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Part I. Synthesis of the C38 to C51 region of halichondrin B.
Part II. An immunoassay for protein-bound levuglandin-derived pyrroles

DiFranco, Elso, Ph.D.

Case Western Reserve University, 1994
PART I. SYNTHESIS OF THE C38 TO C51 REGION
OF HALICHONDрин B

PART II. AN IMMUNOASSAY FOR PROTEIN-BOUND
LEVUGLANDIN-DERIVED PYRROLES

by

ELSO DIFRANCO

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

Thesis Advisor: Professor Robert G. Salomon

Department of Chemistry
CASE WESTERN RESERVE UNIVERSITY
January, 1994
CASE WESTERN RESERVE UNIVERSITY

GRADUATE STUDIES

We hereby approve the thesis of

Elso DiFranco

candidate for the Ph.D.
degree.*

(signed) Philip P. Darsen
(chair)

Malcolm 2 Kennedy
Charlotte A. Kaitz
Michael A. Jazinski
Robert T. Clark

date 8/11/93

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Elio R. Franco
PART I. SYNTHESIS OF THE C38 TO C51 REGION OF HALICHONDRLN B

A highly convergent, stereoselective synthesis of the C38 to C51 fragment of halichondrin B was accomplished starting from D-mannitol (8). The acid-catalyzed dehydrocyclization product 7 from D-mannitol provided 5 of the stereocenters, C40, C41, C47, C48, and C50, (halichondrin numbering system) of this fragment. Removal of the extra hydroxyl group in 7 and conversion to aldehyde 6a proceeded in excellent yields. The α,β-unsaturated acyl silane 69, obtained by
Horner-Wadsworth-Emmons reaction of aldehyde 6a and a ketophosphonate, was a key intermediate for both the introduction of the methyl substituent at C42 (or C46) via conjugate addition and for obtaining the ketoylde 58 through acid 61. Reaction of ketoylde 58 with aldehyde 6a or 6e provided the enones 26 and 78 respectively that were used to introduce the second methyl substituent at C46 (or C42). The tetracyclic intermediates 74 and 80, were then generated by diastereoselective spiroketalization.

![Chemical structures](image)

**PART II. AN IMMUNOASSAY FOR PROTEIN-BOUND LEVUGLANDIN-DERIVED PYRROLES**

Levuglandins (LGs) arise from rearrangement of the strained dialkyl peroxide nucleus of the prostaglandin endoperoxide (PGH2). To provide a method sensitive enough to detect the tiny quantities of LGs that would be found in vivo, an immunoassay was developed.

Because pyrroles derived from LGE2 and simple amines are unstable, we presumed that analogous protein-derived pyrroles are unsuitable as antigens. Therefore, a pyrazole isostere 105 was used to
reductively alkylate Keyhole Limpet Hemocyanin (KLH). Rabbits were immunized with this antigen. High levels of antibody titres were obtained. The IgG fraction of proteins (that contains the antibody) was separated from the crude antibody serum. LGE2-derived pyrroles and other compounds that might be found in biological systems were tested for crossreactivity with the antibody. Strong cross reactivity was found only for compounds that contain both PG side chains appended to vicinal carbons in a 5-membered ring with unsaturation between the vicinal carbons. The greatest cross reactivity was observed for a pyrrole analogue of the isostere 105. The assay will be useful for studying protein-LGE2 adducts.
This thesis is dedicated to Nancy.
ACKNOWLEDGEMENTS

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I would also like to thank my fiance, Nancy, who has been my biggest supporter throughout my graduate studies, both in encouraging me in my studies and in providing many luxuries not within my means alone. I am also very appreciative of my special relationship with Adam and Sean, who have been a big part of my life for the past seven years. I would like to extend my gratitude to the members of our research group, both past and present, who were always willing to provide advice and assistance as well as their friendship. Special thanks to Alisa Lee-Lewis for all of her help.

I am also grateful to the research group of Dr. Vincent Monnier, especially Dr. Shinji Taneda, for their assistance and guidance in raising the polyclonal antibodies and in performing the immunoassays. Special thanks to Dr. Charlotte Kaetzel for providing the equipment and expertise for purifying the antibody.

Finally, I wish to acknowledge my friends and grandparents who have troubled themselves unselfishly in my behalf on many occasions. Funding provided by the NIH and the Monsanto Corporation as well as support from the CWRU Chemistry Department was greatly appreciated.
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<td></td>
</tr>
<tr>
<td>PGF₂α</td>
<td>Prostaglandin F₂α</td>
<td></td>
</tr>
<tr>
<td>PGH</td>
<td>Prostaglandin Endoperoxide</td>
<td></td>
</tr>
</tbody>
</table>
PhSH  Thiophenol
Piv   Pivaloyl
PPTS Pyridinium p-Toluenesulfonate
py   Pyridine
TBDMS t-Butyldimethylsilyl
Tf   Trifluormethanesulfonate
TFA Trifluoroacetic Acid
THF Tetrahydrofuran
TLC Thin Layer Chromatography
TMEDA Tetramethylethylenediamine
TMS Trimethylsilyl
p-TsImd (p-Toluenesulfonyl)imidazole
p-TsOH p-Toluenesulfonic Acid
PART I. SYNTHESIS OF THE C38 TO C51 REGION OF HALICHONDRIN B
INTRODUCTION

I. Background. During studies on *Halichondria okadai* *Kadota*, a marine sponge, Uemura et al.\textsuperscript{1a} found that extracts from the animal exhibit remarkable in vivo antitumor activity. This bioactivity was not attributable to okadoic acid which had been the main focus of studies on the sponge extracts. Using bioassays as a guideline, the isolation of biologically active compounds, subsequently named halichondrins, was effected. The sponge was collected on the coast of Aburatsubo in the Miura Peninsula south of Tokyo. After crushing 600 kg of the sponge, extracting with methanol, and filtration, 8 different compounds that were active against B-16 melanoma were eventually isolated (figure 1). Of these compounds, the most potent was halichondrin B. However, only 12.5 mg were isolated from the 600 kg of sponge harvested. The structure of the halichondrins was obtained from an x-ray crystal structure of the p-bromophenacyl ester of norhalichondrin A (see figure 1). The structure of halichondrin B was then elucidated by comparison of the $^1$H and $^{13}$C NMR spectra including COSY, $^1$H-$^{13}$C heteronuclear correlation, and decoupling experiments. A total synthesis of halichondrin B has been reported by Kishi et al.\textsuperscript{1c} confirming the proposed structure.

Interest in this very complex molecule arises from its remarkable in vivo activity. It has an IC$_{50}$ (concentration that inhibits cell growth by 50% after exposure for 20 min at 37 °C) of 0.093 ng/mL against B-16 melanoma cells. A few doses of halichondrin B at 10 µg/kg body weight provided T/C > 200 against B-16 melanoma and T/C > 300 against P-
Figure 1. Structures of the halichondrins.

Norhalichondrin A, B, C

Homohalichondrin A, B, C

Halichondrin B, C

A $R_1 = R_2 = OH$
B $R_1 = R_2 = H$
C $R_1 = OH, R_2 = H$
388 leukemia in mice (T/C refers to survival time of the treated versus control group). The low yield from the natural source as well as the remarkable in vivo bioactivity at such low concentrations have made this molecule a synthetic target despite its intricate structure that includes 32 chiral centers which give rise to over 4 billion possible stereoisomers.

Most recently, studies were performed to determine the mechanism of halichondrin B bioactivity. It was shown to be an inhibitor of tubulin polymerization. These studies were limited by short supplies of the molecule. Synthesis is important not only as a potential practical source for natural halichondrin B, but also because synthetic studies may be essential for the development of clinically effective drugs based on the natural template.

II. Biosynthetic Considerations. Before beginning to plan a strategy for such a complex target, it may be very useful to consider the possible biosynthesis of the molecule in order to gain insight into

![Hypothetical biosynthesis of halichondrin B.](image)

**Figure 2.** Hypothetical biosynthesis of halichondrin B.
approaches for assembling the multicycles. It is very likely that the biosynthesis involves heterocyclization of a polyoxygenated hydrocarbon precursor such as that in figure 2. It is also likely that the JK and LM ring junctions as well as the ABCDE-multicycle self-assemble with the generation of thermodynamically preferred configurations (see Figure 1). This is clearly evident for the LM spiro ring junction that is favored by a double anomeric effect⁴ as well as the diequatorial disposition of the two methyl substituents at C42 and C46. These possible biosynthetic sequences along with the C₂ symmetry of the KLMN fragment of the molecule inspired our strategy for this fragments synthesis, vide infra (Scheme I).

III. Retrosynthetic Analysis. In order to achieve a practical synthesis of a molecule of such complexity, it is necessary to control the generation of stereocenters. To accomplish this, we planned to assemble the molecule from several relatively simple fragments. Many factors can influence how the target can be simplified, but with 32 chiral centers to consider, it is essential that the fragments can be synthesized with the same absolute stereochemistry as the target. It was our strategy to use chiral nonracemic sugars as the building blocks for the fragments. Halichondrin B was dissected into fragments that had sections with the same stereochemistry as various sugars. Such a strategy was implemented previously in our laboratories for the syntheses of C1-15 and C27-35 segments of halichondrin B from D-ribose and D-glucose respectively.⁵

Our strategy for the KLMN tetracycle envisions using D-
Scheme I

Vinyl nucleophile synthon

1

1a

1b

2

3

4

5

6

7

D-mannitol
mannitol to provide five of the sterocenters in this fragment of halichondrin B (1, Scheme I). The JK ring spiro-junction could arise from a keto diol precursor 1a in a biomimetic fashion. The intramolecular Michael addition product from the hydroxyl on C32 of 1b adding to the enone would be in equilibrium with intermediate 1a. The precursor to 1a and 1b could be formed from reaction of a vinyl nucleophile synthon and a lactone 2. Lactone 2 could be obtained from intermediate 3 which has a C2 center of symmetry provided that both P groups were the same. The LM spiro ring junction could arise from a keto diol precursor, intermediate 4, also in a biomimetic fashion.

An important observation is that intermediate 4 has two methyl substituents on carbons that are β to a carbonyl group with γ-alkoxy substituents present. It follows that a logical precursor could be dienone 5 and two methyl nucophile based on well precedented stereospecific conjugate additions to α,β-unsaturated carbonyl systems with γ-alkoxy substituents.5 Dienone 5 is also symmetrical and could arise from 2 equivalents of aldehyde 6 condensed with a "dinucleophilic" acetone synthon. Aldehyde 6 has 3 stereocenters that are identical with centers in tetraol 7, a known product of acid catalyzed dehydration of D-mannitol 8.6 The extra hydroxyl group in 7 can be removed by the Barton protocol.7 D-mannitol 8 is also symmetrical and, therefore, the same product arises from SN2 substitution at C5 by the C2 hydroxyl or SN2 substitution at C2 by the C5 hydroxyl.

In summary, D-mannitol 8 would be used to derive aldehyde 6 and thus provide 6 stereocenters of the KLMN tetracycle subtarget 3.
The methyl groups at C42 and C46 could be introduced by stereospecific conjugate addition to a dienone 5 derived from 2 equivalents of aldehyde 6. Finally, the LM ring junction should be the thermodynamically favored product from spiroketalization of a keto diol precursor 4 in a biomimetic sequence.
RESULTS AND DISCUSSION

I. Synthesis of aldehyde 6a. Acid catalyzed dehydration of D-mannitol gives rise to three main tetraol compounds, 7, 9, and 10, two of which are furanose compounds and one, a pyranose compound. Upon treatment with p-methoxybenzaldehyde, the corresponding acetals 11 - 13 were formed. The product mixture was a very viscous brown syrup. Fortunately, chromatographic isolation of 11 was fairly simple owing to useful differences in product polarities.

Scheme II

D-Mannitol (8)

HCl, reflux, 24 h

7

9

10

p-MeOC₆H₄CHO, p-TsOH, 3 A sieves, MeOH, 40 h

11

p-MeOPh

12

p-MeOPh

13 PhOMe-p

9
The desired product, 11, is a diol. It was not converted into a diacetal because the formation of a trans fused pyranofuran is stereochemically unfavorable. This is not the case for the trans fused pyranopyran in 13 that is relatively strain free. Product 9 easily forms a diacetal 12 as both sets of vicinal hydroxyl groups can easily adopt a syn relationship. The significance of these facts is that a large difference in polarity has been created between the desired product 11 and byproducts 12 and 13. With a Waters 500A Prep LC System, up to 20 g of the crude product mixture can be loaded onto the column in one injection. By using EtOAc as eluting solvent a sufficient separation was attained and solvent recovery is readily achieved. Recrystallization from EtOAc/hexanes gave the desired diol 11 as a white solid in 19% overall yield from D-mannitol. Although this yield is low, it is practical based on the simplicity and efficiency of the procedures and the low cost of the starting material.

As previously mentioned, there is an unneeded hydroxyl group in tetraol 7. Before this hydroxyl could be removed, it was necessary to selectively mask the primary hydroxyl group in 11. This was easily accomplished by selective conversion of the primary hydroxyl to a silyl ether 14. Although some disilylated product 15 was formed, this compound was easily converted back to diol 11 by treatment with Bu4NF (Scheme III). The secondary hydroxyl in 14 was then converted to a xanthate to give 16. This product was a yellow solid that could be used without purification for treatment with freshly prepared Bu3SnH in refluxing toluene to accomplish Barton deoxygenation. It was
essential that the Bu3SnH be freshly distilled and less than three weeks old to obtain a high yield of 17 from these two steps (92% from alcohol 14).

Having removed the unnecessary hydroxyl group, the next challenge was to distinguish between the two hydroxyl groups masked by the p-methoxybenzylidene acetal. Upon treatment of acetal 17 with DIBAIH a single product, alcohol 18, was obtained in which the silyl ether had been cleaved and the acetal was totally unscathed. A similar result has recently been reported by Corey et al. Since the silyl ether was not stable to these acetal cleavage conditions, it was converted to
the corresponding benzyl ether 19. Although the yield was excellent for desilylation using DIBAlH (96%), the use of Bu₄NF was more convenient and proceeded in similar yield. Reductive cleavage of acetal 19 with DIBAlH now proceeded smoothly to give a mixture of alcohols 20 and 21 in a 1.5:1 ratio and a high overall yield (Scheme IV).

Initially, this ratio of products was a cause for concern. The desired primary alcohol 20 was only slightly favored over the secondary alcohol 21. The reaction is run at -10 °C and attempts to influence product ratio by lowering the temperature led only to a slower reaction which ultimately had to be warmed to induce completion. However, either alcohol 20 or 21 can be recycled to the starting acetal 19 in excellent yield by treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) under anhydrous conditions.¹¹

Alcohol 20 was oxidized by the mild conditions of the Swern procedure¹² to give the desired aldehyde 6a in excellent yield. A trace
procedure\textsuperscript{12} to give the desired aldehyde 6a in excellent yield. A trace of a secondary alcohol formed by cleavage of the MBn group is obtained as evidenced by TLC and the absence of MBn signals in its $^1\text{H}$ NMR spectrum. Although aldehyde 6a is relatively stable to purification (silica gel or HPLC), care was taken to avoid exposure to air. \textit{The compound showed significant decomposition after two weeks even when stored at -80 °C}. For this reason, the aldehyde (as well as subsequent similar aldehydes) was prepared as needed and used promptly. The bulk of the material was stored as the primary alcohol 20 which is a stable solid.

\textbf{II. Dienone strategy for the tetracycle.} As mentioned in the retrosynthetic analysis, aldehyde 6a will provide six of the stereocenters of the tetracycle provided that two equivalents are joined in a condensation reaction with an acetone bisnucleophile synthon. The resulting dienone 5 would provide the substrate for the introduction of the methyl groups at C42 and C46 (Scheme I, p.5). As a model reaction, dibenzylidene acetone (23) was shown to undergo conjugate addition of a methyl nucleophile to give 24 (Scheme V).

Dienone 25 was then prepared by a one pot condensation of aldehyde 6a with bis-phosphonium bromide salt 22.\textsuperscript{13} Although the 45\% yield of this reaction was modest, the highly convergent biscondensation provides a very short and direct route to subtarget 5 by
a process that, to this point, was very efficient. The dienone 25,

Scheme \( \text{V}^{6b} \)

but however, was unreactive towards conjugate addition with Me\( \text{2CuLi} \).

The use of TMSCl in an attempt to promote the conjugate addition\(^{14} \) led only to a complex mixture of products not containing the methyl groups expected in the desired product 26 or alkene bonds of the starting dienone 25. These products are presumed to arise by acid catalyzed Nazarov-type cyclization.\(^{15} \) Given the success that was achieved in preparing aldehyde 6a, a different approach was envisioned for joining the two halves of the tetracycle.

**III. Aldol approach to the tetracycle:** It was now established that the methyl groups at C42 and C46 could not be introduced by sequential conjugate methylations of a dienone 5. However, an intermediate such as 26 might be a viable alternative since one of the
methyl substituents is already in place (Scheme VI). Enone 26 could be

formed by aldol condensation of the kinetic enolate 28k from ketone 28a with aldehyde 6a. Ketone 28a could be obtained from aldehyde 6a by a Wittig reaction with a stabilized ylide 30 followed by conjugate addition of a methyl nucleophile.

Reaction of aldehyde 6a with 1-triphenylphosphoranylidene-2-propanone (30) gave a 6:1 mixture of trans and cis alkene isomers 29t and 29c (Scheme VII). The overall yield of this reaction was high (91%). The yield of the trans isomer could possibly be improved by isomerization of the cis alkene if so desired.16 These isomers were also easily separated by column chromatography. Trans enone 29t did not
undergo conjugate addition upon treatment with Me$_2$CuLi. However, use of a 12-15 fold excess of TMSCl promoted a clean reaction which gave a single stereoisomer 28a. Cis enone 29c did not undergo conjugate addition even in the presence of TMSCl. The requisite stereoselective generation of the newly formed stereocenter is predicted by precedents. Furthermore, Corey and Boaz studied similar conjugate additions in both the presence and absence of TMSCl (Equation 1). They found that TMSCl traps the anti π-complex which
goes on to form product. In the absence of TMSCl, the syn π-complex goes on to a cis copper (III) β-adduct and then to product more rapidly than the predominating anti π-complex, presumably owing to steric factors.

All attempts to generate enone 26 by aldol condensation of the kinetic enolate (large excess of LDA at -78 °C and use of a syringe pump for the very slow addition of 28a) and aldehyde 6a failed to produce any of the desired enone 26. Attempts to trap and subsequently dehydrate an intermediate aldol 27 with Burgess reagent or methanesulfonyl-chloride also failed to give any desired enone 26. Since dienone 5 is unsuitable as a substrate for conjugate methylation and enone 26 was not available by aldol condensation between ketone 28 and aldehyde 6a, a less direct route to subtarget 4 was explored.

IV. Dithiane-alkyl iodide strategy: An alternative approach to intermediate 4 could be the coupling of two fragments, possibly both derived from 28a, with both of the methyl substituents at C42 and C46 already incorporated, e. g. by stereospecific conjugate methylation of enone 29t (Scheme VIII). The linking chain between the furanose rings in 4 has only five carbons. To forge this chain from the methyl ketone sidechains of two molecules of 28a, three carbons must be discarded. One possible degradation of the methyl ketone sidechain is a Baeyer-Villiger oxidation that is expected to deliver acetate 31 regioselectively. A variety of alkylating agents such as iodide 33 could be prepared from the derived alcohol 32.
The presumed ready availability of 33 requires conjunction with an acyl carbanion synthon 34 to generate ketodiol 4 (Scheme IX). Dithiane 35 is a well precededented synthetic equivalent of an acyl carbanion. Dithiane 35 could be obtained by masking the aldehyde funtional group in 36. Aldehyde 36 should be available by condensing aldehyde 6a with an ylide 38 and subsequent conjugate methylation of the resulting enal 37 in a sequence similar to that used in synthesizing ketone 28. Thus, fragments 33 and 34 would both be produced from aldehyde 6a and the methyl substituents at C42 and C46 would both be introduced by stereoselective conjugate addition.

A) Dithiane synthesis: Synthesis of the nucleophilic fragment was fairly straightforward. Aldehyde 6a was reacted with (triphenyl-phosphoranylidene) acetaldehyde 38 in gently refluxing benzene to give
enal 37t (Scheme X).22 The yield was good (70-80%) and the ratio of trans 37t to cis 37c isomers was approximately 15:1 based on the 1H NMR integral. The time of reaction was important as evidenced by decreasing yield of the desired product if the reaction was allowed to proceed more than 3 h. Conjugate addition of the methyl group proved to be more difficult than with the corresponding ketone analogue 29t. When the cuprate reagent was prepared from CuI, a number of byproducts were forming in which the MBn ether was being cleaved or
apparent 1,2 addition was predominating (as evidenced by a more polar spot on TLC and a methyl doublet at ~1.20 ppm as opposed to 0.80 ppm for the desired product). CuI was purified immediately before use by precipitation from a saturated potassium iodide solution followed by washing with H₂O, EtOH, and Et₂O. Additives such as HMPA and TBDMSCl were used but the yield of desired product 36 remained low. CuBr·Me₂S was also used to prepare the organocuprate reagent but no desired product was isolated from this procedure. Subsequently, it was found that use of a higher order, mixed organocuprate prepared from CuCN, Me₂Cu(CN)Li₂, avoided side reactions and aldehyde 36 was obtained in fair yield (72%). TMSCl was again necessary in order for the addition to take place. Although compounds 36 and 37t were stable to purification, they were still aldehydes at this stage and care was taken as in the case of aldehyde 6a. Masking of the aldehyde
moiety in 36 as a 1,3 dithiane\textsuperscript{26} proceeded in high yield (90\%) but the MBn ether also was cleaved to give the secondary alcohol 35. This was a minor inconvenience as the alcohol was converted to the corresponding silyl ether 39 in excellent yield by treatment with TBDMSOTf.\textsuperscript{27}

**B) Electrophile synthesis. 1) Baeyer-Villiger strategy:** As noted above, a possible strategy to synthesize iodide 33 involved using ketone 28a in a Baeyer-Villiger oxidation to give acetate 31. Treatment of 28a (P = MBn) with m-CPBA in CH\textsubscript{2}Cl\textsubscript{2} in the presence of solid NaHCO\textsubscript{3} for 16 h at room temperature\textsuperscript{28} resulted in the recovery of starting material 28a and a secondary alcohol 40a (vide infra) formed from cleavage of the MBn group (as evidenced by \textsuperscript{1}H NMR). None of the desired acetate 31 (P = MBn) was formed. Heating the reaction to 42 °C resulted only in more MBn ether cleavage. For this reason several different protecting groups, 28b-d (Equation 2), were substituted for the acid labile MBn group. Compounds 28b and 28c were synthesized from the corresponding enones 43t and 48t. Conjugate addition reactions proceeded in similar yields on the corresponding α,β-unsaturated ketone precursors of 28b and 28c using CuI to prepare the organocuprate reagent. 28d (P = MEM) was synthe-
sized from the equilibrium mixture of alcohol 40a and hemiketal 40b (Scheme XI) by reaction with MEMCl and (i-Pr)2NEt.29 Compounds 23b and 23d did not undergo any reaction when subjected to the m-CPBA oxidation conditions. Compound 23c reacted to give only alcohol 40a by hydrolysis of the silyl group.

It is noteworthy that 40 exists as an equilibrium mixture of 40a and 40b. This equilibrium provides a precedent for the generation of the pyranofuran unit that must be formed in the last step of the proposed tetracycle synthesis. Although all peaks were not assigned for the very complex spectrum of this mixture, characteristic methyl peaks of the two compounds were assigned (table 1). The equilibrium mixture

<table>
<thead>
<tr>
<th>Me group</th>
<th>compound 40a</th>
<th>compound 40b</th>
</tr>
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<tbody>
<tr>
<td>C42 Me</td>
<td>0.88 (d, 3H, J = 6.7 Hz)</td>
<td>1.00 (d, 3H, J = 7.0 Hz)</td>
</tr>
<tr>
<td>C44 Me</td>
<td>2.10 (s, 3H)</td>
<td>1.22 (s, 1.5H)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.40 (s, 1.5H)</td>
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of 40a and 40b was treated with m-CPBA and a catalytic amount of p-TsOH28b to promote interconversion of the ketone 40a and the lactol 40b. A mixture of products was obtained, one of which was apparently an isomeric mixture of benzoates 45. Less than a 20% yield of a compound was isolated that appeared to be the desired Baeyer-Villiger oxidation product 31a (P = H). The secondary hydroxyl group of 31a was converted to the corresponding silyl ether27 31b (P = TBDMS) and treated with Ba(OMe)2 in MeOH. The 1H NMR spectrum of the product agreed with that expected for the desired primary alcohol 32b (disappearance of the acetate methyl) but it was difficult to assign peaks because of the small sample available. The last two steps had
been performed on less than 1 mg of compound. Attempts to reproduce this synthesis of acetate 31 failed.

2) Ozonolysis strategy. Another possible method for generating primary alcohol 32 is oxidative cleavage of an enol derivative of ketone 28b. The challenge would be to generate the regioisomerically correct enol. The requisite regioselective enol generation had already been accomplished during the conjugate addition reactions that produce compounds 28a-c. Thus, the enol silyl ethers 44 that are formed during silyl chloride-promoted cuprate addition, are hydrolyzed to the saturated ketone during workup. If these enol silyl ethers could be isolated, they could be subjected to ozonolysis followed by reductive workup to give the desired primary alcohol 36.

Therefore, enone 43t was treated with Me2CuLi in the presence
of TMSCl and instead of the normal workup involving the addition of a saturated NH₄Cl solution, Et₃N was added and enol silyl ether 44b was isolated and purified. During column chromatography and HPLC purification, the very sensitive TMS silyl ether was not hydrolyzed as long as Et₃N (< 2%) was present in the solvent systems. The conditions for ozonolysis²⁸ were standardized on the enol silyl ether derived from benzylidene acetone by methyl cuprate addition.

Because it was desirable to have a different protecting group than benzyl on the secondary hydroxyl, a TBDMS analogue 44c of 44b was prepared from alcohol 21 (Scheme XII). Thus, the secondary hydroxyl group of 21 was silylated using TBDMSOTf²⁷ to give 46.
Cleavage of the MBn group with the single electron transfer agent, DDQ,\textsuperscript{31} gave alcohol 47. Reaction of ceric ammonium nitrate\textsuperscript{32} (CAN)
with 46 resulted in the formation of a diol owing to cleavage of both the TBDMS and MBn groups and also generated some acetal 19 (22% yield). Oxidation of alcohol 47 by the Swern protocol produced aldehyde 6c. All of the reactions in the preparation of 6c from 21 proceeded in greater than 90% yield. Reaction of aldehyde 6c with ylide 30 gave a mixture of trans enone 48t and cis enone 48c in a ratio of 6.5:1. The trans isomer was reacted with Me2CuLi in the presence of TMSCl, and by using Et3N in the workup, enol-silyl ether 44c was obtained in excellent yield (91%). This compound was very pure according to 1H NMR and TLC analyses and, owing to the sensitivity of the TMS ether, no further purification was attempted. Enol ether 44c was dissolved in MeOH/CH2Cl2 and ozone was bubbled through the solution until a blue color persisted. NaBH4 was added, and workup with cold 5% HCl gave alcohol 32c. The overall yield for the ozonolysis and reductive workup was quite low (37%). Nevertheless, since the dithiane 39 (see p. 20) was already available, the iodide-dithiane coupling reaction was attempted with a view toward subsequent optimization of the ozonolysis yield. Therefore, the hydroxyl group of 32c was converted into the corresponding mesylate 50 and finally the iodide 33c in 68% overall yield.

We now expected that the iodide 33c and dithiane 39 could be converted to ketone 4 by coupling and subsequent unmasking of the carbonyl group. Generation of a dithiane anion and coupling with an iodide33 were first performed on a simple model system using a dithiane prepared from iso-butyraldehyde as the nucleophile and ethyl
iodide as the electrophile. An 89% yield was obtained in this model system. Dithiane 39 was then stirred for 20 h with n-BuLi and TMEDA in THF at -10 °C. A 5 fold excess of the dithiane was used relative to

Scheme XIII-a

1) n-BuLi, TMEDA, -10° C
20 h.

33c

+ 

52

Scheme XIII-b

39

→

52

the iodide 33c because of the difficulty of preparing the iodide and the presumption that unreacted 39 would be readily recovered and recycled. Addition of the iodide 33c followed by workup gave a complex mixture of products, none of which were desired compound 51 (Scheme XIIIa). Two main components of the mixture were isolated and purified. These were the iodide 33c, recovered quantitatively, and
compound 52 that formed by a Wittig rearrangement (Scheme XIII-b).

