SYNTHESIS AND CYTOTOXICITY OF OXYSTEROLS:

Studies on the A, B ring polyoxygenated sterols

Kejun Zhao

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Abstract: Oxysterols (OS), the polyoxygenated sterols, represent a class of potent regulatory molecules for important biological actions. Cytotoxicity of OS is one of the most important aspects in studies of OS bioactivities. However, studies, the structure-activity relationship (SAR) study in particular, have been hampered by the limited availability of structurally diverse OS in numbers and amounts.

The aim of this project was to develop robust synthetic methods for the preparation of polyhydroxyl sterols, thereof, evaluate their cytotoxicity and establish structure-activity relationship. First, we found hydrophobicity of the side chain is essential for 7-HC's cytotoxicity, and a limited number of hydroxyl groups and a desired configuration on the A, B ring are required for a potent cytotoxicity of an OS, after syntheses and tests of a number of 7-HC's analogues against cancer cell lines.

Then polyoxygenation of cholesterol A, B rings was explored. A preparative method for the synthesis of four diastereomerically pure cholest-4-en-3,6-diols was developed. Epoxidation on these cholest-4-en-3,6-diols showed that an allyl group exerts an auxiliary role in producing products with desired configuration in syntheses of the eight diastereomerically pure 45-epoxycholestane-3,6-diols. Reduction of the eight 45-epoxycholestane-3,6-diols produced all eight isomers of the cytotoxic 5α-cholestane-3β,5,6β-triol (CT) for the first time. Epoxide ring opening with protic or Lewis acids on the eight 45-epoxycholestane-3,6-diols are carefully studied. The results demonstrated a combination of an acid and a solvent affected the outcomes of a reaction dramatically. Acyl group participation and migration play an important role with numbers of substrates under certain conditions. All the eight 4,5-*trans* cholestane-3,4,5,6-tetrols were synthesised through manipulation of acyl participation. Furthermore these reaction conditions were tested when a number of cholestane-3,4, 5,6,7-pentols and other C_3-C_7 oxygenated sterols were synthesised for the first time.

Introduction of an oxygenated functional group through cholest-2-ene derivatives was studied. The elimination of 3-(4-toluenesulfonate) esters showed the interaction between the existing hydroxyls or acyls with the reaction centre often resulted in different products. The allyl oxidation, epoxidation and Epoxide ring opening reactions are investigated with these cholest-2-enes.

Keywords: Oxysterols, Synthesis, Epoxide, Epoxidation, Cytotoxicity.

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Abbreviations

AA	arachidonic acid
Ac	acetyl
Apo	apolipoprotein
ATP	adenosine triphosphatase
Bz	benzoyl
CE	cholesterol epoxide
CH(Ch)	cholesterol
CN-TPBP	cytosolic-nuclear tumour promoter-specific binding protein
mCPBA	*m*-chloroperbenzoic acid
CT	5α-cholestane-3β,5,6β-triol
DMAP	4-dimethylaminopyridine
DMP	3,5-dimethylpyrazole
DNA	deoxyribonucleic acid
ES	electrospray
FC	free cholesterlol
HC	hydroxycholesterol
HDCA	hyodeoxyacid
HDL	high density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HCE	HMG-CoA reductase
KC	ketocholesterol
LDL	low density lipoprotein
LXR	liver X receptor
MS	mass spectrometry
NBS	*N*-bromosuccinimide
OS	oxysterol(s)
OSBP	oxysterol binding protein

oxLDL	oxidised low density lipoprotein
PCC	pyridinium dichromate
PL	Phospholipids
PKC	proteinkinase C
SAR	structural activity relationship
SCAP	SREBP cleavage activating protein
SF-1	steroidogenic factor-1
SRE	sterol regulatory element
SREBP	sterol regulatory element binding proteins
StAR	steroidogenic acute regulatory protein
TLC	thin layer chromatography
TBHP	*t*-butyl hydroperoxide
Ts	4-toluenesulfonyl
TXA2	thromboxane 2
$VO(acac)_2$	vanadyl acetylacetonate
VSMC	vascular smooth muscle cells

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Chapter 1. Introduction to bioactivities of oxysterols

Introduction

Oxysterols (OS) comprise a large number of compounds formed from the oxidation of naturally occurring sterols. They are mainly derived from cholesterol (**Figure 1-1**) in mammalian cells. These molecules are characterised by a steroidal skeleton and more than one oxygenated functional groups. In the human body, they are produced either through *in vivo* enzymatic processes from the mevalonate biosynthetic pathway products, the cholesterol and lanosterol, or through nonenzymatic processes (**Table 1-1** and **Table 1-2**) (Guardiola et al 1996). The oxysterols in human blood may be also derived from food by auto-oxidation (Smith 1996). Other than that from human and animal, thousands of natural oxysterols were found from plant and marine source (Dauria et al 1993 and Faulkner 2001).

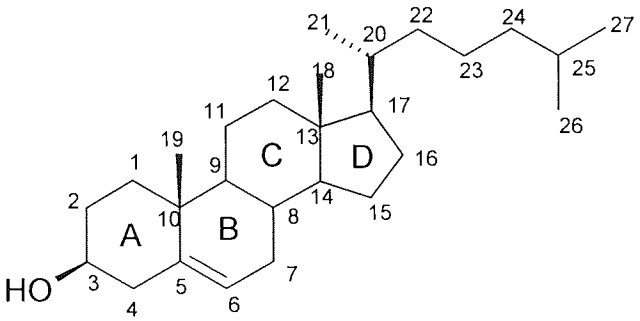


Figure 1-1 Cholesterol

In recent years, oxysterols have attracted a great deal of interest from biological scientists. OS represent a class of potent regulatory molecules, with remarkably diverse important biological actions that demand further investigation. A number of bioactivities of OS have been revealed, including gene regulation, alteration of cellular membrane properties, inhibition of 3-hydroxy-3-methylglutar coenzyme A reductase (HMG-CoA reductase), induction of apoptosis, cytotoxicity, atherogenesis, mutagenesis, carcinogenesis, antiviral activity, etc. The cytotoxicity has been the focus of biological scientists in the past decades. Two mechanisms for OS cytotoxicity have been postulated: the inhibition of endogenous cholesterol synthesis and alteration of membrane properties. Though direct evidence of these mechanisms

remains obscure, attempts to develop antitumour agents from OS have never stopped (Guardiola et al 1996).

For an understanding of the bioactivities of OS, the results of the recent research on OS's fundamental bioactivities: gene regulation and membrane effects are reviewed; then the cytotoxicity studies of OS are discussed, the published results on other activity studies related to these three main bioactivities are summarised.

Table 1-1 Oxysterols from human enzymatic origin

Systematic name	Trivial name	Enzyme
Cholest-5-en-3β,7α-diol*	7α–Hydroxycholesterol (7α–HC)	7α-HC synthetase (Schwarz et al 1998)
3b-Hydroxycholest-5-en-7-one*	7–oxocholesterol (7-KC)	7α-HC dehydrogenase (liver) (Song *et al 1996*)
(24S)-Cholest-5-en-3β,24-diol*	24(S)-Hydroxycholesterol (24S-HC)	Cholesterol 24-hydroxylase in brain (Lund et al 1999)
Cholest-5-en-3β,25-diol*	25-Hydroxycholesterol (25-HC)	Cholesterol 25-hydroxylase (Lund et al 1998)
(25S)-Cholest-5-en-3β,27-diol* and (25R)-Cholest-5-en-3β,27-diol*	(25S)-27-Hydroxycholesterol (25S-27-HC) and (25R)-27-Hydroxycholestero l (25R-27-HC), mixture as 27-HC	Sterol 27-hydroxylase in human lung (Babiker et al 1999)
Cholest-5-en-3β,7α,25-triol		In liver (Toll et al 1994)
(20S)-Cholest-5-en-3β,20-diol**	20α-Hydroxycholesterol(20-HC)	
(22R)Cholest-5-en-3β,22-diol**	(22R)-22-Hydroxycholesterol (22R-HC)	
(20R,22R)-Cholset-5-en-3β,20,22–triol**		

*Bile acid biosynthesis intermediate. ** Steroid hormone biosynthesis intermediate.

Table 1-2 Oxysterols from human nonenzymatic origin

Systematic name	Trivial name
Cholest-5-en-3β,7β-diol	7β–Hydroxycholesterol(7β–HC, 7-HC)
5,6α-Epoxy-5α-cholestan-3β-ol	Cholesterol-5α,6α-epoxide(α-CE)
5,6β-Epoxy-5β-cholestan-3β-ol	Cholesterol-5β,6β-epoxide(β-CE)
5α-cholestane-3β,5,6β-triol	Cholestanetriol (CT)
Cholest-5-en-3β,4β(α)-diol (Breuer 1996)	
	7α–HC, 7-KC, 20-HC and 25-HC

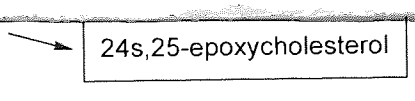
1.1 Oxysterol and gene regulation

1.1.1 Cholesterol in human body

Cholesterol (CH) is an essential constituent of all mammalian cell membranes, and its availability is therefore a prerequisite for cellular growth and other functions. *De novo* cholesterol synthesis is required for DNA synthesis, cell growth and cell proliferation. Quiescent cells synthesise little cholesterol. When cells are stimulated to proliferate, a cycle of sterol synthesis can be detected in the G1 phase of the cell cycle. Total blockage of the HMG-CoA reductase by oxysterols such as 25-HC leads to inhibition of DNA synthesis and of cellular proliferation (Kandutsch et al 1978 and Chen 1984). The cholesterol synthesis intermediates, such as lanosterol, may be also important in maintaining normal cell functions (Sinensky et al 1987). The biosynthesis pathway of cholesterol is shown in **Figure 1-2**.

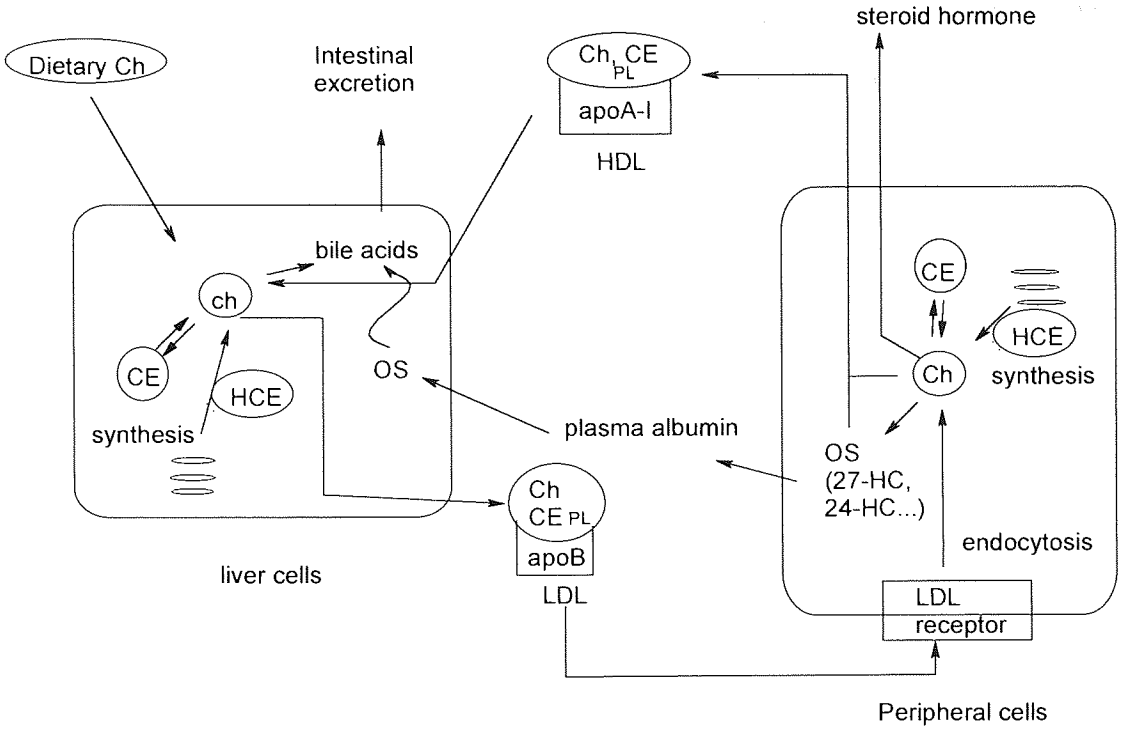
Both liver and peripheral cells synthesise cholesterol. The liver CH produced endocellularly or taken from food are transported mostly through the low density lipoprotein (LDL) to other cells, so called "bad cholesterol". The excess CH in peripheral cells is loaded to high density lipoprotein (HDL) and sent back to liver as "good cholesterol". Most cells can take in exogenous cholesterol from low-density lipoprotein (LDL) through its receptor and suppress the endogenous cholesterol synthesis at some extent, but it cannot totally replace endogenous cholesterol to support

cell growth and other function (Quesney-Huneeus et al 1983, Reimann et al 1991). Liver cells play an important role in the cholesterol homeostasis not only as they pack the CH and CH esters in LDL and send them to other cells; also, excess cholesterol transported back in HDL is metabolised to bile acids. OS represents another form of excess CH transportation as they can also be transferred by plasma proteins (**Figure 1-3**).



(1) HMG-CoA synthase, (2) HMG-CoA reductase, (3) Mevalonate kinase, (4) Phosphomevalonate kinase, (5) Isopentenyldiphosphate isomerase, (6) Dimethylallyl-*trans*-transferase, (7) Geranyl-*trans*-transferase, (8) Farnesyl-PP farnesyl transferase, 9) Squalene Monooxygenase, (10) Lanosterol synthase

Figure 1-2 the cholesterol biosynthesis pathway (Mathews and van Holde, 1996)



Ch cholesterol; CE cholesterol esters; apo apolipoprotein;

LDL low density lipoprotein; HDL high density lipoprotein;

OS oxysterols; PL phospholipids; HCE HMG-CoA reductase.

Figure 1-3 The concise general view of cholesterol homeostasis

1.1.2 Oxysterols, cholesterol homeostasis and cell growth

Balanced cholesterol metabolism in mammalian cells is maintained through the feedback regulation of key proteins involved in its cellular uptake, biosynthesis and metabolism. The major control points of cellular cholesterol homeostasis are at the level of transcription for genes that encode important proteins of these processes, such as HMG-CoA reductase and LDL receptor (Osborne 1995, Goldstein and Brown 1990).

Oxysterols are regulators of *de novo* cholesterol synthesis and metabolism. The regulation of gene expression by OS is involved in cholesterol and lipid metabolism (Wolf 1999). Recent studies showed oxysterols are both positive and negative

regulators of gene expression. As positive effectors, they bind to and activate the nuclear receptor LXR (Janowski et al 1996), which in turn increases transcription of the cholesterol 7α-hydroxylase gene, the rate-limiting enzyme in the formation of bile acids (Lehmann et al 1997). This activation stimulates the conversion of cholesterol into bile acids (Russell et al 1992). Excessive dietary cholesterol leads to increased oxysterol formation. Oxysterol binds to LXR and thereby induces transcription of cholesterol 7α-hydroxylase, thus increasing the removal of cholesterol as bile acids (Brown AJ et al 1997). Two subtypes of LXR were found, the LXRα and LXRβ. The LXRα is located mainly in liver cells and functions as above mentioned; it may also regulate the cholesterol homeostasis in some peripheral cells like macrophage. LXRβ is scattered in most type of cells. However, the researches on their roles in these peripheral cells are just at the beginning.

As negative regulators, oxysterols suppress the cleavage of two transcription factors known as sterol regulatory element binding proteins-1 and -2 (SREBP-1 and -2) (Brown MS et al 1997). These proteins are synthesised as inactive precursors in the membrane compartment of the cell. When intracellular cholesterol levels decline, SREBPs are proteolytically cleaved to release amino-terminal fragments that migrate to the nucleus where they bind with a high affinity to sterol regulatory element (SRE), thus activating the transcription of a network of genes involved in cholesterol synthesis and supply, for example, the gene for the synthesis of HMG-CoA reductase. This activation in turn restores intracellular cholesterol levels (Horton and Shimomura 1999). An interaction between SREBPs and another membrane-embedded protein, the SREBP cleavage activating protein (SCAP), is required for the cleavage to occur. Recent studies shows that sterols such as 25-HC can inhibit the cycling of SCAP between endoplasmic reticulum and Golgi apparatus (Nohturfft et al 1999).

The SREBPs (SREBP1 and 2) are thought to ensure the "concerted regulation of sterol-sensitive genes, particularly that of HMG CoA reductase and LDL receptor, and that of HMG CoA synthase, squalene synthase" (Kisseleva et al 1999, Vallett et al 1996). It is known that the HMG-CoA reductase synthesis is inhibited by oxysterols, in

particular, OS possessing hydroxyl groups at D ring and side chain, and the 6,7 oxygenated are most potent (Guardiola et al 1996, Schroepfer 2000).

Kisseleva et al reported recently that 15-ketosterols do not repress the expression of the LDL receptor gene in Hep G2 cells, as 25-HC does (Kisseleva et al 1999); though both reduce the level of HMG CoA reductase mRNA. The SRE segment exists in both HMG CoA reductase and LDL receptor genes, but it is possible that the cholesterol uptake is regulated by a mechanism independent from its biosynthesis. Both kinds of oxysterols also may repress the HMG CoA reductase gene expression via a different route. Unlike the studies on HMG-CoA reductase, the structure requirement on LDL receptor repression is far from clear as only 25-HC has been studied on this subject.

The oxysterol binding protein (OSBP) is a cytosolic receptor to which a variety of oxysterols binds, but its role in the regulation of SREBPs is still under scrutiny (Storey et al 1998). It undergoes ligand-induced binding to the Golgi apparatus and may respond to or sense altered cellular sterol content and transport in the regulation of cellular cholesterol metabolism (Mohammadi et al 2001).

Oxysterols from enzymatic origin represent a mechanism of reverse cholesterol transport, by which excess sterol is returned from the periphery to the liver for catabolism. They are transported through HDL, albumin and LDL (Babiker et al 1998, Morel et al 1996), so does the auto-oxidised OS. The bile acid synthesis pathway from the side chain hydroxylated sterols starting with the oxysterol 7α-hydroxylase, is a different system compared with that of CH (through cholesterol 7α-hydroxylase). These include 24S-HC, 25-HC and 27-HC. The 27-HC is the most abundant OS in blood. The sterol 27-hydroxylase is a mitochondrial cytochrome P450s enzyme (Cali and Russell 1991). Brain, which cannot transfer excess cholesterol from cells to circulating lipoprotein particles due to the blood-brain barrier, uses cholesterol 24-hydroxylase as a mediator of cholesterol homeostasis, and the 24S-HC is readily secreted from the central nervous system into the plasma (Lund et al 1999, Lutiohann et al 1996).

These side chain oxygenated sterols generated *in vivo* serve as gene regulators. 20S-HC, 22R-HC and 24S-HC show significant activation of LXRα and LXRβ, while 25- and 27-HC have no effect. The 24S, 25 epoxycholesterol, which is derived from a shunt in the mevalonate pathway through squalene epoxide as shown in **Figure 1-2** (Nelson et al 1981), may function as endogenous activators of LXR in liver and 24S-HC does the same in brain (Lehmann et al 1997). Potent gene-regulating properties through SREBPs are ascribed to 25-HC. Unlike other sterol hydroxylases, cholesterol 25-hydroxylase is not a cytochrome P450, but rather it is a member of a small family of microsomal enzymes that utilize di-iron cofactors to catalyse the hydroxylation of hydrophobic substrates (Lund et al 1998).

Binding to LXR is structural specific, and position-specific mono-oxidation of the sterol side chain is requisite for LXR high-affinity binding and activation. Enhanced binding and activation can also be achieved through the use of 24-oxo groups that act as hydrogen bond acceptors in the side chain. In addition, introduction of an oxygen atom on the sterol B-ring results in a ligand with LXRα selectivity. These results support the hypothesis that naturally occurring oxysterols are physiological ligands for LXRs and show that a rational, structure-based approach can be used to design potent LXR ligands for pharmacological use (Janowski et al 1999).

The gene regulation properties of other A, B ring OS to LXR and SREBP, together with the synthetic-bioactivity study are totally scarce.

Another receptor, the steroidogenic factor-1 (SF-1), which was originally thought to be an orphan receptor, is postulated to use oxysterols as activators (Christenson et al 1998, Lala et al 1997). SF-1 is a key transcription factor controlling the expression of steroidogenic enzymes. The steroidogenic acute regulatory protein (StAR) plays an essential role in steroid hormone synthesis by enhancing the delivery of cholesterol to the inner mitochondrial membrane, where the cholesterol side-chain cleavage enzyme (P450scc) system resides. 22, 25, 27-HCs can modulate the SF-1 mediated enzymes like the StAR.

The details of the gene regulation by OS are far from clear. The reports on the bioactive role of these nuclear receptors and their ligands are seldom. Besides the LXRs and SREBPs, a variety of cholesterol derivatives, including steroid hormones and vitamin D, exert effects on gene expression through interactions with members of the nuclear receptor superfamily (Mangelsdorf et al 1995). Members of this family function as ligand-activated transcription factors by binding to short stretches of DNA, termed hormone response elements, present in the regulatory regions of target genes. In addition to the nuclear receptors with known ligands, this superfamily includes a large number of structurally related members that contain DNA binding domains and putative ligand binding domains but lack identified ligands, the so-called "orphan receptors". The biological effects of OS, such as action on DNA synthesis, cholesterol metabolism, cell growth and cell proliferation, may be mediated by these orphan receptors (Lehmann et al 1997). It is speculated that more nuclear receptors modulated by OS will be found in future.

1.1.3 The mevalonate pathway outside the cholesterol synthesis and cell growth

Some evidence suggested that, in addition to cholesterol, the synthesis of several nonsterol isoprenoid compounds may also be required for cell growth and differentiation (Chen 1984).

In 1983, it was found that cholesterol is essential in early G1 and either mevalonate or isopentenyl adenine in late G1 permitted progression through the G1 and S phase DNA synthesis (Quesney-Huneeus et al 1983). Trentalance et al (1984) found that the regulation of the three processes: cell cycling, HMG-CoA reductase activity and cholesterol synthesis appeared uncoupled. The increased levels of in vitro expressed HMG-CoA reductase activity comparing with the decreases in the rate of both cholesterol and squalene biosynthesis suggested diversion of mevalonate into products other than squalene or sterols. It implies that this may reflect the needs of the cell for a nonsterol metabolite of mevalonate necessary for entry of cells into S phase. Many metabolism studies suggest that other compounds through the mevalonate pathway are

correlated with cell growth but with little direct evidence and mechanism supporting (Castellano et al 1994). Recently studies showed that the farnesol and geranylgeraniol derived from the mevalonate pathway were involved in the cell proliferation through farnesylated and geranylgeranylated proteins (Raiteri et al 1997). Also it has been found that continuous farnesylation due to stimulation of the cholesterol synthesis pathway causes the activation of protooncogenes, so OS which inhibit the mevalonate pathway can also served as chemo-prevention agents (Rao 1995).

1.1.4 Oxysterols and apoptosis

Apoptosis is a morphologically distinct type of cell death, which is believed to be programmed and under the control of natural cell genes, as opposed to cell death induced in nonspecific ways by toxic substances. Cells undergoing apoptosis show distinctive morphologic changes, including shrinkage, membrane budding, condensation of cytoplasm and nuclei and formation of apoptotic bodies, the particles of condensed cellular materials. Apoptosis can be initiated by internal messages or by extracellular signals (Kerr et al 1972).

The apoptosis induced by OS is attributed to their anti-proliferative, immunosuppressive activities. 25-HC can kill murine lymphoma cells in vitro as well as thymocytes (Christ et al 1993), human leukemic CEM cells (Ayala-Torres et al 1997) and microglial cells (Chang et al 1998) by apoptosis. 25-HC in macrophage membrane can kill the murine T cell lymphoma by apoptosis, suggesting that this oxysterol expressed in the macrophage cell membrane may participate in the regulation of cell growth through cell contact (Kato et al 1998). 7-HC was first found to induce apoptosis in human monocytic cell lines in 1995 (Aupeix et al 1995). Prolonged cell-mediated oxidation of LDL formed oxidised low density lipoprotein (oxLDL) (Kritharides et al 1995). 7-HC is the major component of this oxLDL which plays a pivotal role in atherosclerosis (Colles et al 1996), and their toxicity is due, at least in part, to the 7-HC and 7-ketocholesterol (Hughes et al 1994). These two oxysterols can induce apoptosis in endothelial and smooth muscle cells and necrosis in fibroblasts, and it is known this

is a stereoselective interaction, as the 7α-HC cannot induce apoptosis (Lizard et al 1999, Lemaire et al 1998, Miyashita et al 1997).

The general mechanism clarified for OS induced apoptosis cell death are down-regulation of c-myc (Thompson et al 1999b) and bcl-2 protein (Nishio et al 1996, Lizard et al 1997), through activating membrane sphingomyelinase and caspases (Harada-Shiba et al 1998) and Ca^{++} influx (Ares et al 1997). In some cases, the results show mixed-mechanism tendency of a single compound (Chang et al 1998, Harada et al 1997).

So far, mechanism studies of the OS induced apoptosis with different cell lines showed a big variation and sometimes controversial conclusions; and a gene regulation, outside the cholesterol metabolism pathway, was also observed. *C-myc* gene is well known to be involved in maintenance of cell cycle and cell viability. The posttranscriptional regulation of the *c-myc* gene, such as the decrease in c-Myc protein levels and an increased rate of mRNA degradation (Ayala-Torres et al 1999), was observed. Another gene which encodes the cellular nucleic acid binding protein (CNBP) is also of interest (Thompson et al 1999a). Using oxysterol sensitive and resistant lymphoid cells as study probes showed that CNBP modulated by OS take a role in oxysterol-induced regulation of cell viability and growth (Ayala-Torres et al 1994). Glucocorticoids and oxysterols kill cells by apoptosis, such as in human leukemic cells, with similar, but not identical patterns of DNA lysis, so they may go the similar way through the steroid receptor super-family before the activation of lethal protein synthesis (Johnson et al 1997).

Oxysterols different in structure such as the configuration and position of oxygenated groups give different results and may act through various mechanisms (Aupeix et al 1995, Lemaire et al 1998). In addition to the gene regulation, inhibitions of cholesterol synthesis alone may not account for apoptosis evoked by OS, but inhibitions of synthesis of mevalonate pathway products by down-regulation of HMG CoA reductase could contribute. This is because it is known some products generated from these by-ways of cholesterol synthesis affect cell viability (Thompson et al 1999a). Oxysterol induced apoptosis is also correlated to oxysterol binding protein (OSBP) (Bakos et al

1993), the protein whose function is not clear yet, supposed to be the lorry of OS transport.

Other substances related to OS, such as the steroidal glycoalkaloids, also were showed with potential to induce cell apoptosis (Chang et al 1998).

Above all, the studies of apoptosis are limited by severe shortage of oxysterols; so far most studies were done only by use of commercially available 7-HC and 25-HC. Few reports described SAR studies (Zhang et al 1997) with a limited number of oxysterols.

1.2 Alteration of cellular membrane structure and functionality by OS

1.2.1 Alterations of membrane structure and properties

Endogenous and exogenous cholesterol is incorporated into plasma membranes and intercalated among phospholipids. When the temperature is below that which can cause a phase transition, cholesterol prevents crystallisation of the lipid chains through blocking binding of the hydrocarbon fatty acyl chains of the membrane lipids to one another; as a result, the fluidity increases. When the temperature is above that for the transition, cholesterol decreases the fluidity by inhibition of the molecular motion of the lipid chains. These two effects expand the temperature range for keeping normal function of the cell membrane compartments, so cholesterol is an essential cell component (Stockton and Smith 1976, Yeagle 1991). In an updated review, it was stated that the hydrophobic part of steroid molecule has a possible role in helping to build the proton and sodium electrochemical gradients that are critical to energy handling at the plasma membranes of all living cells (Haines 2001).

In *in vitro* assays, OS can also be incorporated into membranes, substituting for cholesterol (Mahfouz et al 1995). OS, especially the A, B ring oxygenated, have more potent affinity with cell membranes than cholesterol. OS molecules can replace more cholesterol molecules in cell membranes and the method used to remove membrane cholesterol cannot drive these OS out (Pannecoucke et al 1994). These seem strange because OS are more polar than cholesterol. The exchange of OS molecules between

membranes is also much faster than cholesterol. It is postulated that 25-HC moves between the plasma membrane and endoplasmic reticulum via a common protein transport system; surprisingly it was observed that 25-HC is moving much more rapidly in this system, 2000-3000 times faster than cholesterol (Lange et al 1995). OS in membranes can also derive from oxidation of cholesterol of the membrane by means of some extracellular agents; it was hypothesised that cholesterol may act as a membrane antioxidant (Theunissen et al 1986).

The membrane effects of OS can vary a lot due to the chemical structures of certain OS. Oxysterols, depending on their molecular structure in particular, may exert a cholesterol-like homogenizing effect in membranes (Li et al 1994). The A, B ring oxygenated OS, to a certain extent, have some structure effects similar to cholesterol such as an increase in molecular order or their condensing effect, which are often less than that of cholesterol and maybe cause the functional change of the membrane. The side chain hydroxylated 25-HC do not give the condensing effect (Verhagen et al 1996, Theunissen et al 1986). In liposomes, C-7 OS decrease packing ability of phospholipid non-polar chains. However, this effect has not been observed for 20-HC, 25-HC, 22S-HC and epimers of 23-HC (Rooney et al 1985). As to the side chain, the results from a number of research groups showed that 22R-HC do not give the cholesterol like effects, but 22S- or 23-HC does (Hagiwara et al 1982). 25-HC, 22S-HC also give order effects using model and method different from above (Pannecoucke et al 1994).

However, in most cases, oxysterols are thought to be potent membrane-destabilizing agents. As a consequence of OS insertion, changes are observed in fluidity, permeability, stability and other properties of the cellular membrane, as well as in cellular growth, morphology and viability. Oxidation in the cholesterol nucleus which is situated closer to the phospholipid head groups at the lipid bilayer-aqueous interface results in a more profound, often disturbing effect on the plasma membrane physical structures (Kucuk et al 1992), so do the side chain oxygenated sterols. Both 7-HC and 25-HC reduce membrane fluidity in HTC hepatoma cells (Richard et al 1984). They can also induce membrane anionic phospholipid exposure but whether it is direct or indirect

action is not known (Aupeix et al 1996). Cholestane-3β,5α,6β-triol (CT), a potent atherogenic agent, is the most active OS in inducing permeability of endothelial cells for albumin (Boissonneault et al 1991). High level of OS can cause disorder of the cell membrane and induce cytolysis (Yuji et al 1985).

1.2.2 Membrane components and OS

OS affect the activities of various proteins such as enzymes, receptors and ion channels that are embedded or inserted into the cell membrane. These effects are mainly through two mechanisms: the alterations of membrane structure as mentioned above that affect the microenvironment of these membrane components, and direct interaction with these proteins.

The modulation effects of membrane properties are different with each individual oxysterol, as the activities of enzymes and receptors embedded in membrane may be mediated differently (Lau and Das 1995). For an example, the change of functional group at C-3 makes them interact differently with rat adipocyte membranes, with cholestanone interacting more with phospholipids located at the inner lipid bilayer (e.g. phosphatidylethanolamine) while cholesterol interacts more with phosphatidylcholine located at the outer lipid bilayer. This differential interaction may cause selective changes in membrane fluidity at different depths of the bilayer and thus may modulate the activities of membrane-bound enzymes and receptors (Lau and Das 1995).

OS act differently from cholesterol when they are placed in the detergent-insoluble, cholesterol / glycosphingolipid-enriched membrane domains (caveolaes) which have been implicated in signal transduction because a variety of signaling proteins as well as phosphatidylinositol bisphosphate (PtdInsP2) are compartmentalized. Vastly different effects were observed with modification of OS structure (Pike et al 1998).

The solid evidence for direct OS-protein interactions is lacking. Cholesterol is just known to covalently bond to a secreted protein very recently and no further study on

this is pursued (Edwards et al 1999). It has been supposed that covalent and non-covalent interactions are all possible in the OS enriched domains.

7-Ketocholesterol and possible other components of oxLDL can equilibrate into glycosphingolipid-rich membranes and increase the activity of src kinases, while 7β-hydroxycholesterol, 7α-hydroxycholesterol and cholesterol epoxide all induce a decrease in the background level of src kinase activity (Myers et al 1999).

7α-HC found in human erythrocytes increases protein helical structure in liposomes (Rooney et al 1985). The modification of membrane PKC activity and the inhibition of the phosphorylation of the substrates of PKC located in the membrane by OS were observed. Oxysterols reduce membrane associated PKC activity in immune cells. This may be partially responsible for their immunosuppressive activity (Moog et al 1991).

CT affects hexose uptake, but not 25-HC on this aspect; however both influence sodium/potassium adenosine triphosphatase (Na/K-ATPase) activity. To explain these differences, it was suggested that CT is able to incorporate into membrane, while 25-HC acts only by inhibiting cholesterol synthesis, because CT alters the activity of certain membrane-bound enzymes, particularly Na^{+}, K^{+}, Mg^{++}-ATPase and Ca^{++}-ATPase (Ramasamy et al 1992).

OS also modify calcium ion flux. It is well known that the cholesterol/phospholipid ratio determines Ca^{++} flux and, therefore, it appears likely that OS can modify it. It has been observed that the influx of Ca^{++} is increased in human erythrocytes by CT, 22S-HC and 26-HC and decreased by 7b-HC, 7-KC, 20-HC and 25-HC. However, in rat hepatocytes and platelets, 7-KC, CT and 26-HC increase Ca^{++} influx (Neyes et al 1985 and Sevanian et al 1986). It was proposed that this is one of the consequences of OS induced change of membrane permeability, then influencing Ca^{++} channels. Studies developed in liposomes also revealed that OS incorporation alter the potential energy barrier to inorganic ion conduction (Krull et al 1985).

1.2.3 Other membrane effects of OS

The oxysterols cause impairment in macrophage cholesterol export (van Reyk and Jessup 1999). 7-KC inhibits cholesterol efflux from macrophage foam cells induced by apolipoprotein A-I (apoA-I). Such oxysterols may promote foam cell formation in atherosclerotic lesions by preventing effective clearance of excess cholesterol (Gelissen et al 1999). 25-HC can also inhibit cholesterol export in a series of cell lines (Kilsdonk et al 1995).

Inhibition of gap junctional communication may be an early sign of oxysterols-induced toxicity on hepatocytes (Guo et al 1993). Intercellular communication is considered to play an essential role in maintaining and controlling cell growth, cell differentiation and homeostasis. Cholesterol oxidation products, instead of pure cholesterol, can be promoting factors in the atherogenesis by influencing gap junction communication between arterial smooth muscle cells, the target cells of atherosclerotic lesions (Zwijsen et al 1992).

The immunosuppressive effects of OS are postulated due to their replacement of cholesterol in membranes, such as the inhibition of cytolytic T lymphocyte activity (Kucuk et al 1994), and NK cell-mediated cytotoxicity (Kucuk et al 1992). In these cases oxidation in the cholesterol nucleus which is situated closer to the phospholipid head groups at the lipid bilayer-aqueous interface results in a more profound effect on the plasma membrane physical structure or protein activity (Moog et al 1991).

1.3 Cytotoxicity and antitumour activity of OS

OS like CT, 7-HC, 7-KC and 25-HC are well known for their cytotoxicity against a number of normal and malignant cells. In the middle of the 1970's, Chen et al first described the inhibition of 7-KC, 25-HC and 20α-HC on the growth of mouse L cells (Chen et al 1974); Cheng et al extracted 7β-HC from *Bombyx cum Botryte*, a traditional Chinese medicine curing tumour, and found this compound is cytotoxic to hepatoma HTC and ZHC *in vitro* at 33 μg/ml, but is not toxic to normal murine fibroblast cell 3T3

at 80 μg/ml (Cheng et al 1977). Following studies showed that 7β-HC is toxic to rat lymphomas at micro M concentration but not toxic to lymphoblasts and normal lymphocytes at 50 times higher concentration (Hietter et al 1986). CT was also found to be cytotoxic to murine L-cells (Higley et al 1984), macrophages and pig arterial smooth muscle cells (Baranowski 1982). 7-KC, 22-HC, 25-HC and some other analogues are also reported to be cytotoxic (Smith et al 1989). In recent 15 years, more naturally occurring oxysterols have been found from plant or marine sources with cytotoxicity to tumour cell lines. **Table 1-3** lists selected examples, and their structures were shown in **Figure 1-4**.

Table 1-3 Antitumour oxysterols from natural sources

Compound code	Cytotoxicity (IC_{50} (μg/ml))	Literature source
1.3-1(CT)	P388 70.3% inhibition by 10μg/ml	Synthesised
1.3-2	P388 (2.4)	Liyanage et al 1996
1.3-3	P388 (1.3)	Liyanage et al 1996
1.3-4	moderately cytotoxic to p388	Sheu et al 1991
1.3-5	L1210 (3.0), KB (1.3)	Zeng et al 1993
1.3-6	P388 (1.0)	Zeng et al 1995
1.3-7 (Xeniasterol)	B16 (5), KB (1.3)	Jutagawam et al 1986
1.3-8	renal E39(11), melanoma(11.3), nonsmall lung(8),	Casapullo et al 1995
	WEHI 164 (36.0±1.4), J 774 (1.68±1.0)	Aiello et al 1995
1.3-9	renal E39(11), melanoma(26.6),nonsmall lung(21)	Casapullo et al 1995
1.3-10(Verr ucoside)	P388 (5.9) Lung cancer A549 (7.2) Colon cancer Ht-29 (6.3)	Kashman et al 1991
1.3-11	Cytotoxic	D'Auria et al 1993
1.3-12	Not cytotoxic compare to 1.3-11	D,Auria et al 1993
1.3-13	KB (0.89)Hepatoma PLC/PRF/5 (1.17)	Lin et al 1991
1.3-14	KB (4.7)	West et al 1989
1.3-15	KB (26), PS (>10)	West et al 1989
1.3-16~1.3-20	Highly cytotoxic to lymphoma (JURCAT)	Andersson et al 1989

Continue of **Table 1-3**

1.3-21	P388 (0.5)	Iguch et al 1989
1.3-22	P388 (69% inhibition at 1μg/ml)	Kobaycish et al 1984
1.3-23	P388 (2.3)	Longley 1996
1.3-24	KB (9.79)	Lin et al 1991
1.3-25	K562 (3)	Loop et al 1994
1.3-26	Cytotoxic	Robert et al 1988
1.3-27	PS (4.9)	Maktoob et al 1988
1.3-28	KB (0.5)	Ktari et al 2000
1.3-29	WEHI 164 (50.4±0.6) J 774 (17.12±0.5)	Aiello et al 1995
1.3-30	WEHI 164 (39.2±0.5)± J 774 (8.64±0.3)	Aiello et al 1995
1.3-31	Mean IC50 (29.5), the theonellasterol without the 7α hydroxyl group (>100)	Qureshi et al 2000
1.3-32	P-388, KB, A549, HT-29: 0.4, 2.1, 2.7, 1.4	Sheu et al 2000
1.3-33	P-388, KB, A549, HT-29: 8.3, 1.9, 10.8, 1.5	Sheu et al 2000
1.3-34	P-388, KB, A549, HT-29: 8.3 1.9 10.8 1.5	Sheu et al 2000
1.3-35	P-388, KB, A549, HT-29: all >50	Sheu et al 1999
1.3-36	P-388, KB, A549, HT-29: 0.6, 5.9, 3.1, 0.4	Sheu et al 1999
1.3-37	P-388, KB, A549, HT-29: 0.8, 4.0, 2.5, 1.4	Sheu et al 1999
1.3-38	P-388, KB, A549, HT-29: 0.9, 4.6, 2.3, 1.2	Sheu et al 1999
1.3-39	P-388, KB, A549, HT-29: 0.4, 1.8, 1.8, 1.7	Sheu et al 1999
1.3-40	L1210 (5.2)	Yoshikawa et al 2000
1.3-41	A549, HT-29, KB, P-388: 0.41, 0.17, 0.60, 0.07	Duh et al 1998
1.3-42	A549, HT-29, KB, P-388: 4.09, 3.34, >50, 0.40	Duh et al 1998
1.3-43	A549, HT-29, KB, P-388: 1.76, 1.31, 1.10, 0.45	Duh et al 1998
1.3-44	A549, HT-29, KB, P-388: 0.81, 0.87, 0.38, 0.42	Duh et al 1998
1.3-45	A549, HT-29, KB, P-388: 0.69, 0.72, 0.58, 0.24	Duh et al 1998
1.3-46	A549, HT-29, KB, P-388: 0.81, 0.93, 0.39, 0.34	Duh et al 1998
1.3-47	P-388, KB (marginal)	Topcu et al 1997

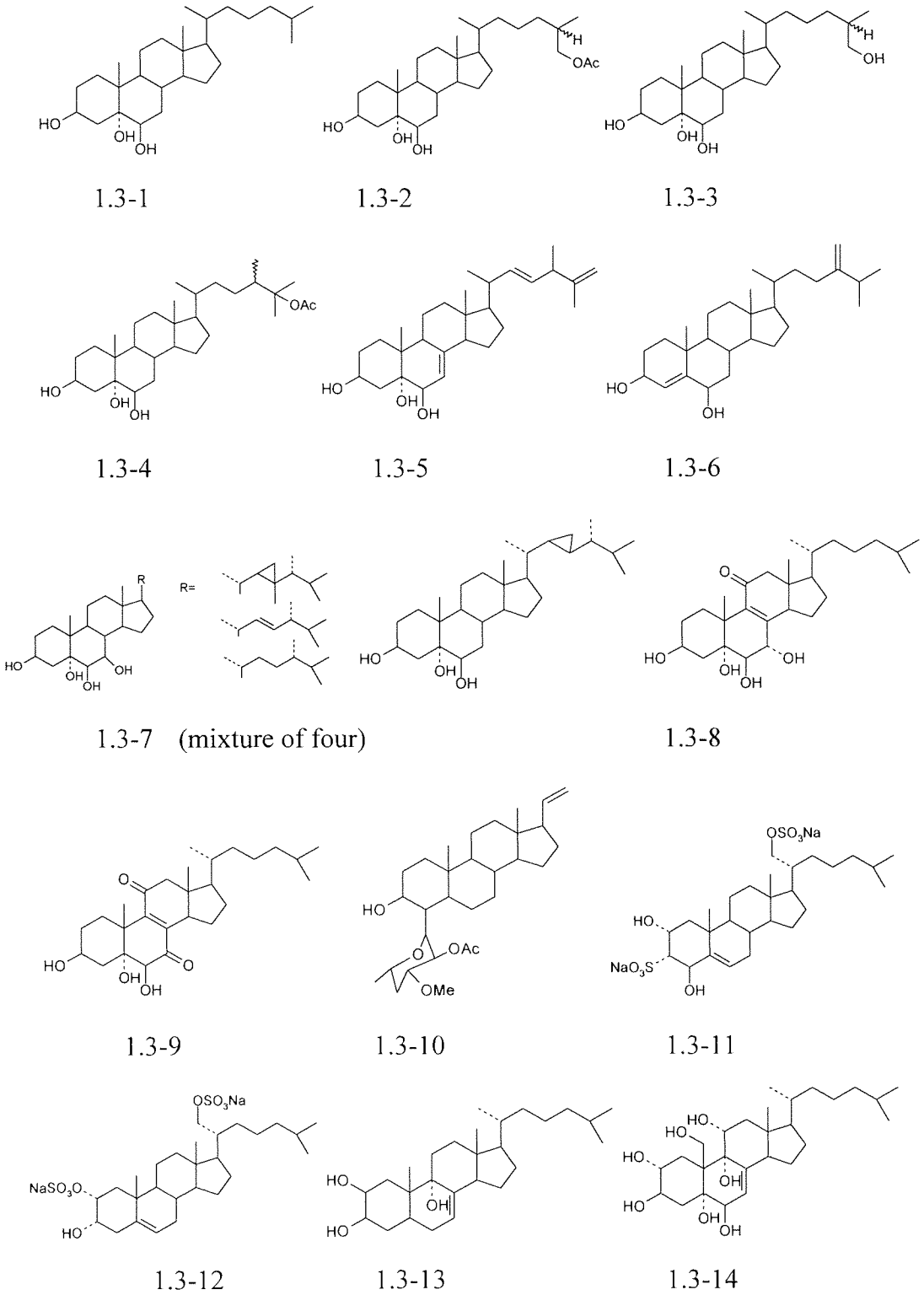
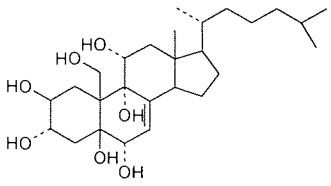
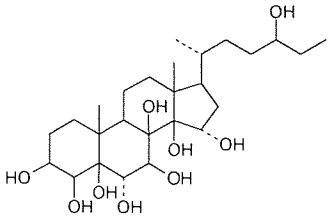


Figure 1-4 The structures of oxysterols from marine and plant sources in Table 1-3

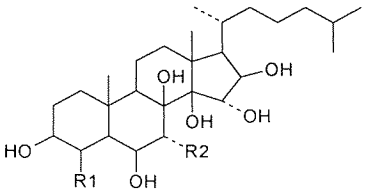
Continue of **Figure 1-4**:



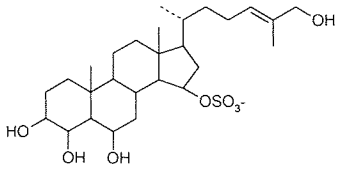
1.3-15



1.3-16



1.3-17 (R1=R2=H), –18(R1=H R2=OH), -19(R1=R2=OH)



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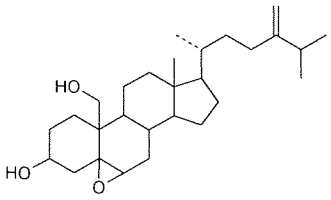

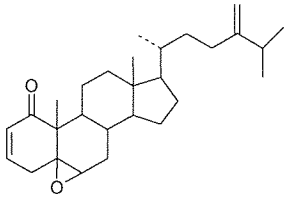

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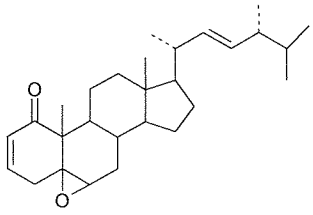
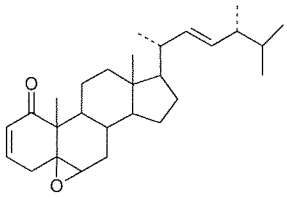
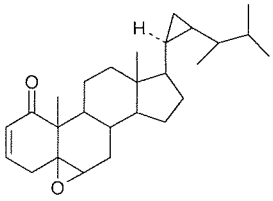
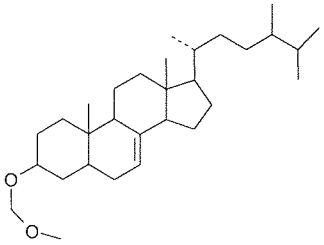






1.3-22 (mixture of the four 2-en-1-ones)



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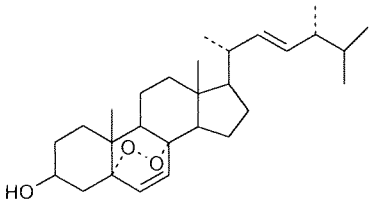

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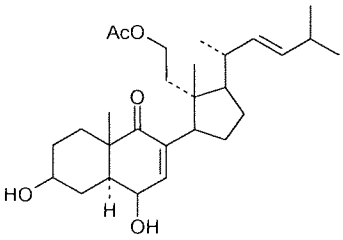

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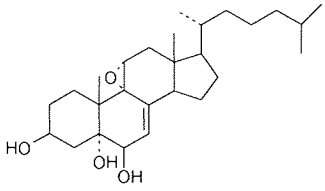

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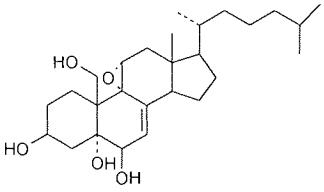

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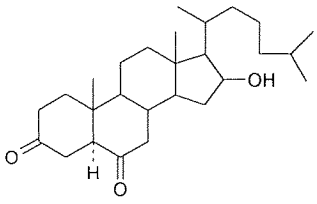

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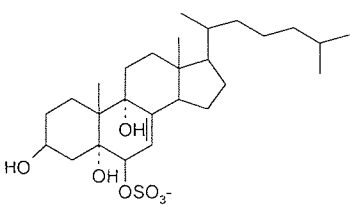

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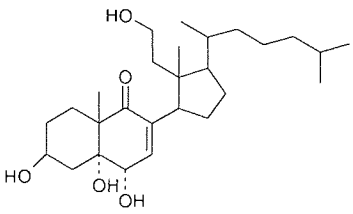

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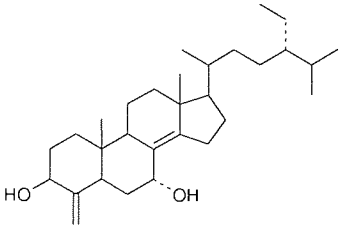
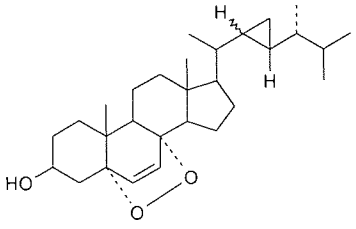


1.3-31

Continue of **Figure 1-4**:



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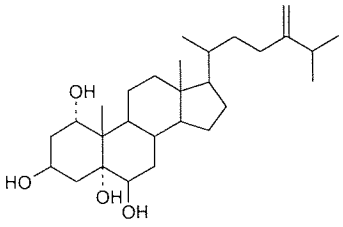

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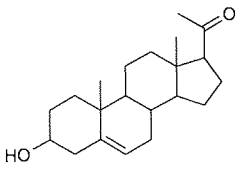

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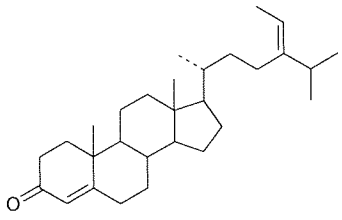

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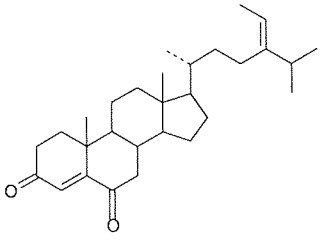

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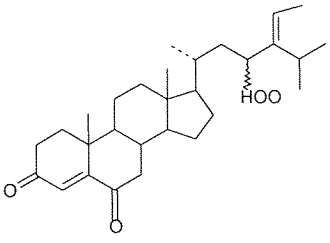

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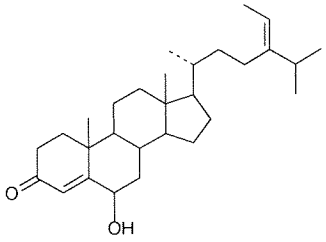

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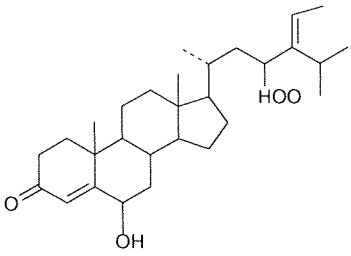

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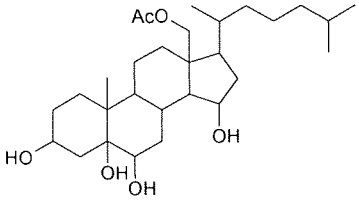

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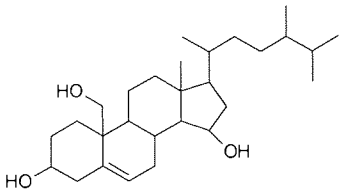

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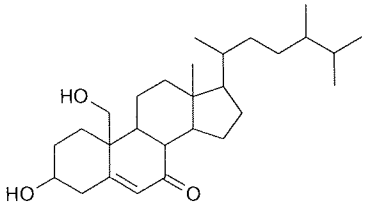

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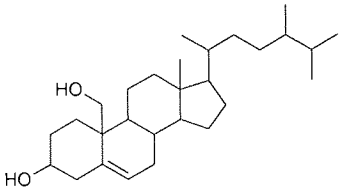

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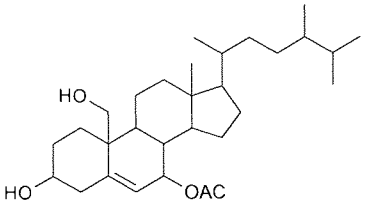

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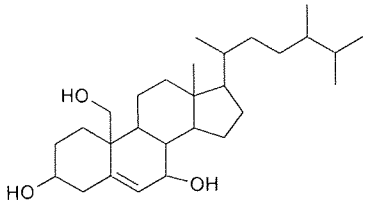

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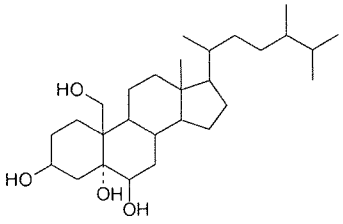

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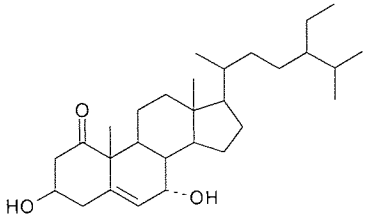
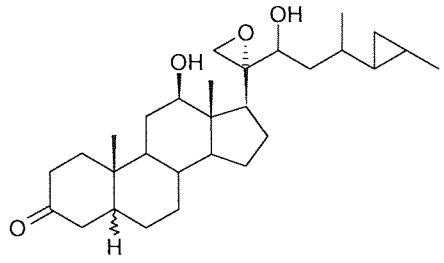
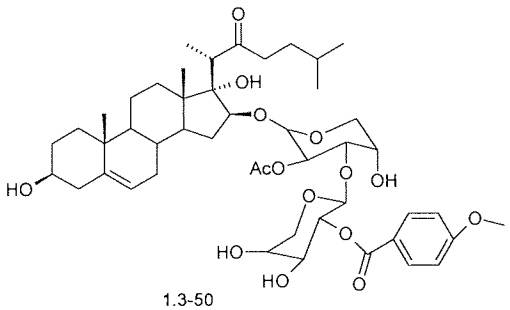


1.3-47

The aragusterol A (1.3-48) (Iguchi et al 1993) and its analogue, synthetic 5β-isomer (1.3-49) (Mitome et al 1997), are the most potent cytotoxic agents among OS against KB and several other cell lines, IC_{50} 10^{-7}~10^{-9} μmol/ml. Another D ring and side chain oxygenated glycoside (1.4-50) discovered by Mimaki et al in 1997 showed potent activity against HL-60 cell (IC_{50} 0.25 μmol/ml) and did not induce haemolytic action with human red blood cells at high concentration (Mimaki et al 1997)



5α 1.3-48 Aragusterol A
5β 1.3-49



1.3-50

It is obvious that structural features of these OS are so different to each other that structure-activity analysis is difficult. The oxygenation at C-6 is an example. In the compound 1.3-35 to 39, oxygenation at C-6 is essential for the cytotoxicity. 24ξ-Hydroperoxy-6 β-hydroxycholesta-4,25-dien-3-one (1.3-51), 25-hydroperoxy-6β-hydroxycholesta-4,23(E)-dien-3-one (1.3-52), 24ξ-hydroperoxycholest-4,25-diene-3,6-dione (1.3-53), and 25-hydroperoxycholesta-4,23(E)-diene-3,6-dione (1.3-54) separated by the same research group with 4-en-3,6-dione or 4-en-6-ol-3-one structure and different side chains also showed IC50 from 0.1 to 2.0 μg/ml (Sheu et al 1997). An earlier report on desmosterols showed the compound 24,25-epoxy-6β-hydroxycholest--4-en-3-one (1.3-55) with cytotoxic effects but not 24,25-epoxycholesterol (1.3-56). However, in the same report, compounds without a C-6 oxygenated group, such as 24-hydroperoxycholesta-5,25-dien-3β-ol (1.3-57), 25-hydroperoxycholesta-5,23(E)-dien-3β-ol (1.3-58), cholesta-5,25-diene-3β,24-diol (1.3-59) are also cytotoxic to the tested tumour cell lines. (Sheu et al 1996). Among the steroidal glycosides reported by Mimaki et al in 1999, the 6-hydroxyl group deactivated the cytotoxicity against HL-60 cells (Mimaki et al 1999). From these available data, the SAR relationship of C-6 functional groups essential for cytotoxicity cannot be established.

From the above overview, the OS are a unique family of compounds and each sub-group may act differently on exerting cytotoxicities. For one series of compounds, the A, B ring oxygenated groups may be essential and the side chain can be changed with little or no effect on their activity; when the D ring and side chain oxygenated functional groups are responsible for the cytotoxicity, as in the aragusterols, the A, B ring features can also be changed with the retention of their antitumor acitvities.

The cytotoxicities of these OS are complicated and far from clear, as several mechanisms have been proposed and individual OS seem to act differently. The cytotoxic effects of OS include inhibition of cell growth and instant or postponed cytolytic effects. Still, their cytotoxic mechanisms derive from the biological effects discussed: the cholesterol synthesis inhibition, apoptosis and membrane effects.

The inhibition of cholesterol synthesis: Common to many malignant cells is an intracellular shortage of 7α, 27-dihydroxy-4-cholesten-3-one caused by a decreased formation or an increased metabolism. This means the fast growing tumour cells need more cholesterol (Axelson et al 1996), and not only OS, potent HMG-CoA reductase inhibitors like 6-nitrocholesterol inhibit cholesterol synthesis and tumour growth as well (Parish et al 1988). It was often found that the cholesterol feedback inhibition mechanism that regulates cholesterol synthesis (Brown et al 1980) is lost in malignant transformation, such as human colon tumour cells, unlike normal colon cells and fibroblasts, exhibit a high endogenous cholesterol synthesis that LDL cannot regulate (Cerda et al 1995). Cancer cells seem to require an increase in the concentrations of cholesterol and of cholesterol precursors, so cholesterol synthesis inhibition may be a selective approach to inhibit tumour cell growth (Buchwald 1992, Dessi et al 1992, Labit-Le Bouteiller et al 1998, Kishinaka et al 1998). But these two effects are often not related. The inhibition of mevalonate pathway other than cholesterol synthesis may be another alternative route (Kishinaka et al 1998).

Apoptosis induced by OS is another mechanism related to their gene regulation properties as mentioned above. Possible differences that can be discriminated between cancer and normal cells have not yet been reported in OS studies.

Disturbance of structure of membrane, such as OS cluster formation, phospholipids redistribution, etc. has often been thought to be the mechanism of the instant cytolytic effects (Yuji et al 1985). The change of membrane protein properties should have great effect on the cell functions such as signal transduction, cell communication and ion exchange that are possibly responsible for the mechanisms (Guardiola et al 1996).

As the cytotoxic properties of OS may be due to a number of mechanisms mentioned above (Sevanian et al 1986 and Parish et al 1989), further studies have been carried out to explore possible mechanism. OS receptor study showed their cytotoxicity resembles that of nonsteroidal antiestrogens in some aspects: (i) the cytotoxic action of both types of compounds is blocked by inhibitions of protein or RNA synthesis, and (ii) both classes of compounds bind with high affinity to the microsomal antiestrogen binding site, a protein which may mediate the cytotoxicity of its ligands. (Low et al 1995). Another protein, cytosolic-nuclear tumour promoter-specific binding protein (CN-TPBP) as a postulated orphan nuclear receptor, was also thought to mediate the cytotoxic effects of OS (Yasuyuki et al 1993). 3β-(2-hydroxyethoxy)-5α-cholest--8(14)-ene-15-one, a synthetic compound, was found to be able to bind to oxysterol binding protein in HepG2 hepatoma cells (Misharin et al 1997) although its function has not been identified.

Oxysterols with oxygenated function on the side chain possibly act through cholesterol synthesis inhibition as described by Defay (Defay et al 1982), together with 7-KC, also a potent HMG-CoA reductase inhibitor. 25-HC and 7KC showed cytotoxicities to two murine cancer cell lines EL4 lymphoma and K36 leukemia, which were blocked by adding inhibitors of protein and RNA synthesis (Hwang 1992). Other evidences are: adding of serum (contains LDL) or exogenous cholesterol can reverse certain OS' inhibitions of cell growth (Brown et al 1974, Chen et al 1974).

The A, B ring oxygenated sterols are more likely to act through membrane because they have more intense membrane effects than the side chain oxygenated one. They induce cytolysis of many cells and this effect can also be partly inhibited by adding cholesterol to the medium *in vitro* (Yuji et al 1985, Bakos et al 1993). This mechanism, due to the

shortage of direct methods to observe membrane and its components, has been explored to its very limit.

The quarrel about whether or not the cytotoxic OS can be developed as antitumour drugs has lasted for a long time; with 7β-HC the selectivity between tumour and normal cells was confirmed (Nordman et al 1989) or denied (Reckewell et al 1987) with different cell lines. Many other OS are cytotoxic to both normal and tumour cells too, but the comparative studies between them are far from enough.

Much work has been carried on with the derivatives of 7β-HC; the water soluble phosphate, diphosphate, dihemisuccinate phosphodiester and glycoside phosphate derivatives are cytotoxic to several cultured tumour cell lines and also prolong life in afflicted mice (Reckewell et al 1987, Christ et al 1991, Moog et al 1993 and Ji et al 1990). Some water soluble esters of 7β-HC display a stronger toxicity towards tumour cells than towards normal cells. They can also prevent or delay the tumour development (Allemand et al 1993). The phosphoric acid diester can induce apoptosis and was considered as a promising soluble analogue of 7β-HC (Hyun et al 1997). There are no further reports on whether these derivatives can go into clinical studies.

The 7β-hydroxycholesteryl-3-oleate attenuated the tumour volume of experimental rat C6 glioblastoma by 80% at 36nm/ml; also it prevented tumour growth when C6 cells and 7β-hydroxycholesteryl-3-oleate were simultaneously injected. It has been shown that a high concentration of this compound with some oleic acid as metabolite abolishes the effect, so the 3-ester along with the 7β-OH are all essential (Werthle et al 1994). Maximal down-regulation of SREBP maturation and the consequent repression of SRE-1 promoters occurs in response to both a regulatory sterol and fatty acid, not ester, and down-regulate the cholesterol synthesis (Thewke et al 1998), so the activity of this compound may not correlate with cholesterol synthesis. Cholesteryl linolenate also shows toxicity to human normal monocyte-macrophages (Hardwick et al 1997).

As to other antitumour activities of OS, it was reported that 5α-Cholest-7-ene--3β,4β,6α,11α,22R-pentol reverses the multidrug resistance of human carcinoma cells (Aoki et al 1999). This kind of action maybe resulted from the cholesterol level

reduction and followed membrane effects (Lenz et al 1997). Ergosterol is recently reported as antiangiogenic substance (Takaku et al 2001).

The antitumour study of OS, on the one hand, is hampered by limits of its biological studies on mechanisms that seem to be a common problem today. On the other hand, the structural activity relationship (SAR) studies are very limited because only 7-HC, 25-HC and a small number of common OS are available. More stereospecific derivatives should help us understand further details of insight about how OS exert their bioactivity. The studies of OS cytotoxicities, gene regulation and certain membrane effects can be carried out *in vitro* with sophisticated methods in recent years. The problems are how to get a sufficient number of OS analogues. Our research interests are development of robust synthetic methodologies for synthesis of stereospecific OS derivatives for studies of their anticancer activities and other bioactivities.

1.4 Oxysterol and atherosclerosis

The atherogenic potential of OS is the most active research area among studies of OS bioactivities in recent years. The earlier studies using a mixture of cholesterol oxidation products gave controversial results on OS atherogenic properties (Schroepfer 2000). More publications of *in vivo* tests with pure OS on a role of OS in atherogenesis are only becoming available in recent years (James et al 1999). Though these reports suggested the atherogenic effects of CT, 7-HC, 7-KC, EC and 27-HC, their role in the process is still not quite clear (Brown and Jessup 1999). Research publications in this area comprise the biggest portion of OS bioactivity studies today.

Oxysterols produced by enzymatic or non-enzymatic reactions are accumulated in large quantity in vascular endothelium and atherosclerotic plaques. A variety of 7-hydroperoxycholesterol and its products were found in human atherosclerotic plaque (Brown et al 1997). Increased plasma 7β-hydroxycholesterol concentration was found significantly associated with progression of carotid atherosclerosis (Salonen et al 1997) and also found in a population with a high risk for cardiovascular disease (Zieden et al

1999). The concentration of oxLDL and OS in plasma is also significantly greater in atherosclerosis patients (Yasunobu et al 2001).

Oxysterols in human atherosclerotic plaque are suggested to play an active role in plaque development. 7-KC and 7-HC appear to be concentrated in foam cells and early lesions compared to advanced lesions and are at least two orders of magnitude higher than plasma levels; a small share of autooxidation generated CT, CEs and 25-HC are also found in the plaques (Brown et al 1999). The characteristic bioeffects of OS mentioned previously may promote atherosclerosis. Cytotoxicity of oxysterols to many cell types has been widely reported as mentioned above, these cells including vascular cells such as endothelial cells (Ramasamy et al 1992), macrophages (Clare et al 1995, Aupeix et al 1995), smooth muscle cells (Hughes et al 1994, Peng et al 1979) and lymphocytes (Christ et al 1993). Death of any of these cell types might stimulate atherogenesis.

Most of the 7-HC and 7-KC are carried within the oxidized low density lipoprotein (oxLDL) as its main toxic components. They are the major factor of the oxLDL's atherogenicity. oxLDL can induce apoptosis in vascular smooth muscle cells (VSMC) and endothelial cells (Björkerud et al 1996, Dimmeler et al 1997, Escargueil-Blanc et al 1997). oxLDL also has been proposed to induce adhesion molecules on surfaces of endothelial cells thereby allowing monocytes to enter the subendothelial space and initiate the early atherosclerotic lesion (Mehta et al 1995). In addition to the toxic effect of 7-HC and 7-KC in oxLDL to VSMC, the effects of OS on vascular permeability, prostaglandin synthesis and platelet aggregation and LDL receptor modification are all possible reasons for their atherogenetic activity (James et al 1999).

Cholesterol efflux inhibition is linked with the foam cell formation by lipid accumulation in macrophages in atherosclerotic plaques. The rapid and unregulated uptake of the cholesterol ester-rich oxLDL by macrophages generates the foam cells. These foam cells are thought to play a critical role in the development of atherosclerosis (von Eckardstein 1996). OS in oxLDL and lesions induced vascular foam cell lesion formation as a major effect of their atherogenicity (James et al 1999). 25-HC can reduce

cellular cholesterol efflux in vitro, a different effect from its HMG-CoA reductase inhibitory activity. This may be partly due to its membrane alteration effect (Kilsdonk et al 1995). Also the 7-KC showed this effect *in vitro* (Gelissen et al 1996). Ability of oxysterols to reduce levels of caveolin mRNA, and a FC-binding protein in fibroblasts should be part of the mechanism (Fielding et al 1997). OS enriched HDL also loses the ability to stimulate removal of cholesterol from the peripheral cells back to the liver for excretion (Gesquiere et al 1997). Cholesterol 5β,6β-epoxide and possibly 3,5-cholestadien-7-one, stimulate cellular sterol accumulation in J774 macrophages and may play an important role in atherogenesis (Cao et al 1995).

The foam cells can produce cytokines, such as IL-8, a potent chemoattractant that may play a role in the recruitment of T lymphocytes and smooth muscle cells into the subendothelial space and may contribute to the formation of atherosclerotic lesions. OS induced production of IL-8 and the apoptosis caused by these molecules in macrophages may contribute to the formation of advanced lesions (Liu et al 1997).

The most abundant OS in the early lesion is the enzyme synthesised 27-HC, which is also more abundant in advanced lesion as compared with 7-KC and 7-HC. 27-Hydroxylase may constitute a protective mechanism for removing cholesterol from macrophages and smooth muscle cells (Shanahan et al 2001). The fact that these OS (and cholesterol) still accumulate in lesions and foam cells indicates that this pathway may be perturbed in atherosclerosis and affords a new opportunity for the development of therapeutic strategies to regress atherosclerotic lesions (Brown et al 2000).

Another side chain OS, the 22R-HC give an interesting effect revealed by recent studies. 22R-HC as LXRα ligand promotes ABCA1 protein expression. These ABCA1 and related proteins are ATP driven pumps on cell membrane to facilitate the cellular cholesterol efflux to extracellular apolipoprotein AI (apoA1) or high density lipoprotein (HDL). It suggested that OS, which are potent LXRα ligands, could be antiatherogenic. As they may prevent the formation of foam cells, this hypothesis need more synthetic and SAR study on oxysterols (**Figure** 1-5) (Venkateswaran et al 2000).

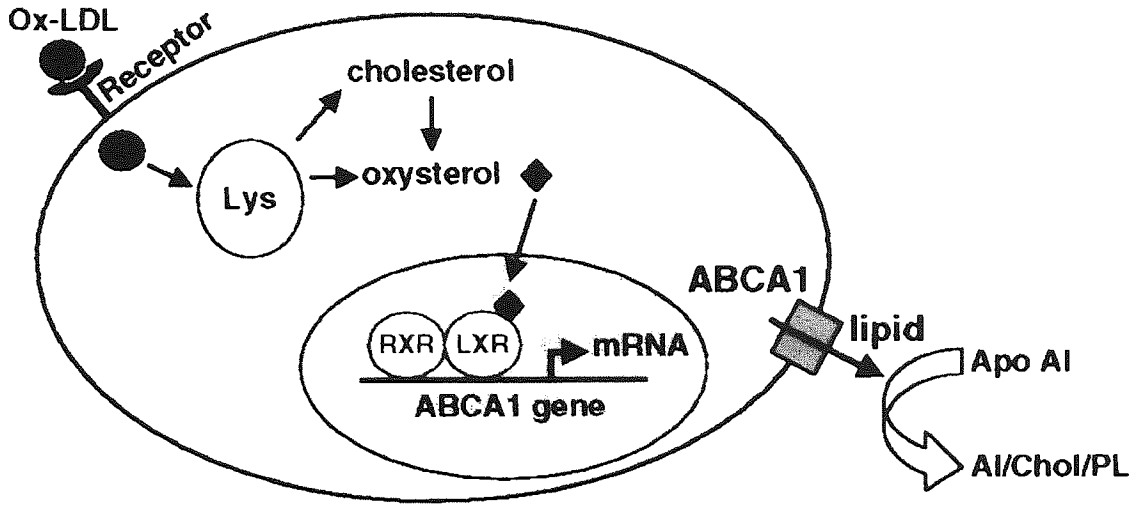


Figure 1-5. The possible role of 22R-HC in the development of atherosclerotic lesions (adopted from Venkateswaran et al 2000).

Above all, there is a mixed story for OS, which are atherogenic or pro-atherogenic, and to some extent, maybe antiatherogenic. More direct evidences are needed to establish a clear chart on the atherogenic effects of OS. Clearly a big number of OS are needed for a proper and intensive SAR study. We hope our synthetic studies can provide plenty of samples for these researches.

1.5 inflammatory and antiinflammatory effects

CT can inhibit the metabolism of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) (Mahfouz, et al 1995), but the OS can enhance thromboxane 2 (TXA2) synthesis by platelets due to activation of phospholipase A2 and the increase of arachidonic acid liberation from the platelet phospholipids (Mahfouz et al 1998). OS potentiated platelet aggregation and increased TXA2 formation in platelets challenged with thrombin, ADP or collagen in the concentration range 5-100 μM (Selley et al 1996).

Oxysterols with the oxidised side chain induced an inhibition of the overall arachidonate conversion and PGI2 synthesis at low concentrations, below the range of cytotoxicity. This inhibition was noted both on the basal and stimulated metabolism. Mechanisms involved in such actions are still to be determined (Seilan et al 1990).

The OS in inflammatory disease and atherosclerosis are linked together by the notion of vascular eicosanoid production induced by oxysterols through cyclooxygenase-2 induction (Wohlfeil et al 1997),

Oxysterols potentiate arachidonic acid (AA) release and prostaglandin (PG) synthesis when NRK cells (fibroblastic clone 49F) are activated by foetal calf serum. As serum is essential for a full oxysterol effect, these compounds could act on one or more of the events triggered by serum growth factor, binding to their specific receptors and leading to PLA2 activation. The oxysterol effect on AA release is synergistic with, but not fully dependent on, protein kinase C (PKC) activity and Ca^{2+} ion fluxes, suggesting that oxysterols could affect early events in the cell signalling pathway (Astruc et al 1994). Therefore oxysterols could affect earlier events triggered by serum growth factor binding to their cell membrane receptors (Lahoua et al 1991, 1989).

One marine oxysterol, the contignasterol, is under preclinical trials as an antiinflammatory agent (**Figure 1-6**) (Jaspars 1999).

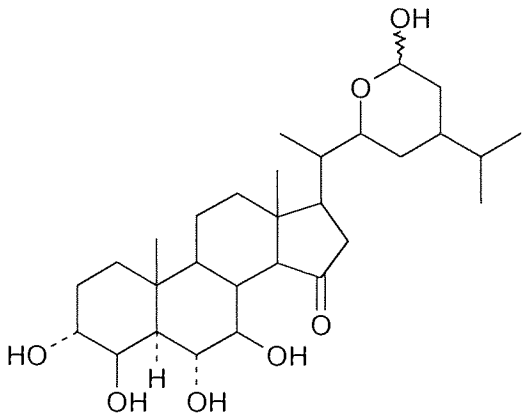


Figure 1-6 Contignasterol

1.6 Mutagenicity

So far it is known only the the epoxide form of OS (cholesterol 56-epoxide, CEs) are mutagenic due to their electrophilic reactivity (Sevanian et al 1986), and a recent report by Woods and O'Brien denied the possibility of genotoxicity that may be caused by OS (Wood and O'Brien 1998).

1.7 Antiviral effects

7β-HC, 25-HC and 7β,25-HC have been tested in vitro on the replication of HIV virus (Moog et al 1998), yielding inhibition with modest but reproducible selectivity indexes. It has been also reported that sulfated polyhydroxysterols isolated from marine organisms have shown antiviral effects on herpes simplex virus (Roccatagliata, et al 1996). Brassinosteroid analogs also have weak to moderate antiviral activity in vitro in Vero cells infected with herpes simplex virus HSV-1 (Ramý´rez et al 2000).

1.8 Availability of OS and research perspectives

As described above, most bioactivity studies on OS focused on several natural products in mammalian cells: 7β(α)-HC, 7-KC, CT, 22-R(S)HC, 23-R(S)HC, 25-HC, 27-HC and CEs. Some studies were also carried out with the naturally occurring OS from plant and marine sources. Synthetic studies of the natural OS with potent activity, such as the aragusterol, were initiated (Mitome et al 1995). A number of synthetic studies of antitumour and receptor binding agents were also carried out. As the bioactivities of oxysterols are varied with delicate changes of their structures, studies with more structurally diverse and structurally more related OS analogues should warrant a better understanding of the structure – activity relationships and biological mechanism.

Recently Schroepfer did an ever detailed review on oxysterols (Schroepfer 2000), which clearly pointed out that: "Despite continuing advances in studies of the chemistry of oxysterols, most investigators have relied on commercial materials that are unfortunately very limited with regard to structural types, available quantities, and reasonable costs. This situation has resulted in the acquisition of a large amount of information on the effects of one oxysterol, 25-OH-Chol, on a wide variety of parameters in cultured mammalian cells. Unfortunately, other oxysterols may be of considerably more physiological importance. Moreover, the results of studies with 25-OH-Chol (or the combination of 25-OH-Chol and Chol) have been frequently generalized to other oxysterols without experimentation. The limited availability of

oxysterols is also a major factor responsible for the very restricted number of studies of their in vivo effects in animals."

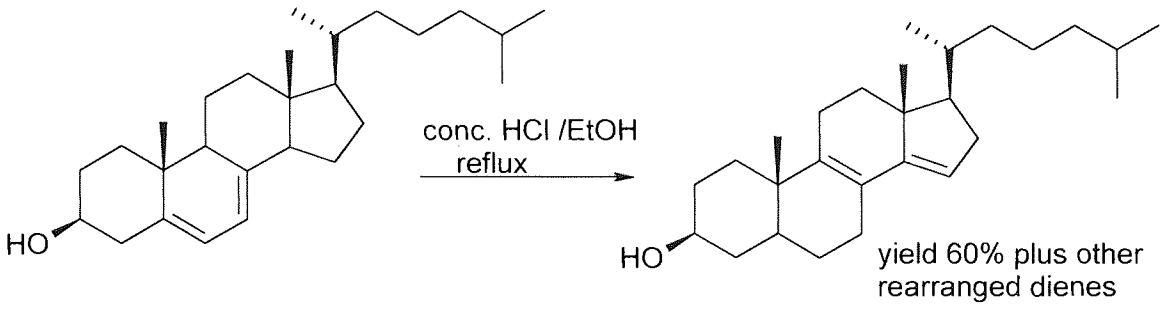
Although studies on the preparation and reaction of OS are recorded since late in the 1930s after the correct cholesterol structure was elucidated, there is not a unified and established synthetic strategy in tackling stereoselective syntheses of a defined oxysterol. Most of the reported syntheses are for diols and their analogues. The regioselective and stereoselective introduction of oxygenated functional groups are not yet studied for structurally related compounds, such as the stereoisomers of certain diol, triol or tetrol. For an example, the synthetic method for CT appeared very simple, but those reported for its isomers are long and involved expensive reagents. Furthermore syntheses of its two isomers (Cholestane– 3α,5β,6β–triol and cholestane – 3α,5β,6α–triol) were not reported. Therefore if we want to do a proper study of cholestan-3,5,6-triols, a better synthetic way has to be developed with easy control of the stereochemistry for obtaining all the isomers.

On the other hand, the SAR studies are very limited even with known compounds. The 3α-isomers of the 7β(α)-HC can be synthesised from the 3,5-diene compound (Ivo and Pavel 1985), but there was no bioactivity study carried with these compounds, while the 7β(α)-HC are intensively studied.

The number of synthetic polyhydroxyl sterols reported is limited. For example among the A,B ring oxygenated cholesterol derivatives about 20~30 tetrols are reported. Only four out the 16 isomers of cholestane-3,5,6,7-tetrol were reported by Warren J et al (Warren et al 1989) in a study of standard sample preparation of cholesterol autooxidation tests. No single synthesis of pentol OS is reported yet. As the polyhydroxyl sterols occurring in natural sources bearing more oxygenated groups are often found with potent bioactivities, systematic research is necessary on gradual introduction of the oxygenated functional groups onto the steroid molecules with the stereochemistry under control.

The bioactive OS can be grouped to A,B ring oxygenated sterols; C,D ring oxygenated sterols, side chain oxygenated sterols and their combinations. The AB ring oxygenated sterols are studied more extensively than the others.

While the C ring (C-11, C-12) oxygenation can be done by using cholic acid as the starting material through multistep reactions (Takashi et al 1990), the introduction of an oxygenated group to the D ring still has not a proper solution. The migration of cholest-5,7-diene to cholest-8, 14-diene (**Scheme 1-1**) can provide a short cut for CD-ring OS (Seto et al 2000). However, very limited study has been carried out on oxygenation of these compounds. The CD ring oxygenated sterols also can be generated either from the side chain or from the B ring. Only selenium oxide was used to introduce 14α-OH by allyl oxidation of the C7-C8 double bond (Valisolalao et al 1983). The 16,17 double bond can be introduced from alkylation of androsterone. The 16,17-epoxide was prepared, but no attempt was made to prepare the 16,17-diol (Helena et al 1994). The allyl oxidation of 16-ene to introduce a 15-oxygenated group was only recently reported (Irene et al 1999).



Scheme 1-1 Migration of the 5,7-diene

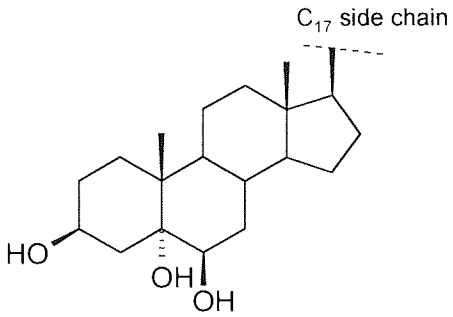
Above all, reported methods for the introduction of oxygenated functional groups to the steroidal skeleton could not satisfy the needs for preparation of a big number of stereo defined OS at low cost. Therefore in order to start a proper study of the bioactivities of oxysterols, a systematic synthetic methodology needs to be developed.

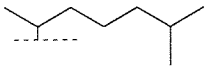
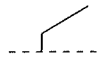
Chapter 2. The initial study on the side chain hydrophilicity and the A, B ring oxygenation

2. The initial study on the side chain hydrophilicity and the A,B ring oxygenation

As mentioned in the introduction, OS' cytotoxicity is the primary interest of our research. The studies of OS cytotoxicity have a relatively short history, about 25 years. It is well known that the 3-esters and water soluble diesters of 7β-HC are potential antitumour agents. Numbers of OS from natural sources with oxygenated groups in the A, B ring showed cytotoxicity to cancer cell lines. A little preliminary analysis of structure-activity relationships could be found in literature, as it was said if the C17 side chain of CT was removed or shortened to two-carbon atoms long, its cytotoxicity to P388 cells was diminished (**Table 2-1**) (Rong et al 1994).

