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Synthesis and Biological Activity of Some Novel Side-Chain Analogues of Brassinolide

by

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Abstract

This Thesis describes the preparation and biological activity of some novel side-chain analogues of brassinolide, a highly potent plant growth promoter. The novel analogues were chosen for the purpose of structure-activity studies and with the intention of blocking certain metabolic pathways by which plants deactivate brassinosteroids.

The novel analogues were derived from the treatment of (*threo*)-(2R,3S,5α,22R,23R,24S)-6,6-ethylenedioxy-2,3-isopropylidenedioxy-23,24-epoxy-26,27-dinorcholestan-22-ol with the appropriate Grignard reagents, followed by Baeyer-Villiger oxidation. The products included three C-24 *n*-alkyl analogues (*n*-dodecyl, *n*-hexyl, *n*-propyl), four C-24 cycloalkyl derivatives with ring size ranging from three to six, 25-azabrassinolide, 25-methoxybrassinolide and 25-fluorobrassinolide. The latter two products were obtained by oxymercuration and hydrofluorination steps after the addition of 2-propenyl magnesium bromide, respectively, to the precursor *threo*-epoxide. The *erythro*-epoxide isomer that had been obtained as a byproduct of the required *threo*-epoxide did not undergo ring-opening and was recovered in all of these preparations. It was recycled by tellurium-mediated deoxygenation to its corresponding allylic alcohol, thereby regenerating the original mixture of *threo*- and *erythro*-epoxides after Sharpless epoxidation.

The products were then subjected to the rice leaf lamina assay. It was found that long alkyl chains at C-24 resulted in loss of bioactivity. Cyclic substituents displayed activity that increased with decreasing ring size. The cyclopropyl and cyclobutyl analogues proved more active than brassinolide itself. Moreover, side-chain analogues with heteroatoms other than oxygen at C-25 were inactive. Thus, 25-fluoro- and 25-azabrassinolide showed no activity, whereas 25-methoxybrassinolide was only slightly less active than brassinolide.

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For my Mom and Dad

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List of Abbreviations

Ac	acetyl
acac	acetylacetonate
Anal.	Elemental analysis
aq.	aqueous
br	broad
Bu	butyl
B-V	Baeyer-Villiger oxidation
^{o}C	degrees Celsius
ca.	*circa* (approximately)
cal	calorie
cat.	catalytic
cm	centimeters
cm^{-1}	reciprocal centimeters-wavenumbers
^{13}C-NMR	carbon-13 nuclear magnetic resonance
C.M.	complex mixture
d	doublet
δ	chemical shift in ppm downfield from tetramethylsilane
°	degrees
dd	doublet of doublets
ddd	doublet of doublet of doublets
DHDQ-CLB	dihydroquinidine *p*-chlorobenzoate
DIBAH	diisobutylaluminum hydride
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylforamide
dq	doublet of quartets
Et	ethyl
^{19}F-NMR	fluorine nuclear magnetic resonance
g	grams
GC	gas chromatography
h	hours
H_3O^+	acid
^{1}H-NMR	proton nuclear magnetic resonance
Hz	Hertz
i	iso

IAA	indole-3-acetic acid
IR	infrared
J	coupling constant
k	kilo
kg	kilograms
LDA	lithium diisopropylamide
L-DET	(L)-(+)-diethyl tartrate
M	molar
m	multiplet
M^+	molecular ion
m/z	mass to charge ratio
Me	methyl
mCPBA	*m*-chloroperoxybenzoic acid
MEK	methyl ethyl ketone
mg	milligrams
MHz	megahertz
min	minutes
mL	millilitres
µL	microlitres
mm	millimeters
mmol	millimoles
mol	moles
mp	melting point
MS	mass spectrometry
Ms	methanesulfonyl
n-	normal
N.R.	no reaction
ng	nanograms
NMO	N-methylmorpholine-N-oxide
Nu	nucleophile
ox.	oxidation
p.	page
p-	para
PCC	pyridinium chlorochromate
Ph	phenyl
pp.	pages

ppm	parts per million
Pr	propyl
py	pyridine
PVPHF	polyvinyl(polyhydrogen fluoride)
quant.	quantitative
R	generalized alkyl group or substituent
RT	room temperature
Δ	reflux temperature
s	singlet
t	triplet
t-	tertiary
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
Ts	toluenesulfonyl

Chapter One

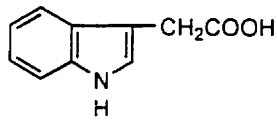
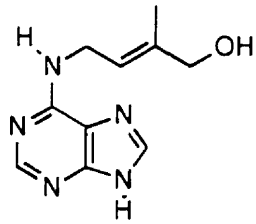
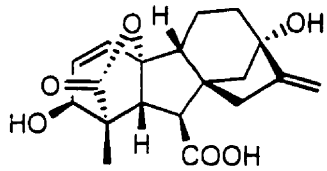
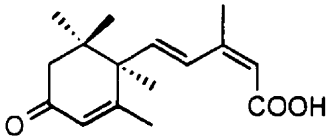
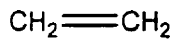
Introduction

1.1 Plant Hormones

Plant growth regulators are used extensively in agriculture to increase crop productivity by modifying plant growth and development. There are numerous types of both synthetic and natural plant growth regulators. Among these, the natural plant hormones are endogenous, organic compounds which influence physiological processes such as growth, differentiation and development at relatively low concentrations.[1a] There are five traditional classes of plant growth hormones that have been identified so far: auxins, cytokinins, gibberellins, abscisic acid, and ethylene. These five classes of compounds can modify plant growth and development in a number of diverse ways, and in the process affect protein synthesis at the gene expression level, and eventually the division, elongation and differentiation of cells. Table 1.1 shows an example of each class, its major function and where it is found in the plant.[1]

In addition to the five traditional classes of plant hormones, another class has recently emerged. These latter compounds have steroidal structures and are called 'brassinosteroids'.[2] They can behave similarly in certain physiological effects, to auxins, cytokinins, and gibberellins.[3,4,5] Although there was initial skepticism as to whether brassinosteroids are true plant hormones, the following evidence suggests that they do indeed meet the required criteria. They occur widely in the plant kingdom, they have a range of physiological effects, some of which are different from the above five traditional classes of plant hormones. They also occur at relatively low concentrations, can be applied to one part of the plant and transported to another location where they elicit a biological response, and they have the ability to regulate gene expression.[6] Brassinosteroids are found in pollen, leaves, flowers, seeds, shoots, galls, and stems in various plant species.[5] Their physiological effects include:[2] increased seed germination, cell elongation and cell division; improved stress resistance to chilling, disease,

TABLE 1.1 Five Traditional Classes of Plant Growth Hormones[1b]

Plant Growth Hormones CLASS: Example	**Physiological effects**	**Where Produced or Found in Plant**
 AUXINS: Indole-3-acetic acid	Stimulate stem elongation, root growth, differentiation and branching, development of fruit	Endosperm and embryo of seeds; meristems of apical buds and young leaves
 CYTOKININS: Zeatin	Affect root growth and differentiation; stimulate cell division and growth, germination, and flowering	Synthesized in many tissues, including roots and transported to other organs
 GIBBERELLINS: Gibberellic acid	Promote seed and bud germination, stem elongation, leaf growth; stimulate flowering and development of fruit; affect root growth and differentiation	Meristems of apical buds, roots, cambial regions young leaves, and embryo
 ABSCISIC ACID: (+)-2-cis-Abscisic acid	Inhibits growth; closes stomata during water stress; counteracts breaking of dormancy	Leaves, stems, green fruit
 ETHYLENE	Promotes fruit ripening; opposes or reduces some auxin effects; promotes or inhibits growth and development of roots, leaves, flowers, depending on species	Tissues of ripening fruits, nodes of stems, senescent leaves

herbicides, drought and salt; decreased fruit abortion and drop; inhibition of root growth and development; and antiecdysteroid activity. These remarkable biological effects quickly stimulated interest in possible agricultural applications, despite the low natural abundance and therefore poor availability, of the brassinosteroids for research use.

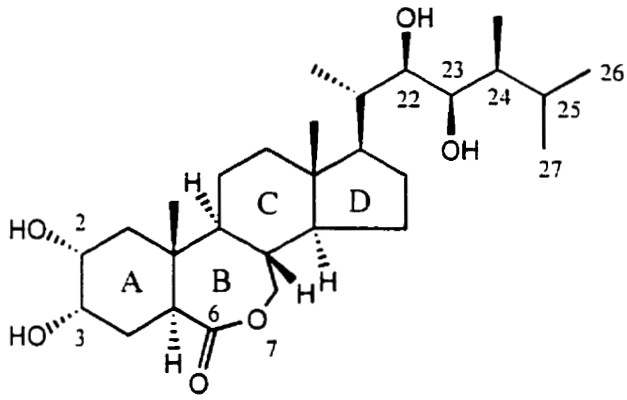
1.2 Brassinolide

1.2.1 History, Isolation and Structural Elucidation

Years before the structure of brassinolide was known, a group of scientists from the U.S. Department of Agriculture (USDA) in Beltsville, Maryland identified a mixture of substances in the pollen of the rape plant *Brassica napus* which was termed 'brassins'. Brassins accelerated plant growth by stimulating both cell elongation and cell division.[7] It was believed then by many that the active component(s) in brassins could comprise a sixth class of plant hormones due to some similarities in their behaviour to that of auxins, cytokinins and gibberellins.[3,4] After laborious efforts to isolate and elucidate the structure of the active ingredient(s) of brassins, the first member of this potential new class of hormones was fully characterized and named brassinolide (**1**) in 1979. From approximately 40 kg of rape pollen gathered by honey bees in Canada, Grove and coworkers[8] isolated 4 mg of pure crystalline brassinolide. X-ray analysis and conventional spectroscopic data confirmed the structure of brassinolide to be (2α,3α,22R,23R,24S)-2,3,22,23-tetrahydroxy-B-homo-7-oxaergostan-6-one (**1**) (Figure 1.1). Brassinolide (**1**) was the first naturally-occurring steroid shown to contain a seven-membered B-ring lactone.[9] In addition to the oxygen atoms in the lactone moiety, the presence of four hydroxyl groups makes this compound highly oxygenated. The presence of four contiguous chiral centers in the side chain makes the structure particularly challenging to the synthetic chemist. Since the discovery of brassinolide, ca. 70 other naturally-occurring brassinosteroids have been reported, of which 36 had been characterized as of 1996, while the existence of the others was inferred from GC-mass spectral evidence.[10] However, brassinolide is the most potent of these with respect to

plant growth-promoting activity. Indeed, effects can be observed in some plant species with the application of ca. 1 ng per individual plant (see Section 1.3).

Figure 1.1 Structure of Brassinolide (**1**)



Brassinolide (1)

1.2.2 Biosynthesis and Metabolism

At the present time, the biosynthesis of brassinolide is not completely understood; however, evidence for two possible biosynthetic pathways has been reported.

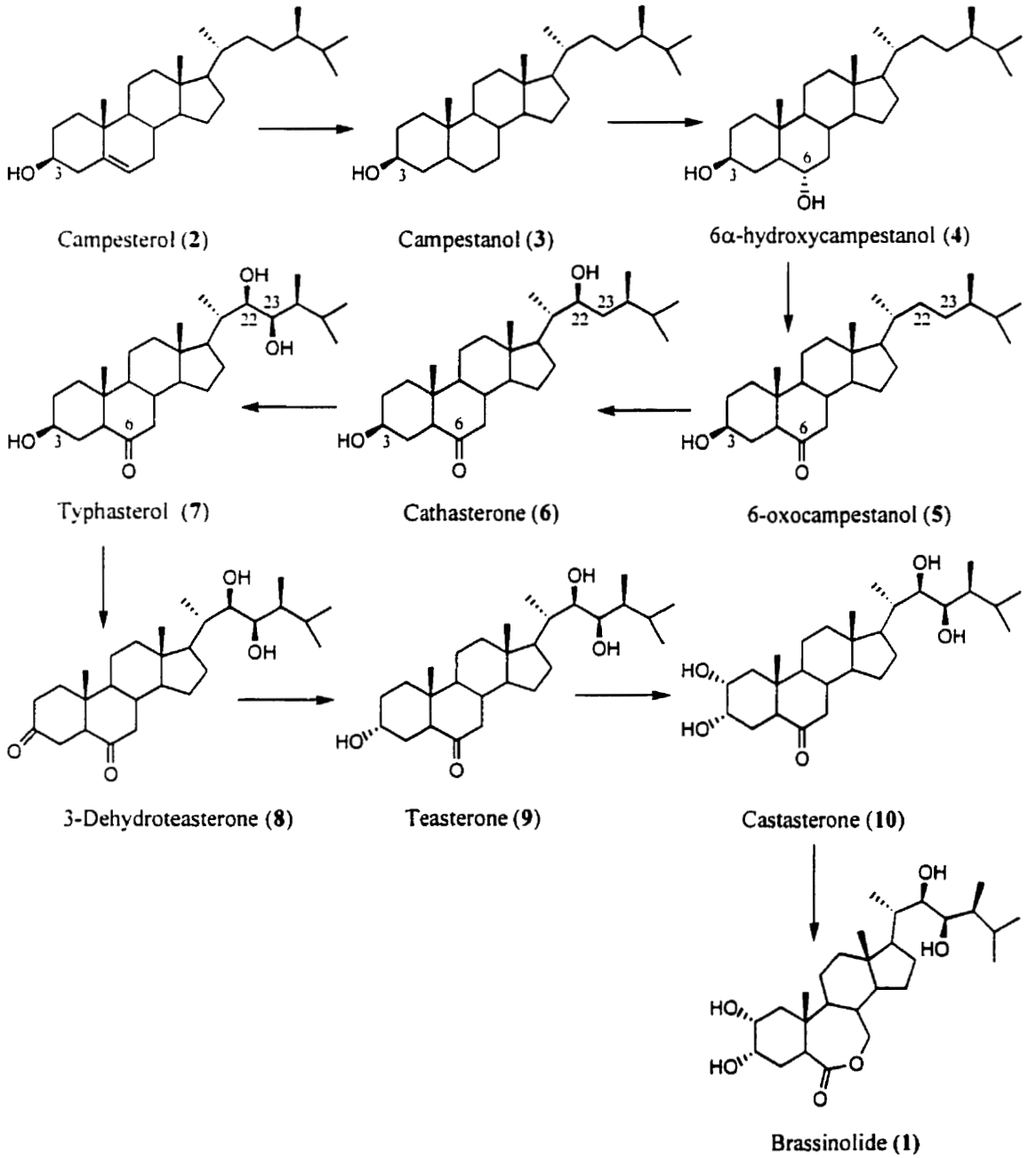
1.2.2.1 Biosynthesis via Teasterone and Typhasterol

In 1991, Yokota et al.[11] proposed a hypothetical biosynthetic pathway leading to brassinolide (**1**) based on the common occurrence and relative biological activities of the naturally-occurring brassinosteroids. That is, it was postulated that the bioactivity of brassinosteroids should increase with each biosynthetic step. In 1995, feeding experiments with isotopically labelled precursors confirmed that the biosynthesis of brassinolide from campesterol (**2**) in cultured cells of *Catharanthus roseus* (Scheme 1.1)[12,13] followed their previously proposed route.

Since campesterol (**2**) was found to be the major component (50%) of the sterol fraction in *Catharanthus roseus* cells, it was assumed to be the primary early precursor of brassinolide. Furthermore, campestanol (**3**), 6α-hydroxycampestanol (**4**), and 6-oxocampestanol (**5**) were identified by GC-mass spectrometry as endogenous compounds and also as metabolites of campesterol (**2**). It was thus postulated that these

precursors of brassinolide (**1**) undergo several reduction, oxidation and epimerization steps to afford **1**. In particular, the conversion of 6-oxocampestanol (**5**) to teasterone (**9**) involves the formation of a vicinal diol in the side chain. The stereospecific introduction of the diol functionality may take place by stepwise hydroxylation at C-22 and C-23, or

Scheme 1.1 Biosynthesis of Brassinolide (**1**) via Teasterone (**9**) and Typhasterol (**7**)

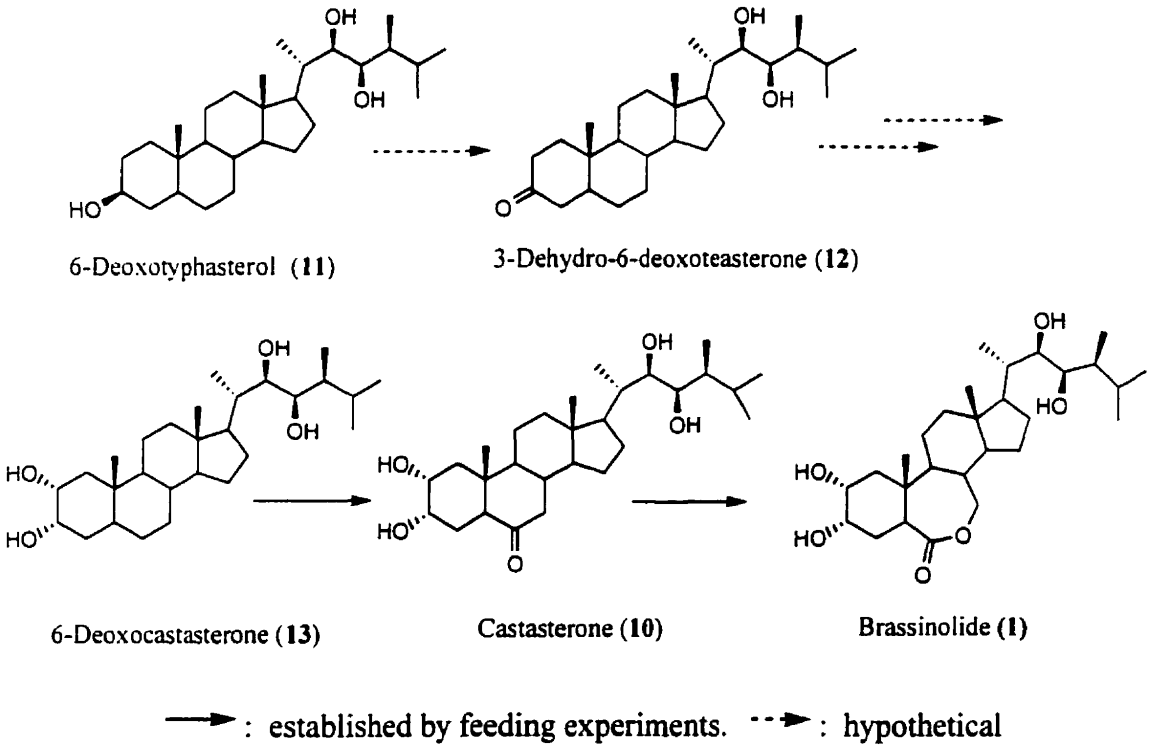


through 22,23-epoxidation and subsequent hydration of the epoxide ring.[12] Epimerization of the hydroxyl group at C-3 presumably takes place via oxidation to the ketone **8**, followed by stereoselective reduction to teasterone (**9**).

1.2.2.2 Biosynthesis via 6-Deoxocastasterone

6-Deoxocastasterone (**13**), a naturally-occurring brassinosteroid with low biological activity, was first found in *Phaseolus vulgaris*. Later, it was also found in several other plants.[14] The widespread occurrence of 6-deoxocastasterone (**13**) and the existence of 6-deoxotyphasterol (**11**) and 3-dehydro-6-deoxoteasterone (**12**) in the pollen of *Robinia pseudo-acacia* prompted Fujioka and coworkers to study further the possibility of **11**, **12**, and **13** being intermediates in the biosynthesis of brassinolide. Using the same plant species, *Cantharanthus roseus*, they were able to identify the presence of **13**. Through deteurium labelling, they were able to demonstrate that **13** is

Scheme 1.2 Biosynthesis of Brassinolide (**1**) via 6-Deoxocastasterone (**13**).



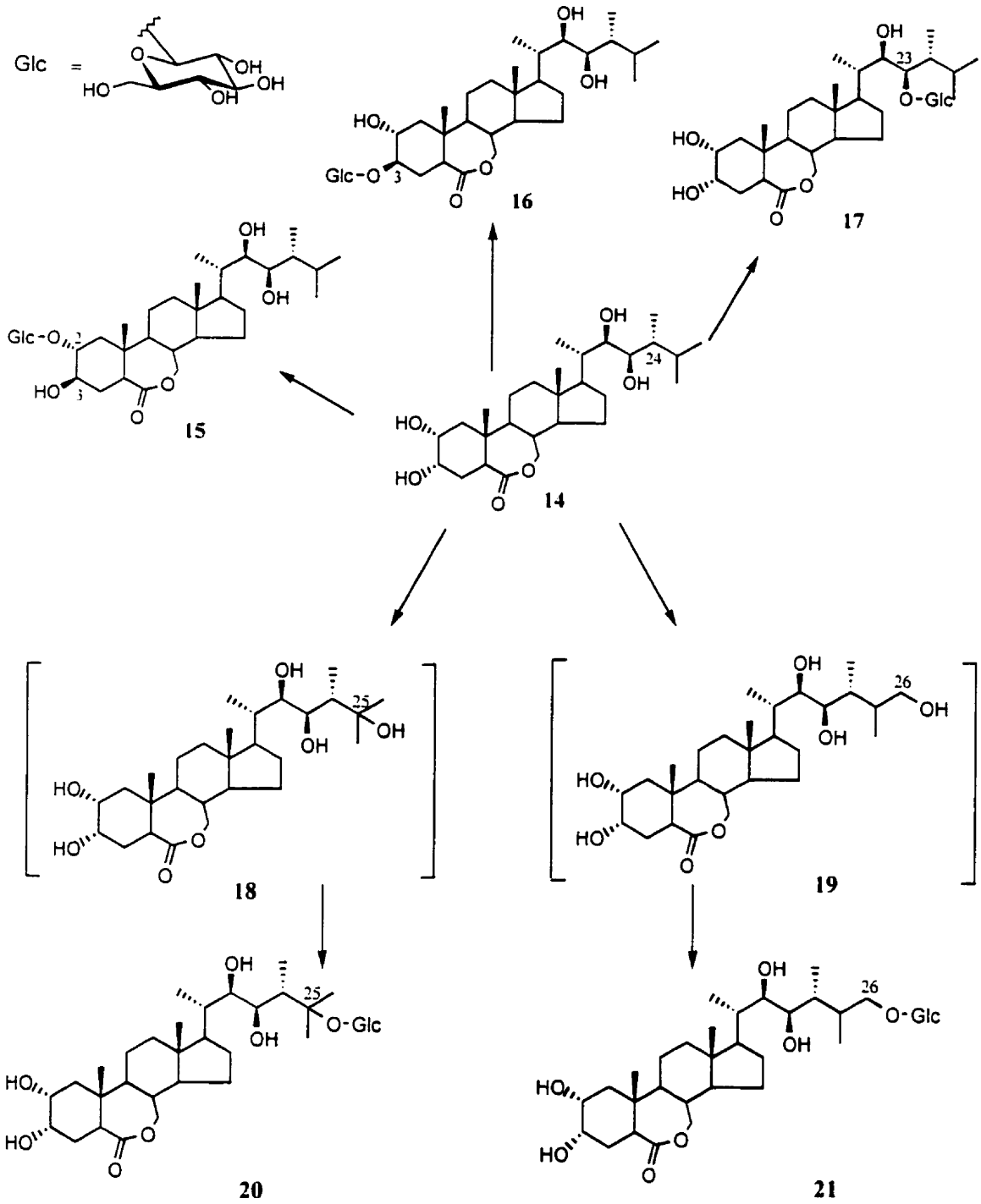
indeed a biosynthetic precursor of castasterone (**10**) (Scheme 1.2).[15] Since the intermediacy of teasterone (**9**) and typhasterol (**7**) had already been established in the biosynthesis of brassinolide (Scheme 1.1), this finding indicates that there are at least two biosynthetic pathways to brassinolide, differing in the order in which oxidation takes place in the side chain and at C-6.

In 1990, a study of *Catharanthus roseus* crown gall cells showed that **10** was the biosynthetic precursor of **1**.[16] However, this is not true for all plant species. For example, in mung beans and rice, it was found that **10** was not converted to **1**, but instead displayed biological activity of its own.[11] Thus, not only are multiple biosynthetic routes to brassinolide possible, but other brassinosteroids such as castasterone, per se, can also exhibit similar biological effects as brassinolide.

1.2.2.3 Metabolism of Brassinosteroids

Yokota et al.[11] and Adam et al.[10,17] demonstrated that glucosylation of brassinosteroids at C-2 (**15**), C-3 (**16**), and C-23 (**17**) can occur in higher plants (Scheme 1.3). Further studies[10] in tomato cell cultures indicated that 24-epibrassinolide (**14**), a naturally-occurring brassinosteroid, is converted into two major metabolites resulting from hydoxylation at C-25 or C-26 to afford **18** and **19**, respectively (Scheme 1.3). Subsequent glucosylations at these sites produce 25-β-D-glucopyranosyloxy-24-epibrassinolide (**20**) and 26-β-D-glucopyranosyloxy-24-epibrassinolide (**21**), respectively. Bioassays of these compounds showed that 25-hydroxy-24-epibrassinolide (**18**) exhibited higher activity than 24-epibrassinolide, whereas 26-hydroxy-24-epibrassinolide (**19**), was less active compared to its C-25 regioisomer. Furthermore, bioassays indicated that the glucosides **15**, **16**, **17**, **20** and **21** were less active than the corresponding free alcohols, which likely indicates that the metabolism of brassinosteroids by glucosylation deactivates the growth-promoting ability of these plant hormones.

Scheme 1.3 Metabolism of 24-Epibrassinolide (**14**)



1.3 Bioassays for Brassinosteroids

A bioassay may be defined as a biological system used to test the activity of a substance with respect to a particular physiological response.[1a] To date there are numerous bioassay systems known for each class of plant hormones and the following sections summarize the three most common bioassays used specifically for brassinosteroids.

1.3.1 Bean Second-Internode Bioassay

The bean second-internode assay was first used to detect brassinosteroids in pollen. When active brassinosteroids are applied to the internode of a decapitated pinto bean (*Phaseolus vulgaris* L.), they cause elongation, swelling, curvature and splitting of the second internode. This effect was termed 'brassin activity'.[18] In this assay, the compound or extract to be tested is dissolved in lanolin. Under a dissecting microscope, the mixture is applied to the second internode of the bean stem of a decapitated 6-day-old bean seedling, at which time the length of the second internode of the stem is about 0.7-1.0 mm. After four days, elongation of the second internode is measured and compared with control plants (treated with lanolin only).[19] The degree of 'brassin activity' in this assay depends on the amount and type of active brassinosteroids present. This bioassay is useful for detecting relatively low concentrations (10 ng) of brassinosteroids. It is also sensitive to gibberellins, but not to auxins and cytokinins. Moreover, the bean second-internode bioassay is highly sensitive to the B-ring lactone-containing brassinosteroids, but not to the 6-keto type.[20]

1.3.2 Wheat Leaf Unrolling Bioassay

The wheat leaf unrolling bioassay was developed in 1985 by Wada et al.[21] In this assay, brassinolide (**1**) and its 6-keto congener, castasterone (**10**), dramatically stimulated leaf unrolling with concentrations as low as 0.5 ng/mL. Typically, wheat seedlings

(*Triticum aestivum* L. cv.) are placed on a wet cotton sheet for 24 h. The germinated seeds are then planted in moist vermiculite and grown in darkness at 26°C for 6 days, and leaf segments (1.5 cm long) are excised from the region 1.5-3.0 cm from the leaf tip under a dim green safe light. The segments are further incubated in dipotassium maleate solution containing the brassinosteroid for 24 h at 30°C in darkness. After the incubation time, the unrolling of the leaf segment is determined by measuring the width with calipers. These measurements showed increases of up to 3.6 cm, whereas control segments grew only 1.2 to 2.0 cm during incubation without the brassinosteroid. Brassinosteroids and zeatin show strong activity in this type of bioassay, whereas gibberellins and certain other cytokinins show only moderate activity. Auxins, on the other hand, inhibited the unrolling of leaf segments. This bioassay is sensitive to all types of brassinosteroids; however, it is only one-tenth as sensitive as the rice leaf lamina assay system (Section 1.3.3). Furthermore, because auxins act as inhibitors in the wheat leaf unrolling test, this bioassay cannot be use to test for synergy between brassinosteroids and auxins.

1.3.3 Rice Leaf Lamina Inclination Bioassay

The rice leaf lamina inclination bioassay was first employed in 1960 by Maeda[22] with auxins and high levels of gibberellins. In the early 1980s, two independent groups reported the use of this bioassay with brassinosteroids.[23,24] Although different types of rice seedlings were used by the two groups, both observed a similar bending response of the rice leaf lamina in rice plants to active brassinosteroids. Wada et al.[23] used normal rice seedlings (*Oryza sativa* L. cv. Kinmaze), and Takeno and Pharis[24] used seedlings of the dwarf rice *Oryza sativa* var., Tan-ginbozu and Waito-C. When active brassinosteroids are applied to rice plants, the angle between the second leaf lamina and the lower part of the stem decreased from an initial angle of ca. 170° in control plants to an acute angle of ca. 70° or even less at high doses of strongly active compounds. Following the procedure devised by Takeno and Pharis,[24] the seedlings of dwarf rice are allowed to germinate in flowing tap water and >95% relative humidity for 3 days.

Selected germinated seeds are then planted on the surface of a 7 mL vial of 0.5% aqueous agar medium and further incubated at 30°C, and 95% relative humidity under the same conditions as above for another 3 days. Once the tested compound is applied, the plants are further incubated for 3 days under the same conditions, after which the angle between the second leaf lamina and the lower part of stem is measured. Gibberellins and auxin have no significant effect on the bending of the second leaf lamina. However, gibberellins significantly induce elongation of the second leaf sheath at very low doses, while brassinosteroids produce only a weak elongation response at high doses. However, even though inactive when applied alone, auxins significantly synergize the effect of brassinosteroids on the rice leaf lamina bending.[24] It should be noted that the rice leaf lamina inclination bioassay has the greatest sensitivity to brassinosteroids, and responses were observed within the concentration range of 0.05 ng/mL to 5 ng/mL.[25] Since the rice leaf lamina inclination bioassay system with seeds of dwarf rice developed by Takeno and Pharis[24] is highly sensitive to brassinosteroids and provides a relatively rapid, simple and convenient assay of brassinosteroid activity, it was the method of choice for testing effects evoked by the novel brassinosteroids that will be described later in this Thesis.

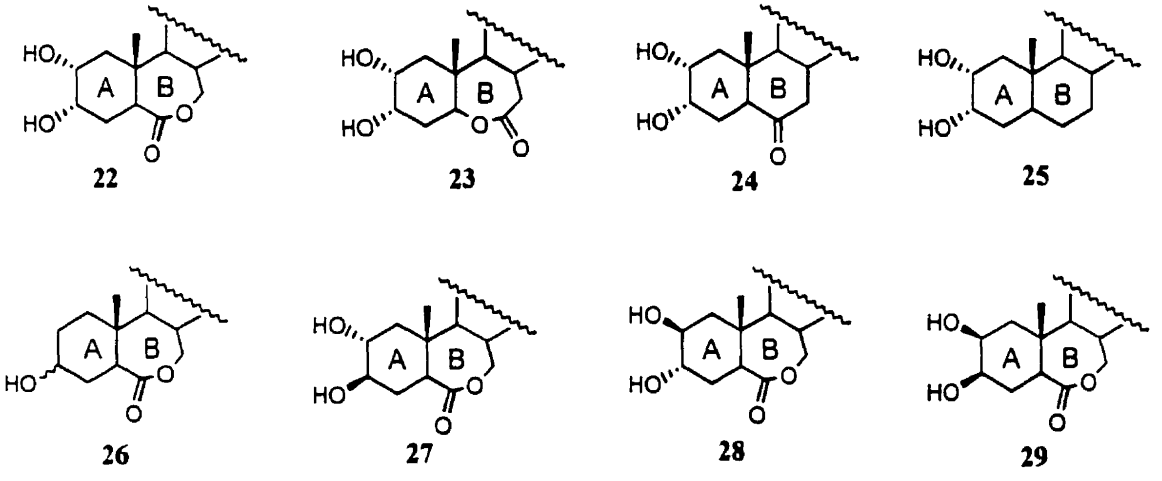
1.4 Structure-Activity Relationships of Brassinosteroids

Since there is a need for a better understanding of the interactions between brassinosteroids and the active sites of putative receptors, extensive structure-activity studies[26-32] have been carried out on a large number of naturally-occurring and synthetic brassinosteroids. Such studies may also provide information that will permit future practical applications of brassinosteroids that have optimal bioactivity with structures that are synthetically more accessible than that of brassinolide itself. The principal findings to date are summarized in sections 1.4.1 and 1.4.2.