To avoid a Wittig rearrangement, we attempted to prepare an analogue of 39 in which the benzyl ether was replaced with a MEM ether (Scheme XIV). Thus, the hydroxyl group of primary alcohol 20

was silylated. Subsequent debenzylation using a W-4 Raney nickel catalyst\(^{34}\) gave primary alcohol alcohol 54. The W-4 Raney nickel catalyst allows selective removal of a benzyl group in the presence of a methoxybenzyl group.\(^{35}\) Alcohol 54 was then converted to a MEM ether followed by cleavage of the TBDMS group to give alcohol 56. The Swern protocol was used to oxidize alcohol 56 to aldehyde 6d in good yield (80%). This yield was significantly lower than that achieved in producing aldehydes 6a-c (>90%). The MEM and TBDMS protecting
groups in this compound are apparently more sensitive to the reaction conditions as evidenced by detection of polar products by TLC analysis of the crude reaction product mixture. Enal 57 was then formed by Wittig reaction of aldehyde 6d and (triphenylphosphoranylidene)-acetaldehyde (38). Attempts to achieve the conjugate addition of methyl cuprate to enal 57 failed to produce any of the desired methylated product. A complex mixture of products was generated (TLC analysis was a streak across the plate) and no attempt was made to isolate any compounds as there was no evidence for the desired product in the \textsuperscript{1}H NMR spectrum of the crude reaction product mixture.

V. Ylide strategy. A strategy closely related to the failed aldol condensation approach to enones like 26 uses an ylide 58 in place of the corresponding "enolate of ketone 28a" (Scheme XV). Based on the
success of previous reactions of the aldehyde 6a with various ylides, this should be a viable scheme to link the K and N rings while also providing an α,β-unsaturated ketone for conjugate addition of the second methyl group. Ylide 58 could be derived from a number of acylating agents 59 and methylenetriphenylphosphorane (60). Furthermore this strategy has the advantage of being much shorter than the dithiane-alkylation approach.

Based on our previous work two possible methods were available for producing acid 61, a precursor for the acylating agents 59 (Scheme XVI). The first involved synthesis of an α,β-unsaturated ester by the reaction of a Wittig reagent with aldehyde 6a. Conjugate addition of a methyl nucleophile followed by hydrolysis of the ester should give the desired acid 61. Ester 62 was synthesized by condensation of aldehyde 6a with methyl (triphenylphosphoranylidene)acetate (49). However, all attempts at conjugate addition to the α,β-unsaturated ester 62 failed to
produce 63 even in the presence of TMSCl.20 The low reactivity of 62 is apparently the result of the ether substituents. This behavior contrasts with precedents in which conjugate additions to α,β-unsaturated esters were achieved.36

A second possible method to produce acid 61 was to oxidize aldehyde 34b (from scheme X, p.19). PDC and m-CPBA proved unsatisfactory as oxidizing agents. Ultimately, a 1.0 M KMnO₄ solution in a phosphate buffer37 provided the desired acid 61. The yield of this reaction was very low (37%) and difficult to reproduce. A byproduct was generated by cleavage of the MBn group and subsequent lactol formation as evidenced in the ¹H NMR spectrum of isolated reaction products (the C42 methyl doublet was shifted downfield to 1.20 ppm compared to 0.80 ppm in the desired acid 61). This strategy was therefore abandoned in favor of another, more exotic, approach.

Thus, according to literature precedents,38a α,β-unsaturated acyl silanes undergo organocuprate conjugate additions (Equation 3).

\[
\text{Equation 3}
\]

Furthermore, unsaturated acyl silanes are readily oxidized to carboxylic acids.38b,c Therefore, it was expected that the saturated acylsilane
obtained from conjugate addition to an \( \alpha,\beta \)-unsaturated acylsilane could provide the corresponding carboxylic acid by oxidative desilylation.

The requisite unsaturated acyl silane 69 was synthesized via Horner-Wadsworth-Emmons modification of the Wittig reaction using aldehyde 6a and phosphonate 64 (Scheme XVIII). Phosphonate 64 was prepared in a short sequence from ethyl-vinyl ether 65 (Scheme XVII) as described in the experimental section.\(^{38}\) A noteworthy point in this

![Scheme XVII]

scheme is the sensitivity of the iodide 68 to light. It is prepared in the dark and used promptly (within a few days). Also, the reaction of the iodide 68 with trimethylphosphophite produces MeI as a byproduct. MeI catalyzes the isomerization of trimethylphosphite to dimethyl methylphosphonate. This can be avoided by insuring that the temperature of the reaction is high enough to allow MeI to be distilled out as it is formed. The phosphonate exists as a tautomeric mixture of the keto and enol forms as evidenced by separate sets of \(^1\)H NMR peaks for each tautomer.
Horner-Wadsworth-Emmons reaction of the phosphonate 64 with aldehyde 6a proceeded smoothly to give the pure trans α,β-unsaturated acyl silane 69 (Scheme XVIII). No cis isomer formed. The yield (80%) was comparable to those mentioned above for Wittig reactions of aldehydes 6a-c with various ylides. Excess NaH that was used to generate the anion of the phosphonate 64 was removed by filtration under N2 through a glass frit prior to addition of the aldehyde solution. It was very important that the anionic solution be cooled with an ice bath if the reaction was larger than a 50 mg scale. When the Wittig reaction was attempted at room temperature, many byproducts were formed, that appear to be cis isomerized alkenes from 1H NMR, and only a minor amount of the desired acyl silane 69 (<22%) was produced.

Attempts at conjugate addition to 69 using CuI failed to give any of the desired product 70, even when the CuI was purified as previously described. Therefore, CuCN was used instead of CuI for generation
of the organocuprate species.\textsuperscript{25} CuCN, when reacted with MeLi (2 eq.)
gives a higher order, mixed organocuprate species, Me\textsubscript{2}Cu(CN)Li\textsubscript{2}. The
CuCN is also more convenient than CuI to prepare for the reaction. It
is simply azeotroped with toluene to remove moisture. Last traces of
solvent are removed into a dry ice-cooled trap with a high vacuum
pump. Addition of the organocuprate derived from CuCN to the acyl
silane \textsuperscript{69} gave the desired diastereomerically pure 1,4 adduct \textsuperscript{70} in fair
yield (70\%). TMSCl was again necessary for reaction to take place.
Treatment of the methylated acyl silane \textsuperscript{70} with NaOH/H\textsubscript{2}O\textsubscript{2}\textsuperscript{39} gave
the desired acid \textsuperscript{61} in excellent yield (89\%) compared to that from
oxidation of aldehyde \textsuperscript{36} using KMnO\textsubscript{4} (37\%).

Having established a viable route to acid \textsuperscript{61}, an acylating agent
for the methylethenetriphenylphosphorane (\textsuperscript{60}) could now be synthesized.
Acid chloride derivatives are a possibility for acylating \textsuperscript{60} but they are
readily hydrolyzed to the corresponding acids and HCl promoted
cleavage of the methoxybenzyl protecting group could ensue. Other
activated acid derivatives should be less susceptible to decomposition
than acid chlorides and react with \textsuperscript{60} in better yield than alkyl
esters.\textsuperscript{40} Thus, carbonyl diimidazole was used to convert the acid
functionality of \textsuperscript{61} to the corresponding imidazolide \textsuperscript{71}.\textsuperscript{41} Although the
imidazolide was produced in high yield (88\%), it was easily hydrolyzed
back to the acid and care had to be exercised during workup. Owing to

\[
\begin{align*}
\text{61} & \xrightarrow{(\text{Im})\textsubscript{2}CO} \text{71} \\
& \quad \quad \text{88\%}
\end{align*}
\]
its hydrolytic instability, 71 was used in the crude form for reaction with ylide 60. However, attempted acylation of 60 with imidazolide 71 failed. The reaction appeared to be proceeding (by TLC analysis) but upon workup, the only identifiable product isolated was recovered acid 61. This may be a result of the imidazolide 71 being impure or the presence of LiBr salts formed during generation of ylide 60 from the reaction of an alkyl lithium base and methylenetriphenylphosphonium bromide. It was decided to synthesize an acylating agent that could be purified and fully characterized before reaction with methylenetriphenylphosphorane (60).

According to precedent,\textsuperscript{42} a thiol ester would be reactive as an acylating agent but more readily purified than imadazolide 71. The acid was therefore converted to thiol ester\textsuperscript{43} 72 in excellent yield (Scheme XIX). Instead of using a solution of methylenetriphenylphosphorane containing LiBr, a salt free solution in toluene was prepared.\textsuperscript{42} Reaction of the thiol ester 72 with salt-free ylide 60 at 105 °C produced ylide 58 in moderate yield (65%). Attempted reaction of aldehyde 6a with ylide 58 in the same manner as with other ylides, i. e.

\begin{center}
\textbf{Scheme XIX}
\end{center}

\begin{center}
\includegraphics[width=\textwidth]{Scheme_XIX.png}
\end{center}
16 h, room temperature, in CH₂Cl₂, failed. When the reaction was run in ClCH₂CH₂Cl at 73 °C, enone 26 was formed in 55% yield. However, the reaction time was greater than 8 h and we suspected that the aldehyde was decomposing under these conditions. In an attempt to reduce reaction time, the Wittig reaction was performed in toluene at reflux. It was complete in less than 3 h, but the yield remained low (ca. 52%). Although the yield of this step was low, reaction of ylide 58 and aldehyde 6a provided intermediate 26 that all other direct routes had failed to deliver.

For the second conjugate addition, CuCN was again used as the copper(I) source for the organocuprate reagent. The addition proceeded smoothly to deliver a methylated species but, interestingly, not the expected symmetrical ketone 73. The ¹H NMR spectrum of this product was more complex than expected for 73 with 2 separate doublets for the methyl substituents and an olefin proton was still present. Careful analysis revealed that the enol silyl ether formed during the conjugate addition had not been hydrolyzed to the saturated ketone during workup with saturated NH₄Cl solution. Treatment of this crude reaction product mixture with cold, dilute HCl (1%) still did not cleave the TMS silyl ether. Finally, treatment with Bu₄NF afforded the desired ketone 73 (Scheme XX) in good yield (79%).

The final steps were generation of a keto diol and ketalization. Either CAN or DDQ could have been used to cleave the MBn groups. CAN was chosen because the acidity of a CAN solution should cause spiroketalization as well as cleave the MBn groups. When 73 was
treated with CAN, a single, diastereomERICally pure product was obtained, the symmetrical KLMN tetracycle 74.

Scheme XX

\[ \text{26} \xrightarrow{\text{1) } \text{Me}_2\text{Cu(CN)Li}_2, \text{TMSCl}} \text{BnO} \]  
\[ \text{2) } \text{Bu}_4\text{NF} \xrightarrow{55\%} \text{73 (75\%)} \]

\[ \text{CAN} \]

In order to link the KLMN tetracycle to the rest of the molecule or add on the triol side chain, it would be necessary to destroy the symmetry of 74 by differentiating the benzyl ethers. This could be done by partial hydrogenation to unmask one hydroxyl group, but this process would most likely lead to a mixture of mono alcohol, diol, and starting diether 74. To avoid the necessity for recycling of byproducts (diol and unreacted starting diether) at this stage of the synthesis, we decided to differentiate the protecting groups prior to coupling the two furan intermediates. We recognized that the secondary alcohol 21 could be exploited to form an aldehyde 6e with different protecting group(s) than those of aldehyde 6a. Aldehyde 6e would then be coupled with ylide 58. In this manner, one half of the tetracycle would be derived
from each of the alcohols 20 and 21 obtained from reductive cleavage of acetal 19.

Silyl ether 46 was available as described above (Scheme XII, p 25). Selective hydrogenolysis of the Bn group in the presence of a MBn group using a W-4 Raney Nickel catalyst gave alcohol 47 (Scheme XXI). Silylation of the primary hydroxyl gave the monobenzyl disilyl ether 76. Demethoxybenzylation using DDQ followed by oxidation using the Swern protocol gave aldehyde 6e. The yield of aldehyde 6e obtained from this procedure varied considerably (60-83%), and it appears that a
milder procedure might give better results. Aldehyde 6e was then condensed with ylide 58 to give the unsymmetrical enone 78. Conjugate addition then produced ketone 79, and unlike the case for symmetrical ketone 73, the NH₄Cl workup of the reaction was sufficient to hydrolyze the enol silyl ether to the saturated ketone. The yields for these reactions were very similar to those of the corresponding symmetrical compounds. For the final spiroketalization, CAN was again used. Both the primary and secondary silyl ethers were cleaved as well as the MBn group. Ketalization also took place in the same reaction mixture but the overall yield was poor (20%). Although this represents the yield for three steps, it was decide to unmask the hydroxyls in a stepwise fashion with milder reagents to increase the yield. DDQ was first used to cleave the MBn group followed by desilylation using Bu₄NF to produce a triol. Treatment of the triol with cold 1% HCl generated the unsymmetrical KLMN tetracycle 80 in 32% overall yield for the three steps. It should also be noted that these final reactions were all done an a small scale and each of the intermediates was isolated by HPLC purification.

Comparison of the ¹H NMR spectrum for the symmetrical tetracycle 74 and ¹³C NMR data for the unsymmetrical tetracycle 80 in CD₃OD to the natural product is presented in tables 2a and 2b. From table 2a, it can be seen that the chemical shifts and hyperfine coupling constants for 74 resemble those for halichondrin B¹a with the exception of H-43 and H-45 at 1.02 that are shifted upfield in 74 owing to shielding by the benzyl substituent (only 1 H is shifted upfield in 80).
**Table 2a.** $^1$H NMR data for halichondrin B and compound 74.

<table>
<thead>
<tr>
<th>Atom(s)</th>
<th>Halichondrin B (360 MHz)</th>
<th>Compound 74 (300 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-40</td>
<td>4.05 (dd, 1H, $J = 2.4$, 3.0 Hz)</td>
<td>3.97 (dd, $J = 2.0$, 4.7 Hz)</td>
</tr>
<tr>
<td>H-48</td>
<td>4.10 (m, 1H)</td>
<td>3.97 (dd, $J = 2.0$, 4.7 Hz)</td>
</tr>
<tr>
<td>Hs-43</td>
<td>1.31 (1H), 1.51 (1H)</td>
<td>1.02 (dd, 2H, $J = 4.5$,13.1 Hz)</td>
</tr>
<tr>
<td>Hs-45</td>
<td>1.43 (1H), 1.50 (1H)</td>
<td>1.23 (dd, 2H, $J = 4.6$,12.8 Hz)</td>
</tr>
<tr>
<td>Me-42</td>
<td>0.94 (d, 3H, $J = 6.9$ Hz)</td>
<td>0.96 (d, 3H, $J = 7.1$ Hz)</td>
</tr>
<tr>
<td>Me-46</td>
<td>1.10 (d, 3H, $J = 6.9$ Hz)</td>
<td>0.96 (d, 3H, $J = 7.1$ Hz)</td>
</tr>
<tr>
<td>H-42</td>
<td>2.28</td>
<td>2.18</td>
</tr>
<tr>
<td>H-46</td>
<td>2.34</td>
<td>2.18</td>
</tr>
<tr>
<td>Hs-39</td>
<td>2.34</td>
<td>1.68 (dd, 2H, $J = 3.2$,13.7 Hz)</td>
</tr>
<tr>
<td>Hs-49</td>
<td>1.83, 2.27</td>
<td>2.18 (2H)</td>
</tr>
<tr>
<td>H-38</td>
<td></td>
<td>4.12 - 4.22 (m,1H)</td>
</tr>
<tr>
<td>H-50</td>
<td>4.00 (ddd, 1H, $J = 9.0$, 4.2, 4.2 Hz)</td>
<td>4.12 - 4.22 (m,1H)</td>
</tr>
</tbody>
</table>

**Table 2b.** $^{13}$C NMR data for halichondrin B and compound 80.

<table>
<thead>
<tr>
<th>Atom(s)</th>
<th>Halichondrin B (90.6 MHz)</th>
<th>Compound 80 (75 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-44</td>
<td>98.4</td>
<td>98.0</td>
</tr>
<tr>
<td>Me-42, Me-46</td>
<td>18.1, 18.3</td>
<td>18.3, 18.3</td>
</tr>
<tr>
<td>C-42, C-46</td>
<td>27.2, 27.1</td>
<td>27.0, 27.0</td>
</tr>
<tr>
<td>C-41, C-47</td>
<td>80.8, 81.3</td>
<td>81.3, 81.5</td>
</tr>
</tbody>
</table>

A total synthesis of halichondrin B involving a totally different strategy for the KLMN-ring segment of the molecule was recently
reported by Aicher et al. at Harvard University. Their strategy involved coupling two halves of the molecule in a Ni(II)/Cr(III)-mediated Nozaki-coupling reaction of iodide 81 and aldehyde 82 followed by oxidation using the Dess-Martin reagent to obtain the trans α,β-unsaturated ketone 83 in 60% overall yield (Scheme XXII). However, since this process required two equivalents of 81, the yield of 83 based on 81 was only 30%. Treatment of 83 with Bu4NF led to formation of the L,M spiro ring junction by hemiketal formation between the C48 hydroxyl and the C44 ketone, and Michael addition of the hemiketal hydroxyl group onto the α,β-unsaturated ketone. A hemiketal of the J
ring was also formed at this step. Demethoxybenzylolation and acid treatment completed the formation of the J,K spirokeetal and thus halichondrin B. The overall yield for these 3 steps was reported as 50-60%.

The rather lengthy synthesis of iodide 81 is shown in Scheme XXIII. L-ascorbic acid was converted into α,β-unsaturated lactone 84.

Scheme XXIII

**D-malic acid**

**91**

**TBDMSOTI**

2) LAH

3) Dess-Martin reagent

4) 92 then 5 steps (see ref. 45)
and conjugate addition provided the C42 methyl group. Conversion of 85 into epoxide 88 was followed by coupling under Yamaguchi conditions\textsuperscript{48} with acetylene 89 to give cis alkene 90 after partial reduction. The yields for the conversion of lactone 85 to epoxide 88 were not reported.\textsuperscript{49} Acetylene 89 was prepared from D-malic acid in 4 steps (25% yield). Sharpless epoxidation\textsuperscript{50} of alkene 90 followed by acid treatment gave the tetrahydrofuran 91 in 61% yield. This sequence provided the N ring with the triol side chain appended. Silylation of the hydroxyl groups in furan 91 was followed by reduction of the pivaloyl ester and oxidation of the resulting alcohol. Reaction of this aldehyde with bromide 92 (prepared in 8 steps from (S)-(+)methyl 3-hydroxy-2-methylpropionate in 40% overall yield) provided iodide 81. The yields for these steps were also not reported. Although this strategy has provided a tetracycle precursor with the triol side chain attached, the length of the synthesis of 81 makes this scheme impractical, especially in view of the poor yield (30%) of 83 produced in the coupling of 81 with 82.
CONCLUSIONS

We have completed an efficient and stereoselective synthesis of tetracycle 80 for the C38-51 segment of halichondrin B. The synthesis is summarized in Scheme XXIV. We started from a known tetraol 7 that is readily available in one step from mannitol,\textsuperscript{6} a very inexpensive starting material. The overall yield for the 18 steps from the tetaol 7 to the tetracycle 74 is approximately 3\% with an 82\% average per-step yield. The conditions of the reaction to produce ylide 58 have yet to be optimized as does the ensuing reaction of the ylide 58 with aldehyde 6e. The final three steps involving demethoxybenzylolation, desilyation, and ketalization were only done on a small scale and each intermediate was isolated by HPLC. Based on the yields of similar reactions in earlier steps, it is reasonable to assume that if these final steps are done on a larger scale, the overall yield can be expected to approach 50-60\%.

This strategy made use of D-mannitol to provide five of the stereocenters in halichondrin B and exploited C\textsubscript{2} symmetry. Two more centers were introduced enantioselectively by conjugate addition. That these two centers are of the correct configuration is supported by spectral comparisons with the natural product (tables 2a and 2b)\textsuperscript{1a} along with the spectrum of spiroketal 74 in C\textsubscript{6}D\textsubscript{6} (see figure 97 in appendix). In this solvent, the peak for H\textsubscript{42} (46) does not overlap with other H atom peaks unlike the spectra taken in CDCl\textsubscript{3} or CD\textsubscript{3}OD. The peaks for all H atoms were unambiguously assigned using the data from decoupling experiments. The coupling constants for H\textsubscript{43} were as follows: J\textsubscript{43axial-43equatorial} = 12.9 Hz, J\textsubscript{43axial-42} = 12.8 Hz, and
$J_{43\text{equatorial-42}} = 4.2$ Hz. Furthermore, when H-42 was irradiated, the 12.8 and 4.2 Hz couplings with Hs-43 as well as the 7 Hz coupling with the C-42 methyl were eliminated (see appendix, figure 98). H-42 must be in the axial position, and thus has a trans-diaxial relation with H43axial, to have the large J value (12.8 Hz) that was observed. Finally, diastereoselective spiroketalization gave the desired target 80. Owing to high convergence, our synthesis is much shorter than that of the Harvard group.

Our successful strategy involved coupling a K-ring fragment with an N-ring fragment by the Wittig reaction of ketoylide 58 with an aldehyde 6e. The ylide was assembled by acylation of methylenetriphenylphosphorane 60. The requisite acylating agent, an activated derivative of acid 61, was not available by conjugate methylation of the corresponding $\alpha,\beta$-unsaturated ester. Thus, use of an $\alpha,\beta$-unsaturated acyl silane as a Michael acceptor followed by oxidative desilylation provided the key intermediate acid 61. Highly stereospecific conjugate addition to a $\gamma$-alkoxy-$\alpha,\beta$-unsaturated acyl silane was a general solution to a major obstacle in our synthesis and this methodology should be applicable to many different natural product syntheses. The practicality of this strategy is bolstered by the ready availability of mutli-gram quantities of alcohols 20 and 21.
General. All melting points are uncorrected and were measured on Thomas Hoover Capillary melting point apparatus. All proton nuclear magnetic resonance (NMR) spectra were recorded either on a Varian XL-200 or a Gemini 300 spectrometer operating at 200 and 300 MHz respectively. Chemical shifts are reported in ppm on the δ scale relative to residual CHCl₃ (δ 7.24) for spectra in CDCl₃. Peaks are designated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. ¹³C NMR spectra were recorded on either a Varian XL-200 spectrometer operating at 50.3 MHz or a Varian Gemini 300 spectrometer operating at 75 MHz. Chemical shifts are reported on the δ scale relative to CDCl₃ (δ 77.0) or CD₂Cl₂ (δ 53.8). Elemental analyses were carried out by Galbraith Laboratories, Inc., Knoxville, TN. High resolution mass spectra (HRMS) or fast atom bombardment (FAB) were done on a Kratos/AEI MS-30 dual beam, double focusing, magnetic sector mass spectrometer with a DS-50S Nova-3 computer. Samples were run with a 200 °C heat source and direct probe insertion. EI spectra were obtained at 20 eV using perfluorokerosene as an internal standard and FAB spectra were run in a glycerol matrix. Optical rotations were measured on a Perkin Elmer 241 Polarimeter at a wavelength of 589 nm using spectrophotometric grade CHCl₃ or CH₃OH.

Thin layer chromatography (TLC) was done with silica gel layered (0.25 mm) glass plates from E. Merck. Plate development was done by viewing under 254 nm UV light, then either placement in an iodine
chamber or spraying with 6% vanillin/10% H$_2$SO$_4$ (w/v) in 95% EtOH, and heating with a hot air gun. Column chromatography was done with silica gel from J. T. Baker Inc., with an average particle size of 40-55 µm. The OD's of columns used are reported and the columns were generally filled with 6 inches of silica gel. UV active eluants were monitored with an ISCO model V$^4$ detector. High pressure liquid chromatography (HPLC) was done on a Waters Model 590 pump and UK6 injector with Whatman Partisil 10 columns. Analytical separations (< 10 mg/injection) were done on a Whatman PXS (4.6 mm ID x 25 cm) column at a flow rate of 2 mL/min. and preparative-scale separations (> 10 mg/injection) were done on a Whatman M9 (9.4 mm ID x 50 cm) column at a flow rate of 10 mL/min. Eluants were detected by a R401 Differential Refractometer.

Materials. All solvents were distilled in an N$_2$ or Ar atmosphere immediately before use. Tetrahydrofuran (THF), benzene, and toluene were distilled from Ph$_2$CO/Na or K. CH$_2$Cl$_2$ was distilled from CaH$_2$. Et$_2$O was distilled over LAH. Acetone was distilled over CaSO$_4$. MeOH was dried by the addition of a small piece of sodium metal and then distilled. Chromatography solvents were used as obtained (ACS grade). HPLC solvents were purchased and used as such (HPLC grade). All reactions, unless in an aqueous media, were carried out in an N$_2$ or Ar atmosphere passed through a CaSO$_4$ drying tube. Water was distilled and deionized through a Millipore filtration system. All reagents were commercially obtained and purified according to literature procedures.$^{51}$ The preparation of Bu$_3$SnH,$^{52}$ methylene-
triphenylphosphorane \((60)\),\(^{42}\) tert-butylmethyldimethylsilylethyltriflate,\(^{27}\) and dimethyl [2-(tert-butylmethyldimethylsilyl)-2-oxoethyl]-phosphonate\(^{38}\) (64) will be described in the experimental section. Alkyl lithium bases were titrated using 4-biphenylmethanol as an indicator.\(^{53}\)

**2,5-Anhydro-1,3-O-anisylidene-D-glucitol (11):**

To concentrated HCl (200 mL) was added D-mannitol 8 (65.0 g, 357 mmol) and the solution was refluxed gently for 24 h. The outlet for the condenser was connected to a pipette that was submerged in oil to prevent HCl vapors from corroding the fume hood. HCl was removed by rotary evaporation at 35 °C followed by high vacuum for 16 h. MeOH (450 mL), p-toluenesulfonic acid (250 mg, 1.31 mmol), p-methoxybenzaldehyde (45.0 g, 330 mmol) and 3 Å molecular sieves (60g) were added and the resulting mixture was stirred at room temperature for 40 h. EtOAc (1 L) was added, stirred for 30 min and the mixture was filtered. Solvent was then removed in vacuo to give a viscous brown oil. The crude compound (12-20 g aliquots) in EtOAc (15 mL) and run through a Waters Prep LC 500 chromatography system using EtOAc as eluant. Recrystallization in EtOAc/hexanes afforded the acetal 11 as a white solid (20.5 g, 19% based on D-mannitol): mp 123-125 °C; \([\alpha]\)^{23}\text{D} 10.4° (c 4.22, CH\text{3}OH); \(^1\text{H NMR}\) (300 MHz, CDCl\text{3}) δ 7.34 (d, 2H, \(J =\)
8.7 Hz), 6.86 (d, 2H, J = 8.7 Hz), 5.40 (s, 1H), 4.40 (d, 1H, J = 13.5 Hz),
4.25 - 4.32 (2H), 4.12 (dd, 1H, J = 1.9, 13.0 Hz), 3.97 - 4.06 (2H), 3.79 -
3.87 (2H), 3.77 (s, 3H), 2.41 (br s, 1H), 2.09 (br s, 1H); 13C NMR (75
MHz, CD2Cl2) δ 160.5, 130.6, 127.7 (2C), 113.9 (2C), 99.7, 88.0, 82.6,
77.9, 73.4, 67.4, 63.2, 55.6; HRMS calcd for C14H18O6 282.1103, found
282.1112; TLC Rf = 0.16 (100% EtOAc).

