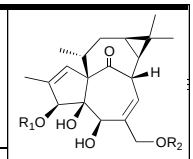
Approaches to the synthesis of ingenol



CHEMICAL SOCIETY REVIEWS

Sanghee Kim† and Jeffrey D. Winkler*

Department of Chemistry, The University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Ingenol is a highly oxygenated tetracyclic diterpene which mimics the function of diacylglycerol, the endogenous activator of protein kinase C. One of the most imposing challenges in the synthesis of ingenol is the establishment of the highly strained 'inside–outside' or *trans*-intrabridgehead stereochemical relationship of the carbocyclic ring system of the ingenanes. The approaches that have been examined in several laboratories towards the synthesis of the ingenanes are summarized. Our own work, using the intramolecular dioxenone photocycloaddition to establish this unique stereochemical feature, is described.

1 Introduction

Ingenol **1** (Fig. 1) is a highly oxygenated tetracyclic diterpene isolated initially from the *Euphorbia ingens* species of the *Euphorbiaceae* plant family.[1] Diverse ingenane types with different oxidation states at C-3, C-4, C-5, C-12, C-13, C-16 or C-20 have also been isolated.[2] Various esters of ingenol are able to substitute for diacylglycerol **2**, the endogenous activator of protein kinase C (PKC), and thereby exhibit antitumour or tumour-promoting activity. Protein kinase C is the phosphorylating enzyme which mediates cellular signal transduction for a large class of hormones and cellular effectors that activate phosphatidylinositol 4,5-bis(phosphate) turnover.[3] In addition to ingenol **1**, several other natural products including teleocidin **3**, asplysiatoxin **4**, bryostatin **6** and esters of phorbol **5** mimic the function of diacylglycerol **2**.[4] Although several proposals for a pharmacophore common to these structurally dissimilar activators of PKC have been described,[5] the requisite structure–activity relationships (SAR), that could lead to new therapeutic agents for the treatment of inflammatory and proliferative diseases, remain to be definitively established.[6,7]

† Current address: The Scripps Research Institute, 10990 North Torrey Pines Road, La Jolla, CA 92037, USA.

The structurally and biologically related diterpene phorbol **5** was recently synthesized by Wender.[8] However, despite the efforts of many groups, ingenol **1** has not yet yielded to total synthesis.[9] While the high degree of oxygenation, notably the *cis*-triol (from C-3 to C-5 located on the β face of A and B rings), represents an important challenge to synthesis, the most imposing obstacle to the synthesis of ingenol is the establishment of the highly strained 'inside–outside' or *trans* intrabridgehead stereochemistry of the B,C ring system. This unique stereochemical feature would appear to play a very important role in the biological properties of the ingenanes, as Paquette[17] has reported that a highly functionalized ingenane analogue **7** (Scheme 1), which has *cis* rather than *trans* intrabridgehead stereochemistry (the C-8 epimer of ingenol), is completely devoid of biological activity.

In this review, the approaches that have been examined in several laboratories towards the synthesis of the ingenanes will be summarized. Also, our own work, in which intramolecular dioxenone photocycloaddition is used to establish the critical inside–outside stereochemical relationship, will be described.

2 Inside–outside stereochemistry of ingenol

Bridged bicyclic systems can exist as three different stereoisomers: an out–out isomer **8**, an in–in isomer **9** and an in–out isomer **10** as shown in Fig. 2.[10] Usually the in–in isomer **9** is most unstable because of the severe repulsive interaction between the inside atoms. However, the energy difference between in–out and out–out isomers varies depending on the system.[10] For example, the out–out isomer of bicyclo[4.4.4]tetradecanes is less stable than the in–out isomer by 12 kcal mol^{-1} (1 cal = 4.184 J), presumably as a consequence of severe eclipsing interactions along each of the three chains of the bridged bicyclic ring system. In the ingenane ring system, the in–out isomer is generally more strained than the out–out isomer.[11] In–out bicyclo[4.4.1]undecane **12** is more strained than its out–out isomer **11** by 6.3 kcal mol^{-1}, whereas the

Sanghee Kim was born in 1966 in Mokpo, Korea. He received his BS degree in pharmacy from Seoul National University in 1988, where he also obtained his MS degree in medicinal chemistry under the supervision of Professor Deukjoon Kim in 1990. After finishing military service as a lieutenant, he worked for a year as an assistant researcher at the Korea Institute of Science and Technology. In 1992, he joined Professor Jeffrey Winkler's group at the University of Pennsylvania and earned his PhD in organic chemistry in 1997. He is now working as a postdoctoral associate at The Scripps Research Institute in the laboratories of Professor K. C. Nicolaou.

Sanghee Kim

Jeffrey D. Winkler graduated cum laude *in Chemistry from Harvard College in 1977, and received his PhD degree with Professor Gilbert Stork. After an American Cancer Society Postdoctoral Fellowship in the laboratories of Professor Ronald C. D. Breslow, he joined the Chemistry Department at the University of Chicago as an Assistant Professor in 1983. He moved to the University of Pennsylvania in 1990, where he is currently Professor of Chemistry and a Member of the University of Pennsylvania Cancer Center.*

Jeffrey D. Winkler

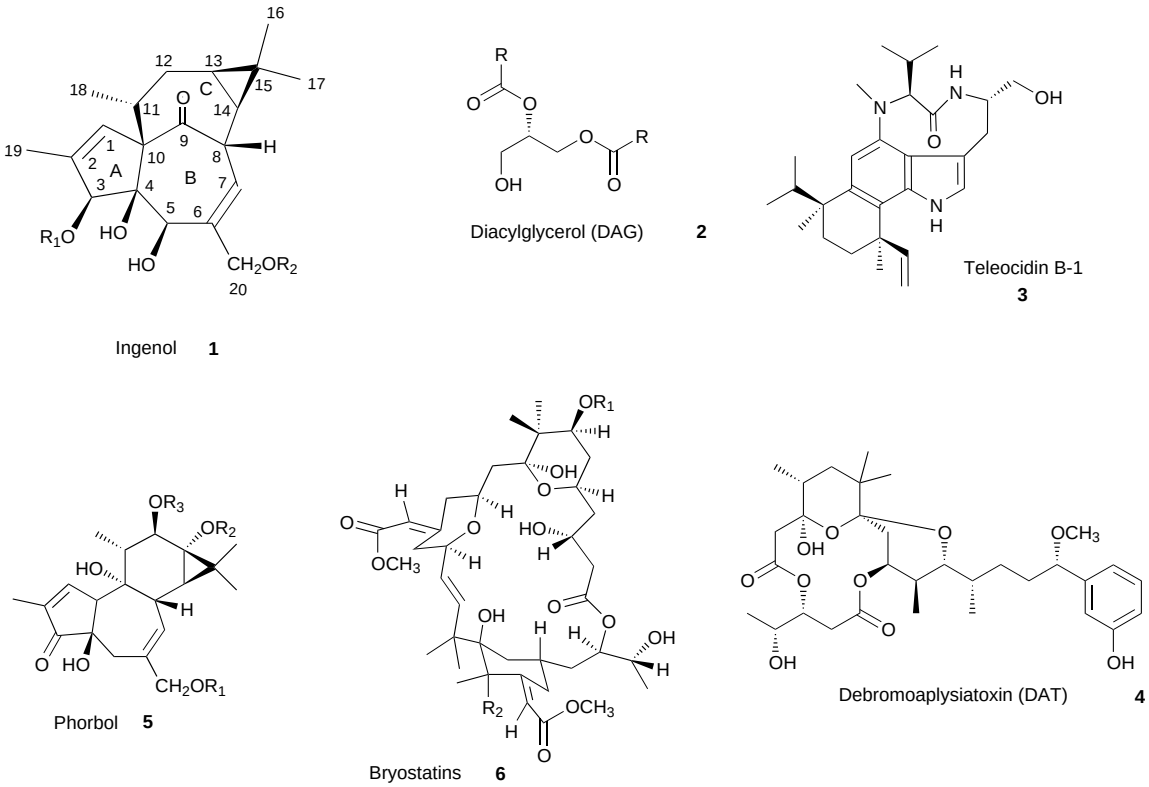
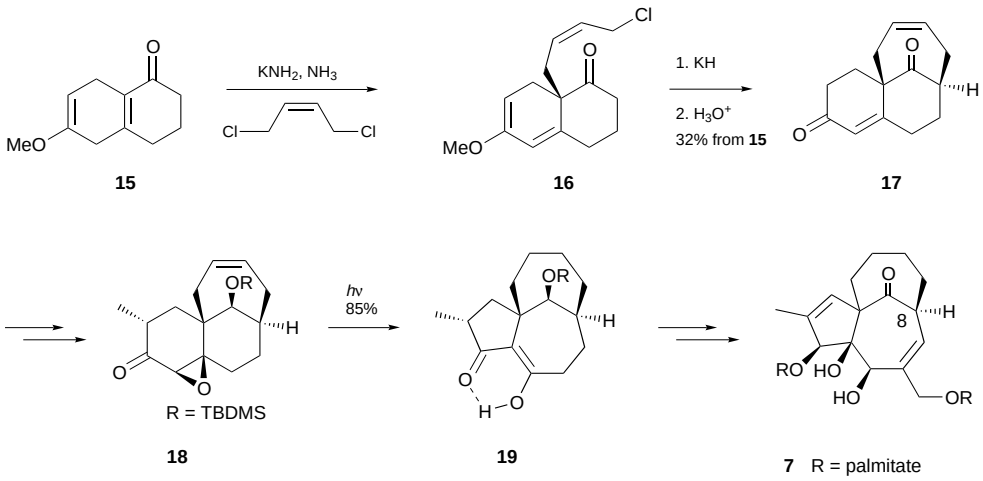


Fig. 1



Scheme 1

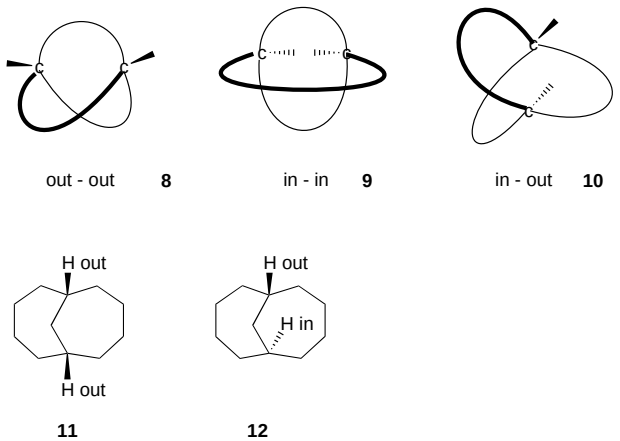


Fig. 2

corresponding in–out and out–out bicyclo[4.4.1]undecan-7-one conformers differ in strain energy by 3.3 kcal mol^{-1}. Ingenol itself is more strained than its C-8 epimer (isoingenol) by 5.9 kcal mol^{-1}.[11]

Only a few other natural products exhibit the phenomenon of 'inside–outside' stereochemistry, such as 3-α-acetoxy-15β-hydroxy-7,6-secotrinervita-7,11-diene and secotrinerviten-2β,3α-diol, macrocycles **13** and **14** in Fig. 3 respectively.[10] The inside–outside intrabridge stereochemical relationship in ingenol **1** is depicted in the MM2 minimized three dimensional picture (Fig. 3).