1.4.1 Structure-Activity Relationships of the Brassinosteroid Nucleus

Structure-activity studies[26,27,29,30] revealed that the structure of the B-ring plays a crucial role in biological activity (Figure 1.2). Highest activity was observed with a 7-oxa lactone B-ring (**22**). Substitution of the 7-oxa lactone function by the isomeric 6-oxa lactone (**23**) resulted in nearly total loss of activity. Significant activity was also observed with the 6-keto B-ring (**24**), but it was lower than with **22**. For example, it was found that castasterone (**10**) gave ca. 50% of the brassinolide (**1**) response in the rice leaf lamina inclination bioassay.[28] Furthermore, when the B-ring contained no functional group, as in compound **25**, it showed almost no activity.[26,28] The number and orientation of hydroxyl groups on the A-ring also determine the activity of brassinosteroids. Monohydroxysteroids **26** were less active than their corresponding dihydroxysteroids. It was also found that α-oriented hydroxyl groups at C-2 and C-3 are needed for high biological activity. Thus, the order of activity for hydroxyl groups at these positions is as follows: 2α, 3α (**22**) > 2α, 3β (**27**) > 2β, 3α (**28**) > 2β, 3β (**29**).[27] It is noteworthy that all naturally-occurring brassinosteroids found so far possess only *trans*-fused A/B ring system. However, Brosa et al.[29] demonstrated that some brassinosteroids with *cis*-fused ring systems do show biological activity. Further studies in this laboratory are currently underway to determine the effects of other B-ring functionalities (e.g. lactam, cyclic ether), as well as the role of conformational features associated with the B-ring lactone.[30]

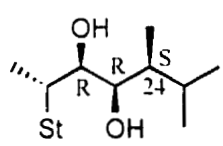
Figure 1.2 Structure Variations of the Brassinosteroidal Nucleus



1.4.2 Structure-Activity Relationships of the Brassinosteroid Side Chain

Structure-activity studies[31,32] of the brassinosteroid side chain indicated that the presence of an alkyl substituent at C-24 is important in obtaining high biological activity. Higher activity was observed with brassinosteroids containing a methyl substituent at C-24 (**30**) than with compounds bearing an ethyl substituent (**31**) or no alkyl substituent (**32**) (Figure 1.3).

Figure 1.3 Structure Variations of the Brassinosteroidal Side-Chain



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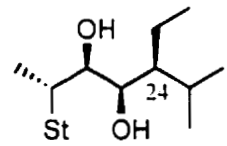

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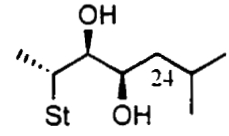
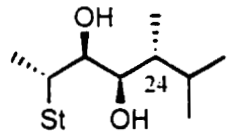
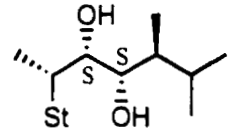


32



33

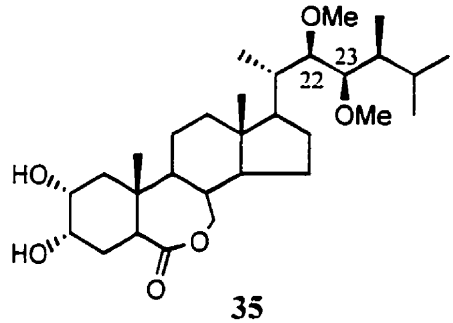


34

St = Various steroid nuclei

The stereochemistry at the C-24 position also plays a crucial role. Brassinosteroids with the S-configuration at C-24 (**30**) exhibit higher activity than those with the R-configuration (**33**).[32] Thus, 24-epibrassinolide (**14**) is only ca. 10% as active as brassinolide (**1**). Furthermore, higher activity is observed with the 22R,23R vicinal diol (**30**) compared to its 22S,23S-isomer (**34**).[32] Very recently, work in this laboratory has demonstrated that free hydroxyl groups on the side chain are not essential for activity, since the C-22,23 ether of brassinolide (**35**) (Figure 1.4) exhibits only ca. 10% of the activity of **1** itself.[33]

Figure 1.4 Dimethyl Ether of Brassinolide



35

In summary, in order for a brassinosteroid to exhibit its highest biological activity on the rice leaf lamina bending, it must possess a 7-oxalactone function in the B-ring, a 2α,3α-diol moiety on the A-ring, a *trans*-fused A/B ring system, as well as a (22R,23R)-vicinal diol moiety and a (24S)-methyl group on the side chain. Indeed, as mentioned earlier, brassinolide, which possesses all of these features, is the most potent of the naturally-occurring brassinosteroids. However, little is known about the effects of chain length, conformational restrictions, or substituents that could prevent metabolic deactivation in the side chain of brassinosteroids upon their bioactivity.

1.5 Practical Applications of Brassinosteroids

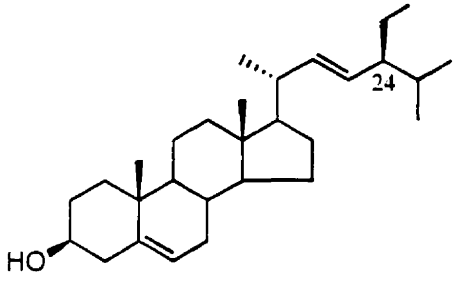
Since the discovery of brassinosteroids, much attention has been devoted to the potential practical application of this new class of plant growth substances in food production. Although **1** is ubiquitous in the plant kingdom, its extremely low abundance makes it very difficult to isolate in useable amounts from natural sources. A number of syntheses of brassinolide have been reported in the literature; [34-36] however, they are generally lengthy and expensive and cannot be used commercially. The unavailability of **1** has made the use of less potent, but more easily synthesized brassinosteroids, such as 24-epibrassinolide (**14**), necessary. Japanese researchers have used compound **14** in large-scale field trials with crops such as wheat, rice, rapeseed and soybeans, which gave increases of ca. 10-20% in crop yields.[37] Even with this less potent analogue, applications of as little as 10-100 mg/hectare often gave significantly higher yields than

controls. Unfortunately, it was found that the results were difficult to reproduce consistently, probably because success depends on factors such as environmental conditions, method of application, and formulation.[38] Even though the preparation of 24-epibrassinolide (**14**) is shorter than that of brassinolide, this compound is only ca. 10% as active as brassinolide; thus, a great deal of interest still remains in developing shorter and more efficient syntheses of brassinolide.

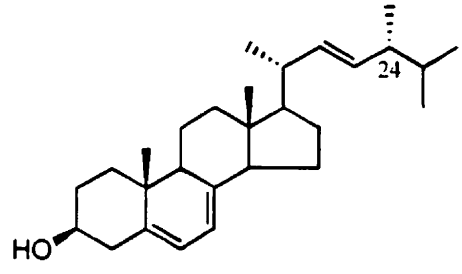
1.6 Previous Syntheses of Brassinolide

The major difficulty in designing a synthesis of brassinolide (**1**) is the generation of the four contiguous chiral centers with the correct stereochemistry in the side-chain. Direct stereoselective *cis*-dihydroxylation of cheap, and naturally-occurring starting materials such as stigmasterol (**36**) from soybean oil, and ergosterol (**37**) from yeast (Figure 1.5) with osmium tetroxide is not a viable route to **1**. This is because the extra

Figure 1.5 Cheap and Naturally-Occurring Potential Precursors of **1**



Stigmasterol (**36**)

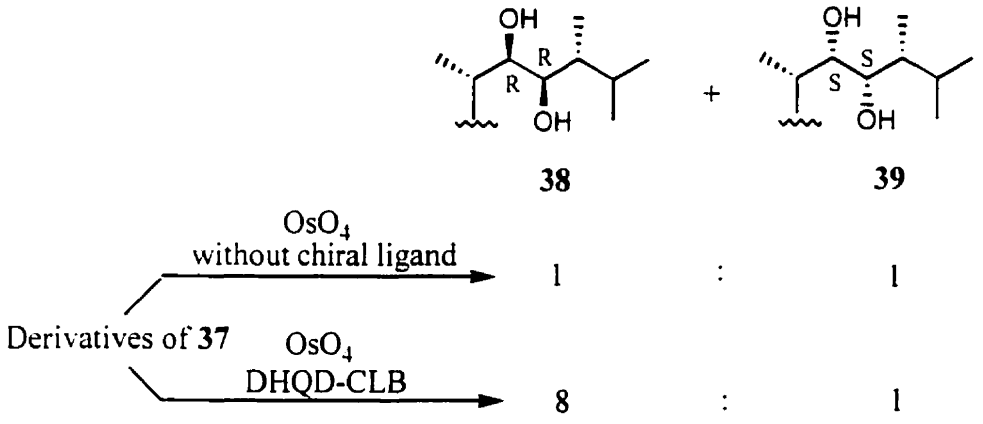


Ergosterol (**37**)

carbon at C-24 in **36** is not easily cleaved and the methyl substituent at C-24 in **37** cannot be epimerized to give the required stereochemistry of the side-chain of **1**. However, direct *cis*-dihydroxylation of derivatives of **37** is a relatively convenient method for obtaining 24-epibrassinolide (**14**), an alternative naturally-occurring brassinosteroid that is being used for large-scale field trials with many crops (see previous section). Investigations of oxidations of existing side-chains of naturally-occurring steroids have

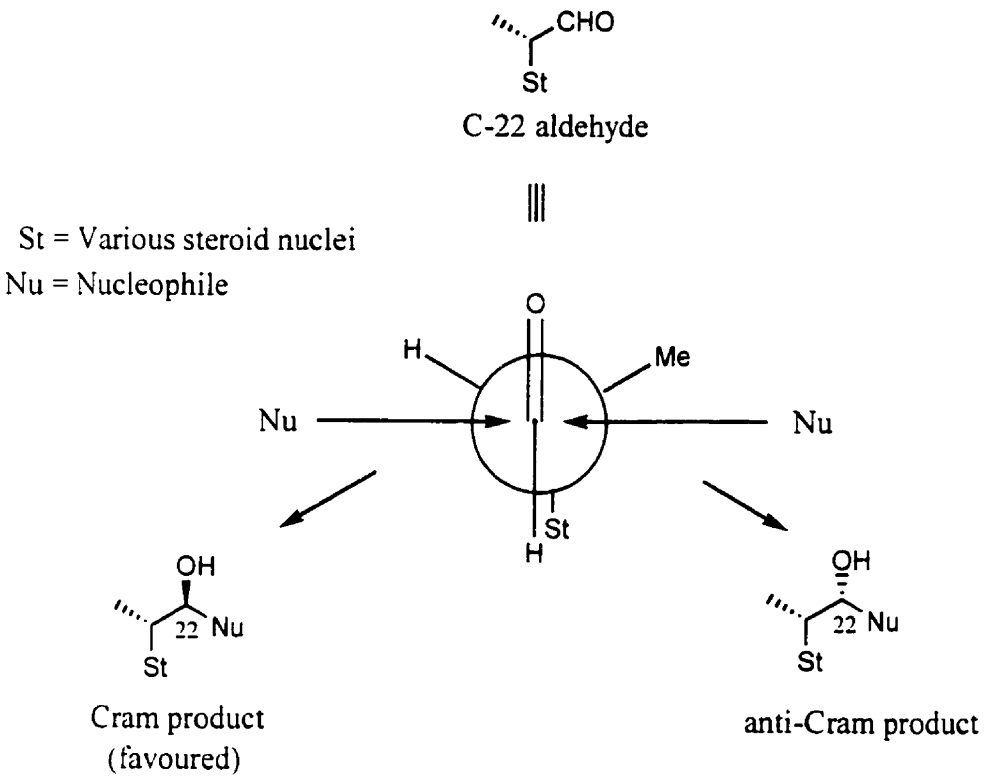
been summarized in a review.[34a] It was found that oxidation of the Δ^{22}-double bond of **37** afforded a 1:1 mixture of the required (22R,23R)-diol (**38**) and the undesired (22S,23S)-diol (**39**) in the absence of chiral ligands. Since diol **38** is a precursor of 24-epibrassinolide (**14**), Sharpless asymmetric dihydroxylation was employed to improve the stereoselectivity of this step. By using dihydroquinidine p-chlorobenzoate (DHQD-CLB) as a chiral ligand, the ratio was increased to 8:1 in favour of **38** (Scheme 1.4).

Scheme 1.4 Direct *cis*-Dihydroxylation



Unfortunately, since no cheap and readily available precursor with the same substituent and configuration at C-24 as brassinolide (**1**) is available, the direct stereoselective *cis*-hydroxylation approach cannot be employed to prepare **1**.[34a] The most frequently used approach is the addition of various nucleophiles to C-22 aldehydes to give predominantly the corresponding Cram products[39] (see Figure 1.6), possessing the brassinolide configuration at C-22. Further modification of the attached nucleophile is then necessary to set up the new stereocenters at C-23 and C-24. These C-22 aldehydes can be easily obtained from ozonolyses of the side chains of suitably protected stigmasterol (**36**) or ergosterol (**37**) derivatives. The following sections illustrate several of the various strategies that have been employed in the past. For more comprehensive treatments of brassinosteroid synthesis, the reader is directed to several reviews,[34] and to two recent reports describing improved syntheses of brassinolide,[35,36] one of which is, in part, the subject of this Thesis.[36]

Figure 1.6 Application of Cram's Rule[39] to Steroidal C-22 Aldehydes

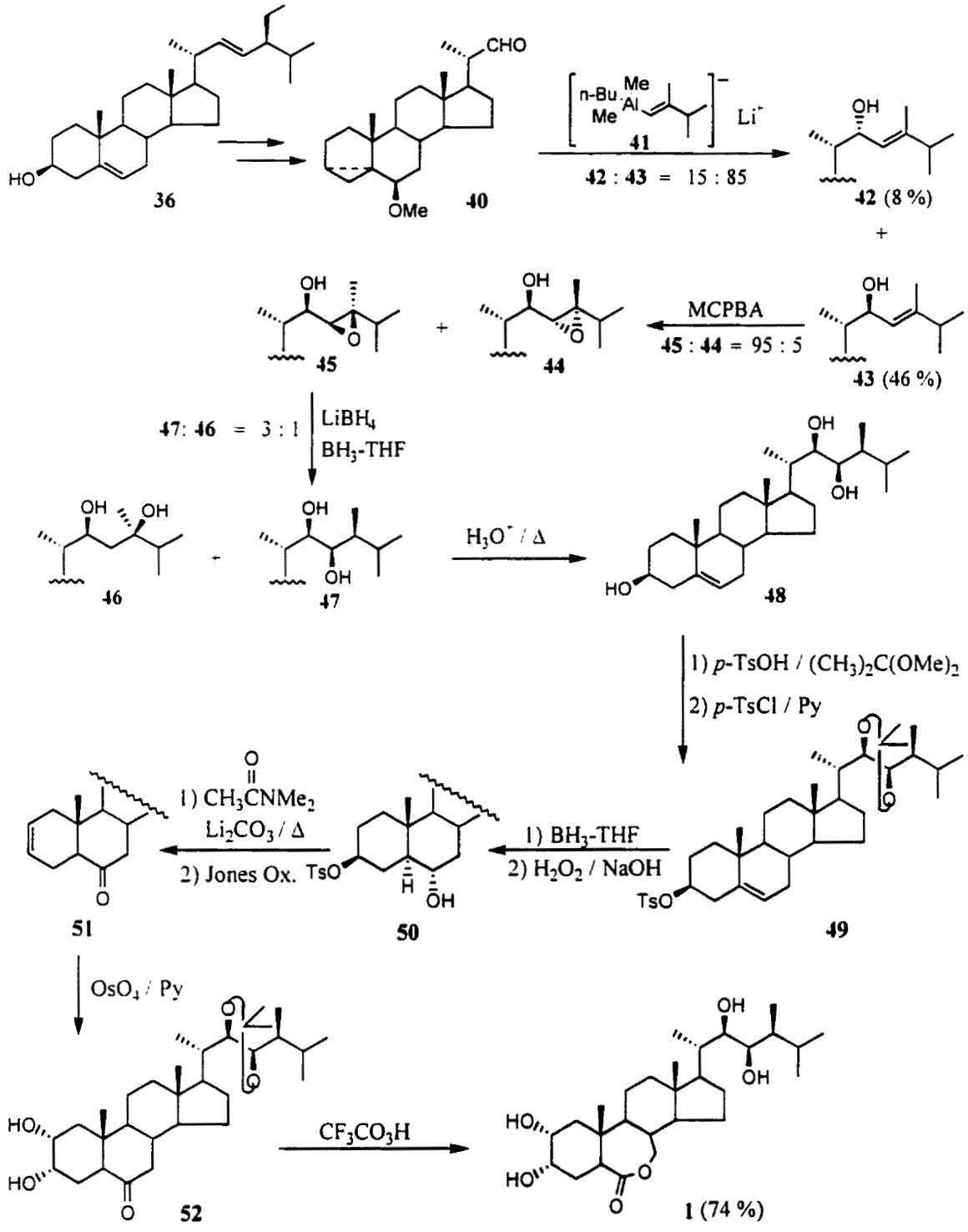


1.6.1 Fung and Sidall's Synthesis

Following the structure elucidation of brassinolide (**1**), the first two syntheses of **1** were reported in 1980 by independent groups.[40,41] Thus, Fung and Sidall (Scheme 1.5)[40] started with stigmasterol (**36**) and converted it into the 3,5-cyclosteroid (20S)-carbaldehyde **40** in three steps. The aldehyde moiety in **40** was elaborated with alanate **41** to furnish a 15:85 mixture of allylic alcohols **42** and **43**. The oxidation of the favoured Cram product **43** proceeded with high stereoselectivity to provide a mixture of the two isomeric epoxy alcohols **44** and **45**. The reaction favoured the formation of *threo* epoxide **45** in the ratio of approximately 95:5. After recrystallization, the desired *threo* epoxide **45** was reduced regioselectively with $LiBH_4/BH_3$-THF to afford a 1:3 mixture of the 1,3-diol **46** and the vicinal diol **47**. Once the elaboration of the side-chain was complete, deprotection of the A and B rings regenerated the 3β-hydroxy and Δ^5 functions in **48**. The vicinal diol of the side chain was then protected as an acetonide, followed by tosylation of the hydroxyl group at C-3, to afford **49**. Compound **50** was produced by the use of a hydroboration-oxidation (BH_3-THF and H_2O_2) process. Elimination was smoothly effected with Li_2CO_3 in dry dimethylacetamide, followed by Jones oxidation to afford enone **51**. The *cis*-hydroxyl groups in C-2 and C-3 were introduced by osmium tetroxide and subsequent Baeyer-Villiger oxidation provided the desired brassinolide (**1**).

Although this synthesis is relatively short, with high stereoselectivity in the epoxidation of **43**, the yield for the alkylation of the C-22 aldehyde **40** with alanate **41** is low. Furthermore, alanate **41** is not commercially available and is difficult to handle, which limits large-scale use.

Scheme 1.5 Fung and Sidall's Synthesis

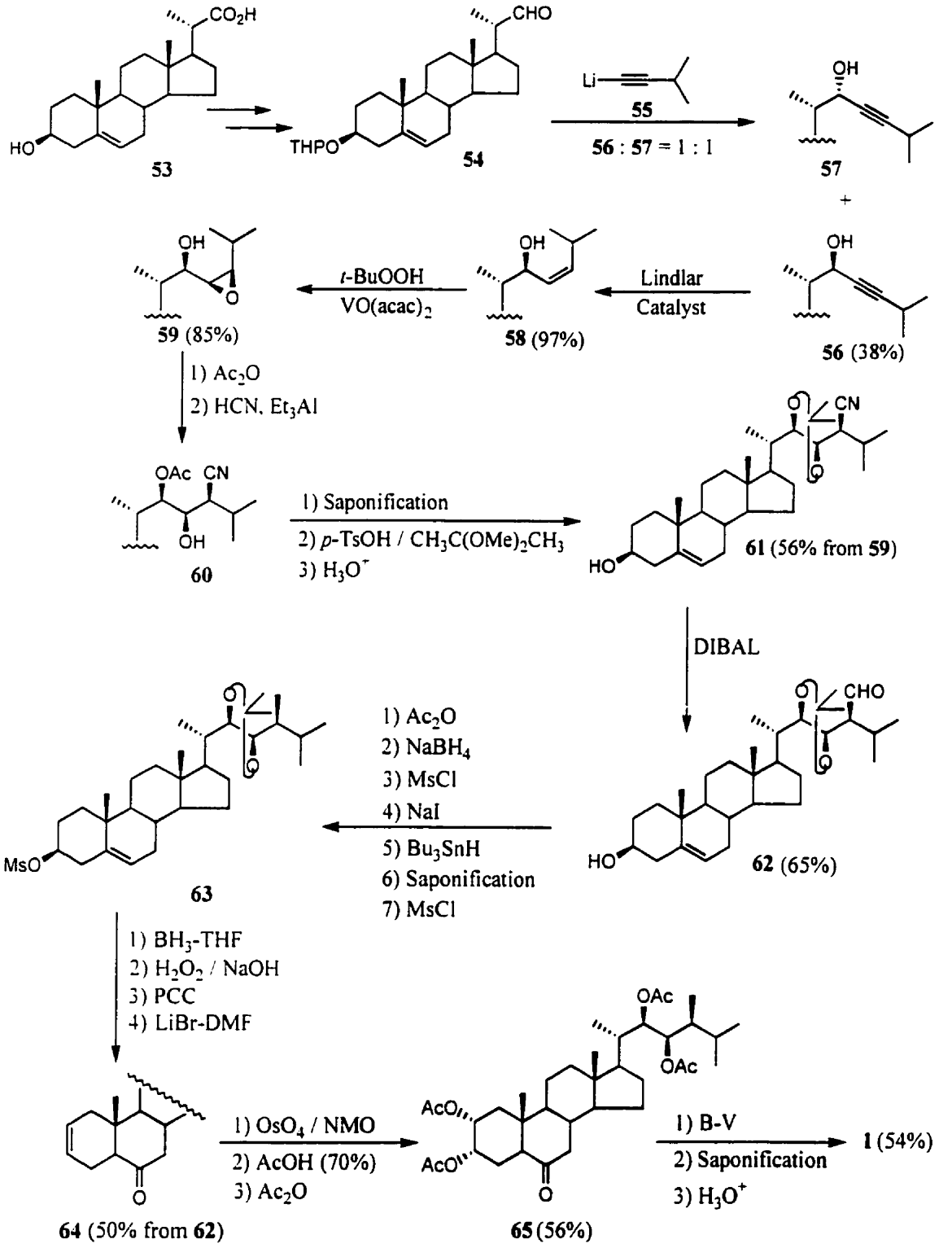


1.6.2 Ikekawa's Synthesis

The other 1980 synthesis of brassinolide by Ikekawa and coworkers (Scheme 1.6)[41] involved the addition of lithium isopropylacetylide **55** to C-22 aldehyde **54**, that was obtained from commercially available dinorcholenic acid **53**. This addition provided a 1:1 mixture of the two epimeric alcohols **56** and **57**. After recrystallization, the desired 22R-isomer **56** (38% yield) was subjected to hydrogenation over Lindlar catalyst, which proceeded with high stereoselectivity to give 97% of the desired *cis*-allylic alcohol **58**. Epoxidation of alcohol **58** with *t*-butyl hydroperoxide-oxovanadium acetylacetonate ($VO(acac)_2$) afforded only the *threo*-epoxide **59** in 85% yield. Epoxide ring opening was achieved with the acetate derivative of **59** by hydrocyanation, with inversion of configuration at C-24 to give **60**. Saponification, followed by acetonide formation and deprotection of the THP group provided intermediate **61** in an overall yield of 56% from **59**. Reduction of the nitrile function of **61** with DIBAL gave the aldehyde **62** in 65% yield. The aldehyde moiety in **62** was converted into the methyl group in five steps (77%), and this was further subjected to saponification and mesylation to give intermediate **63**. The mesylate **63** was regio- and stereoselectively hydroborated, followed by oxidation with PCC and elimination with LiBr-DMF to produce the ketone **64** in an overall yield of 50% from **62**. Osmylation of the Δ^2-double bond with subsequent deprotection and protection reactions provided the tetraacetate **65** in an overall yield of 56%. Finally, Baeyer Villiger oxidation of **65** (80%) followed by removal of the acetate groups (68%) afforded brassinolide (**1**).

This synthesis is very lengthy compared with Fung and Sidall's synthesis. Also, no stereoselectivity was observed in the addition step to the C-22 aldehyde **54** with acetylide **55**. The epoxidation of *cis*-allylic alcohol **58** was, however, carried out effectively to provide only the desired epoxide **59** in high yield.

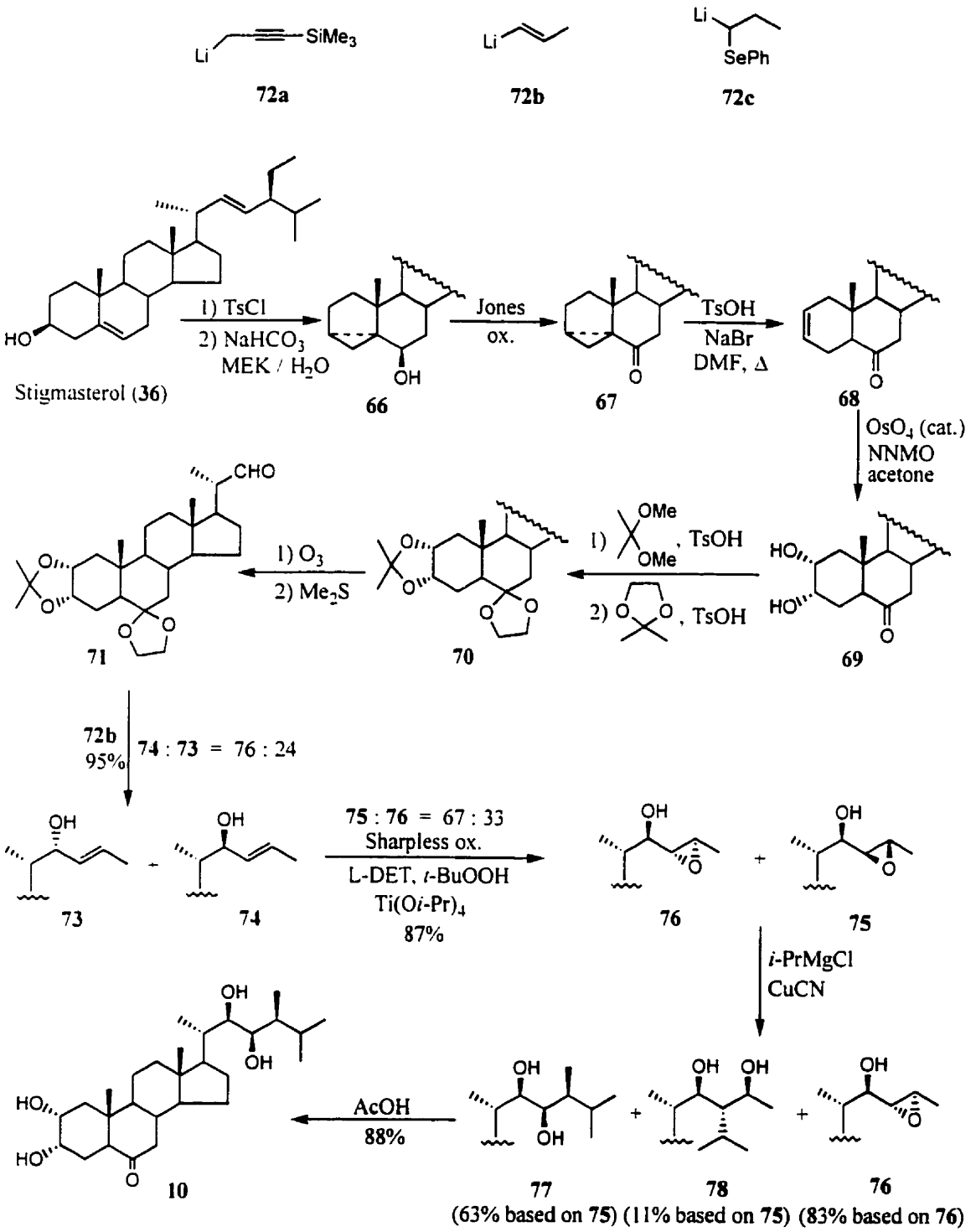
Scheme 1.6 Ikekawa's Synthesis

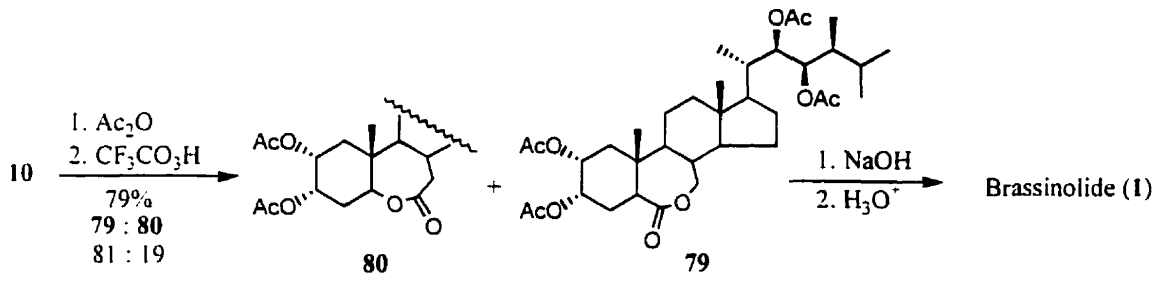


1.6.3 Back's Synthesis

Back and coworkers[36,42-45] have employed several strategies in their synthesis of brassinolide (**1**). Back's first route[42,43] to the brassinolide side chain involved the addition of the propargyllithium **72a** to C-22 aldehyde **71**. Unfortunately, this resulted in poor stereoselectivity with respect to the desired Cram addition product. In an improvement of this synthesis, Back and coworkers later reported[44,45] a more stereoselective addition of vinyllithium **72b** to aldehyde **71**. A description of this synthesis is outlined in Scheme 1.7. The tosylate of stigmasterol (**36**), was subjected to alkaline hydrolysis to give the 3,5-cyclosterol **66**. Jones oxidation of **66** followed by isomerization of the cyclopropane ring in **67** with an acid catalyst provided the enone **68**. The Δ^2 double bond was stereoselectively *cis*-dihydroxylated with a catalytic amount of osmium tetroxide to afford the 2α,3α-dihydroxy moiety of **69**. Protection of the *cis*-diol and the ketone was done by using 2,2-dimethoxypropane and acetone ethylene ketal, respectively, to give intermediate **70**. Ozonolysis and subsequent reductive work up with dimethyl sulfide furnished the (22S)-carbaldehyde **71**. The preceding steps were minor variations of literature procedures[46a,b] that produced aldehyde **71** in an overall yield of 42% from **36**. Addition of (E)-propenyllithium (**72b**) to aldehyde **71** provided both the anti-Cram (**73**) and the Cram (**74**) products in the ratio of 24:76. Allylic alcohol **74** underwent a Sharpless epoxidation to yield two isomeric epoxy alcohols **75** and **76** in the ratio of 67:33. This inseparable epoxy alcohol mixture was further treated with *i*-PrMgCl and a catalytic amount of CuCN to provide the desired 22,23-diol **77** (63% based on *threo*-epoxide **75**) and the by product 22,24-diol **78** (11%). In addition, the *erythro*-epoxide **76**, which is considerably less reactive that the *threo* isomer **75**, remained unchanged under these conditions and was easily separated at this stage, and was recovered in 83% yield. The 22,23-diol **77** was treated with aqueous acetic acid to give **10** in 88% yield. The tetraacetate derivative of **10** was further subjected to trifluoroperoxyacetic acid (generated *in situ* from trifluoroacetic anhydride and 30% hydrogen peroxide) to provide a mixture of regioisomers **79** and **80** in the ratio of 81:19.

Scheme 1.7 Back's Synthesis

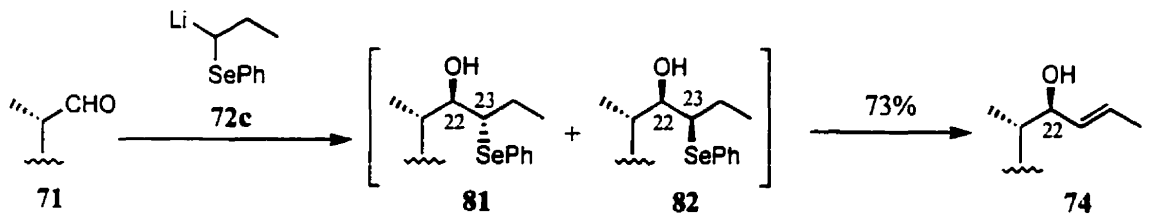




Fortunately, the reaction favoured the formation of 7-oxa-6-oxo regioisomer **79**, an unexpected Baeyer-Villiger product due to the migration of the less substituted carbon atom. This unusual regioselectivity of the oxidation is caused by the presence of electron-withdrawing substituents at the C1, C-2 and/or C-3 positions.[47,48] Ikekawa and coworkers[48] suggested that this observed anomaly is probably the result of inductive effects of electron-withdrawing substituents on the transition state of the migration. Finally, the separated compound **79** was converted to brassinolide (**1**) by saponification and acidification with dilute acid.

