MOLECULAR LIBRARY SYNTHESIS USING NATURAL PRODUCTS:
EXPANDING THE FRAMEWORK OF STEROIDS AND PENTACYCLIC
TRITERPENOIDS

by

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</table>
Abstract

Triterpenoids are a broad and structurally diverse class of natural products primarily derived from the plant kingdom. The triterpenoid family consists of nearly thirty thousand members with over two hundred unique carbocyclic skeletons. Consequently, triterpenoids are known to have a wide array of biological activities, including antifungal, anti-inflammatory, anti-cancer, as well as anti-viral and anti-bacterial properties. Considerable efforts in drug discovery have focused on the isolation and structural elucidation of novel triterpenoid molecules from the plant sources. Moreover, semisynthetic triterpenoids, created by further manipulation of the exterior functional groups, have been shown to enhance the potency of their natural precursors. In this regard, the increase in structural complexity of triterpenoid-like molecular libraries through alteration of the carbocyclic core skeleton of the parent natural product can be viewed as a promising tool to study the chemical biology and medicinal chemistry of this natural product family. This work represents a general synthetic strategy for remodeling of a triterpenoid skeleton based on the reactivity patterns of lanosterol, and application of the devised strategy to pentacyclic triterpenoid bryonolic acid. Lanosterol was chosen because of the unsaturated B/C ring fusion, which can undergo iterative allylic oxidation/oxidative cleavage to produce transannular polyketones. These polyketones, in turn, can form distinct molecular skeletons via regio- and stereoselective aldol addition and Norrish-Yang photocyclization. The main advantage of this approach is that instead of relying on reaction development and catalysis to impart stereochemical and regiochemical selectivity, the inherent complexity of the natural product-derived substrates drives stereoselective and regioselective reactions. The central finding
described herein is that the subtle changes in the parent triterpenoid, which is subjected to the devised diversification strategy, imparts dramatic effects on the resultant products. Consequently, the devised diversification strategy can be applied to many members of this natural product family with significantly different composition of the resultant molecular library.
Chapter 1. General Introduction

1.1 Natural Products in Modern Drug Discovery

Drugs can be broadly defined as chemical substances that are used to either cure or prevent various diseases in living organisms, including humans, animals and plant species.1 Drugs can be categorized by their chemical structure, biological target, or the disease which they aim to combat. The necessity for the development of new drug molecules continues to exist, to: (1) overcome organisms’ resistance to existing drugs; (2) mitigate undesirable side effects of current drugs; (3) facilitate advanced treatment of known diseases; and (4) provide cures for newly identified diseases. The process of development of new drug molecules and understanding their molecular mechanisms of action is known as drug discovery. Both academic and industrial drug discovery efforts aim to improve our society’s standard of living, and require input from experts in various branches of science, including, but not limited to, medicine, biology, biochemistry, chemistry, pharmacology, toxicology, physiology, mathematics and computing.

Historically, drug discovery originated with aqueous extraction of samples from various natural sources, including animals, plants, trees, fungi, microorganisms and minerals. Since ancient times peoples all over the world have used such extracts for medicinal purposes, when they were seeking relief from pain and disease.2-3 These extracts were the first pharmaceutical formulations, containing active compounds along with many other constituents. The development of chemical science in the 19th and 20th centuries allowed for the systematic investigation and isolation of pure active ingredients from many natural materials. This strategy has led to the discovery of many molecules that were used as therapeutic agents. The most prominent examples are quinine,4 an
antimalarial drug isolated from the bark of the cinchona tree, and salicylic acid, a traditional analgesic and antipyretic molecule from the bark of the willow tree. One of the most recent examples is taxol,\textsuperscript{5} isolated from the pacific yew tree and used in cancer chemotherapy.

![Structures of quinine, salicylic acid, and paclitaxel (taxol)]

\textbf{Figure 1.1.1.} Selected examples of natural product drugs

The development of organic chemistry in the last third of the 19\textsuperscript{th} century resulted in the serendipitous discovery of synthetic dyes.\textsuperscript{6-8} This research was further facilitated by the rapidly expanding textile industry, for which the high price of natural dyes was a serious concern. Academic universities were willing to provide the supply for the demand, training skillful organic chemists who were able to discover new reactions and provide routes to many novel dyes. One of the most notable pharmaceutical achievers of the past, Paul Ehrlich\textsuperscript{9-10} started his scientific career studying the distribution of dyes between different tissues in living animals. He then discovered that methylene blue stained the plasmodia that caused malaria, and he successfully administered this dye to patients diagnosed with malaria, resulting in their recovery after the treatment.\textsuperscript{10} This was the first example of a synthetic drug being used with success to cure a specific disease. Encouraged by this success, Ehrlich tested 100 randomly chosen synthetic dyes by
injecting them into mice infected with trypanosome, which was known to cause nagana in cattle, and identified an active compound, Nagana Red. This study became the first example of the phenotypic screening of non-targeted molecular library, resulting in the identification of a lead compound. Further derivatization of Nagana Red to form Trypan Red was the first example of lead optimization.¹¹

![Figure 1.1.2. Selected examples of bioactive synthetic dyes discovered by Ehrlich](image)

The screening of dyes, pioneered by Ehrlich, resulted in the development of many profound therapeutic agents, such as antibacterial aminoacridines and sulfonamides. The pharmaceutical industry developed directly out of the dye industry, and during the 20th century became completely independent of its origins, eventually evolving into the modern science of drug discovery.¹¹

Contemporary drug discovery is a structured scientific investigation.¹ In this model, a team of pharmaceutical professionals gather the information about the pathology of the disease of interest. This information is used to further identify what intervention is most likely to cause the desired outcome. A specific molecular hypothesis is formulated, and specific macromolecular targets are chosen. The team then designs a lead compound. This design, or simply an inspiration for the lead compound, may be facilitated by computer-aided techniques or X-ray crystallography. At this stage, an organic chemist designs the route and performs the synthesis of the compound with the desired structure.
Alternatively, the team may choose to synthesize or purchase for screening a library of chemical compounds that is hypothesized to contain a potential lead. Phenotypic or target-based screening approach is chosen by the team at this stage. Once the lead compound is identified, the team determines its therapeutic index, and, if the latter is sufficiently high, the medicinal chemist performs the optimization of the lead compound by synthesizing a number of its analogues. The analysis of SAR or QSAR may be employed by the team at this stage. The ultimate goal of the team is to forward the analogue with the most promising activity to the clinical trials.

In a recent review, Proudfoot\textsuperscript{12} concluded that the structures of the marketed drugs are frequently highly similar to the structures of the lead compounds from which they were derived. Therefore, the identification of the lead molecule is the critical step in any drug discovery program. Despite the success of synthetic drugs at the early days of the pharmaceutical industry, natural products have always been the main source of inspiration for pharmaceutical researchers.\textsuperscript{13-15} As a result, by 1990, about 80\% of marketed drugs were either natural-product-derived or natural-product-inspired molecules.\textsuperscript{16}

At the same time, the remarkable advancement of molecular biology and biochemistry in the 1980s has resulted in the development of rapid and efficient drug testing systems. In these systems, various protein targets can be simultaneously purified and prepared for automated in vitro assays. The techniques used for these systems are collectively known as high-throughput screening, and allow for the simultaneous screening of hundreds of thousands of small molecules.\textsuperscript{17} The development of such a
“voracious process” necessitated the rapid production of massive numbers of chemical substances for testing as potential leads.\textsuperscript{18}

Taking into account the long history of success of natural products in drug discovery and the large diversity of potential natural sources, the screening of natural materials in search for lead candidates theoretically provided a good opportunity to satisfy the need of high-throughput screening campaigns.\textsuperscript{19} However, high-throughput screening of natural sources also presents many technical difficulties.\textsuperscript{16} First, the challenges of reliable access and continuous supply, especially with respect to marine species, are coupled with the intellectual property concerns of local governments. Second, the seasonal and environmental variations in the chemical composition of living organisms reduce the chance of reproducibility of isolation of active ingredients, especially from the plant kingdom, for which chemical constituents are the only source of intercellular communication. Third, the concentration of an active compound may be insufficiently low for the initial screening. The synergistic or antagonistic effects of several constituents may lead to mistaken conclusions that may be detrimental to the drug discovery program. Furthermore, the complete structural characterization of an active component may be very challenging and time-consuming. And finally, the probability of rediscovery of a known compound is estimated to be 99%; that is, the screening campaign is most likely to result in the identification of a previously published molecule. For the aforementioned reasons, many pharmaceutical organizations have discontinued their research programs associated with the isolation of new secondary metabolites from living organisms.

Another solution to the demand of massive numbers of new chemical entities was the discovery and rapid development of combinatorial chemistry.\textsuperscript{20-21} This scientific
discipline originated from solid-phase synthesis of a tetrapeptide (Leu-Ala-Gly-Val) in 1963,\textsuperscript{22} for which its discoverer Robert Merrifield received a Nobel Prize in chemistry in 1984. The basic principles behind this type of synthesis remain the same to this day. First, a linker is covalently attached to the polymer bead, which is carefully chosen such that it is insoluble in any of the used solvents. A starting material is then coupled with the linker by the appropriate chemical reaction, and the final product is cleaved from the bead after the completion of the synthesis. Ideally, no further purification of the final product is necessary after the cleavage step. In 1985 Richard Houghten\textsuperscript{23} published one of the first combinatorial chemical libraries, whereby 248 different 13-amino-acid-long peptides were synthesized by the “tea bag” method. In this method, protected amino acid resin was placed in several polypropylene bags. Each individual bag was subsequently immersed in different solutions of appropriate activated amino acids, while deprotections and washings were done when all the bags were pooled together. The bags were carefully tagged such that the structure of the final product could be easily deconvoluted. Since then, solid-phase combinatorial chemistry has seen rapid advancement, especially with respect to the various polymer beads and linkers, general experimental techniques and the reaction conditions that were optimized specifically for this type of chemical synthesis. Thus, a combinatorial molecular library synthesis of two million compounds using the “split-pool” technique was successfully carried out in the Schreiber laboratory in 1998.\textsuperscript{24}

Although the solid-phase combinatorial approach leads to the synthesis of large-sized libraries, it is considerably restrictive in terms of the reactions available for this chemistry. However, solution-based combinatorial synthesis, despite the multitude of reactions developed and optimized for this medium, has many disadvantages when
compared to its solid-phase counterpart. These limitations, such as the necessity for purification of the products and intermediates, frequent incomplete conversion of the starting materials and difficulty of the solvent removal, restricted the use of solution-phase combinatorial chemistry in drug discovery endeavors. Introduction of the parallel synthesis experimental technique, automation of the parallel synthesis process, and the development of the fluorous tags to simplify work-up and purification, have made solution-phase combinatorial synthesis more attractive to medicinal chemists. Nevertheless, superiority with respect to the library size and the necessity to feed the “voracious” high-throughput screening process steered the majority of combinatorial chemistry toward the solid phase.

As a result, solid-phase combinatorial synthesis yielding large unfocused libraries of compound mixtures dominated drug discovery efforts in the 1980s and 1990s. This approach for the massive production of new chemical compounds promised to rapidly deliver and forward new drug candidates to clinical trials. By the early 2000s, it became clear that after high expectations and huge investments, combinatorial chemistry has failed to deliver on its promises, and the approaches for the massive production of new active substances needed to be reconsidered. Sorafenib, developed by Bayer for cancer treatment, is the only therapeutic agent resulting from combinatorial drug discovery that has been approved for use by any regulatory agency.\textsuperscript{25} It is noteworthy that Sorafenib was developed as a multikinase inhibitor, targeting several serine/threonine and receptor tyrosine kinases. These proteins constitute molecular targets for which the high-throughput screening of small molecule fragments is an efficient way of lead identification.
This fact led to the realization that combinatorial-type molecules are substantially less diverse in their chemical structures as compared to the molecules from natural sources. Several statistical investigations were undertaken to understand the property distributions between marketed drugs, natural products and combinatorial-type compounds. On average, natural products have higher molecular weights than the synthetic compounds. Moreover, the weight distribution of natural products is similar to that of marketed drugs. Natural molecules contain many sp$^3$-hybridized carbon atoms, whereas the combinatorial compounds are of high sp$^2$-character. The planar sp$^2$-hybridized molecules are only drug-like with respect to kinase targets, which are commonly inhibited at the ATP binding site by organic compounds with no stereogenic centers and high aromatic content, as exemplified by the success story of Sorafenib. However, drugs for which the molecular mechanism of action includes the inhibition of transcription factors, or the interruption of protein-protein interaction, are considerably more complex. The distribution of sp$^3$ carbons within such drugs is highly similar to the distribution of sp$^3$ carbons within natural products. Moreover, natural products frequently have four or more chiral centers, as do many marketed drugs. In sharp contrast, over 71% of unfocused combinatorial libraries contain molecules with no chiral centers. Additionally, natural products are different from synthetic molecules and similar to existing drugs with respect to the number of rotatable bonds, unsaturation, ring systems, side chains and distribution of heteroatoms. Natural products contain a lower number of nitrogen, halogen and sulfur atoms than synthetic compounds, whereas the content of oxygen atoms is nearly twice as high in natural products. To summarize, natural products can be described as structurally complex and diverse, and the creation of
libraries of just such compounds to replace simple and planar combinatorial-type molecules became the paramount goal of many drug discovery campaigns at the lead identification stage. At the same time, combinatorial chemistry remains a powerful tool for the structural optimization once an active scaffold has been identified.

Several approaches to achieve the desired complexity and diversity of potential leads have been developed recently, and the next section of this chapter will discuss the strategies for the construction of natural product-like chemical libraries.

1.2 Natural product-like chemical libraries

1.2.1 Synthesis around privileged scaffolds

From a purely chemical point of view, the wide variety of secondary metabolites collected from various living organisms may be considered to be a large non-targeted chemical library. The traditional solution-phase total synthesis of natural products using well-defined chemical transformations can theoretically provide access to these products, and, consequently, the biological properties of the resultant library could be further explored. Historically, the total synthesis of natural products has been driven forward by the potential for discovering novel chemical transformations, which could be widely applicable in organic chemistry.\textsuperscript{30-31} Today, however, most total syntheses endeavors begin with evaluation of the bioactivity of the target natural product, and the key disconnections during the retrosynthetic analysis are chosen such that they facilitate the synthesis of the analogues of the target natural product.\textsuperscript{32} This approach can be exemplified by the story of taxol.\textsuperscript{33} After its isolation from the bark of the Pacific yew
tree, taxol has become a blockbuster drug in cancer chemotherapy due its ability to disrupt microtubules and inhibit mitosis. Efforts to provide a continuous supply of this drug by extraction from the tree bark were challenging and raised many environmental concerns. As a result, several competing total synthetic strategies were developed by the groups of Holton, Nicolaou, Danishefsky, Wender, Kuwajima, Mukaiyama and Takahashi, to provide a continuous supply of this molecule. If a molecular library is being designed for taxol, one would consider not only the most efficient way to construct the taxane ring system, but also the ability to incorporate diverse peripheral substituents within this ring system, such that many analogues of taxol would be produced.

A molecular library of this type can be considered a targeted (focused) chemical library. Intuitively, such a library would emphasize the structure and composition of targeted end products. The synthesis of targeted libraries of complex natural product analogues is challenging and takes years of hard work by dedicated organic chemists. For that reason, pharmaceutical achievers have made a note of recurring molecular frameworks in natural products and proposed that these common molecular scaffolds could exist across diverse drug candidates. Based on these observations, David Evans formulated the concept of “privileged structures” in 1986, which were hypothesized to have an inherent tendency for biological activity and, thus, could be exploited through liberal modification of peripheral functional groups, to provide ligands for a wide range of macromolecular targets. These privileged molecular scaffolds were collectively summarized in a recent comprehensive review by Scott Snyder and Brent Stockwell, and include, but are not limited to, benzodiazepines, purines and pyrimidines, indoles,
benzofurans, benzothiophenes, steroids, benzopyrans, and many others. The taxane ring system of taxol is another example of a privileged structure.

The current use of natural product-based privileged structures as leads to novel bioactive compounds is best demonstrated by three back-to-back papers from the K.C. Nicolaou laboratory43-45 published in 2000, describing the synthesis of a 10,000-member library of natural product-like molecules containing a benzopyran privileged motif (Figure 1.2.1.A). The argument for benzopyrans being considered privileged was logical when the diversity of bioactive benzopyran-containing natural products was noted. Nicolaou and co-workers were able to identify more than four thousand examples of such products exhibiting activity in a wide range of biological systems. Therefore, the authors hypothesized that a large targeted combinatorial library centered around benzopyran privileged scaffold, when subjected to a target-based high-throughput screening assay, should yield lead compounds with activity against many macromolecular targets.

The library synthesis began by loading a substituted ortho-prenylated phenol onto a polystyrene-based selenenyl bromide resin (Figure 1.2.1.B). The benzopyran was then formed via an intramolecular 6-endo-trig cyclization. At this point, the benzopyran core structure was covalently linked to the polymer bead via a selenoether. The library was then elaborated; in essence, four separate focused libraries were synthesized in which the aryl moiety was substituted using glycosidation, annulation, condensation, and cross-coupling reactions. The products of this solid-phase synthesis were cleaved from the resin via the oxidation of the selenoether with hydrogen peroxide to form selenoxide, which spontaneously underwent syn-elimination at room temperature to yield an unsaturated benzopyran. This final product was used as a starting material for further diversification.
in the solution phase through chemical reactions of the carbon-carbon double bond. Specifically, this unsaturation was exploited via epoxidation and subsequent nucleophilic ring opening using automated parallel synthesis systems.

**Figure 1.2.1. A.** Examples of natural products and drugs containing benzopyran motif. **B.** Strategy for the solid-phase library synthesis. **C.** Leads identified from the library screen.

Initial screening of the benzopyran library led to the identification of several potent bioactive small molecules (Figure 1.2.1.C). This initial success inspired the construction of second-generation targeted libraries to maximize the specific activity of leads identified after the primary screening. The first compound was arrived at after identification of library members capable of inhibiting the growth of methicillin-resistant
Staphylococcus aureus. Structural refinement generated a significantly more active lead displaying an MIC of 4 μg/mL. The second compound was identified as a potent inhibitor of cellular proliferation with an IC\textsubscript{50} of 0.019 μM without nonspecific cytotoxic side effects. The third compound, rac-fexachloramide, was developed as a potent agonist of the farnesoid X receptor (FXR), a transcriptional sensor for bile acids ultimately controlling cholesterol metabolism. (EC\textsubscript{50} = 0.19 μM)

The leads identified through high-throughput screening of just such targeted libraries constructed around privileged scaffolds currently dominate industrial pharmaceutical discovery efforts. Between 1999 and 2008, the FDA approved 259 therapeutic agents, out of which only 50 were first-in-class small molecule drugs with new molecular mechanisms of action.\textsuperscript{46} All of these drugs were discovered after either phenotypic or target-based high-throughput screening of molecular libraries. Such low productivity and output in pharmaceutical research necessitated further development of conceptually different strategies for the identification of new lead compounds.

1.2.2 Chemical modification of crude natural product extracts

Chemical alteration of the components of crude natural extracts originated from the serendipitous discovery of the formation of solvent artifacts resulting in changes of bioactivities of several natural products.\textsuperscript{47} The preparation of “chemically engineered extracts”, as formulated by Ricardo Furlan,\textsuperscript{48-51} represents an alternative strategy for the generation of biologically active molecules through chemical diversification of known and unknown natural product scaffolds by chemical reagents. In this approach, simple chemical reagents, such as NaBH\textsubscript{4}, mCPBA or bromine, react with the functionalities
ubiquitously present within natural product scaffolds, and subsequently result in chemical alteration of most of the components of the starting extract to produce a library of semi-synthetic natural product analogues (Figure 1.2.2). This strategy was hypothesized to overcome the limitation of high re-discovery rate in natural products-associated drug discovery programs.

Figure 1.2.2 Chemical modification of crude natural product extracts

Furlan and co-workers analyzed the variety of natural products found in the 2001 edition of *Dictionary of Natural Products*, and found that certain functional groups, such
as hydroxyl and carbonyl moieties, are present in more than 70% of the structures. Further investigation disclosed that the proportion of structures containing an amino group, a double bond, an aromatic or heteroaromatic ring, is quite high and lies in a range of 40% and 65%. The authors further proposed that by the choice of a complimentary chemical reagent most of the structures with these functional groups can be converted to novel natural product derivatives. If the constituents of the crude extract were previously discovered or simply inactive, this strategy would raise the possibility of identifying new drug leads.

This approach resulted in the identification of several bioactive molecules. One such example is the formation of the antifungal 3,5-diaryl substituted pyrazole, which was observed after an extract of the knotweed herb was treated with an aqueous solution of hydrazine monohydrate (Figure 1.2.2). The drastic changes in the chemical composition were noticed by TLC, HPLC and NMR. This chemical modification also introduced antifungal bioactivity, which was not initially detected during the screening of the crude extract. Bioactivity-guided fractionation of the semi-synthetic mixture resulted in the isolation of the active pyrazole, which was formed by the reaction of hydrazine with previously known and inactive flavone. Another representative example from the Furlan laboratory is the discovery of a brominated furanocoumarin derivative with acetylcholine esterase inhibitory activity comparable to that of physostigmine. The rationale behind the use of bromine as a modifying reagent was that the brominated natural products isolated from marine natural sources have been found to show a diverse profile of bioactivities. Conversely, natural products from terrestrial sources are rarely brominated, but often possess double bonds and unsaturated ring systems, thus allowing
for the bromination of the multiple natural product scaffolds by the use of addition and substitution reactions. Additionally, incorporation of halogen atoms is a common strategy in lead optimization and allows for desirable changes in lipophilicity and stereoelectronic properties of the analogues. The crude methanolic extract of the hemlock herb was not found to possess anti-acetylcholinesterase activity; however, the screening of a chemically engineered extract was shown to inhibit this enzyme. Bioactivity-guided chromatographic separation of the extract resulted in the isolation of active coumarin, which was formed by reaction of bromine with a known furanocoumarin derivative xanthotoxin.

This concept for producing natural product-like libraries from the collections of natural products, formulated by Furlan and further developed by the Imoto laboratory and other groups, resulted in the discovery of many bioactive compounds from otherwise inactive natural product extracts. However, the further use of this methodology may be limited by the inherent shortage of types of functional groups that are available for quick and efficient transformations. Therefore, only a limited number of reactions and reagents can theoretically be applied to crude extracts. In addition, this methodology implies the modification of peripheral molecular appendages, while the core pharmacological scaffolds in most cases remain unchanged. The diversity-oriented synthesis strategy was developed to overcome these obstacles.

1.2.3 Diversity-Oriented Synthesis

As mentioned briefly in the section 1.2.1, only 50 first-in-class small molecule drugs with new molecular mechanisms of action were approved by FDA between 1999 and
It is noteworthy that out of these 50 molecules, only 17 were arrived at after molecular target-based screening, and 28 were identified after phenotypic screening. During a phenotypic screening campaign pharmaceutical researchers test the ability of a molecule to modulate disease pathology in cells or organisms often without prior knowledge about the specific protein target. Ironically, drug discovery originated with the phenotypic screening approach, simply because of the absence of an alternative. Many scientists put forth an argument that phenotypic screening is also the future of drug discovery, especially after the development of chemical biology tools, such as chemical proteomics, for macromolecular target identification. In chemical proteomics, synthetic small molecule probes are utilized to selectively capture and enrich particular protein targets from complex biological samples. Such chemical probes are specifically engineered and contain an active ligand, an enrichment group (such as a polymer bead) for isolation and detection of the probe-bound proteins, and a linker for the attachment for an active compound to the enrichment group. These studies can be conducted with both cell and tissue extracts, and the target proteins can be further identified with the aid of fast-scanning mass spectrometers and bioinformatics. Intuitively, if many future screening programs utilize the phenotypic approach, targeted libraries will need to be replaced with non-targeted libraries covering many natural product-like scaffolds with immense variation of external appendages and functional groups in order to provide ligands for a wide range of macromolecular targets. Ideally, a collection of all possible organic compounds would be repeatedly tested in all specifically designed disease-based phenotypic screens.
The concept of chemical space was then formulated. If one were to take three carefully chosen descriptors of either biological or a chemical nature, and use each of those descriptors to represent axes X, Y and Z, respectively, the area between these axes will then represent three-dimensional chemical space. A target-oriented synthesis usually results in the formation of a single molecule. When the descriptors’ values are determined or calculated for that molecule, it will occupy a discrete point in the chemical space (Figure 1.2.3.A). A targeted library centered on a single privileged scaffold will contain a multitude of unique molecules. However, when the members of the library are described by a set of the same descriptors, such a library will only cover a very dense region of the chemical space (Figure 1.2.3.B). As previously mentioned in this chapter, this limitation of combinatorial libraries at the lead identification stage turns into a great advantage during the lead optimization stage.

Figure 1.2.3 Chemical space occupied as the result of: A. Target-oriented synthesis. B. Combinatorial chemistry. C. Diversity-oriented synthesis.

Stuart Schreiber in early 2000s postulated that the broad goal of generating non-targeted libraries covering many natural product-like scaffolds can be achieved by diversity-oriented synthesis (DOS). It was further hypothesized that these non-targeted libraries would simultaneously cover multiple areas of chemical space (Figure 1.2.3.C).
The central tenet behind this concept was to use simple and similar starting materials to create complex and diverse resultant structures. The concept of diversity is somewhat subjective, and Schreiber subdivided this term into four distinct categories: (1) appendage diversity, which can be incorporated by combinatorial variation in the building blocks used; (2) stereochemical diversity, which can be incorporated using asymmetric reactions; (3) functional group diversity, which can be incorporated using chemical reagents; and (4) skeletal diversity. Achieving skeletal diversity is a non-trivial and challenging task, and the progression of DOS necessitated the development of specific synthetic strategies that would enable the construction of such libraries.

In target-oriented synthesis, retrosynthetic planning is used to devise pathways to a desired structure by a thoughtful choice of appropriate “transforms”\textsuperscript{67}. To use the transform, a synthetic chemist must identify the appropriate structural subunit in the target molecule. These structural subunits are frequently called “retrons”. In DOS, analysis needs to be performed in a forward sense, and the appropriate structural motif has to be identified in the starting material. Schreiber proposed two contrasting strategies to achieve this goal.\textsuperscript{66} The reagent-based branching strategy bifurcates, to utilize: (1) a substrate with pluripotent functionality, whereby the same part of the molecule is subjected to different chemical transformations induced by different regents; or (2) a densely functionalized substrate, whereby different functionalities within the same molecule are transformed by various reagents. The substrate-based folding strategy implies the attachment of different appendages with pre-encoded skeletal information to a single scaffold. The appendages are carefully chosen such that the initial scaffold is transformed to distinct molecular skeletons under the same or similar reaction conditions.
The branching strategy utilizing a substrate with pluripotent functionality is best exemplified by a report from the Robert Stockman laboratory.\(^6\) In this report, Stockman and co-workers used a simple tridecane derivative containing only three functional groups: a keto group, a double bond, and an ester carbonyl (Figure 1.2.4).

![Figure 1.2.4 Example of branching DOS strategy utilizing a substrate with pluripotent functionality](image)

Using hydroxylamine alone, this derivative was converted into a planar isoxazole-containing tricyclic structure. When hydroxylamine was used in combination with heat and microwave irradiation, a more complex bridging isoxazole-containing molecule was formed. When the same reagent was accompanied by a base, such as sodium ethoxide, the starting material was converted to a tricyclic pyrrolidine derivative. The use of ammonia and NaBH\(_4\) initiated a cascade of aza-Michael reactions leading to a quinolizine. Aniline in combination with TiCl\(_4\) triggered a Michael/aza-Michael cascade giving a hydroquinoline. The simple amino acid glycine in the presence of the base led to
the formation of penta[b]pyrrole. The use of tosylhydrazine yielded tricyclic pyrazole. The use of NaH initiated a cascade of Michael reactions leading to the trans-decalin core structure. SmI₂ initiated a cascade of radical cyclization processes resulting in either a bicyclic lactone or a simple cyclopentane, when 2 or 5 equivalents of the reagent were used, respectively. Finally, the use of super-hydride led to the formation of a pyran structure via a reduction of ketone followed by an oxy-Michael reaction. In summary, the use of 8 simple reagents resulted in the formation of 11 privileged scaffolds with tremendous potential for further functionalization.

![Diagram](image)

**Figure 1.2.5** Example of branching DOS strategy utilizing a densely functionalized substrate

The branching strategy utilizing a densely functionalized substrate may be exemplified by a recent report from the Viswanathan laboratory (**Figure 1.2.5**). In this work, the authors prepared a reverse prenyl group-containing amide by Meerwein-Eschenmoser-Claisen rearrangement of the easily accessible allyl imidate. This amide, containing reactive functionalities, such as an aryl halogen, a carbon-carbon double bond and an amide nitrogen, was found to show completely different reactivity when treated with different transition metal catalysts. Thus, the use of Cu(I) resulted in the conversion
of the amide to a reverse prenyl group-containing oxindole through a C-N bond forming Goldberg reaction. In contrast, the use of Pd(0) led to the formation of an indene privileged scaffold through a C-C bond forming intramolecular Heck process. Both oxindole and indene scaffolds could be further diversified by the amide-enolate alkylation, potentially in a stereoselective fashion.

