Chapter

Steroid-Based Supramolecular Systems and their Biomedical Applications: Biomolecular Recognition and Transportation

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Abstract

In this chapter, the biomedical application of steroid-based compounds at "beyond the molecule"—supramolecular level—is reviewed. The renewable and economic natural steroid compounds could be employed as building blocks in the design and construction of steroid-based supramolecular systems. The specific physicochemical features (size, shape, topology, hydrophobicity, chemical modifiability, etc.) and biological properties (biocompatibility, biodegradability, bioaffinity, etc.) could be integrated into functional supramolecular systems by chemical synthesis, modification and intermolecular interactions (such as hydrogen bonding, π-π stacking, van der Waals forces, inclusion interactions, chiral interactions, electrostatic interactions, and so on). The steroid-based (supra)molecules could be employed for molecular recognition and/or be self-assembled into various functional supramolecular assemblies for biomedical applications. The specific physicochemical and biological properties, good biocompatibility, and biological activity endow the steroid-based supramolecular systems good feasibility to be employed in biomolecular recognition/sensing and biomolecular transportation (gene/drug delivery). The examples in this chapter are exemplificative of the transformation of natural steroid-based compounds into functional steroid-based supramolecular systems through molecular and supramolecular engineering technology, moreover, which may inspire the systematic study of natural product-based supramolecular (nano)materials toward future pharmaceutical and biomedical industry.

Keywords: steroid, supramolecular, biomolecular recognition, biomolecular transportation, gene delivery, drug delivery

1. Introduction

Transformation of renewable and biocompatible natural products [1] into a variety of molecular building blocks to construct functional molecular systems and then following the molecular assembly processes to create new functional materials has been highly focused for nurturing the sustainable development. Steroids, a large natural lipid family known as "keys of life," played vital roles including membrane formation, hormone metabolism, and cell signal transduction in organelles. Some steroidal compounds possess special physicochemical features

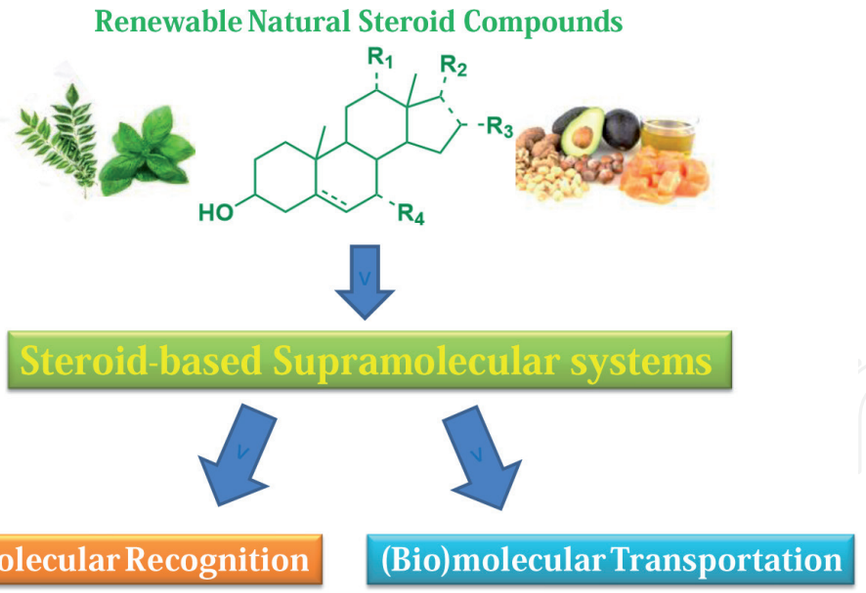

Figure 1.
Steroid-based supramolecular systems for biomolecular recognition and biomolecular transportation.

such as hydrophobicity, rigidity, mesogenic behaviors, and so on, which made them the functional building blocks for the construction of supramolecular architectures [2] and soft nanomatters toward biomaterial application [3].

In general, the functions of supramolecules mainly cover molecular recognition, molecular transportation, and molecular catalysis [4]. Molecular recognition is a fundamental process that integrates molecular information (size, shape, charge, etc.) by interacting (host) molecules with certain (guest) molecular species [5]. Molecular transportation is the use of supramolecules to translocate bounded/loaded molecular species (such as anions [6]) through membranes (especially cell membranes [7]), which could be coupled with chemical potentials [8]. Biomolecular recognition (detecting/sensing of certain biomolecules) and biomolecular transportation (administration/delivery of bioactive molecules into the cells/organs) have been regarded as two important fields in biomedical-orientated supramolecular (medicinal) chemistry [9]. The steroid-based supramolecular systems could be divided into two groups according to their function: (1) steroid-based supramolecular system for biomolecular recognition and (2) steroid-based supramolecular system for biomolecular transportation (**Figure 1**).

2. Steroid-based supramolecular system for biomolecular recognition

Recognition/sensing of biomedically important substances such as specific ions (cations/anions), nucleic acids, peptides, proteins/enzymes, volatile bioorganic molecules, biometabolites, as well as tumor biomarkers is very essential for the deep understanding of biochemical mechanisms. Earlier analytical tools, including chemiluminescence, amperometry, electrochemistry, spectrophotometry, high-performance liquid chromatography, etc., have been developed for the detection of biomedically important substances. However, these traditional methods have some drawbacks such as requirement of expensive instruments and complicated pre-treatment processes, which largely restricted their practical application. Rapid development of artificial molecular receptors or molecular sensors may provide powerful tools for the recognition/sensing of chemical species/analytes, which can be attributed to their advantages of easy-to-manipulate, high-sensitivity, fast-response, high-temporal, and spatial resolution [5].