2,5-Anhydro-1,3-O-anisylidene-6-O-(tert-butyl-dimethylsilyl)-D-
glucitol (14):

\[
\text{H}_3\text{CO} \quad \text{11a} \quad \xrightarrow{\text{TBDMSCI, Im}} \quad \text{H}_3\text{CO} \quad \text{14}
\]

To a stirred solution of 11 (5.6 g, 20 mmol) and imidazole (3.0 g, 44
mmol) in CH2Cl2 (60 mL) at O°C was added TBDMSCI (3.0 g, 20 mmol)
in CH2Cl2 (15 mL). After 5 h, solvent was removed in vacuo and the
residue was extracted with ether (6 x 100 mL). The compound was then
chromatographed using 35% EtOAc/hexanes as eluant to give
disilylated product 15 (510 mg, 5%), and monosilylated product 14 (6.92
g, 87%) as a white solid: mp 72-73 °C; [α]23D 2.88° (c 2.83, CHCl3); 1H
NMR (300 MHz, CDCl3) δ 7.35 (d, 2H, J = 8.7 Hz), 6.95 (d, 2H, J = 8.5
Hz), 5.36 (s, 1H), 4.36 (d, 1H, J = 13.2 Hz), 4.26 (s, 2H), 4.08 (d, 1H, J =
13.2 Hz), 3.74-3.98 (4H), 3.78 (s, 3H), 2.15 (br s, 1H), 0.87 (s, 9H), 0.04
(s, 6H); 13C NMR (75 MHz, CDCl3) δ 156.0, 130.4, 127.4 (2C), 113.5
(2C), 99.2, 87.7, 81.8, 77.97, 72.9, 68.8, 67.1, 55.2, 25.9 (3C), 18.4, -5.2,
-5.4; Anal. calcd for C26H32O6Si: C, 60.58; H, 8.14. Found: C, 60.50; H,
2,5-Anhydro-1,3-O-anisylidene-6-O-(tert-butyl-dimethylsilyl)-4-O-(methylthio thiocarbonyl)-D-glucitol (16):

A suspension of NaH (649 mg, 1.08 g of 60% oil dispersion rinsed with pentane, 27.0 mmol) and imidazole (59.0 mg, 7.0 mmol) in THF (25 mL) was cooled with an ice-water bath and 14 (8.98 g, 22.6 mmol) in THF (50 mL) was added dropwise. The ice bath was removed after the addition and the solution was stirred for 1 h. Dry CS$_2$ (3.39 mL, 56.0 mmol) diluted with THF was then added dropwise and the reaction was stirred for 2.5 h. After cooling under ice once again, MeI (3.54 mL, 56.0 mmol) was added dropwise and the reaction was allowed to reach room temperature and stirred for 16 h. Volatiles were removed in vacuo and ether (100 mL) and water (25 mL) were added. The aqueous layer was extracted with ether (3 x 100 mL). The organic layers were dried with MgSO$_4$, filtered, and concentrated to give 16 (11.0 g) as a yellow solid that was used directly for the deoxygenation: mp 106-107 °C; [α]$^{23}_D$ = -49.90 (c 1.03, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.38 (d, 2H, J = 8.7 Hz), 6.86 (d, 2H, J = 8.8 Hz), 5.85 (s, 1H), 5.39 (s, 1H), 4.49 (s, 1H), 4.38 (d, 1H, J = 13.1 Hz), 4.23 (t, 1H), 4.08 (d, 1H, J = 13.1 Hz), 3.98 (s, 1H), 3.92 (d, 2H), 3.78 (s, 3H), 2.55 (s, 3H), 0.86 (s, 9H), 0.05 (s, 6H);
13C NMR (75 MHz, CDCl3) δ 214.1, 160.1, 130.1, 127.4, 113.5, 99.4, 86.9, 84.8, 78.8, 73.4, 66.8, 63.8, 55.2, 25.9, 19.3, 18.3, -5.2, -5.3; Anal calcd. for C22H34O6S2Si: C, 54.30; H, 7.05. Found: C, 54.27; H, 7.15; TLC Rf = 0.34 (20% EtOAc/hexanes).

**Preparation of Bu3SnH:**

\[
\text{Bu}_3\text{SnCl} \xrightarrow{\text{LiAlH}_4} \text{Bu}_3\text{SnH}
\]

Dry Bu3SnCl (20 g, 16.7 mL, 0.061 mol) in Et2O (20 mL) was added dropwise to a stirred suspension of LiAlH4 (2.32 g, 0.061 mol) in Et2O (100 mL). The mixture was refluxed for 3 h and then cooled with an ice-water bath. Water (4 mL) was added very slowly followed by 20% sodium potassium tartarate solution (100 mL). Stirring was continued for 15 min at 0 °C followed by 20 min at 21 °C. The ether layer was removed and the aqueous layer was extracted with ether (3 x 50 mL). The combined ether layers were washed with brine (40 mL), dried with MgSO4, filtered, and concentrated to give a murky, dull white compound. Fractional distillation afforded Bu3SnH (15.3 g, 86%); bp 52 - 55 °C/ 0.05 Torr.

2,5-Anhydro-1,3-O-anisylidene-6-O-(tert-butyl-dimethylsilyl)-4-deoxy-D-glucitol (17):
Freshly prepared Bu₃SnH (12.0 mL, 44.6 mmol) was added to crude xanthate 16 (11.0 g, 22.7 mmol) in toluene (200 mL) and the solution was gently refluxed for 6 h. Removal of the solvent in vacuo followed by silica gel chromatography on a 45 mm column using a step gradient of 100% hexanes, 5, 10, and 20% EtOAc/hexanes gave 17 (7.92 g, 92% from the alcohol): [α]D⁻²³ 14.3° (c 0.86, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.38 (d, 2H, J = 8.7 Hz), 6.86 (d, 2H, J = 8.8 Hz), 5.35 (s, 1H), 4.31-4.42 (2H), 4.13-4.23 (m, 1H), 4.06 (d, 1H, J = 13.1 Hz), 3.89 (dd, 1H, J = 6.3, 9.9 Hz), 3.77 (s, 3H), 3.67-3.76 (2H), 2.17-2.22 (m, 1H), 1.99-2.05 (m, 1H), 0.88 (s, 9H), 0.06 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 159.9, 130.7, 127.4 (2C), 113.4 (2C), 99.5, 79.3, 76.4, 74.7, 67.2, 66.5, 55.1, 35.7, 25.9 (3C), 18.3, -5.2, -5.4; HRMS calcd. for C₁₆H₂₃O₅Si (M-C₄H₉) 323.1315, found 323.1316; TLC Rf = 0.23 (20% EtOAc/hexanes).

2,5-Anhydro-1,3-O-anisylidene-4-deoxy-D-glucitol (18):

![Chemical structures](image)

Silyl ether 17 (5.04 g, 13.2 mmol) in THF (55 mL) was cooled to 0 °C. Bu₄NF (27 mL of 1.0 M in THF, 5% w/w H₂O) was then added dropwise and the solution was stirred for 1 h. TLC analysis revealed a trace of starting material. Additional Bu₄NF (500 μL) was added and the reaction was stirred for another 2 h at room temperature. The solvent was removed in vacuo and the crude mixture was purified on a 35 mm
silica gel column using 60%, 75%, and 85% EtOAc/hexanes as eluants to obtain alcohol 18 (3.45 g, 98%): [α]²³⁺D 38.3° (c 3.53, CHCl₃; ¹H NMR (300 MHz, CDCl₃) δ 7.37 (d, 2H, J = 8.7 Hz), 6.86 (d, 2H, J = 8.7 Hz), 5.41 (s, 1H), 4.38-4.44 (2H), 4.26-4.33 (m, 1H), 4.10 (d, 1H, J = 13.1 Hz), 3.76 (s, 3H), 3.66-3.80 (3H), 2.50 (br, s, 1H) 2.24-2.35 (m, 1H), 1.99-2.06 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 159.8, 130.2, 127.2 (2C), 113.4 (2C), 99.4, 78.8, 76.3, 74.4, 66.8, 64.9, 55.0, 34.9; HRMS calcd for C₁₄H₁₈O₅ 266.1154, found 266.1130; TLC Rf = 0.10 (60% EtOAc/hexanes).

2,5-Anhydro-1,3-O-anisylidene-6-O-benzyl-4-deoxy-D-glucitol (19):

To a stirred suspension of NaH (24.0 mg, 1.60 mmol, 60% dispersion in oil rinsed with pentane) in THF (1 mL) was added 18 (137 mg, 0.50 mmol) in THF (1.5 mL). After 1 h, benzyl bromide (120 mg, 0.50 mmol) in THF (1.5 mL) was added and stirring was continued for 16 h. Ether (10 mL) was added and the mixture was washed with water (5 mL). The aqueous layer was extracted with ether (4 x 20 mL) and the combined organic layers were dried with MgSO₄, filtered, and concentrated. Purification on a 20 mm silica gel column that was eluted with 20% and 50% EtOAc/hexanes gave 19 as a white solid.
(180 mg, 98%): mp 119-121 °C; [α]23D 9.40° (c 1.23, CHCl3); 1H NMR (300 MHz, CDCl3) δ 7.27-7.36 (7H), 6.86 (d, 2H, J = 8.3 Hz), 5.36 (s, 1H), 4.66 (d, 1H, J = 12.2 Hz), 4.59 (d, 1H, J = 12.2 Hz), 4.33-4.44 (3H), 4.07 (d, 1H, J = 13.1 Hz), 3.80-3.83 (1H), 3.78 (s, 3H), 3.57-3.65 (2H), 2.23-2.32 (m, 1H), 1.93-1.99 (m, 1H); 13C NMR (75 MHz, CDCl3) δ 159.8, 138.2, 130.6, 128.1 (2C), 127.6 (2C), 127.4 (2C), 127.3, 113.4 (2C), 99.4, 77.6, 76.2, 74.7, 73.3, 73.1, 67.0, 55.1, 36.1; Anal calcd for C21H24O5: C, 70.75; H, 6.79. Found: C, 70.92; H, 6.97; TLC Rf = 0.52 (60% EtOAc/hexanes).

2,5-Anhydro-6-O-benzyl-4-deoxy-3-O-(p-methoxybenzyl)-D-glucitol (20) and 2,5-anhydro-6-O-benzyl-4-deoxy-1-O-(p-methoxybenzyl)-D-glucitol (21):

Acetal 19 (1.10 g, 3.09 mmol) was dissolved in toluene (22 mL) and cooled to -10 °C under an ice-salt bath. DIBAlH (5.20 mL of 1.50 M in toluene, 7.80 mmol) was then added and the reaction was stirred for 20 min. MeOH (3.60 mL) was added and the reaction was allowed to reach room temperature and then stirred until a clear gel formed. The gel was diluted with EtOAc (50 mL) and transferred to a separatory funnel. Saturated sodium potassium tartrate solution (80 mL) was added and the organic layer was removed. The aqueous layer was exhaustively extracted with ethyl acetate (10 x 30 mL) and the combined
organic layers were dried with MgSO₄, filtered, and concentrated. The crude product was loaded on a 40 mm silica gel column that was eluted with 40% and 60% EtOAc/hexanes followed by 100% EtOAc⁵⁴ to obtain primary alcohol 20 (646 mg, 58%) as a white solid and secondary alcohol 21 (429 mg, 39%) as an oil: 20: mp 46-48 °C; [α]²³ºD 26.1° (c 0.86, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.24-7.33 (5H), 7.19 (d, 2H, J = 8.4 Hz), 6.85 (d, 2H, J = 8.5 Hz), 4.53-4.61 (2H), 4.50 (d, 1H, J = 11.1 Hz), 4.31 (d, 1H, J = 11.5 Hz), 4.19-4.25 (m, 1H), 4.07-4.13 (m, 1H), 3.91-3.97 (m, 1H), 3.76-3.81 (2H), 3.78 (s, 3H), 3.54-3.58 (2H), 2.55 (br, s, 1H), 2.20-2.22 (m, 1H), 1.86-1.95 (m, 1H); ¹³C NMR (300 MHz, CDCl₃) δ 158.9, 137.8, 129.5, 128.8 (2C), 128.0 (2C), 127.4 (2C), 127.2, 113.5 (2C), 81.0, 78.7, 76.4, 73.0, 72.2, 70.8, 61.6, 54.8, 34.0; Anal. calcd for C₂₁H₂₆O₅: C, 70.36; H, 7.47. Found: C, 70.26; H, 7.47; TLC Rf = 0.20 (60% EtOAc/hexanes).

21: [α]²³ºD 16.1° (c 1.12 , CHCl₃); ¹H NMR (300MHz, CDCl₃) δ 7.30 (s, 5H), 7.26 (d, 2H, J = 8.5 Hz), 6.84 (d, 2H, J = 8.5 Hz), 4.43-4.65 (4H), 4.22-4.25 (m, 1H), 4.11 (m, 1H), 3.84-3.87 (2H), 3.76 (s, 3H), 3.62-3.73 (3H), 3.42 (d, 1H), 2.24-2.30 (m, 1H), 1.85-1.91 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 159.1, 137.1, 130.2, 129.4 (2C), 128.4 (2C) 127.9 (2C), 127.8, 113.6 (2C), 82.9, 76.7, 73.6, 73.1, 71.9, 71.7, 69.0, 55.1, 37.0; TLC Rf = 0.32 (60% EtOAc/hexanes).

(2R,3S,5S)-2-Carboxaldehyde-3-(p-methoxyphenylmethoxy)-5-(phenylmethoxymethyl)-tetrahydrofuran (6a):

\[
\text{HOO} \quad 20 \quad \xrightarrow{1)} \quad (\text{COCl})₂, \text{DMSO} \quad \xrightarrow{2)} \quad \text{Et₃N} \quad \rightarrow \quad \text{HOCH} \quad 6a
\]
Oxalyl chloride (449 μL, 4.69 mmol) in CH₂Cl₂ (12 mL) was cooled to -75 °C. Using an addition funnel, DMSO (850 μL, 12.0 mmol) in CH₂Cl₂ (12 mL) was added and the mixture was stirred for 20 min. Primary alcohol 20 (499 mg, 1.39 mmol) in CH₂Cl₂ (10 mL) was added via the addition funnel and the reaction was stirred for 1 h at -75 °C. Et₃N (2.00 mL, 14.3 mmol) was then added and the reaction was stirred an additional 10 min at -75 °C and 20 min at room temperature. The reaction was diluted with ether (100 mL) and washed with saturated NH₄Cl solution (5 mL), saturated NaHCO₃ solution (5 mL) and brine (5 mL). The organic layer was dried with MgSO₄, filtered, and concentrated. The crude product was then purified on a 35 mm silica gel column using 30% EtOAc/hexanes as eluant to give aldehyde 6a as a clear oil (490 mg, 98%): [α]₂³ discipline 39.8° (c 1.66, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 9.65 (s, 1H), 7.26-7.34 (5H), 7.14 (d, 2H, J = 7.3 Hz), 6.84 (d, 2H, J = 7.1 Hz), 4.62 (d, 1H, J = 12.1 Hz), 4.55 (d, 1H, J = 12.1 Hz), 4.21-4.41 (5H), 3.74 (s, 3H), 3.69-3.72 (m, 1H), 3.57-3.61 (m, 1H), 2.10-2.18 (m, 1H, 1.94-2.00 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 200.8, 159.0, 137.9, 129.0, 128.9 (2C), 128.1 (2C), 127.5 (2C), 127.3, 113.5 (2C), 85.6, 80.4, 78.6, 73.0, 72.3, 71.0, 54.9, 34.1; HRMS calcd. for C₂₁H₂₄O₅ 356.1623, found 356.1632; TLC Rf = 0.33 (50% EtOAc/hexanes).

Enones 29t and 29c:
Aldehyde 6a (96 mg, 0.27 mmol) and 1-triphenylphosphoranylidene-2-propanone (30, 170 mg, 0.534 mmol) in CH$_2$Cl$_2$ (5 mL) were stirred at room temperature for 16 h. The solvent was then removed in vacuo and to the residue was added the minimum amount of CH$_2$Cl$_2$ required to dissolve all the solid. This solution was then loaded onto a 20 mm silica gel column that was eluted with 20% EtOAc/hexanes to obtain trans enone 29t (82 mg, 77%) and cis enone 29c (15 mg, 14%): 29t: $[\alpha]_{23}^{23\text{D}}$ -4.80° (c 1.25, CHCl$_3$); $^1$H NMR (300 MHz, CDC$_3$) $\delta$ 7.24 - 7.33 (5H), 7.15 (d, 2H, $J = 8.6$ Hz), 6.80 - 6.87 (1H), 6.83 (d, 2H, $J = 8.8$ Hz), 6.29 (dd, 1H, $J = 1.5$, 16.2 Hz), 4.60 (d, 1H, $J = 12.1$ Hz), 4.53 (d, 1H, $J = 12.1$ Hz), 4.40 - 4.50 (1H), 4.44 (d, 1H, $J = 11.8$ Hz), 4.30 (d, 1H, $J = 11.8$ Hz), 4.12 - 4.24 (2H), 3.77 (s, 3H), 3.62 (dd, 1H, $J = 6.34$, 9.95 Hz), 3.54 (dd, 1H, $J = 4.9$, 9.9 Hz), 2.15 - 2.26 (m, 1H), 2.22 (s, 3H), 1.88 - 1.97 (m, 1H); $^{13}$C NMR (75 MHz, CDC$_3$) $\delta$ 198.3, 159.2, 143.2, 138.1, 131.4, 129.7, 129.0, 128.3, 127.7, 127.5, 113.7, 81.2, 79.8, 77.2, 73.3, 72.7, 71.1, 55.2, 34.5, 27.0; HRMS calcd for C$_{24}$H$_{28}$O$_5$ 396.1937, found 396.2012; TLC R$_f$ = 0.30 (40% EtOAc/hexanes).

29c: $[\alpha]_{23}^{23\text{D}}$ 178° (c 1.07, CHCl$_3$); $^1$H NMR (300 MHz, CDC$_3$) $\delta$ 7.24 - 7.32 (5H), 7.11 (d, 2H, $J = 8.6$ Hz), 6.80 (d, 2H, $J = 8.6$ Hz), 6.27 (d, 1H, $J = 2.1$ Hz), 5.04 - 5.10 (m, 1H), 4.60 (d, 1H, $J = 12.2$ Hz), 4.52 (d, 1H, $J = 12.2$ Hz), 4.36 - 4.46 (2H), 4.33 (d, 1H, $J = 11.6$ Hz), 4.26 (d, 1H, $J = 11.7$ Hz), 4.04 - 4.15 (m, 1H), 3.77 (s, 3H), 3.58 - 3.69 (m, 1H), 3.52 (dd, 1H, $J = 4.7$, 9.9 Hz), 2.15 - 2.27 (m, 1H), 2.20 (s, 3H), 1.77 - 1.86 (m, 1H); TLC R$_f$ = 0.45 (40% EtOAc/hexanes).
2,5-Anhydro-3,6-di-O-benzyl-4-deoxy-1-O-(p-methoxybenzyl)-D-glucitol (41):

Alcohol 21 (96 mg, 0.27 mmol) in THF (900 μL) was added dropwise to a suspension of NaH (22 mg of 60% oil dispersion, 0.56 mmol) in THF (900 μL) at 0 °C. The temperature was maintained at 0 °C for 20 min followed by 2 h at room temperature. After cooling to 0 °C, BnBr (66 μL, 0.56 mmol) in THF (1 mL) was added and the reaction was stirred for 16 h while reaching room temperature. After solvent removal in vacuo, the residue was loaded onto a 15 mm silica gel column that was eluted with 20% EtOAc/hexanes to remove excess BnBr (into a closed container to prevent the BnBr vapors from escaping into the room) and 41 (61 mg, 84% based on amount of 21 consumed) followed by elution with 40% EtOAc/ hexanes to recover unreacted 21 (38 mg). Note: the purity of the NaH was questionable as evidenced by the large amount of unreacted alcohol. The benzyl ether 41 showed: 1H NMR (200 MHz, CDCl3) δ 7.15 - 7.37 (12H), 6.80 (d, 2H, J = 8.6 Hz), 4.29 - 4.60 (6H), 3.93 - 4.19 (3H), 3.73 (s, 3H), 3.39 - 3.80 (4H), 2.05 - 2.25 (m, 1H), 1.81 - 2.00 (m, 1H); TLC Rf = 0.30 (40% EtOAc/hexanes).

2,5-Anhydro-3,6-di-O-benzyl-4-deoxy-D-glucitol (42):
CAN (200 mg, 0.364 mmol) was added to the MBn ether 41 (46 mg, 0.10 mmol) in CH₃CN/H₂O (9/1, v/v, 2 mL) and the mixture was stirred for 2 h. After solvent removal, the residue was loaded onto a 10 mm silica gel column that was eluted with 25, 40, and 50% EtOAc/hexanes to give alcohol 42 (25 mg, 74%). There were also very polar byproducts formed (presumably diols) but they were not fully characterized: ¹H NMR (200 MHz, CDCl₃) δ 7.10 - 7.34 (10H), 4.41 - 4.59 (3H), 3.98 - 3.38 (3H), 3.90 (m, 1H), 3.78 (br s, 1H), 3.43 - 3.53 (2H), 2.41 (br s, 1H), 2.05 - 2.22 (m, 1H), 1.79 - 1.95 (m, 1H); TLC Rf = 0.07 (50% EtOAc/hexanes).

**Enone 43t from alcohol 42:**

![Diagram of the reaction](image)

The aldehyde 6b was prepared according to the same procedure as aldehyde 6a and was reacted immediately with 1-triphenylphosphoranylidene-2-propanone (30) in CH₂Cl₂ at room temperature for 16 h to give the mixture of cis enone 43c (6 mg, 21%) and trans enone 43t (14 mg, 54%) in an overall yield of 75% from the alcohol. 43t: ¹H NMR (200 MHz, CDCl₃) δ 7.20 - 7.35 (10H), 6.82 (dd, 1H, J = 5.8, 16.2 Hz), 6.26 (dd, 1H, J = 1.3, 16.0 Hz), 4.27 - 4.60 (5H), 4.05 - 4.21 (2H), 3.46 - 3.64 (2H), 2.18 (s, 3H), 2.07 - 2.26 (m, 1H), 1.79 - 1.95 (m, 1H); TLC Rf = 0.32 (40% EtOAc/hexanes).

**Conjugate addition to enones 29t, 43t, and 48t:**
CuI (96 mg, 0.51 mmol) was suspended in THF (1.0 mL) and cooled to 0 °C. MeLi (682 μL of 1.40 M in ether, 0.954 mmol) was added until a pale grey color persisted. This solution was stirred for 20 min and then cooled to -78 °C. TMSCl (317 μL, 2.50 mmol) was added and the resulting cloudy mixture was stirred another 20 min. Enone 29 (48 mg, 0.12 mmol) in THF (1.0 mL) was then added and the resulting brown/yellow solution was stirred for 2 h at -78 °C. Saturated NH₄Cl (1.5 mL) was added and the temperature was maintained at -78 °C for 20 min before allowing it to warm to room temperature. Ether (30 mL) was added and the resulting mixture was washed with water (10 mL) and brine (10 mL). After drying with MgSO₄, filtration, and solvent removal, the residue was passed through 3 inches of silica gel on a 3 mm column eluted with EtOAc to obtain a yellow residue. The crude compound was then loaded onto a 15 mm column that was eluted with 20% EtOAc/hexanes to obtain starting enone 29t (5 mg) and 28a (33 mg, 74%) as a pale yellow oil. ¹H NMR (200 MHz, CDCl₃) δ 7.20 - 7.52 (5H), 7.20 (d, 2H, J = 8.7 Hz), 6.85 (d, 2H, J = 8.6 Hz), 4.60 (d, 1H, J = 12.1 Hz), 4.51 (d, 1H, J = 12.1 Hz), 4.47 (d, 1H, J = 11.3 Hz), 4.22 (d, 1H, J = 11.4 Hz), 4.06 - 4.20 (m, 1H), 3.92 - 4.00 (m, 1H), 3.80 (s, 3H), 3.59 (dd, 1H, J = 6.3, 9.7 Hz), 3.47 (dd, 1H, J = 5.6, 9.8 Hz), 3.38 (dd, 1H, J = 3.8, 9.3 Hz), 2.85 (dd, 1H J = 4.2, 15.8 Hz), 2.48 - 2.67 (m, 1H), 2.04 -
2.32 (2H), 2.11 (s, 3H), 1.88 - 2.02 (m, 1H), 0.86 (d, 3H, J = 6.6 Hz); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 209.1, 159.1, 138.4, 130.1, 129.3 (2C), 128.3 (2C), 127.7 (2C), 127.5, 113.7 (2C), 87.2, 77.2, 73.2, 70.2, 70.1, 55.2, 48.5, 34.1, 30.3, 30.2, 29.4, 16.6; HRMS calcd for C$_{25}$H$_{31}$O$_4$ (M-OH) 395.2222, found 395.2219; TLC R$_f$ = 0.49 (50% EtOAc/hexanes).

Analogues 28b and 28c were prepared were prepared under the same conditions as 28a from the corresponding enones 43t and 48t in 78% and 74% yields respectively. 28b: $^1$H NMR (200 MHz, CDCl$_3$) δ 7.20 - 7.37 (10H), 4.42 - 4.51 (3H), 4.23 (d, 1H, J = 11.8 Hz), 4.00 - 4.14 (m, 1H), 3.85 - 3.95 (m, 1H), 3.24 - 3.59 (3H), 2.80 (dd, 1H, J = 4.2, 16.0 Hz), 2.40 - 2.62 (m, 1H), 1.80 - 2.24 (3H), 2.05 (s, 3H), 0.81 (d, 3H, J = 6.6 Hz).

28c: $^1$H NMR (200 MHz, CDCl$_3$) δ 7.24 - 7.29 (5H), 4.51 (2H), 4.19 - 4.22 (m, 1H), 4.00 - 4.17 (m, 1H), 3.56 (dd, 1H, J = 6.6, 9.4 Hz), 3.40 (dd, 1H, J = 5.6, 9.6 Hz), 3.29 (dd, 1H, J = 3.3, 9.1 Hz), 2.80 (dd, 1H, J = 4.2, 15.8 Hz), 2.36 - 2.55 (m, 1H), 2.10 - 2.29 (2H), 2.07 (s, 3H), 1.62 - 1.76 (m, 1H), 0.77 - 0.85 (12H), 0.01 (s, 3H), 0.00 (s, 3H).

Analogue 28d was prepared by adding (i-Pr)$_2$NEt (7.2 μL, 0.042 mmol) to 40a and 40b (8.1 mg, 0.028 mmol) in CH$_2$Cl$_2$ (80 μL). The mixture was stirred for 20 min and then MEMCl (10 μL, 0.084 mmol) was added and the resulting mixture was stirred for 16 h at room temperature. The mixture was then concentrated and the residue was passed through a pipette with one inch of silica gel that was eluted with 50% EtOAc/hexanes (15 mL). The crude product was purified by HPLC using 30% EtOAc/hexanes as eluant to obtain 28d (2.5 mg, 24%): $^1$H
NMR (200 MHz, CDCl₃) δ 7.26 - 7.40 (5H), 4.71 (d, 1H, J = 7.4 Hz), 4.58 (d, 1H, J = 12.2 Hz), 4.48 (d, 1H, J = 12.2 Hz), 4.00 - 4.29 (2H), 3.29 - 3.81 (10H), 2.85 (dd, 1H, J = 4.1, 16.1 Hz), 2.43 - 2.64 (m, 1H), 2.10 - 2.36 (2H), 2.11 (s, 3H), 1.81 - 1.98 (m, 1H), 0.88 (d, 3H, J = 6.6 Hz). TLC Rf = 0.76 (50% EtOAc/hexanes).

Enal 37t:

(Triphenylphosphoranylidene)acetaldehyde⁰²² (79 mg, 0.252 mmol) was added to 6a (60 mg, 0.17 mmol) in dry benzene (6 mL) and the resulting mixture was heated at 74 to 76 °C for 2.5 h. The solvent was then removed in vacuo and the residue was loaded onto a 15 mm silica gel column that was eluted with 30% EtOAc/hexanes to obtain cis enal 37c (3.2 mg) and trans enal 37t as a clear yellow oil (52 mg, 80%). Trans:cis = 15:1 based on the olefin peak integrals from the ¹H NMR spectrum of the crude reaction mixture. 37t: [α]²³D -16.9° (c 1.70, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 9.54 (d, 1H, J = 7.9 Hz), 7.23 - 7.33 (5H), 7.15 (d, 2H, J = 8.6 Hz), 6.89 (d, 1H, J = 5.4 Hz), 6.84 (d, 2H, J = 8.6 Hz), 6.33 (ddd, 1H, J = 1.5, 8.0, 15.8 Hz), 4.59 (d, 1H, J = 12.0 Hz), 4.53 (d, 1H, J = 12.0 Hz), 4.45 (d, 1H, J = 11.8 Hz), 4.30 (d, 1H, J = 11.7 Hz), 4.09 - 4.26 (2H), 3.77 (s, 3H), 3.46 - 3.51 (3H), 2.09 - 2.26 (m, 1H), 1.83 - 1.97 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 193.4, 159.3, 153.4, 138.0,
132.7, 129.5, 129.1 (2C), 128.3 (2C), 127.7 (2C), 127.6, 113.8 (2C), 80.7, 79.7, 77.4, 73.3, 72.6, 71.2, 55.2, 34.3; HRMS calcd for C_{23}H_{27}O_{5} 382.1780, found 382.1787; TLC Rf = 0.44 (50% EtOAc/hexanes).

37c: ^1^H NMR (300 MHz, CDCl_3) δ 10.1 (d, 1H, J = 7.1 Hz), 7.26-7.33 (5H), 7.13 (d, 2H, J = 8.4 Hz), 6.83 (d, 2H, J = 8.1 Hz), 6.67 (dd, 1H, J = 6.8, 11.5 Hz), 6.08 (dd, 1H, J = 7.1, 11.6 Hz), 5.00 (apparent t, 1H, J = 6.0 Hz), 4.59 (d, 1H, J = 12.3 Hz), 4.52 (d, 1H, J = 12.2 Hz), 4.42 (d, 1H, J = 11.7 Hz), 4.28 (d, 1H, J = 11.8 Hz), 4.15 - 4.26 (2H), 3.77 (s, 3H), 3.64 (dd, 1H, J = 6.2, 9.8 Hz), 3.54 (dd, 1H, J = 5.0, 9.9 Hz), 2.19 - 2.29 (m, 1H), 1.89 - 1.98 (m, 1H).