![Diagram of substrate-based folding DOS strategy](image)

**Figure 1.2.6** Example of substrate-based folding DOS strategy

To demonstrate the diversity-generating potential of the substrate-based folding methodology, Schreiber and co-workers have used the Achmatowicz reaction, a process, by which the oxidative opening of furfuryl alcohol leads to dihydropyran. A common furaldehyde starting material was transformed into three furan derivatives, having zero, one or two appended hydroxyl groups, respectively (Figure 1.2.6). The substrate with one hydroxyl group was prepared by a Grignard reaction. An olefin was then synthesized from 5-methyl furfural by a Witting reaction. Substrates with zero and two hydroxyl groups were prepared from this olefin by catalytic hydrogenation and dihydroxylation, respectively. When the prepared substrates were subjected to the same set of “folding” conditions (oxidation with NBS), they were transformed into products having three different skeletons. The substrate with two hydroxyl groups was transformed into [3.2.1]bicyclic ketal, while the substrates with one and zero such groups were
transformed into the expected pyran and a linear $\alpha,\beta$-unsaturated ketone, respectively. This methodology was further expanded to generate skeletal diversity in a combinatorial manner.

Since the first reports from the Schreiber laboratory, DOS has proven to be a widely applicable concept with new examples continuously being added to the chemical literature. These examples include the synthesis and screening of chemical libraries, as well as the development of new branching and folding synthetic strategies that enable the construction of the aforementioned libraries. However, library development of this type is struggling with the necessity to use reagent development and catalysis, especially for the introduction of stereochemical and regiochemical selectivity. This necessity represents a tremendous challenge, for example when different stereogenic reactions are used at the skeletal diversity generation step.

Intuitively, many natural products themselves are densely functionalized molecules, and many have pluripotent functionalities. Consequently, the use of readily available abundant natural products with easy and guaranteed supply for the development of diversity-oriented methodologies became a new and evolving approach. The central hypothesis behind this approach was that instead of relying on reaction development and catalysis to impart stereochemical and regiochemical selectivity, the inherent complexity of the natural product-derived substrates can drive stereo- and regioselective reactions.

One of the pioneering reports from the Olov Sterner laboratory in 2001 described the use of the divergent reaction of $\alpha,\beta$-unsaturated ketone with an epoxide ring, present in a 5/11/3-fused lathyrane diterpene, for the diversity-oriented construction of diterpene analogues with 5/7/7/4-, 5/6/6/4-, 5/7/5- and 5/8/5-fused ring systems. Another report
from the de la Torre laboratory\textsuperscript{73} described the diversity-oriented access to terpene-like compounds from the readily available natural product sclareolide. In this report, the keto group, the $\alpha,\beta$-unsaturated ketone and the double bond within the same core structure were positioned using chemical reactions such that the 6/6/4/4-, 6/6/6/4/5, 6/6/4- and 6/6/5/4/5-fused terpene analogues were synthesized in a divergent manner via Paterno-Buchi and [2+2] cycloaddition reactions. In 2005, a report from the Schreiber laboratory\textsuperscript{74} described the combinatorial synthesis of 4,000 small molecules having three distinct skeletal frameworks among other unique structural features. The triterpenoid ergosterol was used as a starting material for this synthesis, whereby the diene system within steroidal ring B allowed for a cascade of Diels-Alder/retro-Diels-Alder reactions leading to polycyclic/macrocyclic steroidal analogues. In a recent review, Kitayama\textsuperscript{75} summarized the use of cyclic sesquiterpene zerumbone, containing the $\alpha,\beta$-unsaturated ketone moiety as well the double bond within its monocyclic structure, for the synthesis of 5/8- and 3/9-fused sesquiterpene analogues using transannular epoxide opening reactions, as well as ring cleavage and ring expansion processes. More recently, Porco and Snyder\textsuperscript{76} have reported the remodeling of the sesterpenoid fumagillol. This natural product, containing two epoxide groups separated by a single carbon atom, was converted to isoindole and isoquinoline privileged scaffolds with excellent control over the regio- and stereoselectivity using Lewis acid catalysis. Furthermore, the most recent publication from the Hergenrother laboratory\textsuperscript{77} described a unifying strategy to construct structurally diverse and stereochemically complex natural product-like libraries using reactions that distort the ring systems of the natural product starting materials. This strategy was exemplified by the application of combinations of ring cleavage, ring expansion, ring
fusion and ring rearrangements to natural products from various families, including gibberellic acid, adrenosterone and quinine.

The following section will introduce the triterpenoid family of natural products, as well as the reasons why a triterpenoid-like library was chosen as a target for the development of the diversity-oriented strategy. The choice of substrates, and the synthetic strategy itself, will be presented in the summary of proposed research.

### 1.3 Diversity of steroids and triterpenoids

Triterpenoids are a broad and structurally diverse class of natural products primarily derived from the plant kingdom. The triterpenoid family consists of nearly 30,000 members with over 200 unique carbocyclic skeletons. Most triterpenoid molecules are 6/6/6/6/6 or 6/6/6/6/5 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic and hexacyclic triterpenoids have also been isolated from natural sources. Steroids possess the recognizable tetracyclic A/B/C/D core skeleton, but with respect to all the other characteristics steroids are a class of triterpenoids.

All triterpenoid molecules consist of 30 carbon atoms. This structural consistency was rationalized by the biogenetic isoprene rule, that is, triterpenoids are built from 6 five-carbon isoprene units. These C5 terpene units are the starting points in the biosynthesis of all members of the terpene class of natural products. The biosynthesis of dimethyl allyl pyrophosphate (DMAPP) and isopentenyl diphosphate (IPP) begins with the conversion of glucose to pyruvate and further bifurcates into mevalonate and non-mevalonate pathways. In the mevalonate pathway (MVA), the pyruvate is converted to...
mevalonic acid, which is then enzymatically transformed to DMAPP and IPP. This pathway is predominant in higher organisms. In the mevalonate-independent (MEP) pathway, the pyruvate is converted to methyl-D-erythritol phosphate en route to the same C5 terpene units (Figure 1.3.1). This pathway is frequently observed in the plastids of plants. DMAPP and IPP are readily interconverted by the enzyme isopentenyl pyrophosphate isomerase.

Regardless of the origin of the C5 units, their fate is similar. Thus, DMAPP and IPP are coupled in a “head-to-tail” fashion to produce C10-containing geranyl pyrophosphate. Geranyl pyrophosphate is further converted to farnesyl pyrophosphate (C15) by the reaction with IPP. Two molecules of farnesyl pyrophosphate are coupled “tail-to-tail” with the release of two equivalents of diphosphate to produce squalene (C30), which, in turn, is further transformed into 2,3-oxidosqualene by the enzyme squalene mono-
oxygenase. 2,3-Oxidosqualene constitutes the starting material in the biosynthesis of all triterpenoids.

In plants, the remarkable structural diversity among the members of this natural product family is achieved through a variety of oxidosqualene cyclase enzymes\textsuperscript{82} that catalyze these reactions, as well as through the further modification of the carbon skeletons by minor rearrangements, including homologation, oxidation, cleavage, and degradation.\textsuperscript{81,83} The molecular diversity among triterpenoids leads to the diversity of their medicinal properties, and triterpenoids are known to have a wide array of biological activities, including antifungal,\textsuperscript{84} anti-inflammatory,\textsuperscript{85} anticancer,\textsuperscript{86} as well as antiviral\textsuperscript{87} and antibacterial\textsuperscript{88} properties. In contrast, lanosterol synthase is the only oxidosqualene cyclase enzyme found in the animal kingdom.\textsuperscript{89} Hence, many steroids that play physiologically important roles in animals, such as the bile acids, sex hormones, cholesterol and D-vitamins, are biosynthesized from a single precursor, lanosterol, using various enzymatic reactions.\textsuperscript{81}

The presence of an unsaturated B/C ring fusion makes lanosterol a valuable starting point for chemical synthesis. The specific diversity-oriented strategy to utilize this reactive functionality for the creation of markedly different steroid-like scaffolds is described in the summary of proposed research.

\textbf{1.4 Summary of proposed research}

Described herein are research projects that include the design and execution of diversity-oriented synthesis of a chemical library of triterpenoid analogues. The
envisioned synthetic strategy constitutes a substrate-based folding approach, whereby the unsaturated B/C ring fusion of lanosterol was hypothesized to undergo iterative allylic oxidation and oxidative cleavage reactions to produce three transannular polyketones. Under the same or similar aldol reaction conditions, the resultant di-, tri-, and tetraketone were expected to form three distinct steroid-like scaffolds (Figure 1.4.1).

**Figure 1.4.1** The proposed substrate-based folding approach

A literature search revealed that bryonolic acid is the major documented pentacyclic triterpenoid with an unsaturated B/C ring fusion. We hypothesized that based on this structural similarity, bryonolic acid will react in a complementary fashion, producing novel pentacyclic triterpenoids, each distinctively different from the others and the prototype structure. In addition, bryonolic acid can be isolated in gram-quantities from the sprouts of *Cucurbita pepo* L. (common zucchini) and is known to have anti-inflammatory properties.
Chapter 2 will describe the oxidation chemistry leading to the synthesis of di-, tri-, and tetraketones from parent triterpenoids lanosterol and bryonolic acid. The outcomes of the stereogenic transannular aldol reactions of these substrates will be subsequently studied. The specific aim of the research described in Chapter 2 is to determine how the inherent complexity of the natural product-derived substrates serves as a regio- and stereocontrolling element for the aldol reaction of these non-symmetrical substrates.

![Figure 1.4.2. A. Norrish-Yang photocyclization. B. Photochemistry of cyclodecane-1,2-dione. C. Proposed divergent reaction strategy](image)

Additionally, tri- and tetraketones derived from lanosterol and bryonolic acid were hypothesized to undergo divergent Norrish-Yang photocyclization. In this reaction, photoexcitation of a reactive keto group is followed by abstraction of a hydrogen atom that is in a position \(\gamma\) to that keto group (Figure 1.4.2). The hydrogen-transfer process leads to the formation of 1,4-hydroxy biradical, which, in turn, may undergo either Yang cyclization, or a Norrish type II cleavage process. A literature search revealed that the 1,2-diketone moiety is known to be an excitable chromophore, and irradiation of cyclodecane-1,2-dione substrates results in the formation of 4/8-fused carbocycles via Yang cyclization as opposed to Norrish type II fragmentation.
Chapter 3 will describe the photochemistry leading to the formation of triterpenoid analogues with unique 6/4/8-fused ring composition from lanosterol- and bryonolic acid-derived polyketones (Figure 1.4.2). The specific aim of the research described in Chapter 3 is to determine the structural elements that dictate the regio- and stereochemical outcome of these transannular photochemical transformations.

This work serves as a foundation for future evaluation of the bioactivity of the resultant chemical library. Chapter 4 will propose the most suitable biological activity, as well as the potential for optimization of a lead molecule.

1.5 References


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Chapter 2. Synthesis of tetra- and pentacyclic triterpenoid analogues using transannular aldol reaction

2.1 Introduction

The first challenge of using natural products for chemical synthesis is the isolation of the starting materials from their natural sources. Lanosterol is obtained on industrial scale from sheep wool. In this process, wool grease is separated from the wool by centrifugation, and then the collected grease is subjected to saponification. Saponification results in the formation of fatty acids and wool wax alcohols. The mixture of wool wax alcohols, formerly known as “isocholesterol”, consists primarily of cholesterol, lanosterol, 24,25-dihydrolanosterol and agnosterol. Lanosterol and 24,25-dihydrolanosterol are separated from this mixture of sterols by chromatographic means.

![Figure 2.1.1 Isolation of lanosterol from sheep wool](image)

Further separation of these compounds by chromatography has proven to be challenging, and the mixture containing lanosterol (60%) and 24,25-dihydrolanosterol (40%) is available from various commercial sources.

Conversely, bryonolic acid is not available commercially. Moreover, this triterpenoid was found in only four plant families. Specifically, bryonolic acid was isolated from the...
root bark of *Anisophyllea dichostyla* and *Tetrameles nudiflora*, and from the fruit null of *Sandoricum indicum*. Nevertheless, bryonolic acid is found in abundance in the *Cucurbitaceae* family of plants. A method for large-scale isolation of bryonolic acid from the sprouts of *Cucurbita Pepo L.* (common zucchini) was recently developed in our laboratory.

**Figure 2.1.2** Isolation of bryonolic acid from sprouts of *Cucurbita Pepo L.*

This method includes germination of the sprouts on wet blotting paper, followed by manual separation of the hairy root system. The dry ground roots are subsequently extracted with methanol using the Soxhlet extraction method. The column chromatography of the resultant crude extract on silica yielded gram-quantities of bryonolic acid, which was sufficiently pure for chemical synthesis.

Section 2.2 of this Chapter will describe the oxidation chemistry leading to lanosterol-derived polyketones. Section 2.3 of this Chapter will describe the oxidation chemistry leading to bryonolic acid-derived polyketones. Comparison of the aldol chemistries of the lanosterol- and bryonolic-acid derived diketones will be presented in
Section 2.4. The aldol chemistry of the tri- and tetraketones will be covered in Sections 2.5 and 2.6, respectively.

2.2 Oxidation of the double bond at the B/C ring fusion of lanosterol

Lanosterol has attracted significant attention in the past primarily due to the potential ability of its derivatives to modulate biosynthesis of cholesterol by inhibition of lanosterol synthase. Therefore, many efforts were dedicated to the efficient separation of the commercially available mixture of lanosterol (60%) and 24,25-dihydrolanosterol (40%).

One approach to this problem is the reduction of the mixture with H₂/Pd/C. This process leads to the rapid formation of pure 24,25-dihydrolanosterol, and many chemistries were developed with this molecule as a starting material.

Alternatively, lanosterol could be separated from 24,25-dihydrolanosterol by chemical means. Specifically, regioselective dibromination or dihydroxylation of the Δ24,25 double bond of lanosterol leads to the formation of the corresponding polar derivatives, which proved easily separable from the 24,25-dihydro counterpart by column chromatography on silica. Further reduction of these polar derivatives results in the formation of pure lanosterol.

The strategy of regioselective oxidation of the Δ24,25 double bond was chosen in this work for technical reasons. Particularly, incorporation of additional oxygen atoms within the hydrophobic steroid molecule was hypothesized to simplify the separation of...
the product mixtures due to the ability of these polar atoms to increase compounds’ adsorption on silica surface.

After initial protection of the hydroxyl group at C-3 of lanosterol to generate acetate 2.1, the Δ24,25 double bond of lanosterol acetate 2.1 was selectively oxidized according to the method developed by Reshetova et al.18 The use of KMnO₄ and NaIO₄ in aqueous tert-BuOH at 60 °C and subsequent cooling to rt over 15 h, followed by treatment of the resultant carboxylic acids with freshly prepared diazomethane in dry THF for 30 min, gave the desired methyl ester 2.2 in 65% yield, accompanied by α,β-unsaturated ketones 2.3 and 2.4; and enedione 2.5 in 9%, 7% and 4% yield, respectively. It is noteworthy that the formation of α,β-unsaturated ketone 2.4 was not observed by Reshetova et al.

Scheme 2.2.1. Synthesis of the starting material 2.2

The examination of ¹H NMR spectrum of 2.3 revealed the uninformative “steroid hump” between 0.5 ppm and 2.4 ppm, accompanied by the expected downfield signals: a singlet (3H) at 3.66 ppm and a doublet of doublets at 4.52 ppm (H-3, J₁ =11.6 Hz, J₂ = 4.4 Hz). In the ¹H NMR spectrum of 2.4 several peaks were shifted downfield from the “steroid hump” and provided good starting points for the structure elucidation. Specifically, a doublet of doublets of doublets at 2.99 ppm was found to be attached to a methylene carbon C-1 (δC 34.1), and a pair of doublets at 2.64 ppm and 2.44 ppm (J =
16.2 Hz) were linked to an isolated methylene carbon C-12 (δ_C 51.9). The ^13^C NMR spectra of 2.3 and 2.4 were almost superimposable, especially with respect to the signals corresponding to the α,β-unsaturated ketone moieties. Thus, the carbon atoms C-7, C-8 and C-9 in 2.3 resonated at 198.8, 139.1 and 164.7 ppm, respectively; while the carbon atoms C-8, C-9 and C-11 in 2.4 resonated at 164.2, 139.5 and 199.1 ppm, respectively.

Figure 2.2.1. Key HMBC correlations of 2.3 and 2.4

HMBC spectroscopy allowed for a reliable distinction between these two structures. Specifically, H-5 (δ_H 1.73) in 2.3 showed cross-peaks with carbonyl at C-7 (δ_C 198.8) and an olefinic carbon C-9 (δ_C 164.7). The methyl groups H-19 (δ_H 1.18) and H-27 (δ_H 0.91) in 2.3 showed correlations with C-9 and C-8, respectively (Figure 2.2.1). In sharp contrast, H-5 (δ_H 0.98) in 2.4 correlated with olefinic C-9 (δ_C 139.5) and the methylene carbon C-7 (δ_C 29.9). Additionally, the isolated multiplet H-1 in 2.4 showed a distinct correlation with C-9. Isolated doublets H-12 correlated with C-11 and C-9. The cross-peaks shown by H-18, H-19 and H-27 further supported the structural assignment.

The formation of diketone 2.6 was next investigated. The starting material 2.2 was oxidized with ruthenium tetraoxide under Sharpless conditions. Under these conditions the bridging double bond at the B/C ring fusion underwent competing oxidative reaction to give diketone 2.6 and non-selective allylic oxidation at C-11 and C-7 to give α,β-
unsaturated ketones 2.4 and 2.3, respectively (Scheme 2.2.2). Complete conversion of the starting material was achieved in only 1 hour. Furthermore, the high selectivity of this promiscuous oxidation\textsuperscript{20} and reasonable reaction yield are noteworthy.

Scheme 2.2.2. Synthesis of the diketone 2.6

With the $\alpha,\beta$-unsaturated ketone 2.3 in hand, further research efforts were directed at the oxidation chemistry leading to the triketone 2.7.

Scheme 2.2.3. Synthesis of the triketone 2.7

In due course, when treated with our modified ruthenium conditions, $\alpha,\beta$-unsaturated ketone 2.3 underwent competing cleavage of the $\Delta 8,9$ double bond to give the desired
triketone 2.7 and regioselective allylic oxidation at C-11 to furnish enedione 2.5, which was further oxidized in the course of the reaction to yield tetraketone 2.8 as a minor reaction product. It is noteworthy that the dominating formation of aldol adduct 2.9 proved to be unavoidable during column chromatography of the crude reaction mixture on silica. The stereochemistry of the formation of 2.9 will be discussed in detail in section 2.4.

Scheme 2.2.4. Synthesis of the tetraketone 2.8

In order to obtain the last polyketone in the desired series, ester 2.2 was further oxidized with KMnO₄ and 18-crown-6 in aqueous DCM at rt to furnish enedione 2.5 in 78% yield after 24 h. Tetraketone 2.8 was prepared from enedione 2.5 by catalytic oxidation with ruthenium tetraoxide under Sharpless conditions.¹⁹ The use of RuCl₃ with NaIO₄ as a stoichiometric reoxidant in a mixture of CCl₄, acetonitrile and water gave the desired product 2.8 in 87% yield after 15 h (Scheme 2.2.4)

2.3 Oxidation of the double bond at the B/C ring fusion of Bryonolic acid

The starting material for the oxidation chemistry 2.11 was prepared from bryonolic acid via methylation of C-29 carboxylic acid with diazomethane to initially form ester 2.10, followed by the protection of the hydroxyl group at C3 to generate acetate 2.11 (Scheme 2.3.1).
In order to gain access to ketones 2.12 and 2.14, protected bryonolic acid 2.11 was subjected to catalytic oxidation with ruthenium tetraoxide under our modified conditions.19, 21-22 Under these conditions, substrate 2.11 underwent competing oxidative cleavage of the Δ8,9 double bond to form the desired diketone 2.12 and regioselective allylic oxidation at C7 to give α,β-unsaturated ketone 2.13 (Scheme 2.3.1). This transformation was fast and robust providing complete conversion of the starting material 2.11 in 20 min as monitored by TLC. However, with extended reaction time (24 h), intermediate 2.13 was further oxidized in situ by ruthenium tetroxide to give the desired triketone 2.14 and the product of competing CH activation 2.15.

Scheme 2.3.1. Oxidation of bryonolic acid-derived substrate 2.11
The dominating CH activation pathway of this reaction leading to $\alpha,\beta$-unsaturated ketone 2.13 and the final low yield of diketone 2.12 were disappointing. However, as a result of these two competing oxidation pathways, the desired diketone 2.12 and triketone 2.14 were obtained in a one-step operation without the necessity of any additional manipulations.

Tetraketone 2.16 was arrived at after oxidative cleavage of the enedione 2.15 (Scheme 2.3.2). As in the case of all other substrates described previously, ruthenium tetraoxide under Sharpless conditions proved to be the reagent of choice for this otherwise stubborn transformation. However, larger amounts of ruthenium catalyst and reoxidant and a longer reaction time were required to afford, after careful column chromatography, tetraketone 2.16 together with the product of oxidative ring-opening 2.17 in 18% and 21% yield, respectively.

![Scheme 2.3.2. Oxidation of bryonolic acid-derived enedione 2.15](image)

The structure of the carboxylic acid 2.17 was tentatively suggested after a thorough NMR study but appeared to be difficult to establish due to slow conformational equilibrium and identical chemical shifts of C-8 and C-29 carbonyls at 179.5 ppm. The structure was established unambiguously after carboxylic acid 2.17 was treated with
diazomethane to form the ester **2.18**, which showed three singlets centered around 3.6 ppm in $^1$H spectrum as well as four ester carbonyls and two keto groups in the $^{13}$C spectrum.

**2.4 Transannular aldol reaction of diketones 2.6 and 2.12**

The research described in this section was initiated with the central premise that the divergent transannular aldol reaction of pseudo-symmetrical substrates **2.6** and **2.12** will result in the formation of tetra- and pentacyclic triterpenoids with contrasting ring compositions. Additionally, the aldol reaction is inherently stereogenic, and each of the resultant products should have two newly formed stereocenters. Given the fact that skeletal and stereochemical diversity are key aspects in the construction of DOS libraries,^{23-24} goals were set to: (1) identify and further reinforce the substrate-dictated regio- and stereoselectivity of aldol additions of **2.6** and **2.12**; and (2) explore the possibility of overturning the substrate-biased selectivity by a thoughtful reagent choice.

The aldol reaction of non-symmetrical substrates to form both possible products has historically proven difficult.$^{25-28}$ With respect to the lanosterol-derived diketone, Snatzke$^{29-31}$ in his earlier work predicted the unfeasibility of formation of the aldol product via *pathway b* due to the presence of a strained trans-fused bicyclo[3.3.0]octane fragment in this product. Using this knowledge, Jagodzinski and Sicinski$^{14,32}$ devised an efficient four-step synthetic route leading to a molecular skeleton with the desired ring-fusion type (**Figure 2.4.1**). Concurrently, Fox and Scott$^{33}$ treated the diketone with neutral alumina to obtain two epimeric aldol adducts via *pathway a*, although the stereochemistry of these products was not investigated.
Molecular modeling revealed the potential existence of two conformations of the cyclodecane ring of diketone 2.6. Specifically, a “boat (with bow at C-5 and stern between C-8 and C-11) –chair –boat (with bow between C-9 and C-7 and stern at C-13)” (BCB) conformer, and a “chair –chair –chair” (CCC) conformer were proposed (Figure 2.4.2).

Energy minimization calculations at the B3LYP 6-311G(d,p) level of theory disclosed that the conformer CCC is thermodynamically more stable than the canonical conformer BCB by 4.3 kcal/mol. Additionally, a set of resonances corresponding to only
one conformer was observed in the $^1\text{H}$ and $^{13}\text{C}$ NMR spectra of diketone 2.6. These facts strongly suggest that diketone 2.6 exists in solution as a single conformer CCC.

Further analysis of the substrate-dictated conformer CCC of diketone 2.6 revealed that the pseudoaxial H-7 is antiperiplanar with the carbonyl at C-8, and the pseudoequatorial H-7 is orthogonal to the C=O bond. Thus, after deprotonation by the loss of the pseudoequatorial $\alpha$-hydrogen, (Z)-enolate should be formed as a key intermediate in the aldol addition reaction (Figure 2.4.3). Under common aldol conditions, such as Brønsted acid catalysis, this enolate would be structurally biased to form product 2.19 with syn configuration at the newly formed B/C ring fusion.

![Image](image.png)

**Figure 2.4.3.** Stereochemical model for the formation of 2.19 and 2.20

Additionally, the power of Lewis acid catalysis to govern the diastereoselectivity of aldol addition is well known.\textsuperscript{34-37} The hypothesis was formulated that upon treatment with a Lewis acid, such as neutral alumina, the key intermediate may engage in a six-membered Zimmerman-Traxler transition state. This transition state would determine the $\beta$-facial position of H-7, as well as $\alpha$-facial position of the keto groups at C-8 and C-9,
thus providing product 2.20 with trans configuration at the B/C ring fusion. It is noteworthy that Fox and Scott\textsuperscript{33} have assumed that products 2.19 and 2.20 would be epimeric with respect to the β-hydroxy group at C-9. In contrast, the proposed stereochemical model suggested that the difference between these epimeric products lies in the absolute stereochemistry of α-hydrogen at C-7.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent $i$</th>
<th>Isolated Yield of Product (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2.19</td>
</tr>
<tr>
<td>1</td>
<td>Pyrrolidine (0.5 equiv)</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>TFA (0.2 equiv)</td>
<td>73\textsuperscript{a}</td>
</tr>
<tr>
<td>3</td>
<td>TFA (0.5 equiv)</td>
<td>71\textsuperscript{b}</td>
</tr>
<tr>
<td>4</td>
<td>TFA (5 equiv)</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Al$_2$O$_3$ neutral (100 equiv)</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Al$_2$O$_3$ basic (100 equiv)</td>
<td>11</td>
</tr>
<tr>
<td>7</td>
<td>LDA (1.1 equiv)</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>TiCl$_4$ (1.2 equiv)</td>
<td>0</td>
</tr>
</tbody>
</table>

Key: \textsuperscript{a}brsm, 65% conversion. \textsuperscript{b}brsm, 91% conversion.
Control A: TFA (5 equiv), 48 h (2.21, 90% yield). Control B: Al$_2$O$_3$ neutral (100 equiv), 48 h (2.21, 17% yield; 2.19, 78% recovery). Control C: Al$_2$O$_3$ neutral (100 equiv), 45 min (2.21, 95% yield).

Table 2.4.1. Formation of 2.19 and 2.20 under standard aldol conditions.

In complete agreement with this rationale, predominant formation of product 2.19 was observed when diketone 2.6 was subjected to the standard aldol conditions. Specifically, the use of pyrrolidine as an organocatalyst for enamine-mediated aldol reaction led to the formation of product 2.19 in 86% yield and no other products were observed (Table 2.4.1, Entry 1). In due course, treatment of diketone 2.6 with 0.2 equiv
of TFA in DCM for 48 h yielded syn-adduct 2.19 and α,β-unsaturated ketone 2.21 in 71% and 7% yield, respectively (Table 2.4.1, Entry 2). The formation of ketone 2.21 in this reaction suggested the possible intermediacy of anti-adduct 2.20, which has antiperiplanar arrangement of H-7 and the hydroxyl group at C-9, thus raising the possibility of the increased production of ketone 2.21 if overall elimination of water is to occur through E2-type transition state. When 0.5 equiv of TFA was used in this reaction, products 2.19 and 2.21 were accompanied by the formation of ketone 2.22. Furthermore, the use of excess TFA led to the predominant formation of ketone 2.22, and no products of aldol addition were detected in the reaction mixture. α-Facial position of H-7 in ketone 2.22 suggests that under the acidic reaction conditions diketone 2.6 was initially converted to syn-adduct 2.19, followed by the elimination reaction to form ketone 2.22, which, in turn, underwent simultaneous double bond migration process leading to α,β-unsaturated ketone 2.21. The control experiment confirmed this suggestion when isolated ketone 2.22 was treated with excess TFA to form α,β-unsaturated ketone 2.21 in 90% yield after 48 h (Table 2.4.1, control A).

Treatment of 2.6 with neutral alumina led to the formation of anti-adduct 2.20, syn-adduct 2.19, and α,β-unsaturated ketone 2.21 in 23%, 35% and 11% yield, respectively (Table 2.4.1, entry 5). The use of basic alumina in this reaction resulted in the increased formation of 2.20, although considerable degradation was observed (Table 2.4.1, entry 6). Given the fact that aldol addition is generally known to be a reversible process, we set out to determine whether the distribution of products 2.19 and 2.20 was the result of thermodynamic reaction control. Thus, when purified syn-adduct 2.19 was subjected to the same reaction conditions, no direct interconversion of 2.19 and 2.20 was indicated.
However, the formation of $\alpha,\beta$-unsaturated ketone \textbf{2.21} in 17% yield was noted (\textbf{Table 2.4.1}, control B). One possible explanation for the formation of \textbf{2.21} in the control experiment is the equilibration between \textit{syn}-adduct \textbf{2.19} and \textit{anti}-adduct \textbf{2.20}, followed by simultaneous dehydration of \textbf{2.20}. Alternatively, intermediate formation of ketone \textbf{2.22} could be suggested. When pure ketone \textbf{2.22} was subjected to the conditions of the same control experiment, \textbf{2.21} was formed in 95% yield after 45 min (\textbf{Table 2.4.1}, control C). The absence of direct interconversion of \textbf{2.19} and \textbf{2.20}, coupled with the rapid formation of \textbf{2.21} from \textbf{2.22} in the control experiment, strongly suggest that the formation of \textit{anti}-adduct \textbf{2.20} is the result of Lewis acid-controlled transition state of the aldol addition of diketone \textbf{2.6}.

A range of Lewis acids of varying strength and atomic radius of the metal cation was screened in an attempt to achieve complete control over the diastereoselectivity of this transannular reaction. The lithium cation is generally known to reliably follow the Zimmerman-Traxler model. Hence, upon reaction with LDA (1.1 equiv, -78 °C to rt), diketone \textbf{2.6} yielded \textit{syn}-adduct \textbf{2.19}, \textit{anti}-adduct \textbf{2.20} and $\alpha,\beta$-unsaturated ketone \textbf{2.21} in 40%, 6% and 6%, respectively (\textbf{Table 2.4.1}, entry 7). The use of TiCl$_4$ in the presence of tertiary amine led to the formation of $\alpha,\beta$-unsaturated ketone \textbf{2.21} in 58% yield and no other products were observed (\textbf{Table 2.4.1}, entry 8).