3 Synthetic approaches toward ingenol

The biological activity of ingenol and its unusual structure have stimulated the interest of many chemists. A number of approaches to this complex ring system have been published since the mid 1980s, but the total synthesis of ingenol has not

yet been accomplished, due to the structural complexity and the instability of these natural products.

The published approaches for the synthesis of ingenol include: (1) both inter- and intra-molecular [6 + 4] cycloadditions of tropones to dienes,[12–14] (2) intramolecular [4 + 3] cycloaddition of cyclic oxyallyls to a tethered furan,[15] (3) Lewis acid catalysed aldol cyclization,[16] (4) photo induced ring expansion-ring contraction,[17] (5) a ring contraction strategy based on the Ireland–Claisen rearrangement,[11,18] (6) transformation of an out–out system to an in–out isomer by [1,5]-hydrogen sigmatropy[19] and (7) intramolecular dioxenone photocycloaddition and fragmentation.[20] Only the latter three strategies provide the requisite inside–outside intrabridgehead stereochemical relationship of ingenol.

3.1 Paquette's approach to *cis*-ingenane

Paquette and co-workers have synthesized the less strained isoingenol **7**, which is epimeric with the natural product at C-8, possessing the fully functionalized AB ring of ingenol.[17] The B,C rings of the ingenol skeleton were generated by sequential inter- and intra-molecular alkylation of the partially reduced tetralone substrate **15** with dichlorobutene yielding the *cis* intrabridgehead stereochemistry instead of the requisite *trans* relationship. The photo-induced isomerization of **18** led to the formation of perhydroazulene **19**, containing the ABC ring system of ingenol. The stereocontrolled elaboration of the *cis*-triol and the unsaturations in the A and B rings of ingenol were successfully accomplished. However, this highly functionalized isoingenol **7**, which has out–out intrabridgehead stereochemistry, was devoid of the biological activity associated with the naturally occurring ingenane esters. These results underscore the importance of the *trans*-intrabridgehead stereochemistry for the biological activity of the ingenanes.

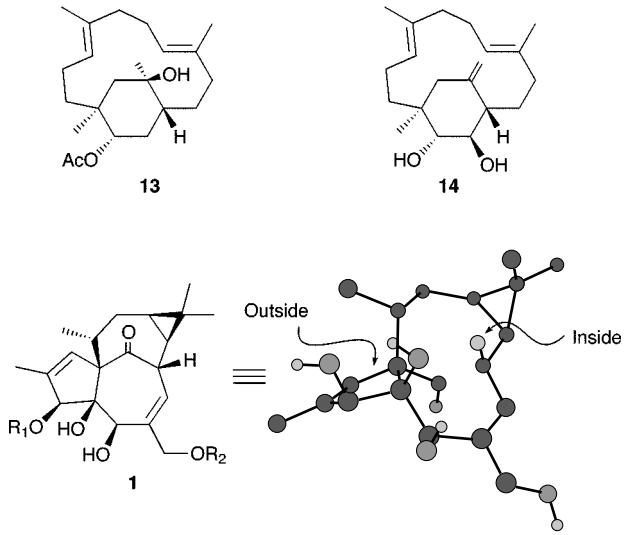


Fig. 3

3.2 Mehta's approach to the isoingenane framework

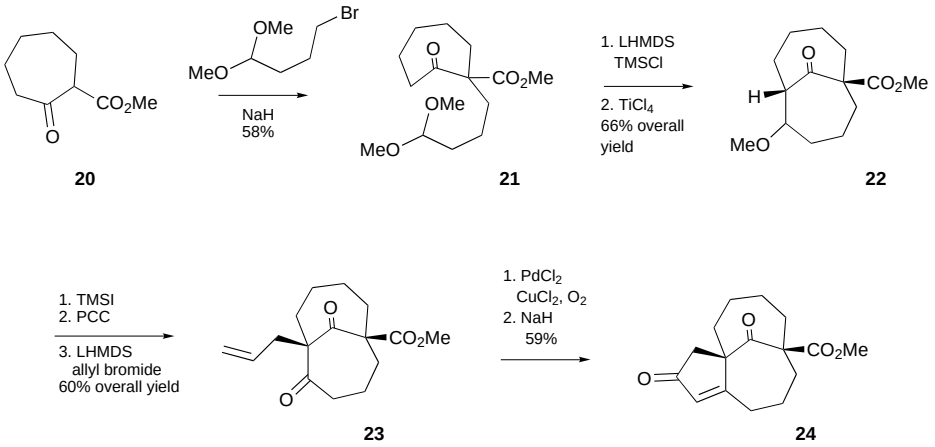
Mehta has reported a construction of the isoingenane ABC framework.[16] He employed a sequential titanium-catalysed intramolecular variant of the Mukaiyama reaction for the synthesis of seven-membered B ring (**21** to **22**), followed by base promoted aldol cyclization for the formation of the 5-membered A ring (**23** to **24**). This approach, however, did not yield the inside–outside chemistry.

3.3 Harmata's approach to the isoingenane framework

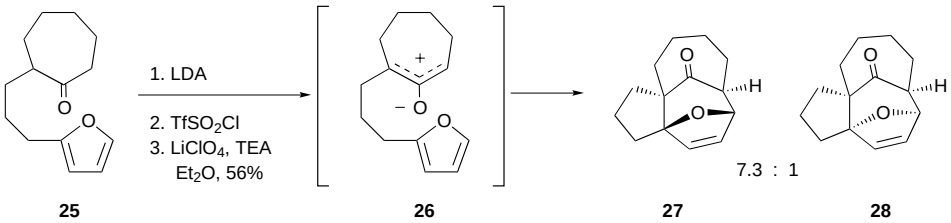
Cyclic oxyallyl cations **26**, derived from cycloheptanone **25** underwent intramolecular [4 + 3] cycloaddition with a tethered furan to give two polycyclic products (**27** and **28**) in a 7.3 to 1 ratio.[15] The major cycloadduct **27** is the result of an *exo* approach of the dienophile to the diene. Both cycloadducts possess the ABC ring structure of isoingenol with *cis* intrabridgehead stereochemistry.

3.4 Rigby's approach to *cis*- and *trans*-ingenane

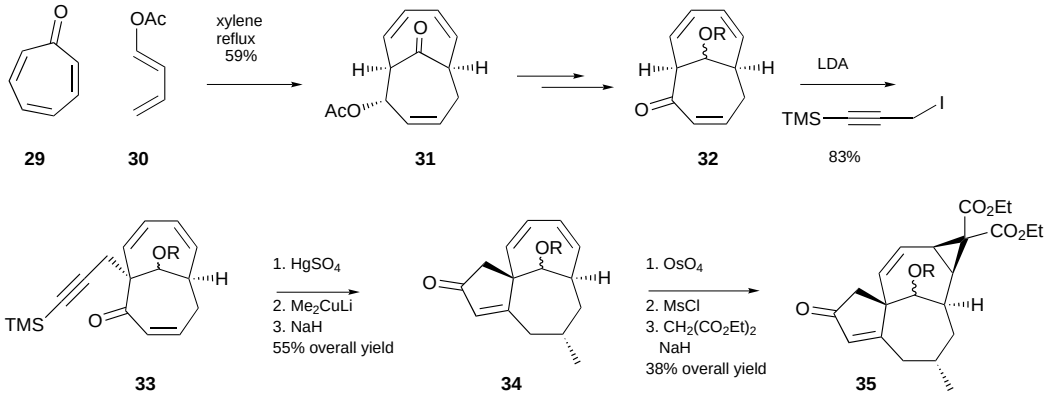
Rigby has employed both inter- and intra-molecular [6π + 4π] cycloadditions of tropones to dienes to construct the *cis* fused bicyclo[4.4.1] system. These thermally allowed cycloadditions produce a functionally rich BC ring building block for the construction of C-8-isoingenol intermediates. In his intermole-



Scheme 2



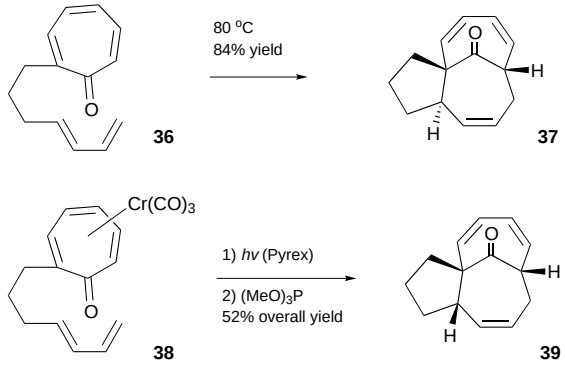
Scheme 3



Scheme 4

cular cycloaddition approach,[13] bridgehead enolate alkylation followed by aldol condensation was used to install the A ring of isoingenol (**32** to **34**). Regioselective dihydroxylation of the C ring followed by activation and treatment with sodiomalonate afforded a tetracyclic isoingenane **35** as shown in Scheme 4.

