation, the residue was purified by flash column chromatography.

Yield ; 2.5g (24%). TLC ; R_F = 0.27 (CH_2Cl_2-MeOH : 10-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.53(3H, s, OCH_3), 3.62(3H, s, OCH_3), 3.71(1H, ddd, J = 18, 18 and 2 Hz, H-6), 4.00(1H, ddd, J = 10, 10 and 2 Hz, H-5), 4.12(1H, dd, J = 10 and 10 Hz, H-4), 4.86(1H, d, J = 3 Hz, H-1), 5.03(1H, dd, J = 10 and 3 Hz, H-2), 5.37(1H, ddd, J = 18, 10 and 10 Hz, H-6), 5.73(1H, dd, J = 10 and 10 Hz, H-3), 7.20-8.00(25H, m, H-aromatic). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 97.4(C-1), 118.3(d, J = 87 Hz, C-6), 162.3(C-carbonyl), 165.5(C-carbonyl).

1,2,3-Tri-O-benzyl-6-bromo-6-deoxy-4-O-methyl-α,β-D-glucopyranoside (46, α/β=1/1)

The procedure for the preparation of **46** was the same as that employed for the preparation of **44**.

Yield ; 71%. TLC ; R_F = 0.38 (CH_2Cl_2).

^{1}H-NMR of α-isomer (300 MHz, $CDCl_3$) δ 3.15(1H, dd, J = 10 and 10 Hz, H-4), 3.42(1H, dd, J = 10 and 4 Hz, H-2), 3.49(2H, d, J = 4 Hz, H-6), 3.52(3H, s, OCH_3), 3.68(1H, dt, J = 10 and 4 Hz, H-5), 3.88(1H, dd, J = 10 and 10 Hz, H-3), 4.44-4.92(6H, m, H-benzyl), 4.76(1H, d, J = 4 Hzz, H-1), 7.20-7.36(15H, m, H-aromatic). ^{13}C-NMR of α-isomer (75.5 MHz, $CDCl_3$) δ 33.5, 61.0, 69.1, 69.8, 73.0, 75.6, 79.8, 81.5, 81.6, 95.5, 127.5-138.7(C-aromatic).

N,N'-bis-tetradecyl-(2R,3R)-tartaric acid diamide (10)

To a solution of L-tartaric acid (7.0g, 46.6 mmole) in methanol (50 ml) were added p-TsOH (0.2g) and anhydrous magnesium sulfate (5g). After stirring at room temperature for 18 hours, sodium bicarbonate (1.0g) was added to the reaction mixture. All precipitate was filtered away using celite and the filtrate was concentrated under reduced pressure. The residue was redissolved and passed through a short silica gel column (CH_2Cl_2-MeOH : 20-1), the suspension was concentrated and the concentrate was treated with 1-tetradecylamine (24g, 0.11 mole) and MeOH (150 ml) at 70°C for 20 hours. After cooling the reaction mixture to room temperature, the precipitated product was collected by filtration. The filter cake was washed twice with MeOH (50 ml) and dried *in vacuo*.

Yield ; 25.3g (97%). TLC ; R_F = 0.32 (CH_2Cl_2-MeOH : 20-1). (For NMR analysis, the product was acetylated in acetic anhydride and pyridine due to its poor solubility in the usual NMR solvents. The diacetate of the product was then analyzed by NMR spectroscopy.) ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 7 Hz, CH_3), 1.20-1.48(48H, m, CH_2), 2.13(6H, s, CH_3), 3.10-3.40(4H, m, NCH_2), 5.56(2H, s, CH). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 39.8($NHCH_2$), 72.2(CH), 166.1(C-carbonyl), 169.5(C-carbonyl).

N,N'-bis-tetradecyl-2,3-O-isopropylidene-(2R,3R)-tartaric acid diamide (47)

To a suspension of **10** (16g, 28.6 mmole) in 2,2-dimethoxypropane (150 ml) was added p-TsOH (0.2g). After stirring at room temperature for 4 hours, 50 ml of the solvent was removed from the reaction mixture by fractional distillation, and the residual solution was cooled to room temperature and treated with anhydrous potassium carbonate (1.0g). After evaporating all 2,2-dimethoxypropane from the reaction mixture, the residue was treated with distilled water (100 ml) and the precipitate formed was collected

by filtration. The filter cake was dissolved in ether (200 ml) and the resultant ethereal solution was filtered through celite to remove a small amount of precipitate in the solution. The filtrate was dried over anhydrous magnesium sulfate and concentrated. The residue was used for the next reaction without further purification.

Yield ; 17.6g (100%). TLC ; R_F = 0.50 (CH_2Cl_2-MeOH : 40-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 6 Hz, CH_3), 1.21-1.55(48H, m, CH_2), 1.47(6H, s, $C(CH_3)_2$), 3.27(4H, td, J = 6 and 6 Hz, NCH_2), 4.48(2H, s, CH).

