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Part 1. Halichondrin B: Synthesis of an H-ring intermediate. Part 2. Levuglandin-protein adducts: Synthesis of an antigen for immunoassay

Kim, Seokchan, Ph.D.

Case Western Reserve University, 1992

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PART 1. HALICHONDRIN B: SYNTHESIS OF AN H-RING INTERMEDIATE

PART 2. LEVUGLANDIN-PROTEIN ADDUCTS: SYNTHESIS OF AN ANTIGEN FOR IMMUNOASSAY

by

SEOKCHAN KIM

Submitted in partial fulfillmemt of the requirements
for the Degree of Doctor of Philosophy

Thesis Advisor: Dr. Robert Gerd Salomon

Department of Chemistry
CASE WESTERN RESERVE UNIVERSITY
January 1992

CASE WESTERN RESERVE UNIVERSITY

GRADUATE STUDIES

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PART 1. HALICHONDRIN B: SYNTHESIS OF AN H-RING INTERMEDIATE
PART 2. LEVUGLANDIN-PROTEIN ADDUCTS: SYNTHESIS OF AN ANTIGEN FOR IMMUNOASSAY

Abstract

by

SEOKCHAN KIM

PART 1. HALICHONDRIN B: SYNTHESIS OF AN H-RING INTERMEDIATE

Halichondrins, a family of macrolide polyethers, were isolated from a sponge, *Halichondria okadai Kadota*, in $4x10^{-7}$ to $5x10^{-6}$% yield. Halichondrin B, the biologically most active member of this new family, is a remarkably effective antitumor agent *in vivo*. The 32 asymmetric carbons in halichondrin B allow more than four billion stereoisomers. Therefore, a practical total synthesis must be highly stereoselective. A practical synthesis of H-ring intermediate **10** which incorporates carbon 27 to 35 of the halichondrin skeleton from D-glucose requires stereocontrolled homologation at C-6 (glucose numbering), epimerization at C-5, replacement of a hydroxyl at C-3 with a Me group, and C-glycosidation at the anomeric center.

Introduction of the Me group at C-3 position was achieved by regioselective opening of the epoxide **13** by axial attack of MeMgCl followed by oxidation of the 2-hydroxyl in **14** and epimerization of the 3-

methyl to the desired configuration. Inversion of the configuration at C-5 (glucose numbering) and elongation of the side chain were accomplished by a C-C bond cleavage-reformation sequence exploiting the pyranose to furanose interconversion which accompanies ketalization of glucose with acetone. Oxidative cleavage of vicinal diol **20** with periodate destroys the center of incorrect chirality at position 5. Entirely stereoselective (>99:1) generation of the requisite configuration at C-5 was achieved by condensation of aldehyde **21** with enol ether **41** in the presence of $TiCl_4$. Hydrolysis and deketalization of **42a** is accompanied by furanose to pyranose interconversion and lactonization to provide cis lactone **26**. Wittig olefination of **26** at anomeric center and heterocyclization of the resulting α,β-unsaturated ester **45** deliver the H-ring intermediate **10**.

PART 2. LEVUGLANDIN-PROTEIN ADDUCTS: SYNTHESIS OF AN ANTIGEN FOR IMMUNOASSAY

Secoprostanoic acid levulinaldehyde derivatives, which we named levuglandins (LGs), are generated along with PGs by rearrangement of PGH_2 under the aqueous environment of its biosynthesis. Our goal is to determine the extent and distribution of LG occurrence *in vivo*. Previous attempts to detect LGs in biological systems failed due to the complicated covalent adduct formation with proteins.

A pyrazole isostere of LGE_2-derived pyrrole **141** was designed and synthesized. Structural assignment of pyrazole isomers, generated in the reaction of 1,3-diketone **149** with 6-

hydroxylhexylhydrazine **150**, was achieved by COSY and NOESY experiments. Coupling of pyrazole isostere **141** with poly-L-lysine was achieved using sodium cyanoborohydride in THF/H_2O to provide an antigen for immunoassay. ^1H NMR analysis showed the presence of 1 pyrazole for every 2 lysyl residues in the modified protein. Antibodies against pyrazole was raised by immunizing rabbits with isostere-protein conjugate **142**. We used the BSA conjugate as a coating reagent for ELISA.

ACKNOWLEDGEMENTS

I would like to thank Professor Robert G. Salomon for his encourgment and guidance throughout this project. His dedication and enthusiasm for the pursuit of knowledge of chemistry had a great influence on me.

I would like to thank Professor Vincent M. Monnier for his advice for doing levuglandin project. I would also like to thank Professor Peter L. Rinaldi for his helping in the structural characterization of pyrazole isomers.

I would also like to thank all the members of the Salomon group for kind words of advice and friendship throught my years at CWRU.

I am greatful for my wife for her support and patience.

Finally, I dedicate this thesis to my wonderful parents for their encouragement and support during the course of my education.

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List of Abbreviations and Acronyms

Abbreviation or Acronym	Equivalent
AA	Arachidonic Acid
Ac	Acetate
$AnLGD_2$	Anhydro Levuglandin D_2
$AnLGE_2$	Anhydro Levuglandin E_2
aq	aqueous
APT	Attached Proton Test
Bn	Benzyl
Boc	tertiary-Butoxycarbonyl
BSA	Bovine Serum Albumin
t-Bu	tertiary-Butyl
COSY	Correlated Spectroscopy
CD_3OD	Methanol-d_4
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance
DCC	N,N-Dicyclohexylcarbodiimide
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	Diethylazodicarboxylate
DIBAL-H	Diisobutylaluminum Hydride
DMAP	4-Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulfoxide

Δ^9-LGD_2	Δ-9-Levuglandin D_2
Δ^9-LGE_2	Δ-9-Levuglandin E_2
ELISA	Enzyme-Linked Immuno-Sorbent Assay
Et	Ethyl
EtOAc	Ethyl Acetate
HHT	12-Hydroxyheptadeca-5(Z),8(E),10(E)-trienoic Acid
HMPA	Hexamethylphosphoric Triamide
^{1}H NMR	Hydrogen Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
IR	Infrared
LGD_2	Levuglandin D_2
LGE_2	Levuglandin E_2
MBn	p-Methoxybenzyl
MDA	Malonaldehyde
Me	Methyl
MEM	2-Methoxyethoxymethyl
MS	Mass Spectrum
Ms	Methanesulfonate
NMO	N-Methylmorpholine-N-oxide
NOE	Nuclear Overhauser Effect

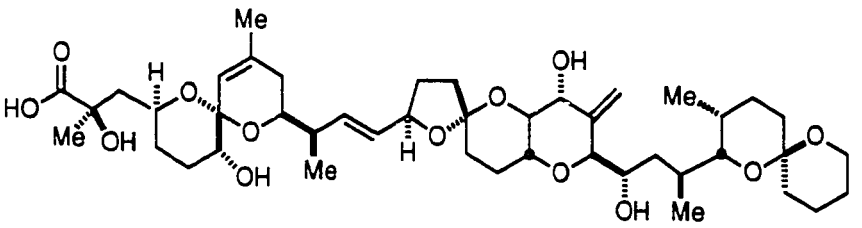
NOESY	Nuclear Overhauser and Exchange Spectroscopy
PBS	Phosphate Buffered Saline
PCC	Pyridinium Chlorochromate
PDC	Pyridinium Dichromate
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
PGI_2	Prostaglandin I_2
Ph	Phenyl
i-PrOH	Isopropanol
PPTS	Pyridinium p-Toluenesulfonate
RPM	Revolutions Per Minute
TBAF	tetra-n-Butylammonium fluoride
TBDMS	t-Butyldimethylsilyl
TES	Triethylsilyl
TfOH	Trifluoromethanesulfonic Acid
TFA	Trifluoroacetic Acid
TFAA	Trifluoroacetic Anhydride
THF	Tetrahydrofuran
THP	Tetrahydropyran
TLC	Thin Layer Chromatography

TMAL	Tetramethylammoniumlevlinate
TMS	Trimethylsilyl
TPAP	tetra-n-Propylammonium Perruthenate
Trityl	Triphenylmethyl
Ts	p-Toluenesulfonate
TX	Thrombooane
UV	Ultraviolet

PART 1. HALICHONDRIN B: SYNTHESIS OF AN H-RING INTERMEDIATE

Introduction

Marine sources continue to provide a wide variety of novel physiologically active substances. A black sponge, *Halichondria Okadai Kadota*, commonly found along the pacific coast of Japan, contains a tiny amount of potently cytotoxic compounds. A macrolide polyether **1**, which was named okadaic acid, was isolated in 10^{-4}% yield.[1] Although **1** is a potent inhibitor of cancer cell growth *in vitro*, it

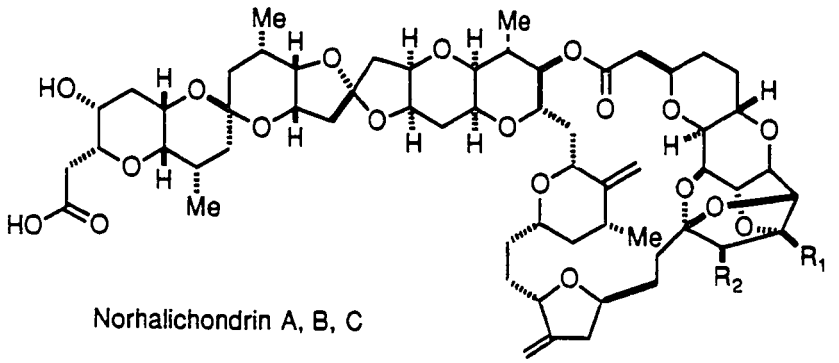


Okadaic Acid (**1**)

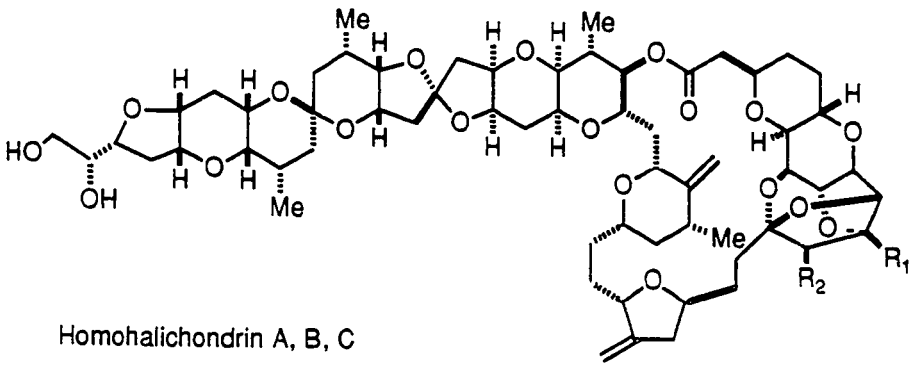
is toxic at doses of ≤ 0.12 mg kg (ip) and showed no tumor inhibition at subtoxic doses when tested *in vivo* against P-388 lymphocytic leukemia. This suggests that okadaic acid was not responsible for the *in vivo* antitumor activity observed for crude extracts of Halichondria okadai.[1c]

Using this unexplained *in vivo* antitumor activity against B-16 melanoma cells as a guide, Hirata and Uemura isolated a new class of natural products with extraordinary *in vivo* antitumor activity which they named halichondrins.[1] Their isolation procedure began with 600 kg of the sponge, which was crushed, extracted with methanol, and filtered. After a series of fractional extractions, followed by careful serial column chromatograpic steps, they were able to achieve the separation and purification of eight halichondrin constituents (Figure

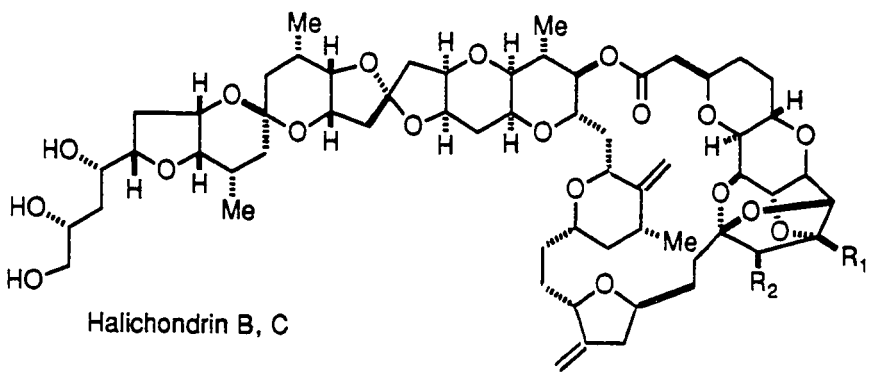
Figure 1



Norhalichondrin A, B, C



Homohalichondrin A, B, C



Halichondrin B, C

A $R_1 = R_2 = OH$

B $R_1 = R_2 = H$

C $R_1 = OH$, $R_2 = H$

1) in milligram quantities, in $4x10^{-7}$ to $5x10^{-6}$% yield.[1c] The halichondrins were tested for cytotoxicity against B-16 melanoma cells (Table 1).[1c]

Table 1. Cytotoxicity against B-16 melanoma cell

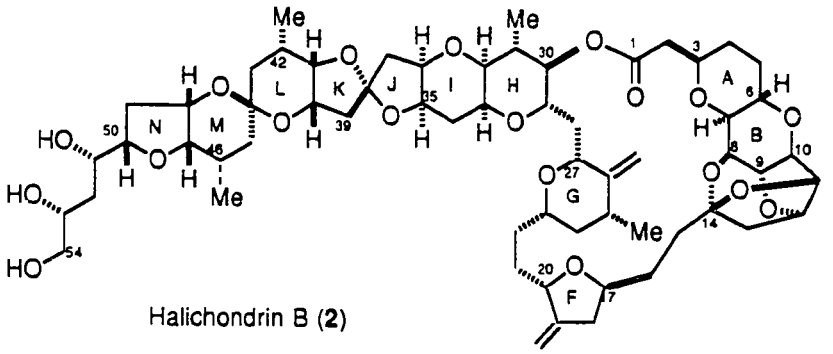
sample	IC_{50} (ng/ml)[a]
Halichondrin B	0.093
Norhalichondrin A	5.2
Homohalichondrin A	0.26
Halichondrin C	0.35
Homohalichondrin B	0.10

[a] Inhibition coefficient

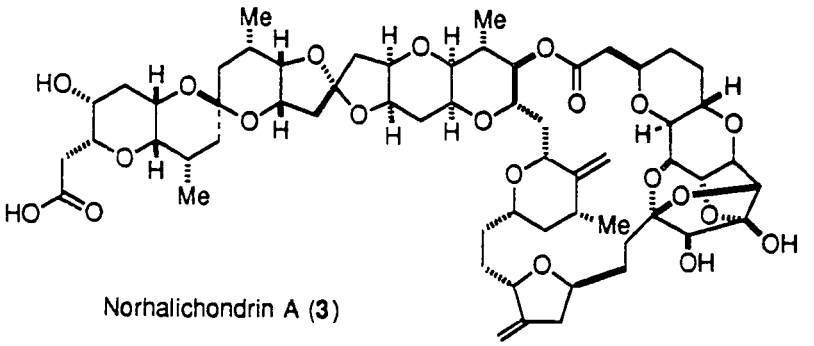
The component that they named halichondrin B (**2**) was the most active. In addition to its superior activity against B-16 melanoma cells, halichondrin B also had a lower acute toxicity than norhalichondrin A (**3**). As such, they selected halichondrin B for *in vivo* tests against a variety of tumor cell lines. Low doses of halichondrin B resulted in a significant improvement in the survival times of mice. Also halichondrin B (**2**) is especially intersting because it is a remarkably effective antitumor agent *in vivo*. A few doses of 10 μg/kg provide T/C >200 against B-16 melanoma and T/C >300 against P-388 leukemia in mice. Unfortunately, further biological evalulation of halichondrin B (**2**) was prevented by lack of material.

Hirata and Uemura[1c] were unable to determine the structure of halichondrin B (**2**) via X-ray analysis. However, in 1985 they were able to determine unambiguously the structure of norhalichondrin A (**3**) through an X-ray crystallographic analysis of its p-bromophenacyl

ester.[1b] Their crystal structure showed a compound rich in interesting and challenging structural features for a synthetic organic



Halichondrin B (**2**)



Norhalichondrin A (**3**)

chemist. It contains a novel polycyclic ring system from C.1-C.15, two exocyclic double bonds, and a macrocyclic ring system. Using the nonemperical dibenzoate chirality method,[1c] in conjuction with anomalous X-ray dispersion, Hirata and Uemura assigned the absolute stereochemistry of norhalichondrin A (**3**).[1b] Structural characterization of the other halichondrins, including halichondrin B, was accomplished by carefully comparing the NMR, mass, IR spectra with those of **3** as well as NMR COSY and decoupling experiments. Evaluation of their biological activity would be facilitated

by a total synthesis. Although any member of the halichondrin family would be an interesting synthetic challenge, we chose halichondrin B (**2**) as our target because it is the most biologically active of the halichondrins.

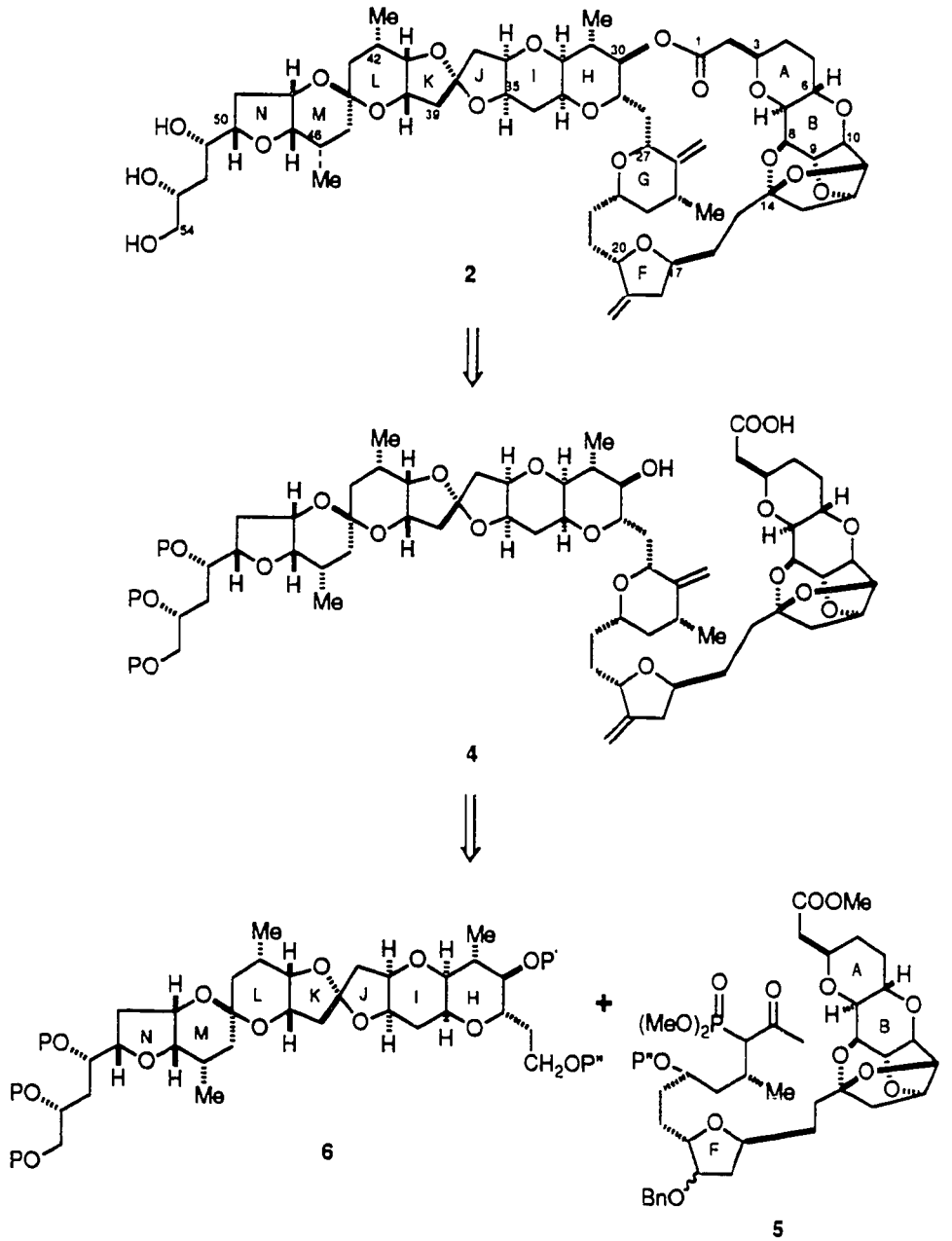
Results and Discussion

1. Retrosynthetic Analysis

Halichondrin B (**2**) is a structurally and functionally complex molecule. The 32 asymmetric carbons in **2** allow more than four billion streoisomers. These asymmetric carbons are located in several regions of the molecule which are apparently insulated from one another. It would be most difficult to generate a desired configuration within one region under a stereocontrolling influence of chirality already present in one of the other sectors. Fortunately most of the functionality of **2** is present in relatively unreactive ether and ketal linkages.

Our overall strategy for construction of halichondrin B (**2**) envisions macrolactonization of a hydroxy acid precursor **4** which will be generated by union of the requisite enantiomers of three main building blocks, **5**, **9**, and **10**, containing respectively the ABCDEF, H, and KLMN ring systems (Figure 2). Each of these building blocks derives absolute configurational information from inexpensive sugar precursors: D-glucose and D-ribose for **5**, D-glucose for **10**, and D-mannitol for **9** as optically active carbon sources. Generation of the G and IJ rings will be accomplished during conjunction of these three fragments. Union of **8** with **9** and creation of the I and J rings will provide an intermediate **7** incorporating the HIJKLMN ring system. In the final steps of the synthesis, this HIJKLMN ring intermediate **6** will be joined with **5** to give **4**.

Figure 2

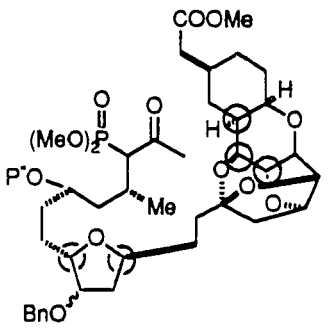


2

4

6

5



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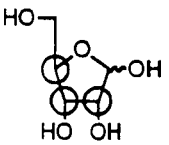
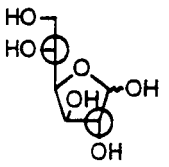
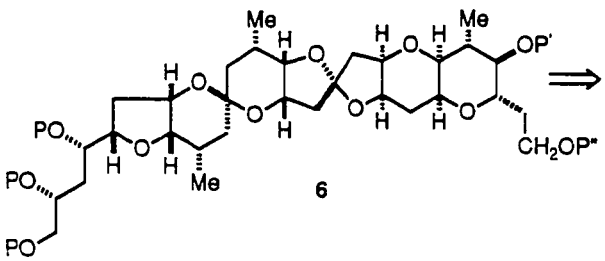


D- ribose



D- glucose



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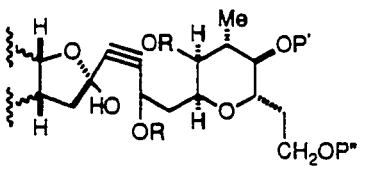

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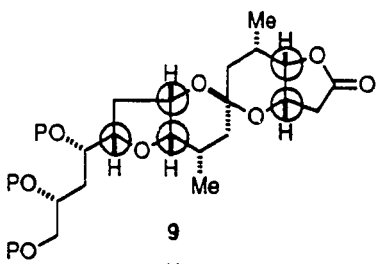

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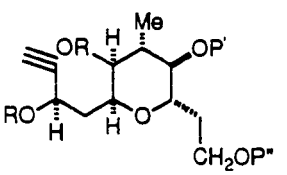
+



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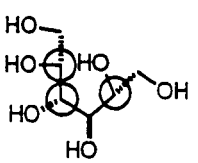
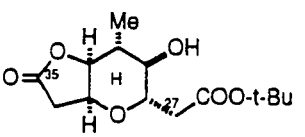
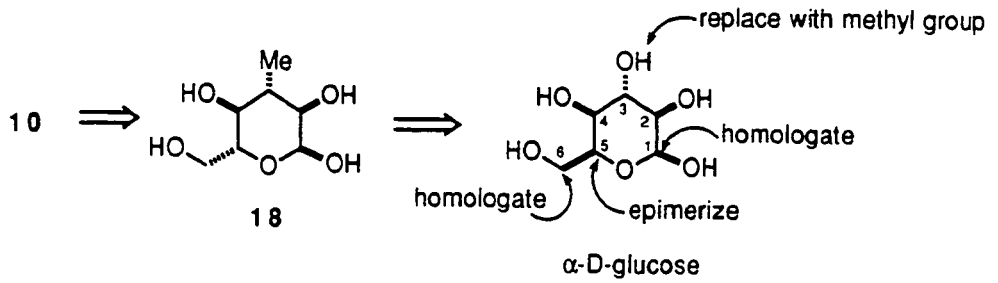


D-manitol



10



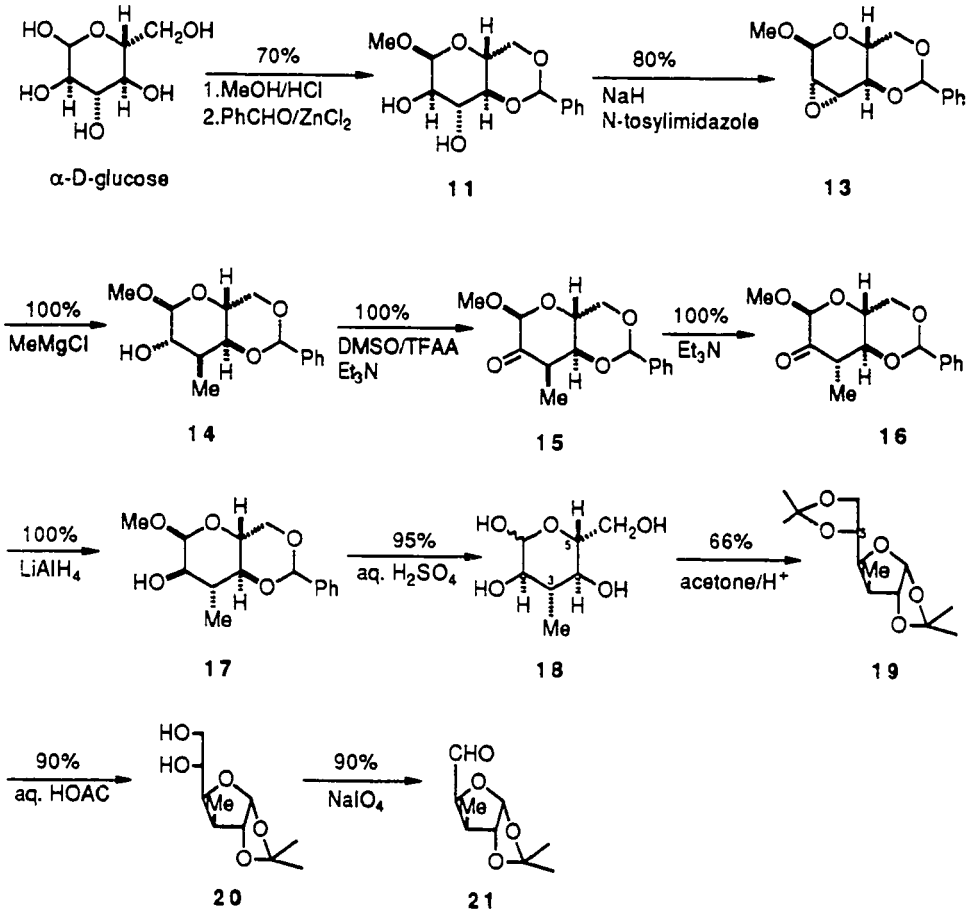
Our strategy for the H-ring pyran intermediate **10**, which incorporates carbons 27 to 35 of the halichondrin skeleton, envisioned preparation via the known 3-deoxy-3-C-methyl-α-D-glucose (**18**)[2] which is readily available in gram quantities from D-glucose. A practical synthesis of H-ring intermediate **10** from D-glucose requires stereocontrolled homologation at C-6 (glucose numbering), epimerization at C-5, replacement of a hydroxyl at C-3 with a Me group, and C-glycosidation at the anomeric center.

2. H-Ring Pyran Intermediate from D-Glucose

Our construction of 3-deoxy-3-C-methyl-α-D-glucose (**18**) are outlined in Scheme I. Introduction of a C-Me group at C-3 (glucose numbering) was intiated by selective masking of the anomeric center with a methoxy group and the hydroxyl groups at positions 4 and 6 with a benzlidene acetal.[3] The remaining trans hydroxyls at positions 2 and 3 were converted to an epoxide **13** by selective activation of the 2-hydroxyl in **11** using N-tosylimidazole (**12**) and excess sodium hydride (2.1 equiv) in dry DMF, and cyclization.[4] Reaction of the key epoxy-mannoside intermediate **13** with a large excess of MeMgCl (20 equiv) for 2 weeks under reflux furnished regiospecifically 3-deoxy-3-C-methyl glucose derivative **14**. Axial attack of MeMgCl on the epoxide gave the expected trans-diaxial product. The configuration of this center (C-3 in glucose) was opposite to that required for the H-ring of halichondrin B (C-31 halichondrin numbering). To accomplish epimerization of this center, the 2-hydroxyl group in **14** was converted in quantitative yield to a ketone carbonyl in **15** by Swern oxidation, without any noticeable epimerization at position 3. Subsequent treatment of this ketone with base, triethylamine, in dry DMF for 36 h delivered the thermodynamically most stable equatorial epimer, C-methyl ketone **16** (>99:1), as a crystalline material. Stereoselective reduction of ketone **16** with $LiAlH_4$ in dry ether gave the equatorial alcohol **17** in almost quantitative yield. Finally, hydrolysis of the masking ketals with 2.5% aqueous sulfuric acid at 110 °C for 2 h provided 3-deoxy-3-C-methyl-α-D-glucose (**18**) as a very hygroscopic

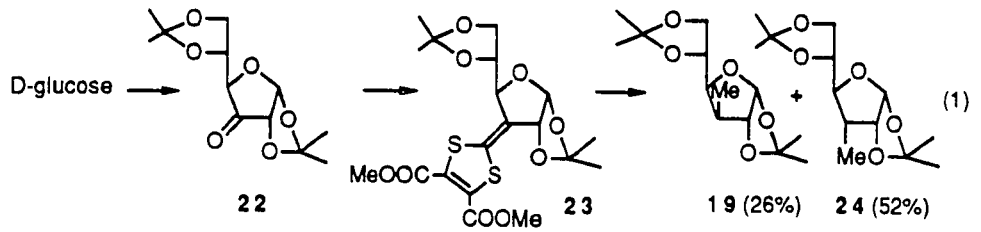
syrup. This sugar **18** is thus available in gram quantities via a nearly quantitative route from the epoxide **13**.

Scheme I



Inversion of the configuration at position 5 in **18** and elongation of the side chain was achieved by a C-C bond cleavage-reformation sequence exploiting the pyranose to furanose interconversion. The glucopyranose derivative **18** was converted to a glucofuranose derivative **19** by ketalization with acetone in the presence of Lewis acid,

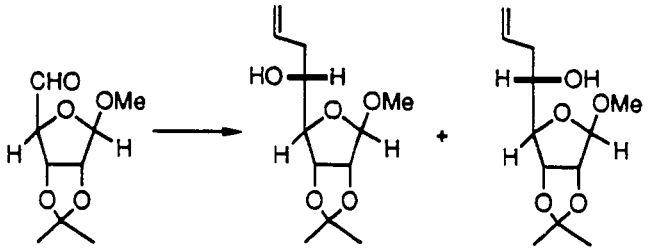
$ZnCl_2$.[5] Diketal **19** was readily monodeketalyzed[7] to provide **20** in 82% yield by treatment with 60% HOAc in H_2O at 34 °C for 5 h.



Even though a short synthesis of this ketalized intermediate **19** from D-glucose was known (Equation 1).[6] Thus, condensation of **22** with CS_2 and dimethyl acetylenedicarboxylate to produce **23** is induced by tributylphosphine. However, the reductive desulfurization of **23** with Raney nickel is nonstereoselective producing twice as much of the epimer **24** as the desired product **19**. Therefore we constructed **19** by a longer route which nevertheless is more efficient (many virtually quantitative steps) and suitable for preparing large quantities of this H-ring precursor.

Oxidative cleavage of the vicinal diol in **20** with periodate destroys the center of incorrect chirality at position 5. A similar process was employed previously to prepare the 3-deoxy glucose analog of **20**, i.e. with Me replaced by H.[8]

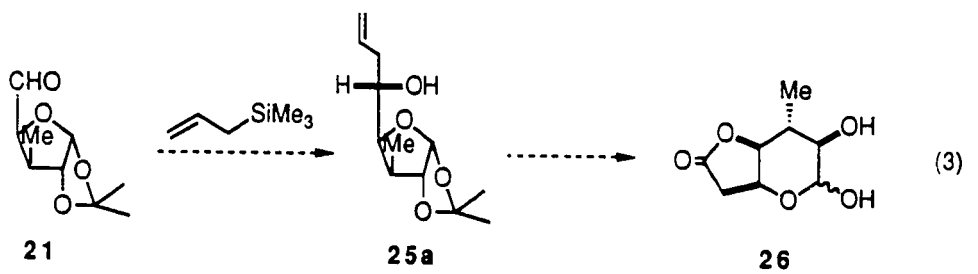
Our original plan for stereoselective generation of the L-configuration at C-5 (glucose numbering) was to use the chelation-controlled titanium(IV) chloride catalyzed allylation introduced by Danishefsky (Equation 2)[9] and subsequent acid-catalysed rearrangement of the furanoside **25a** to the thermodanamically



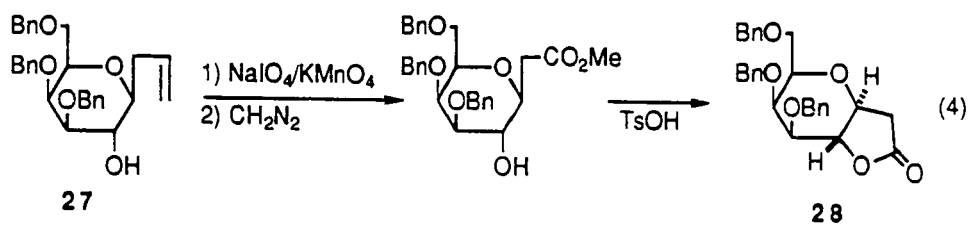
(2)

Nucleophile			
MgX	(70%)	2 :	1
$SiMe_3$ / BF_3OEt_2	(80%)	90 :	1
$SiMe_3$ / $TiCl_4$	(89%)	1 :	20

favored pyranoside, oxidative cleavage of the terminal C=C bond, and lactonization to provide the cis lactone **26** (Equation 3). Similar steps were employed previously to prepare a trans lactone **28** from the allylated sugar derivative **27** (Equation 4).[10]



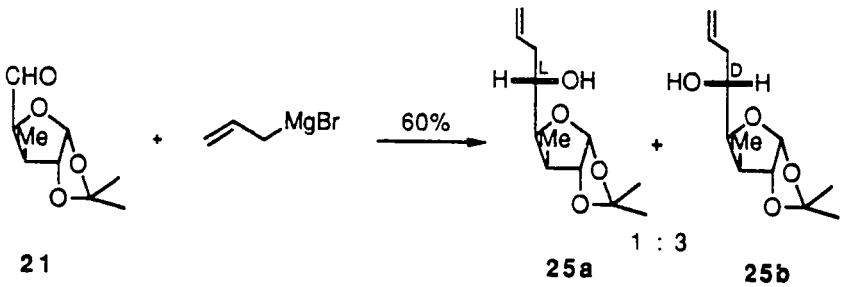
21 **25a** **26** (3)



27 **28** (4)

To attempt the abovementioned strategy, we treated aldehyde **21** with allyltrimethylsilane in the presence of titanium(IV) chloride.

Unfortunately all the aldehyde **21** decomposed. Further attempts with varying reaction conditions gave the same results. This approach was not pursued further. As an alternative approach, we planned to use allylmagnesium bromide instead of allyltrimethylsilane. Condensation of **21** with the Grignard reagent delivered two isomers **25a** and **25b** in 60% isolated yied (Equation 5). After HPLC purification,



(5)

21 **25a** **25b**

the ratio of the desired isomer to its epimer was 1:3, assuming that attack by the Grignard reagent on aldehyde **21** occurred preferentially at the si-face (Felkin-Cram products).[11] As expected by analogy with equation 2[9], the reaction of allylmagnesium bromide with aldehyde **21** exhibted poor stereoselectivity. A D-configuration is presumed for the major isomer **25b** in analogy with equation 2. Because of low yield and poor diastereoselectivity, we moved to third a approach, involving a direct aldol condensation. Addition of the lithium enolate of t-butyl acetate[12] to aldehyde **21** at -78 °C followed by quenching with 20% hydrochloric acid gave aldol products **29a** and **29b** in 87% isolated yield. A high diastereoselectivity favoring **29b** over **29a** (>99:1) was observed (Equation 6). The major isomer **29b** was recrystallized from ethyl acetate and hexane. The structure of **29b** was firmly established by X-ray crystallographic analysis,[13] which showed the D-configuration at

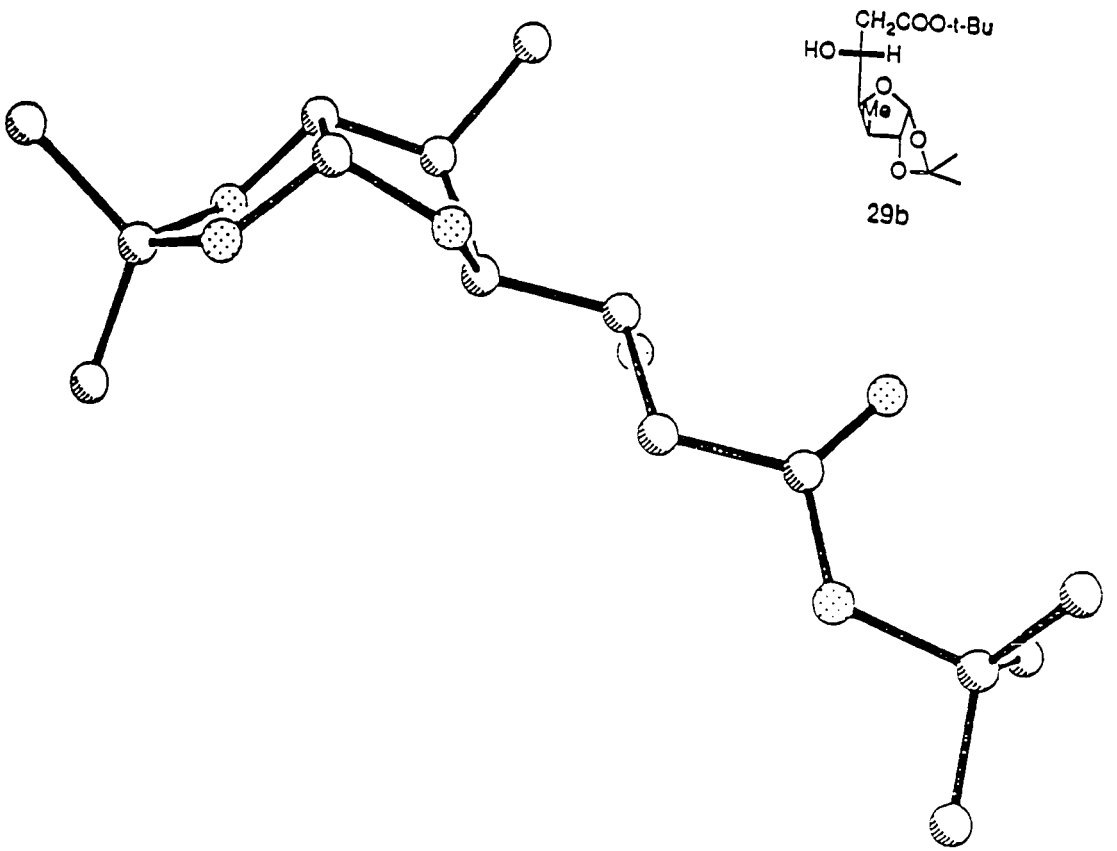
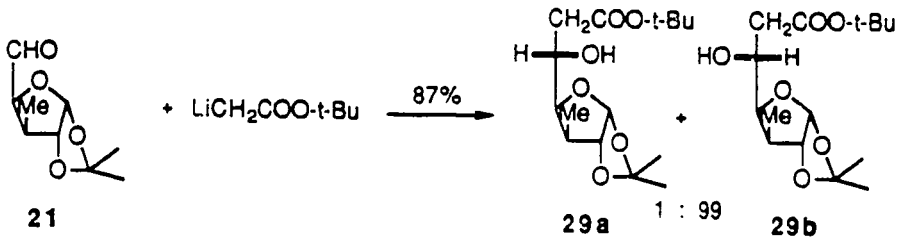


Figure 3. X-ray structure of **29b**.

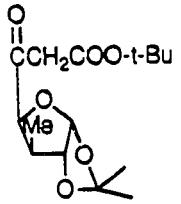


(6)

C-5. We rationalize this selectivity as follows. Without chelation, addition of any nucleophile to aldehyde **21** mostly occurs from the si-face which is less hindered (Felkin-Cram products). This si-face addition delivers the undesired D-configuration in **29b**. To produce the requisite L-configuration at C-5 (glucose numbering), the si-face must be blocked by chelation. We felt that a Reformatsky reaction[14] would provide the right aldol product **29a**, if zinc would chelate the carbonyl and ring oxygens. Unfortunately, the reaction of aldehyde **21** with zinc and t-butyl α-bromoacetate, again showed high diastereoselectivity favoring the wrong isomer **29b** over **29a** (>99 :1) in 65% yield.

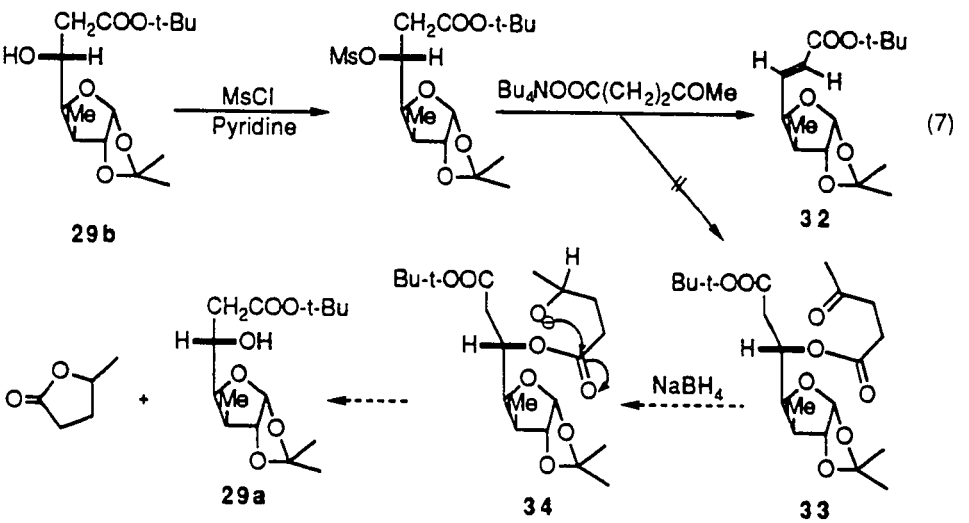
At this juncture we explored inversion of the C-5 center in the wrong isomer **29b**. Swern oxidation of **29b** (DMSO, TFAA, Et_3N, -78 °C) or PCC oxidation afforded the keto-ester **30** in almost quantitative yield.

Table 2. Reduction of **30** with various reduing agents

entry	sample	reducing agents[a]	yield(%)[c]	ratio (**29a/29b**)
1		$NaBH_4$	90	1:5
2		DIBAL-H	75	1:4
3		$Zn(BH_4)_2$	85	1:3.8
4		$NaBH_3CN$[b]	69	1:5.5
5		BH_3 / (+)-α-pinene	58	1:6.8
6	**30**	BH_3 / (-)-α-pinene	60	1:4.3
7		K-Selectride	75	1:4.4

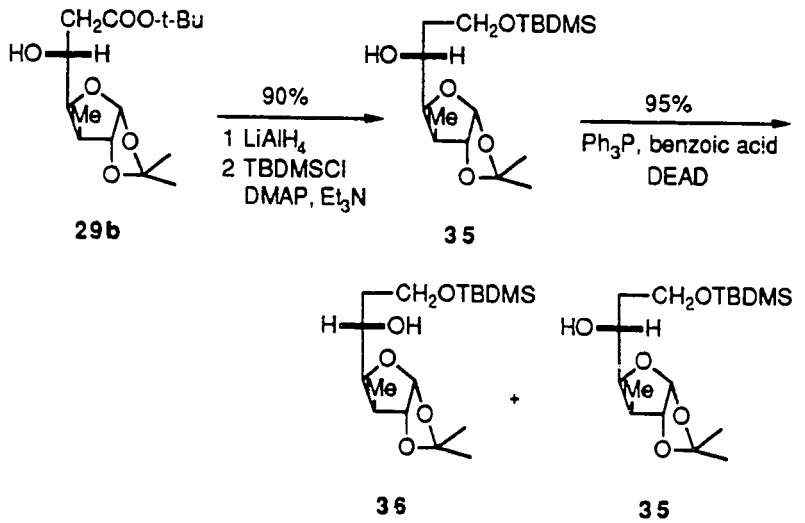
[a]1 equiv [b]acidic condition [c]based on consumed **30**

Reduction of **30** with various reducing agents gave unfavorable stereoselectivities (Table 2). Having these unsuccesful results with oxidation-reduction sequences, we tried another inversion method. Activation of the C-5 hydroxyl group with methylsulfonyl chloride-pyridine and subsequent treatment with tetramethyl-amoniumlevulinate (TMAL) in DME delivered the trans-α,β-unsaturated ester **32** in 80% islated yield owing to elimination rather than the desired substitution (Equation 7).



Our plan for inversion required that nucleophilic substitution by TMAL occurs at the activated C-5 center to yield an S_N2 adduct **33** with inversion at C-5. Sodium borohydride reduction would then form intermediate **34** which will liberate a γ-lactone and inverted product **29b**. But for our substrate TMAL acted as a base rather than a nucleophile. This preference is understandable in terms of the stabilization provided to the ester group by conjugation.

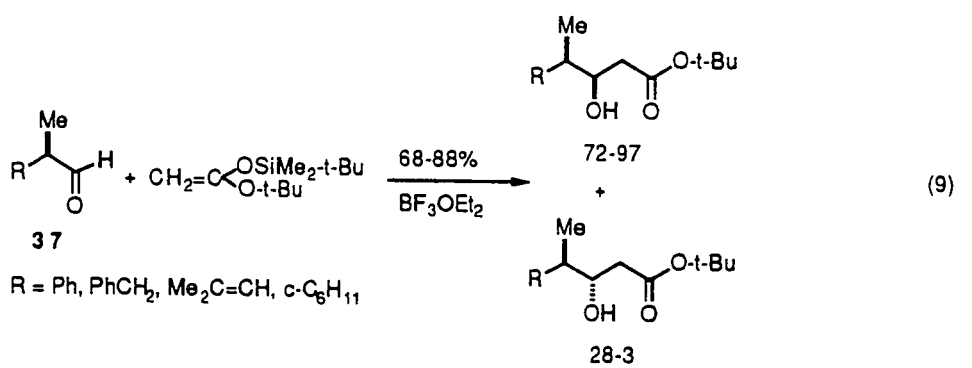
Finally, the well-known Mitsunobu reaction[15] (benzoic acid, triphenylphosphine, diethylazodicarboxylate) was also attempted for the inversion. Treatment of **29b** with $LiAlH_4$ followed by selective protection of resulting diol with a single TBDMS group afforded **35** in 90% yield (two steps). Reaction of **35** under Mistunobu conditions gave only 10% conversion to the desired product **36** and mostly recovered **35** (Equation 8). Despite considerable adjustment of conditions, the yield of **36** could not be improved further.