2.1 Steroid-based macrocyclic molecular receptors

Artificial/synthetic macrocyclic molecular receptors are important supramolecular architectures, which can be used as a host molecule to recognition-specific guest molecules [10]. They can also be used to mimic complex biological host-guest systems, e.g., cell surface receptors, nuclear receptors, as well as enzymes for substrate recognition. Typical macrocyclic molecular receptors bind guest molecules inside their designated cavity. During the past decades, many steroids were developed to construct molecular receptors. Among them, bile acids, a family of molecules with facial amphiphilicity, specific molecular chirality, and multiple reactive sites (hydroxyl and carboxylic acid groups), are often employed as molecular skeletons/scaffolds in the construction of supramolecular architectures for molecular recognition [11, 12].

In an early work, Davis et al. synthesized a neutral and lipophilic system from the steroid cholic acid (**Figure 2**). It forms 1:1 complexes with fluoride, chloride, and bromide ions and shows good discrimination of $Cl^- > Br^- > I^-$ [13]. In this work, the anion recognition process was carried out in organic solvents.

Also for anion recognition, more recently Peng et al. synthesized cholate-based cage amphiphilic systems with combination of structural rigidity and flexibility. These cage compounds with extending and bridging three polar chains were prepared by click reaction. The connecting chains composed of oligo(ethylene glycol) units or chains containing 1,2,3-triazole units to present flexibility, for example, a model compound (triazole **21a**), could recognize halide anions with a binding sequence of $Cl^- > Br^- > I^- \sim F^-$, which makes them potential anions receptors/sensors [14].

Recently, steroid-based macrocyclic molecular receptors with the combination of multifunctions (e.g., chiral recognition-optical properties) emerged as a new trend of research. In this context, Wu et al. synthesized a deoxycholic acid-based macrocycle receptor **CDTB**, which selectively recognized Hg^{2+} involving 1,2,3-triazole motifs as binding sites. The as-formed [**CDTB**·Hg^{2+}] complex could be used to perform enantioselective recognition of amino acids (especially cysteine) in aqueous solution (**Figure 3**), leading to difference in fluorescence enhancement of the chiral BINOL macrocyclic structure at ~358 nm. This research provided cascade recognition of chiral amino acids and bestows the future design of steroid-based dual-functional macrocyclic molecular receptor models for chiral natural product discrimination/recognition [15].

Although some progresses had been made in this field, the synthesis of steroid-based macrocyclic molecular receptors is still mainly focused on the mono steroid-containing macrocyclics and C_2-symmetric macrocyclics; the facile and low-cost

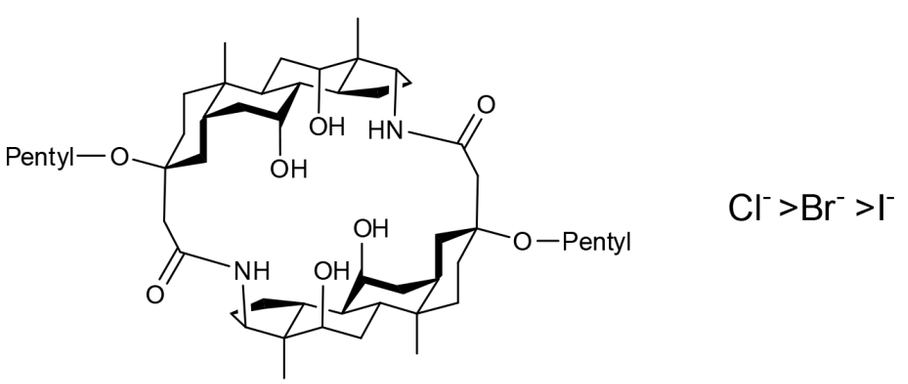

Figure 2.
Cholic acid-based macrocyclic receptor for halides Cl^-, Br^-, and I^- recognition.

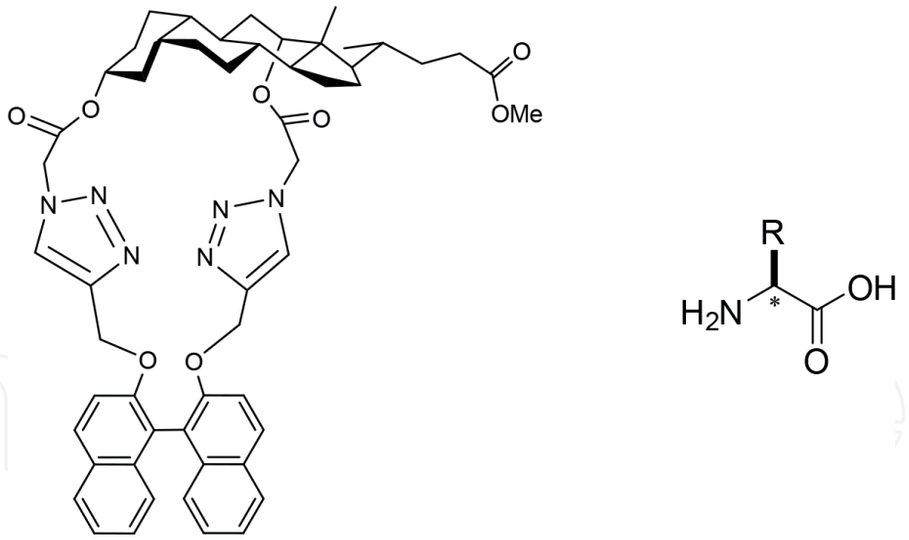

Figure 3.
Cholic acid-BINOL-based fluorescent macrocyclic receptor for chiral amino acid recognition.

preparation of macrocyclics need to be developed. Notably, the steroid-based macrocyclics with higher-order symmetric elements (such as C_3, C_4, D_{xh}, etc.), modifiable and derivable sites, various topological diversities [16], as well as chiral/asymmetric features (giant chiral macrocyclics) are rare. Moreover, for practical application, the functionalities (such as optical, radioactive, paramagnetic, etc.) of the steroid-based macrocyclic molecular receptors need to be largely expanded.