**Conjugate addition to enal 37t:**

![Conjugate addition to enal 37t](image)

CuCN (47 mg, 0.52 mmol) was suspended in THF (1 mL) and cooled to -70 °C. MeLi (750 μL of 1.40 M in ether, 1.04 mmol) was added and the resulting pale brown solution was allowed to warm to -5 °C over 30 min. After cooling back to -70 °C, TMSCl (132 μL, 1.04 mmol) was added followed by 37t (40 mg, 0.10 mmol) in THF (750 μL) and the resulting mixture was allowed to stir between -70 and -50 °C for 1.75 h. Saturated NH_4Cl (1.6 mL) was then added and the resulting mixture was stirred for 10 min at low temperature and for 20 min while warming to room temperature. Ether (50 mL) was added and the ether
extract was washed successively with saturated NaHCO₃ (5 mL), H₂O (5 mL), and brine (5 mL), and then dried with MgSO₄, filtered, and concentrated. The residue was purified by HPLC using 30% EtOAc/hexanes as eluant to give 36 as a pale yellow oil (30 mg, 72%): \([\alpha]^{23}_{D} 28.9^\circ (c 1.50, \text{CHCl}_3); 1^H \text{NMR} (300 \text{ MHz, CDCl}_3) \delta 9.71 \text{ (t, 1H, } J = 2.3 \text{ Hz)}, 7.25 - 7.32 \text{ (5H)}, 7.19 \text{ (d, 2H, } J = 8.6 \text{ Hz)}, 6.84 \text{ (d, 2H, } J = 8.6 \text{ Hz)}, 4.57 \text{ (d, 1H, } J = 12.1 \text{ Hz)}, 4.50 \text{ (d, 1H, } J = 12.1 \text{ Hz)}, 4.47 \text{ (d, 1H, } J = 11.4 \text{ Hz)}, 4.20 \text{ (d, 1H, } J = 11.4 \text{ Hz)}, 4.00 - 4.17 \text{ (m, 1H), 3.86 - 3.94 (m,1H), 3.79 (s, 3H), 3.57 (dd, 1H, } J = 6.2, 9.8 \text{ Hz)}, 3.47 \text{ (dd, 1H, } J = 5.7, 9.8 \text{ Hz), 3.39 (dd, 1H, } J = 3.8, 9.2 \text{ Hz), 2.54 - 2.74 (2H), 2.05 - 2.66 (2H), 1.92 - 1.99 (m, 1H), 0.88 \text{ (d, 3H, } J = 6.6 \text{ Hz); } ^1^C \text{ NMR} \text{ (300 MHz, CDCl}_3) \delta 203.2, 159.2, 138.3, 130.0, 129.3 \text{ (2C), 128.3 \text{ (2C), 127.7 \text{ (2C), 127.5, 113.7 \text{ (2C), 87.2, 77.3, 76.9, 73.3, 73.1, 70.2, 55.2, 48.8, 34.1, 28.4, 16.8; HRMS calcd for C}_{24}H_{30}O_{5} 398.2093, found 398.2101; TLC } R_f = 0.29 \text{ (30% EtOAc/hexanes).}

1,3 Dithiane 35:

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\begin{align*}
\text{BF}_3 \cdot (\text{OEt})_2 \text{ (32 } \mu\text{L, 0.14 mmol) and propanedithiol (10 } \mu\text{L, 0.10 mmol) were added to aldehyde 36 (27 mg, 0.07 mmol) in CH}_2\text{Cl}_2 \text{ (1 mL) at 0 } ^\circ\text{C. The resulting solution was stirred for 1.75 h. Then saturated NaHCO}_3 \text{ (1.0 mL) and CH}_2\text{Cl}_2 \text{ (30 mL} \text{) were added. After extraction}
\end{align*}
\]
with CH₂Cl₂ (3 x 10 mL) and drying with MgSO₄, the residue was purified on a 15 mm silica gel column that was eluted with 20% EtOAc/hexanes to obtain 35 (22 mg, 88%) in which the methoxybenzyl group had also been cleaved: [α]D²³ 4.55° (c 2.20, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.25 - 7.34 (5H), 4.65 (d, 1H, J = 12.0 Hz), 4.56 (d, 1H, J = 12.0 Hz), 4.15 - 4.21 (2H), 3.99 - 4.05 (m, 1H), 3.83 (d, 1H, J = 11.4 Hz), 3.63 (dd, 1H, J = 2.0, 10.3 Hz), 3.39 (dd, 1H, J = 1.8, 10.3 Hz), 3.25 (dd, 1H, J = 2.3, 9.5 Hz), 2.76 - 2.96 (4H), 2.03 - 2.40 (4H), 1.80 - 1.97 (2H), 1.54 - 1.60 (m, 1H), 0.95 (d, 3H, J = 6.6 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 137.3, 128.5 (2C), 127.8, 127.7 (2C), 89.0, 76.3, 73.7, 72.0, 70.9, 45.6, 39.9, 37.0, 30.4, 30.3, 30.1, 26.2, 15.7; HRMS calcd for C₁₉H₂₈O₃S₂ 368.1480, found 368.1477; TLC Rf = 0.31 (40% EtOAc/hexanes).

Silylation of dithiane 35:

![Silylation diagram]

TBDMSCl (25 μL, 0.11 mmol) was added to 35 (18 mg, 0.05 mmol) and Et₃N (23 μL, 0.16 mmol) in CH₂Cl₂ (2 mL) at 0 °C and the resulting mixture was allowed to warm to room temperature over 1 h. CH₂Cl₂ (20 mL) was then added and the organic extract was washed with saturated NaHCO₃ solution (5 mL). The organic layer was dried with MgSO₄, filtered, and concentrated. The residue was purified on a 10
mm silica gel column using 30% EtOAc/hexanes as eluant to obtain 39 as a pale yellow oil (23 mg, 95%): $^1$H NMR (200 MHz, CDCl$_3$) δ 7.26 - 7.39 (5H), 4.55 (s, 2H), 4.04 - 4.29 (3H), 3.59 (dd, 1H, $J = 6.5$, 9.8 Hz), 3.44 (dd, 1H, $J = 5.7$, 9.6 Hz), 3.29 (dd, 1H, $J = 3.3$, 9.0 Hz), 2.69 - 2.90 (4H), 1.48 - 2.40 (7H), 0.69 - 1.00 (12H), -0.01 - -0.04 (6H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 188.5, 128.3 (2C), 127.7, 127.4 (2C), 88.7, 73.7, 73.3, 71.9, 45.6, 39.5, 38.4, 30.3, 30.0, 29.7, 26.2, 25.8, 17.9, 16.1, -4.0, -5.2; HRMS calcd for $C_{25}H_{42}O_3S_2Si$ 482.2344, found 482.2333; TLC Rf = 0.59 (40% EtOAc/hexanes).

Preparation of TBDMS trflate:\textsuperscript{27}

\[ \text{Si-Cl} + \text{HOSO}_2\text{CF}_3 \rightarrow \text{Si-OSO}_2\text{CF}_3 \]

Triflic acid (7 mL, 0.08 mol) was added dropwise to TBDMSCl (12 g, 0.08 mol) at room temperature and the resulting solution was heated at 60 °C for 10 h until HCl evolution ceased. The mixture was distilled directly from the reaction flask under water aspirator vacuum at 60 °C to obtain a clear liquid (19 g, 90%).

2,5-Anhydro-6-O-benzyl-3-O-(tert-butyldimethylsilyl)-4-deoxy-1-O-(p-methoxybenzyl)-D-glucitol (46):
Et$_3$N (300 µL, 2.14 mmol) was added to alcohol 21 (255 mg, 0.71 mmol) in CH$_2$Cl$_2$ (10 mL) and the solution was cooled to 5 °C. TBDMSOTf (330 µL, 1.43 mmol) was added and the reaction was then stirred at 5 °C for 10 min and then at room temperature for 30 min. Et$_2$O (35 mL) was then added and the organic extract was washed with saturated NaHCO$_3$ (5 mL) followed by brine (5 mL). After drying with MgSO$_4$ and solvent removal, the product was purified on a 35 mm silica gel column with 6 inches of gel using 30% EtOAc/hexanes as eluant to obtain a yellow oil 46 (316 mg, 94%): [$\alpha$$^2$$_D$]$_{23}$ 16.7° (c 2.80, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.32-7.33 (5 H), 7.25 (d, 2 H, J = 8.5 Hz), 6.85 (d, 2 H, J = 8.4 Hz), 4.58 (d, 1 H, J = 12.1 Hz), 4.53 (d, 1 H, J = 12.1 Hz), 4.49 (d, 1 H, J = 11.1 Hz), 4.44 (d, 1 H, J = 11.5 Hz), 4.27-4.34 (m, 1 H), 4.11-4.23 (m, 1 H), 3.86-3.94 (m, 1 H), 3.77 (s, 3 H), 3.56-3.68 (3 H), 3.45-3.50 (m, 1 H), 2.15-2.22 (m, 1 H), 1.69-1.76 (m, 1 H), 0.85 (s, 9 H), 0.02 (s, 6 H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 162.8, 142.1, 134.1, 133.3 (2C), 132.0 (2C), 131.5 (2C), 131.2, 117.4 (2C), 86.1, 81.2, 81.1, 80.8, 80.6, 76.0, 72.7, 58.9, 42.2, 29.5, 21.7, -1.0, -1.5; FABMS calcd for C$_{27}$H$_{40}$O$_5$Si 472.2645, found 472.2690; TLC R$_f$ = 0.23 (40% EtOAc/hexanes).

2,5-Anhydro-6-O-benzyl-3-O-(tert-butyl-dimethylsilyl)-4-deoxy-D-glucitol (47):

![Chemical Structure](image)
DDQ (229 mg, 1.01 mmol) was added to 46 (369 mg, 0.78 mmol) in CH₂Cl₂/H₂O (18/1, v/v, 4.4 mL) at 0 °C. The blue-green solution was stirred for 10 min at 0 °C and then 50 min at room temperature as it gradually became yellow-brown. Saturated NaHCO₃ (10 mL) and H₂O (12 mL) were added and the organic layer was removed. The aqueous layer was washed with CH₂Cl₂ (5 x 20 mL). The combined organic layers were dried with MgSO₄, filtered and concentrated. The residue was loaded onto a 35 mm silica gel column that was eluted with 20% EtOAc/hexanes to remove MBn aldehyde and unreacted starting material (41 mg). Elution with 40-50% EtOAc/hexanes then afforded the alcohol 47 (225 mg, 92%): ¹H NMR (200 MHz, CDCl₃) δ 7.25 - 7.30 (5H), 4.54 (d, 1H, J = 10.0 Hz), 4.48 (d, 1H, J = 10.0 Hz), 4.41 (dd, 1H, J = 5.3, 11.8 Hz), 4.07 (apparent quintet, 1H, J = 5.7 Hz), 3.83 (q, 1H, J = 5.0 Hz), 3.65 - 3.75 (2H), 3.42 - 3.58 (2H), 1.96 - 2.22 (2H), 1.62 - 1.78 (m, 1H), 0.82 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H); ¹³C NMR (50 MHz, CDCl₃) δ 128.3, 127.8, 127.6, 81.7, 77.6, 76.5, 76.4, 73.4, 72.8, 62.6, 38.1, 25.7, -4.7, -5.2; HRMS calcd for C₁₉H₃₂O₃Si 352.2070, found 352.2078; TLC Rf = 0.33 (40% EtOAc/hexanes).

(2R,3S,5S)-2-Carboxaldehyde-3-(tert-butyl-dimethylsiloxy)-5-(phenylmethoxymethyl)-tetrahydrofuran (6c):

![Chemical structure image]

Oxalyl chloride (120 μL, 1.38 mmol) in CH₂Cl₂ (6 mL) was cooled to -75 °C and DMSO (250 μL, 3.13 mmol) in CH₂Cl₂ (4 mL) was added
dropwise and the resulting mixture was stirred for 20 min. Primary alcohol 47 (150 mg, 26 mmol) in CH$_2$Cl$_2$ (3 mL) was then added dropwise and the resulting solution was stirred for 1 h at -75 °C. Et$_3$N (750 μL, 5.40 mmol) was then added and the solution was stirred at -75 °C for 10 more min, then allowed to warm to room temperature over 20 min. Volatiles were removed by evaporation with a stream of dry N$_2$ followed by high vacuum. The residue was then dissolved in a minimum of CH$_2$Cl$_2$ and loaded onto a 20 mm column of silica gel. Elution with 30% EtOAc/hexanes gave aldehyde 6c (143 mg, 96%) as a pale yellow oil: [α]$^{23}$D 71.4° (c 0.80, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 9.56 (d, 1H, J = 2.2 Hz), 7.23 - 7.32 (5H), 4.60 - 4.67 (1H), 4.57 (s, 2H), 4.29 - 4.51 (m, 1H), 4.11 (dd, 1H, J = 2.3, 4.9 Hz), 3.74 (dd, 1H, J = 7.0, 9.7 Hz), 3.55 (dd, 1H, J = 5.1, 9.8 Hz), 2.15 - 2.26 (m, 1H), 1.73 - 1.85 (m, 1H), 0.80 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 202.0, 138.0, 128.3 (2C), 127.7 (2C), 127.6, 86.9, 79.0, 75.0, 73.3, 72.8, 38.2, 25.7, 25.4 (3C), 17.7, -5.0, -5.5; HRMS calcd for C$_{19}$H$_{30}$O$_4$Si 350.1913, found (M-CHO) 321.1881, (M-C$_4$H$_9$) 293.1207; TLC R$_f$ = 0.36 (30% EtOAc/hexanes).

Enones 48t and 48c:

![Enones 48t and 48c](image)

A solution containing aldehyde 6c (95 mg, 0.27 mmol) and (1-triphenylphosphoranyliden)e-2-propanone (30, 173 mg, 0.54 mmol) in
CH$_2$Cl$_2$ (15 mL) was stirred for 16 h at room temperature. Solvent was then removed in vacuo and the residue was dissolved in a minimum of CH$_2$Cl$_2$ and loaded onto a 30 mm x 6 inch silica gel column. Elution with 20% EtOAc/hexanes gave trans enone 48t (76 mg, 72%) and cis enone 48c (12 mg, 11%); 48t: [α]$^{23}_D$ 8.1° (c 1.12, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.27 - 7.36 (5H), 6.77 (dd, 1H, J = 5.6, 16.2 Hz), 6.30 (d, 1H, J = 16.5 Hz), 4.61 (d, 1H, J = 12.2 Hz), 4.56 (d, 1H, J = 12.1 Hz), 4.39 - 4.41 (2H), 4.26 (quintet, 1H, J = 7.4 Hz), 3.67 (dd, 1H, J = 6.7, 9.7 Hz), 3.53 (dd, 1H, J = 5.1, 9.7 Hz), 2.21 - 2.32 (m, 1H), 2.25 (s, 3H), 1.74 - 1.81 (m, 1H), 0.84 (s, 9H), 0.03 (s, 3H), 0.01 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 198.3, 144.2, 138.1, 131.7, 128.3, 127.8, 127.6, 82.7, 74.3, 73.2, 73.2, 38.3, 26.7, 25.6, 17.9, -4.8, -5.1; HRMS calcd for C$_{18}$H$_{25}$O$_4$Si (M - Bu-t) 333.1522, found 333.1519; TLC R$_f$ = 0.23 (30% EtOAc/hexanes).

48c: [α]$^{23}_D$ 138° (c 0.79, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.24 - 7.32 (5H), 6.13 - 6.26 (2H), 4.99 (dd, 1H, J = 3.4, 6.1 Hz), 4.60 (d, 1H, J = 12.1 Hz), 4.53 (d, 1H, J = 12.1 Hz), 4.14 - 4.24 (m, 1H), 3.67 (dd, 1H, J = 7.1, 9.7 Hz), 3.50 (dd, 1H, J = 5.0, 9.7 Hz), 2.20 - 2.33 (m, 1H), 2.19 (s, 3H), 1.54 - 1.70 (m, 1H), 0.78 (s, 9H), -0.06 (s, 3H), -0.12 (s, 3H); TLC R$_f$ = 0.32 (30% EtOAc/hexanes).