29b **35** **36** **35** (8)

Having these unsuccessful results, we started to explore another chelate controlled aldol type reaction. The Lewis acid mediated addition of silyl ketene acetals to chiral aldehydes is a well established methodology[16] for carbon chain elongation with diastereofacial selectivity. In 1983 Heathcock and Flippin reported that enolsilanes show exceptional diastereofacial preference in their Lewis acid mediated reactions with chiral α-methyl aldehydes **37**. The

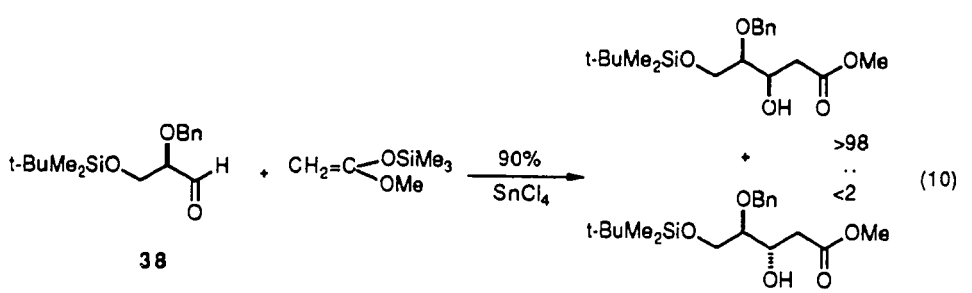
most selective and preparatively useful reagent described in that study is the t-butyldimethyl enolsilane derived from t-butyl acetate (Equation 9).[17] This high selectivity may be the result of an approach trajectory of the nucleophile which is closer to the chiral center when the carbonyl group is bound to the Lewis acid.[17] With chiral α,β-dialkoxy aldehydes the methyl acetate derived silyl ketene acetal was reported to give remarkable chelation-controlled diastereofacial selectivities (Equations 10 and 11).[18] Reetz and Kesseler showed that excellent diastereofacial preference in favor of the syn isomer can be achived with aldehyde **38** and tin tetrachloride through the formation of the α-chelated complex.[18a] Kita and coworkers showed that 2,3-O-isopropylidine glyceraldehyde **39** and catalytic ZnI_2 give high ratios of



37

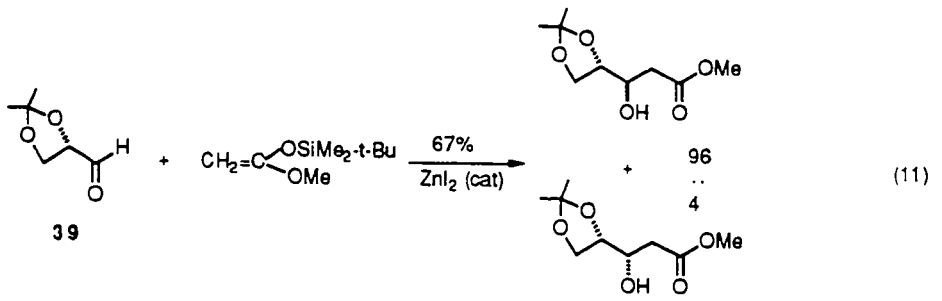
R = Ph, $PhCH_2$, $Me_2C{=}CH$, c-C_6H_{11}

(9)

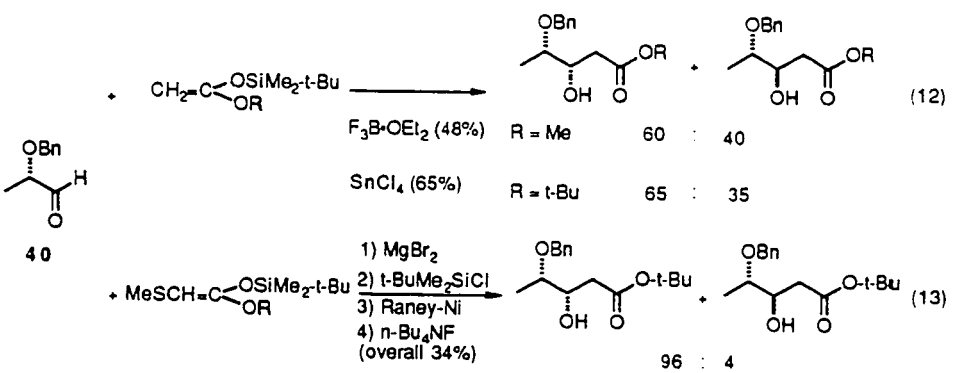


38

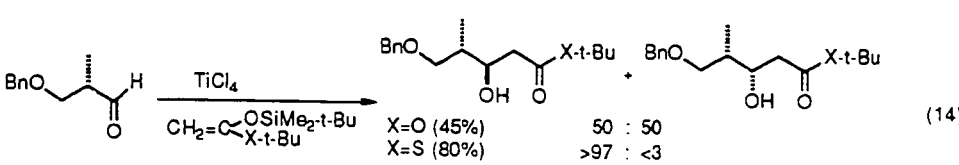
(10)

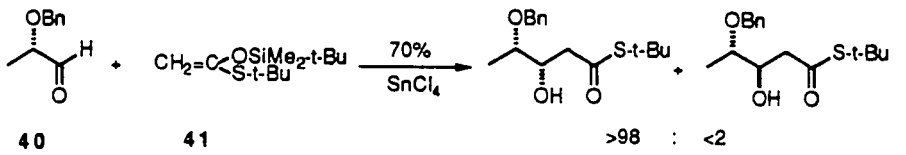


the anti isomer, possibly through the formation of the β-chelated complex.[18b] Unfortunately this high selectivity cannot be extended to chiral α-alkoxy aldehyde **40** (Equation 12).[19] A somewhat circuitous solution to this problem was proposed with the use of a methylthio-substituted silyl ketene acetal followed by protection, reductive desulfurization and deprotection (Equation 13).[20]



Meanwhile, in 1988 Gennari and Cozzi showed that the t-butyldimethyl enolsilane derived from t-butylthioacetate is a very stereoselective reagent where the corresponding acetate is not (Equation 14).[21]

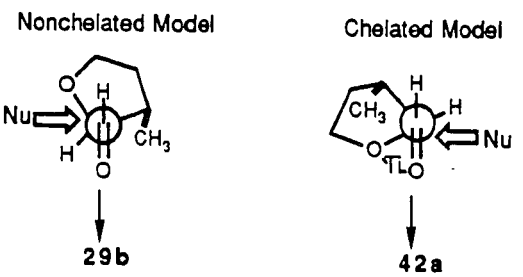




(15)

40 41 >98 : <2

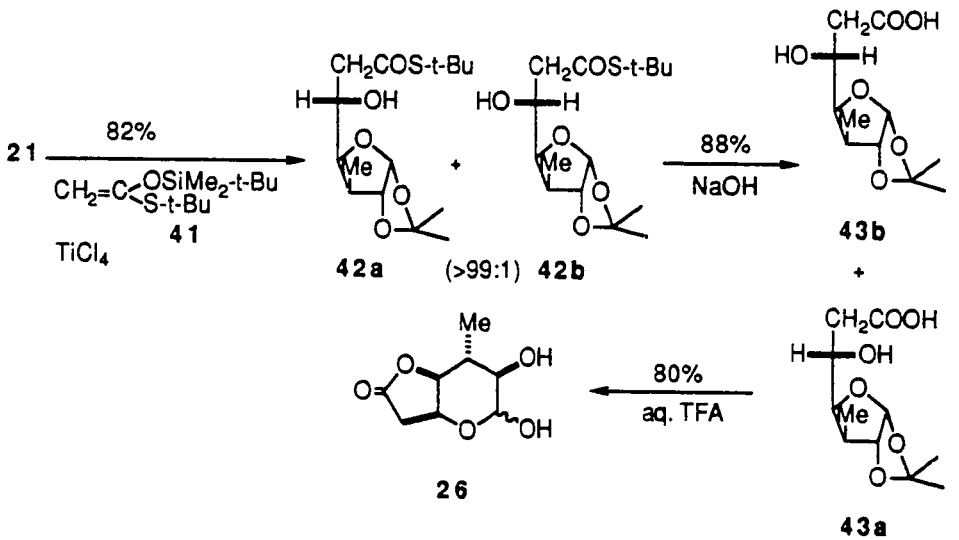
It is possible that the two oxygens of the acetate derived silyl ketene acetal somehow compete with the alkoxy aldehyde in the chelation of the Lewis acid with consequent loss of stereoselectivity. With the thio analog **41** this undesirable effect is avoided and high chelation is restored (Equation 15),[19] in analogy with the similar behavior of propionates and thiopropionates.[19] Encouraged by this literature survey, we planned to attempt the aldol addition to aldehyde **21** to produce the right isomer at C-5 (glucose numbering) using the t-butyl(dimethyl)silyl enol ether **41** of t-butyl thioacetate in the presence of titanium(IV) tetrachloride. To our delight, this condensation generates only the requisite configuration at this center in 82% isolated yield (Scheme II). High stereoselectivity (99>1) could be explained in terms of chelation between titanium and the carbonyl and ring oxygens in **21**. This chelation presumably blocks the re-face and encourages attack by the nucleophile on the si-face.



Hydrolysis of the thioester **42** was achieved with 0.2 N NaOH in THF in 90% yield without interferance of retro-aldol reaction.[22]

Deketalization of the resulting carboxylic acid **43a** with aqueous

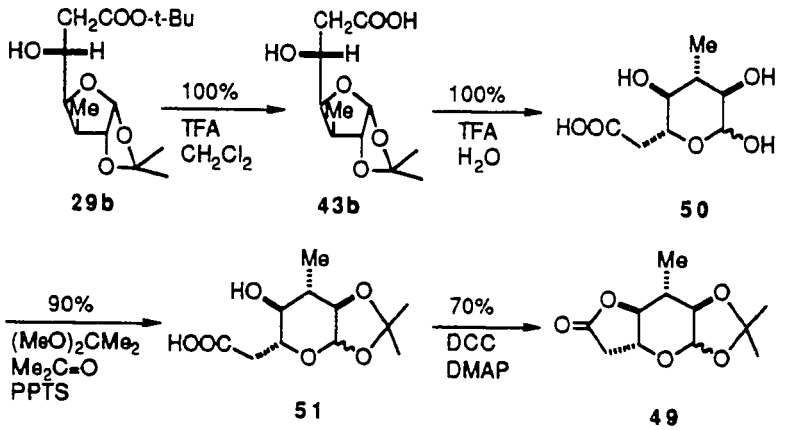
Scheme II



trifluoroacetic acid was accompanied by furanose to pyranose interconversion and lactonization to provide the cis lactone **26** in 80% yield for two steps. This lactone formation even in aqueous trifluoroacetic acid is remarkable. It suggests a cis disposition of the hydroxy and carboxylic acid substituents in **26**. Thus, there was a contrast in the ease of formation of cis lactone **26** and trans lactone **49** (vide infra). The trans lactone **49** was obtained from the hydroxyl ester **29b**. As shown in scheme III, the t-butyl ester in **29b** was hydrolyzed with trifluoroacetic acid in methylene chloride in 100% yield. The resulting hydroxy acid **50** did not lactonize in trifluoroacetic acid. Without purification, reaction of **50** with acetone and 2,2-dimethoxypropane in the presence of a catalytic amount of PPTS delivered pyranose acetonide **51** in 70% yield.[23] The trans lactone **49** was obtained from acetonide **51** upon treatment with N,N'-

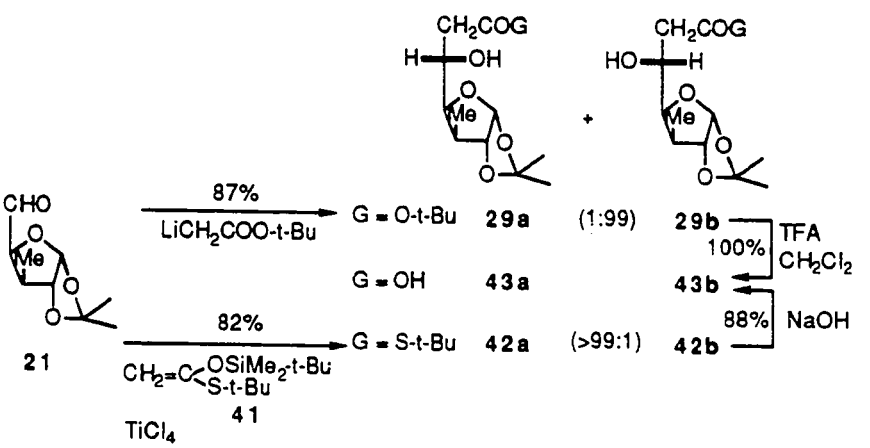
dicyclohexylcarbodiimide in the presence of p-(N,N'-dimethylamino)pyridine.[24]

Scheme III

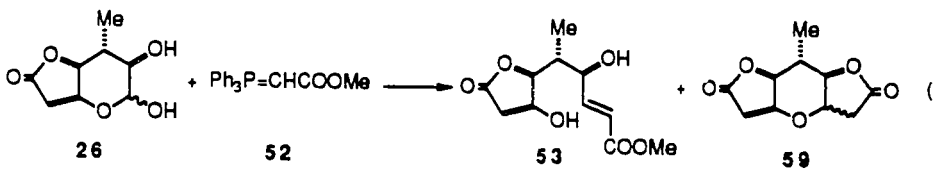


The four aldol products **29a**, **29b**, **42a**, and **42b** derived from aldehyde **21** were chemically correlated (Table 3). The hydroxy acids **43a** derived from **29a** and **42a** and the hydroxy acids **43b** derived from **42b** and **29b** were identical with each other by ^{1}H NMR and optical rotation.

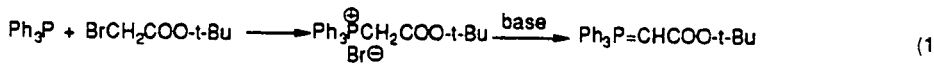
Table 3. Correlation of aldol condensation stereochemistries



Our plan for generation of the H-ring pyran envisioned Wittig olefination at the anomeric center[25] in pyranose **26** and heterocyclization by Michael addition to an intermediate α,β-unsaturated ester. Reaction of methyl(triphenylphosphoranylidene)-acetate (**52**) with **26** in acetonitrile gave only a 50% yield of Wittig product **53**. The dilactone structure **59** was tentatively assigned to a byproduct of this reaction (Equation 16). To reduce the proclivity towards lactonization, the methyl ester was replaced with a more sterically encumbered t-butyl ester. t-Butyl(triphenylphosphoran-ylidene)acetate (**44**) was prepared as outlined in equation 17. Reaction of cis-fused lactone **26** with ylide **44** stereoselectively furnished Wittig products **45a**, **45b** in 80% isolated yield, as anticipated[25] favoring the trans isomer by 13:1 (Scheme IV).



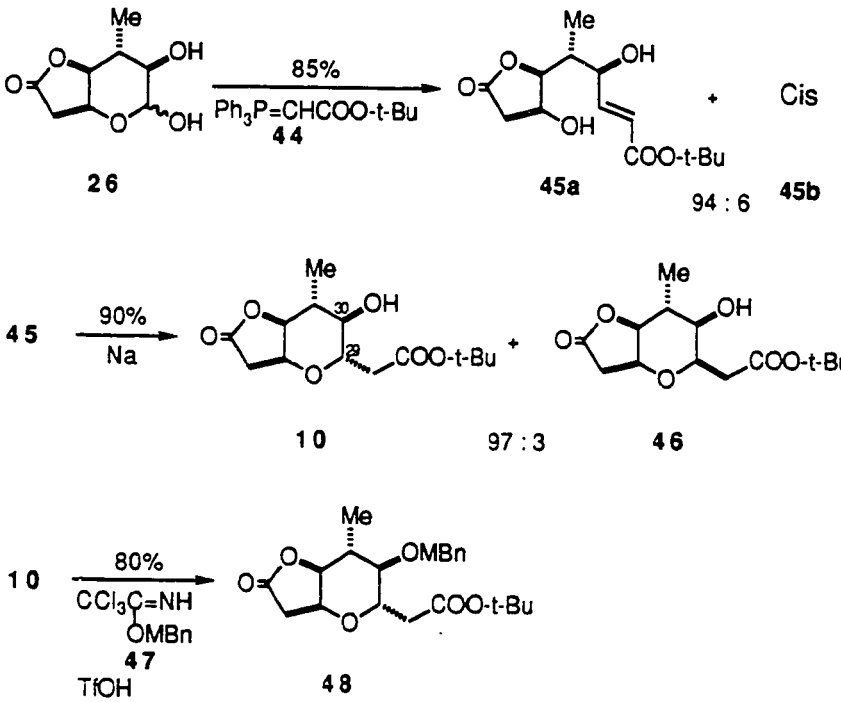
26 52 53 59



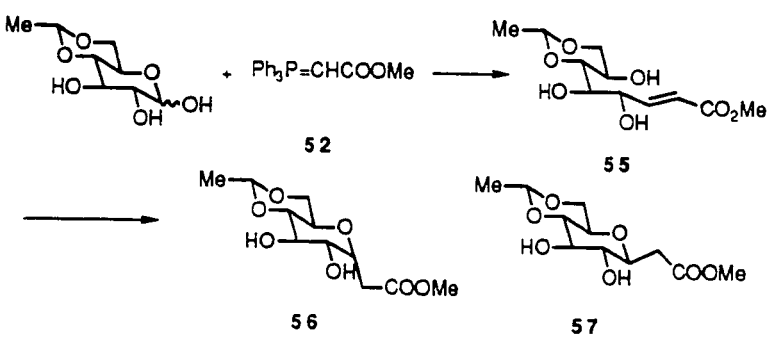
44

Intramolecular hetro Michael addition was exploited for construction of the H-ring in intermediate **10** for halichondrin B (Scheme IV). This process was expected to provide the required configuration at C-29 (halichondrin numbering) owing to a preference for the less sterically encumbered equatorial disposition of the carbo-t-butoxymethyl substituent and facile equilibration of C-29 epimers.

Scheme IV



There is pertinent precedent for such chain extension by Wittig reaction and stereoselective cyclization of the resulting unsaturated ester (Equation 18).[26] Thus, cyclization of **55** in the presence of dilute base initially produces a 1:1 mixture of products **56** and **57**. However, equilibration generates the thermodynamically favored trans isomer with carbomethoxy methyl group equatorial in 70% yield.



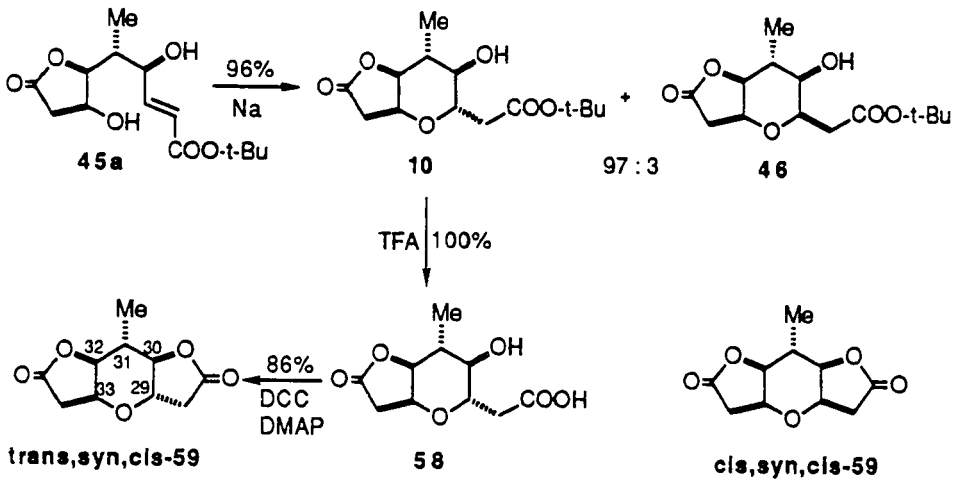
(18)

We treated Wittig products **45** with a catalytic amount of potassium carbonate in methanol at room temperature. However, only a small amount of cyclized products (less than 20%) was formed and the ^1H NMR spectrum of the crude product mixture showed four cyclized products owing to the partial transesterification by methanol. With potassium t-butoxide in t-BuOH at room temperature, less than 10% conversion occurred. Even prolonged reaction gave the same result. Finally, we found that a small pinch of sodium metal in undistilled THF initiated the cyclization. Within 30 min in THF at room temperature, the conversion of **45** to the epimeric pyrans **10** and **46** was complete. After HPLC separation the ratio of major epimer **10** to minor epimer **46** was 97:3 in 96% isolated yield (Scheme IX). Thus, presuming that a chair conformation of the pyran ring is favored for the cyclization products **10** and **46** from **45**, the required trans isomer **10** with an equatorial carbo-t-butoxymethyl substituent is favored over cis **46** with that substituent axial.

Further stereochemical characterization of the major cyclization product **10** from **45** was accomplished as outlined in Scheme V. De-t-butylation upon treatment with trifluoroacetic acid delivered a γ-hydroxy acid **58** which did not lactonize under these conditions in contrast with the intermediate γ-hydroxy acid derived from acetonide **43a** which produces the cis butyrolactone **26** even in the aqueous TFA (Scheme II). This reluctance of **58** to lactonize is consistent with a trans disposition of the γ-hydroxy and carboxymethyl substituent in **58**. Lactonization was accomplished upon treatment

with N,N-dicyclohexylcarbodiimide in the presence of p-(N,N-dimethylamino)pyridine.[24] That the resulting dilactone (87% isolated yield from **10**) was the unsymmetrical isomer **trans,syn,cis-59** rather than the symmetrical isomer **cis,syn,cis-59** was readily apparent from the appearance of the five distinct resonances corresponding to the nonequivalent hydrogens at positions 29 to 33 in its ^{1}H NMR spectrum.

Scheme V

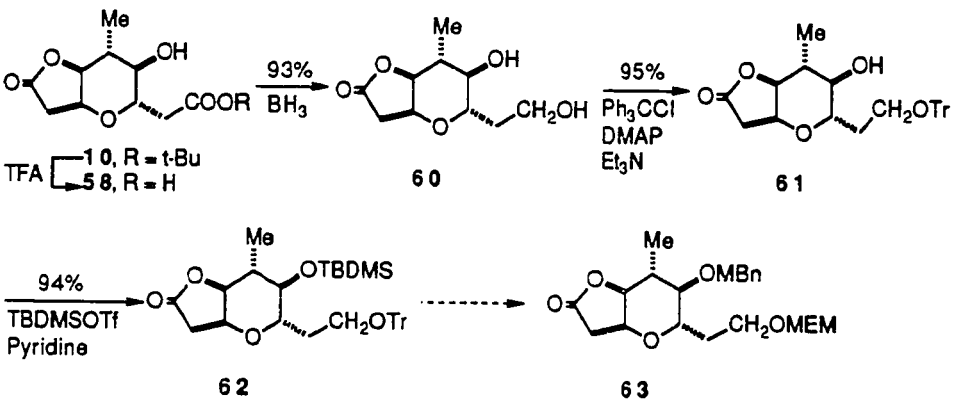


Protection of the hydroxy function in **10** with a methoxybenzyl (MBn) group was readily achived by the method of Yonemitsu and coworkers[27] which is useful for methoxybenzylation under mild acidic conditions under which alkali and even some acid sensitive groups are completely unaffected. Their reagent, p-methoxybenzyl trichloroacetimidate (**47**), is easily prepared from p-methoxybenzyl alcohol and trichloroacetonitrile in the presence of sodium hydride. In the event, methoxybenzylation of **10** with imidate **47** in the presence of trifluoromethanesulfonic acid delivered target **48** in 80% isolated yield (Scheme IV).

3. Model Study for Chain Extension

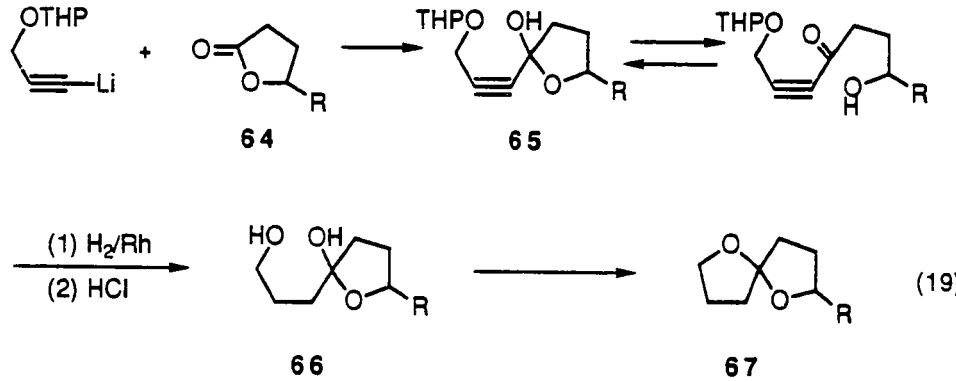
Our strategy for assembling the HIJKLMN-ring system (see Figure 2 on page 8) involves union of H-ring and KLMN-ring intermediates (**8** and **9** respectively) of the correct absolute configuration and subsequent generation of the I and J ring between these two fragments. As a prelude to generating **8** from **10** the ester and lactone in **10** were differentiated (Scheme VI).

Scheme VI



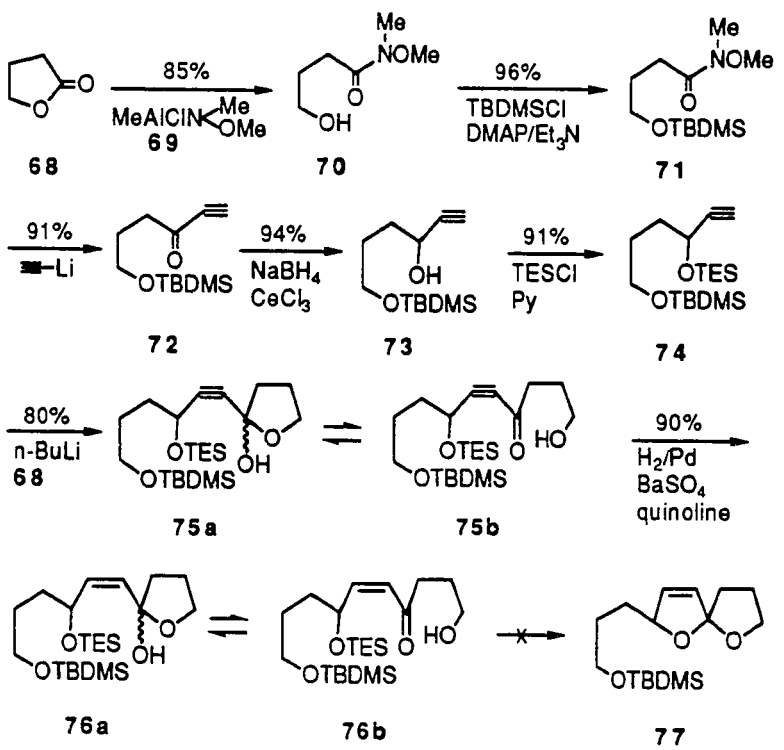
Selective reduction of the carboxylic acid in **58** with borane[28] delivered the diol **62** in 93% isolated yield. Differentiation of the hydroxyls in this diol **62** was accomplished by etherification with triphenylmethyl chloride affording **61** in 95% isolated yield. The remaining hydroxy was protected with TBDMSOTf in pyridine providing **62** in 93% yield. In the future, reductive detritylation will be followed by reprotection of the primary hydroxyl as a MEM ether[29] to give **63**. The TBDMS-ether will then be removed and the secondary hydroxyl will finally be masked with a p-methoxylbenzyl group (Scheme VI).

Our plan for generating the J,K spirofuran rings is a modification of a known synthesis of spirofurans from butyrolactones which is exemplified by the **64** to **67** conversion (Equation 19).[30]

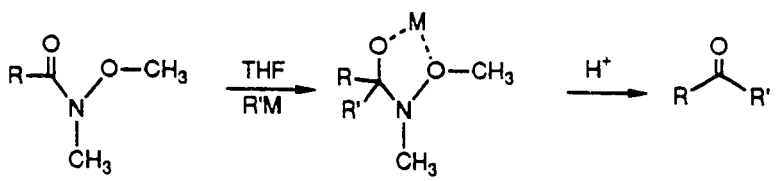


Thus, we chose γ-butyrolactone (**68**) as a readily available model for **63** (Scheme VII).

Scheme VII



Reaction of lactone **68** with a N-methoxy-N-methylamino methylchloroaluminum amide[31] (**69**) generated from trimethyl aluminum and N,O-dimethylhydroxylamine hydrochloride delivered amide **70** in 85% isolated yield. Silylation of the resulting primary alcohol with TBDMSCl, Et_3N in the presence of DMAP provided t-butyldimethylsilyl (TBDMS) derivative **71** in quantitative yield. It is known that N-methoxy-N-methylamides **78** (Equation 20) combine cleanly with both Grignard reagents and organolithium species in THF to form ketones.[32] Significantly, these reactions do not produce tertiary alcohols even when a large excess of the organometallics are used. Presumably, this conversion procedes through a very stable metal-chelated intermediate (Equation 20), which probably accounts for the observed resistance to over-addition.



78 (20)

N-methoxy-N-methyl amide **71** was converted in 91% isolated yield to propargyl ketone **72** via reaction with a lithium acetylide.[33] Asymmetric reduction of this propagyl ketone with chiral reducing agent[34] is expected to deliver the requisite R-configuration enantioselectively at position C-35 (halichondrin numbering). In our model study no effort was made to achieve asymmetric induction. Thus, reduction of propargyl ketone **72** under Luche conditions ($NaBH_4/CeCl_3$)[35] afforded racemic propargyl alcohol **73** in 94% isolated

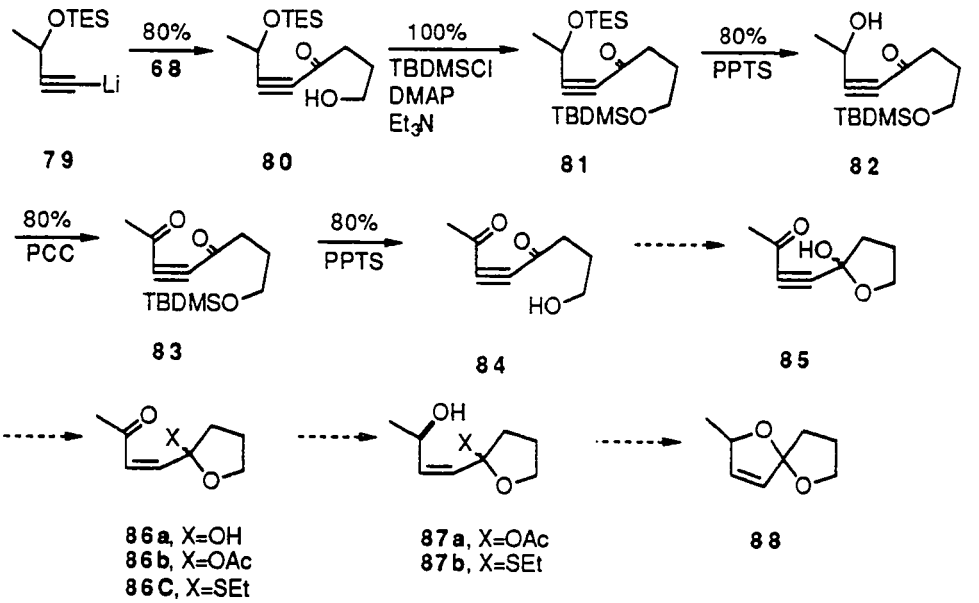
yield. Protection of the resulting propargyl alcohol **73** was achieved in 92% yield with TESCl in pyridine.[36]

Union of the H-ring model **74** with a second molecule of γ-butyrolactone (**68**), this time serving as a model for K-ring lactone in KLMN-ring fragment **9**, delivered an adduct **75** in 80% isolated yield. Stereospecific cis catalytic partial hydrogenation of **75** provided a single alkene **76** in 90% isolated yield (Scheme VII). To generate the spirofuran **77**, we treated **76** with a variety of acidic conditions.[30] None of these gave the desired spirofuran **77**. Instead it afforded a different product. A ^{13}C NMR spectrum of **76** showed that it exists in a ketone form **76b** rather than in hemiketal **76a** in contrast with the saturated analogue **66**.[30] This preference is understandable in terms of the stabilization provided to the carbonyl group by conjugation.

4. Future Work

We now realize that the formation of spirofuran **77** is hampered by the preference of the ketone form **76b**. A plan for overcoming this preference exploits conjugation to the destabilize the same carbonyl group (vinylogous α-diketone, Scheme VIII). Graduate student Wenxi Pan is presently developing the conversion of **84** to target **88**.

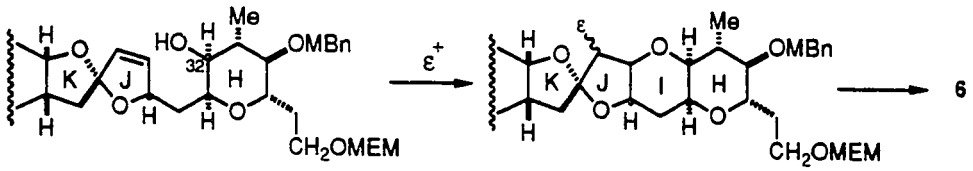
Scheme VIII



Conjugation with a second carbonyl in **84** should destabilize the keto form relative the hemiketal form **85** (Scheme VIII). Selective partial reduction of the C≡C bond and activation of the hemiketal **86a** as an acylal[37] **86b** or hemithioketal[38] **86c** followed by reduction of the remaining carbonyl should provide **87**. Ample precedent exists for generating ketals from acylals[39] or hemithioketals[40] which should provide **88** from **87**. Stereoselective generation of the correct absolute

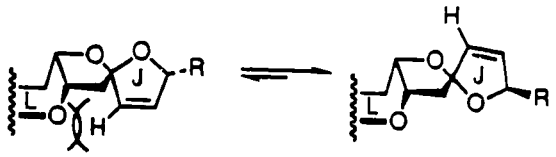
configuration at the allylic hydroxyl group at C-35 (halichondrin B numbering) could be explored in the **86** to **87** conversion.

The I-ring pyran will then be formed by nucleophile capture of a J-ring electrophile by the C-32 hydroxyl on the H-ring of **89**. The requisite J-ring electrophile can be generated by addition of an electrophile, ε^+ to the C=C bond in **89**. Possible electrophiles include $PhSe^+$, I^+, or XHg^+.



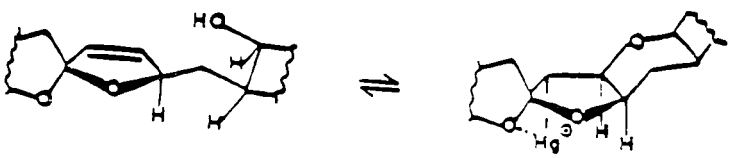
89 **90**

There are ample precedents for generating pyran rings by cyclization of δ-hydroxy alkenes with selenium,[41] halogen,[42] or mercury[43] electrophiles. Reductive cleavage of the vestigial ε group in **90** will produce the I-ring pyran. There are good reasons for expecting that the cyclizations which generate the I and J-rings will be stereoselective for the configurations required at C-36 and C-38 in **6**. Thus, the intramolecular ketalization leading to **89** will be readily reversible. The equilibrium between **89** and the diastereomeric spiroketal **91** will clearly favor the former owing to the unfavored steric congestion in **91** involving a vinyl hydrogen on the J-ring and the cis-fused L-ring. A trans ring fusion between the I and J rings in **90** would undoubtedly incorporate greater ring strain than the cis fusion found in the natural products.



91 89

If the electrophile ε^+ is a mercuric cation two additional factors can favor the generation of the correct configuration at C-36 (halichondrin numbering) in **90**. Thus, coordination of the K-ring tetrahydrofuran oxygen with Hg^+ can stabilize the required oxymercuration product **92** and a judicious choice of counter ion for mercury can facilitate equilibration of isomeric cyclization products by a deoxymercuration-oxymercuration process.



89 92

Experimental

General. All proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-200 spectrometer at 200 MHz or Gemini 300 spectrometer at 300 MHz and are reported in parts per million (ppm) on the δ scale relative to chloroform-d (δ 7.236) or tetramethylsilane (δ 0.00). Significant ^1H NMR spectral data are tabulated in the order: number of protons, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), and coupling constant(s) in Hertz. The use of "apparent" in proton multiplicity description implies a hyperfine splitting necessarily more complex than first order. All NMR samples were analyzed as solutions in $CDCl_3$. Carbon NMR spectra were recorded on a Varian XL-200 spectrometer at 50.3 MHz or Gemini 300 spectrometer at 75.0 MHz in the FT mode and are reported in parts per million on the δ scale relative to chloroform-d (δ 77.0). Carbon atoms determined by the Attached Proton Test (APT) to have one or three appended protons are indicated with a minus sign (-). Carbon atoms bearing two or no attached protons are denoted by a plus sign (+).

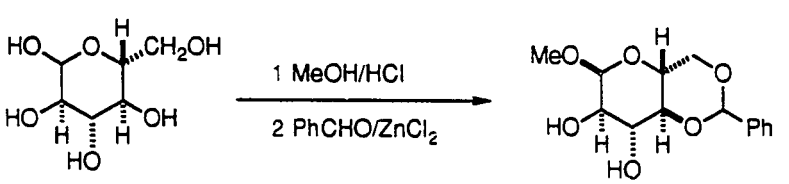
All melting point determinations are uncorrected and were recorded on a Thomas Hoover Capillary Melting Point Apparatus. High resolution mass spectra were recorded on a Kratos AEI MS25 RFA double sector high resolution mass spectrometer with a DS-50S Nova-3 computer. Samples were run to 70 eV. The heat source was at 200 °C with direct probe insertion. Analytical thin layer chromatography (TLC) was performed with E. Merck pre-coated TLC

plates, silica gel 60F-254, layer thickness 0.25 mm. TLC plates were developed by spraying the dried plate with a vanillin indicator (6% w/v vanillin in 10% v/v ethanolic sulfuric acid) or sometimes anisaldehyde indicator (6% w/v anisaldehyde in 10% v/v ethanolic sulfuric acid) and heating with a hot air gun until spots appear. Flash chromatography was performed on 230-400 mesh silica gel 60 supplied by E. Merck. Preparative high resolution liquid chromatography (HPLC) was performed using a Waters Associates System consisting of a Waters M6000A or M590 solvent delivery system and a Waters U6K injector. Column eluents were monitored with an Instrumentation Specialities Company model 1840 variable wavelength UV absorbance detector or a Waters model R401 Differential Refractomerer. Optical rotations were measured using a Perkin-Elmer 241 polarimeter at room temperature using the sodium D line. Elemental Analysis was performed by Galbraith Laboratories, Knoxville, Tennessee.

All reactions were performed in an inert moisture-free atmosphere under a positive pressure of nitrogen or argon except when working in aqueous media. Purification and handling of all solvents was conducted under nitrogen. All solvents were reagent grade. Acetone was distilled from anhydrous potassium carbonate prior to use. Acetonitrile was distilled from calcium hydride and stored over 4Å molecular sieves. Allyltrimethylsilane was distilled prior to use. Benzene was boiled under reflux over potassium benzophenone ketyl followed by distillation. Chloroform was freshly distilled from phorphous pentoxide. Chlorotriethylsilane was distilled

under reduced pressure. Diisopropylamine and triethylamine were freshly distilled over calcium hydride, and stored over 4Å molecular sieves. N,N-Dimethylforamide and dimethylsulfoxide were distilled from calcium hydride under reduced pressure. Ethyl acetate was boiled under reflux over phosphorus pentoxide followed by distillation. Ethyl ether was boiled under reflux over lithium aluminum hydride followed by distillation. Hexamethylphosphoric triamide was distilled over calcium hydride, and the constant boiling fraction 70 °C (1 mmHg) was collected and used. Hexane was distilled over sodium hydride. Methanesulfonyl chloride was distilled under reduced pressure prior to use. Methylene chloride was boiled under reflux over phosphorous pentoxide followed by distillation. Pyridine was distilled from potassium hydroxide. Tetrahydrofuran was boiled under reflux over potassium benzophenone ketyl followed by distillation. p-Toluenesulfonyl chloride was recrystallized from benzene. Trifluoroaetic anhydride was distilled prior to use. Triphenylphosphine was recrystallized from hexane. Zinc powder was heated with a solution of sulfuric acid and nitric acid and washed with water, ether, acetone, then dried under vacuum in an oven. Zinc chloride was fused and pulverized prior to use.

Methyl 4,6-O-Benzylidene-α-D-glucopyranoside (11).[3]

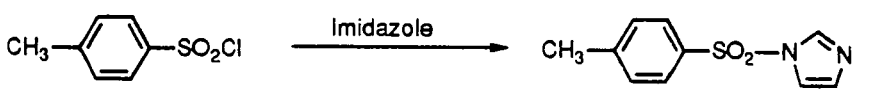


α-D-glucose

11

A mixture of methyl α-D-glucopyranoside (133 g, 0.685 mol) and freshly fused $ZnCl_2$ (100 g, 0.734 mol) and freshly distilled benzaldehyde (333 mL, 3.28 mmol) was shaken in a glass bottle for 2 days. The mixture was slowly poured into cold water (2.5 L) and the mixture was refrigerated overnight. Petroleum ether (150 mL) was then added and the mixture stirred 0.5 h to aid in removing excess benzaldehyde and the product was then separated on a Büchner funnel, washed twice with cold water (200 mL), twice with petroleum ether (200 mL), and again with cold water (200 mL). The crude product was dried overnight in the air and then in a vacuum oven at 70 °C. The crude benzylidene compound was purified by recrystallization from hot water (1.5 L) to give colorless crystals **11** (102 g) and further recrystalliation from mother liquid afford more **11** (34 g, 70% total yield): mp 160 - 162 °C; $[\alpha]_D^{25}$ +105° (c 1, $CHCl_3$); (lit[3] mp 163 - 164 °C, $[\alpha]_D^{20}$ +110° (c 2, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.45 (m, 5 H), 5.5 (s, 1 H), 4.8 (d, 1 H, J = 3.9 Hz), 4.30 (dd, 1 H, J = 9.0, 3.6 Hz), 3.75 (m, 5 H), 3.43 (s, 3 H).

N-p-Tolylsulphonylimidazole (12).[4]

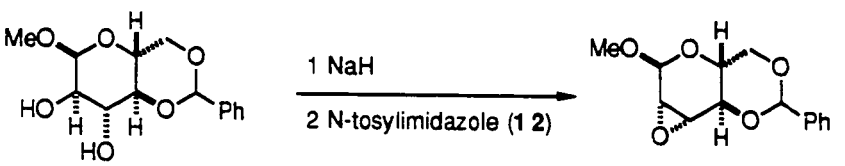


12

Imidazole (61.2 g, 0.90 mol) was dissolved in chloroform (660 mL) and p-tolunesulfonyl chloride (85.6 g, 0.460 mol) was added in portions to the stirred solution. After standing for 1 h at room temperature, the precipitated imidazole hydrochloride was removed by

filtration, and the filtrate was washed with saturated sodium bicarbonate solution (400 mL) and water (400 mL) and then dried over anhydrous sodium sulphate. The residue, obtained upon evaporation of solvents, was dissolved in benzene (500 mL) and petroleum ether (30 - 60 °C) was added until crystals came out. After standing overnight, the crystals were collected on a Büchner funnel (84 g, 85% yield): mp 74 - 76 °C; (lit[4] mp 74.5 - 76.0 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.02 (s, 1 H), 7.83 (d, 2 H, J = 8.2 Hz), 7.31 (m, 4 H), 2.45 (s, 3 H).

Methyl 2,3-Anhydro-4,6-O-benzylidene-α-D-mannopyranoside (13).[4]

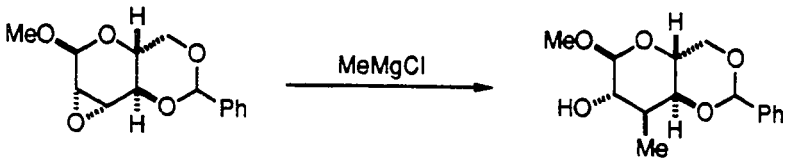


11 13

Sodium hydride (50% oil dispersion, 8.06 g, 0.168 mol) was placed in a dry 1 L flask equipped with a magnetic stirrer and drying tube, and washed free of oil with pentane (3 x 70 mL). Dry dimethylformamide (800 mL) was added followed by methyl 4,6-O-benzylidene-α-D-glucopyranoside (**11**, 22.32 g, 0.080 mol), and the mixture was stirred for 0.5 h at room temperature. N-tosylimidazole (**12**, 19 g, 0.088 mol) was then added and the suspension stirred further for 1 h. The reaction mixture was then poured with stirring into ice-cold water (1.0 L) and the resulting crystals were filtered with suction and washed with water until the washings were colorless. The product was recrystallized from methanol (17 g, 80% yield): mp 145.5 - 146.5 °C; (lit[4] 145 -146 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48 (m, 2 H),

7.36 (m, 3 H), 5.53 (s, 1 H), 4.78 (d, 1 H, J = 3.9 Hz), 4.29 (dd, 1 H, J = 9.0, 3.6 Hz), 3.75 (m, 5 H), 3.46 (s, 3 H).

Methyl 4,6-O-Benzylidene-3-deoxy-3-C-methyl-α-D-altropyranoside (14).[2]

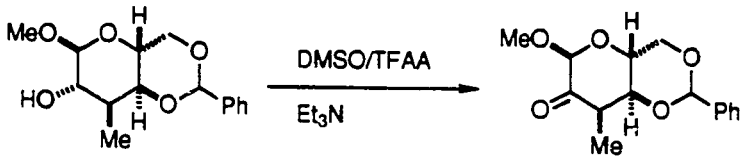


13 14

Methylmagnesium chloride (3.0 M in THF, 30.0 mL, 10 equiv) was added via syringe into a suspension of the epoxide **13** (2.65 g, 10 mmol) in dry ether (300 mL) and the mixture was boiled under reflux under nitrogen for one week. TLC analysis with 50% ethyl acetate in hexane showed a product (R_f 0.38) and some unreacted epoxide **13** (R_f 0.56). After a second addition of methylmagnesium chloride (3.0 M in THF, 30.0 mL), reflux was resumed for an additional week. After cooling, the reaction mixture was poured into an aqueous saturated ammonium chloride solution (600 mL). The layers were separated and the aqueous layer was extracted with ether (3 x 30 mL). The combined organic layers were dried over anhydrous magnesium sulfate. The filtrate was concentrated by rotary evaporation to give a yellow syrup. This crude material was recrystallized from ether-petroleum ether to give **14** as needles (2.73 g, 98% yield): mp 114 - 115 °C; $[\alpha]_D^{20}$ +116.5° (c 1.0, $CHCl_3$); (lit[2] mp 115 - 11.5 °C $[\alpha]_D$ +120° (c 1.7, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.49 (m, 2 H), 7.35 (m, 3 H), 5.60

(s, 1 H), 4.60 (s, 1 H), 4.29 (m, 1 H), 4.04 (m, 2 H), 3.78 (m, 2 H), 3.39 (s, 3 H), 2.40 (m, 1 H), 1.22 (d, 3 H, J = 7.49 Hz).

Methyl 4,6-O-Benzylidene-3-deoxy-3-C-methyl-α-D-ribo-hexpyranosid-2-ulose (15).[2]

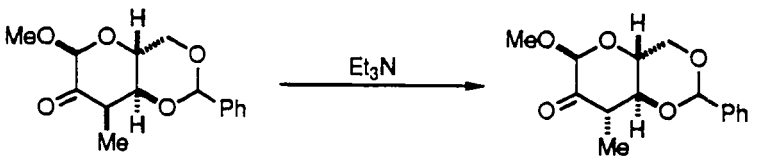


14 15

A solution of trifluoroacetic anhydride (1.85 mL, 13.1 mmol, 1.5 equiv) in dry dichloromethane (5 mL) was added dropwise under nitrogen over 10 min to a cooled mixture (-78 °C) of dry dichloromethane (18 mL) and dimethylsulfoxide (1.23 mL, 17.6 mmol, 2 equiv) 10 min after the end of addition, a solution of the alcohol **14** (2.47 g, 8.80 mmol) in dry dichloromethane (30 mL) was added by syringe and the mixture kept for 1 h at -78 °C. Triethylamine (3.5 mL) was slowly added then the solution was warmed to room temperature. The reaction mixture was diluted with ether (300 mL), washed with 1 N HCl (35 mL), washed with water (30 mL), and washed with saturated aqueous sodium bicarbonate (30 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered with suction, and the filtrate was concentrated under reduced pressure to afford a slightly yellow syrup (2.4 g, 96% yield). This syrup was used for the next reaction without further purification: $[\alpha]_D^{25}$ +51° (c 1.5, $CHCl_3$); (lit[2] $[\alpha]_D$ +53° (c 1.0, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.50 (m, 2 H), 7.34 (m, 3 H), 5.53 (s, 1 H), 4.59 (s, 1 H), 4.36 (dd, 1 H, J = 10.10, 4.80

Hz), 4.21 (m, 1 H), 3.75 (t, 2 H, J = 10.19 Hz), 3.45 (s, 3 H), 3.04 (m, 1 H), 1.35 (d, 3 H, J = 7.65 Hz).