2.2 Steroid-based molecular clefts/tweezers

Another type of artificial/synthetic molecular receptors is open-structured molecular clefts/tweezers, which can recognize guest molecules by forming a sandwich-type structure through π-π stacking, hydrogen bonding, and/or ionic and electrostatic interactions. For the recognition of aromatic molecules, the arms of the molecular clefts/tweezers were generally designated to be aromatic and with special geometrical arrangements. Taking the advantages of low cost, head-tail-modifiable molecular groups, rigidity, chemically different hydroxyl groups, unique amphiphilicity, and natural chiral microenvironment, bile acids and their derivatives are mostly employed to construct steroid-based molecular clefts/tweezers [17].

For the steroid-based molecular clefts/tweezers toward anion recognition, acidic amide groups (such as NH in ureas or thioureas) were always used to achieve higher affinities [17]. In this context, Davis et al. constructed anion receptor by placing squaramide groups in axial positions at the hydroxyl groups of steroid (cholic acid) skeleton, which could fix the NH groups on squaramide at certain locations for cooperatively bind anions (**Figure 4**). By using the steroid-squaramide receptor, anions Cl^- and AcO^- could be transferred from water to organic solvent by liquid-phase extraction. The binding constants of the steroid-squaramide receptor to Cl^- and AcO^- of tetraethylammonium salts exceeding $10^{14}\ M^{-1}$ in chloroform solution have been measured. The results indicated that these anion receptors might serve as transmembrane anion carriers or artificial cell surface receptors for biomedical application [18].

The synthesized molecular tweezers for small biomolecule recognition mainly have charge-bearing moieties/groups such as carboxylic acids and amine/guanine groups. As an example, Rao et al. have designed and synthesized a bile acid-based molecular tweezer with two carboxylic acid groups attached to the C-3 and C-12

hydroxyl groups, which could complex 9-*N*-butyladenine and biotin methyl ester [19] by π-π and electrostatic interactions along with restricted rotation effects (**Figure 5**). Notably, the sensitivity and selectivity of this kind of receptors are not high enough to distinguish biomolecules with similar structures. To design highly selective molecular tweezers, a possible strategy is to mimic the microchemical environment of protein (or sugar) domains responsible for enzyme-substrate recognition or cell receptor-ligand interactions [4].

For chiral amino acid recognition, Davis et al. [20] prepared guanidinium-bearing steroidal molecular tweezers, which could recognize and extract *N*-acetyl-amino acids (**Figure 6**) from aqueous solution into the organic phase ($CHCl_3$) by electrostatic interactions between guanidinium moiety and carboxylic acid groups, with enantiomeric excesses (ee%) of about 80% [21]. In general, the association constant for these acceptors should be around ~10^{-4}–10^{-5}.

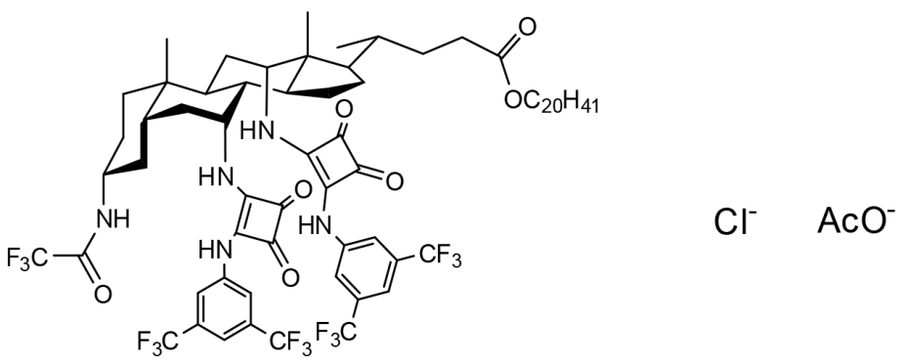

Figure 4.
Cholic acid-squaramide conjugates as a molecular tweezer for anions Cl^- and AcO^- recognition.

