Steroid Dimers

Chemistry and Applications in Drug Design and Delivery

Lutfun Nahar | Satyajit D. Sarker

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Dedicated to our parents

Mariam Sattar *Sadhan Sarker*
Abdus Sattar *Madhuri Sarker*

Contents

Preface

Steroid dimers form an important group of pharmacologically active compounds that are predominantly biosynthesized by various marine organisms, and also synthesized in laboratories. These dimers can also be used to create *'molecular umbrella'* for drug delivery. While there are hundreds of such compounds and numerous research papers on these compounds available to date, there is no book documenting the chemistry and applications of these compounds. However, there are two reviews, one by Li and Dias, and the other one by us (Nahar, Sarker and Turner) published, respectively, in 1997 and 2007. We believe that this is the right time to publish a book on steroid dimers covering their chemistry and applications. This book will be a handy reference for the organic synthetic, medicinal and natural-products chemists working in the area of steroids, and drug design, discovery and development in general.

The primary readership of this book is expected to be the postgraduate synthetic organic, medicinal and natural-product chemists working either in academia or industries, especially in the area of drug design, discovery and delivery. This book will also be suitable for the postgraduate students (and undergraduate students to some extent) within the subject areas of Chemistry, Pharmacy, Biochemistry, Food Sciences, Health Sciences, Environmental Sciences and Life Sciences.

This book comprises six chapters. *Chapter 1* introduces the topic, 'steroid dimers', and builds the foundation of the subsequent chapters. *Chapters 2* and *3* deal with the synthesis and the chemistry of various classes of steroid dimers, including cyclic and acyclic dimers, placing particular emphasis on the types of connectivities. *Chapter 4* presents an overview on the naturally occurring steroidal dimers, e.g., cephalostatins, crellastatins and ritterazines. *Chapter 5* discusses the synthesis of cephalostatin and ritterazine analogues, as well as the total synthesis of the naturally occurring extremely cytotoxic steroidal dimer cephalostatin 1. *Chapter 6* looks into the applications of both synthetic and natural steroid dimers, and evaluates the importance of these dimeric compounds in drug design, discovery and delivery. It also elaborates the concept of *'molecular umbrella'* in the context of steroid dimers.

The major features of this book include easy-to-follow synthetic protocols for various classes of important dimeric steroids, source details, valuable spectroscopic data and depiction of unique structural features of natural steroidal dimers, applications of steroidal dimers, especially in relation to drug design, development and delivery, and the Structure-Activity-Relationships (SARs) of some pharmacologically active dimeric steroids.

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October 2011

List of Abbreviations

Å	Angstrom
AcCl	Acetyl chloride
Ac_2O	Acetic anhydride
AcOH	Acetic acid
$AgBF_4$	Silver tetrafluoroborate
$AgNO_3$	Silver nitrate
Ag_2O	Silver oxide
AIBN	Azobisisobutyronitrile
Al(O-*t*-Bu)$_3$	Aluminum *tert*-butoxide
Ar	Argon
$BaCO_3$	Barium carbonate
BF_3	Boron trifluoride
$BF_3.Et_2O$	Boron trifluoride etherate
BH_3	Borane
$BH_3{\cdot}SMe_2$	Borane-dimethyl sulphide
BnBr	Benzylbromide
BnOH	Benzyl alcohol
$(Boc)_2O$	Di-*tert*-butyl dicarbonate
$BrCH_2COBr$	Bromoacetyl bromide
n-BuLi	Butyllithium
Bu_4NOAc	Tetrabutylammonium acetate
2-BuOH	2-Butanol
t-BuOH	*tert*-Butanol or *tert*-butyl alcohol
t-BuOK	Potassium *tert*-butoxide
Bu_3Sb	Tributylstibine
Bu_2SnCl_2	Dibutyltin dichloride or dichlorodibutylstannane
Bu_3SnH	Tributyltin hydride or tributylstannane
^{13}C NMR	Carbon Nuclear Magnetic Resonance
$CaCO_3$	Calcium carbonate
CaH_2	Calcium hydride
$CaSO_4$	Calcium sulphate
m-CBPA	*meta*-Chloroperoxybenzoic acid
CC	Column chromatography
CCl_4	Carbon tetrachloride
C_5D_5N	Deuterated pyridine
$CeCl_3$	Cerium trichloride
$CeCl_3.7H_2O$	Cerium trichloride heptahydrate

