

A Review on Steroid Dimers: 2011-2019

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Running title: Steroid Dimers: 2011-2019

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Highlights

- Steroid dimers reported during 2011-2019
- Over 200 new synthetic steroidal dimers
- Ring A-ring A connection formed the major group of dimers
- Only one natural steroid dimer
- Steroid dimers with anticancer, antitumor and antimicrobial properties

ABSTRACT

The first review article on steroid dimers by Li and Dias in 1997, followed by the second review and a book on steroid dimers by Nahar and Sarker in 2007 and 2012, respectively, covered steroid dimers reported until the end of 2010. Since then, there have been considerable amounts of research carried out on steroid dimers, prompting the need for another comprehensive review on this topic. Therefore, this present review appraises the literature published during the period 2011-2019 on various aspects of steroid dimers, including isolation from natural sources, synthesis and applications. A structured and systematic literature search was performed, using the key words: steroid dimer, steroidal dimer, dimeric steroid, bis-steroid, bis-steroidal conjugates, molecular umbrella, cephalostatins, ritterazines and crellastatins. Several databases like Web of Knowledge, Science Direct, PubMed and Google Scholar were consulted. During the period covered in this review, well over 200 new synthetic steroidal dimers, ring A-ring A connection being the major group, have been reported, only one natural steroid dimer has been isolated, and potential applications of steroid dimers in the treatment of cancers and tumors, and microbial infections have been indicated.

Keywords:

Steroids dimer, Bis-steroid, Synthesis, Natural products, Molecular umbrella, Anticancer, Cephalostatins, Ritterazines, Crellastatins

Abbreviations: DCC, dicyclohexylcarbodiimide; DIPEA, diisopropylethylamine; 5-FU, 5-fluorouracil; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylethanaminium hexafluorophosphate *N*-oxide; HMDO, hexamethyldisiloxane, PASS, Prediction of Activity Spectra for Substances; TBAI, tetrabutylammonium iodide; TMSOTf, trimethylsilyl trifluoromethanesulfonate;

1. Introduction

Steroids, 1,2-cyclopentanoperhydrophenanthrenes or simply, cyclopentanophenanthrenes, are metabolic derivatives of terpenes [1-3]. They are an important group of biologically active lipophilic molecules, *e.g.*, cholesterol, steroidal hormones, bile acids and phytosterols, and are biosynthesized by plants as well as animals including humans. Steroids play a pivotal role in biological systems, particularly, by offering membrane fluidity of cells and acting as signalling molecules [1, 2]. Any changes in the functional groups attached to the steroid skeleton may render marked changes in biological functions of steroidal compounds [2], and because of this, structural modification of steroids has always been an attractive prospect for synthetic chemists working in the area of drug discovery, design and delivery. Steroid dimers are one of such modified groups of steroids, synthesized in the laboratory and/or biosynthesized mainly by marine sponges.

Steroid dimers, for example, the first synthetic steroid dimer bisergostatrienol (**1**) (Figure 1), are rigid, predictable and have asymmetric architecture [1]. The first review article on steroid dimers was published by Li and Dias in 1997 [3]. Then the second review article and a book on steroid dimers by Nahar and Sarker were published in 2007 [2] and 2012 [1], respectively, which covered almost all steroid dimers known to us until the end of 2010. Since then, there have been significant amounts of work published on steroid dimers, which justify for a compilation of another review article on this topic to cover the recent progress. Therefore, this review article provides a systematic and comprehensive appraisal of synthetic and naturally occurring steroid dimers, their reactions and applications reported during 2011-2019.

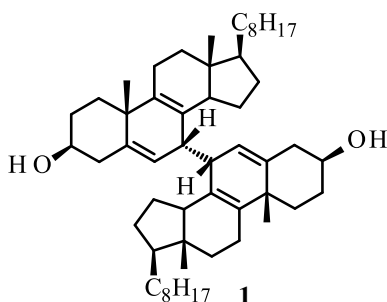


Figure 1. Structure of bisergostatrienol (**1**)

Steroid dimers are classified mainly into acyclic dimers (*'linear dimers'*) and cyclic dimers [1]; acyclic dimers involve connections between A, B, C or D rings, or *via* C-19, direct or through spacers, whilst cyclic steroid dimers are formed through direct connections or

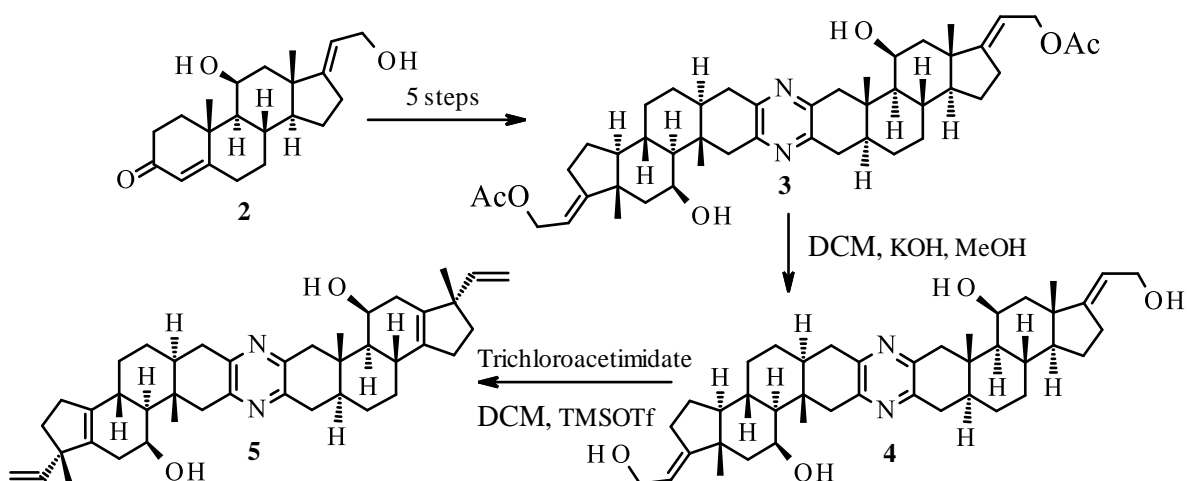
through spacers by head-head or head-tail and form new ring systems or macrocyclic structures, *e.g.*, cyclocholates or cholaphanes, respectively [1]. However, steroid dimers can also be grouped into symmetrical and unsymmetrical dimers, and into natural and synthetic dimers. In this review, all steroid dimers, reported over the last nine years, are discussed under two major sections, synthetic steroid dimers (further subdivided according to connectivity), and naturally occurring steroid dimers.

2. Synthetic steroid dimers

Since the synthesis of bisergostatrienol (**1**), several steroid dimers, connecting two steroidal molecules through spacers or through direct connections (ring to ring or ring to side chains), have been synthesized and appeared in the literature. The following sections present the synthesis of several steroid dimers reported over the last nine years.

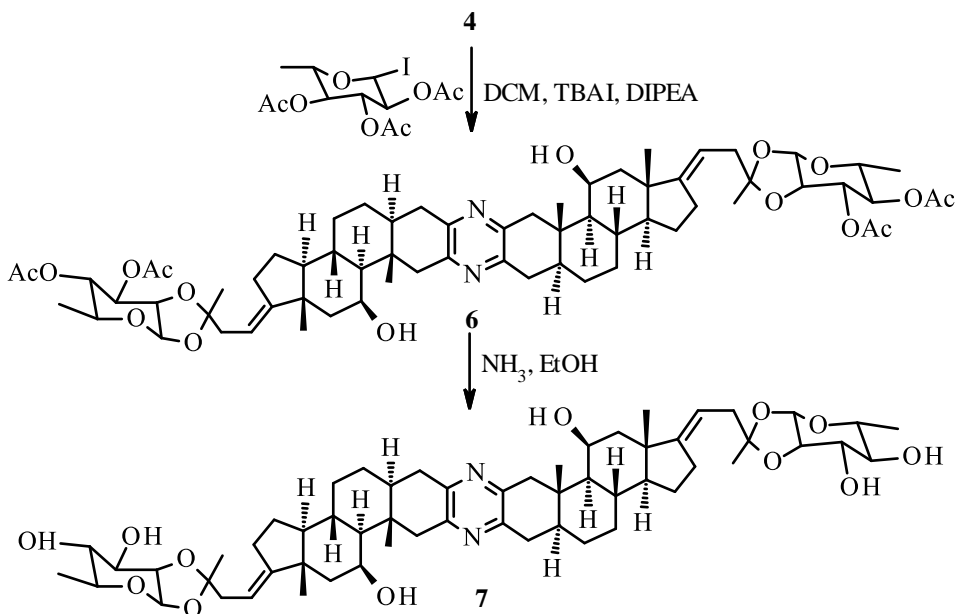
2.1 Dimers via ring A-ring A connection

Ring A-ring A connection, with or without a spacer, appears to be a popular option for the synthesis of steroid dimers. Sometimes spacers containing heteroatoms are brought in to enhance biological functions of steroid dimers. Ring A-ring A connection through various spacer groups is one of the most preferred routes for the synthesis of steroid dimers or bis-steroids from monomeric steroidal units. The synthesis of bis-steroidal pyrazines is well documented and generally involves reduction of 2-azido-3-oxo monomeric steroid [4]. In the synthesis of several of such steroid dimers, 3-oxo-11,21-dihydroxy-pregna-4,17(20)-diene (**2**) was employed as the starting material for the synthesis of diacetate pyrazine dimer **3** (77%) in five-steps (Scheme 1). Deacetylation of diacetate pyrazine dimer **3** in dry DCM with methanolic KOH provided a diol pyrazine dimer **4** (80%). Glycosylation of diol pyrazine dimer **4** with trichloroacetimidate and trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dry DCM afforded a rearrangement product as diene pyrazine dimer **5** (36%) [5].



Scheme 1. Synthesis of diene pyrazine dimer **5** from 3-oxo-11,21-dihydroxy-pregna-4,17(20)-diene (**2**) in five steps

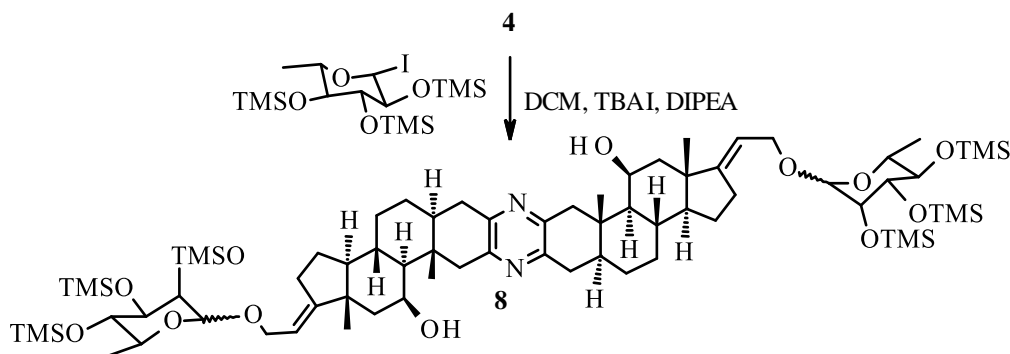
Coupling of diol pyrazine dimer **4** with 2,3,4-*O*-acetyl- α -L-rhamnopyranosyl iodide in tetrabutylammonium iodide (TBAI) and diisopropylethylamine (DIPEA) gave *ortho*-ester rhamnoside pyrazine dimer **6** (87%), which upon deacetylation with ethanolic ammonia provided diol rhamnoside pyrazine dimer **7** (77%) (Scheme 2) [5].



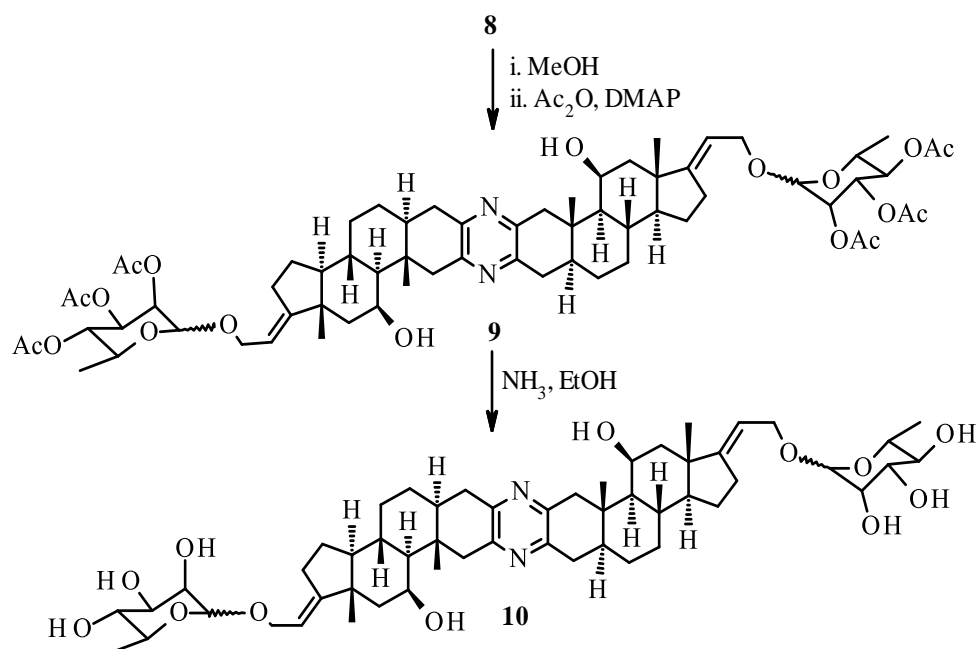
Scheme 2. Synthesis of diol rhamnoside pyrazine dimer **7**

A mixture of rhamnoside pyrazine dimer **8** was obtained from diol pyrazine dimer **4** by the treatment with 2,3,4-*O*-trimethylsilyl- α -L-rhamnopyranosyl iodide, TBAI and DIPEA in dry DCM. The rhamnoside pyrazine dimer **8** was acetylated using the classical method and

obtained polyacetoxy rhamnoside pyrazine dimer **9** (23%), which was deacetylated with ethanolic NH_3 to afford polyhydroxy rhamnoside pyrazine dimer **10** (56%) (Scheme 4) [5].



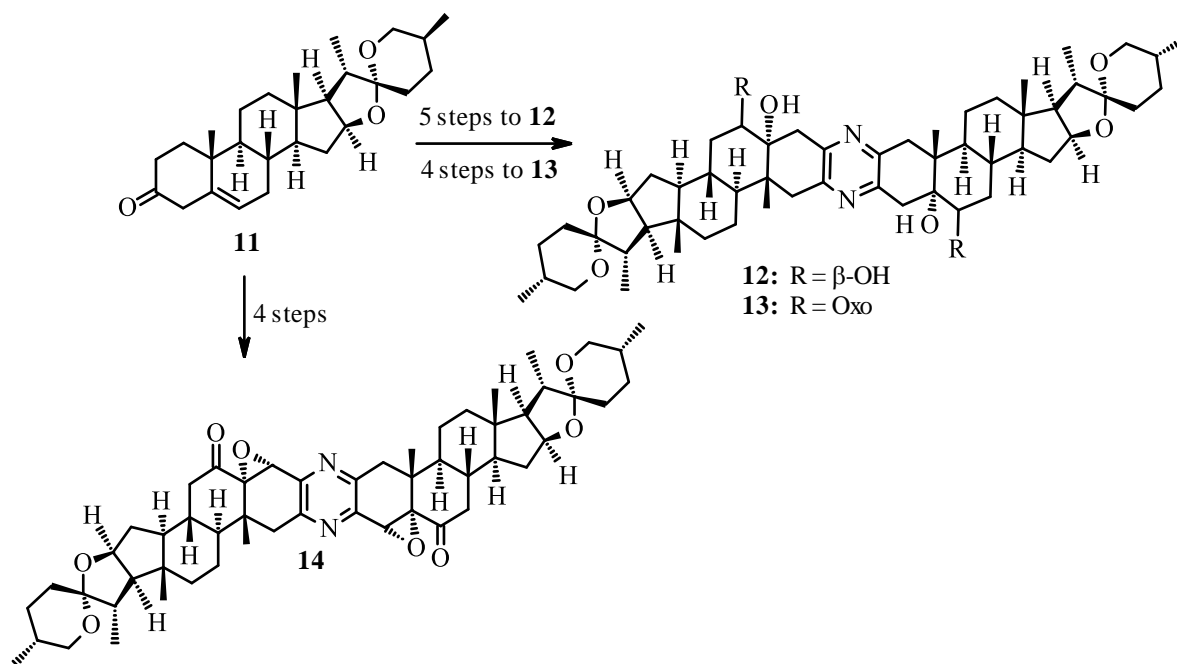
Scheme 3. Synthesis of a mixture of rhamnoside pyrazine dimer **8**



Scheme 4. Synthesis of polyhydroxy rhamnoside pyrazine dimer **10**

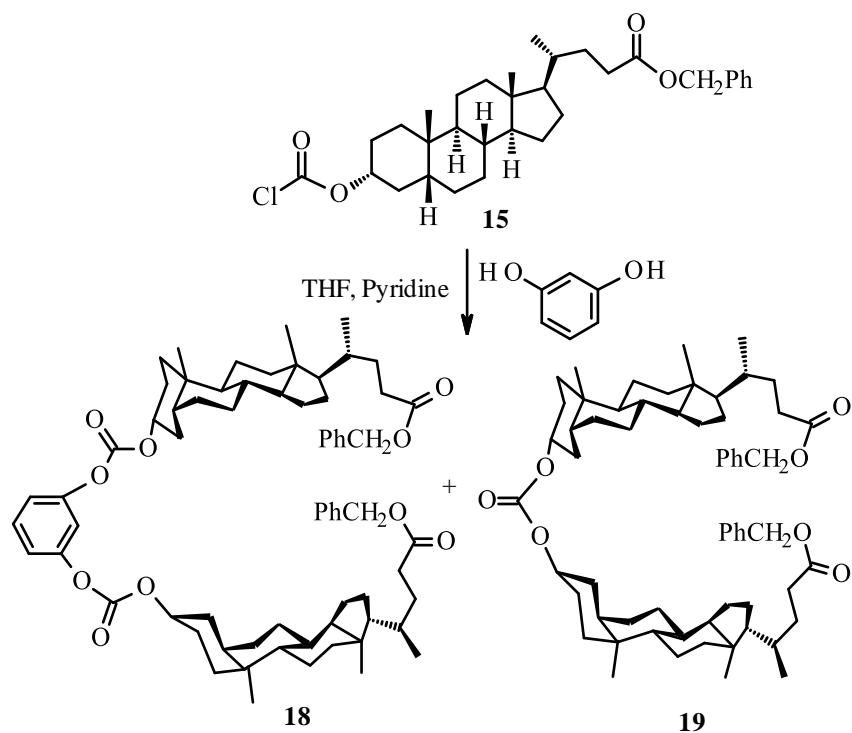
Polyoxygenated bis-steroidal pyrazine analogs are known to possess important biological properties because of the increased presence of polar functional groups. Three polyoxygenated pyrazine dimers **12-14**, analogues of marine steroidal dimers cephalostatins and ritterazines, were synthesized from readily available steroidal monomer, diosgenin (**11**) (Scheme 5) [4, 6]. The steroidal pyrazine dimers **12-14** were obtained by classical condensation of α -amino ketones, which is considered the most efficient method of pyrazine ring formation [4]. The steroidal pyrazine dimers **12** (64%) and **13** (62%) were produced from diosgenin (**11**),

respectively, in five- and four-steps. In a similar fashion, the epoxy pyrazine dimer **14** (65%) was synthesized from the same starting material (**11**) in four-steps [6].

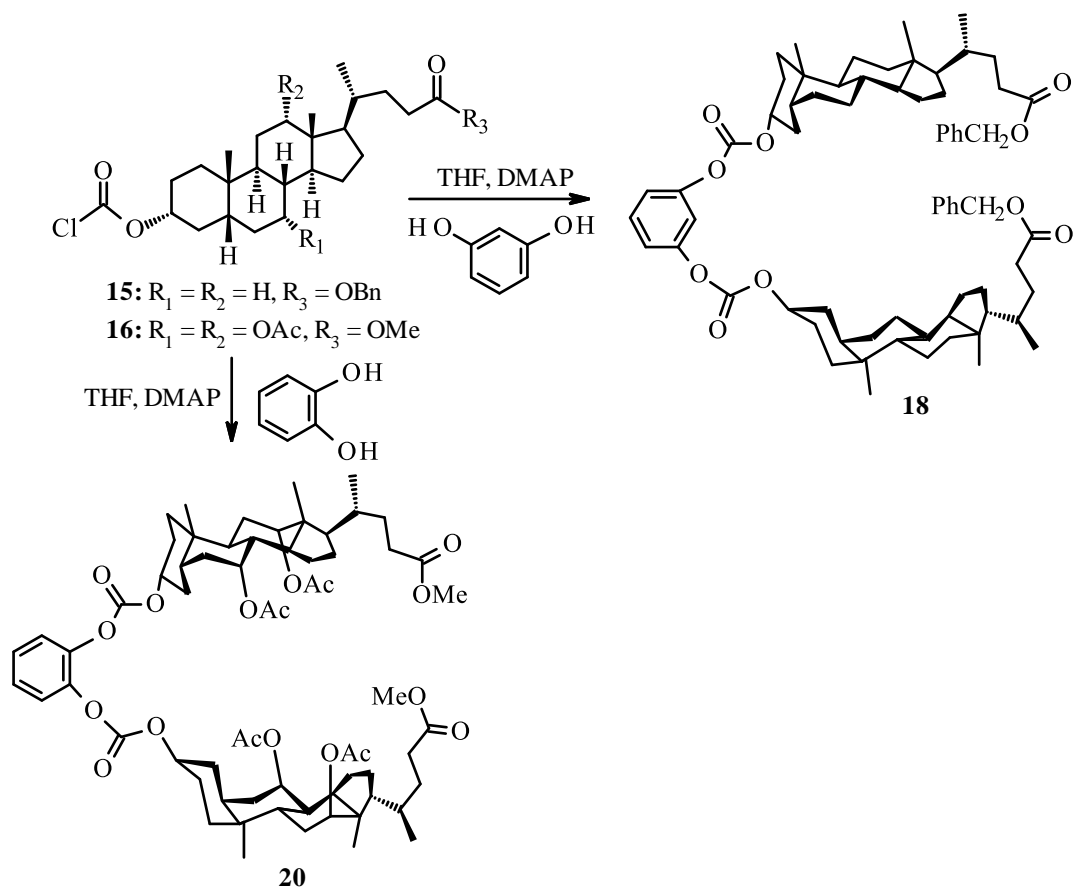


Scheme 5. Synthesis of polyoxygenated steroidal pyrazine dimers **12-14**

The unique characteristics of the bile acids in relation to their chiral, rigid and curved framework and chemically diverse hydroxyl groups have made them important building blocks in tailoring supramolecular hosts [2]. An efficient procedure for the preparation of such supramolecular hosts produced head-to-head bile acid-based dimers **18-27** bearing either carbamate or carbonate or monothiocarbonate spacer group with bile acid methyl or benzyl chloroformates **15-17**. The reaction of lithocholic acid benzyl chloroformate **15** with 1,3-dihydroxybenzene in THF catalyzed by pyridine yielded lithocholic acid carbamate dimer **18** (32%) and lithocholic acid carbonate dimer **19** (38%) (Scheme 6) [7]. Interestingly, it was observed that the DMAP catalyzed reaction of lithocholic acid benzyl chloroformate **15** with 1,3-dihydroxybenzene produced only lithocholic acid carbamate dimer **18** (92%). Similarly, the DMAP catalyzed reaction of diacetate bile acid methyl chloroformate **16** with 1,2-dihydroxybenzene produced diacetate cholic acid carbamate dimer **20** (90%) (Scheme 7) [7].

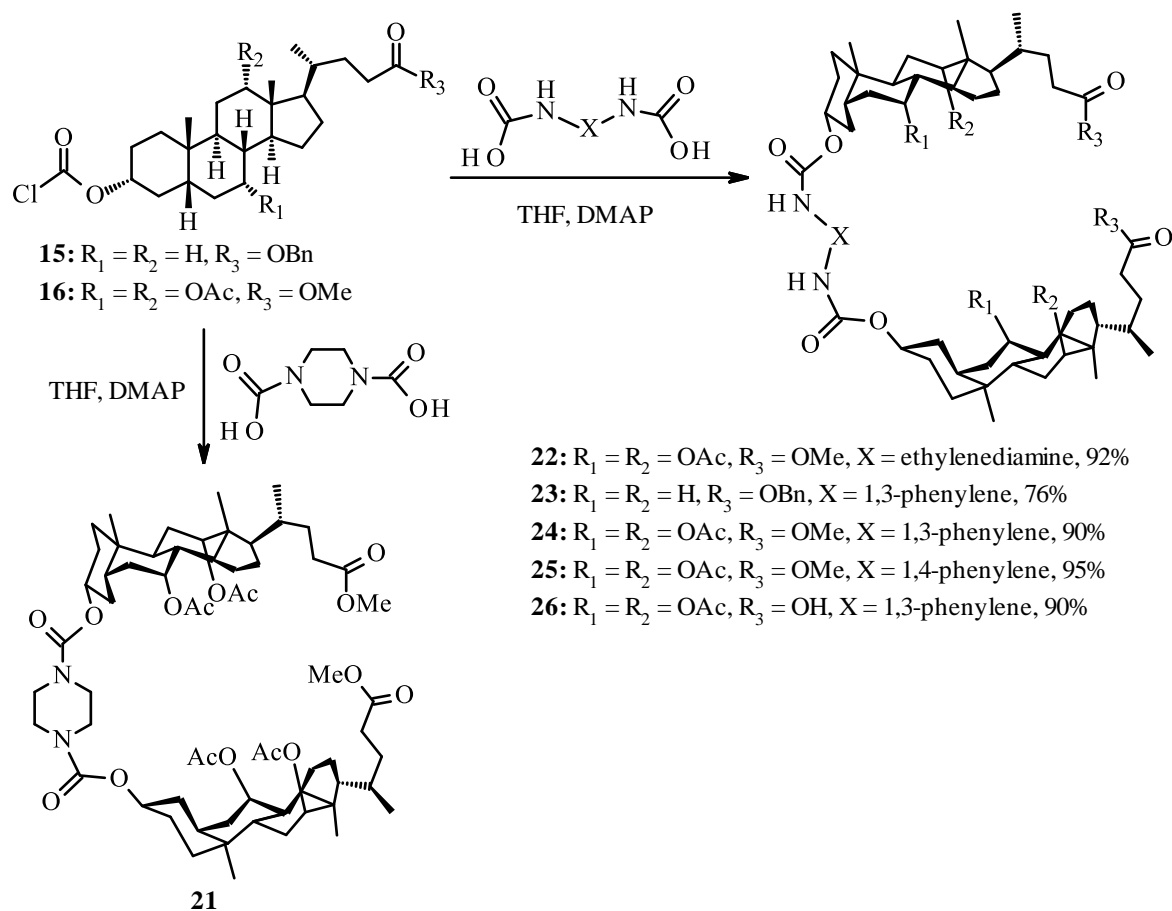


Scheme 6. Synthesis of lithocholic acid carbamate dimer **18** and carbonate dimer **19**



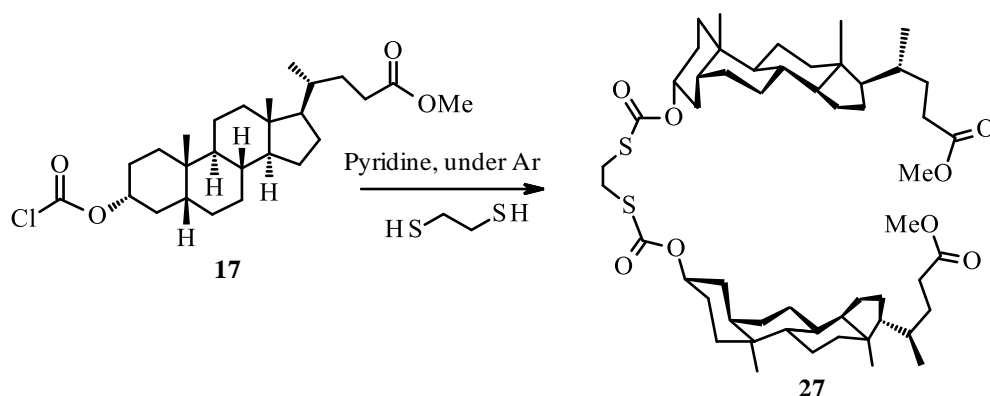
Scheme 7. Synthesis of diacetate cholic acid carbamate dimer **20**

Following the same procedure, further six bis-carbamates **21-26** (76-95%) (Scheme 8) based on bile acid derivatives with *N,N'*-dinucleophiles were prepared, respectively, by the DMAP catalyzed reactions of lithocholic acid benzyl chloroformate **15** or diacetate bile acid methyl chloroformate **16** with acyclic or acyclic aliphatic diamines and aromatic diamines in THF [7].