Table 2-1 The cytotoxic effects to murine leukaemia P388



Side chain	IC_{50} μg/ml to P388 cells
	10-15
	70-100
No side chain	>100

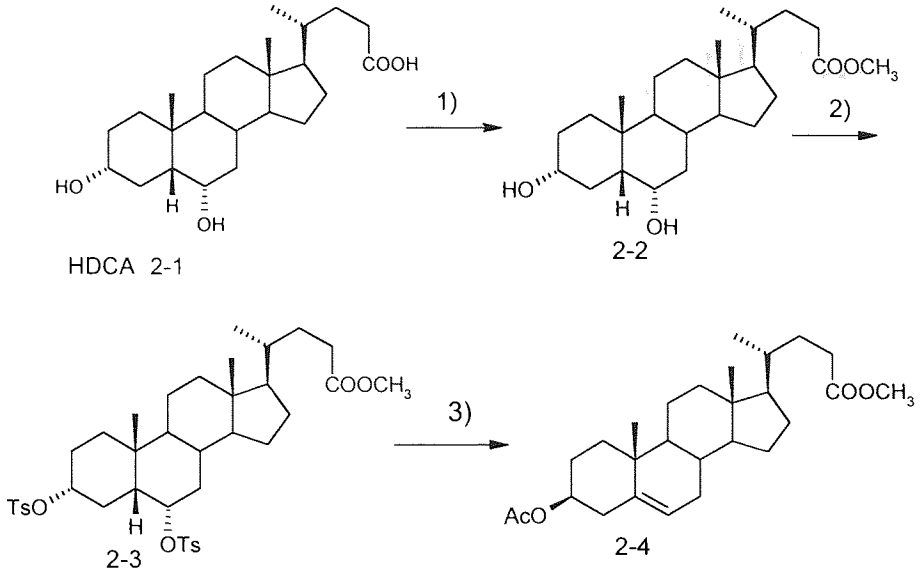
It was also described that antitumour activity of the water soluble esters of 7β-HC is better than that of 7β-HC both *in vitro* and *in vivo*, possibly due to a better water solubility of the esters. However, there is no report on the bioactivity of the compounds with a hydrophilic C17 side chain. We took this as our start point in our studies of OS cytotoxicity. We planned first to synthesise OS having a hydrophilic functional group on the C17 side chain. A carboxylic group is a good choice for this purpose, as its metal salt implies the best water solubility and a good comparison with acid form and esters. Another consideration is that we need to keep free hydroxyl groups on the A and

B rings because if the hydroxyls were protected as ester they may not be completely hydrolysed into the free OS in the cell culture.

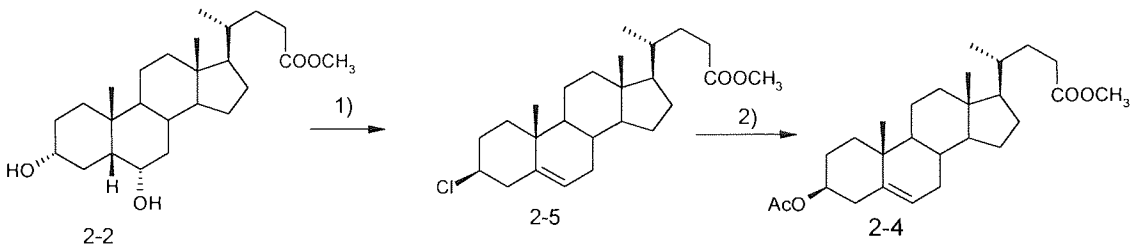
We selected the chol-24-oic acid as starting material as its side chain is similar to that of cholesterol in length. The most conveniently available starting material with a carboxylic group on the side chain is the hyodeoxyacid (HDCA, 2-1).

Ziegler has reported a one-pot process to synthesize methyl 3β-acetoxychol-5--en-24-oate (2-4) from (2-1) using 4-toluenesulfonyl (Ts) ester as a leaving group (**Scheme 2-1**) (Ziegler 1959). The major problem for this transaction is that both industrial and reagent grade HDCA (2-1) contains about 20% hyocholic acid (HCA), which has an additional α-hydroxyl group at C-7. This made the purification of compound 2-4 from reaction mixture by chromatography almost impossible. To overcome this problem, we modified the route developed by Ushizawa (**Scheme 2-2**) (Ushizawa 1960), which goes through 3β-chloro derivative 2-5. In preparation of 2-5 we reduced the amount of reagents and performed addition of the starting material 2-2 dropwise at optimized temperature (75°C) in a shortened time. Compound 2-5 was easily purified by recrystallization from ethanol and water in a moderate yield of 44% on 50g scale. Then compound 2-5 was converted to the 2-4 at nearly quantitative yield.

Introduction of an oxygen atom to C-7 of steroidal 5-enes is often fulfilled by use of a chromium trioxide complex, and the CrO_3-DMP (3,5-dimethylpyrrzole) often gave the best result (Salmond et al 1978). Though one report claimed pyridinium dichromate (PCC) gave a higher yield (Chidambaram et al 1987), we failed in repeating it. Shoda (1993) reported a direct introduction of 7-hydroxyl group to compound 2-4 using tert-butylperbenzoate catalysed by $CuBr_2$.

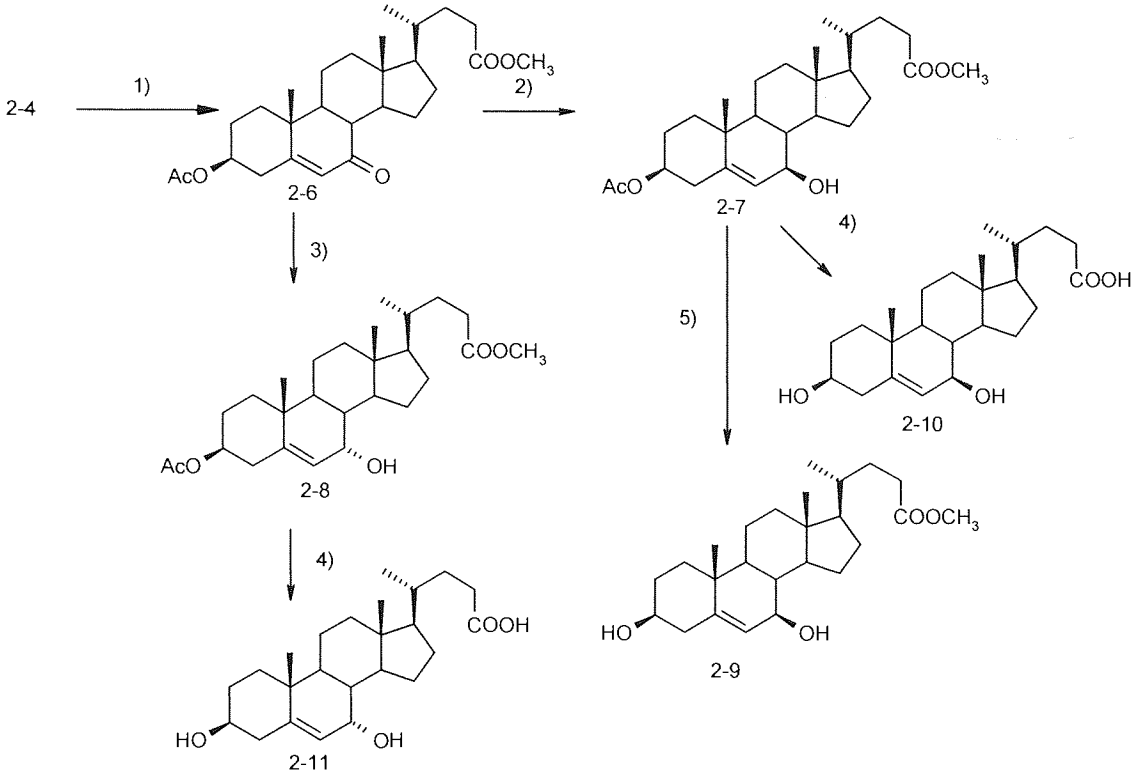


Scheme 2-1 Preparation of methyl 3β-acetoxychol-5-en-24-oate *via* Ts ester



Scheme 2-2 Preparation of methyl 3β-acetoxychol-5-en-24-oate *via* 3-chloride

In our experiment on the oxidation of 2-4 by chromium trioxide complexes, only CrO_3-DMP complex in CH_2Cl_2 at -25°C gave a pure product directly. Ketone 2-6 was reduced stereoselectively to the 7β-hydroxy compound 2-7 using a mixture of sodium borohydride, methanol and Cerium trichloride. Though reduction of 2-6 with L-selectride produced the 7α–hydroxy isomer 2-8, the pure product was obtained only after several recrystallisations. Sodium borohydride reduction of 2-6 in THF/water gave the β/α ratio at 4.5 /1. The removal of 3-acetyl group in 2-7 and 2-8 was completed with NaOMe/MeOH at reflux, while undergoing exchange of the 24-methyl ester group with solvent. Total hydrolysis with NaOH-EtOH-H_2O gave the target compounds 2-10 and 2-11 (**Scheme2-3**).



1) DMP, CrO_3, 70%; 2) $CeCl_3.7H_2O$, $NaBH_4$, MeOH , 88%; 3) L-selectride , THF; 60%
4) NaOH, EtOH, H_2O 99% 5) NaOMe, MeOH; 79%

Scheme 2-3 Preparation of 7-HC analogues from compound 2-4

The *in vitro* antitumour tests were performed on compounds 2-7, 2-8, 2-9 and 2-10. They showed that the compound with a free carboxylic acid group on the side chain (2-10) has no activities against any tested tumour cell lines, while compound 2-9, the analogue of 7β-HC, and 3-acetyl esters 2-7 and 2-8 demonstrated weak to medium cytotoxic effects against some cancer cell lines (Table 2-2). The sodium salt of compound 2-10 gave no activities too. This seemed to indicate that the hydrophobicity of C17 side chain is essential to the cytotoxicity on this aspect. Therefore, we decided to use the cholesterol as the starting material to develop series of A and B ring-oxygenated sterols.

As mentioned in the introduction, small change in OS structures can alter their bioactivity significantly. On the other hand, naturally occurring sterols from a number of different sources have shown similar cytotoxicity, and compound 2-7 and 2-8 are additional examples.

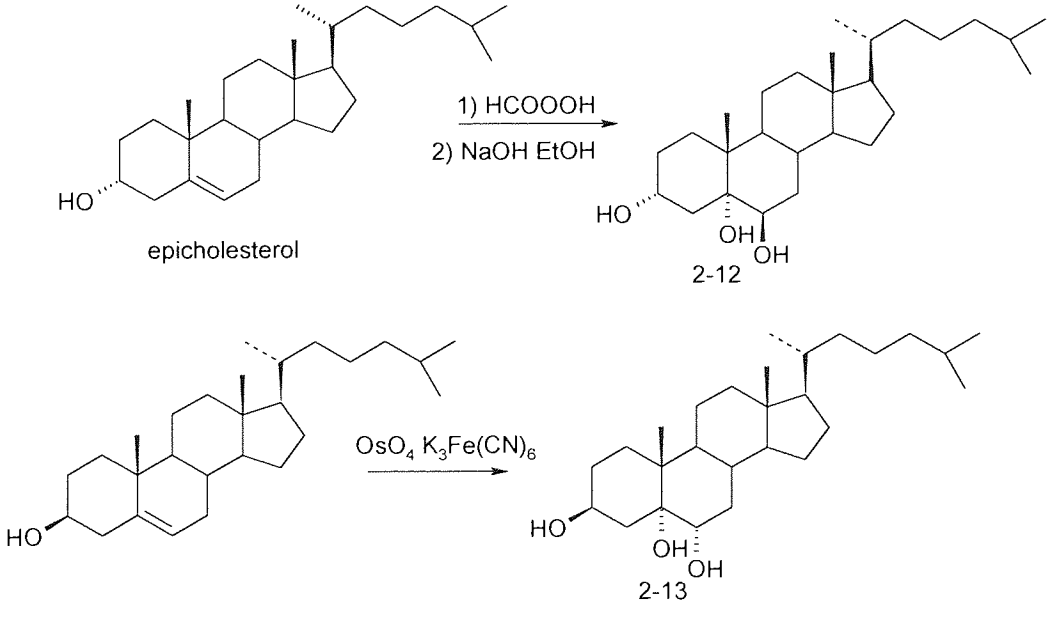
Table 2-2 Cytotoxicities of compounds 2-7 to 2-10 [log GI_{50} (Molar)]

Cancer cell lines	2-7	2-8	2-9	2-10
leukemia K-562	-4.96	-5.37	-5.20	>-4.00
MOLT-4	-4.75	-5.08	-4.81	>-4.00
SR	-4.57	-5.32	-5.41	>-4.00
NSC Lung Cancer A549/ATCC	-4.63	-4.33	-4.72	>-4.00
HOP-62	-4.69	-4.71	-4.79	>-4.00
NCI-H522	-4.54	-4.98	-4.97	>-4.00
Colon Cancer COLO-205	-4.79	-4.86	-4.60	>-4.00
HT-29	-4.83	-5.63	-5.80	>-4.00
CNS Cancer SF-295	-4.78	-4.84	-4.83	>-4.00
SNB-19	-4.71	-4.77	-4.74	>-4.00
U251	-4.71	-4.84	-4.89	>-4.00
Melanoma LOX IMVI	-4.69	-5.25	-4.80	>-4.00
M14	-4.80	-4.91	-5.00	>-4.00
UACC-257	-4.73	-4.21	-4.72	>-4.00
Ovarian Cancer IGROV1	-4.59	-4.66	-4.78	>-4.00
Renal Cancer 786-0	-4.74	-4.77	-4.67	>-4.00
Prostate Cancer PC3	-4.61	-4.61	-4.74	>-4.00
Breast Cancer MCF7	-4.56	-4.17	-4.50	>-4.00
BT549	-4.54	-4.69	-4.77	>-4.00

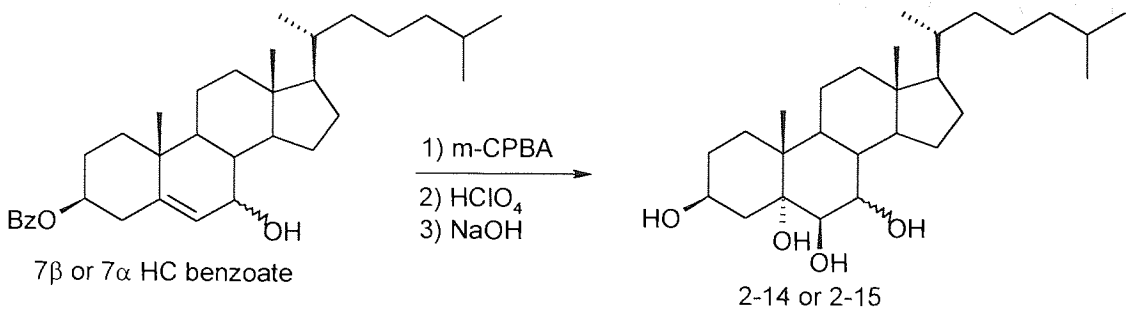
In order to know whether or not changes in stereochemistry of one or more hydroxyl groups in OS can affect its cytotoxic effect significantly, we selected CT as template and synthesized its isomers 2-12 and 2-13 (**Scheme 2-4**). We also prepared cholest-3β,5α,6β,7β-tetrol 2-14 and its 7α-isomer 2-15 (**Scheme 2-5**) as compounds that have the hydroxyl groups with the same position and configuration to those in 7-HC and CT together.

Synthesis of the intermediate 3α-hydroxycholest-5-ene (epicholesterol) has been a troublesome task for a long time. The most convenient method described in literature is the catalytic hydrogenation of cholest-5-en-3-one (Ishige et al 1980). Problem for this approach is that a special catalyst is needed, and isolation and purification of epicholesterol is sluggish. We adopted Houminer's method (Houminer et al 1975) in preparation of a small quantity of epicholesterol. The 3α-isomer of CT is synthesised from epicholesterol. First is epoxidation and Epoxide ring opening of the 5, 6 double bond in formic acid with performic acid generated *in situ* by adding hydrogen peroxide, then hydrolysis to give the 5α-cholestane-3α,5α,6β-triol (2-12). A small amount of sample 5α-cholestane-3β,5α,6α-triol (2-13) was prepared directly by using potassium hexacyanoferrate and catalytic amount of OsO_4 (Minato et al 1990). This oxidation is very slow. After being stirred for 72hr at 40°C, only 15% of the cholesterol is converted to 2-13. Goto et al developed a long route to 2-13 from cholesterol. The crucial step is the introduction of a 7α-bromo group as the steric hindrance group to block the α-side attack of borohydride to the 6-one (Goto et al 1961,1962). Unfortunately these methods are not feasible for preparation of a small amount of sample in our laboratories.

The two tetrols are prepared from the 7α and 7β-HC through epoxidation and hydrolysis in butanone using perchloric acid as the catalyst (**Scheme 2-5**).



Scheme 2-4 Preparation of CT's isomers



Scheme 2-5 Preparation of CT & 7HC's analogues

A significant diversity in cytotoxic effects of the prepared derivatives (CT, and compound 2-12 to 2-13) was observed in a preliminary test (**Table 2-3**). It seems that the hydroxyl groups on A, B ring in β-configuration enhance the cytotoxicities. It is in line with the report that the natural OS 1.3.1-11 lost its activity when its 4β-OH was removed (compound 1.3.1-12). As the first cytotoxic tetrol with the cholesterol skeleton, compound 2-14 (**Table 2-4**) is an exciting discovery. This is also direct evidence supporting the idea that OS bearing more oxygenated groups are worth further study.

It is not difficult to imagine how many combinations are possible of oxygenated functional groups on A, B ring of OS by taking account of locations and stereochemistry, and it seems impossible to synthesize all these compounds though it looks very attractive and exciting. However without all necessary derivatives, how we can get a proper picture of SAR for this group of compounds? By application of medicinal chemistry principles we decided to tackle this goal from the following range of the object compounds:

Syntheses should start with bulk materials that can be prepared from cholesterol conveniently on big scale;

Ensure every type of the oxygenated groups-the hydroxyl group and epoxide with α or β configurations as well as ketone group-to appear at each position of A and B rings at least once. QSAR analysis should be carried out;

Synthesise OS with four, five or more oxygenated groups located from C1 to C9 as chemical and biological study of these compounds are interesting, and studies in this field are nearly blank.

Table 2-3 the different cholestan-3,5,6-triols and their IC_{50}(μg/ml) to L1210 cells

Compound	IC_{50}
CT	20-35
2-12	50~100
2-13	>100

Table 2-4 Cytotoxicities of compounds 2-14 and 2-15 [log GI_{50} (Molar)]

Cancer cell lines	2-14	2-15
leukemia K-562	-7.36	-5.46
MOLT-4	-7.25	-5.49
SR	-7.75	-5.53
NSC Lung Cancer A549/ATCC	-6.61	-5.46
HOP-62	-6.33	-4.89
NCI-H522	-6.78	-5.30
Colon Cancer COLO-205	-6.55	-4.85
HT-29	-6.61	-4.27
CNS Cancer SF-295	-7.02	-4.81
SNB-19	-6.70	-4.96
U251	-6.79	-4.84
Melanoma LOX IMVI	-4.69	-5.48
M14	-7.12	-6.91
UACC-257	-4.99	-4.81
Ovarian Cancer IGROV1	-6.57	-5.39
Renal Cancer 786-0	-6.86	-5.11
Prostate Cancer PC3	-6.55	-5.00
Breast Cancer MCF7	-7.34	-5.08

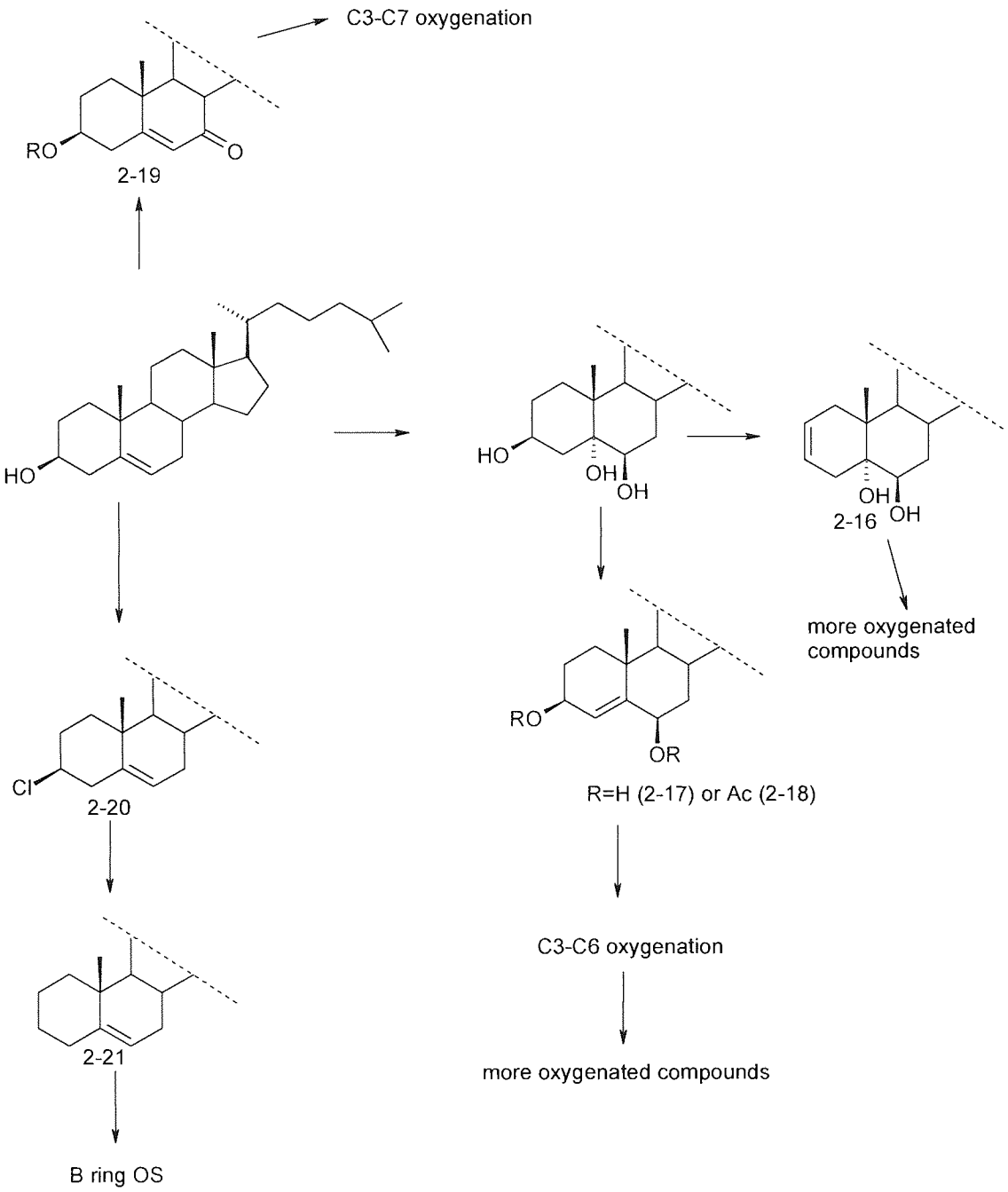
To achieve these goals, first we need to develop a robust synthetic strategy, which can produce a desired product in high yield with low costs, and which can be easily amplified since the synthetic route is long and a big amount of starting materials often is needed.

We started with manipulation of the cholesterol's 3β-OH and 5,6-double bond (**Scheme 2-6**).

CT can be prepared from cholesterol through epoxidation followed by Epoxide ring opening in acidic conditions in a high yield (85%~93%) (Fieser and Rajacopalan 1948). This compound can serve as the starting material as it gives a quantitative yield of 5α-cholest-2-en-5,6β-diol after elimination of its 3-(toluene-4-sulfonate). Cholest-4-en-3β,6β-diol also can also be prepared in a high yield through the elimination of 5a-hydroxyl group in CT's acetates (Elils et al 1939). These two typical compounds with alkene and allyl functional groups open a door for further oxygenation (**Figure 2-1**) to synthesise polyoxygenated OS.

The reduction of cholesteryl chloride with Li in liquid NH_3 gave cholest-5-ene, a starting material for introduction of an oxygenated group on B ring. When sodium was used in ethanol at -50°C, a low yield was obtained, caused by low solubility of the starting material. With increased temperature, more impurities were generated. When the reaction was performed in a mixture of THF and ethanol as the solvent at -50°C , the product yield was over 95%.

7-KC esters can be prepared via the allyl oxidation with DMP/CrO_3. This compound also can serve as the starting material with an additional functional group at C-7.



Scheme 2-6 Intermediates for polyhydroxysterols

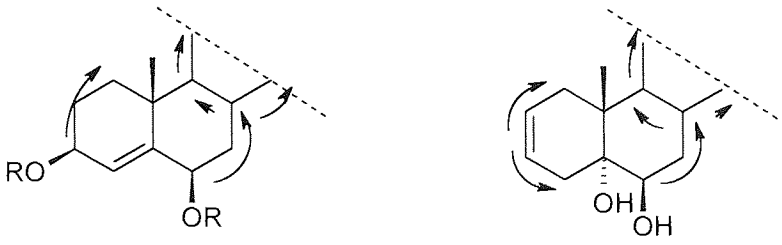


Figure 2-1 Possible direction of hydroxylation

Chapter 3. Cholest-4-ene-3,6-diols: Preparation and epoxidation.

3. Cholest-4-en-3, 6-diols

3.1 Preparation of the four cholest-4-ene-3, 6-diols

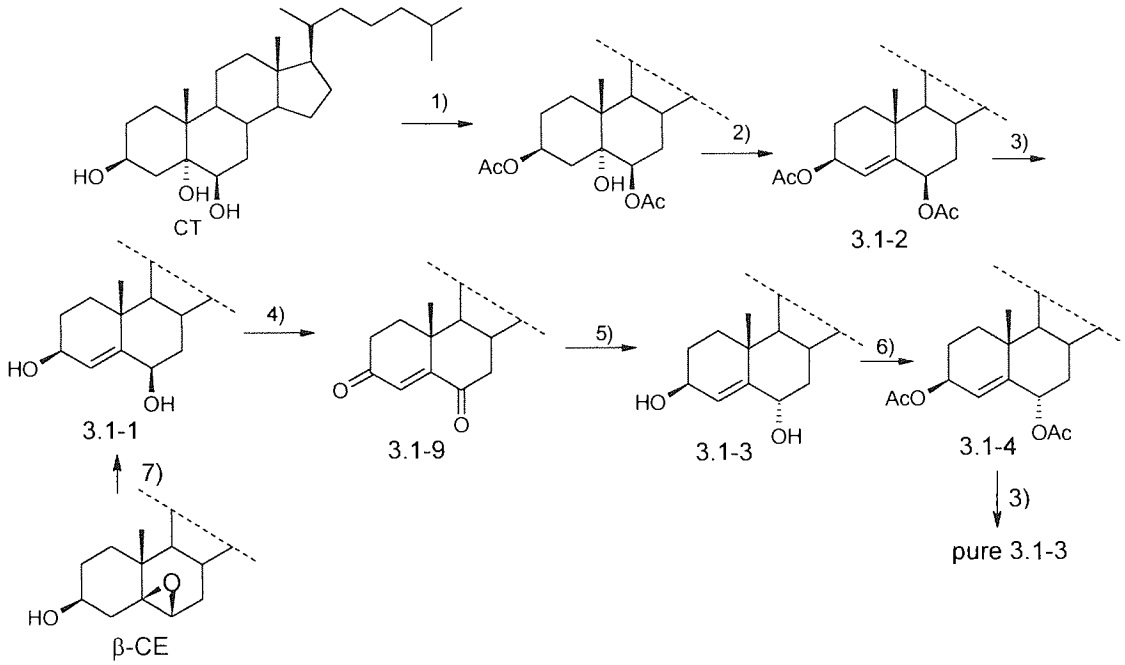
Cholest-4-en-3, 6-diols are the key precursors for synthesis of cholestane-3,4,5,6 tetrols, the prime targets of the project. In literature, syntheses of two 3β-isomers of cholest-4-en-3, 6-diols were described. By adopted literature methods cholest-4-en--3β,6β-diol (3.1-1) and its diacetate (3.1-2) are prepared from CT with ~80% overall yield (Elils et al 1939). The rearrangement of 5,6β-epoxy-5β-cholestan- -3β-ol (β–CE) is a shorter route but the availability of pure β–CE from cholesterol is limited (**Scheme3.1-1**).

The reduction of steroidal 6-one with existence of 4, 5 double bond formed preferably 6α-ol, due to the orbital overlap effect of the double bond when the hydrogen anion attacks the 6-carbon atom (You and Koreeda 1993). In our experiments, 3.1-1 was oxidized to 3, 6-dione (3.1-9), followed by reduction with $NaBH_4$ or $LiAlH_4$ to give the same result, 3β, 6α-diol (3.1-3) as a major product with several minor by-products. The polarity of these compounds is similar and 3.1-3 cannot be separated by common chromatography method. After acetylation, the pure diacetate of cholest-4-en-3β,6α--diol (3.1-4) can be crystallized from methanol, and hydrolysis gave the pure 3.1-3 (**Scheme3.1-1**). The total yield is around 65% from Cholest-4-en-3β,6β-diol.

Another route via the reduction of 3β-acetoxycholest-4-en-6-one (Kocovsky et al 1977) using sodium borohydride give a better total yield of 3β,6α isomer 3.1-3. (~70% from cholesterol) (**Scheme 3.1-2**).

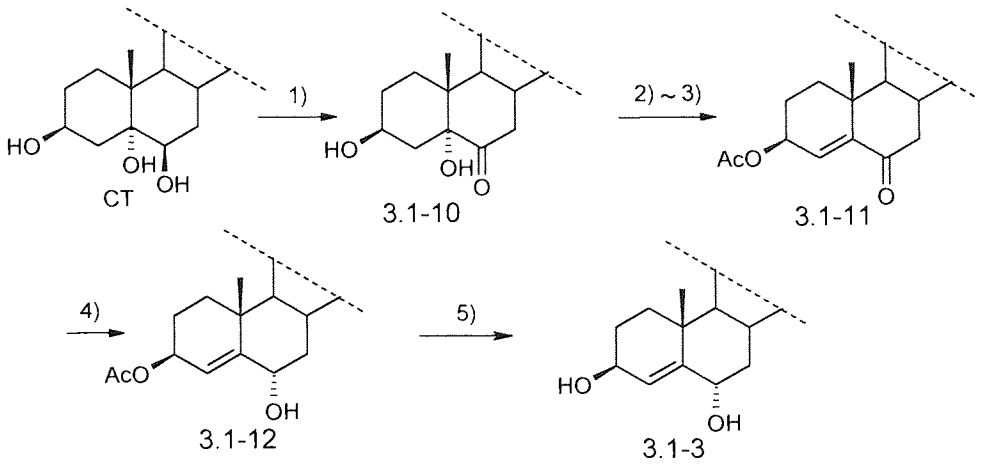
Not like the 3β isomers, there are no feasible preparative methods for the two 3α isomers of cholest-4-en-3,6-diols described in literature. Though a number of approaches to 3α,6β- and 3α,6α-cholest-4-en-3,6-diols were mentioned, each of them is limited by availability of starting material (Ishige and Shiota 1980, Fischli 1982) and low yield (You and Koreeda 1993) for preparation of multigram products economically. It is worth mentioning that 5β,6β- and 4α,5α-epoxy steroidals with 3α-OH refused to rearrange with bases (Holland and Jahangir, 1983). In order to

economically obtain 3α,6β- and 3α,6α-cholest-4-en-3,6-diols in multigram scale, obviously, we need (a) to develop an efficient methodology to establish α configuration of 3-OH, which was done either through multistep (Pattner 1944) or by use of expensive reagents (Lee et al 1994)in the past, (b) to reduce numbers of synthetic steps to achieve a high yield.



1) Ac_2O, Py ; 2)$SOCl_2$, Py ; 3) NaOH/EtOH 4) CrO_3, AcOH;
5) $NaBH_4$ or $LiAlH_4$; 6)Ac_2O, Py; 7) LDA

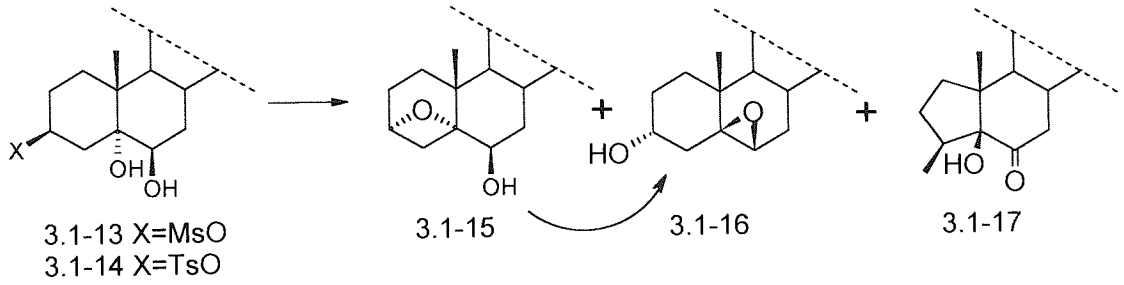
Scheme 3.1-1 Synthesis of 6α-cholest-4-en-3,6-diol 3.1-1 and 3.1-3



1) NBS, ether,MeOH; 2) Ac_2O, Py; 3) $SOCl_2$, Py; 4)$NaBH_4$; 5) NaOH, EtOH, H_2O.

Scheme 3.1-2 Synthesis of 3.1-3 *via* 3β-acetoxycholest-4-en-6-one

When they treated 3β-mesylatecholestan-5α,6β-diol (3.1-13) with potassium *t*-butoxide to make 3,5-oxitane(3.1-15), Tsui and Just (1973) observed a formation of 3α-hydroxy- -5β,6β-epoxycholestane (3.1-16) as a by-product (**Scheme 3.1-3**), obviously which was the subsequent product of 3.1-15. While using TsO as the leaving group, Marples and Spilling (Marples and Spilling 1992) obtained a ring-contracted by-product (3.1-17). We felt if using a relatively inert leaving group such as Cl, this transformation could be exploited to be an effective way to establish α configuration of 3-OH.



3.1-13 X=MsO
3.1-14 X=TsO

3.1-15 3.1-16 3.1-17

Scheme 3.1-3 Strong base catalysed rearrangement of 3.1-13 and 3.1-14

Cholesteryl chloride (2-20) can be converted to cholesterol acetate with the preservation of C3 configuration by reflux with AcOK in AcOH. This is attributed to the homoallyl effect of the 5,6 double bond which prevents the nuclear attack from the down side (**Figure 3.1-1**). Using THF and DCM as solvent at ambient temperature we synthesized 3β-chlorocholestan-5α,6β-diol (3.1-18) at quantitative yield. This compound, without the 5,6-double bond, gave no reaction when refluxed with AcOK in AcOH or DMF. We treated this compound with a variety of basic conditions and isolated several structurally different products as showed in **Table 3.1-1** and **Scheme 3.1-4**. We changed the reaction conditions for 3.1- 4 to sodium hydroxide and ethanol and successfully isolated 5,6β-epoxycholestan-3α-ol 3.1-16 in 80% yield. By this way the product can be precipitated as crystals directly from the reaction mixture without chromatography. Combined with the easy convertion of cholesterol to cholesteryl chloride, to our best knowledge this is the most efficient method for the preparation of this compound from cholesterol.

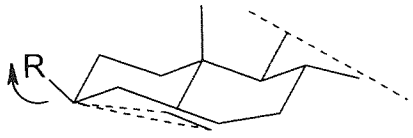
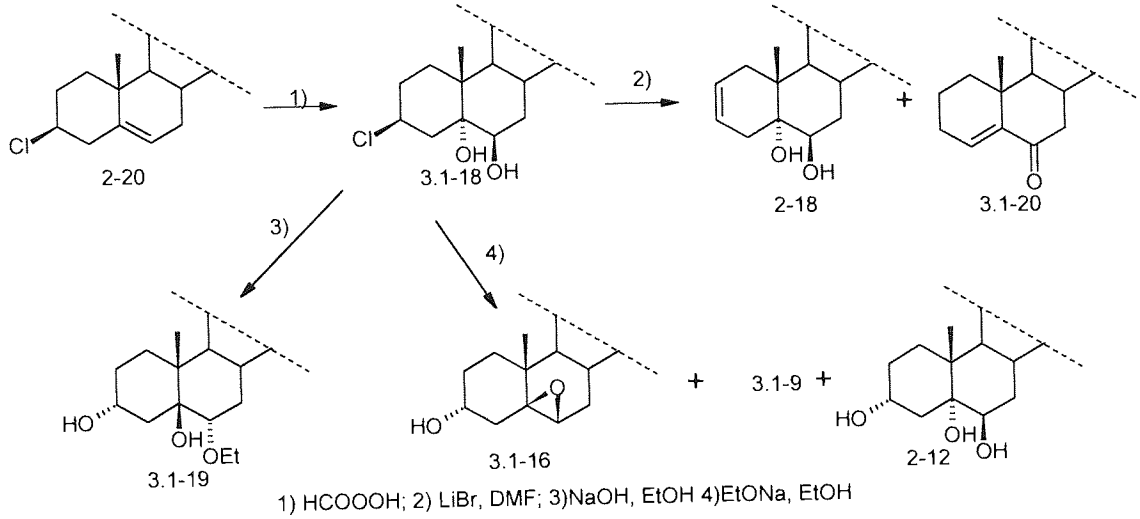


Figure 3.1-1 the homoallyl effect of steroidal 5-ene

Table3.1-1 The reaction of compound 3.1-18 in **Scheme 3.1-4**

Reaction condition	Products
Excess EtONa, in EtOH reflux 2hr	3.1-19 (33%), 3.1-16 (40%), 2-12 (5%)
Excess LiBr and Li_2CO_3 in DMF reflux for 1hr	2-18 (54%), 3.1-20 (11%)
NaOH (30 times excess) in EtOH reflux for 36hr	3.1-19 (77%)
NaOH (30 times excess) in EtOH reflux for 5hr	3.1-19 (25%), 3.1-16 (53%)
NaOH (20 times excess) in EtOH/H2O (2:1, v/v) reflux for 5 hr	3.1-16 (80%)

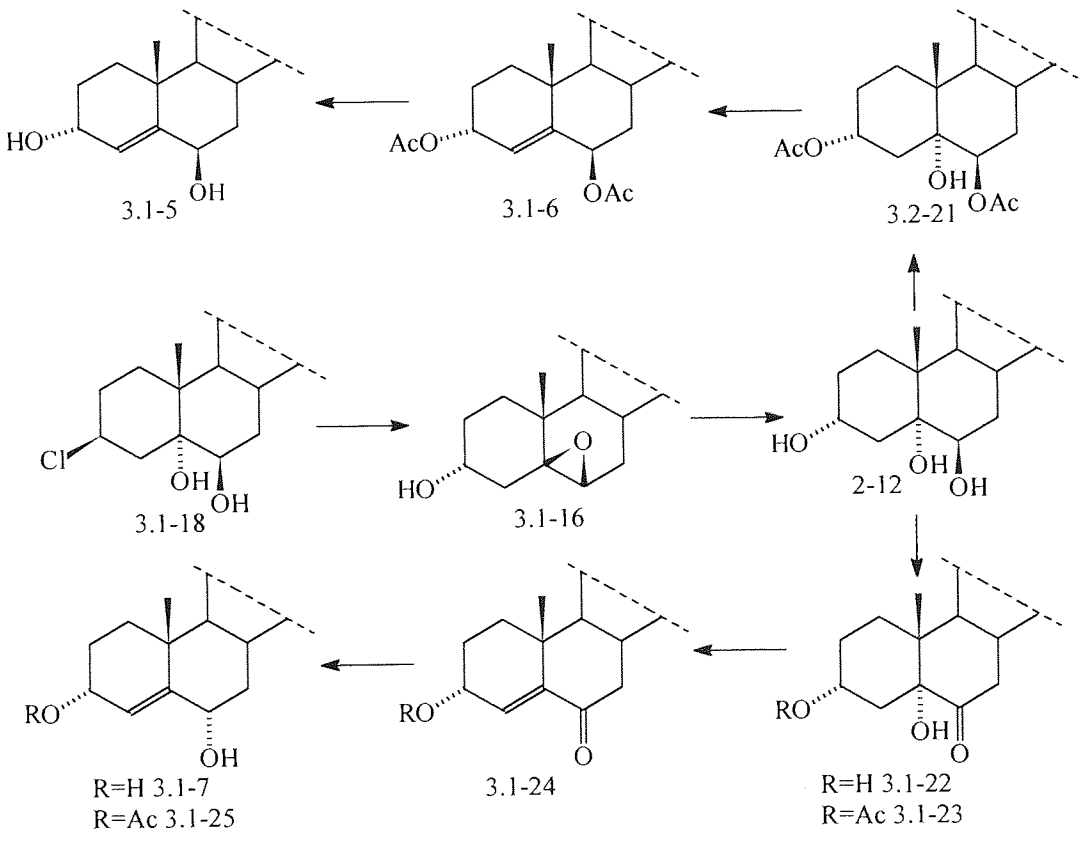


Scheme 3.1-4 The 3β-chloro-5α-cholestane-5,6β-diol 3.1-18 with bases

Under acidic condition, epoxide 3.1-16 was converted to cholestan-3α,5α,6β-triol (2-12) stereospecifically. The less stereo hindered 3α- and 6β-OH were protected with acetic anhydride to give diacetate 3.1-21. The remaining free 5α-OH was eliminated with $SOCl_2$ in pyridine at -5°C, followed by removal of acyl groups with NaOH in

aqueous ethanol to give the desired cholest-4-en-3α,6β-diol 3.1-5 as a single compound in 70% yield.

The α configuration of 6-OH in cholest-4-en-3β, 6α-diol (3.1-7) could be established through an oxidation-reduction process according to literature (You and Koreeda 1993). N-Bromosuccinimide (NBS) was used in oxidation of steriodal axial OH to ketone when both horizontal and axial OH exist (Fisher and Rajagopalan 1949). In case of cholestan-3α,5α,6β-triol (2-12), all three hydroxyls are axial. We found a noticeable difference in speed of oxidation of 3α- and 6β-OH with NBS after numerous attempts. Having taken this advantage, a selective oxidation of 6β-OH into 6-one 3.1-22 with NBS was accomplished successfully in methanol and ether in 90% yield. Selective protection of 3α-OH was followed by the standard elimination with $SOCl_2$ to create 4,5 double bond. $NaBH_4$ reduction of 6-one afforded 6α-OH compound 3.1-25 stereospecifically. After removal of 3-acetyl with NaOH in aqueous ethanol, cholest-4-en-3α,6α-diol 3.1-7 was obtained in 65% overall yield from 2-12 (**Scheme 3.1-5**).



Scheme 3.1-5. Synthetic sequence to 3.1-5 and 3.1-7

Mechanisms of oxidation and reduction reactions are often quite hard to interpret, as is that of the NBS oxidation. The NBS oxidation is often accelerated by ultraviolet or even visible light as that in allyl bromation, providing evidence in support of a radical pathway. But in the oxidation of alcohols, the ionic pathway is more often suggested and the hypobromite (i) or halohydrine (ii) are possible intermediates (**Figure 3.1-2**) (Filler 1963). Cossy and Furet reported in 1995 that NBS oxidation of cyclopropylcarbinols is most likely to go through the hypobromite pathway; the result was supported by free radical trap and isotopic rate ratio (k_H/k_D) experiments (Cossy and Furet 1995).

It seems that the congested 6β-OH in 2-12 is not a favoured site for the bromine to attack. Preliminary tests were carried with CT in aqueous THF and the results shows that: 1) acid (pH=1) catalyses the reaction and it takes 8~10 minutes to be finished at room temperature; while a reaction without an acid finished in 15 min with heating at 40°C, and the resulting mixture is acidic suggesting the hydrobromic acid generated *in situ* served as the catalyst; 2) when all the three hydroxyl groups are deuterated, the reaction is faster in the first 3 minutes, but the completion time is almost same as the reaction with CT. Therefore it is hard to suggest which of i or ii is the intermediate in this case.

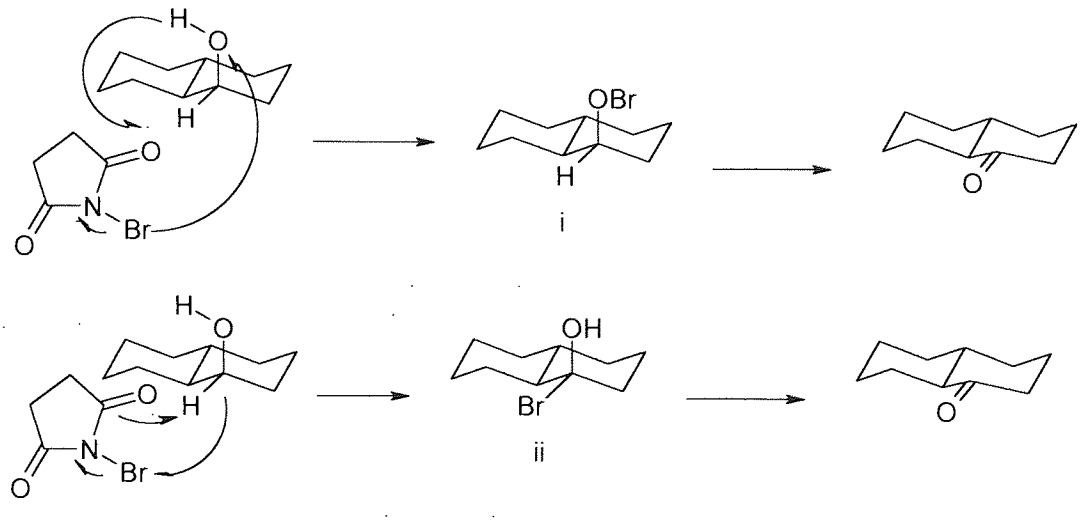


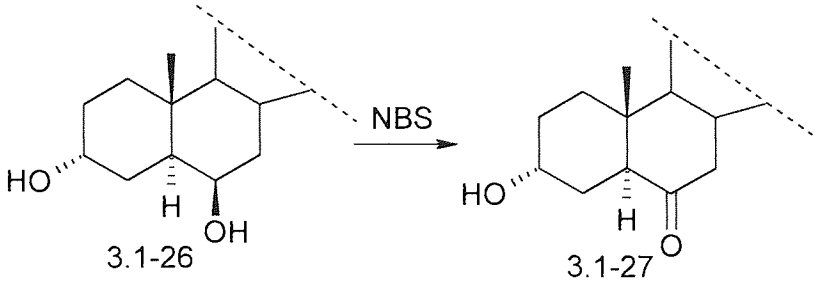
Figure 3.1-2 The ionic mechanism of NBS oxidation

Velgova et al (1969) reported that the NBS oxidation of 5α–cholestan-3,6-diol to 3-hydroxyl-6-one in 1,4-dioxane under similar conditions was complete within 15 min .

When we treated the 3α isomer (3.1-26) in 1,4-dioxane or ether / methanol (**Scheme 3.1-6),** a quite different result was obtained. Reaction with 4 molar excess NBS after 2days gave the 6-one (3.1-27) in 55% yield, mixed with the starting material and several minor impurities. As demonstrated by several repeated reactions, the reaction is faster at the beginning and seems to slow down or stop at a later stage. It was speculated that possibly the succinimide, as the major by-product, retards the reaction or interferes with the reaction progress. In order to prove this speculation a new condition was designed for the reaction. This time the reaction was performed in water / ether bi-layer system, therefore, the water soluble by-product succinimide will be transferred into the water layer once it has been formed. An interesting result was afforded as shown in **Table 3.1-2**. The 3β-hydroxyl group showed stronger steric effect, so the hypobromite (i) more likely is the intermediate. All reactions gave 6-ones only, confirmed by ^{1}H NMR of the resulting mixtures. It seems NBS oxidation deserves theoretical study on a variety of polyhydroxyl systems to make it to be a useful stereoselective oxidation method for the polyhydroxyl sterol synthesis.

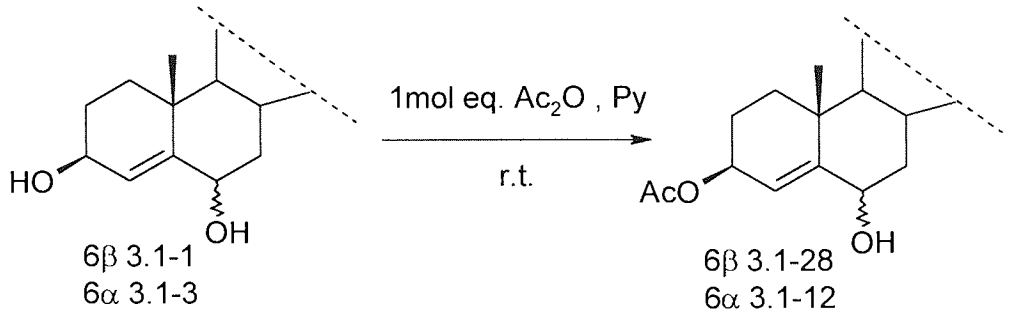
Table 3.1-2 The NBS reaction speed tests

Starting material	Reaction time (min)
5α–Cholestane-3β,5,6β-triol (CT)	14
5α–Cholestane-3β, 6β-diol	11
5α–Cholestane-3α,5,6β-triol (2-12)	8
5α–Cholestane-3α, 6β-diol (3.1-26)	5

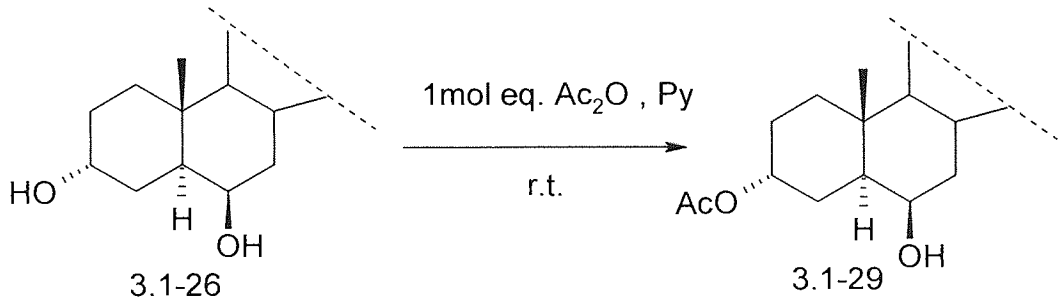


Scheme 3.1-6 The NBS oxidation of 3.1-26

The monoesters of these diols are prepared, besides the 3-monoesters of two 6α isomers (3.1-12, 3.1-25) which could be prepared from reduction of 6-one; the 3β-acetoxycholest-4-en- -6β-ol (3.1-28) is also available by acetylation in pyridine with equal molar acetic anhydride. 3.1-12 was afforded from the diol 3.1-3 using the same condition (Scheme 3.1-7). When the same condition was applied on the 3α isomer 3.1-5, a mixture was produced. The ratio of 3- and 6- monoesters is 1:1 as there is no selectivity from the two hydroxy groups, the 3α, 6α – diol 3.1-7 acts as the same with 3.1-5. However, when the double bond in 3.1-5 was saturated (compound 3.1-26), the selective 3-acetylation happened (**Scheme 3.1-8**).



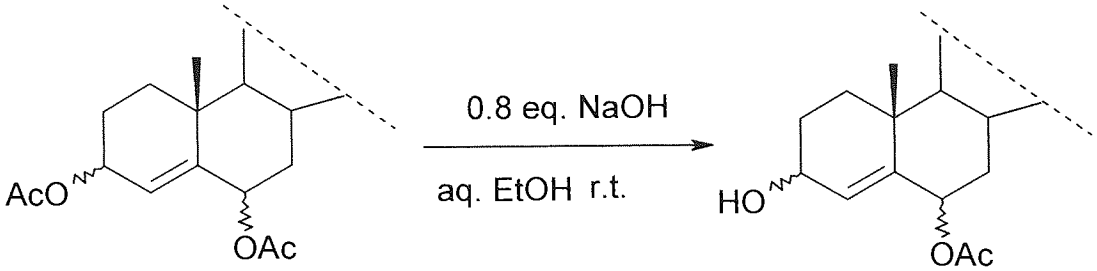
Scheme 3.1-7 Preparation of the 3-monoesters by acetylation



Scheme 3.1-8 Preparation of the 3-acetate 3.1-29 from 3.1-26

The diesters of these compounds were subjected to hydrolysis with 0.8 equal molar sodium hydroxide in aqueous ethanol; only compound with 6β-configuration gave the 6-monoesters as the main product (**Scheme 3.1-9**). The cholest-4-en-3β, 6α-diol diacetate (3.1-4) also gave 6-monoacetate 3.1-32 with more than 10% free diol 3.1-3.

Cholest-4-en-3α,6α-diol diacetate (3.1-8) only give the free diol 3.1-7 with some remaining starting materials.



3β,6β 3.1-2
3α,6β 3.1-6
3β,6α 3.1-4

3β,6β 3.1-30
3α,6β 3.1-31
3β,6α 3.1-32

Scheme 3.1-9 Preparation of the 6-monoesters by hydrolysis

3.2 Epoxidation of the 4,5-double bonds

After developing efficient preparative syntheses for cholest-4-en-3β,6β-; -3β,6α-; -3α,6β- and -3α,6α-diols (3.1-1, 3.1-3, 3.1-5 and 3.1-7) (**Table 3.2-1**), studies of the possible stereocontrolled epoxidations to gain the eight diastereomerically pure epoxides in gram scales are considered. These epoxides, 4α,5-epoxy-5α-cholestane--3β,6β-diol (3.2-1), 4β,5-epoxy-5β-cholestane-3β,6β-diol (3.2-2), 4α,5-epoxy-5α--cholestane-3β,6α-diol (3.2-3), 4β,5-epoxy-5β-cholestane-3β,6α-diol (3.2-4), 4α,5--epoxy-5α-cholestane-3α,6β-diol (3.2-5), 4β,5-epoxy-5β-cholestane-3α,6β-diol (3.2-6), 4α,5-epoxy-5α-cholestane-3α,6α-diol (3.2-7) and 4β,5-epoxy-5β-cholestane--3α,6α-diol (3.2-8) are important precursors in the stereoselective syntheses for study of oxysterols with hydroxyl groups on C-3 ~ C-6. The most frequent oxygenation occurs on steroidal A and B rings in natural products.

The syn-directive effect of the hydroxyl of allylic and alicyclic alcohols is well known (Rossiter et al 1979), and disappearance of this effect after alkylation to ethers (Morrison and Wilkinson 1990) or acylation to esters is also well documented (Bartlett 1950). In contrast to allylic alcohols, there have only been sporadic reports of epoxidations of bis-allylic and bis-alicyclic alcohols with examples of *cis* or *trans* selectivity. Rosenheim and Starling (1973) reported epoxidation of

3β, 6β-dihydroxy-cholest- -4-ene (3.1-1) with perbenzoic acid, showing that the *syn* directing effect of the hydroxyls is strong enough to drive the epoxidation to occur predominantly from the β-side. Later, similar findings were also reported by other groups (Henbest and Wilson 1957, Huang et al 1994). However, no further systematic investigation on the co-ordination effect of di-hydroxyl groups in bis-allylic and bis-alicyclic alcohol systems, as well as effects of hydroxyl protecting groups and oxidants on the stereocontrol of such epoxidations has been reported. We investigated the effects on the epoxidations of bis-alicyclic alcohols 3.1-1, 3.1-3, 3.1-5 and 3.1-7 and their acylated derivatives to gain the targets.

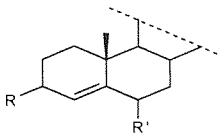
The monoacetates 3.1-28, 3.1-31, 3.1-32, 3.1-33, 3.1-12 and 3.1-25 were prepared as described above. The benzoyl esters 3.2-9 ~ 3.2-12 and acetyl benzoyl esters 3.2-13, 3.2-14 were prepared by reaction of benzoyl chloride with appropriate diols and their monoesters in pyridine at room temperature.

Three typical epoxidation conditions, mCPBA (Paquette and Barrett 1973), $VO(acac)_2$ / TBHP (Sharpless 1973) and Sharpless method (Katsuki and Sharpless 1980), have been investigated with these cholest-4-en-diols, their diacetates, benzoates and acetate-benzoates listed above. The results are summarised in **Table 3.2-1** and **Table 3.2-2**.

In this particular group of substrates, mCPBA showed superior reactivity to $VO(acac)_2$/TBHP and Sharpless conditions and exerted satisfactory stereoselectivity. mCPBA affected all substrates and gave good to excellent yields. The reaction rates with di-acylated substrates 3.1-2, 3.1-4, 3.1-6, 3.1-8 and 3.2-9~3.2-14 were dramatically decreased, because of increased steric-hindrance and lack of co-ordination between mCPBA and free hydroxyl groups. $VO(acac)_2$ / TBHP generally gave a better stereoselectivity than mCPBA with the substrates bearing one or two free hydroxyl groups, 3.1-1, 3.1-3, 3.1-5, 3.1-7 3.1-28, 3.1-31, 3.1-32, 3.1-33, 3.1-12 and 3.1-25, but failed to react with di-acylated substrates 3.1-2, 3.1-4, 3.1-6, 3.1-8 and 3.2-9~3.2-14. This demonstrates the importance of the co-ordination between the oxidant and the OH in $VO(acac)_2$ / TBHP epoxidation. Diol 3.1-5 with two axial hydroxyl groups reacts

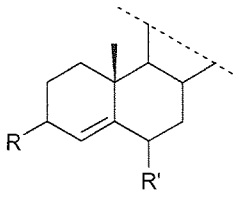
faster than the others despite the increased steric hindrance effects showed on acetylation with these hydroxyl groups in section 3.1 (**Scheme 3.1-7 and 3.1-9**).

Table 3.2-1 The summary of the results of the steroidal 4-ene epoxidations



	3R	6R'	MCPBA α/β Ratio[c]	MCPBA Yield[b] (%)	MCPBA Time (h)	$VO(acac)_2$ / TBHP α/β Ratio[c]	$VO(acac)_2$ / TBHP Yield[b] (%)	$VO(acac)_2$ / TBHP Time (h)
3.1-1	β-OH	β-OH	0:100	98	2	0:100	95	3
3.1-28	β-OAc	β-OH	7:93	94	4	0:100	90	5
3.1-31	β-OH	β-OAc	2:98	94	4	0:100	90	5
3.1-2	β-OAc	β-OAc	82:18	71	288	-[d]		
3.2-9	β-OBz	β-OBz	75:25	75	96	-[d]		
3.1-5	α-OH	β-OH	38:62	70	1	33:67	93	0.5
3.1-32	α-OH	β-OAc	91:9	84	5	100:0	90	1
3.1-6	α-OAc	β-OAc	76:34	73	120	-[d]		
3.2-11	α-OBz	β-OBz	50:50	80	48	-[d]		
3.1-3	β-OH	α-OH	12:88	69	4	0:100	98	2
3.1-12	β-OAc	α-OH	42:58	88	11	13:87	82	5
3.1-33	β-OH	α-OAc	2:98	78	11	0:100	86	5
3.1-4	β-OAc	α-OAc	73:27	74	120	-[d]		
3.2-13	β-OAc	α-OBz	95:5	77	48	-[d]		
3.2-10	β-OBz	α-OBz	87:13	81	48	-[d]		
3.1-7	α-OH	α-OH	100:0	98	2	100:0	82	3
3.1-25	α-OAc	α-OH	27:73	75	4	0:100	95	5
3.1-8	α-OAc	α-OAc	25:75	80	72	-[d]		
3.2-14	α-OAc	α-OBz	52:48	83	48	-[d]		
3.2-12	α-OBz	α-OBz	75:25	84	48	-[d]		

Table 3.2-2 The summary of the further test with Sharpless method

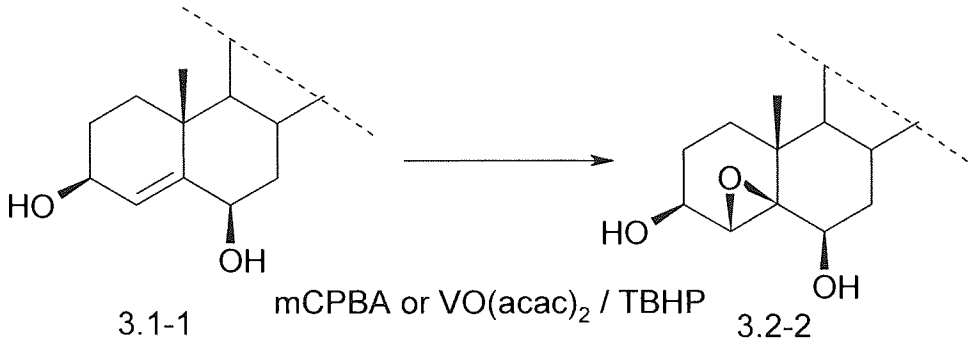
			Ti(OiPr)4/TBHP/(−)DET			Ti(OiPr)4/TBHP/(+)DET		
	3R	6R'	α/β Ratio[c]	Yield[b] (%)	Time (h)	α/β Ratio[c]	Yield[b] (%)	Time (h)
3.1-1	β-OH	β-OH	–[d]			–[d]		
3.1-5	α-OH	β-OH	–[d]			–[d]		
3.1-3	β-OH	α-OH	0:100	52	12	94:6	60	12
3.1-12	β-OAc	α-OH	–[d]			–[d]		
3.1-7	α-OH	α-OH	100:0	43	12	100:0	55	12
3.1-25	α-OAc	α-OH	–[d]			–[d]		

For both table 3.1-1 and 3.1-2: [a] Epoxidations were performed using the standard protocols; [b] yields of a+b; [c] Calculated from 1H NMR spectra; [d] No reaction after 72 hours.

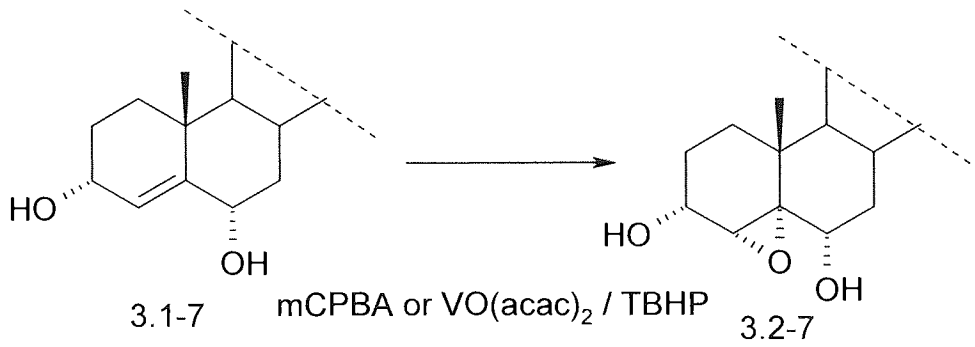
Sharpless conditions affected diols with 6α configuration (3.1-3 and 3.1-7) only. The reactions are slower than that of $VO(acac)_2$/TBHP and with lower yields, possibly due to steric hindrance of the steroidal skeletons blocking approach of the complex oxidant to the double bond. The 6β-hydroxyl group may exert a crucial blocking effect rather than facilitating the reaction as 3.1-1 and 3.1-5 did not react. The 6α-hydroxyl group also does not participate in the reactions as the blocking of the 3-hydroxyl group in 3.1-3 and 3.1-7 inhibited the reaction.

When the 3- and 6-OH are in the same configuration β or α, 3.1-1 and 3.1-7, mCPBA and $VO(acac)_2$/TBHP afforded 100% *cis*-epoxidation products, resulting from an enhanced *syn* directing effect (**Scheme 3.2-1** and **Scheme 3.2-2**). The result implies that the two OH, in the same configuration, are involved in anchoring the active oxygen of mCPBA or $VO(acac)_2$/TBHP to approach the C-C=C plane from one side, otherwise there should be less *cis*-epoxidation because of the increased *cis*-side congestion. When the 3- and 6-OH are in the opposite configuration, 3.1-3 and 3.1-5, the outcomes of epoxidations with all three oxidants took us by surprise because there is a clear stereoselectivity. 3.1-5 furnished a mixture of α-isomer: β-isomer 1:2 with both

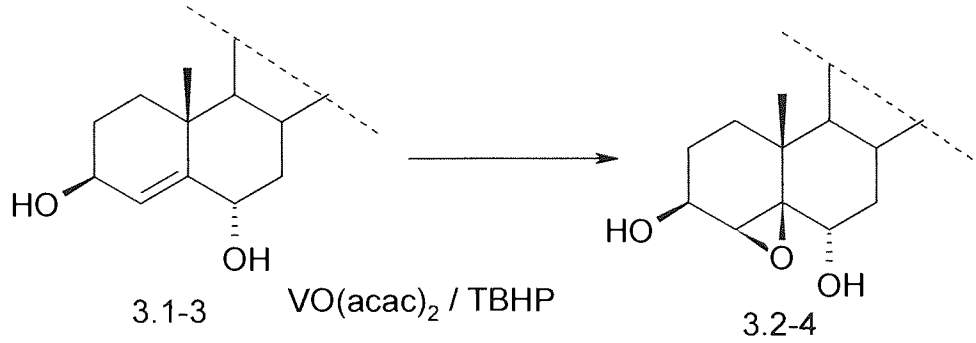
mCPBA and $VO(acac)_2$/TBHP; while 3.1-3 gave rise to 88% β-isomer with mCPBA and 100% with $VO(acac)_2$/TBHP (**Scheme 3.2-3**). These results indicate that the α configuration OH exerts less *syn* directive effect than the β one. The 6α-OH in particular exerts minimal influence. This is reminiscent of Lavie's findings that both 4α-hydroxycholest-5-ene and 6β-hydroxycholest-4-ene underwent *cis*-epoxidation with a peracid, but 6α-hydroxycholest-4-ene gave a mixture of products (Lavie et al 1966, Greenfield et al 1967).



Scheme 3.2-1 Epoxidation of 3.1-1



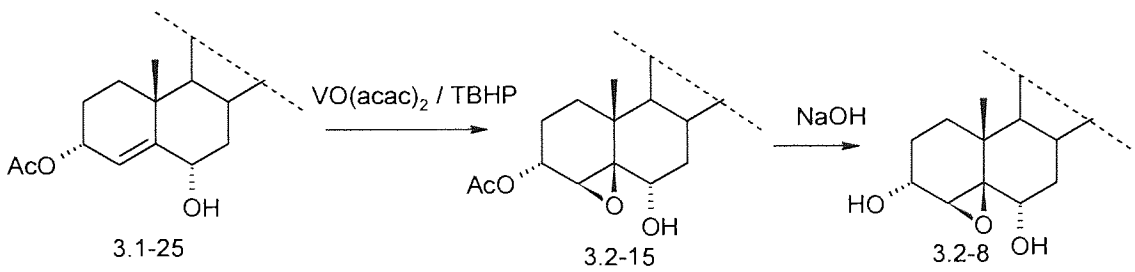
Scheme 3.2-2 Epoxidation of 3.1-7



Scheme 3.2-3 Epoxidation of 3.1-3

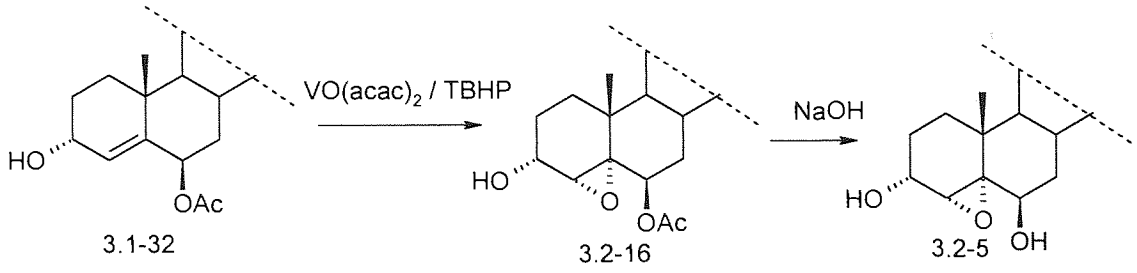
It has been observed that acylation of the OH in alicyclic alcohols, cyclohexenol (Henbest and Wilson 1957) and oxysteroids (Greenfield et al 1967), resulted in a change in orientation of peracid epoxidations from *cis*-stereoselection to *trans*. This is generally attributed to steric hindrance created on the normally favoured *cis*-side by the acetate group and to prevention of the coordination between the oxidant and the hydroxyl group of the substrate (Katsuki and Sharpless 1980).

In this study we found the effect of acylation is much more complicated. When 3- and 6-hydroxyl groups are β configuration, acylation of one of them with Ac, 3.1-28 and 3.1-31, only reduced mCPBA stereoselectivity slightly. However, when 3 and 6-hydroxyl groups are both α configured, reaction of a monoacylated substrate 3.1-25 with $VO(acac)_2$ /TBHP surprisingly led to a total reorientation of stereoselectivity from α-side to β-side, furnishing a single β-isomer (**Scheme 3.2-4**). In the cases of 3α and 6β-hydroxyl substrates, after acylation of the 6β-OH, e.g. 3.1-32, the 3α-OH directed *cis*-epoxidation product dominated, producing 91% of the α-epoxide with mCPBA and 100% with $VO(acac)_2$/TBHP (**Scheme 3.2-5**). In contrast, in the substrates with the 3β and 6α-OH, acylation of the 3β-OH (3.1-12), failed to make α-side epoxidation predominant. Again, this demonstrates the 6α-OH exerts little α-side directive effect. Conversely, with the 6α-O-acylated substrate 3.1-33, the β-side epoxidation was predominant under the 3β-OH directing effect.



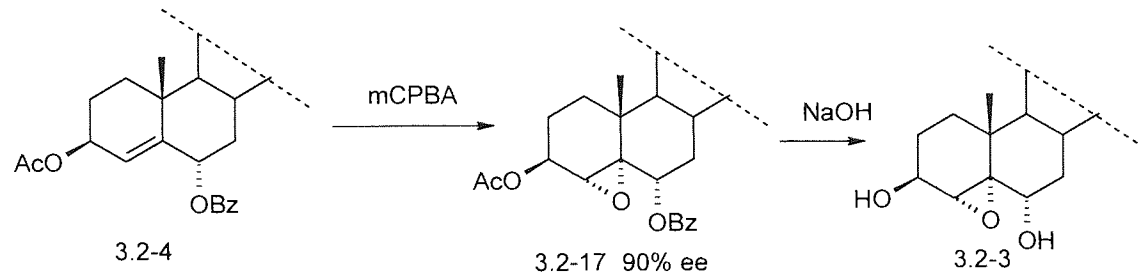
Scheme 3.2-4 Preparation of epoxide 3.2-8

By and large, when both 3- and 6-OH were acetylated, epoxidation occurred selectively from the opposite side to that of its free hydroxy congener, such as the epoxidations of 3.1-2, 3.1-4, 3.1-6 and 3.1-8, which were opposite to that of 3.1-1, 3.1-3, 3.1-5 and 3.1-7.



Scheme 3.2-5 Preparation of epoxide 3.2-5

The replacement of the acetyl group with the benzoyl (Bz) group was carried out and the effect on the epoxidation by mCPBA was investigated with an expectation to maximise the trend of reorientation of the epoxidations. The experiments showed Bz apparently exerted less reorientation effect than Ac, e.g.3.2-9, 3.2-12 furnished less of the stereoselectively re-oriented epoxides compared with 3.1-2 and 3.1-8. Surprisingly, mix-acylation of the 3- and 6-OH with Ac and Bz in compound 3.2-13 resulted in high levels of the stereoselectivity re-oriented epoxides, afforded almost a single isomer with mCPBA (**Scheme 3.2-6**).

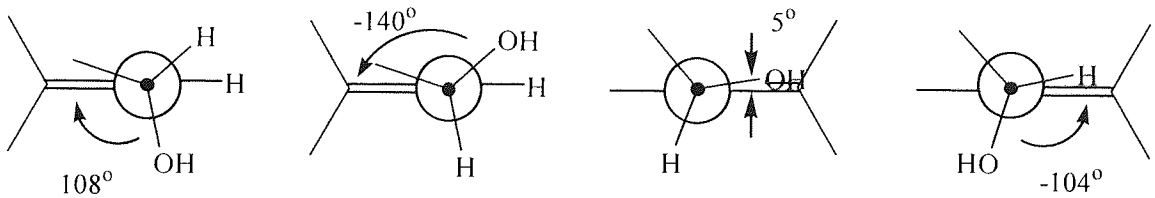


Scheme 3.2-6 Preparation of epoxide 3.2-3

The importance of transition state geometries of allylic (Rossiter et al 1979) and alicyclic alcohols (Itoh et al 1979), torsion (dihedral) angle of O-C-C=C, in determination of orientations of epoxidations has been demonstrated with peracids (Chamberlin et al 1970) and $VO(acac)_2$/TBHP (Rossiter et al 1979). Chamberlin et al first proposed a transition state geometry for peracid epoxidations of alicyclic alcohols. In his cyclohexenol model, the optimal arrangement for a higher *cis*-stereoselectivity and a faster reaction is that the OH occupies pseudo-equatorial conformation, with a torsion angle at about 150°. Later with the same group of compounds Dehnel and Whitham (1979) found a pseudo-axial OH, with a torsion angle at about 90°,

responsible for $VO(acac)_2$/TBHP high *cis*-epoxidation and a rapid reaction. A number of epoxidations of an allylic fragment within steroids have been reported, some of which were clearly *cis*-stereoselective, while some were not.

We calculated torsion angles of $O\text{-}C_3\text{-}C_4\text{=}C_5$ and $O\text{-}C_6\text{-}C_5\text{=}C_4$ in the substrates 3.1-1, 3.1-3, 3.1-5, 3.1-7 and some of their acylated derivatives (**Figure 3.2-1**) by semi-empirical method AM1. The torsion angle of the 3α-OH is about 108°, while 3β is about −140° (on opposite side). The torsion angle of the 6α–OH is very small about 5°, implying four atoms $O\text{-}C_6\text{-}C_5\text{=}C_4$ are almost on the same plane; while 6β is about -105°. Mono- or di-acylation has little effect on the torsion angles. None of these torsion angles matches to those for *cis*-stereoselectivity proposed above. However, the answer for the 6α-OH deviant behaviour is clear. Because the oxygen of the 6α-OH is in the same plane with the $C_6\text{-}C_5\text{=}C_4$ fragment, as a result, its transition state geometry is out of line with all speculations. Still, its predominant *trans*-directive effect in the $VO(acac)_2$/TBHP epoxidations of compound 3.1-12 and 3.1-25 remains a question, because, in this case, the transition state geometry is perfect for oxidation of an alicyclic alcohol into its enone form according to Ternaishi's model (Itoh et al 1979). The dominant *cis*-directive effect of the 3α, 3β and 6β-OH in the epoxidations of this group of substrates with mCPBA and $VO(acac)_2$/TBHP means the torsion angles of their transition state geometry are not that restrictive.

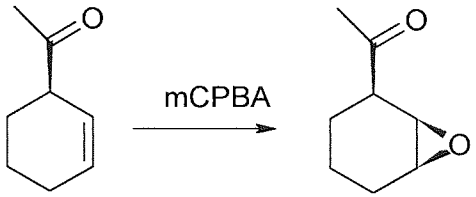


3α-, O-C3-C4=C5 3β-, O-C3-C4=C5 6α-, O-C6=C5-C4 6β-, O-C6=C5-C4

Figure 3.2-1 The calculated torsion angle of cholest-4-en-3,6-diols

There are more questions on the mCPBA epoxidation with these rigid systems. The effects of allyl ester groups cannot be totally explained by steric interaction, especially the 6α-group. Reaction of the 3β-hydroxyl compounds 3.1-3 and 3.1-33 shows the acetylation of 6α-OH give rise to more β-epoxide; while when the 3β-acetoxy group exists as in compound 3.1-12 and 3.1-4, acetylation of 6α-OH significantly increased

the portion of α-epoxide. The ketone directed mCPBA epoxidation reported by Armstrong et al (1994) shows that not only hydroxyl groups have co-ordination effects on epoxidation (**Scheme 3.2-7**). The reaction examples listed in **Table 3.2-1** and **Table 3.2-2** are not enough to explain whether the possible co-ordination of ester groups is also a factor beside the steric effects, and the influence of difference hydroxyl groups, as they have different torsion angles, on these effects. More examples on different esters are needed to develop more subtle models. Some of them are in the following sections.



Scheme 3.2-7 Ketone directed epoxidation

A number of other epoxidation conditions, e.g. chloral hydrate plus hydrogen peroxide (Kasch 1996) and dioxirane plus oxone-acetone (Scott and Wu 1997), have been tested on this bis-alicyclic alcohol system, but none of them is effective.

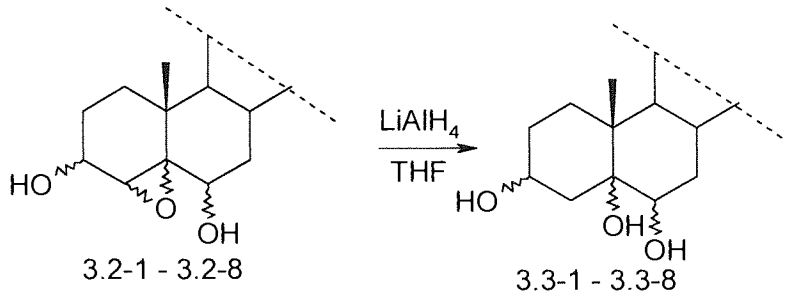
In summary, the eight target epoxides (3.2-1~3.2-8) have been synthesised in diastereomerically pure form: four from the free alcohols: 3.1-1, 3.1-32, 3.1-3, 3.1-7, two from monoacetate 3.1-25 and diacetate 3.2-12. The remaining two were made indirectly, 4α,5-epoxy-5α-cholest-3β,6β-diol (3.2-1) from 3.1-2 after recrystallisation from methanol followed by hydrolysis, and 4β, 5-epoxy-5β-cholest-3α,6β-diol (3.2-6) from the crude product of 3.1-5 *via* di-acetylation, recrystallysation and deprotection. The results show, in this particular group of bis-alicyclic steroidal alcohols, the 3α, 3β and 6β-OH exert *syn* directive effect with mCPBA and $VO(acac)_2$/TBHP, and the enhanced *syn* directive effect was observed with two OH in the same configuration. In directing orientations of epoxidations, the 6α-OH behaves differently, *trans*-directing with $VO(acac)_2$/TBHP in particular, e.g.3.1-12 and 3.1-25. The study shows that by manipulating the acylation of the hydroxyl groups, the directing effects of the

substituents can be adjusted and with careful selection of oxidant high stereoselectivity can be achieved in the epoxidation reactions of these steroid substrates.

These eight epoxides (3.2-1~3.2-8) are invaluable starting materials for synthesis of 3,4,5,6-tetrols, 3,4,6-triols and 3,5,6-triols and more important they will serve as an unique platform for a comprehensive study on stereoselective opening or cleavage of oxiranes on steroidal skeleton.

3.3 Cholestane-3,5,6-triols

The cholestane-3,5,6-triols (3.3-1 to 3.3-8) were synthesised from the relevent epoxides by reduction using lithium aluminium hydride in dry THF (**Scheme 3.3-1** and **Table 3.3-1**).



Scheme 3.3-1 Preparation of the eight cholestane-3,5,6-triols

This is the first synthesis of all the eight enantiomers in one time. All the reactions proceed smoothly, as shown in **Table 3.3-1**. The yields are lower when the starting material is bearing 3α-hydroxyl groups due to more impurities; the reason is unknown.

3,6-Diacetates of these triols (3.3-1a – 3.3-8a) were also prepared.

Table 3.3-1 the cholestane-3, 5,6- triols[a]

Starting material	Product	Yield (%)	Reaction time
4α,5-epoxy-5α-cholestan-3β, 6β-diol 3.2-1	5α-Cholestan-3β,5,6 β-triol 3.3-1(CT)	81	8 hr
4β,5-epoxy-5β-cholestan-3β,6 β-diol 3.2-2	5β-Cholestan-3β,5,6β -triol 3.3-2	96	14 hr
4α,5-epoxy-5α-cholestan-3β, 6α-diol 3.2-3	5α-Cholestan-3β,5,6 α-triol 3.3-3	84	10 hr
4β,5-epoxy-5β-cholestan-3β,6 α-diol 3.2-4	5β-Cholestan-3β,5,6 α-triol 3.3-4	89	14 hr
4α,5-epoxy-5α-cholestan-3α, 6β-diol 3.2-5	5α-Cholestan-3α,5,6 β-triol 3.3-5	79	14 hr
4β,5-epoxy-5β-cholestan-3α,6 β-diol 3.2-6	5β-Cholestan-3α,5,6 β-triol 3.3-6	93	20 hr
4α,5-epoxy-5α-cholestan-3α, 6α-diol 3.2-7	5α-Cholestan-3α,5,6 α-triol 3.3-7	68	24 hr
4β,5-epoxy-5β-cholestan-3α,6 α-diol 3.2-8	5β-Cholestan-3α,5,6 α-triol 3.3-8	84	14 hr

[a]Melting points and spectra data in experimental part.