$(CF_3CO)_2O$	Trifluorocacetic anhydride
C_6F_5OH	Pentafluorophenol
C_6F_5SH	Pentafluorothiophenol
C_6H_6	Benzene
C_6H_{12}	Cyclohexane
$CHCl_3$	Chloroform
CH_2I_2	Diiodomethane
CH_2N_2	Diazomethane
C_5H_5N	Pyridine
CH_3NO_2	Nitromethane
$C_2H_6O_2$	Ethane-1,2-diol or ethylene glycol
$CH(OMe)_3$	Trimethoxymethane or trimethyl orthoformate
$(C_3H_5O)_2O$	Propionic anhydride
CH_3ReO_3	Methyltrioxorhenium
CIMS	Chemical Ionization Mass Spectroscopy
$ClCH_2CO_2H$	Chloroethanoic acid
$(Cl_3CO)_2CO$	Triphosgene
$Cl_2P(O)OEt$	Dichloroethylphosphate
CME	Chloromethyl ethyl ether
CO_2	Carbon dioxide
$(COCl)_2$	Oxalyl chloride
COSY	Correlation Spectroscopy
CrO_3	Chromium trioxide
CSA	Camphorsulfonic acid
$Cu(AcO)_2$	Copper acetate
CuCN	Copper cyanide
CuI	Copper Iodide
$Cu(OTf)_2$	Copperbistrifluoromethanesulfonate or copper triflate
d	Day (s)
DABCO	1,4-diazabicyclo[2.2.2]octane
DBE	Di-*n*-butyl ether
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCBC	2,6-Dichlorobenzoyl chloride
DCC	Dicyclohexyl carbodiimide
DCCC	Droplet counter current chromatography
DCE	Dichloroethene or dichloroethylene
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DEG	Diethylene glycol
DEPC	Diethylphosphoryl cyanide
DEPT	Distortionless Enhancement by Polarisation Transfer
DHEA	Dehydro-*epi*-androsterone
DHP	2,3-Dihydropyran
DHT	Dihydrotestosterone
$(DHQ)_2PHAL$	Hydroquinine 1,4-phthalazinediyl diether
DIAD	Diisopropyl azodicarboxylate

DIB	Diacetoxyiodobenzene
Dibromo-PEG	Dibromo-polyethyleneglycol
DIEA	*N*,*N*-diisopropylethylamine
DIPA	Diisopropylamine
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMDO	Dimethyldioxirane
DME	Dimethyl ether
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
2,4-DNP	2,4-Dinitrophenylhydrazine
D_2O	Deuterated water or heavy water
DTBB	4,4′-Di-*tert*-butylbiphenyl
EDC	1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide hydrochloride
EDTA	Ethylenediaminetetraacetic acid
EEDQ	*N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
EIMS	Electron Ionisation Mass Spectroscopy
ESIMS	Electron Spray Ionisation Mass Spectroscopy
$EtCO_2Cl$	Ethyl chloroformate
Et_3N	Triethyl amine
$EtNO_2$	Nitroethane
Et_2O	Ether
EtOAc	Ethyl acetate
$EtO_2CC(N_2)PO(OEt)_2$	Ethyldiazophosphonate
$(EtO)_3CH$	Triethyl orthoformate
EtOH	Ethanol
$EtPPh_3Br$	Ethyltriphenylphosphonium bromide
FABMS	Fast Atom Bombardment Mass Spectroscopy
FCC	Flash column chromatography
$FeCl_3.Et_2O$	Ferric chloride etherate
$Fe(ClO_4)_3$	Ferric perchlorate
FTIR	Fourier-Transfer Infrared Spectroscopy
h	Hour (s)
H_2	Hydrogen
H_3BO_3	Boric acid
HBr	Hydrobromic acid or hydrogen bromide
HCl	Hydrochloric acid or hydrogen chloride
$HClO_4$	Perchloric acid
HCO_2H	Formic acid
H_2CrO_4	Chromic Acid
HDTC	Ethylene glycol
HF	Hydrofluoric acid or Hydrogen fluoride
HgO	Mercury oxide
HIO_4	Periodic acid
HMBC	Heteronuclear Multiple Bond Correlation

$(HMe_2Si)_2O$	Tris(dimethylsilyl)methane
$H_2N(CH_2)_2NH_2$	Ethylenediamine
HN_3	Hydrogen azide or hydrazoic acid
^1H NMR	Proton Nuclear Magnetic Resonance
H_2O	Water
H_2O_2	Hydrogen peroxide
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Mass Spectroscopy
HRFABMS	High Resolution Fast Atom Bombardment Mass Spectroscopy
H_2SO_4	Sulphuric acid
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
I_2	Iodine
IR	Infrared Spectroscopy
KBr	Potassium bromide
K_2CO_3	Potassium carbonate
K_2CrO_4	Potassium chromate
$K_3Fe(CN)_6$	Potassium ferricyanide
KH	Potassium hydride
$KHCO_3$	Potassium bicarbonate or potassium hydrogen carbonate
KHMDS	Potassium hexamethyldisilazane
KI	Potassium iodide
$KMnO_4$	Potassium permanganate
KOAc	Potassium acetate
K_2OsO_4	Potassium osmate
$K_2S_2O_5$	Potassium metabisulphite
LDA	Lithium diisopropylamide
Li	Lithium
$LiAlH_4$	Lithium aluminium hydride
$LiBH_4$	Lithium borohydride
LiBr	Lithium bromide
$LiClO_4$	Lithium perchlorate
Li_2CO_3	Lithium carbonate
LiOH	Lithium hydroxide
LSIMS	Liquid secondary ion mass spectrometry
MALDI-TOF	Matrix-assisted laser desorption ionisation-time of flight
MeCN	Acetonitrile
MeCO	Acetyl or Ac
Me_2CO	Acetone
MeI	Methyl iodide
MeLi	Methyllithium
MeMgBr	Methyl magnesium bromide
Me_2NEt	*N*,*N*-Dimethylethylamine
MeOH	Methanol
$MeONH_2.HCl$	*O*-Methylhydroxylamine hydrochloride
MsCl	Methanesulfonyl chloride