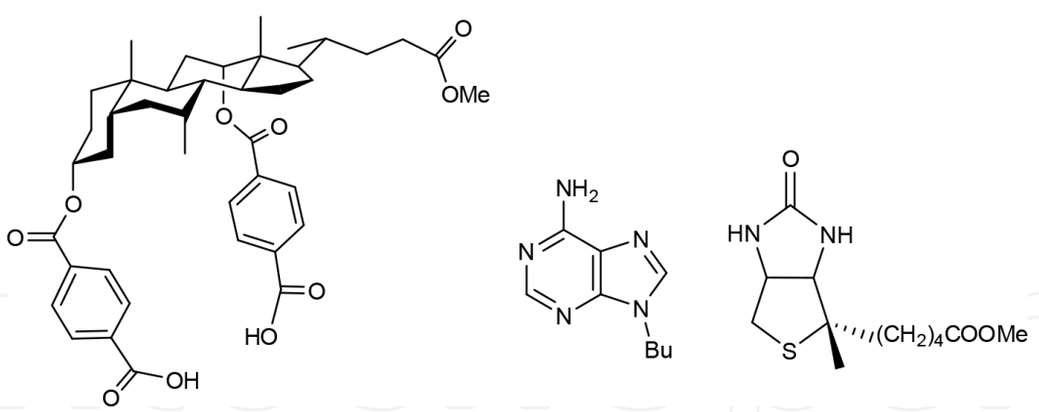

Figure 5.
Cholic acid-based molecular tweezer for N-butyladenine and biotin methyl ester recognition.

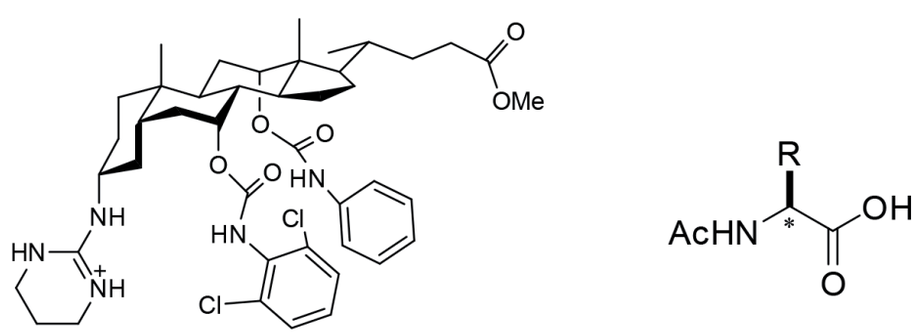

Figure 6.
Cholic acid-guanidinium molecular tweezer for N-acetyl-amino acid recognition.

Bile acid-based receptors containing 2,6-diaminopyridine and the dioctylamide of 2,6-diaminopyridine were also used to bind 7,8-dimethyl flavin analogues. The association constants increased with increasing electron-donating capacity of the substituents at the 7 and 8 positions of the flavin analogues [22].

To our knowledge, up to date, the molecular recognition of the steroid-based molecular tweezers mainly focuses on several simple molecules including anions, nucleosides, and amino acids. Their recognition properties toward more biomolecular analytes/substrates (such as oligosaccharides, peptides, biometabolites, as well as pharmaceuticals) need to be continuously explored. Further improvements on the sensitivity and selectivity, possibility to perform quantitative detection/recognition, increasing signal-noise ratios, as well as developing portable in situ test kit/membrane also need to be taken into consideration. Notably, the cell biological behaviors such as uptake, metabolism, and pharmacological applications of these steroid molecular tweezers are far from being understood. Moreover, the emergence of natural compound such as coumarin [23–25]-based fluorescent molecular receptors/sensors may inspire further development of steroid-based multichannel molecular receptors [4].

3. Steroid-based supramolecular system for biomolecular transportation

Transportation/delivery technology of biomolecular species (especially therapeutic agents) across cell membranes and other biological barriers emerged and rapidly developed as a pivotal area in pharmaceutical and clinical biomedicine, since many biological barriers prevent the implementation of clinically effective therapeutic agents (e.g., genes, antitumor drugs, cell signal inhibitors, neuron modulators, etc.). Therefore, developing functional therapeutic (gene/drug) transportation/delivery systems with the merit of low cost, facile-to-prepare, high storage stability, low cytotoxicity, high gene/drug-loading/delivery capacity, as well as controllable releasing/targeting features has attracted much attention in recent years [26–32].

3.1 Steroid-based supramolecular system for gene delivery

Using renewable and biocompatible natural-based resources to construct supramolecular biomaterials has attracted great attentions in recent years. As a hot spot in biomaterial research, developing new cationic lipids as non-viral gene (DNA, oligo DNA, SiRNA, etc.) carriers toward gene therapy has been achieved increasing attentions in the past few decades [33, 34]. An ideal lipid gene carrier should be highly biocompatible [35] and could efficiently load and release therapeutic gene substances [36] into target cells. In this context, recent researches revealed that the introduction of some steroidal hydrophobic molecules in gene carriers could enhance gene loading capacity and delivery efficiency [37], improve estrogen receptor (ER) affinity [38], lower cytotoxicity and membrane disruption [39], and so on, making the steroid-based cationic amphiphiles/lipids promising candidates for gene delivery/transfection (**Figure** 7).

Among the steroid compounds, cholesterol was the most commonly used steroidal compounds in the construction of functional gene/drug [40] carriers. As an example, Bhattacharya and Bajaj developed a series of cholesterol cationic lipids [41] and gemini-lipids [42–46] with remarkably high gene transfection efficiency and transfected p53-EGFP-C3 plasmid DNA to induce tumor apoptosis [47]. In another example, Rana et al. [48] prepared some cholesterol-hybridized cationic lipids with enhanced SiRNA delivery efficiencies and lower cytotoxicity.

In addition, Zenkova et al. [49–51] disclosed a series of cholesterol cationic lipids modified with heterocyclic (pyridine, methylimidazole, etc.) or polyamine headgroups having low cytotoxicity and high transfection efficiency, and some cholesterol-based cationic glucosidal lipids also have similar properties [52].