Chapter 4. The chemistry of 4,5-epoxycholestane-3,6-diols and preparation of cholestane-3,4,5,6-tetrols

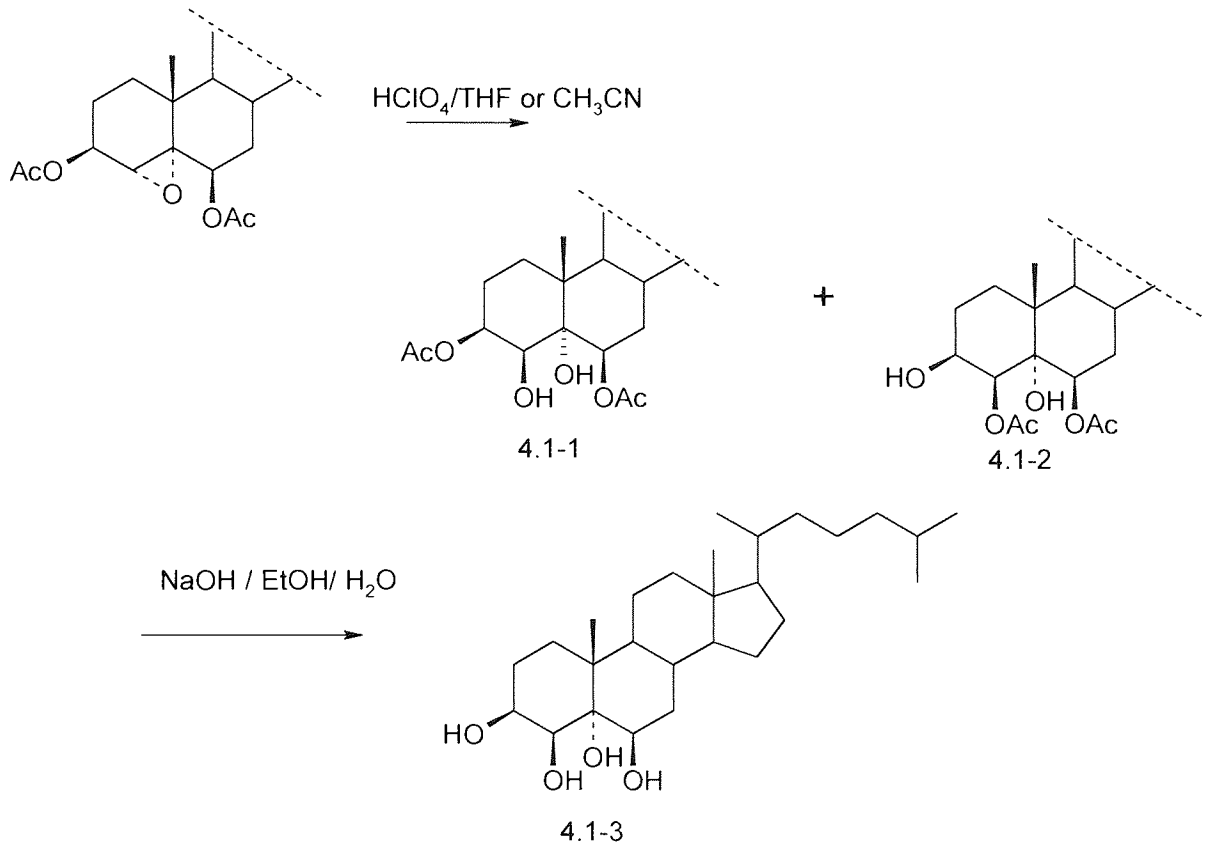
4. The chemistry of 4,5-epoxycholestane-3,6-diols and preparation of cholestane-3,4,5,6-tetrols

It is well known that oxirane rings open to give diols under acidic conditions. And normally a trans-diol is produced, if there is no neighbourhood group participation or solvent effects. It is obvious that substituent groups, hydroxy, acetate or benzoylate at position 3 and 6 of 4,5-epoxycholestane-3,6-diols (3.2-1~3.2-8) will influence the outcome of the oxirane ring openings, weak or strong depending how deeply it is involved in the reaction process. Therefore in order to prepare the desired 3,4,5,6-tetrols, it's wise to test various reaction conditions. Part 4.1 ~ 4.4 describes acid catalysed reactions of these epoxides.

4.1 The 4, 5-epoxycholestane-3β,6β-diol and its acetates

4.1.1 4α,5–Epoxy-5α–cholestane–3β,6β-diol diacetate (3.2-1a)

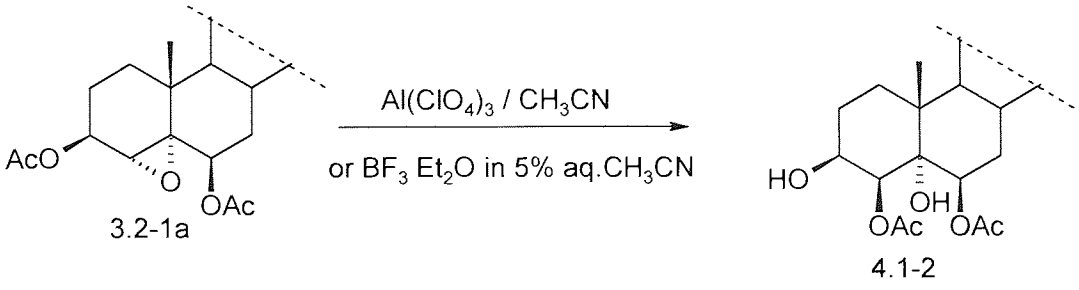
Scheme 4.1-1a describes ring opening reaction of 4α,5–epoxy-5α--cholestane–3β,6β-diol 3,6-diacetate (3.2-1a) under perchloric acid conditions.



Scheme 4.1-1a Epoxide 3.2-1a with perchloric acid

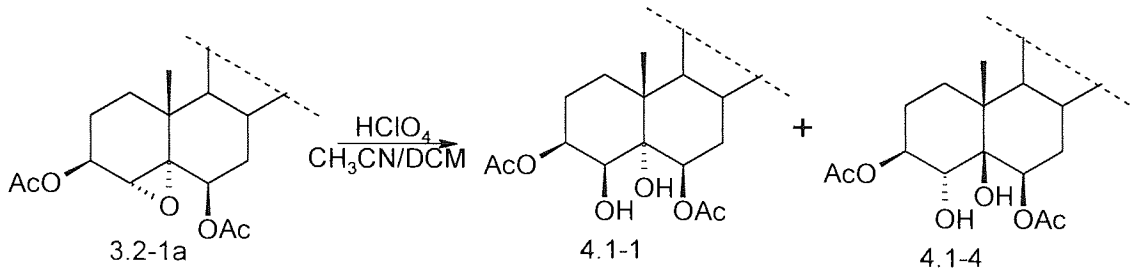
With the same substrate and same acid, perchloric acid, Ishiguro et al (1980) obtained the two diacetates 4.1-1 and 4.1-2 as a 1:2 mixture when THF was used as the solvent. Following this reaction process, first we switched to acetonitrile as the solvent and found acetonitrile accelerated the reaction dramatically. In order to make one product dominate, a careful study of the reaction condition was carried out in acetonitrile. When catalytic amount of perchloric acid (75% aq, 0.1~0.2 molar ratio) and more water (5~10%) was added, the reaction took more than 5 hr to complete. The major product is the 4-acetate 4.1-2 with minor 4.1-1 at about 5%. When more perchloric acid (4 molar excess) was used without additional water the reaction finished in 10 minutes. This time, the 3-acetate 4.1-1 is the only product. It seems that under lower acid concentration with help from water, the C_3-O to C_4-O acetyl migration product of the ring opening predominated, while under concentrated acidic condition with less water, the ring cleavage happened instantly with no opportunity for 3-acetate to migrate. This implies a potential practical strategy for utilizing the 3-acetate in a defined opening of the oxirane as desired. We called these two processes the slow procedure and the fast procedure in the oxirane ring opening, and they will be discussed with other substrates below.

To expend this idea further, aluminium perchlorate was chosen for this ring opening as it generates a much weaker acidic condition. The reactions were carried out in THF and acetonitrile respectively with 10~20 fold excess of aluminium perchlorate. The reaction happened in acetonitrile only and gave 4.1-2 as the only product, but the reaction is very slow (overnight). Because of the low speed of reaction and a big excess of aluminium prechlorate needed, the scale-up of the reaction failed (**Scheme 4.1-1b**).



Scheme 4.1-1b Epoxide 3.2-1a with different acidic conditions in acetonitrile

Under a different combination of the solvent, perchloric acid and acetonitrile/DCM, Narayanan and Landge (1993) furnished the 4.1-1 and a 4α, 5β–isomer 4.1-4 at a 3:1 ratio (3:1) from (5α)-4α,5–epoxycholestane–3β,6β-diol 3,6-diacetate (3.2-1a) (**Scheme 4.1-2**). We repeated this reaction and found this is not a clean reaction with a lot of impurities. However we noticed the 4α, 5β–isomer (4.1-4) is the major product (the ratio of 4.1-1 to 4.1-4 is 1:1.5), not like that they claimed. It seems the reason for introduction of DCM is because the epoxide diacetate 3.2-1a is almost insoluble in acetonitrile. However, we found the solubility virtually is not a problem for this reaction. Because both the starting material and the product have poor solubility in acetonitrile, the reaction goes *via* a fast dissolution and a fast precipitation process when treated with perchloric acid in acetonitrile with solid visible all the time during the reaction. What we need to control is the end of reaction by TLC.

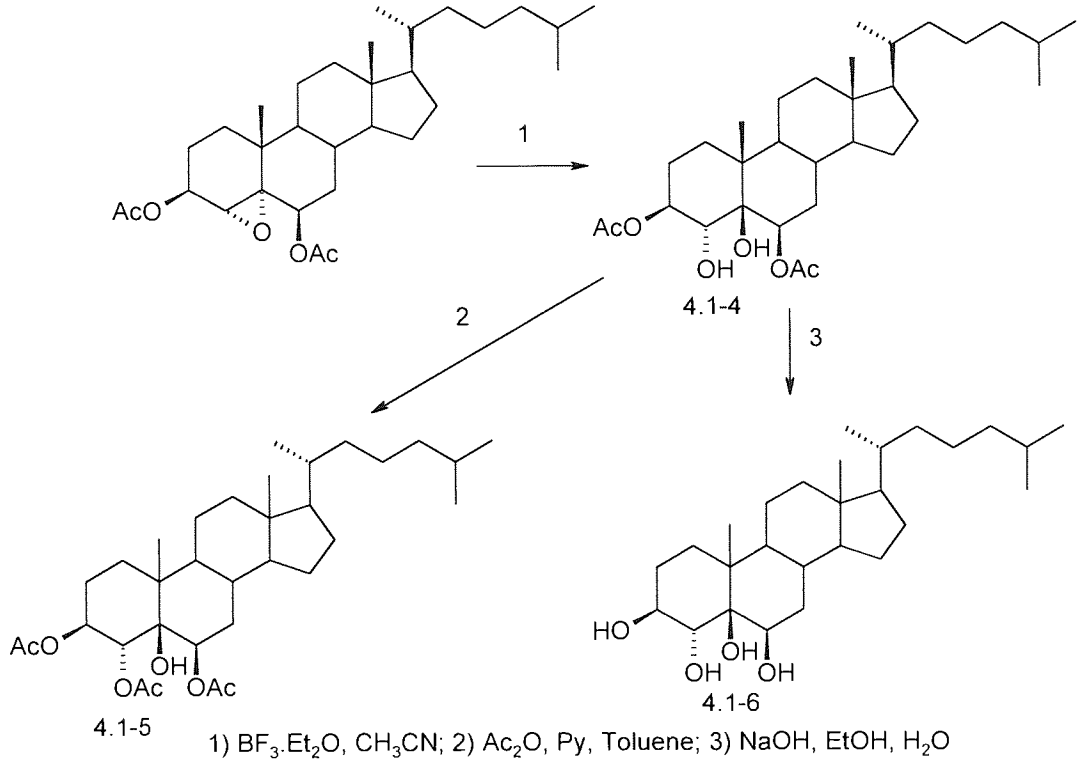


Scheme 4.1-2 Epoxide 3.2-1a with perchloric acid in acetonitrile and DCM

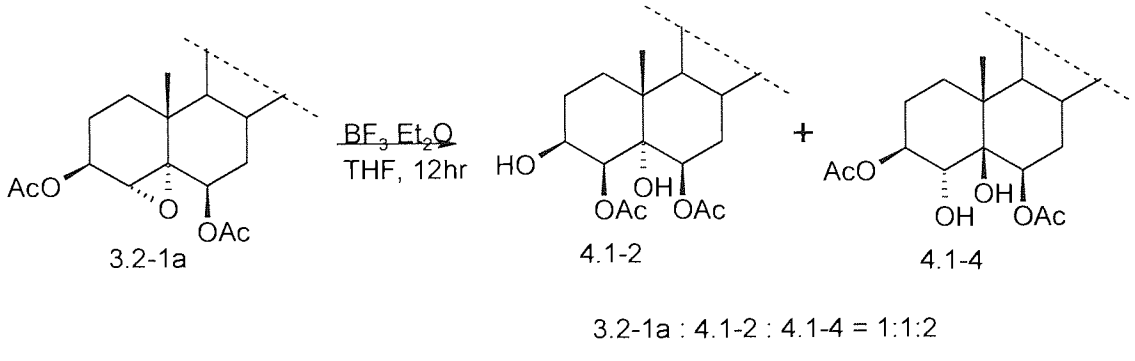
A Lewis acid is not regularly used in epoxide ring openings for the preparation of diols. When we tested its ability in catalytic oxirane ring openings of our bis-allyl acetoxy substrates, we found they acted very differently from a protic acid. For example, when (5α)-4α,5–epoxycholestane–3β,6β-diol 3,6-diacetate (3.1-2a) was treated with boron trifluoride etherate in acetonitrile, a quantitative yield of the 4α, 5β–isomer 4.1-4 (**Scheme 4.1-3**) was obtained within 30 min, however if the reaction was carried out in THF, after 12 hours a mixture was detected: the ratio of the starting material: 4.1-1 : 4.1-4 is 1 : 1 : 2 (**Scheme 4.1-4**).

When we added water in the reaction system, the outcomes were changed greatly: (1) Lewis acid $BF_3{\cdot}Et_2O$ in CH_3CN / water (95/5), overnight, the 4-acetyl compound 4.1-2 (**Scheme 4.1-1b**) was the only product; in aqueous THF, no reactions were observed

after 4 days. This result showed the combination of Lewis acid $BF_3{\cdot}Et_2O$ with water acted like a weak protic acid. This again confirmed the importance of water in facilitating 3-acyl group migration in the neighbouring-group participation of the epoxide ring opening.



Scheme 4.1-3 Epoxide 3.2-1a with $BF_3{\cdot}Et_2O$ in acetonitrile



Scheme 4.1-4 Epoxide 3.2-1a with $BF_3{\cdot}Et_2O$ in THF

In contrast, in the absence of water, the Lewis acid $BF_3{\cdot}Et_2O$ seemed to facilitate 6-acetate participation in the ring opening to give a less favoured A, B ring *cis* fused

steroidal skeleton product. This clearly dominated fashion of the oxirane cleavage was only observed previously when the C_4-O bond in the oxirane was deactivated by C_3-OMe donated inductive effect (Morrison 1990). This observation is controversial to the previous understanding: participation of 3β-acetoxy is faster than that of 6β-acetoxy in this type of ring opening (Ishiguro et al 1980). In referring to the mechanism described for 6β-acetoxy participation when a protic acid is the catalyst, a postulated mechanism for $BF_3{\cdot}Et_2O$ catalysed ring opening is described in **Figure 4.1-1**. However, it is not clear why and how $BF_3{\cdot}Et_2O$ could make energetically less favoured intermediate (b) more stable than the favoured (a) in this reaction process to give the less favoured *cis* product.

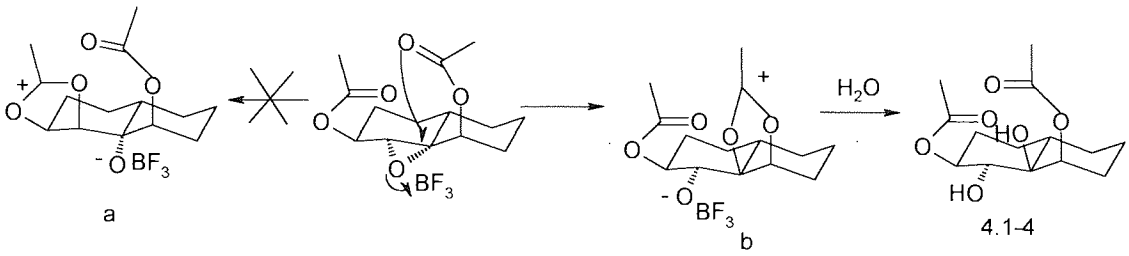


Figure 4.1-1 Lewis catalysed epoxide ring opening

When another Lewis acid, aluminium chloride was used in THF, 4.1-4 was obtained as the major product with about 10% percent unidentified non-polar by-product; after switching to acetonitrile as the solvent the reaction is fast and impurities were decreased dramatically to give 95% isolated yield of 4.1-4.

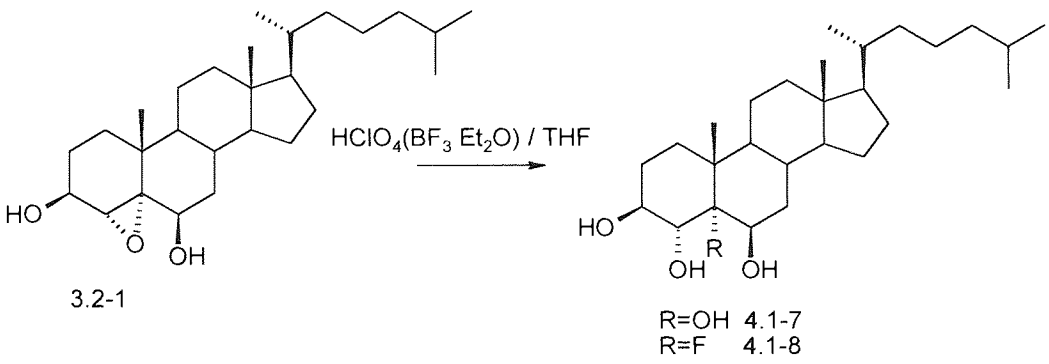
The same as the observation with $BF_3{\cdot}Et_2O$, when water was introduced into the aluminium chloride reaction system with acetonitrile as the solvent, the reaction became very slow and the 4β,5α compound 4.1-2 was the only product.

Furthermore when the reaction was performed in inert solvent DCM with perchloric acid a messy mixture was produced and no single product isolated. The $BF_3{\cdot}Et_2O$ in DCM give a lower yield of 4.1-4 (88%) than that in acetonitrile. It suggests that Lewis acid catalysed ring opening is less dependant on the polarity of the solvent than that of protic acid.

4.1.2 4α,5–Epoxy-5α–cholestane–3β,6β-diol (3.2-1)

It has been known for a long time that behaviour of a hydroxyl group participating in acid catalysed oxirane openings is different from its acylated or alkylated congeners. When we treated 4α,5-epoxy-5α-cholestane-3β,6β-diol (3.2-1) with a perchloric acid, it underwent a "*cis*-cleavage" to give 4α, 5α -isomer, cholestane-3β,4α,5α,6β-tetrol 4.1-7 (**Scheme4.1-5**). Clearly it is the result of the participation of the 6β-hydroxyl group, epoxide migration to yield the 5β,6β-epoxide followed by a normal diaxial scission (Morrison and Wilkinson 1990). This type of overall *cis*-cleavage was observed with 3β-methoxy-4α,5-epoxy-5α-cholestan-6β-ol and 3β,6β-dimethoxy--4α,5-epoxy- 5α-cholestane, resulting from 3β-methoxy donating inductive effect. When treated with aqueous perchloric acid and methanolic toluene-p-sulphonic acid, this *cis*-cleavage did not happen to (5α)-4α,5–epoxycholestan-6β–ol. This probably is due to no presence of 3β-methoxy donating inductive effect; nevertheless, it did happen when $BF_3{\cdot}Et_2O$ was used to give a 5α-fluoro product(Morrison and Wilkinson 1990). We observed the same phenomena: when we replaced perchloric acid with boron trifluoride etherate, the 5α-fluoro compound 4.1-8 was obtained (**Scheme 4.1-5**).

The total *cis*-cleavage of (5α)-4α,5-epoxycholestane-3β,6β-diol (3.2-1) with perchloric acid clearly demonstrated the 6β-OH is more competitive than the 3β-OH.



Scheme 4.1-5 Epoxide 3.2-1 with $HClO_4$ or $BF_3{\cdot}Et_2O$ in THF

The reaction may take several days to complete at 0°C with catalytic amount of perchloric acid. We found when 1.0g 3.2-1 in 5ml THF was treated with 1ml 70% aq. perchloric acid, if the reaction mixture is heated to 50°C first and then cooled down to

room temperature gradually, the reaction time could be cut to several hours. Though this process generated a certain amount of hydrophobic by-products, it is still more friendly since the by-product is easily removed by recrystallisation with DCM and hexane.

When the reaction was carried out in CH_3CN, surprisingly, a Ritter reaction happened (Ritter and Minieri, 1948). The generation of acetamide is shown in **Figure 4.1-2**.

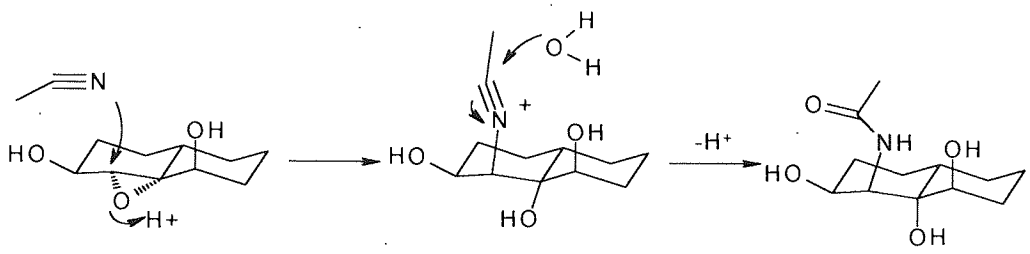
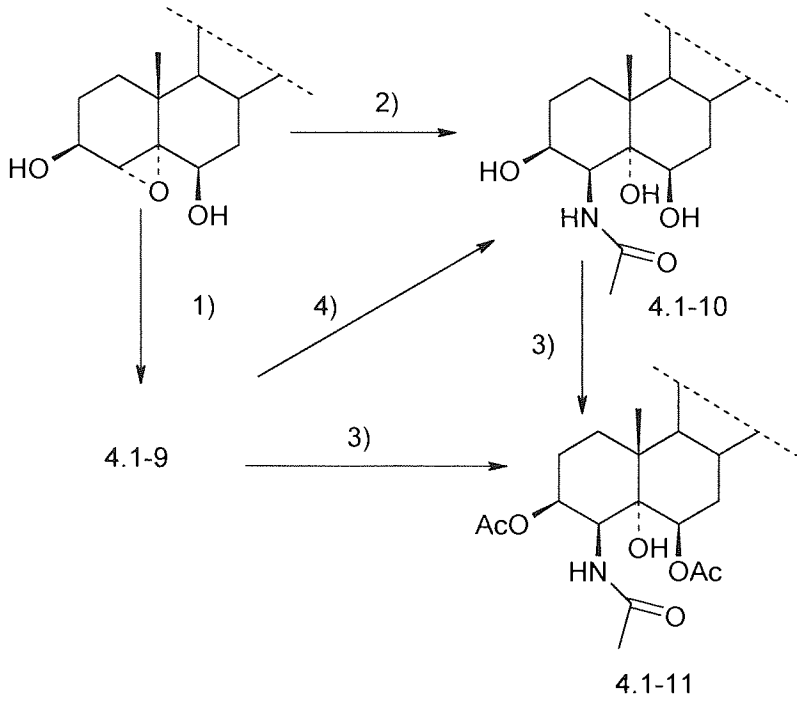


Figure 4.1-2 Ritter reaction.

It was reported that under Ritter reaction conditions (5β)-4β,5-epoxycholestan-6β-ol underwent a rearrangement to give (5α)-cholestan-6-one, while (5α)-3β,6β-diacetoxy-4α,5-epoxycholestane underwent a sluggish cleavage to give (5β)-3β,6β-diacetoxy--cholestane-4α,5β-diol and (5α)-3β,6β-diacetoxy-cholestane- 4β,5α-diol as the major products (Narayanan and Landge, 1993).

In contrast to the above report we observed a clear Ritter reaction when (5α)-4α,5–epoxycholestane–3β,6β-diol (3.2-1) was treated with $BF_3{\cdot}Et_2O$ to give a single product 4.1-10; the nitrogen attacked the less substituted carbon (**Scheme 4.1-6**). However, when the reaction was performed with $HClO_4$, an interesting result was obtained. The initial product 4.1-9 is a solid with a similar ^{1}HNMR spectrum to 4.1-10, the difference appearing in low field. In contrast to 4.1-10, this product does not dissolve well in DCM. Furthermore it has the same acetylation product, diacetate 4.1-11, as 4.1-10, after a treatment with acetic anhydride and pyridine. This product was converted to 4.1-10 as a single product after treated with an acid or a base: aq. NaOH in ethanol or concentrated HCl in methanol at room temperature over 2hr. Based

on these observations, the structure of 4.1-9 should be closely related with that of 4.1-10 (**Scheme 4.1-6**).



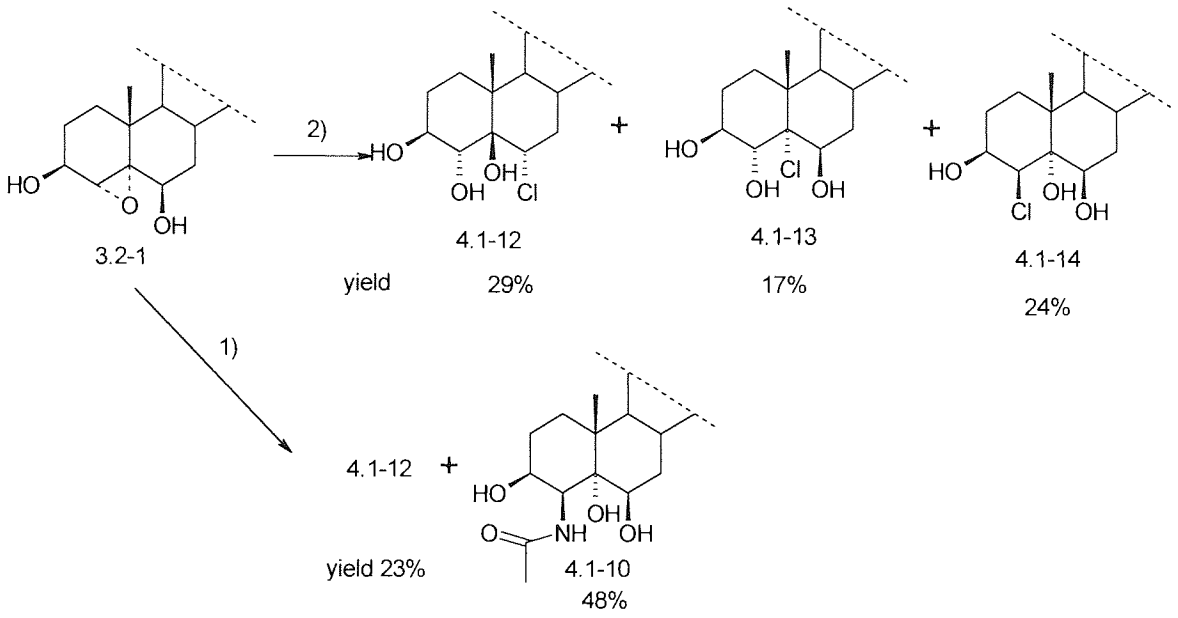
1) $HClO_4$ / CH_3CN, 2) BF_3 Et_2O / CH_3CN, 3) Ac_2O /Py,
4) NaOH/EtOH or HCl/ MeOH

Scheme 4.1-6 Ritter reaction of Epoxide 3.2-1

The other conditions used on 3β,6β–diacetoxy (3.2-1a) also were tried on (5α)-4α,5–epoxycholestane–3β,6β-diol (3.2-1). When aluminium chloride was applied in THF, the reaction performed less stereoselectively, and chlorinated oxysterols 4.1-12, 4.1-13 and 4.1-14 were obtained as the only main products in low to moderate yield totally; no tetrols were found (**Scheme 4.1-7**). In acetonitrile, the solvent participation dominated and gave the Ritter reaction product 4.1-10 as the main one. Not like the boron trifluoride, the reaction speeds are not very different between the two solvents. The configurations of these compounds were assigned on the bases of spectroscopic data of relative 3,4,5,6-tetrols. This result is so different from that of the diacetate 3.2-1a under the same condition, where the reaction gave only the diequatorial opening tetrol products only in a high yield (95%) (**Scheme 4.1-2**). It is clear that 4.1-12 and 4.1-13 are the results of opening of 5,6β-epoxy-5β- -cholestane-3β,4α-diol,

the intermediate resulted from epoxide migration, by chloride on C5 and C6; and 4.1-14 is the product of direct diaxial opening by chloride from β-face.

Above all, the Lewis acid ring opening is normally carried out in dry solvent before working up with water. Without the facilitation of allyl acetoxy groups to form a stable intermediate, the direct attack by chloride or fluoride happens and no tetrol was given.



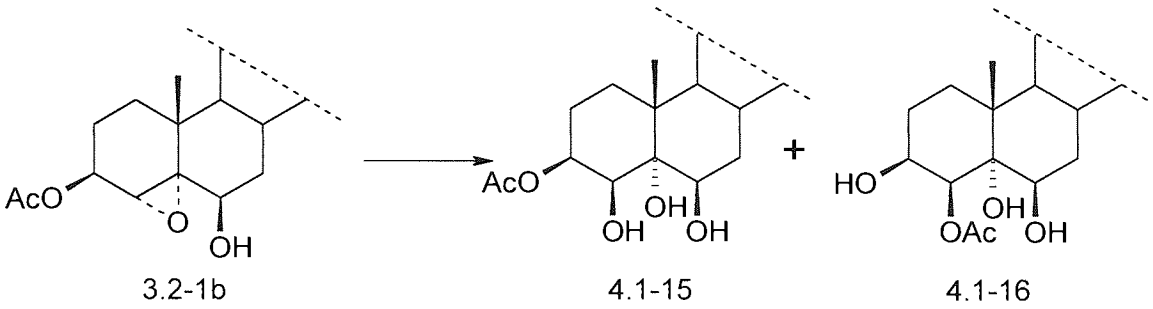
Scheme 4.1-7 Epoxide 3.2-1 with aluminium chloride

4.1.3 Monoacetates of 4α,5–epoxy-5α–cholestane–3β,6β-diol (3.2-1b, c)

As shown above, 3-acetate, 6-acetate and 6-OH are the major factors in affecting orientation of oxirane openings of (5α)-4α,5–epoxycholestane–3β,6β-diol 3,6-diacetate (3.2-1a) and (5α)-4α,5–epoxycholestane–3β,6β-diol (3.2-1). In order to define the more precisely the role of 3-acetate and 6-acetate in this process, the two mono acetates (5α)-4α,5–epoxycholestane–3β,6β-diol 3-acetate (3.2-1b) and (5α)-4α,5- epoxycholestane-3β,6β-diol 6-acetate (3.2-1c) were prepared following the procedure described for preparation of the 4-en-3,6-diols monoacetate in section 3.1.

The results of (5α)-4α,5–epoxycholestane–3β,6β-diol 3-acetate (3.2-1b) treated with four different combinations of an acid and a solvent (**Scheme 4.1-8**), perchloric acid or boron triflouride in THF or acetonitrile, are summarised in **Table 4.1-1**. Overall a

diaxial opening dominated in this case to give a 4β,5α-tetrol. So it can be concluded that the 3-acetyl group has a stronger influence than the 6-hydroxy on the orientation of the oxirane opening of (5α)-4α,5–epoxycholestane–3β,6β-diol 3-acetate (3.2-1b). However we cannot ignore that the reaction time of 3.2-1b with perchloric acid in THF (40min) is much less than that of 3.2-1a (5hr), and the slower reaction with boron trifluoride in acetonitrile compared with that of the diacetate 3.2-1a. As we have described, 3.2-1a under $BF_3{\cdot}Et_2O$ in acetonitrile gave the less energetically favoured *cis*-tetrol, resulting from 6-acetoxy participation (**Scheme 4.1-3**). Here again we saw that $BF_3{\cdot}Et_2O$ did not work with 3-acetoxy, at least not as well as perchloric acid did. The 6-acetyl migration favoured by $BF_3{\cdot}Et_2O$ may be explained by the slowdown while other reasons are not excluded as possible.



3.2-1b 4.1-15 4.1-16

Scheme 4.1-8 Epoxide 3.2-1b with perchloric acid or $BF_3{\cdot}Et_2O$

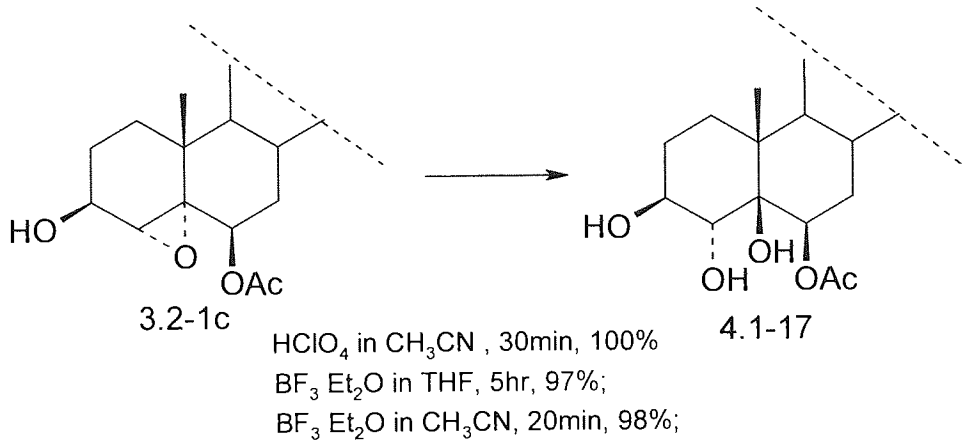
Table 4.1-1 Reactions in **Scheme 4.1-8** (all yield >95%)

Reaction conditions	Products	Reaction time
$HClO_4$ in THF	4.1-15 / 4.1-16 : 10/1	Reaction time 40min
$HClO_4$ in CH_3CN	4.1-15 / 4.1-16 : 9/1	Reaction time 20 min
$BF_3{\cdot}Et_2O$ in THF	4.1-15 / 4.1-16 : 1/20	1.5hr with 50% converted.
$BF_3{\cdot}Et_2O$ in CH_3CN	4.1-15 / 4.1-16 : 1/5	Reaction time 1hr

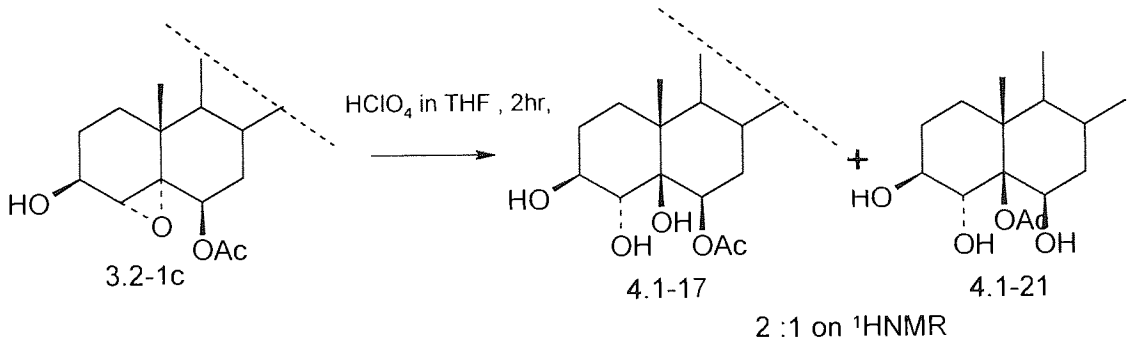
A protic acid favours 3-acetoxy, while a Lewis acid favours 6-acetoxy. This is approved in the following reaction of 6-acetoxy congener 3.2-1c (**Scheme 4.1-9**).

When 6-acetoxy isomer (3.2-1c) was treated with $HClO_4$ in CH_3CN, $BF_3{\cdot}Et_2O$ in THF or CH_3CN, almost 100% yield of 4.1-17 was produced (**Scheme 4.1-9**). When switched

to $HClO_4$ in THF (slow and fast procedures give the same results), though the reaction was not as clean as the previous ones, the only product isolated was 3,4,6-triacetate of 4.1-17 in overall 90% yield after acetylation (**Scheme 4.1-10**). It is clearly that the 3-OH played little role in the ring opening reactions.



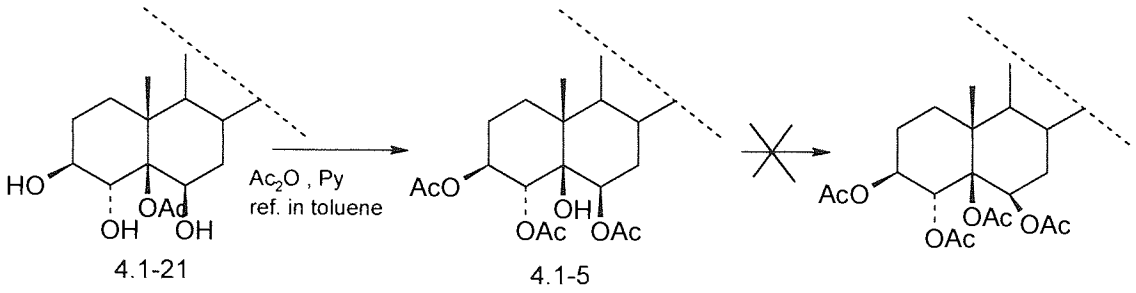
Scheme 4.1-9 Epoxide 3.2-1c with perchloric acid or $BF_3{\cdot}Et_2O$



Scheme 4.1-10 Epoxide 3.2-1c with perchloric acid in THF

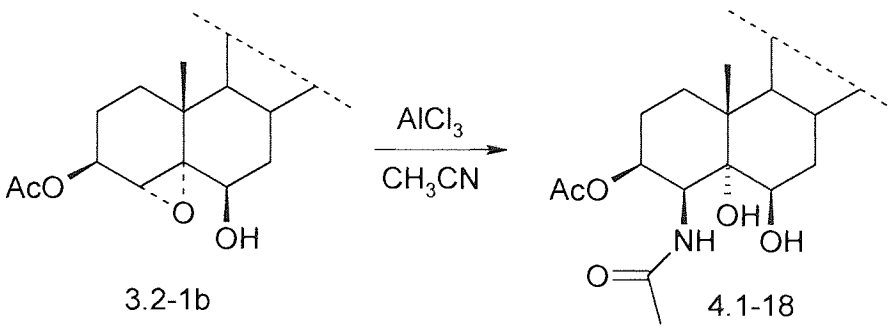
In 1980 Ishiguro et al reported the reaction of 3.2-1c in THF with perchloric acid to give the 6-acetate 4.1-17 as the only product. Earlier than that in 1976 Campion et al reported the 3-methoxy analogue under the same reaction conditions performing a ring opening in the same fashion. They postulated the second compound rather than the 6-acetate is the 4-acetate by ^1H NMR. However, in our experiments, if the acetyl group is moved to the 5β-hydroxyl group, the C4-β proton signal will give the same migration (1.0~1.2ppm) to low field just as if the 4α-OH has been acetylated. On acetylation the

5-acetyl group of 4.1-21 migrated to C-6 or C-4 hydroxyls to yield the 3,4,6-triacetate 4.1-5. This migration is more reasonable than that postulated by Campion who did not expand the discussion. Due to the highly congested environment of 5β, 6β-hydroxyl groups, attempts to make 3,4,5,6-tetraacetate failed even with very vigorous conditions like heating in acetic anhydride with excess $BF_3 \cdot Et_2O$ (**Scheme 4.1-11**).



Scheme 4.1-11 Acetylation of 5-acetate 4.1-21

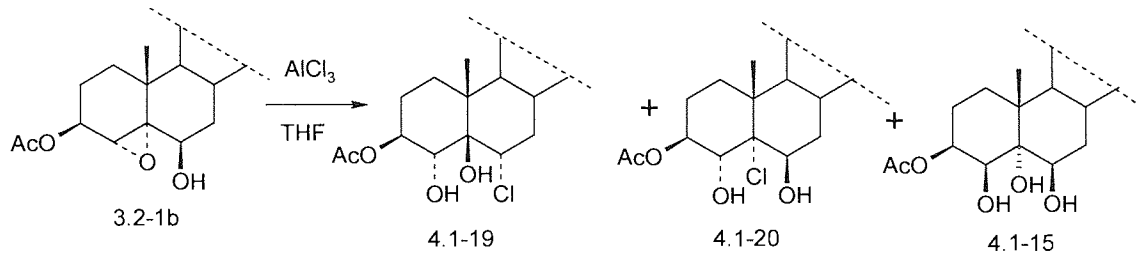
When aluminium trichloride was used, in acetonitrile the oxirane opening of the 6-acetate 3.2-1c gave the same major compound 4.1-17 in a yield of 72% with at least four impurities under further study. Interesting is that the 3-acetoxy congener 3.2-1b underwent a Ritter reaction to give a quantitative yield of 4-acetamide product 4.1-18 (**Scheme 4.1-12**). In THF the reactions of 3.2-1b (**Scheme 4.1-13**) and 3.2-1c (**Scheme 4.1-14**) with $AlCl_3$ are quite complicated; each gave more than five products and many impurities. In general, compounds like tetrol and chloro alcohols can be found in each case.



Scheme 4.1-12 Epoxide 3.2-1b with $AlCl_3$ in acetonitrile

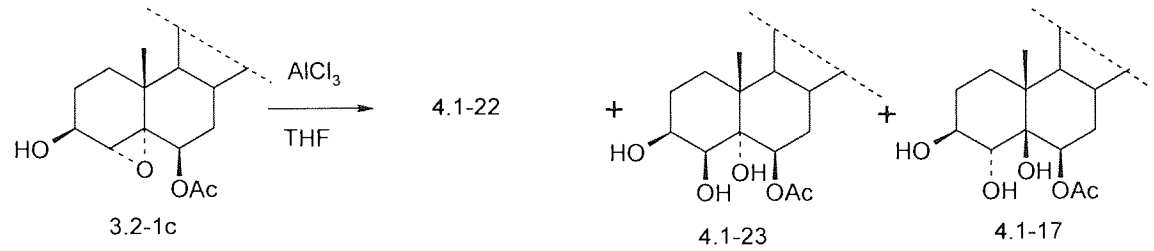
It is interesting that in **Scheme 4.1-13**, the only separated tetrol ester is the 3-acetate of 4β, 5α–isomer 4.1-15, so it is rational to postulate that the acetyl participation is not as

effective as that in protic acid catalysed ring opening. The reaction of 6-acetate 3.2-1c with aluminium chloride in THF gave less products (**Scheme 4.1-14**). The main product is a compound with an α,β-unsaturated ketone structure without alkene hydrogens shown by ^{1}H NMR , ^{13}C NMR and $^{1}H^{1}H$ COSY spectra. The two tetrol products generated are of special interest as the ratio of 4β,5α-isomer (4.1-23) to 4α, 5β-isomer (4.1-17) is 1: 0.6, compared with those reactions using $BF_3{\cdot}Et_2O$ (**Scheme 4.1-8** and **Scheme 4.1-9**). We can say that the two Lewis acids act quite differently with both monoesters 3.2-1b and 3.2-1c; it seems that $AlCl_3$ drives an 1,3-acetyl migration.



yield after chromatography, 4.1-19 15.6% , 4.1-20 13.2%, 4.1-15 27%, other unidentifiable residue 36% by weight

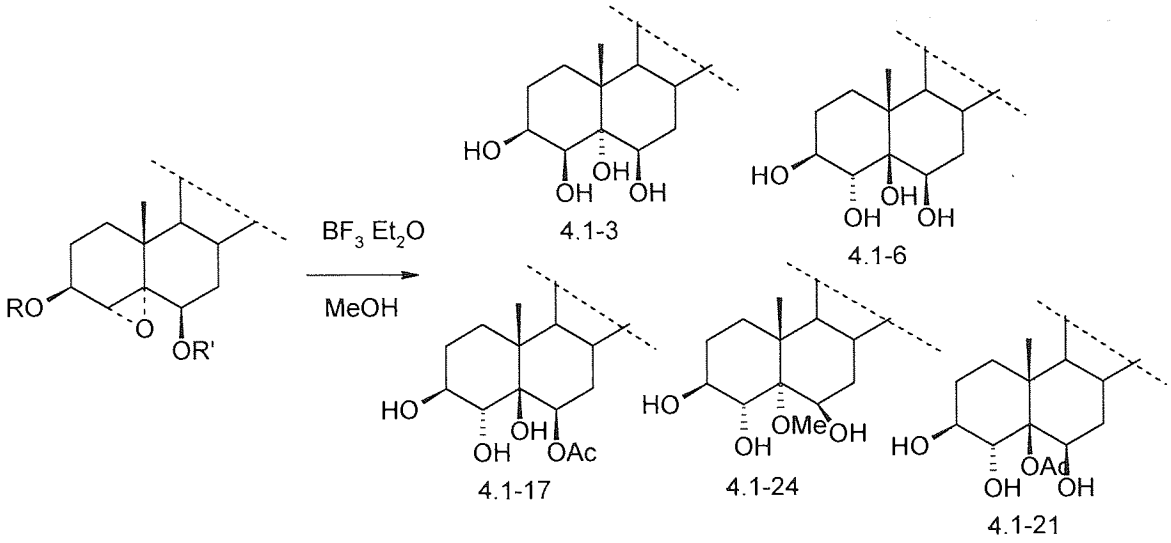
Scheme 4.1-13 Epoxide 3.2-1b with $AlCl_3$ in THF



yield after chromatography, 4.1-22 61.5% , 4.1-23 22.1%, 4.1-17 12.0%.

Scheme 4.1-14 Epoxide 3.2-1b with $AlCl_3$ in THF

To further understand the roles of the solvent in the combination with a Lewis acid in the oxirane opening reactions of 3.2-1 and its acetates 3.2-1a to 3.2-1c, methanol was also tried with $BF_3{\cdot}Et_2O$. The results were summarised in **Scheme 4.1-15** and **Table 4.1-2**.



Scheme 4.1-15 Epoxides 3.2-1(a-c) with $BF_3{\cdot}Et_2O$ in CH_3OH

Table 4.1-2 Reactions with boron trifluoride etherate (**Scheme 4.1-15**)

Starting material	Products	Reaction time
3.2-1a R=R'=Ac	4.1-3(90%)	1hr
3.2-1b R=Ac, R'=H	4.1-3(90%)	20min
3.2-1c R=H, R'=Ac	4.1-21(6.3%), 4.1-17(26.4%),	12hr
	4.1-24(6.1%), 4.1-6(40.3%)	
3.2-1 R=R'=H	4.1-24(85%)	4hr

In case of 3β,6β-diacetoxy 3.2-1a and 3β-acetoxy 3.2-1b, the results were similar to the previous observations with THF and acetonitrile. In case of 6β-acetoxy 3.2-1c, the result seemed messy; however it was clear that 6β-acetoxy participation is the main action. This result provided further evidence that 6-Ac participation is less effective than that of 3-Ac. The result of 3β,6β-diol was equal to its result in THF except the nucleophilic reagent is methanol.

The above studies of the oxirane opening of 4α, 5-epoxy-5α-cholestan-3β,6β-diol (3.2-1) and its esters (3.2-1a to 1c) with a different combination of a protic acid or a Lewis acid with a solvent told us that the orientation of this opening is affected by the acid and the solvent greatly. A careful manipulation of the conditions can produce

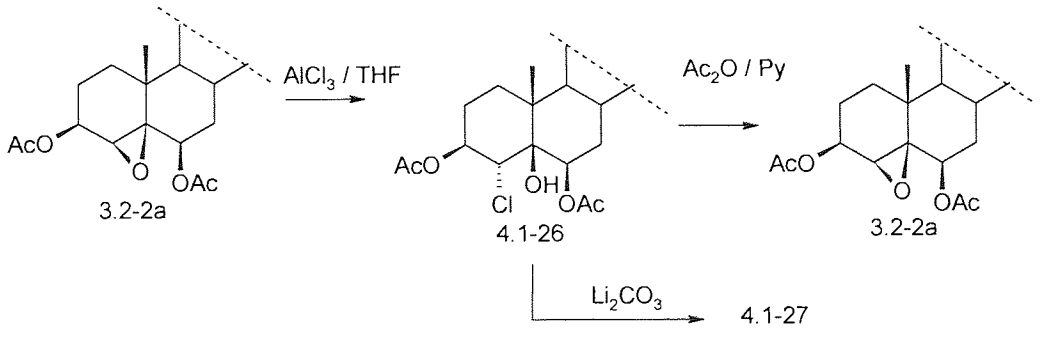
desired products in a good yield. In the following sections, we shall apply this first hand knowledge into studies of the remaining epoxide substrates.

In summary, the seven combinations of an acid and a solvent studied in **4.1.1** to **4.1.3** could be defined as follows and used in further discussions:

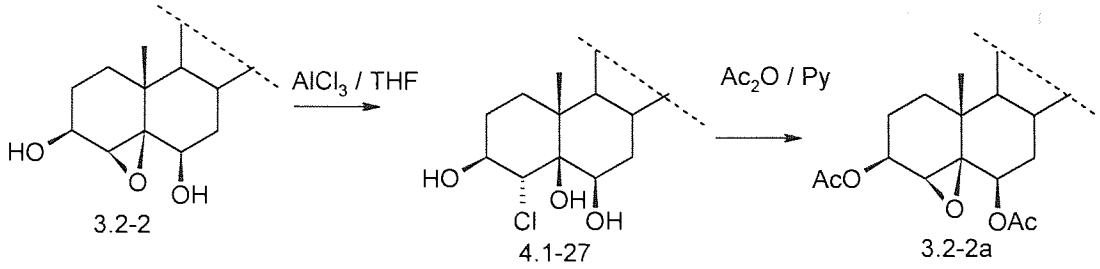
1. $HClO_4$/THF; **2**. $HClO_4/CH_3CN$; **3**. $BF_3{\cdot}Et_2O$/THF; **4**. $BF_3{\cdot}Et_2O/CH_3CN$; **5**. $AlCl_3$/THF; **6**. $AlCl_3/CH_3CN$; **7**. BF_3-Et_2O/CH_3OH.

4.1.4 the 4β , 5-epoxy-5β-cholestane-3β,6β-diol (3.2-2) and its acetates

As the THF and acetonitrile affected the reaction differently, here the combinations **1**, **3** and **5** were discussed separately from combinations **2**, **4** and **6** on the β-epoxides. The 4β,5–epoxy-5β–cholestane–3β,6β-diol 3,6-diacetate (3.2-2a) refused to react with the combinations **1** and **3** because there is no opportunity for neighbouring group participation and the two acetoxy groups exert a strong steric hindrance effect. Under vigorous conditions, such as heating over 50°C, a decomposed mess was created. The reaction with combination **5** for 28 days shows only one third of the starting material was consumed and the identified product was 4α-chlorinated compound 4.1-26 (**Scheme 4.1-16**), which was converted to chloro-triol 4.1-27 and the epoxide 3.2-2 under a carefully controlled hydrolysis. 4.1-27 could be also produced from 4β,5-epoxy-5β-cholestane-3β,6β-diol (3.2-2) with conditions **5** in 89% yield in 3hr. Upon acetylation with acetic anhydride and pyridine in toluene at reflux 4.1-26 and 4.1-27 afforded the same product 4β,5-epoxy-5β-cholestane-3β,6β-diol 3,6-diacetate (3.2-2a) (**Scheme 4.1-17**).

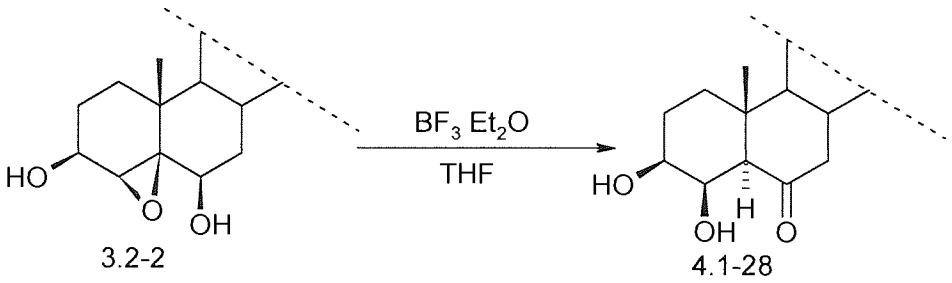


Scheme 4.1-16 Epoxide 3.2-2a with combination **5**



Scheme 4.1-17 Epoxide 3.2-2 with combination **5**

Even the free diol 3.2-2 offered no reaction with perchloric acid in THF (combination **1**). When 3.2-2 was treated with combination **3**, the migration of C-6 H to C-5 H happened slowly, giving less than 20% 3β,4β-dihydroxy-5α-cholestan-6-one (4.1-28) and 80% starting material after 5hr (**Scheme 4.1-18**). .

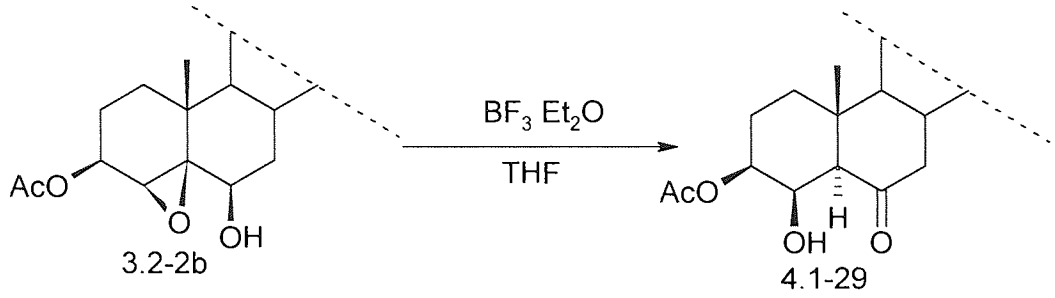


Scheme 4.1-18 Epoxide 3.2-2 with combination **3**

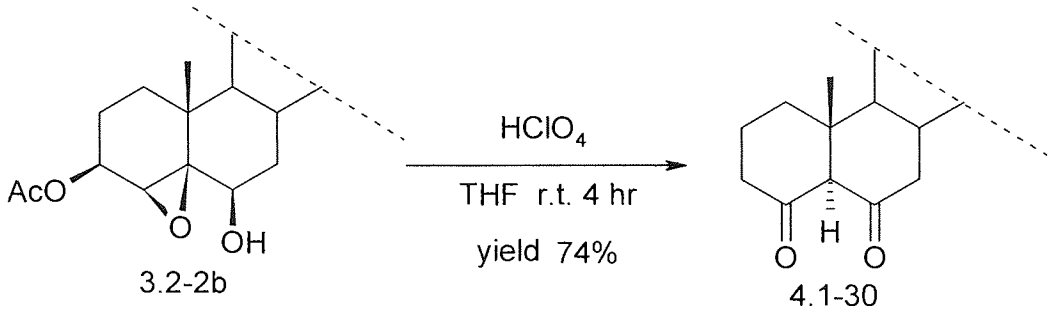
The two monoesters, the 4β, 5-epoxy-5β-cholestane-3β,6β-diol 3-acetate (3.2-2b) and 6-acetate (3.2-2c), were also tested with these conditions. Similar to the diacetate 3.2-2a, the 6-acetate 3.3-2c gave no reaction with combinations **1** and **3**. The 3-acetate 3.2-2b gave interesting results. With combination **3** the result is like that of the free diol 3.2-2, with 15% yield of the 6-one compound 4.1-29, the 3-acetate of 4.1-28 in 5 hrs (**Scheme 4.1-19**), however with combination **1**, a known compound, 5α-cholestane-4,6-dione was afforded in considerable yield (**Scheme 4.1-20**). It seems that with perchloric acid, the 3β-acetoxy group can serve as a good leaving group and triggered the hydrogen migration from C-4 to C-3 after the epoxide ring opened and C-6 H moved to C-5.

As we changed the solvent to acetonitrile, the 3β,6β-diacetate 3.2-2a under conditions of both combinations **2** and **4** reacted very slowly and gave numerous products. The compound 5α-cholestane-4,6-dione (4.1-30) was the only one isolated in 20~25%

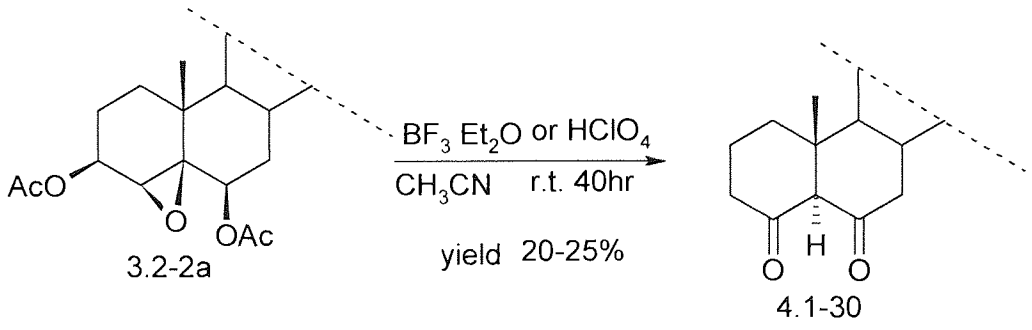
yields in both cases (**Scheme 4.1-21**). Obviously the 6-acetate hampered the formation of 6-one and low yield of the 4.1-30 was given.



Scheme 4.1-19 Epoxide 3.2-2b with combination **3**

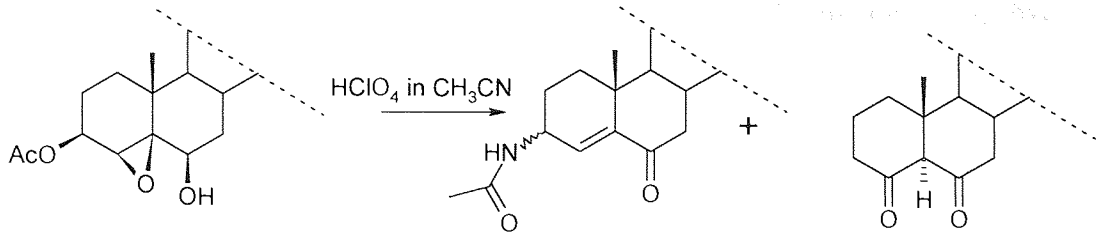


Scheme 4.1-20 Epoxide 3.2-2b with combination **1**



Scheme 4.1-21 Epoxide 3.2-2a with combination **2**, **4**

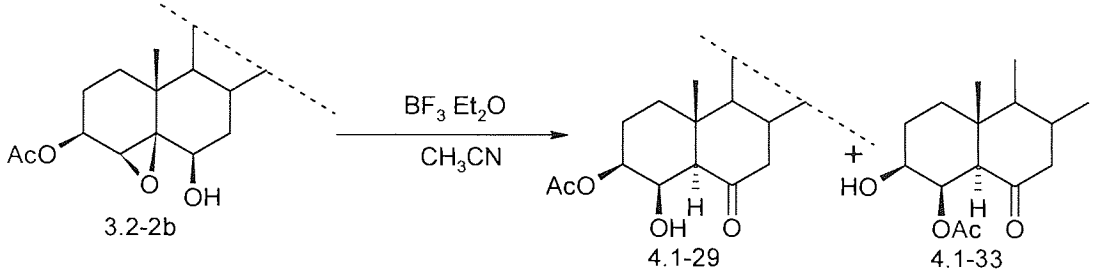
The 3-monoacetate (3.2-2b) gave the dione 4.1-30 in 64% yield with combination **2**, plus the acetamide products 4-en-6-one 4.1-31 and 4.1-32 in a combined yield 24% (**Scheme 4.1-22**). The ratio of 4.1-31 and 4.1-32 is 2:1 on ^{1}H NMR.



3.2-2b 24% α(4.1-31) : β(4.1-32)=2:1 64% 4.1-30

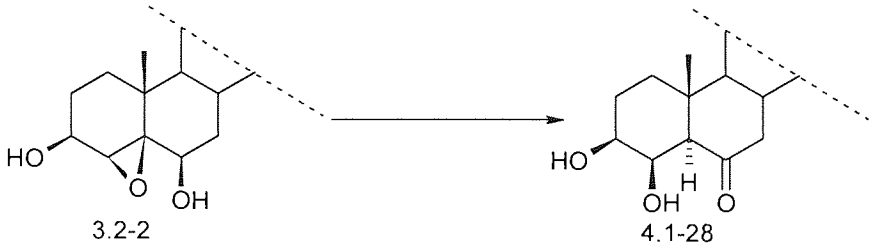
Scheme 4.1-22 Epoxide 3.2-2b with combination **2**

Reaction of 3-acetate 3.2-2b with $BF_3 \cdot Et_2O$ in acetonitrile give the two 6-one compound 4.1-29 and 4.1-33 in good yield (**Scheme 4.1-23**), the free diol 3.2-2 also give the 3β.4β-dihydroxy-5α-cholestan-6-one 4.1-28 in good yield with combinations **2** and 4 (**Scheme 4.1-24**).



r.t. 30 min give 27% 4.1-29 and 50% 4.1-33

Scheme 4.1-23 Epoxide 3.2-2b with combination **4**

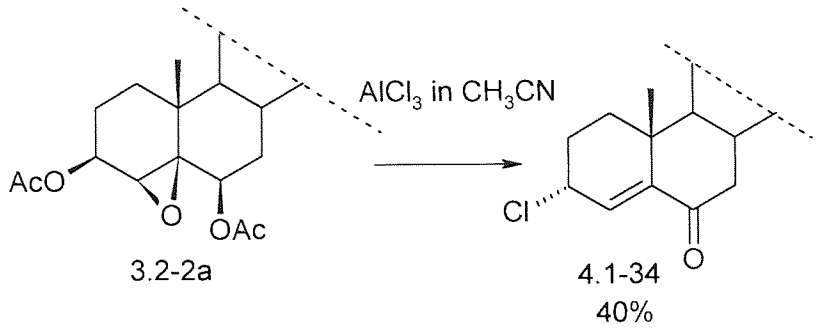


BF_3 Et_2O in CH_3CN 20min , yield 97%
$HClO_4$ in CH_3CN 30min , yield 86%

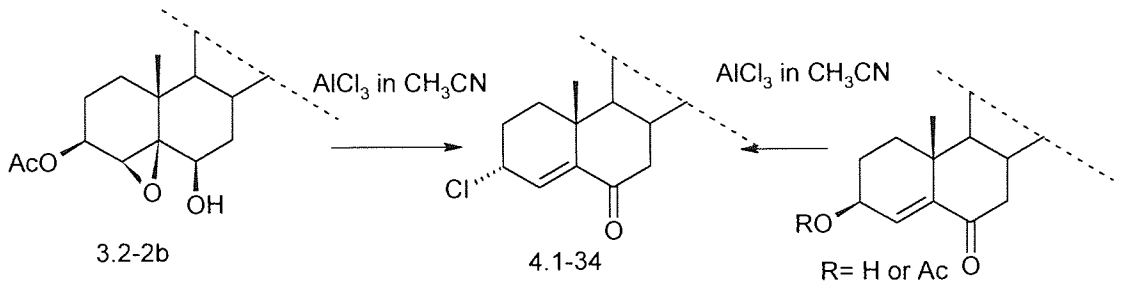
Scheme 4.1-24 Epoxide 3.2-2 with combinations **2**, **4**

Combination **6** was also applied to these epoxides, 3.2-2a give 40% 3α–chlorocholest -4-en-6-one (4.1-34) (**Scheme 4.1-25**), while the 3-monoacetate give 4.1-34 in 95% yield after 4hrs (**Scheme 4.1-26**). Compound as 3-hydroxyl or 3-acetoxycholest-4-en--6-one give the 4.1-34 in high yield with the same conditions, so we postulate that the

reaction happens first with the formation of 6-one and elimination of 4β-hydroxyl group thereafter, the substitution on C-3 is the last step.



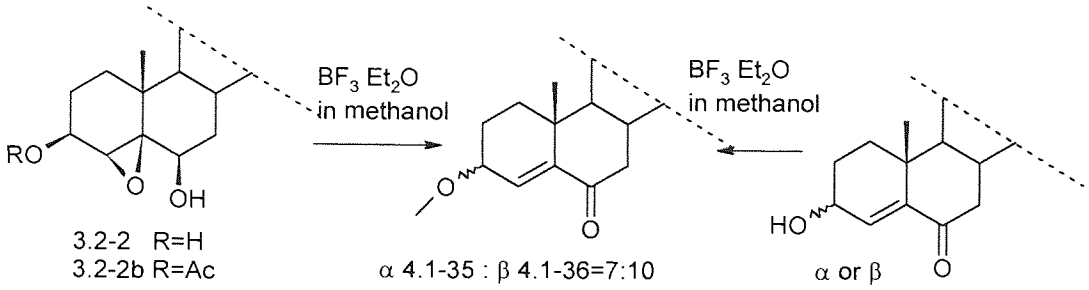
Scheme 4.1-25 Epoxide 3.2-2a with combination **6**



Scheme 4.1-26 Epoxide 3.2-2b with combination **6**

With combination **6**, the free diol 3.2-2 gave a mess hard to be analysed, the 6-monoacetate 3.2-2c did give an unidentified single compound in 90% yield.

When treated with boron trifluoride in methanol 3-methoxy-cholest-4-en-6-ones 4.1-35 (3α hydroxyl isomer) and 4.1-36 (3β hydroxyl isomer) were created with the 3.2-2 and 3.2-b which have free 6-OH. The contrasting reaction of 3β or 3α-hydroxycholest-4-en-6-one with the same condition and product shows the reactions proceed similarly to that of the combination **6** (**Scheme 4.1-27**). Not like the α-epoxide in **Scheme 4.1-15**, 3.2-2a and 3.2-2c do not react in 24 hr and give no reaction with $BF_3{\cdot}Et_2O$ in methanol. The 3-configuration difference of combination **6** and combination **7** can be explained that the chloride is not a good leaving group and the substitution is a S_N2 reaction, as the $BF_3{\cdot}Et_2O$ in methanol is a clearly S_N1 condition.



Scheme 4.1-27 Epoxides 3.2-2(b) with combination **7**

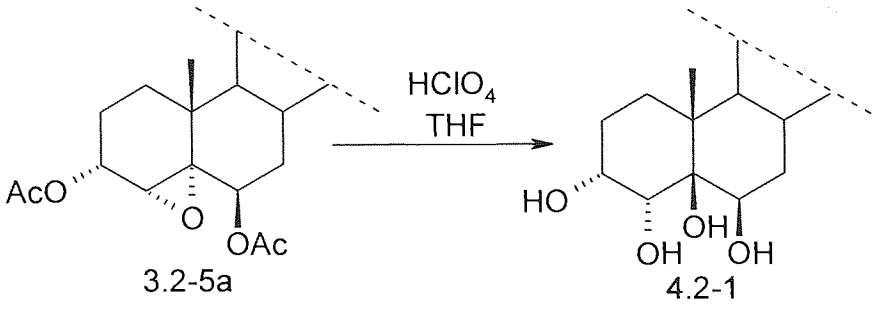
In summary, the oxirane openings of 4β,5-epoxy-5β-cholestane-3β,6β-diol diacetate (3.2-2a) and its 6-acetate (3.2-2c) were difficult to conduct, and difficult to predict the products; in general, they gave a messy mixture. The reaction of 3-acetate 3.2-2b shows three different results. With perchloric acid, the abstraction of C4-H is the major effect to yield the 4,6-dione. With boron trifluoride the C-4 hydrogen was intact and resulted in the dihydroxyl ketone compounds; while aluminium chloride in acetonitrile or boron trifluoride in methanol induced the elimination of 4- hydroxyl to form the 4,5-double bond as the main product. These results showed us the complication of both protic and Lewis acids catalysed epoxide ring opening, the difference between those solvent-acid combinations needs further study as useful tools for epoxide ring opening reactions.

4.2 The 4,5-epoxycholestane–3α,6β-diol and its acetates

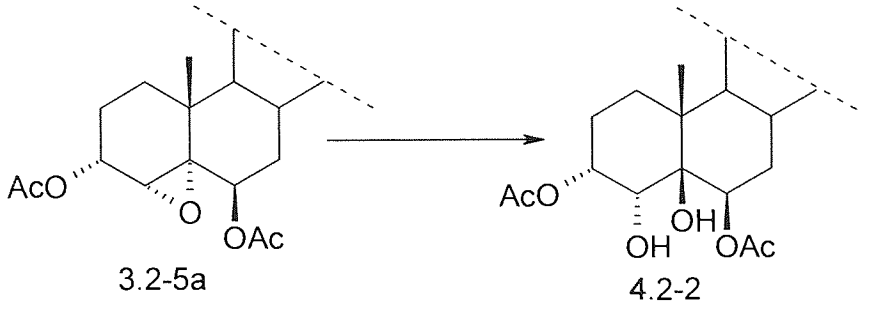
4.2.1 The 4α,5-epoxy-5α-cholestane–3α,6β-diol (3.2-5) and its acetates

The reaction of 4,5-epoxycholestane-3α,6β-diol 3,6-diacetate (3.2-5a) with catalytic amount of perchloric acid in THF proceeded very slowly, finished in 8 days and gave 5α-cholestane-3α,4α,5β,6β-tetrol (4.2-1) with no acetyl group remaining in over 80% yield. When the reaction was performed with a large excess of perchloric acid in THF, it finished in 3 hr, but as the consequences, more impurities were produced (**Scheme 4.2-1**).

With all the combinations from **2** to **6**, 4,5-epoxycholestane-3α,6β-diol 3,6-diacetate (3.2-5a) gave the same 5β-cholestane-3α,4α,5β,6β-tetrol 3,6-diacetate (4.2-2) as the major product (**Scheme 4.2-2** and **Table 4.2-1**).



Scheme 4.2-1 Epoxide 3.2-5a with combination **1**



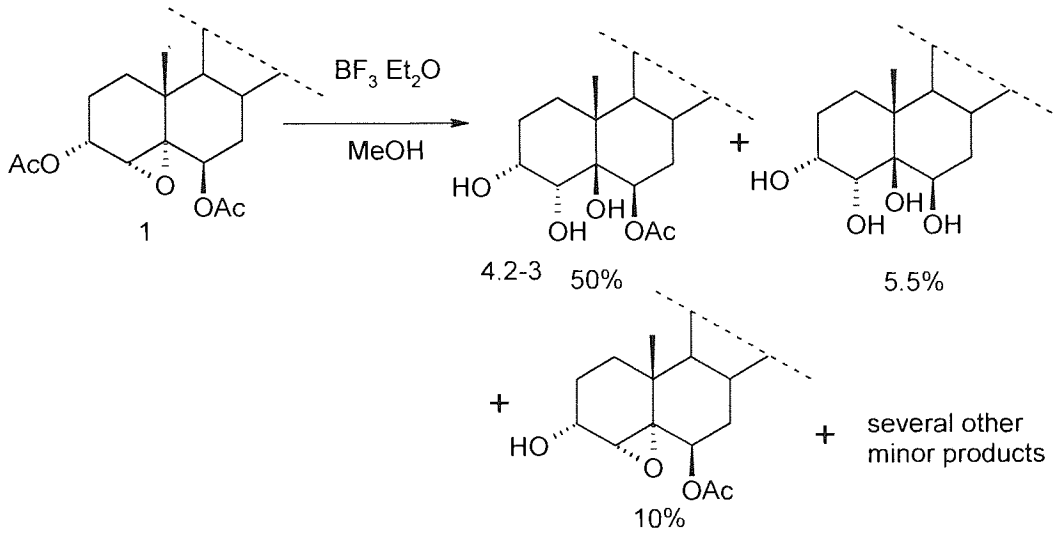
Scheme 4.2-2 Epoxide 3.2-5a with combination **2-6**

Table 4.2-1 Reactions in **Scheme 4.2-2**

Procedure	Yield and purity	Reaction time
$HClO_4$ in CH_3CN	Yield >95%	30min
BF_3 in THF	Yield >95%	20hr
BF_3 in CH_3CN	with minor by-products on TLC Yield after recrystallisation 83%	30min
$AlCl_3$ in THF	Yield 71% with some less polar impurities	16hr
$AlCl_3$ in CH_3CN	Yield 90%	3hr

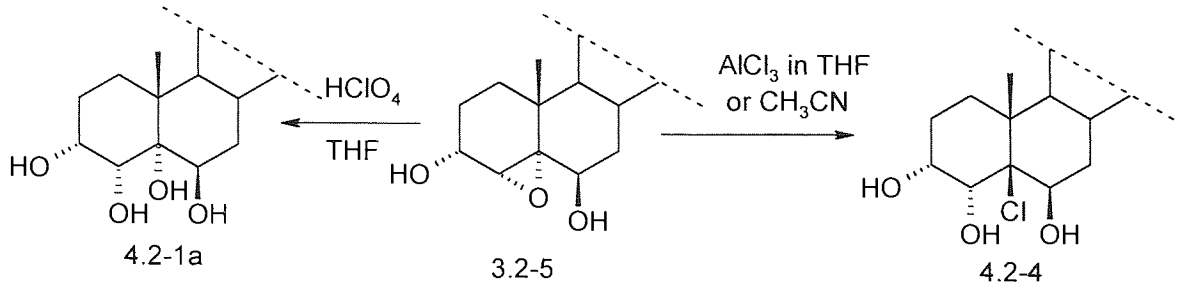
It is well known that the electronic effects assist the cleavage of epoxide on the more substituted carbon side. A combined stereoelectronic effect favours the oxirane opening to form axial hydroxyls. With the 4,5-epoxysterols, the two effects favour a different product. In literature, 4α,5-epoxy-5α-cholestane (Ma et al 1992, Davey et al 1968) give 4β, 5α diol using protic acid, showing a domination of the stereo-electronic effect (Hanson and Yildirim 1999). Its 3β-acyloxy derivatives also

give 4β, 5α diols (D'Auria 1989 and Tomas et al 1999), the 3-acetyl participation as discussed in part 4.1 can be another factor. The only exception is the 3β-methoxy -4α,5-epoxy-5α-cholestane that gave the 3β-methoxy-5β-cholestane-4α,5-diol using perchloric acid (Morrison and Wilkinson 1990). Only one report described the use of aluminium oxide that reacted with 4α,5-epoxy-5α-cholestane, the electronic favoured 4α,5β-isomer was generated (Davey et al 1968). It seems that the stereoelectronic effect is more important. In the case of 3,6-diacetates 4.2-2a, the 6β-acetoxy group is the probe in spinning the orientation of the oxirane opening through participation to yield the 5β-isomer 4.2-2 as discussed in part 4.1. The participation is the major driving force, because when reacted with boron trifloride in methanol, there is no solvent influence on the C-5, the major products are still the 5β–tetrols (**Scheme 4.2-3**). The 3α–acetoxy gave no participation to the ring opening.



Scheme 4.2-3 Epoxide 3.2-5a with combination 7

Similar to the 3β-isomer in Scheme 4.1-5, 4α,5-epoxy-5α-cholestane-3α,6β-diol (3.2-5) gave 79% "*cis*-cleavage" product 5α–cholestane–3α,4α,5α,6β–tetrol 4.2-1a with perchloric acid in THF. Perchloric acid in acetonitrile or boron trifloride in both THF and acetonitrile gave messy mixtures hardly to be separated. In contrast, aluminium chloride in both THF and acetonitrile furnished high yields of 5β-chloro product 4.2-4 (**Scheme 4.2-4**).

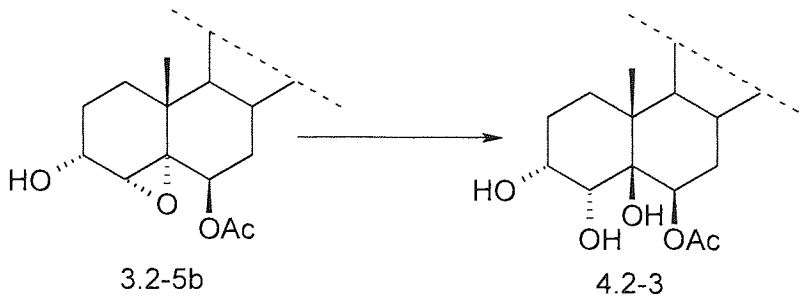


Scheme 4.2-4 Epoxide 3.2-5a with combination **1**, **5**, **6**

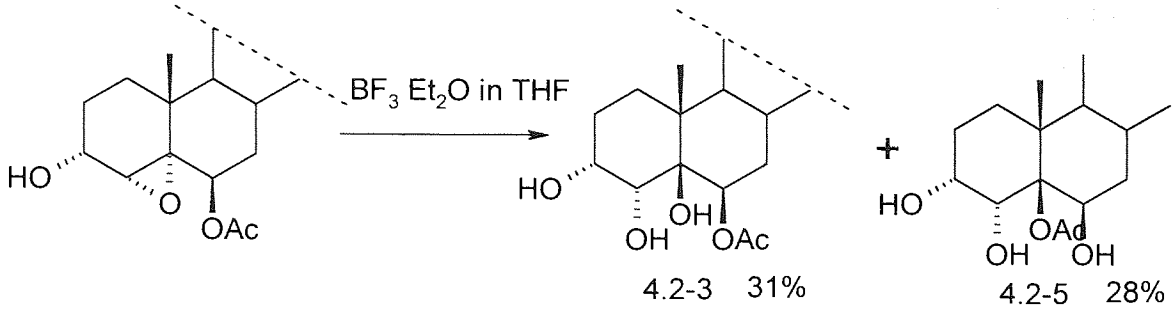
The preparation of 3-monoacetate failed due to the non-selectivity of the two hydroxyl groups and the two monoacetates generated in 1:1 ratio cannot be separated by chromatography. The 6-monoacetate 3.2-5b was prepared by the hydrolysis of the diacetate 3.2-5a.

When 3.2-5b was treated with perchloric acid in THF, the reaction occurred faster than that of diacetate 3.2-5a to give 6-acetate 4.2-3 in over 95% yield in 5hrs. In comparison with the result of 3,6-diacetate, where total hydrolysed product 4.2-1 is the major product (**Scheme 4.2-1**), this result implies the 3α-acetoxy group play a significant steric blockage in the diacetate case. Both combinations, perchloric acid and boron trifluoride in acetonitrile furnished the same product 4.2-3 in 82% and 85% yield respectively in 15min. In contrast, with the combination of boron trifloride with THF over 12hr, the acetyl migration product 4.2-5 was isolated in 28% yield (**Scheme 4.2-6**). No doubt 4.2-5 is the product resulting from the 6-acetoxy participation.

The 4.2-3 was also found as the major product with the combinations of $AlCl_3$ in THF or CH_3CN, in acetonitrile the yield is over 95% and in THF the yield is 86% with some non-polar impurities.



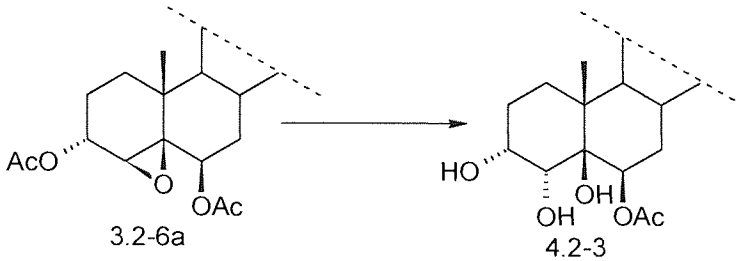
Scheme 4.2-5 Epoxide 3.2-5b with combination **1**, **2**, **4**, **5**, **6**.



Scheme 4.2-6 Epoxide 3.2-5b with combination **3**.

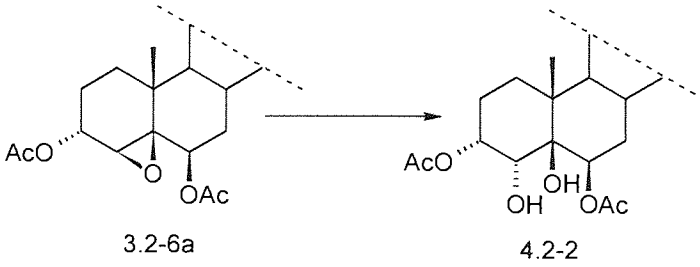
4.2.2 The 4β,5-epoxy-5β-cholestane–3α,6β-diol (3.2-6) and its acetates

For oxirane opening of the 4β,5-epoxy-5β-cholestane–3α,6β–diol 3,6-diacetate (3.2-6a), the 3-acetyl participation is significant and the resulting product is the 3α,4α,5β,6β-tetrol ester same to the 6-acetyl facilitated ring opening of the epoxide 3.2-5a above. When the reaction occurred in methanol with boron trifluoride no product from direct methoxy attack was found (**Scheme 4.2-7**). Diacetate 4.2-2 was afforded with combination **2** and **3** (**Scheme 4.2-8**).