The use of thermal, metal-free, and metal-promoted intramolecular higher-order cycloadditions has been reported recently by Rigby.[12] He found that tropone with various diene tethers underwent *exo*-selective [6π + 4π] cycloaddition under thermal conditions (**36** to **37**). He also reported that the metal-promoted reactions proceeded *via* an exclusive *endo*-selective pathway (**38** to **39**).

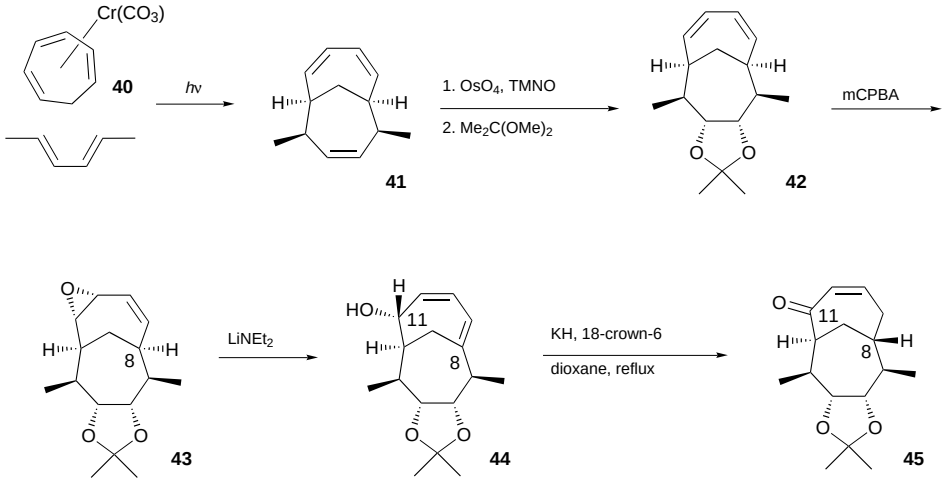


Scheme 5

More recently, he reported an elegant protocol for the conversion of the out–out bicyclo[4.4.1]undecane system into the more strained in–out stereoisomer by using an internal delivery approach to the establishment of the C-8β bridghead hydrogen stereochemistry.[19] The out–out bicyclo[4.4.1]undecane **41** was produced as a single (*endo*) isomer by chromium(0)-mediated higher-order cycloaddition between complex **40** and 2,4-hexadiene. Selective dihydroxylation of **41** and protection of the resulting diol as an acetonide gave **42**. *Exo*-face selective epoxidation of **42**, followed by epoxide opening with a lithium base led to the exclusive formation of dienol **44**. This regioselective elimination process can be rationalized by the fact that the C-8 hydrogen is the only one properly aligned to achieve ring opening due to the conformation of the bicyclic undecane system. An alkoxide accelerated [1,5]-hydrogen sigmatropic rearrangement was employed for delivering the β-H on the alkoxide carbon (C-11) to the bridgehead position (C-8) with retention of stereochemistry to yield the in–out bicyclo[4.4.1]undecane **45**.

3.5 Funk's approach to *trans*-ingenane

Funk has reported a clever solution to the problem of the inside–outside stereochemistry of ingenol, in which an intramolecular version of the Ireland–Claisen rearrangement leads to the transformation of 12-membered lactone **50** to a more strained *trans* fused bicyclo[4.4.1]undecane ring system **52**.[11] It should be noted that the crucial *trans* relationship of C-8 and C-10 (ingenol numbering) was set early in the sequence by stereoselective alkylation of a chiral homocarene enone–ester **47** obtained from (+)-3-carene **46**. Both conjugate addition of methyl cuprate to **47** at C-11 and alkylation of **48** with the butenol chain at C-8 occurred opposite to the cyclopropane ring. It is noteworthy that the resulting macrobicyclic lactone **50** does not suffer from the ring strain present in ring-contracted *trans*-bridged bicyclo[4.4.1] system, *i.e.* **52**, which is obtained on



Scheme 6

rearrangement of the ketene acetal **51** derived from lactone **50**, *via* a boatlike transition state.

Unfortunately, the first approach, shown above, afforded an ingenane nucleus in which the functionality is not optimally situated for the construction of the A-ring. A subsequent approach using the same strategy delivered a more readily elaborated Claisen product.[18] The new version makes use of an *n* to *n* − 2 ring contraction (**53** to **54**) instead of an *n* to *n* − 4 rearrangement (**50** to **52**) as in Scheme 7, and leads to the first synthesis of the tetracyclic ring system with the inside–outside stereochemistry.

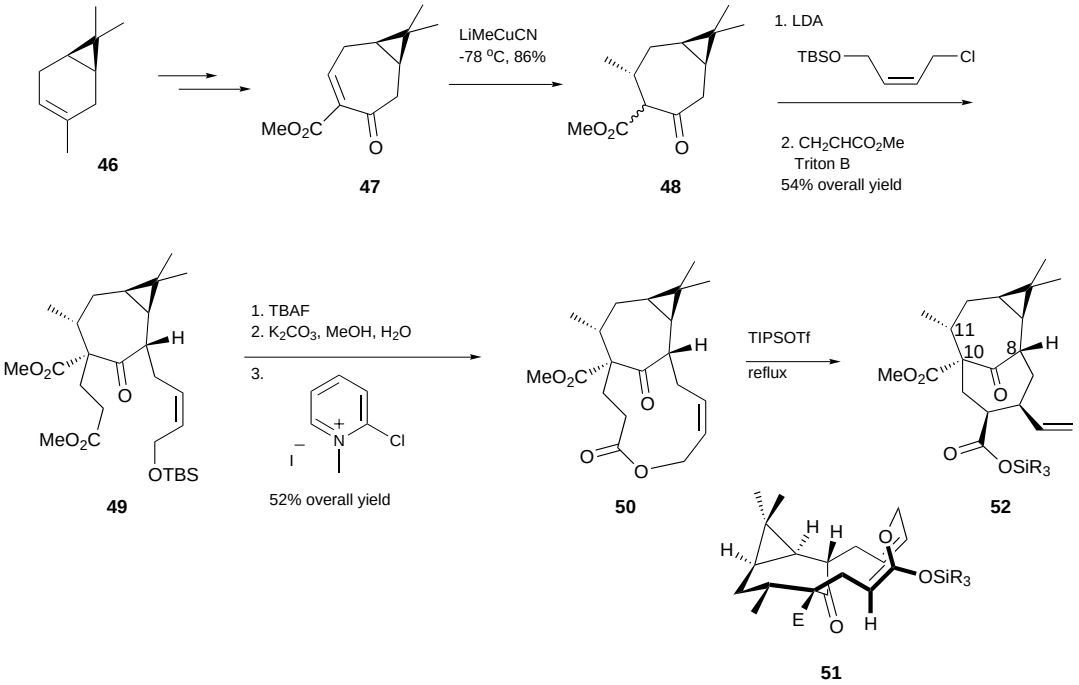
4 Construction of the tricyclic nucleus of the ingenane diterpenes by dioxenone photocycloaddition and fragmentation methodology

Winkler and co-workers reported the first synthesis of a tricyclic ingenane ring system having the correct *trans*-intrabridgehead stereochemical relationship.[21] In order to construct the inside–outside ring system, they employed the intramolecular version of the modified de Mayo reaction, the dioxenone photoaddition–retroaldol fragmentation.[22,23]

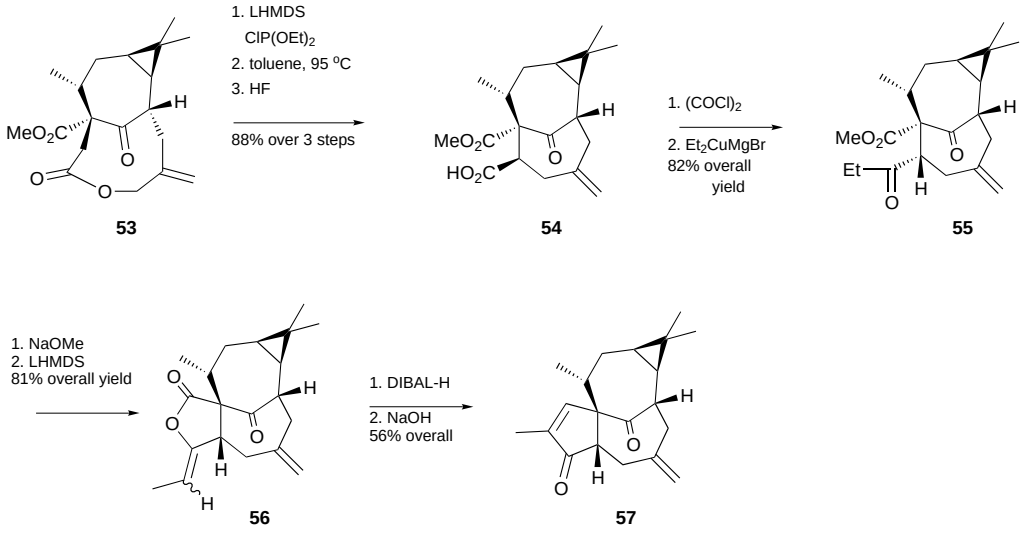
4.1 The de Mayo reactions

de Mayo found that irradiation of the enol form **59** of a β-diketone **58** in the presence of an alkene produced a four-membered ring aldol product **60**.[24] This photoadduct **60** underwent a facile retro-aldol reaction *in situ* to produce a 1,5-diketone **61**, thereby alleviating the strain energy of the cyclobutane intermediate. Attempted extension of these results to β-keto esters gave a different [2 + 2] photocycloaddition, yielding oxetane products.[34] A solution to this problem was reported by Baldwin,[25] who used dioxenone heterocycles **62** as covalently locked enol tautomers of the corresponding β-keto ester. However, it still proved too difficult to predict the regiochemical outcome of the photocycloaddition with unsymmetrical alkenes.