In our earlier work, we prepared a series of bioreduction-responsive cholesterol disulfide cationic (CHOSS) lipids [53], which possessed low cytotoxicity, high pDNA transfection efficiency, as well as perinucleic localization effect (**Figure 8**). Afterward, we studied the structure-gene transfection relationship of some cholesterol-based cationic lipids bearing versatile amino acid headgroups and chemical linkage bonds [54], and it was found that the physicochemical features and gene transfection-related properties of the cholesterol-based lipids relied greatly on the cationic headgroups [54].

Besides cholesterol, some other steroidal compounds such as diosgenin (a phytosteroid sapogenin used in the preparation of different steroids, e.g., cortisone), bile acids, etc. were employed to construct lipid gene carriers. As an example, Regen et al. developed a series of "molecular umbrella" amphiphiles [55] and disulfide-containing bile acid-SiRNA conjugates [56] for intracellular SiRNA delivery. In addition, Yi et al. [57–59] synthesized some diosgenin-based cyclen cationic lipids with the merit of low cytotoxicity and high transfection efficiency. In a previous work, we also synthesized some cholesterol and lithocholate-derived cationic lipids via CuAAC "click" approach and disclosed that their gene transfection efficiency relied greatly on the steroid structures [60].

It has been known that the endocytosis mechanism greatly affects the intracellular gene transfection efficacy and subcellular distribution of gene carriers [61]. For the endocytosis pathways of steroid-containing gene carriers, only a few cases were

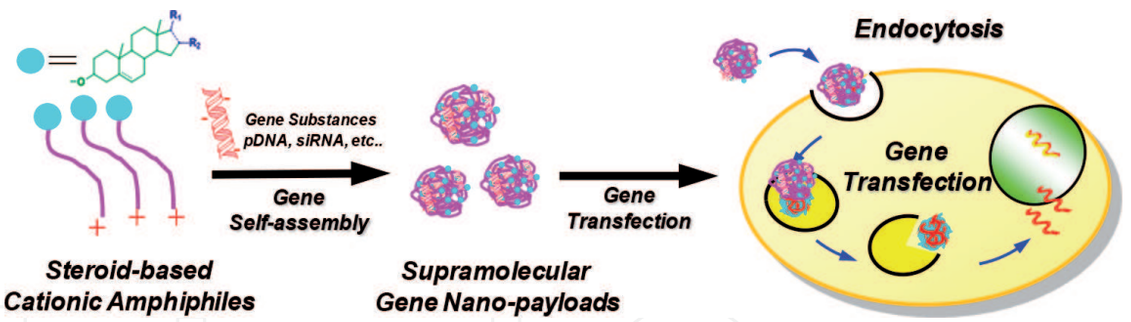

Figure 7.
Steroid-based cationic amphiphiles/lipids for gene delivery/transfection.

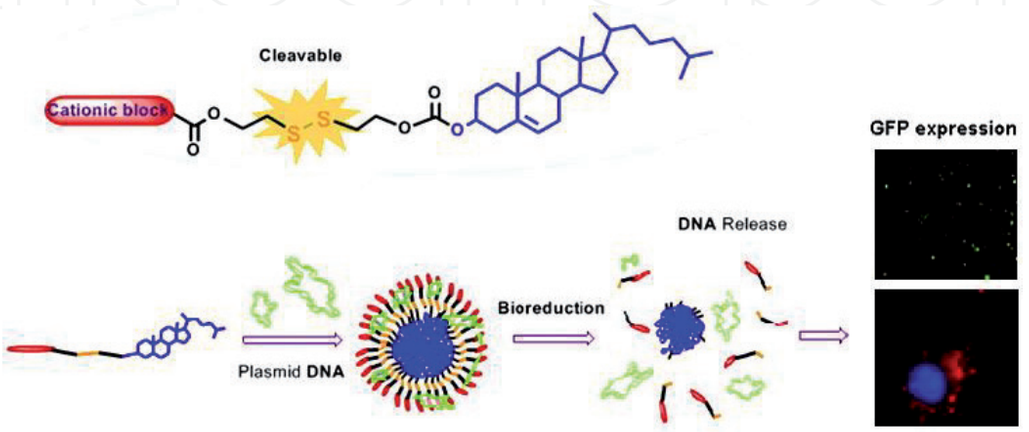

Figure 8.
Bioreduction-responsive cholesterol-based disulfide cationic lipids/pDNA supramolecular payloads as efficient gene delivery carriers.

investigated. In this context, Bae et al. [62] found that clathrin-mediated endocytosis is the dominant pathway for cholesterol-based (CHOL-E) liposomes. On the other hand, Pozzi et al. [63] disclosed that macropinocytosis is the only endocytosis pathway of a cholesterol cationic lipid (DC-Chol) containing multicomponent envelope-type nanoparticle system (MENS). Besides, Jeong et al. [64] disclosed that clathrin, caveolae, and pinocytosis pathways are involved in the cellular uptake mechanism of hydrophobic 5β-cholanic acid containing glycol chitosan (HGC) nanoparticles.