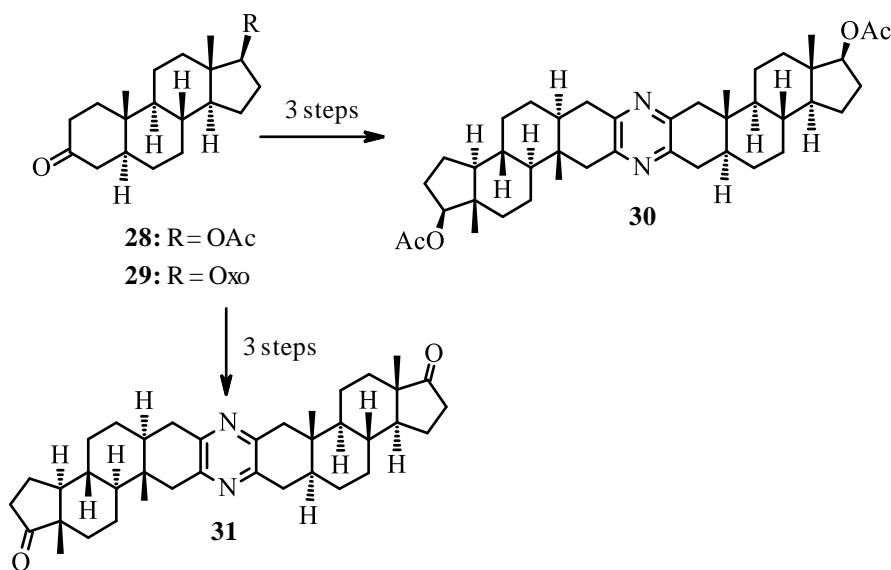
Scheme 8. Synthesis of bis-carbamates **21-26**

It was observed that the dimerization of chloroformate with *S,S*-dinucleophile was not that effective. So, as an alternate, the reaction of lithocholic acid methyl chloroformate **17** with 1,2-ethanedithiol in pyridine under argon afforded lithocholic acid monothiocarbonate dimer **27** (55%) along with small quantity of unreacted starting steroidal monomer **17** (15%) (Scheme 9) [7].



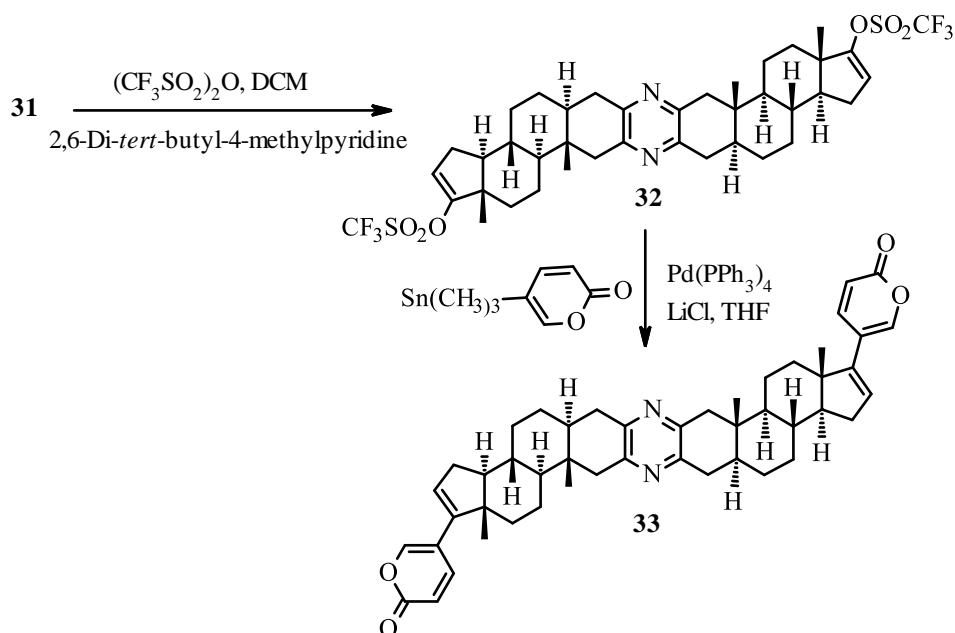
Scheme 9. Synthesis of lithocholic acid monothiocarbonate dimer **27**

Steroidal pyrazine dimers **29-34** were synthesized (Schemes 10-12) and screened for the cancer cell growth inhibitory activity [8]. First two steroidal pyrazine dimers, diacetate pyrazine dimer **30** (45%) and dione pyrazine dimer **31** (45%) were obtained, respectively, from 5 α -dihydrotestosterone acetate (**28**) and 5 α -androstanedione (**29**) in three-steps.



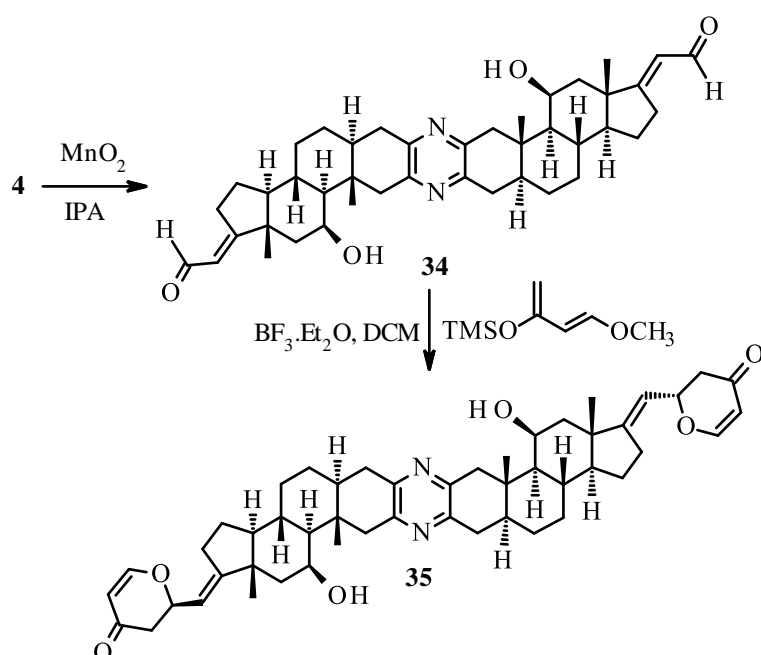
Scheme 10. Synthesis of steroidal pyrazine dimers **29-34**

Dione pyrazine dimer **31** was converted to enol triflate pyrazine dimer **32** (84%) by the reaction with triflic anhydride and 2,6-di-*tert*-butyl-4-methylpyridine in DCM. The bis-bufadienolide pyrazine **33** (31%) was prepared from enol triflate pyrazine dimer **32** in THF by reacting with freshly prepared stannyl pyrone and then added LiCl and Pd(PPh₃)₄ (Scheme 11) [5, 8].



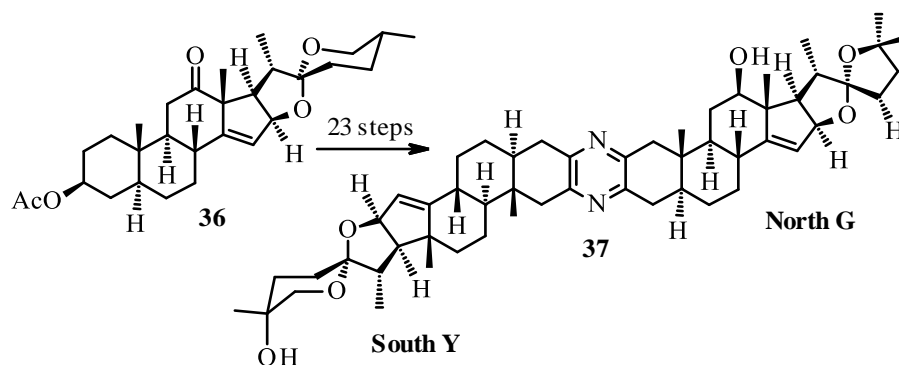
Scheme 11. Synthesis of bis-bufadienolide pyrazine **33** from dione pyrazine dimer **31**

Selective oxidation of diol pyrazine dimer **4** using MnO_2 in isopropyl alcohol (IPA) provided dialdehyde pyrazine dimer **34** (66%). Finally, the synthesis of dihydro-4-pyrone pyrazine dimer **35** (17%) was achieved via a Diels-Alder-type cycloaddition reaction of dialdehyde pyrazine dimer **34** with Danishefsky's diene by reacting with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in DCM (Scheme 12) [8].



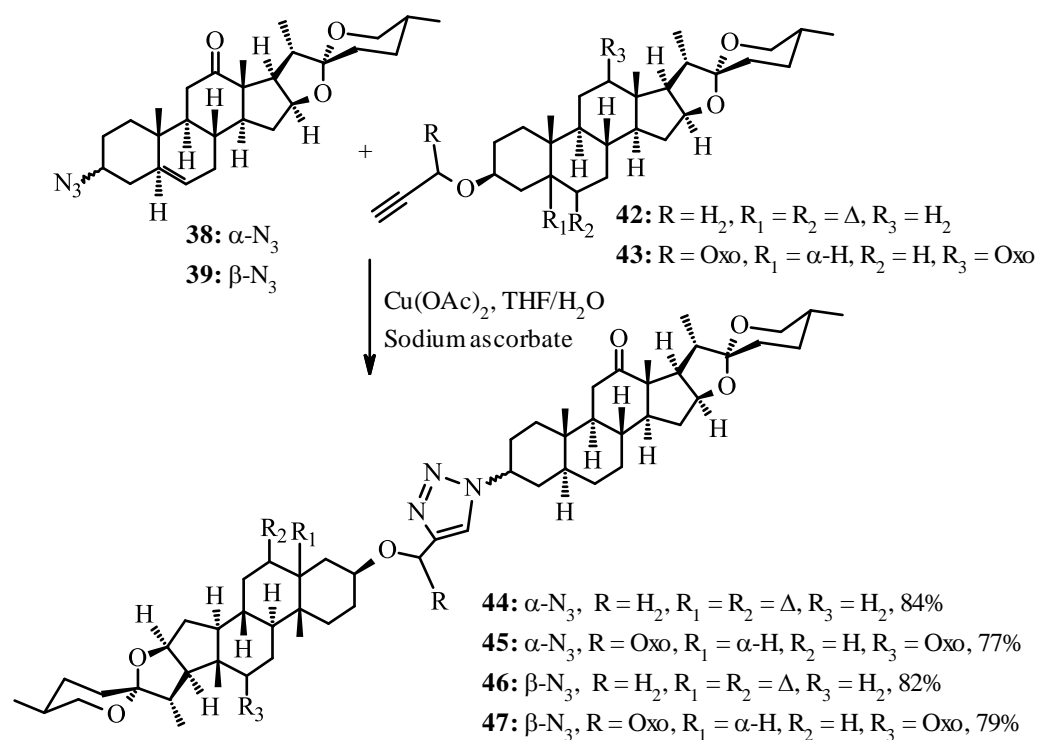
Scheme 12. Synthesis of dihydro-4-pyrone pyrazine dimer **35**

It is well-known that the ritterazine-cephalostatin family of natural products displays potent antitumor activities, and because of this, these compounds have long been considered as target molecules for total synthesis as well as synthesis of their analogs. A ritterazine Y analog known as 14',15'-dehydro-ritterazine Y **37** (24%) was synthesized from hecogenin acetate (**36**) in 23-steps by involving some classical synthetic methods as well as employing the Guo-Fuchs asymmetric pyrazine coupling of South Y and North G units and removal of the protective benzoyl groups by basic hydrolysis in the final stage [9].

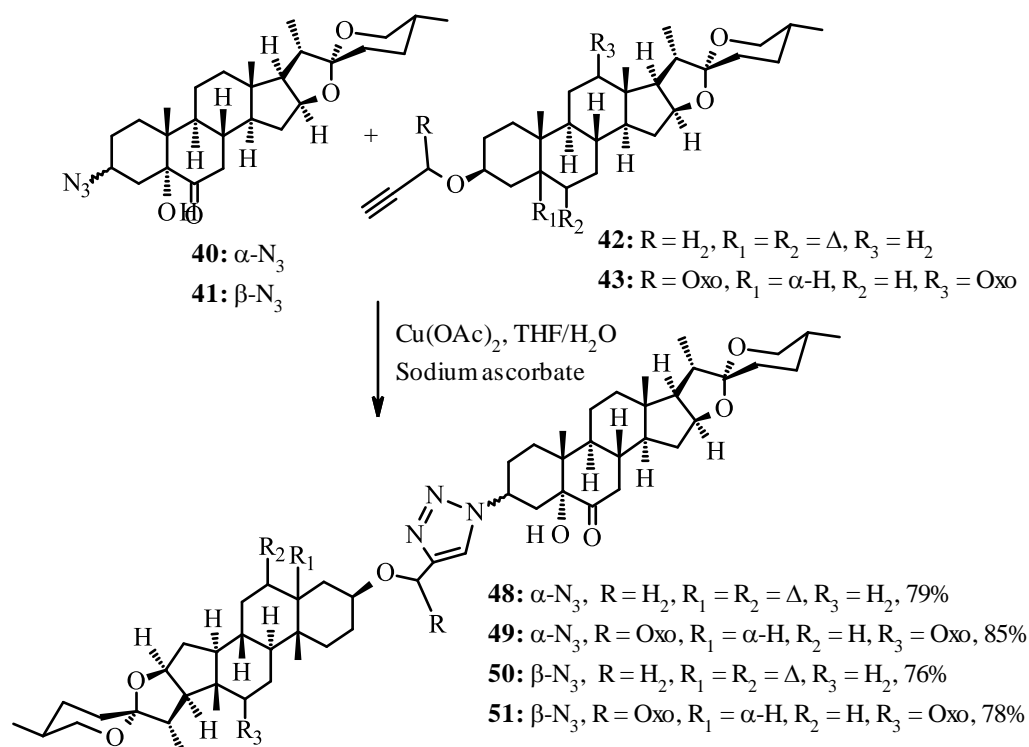


Scheme 13. Synthesis of 14',15'-dehydro-ritterazine Y **37** from hecogenin acetate (**36**)

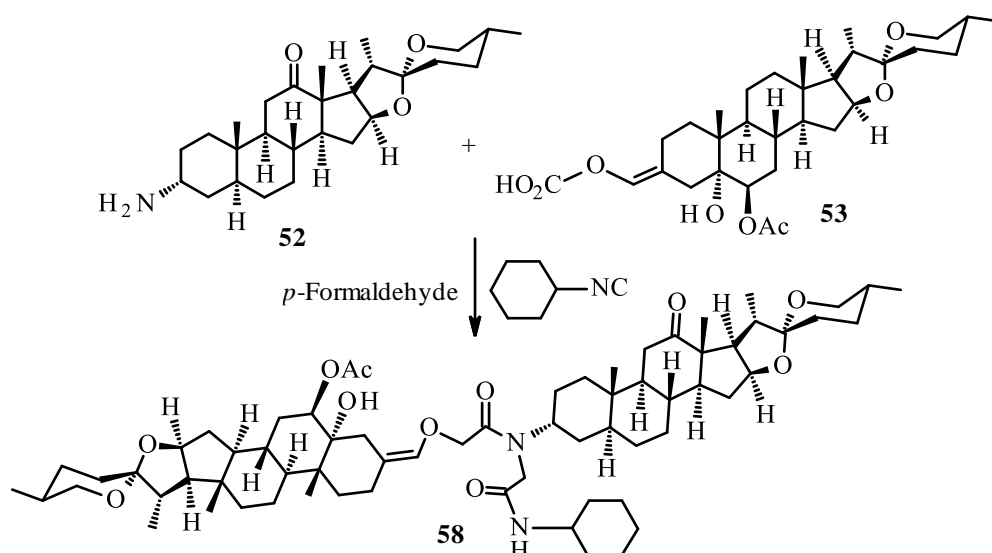
A series of triazole-linked spirostane dimers **44-51** were prepared (Schemes 14 and 15) and their conformational characteristics were extensively studied. The spirostane dimers **44-47** (77-84%) were synthesized, respectively, from 3 α -hecogenyl azide **38** and 3 β -hecogenyl azide **39** either reacting with diosgenyl alkyne **42** or hecogenyl alkyne **43** by Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction using Cu(AcO)₂ and sodium ascorbate in THF and H₂O [10]. Similarly, the spirostane dimers **48-51** (76-85%) were obtained, respectively, from 3 α -laxogenyl azide **40** and 3 β -laxogenyl azide **41** and either reacting with diosgenyl alkyne **42** or hecogenyl alkyne **43** using the same reaction conditions (Scheme 15). The same group of researchers synthesized further spirostane dimers **58-65** connected via ring A-ring A employing a one-pot condensation reaction employing the Ugi four component reactions (Ugi-4CR). Multicomponent nature as well as conformational characteristics of these compounds were evaluated. 3 α -Hecogenyl amine **52** and spirostane acid **53** in *p*-formaldehyde with cyclohexylisocyanide yielded spirostane dimer **58** (68%) (Scheme 16) [11].



Scheme 14. Synthesis of triazole-linked spirostane dimers **44-47**

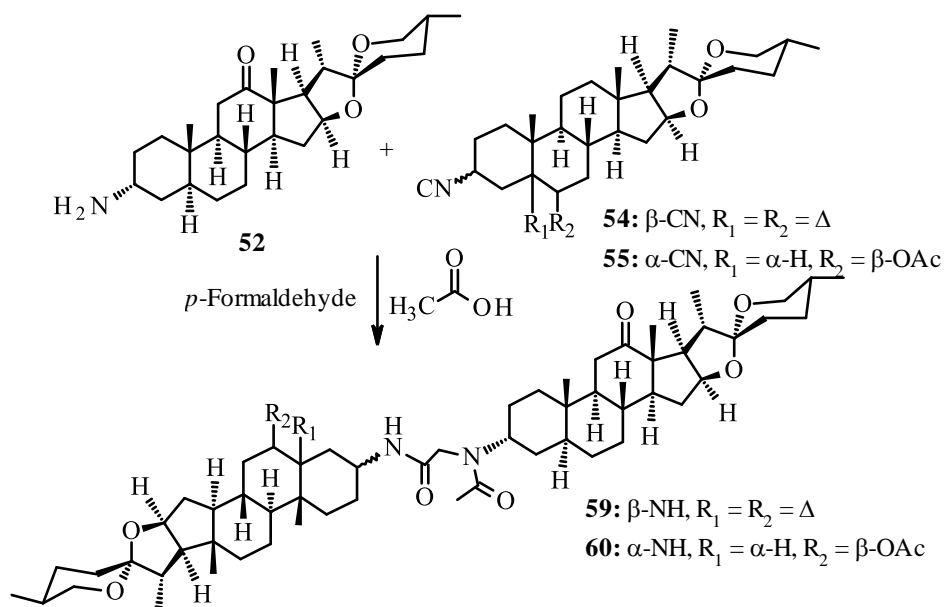


Scheme 15. Synthesis of triazole-linked spirostane dimers **48-51**



Scheme 16. Synthesis of spirostane dimer **58**

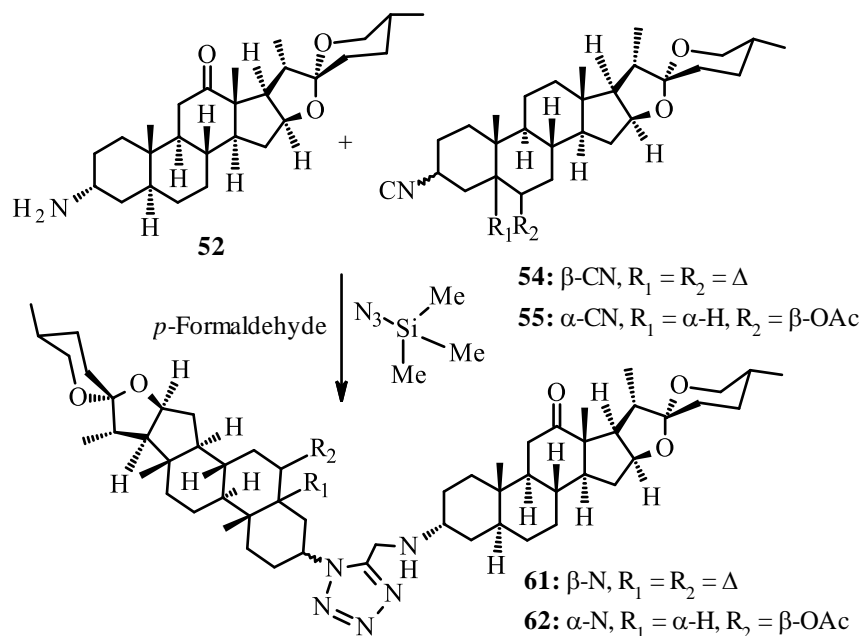
In a similar manner, two other spirostane dimers **60** (62%) and **61** (64%) were synthesized (Schemes 17 and 18) from 3α-hecogenyl amine **52** and acetic acid by reacting with α-spirostanic isocyanide **54** in *p*-formaldehyde or β-spirostanic isocyanide **55** in *p*-formaldehyde [11].



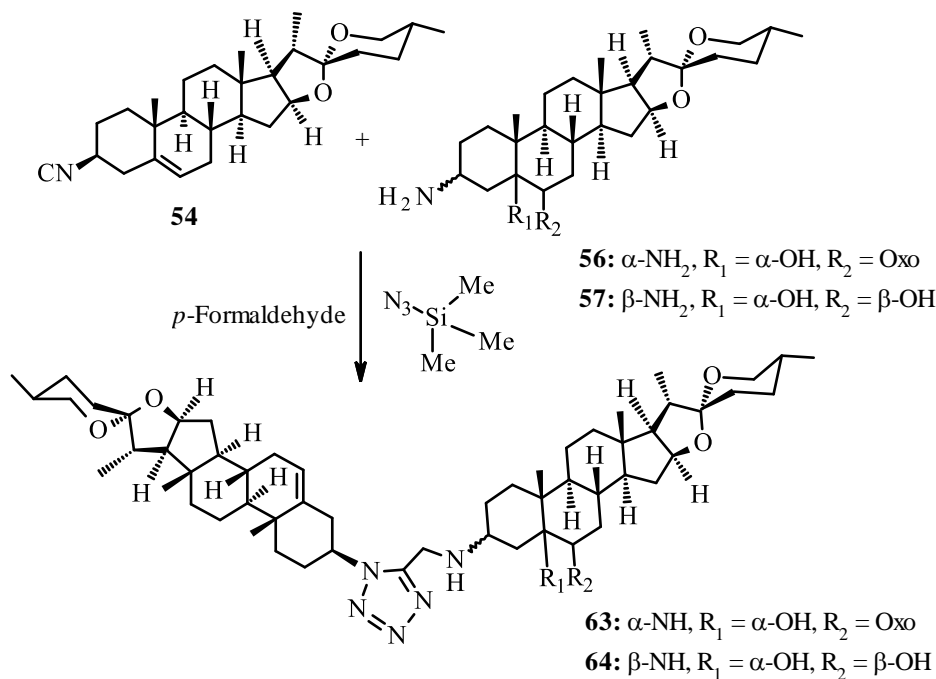
Scheme 17. Synthesis of spirostane dimers **59** and **60**

Spirostanic dimers **61-64** (Schemes 18 and 19) were synthesized using slightly different reagent. The 3α-hecogenyl amine **52** in *p*-formaldehyde reacted with α-spirostanic isocyanide **54** and azidotrimethylsilane or β-spirostanic isocyanide **55** and azidotrimethylsilane to afford

spirostane dimer **61** (63%) or spirostane dimer **62** (66%). Similarly, α -spirostanic isocyanide **54** reacted either with α -spirostanic amine **56** or β -spirostanic amine **57** and azidotrimethylsilane in *p*-formaldehyde to provide spirostane dimer **63** (58%) or spirostane dimer **64** (84%) (Scheme 19) [11]

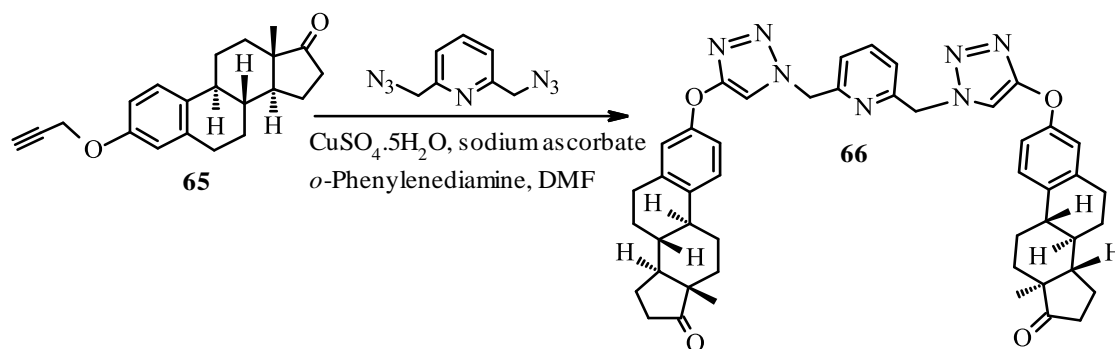


Scheme 18. Synthesis of spirostane dimers **61** and **62**



Scheme 19. Synthesis of spirostane dimers **63** and **64**

A “ribbon” type head-to-head etinic acid dimer **66** (77%) was produced by 1,3-dipolar cycloaddition reaction from estrone propargyl ether (**65**) using 1,3-di(azidomethyl)benzene, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate and *o*-phenylenediamine in DMF (Scheme 20), and its cytotoxicity effect was studied [12].