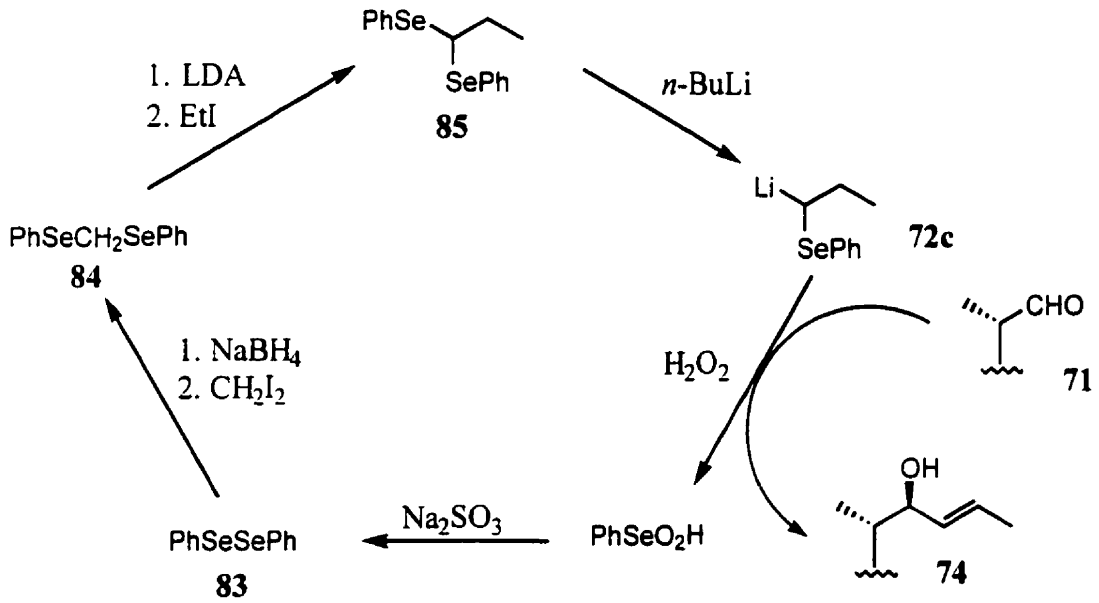
While this method elaborates the brassinolide side chain from the C-22 aldehyde **71** in just three steps, the stereoselectivity for two steps, the propenylation of **71** with **72b**, and the epoxidation of allylic alcohol **74**, is only moderate. Moreover, the last five steps of the synthesis are somewhat lengthy and needless. Further improvements of the stereoselectivity of the propenylation of **71** and shortening the last five steps of the above mentioned synthesis[45] were recently reported by Back and coworkers.[36] Back's improved approach to the propenylation of **71** (Scheme 1.8) involved the use of selenium-stabilized anion **72c**. Addition of anion **72c** to aldehyde **71** furnished a mixture of selenide stereoisomers **81** and **82** at C-23. High Cram selectivity at C-22 was achieved with no detectable amount (by NMR) of the anti-Cram product being formed. The unseparated

Scheme 1.8 Propenylation of Aldehyde **71**



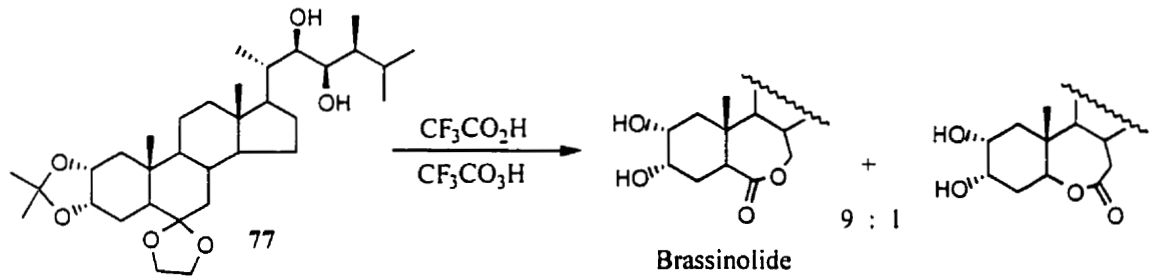
mixture was subjected to oxidative workup with hydrogen peroxide, which resulted in *syn* selenoxide elimination of each isomer to form *trans*-allylic alcohol **74**. As expected, *syn* selenoxide elimination proceeded with high regioselectivity away from the oxygen atom and favoured the *trans* isomer **74**, since the product is a 1,2-disubstituted olefin.[49,50] The preparation of anion **72c** is outlined in Scheme 1.9. Unfortunately, anion **72c** cannot be obtained directly by treating phenyl propyl selenide (n-PrSePh) with LDA because, with only one phenylseleno group present, the α proton is not acidic enough to be abstracted, even by strong bases. Alternatively, reduction of diphenyl diselenide (**83**) with sodium borohydride ($NaBH_4$), followed by treatment of the resulting selenolate with diiodomethane, provided selenoacetal **84**. Compound **84** was further alkylated with ethyl iodide to give the selenoacetal **85**. Finally, treatment of **85** with one equivalent of n-BuLi resulted in cleavage of one of the carbon-selenium bonds to afford the desired anion **72c**. The byproduct, benzeneseleninic acid ($PhSeO_2H$), of the selenoxide elimination regenerated the diselenide **83** after reductive work-up with sodium sulfite, permitting its recovery in 70%-90% yield. The use of cumene hydrogen peroxide instead of *t*-butyl hydrogen peroxide in the Sharpless epoxidation step improved the stereoselectivity to ca.

Scheme 1.9 Preparation of Selenium Stabilized Anion **72c**



70:30 in favour of the *threo*-epoxide **75**. Back's earlier synthesis[45] was further improved by shortening the last five steps to one. Similarly to the procedure employed by McMorris et al.,[35] Back and coworkers directly treated their 22,23-diol **77** with trifluoroacetic and trifluoroperoxyacetic acids, which resulted in the removal of the protecting groups, followed by Baeyer-Villiger oxidation to afford a mixture of brassinolide and its 6-oxa-7-oxo regioisomer in the ratio of 9:1 (Scheme 1.10). Finally, pure brassinolide (**1**) was obtained by recrystallization. This represents the most concise synthesis of brassinolide reported to date, affording the product in just 12 steps from stigmasterol (**36**).

Scheme 1.10 Baeyer-Villiger Oxidation of Intermediate **77**



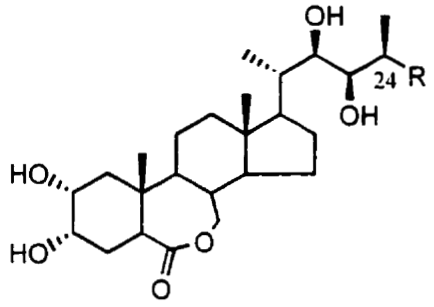
Brassinolide

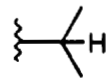
1.7 Previous Related Synthetic Analogues

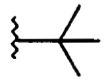
Since the discovery of brassinolide (**1**), a large number of synthetic brassinosteroids have been prepared for the investigation of structure-activity relationships (see section 1.4). Side-chain analogues that differ in the substituent R at C-24, as shown in Figure 1.7, are the subject of this Thesis. Therefore, some further discussion of previously reported variants of this structure (**86** and **87**) follows.

The observation that 25-methyldolichosterone (**89**) (Figure 1.8), isolated from *Phaseolus vulgaris*, was more potent than dolichosterone (**88**) prompted Mori and Takeuchi[51] to synthesize 25-methylbrassinolide (**86**), an unnatural brassinosteroid, to compare its activity against that of brassinolide in the rice leaf lamina bioassay. As expected from the increased activity of **89** vs. **88**, **86** proved to be slightly more potent than brassinolide (**1**) and was reported to be the most active brassinosteroid known at the time.

Figure 1.7 C-24 Substituted Analogues of Brassinolide



R =  Brassinolide (**1**)

 25-Methylbrassinolide (**86**)

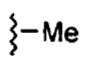
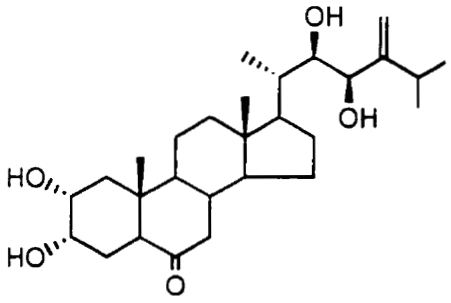
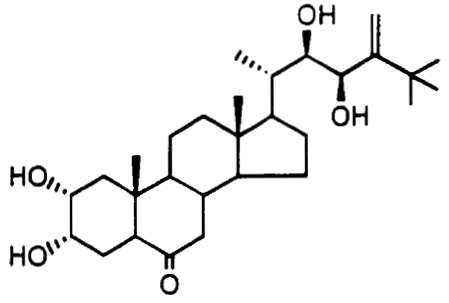
 26,27-Bisnorbrassinolide (**87**)

Figure 1.8 Structures of Dolichosterone and 25-Methyldolicholesterone



Dolichosterone (**88**)



25-Methyldolichosterone (**89**)

26,27-Bisnorbrassinolide (**87**) is another unnatural brassinosteroid and it has one fewer chiral center in the side chain than brassinolide. It was determined from the rice leaf lamina inclination test that this compound is almost as active as brassinolide (**1**).[52] The findings that the extra carbon atom in **86** confers slightly greater activity relative to **1**, but the removal of two carbon atoms from the side-chain of **87** has little effect appeared somewhat contradictory and worthy of further investigation.

1.8 Conclusion

There has been a great deal of progress in the study of brassinosteroids since the discovery of brassinolide in 1979. At the present time, however, cheap commercial production of brassinolide is not possible, and improved syntheses are still needed. Extensive studies[26-33] have been peformed on structure-activity relationships, but the structural requirements of the side chain, apart from the functionalization and stereochemistry at C-22, C-23, and C-24, remain unclear.

1.9 Objectives

Since Back's synthesis[36,44,45] permits the introduction of any group R (Figure 1.7) by means of an appropriate choice of Grignard reagent in the penultimate step of the synthesis, it provides a good opportunity to study structure-activity relationships of R. This is especially interesting because of the reported greater bioactivity of **86**.[51] This Thesis will focus on groups R of different chain length and cyclic substituents of varying ring-size. In addition, since hydroxylation and glucosylation at C-25 deactivates brassinosteroids (section 1.2.2.3), the above synthesis also provides the opportunity to introduce modified groups R that can't be oxidized or glucosylated. Our objective was to study (1) the effects of chain length to determine steric and hydrophobic requirements of the side chain, (2) the effects of conformational restriction through the introduction of cyclic substituents and the effects of various ring sizes, and (3) the effects of introducing heteroatoms at C-25 to see how bioactivity would be affected by blocking metabolic oxidation and/or glucosylation at that position (Scheme 1.3).

In addition, since the preparation of the *threo*-epoxide **75** was accompanied by the unwanted *erythro*-epoxide **76** in Back's synthesis, different methods were examined to recycle **76** back to the *trans*-allylic alcohol **74**.

Chapter Two

Brassinolide Side Chain Analogues

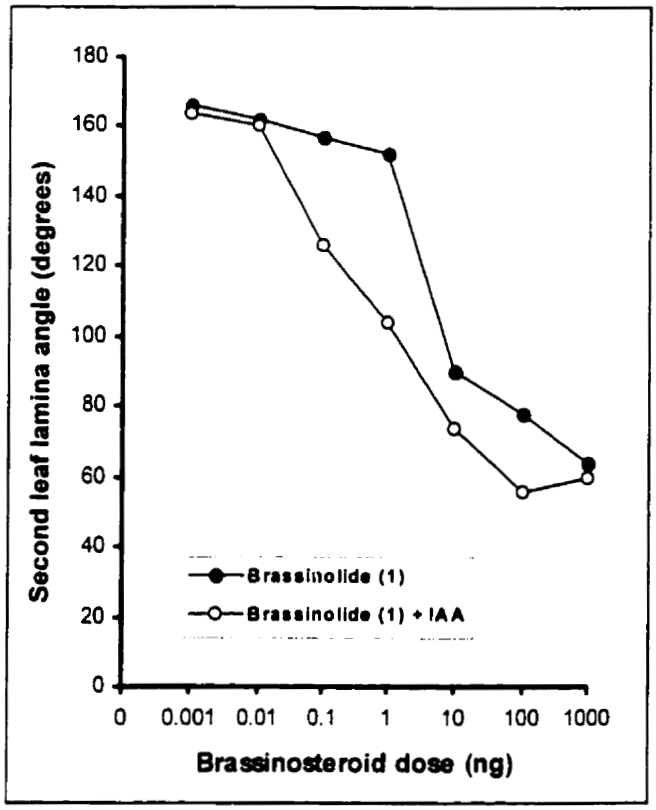
2.1 Introduction

For reasons that were discussed in Chapter One, we wanted to prepare and study side-chain analogues with different groups R in Figure 1.7. All the novel side-chain analogues presented in this Thesis were prepared via epoxide **75** that was generated following Back's 10-step procedure.[36] The preparation and biological activities of different types of side-chain analogues will be detailed in section 2.2. The bioactivities of the novel analogues shown in this Chapter were assayed by Dr. R.P. Pharis and his group using the rice leaf lamina inclination assay with dwarf rice seedling *Oryza sativa* var. Tan-ginbozu (Section 1.3.3).

The bioactivities are expressed graphically as in the example in Figure 2.1. The y-axis represents the angle of the second leaf lamina in degrees and the x-axis shows the logarithm of the brassinosteroid dose applied per plant in nanograms (ng). Each data point on the graph is the mean of the leaf angles from 36 plants for doses up to 100 ng, and from 24 plants for the 1000 ng and higher doses. Furthermore, it was reported by Takeno and Pharis[24] that auxins such as indole-3-acetic acid (IAA) significantly synergized the effect of brassinosteroids on the bending of the second leaf lamina. As illustrated in Figure 2.1, application of brassinolide (**1**) without IAA caused significant bending of the second leaf lamina at doses above ca. 1 ng/plant. However, in the presence of IAA, activity was observed at doses above ca. 0.01 ng/plant level, ca. two orders of magnitude lower than the minimal dosage required to elicit a response with brasssinolide alone. Since IAA significantly enhanced the effect of brassinolide on the bending of the second leaf lamina, we also wanted to see if similar synergy would be observed between IAA and our novel side-chain analogues. Thus, in these studies, 1000 ng of IAA was applied simultaneously with the brassinosteroid. These results will be discussed in section 2.2.

Finally, the accumulation of the undesired *erythro*-epoxide **76** after the ring-opening step in Back's synthesis[36] (see section 1.6.3) and in the preparation of novel side-chain analogues prompted us to search for an effective way to recycle it back to the *trans*-allylic alcohol **74**, from which the desired threo-epoxide could be generated. The results of this investigation will be described in Section 2.3.

Figure 2.1 The Effect of **1** With and Without IAA
on The Rice Leaf Lamina Assay

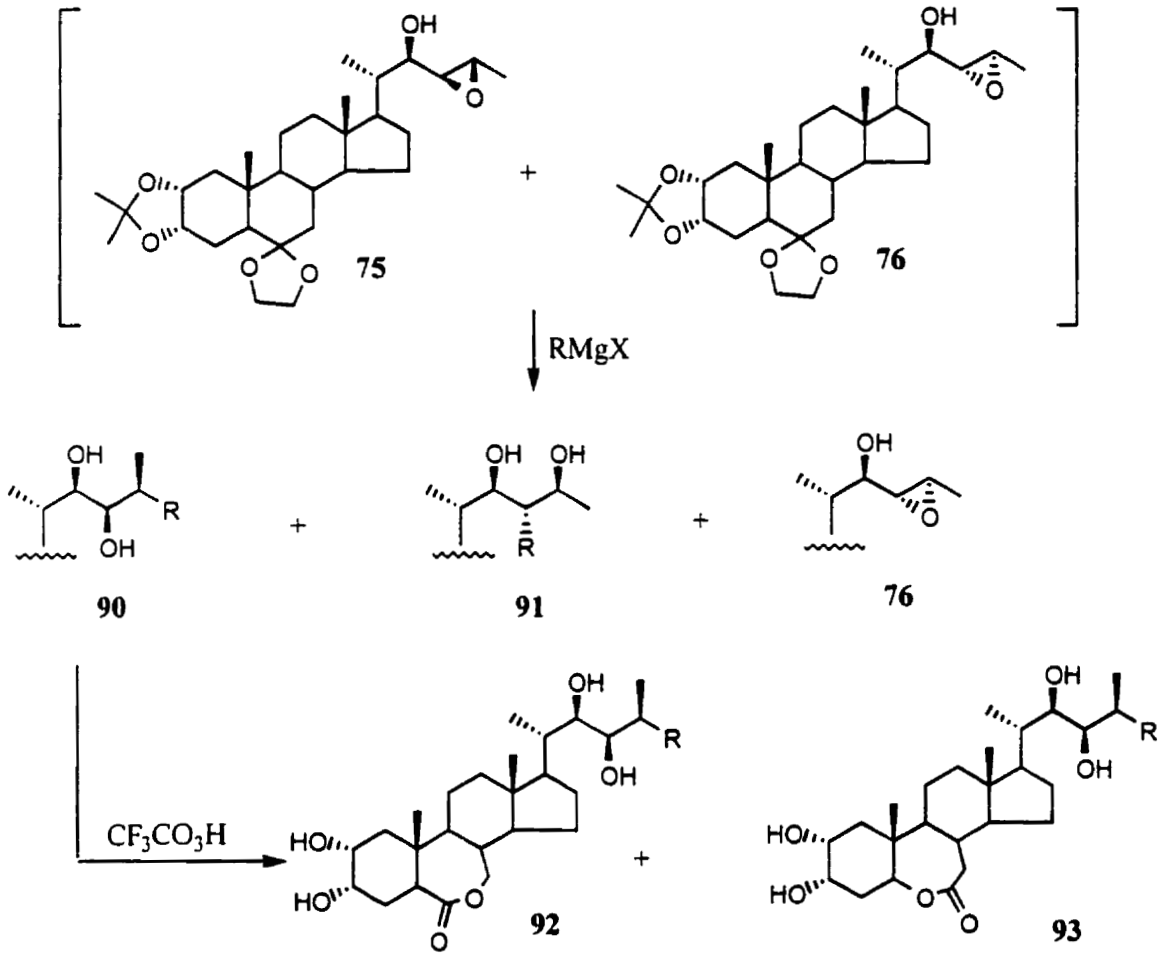


2.2 Preparation and Biological Activities of Novel Side-Chain Analogues

The preparation of most of the novel side-chain analogues in this Thesis is shown generally in Scheme 2.1. The construction of the four chiral centers on the side-chain of **90** was accomplished by the addition of appropriate Grignard reagents to the inseparable mixture of epoxides **75** and **76**. It will be recalled from Section 1.6.3 that *erythro*-epoxide **76** is less reactive than the *threo*-epoxide **75**; therefore, it was recovered after the

reaction was completed. A one pot reaction was used in the final step, where the acidic conditions of the Baeyer-Villiger reaction first removed the two ketal groups, following which the deprotected C-6 ketone reacted to afford the usual mixture of lactone isomers **92** and **93**. The desired compounds **92** were purified by recrystallization.

Scheme 2.1 Preparation of Side-Chain Analogues



2.2.1 Novel C-24 *n*-Alkyl Analogues

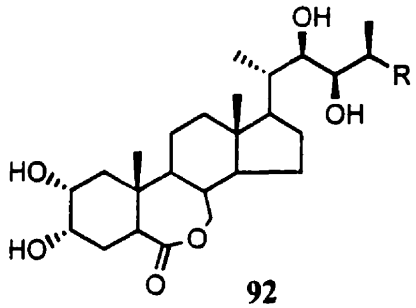
Since lipophilic side chains might permit brassinosteroids to penetrate the waxy cuticles of leaves more effectively, we prepared novel analogue **92a**, where R is a twelve carbon aliphatic chain (R = *n*-$C_{12}H_{25}$). In addition, as will be discussed later, the inactivity of analogue **92a** prompted us to prepare other novel side-chain analogues with

different *n*-alkyl substituents at C-24, **92b** (R = *n*-C_6H_{13}) and **92c** (R = *n*-C_3H_7), for structure-activity study purposes. The yields for the preparation of these compounds, as well as their precursors **90a-c**, are listed in Table 2.1. These compounds are novel and the structures were determined by spectroscopic methods and elemental analyses. The presence of hydroxyl groups, as well as the lactone moiety, were apparent in their IR spectra. DEPT experiments confirmed the number of methyl, methylene, methine and quaternary carbons in each structure. The significant competing formation of 1,3-diols **91** from **75** with Grignard reagents was not observed, and these regioisomers were not isolated. The chemical shift of the C-28 methyl group appeared as a doublet at ca. δ 0.9 ppm in each of **92a-c**, and is in good agreement with that of the C-28 methyl group of brassinolide (**1**) at 0.95 ppm, and is considerably further upfield than the 1,3-diol isomer **91**, where the C-28 methyl group appeared at δ 1.29 ppm.[53] In addition, the two regioisomers **92** and **93** were distinguished based on the chemical shifts of the methine protons α to the carbonyl group in **92** and α to the lactone oxygen atom in **93**, appearing as doublets of doublets at δ 3.1 ppm and 4.6 ppm, respectively.

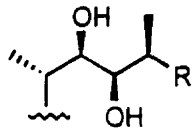
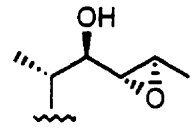
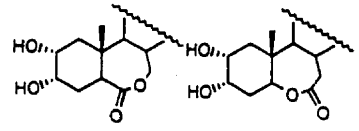
Interestingly, as shown in Figure 2.2, compound **92a** displayed no biological activity at any dose level, as compared with brassinolide (**1**), where bioactivity was observed as usual above ca. 1 ng/plant level. Even in the presence of IAA, no response was observed with analogue **92a** (Figure 2.3). Since the long-chain analogue **92a** was inactive, we next investigated the threshhold of chain length where activity resumed. This study was also intended to provide information on the requirements of the active site with respect to its ability to accommodate various lengths of side-chain at C-24. Similarly to **92a**, analogue **92b** (R = *n*-C_6H_{13}) also displayed no activity in the absence of IAA at low doses. Slight activity was observed with high doses in the range 100-1000 ng (Figure 2.4). However, in the presence of IAA, activity was observed at doses of 100 ng/plant and reached ca. 90 degrees at higher doses (≥ 1000 ng/plant) (Figure 2.5). Activity more closely resembling that of **1** resumed with a three carbon chain length (R = *n*-C_3H_7) (**92c**), which is an isomer of brassinolide. According to Figure 2.6, without IAA, the second leaf lamina started to bend at a dose of 10 ng/plant; ca. one order of magnitude higher than **1**. The bending of the second leaf lamina was noticeable at a dose of ca. 0.1

ng/plant when **92c** was applied together with IAA (Figure 2.7), as in the case of brassinolide. Interestingly, IAA synergized the effect of analogue **92c** more than the effect of brassinolide, at doses higher than 1 ng/plant, making **92c** more potent than **1**. Based on this investigation, we concluded that long lipophilic side chains destroy the biological activity, possibly because the side chains of compounds **92a** and **92b** are too big to fit into the active site of a receptor. This study further indicated that a three carbon chain (**92c**) might be the longest chain length at C-24 that the active site will tolerate, since in the absence of IAA, this analogue is only 10% as active as brassinolide itself.

Table 2.1 Yields of Novel C-24 *n*-Alkyl Analogues



92

RMgBr	 **90**, yield (%) based on **75**	 **76**, yield (%) (recovery)	 **92** : **93** (ratio)[a], yield (%)[b]	**92** yield (%)[c]
R= *n*-$C_{12}H_{25}$	**90a**, 67%	88%	**92a** : **93a** (9 : 1), 86%	**92a**, 67%
R= *n*-C_6H_{13}	**90b**, 67%	86%	**92b** : **93b** (8 : 1), 60%	**92b**, 46%
R= *n*-C_3H_7	**90c**, 65%	96%	**92c** : **93c** (8.5 : 1.5), 66%	**92c**, 40%

(a) Ratio determined by NMR integration of the crude product mixture.
(b) Yields of the unseparated mixtures of regioisomers after column chromatography.
(c) Yields of pure **92** obtained after recrystallization.

Figure 2.2 The Effect of **1** and **92a** on The Rice Leaf Lamina Assay

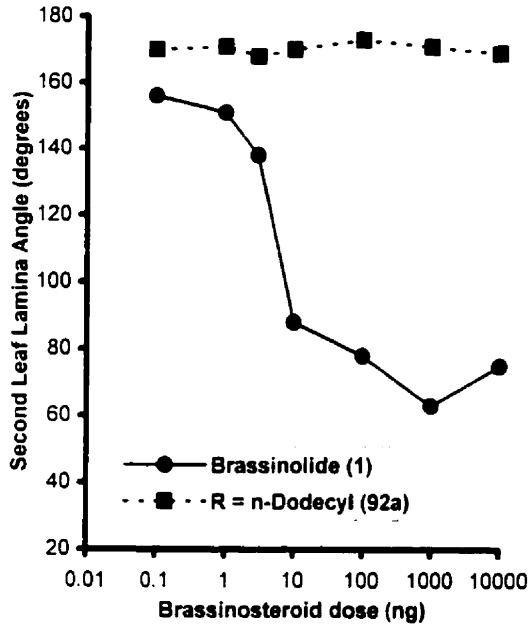


Figure 2.3 The Effect of **1** and **92a** With **IAA** on The Rice Leaf Lamina Assay

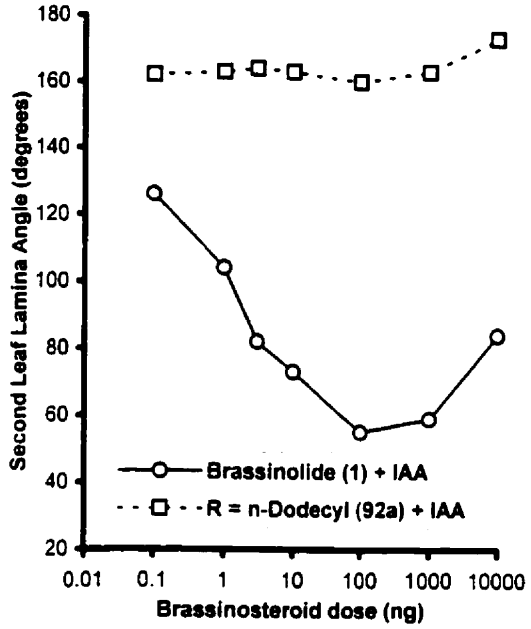


Figure 2.4 The Effect of **1** and **92b** on The Rice Leaf Lamina Assay

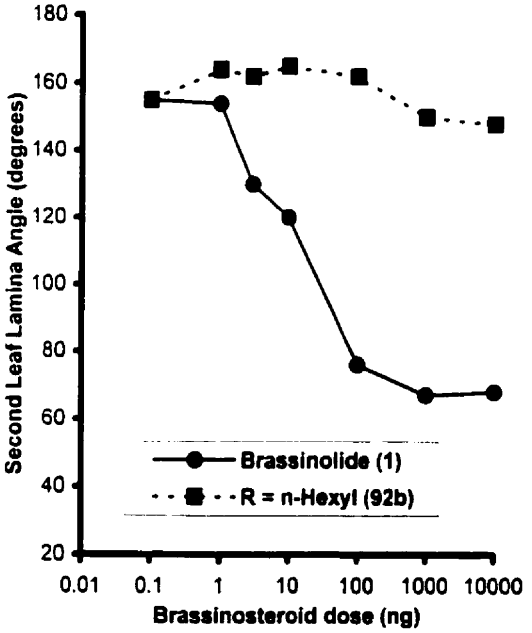


Figure 2.5 The Effect of **1** and **92b** With **IAA** on The Rice Leaf Lamina Assay

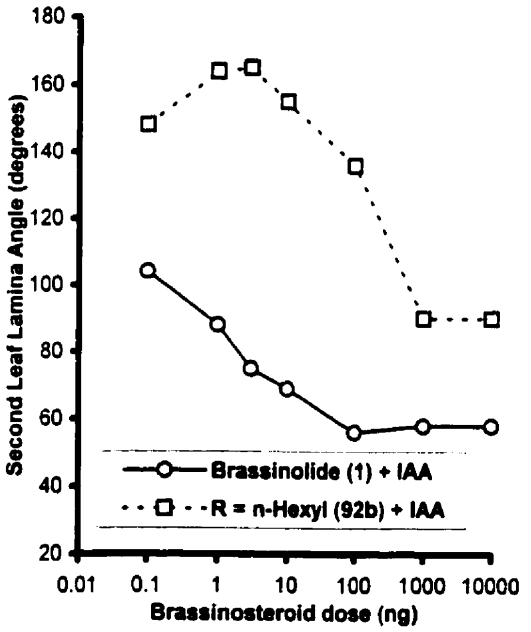


Figure 2.6 The Effect of **1** and **92c** on The Rice Leaf Lamina Assay

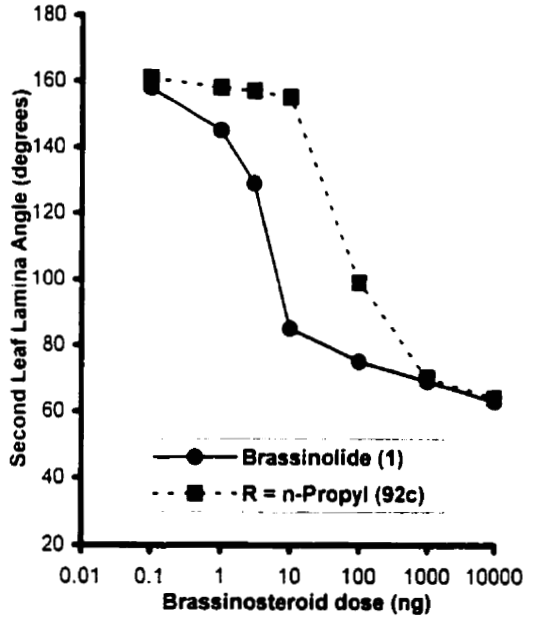
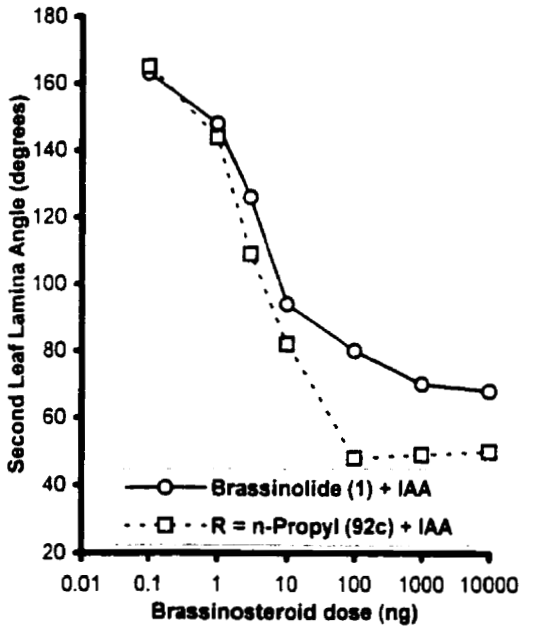


Figure 2.7 The Effect of **1** and **92c** With **IAA** on The Rice Leaf Lamina Assay



2.2.2 Cyclic Side-Chain Analogues

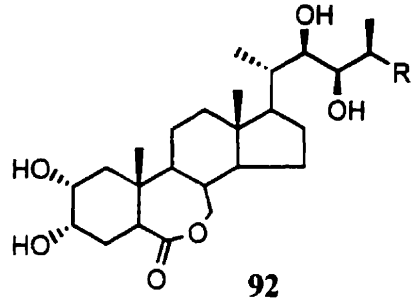
As part of our efforts to establish the relationship between side-chain structure and biological activity, as well as to find new analogues with high activity, we decided to restrict the conformational mobility of the side-chain by locking the two terminal methyl groups (C-26 and C-27) into small cyclic systems. Restricting the degrees of freedom of the side-chain might result in a greater probability of an active conformation coming into contact with the active site of the receptor.[54] Moreover, we wanted to examine the effect of ring size on biological activity in a more general sense to see if there was a similar loss of activity with increasing ring size as had been observed with increasing chain length. Consequently, the cyclohexyl, cyclopentyl, cyclobutyl and cyclopropyl derivatives **92d**, **92e**, **92f**, and **92g**, respectively, were prepared in the usual manner by the addition of the appropriate Grignard reagents to a mixture of *threo*-epoxide **75** and *erythro*-epoxide **76**, to afford **90d-g**, followed by Baeyer-Villiger oxidation. Moreover, as will be discussed

later, several of these products proved to be more active brassinosteroids than brassinolide itself. Since the previously known 25-methyl derivative **86** had also been reported to be more active than brassinolide,[51] it was also prepared in order to permit a comparison of its activity with those of our novel cyclic side-chain analogues **92d-g**. Therefore, the last entry in Table 2.2 includes **86**, even though it is not itself a cyclic side-chain analogue.