In a recent work, our research team successfully prepared a series of steroid-based cationic lipids by integrating various hydrophobic steroid skeletons with (*l*-)-arginine headgroups via a facile and efficient synthetic approach. We found that the plasmid DNA (pDNA)-binding affinity of the steroid-based cationic lipids, average particle sizes, surface potentials, morphologies, as well as stability of the steroid-based cationic lipids/pDNA lipoplexes depend largely on the steroid skeletons. Cellular evaluation results revealed that cytotoxicity and gene transfection efficiency of the steroid-based cationic lipids in H1299 and HeLa cells strongly relied on the steroid. Interestingly, the steroid lipids/pDNA lipoplexes seemed to enter H1299 cells mainly through caveolae- and lipid-raft-mediated endocytosis pathways, and an intracellular trafficking route of "lipid-raft-mediated endocytosis→lysosome→cell nucleic localization" was accordingly proposed (**Figure 9**). The study provided possible approach for developing high-performance steroid-based lipid gene carriers, in which the cytotoxicity, gene transfection capability, endocytosis pathways, as well as intracellular trafficking/localization manners could be tuned/controlled by introducing proper steroid skeletons/hydrophobes. Noteworthy, among the lipids, Cho-Arg showed remarkably high gene transfection efficacy even under high serum concentration (50% FBS), making it an efficient gene transfection agent for practical application [65].

Although many remarkable achievements have been made in the steroid-based gene delivery systems, the working performance such as biocompatibility, gene transfection efficiency, serum compatibility, cell membrane permeability, as well as the in vivo transfection of the most of steroid-based gene carriers were still far from their maximum value, especially far below from their natural virus (adenovirus, SV40, etc.) counterparts. The correlation between steroid-based molecular structures and their transfection efficiency is not well known, and, notably, the correlation between molecular structures and endocytosis pathways, endonucleasis gateways, and intracellular trafficking and subcellular targeting/localization for the most of steroid-based gene delivery systems still remains unclear. Elucidating these correlations may offer new routes to further design steroid-based supramolecular systems with "endocytosis pathway selection" and "subcellular organelle targeting/localization" features.

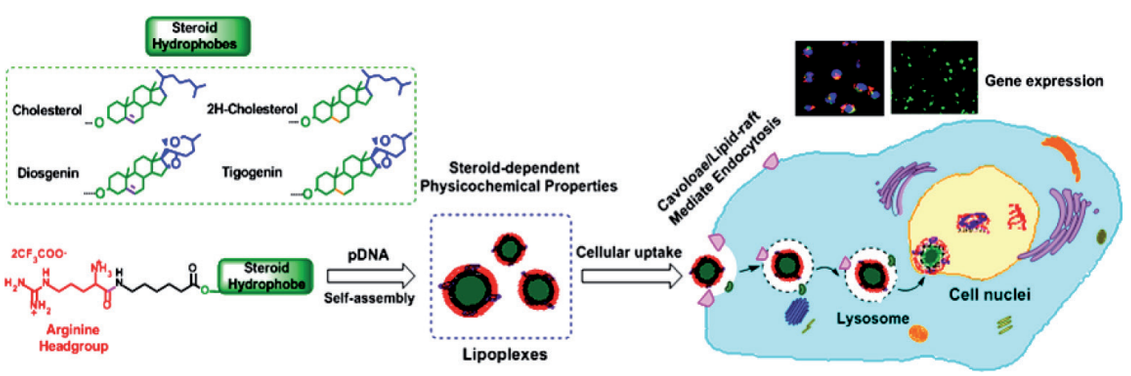

Figure 9.
Steroid-based cationic lipids/pDNA supramolecular payloads as efficient gene delivery carriers and the caveolae/lipid-raft-mediated cellular uptake pathway.

Moreover, to achieve combo-chemotherapy and high theranostic performance, remote [66] factors (e.g., near-infrared light, ultrasonic, X-ray, or γ-ray)—induced controllable gene releasing and (optical and radioactive) imaging agents—which incorporated steroid-based supramolecular gene carriers need to be taken into consideration. For future research, we envisioned that "smart" features such as enzyme-responsive [67], self-programmable [68], self-replicable, as well as self-evolution technology could be implemented on the steroid-based supramolecular gene carriers by designing/optimizing the steroid-based molecular structures or supramolecular architectures through molecular or supramolecular engineering approaches.

3.2 Steroid-based supramolecular system for small molecule/drug delivery

Similar to gene delivery, controllable delivering of small molecules, including drugs and other bioactive compounds by steroid-based supramolecular systems, is another important field. Some steroids such as bile acids and diosgenin were utilized to prepare drug delivery carriers. In an early study, Regen et al. developed some cholic acid-based molecular umbrellas, which were utilized to transport small biomolecules such as adenosine 5-triphosphate (ATP) [69], glutathione (GSH) [70], as well as an oligonucleotide (S-dT16) [71] across phospholipid bilayer membranes prepared from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylglycerol.

To improve the hydrophilic, long-retention/stealth effect, and biocompatibility, polyethylene glycol (PEG) was often introduced to steroid scaffolds [72]. In fact, PEGylated bile acids were synthesized to further prepare self-emulsifying drug delivery systems (SEDDSs), which could enhance the solubility and absorption of poor water-soluble antitumor agent (doxorubicin [73]) or antibiotics (itraconazole [74]), thus providing a significant enhancement of solubility and bioavailability of these small molecular drugs. The emulsions consisted of spherical micelles with a mean hydrodynamic diameter around 100–220 nm, with good biocompatibility (low cytotoxic and hemolytic effect).