In due course, treatment of diketone \textbf{2.6} with BF$_3$·Et$_2$O unexpectedly resulted in the formation of aliphatic ketones \textbf{2.23-2.26} via \textit{pathway b}, accompanied by the products of \textit{pathway a} \textbf{2.22} and \textbf{2.21} (\textbf{Table 2.4.2}, entry 1). Similar product distribution was observed when 0.2 equiv of Al(OTf)$_3$, Sc(OTf)$_3$ and In(OTf)$_3$ were used to catalyze the aldol addition of \textbf{2.6} at rt (\textbf{Table 2.4.2}, entries 2-4).
<table>
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</tr>
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<td>17</td>
</tr>
<tr>
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<td>11&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>16</td>
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</table>

Key: "Unless otherwise indicated, all reactions were performed in 0.1M solutions of DCM with 0.2 equiv of the catalyst. "Yields of 7,9-12 were determined by <sup>1</sup>H NMR. "Reaction performed with 1.1 equiv of the reagent (-78 °C to rt). "brsm, 74% conversion. "brsm, 55% conversion. "brsm, 90% conversion.

Table 2.4.2. Formation of 2.23-2.26 under strong Lewis acidic conditions

The use of InCl<sub>3</sub> and Cu(OTf)<sub>2</sub> at rt led to the full recovery of 2.6. Increasing the temperature to 50 °C significantly improved the efficiency of this transformation (Table 2.4.2, entries 5-8). Alternative catalysts such as Zn(OTf)<sub>2</sub>, Yb(OTf)<sub>3</sub> and La(OTf)<sub>3</sub> did not show any reactivity after 24 h under both of the optimized reaction conditions. The fact that the formation of the desired anti-adduct 2.20 was not observed under our Lewis acidic conditions was disappointing. However, the unexpected formation of 2.23-2.26 with 6/7/5/5 ring composition is important because it constitutes the discovery of a formerly intractable pathway of a well-known reaction and, therefore, provides direct access to the area of chemical space which has not yet been explored.
The absolute stereochemistry of H-11 in 2.23-2.26 suggested the following mechanistic rationale for the formation of these molecules (Figure 2.4.4).

Figure 2.4.4. Mechanistic rationale for the formation of 2.23-2.26 from 2.6 via pathway b

Presumably, the Z-enolate of diketone 2.6 can be formed upon deprotonation by the loss of pseudoequatorial α-hydrogen at C-11. This enolate is geometrically predisposed for the formation of intermediate aldol adduct via pathway b with syn configuration at the B/C ring fusion. Upon further in situ reaction with a strong Lewis acid, such as BF₃·Et₂O, the syn-adduct engages in E1-type transition state to form a carbocationic intermediate, which, in turn, may be followed by either elimination of H-7 to give 2.23, or C-30 methide shift and elimination of H-15 to give 2.24. Additionally, the carbocation can be followed by a cascade of C-30 and C-18 methide shifts and an elimination of H-17 to form 2.25.

The formation of 2.26 deserves a separate comment. Analogously with ketones 2.24 and 2.25, formation of the Δ16,17 double bond in 2.26 may result from sequential C-30 and C-18 methide shifts, H-17 hydride shift and an elimination of H-16. However, the α-facial position of H-17 in the intermediate carbocation, and the resultant β-facial
orientation of H-13 in ketone 2.26 led to a conclusion that the Δ16,17 double bond in 2.26 is the result of allylic shift and migration of the β-oriented pseudoaxial H-16 of ketone 2.25.

Transannular aldol addition of bryonolic acid-derived diketone 2.12 was next investigated. Examination of the structure of 2.12 revealed two possible enolization sites, potentially leading to regioisomeric aldol adducts with 6/7/5/6/6- and 6/5/7/6/6-fused ring composition via pathways a and b, respectively. By direct comparison with lanosterol-derived diketone 2.6, the formation of both possible products from 2.12 seemed equally plausible due to the absence of highly strained bicyclic systems in either one of the possible structures.

**Figure 2.4.5.** Conformational analysis of diketone 2.12

By virtue of substrate bias, the lowest energy conformation of the cyclodecane ring of 2.12 is “boat (with bow at C-5 and stern between C-8 and C-11) -chair-chair” (BCC) which determines the α-facial position of the carbonyl group at C-8 and β-facial position of the carbonyl at C-9 (**Figure 2.4.5**). In both pathways a and b the pseudo-axial α-hydrogen is antiperiplanar with the carbonyl and the pseudo-equatorial α-hydrogen is orthogonal to the C=O bonds, thus producing the trans (Z)-enolate of 2.12 as the key intermediate after deprotonation by loss of the pseudo-equatorial hydrogen. As shown in
Figure 2.4.6 for pathway a, the intermediate enolate will be geometrically predisposed for the formation of transannular C-C bonds with syn configuration at the ring fusion.

![Stereochemical model for the formation of 2.27 and 2.29 via pathway a.](image)

By direct analogy with lanosterol-derived substrate 2.6, it was further hypothesized that when treated with a Lewis acid, the key intermediate may engage in the Zimmerman-Traxler transition state,\(^{38}\) (Figure 2.4.6) which determines the β-facial position of H-11 and α-facial position of the keto groups at C-8 and C-9. Hence, product 2.29 should be formed with trans-configuration at the newly formed B/C ring fusion.

Initial efforts were focused on the standard aldol protocols. The use of catalytic TFA at room temperature led to the formation of a mixture of syn product from pathway a (2.27) and syn product from pathway b (2.28) in good overall yield (85%, Table 2.4.3, entry 1). The use of sodium hydride as well as pyrrolidine as an organocatalyst for enamine-mediated aldol reaction gave a similar distribution of products 2.27 and 2.28, although reaction with sodium hydride led to some degradation (Table 2.4.3, entries 2 and 3). Predominant formation of aldol adduct 2.27 suggested that it was the more
thermodynamically stable product of the reaction. It is noteworthy that no products of aldol condensation were observed in any of the reactions performed.

<table>
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<tr>
<th>Entry</th>
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<th>Isolated yield (%)</th>
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</tr>
<tr>
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<tr>
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<td>8</td>
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<tr>
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<td>TiCl₄</td>
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</table>

Table 2.4.3. Formation of 2.27-2.30 from bryonolic acid-derived diketone 2.12

Lithium amide bases were then used to force the transannular aldol reaction of diketone 2.12 into pathway b under kinetic conditions. Upon reaction of diketone 2.12 with LDA (1.1 equiv., THF, -78°C to rt), aldol adducts 2.27 and 2.28 were formed in 71% and 22% yield, respectively (Table 2.4.3, entry 4). Increase in the steric bulk of the amide base led to increased formation of aldol adduct 2.28 via pathway b. Thus, the use of LiHMDS produced 2.28 in 25% yield and LiTMP proved to be the reagent of choice, giving the highest yield of aldol adduct 2.28 (43%, Table 2.4.3, entry 6).
Upon treatment of 2.12 with Al₂O₃ (basic) in DCM, aldol adduct 2.27 was accompanied by the formation of anti product of pathway a (2.29) in 7% yield (Table 2.4.3, entry 8). When purified aldol adduct 2.27 was resubjected to the same reaction conditions (100 equiv Al₂O₃, DCM, rt, 16 h), no interconversion of these products was observed. The absence of product interconversion under the same set of conditions led to the conclusion that the relative stereochemistry of anti product 2.29 is the result of Lewis acid-controlled transition state of the aldol addition reaction of the diketone 2.12.

It is noteworthy that the lithium cation did not give any anti products in reactions with amide bases (Table 2.4.3, entries 4-6). The reaction of 2.12 with TiCl₄ in the presence of tertiary amine (1.1 equiv, -78 °C to rt) led to the formation of aldol adducts 2.27 and 2.28 in 1.6:1 ratio and no anti products were observed (Table 2.4.3, entry 9). In due course, treatment of diketone 2.12 with BF₃·Et₂O (1.1 equiv, -78 °C to rt) resulted in the formation of aliphatic ketones 2.31 and 2.32 and α,β-unsaturated ketone 2.33 (Scheme 2.4.1, pathway c).

The absolute stereochemistry of H-11 suggested that ketone 2.31 was the result of aldol addition via pathway a to form aldol adduct 2.27 followed by a cascade of methide shifts and an elimination of H-18 to form the internal double bond at Δ13,18. The control experiment (Scheme 2.4.1, pathway d) confirmed our prediction when isolated aldol adduct 2.27 was treated with BF₃·Et₂O to form aliphatic ketone 2.31 in 92% yield. The thermodynamic stability of 2.27 and trans arrangement of the C-8 hydroxyl group and the methyl at C-26 add to the robustness of this transformation. A similar rationale was then proposed for the formation of ketones 2.32 and 2.33 via pathway c in 1:4 ratio. β-Facial position of H-7 in ketone 2.32 suggested that the formation of aldol adduct 2.28 via
pathway $b$ would be followed by either a C-25 methide shift or an elimination of H-7 through a common carbocationic intermediate and E1-type transition state to yield ketones 2.32 or 2.33, respectively.

Both of these possibilities seem equally plausible due to the fact that both the C-25 methyl group and H-7 are cis relative to the hydroxyl group at C-9. The control experiment (Scheme 2.4.1, pathway $d$) with isolated aldol adduct 2.28 yielded compounds 2.32 and 2.33 in 2.4:1 ratio. The reversal of selectivity observed for the production of ketone 2.32 over 2.33 via pathway $d$ (Scheme 2.4.2) led to the conclusion that the reaction through pathway $c$ gives intermediate aldol adduct 2.28 and an anti product of pathway $b$ (2.30), which has the antiperiplanar arrangement of H-7 and the hydroxyl group at C-9 (Table 2.4.3), thus raising the possibility of increased production.

Key: (a) BF$_3$·Et$_2$O (1.1 equiv.), DCM, -78 °C to rt, 4 days; (b) BF$_3$·Et$_2$O (10 equiv.), DCM, -78 °C to rt, 18 h

**Scheme 2.4.1** Aldol addition/condensation of diketone 2.12 via pathways $c$ and $d$
of ketone 2.33, if the overall elimination of water is to occur through an E2-type transition state.

Based on the above results, the diastereoselectivity of the aldol reaction of lanosterol- and bryonolic acid-derived diketones can be predicted based on the analysis of their conformations. Moreover, some degree of control over the reaction pathway of these non-symmetrical substrates was achieved using judicious reagent choice. A similar strategy was applied to the triterpenoid-derived tri- and tetraketones (sections 2.5 and 2.6).

**Scheme 2.4.2** Mechanistic considerations for the formation of ketones 2.31, 2.32 and 2.33
2.5 Transannular aldol reaction of triketones 2.7, 2.14 and 2.34

As briefly mentioned in section 2.2, the formation of aldol product 2.9 has proven unavoidable during the purification of triketone 2.7 on silica. This fact can be explained by analysis of the possible conformations of triketone 2.7.

Figure 2.5.1 Conformational analysis of 2.7

Energy minimization at the B3LYP 6-311G(d,p) level of theory revealed that the “chair −chair −chair” conformer (CCC) is thermodynamically more stable than the canonical “boat (with bow at C-5 and stern between C-8 and C-11) −chair −boat (with bow between C-9 and C-7 and stern at C-13)” (BCB) conformer by 2.6 kcal/mol.

Figure 2.5.2 Stereochemical model for the formation of 2.9

Consequently, deprotonation of the CCC conformer by loss of the pseudoequatorial α-hydrogen at C-11 results in the formation of (Z)-enolate. This enolate is geometrically predisposed to engage in a six-membered transition state, which allows for the retention of the β-facial position of H-11, as well as α-facial orientation of the keto groups at C-7 and C-9. Hence, aldol adduct 2.9 is formed with trans configuration at the B/C ring.
fusion. The above mentioned geometric predisposition, and the fact that both rings B and C have energetically favorable “chair” conformation, provide an explanation for the instantaneous formation of $2.9$ upon interaction of $2.7$ with silica.

A similar hypothesis was then formulated for prediction of the stereoselectivity of the aldol reaction of triketone $2.34$ resulting from the oxidation of $\alpha,\beta$-unsaturated C-11 ketone $2.4$.

![Figure 2.5.3 Conformational analysis of 2.34](image)

Energy minimization at the B3LYP 6-311G(d,p) level of theory revealed that the “chair –chair –chair” conformer (CCC) of such a triketone would be thermodynamically more stable than the “boat (with bow at C-5 and stern between C-8 and C-11) –chair -boat (with bow between C-9 and C-7 and stern at C-13)” (BCB) conformer by 4.1 kcal/mol.

![Figure 2.5.4 Stereochemical model for the formation of 2.35](image)

Deprotonation of the CCC conformer at C-7 was envisioned to produce trans (Z)-enolate. The six-membered transition state leading to the aldol product $2.35$ was
envisioned to retain the β-facial position of the keto groups at C-8 and C-11, as well as the α-facial orientation of H-7. This geometric bias, coupled with the “chair” conformation of rings B and C, allowed for the prediction of instantaneous formation of 2.35.

The separation of α,β-unsaturated ketones 2.3 and 2.4 on silica proved challenging. Compounds 2.3 and 2.4 were obtained as one fraction, in approximately 1:1 ratio, after oxidation of lanosterol acetate 2.1 with ruthenium tetraoxide. To obtain pure 2.3 and 2.4 for the purpose of their structural analysis (described in detail in section 2.2 of this Chapter), several repetitive chromatography columns were needed. In this method, pure 2.4 was isolated after each step, followed by the mixture which was enriched in 2.3, finally resulting in the complete separation of these compounds.

Scheme 2.5.1 Synthesis of 2.9 and 2.35

When the mixture of 2.3 and 2.4 (1:1, as determined by 1H NMR) was subjected to catalytic oxidation with ruthenium tetraoxide under our modified conditions, polyketones 2.7, 2.8 and 2.34 were formed. Treatment of the mixture of these substrates with basic Al2O3 in DCM resulted in production of the desired aldol products 2.9 and 2.35 in 38%
and 2% yield, respectively. The regiochemistry of these products and the absolute configuration of the newly formed stereocenters at the B/C ring fusions were in complete accordance with the above proposed conformation-based rationale. The formation of 2.8, and concomitantly 2.36 in 23% yield, was not desired but anticipated. The chemistry leading to 2.36 will be discussed in detail in the following section.

The chemistry leading to the aldol product 2.37 was rationalized in a similar manner. In sharp contrast with the lanosterol-derived substrates, the lowest energy conformation of 2.37 appeared to be downward-facing “boat (with bow at C-5 and stern between C-8 and C-11) -chair-chair” (BCC) conformation. According to B3LYP 6-311G(d,p) energy minimization calculations, BCC was more stable than its upward-facing counterpart “chair-chair-boat (with bow between C-7 and C-9 and stern at C-13)” (CCB) by 4.99 kcal/mol (Figure 2.5.5).

![Figure 2.5.5 Conformational analysis of 2.14](image)

Abstraction of the pseudo-equatorial α-hydrogen at C-11 of 2.14 was envisioned to result in the production of (Z)-enolate, which is structurally biased to adopt the six-membered Zimmerman-Traxler transition state with β-oriented C-7 carbonyl thus forming anti adduct 2.37. In complete agreement with this rationale, the desired aldol adduct 2.37 was formed in 80% yield upon treatment of triketone 2.14 with basic Al₂O₃ in DCM at room temperature (Figure 2.5.6, Scheme 2.5.2).
Figure 2.5.5 Stereochemical model for the formation of 2.37

Scheme 2.5.2 Synthesis of 2.37

The differences and similarities in aldol reactivity of lanosterol- and bryonolic acid-derived tetraketones will be discussed in the following section.

2.6 Transannular aldol reaction of tetraketones 2.8 and 2.16

Marsaioli and co-workers\textsuperscript{39-40} studied the aldol reactivity of the tetraketone derived from 24,25-dihydrolanosterol in 1992. In their work, the authors determined that under various aldol reaction conditions the tetraketone undergoes site-selective deprotonation at C-6 to form corresponding enolate, followed by aldol addition with the keto group at C-11. The newly formed hydroxyl group at C-11 then simultaneously forms the bridging hemiketal with the C-8 carbonyl. The authors determined the structure of this product
based on analysis of the homonuclear and heteronuclear 2-D NMR spectra, and further confirmed their structural assignment by X-ray crystallography (Scheme 2.6.1).

Scheme 2.6.1 Synthesis of 2.36

The authors suggested that this reactivity pattern is due to conformation-dictated special proximity of the C-6 and the C-11 carbonyl of the tetraketone, as well as concomitant closeness of the newly formed hydroxyl group at C-11 to C-8 carbonyl and the thermodynamic stability of the fused furano-pyran bicyclic system. Predictably, subtle modification of the side-chain did not change the outcome of this reaction. When tetraketone 2.8 was treated with basic Al₂O₃ in DCM at room temperature, the product of aldol addition 2.36 was formed in 88% yield after 24 h.

Scheme 2.6.1 Synthesis of 2.38 and 2.39

However, when bryonolic acid-derived tetraketone 2.16 was subjected to the conditions of aldol addition reaction (LDA, 1.1 equiv, THF, -78 °C to rt), the aldol
adduct 2.38 was formed only in 9% yield, and the product of transannular hemiketalization 2.39 was obtained in 64% yield (Scheme 2.6.1).

Figure 2.6.1 Structural analysis of 2.39

The structure elucidation of 2.39 deserves a separate comment (Figure 2.6.1). Examination of the routine $^{13}$C NMR spectrum of 2.39 disclosed the signals of two carbonyl carbons (C-8, $\delta_C$ 207.6; C-11, $\delta_C$ 205.1), as well as two quaternary carbons (C-7, $\delta_C$ 100.9; C-9, $\delta_C$ 99.0) in the region downfield from 80 ppm. Further study of the NMR spectra of 2.39 revealed an AX system of two doublets at 3.14 and 2.18 ppm ($J = 12$ Hz) attached to an isolated methylene carbon at 43.6 ppm. These protons correlate, as observed by HMBC, with the carbonyl at C-11, as well as with quaternary carbons C-9, C-13 ($\delta_C$ 44.6), C-14 ($\delta_C$ 55.0), and a methyl carbon C-27 ($\delta_C$ 16.88). These correlations allowed the assignment of the AX system to position 12 of 2.39. In the HMBC spectrum, H-5 showed cross-peaks with quaternary carbons C-7, C-9, C-10 ($\delta_C$ 40.1) and C-4 ($\delta_C$ 37.3), while H-6 correlated with quaternary carbons C-4, C-7, C-10, methine carbon C-5 ($\delta_C$ 40.5) and a carbonyl carbon C-8. The atom connectivities were further confirmed by the HMBC correlations of H-25, H-26 and H-27 (see Figure 2.6.1).
The relative stereochemistry of 2.39 was confirmed by NOESY spectroscopy, which showed enhancement between the hydroxyl group at C-7 and the methyl proton H-26. H-26, in turn, showed correlations with Hβ-12 and H-18 (Figure 2.6.1). The hydroxyl group at C-9 exhibited a correlation with β-oriented methyl proton H-25. These NOESY data are consistent with the β-facial environment of the newly formed rings B and C. The α-facial environment of these rings was determined by the enhancement between H-5 and H-27.

Formation of 2.39 suggested the following mechanism (Figure 2.6.2). In the ground-state conformation of tetraketone 2.16 ("boat (with bow at C-5 and stern between C-8 and C-11) –chair-chair", BCC; see Chapter 3 for detailed analysis) pseudo-axial H-6 is coplanar with the carbonyl at C-7 and pseudo-equatorial H-6 is orthogonal to the C=O bond. Thus, the cis (E)-enolate is formed as the key intermediate after the abstraction of the pseudo-equatorial α-hydrogen by the base.

![Figure 2.6.2 Mechanistic rationale of the formation of 2.38 and 2.39](image)

The higher yield of 2.39 over the aldol adduct 2.38 can be explained in view of the fact that the transition state leading to the aldol adduct 2.38 seems to be markedly higher
in energy in contrast with the six-membered chair-like transition state leading to the product of transannular hemiketalization \(2.39\). The steps leading to \(2.39\) presumably include the formation of an intermediate with the charge localized on the oxygen of the pyranyl ring.\(^{41}\) This proposed mechanism was confirmed when the reaction was quenched with \(\text{D}_2\text{O}\), resulting in a partial deuterium incorporation at C-6.

Marsaioli and co-workers have suggested that 24,25-dihydrolanosterol-derived tetraketone exists as a mixture of conformational isomers. Based on this observation, it was envisioned that the least-populated of the suggested conformers (“chair-chair-chair”, CCC; see Table 2.6.1) may exhibit reactivity similar to that of tetraketone \(2.16\) leading to the product of transannular hemiketalization \(2.40\). Conformational equilibrium was expected to be slower at low temperature, thus allowing for the formation the enolate of CCC that would be predisposed for the formation of the desired hydroxypyran structure through the six-membered boat-like transition state.

![Image of reaction pathway](image)

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*Table 2.6.1* Aldol addition and hemiketalization of tetraketone \(2.8\)
Upon treatment of 2.8 with LDA (1.1 equiv, THF, -78 °C to rt), the expected product 2.36 was accompanied by the structure 2.41 having a hydrogen in position 8, but the desired hemiketal 2.40 was not observed. The formation of 2.41 could be explained by the hydride-donating properties of LDA.\(^4\) Hence, the use of the amide bases that lack a hydrogen alfa to the nitrogen, such as LiTMP and LiHMDS, under the same reaction conditions resulted in the exclusive formation of 2.36, and no other products were detected in the reaction mixture (Table 2.6.1). The fact that the desired product of transannular hemiketalization 2.40 was not observed in these reactions could be explained by predictable instability of the boat-like transition state, as well as by possible reversibility of the formation of the intermediate vinylic hemiketal.

In summary, the development of aldol chemistries of the lanosterol- and bryonolic acid-derived tetraketones 2.8 and 2.16 brought two novel triterpenoid structures 2.41 and 2.39 into the chemical library by virtue of unanticipated reduction and hemiketalization, respectively.

### 2.7 Conclusions

In this Chapter, the preparation of triterpenoids lanosterol and bryonolic acid from their natural sources was described. The unsaturated B/C ring fusion in these molecules underwent competing allylic oxidation and oxidative cleavage reactions leading to transannular di-, tri- and tetraketone substrates. It is noteworthy that ruthenium tetraoxide under Sharpless conditions proved to be the reagent of choice for all of the desired transformations.
Lanosterol-derived diketone was used as a substrate for divergent aldol reaction leading to 6/5/7/5- and 6/7/5/5-fused triterpenoids via pathways a and b, respectively. The diastereoselectivity of this reaction via pathway a was predicted using conformational analysis. The screening of various Lewis acids unexpectedly resulted in the formation of reaction products via pathway b, which was initially assumed to be unfeasible on the basis of a literature review. The aldol addition of bryonolic acid-derived diketone was shown to follow three different reaction pathways leading to 6/5/7/6/6- and 6/7/5/6/6-fused molecules. Some control over the pathway selectivity was achieved using kinetic and thermodynamic reaction conditions.

The aldol addition of lanosterol- and bryonolic acid-derived triketones led to unifying results despite contrasting conformational preferences of these molecules. Specifically, linear triterpenoid analogues were formed in a predictable and straightforward manner via similar six-membered transition states.

The aldol chemistries of the tetraketones derived from lanosterol and bryonolic acid were contrastingly illustrative that even subtle structural differences between the parent molecules may result in completely different reaction outcomes. Thus, the lanosterol-derived tetraketone exclusively yielded the product of aldol addition, while the bryonolic acid-derived tetraketone produced a novel bridging hemiketal as the major reaction product. These results were rationalized based on opposing reactive conformations of the tetraketones, and attempts to control the chemoselectivity were unsuccessful.
2.8 Experimental part

2.8.1 General experimental details

All reactions were run in atmosphere of dry argon unless otherwise stated. RuCl$_3$ (99%, anhydrous), NaIO$_4$, pyrrolidine, diisopropylamine, TiCl$_4$ (99.9%) were purchased from Acros. BF$_3$·Et$_2$O (purified, redistilled), n-butyllithium (2.5M in hexanes) were purchased from Sigma-Aldrich. Trifluoroacetic acid (TFA), neutral and basic alumina, were purchased from Fisher Scientific. Diisopropylamine and pyrrolidine were distilled from P$_2$O$_5$ prior to use. Diazomethane was prepared with Diazald kit from Sigma-Aldrich.

Dry pyridine and dichloromethane (DCM) were purchased from Acros in Acroseal bottles. THF was distilled from benzophenone ketyl solution with sodium prior to use. Acetonitrile and carbon tetrachloride were purchased from Acros and were used without further purification. Quick syringe transfers were done with disposable syringes and needles.

Column chromatography was performed with Purasil Silica Gel 60A 230-400 Mesh. Ethyl acetate and hexanes ACS grade were purchased from Fisher Scientific and were used without any purification. Analytical and semi-preparative HPLC separations were performed with Agilent Technologies 1200 Series HPLC instrument using acetonitrile and water CHROMASOLV Plus grade (for HPLC, 99.9%) purchased from Sigma-Aldrich. Analytical thin-layer chromatography (TLC) was carried out using Partisil K6F Silica Gel 60A 0.25 mm plates with fluorescent indicator. All reactions that were monitored by TLC were visualized with a 254 nm UV-lamp or using phosphomolybdic
acid (PMA) and 1,4-dinitrophenylhydrazine (DNP) stain solutions prepared by well-known protocols.

NMR spectra were recorded with either Varian Gemini (\(^{1}\text{H} 400 \text{ MHz}, \quad ^{13}\text{C} 100 \text{ MHz}\)) spectrometer or Varian Inova (\(^{1}\text{H} 600 \text{ MHz}, \quad ^{13}\text{C} 150 \text{ MHz}\)) spectrometer. Chemical shifts reported in \(\delta\) units, part per million (ppm) with reference to the residual solvent peak (CDCl\(_3\), 7.26 ppm for \(^{1}\text{H} \text{NMR}\) and 77.16 ppm, center of triplet, for \(^{13}\text{C} \text{NMR}\)). DEPT, COSY, NOESY, HMQC, HMBC spectra were recorded using standard 2-D VNMR pulse sequences. High-resolution mass spectra (HRMS) were obtained using a PE/Sciex Elan 6000 ICP-MS spectrometer (ESI).

2.8.2 Oxidation of lanosterol and its derivatives

Preparation of lanosterol acetate (2.1)

\((3S,5R,10S,13R,14R,17R)-4,4,10,13,14-Pentamethyl-17-((R)-6-methylhept-5-en-2-yl)-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl acetate (2.1)\) In a round-bottom flask, lanosterol (10g, 23.4 mmol; 60% in the mixture with 24,25-dihydrolanosterol, purchased from various commercial sources) and DMAP (143 mg, 1.17 mmol) were dissolved in 47 mL of dry pyridine. Acetic anhydride (7.18 g, 70.3 mmol, 6.6 mL) was added to the resulting solution by a quick syringe transfer. The reaction mixture was stirred at 50 °C overnight, after which time pyridine was removed at reduced pressure and the crude mixture was taken up in DCM. The organic layer was washed successively with dilute HCl, saturated solution of sodium bicarbonate and brine. It was subsequently dried over Na\(_2\)SO\(_4\) and the
solvent removed in vacuo. The crude product was purified by column chromatography to give 2.1 (60% in the mixture with 24,25-dihydrolanosterol acetate) as a white solid (9.7 g, 88%). R_f = 0.55 (EA/hex = 10/90). Identity and purity of 2.1 were confirmed by ^1H and ^13C NMR. Spectroscopic data were found to match lit. 43 data.

**Oxidation of 2.1 with KMnO4. Preparation of products (2.2-2.5)**

Using modified literature protocol, 18 lanosterol acetate 2.1 (10 g, 60% in the mixture with 24,25-dihydrolanosterol acetate, 12.8 mmol) was dissolved in 1 L of tert-BuOH at 60 °C, followed by the addition of K_2CO_3 (10 g, 72.4 mmol). Meanwhile, a solution of KMnO_4 (1.42 g, 9 mmol) and NaIO_4 (24.1 g, 0.113 mol) in 800 mL of H_2O was heated to 60 °C and subsequently added to the resulting suspension in one portion. The flask was removed from heat, and the reaction mixture was allowed to cool to rt and stirred at rt overnight, at which time the reaction was interrupted by the addition of saturated solution of sodium thiosulfate until complete discoloration was observed (about 250 mL). After the organic layer was separated and the solvent removed in vacuo, the crude residue was acidified with dilute HCl and extracted with DCM. The organic layer was washed successively with water and brine. It was subsequently dried over Na_2SO_4 and the solvent removed in vacuo. The crude mixture of products was separated by gradient elution column chromatography on silica with ethyl acetate-hexanes mixtures. The use of hexanes as an eluting solvent yielded pure 24,25-dihydrolanosterol acetate as a white solid (3.36 g, 84%). R_f = 0.75 (EA/hex = 20/80). mp: 118-120 °C (lit. 44 mp 120-121 °C). Elution with progressively more polar combinations of eluting solvent, followed by the use of ethyl acetate as an eluting solvent, gave a crude mixture (6.5 g) of carboxylic acids. In a round-bottom flask open to atmosphere, the mixture of carboxylic acids (6.5 g)
was dissolved in 50 mL of freshly distilled THF. Freshly prepared diazomethane in diethyl ether (15 mmol) was added dropwise to the resulting solution by a pipette, and the reaction mixture was stirred at rt until full conversion was detected by TLC (approximately 30 min). The reaction was quenched by dropwise addition of glacial acetic acid until the yellow color of the solution disappeared. The solvent was removed in vacuo and the crude mixture of products was separated by careful column chromatography on silica to yield \textbf{2.2} as a white solid (3.9 g, 65\%), \textbf{2.3} as a white solid (610 mg, 9\%), \textbf{2.4} as a white solid (420 mg, 7\%), and \textbf{2.5} as a yellow solid (270 mg, 4\%).