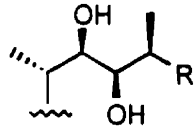
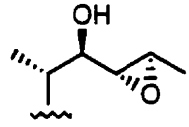
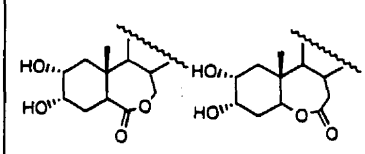
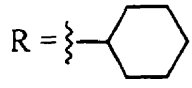
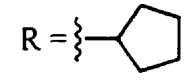
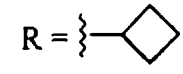
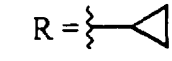
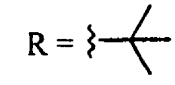
The yields of **92d**, **92e**, **92f** and **92g** and their precursors **90d-g** are recorded in Table 2.2. Their identities were evident from the hydroxyl and lactone absorptions in their IR spectra. DEPT experiments verified the number of C, CH, CH_2, and CH_3 in each structure. Similar to the preparation of C-24 *n*-alkyl analogues, the competing formation of 1,3-diol **91** was also not observed and these regioisomers were not isolated. Therefore, the chemical shifts of the C-28 methyl group and the methine proton α to the carbonyl group appeared at ca. δ 0.9 and 3.1 ppm, repectively, in their ^{1}H-NMR spectra. For analogue **92g**, two distinct signals were observed at δ 0.5 and 0.2 ppm, which correspond to the methylene protons of the cyclopropane ring. All of these compounds gave satisfactory elemental analyses or ^{13}C-NMR spectra and low and high resolution mass spectra consistent with their structures. The Grignard reagents for analogues **92f** and **92g** were freshly prepared in tetrahydrofuran (THF) according to a literature procedure,[55] since these Grignard reagents are not commercially available. All of these reactions proceeded in a straight-forward manner similar to those used for preparing the *n*-alkyl analogues **92a-c**, except that an unexpected byproduct was obtained during the preparation of **92g**. This will be further described in Section 2.2.2.1.

Bioassays of these analogues indicated a relationship between the ring size and the biological activity, where the smaller the ring system, the higher the activity that was obtained. According to Figure 2.8, the cyclohexyl analogue **92d** displayed activity above 10 ng/plant. The leaf lamina angle reached ca. 110 degrees at a dose of 1000 ng/plant and **92d** was therefore less than 10% as active as brassinolide (**1**). The effect of **92d** was also synergized by the addition of IAA (Figure 2.9); where activity was observed at a dose of 10 ng/plant. Decreasing the ring size from cyclohexyl (**92d**) to cyclopentyl (**92e**) resulted

Table 2.2 Yields of Cyclic Side-Chain Analogues



92

RMgX	 **90**, yield (%) based on **75**	 **76**, yield (%) (recovery)	 **92** : **93** (ratio), yield (%)	**92** yield (%)
R = 	**90d**, 73%	81%	**92d** : **93d** (9 : 1), 69%	**92d**, 51%
R = 	**90e**, 81%	89%	**92e** : **93e** (10 : 1), 65%	**92e**, 53%
R = 	**90f**, 78%	85%	**92f** : **93f** (9 : 1), 62%	**92f**, 45%
R = 	**90g**, 76%	90%	**92g** : **93g** (9 : 1), 67%	**92g**, 49%
R = 	**90h**, 25%	93%	**86** : **93h** (9 : 1), 68%	**86**, 38%

(a) Ratio determined by NMR integration of the crude product mixture.
(b) Yields of the unseparated mixtures of regioisomers after column chromatography.
(c) Yields of pure **92** obtained after recrystallization.

in significantly stronger activity, comparable to that of brassinolide (Figure 2.10), and the bioactivity of **92e** was again significantly increased by simultaneous application of IAA (Figure 2.11). Remarkably, the cyclobutyl and cyclopropyl analogues **92f** and **92g** exhibited greater bioactivity than **1**. At dose levels in the range of 1-100 ng, the effect of **92f** on the second leaf lamina angle was more pronounced than the effect of **1**, where **92f** at 10 ng/plant showed ca. three times greater activity than **1** (Figure 2.12). That is, ca. three times as much **1** was required to elicit the same response as 10 ng of **92f**. The cyclopropyl analogue **92g** was the most potent brassinosteroid of all, and proved to be up to ca. seven times more active than brassinolide at mid-range dosage levels. Based on Figure 2.14, in order for the second leaf lamina to bend to ca. 140 degrees from its base value of 160-165 degrees, it required a dose of ca. 7 ng of **1**, but only a dose of ca. 1 ng of compound **92g**. Synergistic activity between IAA and **92f** or **92g** is shown in figures 2.13 and 2.15. In particular, it is worth noting that both **92f** and **92g** are again synergized by IAA. Whereas the combination of **92f** plus IAA proved less potent than brassinolide plus IAA, **92g** plus IAA remained more effective than brassinolide plus IAA. When we prepared the 25-methyl analogue **86** and studied its bioactivity, it proved comparable in activity to brassinolide (Figure 2.16). The effect of IAA upon the activity of **86** was slightly less than upon that of **1** (Figure 2.17).

All four cyclic systems thus showed significant activity when tested, with and without IAA, in the rice leaf lamina inclination assay. A remarkable enhancement of activity was observed as the ring size decreased. Apart from the fact that the small-ring analogues are conformationally more rigid, one can also speculate that as the ring size decreased, the C-26 and C-27 carbon atoms are forced closer to each other; therefore allowing the side-chain to fit into the binding site of the receptor better, giving rise to greater activity. Thus, analogues **92f** and **92g** are the most potent brassinosteroids reported to date, surpassing the activity of either brassinolide itself or the synthetic analogue **86**, that had previously been claimed to be the most potent brassinosteroid known.

Figure 2.8 The Effect of **1** and **92d** on The Rice Leaf Lamina Assay

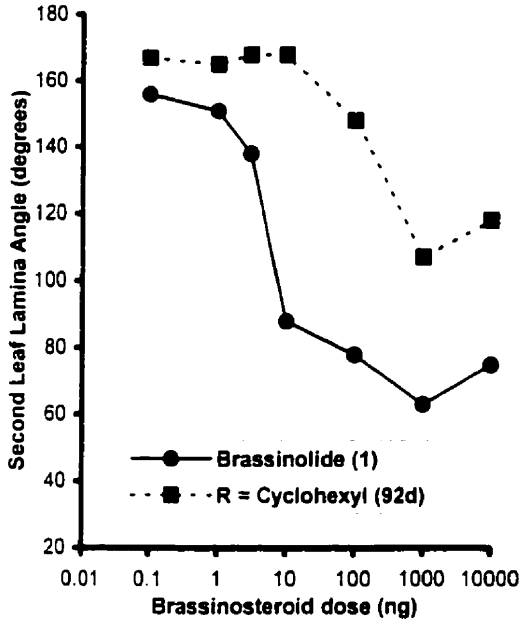


Figure 2.9 The Effect of **1** and **92d** With **IAA** on The Rice Leaf Lamina Assay

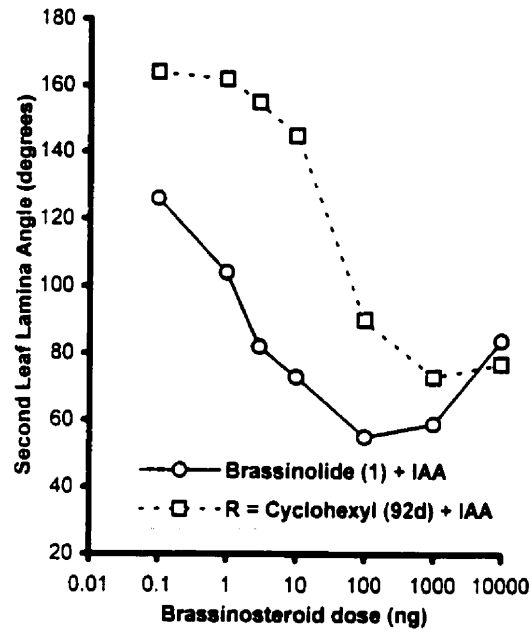


Figure 2.10 The Effect of **1** and **92e** on The Rice LeafLamina Assay

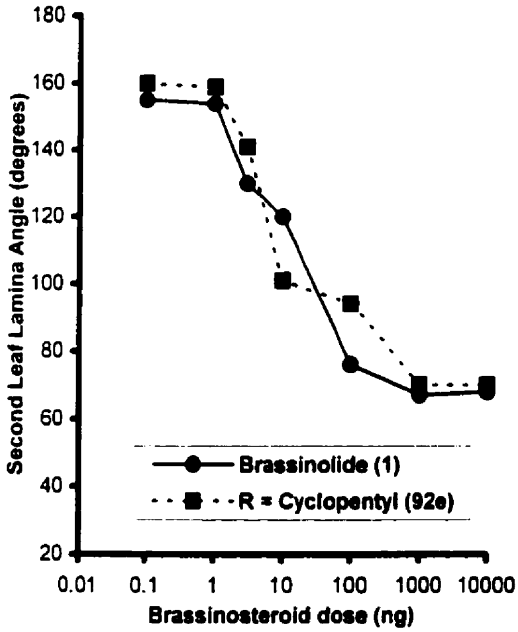


Figure 2.11 The Effect of **1** and **92e** With **IAA** on The Rice Leaf Lamina Assay

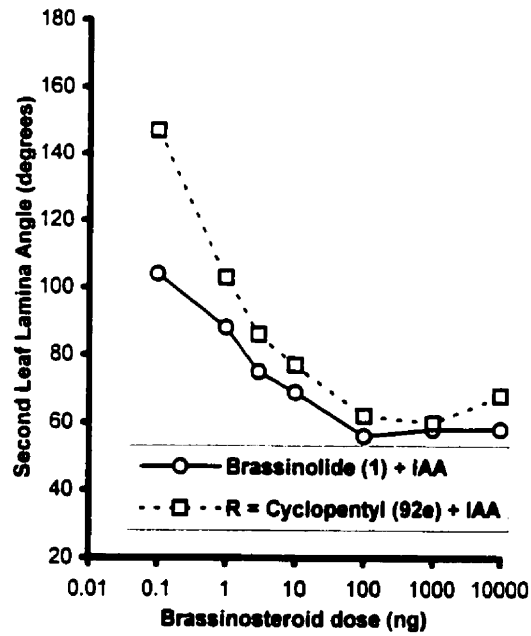


Figure 2.12 The Effect of **1** and **92f** on The Rice Leaf Lamina Assay

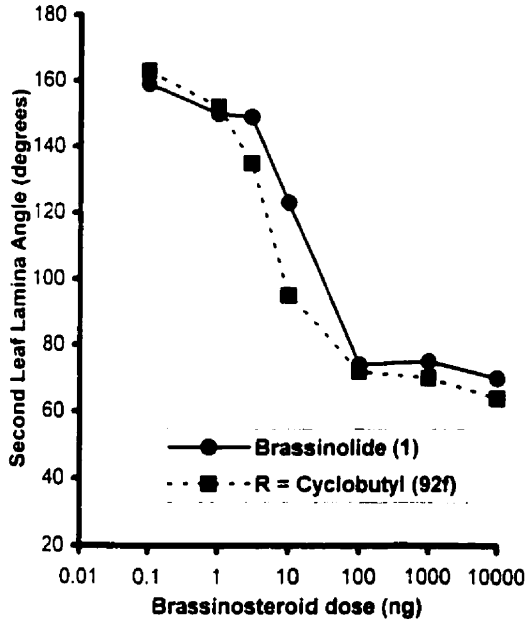


Figure 2.13 The Effect of **1** and **92f** With **IAA** on The Rice Leaf Lamina Assay

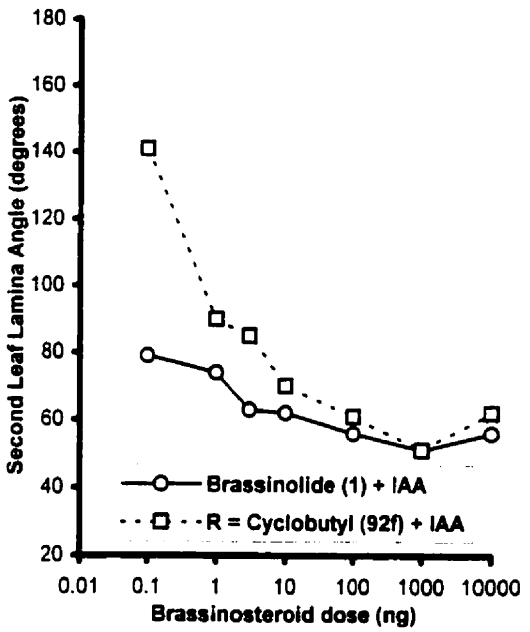


Figure 2.14 The Effect of **1** and **92g** on The Rice Leaf Lamina Assay

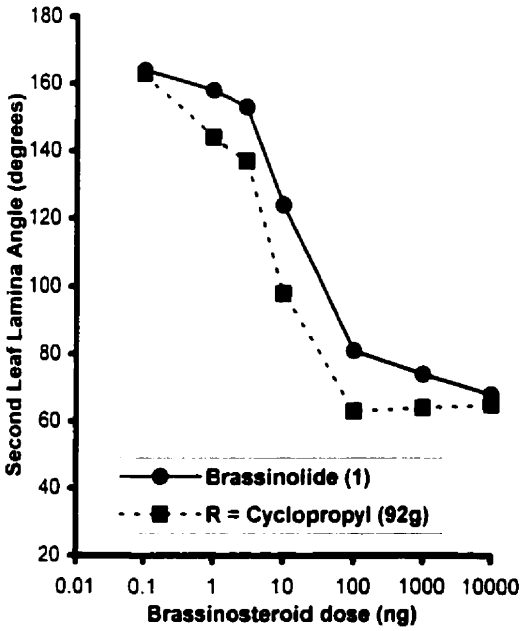


Figure 2.15 The Effect of **1** and **92g** With **IAA** on The Rice Leaf Lamina Assay

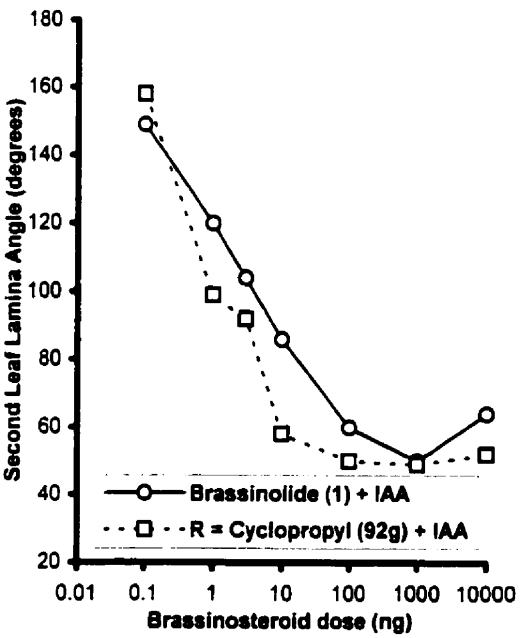


Figure 2.16 The Effect of **1** and **86** on The Rice Leaf Lamina Assay

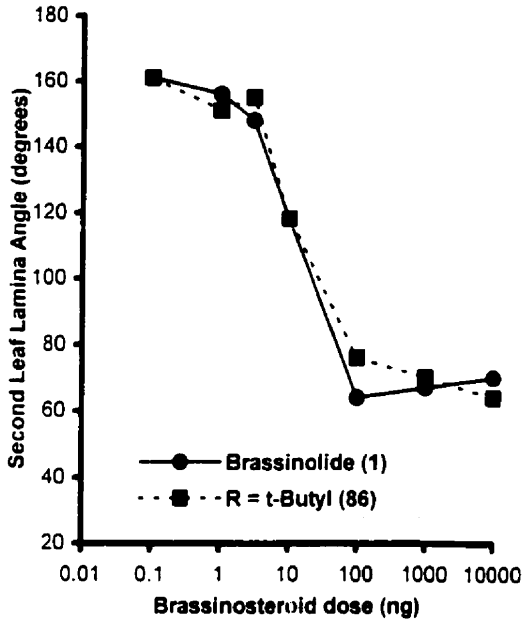
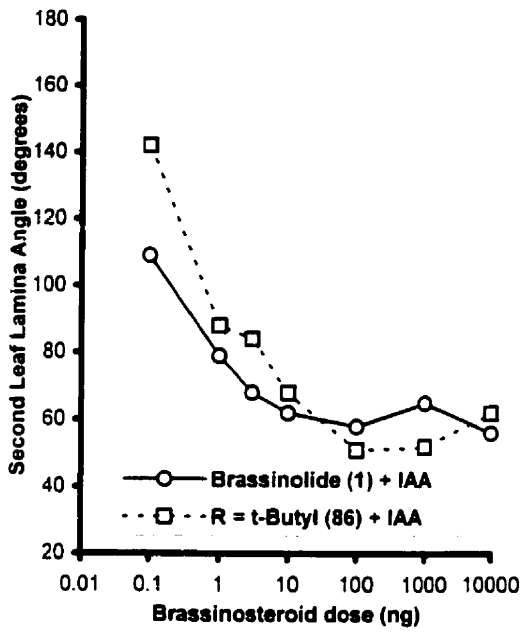


Figure 2.17 The Effect of **1** and **86** With **IAA** on The Rice Leaf Lamina Assay

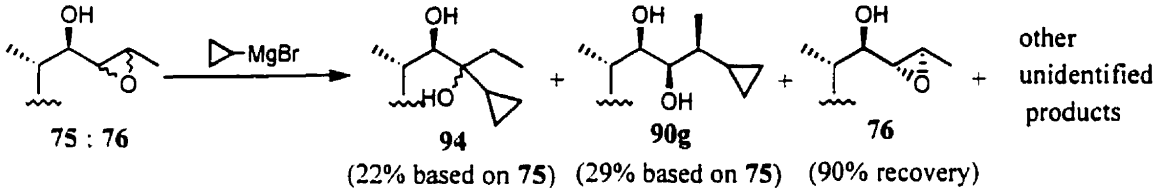


2.2.2.1 Unexpected Rearrangement During the Reaction of Epoxides 75 and 76 with Cyclopropylmagnesium Bromide

An interesting and unexpected reaction was observed during the preparation of intermediate **90g**. When the cyclopropyl Grignard reagent was prepared in diethyl ether instead of THF, a significant amount of compound **94** formed along with the desired product **90g**, recovered **76** and other minor unidentified products. The DEPT ^{13}C-NMR spectrum of **94** indicated one extra quaternary carbon atom, one extra CH_2 and two fewer CH signals than the expected product **90g** (Scheme 2.2). In addition, when the ^{1}H-NMR spectrum was obtained at 400 MHz, a distinct triplet signal was observed at ca. 1.0 ppm which corresponded to the methyl group of an ethyl moiety. All other spectroscopic and analytical data confirmed the structure of **94**. A possible explanation for the formation of **94** is illustrated in Scheme 2.3 and 2.4. There is an equilibrium that exists between

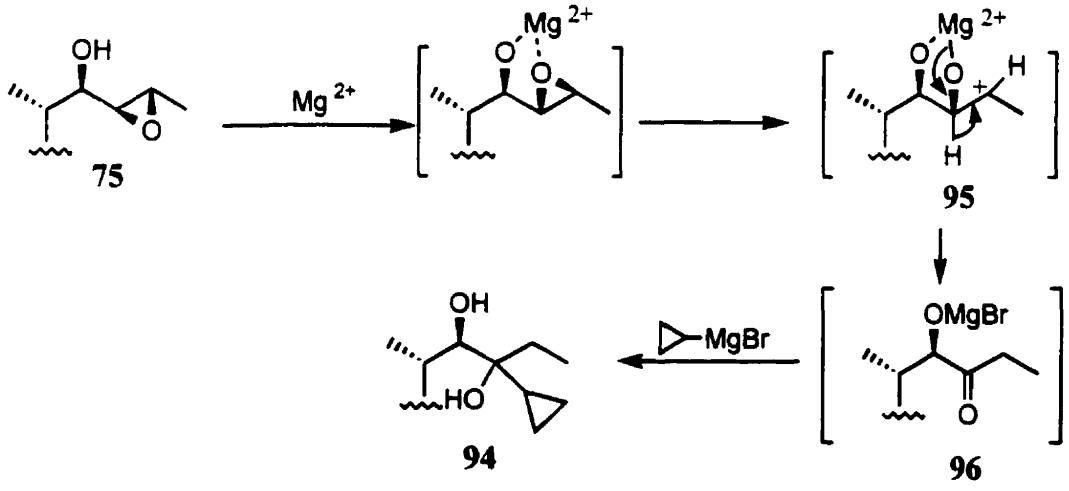
Grignard reagents and the corresponding magnesium halides and dialkylmagnesiums (Scheme 2.3). The solvent and the identity of specific organic group dictate the position of the equilibrium.[56] When the organic group is cyclopropyl and the solvent is diethyl ether, the equilibrium apparently lies further to the right; therefore more of the magnesium ion (Mg^{2+}) is present, which act as a Lewis acid that is capable of complexing to both the hydroxyl and the epoxy oxygens of **75** (Scheme 2.4). Lewis acid-catalyzed ring-opening of the epoxide, with a hydride shift in carbocation **95**, affords ketone **96**. Further addition of cyclopropyl Grignard reagent to **96** thus provided the byproduct **94** in 22% yield. When THF was used instead of diethyl ether, a much cleaner reaction was obtained to give the desired product **90g** in 76% yield based on *threo*-epoxide **75**, as shown in Table 2.2.

Scheme 2.2 Formation of **94**



Scheme 2.3 Equilibrium of Cyclopropylmagnesium Bromide

Scheme 2.4 Mechanism for the Formation of **94**



2.2.3 C-25 Substituted Analogues

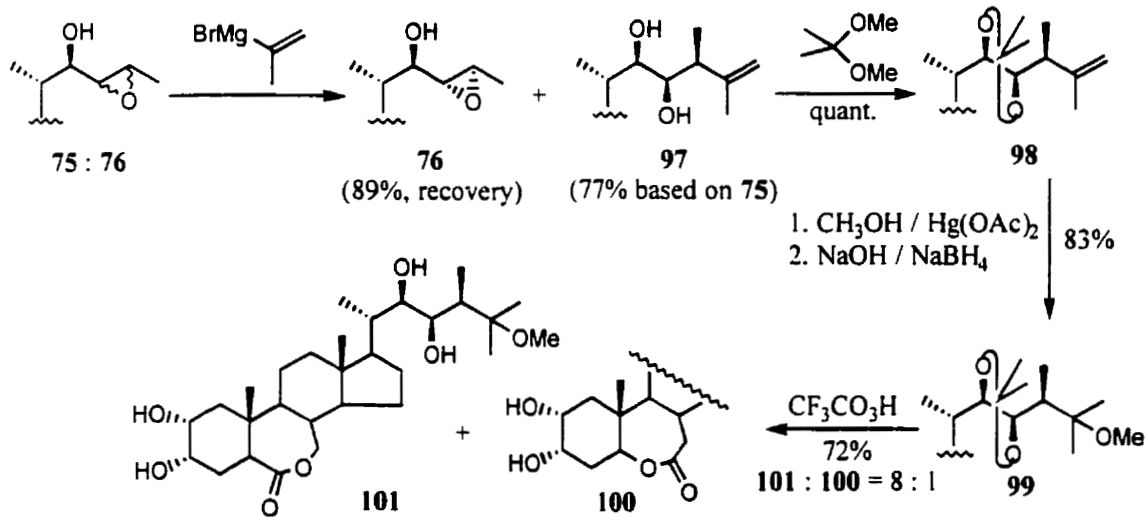
As previously discussed in Chapter One, 25-hydroxylation of brassinosteroids results in active metabolites, while subsequent glucosylation deactivates them (Section 1.2.2.3). Thus, we were interested in making analogues that would either prevent the glucosylation step or block the preceding oxidation pathway in order to produce longer-lasting compounds that would not undergo metabolic deactivation, and that would therefore have greater potential for agricultural applications. To stop the glucosylation process at C-25, we decided to prepare the novel analogue 25-methoxybrassinolide (**101**). Since activity was retained after changing the C-22 and C-23 hydroxyl groups to methoxy moieties,[33] we similarly hoped that **101** would retain its bioactivity. To completely block the above mentioned metabolic pathway, we also prepared two new compounds, 25-fluorobrassinolide (**104**), and 25-azabrassinolide (**109**). Fluorinated analogues have been recognized as useful tools for the study of structure-activity relationships because of the unique physical and biological properties imparted by the fluorine atom.[57] Selective fluorination of steroids has been employed to block enzymatic hydroxylation or other processes by virtue of the stronger[58] bond that exists between the fluorine and the carbon atoms compared to the carbon-hydrogen bond (C-F bond is ca. 116 kcal/mole, while C-H bond is ca. 99 kcal/mole).[59] Thus, we predicted that introducing a fluorine atom at the C-25 position would prevent C-25 hydroxylation and allow the steroid to have a longer-lasting effect upon plants. The preparation and biological activity results of these novel analogues are described in the following sections. In addition, since 25-fluorocastasterone (**102**) was obtained as a precursor of 25-fluorobrassinolide (**104**), its biologically activity was compared with castasterone (**10**), a brassinosteroid with ca. 50% of the activity of brassinolide (Section 1.4.1).

2.2.3.1 Preparation of 25-Methoxybrassinolide (101)

The preparation of analogue **101** is shown in Scheme 2.5. Intermediate **97** was obtained by the addition of freshly prepared 2-propenylmagnesium bromide[55] to the

mixture of epoxides **75** and **76**. Before the oxymercuration reaction was carried out, the diol on the side-chain of **97** was protected as the acetonide **98** due to the ease with which intramolecular alkoxymercuration proceeds when a five-membered or six-membered cyclic ether is formed.[60] Compound **98** was then subjected to oxymercuration conditions to afford the desired intermediate **99**. Finally, a one pot deprotection and Baeyer-Villiger oxidation was carried out to provide **100** and **101** in a ratio of 1:8 with an overall

Scheme 2.5 Preparation of **101**



combined yield of 72% from **99**. Compound **101** was purified by recrystallization (56% yield) and was identified from its ^{1}H-NMR signals for the methoxy group at δ 3.23 ppm and also from the two singlets for the C-26 and C-27 methyl groups at δ 1.33 and 1.22 ppm, respectively (shifted to lower field due to the inductive effect of the adjacent methoxy group). The ^{13}C-NMR spectrum showed a new signal from the methoxy group at δ 49.0 ppm, as well as a quaternary carbon (COMe) signal at δ 78.6 ppm. Complete characterization of this novel analogue is further described in the Experimental Section.

The rice leaf lamina assay indicated that analogue **101** is only slightly less active than brassinolide (see Figure 2.18). A significant change in the inclination of the leaf lamina at dose levels in the range of 10-100 ng was observed. This angle reached ca. 65 degrees at a dose of 10000 ng/plant. As shown in Figure 2.19, addition of IAA lowered

the dose levels required to achieve a given leaf lamina angle by about one order of magnitude in the mid-range dose levels of 1-100 ng/plant. Since analogue **101** is expected to prevent glucosylation at C-25, and since it also displayed significant activity, it is a good candidate for field trials to test for persistence.

Figure 2.18 The Effect of **1** and **101** on The Rice Leaf Lamina Assay

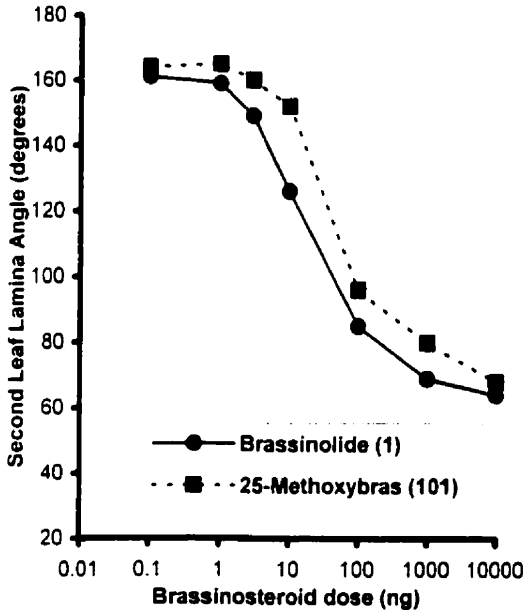
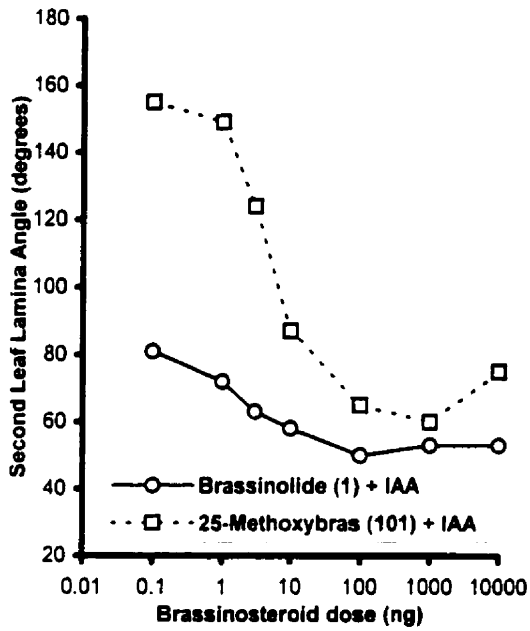


Figure 2.19 The Effect of **1** and **101** With **IAA** on The Rice Leaf Lamina Assay



2.2.3.2 Preparation of 25-Fluorobrassinolide (104)

We next turned our attention to the preparation of the 25-fluoro analogue **104** in order to block enzymatic hydroxylation at C-25. First, we treated **98** with polyvinyl-poly(hydrogen fluoride) (PVPHF),[61] a solid polymeric hydrofluorinating reagent; however, no reaction was observed with this method. Replacing the solid reagent with pyridinium poly(hydrogen fluoride)[62] afforded the desired 25-fluorocastasterone **102**. As a result of the acidic conditions in this procedure, all the protecting groups were also removed, which set up the Baeyer-Villiger oxidation. Analogue **104** was obtained in 57%

yield after recrystallization. The identity of this compound was evident by the presence of a fluorine signal in its ^{19}F-NMR spectrum at δ -142 ppm, which is typical for a fluorine atom attached to a quaternary carbon center.[61] In addition, two doublets with coupling constants of 9.2 and 13.2 Hz (J ^{1}H-^{19}F), attributed to the C-26 and C-27 methyl groups, were shifted downfield in the ^{1}H-NMR spectrum, due to the inductive effect of the adjacent fluorine atom. Surprisingly, both 25-fluorocastasterone (**102**) and 25-fluorobrassinolide (**104**) displayed no activity with or without IAA in the rice leaf lamina assay (see Figures 2.20 to 2.23). These results were unexpected, since we had earlier predicted that analogues **102** and **104** would show similar activity to castasterone (**10**) and brassinolide (**1**), respectively.