Taking advantage of organotropism effect of certain steroid compounds (such as cholesterol and cholic acid), steroid-drug conjugates enable enhanced active targeting of drug delivery into certain organelles to improve their bioavailability. Some bile acid-based prodrugs are prepared by conjugating drugs through degradable bonds, either direct or via spacer molecules to the carboxylic group or to the chemically different (C-3, C-7, and C-12) hydroxyl groups [75]. Tolle-Sander et al. found that cholic acid-acyclovir conjugated prodrugs could target human apical sodium-dependent bile acid transporter (ASBT) to enhance acyclovir bioavailability. In this case, a valine linker between cholic acid and acyclovir could be cleaved upon esterase hydrolysis and release acyclovir [76]. Later, other bile acid-based prodrugs such as cholic acid-cytarabine conjugates [77], cholic acid-5-fluorouracil (FU) conjugates [78], and bile acid-tamoxifen conjugates [79] were developed. The bile acid-based prodrug transport systems showed improved drug absorption, membrane permeation, as well as the "trojan horse" effect [80] that largely increased the bioavailability of the antitumor drugs. In 2009, Regen et al. reported molecular umbrella-hydrophobic drug conjugates, which exhibit enhanced uptake capability to enter living (such as HeLa) cells and increased drug activity, suggesting the conjugates could be used as drug carriers [81]. Besides, the organ-specific targeting properties, especially the liver and small intestine distribution effect, were making the bile acid-based prodrug transport systems efficient candidates for the delivery of low-bioavailability molecular pharmaceutics [82]. The bile acid-based prodrugs provide efficient building blocks for constructing and developing supramolecular prodrug drug delivery systems (SPDDS), which also inspired the extensive R&D of other steroid-based

SPDDS [83]. It could be envisioned that, by choosing certain functional moieties to construct steroid-based prodrugs and followed by self-assembly, efficient SPDDS toward controllable chemotherapy could be achieved (**Figure 10**).

Recently Wei et al. designed and prepared a novel diosgenin-PEG (derivative)-based prodrug nanocarrier for inhibiting thrombosis. The steroid diosgenin was conjugated to PEG by means of a pH-sensitive Schiff base bond to prepare the prodrug, then which was self-assembled into nanomicelles in aqueous solution. Under acidic condition (around thrombosis places), the diosgenin-PEG-containing micelles could be cleaved and released and could improve the blood diosgenin concentration to efficiently inhibit thrombosis. Moreover, the diosgenin-PEG micelles without bleeding risk prevented thrombosis by inhibiting activation and apoptosis of platelet. In this study, the observed efficiency of diosgenin-PEG was better than that of the nonsteroid antithrombotic agent aspirin [84].

Multicomponent nanotherapeutic (by combining two or more drugs/prodrugs into a single system) drug delivery systems (MCNDDS) and related formulations have attracted more and more attention. With the merit of easy-to-manipulate, good storage stability, high drug-loading capacity, low cytotoxicity, as well as controllable drug-releasing features, R&D on MCNDDS could be expected to serve as a promising field in nanopharmaceutics and clinical medicine [85]. As mentioned above, cholesterol has been known to play important roles in membrane property regulation, cell adhesion, and signal transduction, regulating lipid bilayer interaction and intracellular trafficking of nanoparticles, thus bringing new potential applications in biomedical engineering. In one case, cholesterol-based adenosine triphosphate has been prepared, which could be efficiently transported across bilayer membranes of liposomes [86]. In recent studies, we prepared a series of combo-nanotherapeutics by controllable incorporation of cholesterol-based/—conjugated doxorubicin prodrug (**Chol-LK-Dox**) with tocopherol polyethylene glycol succinate (**TPGS**), a helper lipid in the construction of functional liposomes or solid lipid nanoparticles, using a thin-film hydration method (**Figure 11**). Among them, we found that a series of **Chol-Dox/TPGS** assemblies (molar ratios 2:1, 1:1, and 1:2) were able to form nanoscaled particles with the average hydrodynamic particle diameter of 100–250 nm and remarkable solution stability (in 0.1 M PBS, 30 days). Notably, the doxorubicin loading and releasing properties could be adjusted by changing the molar ratio of **Chol-Dox** and **TPGS**, thus leading to controllable tumor cell inhibition properties to breast cancer (MCF-7 and MDA-231) cells. Likewise, the physicochemical properties and bioactivity of another cholesterol-based nanodelivery system (**Chol-LK-Dox/TPGS**) could also be tuned by changing the (bioresponsive) linkers and molar ratio of **Chol-LK-Dox** and **TPGS**. The cellular biological properties of **Chol-LK-Dox/TPGS** systems in other cancer cell lines and in vivo therapeutic properties in xenograft mice models will be deeply investigated (project ongoing in our lab).

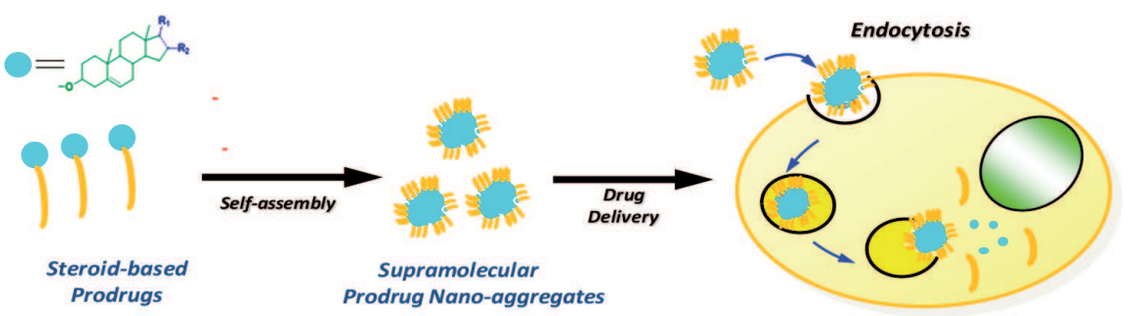

Figure 10.
Self-assembly of steroid-based prodrugs into supramolecular payloads for drug delivery application.