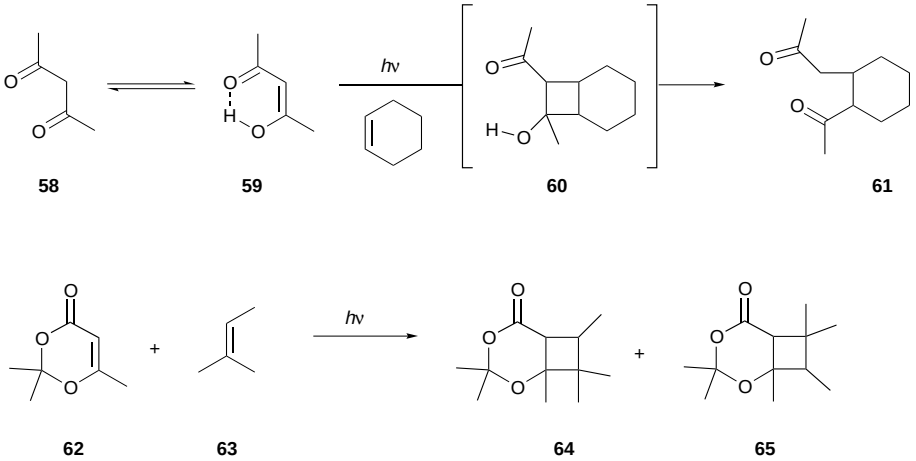
Winkler and co-workers found that regiochemical control of the dioxenone photocycloaddition can be greatly improved using the intramolecular version of this reaction.[26] Irradiation of



Scheme 7

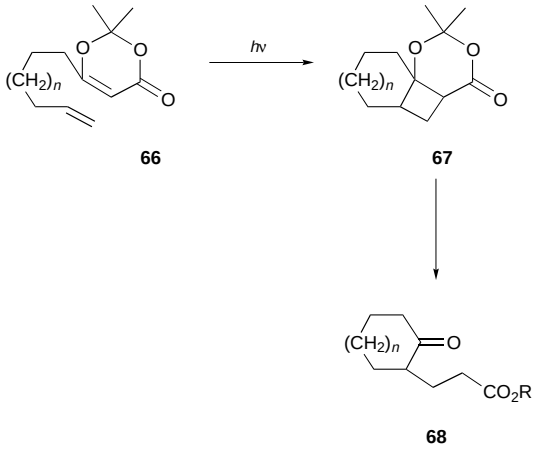


Scheme 8



Scheme 9

a tethered dioxenone **66** produced photoadduct **67** which on retro-aldol fragmentation provided six-, seven- and eight-membered ring esters **68** in excellent yield with exceedingly high (> 50 : 1) levels of regiochemical control.



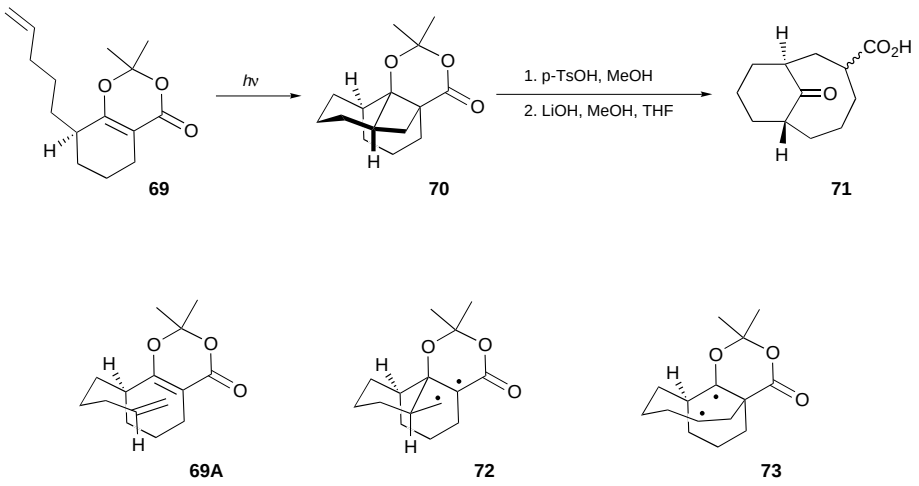
Scheme 10

4.2 Construction of bridged bicyclic ring systems by intramolecular dioxenone photocycloaddition and fragmentation methodology

This intramolecular dioxenone photocycloaddition–fragmentation methodology can be used for the construction of various bridged bicyclic ring systems such as those of paclitaxol and ingenol which are not accessible using standard de Mayo reaction conditions. Irradiation of dioxenone **69** led to the exclusive formation of photoadduct **70**, which on fragmentation produced *trans*-bicyclo[5.3.1]undecan-11-one-3-carboxylic acid **71**, the smallest bridged bicycloalkanone with 'inside–outside' stereochemistry at that time. We proposed that the unusual *trans* intrabridgehead stereochemical relationship could be a consequence of the chairlike folding of the nascent six-membered ring, as shown in conformation **69A**. Photocycloaddition of conformer **69A** should lead to the intermediate cyclohexane diyl **72** (β-bond formation) instead of diyl **73**, based on recent mechanistic studies from our laboratory.[35] The alternative formation of the eight-membered ring diyl **73** (α-bond formation) would be expected to produce the less strained *cis*-ring-fusion stereochemistry in the cycloaddition, since the rate of intersystem crossing for the triple diyl is slower than ring flipping.[27]

This methodology has been extended to the construction of the *trans*-bicyclo[4.4.1]undecane moiety which constitutes the BC ring system of the ingenanes. The dioxenone **76** was derived from retrosynthetic analysis of the ingenane **74** which lacks most of the functionality of ingenol except for the C-9 carbonyl, the C-20 oxygen functionality and the critical C-8β hydrogen.

A model system was first examined to establish the feasibility of this methodology, shown in Scheme 12.[28] In contrast to previous results which proceeded to give a single photoadduct in high yield (Scheme 11), irradiation of **77** under the usual conditions (4.8 mM in 1 : 9 acetone–acetonitrile, Pyrex immersion well, 0 °C, 450 W, medium-pressure Hg lamp) led to the



Scheme 11

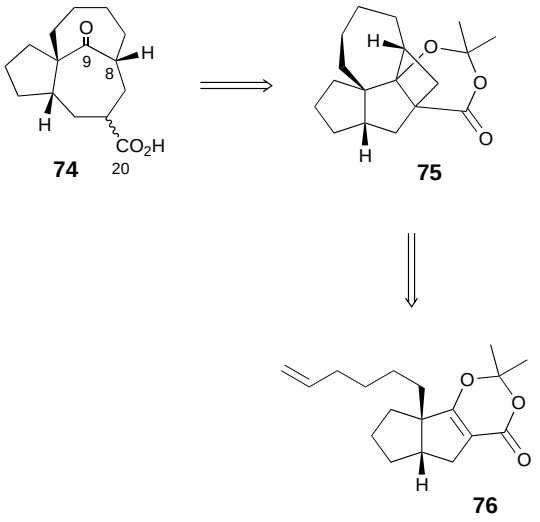
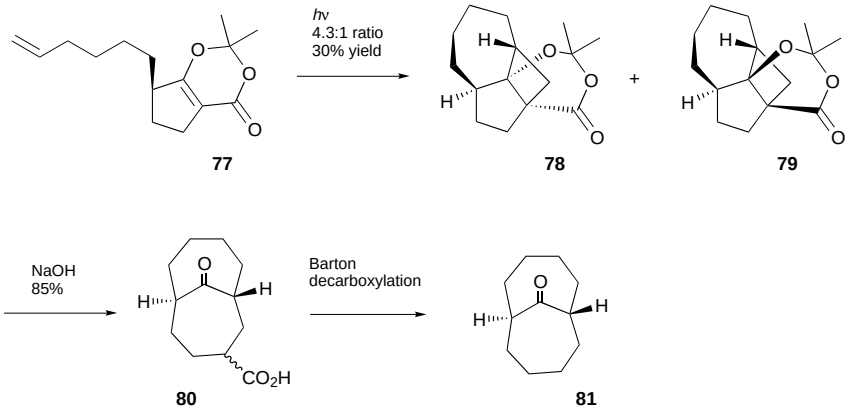


Fig. 4

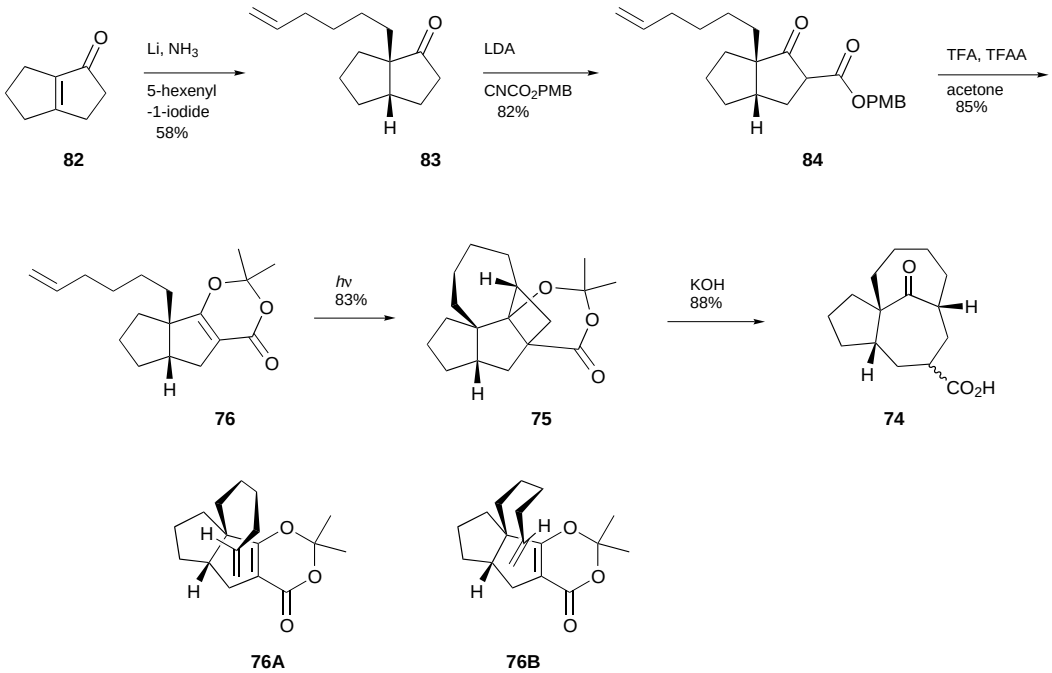
formation of two photoadducts, **78** and **79**, in a 4.3 : 1 ratio in 30% yield. The diastereomeric photoadducts **78** and **79**, which result from the addition of alkene from either face of dioxenone, were subjected to fragmentation and Barton decarboxylation to give a single bicycloundecanone **81** with the highly strained *trans* intrabridgehead stereochemistry. The diastereomeric ketoacids **80** become enantiomeric ketones **81** on decarboxylation.