Scheme 2.6 Preparation of **104**

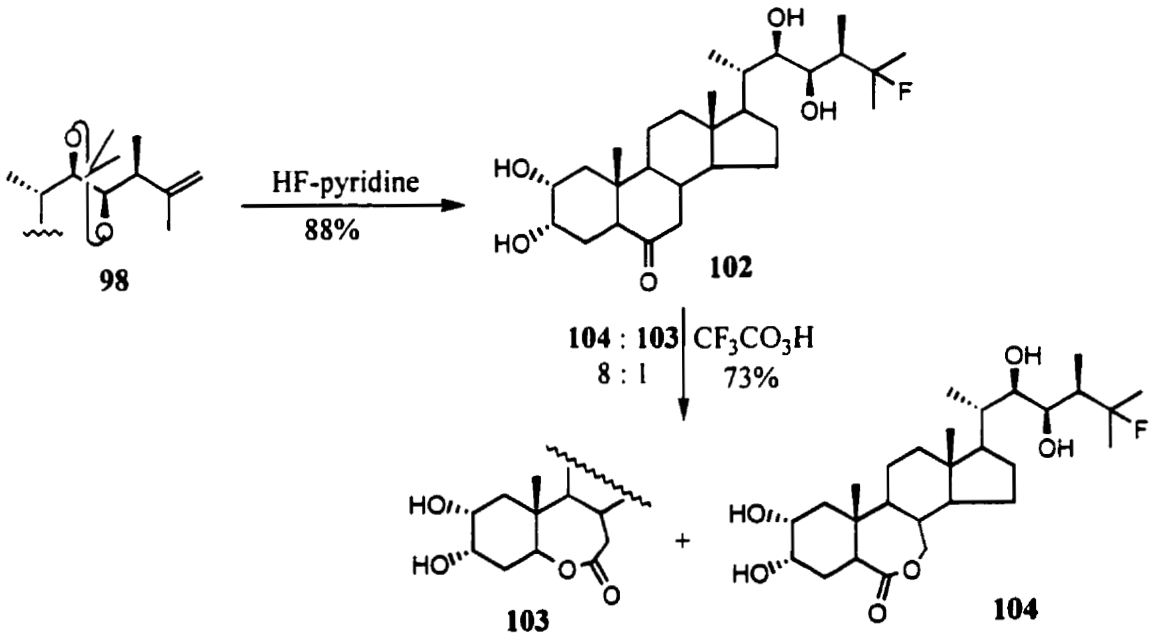


Figure 2.20 The Effect of **1**, **10** and **102** on The Rice Leaf Lamina Assay

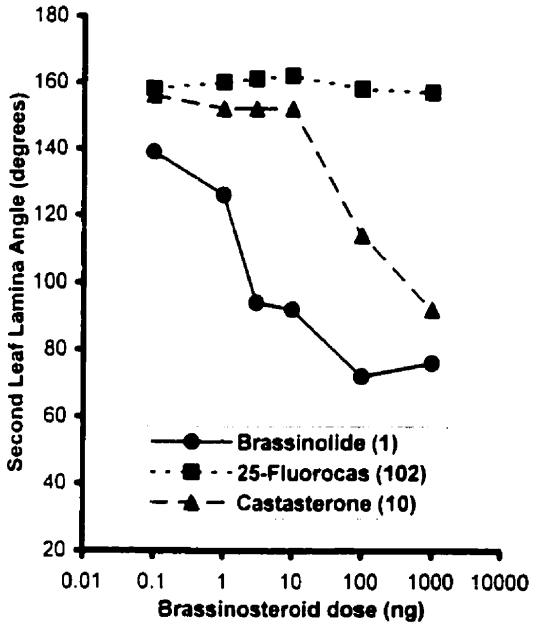


Figure 2.21 The Effect of **1**, **10** and **102** With **IAA** on The Rice Leaf Lamina Assay

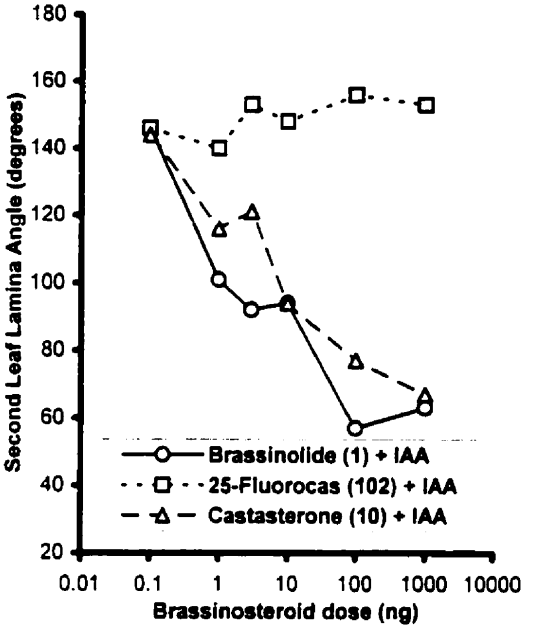


Figure 2.22 The Effect of **1** and **104** on The Rice Leaf Lamina Assay

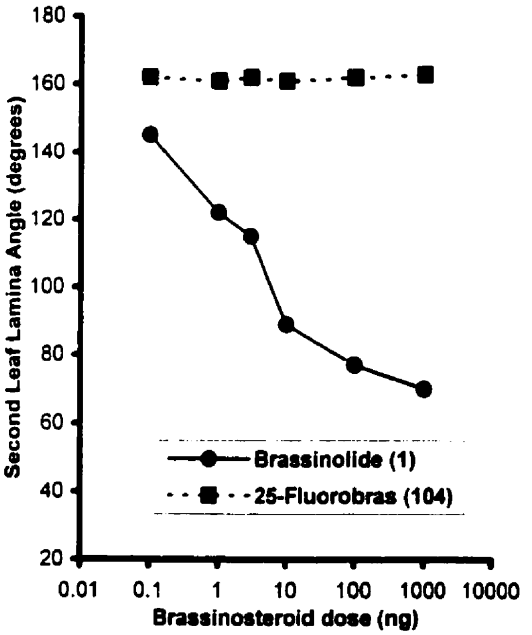
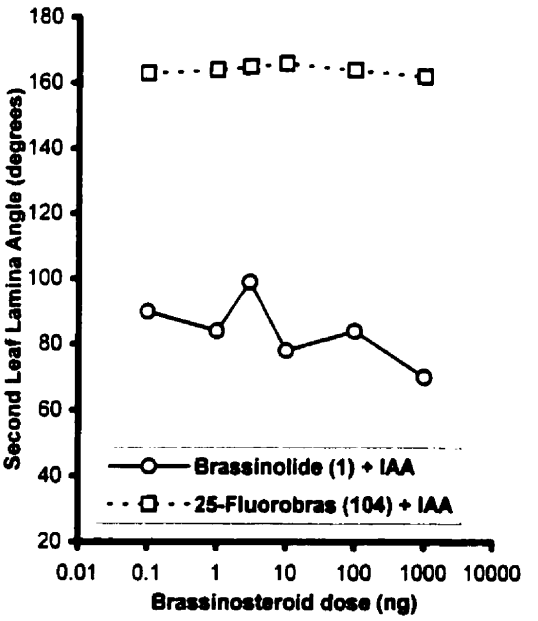


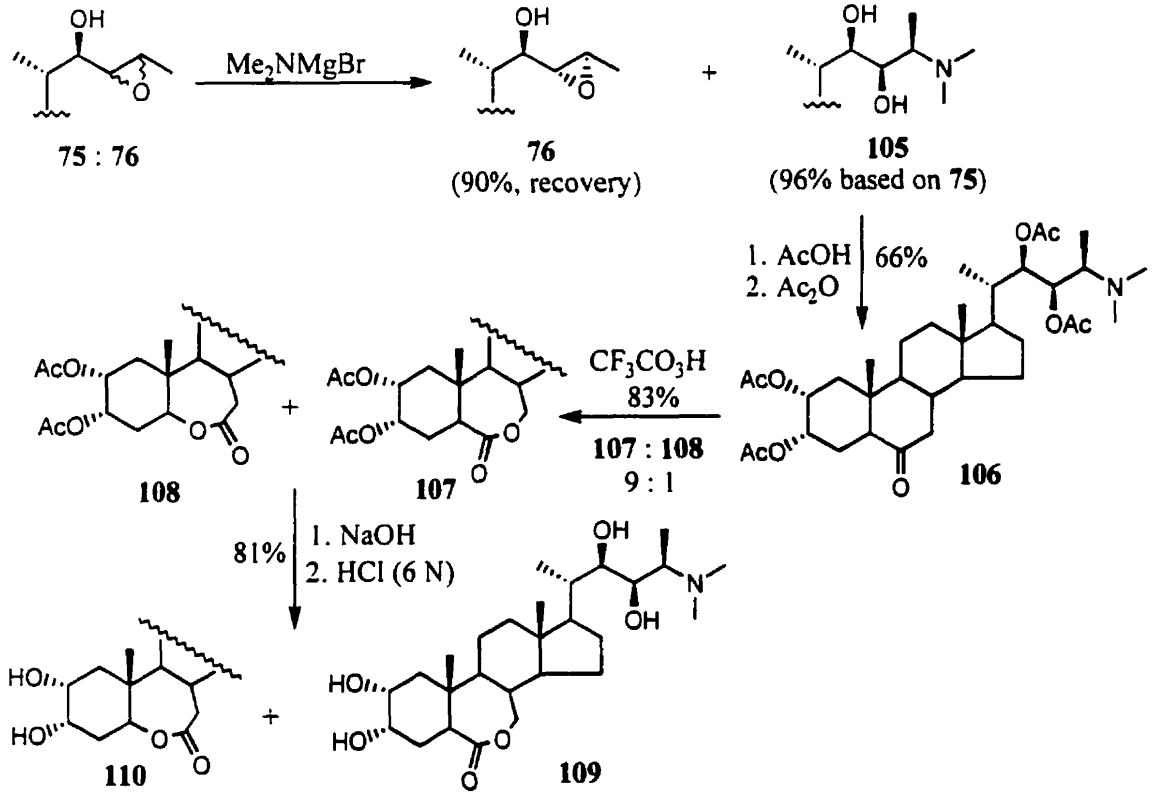
Figure 2.23 The Effect of **1** and **104** With **IAA** on The Rice Leaf Lamina Assay



2.2.3.3 Preparation of 25-Azabrassinolide (109)

We were unsuccessful in obtaining compound **109** by following the literature method of heating the corresponding epoxide with dimethylamine.[63] Alternatively, **109** was obtained by adding the mixture of epoxides **75** and **76** to the corresponding magnesium amide[64] (Scheme 2.7). Diol **105** was obtained in 96% yield by this method. Intermediate **105** was treated with aqueous acetic acid followed by acetylation with acetic anhydride and DMAP to furnish **106** in an overall yield of 66%. Intermediate **106** underwent Baeyer- Villiger oxidation to afford a mixture of **107** and **108** in the ratio of 9:1 in an overall yield of 83%. The unseparated mixture of **107** and **108** was further treated with NaOH followed by 6 N HCl and basic work-up with 10% NaOH solution to give a mixture of **109** and **110** (81%). Finally, the desired analogue **109** was obtained after recrystallization (61%). The structure of analogue **109** was evident from the presence of a new singlet in its ^{1}H-NMR spectrum at δ 2.10 ppm, which corresponds to

Scheme 2.7 Preparation of **109**



the two N-methyl groups, as well as, a signal for the proton α to the nitrogen atom at δ 2.2 ppm. Full characterization of this compound is reported in the Experimental Section. As shown in Figure 2.24 and 2.25, no activity in the rice leaf lamina bioassay was observed with analogue **109**, with or without IAA.

Figure 2.24 The Effect of **1** and **109** on The Rice Leaf Lamina Assay

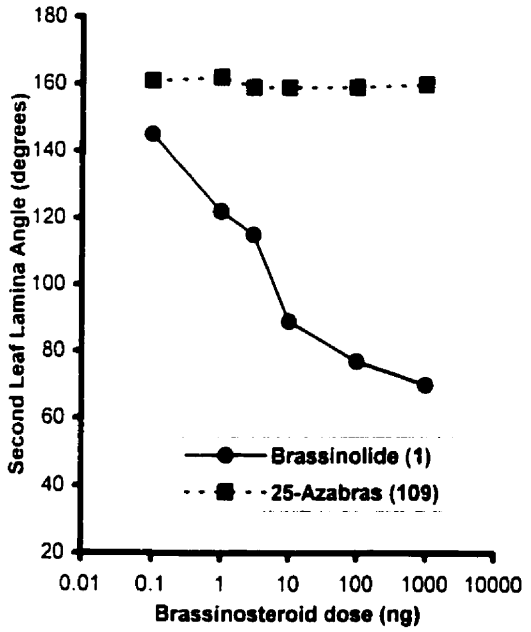
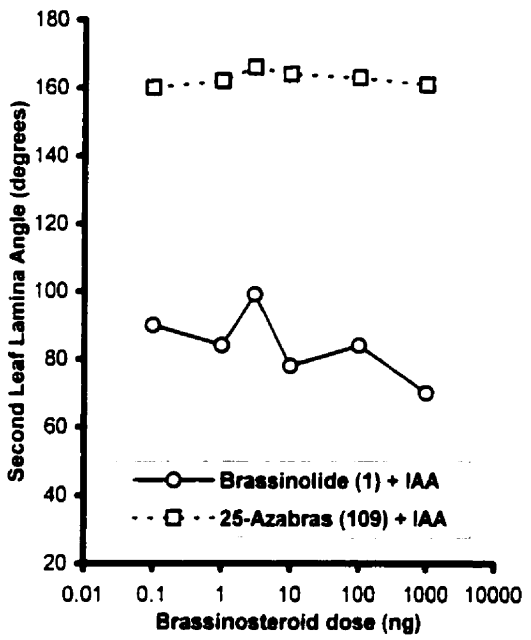


Figure 2.25 The Effect of **1** and **109** With **IAA** on The Rice Leaf Lamina Assay



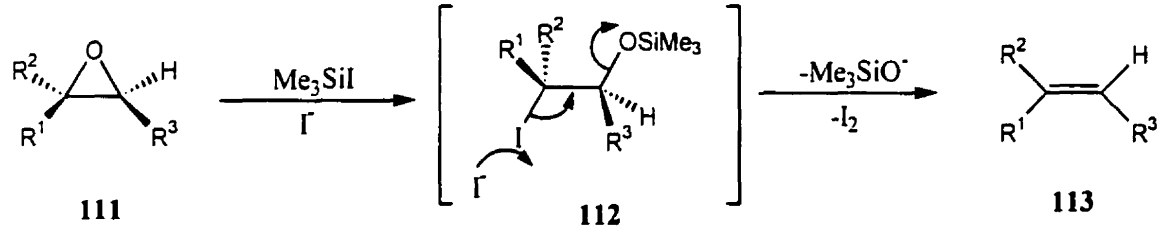
2.3 Recycling of *erythro*-Epoxide 76

It will be recalled that the Sharpless epoxidation step in our synthesis of brassinolide[36] and of all the novel analogues described in this Thesis produced a significant amount of the undesired *erythro*-epoxide **76** along with the required *threo*-epoxide **75**. This undesired epoxide was recovered in high yield after each reaction with a Grignard reagent. Since epoxide **76** is an advanced intermediate that cannot be used further in our synthesis, we needed an effective method to recycle **76** back to the *trans*-allylic alcohol **74** which could then be epoxidized back to the 70:30 mixture of *threo* and

erythro epoxides. Several methods of deoxygenating epoxides have been reported in the literature and were investigated for the purpose of deoxygenating **76**.

The first method investigated employs NaI and Me_3SiCl and was reported by Caputo et al.[65] As shown in Scheme 2.8, iodotrimethylsilane is generated *in situ* from chlorotrimethylsilane and sodium iodide. Iodotrimethylsilane reacts with a given substrate epoxide **111** to afford the corresponding silylated iodohydrin **112**. Further attack of excess iodide ion results in reductive elimination of the iodohydrin to furnish alkene **113** with retention of configuration. However, our substrate **76** did not undergo deoxygenation, but resulted in complex mixtures with the absence of both allylic alcohol **74** and starting material **76** (by NMR).

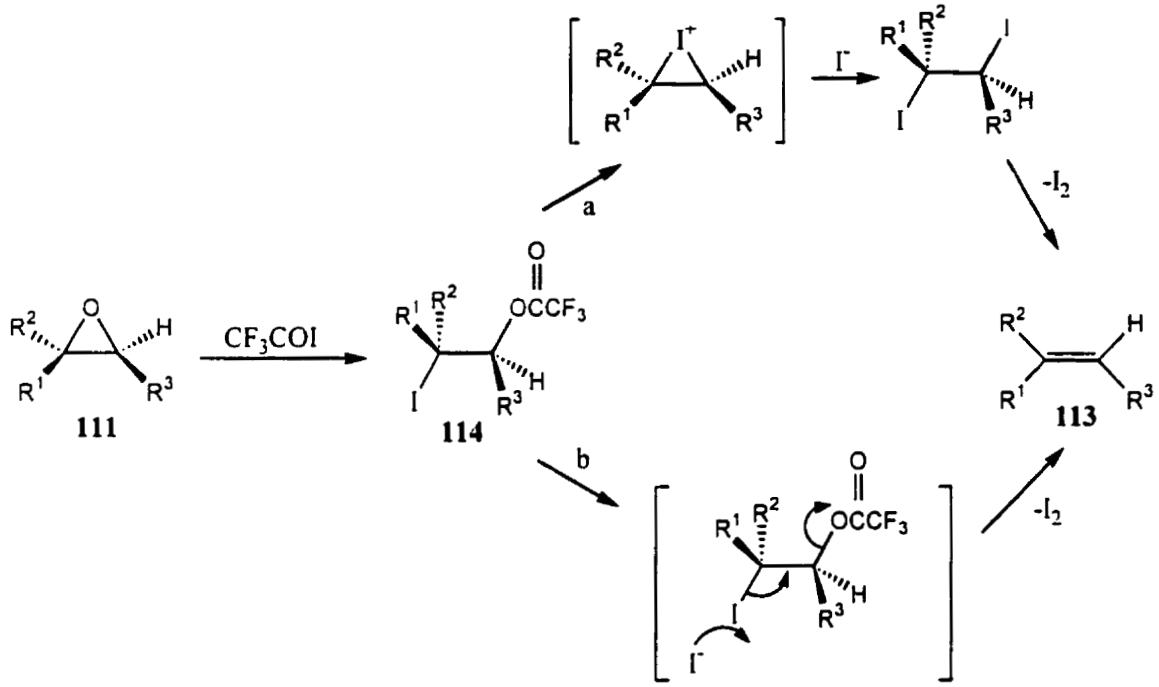
Scheme 2.8 Deoxygenation of Epoxides with Me_3SiI



111 **112** **113**

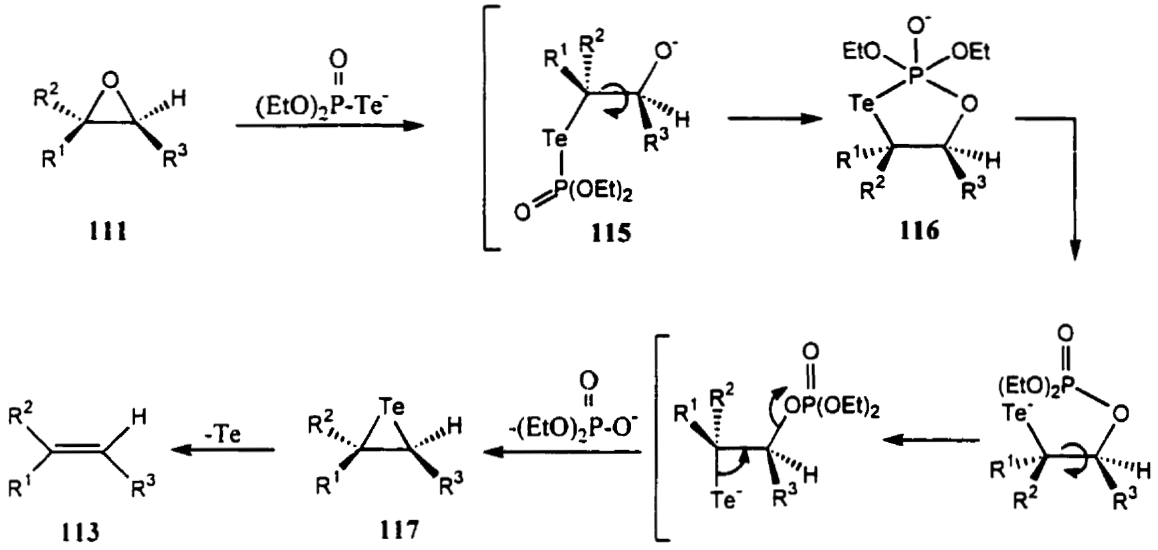
Our next attempt was the use of sodium iodide and trifluoroacetyl iodide prepared *in situ* from trifluoroacetic anhydride and sodium iodide, as reported by Sonnet[66] (Scheme 2.9). Similarly to Caputo's method,[65] the trifluoroacetyl iodide reacts with epoxide **111** to produce a β-iodotrifluoroacetate **114**. Further reaction is assumed to take place either via an iodonium ion (path a), or by a similar reductive elimination (path b) to that shown in Scheme 2.8. Both pathways would lead to the same product with retention of configuration. Unfortunately, again this method gave us complex mixtures of unidentifiable products with no detectable amount (by NMR) of the desired *trans*-allylic acohol **74** being observed when applied to epoxide **76**.

Scheme 2.9 Deoxygenation of Epoxides with Trifluoroacetyl Iodide



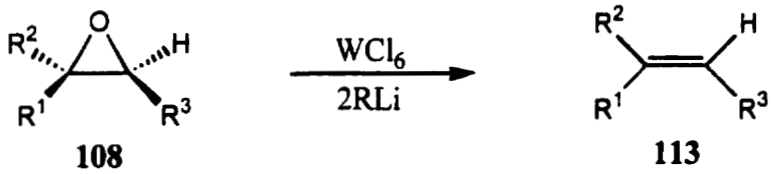
Clive and Menchen[67] reported a method for the deoxygenation of epoxides using alkali metal O,O-diethyl phosphorotelluroates as shown in Scheme 2.10. Nucleophilic attack upon epoxide **111** by O,O-diethyl phosphorotellurate anion, generated from elemental tellurium and sodium diethyl phosphite, provides intermediate **115**. The conversion of **115** to epitelluride **117** is achieved via the postulated cyclic intermediate **116**. Finally, extrusion of elemental tellurium results in alkene **113** with retention of configuration. When we tried this method with our *erythro*-epoxide **76**, we were unsuccessful in obtaining the desired *trans* olefin **74**. A mixture of unidentifiable products was obtained instead (NMR).

Scheme 2.10 Deoxygenation of Epoxides with a Phosphorotelluroate



Our fourth attempt employed a tungsten halide-based method reported by Sharpless et al.[68] As illustrated in Scheme 2.11, *in situ* reduction of tungsten (VI) hexachloride (WCl_6) with an alkyllithium in THF generates tungsten (IV) tetrachloride ($[WCl_6]^{2-}$). Subsequent deoxygenation of epoxide **111** with this species furnishes olefin **113** with retention of configuration. Unlike the previously mentioned methods (Scheme 2.8-2.10), this approach was employed with substrates containing hydroxyl groups, along with the epoxide moiety.[69] Unfortunately, this method also resulted in complex mixtures (NMR) when applied to our system.

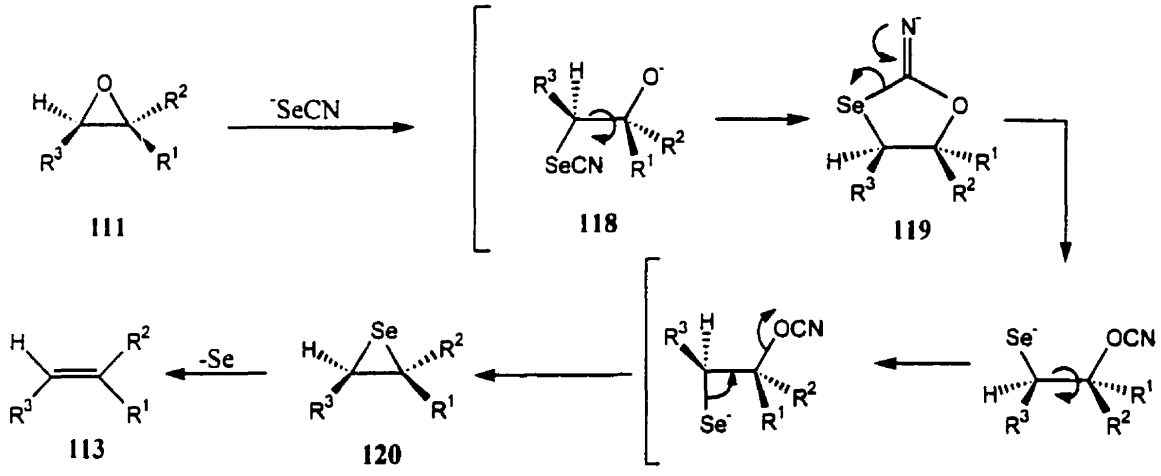
Scheme 2.11 Deoxygenation of Epoxides with WCl_6



The use of potasium selenocyanate[70] to convert epoxide **76** into allylic alcohol **74** proved partly successful, but proceeded in low yield. As illustrated in Scheme 2.12, the

selenocyanate anion attacks the less sterically hindered side of oxiranes to give intermediate **118**. Similarly to Clive and Menchen's method,[67] an episelenide species **120** is formed via cyclic intermediate **119**. Subsequently, extrusion of elemental selenium provides alkene **113**. An initial experiment with **76** resulted in the conversion of the epoxide to the desired allylic alcohol in 50% yield, providing that a large excess of potassium selenocyanate (ca. 30 eq.) was used. We thought that by adding potassium cyanide to the reaction mixture, it would regenerate potassium selenocyanate from the byproduct selenium,[71] thereby permitting less of the latter to be employed. Unfortunately, this resulted in complex mixtures and only trace amounts of the desired product as evident from NMR analysis of the crude reaction mixture.

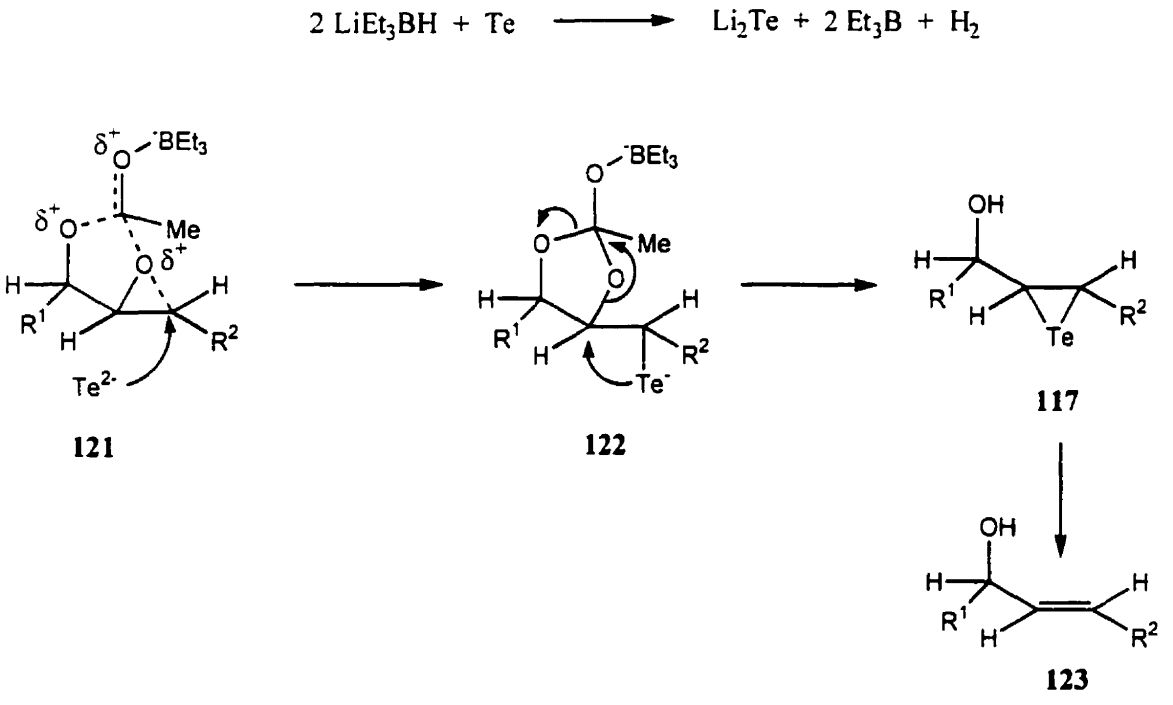
Scheme 2.12 Deoxygenation of Epoxides with Selenocyanate



All of the above methods, except one (Scheme 2.12) that proceeded in low yield, failed to deoxygenate the *erythro*-epoxide **76**. One possible reason could be the interference of the neighboring C-22 hydroxyl group in our substrate **76**. Therefore, it became necessary to deoxygenate the epoxide with protection of the C-22 hydroxyl group. This would introduce two additional steps, including the final deprotection of the hydroxyl moiety. However, in 1994, Dittmer and coworkers[72] reported a method that successfully deoxygenated epoxides derived from allylic acetates that appeared particularly suitable to our requirements. The procedure permits a one pot deoxygenation of epoxides with retention of configuration, along with deacetylation of the acetate group

using telluride ion. As shown in Scheme 2.13, reduction of elemental tellurium with lithium triethylborohydride provides telluride ion (Te^{2-}) and triethylborane. The boron species acts as a Lewis acid and complexes to the carbonyl oxygen atom of glycidyl acetate **121**. Telluride ion (Te^{2-}) then attacks intermediate **121** to form a relatively strain-free five-membered 1,3-dioxolane intermediate **122** from the strained three-membered epoxide. Further intramolecular attack of telluride ion (Te^{-}) provides epitelluride **117**. Finally, extrusion of elemental tellurium affords the desired *trans* allylic alcohol **123**.

Scheme 2.13 Deoxygenation of Epoxides with Dittmer's Method



In order to apply this method to our substrate **76**, we needed to convert the hydroxyl group to the corresponding acetate. Subsequently, glycidyl acetate **124** was treated with telluride ion (Te^{2-}), produced by reduction of elemental Te with lithium triethylborohydride ($LiEt_3BH$), to afford the desired *trans* allylic acohol **74** in an overall yield of 75% from **76** (Scheme 2.14). The *trans* allylic alcohol **74** was then epoxidized back to the 70:30 mixture of *threo* and *erythro* epoxides in the usual manner.

The results of our attempts to deoxygenate epoxy alcohol 76 via the above methods are summarized in Table 2.3.

Scheme 2.14 Recycling of Epoxide **76**

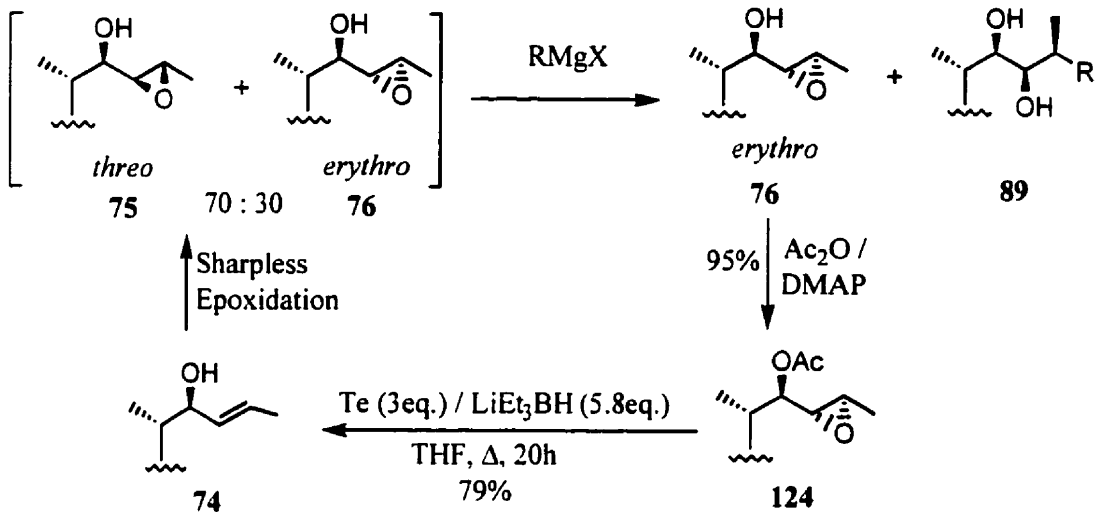


Table 2.3 Conditions for Recycling Epoxide **76**

Entry	Reagent(s)	Substrate	Conditions	Yield %
1	$(CH_3)_3SiCl$ + NaI	**76**	24 h, RT, THF-CH_3CN	C.M.[a,b]
2	$(CF_3CO)_2O$ + NaI	**76**	24 h, RT, THF-CH_3CN	C.M.[a,b]
3	$(EtO)_2P(O)TeNa$	**76**	24 h, RT, N_2	C.M.[a,b]
4	WCl_6 + *n*-BuLi	**76**	0.5 h, -78°C, THF, N_2	C.M.[a,b]
5	KSeCN (1.5eq)	**76**	1.5 h, 55°C, MeOH-H_2O	N.R.[a,b]
6	KSeCN (3.0eq)	**76**	84 h, 55°C, MeOH-H_2O	15%[c]
7	KSeCN (30eq)	**76**	48h, 55°C, MeOH-H_2O	50%[c]
8	KSeCN (5eq) + KCN (5eq)	**76**	48h, 55°C, MeOH-H_2O	C.M.[a,b]
9	Te (3eq) / $LiEt_3BH$ (5.8eq)	**124**	2h, RT, THF	12%[c]
10	Te (6eq) / $LiEt_3BH$ (11eq)	**124**	24h, RT, THF	65%[d]
11	Te (3eq) / $LiEt_3BH$ (5.8eq)	**124**	20h, Δ, THF	79%[d]

a) Based on NMR and TLC. b) C.M. = Complex mixture; N.R. = No reaction. c) Product was obtained as a mixture with starting material and the yield was determined based on NMR. d) Isolated yield.