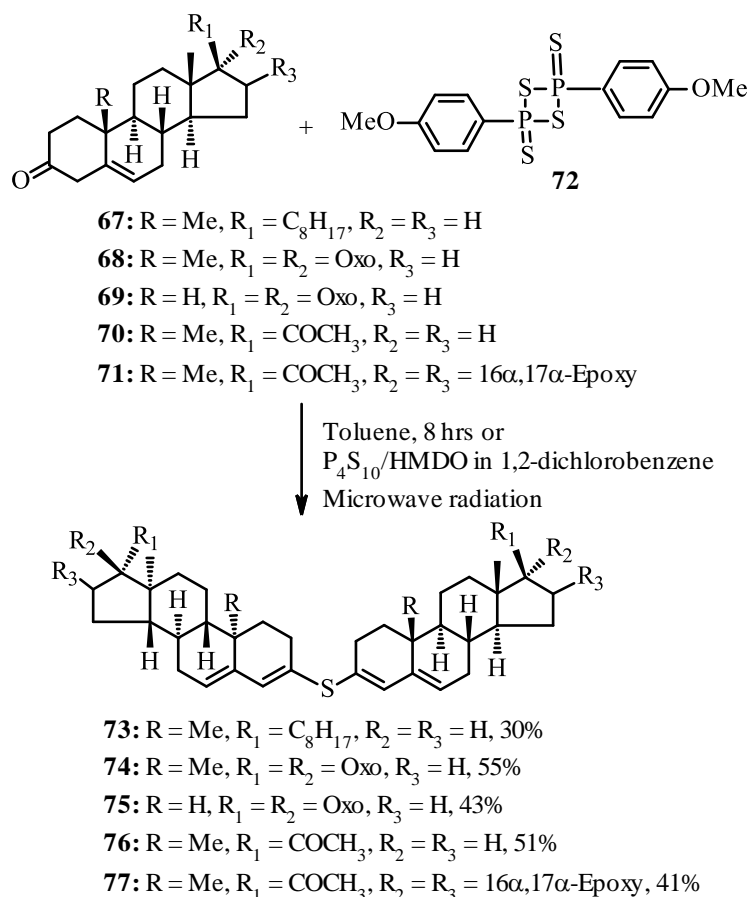


Scheme 20. Synthesis of etinic acid dimer **66**

Steroidal dimers **73-86** connected via ring A-ring A were synthesized and their *in vitro* cytotoxicity and antimicrobial activities were evaluated. Bis-steroids **73-86** were prepared (Schemes 21 and 22), respectively, from α,β -unsaturated cholestane, androstane and pregnane carbonyl derivatives **67-71** using Lawesson’s reagent (**72**) either in toluene or in DCM and with a combination of P_4S_{10} /HMDO in 1,2-dichlorobenzene under microwave irradiation [13]. It was found that when the reaction was carried out with Lawesson’s reagent (**72**) in toluene for 8h or with a combination of P_4S_{10} /HMDO in 1,2-dichlorobenzene under microwave irradiation, only sulfide dimers **73-77** (30-51%) (Scheme 21) and some unreacted starting steroidal monomers **67-71** (26-52%) were obtained from α,β -unsaturated cholestane, androstane and pregnane carbonyl derivatives **67-71**. When the reactions were performed in DCM for 8h, a mixture of 3,3’-sulphide dimers **78-82** (Scheme 22) (11-79%) and 3,3’-phosphorotrithioate dimers **83-86** (8-36%) (Scheme 22) with some unreacted starting steroidal monomers **67-71** (8-27%) were produced, but no 3,3’-phosphorotrithioate cholestane dimer was formed [13].

A number of bis-androstanes **88a-95a** (32-42%) with 3,3’-dicarboxamide spacers were synthesized in a simple one-step reaction via palladium-catalyzed aminocarbonylation of 3-iodo-17-ethylene ketal (**87**). The lower yields could be explained by the formation of the other minor dimers **88b-95b** and **88c-95c** due to isomerization of 3,5-diene to the corresponding 2,4-diene (Scheme 23) [14].

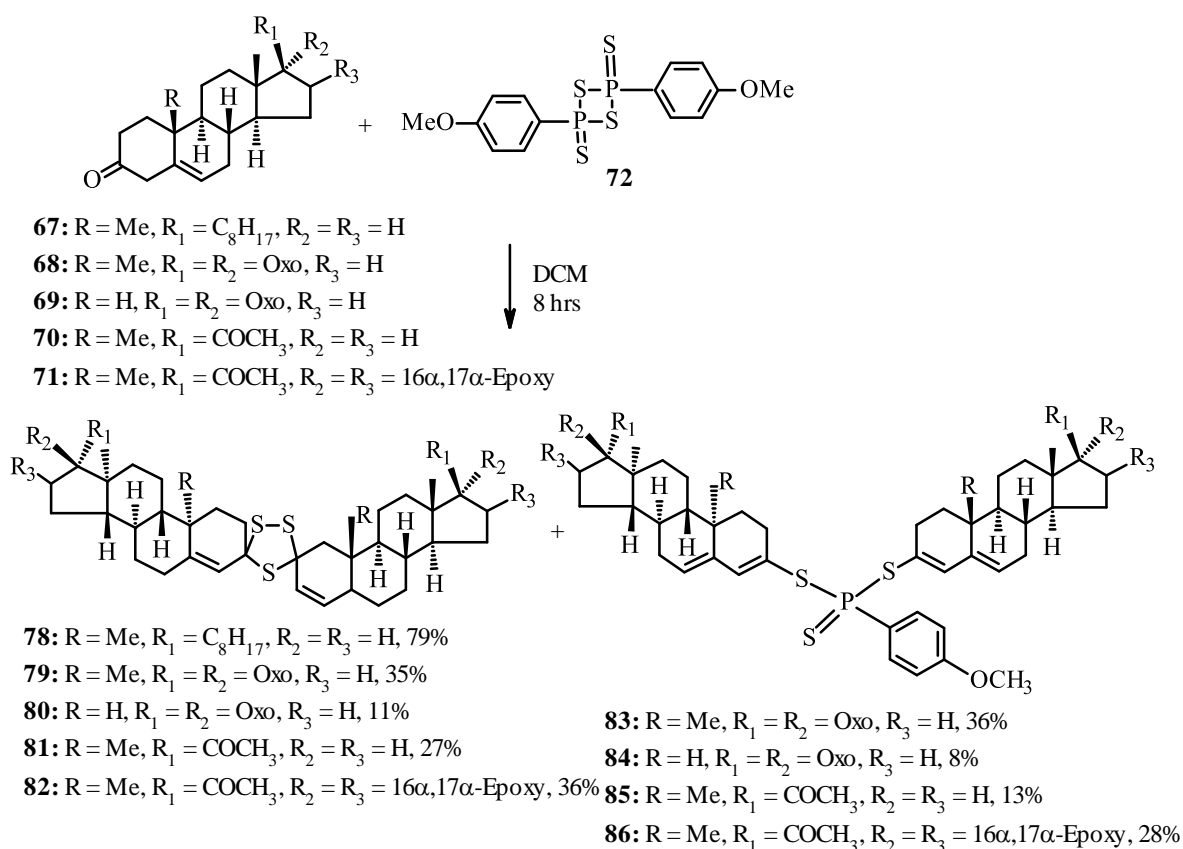
Dimeric steroidal terephthalates **98** and **99** were obtained, respectively, from epimeric 4,5-*seco*-cholest-3-yn-5-ols **96** and **97**. The crystallographic data of these dimers were analyzed. Treatment of alkynol **96** with terephthaloyl chloride, DMAP and triethylamine in dry refluxing toluene, afforded the symmetrical dimer **98** (57.8%). Similar treatment of **97** provided **99** (50.9%) (Scheme 24) [15].



Scheme 21. Synthesis of steroid sulfide dimers **73-77**

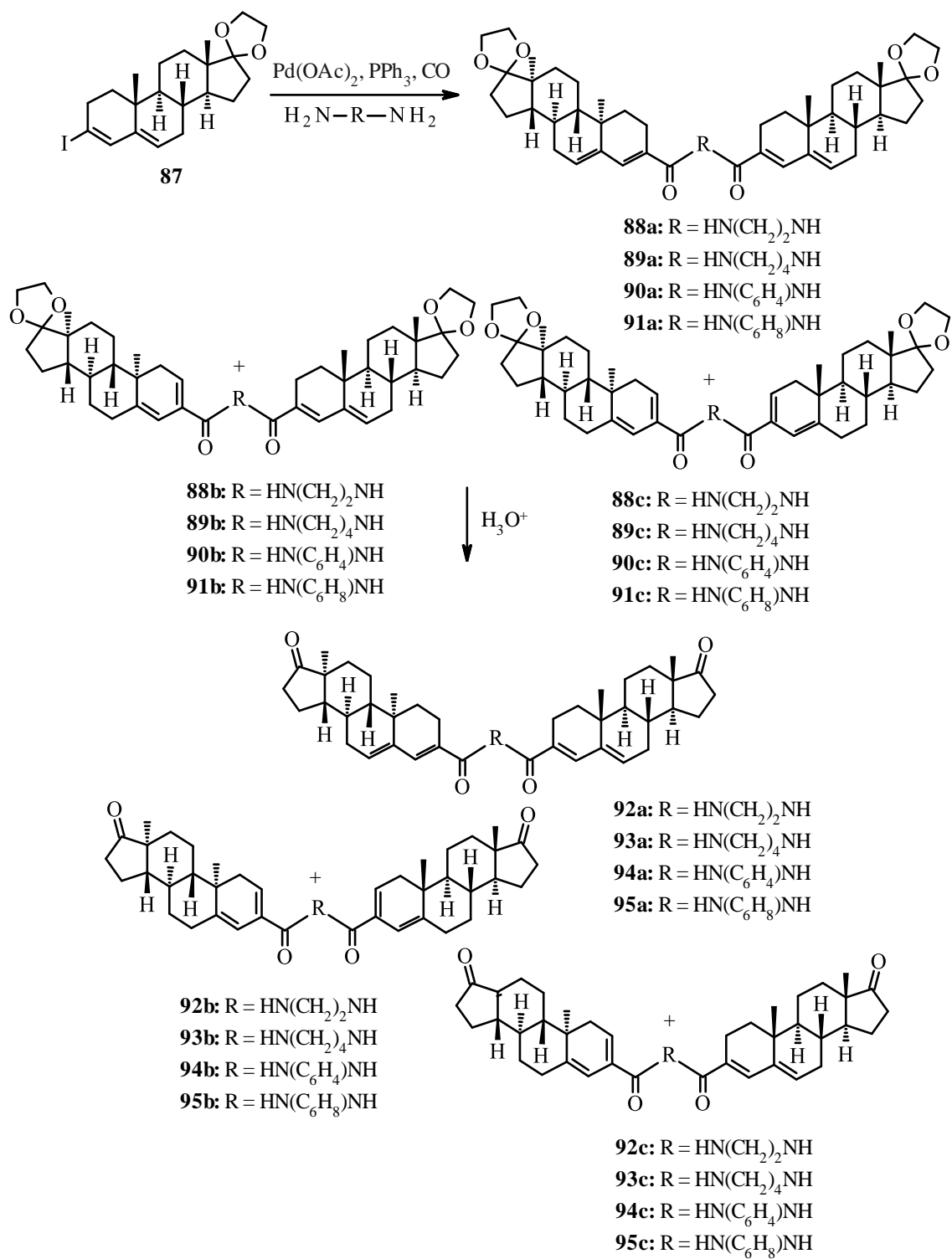
A DHEA dimer **102** (38%) was synthesized by ‘click chemistry’ via molecular hybridization approach and its antiproliferative activity was evaluated against several cancer cell lines. It can be mentioned here that the term ‘click chemistry’, introduced by K. B. Sharpless in 2001, refers to the reactions that are high yielding, wide in scope, produce only the by-products that can be removed without the use of any chromatographic techniques, are stereospecific, simple to carry out, and can be achieved using easily removable or benign solvents. In fact, ‘click chemistry’ is not a single specific reaction, but it describes a way of producing products that follow examples from nature and generates molecules by joining small modular units. The dehydroandrosterone dimer **102** was obtained from DHEA azidoacetate

100 and dipropargyl substituted 5-FU alkyne **101** reacting with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate *t*-BuOH, MeOH, THF and H_2O (Scheme 25) [16].

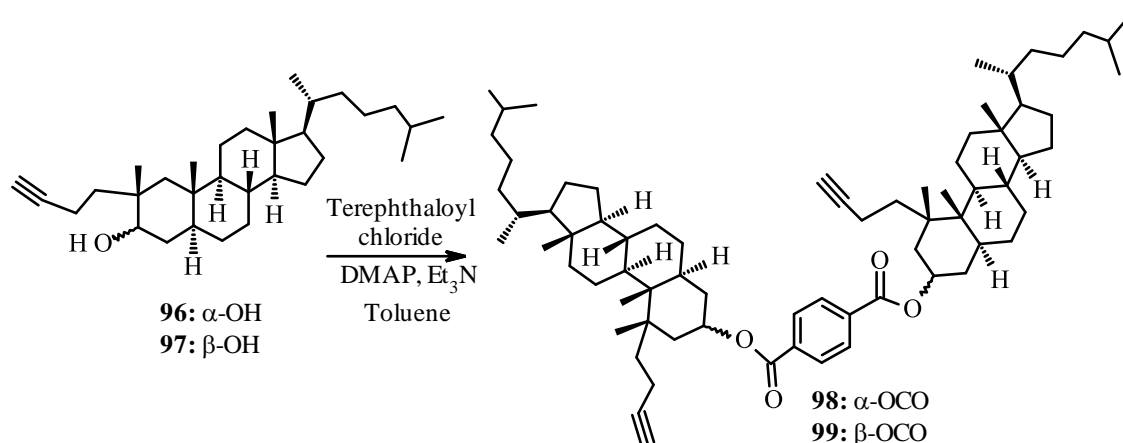


Scheme 22. Synthesis of a mixture of 3,3'-sulphide dimers **78-82** and 3,3'-phosphorotrithioate dimers **83-86**

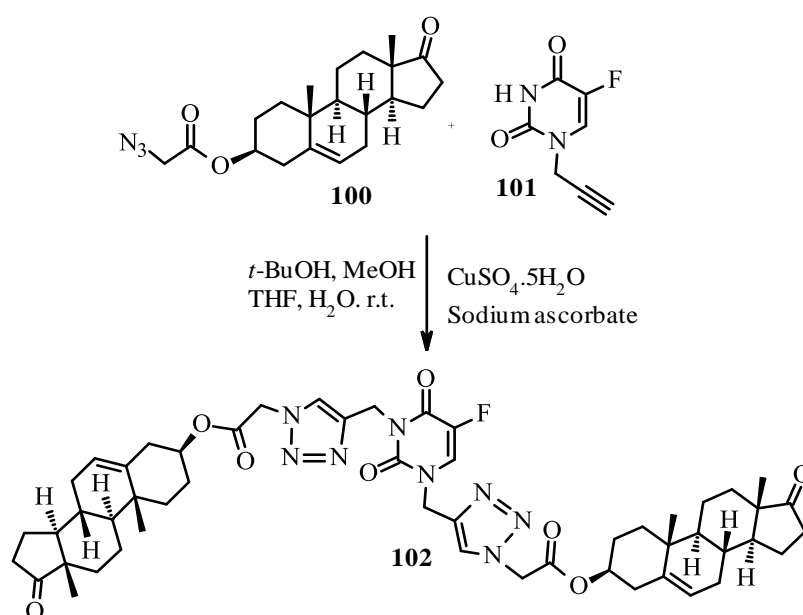
Two highly effective immunoaffinity fluorescent (IAF) probes **105** and **106** were prepared and their structure-activity-relationships as well as various antiproliferative activities were studied. Immunoaffinity fluorescent (IAF) probes are generally used to understand mode of biological actions of small molecules in relation to new drug discovery, and they allow simultaneous parallel studies at both the cellular and molecular levels. The IAF probes **105** (40%) and **106** (75%) were afforded by coupling the IAF at the C-25 position of ritterostatin $\text{G}_{\text{N}1\text{N}}$ (**103**) and 25-*epi*-ritterostatin $\text{G}_{\text{N}1\text{N}}$ (**104**), respectively, by reacting with peptide coupling reagent HATU in DIPEA and DMF (Scheme 26) [17, 18].



Scheme 23. Synthesis of bis androstanes **88a-95a**, **88b-95b** and **88c-95c**

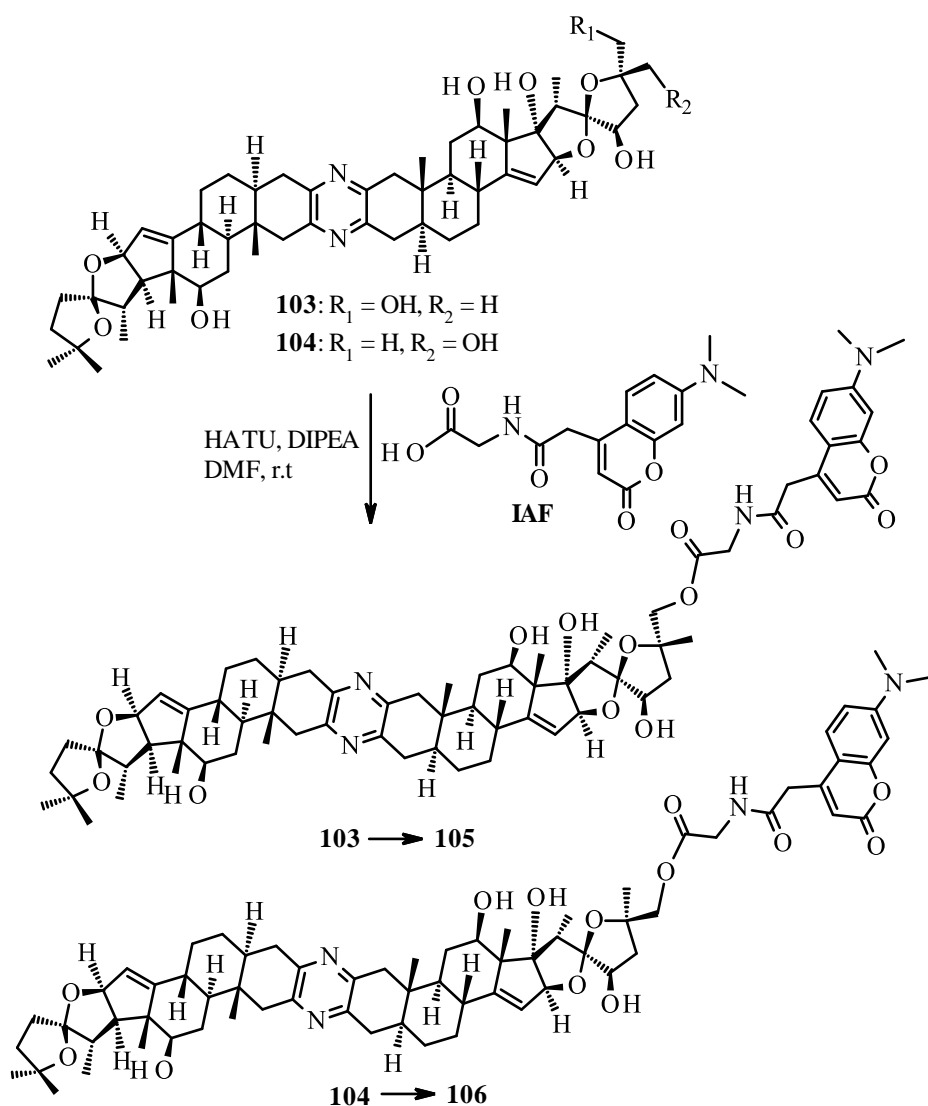


Scheme 24. Synthesis of dimeric steroidal terephthalates **98** and **99**



Scheme 25. Synthesis of dehydroandrosterone dimer **102**

Cortisol and progesterone are naturally occurring steroid hormones that play important roles in the immune system and pregnancy cycle as well as blood pressure and blood glucose concentration regulation. Cortisol and progesterone-based dimers **109-112** (25-68%) (Scheme 27) were synthesized using oxime click chemistry, respectively, from cortisol (**107**) and progesterone (**108**) by Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The acidic hydrolysis of oxime bond degradation of **109** with TFA in acetone was also studied [19].

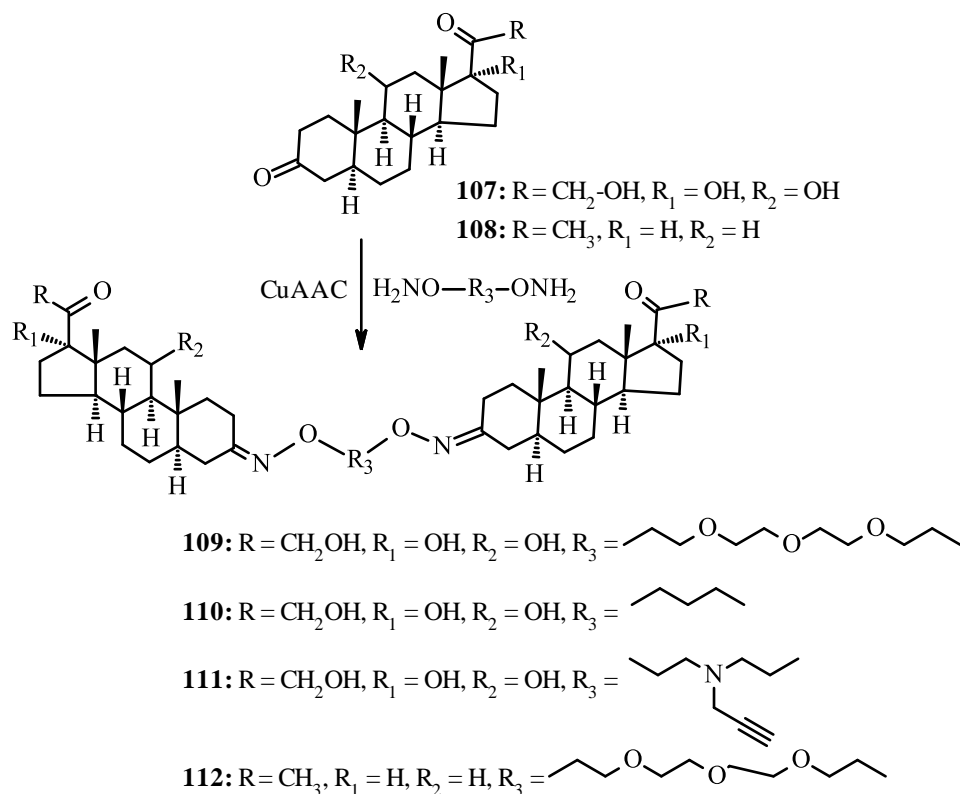


Scheme 26. Synthesis of immunoaffinity fluorescent (IAF) probes **105** and **106**

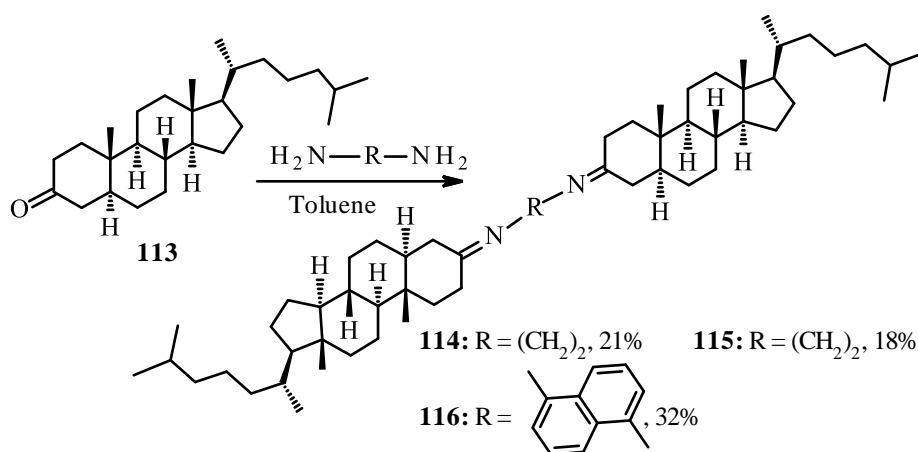
Head-to-head cholestanone ketimine dimers **114-116** (18-32%) (Scheme 28) were prepared from cholestanone (**113**) in toluene by reacting with three different amines namely 1,2-ethylenediamine, 1,4-diaminobutane and 1,5-diaminonaphthalene, respectively [20].