$HClO_4$ (catalytic amount) in THF, 10hr, yield 90%
BF_3 Et_2O in CH_3OH 10min, yield 88%

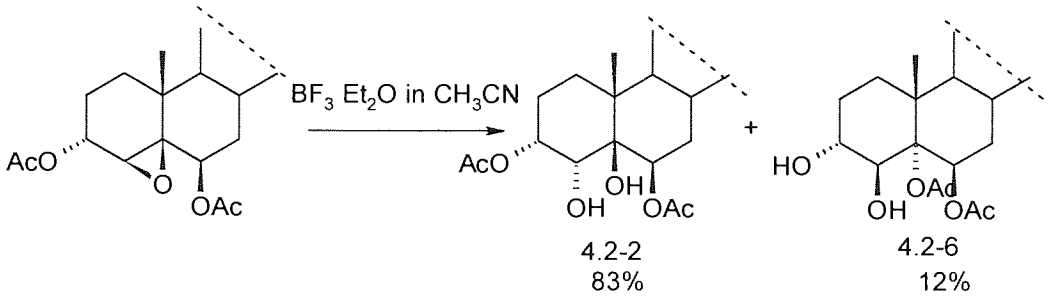
Scheme 4.2-7 Epoxide 3.2-6a with combinations **1**, **7**



$HClO_4$ in CH_3CN 30min 81%
BF_3 Et_2O in THF 20hr 85%

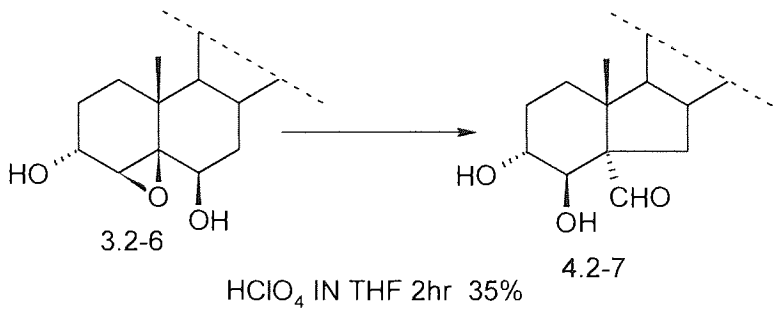
Scheme 4.2-8 Epoxide 3.2-6a with combinations **2**, **3**

The result from the $BF_3{\cdot}Et_2O$ in acetonitrile was interesting. Though TLC showed two spots, the 4.2-2 was obtained in 83% yield after separation. The minor one (4.2-6) is the product of acetyl migration from C-3 OH to C-5 OH, and the 5α-OAc was formed as the consequence (**Scheme 4.2-9**). The 1,3-acetyl migration was also postulated for the configuration of the minor products from the reactions of 3.2-2b and 3.2-2c with $AlCl_3$ / THF (**Scheme 4.1-13** and **Scheme 4.1-14**). The mechanism is not clear.

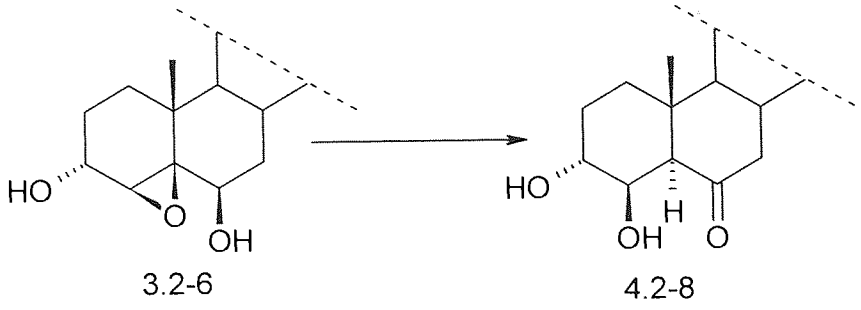


Scheme 4.2-9 Epoxide 3.2-6a with combination **4**

When the dihydroxyl β-epoxide 3.2-6 was treated with the $HClO_4$ in THF, several unidentified compounds were generated, one of which can be separated as pure crystals in 35% yield. This is a ring contracted aldehyde with two hydroxyl groups. From $^1H^1H$ cosy spectrum the two hydrogens on the hydroxylated carbons show a big coupling effect. Combined with the carbon spectrum the structure was postulated as 4.2-7 (**Scheme 4.2-10**). Under combinations **2~4** the 3α,4β-dihydroxy-5α-cholestan-6-one 4.2-8 was afforded (**Scheme 4.2-11** and **Table 4.2-2**).



Scheme 4.2-10 Epoxide 3.2-6 with combination **1**

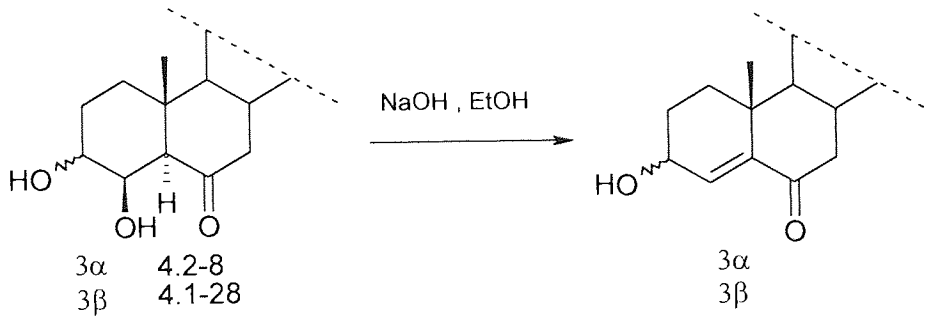


Scheme 4.2-11 Epoxide 3.2-6 with combination **2-4**

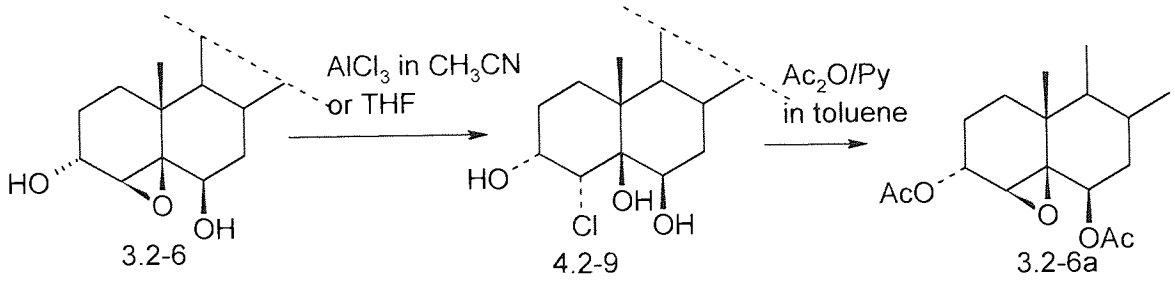
Table 4.2-2 Reactions for **Scheme 4.2-11**

Reagents	Yield	Reaction time
$HClO_4$ IN CH_3CN	75%	1hr
$BF_3{\cdot}Et_2O$ in CH_3CN	78%	30min
$BF_3{\cdot}Et_2O$ in THF	66%	6hr

Structures of 4.2-8 and the 3β-isomer 4.1-28 (**Scheme 4.1-24**) were confirmed by the reaction with NaOH in ethanol, the known compound 3α(β)- hydroxycholest-4-en-6--one was generated (**Scheme 4.2-12**). 4α-Chloro triol 4.2-9 was given from 3.2-6 in over 90% yield with combination **5** or **6** (**Scheme 4.2-13**). The reaction with aluminium chloride gave the electronic effect dominated 5β-isomer for both α and β epoxide.



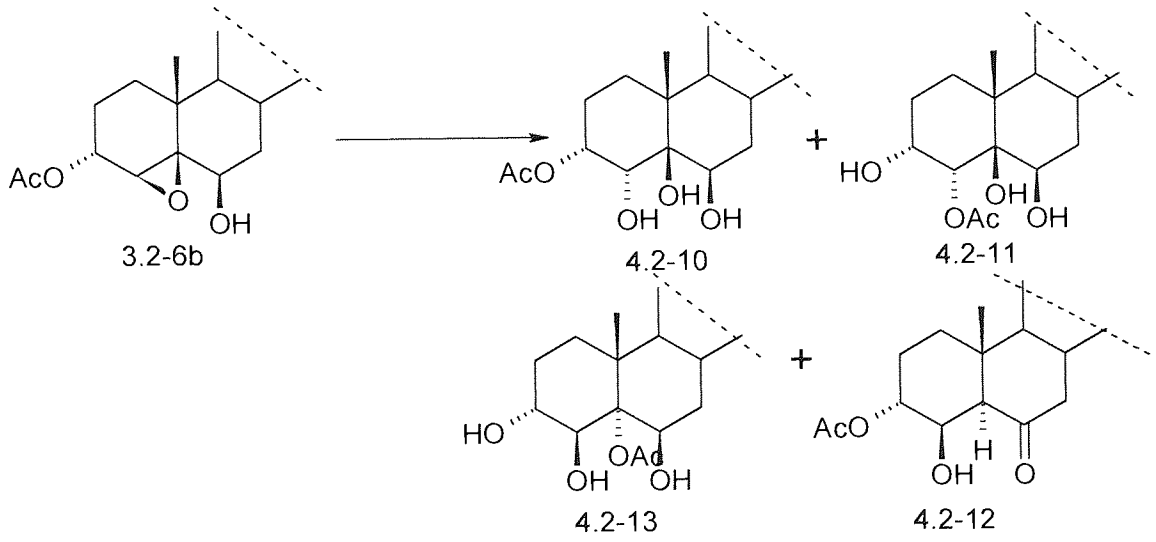
Scheme 4.2-12 Elimination of 4-hydroxyl groups in 3,4-dihydroxy-6-one



Scheme 4.2-13 Epoxide 3.2-6 with combination **5**, **6**

The two mono-acetates 3.2-6b (3-acetate) and 3.2-6c (6-acetate) were prepared following the established protocols. The reactions of 3-monoacetate (3.2-6b) may be conducted with two directions: the 3-acetyl participation or the formation of 6-one compound (migration of C-6 hydrogen to C-5). The results afforded using combination **1** to **6** are shown in **Scheme 4.2-14** and **Table 4.2-3**. Those reactions of 3.2-6b in THF gave complicated mixtures, as compared to that of diacetate 3.2-6a; in combination **3** and **5** it was hard to get a pure product out. When the reactions were carried out with other acidic conditions **2**, **4** and **6**, the 3-acetyl migration to C4 gave the 5β-isomer 4.2-11 with considerable or high yield. Formation of tetrol 4.2-1 with loss of the 4-acetyl group were observed in combination **7** (**Table 4.2-3**). All these results show that the 3-acetyl participation dominates the reaction.

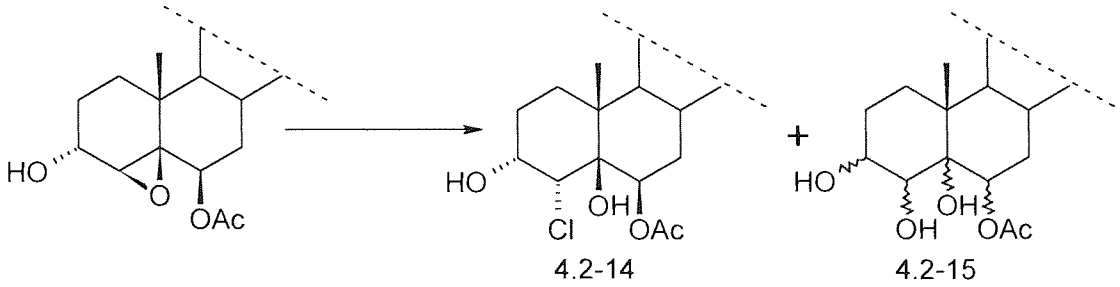
The 6-monoacetate (3.2-6c) lacks both the facilitation factors in 3.2-6b. It also gave a messy mixture with combination **1**, **2** or **4**, while no reaction happened with combination **3**. In the reaction with $AlCl_3$ in THF and acetonitrile, two products were generated, 4a-chlorocompound 4.2-14 and 4.2-15. The structure of the 4.2-15 may contains three hydroxyl and one acetoxyl group as shown in MS spectrum with m/z 479 (M+1) peak and in ^{13}C-NMR, however, from the ^{1}H NMR coupling effects the structure can not be deduced on its stereochemistry (**Scheme 4.2-15**).



Scheme 4.2-14 Epoxide 3.2-6b with combination **1-7**

Table 4.2-3 Reactions for **Scheme 4.2-14**

Reagents	Products and yields	Reaction time
$HClO_4$ in CH_3CN	4.2-10 as main product 50%, 4.2-11 10% with many other impurities, no 6-one compound observed	4hr
$HClO_4$ in CH_3CN	4.2-11 88%	40min
$BF_3{\cdot}Et_2O$ in THF	Give many products, from ^1H NMR, the 4.2-10 and 4.2-11 are the main one, only small part of 4.2-12 was found in the mixture	5hr
$BF_3{\cdot}Et_2O$ in CH_3CN	Give 4.2-11 over 70% yield, another compound not separated, possible 4.2-13, about 10%	6hr
$AlCl_3$ in THF	Give a mess but the peak of 4.2-11 still significant on the ^1H NMR of the resulting mixture.	10 min
$AlCl_3$ in CH_3CN	Give 4.2-11 95% yield	5 min
$BF_3{\cdot}Et_2O$ in methanol	Give the free diol 4.2-1 (as in scheme 4.2-1) around 60-70% yield many by-products.	4hr



4.2-14 4.2-15

$AlCl_3$ in THF 2hr, 4.2-14 : 4.2-15=3:7 total yield 90%
$AlCl_3$ in CH_3CN 20min, 4.2-14 : 4.2-15=7:1 total yield 90%

Scheme 4.2-15 Epoxide 3.2-6c with combination **5, 6**

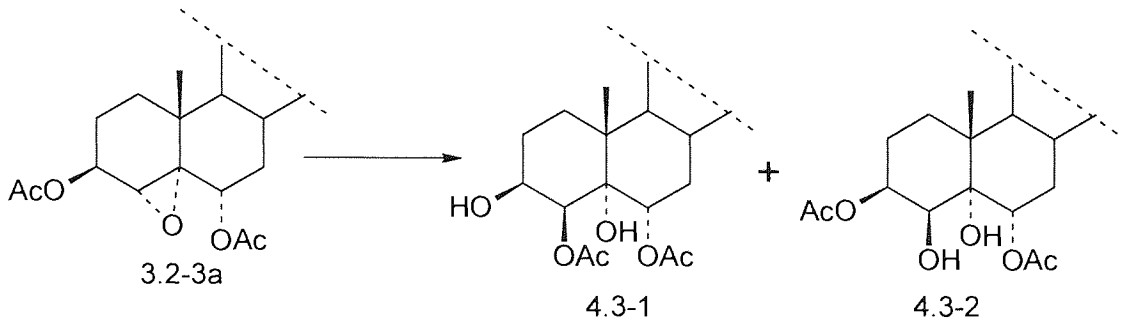
4.3 The 4,5-epoxycholestane-3β,6α-diol and its acetates

As discussed in the previous two sections, the axial 3α and 6β- hydroxyl group and acetates all exerted, to some extent, weak or strong influence on the orientation of ring

openings of the epoxides under a number of acidic conditions. In this part, we will discuss oxysterol epoxides with two equatorial hydroxyl groups and their acetate esters.

4.3.1 Formation of 5α-cholestane-3β,4β,5,6α-tetrol (4.3-6) and its esters:

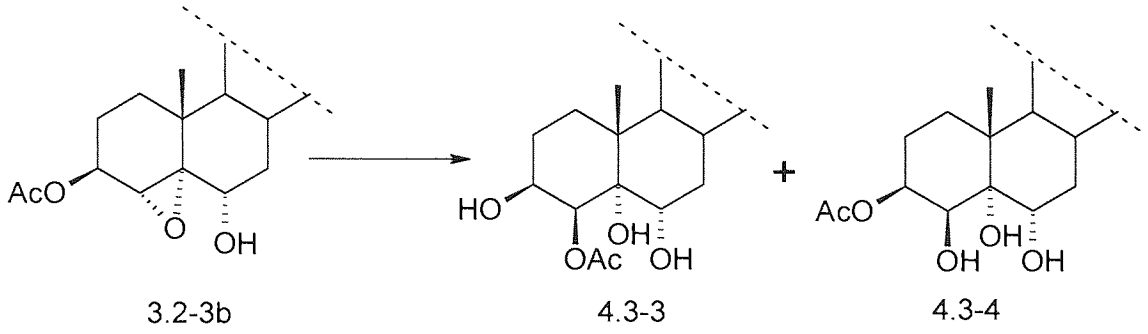
The reaction of perchloric acid in acetonitrile with 4α,5-epoxy-5α-cholestane-3β,6α-diol diacetate (3.2-3a) gave the 4β, 5α-tetrols diacetates 4.3-1 and 4.3-2 as the major product as shown in **Scheme 4.3-1**. Clearly, they are the products resulting from 3-acetyl participation. Combinations **1**, **3**, and **5** give the same results. Without the blockage of 6β-acetoxy group as that in compound 3.2-1a, the reaction of 3.2-3a with $HClO_4$ in THF is significantly accelerated, finished in 10 min with catalytic amount of perchloric acid (**Scheme 4.3-1** and **Table 4.3-1**). As to the 3-monoacetate (3.2-3b) prepared through epoxidation of the 3β-acetoxycholest-4-en-6α-ol, this type of epoxide cleavage occurs more frequently as combinations **1**~**5** all give the 4β, 5α-isomers 4.3-3 and 4.3-4 (**Scheme 4.3-2** and **Table 4.3-2**). When treated with boron trifluoride etherate in methanol, no solvent addition happens with 3.2-3a and 3.2-3b (**Scheme 4.3-3**).



Scheme 4.3-1 Epoxide 3.2-3a with combination **1,2,3,5**

Table 4.3-1 Reactions in **Scheme 4.3-1**

Procedure	Yield and purity	Reaction time
$HClO_4$ in THF	4.3-1 yield 77%	10 min
$HClO_4$ in CH_3CN	4.3-1 and 4.3-2 (5:4) combined yield 98%	10 min
BF_3 in THF	4.3-1 and 4.3-2 (1:2) combined yield 90%	90 min
$AlCl_3$ in THF	3.2-3a 25%, 4.3-1 51%, 4.3-2 5%	60 min

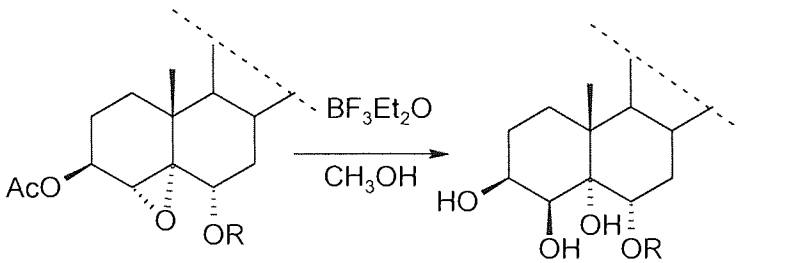


3.2-3b 4.3-3 4.3-4

Scheme 4.3-2 Epoxide 3.2-3b with combination **1-5**

Table 4.3-2 the reaction in **Scheme 4.3-2**

Procedure	Yield and purity	Reaction time
$HClO_4$ in THF	4.3-3 and 4.3-4 (4:6) combined yield 95%	10 min
$HClO_4$ in CH_3CN	4.3-3 and 4.3-4 (1:1) combined yield 98%	10 min
BF_3 in THF	4.3-3 yield 77%	30 min
BF_3 in CH_3CN	4.3-3 yield 82%	10 min
$AlCl_3$ in THF	4.3-3 and 4.3-4 (3:1) combined yield 83%	10 min



R=Ac 3.2-3a
R=H 3.2-3b

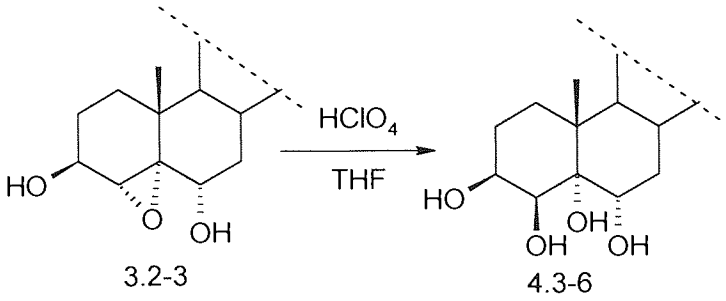
R=Ac 4.3-5 yield 85% 4hr
R=H 4.3-6 yield 91% 45min

Scheme 4.3-3 Epoxides 3.2-3a(b) with combination **7**

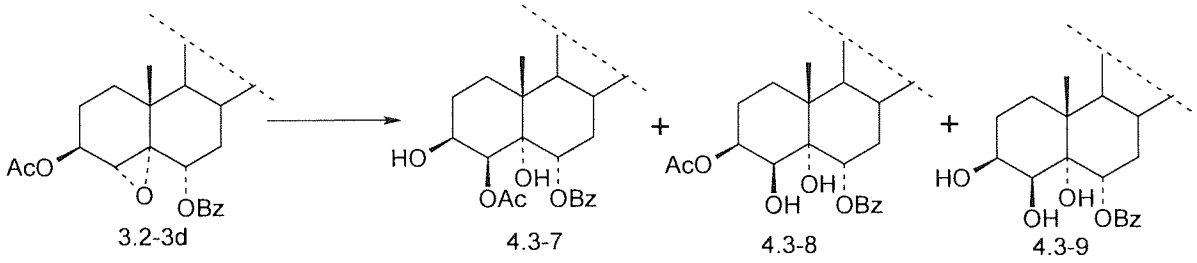
Without the 3β-acetyl group, the 4α,5-epoxy-5α-cholestane-3β,6α-diol (3.2-3) also furnished the stereo and electronic favoured tetrol 4.3-6 as the only product with more perchloric acid (more than 1 molar ratio) in THF and over a period of 4 days, the yield is around 70~75% (**Scheme 4.3-4**).

The 6-monoacetate congener of 3.2-3 (3.2-3c) cannot be prepared through selective acylation of 3.2-3 and selective hydrolysis of the 3,6-acetates 3.2-3a. The 4α,5-epoxy-5α-cholestane-3β,6α-diol 3-acetate 6-benzoate (3.2-3d) acts similar to the

diacetate 3.2-3a (**Scheme 4.3-5** and **Table 4.3-3**), however, with lower reaction speed. It is possible that the 6-benzoxy group exerts stronger steric hindrance effect than the acetate.



Scheme 4.3-4 Epoxide 3.2-3 with combination **1**



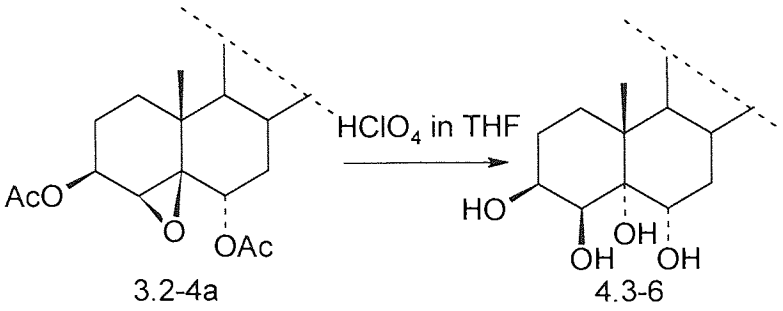
Scheme 4.3-5 Epoxide 3.2-3 with combination **1**, **2**, **3**, **5**, 7

Table 4.3-3 Reactions in **Scheme 4.3-5**

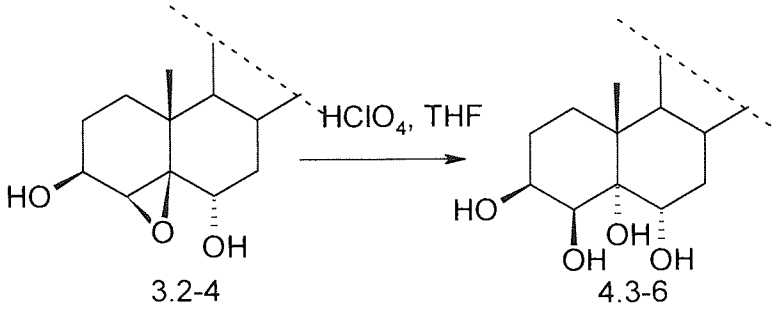
Procedure	Yield and purity	Reaction time
$HClO_4$ in THF	4.3-9 and 4.3-7 (6:1) combined yield 77%	4 days
$HClO_4$ in CH_3CN	4.3-9 yield 86%	3 hr
BF_3 in THF	3.2-3d 20% and 4.3-7 yield 80%	40 hr
$AlCl_3$ in THF	4.3-8 yield 20% and 4.3-7 yield 72	2 hr
BF_3 in CH_3OH	4.3-9 yield 99%	5 hr

In contrast, 4β,5-epoxy-5β-cholestane-3β,6α-diol diacetate (3.2-4a) furnished the 3β,4β,5α,6α-tetrol 4.3-6 as the only product in 86% yield after treating with perchloric acid in THF for 3 days (**Scheme 4.3-6**). Combination **3** and **5** gave no reaction with 3.2-4a. The free diol 3.2-4 also gave 83% yield 4.3-6 under the same condition (**Scheme 4.3-7**). Using combination 7 the 5α–methoxy triols were separated as main

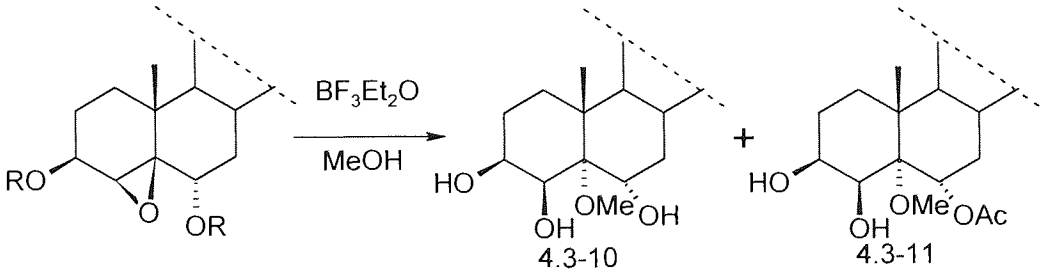
products (**Scheme 4.3-8** and **Table 4.3-4**). These results suggest that no acetyl participation occurred in cases of the β-epoxide ring opening.



Scheme 4.3-6 Epoxide 3.2-4a with combination **1**



Scheme 4.3-7 Epoxide 3.2-4 with combination **1**



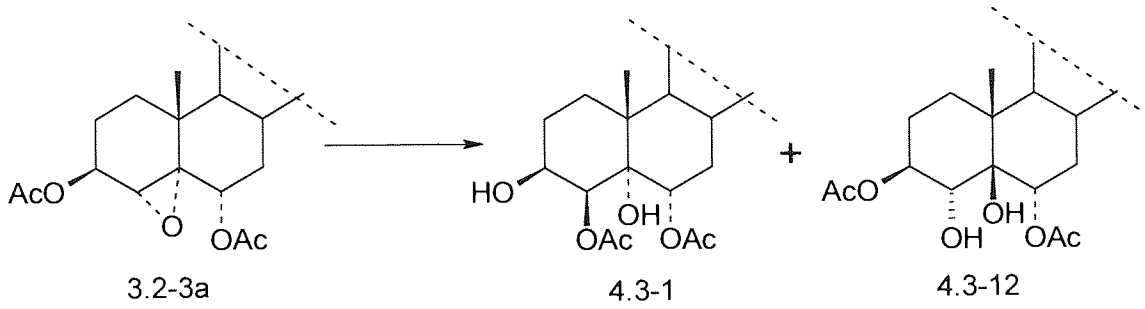
Scheme 4.3-8 Epoxides 3.2-4(a) with combination **7**

Table 4.3-4 Reactions in **Scheme 4.3-8**

Starting material	Yield and purity	Reaction time
3.2-4a (R=Ac)	4.3-10 and 4.3-11 (7:3) combined yield 82%	2 days
3.2-4 (R=H)	4.3-10 yield 85%	1 hr

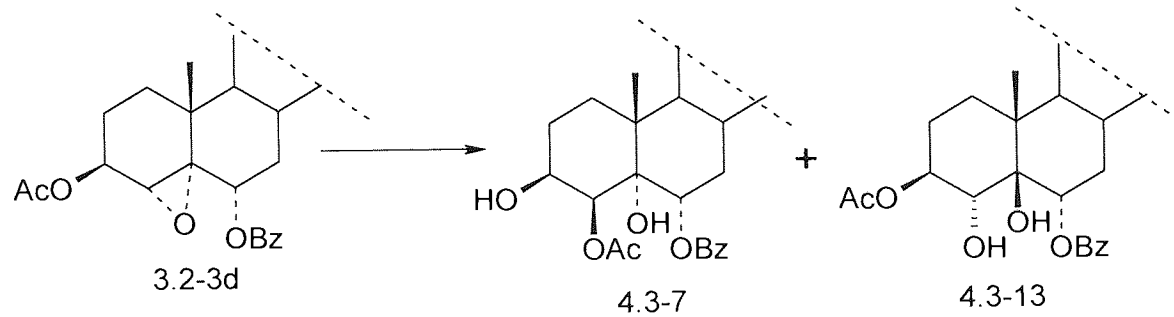
4.3.2 Reaction using Lewis acid in acetonitrile

By using $BF_3{\cdot}Et_2O$ or $AlCl_3$ in acetonitrile, the 4α,5-epoxy-5α-cholestane-3β,6α-diol diacetate (3.2-3a) gave a mixture of the 3β-acetoxy migration product 5α–tetrol diacetate 4.3-1 and the 3,5 acetyl participation product 4α,5β–isomer 4.3-12. With only the presence of 3-acetyl group (3.2-3b), no 5β-isomer was observed as shown in Scheme 4.3-2. Results with aluminium chloride are similar, except 3,4 acetyl participation dominated (**Scheme 4.3-9**). The 6-benzoyl ester 3.2-3d also give similar results (**Scheme 4.3-10**). Reaction in Scheme 4.3-9 and 4.3-10 are listed in **Table 4.3-5**.



3.2-3a 4.3-1 4.3-12

Scheme 4.3-9 Epoxide 3.2-3a with combinations **2**, **4**



3.2-3d 4.3-7 4.3-13

Scheme 4.3-10 Epoxide 3.2-3d with combinations **2**, **4**

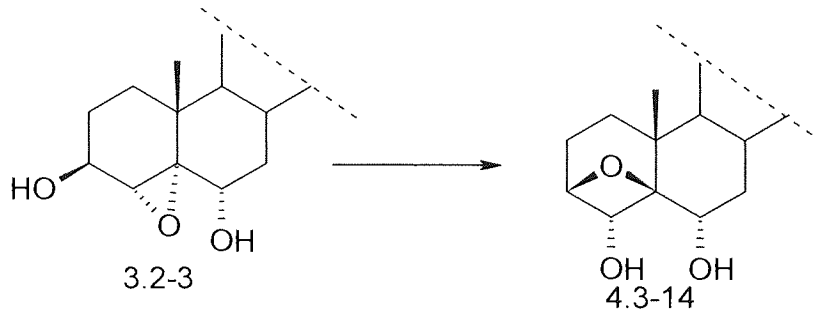
Table 4.3-5 Reactions in **Scheme 4.3-9** and **4.3-10**

Starting material	Yield and purity	Reaction time
3.2-3a, BF_3 in CH_3CN	4.3-1 and 4.3-12 (1:1) combined yield 95%	10 min
3.2-3a,$AlCl_3$ in CH_3CN	4.3-1 and 4.3-12 (20:7) combined yield 90%	1 hr
3.2-3d, BF_3 in CH_3CN	4.3-7 and 4.3-13 (21:48) combined yield 95%	1hr
3.2-3d,$AlCl_3$in CH_3CN	4.3-7 and 4.3-13 (7:1) combined yield 83%	2 hr

A careful study on the reaction of α-epoxide 3-acetate 6-benzoate 3.2-4d with boron trifluoride etherate in acetonitrile was carried out. When commercial ACS grade acetonitrile with additional 0.5% water was used, the only major product is the 5α-isomer 4.3-7 in over 80% yield with trace impurities. Using acetonitrile mixing with less than 0.2% water, a mixture of 5β-isomer 4.3-13 (28.0%) and the 4.3-7 (28.9%) was afforded. The result in Table 4.3-5 is by using commercially dried acetonitrile. When dry DCM was used, 4.3-13 (12.9%) and 4.3-7 (30.6%) was afforded.

The diacetate α-epoxide 3.2-3a is not as sensitive as this benzoate 3.2-3d toward water in this reaction. In commercial ACS reagent grade acetonitrile, the result is the same as that in the dry one.

The free diol α-epoxide 3.2-3 gave the 3,5-epoxide 4.3-14 in moderate yield with combinations **3**, **5** and **6** (**Scheme 4.3-11** and **Table 4.3-6**). This does not happen when 6β-hydroxyl exists, as it's a slow procedure compared to the formation of 5,6β-epoxides described in the two parts 4.1 and 4.2.



Scheme 4.3-11 Epoxide 3.2-3 with combination **2-6**

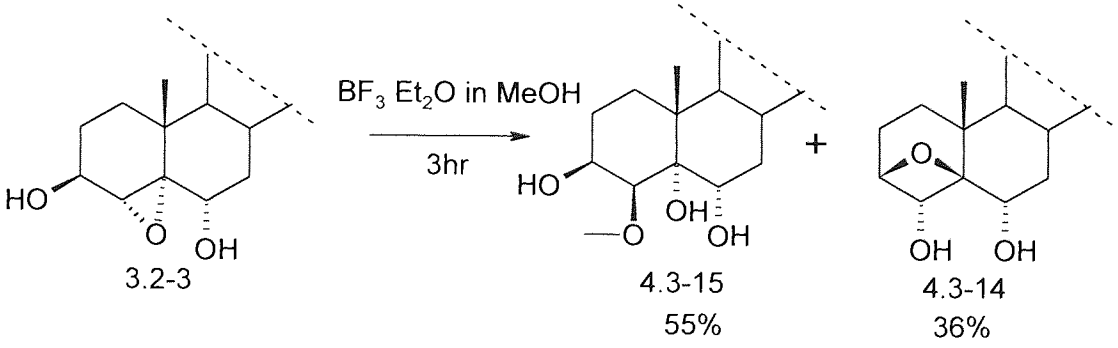
Table 4.3-6 Reactions in **Scheme 4.3-11**

Starting material	Yield and purity	Reaction time
$HClO_4$ in CH_3CN	Mess	30 min
$BF_3{\cdot}Et_2O$ in THF	48%	3 hr
$BF_3{\cdot}Et_2O$ in CH_3CN	Mess with trace of 4.3-14 on ^1H NMR	10min
$AlCl_3$ in THF	Give 67% 4.3-14	30 min
$AlCl_3$ in CH_3CN	Give 80% 4.3-14	30 min

As we discussed above, no reaction occurred when the β–epoxide diacetate 3.2-4a was treated with Lewis acid in THF; while in acetonitrile the diacetate 3.2-4a with combination **2**, **4** and **6** give complicated mixtures including mainly Ritter reaction products. The free diol β-epoxide 3.2-4 showed a very similar result to that of its 6β-hydroxyl 4α,5α-epoxide congener 3.1-1 in **Scheme 4.1-6**. The exact structure was not established yet.

4.3.3 Summary of the reaction with boron trifloride in methanol:

In case of 4α,5α-epoxide, with the presence of 3β-acetoxy group, 3.2-3a and 3.2-3b, the only products are 3-acetoxy participation products, see **Scheme 4.3-3**. In contrast when 3β-hydroxyl free compound 3.2-2 was treated with boron trifloride etherate in methanol, a mixture of products were generated, including methanol directly attacked product 4.3-17 and oxitane 4.3-13 (**Scheme 4.3-12**). In comparison with the reactions in **Scheme 4.3-1, 4.3-3** and **4.3-11,** it is concluded that the 3,5 hydroxyl participation is weaker than those acetyl participation, which totally block the solvent addition. In case of 4β,5β-epoxide, no acetyl or hydroxyl participation occurred as shown in **Scheme 4.3-8.**



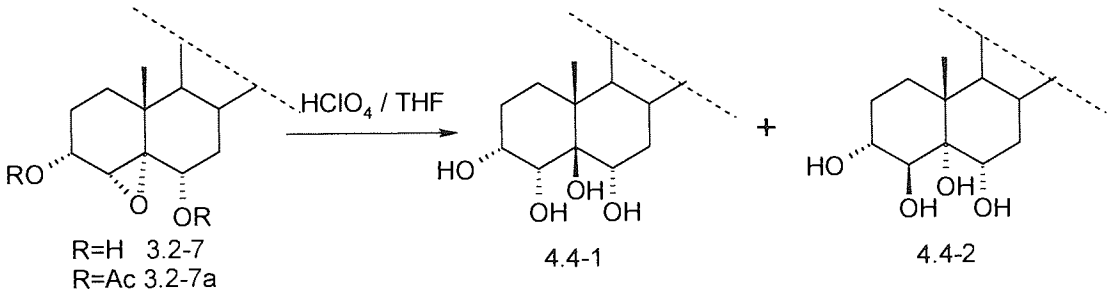
Scheme 4.3-12 Epoxide 3.2-3 with combination 7

From the results of part 4.3-1 to 4.3-3 it is concluded that the equatorial 3-acetoxy group gives strong participation to the α-epoxide ring opening reaction while the equatorial 6-acyl moiety give no participation effects and less steric effects than that of axial 6β-acyl group.

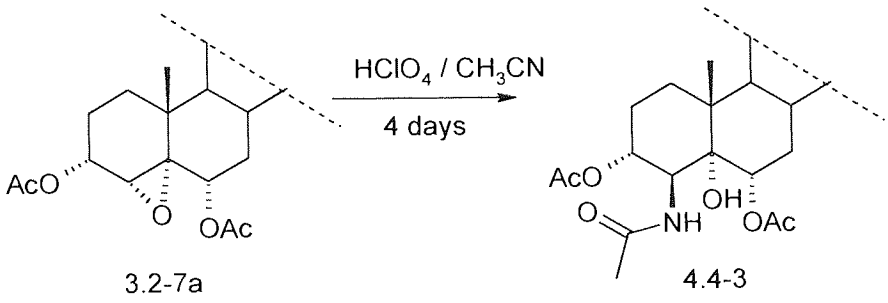
4.4 The 4,5-epoxycholestane-3α,6α-diol and its acetates

4.4.1 Ring opening with perchloric acid:

In THF, the reaction of the 4α,5-epoxy-5α-cholestane-3α,6α-diol (3.2-7) α-oxirane opening happened very slowly, in four days yielded a 9:2 mixture of 5β (4.4–1) and 5α tetrols (4.4-2). The overall yield of the two tetrols is 80%. The diacetate (3.2-7a) reacted much slower, in 10 days ended at the same products as from diol 3.2-7. The acetates may be hydrolysed first, before the ring opening (**Scheme 4.4-1**). In contrast, when the reaction was performed in acetonitrile the Ritter reaction product 4.4-3 is the only main one in 65% yield (**Scheme 4.4-2**).



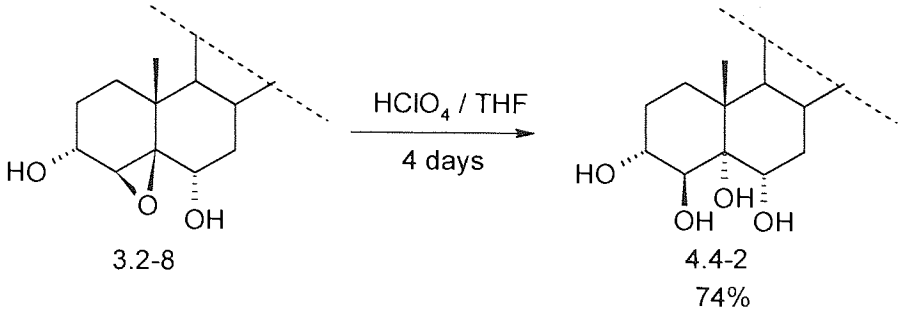
Scheme 4.4-1 Epoxides 3.2-7(a) with combination **1**



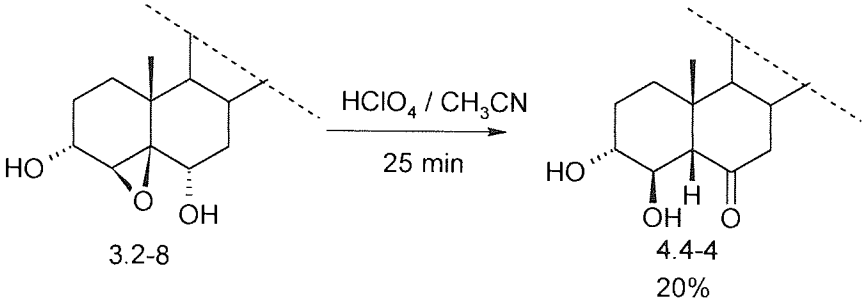
Scheme 4.4-2 Epoxide 3.2-7a with combination **2**

In THF the β-epoxide diol 3.2-8 gave the 5α isomer tetrol 4.4-2 only in 74% yield (**Scheme 4.4-3**). In acetonitrile 3.2-8 furnished a number of products. One of them is 3α,4β-dihydroxy-5β-cholestan-6-one (4.4-4) in 20% yield (**Scheme 4.4-4**).

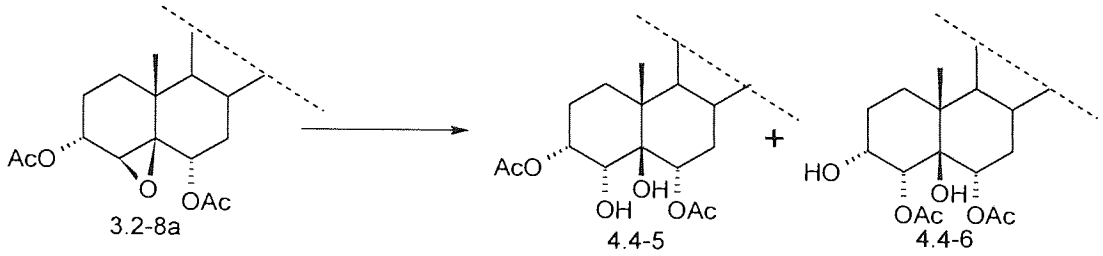
The β-epoxide 3,6-diacetate 3.2-8a gave the 5β-isomer product and the 3-acetoxy migration to C_4-OH was observed, especially when the reaction was carried out in acetonitrile (**Scheme 4.4-5** and **Table 4.4-1**).



Scheme 4.4-3 Epoxide 3.2-8 with combination **1**



Scheme 4.4-4 Epoxide 3.2-8 with combination **2**



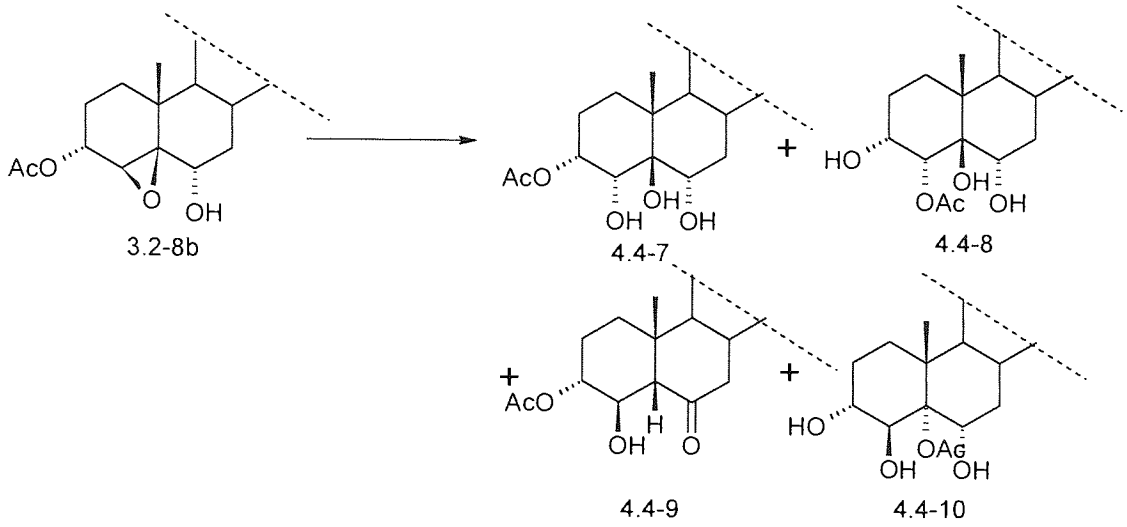
Scheme 4.4-5 Epoxide 3.2-8a with combination **1-2**

Table 4.4-1 Reactions in **Scheme 4.4-5**

Combinations	Products and yields	Time
$HClO_4$ / THF	4.4-5 92%, 4.4-6 3%	20min
$HClO_4$ / CH_3CN	4.4-5 56.5%, 4.4-6 33.5%	30min

When treated with $HClO_4$ in CH_3CN, the β-epoxide 3-monoacetate 3.2-8b and 6-monoacetate 3.2-8c both gave more complicated mixtures of products. For 3.2-8b, the 3-acetoxyl group exerts significant 3,4 and 3,5 participation effects and acetates of 4.4-1 and 4.4-2 were generated as main products. Products from 3.2-8c include

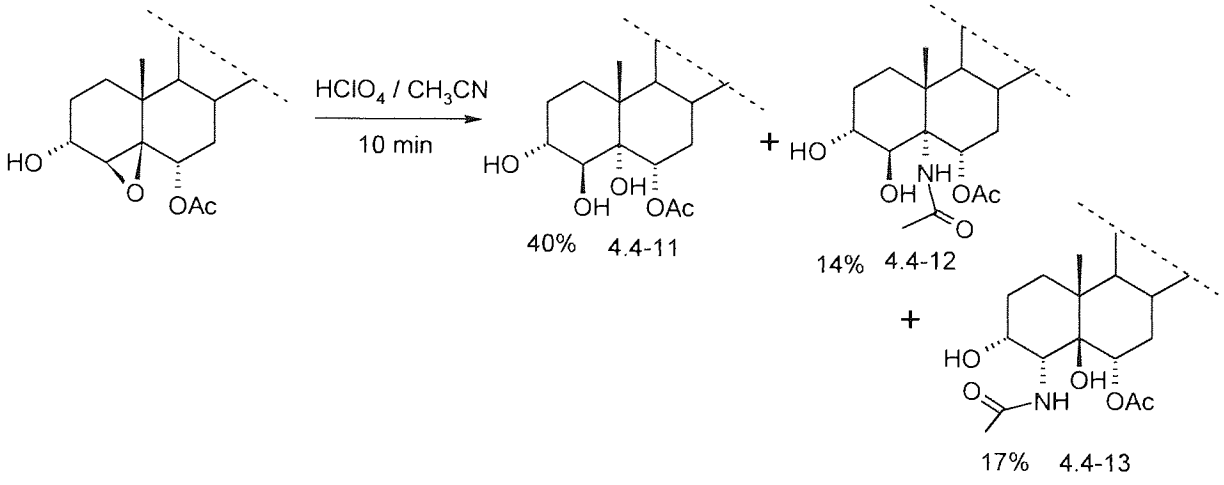
acetamides from Ritter reaction. In THF the reaction of 3.2-8b only gave the 5β-tetrol derivatives (**Scheme 4.4-6, 4.4-7** and **Table 4.4-2**).



Scheme 4.4-6 Epoxide 3.2-8b with combination **1-2**

Table 4.4-2 Reactions in **Scheme 4.4-6**

Combinations	Products and yields	Time
$HClO_4$ / THF	4.4-7 87%, 4.4-8 13%	10min
$HClO_4$ / CH_3CN	4.4-7 24%, 4.4-8 22%, 4.4-9 12%, 4.4-10 21%	10min

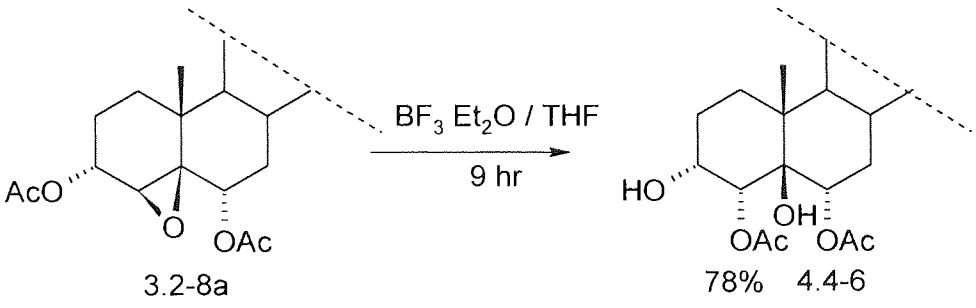


Scheme 4.4-7 Epoxide 3.2-8c with combination **2**

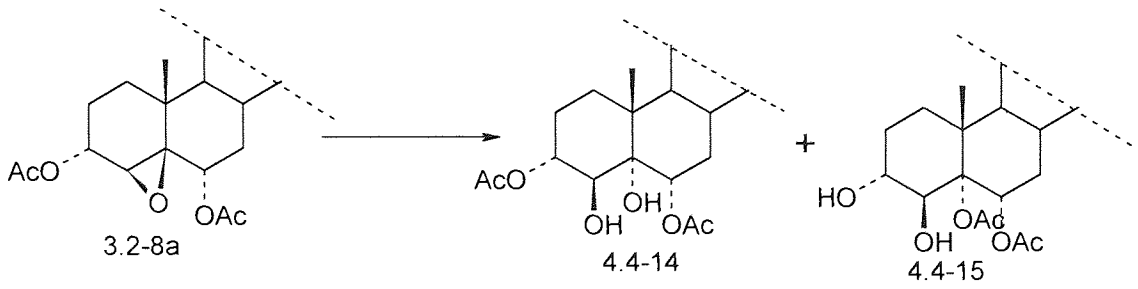
4.4.2 Reaction with boron trifloride etherate or aluminum chloride:

The α–epoxide diacetate 3.2-7a did not respond to both Lewis acids, BF_3 and $AlCl_3$, in THF. The α–epoxide free diol 3.2-7, on other hand, gave messy mixtures under the same conditions. While in acetonitrile, both starting materials, 3.2-7 and 3.2-7a, furnished a Ritter reaction mixture of acetamides.

In THF with boron trifloride etherate, the β-epoxide 3α,6α-diacetate (3.2-8a) gave the 5β-tetrol diacetate 4.4-6 in 78% yield (**Scheme 4.4-8**). When the THF was replaced by acetonitrile, the 3,5-participation dominated the reaction and the 5α-tetrol diacetates 4.4-14 and 4.4-15 were given. With aluminium chloride in acetonitrile over a period of 1hr, over 90% yield of 3,6-diacetate 5α-tetrol 4.4-14 was obtained from 3.2-8a (**Scheme 4.4-9** and **Table 4.4-3**). Furthermore, with aluminium chloride in THF, 3.2-8a reacts very slowly, 70% starting material left over 8hr. It gave a mixture of 5α and 5β-tetrol esters 4.4-5 (10%), 4.4-6 (9%) and 4.4-14 (2%) (**Scheme 4.4-10**). It means that the acetonitrile is important in compelling the 3,5-acetyl participation.



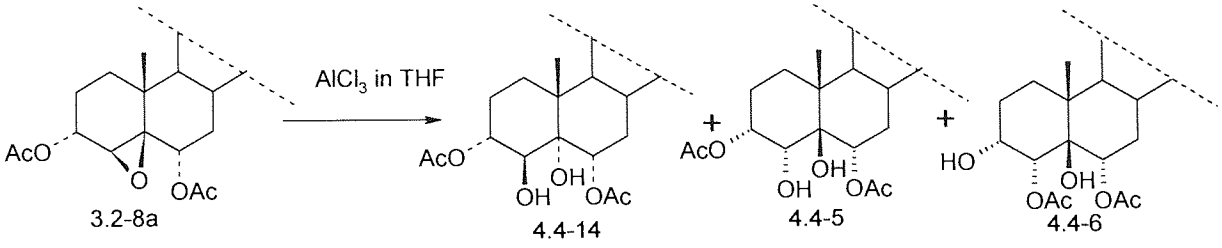
Scheme 4.4-8 Epoxide 3.2-8a with combination **3**



Scheme 4.4-9 Epoxide 3.2-8a with combination **4**, **6**

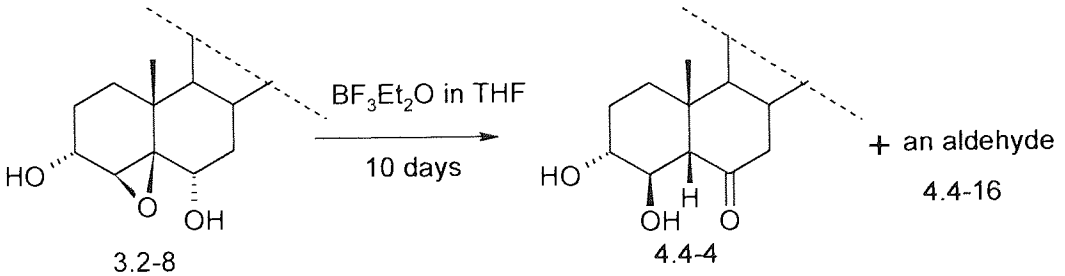
Table 4.4-3 Reactions in **Scheme 4.4-9**

Combinations	Products and yields	Time
$BF_3 \cdot Et_2O$ / CH_3CN	4.4-14 60%, 4.4-15 36%	30min
$AlCl_3$ / CH_3CN	4.4-14 over 90%	1hr



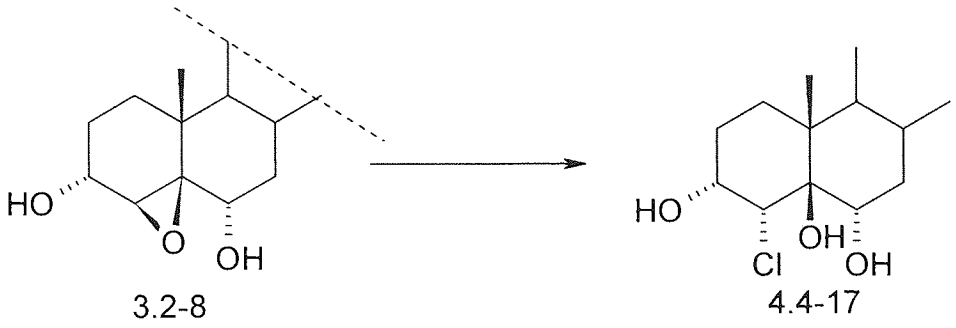
Scheme 4.4-10 Epoxide 3.2-8a with combination **5**

The free diol 3.2-8 was also tested with these Lewis acids. In THF the boron trifluoride etherate give higher yield of the 6-one 4.4-4 (50%) compared with that of the perchloric acid one in **Scheme 4.4-4**. The reaction speed is quite slow as still 5% starting material was found in the resulting mixture over 10 days. Another product in 33% yield was postulated from NMR spectra to be a ring contracted product aldehyde (4.4-16) (**Scheme 4.4-11**). In acetonitrile with $BF_3 \cdot Et_2O$ only 14% 6-one 4.4-4 was given with many unidentified Ritter reaction products. As compared to the high yields of 6-one from 6β-isomers under the same condition, it is concluded that the 6-5 *cis* hydrogen migration is much less favoured.



Scheme 4.4-11 Epoxide 3.2-8 with combination **3**

High yield of the 4α-chlorotriol 4.4-17 was give when 3.2-8 was treated with $AlCl_3$ in THF or acetonitrile (**Scheme 4.4-12** and **Table 4.4-4**). 4.4-17 is not stable on silica, about 20% of it converted back to the starting material 3.2-8 after chromatography. Pure sample was afforded from recrystallisation twice from methanol.



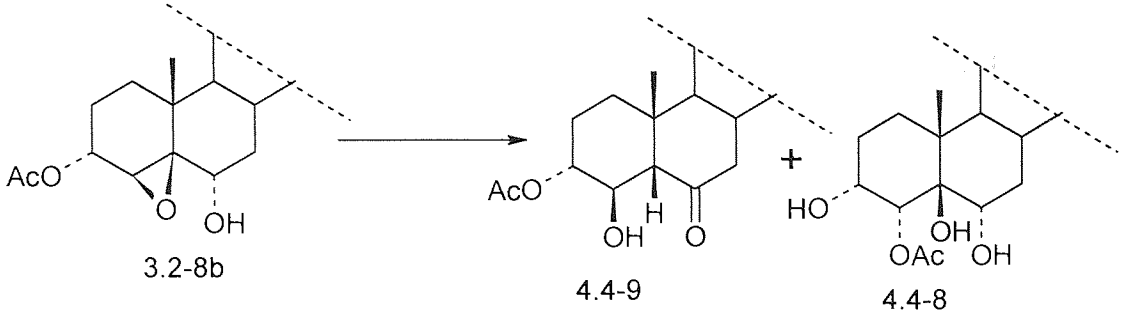
Scheme 4.4-12 Epoxide 3.2-8 with combination **5,6**

Table 4.4-4 Reactions in **Scheme 4.4-12**

Conditions	Products and yields	Time
$AlCl_3$ / THF	4.4-17 90%	30min
$AlCl_3$ / CH_3CN	4.4-17 80%	30min

Compared with the results of other isomers, 3.2-3a and 3.2-5a, reactions (**Scheme 4.4-5, 4.4-9**) show the only complete overturn from 3,4-acetyl migration to a 3,5 migration happened with 3α and 6α-acetoxyl β-epoxide, when a protic acid was changed to a Lewis acid in acetonitrile or THF was replaced by acetonitrile in Lewis acid catalysed reactions. The possible reason is that both the lesser steric effects of the 6α-acetoxyl group and the 3α-axial configuration of the 3-acetoxyl group exerted a combined effect. To further explain this, the monoacetates are treated with the same conditions.

The 4β,5β-epoxide 3-acetate (3.2-8b) showed the competition of the 6-one (4.4-9) formation with the 3,4 acetyl participation (generation of 4.4-8) when treated with boron trifloride etherate in THF. In acetonitrile, the 6-one formation was still the main product, however several Ritter acetamide products emerged. The aluminium chloride gave only the 6-one 4.4-9, yield 82% and 73% in THF and acetonitrile respectively (**Scheme 4.4-13** and **Table 4.4-5**).

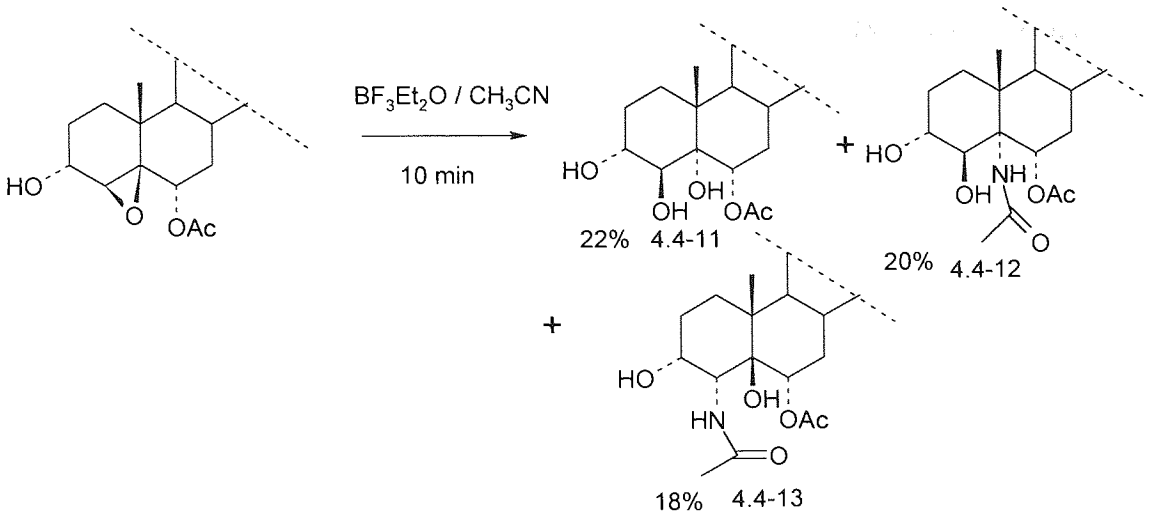


Scheme 4.4-13 Epoxide 3.2-8b with combination **3-6**

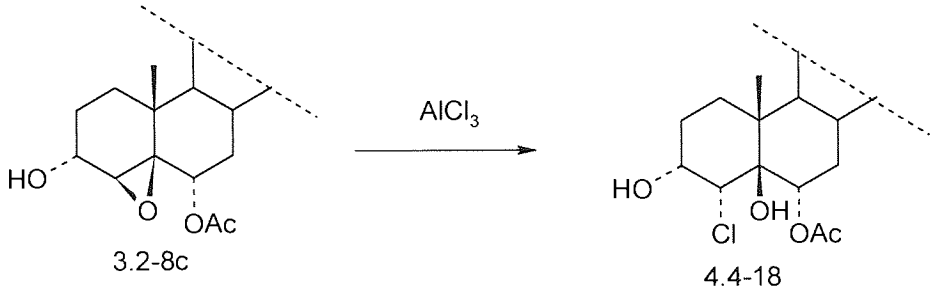
Table 4.4-5 Reactions in **Scheme 4.4-13**

Conditions	Products and yields	Time
$BF_3{\cdot}Et_2O$ / THF	4.4-9: 4.4-8 = 89:11 combined yield 90%	24hr
$BF_3{\cdot}Et_2O$ / CH_3CN	4.4-9 yield 34% , 4.4-8 yield 8% acetamides 40-50%	10min
$AlCl_3$ / THF	4.4-9 82%	20min
$AlCl_3$ / CH_3CN	4.4-9 73%	20min

The β-epoxide 6-monoacetate (3.2-8c) did not react with boron trifloride in THF; while in acetonitrile it gave the 5α-tetrol ester 4.1-11 and Ritter products 4.1-12, 4.1-13 (**Scheme 4.4-14**). This reaction is similar to that of protic acid (**Scheme 4.1-7**), except the acetyl migration product decreased. A possible 6-5 acetyl participation may be responsible for the formation of the tetrol ester 4.1-11, otherwise only acetamides can be generated. However, with aluminium chloride, 3.2-8c furnished the 4α-chloro compound only (**Scheme 4.4-15**). This reaction of 3.2-8c is quite similar to that of the free diol 3.2-8. And it suggested that the 6-acetyl group exerted little effects in this case. Above all, the 3-4, 3-5 and a possible 6-5 acetyl migration or participation can be affected greatly by both the type of acid catalysts and solvents. This is a good starting point that new acid – solvent combinations can be developed to control the direction of epoxide ring open. The 3 acetyl group also gives steric block to prevent the formation of chloride.



Scheme 4.4-14 Epoxide 3.2-8c with combination **4**



Scheme 4.4-15 Epoxide 3.2-8c with combination **5,6**

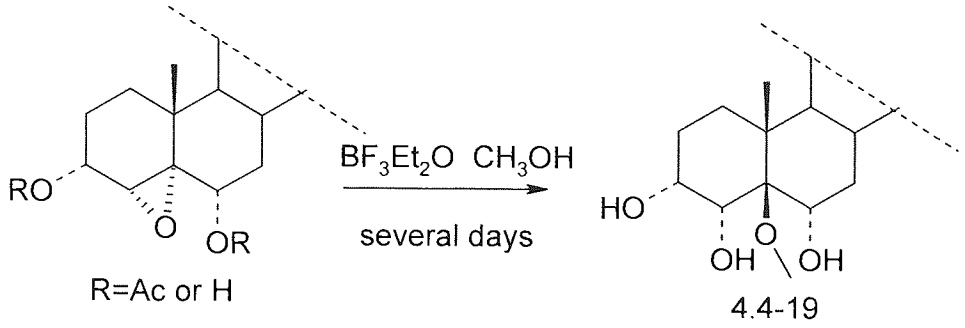
Table 4.4-6 Reactions in **Scheme 4.4-15**

Conditions	Products and yields	Time
$AlCl_3$ / THF	4.4-18 83%	90min
$AlCl_3$ / CH_3CN	4.4-18 93%	10min

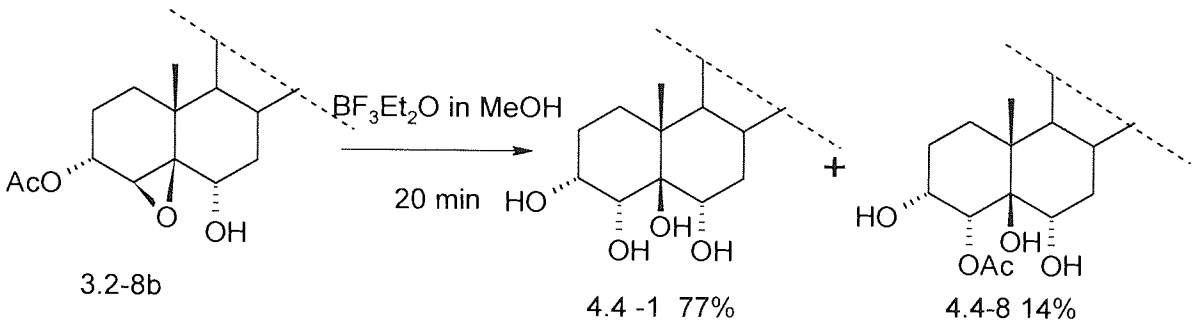
4.4.3 With boron trifloride etherate in methanol

The α-epoxide diol 3.2-7 and its diacetate 3.2-8 gave the 5β-methoxy product 4.4-19 (**Scheme 4.4-16**), also the β-epoxide 3-acetate 3.2-8b gave the 5β tetrol products 4.4-1 and 4.4-8 in high yields (**Scheme 4.4-17**). Diacetate 3.2-8a gave 6-monoacetate 4.4-20 as the only product in 4 hr (**Scheme 4.4-18**). The 3,5-acetyl participation did not happen in this case. When the 3-hydroxyl group is free, a direct attack of solvent methanol

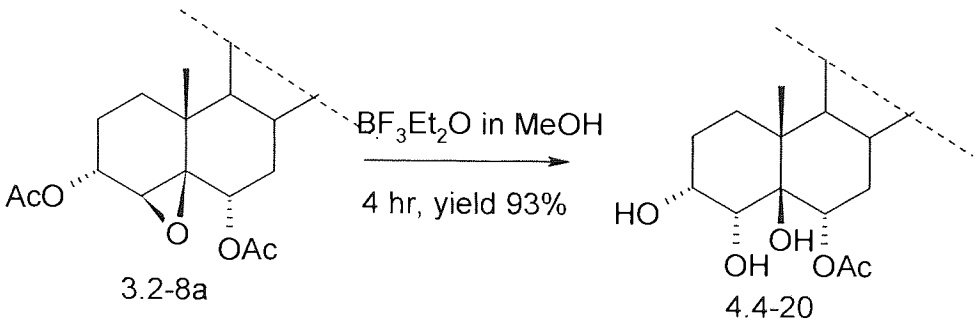
furnished the 5α-methoxyl compound 4.4-21 (**Scheme 4.4-19**). These reactions demonstrated more about the 3-acetyl participation.



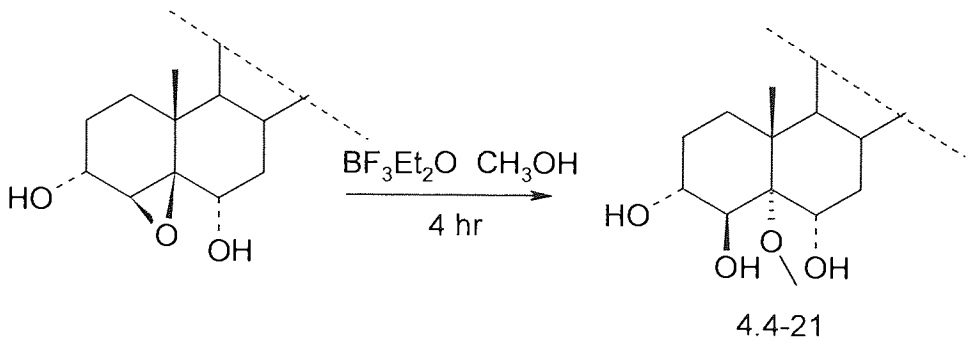
Scheme 4.4-16 Epoxides 3.2-7(a) with combination 7



Scheme 4.4-17 Epoxide 3.2-8b with combination 7



Scheme 4.4-18 Epoxide 3.2-8a with combination 7

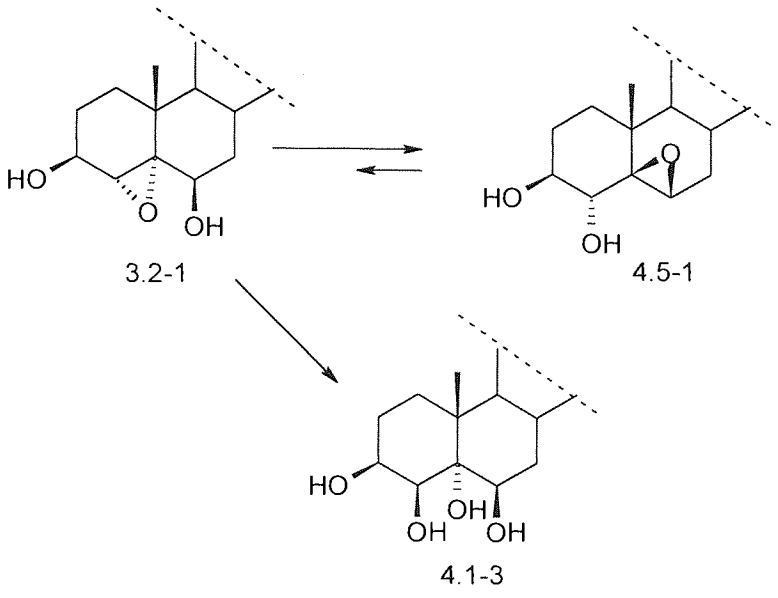


Scheme 4.4-19 Epoxide 3.2-8 with combination 7

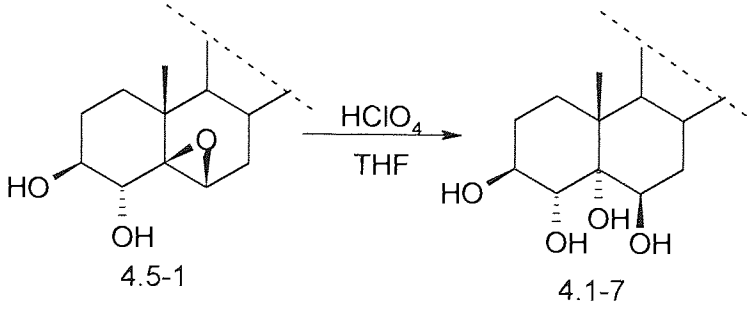
4.5 The Payne rearrangements of the 4,5-epoxycholestane-3, 6-diols (3.2-1 ~3.2-8):

Normally, the hydrolysis of the acetates of these 4,5-epoxides 3.2-1a ~ 3.2-8a into free diols 3.2-1~3.2-8 at room temperature finished in one hour. When we do the hydrolysis of the diacetate of 4α,5-epoxy-5α-cholestane-3β,6β-diol (3.2-1) by heating at reflux with excess sodium hydroxide in ethanol and water over a period of 30 minutes, the major product was a new epoxide: the 5,6β-epoxy-5β-cholestane-3β,4α-diol (4.5-1) (**Scheme 4.5-1**). As mentioned in part 4.1, the perchloric acid catalysed *cis*-cleavage is believed to go through the intermediate 4.5-1, which was not separated in that case (**Scheme 4.1-5**). 4.5-1 did give the ring opened product 4.1-7 in high yield when it was treated with perchloric acid in THF this time (**Scheme 4.5-2**).

The epoxide rearrangement of this type is called the Payne rearrangement. (Payne 1962, Page et al 1990).



Scheme 4.5-1 Payne rearrangement of epoxide 3.2-1 in NaOH /aq. EtOH

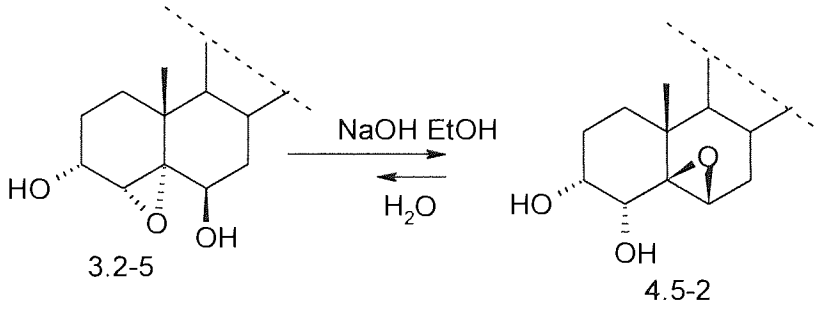


Scheme 4.5-2 Epoixde 4.5-1 with combination **1**

The basic media of 5,6β-epoxy-5β-cholestan-3α-ol (3.1-16) preparation used in the further studies on the temperature effects to this rearrangement were NaOH (7.5mmol) in ethanol (4.0ml) and water (2.0ml) with the 0.4 mmol epoxide. We observed:

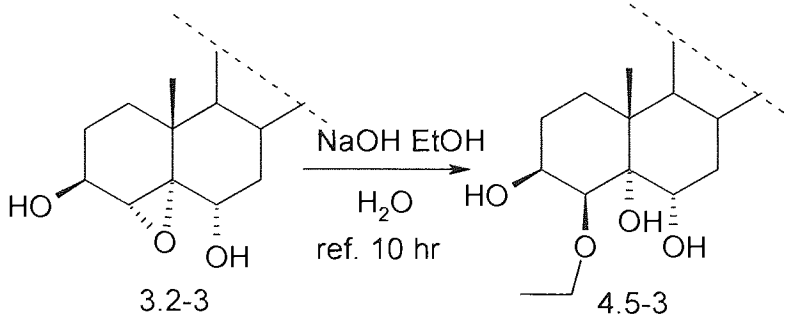
(1) At room temperature, the reaction is quite slow and the equilibrium of the two epoxide (4,5-epoxide 3.2-1: 5,6-epoxide 4.5-1 = 1:9 molar ratio on HNMR) was reached after 2 weeks, with only trace tetrol on TLC; (2) when the reaction mixture was heated at reflux, the equilibrium was set up in 15minutes, with less than 5% tetrol generated, after 5 hours over 60% in the reaction system is the tetrol and other non-polar impurities appeared.

When the C-3 hydroxyl group is α configuration, the balance is reached at 1hr at reflux and there is no sign of tetrols, the molar ratio is 15:1 (5,6 epoixde 4.5-2 : 4,5 epoxide 3.2-5). After prolonged reflux (over 5hr), trace tetrol 4.2-1a was detected and many none polar impurities also generated. After 20 hrs, the reaction mixture turned dark, and NMR told us that the impurities became the main components (**Scheme 4.5-3**).



Scheme 4.5-3 Payne rearrangement of epoxide 3.2-5 in NaOH /aq. EtOH

In case of *cis* 6α-hydroxyl group in 3.2-3, the epoxide only gave the 4β-ethoxy compound 4.5-3 in 60% yield (**Scheme 4.5-4**).



Scheme 4.5-4 Epoxide 3.2-3 in NaOH /aq. EtOH

4.6 Preparation of 3,4,5,6-tetrol with *cis* 4,5-dihydroxyl group

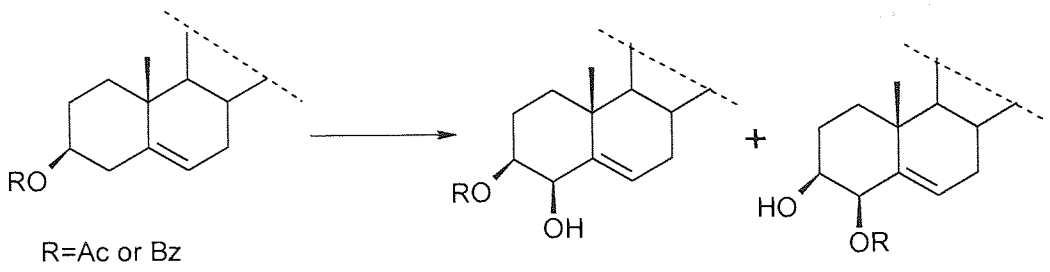
4.6.1 From cholest-5-ene-3β(α), 4β-diol

In the parts 4.1-4.4, all the eight 4,5-*trans* cholestan-3,4,5,6-tetrols were prepared by using the acyl or hydroxyl participation effects when performing the epoxide ring opening reactions of 4,5-epoxycholestane-3,6-diols (3.2-1~3.2-8) and their esters. The two 4α,5α-tetrols with a 6β-hydroxyl group (4.1-7 and 4.2-4) are also synthesised by using the 6β-hydroxyl group participation cleavage of the relevant 4α,5α–epoxides. The 3α-axial OH may also give this effect but it has not been determined (**Scheme 4.2-15**). So the preparation of 4,5-*cis* cholestan-3,4,5,6-tetrols from starting material other than these 4,5-epoxycholestane-3,6-diols is worthy to be studied.

As we are looking for an alternative way to reach the 4β,5β-configuration cholestan-3,4,5,6-tetrols, the cholest-5-en-3β,4β-diol derivatives can serve as the ideal starting material if the ring opening of 5,6-epoxy gives the 5β,6α configuration and the oxidation – reduction at C-6 gives the 6(α)β hydroxyl group that we want.

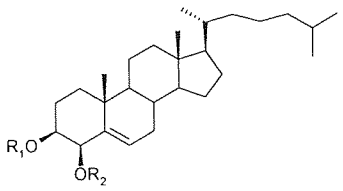
The selenium oxide oxidations of cholesterol and their esters are well documented, but like other oxidation-reduction, the mechanism is not fully understood yet. Shibuya et al (1994) showed that when formic acid served as the catalyst, the reaction time was shortened significantly with improvement in yield. A series of compounds were prepared with this method (**Scheme 4.6-1**).

The changes of NMR spectra upon the esterification of the two hydroxyls, C-3 OH and C-4 OH, were studied. As shown in **Table 4.6-1**, the signal of 19-CH_3 shifted to high field when the 4-hydroxyl group was esterified, especially with acetate; the 3-ester group shifted the signal of 19-CH_3 to lower field with the benzoyl group as the more effective one. The shift of C3-C6 signals also happened regularly and 3-monoesterification in 4.6-2 and 4.6-5 rendered the relative carbon low field shift, but the effect is blocked by double esterification of both hydroxyls.



Scheme 4.6-1 the selenium oxide hydroxylation of cholesterol esters

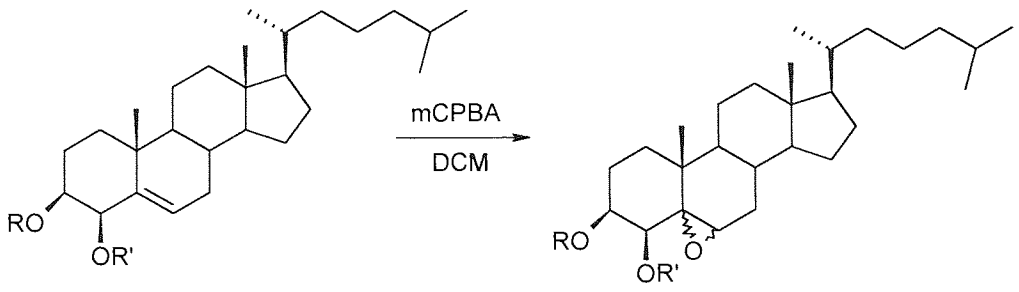
Table 4.6-1 The NMR shift (ppm) of the cholest-5-ene-3β,4β-diol derivatives



	R1	R2	C3	C4	C5	C6	19-CH_3
4.6-1	H	H	-	-	-	-	1.15
4.6-2	Ac	H	75.3	75.4	141.4	129.2	1.20
4.6-3	H	Ac	71.4	79.0	138.8	131.2	1.07
4.6-4	Ac	Ac	72.7	75.8	138.1	131.5	1.11
4.6-5	Bz	H	75.6	76.0	141.4	129.6	1.24
4.6-6	H	Bz	71.8	79.8	138.8	131.7	1.18
4.6-7	Ac	Bz	72.8	76.4	138.1	132.0	1.22
4.6-8	Bz	Ac	73.5	75.9	138.2	131.7	1.16
4.6-9	Bz	Bz	-	-	-	-	1.25

As observed on the cholest-4-en-3,6-diols that the benzoyl ester exerts a less bulky or maybe co-ordination effect, so different acetyl and benzoyl esters were also prepared and the epoxidation was carried out with mCPBA (**Scheme 4.6-2**). The result is showed in the **Table 4.6-2**. It clearly indicated that on the epoxidation of the 5,6-double bond, the benzoyl group exerted the same effects as acetyl esters on the 4β–hydroxyl group; however, an additional ester group on the 3β-OH increased the β-epoxide content significantly. The acetyl gave a little higher β percent than the benzoyl one. This change

is possible due to the lost of hydrogen bonding and the 4-acyl group becomes more flexible.



Scheme 4.6-2 Epoxidation of cholest-5-en-3β,4β-diol and esters

Table 4.6-2 The mCPBA epoxidation of cholest-4-en-3β,4β-diol derivatives.