2.4 Conclusions

It has been shown that most of the novel side-chain analogues reported in this Thesis can be prepared in two steps from the mixture of epoxy alcohols **75** and **76**, except for the C-25 substituted analogues **101** (25-methoxy), **104** (25-fluoro), and **109** (25-aza), which required further elaboration after the epoxide ring-opening step with 2-propenyl Grignard reagent. This mixture of epoxides was synthesized from stigmasterol (**36**) in ten steps in an overall yield of 26% in the ratio of 70:30.[36] Since *erythro* epoxide **76** was relatively less reactive than *threo* epoxide **75**, it was recovered in high yield after the addition of various Grignard reagents. Therefore, a procedure was developed, based on Dittmer's telluride anion deoxygenation,[72] in which the undesired advanced intermediate **76** was effectively recycled back to the *trans* allylic alcohol **74** in two steps in an overall yield of 75%. Thus, the only moderate stereoselectivity of the epoxidation of allylic alcohol **74** in Back's most recent synthesis of brassinolide[36] was compensated by this recycling process.

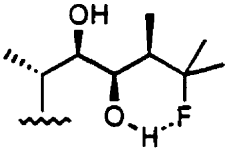
The rice leaf lamina bioassay revealed that long lipophilic side-chains, as in the *n*-dodecyl and *n*-hexyl analogues **92a** and **92b**, respectively, resulted in loss of biological activity. Activity resumed with a three carbon chain at C-24 (**92c**). It can be concluded that a twelve or six carbon substituent at C-24 is too big to fit into the active site of the putative receptor. The maximum chain length at C-24 that can be accommodated appears to be between three and six carbon atoms in length, since slight activity was observed with the *n*-hexyl derivative **92b** at high doses and pronounced activity was observed with the *n*-propyl analogue **92c**.

In contrast to the *n*-alkyl analogues, cyclic substituents are better tolerated at C-24, although it was found that ring size greatly affects the activity. Activity was dramatically enhanced as the ring systems become smaller; thus highest activity was observed with cyclopropyl analogue **92g**. Remarkably, both the cyclopropyl (**92g**) and cyclobutyl (**92f**) analogues produced greater responses than brassinolide itself, and both compounds displayed higher activity than 25-methylbrassinolide (**86**), a compound that had been previously claimed to be the most potent brassinosteroid.[51] These analogues are

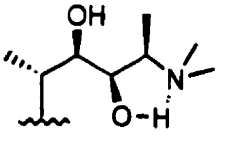
therefore the most potent brassinosteroids reported to date in this bioassay, with their enhanced activity attributed to conformational restriction of the side-chain. We also demonstrated that all of the active brassinosteroids described here showed strong synergy with the auxin IAA. This permits doses of brassinosteroids to be decreased by 1-2 orders of magnitude. Thus, doses of expensive brassinosteroids can be reduced by applying them with an inexpensive auxin. This may make brassinosteroids more economical in future agricultural applications.

Significant activity was observed with 25-methoxybrassinolide (**101**) which suggests that this analogue is a worthy candidate for further investigation of persistence in the field, since it is expected to block metabolic deactivation by glucosylation at C-25 after enzymatic hydroxylation by the plant at that position. Since the 25-methoxy analogue **101** is active, but the 25-fluoro **104** and the 25-aza **109** analogues are not, these results suggest that hydroxylation at C-25 may be required for biological activity, and substituents that prevent hydroxylation render the compound inactive. This in turn suggests the interesting possibility that brassinolide might not be the principal endogenous plant growth promoter after all, but that it is merely the precursor of 25-hydroxybrassinolide (**18**), which is the true plant hormone. In other words, in order for a brassinosteroid to be biological active, it may require an oxidizable site or an existing oxygen substituent at C-25. Alternatively, the fluorine or nitrogen atoms in **104** and **109**, respectively, change the conformation of the side chain by means of intramolecular hydrogen-bonding as in structures **125** and **126a** or **126b**, thereby preventing these modified brassinosteroids from binding to the active site of the putative receptor.

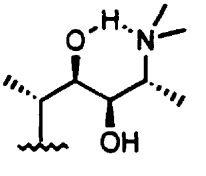
Figure 2.26 Hydrogen-Bonding in the Side Chains of **104** and **109**



125



126a



126b

Further investigation is required to test these hypotheses, as well as to explain why 25-methylbrassinolide (**86**) is comparable in activity to **1** despite having the C-25 site blocked by a methyl group.

Chapter Three

Experimental Section

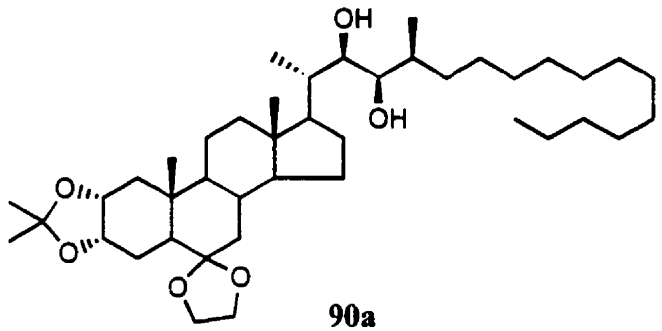
3.1 General Comments

Melting points were determined on a A.H. Thomas hot-stage apparatus and are uncorrected. IR spectra were recorded on a Mattson 4030 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ACE 200, Bruker AM 400, Varian XL 300 or Varian XL 200 spectrometer, with deuteriochloroform as the solvent and, where indicated, deuteriomethanol as the co-solvent, and chloroform as the internal standard. ^{19}F NMR spectra were obtained on a Bruker AM 400 or Varian XL 300 spectrometer, with deuteriochloroform as the solvent, and hexafluorobenzene (C_6F_6) as the internal standard. Assignment of signals as CH_3, CH_2, CH, or C, where so indicated in ^{13}C NMR spectra, was performed by DEPT experiments.[73] Elemental analyses and mass spectra were obtained by Ms. D. Fox and Ms. Q. Wu at the University of Calgary. Analytical TLC was carried out with aluminum sheets coated with Merck silica gel 60 F-254, and the spots were visualized by spraying with a 2% ceric sulfate solution in 12% aqueous sulfuric acid, or by dipping in 10% ammonium molybdate solution in aqueous sulfuric acid, followed by heating for several seconds. Flash chromatography[74] was performed using Merck silica gel, 230-400 mesh.

The mixture of epoxides **75** and **76** was prepared according to the procedure of Back et al.[36], and the ratio obtained for this mixture was ca. 70:30 (by NMR integration). Noncommercial Grignard reagents were prepared[55] in THF at the time of the reaction. All Grignard reagents were titrated with 1 M sec-butyl alcohol in xylene and 1,10-phenanthroline as the indicator.[75] Trifluoroperoxyacetic acid was generated *in situ* with hydrogen peroxide and trifluoroacetic anhydride at 0°C.[76] All other reagents were obtained from commercial sources and were used without further purification. Anhydrous THF, diethyl ether and methylene chloride were obtained by distillation from lithium aluminum hydride, calcium hydride and phosphorus pentoxide, respectively.

Solutions of common inorganic reagents refer to saturated aqueous solutions unless otherwise indicated.

3.2 (2R,3S,5α,22R,23R,24S)-24-*n*-Dodecyl-6,6-(ethylenedioxy)-2,3-(isopropylidene-dioxy)-26,27-dinorcholestane-22,23-diol (90a)

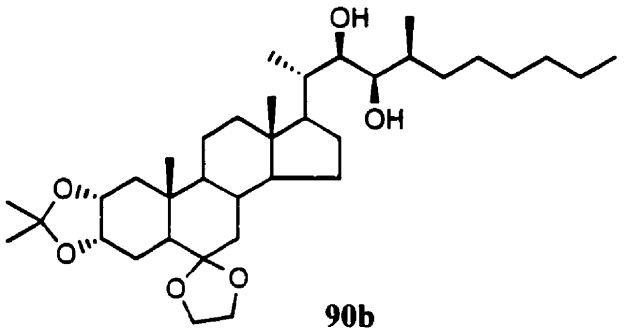


90a

Similarly to the procedure reported by Back and coworkers[36] for the preparation of brassinolide (**1**), *n*-dodecylmagnesium bromide (11 mL of a 0.82 M solution in diethyl ether, 8.4 mmol) was added to a suspension of CuCN (63.0 mg, 0.703 mmol) in 10 mL of diethyl ether at -78 °C. The mixture was stirred for 1 h, followed by the slow addition of the mixture of epoxides **75** and **76** (710 mg, 1.41 mmol; *threo/erythro* ratio of 67:33) in 10 mL of ether. The reaction mixture was stirred for 1 h at -78 °C and for 4 h at 0 °C, followed by the addition of 20% aqueous NH_4Cl solution, and extraction three times with ether. The organic extracts were washed twice with $NaHCO_3$ solution and once with NaCl solution, dried ($MgSO_4$), and concentrated under vacuum. The crude product was chromatographed over silica gel (elution with 25-60% ether-hexanes) to afford 275 mg (88%) of recovered *erythro*-epoxy alcohol **76** and 428 mg (67%) (based on *threo*-epoxide **75**) of the 22,23-diol **90a** as a colourless oil: IR (KBr) 3409 (OH), 1238, 1218, 1082, 1051 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 1 H), 3.55 (br s, 2 H, 22 and 23-H), 1.49 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 0.91 (d, J = 5.6 Hz, 3 H, 21-Me), 0.89 (d, J = 6.1 Hz, 3H, 28-Me) 0.85 (t, J = 7.9 Hz, 3 H, *n*-dodecyl-Me), 0.84 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.9 (C), 75.0 (CH), 74.4 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8

(CH), 53.0 (CH), 52.3 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (C), 40.9 (CH_2), 39.7 (CH_2), 38.0 (C), 37.0 (CH), 34.4 (CH_2), 33.8 (CH), 32.9 (CH), 31.9 (CH_2), 29.8 (CH_2), 29.6 (five CH_2), 29.3 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 27.3 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 22.6 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 14.1 (CH_3), 13.4 (CH_3), 12.7 (CH_3), 12.0 (CH_3), 11.9 (CH_3); mass spectrum, *m/z* (relative intensity %) 674 (M^+, 2), 659 (M^+-CH_3, 6), 431 (29), 235 (85), 58 (100). Exact mass calculated for $C_{42}H_{74}O_6$: 674.5485. Found: 674.5521.

3.3 (2R,3S,5α,22R,23R,24S)-24-*n*-Hexyl-6,6-(ethylenedioxy)-2,3-(isopropylidene-dioxy)-26,27-dinorcholestane-22,23-diol (90b)

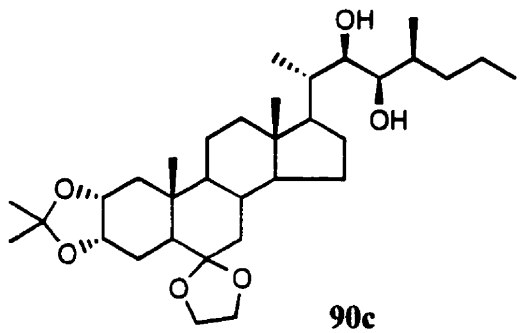


90b

The procedure for the preparation of compound **90a** was followed with 0.991 mmol of **75/76** (1.5:1), employing *n*-hexylmagnesium bromide instead of the *n*-dodecyl derivative. Flash chromatography (elution with 40-70% ether-hexanes) afforded 86% of recovered *erythro*-epoxy alcohol **76** and 67% (based on *threo*-epoxide **75**) of the 22,23-diol **90b** as a colourless oil: IR (KBr) 3476 (OH), 1456, 1378, 1231, 1056 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.77 (m, 1 H), 3.55 (br s, 2 H, 22 and 23-H), 1.48 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 0.91 (d, J = 5.9 Hz, 3 H, 21-Me), 0.90 (d, J = 4.4 Hz, 3 H, 28-Me), 0.84 (s, 3 H, 19-Me), 0.85 (t, J = 4.3 Hz, 3 H, *n*-hexyl-Me), 0.68 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.5 (C), 75.0 (CH), 74.4 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8 (CH), 52.9 (CH), 52.4 (CH), 45.4 (CH), 42.7 (C), 42.3 (CH_2), 40.9 (CH_2), 39.7 (CH_2), 38.0 (C), 37.0 (CH), 34.5 (CH_2), 33.8 (CH), 32.9 (CH), 31.8 (CH_2), 29.5 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 27.3 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 22.6 (CH_2), 21.9 (CH_2), 20.8 (CH_2), 14.1 (CH_3), 13.4

(CH_3), 12.7 (CH_3), 12.0 (CH_3), 11.9 (CH_3); mass spectrum, *m/z* (relative intensity %) 590 (M^+, 1), 575 (M^+-CH_3, 5), 557 (3), 431 (56), 235 (100). Exact mass calculated for $C_{36}H_{62}O_6$: 590.4546. Found: 590.4489.

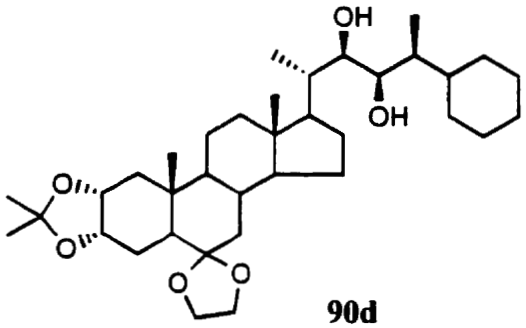
3.4 (2R,3S,5α,22R,23R,24S)-24-*n*-Propyl-6,6-(ethylenedioxy)-2,3-(isopropylidene-dioxy)-26,27-dinorcholestane-22,23-diol (90c)



90c

The procedure for the preparation of compound **90a** was followed with 0.793 mmol of **75/76** (1.5:1), employing *n*-propylmagnesium chloride instead of the *n*-dodecyl derivative. Flash chromatography (elution with 40-60% ether-hexanes) afforded 96% of recovered *erythro*-epoxy alcohol **76** and 65% (based on *threo*-epoxide **75**) of the 22,23-diol **90c** as a colourless oil: IR (KBr) 3434 (OH), 1456, 1376, 1228, 1055 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 1 H), 3.56 (br s, 2 H, 22 and 23-H), 1.49 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 0.93 (d, J = 6.9 Hz, 3 H, 21-Me), 0.91 (d, J = 5.4 Hz, 3H, 28-Me), 0.84 (t, J = 3.3 Hz, 3 H, *n*-propyl-Me), 0.84 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.6 (C), 75.1 (CH), 74.5 (CH), 73.0 (CH), 72.9 (CH), 65.5 (CH_2), 64.2 (CH_2), 55.9 (CH), 53.0 (CH), 52.4 (CH), 45.5 (CH), 42.7 (C), 42.3 (CH_2), 41.0 (CH_2), 39.7 (CH_2), 38.0 (C), 37.0 (CH), 36.7 (CH_2), 33.6 (CH), 33.0 (CH), 28.6 (CH_3), 27.7 (CH_2), 26.6 (CH_3), 24.1 (CH_2), 22.0 (CH_2), 20.8 (CH_2), 20.4 (CH_2), 14.3 (CH_3), 13.4 (CH_3), 12.6 (CH_3), 12.0 (CH_3), 11.9 (CH_3); mass spectrum, *m/z* (relative intensity %) 548 (M^+, 28), 533 (M^+-CH_3, 63), 431 (45), 337 (100), 235 (74). Exact mass calculated for $C_{33}H_{56}O_6$: 548.4077. Found: 548.4061.

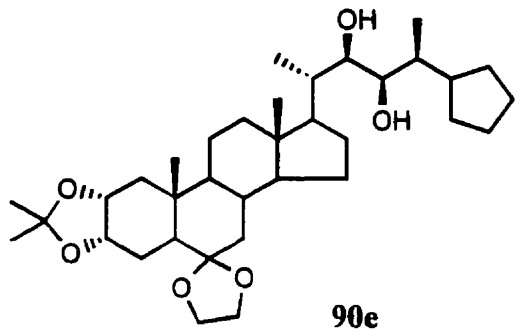
3.5 (2R,3S,5α,22R,23R,24S)-24-Cyclohexyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (90d)



90d

The procedure for the preparation of compound **90a** was followed with 2.28 mmol of **75/76** (0.8:1), employing cyclohexylmagnesium chloride instead of the *n*-dodecyl derivative. Flash chromatography (elution with 25-60% ether-hexanes) afforded 81% of recovered *erythro*-epoxy alcohol **76** and 73% (based on *threo*-epoxide **75**) of the 22,23-diol **90d** as a colourless oil: IR (KBr) 3458 (OH), 1240, 1217, 1083, 1051, 972, 753 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.27 (m, 1 H), 4.10 (m, 1 H), 3.9 (m, 3 H), 3.73 (m, 2 H), 3.55 (br d, J = 8.5 Hz, 1 H, 22 or 23-H), 1.47 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 0.88 (d, J = 5.8 Hz, 3 H, 21-Me), 0.83 (d, J = 6.0 Hz, 3 H, 28-Me), 0.83 (s, 3 H, 19-Me), 0.67 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.5 (C), 74.7 (CH), 72.9 (CH), 72.8 (CH), 72.4 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8 (CH), 52.9 (CH), 52.3 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (C), 40.9 (CH_2), 40.4 (CH), 39.7 (CH_2), 38.8 (CH), 38.0 (C), 36.8 (CH), 32.9 (CH), 31.0 (CH_2), 30.9 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 26.6 (CH_2), 26.6 (CH_3), 24.0 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 13.4 (CH_3), 11.9 (two CH_3), 10.0 (CH_3); mass spectrum, *m/z* (relative intensity %) 588 (M^+, 2), 573 (M^+-CH_3, 8), 446 (16), 431 (60), 235 (100). Exact mass calculated for $C_{36}H_{60}O_6$: 588.4390. Found: 588.4371.

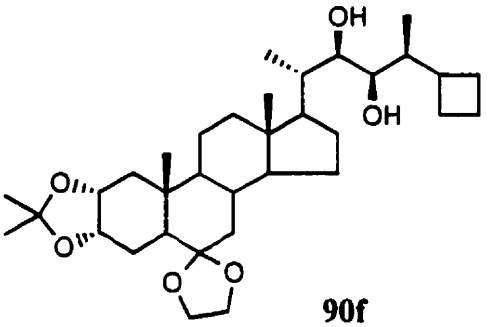
3.6 (2R,3S,5α,22R,23R,24S)-24-Cyclopentyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (90e)



90e

The procedure for the preparation of compound **90a** was followed with 0.991 mmol of **75/76** (1.5:1), employing cyclopentylmagnesium chloride instead of the *n*-dodecyl derivative. Flash chromatography (elution with 40-70% ether-hexanes) afforded 89% of recovered *erythro*-epoxy alcohol **76** and 81% (based on *threo*-epoxide **75**) of the 22,23-diol **90e** as a colourless oil: IR (KBr) 3439 (OH), 1456, 1378, 1230, 1056 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.95 (m, 3 H), 3.75 (m, 1 H), 3.66 (br d, J = 8.6 Hz, 1 H, 22 or 23-H), 3.55 (br d, J = 8.5 Hz, 1 H, 22 or 23-H), 1.49 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 0.90 (d, J = 6.2 Hz, 3 H, 21-Me), 0.87 (d, J = 6.7 Hz, 3 H, 28-Me), 0.84 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.6 (C), 74.65 (CH), 74.57 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.2 (CH_2), 55.9 (CH), 52.9 (CH), 52.4 (CH), 45.5 (CH), 43.6 (CH), 42.7 (CH_2), 42.3 (C), 41.0 (CH_2), 39.8 (CH), 39.7 (CH_2), 38.0 (C), 36.6 (CH), 33.0 (CH), 31.5 (CH_2), 31.1 (CH_2), 28.6 (CH_3), 27.7 (CH_2), 26.6 (CH_3), 25.1 (CH_2), 25.0 (CH_2), 24.0 (CH_2), 22.0 (CH_2), 20.8 (CH_2), 13.4 (CH_3), 11.92 (CH_3), 11.87 (CH_3), 11.5 (CH_3); mass spectrum, *m/z* (relative intensity %) 574 (M^+, 2), 559 (M^+-CH_3, 9), 446 (17), 431 (51), 235 (100). Exact mass calculated for $C_{35}H_{58}O_6$: 574.4233. Found: 574.4248.

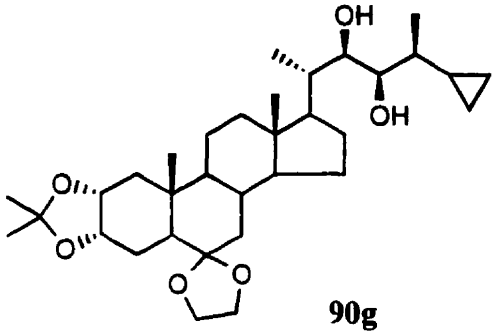
3.7 (2R,3S,5α,22R,23R,24S)-24-Cyclobutyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (90f)



90f

The procedure for the preparation of compound **90a** was followed with 0.793 mmol of **75/76** (1.5:1), employing cyclobutylmagnesium chloride instead of the *n*-dodecyl derivative. Flash chromatography (elution with 40-70% ether-hexanes) afforded 85% of recovered *erythro*-epoxy alcohol **76** and 78% (based on *threo*-epoxide **75**) of the 22,23-diol **90f** as a colourless oil: IR (KBr) 3439 (OH), 1454, 1375, 1228, 1051 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.95 (m, 3 H), 3.75 (m, 1 H), 3.52 (br s, 2 H, 22 and 23-H), 1.50 (s, 3 H, acetonide), 1.35 (s, 3 H, acetonide), 0.90 (d, J = 5.9 Hz, 3 H, 21-Me), 0.84 (s, 3 H, 19-Me), 0.73 (d, J = 6.8 Hz, 3 H, 28-Me), 0.69 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.6 (C), 74.5 (CH), 73.0 (CH), 72.9 (CH), 72.2 (CH), 65.5 (CH_2), 64.2 (CH_2), 55.9 (CH), 52.9 (CH), 52.4 (CH), 45.5 (CH), 42.7 (CH_2), 42.3 (C), 41.0 (CH), 39.7 (CH_2), 38.9 (CH), 38.0 (C), 36.8 (CH), 33.0 (CH), 28.6 (CH_3), 27.7 (CH_2), 27.5 (CH_2), 27.0 (CH_2), 26.6 (CH_3), 24.0 (CH_2), 22.0 (CH_2), 20.8 (CH_2), 17.5 (CH_2), 13.4 (CH_3), 11.94 (CH_3), 11.85 (CH_3), 9.0 (CH_3); mass spectrum, *m/z* (relative intensity %) 560 (M^+, 11), 545 (M^+-CH_3, 33), 446 (18), 431 (58), 235 (100). Exact mass calculated for $C_{34}H_{56}O_6$: 560.4077. Found: 560.4101.

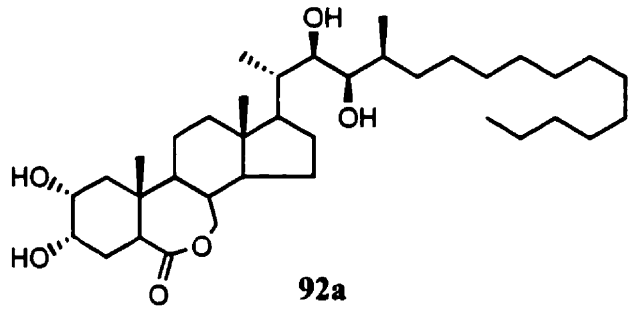
3.8 (2R,3S,5α,22R,23R,24S)-24-Cyclopropyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (90g)



90g

The procedure for the preparation of compound **90a** was followed with 1.14 mmol of **75/76** (1.2:1), employing cyclopropylmagnesium bromide instead of the *n*-dodecyl derivative. Flash chromatography (elution with 30-60% ether-hexanes) afforded 90% of recovered *erythro*-epoxy alcohol **76** and 76% (based on *threo*-epoxide **75**) of the 22,23-diol **90g** as a colourless oil: IR (KBr) 3478 (OH), 1456, 1378, 1229, 1055 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.29 (m, 1 H), 4.14 (m, 1 H), 3.95 (m, 3 H), 3.74 (m, 2 H), 3.58 (br d, J = 7.0 Hz, 1 H, 22 or 23-H), 1.48 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 0.96 (d, J = 6.6 Hz, 3 H, 21-Me), 0.88 (d, J = 6.4 Hz, 3 H, 28-Me), 0.84 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me), 0.49 (br t, J = 7.0 Hz, 2 H, cyclopropane), 0.13 (br d, J = 4.9 Hz, 2 H, cyclopropane); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.5 (C), 76.1 (CH), 73.9 (CH), 72.9 (CH), 72.8 (CH), 65.4 (CH_2), 64.1 (CH_2), 55.8 (CH), 52.9 (CH), 52.3 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (C), 40.9 (CH_2), 40.3 (CH), 39.7 (CH_2), 37.9 (C), 37.1 (CH), 32.9 (CH), 28.5 (CH_3), 27.7 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 15.7 (CH), 13.3 (CH_3), 12.5 (CH_3), 11.9 (CH_3), 11.8 (CH_3), 4.3 (CH_2), 4.0 (CH_2); mass spectrum, *m/z* (relative intensity %) 546 (M^+, 2), 531 (M^+-CH_3, 4), 446 (9), 431 (46), 235 (100). Exact mass calculated for $C_{33}H_{54}O_6$: 546.3920. Found: 546.3904.

3.9 (2R,3S,5α,22R,23R,24S)-24-*n*-Dodecyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92a)

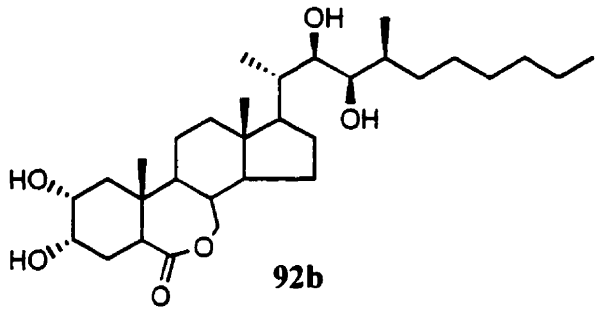


92a

Following the procedure by Back et al.,[36] aqueous hydrogen peroxide (0.19 mL of a 30% solution, ca. 2.1 mmol) was slowly added to trifluoroacetic anhydride (1.54 mL, 10.9 mmol) at 0 °C, and was allowed to stir for 30 min. In a separate vessel, trifluoroacetic acid (1.3 mL) was added to a solution of diol **90a** (175 mg, 0.259 mmol) in 10 mL of chloroform. The latter solution was stirred at room temperature for 40 min and was then added slowly to the pregenerated trifluoroperoxyacetic acid solution at 0 °C, followed by warming to room temperature and stirring for an additional 1.5 h. The mixture was diluted with chloroform and was washed twice with 10 mL of water and twice with 10 mL of 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$) and concentrated under vacuum. The resulting yellow-white solid was chromatographed over silica gel (elution with 5-10% methanol-chloroform) to provide 136 mg (86%) of a mixture of **92a** and its 6-oxa regioisomer (**93a**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 106 mg (67%) of pure **92a**: mp 208-211 °C; IR (KBr) 3440 (OH), 1695 (lactone), 1187, 1062, 1044, 981 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (m, 1 H), 3.74 (m, 1 H), 3.57 (br s, 2 H, 22 and 23-H), 3.13 (dd, J = 12.2, 4.1 Hz, 1 H), 0.93 (s, 3 H, 19-Me), 0.91 (d, J = 6.6 Hz, 3 H, 21-Me), 0.90 (t, J = 7.0 Hz, 3 H, *n*-dodecyl-Me), 0.84 (d, J = 6.8 Hz, 3 H, 28-Me), 0.72 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 177.3 (C), 74.8 (CH), 73.9 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.1 (CH), 51.2 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 39.5 (CH_2), 39.0 (CH), 38.1 (C), 37.0 (CH), 34.3 (CH_2), 33.8 (CH), 31.8 (CH_2), 31.1 (CH_2), 29.8 (CH_2), 29.6 (five CH_2), 29.2

(CH_2), 27.4 (CH_2), 27.2 (CH_2), 24.6 (CH_2), 22.6 (CH_2), 22.1 (CH_2), 15.3 (CH_3), 14.0 (CH_3), 12.5 (CH_3), 11.8 (CH_3), 11.6 (CH_3); mass spectrum, *m/z* (relative intensity %) 603 (5), 577 (3), 380 (6), 55 (100). Anal. Calculated for $C_{37}H_{66}O_6$: C, 73.22; H, 10.96. Found: C, 73.00; H, 10.70.

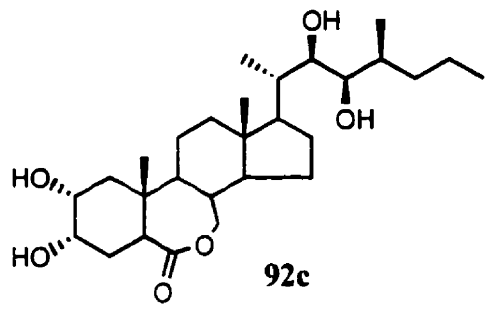
3.10 (2R,3S,5α,22R,23R,24S)-24-*n*-Hexyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92b)



92b

Compound **90b** (0.330 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 60% of a mixture of **92b** and its 6-oxa regioisomer (**93b**) in the ratio of 8:1 (NMR integration). Recrystallization from methanol afforded 46% of pure **92b**: mp 228-230 °C; IR (KBr) 3442 (OH), 1709 (lactone), 1456, 1390, 1063, 977 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.11 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 1 H), 3.55 (br s, 2 H, 22 and 23-H), 3.13 (dd, J = 7.6, 4.5 Hz, 1 H), 0.93 (s, 3 H, 19-Me), 0.91 (d, J = 6.5 Hz, 3 H, 21-Me), 0.89 (t, J = 6.8 Hz, 3 H, *n*-hexyl-Me), 0.84 (d, J = 6.7 Hz, 3 H, 28-Me), 0.72 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 176.3 (C), 75.2 (CH), 74.2 (CH), 70.4 (CH_2), 68.14 (CH), 68.12 (CH), 58.1 (CH), 52.3 (CH), 51.4 (CH), 42.4 (C), 41.4 (CH_2), 40.9 (CH), 39.7 (CH_2), 39.2 (CH), 38.3 (C), 37.1 (CH), 34.5 (CH_2), 33.9 (CH), 31.8 (CH_2), 31.1 (CH_2), 29.5 (CH_2), 27.6 (CH_2), 27.3 (CH_2), 24.7 (CH_2), 22.6 (CH_2), 22.2 (CH_2), 15.5 (CH_3), 14.1 (CH_3), 12.6 (CH_3), 12.0 (CH_3), 11.7 (CH_3); mass spectrum, *m/z* (relative intensity %) 465 (1), 380 (8), 333 (13), 229 (23), 81 (100). Anal. Calculated for $C_{31}H_{54}O_6$: C, 71.23; H, 10.41. Found: C, 71.49; H, 10.37.