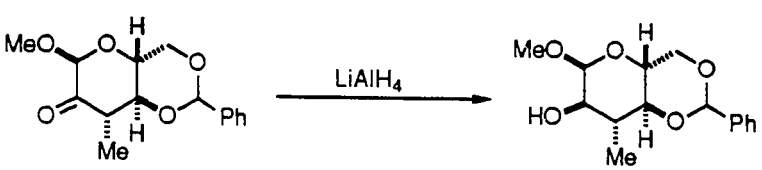
Methyl 4,6-O-Benzylidene-3-deoxy-3-C-methyl-α-D-arabinohexo-pyranosid-2-ulose (16).[2]



15 16

A solution of ketone **15** (24 g, 8.6 mmol) in dry dimethylformamide (9 mL) and triethylamine (2.7 mL) was kept at room temperature for 36 h. The reaction mixture was then poured into water (15 mL) and extracted with ether (3 x 20 mL). The organic layers were dried over anhydrous magnesium sulfate, filtered, and the solvent removed from the filtrate by rotary evaporation to give crude crystals. This crude material was recrystallized from hexane (2.35 g, 98% yield): mp 125 - 126 °C; $[\alpha]_D^{25}$ +54° (c 1.0, $CHCl_3$); (lit[2] mp 125.5 - 126 °C, $[\alpha]_D$ +56° (c 0.77, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.50 (m, 2 H), 7.38 (m, 3 H), 5.52 (s, 1 H), 4.62 (s, 1 H), 4.38 (dd, 1 H, J = 10.26, 4.88 Hz), 4.22 (m, 1 H), 3.76 (t, 2 H, J = 10.10 Hz), 3.49 (s, 3 H), 3.06 (m, 1 H), 1.21 (d, 3 H, J = 6.34 Hz).

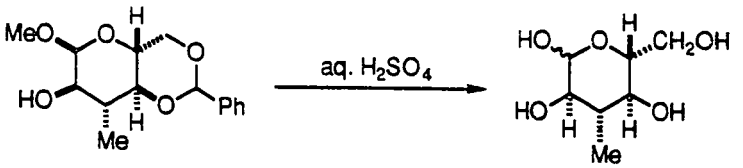
Methyl 4,6-O-Benzylidene-3-deoxy-3-C-methyl-α-D-glucopyranoside (**17**).[2]



16 17

Lithium aluminum hydride (321 mg, 846 mmol) was added to a solution of the ketone **16** (2.35 g, 8.46 mmol) in dry ether (65 mL). After 1 h stirring at room temperature, the excess hydride was destroyed with water (5.0 mL). The organic layer was separated and aqueous layer was extracted with ether (3 x 15 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and the solvent removed from the filtrate by rotary evaporation to give an amphorous solid **17** (2.26 g, 96% yield): mp 149.5 - 151 °C; $[\alpha]_D^{25}$ +104° (c 1.0, $CHCl_3$); (lit[2] mp 153.5 °C $[\alpha]_D$ +107° (c 0.48, $CHCl_3$)); ^1H NMR (200 MHz, $CDCl_3$) δ 7.45 (m, 2 H), 7.35 (m, 3 H), 5.48 (s, 1 H), 4.67 (d, 1 H, J = 3.58 Hz), 4.24 (m, 1 H), 3.71 (m, 2 H), 3.46 (s, 3 H), 3.34 (m, 2 H), 3.13 (t, 1 H, J = 10.42 Hz), 1.93 (m, 1 H), 1.16 (d, 3 H, J = 6.35 Hz).

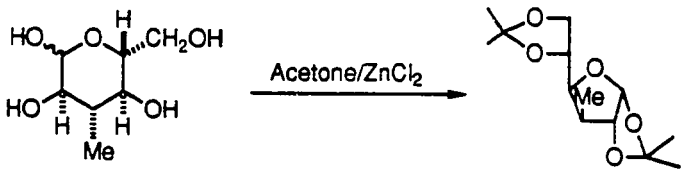
3-Deoxy-3-C-methyl-D-glucopyranose (18).[2]



17 **18**

Alcohol **17** (2.26 g, 8.04 mmol) was stirred in 2.5% aqueous sulfuric acid (210 mL) at 110 °C for 2 h, then cooled, diluted with water (250 mL) and extracted with ether (3 x 30 mL). The aqueous solution was then neutralized with barium carbonate, filtered and evaporated to give a deprotected product **18** (1.4 g, 98% yield) as a very hygroscopic syrup. Without further purification this crude product was used for the next reaction.

1,2,5,6-Diisopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (19).

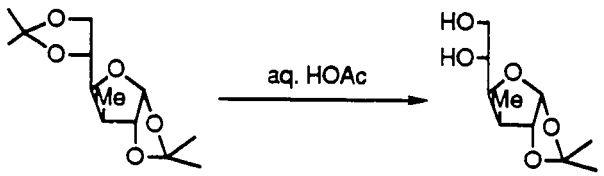


18 19

To an efficiently mechanically stirred suspension of **18** (1.40 g, 7.86 mmol) in freshly distilled acetone (10 mL) was added anhydrous pulverized zinc chloride (1.16 g), followed by 85% phosphoric acid (75 mg). This mixture was stirred at room temperature for 30 h and then the solution was made slightly alkaline with 50% aqueous sodium hydroxide solution. Insoluble inorganic material was removed by filtration and washed with acetone. The almost colorless filtrate and washings were concentrated and the residue was diluted with water (15 mL) and extracted with chloroform (3 x 20 mL). The combined chloroform extracts were washed with water (10 mL) and dried over anhydrous magnesium sulfate, filtered, and the filtrate was concentrated by rotary evaporation to give a crude product (1.72 g). The crude product was purified by flash chromatography eluting with 20% ethyl acetate in hexane. The fractions containing the product (R_f 0.30) were pooled, and the solvent was removed by rotary evaporation to give a product **19** (1.42 g, 70% yield) as a syrup: $[\alpha]_D^{25}$ -8.4° (c 2.5, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.69 (d, 1 H, J = 3.47 Hz), 4.27 (d, 1 H, J = 3.47 Hz), 3.94 (m, 4 H), 2.35 (m, 1 H), 1.43 (s, 3 H), 1.32 (s, 3 H), 1.26 (s, 3 H), 1.22 (s, 3 H), 0.87 (d, 3 H, J = 7.48 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.29, 109.20, 104.76, 86.53, 80.71, 73.53, 68.40, 40.56, 26.82,

26.75, 26.08, 25.34, 11.06; mass spectrum m/z (M^+) for $C_{13}H_{22}O_5$ calcd 258.1467, found 258.1458.

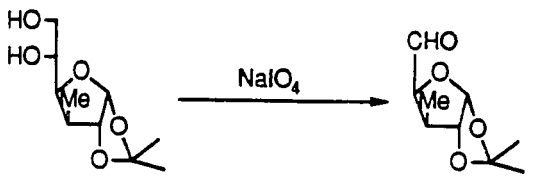
1,2-Isopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (20).



19 20

A solution of diacetonide **19** (3.0 g, 11.6 mmol) in HOAc (12.24 mL) and H_2O (7.2 mL) was warmed to 34 °C. The reaction mixture was stirred for 5 h at 34 °C. After 5 h, TLC analysis with 50% ethyl acetate in hexane showed no starting material. The reaction mixture was neutralized with powdered anhydrous K_2CO_3 and filtered. The solid on the filter was washed with ethyl acetate (15 mL). Then the filtrate was extracted with EtOAc (3 x 25 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Slightly yellow crude product (2.45 g) was purified by flash chromatography with 50% ethyl acetate in hexane as eluent. Product was obtained as a colorless oil (2.28 g, 90% yield). Upon prolonged standing in the refrigerator, this oil forms a white solid: mp 46 - 47 °C; $[\alpha]_D^{25}$ -20.0° (c 0.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.77 (d, 1 H, J = 3.69 Hz), 4.35 (d, 1 H, J = 3.52 Hz), 4.10 (m, 1 H), 3.67 (m, 3 H), 2.43 (m, 1 H), 1.49 (s, 3 H), 1.28 (s, 3 H), 0.94 (d, 3 H, J = 7.38); ^{13}C NMR (50 MHz, $CDCl_3$) δ 111.20, 104.62, 86.32, 79.08, 69.73, 64.83, 40.36, 26.61, 25.98, 10.89. Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.02; H, 8.32. Found: C, 55.27; H, 8.46.

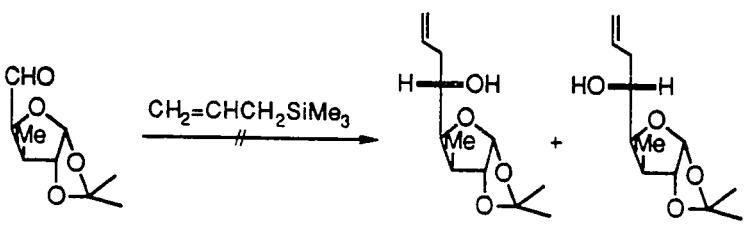
1,2-O-Isopropylidene-3-deoxy-3-C-methyl-erythro-pentodialdofuranose (21).



20 **21**

To a well-stirred solution of diol **20** (2.20 g, 10.08 mmol) in water (52 mL) was added $NaIO_4$ (2.59 g, 12.10 mmol) and the resulting solution was stirred for 1 h at room temperature. After 1 h, the reaction mixture was extracted with $CHCl_3$ (4 x 50 mL) and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. This material was sufficiently pure for the next step. This aldehyde was a viscous syrup (1.68 g, 90% yield) which gradually hardened on standing: $[\alpha]_D^{25}$ -100.4° (c 0.49, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 9.68 (d, 1 H, J = 1.28 Hz), 5.92 (d, 1 H, J = 3.45 Hz), 4.56 (m, 1 H), 4.36 (d, 1 H, J = 3.28 Hz), 2.66 (m, 1 H), 1.45 (s, 3 H), 1.26 (s, 3 H), 0.85 (d, 3 H, J = 7.32 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 201.38, 111.79, 105.02, 86.00, 84.11, 41.99, 26.72, 26.03, 11.17; mass spectrum m/z (M^+) for $C_9H_{16}O_4$ calcd 188.1048, found 188.0680.

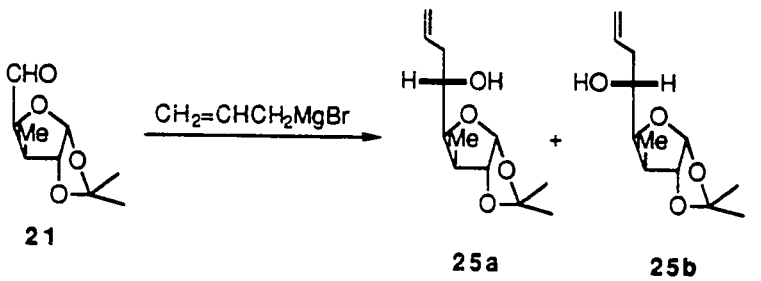
Attempted Allylation of 21 with Allyltrimethylsilane.



21 **25a** **25b**

Titanium(IV) chloride in methylene chloride (1.0 M, 0.32 mL, 0.32 mmol) was added at -78 °C to a solution of allyltrimethylsilane (50 μL, 0.32 mmol) and aldehyde **21** (50 mg, 0.27 mmol) in methylene chloride (1.5 mL) under nitrogen. The mixture was stirred at -78 °C for 1 h, then quenched with water (50 μl), and warmed to room temperature. It was diluted with methylene chloride (3 mL), neutralized with saturated $NaHCO_3$, washed with water (3 mL), brine (3 mL), and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure. TLC analysis and ^1H NMR spectra of the residue showed decomposition of aldehyde **21.**

Allylation Products 25a and 25b from Allylmagnesium Bromide and 21.



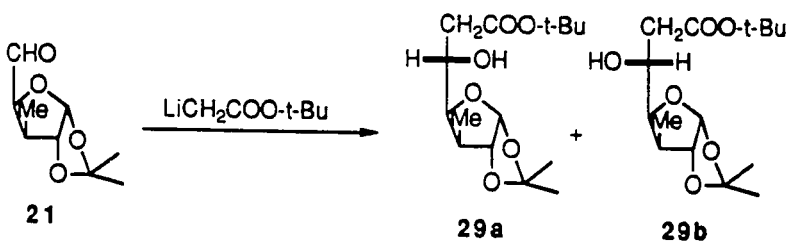
21 **25a** **25b**

To a solution of the aldehyde **21** (50 mg, 0.27 mmol) in ether (2 mL) was added at 0 °C allylmagnesium bromide in ether (0.6 M, 0.54 mL, 0.32 mmol) under nitrogen pressure. After 1 h stirring, the solution was quenched with water (0.5 mL) at 0 °C and warmed to room temperature. The organic layer was separated and the water layer was extracted with ether (3 x 5 mL). The combined organic extracts were dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to afford a crude product (40

mg). The two isomers were separated by HPLC with 10% acetonitrile in methylene chloride as eluent to deliver **25a** (9.3 mg, 15%) and **25b** (27.8 mg, 45%) as colorless oils. See text for a discussion of stereochemistry. Minor product **25a**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.80 (m, 1 H), 5.78 (d, 1 H, J = 3.28 Hz), 5.21 (m, 2 H), 4.35 (d, 1 H, J = 3.58 Hz), 3.98 (dd, 1 H, J = 8.79, 3.87 Hz), 3.67 (m, 1 H), 2.57 (m, 1 H), 2.43 (m, 1 H), 2.21 (m, 2 H), 1.50 (s, 3 H), 1.29 (s, 3 H), 0.94 (d, 3 H, J = 7.45 Hz).

Major product **25b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.88 (m, 1 H), 5.80 (d, 1 H, J = 3.52 Hz), 5.13 (m, 2 H), 4.36 (d, 1 H, J = 3.75 Hz), 4.04 (dd, 1 H, J = 8.21, 3.88 Hz), 3.73 (m, 1 H), 2.22 (m, 3 H), 1.50 (s, 3 H), 1.29 (s, 3 H), 0.86 (d, 3 H, J = 7.32 Hz).

t-Butyl Esters 29a and 29b by Aldol Condensation.



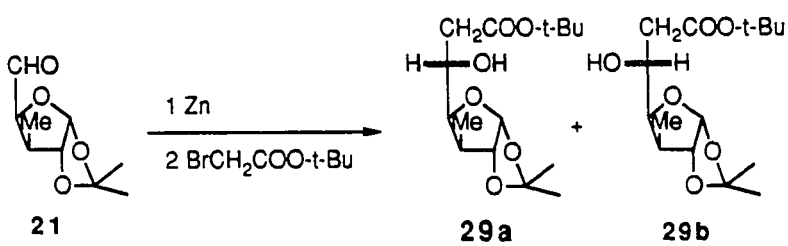
21 **29a** **29b**

A dry round-bottomed flask (10 mL), equipped with magnetic stirring, was flushed with nitrogen and immersed in an ice-water bath. The flask was charged with n-BuLi in hexane (1.6 M, 0.83 ml, 1.33 mmol) and diisopropylamine (0.19 mL, 1.33 mmol) was added over 10 min at 0 °C. The flask was then immersed in a dry-ice acetone bath and t-butyl acetate (0.18 mL, 1.33 mmol) was added over 15 min. The reaction mixture was stirred for an additional 30 min at -78 °C and then a solution of aldehyde **21** (0.2 g, 1.1 mmol) was added in THF (1.5

mL). The reaction mixture was stirred at -78 °C for 30 min. The solution was then quenched with 20% HCl (0.30 mL). Then the solution was allowed to warm to room temperature. The reaction mixture was extracted with ethyl acetate (3 x 10 mL), washed with water (4.0 mL) and dried over anhydrous $MgSO_4$. The crude product was purified by flash chromatography with 10% acetonitrile in methylene chloride to give a major isomer **29b** as white crystals (262 mg, 80% yield) and a minor isomer **29a** (5.0 mg, 1.5% yield). Major isomer **29b**: mp 81 - 85 °C; $[\alpha]_D^{25}$ -10.0° (c 0.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.77 (d, 1 H, J = 3.58 Hz), 4.36 (d, 1 H, J = 3.58 Hz), 3.99 (m, 2 H), 2.75 (dd, 1 H, J = 16.93, 2.5 Hz), 2.42 (dd, 1 H, J = 16.93, 8.31 Hz), 2.48 (m, 1H), 1.47 (s, 3 H), 1.44 (s, 9H), 1.28 (s, 3 H), 0.94 (d, 3 H, J = 7.43 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.97, 111.10, 104.43, 86.57, 81.45, 80.98, 66.48, 40.25, 39.79, 28.04, 26.65, 26.10, 10.71. Anal Calcd for $C_{15}H_{26}O_6$: C, 59.57; H, 8.67. Found C, 59.54; H, 8.62.

Minor isomer **29a**: $[\alpha]_D^{25}$ -25.0° (c 0.2, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.82 (d, 1 H, J = 3.58 Hz), 4.35 (d, 1 H, J = 3.63 Hz), 4.06 (m, 2 H), 2.37 (m, 2 H), 2.25 (m, 1 H), 1.49 (s, 3 H), 1.45 (s, 9 H), 1.29 (s, 3 H), 0.89 (d, 3 H, J = 7.33 Hz). Anal Calcd for $C_{15}H_{26}O_6$: C, 59.57; H, 8.67. Found C, 59.56; H, 8.64.

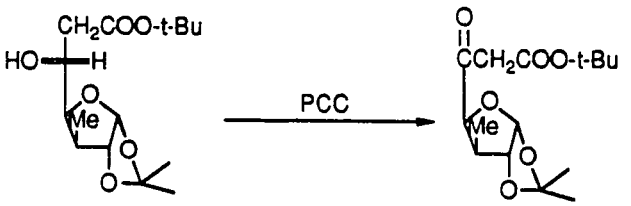
t-Butyl Esters 29a and 29b by Reformatsky Reaction.



21 **29a** **29b**

Anhydrous zinc (53 mg, 0.81 mmol), a small crystal of iodine, and THF (2 mL) were stirred and heated under reflux. A solution of t-butyl α-bromoacetate (65 μL, 0.41 mmol) and aldehyde **21** (50 mg, 0.27 mmol) in THF (1.5 mL) was added under a blanket of dry nitrogen. Within a few minutes, the solution became cloudy and the iodine color disappeared, indicating that the reaction started. The remainder of the solution was added to the zinc over 10 min, after which the reactants were stirred under reflux. The course of the reaction was monitored by TLC with 30% ethyl acetate in hexane. After 1 h reflux TLC showed disappearence of **21** (R_f 0.40) and the appearance of a new spot (R_f 0.24). The cooled solution was poured into 0.1 N HCl (1.0 mL). The excess zinc was filtered off and the mixture was extracted with ethyl acetate (5 x 5 mL). The organic layer was washed with saturated sodium bicarbonate, then water, and dried over anhydrous magnesium sulfate. The filtrate was concentrated under reduced pressure to yield crude **29** as a pale yellow oil that was flash chromatographed with 30% ethyl acetate in hexane. The major product **29b** (50 mg, 60% yield) exhibited a ^{1}H NMR spectrum identical with that found for the major product from reaction of t-butyl acetate with **21**. The minor product **29a** (0.4 mg, 0.48% yield) was also obtained.

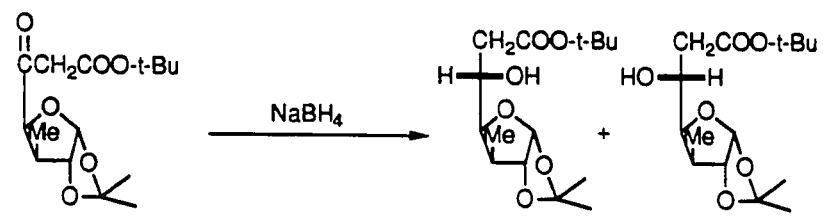
Ketoester 30.



29b **30**

To the solution of the **29b** (25.0 mg, 0.08 mmol) in methylene chloride (2.0 mL) was added PCC (34 mg, 0.16 mmol) at room temperature. The reaction was stirred for 24 h under nitrogen. After 24 h, TLC analysis with 30% ethyl acetate in hexane showed disappearance of **29b**. Filtration through a bed of florisil and evaporation of solvent afforded crude product. Flash chromatography with 30% ethyl acetate in hexane gave a ketoester **30** (20.0 mg, 80% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.85 (d, 1 H, J = 3.5 Hz), 4.74 (d, 1 H, J = 5.24 Hz), 4.39 (d, 1 H, J = 3.47 Hz), 3.48 (d, 2 H, J = 5.8 Hz), 2.70 (m, 1 H), 1.50 (s, 3 H), 1.46 (s, 9 H), 1.30 (s, 3 H), 0.90 (d, 3 H, J = 7.5 Hz); ^{13}C NMR (50 MHZ, $CDCl_3$) δ 190.97, 137.76, 129.49, 112.14, 111.94, 104.46, 86.35, 85.09, 81.95, 50.15, 43.15, 42.94, 29.86, 29.13, 27.02, 26.79, 11.84.

Alcohols 29a and 29b from Reduction of Ketone 30.

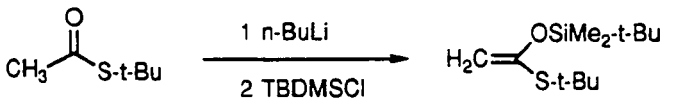


30 **29a** **29b**

The ketoester **30** (15.0 mg, 0.047 mmol) in dioxane (0.95 mL) and H_2O (0.2 mL) was treated with $NaBH_4$ (3.5 mg, 0.094 mmol) at 0 °C. The reaction mixture was warmed to room temperature, stirred for 1

h, and monitored by TLC with 25% ethyl acetate in hexane as developing solvent. Then, water (1.0 mL) was added very slowly. All solvent was evaporated under reduced pressure to afford a crude alcohol **29**. HPLC separation with 25% ethyl acetate in hexane as solvent gave two isomers **29a** (2.25 mg, 18% yield) and **29b** (13.5 mg, 72% yield). The **29a** to **29b** ratio was 1:5.

t-Butyldimethylsilyl Ketene Acetal 41.[21]

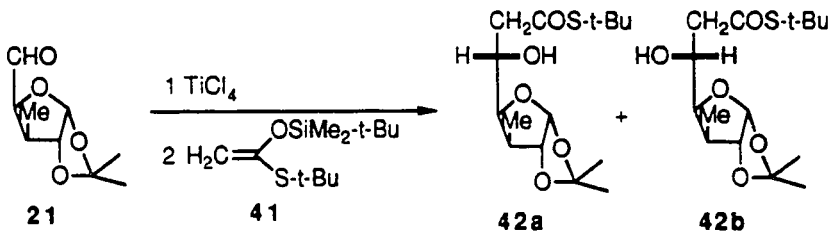


41

t-Butyl thioacetate was prepared from acetyl chloride and t-butyl mercaptan and fractionally distilled.[21] The yield was 80% with a bp of 135 °C (760 mmHg): ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3 H), 1.46 (s, 9 H). A solution of diisopropylamine (5.9 mL, 42.16 mmol) in THF (50 mL) was treated with a n-BuLi in hexane (1.6 M, 26 mL, 42.16 mmol) at 0 °C, under nitrogen, with stirring. After 20 min at 0 °C the solution was cooled to -78 °C and a solution of t-butyl thioacetate (5 mL, 35.13 mmol) in HMPA (15 mL) was slowly added over 5 min. After 30 min at -78 °C a solution of t-butyldimethylsilyl chloride (6.3 g, 42.16 mmol) in HMPA (15 mL) and hexane (7 mL) was added. Then the mixture was warmed to room temperature during 30 min, diluted with ice-cold pentane (200 mL), and washed with water (2 x 25 mL). The organic phase was separated and dried over anhydrous magnesium sulfate. The filtrate was concentrated under reduced pressure and the resulting crude product was purified by distillation

to give a colorless liquid **41** (6.0 g, 70% yield): bp 145 - 149 °C (20 mmHg); (lit[21] bp 145 (20 mmHg)); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.68 (s, 1 H), 4.66 (s, 1 H),1.36 (s, 9 H), 0.92 (s, 9 H), 0.18 (s, 6 H).

Thioesters 42a and 42b.

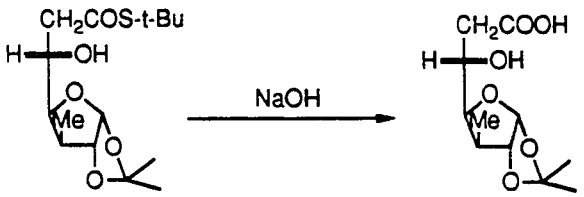


A solution of aldehyde **21** (1.40 g, 7.52 mmol) in methylene chloride (17.0 mL) was treated with $TiCl_4$ in methylene chloride (1.0 M, 7.52 mL, 7.52 mmol) at -78 °C, under nitrogen with stirring. After a few seconds, the t-butyldimethylsilyl ketene acetal **41** (2.98 g, 11.28 mmol) was added. After 1.5 h at -78 °C, the mixture was quenched with 1 N KOH and the organic phase was washed with saturated brine, dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure to give crude product **42** (2.8 g) which was purified twice by flash chromatography with 30% ethyl acetate in hexane as eluting solvent. **42a** (1.93 g, 80% yield) and **42b** (1.2 mg, 0.05% yield) were obtained. Major isomer **42a**: mp 48-49 °C; $[\alpha]_D^{25}$ -26.3° (c 1.15, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.83 (d, 1 H, J = 3.42 Hz), 4.37 (d, 1 H, J = 3.64 Hz), 4.11 (m, 2 H), 2.67 (dd, 1 H, J = 15.03, 8.19 Hz), 2.55 (dd, 1 H, J = 15.09, 3.21 Hz), 2.27 (m, 1 H), 1.51 (s, 3 H), 1.47 (s, 9 H), 1.31 (s, 3 H), 0.91 (d, 3 H, J = 7.38 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 198.18, 111.49, 104.28, 86.87, 82.00, 67.98, 48.62, 47.52, 40.42, 29.74, 26.72,

26.26, 11.01. Anal Calcd for $C_{15}H_{26}O_5S$: C, 56.58; H, 8.24. Found C, 56.51; H, 8.19.

Minor isomer **42b**: ^1H NMR (200 MHz, $CDCl_3$) δ 5.74 (d, 1 H, J = 3.42 Hz), 4.30 (d, 1 H, J = 3.42 Hz), 3.97 (m, 2 H), 2.65 (m, 2 H), 2.40 (m, 1 H), 1.54 (s, 3 H), 1.44 (s, 9 H), 1.27 (s, 3 H), 0.94 (d, 3 H, J = 7.43 Hz).

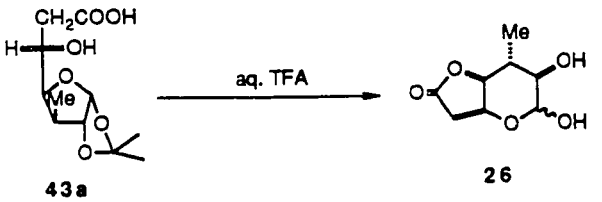
Hydroxy Acid 43a.



42a **43a**

Thioester **42a** (1.90 g, 5.97 mmol) was dissolved in 0.2 N NaOH (90 mL) and THF (90 mL). The solution was stirred at room temperature. After 20 min TLC showed a very polar product with ethyl acetate as developing solvent. The solution was neutralized by adding acetic acid and then evaporated under reduced pressure. Then the solution was diluted with aqueous NaCl (20 mL) and extracted with ethyl acetate (10 x 25 mL). The combined extracts were dried over anhydrous magnesium sulfate and concentrated. Flash chromatography with ethyl acetate afforded acid **43a** (1.29 g, 88% yield) as white crystals: mp 148 - 150 °C; $[\alpha]_D^{25}$ -16.0° (c 0.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.83 (d, 1 H, J = 3.47 Hz), 4.37 (d, 1 H, J = 3.58 Hz), 4.12 (m, 2 H), 2.48 (m, 2 H), 2.28 (m, 1 H), 1.50 (s, 3 H), 1.29 (s, 3 H), 0.89 (d, 3 H, J = 7.16 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 174.69, 111.64, 104.25, 86.94, 81.88, 67.54, 40.27, 37.54, 26.67, 26.17, 10.88. Anal Calcd for $C_{11}H_{18}O_6$: C, 53.63; H, 7.37. Found C, 53.55; H, 7.32.

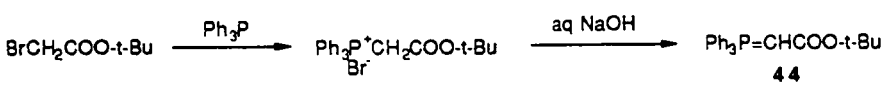
Lactone 26.



43a 26

To a solution of hydroxy acid **43a** (1.25 g, 5.08 mmol) and H_2O (2.0 mL) was added TFA (8.0 mL) in one portion. The reaction mixture was stirred at room temperature. After 10 min TLC showed disappearance of starting material and a new spot (R_f 0.25) with ethyl acetate as developing solvent. Evaporation under reduced pressure afforded crude product (0.82 g) as slightly yellow crystals. Flash chromatography with ethyl acetate gave lactone **26** (0.77 g, 80% yield) as white crystals: mp 145 - 146 °C; $[\alpha]_D^{25}$ -91.0° (c 0.5, CH_3CN_3); ^1H NMR (200 MHz, CD_3CN) δ 4.76 (d, 1 H, J = 5.04 Hz), 4.52 (dd, 1 H, J = 6.4, 4.5 Hz), 4.17 (dd, 1 H, J = 7.34, 4.34 Hz), 3.20 (dd, 1 H, J = 10.85, 5.16 Hz), 2.84 (dd, 1 H, J = 18.23, 6.67 Hz), 2.34 (d, 1 H, J = 19.05 Hz), 1.76 (m, 1 H), 1.14 (d, 3 H, J = 6.78 Hz); ^{13}C NMR (50 MHz, CD_3CN) δ 176.39, 98.38, 85.85, 73.44, 66.74, 37.33, 36.33 15.42. Anal.Calcd for $C_8H_{12}O_5$: C, 51.05; H, 6.43. Found: C, 51.26; H, 6.42.

t-Butyl (Triphenylphosphoranylidene)acetate (44).

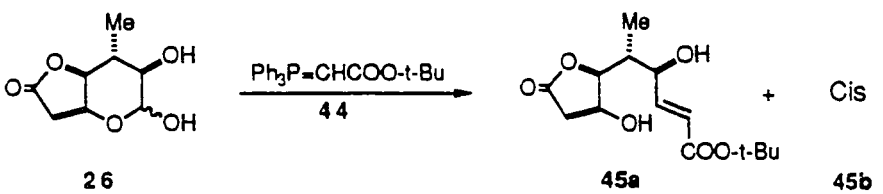


44

To a stirred solution of triphenylphosphine (8.12 g, 30.96 mmol) in THF (17 mL) was added t-butyl α-bromoacetate (5.0 mL, 30.96 mmol) at room temperature. Colorless crystals (phosphonium salt) were

obtained from the reaction mixture within a few minutes. This salt was collected by suction filtration and dried under vacuum. A solution of NaOH (1.24 g, 30.96 mmol) in water (5 mL) was added rapidly to a solution of phosphonium salt (14.0 g, 30.20 mmol) in methanol (10 mL). The mixture was then diluted with water (15 mL) and allowed to stand for 30 min at room temperature. The white precipitate was collected and dried in a vacuum oven at room temperature (11 g, 95% yield): mp 156 - 158 °C; (lit[44] mp 152 - 153 °C);^{1}H NMR (200 MHz, $CDCl_3$) δ 7.45 (m, 15 H), 2.60 (d, 1 H, J = 30.5 Hz), 1.23 (s, 9 H).

Wittig Products 45a and 45b.



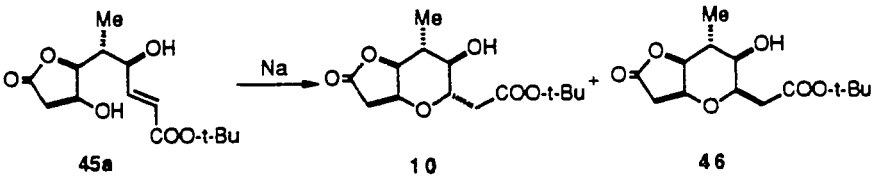
26 45a 45b

A solution of lactone **26** (0.75 g, 3.99 mmol) and ylide **44** (1.65 g, 4.39 mmol) in CH_3CN (15 mL) was heated to reflux under nitrogen for 5 h. Removal of solvent afforded crude product (1.4 g). Flash chromatography with 5% methanol in chloroform gave pure trans product **45a** (0.929 g, 80% yield) as a syrup. Upon prolonged standing, it formed a white crystalline mass. The remaining fractions containing cis product **45b** and heterocyclization products **10** and **46**, and triphenylphosphine oxide were subjected to flash chromatography with 30% acetonitrile in methylene chloride to remove the

triphenylphosphine oxide (R_f 0.24). A mixture of cis product **45b** and heterocyclization products **10** and **46** were then isolated from appropriate fractions by HPLC with 40% ethyl acetate in hexane. After HPLC, cis product **45b** (57.0 mg, 5% yield) was obtained as a syrup. On standing, it formed a white crystalline mass. Also obtained were the major heterocyclization product **10** (11.4 mg, 1.0% yield) and a tiny amount of minor heterocyclization product **46** (4.0 mg, 0.35% yield). Trans α,β-unsaturated ester **45a**: mp 119 - 121 °C; $[\alpha]_D^{25}$ -37.9° (c 1.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 6.90 (dd, 1 H, J = 15.6, 5.0 Hz), 6.05 (dd, 1 H, J = 15.6, 1.8 Hz), 4.50 (m, 2 H), 4.07 (dd, 1 H, J = 10.4, 3.0 Hz), 2.78 (dd, 1 H, J = 17.84, 5.26 Hz), 2.58 (m, 2 H), 1.51 (s, 9 H), 1.20 (d, 3 H, J = 6.94 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.42, 165.11, 143.73, 124.31, 86.52, 81.32, 73.19, 68.34, 38.40, 37.52, 28.06, 14.34. Anal. Calcd for $C_{14}H_{22}O_6$: C, 58.71; H, 7.75. Found: C, 58.41; H, 7.62.

Cis α,β-unsaturated ester **45b**: mp 57 - 59 °C; $[\alpha]_D^{25}$ -85.0° (c 0.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 6.29 (dd, 1 H, J = 11.99, 6.19 Hz), 5.83 (dd, 1 H, J = 11.99, 1.85 Hz), 5.03 (br, 1 H), 4.54 (br, 2 H), 4.31 (br, 1 H), 4.23 (dd, 1 H, J = 11.99, 1.85 Hz), 2.71 (dd, 1 H, J = 17.58, 4.88 Hz), 2.53 (d, 1 H, J = 17.47 Hz), 2.41 (m, 1 H), 1.45 (s, 9 H), 1.13 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.79, 166.83, 148.58, 123.35, 86.31, 82.57, 68.66, 68.13, 38.65, 37.31, 27.99, 12.20. Anal. Calcd for $C_{14}H_{22}O_6$: C, 58.71; H, 7.75. Found C, 58.44; H, 7.78.

Michael Addition Products 10 and 46.

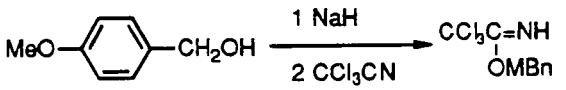


To a stirred solution of α,β-unsaturated ester **45** (0.90 g, 3.14 mmol) in THF (4 mL) was added a pinch of sodium. The resulting reaction mixture was stirred under nitrogen. After 1 h, TLC showed completion of the reaction. The reaction mixture was filtered with ethyl acetate through a small pipette column packed with silica gel. Evaporation of solvent afforded a crude product (0.91 g). The crude product was purified twice by flash chromatography with 50% ethyl acetate in hexane to give the major isomer **10** (0.79 g, 88%) as white crystals and the minor isomer **46** (18.0 mg, 2%) as white crystals. Major tetrahydropyran product **10**: mp 95 - 96 °C; $[\alpha]_D^{25}$ -21.0° (c 0.1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 4.49 (dd, 1 H, J = 12.48, 6.08 Hz), 4.19 (dd, 1 H, J = 8.08, 5.83 Hz), 3.84 (ddd, 1 H, J = 12.45, 8.14, 4.93 Hz), 3.24 (ddd, 1 H, J = 12.56, 9.82, 5.74 Hz), 2.61 (m, 4 H), 1.92 (m, 1 H), 1.44 (s, 9 H), 1.23 (d, 3 H, J = 6.62 Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 174.45, 170.88, 83.13, 81.54, 73.82, 72.45, 68.92, 39.21, 39.06, 32.86, 28.03, 15.10. Anal. Calcd for $C_{14}H_{22}O_6$: C, 58.71; H, 7.75. Found C, 58.88; H, 7.78.

Minor tetrahydropyran product **46**: mp 71 - 73 °C; $[\alpha]_D^{25}$ -32.3° (c 0.14, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 4.77 (m, 2 H), 3.87 (m, 1 H), 3.79 (dd, 1 H, J = 9.17, 4.07 Hz), 3.37 (d, 1 H, J = 3.85 Hz), 2.69 (m, 3 H), 2.34 (dd, 1 H, J = 16.82, 8.35 Hz), 1.61 (m, 1 H), 1.44 (s, 9 H), 1.02 (d, 3 H, J = 7.43 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.75, 172.72, 114.06, 90.05,

81.76, 81.55, 66.70, 40.17, 39.85, 36.46, 28.12, 10.48. Anal. Calcd for $C_{14}H_{22}O_6$: C, 58.71; H, 7.75. Found C, 58.82; H, 7.73.

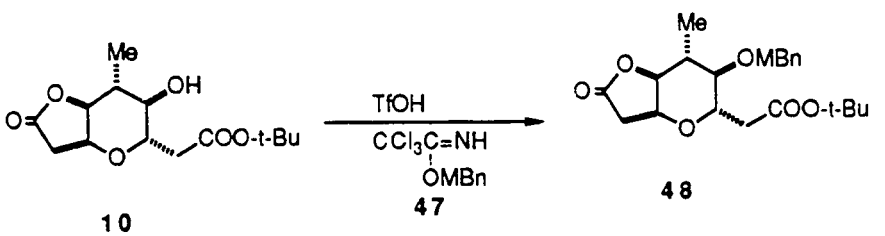
4-Methoxybenzyl Trichloroacetimidate (47).[27]



47

4-Methoxybenzyl alcohol (20 g, 145 mmol) was dissolved in ether (40 mL) and added dropwise to a suspension of NaH (50% oil dispersion, 0.35 g, 14.5 mmol) in ether (15 mL). Trichloroacetonitrile (19.7 g, 137 mmol) was added over 20 min at 5 °C, then the resulting solution was allowed to warm to room temperature and stirred for 1 h. The solution was concentrated under reduced pressure and pentane (15 mL) and methanol (1.0 mL) were added. Adding more pentane caused a dark oil phase which was filtered using a course frit. Solvent was evaporated to give a brown sticky oil which was purified by vacuum distillation. (bp 150 - 155 °C/0.1 torr) (lit[27] bp 135 - 137 °C/0.7 torr). Imidate **47** (30 g, 80% yield) was obtained: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.4 (br, 1 H), 7.25 (m, 2 H), 6.97 (m, 2 H), 5.23 (s, 2 H), 3.80 (s, 3 H).

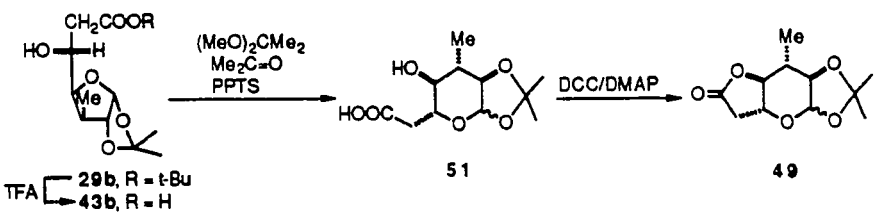
MBn Ether 48.



10 47 48

To a stirred solution of the major tetrahydropyran **10** (100 mg, 0.35 mmol) and imidate **47** (200 mg, 0.70 mmol) in CH_2Cl_2 (3 mL) was added TfOH (3μL, 0.035 mmol). After a few seconds, the reaction mixture became hazy. After 1 h, TLC with 30% ethyl acetate in hexane showed disappearance of **10** and appearance of a new spot (Rf 0.20). Filtration and evaporation of solvent afforded the crude product which was purified twice by flash chromatography to give pure MBn ether **48** as white crystals (114 mg, 80% yield): mp 96 - 98 °C; $[\alpha]_D^{25}$ -38.1° (c 0.21, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.22 (m, 2 H), 6.87 (m, 2 H), 4.50 (m, 3 H), 4.26 (dd, 1 H, J = 7.44, 6.46 Hz), 3.92 (m, 1 H), 3.79 (s, 3 H), 3.06 (t, 1 H, J = 8.68 Hz), 2.62 (m, 3 H), 2.32 (dd, 1 H, J = 15.30, 9.34 Hz), 2.06 (m, 1 H), 1.42 (s, 9 H), 1.21 (d, 3 H, J = 6.84 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 175.24, 169.86, 159.49, 129.84, 129.60, 113.94, 82.77, 81.01, 78.98, 73.27, 72.22, 69.14, 55.32, 38.96, 38.15, 32.91, 28.12, 16.07. Anal. Calcd for $C_{22}H_{30}O_7$: C, 64.99; H, 7.44. Found C, 65.02; H, 7.35.

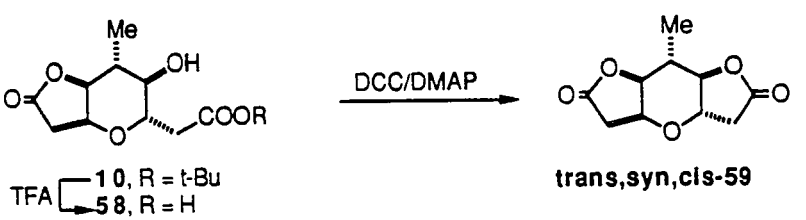
Trans Lactone 49.



A solution of the **29b** (50.0 mg, 0.17 mmol) and a few drops of TFA in CH_2Cl_2 (3.0 mL) was stirred at room temperature. After 6 h, TLC with ethyl acetate showed disappearance of **29b** and appearance of

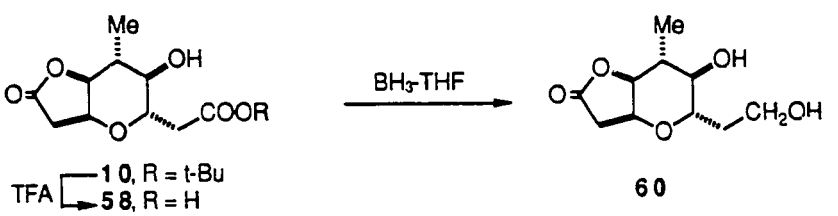
a polar product **43b**. Removal of TFA and CH_2Cl_2 under reduced pressure and addition of TFA (few drops) and H_2O (1.5 mL) and stirring overnight afforded a very polar product. The TFA and H_2O were removed by rotary evaporation. Stirring of this polar compound overnight with 2,2-dimethoxypropane (42 μL, 0.34 mmol), acetone (1.0 mL), PPTS (4.5 mg, 0.018 mmol) gave two products (pyranose **51** and furanose **43b**, ratio 7:1 from ^1H NMR). After removal of all solvents, the crude product was subjected to lactonization by adding DCC (42 mg, 0.20 mmol), and DMAP (2.0 mg, 0.017 mmol) in CH_2Cl_2 (3.0 mL). After 4 h stirring at room temperature under nitrogen, evaporation of solvent afforded a crude product. Flash chromatography with 30% ethyl acetate in hexane gave pure trans lactone **49** as white crystals (27.20 mg, overall 70% yield from **29b**): mp 83 - 85 °C; $[\alpha]_D^{25}$ -10.0° (c 0.4, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 5.51 (d, 1 H, J = 4.34 Hz), 4.36 (m, 1 H), 3.80 (dd, 1 H, J = 5.48, 4.34 Hz), 3.56 (dd, 1 H, J = 11.28, 9.16 Hz), 2.79 (dd, 1 H, J = 16.11, 7.32 Hz), 2.61 (dd, 1 H, J = 16.11, 11.72 Hz), 2.13 (m, 1 H), 1.52 (s, 3 H), 1.36 (s, 3 H), 1.25 (d, 3 H, J = 6.72 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 172.54, 108.16, 98.43, 82.59, 78.33, 70.87, 39.36, 35.52, 27.88, 26.61, 16.94. Anal. Calcd for $C_{11}H_{16}O_5$: C, 57.87; H, 7.07. Found C, 57.75; H, 7.04.

Dilactone 59.



A solution of cis lactone **10** (30 mg, 0.10 mmol) and a few drops of TFA in CH_2Cl_2 (2.5 mL) was stirred at room temperature. After 4 h, TLC with ethyl acetate showed disappearance of **10** and appearance of a new polar spot. Evaporation of CH_2Cl_2 and TFA afforded crude acid **58**. The crude acid **58** was subjected to lactonization by adding DCC (25.0 mg, 0.12 mmol), DMAP (1.2 mg, 0.01 mmol), and CH_2Cl_2 (3.0 mL). The reaction mixture was stirred at room temperature under nitrogen. After 3 h, filtration and evaporation afforded crude product. Flash chromatography with 40% ethyl acetate in hexane gave dilactone **59** as white crystals (18.3 mg, 86% yield): mp 101 - 103 °C; $[\alpha]_D^{25}$ -38.1° (c 0.21, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 4.86 (ddd, 1 H, J = 10.25, 8.29, 1.99 Hz), 4.29 (dd, 1 H, J = 8.19, 7.11 Hz), 4.00 (ddd, 1 H, J = 11.61, 9.12, 2.49 Hz), 3.56 (dd, 1 H, J = 11.56, 9.12 Hz), 2.76 (m, 4 H), 2.05 (m, 1 H), 1.32 (d, 3 H, J = 6.34 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 171.98, 171.56, 81.34, 80.64, 72.72, 71.84, 40.02, 35.49, 29.78, 15.62. Anal. Calcd for $C_{10}H_{12}O_5$: C, 56.59; H, 5.70. Found C, 56.61; H, 5.73.

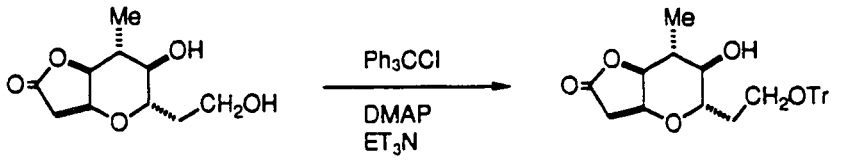
Diol 60.