We anticipated that annulation of the ingenol A ring onto the photosubstrate **77** would reduce the degrees of freedom of the alkyl chain and could lead to a higher facial selectivity as well as yield. The requisite photosubstrate **76** was prepared as outlined in Scheme 13. Reductive alkylation of bicyclo-[3.3.0]octenone **82** with hexenyl iodide followed by carboxylation and dioxenone formation provided photosubstrate **76**. Irradiation of **76** through a Pyrex filter afforded a single photoadduct **75** in high yield. The adduct was then fragmented to keto acid **74** with potassium hydroxide.[28] The exclusive formation of the inside–outside isomer **74** can be explained by approach of the alkene to the dioxenone in the pseudochair conformation **76A**. The other possible conformation is a pseudoboat, **76B**, which suffers from transannular eclipsing non-bonded interactions which are not present in **76A**.

Two different approaches for the synthesis of C-3 oxygenated analogues of ingenol have been studied by Winkler and co-workers.[20] In the first approach, the angular functionalization of C-3 (ingenol numbering) oxygenated enone **87** was achieved by [2 + 2] photocycloaddition with allene. Ozonolysis of the



Scheme 12



Scheme 13

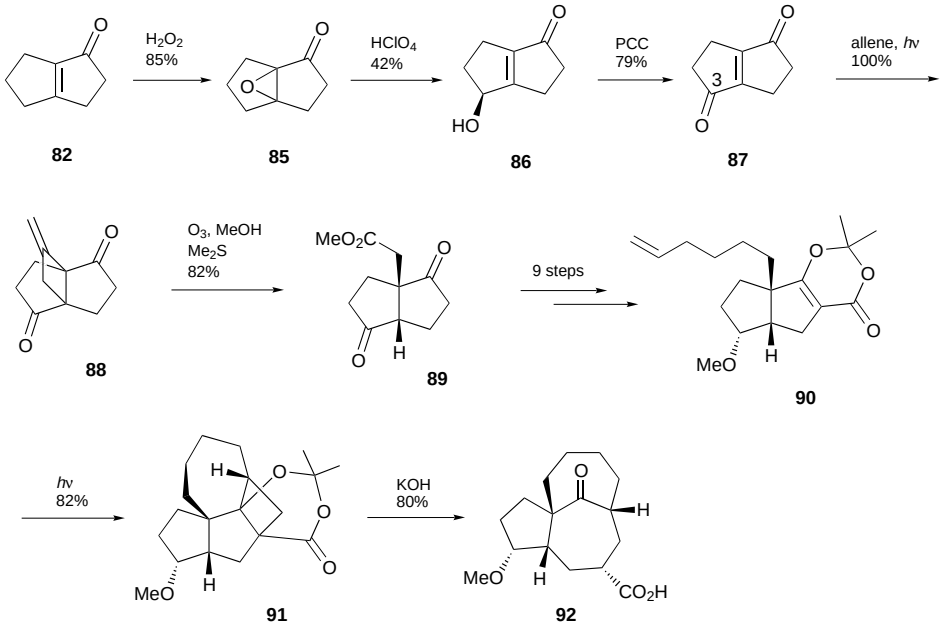
photoadduct **88** in methanol gave the keto–ester **89**, which was transformed to the desired photosubstrate **90** through a nine step sequence. However the length of this sequence and low overall yield demanded a more efficient route.

The second approach is outlined in Scheme 15. The reductive alkylation of **86** with hex-5-enyl iodide produced the desired angularly alkylated product **93** in 45–52% yield. This result reduced the length of the sequence by six steps and delivered C-3β oxygen stereochemistry found in the natural product. Carboxylation of **93** followed by ester exchange with anisyl alcohol, and condensation with acetone under acidic conditions led to the formation of dioxenone **94**. Irradiation of **94** gave the desired photoadduct **95** in 61% yield. Fragmentation of **95** with *p*-toluenesulfonic acid in refluxing methanol gave the ingenane tricycle **96** with the correct C-8β hydrogen stereochemistry as a 3 : 2 mixture of C-6 α : β ester epimers **96a** and **96b**, respectively. Basic fragmentation condition[30] (1% K_2CO_3, MeOH) of **95** provided the 3β-hydroxy ingenane tricycle **97** as a mixture of C-6 β and α ester epimers in a ratio of 6 to 1, respectively.

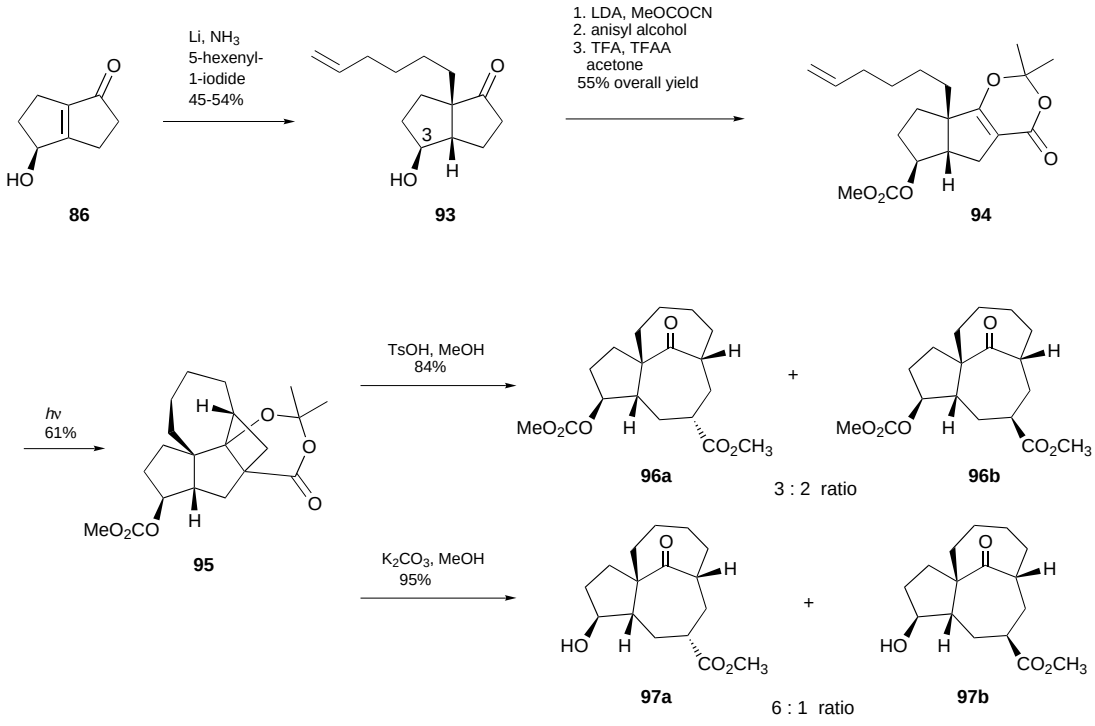
4.3 Advanced ingenol analogues

With an improved method for the preparation of C-3 oxygenated ingenane tricycles in hand, we began to investigate the elaboration of the functionality in the A/B ring system and prepared a series of functionalized analogues, some of which were found to be biologically active *in vitro*.[31]

In an effort to prepare the first C-3, C-4 dihydroxylated ingenane analogues *via* dihydroxylation of the $\Delta^{3,4}$ alkene, the elimination reaction of C-3 hydroxy group of various ingenanes