3.11 (2R,3S,5α,22R,23R,24S)-24-*n*-Propyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92c)

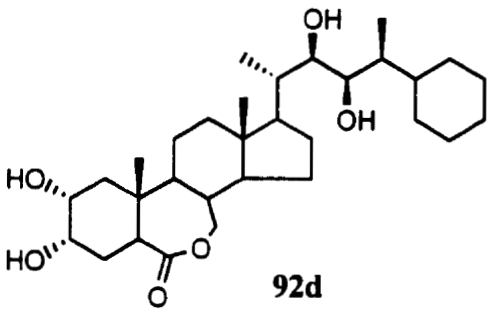


92c

Compound **90c** (0.270 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 66% of a mixture of **92c** and its 6-oxa regioisomer (**93c**) in the ratio of 8.5:1.5 (NMR integration). Recrystallization from methanol afforded 40% of pure **92c**: mp 237-240 °C; IR (KBr) 3405 (OH), 1722 (lactone), 1452, 1381, 1062, 979 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.12 (m, 2 H), 4.02 (m, 1 H), 3.72 (m, 1 H), 3.55 (br s, 2 H, 22 and 23-H), 3.12 (dd, J = 7.7, 4.5 Hz, 1 H), 0.94 (t, J = 6.0 Hz, 3 H, *n*-propyl-Me), 0.93 (s, 3 H, 19-Me), 0.92 (d, J = 6.5 Hz, 3 H, 21-Me), 0.85 (d, J = 6.8 Hz, 3 H, 28-Me), 0.73 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 176.7 (C), 74.9 (CH), 74.0 (CH), 70.5 (CH_2), 67.9 (2 CH), 58.1 (CH), 52.2 (CH), 51.2 (CH), 42.4 (C), 41.3 (CH_2), 40.9 (CH), 39.6 (CH_2), 39.1 (CH), 38.2 (C), 37.0 (CH), 36.6 (CH_2), 33.6 (CH), 31.0 (CH_2), 27.5 (CH_2), 24.7 (CH_2), 22.2 (CH_2), 20.3 (CH_2), 15.4 (CH_3), 14.2 (CH_3), 12.5 (CH_3), 11.9 (CH_3), 11.6 (CH_3); mass spectrum, *m/z* (relative intensity %) 462 (M^+-H_2O, 1), 409 (4), 380 (38), 333 (26), 81 (100). Anal. Calculated for $C_{28}H_{48}O_6$: C, 69.96; H, 10.06. Found: C, 68.97; H, 9.83 (recrystallization was carried out three times, and this was the best analytical result obtained). Since its elemental analysis was not satisfactory, **92c** was converted to the corresponding tetraacetate by treatment with acetic anhydride and DMAP in pyridine for 5 h. The following spectra are for the tetraacetate derivative of **92c**: ^{1}H-NMR (200 MHz) δ 5.36 (m, 1 H, 2 or 3-H), 5.17 (br s, 2 H, 22 and 23-H),

4.90 (m, 1 H, 2 or 3-H), 4.09 (m, 2 H), 2.99 (dd, J = 7.4, 4.6 Hz, 1 H), 2.12 (s, 3 H, Me-C=O), 2.02 (s, 6 H, Me-C=O), 2.01 (s, 3 H, Me-C=O), 1.01 (d, J = 8.2 Hz, 3 H, 21-Me), 0.99 (3 H, 19-Me), 0.95 (d, J = 6.8 Hz, 3 H, 28-Me), 0.89 (t, J = 7.1 Hz, 3 H, *n*-propyl-Me), 0.74 (s, 3 H, 18-Me); mass spectrum, *m/z* (relative intensity %) 589 (M^+-AcOH, 1), 528 (M^+-2AcOH, 2), 506 (18), 463 (100). Exact mass calculated for $C_{32}H_{48}O_6$ (M^+-2AcOH): 528.3451. Found: 528.3405.

3.12 (2R,3S,5α,22R,23R,24S)-24-Cyclohexyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92d)

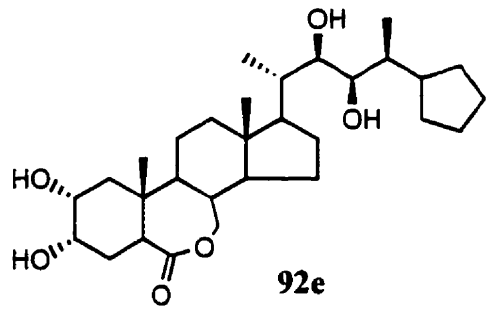


92d

Compound **90d** (0.170 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 69% of a mixture of **92d** and its 6-oxa regioisomer (**93d**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 51% of pure **92d**: mp 277-280 °C; IR (KBr) 3452 (OH), 1697 (lactone), 1185, 1066, 1027, 979 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (m, 1 H), 3.74 (m, 2 H), 3.55 (br d, J = 8.0 Hz, 1 H, 22 or 23-H), 3.15 (dd, J = 7.7, 4.5 Hz, 1 H), 0.93 (s, 3 H, 19-Me), 0.90 (d, J = 6.6 Hz, 3 H, 21-Me), 0.85 (d, J = 6.5 Hz, 3 H, 28-Me), 0.72 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 177.3 (C), 74.2 (CH), 72.6 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.1 (CH), 51.2 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 40.2 (CH), 39.5 (CH_2), 39.0 (CH), 38.9 (CH), 38.1 (C), 36.8 (CH), 31.1 (CH_2), 30.8 (two CH_2), 27.4 (CH_2), 26.5 (two CH_2), 26.4 (CH_2), 24.6 (CH_2), 22.1 (CH_2), 15.3 (CH_3), 11.7 (CH_3), 11.6 (CH_3), 9.9 (CH_3); mass spectrum, *m/z* (relative intensity %) 502 (M^+-H_2O, 1), 380 (17),

361 (18), 123 (42), 55 (100). Anal. Calculated for $C_{31}H_{52}O_6$: C, 71.50; H, 10.07. Found: C, 71.16; H, 10.14.

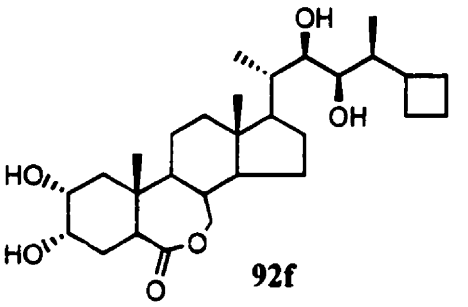
3.13 (2R,3S,5α,22R,23R,24S)-24-Cyclopentyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92e)



92e

Compound **90e** (0.304 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 65% of a mixture of **92e** and its 6-oxa regioisomer (**93e**) in the ratio of 10:1 (NMR integration). Recrystallization from methanol afforded 53% of pure **92e**: mp 283-286 °C; IR (KBr) 3451 (OH), 1696 (lactone), 1391, 1327, 1064 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.10 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 2 H), 3.54 (br d, J = 8.5 Hz, 1 H, 22 or 23-H), 3.13 (dd, J = 7.5, 4.5 Hz, 1 H), 0.93 (s, 3 H, 19-Me), 0.91 (d, J = 6.0 Hz, 3 H, 21-Me), 0.87 (d, J = 6.7 Hz, 3 H, 28-Me), 0.73 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 177.0 (C), 74.3 (CH), 74.1 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.1 (CH), 51.2 (CH), 43.5 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 39.8 (CH), 39.6 (CH_2), 39.0 (CH), 38.1 (C), 36.5 (CH), 31.3 (CH_2), 31.04 (CH_2), 31.01 (CH_2), 27.4 (CH_2), 25.0 (CH_2), 24.6 (CH_2), 22.1 (CH_2), 20.9 (CH_2), 15.3 (CH_3), 11.7 (CH_3), 11.6 (CH_3), 11.3 (CH_3); mass spectrum, *m/z* (relative intensity %) 409 (1), 380 (11), 361 (11), 333 (7), 55 (100). Anal. Calculated for $C_{30}H_{50}O_6$: C, 71.11; H, 9.95. Found: C, 71.13; H, 10.37.

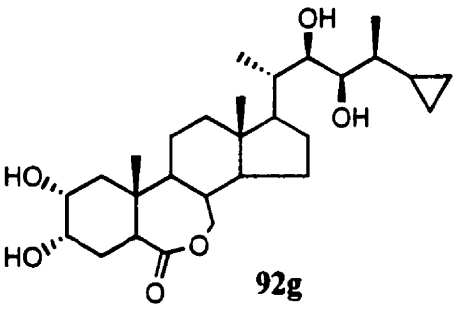
3.14 (2R,3S,5α,22R,23R,24S)-24-Cyclobutyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92f)



92f

Compound **90f** (0.373 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 62% of a mixture of **92f** and its 6-oxa regioisomer (**93f**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 45% of pure **92f**: mp 284-286 °C; IR (KBr) 3415 (OH), 1714 (lactone), 1457, 1389, 1065 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.12 (m, 2 H), 4.03 (m, 1 H), 3.72 (m, 1 H), 3.53 (br s, 2 H, 22 and 23-H), 3.13 (dd, J = 7.7, 4.5 Hz, 1 H), 0.94 (s, 3 H, 19-Me), 0.92 (d, J = 6.6 Hz, 3 H, 21-Me), 0.74 (s, 3 H, 18-Me), 0.73 (d, J = 6.8 Hz, 3 H, 28-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 177.2 (C), 73.8 (CH), 71.7 (CH), 70.5 (CH_2), 67.7 (two CH), 58.0 (CH), 52.0 (CH), 51.1 (CH), 42.2 (C), 41.03 (CH_2), 41.0 (CH), 40.8 (CH), 39.5 (CH_2), 39.0 (CH), 38.7 (CH), 38.1 (C), 36.6 (CH), 31.0 (CH_2), 27.33 (CH_2), 27.25 (CH_2), 26.9 (CH_2), 24.5 (CH_2) , 22.1 (CH_2), 17.3 (CH_2), 15.2 (CH_3), 11.5 (two CH_3), 8.7 (CH_3); mass spectrum, *m/z* (relative intensity %) 406 (1), 380 (10), 361 (23), 107 (67), 81 (100). Anal. Calculated for $C_{29}H_{48}O_6$: C, 70.70; H, 9.82. Found: C, 70.88; H, 10.03.

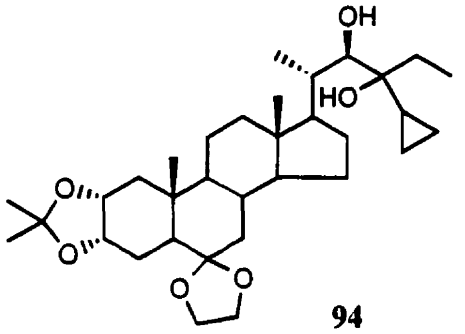
3.15 (2R,3S,5α,22R,23R,24S)-24-Cyclopropyl-2,3,22,23-tetrahydroxy-B-homo-26,27-dinor-7-oxacholestan-6-one (92g)



92g

Compound **90g** (0.476 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 67% of a mixture of **92g** and its 6-oxa regioisomer (**93g**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 49% of pure **92g**: mp 272-274 °C; IR (KBr) 3418 (OH), 1711 (lactone), 1456, 1387, 1066 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.08 (m, 3 H) 3.72 (m, 2 H), 3.57 (br d, J = 9.1 Hz, 1 H, 22 or 23-H), 3.13 (dd, J = 6.9, 4.7 Hz, 1 H), 0.97 (d, J = 6.4 Hz, 3 H, 21-Me), 0.93 (s, 3 H, 19-Me), 0.89 (d, J = 6.6 Hz, 3 H, 28-Me), 0.70 (s, 3 H, 18-Me), 0.54 (t, J = 7.4 Hz, 2 H, cyclopropane), 0.15 (d, J = 4.6 Hz, 2 H, cyclopropane); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 177.2 (C), 75.8 (CH), 73.4 (CH), 70.4 (CH_2), 67.7 (two CH), 57.9 (CH), 52.0 (CH), 51.1 (CH), 42.2 (C), 41.0 (CH_2), 40.8 (CH), 40.3 (CH), 39.5 (CH_2), 38.9 (CH), 38.0 (C), 37.0 (CH), 31.0 (CH_2), 27.3 (CH_2), 24.5 (CH_2), 22.0 (CH_2), 15.5 (CH), 15.2 (CH_3), 12.3 (CH_3), 11.6 (CH_3), 11.5 (CH_3), 4.1 (CH_2), 3.9 (CH_2); mass spectrum, *m/z* (relative intensity %) 478 (M^+, 1), 460 (M^+-H_2O, 2), 409 (M^+-cyclopropyl), 379 (16), 350 (42), 41 (100). Anal. Calculated for $C_{28}H_{46}O_6$: C, 70.26; H, 9.69. Found: C, 70.26; H, 9.60.

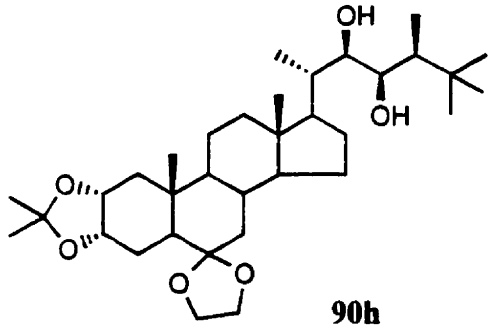
3.16 (2R,3S,5α,22R)-23-Cyclopropyl-23-ethyl-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (94)



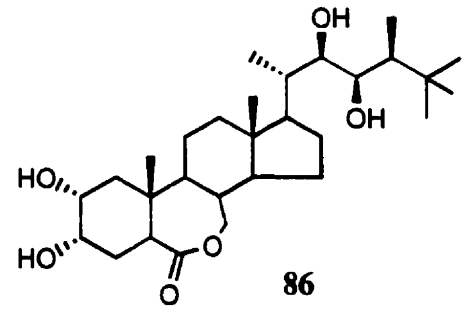
94

When cyclopropylmagnesium chloride was prepared in diethyl ether instead of THF in the preceding procedure with 1.19 mmol of **75/76** (2.3:1), 22% (based on *threo*-epoxide **75**) of the less polar (relative to **90g**) compound **94** was produced as a colourless oil, along with 90% of recovered *erythro*-epoxy alcohol **76** and 29% (based on *threo*-epoxide **75**) of the 22,23-diol **90g**. Compound **94** had: IR (film) 3480 (OH), 1453, 1376, 1228, 1151, 1054 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.29 (m, 1 H), 4.10 (m, 1 H), 3.92 (m, 3 H), 3.76 (m, 1 H), 3.61 (br s, 1 H, 22-H), 1.48 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 1.05 (d, J = 6.0 Hz, 3 H, 21-Me), 0.98 (t, J = 6.0 Hz, 3 H, ethyl-Me), 0.85 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me), 0.36 (m, 4 H, cyclopropane); ^{13}C-NMR (100 MHz) δ 109.7 (C), 107.5 (C), 77.7 (CH), 73.7 (C), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 56.0 (CH), 53.4 (CH), 52.9 (CH), 45.4 (CH), 42.7 (CH_2), 42.4 (C), 41.0 (CH_2), 39.8 (CH_2), 38.0 (C), 36.7 (CH), 32.9 (CH), 29.9 (CH_2) 28.6 (CH_3), 28.3 (CH_2), 26.5 (CH_3), 24.1 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 17.3 (CH), 13.4 (CH_3), 13.3 (CH_3), 11.7 (CH_3), 8.1 (CH_3), -0.3 (CH_2), -0.8 (CH_2); mass spectrum, *m/z* (relative intensity %) 546 (M^+, 1), 528 (M^+-H_2O, 16), 513 (M^+-H_2O and CH_3, 37), 431 (43), 390 (71), 235 (84), 55 (100). Exact mass calculated for $C_{33}H_{54}O_6$: 546.3920 Found: 546.3903.

3.17 (2R,3S,5α,22R,23R,24S)-25-Methyl-(6,6)-(ethylenedioxy)-(2,3)-(isopropylidene-dioxy)ergostane-22,23-diol (90h) and 25-Methylbrassinolide (86)



90h

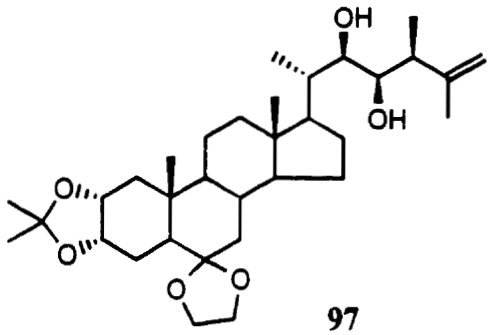


86

The procedure for the preparation of compound **90a** was followed with 0.594 mmol of **75/76** (1.6:1), employing *tert*-butylmagnesium chloride instead of the *n*-dodecyl derivative. Flash chromatography (elution with 40-60% ether-hexanes) afforded 93% of recovered *erythro*-epoxy alcohol **76** and 25% (based on *threo*-epoxide **75**) of the 22,23-diol **90h** as a colourless oil: ^{1}H-NMR (200 MHz) δ 4.29 (m, 1 H), 4.11 (m, 1 H), 3.95 (m, 3 H), 3.76 (m, 2 H), 3.48 (br d, J = 8.6 Hz, 1 H, 22 or 23-H), 1.47 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 0.95 (s, 9 H, *t*-butyl-Me), 0.90 (d, J = 5.9 Hz, 3 H, 21-Me), 0.84 (d, J = 5.3 Hz, 3 H, 28-Me), 0.83 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me); ^{13}C-NMR (50 MHz) δ 109.7, 107.6, 75.0, 72.94, 72.85, 72.2, 65.5, 64.2, 55.9, 53.0, 52.6, 45.5, 42.7, 42.4, 41.0, 39.7, 38.0, 36.8, 33.3, 33.0, 29.7, 28.6, 28.3, 27.9, 26.6, 24.1, 22.0, 20.8, 13.4, 11.9, 7.9. This compound was used directly in the next stage. Compound **90h** (0.11 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 10% methanol-chloroform) provided 68% of a mixture of **86** and its 6-oxa regioisomer (**93h**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol afforded 38% of pure **86**: mp 273-275 °C; ^{1}H-NMR (400 MHz) δ 4.11 (m, 2 H), 4.03 (br s, 1 H), 3.82 (br d, J = 7.8 Hz, 1 H, 22 or 23-H), 3.72 (m, 1 H), 3.47 (br d, J = 9 Hz, 1 H, 22 or 23-H), 3.13 (dd, J = 7.7, 4.8 Hz, 1 H), 0.96 (s, 9 H, *t*-butyl-Me), 0.94 (s, 3 H, 19-Me), 0.92 (d, J = 6.6 Hz, 3 H, 21-Me), 0.86 (d, J = 7.1 Hz, 3 H, 28-Me), 0.73 (s, 3 H, 18-Me).

The following melting point and proton NMR spectrum for **86** were reported in the literature:[51] mp 274-276 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.06-4.11 (m, 2 H), 4.03 (dd, J = 2.0, 4.8 Hz, 1 H), 3.82 (dd, J = 0.7, 9.0 Hz, 1H), 3.72 (ddd, J = 2.9, 4.8, 12.2 Hz, 1 H), 3.47 (dd, J = 1.0, 9.0 Hz, 1 H), 3.12 (dd, J = 4.8, 12.2 Hz, 1 H), 0.96 (s, 9 H), 0.93 (s, 3 H), 0.91 (d, J = 6.5 Hz, 3 H), 0.85 (d, J = 7.0 Hz, 3 H), 0.72 (s, 3 H).

3.18 (2R,3S,5α,22R,23R,24S)-24-(2-Propenyl)-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholestane-22,23-diol (97)

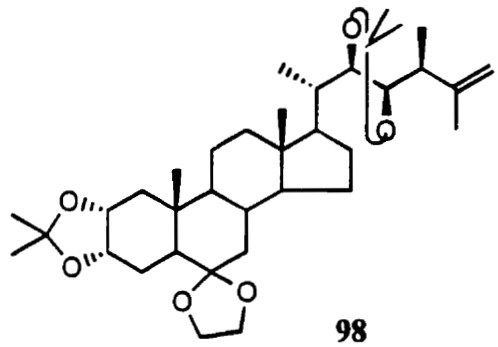


97

The procedure for the preparation of compound **90a** was followed with 0.991 mmol of **75/76** (1.5:1), employing 2-propenylmagnesium bromide instead of the *n*-dodecyl derivative. Flash chromatography (elution with 45-60% ether-hexanes) afforded 89% of recovered *erythro*-epoxy alcohol **76** and 77% (based on *threo*-epoxide **75**) of the 22,23-diol **97** as a colourless oil: IR (KBr) 3442 (OH), 1640 (C=C), 1451, 1376, 1228, 1055, 754 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.93 (br s, 1 H, HC=C), 4.81 (br s, 1 H, HC=C), 4.29 (m, 1 H), 4.09 (m, 1 H), 3.92 (m, 3 H), 3.76 (m, 1 H), 3.60 (br s, 2 H, 22 and 23-H), 1.78 (s, 3 H, Me-C=C), 1.47 (s, 3 H, acetonide), 1.32 (s, 3 H, acetonide), 1.01 (d, J = 7.0 Hz, 3 H, 21-Me), 0.96 (d, J = 6.0 Hz, 3 H, 28-Me), 0.83 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 147.9 (C), 111.6 (CH_2), 109.7 (C), 107.5 (C), 73.7 (CH), 73.0 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.8 (CH), 52.9 (CH), 52.4 (CH), 45.5 (CH), 42.7 (CH_2), 42.3 (C), 41.8 (CH), 41.0 (CH_2), 39.7 (CH_2), 38.0 (C), 37.5 (CH), 32.9 (CH), 28.6 (CH_3), 27.7 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 22.1 (CH_3), 22.0 (CH_2), 20.8

(CH_2), 13.4 (CH_3), 12.2 (CH_3), 11.9 (CH_3), 11.7 (CH_3); mass spectrum, *m/z* (relative intensity %) 546 (M^+, 10), 531 (M^+-CH_3, 52), 446 (29), 431 (72), 335 (100) Exact mass calculated for $C_{33}H_{52}O_6$: 546.3920. Found: 546.3947.

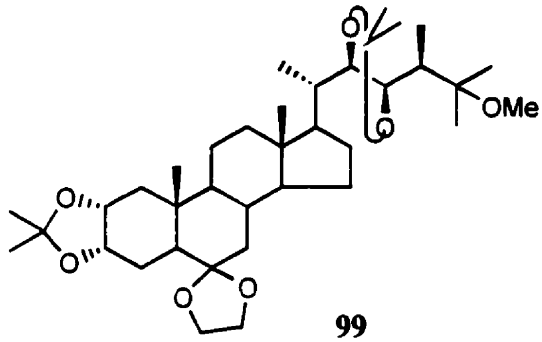
3.19 (2R,3S,5α,22R,23R,24S)-24-(2-Propenyl)-6,6-(ethylenedioxy)-(2,3),(22,23)-bis(isopropylidenedioxy)-26,27-dinorcholestane (98)



98

Dimethoxypropane (0.41 mL, 3.3 mmol) was added to a solution containing compound **97** (183 mg, 0.335 mmol) in CH_2Cl_2, followed by p-TsOH (8.4 mg, 0.04 mmol) at room temperature. After 20 min, ~15 mg of potassium carbonate was added, and the solution was allowed to stir for another 5 min. The mixture was then poured into water, which was extracted three times with CH_2Cl_2 . The organic extracts were washed with $NaHCO_3$ solution, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography (elution with 10% ethyl acetate-hexane) provided a quantitative yield of **98** as a colourless oil: IR (KBr) 1453, 1375, 1167, 1053 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.79 (br s, 1 H, HC=C), 4.76 (br s, 1 H, HC=C), 4.29 (m, 1 H), 4.11 (m, 1 H), 3.92 (m, 4 H), 3.68 (m, 2 H), 1.66 (s, 3 H, Me-C=C), 1.48 (s, 3 H), 1.38 (s, 3 H), 1.36 (s, 3 H), 1.33 (s, 3 H), 1.13 (d, J = 6.9 Hz, 3 H), 0.94 (d, J = 5.8 Hz, 3 H), 0.83 (s, 3 H), 0.62 (s, 3 H). This compound was used directly in the next stage.

3.20 (2R,3S,5α,22R,23R,24S)-25-Methoxy-6,6-(ethylenedioxy)-(2,3),(22,23)-bis(isopropylidenedioxy)ergostane-22,23-diol (99)

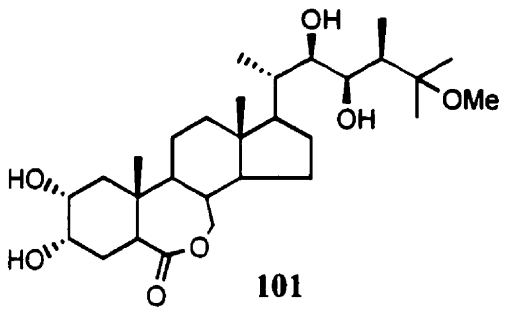


99

Compound **98** (100 mg, 0.170 mmol) in 1 mL of MeOH was added to a vigorously stirred suspension of mercuric acetate (109 mg, 0.341 mmol) in 1 mL of MeOH. The mixture was allowed to stir for 2 h at room temperature. After this time, 2 mL of 3M NaOH were added to the mixture and it was further stirred for 5 min before the addition of 2 mL of 0.5M $NaBH_4$ (prepared from 94.6 mg of $NaBH_4$ in 5 mL of 3M NaOH). The mixture was stirred for an additional 2 h at room temperature. It was then filtered through a pad of Celite and the solution was extracted three times with diethyl ether. The organic extracts were washed with $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated under vacuum. Flash chromatography over silica gel (elution with 10% ethyl acetate-hexanes) afforded compound **99** (88 mg, 83%) as a colourless oil: IR (KBr) 1457, 1376, 1232, 1151, 1054 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.29 (m, 1 H), 4.02 (m, 5 H), 3.72 (m, 2 H), 3.65 (s, 3 H, O-Me), 1.48 (s, 3 H, acetonide), 1.33 (br s, 9 H, acetonide), 1.13 (s, 6 H, 26 and 27-Me), 0.98 (d, J = 6.0 Hz, 3 H, 21-Me), 0.90 (d, J = 7.0 Hz, 3 H, 28-Me), 0.83 (s, 3 H, 19-Me), 0.66 (s, 3 H, 18-Me); ^{13}C-NMR (50 MHz) δ 109.7 (C), 107.5 (2 C), 80.0 (C and CH), 75.0 (CH), 72.9 (CH), 72.8 (CH), 65.5 (CH_2), 64.1 (CH_2), 55.7 (CH), 53.4 (CH), 52.9 (CH), 48.6 (CH_3), 45.5 (CH), 42.7 (CH_2), 42.4 (C), 41.0 (CH_2), 40.3 (CH), 39.5 (CH_2), 38.0 (C), 35.1 (CH), 32.9 (CH), 28.6 (CH_3), 27.9 (CH_2), 27.3 (CH_3), 27.2 (CH_3), 26.5 (CH_3), 24.0 (CH_2), 22.8 (CH_3), 22.0 (CH_2 and CH_3), 20.7 (CH_2), 13.3 (CH_3), 12.5 (CH_3), 11.8 (CH_3), 8.7 (CH_3); mass spectrum, *m/z* (relative intensity %) 618 (M^+,

0.2), 603 (M^+-CH_3), 517 (7), 489 (20), 407 (16), 293 (81), 73 (100). Exact mass calculated for $C_{37}H_{62}O_7$: 618.4496. Found: 618.4496.

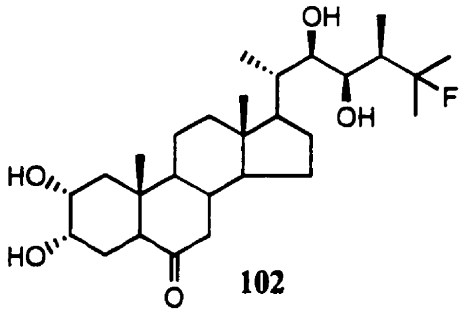
3.21 (2R,3S,5α,22R,23R,24S)-25-Methoxy-2,3,22,23-tetrahydroxy-B-homo-7-oxa-ergostan-6-one (101)



101

Compound **99** (0.14 mmol) was treated with trifluoroperoxyacetic acid as in the procedure for the preparation of **92a**. Flash chromatography (elution with 5-10% methanol-chloroform) provided 72% of a mixture of **101** and its 6-oxa regioisomer (**100**) in the ratio of 8:1 (NMR integration). Recrystallization from methanol afforded 56% of pure **101**: mp 228-230 °C; IR (KBr) 3481 (OH), 1707 (lactone), 1457, 1383, 1132, 1066 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.03 (m, 4 H) 3.73 (m, 1 H), 3.56 (br d, J = 7.0 Hz, 1 H, 22 or 23-H), 3.23 (s, 3 H, O-Me), 3.13 (dd, J = 7.3, 4.5 Hz, 1 H), 1.33 (s, 3 H, 26 or 27-Me), 1.22 (s, 3 H, 26 or 27-Me), 0.96 (d, J = 6.9 Hz, 3 H, 21-Me), 0.94 (s, 3 H, 19-Me), 0.92 (d, J = 3.9 Hz, 3 H, 28-Me), 0.71 (s, 3 H, 18-Me); ^{13}C-NMR (75 MHz, $CDCl_3$-CD_3OD) δ 177.2 (C), 78.6 (C), 74.0 (CH), 72.2 (CH), 70.5 (CH_2), 67.8 (two CH), 58.0 (CH), 52.0 (CH), 51.1 (CH), 48.9 (CH_3), 42.5 (CH), 42.3 (C), 41.1 (CH_2), 40.9 (CH), 39.5 (CH_2), 39.1 (CH), 38.1 (C), 36.9 (CH), 31.1 (CH_2), 27.5 (CH_2), 24.6 (CH_2), 23.4 (CH_3), 22.8 (CH_3), 22.1 (CH_2), 15.3 (CH_3), 11.8 (CH_3), 11.6 (CH_3), 7.2 (CH_3); mass spectrum, *m/z* (relative intensity %) 503 (1), 442 (17), 409 (16), 379 (44), 350 (46), 40 (100). Anal. Calculated for $C_{29}H_{50}O_7$: C, 68.20; H, 9.87. Found: C, 68.14; H, 9.88.

3.22 (2R,3S,5α,22R,23R,24S)-25-Fluoro-2,3,22,23-tetrahydroxyergostan-6-one (102)

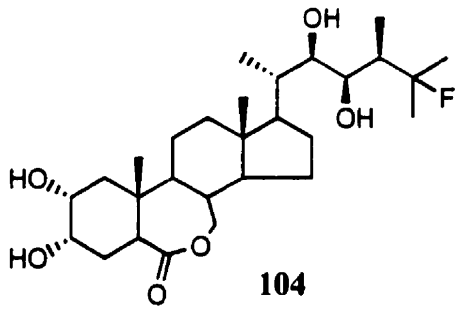


102

Alkene **98** (175 mg, 0.298 mmol), dissolved in 5 mL of THF, was slowly added to 11 mL of a solution of 70% HF-pyridine in a plastic vessel under nitrogen at 0 °C. The mixture was allowed to stir for 1 h, followed by extraction three times with chloroform. The combined organic extracts were washed twice with water, and twice with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and evaporated to dryness. The powdery white solid was chromatographed over silica gel (elution with 10% methanol-chloroform) to furnish 125 mg of compound **102** (88%): mp 318-320 °C; IR (KBr) 3483 (OH), 1693 (ketone), 1446, 1376, 1226, 1062, 993 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.06 (m, 1 H), 3.97 (br d, J = 8.7 Hz, 1 H, 22 or 23-H), 3.77 (m, 1 H), 3.59 (br d, J = 8.5 Hz, 1 H, 22 or 23-H), 2.70 (dd, J = 9.1, 3.4 Hz, 1 H), 1.36 (d, J = 9.3 Hz, 3 H, Me-CF), 1.25 (d, J = 7.0 Hz, 3 H, Me-CF), 0.96 (d, J = 6.6 Hz, 3 H, 21-Me), 0.85 (d, J = 7.1 Hz, 3 H, 28-Me), 0.77 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me); ^{19}F-NMR (400 MHz, $CDCl_3$-C_6F_6) δ -142.0 (m, 1 F). Product **102** was insufficiently volatile to provide a satisfactory electron impact mass spectrum. It was therefore converted to the corresponding tetraacetate by treatment with acetic anhydride and DMAP in pyridine for 10 h at room temperature. The following spectra are for the tetraacetate derivative of **102**: ^{1}H-NMR (400 MHz) δ 5.44 (br d, J = 9.3 Hz, 1 H, 22 or 23-H), 5.39 (m, 1 H), 5.11 (br d, J = 9.2 Hz, 1 H, 22 or 23-H), 4.97 (m, 1 H), 2.57 (dd, J = 6.9, 4.5 Hz, 1 H), 2.33 (dd, J = 8.7, 4.5 Hz, 1 H), 2.09 (s, 3 H, Me-C=O), 2.04 (s, 3 H, Me-C=O), 2.00 (s, 6 H, Me-C=O), 1.34 (d, J = 21.9 Hz, 3 H, Me-CF), 1.31 (d, J = 22.2 Hz, 3 H, Me-CF), 1.09 (d, J = 7.1 Hz, 3 H, 21-Me), 1.06 (d, J = 6.8 Hz, 3 H,

28-Me), 0.84 (s, 3 H, 19-Me), 0.71 (s, 3 H, 18-Me); ^{19}F-NMR (300 MHz) δ -135.6 (doublet of septets, J = 22.0, 13.0 Hz, 1 F); mass spectrum, *m/z* (relative intensity %) 631 (M^+-F, 0.1), 590 (M^+-AcOH, 5), 561 (14), 510 (19), 417 (50), 387 (100). Exact mass calculated for $C_{36}H_{55}O_9$ (M^+-F): 631.3846. Found: 631.3875.