Me_3SI	Trimethylsulfonium iodide
$MeSO_2NH_2$	Methane sulfonamide
Mg	Magnesium
$MgSO_4$	Magnesium sulphate
MHz	Megahertz
Mp	Melting point
MS	Mass Spectroscopy
MsCl	Methanesulfonyl chloride or mesyl chloride
MTBE	Methyl tertiary butyl ether
MTO	Methyltrioxorhenium
MWAM	Microwave assisted metathesis
m/z	Mass to charge ratio
N_2	Nitrogen
$NaBH_4$	Sodium borohydride
$NaBH(OAc)_3$	Sodium triacetoxyborohydride
$NaClO_2$	Sodium chlorite
$NaClO_4$	Sodium perchlorate
$NaCNBH_3$	Sodium cyanoborohydride
Na_2CO_3	Sodium carbonate
$Na_2Cr_2O_7$	Sodium dichromate
NaH	Sodium hydride
$NaHCO_3$	Sodium bicarbonate or sodium hydrogen carbonate
Na-Hg	Sodium-mercury or sodium amalgam
NaI	Sodium iodide
$NaIO_4$	Sodium periodate or sodium metaperiodate
NaN_3	Sodium azide
NaOAc	Sodium acetate
$NaOAc.3H_2O$	Sodium acetate trihydrate
NaOH	Sodium hydroxide
NaOMe	Sodium methoxide
Na_2SO_4	Sodium sulphate
$Na_2S_2O_3$	Sodium thiosulphate
$Na_2S_2O_5$	Sodium metabisulphite
NaTeH	Sodium hydrogen telluride
NBS	*N*-Bromosuccinimide
NH_3	Ammonia
N_2H_4	Hydrazine
NH_4Cl	Ammonium chloride
$N_2H_4.H_2O$	Hydrazine hydrate
NH_4OAc	Ammonium acetate
NH_4OH	Ammonium hydroxide
$NH_2OH.HCl$	Hydroxylamine hydrochloride
$NH_2OMe{\cdot}HCl$	Methoxyamine hydrochloride
$(NH_4)_2SO_4$	Ammonium Sulphate
NH_2SO_3H	Sulfamic acid
NMO	*N*-Methylmorpholine-*N*-oxide

NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
O_3	Ozone
OsO_4	Osmium tetroxide
OTs	Tosylate
$Pb(OAc)_4$	Lead tetraacetate
PCC	Pyridinium chlorochromate
Pd-C	Palladium on carbon
PDC	Pyridinium dichromate
$Pd(MeCN)_2Cl_2$	Bis(acetonitrile)dichloropalladium
Petroleum ether	Petroleum ether (40–60 °C)
PFPOH	Pentafluorophenol
Ph	Phenyl
$PhI(OAc)_2$	Diacetoxyiodobenzene
PhLi	Phenyllithium
$PhMe_3NBr_3$	Phenyltrimethylammonium tribromide
Ph_3P	Triphenylphosphine
Ph_3PAuCl	Chloro(triphenylphosphine)gold
$Ph_3P{=}CH_2Br$	Triphenyl(methyl)phosphonium bromide
PPh_3Cl	Dichlorotriphenylphosphane
Ph_3PO	Triphenylphosphine oxide
PPh_3Cl	Dichlorotriphenylphosphane
$(Ph_3P)_4Pd$	Tetrakis(triphenylphosphine)palladium
PhSeBr	Phenylselenyl bromide
$(PhSeO)_2O$	Phenylseleninic anhydride
$PhSO_2Cl$	Phenylsulphonyl chloride
PMP	*p*-Methoxyphenol
PPTS	Pyridinium *p*-toluenesulfonate
PTAB	Phenyltrimethylammoniumbromide tribromide
PTAP	Phenyltrimethylammonium perbromide
PTLC	Preparative Thin Layer Chromatography
PtO_2	Platinum dioxide or Adams' catalyst
P_2O_5	Phosphorus pentoxide
POPC	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
i-Pr_2NEt	*N*,*N*-Diisopropylethylamine
n-$PrNH_2$	*n*-propylamine
i-Pr_2O	Diisopropyl ether
i-PrOH	*iso*-Propanol or propan-2-ol
PVP	Polyvinylpyridine
RCM	Ring-closing metathesis
$Rh_2(OAc)_4$	Rhodium acetate
ROSEY	Rotating-frame Overhauser Effect SpectroscopY
r.t.	Room temperature
$RuCl_3$	Ruthenium trichloride
$RuCl_3.H_2O$	Ruthenium trichloride hydrate

L-Selectride	Lithium tris(*sec*-butyl)hydroborate
SiO_2	Silica or silicon dioxide
SO_2	Sulphur dioxide
SO_3	Sulphur trioxide
$SOCl_2$	Thionyl chloride
SPHRSIMS	Super Probe High Resolution Secondary Ion Mass Spectrometry
TBAF	Tetrabutylammonium fluoride
TBAHS	Tetrabutylammonium hydrogen sulphate
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPS	*tert*-Butyldiphenylsilyl
TBDPSCl	*tert*-Butylchlorodiphenylsilane
TBMS	*tert*-Butyldimethylsilyl
TBDMSOTf	*tert*-Butyldimethylsilyl trifluoromethanesulfonate
TBSOTf	*tert*-Butyldimethylsilyl triflate
TEA	Triethanolamine
TEG	Triethylene glycol
TFAT	Trifluoroacetyl trifluoromethanesulfonate
THF	Tetrahydrofuran
THP	Tetrahydropyran
$TiCl_3$	Titanium trichloride
$TiCl_4$	Titanium tetrachloride
TIPS	Triisopropylsilyl
TIPSCl	Triisopropylsilyl chloride
TLC	Thin Layer Chromatography
TMGA	Tetramethylguanidinium azide
TMS	Trimethylsilyl
TMSCN	Trimethylsilyl cyanide
$(TMS)_2O_2$	Bis(trimethylsilyl) peroxide
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TPAP	Tetrapropylammonium perruthenate
TPSCl	*tert*-Butylchloro diphenylsilane
TrCl	Triphenylmethylchloride
p-TsCl	*p*-Toluenesulfonyl chloride or tosyl chloride
p-TsOH	*p*-Toluenesulfonic acid
p-TsOH.H_2O	*p*-Toluenesulfonic acid hydrate
UV	Ultra violet
VLC	Vacuum Liquid Chromatography
Zn	Zinc
$ZnCl_2$	Zinc chloride