**Methylated enol silyl ether 44c:**

```
\[
\text{BnO} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{CH}_3 \quad \text{OTMS} \\
\text{48t} \quad \text{H} \quad \text{OTBDMS} \\
\text{1} \quad \text{CuI, MeLi, TMSCl} \\
\text{BnO} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{CH}_3 \quad \text{OTMS} \\
\text{44c} \quad \text{H} \quad \text{OTBDMS} \\
\text{2} \quad \text{Et}_3\text{N}
\]```
MeLi (864 µL of 1.40 M in ether, 1.21 mmol) was added to a suspension of CuI (115 mg, 0.605 mmol) in THF (900 µL) at 0 °C and the resulting mixture was stirred for 20 min. A solution of TMSCl (383 µL, 3.03 mmol) in THF (200 µL) was added and the reaction was cooled to -70 °C and stirred for 10 min. A solution of 48t (47 mg, 0.12 mmol) in THF (900 µL) was then added and the temperature was kept between -40 and -50 °C for 2 h. After cooling back to -70 °C, Et3N (422 µL, 3.03 mmol) was added and the mixture was allowed to warm to room temperature. Dry ether (50 mL) was added and the resulting mixture washed with pH 9.5 1.0 M phosphate buffer (8 mL). The aqueous layer was extracted with ether (2 x 20 mL) and the organic extracts were combined, dried with MgSO4, filtered, and concentrated. The residue was passed through 1 inch of silica gel in a pipette using 50% EtOAc/hexanes (20 mL) with Et3N (200 µL) as eluant. Solvent removal gave the crude enol silyl ether 44c (53 mg, 91%): 1H NMR (200 MHz, CDCl3) δ 7.18- 7.28 (5H), 4.34 - 4.52 (3H), 4.00 - 4.19 (2H), 3.54 (dd, 1H, J = 5.9, 9.1 Hz), 3.38 (dd, 1H, J = 7.0, 9.2 Hz), 3.28 (dd, 1H, J = 3.0, 9.9 Hz), 2.79 - 3.00 (m, 1H), 2.00 - 2.19 (m, 1H), 1.63 - 1.82 (m, 1H), 1.70 (s, 3H), 0.71 - 0.88 (12H), 0.06 - 0.15 (9H) -0.01 (s, 6H); TLC Rf = 0.68 (40% EtOAc/hexanes with < 2% Et3N).

**Alcohol 32c by ozonolysis of enol silyl ether 44c:**

![Chemical structure](image-url)
Enol ether 44c (48 mg, 0.10 mmol) in CH₃OH (480 μL) and CH₂Cl₂ (120 μL) in a 2 neck pear-shaped flask was cooled to -75 °C. O₃ was bubbled through the solution until a blue color persisted (< 10 min). The reaction vessel was then purged with N₂ and NaBH₄ (14 mg, 0.37 mmol) was added. The resulting mixture was stirred 1 h at -75 °C and then an additional 14 mg of NaBH₄ was added. The reaction was allowed to warm to room temperature over 1 h and then CHCl₃ (20 mL) was added and the organic extract was washed with cold 5% HCl (7 mL). The aqueous layer was extracted with CHCl₃ (2 x 10 mL) and the organic extracts were combined, dried with MgSO₄, filtered, and concentrated. The residue was loaded onto a 20 mm silica gel column that was eluted with 40% EtOAc/hexanes to give 19 mg of crude product. The product was purified further by HPLC using 30% EtOAc/hexanes to give the alcohol 32c (14 mg, 37%): 1H NMR (200 MHZ, CDCl₃) δ 7.26 - 7.46 (5H), 4.55 (s, 2H) 4.17 - 4.33 (2H), 3.19 - 3.76 (5H), 2.04 - 2.31 (2H), 1.68 - 1.84 (m, 1H), 0.85 (s, 9H), 0.78 (d, 3H, J = 6.9 Hz), 0.04 (s, 6H); HRMS calcd for C₁₇H₂₇O₄Si (M - Bu-t) 323.1678, found 323.1685; TLC Rf = 0.31 (40% EtOAc/hexanes).

**Mesylate 50 from alcohol 32c:**

\[
\text{BnO} \quad \text{H} \quad \text{H} \quad \text{CH₃} \quad \text{H} \quad \text{OTBDMS} \quad \text{MsCl, Et₃N} \quad \text{BnO} \quad \text{H} \quad \text{H} \quad \text{CH₃} \quad \text{OSO₂CH₃} \quad \text{OTBDMS}
\]

MsCl (6.0 μL, 0.078 mmol) was added to a solution of 32c (9.8 mg, 0.026 mmol) and Et₃N (15 μL, 0.11 mmol) in CH₂Cl₂ (370 μL) at 0 °C. The reaction was complete in less than 1 h as evidenced by TLC analysis. It
was then diluted with CH₂Cl₂ (25 mL) and the organic solution was washed with saturated NaHCO₃ solution (5 mL), dried with MgSO₄, filtered, and concentrated. The residue was passed through a pipette with one inch of silica gel eluting with 50% EtOAc/hexanes. The product was further purified by HPLC using 30% EtOAc/hexanes to obtain mesylate 50 (11 mg, 90%): ¹H NMR (200 MHz, CDCl₃) δ 7.26 (s, 5H), 4.49 (s, 2H), 4.29 - 4.36 (2H), 4.22 - 4.28 (m, 1H), 4.01 - 4.17 (m, 1H), 3.53 (dd, 1H, J = 6.8, 9.5 Hz), 3.37 - 3.49 (2H), 2.92 (s, 3H), 2.09 - 2.34 (2H), 1.63 - 1.78 (m, 1H), 0.94 (d, 3H, J = 6.8 Hz), 0.80 (s, 9H), 0.00 (s, 6H); TLC Rf = 0.50 (40% EtOAc/hexanes).

**Conversion of mesylate in 50 to iodide 33c**

NaI (16 mg, 0.11 mmol) was added to 50 (10.7 mg, 0.023 mmol) in dry 2-butanone (500 μL). The solution was heated in a sealed vial for 9 h at 55 to 63 °C. It was then diluted with ether and washed with water (5 mL) followed by sodium thiosulfate (5 mL) solution and shaken until colorless. The organic layer was dried with MgSO₄, filtered, and concentrated. The crude product was purified by HPLC using 7% EtOAc/hexanes to obtain the iodide 33c (8.1 mg, 75%): ¹H NMR (200 MHz, CDCl₃) δ 7.30 (s, 5H), 4.53 (s, 2H), 4.09 - 4.30 (2H), 3.58 (dd, 1H, J = 6.5, 9.5 Hz), 3.29 - 3.55 (4H), 2.10 - 2.29 (m, 1H), 1.65 - 1.81 (m, 1H), 0.92 (d, 3H, J = 6.5 Hz), 0.83 (s, 9H), 0.03 (s, 6H); TLC Rf = 0.67 (40%
n-BuLi (40 μL of 2.24 M in hexane, 0.095 mmol) was added to 39 (33 mg, 0.079 mmol) and tetramethylethlenediamine (36 μL, 0.237 mmol) in THF (400 μL) and the resulting mixture was cooled to -10 °C. The solution was stirred for 20 h at -10 °C. Iodide 33c (7.9 mg, 0.017 mmol) in THF (100 μL) was then added and the resulting mixture was stirred for 20 min. The reaction was quenched with saturated NH₄Cl solution (500 μL) and extracted with ether (3 x 15 mL). The product mixture was loaded onto a 20 mm silica gel column that was eluted with 30% EtOAc/hexanes to remove the more polar components. This fraction was then purified by HPLC to obtain 2 major fractions. One was 7.5 mg of the iodide and the other was a Wittig rearrangement product 52 (6.0 mg, 16%): [α]²³D 20.3° (c 0.40, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.18 - 7.41 (5H), 4.89 (dd, 1H, J = 2.93, 9.53 Hz), 3.84 (br, s, 1H), 3.30 (dd, 1H, J = 3.2, 9.2 Hz), 2.75 - 2.94 (4H), 1.97 - 2.47 (4H), 1.76 - 1.96 (2H), 1.53 - 1.71 (2H), 1.20 - 1.37 (m, 1H), 0.83 - 0.95 (12H), 0.05 (s, 3H), 0.02 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 144.8, 128.2, 127.1, 125.9, 88.3, 77.9, 77.2, 74.4, 71.9, 46.3, 45.2, 41.9, 40.0, 30.1, 29.9, 29.7, 26.1, 25.8, 18.0, 16.3, -3.9, -5.2; HRMS calcd for C₂₅H₄₂O₃S₂Si 482.2344,
found 482.2376.

2,5-Anhydro-6-O-benzyl-1-O-(tert-butyl-dimethylsilyl)-4-deoxy-3-O-(p-methoxybenzyl)-D-glucitol (53):

\[
\begin{align*}
\text{TBDMSCl (50 mg, 0.34 mmol) was added to 20 (95 mg, 0.28 mmol) and imidazole (23 mg, 0.34 mmol) in CH}_2\text{Cl}_2 (1 mL). A white precipitate formed immediately and the reaction was stirred 1 h at room temperature. More CH}_2\text{Cl}_2 (20 mL) was then added and then half saturated NaHCO}_3 in water (10 mL). The aqueous layer was extracted with CH}_2\text{Cl}_2 (2 x 5 ml) and the organic layers were combined and dried with MgSO}_4, filtered and concentrated. The residue was passed through a one-half inch plug of silica gel in a pipette that was eluted with 50% EtOAc/hexanes (15 mL) to obtain a clear oil (127 mg, 96%): \\
[\alpha]^{23}_D 14.1^\circ (c 1.82, CHCl}_3); {^1}\text{H NMR (300 MHz, CDCl}_3) \delta 7.26 - 7.40 (5H), 7.21 (d, 2H, J = 8.5 Hz), 6.85 (d, 2H, J = 8.6 Hz), 4.60 (d, 1H, J = 12.2 Hz), 4.52 (d, 1H, J = 12.0 Hz), 4.48 (d, 1H, J = 10.7 Hz), 4.39 (d, 1H, J = 11.7 Hz), 4.12 - 4.21 (m, 1H), 4.03 - 4.17 (m, 1H), 3.73 - 4.00 (3H), 3.78 (s, 3H), 3.61 (dd, 1H, J = 6.5, 9.8 Hz), 3.49 (dd, 1H, J = 5.3, 9.8 Hz), 2.10 - 2.21 (m, 1H), 1.82 - 1.97 (m, 1H), 0.90 (s, 9H), 0.06 (s, 6H); {^{13}}\text{C NMR (75 MHz, CDCl}_3) \delta 158.9, 138.3, 130.4, 128.8 (2C), 128.2 (2C), 127.6 (2C), 127.4, 113.5 (2C), 83.2, 77.7, 76.7, 73.2, 73.1, 70.8, 61.34, 55.1, 34.5, 25.9, 18.2, -5.4, -5.5; TLC Rf = 0.31 (40% EtOAc/hexanes).}
\end{align*}
\]
Preparation of W-4 Raney nickel:\(^{34}\)

NaOH (25.6 g, 0.64 mol) was added to \(\text{H}_2\text{O} (100 \text{ mL})\) in a 250-mL Erlenmeyer flask and allowed to dissolve. When the solution had cooled to 50 \(^{\circ}\)C, Ni-Al alloy (20g) was added in small portions with vigorous stirring. If the solution began to effervesce too much during the additions, a few drops of 100\% EtOH were added. A warm water bath was used to maintain the suspension temperature at 50 \(^{\circ}\)C for a 1 h digestion period. The mixture should be a thick suspension which remains at the bottom of the Erlenmeyer flask. After 1 h, the suspension was washed with water until the pH of the washings was neutral. As the washings proceed, fine particles become suspended necessitating a short pause for resettling before decanting the water. The black granules were then washed with 95\% EtOH (3 x 30 mL) followed by absolute EtOH (3 x 30 mL). The catalyst is then stored under absolute EtOH.

2,5-Anhydro-1-O-(tert-butyl-dimethylsilyl)-4-deoxy-3-O-(p-methoxybenzyl)-D-glucitol (54):

\[
\begin{align*}
\text{BnO} & \quad \text{OTBDMS} \\
\text{OMBn} & \quad \text{W-4 Raney Ni} \\
\end{align*}
\]

W-4 Raney nickel (~50 mg) was added to 53 (265 mg, 0.56 mmol) in absolute EtOH (2 mL). An H\(_2\) balloon was attached and the mixture was heated with an oil bath at 70 to 75 \(^{\circ}\)C until the reaction was complete (~ 2 h). The reaction mixture was filtered through a plug of silica gel (1 inch in a pipette) that was eluted with EtOAc. After
concentration, the residue was loaded onto a 20 mm silica gel column that was eluted with 20% and 37% EtOAc/hexanes\textsuperscript{54} to obtain 54 (189 mg, 88%) as an oil: \textsuperscript{1}H NMR (200 MHz, CDCl\textsubscript{3}) \(\delta\) 7.22 (d, 2H, \(J = 8.4\) Hz), 6.84 (d, 2H, \(J = 8.7\) Hz), 4.52 (d, 1H, \(J = 11.5\) Hz), 4.36 (d, 1H, \(J = 11.4\) Hz), 4.05 - 4.26 (2H), 3.68 - 3.98 (5H), 3.77 (s, 3H), 3.51 (dd, 1H, \(J = 4.0, 11.7\) Hz), 2.04 - 2.15 (2H), 0.87 (s, 9H), 0.03 (s, 6H); TLC \(R_f = 0.35\) (40% EtOAc/hexanes).

\textbf{2,5-Anhydro-1-O-(tert-butyl-dimethylsilyl)-4-deoxy-3-O-(p-methoxybenzyl)-6-O-(methoxyethoxy methyl)-D-glucitol (55)}:

\begin{center}
\includegraphics[width=0.5\textwidth]{diagram.png}
\end{center}

MEMCl (47 \(\mu\)L, 0.41 mmol) was added to 54 (104 mg, 0.272 mmol) and (i-Pr)\textsubscript{2}NEt (95 \(\mu\)L, 0.54 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (1.0 mL) at room temperature. After 4 h, there was still a trace of unreacted alcohol. Therefore, additional (i-Pr)\textsubscript{2}NEt (50 \(\mu\)L) and MEMCl (20 \(\mu\)L) were added. The reaction was complete in an additional hour whereupon it was diluted with CH\textsubscript{2}Cl\textsubscript{2} (50 mL), washed with saturated NaHCO\textsubscript{3} solution (10 mL), dried with MgSO\textsubscript{4}, filtered, and concentrated. The residue was loaded onto a 20 mm silica gel column that was eluted with 20% and 30% EtOAc/hexanes\textsuperscript{54} to obtain the MEM ether 55 (110 mg, 86%): \textsuperscript{1}H NMR (200 MHz, CDCl\textsubscript{3}) 7.21 (d, 1H, \(J = 8.8\) Hz), 6.84 (d, 1H, \(J = 8.7\) Hz), 4.70 (s, 2H), 4.49 (d, 1H, \(J = 11.7\) Hz), 4.39 (d, 1H, \(J = 11.7\) Hz), 4.04 - 4.23 (2H), 3.78 - 3.91 (3H), 3.78 (s, 3H), 3.48 - 3.76 (6H), 3.35 (s, 3H), 2.02 - 2.23 (m, 1H), 1.73 - 1.88 (m, 1H), 0.86 (s, 9H), 0.02 (s, 6H);
TLC Rf = 0.39 (40% EtOAc/hexanes).

2,5-Anhydro-4-deoxy-3-O-(p-methoxybenzyl)-6-O-(methoxyethoxymethyl)-D-glucitol (56):

Bu₄NF (565 μL of 1.0 M in THF with 5% w/w H₂O) was added to 55 (110 mg, 0.233 mmol) in THF (500 μL) and the reaction mixture was stirred for 1 h at room temperature. The reaction was then diluted with ether (50 mL) followed by washing with H₂O (5 mL) and drying with MgSO₄. After filtration and concentration, the residue was loaded onto a 20 mm silica gel column that was eluted with 30% EtOAc/hexanes to remove the less polar components followed by 100% EtOAc⁵⁴ to elute the primary alcohol 56 (74 mg, 89%): ¹H NMR (200 MHz, CDCl₃) δ 7.20 (d, 2H, J = 8.7 Hz), 6.85 (d, 2H, J = 8.7 Hz), 4.73 (s, 2H), 4.52 (d, 1H, J = 11.5 Hz), 4.32 (d, 1H, J = 11.5 Hz), 4.23 (q, 1H, J = 5.9 Hz), 4.05 - 4.20 (m, 1H), 3.95 (q, 1H, J = 5.5 Hz), 3.78 (s, 3H), 3.65 - 3.77 (5H), 3.52 - 3.60 (3H), 3.36 (s, 3H), 2.55 (br s, 1H), 2.12 - 2.25 (m, 1H), 1.77 - 1.90 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 159.3, 129.6, 129.2 (2C), 113.9 (2C), 95.6, 80.8, 79.1, 71.7, 71.3, 70.2, 66.8, 62.1, 59.0, 55.2, 34.2; TLC Rf = 0.16 (70% EtOAc/hexanes).

(2R,3S,5S)-2-Carboxaldehyde-5-(methoxyethoxymethoxymethyl)-3-(p-methoxyphenylmethoxy)-tetrahydrofuran (6d):
DMSO (37 µL, 0.52 mmol) in CH₂Cl₂ (250 µL) was added to oxalyl chloride (32 µL, 0.36 mmol) in CH₂Cl₂ (900 µL) at -75 °C and the mixture was stirred for 20 min. Primary alcohol 56 (37 mg, 0.10 mmol) in CH₂Cl₂ (600 µL) was then added dropwise and the mixture was stirred for 1 h at -75 °C. Et₃N (101 µL, 0.728 mmol) was then added and the reaction was allowed to reach room temperature. Ether (15 mL) was added and the mixture was washed with saturated NaHCO₃ (10 mL), saturated NaCl (10 mL), and dried with MgSO₄. After filtration and concentration, the residue was loaded onto a 10 mm silica gel column that was eluted with 70% EtOAc/hexanes to obtain aldehyde 6d (30 mg, 80%): ¹H NMR (200 MHz, CDCl₃) δ 9.64 (d, 1H, J = 2.3 Hz), 7.15 (d, 2H, J = 8.6 Hz), 6.84 (d, 2H, J = 8.7 Hz), 4.75 (s, 2H), 4.21 - 4.57 (5H), 3.77 (s, 3H), 3.67 - 3.95 (4H), 3.50 - 3.60 (2H), 3.35 (s, 3H), 2.12 - 2.29 (m, 1H), 1.88 - 2.10 (m, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 201.3, 159.0, 129.2 (2C), 119.9, 113.8 (2C), 95.7, 85.9, 80.6, 78.7, 71.7, 71.4, 70.2, 66.8, 59.0, 55.2, 34.4; TLC Rf = 0.21 (70% EtOAc/hexanes).

Enal 57:

Aldehyde 6d (155 mg, 0.439 mmol) and triphenylphosphoranylidenec acetaldehyde²² (200 mg, 0.657 mmol) in dry benzene (8 mL) were heated at 74 to 76 °C for 2.5 h. Solvent was removed in vacuo and the residue was purified on a 20 mm silica gel column with 60% EtOAc/hexanes as eluant. Triphenylphosphine oxide and the product
were separated by HPLC using 60% EtOAc/hexanes as eluant to obtain trans enal 57 (111 mg, 66%): $^1$H NMR (200 MHz, CDCl$_3$) δ 9.52 (d, 1H, J = 7.8 Hz), 7.21 (d, 2H, J = 8.6 Hz), 6.86 (d, 2H, J = 8.7 Hz), 6.71 (dd, 1H, J = 4.4, 15.7 Hz), 6.30 (ddd, 1H, J = 1.6, 7.9, 15.6 Hz), 4.67 (s, 2H), 4.63 - 4.69 (m, 1H), 4.49 (d, 1H, J = 11.5 Hz), 4.41 (d, 1H, J = 11.4 Hz), 4.27 - 4.39 (m, 1H), 3.86 - 3.98 (m, 1H), 3.78 (s, 3H), 3.50 - 3.72 (6H), 3.36 (s, 3H), 2.10 - 2.29 (m, 1H), 1.79 - 1.92 (m, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 193.4, 159.3, 153.2, 132.7, 129.1(2C), 113.8 (2C), 95.6, 80.8, 79.7, 77.2, 71.7, 71.3, 70.3, 66.9, 39.0, 35.2, 34.3; TLC $R_f$ = 0.33 (70% EtOAc/hexanes).

**KMnO$_4$ oxidation of aldehyde 36:**

NaH$_2$PO$_4$ buffer (pH 4.4, 50 μL) was added to aldehyde 36 (5.0 mg, 0.012 mmol) in t-BuOH (750 μL) followed by the addition of KMnO$_4$ (75 μL of 1.0 M, 0.075 mmol). The mixture was stirred at room temperature for 5 min and then saturated Na$_2$SO$_3$ (200 μL) was added. After dilution with ether (50 mL), the reaction was acidified with cold dilute HCl until all of the brown suspension was dissolved (pH 3 to 4). The aqueous layer was extracted with ether (3 x 10 mL) and the combined organic layers were washed with saturated NaCl solution (5 mL) and dried with MgSO$_4$. After filtration and concentration, the crude product was purified by HPLC using 10% i-PrOH/hexanes as
eluant to obtain the acid 61 (1.9 mg, 37%). The data for this product was identical to that for the acid produced by oxidative desilylation of 70 (see pp.87-88).

**t-Butyl dimethyl silane 66 of ethyl vinyl ether:**

\[
\begin{align*}
\text{OCH}_2\text{CH}_3 & \quad 1) \text{t-BuLi} \\
\text{65} & \quad 2) \text{TBDMSCl} \\
\end{align*}
\]

\[
\text{Si} \quad \text{66}
\]

A 250-mL, 3-neck flask equipped with a glass stopper, thermometer, and addition funnel was charged with ethyl vinyl ether (9.9 mL, 0.104 mmol) in THF (50 mL) and cooled to -75 °C. t-BuLi (50 mL, 1.7M in pentane, 0.085 mmol) was added via the addition funnel. When handling the t-BuLi, the syringe tip was kept in a glass tube with a rubber septum at each end to prevent exposure to air both when pipetting t-BuLi from the Sure Seal™ bottle and adding it to the funnel. The reaction was allowed to warm to -5 °C over 2.5 h and then maintained between 0 and 5 °C for 30 min (yellow solution). After cooling back to -75 °C, TBDMSCl (10.4 g, 0.067 mmol) in THF (5 mL) was added and the mixture was allowed to warm to room temperature over 2 h and then stirred for an additional 3 h (cloudy white mixture). After cooling the solution with an ice bath, half-saturated NH₄Cl (50 mL) was added via the addition funnel. The organic layer was removed and the aqueous layer was washed with ether (25 mL). The combined organic layers were washed with brine (300 mL), dried with MgSO₄, filtered, and concentrated. Fractional distillation of the residue at reduced pressure afforded vinyl silane 66 as a colorless oil (8.9 g, 46%);
bp 60 - 80 °C / 35-45 Torr.

**Bromoacylsilane 67**: 38a

![Chemical Structure](image)

A 250-mL, 3-neck flask equipped with thermometer, glass stopper, and septum was charged with NBS (8.54 g, 0.048 mmol) in CH₃CN (200 mL) and H₂O (12 mL) and cooled to -40 °C. Enol ether 66 (8.88 g, 0.048 mmol) was added neat and stirred at -40 °C for 2 h and then warmed to -10 °C over 1 h. The reaction mixture was then poured into a separatory funnel containing H₂O (600 mL) and pentane (200 mL). The organic layer was removed and washed with H₂O (800 mL). Each aqueous layer was washed with pentane (2 x 100 mL) and the combined organic layers were dried with MgSO₄ (at which point the solution became bright yellow), filtered and concentrated. The crude bromide 67 (10.14 g, 89%) was used directly for the conversion to iodide 68.

**1-(tert-Butyldimethylsilyl)-2-iodo-1-ethanone 68**: 38a

![Chemical Structure](image)

**Note:** This reaction is done in the dark as the iodide 68 is light sensitive. NaI (9.6 g, 0.064 mmol) in acetone (100 mL) was cooled with an ice bath and bromide 67 (10.1 g, 0.043 mmol) was added over 5 min. The resulting mixture was stirred for an additional 10 min. Pentane
(300 mL) and H₂O (900 mL) were added and the organic layer was removed. The aqueous layer was washed with pentane (2 x 100 mL) and the combined organic layers were dried with MgSO₄ and concentrated to obtain a light brown oil 68 (11.2 g, 92%) that was used directly for conversion to phosphonate 64.

**Dimethyl [2-(tert-Butyldimethylsilyl)-2-oxoethyl]phosphonate:**

\[ \text{I} \xrightarrow{(\text{MeO})_3\text{P}} \xrightarrow{\text{OH}} \text{Si} \quad \xrightarrow{(\text{MeO})_2\text{P}} \xrightarrow{\text{O}} \xrightarrow{\text{OH}} \text{Si} \]

A 250-mL, 3-neck flask was equipped with a magnetic stirring bar, glass stopper, addition funnel, and short path distillation condenser connected to an argon inlet and a receiving flask. The reaction flask was charged with dimethylphosphite (111 mL) and heated until the phosphite began to distill. The iodide 68 (11.2 g, 0.039 mmol) was added over 15 min via the addition funnel. Finally, the addition funnel was rinsed with Et₂O (2 mL) while distillation was continued. Heating was continued for 15 min after the addition was complete and then the mixture was cooled to room temperature. The reaction mixture was then distilled under reduced pressure (25 mm) from the 3 neck flask until no more phosphite remained (105 °C). The residue was then transferred to a 25 mL flask and fractionally distilled. The pale yellow oil product 64 condensed at 105 to 108 °C/0.55 Torr (10 g, 95%) as a 1:1 mixture of the keto and enol tautomers (see ref. 38a).

**α,β- Unsaturated acyl silane 69 from aldehyde 6a:**
To NaH (13 mg, 0.32 mmol, 60% in oil rinsed with pentane) in THF (1.4 mL) was added phosphonate 64 (70 μL, 0.29 mmol) dropwise. The mixture was stirred until it became clear and H₂ evolution ceased. The solution was then cooled with an ice-water bath to 0 °C. Aldehyde 6a (85 mg, 0.24 mmol) in THF (500 μL) was then added slowly and the solution became bright yellow. The reaction was complete in 30 min as evidenced by TLC analysis. Half saturated NH₄Cl solution (1.5 mL) was added and the mixture was allowed to reach room temperature. Ether (30 mL) was added and the mixture was washed with H₂O (10 mL) followed by brine (10 mL). The organic layer was dried with MgSO₄, filtered, and concentrated to deliver a bright yellow oil. HPLC purification using 30% EtOAc/hexanes yielded α,β-unsaturated acyl silane 69 (94 mg, 80%) as a yellow oil: [α]²³ D -18.1° (c 0.31, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.26-7.30 (5H), 7.14 (d, 2H, J = 8.2 Hz), 6.81 (d, 2H, J = 8.4 Hz), 6.71-6.78 (dd, 1H, J = 16.0 Hz), 6.57 (d, 1H, J = 15.9 Hz), 4.60 (d, 1H, J = 12.2 Hz), 4.53 (d, 1H, J = 12.1 Hz), 4.44-4.49 (m, 1H), 4.40 (d, 1H, J = 11.7 Hz), 4.29 (d, 1H, J = 11.5 Hz), 4.14-4.20 (2H), 3.74 (s, 3H), 3.51-3.68 (2H), 2.13-2.20 (m, 1H), 1.84-1.90 (m, 1H), 0.89 (s, 9H), 0.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 159.2, 141.4, 138.2, 135.9, 129.8, 129.1 (2C), 128.3 (2C), 127.7 (2C), 127.6, 113.7 (2C), 81.5, 79.8, 77.3, 73.4, 72.8, 71.1, 55.2, 34.5, 26.6 (3C), 16.6, -6.0, -6.1; HRMS calcd for C₂₉H₄₀O₅Si 496.2645, found 496.2639; TLC Rf = 0.52 (50%
Conjugate addition to α,β unsaturated acyl silane 69:

CuCN (313 mg, 3.49 mmol) dried azeotropically with toluene was suspended in THF (7 mL) and cooled to -75 °C. MeLi (5.0 mL of 1.4 M in ether, 7.0 mmol) was then added and the solution was allowed to warm over 30 min to -5 °C, then held there for 2 min and then cooled back to -75 °C. TMSCl (1.33 mL, 10.5 mmol) in THF (3 mL) was then added followed by acyl silane 69 (428 mg, 0.860 mmol) in THF (7 mL). The reaction was allowed to warm to -65 °C over 1 h 45 min and then quenched with saturated NH₄Cl solution (7 mL) and allowed to warm to room temperature over 20 min. Distilled ether (90 mL) was added and the mixture was washed with H₂O (10 mL) and then brine (10 mL). The organic layer was dried with MgSO₄, filtered, and concentrated. The crude product was loaded onto a 35 mm silica gel column with 6 inches of gel that was eluted with 30% EtOAc/hexanes to obtain 70 (308 mg, 70%) as a clear oil: [α]²³ D 16.7° (c 1.29, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 7.25-7.31 (5H), 7.17 (d, 2H, J = 8.7 Hz), 6.82 (d, 2H, J = 8.7 Hz), 4.56 (d, 1H, J = 12.0 Hz), 4.47 (d, 1H, J = 12.1 Hz), 4.44 (d, 1H, J = 11.5 Hz), 4.18 (d, 1H, J = 11.5 Hz), 4.07 (m, 1H), 3.88 (m, 1H), 3.77 (s, 3H), 3.29-3.63 (4H), 2.98 (dd, 1H, J = 2.8, 16.5 Hz), 2.54-2.71 (m, 1H), 2.45 (dd, 1H), 1.83-2.17 (2H), 0.88 (s, 9H), 0.77 (d, 3H, J = 6.4 Hz), 0.14
(s, 3H), 0.11 (s, 3H); $^{13}$C NMR (75 MHz, CDCl3) δ 159.0, 138.5, 130.3, 129.1 (2C), 128.3 (2C), 127.7 (2C), 127.5, 113.7 (2C), 87.2, 76.7, 73.3, 70.2, 55.2, 54.55, 34.2, 27.7, 26.5 (3C), 16.9, 16.6, -1.1, -6.9, -7.0; FABMS calcd for C$_{30}$H$_{44}$O$_{5}$Si 512.2958, found 512.2944; TLC $R_f$ = 0.52 (40% EtOAc/hexanes).

**Oxidative desilylation of acyl silane 70 to acid 61:**

![Chemical structure](image)

To acyl silane 70 (419 mg, 0.817 mmol) in THF (10 mL) was added 3N NaOH (2 mL) followed by 30% H$_2$O$_2$ (2 mL) and the mixture was heated at 40 °C with an oil bath until reaction appeared complete by TLC analysis (~1 h). Ether (20 mL) was added and the aqueous layer was acidified with concentrated HCl to pH ~ 3. The aqueous layer was then extracted with ether (2 x 15 mL). The organic layers were combined and washed with brine (5 mL) and dried with MgSO$_4$. The residue was loaded onto a 35 mm silica gel column with 6 inches of gel that was eluted with 50% EtOAc/hexanes to obtain 61 (301 mg, 89%) as a low melting, white solid: $[\alpha]^{23}_{D}$ 16.7° (c 1.29, CHCl3); $^1$H NMR (300 MHz, CDCl3) δ 7.25-7.32 (15H), 7.17 (d, 2H, $J$ = 8.5 Hz), 6.84 (d, 2H, $J$ = 8.7 Hz), 4.58 (d, 1H, $J$ = 12.0 Hz), 4.48 (d, 1H, $J$ = 12.0 Hz), 4.46 (d, 1H, $J$ = 11.5 Hz), 4.20 (d, 1H, $J$ = 11.4 Hz), 4.14-4.20 (m, 1H), 3.92 (t, 1H), 3.78 (s, 3H), 3.58 (dd, 1H, $J$ = 6.8, 9.7 Hz), 3.42-3.49 (3H), 2.74 (dd, 1H,
$J = 5.4, 15.3 \text{ Hz}$, 2.40-2.57 (m, 1H), 2.21-2.88 (m, 1H), 2.05-2.15 (m, 1H), 1.92-1.97 (m, 1H), 0.90 (d, 3H, $J = 6.7 \text{ Hz}$); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 177.9, 159.1, 138.2, 130.0, 129.3 (2C), 128.3 (2C), 127.8 (2C), 127.6, 113.7 (2C), 87.1, 77.2, 73.3, 73.1, 70.2, 55.2, 39.2, 33.9, 29.8, 16.7; HRMS calcd for C$_{24}$H$_{29}$O$_6$ (M-H) 413.1964, found 413.1957; TLC $R_f = 0.30$ (50% EtOAc/hexanes).

**Conversion of acid 61 to imidazolide 71:**

Carbonyl diimidazole (4.3 mg, 0.026 mmol) was added to acid 61 (10 mg, 0.024 mmol) in THF (600 μL). The mixture was heated at 42 °C with an oil bath. Reaction was complete in less than 1 h as evidenced by TLC analysis. After cooling to room temperature, CHCl$_3$ (20 mL) was added and the mixture was washed with H$_2$O (5 mL) and then brine (5 mL). After drying with MgSO$_4$, filtration, and solvent removal in vacuo, the residue was passed through a pippette with one inch of silica gel using 20% i-PrOH/EtOAc as eluant to afford the imidazolide 71 (9.8 mg, 88%):

$^{1}$H NMR (300 MHz, CDCl$_3$) δ 8.17 (s, 1H), 7.47 (s, 1H), 7.28 - 7.37 (5H), 7.14 (d, 2H, $J = 8.6 \text{ Hz}$), 7.01 (s, 1H), 6.86 (d, 2H, $J = 8.6 \text{ Hz}$), 4.40 - 4.60 (3H), 4.19 (d, 1H, $J = 11.4 \text{ Hz}$), 4.12 (m, 1H), 3.95 (m, 1H), 3.79 (s, 3H), 3.30 - 3.67 (3H), 2.47 - 2.79 (2H), 2.21 - 2.35 (m, 1H), 2.05 - 2.19 (m, 1H), 1.88 - 2.00 (m, 1H), 0.98 and 0.90 (d, 3H, $J = 6.6 \text{ Hz}$); TLC $R_f = 0.35$ (5% i-PrOH/CHCl$_3$).
Attempted acylation of methyltriphenylphosphonium bromide with imidazolidine 71:

MeLi (55 µL, 1.4 M in Et2O, 0.78 mmol) was added to a -10 °C solution (ice/salt bath) of methyltriphenylphosphonium bromide in Et2O (900 µL) and the resulting yellow suspension was stirred for 50 min. Imidazolidine 71 (7.2 mg, 0.016 mmol) in Et2O (200 µL + 200 µL rinse) was added and the suspension was stirred for 45 min at -10 °C. H2O (~60 µL) was then added dropwise until the yellow color disappeared and the reaction was allowed to warm to room temperature. CHCl3 (20 mL) was added and the mixture was washed with H2O (5 mL). The aqueous layer was washed with CHCl3 (2 x 5 mL) and the combined organic layers were dried with MgSO4, filtered and concentrated. The crude product was then loaded onto an 8 mm silica gel column that was eluted with 10% i-PrOH/CHCl3. No desired product was obtained. Rather, acid 61, the precursor of imidazolidine 71 was obtained.

Thiolester 72 from acid 61:
A solution of acid 61 (213 mg, 0.510 mmol) in DME (5 mL) was cooled to 0 °C with an ice-water bath. Pyridine (130 μL, 1.61 mmol), PhSH (110 μL, 11.0 mmol), and PhOP(O)Cl₂ (110 μL, 0.770 mmol) were added sequentially. The solution was then stirred 16 h while warming to room temperature. The mixture was then diluted with CHCl₃ (50 mL) and washed with 1N NaOH (5 mL) followed by brine (5 mL). The organic layer was dried with MgSO₄, filtered, and concentrated. The product was purified on a 35 mm silica gel column with 6 inches of gel that was eluted with 20% EtOAc/hexanes to obtain 72 (238 mg, 91%): [α]²³D 4.68° (c 0.15, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 7.23-7.36 (10H), 7.17 (d, 2H, J = 8.60 Hz), 6.82 (d, 2H, J = 8.66 Hz), 4.48-4.62 (2H), 4.45 (d, 1H, J = 11.6 Hz), 4.19 (d, 1H, J = 11.6 Hz), 4.11 (m, 1H), 3.92 (m, 1H), 3.76 (s, 3H), 3.39-3.63 (4H), 3.12 (d, 1H, J = 11.4 Hz), 2.44, 2.56 (2H), 2.02-2.08 (m, 1H), 1.89-1.96 (m, 1H), 0.92 (d, 3H, J = 6.32 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 197.1, 159.1, 138.4, 134.5 (2C), 130.4, 129.2 (2C), 129.1 (2C), 128.4 (2C), 127.8 (2C), 127.6, 113.7 (2C), 86.7, 77.2, 73.3, 73.2, 70.2, 55.3, 47.85, 34.2, 30.9, 16.1; TLC Rf = 0.48 (40% EtOAc/hexanes).

Salt-free methylenetriphenylphosphorane solution in toluene:⁴²

\[
\text{NaH/NH}_3 + \text{Ph}_3\text{P(Br)CH}_3 \xrightarrow{\text{Fe(NO}_3)_3} \text{Ph}_3\text{PCH}_2
\]

NH₃ (50 mL) was condensed at -78 °C over Na metal and then redistilled into another flask containing Na metal (167 mg). Several crystals of ferric nitrate were added and when the color changed from
blue to gray/black, Ph3P(Br)CH3 (2.61 g, 7.30 mmol) was added, the condenser was removed, the acetone-dry ice bath was replaced with a water bath and the NH3 was allowed to evaporate. Toluene (33 mL) was added and the mixture was boiled under reflux for 30 min to insure the removal of residual NH3. The dark solution was then filtered under N2 to give a yellow solution (0.22 M).