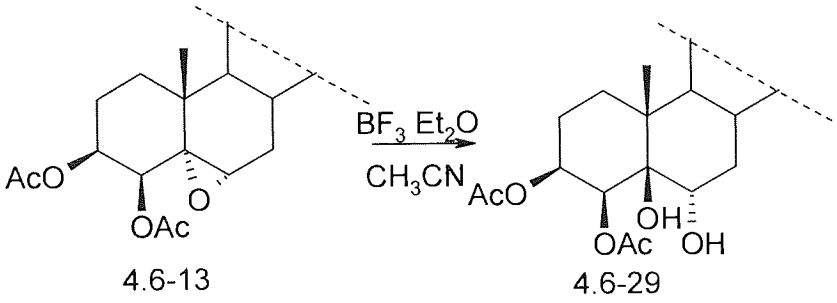
	R1	R2	α:β ratio	Product codes* α	β	Total yield	Products overview
4.6-1	H	H	31:69	4.6-10	4.6-19	87%	
4.6-2	Ac	H	28:72	4.6-11	4.6-20	>90%	With acetyl migration 3ac:4ac 6:1 (the β-epoxide)
4.6-3	H	Ac	92:8	4.6-12	4.6-21	98%	With acetyl migration 3ac:4ac 13:87 for α epoxide, 3ac observed as the main one with β-epoxide
4.6-4	Ac	Ac	80:20	4.6-13	4.6-22	98%	
4.6-5	Bz	H	30:70	4.6-14	4.6-23	95%	No acyl migration occurred with the benzoyl group.
4.6-6	H	Bz	93:7	4.6-15	4.6-24	98%	As above
4.6-7	Ac	Bz	86:14	4.6-16	4.6-25	95%	
4.6-8	Bz	Ac	81:19	4.6-17	4.6-27	95%	
4.6-9	Bz	Bz	86:14	4.6-18	4.6-28	90%	

* The product with acetyl migration not considered

The compounds among 4.61-4.6-9 with free 4β–OH gave a rise of a β-epoxide only in a high yield with TBHP/$VO(acac)_2$. However, when only 3β-OH is free and 4β–OH

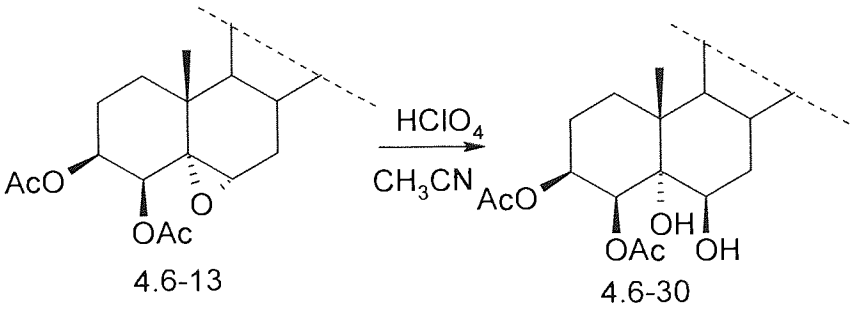
was esterified, the reaction became sluggish with many impurities. The α-epoxides were prepared after flash chromatography from the products of mCPBA epoxidation on the 4-acetoxy compounds 4.6-3 or 4-benzoyl ester 4.6-6.

The 5α,6α- and 5β,6β-epoxide diacetates 4.6-13 and 4.6-22 were chosen for the studies of the epoxide ring opening. The reaction of 5α,6α-epoxide always happened faster than 5β,6β-epoxide when the reaction was carried out in acetonitrile. Only the 5α,6α–epoxide was treated with boron trifluoride etherate in acetonitrile or acetonitrile / acetic acid (1/1) to give a single product and was assigned with the 5β,6α-dihydroxyl, the isomer we want (Scheme 4.6-3). When the solvent changed to acetic acid only, the result was a messy mixture.



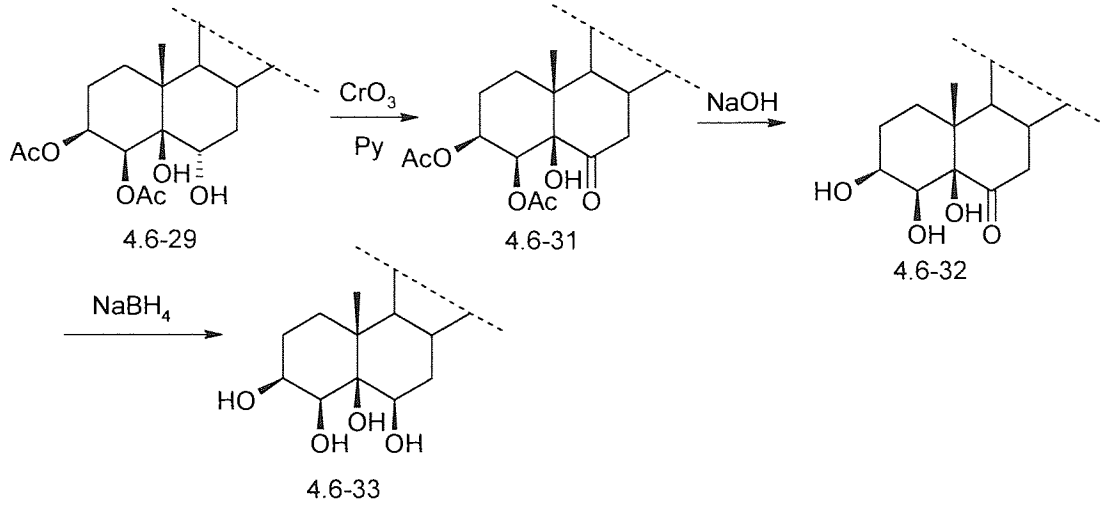
Scheme 4.6-3 Epoxide 4.6-13 with combination **4**

The reaction performed with $HClO_4$ in acetonitrile gave the major tetrol (4.6-30) with 5α , 6β-dihydroxyl group in 65% yield, minor by-products were unidentified (**Scheme 4.6-4**).



Scheme 4.6-4 Epoxide 4.6-13 with combination **1**

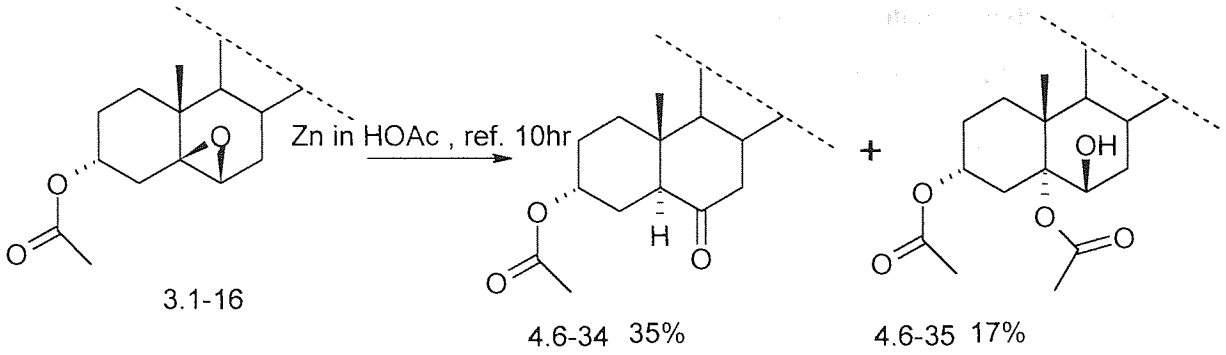
The 3β,4β,5β,6β-tetrol was difficult to obtained directly. However, it was prepared in a good yield through oxidation of 6-hydroxyl of 4.6-30 to the ketone followed by hydrolysis and $NaBH_4$ reduction (**Scheme 4.6-5**).



Scheme 4.6-5 Preparation of the 5β-cholestane-3β,4β,5,6β-tetrol

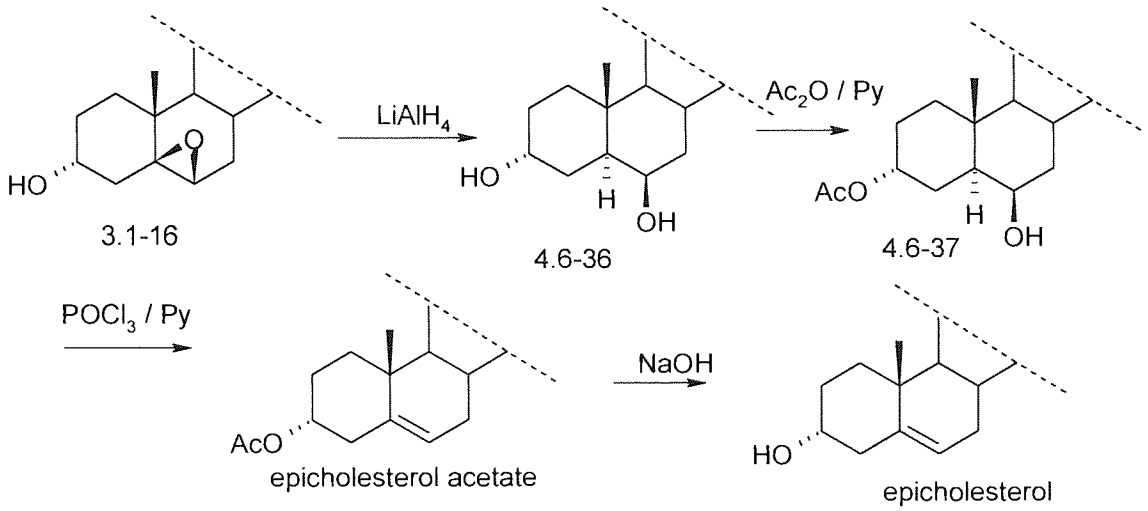
As described in Part 2, the preparation methods reported in literature for epicholesterol generally are not suitable for the laboratory scale preparation due to expensive or specially prepared reagent. The method we developed for preparation of 5, 6β-epoxy–5β–cholestan-3α-ol (3.1-16) filled this gap as it convenient and productive; furthermore, 5, 6β-epoxy–5β–cholestan-3α-ol can serve as the starting material to synthesize a pool of epicholesterols.

As it was described, a number of reagents can be used for the reduction of the epoxide to the double bond; we tested nearly all of them on 5, 6β-epoxy–5β–cholestan-3α-ol (3.1-16). None of them gave a clean reaction, including the iodine / Ph_3P, $AgNO_3$ on neutral alumina and chlorotrimethylsilane, sodium iodide. The classical method, Zn/HOAc, did give two separable products as shown in the **Scheme 4.6-6**, no epicholesterol generated. The structure of the 6-one was determined by the reduction to 6β-ol, which was a known standard sample.



Scheme 4.6-6 Attempt reduction of 3.1-16 by Zinc dust in acetic acid

After a process of trial and error, finally the two-step procedure was furnished for the preparation of epicholesterol (**Scheme 4.6-7**).



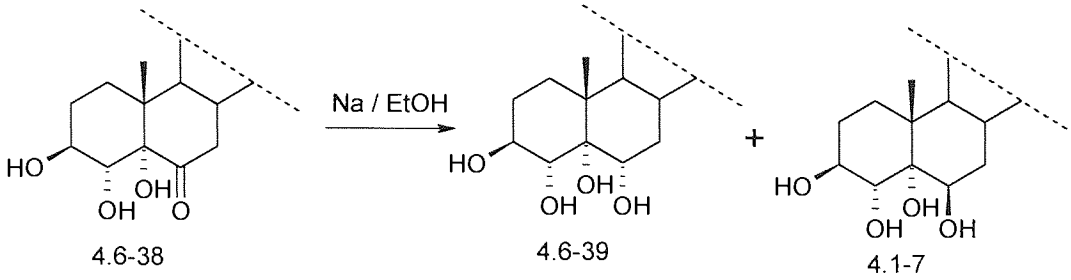
Scheme 4.6-7 Preparation of epicholesterol from 3.1-16

To our surprise, the selenium oxide oxidation of epicholesterol failed to give the desired cholest-5-en-3α,4β-diol, so did epicholesterol acetate in attempt to prepare the 4β-hydroxyl epicholesterol or epicholesterol acetate. Yellow brown oil generated as the resultant contained at least 15 compounds from TLC.

4.6.2 The reduction of ketone in preparation of cholestane-3,4,5,6-tetrols

Oxidation and reduction on a single hydroxyl group were carried to give other cholestane-3,4,5,6-tetrols. The two 6-one sterols 4.6-38 and 4.6-40 are prepared from the tetrols 4.1-7 and 4.2-1a with NBS oxidation in aqueous THF with yield over 80% in

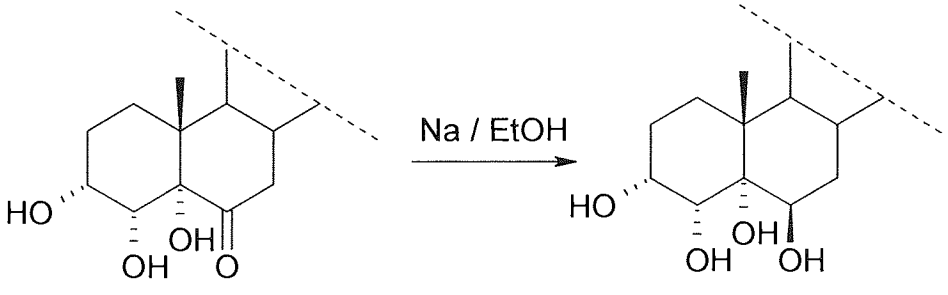
each case. Reduction by sodium in alcohols, which often generated a hydroxyl in different configuration from that from sodium borohydride reduction, did not give desired 6α-tetrols in good yield or not at all (**Scheme 4.6-8** and **Scheme 4.6-9**).



4.6-38 4.6-39 4.1-7

total yield 60% , mole ratio of 4.6-39 and 4.1-7 is 1:1

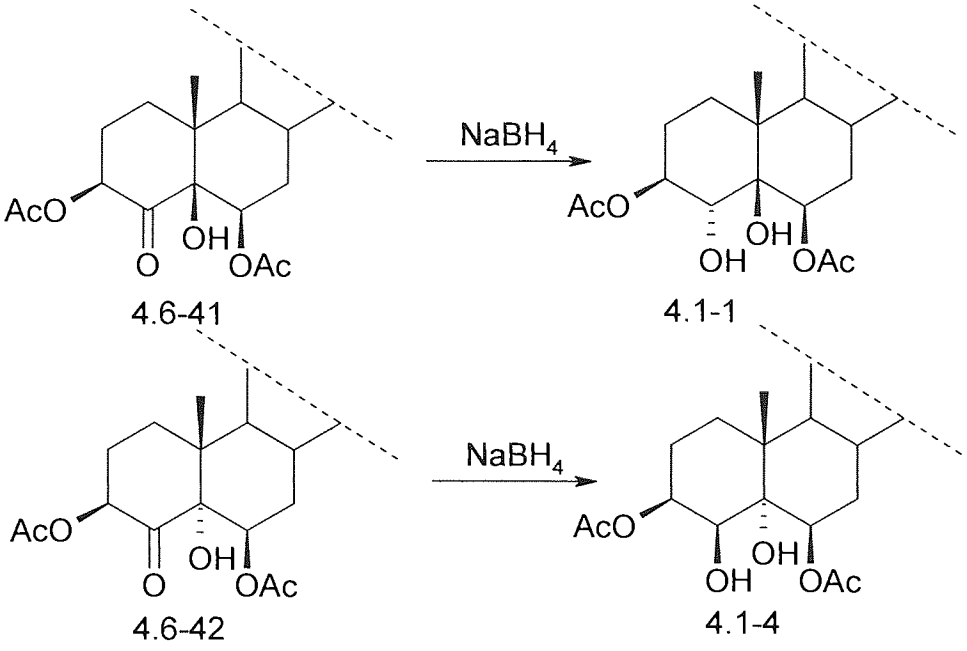
Scheme 4.6-8 Preparation of the 5α-cholestane-3β,4α,5,6α-tetrol



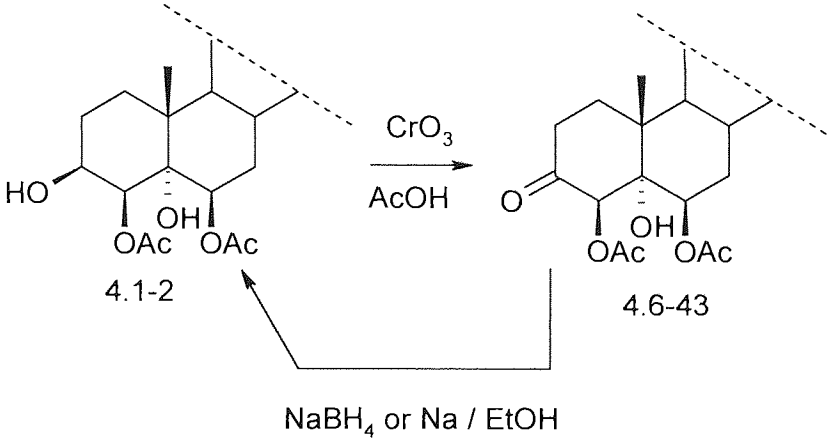
4.6-40 4.2-1a yield 16%

Scheme 4.6-9 Reduction of 4.6-40 by sodium in ethanol

Several attempts made on the sodium borohydride reduction of the 3-one in 4.6-43 to the 3-α group failed, as only β-isomer was generated; furthermore the 4β-hydroxyl in 5β configuration oxysterols also can not be afforded simply by reduction of 4-one in 4.6-41 (**Scheme 4.6-10** and **Scheme 4.6-11**).



Scheme 4.6-10 Reduction of 4-one 4.6-41 and 4.6-42



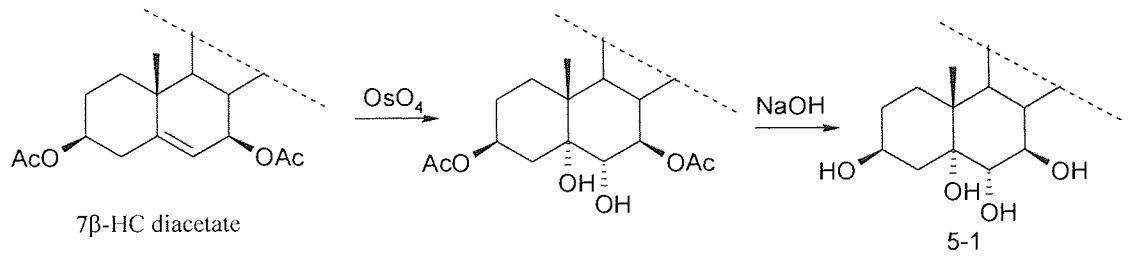
Scheme 4.6-11 Reduction of 3-one 4.6-43

Chapter 5: Cholestane-3,5,6,7-tetrols and cholestane-3,4,5,6,7-pentols

5 Cholestane-3,5,6,7-tetrols and cholestane-3,4,5,6,7-pentols

As mentioned previously in section 2, in a test of cytotoxicities against cancer cells, 5α–cholestane-3β,5,6β,7β-tetrol (2-14) and 5α–cholestane-3β,5,6β,7α-tetrol (2-15) showed a big difference. One could speculate that this difference in cytotoxicity resulted from their C7 stereochemistry. With this in mind, the other stereoisomeric cholestane-3,5,6,7-tetrols have become the most wanted analogues for a comprehensive cytotoxicity test. In addition, these tetrols are not only final products but also can serve as starting materials for preparation of oxysterols bearing more hydroxyl group, such as 3,4,5,6,7-pentol. Here we will describe the studies of these compounds. Certainly, the synthetic methodologies developed in the previous sections are very useful here.

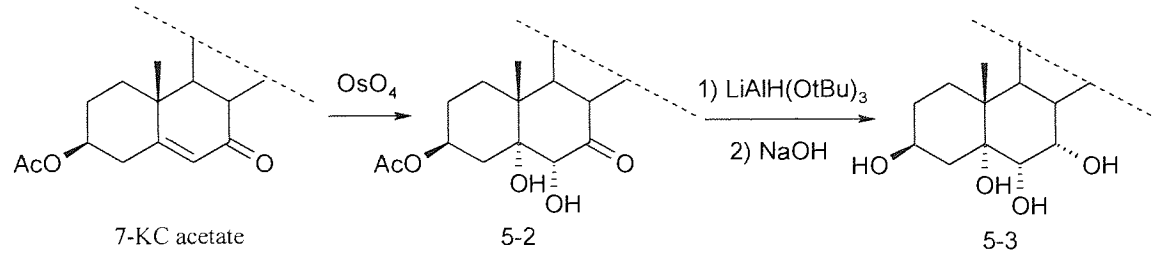
In literature, 2–14 and 2-15 were synthesized using the conventional epoxidation and epoxide ring opening reaction methods, as mentioned before. The two 5α,6α-isomers, 5α-cholestane-3β,5,6α,7β-tetrol (5-1) and 5α-cholestane-3β,5,6α,7α-tetrol (5-3), are also synthesised by the Osmium tetroxide (OsO_4) oxidation of the related 5,6-double bonds (**Scheme 5-1**), and following reduction of the 7-ketone to give the 7α –isomer (**Scheme 5-2**) (Warren et al 1989).



7β-HC diacetate

5-1

Scheme 5-1 Preparation of 5α-cholestane-3β,5,6α,7β-tetrol (5-1)



7-KC acetate

5-2

5-3

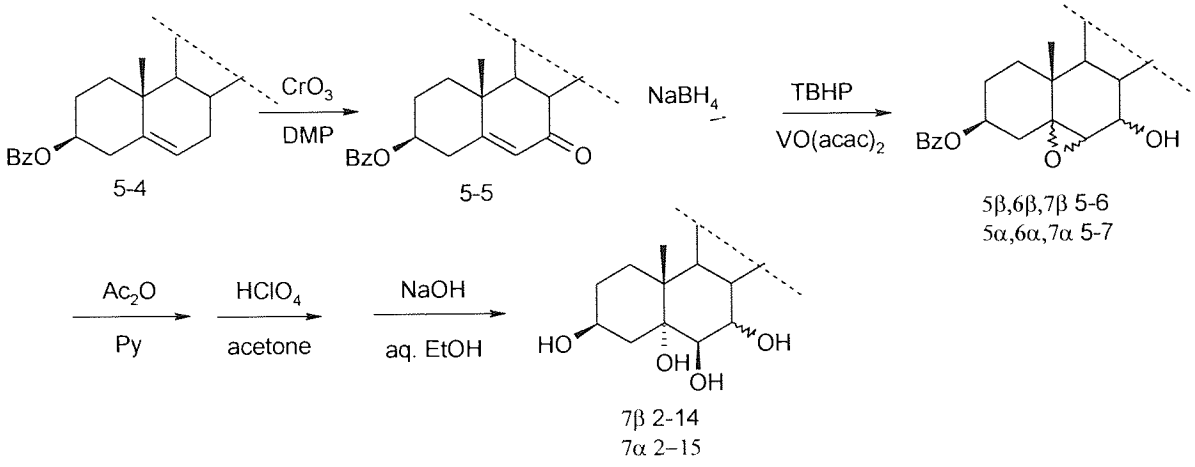
Scheme 5-2 Preparation of 5α-cholestane-3β,5,6α,7α-tetrol (5-3)

As we knew, some approaches used in literature are not suitable to our needs. For example, OsO_4 oxidation is a convenient approach in preparation of a small amount of samples in laboratories, but not for a gram scale preparation; and the selective reduction of 7-one to 7α-ol always gives a low to moderate yield, most of the valuable 7-KC acetate being wasted.

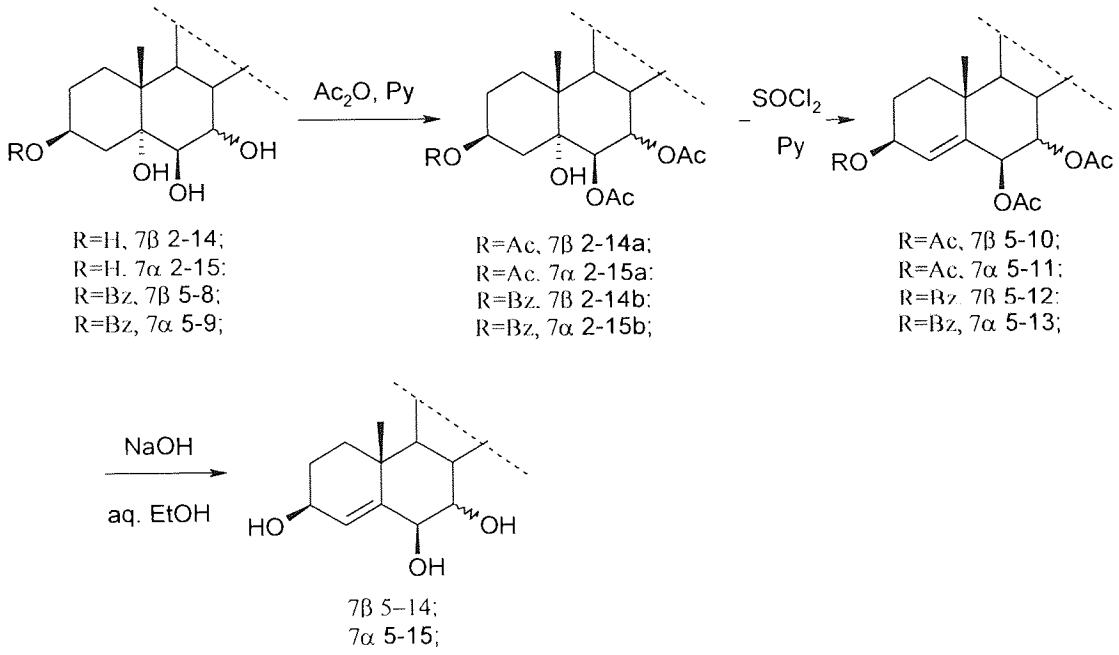
Therefore in order for us to get the desired 3,5,6,7-tetrols in a good quantity, new approaches have to be developed. In this section, I discussed a series of synthetic routes designed and developed for the preparation of the stereoisomers relative to cholestane-3β,5α,6β,7β-tetrol and cholestane-3β,5α,6β,7α-tetrol. Analogues like related cholestane-3,4,6,7-tetrols are also synthesised. Due to time limit, not all the isomers have been synthesized; however, the key synthetic steps were studied, the typical isomers were synthesised, and the potential synthetic routes to all desired compounds were discussed.

Some improvements were made on preparation of cholestane-3β,5α,6β,7β-tetrol (2-14) and cholestane-3β,5α,6β,7α-tetrol (2-15). Thus, cholesterol benzoate (5-4) was oxidaized with 6 molar excess CrO_3 / DMP complex to yield the 7-one compound (5-5) in 78~82% yield. The separation of 5-5 from the resulting mixture was performed on silica filtration with ether. After reduction with sodium borohydride in THF/water and epoxidation with TBHP/$VO(acac)_2$, the 5,6β–epoxy-5β-cholestane-3β,7β-diol 3-benzoate (5-6) was separated by recrystallisation from methanol. The concentrated methanolic filtrate gave the 5,6α–epoxy-5α-cholestane-3β,7α-diol 3-benzoate (5-7) after standing overnight at room temperature. These two compounds 5-6 and 5-7 are acetylated with acetic anhydride, followed by treatment with perchloric acid in acetone, and hydrolysed to yield the cholestane-3β,5α,6β,7β-tetrol (2-14) and cholestane-3β,5α,6β,7α-tetrol (2-15) separately. Acetylation of 2-14 and 2-15 or their 3-benzoyl esters 5-8 and 5-9 gave the triacetates 2-14a, 2-15a or the benzoyl diacetates 2-14b and 2-15b, which were reacted with thionyl chloride to yield the 4-ene compounds 5-10 ~ 5-13. Hydrolysis at room temperature gave the 4en-3,6,7 triols:

Cholest-4-ene-3β,6β,7β-triol (5-14) and cholest-4-ene-3β,6β,7α-triol (5-15) (**Scheme 5-3** and **Scheme** 5-4).



Scheme 5-3 Improved preparation of 5α-cholestane-3β,5,6β,7-tetrol

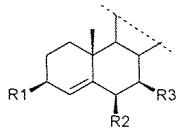


Scheme 5-4 Preparation of Cholest-4-ene-3β,6β,7-triols

The epoxidation of triacyl derivatives 5-10 ~ 5-13 by using mCPBA happened even slower in comparison with that of the cholest-4-en-3β,6β-diol esters 3.1-2 and 3.1-9 in Table 3.2-1. Furthermore the α selectivity in 5-12 was quite low when the 3-benzoyl ester was introduced. To explore this further, two mixed esters of cholest-4-en-3β,6β-diol (3.1-1) the 3-benzoate-6-acetate (5-16) and 3-acetate-6-benzoate (5-17) were prepared and tested with the same condition, it shows

clearly that without the 7β-hydroxyl group, increase of β-isomer ratio is much less (**Table 5-1**).

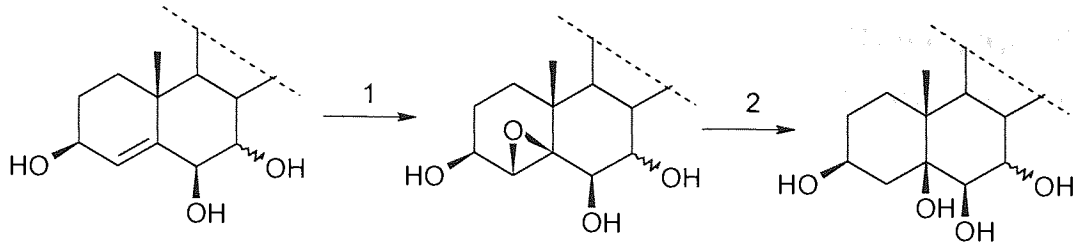
Table 5-1 mCPBA epoxidation of compound 5-10, 5-12, 5-16 and 5-17

		α:β ratio	Yield	Reaction time
5-10	R1=R2=R3=AcO	76:24 (5-18:5-19)	77%	24days
5-12	R1=BzO, R2=R3=AcO	46:54 (5-20:5-21)	82%	17days
5-16	R1=BzO R2=AcO, R3=H	77:23 (5-22:5-23)	>90%	6days
5-17	R1=AcO R2=BzO R3=H	82:18 (5-24:5-25)	>90%	5days

As showed in **Table 3.2-1** and **Table 5-1**, the benzoyl group protected compounds furnished quite different results. In conjunction with the results from cholest-5-en-3β,4β-diol esters 4.6-2~4.6-9, a preliminary conclusion is drawn: The benzoyl group of the axial allyl hydroxyl group exerts a similar steric effect to the acetyl group, as shown in the examples containing 4β, and 6β acetoxy (benzoxy) groups 4.6-3,4, 6, 7, 9 and 3.1-2, 9; 5-16, 17; an acetyl group replaced by benzoyl group on the *cis*-homoallyl hydroxyl can significantly reduce the steric hindrance effects.

Due to need of prolonged reaction time for the completion of the reactions of the 7α isomer, cholest-4-ene-3β,6β,7α-triol triacetate 5-11 or 3-benzoate-6,7-diacetate 5-13, inevitably, many impurities were generated which made the final work-up more difficult. Therefore further experimental work was needed for conducting a good exploitation of these compounds.

The two free triols 5-14 and 5-15 reacted with mCPBA very fast, both completed in 15 minutes. The yields are 83% for 4β,5-epoxy-5β-Cholestane-3β,6β,7β-triol (5-26) and 75% for 4β,5-epoxy-5β-Cholestane-3β,6β,7α-triol (5-27). On reduction with $LiAlH_4$ 5β tetrols 5-28 and 5-29 were given (**Scheme 5-5**).

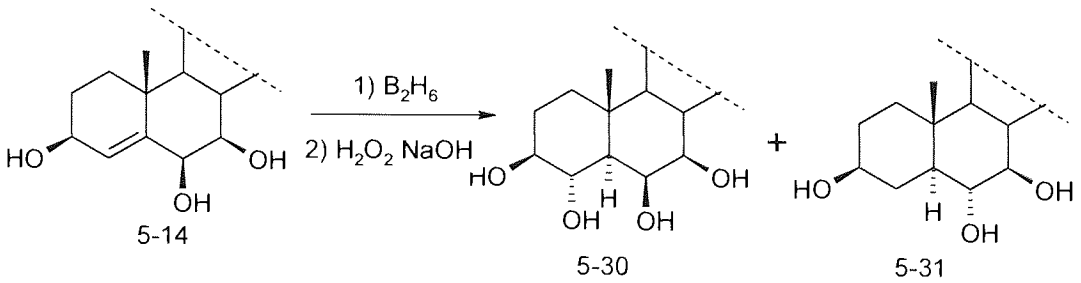


7β 5–14; 7α 5-15; 7β 5–26; 7α 5-27; 7β 5–28; 7α 5-29;

1. mCPBA, DCM; 2. $LiAlH_4$, THF.

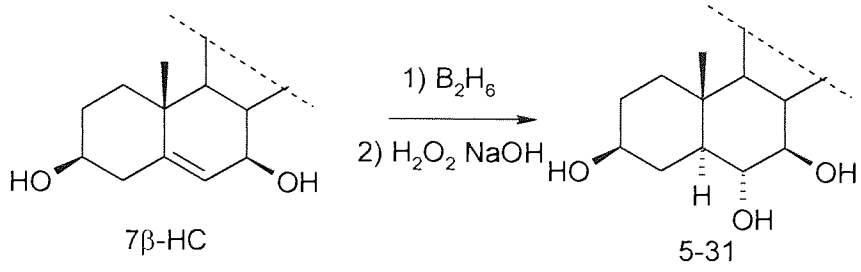
Scheme 5-5 Preparation of 5β-cholestane-3β,5,6β,7-tetrol

The hydroboration – oxidation of the cholest-4-ene-3β,6β,7β-triol (5-14) gave two compounds, the polar one is the 5α–cholestane–3β,4α,6β,7β–tetrol (5-30) in yield of 61% and the non-polar one (23%) is a triol (5-31) (**Scheme 5-6**), the same product of reaction of 7β–HC with borane followed by hydrogen peroxide with alkali (**Scheme 5-7**). The structure of the second compound 5-31 was identified as the 5α–cholestane-3β,6α,7β-tetrol because the C_3-H showed the same coupling feature to other 3β–hydroxyl–5α–steroids. A recently published paper also indicated the same compound can be made from 7-HC 3,7-diacetate using a similar condition (Jung and Johnson 2001).



5-14 5-30 5-31

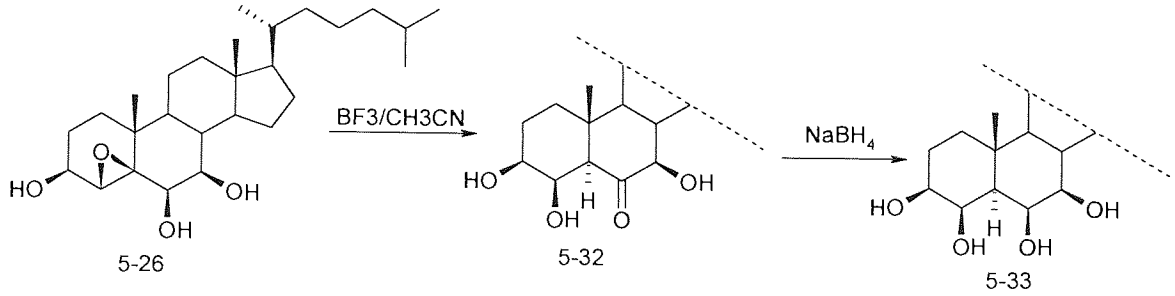
Scheme 5-6 Hydroboration and oxidation of 5-14



7β-HC 5-31

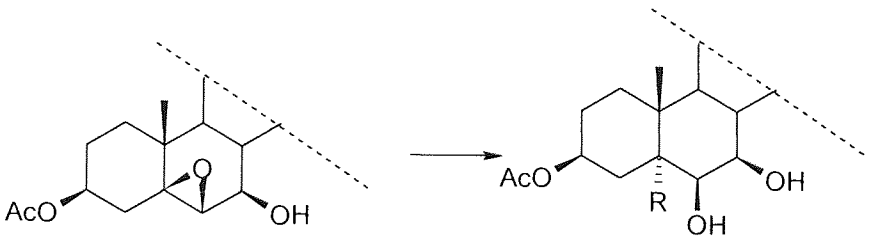
Scheme 5-7 Hydroboration and oxidation of 7-HC

The 4β-isomer (5-33) of 5α–cholestane–3β,4α,6β,7β–tetrol (5-31) was prepared by the BF_3 induced rearrangement to 6-one 5-32, followed by $NaBH_4$ reduction to generate 6β-hydroxyl (**Scheme 5-8**).



Scheme 5-8 Epoxide 5-26 with combination **4**

The oxirane opening reactions of the 5,6-epoxide 3,7-diol 3-acetate 5-34 were studied further to see if the direct ring opening to 5β-isomer can be processed. Under variable conditions (**Scheme 5-9** and **Table 5-2**), only the 5α isomer was produced in reasonable yields. Other 3,7-dihydroxy-5,6 epoxides with Lewis acid all give unidentified mixtures.

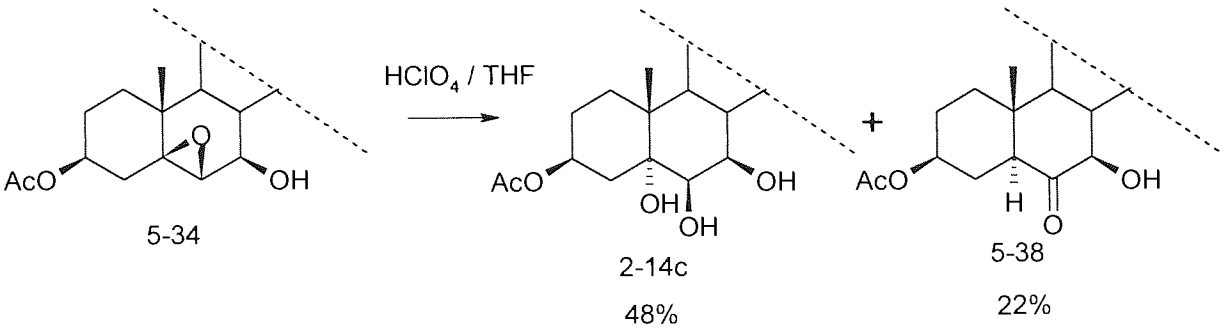


Scheme 5-9 Epoxide 5-34 with combinations **3**,**4**,**7**

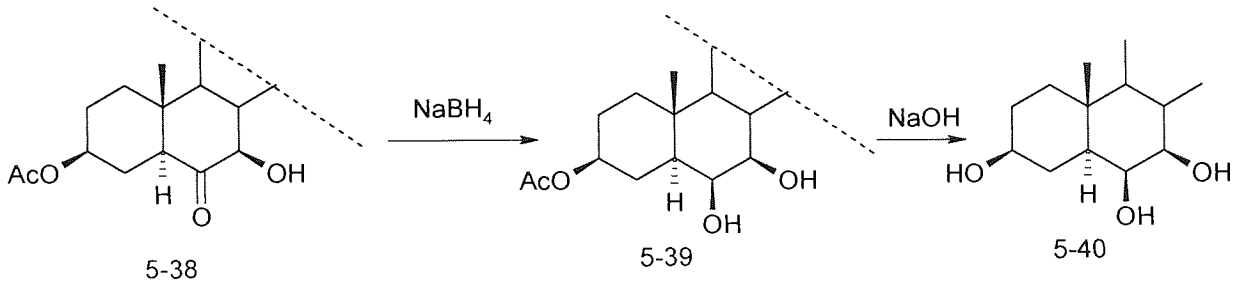
Table 5-2 Reactions in Scheme 5-9

Reagents	Product	Yield	Reaction time
$BF_3{\cdot}Et_2O$, THF	5-35	60%	2hr
$BF_3{\cdot}Et_2O$, CH_3CN	5-36	82%	2hr
$BF_3{\cdot}Et_2O$, MeOH	5-37	75%	5hr

Compound 5-34, if which was not acetylated as in **Scheme 5-3**, can give the 6-one compound 5-38 as the second compound (**Scheme 5-10**). 5-38 was reduced by sodium borohydride and hydrolysed to yield the 5α-cholestane-3β,6β,7β-triol (5-40) (**Scheme 5-11**).



Scheme 5-10 Epoxide 5-34 with combination **1**

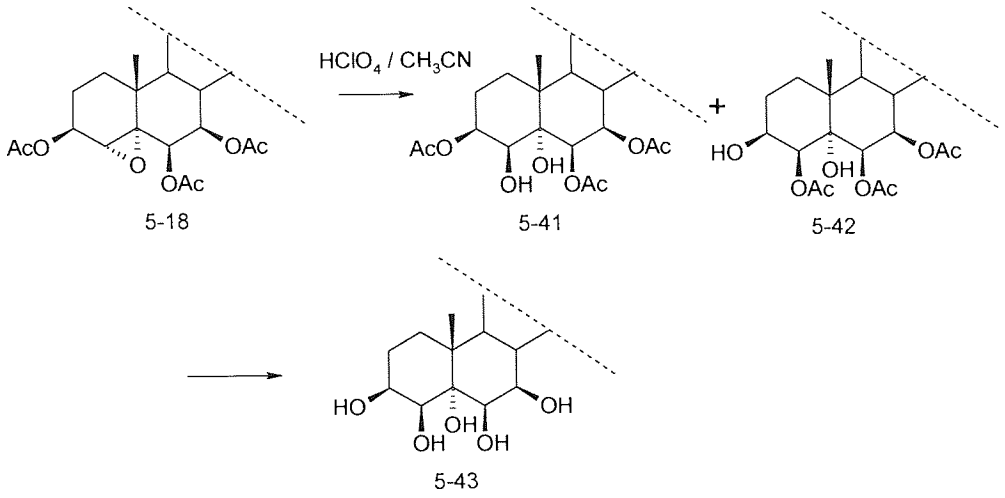


Scheme 5-11 Reduction of 5-38

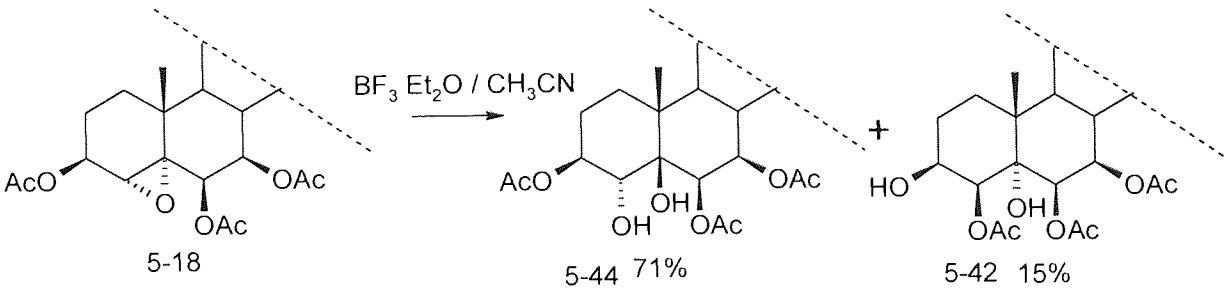
The ring openings of the 4,5-epoxycholest-3,6,7-triol triacetate 5-18 gave a similar result to that of cholest-4-en-3,6-diol diacetates (3.2-1a) under the same conditions. The $HClO_4$ in acetonitrile give a mixture of 3,6,7-triacetate 5-41 and 4,6,7-triacetate 5-42 with some other impurities. After hydrolysis and purification with chromatography it gave a total yield of 69% of the final product, the 5α-cholestane-3β,4β,5α,6β,7β-pentol (5-43) (**Scheme 5-12**). 5-43 is the first A, B rings oxygenated cholestane-pentol ever synthesised.

However, presence of the 7β-acetoxyl group rendered an interaction between the 7β-acetoxy and the 6β-acetyl possible; as a result, the treatment with BF_3 in CH_3CN also afforded 15~20% of 4β, 5α isomers 5-42 (**Scheme 5-13**). This again confirmed that the 6β-acetyl participation is necessary for the preparation of 5β-isomers with a

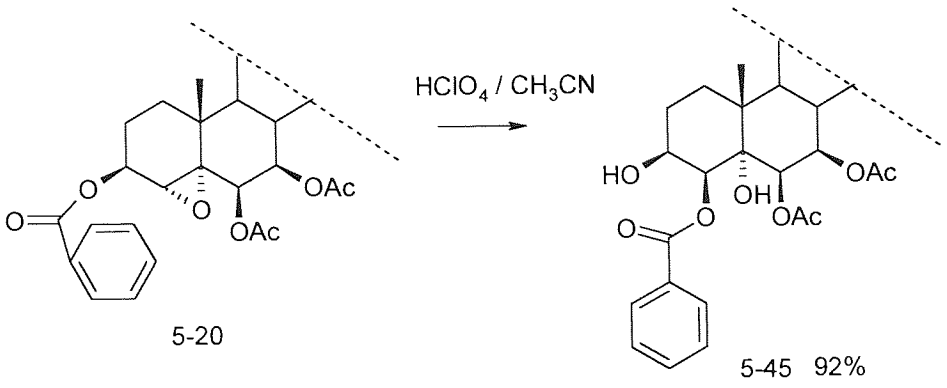
Lewis acid. Interestingly, when the 3-acetoxyl group of 5-18 was replaced by benzoxyl group, treatment with perchloric acid in acetonitrile gave 92% yield of cholestane-3β,4β,5α,6β,7β-pentol 4-benzoate-6,7-diacetate 5-45 (**Scheme 5-14**). While with boron trifluoride in acetonitrile, the 4α,5β-isomer 5-46 was afforded with a higher yield than that of the 3-acetate 5-42, no significant 4β,5α-isomer was found. Pentol 5-47 was prepared by direct hydrolysis without further purification (**Scheme 5-15**).



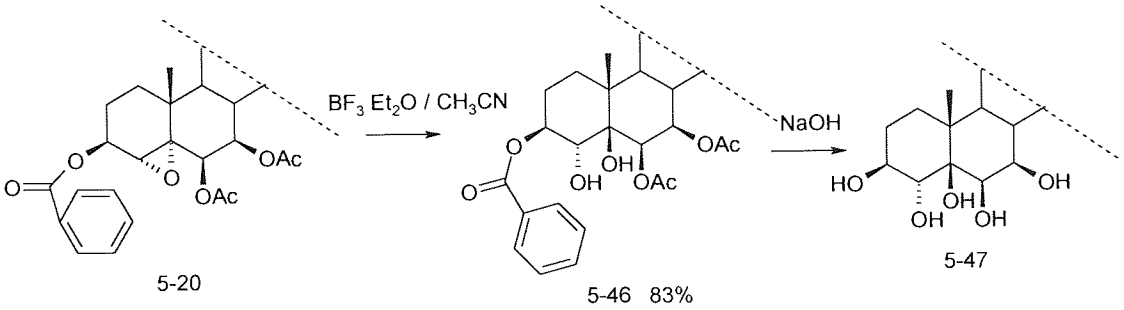
Scheme 5-12 3-Acetoxy Epoxide 5-18 with combination **2**



Scheme 5-13 3-Acetoxy Epoxide 5-18 with combination **4**



Scheme 5-14 3-Benzoyloxy Epoxide 5-20 with combination **2**



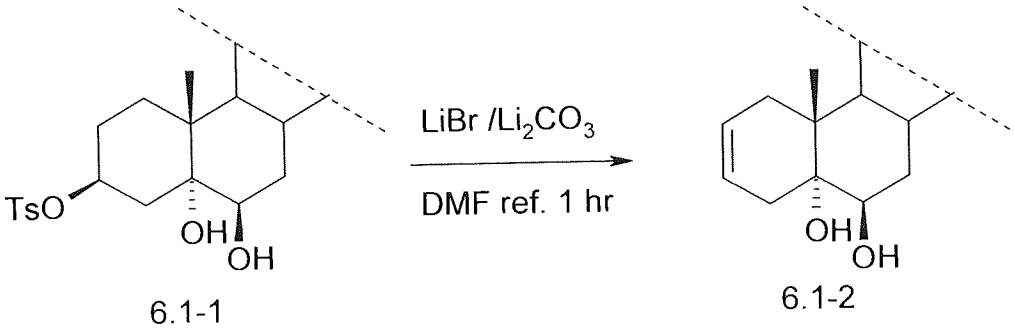
Scheme 5-15 3-Benzoyloxy Epoxide 5-20 with combination **4**

Chapter 6: The preparation and reaction of steroidal 2-enes

6 The preparation and reaction of steroidal 2-enes

6.1 preparation of the 2-enes

The 3β-(toluene-4-sulfonyl)-5α-cholestane-5,6β-diol (6.1-1) gave quantitative yield of 5,6β-dihydroxy-5α-cholest-2-ene (6.1-2) (**Scheme 6.1-1**). There are the two allyl positions C1 and C4, and oxygenated groups can be introduced on to the C1-C4 via a proper oxidation. Another way to conduct the C1-C4 oxidation is through compounds that already have a 4-hydroxyl group, as the tetrol and triols discussed previously. These two approaches can be complementary to each other and the presence of a hydroxyl group with the certain configuration on C-4 is helpful to the product structure discrimination.



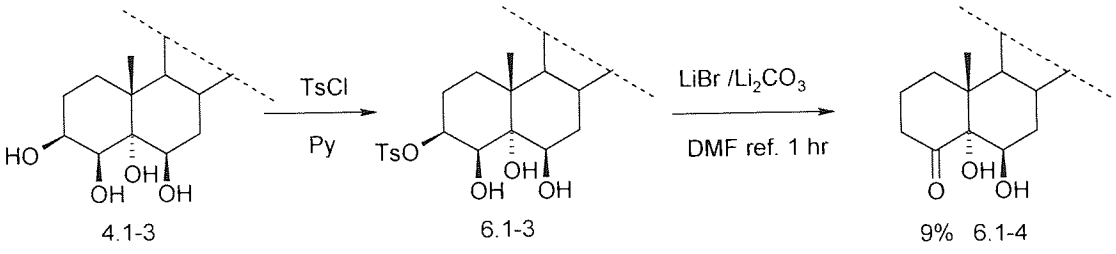
6.1-1 6.1-2

Scheme 6.1-1 Preparation of 5,6β–dihydroxy–5α–cholest-2-ene (6.1-2)

The preparation of toluene-4-sulfonyl ester of oxysterols was performed in pyridine with p-toluenesulfonyl chloride (TsCl) at ambient temperature. The free triol 6.1-3 with additional 4β-OH as compared to 6.1-1 was prepared from tetrol 4.1-3. It was treated under the same conditions. The starting material disappeared on TLC in 1 hr, however more than ten products were seen. Among them, the only determined structure is the 5,6β–dihydroxy-5α-cholestan-4-one 6.1-4 as the C4-α H abstracted product with only 9% separated yield (**Scheme 6.1-2**).

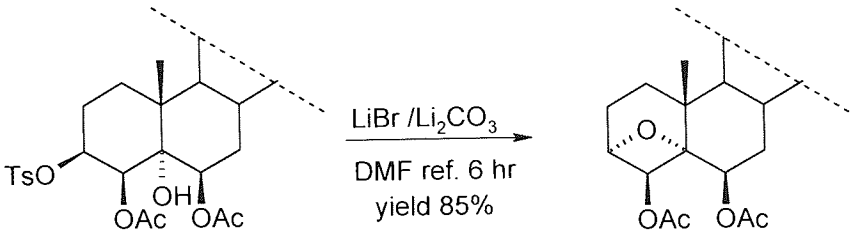
To avoid the side reactions, the C-4 and C-6 hydroxy groups in 6.1-3 need to be protected by acetyl groups. Diacetate 6.1-5 was prepared either by heating 6.1-3 in toluene with acetic anhydride and pyridine, or from the reaction of 4.1-2 with TsCl.

Under the same conditions, $LiBr/Li_2CO_3$, the 3α,5α-oxitane 6.1-6 was formed in a considerable high yield (**Scheme 6.1-3**).



4.1-3 6.1-3 9% 6.1-4

Scheme 6.1-2 Elimination of 3-Ts ester of 4.1-3



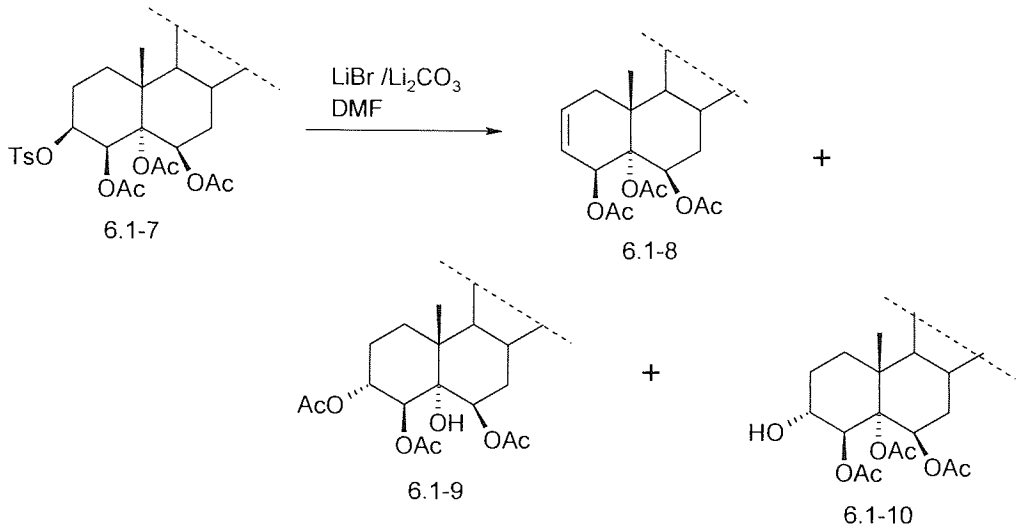
6.1-5 6.1-6

Scheme 6.1-3 Elimination of 3-Ts ester 6.1-5

The 3,5-hydroxyl participation is not wanted. As the next step, the 3β–(toluene-4-sulfonyl)–5α–cholestane–4β,5,6β–triol 6.1-3 or its 4,6-diacetate 6.1-5 was converted to its 4,5,6-triacetate 6.1-7 with acetic anhydride and boron trifloride as the catalyst at room temperature overnight, or heating in Ac_2O with p-toluenesulfonic acid at 70°C over 24hr.

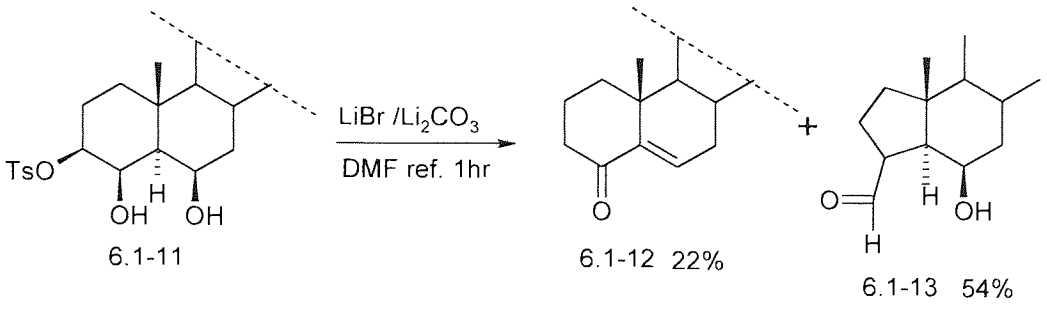
The resulting 4,5,6-triacetate 3-tosylate 6.1-7 was treated with LiBr/LiCO3 in DMF offering three products 6.1-8, 6.1-9 and 6.1-10 (**Scheme 6.1-4**). It is clear the participation of the 5-acetyl group is significant in this reaction. We also noticed that quality of DMF used affected the outcomes of the reaction greatly, probably due to presence of different percentage of water. With dried distilled commercial DMF marked water<0.02%, the yields are 67% of 6.1-8, 18% of 6.1-9, 4% of 6.1-10. While with the A.C.S. reagent marked water below 0.15%, the yield is 25% of 6.1-8 and 69% of 6.1-9. Compound 6.1-10 was hydrolysed to its 5-acetate (6.1-10a).

The amount of lithium carbonate, as an acid scavenger, seemed not to affect the reaction significantly over a range from 2 fold to 10 fold excess of the starting material. However, the lithium bromide should be 5-fold molar excess to the tosylate 6.1-7, to generate over 60% yield and less impurities. Compared with the prototype reaction in **Scheme 6.1-1**, the reaction of 6.1-7 happened quite slowly, normally more than 16 hrs was needed to make it complete; while reaction of 6.1-1 without the 4β-acetoxy group finished in 45min ~ 1hr.



Scheme 6.1-4 Elimination of 3-Ts ester 6.1-7

As participation of the 5-hydroxyl or 5-acetyl cannot be avoided in these reactions of compound 6.1-5, 6.1-7 and possibly 6.1-3, compounds without 5-OH were also studied. The 3β–(toluene-4-sulfonyl)–5α–cholestane–4β,6β–diol 6.1-11 gave the ring contracted compound 6.1-13 as the major product, along with the cholest-5-en-4-one 6.1-12 as the C4-α H joined reaction product. No 2-en compound was detected in the resulting mixture (**Scheme 6.1-5**).

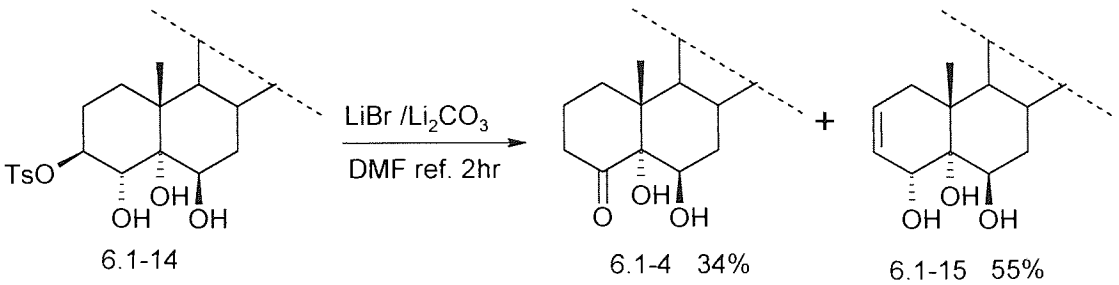


Scheme 6.1-5 Elimination of 3-Ts ester 6.1-11

It is very strange that the diacetate of 6.1-11 gave a messy result with LiBr / Li_2CO_3, in which no major product could be isolated.

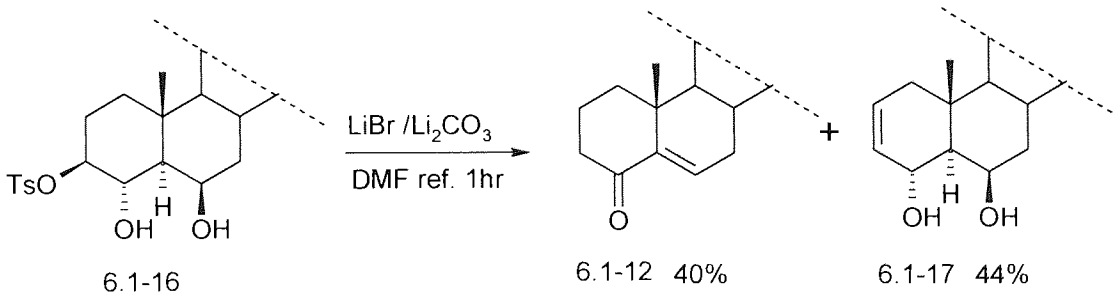
The 4β-OH is somehow an obstacle for the reaction. The reaction is much slower in the presence of the 4β-acetoxy group. When the 4β-OH is free, the abstraction of C-2H or 4α-H was accompanied with many other by-products. To study further on the effect of C-4 OH, compounds with 4α –hydroxyl group were prepared and tested.

Without the presence of 5α hydroxyl group, compound with the 4α hydroxyl group 6.1-16 gave nearly equally C2 and C4 hydrogen eliminated products (**Scheme 6.1-7**); while with the presence of 5α hydroxyl group (6.1-14) the ratio of the products 6.1-15 to 6.1-4 is 1.7:1 (**Scheme 6.1-6**).



6.1-14 6.1-4 34% 6.1-15 55%

Scheme 6.1-6 Elimination of 3-Ts ester 6.1-14

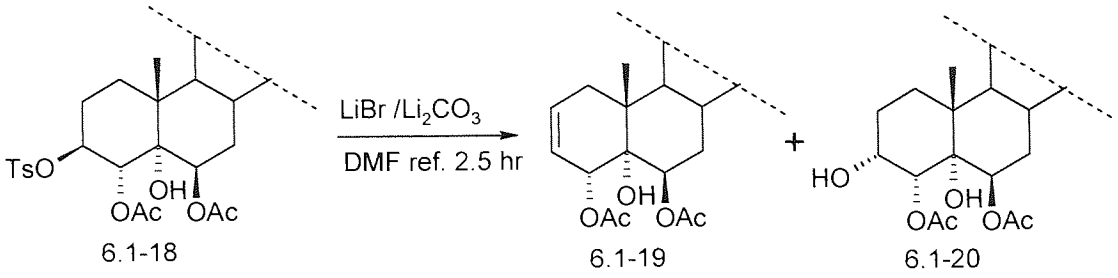


6.1-16 6.1-12 40% 6.1-17 44%

Scheme 6.1-7 Elimination of 3-Ts ester 6.1-16

When the 4α and 6β hydroxyl groups were protected by acetylation (compound 6.1-18), a yield over 90% of the 2-ene compound 6.1-19 was afforded (**Scheme 6.1-8**). When DMF was mixed with about 0.5% water and used as solvent, the acyl participation from C4 acetoxy group to C3 generates the 5α-cholestane-3α,4α,5,6β-tetrol 4,6-diacetate 6.1-20 as a minor product (yield ~10%). The preparation of relative triacetates of 6.1-14

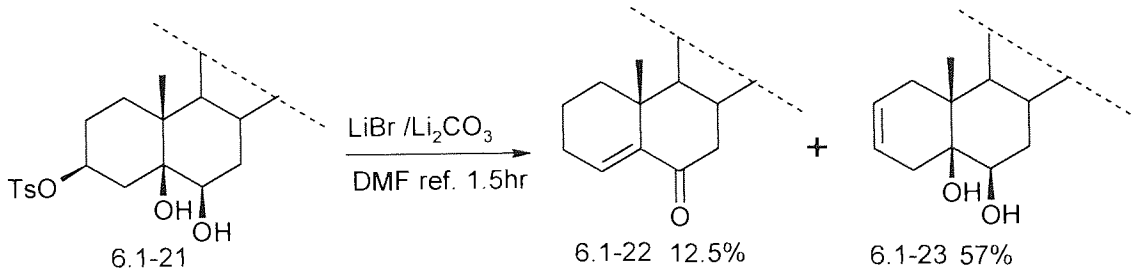
failed under an acidic condition, so whether or not there is participation of the 5-acetyl group is unknown.



Scheme 6.1-8 Elimination of 3-Ts ester 6.1-18

In summary, these 3β-p-toluenesulfonyloxy-5α-sterols give quite different results when treated with LiBr / Li_2CO_3 in DMF at reflux. The abstraction of 4β-H is easier than 4α-H, while acetylation of the 4-hydroxyl group prohibits this abstraction. C4α-OH and acetoxy group block the 3,5-participation of hydroxyl group. It was also postulated that the electronegative effect of the group on C-4 helps the abstraction of the C4-H, which is a disadvantage as we want the cholest-2-ene with C-4 oxygenated. The favoured 3,4 *cis*-elimination is interesting, which hydrogen was abstracted on C-2 when 2-ene compound formed was still unknown.

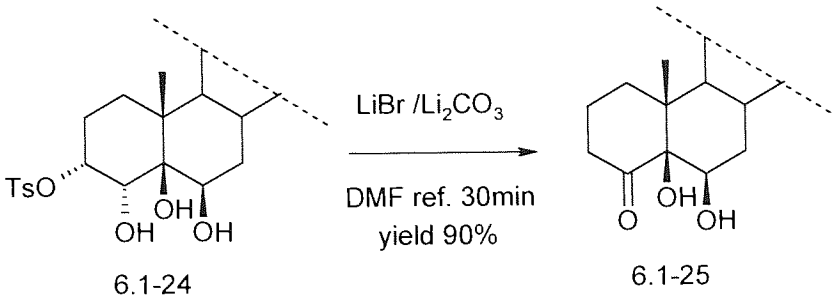
The 5β-isomer (6.2-21) of the prototype reaction starting material 6.1-1 does undergo C-4 H loss as shown in **Scheme 6.1-9**. The rearranged compound cholest-4-en-6-one 6.1-22 yield 12.5%, the 2-ene-5β,6β-diol 6.1-23 yield 57%.



Scheme 6.1-9 Elimination of 3-Ts ester 6.1-21

Preparation of the 3β-tosylate derivative of 5β–cholestane–3β,4α,5,6β–tetrol failed due to messy products. From the 3α–tosylate–5β–cholestane–4α,5,6β–triol 6.1-24,

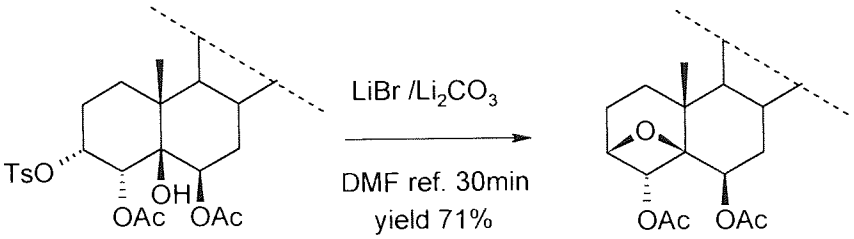
under the same reaction conditions only the 5,6β–dihydroxy–5β–cholestan-4-one (6.1-25) was afforded with high yield (**Scheme 6.1-10**).



6.1-24 6.1-25

Scheme 6.1-10 Elimination of 3-Ts ester 6.1-24

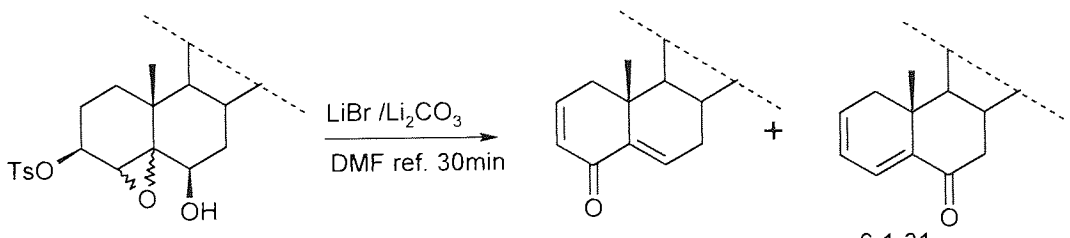
The 5β-hydroxyl group of 6.1–24 cannot be acetylated by acid conditions. The 4,6-diacetate of 3α–tosylate–5β–cholestane–4α,5,6β–triol (6.1-26) gave the 3,5β-oxitane 6.1-27 in a moderate yield (**Scheme 6.1-11**).



6.1-26 6.1-27

Scheme 6.1-11 Elimination of 3-Ts ester 6.1-26

The preparation of 4,5-epoxycholestan-3β,6β-diol sulfonates usually gave a mixture of 3-monosulfonate and 3,6-disulfonate together. The 3-monosulfonates 6.1-28 and 6.1-29 were treated with LiBr in DMF to give a separable mixture of conjugate ketone-4 6.1-30 and ketone-6 6.1-31 (**Scheme 6.1-12**).



α epoxide 6.1-28
β epoxide 6.1-29

6.1-30
54%
24%

6.1-31
26%

Scheme 6.1-12 Elimination of 3-Ts 45-epoxy ester 6.1-28 &29

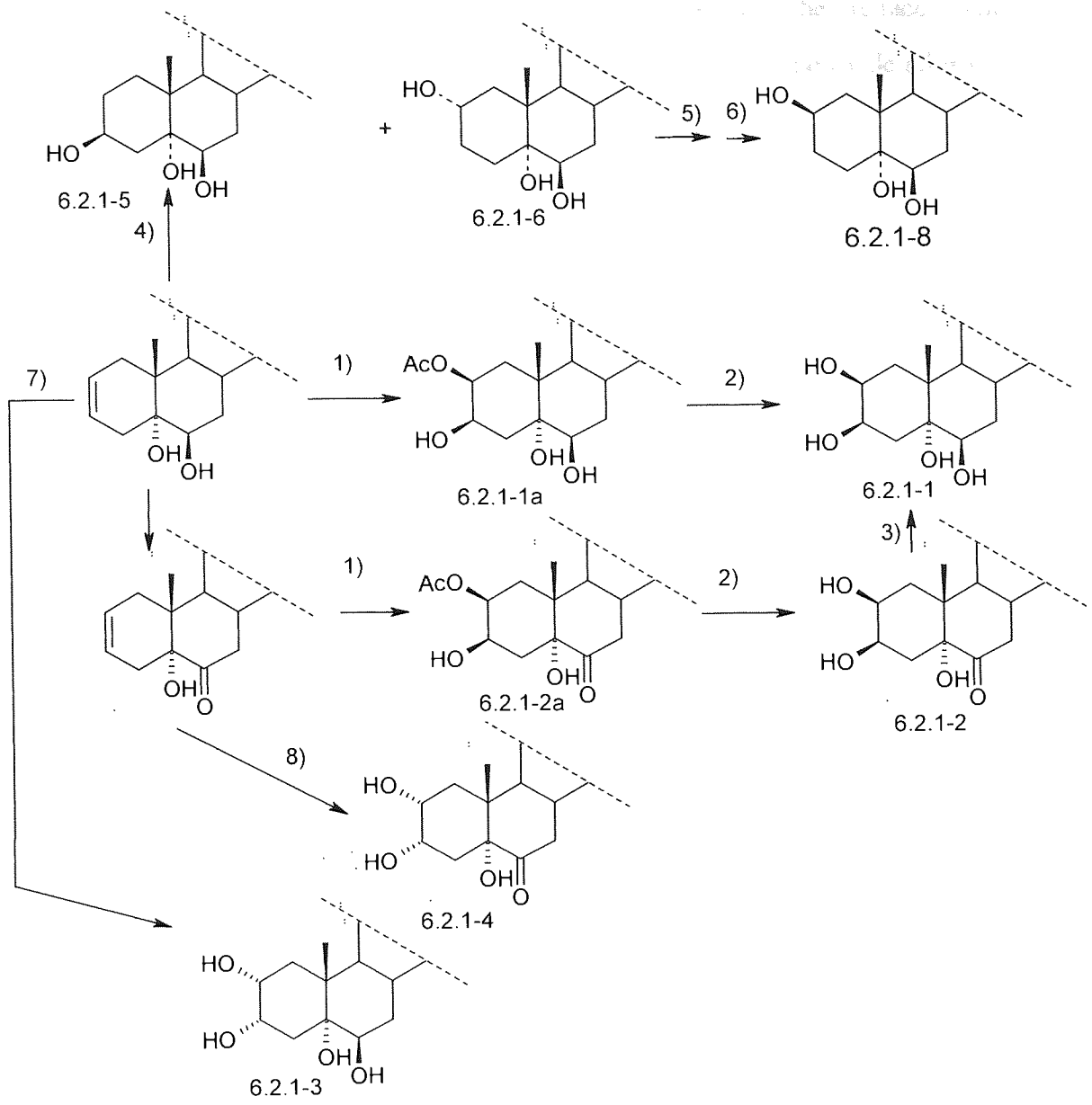
Above all, the 3-TsO elimination can be used to yield cholest-2-enes in some cases. The functional group induced C-4 H abstraction and other participation effects may be used in the synthesis of other compounds like 3-configuration inverted (**Scheme 6.1-4**) or C-3 oxygen eliminated (**Scheme 6.1-10**) products.

6.2 Chemistry of the 4-substituted cholest-2-enes: a comparative study with 2-en-5,6-diol.

6.2.1 The chemistry of cholest-2-en-5α,6β-diol

Cholest-2-en-5α,6β-diol may serve as the starting material on A ring oxygenated sterols. The 2β,3β,5α,6β-tetrol 6.2.1-1 was prepared according to literature Woodward addition method, so did the preparation of 2β,3β,5α- hydroxycholestan-6-one (6.2.1-2) (Kocovsky and Cerny 1976, Alston et al 1976). The 2α,3α,5α,6β-tetrol (6.2.1-3) was prepared by OsO_4-cooxidant method as described for compound 2-13. Its 6-one analogue 6.2.1-4 was synthesised with $KMnO_4$ and after several unsuccessful attempts, the best conditions for this reaction were found to be THF as solvent at -60°C in 33% yield.

The hydroboration oxidation of choles-2-en-5α,6β-diol gave only two products 6.2.1-5 (CT) and 6.2.1-6 with equatorial 2α and 3β hydroxyl group respectively (yield 36% and 42.7%), possibly due to the 19-methyl and 5α–hydroxyl groups' stereo-effect. The structure of the 3β isomer was determined by a comparison with the authentic sample. The 2α isomer 6.2.1-6 was oxidised with CrO_3 /DMP to afford the 2,6-dione 6.2.1-7, and reduced with $NaBH_4$ to give two products: the starting 2α isomer 6.2.1-6 and the 2β-OH isomer 6.2.1-8 (mole ratio 69:31) without 6α–isomers. The configuration was determined by the two big coupling effects of the 2β-hydrogen in the 2α isomer with the 1β and 3β hydrogen atoms on the $^1H^1H$COSY spectrum (**Figure 6.2-1**). The C-2 H of 2β isomer 2.6-8 give a broad single peak and weak coupling peaks in the $^1H^1H$COSY spectrum. The reactions discussed are shown in **Scheme 6.2-1**.

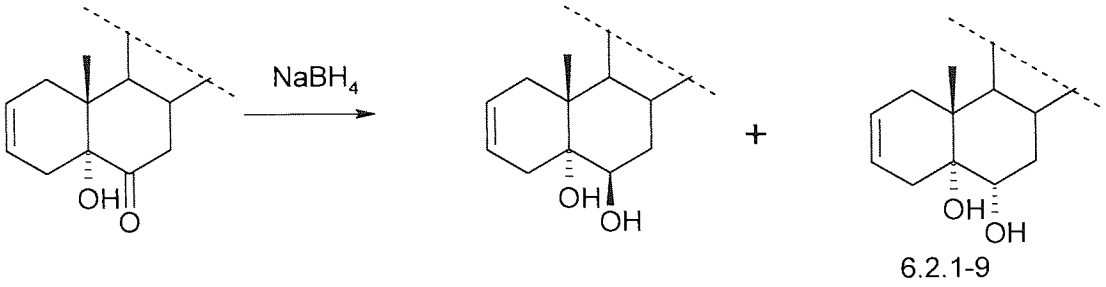


1) I_2, AcOAg;2) NaOH; 3) $NaBH_4$;4)B_2H_6 , H_2O_2/NaOH; 5)CrO_3,HOAc
6) $NaBH_4$;7)OsO_4; 8)$KMnO_4$;

Scheme 6.2-1 Chemistry of 5α-Cholest-2-en-5α,6β-diol

The reduction of the two 2,3,5-trihydroxy-6-ones 6.2.1-2 and 6.2.1-4 gave only the 6β-tetrols 6.2.1-1 and 6.2.1-3 with sodium borohydride. However, the 6-one group of 5-hydroxy-5α-cholest-2-en-6-one, when reacted with sodium borohydride, gave 6α isomer (6.2.1-9) and Choles-2-en-5α,6β-diol at 11% and 74% yields after chromatographic separation (**Scheme 6.2-2**). This may be caused by the conformational change of the A and B rings.

The epoxidation of cholest-2-en-5α,6β-diol always favours the α-face. The epoxidation with peracid, or metal catalysed peroxide or hydrogen peroxide all gives the α-epoxide 6.2.1-10 as the major product (**Scheme 6.2-3**).



6.2.1-9

Scheme 6.2-2 Reduction of 5-hydroxy-5α-cholest-2-en-6-one

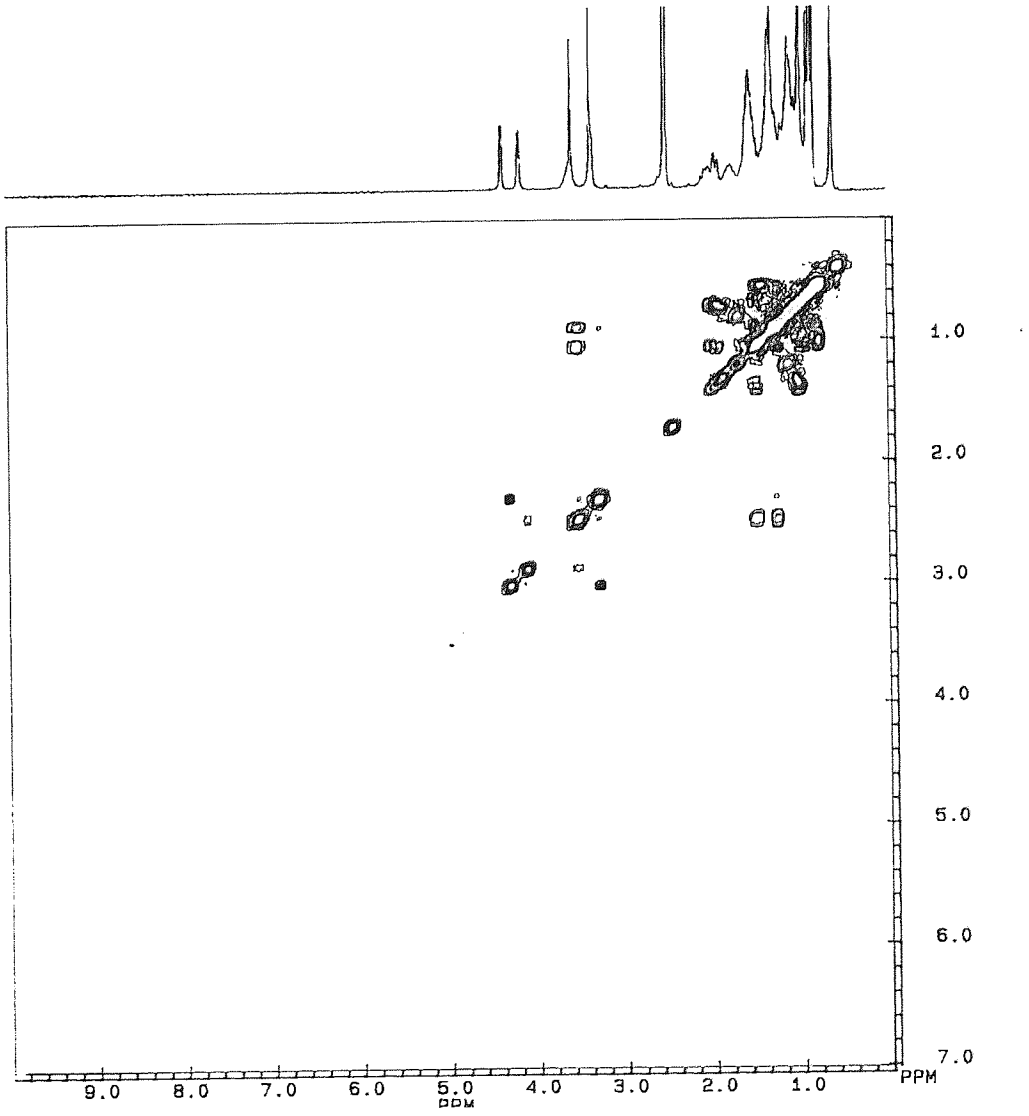
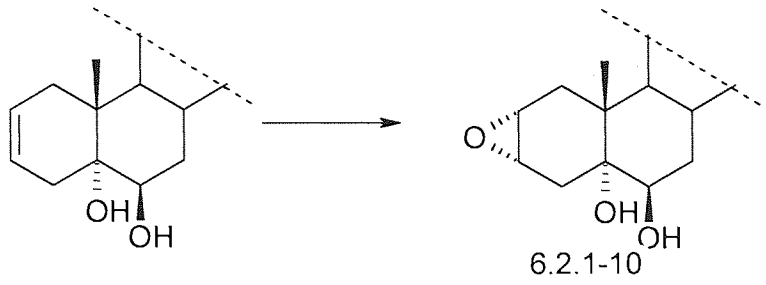
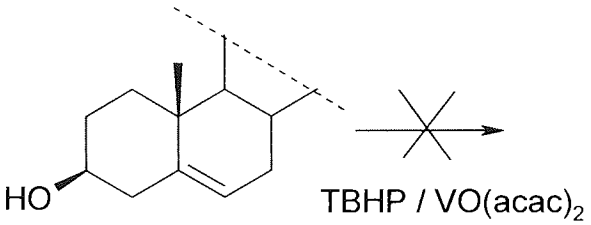


Figure 6.2-1 HHCOSY spectrum of 6.2.1-6



Scheme 6.2-3 Epoxidation of 5α-Cholest-2-en-5α,6β-diol

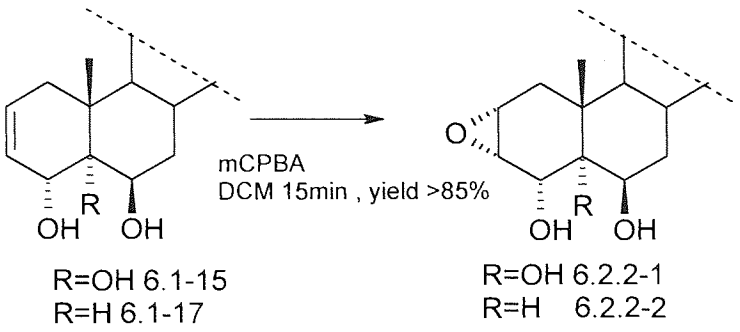
In the metal catalysed reactions, the reaction was sluggish. Such as with TBHP and $VO(acac)_2$, more than 10 molar ratio excess reagent was used with less than 50% yield. We consider that the 3β-hydroxyl group in cholesterol does not facilitate this type of reaction as we tested for several times. This may be due to the difficulty of generating homoallyl hydroxyl participation (**Scheme 6.2-4**).



Scheme 6.2-4 Attempted epoxidation on cholesterol

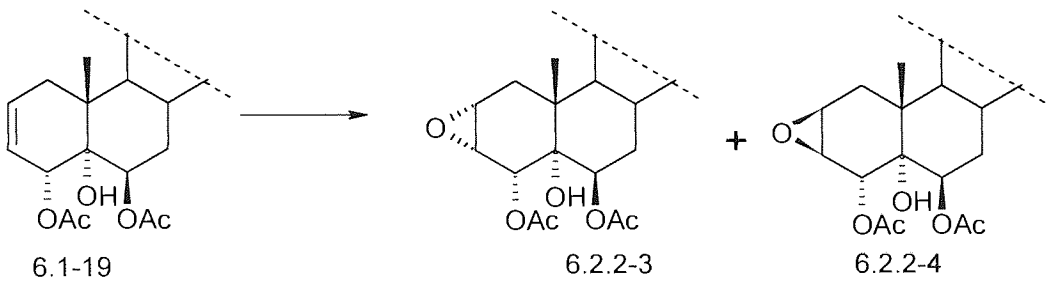
6.2.2 The reaction of 4α(β) hydroxyl cholest-2-ene derivatives.