1

Introduction

1.1 Steroids and Steroid Dimers

Steroids are a family of biologically active lipophilic molecules that include cholesterol, steroidal hormones, bile acids and plant sterols (also known as phytosterols). These metabolic derivatives of terpenes are biosynthesized by plants as well as animals including humans, and play an important role in biological systems (Li and Dias, 1997; Nahar *et al*., 2007a). Structurally, a steroid is a lipid molecule having a carbon skeleton with four fused rings; three fused cyclohexane rings, known as phenanthrene, are fused with a cyclopentane ring (Sarker and Nahar, 2007). The basic tetracyclic seventeen carbon steroidal ring system is known as *1,2-cyclopentano-perhydrophenanthrene* or simply *cyclopentaphenanthrene* (*Figure 1.1.1*). All steroids are derived from the acetyl CoA biosynthetic pathway. The four rings are lettered A, B, C, and D, and the carbon atoms are numbered beginning in the A ring. In steroids, the B, C, and D rings always are *trans*-fused, and in most natural steroids, rings A and B also are *trans*-fused. Each member of the steroid family has a structure that differs from the basic cyclopentaphenanthrene skeleton in the degrees of unsaturation within the rings and the identities of the hydrocarbon side chain substituents, *e.g*., alkyl, alcohol, aldehyde, ketone or carboxylic acid functional groups, attached to the rings.

Even minor changes in the functionalities attached to the steroid skeleton can lead to significant changes in their biological and pharmacological activities (Nahar *et al*., 2007a). That is why synthetic chemists have always been keen to carry out structural modifications of steroids to optimize their biological and pharmacological properties or to discover new properties. Steroid dimers are one of such group of modified steroids that are well known for their rigid, predictable and inherently asymmetric architecture.

Steroid dimer formation was first noticed during photochemical studies on steroids. During the investigation of the effect of sensitized light on the activation of ergosterol (**1**) in the absence of oxygen, it was discovered that in an alcoholic solution containing sensitizer, ergosterol on exposure to sunlight had undergone dehydrogenation to form a strongly

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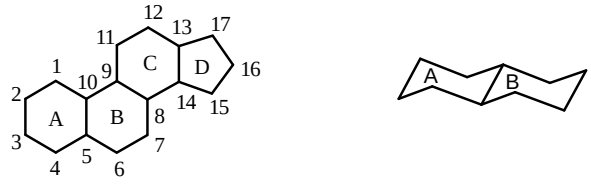


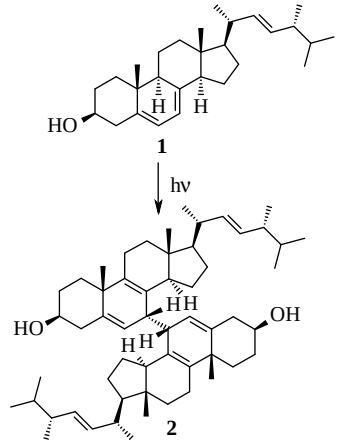
Figure 1.1.1 *Cyclopentaphenanthrene skeleton (left) and* trans*-fused rings (right)*

levorotatory substance ($[\alpha]_D$: $-209°$, mp: 205 °C) having double the original molecular weight and two hydroxyl groups. This bimolecular product was named bisergostatrienol (**2**) (*Scheme 1.1.1*) (Windaus and Borgeaud, 1928). Since this discovery, several dimeric steroids have been found in nature, particularly from marine sponges, and also have been synthesized in the laboratory (Nahar *et al*., 2007a).

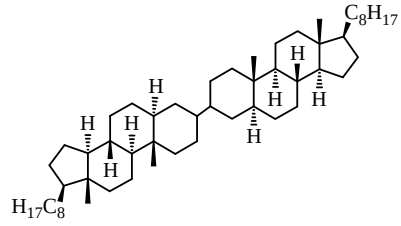
Steroid dimers can be classified broadly into acyclic dimers (also known as *'linear dimers'*) and cyclic dimers (*Figure 1.1.2*). Acyclic dimers involving connections between A, B, C or D rings, or *via* C-19, direct or through spacers, form the major group of steroid dimers (see *Chapter 2*). In the cyclic steroid dimers, dimerization of steroids, direct or through spacers, leads to formation of new ring systems or macrocyclic structures, *e.g*., cyclocholates or cholaphanes, respectively (see *Chapter 3*). Steroid dimers can also be classified as symmetrical and unsymmetrical dimers; when a dimer is composed of two identical steroid monomeric units, it is called a symmetrical dimer, and when two different monomeric steroid units are involved or two identical monomeric steroid units are joined in a way that there is no symmetry in the resulting dimer, the dimer is known as an unsymmetrical dimer (*Figure 1.1.2*). One other way of classifying steroid dimers is to divide them into natural and synthetic dimers (*Figure 1.1.2*).