Scheme 14

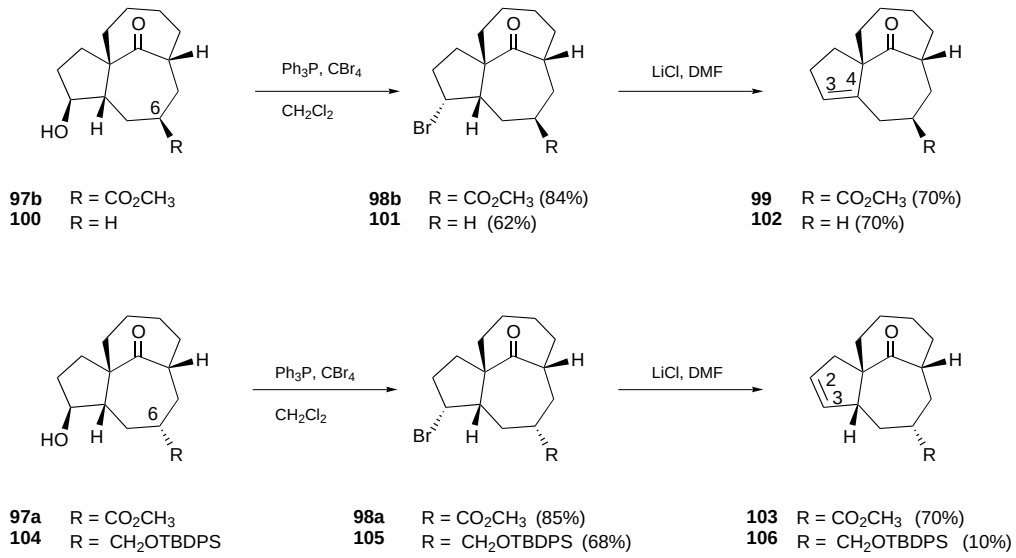


Scheme 15

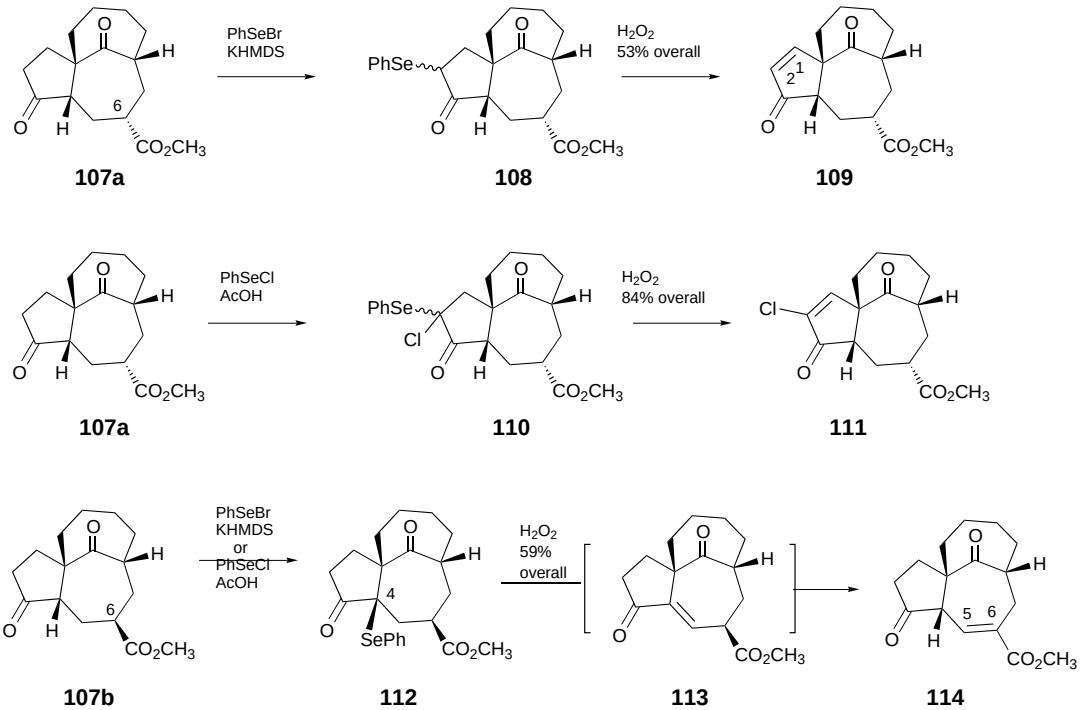
was examined.[5] The Mitsunobu bromination of **97b** (C-6β ester), **97a** (C-6α ester), **100** and **104** proceeded to give the inverted C-3α bromides **98b**, **98a**, **101** and **105**, respectively. These compounds were submitted to standard elimination conditions (LiCl, DMF, reflux) to produce the corresponding alkenes. Bromide **98b** led to the exclusive formation of the $\Delta^{3,4}$ alkene product **99**. The compound **101**, which lacks the ester substituent at C-6, also produced only the $\Delta^{3,4}$ alkene **102**. In contrast, it was found that the C-6α ester **98a** yield only the $\Delta^{2,3}$ alkene **103** in the same reaction conditions (unlike the C-6β isomers, elimination of the C-6α isomers needed long periods of refluxing). The elimination reaction of a silyl ether **105** was examined to probe the involvement of the C-6α ester functionality during the reaction. Under the same conditions, the $\Delta^{2,3}$ product **106** was the only alkene product observed, albeit in low yield, indicating that the ester functionality was not involved during the reaction.

The regioselectivity of selenation for ketone **107** was also examined.[30] The selenation of C-6α ester ketone **107a** with different bases such as LDA, LHMDS, KHMDS also occurred at C-2 to give exclusively $\Delta^{1,2}$ enone **109** in good yield after oxidative elimination. However, treatment of ketone **107a** with excess phenylselenyl chloride in acetic acid, followed by oxidation with hydrogen peroxide resulted in the 2-chloro $\Delta^{1,2}$ enone **111**. Different regioselectivity was observed when the C-6β ester ketone **107b** was treated with phenylselenyl halide. Selenation, in both acetic acid and basic conditions (KHMDS in THF), occurred at the more substituted carbon (C-4). Surprisingly, after oxidative elimination of the selenide **112** with hydrogen peroxide, the $\Delta^{5,6}$ unsaturated ester **114**, which resulted from enone **113** in the reaction conditions, was obtained.

These results suggest the importance of the stereochemistry of the C-6 substituent in the regioselective formation of alkenes



Scheme 16



Scheme 17

or enones. The C-6α substituent induces the formation of the less substituted alkene products, whereas the C-6β substituent has the opposite effect. We reasoned that a C-6 substituent induced the different conformation of the ingenane tricycle nucleus as shown in Fig. 5.

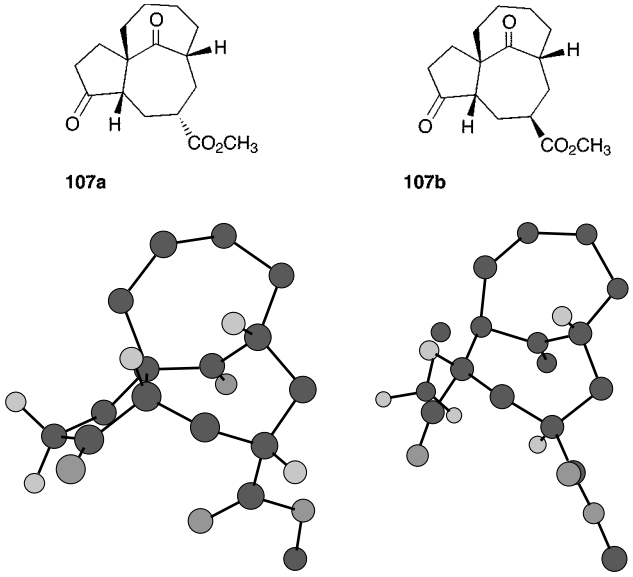


Fig. 5

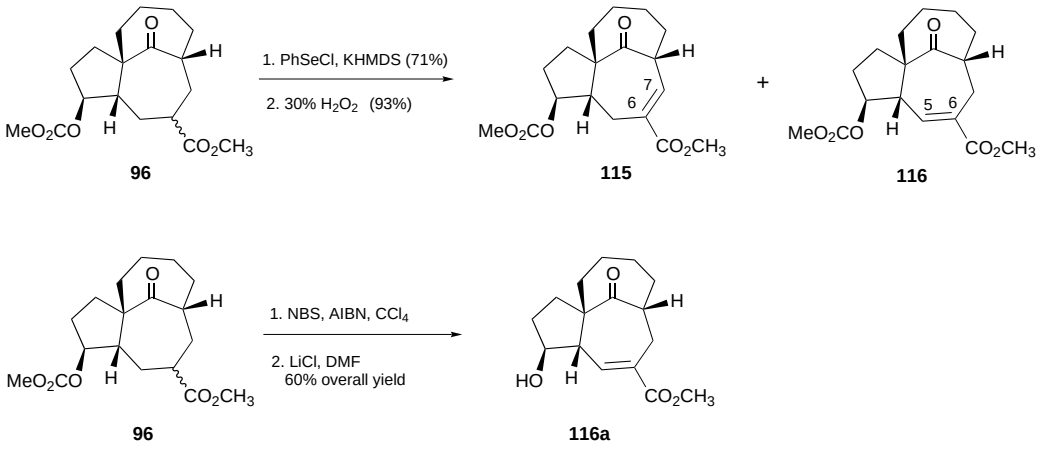
After extensive investigation, we could introduce the ingenane B ring $\Delta^{6,7}$ alkene into **96** as outlined in Scheme 18. Selenation (KHMDS, PhSeCl) of a mixture of C-6 ester isomers **96** gave a single selenide; oxidative elimination of the resulting selenide using H_2O_2–CH_2Cl_2 produced a mixture of products consisting of a 5 : 1 ratio of the $\Delta^{6,7}$ **115** and $\Delta^{5,6}$ unsaturated ester **116** respectively.[20,30] While selenation–oxidation of C-6 esters led to the formation of the $\Delta^{6,7}$ unsaturated ester **115**, it was found that reaction of **96** with NBS–AIBN in refluxing carbon tetrachloride, followed by treatment of the derived mixture of α-bromoesters with excess lithium chloride in refluxing DMF led to the exclusive formation of the $\Delta^{5,6}$ unsaturated ester **116a**, the first example of the selective functionalization of C-5 as the $\Delta^{5,6}$ alkene. Compound **116a** is the key intermediate for the completion of the B ring functionalization, which will be discussed in the following sections.

The synthesis of advanced ingenol analogue **122** is outlined in Scheme 19.[5] The final compound **122** has all the functionality of the AB rings of ingenol except the C-2 methyl and C-5 hydroxy groups. It is noteworthy in that stereoselective dihydroxylation (**99** to **117**) at C-3 and C-4, and regioselective introduction of $\Delta^{6,7}$ unsaturation (**117** to **118**) have been achieved. However, this key intermediate could be obtained only from the C-6β ester of **96** or **97**, which are the minor isomers from retro-aldol fragmentation. The isomerization of the C-6α ester **96a** to the β ester **96b** has not been achieved under a variety of conditions.

