Abstract

Gorgosterol and its derivative demethylgorgosterol are two long known marine steroids with unusual properties and a unique structure, containing a cyclopropane moiety in the side chain. Several semisyntheses for these molecules are known, which however suffer drawbacks, such as long and low yielding synthetic sequences and low stereoselectivity. Gorgosterol acts as a growth inhibitor in human colon cancer cell lines, and it was also identified as a new chemotype of farnesoid-X-receptor (FXR) antagonist, making it a potential target for the treatment of cholestasis. The biological function of gorgosterol is unknown, and so is the exact mechanism of the biosynthesis of these steroids, despite ongoing research. Various derivatives of gorgosterol are known, with diverse structural motives and various biological activities. However, they have not been explored synthetically. Moreover, corals and coral reefs have a tremendous ecological and economic impact but are endangered by climate change. A better understanding of corals can therefore aid their protection and conservation.

To achieve all these goals, this work aimed to develop novel synthetic routes and methods for the synthesis of demethylgorgosterol and gorgosterol. They should be concise, high-yielding, stereoselective, and provide a modular basis for synthetic analogs.

A novel, short and high yielding formal semisynthesis for the marine steroid demethylgorgosterol was developed in this work. It was centered on stereoselective cyclopropanation. A known ketone intermediate was synthesized in ten steps in a linear sequence yielding 27% and with excellent stereocontrol. Further steps were taken to complete the semisynthesis, with the most promising approach being the decarboxylative coupling of active esters synthesized from cyclopropanecarboxylic acids. A variety of demethylgorgosterol analogs were synthesized for biological applications. These include hydrocarbon analogs, diversely functionalized analogs which could also be derivatized even further, and fluorophore-steroid conjugates to track and visualize steroids *in vivo*. Finally, a new method for the synthesis of 3-cyclopropylacrylates was developed. Here, a vinylogous diazoester was utilized to cyclopropanate alkenes. The observed *cis*-selectivity was explained with π-π-interactions in the transition state.

Kurzzusammenfassung

Gorgosterol und sein Derivat Demethylgorgosterol sind zwei lange bekannte marine Steroide mit außergewöhnlichen Eigenschaften und einer einzigartigen Struktur, die eine Cyclopropan-Einheit in der Seitenkette aufweist. Es sind mehrere Semisynthesen für diese Moleküle bekannt, die jedoch verschiedene Nachteile aufweisen. Gorgosterol wirkt als Wachstumsinhibitor menschlicher Darmkrebszelllinien und wurde als neuer Chemotyp von Farnesoid-X-Rezeptor-Antagonisten identifiziert, was es zu einem potenziellen Ziel für die Behandlung von Cholestase macht. Die biologische Funktion von Gorgosterol ist unbekannt, ebenso der Mechanismus der Biosynthese dieser Steroide. Es sind verschiedene Derivate des Gorgosterols bekannt, mit unterschiedlichen strukturellen Motiven und vielfältigen biologischen Aktivitäten, die jedoch synthetisch nicht erschlossen sind. Darüber hinaus haben Korallen und Korallenriffe eine enorme ökologische und wirtschaftliche Bedeutung, sind aber unter anderem durch den Klimawandel gefährdet. Ein besseres Verständnis der Korallen kann daher zu ihrem Schutz beitragen.

Um diese Ziele zu erreichen, war diese Arbeit darauf ausgerichtet, neuartige Routen und Methoden für die Synthese von Demethylgorgosterol und Gorgosterol zu entwickeln. Diese sollten kurz, ertragreich und stereoselektiv sein und darüber hinaus eine modulare Basis für die Synthese von synthetischen Analoga bieten.

In dieser Arbeit wurde eine kurze und ausbeutereiche formale Semisynthese für das marine Steroid Demethylgorgosterol entwickelt, basierend auf einer stereoselektiven Cyclopropanierung. Ein bekanntes Keton-Intermediat wurde in insgesamt zehn linearen Schritten in einer Ausbeute von 27% und mit ausgezeichneter Stereokontrolle synthetisiert. Weitere Schritte wurden unternommen, um die Semisynthese zu vervollständigen, wobei der vielversprechendste Ansatz die decarboxylierende Kupplung von Aktivestern von Cyclopropancarbonsäuren war. Es wurde eine Vielzahl von Demethylgorgosterol-Analoga für biologische Anwendungen synthetisiert. Dazu gehören sowohl simple, als auch vielfältig funktionalisierte Analoga, die weiter derivatisiert werden können, sowie Fluorophor-Steroid-Konjugate zur Verfolgung und Visualisierung von Steroiden *in vivo*. Schließlich wurde eine neue Methode zur Synthese von 3-Cyclopropylacrylaten entwickelt. Ein vinyloger Diazoester wurde zur Cyclopropanierung von Alkenen verwendet. Die beobachtete *cis*-Selektivität wurde mit π-π-Wechselwirkungen im Übergangszustand erklärt.