\textbf{(R)-Methyl-4-((3S,5R,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (2.2).} Mp: 174-177 °C (lit.$^{18}$ mp 226-227 °C, lit.$^{45}$ mp 174-176 °C). $R_f = 0.58$ (EA/hex = 20/80). Spectroscopic data did not duplicate lit.$^{18}$ data. $^1$H NMR (400 MHz, CDCl$_3$, $\delta$): 4.50 (dd, $J_1 = 11.6$ Hz, $J_2 = 4.4$ Hz, 1H), 3.66 (s, 3H), 2.37 (m, 1H), 2.22 (m, 1H), 2.05 (s, 3H), 1.00 (s, 3H), 0.89 (d, $J = 6$ Hz, 3H), 0.88 (s, 3H), 0.88 (s, 3H), 0.87 (s, 3H), 0.68 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 174.9, 171.1, 134.6, 134.4, 81.1, 51.6, 50.6, 50.4, 50.0, 44.7, 38.0, 37.0, 36.2, 35.4, 31.41, 31.39, 31.1, 30.9, 28.2, 28.1, 26.5, 24.4, 24.3, 21.5, 21.1, 19.3, 18.4, 18.3, 16.7, 15.9. HRMS (ESI) $m/z$ calcd for C$_{30}$H$_{48}$O$_4$Na$^+$ [M+Na]$^+$ 495.34448, found 495.34456.

\textbf{(R)-Methyl-4-((3S,5R,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-7-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (2.3).} Mp: 200-203 °C (lit.$^{18}$ mp 255-258 °C). $R_f = 0.28$ (EA/hex = 20/80). $R_f = 0.39$ (EA/hex = 25/75). Identity of \textbf{2.3} was confirmed by $^1$H and $^{13}$C NMR and HRMS. $^1$H NMR data did not duplicate lit.$^{18}$ data. $^1$H
NMR (600 MHz, CDCl₃, δ): 4.52 (dd, J₁ = 11.6 Hz, J₂ = 4.4 Hz, 1H), 3.66 (s, 3H), 2.06 (s, 3H), 1.18 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.91 (s, 3H), 0.88 (s, 3H), 0.65 (s, 3H).

¹³C NMR (150 MHz, CDCl₃, δ): 198.8 (CO), 174.8 (CO), 171.0 (CO), 164.7 (C), 139.1 (C), 79.7 (CH), 51.6 (CH₃), 50.0 (CH), 48.9 (CH), 47.9 (C), 45.1 (C), 39.8 (C), 37.9 (C), 36.6 (CH₂), 36.1 (CH), 34.6 (CH₂), 32.1 (CH₂), 31.4 (CH₂), 31.4 (CH₂), 30.2 (CH₂), 28.8 (CH₂), 27.5 (CH₃), 25.1 (CH₃), 24.0 (CH₂), 23.8 (CH₂), 21.4 (CH₃), 18.6 (CH₃), 18.5 (CH₃), 16.5 (CH₃), 15.9 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₇O₅⁺ [M+H]⁺ 487.34180, found 487.34188.

(R)-Methyl-4-((3S,5R,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-11-oxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)pentanoate (2.4). mp: 144-147 °C (lit. 145-149 °C). R_f = 0.33 (EA/hex = 20/80). R_f = 0.42 (EA/hex = 25/75). ¹H NMR (600 MHz, CDCl₃, δ): 4.49 (dd, J₁ = 10.2 Hz, J₂ = 6 Hz, 1H), 3.65 (s, 3H), 2.99 (ddd, J₁ = 13.8 Hz, J₂ = J₃ = 3.6 Hz, 1H), 2.64 (d, J = 16.2 Hz, 1H), 2.44 (d, J = 16.2 Hz, 1H), 2.03 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 0.88 (s, 3H), 0.85 (d, J = 6.6 Hz, 3H), 0.80 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 199.1 (CO), 174.6 (CO), 171.1 (CO), 164.2 (C), 139.5 (C), 80.7 (CH), 52.0 (CH), 51.9 (CH₂), 51.7 (CH₃), 51.6 (C), 50.1 (CH), 47.4 (C), 38.0 (C), 37.6 (C), 35.8 (CH), 34.1 (CH₂), 31.2 (CH₂), 31.0 (CH₂), 29.9 (CH₂), 28.4 (CH₃), 27.0 (CH₂), 25.9 (CH₃), 24.3 (CH₂), 21.4 (CH₃), 19.1 (CH₃), 18.1 (CH₃), 17.3 (CH₂), 16.9 (CH₃), 16.8 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₇O₅⁺ [M+H]⁺ 487.3423, found 487.3426.

(R)-Methyl 4-((3S,5R,10S,13R,14R,17R)-3-acetoxy-4,4,10,13,14-pentamethyl-7,11-dioxo-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-
**Cyclopenta[a]phenanthren-17-ylpentanoate (2.5).** A 100 mL round-bottom flask open to atmosphere was charged with KMnO₄ (2.67 g, 16.92 mmol) and 18-crown-6 (4.47 g, 16.92 mmol), followed by the addition of 33.9 mL of water. A solution of 2.2 (1.6 g, 3.385 mmol) in 33.9 mL of DCM was then added to the resulting suspension by a quick syringe transfer. The flask was sealed with a glass stopper, and the reaction mixture was vigorously stirred at rt for 24 h, at which time the mixture was filtered over a fine sinter funnel and washed with DCM. Layers were separated, and the aqueous layer was extracted with DCM. The organic layer was dried over Na₂SO₄ and the solvent removed in vacuo. The crude product was purified by column chromatography on silica to give 2.5 as a yellow solid (1.32 g, 78%). Mp: 142-145 °C (lit. mp 178-179 °C, lit. mp 141-143 °C). R_f = 0.25 (EA/hex = 20/80). R_f = 0.37 (EA/hex = 25/75). ¹H NMR (400 MHz, CDCl₃, δ): 4.51 (dd, J₁ = 11.2 Hz, J₂ = 5.2 Hz, 1H), 3.65 (s, 3H), 2.88 (ddd, J₁ = 13.6 Hz, J₂ = J₃ = 3.6 Hz, 1H), 2.75 (d, J = 16 Hz, 1H), 2.58 (d, J = 16 Hz, 1H), 2.04 (s, 3H), 1.31 (s, 3H), 1.15 (s, 3H), 0.94 (s, 3H), 0.88 (s, 3H), 0.87 (d, J = 5.2 Hz, 3H), 0.78 (s, 3H). ¹³C NMR data was found to match lit. data. HRMS (ESI) m/z calcd for C₃₀H₄₅O₆⁺ [M+H]⁺ 501.32107, found 501.32111.

**Oxidation of 2.2 with RuO₄. Preparation of diketone (2.6)**

In a 200 mL single-neck round-bottom flask, RuCl₃ (87 mg, 0.42 mmol) was added in one portion to a solution of NaIΟ₄ (1.89 g, 8.82 mmol) in 63 mL of H₂O, and the resulting suspension was stirred open to atmosphere for 15 min, followed by the addition of 42 mL of acetonitrile. The solution of starting material 2.2 (1 g, 2.1 mmol) in 42 mL of CCl₄ was then added dropwise to the reaction mixture by a syringe-pump. The flask was sealed with a glass stopper and the resulting biphasic mixture was vigorously stirred for 1
hour, at which time 5 mL of ethanol were added to the solution. The layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried over Na$_2$SO$_4$, concentrated under vacuum and the crude mixture of products was further separated by careful column chromatography on silica to yield $\alpha,\beta$-unsaturated ketone 2.4 as a white solid (48 mg, 5%), $R_f = 0.31$ (EA/hex = 25/75), $\alpha,\beta$-unsaturated ketone 2.3 as a white solid (140 mg, 14%), $R_f = 0.28$ (EA/hex = 25/75), and diketone 2.6 as a white solid (480 mg, 45%), $R_f = 0.25$ (EA/hex = 25/75).

(R)-Methyl 4-((1R,3aR,6aS,8S,10aS,13aR)-8-acetoxy-3a,7,7,10a,13a-pentamethyl-4,11-dioxohexadecahydro-1H-benzo[a]cyclopenta[f][10]annulen-1-yl)pentanoate (2.6): Mp: 180-183 °C (lit. $^3$ mp 175-177 °C). $^1$H NMR (400 MHz, CDCl$_3$, $\delta$): 4.54 (dd, $J_1 = 11.6$ Hz, $J_2 = 4.4$ Hz, 1H), 3.64 (s, 3H), 2.02 (s, 3H), 1.24 (s, 3H), 1.16 (s, 3H), 0.93 (d, $J = 6.4$ Hz, 3H), 0.87 (s, 3H), 0.87 (s, 3H), 0.80 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 217.1, 215.8, 174.5, 170.7, 79.8, 61.7, 54.6, 51.9, 51.6, 51.5, 50.9, 40.8, 39.5, 37.3, 35.0, 32.8, 31.51, 31.48, 31.1, 28.0, 25.8, 24.6, 23.3, 21.3, 20.6, 19.8, 19.3, 16.8, 16.7, 15.8. HRMS (ESI) $m/z$ calcd for C$_30$H$_{48}$O$_6$Na$^+$ [M+Na]$^+$ 527.33431, found: 527.33436.

Oxidation of 2.3 with RuO$_4$. Preparation of triketone (2.7)

In a 100 mL single-neck round-bottom flask, RuCl$_3$ (30 mg, 0.144 mmol) was added in one portion to a solution of NaIO$_4$ (646 mg, 3.02 mmol) in 22 mL of H$_2$O, and the resulting suspension was stirred open to atmosphere for 15 min, followed by the addition of 14.5 mL of acetonitrile. The solution of 2.3 (350 mg, 0.719 mmol) in 14.5 mL of CCl$_4$ was then added dropwise to the reaction mixture by a syringe-pump. The flask was sealed with a glass stopper and the resulting biphasic mixture was vigorously stirred for 6 hours,
at which time 3 mL of ethanol was added to the solution. The layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried over Na₂SO₄, concentrated under vacuum and the crude mixture of products was further separated by column chromatography on silica to yield tetraketone 2.8 as a yellow solid (24 mg, 6%), Rₜ = 0.39 (EA/hex = 25/75), triketone 2.7 as a yellow oil (103 mg, 28%), and aldol adduct 2.9 as a white solid (238 mg, 64%).

(R)-Methyl 4-((1R,3aR,6aS,8S,10aS,13aR)-8-acetoxy-3a,7,7,10a,13a-pentamethyl-4,5,11-trioxohexadecahydro-1H-benzo[a]cyclopenta[f][10]annulen-1-yl)pentanoate (2.7). Rₜ = 0.26 (EA/hex = 25/75). ¹H NMR (400 MHz, CDCl₃, δ): 4.66 (dd, J₁ = 11.6 Hz, J₂ = 4.4 Hz, 1H), 3.67 (s, 3H), 2.81 (dd, J₁ = 16.4 Hz, J₂ = 7.6 Hz, 1H), 2.06 (s, 3H), 1.37 (s, 3H), 1.30 (s, 3H), 0.98 (s, 3H), 0.96 (d, J = 6.8 Hz, 3H), 0.90 (s, 3H), 0.79 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 214.0, 209.2, 203.2, 174.4, 170.7, 79.4, 59.0, 53.4, 53.1, 52.2, 51.7, 50.4, 39.2, 36.2, 35.5, 34.3, 33.8, 33.2, 31.7, 30.8, 28.0, 26.1, 25.1, 23.4, 21.3, 20.3, 19.4, 17.7, 17.3, 16.8. HRMS (ESI) m/z calcd for C₃₀H₄₇O₇Na⁺ [M+H]⁺ 519.33163, found 519.33173.

Oxidation of 2.5 with RuO₄. Preparation of tetraketone (2.8)

(R)-Methyl-4-((1R,3aR,6aS,8S,10aS,13aR)-8-acetoxy-3a,7,7,10a,13a-pentamethyl-4,5,11,12-tetraoxohexadecahydro-1H-benzo[a]cyclopenta[f][10]annulen-1-yl)pentanoate (2.8). Using modified literature protocol,³⁹ RuCl₃ (62 mg, 0.299 mmol) was added in one portion to the solution of NaIO₄ (539 mg, 2.52 mmol) in 18 mL of water, and the resulting suspension was stirred open to atmosphere for 15 min, followed by the addition of 12 mL of acetonitrile. The solution of 2.5 (300 mg, 0.599 mmol) in 12 mL of CCl₄ was then added to the reaction mixture by a
quick syringe transfer. The flask was sealed with a glass stopper, and the resulting biphasic mixture was vigorously stirred at rt for 15 h, at which time the reaction was interrupted by the addition of 5 mL of ethanol. The layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried over Na₂SO₄ and the solvent removed in vacuo. The crude product was purified by column chromatography on silica to give 2.8 as a yellow solid (278 mg, 87%). Mp: 140-143 °C. R_f = 0.39 (EA/hex = 25/75). ¹H NMR (600 MHz, toluene-d₈, 273.15 K) major conformer, δ: 4.82 (dd, J₁ = 11.4 Hz, J₂ = 4.2 Hz, 1H), 3.54 (d, J = 13.2 Hz, 1H), 3.42 (s, 3H), 3.21 (dd, J₁ = J₂ = 3.6 Hz, 1H), 2.69 (ddd, J₁ = J₂ = 12.6 Hz, J₃ = 6.6 Hz, 1H), 1.67 (s, 3H), 1.34 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.75 (s, 3H), 0.71 (d, J = 6.6 Hz, 3H), 0.64 (s, 3H); minor conformer, δ: 4.60 (dd, J₁ = 11.4 Hz, J₂ = 3.6 Hz, 1H), 3.48 (d, J = 13.8 Hz, 1H), 3.40 (s, 3H), 3.03 (dd, J₁ = 15.6 Hz, J₂ = 9.6 Hz, 1H), 1.65 (s, 3H), 1.42 (s, 3H), 1.30 (s, 3H), 0.87 (s, 3H), 0.77 (d, J = 6.6 Hz, 3H), 0.63 (s, 3H). ¹³C NMR (150 MHz, toluene-d₈, 273.15 K) major conformer, δ: 208.7, 206.1, 205.1, 203.2, 173.7, 169.8, 79.6, 62.3, 55.1, 53.1, 51.5, 49.7, 48.2, 44.3, 38.9, 37.6, 36.3, 33.8, 31.7, 31.61, 31.59, 30.9, 28.6, 24.3, 23.9, 20.3, 20.2, 19.6, 17.9, 16.5; minor conformer, δ: 207.3, 207.1, 202.6, 201.3, 173.3, 169.2, 78.7, 60.6, 52.3, 50.98, 50.0, 49.4, 46.9, 41.4, 38.7, 35.6, 35.2, 34.8, 34.5, 30.7, 27.3, 24.4, 22.7, 19.64, 18.9, 17.8, 17.0, 16.2. HRMS (ESI) m/z calcd for C₃₀H₄₄O₈Na⁺ [M+Na]⁺ 555.29284, found 555.29296.

2.8.3 Oxidation of bryonolic acid and its derivatives

Preparation of bryonolic acid

2R,4aS,6aS,8aR,10S,12aS,14aS,14bR)-10-Hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,13,14,14a,14b-icosahydropicene-
2-carboxylic acid. Bryonolic acid was isolated in gram-quantities from the sprouts of “Spineless beauty” zucchini squash by previously published method\textsuperscript{35}. mp 274-278 °C (lit\textsuperscript{35}. 274-278 °C).

**Preparation of bryonolic acid methyl ester (2.10)**

(2R,4aS,6aS,8aR,10S,12aS,14aS,14bR)-Methyl 10-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12a,13,14,14a,14b-icosahydropicene-2-carboxylate (2.10). In a round-bottom flask open to atmosphere, bryonolic acid (3 g, 6.57 mmol) was dissolved in 65 mL of freshly distilled THF. Freshly prepared diazomethane in diethyl ether (15 mmol) was added dropwise to the resulting solution by a pipette and the reaction mixture was stirred until full conversion detected by TLC (approximately 5 min). The reaction was quenched by dropwise addition of glacial acetic acid until the yellow color of the solution disappeared. The reaction mixture was concentrated under vacuum and the crude product was purified by column chromatography on silica to give 2.10 as a white solid (2.72 g, 88%). Mp 142-144 °C. \( R_f = 0.5 \) (EA/hex, 2/3). \(^1\)H NMR (400 MHz, CDCl\textsubscript{3}, \( \delta \)): 3.61 (s, 3H), 3.23 (dd, \( J_1 = 12 \) Hz, \( J_2 = 4 \) Hz, 1H), 2.37 (d, \( J = 12 \) Hz, 1H), 2.19 (m, 1H), 2.1 (s, 3H), 1.02 (s, 3H), 0.99 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.79 (s, 3H), 0.74 (s, 3H). \(^13\)C NMR (150 MHz, CDCl\textsubscript{3}, \( \delta \)): 179.4, 134.1, 133.9, 79.0, 51.6, 50.5, 44.8, 41.9, 40.5, 38.9, 37.6, 37.2, 37.1, 35.1, 34.5, 33.0, 31.3, 31.0, 30.9, 30.4, 30.0, 28.1, 27.9, 27.7, 25.1, 22.1, 20.8, 20.0, 19.3, 17.2, 15.7. HRMS (EI) \( m/z \) calcd for C\textsubscript{31}H\textsubscript{50}O\textsubscript{3}\textsuperscript{+} [M\textsuperscript{+}] 470.37600, found: 470.37553.

**Preparation of bryonolic acid methyl ester acetate (2.11)**

(2R,4aS,6aS,8aR,10S,12aS,14aS,14bR)-Methyl 10-acetoxy-2,4a,6a,9,9,12a,14a-heptamethyl-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12a,13,14,14a,14b-icosahydropicene-
**2-carboxylate (2.11):** In a round-bottom flask, bryonolic acid methyl ester 2.10 (2.35 g, 5 mmol) was dissolved in 10 mL of dry pyridine. Acetic anhydride (1.02 g, 10 mmol) was added to the resulting solution by a quick syringe transfer. The reaction mixture was stirred at 50 °C for 24 h, after which time pyridine was removed at reduced pressure and the crude mixture was taken into ethyl acetate. The organic layer was washed successively with dilute HCl, water and saturated solution of sodium bicarbonate. It was subsequently dried with Na2SO4 and evaporated in vacuo. The crude product was purified by column chromatography to give 2.11 as a white solid (2.16 g, 84%). Mp 164-167 °C. Rf = 0.62 (EA/hex = 3/7). 1H NMR (400 MHz, CDCl3, δ): 4.47 (dd, J1 = 12 Hz, J2 = 4 Hz, 1H), 3.62 (s, 3H), 2.37 (d, J = 16 Hz, 1H), 2.19 (d, J = 12 Hz, 1H), 2.05 (s, 3H), 1.17 (s, 3H), 1.02 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.87 (s, 3H), 0.86 (s, 3H), 0.73 (s, 3H). 13C NMR (100 MHz, CDCl3, δ): 179.3, 171.1, 134.1, 134.0, 81.0, 51.7, 50.7, 44.9, 41.9, 40.6, 37.8, 37.5, 37.3, 37.1, 34.8, 34.5, 33.0, 31.3, 31.0, 30.9, 30.4, 30.0, 28.1, 27.5, 25.1, 24.3, 22.2, 21.5, 20.8, 20.1, 19.2, 17.1, 16.8. HRMS (EI) m/z calcd for C33H52O4+ [M]+ 512.38656, found: 512.38532.

**Oxidation of 2.11 with RuO4. Preparation of products (2.12, 2.14 and 2.15)**

In a 200 mL single-neck round-bottom flask, RuCl3 (81 mg, 0.39 mmol) was added in one portion to the solution of NaIO4 (2.09 g, 9.75 mmol) in 59 mL of H2O, and the resulting suspension was stirred open to atmosphere for 15 min, followed by the addition of 39 mL of acetonitrile. The solution of 2.11 (1 g, 1.95 mmol) in 39 mL of CCl4 was then added dropwise to the reaction mixture by a syringe-pump. The flask was sealed with a glass stopper and the resulting biphasic mixture was vigorously stirred for 24 h, at which time 10 mL of ethanol was added to the solution, layers were separated and the
aqueous layer was extracted with DCM. The organic layer was dried with Na$_2$SO$_4$, concentrated under vacuum and the products further separated by chromatography. Silica column chromatography yielded an inseparable mixture (351 mg) of 2.12 and 2.15, R$_f$ = 0.45 (EA/hex = 25/75); and pure 2.14 as yellow solid (416 mg, 38%). Mp 168-171 °C. R$_f$ = 0.38 (EA/hex = 25/75). 2.12 and 2.15 were further separated by semi-prep HPLC (Agilent C18 column 21.2×250 mm, isocratic elution CH$_3$CN/H$_2$O = 9/1, flow rate 5 mL/min) to yield 2.15 as a yellow oil (96 mg, 9%), R$_t$ = 52 min; and 2.12 as white foam (231 mg, 22%). R$_t$ = 66 min.

(2R,4aS,6aS,9aS,11S,13aS,16aS,16bR)-Methyl 11-acetoxy-2,4a,6a,10,10,13a,16a-heptamethyl-7,14-dioxodocosahydrobenzo[6,7]cyclodeca[1,2-a]naphthalene-2-carboxylate (2.12). $^1$H NMR (400 MHz, CDCl$_3$, δ): 4.61 (m, 1H), 3.67 (s, 3H), 2.65-2.57 (2H), 2.39 (d, J = 16 Hz, 1H), 2.22 (d, J = 16 Hz, 1H), 2.08 (s, 3H), 1.174 (s, 3H), 1.170 (s, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 1.04 (s, 3H), 0.94 (s, 3H), 0.87 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$, δ): 218.1, 216.6, 179.0, 171.1, 80.2, 54.1, 52.2, 52.0, 45.52, 45.51, 44.2, 40.8, 38.7, 36.4, 36.3, 35.2, 34.7, 32.9, 31.7, 31.6, 31.5, 29.83, 29.79, 29.2, 28.5, 28.4, 28.1, 23.5, 21.4, 19.3, 17.4, 16.4, 16.0. HRMS (EI) m/z calcd for C$_{33}$H$_{52}$O$_6$ $^+$ [M]$^+$ 544.37639, found: 544.37685.

(2R,4aS,6aS,9aS,11S,13aS,16aS,16bR)-Methyl 11-acetoxy-2,4a,6a,10,10,13a,16a-heptamethyl-7,8,14-trioxodocosahydrobenzo[6,7]cyclodeca[1,2-a]naphthalene-2-carboxylate (2.14). $^1$H NMR (400 MHz, CDCl$_3$, δ): 4.67 (dd, J$_1$ = 12 Hz, J$_2$ = 4 Hz, 1H), 3.67 (s, 3H), 2.78 (dd, J$_1$ = 20 Hz, J$_2$ = 8 Hz, 1H), 2.71 (dd, J$_1$ = 16 Hz, J$_2$ = 4 Hz, 1H), 2.32 (d, J = 16 Hz, 1H), 2.27 (dd, J$_1$ = 8 Hz, J$_2$ = 4 Hz, 1H), 2.23 (d, J = 12 Hz, 1H), 2.06 (s, 3H), 1.79 (dd, J$_1$ = 16 Hz, J$_2$ = 4 Hz, 1H), 1.73 (dd, J$_1$ = 16 Hz, J$_2$ = 8 Hz, 1H), 1.54
(d, J = 8 Hz, 1H), 1.25 (s, 3H), 1.18 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.91 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$, δ): 214.1, 212.6, 203.1, 179.2, 171.0, 79.6, 53.1, 52.0, 50.7, 46.3, 44.4, 43.5, 40.8, 39.3, 39.2, 35.4, 33.6, 33.0, 32.3, 31.9, 31.8, 31.7, 31.2, 30.9, 29.6, 28.5, 27.5, 23.4, 21.4, 17.6, 16.9, 16.6, 16.1. HRMS (ESI) m/z calcd for C$_{33}$H$_{50}$O$_7$Na$^+$ [M+Na]$^+$ 581.3454, found: 581.3465.

(2R,4aS,6aS,8aR,10S,12aS,14aS,14bR)-Methyl 10-acetoxy-2,4a,6a,9,9,12a,14a-heptamethyl-7,13-dioxo-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,13,14,14a,14b-icosahydricene-2-carboxylate (2.15). $^1$H NMR (400 MHz, CDCl$_3$, δ): 4.56 (m, 1H), 3.66 (s, 3H), 2.58 (ddd, $J_1$ = 12 Hz, $J_2$ = $J_3$ = 4 Hz, 1H), 2.51-2.47 (3H), 2.07 (s, 3H), 1.93 (ddd, $J_1$ = $J_2$ = 12 Hz, $J_3$ = 4 Hz, 1H), 1.28 (s, 3H), 1.24 (s, 3H), 1.20 (s, 3H), 1.06 (s, 3H), 0.989 (s, 3H), 0.987 (s, 3H), 0.96 (s, 3H), 0.89 (s, 3H). $^{13}$C NMR (100 MHz, CDCl$_3$, δ): 200.0, 199.8, 179.1, 171.1, 154.9, 152.7, 79.6, 52.1, 52.0, 48.1, 44.2, 42.1, 40.5, 39.1, 38.1, 37.6, 36.8, 36.3, 34.1, 33.6, 32.5, 31.0, 30.9, 30.6, 29.8, 29.6, 27.5, 25.3, 23.9, 21.4, 21.0, 20.6, 17.9, 16.1. HRMS (ESI) m/z calcd for C$_{33}$H$_{48}$O$_6$Na$^+$ [M+Na]$^+$ 563.3348, found: 563.3352.

**Oxidation of 2.15 with RuO$_4$. Preparation of products (2.16-2.18)**

A round-bottom flask open to atmosphere was charged with RuCl$_3$ (161 mg, 0.777 mmol) and NaIO$_4$ (1.66 g, 7.77 mmol), followed by the addition of H$_2$O (11.7 mL) and CH$_3$CN (7.8 mL). To the resulting dark suspension, a solution of 2.15 (420 mg, 0.777 mmol) in CCl$_4$ (7.8 mL) was added by a quick syringe transfer. The flask was sealed with a glass stopper and the resulting biphasic mixture was vigorously stirred for 7 days, at which time 5 mL of ethanol was added to the solution. Subsequently, layers were separated and aqueous layer was extracted with DCM. The organic layer was dried with
Na\textsubscript{2}SO\textsubscript{4}, concentrated under vacuum and the crude mixture of products was separated by flash column chromatography with gradient elution to give \textbf{2.16} as a yellow solid (75 mg, 18%), Mp 186-188 °C, \(R_f = 0.76\) (EA/hex = 1/1); unreacted \textbf{2.15} (18 mg), \(R_f = 0.76\) (EA/hex = 1/1); \textbf{2.39} as a transparent oil (26 mg, 6%), \(R_f = 0.56\) (EA/hex = 1/1); \textbf{2.17} as a yellow solid (92 mg, 21%). Mp 93-96 °C. \(R_f = 0.1\) (EA/hex = 1/1).

\textbf{(2R,4aS,6aS,9aS,11S,13aS,16aS,16bR)-Methyl 11-acetoxy-2,4a,6a,10,10,13a,16a-heptamethyl-7,8,14,15-tetraoxodocosahydrobenzo[6,7]cyclodeca[1,2-a]naphthalene-2-carboxylate (2.16).} \(^1\)H NMR (600 MHz, CDCl\textsubscript{3}, \(\delta\)): 4.74 (dd, \(J_1 =12\) Hz, \(J_2 = 3.6\) Hz, 1H), 3.74 (s, 3H), 3.51 (d, \(J = 13.2\) Hz, 1H), 3.28 (dd, \(J_1 =14.4\) Hz, \(J_2 = 4.2\) Hz, 1H), 2.76 (ddd, \(J_1 = J_2 = 13.8\) Hz, \(J_3 = 3.6\) Hz, 1H), 2.59 (dd, \(J_1 = 10.2\) Hz, \(J_2 = 3.6\) Hz, 1H), 2.34 (d, \(J = 16.2\) Hz, 1H), 2.28 (d, \(J = 14.4\) Hz, 1H), 2.07 (s, 3H), 1.48 (s, 3H), 1.26 (s, 3H), 1.22 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.96 (s, 3H). \(^{13}\)C NMR (150 MHz, CDCl\textsubscript{3}, \(\delta\)): 207.4 (CO), 205.9 (CO), 203.6 (CO), 201.1 (CO), 178.7 (CO), 170.6 (CO), 79.1 (CH), 53.3 (C), 52.1 (CH\textsubscript{3}), 51.1 (C), 45.8 (CH), 44.0 (C), 42.5 (CH\textsubscript{2}), 41.6 (CH), 40.6 (C), 38.7 (C), 38.5 (CH\textsubscript{2}), 35.1 (CH\textsubscript{2}), 34.7 (CH\textsubscript{2}), 33.7 (CH\textsubscript{2}), 32.7 (CH\textsubscript{3}), 32.0 (C), 31.6 (CH\textsubscript{2}), 31.4 (CH\textsubscript{3}), 29.69 (CH\textsubscript{2}), 29.67 (CH\textsubscript{2}), 27.7 (CH\textsubscript{3}), 23.4 (CH\textsubscript{2}), 21.4 (CH\textsubscript{3}), 18.0 (CH\textsubscript{3}), 17.4 (CH\textsubscript{3}), 17.2 (CH\textsubscript{3}), 16.5 (CH\textsubscript{3}). HRMS (ESI) \(m/z\) calcd for C\textsubscript{33}H\textsubscript{48}O\textsubscript{8}Na\(^+\) [M+Na\(^+\)] 595.3247, found: 595.3256.