A solution of **10** (20 mg, 0.07 mmol) and a few drops of TFA in CH_2Cl_2 (2 mL) was stirred at room temperature. After 3 h, TLC analysis with 50% ethyl acetate in hexane showed disappearance of **10**

and appearance of the polar acid **58** (R_f 0.06). TFA and CH_2Cl_2 were completely removed under reduced pressure. The crude residual acid **58** was used for reduction with borane without further purification. A solution of **58** in THF (2 mL) was cooled to 0 °C under nitrogen. Then borane in THF (1.0 M, 70 μL, 0.07 mmol) was added slowly. There was hydrogen evolution during the course of the addition. The ice-bath was removed and replaced by a water bath. After 30 min, TLC analysis with 7% methanol in chloroform showed a new less polar spot (R_f 0.23) and the disappearance of **58**. The reaction mixture was hydrolyzed with water (0.5 mL), and diluted with ethyl acetate (3 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 3 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. The crude residual product was purified by flash chromatography with 7% methanol in chloroform to give diol **60** (14.0 mg, 93% yield) as white crystals: mp 156 - 157 °C; $[\alpha]_D^{25}$ -67.5° (c 1.34, CH_3CN); ^1H NMR (200 MHz, CD_3CN) δ 4.43 (m, 1 H), 4.18 (dd, 1 H, J = 8.19, 5.48 Hz), 3.54 (m, 3 H), 3.09 (m, 1 H), 2.79 (t, 1 H, J = 5.21 Hz), 2.66 (dd, 1 H, J = 17.9, 7.11 Hz), 2.51 (dd, 1 H, J = 17.9, 4.72 Hz), 1.74 (m, 3 H), 1.14 (d, 3 H, J = 6.62 Hz); ^{13}C NMR (75 MHz, CD_3CN) δ 176.04, 85.01, 76.42, 72.90, 69.17, 59.52, 39.95, 35.42, 34.08, 15.51. Anal. Calcd for $C_{10}H_{16}O_5$: C, 55.53; H; 7.46. Found C; 55.56, H; 7.49.

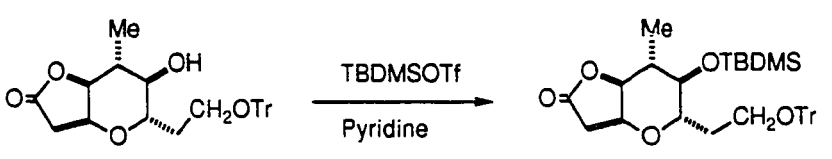
Trityl Ether 61.



60 61

To a stirred solution of diol **60** (14 mg, 0.065 mmol) and Et_3N (16.0 μL, 0.065 mmol) was added DMAP (0.80 mg, 0.006 mmol) and TrCl (20 mg, 0.07 mmol) in CH_2Cl_2 (2.0 mL) under nitrogen, and the resulting mixture was stirred at room temperature. After stirring overnight, TLC analysis showed disappearance of diol **60** and appearance of a new spot (R_f 0.35) with 60% ethyl acetate in hexane. The solvent was removed by rotary evaporation. The residue was diluted with water (2.0 mL) and ethyl acetate (4.0 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 x 4 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography with 60% ethyl acetate in hexane as eluent to furnish the trityl ether **61** (26.8 mg, 90% yield) as white crystals: ^1H NMR (200 MHz, $CDCl_3$) δ 7.35 (m, 15 H), 4.40 (q, 1 H, J = 6.24 Hz), 4.14 (dd, 1 H, J= 8.19, 5.81 Hz), 3.58 (m, 2 H), 3.25 (m, 2 H), 2.63 (dd, 2 H, J = 17.24, 5.32 Hz), 1.94 (m, 3 H), 1.22 (d, 3 H, J = 6.62 Hz).

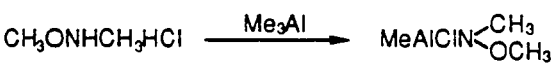
TBDMS Ether 62.



61 62

To a stirred solution of **61** (26.8 mg, 0.059 mmol) in CH_2Cl_2 (2.0 mL) was added pyridine (9.38 μL, 0.118 mmol) under nitrogen, and the resulting reaction mixture was cooled to 0 °C. TBDMS-OTf (26.8 μL, 0.118 mmol) was added slowly, and the reaction mixture was stirred 1 h at 0 °C and then warmed to room temperature. The solvents were removed by rotary evaporation and the residue was purified by flash chromatography with 20% ethyl acetate in hexane. The silyl ether product **62** (32.1 mg, 95% yield) was obtained as white crystals: ^1H NMR (200 MHz, $CDCl_3$) δ 7.30 (m, 15 H), 4.30 (m, 1 H), 4.13 (dd, 1 H, J = 7.86, 5.81 Hz), 3.71 (m, 1H), 3.16 (m, 3 H), 2.53 (dd, 2 H, J = 18.12, 5.32 Hz), 2.00 (m, 2 H), 1.62 (m, 1 H), 1.13 (d, 3 H, J = 6.84 Hz), 0.94 (s, 9 H), 0.11 (s, 3 H), 0.10 (s, 3 H).

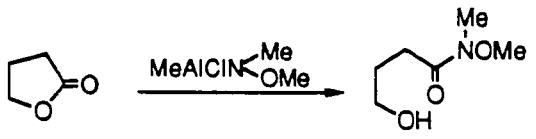
Preparation of 0.68 M Stock Solution of N-Methoxy-N-methylmethylchloroaluminum Amide (69).[31]



69

To a suspension of N,O-dimethylhydroxylamine hydrochloride (3 g, 30.75 mmol) in freshly distilled benzene (30 mL) at 5 °C was slowly added trimethylaluminum in toluene (2 M, 15.4 mL, 30.80 mmol). After addition was complete, the reaction mixture was allowed to warm to room temperature and was stirred for 1.5 h until gas evolution had ceased.

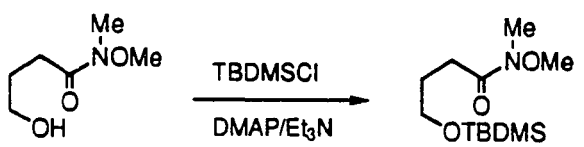
Amide 70.



70

To a solution of γ-butyrolactone (1.0 mL, 13.00 mmol) in freshly distilled benzene (130 mL) was added aluminum amide **69** in benzene (0.68 M, 39 mL, 26.00 mmol) under nitrogen. This mixture was refluxed under nitrogen for 3 h. The reaction mixture was cooled to room temperature and quenched with 5% HCl (30 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 30 mL). The combined organic extracts were dried over anhydrous magnesium sulfate. Filtration and concentration by rotary evaporation gave a crude product (2.3 g). Flash chromatography (R_f 0.18) with ethyl acetate as eluting solvent afford pure amide **70** (1.62 g, 85% yield) as a colorless oil: ^1H (200 MHz, $CDCl_3$) δ 3.71 (s, 3 H), 3.67 (t, 2 H, J = 4.60 Hz), 3.20 (s, 3 H), 2.62 (t, 2 H, J = 6.67 Hz), 1.90 (m, 2 H).

TBDMS Ether 71.

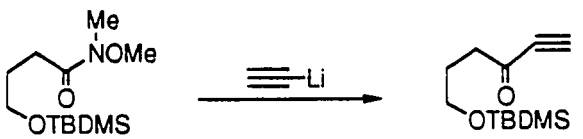


70 **71**

To a stirred solution of amide **70** (0.5 g, 34 mmol) in dry methylene chloride (10 mL) was added triethylamine (0.56 mL, 1.2 equiv) and N,N-dimethylaminopyridine (42 mg, 0.1 equiv). The resulting reaction mixture was stirred for 5 min at room temperature and TBDMS-Cl (0.62 g, 1.2 equiv) was slowly added. The reaction was

monitored by TLC (product R_f 0.36 with 30% ethyl acetate in hexane). Formation of product was quantitative after 3 h. After addition of water (5 mL), the organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate. Filtration and conconcentration by rotary evaporation gave a crude product (0.92 g). Flash chromatography eluting with 30% ethyl acetate in hexane afforded a pure product **71** (0.854 g, 96% yield) as a colorless oil: ^{1}H (200 MHz, $CDCl_3$) δ 3.63 (s, 3 H), 3.62 (t, 2 H, J = 4.56 Hz), 3.12 (s, 3 H), 2.46 (t, 2 H, J = 7.7 Hz) 1.80 (m, 2 H), 0.85 (s, 9 H), 0.012 (s, 6 H).

Propargyl Ketone 72.

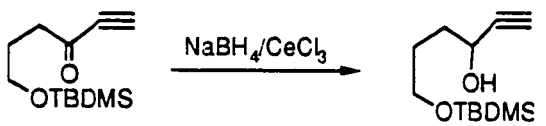


71 **72**

Dry tetrahydrofuran (8 mL) was placed into a nitrogen-filled 25 mL flask fitted with a septum, and a magnetic stirring bar. Cylinder acetylene was purified before use by passing through saturated aqueous sodium bisulfite ($Na_2S_2O_5$) and sulfuric acid and a dry-ice acetone trap and calcium chloride drying tube. Acetylene was bubbled through the THF. After 30 min bubbling, n-BuLi in hexane (2.5 M, 0.54 mL, 1.35 mmol) was added dropwise at 0 °C under nitrogen. Then bubbling of acetylene was maintained for an additional 1 h. The amide **71** (70 mg, 027 mmol) in tetrahydrofuran (2.0 mL) was added dropwise to the solution of lithium acetylide at 0 °C. Stirring was continued for 2 h at room temperature. Addition of saturated ammonium chloride

(5.0 mL) and evaporation of solvent gave an oily residue which was suspended in water (5.0 mL) and then extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and solvents were evaporated to give a crude product (62 mg). This crude product (R_f 0.20) was purified by flash chromatography with 5% ethyl acetate in hexane as eluting solvent to give pure ketone **72** (55 mg, 91% yield): ^1H (200 MHz, $CDCl_3$) δ 3.61 (t, 2 H, J = 6.0 Hz), 3.18 (s, 1 H), 2.65 (t, 2 H, J = 7.0 Hz) 1.88 (m, 2 H), 0.86 (s, 9 H), 0.013 (s, 6 H).

Propargyl Alcohol 73.

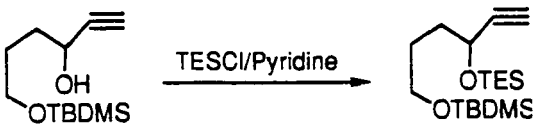


72 73

The propargyl ketone **72** (50 mg, 0.22 mmol) was dissolved in a solution of cerium chloride heptahydrate in methanol (0.4 M, 0.61mL, 1.1 equiv) and sodium borohydride (8.3 mg, 1 equiv) was slowly added with stirring at 0 °C. The mixture was allowed to react for 30 min. Excess hydride was then destroyed by addition of aqueous ammonium chloride (2.0 mL). The mixture was extracted with ethyl acetate (3 x 5 mL) and dried over anhydrous magnesium sulfate. Filtration and concentration by rotary evaporation gave a crude product (52 mg). Flash chromatography (R_f 0.22) with 15% ethyl acetate in hexane as eluting solvent gave pure propargyl alcohol **73** (47 mg, 94% yield) as a colorless oil: ^1H (200 MHz, $CDCl_3$) δ 4.42 (m, 1 H), 3.67 (m, 2 H), 3.37 (d,

1 H, J = 6.18 Hz), 2.41 (d, 1 H, J = 2.11 Hz), 1.75 (m, 4 H), 0.88 (s, 9 H) 0.053 (s, 6 H).

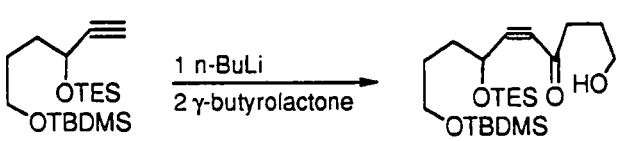
TES Ether 74.



73 **74**

The propargyl alcohol **73** (44 mg, 0.19 mmol) was dissolved in pyridine (4.0 mL) and triethylsilyl chloride (38 μL, 1.2 equiv) was slowly added at room temperature under nitrogen. Then, the mixture was refluxed at 50 - 60 °C for 1 h. The reaction was monitored by TLC. Formation of product was quantitative after 1 h. After cooling to room temperature, the mixture was diluted with water (10 mL) and ethyl acetate (15 mL). The ethyl acetate layer was separated and washed with water (2 x 5 mL) to remove pyridine and then dried over magnesium sulfate. The filtrate was concentrated by rotary evaporation to give a crude product (0.82 g). Flash chromatography (R_f 0.66) with 15% ethyl acetate in hexane as eluting solvent gave **74** (60 mg, 91% yield) as a colorless oil: ^{1}H (200 MHz, $CDCl_3$) δ 4.37 (m, 1 H), 3.62 (t, 2 H, J = 6.02 Hz), 2.35 (d, 1 H, J = 2.06 Hz), 1.65 (m, 4 H), 0.95 (t, 9 H, J = 8.03 Hz), 0.869 (s, 9 H), 0.664 (q, 6 H, J = 7.94 Hz), 0.024 (s, 6 H).

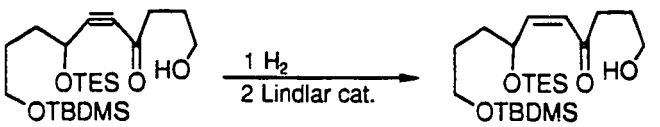
Ketone 75b.



74 **75b**

Acetylene **74** (33.6 mg, 0.098 mmol) and dry THF (200 μL) were placed in a 5 mL flask under nitrogen. n-BuLi in hexane (1.6 M, 65 μL, 1.0 equiv) was added dropwise (rapidly) at 0 C°. After 5 min, the soltuion was transferred into a second nitrogen-filled flask containing a magnetically stirred solution of γ-butyrolactone (7.3 μL, 1.0 equiv) in THF (200 μL). After the resulting solution was stirred for 2 h at 0 °C, TLC showed no more progress of the reaction. The reaction was quenched with saturated ammonium chloride (10 μL). All solvent was evaporated by rotary evaporation. The residue was diluted with water (1 mL) and extracted with ethyl acetate (3 x 3 mL). The ethyl acetate extracts were dried over anhydrous magnesium sulfate, filtered, and the filtrate was concentrated by rotary evaporation to give a crude product (44.5 mg). The crude product was purified by flash chromatography eluting with 15% ethyl acetate in hexane. The fractions containing the starting material **74** (R_f 0.90) were collected, and the solvent was removed by rotary evaporation to give starting material **74** (23.4 mg). The product **75b** (R_f 0.17) was collected and concentrated by rotary evaporation to give ketone **75b** (10.2 mg, 80% yield based on consumed starting material) as a colorless oil: ^{1}H (200 MHz, $CDCl_3$) δ 4.53 (t, 1 H, J = 6.08 Hz), 3.64 (m, 4 H), 2.68 (t, 2 H, J = 7.11 Hz), 1.76 (m, 6 H), 0.95 (t, 9 H, J = 8.09 Hz), 0.87 (s, 9 H), 0.62 (q, 6 H, J = 7.97 Hz), 0.024 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.38, 93.40, 82.89, 62.55, 62.33, 61.56, 41.96, 34.49, 28.27, 26.55, 25.85, 18.23, 6.64, 4.59. -5.40; mass spectrum m/z (M^+) for $C_{22}H_{44}O_4Si_2$ calcd 428.2778, found 428.2800.

cis-Alkene 76b.



75b **76b**

Acetylenic ketone **75b** (20 mg, 0.047 mmol) was dissolved in methanol (2.0 mL) and hydrogenated over 5% palladium on barium sulfate (2.5 mg) after addition of synthetic quinoline (2 μL). Slightly less than one equivalent of hydrogen (1.0 mL) was absorbed in 5 min. TLC analysis with 15% ethyl acetate in hexane showed a small amount of starting **75b** (R_f 0.26) and the alkene product **76b** (R_f 0.21). The reaction mixture was filtered through a bed of celite to remove the catalyst and rinsed with additional methanol (5 mL). The filtrate was concentrated by rotary evaporation to afford a viscous, dark yellow liquid. This crude alkene was purified by flash chromatography eluting with 15% ethyl acetate in hexane to deliver alkene product **76b** (17.8 mg, 90% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.03 (dd, 2 H, J = 11.5, 6.35 Hz), 5.20 (m, 1 H), 3.60 (m, 4 H), 2.61 (t, 2 H, J = 7.14 Hz), 1.85 (m, 2 H), 1.53 (m, 4 H), 0.886 (t, 9 H, J = 7.93 Hz), 0.848 (s, 9 H), 0.50 (q, 6 H, J = 7.98 Hz), 0.003 (s, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 201.10, 152.18, 124.07, 68.63, 63.28, 62.15, 40.85, 33.62, 28.54, 26.55, 25.95, 18.33, 6.77, 4.74, -5.26; mass spectrum m/z (M^+) for $C_{22}H_{46}O_4Si_2$ calcd 430.2934, found 430.2941.

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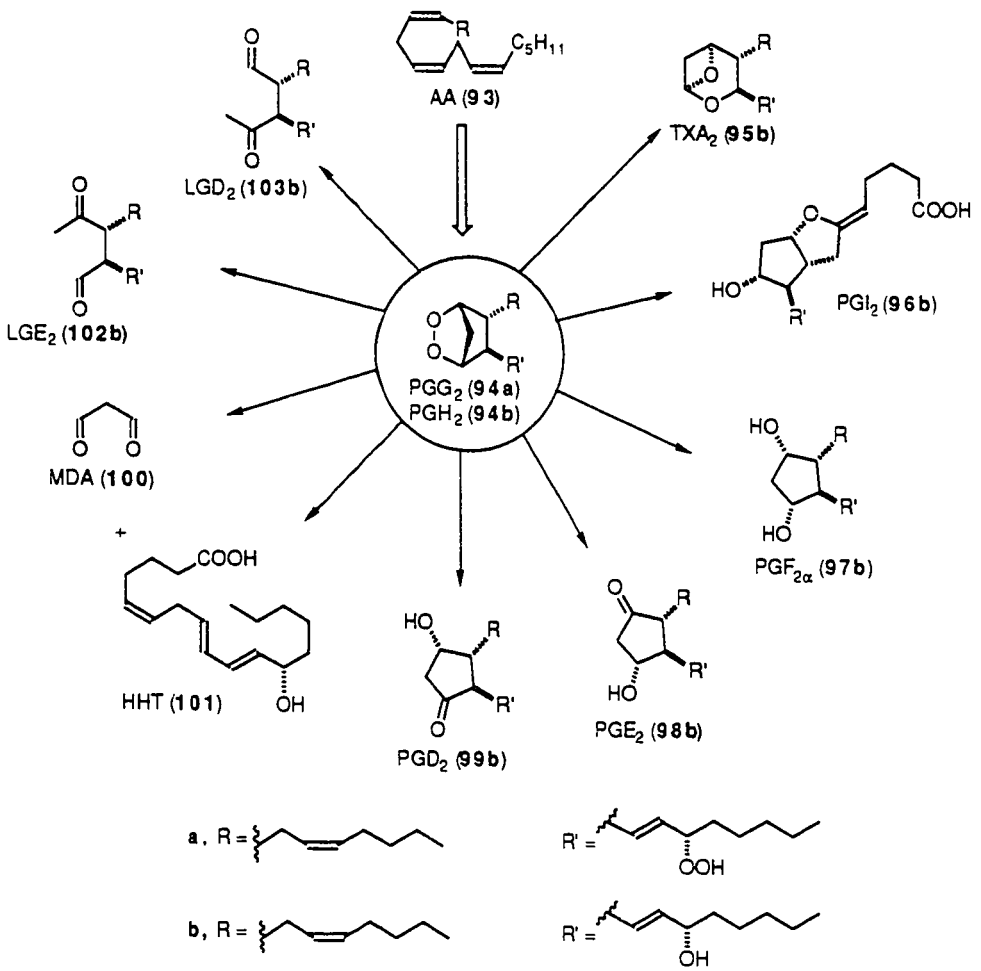
PART 2. LEVUGLANDIN-PROTEIN ADDUCTS: SYNTHESIS OF AN ANTIGEN FOR IMMUNOASSAY

Introduction

History. In the biosynthesis of a diverse array of biologically active oxidative metabolites of essential fatty acids (Scheme IX), two atoms of molecular oxygen are streospecifically introduced into arachidonic acid (AA, **93**), producing the highly reactive prostaglandin endoperoxides PGG_2 (**94a**) and PGH_2 (**94b**).[45]

Scheme IX

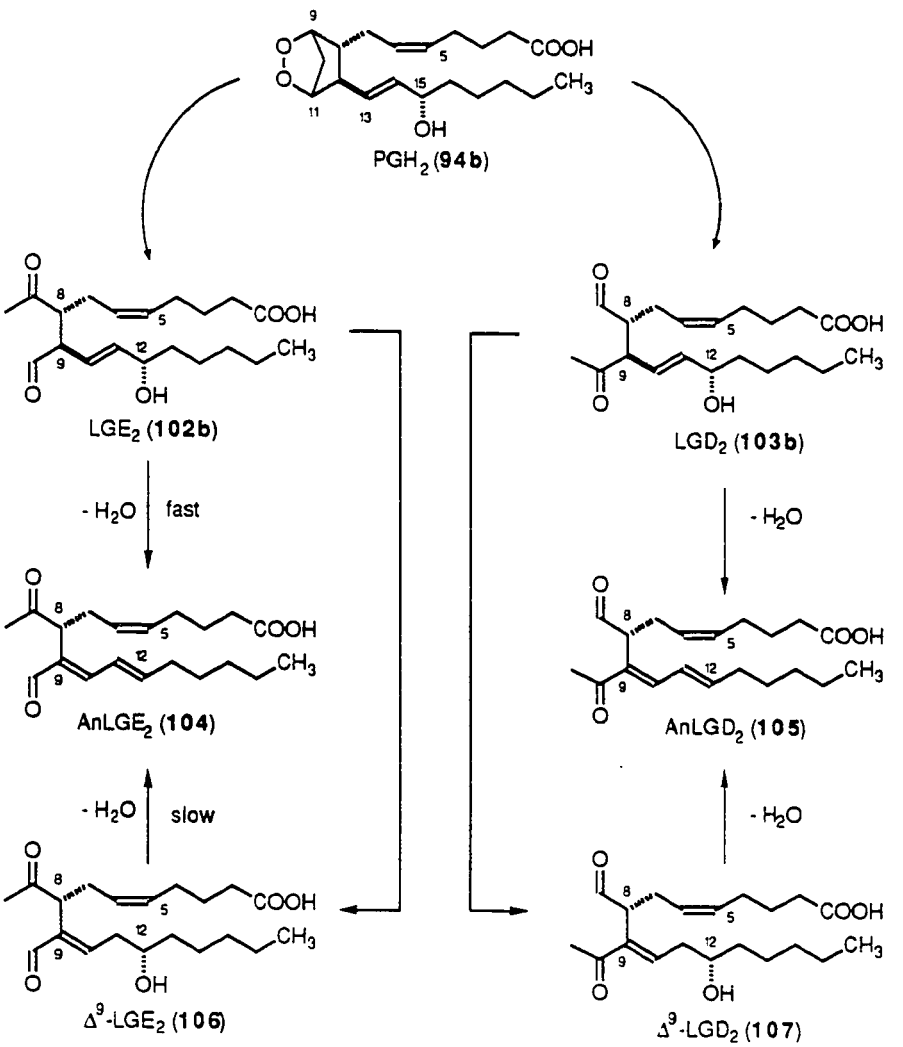


The endoperoxide PGH_2 (**94b**) is a pivotal intermediate in the

biosynthesis of thromboxane A_2 (TXA_2, **95b**), prostacyclin (PGI, **96b**), prostaglandins (PGs),[46] and levuglandins (LGs).[47]

Recently we discovered a new rearrangement pathway of PGH_2 (**94b**) leading to levuglandins.[47,48] Levuglandin E_2 (LGE_2, **102b**) is a 1,4-ketoaldehyde with a 10,11-seco prostanoic acid structure, while levuglandin D_2 (LGD_2, **103b**) is a 1,4-ketoaldehyde and possesses a 9,10-seco prostanoic acid structure.

Scheme X



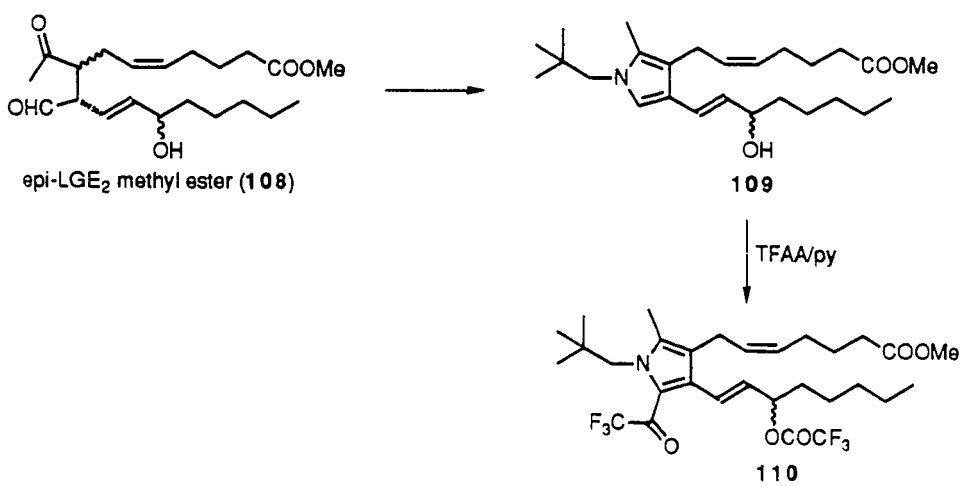
These vinylogous β-hydroxy carbonyl compounds undergo a facile dehydration (Scheme X) to provide highly UV active products, anhydro levuglandin E_2 ($AnLGE_2$, **104**) and anhydro levuglandin D_2 ($AnLGD_2$, **105**). LGE_2 (**102b**) and LGD_2 (**103b**) also undergo an allylic rearrangement to Δ^9-levuglandins, Δ^9-LGE_2 (**106**) and Δ^9-LGD_2 (**107**). The nomenclature of these compounds is based on their ***hypothetical*** chemical relationship to prostaglandins PGE_2 (**98b**) and PGD_2 (**99b**) by aldol condensations.

Covalent adduct formation between proteins,[49] peptides,[50,51] or DNA[52,53] and unidentified electrophilic products from PGH_2 is widely documented. The difficulty of characterizing the molecular structures of the adducts with proteins was further complicated by the fact that binding of the unidentified electrophiles with proteins results in crosslinking. **We postulate that the unidentified electrophilic products are LGs.** Presumptive evidence supporting this hypothesis is provided by our group's observations that LGE_2 binds covalently with proteins resulting in intermolecular crosslinking.[53,54]

Covalent adduct formation with endogeneous nucleophiles will complicate detection and quantification of levuglandins *in vivo*. The reaction of LGE_2 (**102b**) with protein is rapid and generates a complex mixture of products. Thus, free LG's generated *in vivo* could be rapidly sequestered from the reaction milieu making their detection difficult. Since LGE_2 (**102b**) incorporates a γ-ketoaldehyde array, it seemed reasonable to expect Paal-Knorr condensation with the primary amino groups of proteins would produce pyrroles.[55,56] Of the

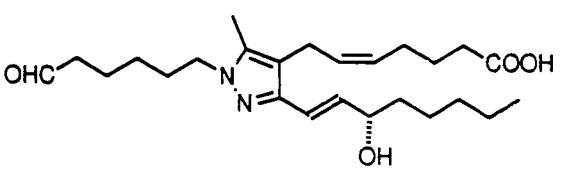
products formed, pyrroles have stereochemically defined structures and little tendency to react further. Our efforts were therefore directed toward detecting protein-bound levuglandins instead of free levuglandins themselves.[57] An immunoassay for such LGE_2-derived pyrroles would provide the sensitivity needed to detect the anticipated low levels of LG-adducts formed *in vivo*.

In model studies, conducted by Raj Iyer,[57] LGE_2-derived pyrrole **109** was generated by condensation of neopentyl amine with **108** in ethanol in >70% yield.[58] Unfortunately on standing at room temperature, complete disappearance of **109** was evidenced by both tlc analysis and loss of characteristic pyrrole resonances in the ^1H NMR spectrum.[57] The high air instabiliy of LG-derived pyrroles suggested that the use of protein conjugates of such π-electron-rich pyrroles, as antigens to raise antibodies for an immunoassay, would be difficult. It was thought that the electron-rich nature of the pyrrole ring was an important factor leading to its decomposition.

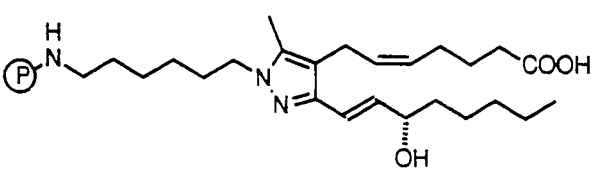


An electron withdrawing substituent, such as a trifluoroacetyl group, might stabilize such pyrroles. In fact, trifluoroacetylated LGE_2-pyrrole adduct **110** was isolated in 15% yield from LGE_2 following *in situ* reaction of the pyrrole **109** with trifluoroacetic acid anhydride.[57] Aside from the low yield of **110** by this procedure,[57] the highly alkylated and conjugated ring in LGE_2-pyrrole adducts like **109** is very nucleophilic.

Pyrazole Isostere of LG-derived Pyrroles. As discussed above, the development of an immunoassay for protein bound LGE_2-derived pyrrole antigen was hindered by the chemical instability owing, presumably, to the high nucleophilicity of LG-derived pyrroles. Therefore, another approach for overcoming these obstacles was explored. This approach involves the use of a stable isostere of the LGE_2-derived pyrroles. Thus, a pyrazole analogue **111** of LGE_2-derived pyrroles, in which the ring CH is replaced by N, was expected to be relatively electron deficient, less prone to oxidation, and therefore a stable isostere of the pyrroles.[59]



Pyrazole isostere **111**



112

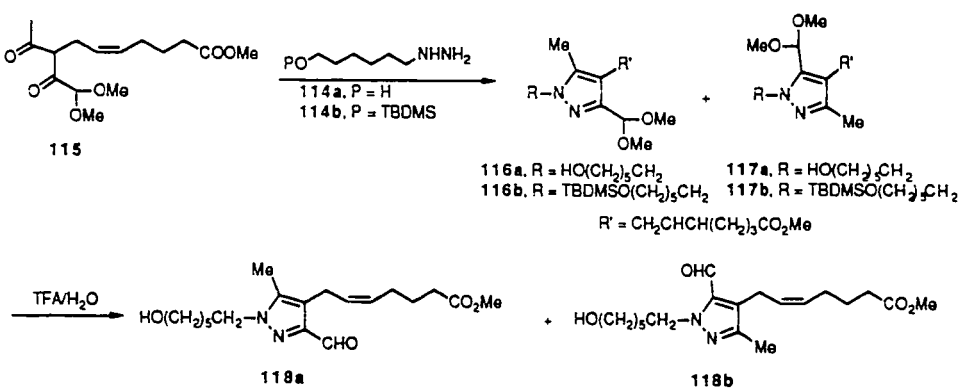
The aldehyde group at the end of a linking tether was incorporated to allow generation of antigen **112** by reductive alkylation of lysyl amino groups of proteins. It was anticipated that antibodies raised against these isostere adducts with proteins would cross react effectively with LGE_2-derived pyrroles.

Prologue. Detection of free LGs *in vivo* and *in vitro* is very difficult because of their chemical instability and competing sequestration by covalent binding with protein. An indirect method, immunoassay with antibodies raised against an LGE_2-pyrrole antigen, was explored by Raj Iyer.[57] Unfortuately, LGE_2-derived pyrroles were very sensitive and difficult to handle and also exhibted high nucleophilicity which interfered with attempts to link them to protein. A pyrazole isostere **111** of LGE_2-derived pyrrole was designed to circumvent these obstacles. This work was initiated by Mike Kobierski[60] who completed a synthesis of the pyrazole alcohol **113** (vide infra: Scheme XI). The present thesis reports a synthesis of the pyrazole aldehyde **111** starting from **113** and coupling of this aldehyde with protein to provide an antigen.

Results and Discussion

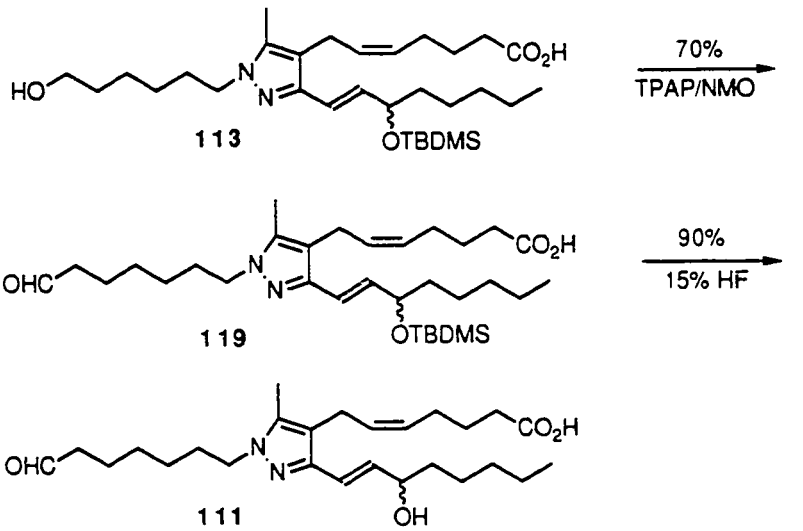
(1) Synthesis of Pyrazole Isostere

Mike Kobierski[60] condensed **114a** and **114b** with diketone **115** to obtain pyrazole isomers **116** and **117** in a 1:1 ratio and high overall yields.[61] The two structural isomers **116, 117** were separated by flash chromatography. The two isomers **116, 117** were characterized after hydrolysis to the corresponding aldehydes. Hydrolysis of each acetal with (TFA/H2O) delivered pure aldehyde **118a** or **118b**. A complete characterization of the isomeric pyrazoles was made by multidimensional NMR experiments. This will be discussed in the next section.



The conversion of **118a** into isostere **113** was accomplished by Mike Kobierski.[60] It was expected that oxidation of the primary alcohol **113** to aldehyde **119** would be simple (Scheme XI). However, reaction of **113** with four equivalents of pyridinium dichromate (PDC) in methylene chloride resulted in the complete consumption of starting material.

Scheme XI



Three products were detected by TLC analysis of the crude reaction mixture, all of them less polar than the starting alcohol. Filtration of the suspension through celite was more difficult than anticipated, owing to the formation of a gummy brown substance. Concentration of the filtrate yielded only few milligrams of residue (from 50.0 mg of starting material), none of which was the desired product. Extraction of the brown residue with numerous solvents (diethyl ether, ethyl acetate, methanol) provided either no further products, or in the case of methanol, solubility of everything including the brown precipitate. PCC oxidation of **113** gave a similar result. When **113** was subjected to Swern oxidation (DMSO, $(COCl)_2$, Et_3N), TLC analysis showed a product less polar than the starting alcohol and disappearance of starting material. After purification, ^1H NMR spectral analysis showed not only an aldehyde hydrogen resonance, but also an additional singlet around δ 3.5 which seemed to be a methyl thioester.

An exceptionally mild new method for oxidation of primary alcohols to aldehydes was reported recently by Griffith et al.[62] In 1987 they developed a novel reagent, tetrapropylammonium perruthenate (TPAP), for oxidation of alcohols to carbonyl compounds. This reagent (TBAP), used in catalytic quantities together with 4-methylmorpholine N-oxide (NMO), oxidizes primary alcohols to aldehydes and secondary alcohols to ketones in a rapid, efficient manner at room temperature. A major advantage of this oxidant is the lack of the many complications (work-up difficulty, generation of obnoxious side products) which sometimes accompany the use of more conventional, stoichometric oxidants such as chromiun reagents and Swern systems. Therefore, oxidation of **113** was performed with TPAP (0.5 mol %), NMO (1.5 equiv.), and 4Å molecular sieves (employed to remove water generated in the reaction) at room temperature. Surprisingly, after 30 min stirring, TLC analysis showed a clean spot to spot reaction. Easy work-up gave a 70% isolated yield of **119**.

Regeneration of the allylic alcohol from silyl ether **119** would deliver the final target pyrazole-aldehyde **111**. When **119** was treated with 2 equivlants of 1.0 M tetrabutylammonium fluoride in THF at room temperature, only very polar non-uv active product was isolated. This seemed to be a polymeric product rather than the final pyrazole-aldehyde **111**. Reaction of **119** with 80% aquous acetic acid (v/v) gave a crude product which showed two major spots of similar intensity by TLC analysis. The two products were separated. The more polar product, the desired final pyrazole-aldehyde **111**, was obtained in 45%

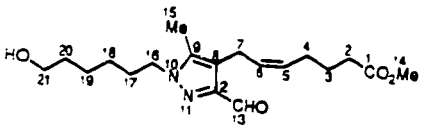
yield. The most efficient reagent found for removal of the TBDMS-ether in **119** was 15% concentrated hydrofluoric acid in acetonitrile.[63] A 90% isolated yield of the allylic alcohol **111** was isolated after treatment of **119** with 15% HF at room temperature.

(2) Characterization of Pyrazole Isomers

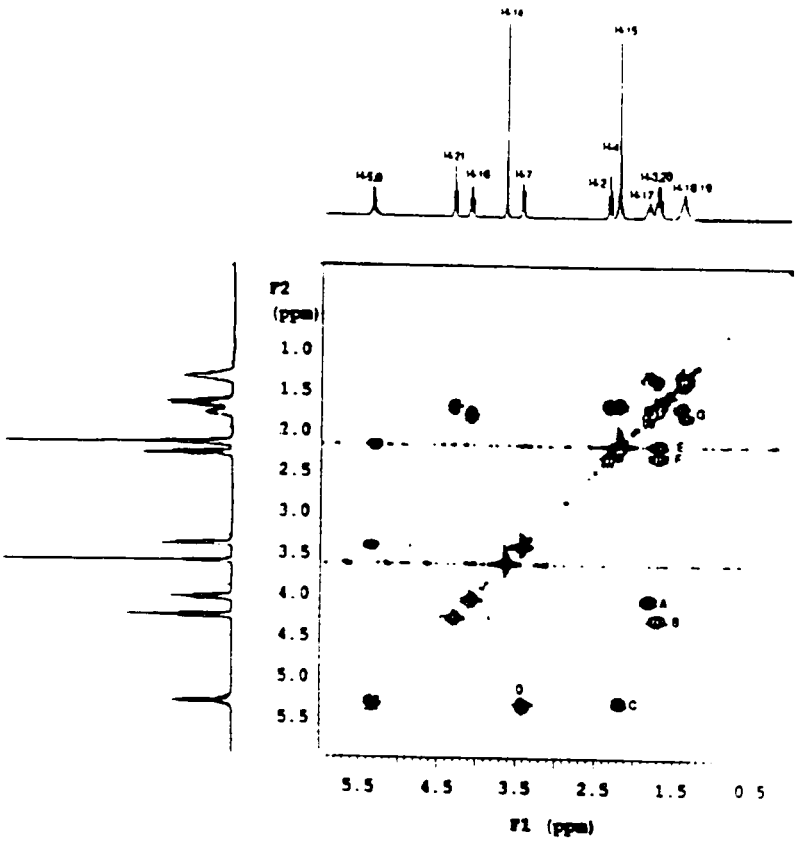
For characterization of the pyrazole isomers **118a** and **118b**, NMR correlation spectroscopy (COSY) and nuclear Overhauser enhancement spectroscopy (NOESY)[64] were used. The COSY experiment was necessary for the assignment of the ^{1}H NMR spectra of the pyrazole isomers **118a** and **118b**. Significantly, the N-methylene (H-16) signal in the ^{1}H NMR spectrum of **118b** appeared at δ 4.21, downfield with respect to the corresponding signal (δ 4.07) in the spectrum of **118a** (Figure 4, 5). The spatial proximity of the N-methylene (H-16) group to the aldehyde carbonyl group in **118b**, would put the methylene group in the deshielding cone of the carbonyl group. This would explain the large downfield shift of the N-methylene signal in **118b** relative to **118a**. All corresponding coupling partners in the COSY spectrum are summarized in figures 4 and 5.

From the NOESY spectra we confirmed the structure assignment of the pyrazole isomers **118a** and **118b**. Especially the NOE's between methyl group proton (H-15) and N-methylene (H-16) proved to be useful for the assignment of isomers. The interring NOE's (Correlation A in Figure 6) between H-15 at δ 2.18 and H-16 at δ 4.07 in **118a** are important because they reveal the spatial relationship between pyrazole ring and N-alkyl side chain. On the other hand, no NOE was observed between H-15 at δ 2.21 and H-16 at δ 4.21 in **118b** (Figure 7) confirming that the methyl group and N-alkyl side chain in **118b** are further away from each other than in **118a**. Also NOE's between H-15 and methylene group (H-7) were observed in **118a**

(Correlation B in Figure 6) and **118b** (Correlation A in Figure 7). This confirms our assignments for the structures of the two isomers **118a** and **118b**. Other observed NOEs of **118a** and **118b** are summarized in figures 6 and 7.



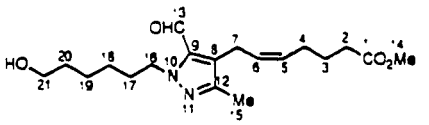
118a



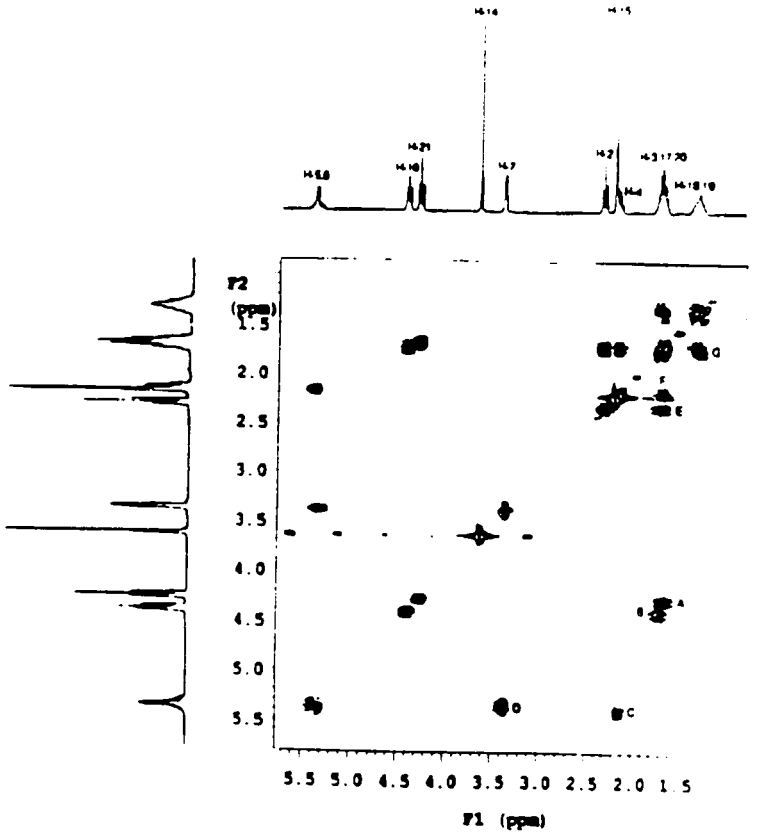
COSY Proton Correlations

Correlations	Protons
A	16-17
B	20-21
C	4-5
D	6-7
E	2-3
F	3-4
G	19-20

Figure 4. COSY Spectrum and Correlations of **118a**



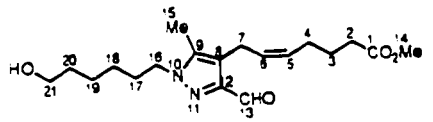
118b



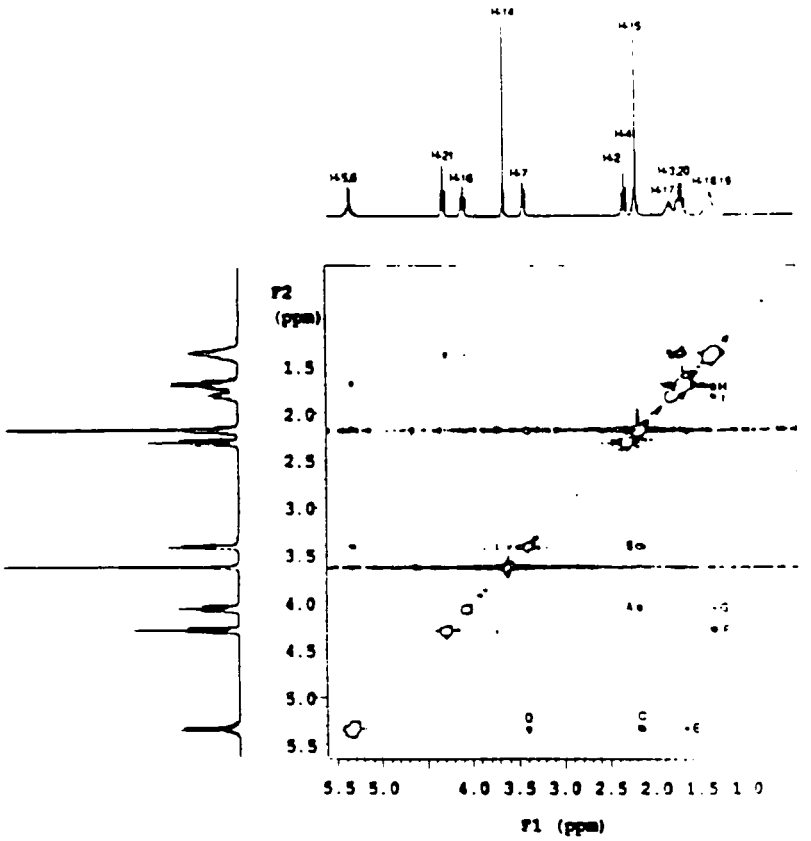
COSY Proton Correlations

Correlations	Protons
A	20-21
B	16-17
C	4-5
D	6-7
E	2-3
F	3-4
G	17-18

Figure 5. COSY Spectrum and Correlations of **118b**



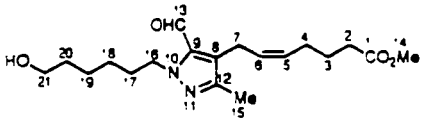
118a



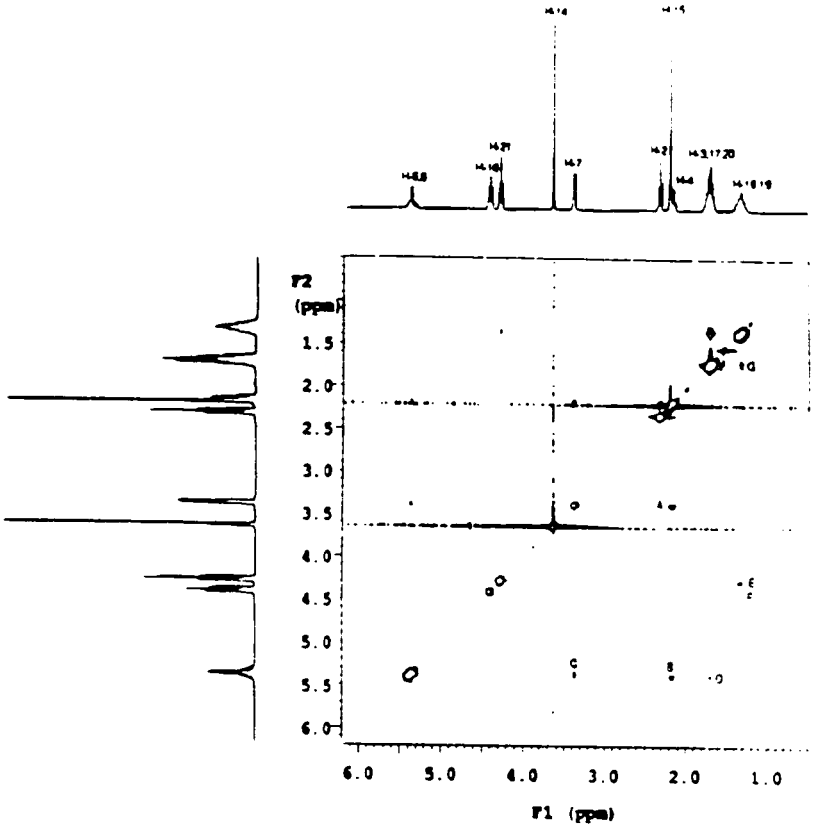
NOESY Proton Correlations

Correlations	Protons
A	15-16
B	7-15
C	4-6
D	5-7
E	3-5
F	19-21
G	16-18
H	18-20
I	17-19

Figure 6. NOESY Spectrum and Correlations of **118a**



118b



NOESY Proton Correlations

Correlations	Proteins
A	7-15
B	4-6
C	5-7
D	3-5
E	19-21
F	16-18
G	17-19

Figure 7. NOESY Spectrum and Correlations of **118b**

(3) Immunoassay of LG-derived Isostere

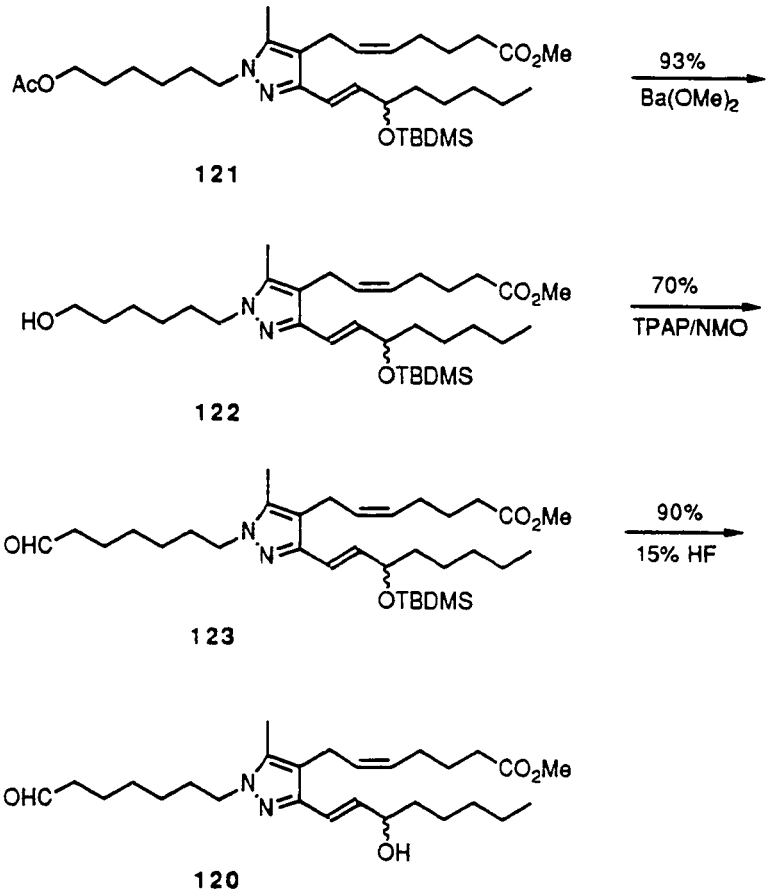
Immunoassay is particularly attractive as a mild method for detecting protein-bound LG-derived pyrroles since it not only allows quantitative determination of the levels of adducts but also their localization in tissues. As discussed in the preceding section, the development of an immunoassay for protein bound LG-derived pyrrole antigen was hindered by the several obstacles. (1) Since the levuglandins-protein reaction generates a complex mixture of products, it is not suitable for producing a specific antigen. Rather, a protein derivatized with many identical LGE_2-derived pyrrole units is need. (2) The carboxyl, and allylic hydroxyl functionality in the LGE_2-derived pyrroles limits the choices available for coupling an appropriate derivative with a protein to produce an antigen. (3) Model studies revealed high nucleophilic reactivity for LGE_2-derived pyrroles, e.g., both the allylic hydroxyl and the pyrrole ring were acylated with trifluoroacetic anhydride. This means that even if such a homogeneously derivatized protein can be prepared, its homogenity may be compromised by reaction with electrophiles or oxygen *in vivo* during the weeks required for antibody generation. (4) The strong nucleophilicity of the highly alkylated and conjugated pyrrole ring found in LGE_2-derived pyrrole would interfere with attempts at linking an appropriate derivative to a protein. A possible solution to these problems is to exploit the high crossreactivity anticipated for antibodies raised against an isostere of the LG-derived pyrrole. A chemically stable isostere was successfully exploited to raise

antibodies which cross react with PGH_2 allowing immunoassay for this unstable ($t_{1/2}$ = 5 min at 37 °C) AA metabolite.[65] Thus, a pyrazole analogue **111** of LGE_2-derived pyrroles in which the ring CH is replaced by N was expected to be relatively electron deficient, less prone to oxidation, and therefore a stable isostere of the pyrroles. The aldehyde group at the end of a linking tether was incorporated to allow generation of antigen **112** by reductive alkylation of lysyl amino groups of proteins.