1 Introduction

Approximately 70% of the earth's surface is covered in water. Corals and coral reefs cover only about 1% of this area. However, they are the most diverse aquatic ecosystems.[1] They are associated with an estimated 25% of all marine species and 32 of the 33 animal phyla. Their biodiversity is rivaled only by rain forests, although these cover roughly twenty times the area.[2]

Their ecological and economic impact is of global scale.[3-4] Reefs act as nurseries for edible fish and have touristic value. Furthermore, they are the source of countless natural products, not limited to any number of substance classes.[5]

Yet, corals are endangered by climate change in multiple ways, such as rising temperatures and pH values.[6-8] Environmental pollution presents an equally important problem, not only in the form of pesticides, heavy metals, and eutrophication, but also increased sediment loads,[6] amongst others.[9-10]

Corals react to these stresses by bleaching, the expulsion of symbiotic algae, leading to their demise if endured too long.[3] A better understanding of this symbiotic relationship might contribute to their protection and conservation.

1.1 Steroids[11]

1.1.1 Introduction

Steroids are compounds occurring ubiquitously, with diverse structures and functions, in plants and animals alike. In humans, in the form of hormones, they serve to control and regulate metabolic processes, menstruation, and pregnancy, to name but a few.[12-13] Furthermore, bile acids play a major role in fat digestion. Cholesterol, which is the most important steroid in humans in terms of quantity and a component of the cell membrane, regulates membrane fluidity. Furthermore, it is the starting point for the entire steroid metabolism, even though it has no biological activity.[12,14] Also worth mentioning are the cardenolides, a series of heart-active plant steroids, and the steroid alkaloids, which are used by many plants and animals as defense poisons.[12]

Chemically the steroids are isoprenoids and thus related to the terpenes. Both substance classes are biosynthetically built up from isoprene units. The basic skeleton of the steroids is a system of four fused rings, perhydro-cyclopenta[a]phenanthrene, also known as gonane (**1**). Because systematic names can become arbitrarily long and complicated for many steroids, a different nomenclature was put in place for them, considering their historically determined numbering.[13,15-16] Figure 1 illustrates this numbering using stigmasterol (**2**) and lanosterol (**3**) as examples, as they highlight several particularities. In stigmasterol (**2**), the smaller chain attached to C-24 is labeled with superscript numbers, as the previously used 28 and 29 are now reserved for C-30 steroids like lanosterol (**3**). The two methyl groups attached to C-4 are labeled 28 and 29, while the methyl group attached to C-14 is labeled 30. Missing atoms do not change the numbering.

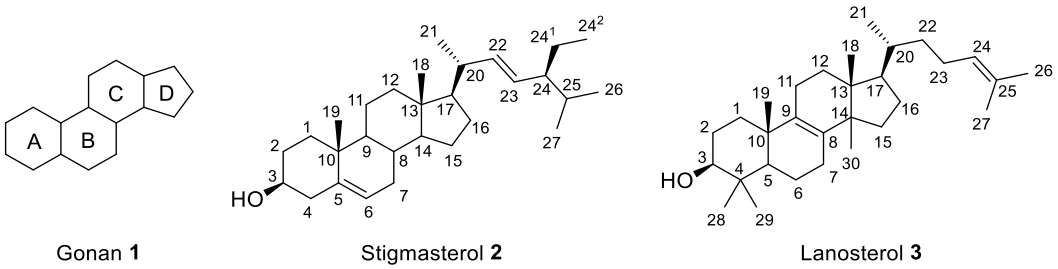
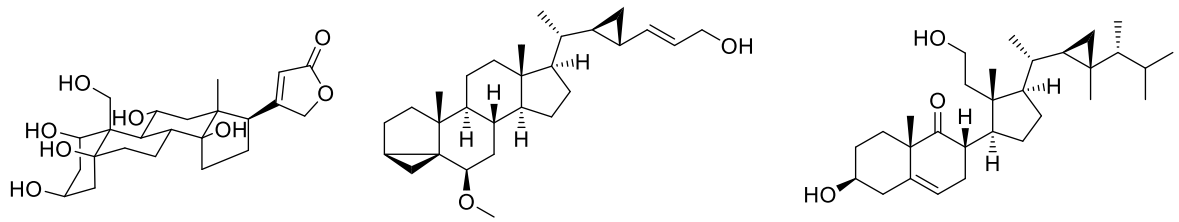


Figure 1: Gonan (**1**), possessing the most basic skeleton of the steroids, and their numbering using stigmasterol (**2**) and lanosterol (**3**) as examples.

The individual rings can be linked in either *cis* or *trans* conformation. However, not all possible ring linkages occur. The A and B rings can be *cis*- or *trans*-linked, but the B and C rings occur

only in *trans* configuration. The C and D rings are also usually *trans*-linked, except for cardenolides, for example.[12] A system of descriptors and prefixes is used to name the stereochemistry and structural deviations found in many steroids.[13,15] Thus, α (alpha) stands for a substituent that is below and β (beta) for one that is above the molecular plane. Among the prefixes, homo- and nor- as well as cyclo- and seco- are particularly noteworthy. Homo- describes an additional methylene group (CH_2) compared to the parent steroid, while nor- describes a missing methylene group. Cyclo- denotes a ring closure between two non-adjacent atoms of the skeleton or side chain. Seco- on the other hand, denotes a ring-opening. Examples are given in Figure 2.



Ouabagenin **4** **5** **6**

Figure 2: *Cis*-linked A/B- and C/D-rings in Ouabagenin (**4**),[12] 6β-methoxy-3β,5-cyclo-23^1,24^1,27-trinorgorgost-24-en-26-ol (**5**) and 9-oxo-9,11-secogorgost-5-en-3β,11-diol **6**.[17]