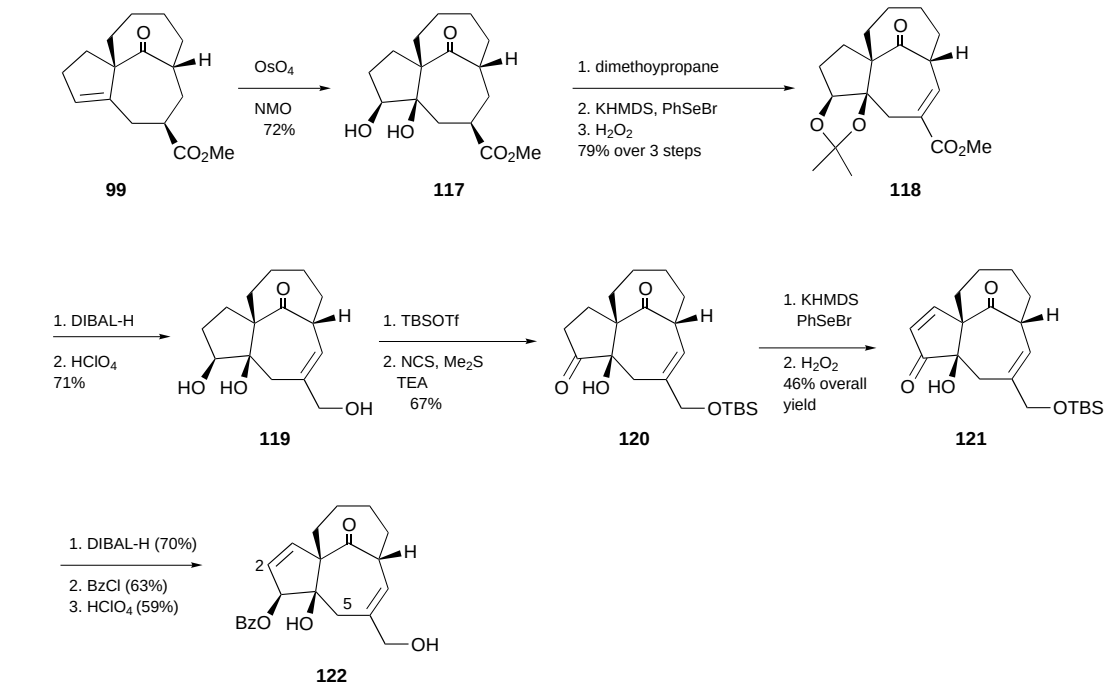
We next turned our attention to the introduction of the $\Delta^{6,7}$ alkene at early stage of the synthetic scheme.[30] Unsaturated ester **115**, obtained as mentioned above (Scheme 18), was reduced with DIBAL-H in THF to give the desired allylic alcohol **123**. Protection of the resulting primary allylic alcohol with *tert*-butyldiphenylsilyl chloride, followed by oxidation of C-3β alcohol with PCC produced ketone **124**. In order to introduce the $\Delta^{1,2}$ alkene, a variety of reaction conditions were investigated. Even though high regioselectivity had been realized with the saturated analogues (Scheme 17), regioselective functionalization of the $\Delta^{6,7}$ alkene ketone **124** (*i.e.* selenation or enol silyl ether formation) could not be achieved. Efforts to introduce the 4-hydroxy group before the unsaturation only led to decomposition of starting material under various reaction conditions. However, we could functionalize C-2 selectively through activation as a β-ketoacid. Carboxylation of **124** followed by treatment with Eschenmoser's salt led to the exclusive formation of α-methylene ketone **125**. Initial attempts to prepare **125** directly from **124** using Eschenmoser's salt have not been successful. Isomerization of **125** to the endocyclic enone **126** was achieved by using rhodium trichloride as a catalyst. The enone **126** was reduced under Luche conditions and resulting α-alcohol was converted to the β-benzoate under Mitsunobu conditions to provide **127**, an analogue of ingenol containing both A and B ring unsaturations.

Having achieved the synthesis of compound **127** which has all the functionality of the A and B rings except the C-4 and C-5 hydroxy groups, we began to investigate possible routes for the introduction of these remaining functionalities.[30] In order to introduce the C-5 hydroxy functionality, allylic oxidation and allylic bromination of **115** were investigated. Neither allylic oxidation with SeO_2 nor allylic bromination with NBS–AIBN produced the desired corresponding C-5 alcohol or bromide.

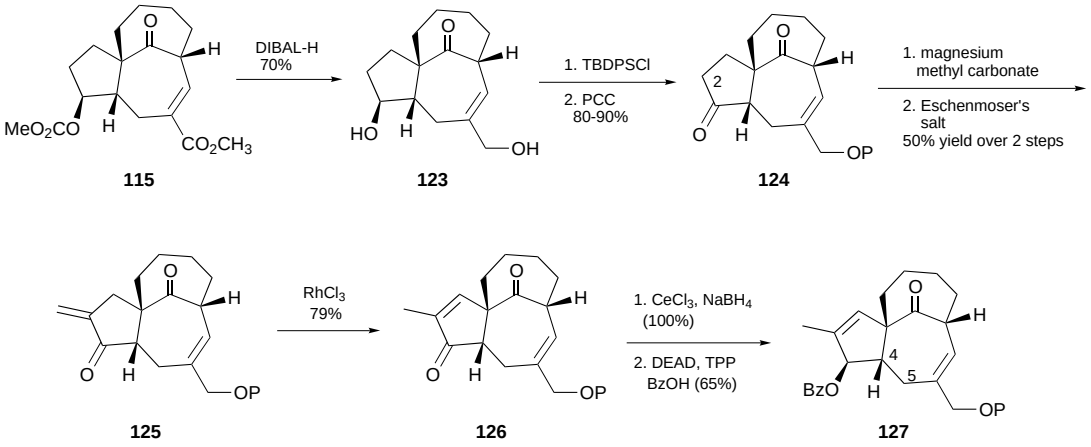
The $\Delta^{6,7}$ unsaturated ester route was abandoned and we began working with the $\Delta^{5,6}$ unsaturated ester **116a**. Unfortunately, it was found that the lack of reactivity of $\Delta^{5,6}$ unsaturated esters (**116** and **116a**) under a variety of reaction conditions (such as ene reaction with singlet oxygen, dihydroxylation with OsO_4 or epoxidation) prevented the introduction of the C-5 hydroxy group. Presumably, the sterically congested environment around C-5 causes the unsaturated esters (**116** and **116a**) to be unreactive. Reaction of C-3 hydroxy $\Delta^{5,6}$ unsaturated ester **116a** with methanesulfonyl chloride followed by treatment with base led to the formation of the diene ester **128** in excellent yield. Epoxidation of **128** with mCPBA occurred with high degree of regio- and stereochemical control to give **129**. Treatment of the epoxy un-



Scheme 18



Scheme 19

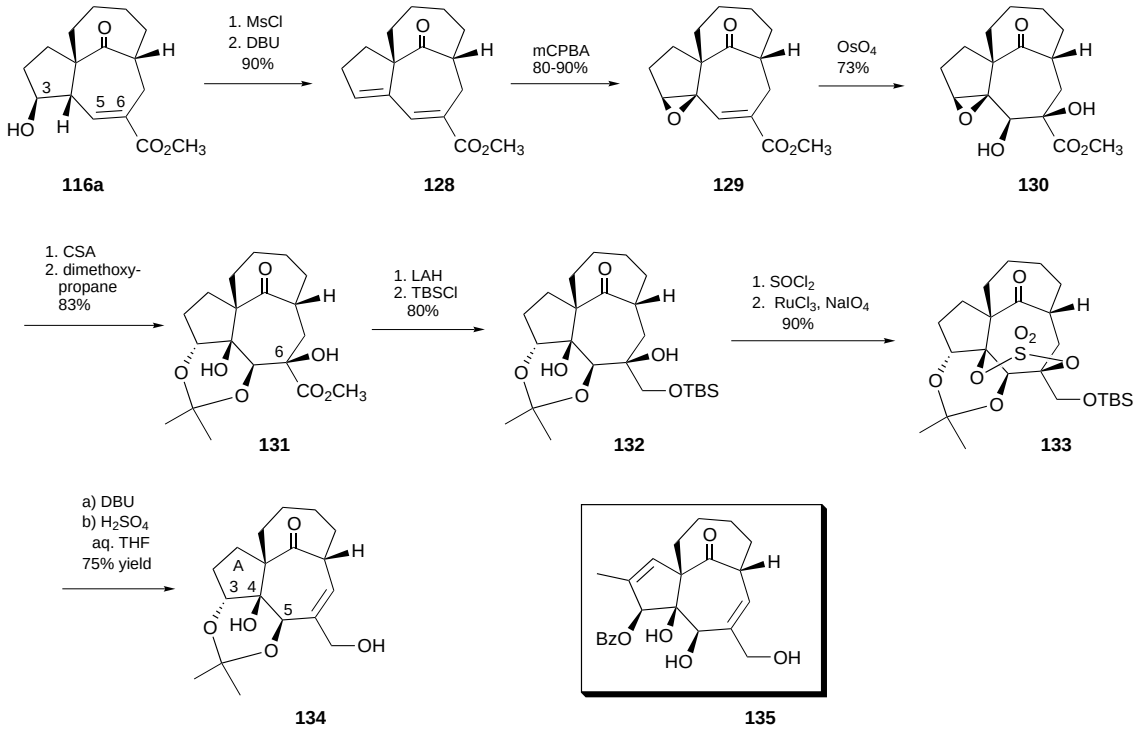


Scheme 20

saturated ester **129** with osmium tetroxide led to the exclusive formation of epoxy diol **130**. The dihydroxylations of the 3,4-dihydroxy $\Delta^{5,6}$ unsaturated ester and the 3-hydroxy unsaturated esters **116** were not successful under the same reaction conditions. We reasoned that the epoxide changed the conformation of the ingenane nucleus to reduce the steric environment around C-5. Epoxy diol **130** was treated with camphorsulfonic acid in wet acetone to yield the tetraol, which on exposure to excess dimethoxypropane produced the acetonide **131** as a single isomer. The structure and relative stereochemistry of **131** have been verified by X-ray crystallographic analysis.

The selective elimination of C-6 hydroxy group of **131** to introduce the $\Delta^{6,7}$ unsaturation was next investigated. Various standard elimination reaction conditions were examined, such as Burgess reagent, Ph_3BiBr_2/I_2, $PPh_3/CCl_4/CH_3CN$, Furukawa reagent, Martin sulfurane, and base treatment of the C-6 triflate or mesylate. None of the above conditions produced the desired $\Delta^{6,7}$ unsaturated ester. We decided to reduce the C-6 ester to the corresponding alcohol, because of the instability of the $\Delta^{6,7}$ unsaturated esters we experienced in many cases. The selective reduction of α-hydroxy ester in the presence of the hindered C-9 ketone was conducted with $LiAlH_4$ in THF. The resulting primary alcohol was selectively protected with TBDPSCl or TBDMSCl. The elimination of the C-6 hydroxy group of **132** was also investigated for the introduction of unsaturation of the B ring. Various standard conditions only resulted in decomposition of the starting compound without formation of the desired allylic alcohol **134**.