We prepared pyrazole-aldehyde **111** as described in the preceding section. Before coupling of pyrazole **111** with poly-L-lysine, model studies were conducted to answer the following questions. (1) Which reagent is effective for reductive alkylation? (2) What solubility problems will be encountered when the relatively nonpolar pyrazole **111** is coupled with the very polar polymeric poly-L-lysine? (3) How can the adduct from coupling of pyrazole aldehyde **111** and poly-L-lysine be purified and characterized? We prepared methyl ester **120** as a very nonpolar model of pyrazole-aldehyde **111** (Scheme XII). Thus, selective removal of the acetyl protecting group was achieved with barium methoxide to afford **122**. Oxidation of **122** and desilylation of the resulting **123** provided **120** in close analogy with our synthesis of the corresponding acid **111**. Sodium cyanoborohydride is widely used for imine reduction and carbonyl amination of complex biological systems in aqueous solution at pH 6-8.[66] To explore the reductive alkylation reaction using sodium cyanoborohydride, **120** and the methyl ester **124** (2 equiv) of N-α-t-Boc-L-lysine (as a model of poly-L-

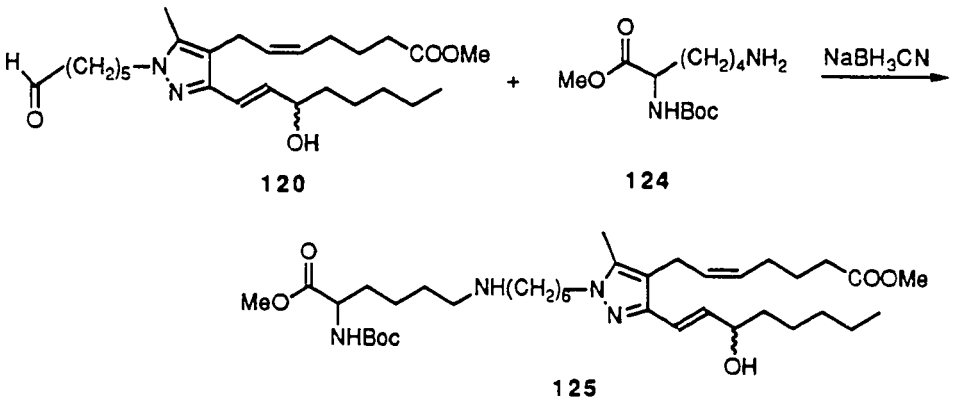
lysine) were reacted with sodium cyanoborohydride (2 equiv) in THF at room temperature.

Scheme XII

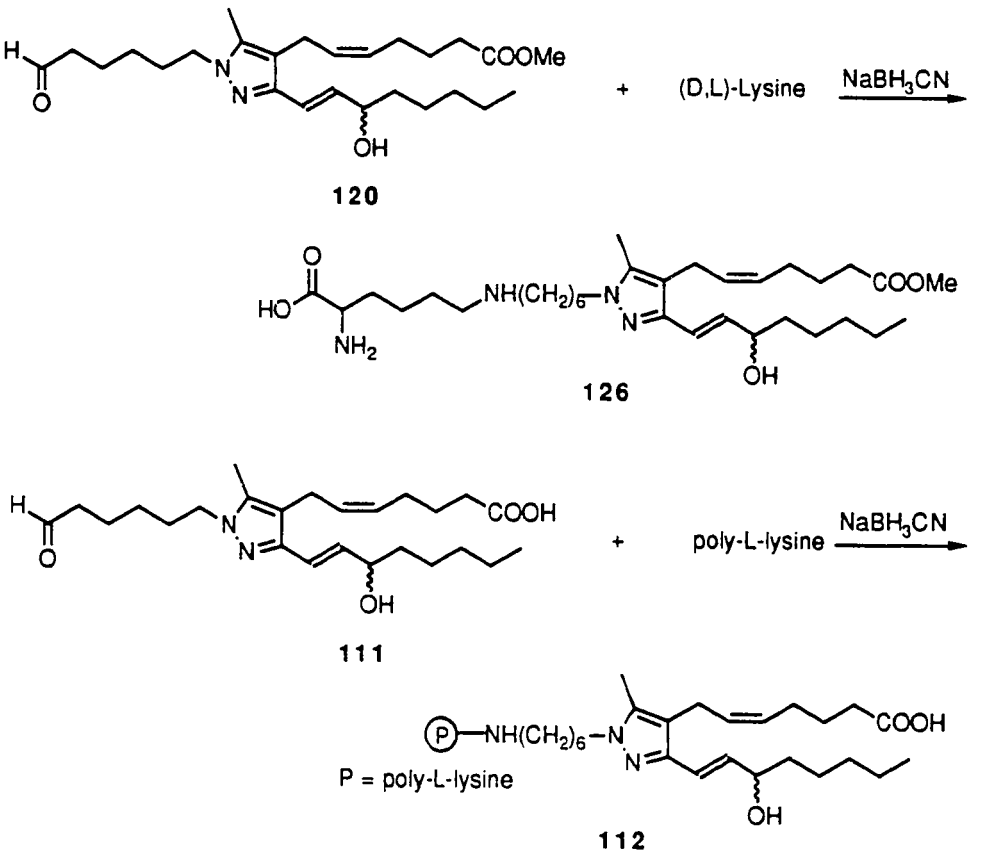


As expected, reductive alkylation was complete in 1 h. The resulting adduct **125** was isolated by chromatography on a small pipette silica gel column eluting with 20% i-PrOH in ethyl acetate. ^{1}H NMR spectroscopy clearly showed a broad absorption at δ 2.63 ppm integrating for 4 hydrogens corresponding to the four methylene hydrogens α to the nitrogen (ε nitrogen of lysine) and no aldehyde peak

in **125**. Before coupling of **111** and poly-L-lysine, we had to find a solvent system which would dissolve both reactants.



Therefore, lysine which has a solubility similar to poly-L-lysine was employed as a model for poly-L-lysine. Lysine dissolves in water and alcohol but not in organic solvents such as THF, EtOAc, $CHCl_3$. On the other hand, pyrazole-aldehydes **111**, **120** dissove in organic solvents and have a partial solubility in water and alcohol. We tried the reductive alkylation in THF to see if alkylation could be achieved under the heterogeneous conditions. No alkylation occured after stirring at room temperature for several hours. When pyrazole aldehyde ester **120** in THF and lysine (2 equiv) in water were mixed in the presence of sodium cyanoborohydride, the initial solution was hazy. Within a 5 min the solution became homogeneous and TLC analysis showed the disappearance of starting material **120** and formation of a polar product. This lysine adduct **126** was isolated by HPLC using a C_{18} μ-Bondpak reverse phase column and a gradient solvent system. The retention time of the unreacted excess lysine was 3 min with 70% aqueous (1% TFA) in methanol.



Changing the solvent system to 20% aqueous (1% TFA) in methanol delivered lysine adduct **126** which had a 2.5 min retention time. Extensive ^{1}H NMR analysis showed the absence of an aldehyde group and evidence for alkylation with a broad resonance at δ 2.79 ppm integrating for 4 hydrogens corresponding to the 4 methylene hydrogens α to the nitrogen (presumably ε nitrogen of lysine) in **126**. Using methanol instead of THF/water as solvent for the reductive alkylation gave virtually the same result. Thus, the spectral data of the adduct **126** obtained in methanol was very similar to the previous one obtained with THF/water as reaction solvent.

With these results as a guide, we tried the coupling reaction of pyrazole aldehyde acid **111** with poly-L-lysine in methanol solution. The heterogeneous reaction mixture became a clear solution as the reaction proceeded. After 1 h TLC analysis 10% methanol in ethyl acetate showed the formation of a polar UV active product and the disappearance of starting material **111**. The adduct **112** was purified by dialysis with 90% water in methanol. The dialysis (Mr cutoff 14,000) removed unreacted starting material, if any, and inorganic salts. After dialysis and concentration of adduct by rotary evaporation, two product fractions were obtained. One is soluble in methanol, the other is insoluble in methanol but soluble in H_2O. The ^1H NMR spectrum of the methanol soluble adduct in CD_3OD shows a broad absorption at about δ 2.85 ppm integrating for 4 hydrogens corresponding to the four methylene hydrogens α to the nitrogen (presumably ε nitrogen of lysine) and also disappearance of aldehydic hydrogen in **112**. Also coupling of pyrazole-aldehyde (1 equiv) **111** with poly-L-lysine (4 equiv) in THF/H_2O and purification by dialysis gave the same adduct. ^1H NMR analysis of the methanol soluble adduct in CD_3OD clearly showed two absorptions between δ 6.1 and 6.4 ppm corresponding to two olefinic hydrogens in the lower side chain of the pyrazole also present is a broad absorption centered at δ 2.85 ppm corresponding to 4 methylene hydrogens α to the NH group (from the alkylated ε lysine amino group) in the adduct **112** and 2 methylene hydrogens α to the NH_2 groups (unalkylated lysine ε amino group). As the relative integral areas of the olefinic and methylene absorptions is 1:4, we

estimate a 1:2 ratio of alkylated versus unalkylated ε amino groups or a 1:3 molar ratio of pyrazole to lysyl residues.

The brevity of the overall coupling reaction makes this approach an outstanding synthesis which was readily adapted to preparation of radiolabeled derivatives that are need to accurately quantify the loading of isostere in protein conjugates. A bovine serum albumin (BSA)-isostere conjugate was also be prepared by Krishna Murthi using the procedure described above for poly-L-lysine. The BSA adduct will be used in an enzyme-linked immsorbent assay (ELISA) for LGE_2-derived pyrroles. Because of the complex NMR spectra of proteins, this analytical technique is not readily applicable to the conjugates of BSA. Rather, radiolabeled hapten was employed. Allylically tritiated isostere containing 1.02 mCi/mmol was generated by substituting $NaBT_4$ for $NaBH_4$ in the preparation of **121**. We will use the BSA conjugate as a coating reagent for ELISA.

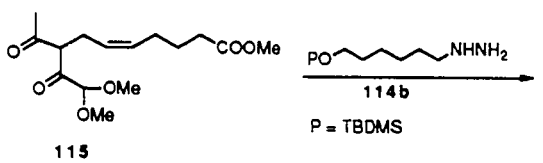
Antibodies against the pyrazole are being raised by immunizing rabbits with the isostere-protein conjugate **112**. Two fractions (methanol soluble and water soluble) of immunogen (1 mg, pyrazole-conjugated poly-L-lysine **112**) were dissolved in PBS (1 mL) pH 7.4. An antigen solution(100 μg/100 μL from 1 mg/mL) was emulsified in complete Freund's adjuvalent (100 μL). Two New Zealand White rabbits were inoculated intradermally into four sites on the back (25 μg/site) with the methanol soluble antigen (100 μg) and one rabbit was inoculated intradermally into four sites on the back with the water soluble antigen (100 μg). After 4, 8, and 11 weeks, blood was collected

from the rabbits and intradermal booster injections in incomplete Freund's adjuvalent were given. Serum was isolated from the blood (see experimental section for details) and kept in the refrigerator for an ELISA assay. The procedure used to develop our ELISA for protein-bound LG-derived pyrroles will be the same as that used by Dr. Monnier in his ELISA for a protein-bound glucose-derived pyrrole.[67] The validity of our immunoassay for the quantification of LG-derived pyrrole hapten will be investigated first using the in vitro model system consisting of human serum albumin (HSA) + LGE_2 used previously by Rajkumar Iyer who quantified pyrrole with Ehrlich reagent.[57] Also to be studied is the binding of purified LG-derived pyrrole haptens prepared from ω-aminohexanoic acid or neopentyl amine and LGE_2 with antibodies raised against the antigen **112**.

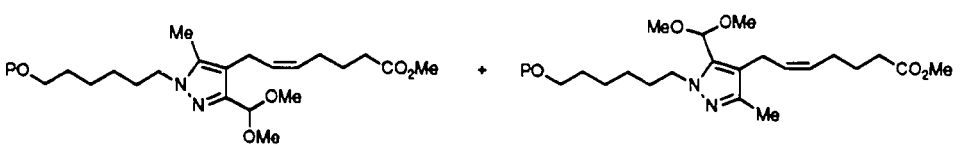
Experimental

General. Sodium cyanoborohydride was obtained from Aldrich Chemical Co. Poly-L-lysine, DL-lysine, N-α-t-Boc-L-lysine and pH 7.4 phosphate buffered saline (PBS) were all purchased from Sigma Chemical Co. Spectrapor membrane tubing (Mr cutoff 14,000 No. 2) for standard dialysis was obtained from Fisher Scientific Co. HPLC separation was performed with a Waters Associates model 6000A solvent pump equipped with a U6K injector and a model 660 solvent programmer, a μ-Bondpak reverse phase C_{18} column. The eluate was monitored with an Instrumentation Specialties Company model 1840 UV absorbance detector.

Pyrazole Isomers 116b and 117b.



115



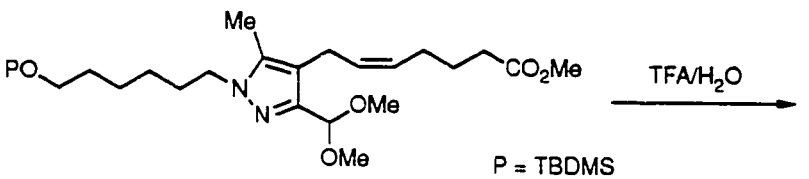
116b 117b

TBDMS-protected hydrazine **114b** (2.2 g, 9 mmol) was slowly added to magnetically stirred solution of diketone **115** (1.8 g, 6 mmol) in absolute ethanol (2.5 mL). The resulting reaction mixture was stirred at room temperature for 1 h. Solvent was then removed by rotary evaporation and the residue was purified by flash chromatography eluting with 20% ethyl acetate in hexane to afford the isomeric

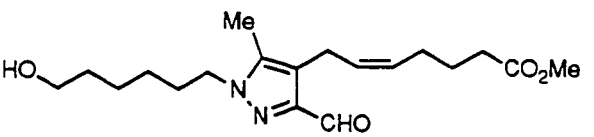
pyrazoles **116b** (1.40 g) and **117b** (1.50 g) in 94% total yield. The ratio of **116b** : **117b** is 1 : 1.07. **116b**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.40 (m, 3 H), 4.06 (t, 2 H, J = 7.4 Hz), 3.67 (s, 3 H), 3.54 (t, 2 H, J=6.4 Hz), 3.42 (s, 6 H), 3.39 (d, 2 H, J = 5.2 Hz), 2.40 (t, 2 H, J = 7.5 Hz), 2.20 (m, 2 H), 2.20 (s, 3 H), 1.55 (m, 10 H), 0.87 (s, 9 H), 0.02 (s, 6 H).

117b: ^{1}H NMR (200MHz, $CDCl_3$) δ 5.40 (m, 2 H), 5.38 (s, 1 H), 4.18 (t, 2 H, J = 7.4 Hz), 3.64 (s, 3 H), 3.60 (t, 2 H, J = 6.4 Hz), 3.35 (s, 6H), 3.21 (d, 2 H, J = 5.2 Hz), 2.38 (t, 2 H, J = 7.5 Hz), 2.16 (m, 2 H), 2.11 (s, 3 H), 1.52 (m, 10 H), 0.88 (s, 9 H), 0.02 (s, 6 H).

Pyrazole 118a.



116b

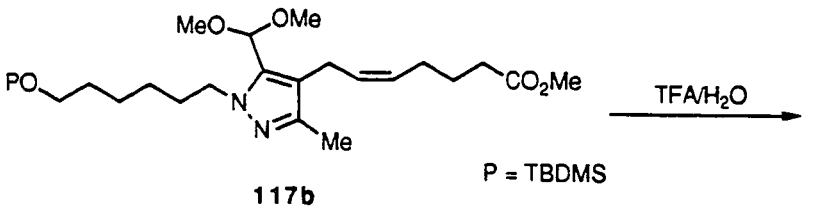


118a

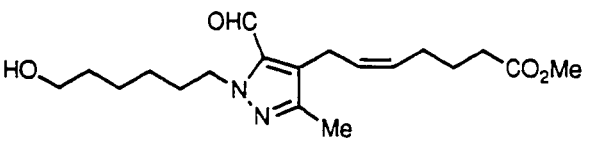
Pyrazole-acetal **116b** (0.5 g, 0.98 mmol) was treated with 90% TFA (11.0 mL) in water and stirred at room temperature 30 min. TLC analysis showed one major UV-active spot (R_f 0.25) with 30% ethyl acetate in hexane. TFA and water were removed by rotary evaporation. The remaining organic residue was purified by flash chromatography with 30% ethyl acetate in hexane as eluting solvent to yield **118a** (0.326 g, 95% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.95 (s, 1

H), 5.40 (m, 2 H), 4.34 (t, 2 H, J = 6.5 Hz), 4.09 (t, 2 H, J = 7.3 Hz), 3.68 (s, 3 H), 3.46 (d, 2 H, J = 5.3 Hz), 2.36 (t, 2 H, J = 7.4 Hz), 2.22 (m, 2 H), 2.21 (s, 3 H), 1.72 (m, 6 H), 1.40 (m, 4 H): ^{13}C NMR (50 MHz, $CDCl_3$) δ 187.04, 173.35, 143.23, 137.10, 128.10, 127.74, 118.42, 67.32, 50.94, 49.21, 32.85, 29.07, 27.31, 26.03, 25.49, 24.57, 24.17, 20.79, 8.66; mass spectrum m/z (M^+) for $C_{19}H_{30}N_2O_4$ cacld 350.2205, found 350.2197.

Pyrazole 118b.



117b

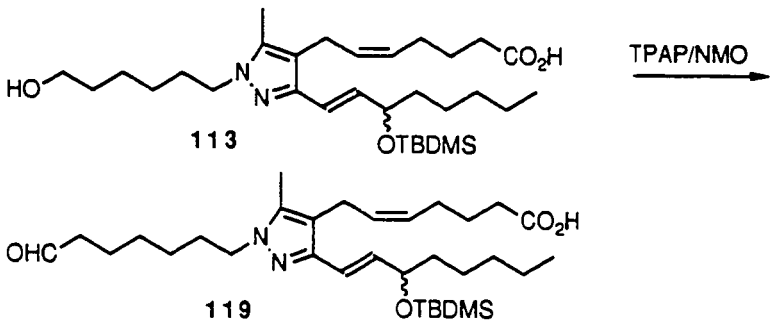


118b

The procedure was the same as used for making **118a**. Pyrazole-acetal **117b** (0.5 g, 0.98 mmol) was reacted to give a product. Crude product was purified by flash chromatography with 30% ethyl acetate in hexane to afford **118b** (0.316 mg, 92% yield): ^1H NMR (200 MHz, $CDCl_3$) δ 9.85 (s, 1 H), 5.39 (m, 2 H), 4.39 (t, 2 H, J = 7.3 Hz), 4.30 (t, 2 H, J = 6.5 Hz), 3.66 (s, 3 H), 3.38 (d, 2 H, J = 5.1 Hz), 2.34 (t, 2 H, J = 7.4 Hz), 2.20 (m, 2 H), 2.19 (s, 3 H), 1.72 (m, 6 H), 1.38 (m, 4 H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 178.96, 173.24, 145.74, 134.33, 128.85, 127.41, 125.70, 67.43, 50.93, 42.80, 32.73, 29.70, 27.27, 26.03, 25.27, 24.45, 23.98,

20.38, 10.82; mass spectrum m/z (M^+) for $C_{19}H_{30}N_2O_4$ calcd 350.2205, found 350.2201.

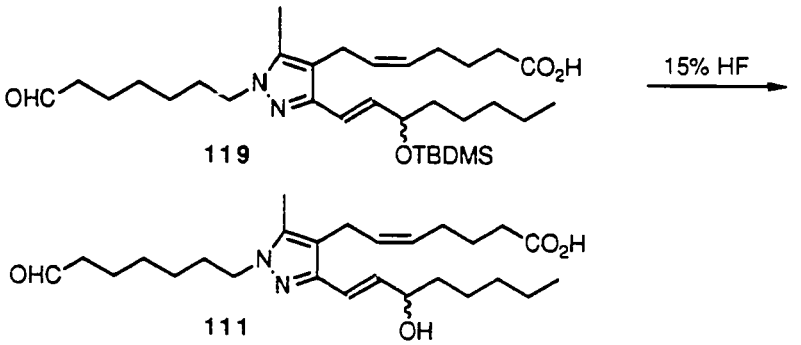
Aldehyde 119.



113 119

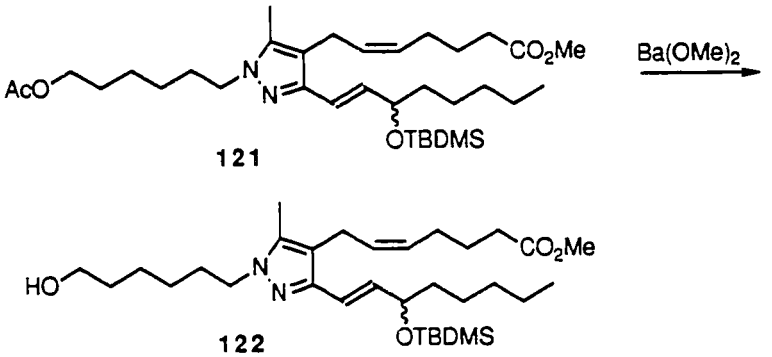
The alcohol **113** (20 mg, 0.036 mmol) was dissolved in dichloromethane (4 mL) containing 4Å molecular sieves and 4-methylmorpholine N-oxide (6.3 mg, 0.054 mmol). Solid tetrapropylammonium perruthenate (2 mg, 0.15 equiv) was then added under nitrogen and the resulting green mixture stirred at room temperature. After 1 h stirring, TLC analysis showed a new spot (R_f 0.3) with 70% ethyl acetate in hexane. Evaporation and filtration (small pipette silica-gel column) eluting with ethyl acetate removed all the inorganic material. Rotary evaporation gave a crude product **119** (14 mg, 70% yield) as an oil. This crude product was used for the next reaction without further purification: ^1H NMR (200 MHz, $CDCl_3$) δ 9.73 (t, 1H, J = 1.6 Hz), 6.40 (d, 1 H, J = 16.0 Hz), 6.16 (dd, 1 H, J = 16.08, 5.96 Hz), 5.34 (m, 2 H), 4.20 (m, 1 H), 3.96 (t, 2 H, J = 7.45 Hz), 3.15 (d, 2 H, J = 4.69 Hz), 2.39 (m, 4 H), 2.20 (m, 2 H), 2.11 (s, 3 H), 1.50 (m, 16H), 0.88 (s, 9 H), 0.85 (t, 3 H, J = 2.24 Hz), 0.042 (s, 3 H), 0.02 (s, 3 H).

Pyrazole Aldehyde 111.



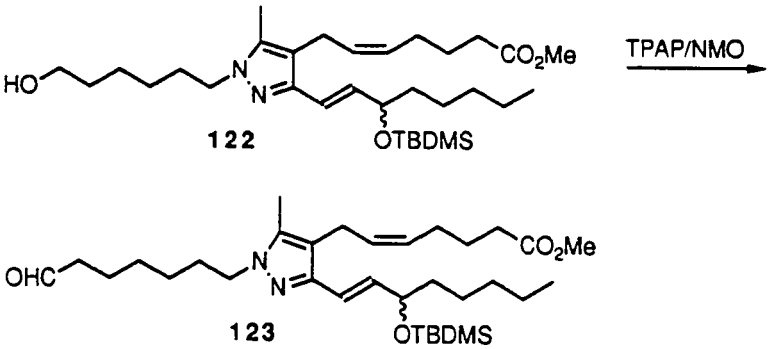
TBDMS ether **119** (14 mg, 0.025 mmol) was treated with concentrated aqueous hydrofluoric acid (0.16 mL, 49% w/v) and acetonitrile (0.34 mL) in a polyethylene vial. The desilylation was followed by TLC with ethyl acetate as developing solvent (R_f starting silyl ether 0.57, desilyated product 0.24). After 20 min, TLC analysis showed no starting material. The reaction mixture was diluted with water (1.5 mL) and extracted with $CHCl_3$ (3 x 5 mL), dried over anhydrous magnesium sulfate. Filtration and evaporation gave a slightly yellow oil (9.6 mg, 87% yield): ^1H NMR (200 MHz, $CDCl_3$) δ 9.76 (t, 1 H, J = 1.6 Hz), 6.48 (d, 1 H, J = 16.2 Hz) 6.19 (dd, 1 H, J = 16.2, 5.99 Hz), 5.36 (m, 2 H), 4.22 (m, 1 H), 3.99 (t, 2 H, J = 7.46 Hz), 3.16 (d, 2 H, J = 4.71 Hz), 2.40 (m, 4 H), 2.24 (m, 2 H), 2.12 (s, 3 H), 1.50 (m, 16 H), 0.86 (t, 3 H, J = 2.25 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.45, 177.14, 145.60, 136.02, 133.09, 129.34, 128.57, 120.70, 115.49, 73.30, 52.67, 49.07, 48.82, 43.65, 37.19, 33.17, 31.79, 30.36, 26.46, 25.18, 24.44, 22.62, 21.69, 14.08, 9.57; mass spectrum m/z (M^+) for $C_{25}H_{40}N_2O_4$ calcd 432.2988, found 432.2899.

Alcohol Methyl Ester122.



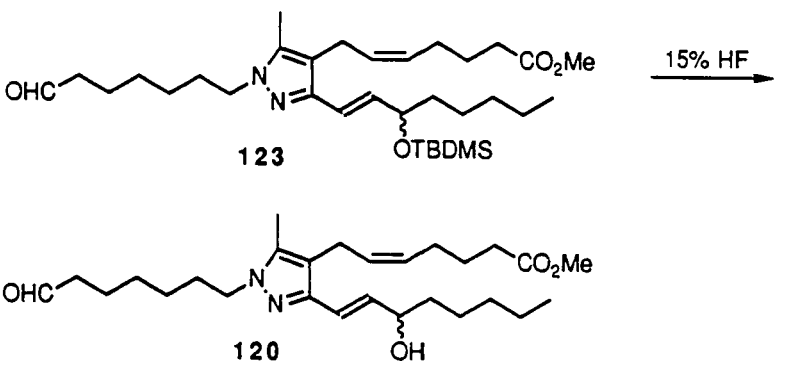
To a stirred solution of acetate **121** (20 mg, 0.033 mmol) and methanol (0.5 mL) was added $Ba(OMe)_2$ in methanol(1.6 M, 20 μL, 0.066 mmol) at 0 °C under nitrogen and stirring was continued for 4 h. TLC analysis with 50% ethyl acetate in hexane showed a new spot (R_f 0.13). All methanol was removed under reduced pressure. The residue was diluted with water water (2.0 mL) and extracted with ethyl acetate (3 x 5 mL). The organic layer was dried over anhydrous $MgSO_4$. The filtrate was concentrated by rotary evaporation. The residue was purified by flash chromatography with 50% ethyl acetate in hexane as eluting solvent to give **122** (17.2 mg, 93% yield): ^1H NMR (200 MHz, $CDCl_3$) δ 6.35 (d, 1 H, J = 16.08 Hz), 6.16 (dd, 1 H, J = 16.11, 5.86 Hz), 5.31 (m, 2 H), 4.18 (q, 1 H, J = 5.6 Hz), 3.96 (t, 2 H, J = 7.3 Hz), 3.65 (s, 3 H), 3.59 (t, 2 H, J = 6.4 Hz), 3.14 (d, 2 H, J = 4.98 Hz), 2.33 (t, 2 H, J = 7.55 Hz), 2.16 (m, 2 H), 2.11 (s, 3 H), 1.71 (m, 4 H), 1.50 (m, 4 H), 1.22 (m, 10 H), 0.87 (s, 9 H), 0.84 (t, 3 H, J = 2.99 Hz), 0.034 (s, 3 H), 0.01 (s, 3 H).

Aldehyde Methyl Ester123.



The procedure was the same as used for making **119** except **122** was used in place of **113**. Thus, primary alcohol **122** (17 mg, 0.03 mmol) gave an aldehyde **123** (11.8 mg, 70% yield): ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.74 (t, 1 H, J = 1.59 Hz), 6.40 (d, 1 H, J = 16.02 Hz), 6.17 (dd, 1 H, J = 16.04, 5.96 Hz), 5.34 (m, 2 H), 4.21 (m, 1 H), 3.96 (t, 2 H, J = 7.5 Hz), 3.66 (s, 3 H), 3.16 (d, 2 H, J = 4.71 Hz), 2.38 (m, 4 H), 2.20 (m, 2 H), 2.11 (s, 3 H), 1.50 (m, 16 H), 0.88 (s, 9 H), 0.86 (t, 3 H, J = 2.23 Hz), 0.04 (s, 3 H), 0.012 (s, 3 H).

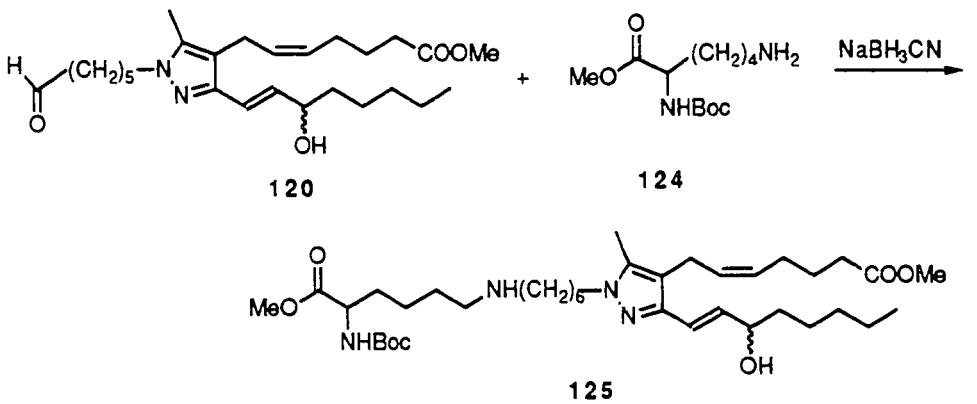
Allylic Alcohol 120.



The procedure was the same as used for making **111** except **123** was used in place of **119**. Thus, silyl ether **123** (11.0 mg, 0.02 mmol) gave allylic alcohol **120** (7.6 mg, 87% yield): ^{1}H NMR (200 MHz, $CDCl_3$)

δ 9.76 (t, 1 H, J = 1.62 Hz), 6.48 (d, 1 H, J = 16.01 Hz), 6.20 (dd, 1 H, J = 16.2, 5.97 Hz), 5.37 (m, 2 H), 4.24 (m, 1 H), 4.0 (t, 2 H, J = 7.45 Hz), 3.67 (s, 3 H), 3.16 (d, 2 H, J = 4.72 Hz), 2.41 (m, 4 H), 2.40 (m, 4 H), 2.24 (m, 2 H), 2.12 (s, 3H), 1.50 (m, 16 H), 0.87 (t, 3 H, J = 2.24 Hz).

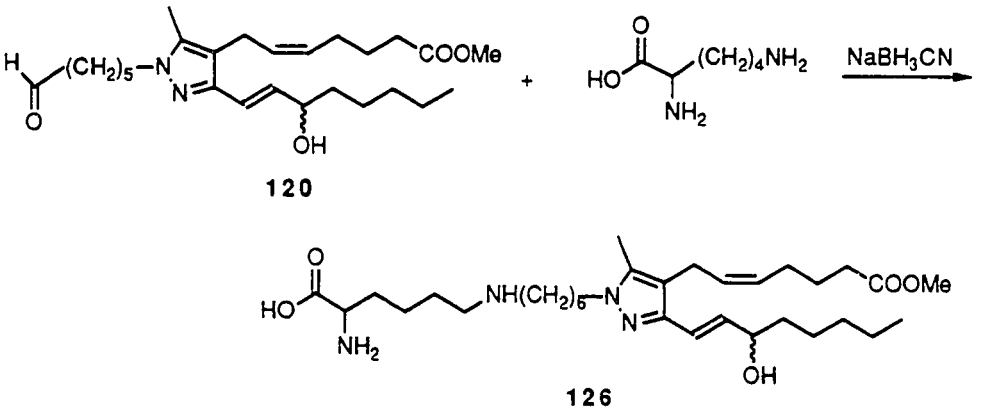
Coupling Product 125 from N-α-Boc-L-lysine Methyl Ester.



To a stirred solution of the methyl ester of N-α-Boc-L-lysine **124** (2.5 mg, 2 equiv) and sodium cyanoborohydride (1.0 mg) in THF (0.5 mL) was added **120** (2.0 mg) in THF (0.5 mL). Stirring was continued overnight at room temperature. TLC analysis with 20% i-PrOH in ethyl acetate showed the formation of a polar product (R_f 0.09). Removal of THF by rotary evaporation afforded crude product which was isolated by chromatography on a small pipette silica gel column eluting with 20% i-PrOH in ethyl acetate. Evaporation of solvent delivered the product **125** (2 mg): ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.46 (d, 1 H, J = 16.41 Hz), 6.23 (dd, 1 H, J = 16.01, 6.45 Hz), 5.33 (m, 2 H), 4.21 (m, 2 H), 3.96 (t, 2 H, J = 7.62 Hz), 3.71 (s, 3 H), 3.66 (s, 3 H), 3.14 (d, 2 H, J =

4.10 Hz), 2.63 (m, 4 H), 2.34 (t, 2 H, J = 7.42 Hz), 2.24-1.17 (26 H), 2.12 (s, 3 H), 1.41 (s, 9 H), 0.86 (t, 3 H, J = 6.12 Hz).

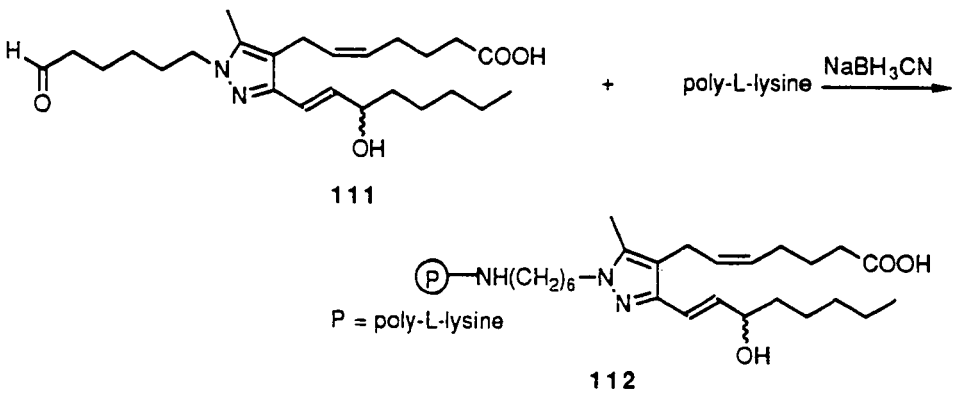
Coupling Product 126 from Lysine.



To a stirred solution of lysine (1.3 mg, 2 equiv) and sodium cyanoborohydride (1.0 mg) in H_20 (0.25 mL) was added **120** (2.0 mg) in THF (0.25 mL) at room temperature. After addition of **120** the resulting solution was cloudy. In less than 5 min the solution became clear, indicating that the reaction had started. Stirring was continued for 1 h. TLC analysis with ethyl acetate showed the disappearance of **120** and the formation of a polar product. Evaporation of THF and H_2O afforded crude product. Isolation of product was achieved by reverse phase HPLC using a C_{18} μ-Bondpak column with a gradient solvent system (from 70% aqueous water (1% TFA) in methanol to 20% aqueous water (1% TFA) in methanol). The eluate was monitored with an Instrumentation Specialties Company model 1840 UV absorbance detector at 245 nm. The retention time of excess lysine was 3 min with 70% aqueous water (1% TFA) in methanol. Changing the

solvent system to 20% aqueous water (1% TFA) in methanol delivered the product **126** which had a 2.5 min retention time. Concentration gave product **126** (2.0 mg): ^{1}H NMR (200MHz, CD_3OD) δ 6.45 (d, 1 H, J = 16.2 Hz), 6.13 (dd, 1 H, J = 16.1, 5.9 Hz), 5.32 (m, 2 H), 4.08 (m, 1 H), 3.95 (m, 2 H), 3.79 (s, 3 H), 3.16 (d, 2 H, J = 4.70 Hz), 2.79 (m, 4 H), 2.29 (t, 2 H, J = 7.5 Hz), 2.20-1.16 (26 H), 2.13 (s. 3 H), 0.84 (t, 3 H, J = 6.27 Hz).

Coupling of Pyrazole Aldehyde 111 with Poly-L-lysine.



Poly-L-lysine (2.7 mg, 4 equiv based on unit base of lysine, Mr = 55,000) and pyrazole aldehyde **111** (2 mg, 0.0046 mmol) were dissolved in methanol (0.4 mL). This solution became a little cloudy. After 5 min stirring, sodium cyanoborohydride (1.0 mg) was quickly added at room temperature. When the addition was complete, the solution became clear. This solution was stirred for 2 h at room temperature. After 2 h, TLC analysis with ethyl acetate showed a new uv-active polar spot and the disappearence of **111** (R_f 0.24). The solution was transferred to a dialysis tube (Mr cutoff 14,000, spectrapor membrane tubing No. 2) and dialyzed twice against 10% water (250 mL) in

methanol for 24 h. The absence of free hapten **111** in the polylysine conjugate **112** was confirmed by TLC with ethyl acetate as developing solvent. After dialysis and concentration of adduct by rotary evaporation, two product fractions were obtained. One (2.3 mg) is soluble in MeOH, the other (2.5 mg) is insoluble in MeOH but soluble in water.

Immunization Procedure. Stock solutions (1 mg/mL) of the two fractions (methanol soluble and water soluble) of the poly-L-lysine-pyrrazole conjugate (antigen) were prepared in PBS pH 7.4. Two New Zealand White rabbits were inoculated with the methanol soluble antigen (100 μg each) and one rabbit was inoculated with the water soluble fraction (100 μg). In the first step of the inocculation procedure, the area to be injected was shaved with an electric razor. To prepare the rabbit for injection with antigen, the shaved area was then cleaned with an antiseptic swabsticks (saturated with a 10% Povidone-Iodine solution). A mixture of antigen (100 μg, 100 μL of the 1 mg/mL stock) and Freund's complete adjuvant (100 μL) was vortexed in an Eppendorf tube until a milky white emulsion formed. This emulsion was then taken up without a needle into a syringe (1 mL) and injected intradermally through a needle at four sites (~25 μg antigen/site). For the intradermal injection the needle is held at a slight angle from the skin with the bevel facing up. If the skin is stretched, it is easier to insert the needle. The appearance of a white bump on the skin after the injection is an indication that the injection is intradermal.

Bleeding and Boosting Procedure. Four, eight and eleven weeks after the first inoculation, the rabbits were given booster injections with the antigen. The blood from the rabbits was collected from the ear four, eight, and twelve weeks after the first inoculation. During the bleeding procedure the rabbit was put in a restrainer and the pusher was clamped in a position such that the rabbit was snugly placed in the restrainer and could not move. The pusher should not be clamped so tightly that the rabbit starts to breathe through its mouth. The ear which was to be bled was first swabbed with xylene to make the main artery stick out. The barrel of the syringe to be used was pulled out and pushed into the syringe a couple of times before use. A butterfly needle was then attached to the syringe via a thin plastic tube. The needle was held at a slight angle above the artery with the bevel up and then carefully inserted into the artery. Once the artery is pierced the needle will slide in easily. It should be advanced most of the way. As the blood started to fill the thin plastic tube, the barrel of the syringe was slowly drawn and the blood (20 mL) was collected into the syringe. The collected blood was transferred to a centrifuge tube. After the blood was collected, a gauze was held over the needle and the needle was pulled out. The pusher was unclamped to release the rabbit and the gauze was held over the ear until the bleeding stopped. The ear was then cleaned with soap and water and a lotion was applied to prevent any burning caused by the xylene.

The procedure for the booster injections was exactly the same as the immunization procedure described above except in this case

Freund's incomplete adjuvant was used instead of Freund's complete adjuvant to emulsify the antigen.

The blood collected in the centrifuge tubes was allowed to clot at room temperature for an hour and then in ice for 45 minutes. The clotted blood was then centrifuged at 2000 rpm for 15 min to separate the serum from the fibrinogen and the red blood cells. If necessary, the supernatent was recentrifuged to remove the last traces of red blood cells. The supernatent serum was then carefully transferred into vials (50 μL and 1.5 mL aliquots) and stored at -78 °C. This serum will be used for to obtain antibody titers and for the ELISA assay.

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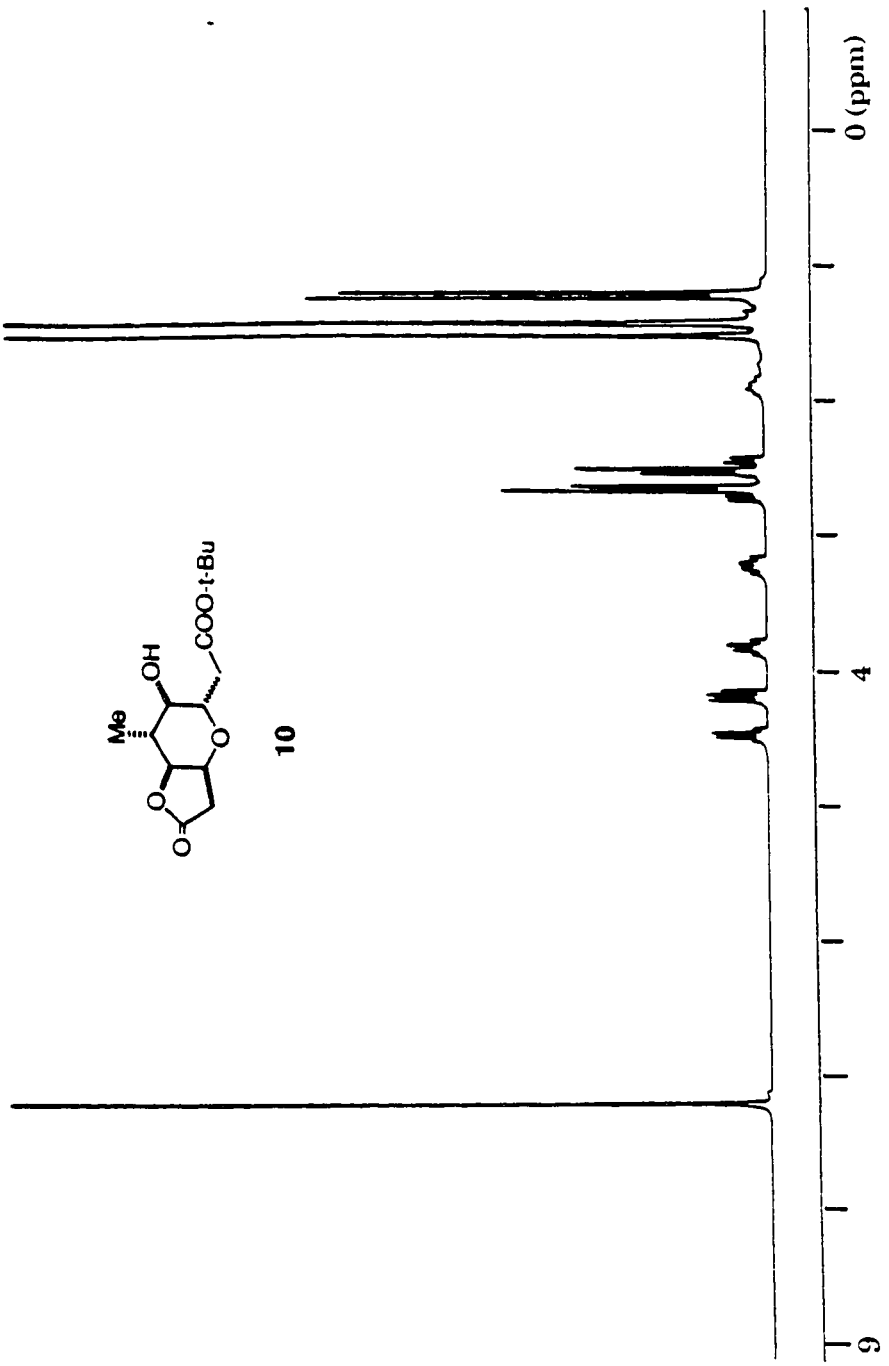
Appendix

This appendix contains ^{1}H and ^{13}C NMR spectra of H-ring intermediate involved in the total synthesis of Halichondrin B and pyrazole isostere of LG-derived pyrroles.