Sterols are multifunctional molecules and can serve as an essential membrane component. It is well-known that cholesterol dimers act as good supramolecular hosts for ions or small molecules, important in drug delivery. Two cholic acid-based dimers **118** and **119** with disulfide spacers via ring A-ring A were synthesized starting from cholic acid (**117**) (Scheme 29) [21-23]. Firstly, diol disulfide dimer **118** (93%) was produced in five-steps from cholic acid (**117**). Then iodination of diol disulfide dimer **118** with I_2 -triphenylphosphine complex in benzene and pyridine yielded diiodide disulfide dimer **119** (55%). These dimers

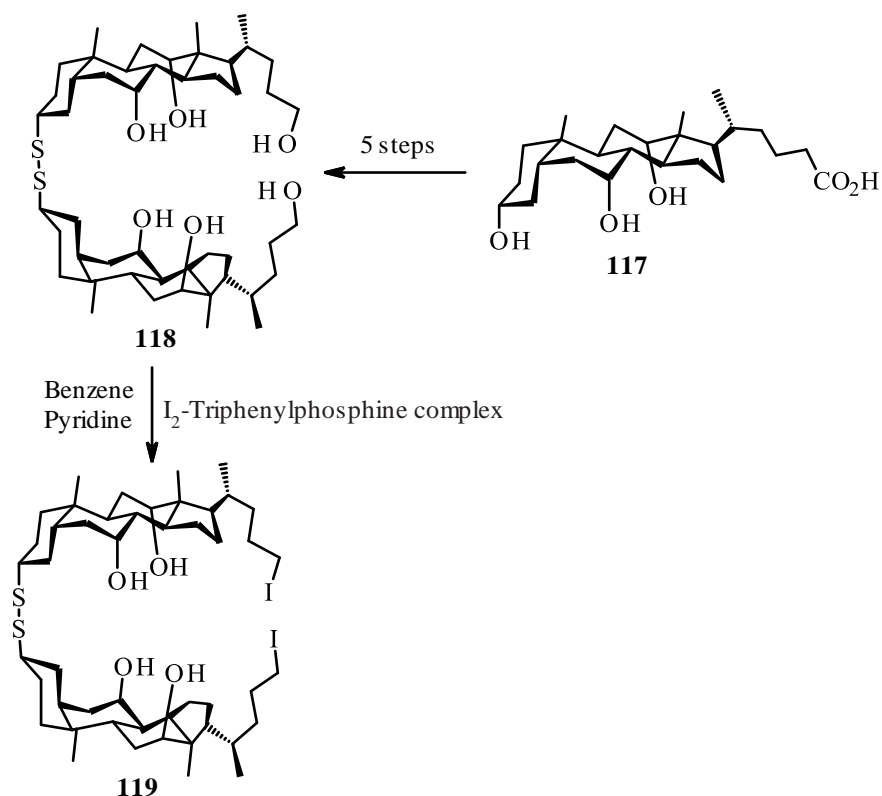
are potentially suitable for molecular or ionic recognition. Compound **119** may not be that stable, and it can be easily cyclized to form cyclic steroidal dimers, the synthesis of which is discussed under the cyclic dimers section later in this review.



Scheme 27. Synthesis of cortisol and progesterone-based dimers **109-112**



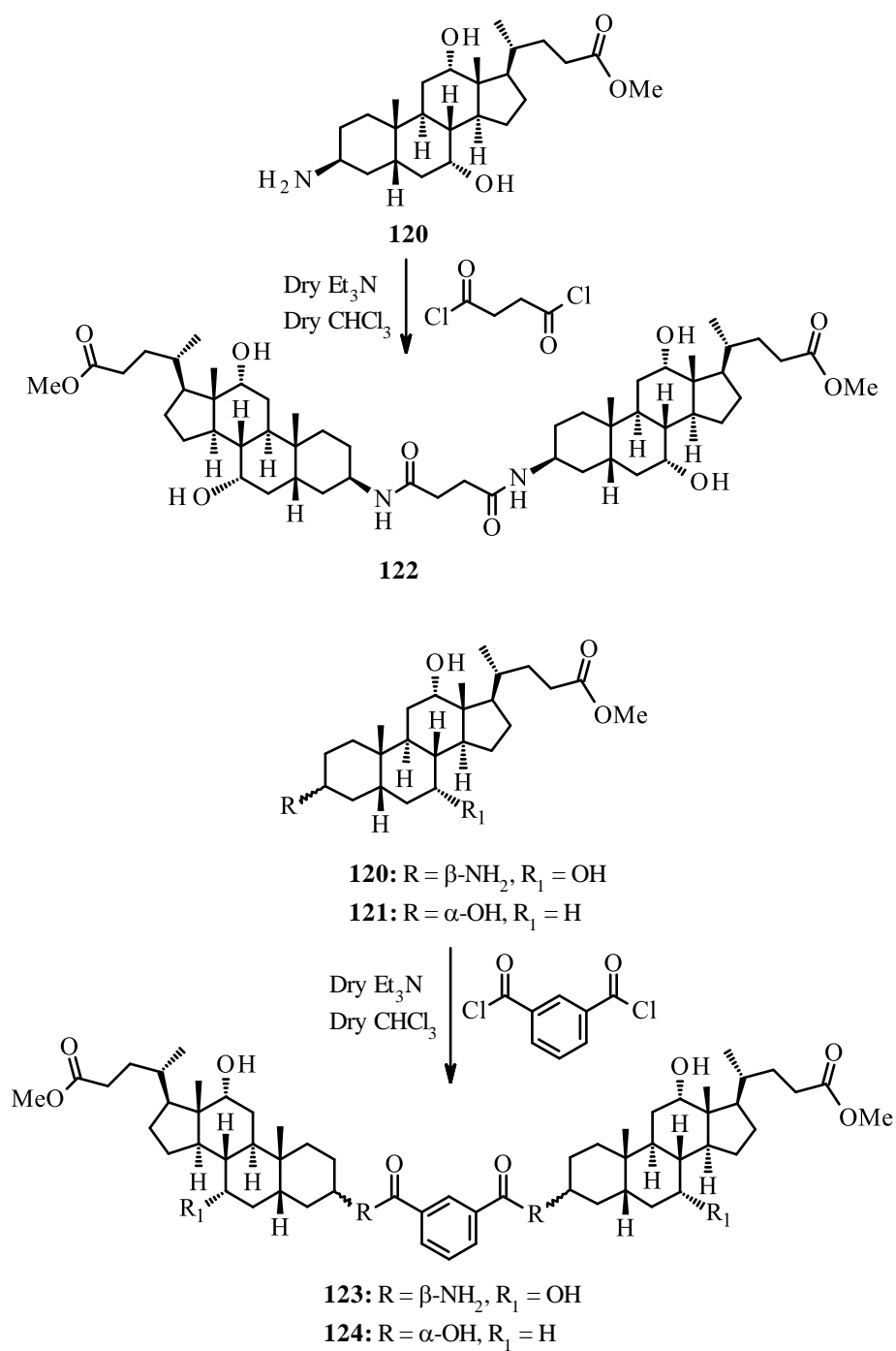
Scheme 28. Synthesis of cholestanone ketimine dimers **114-116**



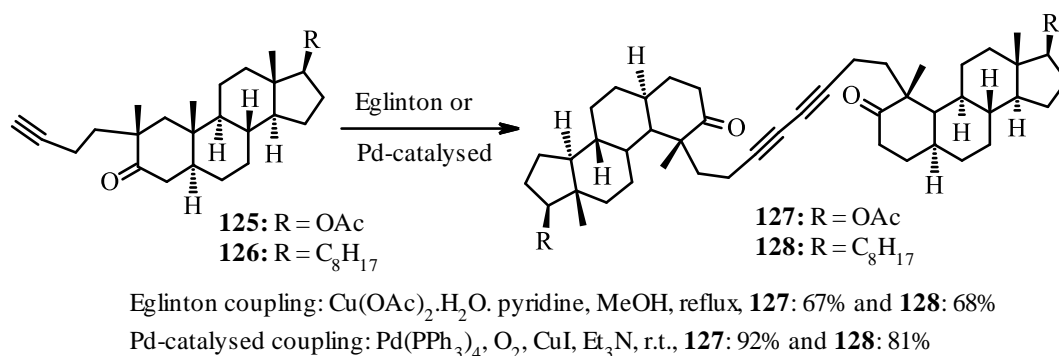
Scheme 29. Synthesis of cholic acid-based dimers **118** and **119** with disulfide spacers

Head-to-head bile acid-based dimers **122-124** connected through ring A-ring A were prepared and their crystal structures such as crystal packing and hydrogen bond network were discussed. The bile acid dimers **122-124** (40-50%) were obtained, respectively, from 3 α -amino methyl cholate (**120**) by reacting either with succinic dichloride in dry Et_3N and dry $CHCl_3$ or 1,3-benzenedicarbonyl dichloride in Et_3N and DCM and deoxy methyl cholate (**121**) by reacting with 1,3-benzenedicarbonyl dichloride in Et_3N and DCM (Scheme 30) [24, 25].

Steroidal dimers **127** (92%) and **128** (81%) were obtained, respectively, from 5-oxo-4,5-seco-3-yne steroidal monomers **125** and **126** by linking with a flexible diyne spacer group either via Eglinton coupling or Pd-catalyzed coupling. It was found that the Pd-catalyzed coupling reactions provided much higher yields (92% and 81%) than the Eglinton coupling reactions (67% and 68%) (Scheme 31) [26]. It is worth mentioning that the Eglinton coupling is an oxidative coupling of terminal alkynes, and it allows the synthesis of symmetric or cyclic bis-acetylenes via reaction of the terminal alkyne, generally, with a stoichiometric amount of a copper (II) salt in pyridine.



Scheme 30. Synthesis of head-head bile acid-based dimers **122-124**

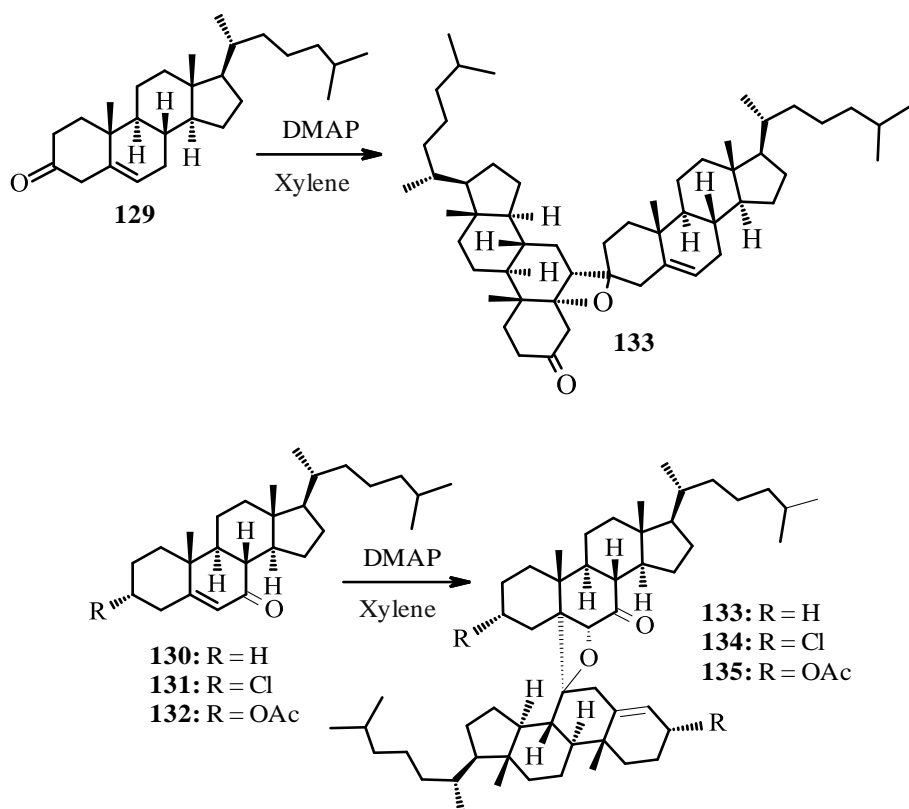


Scheme 31. Synthesis of dimers **127** and **128** - an example of Eglinton coupling

2.2 Dimers via ring A-ring B connection

Dimers via ring A-ring B connection through spacer groups can be synthesized using a suitable spacer. Ring A-ring B connected cholestenone-based dimers **133-135** (52-62%) were prepared by DMAP catalyzed dimerization, respectively, from cholest-5-en-3-one (**129**), cholest-5-en-7-one (**130**), 3 β -chlorocholest-5-en-7-ones (**131**) and 3 β -acetoxcholest-5-en-7-ones (**132**) using DMAP in xylene (Scheme 32) [27]. One might think that as the compounds **133-135** have a constrained 4-membered ring, which is entropically unfavored, the synthesis of these compounds would be difficult or even impossible. However, these compounds were synthesized using the reaction conditions shown in Scheme 32, isolated as stable dimers, crystallized and the structures of these compounds were proven conclusively by NMR and MS analyses.

Earlier several spirostanoic dimers connected via ring A-ring A were prepared in one-pot condensation reactions utilizing the Ugi four component reactions (Ugi-4CR) and following the same procedure three more ring A-ring B connected spirostanoic dimers **138-140** were afforded (Schemes 33 and 34). The spirostanoic dimer **138** (85%) was synthesized from spirostanoic acid **136** and α -spirostanoic isocyanide **55** in *p*-formaldehyde and *tert*-butyl isocyanide. In a similar fashion, the spirostanoic dimers **139** (60%) and **140** (87%) were obtained either by reacting spirostanoic acid **136** with spirostanoic amine **56** and *tert*-butyl isocyanide in *p*-formaldehyde or spirostanoic amine **137** and *tert*-butyl isocyanide in *p*-formaldehyde. [11].



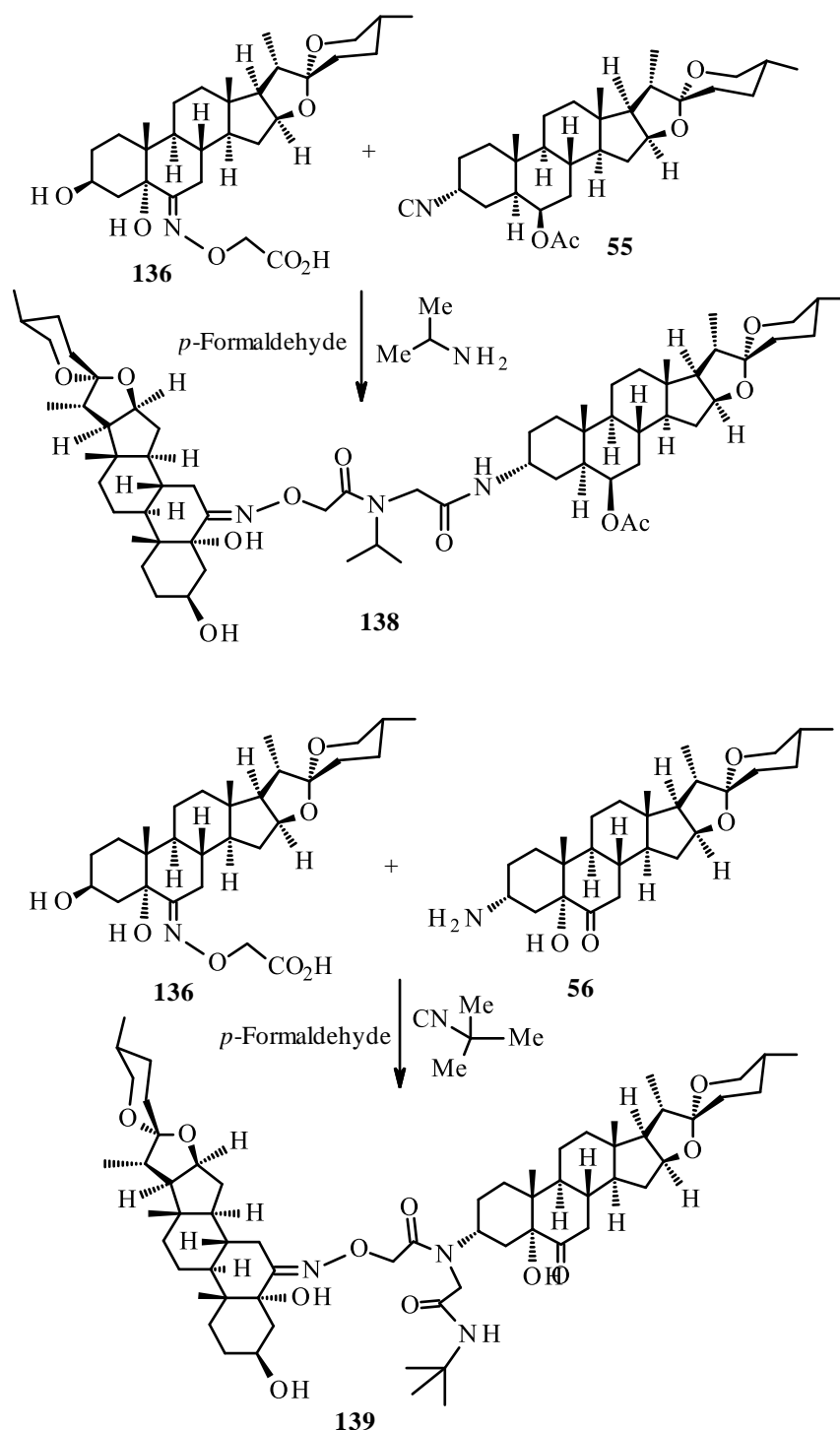
Scheme 32. Synthesis of ring A-ring B connected cholestenone-based dimers **133-135**

2.3 Dimers via ring A-ring C connection

Ring A-ring C connected spirostanoic dimers **142-144** were synthesized (Scheme 35) in one-pot condensation reactions utilizing the Ugi four component reactions (Ugi-4CR) following the same procedure described earlier [11]. The spirostanoic dimers **142** (63%) and **143** (61%) were synthesized reacting spirostanoic acid **141** either with 3 α -hecogenyl amine **52** and *tert*-butyl isocyanide in *p*-formaldehyde or spirostanoic amine **56** and *tert*-butyl isocyanide in *p*-formaldehyde. Finally, in a similar fashion spirostanoic dimer **144** (84%) was synthesized from spirostanoic acid **141** and α -spirostanoic isocyanide **55** reacting with isopropyl amine in *p*-formaldehyde [11].

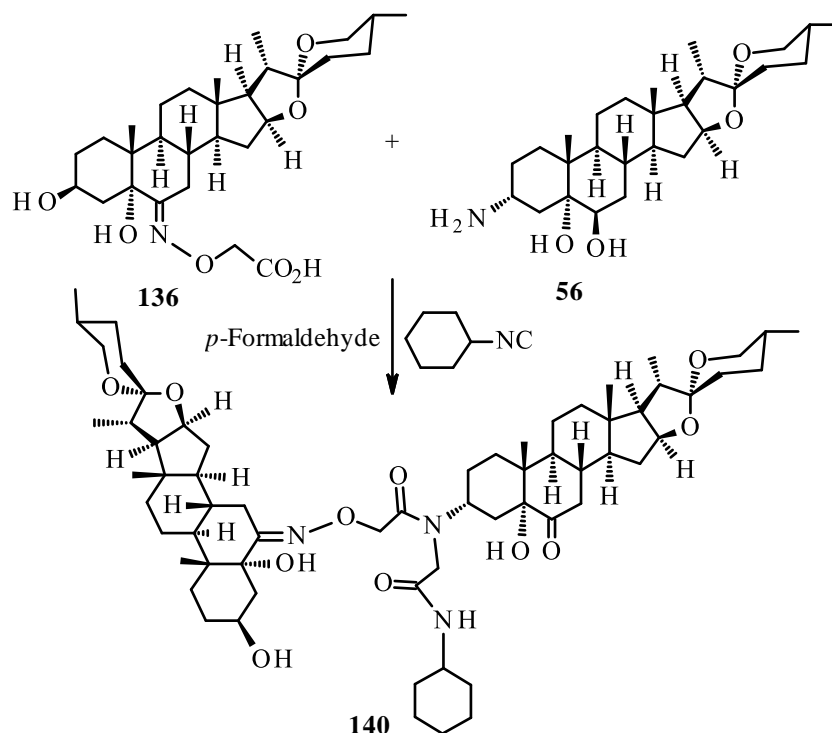
2.4 Dimers via ring A-ring D connection

The synthesis of cholic acid-based dimers **145** (95%) and **146** (43%) with disulfide spacers via ring A-ring D was achieved, starting from cholic acid (**117**) (Scheme 36) [21-23]. Firstly, 3,24-disulfide dimer **145** was produced in five-steps from cholic acid (**117**). Then the 3,24-disulfide dimer **145** was reacted with I₂-triphenylphosphine complex in benzene and pyridine to yield 3,24-diiodide dimer **146**.



Scheme 33. Synthesis of ring A-ring B connected spirostane dimers **138** and **139**

A head-tail cholic acid dimer **148** linked via a 1,2,3-triazole ring was obtained by 1,3-dipolar cycloaddition reaction of cholic acid derivative **147** in the presence of CuSO₄·5H₂O and sodium ascorbate in *t*-BuOH and H₂O or DMF and H₂O (Scheme 37) [28]. The reaction produced a mixture of unreacted substrate **147**, acyclic dimer **148** and some oligomeric compound, which were separated by silica column chromatography.



Scheme 34. Synthesis of ring A-ring B connected spirostane dimer **140**

Enantiomeric head-to-tail spirostanol-based dimers **152-155** (Scheme 38) were obtained by $\text{BF}_3 \cdot \text{Et}_2\text{O}$ -catalyzed Aldol condensation, respectively, from tigogenin acetate (**149**) and sarsasapogenin acetate (**150**) reacting with 2-formyl-estradiol diacetate **151** in DCM [29].

2.5 Dimers via ring B-ring B connection

A number of testosterone-based dimers **164-167** (45%) and **168-170** (30%) via ring B-ring B connection (Scheme 39) were synthesized from testosterone ω -hydroxy-alkyl esters **156-159** in DCM and treated with testosterone acid **163**, dicyclohexylcarbodiimide (DCC) and 4-pyrrolidinopyridine, and testosterone *para*-, *meta*- or *ortho*-hydroxymethyl-benzyl esters **160-162** in DCM and treated with testosterone acid **163**, DCC and 4-pyrrolidinopyridine [30].

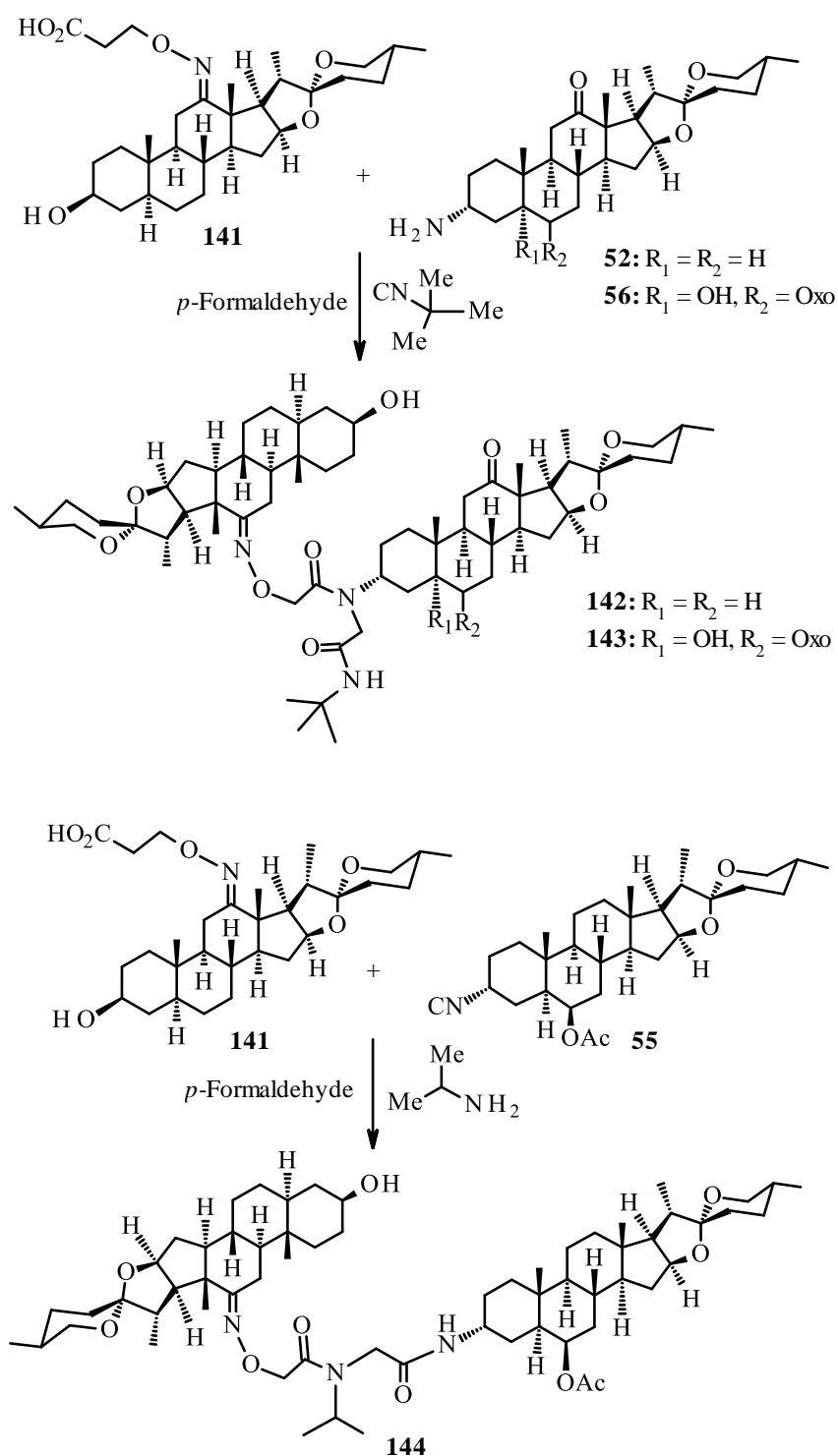
2.6 Dimers via ring D-ring D connection

There are steroid dimers formed from ring D-ring D connections, mainly linking through spacer groups, as well as involving side chains and the spacer groups.