The lack of literature procedures for the efficient transformation of a diol or a triol to an allylic alcohol led us to investigate new methodology for achieving this transformation. We turned our attention to cyclic sulfates,[32] the chemistry of which is analogous to that of an epoxide, only more reactive. The elimination of an unactivated cyclic sulfate had not been reported in the literature, whereas some examples of elimination reactions with activated cyclic sulfites were known.[33] The C-4/C-6 cyclic sulfate **133** was prepared by treatment of diol **132** with thionyl chloride in pyridine, followed by oxidation with $RuCl_3$ and $NaIO_4$.[32] Reaction of the cyclic sulfate **133** with DBU in refluxing toluene, with subsequent hydrolysis in mild acidic conditions afforded the desired allylic alcohol **134** in 75% yield. To the best of our knowledge, this is the first example of an elimination reaction of a non-activated cyclic sulfate.[29] The eliminated product **134** has all the functionality of the B ring of ingenol, including the C-4, C-5 hydroxy groups



Scheme 21

with the correct stereochemistry, as well as the C-3 hydroxy group, which might be utilized for full functionalization of the A ring.

5 Biological evaluation of ingenol analogues

The process of PKC activation is complex, requiring multiple components. At this time, neither the basis for the binding of a wide variety of structural types (Fig. 1) to the PKC receptor nor the pharmacophore of PKC activators has been unambiguously established.[4,5,20] Even though the crystal structure of phorbol-bound PKC confirmed some predictions of tumour promoter models,[6] the results for phorbol esters do not necessarily translate to *in vivo* and to other type of tumour promoters.

Several groups have studied the pharmacophore of the seemingly structurally unrelated activators of PKC, using synthetic structure–activity and molecular modelling studies.[5] The molecular modelling approach is used to find similarities in the three-dimensional array of homologous functional groups.[5] A synthetic structure–activity study is used to find the structural requirements for activation of PKC, focused on the synthesis of specifically modified derivatives of natural products. Recently, we evaluated the biological potency of synthetic ingenane analogues which contain some oxygen functionalities along with correct inside–outside intrabridgeheaded stereochemistry present in the natural product.[5,20,31] The C-3 monobenzoate of ingenol **136** and its analogues were evaluated for their ability to interact with the regulatory site on PKC, as quantitated by the inhibition of [^{3}H]PDBU binding to the enzyme reconstituted in the presence of phosphatidylserine and assayed for 5 min at 37 °C. Ingenol 3-monobenzoate **136** yielded an apparent K_i of 0.15 ± 0.03 nM (mean ± SEM, n = 4) for protein kinase C-α, the C-4 deoxy ingenane analogue **137** had a K_i of 165 ± 21 nM (mean ± SEM, n = 3) and the C-4 hydroxylated analogue **122**, assayed in parallel, had a K_i of 561 ± 94 nM (mean ± SEM, n = 3). Ingenol analogue **7** which has *cis* rather than *trans* intrabridgehead stereochemistry (C-8 epimer of ingenol, iso-ingenol) is completely inactive as mentioned before. These data have important implications for the understanding of structure–activity relations for the ligand binding site on the protein kinase C. Further testing of these and related compounds, as well as the synthesis and biological evaluation of more highly functionalized ingenol congeners such as **138** (which can be obtained by deprotection of **127**, Scheme 20), **122** (prepared in Scheme 19) and **135**, are currently underway in our laboratory and our results will be reported in due course.

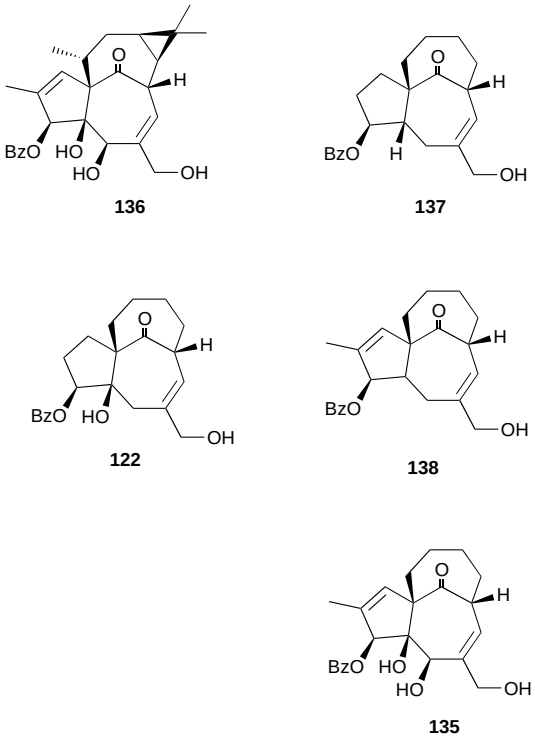


Fig. 6

6 References

1 H. Opferkuch and E. Hecker, *Tetrahedron Lett.*, 1974, **15**, 261.
2 R. R. Upadhyay and G. Mohaddes, *Curr. Sci.*, 1987, **56**, (**20**), 1058.

3 For recent reviews, see P. J. Blackshear, *J. Biol. Chem.*, 1993, **268**, 1501; L. V. Decker and P. J. Parker, *Trends in Biochem. Sci.*, 1994, **19**, 73.
4 R. Schmidt and A. Aitken, in *Naturally Occuring Phorbol Esters*, ed. F. Evans, CRC Press, Inc., Boca Raton, 1986, pp. 245; E. Hecker, in *Carcinogenesis: A Comprehensive Survey, Mechanisms of Tumor Promotion and Cocarcinogenesis*, vol. 2; ed. T. J. Slaga, A. Sivak and R. K. Boutwell, Raven, New York, 1987, p. 11.
5 J. D. Winkler, B.-C. Hong and S. Kim, *Synlett*, 1995, 533, and references cited therein.
6 Recently X-ray crystal structure of PKC–phorbol ester complex have been advanced to explain the pharmacophore of the PKC activators, see *Chem. Eng. News*, 1995, Oct. 23, p. 21; and references cited therein.
7 B. Shearer, B. Sullivan, J. Carter, R. Mathew, P. Waid, J. Connor, R. Patch and R. Buch, *J. Med. Chem.*, 1991, **34**, 2931; and for a review of PKC regulation; P. C. Gordge and W. J. Ryves, *Cellular Signalling*, 1994, **6**, 871.
8 P. Wender and F. McDonald, *J. Am. Chem. Soc.*, 1990, **112**, 4956.
9 For recent review of the synthesis of tumour-promoting diterpenes, see: J. H. Rigby, in *Studies in Natural Products Chemistry*, ed. A.-u. Rahman; Elsevier Science Publishers, Amsterdam, 1993, **12**, p. 233.
10 For a recent review of inside–outside chemistry, see: R. W. Alder and S. P. East, *Chem. Rev.* 1996, **96**, 2097.
11 R. Funk, T. A. Olmstead and M. Parvez, *J. Am. Chem. Soc.*, 1988, **110**, 3298.
12 J. H. Rigby, S. D. Rege, V. P. Sandanayaka and M. Kirova, *J. Org. Chem.*, 1996, **61**, 842.
13 J. H. Rigby and S. V. Cuisiat, *J. Org. Chem.*, 1993, **58**, 6286.
14 R. Funk and G. Bolton, *J. Am. Chem. Soc.*, 1986, **108**, 4655.
15 M. Harmata, S. Elahmad and C. L. Barnes, *Tetrahedron Lett.*, 1995, **36**, 1397.
16 G. Mehta and V. Pathak, *J. Chem. Soc., Chem. Commun.*, 1987, 876.
17 L. Paquette, R. Ross and J. Springer, *J. Am. Chem. Soc.*, 1988, **110**, 6192 and references cited therein.
18 R. Funk, T. A. Olmstead, M. Parvez and J. B. Stallman, *J. Org. Chem.*, 1993, **58**, 5873.
19 J. H. Rigby, V. S. Claire, S. V. Cuisiat and M. J. Heeg, *J. Org. Chem.*, 1996, **61**, 7992.
20 J. D. Winkler, B.-C. Hong, A. Bahador, M. G. Kazanietz and P. M. Blumberg, *J. Org. Chem.*, 1995, **60**, 1381 and references cited therein.
21 J. D. Winkler, K. Henegar and P. Williard, *J. Am. Chem. Soc.*, 1987, **109**, 2850.
22 For a review describing this general strategy, see J. D. Winkler, C. M. Bowen and F. Liotta, *Chem. Rev.*, 1995, **95**, 2003.
23 For other reviews of [2 + 2] photocycloaddition, see: M. T. Crimmins, *Chem. Rev.*, 1988, **88**, 1453 and D. I. Schuster, G. Lem and N. A. Kaprinidis, *Chem. Rev.*, 1993, **93**, 3.
24 P. de Mayo, *Pure Appl. Chem.*, 1964, **9**, 597.
25 S. W. Baldwin and J. M. Wilkinson, *J. Am. Chem. Soc.*, 1980, **102**, 3634.
26 J. D. Winkler, J. P. Hey, F. J. Hannon and P. G. Williard, *Heterocycles*, 1987, **25**, 55.
27 J. D. Winkler, B.-C. Hong, J. P. Hey and P. G. Williard, *J. Am. Chem. Soc.*, 1991, **113**, 8839.
28 J. D. Winkler, K. E. Henegar, B.-C. Hong and P. G. Williard, *J. Am. Chem. Soc.*, 1994, **116**, 4183.
29 J. D. Winkler, unpublished results.
30 J. D. Winkler, S. Kim and B.-C. Hong, 212th *American Chemical Society National Meeting*, 1996, Aug., ORGN, No. 161.
31 For the first synthesis of a biologically active analogue of ingenol, see J. D. Winkler, B.-C. Hong, A. Bahador, M. G. Kazanietz and P. M. Blumberg, *Bioorg. Med. Chem. Lett.*, 1993, **3**, 577; and ref. 20.
32 B. B. Lohray, *Synthesis*, 1992, 1035.
33 (*a*) S. K. Kang, D. H. Lee, Y. S. Kim and S. C. Kang, *Synth. Commun.*, 1992, **22**, 1109; (*b*) Y. L. Bennani and K. B. Sharpless, *Tetrahedron Lett.*, 1993, **34**, 2083.
34 M. Tada, T. Kokubo and T. Sato, *Bull. Chem. Soc. Jpn.*, 1970, **43**, 2162.
35 J. D. Winkler and B. Shao, *Tetrahedron Lett.*, 1993, **34**, 3355.

Received, 24th April 1997
Accepted, 16th June 1997