\textbf{(1S,2S,4aR,7R,8aR)-1-(3-((1S,2S,4S)-4-Acetoxy-2-(carboxymethyl)-1,3,3-trimethylcyclohexyl)-2,3-dioxopropyl)-7-(methoxycarbonyl)-1,2,4a,7-tetramethyldecahydronaphthalene-2-carboxylic acid (2.17).} \(^1\)H NMR (600 MHz, CDCl\textsubscript{3}, \(\delta\)): 9.65 (br. s), 4.73 (dd, \(J_1 =11.4\) Hz, \(J_2 = 4.2\) Hz, 1H), 3.62 (s, 3H), 3.52 (d, \(J = 19.8\) Hz, 1H), 2.80 (dd, \(J_1 = J_2 = 4.8\) Hz, 1H), 2.64 (d, \(J = 19.8\) Hz, 1H), 2.47 (dd, \(J_1 = 11.4\) Hz, 1H), 2.16 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.85 (s, 3H), 1.81 (s, 3H), 1.78 (s, 3H), 1.73 (s, 3H), 1.70 (s, 3H), 1.50 (s, 3H), 1.49 (s, 3H), 1.44 (s, 3H), 1.42 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H), 1.36 (s, 3H), 1.34 (s, 3H), 1.32 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H), 1.27 (s, 3H), 1.26 (s, 3H), 1.24 (s, 3H), 1.22 (s, 3H), 1.21 (s, 3H), 1.19 (s, 3H), 1.18 (s, 3H), 1.16 (s, 3H), 1.14 (s, 3H), 1.13 (s, 3H), 1.12 (s, 3H), 1.11 (s, 3H), 1.10 (s, 3H), 1.09 (s, 3H), 1.08 (s, 3H), 1.07 (s, 3H), 1.06 (s, 3H), 1.05 (s, 3H), 1.04 (s, 3H), 1.03 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 1.00 (s, 3H), 0.99 (s, 3H).
=17.4 Hz, \( J_2 = 5.4 \) Hz, 1H), 2.18 (dd, \( J_1 = 17.4 \) Hz, \( J_2 = 5.4 \) Hz, 1H), 2.07 (s, 3H), 1.32 (s, 3H), 1.23 (s, 3H), 1.20 (s, 3H), 1.13 (s, 3H), 0.97 (s, 3H), 0.94 (s, 3H), 0.94 (s, 3H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\), \( \delta \)): 204.6 (CO), 199.7 (CO), 183.9 (CO), 179.5 (CO), 179.5 (CO), 170.8 (CO), 79.1 (CH), 51.8 (CH\(_3\)), 50.4 (C), 47.2 (C, br.), 45.8 (CH\(_2\), br.), 43.6 (CH), 42.9 (C, br.), 42.6 (C, br.), 42.4 (C), 40.4 (CH, br.), 38.9 (C), 37.9 (CH\(_2\), br.), 33.7 (C), 32.9 (CH\(_2\), br.), 32.4 (CH\(_2\)), 32.2 (CH\(_2\), br.), 30.5 (CH\(_3\)), 29.8 (CH\(_2\), br.), 29.4 (CH\(_2\)), 28.6 (CH\(_3\), br.), 27.7 (CH\(_3\)), 24.5 (CH\(_3\), br.), 23.1 (CH\(_2\)), 22.7 (CH\(_3\), br.), 21.4 (CH\(_3\)), 20.5 (CH\(_3\), br.), 17.6 (CH\(_3\), br.), 17.3 (CH\(_3\), br.). HRMS (ESI) \( m/z \) calcd for C\(_{33}\)H\(_{50}\)O\(_{10}\)Na\(^+\) \([\text{M+Na}]^+\) 629.3301, found: 629.3299.

(1S,2S,4aR,7R,8aR)-Dimethyl 1-(3-((1S,2S,4S)-4-acetoxy-2-(2-methoxy-2-oxoethyl)-1,3,3-trimethylcyclohexyl)-2,3-dioxopropyl)-1,2,4a,7-tetramethyldecahydronaphthalene-2,7-dicarboxylate (2.18): Solution of 2.17 (30 mg, 0.05 mmol) in diethyl ether (1 mL) in a round-bottomed flask was cooled down to \( 0 \) °C, followed by a dropwise addition of solution of diazomethane in ether (about 2 mL total), and the reaction mixture was stirred at \( 0 \) °C until full conversion of the starting material was detected by TLC (approximately 15 min). The excess of diazomethane was quenched by a drop of glacial acetic acid, the solvent was removed in vacuo and the crude product was further purified by column chromatography on silica to give trimethyl ester 2.18 as a white solid (29.5 mg, 93%). Mp 114-117 °C. \( R_f = 0.7 \) (EA/hex = 1/1). \(^1\)H NMR (400 MHz, CDCl\(_3\), \( \delta \)): 4.69 (dd, \( J_1 = 11.6 \) Hz, \( J_2 = 4 \) Hz, 1H), 3.66 (s, 3H), 3.63 (s, 3H), 3.62 (s, 3H), 3.51 (d, \( J = 20 \) Hz, 1H), 2.77 (dd, \( J_1 = 6.4 \) Hz, \( J_2 = 5.2 \) Hz, 1H), 2.72 (d, \( J = 20 \) Hz, 1H), 2.44-2.31 (3H), 2.06 (s, 3H), 1.33 (s, 3H), 1.26 (s, 3H), 1.20 (s, 3H), 1.08 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\), \( \delta \)): 205.5,
200.9, 179.5, 177.9, 174.0, 170.7, 79.1, 52.0, 46.9, 46.1, 44.2, 43.0, 42.6, 40.1, 38.8, 38.2, 33.7, 33.6 (br.), 32.4, 31.9, 30.7, 30.0, 29.5, 28.4 (br.), 27.7, 24.4, 23.1, 23.0, 21.4, 19.9 (br.), 17.31 (br.), 17.28 (br.). HRMS (EI) m/z calcd for C\textsubscript{35}H\textsubscript{54}O\textsubscript{10} \([M]^+\) 634.37170, found: 634.36992.

2.8.4 Aldol addition of 2.6 under common conditions. Preparation of (2.19-2.22)

Table 2.4.1 (Entry 1). Pyrrolidinone (7 mg, 0.099 mmol, 8.1 μL) was added dropwise to the solution of diketone 2.6 (100 mg, 0.198 mmol) in DCM (2 mL). The flask was then sealed with a glass stopper and the resulting solution was stirred vigorously at rt for 48 h, at which time water was added to the reaction mixture. Layers were separated and the aqueous layer was extracted with DCM. The organic layer was washed with brine and subsequently dried over Na\textsubscript{2}SO\textsubscript{4}. The solvent was removed in vacuo and the crude product was purified by column chromatography on silica to give 2.19 as a white solid (86 mg, 86%).

General procedure for the reaction of 2.6 with TFA

A specified amount of TFA was added dropwise to the solution of the diketone 2.6 (1 equiv) in DCM (0.05M in starting material). A flask was then sealed with the glass stopper and the resulting solution was stirred vigorously at rt for a specified amount of time, until the starting material was consumed or decomposition was observed, as determined by TLC. After removal of the solvent in vacuo, products 2.19, 2.22, 2.21, and unreacted diketone 2.6 were isolated by column chromatography on silica with the mixture of ethyl acetate and hexanes (20/80) as eluting solvent.

Table 2.4.1 (Entry 2). Following the general procedure for the reaction of 2.6 with TFA, the use of diketone 2.6 (100 mg, 0.198 mmol), TFA (4.5 mg, 0.0396 mmol, 3 μL)
and DCM (4 mL) yielded 2.21 (4.2 mg, 7% brsm), unreacted 2.6 (35.4 mg, 65% conversion), and 2.19 (47.1 mg, 74% brsm).

Table 2.4.1 (Entry 3). Following the general procedure for the reaction of 2.6 with TFA, the use of diketone 2.6 (100 mg, 0.198 mmol), TFA (11.4 mg, 0.0991 mmol, 7.6 μL) and DCM (4 mL) yielded 2.22 (8.4 mg, 10% brsm), 2.21 (3.4 mg, 4% brsm), unreacted 2.6 (9.3 mg, 91% conversion), and 2.19 (64.7 mg, 71% brsm).

Table 2.4.1 (Entry 4). Following the general procedure for the reaction of 2.6 with TFA, the use of diketone 2.6 (100 mg, 0.198 mmol), TFA (112.9 mg, 0.99 mmol, 76 μL) and DCM (4 mL) yielded 2.22 (46.1 mg, 48% brsm) and 2.21 (31.2 mg, 32% brsm).

Table 2.4.1 (Entry 5). A round-bottom flask open to atmosphere was charged with neutral alumina (2 g, 19.8 mmol), followed by the addition of DCM (1 mL). A solution of 2.6 (100 mg, 0.198 mmol) in DCM (1 mL) was then added to the resulting suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 48 hours, at which time the mixture was filtered over a fine sinter funnel and washed successively with ethyl acetate. After the removal of the solvent in vacuo, careful column chromatography on silica yielded 2.21 as a white solid (10.8 mg, 11%), R_f = 0.32 (EA/hex = 25/75), 2.20 as a white solid (22.9 mg, 23%), R_f = 0.15 (EA/hex = 25/75), and 2.19 as a white solid (35.3 mg, 35%), R_f = 0.14 (EA/hex = 25/75).

Table 2.4.1 (Entry 6). A round-bottom flask open to atmosphere was charged with basic alumina (2 g, 19.8 mmol), followed by the addition of DCM (1 mL). A solution of 2.6 (100 mg, 0.198 mmol) in DCM (1 mL) was then added to the resulting suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 48 hours, at which time the mixture was filtered over a fine sinter funnel and
washed successively with ethyl acetate. After the removal of the solvent in vacuo, careful column chromatography on silica yielded \textbf{2.21} as a white solid (8.5 mg, 9\%), \(R_f = 0.32\) (EA/hex = 25/75), \textbf{2.20} as a white solid (29.8 mg, 30\%), \(R_f = 0.15\) (EA/hex = 25/75), and \textbf{2.19} as a white solid (10.7 mg, 11\%), \(R_f = 0.14\) (EA/hex = 25/75).

Table 2.4.1 (Entry 7). The solution of diisopropyl amine (11 mg, 0.109 mmol, 15 \(\mu\)L) in dry THF (3 mL) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 \(^\circ\)C, followed by a dropwise addition of n-butyl lithium (2.5M in hexanes, 43.6 \(\mu\)L). The reaction mixture was warmed up to 0 \(^\circ\)C, stirred at this temperature for 5 min and subsequently cooled to -78 \(^\circ\)C. A solution of \textbf{2.6} (50 mg, 0.0991 mmol) in dry THF (2 mL) was then added dropwise to the solution of LDA by a syringe at -78 \(^\circ\)C. The cooling bath was then removed and the reaction mixture was allowed to warm up to rt and stirred at rt for the total of 15 hours. After removing solvent in vacuo the residue was taken up in DCM, washed with water and brine. The organic layer was dried over Na\(_2\)SO\(_4\). Evaporation of the solvent in vacuo gave crude product mixtures that were further separated by careful column chromatography on silica to give \textbf{2.21} as a white solid (3.1 mg, 6\%), \(R_f = 0.32\) (EA/hex = 25/75), \textbf{2.20} as a white solid (3.2 mg, 6\%), \(R_f = 0.15\) (EA/hex = 25/75), and \textbf{2.19} as a white solid (19.8 mg, 40\%), \(R_f = 0.14\) (EA/hex = 25/75).

Table 2.4.1 (Entry 8). Dry DCM (0.5 mL) was added to a flame-dried (under vacuum) round-bottomed flask. The flask was cooled to -78 \(^\circ\)C and TiCl\(_4\) (22.6 mg, 0.1189 mmol, 13 \(\mu\)L) was added at that temperature by a quick syringe transfer, followed by a dropwise addition of diisopropyl ethyl amine (17.9 mg, 0.139 mmol, 24 \(\mu\)L). A solution of diketone \textbf{2.6} (50 mg, 0.0991 mmol) in dry DCM (0.5 mL) was added to the reaction mixture at -78 \(^\circ\)C, and the reaction mixture was allowed to warm to rt and stirred at rt overnight, at
which time water (1 mL) was added to the solution, layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried with Na₂SO₄, concentrated under vacuum and the crude product was purified by column chromatography on silica to give 2.21 (28 mg, 58%) as a white solid.

(R)-Methyl 4-((1R,3aR,4aR,5aS,7S,9aS,9bR,11aR)-7-acetoxy-9b-hydroxy-3a,6,6,9a,11a-pentamethyl-4-oxohexadecahydro-1H-benzo[a]cyclopenta[f]azulen-1-yl)pentanoate (2.19). Mp: 206-208 °C. ¹H NMR (600 MHz, CDCl₃, δ): 4.53 (m, 1H), 3.65 (s, 3H), 3.34 (dd, J₁ = J₂ = 9 Hz, 1H), 2.04 (s, 3H), 1.35 (s, 3H), 0.99 (s, 3H), 0.92 (d, J = 6 Hz, 3H), 0.91 (s, 3H), 0.76 (s, 3H), 0.68 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 212.2 (CO), 174.6 (CO), 171.2 (CO), 81.9 (COH), 81.1 (CH), 63.4 (C), 57.6 (CH), 51.7 (CH₃), 51.3 (CH), 49.1 (CH), 48.7 (C), 46.7 (C), 37.1 (C), 35.4 (CH), 31.4 (CH₂), 31.4 (CH₂), 30.6 (CH₃), 29.64 (CH₃), 29.56 (CH₂), 29.4 (CH₂), 28.5 (CH₂), 27.2 (CH₂), 24.6 (CH₂), 23.2 (CH₂), 21.9 (CH₃), 21.4 (CH₃), 19.3 (CH₃), 17.6 (CH₃), 16.8 (CH₃), 16.2 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₈O₆Na⁺ [M+Na]⁺ 527.3348, found: 527.3340.

(R)-Methyl 4-((1R,3aR,4aR,5aS,7S,9aS,9bR,11aR)-7-acetoxy-9b-hydroxy-3a,6,6,9a,11a-pentamethyl-4-oxohexadecahydro-1H-benzo[a]cyclopenta[f]azulen-1-yl)pentanoate (2.20). Mp: 172-174 °C. ¹H NMR (600 MHz, CDCl₃, δ): 4.49 (dd, J₁ = 11.4 Hz, J₂ = 4.2 Hz, 1H), 3.67 (s, 3H), 3.23 (dd, J₁ = 11.4 Hz, J₂ = 5.4 Hz, 1H), 2.05 (s, 3H), 1.30 (s, 3H), 0.99-0.98 (9H), 0.95 (s, 3H), 0.90 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 215.7 (CO), 174.6 (CO), 171.2 (CO), 83.5 (COH), 81.3 (CH), 63.0 (C), 58.6 (CH), 51.7 (CH₃), 51.4 (CH), 50.0 (C), 48.2 (CH), 45.5 (C), 37.3 (C), 35.1 (CH), 34.2 (CH₂), 31.6 (CH₂), 31.1 (CH₂), 29.7 (CH₂), 29.03 (CH₃), 28.95 (CH₂), 28.0 (CH₂), 24.8 (CH₂), 24.6 (CH₂), 22.9 (CH₃), 21.7 (CH₂), 21.3 (CH₃), 20.0 (CH₃), 17.2 (CH₃), 16.45

(R)-Methyl 4-((1R,3aR,5aR,7S,9aS,11aR)-7-acetoxy-3a,6,6,9a,11a-pentamethyl-4-oxo-2,3,3a,4,5,5a,6,7,8,9,9a,10,11,11a-tetradecahydro-1H-benzo[a]cyclopenta[f]azulen-1-yl)pentanoate (2.21). Mp: 154-156 °C (lit. mp 155-158 °C). ¹H NMR (400 MHz, CDCl₃, δ): 4.54 (dd, J₁ = 11.2 Hz, J₂ = 4.8 Hz, 1H), 3.66 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 0.96 (d, J = 6.4 Hz, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.80 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 206.1 (CO), 174.6 (CO), 171.2 (CO), 160.7 (C), 135.8 (C), 80.9 (CH), 62.7 (C), 54.9 (CH), 51.7 (CH₃), 51.6 (CH), 50.4 (C), 45.2 (C), 37.3 (C), 34.6 (CH), 33.7 (CH₂), 31.7 (CH₂), 31.2 (CH₂), 30.9 (CH₂), 30.4 (CH₂), 30.3 (CH₂), 28.7 (CH₃), 25.2 (CH₂), 24.4 (CH₂), 24.1 (CH₃), 22.8 (CH₂), 21.4 (CH₃), 20.1 (CH₃), 17.1 (CH₃), 17.0 (CH₃), 15.0 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₆O₅Na⁺ [M+Na]⁺ 509.32375, found: 509.32372.

(R)-Methyl 4-((1R,3aR,4aS,5aR,7S,9aS,11aR)-7-acetoxy-3a,6,6,9a,11a-pentamethyl-4-oxo-2,3,3a,4,4a,5,5a,6,7,8,9,9a,11,11a-tetradecahydro-1H-benzo[a]cyclopenta[f]azulen-1-yl)pentanoate (2.22): White foam. ¹H NMR (400 MHz, CDCl₃, δ): 5.25 (ddd, J₁ = 9.2 Hz, J₂= J₃=3.5 Hz, 1H), 4.52 (dd, J₁ = 11.5 Hz, J₂ = 4.8 Hz, 1H), 3.94 (m, 1H), 3.66 (s, 3H), 2.05 (s, 3H), 1.30 (d, J = 0.7 Hz, 3H), 0.97 (d, J = 6.7 Hz, 3H), 0.95 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.62 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 210.1 (CO), 174.6 (CO), 171.2 (CO), 150.3 (C), 116.0 (CH), 81.2 (CH), 62.9 (C), 54.0 (CH), 51.7 (CH₃), 50.9 (CH), 50.6 (CH), 49.4 (C), 45.4 (C), 37.5 (C), 34.98 (CH), 34.92 (CH₂), 32.0 (CH₂), 31.6 (CH₂), 31.1 (CH₂), 30.1 (CH₂), 28.9 (CH₃), 25.6 (CH₂), 25.3 (CH₂), 23.0 (CH₃), 22.5 (CH₂), 21.4 (CH₃), 21.4 (CH₃), 19.7 (CH₃), 16.9

Table 2.4.1. Control experiments A, B, C.

**Control A**: TFA (23.4 mg, 0.206 mmol, 16 μL) was added dropwise to the solution of the ketone 2.22 (20 mg, 0.041 mmol) in DCM (0.8 mL). The flask was then sealed with a glass stopper and the resulting solution was stirred vigorously at rt for 48 h. After removal of the solvent in vacuo, column chromatography of the crude mixture on silica yielded 2.21 as a white solid (18.1 mg, 90%), R_f = 0.32 (EA/hex = 25/75).

**Control B**: A round-bottom flask open to atmosphere was charged with neutral alumina (404 mg, 4 mmol), followed by the addition of DCM (0.2 mL). A solution of 2.19 (20 mg, 0.04 mmol) in DCM (0.2 mL) was then added to the resulting suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 48 h, at which time the mixture was filtered over a fine sinter funnel and washed successively with ethyl acetate. After the removal of the solvent in vacuo, careful column chromatography on silica yielded 2.21 as a white solid (3.5 mg, 17%), R_f = 0.32 (EA/hex = 25/75); and unreacted 2.19 as a white solid (15.6 mg, 78% recovery), R_f = 0.14 (EA/hex = 25/75).

**Control C**: A round-bottom flask open to atmosphere was charged with neutral alumina (419 mg, 4.11 mmol), followed by the addition of DCM (0.2 mL). A solution of 2.22 (20 mg, 0.041 mmol) in DCM (0.2 mL) was then added to the resulting suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 45 min, at which time the mixture was filtered over a fine sinter funnel and washed successively with ethyl acetate. After the removal of the solvent in vacuo, careful
column chromatography on silica yielded \textbf{2.21} as a white solid (19 mg, 95%), \( R_f = 0.32 \) (EA/hex = 25/75).

2.8.5 \textbf{Aldol addition of 2.6 under strong Lewis acidic conditions.}

\textbf{Preparation of (2.23-2.26)}

\textit{Table 2.4.2 (Entry 1).} Solution of diketone \textbf{2.6} (100 mg, 0.198 mmol) in dry DCM (1 mL) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 °C, followed by a dropwise addition of BF$_3$·Et$_2$O (30.9 mg, 0.218 mmol, 27 \( \mu \)L) at this temperature. The reaction mixture was allowed to warm up to rt overnight and stirred at this temperature for the total of 48 h, at which time the solvent was removed under vacuum, water (2 mL) was added to the solution, layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried with Na$_2$SO$_4$, concentrated under vacuum and the crude mixture of products was separated by column chromatography on silica to give an inseparable mixture (43 mg), containing \textbf{2.22} (9.9 mg, 10%), \textbf{2.23} (6.4 mg, 7%), \textbf{2.24} (2 mg, 2%), \textbf{2.25} (6.9 mg, 7%) and \textbf{2.26} (14.3 mg, 15%) as determined by \textit{1H} NMR, \( R_f = 0.32 \) (EA/hex = 20/80), and pure \( \alpha,\beta \)-unsaturated ketone \textbf{2.21} (38 mg, 39%), \( R_f = 0.25 \) (EA/hex = 20/80).

\textit{General procedure for reaction of diketone 2.6 with Lewis Acid Catalysts at rt:}

A specified Lewis Acid catalyst (0.2 equiv) was added in one portion to the solution of diketone \textbf{2.6} (1 equiv) in DCM (0.1M in diketone) in a flame-dried round-bottom flask at rt. The flask was sealed with a glass stopper, and the resulting solution was stirred vigorously at rt for a specified amount of time, until the starting material was consumed or decomposition was observed, as determined by TLC. After the solvent was removed in
vacuo, the crude mixture of products was separated by column chromatography on silica with the specified ethyl acetate-hexanes mixtures.

Table 2.4.2 (Entry 2). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at rt, the use of diketone 2.6 (50 mg, 0.0991 mmol), aluminum triflate (9.4 mg, 0.0198 mmol) and DCM (1 mL) for 96 h yielded inseparable mixture (20.2 mg), containing 2.22 (10.7 mg, 30% brsm), 2.23 (3.9 mg, 11% brsm), 2.24 (3.9 mg, 11% brsm) and 2.25 (1.8 mg, 5% brsm), as determined by $^1$H NMR, $R_f = 0.32$ (EA/hex = 20/80), pure α,β-unsaturated ketone 2.21 (5.1 mg, 14% brsm), $R_f = 0.25$ (EA/hex = 20/80), and unreacted diketone 2.6 (12.8 mg, 74% conversion), $R_f = 0.17$ (EA/hex = 20/80).

Table 2.4.2 (Entry 3). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at rt, the use of diketone 2.6 (50 mg, 0.0991 mmol), scandium triflate (10 mg, 0.01982 mmol) and DCM (1 mL) for 48 h yielded inseparable mixture (7.7 mg), containing 2.22 (2.3 mg, 9% brsm), 2.23 (1.8 mg, 7% brsm), 2.24 (1.3 mg, 5% brsm) and 2.25 (1.7 mg, 6% brsm), as determined by $^1$H NMR, $R_f = 0.32$ (EA/hex = 20/80), and pure unreacted diketone 2.6 (22.3 mg, 55% conversion), $R_f = 0.17$ (EA/hex = 20/80).

Table 2.4.2 (Entry 4). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at rt, the use of diketone 2.6 (50 mg, 0.0991 mmol), indium triflate (11 mg, 0.0198 mmol) and DCM (1 mL) for 48 h yielded inseparable mixture (22.3 mg), containing 2.22 (8.1 mg, 17%), 2.23 (3.9 mg, 8%), 2.24 (5.3 mg, 11%), 2.25 (3.1 mg, 7%) and 2.26 (0.8 mg, 2%), as determined by $^1$H NMR, $R_f = 0.32$ (EA/hex = 20/80), and pure α,β-unsaturated ketone 2.21 (8 mg, 17%), $R_f = 0.25$ (EA/hex = 20/80).
General Procedure for reaction of diketone 2.6 with Lewis Acid Catalysts at 50 °C:

A specified Lewis Acid catalyst (0.2 equiv) was added in one portion to the solution of diketone 2.6 (1 equiv) in DCM (0.1M in diketone) in a 5 mL Schlenk tube at rt. The tube was filled with argon and subsequently sealed with a Teflon stopper. The resulting solution was stirred vigorously at 50 °C for a specified amount of time, until the starting material was consumed or decomposition was observed, as determined by TLC. The solvent was removed in vacuo and the crude mixture of products was separated by column chromatography on silica with the specified ethyl acetate-hexanes mixtures.

Table 2.4.2 (Entry 5). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at 50 °C, the use of diketone 2.6 (50 mg, 0.0991 mmol), scandium triflate (10 mg, 0.01982 mmol) and DCM (1 mL) for 72 h yielded inseparable mixture (24.1 mg), containing 2.22 (4.7 mg, 11% brsm), 2.23 (4.9 mg, 11% brsm), 2.24 (4.1 mg, 9% brsm), 2.25 (6.1 mg, 14% brsm) and 2.26 (2.6 mg, 6% brsm), as determined by $^1$H NMR, $R_f = 0.32$ (EA/hex = 20/80), pure $\alpha,\beta$-unsaturated ketone 2.21 (7 mg, 16% brsm), $R_f = 0.25$ (EA/hex = 20/80), and unreacted diketone 2.6 (5 mg, 90% conversion), $R_f = 0.17$ (EA/hex = 20/80).

Table 2.4.2 (Entry 6). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at 50 °C, the use of diketone 2.6 (50 mg, 0.0991 mmol), indium triflate (11 mg, 0.01982 mmol) and DCM (1 mL) for 7 h yielded inseparable mixture (20 mg), containing 2.22 (4.2 mg, 9%), 2.23 (3.5 mg, 7%), 2.24 (5.6 mg, 12%), 2.25 (3.1 mg, 6%) and 2.26 (1.6 mg, 3%), as determined by $^1$H NMR, $R_f = 0.32$ (EA/hex = 20/80), and pure $\alpha,\beta$-unsaturated ketone 2.21 (7 mg, 21%), $R_f = 0.25$ (EA/hex = 20/80).
Table 2.4.2 (Entry 7). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at 50 °C, the use of diketone 2.6 (50 mg, 0.0991 mmol), indium (III) chloride (4.4 mg, 0.01982 mmol) and DCM (1 mL) for 54 h yielded inseparable mixture (19.5 mg), containing 2.6 (1.6 mg, 3%), 2.23 (0.6 mg, 1%), 2.25 (4.6 mg, 10%) and 2.26 (10 mg, 21%), as determined by ¹H NMR, \( R_f = 0.32 \) (EA/hex = 20/80), and pure \( \alpha,\beta \)-unsaturated ketone 2.21 (15.8 mg, 33%), \( R_f = 0.25 \) (EA/hex = 20/80).

Table 2.4.2 (Entry 8). Following the general procedure for the reaction of 2.6 with Lewis Acid catalysts at 50 °C, the use of diketone 2.6 (50 mg, 0.0991 mmol), copper (II) triflate (7.2 mg, 0.01982 mmol) and DCM (1 mL) for 16 h yielded inseparable mixture (20 mg), containing 2.24 (4.6 mg, 10%), 2.25 (4.4 mg, 9%) and 2.26 (8 mg, 17%), as determined by ¹H NMR, \( R_f = 0.32 \) (EA/hex = 20/80), and pure \( \alpha,\beta \)-unsaturated ketone 2.21 (20 mg, 42%), \( R_f = 0.25 \) (EA/hex = 20/80).

(R)-Methyl 4-((1R,3aR,5aS,7S,9aS,10aR,11aR)-7-acetoxy-3a,6,6,9a,11a-pentamethyl-10-oxo-2,3,3a,5,5a,6,7,8,9,9a,10,10a,11,11a-tetradecahydro-1H-benzo[f]cyclopenta[a]azulen-1-yl)pentanoate (2.23). White foam. ¹H NMR (600 MHz, C₆D₆, δ): 5.37 (m, 1H), 4.60 (dd, \( J_1 = 12 \) Hz, \( J_2 = 4.6 \) Hz, 1H), 4.29 (ddd, \( J_1 = J_2 = 8 \) Hz, \( J_1 = 1.6 \) Hz, 1H), 3.40 (s, 3H), 2.64 (dd, \( J_1 = 12 \) Hz, \( J_2 = 9 \) Hz, 1H), 1.71 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H), 0.76 (d, \( J = 6.4 \) Hz, 3H), 0.71 (s, 3H). ¹³C NMR (150 MHz, C₆D₆, δ): 206.7 (CO), 173.8 (CO), 169.6 (CO), 148.5 (C), 120.1 (CH), 79.4 (CH), 61.9 (C), 55.15 (CH), 55.14 (CH), 54.3 (C), 52.3 (C), 51.0 (CH₃), 46.0 (CH), 40.1 (C), 35.3 (CH), 33.9 (CH₂), 33.4 (CH₂), 32.6 (CH₂), 31.5 (CH₂), 30.9 (CH₂), 28.5 (CH₂), 28.1 (CH₃), 24.8 (CH₃), 24.0 (CH₂), 23.7 (CH₂), 20.8 (CH₃), 19.8 (CH₃), 18.0
(R)-Methyl (3aR,3bS,5aS,7S,9aS,10aR,11aR)-7-acetoxy-3b,6,6,9a,11a-pentamethyl-10-oxo-2,3b,4,5,5a,6,7,8,9,9a,10,10a,11,11a-tetradecahydro-1H-benzo[f]cyclopenta[a]azulen-1-yl)pentanoate (2.24). White foam. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 5.13 (dd, $J_1 = 3.6$ Hz, $J_2 = 1.2$ Hz, 1H), 4.46 (m, 1H), 3.79 (dd, $J_1 = 12$ Hz, $J_2 = 4.9$ Hz, 1H), 3.66 (s, 3H), 2.05 (s, 3H), 1.16 (s, 3H), 0.95 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.86 (s, 3H), 0.84 (d, $J = 6.8$ Hz, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 212.6 (CO), 174.8 (CO), 170.3 (C), 115.7 (CH), 80.3 (CH), 60.1 (CH), 56.6 (CH), 54.6 (C), 51.7 (CH$_3$), 49.4 (C), 45.4 (CH), 40.4 (C), 40.3 (C), 39.7 (CH$_2$), 39.5 (CH$_2$), 38.9 (CH$_2$), 32.8 (CH), 31.8 (CH$_2$), 31.4 (CH$_2$), 31.0 (CH$_3$), 30.3 (CH$_2$), 28.0 (CH$_3$), 23.6 (CH$_2$), 23.5 (CH$_2$), 21.4 (CH$_3$), 18.6 (CH$_3$), 18.0 (CH$_3$), 17.1 (CH$_3$), 16.4 (CH$_3$). HRMS (ESI) $m/z$ calcd for C$_{30}$H$_{46}$O$_5$Na$^+$ [M+Na]$^+$ 509.32375, found: 509.32392.