1.2 General Physical and Spectroscopic Properties of Steroid Dimers

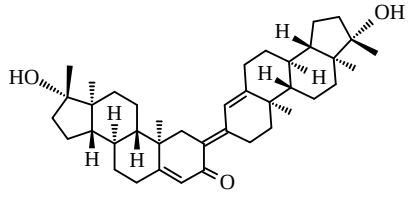
In general, like most monomeric steroids, steroid dimers are lipophilic in nature and are not water soluble. However, depending on the monomeric steroid, spacer group or other



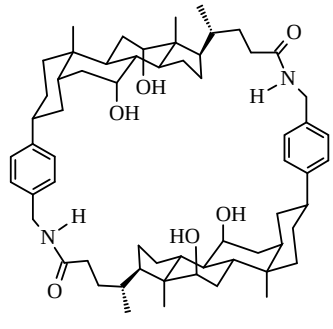
Scheme 1.1.1 *Conversion of ergosterol (**1**) to bisergostatrienol (**2**)*



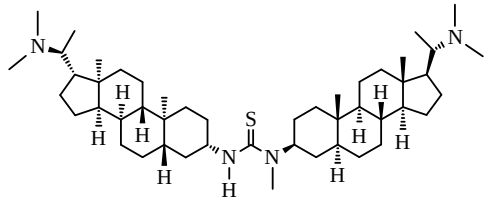
Bischolestane, a synthetic symmetrical acyclic (linear) steroid dimer



17α-methyltestosterone dimers, a synthetic acyclic unsymmetrical steroid dimer



Cholaphane, a synthetic cyclic (macrocyclic) steroid dimer

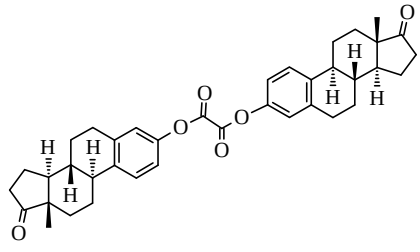


Japindine, a natural symmetrical steroid dimer

Figure 1.1.2 *Classification of steroid dimers*

functionalities present on the dimeric steroid skeleton, the solubility of such molecules can be quite variable. For example, steroid dimers composed of two sterol (steroid alcohol) units where the hydroxyl groups are not altered, as in bisergostatrienol (**2**), will retain some degree of polar character due to the hydroxyl groups, while keeping its nonpolar or hydrophobic nature because of the ring systems and other alkyl substituents or aliphatic side chains, and thus, these dimers will have properties like amphipathic lipids.

Most dimeric steroids are solids and can be transformed into well-formed crystals from various solvents (see *Chapters 2* and *3*), *e.g.*, bis[estra-1,3,5(10)-trien-17-on-3-yl]oxalate (**3**) was crystallized from $CHCl_3$-EtOAc (2:1) (Nahar, 2003).



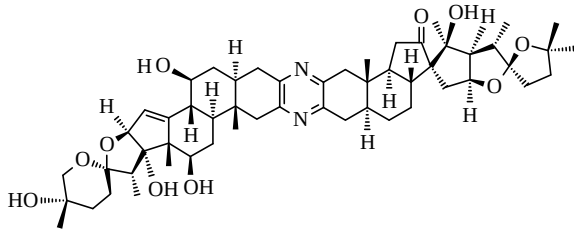
Bis[estra-1,3,5(10)-trien-17-on-3-yl]oxalate (**3**)

The melting points of steroid dimers are quite variable and depend on the monomer, the spacer groups and other functionalities. The UV absorption spectra of steroid dimers depend on the presence or absence of chromophores, *e.g.*, conjugated double bonds. The IR spectra can be different from dimer to dimer based on the functional groups present. Details on these spectral data of various steroid dimers will be presented in *Chapters 2–5*. Like the monomeric steroids, the dimeric steroids have several chiral centres in the molecule that make these molecules optically active. Therefore, specific rotation $[\alpha]_D$ data can provide additional characteristic information for any dimer.

To determine the molecular weight and molecular formula of steroid dimers, it is often essential to employ soft ionization techniques like fast-atom bombardment (FAB), electrospray ionization (ESI) or chemical ionization (CI) mass spectroscopy. The use of the MALDI–TOF technique has also been observed for some dimers very recently. MS information is particularly important for the symmetrical dimers composed of two identical steroid momoners without any spacer groups, where the information obtained from the nuclear magnetic resonance (NMR) spectroscopy may not be adequate to confirm the structure.

A range of 2D NMR techniques, particularly, correlation spectroscopy (COSY), nuclear Overhauser spectroscopy (NOESY), heteronuclear multiple bond coherence (HMBC) and heteronuclear single quantum coherence (HSQC), could be useful to confirm the structures of a number of dimeric steroids (Nahar, 2003; Nahar and Turner, 2003; Nahar *et al.*, 2006, 2007b). Sometimes, the use of the rotating frame Overhauser effect spectroscopy (ROESY) could be useful in establishing the relative stereochemistry, as in the case of crellastatins (D'Auria *et al.*, 1998; see *Chapter 4*). Fuzukawa *et al.* (1996) used ^{15}N-HMBC NMR technique to determine the orientation of the steroidal

units about the pyrazine ring in ritterazine A (**4**). However, the use of the ^{15}N-HMBC NMR technique is rather limited.