**Acylation of methylenetriphenylphosphorane 60 with thiol ester 72:**

![Chemical structure](image)

To thiol ester 72 (23 mg, 0.046 mmol) in toluene (775 μL) was added Ph3PCH2 (477 μL of 0.22 M in toluene) and the solution was heated at 104 to 107 °C for 1 h 30 min. More Ph3PCH2 (700 μL) was added during the reaction until the pale-yellow color persisted and starting material was no longer present as evidenced by TLC analysis. The reaction mixture was allowed to cool to room temperature and then diluted with dry toluene (35 mL) and washed with 0.5 N NaOH (2 mL) followed by H2O (2 mL). The organic layer was dried with MgSO4, filtered, and concentrated. The product was purified on a 12 mm silica gel column with 4 inches of gel. 10% i-PrOH/CHCl3 was used to elute unreacted starting material 72 and 30% i-PrOH/CHCl3 to elute the ylide 58 (18 mg, 65% yield based on unrecovered starting material):
[α]$^{23}_D$ 8.55° (c 2.80, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 7.37-7.67 (16H), 7.23-7.29 (4H), 7.19 (d, 2H, J = 8.7 Hz), 6.80 (d, 2H, J = 8.7), 4.41-4.64 (2H), 4.44 (d, 1H, J = 11.5 Hz) 4.22 (d, 1H, J = 11.4 Hz), 4.07-4.11 (m, 1H), 3.92 (t, 1H), 3.76 (s, 3H), 3.40-3.59 (4H), 2.85 (dd, 1H J = 3.5, 13.4 Hz), 2.26-2.60 (m, 1H), 1.83-2.16 (3H), 0.95 (d, 3H, J = 6.5 Hz); ¹³C NMR (75 MHz, CDCl₃) δ 193.5, 158.9, 138.6, 133.2, 133.0, 131.8, 130.5, 129.2, 128.8, 128.6, 128.2, 127.8, 127.5, 127.4, 127.0, 113.6, 87.8, 77.9, 73.4, 73.2, 70.2, 55.2, 46.1, 45.96, 45.9, 34.1, 31.9, 25.3, 16.2; HRMS calcd for C₄₃H₄₅O₅P 672.3004, found 672.3046; TLC Rf = 0.18 (10% i-PrOH/CHCl₃).

**Wittig reaction of aldehyde 6a with ylide 58:**

![Wittig reaction diagram](image)

To ketophosphonium ylide 58 (18 mg, 0.027 mmol) was added aldehyde 6a (11 mg, 0.031 mmol) in dichloroethane (700 µL). The solution was warmed with an oil bath at 73 °C for 8.5 h. Solvent was then removed in vacuo and the product was purified by HPLC using 40% EtOAc/hexanes to obtain enone 26 (11 mg, 55%). The use of toluene as the solvent and conducting the reaction at reflux decreased the reaction time greatly (<3 h) but did not improve the yield (~52%); [α]$^{23}_D$ -8.90° (c 0.72, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 7.28 (s, 5H), 7.26 (s, 5H), 7.09-7.22 (4 H), 6.89 (d, 1H, J = 15.9 Hz), 6.77-6.87 (4H), 6.31 (d,
Conjugate addition to enone 26:

CuCN (5.0 mg, 0.05 mmol) was dried azeotropically with toluene, then suspended in THF (150 mL) and cooled to -75 °C. MeLi (75 µL of 1.4 M in ether, 0.10 mmol) was added and the solution was allowed to warm to -5 °C until it became almost clear. The solution was then cooled back to -75 °C and TMSCl (32 µL, 0.25 mmol) was added. After 10 min, 26 (7.6 mg, 0.010 mmol) in THF (150 µL) was added and the reaction as allowed to warm to -40 °C over 1 h 45 min. After cooling back to -65 °C, saturated NH₄Cl solution (90 µL) was added. The mixture was then allowed to warm to room temperature over 30 min.
and then diluted with dry ether (20 mL) and washed with H₂O (3 mL) followed by brine (3 mL). The ether layer was dried with MgSO₄, filtered, and concentrated. TLC and NMR analyses showed that only the enol silyl ether of the desired product was present. The crude product was then dissolved in THF (30 µL) and Bu₄NF (30 µL of 1.0M, 5% w/w H₂O) was added. The mixture was stirred until the reaction was complete as evidenced by TLC analysis (~20 min). Volatiles were removed by evaporation with a stream of dry N₂ and the crude mixture was passed through a pipette of silica gel using 60% EtOAc/hexanes. The product was further purified by HPLC using 40% EtOAc/hexanes to obtain 73 (6.1 mg, 79%): [α]²³D 22.9° (c 0.70, CHCl₃); ¹H NMR (200 MHz, CDCl₃) δ 7.25-7.31 (10H), 7.17 (d, 4H, J = 8.6 Hz), 6.82 (d, 4H, J = 8.7 Hz), 4.56 (d, 2H, J = 12.0 Hz), 4.47 (d, 2H, J = 12.1 Hz), 4.44 (d, 2H, J = 11.5 Hz), 4.18 (d, 2H, J = 11.6 Hz), 4.00-4.15 (m, 2H), 3.87 (t, 2H), 3.77 (s, 6H), 3.29-3.56 (6H), 2.80 (dd, 2H, J = 3.4, 16.3 Hz), 2.39-2.65 (m, 2H), 2.20 (dd, 2H, J = 9.7, 16.3 Hz), 1.96-2.08 (m, 2H), 0.79 (d, 6H, J = 6.5 Hz); ¹³C NMR (300 MHz, CDCl₃, all peaks are for 2C unless otherwise noted) δ 210.4 (1C), 159.0, 138.4, 130.2, 129.2 (4C), 128.3 (4C), 127.8 (4C), 127.5, 113.7 (4C), 87.2, 76.3, 73.3, 70.2, 55.3, 47.6, 34.2, 29.0, 16.5; TLC Rf = 0.25 (40% EtOAc/hexanes).

**Spiroketal 74:**

![Spiroketal 74](image)
Ceric ammonium nitrate (33 mg, 0.061 mmol) was added to 73 (5.8 mg, 0.0076 mol) in 9:1 CH3CN/H2O (270 µL, v/v). The reaction was stirred 1.5 h at room temperature and then solid NaHCO3 (30 mg) was added. The entire reaction mixture was passed through a pipette of silica gel that was eluted with EtOAc (20 mL). TLC analysis revealed a UV active spot (p-methoxybenzaldehyde) with the same TLC Rf value as the product. Air was blown over the crude compound to oxidize the p-methoxybenzaldehyde to p-methoxybenzoic acid. The mixture was dissolved in 30% EtOAc/hexanes (the p-methoxbenzoic acid was not soluble in this solvent system) and passed through another pipette of silica gel to yield a dark yellow oil. The product was purified further by HPLC using 30% EtOAc/hexanes as eluant to obtain the symmetrical spiroketal 74 (2.1 mg, 55% yield): [α]23D -55.9° (c 0.43, CHCl3); 1H NMR (200 MHz, CDCl3) δ 7.27-7.33 (10H), 4.56 (s, 4H), 4.19 (m, 2H), 3.93 (dd, 2H, J = 1.9, 4.7 Hz), 3.35-3.56 (6H), 2.13-2.18 (4H), 1.70 (dd, 2H, J = 3.3, 13.7 Hz), 1.06 (dd, 2H, J = 4.7, 13.2 Hz), 0.97 (d, 6H, J = 7.1 Hz); 13C NMR (75 MHz, CDCl3, all peaks are for 2C unless otherwise noted) δ 138.6, 128.3 (4C), 127.7 (4C), 127.5, 96.6, 80.1, 77.2, 73.4, 73.0, 71.8, 37.1, 35.9, 25.6, 18.0; HRMS calcd for C32H40O6 508.2825, found 508.2824; TLC Rf = 0.62 (60% EtOAc/hexanes).

2,5-Anhydro-3-O-(tert-butyl-dimethyisilyl)-4-deoxy-1-O-(p-methoxybenzyl)-D-glucitol (75):
Freshly prepared W-4 Raney nickel (~100 mg) was added to 46 (439 mg, 1.93 mmol) in absolute EtOH (2.5 mL). A balloon filled with H₂ attached and the reaction was stirred until TLC analysis revealed completion (~ 8 h). The mixture was filtered through a plug of silica gel (1 inch in a pipette) and chromatographed on a 35 mm silica gel column with 6 inches of gel using 40% EtOAc/hexanes to obtain alcohol 75 (300 mg, 84%): [α]^{23}_D 29.0° (c 0.78, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.23 (d, 2H, J = 8.6 Hz), 6.83 (d, 2 H, J = 8.7 Hz), 4.48 (d, 1H, J= 11.5 Hz), 4.42 (d, 1H, J = 11.5 Hz), 4.28-4.31 (m, 1H), 4.14-4.18 (m, 1H), 3.85-3.90 (m, 1H), 3.77 (s, 3H), 3.70-3.75 (m, 1H), 3.49-3.63 (3H), 2.60 (t, 1H), 2.14-2.23 (m, 1H), 1.78-1.85 (m, 1H), 0.85 (s, 9H), 0.031 (d, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 159.0, 129.9, 129.4 (2C), 113.6 (2C), 81.9, 78.2, 73.0, 72.36, 68.6, 64.6, 55.1, 36.9, 25.6 (3C), 17.9, -4.9, -5.4; FABMS calcd for C₂₀H₃₄O₅Si 382.2175, found 382.2178; TLC Rₚ = 0.28 (50% EtOAc/hexanes).

2,5-Anhydro--3,6-di-O-(tert-butyl-dimethylsilyl)-4-deoxy-1-O-(p-methoxybenzyl)-D-glucitol (76):

\[
\begin{align*}
\text{TBDMSO} & \quad \text{75} \\
\text{MBnO} & \quad \text{O} \\
\text{TBDMSO} & \quad \text{76} \\
\end{align*}
\]

TBDMSCl (290 mg, 1.920 mmol) in CH₂Cl₂ (500 μL) was added to imidazole (160 mg, 0.320 mmol) and 75 (443 mg, 1.160 mmol) in CH₂Cl₂ (2.60 mL). The solution was stirred for 4 h. The solvent was then removed in vacuo. The residue was loaded onto a 15 mm silica gel
column with 6 inches of gel and eluted with 40% EtOAc/hexanes to obtain a clear oil 76 (530 mg, 92%); \([\alpha]^{23}_D\) 19.8° (c 0.47, CHCl3); \(^1\)H NMR (300 MHz, CDCl3) \(\delta\) 7.24 (d, 2H, \(J = 8.7\) Hz), 6.83 (d, 2H, \(J = 8.7\) Hz), 4.49 (d, 1H, \(J = 11.4\) Hz), 4.42 (d, 1H, \(J = 11.5\) Hz), 4.26-4.30 (m, 1H), 3.97-4.03 (m, 1H), 3.87-3.92 (m, 1H), 3.75-3.79 (m, 1H), 3.77 (s, 3H), 3.62 (dd, 1H, \(J = 4.7, 9.7\) Hz), 3.51-3.58 (2H), 2.09-2.18 (m, 1H), 1.71-1.78 (m, 1H), 0.86 (s, 9H), 0.85 (s, 9H), 0.03 (s, 6H), 0.01 (s, 6H); \(^{13}\)C NMR (75 MHz, CDCl3) \(\delta\) 159.0, 130.2, 129.3 (2C), 113.5 (2C), 82.1, 78.5, 72.9, 72.2, 69.0, 66.3, 54.9, 37.8, 25.8 (3C), 25.6 (3C), 18.2, 17.8, 4.9 (2C), 5.3, 5.4; FABMS calcd for C\(_{26}\)H\(_{48}\)O\(_5\)Si\(_2\) 496.3040, found 496.3140; TLC Rf = 0.58 (40% EtOAc/hexanes).

2,5-Anhydro-4-deoxy-3,6-di-O-(tert-butyl-dimethylsilyl)-D-glucitol (77):

\[
\begin{align*}
\text{MBnO} & \quad \text{TBDMSO} \\
\text{TBDMSO} & \quad \text{DDQ} \\
\text{76} & \quad \text{DDQ} \\
\text{HO} \quad \text{TBDMSO} & \quad \text{77}
\end{align*}
\]

DDQ (173 mg, 0.764 mmol) was added to 76 (190 mg, 0.382 mmol) in CH\(_2\)Cl\(_2\)/H\(_2\)O (18/1, v/v, 3 mL) at 0 °C and the reaction was stirred at 0 °C for 30 min, then at room temperature until complete (~1 h). Saturated NaHCO\(_3\) solution (5 mL) and H\(_2\)O (6 mL) were then added. The aqueous layer was extracted with CH\(_2\)Cl\(_2\) (5 x 10 mL). The organic extracts were dried with MgSO\(_4\), filtered, and concentrated. The crude product was loaded onto a 35 mm silica gel column (6 inches of gel) and eluted with 20% EtOAc/hexanes to remove the less polar components.
followed by 50% EtOAc /hexanes to elute the product, a low melting white solid 77 (26 mg, 88%): [α]$_D^{23}$ =9.26° (c 2.26, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 4.44 (q, 1H), 3.91-4.02 (quintet, 1H), 3.87 (q, 1H), 3.61-3.72 (4H), 2.43 (t, 1H), 2.05-2.22 (m, 1H), 1.78-1.85 (m, 1H), 0.86 (s, 18H), 0.04 (s, 6H), 0.03 (s, 6H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 81.4, 78.1, 73.4, 65.8, 62.6, 37.3, 25.9 (3C), 25.7 (3C), 18.4, 17.9, -4.7 (2C), -5.2, -5.4; FABMS calcd for C$_{18}$H$_{40}$O$_4$Si$_2$ 376.2465, found 376.2538; TLC R$_f$ = 0.15 (15% EtOAc/hexanes).

(2R,3S,5S)-2-carboxaldehyde-3,5-di-(tert-butyl-dimethylsiloxy)-tetrahydrofuran (6e):

![Chemical Structure](image)

DMSO (132 µL, 1.860 mmol) in CH$_2$Cl$_2$ (1.0 mL) was added to oxalyl chloride (79 µL, 0.90 mmol) at -75 °C in CH$_2$Cl$_2$ (2.0 mL). The resulting solution was stirred at -75 °C for 20 min and 77 (75 mg, 0.20 mmol) in CH$_2$Cl$_2$ (1.0 mL) was then added dropwise and the mixture was stirred for 1 hr at -75 °C. Et$_3$N (400 µL, 2.870 mmol) was added and the mixture was stirred while the reaction was allowed to warm to room temperature over 20 min. After removal of the solvent, the residue was dissolved in a minimum of CH$_2$Cl$_2$. This solution was loaded onto a 15 mm column with 6 inches of silica gel that was eluted with 20% EtOAc/hexanes. It was purified further by HPLC using 15% EtOAc/hexanes to obtain 6e (62 mg, 83%) as a yellow oil. **Note:** the
yield of this reacton varied unlike the yield obtained in preparing the aldehydes 6a-d. It may be advisable to use a milder oxidation procedure, perhaps (CF₃CO)₂O⁵⁵ in place of (COCl)₂: [α]²³_D 59.3° (c 2.55, CHCl₃); ^1H NMR (300 MHz, CDCl₃) δ 9.58 (d, 1H, J = 2.3 Hz), 4.63-4.68 (m, 1H), 4.10 (dd, 1H, J = 2.3, 4.9 Hz), 3.86 (dd, 1H, J = 6.2, 10.1Hz), 3.69 (dd, 1H, J = 6.0, 10.2 Hz), 2.13-2.22 (m, 1H), 1.84-1.91 (m, 1H), 0.88 (s, 9H), 0.82 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H), 0.00 (s, 3H); ^13C NMR (75 MHz, CDCl₃) δ 202.3, 87.0, 81.0, 75.2, 66.0, 37.8, 25.9 (3C), 25.6 (3C), 18.4, 17.9, -4.8, -5.2, -5.3, -5.4; HRMS calcd for C₁₇H₃₇O₃Si₂ (M-CHO) 345.2281, found 345.2288; TLC Rf = 0.19 (50% EtOAc/hexanes).

Wittig reaction of aldehyde 6e with ylide 58:

Ketophosphonium ylide 58 (85 mg, 0.26 mmol) and aldehyde 6e (55 mg, 0.15 mmol) in toluene (3 mL) were heated at 110 °C for 3 h. Solvent was then removed in vacuo and the residue was loaded onto a 15 mm silica gel column that was eluted with 20% EtOAc/hexanes to obtain enone 78 as a pale yellow oil (55 mg, 57%): [α]²³_D 4.71° (c 1.49, CHCl₃); ^1H NMR (300 MHz, CDCl₃) δ 7.23-7.37 (5H), 7.18 (d, 2H, J = 8.6 Hz), 6.82 (d, 2H, J = 8.6 Hz), 6.75 (dd, 1H, J = 5.6, 16.1 Hz), 6.28 (d, 1H, J = 16.1 Hz), 4.50-4.63 (2H), 4.44 (d, 1H, J = 11.4 Hz), 4.32 (d, 2H, J = 4.1
Hz), 4.20 (d, 1H, J = 11.5 Hz), 4.00-4.14 (2H), 3.86-3.94 (1H), 3.78 (s, 3H), 3.71-3.77 (1H), 3.51-3.67 (2H), 3.31-3.49 (2H), 2.98 (dd, 1H, J = 3.0, 16.0 Hz), 2.50-2.64 (m, 1H), 2.39 (dd, 1H, J = 9.9, 16.1 Hz), 2.00-2.23 (2H), 1.86-1.97 (m, 1H), 1.71-1.95 (m, 1H), 0.71-0.94 (21H), 0.04 (s, 6H), 0.00 (s, 3H), -0.04 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 199.7, 159.0, 142.3, 138.4, 131.0, 130.1, 129.3 (2C), 128.3 (2C), 127.7 (2C), 127.5, 113.6 (2C), 87.0, 82.8, 79.2, 77.3, 74.3, 73.2, 70.12, 66.4, 55.2, 44.8, 38.0, 34.2, 29.2, 25.9, 25.6 (3C), 25.5 (3C), 18.4, 17.9, 16.3, -4.8, -5.1, -5.2, -5.3; HRMS calcd for C$_{43}$H$_{68}$O$_8$Si$_2$ 768.4452, found 762.4408; TLC R$_f$ = 0.24 (30% EtOAc/hexanes).

Conjugate addition to enone 78:

MeLi (167 µL of 1.4 M in diethyl ether, 0.23 mmol) was added to a suspension of azeotropically dried (with toluene) CuCN (10.5 mg, 0.117 mmol) in THF (300 µL) at -70 °C. The mixture was allowed to warm to -5 °C over 30 min at wherupon it became light tan. It was cooled back to -70 °C and TMSCl (60 µL 0.472 mmol) followed by enone 78 (18 mg, 0.023 mmol) in THF (300 mL) were added. The solution was allowed to warm to -45 °C over 1 h 45 min. It was then cooled back to -70 °C, then saturated NH$_4$Cl solution (230 µL) was added, and the resulting
mixture was allowed to warm to room temperature over 30 min. The mixture was then diluted with ether (20 mL) and washed with H$_2$O (5 mL) followed by brine (5 mL). The organic layer was dried with MgSO$_4$, filtered, and concentrated. The residue was purified on a 15 mm silica gel column with 6 inches of gel using 20% EtOAc/hexanes as eluant to obtain 79 as a clear oil (7.5 mg, 75%); $[\alpha]^{23}_D$ 13.4$^\circ$ (c 1.05, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.25-7.31 (5 H), 7.17 (d, 2 H, J = 8.6 Hz), 6.82 (d, 2 H, J = 8.6 Hz), 4.56 (d, 1 H, J = 12.2 Hz), 4.49 (d, 1 H, J = 12.2 Hz), 4.44 (d, 1 H, J = 11.5 Hz), 4.19 (d, 1 H, J = 11.5 Hz), 4.16-4.23 (1 H), 4.06-4.10 (m, 1 H), 3.87-3.94 (m, 1 H), 3.78 (s, 3 H), 3.70 (dd, 1 H, J = 6.3, 10.0 Hz), 3.40-3.60 (4 H), 3.27-3.36 (2 H), 2.82 (t, 1 H), 2.77 (t, 1 H), 2.50-2.72 (m, 1 H), 2.35-2.50 (m, 1 H), 2.18-2.28 (2 H), 2.00-2.25 (2 H), 1.90 (m, 1 H), 1.73 (m, 1 H), 0.87 (s, 9 H), 0.85 (s, 9 H), 0.80 (d, 6 H, J = 6.5 Hz), 0.05 (s, 3 H), 0.04 (s, 3 H), 0.01 (s, 6 H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 210.4, 159.0, 138.4, 130.2, 129.2 (2 C), 128.3 (2 C), 127.8 (2 C), 127.5, 113.6 (2 C), 87.8, 87.2, 78.2, 73.3, 71.7, 70.2, 66.77, 55.2, 47.6, 38.2, 34.2, 28.9, 25.9 (3 C), 25.8 (3 C), 18.4, 18.0, 16.5, -4.1, -5.2, -5.3 (2 C); HRMS calcd for C$_{44}$H$_{72}$O$_8$Si$_2$ 784.4765, found 784.4645; TLC $R_f$ = 0.54 (30% EtOAc/hexanes).

**Spiroketal 80:**

\[
\text{DDQ (4.5 mg, 0.019 mmol) was added to 79 (11.7 mg, 0.015 mmol) in CH}_2\text{Cl}_2/H_2O (18/1, v/v, 50 \mu L). The reaction was stirred for 10 min at 5} \]
°C then 50 min at room temperature. It was then passed through a plug of silica gel eluted with EtOAc (20 mL). The residue was taken up in THF (500 μL), Bu4NF (30 μL, 1.0 M in THF, 5% w/w H2O) was added, and the resulting mixture was stirred at room temperature for 10 min. Removal of the THF by evaporation with a stream of dry N2 followed by filtration through a 1 inch plug of silica gel in a pipette eluted with EtOAc (20 mL) gave a yellow oil. This oil was dissolved in THF (500 μL) and 1% HCl (50 μL) was added and the mixture was stirred for 10 min. EtOAc (20 mL) was then added and the mixture was washed with H2O (2 mL) followed by brine (2 mL). Drying of the organic solution with MgSO4 and filtration followed by HPLC with 70% EtOAc/Hexanes gave the unsymmetrical spiroketal 80 (2 mg, 32%).

Note: Each of the intermediate compounds was purified by HPLC.

When ceric ammonium nitrate was used for this reaction, spiroketal 80 was obtained in a single step from 79, but the yield was much lower (~20%): [α]23D -53.6° (c 0.46, CHCl3); 1H NMR (300 MHz, CDCl3) δ 7.25-7.36 (5H), 4.56 (s, 2H), 4.21 (m, 2H), 3.99 (m, 2H), 3.75 (d, 1H, J = 12.2 Hz), 3.45-3.62 (4H), 3.39 (dd, 1H, J = 5.4, 9.6 Hz), 2.83 (br s, 1H), 2.10-2.33 (4H), 1.87 (dd, 1H, J = 3.2, 14.2 Hz), 1.73 (dd, 1H, J = 3.3, 13.7 Hz), 1.40 (dd, 2H, J = 13.2 Hz), 1.28 (dd, 1H, J = 4.9, 13.1 Hz), 1.14 (dd, 1H, J = 4.5, 13.2 Hz), 1.00 (d, 3H, J = 7.0 Hz), 0.99 (d, 3H, J = 6.8 Hz); 13C NMR (75 MHz, CDCl3) δ 138.5, 128.3 (2C), 127.7 (2C), 127.5, 97.2, 79.9, 77.2, 73.3, 73.1, 72.1, 72.1, 71.9, 71.9, 65.0, 37.0, 36.8, 35.9, 34.7, 25.7, 25.6, 17.9; HRMS calcd. for C24H34O6 418.2355, found 418.2329; TLC Rf = 0.37 (100% EtOAc).
REFERENCES AND NOTES


6. (a) Koerner, Jr., T. A. W.; Voll, R. J.; Younathan, E. S. Carbohydrate Res. 1977, 59, 403. (b) These reactions were first done by former postdoctoral research associate Dr. V.T. Ravikumar.


10. A similar result has also been reported recently. Corey, E. J.; Jones, G. B. J. Org. Chem. 1992, 57, 1028-1029.


16. This reaction was not attempted because this substrate was not used to synthesize compound 74 or 80.


19. These reactions were done exclusively by former postdoctoral research associate Dr. V. T. Ravikumar.


54. When a solvent gradient was used for silica gel column chromatography, the eluants were closely monitored by TLC analysis. When a particular spot from the reaction mixture TLC had been eluted, the elution solvent was then changed to the next more polar solvent system.

PART II. AN IMMUNOASSAY FOR PROTEIN-BOUND LEVUGLANDIN-DERIVED PYRROLES
INTRODUCTION

I. Background. Oxidative metabolites of essential fatty acids derived from arachidonic acid (AA) in the cyclooxygenase pathway are known to mediate many cellular activities. One branch of this pathway involves production of prostaglandins, \textsuperscript{56} thromboxanes, and prostacyclins, through a prostaglandin endoperoxide intermediate \textsuperscript{93} (PGH\textsubscript{2}). During studies on the chemistry of the strained dialkyl peroxide nucleus of the PG endoperoxides, Salomon et al.\textsuperscript{57} hypothesized that additional rearrangement products should be formed. Recently, \textsuperscript{1}H NMR evidence was obtained showing that \textgamma-ketoaldehydes are produced upon solvent induced rearrangement of PGH\textsubscript{2} (93) in DMSO-d\textsubscript{6} at 37 °C\textsuperscript{58} (Scheme XXV). These compounds were named

Scheme XXV
levuglandins (LGs) based on their relationships to PGE$_2$ (94) and PGD$_2$ (95) by hypothetical aldol condensations.

The formation of LGs was demonstrated during the in vitro biosynthesis of PGs from AA in the presence of ram seminal vesicle microsomes.$^{59}$ The LGs produced are, LGE$_2$ (96, a 10,11-seco PG) and LGD$_2$ (97, a 9,10-seco PG). The structures of these molecules were confirmed by a total synthesis$^5$ which provided ample supplies for biological and chemical studies. These vinylogous β-hydroxyl carbonyl compounds readily eliminate water giving rise to the anhydro forms, AnLGE$_2$ (99) and AnLGD$_2$ (100).$^5$ The kinetics of solvent and buffer-induced decompositon of LGE$_2$ was studied in detail.$^{60}$ These LGs can also rearrange to conjugated isomers, e. g. $\Delta^9$-LGE$_2$ (98).

**II. Studies on LGs.** Biological studies revealed that LGs exert potent effects on the contractile activity of uterine muscle in rats.$^{61}$ The AnLGs appear to be specific antagonists of PGs. It was also shown that covalent binding of LGE$_2$ with protein (tubulin) inhibits microtubule assembly and therefore mitosis in sea urchin eggs.$^{62}$ LGE$_2$ (96) also caused damage to the blood-brain barrier when injected into rats.$^{60}$

No method was available for detecting or quantifying LGs formed *in vivo*. Covalent adduct formation between proteins,$^{63}$ peptides,$^{64}$ or DNA,$^{65}$ and unidentified electrophilic products from PGH$_2$ (93) is widely documented. The difficulty of characterizing the molecular structures of the adducts with proteins is complicated by the fact that crosslinking of the proteins occurs. *Dr. Salomon postulated that the unidentified electrophilic products from PGH$_2$ are LGs.* Further
studies revealed that LGE2 (96) binds covalently with proteins resulting in intermolecular crosslinking (Scheme XXVI). It is these reactions that will complicate detection and quantification of free LGs generated in vivo. In this study, glycine was found to bind to the adduct formed between LGE2 (96) and ovalbumin (OA). Over time, the ability of the LGE2-OA adduct to bind glycine decreased. This can be explained by a dihydroxypyrrolidine (101, DHP) intermediate formed from LGE2 and the ε-amino group of a lysyl residue in OA. Reaction of DHP (101) with a second lysyl residue of OA would give the crosslink product 103 whereas loss of two molecules of water would give pyrrole
102. Of the products formed, protein-bound LG-derived pyrroles have stereoisomerically well defined structures and no tendency to react further with nucleophiles owing to low electrophilicity. Therefore, our efforts were directed towards detection of these LG$E_2$-derived pyrroles.

In order to be able to detect the tiny amounts of these compounds found in vivo, a particularly sensitive method would be necessary. Immunoassay is a mild method for detecting these protein-bound LG$E_2$-derived pyrroles that would not only allow quantitative determination of the levels of adducts but also their localization in tissue. For an immunoassay, it would be necessary to raise antibodies against a pyrrole-derived antigen that could be used for detecting pyrroles in tissues and blood.

Model studies in our laboratories$^{60}$ found that various pyrroles could be synthesized from Paal-Knorr condensation of primary amino groups$^{67}$ with LG$E_2$. Unfortunately, these pyrroles were unstable and began decomposing immediately on standing at room temperature. Storage of the pyrroles in either EtOH or CD$_3$CN at low temperature (-80 °C) prolonged their shelf life for only a short time as an apparent polymeric precipitate formed. It therefore appeared unlikely that these types of compounds would be suitable as antigens for raising antibodies. The instability of the pyrroles is presumably caused by the electron-rich nature of the ring.

To overcome the obstacle of pyrrole instability, a short and efficient synthesis of a pyrrole isostere was developed. Replacement of the ring CH with N would give a pyrazole compound that was expected
to be relatively electron deficient, less prone to oxidation, and therefore more stable than the pyrrole. Pyrazole isostere 105 was synthesized with an aldehyde tether to allow coupling with a carrier protein by reductive alkylation. Poly-L-lysine, used initially as the carrier protein, was reacted with aldehyde 105. Antigen 106 was obtained and used in an attempt to raise antibodies.

The second part of this thesis will describe the development of an immunoassay using an antibody raised against a pyrazole isostere of LGE2-derived protein-bound pyrroles, purification of the antibody, and determination of the crossreactivity of various compounds with the antibody.
RESULTS AND DISCUSSION

I. Polyclonal antibodies. A) Attempts to raise antibodies using a poly-L-lysine-pyrazole antigen. As mentioned in the introduction to this section, the poly-L-lysine-pyrazole isostere antigen 106 was used in an attempt to raise antibodies. Three New Zealand white rabbits (pasteurella free) were used as the subjects. Initial immunizations consisted of an equal volume of antigen dissolved in pH 7.4 phosphate buffered saline solution (PBS)\textsuperscript{70} and Freund's complete adjuvant, a commercially available reagent consisting of paraffin oils and mycobacteria. The adjuvant was used to form an emulsion and thus achieve a time released effect of the antigen. The antigen and adjuvant were both measured into a small plastic Eppendorf tube and vortexed to give an oily, white emulsion. The area injected was several sites on the animals back intradermally (between the epidermis and dermis). Later, both the back and back leg muscle (intramuscular) were injected. Booster shots were done with an equal volume of the antigen solution and Freund's incomplete adjuvant. Injections and blood collection were done simultaneously at 4 week intervals. The crude serum was stored at -80 °C in order to prevent enzymes that may be present from affecting the antibody. Specific details of these procedures are given in the Experimental section.

After several months of booster shots with the poly-L-lysine-pyrazole antigen, the immunogenic response was still very low as evidenced by ELISA. The level of antibody titre had increased slightly after intramuscular injections were initiated, however, it again reached
a plateau. Before describing experiments to determine the efficacy of the immunization procedures, a background of the ELISA procedure will be given.

**ELISA Background:** The plastic plates used for these immunoassays have 96 sample wells (400 µL volume) in an 8 row, 12 column array. In the initial stage, the bottoms of the wells are coated with a bovine serum albumin-pyrazole conjugate (1:6.6 molar ratio). The rest of the well is then coated with a blocking agent (1% chicken egg ovalbumin in PBS). The coating agent provides the substrate for the antibody to bind with and the blocking agent prevents non-specific binding of the antibody to the walls of the plate wells. Blood serum (containing the antibody) is diluted to three concentrations with 0.2% chicken egg ovalbumin in PBS (1:100, 1:1,000, and 1:10,000) and loaded into the wells. The next stage involves the use of Goat anti-rabbit IgG alkaline phosphatase conjugate which binds to the antibody. Thus, a chain is formed with coating agent, followed by antibody, and anti-rabbit IgG. For development of the plate, p-nitrophenyl phosphate tablets are dissolved in a pH 9.6 solution containing glycine (50 mM) and MgCl₂ (1 mM). This solution contains the substrate for the enzyme, alkaline phosphatase, that causes the formation of an anion that is yellow. The intensity of the color is proportional to the amount of the conjugates which bind to the well. The absorbance of these solutions is measured at 450 nm using a Bio Rad microplate reader.

As mentioned above, the level of antibody titre from the poly-L-lysine-pyrazole antigen was very low even at the 1:100 dilution. To
determine the efficacy of the immunization procedures, a modification of the ELISA protocol was done. A poly-L-lysine solution was substituted for the BSA-pyrazole conjugate as the coating agent. The reason for this change is that the rabbit will produce antibodies when injected with poly-L-lysine alone. Therefore, if our immunization procedures were effective, we should be able to detect the antibodies to the protein. When the assay was carried out with poly-L-lysine as the coating agent, the titre was still very low. These findings indicated the need for a different immunization procedure and/or a different carrier protein.

B) Antibodies raised against a KLH-pyrazole antigen. The use of poly-L-lysine as the carrier protein was based on the precedent of an antibody preparation reported by Monnier et al.\textsuperscript{72} Another protein expected to evoke high immunogenic response is keyhole limpet hemocyanin (KLH).\textsuperscript{73} Our efforts were, therefore, focused on preparing an antigen in which KLH was reductively alkylated with pyrazole isostere 105. One obstacle to accomplishing this goal was the problem of determining the amount of hapten (pyrazole) which was incorporated onto the protein and the low solubility of KLH. KLH has a molecular weight of 3-5 million Daltons and its structure has not been totally elucidated. The poly-L-lysine antigen had a fairly simple $^1$H NMR spectrum in which the alkylated versus unalkylated amino residue ratio could be determined from the integral. KLH has a structure that is much too complex for NMR determination of the level of hapten incorporation. To overcome this problem, a radiolabel was incorporated
at C12 (LG numbering system) in the pyrazole (Scheme XXVII). This method allows the use of a liquid scintillation (LS) counter for determining the amount of hapten that had bound to the protein after dialyzing away unreacted 109 and other reagents.