(R)-Methyl 4-((3aR,3bS,5aS,7S,9aS,10aR)-7-acetoxy-3a,3b,6,6,9a-pentamethyl-10-oxo-3a,3b,4,5,5a,6,7,8,9,9a,10,10a,11-tetradecahydro-2H-benzo[f]cyclopenta[a]azulen-1-yl)pentanoate (2.25). White foam. $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 4.47 (dd, $J_1 = 11.8$ Hz, $J_2 = 3.9$ Hz, 1H), 3.84 (dd, $J_1 = J_2 = 9$ Hz, 1H), 3.65 (s, 3H), 2.64 (ddd, $J_1 = 17.5$ Hz, $J_2 = 9$ Hz, $J_3 = 1.7$ Hz 1H), 2.05 (s, 3H), 1.16 (s, 3H), 1.08 (s, 3H), 0.98 (d, $J = 6.8$ Hz, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.64 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 213.2 (CO), 174.5 (CO), 170.9 (CO), 147.2 (C), 132.8 (C), 80.2 (CH), 64.2 (C), 54.8 (CH), 51.6 (CH$_3$), 49.1 (C), 48.7 (CH), 44.9 (C), 40.2 (C), 35.2 (CH$_2$), 33.1 (CH$_2$), 33.0 (CH), 32.7 (CH$_2$), 31.9 (CH$_2$), 31.2 (CH$_2$), 30.6 (CH$_2$), 27.7 (CH$_3$), 24.9 (CH$_3$), 24.3 (CH$_2$), 23.6 (CH$_2$), 22.1 (CH$_2$), 21.4 (CH$_3$), 20.4 (CH$_3$), 19.3
(CH₃), 18.5 (CH₃), 16.5 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₆O₅Na⁺ [M+Na]⁺ 509.32375, found: 509.32394.

(R)-Methyl 4-((3aS,3bS,5aS,7S,9aS,10aR,11aR)-7-acetoxy-3a,3b,6,6,9a-pentamethyl-10-oxo-3a,3b,4,5,5a,6,7,8,9,9a,10,10a,11,11a-tetradecahydro-3H-benzo[f]cyclopenta[a]azulen-1-yl)pentanoate (2.26). White foam. ¹H NMR (600 MHz, CDCl₃, δ): 5.15 (m, 1H), 4.46 (dd, J₁ = 11.8 Hz, J₂ = 4.4 Hz, 1H), 3.65 (s, 3H), 3.41 (dd, J₁ = 12.2 Hz, J₂ = 6.4 Hz, 1H), 2.72 (dd, J₁ = J₂ = 8.3 Hz, 1H), 2.37 (dd, J₁ = 17.2 Hz, J₂ = 2.2 Hz, 1H), 2.05 (s, 3H), 1.20 (s, 3H), 1.19 (s, 3H), 1.04 (d, J = 6.8 Hz, 3H), 0.91 (s, 3H), 0.89 (s, 3H), 0.70 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 213.7 (CO), 174.6 (CO), 170.8 (CO), 150.6 (C), 122.4 (CH), 80.1 (CH), 57.7 (CH), 56.2 (C), 52.6 (CH), 51.7 (CH₃), 50.9 (CH), 49.3 (C), 47.6 (C), 40.7 (CH₂), 40.2 (C), 37.7 (CH₂), 31.9 (CH), 31.5 (CH₂), 31.2 (CH₂), 30.3 (CH₂), 28.3 (CH₂), 27.7 (CH₃), 27.3 (CH₃), 23.58 (CH₂), 23.57 (CH₂), 23.4 (CH₃), 21.4 (CH₃), 18.6 (CH₃), 18.1 (CH₃), 16.5 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₆O₅Na⁺ [M+Na]⁺ 509.32375, found: 509.32385.

2.8.6 Aldol addition of diketone 2.12.

Preparation of (2.27-2.29)

Table 2.4.3 (entry1): In a round-bottom flask open to atmosphere, trifluoroacetic acid (1 μL, 35 mol%) was added to the solution of 2.12 (20 mg, 0.0367 mmol) in 1 mL of DCM. The flask was sealed with a glass stopper and the mixture was stirred vigorously for 72 h, at which time the solvent was removed in vacuo and the mixture of products was separated by column chromatography on silica without additional work-up, to yield 2.27 (9 mg, 45%) and 2.28 (8 mg, 40%).
Table 2.4.3 (entry 2). NaH (95%, 2 mg, 0.0792 mmol) was placed in a flame-dried (under vacuum) round-bottom flask, followed by the addition of 0.5 mL of dry THF. The resulting suspension was cooled to 0 °C and the solution of 2.12 (36 mg, 0.066 mmol) in 0.5 mL of dry THF was added dropwise at this temperature. The reaction mixture was stirred at 0 °C for 30 min, then warmed up to rt and stirred at rt for 19 h, at which time the solvent was removed in vacuo and the crude mixture was treated with 5% acetic acid in H₂O and the aqueous layer was extracted with DCM. The organic layer was dried with Na₂SO₄, concentrated under vacuum and the crude mixture of products was separated by column chromatography on silica to give 2.27 (20 mg, 56%) and 2.28 (2 mg, 6%).

Table 2.4.3 (entry 3). To a solution of 2.12 (25 mg, 0.0459 mmol) in 0.5 mL of DCM in a flame dried (under vacuum) round-bottom flask, pyrrolidine (1.9 μL, 1.63 mg, 0.023 mmol) was added dropwise at rt. The resulting mixture was stirred at rt for 24 h, at which time the solvent was removed under vacuum and the crude mixture of products was separated by column chromatography on silica without additional work-up, to give 2.27 (20 mg, 80%) and 2.28 (2 mg, 8%).

General procedure for reaction of 2.12 with amide bases:

Solution of a secondary amine (1.1 eq.) in dry THF (0.027M in amine) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 °C, followed by a dropwise addition of n-butyl lithium (2.5M in hexanes, 1.1 eq.). The reaction mixture was warmed up to 0 °C, stirred at this temperature for 5 min and subsequently cooled to -78 °C. A solution of 2.12 (1 eq.) in dry THF (0.07M in 2.12) was then added dropwise to the solution of lithium amide by syringe at -78 °C. The reaction mixture was allowed to warm up to rt overnight and stirred at rt for the total of 24 h. After removing solvent in
vacuo the residue was taken up in DCM, washed with water and brine, the organic layer was dried over Na₂SO₄. Evaporation of the solvent in vacuo gave crude product mixtures that were further separated by chromatography.

Table 2.4.3 (entry 4). Following the general procedure, the use of diisopropylamine (20 mg, 29 μL, 0.202 mmol), n-butyl lithium (0.202 mmol, 81 μL), 2.23 (100 mg, 0.1836 mmol) and THF (10 mL) gave, after column chromatography on silica, 2.27 (71 mg, 71%) and 2.28 (22 mg, 22%).

Table 2.4.3 (entry 5). Following the general procedure, the use of hexamethyldisilazane (6.5 mg, 8.5 μL, 0.0404 mmol), n-butyl lithium (0.0404 mmol, 16.2 μL), 2.12 (20 mg, 0.0367 mmol) and THF (2 mL) gave, after column chromatography on silica, 2.27 (13 mg, 65%) and 2.28 (5 mg, 25%).

Table 2.4.3 (entry 6). Following the general procedure, the use of 2,2,6,6-Tetramethylpiperidine (5.7 mg, 0.0404 mmol), n-butyl lithium (0.0404 mmol, 16.2 μL), 2.12 (20 mg, 0.0367 mmol) and THF (2 mL) gave, after column chromatography on silica, 2.27 (9.6 mg, 48%) and 2.28 (8.6 mg, 43%).

Table 2.4.3 (entry 7). Following the general procedure for amide bases, the use of Ph₃CH (9.9 mg, 6.9 μL, 0.0404 mmol), n-butyl lithium (0.0404 mmol, 16.2 μL), 2.12 (20 mg, 0.0367 mmol) and THF (2 mL) gave, after column chromatography on silica, 2.27 (16 mg, 79%) and 2.28 (1 mg, 5%).

Table 2.4.3 (entry 8). A round-bottom flask open to atmosphere was charged with basic alumina (1.9 g, 18.4 mmol), followed by the addition of DCM (1 mL). A solution of 2.12 (100 mg, 0.184 mmol) in DCM (1 mL) was then added to the resulting
suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 16 h, at which time the mixture was filtered over a fine sinter funnel and washed successively with ethyl acetate. After the removal of the solvent in vacuo, the column chromatography on silica yielded an inseparable mixture (90 mg) of 2.27 and 2.29. \( R_f = 0.4 \) (EA/hex = 25/75). 2.27 and 2.29 were further separated by semi-prep HPLC (Agilent C18 column 21.2×250 mm, isocratic elution CH\(_3\)CN/H\(_2\)O = 95/5, flow rate 5 mL/min) to yield 2.27 (81 mg, 81%), \( R_t = 53 \) min; and 2.29 (7 mg, 7%). \( R_t = 68 \) min.

Table 2.4.3 (entry 9). Dry DCM (1 mL) was added to a flame-dried (under vacuum) round-bottomed flask. The flask was cooled to -78 °C and TiCl\(_4\) (41.8 mg, 0.22 mmol, 24.2 μl) was added at that temperature by a quick syringe transfer, followed by a dropwise addition of diisopropyl ethyl amine (33.2 mg, 0.257 mmol, 44.8 μl). A solution of 2.12 (100 mg, 0.1836 mmol) in dry DCM (1 mL) was added to the reaction mixture at -78 °C, and the reaction mixture was allowed to warm to rt and stirred at rt for 24 h, at which time water (2 mL) was added to the solution, layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried with Na\(_2\)SO\(_4\), concentrated under vacuum and the crude mixture of products was separated by column chromatography on silica to give 8 (49.5 mg, 50%) and 10 (31.5 mg, 32%).

\( (2R,4aS,6aS,6bR,8aS,10S,12aS,13aS,14aS,14bR)\)-Methyl 10-acetoxy-6b-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-13-oxodocosahydrobenzo[f]naphtho[2,1-a]azulene-2-carboxylate (2.27). White solid. Mp 235-238 °C. \( R_f = 0.53 \) (EA/hex = 3/7). \( R_f = 0.4 \) (EA/hex = 25/75). \(^1\)H NMR (600 MHz, CDCl\(_3\), δ): 4.45 (m, 1H), 3.62 (s, 3H), 3.57 (dd, \( J_1 = J_2 = 12 \) Hz, 1H), 2.43 (dd, \( J_1 = 12.6 \) Hz, \( J_2 = 4.2 \) Hz, 1H), 2.17 (2H), 2.04 (s, 3H),
1.18 (s, 3H), 1.16 (s, 3H), 1.08 (s, 3H), 1.05 (s, 3H), 0.96 (s, 3H), 0.95 (s, 3H), 0.59 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 215.4 (CO), 179.3 (CO), 171.0 (CO), 84.5 (COH), 80.2 (CH), 56.8 (CH), 52.8 (C), 51.8 (CH$_3$), 49.6 (C), 46.8 (C), 46.0 (CH), 44.1 (CH), 40.7 (C), 38.5 (CH$_2$), 37.8 (C), 37.4 (CH$_2$), 35.9 (CH$_2$), 34.9 (CH$_2$), 33.0 (CH$_2$), 32.7 (CH$_3$), 31.4 (C), 31.1 (CH$_3$), 30.6 (CH$_2$), 30.3 (CH$_2$), 27.8 (CH$_3$), 24.0 (CH$_2$), 23.7 (CH$_2$), 21.4 (CH$_3$), 21.2 (CH$_3$), 19.7 (CH$_2$), 17.8 (CH$_3$), 16.6 (CH$_3$), 16.1 (CH$_3$). HRMS (ESI) m/z calcd for C$_{33}$H$_{52}$O$_6$Na$^+$ [M+Na]$^+$ 567.3661, found: 567.3660.

$^{(2R,4aS,6aS,7aR,8aS,10S,12aS,12bS,14aS,14bR)}$-Methyl 10-acetoxy-12b-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-7-oxodocosahydrobenzo[a]naphtho[2,1-f]azulene-2-carboxylate (2.28). White solid. Mp 187-190 °C. R$_f$ = 0.46 (EA/hex = 3/7).

$^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 4.45 (dd, $J_1 = 12$ Hz, $J_2 = 4.8$ Hz, 1H), 3.64 (s, 3H), 3.14 (d, $J = 9.6$ Hz, 1H), 2.42-2.38 (2H), 2.22 (d, $J = 14.4$ Hz, 1H), 2.16 (ddd, $J_1 = J_2 = 14.4$ Hz, $J_3 = 4.8$ Hz, 1H), 2.06 (ddd, $J_1 = J_2 = 14.4$ Hz, $J_3 = 4.8$ Hz, 1H), 2.05 (s, 3H), 1.84 (dd, $J_1 = J_2 = 13.2$ Hz, 1H), 1.75-1.47 (12H), 1.45 (s, 3H), 1.42-1.27 (4H), 1.18 (s, 3H), 1.13 (dd, $J_1 = 13.8$ Hz, $J_2 = 6$ Hz, 1H), 1.08 (s, 3H), 1.04 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.64 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 214.6 (CO), 179.2 (CO), 171.2 (CO), 82.1 (C), 81.0 (CH), 58.5 (CH), 56.3 (C), 52.0 (CH$_3$), 50.5 (CH), 49.7 (C), 45.7 (CH), 40.8 (C), 39.1 (C), 37.5 (C), 36.5 (CH$_2$), 34.0 (CH$_2$), 33.0 (CH$_3$), 32.8 (CH$_2$), 31.8 (C), 31.40 (CH$_3$), 31.35 (CH$_2$), 31.0 (CH$_2$), 30.8 (CH$_2$), 29.8 (CH$_2$), 29.0 (CH$_3$), 27.6 (CH$_2$), 25.0 (CH$_2$), 22.8 (CH$_2$), 21.4 (CH$_3$), 18.0 (CH$_3$), 17.8 (CH$_3$), 17.0 (CH$_3$), 16.1 (CH$_3$). HRMS (ESI) m/z calcd for C$_{33}$H$_{52}$O$_6$Na$^+$ [M+Na]$^+$ 567.3661, found: 567.3647.

Preparation of 8, 9 and 10. (2R,4aS,6aS,6bR,8aS,10S,12aS,13aR,14aS,14bR)-Methyl 10-acetoxy-6b-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-13-
oxodocosahydrobenzo[f]naphtho[2,1-a]azulene-2-carboxylate (2.29). White foam. \( R_f = 0.4 \) (EA/hex = 25/75). \(^1\)H NMR (600 MHz, CDCl\(_3\), \( \delta \)): 4.48 (dd, \( J_1 = 17.4 \) Hz, \( J_2 = 6 \) Hz, 1H), 3.70 (s, 3H), 3.60 (dd, \( J_1 = 16.2 \) Hz, \( J_2 = 4.2 \) Hz, 1H), 2.35 (m, 1H), 2.04 (s, 3H), 1.18 (s, 3H), 1.10 (s, 3H), 1.07 (s, 3H), 1.04 (s, 3H), 0.91 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H). \(^{13}\)C NMR (150 MHz, CDCl\(_3\), \( \delta \)): 212.4 (CO), 179.1 (CO), 170.8 (CO), 87.7 (COH), 80.4 (C), 53.3 (CH), 53.0 (C), 52.1 (CH\(_3\)), 49.5 (C), 48.5 (C), 47.1 (CH), 44.2 (CH), 40.7 (C), 40.0 (C), 37.6 (CH\(_2\)), 37.4 (CH\(_2\)), 35.8 (CH\(_2\)), 35.7 (CH\(_2\)), 32.8 (CH\(_3\)), 32.6 (CH\(_3\)), 32.3 (CH\(_2\)), 31.5 (C), 31.3 (CH\(_3\)), 30.2 (CH\(_2\)), 28.0 (CH\(_3\)), 24.2 (CH\(_2\)), 23.7 (CH\(_2\)), 23.5 (CH\(_2\)), 21.7 (CH\(_3\)), 21.5 (CH\(_3\)), 21.2 (CH\(_3\)), 18.1 (CH\(_3\)), 16.4 (CH\(_3\)). HRMS (ESI) \( m/z \) calcd for C\(_{33}\)H\(_{52}\)O\(_6\)Na\(^{+}\) [M+Na]\(^{+}\) 567.3661, found: 567.3654.

**Preparation of (2.31-2.33)**

Solution of 2.12 (30 mg, 0.055 mmol) in dry DCM (1 mL) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 °C, followed by a dropwise addition of BF\(_3\)-Et\(_2\)O (8.6 mg, 0.0605 mmol, 7.6 μL) at this temperature. The reaction mixture was allowed to warm up to rt overnight and stirred at rt for the total of 4 days, at which time the solvent was removed under vacuum, water (2 mL) was added to the solution, layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried with Na\(_2\)SO\(_4\), concentrated under vacuum and the crude mixture of products was separated by column chromatography on silica to give 2.32 as transparent oil (2.5 mg, 9%), \( R_f = 0.3 \) (EA/hex = 15/85); 2.31 as white solid (13 mg, 45%), mp 214 °C, \( R_f = 0.27 \) (EA/hex = 15/85); and 2.33 as white solid (10 mg, 35%). Mp 198-202 °C. \( R_f = 0.22 \) (EA/hex = 15/85).
(2R,4aS,6aS,6bR,8aS,10S,12aS,13aS)-Methyl 10-acetoxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,13,13a,14-icosahydrobenzo[f]naphtha [2,1-a] azulene-2-carboxylate (2.31). $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 4.52 (dd, $J_1 = 10.2$ Hz, $J_2 = 6$ Hz, 1H), 3.65 (dd, $J_1 = J_2 = 9$ Hz, 1H), 3.57 (s, 3H), 2.92 (dd, $J_1 = 18$ Hz, $J_2 = 8.4$ Hz, 1H), 2.74 (dd, $J_1 = 13.8$ Hz, $J_2 = 2.4$ Hz, 1H), 2.14 (dd, $J_1 = 17.4$ Hz, $J_2 = 9.6$ Hz, 1H), 2.05 (s, 3H), 1.25 (s, 3H), 1.17 (s, 3H), 0.99 (s, 6H), 0.96 (s, 3H), 0.89 (s, 3H), 0.48 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 217.4 (CO), 177.4 (CO), 171.0 (CO), 138.3 (C), 130.2 (C), 80.1 (CH), 51.5 (C), 51.33 (CH$_3$), 51.26 (CH), 50.7 (CH), 49.8 (C), 49.7 (C), 45.9 (C), 39.7 (CH$_2$), 38.8 (C), 36.8 (CH$_2$), 36.6 (CH$_2$), 36.1 (CH$_2$), 35.7 (CH$_2$), 34.1 (C), 32.2 (CH$_2$), 28.2 (CH$_3$), 28.1 (CH$_3$), 26.8 (CH$_2$), 25.1 (CH$_2$), 24.8 (CH$_3$), 23.7 (CH$_2$), 22.7 (CH$_3$), 21.8 (CH$_2$), 21.4 (CH$_3$), 19.0 (CH$_3$), 17.5 (CH$_3$), 16.9 (CH$_3$). HRMS (ESI) m/z calc'd for C$_{33}$H$_{50}$O$_5$Na$^+$ [M+Na]$^+$ 549.3556, found: 549.3551.

(2R,4aS,6aS,7aR,10S,12bR,14aS,14bR)-Methyl 10-acetoxy-2,4a,6a,9,9,12b,14a-heptamethyl-7-oxo-1,2,3,4,4a,5,6,6a,7a,8,9,10,11,12,12b,13,14,14a,14b-icosahydrobenzo[a]naphtho[2,1-f]azulene-2-carboxylate (2.32). $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 4.75 (ddd, $J_1 = 10.2$ Hz, $J_2 = 3$ Hz, $J_3 = 1.8$ Hz, 1H), 3.58 (s, 3H), 3.32 (ddd, $J_1 = 9$ Hz, $J_2 = 3$ Hz, $J_3 = 1.2$ Hz, 1H), 2.96 (m, 1H), 2.42 (d, $J = 15.6$ Hz, 1H), 2.05 (s, 3H), 1.32 (s, 3H), 1.25 (s, 3H), 1.17 (s, 3H), 1.07 (s, 6H), 1.00 (s, 3H), 0.67 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 214.8 (CO), 179.1 (CO), 171.2 (CO), 137.5 (C), 137.3 (C), 78.2 (CH), 55.5 (C), 54.5 (CH), 51.8 (CH$_3$), 50.9 (C), 45.3 (CH), 40.7 (C), 39.4 (C), 36.7 (CH$_2$), 36.2 (C), 34.9 (CH$_2$), 32.9 (CH$_3$), 32.2 (CH$_2$), 31.8 (CH$_2$), 31.7 (CH$_3$), 31.5 (C), 31.4 (CH$_2$), 30.4 (CH$_2$), 29.9 (CH$_2$), 27.0 (CH$_2$), 26.2 (CH$_3$), 25.6 (CH$_3$), 24.5 (CH$_2$), 21.6
(CH₃), 21.4 (CH₃), 19.7 (CH₂), 19.3 (CH₃), 17.5 (CH₃). HRMS (ESI) m/z calcd for C₃₃H₅₀O₅Na⁺ [M+Na]⁺ 549.3556, found: 549.3544.

(2R,4aS,6aR,10S,12aS,14aS,14bR)-Methyl 10-acetoxy-2,4a,6a,9,9,12a,14a-heptamethyl-7-oxo-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,13,14,14a,14b-icosahydrobenzo[a]naphtho[2,1-f]azulene-2-carboxylate (2.33). ¹H NMR (600 MHz, CDCl₃, δ): 4.52 (dd, J₁ = 11.4 Hz, J₂ = 4.8 Hz, 1H), 3.59 (s, 3H), 2.68 (dd, J₁ =15 Hz, J₂ = 6.6 Hz, 1H), 2.35 (d, J = 16.2 Hz, 1H), 2.05 (s, 3H), 1.19 (s, 3H), 1.08 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H), 0.97 (s, 3H), 0.89 (s, 3H), 0.77 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 206.9 (CO), 179.4 (CO), 171.1 (CO), 158.9 (C), 135.5 (C), 80.9 (CH), 56.2 (CH), 55.6 (C), 51.8 (CH₃), 50.8 (C), 46.1 (CH), 40.8 (C), 39.3 (C), 37.2 (C), 36.4 (CH₂), 34.0 (CH₂), 33.0 (CH₃), 32.7 (C), 32.6 (CH₂), 32.5 (CH₂), 32.0 (CH₂), 31.3 (CH₃), 29.6 (CH₂), 29.3 (CH₂), 28.5 (CH₃), 26.1 (CH₂), 24.9 (CH₂), 24.0 (CH₂), 21.9 (CH₃), 21.4 (CH₃), 17.2 (CH₃), 17.1 (CH₃), 16.7 (CH₃). HRMS (ESI) m/z calcd for C₃₃H₅₁O₅⁺ [M+H]⁺ 527.3737, found: 527.3736.

*General procedure for reaction via pathway d:*

A flame-dried (under vacuum) round-bottom flask was cooled to -78 °C and charged with BF₃·Et₂O (10 equiv) followed by the addition of DCM (0.14M in starting material). The solution of aldol adduct (1 equiv) in DCM (0.14M in starting material) was then added dropwise to the reaction mixture at -78 °C, allowed to warm up to rt overnight and stirred at rt for the total of 18 h, at which time the solvent was removed under vacuum, water (2 mL) was added to the solution, layers were separated and aqueous layer extracted with DCM. The organic layer was dried with Na₂SO₄, concentrated under
vacuum and the crude mixture of products was separated by column chromatography on silica.

_Reaction of 2.27 via pathway d._ Following the general procedure for reaction via pathway d, the use of 2.27 (75 mg, 0.1377 mmol), BF$_3$·Et$_2$O (195.4 mg, 1.377 mmol, 173 μL) and DCM (2 mL) gave, after column chromatography, 2.31 (67 mg, 92%). $R_f = 0.58$ (EA/hex = 3/7).

_Reaction of 2.28 via pathway d._ Following the general procedure for reaction via pathway d, the use of 2.28 (37 mg, 0.068 mmol), BF$_3$·Et$_2$O (96.5 mg, 0.68 mmol, 85 μL) and DCM (1.4 mL) gave, after column chromatography, 2.32 (19 mg, 53%), $R_f = 0.5$ (EA/hex = 25/75); and 2.33 (8 mg, 22%), $R_f = 0.43$ (EA/hex = 25/75).

2.8.7 _Aldol reaction of lanosterol- and bryonolic acid-derived triketones_

_Preparation of (2.9 and 2.35)_

In a 50 mL single-neck round-bottom flask, RuCl$_3$ (17 mg, 0.0822 mmol) was added in one portion to a solution of NaIO$_4$ (369.2 mg, 1.726 mmol) in 12.3 mL of H$_2$O, and the resulting suspension was stirred open to atmosphere for 15 min, followed by the addition of 8.2 mL of acetonitrile. The solution of 2.3 and 2.4 (1:1 mixture, 200 mg, 0.411 mmol) in 8.2 mL of CCl$_4$ was then added dropwise to the reaction mixture by a syringe-pump. The flask was sealed with a glass stopper and the resulting biphasic mixture was vigorously stirred for 15 h, at which time 1 mL of ethanol was added to the solution. The layers were separated and the aqueous layer was extracted with DCM. The organic layer was dried over Na$_2$SO$_4$ and concentrated under vacuum. The crude residue was passed through a short plug of silica to get rid of inorganic impurities. The crude mixture of
products further dissolved in 4 mL of DCM, and basic alumina (4.2 g, 41.1 mmol) was subsequently added to the resulting solution. The reaction mixture was stirred at rt overnight, at which time the crude mixture of products was separated by column chromatography on silica to yield 2.35 as a white foam (5 mg, 2%), 2.9 as a white solid (80 mg, 38%), and 2.36 as a white solid (65 mg, 23%).

**(R)-Methyl-4-((1R,3aR,4aR,5aS,7S,9aS,10aR,11aR)-7-acetoxy-4a-hydroxy-3a,6,6,9a,11a-pentamethyl-4,10-dioxohexadecahydro-1H-cyclopenta[b]anthracen-1-yl)pentanoate (2.9).** Mp: 260-264 °C. R<sub>f</sub> = 0.15 (EA/hex = 25/75). <sup>1</sup>H NMR (600 MHz, CDCl₃, δ): 4.48 (dd, J₁ = 11.4 Hz, J₂ = 4.6 Hz, 1H), 3.65 (s, 3H), 3.10 (dd, J₁ = 12.4 Hz, J₂ = 2.6 Hz, 1H), 2.13 (d, J = 13.3 Hz, 1H), 2.04 (s, 3H), 1.28 (s, 3H), 1.12 (s, 3H), 0.99 (s, 3H), 0.95 (d, J = 6.4 Hz, 3H), 0.88 (s, 3H), 0.68 (s, 3H). <sup>13</sup>C NMR (150 MHz, CDCl₃, δ): 211.8 (CO), 211.6 (CO), 174.6 (CO), 171.0 (CO), 80.0 (CH), 79.2 (C), 59.3 (C), 51.7 (CH₃), 50.2 (CH), 49.5 (CH), 47.9 (C), 47.3 (C), 45.5 (CH), 38.5 (C), 35.3 (CH), 31.2 (CH₂), 31.1 (CH₂), 30.7 (CH₂), 29.3 (CH₂), 28.9 (CH₂), 28.4 (CH₂), 28.1 (CH₃), 26.9 (CH₂), 23.6 (CH₂), 22.8 (CH₃), 21.3 (CH₃), 19.5 (CH₃), 18.7 (CH₃), 18.6 (CH₃), 17.3 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₆O₇Na⁺ [M+Na⁺] 541.31357, found 541.31373.