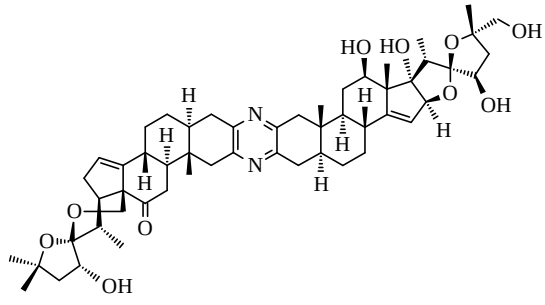
Ritterazine A (**4**)

1.3 Chromatographic Behaviour of Steroid Dimers

Most steroid dimers are nonpolar in nature and can be separated by normal-phase column, flash or thin layer chromatography (FCC or TLC) on silica gel (SiO_2) as the stationary phase and using various solvent mixtures, *e.g.*, *n*-hexane-EtOAc or $CHCl_3$-MeOH, as the mobile phase or eluent (Nahar, 2003). However, alumina or celite as the stationary phase has also been utilized for the separation of several steroid dimers.

On the TLC plates, steroid dimers can be detected by I_2 vapour, or using various sprays reagents, *e.g.*, vanillin-H_2SO_4 and Liebermann–Burchard reagents. For the detection of steroidal alkaloid dimers, *e.g.*, cephalostatin 1 (**5**), any alkaloid-detecting reagents, *e.g.*, Dragendorff's reagent, may be used.

The use of the reversed-phase high-performance liquid chromatography (HPLC) can equally be useful, and generally, MeOH-H_2O or MeCN-H_2O as the mobile phase, and a C_{18} reversed-phase column as the stationary phase can be used (Nahar, 2003). However, for the purification of some cephalostatins and ritterazines, a C_8 reversed-phase column was reported to be used (see *Chapter 4*).



Cephalostatin 1 (**5**)

In some cases, for the initial separation of naturally occurring cytotoxic steroid dimers, *e.g.*, cephalostatins or ritterazines, solvent partitioning methods and droplet countercurrent chromatography (DCCC) have been regularly employed (see *Chapter 4*).

1.4 Applications of Steroid Dimers

Dimerization of steroid skeleton renders some unique characteristics that are applicable to different areas. Dimeric steroids have miceller, detergent, and liquid-crystal properties, and have been used as catalysts for different types of organic reactions. A number of dimeric steroids, *e.g.*, cephalostatins [*e.g.*, cephalostatin 1 (**5**)], are among the most potent natural cytotoxins. It has been suggested that a polyamine dimeric steroid binds to DNA due to the presence of two parts, one hydrophilic (positively charged nitrogen) and the other is hydrophobic steroid skeleton. Steroid dimers have also found their applications as *'molecular umbrella'* for drug delivery. Applications of steroid dimers are discussed further in *Chapter 6*.

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2

Synthesis of Acyclic Steroid Dimers

Several steroid dimers have been synthesized over the years (Li and Dias, 1997a; Nahar *et al*., 2007a), and acyclic dimers involving connections between A, B, C or D rings, direct or through spacers, form the major group of such molecules. These dimers are also referred to as *'linear dimers'*. In this chapter, dimers connected *via* ring A–ring A, ring B–ring B, ring C–ring C, ring D–ring D, ring A–ring D, dimers *via* C-19 and *'molecular umbrellas'* are discussed.

2.1 Dimers *via* Ring A–Ring A Connection

2.1.1 Direct Connection

Direct ring A–ring A connection between two steroid units can be achieved by using an active metal reduction or a photochemical condensation. Several dimers were synthesized from steroidal 4-en-3-one by photochemical, electrolytic, and metal reduction (Squire, 1951; Lund, 1957; Bladon *et al*., 1958). Squire (1951) reported the synthesis of bicholestane (**5**) in three steps by chlorination of cholesterol (**1**, cholest-5-en-3β-ol), hydrogenation of cholesteryl chloride (**2**, 3β-chlorocholest-5-ene) and finally the coupling between cholestanyl chloride (**3**, 3β-chloro-5α-cholestane) and cholestenyl magnesium chloride (**4**, 3β-magnesium chloro-5α-cholestane) (prepared *in situ*) (*Scheme 2.1.1*).

Cholesteryl chloride (**2**) was first obtained from cholesterol (**1**) using the method described in the literature (Daughenbaugh, 1929). To a stirred solution of **1** (5.0 g, 12.93 mmol) in dry C_5H_5N (1 mL), $SOCl_2$ (10 mL) was added and the reaction mixture was refluxed for 60 min (*Scheme 2.1.1*). After complete liberation of SO_2, the reaction mixture was cooled, H_2O was added, and extracted with Et_2O. The etheral solution was dried (Na_2CO_3) and evaporated under pressure to a semicrystalline residue, which was recrystallized from EtOH and identified as cholesteryl chloride (**2**, 3.3 g, 63%, mp: 95–96 °C) (Daughenbaugh, 1929). Compound **2** was then utilized for the synthesis of cholestanyl chloride (**3**) (Squire, 1951).