When a 4α group exists, the reaction of 6.1-15 with TBHP and $VO(acac)_2$ gave many oxidised by-products and the yield of the epoxide 6.2.2-1 was between 20-30%. With mCPBA, the reaction of 6.1-15 and 6.1-17 gave high yield of epoxides 6.2.2-1 and 6.2.2-2 and finished in several minutes (**Scheme 6.2-5**).



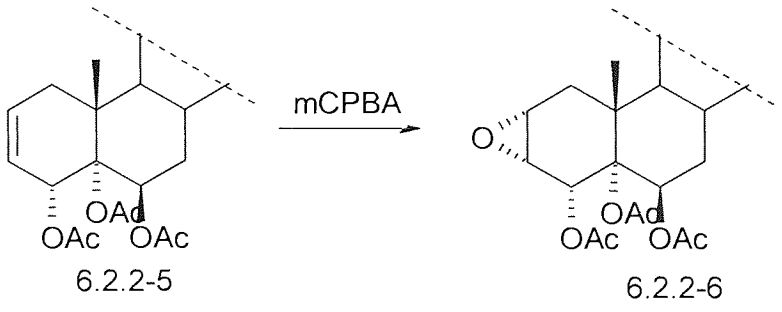
Scheme 6.2-5 Epoxidation of 4α-hydroxyl compounds 6.1-15 & 17

The 4α hydroxy may not give strong effects on the stereochemistry of the epoxidation, while it does accelerate the reaction. When the triol 4,6-diacetate 6.1-19 was reacted with mCPBA for 24hr, there was still no significant β-epoxide shown in the product mixture's 1HNMR. Careful chromatography gave 50mg β–epoxide (6.2.2-3) (4%) from the resulting mixture of 1500mg starting material (**Scheme 6.2-6**).

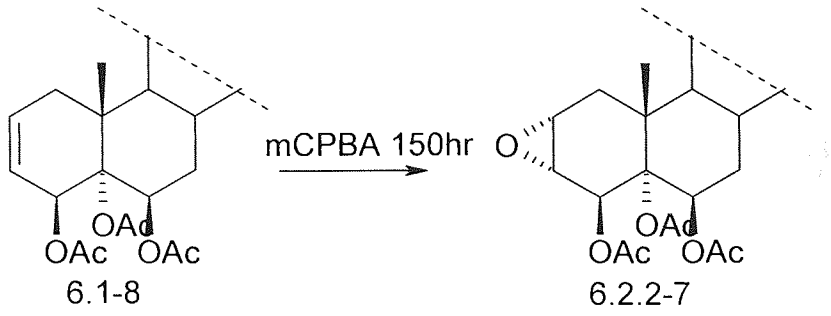


Scheme 6.2-6 Epoxidation of 4α-acetoxyl compound 6.1-19

The 4,5,6-triacetate compound 6.2.2-5 reacted even slower than that of 4β-congener 6.2.2-7, and after 1 month, the starting material still remained over 20% with many by-products. α-Epoxide 6.2.2-6 was afforded in 30% yield (**Scheme 6.2-7** and **Scheme 6.2-8**).

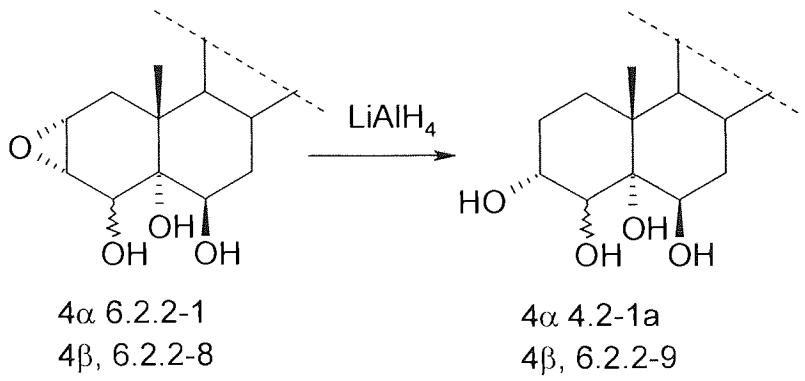


Scheme 6.2-7 Epoxidation of 4α–acetoxyl compound 6.2.2-5



Scheme 6.2-8 Epoxidation of 4β-acetoxyl compound 6.1-8

The reduction of these α epoxides only gave the product with the axial 3α hydroxyl group compound (**Scheme 6.2-9**). This may provide another method for the reversion of 3β-hydroxyl group to 3α-configuration by sulfonation, elimination to generate the 2-ene, followed by epoxidation and lithium aluminium hydride reduction. This is an alternative method to the 5-acetyl participation as discussed in **Section 6.1**. To convert the 3α-hydroxyl group to 3β-OH, however, there are a few factors needed to consider in this procedure such as, the sulfonation of 3α-hydroxyl group often occurs with low reactivity and less selectivity, the β-epoxide is not favoured and the reduction of β-epoxide may give 2β-hydroxyl group compound.



Scheme 6.2-9 Reduction of 2a,3a-epoxide 6.2.2-1 and 6.2.2-8

Compound 6.2.2-9 was also afforded from the hydrolysis of 4,5-epoxy ring opened product 4.2-6, 4.2-11, 4.2-13 and the 5,3-acetyl participation rearrangement product 6.1-9 and 6.1-10, therefore it is necessary to confirm the structure further from NMR spectra and X-ray crystallography. Crystals were made by slow evaporation of ethanol containing 6.2.2-9. The crystal structure is shown in **Figure 6.2-2**.

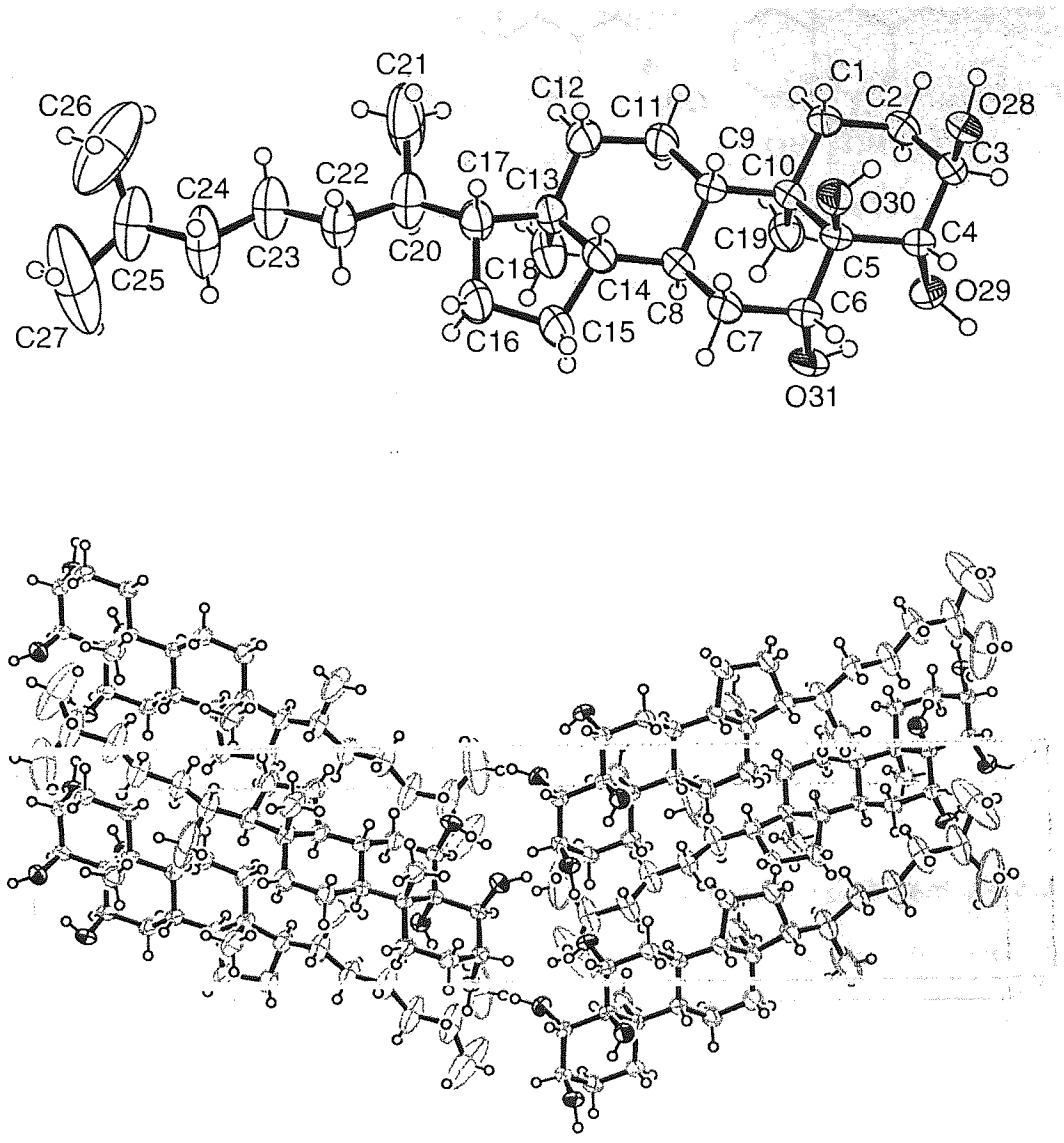
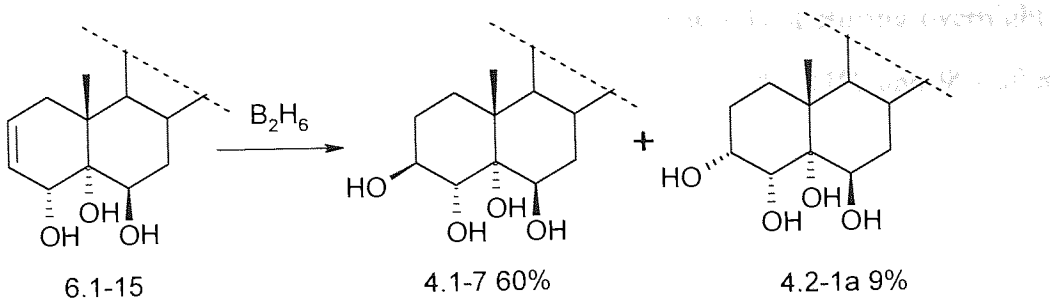


Figure 6.2-2 The X-ray crystal structure of 5α-Cholestane-3α,4β,5,6β-tetrol (6.2.2-9)

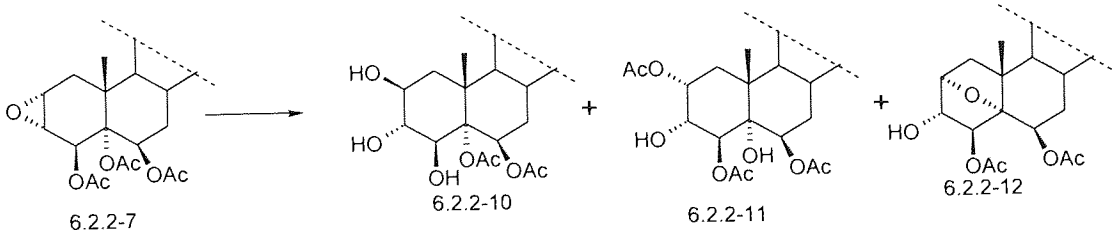
As described previously, the borane accesses C2=C3 double bond from equatorial orientation of the substrate. To our surprise, when the 4α-OH exists (6.1-15), attack only happened at position 3 (**Scheme 6.2-10**).



Scheme 6.2-10 Hydroboration and oxidation of 6.1-15

Clearly here the 4α-OH not only exerted a steric hindrance effect as in the case of the 4en-3,6-diols, but also was involved in coordination with borane.

The epoxide 6.2.2-7 was treated with 95% aq perchloric acid (5mole excess) in THF at 50°C, the 5-acetyl migrated compound 6.2.2-11 was furnished as the major product, and the reaction took only 30min to finish (**Scheme 6.2-11**). When more water was added (1g starting material, 5ml THF with 0.5ml water), the reaction became slow even with 12-molar excess perchloric acid. Because the reaction was happened at 50° C, many by-products appeared; so later the reaction was repeated at room temperature for 6hr with the compound with 4-acetyl replaced by hydroxyl as the dominant product. The 2,5-oxygen bridge product only separated from the first procedure at a yield of 3-4%. The diaxial epoxide ring opening is documented to happen when the stereoelectronic effect took precedence over other steric and electronic effects, while there was no 5-acetyl migration reported.

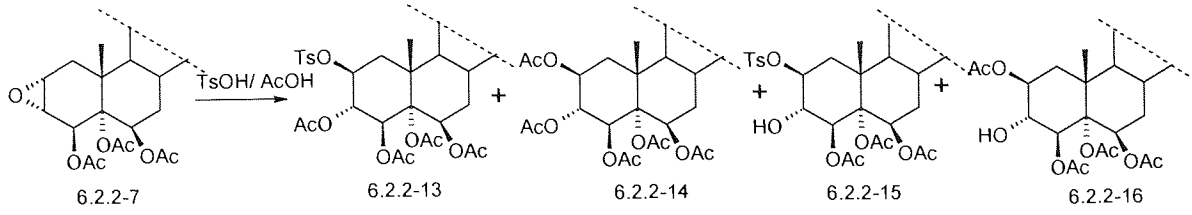


Procedure 1:	10%	59%	3%
Procedure 2: (more water)	53 %	9%	–

Scheme 6.2-11 Epoxide ring open of 6.2.2-7 by perchloric acid

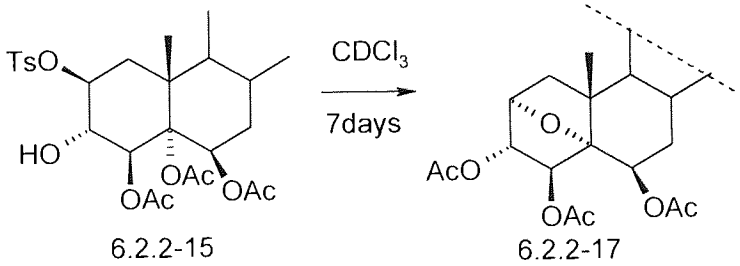
To investigate further, the reaction was carried out in acetic acid. When only acetic acid was used as the solvent, there was no reaction up to 70°C. Then 5 molar excess

toluene-4-sulfonate was added and the reaction mixture was kept stirring overnight, four products were given 6.2.2-13 ~ 6.2.2-16, yield 24%, 42%, 11% and 9% after separation (**Scheme 6.2-12**).



Scheme 6.2-12 Epoxide ring opening of 6.2.2-7 by TsOH in acetic acid

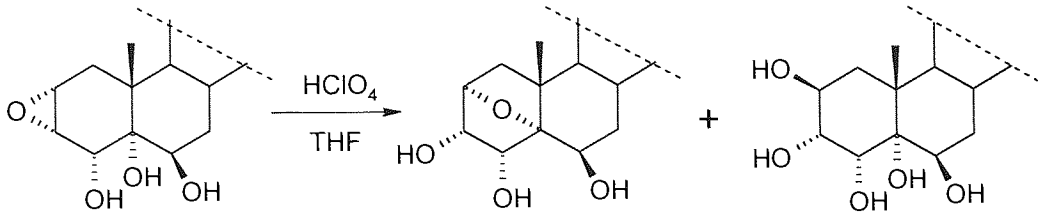
The third compound 6.2.2-15 in $CDCl_3$ in a NMR tube, after standing at room temperature for 7 days, was turned to the 2,5-oxygen bridge 6.2.2-17 (**Scheme 6.2-13**). The 5-acetyl migration to 3α-OH, the familiar type of acetyl participation shown in **Scheme 6.1-4** also happened in this case between the C3 hydroxyl group and 5-acetyl group. It is rational to postulate that a strong tendency of 2,5-epoxide formation exists. This may cause problems for further synthesis on 2,3,4,5,6-polyhydroxyl sterols. When the 3α-hydroxyl group is protected as in 6.2.2-13, the reaction was blocked.



Scheme 6.2-13 Formation of 2,5-oxygen bridge by 6.2.2-15

Free epoxy triol 6.2.2-1 give the 2,5-oxygen bridge compound 6.2.2-18 as the main separated product (**Scheme 6.2-14**), a small fraction of the polar by-products is the pentol 6.2.2-19, yield 7%

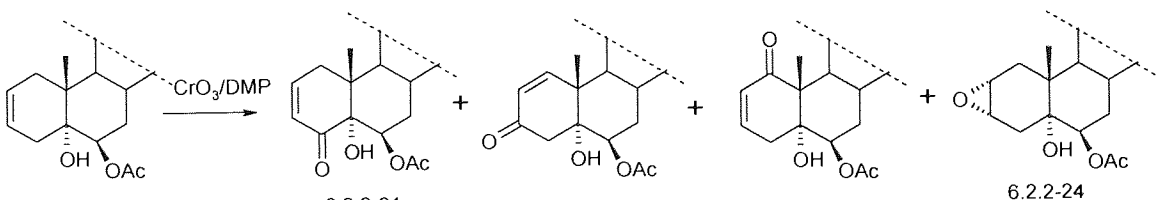
Compounds 6.2.2-10 to 6.2.2-12 were acetylated to yield 6.2.2-14, 5α-cholestane-2α,3α,4β,5,6β-pentol 2,3,5,6 tetraacetate (6.2.2-11a) and 6.2.2-17 to facilitate their purifications by column chromatography.



6.2.2-1 6.2.2-18 6.2.2-19

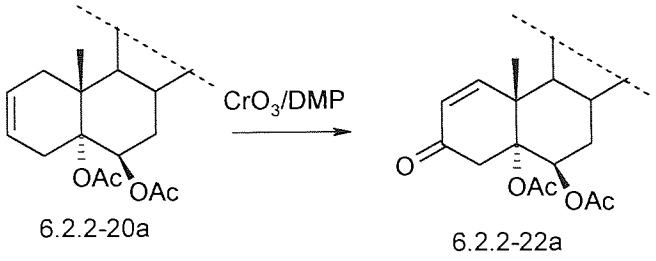
Scheme 6.2-14 Epoxide ring opening of 6.2.2-1 by perchloric acid

In the preparation of 2-enes with a 4-hydroxyl group, often hydroxyl or acetyl participation products were observed. Therefore the allyl oxidation was considered as an alternative approach to introduce the oxygenated functional group to C-1 and C-4 from 5α-cholest-2-en-5,6β-diol acetates. The CrO_3 / DMP oxidation of the 6-acetate 6.2.2-20 gave four products (6.2.2-21 to 6.2.2-24 9:3:2:1) in an overall yield of 69% (**Scheme 6.2-15**), while the 5,6-diacetate only gave the rearrangement compound 1-ene-3-one (**Scheme 6.2-16**). This may be explained by sequencing the oxidation process of intermediate ii in **Figure 6.2-3** other than one suggested by Salmond et al (Salmond et al 1978). The reason is that ii is highly congested with steric functional groups on both sides of the ring system. The reaction in **Scheme 6.2-16** required one week to reach 50% conversion, while the oxidation of the compound 6.2.2-20 needed 10hr to complete. Oxidation of 6.2.2-20 with CrO_3 in aqueous acetic acid gave 4-one 6.2.2-21 only in 50% yield (**Scheme 6.2-17**).



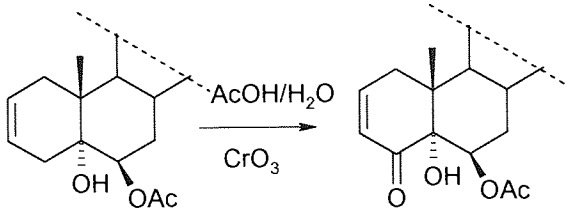
6.2.2-20 6.2.2-21 6.2.2-22 6.2.2-23 6.2.2-24

Scheme 6.2-15 Allyl oxidation of 6.2.2-20



6.2.2-20a 6.2.2-22a

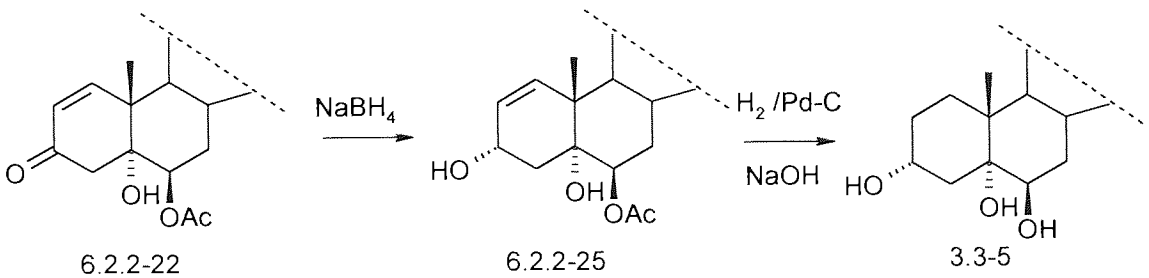
Scheme 6.2-16 Allyl oxidation of 6.2.2-20a



6.2.2-20 6.2.2-21

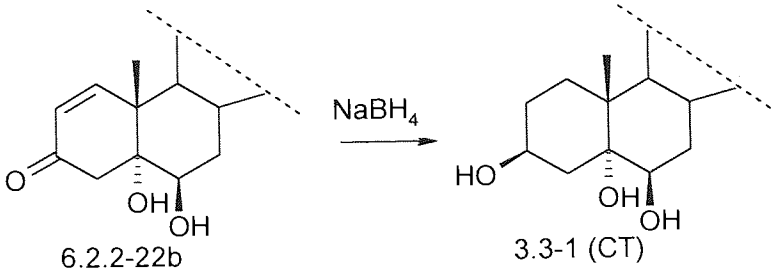
Scheme 6.2-17 Allyl oxidation of 6.2.2-20 in acidic media

5,6β-Diacetoxy-5α-cholest-1-en-3-one (6.2.2-22a) cannot be reduced by sodium borohydride under normal conditions. The 6-monoacetate (6.2.2-22) and the free diol (6.2.2-22b) gave different results as shown in **Scheme 6.2-18** and **Scheme 6.2-19**. Obviously the 6-acetoxy group prevented the attack of hydride on C-1. Structure of 6.2.2-21 is confirmed through its hydrogenation to the known compound 6.1-4 (**Scheme 6.2-20**). On epoxidation with hydrogen peroxide under basic conditions followed by reduction with sodium borohydride, compound 6.2.2-21 gave 2β,3β-epoxy-5α-cholestane-4β,5,6β-triol 6.2.2-27 (**Scheme 6.2-21**). This compound was also obtained from the epoxidation of the compound with a co-ordinate 4β-hydroxyl group as shown in **Scheme 6.2-22**.



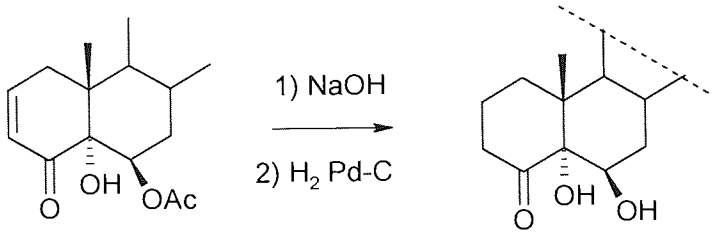
6.2.2-22 6.2.2-25 3.3-5

Scheme 6.2-18 Reduction of 6.2.2-22



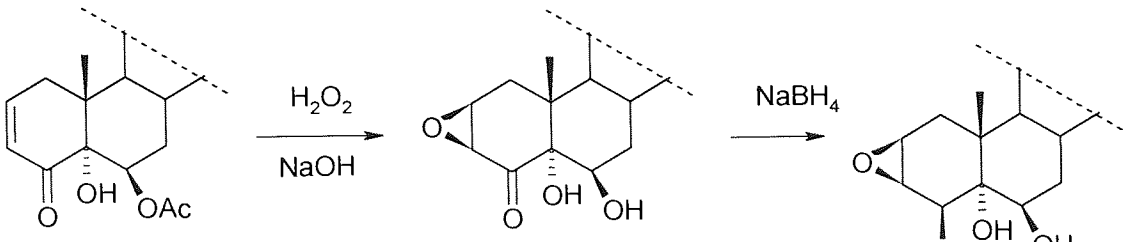
6.2.2-22b 3.3-1 (CT)

Scheme 6.2-19 Reduction of 6.2.2-22b



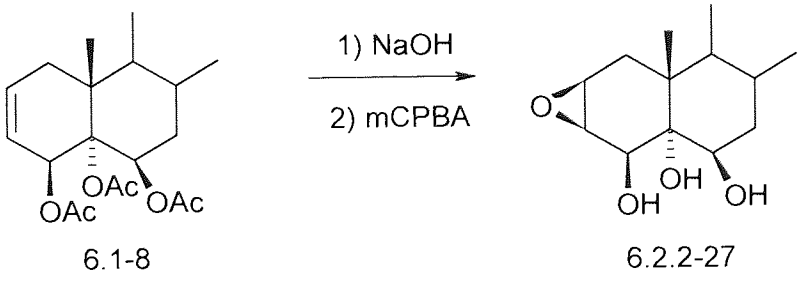
6.2.2-21 6.1-4

Scheme 6.2-20 Hydrogenation of 6.2.2-22



6.2.2-21 6.2.2-26 6.2.2-27

Scheme 6.2-21 Epoxidation and reduction from 6.2.2-21



6.1-8 6.2.2-27

Scheme 6.2-22 Hydrolysis and epoxidation from 6.1-8

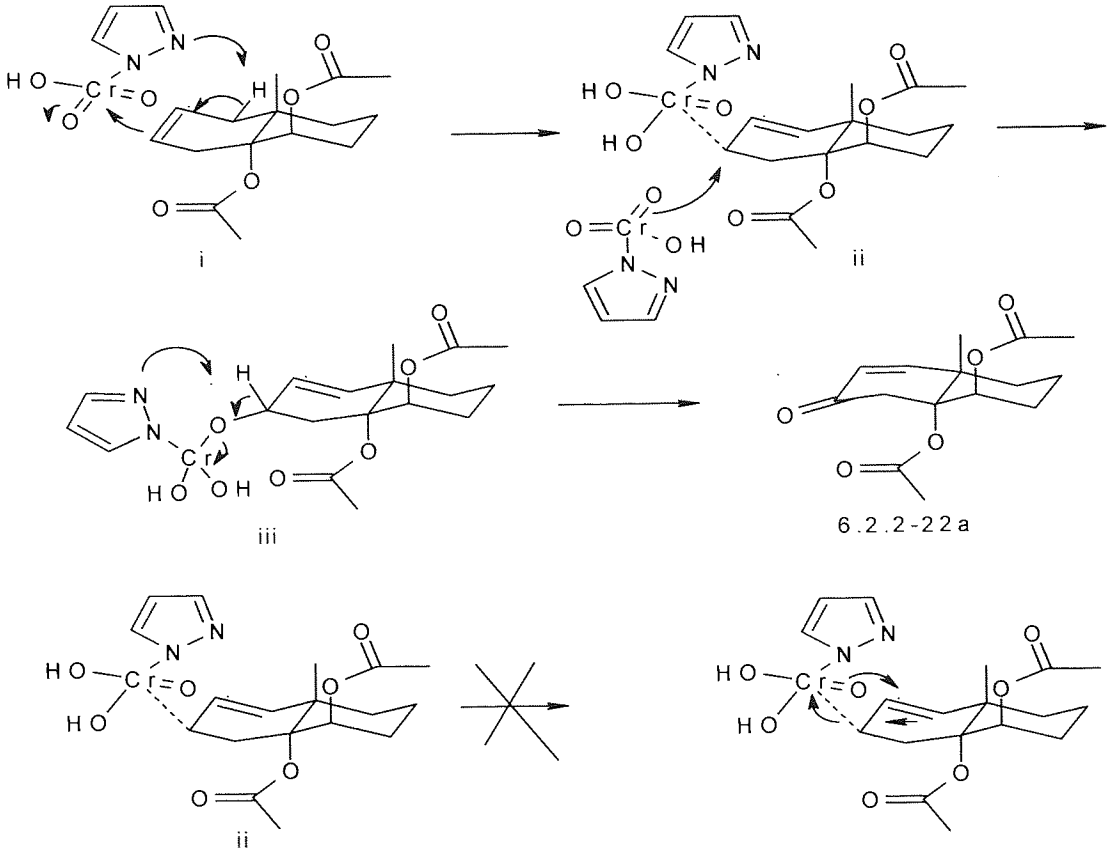
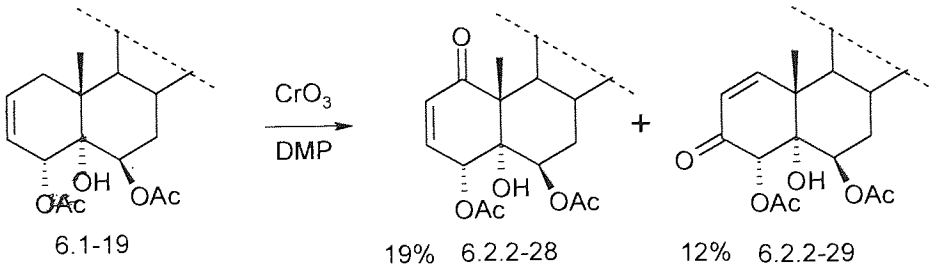


Figure 6.2-3 The intermolecular and intramolecular process on CrO_3 / DMP oxidation.

An attempt was made on CrO_3/DMP oxidation of the compound with 4α-acetoxy group, as shown in **Scheme 6.2-23**. The steric effect of the 4α-acetoxy group changed the molar ratio of 2-ene-1-one to 1-en-3-one from 1:3 to 2:1. This opened a possible route to preparations of 1-oxygenated polyoxygenated sterols by manipulating the steric effects on C-4 functional groups. However, the reaction is too slow to be a practical method because more than half the starting material was left after 3 weeks. Therefore more work is needed to develop effective allyl oxidation reagents for synthesis of these compounds.



Scheme 6.2-23 Allyl oxidation of 6.1-19

7. Experimental

Unless otherwise stated the following procedures were adopted:

Melting points were taken on Reichert-Jung Microthermal, uncorrected. I.R. spectra were recorded on a Mattson 3000 instrument and N.M.R. spectra at 250 MHz on a Bruker AC-250 or at 300 MHz on a Bruker AMX-300 instrument. Mass spectra were obtained on a HP G1034C GC/LC-MS Chemstation using atmospheric chemical ionisation (ACPI) and electrospary (ES) method. High-resolution mass spectra (HRMS) were measured on a Finnigan MAT 900 XLT high-resolution double-focusing mass spectrometer using electrospray method.

Solvents and chemicals used for reactions were purchased from commercial suppliers and used without further purification. All column chromatographic purifications were accomplished on silica gel 60 (200-400 mesh) with the appropriate solvent gradients. Thin-layer chromatography (TLC) was performed using 0.25 mm Merck Kieselgel 60 F254 precoated silica gel plates.

In a ^{13}C NMR spectrum a chemical shift marked with * indicates it represents two carbons.

General procedures:

Epoxidation:

A1: General procedure for mCPBA epoxidation

The steroidal alkene (1.00 mmol) and mCPBA (207 mg, 1.20 mmol) was dissolved in DCM (10 ml) and the mixture was stirred at room temperature for a given period. The resulting mixture was washed with 10% aqueous sodium hydroxide and water, dried over sodium sulfate. Removal of the solvent gave the crude product for further purification (see individual compounds for details).

A2: General procedure for VO (acac) $_2$ /TBHP epoxidations

To a solution of the steroidal alkene (1.00 mmol) and vanadyl acetylacetonate (5.3 mg, 0.02mmol) in DCM (10 ml) was added dropwise the TBHP/toluene solution (4.0 M,

0.75 ml, 3.00 mmol). The resulting homogeneous solution was stirred at room temperature for a given period. When TLC showed completion of the reaction, the mixture was washed with saturated sodium bicarbonate (2×20ml) and brine (2×20 ml), dried over $MgSO_4$. After removal of the solvent, the residue was subjected to standard flash column chromatography purification.

A3 General procedure for Sharpless epoxidations

Freshly distilled titanium tetra-*iso*-propoxide (426 mg, 1.50mmol) was added to DCM (2 ml), with pre-activated and powdered molecular sieves (400mg). The resulting mixture was cooled to -20°C. Freshly distilled (S,S) - (-) -di-*iso*-propyl tartrate (-DIPT) or (R,R) - (+) - di-*iso*-propyl tartrate (+DIPT) (352mg, 1.50 mmol) was then added. The resulting mixture was stirred for 15 min. A solution of the steroidal alkene (1.0 mmol) in DCM (10ml) was added. After 10 min of stirring, *tert*-butylhydroperoxide solution (4M, 0.75ml, 3.0 mmol) was added dropwise, and the reaction mixture was then stirred at a temperature between -10 and 0°C for 48 h. The resulting mixture was poured into a pre-cooled (0°C) aqueous solution of tartaric acid (10% w/v) (20 ml). The resulting mixture was vigorously stirred for 30 min, followed by dilution with diethyl ether (50 ml), washing with 10% aqueous tartaric acid (2×25 mL), brine (2×40 ml), and dry over $MgSO_4$. After the solvent was removed, the resulting crude product was subjected to a standard flash column chromatography purification.

The general procedures for ring opening of the epoxides:

B1. $HClO_4$ in THF: Slow procedure:

To the steroidal epoxide (2.0mmol) in THF (10ml) was added perchloric acid (0.3ml, 70%aq) at 0°C. The reaction was kept at 0°C for 20min and room temperature over the given period. The resulting mixture was diluted with water (20ml), extracted with ether or DCM. The organic layer was treated conventionally to yield the crude product that was subjected to a standard flash column chromatography purification to obtain the pure compounds.

B2. $HClO_4$ in THF: Fast procedure:

To the steroidal epoxide (2.0mmol) in THF (10ml) and water (0.4ml) was added perchloric acid (2.5ml, 70%aq) at room temperature. The reaction temperature rose to 50-55°C. Cooling water bath was necessary if the temperature exceeded 60°C. The reaction mixture was stirred while cooling down in the air over the given period. The resulting mixture was worked up as described in the slow procedure.

B3. $HClO_4$ in CH_3CN

To the steroidal epoxide (2.0mmol) dissolved in acetonitrile (20ml) was added perchloric acid (75%, 0.8ml) dropwise with constant stirring under in an ice bath. After stirred for the given period at room temperature, the resulting mixture was diluted with water, and treated conventionally.

The specified "fast" and "slow" procedures in acetonitrile were similar to that in THF (procedure B1 and B2).

B4. $BF_3{\cdot}Et_2O$ in THF

To the steroidal epoxide (0.5mmol) dissolved in dry THF (4ml) was added boron trifluoride etherate (0.15ml, 1.20mmol) dropwise at room temperature. After being stirred for the given period at room temperature, the resulting mixture was diluted with water and treated conventionally after.

B5. $BF_3{\cdot}Et_2O$ in CH_3CN

Same as the procedure for **B4. $BF_3{\cdot}Et_2O$ in THF** except using dry acetonitrile (4ml) to replace the THF.

B6. $AlCl_3$ in THF

Anhydrous aluminium chloride (0.30g, 2.2mmol) was dissolved in THF (4ml), the steroidal epoxide (0.5mmol) was added and the mixture was stirred at room temperature over the given period. The resulting mixture was diluted with water, and treated conventionally.

B7. $AlCl_3$ in CH_3CN

Same as the procedure for **B6. $AlCl_3$ in THF** except using dry acetonitrile (4ml) to replace the THF.

B8. $BF_3{\cdot}Et_2O$ in CH_3OH

Same as the procedure for **B6. $BF_3{\cdot}Et_2O$ in THF** except using methanol (4ml) to replace the THF and DCM was added after 5hr if the solid epoxide did not dissolve properly.

C1. General procedure for allylic oxidation with CrO_3 and DMP

3,5-Dimethylpyrazole (DMP) (2.05g, 21mmol) was dissolved in DCM (30ml), the solution was cooled to -20C. Chromium trioxide (2.1g, 21mmol) was added by small portions, the mixture was stirred at this temperature for 30min. Steroidal alkylene (4mmol) was added and the solution was kept at –18~-28°C for 5hr. The resulting dark mixture was stirred with 10% HCl (20ml) in an ice water bath for 5min, the organic layer was separated and washed with water (10ml), half saturated NaCl water solution, dried over sodium sulfate. The product was absorbed on silica gel (4g), then the mixture was fed to a column of dry silica (10g) and washed thoroughly with ether. The ether was evaporated and the residue solidified to give the product, using appropriate solvent (commonly methanol or ethanol) to do recrystallisation if necessary, yield 50-80%.

C2. General procedure for the reduction of steroidal carbonyl group to hydroxyl group with $NaBH_4$

The steroidal ketone (10mmol) was dissolved in THF (40ml), methanol (8ml) and water (4ml). Sodium borohydride (1.6g, 42.3mmol) was added and the mixture was stirred at room temperature for 1hr. TLC was used to check the end of the reaction. The reaction was quenched by adding acetic acid (or 10%HCl) till the gas release ceased. The resulting mixture was poured into water, the solid was filtered out and dried to give the crude product. Oil product was extracted with appropriate solvents.

C3. General procedure for the reduction of steroidal functional groups with $LiAlH_4$

The $LiAlH_4$ (76mg, 2.0mmol) was dissolved in dry THF (4ml), followed by the addition of the steroid. The mixture was stirred at room temperature until TLC shows the reaction completed. The resulting mixture was stirred with ice water bath cooling and methanol was added dropwise until the gas release ceased. Hydrochloric acid (10% aq., 15ml) was added and the product was filtered off or extracted with appropriate solvent and conventionally treated.

C4. General procedure for steroidal alkene hydroboration-oxidation:

Steroidal alkene (2.5mmol) was added to dry THF (15ml), followed by $NaBH_4$ (0.4g, 10.7mmol) and the mixture was stirred in an ice water bath under N_2 atmosphere. Boron trifloride etherate (1.2ml, 10.6mmol) was added dropwise during 1hr, then the mixture was stirred at room temperature for 2.5hr. Water was added slowly to destroy the excess borane; after the gas evolution ceased, 10% NaOH (15ml) was added and the mixture was returned to the ice water bath with stirring, hydrogen peroxide (30%, 5ml) was added dropwise and the mixture was stirred for an further hour. Diluted with water and ether, the organic layer was washed with sodium sulfate (20% aq) and water dried over sodium sulfate and evaporated to afford the crude product. The crude product was purified by passing through a silica column and recrystallised in appropriate solvent to afford the product.

D1. Hydrolysis of a sterol acetate or benzoate in a protic solvent with NaOH under heating

The oxysterol acetate (2.0mmol) was dissolved in ethanol (20ml) (if it was not dissolved at room temperature heating the mixture to 40-50 °C), a solution of sodium hydroxide (0.4g, 10mmol) in water (4ml) was added and the mixture was heated at reflux (for substance not stable under heating lower temperatures are applied) for 1 hr. After cooling down the mixture was poured into water and the solid was collected by

filtration, if the product is oil then extracted with suitable solvent followed by normal working-up.

D2. Hydrolysis of a sterol acetate or benzoate in a mixed solvent with NaOH at room temperature

The oxysterol acetate (2.0mmol) was dissolved in DCM and ethanol (make as small volume as possible), a solution of sodium hydroxide (0.4g, 10mmol) in water (2ml) was added and the mixture was kept at room temperature for 1 hr. After cooling down the mixture was poured into water and the solid was collected by filtration. If the product is oil then extracted with suitable solvent followed by normal working-up.

D3. Acetylation with basic catalyst:

Sterol (2.00mmol), acetic anhydride (1.0ml for every hydroxyl group) and pyridine (0.05ml) were heated at reflux in toluene (10.0ml) for the given period. For more stereo hindered sterols, DMAP (0.10mmol) was used additionally. After cooling down, the toluene was evaporated and the residue was taken up with DCM, washed with 5% HCl and dried over sodium sulfate. Removal of the solvent gives the crude product for appropriate further purification.

D4. Acetylation with an acidic catalyst:

Sterol (2.00mmol) and acetic anhydride (5ml) were stirred in room temperature, following with the addition of boron trifloride etherate (0.40mmol). After a given period, the reaction was quenched by pouring the resulting mixture into ice water (40ml), followed by normal working-up.

D5. General procedure for the sterol p-methylsulfonation at room temperature

The oxysterol (1.00mmol) was dissolved in pyridine (5.0ml), toluene-4-sulfonyl chloride (0.23g, 1.20mmol for every hydroxyl group) was added and the solution was kept at room temperature for 16hr. The resulted mixture was poured into ice cooled 10% hydrochloric acid, stirred, filtered or extracted with appropriate solvents, purified by normal working-up.

E1. General procedure for the elimination of steroidal 5-hydroxyl group in pyridine with thionyl chloride:

The steroid (4.0 mmol) was dissolved in pyridine (6ml) and the solution was cooled to –20°C. Thionyl chloride (0.40ml, 5.5mmol) was added in several portions within one minute under shaking. The mixture was allowed to stand at –20°C for 5 min, and at 0°C for 10min, then poured into ice water. The product was filtered out or extracted with appropriate solvent followed by normal working-up.

E2. General elimination procedure by use of LiBr / Li_2CO_3 in DMF.

The steroidal toluene-4-sulfonate (1.5 mmol), LiBr (1.2g, 13.8mmol), Li_2CO_3 (150mg, 2.0mmol) was heated at reflux in DMF(5ml). Working up: diluted with water and treated conventionally.

Methyl 3β–acetoxychol-5-en-24-oate (2-4)

Glacial acetic acid (200ml), acetic anhydride (10ml) and potassium acetate (40.0g, 410mmol) was heated until all the solids dissolved. Methyl 3β–chlorochol -5-en-24-oate (2-5) (12.0g, 30mmol) was added and the mixture was heated at reflux for 5 h. After cooling down to room temperature, the resultant mixture was poured into cold water (200ml) and kept at room temperature for a while, filtered and dried to afford 12.6g white solid, recrystallisation from ethanol-water gave white platelet crystals 11.9g (94%). M.p. 155-157°C (Lit. M.p.155-156°C, Ushizawa 1960); IR: ν_{max} 3500-3300, 2958, 2937, 2888, 2863, 2852, 1734, 1435, 1372, 1251 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.35 (m, 1H, H-6), 4.56 (m, 1H, H-3), 3.64 (s, 3H, 24-OCH_3), 1.00 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); m/z 453 ($M+Na^+$, 100).

Methyl 3β–chlorochol-5-en-24-oate (2-5)

To a three-necked flask was added anhydrous pyridine (120ml) and phosphorus oxychloride (60.0ml, 660mmol), the mixture was stirred and heated at 70°C. HDCA methyl ester (2-2) (30.0g, 62mmol) in pyridine (60ml) was added dropwise during 1 h at 75°C. Stirring was continued at 75°C for 2.5h. After cooling down, the resulting mixture was poured slowly into ice water (3L), the solid was filtered out and

dissolved in ether (250ml). The ether layer was washed with 5% HCl, 5% NaOH and water, dried over sodium sulfate and evaporated to give an oil which on recrystallisation from ethanol-water gave light yellow crystals, yield 12.6g (44%). M.p. 114-115°C (Lit. 117-118°C, Ushizawa, 1960); IR: ν_{max} 3500-3300, 2954, 2937, 2906, 2868, 2846, 1743, 1463, 1437, 1377, 1197, 1161 and 804 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.34 (d, 1H, H-6), 3.67-3.78 (m, 1H, H-3), 3.64 (s, 3H, 24-OCH_3), 1.00 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 18.2, 19.1, 51.4; (CH_2): 20.8, 24.1, 28.0, 30.8, 30.9, 31.6, 33.3, 39.0, 39.5, 43.3, (CH): 31.6, 35.2, 49.9, 55.6, 56.5, 60.1, 122.3; (C): 36.2, 42.2, 140.6, 174.5; MS (ES^+): m/z 429 ($M+Na^+$, 100).

Methyl 3β–acetoxychol-5-en-7-one-24-oate (2-6)

Compound 2-6 was prepared using the general procedure C1 for allylic oxidation from Methyl 3β–acetoxychoc-5-en-24-oate (2-4) using CrO_3 and DMP. The crude product was recrystallised from methanol and a white solid was given, yield 70%. M.p. 176-177°C (Lit. 177.5-179°C, Greenhalgh et al 1952) IR: ν_{max}: 2942, 2873, 1741, 1665, 1471, 1441, 1371, 1305, 1243, 1182, 1164 and 1037 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.67 (d, J=1.3 Hz, 1H, H-6), 4.64-4.73 (m, 1H, H-3), 3.64 (s, 3H, 24-OCH_3), 1.18 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 17.1, 18.3, 21.1, 51.3; (CH_2): 21.0, 26.1, 27.2, 28.3, 30.8, 30.9, 35.9, 37.6, 38.5, (CH): 35.1, 45.2, 49.6, 49.8, 54.3, 72.0, 126.5; (C): 38.2, 43.0, 163.7, 170.1, 174.6, 201.6; MS (ES^+): m/z 467 ($M+Na^+$, 45), 385 (100).

Methyl 3β–acetoxy-7β-hydroxychol-5-en-24-oate (2-7)

Methyl 3β–acetoxychoc-5-en-7-one-24-oate (2-6) (1.00g, 2.25mmol) was dissolved in methanol (20ml). Cerium trichloride hydrate ($CeCl_3 \cdot 7H_2O$, 1.50g, 4.00mmol) in water (4ml) was added following by sodium borohydride (0.15g, 3.70mmol), and the mixture was stirred at room temperature for 1h. TLC was used to monitor the reaction. The reaction was quenched by adding acetic acid (or 10%HCl) till the gas release ceased. The resulting mixture was poured into water, the solid was filtered out, dried to give the crude product which was recrystallised from ethanol-water to afford white

crystals, yield 0.68g (67%). M.p. 121-123°C; IR: ν_{max}: 3432, 2964, 2933, 2892, 2858, 1729, 1712, 1438, 1387, 1263, 1203, 1172 and 1043 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.29 (s, 1H, H-6), 4.60 (m, 1H, H-3), 3.83 (dd, 1H, H-7), 3.64 (s, 3H, 24-OCH_3), 1.04 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 18.3, 19.0, 21.3, 51.4; (CH_2): 20.9, 26.2, 27.6, 28.3, 30.9*, 36.4, 37.5, 39.3, (CH): 35.2, 40.6, 48.0, 55.0, 55.8, 73.0, 73.3, 126.3; (C): 36.5, 42.8, 142.1, 170.4, 174.6; MS (ES^+): m/z 469 ($M+Na^+$, 12), 369 (75), 690 (100).

Methyl 3β–acetoxy-7α-hydroxychol-5-en-24-oate (2-8)

Methyl 3β–acetoxychoc-5-en-7-one-24-oate (2-6) (1.00g, 2.25mmol) was dissolved in THF (20ml). L-selectride (3.00ml, 3.00mmol) was added dropwise at 15°C, the mixture was stirred at room temperature for 1h. The reaction mixture was treated as described for the preparation of compound 2-7 except that the recrystallisation was three times from methanol and the yield is 0.44g (44%). M.p. 165-166°C (Lit. 164-165°C, Tohma et al 1986). IR: ν_{max}: 3502, 2968, 2933, 2879, 1736, 1722, 1461, 1442, 1405, 1373, 1242, 1201, 1182 and 1030 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.60 (d, J=5.0 Hz, 1H, H-6), 4.60 (m, 1H, H-3), 3.83 (br s, 1H, H-7), 3.61 (s, 3H, 24-OCH_3), 0.98 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.5, 18.0, 18.2, 21.2, 51.3; (CH_2): 20.5, 24.0, 27.4, 28.0, 30.8*, 36.5, 37.7, 38.9, (CH): 35.2, 37.3, 41.9, 49.2, 51.3, 64.8, 73.2, 124.7, (C): 37.3, 42.0, 144.6, 170.2, 174.5; MS (ES^+): m/z 469 ($M+Na^+$, 11), 369 (100).

Methyl 3β, 7β-dihydroxychol-5-en-24-oate (2-9)

Sodium (0.2g, 8.7mmol) was dissolved in anhydrous ethanol (12ml). To this solution compound 2-7 (0.4g, 1.0mmol) was added and the solution was heated instantly at reflux for 9 min. the resultant mixture was cooled to room temperature and poured to water (10ml). The solid was filtered out and recrystallised from methanol twice to give white crystals as the product, yield 0.22g (61%). M.p.144-146°C; ^1H NMR ($CDCl_3$): δ_H 5.25 (s, 1H, H-6), 3.80 (dd, 1H, H-7), 3.62 (s, 1H, H-3), 3.62 (s, 3H, 24-OCH_3), 1.02 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 427 ($M+Na^+$, 100).

3β, 7β-dihydroxychol-5-en-24-oic acid (2-10) and **3β, 7α-dihydroxychol -5-en-24-oic acid (2-11)** was prepared using the general hydrolysis method at ambient temperature (procedure D2) and the yields are 92% and 91% separately.

2-10: M.p. 231-234°C (Lit. 237 °C, Tohma et al 1986); IR: ν_{max}: 3500-3300, 2933, 2870, 1699, 1652, 1644, 1462, 1436, 1381, 1259, 1099, 1061 and 806 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 5.38 (d, 1H, H-6), 3.57 (m, 1H, H-7), 3.30 (m, 1H, H-3), 0.89 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^+): m/z 413 ($M+Na^+$, 100).

2-11: M.p. 203-204°C (Lit. 200-201°C, Tohma et al 1986); IR: ν_{max}: 3500-3300, 2932, 2860, 1710, 1639, 1461, 1374, 1255, 1160, 1097 and 806 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 5.34 (d, 1H, H-6), 3.35 (m, 1H, H-7), 3.26 (m, 1H, H-3), 0.93 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); MS (ES^+): m/z 413 ($M+Na^+$, 100).

5α–Cholestane-3β,5,6β-triol (CT)

A refined procedure developed from the published method (Fieser and Rajacopalan 1948) was tested repeatedly as following:

To cholesterol (50g, 129mmol) was added formic acid (98%, 350ml), the mixture was heated to 75°C with stirring and standing at this temperature for 5min. After cooling to room temperature, aqueous hydrogen peroxide (30%, 70ml, 620mmol) was dropped in during 15min with constant stirring. The mixture was stirred at room temperature overnight. The resulting foam product was diluted with hot water (760ml) and the solid was filtered out after cooling to room temperature, washed with saturated sodium carbonate and dried. This solid was dissolved in methanol (1500ml), sodium hydroxide (25%, 50ml) was added and the result solution was refluxed for 1h. Water was added slowly to make the precipitation complete. The mixture was cooled to room temperature, the solid product was filtered out and dried to give a white solid, yield 50.6g(93%). The analytical data was listed in 3.3-1.

5α–Cholestane-3α,5,6β-triol (2-12)

The method described for the preparation of CT was used with epicholesterol as the starting material. Yield 84%. The preparative method used in section 3.1 is as follows:

5β,6β–Epoxycholestan–3α–ol (3.1-16) (15.6g, 38.7mmol) was dissolved in acetone (100ml) and water (10ml) at 0°C with stirring. Perchloric acid (15ml) was added and the reaction mixture was stirred at room temperature for 2h. NaOH was added to quench the reaction and the acetone was removed under reduced pressure. The residual was extracted with ether and dried over Na_2SO_4. Removal of the solvents gave the triol (15.0g, 95%) as a white solid. The analytical data was listed in 3.3-5.

5α–Cholestane-3β,5,6α-triol (2-13)

Published method (Minato et al 1990) based on catalytic amount osmium with $K_3Fe(CN)_6$ was used to afford the title compound in 15% yield from cholesterol. The analytical was listed in 3.3-3.

5α–Cholestane–3β,5,6β,7β-tetrol (2-14)

7β-Hydroxycholesterol 3-benzoate (2.00g, 3.95mmol) was dissolved in DCM (10ml), a solution containing perbenzoic acid (2.00g, 14.5mmol) in DCM (10ml) was added and the reaction mixture was kept at 0°C overnight. The resulting mixture was washed with 10% aqueous $NaHSO_3$ (20ml), 10% NaOH (20ml) and brine, dried over sodium sulfate. The solid impurity in the solution was filtered off through Celite, the clear solution was evaporated to give 2.0 g α,β epoxide mixture as a white solid. This mixture was dissolved in butanone (60ml) with stirring in ice water bath. Perchloric acid (70%, 1ml) was added dropwise and the mixture was stirred for further 30min at 0°C and 5h at room temperature. The resulting mixture was poured into water, filtered, the solid was dried and purified over silica column with chloroform as eluent to remove the less polar impurities and chloroform/ acetone (30:1) as eluent to afford a white solid, yield 1.40g (65%). The 3-benzoate was hydrolysed using general method to afford the title compound as white crystals, yield 1.30g (99%). Mp 220-221°C (Lit. 219-222°C, Kawata et al, 1976); IR: ν_{max} 3500-3300, 2937, 2861, 1465,1390 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.06 (m, 1H, H-3), 3.80(m, 1H, H-7), 3.48 (m, 1H, H-6) 1.10 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^-): m/z 437($M+H^+$, 36), 497(100).

3,6,7-Triacetate (Warren et al 1989):

Obtained as foam. IR: ν_{max} 3500-3300, 2954, 2867, 1741, 1469,1377, 1240 and 1027 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.10-5.25 (dd, J1=3.9Hz, J2=10.7Hz, 1H, H-7; m, 1H, H-3), 4.98 (d, J=3.9Hz, 1H, H-6), 1.14 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 17.0, 18.6, 21.0, 21.2, 21.3, 22.5, 22.7; (CH_2): 21.1, 23.7, 25.2, 26.9, 28.5, 32.1, 36.0, 36.7, 39.4, 39.9, (CH): 27.9, 35.4, 35.6, 43.9, 54.3, 55.1, 70.4, 73.0, 74.6,(C): 37.8, 43.4, 75.1, 170.3, 170.6, 171.1;

5α–Cholestane–3β,5,6β,7α-tetrol (2-15)

This compound was prepared from 7α-hydroxycholesterol benzoate using the same method as the 7β isomer. Mp 248-249°C (Lit. 250-251°C, Kawata et al 1976); IR: ν_{max} 3500-3300, 2935, 2871, 1463,1378 and 1040 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 5.34(d, J=5.8hz, 1h, C-6OH), 4.92(s, 1H, C-5OH), 4.86(d, J=4.6Hz, 1H, C-7OH), 4.21(d, J=5.7Hz, 1H, C-3OH), 3.74 (m, 1H, H-3), 3.46(m, 1H, H-7), 3.29 (m, 1H, H-6) 0.99 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^-): m/z 437($M+H^+$, 100), 497(70).

3,6,7-Triacetate (Warren et al 1989):

Mp 139-144°C; IR: ν_{max} 3500-3300, 2948, 2867, 1738, 1463,1371, 1243 and 1031 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.15 (m, 1H, H-3), 4.84 (t, 1H, H-7), 4.77 (d, 1H, H-6), 1.13 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 16.3, 18.5, 20.9, 21.0, 21.3, 22.5, 22.7; (CH_2): 20.7, 23.5, 23.7, 26.4, 27.8, 31.5, 35.7, 36.0, 39.1, 39.3, (CH): 27.9, 34.0, 35.6, 39.0, 45.4, 55.8, 69.9, 72.1, 72.8,(C): 38.7, 42.6, 75.4, 168.6, 168.7, 170.3; MS (ES^+): m/z 580 ($M+NH_4^+$,100).

3β-Benzoxycholest-5-en-7-one (2-19, R=Bz)

DMP (100.0g, 1.04mol) was dissolved in dry DCM (400ml). The solution was stirred at -25~-35°C and chromium trioxide (104g, 1.04mol) was added by several portions. The solution was stirred at this temperature until all solid dissolved. Cholesterol benzoate (65g, 132mmol) was added in one portion and the mixture was stirred at this temperature for another 10min. the resulting solution was kept in the freezer (-20~-25°C) for 72h. After the reaction finished, monitored by TLC (DCM/Acetone 40/1),

the resulting mixture was washed with ice-cooled hydrochloric acid (20% 300ml) and half –saturated brine (200ml). Silica gel (240g) was added and the mixture was evaporated below 35 °C. the residue was added to a column of 480g silica gel and washed with 3~4l ether. Evaporation of the ether gave a solid which give off-white solid by treating with methanol (95%aq, 80ml). The purity is enough to do NMR analysis (with no impurities peak). The yield is 55g (82%).

Cholesteryl chloride (3β-chlorocholest-5-ene) (2-20)

Cholesterol (38.7g, 100mmol) was mixed with thionyl chloride (35ml) and the mixture was kept at room temperature for 20 hr. The resulting dark liquid was diluted with acetone (400ml), and the excess reagent destroyed by adding water. The solid was filtered out and washed by acetone to yield 37.0g light yellow crystals, yield 90%.

Cholest-5-ene (2-21)

Cholesteryl chloride (4.0g, 10mmol) was dissolved in ethanol (40ml) and THF (55ml). The solvent was cooled to –50°C with stirring. Sodium chips (3.0g) was added and the mixture was stirred for 5hr at this temperature. The sodium chips left were filtered off and the filtrate was poured into 200ml ice water. Filtration and drying in vacuum give 3.6g white solid, yield 99%.

Cholest-4-en-3β,6β-diol (3.1-1)

Cholest-4-en-3β,6β-diol 3,6-diacetate (3.1-2) was hydrolysed using general procedure D1 to afford the title compound as granular crystals with quantitative yield. M.p. 254-256°C (Lit. 253-256°C, Teng et al 1973); IR: ν_{max}: 3500-3300, 2935, 2867, 1635, 1465, 1378 and 1037 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.52 (s, 1H, H-4), 4.21 (s, 1H, H-6), 4.08 (m, 1H, H-3), 1.24 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); MS(ES^+): m/z 425 ($M+Na^+$,100).

Cholest-4-en-3β,6β-diol 3,6-diacetate (3.1-2)

5α–Cholestane-3β,5,6β-triol (CT) was converted to its 3,6-diacetate with procedure D3 in 91% yield and the 5-hydroxyl group was eliminated (protocol E1) to generate

the 4,5-double bond and give the title compound as white solid, yield 96%. M.p. 133-135 °C (Lit. 134-135°C, Ellington et al 1966); IR: ν_{max}: 3500-3300, 2952, 2871, 1753, 1471, 1383, 1240, 1008, 705 and 609 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.58 (s, 1H, H-4), 5.26 (s, 1H, H-6), 5.19 (m, 1H, H-3), 1.17 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.4, 20.2, 20.9, 21.2, 22.3, 22.6; (CH_2): 20.6, 23.6, 23.9, 24.5, 27.9, 35.9, 36.3, 36.9, 39.3, 39.5; (CH): 27.7, 30.5, 35.6, 53.6, 55.7, 55.9, 69.9, 74.6, 127.4; (C): 36.3, 42.2, 143.5, 169.1, 169.9; MS (ES^+): m/z 509 ($M+Na^+$, 100).

Cholest-4-en-3β,6α-diol (3.1-3)

Cholest-4-en-3β,6α-diol 3,6-diacetate (3.1-4) was hydrolysed using general procedure D1 to afford the title compound as white solid with quantitative yield. M.p. 176-177°C (Lit. 174-177°C, Kulig et al 1973); IR: ν_{max}: 3500-3300, 2870, 1654, 1463, 1378 and 1076 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.63 (s, 1H, H-4), 4.15-4.21 (m, 2H, H-3 & H-6), 1.02 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 425 ($M+Na^+$, 100).

Cholest-4-en-3β,6α-diol 3,6-diacetate (3.1-4)

Cholest-4en-3,6-dione (3.1-9) was reduced with sodium borohydride (protocol C2) and the crude product was acetylated (protocol D3) and recrystallised twice from methanol to yield the title compound as plate crystals. Yield 70%. M.p. 161-162°C (Lit. 162-163°C, Hartshorn et al 1966); IR: ν_{max}: 3500-3300, 2937, 2870, 1738, 1475, 1383, 1245 and 1029 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.41 (s, 1H, H-4), 5.28 (m, 2H, H-3 & H-6), 1.07 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 19.3, 21.1, 21.2, 22.4, 22.7; (CH_2): 20.7, 23.7, 23.9, 24.6, 28.0, 35.3, 36.0, 38.5, 39.3, 39.4; (CH): 27.8, 34.0, 35.6, 53.7, 55.6, 56.0, 70.2, 70.3, 116.3; (C): 38.0, 42.4, 146.0, 169.8, 170.6; MS (ES^+): m/z 509 ($M+Na^+$, 100).

Cholest-4-en–3α,6β–diol (3.1-5).

Cholest-4-en–3α,6β–diol, 3,6-diacetate (13.6g, 27.9mmol) was dissolved in ethanol, then NaOH (4.0g) dissolved in water (16ml) was added. The resulting clear solution

was heated at reflux for 30min, then diluted with water (200ml) and extracted with ether (160ml). The ether layer was dried over sodium sulfate. Removal solvent gave the title compound 3.1-5 (11.2g, 99%). M.p. 235-237°C (Lit. 248°C, Wahidulia et al 1998); IR: ν_{max}: 3500-3300, 2937, 2871, 1677, 1469, 1378 and 1021 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.68 (d, J=4.5Hz, 1H, H-4), 4.21 (br s, 1H, H-6), 4.10 (m, 1H, H-3), 1.15 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.6, 20.4, 22.5, 22.7; (CH_2): 21.3, 23.8, 24.1, 27.7, 28.1, 32.4, 36.0, 39.0, 39.4, 39.7; (CH): 27.9, 29.8, 36.0, 53.6, 56.0, 56.1, 63.6, 74.4, 125.8; (C): 36.8, 42.6, 149.6; MS (ES^+): m/z 385 (M-OH, 100).

Cholest-4-en–3α,6β–diol, 3,6-diacetate (3.1-6)

Prepared by using general procedures D3 and E1 with triol 2-12 as the starting material in a combined yield of 90%. M.p. 104-106°C (Lit. 102.5-103.5°C, Urushibara and Mori 1958); IR: ν_{max}: 3500-3300, 2948, 2875, 1745, 1475, 1382, 1245 and 1012 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.76 (d, J=4.8Hz, 1H, H-4), 5.36 (m, 1H, H-6), 5.19 (m, 1H, H-3), 1.06 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.5, 19.4, 21.1, 21.4, 22.4, 22.7; (CH_2): 21.2, 23.7, 24.0, 24.5, 28.0, 33.0, 36.0, 36.7, 39.3, 39.6; (CH): 27.8, 30.4, 35.6, 53.1, 55.7, 56.0, 66.3, 75.3, 124.8; (C): 36.6, 42.4, 146.7, 169.4, 170.0; MS (ES^+): m/z 509 ($M+Na^+$,100) 427(80), 385(30).

Cholest-4-en–3α,6α–diol (3.1-7)

General procdure D2 was used on hydrolysis of 3.1-25, yield 89%. M.p. 170-171°C (Lit. 166-167°C, Fudge et al 1954); IR: ν_{max}: 3500-3300, 2941, 2871, 1685, 1463, 1378, 1080and 999 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.81 (s, 1H, H-4), 4.17 (m, 2H, H-3 & H-6), 0.95 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 425 ($M+Na^+$, 30).

Cholest-4-en–3α,6α–diol, 3,6-diacetate (3.1-8)

The 3-monoacetate 3.1-25 was acetylated using the general procedure D3 to give an oil product in 95% yield, solidified on long time standing in room temperature. M.p. 101-104°C (Lit. 108-109°C, Hartshorn et al 1966); IR: ν_{max}: 3500-3300, 2945, 2865,

1735, 1469, 1371, 1226 and 1012 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.54 (d, J=5.0Hz, 1H, H-4), 5.26 (m, 1H, H-6), 5.14 (br s, 1H, H-3), 0.99 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 18.5, 20.9, 21.1*, 22.4, 22.6; (CH_2): 21.2, 23.6, 23.9, 24.2, 28.0, 32.5, 35.9, 38.1, 39.3, 39.4; (CH): 27.8, 33.8, 35.5, 53.2, 55.5, 55.9, 66.4, 70.3, 113.6; (C): 37.9, 42.4, 148.5, 169.5, 170.2; MS (ES^+): m/z 509 ($M+Na^+$,100).

Cholest-4en-3,6-dione (3.1-9)

DMP (10.0g, 96mmol) was dissolved in DCM (150ml) and cooled to –5°C, chromium trioxide (10.0g, 99mmol) was added portionwise and the dark solution was stirred at this temperature for 20min. Cholest-4-en-3β,6β-diol (16g, 39.8mmol) was added and the solution was stirred at –5°C for 2 hr. The resultant DCM solution was washed with 10% HCl, brine and dried over sodium sulfate, then mixed with silica gel (20g) and evaporated to dryness. The dark solid was triturated with ether and added to the top of a column with 60g silica gel in it, eluted with ether (300ml). The ether was removed by distillation to give the title compound, yield 15.0g(95%). M.p. 124-125°C (Lit. 124-125°C, Dauben et al 1960); IR: ν_{max}: 2944, 2865, 1685, 1467, 1382, 1242 and 1225 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.14 (s, 1H, H-4), 1.14 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.3, 17.3, 18.5, 22.4, 22.7; (CH_2): 20.7, 23.6, 23.8, 27.9, 33.8, 35.3, 35.9, 39.0, 39.3, 46.5; (CH): 27.8, 34.0, 35.5, 50.7, 55.8, 56.3, 125.2; (C): 39.6, 42.3, 160.8, 199.1, 201.9; MS (ES^+): m/z 399 ($M+H^+$,100).

3β,5-Dihydroxy-5α-cholestan-6-one (3.1-10)

Cholestan-3β,5α,6β-triol (CT) (1.0g, 2.5mmol) was dissolved in ether (14ml), methanol (5ml) and water (2ml), the mixture was stirred at 28-30°C. To this solution, N-bromosuccinamide (0.55g, 3.1mmol) was added and the reaction mixture was stirred for 20 min, a white precipitate was formed during this period. 5% $NaHSO_3$ was added to quench the reaction. The white precipitate was filtered out and dried at room temperature to give the product, yield 0.73g(73%). The ether layer of the filtrate was separated and evaporated. The residue was recrystallised with hexane / DCM to give another 0.21g product, the total yield is 94%. M.p. 229-231°C (Lit. 230-231°C,

Kaminski and bodor 1976); IR: $\nu_{max:}$ 3500-3300, 2948, 2871, 1706, 1465, 1375, 1248 and 975 cm^{-1}; ^1H NMR ($CDCl_3$): 3.95 (m, 1H, H-3), 0.78 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^+): m/z 441 ($M+Na^+$,100).

3β-Acetoxycholest-4-en-6-one (3.1-11)

3β,5-dihydroxy-5α-cholestan-6-one (3.1-10) was converted to its 3-acetate using protocol D3. The 5-hydroxyl group was eliminated using the procedure E1 to yield the title compound in 85%. M.p. 108-110°C (Lit. 109-110°C, Fieser, Yuan et al 1960); IR: ν_{max}: 2956, 2865, 1739, 1691, 1640, 1467, 1382, 1228, 1033 and 1014 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.04 (s, 1H, H-4), 5.27-5.33 (m. 1H, H-3), 1.00 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 19.4, 20.9, 22.4, 22.7; (CH_2): 20.6, 23.6, 23.7, 23.9, 27.9, 35.3, 34.2, 35.9, 39.1, 39.3, 46.1; (CH): 27.8, 33.9, 35.5, 51.0, 55.8, 56.4, 69.0, 128.4; (C): 38.0, 42.4, 147.8, 170.2, 201.9; MS (ES^+): m/z 465 ($M+Na^+$, 90), 504(100).

Cholest-4-en-3β,6α-diol, 3-acetate (3.1-12)

3β-acetoxycholest-4-en-6-one (3.1-11) was treated with sodium borohydride (procedure C2). The yield is 88%. M.p.116-117°C; IR: ν_{max}: 3500-3300, 2952, 2865, 1733, 1708, 1469, 1373, 1236 and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.57 (s, 1H, H-4), 5.27 (m. 1H, H-3), 4.11-4.18 (m, 1H, H-6), 1.03 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 19.4, 21.3, 22.4, 22.7; (CH_2): 20.8, 23.7, 24.1, 24.7, 28.0, 35.5, 36.0, 39.3, 39.5, 41.9; (CH): 27.8, 34.2, 35.6, 53.8, 55.7, 56.0, 68.2, 70.8, 115.8; (C): 37.6, 42.3, 150.6, 170.8; MS (ES^+): m/z 467 ($M+Na^+$, 100).

5β,6β-Epoxy-3α-cholestanol (3.1-16)

3β-Chlorocholestan-5α,6β-diol (3.1-18) (20.0g, 45.5mmol) was dissolved in ethanol (400ml). To this solution aqueous NaOH (30.0g, 750mmol in 200ml water) was added. The resulted mixture was heated at reflux for 5h. Water (300ml) was added slowly when it was still hot. After cooled down to room temperature 15.6g (85%) of the crystal 3.1-16 was collected. m.p.157-158°C (Lit. 156-159°C, Holland and

Jahangir 1983); IR: ν_{max}: 3413, 2933, 2863, 1469, 1384 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.18 (s, 1H, H-3), 3.06 (s. 1H, H-6), 0.95 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); MS (ES^+): m/z 425 ($M+Na^+$, 100).

3β-Chloro-5α-cholestane-5,6β-diol (3.1-18)

Cholesteryl chloride (9.6g, 24.0mmol) was dissolved in THF (130ml) and DCM (100ml), a mixture of formic acid (90ml) and hydrogen peroxide (30%, 10ml) was added slowly with ice water cooling. The solution was stand at room temperature for 16hr. water (200ml) was added and the DCM layer separated out. The organic layer was washed twice with water and dried over sodium sulfate, evaporation of the solvent gave an oil which was dissolved in ethanol (150ml), sodium hydroxide solution (25%, 20ml) was added and the solution was kept in a 55°C water bath for 50min. the reaction mixture was poured into water and filtered to give a white solid, this solid was dissolved in acetone (150ml) and water (8ml), perchloric acid (75%, 5ml) was added and the solution was stirred for 1hr., the resultant mixture was poured into water and extracted with DCM , washed with water and dried , removal of the solvent gave an oil which on recrystallisation from DCM and petroleum spirit gave a white solid, yield 10.1g(97%). M.p.125-126°C (Lit. 127°C, Hanson and Premuzic 1967); IR: ν_{max}: 3500-3300, 2943, 2870, 1465, 1374, 1037 and 756 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.33 (m, 1H, H-3), 3.52 (s. 1H, H-6), 1.18 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.7, 18.6, 22.5, 22.7; (CH_2): 20.9, 23.8, 24.1, 28.1, 32.2, 33.8, 34.6, 36.0, 39.4, 39.8, 42.2; (CH): 27.9, 30.0, 35.7, 45.8, 55.8, 56.1, 56.9, 75.8; (C): 38.1, 42.6, 76.2;MS (ES^+): m/z 461M+Na^+, 100).

6-Ethoxy-5β-cholestan-3α,5-diol (3.1-19)

Afforded as described in table 3.1-1. IR: ν_{max}: 3500-3300, 2935, 2865, 1461, 1377, 1103, 1084, 1029 and 968 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.99 (m, 1H, H-3), 3.59-3.68 (m, 1H, H-6), 3.27-3.38 (m, 2H, O-CH_2-), 0.89 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.6, 16.4, 18.5, 22.5, 22.7; (CH_2): 20.9, 23.7, 24.2, 28.1, 29.7, 30.1, 32.2, 36.0, 36.3, 39.4, 39.7, 64.5; (CH): 27.9, 33.9, 35.7, 42.6,

56.0, 56.1, 67.8, 80.6; (C): 39.5, 42.6, 77.3; MS ($APCI^+$): m/z 431 ($M\text{-}OH^+$, 12), 385 (100).

Cholest-4-en-6-one (3.1-20)

Afforded as described in table 3.1-1 M.p.106-107°C (Lit. 106-107°C, Pinhey et al 1978); IR: ν_{max}: 3500-3300, 2952, 2865, 1691, 1616, 1473, 1371, 1267, 1003 and 638 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.37 (m, 1H, H-4), 0.94 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 20.1, 22.4, 22.7; (CH_2): 17.7, 21.2, 23.7, 23.8, 25.3, 27.9, 35.3, 36.0, 39.3*, 45.8; (CH): 27.9, 33.5, 35.6, 50.9, 55.9, 56.6, 132.2; (C): 37.5, 42.4, 145.7, 202.8; MS (ES^+): m/z 407 ($M+Na^+$, 100).

5α-Cholestane-3α,5,6β-triol 3,6-diacetate (3.1-21)

The 5α-cholestane-3α,5,6β-triol was acetylated by using the general protocol D3. Analytical data was shown in **3.3-5a.**

3α,5α–Dihydroxycholestan-6-one (3.1-22).

Cholestane–3α,5α,6β–triol (2.1-12) (15.0g, 36.8mmol) was dissolved in methanol (80ml), ether (220ml) and water (40ml). NBS was added and the mixture was stirred at room temperature for 45min. The reaction was quenched by addition of sodium bisulfite untill the yellowish colour disappeared. The ether was removed under reduced pressure and the product precipitated as white crystals (13.2g, 90%). M.p. 190-191°C (Lit. 192-194°C, Valisolalao et al 1983); IR ν_{max}: 3500-3300,2944, 2865, 1715, 1465, 1383, 1238 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.30(m, 1H, H-3), 0.75 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^-): m/z 417 (M-H, 100).

3α-Acetoxy-5α–hydroxycholestan-6-one (3.1-23).

General acetylation protocol D3 was used starting from diol 3.1-22. Yield 91%. M.p. 159-160°C (Lit. 155-156°C, Schultz 1959); IR ν_{max}: 3500-3300, 2946, 2868, 1720, 1463, 1379, 1274, 1242 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.25 (m, 1H, H-3), 0.74 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 13.5, 18.5, 21.3, 22.4, 22.7; (CH_2): 20.9, 23.7*, 24.8, 25.5, 27.9, 29.9, 36.0, 39.3, 39.5, 41.4;

(CH): 27.9, 35.6, 37.4, 44.4, 56.0, 56.3, 69.9; (C): 43.0, 43.2, 79.2, 168.8, 210.8; MS (ES^+): m/z 483 ($M+Na^+$, 100).

3α-Acetoxycholest-4-en-6-one (3.1-24)

General acetylation protocol E1 was used starting from 3.1-23. M.p. 88-89°C (Lit. 86-88°C, Glotter et al 1991). IR: ν_{max}: 3500-3300, 2944, 2865, 1737, 1691, 1469, 1377, 1234 and 1018 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.19 (d, J=4.7 Hz, 1H, H-4), 5.25(m, 1H, H-3), 0.93 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.1, 18.5, 21.1, 22.4, 22.7; (CH_2): 21.2, 23.7, 23.8, 24.1, 27.9, 30.6, 35.9, 39.2, 39.3, 46.2; (CH): 27.8, 33.8, 35.6, 50.7, 55.9, 56.5, 65.4, 125,7; (C): 38.5, 42.4, 150.7, 170.0, 203.0; MS (ES^+): m/z 465 ($M+Na^+$, 100).

3α-Acetoxycholest-4-en–3α,6α–diol (3.1-25).

3α-Acetoxycholest-4-en-6-one (3.1-24) was treated with sodium borohydride (2 equiv) in THF/H_2O (10:1, v:v) at room temperature, followed by extraction to give a gum as the product in a quantitative yield. Analytical sample was prepared by chromatography. IR: ν_{max} 3500-3300, 2937, 2865, 1732, 1469, 1373, 1246 and 1016 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.76 (dd, 1H, H-4), 5.21(m, 1H, H-3), 4.18(1H, m, H-6), 0.96 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 18.8, 21.4, 22.5, 22.7; (CH_2): 21.3, 23.7, 24.1, 24.5, 28.0, 32.6, 36.0, 39.4, 39.6, 41.8; (CH): 27.9, 34.1, 35.6, 53.4, 55.6, 56.0, 67.0, 68.5, 113.2; (C): 37.8, 42.4, 153.5, 170.8; MS (ES^+): m/z 467 ($M+Na^+$, 100).

4α,5-Epoxy-5α-cholestane-3β,6β-diol (3.2-1)

4α,5α-Epoxycholestane-3β,6β-diol 3,6-diacetate (2.0g, 4.0mmol) was dissolved in DCM (15ml) and ethanol (50ml). 15%NaOH aqueous solution (8ml) was added with stirring. The solution was stirred at room temperature for 1.5hr, poured into water (40ml). DCM layer was separated and washed with water, then dried over sodium sulfate. The solvent was removed under reduced pressure to give a solid product, which was recrystallised from ether-petroleum ether, yield 1.70g (91%). Mp 166 – 167°C; $[\alpha]_D^{15}$= +23° (*c* 10.0, $CHCl_3$); IR: ν_{max} 3500-3300, 2949, 2865, 1469,1385

and 1051 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.02 (dd, *J*=8.0 and 0.5Hz, 1H, H-3), 3.22 (s, 1H, H-6), 2.93 (s, 1H, H-4), 1.26 (s, 3H, C*H*$_3$-19), 0.67 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 12.0, 17.7, 18.6, 22.5, 22.7; (CH_2): 20.6, 23.8, 24.1, 26.5, 28.1, 29.4, 35.9, 36.1, 39.4, 39.5; (CH): 27.9, 30.4, 35.7, 49.7, 55.4, 56.1, 63.6, 64.6, 73.5; (C): 34.7, 42.5, 65.9; MS (ES^+): m/z 441($M+Na^+$,100).

4α,5–Epoxy–5α–cholestane–3β,6β–diol, 3,6-diacetate (3.2-1a)

Obtained *via* recrystallization from methanol as white needles. $[\alpha]_D^{15}$= +18° (*c* 10.0, $CHCl_3$); Mp 157-158°C; IR: ν_{max} 2954, 2850, 1743, 1465,1367, 1240 and 1027 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.93 (dd, *J* = 8.2 and 0.6Hz, 1H, H-3), 4.28 (m, 1H, H-6), 3.16 (s, 1H, H-4), 1.19 (s, 3H, C*H*$_3$-19), 0.70 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 17.0, 18.5, 20.4, 20.9, 22.4, 22.6; (CH_2): 20.4, 22.8, 23.7, 23.9, 28.0, 29.3, 33.6, 36.0, 39.3, 39.4; (CH): 27.8, 30.8, 35.6, 49.4, 55.2, 56.0, 62.2, 66.9, 74.2; (C): 34.5, 42.4, 63.2, 169.5, 169.7; MS (ES^+): m/z 503($M+H^+$, 40), 525 ($M+Na^+$,100).

4β,5-Epoxy-5β-cholestane-3β,6β-diol (3.2-2)

Obtained directly from the reaction without further purification. Mp 162–163°C (Lit. 164-165°C, Rosenheim and Starling 1937); $[\alpha]_D^{15}$= -7° (*c* 10.0, $CHCl_3$) (Lit. $[\alpha]_D^{20}$= -7.5°, *c* 0.6, $CHCl_3$); IR: ν_{max} 3500-3300, 2960, 2848, 1463,1380 and 1059; ^1H NMR ($CDCl_3$): δ_H 3.96 (m,1H, H-3), 3.32 (m, 1H, H-6), 3.22 (d, *J*=3.2Hz, 1H, H-4), 1.16 (s, 3H, C*H*$_3$-19), 0.70 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 18.5, 19.6, 22.5, 22.7; (CH_2): 21.3, 23.7, 24.1, 25.5, 28.7, 32.5, 36.0, 36.8, 39.4, 39.7; (CH): 27.9, 29.2, 35.6, 50.6, 55.9, 56.1, 65.1, 66.1, 74.0; (C): 35.1, 42.4, 69.4; MS (ES^+): m/z 441($M+Na^+$,100).

4β,5-Epoxy-5β-cholestane-3β,6β-diol 3,6-diacetate (3.2-2a)

Obtained *via* recrystallization from methanol as off-white needles. Mp 160-161 °C (Lit. 154-155°C, Rosenheim and Starling 1937); $[\alpha]_D^{15}$= -60° (*c* 10.0, $CHCl_3$) (Lit. $[\alpha]_D^{19}$= -58.5°, *c* 1.0, $CHCl_3$); IR: ν_{max} 2948, 2871, 1743, 1463,1369, 1239 and 1027 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.01 (m, 1H, H-3), 4.51(m, 1H, H-6), 3.27 (d, *J* = 3.0 Hz, 1H, H-4), 1.14 (s, 3H, C*H*$_3$-19), 0.68 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3):

11.8, 18.4, 18.9, 20.7, 21.0, 22.4, 22.6; (CH_2): 21.9, 22.3, 23.6, 24.0, 27.9, 32.2, 35.2, 35.9, 39.2, 39.4; (CH): 27.7, 29.9, 35.5, 50.0, 55.4, 55.9, 60.8, 68.6, 75.4; (C): 35.6, 42.3, 64.8, 169.3, 170.1; MS (ES^+): m/z 525 ($M+Na^+$, 100).