**(R)-Methyl-4-((1R,3aR,4aS,5aS,7S,9aS,10aS,11aR)-7-acetoxy-10a-hydroxy-3a,6,6,9a,11a-pentamethyl-4,10-dioxohexadecahydro-1H-cyclopenta[b]anthracen-1-yl)pentanoate (2.35).** <sup>1</sup>H NMR (600 MHz, CDCl₃, δ): 4.44 (dd, J₁ = 11.5 Hz, J₂ = 4.7 Hz, 1H), 3.67 (s, 3H), 2.71 (dd, J₁ = 11.6 Hz, J₂ = 2.1 Hz, 1H), 2.42 (d, J = 13.7 Hz, 1H), 2.05 (s, 3H), 1.33 (s, 3H), 1.13 (s, 3H), 1.02 (s, 3H), 0.97 (d, J = 6.4 Hz, 3H), 0.93 (s, 3H), 0.91 (s, 3H). <sup>13</sup>C NMR (150 MHz, CDCl₃, δ): 211.3 (CO), 211.1 (CO), 174.6 (CO), 171.0 (CO), 83.8 (C), 79.9 (CH), 59.7 (C), 54.6 (CH), 53.4 (CH), 51.7 (CH₃), 51.2 (CH), 48.7
Preparation of (2.37)

(2R,4aS,6aS,7aS,8aS,10S,12aS,13aS,14aS,14bR)-Methyl 10-acetoxy-7a-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-7,13-dioxodocosahydrobenzo[a]tetracene-2-carboxylate (2.37). A round-bottom flask open to atmosphere was charged with basic alumina (11 g, 107.4 mmol), followed by the addition of DCM (5.7 mL). A solution of 2.11 (600 mg, 1.074 mmol) in DCM (5 mL) was then added to the resulting suspension. The flask was sealed with a glass stopper and the reaction mixture was vigorously stirred at rt for 24 h, at which time the mixture was filtered on a fine sinter funnel and washed successively with ethyl acetate. After the removal of the solvent in vacuo, column chromatography on silica yielded 2.37 as white solid (480 mg, 80%). Mp: 292 °C. Rf = 0.37 (EA/hex = 3/7). 1H NMR (600 MHz, CDCl3, δ): 4.52 (m, 1H), 3.59 (s, 3H), 2.90 (m, 1H), 2.61(dd, J1 = 16 Hz, J2 = 8 Hz, 1H), 2.43 (m, 1H), 2.15 (m, 1H), 2.05 (s, 3H), 1.96 (ddd, J1 = J2 = 16 Hz, J3 = 4 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 1.15 (s, 3H), 1.06 (s, 3H), 0.97 (s, 3H), 0.91 (s, 3H), 0.62 (s, 3H). 13C NMR (150 MHz, CDCl3, δ): 216.6 (CO), 213.8 (CO), 179.1 (CO), 171.0 (CO), 80.2 (CH), 75.5 (COH), 52.7 (C), 51.9 (CH3), 47.3 (CH), 46.3 (C), 45.9 (CH), 43.8 (CH), 40.7 (C), 39.7 (C), 38.2 (C), 35.6 (CH2), 35.4 (CH2), 33.9 (CH2), 32.7 (CH3), 31.8 (CH3), 30.9 (CH2), 30.8 (C), 30.4 (CH2), 29.9 (CH2), 28.1 (CH2), 27.7 (CH3), 24.7 (CH2), 23.8 (CH2), 21.3 (CH3), 21.0 (CH3), 20.3 (CH3), 19.8

(C), 48.4 (C), 40.0 (CH2), 39.1 (C), 35.1 (CH), 32.6 (CH2), 31.3 (CH2), 31.1 (CH2), 28.5 (CH3), 27.7 (CH2), 27.2 (CH2), 23.4 (CH3), 23.3 (CH2), 21.4 (CH3), 20.7 (CH3), 19.8 (CH3), 18.6 (CH3), 17.4 (CH3), 16.1 (CH2). HRMS (ESI) m/z calcd for C30H46O7Na+ [M+Na]+ 541.31357, found 541.31369.
(CH₃), 16.7 (CH₃). HRMS (EI) m/z  caded for C₃₃H₅₀O₇ [M]+ 558.35565, found: 558.35386.

2.8.8 Aldol reaction of the lanosterol- and bryonolic acid-derived tetraketones

Preparation of (2.38 and 2.39)

Solution of diisopropylamine (10.7 mg, 0.1056 mmol, 15 μL) in dry THF (2 mL) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 °C, followed by a dropwise addition of n-butyl lithium (2.5M in hexanes, 0.1056 mmol, 42 μL.). The reaction mixture was warmed to 0 °C, stirred at this temperature for 5 min and then cooled to -78 °C. A solution of 2.16 (55 mg, 0.096 mmol) in dry THF (3 mL) was then added dropwise to the solution of LDA by syringe at -78 °C. The reaction mixture was stirred at this temperature for 30 min, at which time the reaction was quenched by H₂O (D₂O) (2 mL). Followed the addition of DCM (2 mL), the layers were separated and the aqueous layer extracted with DCM. Organic fractions were combined, washed with water and brine and dried over Na₂SO₄. Evaporation of the solvent in vacuo gave crude product mixtures that were further separated by chromatography. Column chromatography on silica yielded an inseparable mixture of compounds (7 mg) containing 2.38, Rᵣ = 0.43 (EA/hex = 4/6); and pure 2.39 as a transparent oil (36 mg, 64%). Rᵣ = 0.31 (EA/hex = 4/6). A mixture of unidentifiable compounds containing 2.38 was further purified by semi-prep HPLC (Agilent C18 column 21.2×250 mm, isocratic elution CH₃CN/H₂O = 8/2, flow rate 5 mL/min) to yield 2.38 as a white foam (5 mg, 9%). Rᵣ = 30 min.

(2R,4aS,6aS,8aR,8bS,10S,12aS,13aS,14aS,14bR)-Methyl 10-acetox-13a-hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-7,8,13-trioxodocosahydrobenzo[a]naphtho[1,2-f]azulene-2-carboxylate (2.38). ^1H NMR
(600 MHz, CDCl₃, δ): 4.49 (dd, J₁ = 12 Hz, J₂ = 4.8 Hz, 1H), 3.62 (s, 3H), 3.40 (d, J = 13.8 Hz, 1H), 2.76 (d, J = 15 Hz, 1H), 2.58 (d, J = 16.2 Hz, 1H), 2.22 (d, J = 13.8 Hz, 1H), 2.06 (s, 3H), 1.33 (s, 3H), 1.24 (s, 3H), 1.22 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 1.02 (s, 3H), 0.51 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 216.1 (CO), 209.9 (CO), 209.7 (CO), 178.9 (CO), 170.8 (CO), 80.0 (CH), 78.9 (COH), 57.6 (CH), 54.2 (C), 52.1 (CH₃), 50.0 (CH), 47.3 (C), 44.5 (CH), 43.6 (CH₂), 40.6 (C), 40.0 (C), 39.9 (C), 35.7 (CH₂), 33.9 (CH₂), 32.5 (CH₃), 32.1 (C), 31.52 (CH₂), 31.45 (CH₃), 30.9 (CH₂), 29.8 (CH₂), 27.3 (CH₃), 23.8 (CH₂), 23.7 (CH₂), 21.4 (CH₃), 21.2 (CH₃), 19.1 (CH₃), 18.0 (CH₃), 17.8 (CH₃). HRMS (EI) m/z calcd for C₃₃H₄₈O₈⁺ [M]⁺ 572.33492, found: 572.33395.

(2R,4aS,6aS,8S,9aS,11S,13aS,14R,16aS,16bR)-Methyl 11-acetoxy-8,14-dihydroxy-2,4a,6a,10,10,13a,16a-heptamethyl-7,15-dioxodocosahydro-8,14-epoxybenzo[6,7]cyclodeca[1,2-a]naphthalene-2-carboxylate (2.39, 6-H): ¹H NMR (600 MHz, CDCl₃, δ): 4.65 (dd, J₁ =11.4 Hz, J₂ = 3.6 Hz, 1H), 3.74 (s, 3H), 3.14 (d, J = 12 Hz, 1H), 3.08 (br. s, 1H), 2.91 (br. S, 1H), 2.75 (2H), 2.37 (d, J = 15.6 Hz, 1H), 2.31-2.23 (2H), 2.18 (d, J = 12 Hz, 1H), 2.05 (s, 3H), 1.82 (s, 3H), 1.19 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H), 0.92 (s, 3H), 0.91 (s, 3H), 0.71 (s, 3H). ¹³C NMR (150 MHz, CDCl₃, δ): 207.6 (CO), 205.1 (CO), 178.6 (CO), 170.9 (CO), 100.9 (COH), 99.0 (COH), 79.9 (CH), 55.0 (C), 52.1 (CH₃), 44.8 (CH), 44.6 (C), 43.6 (CH₂), 40.6 (C), 40.5 (CH), 40.1 (C), 37.3 (C), 35.8 (CH₂), 34.7 (CH₂), 32.7 (CH₃), 31.6 (CH₂), 31.54 (CH₃), 31.47 (C), 29.8 (CH₂), 29.4 (CH₂), 29.0 (CH₂), 28.7 (CH₂), 27.8 (CH₃), 23.6 (CH₂), 21.4 (CH₃), 16.92 (CH₃), 16.88 (CH₃), 16.5 (CH₃), 16.0 (CH₃). HRMS (ESI) m/z calcd for C₃₃H₅₀O₉Na⁺ [M+Na]⁺ 613.3353, found: 613.3352.
Hemiketal (2.39, 6-D): $^2$H NMR (600 MHz, CHCl$_3$, $\delta$): 1.35 (br. m). HRMS (ESI) $m/z$ calcd for C$_{33}$H$_{49}$DO$_9$Na$^+$ [M+Na]$^+$ 614.34098, found: 614.34077.

Preparation of (2.36 and 2.41)

*General procedure for reaction of tetraketone 2.8 with amide bases:*

Solution of a secondary amine (1.1 equiv) in dry THF (4 mL) in a flame-dried (under vacuum) round-bottom flask was cooled to -78 °C, followed by a dropwise addition of n-butyl lithium (2.5M in hexanes, 1.1 equiv). The reaction mixture was warmed up to 0 °C, stirred at this temperature for 15 min and subsequently cooled to -78 °C. A solution of tetraketone (50 mg, 0.094 mmol) in dry THF (0.7 mL) was then added dropwise to the solution of the amide base by a syringe at -78 °C. The reaction mixture was allowed to warm up to rt overnight and stirred at rt for the total of 15 h. After removing solvent in vacuo the residue was taken up in DCM, washed with water and brine. The organic layer was dried over Na$_2$SO$_4$. Evaporation of the solvent in vacuo gave crude product mixtures that were further separated by careful column chromatography on silica to give the products 2.36 and 2.41.

*Table 2.6.1 (Entry 1).* Following the general procedure, the use of diisopropyl amine (10.4 mg, 0.1 mmol, 14.6 $\mu$L), n-Butyl lithium (41.4 $\mu$L), THF and 2.8 gave, after column chromatography on silica, aldol adduct 2.36 as a white solid (23.3 mg, 47%), $R_f = 0.43$ (EA/hex =40/60), and aldol adduct 2.41 as transparent oil (3 mg, 6%), $R_f = 0.18$ (EA/hex =40/60).

*Table 2.6.1 (Entry 2).* Following the general procedure, the use of tetramethylpiperidine (14.6 mg, 0.1 mmol, 17.6 $\mu$L), n-Butyl lithium (41.2 $\mu$L), THF and
2.8 gave, after column chromatography on silica, aldol adduct 2.36 as a white solid (32.1 mg, 64%), R<sub>f</sub> = 0.43 (EA/hex =40/60).

Table 2.6.1 (Entry 3). Following the general procedure, the use of hexamethyldisilazane (16.7 mg, 0.1 mmol, 21.6 μL), n-Butyl lithium (41.3 μL), THF and 2.8 gave, after column chromatography on silica, aldol adduct 2.36 as a white solid (34.3 mg, 69%), R<sub>f</sub> = 0.43 (EA/hex =40/60).

2.9 References


34. Mahrwald, R., Diastereoselection in Lewis-Acid-Mediated Aldol Additions. 


3.1 Introduction

Considerable effort in drug discovery has focused on the isolation and structural elucidation of novel triterpenoid molecules from plant sources. The recent study of the biologically active constituents of *Ganoderma sinense* led to the isolation of Ganosinensates, unique cyclobutane-containing triterpenoids of lanostane type, which were shown to possess some antitumor activity. Qiu and co-workers\(^1\) hypothesized that Norrish-Yang photocyclization was the key step in the biogenetic pathway leading to these unique triterpenoids. The authors proposed that photoexcitation of the keto group at C-11 in lucidenic acid A leads to the abstraction of the $\gamma$-hydrogen at position 1, followed by stereospecific formation of the cyclobutanol, thus providing a linkage between C-1 and C-11.

![Figure 3.1.1 Biosynthetic route leading to Ganosinensate A](image)

The use of photochemical transformations for target-oriented synthesis of complex natural products has proven an effective approach.\(^2\)\(^-\)\(^3\) The advantages of such transformations are the sunlight and low-cost nature of the reagent, as well as the fact that such transformations allow for the rapid construction of strained systems that otherwise would be very difficult to synthesize. Many applications of photochemical reactions in
natural product synthesis rely on either forming directly complex natural products with a
cyclobutane scaffold or on accessing natural products through selective cyclobutane
cleavage reactions within a polycyclic skeleton.\textsuperscript{4}

The use of light as a reagent in diversity oriented synthesis appears to be limited.\textsuperscript{5-6}
In relation to natural products, a single report by the group of de la Torre\textsuperscript{7} describes the
synthesis of diverse polycyclic terpene-like structures from the readily available
sesquiterpene Sclareolide.

\begin{center}
\textbf{Figure 3.1.2} Divergent photoreactivity of Sclareolide
\end{center}

In this example, the ester functionality in Sclareolide was manipulated to convert the
parent natural product to the corresponding Weinreb’s amide. This amide further reacted
with the appropriate organolithium reagents to produce either saturated or $\alpha,\beta$-unsaturated
ketones. Upon irradiation, the saturated substrates yielded oxetane-containing $6/6/4$-fused
products via Paterno-Buchi reaction, while the $\alpha,\beta$-unsaturated counterparts resulted in
$6/6/6/4$-fused sesquiterpenes via $[2+2]$ cycloaddition.

The application of photochemical reactions to the construction of additional four-
membered rings in steroids has been reviewed by Kamernitskii et al.\textsuperscript{8} These earlier
approaches bifurcated and utilized either Norrish-Yang photocyclization or $[2+2]$
cycloaddition. The former process commonly occurred between the methyl groups at ring fusions with \( \gamma \)-positioned ketones thus yielding bridging cylobutanols. In the latter approach, various olefins reacted with the unsaturated steroid substrates leading to steroid analogues with an appended cyclobutane fragment within their ring systems. Both of the reviewed methodologies converge in using functionalities located on the periphery of the common steroidal skeleton.

![Figure 3.1.3 Divergent Norrish-Yang reactivity of pseudo-symmetrical substrates](image)

The diversity-oriented strategy described in this Chapter is different from all previous approaches in that the pseudo-symmetrical polyketones derived from lanosterol and bryonolic acid were envisioned to undergo divergent Norrish-Yang cyclization leading to regioisomeric products with newly formed 6/4/8 ring systems (Figure 3.1.3). The following sections constitute a systematic study of the structural elements dictating the selectivity during generation of these ring systems. Section 3.2 will describe the application of this methodology to bryonolic-acid derived polyketones. Section 3.3 will cover the photoreactivity of lanosterol-derived polyketones.
3.2 Norrish-Yang photoreactivity of bryonolic acid-derived polyketones

Early analysis of the bichromophoric structure of bryonolic acid-derived tetraketone 2.16 revealed that the ‘bowsprit’ hydrogen H-5 is γ- to the keto groups at C-8 and C-11. Thus, photoexcitation of the C-8 carbonyl bond could lead to the formation of cyclobutanol 3.1 via pathway b, and, concurrently, photoexcitation of the C-11 carbonyl would result in the formation of cyclobutanol 3.2 via pathway a (Table 3.2.1). Taking into account this rationale, a solution of tetraketone 2.16 in dry deoxygenated C₆D₆ was irradiated in a quartz cuvette with 254 nm UV light until complete consumption of the starting material was indicated by TLC (45 min, Table 3.2.1, entry 1). Further investigation of the reaction mixture disclosed the formation of two products, which were separable by column chromatography on silica.

<table>
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<th>Entry</th>
<th>λ_{max} (nm)</th>
<th>Time (min)</th>
<th>Isolated yield of product (%)</th>
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<tr>
<td>4</td>
<td>300\textsuperscript{a}</td>
<td>45</td>
<td>56</td>
</tr>
</tbody>
</table>

Key: \textsuperscript{a}Reaction was carried out in the presence of 1.0 equiv of benzophenone

Table 3.2.1 Divergent Norrish-Yang reactivity of 2.16
The NMR spectra of the major reaction product revealed an AX system of two doublets at 3.40 and 2.75 ppm ($J = 12$ Hz) attached to the isolated methylene carbon at 41.2 ppm. These protons correlate, as observed by HMBC, with two carbonyl carbons (C-7, $\delta_C$ 210.0; C-8, $\delta_C$ 211.5), as well as with four quaternary carbons (C-4, $\delta_C$ 43.8; C-5, $\delta_C$ 56.9; C-10, $\delta_C$ 62.3; C-11, $\delta_C$ 92.2). These correlations allowed the assignment of the AX system to position 6 of the cyclobutanol 3.2 and ruled out structure 3.1, in which H-6 correlates to only one carbonyl carbon by HMBC spectroscopy. Further investigation of the structure of cyclobutanol 3.2 disclosed an AX system of two doublets at 2.25 and 1.73 ppm ($J = 18$ Hz), attached to another isolated methylene carbon C-12 ($\delta_C$ 42.4). In the HMBC spectrum, H-12 showed cross-peaks with one carbonyl carbon (C-9, $\delta_C$ 218.9), as well as one methine carbon (C-18, $\delta_C$ 47.0), one methyl carbon (C-27, $\delta_C$ 19.0) and four quaternary carbons (C-5, C-11, C-13, $\delta_C$ 40.6; C-14, $\delta_C$ 55.4). Consequently, the NMR, as well as the HRMS data were consistent with the proposed structural framework of cyclobutanol 3.2 (Figure 3.2.1).

![Figure 3.2.1 Key HMBC and COSY correlations of 3.2 and 3.3](image)

The routine NMR spectra of the minor reaction product were inconsistent with the structure of a product of Yang cyclization of tetraketone 2.16. Specifically, examination of $^{13}$C NMR spectrum revealed the signals of two carbonyl carbons (C-8, $\delta_C$ 212.6; C-9,
\[ \delta_C 212.4 \), as well as three quaternary carbons (C-5, \( \delta_C 90.6 \); C-7, \( \delta_C 90.3 \); C-11, \( \delta_C 77.3 \)) in the region downfield from 50 ppm. Extensive 2D-NMR experiments allowed us to propose structure 3.3 for the minor product of the reaction. Especially useful were the cross-peaks shown by two doublets at 2.69 and 1.88 ppm (H-6, \( J = 14 \) Hz), attached to an isolated methylene carbon C-6 (\( \delta_C 39.8 \)). In the HMBC spectrum, H-6 correlated to C-5, C-7, C-8, as well as C-10 (\( \delta_C 48.7 \)) and C-11. Additionally, the correlations of two doublets at 2.20 and 1.97 ppm (H-12) in the HMBC spectrum supported the elucidation of the carbon framework of structure 3.3 by showing the cross-peaks with C-7, C-9, C-11, as well as C-13 (\( \delta_C 40.4 \)), C-14 (\( \delta_C 52.5 \)), C-18 (\( \delta_C 44.3 \)) and C-27 (\( \delta_C 22.3 \)) (Figure 3.2.1). The structural assignment was also supported by HRMS data.

After elucidation of the structures of the reaction products, the yields of cyclobutanol 3.2 and the minor product of the photolysis 3.3 were found to be 33% and 10%, respectively (Table 3.2.1, entry 1). Changing the light sources did not lead to substantial difference in product distribution; however, the highest combined yield of 3.2 and 3.3 was achieved with a 300 nm UV lamp (76%, Table 3.2.1, entry 2). It is noteworthy that no products of the type II photofragmentation were observed in any reactions performed.

To gain insight into the regio- and, more importantly, stereochemical outcome of the Yang cyclization of tetraketone 2.16, a plausible mechanism for the process was examined. It has been determined by the Scheffer Group\(^{9-11}\) that in order for a ketone to undergo successful Norrish type II hydrogen abstraction reaction, the distance between the oxygen of the carbonyl and the \( \gamma \)-H has to be less than the sum of Van der Waals radii for H and O (2.72 Å).
The lowest energy conformation of 2.16 appeared to be a downward-facing “boat (with bow at C-5 and stern between C-8 and C-11) -chair-chair” (BCC) conformation. According to B3LYP 6-311G(d,p) energy minimization calculations, BCC was more stable than its upward-facing counterpart “chair-chair-boat (with bow between C-7 and C-9 and stern at C-13)” (CCB) by 6.87 kcal/mol (Figure 3.2.2). Using this B3LYP/6-311G(d,p) calculated geometry of tetraketone 2.16, it was determined that only C-11 keto group met Scheffer’s interatomic distance requirement ($d_1 = 2.29 \text{ Å}$, Figure 3.2.3), while the carbonyl at C-8 exceeded the suggested interval ($d_2 = 2.95 \text{ Å}$).

Consequently, irradiation of tetraketone 2.16 led exclusively to the formation of 1(C-5),4(C-11)-hydroxy biradical, which is geometrically predisposed to cyclize into cis-
cyclobutanol 3.2. The intermediate hydroxy biradical can concurrently undergo photopinacolization\textsuperscript{12-15} with the C-7 carbonyl leading to a C-5,O-7-centered biradical, which presumably can experience intermolecular H-exchange, followed by a ring-closure to from bridged structure 3.3 (Figure 3.2.3).

The mechanistic rationale of the reaction is in complete agreement with the observed stereochemistry of the products 3.2 and 3.3, as determined by NOE correlation spectroscopy (Figure 3.2.4), demonstrating a memory of chirality effect, whereby the chiral information of the tetraketone 2.16 is retained by the conformation of the biradical intermediate.\textsuperscript{16-19}

![NOESY Key correlations of compounds of 3.2 and 3.3.](image)

In the NOESY spectrum of cyclobutanol 3.2, H\textsubscript{β}-6 correlates with H-25 and H-24, while H\textsubscript{α}-6 correlates with H-23 and H-27, and the NOE interactions of H\textsubscript{β}-12 with H-25 and H-26 indicate α-facial orientation of the hydroxyl group at C-11. In the case of the bridged structure 3.3, H\textsubscript{α}-6 correlates with H-24 and H-27, while H\textsubscript{β}-6 correlates with H-25, as well as with the C7-OH hydroxyl group, which, in turn, shows an interaction with H-26. This provided evidence for the β-facial orientation of this hydroxyl group.
In an effort to force Yang cyclization in the alternative direction to form cyclobutanol 3.4, triketone 2.14 was taken as a substrate for the photoreaction. Synthesis and conformational analysis of triketone 2.14 were described in Chapter 2, sections 2.3 and 2.5, respectively. The structure of triketone 2.14 preserves the main structural features of tetraketone 2.16, but lacks the keto group at C-11 (Table 3.2.2).

![Diagram of molecules](image)

<table>
<thead>
<tr>
<th>Entry</th>
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<th>Time (h)</th>
<th>Yield$^a$ of 3.5 (% brsm)</th>
<th>Conversion$^a$ of 2.14 (%)</th>
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<td>254$^b$</td>
<td>72</td>
<td>18</td>
<td>72</td>
</tr>
</tbody>
</table>

$^a$Determined by $^1$H NMR  
$^b$Reaction was carried out in the presence of 1.0 equiv of benzophenone  
$^c$Isolated yield

**Table 3.2.2 Norrish-Yang photoreactivity of 2.14**

The B3LYP/6-311G(d,p) computed distance between H-5 and the oxygen of the C-8 carbonyl of triketone 2.14 is 2.56 Å, suggesting the high possibility of the desired transformation. In addition, the 1(C-7),2(C-8)-dione system becomes the major chromophore of the polyketone. Accordingly, irradiation of triketone 2.14 with 254 nm
UV light for 72 hours yielded a single reaction product (6% isolated yield, Table 3.2.2, entry 1). However, the structure of the product of photolysis of triketone 2.14 was formulated as 3.5 on the basis of a thorough NMR investigation (Figure 3.2.5).

![Figure 3.2.5 Key HMBC and COSY correlations of 3.5.](image)

The $^{13}$C NMR spectrum of 3.5 showed the presence of one carbonyl carbon C-8 ($\delta_c$ 212.9) as well as newly formed quaternary carbons C-7 ($\delta_c$ 87.3) and C-9 ($\delta_c$ 78.9), and a methylene carbon C-26 ($\delta_c$ 72.6) in the region downfield from 50 ppm. This observation alone excludes structure 3.4, which would be expected to have two ketone signals in the $^{13}$C spectrum. The $^1$H NMR of 3.5 exhibited a pair of doublets at 4.29 and 4.04 ppm ($J = 10$ Hz), corresponding to a methylene carbon C-26 ($\delta_c$ 72.6). In the HMBC spectrum, H-26 showed cross-peaks with C-7, C-8, C-13 ($\delta_c$ 39.7), C-14 ($\delta_c$ 53.9) and a methylene carbon C-15 ($\delta_c$ 25.6). Structural assignment was completed by further analysis of cross-peaks shown by H-5, H-11 and H-25 (Figure 3.2.5), as well as HRMS data.

The formation of structure 3.5 was not anticipated. Presumably, the initial photoexcitation of the C-7 keto group is followed by a new carbon-carbon bond formation via pinacol-type coupling reaction with the C-9 carbonyl. The resulting oxygen-centered biradical intermediate, formation of which is commonly associated with the photochemical cleavage of cyclic peroxides, undergoes abstraction of $\varepsilon$-hydrogen.
H-26 by the oxygen of the C-9 carbonyl ($d_2 = 2.31 \text{ Å}$), followed by a radical cyclization process to form a furan ring (Figure 3.2.6).

![Figure 3.2.6](image)

**Figure 3.2.6** Mechanistic considerations for the formation of 3.5.

The relative stereochemistry of 3.5 was confirmed by NOESY spectroscopy, which showed enhancements between the hydroxyl group at C-9 with H$_\beta$-26, H-25 and H$_\beta$-12. H$_\beta$-12 in turn showed correlations with H-18 and H$_\beta$-26, while H$_\alpha$-26 showed a cross-peak with H$_\beta$-16 (Figure 3.2.6). These NOESY data have determined the β-facial environment of the newly formed rings B and C.

Changing the light source did not significantly improve the efficiency of this reaction; however, almost complete conversion of the starting material was achieved by the use of benzophenone in combination with a 300 nm UV light (Table 3.2.2, entry 4). Low efficiency and long reaction times of the photolyses of triketone 2.14 are caused, perhaps, by the known reversibility of light-induced pinacolization and hydrogen-transfer processes.

After establishing the reactivity patterns of the polyketones derived from bryonolic acid, attention was turned to the lanosterol-derived substrates. It was determined in Chapter 2 that the subtle structural differences between bryonolic acid and lanosterol led to dramatic differences in the aldol reactivity of the polyketones derived from these molecules. The following section will discuss the analysis of the possibility of formation.
of the alternative product of Yang cyclization via pathway b from lanosterol-derived polyketones.

### 3.3 Norrish-Yang photoreactivity of lanosterol-derived polyketones

The oxidation chemistry leading to tetraketone 2.8 was discussed in Chapter 2, section 2.2. In Chapter 2, section 2.6, it was mentioned that Marsaioli and co-workers have previously studied the aldol reactivity of a similar tetraketone derived from 24,25-dihydrolanosterol. Based on analysis of the $^1$H and $^{13}$C NMR spectra of the tetraketone the authors suggested that this substrate exists as a mixture of two conformers (Chapter 2, Table 2.6.1). Rigorous verification of this suggestion was critical to our analysis and prediction of the photochemical behavior of tetraketone 2.8. Therefore, we conducted a variable temperature NMR study of 2.8, which indeed was isolated as an apparent mixture of two products (ca. 2.5:1 at 303.15 K), inseparable by various chromatographic means (Figure 3.3.1).

$^1$H signals were assigned by the analysis of COSY, HMQC and HMBC spectra of tetraketone 2.8. $^1$H NMR experiments carried out in toluene-$d_8$ at 0 °C revealed that H-3 signal exists as a doublet of doublets at 4.82 ppm ($J_1 = 11.4$ Hz, $J_2 = 4.2$ Hz) and a doublet of doublets at 4.60 ppm ($J_1 = 11.4$ Hz, $J_2 = 3.6$ Hz), H-12 splits into two doublets at 3.54 ppm ($J = 13.2$ Hz) and 3.48 ppm ($J = 13.8$ Hz), H-26 shows two singlets at 3.42 ppm and 3.40 ppm, while H-5 appears as a doublet of doublets at 3.21 ppm ($J_1 = J_2 = 3.6$ Hz) and a doublet of doublets at 3.03 ppm ($J_1 = 15.6$ Hz, $J_2 = 9.6$ Hz). With increasing temperature, all peaks broaden and at temperatures at and above 80 °C the doubled peaks merge and only time-average resonances are observed. The coalescence of the doubled
peaks at higher temperatures confirms that tetraketone 2.8 exists as an equilibrium mixture of two conformational isomers. Lowering the temperature to -30 °C does not lead to any difference in the line shapes, indicating that the conformational equilibrium has reached the slow exchange rate.

![Figure 3.3.1](image)

**Figure 3.3.1** Variable temperature ¹H NMR study of tetraketone 2.8.

Marsaioli and co-workers suggested the structures of the four possible conformers of the lanosterol-derived cyclodecatetraone based on empirical calculations using the torsional angles in the ten-membered ring (**Figure 3.3.2**).