Figurre 8. The 200 MHz ^1H NMR (in $CDCl_3$) of tetrahydropyran **10**

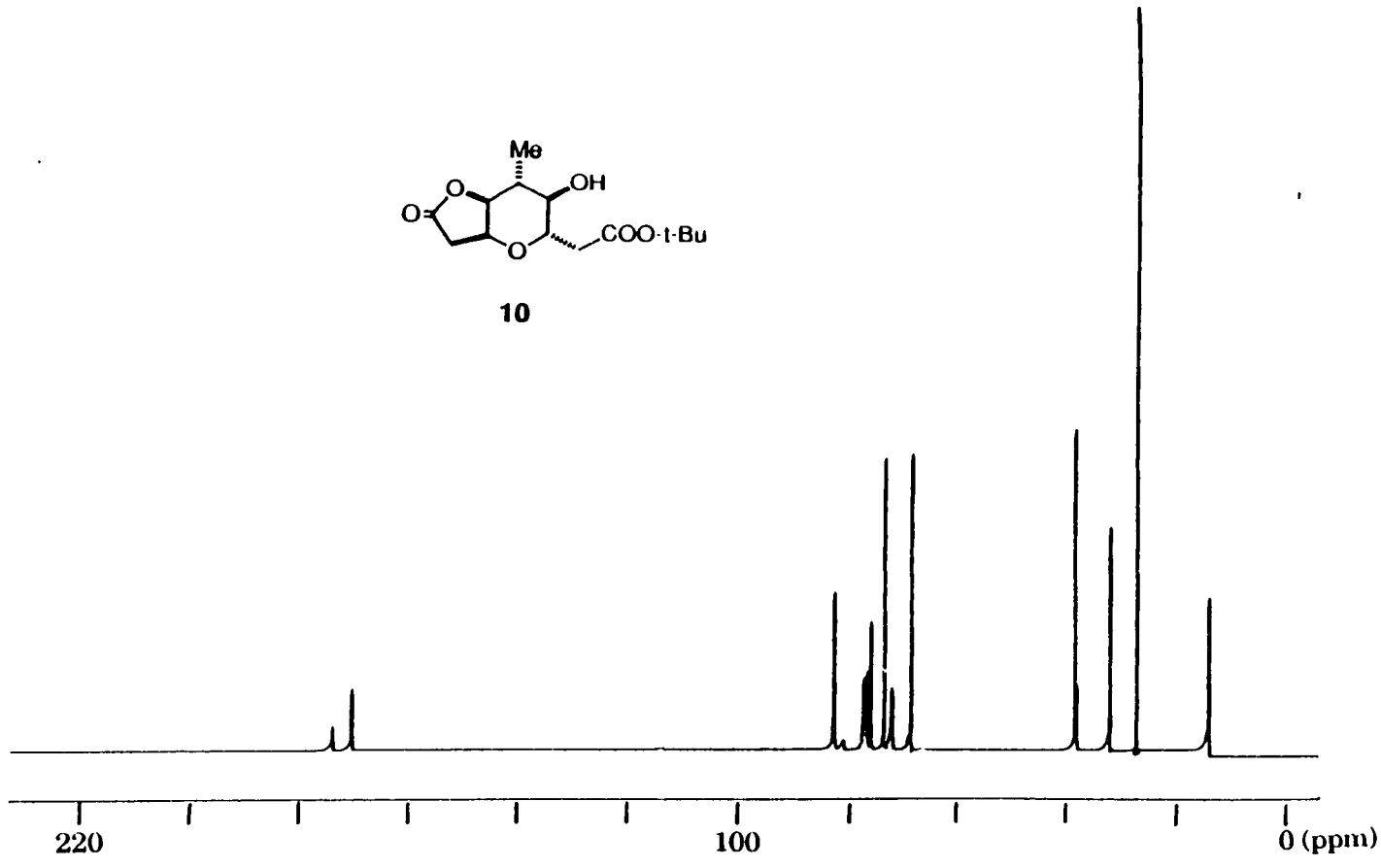


Figure 9. The 50 MHz ^{13}C NMR (in $CDCl_3$) of tetrahydropyran **10**

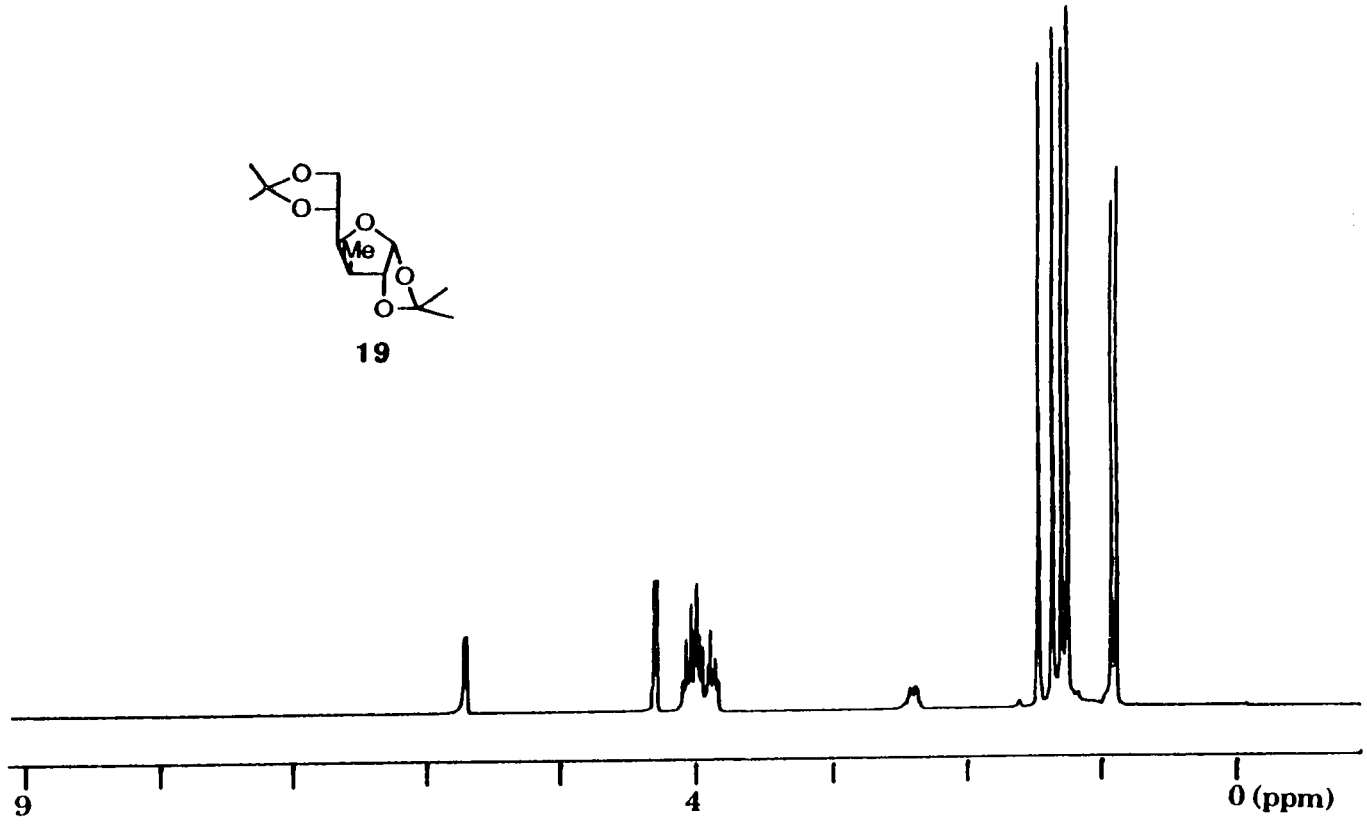


Figure 10. The 200 MHz ^1H NMR (in $CDCl_3$) of 1,2,5,6-Isopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (**19**)

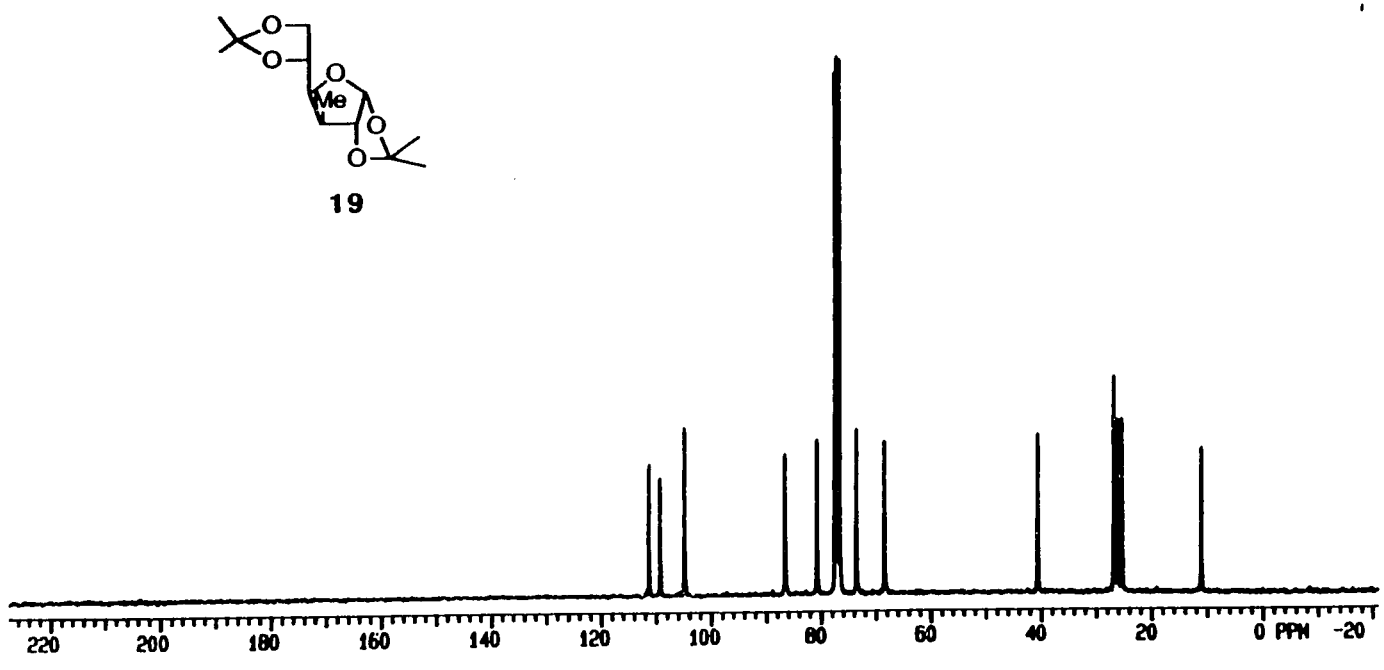


Figure 11. The 75 MHz ^{13}C NMR (in $CDCl_3$) of 1,2,5,6-Isopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (**19**)

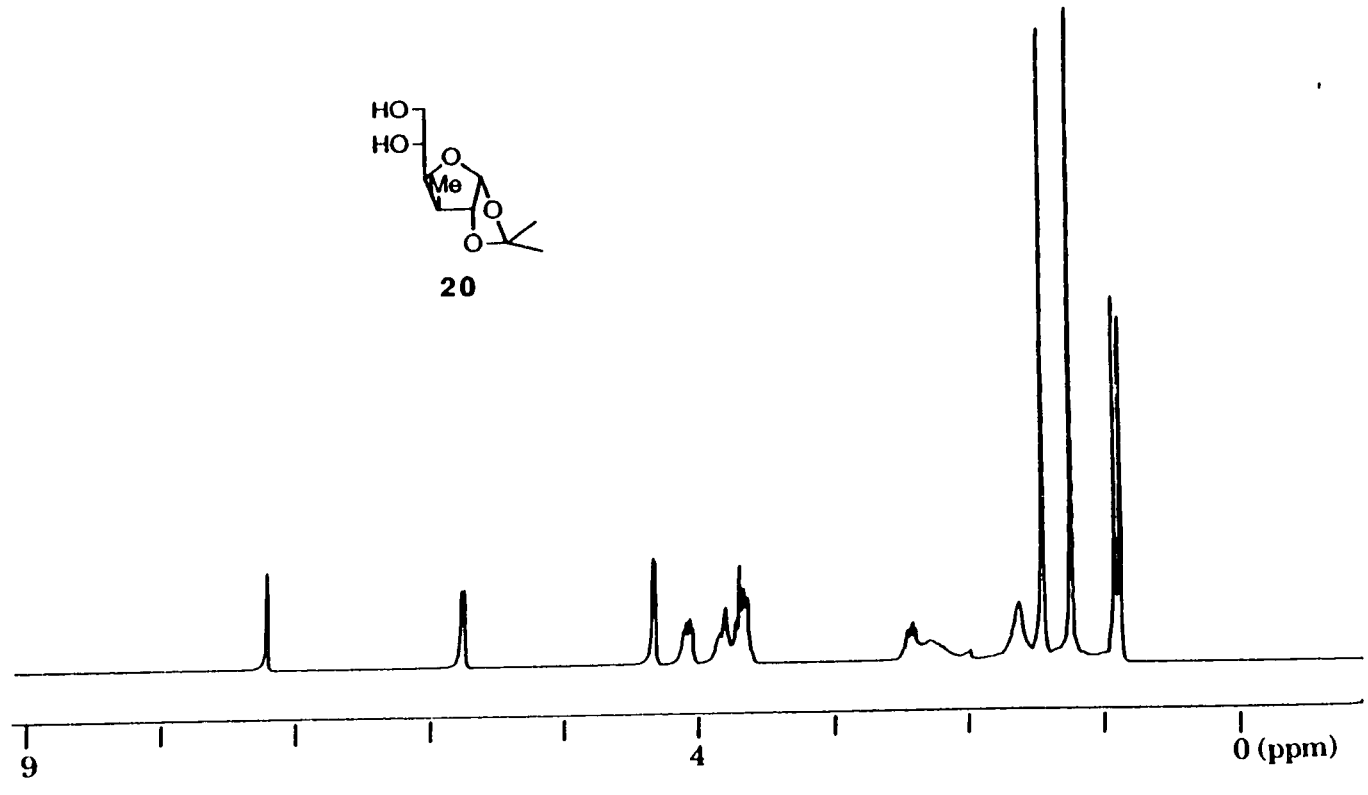


Figure 12. The 200 MHz ^1H NMR (in $CDCl_3$) of 1,2-Isopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (**20**)

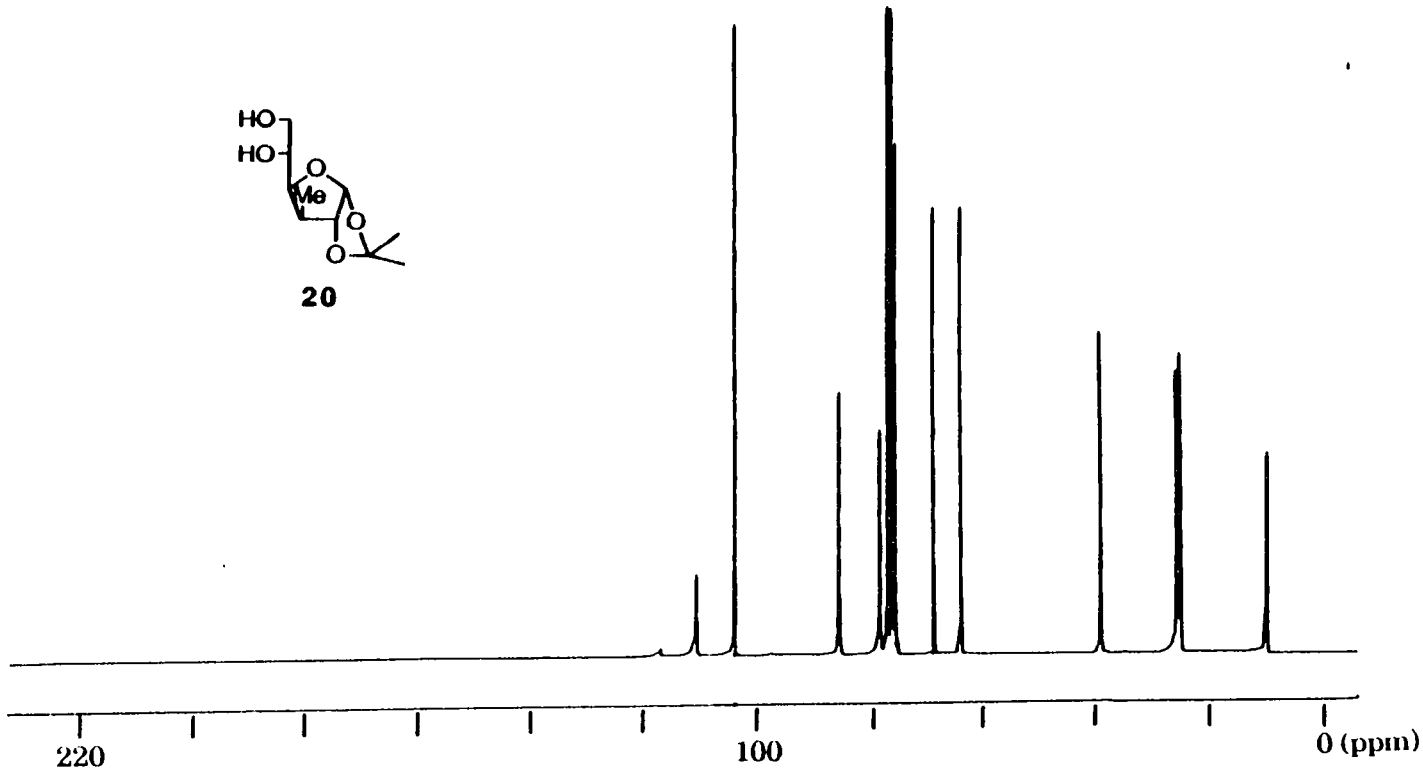


Figure 13. The 50 MHz ^{13}C NMR (in $CDCl_3$) of 1,2-Isopropylidene-3-deoxy-3-C-methyl-gluco-furanoside (**20**)

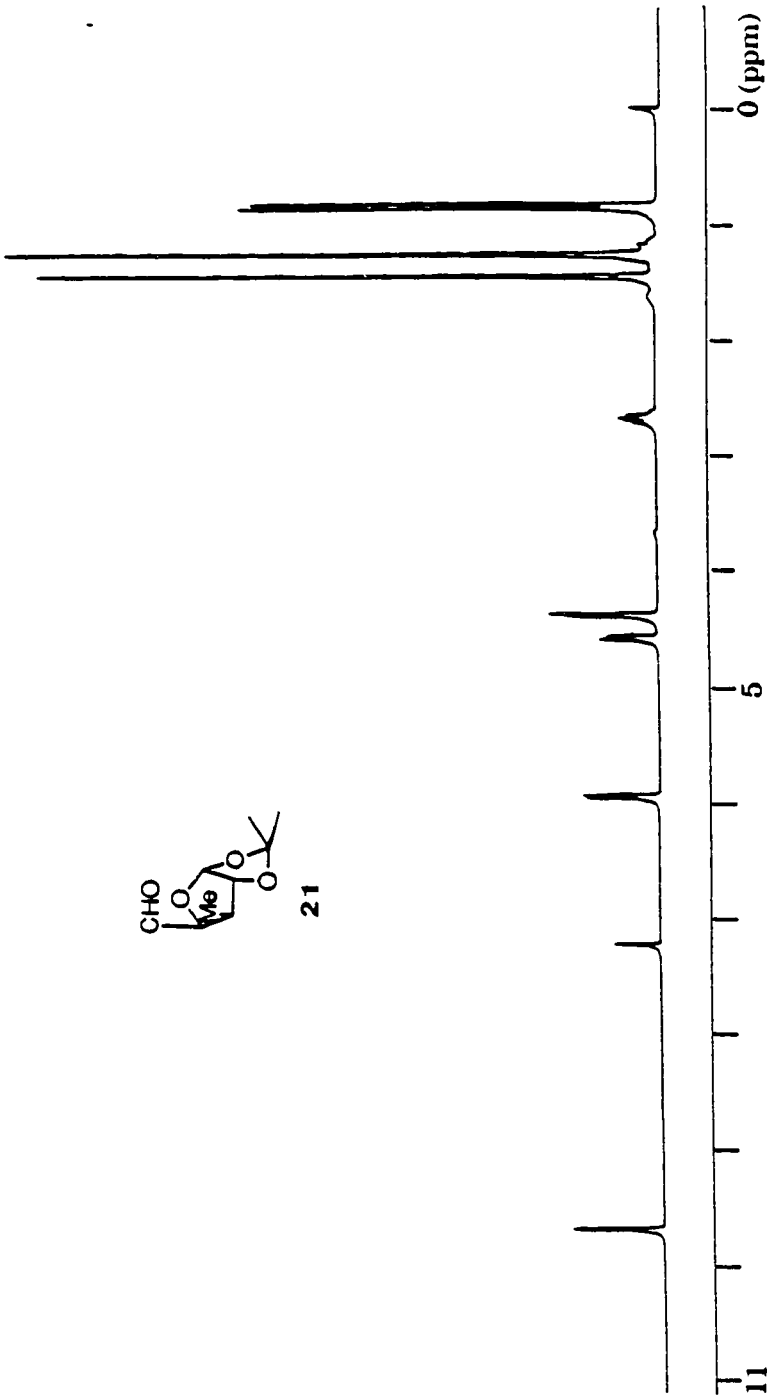


Figure 14. The 200 MHz ^{1}H NMR (in $CDCl_3$) of 1,2-Isopropylidene-3-deoxy-3-C-methyl-erythro-pentodialdfouranose (**21**)

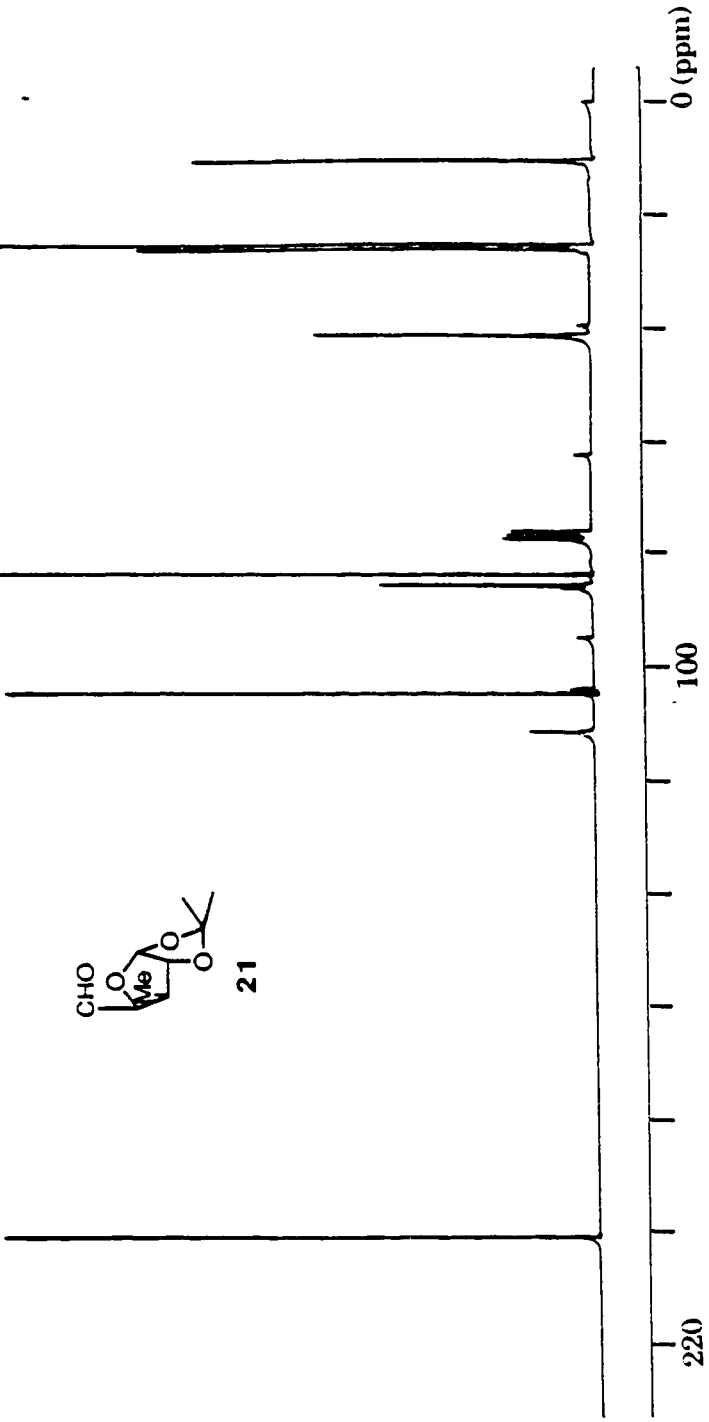


Figure 15. The 50 MHz ^{13}C NMR (in $CDCl_3$) of 1,2-Isopropylidene-3-deoxy-3-C-methyl-erythro-pentodialdofuranose (**21**)