4α,5-Epoxy-5α-cholestane-3β,6α-diol (3.2-3),

Obtained *via* hydrolysis of 3β–Acetoxy–6α–benzoxy-4α,5–epoxy–5α–cholestane as white solid. Mp 180 – 181 °C; $[\alpha]_D^{20}$= +49° (c 1.0, $CHCl_3$); HRMS (ES^+): m/z 436.3788 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500-3300, 2937, 1469,1382 and 1074; ^1H NMR ($CDCl_3$): δ_H 3.95 (m, 2H, H-3 & H-6), 3.39 (s, 1H, H-4), 1.07 (s, 3H, C*H*$_3$-19), 0.65 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 17.1, 18.5, 22.5, 22.7; (CH_2): 20.7, 23.9, 24.1, 25.7, 28.1, 29.0, 36.0, 38.1, 39.4, 39.5; (CH): 27.9, 34.3, 35.7, 49.3, 55.4, 56.2, 60.1, 64.9, 65.4; (C): 36.2, 42.6, 69.3; MS (ES^+): m/z 441 ($M+Na^+$, 100).

4α,5-Epoxy-5α-cholestane-3β,6α-diol 3,6-diacetate (3.2-3a),

Obtained *via* acetylation of the correspondent diol as a colourless gum. $[\alpha]_D^{20}$= +47° (*c* 2.9, $CHCl_3$); HRMS (ES^+): m/z 503.3738 ($M+H^+$, C31H51O5 requires 503.3736); IR: ν_{max} 2948, 2865, 1733, 1465,1379, 1246 and 1033; ^1H NMR ($CDCl_3$): δ_H 5.23 (dd, *J* = 11.9 and 4.7Hz, 1H, H-6), 4.93 (m, 1H, H-3), 3.08 (s, 1H, H-4), 1.11 (s, 3H, C*H*$_3$-19), 0.60 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 17.0, 18.5, 21.0, 21.2, 22.4, 22.7; (CH_2): 20.6, 22.8, 23.7, 23.9, 27.9, 28.4, 34.0, 35.9, 39.3*; (CH): 27.8, 34.1, 35.6, 49.2, 55.1, 55.9, 57.5, 67.1, 67.2; (C): 36.4, 42.5, 66.0, 169.2, 169.6; MS (ES^+): m/z 525 ($M+Na^+$, 100).

4β,5-Epoxy-5β-cholestane-3β,6α-diol (3.2-4)

Obtained *via* flash chromatography (ether as elutent) as white solids. Mp 66-67 °C; $[\alpha]_D^{20}$ = +30° (*c* 1.0, $CHCl_3$); HRMS (ES^+): m/z 436.3787 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500-3300, 2938, 2865, 1463,1378 and 1066; ^1H NMR ($CDCl_3$): δ_H 3.95(m, 2H, H-3 & H-6), 3.65 (s, 1H, H-4), 0.95(s, 3H, C*H*$_3$-19), 0.62(s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.8, 18.5, 19.0, 22.5, 22.7; (CH_2): 21.3,

23.7, 24.1, 25.3, 27.9, 28.0, 36.0, 37.8, 39.3, 39.5; (CH): 27.9, 29.4, 35.7, 46.4, 55.9, 56.1, 65.8, 66.1, 74.3; (C): 36.4, 42.4, 71.0; MS (ES^+): m/z 441($M+Na^+$, 100).

4β,5-Epoxy-5β-cholestane-3β,6α-diol 3,6-diacetate (3.2-4a),

Obtained *via* acetylation of the correspondent diol as a colourless gum. $[\alpha]_D^{20}$ = +55° (*c* 0.18, $CHCl_3$); HRMS (ES^+): m/z 503.3733 ($M+H^+$, $C_{31}H_{51}O_5$ requires 503.3736); IR: ν_{max} 2948, 2865, 1733, 1465,1378, 1246 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.20 (dd, *J* = 12.2 and 4.8 Hz, 1H, H-6), 5.10 (m, 1H, H-3), 3.54 (d, *J* = 3.6 Hz, 1H, H-4), 1.06 (s, 3H, C*H*$_3$-19), 0.65 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.7, 18.4, 18.6, 20.7, 20.8, 22.3, 22.6; (CH_2): 21.2, 22.2, 23.6, 23.9, 27.9, 28.9, 35.9, 36.0, 39.2, 39.3; (CH): 27.7, 33.6, 35.5, 47.8, 54.6, 55.5, 55.9, 66.3, 66.9; (C): 36.7, 42.4, 66.3, 169.5, 170.3; MS (ES^+): m/z 525 (100).

4α,5-Epoxy-5α-cholestane-3α,6β-diol (3.2-5)

Obtained *via* hydrolysis of its 6-acetate, then recrystallisation from chloroform/hexane as granular crystals. Mp 152 – 154 °C; $[\alpha]_D^{20}$ = +57° (*c* 10.0, $CHCl_3$); HRMS (ES^+): m/z 419.3528 ($M+H^+$, $C_{24}H_{47}O_3$ requires 419.3525); IR: ν_{max} 3500-3300, 2939, 2865, 1469,1380 and 1034 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.06 (m, 1H, H-3), 3.26 (m, 2H, H-6 & H-4), 1.18 (s, 3H, C*H*$_3$-19), 0.68 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 12.0, 17.3, 18.5, 22.5, 22.7; (CH_2): 20.6, 23.8, 24.1, 26.8, 28.1, 29.5, 35.7, 36.0, 39.4, 39.6; (CH): 27.9, 30.3, 35.7, 50.2, 55.5, 56.1, 62.8, 63.3, 73.8; (C): 34.6, 42.5, 67.7; MS (ES^+): m/z 436 ($M+NH_4^+$, 55), 236(50), 214(100).

4α,5-Epoxy-5α-cholestane-3α,6β-diol 3,6-diacetate (3.2-5a)

Obtained *via* acetylation of the crude 6-acetate with acetic anhydride and pyridine in toluene at reflux for 2h. Recrystallisation from methanol as white crystals. Mp 98-99 °C; $[\alpha]_D^{20}$ = +60° (*c* 10.0, $CHCl_3$); HRMS (ES^+): m/z 520.4002 ($M+NH_4^+$, C31H54NO5 requires 520.4002); IR: ν_{max} 2942, 1753, 1467,1383, 1220 and 1026; ^1H NMR ($CDCl_3$): δ_H 5.15 (m, 1H, H-3), 4.30 (m, 1H, H-6), 3.44 (d, *J* = 3.5 Hz, 1H, H-4), 1.09 (s, 3H, C*H*$_3$-19), 0.70 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 12.0, 16.8, 18.5, 21.0, 21.3, 22.5, 22.7; (CH_2): 20.5, 23.6, 23.7, 24.0, 28.0, 30.0, 33.8, 36.0,

39.4, 39.5; (CH): 27.9, 30.8, 35.7, 49.9, 55.3, 55.9, 60.4, 66.4, 74.8; (C): 34.8, 42.5, 63.8, 169.8, 170.5; MS (ES^+): m/z 525 ($M+Na^+$, 100).

4β,5-Epoxy-5β-cholestane-3α,6β-diol (3.2-6)

Obtained *via* hydrolysis of its 3,6-diacetate and recrystallised from DCM/hexane as white solids. Mp 137 – 138 °C; $[\alpha]_D^{20}$ = +10° (*c* 1.0, $CHCl_3$); HRMS (ES^+): m/z 419.3517 ($M+H^+$, $C_{24}H_{47}O_3$ requires 419.3525); IR: ν_{max} 3500-3300, 2942, 2865, 1463,1378 and 1058; ^1H NMR ($CDCl_3$): δ_H 3.94 (dd, *J* = 9.1, 2.6 Hz, 1H, H-3), 3.35 (m, 1H, H-6), 2.93 (s, 1H, H-4), 1.14 (s, 3H, C*H*$_3$-19), 0.69 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 18.5, 20.6, 22.5, 22.7; (CH_2): 21.0, 23.8, 24.2, 25.3, 27.3, 28.1, 35.8, 36.0, 39.4, 39.6; (CH): 27.9, 29.4, 35.7, 46.4, 55.9, 56.1, 65.8, 66.1, 74.3: (C): 35.8, 42.5, 67.6; MS (ES^+): m/z 441 ($M+Na^+$, 100), 419 ($M+H^+$, 39), 236 (61), 214 (37).

4β,5-Epoxy-5β-cholestane-3α,6β-diol 3,6-diacetate (3.2-6a)

Recrystallised from methanol as wihte tiny plates. Mp 72-73°C; $[\alpha]_D^{20}$ = -24° (*c* 8.0, $CHCl_3$); HRMS (ES^+): m/z 520.3988 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); IR: ν_{max} 2946, 2871, 1745, 1463,1365, 1236 and 1034 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.85(dd, *J* = 9.8 and 2.0 Hz, 1H, H-3), 4.52 (m, 1H, H-6), 2.92 (s, 1H, H-4), 1.11 (s, 3H, C*H*$_3$-19), 0.70 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 18.5, 20.0, 21.0, 21.3, 22.4, 22.7; (CH_2); 20.9, 21.8, 23.6, 24.1, 26.6, 28.0, 35.1, 36.0, 39.3, 39.4; (CH): 27.9, 30.1, 35.6, 46.1, 55.6, 56.0, 62.1, 68.0, 75.7; (C): 36.0, 42.5, 64.2, 169.9, 170.1; MS (ES^+): m/z 525 ($M+Na^+$, 100).

4α,5-Epoxy-5α-cholestane-3α,6α-diol (3.2-7)

Obtained *via* recrystallistion from chloroform/hexane as white solid. Mp 173-174 °C; $[\alpha]_D^{20}$ = +64° (*c* 4.0, $CHCl_3$); HRMS (ES^+): m/z 436.3790 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500-3300, 2943, 1469,1376 and 1042 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.04 (m, 1H, H-3), 3.87 (m, 1H, H-6), 3.71(d, *J* = 4.4 Hz, 1H, H-4), 0.98 (s, 3H, C*H*$_3$-19), 0.65 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 16.6, 18.5, 22.5, 22.7; (CH_2): 20.6, 23.8, 24.1, 26.6, 28.0, 28.1, 36.0, 38.6, 39.4, 39.5; (CH): 27.9,

34.3, 35.7, 49.8, 55.4, 56.1, 57.7, 62.5, 64.8; (C): 36.2, 42.5, 70.5; MS (ES^+): m/z 441 ($M+Na^+$, 100), 401 (55), 214 (71), 114 (43).

4α,5-Epoxy-5α-cholestane-3α,6α-diol 3,6-diacetate (3.2-7a)

Obtained *via* acetylation of the correspondent diol as white solid. Mp 160-161 °C; $[\alpha]_D^{20}$ = +94° (*c* 10.0, $CHCl_3$); HRMS (ES^+): m/z 520.4000 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002);IR: ν_{max} 2937, 2861, 1735, 1465,1379, 1236 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.20 (dd, *J* = 12.0 and 4.8 Hz, 1H, H-6), 5.12 (m, 1H, H-3), 3.44 (d, *J* = 4.0 *Hz* 1H, H-4), 1.06 (s, 3H, C*H*$_3$-19), 0.65 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 16.7, 18.5, 20.9, 21.0, 22.4, 22.7: (CH_2): 20.5, 23.4, 23.7, 23.9, 28.0, 28.8, 34.2, 36.0, 39.3, 39.4; (CH): 27.9, 34.3, 35.6, 49.7, 55.0, 55.2, 56.0, 66.1, 66.8; (C): 36.8, 42.5, 66.9, 170.3, 170.5; MS (ES^+): m/z 525 ($M+Na^+$, 100).

4β,5-Epoxy-5β-cholestane-3α,6α-diol (3.2-8)

Obtained *via* hydrolysis of the 3-acetate and flash chromatography as white solid. Mp 99-100 °C; $[\alpha]_D^{20}$= +25° (c 3.0, $CHCl_3$); HRMS (ES^+): *m/z* 436.3793 ($M+NH_4^+$, $C_{24}H_{50}NO_3$ requires 436.3791); IR: ν_{max} 3500-3300, 2937, 1467,1365 and 1078; ^1H NMR ($CDCl_3$): δ_H 4.01 (m, 2H, H-3 and H-6), 3.36 (s, 1H, H-4), 0.96 (s, 3H, C*H*$_3$-19), 0.65 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.8, 18.5, 18.9, 22.4, 22.7; (CH_2): 21.1, 23.8, 24.2, 24.6, 26.4, 28.1, 36.0, 37.7, 39.4, 39.5; (CH): 27.9, 34.0, 35.7, 45.5, 55.6, 56.2, 59.8, 65.9*; (C): 36.0, 42.5, 69.8; MS (ES^+): m/z 436 ($M+NH_4^+$, 62), 401 (100), 236 (15), 214 (18).

4β,5-Epoxy-5β-cholestane-3α,6α-diol 3,6-diacetate (3.2-8a)

Obtained *via* acetylation of the correspondent diol as white solid. Mp 63-64°C; $[\alpha]_D^{20}$ = +36° (*c* 1.0, $CHCl_3$); HRMS (ES^+): m/z 520.4000 ($M+NH_4^+$, $C_{31}H_{54}NO_5$ requires 520.4002); IR: ν_{max} 2952, 2861, 1741, 1465,1379, 1234 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.21(dd, *J* = 12.2, 4.8 Hz, 1H and H-6), 4.88 (dd, J = 9.2 and 0.4 Hz,1H and H-3), 3.19 (s, 1H, H-4), 1.02 (s, 3H, C*H*$_3$-19), 0.63 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.8, 18.5, 18.8, 20.9, 21.0, 22.4, 22.7; (CH_2): 21.0, 21.6, 23.6,

24.0, 25.8, 27.9, 35.9, 36.0, 39.3, 39.4; (CH): 27.8, 33.9, 35.5, 45.6, 55.6, 56.0, 57.0, 67.2, 68.1; (C): 37.1, 42.5, 66.1, 169.8*; MS (ES^+): *m/z* 525 ($M+Na^+$, 100).

4α,5-Epoxy-5α-cholestane-3β,6α-diol 3-acetate 6-benzoate (3.2-17)

Obtained *via* recrystallization from acetone as white flakes. Mp 145-146°C; $[\alpha]_D^{20}$= +82° (*c* 10.0, $CHCl_3$); HRMS (ES^+): m/z 582.4160 ($M+NH_4^+$, $C_{36}H_{56}NO_5$ requires 582.4158);IR: ν_{max} 2946, 2869, 1730, 1724, 1447,1378, 1272, 1240, 1110, 1032 and 715 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.48 (dd, *J* = 12.2 and 4.8 Hz, 1H and H-6), 4.98 (m,1H, H-3), 3.21 (s, 1H, H-4), 1.20 (s, 3H, C*H*$_3$-19), 0.70 (s, 3H, C*H*$_3$-18); ^{13}C NMR ($CDCl_3$): δ_C: (CH_3): 11.9, 17.1, 18.6, 21.0, 22.5, 22.7; (CH_2): 20.7, 22.9, 23.8, 24.0, 28.0, 28.6, 34.1, 36.0, 39.3, 39.4; (CH); 27.9, 34.3, 35.7, 49.3, 55.2, 56.0, 57.6, 67.3, 67.6, 128.3*, 129.6*, 133.0; (C): 36.7, 42.6, 66.7, 129.8, 165.5, 169.8; MS (ES^+): *m/z* 587 ($M+Na^+$, 100).

Compounds **3.3-1** to **3.3-8** are prepared using the general $LiAlH_4$ reduction method C3, the starting material, yields and reaction time are listed in **Table 3.3-1**.

5α-Cholestan-3β,5,6β-triol (3.3-1)

M.p.239-241°C (Lit. 237-239°C, Kimura et al 1976); IR: ν_{max} 3500-3300, 2937, 2865, 1465,1375 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): 4.08(m, 1H, H-3) 3.52(s, 1H, H-6), 1.16 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 4.39(d, J=4.1Hz, 1H, OH-6), 4.16 (d, J=5.7Hz, 1H, OH-3), 3.79(m,1H, H-3) 3.63(s, 1H, OH-5), 3.28(m, 1H, H-6), 1.00 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); MS (ES^-): m/z 419 (M-H, 79), 840 (100).MS (ES^+): m/z 443 ($M+ Na^+$, 63), 385 (100), 438 (46).

5α-Cholestan-3β,5,6β-triol 3,6-diacetate (3.3-1a)

M.p. 167-169°C (Lit. 169-170°C, Kimura et al 1972); IR: $\nu_{max:}$ 3485, 2941, 2871, 1736, 1712, 1465, 1378, 1267 and 1027 cm^{-1}; ^1H NMR ($CDCl_3$): 5.11 (m,1H, H-3) 4.67 (s, 1H, H-6), 2.04, 1.99 (both s, 6H, the acetyl CH_3), 1.12 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.1, 18.5, 21.3, 21.4, 22.4, 22.7; (CH_2): 20.9, 23.9, 24.0, 26.6, 28.1, 31.2, 31.7, 36.1, 36.4, 39.4, 39.8, (CH): 27.9, 30.6,

35.8, 44.6, 55.6, 56.2, 70.9, 76.2,(C): 38.2, 42.6, 74.7, 170.2, 170.7; MS (ES^+): m/z 522 (M+ NH_4^+, 100).

5β-Cholestan-3β,5,6β-triol (3.3-2)

M.p. 128-130°C (Lit.126-128°C, Rowland 1964) IR: ν_{max} 3500-3300, 2931, 2865, 1463,1385 and 1052 cm^{-1}; ^1H NMR ($CDCl_3$): 4.12(s, 1H, H-3) 3.54(m, 1H, H-6), 1.06 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18). MS (ES^-): m/z 419 ($(M-H)^-$, 100); MS (ES^+): m/z 443 (M+ Na^+, 27), 385 (27), 864 (100).

5β-Cholestan-3β,5,6β-triol 3,6-diacetate (3.3-2a)

M.p. 164-166°C (Lit. 165-167°C, Henbest and Wilson 1957); IR: ν_{max}: 3500-3300, 2942, 2865, 1735, 1463, 1429, 1375, 1253, 1243, 1169 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): 5.15 (s,1H, H-3) 4.82 (s, 1H, H-6), 1.07 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.3, 18.5, 21.3, 21.4, 22.4, 22.7; (CH_2): 21.2, 23.7, 24.1, 26.1, 28.1*, 33.3, 35.3, 36.0, 39.4, 39.7; (CH): 27.9, 30.3, 35.6, 43.0, 56.0, 56.1, 70.0, 76.0; (C): 39.4, 42.5, 169.5, 170.6; MS (ES^+): m/z 527 (M+ Na^+, 85), 487 (100).

5α-Cholestan-3β,5,6α-triol (3.3-3)

M.p. 233-235°C (Lit. 232-234°C, Bowers et al 1959) IR: ν_{max} 3500-3300, 2935, 2865, 1465,1377, 1051 and 978 cm^{-1}; ^1H NMR ($CDCl_3$): 4.05(m, 1H, H-3) 3.64(dd, 1H, H-6), 0.94 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 3.77 (m, 1H, H-3), 3.28 (m, 1H, H-6), 0.99 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); MS (ES^-): m/z 419 (M-H, 100). MS (ES^+): m/z 438 (M+ NH_4^+, 69), 864 (100).

5α-Cholestan-3β,5,6α-triol 3,6-diacetate (3.3-3a)

M.p. 188-190°C (Lit. 187-189°C, Ellington 1966) IR: $\nu_{max:}$ 3444, 3395, 2937, 2865, 1729, 1714, 1465, 1442, 1363, 1269, 1247 and 1029 cm^{-1}; ^1H NMR ($CDCl_3$): 5.08 (m, 1H, H-3) 4.88 (dd, 1H, H-6), 2.04, 1.99 (both s, 6H, the acetyl CH_3), 0.99 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.5, 18.5, 21.1, 21.3, 22.5, 22.7; (CH_2): 21.0, 23.8, 23.9, 26.4, 28.0, 30.4, 31.3, 34.7, 36.0, 39.4, 39.6,

(CH): 27.9, 33.4, 35.7, 44.0, 55.6, 56.1, 70.5, 74.1,(C): 39.6, 42.6, 75.7, 170.2, 170.5; MS (ES^+): m/z 522 (M+ NH_4^+, 100).

5β-Cholestan-3β,5,6α-triol (3.3-4)

M.p. 87-91°C IR: ν_{max} 3500-3300, 2942, 2865, 1463,1383, 1097,1050 and 1001 cm^{-1}; ^1H NMR ($CDCl_3$): 4.21(s, 1H, H-3) 3.75(dd, 1H, H-6), 0.89 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^-): m/z 419 ($(M-H)^-$, 100). MS (ES^+): m/z 443 (M+ Na^+, 25), 385 (30), 403 (33), 864 (100).

5β-Cholestan-3β,5,6α-triol 3,6-diacetate (3.3-4a)

M.p. 121-122°C; IR: ν_{max}:; ^1H NMR ($CDCl_3$): 5.23 (s,1H, H-3) 4.99-5.06 (dd, J1=12.1Hz, J2=5.2Hz, 1H, H-6), 2.04, 2.02 (both s, 6H, the acetyl CH_3), 0.97 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 16.9, 18.5, 21.3, 21.4, 22.4, 22.7; (CH_2): 21.3, 23.6, 23.9, 24.0, 25.5, 28.0, 29.8, 33.6, 36.0, 39.3, 39.6, (CH): 27.8, 33.4, 35.6, 42.7, 56.0, 56.2, 70.1, 74.3; (C): 41.4, 42.5, 75.1, 169.4, 170.6; MS (ES^+): m/z 527 (M+ Na^+, 65), 427 (100).

5α-Cholestan-3α,5,6β-triol (3.3-5)

M.p. 205-206°C (Lit. 205-206°C, Coxon et al 1970); IR: ν_{max} 3500-3300, 2942, 2871, 1469,1376 and 1042 cm^{-1}; ^1H NMR ($CDCl_3$): 4.24(s,1H, H-3) 3.56(s, 1H, H-6), 1.09 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 5.34 (d, 1H, OH-6), 5.05 (s, 1H, OH-5), 4.38 (d, J=3.9Hz, 1H, OH-3), 4.02(s,1H, H-3), 3.27(m, 1H, H-6), 0.99 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); MS (ES^+): m/z 443 ((M+Na^+, 75), 385 (12), 122 (100).

5α-Cholestan-3α,5,6β-triol 3,6-diacetate (3.3-5a)

M.p. 87-88°C (Lit. 86-88°C, Tsui and Jast 1973); IR: ν_{max}: 3588, 2944, 2865, 1737, 1465, 1442, 1371, 1243, 1172 and 1024 cm^{-1}; ^1H NMR ($CDCl_3$): 5.23 (s,1H, H-3) 4.71 (s, 1H, H-6), 1.06 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.0, 18.5, 21.3*, 22.4, 22.7; (CH_2): 20.6, 23.7, 23.9, 25.3, 28.1, 28.2,

30.8, 34.0, 36.0, 39.4, 39.8; (CH): 27.8, 30.5, 35.7, 44.7, 55.7, 56.0, 70.9, 75.5; (C): 39.0, 42.6, 168.9, 169.8; MS (ES^+): m/z 522 ($M+H_3O^+$, 100), 427 (41).

5β-Cholestan-3α,5,6β-triol (3.3-6)

M.p. 172-174°C. IR: ν_{max} 3500-3300, 2942, 2865, 1457,1370, 1054 and 1029 cm^{-1}; ^1H NMR ($CDCl_3$): 3.92(m,1H, H-3) 3.55(s, 1H, H-6), 0.99 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^-): m/z 419 (M-H, 100); MS (ES^+): m/z 443 ($M+ Na^+$, 100), 385 (66).

5β-Cholestan-3α,5,6β-triol 3,6-diacetate (3.3-6a)

M.p. 61-63°C; IR: $\nu_{max:}$ 3500-3300, 2944, 2867, 1737, 1463, 1365, 1240, 1162, 1059 and 1032 cm^{-1}; ^1H NMR ($CDCl_3$): 4.95-5.11 (m,1H, H-3), 4.70 (s, 1H, H-6), 2.07, 2.00 (both s, 6H, the acetyl CH_3), 0.97 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.5, 18.5, 21.2, 21.3, 22.4, 22.7; (CH_2): 20.8, 23.7, 24.0, 25.8, 28.0, 30.0, 33.0, 36.0, 37.7, 39.3, 39.6, (CH): 27.8, 30.5, 35.6, 42.9, 56.0, 56.1, 70.6, 77.2,(C): 39.6, 42.4, 74.8, 170.2, 170.9; MS (ES^+): m/z 522 ($M+ NH_4^+$, 100).

5α-Cholestan-3α,5,6α-triol (3.3-7)

M.p. 163-164°C (Lit. 168-172°C, Fudge et al 1954); IR: ν_{max} 3500-3300, 2935, 2865, 1469, 1383, 1050 and 1014 cm^{-1}; ^1H NMR ($CDCl_3$): 4.18 (s, 1H, H-3), 3.47 (dd, 1H, H-6), 0.88 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); MS (ES^-): m/z 419 (M-H, 100); MS (ES^+): m/z 443 ($M+ Na^+$, 24), 403 (36), 864 (100).

5α-Cholestan-3α,5,6α-triol 3,6-diacetate (3.3-7a)

Afforded as colourless gum. IR: ν_{max}: 2951, 2865, 1735, 1462, 1436, 1375, 1254, 1033 and 1020 cm^{-1}; ^1H NMR ($CDCl_3$): 5.18 (s, 1H, H-3), 4.84 (dd, 1H, H-6), 0.95 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): □$_C$ (CH_3): 11.9, 15.5, 18.5, 21.1, 21.3, 22.4, 22.7; (CH_2): 20.6, 23.7, 23.8, 25.0, 26.7, 28.0, 30.9, 32.3, 36.0, 39.3, 39.7; (CH): 27.8, 33.6, 35.6, 44.3, 55.6, 56.0, 69.6, 73.7; (C): 40.5, 42.5, 74.2, 169.0, 170.7; MS (ES^+): m/z 527 ($M+Na^+$, 100), 385 (48), 487 (49).

5β-Cholestan-3α,5,6α-triol (3.3-8)

M.p. 104-106°C; HRMS (ES^+): m/z 438.3949 ($M+NH_4^+$, $C_{27}H_{52}NO_3$ requires 438.3947); IR: ν_{max} 3500-3300, 2934, 2865, 1463,1377, 1050 and 970 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.96 (m, 1H, H-3), 3.72 (dd, 1H, H-6), 0.85 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^-): m/z 419 ($(M-H)^-$, 100), 435 (56). MS (ES^+): m/z 443 ($M+ Na^+$, 57), 385 (66), 864 (100).

5β-Cholestan-3α,5,6α-triol 3,6-diacetate (3.3-8a)

Afforded as colourless gum. IR: ν_{max}: 2951, 2867, 1737, 1462, 1381, 1369, 1239 and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): 4.99-5.10 (m, 1H, H-3), 4.86-4.99 (dd, 1H, H-6), 2.04, 2.00 (both s, 6H, the acetyl CH_3), 0.90 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 16.2, 18.5, 21.2, 21.3, 22.4, 22.7; (CH_2): 21.0, 23.7, 24.0, 25.9, 28.0, 29.4, 32.3, 33.3, 36.0, 39.3, 39.6; (CH): 27.9, 33.7, 35.6, 42.7, 56.0, 56.1, 70.9, 76.2; (C): 40.6, 42.5, 76.5, 170.4, 171.4; MS (ES^+): m/z 522 ($M+ NH_4^+$, 100), 487 (52).

5α–Cholestane–3β,4β,5,6β–tetrol 3,6–diacetate (4.1-1)

The fast epoxide ring opening procedure (general procedure B2 with acetonitrile as solvent) was performed on 4α,5–Epoxy–5α–cholestane–3β,6β–diol, 3,6-diacetate (3.2-1a), yield 94%. M.p. 144-146°C (Lit. 160-165°C, Narayanan and Landge 1993); IR: ν_{max}: 3500-3300, 2949, 2867, 1724, 1465, 1378, 1269 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): 5.20 (m, 1H, H-3) 4.98 (s, 1H, H-6), 3.46 (d, 1H, H-4), 2.03 (s, 6H, the acetyl CH_3), 1.36 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 543 ($M+Na^+$,100).

5α–Cholestane–3β,4β,5,6β–tetrol 4,6–diacetate (4.1-2)

The slow epoxide ring opening procedure (general procedure B1 with acetonitrile as solvent) was performed on 4α,5–Epoxy–5α–cholestane–3β,6β–diol, 3,6-diacetate (3.2-1a), yield 90%. M.p. 191-193 °C IR: ν_{max}: 3500-3300, 2944, 2871, 1734, 1465, 1373, 1269 and 1029 cm^{-1}; ^1H NMR ($CDCl_3$): 5.11 (d, J=3.4Hz, 1H, H-4), 4.93 (s, 1H, H-6), 4.30 (m, 1H, H-3), 2.08, 1.99 (both s, 6H, the acetyl CH_3), 1.28 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); MS (ES^+): m/z 538 ($M+NH_4^+$,100).

5α–Cholestane–3β,4β,5,6β–tetrol (4.1-3)

Diacetate 4.1-1 or 4.1-2 was hydrolysed with aqueous sodium hydroxide in ethanol at 50 °C for 20min. yield 99%. M.p. 179-181°C (Lit. 173-176°C, Fieser et al 1960); IR: $\nu_{max.}$ 3374, 2937, 2865, 1468, 1380, 1204, 1159 and 1018 cm^{-1}; ^1H NMR ($CDCl_3$): 4.08 (m, 1H, H-3), 3.81 (br s, 2H, H4 & H-6), 4.30 (m, 1H, H-3), 1.39 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 5.23 (d, 1H, -OH), 4.92 (s, 1H, -OH), 4.20 (d, 1H, -OH), 3.93 (s, 1H, -OH), 3.83 (m, 1H, H-3), 3.63 (br s, 2H, H4 & H-6), 1.26 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR (DMSO -d_6): δ_C (CH_3): 11.9, 15.3, 18.5, 22.4, 22.6; (CH_2): 20.0, 23.5, 23.9, 26.1, 27.9, 32.0, 34.0, 35.8, 39.1, 39.8; (CH): 27.5, 30.1, 35.4, 45.3, 55.7, 55.8, 66.9, 76.5, 79.2; (C): 37.5, 42.3, 71.9; MS (ES^+): m/z 454 ($M+NH_4^+$, 100), 401 (55), 214 (60).

3,4,6-Triacetate:

M.p. 215-217 °C (Lit. 213-214.5 °C, Ishiguro et al 1980); IR: $\nu_{max.}$ 3436, 2941, 2873, 2848, 1759, 1735, 1720, 1469, 1377, 1272, 1242 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): 5.34 (m, 1H, H-3), 5.17 (d, J=3.3Hz, 1H, 4-H), 4.80 (s, 1H, H-6), 2.07, 2.00, 1.94(all s, 9H, acetyl CH_3), 1.34 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.7, 18.5, 21.0, 21.1, 21.4, 22.5, 22.7; (CH_2): 20.2, 22.4, 23.8, 24.0, 28.1, 31.9*, 36.0, 39.4, 39.7; (CH): 27.9, 30.4, 35.7, 46.0, 55.5, 56.0, 70.0, 73.3, 74.7; (C): 38.6, 42.5, 76.5, 169.5, 169.6, 170.5; MS (ES^+): m/z 580 ($M+NH_4^+$, 100).

5β–Cholestane–3β,4α,5,6β–tetrol 3,6–diacetate (4.1-4)

Epoxide ring opening procedure B4 was performed on 4α,5–Epoxy–5α–cholestane –3β,6β–diol, 3,6-diacetate (3.2-1a), yield 96% M.p. 179-181°C (Lit. 150-151°C, Narayanan and Landge 1993); IR: $\nu_{max.}$ 3500-3300, 2948, 2871, 1722, 1469, 1378, 1263 and 1031 cm^{-1}; ^1H NMR ($CDCl_3$): 5.06 (br s, 1H, H-6), 4.80 (s, 1H, H-3), 3.75 (dd, 1H, H-4), 1.02 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.4, 18.5, 21.3, 21.5, 22.5, 22.7; (CH_2): 20.7, 22.4*, 23.7, 24.1, 28.1, 34.3*, 36.0, 39.4, 39.6; (CH): 27.9, 30.4, 35.7*, 55.9, 56.3, 74.1, 74.6, 76.4; (C): 39.8, 42.3, 74.7, 170.7, 170.9; MS (ES^+): m/z 539 ($M+H_3O^+$, 50), 504 (100).

5β–Cholestane–3β,4α,5,6β–tetrol 3,4,6–triacetate (4.1-5)

M.p. 167-169°C (Lit. 167-168 °C, Ishiguro et al 1980); IR: $\nu_{max.}$ 3500-3300, 2954, 2867, 1735, 1469, 1374, 1245 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): 4.91 (br s, 2H, H-4 & H-6), 4.80 (m, 1H, H-3), 1.07 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.3, 18.5, 21.1, 21.3, 21.4, 22.4, 22.7; (CH_2): 20.7, 21.0, 22.4, 23.7, 24.1, 28.1, 34.2*, 36.0, 39.4, 39.7; (CH): 27.9, 29.8, 35.6*, 55.9, 56.9, 70.4, 74.8, 75.1; (C): 39.4, 42.4, 73.5, 168.8, 169.2, 170.4; MS (ES^+): m/z 581 ($M+H_3O^+$, 100).

5β–Cholestane–3β,4α,5,6β–tetrol (4.1-6)

Diacetate 4.1-4 was hydrolysed using procedure D2 to afford the title tetrol. Yield 98%. M.p. 153-155 °C; IR: $\nu_{max.}$ 3500-3300, 2945, 2865, 1463, 1380 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): 3.92 (br s, 1H, H-4), 3.74 (br s, 1H, H-6), 3.68 (br s, 1H, H-3), 1.01 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 5.18 (d, 1H, -OH), 5.04 (d, 1H, -OH), 4.67 (s, 1H, -OH), 3.85 (br s, 1H, H-6), 3.61 (m, 1H, H-3), 3.51 (m, 1H, H-4), 0.94 (s, 3H, CH_3-19), 0.61 (s, 3H, C H_3-18); MS (ES^+): m/z 459 ($M+Na^+$, 100).

5α–Cholestane–3β,4α,5,6β–tetrol (4.1-7)

4α,5-Epoxy-5α-cholestane-3β,6β-diol(3.2-1) was treated with general procedure B2 with THF as the solvent. The crude product was recrystallised from hexane /DCM to give the title compound as white crystals. Yield 78%. M.p. 224-226 °C; IR: $\nu_{max.}$ 3500-3300, 2941, 2871, 1463, 1378 and 1070 cm^{-1}; ^1H NMR (DMSO-d_6): 4.23 (dd, 2H, -OH), 4.09 (d, 1H, -OH), 3.78 (m, 1H, H-6), 3.60 (dd, 1H, H-4), 3.51 (m, 1H, H-3), 3.31 (s, 1H, -OH), 1.02 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); MS (ES^+): m/z 459 ($M+Na^+$, 70), 214(100).

3,4,6-Triacetate:

M.p. 149-151°C (Lit. 148-149.2°C, Warren et al 1989); IR: $\nu_{max.}$ 3472, 2944, 2867, 2848, 1739, 1465, 1367, 1251, 1049 and 867 cm^{-1}; ^1H NMR ($CDCl_3$): 5.36 (d, J=9.5Hz, 1H, H-4), 5.17 (m, 1H, H-3), 4.78 (s, 1H, H-6), 2.01, 2.00, 1.97 (all s, 9H,

acetyl CH_3), 1.19 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.5, 18.5, 20.5, 20.9, 21.2, 22.4, 22.7; (CH_2): 20.5, 23.8, 23.9, 25.2, 28.1, 30.5, 31.1, 36.0, 39.3, 39.7; (CH): 27.8, 30.1, 35.7, 44.4, 55.5, 56.0, 69.6, 72.2, 72.9; (C): 40.0, 42.4, 75.8, 170.2, 170.3, 170.6; MS (ES^+): m/z 585 ($M+Na^+$, 100).

5-Fluoro–5α–cholestane–3β,4α,6β–triol (4.1-8)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with boron trifluoride etherate in THF (protocol B4) in 2h. Yield 73% after chromatography (ether /acetone 5/1 as mobile phase). M.p. 239-242 °C; IR: ν_{max}: 3500-3300, 2941, 2865, 1468, 1385 and 1070 cm^{-1}; ^1H NMR ($CDCl_3$): 4.26 (s, 1H, H-6), 3.75-3.97 (m, 2H, H-3 & H-4), 1.14 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 461 ($M+Na^+$, 100), 236(60), 214(70).

3,4,6-Triacetate

Afforded by acetylation of 4.1-8 (procedure D3). Foam. IR: ν_{max}: 3470, 2954, 2871, 1753, 1473, 13711245, 1225 and 1050 cm^{-1}; ^1H NMR ($CDCl_3$): 5.25-5.45 (dd, 1H, H-4), 5.08 (br s, 2H, H-3 & H-6), 1.18 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.4 / 15.5, 18.5, 20.9, 21.1, 21.4, 22.4, 22.7; (CH_2): 20.5, 23.7, 23.9, 25.0, 28.0, 30.8, 31.4, 36.0, 39.3, 39.4; (CH): 27.8, 29.5, 35.6, 45.4, 55.3, 55.9, 66.1 / 66.7, 69.9 / 70.1, 72.2 / 72.3; (C): 40.2 / 40.4, 42.3, 96.3 / 99.1, 169.7, 170.0, 170.3; ^{19}F NMR ($CDCl_3$): 1.99, 2.11.

4β-Acetamido-5α-cholestane–3β,5,6β–triol (4.1-10)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with boron trifluoride etherate in acetonitrile (protocol B5) for 20-30 min. Yield 91%. M.p. 253-255 °C; IR: ν_{max}: 3500-3300, 2949, 2865, 1654, 1457, 1378 and 1049 cm^{-1}; ^1H NMR ($CDCl_3$): 4.50 (d, 1H, H-4), 3.48 (m, 1H, H-3), 3.36 (s, 1H, H-6), 1.13 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d_6): δ_C (CH_3): 11.9, 14.9, 18.4, 19.2, 22.3, 22.5; (CH_2): 20.6, 21.0, 23.6, 23.9, 28.0, 30.6, 34.1, 35.7, 39.1, 39.3; (CH): 27.5, 30.4, 35.7, 45.9, 55.5, 55.9, 70.0, 73.3, 85.4; (C): 38.5, 42.3, 77.3, 163.2; MS (ES^+): m/z 460 (85), 426(100).

4β-Acetamido-5α-cholestane–3β,5,6β–triol, 3,6-diacetate (4.1-11)

Afforded by acetylation of **4.1-10**. M.p. 154-155 °C; IR: $\nu_{max:}$ 3500-3300, 2960, 2933, 2890, 2873, 1731, 1654, 1463, 1431, 1375, 1228 and 1036 cm^{-1}; ^1H NMR ($CDCl_3$): 4.62 (m, 1H, H-3), 4.46 (s, 1H, H-6), 4.23 (d, J=5.6 Hz, 1H, H-4), 1.11 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d_6): δ_C (CH_3): 12.0, 14.9, 18.4, 19.1, 20.9, 21.2, 22.3, 22.6; (CH_2): 20.6, 23.3, 23.7, 23.8, 28.0, 30.0, 30.8, 36.1, 39.2, 39.4; (CH): 27.7, 30.8, 35.6, 45.8, 55.0, 55.9, 72.1, 76.6, 81.7; (C): 35.9, 42.5, 76.6, 165.3, 169.6, 169.8; MS (ES^+): m/z 544 (100).

6α-Chloro-5β-cholestane–3β,4α,5–triol (4.1-12)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with aluminium chloride in THF (protocol B6) or in CH_3CN (protocol B7) for 90 min. Yield 29% and 23% separately. M.p. 113-116 °C; IR: $\nu_{max:}$ 3500-3300, 2944, 2865, 1465, 1374 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): 4.31-4.39 (dd, J=12.3 and 6.9 Hz, 1H, H-6), 4.25 (s, 1H, H-3), 3.87 (s, 1H, H-4), 0.96 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 17.5, 18.5, 22.5, 22.7; (CH_2): 21.1, 22.6, 23.7, 24.0, 25.2, 28.1, 36.0, 38.9, 39.4, 39.6; (CH): 27.9, 35.0, 35.6, 40.4, 55.9*, 69.4, 72.1, 72.7; (C): 42.5, 42.7, 76.2; MS (ES^+):m/z 472 ($M+NH_4^+$, 5), 436(15), 401(20), 214(100).

5-Chloro-5α-cholestane–3β,4α,6β–triol (4.1-13)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with aluminium chloride in THF (protocol B6) for 90 min. Yield 17%. M.p. 190-193 °C; IR: $\nu_{max:}$ 3500-3300, 2942, 2865, 1458, 1380 and 1068 cm^{-1}; ^1H NMR ($CDCl_3$): 4.28 (s, 1H, H-6), 4.07 (d, J=8.6Hz, 1H, H-4), 3.95 (m, 1H, H-3), 1.30 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+):m/z 472 ($M+NH_4^+$, 20), 214(100).

4β-Chloro-5α-cholestane–3β,5,6β–triol (4.1-14)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with aluminium chloride in THF (protocol B6) for 90 min. Yield 24%. M.p. 155-158 °C; IR: $\nu_{max:}$ 3500-3300, 2937, 2861, 1466, 1380 and 1012 cm^{-1}; ^1H NMR ($CDCl_3$): 4.33 (m, 1H, H-3), 4.18 (d, J=4.0Hz, 1H, H-4), 3.81 (s, 1H, H-6), 1.44 (s, 3H, CH_3-19), 0.66 (s, 3H,

CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 15.9, 18.6, 22.5, 22.7; (CH_2): 20.3, 23.8, 24.0, 26.2, 28.1, 32.8, 34.4, 36.0, 39.4, 39.7; (CH): 27.9, 29.9, 35.7, 47.5, 55.9, 56.1, 67.7, 70.8, 76.9; (C): 38.8, 42.6, 75.8; MS (ES^+): m/z 214(100).

5α–Cholestane–3β,4β,5,6β–tetrol 3-acetate (4.1-15)

The title compound was afforded as described in scheme 4.1-8 and 4.1-10. ^1H NMR ($CDCl_3$): 5.04 (s, 1H, H-3), 4.28 (s, 1H, H-6), 4.23 (d, 1H, H-4) 2.07 (s, 3H, acetyl CH_3), 1.29 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); compound was hydrolysed to give the tetrol 4.1-3.

5β–Cholestane–3β,4α,5,6β–tetrol 6-acetate (4.1-17)

The title compound was afforded as described in scheme 4.1-9. ^1H NMR ($CDCl_3$): 4.98 (m, 1H, H-6), 3.78 (d, 1H, H-4), 3.71 (m, 1H, H-3), 2.07 (s, 3H, acetyl CH_3), 1.01 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); compound was hydrolysed to give the tetrol 4.1-6.

4β-Acetamido-5α-cholestane–3β,5,6β–triol, 3-acetate (4.1-18)

Afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol 3-acetate (3.2-1b) with aluminium chloride in CH_3CN (protocol B6) for 2h. Yield 95%. Gum; IR: ν_{max}: 3500-3300, 2935, 2857, 1733, 1643, 1465, 1377, 1234, 1047 and 728 cm^{-1}; ^1H NMR ($CDCl_3$): 4.60 (m, 2H, H-3 & H-4), 3.33 (s, 1H, H-6), 2.04, 1.93 (both s, 6H, acetyl CH_3), 1.22 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 15.0, 18.5, 19.5, 21.2, 22.4, 22.7; (CH_2): 20.8, 23.5, 23.8, 24.0, 28.2, 30.3, 34.4, 36.0, 39.3, 39.5; (CH): 27.8, 30.3, 35.7, 46.2, 55.2, 56.1, 71.1, 77.0, 82.0; (C): 35.9, 42.6, 77.9; MS (ES^+): m/z 519(M-OH^-)(100), 214(45).

6α-Chloro-5β-cholestane–3β,4α,5–triol 3-acetate (4.1-19)

The title compound was afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol 3-acetate (3.2-1b) with general procedure B6. Yield 15.6%. Gum; ^1H NMR ($CDCl_3$): 4.95 (s, 1H, H-3), 4.31-4.39 (dd, J=12.2 and 7.0Hz, 1H, H-6), 4.20 (s, 1H, H-4), 2.07

(s, 3H, acetyl CH_3), 0.96 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); compound was hydrolysed to give the triol 4.1-12.

5-Chloro-5α-cholestane–3β,4α,6β–triol 3-acetate (4.1-20)

The title compound was afforded in procedure described for 4.1-19. Yield 13.2%. M.p. 147-150 °C; ^{1}H NMR ($CDCl_3$): 5.21 (m, 1H, H-3), 4.28 (s, 1H, H-6), 4.23 (d, 1H, H-4) 2.07 (s, 3H, acetyl CH_3), 1.29 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); compound was hydrolysed to give the triol 4.1-13.

5β–Cholestane–3β,4α,5,6β–tetrol 5-acetate (4.1-21)

The title compound was afforded as described in scheme 4.1-10. ^{1}H NMR ($CDCl_3$): 4.79 (s, 1H, H-4), 3.81 (br s, 1H, H-6), 3.61 (m, 1H, H-3), 2.12 (s, 3H, acetyl CH_3), 1.07 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); compound was reduced by $LiAlH_4$ to give the tetrol 4.1-6.

5α–Cholestane–3β,4β,5,6β–tetrol 6-acetate (4.1-23)

The title compound was afforded as described in scheme 4.1-14. ^{1}H NMR ($CDCl_3$): 5.03 (m, 1H, H-6), 4.30 (m, 1H, H-3), 4.04 (dd, J=3.8Hz, 1H, H-4), 2.08 (s, 3H, acetyl CH_3), 1.37 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); compound was hydrolysed to give the tetrol 4.1-3.

5-Methoxy-5α-cholestane–3β,4α,6β–triol (4.1-24)

The title compound was afforded by treating 4α,5–epoxy-5α-cholestane–3β,6β-diol (3.2-1) with general procedure B8. Yield 85%. Gum; IR: ν_{max}: 3500-3300, 2946, 2857, 1464, 1378, 1064 and 1015 cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.52 (s, 1H, H-6), 4.05 (d, J=9.6Hz, 1H, H-4), 3.89 (m, 1H, H-3), 3.41 (s, 3H, 5-OCH_3), 1.22 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); MS (ES^+): m/z 473 ($M+Na^+$, 70);

4α-Chloro-5β-cholestane–3β,5,6β–triol 3,6-diacetate (4.1-26)

Afforded from 4β,5–epoxy-5β-cholestane–3β,6β-diol 3,6-diaceate (3.2-2a) with aluminium in THF (general procedure B5). Yield 28% after 28days. Also afforded by acetylation of 4.1-27 in acid media (general procedure D4). Gum; IR: ν_{max}: 3450, 2954,

2865, 1739, 1469, 1440, 1374, 1253 and 1036 cm^{-1}; ^1H NMR ($CDCl_3$): 5.01-5.08 (m, 2H, H-3 & H-6), 4.08 (d, 1H, H-4), 1.06 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+):m/z 556 ($M+NH_4^+$, 100).

4α-Chloro-5β-cholestane–3β,5,6β–triol (4.1-27)

The title compound was afforded by treating 4β,5–epoxy-5β-cholestane–3β,6β-diol (3.2-2) with general procedure B6. Yield 89%. M.p. 110-112°C; IR: $\nu_{max.}$ 3450, 2954, 2865, 1465, 1375 and 1038 cm^{-1}; ^1H NMR ($CDCl_3$): 4.09 (s, 1H, H-3), 3.92 (s, 1H, H-4), 3.70 (d, J=3.1Hz, 1H, H-6), 1.10 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.6, 19.6, 22.5, 22.7; (CH_2): 21.4, 21.9, 23.7, 24.0, 25.1, 28.2, 35.1, 36.0, 39.4, 39.7; (CH): 27.9, 29.5, 35.6, 41.3, 55.9, 56.3, 62.4, 72.8, 73.8; (C): 40.8, 42.4, 75.9. MS (ES^+): m/z 472 ($M+NH_4^+$, 100).

3β,4β-Dihydroxy-5α-cholestan-6-one (4.1-28)

Afforded by treating 4β,5–epoxy-5β-cholestane–3β,6β-diol (3.2-2) with general procedure B2 or B4, yield of 86% and 97% separately. Mp151-153°C. IR: ν_{max} 2942, 2865, 1704, 1469,1385, 1224, 1072 and 979 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.26 (s, 1H, H-4), 3.37 (m, 1H, H-3), 0.97 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); MS: (ES^+):m/z 436 ($M+NH_4^+$, 100).

3β,4β-Dihydroxy-5α-cholestan-6-one 3-acetate (4.1-29)

Afforded by treating 4β,5–epoxy-5β-cholestane–3β,6β-diol 3-acetate (3.2-2b) with general procedure for B4, yield of 27%. Foam. IR: $\nu_{max.}$ 3500-3300, 2944, 2861, 1714, 1469, 1375, 1257, 1047 and 977 cm^{-1}; ^1H NMR ($CDCl_3$): 4.60 (m, 1H, H-3), 4.40 (S, 1H, H-4), 3.31 (s, 1H, OH-4), 1.02 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.7, 18.5, 21.1, 22.4, 22.7; (CH_2): 20.6, 21.6, 23.6, 23.8, 27.8, 35.9, 36.2, 39.1, 39.3, 46.8; (CH): 27.8, 35.5, 38.0, 54.4, 55.9, 56.6, 59.4, 66.3, 73.5; (C): 41.1, 42.8, 170.4, 213.0. MS: (ES^+):m/z 478 ($M+NH_4^+$, 100).

5α-Cholestane-4,6-dione (4.1-30)

The title compound was afforded by treating 4β,5–epoxy-5β-cholestane–3β,6β-diol 3-acetate (3.2-2b) with general procedure B1. Yield 74%. M.p. 100-102°C; IR: ν_{max}: 3403, 2952, 2867, 1712, 1465, 1380, 1257 and 1236 cm^{-1}; ^1H NMR ($CDCl_3$): 0.93 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 12.4, 18.5, 22.4, 22.7; (CH_2): 21.5, 23.7, 23.9, 27.9, 35.9, 36.8, 37.2, 37.9, 39.2, 39.3, 46.4; (CH): 27.8, 35.5, 37.9, 53.2, 56.0, 56.4, 57.3; (C): 41.1, 42.8, 208.9, 211.0; MS: (ES^+):m/z 423 ($M+Na^+$, 100).

3β,4β-Dihydroxy-5α-cholestan-6-one 4-acetate (4.1-33)

Afforded by treating 4β,5–epoxy-5β-cholestane–3β,6β-diol 3-acetate (3.2-2b) with general procedure B4. Yield 50%. Gum. IR: ν_{max}: 3500-3300, 2944, 2865, 1751, 1725, 1714, 1463, 1383, 1247, 1077 and 727 cm^{-1}; ^1H NMR ($CDCl_3$): 5.48 (s, 1H, H-4), 3.60 (m, 2H, H-3 & H-6), 0.93 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 15.4, 18.5, 21.1, 22.4, 22.7; (CH_2): 20.6, 23.6, 23.8, 24.9, 27.9, 35.9*, 39.1, 39.3, 46.6; (CH): 27.8, 35.5, 36.6, 54.1, 55.9, 56.5, 58.1, 69.0, 70.5; (C): 40.1, 42.6, 171.6, 207; MS (ES^+): m/z 461 ($M+H^+$, 100).

5α-Cholestane-3α,4α,5β,6β-tetrol (4.2-1)

Afforded by hydrolysis of its 3,6-diacetate (4.1-2). M.p. 179-181°C; HRMS(ES^+): m/z 454.3894 ($M+NH_4^+$, C27H52NO4 requires 454.3896); ^1H NMR ($CDCl_3$): 3.95 (m, 1H, H-3), 3.78 (s, 1H, H-6), 3.60 (s, 1H, H-4), 0.98 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^1H NMR (DMSO-d_6): 4.51 (d, 1H, OH-6), 4.40 (d, 1H, OH-4), 4.20 (d, J=6.6Hz, 1H, OH-3), 3.77 (s, 1H, OH-5), 3.63 (m, 1H, H-3), 3.47 (s, 1H, H-6), 3.33 (s, 1H, H-4), 0.83 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d_6): δ_C (CH_3): 11.7, 18.4, 18.5, 22.4, 22.6; (CH_2): 20.8 23.4 23.9 24.0 28.0, 30.7, 35.8, 36.2, 39.1, 39.8; (CH): 27.5, 29.2, 35.4, 39.7, 55.8, 56.2, 68.1, 73.6, 77.3; (C): 38.8, 42.0, 74.4; MS (ES^+):m/z 454 ($M+NH_4^+$ 28), 419 (100).

3,4,6-Triacetate:

Recrystallised from methanol. M.p. 193-194°C; IR: ν_{max}: 3500-3300, 2952, 2873, 1753, 1463, 1371, 1238 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): 5.16-5.22 (m, 1H, H-3),

5.18 (s, 1H, H-4), 4.78 (s, 1H, H-6), 2.12, 2.07, 1.92 (all s, 9H, acetyl CH_3), 1.00 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 17.9, 18.5, 20.9, 21.2, 21.4, 22.4, 22.7; (CH_2): 21.0*, 23.7 24.1, 28.1, 29.8, 34.0, 36.0, 39.4, 39.8; (CH): 27.9, 29.7, 35.6, 40.4, 56.0, 56.8, 70.1, 74.7, 75.9; (C): 39.8, 42.5, 74.8; MS (ES^+): m/z 580 ($M+NH_4^+$ 100).

5β-Cholestane-3α,4α,5,6β-tetrol 3,6-diacetate (4.2-2)

Afforded by applying the ring open procedure for 4α,5-Epoxy-5α-cholestane- 3α,6β-diol 3,6-diacetate (3.2-5a) or the β-epoxide 3.2-6a as described in section 4.2. M.p. 172-174°C; IR: $\nu_{max:}$ 3500-3300, 2952, 2871, 1722, 1641, 1463, 1371, 1258 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): 5.05-5.11 (m, 1H, H-3), 4.93 (s, 1H, H-6), 3.77 (d, J=2.8Hz, 1H, H-4), 2.08, 2.04 (both s, 6H, acetyl CH_3), 0.97 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.8, 18.5, 21.2, 21.4, 22.4, 22.7; (CH_2): 20.4, 20.8, 23.7 24.1, 28.2, 29.8, 34.1, 36.0, 39.3, 39.7; (CH): 27.8, 29.8, 35.6, 40.0, 56.0*, 73.2, 75.0, 78.0; (C): 39.7, 42.3, 74.8; MS (ES^+): m/z 543 ($M+Na^+$, 100).

5β-Cholestane-3α,4α,5,6β-tetrol 6-acetate (4.2-3)

Afforded by applying the ring open procedure for 4α,5-Epoxy-5α-cholestane -3α,6β-diol 6-diacetate (3.2-5b) as described in section 4.2. Foam. ^1H NMR ($CDCl_3$): 4.97 (s, 1H, H-6), 4.03 (m, 1H, H-3), 3.65 (d, 1H, H-4), 2.08 (s, 3H, acetyl CH_3), 0.96 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18). The crude product was hydrolysed directly to give pure tetrol **4.2-1** after recrystalisation.

5-Chloro-5β-cholestane–3α,4α,6β–triol (4.2-4)

Afforded by applying the ring open procedure B6 or B7 to 4α,5-Epoxy-5α-cholestane-3α,6β-diol (3.2-5). Yield 95% and 91% separately. Foam. IR: $\nu_{max:}$ 3500-3300, 2935, 2871, 1463, 1378 and 1076 cm^{-1}; ^1H NMR ($CDCl_3$): 4.31 (s, 1H, H-4), 4.26 (br s, 1H, H-3), 4.02 (s, 1H, H-6), 1.23 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 17.7, 18.6, 22.5, 22.7; (CH_2): 20.6, 23.7 23.9, 27.4, 28.1, 28.2, 32.7, 36.0, 39.4, 39.7; (CH): 27.9, 29.9, 35.7, 46.0, 55.9, 56.0, 66.7, 69.1, 70.1; (C): 41.4, 42.5, 89.5; MS (ES^+): m/z 472 ($M+NH_4^+$, 13), 383 (100). The

title compound was treated with acetylation procedure D3 to give the 3,4,6-triacetate: ^{1}H NMR ($CDCl_3$): 5.42 (d, 1H, H-4), 5.25 (m, 1H, H-3), 5.17 (d, 1H, H-6), 1.25 (s, 3H, CH_3-19), 0.59 (s, 3H, CH_3-18).

5β-Cholestane-3α,4α,5,6β-tetrol 5-acetate (4.2-5)

Afforded as the 6,5-acetyl migration product of 4α,5-Epoxy-5α-cholestane-3α,6β-diol 6-acetate (3.2-5b) when treating with procedure B4. Yield 28%. Foam. IR: $\nu_{max:}$ 3500-3300, 2929, 2865, 2846, 1718, 1467, 1372, 1267 and 975 cm^{-1}; ^{1}H NMR ($CDCl_3$): 4.87 (s, 1H, H-4), 4.15-4.21 (m, 2H, H-3 & H-6), 1.04 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.6, 18.5, 18.6, 21.0, 22.4, 22.7; (CH_2): 23.7*, 23.9, 25.7, 28.3, 29.4, 29.6, 35.9, 36.6, 39.3, 40.9; (CH): 27.9, 31.7, 35.7, 51.7, 56.0, 56.6, 77.3, 78.9, 79.5; (C): 40.9, 43.5, 80.6; MS (ES^+):m/z 472 ($M-OH^-$, 80), 383 (100).

5α-cholestane-3α,4β,5,6β-tetrol 5,6-diacetate (4.2-6)

Afforded as the 3,5-acetyl migration product of 4β,5-Epoxy-5β-cholestane-3α,6β-diol 3, 6-acetate (3.2-6a) when treating with procedure B5. Yield 12%. M.p. 200-201°C; IR: $\nu_{max:}$ 3500-3300, 2929, 2865, 2856, 1730, 1467, 1380, 1282, 1259 and 1039 cm^{-1}; ^{1}H NMR ($CDCl_3$): 6.38 (s, 1H, H-6), 4.80 (d, 1H, H-4), 4.26 (s, 1H, OH-3), 4.06 (d, 1H, OH-4), 3.90 (s, 1H, H-3), 1.38 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.2, 15.7, 18.6, 21.7, 22.4, 22.7*; (CH_2): 20.1, 23.0, 23.6, 23.9, 28.1, 28.8, 32.1, 36.0, 39.4, 39.8; (CH): 27.9, 30.0, 35.6, 46.7, 56.0*, 68.0, 69.5, 72.4; (C): 40.0, 42.6, 84.4, 172.1, 172.3; MS (ES^+): m/z 543 ($M+Na^+$, 100).

3α,4β-Dihydroxy-5α-cholestan-6-one (4.2-8)

4β,5-Epoxy-5β-cholestane-3α,6β-diol 3, 6-acetate (3.2-6) treated with general procedure B3-B5 give the title compound with the best yield 78%. M.p. 165-167 °C ; IR: ν_{max} 2940, 2865, 1706, 1701, 1461,1382, 1083 and 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 4.08 (s, 1H, H-4), 3.93 (d, 1H, H-3), 2.57 (s, 1H, H-5), 0.98 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.4, 18.5, 22.4, 22.7; (CH_2):

20.4, 23.7, 23.8, 23.9, 27.9, 31.0, 36.0, 39.2, 39.4, 47.1; (CH): 27.9, 35.6, 38.3, 54.4, 55.6, 55.9, 56.7, 68.2, 69.7; (C): 41.6, 42.9, 216.22; MS (ES^+): m/z 419 ($M+H^+$, 100).

4α-Chloro-5β-cholestane–3α,5,6β–triol (4.2-9)

4β,5-Epoxy-5β-cholestane-3α,6β-diol 3, 6-acetate (3.2-6) treated with general procedure B6 or B7 give the title compound as white foam.Yield 90%. IR: ν_{max} 3500-3300, 2952, 2865, 1463, 1383, 1267, 1066 and 765 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.15 (m, 2H, H-3 & H-4), 3.75 (s, 1H, H-6), 0.98 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); MS (ES^+): m/z 472 ($M+NH_4^+$, 100)

5β-Cholestane-3α,4α,5,6β-tetrol 3-acetate (4.2-10)

Afforded as the product of 4β,5-Epoxy-5β-cholestane-3α,6β-diol 3-acetate (3.2-6b) when treating with procedure B2. Yield 50%. ^1H NMR ($CDCl_3$): 5.03-5.10 (m, 1H, H-3), 3.71 (m, 2H, H4 & H-6), 0.98 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.6*, 21.2, 22.5, 22.7; (CH_2): 20.1, 21.0, 23.7, 24.1, 28.2, 30.9, 36.1, 36.3, 39.4, 39.7; (CH): 27.9, 29.2, 35.7, 40.2, 56.1, 56.3, 73.5, 75.0, 75.9; (C): 39.8, 42.4, 75.2, 169.9; MS (ES^+): m/z 496 ($M+NH_4^+$, 100).

5α-Cholestane-3β,4β,5,6α-tetrol 4, 6-diacetate (4.3-1)

M.p. 163-166°C; IR: $\nu_{max.}$ 3463, 2948, 2867, 1739, 1716, 1463, 1379, 1255 and 1045 cm^{-1}; ^1H NMR ($CDCl_3$): 5.09-5.18 (d & dd, 2H, H-4 & H-6), 4.21 (m, 1H, H3), 1.13 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 14.6, 18.5, 21.0*, 22.4, 22.7; (CH_2): 20.3, 23.7, 23.9, 25.2, 28.0, 30.5, 31.4, 36.0, 39.3, 39.6; (CH): 27.8, 32.3, 35.6, 43.7, 55.7, 56.0, 67.1, 69.9, 71.9; (C): 39.6, 42.6, 76.3, 170.2, 171.4; MS (ES^+): m/z 521 ($M+H^+$, 24), 461 (100), 502 (26), 562 (89).

5α-Cholestane-3β,4β,5,6α-tetrol 6-acetate (4.3-5)

Afforded as white solid. M.p. 224-226°C; ^1H NMR ($CDCl_3$): 5.30-5.36 (dd, 1H, H-6), 3.98 (m, 1H, H3), 3.44 (d, J=3.9Hz, 1H, H-4), 1.16 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 14.6, 18.5, 21.1, 22.5, 22.7; (CH_2): 20.1,

23.7, 23.9, 25.9, 28.1, 30.7, 31.5, 36.0, 39.4, 39.6; (CH): 27.9, 33.4, 35.7, 45.0, 55.8, 56.0, 67.9, 71.8, 72.4; (C): 39.4, 42.6, 76.2, 172.4; MS (ES^+): m/z 496 ($M+NH_4^+$, 100).

5α-cholestane-3β,4β,5,6α-tetrol (4.3-6)

M.p. 283-284°C (lit. 286-288°C, Warren et al 1989); IR: $\nu_{max.}$ 3500-3300, 2949, 2867, 1463, 1381, 1051 and 968 cm^{-1}; ^1H NMR (DMSO-d_6): 4.06 (d, 1H, -OH), 3.96(d, 1H, -OH), 3.90(d, 1H, -OH), 3.87 (dd, 1H, H-6), 3.80 (m, 1H, H-3), 3.71 (d, 1H, H-4), 3.28 (s, 1H, OH-5), 1.00 (s, 3H, CH_3-19), 0.58 (s, 3H, CH_3-18); MS (ES^+): m/z 454 ($M+NH_4^+$, 100).

3,4,6-triacetate.

M.p. 152-154°C (lit. 151-152°C, Warren et al 1989); IR: $\nu_{max.}$ 3500-3300, 2944, 2865, 1745, 1463, 1379, 1253, 1041 and 737 cm^{-1}; ^1H NMR ($CDCl_3$): 5.25-5.35 (m, 1H, H-3), 5.17 (d, 1H, H-4), 5.06-5.14 (dd, 1H, H-6), 1.99, 1.95, 1.90 (all s, 9H, acetyl CH_3), 1.12 (s, 3H, CH_3-19), 0.58 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 14.6, 18.5, 20.7, 20.8*, 22.4, 22.7; (CH_2): 20.2, 22.3, 23.7, 23.9, 28.1, 30.3, 31.3, 36.0, 39.3, 39.5; (CH): 27.8, 33.3, 35.6, 44.6, 55.6, 56.0, 69.0, 69.4, 69.5; (C): 39.6, 42.6, 76.0, 169.8, 170.2, 170.3; MS (ES^+): m/z 581 ($M+H_2O^+$, 100).

5α-cholestane-3β,4β,5,6α-tetrol 4-acetate-6-benzoate (4.3-7)

M.p. 248-250°C; IR: $\nu_{max.}$ 3500-3300, 2952, 2865, 1734, 1705, 1463, 1375, 1272, 1246 and 717 cm^{-1}; ^1H NMR ($CDCl_3$): 7.38-7.96 (m, 5H, aryl H), 5.40-5.47 (dd, J=11.0 and 5.2Hz, 1H, H-6), 5.13 (d, J=3.9Hz, 1H, H-4), 4.23 (m, 1H, H-3), 1.99 (s, 3H, acetyl CH_3), 1.20 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.7, 18.6, 21.0, 22.5, 22.7; (CH_2): 20.3, 23.8, 23.9, 25.1, 28.1, 30.4, 31.5, 36.0, 39.4, 39.6; (CH): 27.9, 33.4, 35.7, 45.1, 55.8, 56.0, 67.4, 70.7, 72.2, 128.3, 129.4, 132.9; (C): 39.8, 42.7, 76.6, 130.0, 165.7, 171.3; MS (ES^+): m/z 601 ($M+H_2O^+$, 100).

5β-cholestane-3β,4α,5,6α-tetrol 3,6-diacetate (4.3-12)

Foam. IR: $\nu_{max.}$ 3500-3300, 2946, 2871, 1733, 1720, 1635, 1463, 1375, 1257 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): 5.12 (dd, 1H, H-6), 4.96 (s, 1H, H-3), 4.07 (d, 1H, H-4), 2.03

(s, 6H, acetyl CH_3), 0.97 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 17.8, 18.5, 21.3, 22.4, 22.7; (CH_2): 20.0, 21.1, 23.7, 24.0, 25.5, 28.1, 33.6, 36.0, 39.3, 39.6; (CH): 27.8, 32.7, 35.6, 40.0, 55.9, 56.1, 70.8, 74.0, 76.8; (C): 41.1, 42.3, 74.9, 170.0, 171.0; MS (ES^+): m/z 521 ($M+H^+$, 80), 443 (100).

5β-cholestane-3β,4α,5,6α-tetrol 3-acetate-6-benzoate (4.3-13)

Foam. IR: $\nu_{max:}$ 3500-3300, 2950, 2865, 1718, 1464, 1371, 1278, 1031 and 709 cm^{-1}; ^1H NMR ($CDCl_3$): 7.38-8.02 (m, 5H, aryl H), 5.38-5.46 (dd, J=11.5 and 6.4Hz, 1H, H-6), 5.02 (s, 1H, H-3), 4.22 (d, 1H, H-4), 1.96 (s, 3H, acetyl CH_3), 1.04 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.0, 18.6, 21.3, 22.5, 22.8; (CH_2): 20.1, 21.2, 23.7, 24.1, 25.6, 28.2, 33.7, 36.0, 39.4, 39.6; (CH): 27.9, 32.9, 35.7, 40.1, 55.9, 56.1, 71.1, 74.2, 77.3, 128.2, 129.6, 132.7; (C): 41.3, 42.4, 75.3, 132.7, 166.3, 170.1; MS (ES^+): m/z 601 ($M+H_2O^+$, 100).

5β-cholestane-3α,4α,5,6α-tetrol (4.4-1)

Hydrolysis of diacetate 4.4-5 provided the title compound as white crystals (small flake). M.p.188.5-189.5 °C; HRMS(ES^+): m/z 454.3893 ($M+NH_4^+$, C27H52NO4 requires 454.3896); IR: ν_{max} 3500-3300, 2949, 2870, 1464,1057 and 987 cm^{-1}; ^1H NMR (DMSO-d6): δ_H 4.02-4.07 (m, 3H, OH-3, 4,6), 3.810(m, 1H, H-4), 3.79 (s, 1H, OH-5) 3.50-3.70(m, 2H, H-3 & H-6), 0.74 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d6): δ_C (CH_3): 11.7, 17.2, 18.4, 22.3, 22.6; (CH_2) 22.0, 23.4, 23.9, 24.5, 27.9, 30.3, 35.7, 36.4, 38.7, 39.7, (CH): 27.5, 33.3, 35.4, 39.6, 55.8, 56.0, 68.1, 72.3, 73.4,(C): * one in DMSO s peak, 42.1, 76.0; MS (ES^+): m/z 454 ($M+NH_4^+$, 100).

3,4,6-Triacetate:

Prepared from 4.4-1 by acetylation (procedure D3). Afforded as small rectangle crystals. M.p. 206-207°C; sublimed at 197 °C to form long crystals; HRMS(ES^+): m/z 580.4220 ($M+NH_4^+$, C33H58NO7 requires 580.4213); IR: ν_{max} 3500-3300, 2950, 2871, 1743, 1463,1367, 1253,1238 and 1047 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.36 (d, 1H, H-4), 5.19(m, 1H, H-3), 4.99 (dd, J1=12.1Hz, J2=6.5Hz, 1H, H-6), 0.96 (s, 3H, CH_3-

19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.3, 18.5, 20.9, 21.1, 21.5, 22.4, 22.7; (CH_2) 21.2*, 23.7, 24.0, 28.1, 29.9, 33.7, 36.0, 39.4, 40.2, (CH): 27.9, 32.8, 35.6, 40.2, 56.0, 56.9, 70.3, 71.0, 76.7,(C): 41.0, 42.5, 75.8, 168.8, 170.2, 172.2; MS (ES^+): m/z 580 ($M+NH_4^+$, 100).

5α-Cholestane-3α,4β,5,6α-tetrol (4.4-2)

Afforded as white crystals (small rectangle). M.p. 258-259°C; HRMS (ES^+): m/z 454.3895 ($M+NH_4^+$, $C_{27}H_{52}NO_4$ requires 454.3896); IR: ν_{max} 3500-3300, 2937, 1463,1387, 1053 and 994; ^1H NMR (DMSO-d6): δ_H 5.41 (d, J=4.2Hz, 1H, OH-4), 4.77(d, J=4.7Hz, 1H, OH-6), 4.47(s,1H, OH-5) 3.74(m, 3H, H-3, 4, 6), 3.62 (d, J=8.2Hz, 1H, OH-3), 1.01 (s, 3H, CH_3-19), 0.59 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d6): δ_C (CH_3): 12.0, 15.0, 18.5, 22.4, 22.7; (CH_2) 19.6, 23.3, 23.7, 24.3, 26.9, 27.9, 34.6, 35.7, 38.3, 39.7, (CH): 27.4, 33.4, 35.3, 45.2, 55.7, 55.9, 65.8, 69.1, 70.6,(C): * one in DMSO s peak, 42.3, 76.2; MS (ES^+): m/z 457 ($M+Na^+$, 2), 419 (80), 391 (100).

3,4,6-Triacetate:

Afforded as granular crystals. Mp 144-146°C; IR: ν_{max} 3500-3300, 2951, 2865, 1739, 1463,1364, 1253 and 1038 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.10 (m, 1H, H-6), 4.95(s, 1H, H-4), 4.90 (m, 1H, H-3), 1.12 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.0, 18.5, 20.8, 21.0, 21.1, 22.4, 22.7; (CH_2) 20.0, 22.0, 23.7, 23.8, 26.4, 28.0, 30.9, 36.0, 39.3, 39.6, (CH): 27.8, 33.4, 35.6, 45.1, 55.6, 56.0, 69.3, 69.9, 70.0; (C): 40.0, 42.6, 74.8, 168.7, 170.0, 170.7; MS (ES^+): m/z 580 ($M+NH_4^+$, 100).

4β-Acetamido-5α-cholestane-3α,5,6α-triol 3,6-diacetate (4.4-3)

Afforded as described in scheme 4.4-2 as white solid from methanol. Mp 126-128°C; IR: ν_{max} 3457, 2948, 2862, 1750, 1739, 1668, 1463, 1379, 1248 and 1043 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.30 (d, J=4.1 Hz, 1H, H-4), 5.15 (dd, J=12.4 and 5.9 Hz, 1H, H-6), 4.25 (br s, 1H, H-3), 0.96 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.4, 18.5, 20.2, 21.2, 21.7, 22.4, 22.7, (CH_2) 21.8, 22.8, 23.7, 23.9,

26.0, 28.1, 32.6, 36.0, 39.3, 40.1, (CH) 27.9, 33.4, 35.6, 41.6, 56.2, 57.0, 67.7, 69.5, 75.0, (C) 42.7, 43.3, 58.5, 156.7, 168.8, 170.2; MS (ES^+): m/z 580 ($M+H_2O^+$, 100).

3α,4β-Dihydroxy-5β-cholestan-6-one (4.4-4)

Afforded as white solids. M.p. 177-179°C; IR: ν_{max} 3500-3300, 2942, 2865, 1707, 1468,1378, and 1064 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.89 (dd, 1H, H-4), 3.35 (m, 1H, H-3), 0.85 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 21.2, 22.4, 22.7, 23.0; (CH_2) 20.9, 23.8, 23.9, 27.2, 28.0, 34.0, 36.0, 39.5, 39.6, 43.2, (CH): 27.9, 35.6, 37.8, 41.6, 56.1, 56.6, 66.4, 73.9, 74.9,(C): 41.8, 43.0, 213.4; MS (ES^+): m/z 436 ($M+NH_4^+$, 29), 418 (100).

5β-Cholestane-3α,4α,5,6α-tetrol 3,6-diacetate (4.4-5)

Afforded as yellow foam from the reaction of 3.2-7a with perchloric acid in THF (general procedure B1). Yield 85%. IR: ν_{max} 3500-3300, 2946, 2867, 1737, 1722, 1463,1371, 1255 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.01-5.08 (m, 2H, H-3&H-6), 4.07 (s, 1H, H-4), 0.93 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 17.1, 18.5, 21.2, 21.3, 22.4, 22.7; (CH_2) 20.5*, 23.7, 24.0, 28.1, 29.4, 33.0, 36.0, 39.4, 39.7, (CH): 27.8, 33.1, 35.6, 40.0, 56.0*, 71.9, 73.3, 77.7, (C): 41.0, 42.4, 75.9, 170.1, 172.1. MS (ES^+): m/z 521 ($M+H^+$, 10), 544 (100).

5β-Cholestane-3α,4α,5,6α-tetrol 4,6-diacetate (4.4-6)

Afforded as a minor product in preparation of **4.4-5**. Yield 5%. M.p. 177-179°C; IR: ν_{max} 3500-3300, 2946, 2867, 1743, 1463,1373, 1247 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.25 (d, J=3.6Hz, 1H, H-4), 4.99 (dd, J1=12.0Hz, J2=6.0Hz, 1H, H-6), 4.10 (m, 1H, H-3) 0.95 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 17.3, 18.5, 21.2, 21.7, 22.5, 22.7; (CH_2): 21.2, 23.4, 23.7, 24.0, 28.1, 29.9, 33.8, 36.0, 39.4, 39.7, (CH): 27.9, 32.8, 36.0, 40.1, 55.9, 56.8, 68.1, 73.7, 76.8; (C): 40.9, 42.5, 76.2, 170.6, 172.3; MS (ES^+): m/z 538 ($M+NH_4^+$, 100).

5β-Cholestane-3α,4α,5,6α-tetrol 3-acetate (4.4-7) and 4-acetate (4.4-8)

Afforded as shown in **Scheme 4.4-6** and **Table 4.4-2**. Compound 4.4-7: ^{1}H NMR ($CDCl_3$): δ_H 4.98 (m, 1H, H-3), 4.26 (br s, 1H, H-4), 3.82 (dd, 1H, H-6), 0.85 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18). Compound 4.4-8: ^{1}H NMR ($CDCl_3$): δ_H 5.23 (d, J=3.3 Hz, 1H, H-4), 4.15 (m, 1H, H-3), 3.74 (dd, 1H, H-6), 0.89 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18). Both were hydrolysed to yield the free tetrol **4.4-1**.

3α,4β-Dihydroxy-5β-cholestan-6-one 3-acetate (4.4-9)

Afforded as white solids. M.p. 185-187°C; IR: ν_{max} 3500-3300, 2950, 2873, 1718, 1468,1378, 1271, 1066 and 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 4.57 (m, 1H, H-3), 4.10 (dd, J1=10.8Hz, J2=9.5Hz, 1H, H-4), 0.84 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.5, 21.2, 22.5, 22.7, 22.8; (CH_2) 20.9, 23.7, 23.9, 24.7, 28.0, 33.6, 35.9, 39.3, 39.4, 43.0, (CH): 27.9, 35.6, 37.8, 41.8, 56.0, 56.7, 66.7, 71.1, 77.5, (C): 41.4, 43.0, 171.1, 211.3; MS (ES^+): m/z 478 ($M+NH_4^+$, 100).

5-Acetamido-5α-cholestane-3α,4β,6α-triol 6-acetate (4.4-12)

Afforded as white foam. IR: ν_{max} 3500-3300, 2951, 2865, 1739, 1720, 1670, 1463, 1375, 1243 and 1029 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 5.27-5.34 (dd, J=11.5 and 4.5 Hz, 1H, H-6), 4.25 (br s, 1H, H-4), 3.68 (d, 1H, OH-3), 3.48 (m, 1H, H-3), 1.27 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.6, 18.6, 20.7, 21.3, 22.5, 22.7; (CH_2): 19.6, 23.7, 23.9, 24.1, 28.1, 29.0, 30.8, 36.0, 39.4*; (CH): 27.9, 34.2, 35.6, 45.9, 55.7, 55.9, 65.8, 72.3*; (C): 42.5, 43.3, 60.0, 155.4, 173.5; MS (ES^+): m/z 538 ($M+H_2O^+$, 100).

4α-Acetamido-5β-cholestane-3α,5,6α-triol 6-acetate (4.4-13)

Afforded as white solids. M.p. 218-220°C; IR: ν_{max} 3500-3300, 2949, 2871, 1720, 1662, 1463, 1378, 1257, 1026 and 975 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 5.09-5.16 (dd, J1=12.5 Hz, J2=5.7 Hz, 1H, H-6), 4.71 (m, 1H, H-3), 4.15 (dd, 1H, H-4), 0.98 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 14.5, 17.2, 18.6, 21.4, 22.5, 22.7; (CH_2): 21.1, 23.4, 23.7, 24.0, 28.1, 28.2, 32.8, 36.0, 39.4, 39.7; (CH): 27.9, 33.6, 35.6, 43.8, 55.8, 55.9, 69.8, 78.0, 78.5; (C): 41.0, 42.4, 75.8, 164.3, 172.5; MS (ES^+): m/z 538 ($M+H_2O^+$, 100).

5α-Cholestane-3α,4β,5,6α-tetrol 3,6-diacetate (4.4-14)

Afforded as white solids. M.p. 152-154°C; IR: ν_{max} 3500-3300, 2944, 2865, 1743, 1469,1379, 1244 and 1029 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.27 (dd, 1H, H-6), 5.12 (m, 1H, H-3), 3.73(d, J=3.9Hz, 1H, OH-4), 3.52(br s, 1H, H-4), 2.76(s, 1H, OH-5), 1.21 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.0, 18.5, 21.2*, 22.4, 22.7; (CH_2): 19.9, 21.6, 23.7, 23.9, 26.9, 28.1, 31.2, 36.0, 39.4, 39.6, (CH): 27.9, 33.5, 35.6, 45.2, 55.7, 56.0, 69.3, 72.2, 73.1,(C): 40.3, 42.6, 75.1, 168.9, 172.8; MS (ES^+): m/z 538 ($M+NH_4^+$, 100).

5α-Cholestane-3α,4β,5,6α-tetrol 5,6-diacetate (4.4-15)

Afforded as white solids. M.p. 123-124°C; IR: ν_{max} ^1H NMR ($CDCl_3$): δ_H 5.30 (dd, 1H, H-6), 5.04 (s, 1H, H-4), 4.01(s, 1H, H-3), 1.26 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.1, 18.6, 21.2, 22.4, 22.7, 23.0; (CH_2) 20.1, 23.6, 23.8*, 26.7, 28.1, 32.4, 36.0, 39.4, 39.7; (CH): 27.9, 33.4, 35.6, 45.3, 55.9, 56.1, 64.7, 69.9, 71.9; (C): 42.2, 42.7, 87.4, 171.0, 173.6; MS (ES^+): m/z 538 ($M+NH_4^+$, 100).

4α-Chloro-5β-cholestane–3α,5,6α–triol (4.4-17)

Afforded as small granular crystals. from the reaction of 3.2-8 with $AlCl_3$ in THF (general procedure B6). M.p. 148-149°C; IR: ν_{max} 3500-3300, 2946, 2865, 1639, 1456,1385 and 1061 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.47 (d, 1H, J=3.0Hz, H-4), 4.18(m, 1H, H-3), 3.90 (dd, J1=12.4 Hz, J2=6.8Hz, 1H, H-6), 0.91 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.6, 18.5, 22.5, 22.7; (CH_2) 21.3, 23.7, 24.0, 24.9, 28.1, 30.6, 36.0, 36.2, 39.4, 39.8, (CH): 27.9, 33.3, 35.6, 40.7, 55.9, 56.0, 65.5, 68.9, 74.8; (C): 40.7, 42.4, 78.4; MS (ES^+): m/z 454 ($M+H^+$, 100).