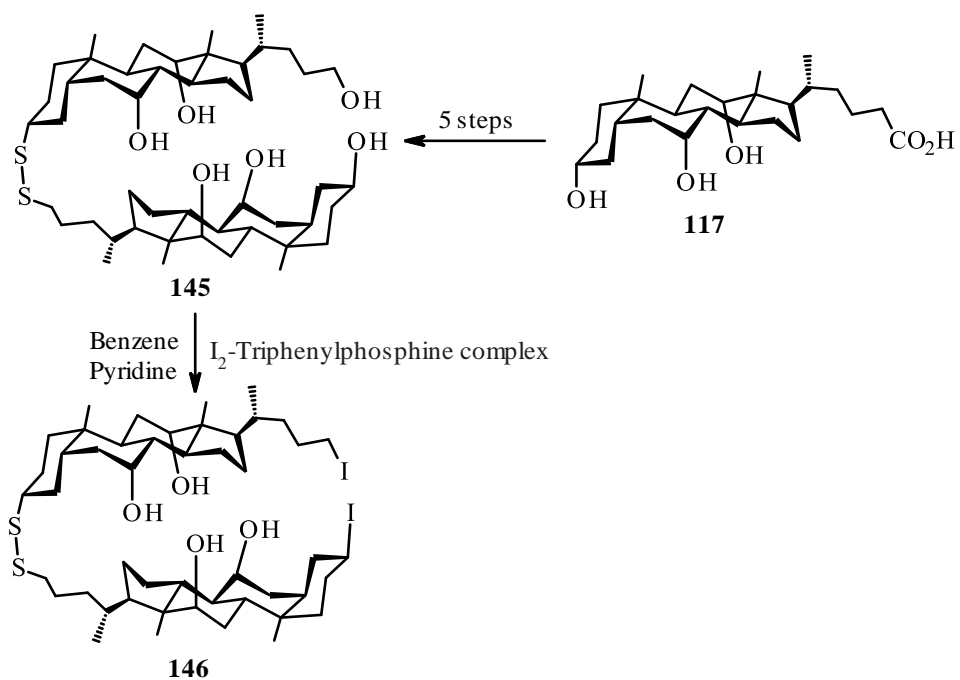
2.6.1 Through spacer groups

Two “ribbon” type etinic acid dimers **173** (82%) and **174** (68%) derived by 1,3-dipolar cycloaddition reaction from etinic acid propargyl ester **171** and etinic acid propargyl amide

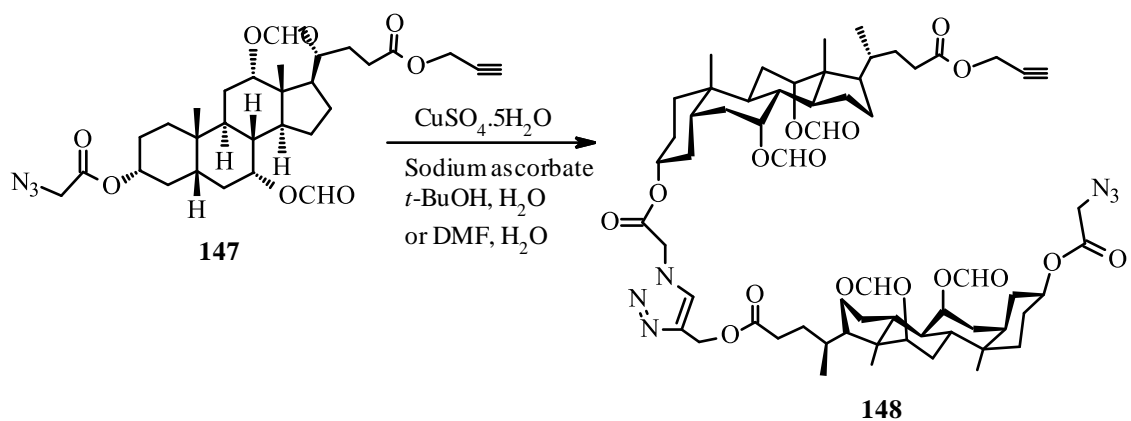
172 using 1,3-di(azidomethyl)benzene, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate and *o*-phenylenediamine in DMF (Scheme 40) [12].



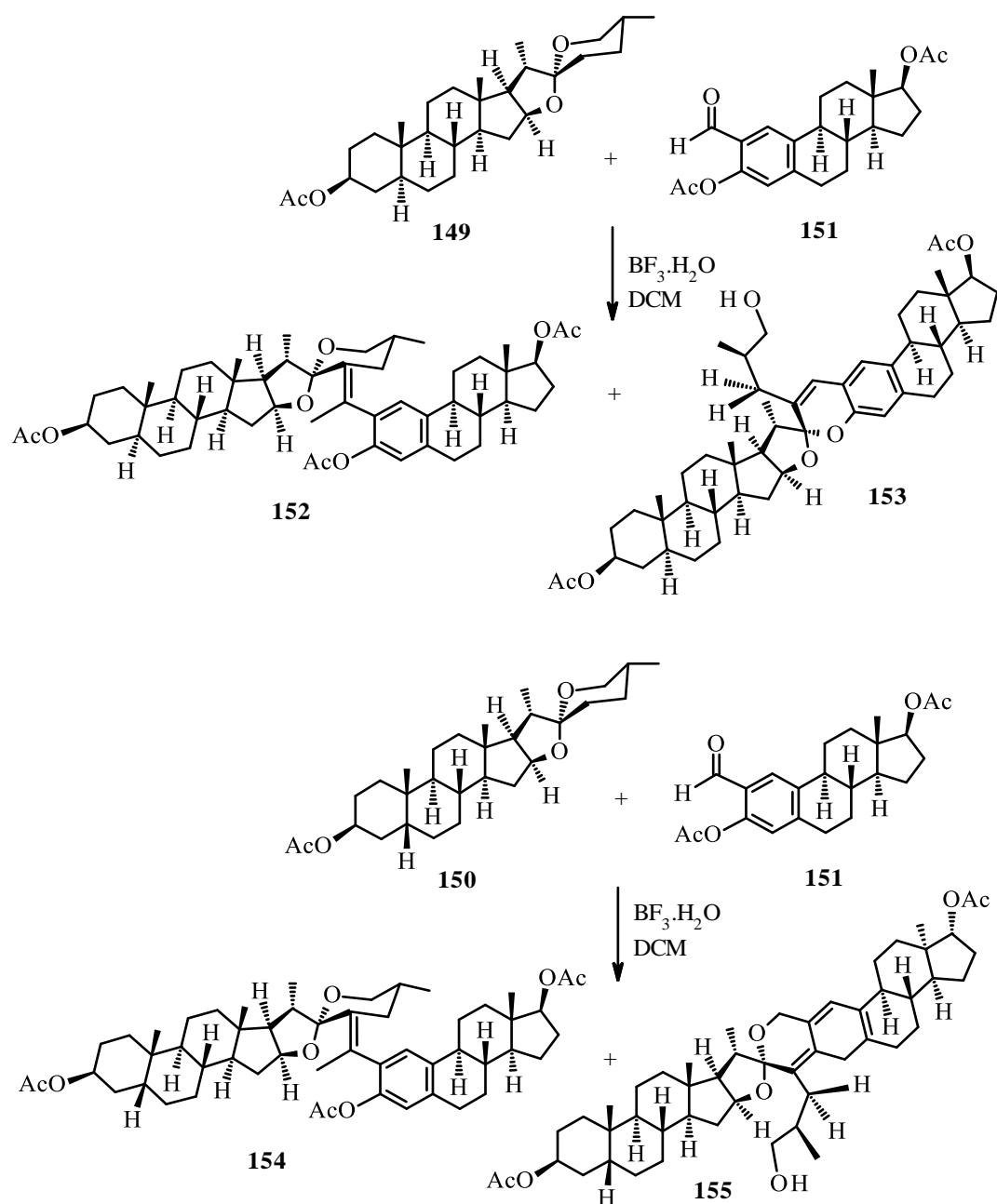
Scheme 35. Synthesis of ring A-ring C connected spirostane dimer **142-144**



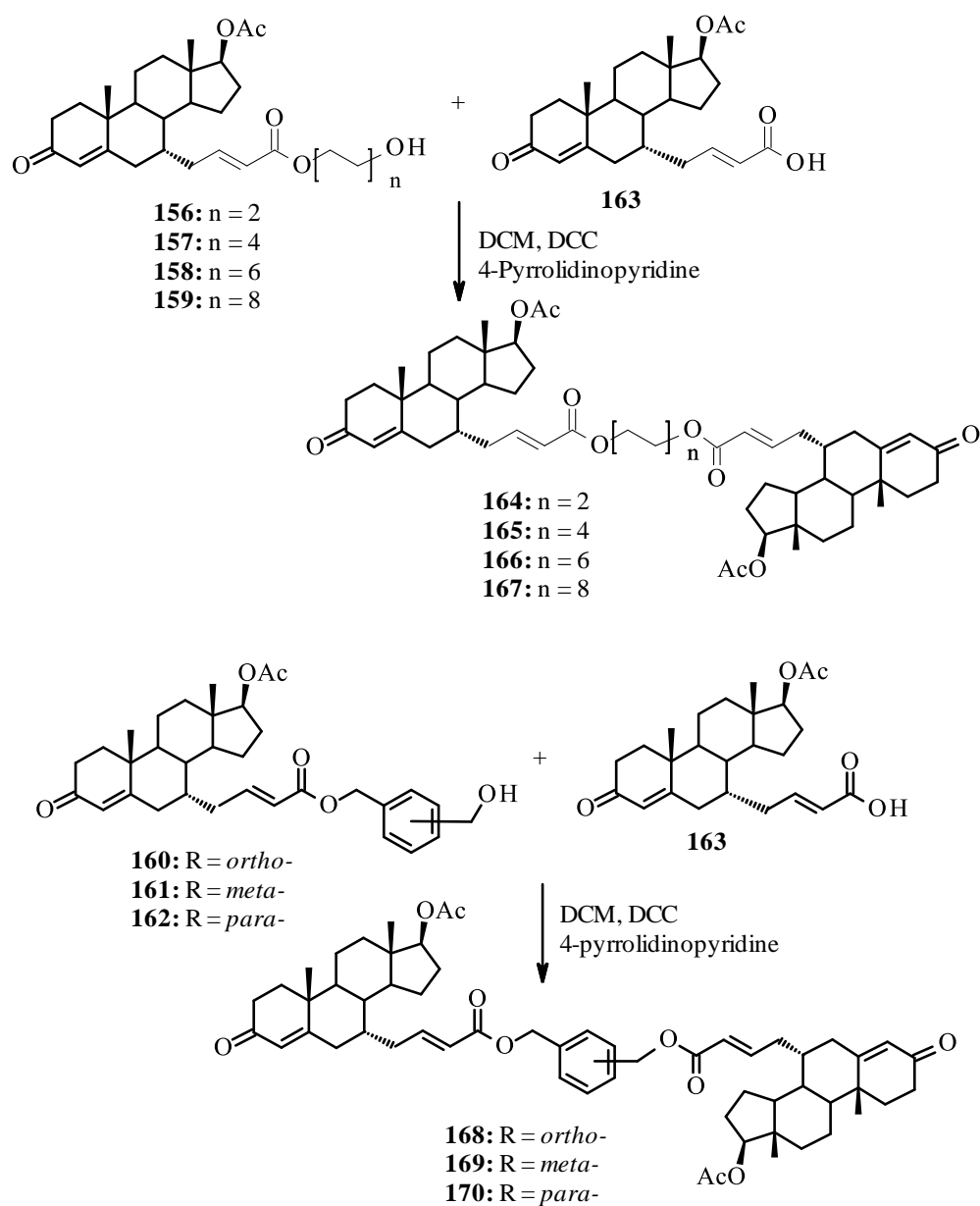
Scheme 36. Synthesis of cholic acid-based dimers **145** and **146** with disulfide spacers via ring A-ring D



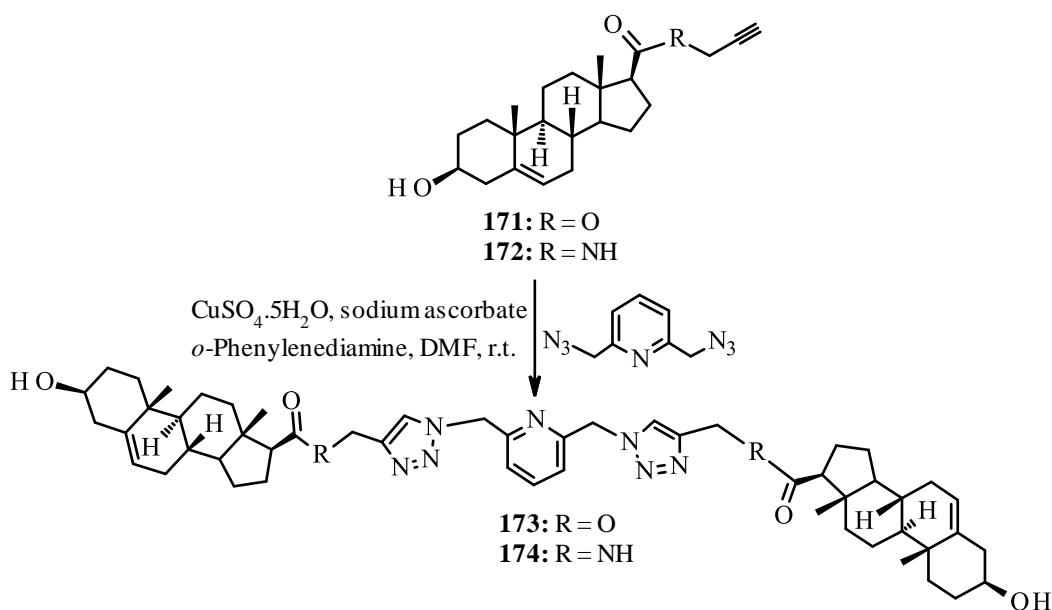
Scheme 37. Synthesis of head-to-tail cholic acid dimer **148** linked via a 1,2,3-triazole ring



Scheme 38. Synthesis of enantiomeric head-to-tail spirostanol-based dimers **152-155**

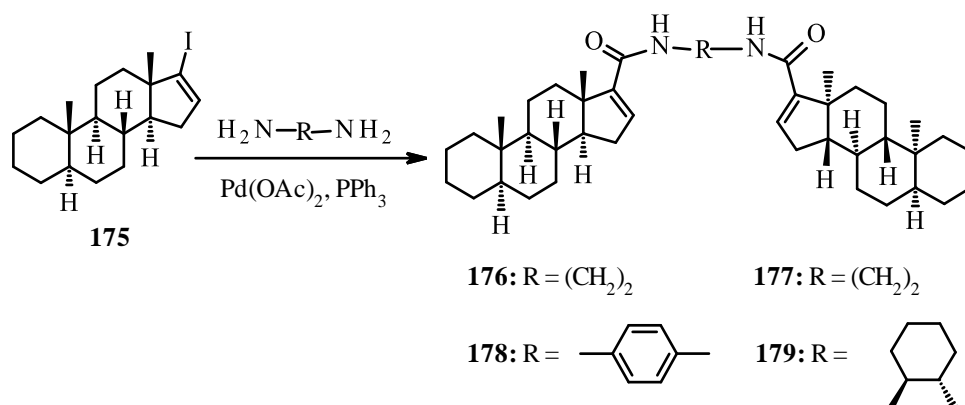


Scheme 39. Synthesis of testosterone-based dimers **164-170**



Scheme 40. Synthesis of “ribbon” type etinic acid dimers **173** and **174**

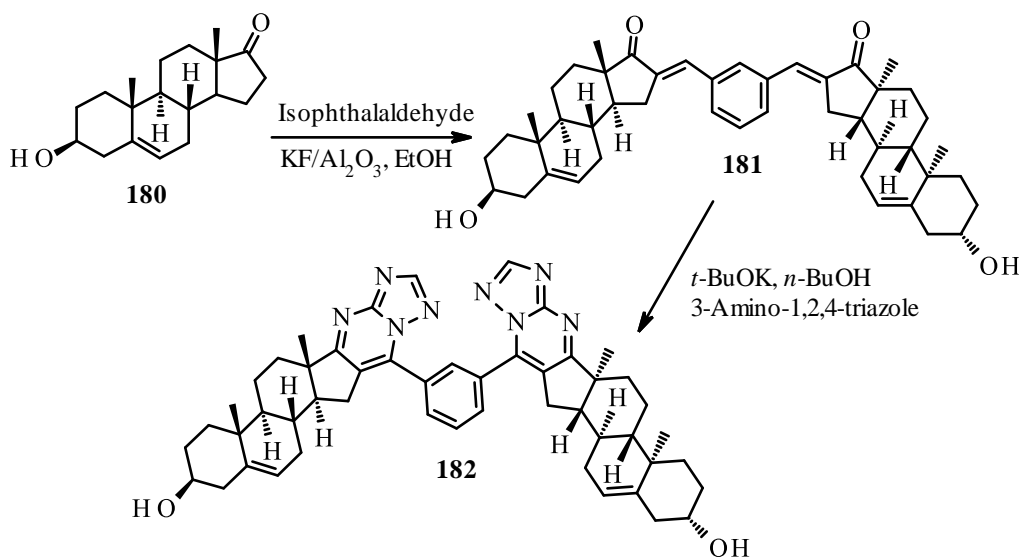
Bis-steroids **176-179** linked through ring D-ring D were obtained via catalytic diaminocarbonylation of 17-iodo-5 α -androst-16-ene (**175**) (Scheme 41), in the presence of palladium-phosphine *in-situ* catalysts and aliphatic or aromatic diamines such as 1,2-diaminoethane, 1,4-diaminobutane, 1,4-diaminobenzene, and (1*S*,2*S*)-1,2-diaminocyclohexane as *N*-nucleophiles *N*-nucleophiles. The dimerization reaction leading to steroidal dicarboxamides **176-179** took place under relatively mild conditions with quantitative conversion of the initial iodo-alkenyl steroid in 5h. Except **178** (48%), all symmetric androst-16-en dicarboxamide dimers were obtained in high yield (81-95%) [31].



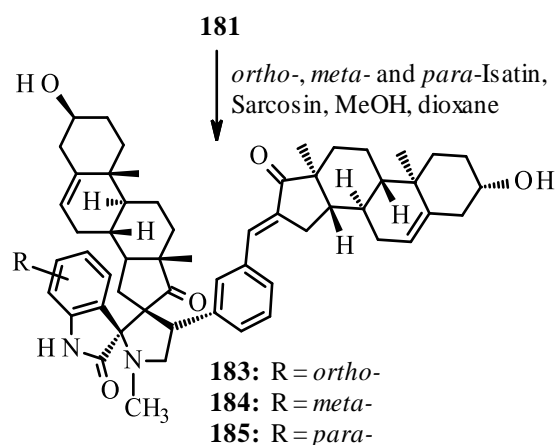
Scheme 41. Synthesis of bis-steroids **176-179**

DHEA dimers **181** and **182** were obtained in a two-steps sequence reaction (Scheme 42) [32]. In the first step, the Claisen-Schmidt condensation of

dehydroepiandrosterone (DHEA, **180**) with isophthalaldehyde and catalyzed by $\text{KF}/\text{Al}_2\text{O}_3$ in EtOH affording a phenyl-linked keto DHEA dimer **181** (90%), which was then reacted via Aza-Michael addition reaction and intramolecular cyclization reaction with 3-amino-1,2,4-triazole in *t*-BuOK and *n*-BuOH to afford [1,2,4] triazolo [1,5-a] pyrimidine-based phenyl-linked DHEA dimer **182** (69%). Later, steroidal spiro-pyrrolidinyl oxindoles **182-185** (68-73%) were obtained from the keto DHEA dimer **181** using *ortho*-, *meta*- and *para*-substituted isatin, sarcosine, MeOH and dioxane (Scheme 43) [33].



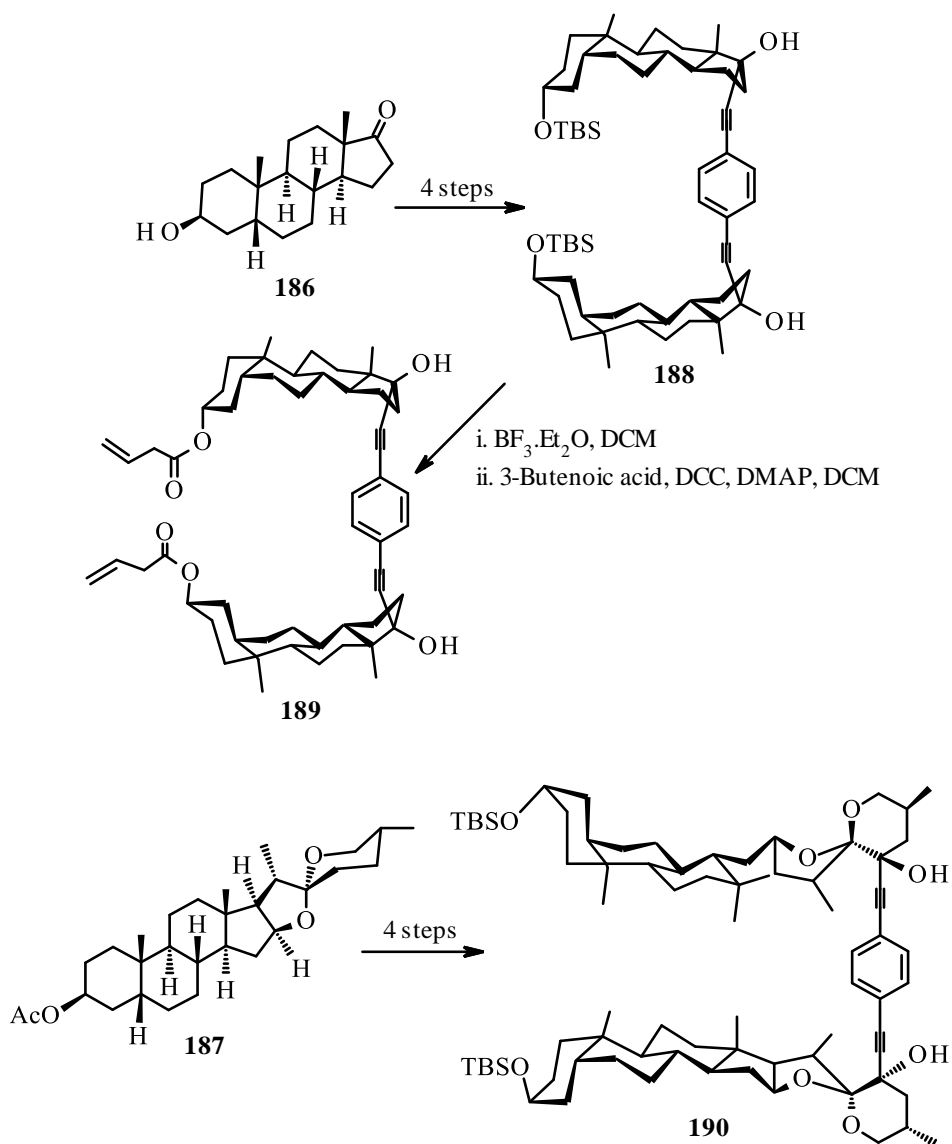
Scheme 42. Synthesis of DHEA dimers **181** and **182**



Scheme 43. Synthesis of steroidal spiro-pyrrolidinyl oxindoles **182-185**

Bis-steroids **188-190** were synthesized from 5β -androsterone (**186**) and sarsasapogenin acetate (**187**) (Scheme 44) [34]. Firstly, 5β -androsterone (**186**) was converted to silyl protected bis-steroid **188** (33%) in four-steps, following silyl protection of 3α -hydroxy, Grignard

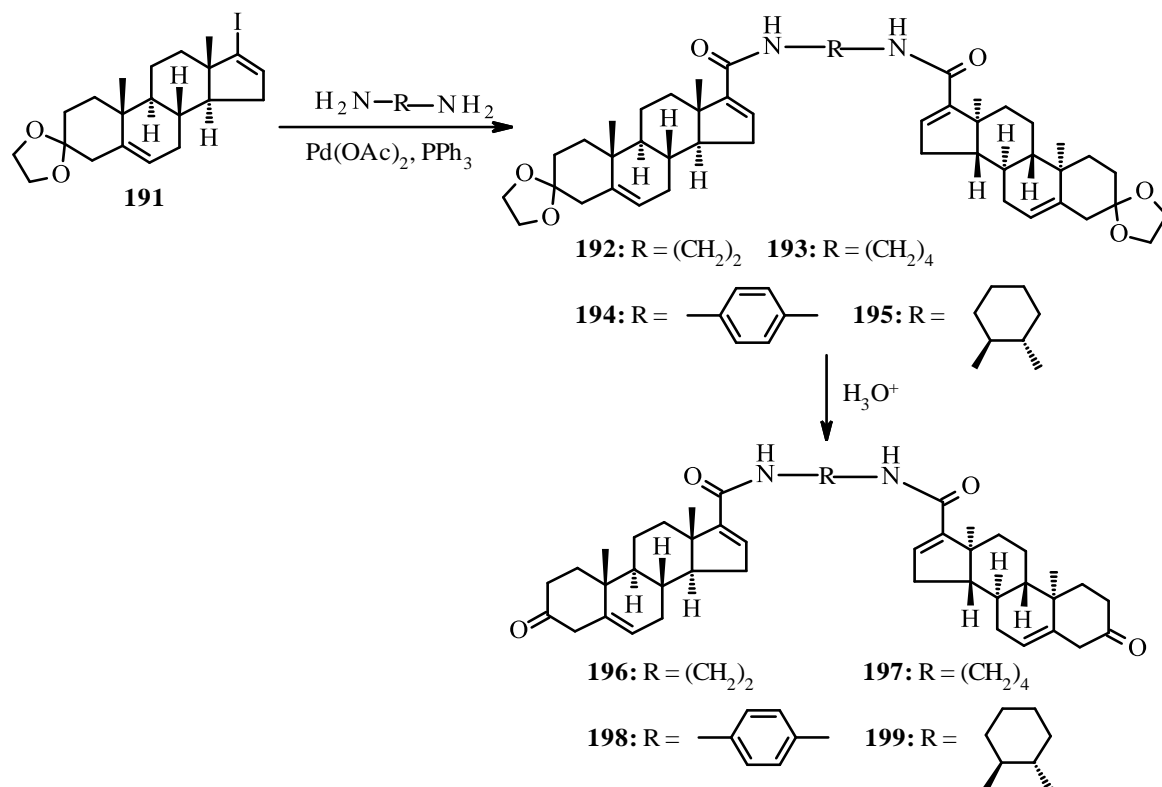
ethynylation and finally, Sonogashira coupling with 1,4-diiodobenzene. The protected dimer **188** was then deprotected with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in DCM, followed by Steglich esterification with 3-butenolic acid, DCC, DMAP in DCM gave the corresponding bis-steroid **189** (98%) with terminal double bonds. Finally, the bis-steroid **190** (52%) was prepared in four-steps starting with sarsasapogenin acetate (**187**).