To obtain high incorporation of hapten by reductive alkylation, it was important to use solutions of KLH that had been solubilized in PBS. Since any organic solvents tended to cause precipitation of KLH, the alkylation was run in PBS with the minimum of MeOH to dissolve the LG-E2. When solubilized KLH, pyrazole 109, and NaCNBH3 were stirred for 16 h at room temperature, antigen 110 was obtained. The μmol ratio of hapten 109 to lysyl residues in KLH was 0.75/1.0 as determined by LS counting (after dialysis in pH 7.4 PBS).

Scheme XXVII

Three young, white, New Zealand male rabbits were obtained for immunizations with antigen 110. The rabbits were injected intradermally in several locations on their backs as well as in the back leg muscle. The schedule for injection and blood collection was changed
from that used with the poly-L-lysine-pyrazole antigen 106. Thus, injections were done every 21 days instead of 28 and blood was collected 10 days after each injection rather than concurrently with the next injection. The reason for collecting blood much sooner was that the peak immunogenic response period is expected to occur 7-14 days after injection. The injection emulsion was also prepared in a different manner. Two separate glass syringes were used to measure the desired volume of adjuvant and antigen solutions (polypropylene syringes will seize if used with adjuvant). The syringes were then connected by a three-way valve and by alternation of plunger depressions, a homogenous, white, turbid emulsion was obtained. This process was much more efficient in generating an emulsion than vortexing the adjuvant and antigen together.

![Graph](image)

**Figure 3.** KLH-pyrazole antibody titre of the 1:10,000 blood serum dilution as determined by ELISA. The plate was coated with BSA-pyrazole conjugate. Absorbance values were measured 20 min after addition of the p-nitrophenol phosphate solution.
With antigen 106, a very strong immunogenic response was seen in all three rabbits almost immediately (figure 3). Rabbits 1 and 3 had especially high titre levels even at the 1:10,000 (10^{-4}) dilution. Booster injections and blood collection were continued until the level of antibody titre remained fairly constant. Once this occurred, the rabbits were exsanguinated (anesthetized and blood was collected from the heart).

II. Crossreactivity of a BSA-LGE2 adduct with the KLH-pyrazole antibody. Having succeeded in raising an antibody against the KLH-pyrazole isostere antigen, we began preliminary studies to determine if the antibody could recognize an LGE2-derived pyrrole. For these experiments, the crude antibody serum was used. The procedure for conducting a crossreactivity assay is slightly modified from that used for monitoring the level of antibody titre. The coating, blocking, anti-rabbit IgG, and development steps are the same as those described on p.114. The sample preparation differs in that various concentrations of inhibitor solutions are prepared and then preincubated with the antibody serum prior to loading on the plate. If the antibody recognizes these inhibitors, it will bind to them and be washed away when the plate is rinsed prior to addition of anti-rabbit IgG. The net effect should be decreased absorbance values for the sample wells where the inhibitor has successfully competed for binding to the antibody.

A) ELISA 1. At the time these studies were initiated, the highest level of antibody titre corresponded to serum collected from all three rabbits after 93 days. This serum was used for all ensuing studies. The first cross reactivity experiment involved incubation of a
PBS solution (1.0 mL) containing BSA (0.1 mM) and LGE2 (0.1 mM) for 30 min at 37 °C. This solution was then serially diluted by a factor of 0.5 and then incubated with an equal volume of antibody serum from each rabbit. For rabbits 1 and 3, a final concentration of 1:5,000 serum was used (since it was diluted with an equal volume of BSA-LGE2 solution) and for rabbit 2, a final concentration of 1:1,667 serum was used in an attempt to match the antibody titre of rabbit 1 (refer to figure 3). All of these solutions were preincubated at 37 °C for 1 h before being loaded into the appropriate sample wells. The absorbance values measured (y-axis) are plotted against log of inhibitor concentration, that was taken to be the concentration of bound LGE2 contained in the BSA-LGE2 adduct, (x-axis) in figures 4-1, 4-2, and 4-3 corresponding to the data for rabbits 1, 2, and 3 respectively. These figures show that at higher concentrations of the BSA-LGE2 solutions, the absorbance values are significantly lower. The IC50 values were 295, 289, and 529 pmol/well for rabbits 1, 2, and 3 respectively.

These values are only a rough estimate, because more data points at both ends of the dilution range are required to insure that minima and maxima have been reached. Nevertheless, these preliminary results indicate that an adduct derived from a protein and LGE2 (presumably a pyrrole) crossreacted with the pyrazole isostere antibody. However, there are other variables to be considered. One variable is the possibility of inhibition of antibody binding to the plate by the protein (BSA) itself. The effect of the protein can be determined by performing a serial dilution using a solution containing only protein.
Figure 4-1

Absorbance

Figure 4-2

Absorbance

Inhibitor (pmol/well)

Inhibitor (pmol/well)
Figures 4-1, 4-2, and 4-3. ELISA inhibition curves for BSA-LGE2 adduct tested with rabbit KLH-pyrazole antibody. The curve represents the ability of the adduct to inhibit antibody binding to immobilized BSA-pyrazole conjugate. A 0.1 mM BSA-LGE2 solution was serially diluted by a factor of 0.5 prior to being incubated with 1:10,000 antibody serum at 37 °C for 1h. The IC50 values were 295, 289, and 529 pmol/well for rabbits 1, 2, and 3 respectively. The ELISA plate was developed for 20 min after addition of the p-nitrophenyl phosphate solution.

(BSA) followed by preincubation with the antibody serum. Another factor that may cause inhibition of antibody binding is that free LGE2, that failed to react with BSA may react with the antibody. This possibility can be eliminated by dialyzing the BSA-LGE2 reaction product mixture prior to conducting the assay. In this way, any unreacted LGE2 will be removed from the sample leaving only the protein and LGE2 that is covalently, tightly bound to it.
B) ELISA 2. An ELISA was run using the antibody serum from rabbits 1 and 3. Once again a PBS solution (1.0 mL) containing BSA (0.1 mM) and LGE2 (0.1 mM) was incubated at 37 °C for 30 min. However, this time the product mixture was then dialyzed against pH 7.4 PBS (2 x 200 mL) for 24 h. The buffer solution was replaced after 8 h. This dialyzed protein adduct solution was then serially diluted by a factor of 0.2. A solution of BSA (1.0 mL, 0.1 mM) was also serially diluted in the same manner. These dilutions are in greater increments than those of the first crossreactivity plate. This was done in order to have a wider concentration range for the inhibitor. The rest of the assay was conducted in the same manner as ELISA 1.

The absorbance data is plotted in figure 5. The BSA solutions showed no crossreactivity as evidenced by fairly constant absorbance values throughout the dilution range. In contrast, the dialyzed BSA-LGE2 adducts (pyrrole) again showed significant inhibition of antibody binding thereby confirming that the crossreactivity is a result of the adduct and not unreacted LGE2 or BSA. The IC50 values were 677 and 420 pmol/well for rabbits 1 and 3 respectively.

Having shown that a protein-LGE2 adduct (pyrrole) is being recognized by the antibody raised against the KLH-pyrazole antigen, we wanted to test a hypothesis regarding catalysis of pyrrole formation. DHP (101, Scheme XXVII, p. 116) is a proposed intermediate in the reaction of proteins with LGE2. We felt that acid treatment might facilitate the dehydration of DHP (101) thereby increasing the amount of pyrrole formed. It would still be necessary to dialyze the reaction
**Figure 5.** ELISA inhibition curves for BSA only and BSA-LGE\textsubscript{2} adduct tested with rabbit KLH-pyrazole antibody. 0.1 mM BSA-LGE\textsubscript{2} and 0.1 mM BSA solutions were serially diluted by a factor of 0.2 prior to incubation with the antibody serum. Other conditions were the same as in figures 4-1 - 4-3. IC\textsubscript{50} values for the BSA-LGE\textsubscript{2} adduct were 677 and 420 pmol/well for rabbits 1 and 3 respectively. The BSA only solutions had no measurable inhibition effect.

product mixtures from BSA-LGE\textsubscript{2} even after acid treatment to insure that free LGE\textsubscript{2} would not be present at potentially affect the antibody binding.

**C) ELISA 3.** For the assay to determine the effect of acid on pyrrole formation, only the antibody serum from rabbit 1 was used. The concentration of coating agent was reduced to increase the sensitivity of the assay. Thus, it will take less inhibitor to prevent binding of the antibody to the plate because the competitive efficiency of
the coating agent was decreased relative to that of the inhibitor. For this plate, two PBS solutions (1.0 mL each) containing BSA (0.1 mM) and LGE2 (0.1 mM) were incubated at 37 °C for 30 min. One of these solutions was dialyzed without further treatment (vide infra). The other BSA-LGE2 solution was treated with 12% trichloroacetic acid (TCAA) (1.0 mL) causing the formation of a white precipitate. The resulting suspension was centrifuged (3000 rpm) for 30 min at 5 °C. A white pellet and a clear supernatant fluid formed. The supernatant was carefully removed and the pellet was dissolved in 0.05 N NaOH and 0.2 M phosphate buffer in a ratio that the resulted in pH ~7.4. A solution of BSA (1.0 mL, 0.1 mM) was also treated with TCAA in the same manner to serve as a control for any affect the acid might have on the BSA. These three solutions (BSA-LGE2, BSA-LGE2-TCAA, BSA-TCAA) were then dialyzed against pH 7.4 PBS (2 x 200 mL) for 24 h. The buffer solution was replaced after the first 6 h. The dialyzed solutions along with a fourth solution containing untreated BSA (1.0 mL, 0.1 mM) were then serially diluted by a factor of 0.3. The rest of this assay was done as described above for ELISA 1.

The results are plotted in figure 6. The solutions do not all have the same maximum absorbance values owing to the fact that their volumes were slightly different after dialysis. The BSA and the BSA-TCAA solutions had no effect on antibody binding as evidenced by the essentially constant absorbance values. The BSA-LGE2 and BSA-LGE2-TCAA solutions had a significant effect on antibody binding as expected from the previous assays. That the TCAA had no effect on
pyrrole formation is evidenced by nearly identical IC₅₀ values of 609 and 548 pmol/well for the BSA-LGE₂ and BSA-LGE₂-TCAA solutions respectively.

![Graph showing absorbance vs. inhibitor (pmol/well)](image)

**Figure 6.** ELISA inhibition curves for BSA, BSA treated with trichloroacetic acid (TCAA), BSA-LGE₂, and BSA-LGE₂ treated with TCAA. Solutions treated with TCAA were dialyzed for 24 h against pH 7.4 PBS buffer (200 mL). All solutions were initially 0.1 mM and were diluted by a factor of 0.3 prior to incubation with the antibody serum. Other conditions were the same as in figures 4-1 - 4-3. IC₅₀ values were 610 and 548 pmol/well for the BSA-LGE₂ and BSA-LGE₂-TCAA solutions respectively. The BSA and BSA-TCAA solutions had no measurable inhibition effect.

**III. Antibody purification.** The assays described above were conducted with unpurified antibody serum, i.e. centrifuged serum from whole blood. To confirm these results, the antibody had to be purified. Thus, the crude serum was delipidated with a commercial reagent followed by column chromatography using a protein-A sepharose
column. This procedure does not isolate the antibody specifically but rather separates out the IgG (secondary response) class of antibodies. The purified antibody was dialyzed against PBS containing NaN₃ (0.02%) at 5°C and then stored at that temperature. The sodium azide is present as an antibacterial agent. Storage of the purified antibody at lower temperature may result in the formation of precipitates.

Since the concentration of the antibody solution had been altered by the purification procedures, it was necessary to restandardize the immunoassay conditions. The sensitivity of the assays could be increased since possible inhibitors present from the crude serum itself, e.g. enzymes and lipids were removed. Therefore, the amount of coating agent was reduced and the dilution of the antibody was increased. Manipulation of these two parameters allowed control of the time required for plate development. In the previous immunoassays high absorbance values, i.e. above 1, were being reached in less than 20 min. Optimal plate development time should be approximately 45 min and the absorbance values should be less than 1.69

A series of ELISA's was conducted to optimize the above mentioned parameters. The amount of coating agent was reduced to approximately one-sixth of the amount used above to determine antibody titre levels in crude antibody serum (see p. 143). Thus, 3.4 μL of the stock solution of BSA-pyrazole (1:6.6 molar ratio) was diluted with PBS (11 mL). For the antibody solution, 10 μL of the purified serum was diluted with 0.2% chicken egg ovalbumin (50 mL). This volume of solution was much more than required for 1 assay but the accuracy of
the experiment would be sacrificed if less than 10 μL of antibody solution was measured out. That the antibody supply is adequate is evidenced by the following; during each blood collection, approximately 20 mL of blood is collected that after centrifugation yields 7-8 mL of crude serum. When 1.5 mL of crude serum was purified, the volume was approximately 3 mL after dialysis. To run 1 ELISA plate, less than 10 μL of this dialyzed solution was needed which means more than 300 assays can be accomplished with only 1.5 mL of crude antibody serum! After exsanguination, 60 mL of serum were obtained from rabbit 1.

IV. Crossreactivity with pyrroles, prostaglandins, prostaglandin side chains, and protein-LGE₂ adducts. As discussed previously, the reason for developing this immunoassay was to use the KLH-pyrazole isostere antibody to detect LGE₂-derived protein-bound pyrroles. Preliminary studies described above had shown that this antibody could recognize an LGE₂-protein pyrrole. Having purified the antibody, we could now study crossreactivity with various compounds. For the assays that will be described subsequently, a 0.1 mM solution of pyrazole 111 was used as a reference standard to compare the cross reactivities of various compounds. We expected this free (i.e. not protein-bound) pyrazole to inhibit antibody binding more than other compounds to be tested because it was the hapten used for raising the polyclonal antibodies.

A) Prostaglandins. We discovered that LGs 96 and 97 are formed as rearrangement products from PGH₂ (Scheme XXV, p. 108). It follows then that owing to similarities in structure, i.e. the upper
and lower side chains, the prostaglandins could be expected to crossreact with our pyrazole isostere antibody in an ELISA. For this assay, 1.0 mM solutions of PGE\textsubscript{2} (94), PGB\textsubscript{2} (112), and PGF\textsubscript{2\alpha} (113), were serially diluted by a factor of 0.1 and then incubated with the purified antibody (10 \( \mu \text{L} \) in 50 \( \mu \text{L} \) of 0.2\% chicken egg ovalbumin). The rest of the assay was conducted as described in the antibody purification section.

![Chemical Structures](image)

The results are plotted in figure 7. As expected, pyrazole 111 had a much greater inhibitory effect than the three prostaglandins tested. Thus, 111 showed \( IC_{50} = 0.11 \) pmol/well while PGB\textsubscript{2} (112), which showed the strongest effect of the prostaglandins, exhibited \( IC_{50} = 38 \) pmol/well. This corresponds to a cross reactivity of 0.3\%. It is reasonable that PGB\textsubscript{2} (112) would have the highest inhibitory effect of the prostaglandins tested because its ring unsaturation gives it the structure most similar to that of pyrazole 111. PGE\textsubscript{2} and PGF\textsubscript{2\alpha} had \( IC_{50} \) values of 165 and 658 pmol/well respectively. These results correspond to crossreactivities of only 0.07\% and 0.02\% respectively.
Figure 7. ELISA inhibition curves for pyrazole 111, PGB₂, PGE₂, and PGF₂α. 0.1 mM pyrazole 111 and 1.0 mM PG solutions were serially diluted by a factor of 0.1 with PBS and incubated with an equal volume of antibody (purified) for 1 h at 37 °C. Coating agent concentration was reduced to 1/6 of that used in figures 3-6. Plate development time was ca. 45 min. IC₅₀ values: pyrazole 111, 0.11 pmol/well; PGB₂, 38 pmol/well, PGE₂, 165 pmol/well, and PGF₂α, 658 pmol/well.

Although the prostaglandins do exhibit some crossreactivity with the antibody, they can removed from the solution by dialysis since they do not form covalent adducts with proteins. This fact will be significant when testing biological samples, e.g. tissues or blood, for the presence of LG-derived protein-bound pyrroles.

**B) PG side chains and pyrroles.** Since the prostaglandins were recognized by the antibody, we wanted to determine to what extent the side chains were responsible for the crossreactivity. The next series of compounds tested were a lower side chain analog, chloride
analog, chloride \(114\), and an upper side chain analog, allylic alcohol \(115\). To provide a basis for the presumption that protein-bound LG-derived pyrrole would cross react with the pyrazole isostere antibodies, an LGE2-derived pyrrole from a simple amine was tested. Thus, neo-pentyl pyrrole \(116\), previously synthesized in our laboratories,\(^6\) was also tested on the same ELISA plate. 1.0 mM solutions of \(114, 115\)

![Chemical structures](image)

and \(116\), were prepared in PBS. These solutions were serially diluted by a factor of 0.1. A 0.1 mM solution of pyrazole \(111\) was also serially diluted in the same manner. The rest of the assay conditions were the same as those described for the prostaglandins.

The results are plotted in figure 8. The pyrazole isostere \(111\) showed IC\(_{50}\) = 0.18 pmol/well. Pyrrole \(116\) showed IC\(_{50}\) = 17 pmol/well which corresponds to a 1.1% crossreactivity. The actual value may be even higher as the exact concentration of the pyrrole solution may be lower than presumed owing to its instability. This result supports the presumption that antibodies raised against a pyrazole isostere can be exploited to detect LGE2-derived pyrroles. Upper side chain analog \(114\) showed IC\(_{50}\) = 6137 pmol/well which is a negligible crossreactivity (0.003%). The lower side chain analog \(115\) gave a similar result, an IC\(_{50}\) = 4579 pmol/well and a crossreactivity of 0.004%. That the side
chains are not solely responsible for crossreactivity is demonstrated by these results.

![Graph](image)

**Figure 8.** ELISA inhibition curves for pyrazole 111, lower PG side chain analog 114, upper PG side chain analog 115, and pyrrole 116. 0.1 mM pyrazole 111 and 1.0 mM side chain and pyrrole solutions were serially diluted by a factor of 0.1 in PBS. Other conditions were the same as described in figure 7. IC50 values: pyrazole 111, 0.18 pmol/well; upper side chain analog 114, 6137 pmol/well, lower side chain analog 115, 4579 pmol/well, and pyrrole 116, 17 pmol/well.

The neo-pentyl pyrrole 116 has the same side chains as pyrazole 111 but it lacks the 6 carbon chain used as a tether in coupling the pyrazole 111 to the carrier protein. Therefore, we felt that if a 6 carbon chain were incorporated during the Paal Knorr condensation of a primary amine and LGE2 (96), the resulting pyrrole might show increased inhibition. Hexanol pyrrole 117 was, therefore, synthesized by reacting 6-amino-1-hexanol with LGE2 (96) in 100% ethanol.
A 1.0 mM solution of pyrrole 117 in PBS and a 0.1 mM solution of pyrazole 111 were serially diluted by a factor of 0.1 and incubated with the antibody at 37 °C for 1 h. The ELISA results are plotted in figure 9.

**Figure 9.** ELISA inhibition curves for pyrazole 111 and hexanol pyrrole 117. 0.1 mM pyrazole 111 and a 1.0 mM pyrrole 117 solution were serially diluted by a factor of 0.1 in PBS. Other conditions were the same as described in figure 7. IC₅₀ values: pyrazole 111, 0.32 pmol/well and pyrrole 117, 15 pmol/well.

Pyrrole 117 had 2.1% crossreactivity based on an IC₅₀ = 15 pmol/well since the pyrazole isostere standard 111 exhibited an IC₅₀ = 0.32 pmol/well on this plate. This is a minimum for the actual crossreactivity owing to the instability of the pyrrole. However, a value
slightly higher than the crossreactivity of pyrrole 116 (1.1%) as reasonable owing to the closer similarity of structure between pyrrole 117 and the pyrazole isostere 111 compared to the pyrrole 116.

Having established that LGE2-derived pyrroles crossreact with the pyrazole isostere antibody, several other pyrroles corresponding to protein-derived pyrroles that might be present in biological samples were synthesized and tested. Condensation of γ-ketomononal 118 with ε-aminocaproic acid or glycine gave pyrroles 119 and 120 respectively.

\[
\begin{align*}
118 & \quad \text{CHO} \\
119 & \quad \text{R=(CH}_2\text{)}_5\text{COOH} \\
120 & \quad \text{R=CH}_2\text{COOH}
\end{align*}
\]

Caproyl pyrraline 121 obtained from Dr. Monnier's laboratories as a dull yellow solid and used as received. 1.0 M PBS solutions of pyrroles 119-121 and a 0.1 M solution of pyrazole 111 were prepared and serially diluted by a factor of 0.1. The results are plotted in figure 10. None of these pyrroles showed any significant crossreactivity as evidenced by IC₅₀ values of 9428, 4990, and 1265 pmol/well for 119, 120, and 121 respectively while the pyrazole standard 111 exhibited an IC₅₀ = 0.18 pmol/well. These results along with the results from the PG side chain analogs indicate that the molecular structure of the inhibitor must be very similar to that of the hapten to be recognized by the antibody.
Figure 9. ELISA inhibition curves for pyrazole 111 and hexanol pyrrole 117. 0.1 mM pyrazole 111 and a 1.0 mM pyrrole 117 solution were serially diluted by a factor of 0.1 in PBS. Other conditions were the same as described in figure 7. IC$_{50}$ values: pyrazole 111, 0.32 pmol/well and pyrrole 117, 15 pmol/well.

A final cross reactivity experiment was run to test the effect of arachidonic acid (AA, 122), the biological precursor to the PGs and LGs, on antibody binding. BSA-LGE$_2$ and HSA-LGE$_2$ adducts were also tested in the same ELISA. A 1.0 mM solution of AA (122) was serially diluted by a factor of 0.1. PBS solutions (1.0 mL) containing LGE$_2$ (0.1 mM) and BSA or HSA (0.1 mM) were incubated for 4 h at 37 °C and then dialyzed against PBS (2 x 250 mL) for 24 h. The buffer solution was replaced after the first 8 h. The dialyzed reaction mixtures and a
0.1 mM solution of pyrazole 111 were diluted by a factor of 0.1. The rest of the assay was conducted as mentioned above. The results are plotted in figure 11. AA (122) showed no cross reactivity as evidenced by IC\textsubscript{50}.

**Figure 11.** ELISA inhibition curves for pyrazole 111, AA (122), BSA-LGE\textsubscript{2}, and HSA-LGE\textsubscript{2} adducts. 0.1 mM pyrazole 111, and dialyzed BSA-LGE\textsubscript{2}, and HSA-LGE\textsubscript{2} solutions were serially diluted by a factor of 0.1 in PBS. A 1.0 mM solution of AA (122) was also serially diluted by a factor of 0.1. Other conditions were the same as described in figure 7. IC\textsubscript{50} values: pyrazole 111, 0.20 pmol/well, AA (122), 5776 pmol/well, BSA-LGE\textsubscript{2}, 31.8 pmol/well, and HSA-LGE\textsubscript{2}, 31.9 pmol/well.

= 5776 pmol/well while the pyrazole isostere standard 111 showed IC\textsubscript{50} = 0.20 pmol/well. The BSA-LGE\textsubscript{2} and HSA-LGE\textsubscript{2} adducts showed very similar results with IC\textsubscript{50}'s = 31.8 and 31.9 pmol/well respectively. Here, the concentration refers to BSA or HSA. The concentration of protein-bound LGE\textsubscript{2}-derived pyrrole is no greater than this. The actual pyrrole concentration is probably much less since the extent of LG
binding may be less than 100%. These values correspond to cross reactivities of 0.6% for both adducts. Thus, the results obtained from the BSA-LGE\(_2\) adducts tested with the crude antibody serum are supported by these ELISAs with purified antibody.
CONCLUSIONS

The results of all inhibition assays done with purified antibody are summarized in Table 3.

Table 3. Inhibition assay results.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration at 50% Inhibition of Binding (pmol/well)</th>
<th>Crossreactivity at 50% Inhibition of Binding (%)</th>
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<tr>
<td>R = HO(CH₂)₈, G = N (111)</td>
<td>0.11</td>
<td>100</td>
</tr>
<tr>
<td>R = HO(CH₂)₈, G = CH (117)</td>
<td>5.2</td>
<td>2.1</td>
</tr>
<tr>
<td>R = (H₃C)₃C, G = CH (116)</td>
<td>16.8</td>
<td>0.65</td>
</tr>
<tr>
<td>n = 1, R¹ = H, R² = C₅H₁₁ (120)</td>
<td>2889</td>
<td>0.0038</td>
</tr>
<tr>
<td>n = 5, R¹ = H, R² = C₅H₁₁ (119)</td>
<td>5458</td>
<td>0.002</td>
</tr>
<tr>
<td>n = 5, R¹ = CH₂OH, R² = CHO (121)</td>
<td>732</td>
<td>0.015</td>
</tr>
<tr>
<td>P = BSA</td>
<td>31.8*</td>
<td>0.06*</td>
</tr>
<tr>
<td>P = HSA</td>
<td>31.9*</td>
<td>0.06*</td>
</tr>
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<td>PGB₂</td>
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</tr>
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</tr>
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<tr>
<td>6137</td>
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</tr>
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</table>

*These values are the protein concentrations, and represent upper limits for protein bound pyrrole. Although protein and LGE₂ were incubated (1:1), the actual concentration of LGE₂-derived pyrrole is probably lower because the yield of pyrrole is not quantitative.

As discussed previously (pp. 127-135), the strongest inhibitors of antibody binding to the BSA-pyrazole coating agent (other than pyrazole 111) were pyrroles 116 and 117. Both of these pyrroles were
obtained from reaction of LGE2 and a primary amine. That dialyzed solutions of LGE2 adducts with BSA and HSA inhibit antibody binding is presumably owing to pyrrole formation.

The stability of a KLH-LGE2 adduct, presumably a pyrrole, has recently been established, as evidenced by constant IC50 values from ELISA, using the antibodies raised against the KLH-pyrazole antigen. This is in contrast to the observation of the instability of pyrroles derived from LGE2 and primary amines. The KLH-LGE2 antigen is being used to raise antibodies that might be more sensitive than the KLH-pyrazole derived antibodies to protein-bound LGs. These antibodies could then be used in an ELISA to test for the protein-bound LGs that may be involved in the tissue damage associated with the burst of fatty acid oxidative metabolism during oxygen reperfusion following the ischemia of heart attacks or stroke.
EXPERIMENTAL

General. Liquid scintillation counting (LSC) was done on a Beckman LS 5801 counter with quench curves made from a Beckman $^3$H standard set. Samples were prepared with 5 mL of xylene-based scintillation fluid (Fisher). Centrifugation was done on a Sorvall centrifuge at 5 °C and 2000 rpm unless otherwise noted. Absorbance values of ELISA's were measured on a Bio Rad Microplate Reader using dual wavelength (405 nm to read the plate and 650 nm as a reference).

Materials. The following materials were commercially available and used as received: chicken egg ovalbumin (grade V, 99%, Sigma); bovine serum albumin (fraction V, 96-99%, Sigma); keyhole limpet hemocyanin (ICN Biochemicals); human serum albumin (fraction V, Sigma); goat anti-rabbit alkaline phosphatase (Boehringer-Mannheim); p-nitrophenol phosphatase tablets (Sigma); sterilized ELISA plates and lids (Baxter). Phosphate buffered saline (PBS) was prepared from a stock solution containing 0.2 M NaPO$_4$, 3.0 M NaCl, and 0.02% NaN$_3$ (w/w). This solution was diluted 20x as needed.

I. Reductive alkylation of KLH with pyrazole 109:

A mixture of $^3$H labeled and unlabeled aldehydes 109 (4.4 mg, 0.010 mmol, specific activity = 0.092 mCi/mmol) in MeOH (400 μL) was added to KLH (10.7 mg, 700 μL of a solution containing 15.3 mg KLH/mL