N,N'-bis-tetradecyl-1,4-diamino-(2S,3S)-dihydroxybutane isopropylidene acetal (48)

To a solution of **47** (9.4g, 15.6 mmole) in dry ether (50 ml) was added lithium aluminium hydride (2.0g, 52.6 mmole). After refluxing for 16 hours, ethylacetate (3 ml) was added to the reaction mixture to quench the reaction. The reaction mixture was treated with additional ether (400 ml) and 20% aqueous NaOH solution (200 ml) at room temperature for 20 hours. The organic layer was isolated and dried over anhydrous magnesium sulfate, filtered and the filtrate concentrated. The concentrate was treated with pet. ether (200 ml). The precipitate formed was removed by filtration and the filtrate was concentrated. The concentrate was subjected to flash column chromatography (CH_2Cl_2-MeOH-$(Et)_2NH$: 40-2-1).

Yield ; 6.9g (77%). TLC ; R_F = 0.20 (CH_2Cl_2-MeOH-$(Et)_2NH$: 40-2-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 7 Hz, CH_3), 1.20-1.50(48H, m, CH_2), 1.36(6H, s, CH_3), 2.61(4H, t, J = 6 Hz, NCH_2), 2.73(2H, dd, J = 10 and 4 Hz, CH_2N), 2.82(2H, dd, J = 10 and 6 Hz, CH_2N), 3.82-3.90(2H, m, CH). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 50.0(CH_2NH), 51.9(CH_2NH), 79.0(CH), 109.0(OCO).

N,N'-bis-tetradecyl-1,4-dimethylamino-(2S,3S)-dihydroxybutane isopropylidene acetal di-methylsulfate salt (TM3 $2CH_3SO_4^-$)

To a solution of **48** (8.7g, 15.0 mmole) in dichloromethane (40 ml) were added dimethyl sulfate (7.2g, 57.1 mmole), anhydrous potassium carbonate (9.46g, 68.6 mmole) and TBAB (0.1g). After stirring at room temperature for 18 hours, the reaction mixture was treated with distilled water (100 ml) for 18 hours. The organic layer was isolated and dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated and the residue was dried *in vacuo*.

Yield ; 4.2g (33%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.80(6H, t, J = 7 Hz, CH_3), 1.15-1.30(44H, m, CH_2), 1.40(6H, s, CH_3), 1.60-1.80(4H, m, CH_2), 3.16(6H, s, NCH_3), 3.18(6H, s, NCH_3), 3.30-3.40(4H, m, NCH_2), 3.60(6H, s, $CH_3SO_4^-$), 3.77(2H, dd, J = 13 and 7 Hz, CH_2N), 3.98(2H, dd, J = 13 and 1 Hz, CH_2N), 4.42(2H, m, CH). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 51.5, 54.2, 58.5, 64.2, 66.0, 72.6(CH), 112.8(OCO).

N,N'-bis-tetradecyl-2,3-O-methylene-(2R,3R)-tartaric acid diamide (49)

To the suspension of **10** (1.32g, 2.35 mmole) in dichloromethane (50 ml) were added anhydrous potassium carbonate (2.2g, 16 mmole) and 18-crown-6 (0.27g, 1.0 mmole). To this solution was added diiodomethane (1.5g, 5.6 mmole) at room temperature. The reaction mixture was refluxed for 18 hours. When the reaction was complete, the reaction mixture was cooled to room temperature and treated with distilled water (50 ml). The organic layer was isolated and dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated and the residue was purified by flash column chromatography.

Yield ; 1.2g (89%). TLC ; R_F = 0.50 (CH_2Cl_2-MeOH : 30-1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 6 Hz, CH_3), 1.20-1.55(48H, m, CH_2), 3.26(4H,m, NCH_2), 4.44(2H, s, CH), 5.11(2H, s, OCH_2O). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 77.5(CH), 96.1(OCH_2O), 169.0(C-carbonyl).

N,N'-bis-tetradecyl-1,4-diamino-(2S,3S)-dihydroxybutane methylene acetal (50)

The procedure for the preparation of **50** was the same as that employed for the preparation of **48**.

Yield ; 79%. TLC ; R_F = 0.20 (CH_2Cl_2-MeOH-$(Et)_2NH$: 40-2-1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 6 Hz, CH_3), 1.20-1.55(48H, m, CH_2), 2.62(4H, t, J = 6 Hz, NCH_2), 2.75(2H, dd, J = 12 and 3 Hz, CH_2N), 2.83(2H, dd, J = 12 and 8 Hz, CH_2N), 3.82(2H, m, CH), 4.99(2H, s, OCH_2O). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 49.8(CH_2NH), 51.0(CH_2NH), 78.5(CH), 94.0(OCO).

N,N'-bis-tetradecyl-1,4-dimethylamino-(2S,3S)-dihydroxybutane methylene acetal di-methylsulfate salt (TM4 $2CH_3SO_4^-$)

The procedure for the preparation of **TM4** was the same as that employed for the preparation of **TM3**.

Yield ; 45%. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85(6H, t, J = 7 Hz, CH_3), 1.20-1.35(44H, m, CH_2), 1.65-1.83(4H, m, CH_2), 3.23(6H, s, NCH_3), 3.25(6H, s, NCH_3), 3.42(4H, t, J = 6 Hz, NCH_2), 3.68(6H, s, $CH_3SO_4^-$), 3.85(2H, dd, J = 12 and 7 Hz, CH_2N), 4.15(2H, dd, J = 12 and 1 Hz, CH_2N), 4.49(2H, m, CH), 5.15(2H, s, OCH_2O). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ 51.2, 51.3, 54.5, 64.0, 66.5, 73.0(CH), 112.8(OCO).

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