To a stirred solution of **2** (630 mg, 1.56 mmol) in Et_2O (15 mL) and EtOH (15 mL), PtO_2 (38 mg) was added (*Scheme 2.1.1*). The reaction mixture was kept under 2 atm of H_2 for

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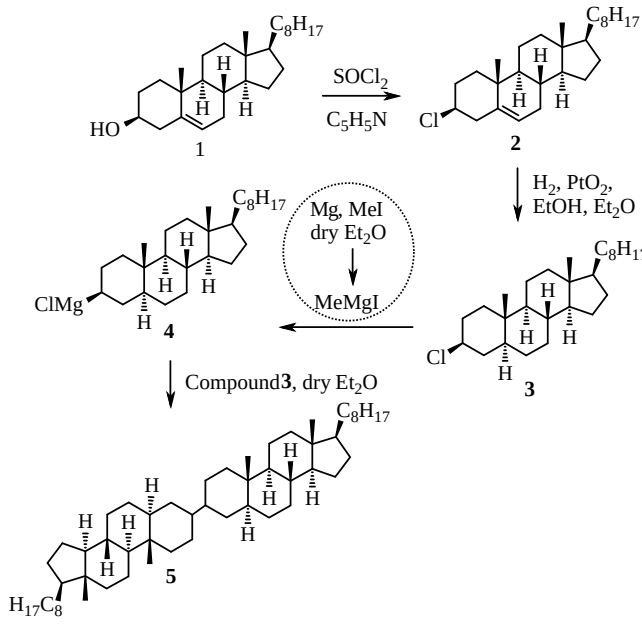
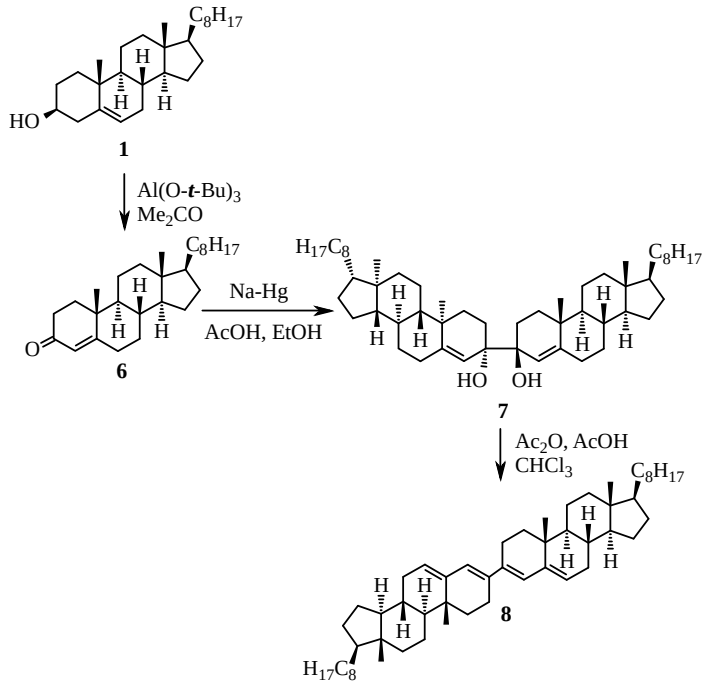


Scheme 2.1.1 *Synthesis of 3β-chlorocholest-5-ene (**2**), 3β-chloro-5α-cholestane (**3**) and bicholestane (**5**)*

60 min with stirring. On completion of the hydrogenation, the reaction mixture was filtered and rotary evaporated to yield cholestanyl chloride (**3**, 510 mg, 80%, mp: 115–115.5 °C), which was employed for the synthesis of bicholestane (**5**) (Squire, 1951).

Compound **3** (5.0 g), Mg powder (10 g, 41.0 mmol) and MeI (10 mL) in dry Et_2O (5 mL) were refluxed under N_2 (*Scheme 2.1.1*). After cholestenyl magnesium chloride (**4**) had formed, a solution of **3** (820 mg, 2.01 mmol) in dry Et_2O (15 mL) was added carefully, and the reaction mixture was stirred for 22 h. The reaction mixture was cooled (0 °C) and treated with CO_2 gas at 1.5 atm pressure for another 18 h, after which, a mixture of ice (100 g) in conc. HCl (20 mL) was added and left for a further 3 h. The ice cooled solution was extracted with Et_2O (100 mL), the Et_2O layer was successively washed with 0.1N NaOH (5 × 20 mL) and H_2O (5 × 10 mL), dried (Na_2SO_4), filtered and evaporated under vacuum. Recrystallization of the residue from Et_2O afforded the ring A-ring A dimer, bischolestane (**5**, 37 mg, 5%, mp: 265 °C) (Squire, 1951).

Cholestenone pinacol, bis(3β-hydroxycholest-4-en-3′α-yl) (**7**), was produced with high yield (85%) from the reaction between cholestenone (**6**, cholest-4-en-3-one) and Na-Hg in AcOH (*Scheme 2.1.2*) (Squire, 1951). Later, the same dimer **7** was also synthesized by electrolytic reduction of **6** (Bladon *et al*., 1958). Cholestenone (**6**) was produced from a stirred solution of **1** (20 g, 51.73 mmol) in Me_2CO by the treatment with $Al(O\text{-}t\text{-}Bu)_3$ according to procedure described in the literature (Oppenauer, 1937) (*Scheme 2.1.2*). The crude dimer was purified initially by TLC on Al_2O_3, and recrystallized from MeOH-petroleum ether to yield **6** (17.9 g, 90%, mp: 78–79 °C), which was employed to prepare cholestenone pinacol **7**.