Scheme 44. Synthesis of bis-steroids **188-190**

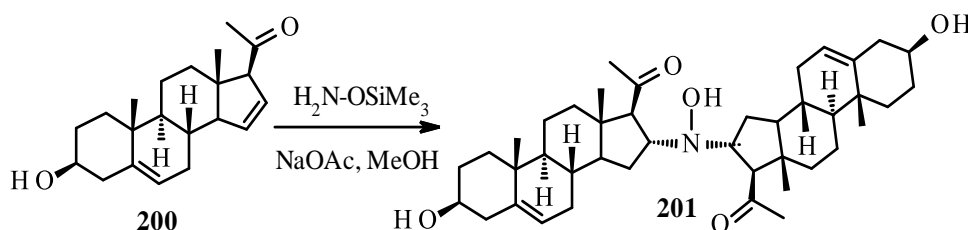
A series of ring D-ring D connected androstene-based dimers **192-195** (32-42%) were synthesized from 17-iodoandrost-5,16-ene-3-(ethylene ketal) (**191**), respectively, reacting with 1,2-diaminoethane, 1,4-diaminobutane, 1,4-diaminobenzene, and (1*S*,2*S*)-1,2-diaminocyclohexane as *N*-nucleophiles in presence of $\text{Pd}(\text{OAc})_2$ in PPh_3 (Scheme 45) [35]. The ketal

dimers **192-195** were hydrolyzed to androst-16-ene-17-carboxamide dimers **196-199** (18-46%) (Scheme 45).



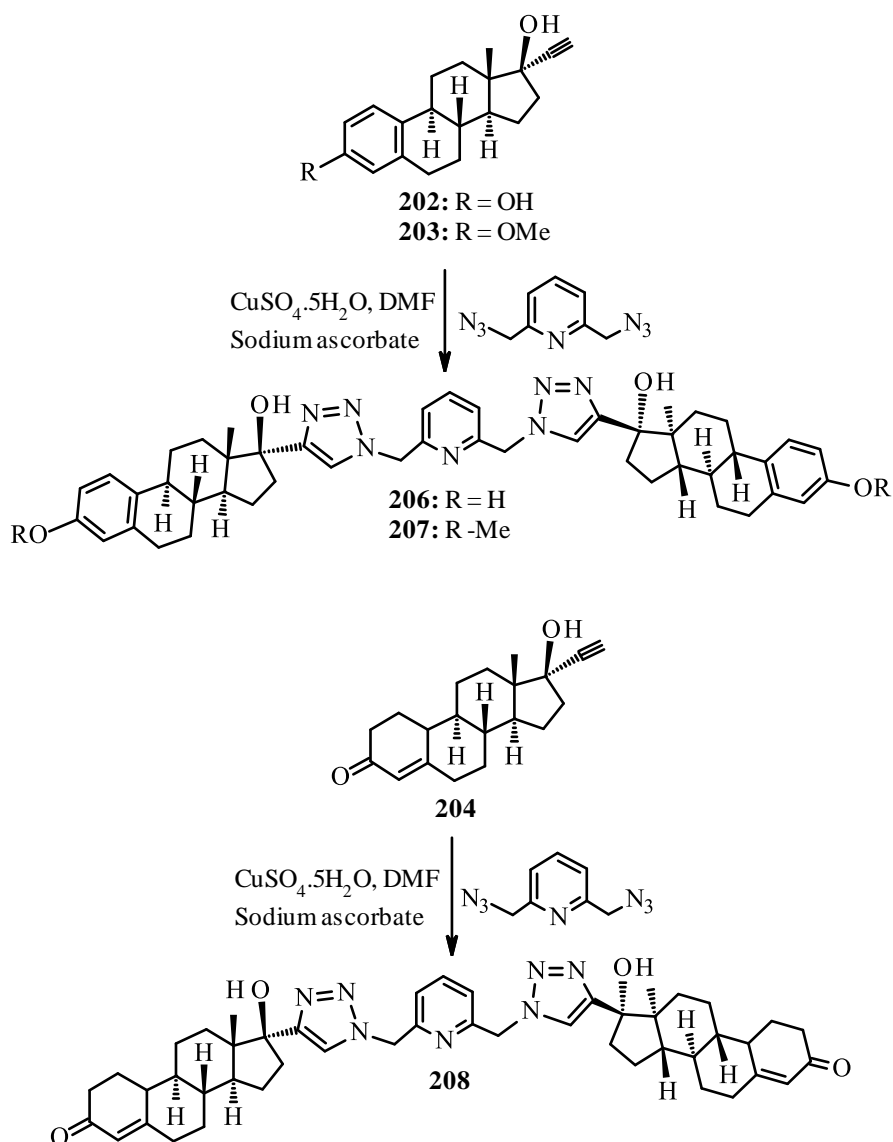
Scheme 45. Synthesis of ring D-ring D connected androstene-based dimers **192-199**

A steroidal dimer **201** (47%) was synthesized in the base-catalyzed reaction of 3 β -acetoxypregn-5,16-dien-20-one (**200**) with *O*-(trimethylsilyl)hydroxylamine in methanolic sodium acetate (Scheme 46) [36].



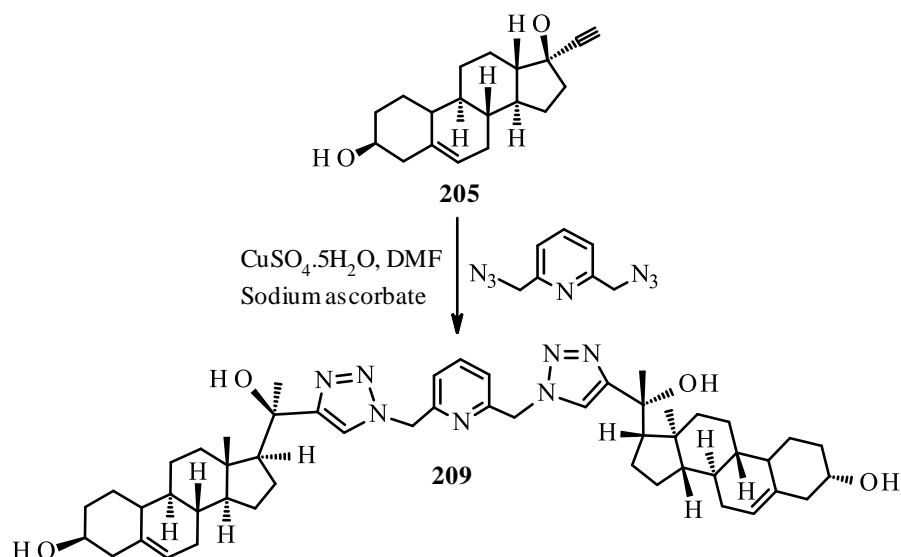
Scheme 46. Synthesis of dimer **201** from 3 β -acetoxypregn-5,16-dien-20-one (**200**)

The synthesis of several steroidal dimers **206-209** (78-87%) via ring D-ring D was achieved by 1,3-dipolar cycloaddition reaction of estradiol (**202**), *O*-methyl estradiol (**203**), testosterone (**204**) and pregnenolone (**205**) using 1,3-di(azidomethyl)benzene, CuSO₄·5H₂O and sodium ascorbate in DMF, respectively (Schemes 47 and 49) [37].

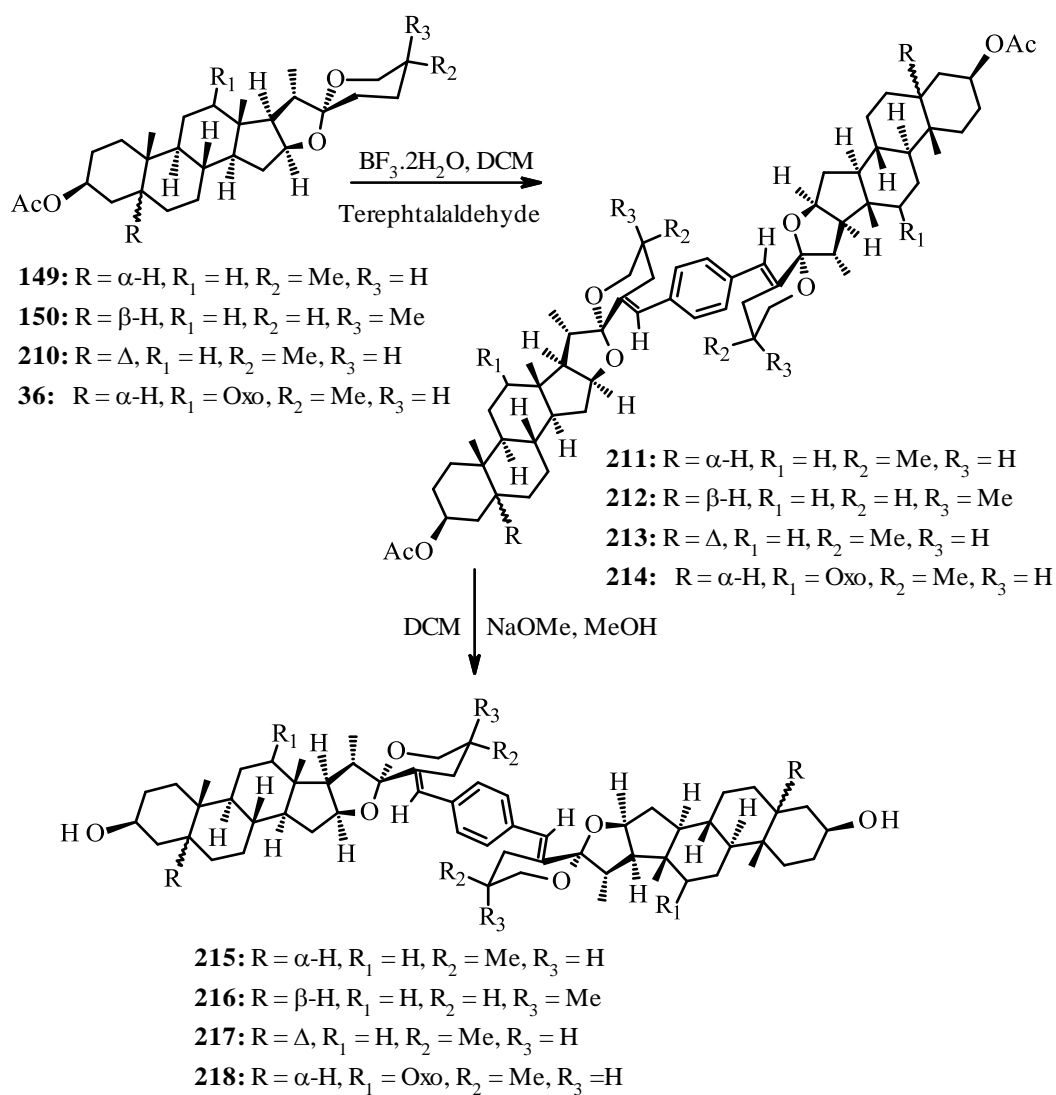


Scheme 47. Synthesis of steroidal dimers **206-208**

Spirostanol-based dimers **211-214** (Scheme 49) were synthesized by BF₃·Et₂O-catalyzed double Aldol condensation. Spirostanol acetates such as tigogenin acetate (**149**), sarsasapogenin acetate (**150**), diosgenin acetate (**210**) and hecogenin acetate (**36**) reacted with BF₃·2H₂O and terephthalaldehyde in DCM to give acetylated dimeric spirostanols **211-214** (52-83%), which followed by saponification with NaOMe in MeOH and DCM provide to the corresponding dimeric spirostanols **215-218** (89-100%) (Scheme 49) [38].



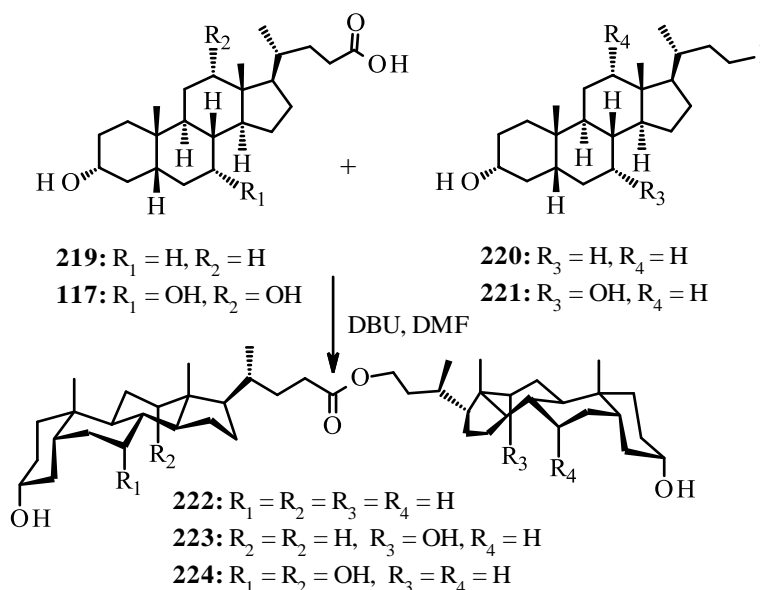
Scheme 48. Synthesis of steroidal dimer **209**



Scheme 49. Synthesis of spirostanol-based dimers **211-218**

2.6.2 Through side chain and spacer groups

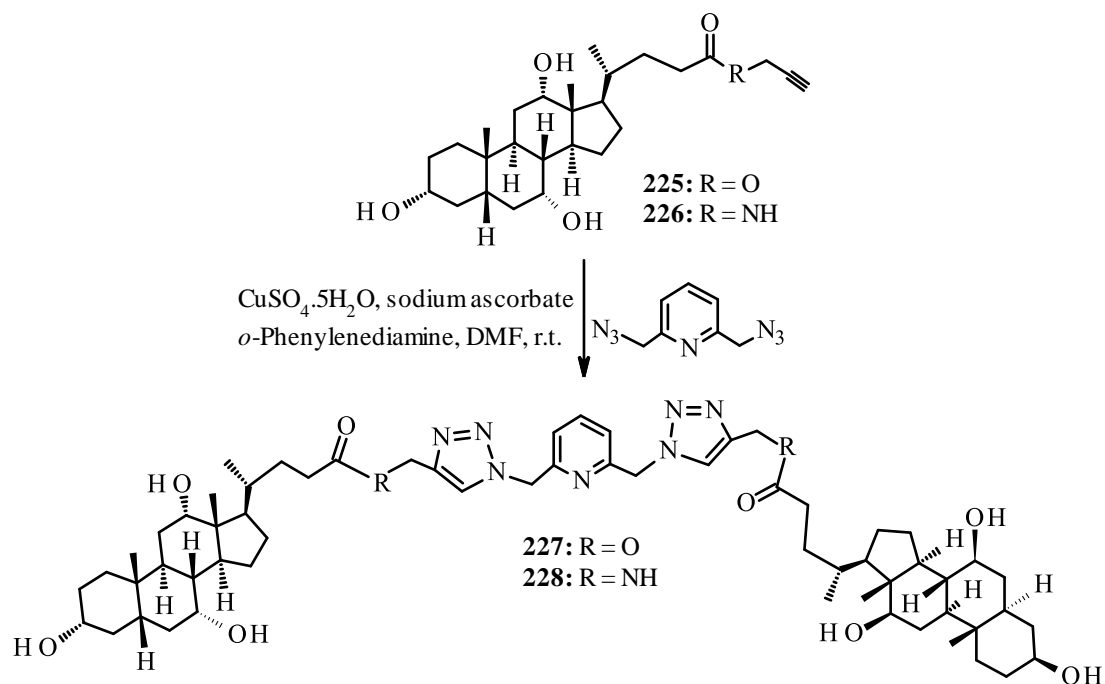
Three unsymmetrical bile acid dimers **222-224** (52-76%) were prepared, respectively, from lithocholic acid (**219**) either coupling with 3 α -hydroxy-5 β -23-iodo-24-norcholane (**220**) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF or 3 α ,12 α -dihydroxy-5 β -23-iodo-24-norcholane (**221**) and DBU in DMF, and finally cholic acid (**117**) coupling with 3 α -hydroxy-5 β -23-iodo-24-norcholane (**220**) and DBU in DMF (Scheme 50) [39].



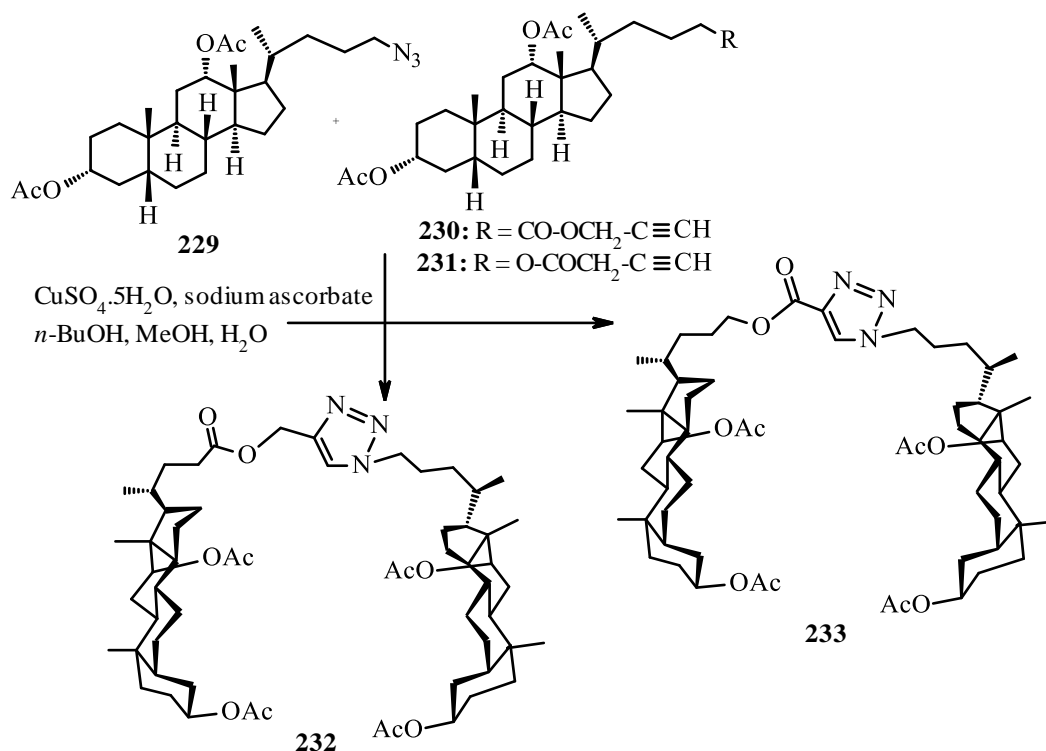
Scheme 50. Synthesis of unsymmetrical bile acid dimers **222-224**

Earlier several “ribbon” type etienic- and estrone-based bis-steroids were synthesized, and following the same methods two other “ribbon” type cholic acid-bases dimers **227** (77%) and **228** (80%) were prepared, respectively, by 1,3-dipolar cycloaddition reactions of cholic acid propargyl ester **225** and cholic acid acetylenic amide **226** using 1,3-di(azidomethyl)benzene, CuSO₄·5H₂O and sodium ascorbate in DMF (Scheme 51) [12].

Unsymmetrical deoxycholic acid-based dimers **232** (94%) and **233** (84%) through ring D-ring D side chains and linked via 1,4-disubstituted 1,2,3-triazole were synthesized by 1,3-dipolar cycloaddition of deoxycholic acid azide **229** either reacting with deoxycholic acid propargyl ester **230** or deoxycholic acid propiolate **231** (Scheme 52) [40].

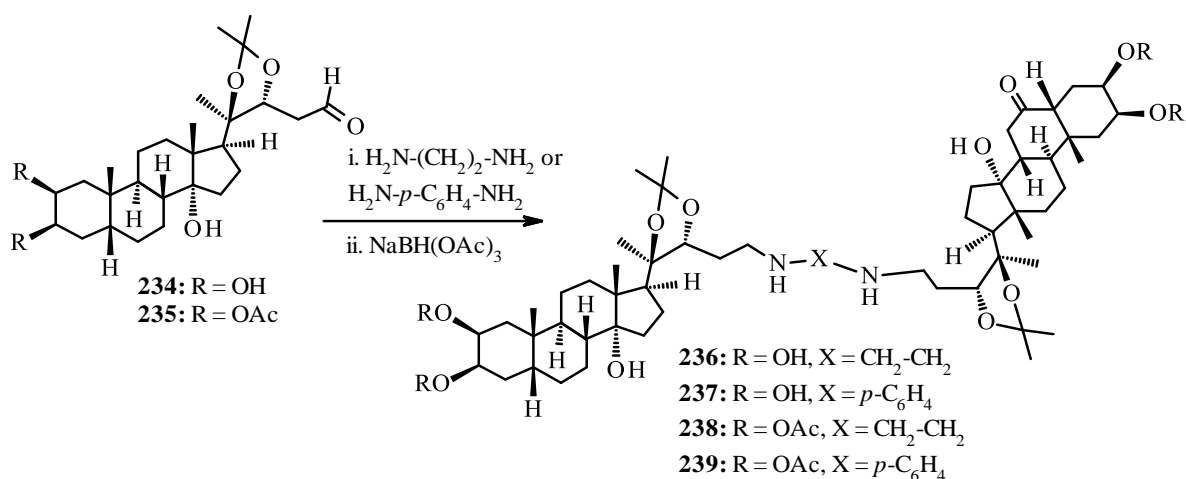


Scheme 51. Synthesis of “ribbon” type cholic acid-bases dimers **227** and **228**



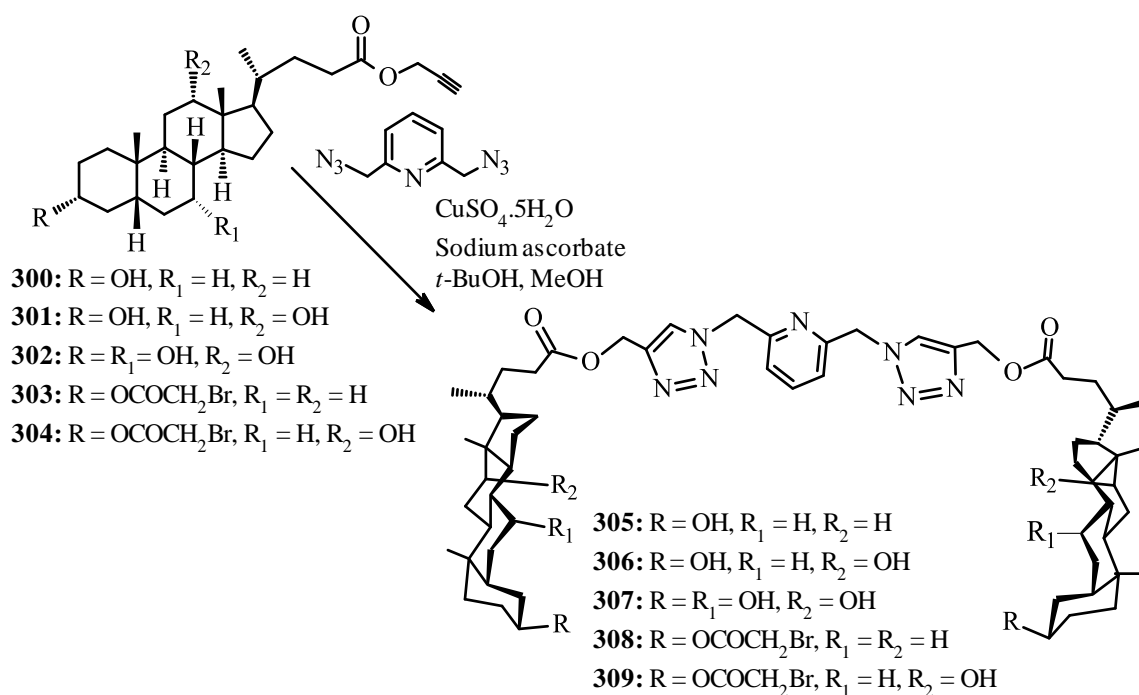
Scheme 52. Synthesis of unsymmetrical deoxycholic acid-based dimers **232** and **233**

N-alkyl- and *N*-arylaminoecdysteroid dimers **236-239** (40-54%) were obtained, respectively, by reductive amination of aldehydes **234** and **235** with either ethylenediamine or *p*-phenylenediamine followed by treatment with $\text{NaBH}(\text{OAc})_3$ (Scheme 53) [41].



Scheme 53. Synthesis of *N*-alkyl- and *N*-arylaminoecdysteroid dimers **236-239**

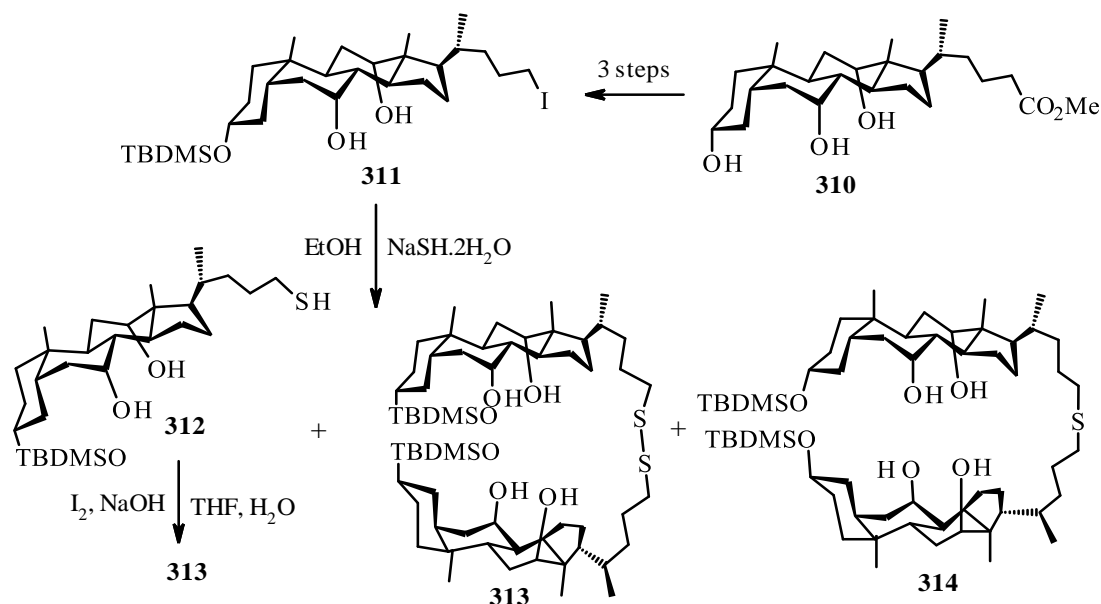
Bile acid-based dimers **305-309** were prepared from bile acid propargyl esters **300-304** (by 1,3-dipolar cycloaddition reactions of 1,3-di(azidomethyl)benzene, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and sodium ascorbate in *t*-BuOH and MeOH (Scheme 54) [42].