4α-Chloro-5β-cholestane–3α,5,6α–triol 6-acetate (4.4-18)

Recrystallised from methanol. M.p. 95-96°C. ^1H NMR ($CDCl_3$): δ_H 5.05-5.13 (dd, 1H, H-6), 4.30(d, 1H, H-4), 4.14 (m, 1H, H-3), 0.96 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 18.8, 21.1, 22.4, 22.7; (CH_2): 20.9, 21.7,

23.6, 24.1, 25.8, 28.0, 36.0, 36.1, 39.4*; (CH): 27.9, 33.9, 35.6, 45.6, 55.6, 56.0, 57.1, 67.3, 68.1; (C): 37.1, 45.6, 66.2, 169.9; MS (ES^+): m/z 518 ($M+Na^+$, 100).

5β-Cholestane-3α,4α,5,6α-tetrol 6-acetate (4.4-20)

Afforded as shown in **Scheme 4.4-18**. ^1H NMR ($CDCl_3$): δ_H 5.07 (dd, 1H, H-6), 3.95 (m, 2H, H-3 & H-4), 0.92 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18). Hydrolysis by general method D2 give the free tetrol 4.4-1.

5–Methoxy–5α-Cholestane-3α,4β,6α-triol (4.4-21)

Afforded as white solids from the reaction of 3.2-8 with boron trifluoride etherate in methanol over 4hr. Mp 215-217°C; IR: ν_{max} 3500-3300, 2929, 2865, 1629, 1463, 1385, 1084, and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.44 (s, 1H, H-4), 4.26 (m, 1H, H-6), 3.82(brs, 1H, H-3), 3.53(s, 3H, Me-O5), 1.11 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); MS (ES^+): m/z 473 ($M+Na^+$, 8), 401 (100).

3,4,6-triacetate

Afforded as oil. IR: ν_{max} 3500-3300, 2929, 2865, 1730, 1463, 1384, 1086, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.42 (s, 1H, H-4), 5.20 (m, 1H, H-6), 4.80(s, 1H, H-3), 3.44(s, 3H, Me-O5), 1.12 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.0, 18.5, 20.9, 21.1, 21.2, 22.4, 22.7, 52.0; (CH_2): 20.4, 22.2, 23.7, 23.8, 26.9, 28.0, 31.8, 36.0, 39.4, 39.7, (CH): 27.9, 34.2, 35.6, 45.2, 55.7, 56.0, 64.5, 69.7, 71.7, (C): 41.6, 42.7, 77.2, 169.3, 169.8, 170.2; MS (ES^+): m/z 594 ($M+NH_4^+$, 100).

5,6β-Epoxy-5β-cholestane-3β,4α-diol (4.5-1)

Afforded as white solid. M.p. 164-165°C; IR: ν_{max} 3500-3300, 2933, 2865, 1643, 1465, 1380 and 1076 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.82 (dd, 1H, H-4), 3.60 (d, 1H, H-6), 3.54 (m, 1H, H-3), 1.05 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 17.5, 18.6, 22.5, 22.7; (CH_2): 21.8, 23.7, 24.1, 27.3, 28.0, 31.8, 35.7, 36.0, 39.4, 39.7, (CH): 27.9, 29.3, 35.6, 51.0, 56.1*, 56.7,72.9, 73.6; (C): 36.1, 42.1, 65.8;

5,6β-Epoxy-5β-cholestane-3α,4α-diol (4.5-2)

Afforded as white solid. M.p. 157-159°C; IR: ν_{max} 3500-3300, 2939, 2870, 1463, 1372, 1065, 1058 and 738 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.16 (m, 1H, H-3), 3.95 (d, 1H, H-4), 3.52 (d, 1H, H-6), 0.96 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 17.3, 18.6, 22.5, 22.7; (CH_2): 21.7, 23.7, 24.1, 26.1, 28.1, 31.5, 32.7, 36.0, 39.4, 39.7, (CH): 27.9, 29.5, 35.6, 50.2, 56.0, 56.1, 67.2 68.9, 69.3; (C): 36.1, 42.1, 65.1.

4β–Ethoxy–5α–cholestane–3β,5,6α–triol (4.5-3)

Afforded as white solid. M.p. 177-178°C; IR: ν_{max} 3500-3300, 2954, 2867, 1465, 1377 and 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.97 (m, 2H, H-3 & H-4), 3.80 (dd, 1H, OCH_2), 3.66 (d, 1H, H-6), 3.62 (dd, 1H, OCH_2), 1.17 (t, 3H, OCH_2CH_3), 1.04 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.6, 15.9, 18.5, 22.5, 22.7, (CH_2): 20.3, 23.7, 24.1, 26.7, 28.2, 31.1, 35.6, 36.1, 39.4, 39.8, 68.5, (CH): 27.9, 33.4, 35.6, 45.4, 55.9, 56.1, 67.9, 68.2, 80.0; (C): 38.8, 42.6, 77.3; MS (ES^+): m/z 465 ($M+H^+$, 100).

5,6α–Epoxy–5α–cholestan–3β,4β–diol, 3,4-diacetate (4.6-13)

M.p. 143-144°C; IR: 2946, 2863, 1735, 1458, 1442, 1379, 1242, 1225, 1039 and 1024 cm^{-1}; ^1H NMR ($CDCl_3$): 4.95-5.04 (bis dd, H-3), 4.47 (d, J=3.0Hz, 1H, H-4), 3.13 (d, J=4.05Hz, 1H, H-6), 2.09(s, 3H, Ac), 1.97 (s, 3H, Ac), 1.13 (s, 3H, CH_3-19), 0.57 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 15.0, 18.5, 20.9, 21.1, 22.4, 22.7; (CH_2): 19.7, 22.7, 23.7, 23.9, 28.0, 32.2, 36.0*, 39.1, 39.3; (CH): 27.9, 29.6, 35.6, 42.9, 55.6, 56.6, 59.9, 70.4, 75.3; (C): 34.4, 42.2, 63.3, 169.7, 169.8.

5,6β–Epoxy–5β–cholestan–3β,4β–diol, 3-acetate (4.6-20)

M.p. 193-194°C; IR: 2941, 2870, 1724, 1461, 1378, 1261, 1039 and 912 cm^{-1}; ^1H NMR ($CDCl_3$): 4.74-4.81 (bis dd, H-3), 3.42 (d, J=2.7 Hz, 1H, H-4), 3.19 (s, 1H, H-6), 2.12(s, 3H, Ac), 1.13 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.5, 17.8, 18.5, 21.2, 22.4, 22.7; (CH_2): 21.0*, 23.7, 24.1, 28.0, 32.3, 36.0,

36.8, 39.3, 39.4; (CH): 27.9, 29.5, 35.6, 51.5, 55.9, 56.0, 63.5, 73.3, 74.8; (C): 34.9, 42.0, 65.0, 170.5.

5,6β–Epoxy–5β–cholestan–3β,4β–diol, 3,4-diacetate (4.6-22)

M.p. 176-178°C; IR: 2956, 2865, 2848, 1741, 1463, 1438, 1375, 1363, 1263, 1226 and 1039 cm^{-1}; ^1H NMR ($CDCl_3$): 4.77-4.84 (bis dd, H-3), 4.78 (s, 1H, H-4), 3.22 (s, 1H, H-6), 2.11 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.12 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.5, 17.3, 18.6, 20.9*, 22.5, 22.7; (CH_2): 21.3, 21.7, 23.7, 24.1, 28.0, 32.2, 36.0, 37.0, 39.4, 39.5; (CH): 27.9, 29.5, 35.6, 51.8, 55.9*, 62.9, 71.4, 75.6; (C): 35.0, 42.0, 62.2, 170.1, 170.2.

5β-Cholestan-3β,4β,5,6α-tetrol 3,4-diacetate (4.6–29)

Afforded as colourless foam. IR: ν_{max} 3500-3300, 2946, 2871, 1741, 1463, 1379, 1240 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.64 (d, J=3.8 Hz, 1H, H-4), 5.25 (br s, 1H, H-3), 3.88 (dd, J= 12.4 and 4.4 Hz, 1H, H-6), 0.96 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 17.0, 18.4, 21.0, 21.1, 22.4, 22.7; (CH_2): 21.4, 23.5, 23.6, 23.9, 25.3, 28.0, 34.5, 35.9, 39.3, 39.6; (CH): 27.8, 34.0, 35.6, 43.3, 55.6, 55.9, 67.3, 72.1, 72.7; (C): 42.4, 43.3, 78.2, 169.1, 169.5; MS (ES^+): m/z 564 ($M+H_3O^+$, 10), 504 (100).

5β-Cholestan-3β,4β,5,6β-tetrol (4.6–33)

Afforded as long crystals.M.p. 145-148°C; IR: ν_{max} 3500-3300, 2947, 2865, 1463, 1380 and 1057 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.27 (br s , 1H, H-6), 3.96 (br s, 1H, H-3), 3.67 (d, J= 3.4 , H-4), 1.08 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.5, 19.0, 22.5, 22.7; (CH_2): 21.1, 23.8, 24.2, 25.3, 26.1, 28.2, 34.6, 36.1, 39.4, 39.8; (CH): 27.9, 29.5, 35.7, 43.3, 56.2, 56.4, 68.6, 69.1, 71.4; (C): 41.4, 42.5, 78.9; MS (ES^+): m/z 437 ($M+H^+$, 100).

Cholest-4-ene-3β,6β,7β-triol triacetate (5-10)

M.p. 116-118°C; IR: ν_{max} 3500-3300, 2954, 2867, 1741, 1645, 1469,1375, 1240 and 1032 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.66 (s, 1H, H-4), 5.44 (d, J=3.8Hz, 1H, H-6), 5.20

(m, 1H, H-3), 4.58 (dd, J1=10.8Hz, J2=3.8Hz, 1H, H-7), 1.18 (s, 3H, CH_3-19), 0.72 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.6, 20.8, 21.1, 21.2, 21.3, 22.5, 22.7; (CH_2) 20.6, 23.6, 24.7, 25.9, 28.4, 35.9, 36.6, 39.2, 39.3, (CH): 27.8, 35.1, 35.4, 51.6, 54.4, 54.9, 70.0, 74.4, 75.6, 129.3, (C): 35.9, 44.3, 141.8, 169.8, 170.1, 170.5; MS (ES^+): m/z 563 ($M+H_3O^+$, 100).

Cholest-4-ene-3β,6β,7α-triol triacetate (5-11)

Afforded as colourless foam. IR: ν_{max} 3500-3300, 2948, 2873, 1743, 1462,1367, 1240 and 1024 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.63 (s, 1H, H-4), 5.27 (m, 1H, H-3), 5.05 (d, J=3.1Hz, 1H, H-6), 4.79 (t, 1H, H-7) 1.13 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.6, 18.5, 20.6, 20.9, 21.1, 21.2, 22.4, 22.7; (CH_2): 20.4, 23.5, 23.6, 24.5, 27.8, 35.9, 36.2, 39.0, 39.3, (CH): 27.8, 34.1, 35.6, 45.6, 49.5, 55.8, 70.0, 71.2, 73.8, 130.6; (C): 36.1, 42.4, 140.6, 168.7, 169.8, 170.6; MS (ES^+): m/z 625 ($M+H_3O^+$, 100).

Cholest-4-ene-3β,6β,7β-triol 3-benzoate-6,7-diacetate (5-12)

M.p. 145-147°C; IR: ν_{max} 3500-3300, 2954, 2863, 1745, 1739, 1716, 1448,1373, 1264,1240, 1111, 1041,1026 and 709 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.80 (s, 1H, H-4), 5.48 (d, J=3.7Hz, 1H, H-6), 5.35-5.50 (m, 1H, H-3), 4.59-4.65 (dd, J1=10.9Hz, J2=3.7Hz, 1H, H-7) 2.08(s, 3H, the Ac CH_3), 1.95(s, 3H, the Ac CH_3), 1.23 (s, 3H, CH_3-19), 0.73 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.7, 20.9, 21.3, 21.4, 22.5, 22.7; (CH_2) 20.6, 21.3, 23.7, 24.9, 25.9, 28.5, 36.0, 36.7, 39.4, (CH): 27.9, 35.1, 35.5, 51.6, 54.5, 54.9, 70.7, 74.5, 75.7, 128.2, 129.3, 129.6, 132.8,(C): 36.2, 43.3, 130.2, 141.9, 166.1, 169.8, 170.2; MS (ES^+): m/z 625 ($M+H_3O^+$, 100).

Cholest-4-ene-3β,6β,7β-triol (5-14)

Cholest-5-en-3β-benzoxy-7-one (55.0g, 109mmol) was dissolved in a mixture of THF (200ml) methanol (20ml) and water (5ml). The resulting mixture was cooled and stirred at 0°C. Sodium borohydride (5.0g, 132mmol) was added by several portion in 10min. The mixture was stirred for 7hr. The resulting solution was poured into water

(400ml) and the mixture was stirred for 20min. the solid was collected by filtration and dried to give about 50g 7α,7β mixture. The ratio on NMR is about 1:4.5.

The 50g compound was dissolved in DCM (280ml) $VO(acac)_2$ (600mg, 2.3mmol) was added and the solution was cooled and stirred at 0°C. TBHP (5.0M in toluene, 25ml, 125mmol) was added in several portion, the resulting dark solution was stirred at 0°C for 1hr and room temperature for 5hr. the DCM solution was washed with 5% aq. sodium hydrogen carbonate and water, dried over magnesium sulfate. After evaporation, the residue was treated with methanol (160ml) to give the 5,6β–epoxy–5β–cholestan–3β,7β–diol 3-benzoate as off-white solid, yield 32 g. An additional part of crystals (8.0g) was separated from the methanol layer after kept in room temperature overnight. They are the mixture of 5, 6β–epoxy–5β-cholestan–3β,7β–diol 3-benzoate and 5,6α–epoxy–5α–cholestan–3β,7α–diol 3-benzoate (mole ratio 1:3.5). The residue after the evaporation of methanol is 11g.

The 32g of 5,6β–epoxy–5β–cholestan–3β,7β–diol 3-benzoate was acetylated by using the general method. The ring opening was carried out in acetone with perchloric acid, also the general procedure. Then the products were acetylated again with the toluene reflux method. The 5α-hydroxyl group was eliminated to generate the 4,5-ene by using the general method with thionyl chloride in pyridine. The 32g solid part finally yield about 25g Cholest-4-en-3β,6β,7β-triol 3-benzoate, 6,7-diacetate. The residue part, finally after hydrolysis and chromatography, give 4g Cholest-4-en-3β,6β,7α-triol as granular crystals. For compound 5-14: M.p. 127-129°C; IR: ν_{max} 3500-3300, 2933, 2870, 1639, 1456,1385, 1072, 1054 and 1031; ^{1}H NMR ($CDCl_3$): δ_H 5.60 (s, 1H, H-4), 4.13 (m, 1H, H-3), 4.06 (d, J=3.7Hz, 1H, H-6), 3.22-3.28(dd, J1=9.9Hz, J2=3.7Hz, 1H, H-7), 1.20 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.8, 21.2, 22.5, 22.7; (CH_2): 20.6, 23.9, 27.2, 28.7, 28.9, 36.1, 37.0, 39.4, 39.6; (CH): 27.9, 35.7, 37.5, 51.5, 55.2, 55.3, 67.5, 76.5, 76.8, 130.4; (C): 36.0, 43.3, 145.2; MS (ES^+): m/z 437 ($M+H_3O^+$, 35), 402 (100).

Cholest-4-ene-3β,6β,7α-triol (5-15)

Prepared from Cholestane – 3β,5α,6β,7α–tetrol 3-benzoate, 6,7-triacetate similar to 5-14. M.p. 180-182°C; IR: ν_{max} 3500-3300, 2949, 2867, 1463, 1375,1334 and 1023; ^{1}H NMR (Pyridine-d_5): δ_H 6.01 (s, 1H, H-4), 4.49 (d, J=2.9Hz, 1H, H-6), 4.40 (m, 1H, H-3), 4.18 (s, 1H, H-7), 1.43 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR (Pyridine-d_5): δ_C (CH_3): 11.9, 18.8, 21.8, 22.5, 22.8; (CH_2): 21.2, 23.8, 24.0, 28.3, 30.5, 36.1, 37.4, 39.5, 40.0, (CH): 28.0, 35.6, 35.9, 45.6, 50.5, 56.4, 67.4, 72.4, 79.0, 132.8 (C): 37.2, 42.5, 145.0; MS (ES^-): m/z 419 (M+1, 100)

4α,5–epoxy–5α–cholestane–3β,6β,7β–triol triacetate (5-18)

Prepared as described in table 5-1, white foam; HRMS(ES^+): m/z 561.3795 ($M+H^+$, C33H53O7 requires 561.3791)IR: ν_{max} 2956, 2871, 1749, 1469, 1371, 1240, 1045 and 1036; ^{1}H NMR ($CDCl_3$): δ_H 4.85-4.95 (m, 2H, H-3 & H-7), 4.55 (d, J=4.0Hz, 1H, H-6), 3.07 (s, 1H, H-4), 1.22 (s, 3H, CH_3-19), 0.72 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 17.5, 18.6, 20.9*, 21.6, 22.4, 22.6; (CH_2) 20.3, 22.9, 23.6, 25.7, 28.4, 29.4, 35.9, 39.3*, (CH): 27.8, 35.4, 35.6, 47.8, 54.2, 55.0, 61.1, 66.6, 73.3, 73.4, (C): 34.0, 43.1, 63.1, 169.5, 169.6, 169.7; MS (ES^+): m/z 561 ($M+H^+$, 100)

4β,5–Epoxy–5β–cholestane–3β,6β,7β–triol triacetate (5-19)

Prepared as described in **Table 5-1**, white foam; IR: ν_{max} 2954, 2871, 1749, 1463, 1375, 1248 and 1029; ^{1}H NMR ($CDCl_3$): δ_H 5.05 (m, 1H, H-3), 4.63-4.70 (m, 2H, H-6 & H-7), 3.40 (d, J=2.7 Hz, 1H, H-4), 1.16 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5, 19.1, 20.7*, 20.9, 22.3, 22.6; (CH_2): 21.1, 21.8, 23.5, 25.8, 28.4, 32.3, 35.8, 39.2*, (CH): 27.7, 34.4, 35.3, 47.0, 54.2, 54.8, 60.7, 68.4, 73.9, 74.9, (C): 34.7, 43.2, 63.6, 169.6, 169.8, 170.2; MS (ES^+): m/z 578 ($M+NH_4^+$, 100)

4α,5–epoxy–5α–cholestane–3β,6β,7β–triol 3-benzoate-6,7-diacetate (5-20)

Prepared as described in table 5-1, white foam; HRMS(ES^+): m/z 640.4220 ($M+NH_4^+$, C38H58NO7 requires 640.4213); IR: ν_{max} 2956, 2871, 1747, 1726, 1469, 1450, 1371, 1269, 1242, 1115, 1026 and 715 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 7.40-8.03 (m, 5H, aryl H), 5.17 (dd, J= 8.4 and 8.6 Hz, 1H, H-3), 4.92 (dd, J=11.0 and 4.0 Hz, 1H, H-7), 4.59

(d, J=4.0 Hz, 1H, H-6), 3.25 (s, 1H, H-4), 1.28 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 17.6, 18.7, 20.9, 21.1, 22.5, 22.7; (CH_2) 20.5, 23.1, 23.7, 25.8, 28.4, 29.5, 36.0, 39.3*, (CH) 27.8, 35.5, 35.7, 47.9, 54.1, 55.0, 61.2, 67.4, 73.4, 73.6, 128.2, 129.5, 133.0, (C) 34.1, 43.4, 63.2, 129.8, 165.3, 169.6*; MS (ES^+): 623 m/z ($M+H^+$, 100).

4β,5–epoxy–5β–cholestane–3β,6β,7β–triol 3-benzoate-6,7-diacetate (5-21)

Prepared as described in **Table 5-1**, white foam; IR: ν_{max} 2954, 2865, 1747, 1718, 1625, 1454, 1375, 1275, 1248, 1110, 1093, 1026 and 709 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.40-8.03 (m, 5H, aryl H), 5.31 (m, 1H, H-3), 4.74 (s, 1H, H-6), 4.68-4.75 (m, 1H, H-7), 3.54 (d, J=2.7 Hz, 1H, H-4), 1.21 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.6, 19.2, 20.8, 21.0, 22.4, 22.7; (CH_2) 21.0, 22.0, 23.6, 25.9, 28.4, 32.6, 35.9, 39.3*, (CH) 27.8, 34.5, 35.7, 47.2, 54.2, 54.9, 60.8, 69.0, 74.0, 75.0, 128.1, 129.6, 132.9, (C) 34.8, 43.2, 63.6, 129.8, 165.6, 169.6, 169.9; MS (ES^+): 640 m/z ($M+NH_4^+$, 100).

4β,5–epoxy–5β–cholestane–3β,6β,7β–triol (5-26)

Prepared from Cholest-4-ene-3β,6β,7β-triol with mCPBA in DCM at room temperature for 15 min. Flake crystals were afforded after crystalised from DCM/Hexane. Yield 83%. Mp 201-202 °C; IR: ν_{max} 3500-3300, 2948, 2867, 1743, 1460,1378 and 1044 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.90-4.00 (m, 1H, H-3), 3.32 (m, 1H, H-6), 3.27 (m, 1H, H-7), 3.22 (d, 1H, H-4) 1.14 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.7, 19.6, 22.4, 22.7; (CH_2): 21.1, 23.8, 25.3, 27.0, 28.6, 31.7, 36.0, 39.4, 39.5, (CH): 27.9, 35.6, 37.2, 46.4, 55.0, 55.2, 63.6, 65.5, 74.9, 76.8; (C): 34.6, 43.4, 68.1; MS (ES^+): m/z 453 ($M+H_3O^+$, 100).

5β–Cholestane–3β,5,6β,7β-tetrol (5-28)

Prepared from 4β,5–epoxy–5β–cholestane–3β,6β,7β–triol with $LiAlH_4$ (procedure C3), yield 72% after eluted with DCM/Acetone 5:1 on silica gel, afforded as white powder. M.p.112-114 °C; HRMS(ES^+): m/z 454.3889 ($M+NH_4^+$, C27H52NO4 requires 454.3896); IR: ν_{max} 3500-3300, 2952, 2871, 1457, 1384, 1095 and 1045 cm^{-1};

^{1}H NMR ($CDCl_3$): δ_H 4.04 (brs, 1H, H-3), 3.54 (d, J=3.7Hz, 1H, H-6), 3.37 (dd, J1=10.1Hz, J2=3.7Hz, 1H, H-7), 1.01 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 18.7, 19.0, 22.5, 22.8; (CH_2): 21.2, 23.8, 25.9*, 27.1, 28.8, 36.1, 36.5, 39.4, 39.8; (CH): 27.9, 35.7, 37.9, 40.8, 55.3, 55.8, 66.9, 73.6, 77.2; (C): 39.6, 43.3, 75.7; MS (ES^+): m/z 459 ($M+Na^+$, 100).

5β–Cholestane–3β,5,6β,7β-tetrol 3,6,7-triacetate

Afforded as foam. HRMS(ES^+): m/z 580.4210 ($M+NH_4^+$, C33H58NO7 requires 580.4213); IR: ν_{max} 3500-3300, 2954, 2867, 1737, 1463,1379, 1259 and 1031 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 5.15 (brs, 1H, H-3), 5.00 (d, J=3.6Hz, 1H, H-6), 4.80 (dd, J1=11.1Hz, J2=3.6Hz, 1H, H-7) 1.08 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.6, 20.9, 21.0, 21.2, 21.3, 22.4, 22.7; (CH_2): 21.2, 23.5, 23.9, 25.7, 26.1, 28.4, 35.0, 35.9, 39.2, 39.5; (CH): 27.8, 35.4, 35.9, 41.1, 54.7, 54.9, 60.4, 72.8, 75.5; (C): 39.7, 43.2, 72.8; MS (ES^+): m/z 580 ($M+NH_4^+$,100).

5β–Cholestane–3β,5,6β,7β-tetrol 3,5,6,7-tetracetate

the 5β–Cholestane–3β,5,6β,7β-tetrol 3,6,7-triacetate was treated by the general acetylation method at room temperature for 2 days. The tetracetate was afforded as a foam after chromatography, yield 49%. ^{1}H NMR ($CDCl_3$): δ_H 5.84 (d, J=3.7Hz, 1H, H-6), 5.06 (s, 1H, H-3), 4.76 (dd, J1=11.0Hz, J2=3.6Hz, 1H, H-7), 3.00(d, 1H, the 4α-H), 1.11 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.6, 18.7, 20.9, 21.2, 21.3, 21.9, 22.4, 22.7; (CH_2): 21.2, 23.6, 23.9, 25.6, 26.4, 28.4, 29.0, 35.9, 39.3, 39.4; (CH): 27.9, 34.8, 35.5, 40.7, 54.7, 54.9, 67.0, 71.5, 72.6; (C): 39.6, 43.3, 81.5; MS (ES^+): m/z 622 ($M+NH_4^+$,100).

5α–Cholestane–3β,4α,6β,7β-tetrol (5-30)

Afforded by the hydroboration and oxidation of Cholest-4-ene-3β,6β,7β-triol, yield 64% wihte solid. M.p.204-206 °C; HRMS(ES^+): m/z 454.3894 ($M+NH_4^+$, C27H52NO4 requires 454.3896); IR: ν_{max} 3500-3300, 2930, 2870, 1457, 1385, 1098 and 1068 cm^{-1}; ^{1}H NMR (Pyridine-d_5): 4.85 (s, 1H, H-6), 4.29 (dd, J1=10.6Hz, J2=8.8Hz, 1H, H-4), 3.71 (m, 1H, H-3), 3.32 (dd, 1H, H-7), 1.23 (s, 3H, CH_3-19),

0.54 (s, 3H, CH_3-18); ($CDCl_3$): δ_H 4.17 (s, 1H, H-6), 3.79 (m, 1H, H-4), 3.45 (m, 1H, H-3), 3.23 (m, 1H, H-7), 1.01 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR (Pyridine-d_5): δ_C (CH_3): 12.3, 17.5, 18.9, 22.5, 22.8; (CH_2): 21.2, 24.0, 27.9, 28.8, 29.9, 36.4, 38.6, 39.5, 40.0, (CH): 28.0, 36.0, 38.3, 52.6, 53.2, 55.6, 56.2, 68.9, 72.0, 77.3, 77.4; (C): 36.8, 43.5; MS (ES^+): m/z 459 ($M+Na^+$, 100).

5α–Cholestane–3β,4α,6β,7β-tetrol 3,4,6,7-tetracetate

Afforded as foam. IR: ν_{max} 3500-3300, 2950, 2871, 1749, 1458, 1365, 1257 and 1051 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.25 (m, 1H, H-6), 5.19 (dd, J1=11.4Hz, J2=9.6Hz, 1H, H-4), 4.73 (m, 1H, H-3), 4.60 (dd, J1=10.9Hz, J2=3.9Hz, 1H, H-7), 1.10 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.9, 18.6,20.5, 20.6, 20.7, 21.0, 22.4, 22.6; (CH_2)*: 23.5, 25.6*, 28.3, 35.9, 36.9, 39.2*; (CH): 27.7, 34.7, 35.4, 48.0, 51.9, 54.5, 54.9, 65.7, 68.5, 74.9, 75.1; (C): 36.5, 43.1, 170.0*,170.3*; MS (ES^+): m/z 622 ($M+NH_4^+$,100).

3β,4β,7β–Trihydroxy–5α–cholestan-6-one (5-32)

This compound was prepared from the rearrangement of 4β,5–epoxy–5β–cholestane –3β,6β,7β–triol 3,6,7-triacetate with boron trifloride etherate in acetonitrile by using the general procedure B5. Yield after chromatography (DCM/acetone 10/1) 62% and a minor product 7%, unrecognised. M.p.198-201 °C; IR: ν_{max} 3500-3300, 2954, 2870, 1696, 1635, 1456,1382, 1120 and 1064 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.28 (t, 1H, H-4), 3.77 (d, J=9.5Hz, 1H, H-7), 3.38 (m, 1H, H-3), 0.95 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.7, 18.7, 22.5, 22.7; (CH_2): 20.7, 23.7, 25.4, 26.0, 28.3, 36.0, 36.4, 39.3, 39.4, (CH): 27.9, 35.6, 46.9, 52.1, 55.4, 56.8, 57.5, 68.0, 70.8, 79.1; (C): 41.3, 43.4, 213.2; MS (ES^-): m/z 433(M-H, 36), 397 (100).

5α–Cholestane–3β,4β,6β,7β-tetrol (5-33)

Afforded from the reduction of 3β,4β,7β–trihydroxy–5α–Cholestan-6-one by using general method. M.p.167-169 °C; HRMS(ES^+): m/z 454.3896 ($M+NH_4^+$, C27H52NO4 requires 454.3896); IR: ν_{max} 3500-3300, 2937, 2870, 1463, 1380, 1259, 1176, 1107 and 1049 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.08 (s, 2H, H-4 &H-6), 3.47 (m,

1H, H-3), 3.23 (m, 1H, H-7), 1.26 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); MS (ES^+): m/z 459 ($M+Na^+$, 100).

5–Fluoro–5α–cholestane–3β,6β,7β-triol 3-acetate (5-35)

Afforded as described in **Table 5-2**, white sold. M.p. 204-205 °C; ^1H NMR ($CDCl_3$): δ_H 5.04 (s, 1H, H-3), 3.66 (m, 2H, H-6 & H-7), 1.12 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3) 12.0, 16.6 (16.7), 18.7, 21.3, 22.5, 22.7; (CH_2) 20.8, 23.8, 26.3, 26.9, 28.6, 31.9*, 35.3, 36.1, 39.4, 39.6; (CH) 27.9, 35.6, 37.8, 43.8, 54.8, 55.1, 70.0, 72.8, 74.5 (75.1); (C) 37.5, 43.5, 98.5 (101.1), 170.5.

5–Acetamido–5α–cholestane–3β,6β,7β-triol 3-acetate (5-36)

Afforded as described in **Table 5-2**, white sold. M.p. 218-219 °C; IR: ν_{max} 3500-3300, 2950, 2865, 1733, 1660, 1510, 1469, 1385, 1238 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.04 (s, 1H, OH-6), 4.79 (m, 1H, H-3), 4.55 (d, J=3.7 Hz, 1H, H-6), 3.48 (dd, J=10.2 and 3.7 Hz, 1H, H-7), 2.90 (dd, 1H, 4α-H), 1.27 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3) 12.2, 17.7, 18.7, 21.3, 22.4, 22.7, 24.7; (CH_2) 21.3, 23.7, 26.1, 26.9, 28.0, 30.0, 31.2, 36.1, 39.4, 39.8; (CH) 27.9, 35.6, 37.9, 45.3, 55.4, 55.6, 70.3, 70.4, 71.7; (C) 37.6, 43.4, 63.3, 170.0, 170.7.

5–Methoxy–5α–cholestane–3β,6β,7β-triol 3-acetate (5-37)

Afforded as described in table 5-2, white sold. M.p.171 °C; IR: ν_{max} 3500-3300, 2947, 2871, 1706, 1628, 1471, 1380, 1264, 1074 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.85 (m, 1H, H-3), 3.81 (dd, 1H, H-6), 3.60 (m, 1H, H-7), 3.24 (s, 3H, 5-OCH_3), 1.16 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3) 12.1, 17.7, 18.7, 21.3, 22.4, 22.7, 48.6; (CH_2) 20.0, 23.8, 26.5, 27.0, 28.0, 30.0, 31.7, 36.1, 39.4, 39.8; (CH) 27.9, 35.6, 38.1, 43.2, 54.9, 55.2, 70.6, 71.8, 72.8; (C) 38.7, 43.6, 79.4, 170.8.

5α–Cholestane–3β,4β,5,6β,7β-pentol (5-43)

Compound 5-41 and 5-42 were hydrolysed to afford the title compound as white solid. M.p.234-236 °C (sublime at 212°C); IR: ν_{max} 3500-3300, 2946, 2865, 1625, 1462, 1387, 1284, 1178, 1139 and 1039 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 5.17 (d, 1H, OH-6), 5.03 (s, 1H, OH-7), 4.12 (d, J=7.2 Hz, 1H, OH-3), 4.08 (s, 1H, OH-4), 3.80 (m, 1H,

H-3), 3.67 (br s, 1H, H-4), 3.60 (br, s, 1H, H-6), 3.50 (s, 1H, OH-5), 3.46 (dd, J= 9.8 and 3.7 Hz, 1H, H-7), 1.24 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d_6): δ_C (CH_3): 12.0, 15.7, 18.7, 22.4, 22.7; (CH_2): 20.8, 23.6, 26.3, 26.9, 28.6, 32.4, 35.9, 38.8, 40.1, (CH): 27.5, 35.5, 38.2, 43.9, 55.1, 55.5, 66.9, 71.9, 78.9, 79.2; (C): 37.0, 43.0, 73.0; MS (ES^-): m/z 451 (M-1, 100).

5α–Cholestane–3β,4β,5,6β,7β-pentol 3,4,6,7-tetracetate

Compound **5-41** and **5-42** were converted to the title compound by general acetylation method D3. M.p.212-214 °C; IR: ν_{max} 3500-3300, 2950, 2875, 2846, 1753, 1724, 1631, 1455, 1373, 1265, 1234 and 1037 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.39 (m, 1H, H-3), 5.15-5.24 (m, 3H, H-4, H6 &H-7), 1.36 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 15.5, 18.7, 20.8*, 20.9, 21.2, 22.5, 22.7; (CH_2): 20.4, 22.4, 23.6, 25.6, 28.5, 32.2, 36.0, 39.4, 39.6, (CH): 27.9, 35.3, 35.5, 44.8, 54.2, 55.0, 69.4, 73.2, 74.1, 74.4; (C): 38.2, 43.4, 73.7, 169.3*, 170.7, 171.1; MS (ES^+): m/z 638 ($M+NH_4^+$, 100).

5α–Cholestane–3β,4β,5,6β,7β-pentol 4-benzoate-6,7-acetate (5-45)

Compound 5-20 treating with $HClO_4$ in acetonitrile (general procedure B3) at room temperature for 1hr give the title compound as colourless foam. Yield 92%; IR: ν_{max} 3500-3300, 2954, 2867, 1745, 1652, 1454, 1371, 1274, 1117, 1035 and 707 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.38-8.05 (m, 5H, aryl H), 5.51 (d, 1H, H-4), 5.36 (d, J=3.8 Hz, 1H, H-6), 5.22 (dd, J=10.6 and 3.8 Hz, 1H, H-7), 4.43 (m, 1H, H-3), 1.42 (s, 3H, CH_3-19), 0.69 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.8, 18.7, 20.5, 21.2, 21.3, 22.5, 22.7; (CH_2): 23.6, 25.7, 25.9, 28.0, 28.4, 30.7, 36.4, 39.4, 39.7, (CH): 27.8, 35.5, 36.0, 44.6, 54.1, 54.9, 66.8, 73.6, 74.4, 77.7, 128.0, 128.2, 132.9; (C): 37.9, 43.3, 74.0, 129.8, 166.4, 170.1, 171.0; MS (ES^+): m/z 658 ($M+NH_4^+$, 100).

5β–Cholestane–3β,4α,5,6β,7β-pentol 3,4,6,7-tetracetate

Compound **5-44** was acetylated to afford the title tetracetate. M.p. 124-126 °C; HRMS(ES^+): m/z 638.4266 ($M+NH_4^+$, C35H60NO9 requires 638.4268); IR: ν_{max} 3500-3300, 2950, 2867, 1739, 1630, 1462, 1436, 1371, 1255, 1076 and 1031 cm^{-1}; ^1H

NMR ($CDCl_3$): δ_H 5.10 (dd, J=10.7 and 4.0 Hz, 1H, H-7), 5.00 (d J=4.0 Hz, 1H, H-6), 4.90 (s, 1H, H-4), 4.85 (br s, 1H, H-3), 2.23, 2.10, 2.06, 1.90 (all s, 12H, the acetyl CH_3), 1.11 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.7*, 20.8, 21.1, 21.2*, 22.4, 22.7; (CH_2): 20.4, 23.6*, 25.7, 28.4*, 36.0, 39.4, 39.7, (CH): 27.8, 34.3, 35.4, 39.3, 55.0, 55.4, 70.0, 73.1, 73.9*, (C): 39.3, 43.2, 77.0, 168.5, 168.8, 170.3, 170.4; MS (ES^+): m/z 638 ($M+NH_4^+$, 100).

5β–Cholestane–3β,4α,5,6β,7β-pentol 3-benzoate-6,7-acetate (5-46)

Compound 5-20 treating with $BF_3{\cdot}Et_2O$ in acetonitrile (general procedure B5) at room temperature for 30 min give the title compound as colourless foam. Yield after chromatography (DCM/acetone 15/1) 83%; IR: ν_{max} 3500-3300, 2952, 2865, 1721, 1631, 1453, 1374, 1283, 1176, 1121, 1065, 1022 and 717 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.32-8.03 (m, 5H, aryl H), 5.43 (m, 1H, H-7), 5.35 (m, 1H, H-6), 5.04 (br s, 1H, H-3), 3.84 (s, 1H, H-4), 1.06 (s, 3H, CH_3-19), 0.70 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 18.7, 20.4, 21.5*, 22.5, 22.8; (CH_2): 20.8, 23.7, 26.1, 26.5, 28.6, 36.0, 39.4, 39.7, (CH): 27.9, 34.6, 35.6, 39.0, 55.0, 55.2, 72.0, 73.7, 75.8*, 128.1, 128.9, 132.5; (C): 38.8, 43.2, 74.7, 130.8, 165.5, 171.1, 173.5; MS (ES^+): m/z 658 ($M+NH_4^+$, 100).

5β–Cholestane–3β,4α,5,6β,7β-pentol (5-47)

Compound was hydrolysed by general method to yield the free pentol as white solid precipitated from DCM / Hexane. M.p.172-174 °C (sublime at 150°C); IR: ν_{max} 3500-3300, 2954, 2933, 2865, 1625, 1462, 1438, 1380, 1076 and 1041 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.84 (d, 1H, H-3), 3.66 (m, 1H, H-7), 3.54 (m, 2H, H-4 & H-6), 0.96 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^-): m/z 451 (M-1, 100).

3β–(toluene-4-sulfonyl)–5α–cholestane–4β,5,6β–triol (6.1-3)

M.p.132-134 °C; IR: ν_{max} 3500-3300, 2944, 2865, 1596, 1467,1361, 1172 and 940 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.78(d, 2H, aryl H), 7.34(d, 2H, aryl H), 4.88-4.97 (m, 1H, H-3), 4.03 (d, J=4.8Hz, 1H, H-4), 3.78 (s, 1H, H-6), 2.43(s, 3H, aryl-CH_3), 1.37 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 15.3, 18.6, 21.6,

22.5, 22.7; (CH_2): 21.6, 22.8, 23.8, 24.0, 28.1, 32.3, 33.9, 36.3, 39.4, 39.8, (CH): 27.9, 30.1, 35.7, 46.3, 55.8, 56.1, 77.0, 78.0, 81.0, 127.7, 129.8; (C): 37.8, 42.6, 73.7, 133.7, 144.8; MS (ES^+): m/z 613 ($M+Na^+$, 100).

5,6β–Dihydroxy–5α–cholestan-4-one (6.1-4)

M.p. 149-152°C; IR: ν_{max} 3500-3300, 2946, 2857, 1704,1469, 1387 and 1014 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.93 (s, 1H, H-6), 3.72 (s, 1H, OH), 3.00-3.10(m, 1H, 3α-H), 2.53(s, 1H, OH), 1.00 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.9, 18.5, 22.5, 22.7; (CH_2): 21.1, 21.7, 23.8, 23.9, 28.1, 31.5, 32.7, 36.0, 36.8, 39.4, 39.9, (CH): 27.9, 29.6, 35.7, 45.3, 55.7, 56.2, 70.7; (C): 42.5, 43.6, 78.2. MS (ES^+): m/z 441 ($M+Na^+$, 100), 419(95).

3β–(Toluene-4-sulfonyl)–5α–cholestane–4β,5,6β–triol 4,6 diacetate (6.1-5)

Foam. IR: ν_{max} 3500-3300, 2944, 2865, 1747, 1467,1375, 1255 and 863 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.73(d, 2H, aryl H), 7.30(d, 2H, aryl H), 5.03 (s, 1H, H-4), 4.95-5.04 (m, 1H, H-3), 4.74 (s, 1H, H-6), 2.40(s, 3H, aryl-CH_3), 2.04, 1.95 (both s, 6H, acetyl CH_3), 1.27 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.5, 18.5, 21.1, 21.3, 21.5, 22.5, 22.7; (CH_2): 20.2, 23.7, 23.8, 24.0, 28.0, 31.6, 31.9, 36.0, 39.4, 39.6; (CH): 27.9, 30.3, 35.6, 45.9, 55.4, 55.9, 74.7, 76.4, 77.9, 124.0, 127.6, 129.6, 148.9; (C): 38.4, 42.5, 73.5, 133.6, 144.4, 169.4, 169.6; MS (ES^+): m/z 754(100), 692 ($M+NH_4^+$, 40).

3α,5–Epoxy–5α–cholestane–4β,6β–diol, 4,6-diacetate (6.1-6)

Foam. IR: ν_{max} 2948, 2865, 1749, 1635, 1462, 1377, 1243, 1101, 1058 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.22 (d, J=5.9Hz, 1H, H-4), 5.01 (m, 1H, H-6), 4.64 (m, 1H, H-3), 2.05, 2.02 (both s, 6H, acetyl CH_3), 1.08 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); MS (ES^+): m/z 503 ($M+H^+$, 78), 443 (100).

3β–(Toluene-4-sulfonyl)–5α–cholestane–4β,5,6β–triol 4,5,6 triacetate (6.1-7)

Afforded as yellow foam by treating 6.1-3 or 6.1-5 with general procedure D4. IR: ν_{max} 2952, 2865, 1755, 1467,1369, 1245 and 1224 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.728-7.80(m, 4H, aryl H), 6.02 (d, J=3.0Hz, 1H, H-4), 5.82 (m, 1H, H-6), 4.31-4.38 (m, 1H,

H-3), 2.43(s, 3H, aryl-CH_3), 2.00, 1.94, 1.86 (all s, 9H, acetyl CH_3), 1.35 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.1, 18.5, 21.0, 21.2, 21.6, 21.8, 22.5, 22.7; (CH_2): 20.3, 23.6*, 23.9, 28.0, 31.6, 32.2, 36.0, 39.4, 39.6; (CH): 27.9, 29.8, 35.6, 46.5, 55.7, 55.9, 68.1, 69.7. 77.0, 126.9, 127.7, 129.8, 130.2; (C): 39.4, 42.5, 84.8, 132.8, 145.1, 167.8, 168.6, 168.7; MS (ES^+): m/z 735 ($M+H_3O^+$, 100).

5α–Cholest-2-ene–4β,5,6β–triol triacetate (6.1-8)

Foam. IR: ν_{max} 2950, 2937, 2871, 1751, 1635, 1462, 1436, 1371, 1261, 1222, 1043 and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.34 (m, 1H, H-6), 6.04 (m, 1H, H-4), 5.80 (m, 1H, H-2), 5.65 (m, 1H, H-3), 2.04(s, 3H, acetyl CH_3), 1.95 (s, 6H, acetyl CH_3), 1.26 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.3, 18.6, 21.0, 21.6, 22.1, 22.5, 22.7; (CH_2): 20.7, 23.6, 23.9, 28.0, 32.5, 36.0, 38.1, 39.4, 39.8; (CH): 27.9, 30.0, 35.6, 47.8, 55.6, 56.0, 67.3, 67.8. 121.6, 129.9; (C): 38.8, 42.6, 83.9, 168.9, 169.1, 169.4; MS (ES^+): m/z 562 ($M+H_3O^+$, 100).

5α-Cholestane-3α,4β,5,6β-tetrol 3,4,6-triacetate (6.1-9)

Afforded as described in **Scheme 6.1-4** by general procedure E2. M.p. 137-139°C; HRMS(ES^+): m/z 580.4210 ($M+NH_4^+$, C33H58NO7 requires 580.4213); IR: ν_{max} 3500-3300, 2952, 2865, 1741, 1465, 1382, 1249 and 1032; ^1H NMR ($CDCl_3$): δ_H 4.86 (m, 3H, H-3, H-4 & H-6), 3.22 (s, 1H, OH-5), 1.29 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.9, 18.5, 21.0*, 21.4, 22.4, 22.7; (CH_2): 20.0, 22.1, 23.7, 23.9, 28.1, 28.3, 31.5, 36.0, 39.3, 39.7; (CH): 27.8, 30.3, 35.7, 46.2, 55.5, 56.1, 71.4, 74.0. 75.2; (C): 38.9, 42.5, 72.2, 168.2, 168.6, 169.3; MS (ES^+): m/z 580 ($M+NH_4^+$, 100).

5α-Cholestane-3α,4β,5,6β-tetrol 5-acetate (6.1-10a)

Afforded as gum. IR: ν_{max} 3500-3300, 2949, 2854, 1738, 1635, 1465, 1382, 1242, 1045, 1020 and 972 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.11 (s, 1H, H-6), 5.03 (d, 1H, H-4), 3.71 (m, 1H, H-3), 2.00 (s, 3H, acetyl CH_3), 1.45 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 17.1, 18.6, 22.3, 22.4, 22.7; (CH_2): 20.4,

23.6, 23.9, 24.9, 28.1, 32.0, 34.1, 36.0, 39.4, 39.9; (CH): 27.9, 29.6, 35.6, 46.5, 56.1, 56.2, 68.1, 69.1. 71.6; (C): 39.3, 42.6, 86.8, 169.8; MS (ES^+): m/z 496 ($M+NH_4^+$, 100).

Cholest-5-en-4-one (6.1-12)

M.p. 108-110°C; IR: ν_{max} 3500-3300, 2944, 2865, 1691, 1615, 1473, 1371 1266, 1002 and 638 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.39 (m, 1H, H-6), 0.92 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.6, 21.2, 22.5, 22.7; (CH_2): 19.2, 21.1, 23.7, 24.0, 28.0, 31.6, 36.0*, 39.4, 39.6, 40.0, (CH): 27.9, 30.9, 35.7, 49.1, 56.0, 56.5, 132.4; (C): 38.5, 42.2, 145.3, 203.1; MS (ES^+): m/z 597($M+Na^+$, 100), 399(36), 385(38).

6β–hydroxy-5α-4-norcholestan-3-aldehyde (6.1-13)

Afforded as gum. IR: ν_{max} 2945, 2865, 1722, 1463,1378 and 1043 cm^{-1}. ^1H NMR ($CDCl_3$): δ_H 9.60 (d, J=3.6Hz, 1H, CHO), 4.06 (d, J=2.6Hz, 1H, H-6), 2.88-3.00 (m, 1H, H-3), 0.97 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 16.8, 18.6, 22.4, 22.7; (CH_2): 22.2, 23.3, 23.7, 24.3, 28.1, 36.1, 39.4*, 39.7*, (CH): 27.9, 31.0, 35.6, 49.0, 54.3, 54.8, 55.7, 56.1, 66.4, 205.3; (C): 42.9, 44.4.

5α–Cholest-2-ene–4α,5,6β–triol (6.1-15)

M.p. 144-145°C; IR: ν_{max} 3500-3300, 2933, 2863, 1625, 1463, 1387, 1073 and 999 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.72 (m, 1H, H-2), 5.52 (d, 1H, H-3), 4.56 (s, 1H, H-4), 3.95 (s, 1H, H-6), 1.06 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); MS (ES^+): m/z 436 ($M+NH_4^+$, 100), 441(90), 236(53), 214(55).

5α–Cholest-2-en–4α,6β–diol (6.1-17)

M.p. 153-156°C; IR: ν_{max} 3500-3300, 2933, 2860, 1654, 1469, 1385 1053, 1024 and 704 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.64 (s, 2H, H-2 & H-3), 4.32(d, 1H, H-4), 4.24 (m, 1H, H-6), 0.95 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.5, 18.6, 22.5, 22.7; (CH_2): 20.4, 23.8, 24.1, 28.1, 36.1, 39.0, 39.4*, 39.8, 41.3, (CH): 27.9, 29.7, 35.7, 52.1, 53.8, 56.2, 64.6, 65.8, 127.4, 129.3; (C): 36.3, 42.4; MS (ES^+): m/z 420 ($M+NH_4^+$, 100).

5α–Cholest-2-ene–4α,5,6β–triol 4,6-diacetate (6.1-19)

Foam. IR: ν_{max} 3477, 2948, 2865, 1739, 1465, 1382, 1239and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.80 (m, 1H, H-2), 5.52 (d, 1H, H-4), 5.35 (d, 1H, H-3), 4.88 (m, 1H, H-6), 2.05(s, 3H, acetyl CH_3), 2.02 (s, 6H, acetyl CH_3), 1.07 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 14.7, 18.5, 20.9, 21.2, 22.4, 22.7; (CH_2): 20.1, 23.7, 24.0, 28.0, 31.6, 36.0, 36.7, 39.4, 39.6, (CH): 27.9, 30.1, 35.7, 44.8, 55.5, 56.1, 69.6, 69.8, 122.4, 130.1; (C): 39.9, 42.3, 73.0, 169.9, 170.1; MS (ES^+): m/z 520 ($M+NH_4^+$, 60), 443 (100).

5α-Cholestane-3α,4α,5,6β-tetrol 4,6-diacetate (6.1-20)

M.p. 120-122°C; IR: ν_{max} 3500-3300, 2942, 2856, 1733, 1709, 1465, 1373, 1280, 1236 and 1035 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.16 (d, J=3.4 Hz, 1H, H-4), 4.98 (br s, 1H, H-6), 4.25 (d, 1H, OH), 4.14 (m, 1H, H-3), 2.09 (s, 3H, acetyl CH_3), 1.98 (s, 3H, acetyl CH_3), 1.20 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 15.8, 18.5, 20.8, 21.2, 22.4, 22.7; (CH_2): 20.5, 23.7, 23.9, 27.1, 27.2, 28.1, 30.6, 36.0, 39.4, 39.6, (CH): 27.9, 30.1, 35.6, 44.5, 55.5, 55.9, 69.2, 69.4, 70.0; (C): 41.1, 42.4, 76.8, 170.0, 170.7; MS (ES^+): m/z 543 ($M+Na^+$, 13), 443(100), 214(89).

3β–(Toluene-4-sulfonyl)–5β–cholestane–5,6β–diol (6.1-21)

Foam. IR: ν_{max} 3500-3300, 2944, 2865, 1467,1363, 1174, 894, 684 and 553 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.76(d, 2H, aryl H), 7.32(d, 2H, aryl H), 4.87 (s, 1H, H-3), 3.45 (s, 1H, H-6), 2.43(s, 3H, aryl-CH_3), 1.06 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 18.5, 18.6, 21.5, 22.5, 22.7; (CH_2): 21.1, 23.7, 24.0, 24.8, 25.7, 28.1, 34.3, 36.0, 39.3*, 39.7, (CH): 27.9, 29.6, 35.6, 43.0, 56.1, 56.3, 74.9, 79.8, 127.6, 129.8; (C): 39.7, 42.4, 74.9, 133.8, 144.8; MS (ES^+): m/z 597($M+Na^+$, 100) , 399(36), 385(38).

Cholest-4-en-6-one (6.1-22)

M.p. 108-109°C; IR: ν_{max} 3500-3300, 2944, 2865, 1691, 1615, 1473, 1371 1266, 1002 and 638 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.36 (m, 1H, H-4), 2.50 (d, J=12.0Hz, 1H, H-3a), 0.94 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.5,

20.1, 22.4, 22.7; (CH_2): 17.7, 21.1, 23.7, 23.8, 25.3, 27.9, 35.3, 36.0, 39.3*, 45.8, (CH): 27.8, 33.5, 35.6, 50.9, 55.9, 56.6, 132.2; (C): 37.5, 42.4, 145.7, 202.8; MS (ES^+): m/z 597($M+Na^+$, 100), 399(36), 385(38).

5β–cholest-2-ene–5β,6β–diol (6.1-23)

Foam. IR: ν_{max} 3500-3300, 2933, 2870, 1469, 1446,1384, 1047 and 665 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.48 (m, 2H, H-2&H-3), 3.60 (s, 1H, H-6), 1.03 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18).

3α–(Toluene-4-sulfonyl)–5β–cholestane–4α,5,6β–triol 4,6-diacetate (6.1-24)

Afforded as yellow foam. IR: ν_{max} 3480, 2948, 2865, 1756, 1463,1377, 1222, 1180, 1027, 867 and 555 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 7.75(d, 2H, aryl H), 7.32(d, 2H, aryl H), 4.90 (d, J=3.1Hz, 1H, H-4), 4.68-4.77 (m, 1H, H-3), 4.70 (s, 1H, H-6), 2.44(s, 3H, aryl-CH_3), 2.10, 2.04 (both s, 6H, acetyl CH_3), 0.92 (s, 3H, CH_3-19), 0.62 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 17.9, 18.5, 21.2, 21.4, 21.6, 22.5, 22.7; (CH_2): 20.9, 23.7, 24.1, 28.1, 29.8, 33.9, 36.0, 39.4*, 39.6, (CH): 27.9, 29.6, 35.6, 40.3, 55.9, 56.7, 74.3, 75.6, 78.0, 127.7, 129.6; (C): 39.6, 42.4, 75.2, 133.5, 144.6, 168.9, 171.1; MS (ES^+): m/z 697 ($M+Na^+$, 100).

5,6β–Dihydroxy–5β–cholestan-4-one (6.1-25)

M.p. 146-149°C; IR: ν_{max} 3500-3300, 2949, 2865, 1708,1463, 1385 and 1044 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.19 (dd, J1=4.4Hz, J2=4.0Hz, 1H, H-6), 2.70-2.78 and 2.27-2.34 (both m, 2H, H-3), 1.01 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18);

3β,5–Epoxy–5β–cholestane–4α,6β–diol, 4,6-diacetate (6.1-27)

Foam. IR: ν_{max} 2948, 2865, 1754, 1463,1377and 1228 cm^{-1}. ^1H NMR ($CDCl_3$): δ_H 5.26 (d, J=5.8Hz, 1H, H-4), 5.13 (m, 1H, H-6), 4.64 (dd, J=5.8Hz, 1H, H-3), 2.10, 2.07 (both s, 6H, acetyl CH_3), 1.23 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 17.0, 18.5, 20.9, 21.2, 22.4, 22.7; (CH_2): 23.6*, 24.0, 24.4, 28.0, 32.9, 33.7, 36.0, 39.4, 39.9; (CH): 27.9, 29.8, 35.6, 51.1, 55.7, 55.9, 71.2, 75.1, 81.7; (C): 38.9, 42.7, 91.4, 169.5, 169.9; MS (ES^+): m/z 525 ($M+Na^+$, 100).

Cholest-2,5-dien-4-one (6.1-30)

M.p. 100-101°C (Lit 97-99°C, Hanna and Kodeih 1981); IR: ν_{max} 3500-3300, 2942, 2865, 1668, 1619, 1607, 1463,1387, 1265 and 796 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.82-6.90 (m, 1H, H-2), 6.77 (dd, J1=5.2Hz, J2=2.6Hz, 1H, H-6), 6.06-6.11 (dd, J1=10.2Hz, J2=2.7Hz, 1H, H-3), 1.10 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 18.6, 21.3, 22.5, 22.7; (CH_2): 20.9, 23.7, 24.0, 28.1, 31.7, 36.0, 39.1, 39.4*, (CH): 27.9, 31.0, 35.6, 49.2, 56.0, 56.4, 129.0, 134.5, 147.5; (C): 38.4, 42.2, 141.5, 188.1.

Cholest-2,4-dien-6-one (6.1-31)

M.p. 124-126°C (Lit 127-130°C, Jagodzinski et al 1981); IR: ν_{max} 3500-3300, 2942, 2865, 1674, 1623, 1554, 1463,1387, 1269 and 698 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.80 (m, 1H, H-2), 6.04 (m, 2H, H-3 &H-4), 0.98 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.7, 17.7, 18.6, 22.4, 22.7; (CH_2): 21.1, 23.7, 23.9, 28.0, 35.8, 37.7, 39.3, 39.4, 45.4, (CH): 27.9, 32.7, 35.6, 50.2, 55.9, 56.4, 123.0, 128.3, 132.7; (C): 35.8, 42.3, 140.5, 199.9.

5α-Cholestane-2α,3α,5,6β-tetrol (6.2.1-3)

Cholest-2-ene-5α,6β-diol (1.0g, 2.5mmol), $K_3Fe(CN_6)$ (8.0g, 23.0mmol) K_2CO_3(3.4g, 24.0mmol) were stirred in t-butanol (50ml) and water (50ml), to this mixture a freshly prepared solution of OsO_4 in t-butanol (1M, 2ml) and DABCO (280mg, 2.5mmol) were added and the mixture was stirred at 40°C for 24hr. When complete conversion of the starting material to the tetrol was confirmed by TLC analysis, the reaction was quenched by adding sodium sulfite (10.0g) and stirring at room temperature for 30min. the organic layer was extracted with ether and the ether layer was washed with water, dried with magnesium sulfate and evaporated to give a solid which was purified by silica gel column with ether:acetone (15:1) to give the title compound as a colorless solid , yield 0.55g (53%). M.p. 244-245°C; HRMS (ES^+): m/z 454.3899 ($M+NH_4^+$, $C_{27}H_{52}NO_4$ requires 454.3896); IR: ν_{max}: 3500-3300, 2932, 2864, 1469, 1378, 1062 1024, and 854; ^1H NMR (DMSO-d_6): δ_H 3.88 (m, 1H, H-3), 3.73 (m, 1H, H-2), 3.32

(s, 1H, H-6), 0.97 (s, 3H, CH_3-19), 0.60 (s, 3H, CH_3-18); MS (ES^+): m/z 459 ($M+Na^+$, 81), 214 (100).

2α,3α,5-Trihydroxy-5α-cholestan-6-one (6.2.1-4)

5α-Hydroxycholest-2-en-6-one (1.0g, 2.38mmol) was dissolved in THF (20ml), this solution was cooled to –60°C with stirring. $KMnO_4$ (0.7g, 9.04mmol) was added and the solution was stirred for 2hr at -60°C. The excess $KMnO_4$ was destroyed by adding aqueous H_2O_2, the solution was poured into ice water and the solid was collected by filtration and purified by silica gel chromatography using ether as elution phase. Afforded as white solid. Yield 0.35g(33%). M.p. 190-193°C; IR: ν_{max}: 3500-3300, 2954, 2865, 1710, 1469, 1384, 1238 and 1041 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 5.60 (s, 1H, -OH), 5.43 (s, 1H, -OH), 4.53 (brs, 1H, -OH), 3.92(s, 1H, H-3), 3.60(m, 1H, H-2), 2.56 (t, J=12.4Hz, 1H, 4β-H), 0.67 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); MS (ES^+): m/z 457 ($M+Na^+$, 90), 214 (100).

5α–Cholestane–2α,5,6β–triol (6.2.1-6)

Cholest-2-ene-5α,6β-diol (13.0g, 32.3mmol) was dissolved in dry THF (140ml) $NaBH_4$ (6.0g, 158mmol) was added and the mixture was stirred in an ice water bath under Argon atmosphere. Boron trifluride etherate (35ml, 285mmol) was added dropwise over a period of 1hr. The resulting mixture was stirred at room temperature for 4hr. Water (5ml) was added slowly into the reaction mixture to destroy the excess borane, followed with aqueous sodium hydroxide solution (10%, 150ml). Hydrogen peroxide (60% 75ml) was added dropwise with ice cooling and constant stirring, the mixture was stirred at room temperature for 1hr. ether was added and the organic layer was washed with 10% $NaHSO_3$, brine, dried over magnesium sulfate. Evaporation gave an oil which on chromatography (ether/acetone 40:1) gave Cholestan-3β,5α,6β-triol (CT) 5.8g (42.7%)and .the title compound 4.9g (36%). M.p.215-217 °C; IR: ν_{max}: 3500-3300, 2949, 2883, 1469, 1383, 1035 and 1952 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 4.34 (d, 1H, OH-6), 4.14 (d, 1H, OH-2), 3.60 (m, 1H, H-2), 3.55 (s, 1H, OH-5), 3.33 (m, 1H, H-6), 0.97 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^1H

NMR ($CDCl_3$): δ_H 3.89 (m, 1H, H-2), 3.57 (s, 1H, H-6), 1.12 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); MS (ES^+): m/z 443 ($M+Na^+$, 46), 385 (100).

2,6-Diacetate:

M.p. 168-170°C; IR: ν_{max}: 3500-3300, 2941, 2868, 1733, 1710, 1469, 1373, 1248 and 1028 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.95 (m, 1H, H-2), 4.72 (s, 1H, H-6), 1.13 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.9, 18.5, 21.3*, 22.4, 22.7; (CH_2): 20.7, 23.7, 24.0, 25.6, 28.1, 29.9, 31.4, 36.0, 37.9, 39.3, 39.7, (CH): 27.8, 29.9, 35.7, 45.0, 55.6, 56.0, 70.2, 75.6; (C): 40.0, 42.5, 71.9, 170.3, 170.6; MS (ES^+): m/z 527 ($M+Na^+$, 56), 258 (100).

5–Hydroxy–5α–cholestane–2,6–dione (6.2.1-7)

Cholestane-2α,5α,6β-triol (0.3g, 0.72mmol) was stirred in acetic acid (20ml) and water (4ml) cooled with an ice water bath. CrO3 (0.3g, 3.0mmol) was added and the mixture was stirred at this temperature for 25min. 20% Aqueous Na_2SO_3 solution was added to quench the reaction and the colourless solution was poured into ice water, filtered, the solid was collected by filtration to give the product, yield 260mg (87%). M.p. 218-220°C (202-204°C) (Kocovsky and Cerny 1977(353)) IR: ν_{max}: 3500-3300, 2944, 2871, 1726, 1699, 1469, 1377, 1294 and 970 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 0.70 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 15.5, 18.5, 22.5, 22.7; (CH_2): 20.9, 23.7, 23.8, 27.9, 28.2, 36.0, 36.1, 39.2, 39.4, 42.2, 47.1; (CH): 27.9, 35.6, 37.2, 44.1, 56.0, 56.2, 77.8; (C): 42.8, 46.9, 211.2, 212.1; MS (ES^+): m/z 434 ($M+NH_4^+$, 20), 214 (100).

5α–Cholestane–2β,5,6β–triol (6.2.1-8)

5α-Hydroxycholestan-2,6-dione (6.2.1-7) was reduced by $NaBH_4$ using the general method to give the 2α-isomer (yield 61%) and 2β-isomer (yield 39%). M.p. 205-207°C; IR: ν_{max}: 3500-3300, 2932, 2865, 1465, 1384 and 995 cm^{-1}; ^1H NMR (DMSO-d_6): δ_H 4.30 (d, 1H, OH-6), 3.98 (d, 1H, OH-2), 3.84 (s, 1H, H-2), 3.45 (s, 1H, OH-5), 3.29 (d, 1H, H-6), 1.19 (s, 3H, CH_3-19), 0.61 (s, 3H, CH_3-18); ^1H NMR ($CDCl_3$): δ_H 4.14 (br s, 1H, H-2), 3.52 (s, 1H, H-6), 1.35 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18);

^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.8, 15.5, 18.5, 22.5, 22.7; (CH_2): 20.9, 23.7, 23.8, 27.9, 28.2, 36.0, 36.1, 39.2, 39.4, 42.2, 47.1; (CH): 27.9, 35.6, 37.2, 44.1, 56.0, 56.2, 77.8; (C): 42.8, 46.9, 211.2, 212.1; MS (ES^+): m/z 443 ($M+Na^+$, 24), 420 (100).

5α–Cholest-2-ene–5α,6α–diol (6.2.1-9)

M.p. 168-170°C (Lit. 167-168°C, Kocovsky and Cerny 1977, 155); IR: ν_{max}: 3500-3300, 3018, 2931, 2870, 1635, 1462, 1377, 1051, 987 and 665 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 5.69, 5.61 (m, 2H, H-2 & H-3), 3.58 (m, 1H, H-6), 0.84 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); MS (ES^+): m/z 420 ($M+NH_4^+$, 90), 367 (100).

2α,3α-Epoxy-5α-cholestane-5,6β-diol (6.2.1-10)

M.p. 108-110°C; IR: ν_{max}: 3500-3300, 2931, 2862, 1469, 1385, 1035 and 805 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 3.55 (s, 1H, H-6), 3.43 (s, 1H, H-2), 3.28 (dd, 1H, H-3), 1.07 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); MS (ES^+): m/z 441 ($M+Na^+$, 29), 365 (100).

2α,3α–epoxy–5α–Cholestane–4α,5,6β–triol (6.2.2-1)

M.p. 177-178 °C; IR: ν_{max} 3500-3300, 2937, 2856, 1469 and 1008 cm^{-1}; ^{1}H NMR ($CDCl_3 + D_2O$): δ_H 4.39 (d, 1H, H-4), 3.94 (br s, 1H, H-6), 3.52 (m, 1H, H-2), 3.44 (m, 1H, H-3), 1.08 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.1, 18.6, 22.5, 22.7; (CH_2): 20.4, 23.8, 24.1, 28.1, 34.3, 34.5, 36.0, 39.4, 39.6; (CH): 27.9, 29.7, 35.7, 45.8, 54.8, 55.5, 56.1, 58.5, 64.5, 67.2; (C): 40.7, 42.3, 77.5. MS (ES^+): m/z 452 ($M+NH_4^+$, 95), 435(100), 399(41), 214(55).

2α,3α–epoxy–5α–Cholestane–4α,6β–diol (6.2.2-2)

M.p. 206-208 °C; IR: ν_{max} 3500-3300, 2941, 2865, 1469, 1385 and 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 4.29 (m, 2H, H-4 & H-6), 3.40 (m, 1H, H-2), 3.30(m, 1H, H-3), 0.96 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.8, 18.6, 22.5, 22.7; (CH_2): 20.6, 23.9, 24.1, 28.1, 36.1, 39.0, 39.2, 39.4, 39.7; (CH): 27.9, 29.4, 35.7, 46.9, 53.7, 54.2, 56.0, 56.2, 64.3, 66.4; (C): 36.7, 42.3; MS (ES^+): m/z 436 ($M+NH_4^+$, 100).

2α,3α–epoxy–5α–Cholestane–4α,5,6β–triol, 4,6-diacetate (6.2.2-3)

Acetylation of the free triol give the title compound as white solid after recrystallised from methanol. M.p. 103-104 °C; IR: ν_{max} 3500-3300, 2944, 2856, 1745, 1240 and 1039 cm^{-1}; ^1H NMR ($CDCl_3 + D_2O$): δ_H 5.48 (d, J=2.1Hz, 1H, H-4), 4.95 (s, 1H, H-6), 3.40 - 3.49 (m, 2H, H-2 & H-3), 2.11, 2.01 (both s, 6H, acetyl CH_3), 1.12 (s, 3H, CH_3-19), 0.64 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 15.9, 18.5, 20.8, 21.3, 22.5, 22.7; (CH_2): 20.3, 23.7, 24.0, 28.0, 30.9, 34.2, 36.0, 39.4, 39.5; (CH): 27.9, 30.1, 35.7, 45.3, 53.7, 55.2, 55.3, 55.9, 66.6, 69.4; (C): 41.4, 42.2, 74.8, 169.6, 171.1; MS (ES^+): m/z 541 ($M+Na^+$, 100).

2β,3β–epoxy–5α–Cholestane–4α,5,6β–triol, 4,6-diac (6.2.2-4)

Afforded as colourless gum. ^1H NMR ($CDCl_3$): δ_H 5.27 (s, 1H, H-4), 4.83 (m, 1H, H-6), 3.27 (br s, 1H, H-2), 2.95 (d, J=3.9Hz, 1H, H-3), 2.10, 2.00 (both s, 6H, acetyl CH_3), 1.09 (s, 3H, CH_3-19), 0.63 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.9, 18.5, 20.7, 21.2, 22.5, 22.7; (CH_2): 20.1, 23.7, 24.0, 28.0, 31.5, 34.0, 36.0, 39.4, 39.6; (CH): 27.9, 29.6, 35.7, 46.4, 53.2, 53.8, 55.5, 56.0, 67.6, 69.7; (C): 37.2, 42.3, 73.5; MS (ES^+): m/z 541 ($M+Na^+$, 100).

4α,5,6β-Triacetoxy-5α-cholest-2-ene (6.2.2-5)

The 5α–Cholest-2-ene–4α,5,6β–triol 4,6-diacetate (6.1-19) was treated with general procedure D4 to give the title compound as oil, yield 95%. IR: ν_{max} 2954, 2867, 1753,1469, 1371, 1245 and 1018 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.48 (s, 1H, H-6), 5.80 (m, 1H, H-2), 5.72 (s, 1H, H-4), 5.30 (d, 1H, H-3), 2.05, 2.01, 1.98 (all s, 9H, acetyl CH_3), 1.14 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.6, 18.5, 20.6, 21.2, 22.0, 22.4, 22.7; (CH_2): 20.3, 23.6, 23.8, 28.0, 31.6, 36.0, 37.4, 39.3, 39.6; (CH): 27.8, 29.6, 35.6, 46.1, 55.7, 55.9, 65.5, 68.7, 122.7, 128.8; (C): 41.8, 42.3, 85.9, 169.2, 169.3, 170.5.

2α,3α–Epoxy–5α–Cholestane–4β,5,6β–triol, 4,5,6-triacetate (6.2.2-7)

Afforded as white solid. M.p. 69-71 °C; IR: ν_{max} 3500-3300, 2952, 2871, 1753,1469, 1364, 1251, 1228, 1053 and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.34 (s, 1H, H-4), 6.16 (br s, 1H, H-6), 3.16 (m, 1H, H-2), 3.10 (m, 1H, H-3), 2.05(s, 6H, acetyl CH_3), 2.00(s,

3H, acetyl CH_3), 1.26 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.7, 18.5, 20.8, 21.5, 22.1, 22.4, 22.7; (CH_2): 20.5, 23.5, 23.8, 27.9, 31.7, 35.2, 35.9, 39.3, 39.6; (CH): 27.8, 30.0, 35.5, 47.4, 49.4, 52.1, 55.4, 55.9, 65.2, 68.2; (C): 38.9, 42.4, 81.8, 168.7, 168.9, 169.1. MS (ES^+): m/z 578 ($M+NH_4^+$, 100).

2α,3α–Epoxy–5α–Cholestane–4β,5,6β–triol (6.2.2-8)

M.p. 144-147 °C; IR: ν_{max} 3500-3300, 2953, 2867, 1468, 1377, 1243 and 1026 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.40 (s, 1H, H-4), 3.95 (br s, 1H, H-6), 3.50 (m, 1H, H-2), 3.45(m, 1H, H-3), 1.09 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.0, 18.6, 22.5, 22.7; (CH_2): 20.4, 23.8, 24.1, 28.1, 34.2, 34.5, 36.0, 39.4, 39.6; (CH): 27.9, 29.7, 35.7, 45.8, 54.8, 55.5, 56.1, 58.4, 64.5, 67.3; (C): 40.7, 42.3, 77.3; MS (ES^+): m/z 435 ($M+H^+$, 100).

5α-Cholestane-3α,4β,5,6β-tetrol (6.2.2-9)

M.p. 219-220 °C; HRMS(ES^+): m/z 454.3892 ($M+NH_4^+$, C27H52NO4 requires 454.3896); IR: ν_{max} 3480, 2948, 2927, 2865, 1465, 1377 and 1033 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 3.95 (m, 1H, H-3), 3.92 (m, 1H, H-4), 3.84 (br s, 1H, H-6), 1.36 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR (DMSO-d_6): δ_C (CH_3): 11.9, 15.2, 18.5, 22.3, 22.6, (CH_2): 19.6, 23.5, 23.9, 24.4, 27.9, 33.7, 35.8, 38.8, 39.4; (CH): 27.5, 29.9, 35.4, 45.6, 55.7, 55.8, 70.5, 76.0, 77.7, (C): 38.4, 42.2, 71.7, MS (ES^+): m/z 454 ($M+NH_4^+$, 43), 214 (100).

5α-Cholestane-2α,3α,4β,5,6β-pentol 2,4,6-triacetate (6.2.2-11)

Afforded as colourless gum by treating **6.2.2-7** with excess perchloric acid (**Scheme 6.2.2-7**), yield 59%. This compound was further acetylated to 2,3,4,6-tetracetate to make sample for analysis: IR: ν_{max} 3500-3300, 2952, 2871, 1747, 1465, 1374, 1238 and 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 5.27-5.34 (m, 1H, H-2), 5.23 (br s, 1H, H-3), 4.99 (d, J=2.5Hz, 1H, H-4), 4.92 (s, 1H, H-6), 2.12, 2.06, 2.00, 1.96 (all s, 12H, acetyl CH_3), 1.39 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.0, 18.5, 20.7, 20.8, 21.0, 21.4, 22.4, 22.7; (CH_2): 20.1, 23.7, 23.9, 28.0, 31.3, 33.8,

36.0, 39.3, 39.6; (CH): 27.8, 29.7, 35.6, 46.1, 55.3, 56.0, 66.5, 70.1, 74.4, 75.1; (C): 41.0, 42.5, 71.8, 168.1, 168.5, 169.2, 169.9; MS (ES^+): m/z 638 ($M+NH_4^+$, 100).

5α-Cholestane-2β,3α,4β,5,6β-pentol 2-(p-tolenesulfonate)-3,4,5,6-tetracetate (6.2.2-13)

Afforded as light yellow oil. ^1H NMR ($CDCl_3$): δ_H 7.74, 7.71, 7.32, 7.29 (4H, aryl H), 6.13 (br s, 1H, H-6), 5.93 (d, J=4.4 Hz, 1H, H-4), 5.07 (m, 1H, H-3), 4.68 (m, 1H, H-2), 2.41, 2.04, 2.01, 1.90, 1.78 (all s, 15H, CH_3), 1.44 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.2, 18.5, 19.9, 20.4, 20.6, 21.4, 21.5, 22.3, 22.4, 22.7; (CH_2): 20.5, 23.6, 23.8, 28.0, 31.7, 36.0, 39.4, 39.5, 39.6; (CH): 27.9, 29.6, 35.6, 48.6, 55.4, 55.9, 67.5, 70.3, 71.5, 76.2, 127.6, 129.7; (C): 40.3, 42.7, 83.4, 133.8, 144.8, 168.6, 168.7, 168.8, 168.9; MS (ES^+): m/z 790 ($M+NH_4^+$, 100).

5α-Cholestane-2β,3α,4β,5,6β-pentol 2,3,4,5,6-pentacetate (6.2.2-14)

M.p. 106-108 °C; HRMS(ES^+): m/z 680.4374 ($M+NH_4^+$, C37H62NO10 requires 680.4374); IR: ν_{max} 2948, 2865, 1747, 1463, 1368,1228 and 1045 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.17 (br s, 1H, H-6), 6.04 (d, J=4.0 Hz, 1H, H-4), 5.13 (m, 1H, H-3), 4.96 (m, 1H, H-2), 1.43 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 18.5, 19.4, 20.6, 20.7, 21.0, 21.4, 22.3, 22.4, 22.7; (CH_2): 20.5, 23.5, 23.8, 27.9, 31.7, 35.9, 37.4, 39.3, 39.6; (CH): 27.9, 29.5, 35.5, 48.3, 55.4, 55.9, 67.9, 68.6, 69.9, 71.8; (C): 39.9, 42.6, 83.1, 168.5, 168.8, 168.9, 169.0, 169.5; MS (ES^+): m/z 680 ($M+NH_4^+$, 100).

2α,5-Epoxy-5α-cholestane-3α,4β,6β-triol 3,4,6-triacetate (6.2.2-17, 3-acetate of 6.2.2-12)

Afforded as colourless oil. ^1H NMR ($CDCl_3$): δ_H 5.24 (br s, 1H, H-6), 5.09 (s, 1H, H-3), 4.65 (d, J=2.3 Hz, 1H, H-4), 4.29 (d, J=6.8 Hz, 1H, H-2), 1.31 (s, 3H, CH_3-19), 0.66 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 14.7, 18.6, 20.8, 20.9, 21.0, 22.5, 22.7; (CH_2): 20.4, 23.7, 24.0, 28.1, 33.3, 36.0, 39.4, 39.7, 41.8; (CH): 27.9, 30.2, 35.7, 49.6, 55.7, 55.9, 72.7, 81.0, 82.8, 84.7; (C): 42.6, 45.0, 84.5, 168.9, 169.3, 170.7; MS (ES^+): m/z 578 ($M+NH_4^+$, 100).

2α,5-Epoxy-5α-cholestane-3α,4α,6β-triol (6.2.2-18)

M.p. 158-160 °C; IR: ν_{max} 3500-3300, 2950, 2871, 1635, 1458, 1380, 1263, 1086 and 982 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.40 (d, J=6.0 Hz, 1H, H-3), 4.33 (s, 1H, H-6), 4.21 (d, J=6.4 Hz, 1H, H-2), 3.87 (d, J=6.0 Hz, 1H, H-4), 1.08 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.0, 16.4, 18.6, 22.4, 22.7; (CH_2): 21.0, 23.8, 24.0, 28.2, 35.4, 36.0, 39.4, 39.8, 41.1; (CH): 27.9, 30.1, 35.7, 48.9, 56.1*, 65.4, 71.8, 75.1, 82.4; (C): 41.9, 42.6, 89.4; MS (ES^+): m/z 452 ($M+NH_4^+$, 100).

5α-Cholestane-2β,3α,4α,5,6β-pentol (6.2.2-19)

M.p. 215-217 °C; IR: ν_{max} 3500-3300, 2927, 2854, 1631, 1463, 1382, 1257 and 1037 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.41 (d, J=6.0 Hz, 1H, H-3), 4.33 (m, 1H, H-6), 4.20 (d, J=6.4 Hz, 1H, H-2), 3.89 (d, J=6.0 Hz, 1H, H-4), 1.09 (s, 3H, CH_3-19), 0.67 (s, 3H, CH_3-18); MS (ES^+): m/z 475 ($M+NH_4^+$, 5), 214 (100).

2β,3β-Epoxy-5α-cholestane-4β,5,6β-triol (6.2.2-27)

M.p. 125-128 °C; IR: ν_{max} 3500-3300, 2948, 2863, 1469, 1378 and 1015 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 4.20 (s, 1H, H-3), 3.99 (br s, 1H, H-6), 3.36 (m, 2H, H-2 & H-4), 1.28 (s, 3H, CH_3-19), 0.65 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 11.9, 16.2, 18.6, 22.5, 22.7; (CH_2): 20.0, 23.8, 24.0, 28.1, 34.5, 35.5, 36.0, 39.4, 39.6; (CH): 27.9, 30.0, 35.7, 47.1, 52.6, 55.5, 56.1, 56.4, 72.8, 75.9; (C): 37.3, 42.4, 71.0; MS (ES^+): m/z 457 ($M+Na^+$, 100).

4α,6β-Diacetoxy-5-hydroxy-5α-cholest-2-en-1-one (6.2.2-28)

Afforded as a gum. IR: ν_{max} 3500-3300, 2950, 2871, 1747, 1693, 1469, 1375, 1242, 1203,1037 and 966 cm^{-1}; ^1H NMR ($CDCl_3$): δ_H 6.20-6.25 (dd, J=10.3 and 2.3 Hz, 1H, H-3), 5.91-5.96 (dd, J=10.3 and 2.2 Hz, 1H, H-2), 5.88 (m, 1H, H-4), 4.87 (m, 1H, H-6), 1.32 (s, 3H, CH_3-19), 0.68 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.3, 14.2, 18.5, 20.6, 21.1, 22.4, 22.7; (CH_2): 22.1, 23.8, 24.0, 27.9, 30.0, 36.0, 39.4, 39.7; (CH): 27.9, 30.1, 35.7, 40.9, 55.6, 56.1, 68.9, 70.4, 130.0, 137.9; (C): 42.5, 52.6, 78.0; 169.8, 169.9, 201.9; MS (ES^+): MS (ES^+): m/z 517 ($M+H^+$, 100).

4α,6β-Diacetoxy-5-hydroxy-5α-cholest-1-en-3-one (6.2.2-29)

Afforded as white crystals. M.p. 158-160 °C; IR: ν_{max} 3500-3300, 2948, 2937, 2865, 1756, 1689, 1463, 1375, 1246 and 1059 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ_H 7.01 (d, J=10.2 Hz, 1H, H-1), 5.95 (d, J=10.2 Hz, 1H, H-2), 5.84 (s, 1H, H-4), 5.00 (m, 1H, H-6), 1.43 (s, 3H, CH_3-19), 0.71 (s, 3H, CH_3-18); ^{13}C NMR ($CDCl_3$): δ_C (CH_3): 12.1, 18.2, 18.5, 20.3, 21.1, 22.5, 22.7; (CH_2): 20.6, 23.7, 23.9, 28.1, 30.6, 36.0, 39.3, 39.4; (CH): 27.9, 30.2, 35.7, 41.2, 55.5, 55.9, 69.4, 73.8, 125.0, 154.5; (C): 42.5, 44.8, 77.8; 169.8, 170.0, 192.8; MS (ES^+): m/z 517 ($M+H^+$, 100).

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Appendices

Publications on this subject:

1. Kejun Zhao, Yongfeng Wang* and David C. Billington. Synthesis of 2,3α- and 2,3β-epoxy-5α-cholestane-4α or 4β,5,6β-triols and studies of stereoselectively opening of the oxiranes to gain diastereomerically pure oxysterols. *British Pharmaceutical Conference, Glasgow 23-26th September* **2001**, Abstract Book, 237.
2. Kejun Zhao, Yongfeng Wang* and David C. Billington. Studies on stereocontrolled epoxidations of bis-alicyclic alcohols in steroidal skeletons: preparation of eight diastereomerically pure epoxides from cholest-4-en-3β,6β; -3β,6α-; -3α,6β- and –3α,6α-diols. *Tetrahedron: Asymmetry* **2001**, *12*, 1211-1217.
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