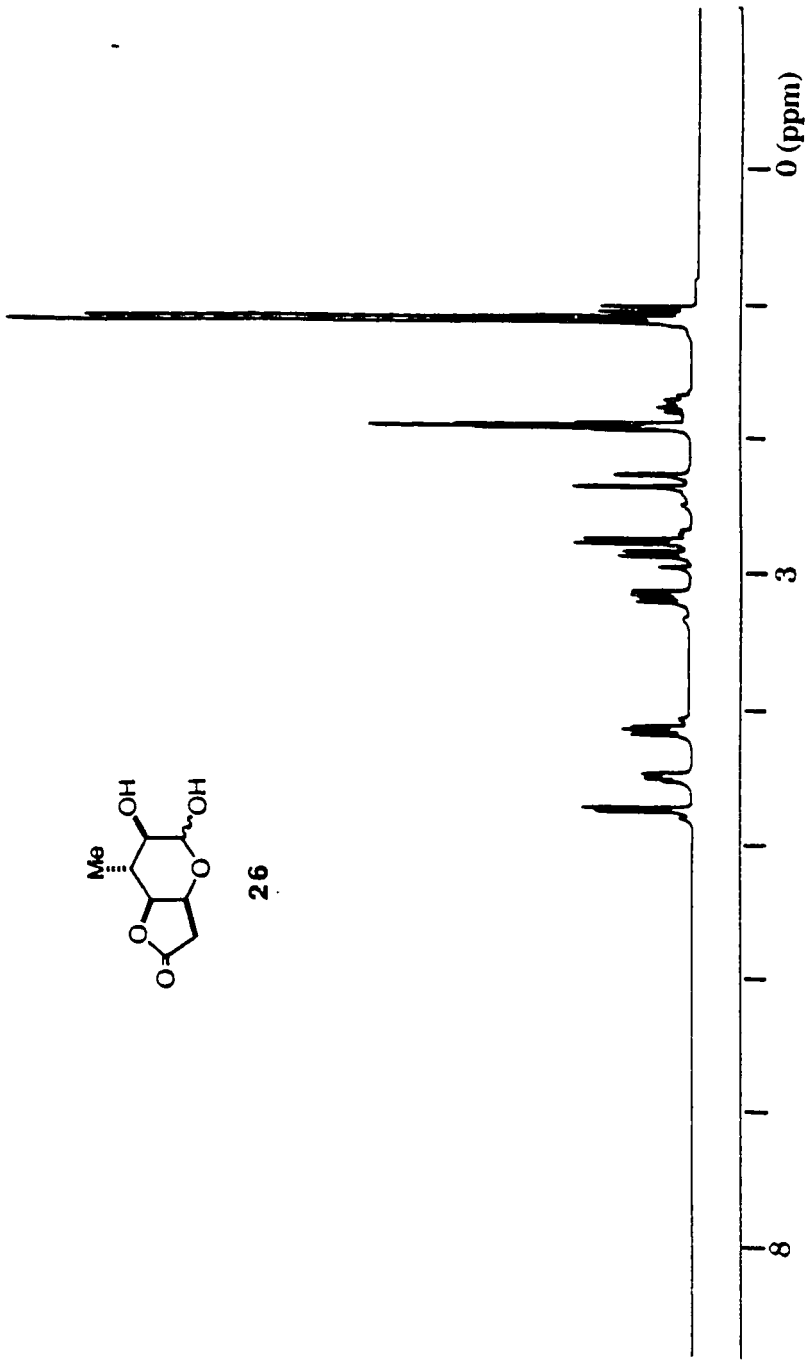


Figure 16. The 200 MHz ^1H NMR (in CD_3CN) of lactone **26**

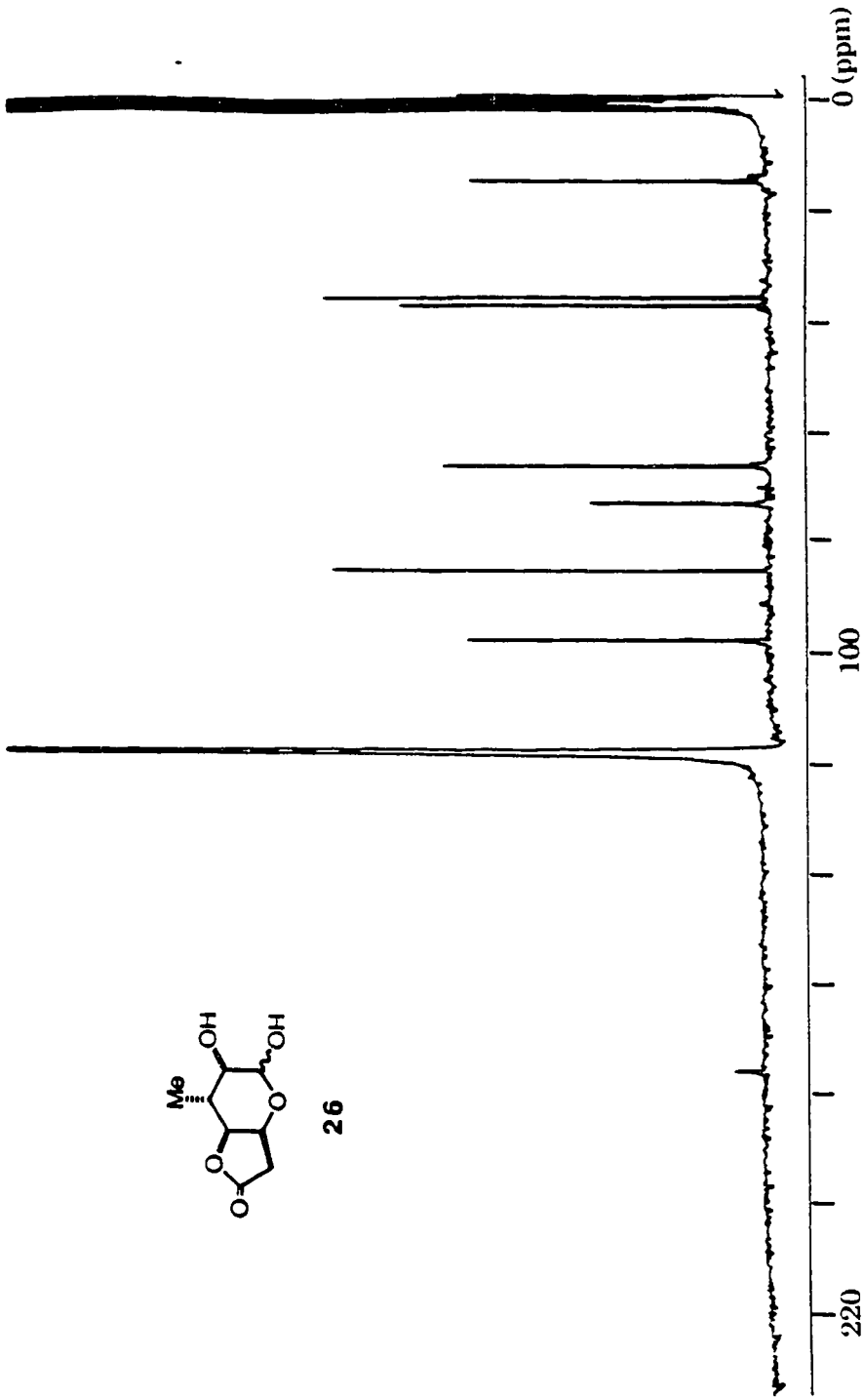


Figure 17. The 50 MHz ^{13}C NMR (in CD_3CN) of lactone **26**

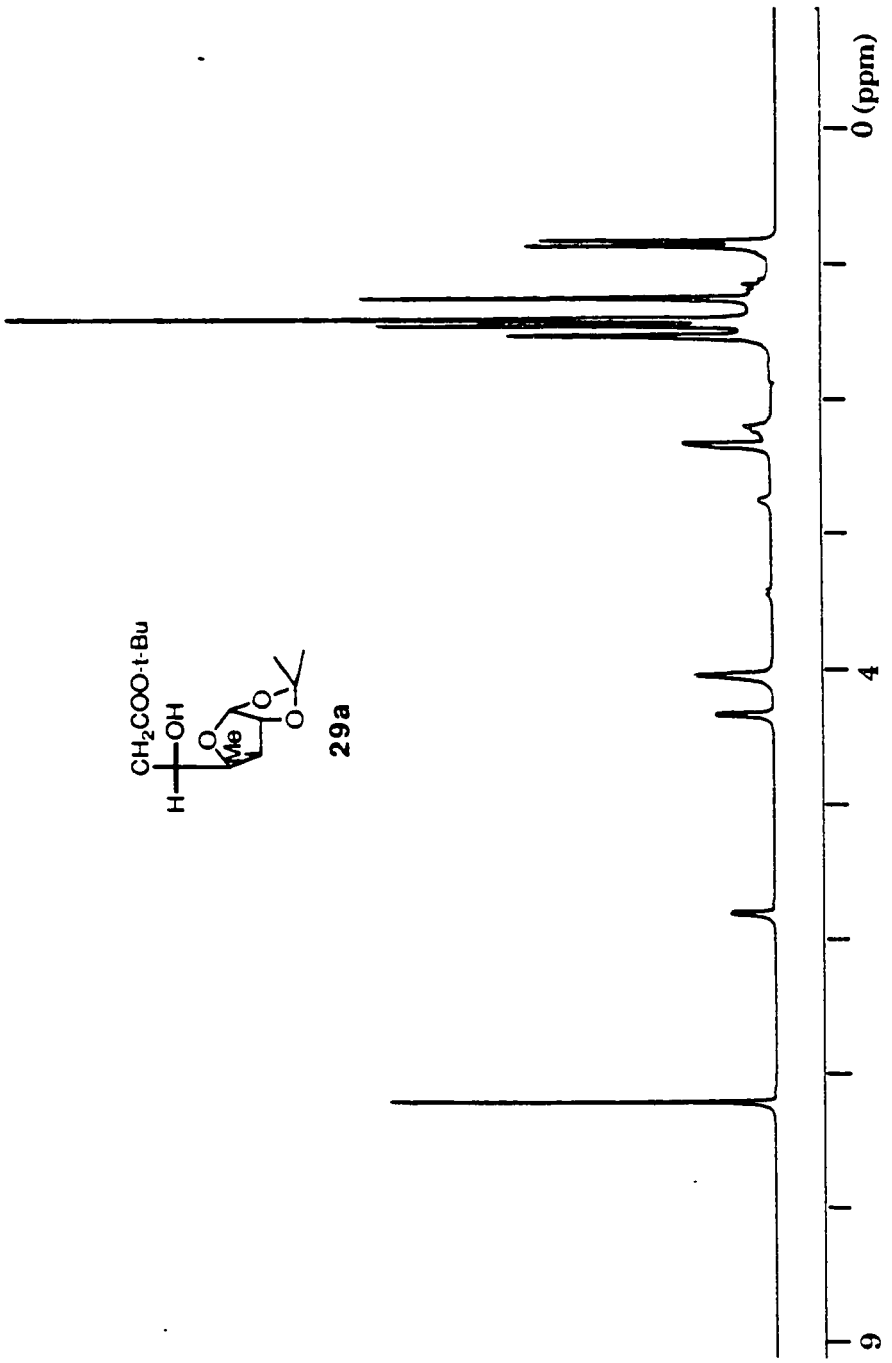


Figure 18. The 200 MHz ^{1}H NMR (in $CDCl_3$) of t-butyl ester **29a**

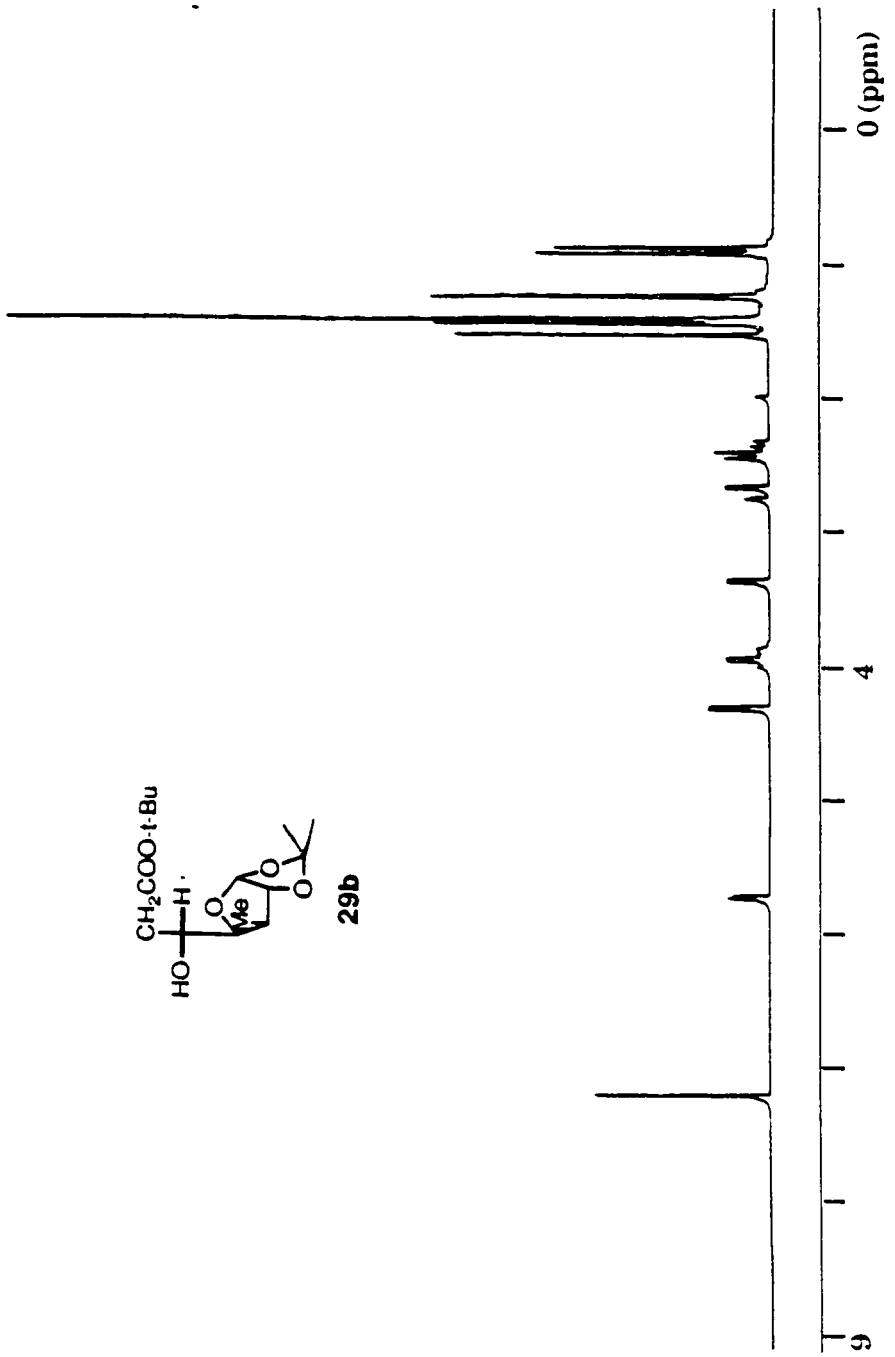


Figure 19. The 200 MHz ^{1}H NMR (in $CDCl_3$) of t-butyl ester **29b**

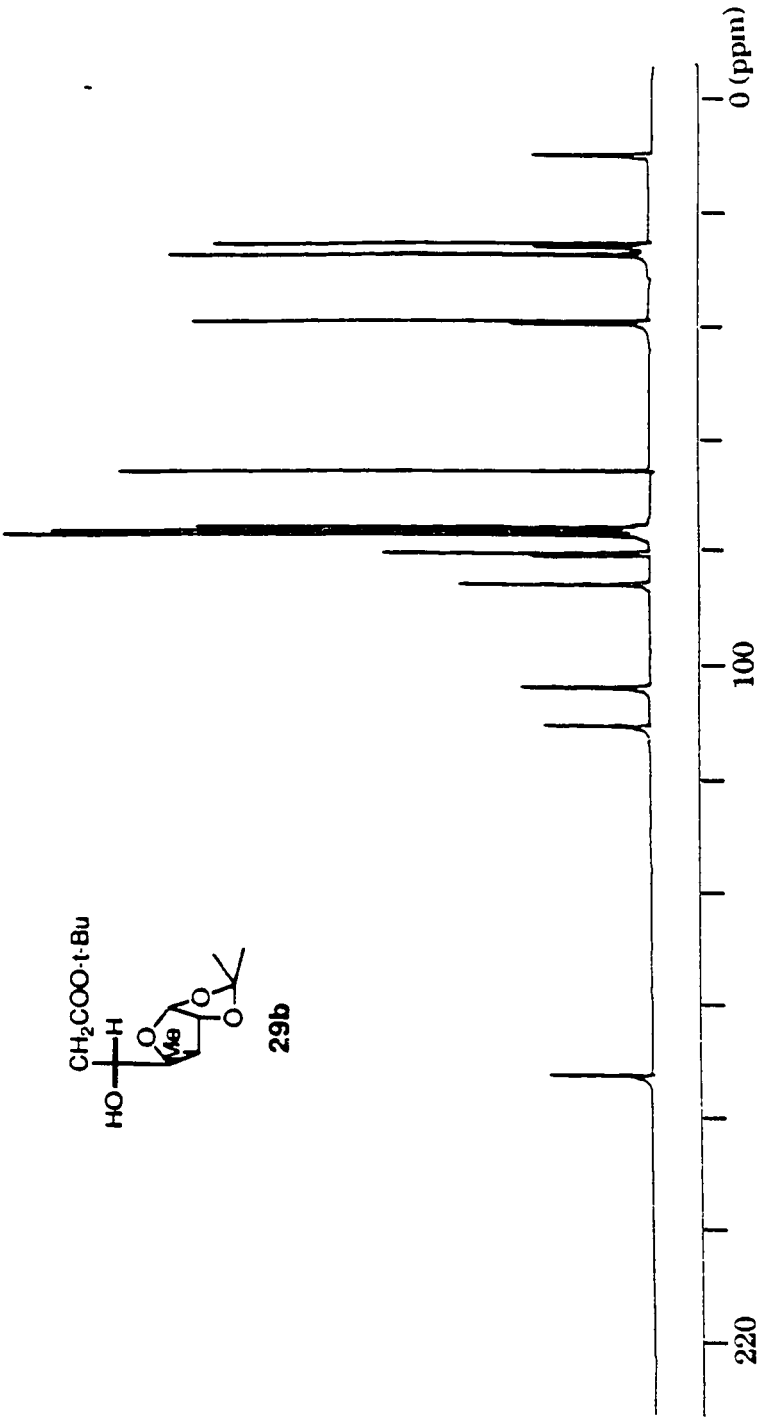


Figure 20. The 50 MHz ^{13}C NMR (in $CDCl_3$) of t-butyl ester **29b**

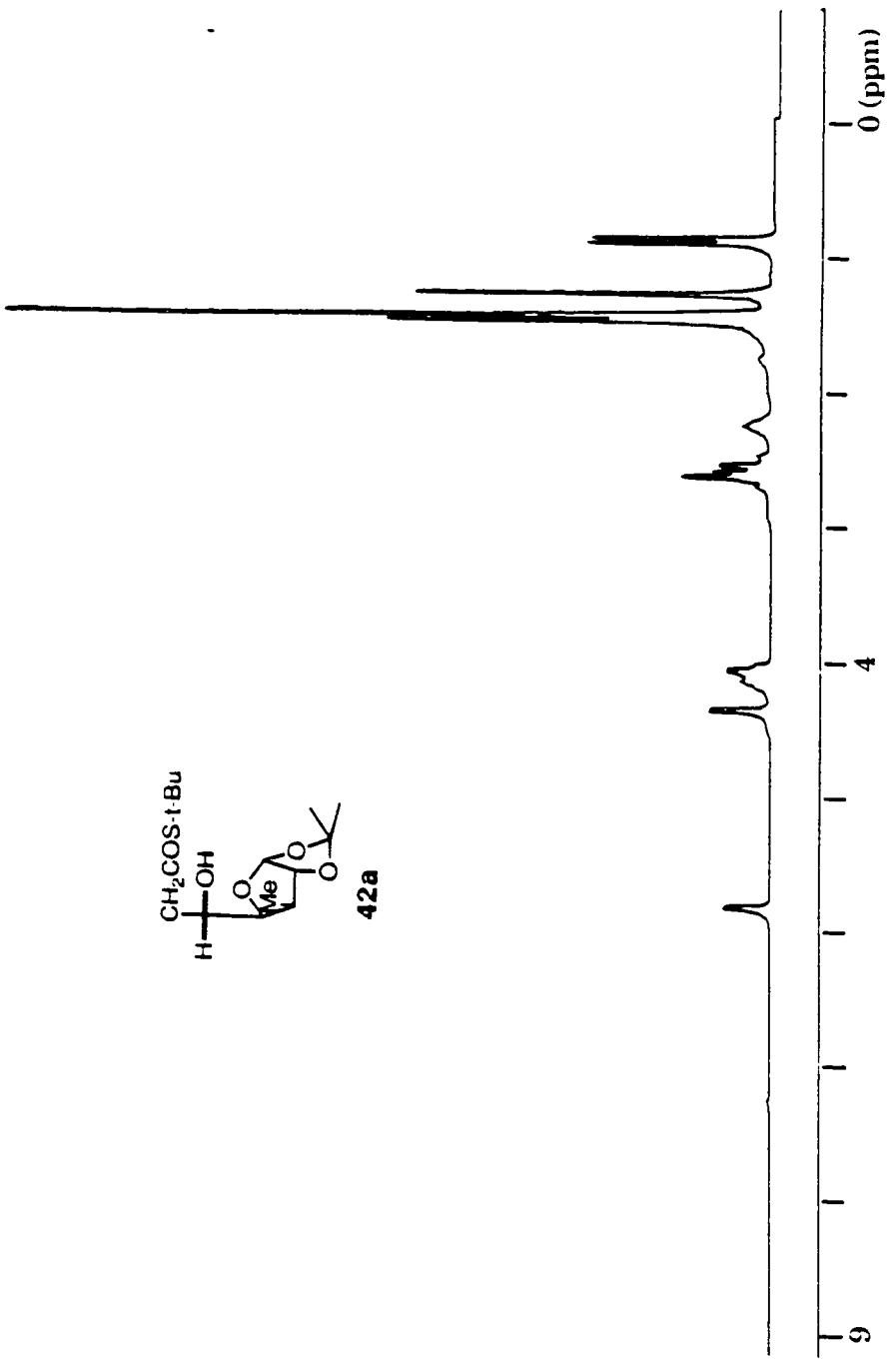


Figure 21. The 200 MHz ^{1}H NMR (in $CDCl_3$) of thioester **42a**

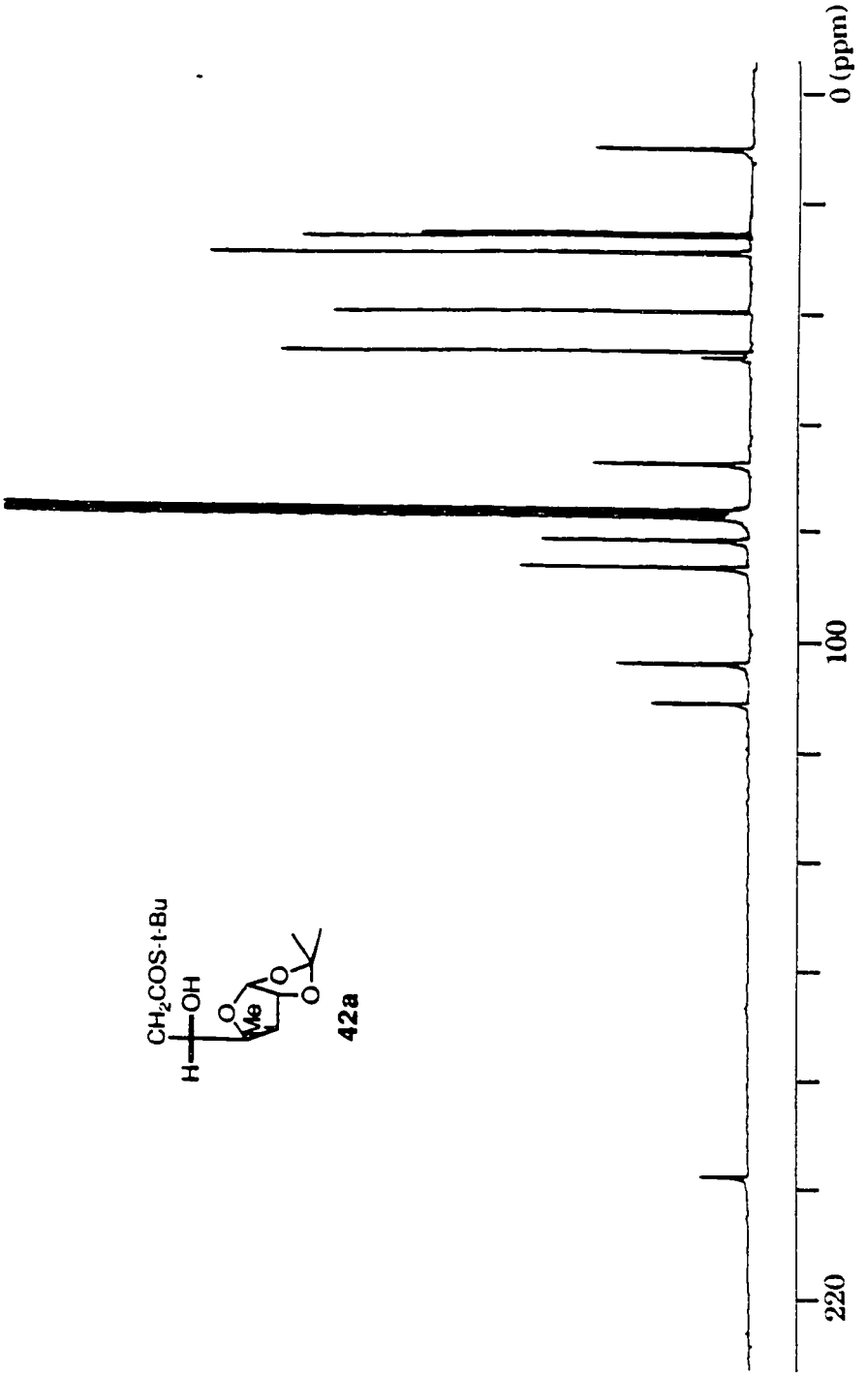


Figure 22. The 50 MHz ^{13}C NMR (in $CDCl_3$) of thioester **42a**

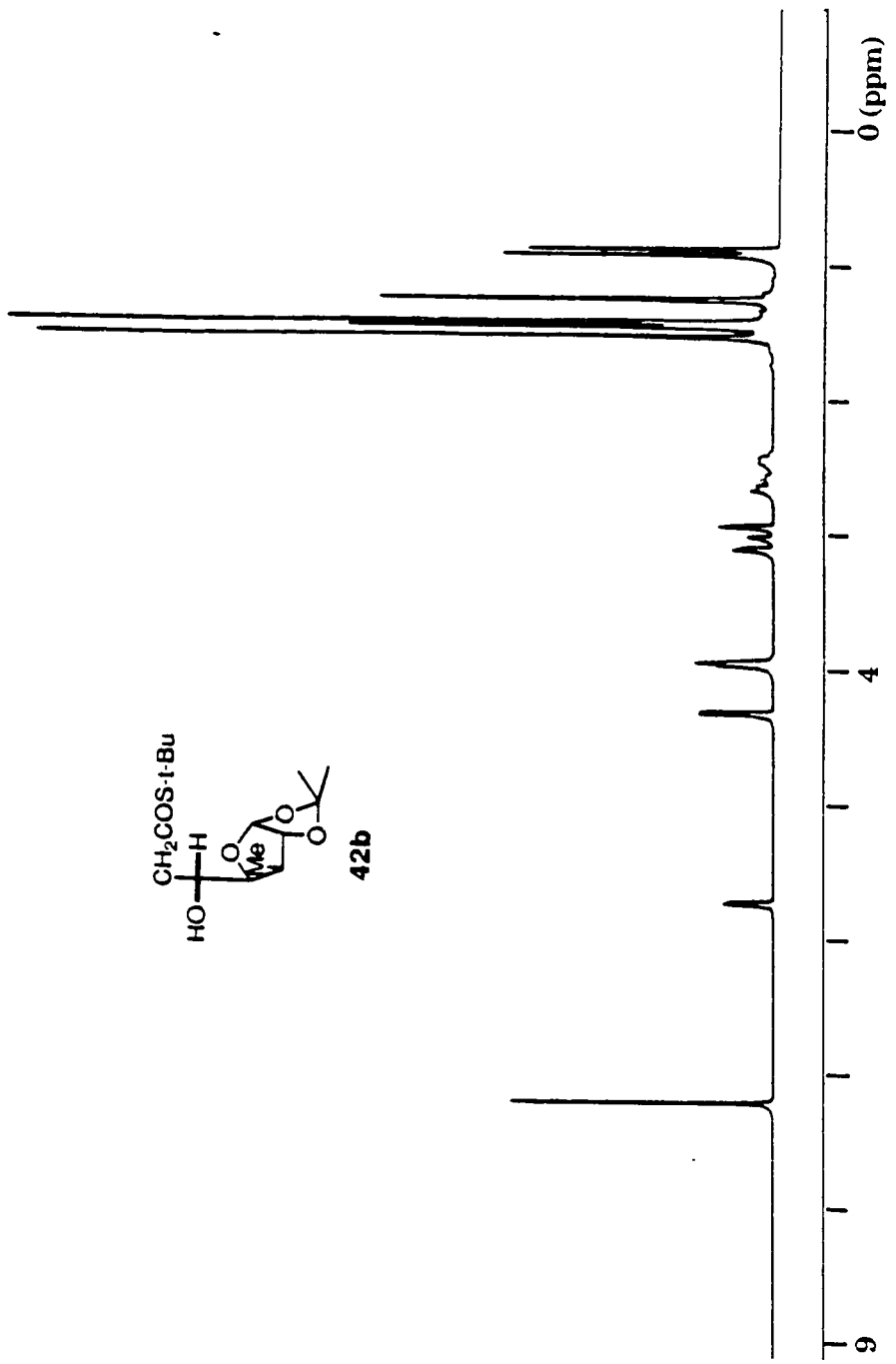


Figure 23. The 200 MHz ^{1}H NMR (in $CDCl_3$) of thioester **42b**

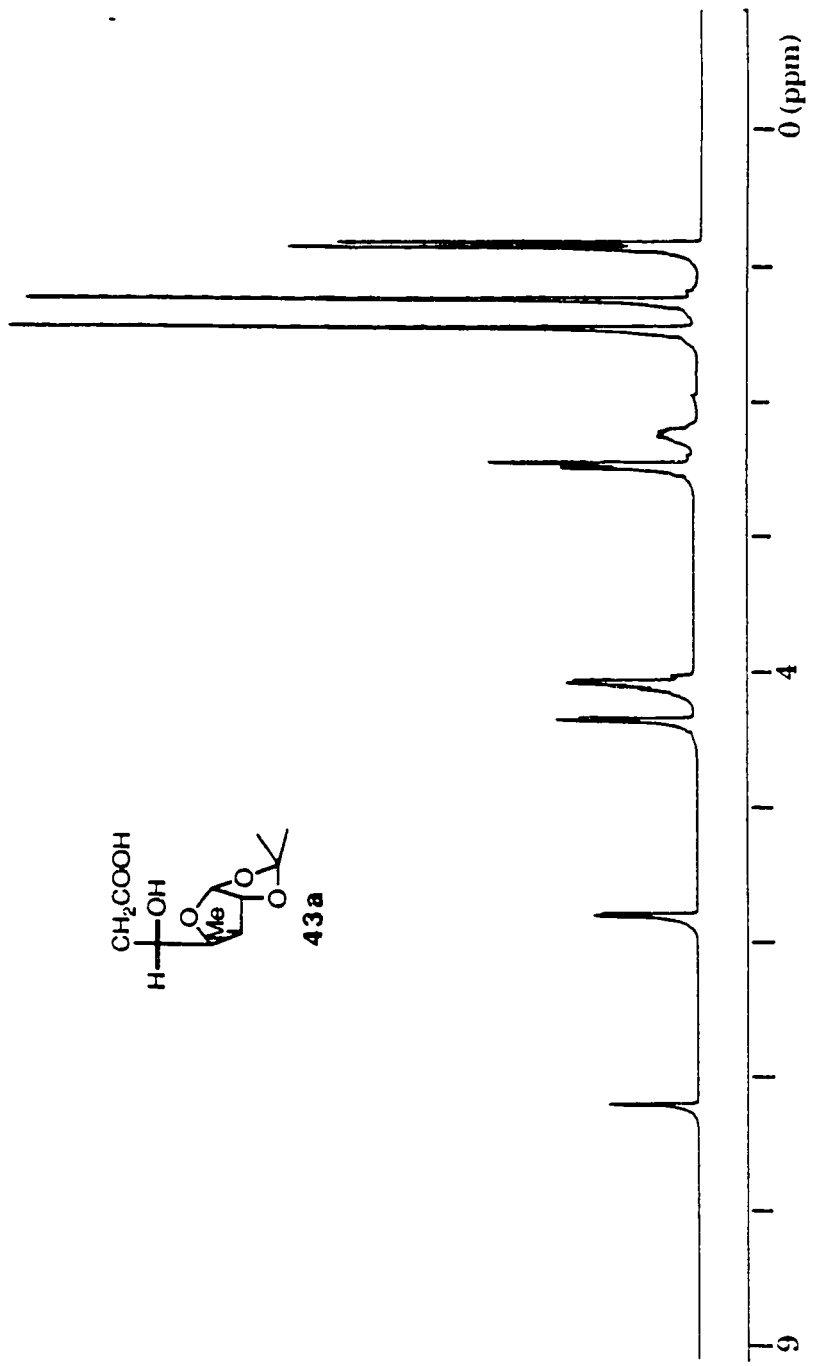


Figure 24. The 200 MHz ^{1}H NMR (in $CDCl_3$) of hydroxy acid **43a**

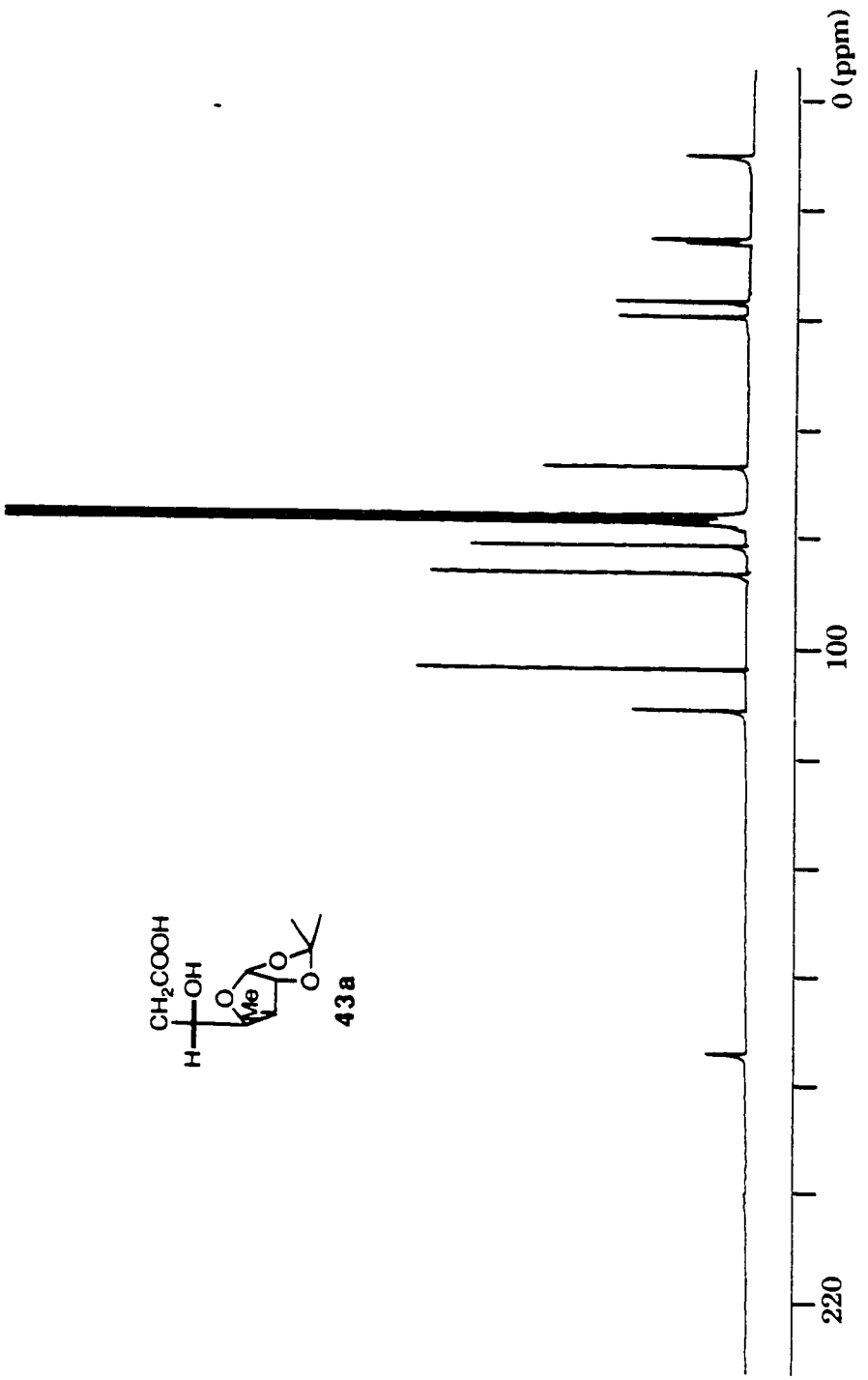


Figure 25. The 50 MHz ^{13}C NMR (in $CDCl_3$) of hydroxy acid **43a**

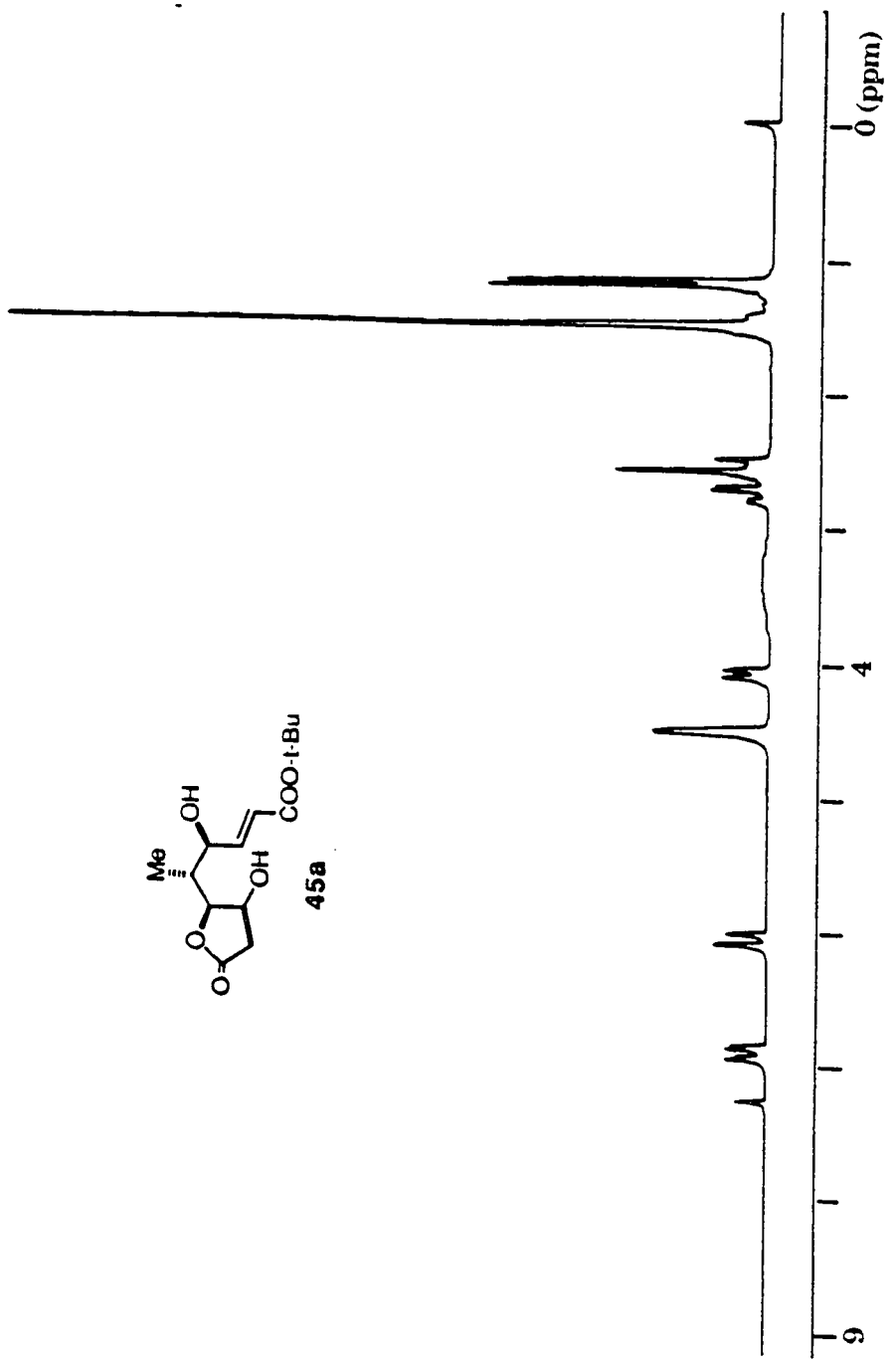


Figure 26. The 200 MHz ^{1}H NMR (in $CDCl_3$) of trans α,β-unsaturated ester **45a**

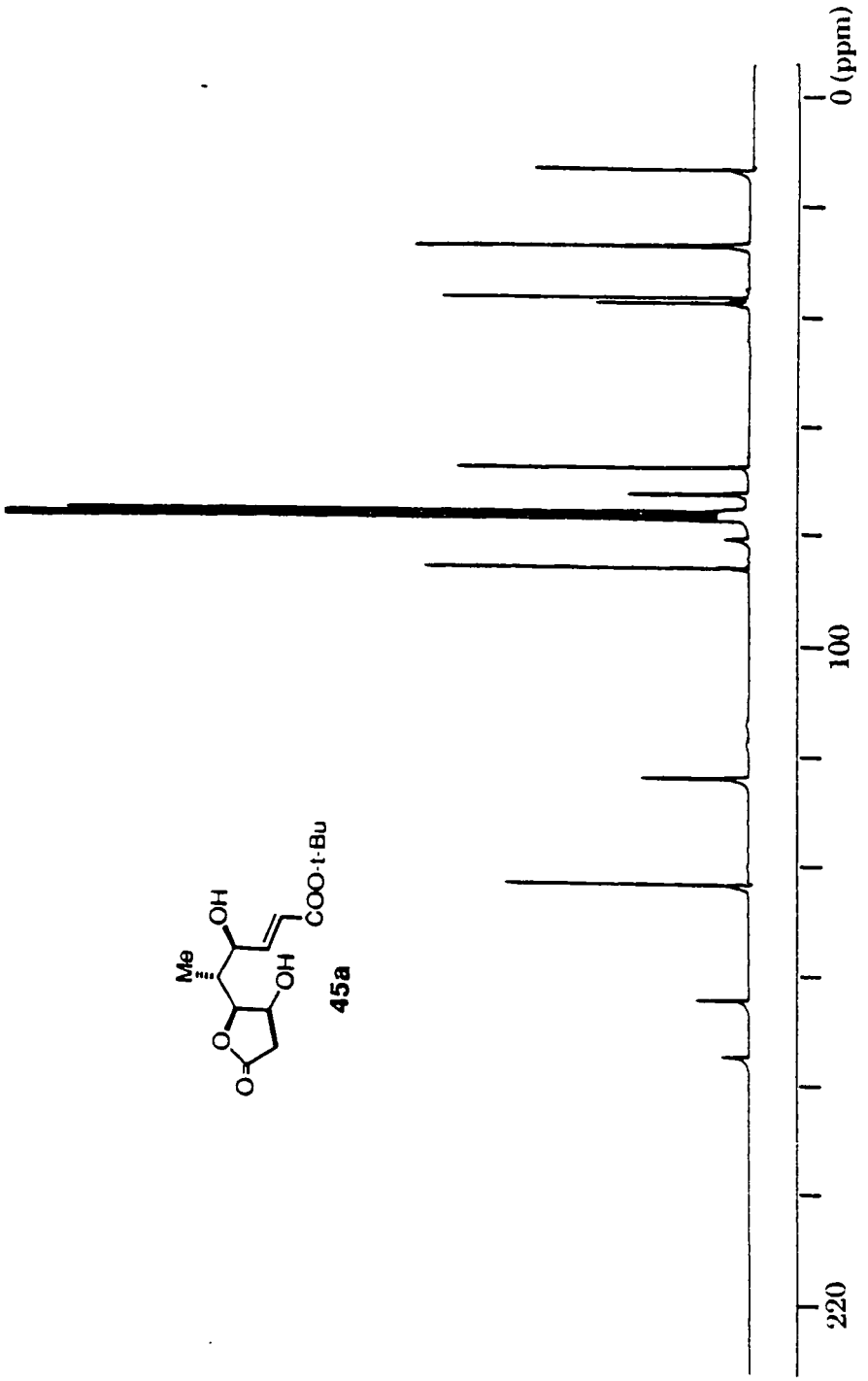


Figure 27. The 50 MHz ^{13}C NMR (in $CDCl_3$) of trans α,β-unsaturated ester **45a**

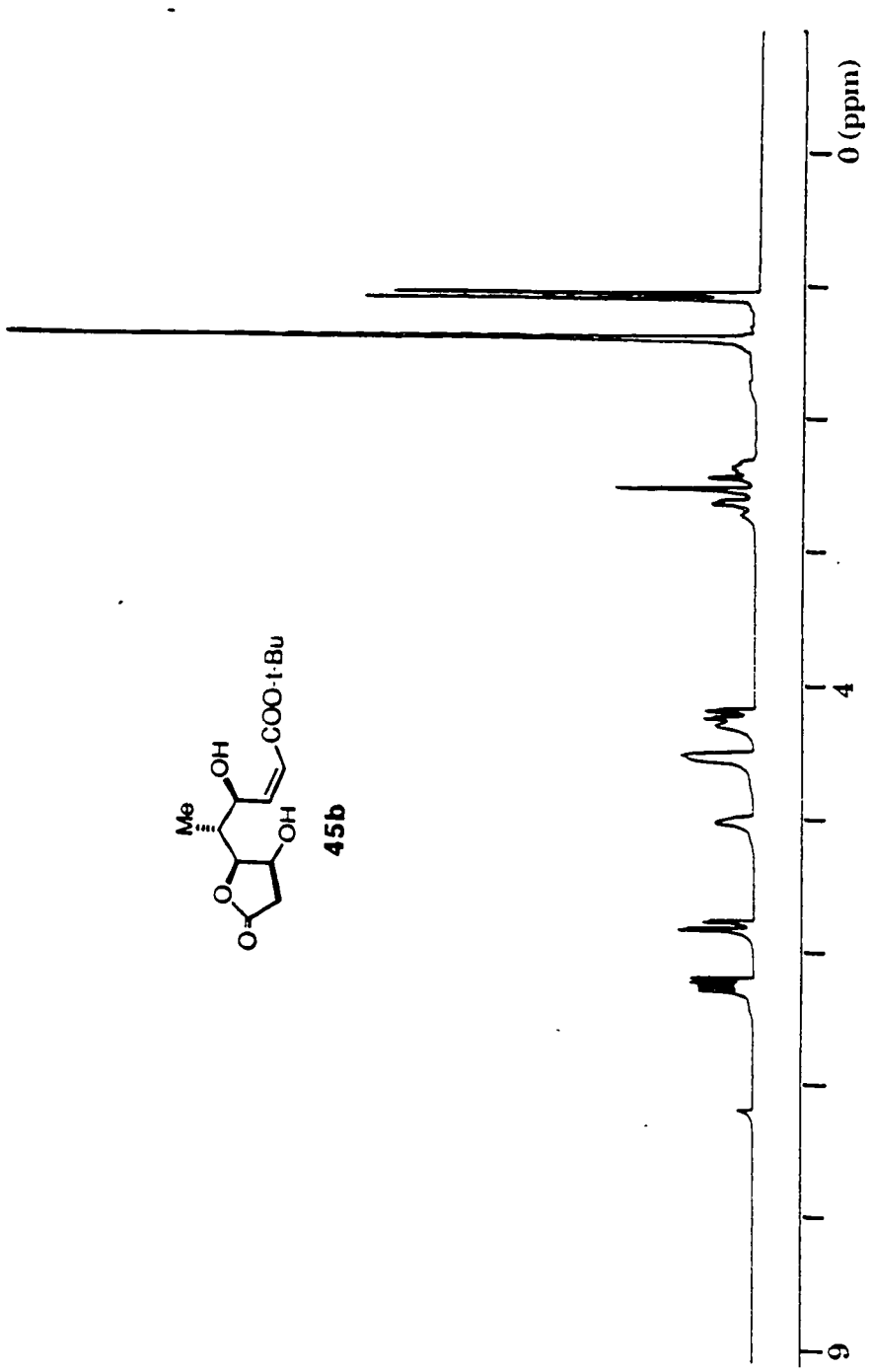


Figure 28. The 200 MHz ^1H NMR (in $CDCl_3$) of cis α,β-unsaturated ester **45b**

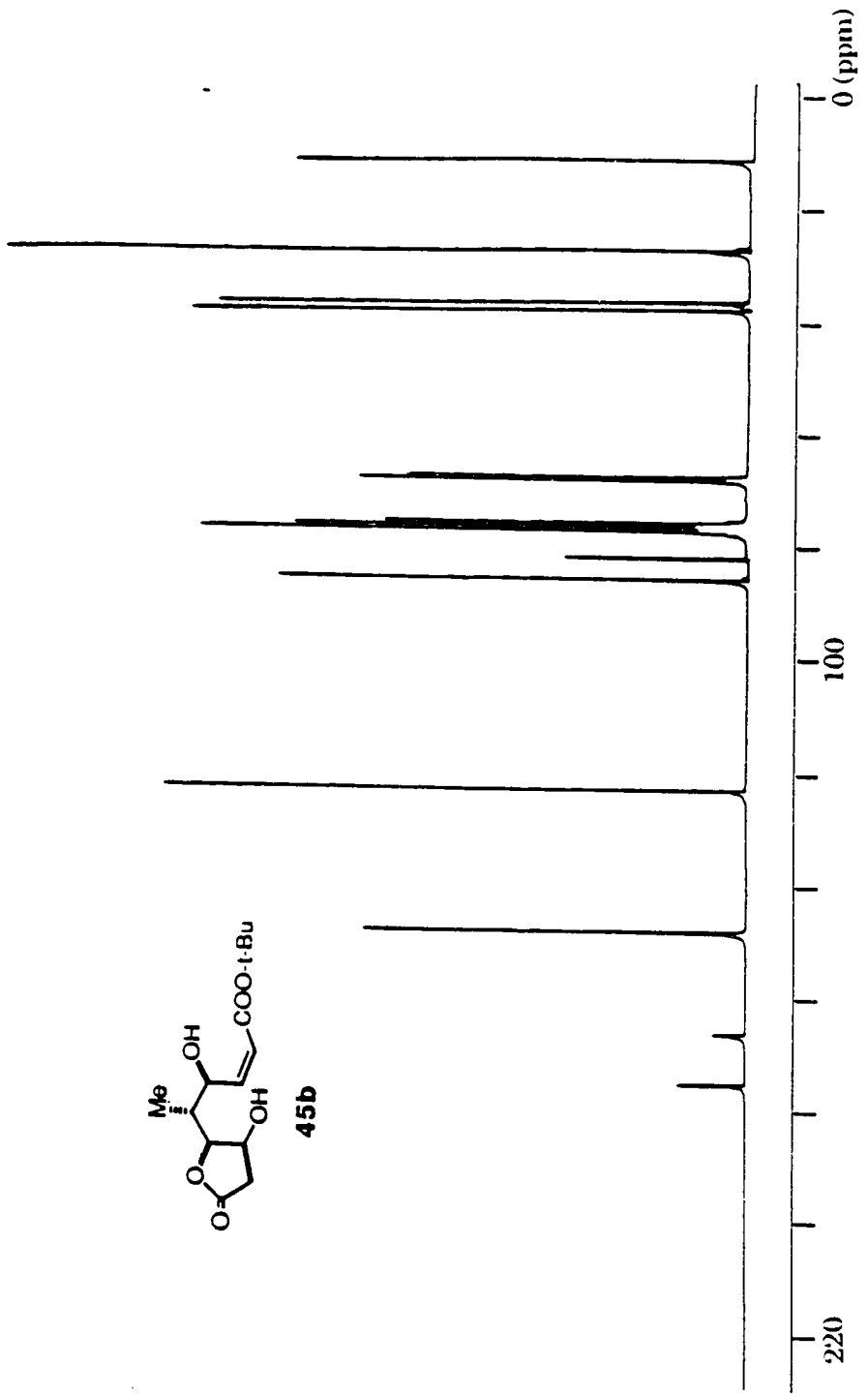


Figure 29. The 50 MHz ^{13}C NMR (in $CDCl_3$) of cis α,β-unsaturated ester **45b**

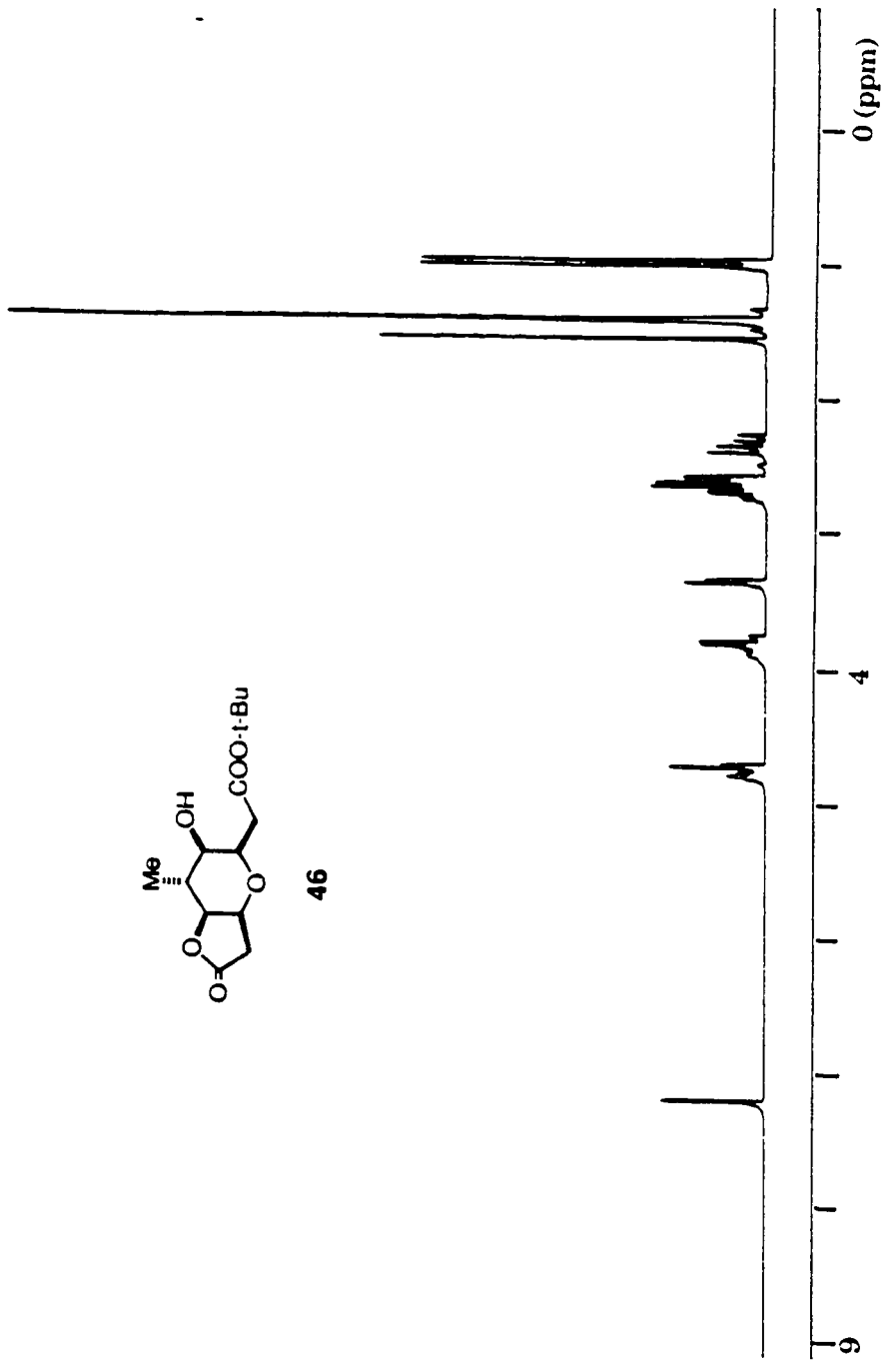


Figure 30. The 200 MHz ^{1}H NMR (in $CDCl_3$) of tetrahydropyran **46**

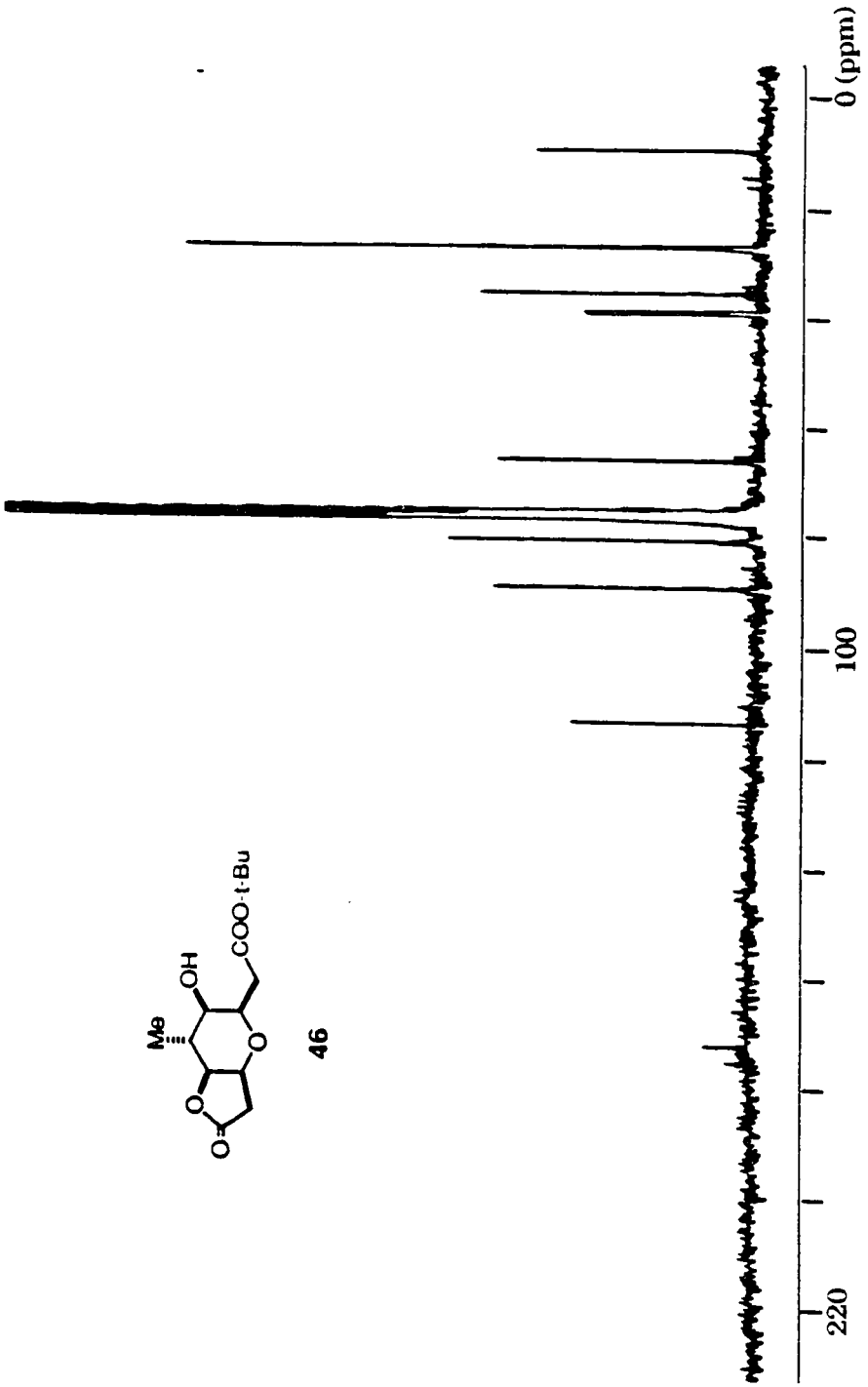


Figure 31. The 50 MHz ^{13}C NMR (in $CDCl_3$) of tetrahydropyran **46**

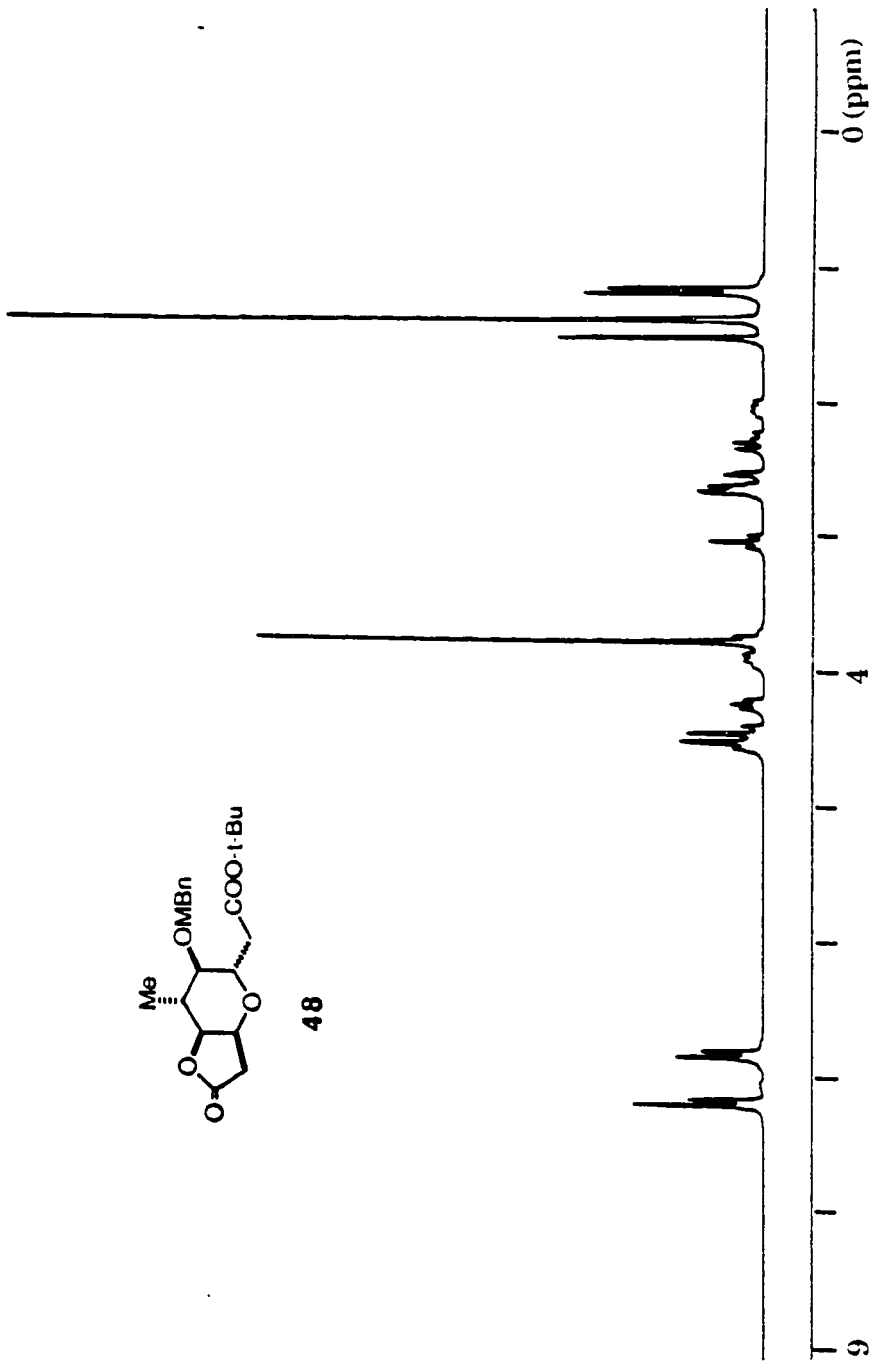


Figure 32. The 200 MHz ^{1}H NMR (in $CDCl_3$) of MBn ether **48**

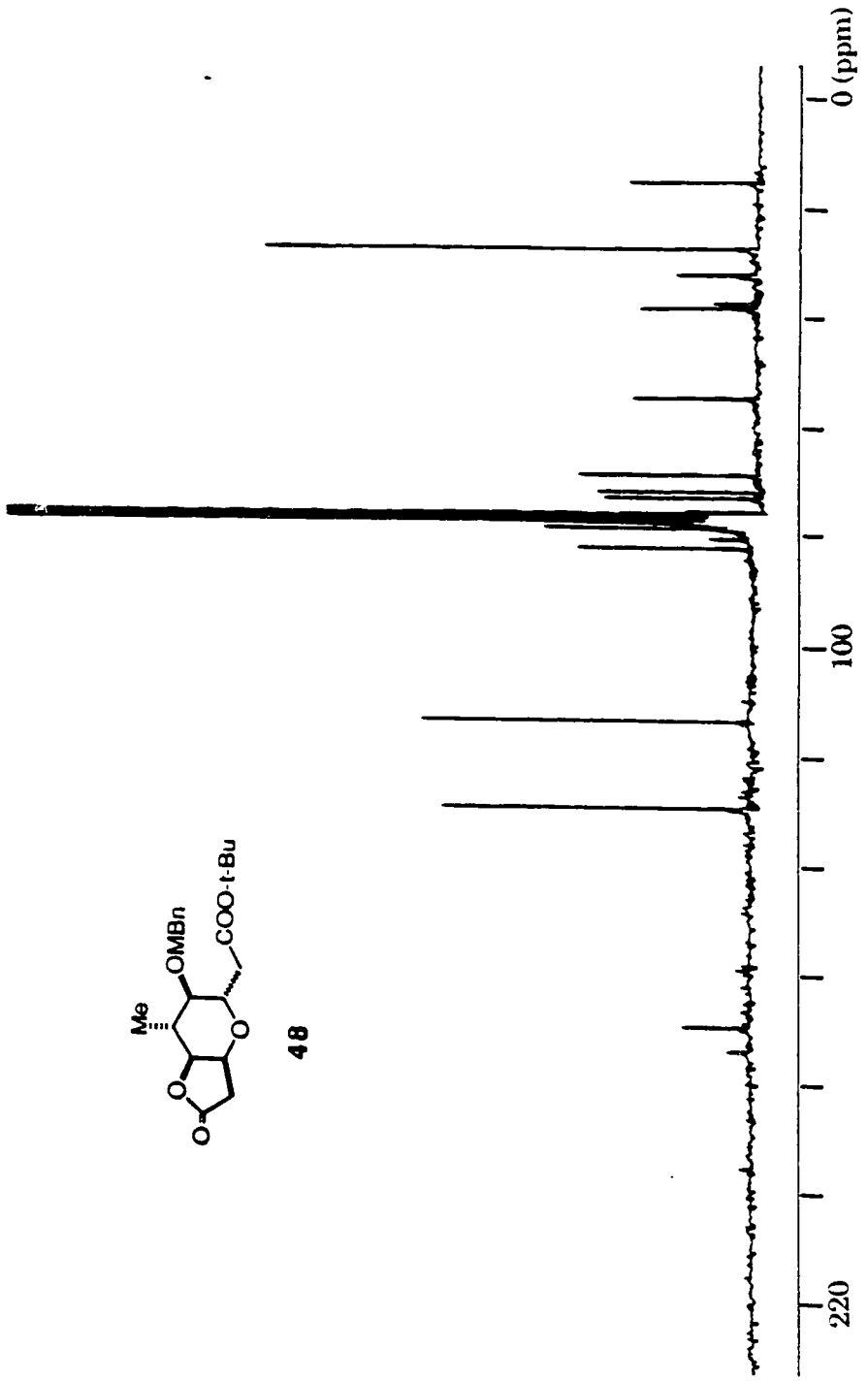
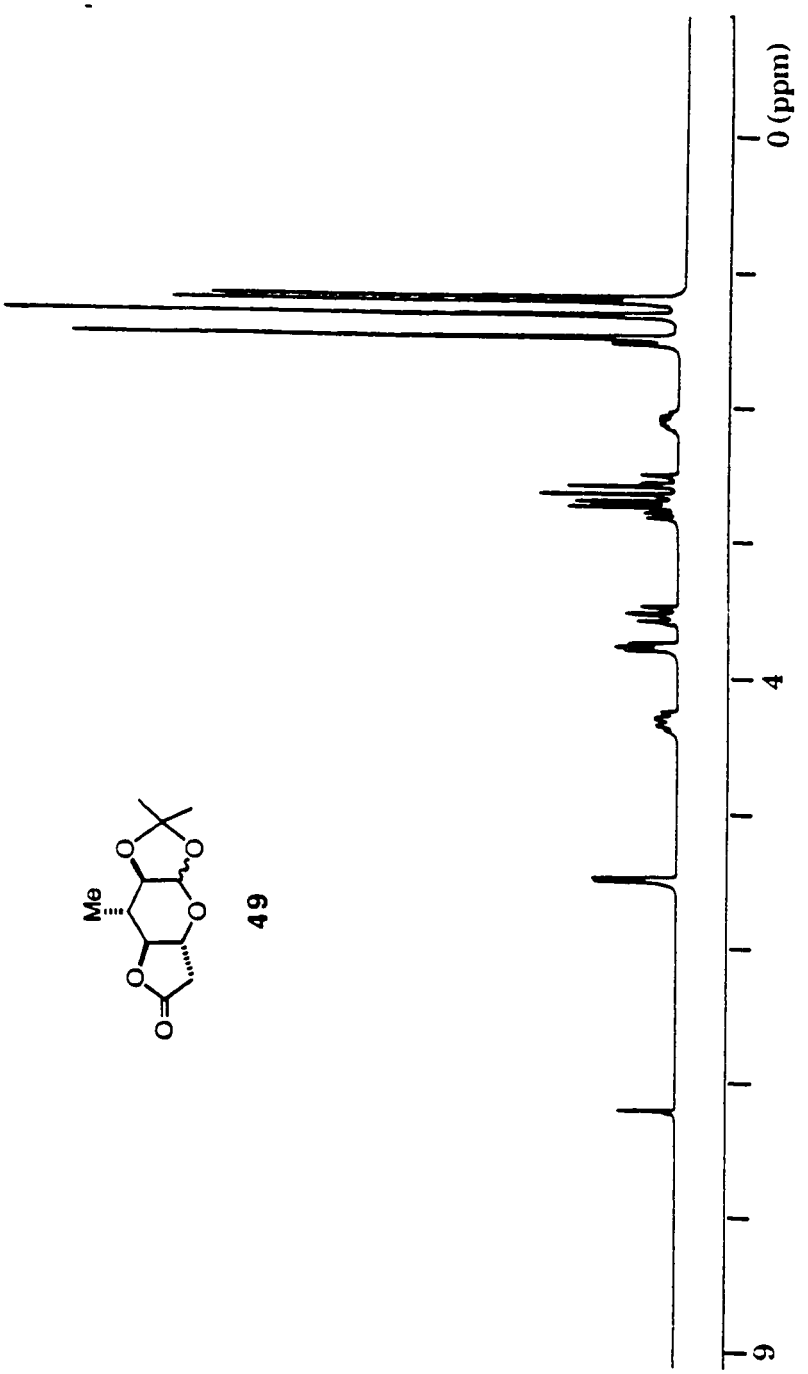
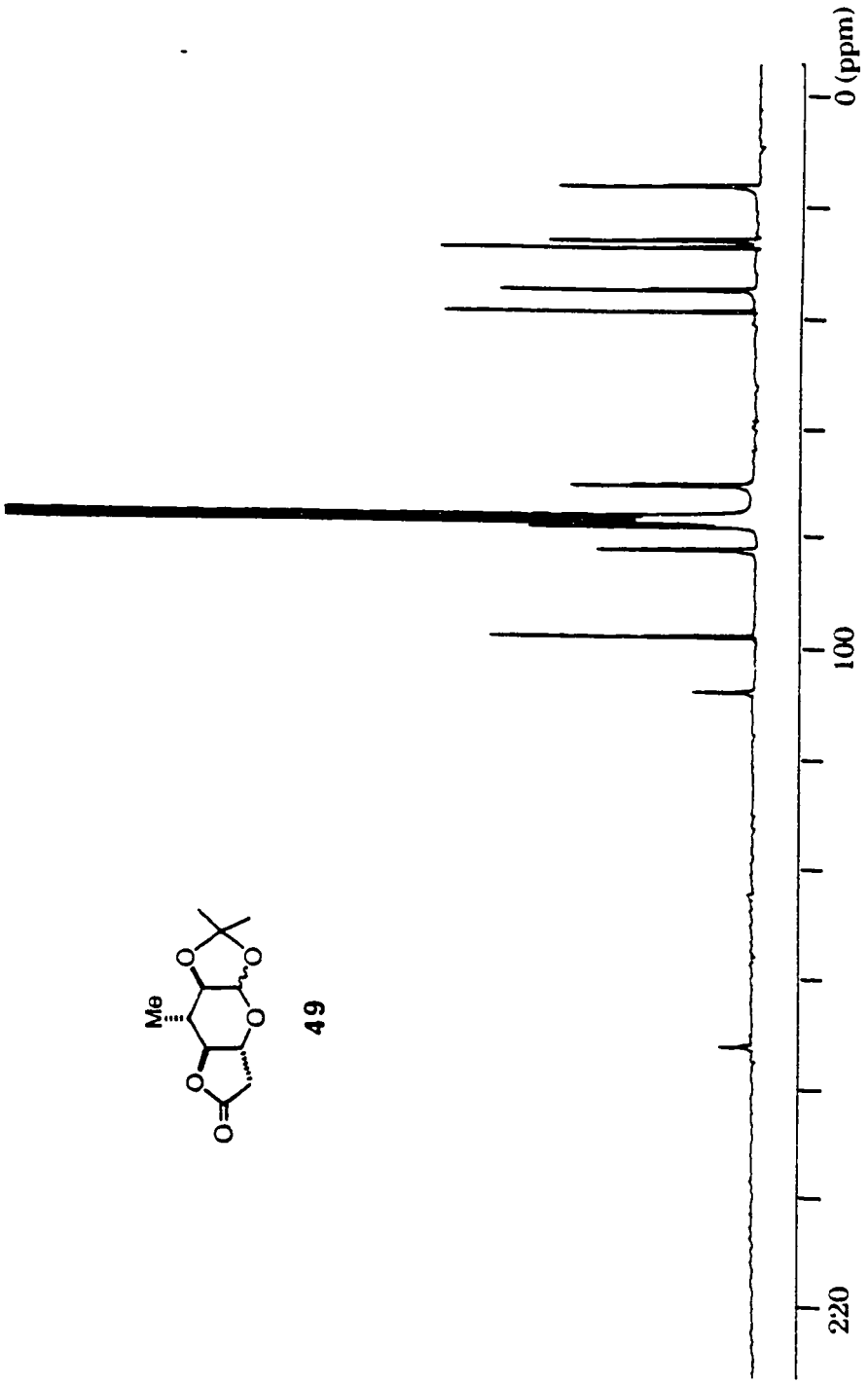