Scheme 54. Synthesis of bile acid-based dimers **305-309**

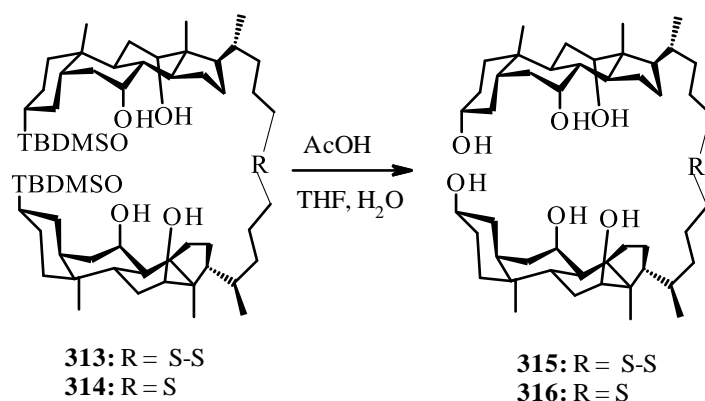
Cholic acid-based dimers **313-319** via ring D-ring D side chains were obtained, respectively, from methyl cholate (**310**) and cholic acid (**117**) following the same procedure employed earlier (Schemes 55-58) [20-22]. Initially, methyl cholate (**310**) was converted to protected $3\alpha\text{-OTBDMS}$ 24-iodide **311** in 3-steps. The protected 24-iodide **311** was reacted

with NaSH.2H₂O in EtOH to give a mixture of protected 24-thiol monomer **312** (29%), protected disulfide dimer **313** (39%) and protected sulfide dimer **314** (31%) [21-23].



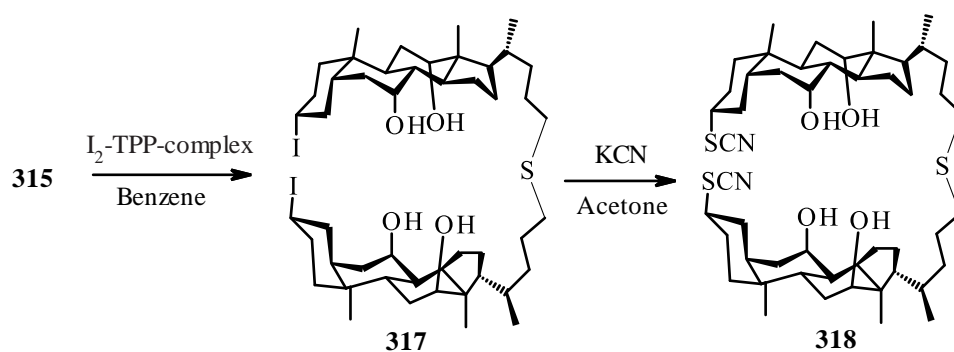
Scheme 55. Synthesis of cholic acid-based dimers **313** and **314**

The protected disulfide dimer **313** (93%) was also obtained in high yield by reacting protected 24-thiol monomer **312** with I₂, NaOH in THF and H₂O. Finally, the deprotected disulfide dimer **315** (96%) and sulfide dimer **316** (98%) (Scheme 56) were produced from protected disulfide dimer **313** and protected sulfide dimer **314**, respectively, using AcOH in THF and H₂O [21-23].



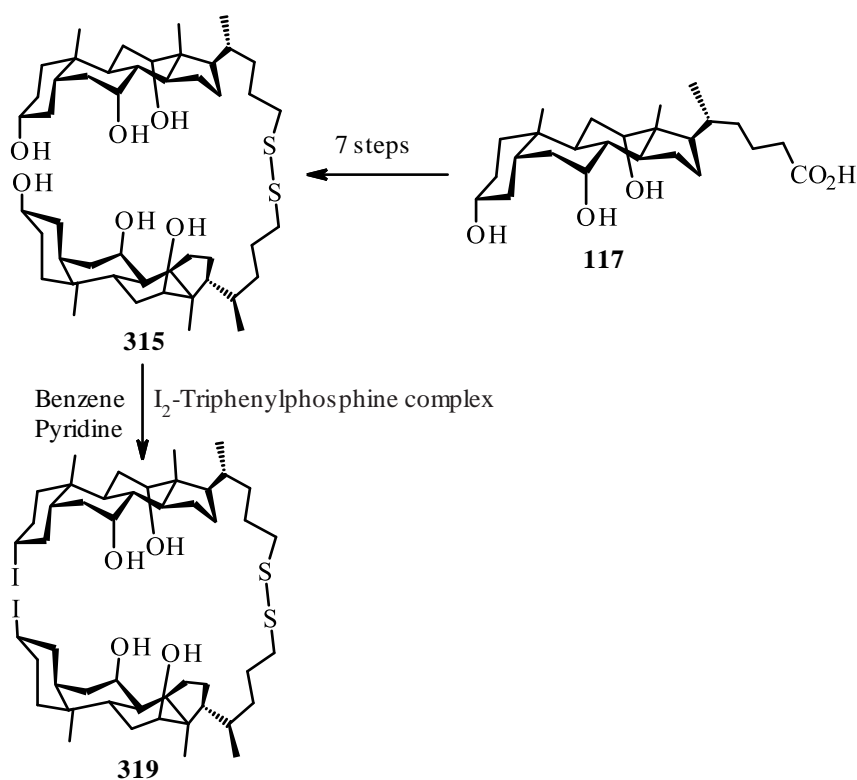
Scheme 56. Synthesis of cholic acid-based dimers **315** and **316**

Finally, the diol disulfide dimer **316** was converted to diiodide sulfide dimer **317** (57%) by using with I₂-triphenylphosphine complex in benzene. The diiodide sulfide dimer **317** was reacted with KCN in acetone to afford dithiocyanate dimer **318** (78%) [21-23].



Scheme 57. Synthesis of cholic acid-based dimers **317** and **318**

The disulfide dimer **315** was also obtained from cholic acid (**117**) in seven-steps utilizing a slightly different synthetic pathway. Iodination of the diol disulfide dimer **315** with iodine-triphenylphosphine complex in benzene and pyridine gave diiodide disulfide dimer **319** (43%) (Scheme 58) [21-23].

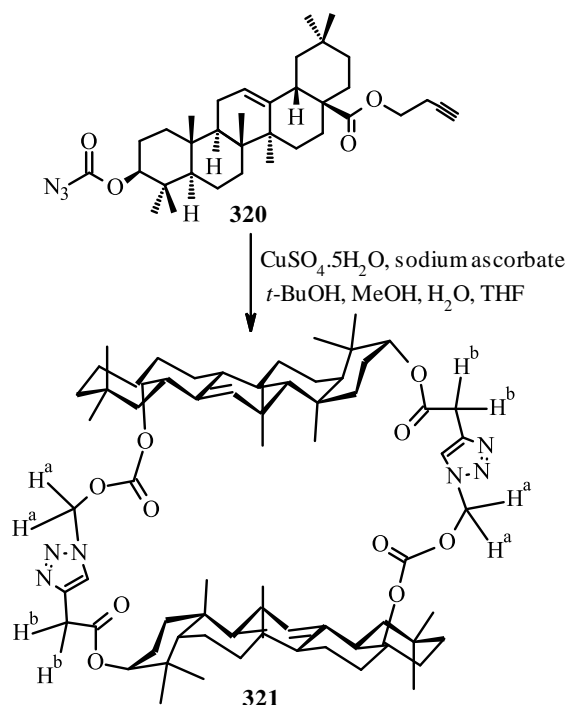


Scheme 58. Synthesis of cholic acid-based dimer **319**

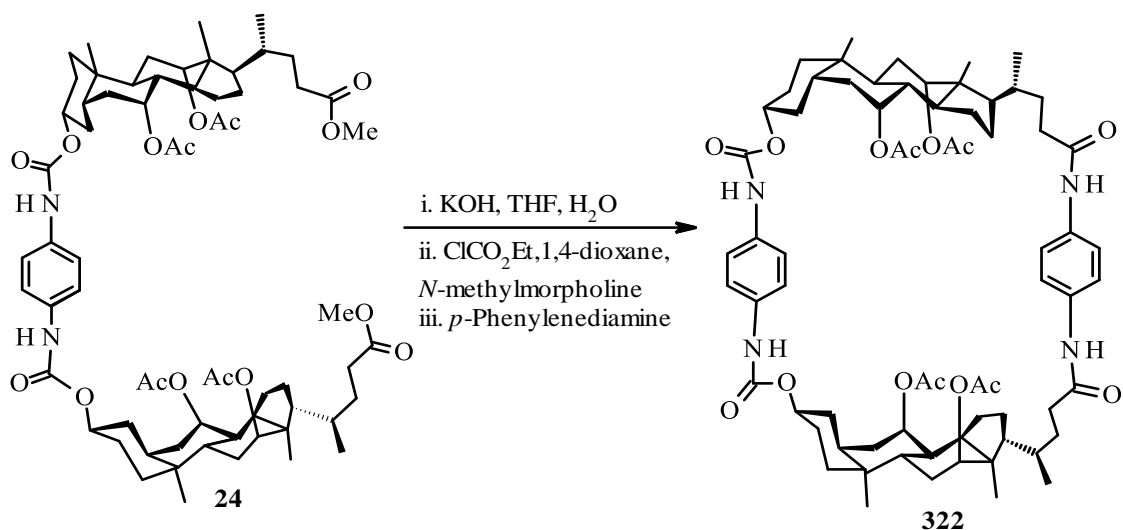
2.7 Cyclic dimers

An oleanolic acid-based cyclic dimer **321** (24%) was prepared by click chemistry from oleanolic acid propargyl ester (**320**) using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, sodium ascorbate *t*-BuOH, MeOH,

THF and H₂O (Scheme 59) [43]. Earlier, a series of cholic acid-based dimers were synthesized and employing the bis-carbamate **24** in a three-step sequence reaction provided a cyclic dimer **322** (35%) (Scheme 60) [7].

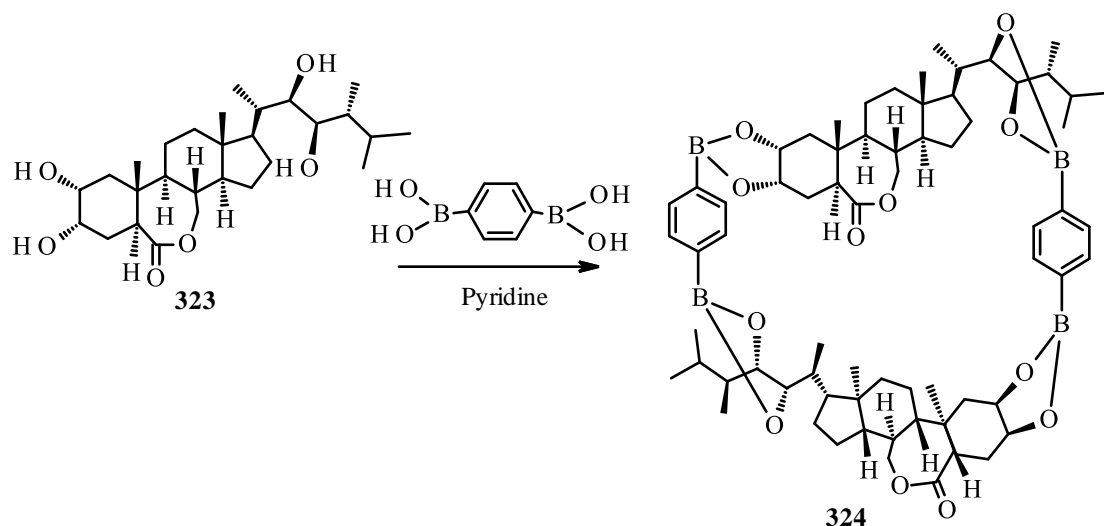


Scheme 59. Synthesis of oleanolic acid-based cyclic dimer **321**



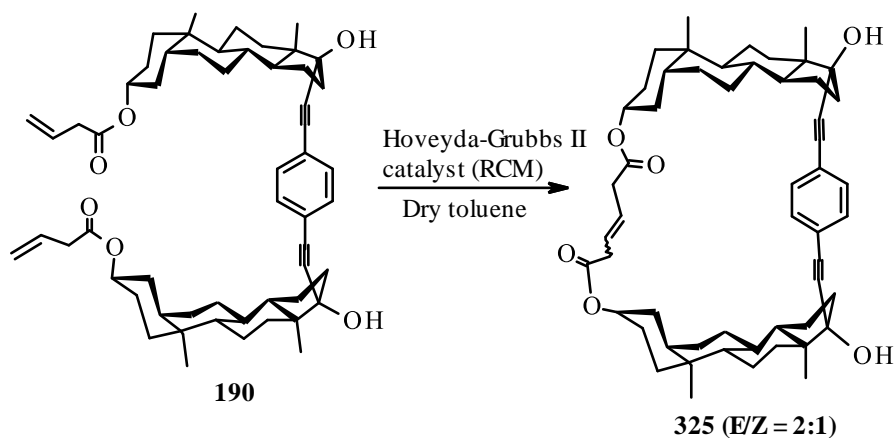
Scheme 60. Synthesis of cholic acid-based cyclic dimer **322**

A cyclic brassinosteroids-based dimer **324** (56%) was obtained via the reaction of 24-epibrassinolide **323** with 1,4-phenylenediboronic acid in pyridine (Scheme 61) [44].



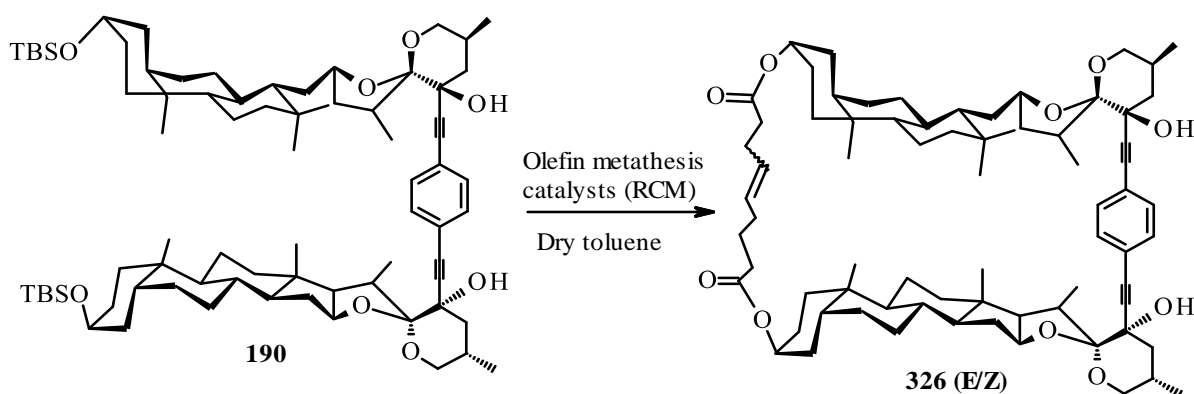
Scheme 61. Synthesis of cyclic brassinosteroids-based dimer **324**

Two diastereoisomeric cyclic dimers **325** and **326** based on 1,4-diethynylphenylene were prepared, respectively, from bis-steroid **190** and bis-steroid **191** via ring-closing metathesis (RCM) (Schemes 62 and 63). The bis-steroid **190** reacted with Hoveyda-Grubbs second-generation catalyst in dry toluene and produced a mixture of diastereoisomeric cyclic dimer **325** (67%, *E/Z* = 2:1), which was separated by a reversed phase-HPLC with CH₃CN/DCM (95:5) [34].



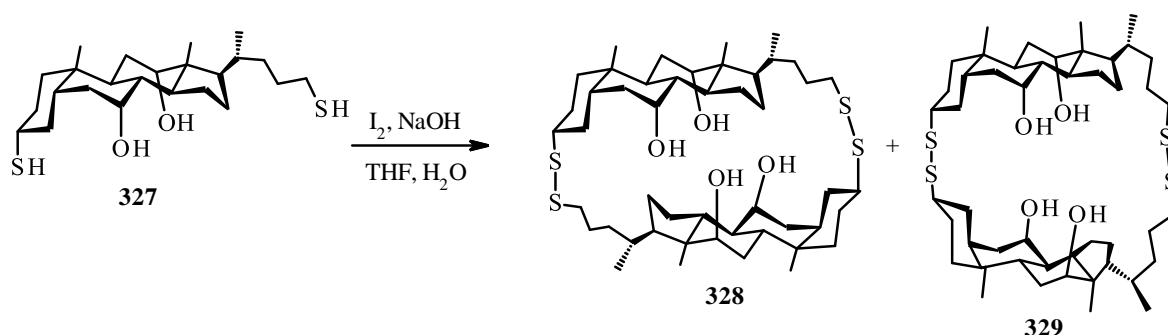
Scheme 62. Synthesis of cyclic steroid dimer **325**

The bis-steroid **190** reacted with various olefin metathesis catalysts (RCM) in dry toluene and produced a mixture of diastereoisomeric cyclic dimer **326** (57-85%, *E/Z*, the yield and the diastereoisomeric ratio depend on the catalyst used). The mixture of the diastereoisomeric cyclic dimer **326** (*E/Z*) was separated by reversed-phase HPLC with CH₃CN/DCM (95:5) (Scheme 63) [34].



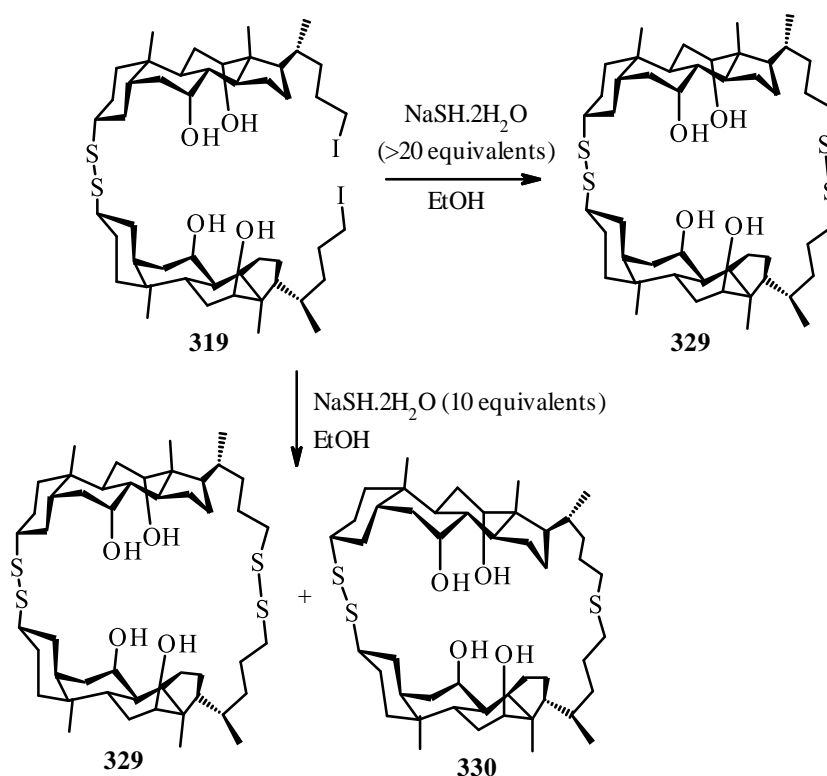
Scheme 63. Synthesis of cyclic steroid dimer **326**

Studies on the synthesis of cholic acid-based molecular receptors have been performed in recent years. In most cases, efforts have been made towards the synthesis of cyclic systems consisting of two steroidal units involving head-head and head-tail combination of cholic acids via suitable spacer [2]. Three cyclic dimers connected via head-tail **328** and head-head **329** as well as another head-head **330** were achieved, respectively, from cholic acid dithiol **327** and diiodide disulfide dimer **319** (Schemes 64 and 65). Firstly, a mixture of cholic acid cyclic dimers via head-tail **328** (15%), and via head-head **329** (30%) with disulfide spacers were synthesized from iodination of cholic acid dithiol monomer **327** using I_2 , NaOH, THF and H_2O [21-23].



Scheme 64. Synthesis of cholic acid-based molecular receptors (cyclic steroid dimers) **328** and **329**

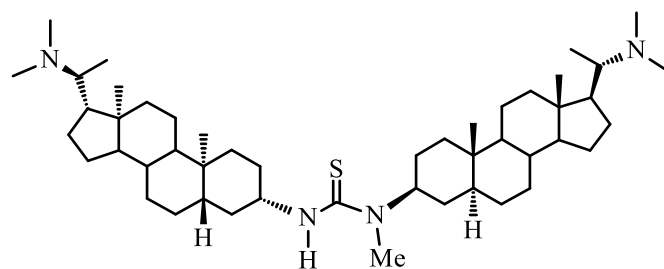
Another mixture of head-head cholic acid cyclic dimer **329** (39%) with disulfide spacer as well as the cholic acid cyclic dimer **330** (48%) containing both disulfide and sulfide spacers were obtained from diiodide disulfide dimer **317** reacting with a large excess of $\text{NaSH} \cdot 2\text{H}_2\text{O}$ (10 equivalents) in EtOH. It was observed that when a large excess of $\text{NaSH} \cdot 2\text{H}_2\text{O}$ (more than 20 equivalents) was used predominately the disulfide cyclic dimer **329** (85%) was formed (Scheme 65) [21-23].



Scheme 65. Synthesis of cholic acid-based molecular receptors (cyclic steroid dimers) **329** and **330**

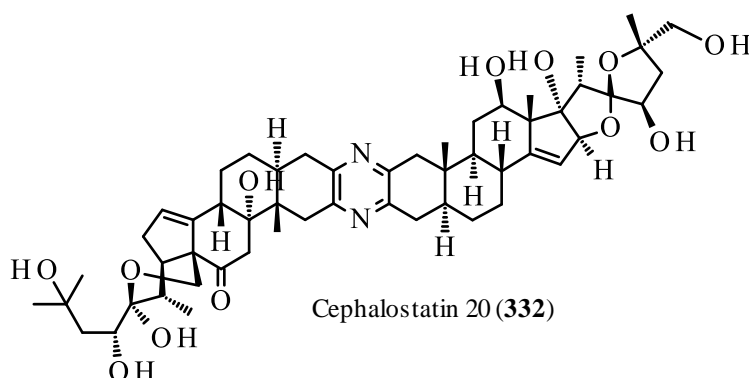
3. Naturally occurring steroid dimers

Since the discovery of the first naturally occurring steroid dimer, japindine (**331**) (Figure 2), from the root bark of *Chonemorpha macrophylla*, several pharmacologically active steroid dimers, *e.g.*, cephalostatins, crellastatins and ritterazines, respectively, from marine sponges *Cephalodiscus gilchristi*, *Crella* sp and *Ritterella tokioka* have been reported [1, 2]. However, the discovery of new naturally occurring steroid dimers within the last nine years appears to be rather limited to only cephalostatin 20 (**332**) (Figure 3) [45]. During 1988-1998, a total of 19 different cephalostatins were reported by Pettit's group [1, 2]. However, there has been no further discovery of cephalostatins until recently, when cephalostatin 20 (**332**) (mol wt: 944, mol formula $\text{C}_{54}\text{H}_{76}\text{N}_2\text{O}_{12}$) was isolated as an amorphous solid from *Cephalodiscus gilchristi* by a combination of various chromatographic techniques, the final technique being a reversed-phase HPLC using a mobile phase comprising acetonitrile, methanol and water [45]. Cephalostatin 20 (**332**) is actually a hydrated product of cephalostatin 2 (**333**) (Figure 3).

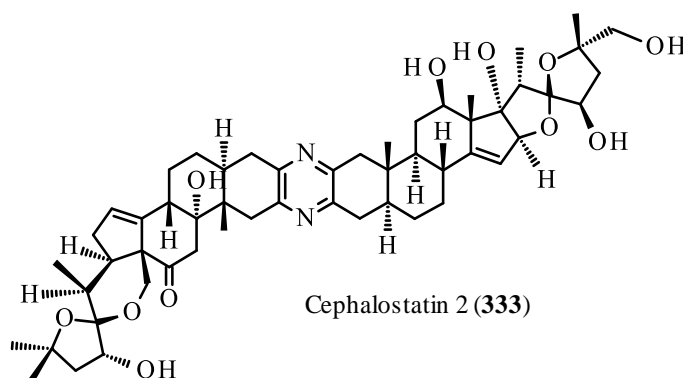


Japindine (**331**)

Figure 2. Structure of japindine (**331**)



Cephalostatin 20 (**332**)



Cephalostatin 2 (**333**)

Figure 3. Structures of cephalostatin 20 (**332**) and cephalostatin 2 (**333**)

4. Applications of steroid dimers

The dimerization of the steroid skeleton generally renders new physicochemical and pharmacological properties leading to new applications of steroid dimers. Dimeric and oligomeric steroids are known to exhibit micellar, detergent and liquid crystal behavior, and pharmacological properties like cytotoxicity against cancer cells [1, 2, 16, 32, 33, 45]. It is also known that biologically important steroid dimers possess enhanced biological activities than that of the corresponding monomers [45]. It has been suggested that steroid dimers could

trigger cellular processes or promote the affinity of ligands to their binding locations by offering new anchoring points to the active sites [46]. The behaviors of steroid dimers as liquid crystals can also be exploited in different types of reactions to generate new pharmaceutically active leads. The following subsections are devoted to specific biological/pharmacological properties of as demonstrated by various steroid dimers reported during the period covered by this review article.

4.1 Cytotoxicity

A majority of the naturally occurring steroid dimers, *e.g.*, cephalostatins, crellastatins and ritterazines and their synthetic analogues possess cytotoxic properties against cancer cells [1, 2]. Cephalostatins represent an important series of cancer cell growth inhibitors, and cephalostatins 1 (**334**), 2 (**333**) and 7 (**335**) (Figures 3 and 4) are among the most potent natural cytotoxins with anticancer and antitumor potential. It has been suggested that the cytotoxic potential of these natural steroid dimers is probably mediated by their ability to target oxysterol bending protein involved in signal transduction [45]. Several cephalostatin analogues including some of them containing sugar moieties (**2-10** and **29-34**), were synthesized and their cytotoxic potential was evaluated [5, 8]. Among them, diol pyrazine dimer **4**, diol rhamnoside pyrazine dimer **7** and polyhydroxy rhamnoside pyrazine dimer **10** were found to inhibit the growth of cancer cells of the murine P388 lymphocytic leukemia cell line as well as against a range of human cancer cell lines, whereas steroid dimers, diacetate pyrazine dimer **30** and dione pyrazine dimer **31** were active against the murine 388 cell line. These studies provided further evidence on the essential structure-activity-relationships (SAR) features required for cephalostatins' cytotoxic properties including the need for a C-14 double bond, C-12 oxygenation (alcohol or ketone), and a 17 α -hydroxy group for optimum activity. It was suggested that there might be several other less obvious molecular features of the cephalostatin-type steroids that are critical to their significant potency against cancer cell growth and mechanisms of biological activity [8].