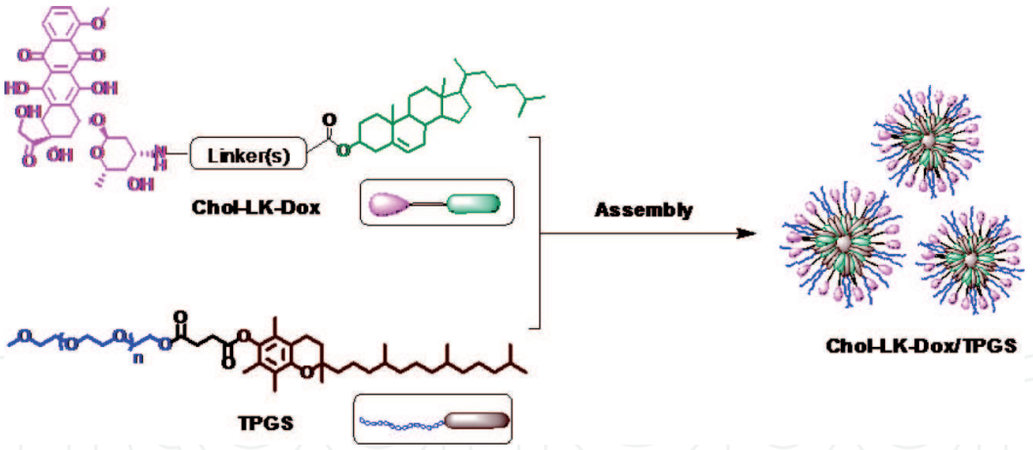

Figure 11.
Self-assembly of steroid (cholesterol)-doxorubicin prodrug (Chol-LK-Dox) with TPGS to prepare MCNDDS for combo-chemotherapy.

Nowadays, for the requirement of "precise biomedical treatment," the steroid-based supramolecular prodrug systems with smart manners such as stimuli-sensitive (temperature, ultrasound, light, electric, pH, redox, biomolecules, and enzyme) features and targeting (cell membrane, subcellular organelles, and cell nuclei) properties need to be further developed.

4. Conclusions

In this chapter, we reviewed the main biomedical application of steroid-based compounds "beyond the molecule"—supramolecular level. The renewable, economic natural steroid compounds could be employed as building blocks in the design and construction of steroid-based supramolecular systems. Based on the specific physicochemical features (size, shape, topology, hydrophobicity, chemical modifiability, etc.) and biological properties (biocompatibility, biodegradability, bioaffinity, etc.), through chemical synthesis, modification, and by means of intermolecular weak interactions (such as hydrogen bonding, π-π stacking, van der Waals forces, inclusion interactions, chiral interactions, electrostatic interactions, and so on), the steroid-based functional molecules could be organized to supramolecules for molecular recognition/sensing and/or be self-assembled into various functional supramolecular assemblies for biomedical applications. The specific physicochemical and biological properties, good biocompatibility, and biological activity endow the steroid-based supramolecular systems good feasibility to be employed in biomolecular recognition/sensing and biomolecular transportation (gene/drug delivery). The examples in this chapter illustrated the transformation of natural steroid-based compounds into functional steroid-based supramolecular systems through molecular and supramolecular engineering technology, which may inspire the systematic study of natural product-based supramolecular (nano) materials toward the future pharmaceutical and biomedical industry.

Although many natural steroid-based supramolecular/nano-systems have been developed and studied, there are still many problems which need to be solved and vast spaces that need to be filled in further extensive research: (1) At molecular level, apart from the natural steroid-based supramolecular shown above, the steroid-based compounds with unique structures (molecular symmetry, geometry and topology, polarity, amphiphilicity, multivalency, etc.), physicochemical (thermal, optical, magnetic, acoustic, radioactive, etc.), properties and biofunctions (biorecognition, targeting, endocytosis, cell signaling, etc.), as well as green synthesis

techniques of the building blocks/units that need to be further developed. (2) At supramolecular level, the self–/forced assembly properties of many natural steroid-based supramolecular/nano-systems were still not well studied; especially their structure–property relationships need to be further explored, realizing the control/adjustment of the steroid-based nanoassemblies with specific physicochemical and/or biological functions. (3) For biomedical application, we need to continue exploring the related biological functions (such as biocompatibility, biometabolic activity, biomimicking manners, etc.) of the steroid-based supramolecular systems and reveal the relationship between the molecular/supramolecular structure and their biological behaviors. Moreover, we anticipated that molecular-level properties of the steroid-based molecules/building blocks would be transferred, enhanced, and/or magnified into supramolecular-level properties, providing a "bottom-up" method to create new renewable resource-derived nanostructures and nanomaterials.

Finally, we need to notice that the steroid-based supramolecular system as aforementioned in this chapter is mostly restricted in low-dimensional 0D and 1D level and, therefore, for real practical application toward complexity systems, higher-ordered steroid-based supramolecular systems (such as 2D and 3D) are needed to be further developed; especially, as for the emergence of natural-based tissue engineering materials and rapid development of 3D bioprinting technology, steroid-based supramolecular system for cell culture and regenerative medicine needs to be taken into consideration and systematically developed in the near future.

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Conflict of interest

No "conflict of interest."

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