Figure 33. The 50 MHz ^{13}C NMR (in $CDCl_3$) of MBn ether **48**



49

Figure 34. The 200 MHz ^{1}H NMR (in $CDCl_3$) of trans lactone **49**



49

Figure 35. The 50 MHz ^{13}C NMR (in $CDCl_3$) of trans lactone **49**

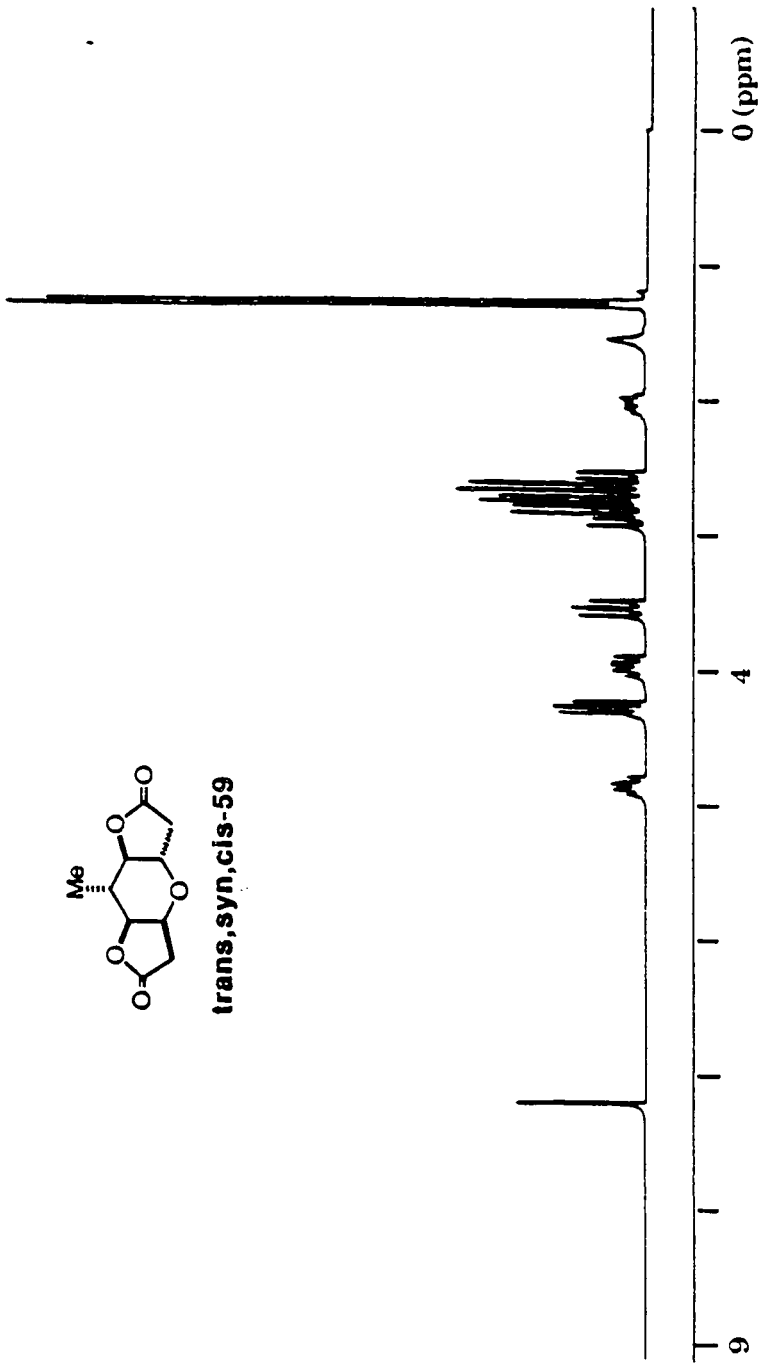


Figure 36. The 200 MHz ^{1}H NMR (in $CDCl_3$) of dilactone **59**

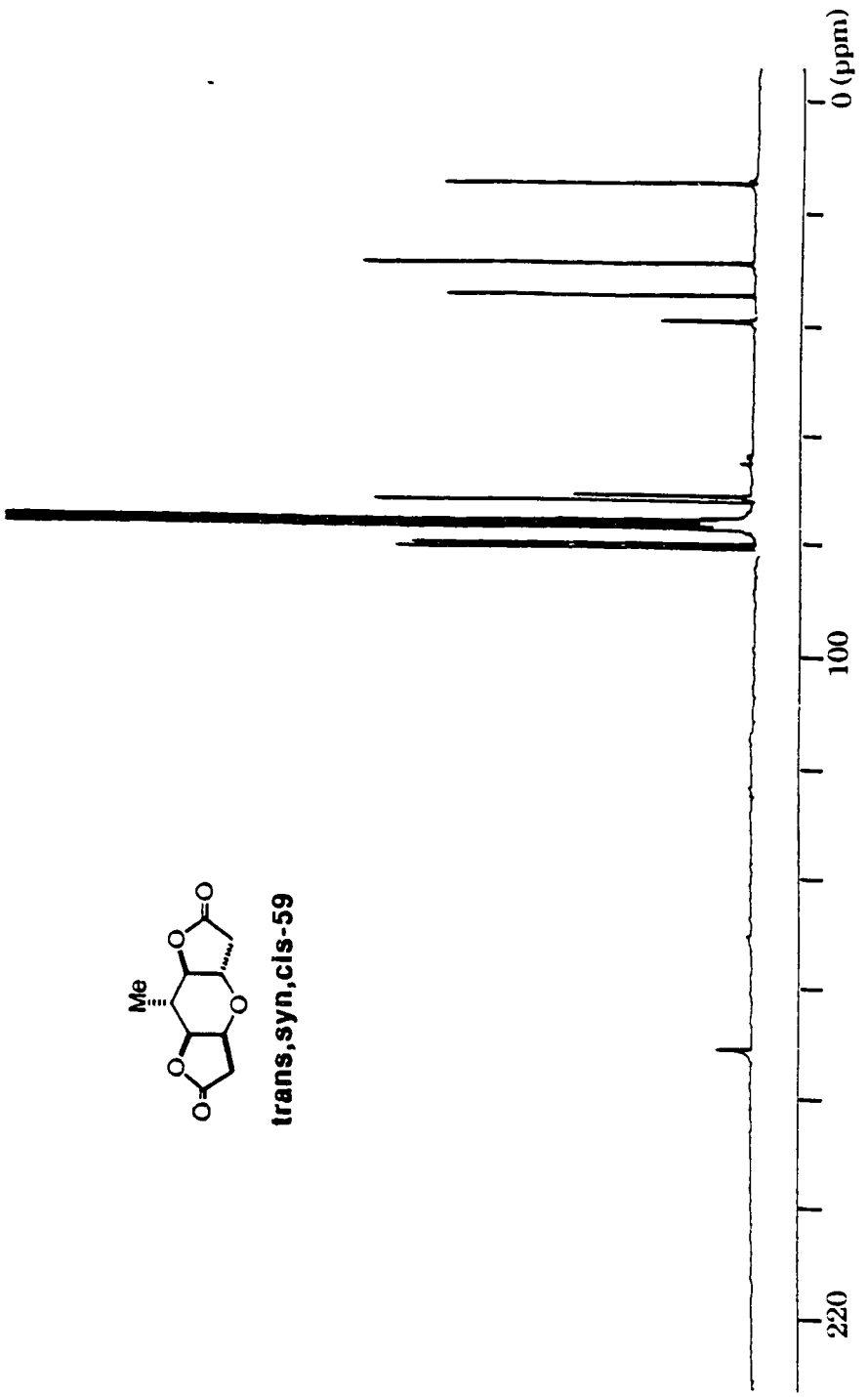
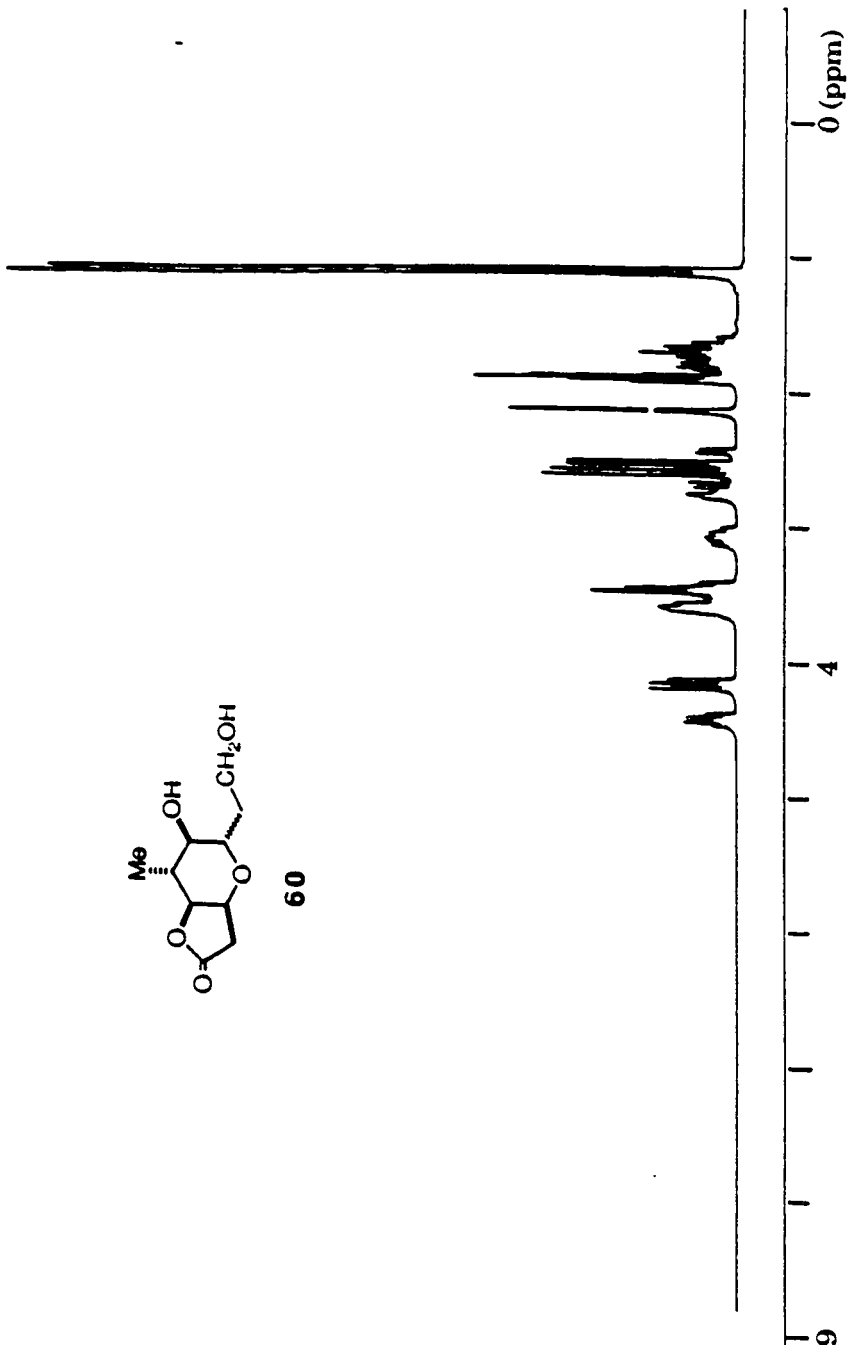


Figure 37. The 50 MHz ^{13}C NMR (in $CDCl_3$) of dilactone **59**



60

Figure 38. The 200 MHz ^{1}H NMR (in CD_3CN) of diol **60**

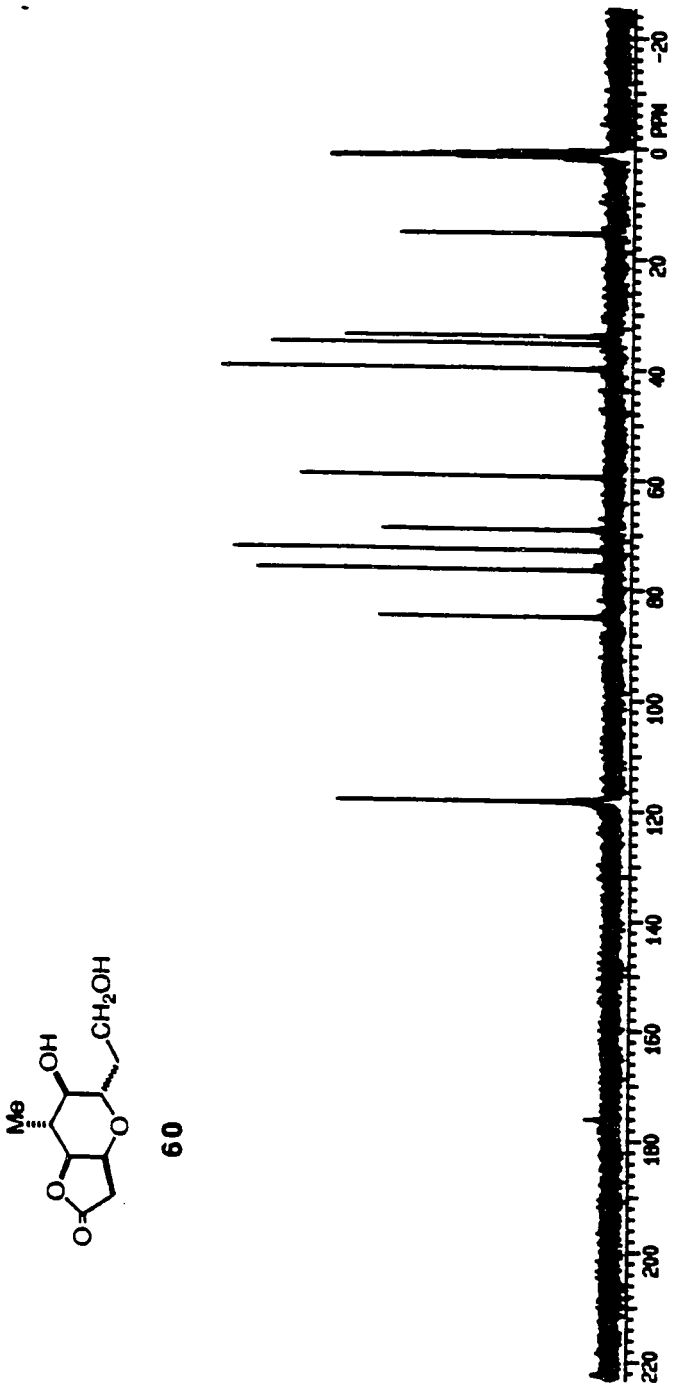


Figure 39. The 75 MHz ^{1}H NMR (in CD_3CN) of diol **60**

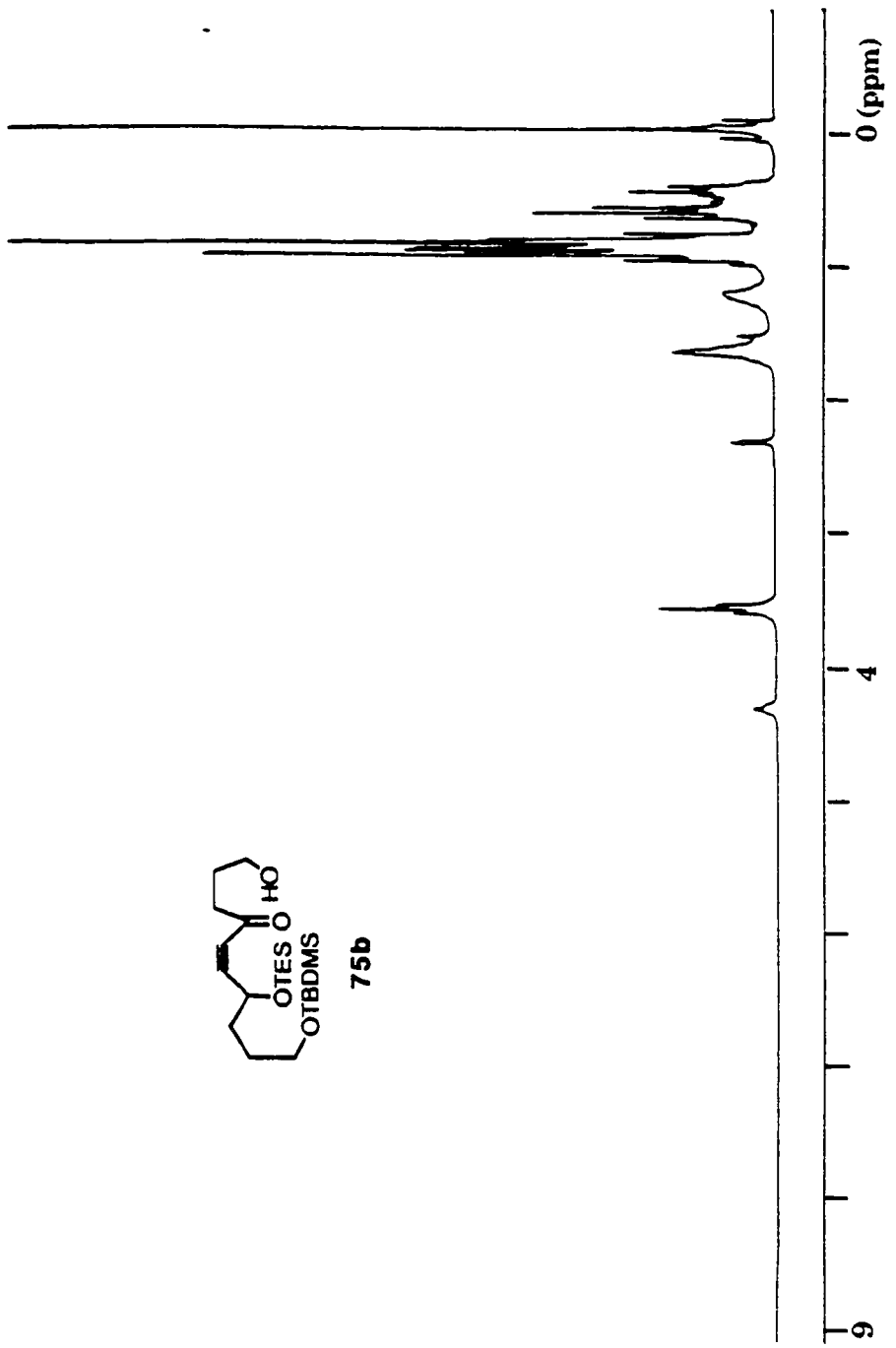


Figure 40. The 200 MHz ^{1}H NMR (in $CDCl_3$) of ketone **75b**

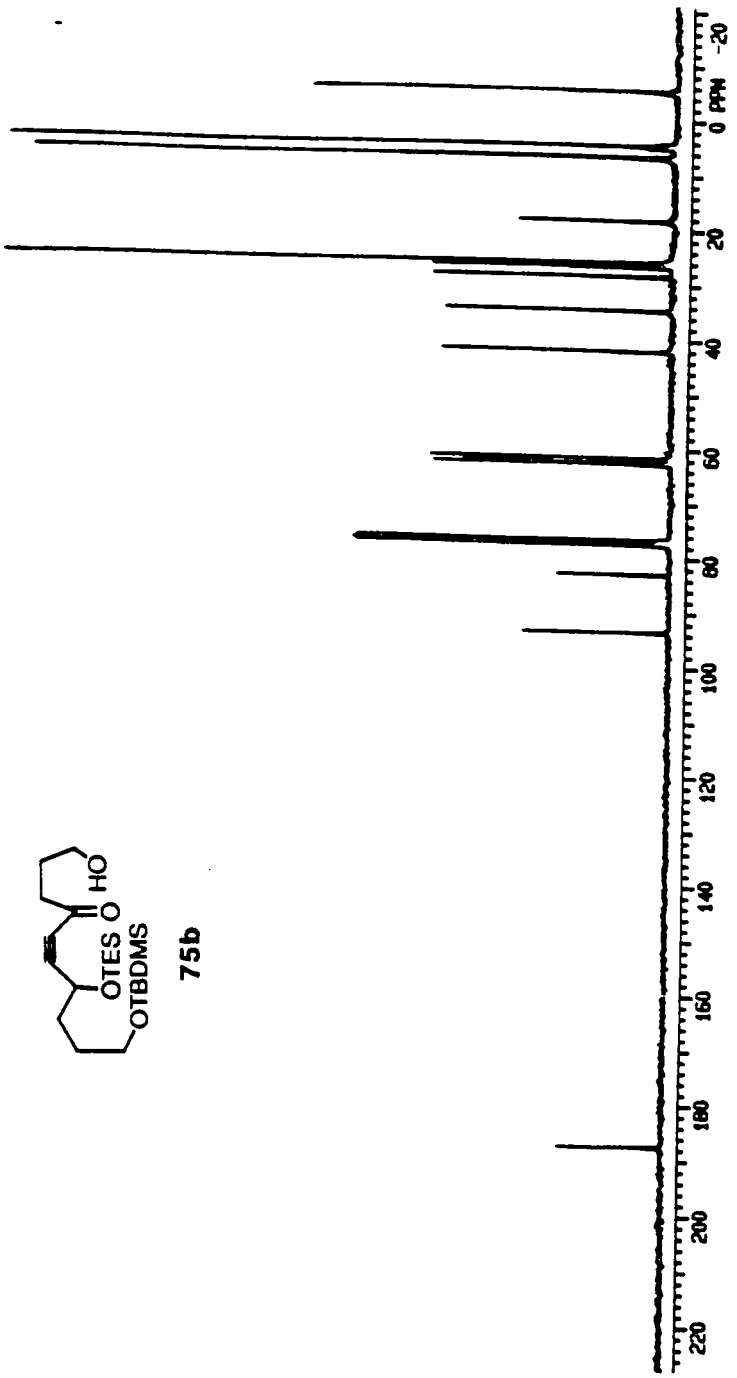


Figure 41. The 75 MHz ^{13}C NMR (in $CDCl_3$) of ketone **75b**

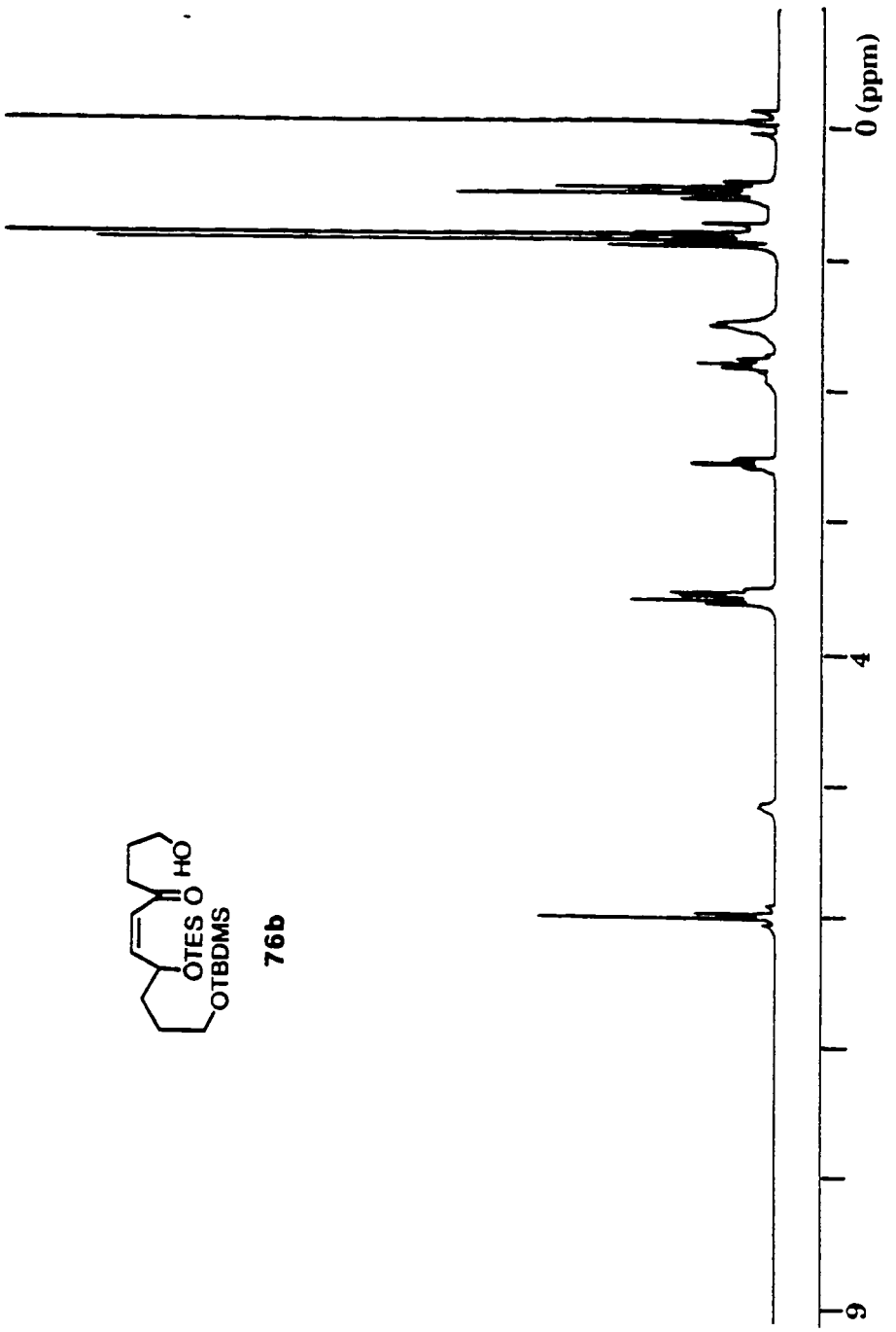


Figure 42. The 200 MHz ^1H NMR (in $CDCl_3$) of cis-alkene **76b**

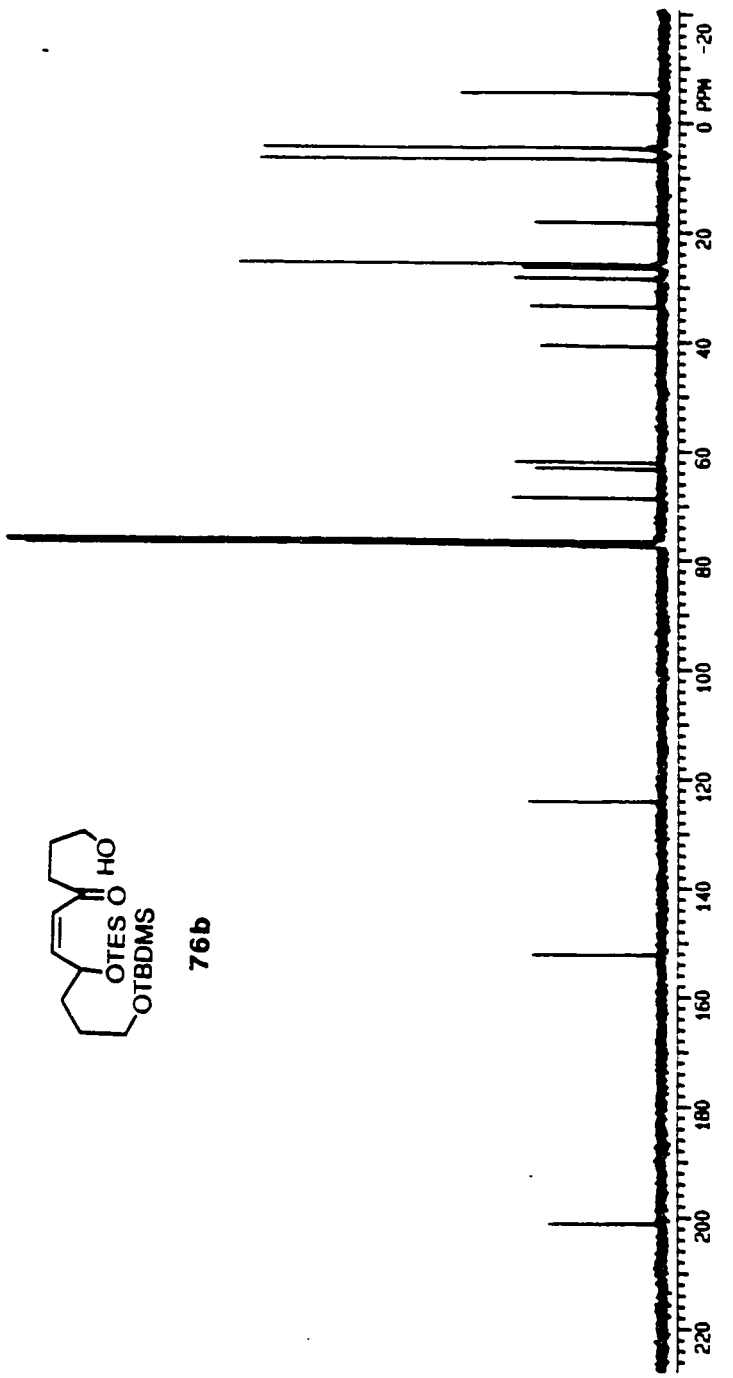


Figure 43. The 75 MHz ^{13}C NMR (in $CDCl_3$) of cis-alkene **76b**

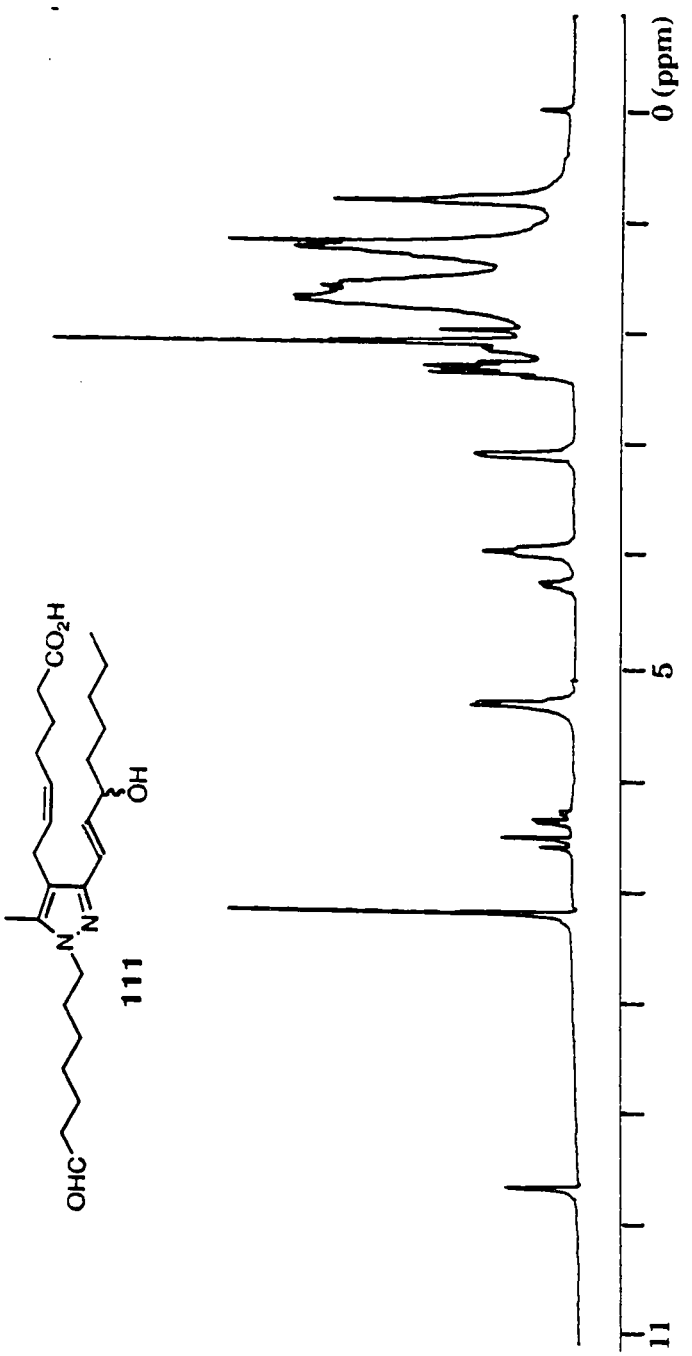


Figure 44. THe 200 MHz ^{1}H NMR (in $CDCl_3$) of pyrazole aldehyde **111**

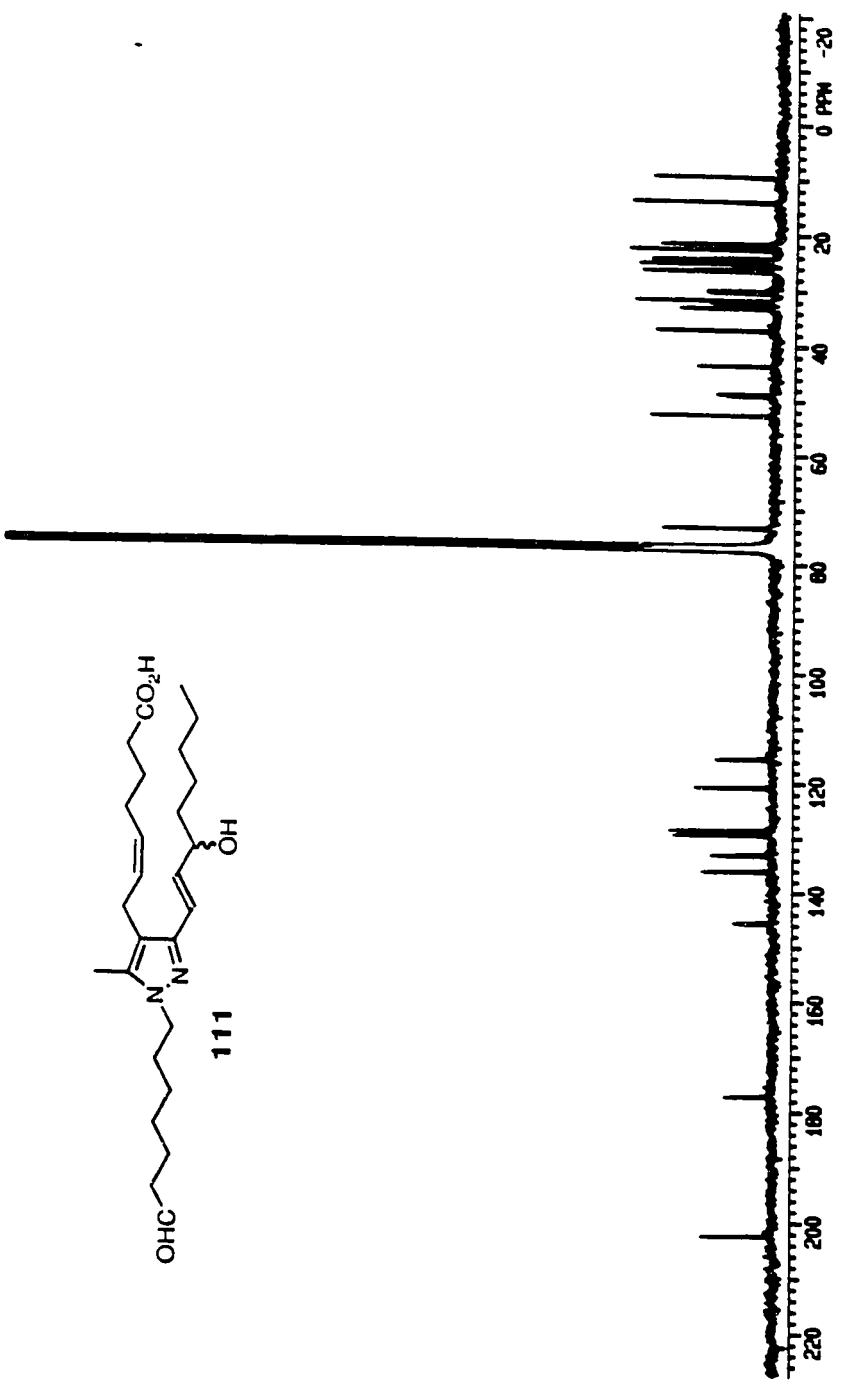


Figure 45. THe 75 MHz ^{13}C NMR (in $CDCl_3$) of pyrazole aldehyde **111**

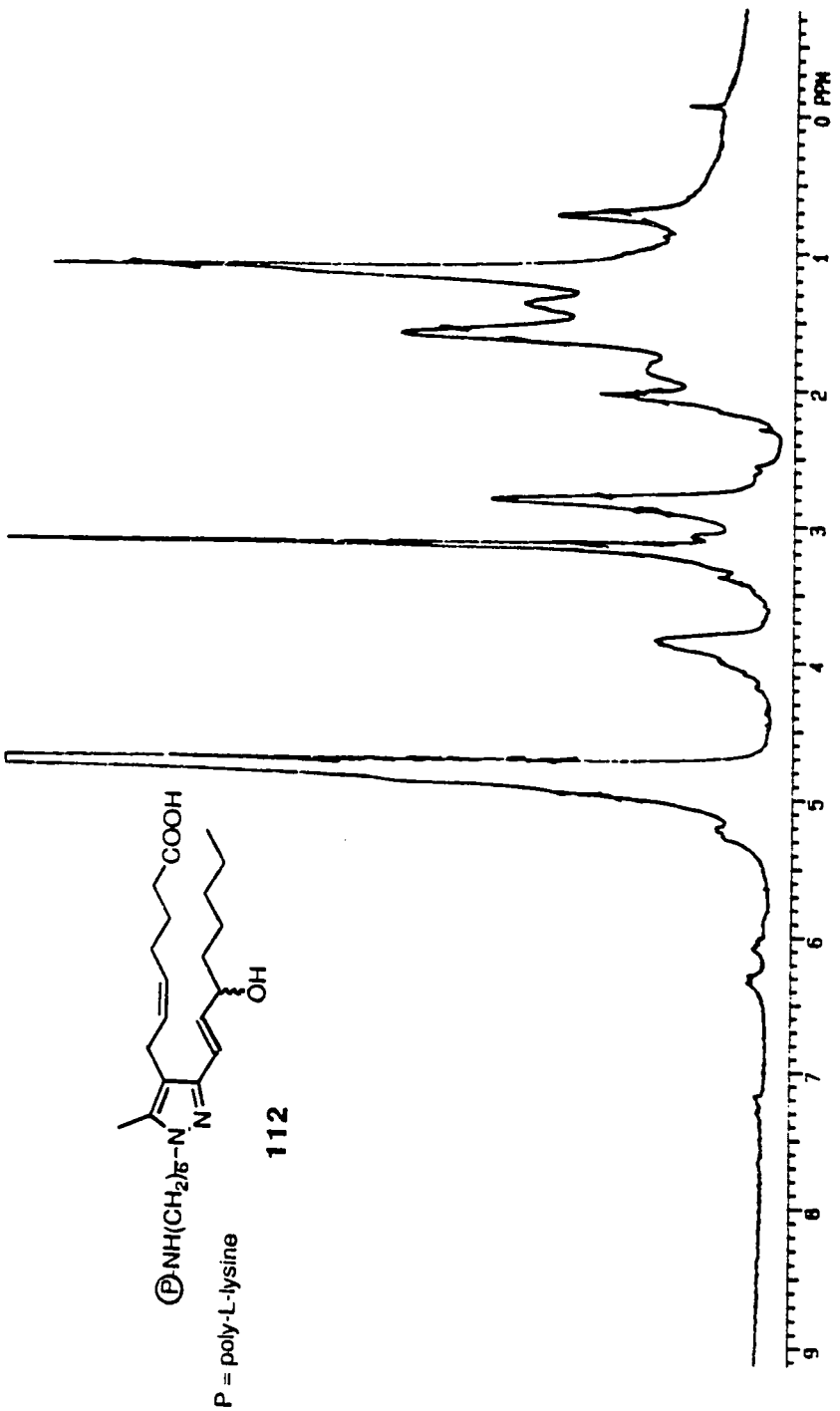


Figure 46. The 300 MHz ^{1}H NMR (in CD_3OD) of coupling product **112**

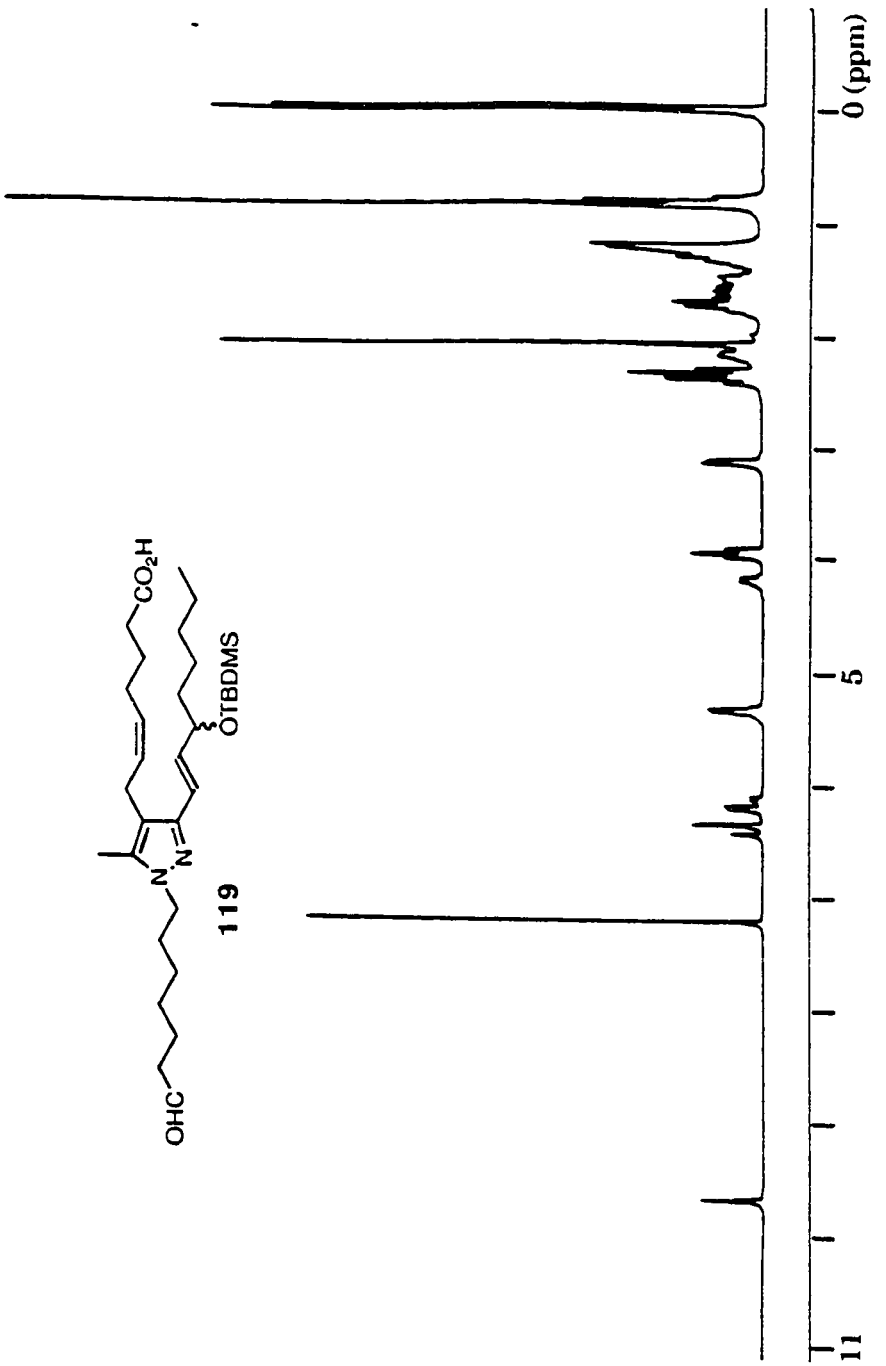


Figure 47. The 200 MHz ^{1}H NMR (in $CDCl_3$) of aldehyde **119**