3.23 (2R,3S,5α,22R,23R,24S)-25-Fluoro-2,3,22,23-tetrahydroxy-B-homo-7-oxa-ergostan-6-one (104)

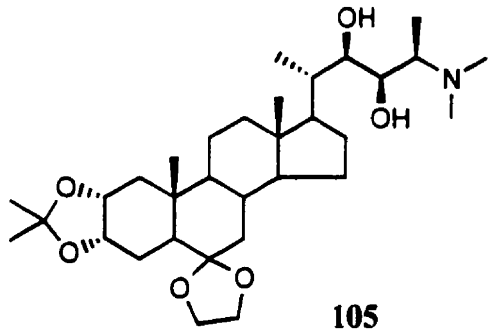


104

A solution of compound **102** (88 mg, 0.18 mmol) in 5 mL of chloroform was added slowly to the pregenerated (as in Section 3.9) trifluoroperoxyacetic acid solution at 0 °C, followed by warming to room temperature and stirring for an additional 2 h. The mixture was diluted with chloroform and washed twice with 2 mL of water and twice with 2 mL of 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$) and concentrated under vacuum. The resulting white solid was chromatographed over silica gel (elution with 5-10% methanol-chloroform) to provide 66 mg (73%) of a mixture of **104** and its 6-oxa regioisomer (**103**) in the ratio of 8:1 (NMR integration). Recrystallization from methanol-dichloromethane solution afforded 52 mg (57%) of pure **104**: mp 339-341 °C; IR (KBr) 3469 (OH), 1695 (lactone), 1376, 1232, 1066 cm^{-1}; ^{1}H-NMR (400 MHz) δ 4.09 (m, 2 H), 4.03 (br s, 1 H), 3.97 (br d, J = 8.4 Hz, 1 H, 22 or 23-H), 3.72 (m, 1 H), 3.58 (br d, J = 9.0 Hz, 1 H, 22 or 23-H), 3.13 (dd, J = 7.8, 4.4 Hz, 1 H), 1.35 (d, J = 9.2 Hz, 3 H, Me-CF), 1.32 (d, J = 13.2 Hz, 3 H, Me-CF), 0.95 (d, J = 6.7 Hz, 3 H, 21-Me), 0.93 (s, 3 H, 19-Me), 0.86 (d, J = 7.1 Hz, 3 H, 28-Me), 0.72 (s, 3 H, 18-Me); ^{19}F-NMR (400 MHz, $CDCl_3$-C_6F_6) δ -142.0 (m, 1 F). Product **104** was insufficiently volatile to provide a satisfactory electron impact mass spectrum. It was therefore converted to the corresponding tetraacetate by treatment with

acetic anhydride and DMAP in pyridine for 10 h at room temperature. The following spectra are for the tetraacetate derivative of **104**: ^{1}H-NMR (400 MHz) δ 5.42 (br d, J = 9.3 Hz, 1 H, 22 or 23-H), 5.35 (br s, 1 H), 5.08 (br d, J = 9.3 Hz, 1 H, 22 or 23-H), 4.86 (m, 1 H), 4.06 (m, 2 H), 2.98 (dd, J = 8.0, 4.3 Hz, 1 H), 2.10 (s, 3 H, Me-C=O), 2.01 (s, 3 H, Me-C=O), 1.99 (s, 3 H, Me-C=O), 1.98 (s, 3 H, Me-C=O), 1.31 (d, J = 21.9 Hz, 3 H, Me-CF), 1.29 (d, J = 22.3 Hz, 3 H, Me-CF), 1.07 (d, J = 7.1 Hz, 3 H, 21-Me), 1.04 (d, J = 6.7 Hz, 3 H, 28-Me), 0.98 (s, 3 H, 19-Me), 0.73 (s, 3 H, 18-Me); ^{19}F-NMR (300 MHz) δ -135.8 (doublet of septets, J = 22.0, 13.3 Hz, 1 F); mass spectrum, *m/z* (relative intensity %) 605 (1), 586 (M^{+}-HF and AcOH, 13), 577 (46), 544 (46), 463 (73), 433 (50), 43 (100). Exact mass calculated for $C_{34}H_{50}O_8$ (M^{+}-HF and AcOH): 586.3506. Found: 586.3471.

3.24 (2R,3S,5α,22R,23R,24S)-25-Aza-6,6-(ethylenedioxy)-2,3-(isopropylidenedioxy)-ergostane-22,23-diol (105)

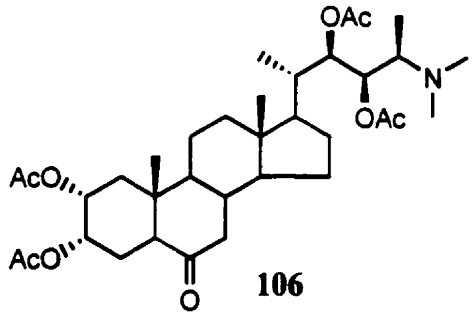


105

A 2.0 M solution of dimethylamine in THF (3.0 mL, 6.0 mmol) was added slowly to a 2.0 M hexylmagnesium bromide solution (2.4 mL, 4.8 mmol) and the mixture was allowed to stir at 35-40 °C for 1 h. The mixture of epoxides **75** and **76** (600 mg, 1.19 mmol; *threo/erythro* ratio of 60:40) in THF was added to the reaction mixture at room temperature and it was again warmed to 35-40 °C for another 1 h. After the solution was cooled to room temperature, the solvent was evaporated and the crude mixture was chromatographed over silica gel (elution with 67% ethyl acetate-28% methanol-5% triethylamine) to give 235 mg (90%) of recovered *erythro*-epoxy alcohol **76** and a white solid residue. The residue was triturated with K_2CO_3 solution, extracted several times with ether, and the combined ether layers were dried ($MgSO_4$), and concentrated under

vacuum to give 375 mg of compound **105** (96%, based on threo-epoxide **75**) as a colourless oil: IR (KBr) 3451 (OH), 1457, 1378, 1289, 1229, 1054, 973 cm^{-1}; ^{1}H-NMR (200 MHz) δ 4.29 (m, 1 H), 4.09 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 2 H), 3.58 (m, 1 H), 2.38 (m, 1 H, CH-N), 2.36 (s, 6 H, N-Me), 1.48 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 1.03 (d, J = 6.0 Hz, 3 H, 21-Me), 0.98 (d, J = 6.0 Hz, 3 H, 28-Me), 0.84 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 109.6 (C), 107.4 (C), 73.0 (CH), 72.8 (CH), 72.7 (CH), 71.6 (CH), 65.4 (CH_2), 64.0 (CH_2), 61.1 (CH, **C**H-N), 55.7 (CH), 52.8 (CH), 52.2 (CH), 45.4 (CH), 42.6 (CH_2), 42.3 (two CH_3, (CH_3)$_2$-N), 42.2 (C), 40.9 (CH_2), 39.6 (CH_2), 37.9 (C), 37.8 (CH), 32.8 (CH), 28.5 (CH_3), 27.7 (CH_2), 26.5 (CH_3), 24.0 (CH_2), 21.9 (CH_2), 20.7 (CH_2), 13.3 (CH_3), 12.3 (CH_3), 11.8 (CH_3), 9.8 (CH_3); mass spectrum, *m/z* (relative intensity %) 549 (M^+, 1), 548 (M^+-H, 1), 534 (M^+-CH_3, 10), 504 (M^+-3CH_3, 14), 489 (35), 293 (56), 72 (100) Exact mass calculated for $C_{32}H_{55}O_6N$: 549.4029. Found: 549.4043.

3.25 (2R,3S,5α,22R,23R,24S)-25-Aza-2,3,22,23-tetrahydroxyergostan-6-one tetraacetate (106)

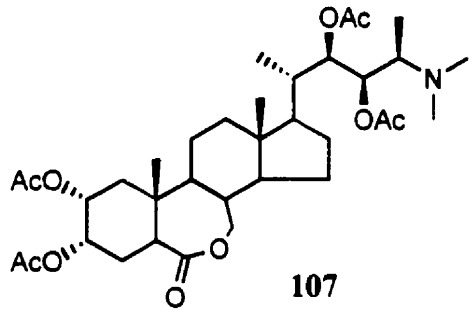


106

Compound **105** (375 mg, 0.682 mmol) was treated with 5 mL of aqueous acetic acid for 30 min. The mixture was concentrated under vacuum and the residue was basified with 10% NaOH solution, followed by extraction five time with chloroform. The combined organic extracts were dried ($MgSO_4$) and concentrated under vacuum to give 255 mg of white powder. This compound was not purified and was directly acetylated with acetic anhydride (0.84 mL, 8.9 mmol), 4-(dimethylamino)pyridine (82 mg, 0.67 mmol) in

pyridine for 5 h at room temperature. The mixture was poured into ice-cold 10% HCl solution, followed by extraction three times with chloroform, and the organic layers were dried ($MgSO_4$), and evaporated to dryness. The crude product was chromatographed over silica gel (elution with 50% ethyl acetate-hexanes) to afford 247 mg (85%) of the corresponding tetraacetate **106** as a colourless oil: ^{1}H-NMR (200 MHz) δ 5.39 (m, 1 H), 5.20 (m, 2 H, 22 and 23-H), 4.96 (m, 1 H), 2.60 (m, 1 H), 2.24 (s, 6 H, N-Me), 2.09 (s, 3 H, Me-C=O), 2.05 (s, 3 H, Me-C=O), 2.03 (s, 3 H, Me-C=O), 1.99 (s, 3 H, Me-C=O), 1.03 (d, J = 6.7 Hz, 3 H, 21-Me), 1.01 (d, J = 6.7 Hz, 3 H, 28-Me), 0.83 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me). This compound was used directly in the next stage.

3.26 (2R,3S,5α,22R,23R,24S)-25-Aza-2,3,22,23-tetrahydroxy-B-homo-7-oxa-ergostan-6-one tetraacetate (107)

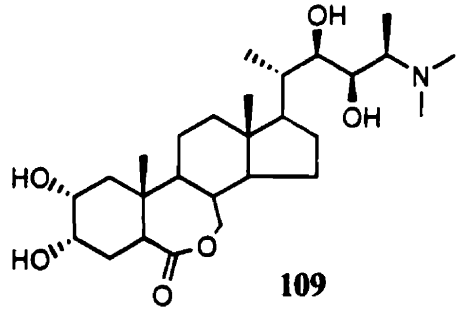


107

A solution of compound **106** (245 mg, 0.375 mmol) in 10 mL of chloroform was added slowly to the pregenerated (as in Section 3.9) trifluoroperoxyacetic acid solution at 0 °C, followed by warming to room temperature and stirring for an additional 2 h. The mixture was diluted with chloroform and washed twice with 5 mL of water and twice with 5 mL of 10% aqueous Na_2SO_3 solution, dried ($MgSO_4$) and concentrated under vacuum. The product was chromatographed over silica gel (elution with 0-2% methanol-chloroform) to provide 209 mg (83%) of a mixture of **107** and its 6-oxa regioisomer (**108**) in the ratio of 9:1 (NMR integration) as a colourless oil: IR (KBr) 1739 (acetate), 1369, 1289, 1244, 1042, 1026 cm^{-1}; ^{1}H-NMR (400 MHz) δ 5.37 (br s, 1 H), 5.20 (m, 1 H, 22 or 23-H), 5.12 (br d, J = 7.7 Hz, 1 H, 22 or 23-H), 4.89 (m, 1 H), 4.48 (m, 0.1 H, 5-H of **108**), 4.08 (m, 2 H), 3.00 (dd, J = 7.9, 4.4 Hz, 0.9 H, 5-H of **107**), 2.65 (m, 1 H, CH-N), 2.26 (s, 6 H, N-

Me), 2.11 (s, 3 H, Me-C=O), 2.04 (s, 3 H, Me-C=O), 2.03 (s, 3 H, Me-C=O), 2.00 (s, 3 H, Me-C=O), 1.02 (d, J = 6.8 Hz, 6 H, 21 and 28-Me), 0.99 (s, 3 H, 19-Me), 0.72 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz) δ 175.0 (C), 170.5 (C), 170.3 (C), 170.2 (C), 169.9 (C), 75.2 (CH), 72.5 (CH), 70.4 (CH_2), 68.9 (CH), 67.9 (CH), 59.4 (CH), 58.3 (CH), 52.7 (CH), 51.3 (CH), 42.5 (C), 42.0 (CH), 41.1 (two CH_3), 39.4 (CH_2), 39.1 (CH), 38.8 (CH_2), 38.4 (C), 37.6 (CH), 29.3 (CH_2), 27.9 (CH_2), 24.7 (CH_2), 22.2 (CH_2), 21.1 (CH_3), 21.0 (two CH_3), 20.8 (CH_3), 15.4 (CH_3), 13.3 (CH_3), 11.5 (CH_3), 9.2 (CH_3); mass spectrum, *m/z* (relative intensity %) 649 (M^+, <<1), 634 (M^+-CH_3, 4), 590 (5), 562 (10), 530 (26), 144 (40), 72 (100). Exact mass calculated for $C_{35}H_{55}O_{10}N$: 649.3826. Found: 649.3772.

3.27 (2R,3S,5α,22R,23R,24S)-25-Aza-2,3,22,23-tetrahydroxy-B-homo-7-oxa-ergostan-6-one (109)

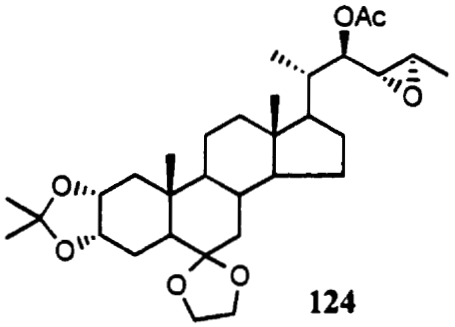


109

The tetraacetate **108** (209 mg, 0.312 mmol) was dissolved in 30 mL of NaOH solution (1.17 g of NaOH, 3.00 mL of H_2O, 45.0 mL of MeOH) and was refluxed for 2.5 h. Thirty mL of THF, followed by 11 mL of 6 N HCl solution, was added to the cooled reaction mixture, which was further stirred for another 2.5 h at room temperature. The solvent was removed under vacuum, and the residue was basified with 10% NaOH solution. The mixture was extracted five times with 10% methanol-chloroform. The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness. The resulting white powder was rinsed with 20 mL of chloroform and dried under high vacuum to afford 121 mg (81%) of a mixture of **109** and its 6-oxa regioisomer (**110**) in the ratio of 9:1 (NMR integration). Recrystallization from methanol-dichloromethane solution afforded 92 mg (61%) of pure

109: mp 273-275 °C; IR (KBr) 3458 (OH), 1691 (lactone), 1455, 1386, 1263, 1181, 1066, 976 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$-CD_3OD) δ 3.89 (m, 2 H), 3.73 (br s, 1 H), 3.51 (dd, J = 4.9, 2.6 Hz, 1 H, 22 or 23-H), 3.38 (m, 1 H), 3.24 (br d, J = 7.2 Hz, 1 H, 22 or 23-H), 2.93 (dd, J = 7.4, 4.6 Hz, 1 H), 2.20 (dq, J = 6.8, 2.7 Hz, 1 H, CH-N), 2.10 (s, 6 H, N-Me), 0.79 (d, J = 6.7 Hz, 3 H, 21-Me), 0.71 (d, J = 6.5 Hz, 3 H, 28-Me), 0.68 (s, 3 H, 19-Me), 0.50 (s, 3 H, 18-Me); ^{13}C-NMR (100 MHz, $CDCl_3$-CD_3OD) δ 176.3 (C), 72.9 (CH), 71.2 (CH), 70.4 (CH_2), 67.5 (CH), 67.4 (CH), 60.5 (CH), 57.8 (CH), 51.9 (CH), 50.9 (CH), 42.3 (C), 41.3 (two CH_3), 40.8 (CH_2), 40.7 (CH), 39.3 (CH_2), 38.8 (CH), 37.9 (C), 37.6 (CH), 30.9 (CH_2), 27.2 (CH_2), 24.3 (CH_2), 21.9 (CH_2), 15.3 (CH_3), 11.8 (CH_3), 11.3 (CH_3), 8.6 (CH_3); mass spectrum, *m/z* (relative intensity %) 481 (M^+, 1), 466 (M^+-CH_3, 1), 432 (7), 349 (20), 331 (31), 177 (44), 72 (100). Anal. Calculated for $C_{27}H_{47}O_6N$: C, 67.33; H, 9.84. Found: C, 67.43; H, 10.05.

3.28 (*erythro*)-(2R,3S,5α,22R,23R,24S)-6,6-(Ethylenedioxy)-2,3-(isopropylidene-dioxy)-23,24-epoxy-26,27-dinorcholestan-22-ol acetate (124)

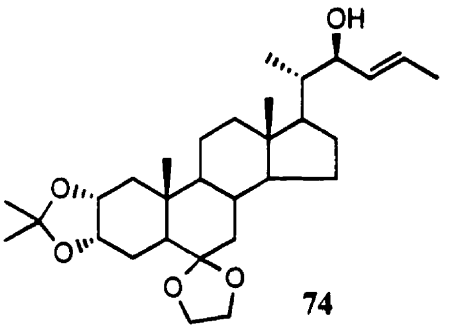


124

Acetic anhydride (8.70 mL, 92.2 mmol) and DMAP (0.845 mg, 6.91 mmol) were added to a solution of *erythro*-epoxide **76** (2.33 g, 4.61 mmol) in 125 mL of 20 % pyridine-dichloromethane solution and the mixture was stirred at room temperature for 1 h. The mixture was poured into ice-cold 10% HCl solution, followed by extraction three times with chloroform. The combined organic extracts were dried ($MgSO_4$), and evaporated to dryness. The crude product was chromatographed over silica gel (elution with 50% ethyl acetate-hexanes) to afford 2.39 g of the corresponding tetraacetate **124** (95%) as a colourless oil: IR (film) 1737 (acetate), 1452, 1374, 1232, 1040, 754 cm^{-1}; ^{1}H-NMR (200

MHz) δ 4.75 (dd, J = 4.1, 1.5 Hz, 1 H, CH-OAc), 4.26 (br s, 1 H), 4.11 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 1 H), 3.02 (dq, J = 3.3, 2.1 Hz, 1 H, 23-H), 2.68 (dd, J = 3.4, 2.2 Hz, 1 H, 22-H), 2.07 (s, 3 H, Me-C=O), 1.47 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 1.29 (d, J = 5.1 Hz, 3 H), 1.07 (d, J = 6.8 Hz, 3 H, 21-Me), 0.83 (s, 3 H, 19-Me), 0.69 (s, 3 H, 18-Me); mass spectrum, *m/z* (relative intensity %) 546 (M^+, 6), 531 (M^+-CH_3, 29), 421 (35), 335 (100). This compound was used directly in the next stage.

3.29 (2R,3S,5α,22S,23E)-6,6-(Ethylenedioxy)-2,3-(isopropylidenedioxy)-26,27-dinorcholest-23-en-22-ol (74)



74

Following the general procedure of Dittmer et al.,[72] elemental tellurium (1.68 g, 13.2 mmol) was stirred in 100 mL of dry THF. The solution was degassed with nitrogen for 5 min, followed by the addition of 1.0 M lithium triethylborohydride (24 mL, 24 mmol) and the mixture was stirred at room temperature for 30 min. The solution turned from gray to purple. In a separate vessel, glicidyl acetate **124** (2.40 g, 4.39 mmol) in 25 mL of THF was degassed with argon for 5 min, and was slowly added to the purple solution. After the solution had been refluxed for 20 h, air was passed through the cooled mixture. Elemental tellurium was filtered through a pad of Celite and the solvent was evaporated to dryness. The mixture was diluted with water and was extracted three times with chloroform, dried ($MgSO_4$), and concentrated under vacuum. The crude product was chromatographed over silica gel (elution with 5-10% acetonitrile-dichloromethane) to furnish 1.70 g (79%) of allylic alcohol **74** as a colourless oil: IR (film) 3470, 1735, 1230, 1040, 965, 765 cm^{-1}; ^{1}H-NMR (200 MHz) δ 5.6 (m, 2 H, 23 and 24-H), 4.29 (m, 1 H), 4.17 (m, 1 H), 4.07 (m, 1 H), 3.92 (m, 3 H), 3.75 (m, 1 H), 1.71 (d, J = 5.2 Hz, 3 H, Me-

C=C), 1.48 (s, 3 H, acetonide), 1.33 (s, 3 H, acetonide), 0.90 (d, J = 6.0 Hz, 3 H, 21-Me), 0.84 (s, 3 H, 19-Me), 0.68 (s, 3 H, 18-Me). The product was identical in all respects (TLC, IR, ^{1}H-NMR) with an authentic sample.[36,45]

References

1. a) Arteca, R.N. *Plant Growth Substances: Principles and Applications*, Chapman & Hall, New York, NY, **1996**, pp. 1-44. b) Campbell, N.A. *Biology*, 2nd Ed., The Benjamin / Cummings Publishing Company, Inc., CA, **1990**, pp.760-767.
2. Cutler, H.C.; Yokota, T.; and Adam, G., eds. *Brassinosteroids-Chemistry, Bioactivity, and Applications*. ACS Symp. Ser. 474, American Chemical Society, Washington, D.C., **1991**.
3. Yopp, J.H.; Mandava, N.B.; Sasse, J.M. *Physiol. Plant.*, **1981**, *53*, 445.
4. Mandava, N.B.; Sasse, J.M., Yopp, J.H. *Physiol Plant.*, **1981**, *53*, 453.
5. Sasse, J.M., Ref. 2, Chapter 13, pp. 158-166.
6. Clouse, S.D.; Zurek, D., Ref. 2, Chapter 11, pp. 122-140.
7. Mitchell, J.W.; Mandava, N.; Worley, J.F.; Plimmer, J.R.; Smith, M.V. *Nature*, **1970**, *225*, 1065.
8. Grove, M.D.; Spencer, G.F.; Rohwedder, W.K.; Mandava, N.; Worley, J.F.; Warthen, J.D.; Steffens, G.L.; Flippen-Anderson, J.L.; Cook, J.C. Jr. *Nature*, **1979**, *281*, 216.
9. Chen, S.C. *Chemistry in Canada*, **1983**, *35*, 13.
10. Adam, G.; Porzel, A.; Schmidt, J.; Schneider, B.; Voigt, B. in *Studies in Natural Products Chemistry*, Atta-ur-Rahman, ed., Elsevier: Amsterdam, **1996**, Vol. 18, pp. 495-549.
11. Yokota,T.; Ogino, Y.; Suzuki, H.;Yoichi, O.; Takahashi, N.; Saimoto, H.; Fujioka, S.; Sakurai, A., Ref. 2, Chapter 8, pp. 86-96.
12. Suzuki, H.; Inoue, T.; Fujioka, S.; Saito, T.; Takatsuto, S.; Yokota, T.; Murofushi, N.; Yanagisawa, T.; Sakurai, A. *Phytochemistry*, **1995**, *40*, 1391.
13. Fujioka, S.; Inoue, T.; Takatsuto, S.; Yanagisawa, T.; Yokota, Y.; Sakurai, A. *Biosci. Biotechnol. Biochem.*, **1995**, *59*, 1543.
14. Yokota, T.; Koba, S.; Kim, S-K.; Takatsuto, S.; Ikekawa, N.;Sakakibara, M.; Okada, K.; Mori, K.; Takahashi, N. *Agric. Biol. Chem.*, **1987**, *51*, 1625.

15. Choi, Y-H.; Fujioka, S.; Harada, A.; Yokota, T.; Takatsuto, S.; Sakurai, A. *Phytochemistry*, **1996**, *43*, 593.

16. Yokota,T.; Yoichi, O.; Takahashi, N.; Saimoto, H.; Fujioka, S.; Sakurai, A. *Agric. Biol. Chem.*, **1990**, *54*, 1107.

17. Schneider, B.; Kolbe, A.; Hai, T.; Porzel, A.; Adam, G. *Proceedings of the 23rd Annual Meeting of the Plant Growth Regulation Society of America*, Calgary, Alberta, **1996**, p. 43.

18. Mandava, N.B., *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **1988**, *39*, 23.

19. Thompson, M.J.; Mandava, N.B.; Meudt, W.J.; Lusby, W.R.; Spaulding, D.W. *Steroids*, **1981**, *38*, 567.

20. Wada, K.; Marumo, S.; Mori, K.; Takatsuto, S.; Morisaki, M.; Ikekawa, N., *Agric. Biol. Chem.*, **1983**, *47*, 1139.

21. Wada, K.; Kondo, H.; Marumo, S. *Agric. Biol. Chem.*, **1985**, *49*, 2249.

22. Maeda, E. *Physiol. Plant.*, **1960**, *13*, 214.

23. Wada, K.; Marumo, S.; Ikekawa, N.; Morisaki, M.; Mori, K. *Plant & Cell Physiol.*, **1981**, *22*, 323.

24. Takeno, K.; Pharis, R.P. *Plant & Cell Physiol.*, **1982**, *23*, 1275.

25. Wada, K.; Marumo, S..; Abe, H.; Morishita, T.; Nakamura, K.; Uchiyama, M.; Mori, K. *Agric. Biol. Chem.*, **1984**, *48*, 719.

26. Yokota, T.; Koba, S.; Kim, S.-K.; Takatsuto, S.; Ikekawa, N.; Sakakibara, M.; Okada, K.; Mori, K.; Takahashi, N. *Agric. Biol. Chem.*, **1987**, *51*, 1625.

27. Kim, S-K., Ref. 2, Chapter 3, pp. 26-33.

28. Adam, G.; Marquardt, V. *Phytochemistry*, **1986**, *25*, 1787.

29. Brosa, C.; Capdevila, J.M.; Zamora, I., *Tetrahedron*, **1996**, *52*, 2435.

30. Back, T.G.; Baron, D.L.; Luo W.; Janzen, L.; Pharis, R.P. unpublished results.

31. Thompson, M.J.; Meudt, W.J.; Mandava, N.B.; Dutky, S.R.; Lusby, W.R.; Spaulding, D.W. *Steroids*, **1982**, *39*, 89.

32. Takatsuto, S.; Yazawa, N.; Ikekawa, N.; Takematsu, T.; Takeuchi, Y.; Koguchi, M. *Phytochemistry*, **1983**, *22*, 2437.

33. Back, T.G.; Luo W.; Janzen, L.; Pharis, R.P. *Phytochemistry*, in press.

34. For reviews of earlier syntheses of brassinolide and related brassinosteroids, see: a) Back, T.G. in *Studies in Natural Products Chemistry*, Atta-ur-Rahman, ed., Elsevier: Amsterdam, **1995**, Vol. 16, pp. 321-364. b) Adam G.; Marquardt, V.; Vorbrodt, H.M.; Horhold, C., Andreas, W.; Gartz, J., Ref. 2, Chapter 7, pp. 74-85. c) Khripach, V.A.; Zhabinskii, V.N.; Litvinovskaya, R.P., Ref. 2, Chapter 5, pp. 43-55. d) McMorris, T.C., Donaubauer, J.R., Silveira, M.H.; Molinski, T.F., Ref. 2, Chapter 4, pp. 36-42.

35. McMorris, T.C.; Chavez, R.G.; Patil, P.A. *J. Chem. Soc., Perkin Trans. 1*, **1996**, 295.

36. Back, T.G.; Baron, D.L.; Luo, W.; Nakajima, S.K. *J. Org. Chem.*, **1997**, *62*,1179.

37. Ikekawa, N.; Zhao, Y-J., Ref. 2, Chapter 24, pp. 280-291.

38. Kamuro, Y.; Takatsuto, S., Ref. 2, Chapter 25, pp. 292-297.

39. a) Cram, D.J.; Abd Elhafez, F.A. *J. Am. Chem. Soc.*, **1952**, *74*, 5828. For a more recent variation of this model, see the Felkin-Anh model: b) Chérest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.*, **1968**, 2199. c) Anh, N.T.; Eisenstein, O. *Nouv. J. Chim.*, **1977**, *1*, 61.

40. Fung, S.; Siddall, J.B. *J. Am. Chem. Soc.*, **1980**, *102*, 6580.

41. Ishiguro, M.; Takatsuto, S.; Morisaki, M.; Ikekawa, N. *J. Chem. Soc., Chem. Commun.*, **1980**, 962.

42. Back, T.G.; Brunner, K.; Krishna, M.V.; Lai, E.K.Y. *Can. J. Chem.*, **1989**, *67*, 1032.

43. Back, T.G.; Krishna, M.V. *J. Org. Chem.*, **1991**, *56*, 454.

44. Back, T.G.; Blazecka, P.G.; Krishna, M.V. *Tetrahedron Lett.*, **1991**, *32*, 4817.

45. Back, T.G.; Blazecka, P.G.; Krishna, M.V. *Can. J. Chem.*, **1993**, *71*, 156.

46. a) Aburatani, M.; Takeuchi, T.; Mori, K. *Synthesis*, **1987**, 181. b) Sakakibara, M.; Okada, K.; Ichikawa, Y.; Mori, K. *Heterocycles*, **1982**, *17*, 301.

47. Cookson, R.C.; Gandhi, R.P.; Southam, R.M. *J. Chem. Soc. (C)*, **1968**, 2494.

48. Takatsuto, S.; Ikekawa, N. *Tetrahedron Lett.*, **1983**, *24*, 917.

49. Paulmier, C. *Selenium Reagents and Intermediates in Organic Synthesis*, Pergamon Press, Oxford, **1986**, Chapter 5.

50. Back, T.G. In *The Chemistry of Organic Selenium and Tellurium Compounds*, Patai, S., ed., Wiley: Chichester, **1987**, Vol. 2, Chapter 3.
51. Takeuchi, T.; Mori, K. *Liebigs Ann. Chem.*, **1988**, 815.
52. Takatsuto, S.; Yazawa, N.; Ikekawa, N. *Phytochemistry*, **1984**, *23*, 525.
53. Blazecka, P.G., *M.Sc. Thesis*, **1992**, University of Calgary.
54. Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action*, Academic Press Inc.: San Diego, **1992**, Chapter 3, pp. 83-84.
55. Owens, F.H., Fellmann, R.P., Zimmerman, F.E. *J. Org. Chem.*, **1960**, *25*, 1808.
56. Ashby, E.C.; Becker, W.E. *J. Am. Chem. Soc.*, **1963**, *85*, 118.
57. Welch, J.T. *Tetrahedron*, **1987**, *43*, 3123.
58. For example, see: Oshima, E.; Sai, H.; Takatsuto, S.; Ikekawa, N.; Kobayashi, Y.; Tanaka, Y.; Deluca, H.F. *Chem. Pharm. Bull.*, **1984**, *32*, 3525.
59. Pine, S.H. *Organic Chemistry*, 5th Ed., McGraw-Hill Book Company: New York, **1987**, Chapter 2, p. 42.
60. Larock, R.C. *Solvomercuration/Demercuration Reactions in Organic Synthesis*, Springer-Verlag, Berlin, **1986**, p. 276.
61. Olah, G.A.; Li, X-Y, *Synlett*, **1990**, 267.
62. Olah, G.A.; Welch J.T.; Vankar, Y.D.; Mosatomo N.; Kerekes I.; and Olah, J.A., *J. Org. Chem.*, **1979**, *44*, 3872.
63. Deyrup, J.A.; Moyer, C.L. *J. Org. Chem.*, **1969**, *34*, 175.
64. Carre, M.C.; Houmounou, J.P.; Caubere, P. *Tetrahedron Lett.*, **1985**, *26*, 3107.
65. Caputo, R.; Mangoni, L.; Neri, O.; Palumbo, G. *Tetrahedron Lett.*, **1981**, *36*, 3551.
66. Sonnet, P.E. *J. Org. Chem.*, **1978**, *43*, 1841.
67. Clive, D.L.; Menchen, S.M. *J. Org. Chem.*, **1980**, *45*, 2347.
68. Sharpless, B.K.; Umbreit, M.A.; Nieh, M.T.; Flood, T.C. *J. Am. Chem. Soc.*, **1972**, *94*, 6538.
69. For example, see: Roush, W.R.; Russo-Rodriguez, S. *J. Org. Chem.*, **1987**, *52*, 598.

70. Behan, J.M.; Johnstone, R.A.W.; Wright, M.J. *J. Chem. Soc., Perkin Trans. 1*, **1975**, 1216.

71. Gunther, W.H.H. In *Organic Selenium Compounds: Their Chemistry and Biology*, Klayman, D.L. and Gunther, W.H.H, eds., Wiley: New York, **1973**, Chapter 3, p. 42.

72. Dittmer, D.C., Zhang, Y.; Discordia R.P. *J. Org. Chem.*, **1994**, *59*, 1004.

73. Lambert, J.B.; Shurvell, H.F.; Lightner, D.A.; Cooks, R.G. *Introduction to Organic Spectroscopy*, Macmillian Publishing Company: New York, **1987**, pp. 115-119.

74. Still, W.C.; Kahn, M.; Mitra, A. *J. Org. Chem.*, **1978**, *43*, 2923.

75. Watson, S.C.; Eastham, J.F. *J. Organometal. Chem.*, **1967**, *9*, 165.

76. Emmons, W.D.; Lucas, G.B. *J. Am. Chem. Soc.*, **1955**, *77*, 2287.