The solution was stirred for 10 min and then NaCNBH₃ was added (5 mg, 0.080 mmol) and the reaction mixture was then stirred for 16 h. **Note:** A minimum of organic solvent must be used to avoid the precipitation of the KLH from solution. The entire reaction mixture was then placed in dialysis tubing (Mw cutoff = 12,000-14,000) and stirred in PBS (3 x 400 mL) for 48 h, replacing the PBS solution after 8 and 24 h. The dialyzed suspension was diluted to a total volume of 3 mL and two aliquots (30 μL) were counted. 40% (0.0040 mmol) of the starting aldehyde 109 had been incorporated into the protein as determined by a count of 3.72 x 10⁻⁴ mCi for the entire mixture.

**II. Immunization procedures.** Pasteurella-free, New Zealand white rabbits (Hazelton) were used as subjects for raising polyclonal antibodies. For the initial immunization of three rabbits, Freund's complete adjuvant (500 μL) and the KLH-pyrazole 110 solution (500 μL) were measured in separate glass syringes (plastic, disposable syringes will seize if used to mix the adjuvant and antigen) and connected by a three-way valve. By alternation of plunger depression for several minutes, the two solutions were mixed until a homogenous, white emulsion was formed.

The areas to be injected were shaved and scrubbed with a Povidone-iodine solution using a swab-stick applicator. The two areas shaved and scrubbed were one of the back legs (1 in²) and an area on either side of the spinal column (3 in²). 250 μL of the emulsion was taken up into a disposable syringe and the "meaty" part of the back leg muscle was injected with 125 μL of the emulsion. The remainder of the
emulsion was injected into several areas (4-5) on the back intradermally. For these injections, the syringe needle is inserted bevel up between the two layers of skin. The appearance of a white bump indicates a successful intradermal injection. For the intramuscular injection, the rabbit is restrained by placing one arm around the far side of the animal and pushing on the knee of the leg to be injected. This will cause the rabbit to extend the leg without having to pull on its foot. For the intradermal injections, the rabbit is restrained by being held at the front and rear shoulders while pressing down gently. This will also draw the skin tighter to allow for an easier injection.

Booster shots were given every 21 days using Freund’s incomplete adjuvant and the KLH-pyrazole solution in the same amounts as the initial immunization described above. The other leg and side of the back were used for the second injection and alternated for subsequent injections.

**III. Blood collection.** Blood was collected from the rabbits ten days after each booster shot. For this procedure, the rabbits are placed in a restrainer which is open on the top half to allow access to the ears. The rabbit should not be in the restrainer so tightly that it begins to breathe through its mouth. The ear to be bled is pulled through the cage and wiped with gauze that is damp with xylene. The ear is then tapped gently to expose the main, central artery. A 20 mL syringe (sterile and disposable) is connected to a butterfly needle (23 gauge) that has a short, plastic tube attached to it. The seal of the syringe plunger must be broken before it is used. The needle is inserted bevel
up into the artery towards the tip of the ear. The reason for entering the artery near the end of the ear is to allow for reinsertion closer to the head if necessary. An index finger is placed under the ear to insure that the needle does not pass through. Another finger is placed above the ear under the needle to insure the needle does not become "stuck" to the top wall of the artery. The syringe plunger is withdrawn at a rate proportional to the blood flow. When approximately 20 mL of blood have been collected, a clean gauze is placed over the point of needle insertion and the needle is withdrawn followed by partial release of the restrainer pressure. The blood is drawn into the barrel of the syringe and then dispensed into a 50 mL conical polypropylene tube. The gauze is held on the ear until the bleeding stops. The ear is then thoroughly washed with a wet soapy towel, rinsed, and lotion is applied to prevent chafing. Opposite ears are used for successive bleedings.

The blood is allowed to coagulate for at least 1 h at 5 °C. It is then centrifuged for 15 min (2000 rpm) at 5 °C in the 50 mL conical tube. The serum (clear yellow or orange upper layer) is then transferred to a 15 mL conical tube and recentrifuged under the above conditions. The clear to slightly red serum is then pipetted into plastic conical vials in 50 μL aliquots (10) and the remainder in 1.5 mL aliquots. The serum is stored at -80 °C and thawed immediately before use.

IV. ELISA Assay for Pyrrole Detection. The following procedure was used for determination of antibody titre levels in crude rabbit blood serum. For all ELISA plates described in this text,
Duplicates of all samples were run on the same plate.

A) Coating: A stock solution of BSA-pyrazole conjugate (1:6.6 molar ratio) containing 2.2 mg BSA/mL PBS (pH 7.4) was prepared. This stock solution (20 μL) was then diluted with PBS (10 mL). Using an Eppendorf dispenser capped with a 250 μL pippette tip, 100 μL of the diluted solution was added to each well of the plate and the sides were tapped to insure all of the liquid reached the bottom of the well. The plate was then incubated at 37 °C for 1 h. The plate was removed from the oven and allowed to cool for a few minutes before being emptied. When emptying the plate, it is quickly turned upside down to avoid cross-contamination between wells and then shaken vigorously. Each well was washed with PBS (3 x 300 μL). Since the maximum volume of the multi-channel pippette used for washings is only 200 μL, it was set at 150 μL and the wells were filled in 2 portions. After each washing, the plate was turned upside down and blotted on paper towels to insure removal of all liquid. If any condensation had formed on the lid, it is dried with a clean Kimwipe towel.

B) Blocking: A 1.0% chicken egg ovalbumin (CEO) solution was prepared by dissolving CEO (500 mg) in PBS (50 mL). Sample wells were then filled with the 1.0% CEO solution (300 μL) and incubated at 37 °C for 1 h. 0.1 % CEO was prepared by diluting 1.0% CEO (10 mL) with PBS (90 mL). The plate was then emptied and the sample wells were washed with 0.1% CEO (1 x 300 μL).

C) Sample Preparation: The samples to be used for the plate were prepared while the plate was incubating with the blocking agent.
It is important not to let the crude serum sit at room temperature for long periods of time. 0.2% CEO was prepared by diluting the 1.0% CEO solution (10 mL) with PBS (40 mL). Three dilutions of each rabbit blood serum sample were prepared as follows:

1. 0.2% chicken egg ovalbumin (1.0 mL) and rabbit serum (10 μL) were used as the 10⁻² dilutions.
2. The 10⁻³ dilutions were prepared with 0.2% CEO (450 μL) and 50 μL of the 10⁻² dilution.
3. The 10⁻⁴ dilutions were prepared with 0.2% CEO (450 μL) and 50 μL of the 10⁻³ dilution.

Each solution was vortexed to insure proper mixing.

**D) Plate loading:** The plate was loaded with 0.2% CEO (100 μL) in each of the blank wells and 100 μL of each dilution in the appropriate sample wells. Samples were loaded from least concentrated (10⁻⁴) to most concentrated (10⁻²) dilutions with the same pipette tip. The tip was then changed between samples. Normal rabbit (not injected with antigen) serum diluted the same as above was run as a negative response control. Incubation was done by shaking the plate (set at 4-5) at room temperature for 1 h. The plate was then emptied and washed with 0.1% CEO (3 x 300 μL).

**E) Incubation with Anti-Rabbit IgG:** 10 μL of goat anti-rabbit IgG-alkaline phosphatase was added to 1.0 % CEO solution (10 mL). 100 μL of this solution was added to each well and the plate was again incubated by shaking at room temperature for 1 h. The plate was then emptied and washed with 0.1% CEO (3 x 300 μL).
F) Plate Development: Two tablets of disodium p-nitrophenol phosphate were dissolved in a pH 9.6 solution (11 mL) containing glycine (50 mM) and MgCl₂ (1 mM). This solution was kept at 4 ºC until it was used. Using an Eppendorf dispenser, 100 µL of this solution was added to each well and, with the poly-L-lysine-pyrazole antibody, the plate was allowed to sit for 1 h at room temperature. Development time for the KLH-pyrazole antibody was under 20 min to avoid being above the maximum absorbance (2.5) read by the microplate reader. Sample absorbances were then measured using dual wavelength on a Bio Rad 450 Microplate reader. On the microplate program, all wells not used on the plate are designated as blanks. Sample wells are designated as standards and the normal rabbit serum is a negative control. The raw data is printed out as an absorbance report.

V. Poly-L-lysine as the coating agent. A modification of the above procedure was performed in order to determine the efficacy of the immunization techniques. Poly-L-lysine was substituted for the BSA-pyrazole conjugate as the coating agent (with the poly-L-lysine-pyrazole antibody). A stock solution was prepared by dissolving poly-L-lysine (4.4 mg) in PBS (2 mL). 20 µL of this solution was further diluted with PBS (10 mL). 100 µL of this solution was then added to each sample well as the coating agent. The rest of this assay was conducted the same as that described above.

VI. Crossreactivity of a BSA-LGE₂ adduct with the crude antibody. A solution containing 0.1 mM BSA and 0.1 mM LGE₂ solutions were incubated at 37 ºC for 30 min simultaneously with the
ELISA plate coating step. The BSA-LGE2 solution was then serially
diluted and mixed with an equal volume of antibody serum (10⁻⁴
dilution). These solutions were then incubated at 37 °C for 1 h
simultaneously with the ELISA plate during the blocking step. In this
way, the time that the unstable LGE₂ solutions were exposed was
minimized. The rest of the assay was conducted in the same manner
as that to determine antibody titre levels, except that the time of plate
development was also reduced to 15 min owing to the KLH-pyrazole
antibody serums having high titres.

VII. Inhibition graphs created with a Sigma Plot
program: The following descriptions and illustrations demonstrate the
process for constructing the inhibition graphs that were given in the
results and discussion section.
Once a new worksheet has been created, the inhibitor concentrations and absorbance values are entered onto the worksheet followed by the column headings.

The curve fit program is now opened.

If the "4 Parameter Logistic Function" equation is not active, it is selected and opened by the following sequence:
The parameters are now modified to fit the data on the worksheet. Parameter $a =$ the maximum absorbance value, $b =$ the slope of the vertical portion of the curve, $c =$ the inhibitor concentration at the 50% absorbance value, and $d =$ the minimum absorbance value.

$\text{[Parameters]}: \lambda \text{Parameter Logistic Function}$

$f = (a-d)/(1+(x/c)b)^d, \text{ if } b>0 \text{ it}$

starts at $a$ and falls to $d$. If $b<0$

starts at $d$ and rises to $a$.

Modify these values for your data

$a = 5.0$ : asymptotic maximum

$b = 1.5$ : slope parameter, $b>0$ gives slope<0

$c = 45$ : value at inflexion point

$d = 0.1$ : asymptotic minimum
The curve fit program is then run and the "dependency" values of the results are checked. If these dependency values are close to 0.99, the program is edited by placing constraints on the parameters, usually the values for "a" and/or "d".

File operations:
[New] [Open] [Save] [Save as] [Revert] [Print] [OK] [Cancel]

Converged, tolerance satisfied.

More iterations

Morse = 0.0793737

Parameter Value StdErr CU(%) Dependency
a 8.536e+01 2.609e+01 3.056e+01 0.985176
b -9.359e+01 9.594e+01 1.025e+02 0.991521
c 1.595e+02 2.978e+02 1.867e+02 0.997681
d 2.036e-00 1.422e+00 6.983e+01 0.997953

Parameter Value StdErr CU(%) Dependency
a 8.536e+01 2.609e+01 3.056e+01 0.985176
b -9.359e+01 9.594e+01 1.025e+02 0.991521
c 1.595e+02 2.978e+02 1.867e+02 0.997681
d 2.036e-00 1.422e+00 6.983e+01 0.997953

[Variables]

x=col(1) :change to appropriate column
y=col(2) :change to appropriate column

[Constraints]

a<1.7

[Equations]
f=(a-d)/(1-(x/c)-b)+d
After running the curve fit program a second time, the dependency values are again checked. If the dependency values are not approaching 0.99, the results are stored in designated columns on the worksheet as shown below.
A Cartesian graph is then created by selecting the requisite x and y axes from the worksheet as shown in the sequence below.

### Pick Columns - Plot 3 - Graph 2

**Plot Type:** Scatter/Line

Plot columns of data as:
- pairwise XY
- Y vs row number
- many Y vs X
- H vs row number
- many H vs Y

Choose "Worksheet" to pick columns from the worksheet. Choose "Pattern" to pick a pattern of columns.

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<th>3</th>
<th>4</th>
<th>5</th>
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</tr>
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</table>

Enter x column.

Click the desired column on the worksheet.

![Graph Diagram](image-url)
The scale of the x-axis is converted to the common log of the absorbance values.

The font of the axes labels is changed from the default "chem" font to "Helvetica". The font size is also adjusted at this point.

The size of the graph is then adjusted as shown in the following illustrations.
The range of the x axis is then adjusted so that the curve occupies the maximum space available. In the same window, the minor tick labels can be activated to give a more appropriately labelled x axis. The labels for the x axis can also be given in a column on the worksheet. By leaving a blank space for every other value, the crowding of the axis label will be reduced.
The following tables give the data used in obtaining figures 4 - 6.

**Table 4-1. Data for figure 4-1.**

<table>
<thead>
<tr>
<th>Inhibitor (pmol/well)</th>
<th>Absorbance</th>
<th>fit absorbance</th>
<th>fit parameters</th>
</tr>
</thead>
<tbody>
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**Table 4-2. Data for figure 4-2.**

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<th>fit absorbance</th>
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</tr>
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**Table 4-3. Data for figure 4-3.**

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<td>Rabbit 1 fit absorbance</td>
<td>Rabbit 1 fit parameters</td>
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<th>fit parameters BSA-LGE-TCAA</th>
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VIII. Antibody Purification: Seroclear\textsuperscript{TM} (1.5 mL) was added to crude antibody serum (1.5 mL) from the 93 day bleeding of rabbit 1 and vortexed for 60 sec. The mixture was then centrifuged for 10 min (3000 rpm) and the upper delipidated layer (aqueous phase) was removed and added to an equal volume of binding buffer. This solution was loaded onto a sample loop (2 mL) and eluted through an FPLC Protein A-Superose (Pharmacia) column with the binding buffer. The eluants were monitored at 280 nm.

After a preset volume of binding buffer had been eluted, the solvent was automatically switched to the elution buffer. The IgG proteins of the crude serum were collected into fractions (1.0 mL) containing pH 8.8 1.0 M tris buffer (160 \(\mu\)L). These fractions were pooled and dialysed against pH 7.4 PBS (0.02% NaN\(_3\)) for 24 h at 5 \(^\circ\)C. The absorbance of 1.0 mL of this solution was measured at 280 nm to determine the concentration of IgGs. The absorbance value was 1.98 which corresponds to 1.38 mg/mL of IgGs.\textsuperscript{75} This concentration is approximately one-ninth of that in the crude serum. The absorbance of a 1.0 mL fraction of the delipidated serum was also measured and had an absorbance of 2.99. This corresponds to 2.09 mg/mL IgG, or roughly one-sixth of the concentration in the crude serum.

IX. Crossreactivity ELISA plates: For the inhibition studies, there were several modifications to the procedures described above.

A) Coating: The coating agent concentration was reduced when running the ELISAs using the purified antibody. The coating agent concentration was adjusted to BSA-pyrazole stock solution (3.4 \(\mu\)L) in
PBS (11 mL). The inhibitor solutions were prepared while the plate was incubating with the coating agent. The initial concentration of pyrazole solutions was 0.1 mM for all of the crossreactivity plates. The various compounds tested all had a 1.0 mM initial concentration except for the BSA-LGE2 and HSA-LGE2 adducts. These solutions were prepared by incubating a PBS solution containing 0.1 mM LGE2 and 0.1 mM BSA or HSA at 37 °C for 4 h. These reaction mixtures were then dialyzed against PBS (2 x 200 mL) for 24 h. The buffer solution was replaced after the first 8 h of dialysis. All inhibitor solutions were diluted ten times by a factor of 0.1 to provide a total of 11 solutions. Each of these solutions (150 μL) was added to an equal volume of the antibody solution prepared from purified serum (10 μL) in 0.2% CEO (50 mL).

B) Blocking. The antibody-inhibitor solutions were incubated at 37 °C for 1 h with the plate during the blocking step.

C) Loading. One column of the plate was used for blank samples (0.2% CEO). The remaining columns were filled with 100 μL of each sample. No negative control (untreated rabbit serum) was used to allow for a greater number of inhibitor dilutions.

D) Incubation with anti-rabbit IgG and Development. These two steps were done the same as in the assay used to measure the antibody titre level. Development time had increased to 45 min based on the above mentioned adjustment of coating agent concentration and antibody dilution. When the absorbance values were 0.5-0.7, the plate was quenched by adding 3 N NaOH (50 μL) to each
0.5-0.7, the plate was quenched by adding 3 N NaOH (50 μL) to each well using an Eppendorf dispenser and the absorbance values were then measured.

The following tables give the data used in obtaining figures 7-11.

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Table 11. Data for figure 11.

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X. Synthesis of pyrrole 117 from LGE₂:

6-amino-1-hexanol (4.3 mg, 0.037 mmol) in 100% EtOH (50 μL) was added to LGE₂ (96) (11 mg, 0.31 mmol) in 100% EtOH (100 μL). The solution was stirred at room temperature for 1 h. Volatiles were then evaporated by a stream of dry N₂ followed by high vacuum. The crude product was purified by HPLC using 60% EtOAc/hexanes as eluant to obtain pyrrole 117 (2.2 mg, 17%) as a brown oil: ¹H NMR (300 MHz, CDCl₃) δ 6.63 (s, 1H), 6.39 (d, 1H, J = 15.8 Hz), 5.74 (dd, 1H, J = 7.2, 15.9 Hz), 5.24 - 5.60 (2H), 4.15 (m, 1H), 3.68 (t, 2H, J = 7.3 Hz), 3.15 - 3.22 (m, 1H), 2.84 - 2.90 (m, 1H), 1.97 - 2.49 (6H), 1.47 - 1.90 (6H), 1.18 - 1.45 (12 H), 0.79 - 1.04 (7H).

XI. Synthesis of pyrroles 119 and 120 from γ-keto nononal:

Crude aldehyde 118 (50 mg, 0.320 mmol) and 6-aminocaproic acid (50 mg, 0.384 mmol) in MeOH/H₂O (500 μL, 19/1, v/v) were stirred for 16 h
at room temperature. Solvents were then removed into a dry ice-cooled trap using high vacuum. EtOAc (1 mL) was added and the insolubles were filtered out. The crude product was then purified by HPLC using 55% EtOAc/hexanes as eluant to obtain pyrrole 119 (11 mg, 11%) as a brown solid: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.54 (t, 1H, $J = 2.3$ Hz), 6.04 (t, 1H, $J = 3.1$ Hz), 5.85 (t, 1H, $J = 1.5$ Hz), 3.76 (t, 2H, $J = 7.3$ Hz), 2.48 (t, 2H, $J = 7.7$ Hz), 2.34 (t, 2H, $J = 7.4$ Hz), 1.56 - 1.79 (6H), 1.26 - 1.44 (8H), 0.85 - 0.96 (3H).

Pyrrole 120 was prepared under the same conditions used in preparing pyrrole 119 by substituting glycine for 6-aminocaproic acid as the primary amine. The crude product was purified by HPLC using 95% EtOAc/hexanes as eluant to obtain 120 (8 mg, 12%) as a brown solid: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.54 (t, 1H, $J = 2.3$ Hz), 6.12 (t, 1H, $J = 3.2$ Hz), 5.92 (t, 1H, $J = 1.2$ Hz), 4.58 (s, 2H), 2.43 (t, 2H, $J = 7.8$ Hz), 1.50 - 1.68 (2H), 1.20 - 1.44 (4H), 0.76 - 0.97 (3H).


69. Initial immunization of the rabbits was done by Seok Chan Kim.

70. Prepared from a 20x Stock solution containing 0.2M NaPO₄, 3.0 M NaCl, and 0.02% NaN₃ (w/w).


APPENDIX

$^1$H and $^{13}$C NMR spectra for new compounds
Figure 12. The $^1$H NMR (in CDCl$_3$) spectrum of 2,5 anhydro-1,3-O-anisylidene-D-glucitol (11).
Figure 13. The 75 MHz $^{13}$C NMR (in CD$_2$Cl$_2$) spectrum of 2,5 anhydro-1,3-O-anisylidene-D-glucitol (11).
Figure 14. The 300 MHz 1H NMR (in CDCl3) spectrum of 2,5-anhydro-1,3-O-anisylidene-6-O-((tert-butyldimethyl)silyl)-D-glucitol (14).
Figure 15. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of 2,5 anhydro-1,3-O-anisylidene-6-O-(tert-butyl-dimethylsilyl)-D-glucitol (14).
Figure 17. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of xanthate 16.
Figure 18. The 300 MHz 1H NMR (in CDCl3) spectrum of acetal 17.
Figure 19. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of acetal 17.
Figure 20. The 300 MHz 1H NMR (in CDCl₃) spectrum of acetal 18.
Figure 21. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of acetal 18.
Figure 22. The 200 MHz 1H NMR (in CDCl3) spectrum of acetal 19.
Figure 23. The 75 MHz 13C NMR (in CDCl3) spectrum of acetal 19.
Figure 24. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of primary alcohol 20.
Figure 25. The 75 MHz 13C NMR (in CDCl3) spectrum of primary alcohol 20.
Figure 26. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of secondary alcohol 21.
Figure 27. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of secondary alcohol 21.
Figure 28. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of aldehyde 6a.
Figure 29. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of aldehyde 6a.
Figure 30. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of enone 29t.
Figure 31. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of enone 29t.
Figure 32. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of enone 29c.
Figure 33. The 300 MHz 1H NMR (in CDCl3) spectrum of 28a.
Figure 35. The 300 MHz 1H NMR (in CDCl3) spectrum of enal 371.
Figure 36. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of enal 374.
Figure 37. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of enal 37c.
Figure 38. The 300 MHz 1H NMR (in CDCl3) spectrum of aldehyde 36.
Figure 39. The 75 MHz 13C NMR (in CDCl3) spectrum of aldehyde 36.
Figure 40. The 300 MHz 1H NMR (in CDCl3) spectrum of dithiane 35.
Figure 41. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of dithiane 35.
Figure 42. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of dithiane 39.
Figure 43. The 50 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of dithiane 39.
Figure 44. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of compound 46.
Figure 45. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of compound 46.
Figure 46. The 200 MHz $^1$H NMR spectrum (in CDCl$_3$) of primary alcohol 47.
Figure 47. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of aldehyde 6c.
Figure 48. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of aldehyde 6c.
Figure 49. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of enone 48t.
Figure 50. The 75 MHz $^{13}\text{C}$ NMR (in CDCl$_3$) spectrum of enone 48t.
Figure 51. The 300 MHz 1H NMR (in CDCl3) spectrum of enone 48c.
Figure 52. The 200 MHz $^1$H NMR (in CDCl$_3$) spectrum of enol silyl ether 44c.
Figure 53. The 200 MHz $^1$H NMR (in CDCl$_3$) spectrum of alcohol 32c.
Figure 54. The 200 MHz 1H NMR (in CDCl3) spectrum of mesylate 50.
Figure 55. The 200 MHz $^1$H NMR (in CDCl$_3$) spectrum of iodide 33c.
Figure 56. The 300 MHz 1H NMR (in CDCl3) spectrum of Wittig rearrangement product 52.
Figure 57. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of Wittig rearrangement product 52.
Figure 58. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of compound 53.
Figure 59. The 75 MHz 13C NMR (in CDCl3) spectrum of compound 53.
Figure 60. The 1H NMR spectrum (in CDCl$_3$) of primary alcohol 54.
Figure 61. The 200 MHz 1H NMR (in CDCl3) spectrum of primary alcohol 56.
Figure 62. The 50 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of primary alcohol 56.
Figure 63. The 200 MHz 1H NMR (in CDCl₃) spectrum of aldehyde 6d.
Figure 64. The 50 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of aldehyde 6d.
Figure 65. The 50 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of enal 57.
Figure 66. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of α,β-unsaturated acyl silane 69.
Figure 67. The 75 MHz 1H NMR (in CDCl₃) spectrum of α,β-unsaturated acylsilane 69.
Figure 69. The 75 MHz 13C NMR (in CDCl3) spectrum of acyl silane 70.
Figure 70. The 300 MHz 1H NMR (in CDCl3) spectrum of acid 61.
Figure 71. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of acid 61.
Figure 72. The 200 MHz 1H NMR (in CDCl₃) spectrum of imidazole 71.
Figure 73. The 200 MHz $^1$H NMR (in CDCl$_3$) spectrum of thiol ester 72.
Figure 74. The 300 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of thiol ester 72.
Figure 75. The $300\text{ MHz}$ $1\text{H NMR (in CDCl}_3$) spectrum of keto ylide 58.
Figure 76. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of keto ylide 58.
Figure 77. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of enone 26.
Figure 78. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of enone 26.
Figure 79. The 200 MHz $^1$H NMR (in CDCl$_3$) spectrum of ketone 73.
Figure 80. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of ketone 73.
Figure 81. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of symmetrical spiroketal 74.
Figure 82. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of symmetrical spiroketal 74.
Figure 83. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of primary alcohol 75.
Figure 84. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of primary alcohol 75.
Figure 85. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of compound 76.
Figure 86. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of compound 76.
Figure 87. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of primary alcohol 77.
Figure 88. The 75 MHz $^1$H NMR (in CDCl₃) spectrum of primary alcohol 77.
Figure 89. The 300 MHz 1H NMR (in CDCl3) spectrum of aldehyde 6e.
Figure 90. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of aldehyde 6e.
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Figure 92. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of enone 78.
Figure 93. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of ketone 79.
Figure 95. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of unsymmetrical spiroketal 80.
Figure 96. The 75 MHz $^{13}$C NMR (in CDCl$_3$) spectrum of unsymmetrical spiroketal 80.
Figure 97. The 300 MHz $^1$H NMR spectrum (in C$_6$D$_6$) of symmetrical spiroketal 74.
Figure 98. The 300 MHz $^1$H NMR (H42,46 decoupled) spectrum (in C6D6) of symmetrical spiroketal 74.
Figure 99. The 300 MHz 1H NMR (in CDCl₃) spectrum of hexanol pyrrole 117.
Figure 100. The 300 MHz 1H NMR (in CDCl3) spectrum of aminocaproic pyrrole 119.
Figure 101. The 300 MHz $^1$H NMR (in CDCl$_3$) spectrum of glycine pyrrole 120.


6. (a) Koerner, Jr., T. A. W.; Voll, R. J.; Younathan, E. S. Carbohydrate Res. 1977, 59, 403. (b) These reactions were first done by former postdoctoral research associate Dr. V.T. Ravikumar.


10. A similar result has also been reported recently. Corey, E. J.; Jones, G. B. J. Org. Chem. 1992, 57, 1028-1029.


16. This reaction was not attempted because this substrate was not used to synthesize compound 74 or 80.


19. These reactions were done exclusively by former postdoctoral research associate Dr. V. T. Ravikumar.


54. When a solvent gradient was used for silica gel column chromatography, the eluants were closely monitored by TLC analysis. When a particular spot from the reaction mixture TLC had been eluted, the elution solvent was then changed to the next more polar solvent system.


69. Initial immunization of the rabbits was done by Seok Chan Kim.

70. Prepared from a 20x Stock solution containing 0.2M NaPO₄, 3.0 M NaCl, and 0.02% NaN₃ (w/w).