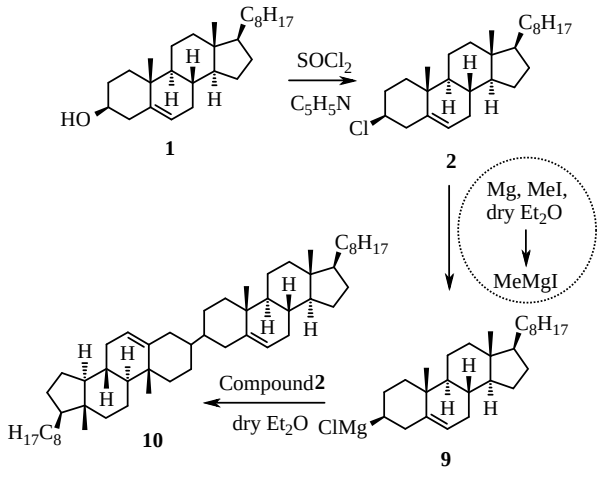


Scheme 2.1.2 *Synthesis of cholest-4-en-3-one (**6**), bis(3β-hydroxycholest-4-en-3′α-yl) (**7**), bicholesta-3,5-dienyl (**8**)*

To a stirred solution of **6** (2.0 g, 5.19 mmol) in AcOH (75 mL) and EtOH (75 mL), 2% Na-Hg (300 g) was added slowly over 30 min under moderate heat (*Scheme 2.1.2*). The reaction mixture was boiled for 5 min, cooled and poured into H_2O (600 mL), resulting in the formation of precipitates, which was filtered under vacuum, washed with H_2O and dried (Na_2SO_4). The crude dimer was dissolved in hot C_6H_6 (100 mL), filtered and concentrated down to 25 mL, to which, hot Me_2CO (100 mL) was added. The resulting mixture was cooled in an ice bath to generate colourless crystals upon standing overnight. The crystals were filtered and dried *in vacuo* to give cholestenone pinacol **7** (1.7 g, 85%, mp: 200–205 °C) (Squire, 1951).

Cholestenone pinacol **7** was employed for the synthesis of bicholesta-3,5-dienyl (**8**) (*Scheme 2.1.2*). Glacial AcOH (5 mL) and Ac_2O (5 mL) were added to a stirred solution of **7** (3.0 g, 3.89 mmol) in $CHCl_3$ (250 mL) under reflux. After 4 h, $CHCl_3$ was slowly evaporated over another 4 h, MeOH (100 mL) was added to the reaction mixture to form precipitation, which was filtered off and dried *in vacuo* to obtain a light yellow solid. Recrystallization of this solid from Me_2CO-$CHCl_3$ yielded **8** (2.6 g, 83%, mp: 244–246 °C) (Squire, 1951; Bladon, 1958).

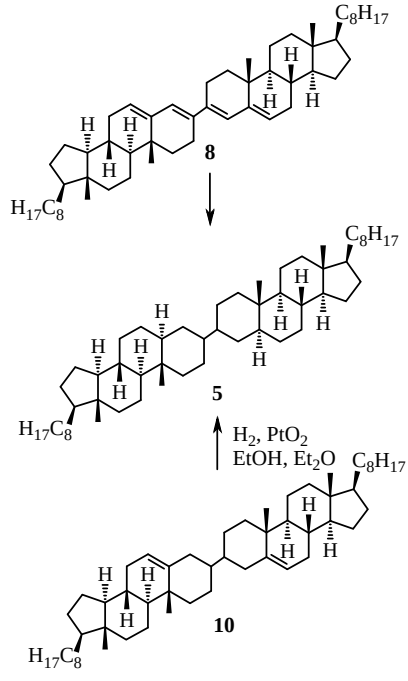
The synthesis of bi(cholest-5-ene) (**10**, bicholesteryl) was accomplished in two steps: by chlorination of **1** forming cholesteryl chloride (**2**), and coupling of **2** and cholesteryl magnesium chloride (**9**) (prepared *in situ*) (*Scheme 2.1.3*). The synthetic protocol for **10** was similar to that described earlier for the dimer **5**. To a solution of **9** (1.30 g, 3.04 mmol) in dry Et_2O, a solution of **2** (1.23 g, 3.04 mmol) in dry 2-BuOH (50 mL) and conc. H_2SO_4



Scheme 2.1.3 *Synthesis of bicholesteryl (**10**)*

(20 drops) were added under N_2 to yield the crude dimer, which was recrystallized from 2-BuOH-Et_2O to afford bicholesteryl (**10**, 195 mg, 17.4%, mp: 266–269 °C) (Squire, 1951).

Bicholestane (**5**) could also be obtained by hydrogenation of either bicholesta-3,5-dienyl (**8**) or bicholesteryl (**10**) (*Scheme 2.1.4*). Finely powdered dimer **8** (100 mg, 0.14 mmol) and PtO_2 catalyst (100 mg) were dissolved in cyclohexane (250 mL) under constant stirring



Scheme 2.1.4 *Synthesis of bicholestane (**5**)*