A cytotoxicity study on cephalostatins [45], including the new cephalostatin 20 (**332**), revealed that cephalostatins 1 (**334**) and 2 (**333**) possessed the strongest cancer cell growth inhibitory activity *in vitro*, and cephalostatin 2 (**225**) was more potent than cephalostatin 1 (**334**) against all cancer cell lines used (particularly the NCI-H460 non-small-cell lung cancer, KM20L2 colon carcinoma, and SF-268 glioblastoma cells), except against the DU-145 prostate cancer cells, where the potency of both compounds were the same. It was postulated that the

hydroxy substitution at C-8' in cephalostatin 2 (**333**) could offer potentiation of antineoplastic property. A similar potentiation was also observed with the new cephalostatin 20 (**332**), where the presence of a hydroxy group at C-8' generally offered 10 times greater cancer cell growth inhibition than that offered by cephalostatin 9 (**336**). However, both cephalostatins 9 and 20 (**336** and **332**) were remarkably less potent cytotoxic agents than cephalostatins 1 (**334**) and 2 (**333**). This study also provided evidence to support the importance of the spirostanol structure for the antineoplastic property [45].

Cephalostatin 1 (**334**), being one of the most cytotoxic cephalostatins, has been the subject of total synthesis and/or synthesis of its analogues, mainly because of its restricted natural sources and extremely poor yield [45]. Two of such cephalostatin 1 analogs, cephalostatins 7 and 9 (**335** and **336**) were studied for their comparative cytotoxicity against several cancer and normal cell lines to have better understanding of their plausible mechanisms of cytotoxicity [45]. It was observed that both analogues selectively killed more cancer cells than normal cells, activated caspase 3 and 9, and induced apoptosis by triggering endoplasmic reticulum stress. In a similar study, it was shown that the cytotoxicity of ritterostatin G_{N1N} probe (**105**), a cephalostatin-ritterazine hybrid, could be due to its ability to trigger unfolding protein response and subsequent apoptotic cell death [16, 17]. In fact, this finding was further supported by a recent study, where it has been demonstrated that cephalostatin 1 analogues could selectively activate apoptosis via the endoplasmic reticulum stress signalling pathway in human cancer cells [47]. Cephalostatin 1 analogues were cytotoxic against all six cancer cell lines, and the cytotoxicity was mediated by apoptosis as evident by DNA fragmentation and exposure of phosphatidylserine on the outside of the plasma membrane. It was also shown that the apoptosis induced by cephalostatin 1 analogues in cancer cells was caspase-dependent [47]. Cephalostatin 1 (**334**) could use the ER stress-induced apoptotic pathway involving caspase 4 independent of cytochrome C release and caspase 8 activation, and it could involve mitochondrial signalling molecules. It was suggested that cephalostatin 1 analogs could induce apoptosis in a highly similar manner to cephalostatin 1 (**334**) rather than through the extrinsic or intrinsic apoptotic pathways.

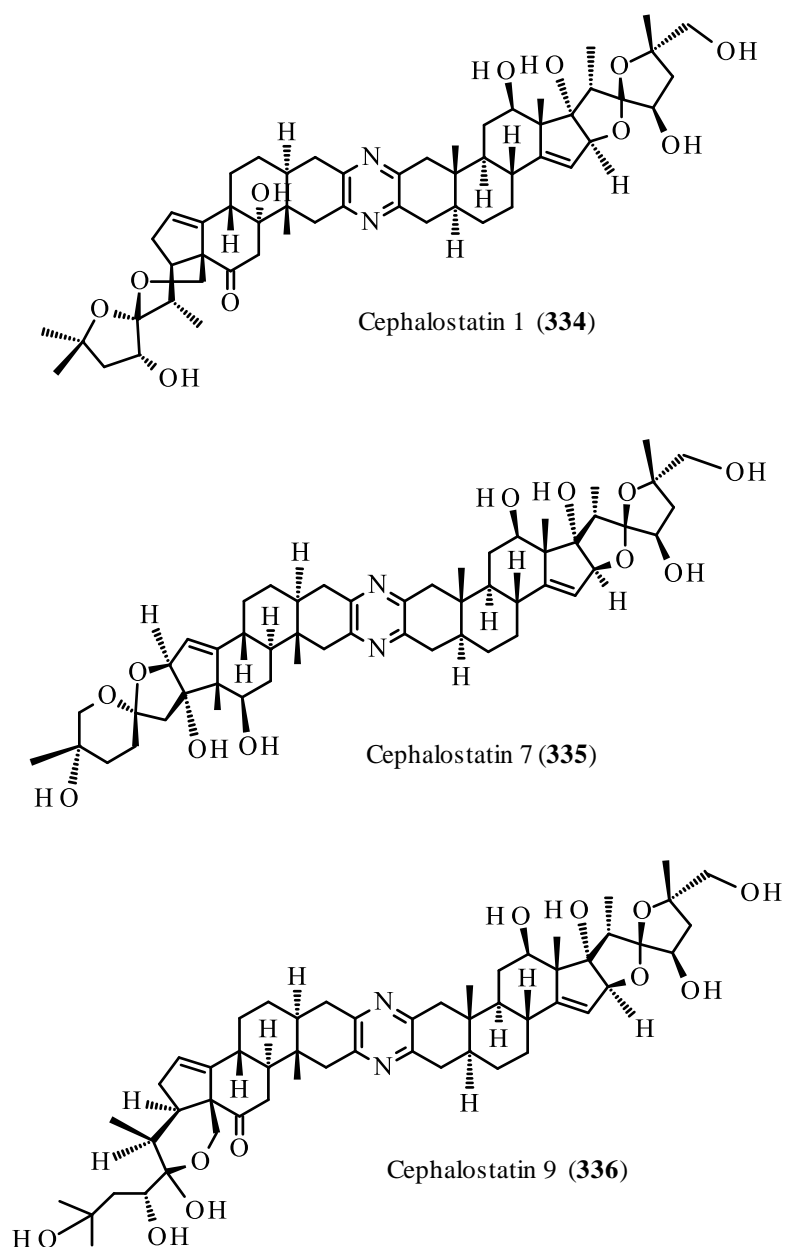


Figure 4. Structures of cephalostatin 1 (**334**), cephalostatin 7 (**335**) and cephalostatin 9 (**336**)

Several cholic acid and etinic acid-based steroidal dimers were tested for their cytotoxic and hormone modulating activity [12, 37]. One of the cholic acid dimer **227** showed significant cytotoxic activity against lymphoblastic and myeloid leukemia cell lines CCRF-CEM and K562 at low micromolar concentrations. It was noted that the potency of this dimer in solid tumors and multidrug resistant leukemia cell lines was lower, and it was comparable to the activity in normal fibroblasts suggesting low therapeutic index. However, the etinic acid dimer **173** was only active against multidrug resistant cells and displayed acceptable therapeutic index, while another etinic acid dimer **66** was only active against the CEM-DNR-BULK cell line, which is a daunorubicin resistant derivative of CCRF-CEM cells overexpressing the

multidrug resistance protein 1. Two other dimers, cholic acid dimer **228** and etinic acid dimer **174** had no cytotoxic activity at test concentrations.

A series of A ring-A ring connected steroid dimers **73-86** were studied for their potential antitumor activity against the human tumor/cancer cell lines, cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562) and two human breast cancer cell lines (MDA-MB-361 and MDA-MB-453) [13]. All dimers exhibited varied levels of cytotoxic activity against those tumor/cancer cell lines at micromolar concentrations, with the dimer sulfides **73-77** being the most potent ones, which exhibited notably higher cytotoxic activities against K562, HeLa and estrogen receptor-negative and progesterone receptor-negative MDA-MB-453 cells than to resting and PHA-stimulated PBMC, suggesting selective anticancer property. The anticancer activity against the leukemia K562 cells was likely to be mediated through apoptosis. The identification of target caspases implicated in the activation of apoptosis implied that those steroid dimers triggered apoptosis in K562 cells employing both the extrinsic and intrinsic signalling pathways [13]. All steroid those steroid dimers induced (at IC₅₀ concentration) G1 phase cell cycle arrest in K562 cells.

Steroid al spiro-pyrrolidinyl oxindoles **182-185** were synthesized and evaluated for their potential antiproliferative property against four human cancer cell lines, and their possible mechanisms of actions were studied [33]. These dimers were tested against human gastric cancer cell line (MGC-803), human breast cancer cell line (MCF-7), human liver cancer cell line (SMMC-7721) and human esophageal cancer cell line (EC-109) using the MTT assay. The DHEA dimers **183-185** did not show any noticeable antiproliferative activities toward the cancer cell lines. Among them, the DHEA dimer **184** had the moderate inhibition against EC-109, SMMC-7721 and MCF-7 with the IC₅₀ values of 7.01, 4.30 and 2.06 μ M, respectively.

Along with a series of steroid hybrid molecules, in an attempt to identify antiproliferative compounds, the DHEA dimer **102** was synthesized and its antiproliferative property was evaluate against several human cancer cell lines with no activity observed at test concentrations [16]. Several testosterone-based steroidal dimers **164-170** via ring B-ring B connection were synthesized and were evaluated for their antiproliferative activity against androgen-dependent and androgen-independent prostate cancer cell lines [30]. The most active dimer **164** showed IC₅₀ values of 3.8, 1.4 and 1.8 μ M, respectively, against the LNCaP, DU-145 and PC3 cancer cell lines, and the dimer was a about 12, 70 and 47 times more powerful than the antiandrogen reference drug cyproterone acetate. The other dimers **165-167** were less

active than **164** but showed selective cytotoxicity against the androgen dependent LNCaP prostate cancer cells.

4.2 Antimicrobial activity

Three cephalostatin analogs were tested for antimicrobial activity against *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterococcus cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, *Neisseria gonorrhoeae* ATCC 49226, *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90112, and the bis-steroidal pyrazine (**3**) was found to inhibit the growth of the nosocomial pathogen *Enterococcus faecalis* with an MIC of 32 mg/mL), which is known to cause urinary and wound infections, endocarditis and bacteremia [5]. Krstic et al. (2014) synthesized A ring-A ring connected steroid dimers **73-86**, and evaluated their potential antimicrobial activity against the Gram-positive *Micrococcus luteus* (ATCC 4698) and the Gram-negative *Escherichia coli* (ATCC 25922) bacterial and fungal strains, *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763) and against *Penicillium* sp. [13], with no detected antibacterial activity against test concentrations. However, the dimers linked with trithiolane ring (**78-82**) showed the best antifungal activity against *Saccharomyces cerevisiae*, suggesting selective antimicrobial activity.

4.3 Steroid dimers in drug delivery

Four cortisol and progesterone-based dimers **109-112** were synthesized using oxime click chemistry linked via hydrophobic, hydrophilic or functional groups, and because of the dynamic nature of the oxime bonds, the steroid-based materials are degradable via acidic hydrolysis and transoximation, potentially making these oxime-based dimers a promising group of materials for the controlled or sustained release steroid hormonal formulations [19]

4.4 Applications in biomaterials and biosensors

The oleanolic acid-based cyclic dimer **321**, exhibited remarkable selectivity and affinity for F⁻ ion through C–H...F⁻ hydrogen bond interactions by the delocalization of proton in methylene, revealing some indications for the potential applications of steroidal cyclic dimers in biomaterials and biosensors [43]. Several acyclic and macrocyclic steroidal dimers, for example, dimers **24** and **322**, were suggested to have their potential applications in molecular

recognition, supramolecular chemistry and broadly in pharmacology [7, 21, 22]. However, no direct experimental evidence is available to date to support this assumption.

Testosterone and its dimers were shown to alter tRNA morphology which may have importance in having insights into hormone-tRNA and drug-tRNA conjugations [48]. Tomkiel et al. (2018) synthesized several steroid dimers with disulphide links (**118**, **119**, **145**, **146**, **313-319**, **328** and **329**) and indicated their plausible role in metal ion complexation and ability to recognise and bind small drug and drug like molecules [23]. However, no further experimental evidence on this has been published yet.

4.5 *Miscellaneous*

A head-tail cholic acid dimer **148** linked via a 1,2,3-triazole ring was synthesized, purified by column chromatography and the biological activity spectra or pharmacotherapeutic potential were predicted *in silico* with the computer-based program, Prediction of Activity Spectra for Substances (PASS) revealing the most predicted types of biological activity being the inhibition of 1-acylglycerol-3-phosphate *O*-acyltransferase, squalenehopene cyclase, peptidoglycan glycosyltransferase, acylglycerol lipase, hypercholesterolemic, *N*-(long-chain-acyl)ethanolamine deacylase, alkenylglycerophosphoethanolamine hydrolase, and cholesterol synthesis [28].

Acknowledgement

Dr L Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

References

- [1] L. Nahar, L., S. D. Sarker, Steroid Dimers: Chemistry and Applications in Drug Design and Delivery, Wiley & Sons: Chichester, UK, 2012.
- [2] L. Nahar, S. D. Sarker, A. B. Turner, A review on synthetic and natural steroid dimers: 1997-2006. *Curr. Med. Chem.* 14 (2007) 1349-1370.
- [3] Y. X. Li, J. R. Dias, Dimeric and oligomeric steroids. *Chem. Rev.* 97 (1997) 283-304.
- [4] K. Q. Shawakfe, N. H. Al-Said, R. M. Al-Zoubi, Synthesis of new symmetrical bis steroidal pyrazine analogues from diosgenin. *Steroids* 73 (2008) 579-584.
- [5] G. R. Pettit, R. F. Mendonça, J. C. Knight, R. K. Pettit, The cephalostatins 21. Synthesis of bis-steroidal pyrazine rhamnosides. *J. Nat. Prods.* 74 (2011) 1922-1930.

- [6] K. Q. Shawakfeh, N. H. Al-Said, Synthesis of new symmetrical bis-steroidal pyrazine analogues from diosgenin. *Steroids* 76 (2011) 232-237.
- [7] Z. Paryzek, R. Joachimiak, M. Piasecka, T. Pospieszny, A new approach to steroid dimers and macrocycles by the reaction of 3-chlorocarbonyl derivatives of bile acids with *O,O*-, *N,N*- and *S,S*-dinucleophiles. *Tet. Lett.* 53 (2012) 6212-6215.
- [8] G. R. Pettit, B. R. Moser, R. F. Mendonça, J. C. Knight, F. Hogan, The cephalostatins 22. Synthesis of bis-steroidal pyrazine pyrones. *J. Nat. Prods.* 76 (2012) 1063-1069.
- [9] Y. Kou, Y. Cheun, M. C. Koag, S. Lee, Synthesis of 14',15'-dehydro-ritterazine Y via reductive and oxidative functionalizations of hecogenin acetate. *Steroids* 78 (2013) 304-311.
- [10] K. Perez-Labrada, C. Morera, I. Brouard, R. Llerena, D. G. Rivera, Synthesis and conformational study of triazole-linked bis-spirostanic conjugates. *Tet. Lett.* 54 (2013) 1602-1606.
- [11] K. Perez-Labrada, C. Morera, I. Brouard, D. G. Rivera. Multicomponent ligation of steroids: Creating diversity at the linkage moiety of bis-spirostanic conjugates by Ugi reactions. *ACS Combi. Sci.* 15 (2013) 320-330.
- [12] M. Jurášek, P. Džubák, D. Sedláček, H. Dvorakova, M. Hajdúch, P. Bartůněk, P. Drašar, Preparation, preliminary screening of new types of steroid conjugates and their activities on steroid receptors. *Steroids* 78 (2013) 356-361.
- [13] N. M. Krstić, I. Z. Matić, Z. D. Juranić, I. T. Novaković, D. M. Sladić, Steroid dimers—*In vitro* cytotoxic and antimicrobial activities. *J. Steroids Biochem. Mol. Biol.* 143 (2014) 365-375.
- [14] M. Kiss, S. Maho, K. Boddi, B. Boros, L. Kollar, Palladium-catalyzed diaminocarbonylation: Synthesis of androstene dimers containing 3,3'- or 17,170-dicarboxamide spacers. *Monatsh Chem.* 146 (2015) 357-364.
- [15] C. Alarcón-Manjarrez, R. Arcos-Ramos, M. F. Álamo, M. A. Iglesias-Artea, Synthesis, NMR and crystal characterization of dimeric terephthalates derived from epimeric 4,5-*seco*-cholest-3-en-5-ols. *Steroids* 109 (2016) 66-72.
- [16] B. Yu, P-P. Qi, X-J. Shi, R. Huang, H. Guo, Y-C. Zheng, D-Q. Yu, H-M. Liu, Efficient synthesis of new antiproliferative steroidal hybrids using the molecular hybridization approach. *Eur. J. Med. Chem.* 117 (2016) 241-255.
- [17] K. A. Kumar, J. J. La Clair, P. L. Fuchs, Synthesis and evaluation of a fluorescent Ritterazine-Cephalostatin hybrid. *Org. Lett.* 12 (2011) 5334-5337.

- [18] A. J. Ambrose, E. A. Santos, P. C. Jimenez, D. D. Rocha, D. V. Wilke, P. Beuzer, J. Axelrod, A. K. Kanduluru, P. L. Fuchs, H. Cang, L. V. Costa-Lotufo, E. Chapman, J. J. La Clair, Ritterostatin G_{N1N}, a cephalostatin-ritterazine bis-steroidal pyrazine hybrid, selectively targets GRP78. *Chembiochem.* 18 (2017) 506-510.
- [19] C. W. Bennett, J. Collins, Z. Y. Xiao, D. Klinger, L. A. Connal, A scalable and versatile synthesis of oxime-based hormone dimers and gels for sustained release. *Chem. - An Asian J.* 12 (2017) 1456-1460.
- [20] H. R. F. Karabulut, H. Ozyildirim, K. Ozaraz, K. Kacan, Synthesis of diimines from cholestanone. *Int. J. Adv. Sci. Eng. Technol.* 5 (2017) 122-124.
- [21] Z. Łotowski, U. Kulesza, Studies on the synthesis of cholane derivatives containing a mercapto group and their dimers with disulfide spacers. Part 1. 24-Mercapto-5 β -cholane-3,7,12-triol and its C(24)-C(24') disulfide dimer. *J. Sulfur Chem.* 31 (2010) 97-102.
- [22] Z. Łotowski, U. Kulesza, A. M. Tomkiel, Studies on the synthesis of cholane derivatives containing a mercapto group and their dimers with disulfide spacers. Part 2. 3 α -Mercapto-5 β -cholane-7 α ,12 α ,24-triol and its C3-C3' disulfide dimer. *J. Sulfur Chem.* 31 (2010) 525-532.
- [23] A. M. Tomkiel, U. Kielczewska, B. Seroka, Z. Łotowski, Dimeric cholaphanes with disulfide spacers. *J. Sulfur Chem.* 39 (2018) 252-266.
- [24] V. H. Soto, M. Alvarez, F. Meijide, J. V. Trillo, A. Antelo, J. Aida, L. Galantini, J. V. Tato, Ice-like encapsulated water by two cholic acid moieties. *Steroids* 77 (2012) 1228-1232.
- [25] F. Meijide, J. V. Trillo, S. de Frutos, L. Galantini, N. V. Pavel, V. H. Soto, A. Jover, J. V. Tato, Crystal structure of head-to-head dimers of cholic and deoxycholic acid derivatives with different symmetric bridges. *Steroids* 78 (2013) 247-254.
- [26] R. M. Valdez-García, C. Alarcón-Manjarrez, R. Arcos-Ramos, M. Flores-Álamo, M. A. Iglesias-Arteaga, Synthesis and characterization of dimeric steroids based on 5-oxo-4,5-seco-yne units linked by a diyne spacer. *Arkivoc* (2018) 13-22. DOI: 10.24820/ark.5550190.p010.459
- [27] S. Zaman, M. G. Alam, T. Siddiqui, Synthesis of steroidal dimers: Selective amine catalysed steroidal dimerization. *J. Chem. Soc.* 4 (2011) 491-495.
- [28] T. Pospieszny, I. Małecka, Z. Paryzek, Synthesis and spectroscopic studies of new bile acid derivatives linked by a 1,2,3-triazole ring. *Tet. Lett.* 53 (2012) 301-305.

- [29] M. A. Ramos-Enríquez, M. A. Iglesias-Arteaga, Synthesis of novel hybrid steroid dimers by $\text{BF}_3 \cdot \text{Et}_2\text{O}$ -catalyzed aldol condensation of 2-formyl-estradiol diacetate and steroid sapogenins. *Steroids* 128 (2017) 46-49.
- [30] A. R. Vesper, J. Lacroix, R. C. Gaudreault, H. A. Tajmir-Rihai, G. Bérubé, Synthesis of novel C2-symmetric testosterone dimers and evaluation of antiproliferative activity on androgen-dependent and -independent prostate cancer cell lines. *Steroids* 115 (2016) 98-104.
- [31] R. M. B. Carrilho, M. M. Pereira, S. M., M. J. Moreno, A. Takács, L. Kollár, A new facile synthesis of steroid dimers containing 17,17'-dicarboxamide spacers. *Tet. Lett.* 54 (2013) 2763-2765.
- [32] B. Yu, X-J. Shi, T-F. Zheng, Y. Fang, E. Zhang, D-Q. Yu, H-M. Liu, A novel [1,2,4] triazolo [1,5- α] pyrimidine-based phenyl-linked steroid dimer: Synthesis and its cytotoxic activity. *Eur. J. Med. Chem.* 69 (2013) 323-330.
- [33] B. Yu, X-J. Shi, P-P. Qi, D-Q. Yu, H-M. Liu, Design, synthesis and biological evaluation of novel steroidal-spiro-oxindoles as potent antiproliferative agents. *J. Steroids Biochem. Mol. Biol.* 141 (2014) 121-134.
- [34] D. Czajkowska-Szczykowska, I. Jastrzebska, R. Santillan, J. W. Morzycki, The synthesis of disteroidal macrocyclic molecular rotors by an RCM approach. *Tetrahedron* 70 (2014) 9427-9435.
- [35] M. Kiss, S. Maho, K. Boddi, B. Boros, L. Kollar, Palladium-catalyzed diaminocarbonylation: synthesis of androstene dimers containing 3,3'- or 17,17'-dicarboxamide spacers. *Monatsh Chem.* 146 (2015) 357-364.
- [36] R. Skoda-Földes, *N,N*-Bis(3 β -acetoxypregn-5(6)-en-20-on-16 α -yl)hydroxylamine. *Molbank* (2015) M847. DOI: <https://doi.org/10.3390/M847>
- [37] M. Jurášek, M. Černohorská, J. Řehulka, V. Spiwok, T. Sulimenko, E. Dráberová, M. Darmostuk, S. Gurská, I. Frydrych, R. Buriánová, T. Ruml, M. Hajdúch, P. Bartůňek, P., Dráber, P. Džubák, P. Drašar, D. Sedlák, Estradiol dimer inhibits tubulin polymerization and microtubule dynamics. *J. Steroids Biochem. Mol. Biol.* 183 (2018) 68-79.
- [38] M. A. Ramos-Enríquez, L. Rárová, M. A. Iglesias-Arteaga, Synthesis of dimeric spirostanols linked through a 1,4-dimethylidenebenzene moiety by double $\text{BF}_3 \cdot \text{Et}_2\text{O}$ -catalyzed aldol condensation of steroid sapogenins and terephthalaldehyde. *Steroids* 140 (2018) 58-61.

- [39] A. Chakrabarty, U. Maitra, Organogels from dimeric bile acid esters: In situ formation of gold nanoparticles. *J. Phys. Chem.* 117 (2013) 8039-8046.
- [40] I. Mądrzak-Litwa, A. Wojciechowska, Z. Paryzek, Synthesis of isomeric dimers of deoxycholic acid derivatives linked by 1,2,3-triazole. *Syn. Comm.* 45 (2015) 1222-1230.
- [41] R. G. Savchenko, S. A. Kostyleva, V. N. Odinokov, Reductive amination of ω -oxoecdysteroids in the synthesis of dimeric ecdysteroids. *Helv. Chim. Acta* 98 (2015) 1292-1301.
- [42] T. Pospieszny, M. K. Pakiet, I. Kowalczyk, B. Brycki, Design, synthesis and application of new bile acid ligands with 1,2,3-triazole ring. *Supramol. Chem.* 29 (2017) 81-93.
- [43] J. Hu, R. Li, J. Lu, Y. Ju, Synthesis and anion recognition of a novel oleanolic acid-based cyclic dimer. *Tet. Lett.* 52 (2011) 4211-4214.
- [44] V. A. Khripach, V. N. Zhabinskii, O. V. Konstantinova, D. V. Tsavlovskii, A. V. Baranovsky, A. S. Lyakhov, P. Drašar, Synthesis, crystal structure and NMR investigation of a new type of cyclic steroidal dimer based on brassinosteroids. *J. Mol. Structure* 1032 (2013) 1-4.
- [45] G. R. Pettit, J-P. Xu, J-C. Chapuis, N. Melody, The cephalostatins. 24. Isolation, structure, and cancer cell growth inhibition of cephalostatin 20. *J. Nat. Prod.* 78 (2015) 1446-1450.
- [46] J. W. Morzycki, Application of olefin metathesis in the synthesis of steroids. *Steroids* 76 (2011) 949-966.
- [47] L. H. Tahtamouni, M. M. Nawasreh, Z. A. Al-Mazaydeh, R. A. Al-Khateeb, R. N. Abdellatif, R. M. Bawadi, J. R. Bamburg, S. R. Yasin, Cephalostatin 1 analogues activate apoptosis via the endoplasmic reticulum stress signaling pathway. *Eur. J. Pharmacol.* 818 (2018) 400-409.
- [48] P. Chanphai, D. Agudelo, A. R. Vesper, G. Bérubé, A. Tajmir-Riahi, H. Testosterone and its dimers alter tRNA morphology. *J. Pharm. Biomed. Anal.* 134 (2017) 269-274.