More accurate energy minimization calculations of the tetraketone 2.8 at the B3LYP/6-311G(d,p) level of theory disclosed that the lowest energy conformers CCC (‘chair-chair-chair’) and TB(‘twist-boat’)-1 are close in energy. Therefore, the energy difference of 0.44 kcal/mol between CCC and TB-1 would correspond well to the equilibrium mixture of two conformers in ca. 2.1:1 ratio at 303.15 K. The assignment of the structures of conformers CCC and TB-1 was further supported by comparison of the experimental J-values for H-5 of both conformers with the J-values predicted using the
dihedral angles ($\phi$) of $-114^\circ$ (TB-1, H$_5$H$_{6\alpha}$), $128^\circ$ (TB-1, H$_5$H$_{6\beta}$) and $-73^\circ$ (CCC, H$_5$H$_{6\alpha}$) and $168^\circ$ (CCC, H$_5$H$_{6\beta}$), which were determined with the GaussView 3.09 program. Unfortunately, NOESY experiments did not provide definitive evidence as to the structures of the conformers.

Figure 3.3.2 Conformational analysis of tetraketone 2.8

Next, we examined the photochemical reactivity of the bichromophoric structure 2.8. Analysis of the less populated conformer CCC (Figure 3.3.2) revealed that the ‘bowsprit’ $\gamma$-hydrogen H-5 is anti-periplanar with C-8 and C-11 keto groups. Disfavorable orientation of reactive functionalities led to a conclusion that CCC must be unreactive in the desired Norrish-Yang photocyclization.

Figure 3.3.3 Mechanistic considerations for the formation of cyclobutanol 3.6
In contrast, examination of the more populated conformer TB-1 revealed that H-5 is anti-periplanar with the pi-bond of C-8 carbonyl, but syn-periplanar with the C-11 keto group (Figure 3.3.3). Thus, favorable spatial position of H-5 and the C-11 carbonyl allows for regiospecific formation of the 1(C-5),4(C-11)-hydroxy biradical, which would be structurally biased to cyclize into cis-cyclobutanol 3.6 via pathway a. In addition, the distance between H-5 and the oxygen of the C-8 carbonyl of TB-1 was determined to be 2.27 Å, increasing the likelihood of the desired reaction. Concomitantly, disfavorable spatial arrangement of H-5 and C(8)=O should exclude the formation of cyclobutanol 3.7 via pathway b (Table 3.3.1).

<table>
<thead>
<tr>
<th>entry</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Time (h)</th>
<th>Yield of 3.6 (%)</th>
<th>Conversion of 2.8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>254</td>
<td>20</td>
<td>48</td>
<td>100</td>
</tr>
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<td>300</td>
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<td>100</td>
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<td>3</td>
<td>254$^a$</td>
<td>6</td>
<td>72$^b$</td>
<td>60</td>
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<td>4</td>
<td>300$^a$</td>
<td>3</td>
<td>66</td>
<td>100</td>
</tr>
</tbody>
</table>

$^a$Reaction was carried out in the presence of 1.0 equiv of benzophenone  
$^b$Yield of 3.6 is based on the recovered 2.8

Table 3.3.1 Norrish-Yang photoreactivity of 2.8

In complete agreement with this rationale, irradiation of a solution of tetraketone 2.8 in dry deoxygenated C$_6$D$_6$ in a quartz cuvette with 254 nm UV light led to the formation of 3.6 as the sole product in 48% yield after complete consumption of the starting material in 20 h. Change of the light source to 300 nm led to a shorter reaction time,
however cyclobutanol 3.6 was isolated in only 27% yield. The addition of 1.0 equiv benzophenone significantly improved the efficiency of the reaction, affording the highest yield of cyclobutanol 3.6 (66%, Table 3.3.1, entry 4). The structure of 3.6 was assigned unambiguously after comprehensive analysis of 2-D NMR experiments and HRMS. It is noteworthy that no other products were observed during the photolysis.

The oxidation reaction leading to triketone 2.7 was discussed in Chapter 2, section 2.2, while the conformational analysis of this molecule is described in section 2.5. Further analysis of the ground-state conformation of triketone 2.7 revealed that despite the fact that the keto group at C-8 is $\gamma$- to H-5, the C-8 carbonyl is anti-periplanar with the C5-H bond (Figure 3.3.4).

![Figure 3.3.4](image)

*Figure 3.3.4 Mechanistic considerations for the formation of cyclobutanol 3.6*

This observation led to a conclusion that analogous to the CCC conformer of tetraketone 2.8, a prediction can be made that triketone 2.7 must be unreactive in the desired Norrish-Yang photocyclization and cyclobutanol 3.8 cannot be formed. However, the possibility of transannular photopinacolization remains plausible for triketone 2.7. By direct comparison with triketone 2.14, excitation of the carbonyl at C-7 can be followed by the formation of an oxygen-centered biradical. Based on the argument of closer spatial proximity, $\epsilon$-hydrogen H-30 may then be abstracted by the oxygen of the C-9 carbonyl.
(Figure 3.3.4, $d_2 = 2.42$ Å), followed by the formation of a furan ring with the oxygen of C-7 carbonyl.

<table>
<thead>
<tr>
<th>Entry</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Time (h)</th>
<th>Yield of 3.9 (% brsm)</th>
<th>Conversion of 2.7 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>254$^a$</td>
<td>24</td>
<td>39</td>
<td>55</td>
</tr>
</tbody>
</table>

$^a$Reaction was carried out in the presence of 1.0 equiv of benzophenone

Table 3.3.2 Norrish-Yang photoreactivity of 2.7

As a result, irradiation of a solution of triketone 2.7 in dry deoxygenated C$_6$D$_6$ in a quartz cuvette with 254 nm UV light for 24 h led to the exclusive formation of furan 3.9 in 18% isolated yield (41% conversion, Table 3.3.2, entry 1), and no other products of the reaction were observed. The change of the $\lambda_{\text{max}}$ alone did not enhance the efficiency of the reaction, however the use of 1.0 equiv of benzophenone with 300 nm UV light yielded photoproduct 3.9 in 25% isolated yield (82% conversion, Table 3.3.2, entry 3). The structure of furan 3.9 was confirmed after extensive 2-D NMR study and by HRMS data.
3.4 Conclusions

In this Chapter, polyketones derived from parent triterpenoids bryonolic acid and lanosterol were used as the substrates for divergent Norrish-Yang photocyclization. Irradiation of bryonolic acid-derived tetraketone 2.16 brought a new 6/6/6-fused structural type 3.3 to the resultant chemical library of triterpenoid analogs by the virtue of an unanticipated photopinacolization. The unexpected structure 3.3 was accompanied by the expected formation of 6/4/8-fused product of Yang cyclization 3.2 via pathway a. The use of bryonolic acid-derived triketone 2.14 to force Yang cyclization into pathway b led to predominant pinacol-type coupling reaction to give 6/5/7-fused structure 3.5, despite the desirable arrangement of the ‘bowsprit’ H-5 and the keto group at C-8. The subtle differences between the parent triterpenoids resulted in completely different conformational preferences of the polyketones and concomitantly, completely different spatial orientations of the reactive functionalities. Consequently, Norrish-Yang photocyclization of lanosterol-derived polyketones 2.8 and 2.7 via pathway b was proven to be unfeasible, but the pinacol-type coupling reaction of triketone 2.7 was proven to be predictable. Thus, under our irradiation conditions, tetraketone 2.8 formed a single photocycloadduct 3.6 via pathway a, while triketone 2.7 yielded the anticipated product of photopinacolization 3.9.
3.5 Experimental part

3.5.1 General experimental details

All reactions were run in an atmosphere of dry argon unless otherwise stated. THF was distilled from benzophenone ketyl solution with sodium prior to use. C₆D₆ was deoxygenated by purging with argon gas under stirring for 15 min. Quick syringe transfers were done with disposable syringes and needles.

Photochemical reactions were performed in a multilamp chamber photoreactor equipped with a cooling fan.

Column chromatography was performed with silica gel (particle size 32-63 μm). Analytical and semi-preparative HPLC separations were performed using acetonitrile and water (for HPLC, 99.9%). Analytical thin-layer chromatography (TLC) was carried out using glass-coated silica gel 0.25 mm plates with fluorescent indicator. All reactions that were monitored by TLC were visualized with a 254 nm UV-lamp or using phosphomolybdic acid (PMA) and 1,4-dinitrophenylhydrazine (DNP) stain solutions prepared by well-known protocols.

Chemical shifts of all ¹H and ¹³C NMR spectra reported in δ units, part per million (ppm) with reference to the residual solvent peak (CDCl₃, 7.26 ppm for ¹H NMR and 77.16 ppm, center of triplet, for ¹³C NMR). DEPT, COSY, NOESY, HMQC, HMBC spectra were recorded using standard 2-D NMR pulse sequences.

For the HRMS measurements, Linear ion Trap Quadrupole (LTQ) mass spectrometer was used with Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass analyzer (100 000 resolving power at m/z 400).
3.5.2 General Procedure for Photolyses of Polyketones 2.7, 2.8, 2.14, and 2.16.

A solution of a specified polyketone (20 mg) in dry C₆D₆ (0.6 mL) in a 4 mL quartz cuvette was deoxygenated by purging with dry argon gas for 15 min while stirring. When specified, benzophenone (1.0 equiv) was added to a reaction mixture. A cuvette was then sealed with a Teflon stopper and placed in the middle of a chamber photoreactor, equipped with six UV-lamps (specified λₘₐₓ) and a cooling fan. Solutions were irradiated at rt for a specified amount of time, until specified polyketone was consumed or decomposition was observed, as determined by either TLC or ¹H NMR. After removal of the solvent in vacuo, photoproducts 3.2, 3.3 3.5, 3.6, and 3.9 were isolated by column chromatography on silica with specified ethyl acetate-hexanes mixtures.

3.5.3 Photolyses of Bryonolic acid-derived polyketones 2.14 and 2.16.

Preparation of photoproducts (3.2) and (3.3).

Table 3.2.1 (Entry 1). Following the general procedure for photolyses, the use of tetraketone 2.16 (0.035 mmol) and 254 nm UV light for 45 min gave 3.2 (7 mg, 33%) and 3.3 (2 mg, 10%).

Table 3.2.1 (Entry 2). Following the general procedure for photolyses, the use of tetraketone 2.16 (0.035 mmol) and 300 nm UV light for 90 min gave 3.2 (12.5 mg, 63%) and 3.3 (2.6 mg, 13%).

Table 3.2.1 (Entry 3). Following the general procedure for photolyses, the use of tetraketone 2.16 (0.035 mmol) and 350 nm UV light for 135 min gave 3.2 (6 mg, 30%) and 3.3 (1 mg, 5%).
Table 3.2.1 (Entry 4). Following the general procedure for photolyses, the use of tetraketone 2.16 (0.035 mmol), benzophenone (6.4 mg, 0.035 mmol) and 300 nm UV light for 45 min gave 3.2 (11.2 mg, 56%) and 3.3 (0.1 mg, <1%).

(2aS,5R,6aR,6bS,7aR,8aS,11S,12aR,15aS)-Methyl-11-acetoxy-7a-hydroxy-2a,5,6b,8a,12,12,15a-heptamethyl-8,14,15-trioxoicosahydro-1H-benzo[1',4']
cyclobuta[1',2':6,7]cycloocta[1,2-a]naphthalene-5-carboxylate (3.2). Transparent oil. R$_f$ = 0.37 (EA/hex = 3/7). $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 5.70 (dd, $J_1$ = $J_2$ = 9 Hz, 1H), 3.60 (s, 3H), 3.40 (d, $J$ = 12.6 Hz, 1H), 3.10 (br. s, 1H), 2.75 (d, $J$ = 12.6 Hz, 1H), 2.35 (d, $J$ = 16.2 Hz, 1H), 2.25 (d, $J$ = 18 Hz, 1H), 2.17 (ddd, $J_1 = J_2 = 14.4$ Hz, $J_3$ = 4.2 Hz, 1H), 2.02 (s, 3H), 1.73 (d, $J$ = 18 Hz, 1H), 1.35 (s, 3H), 1.32 (s, 3H), 1.23 (s, 3H), 1.16 (s, 3H), 1.06 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.95 (m, 1H). $^{13}$C NMR (150 MHz, CDCl$_3$, $\delta$): 218.9 (CO), 211.5 (CO), 210.0 (CO), 178.7 (CO), 170.4 (CO), 92.2 (COH), 72.4 (CH), 62.3 (C), 56.9 (C), 55.4 (C), 52.4 (CH$_3$), 47.0 (CH), 43.8 (C), 42.4 (CH$_2$), 41.2 (CH$_2$), 40.7 (C), 40.6 (C), 35.4 (CH$_2$), 33.4 (CH$_2$), 32.8 (CH$_3$), 32.7 (C), 31.22 (CH$_3$), 31.20 (CH$_2$), 29.8 (CH$_2$), 29.1 (CH$_2$), 24.5 (CH$_2$), 23.2 (CH$_2$), 22.4 (CH$_3$), 21.8 (CH$_3$), 21.6 (CH$_3$), 21.3 (CH$_3$), 19.0 (CH$_3$), 17.2 (CH$_3$). HRMS (ESI) m/z calcd for C$_{33}$H$_{48}$O$_8$Na$^+$ [M+Na]$^+$ 595.3247, found 595.3236.

(2R,4aS,6aS,7aS,8aR,10S,12aS,13aR,14aS,14bR)-Methyl-10-acetoxy-7a-
hydroxy-2,4a,6a,9,9,12a,14a-heptamethyl-7,13-dioxoicosahydro-8a,13a-
epoxybenzo[a]tetracene-2-carboxylate (3.3). Transparent oil. R$_f$ = 0.32 (EA/hex = 3/7). $^1$H NMR (600 MHz, CDCl$_3$, $\delta$): 4.81 (dd, $J_1$ =10.8 Hz, $J_2$ = 4.8 Hz, 1H), 3.61 (s, 3H), 2.69 (d, $J$ = 14.4 Hz, 1H), 2.36 (d, 15 Hz, 1H), 2.05 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H), 1.19 (s, 3H), 1.07 (s, 3H), 1.00 (s, 3H), 0.92 (s, 3H), 0.77 (s, 3H). $^{13}$C NMR (150 MHz,
CDCl₃, δ): 212.6 (CO), 212.4 (CO), 178.6 (CO), 170.6 (CO), 90.6 (C), 90.3 (C), 77.3 (C), 76.5 (CH), 52.7 (C), 51.6 (CH₃), 48.7 (C), 44.3 (CH), 40.4 (C), 40.4 (C), 39.8 (CH₂), 38.5 (C), 35.4 (CH₂), 33.9 (CH₂), 32.5 (CH₃), 31.5 (CH₃), 31.3 (C), 31.1 (CH₂), 30.7 (CH₂), 29.91 (CH₂), 29.87 (CH₂), 24.2 (CH₂), 23.1 (CH₂), 22.3 (CH₃), 21.4 (CH₃), 20.8 (CH₃), 19.4 (CH₃), 18.5 (CH₃), 17.7 (CH₃). HRMS (ESI) m/z calcd for C₃₃H₄₈O₈Na⁺ [M+Na]⁺ 595.32414, found 595.32423

**Preparation of bridged furan (3.5).**

*Table 3.2.2 (Entry 1).* Following the general procedure for photolyses, the use of triketone 2.14 (0.036 mmol) and 254 nm UV light for 72 h yielded an inseparable mixture (10 mg) of 3.5 (1.4 mg, 12% brsm, determined by ¹H NMR) and unreacted 2.14 (8.6 mg, 57% conversion, determined by ¹H NMR). Rₓ = 0.4 (EA/hex = 3/7).

*Table 3.2.2 (Entry 2).* Following the general procedure for photolyses, the use of triketone 2.14 (0.036 mmol) and 300 nm UV light for 72 h yielded an inseparable mixture (8.3 mg) of 3.5 (2.1 mg, 15% brsm, determined by ¹H NMR) and unreacted 2.14 (6.2 mg, 69% conversion, determined by ¹H NMR). Rₓ = 0.4 (EA/hex = 3/7).

*Table 3.2.2 (Entry 3).* Following the general procedure for photolyses, the use of triketone 2.14 (0.036 mmol) and 350 nm UV light for 72 h yielded unreacted 2.14 (20 mg, 0% conversion). Rₓ = 0.4 (EA/hex = 3/7).

*Table 3.2.2 (Entry 4).* Following the general procedure for photolyses, the use of triketone 2.14 (0.036 mmol), benzophenone (6.5 mg, 0.036 mmol) and 300 nm UV light for 72 h yielded 3.5 (7.8 mg, 39%). Rₓ = 0.4 (EA/hex = 3/7).

*Table 3.2.2 (Entry 5).* Following the general procedure for photolyses, the use of triketone 2.14 (0.036 mmol), benzophenone (6.5 mg, 0.036 mmol) and 254 nm UV light
for 72 h yielded an inseparable mixture (8.2 mg) of 3.5 (2.5 mg, 18% brsm, determined by $^1$H NMR) and unreacted 2.14 (5.7 mg, 72% conversion, determined by $^1$H NMR). $R_f = 0.4$ (EA/hex = 3/7).

(2R,4aS,6aS,8aS,9aS,11S,13aS,13bR,15aS,15bR)-Methyl-11-acetoxy-13b-hydroxy-2,4a,10,10,13a,15a-hexamethyl-16-oxoicosahydro-6a,8a-methanoindeno[2,1-b]naphtho[1,2-f]oxocine-2-carboxylate (3.5). Analytically pure 3.5 was obtained by the following procedure. A round-bottom flask open to atmosphere was charged with basic alumina (365 mg, 3.58 mmol), followed by the addition of DCM (0.5 mL). A mixture (20 mg), containing 2.14 (17.2 mg, 0.031 mmol) and 3.5 (2.8 mg, 5 μmol) as determined by $^1$H NMR, was dissolved in DCM (0.5 mL) and subsequently added to the resulting suspension. The flask was sealed with a glass stopper and vigorously stirred at rt overnight, at which time the suspension was filtered over a fine sinter funnel and washed successively with ethyl acetate. After removal of the solvent in vacuo, the column chromatography on silica yielded 3.5 as a transparent oil (2 mg, 71%). $R_f = 0.38$ (EA/hex = 25/75). $^1$H NMR (600 MHz, CDCl$_3$, δ): 4.51 (dd, $J_1 = 11.4$ Hz, $J_2 = 4.2$ Hz, 1H), 4.29 (d, $J = 9.6$ Hz, 1H), 4.04 (d, $J = 9.6$ Hz, 1H), 3.64 (s, 3H), 3.07 (br.s, 1H), 2.05 (s, 3H), 1.19 (s, 3H), 1.00 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.85 (s, 3H). $^{13}$C NMR (150 MHz, CDCl$_3$, δ): 212.9 (CO), 179.3 (CO), 171.1 (CO), 87.3 (C), 80.5 (CH), 78.9 (C), 72.6 (CH$_2$), 53.9 (C), 52.0 (CH$_3$), 48.3 (CH), 47.4 (C), 46.7 (CH), 40.7 (C), 39.7 (C), 37.3 (C), 36.8 (CH$_2$), 34.0 (CH$_2$), 33.5 (CH$_2$), 32.2 (CH$_2$), 31.8 (C), 31.3 (CH$_3$), 30.96 (CH$_2$), 30.90 (CH$_3$), 29.3 (CH$_2$), 29.2 (CH$_2$), 29.1 (CH$_3$), 29.0 (CH$_2$), 25.6 (CH$_2$), 25.3 (CH$_2$), 21.4 (CH$_3$), 17.5 (CH$_3$), 17.2 (CH$_3$), 16.6 (CH$_3$). HRMS (ESI) m/z calcd for C$_{33}$H$_{50}$O$_7$Na$^+$ [M+Na]$^+$ 581.34488, found 581.34503.
3.5.4 Photolyses of Lanosterol-derived polyketones 2.7 and 2.8.

Preparation of cyclobutanol (3.6).

Table 3.3.1 (Entry 1). Following the general procedure for photolyses, the use of tetraketone 2.8 (0.0375 mmol) and 254 nm UV light for 20 h yielded 3.6 (9.6 mg, 48%).

Table 3.3.1 (Entry 2). Following the general procedure for photolyses, the use of tetraketone 2.8 (0.0375 mmol) and 300 nm UV light for 12 h yielded 3.6 (5.3 mg, 27%).

Table 3.3.1 (Entry 3). Following the general procedure for photolyses, the use of tetraketone 2.8 (0.0375 mmol), benzophenone (6.8 mg, 0.0375 mmol) and 254 nm UV light for 6 h yielded unreacted 2.8 (8.1 mg, 60% conversion) and 3.6 (8.6 mg, 72% brsm).

Table 3.3.1 (Entry 4). Following the general procedure for photolyses, the use of tetraketone 2.8 (0.0375 mmol), benzophenone (6.8 mg, 0.0375 mmol) and 300 nm UV light for 3 h yielded 3.6 (13.1 mg, 66%).

(R)-Methyl-4-(((1aS,4S,5aR,8aR,11R,11aR,12aR)-4-acetoxy-12a-hydroxy-1a,5,5,8a,11a-pentamethyl-1,7,8-trioxohexadecahydrobenzo[1,4]cyclobuta[1,2-a]cyclopenta[d][8]annulen-11-yl)pentanoate (3.6). Transparent oil. R\textsubscript{f} = 0.25 (EA/hex = 25/75). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}, \(\delta\)): 5.65 (m, 1H), 3.69 (s, 3H), 2.90 (d, J = 13.9 Hz, 1H), 2.83 (br s, 1H), 2.60 (d, J = 13.9 Hz, 1H), 2.07 (s, 3H), 1.37 (s, 3H), 1.28 (s, 3H), 1.16 (s, 3H), 1.16 (s, 3H), 1.10 (d, J = 6.7 Hz, 3H), 0.93 (s, 3H). \textsuperscript{13}C NMR (150 MHz, CDCl\textsubscript{3}, \(\delta\)): 213.3 (CO), 210.0 (CO), 209.5 (CO), 174.3 (CO), 171.3 (CO), 94.9 (C), 78.7 (CH), 63.4 (C), 59.5 (C), 56.2 (C), 53.9 (CH), 51.8 (CH\textsubscript{3}), 47.8 (C), 43.1 (CH\textsubscript{2}), 40.9 (CH\textsubscript{2}), 40.0 (C), 35.6 (CH\textsubscript{2}), 34.2 (CH), 31.9 (CH\textsubscript{2}), 30.9 (CH\textsubscript{2}), 29.3 (CH\textsubscript{2}), 23.0 (CH\textsubscript{2}), 22.7 (CH\textsubscript{2}), 21.6 (CH\textsubscript{3}), 21.4 (CH\textsubscript{3}), 20.5 (CH\textsubscript{3}), 20.3 (CH\textsubscript{3}), 19.8 (CH\textsubscript{3}), 19.5 (CH\textsubscript{3}), 17.7 (CH\textsubscript{3}). HRMS (ESI) m/z calcd for C\textsubscript{30}H\textsubscript{44}O\textsubscript{8}Na\textsuperscript{+} [M+Na]\textsuperscript{+} 555.2934, found 555.2933.
Preparation of bridged furan (3.9).

Table 3.3.2 (Entry 1). Following the general procedure for photolyses, the use of triketone 2.7 (0.0386 mmol) and 254 nm UV light for 24 h yielded unreacted 2.7 (11.8 mg, 41% conversion) and 3.9 (3.6 mg, 44% brsm).

Table 3.3.2 (Entry 2). Following the general procedure for photolyses, the use of triketone 2.7 (0.0386 mmol) and 300 nm UV light for 24 h yielded unreacted 2.7 (6.3 mg, 69% conversion) and 3.9 (2.6 mg, 19% brsm).

Table 3.3.2 (Entry 3). Following the general procedure for photolyses, the use of triketone 2.7 (0.0386 mmol), benzophenone (7 mg, 0.0386 mmol) and 300 nm UV light for 24 h yielded unreacted 2.7 (3.7 mg, 82% conversion) and 3.9 (5 mg, 31% brsm).

Table 3.3.2 (Entry 4). Following the general procedure for photolyses, the use of triketone 2.7 (0.0386 mmol), benzophenone (7 mg, 0.0386 mmol) and 254 nm UV light for 24 h yielded unreacted 2.7 (9 mg, 55% conversion) and 3.9 (4.3 mg, 39% brsm).

(R)-Methyl-4-((1R,3aR,5aR,6aS,8S,10aS,10bS,12aR)-8-acetoxy-10b-hydroxy-7,7,10a,12a-tetramethyl-13-oxotetradecahydro-1H-3a,5a-methanocyclopenta[f]indeno[2,1-b]oxocin-1-yl)pentanoate (3.9). Transparent oil. R_f = 0.23 (EA/hex = 25/75). 1H NMR (600 MHz, CDCl3, δ): 4.56 (dd, J1 = 11.9 Hz, J2 = 4.7 Hz, 1H), 4.26 (d, J = 9.3 Hz, 1H), 3.80 (d, J = 9.3 Hz, 1H), 3.66 (s, 3H), 3.63 (s, 1H), 2.05 (s, 3H), 0.96 (s, 3H), 0.92 (d, J = 6.4 Hz, 3H), 0.88 (s, 3H), 0.86 (s, 3H), 0.85 (s, 3H). 13C NMR (150 MHz, CDCl3, δ): 209.9 (CO), 174.5 (CO), 171.1 (CO), 89.5 (C), 80.89 (CH), 80.88 (C), 74.1 (CH2), 61.4 (C), 53.5 (CH), 51.7 (CH3), 49.9 (C), 49.2 (CH), 49.1 (C), 37.0 (C), 34.8 (CH), 31.3 (CH2), 31.2 (CH2), 30.4 (CH2), 29.8 (CH2), 29.7 (CH2), 29.6 (CH2), 29.4 (CH3), 27.4 (CH2), 26.2 (CH2), 24.4 (CH2), 21.4 (CH3), 19.0 (CH3), 16.8
(CH₃), 15.4 (CH₃), 15.3 (CH₃). HRMS (ESI) m/z calcd for C₃₀H₄₆O₇Na⁺ [M+Na]⁺ 541.3141, found 541.3142.

3.6 References


Chapter 4. Thesis summary and future directions

4.1 Thesis summary

In Chapter 1, a history of natural products in drug discovery was introduced. The evolution of synthetic molecules in pharmaceutical research was subsequently described, followed by the discussion of various techniques for the synthesis of molecular libraries. The concept of diversity-oriented synthesis for the construction of chemical libraries concluded the discussion. Based on the literature review, the use of natural products as starting materials for the development of complex and diverse collections of molecules was formulated to stand at the forefront of contemporary medicinal chemistry. The major advantage of this approach is that the inherent complexity of natural product-derived substrates drives the regio- and stereoselectivity of reactions. The choice of a triterpenoid family of natural products as an archetype for the synthesis of a library of analogues was justified by the range of skeletal and subsequent medicinal properties of the members of this natural product family.

The proposed synthetic strategy originated from triterpenoids lanosterol and bryonolic acid. Despite the different natural origins of these molecules, both triterpenoids have the common bridging double bond at the fusion of rings B and C. Under the conditions of ruthenium tetroxide oxidation (Sharpless catalytic protocol) both triterpenoids yielded a different distribution of oxidation products. Thus, oxidation of the unsaturated B/C ring fusion of lanosterol resulted in predominant formation of the product of oxidative cleavage 2.6, accompanied by α,β-unsaturated ketones 2.3 and 2.4. Conversely, oxidation of the unsaturated B/C ring fusion of bryonolic acid primarily
followed a C-H activation route leading to triketone 2.14. The targeted product of cleavage of the double bond of 2.12 was obtained as a minor product.

The pseudo-symmetrical diketones 2.6 and 2.12 were hypothesized to undergo divergent transannular aldol addition reactions. On the basis of a literature review, lanosterol-derived diketone 2.6 was envisioned to undergo this reaction only in one direction, while diketone 2.12 was expected to form both possible products. Accordingly, diketone 2.12 yielded two aldol adducts via pathway a, with syn and anti ring fusion, and a single syn-adduct via pathway b. The use of diketone 2.6 resulted in the formation of the expected syn- and anti-adducts via pathway a (2.19 and 2.20, respectively), as well as the unexpected products 2.23-2.26 with previously intractable 6/7/5/5 steroidal ring composition.

The use of lanosterol-derived triketones 2.7 and 2.34 resulted in the production of expected linear steroid analogues 2.9 and 2.35, respectively. Both aldol adducts had anti configuration at the newly formed ring junctions. The use of bryonolic acid-derived triketone 2.14 yielded linear 6/6/6/6/6-fused triterpenoid 2.35 also with anti configuration at the newly formed B/C-ring fusion. Despite contrasting conformational preferences of lanosterol- and bryonolic acid-derived triketones, the resultant products were unifying in that the desired linear adducts were formed in a similar and predictable fashion via six-membered chair-like transition states.

The aldol reactivity of lanosterol-derived tetraketone 2.8 leading to bridged product 2.36 was previously rationalized by another group. Under the standard aldol conditions, bryonolic acid-derived tetraketone 2.16 was expected to yield a similar structure via aldol addition reaction. In sharp contrast with its lanosterol-derived counterpart, tetraketone
2.16 formed the product of transannular hemiketalization 2.39. This result became evident after careful examination of the ground state conformation of 2.16. Specifically, the intermediate enolate was shown to be geometrically predisposed for the formation of transannular vinylic hemiketal, which was easily hydrolyzed into the resultant product 2.39. The conformational analysis of tetraketone 2.8 resulted in the identification of two co-existent conformations of this molecule. While the most-populated conformer predictably underwent aldol addition reaction leading to 2.36, its least-populated counterpart preserved the capability for transannular hemiketalization to yield product 2.41, which would be structurally similar to 2.39. However, the attempts to control the chemoselectivity of this reaction using the differential reactivity of these two conformers were unsuccessful.

Pseudo-symmetrical tetraketones 2.8 and 2.16 were envisioned to constitute bichromophoric substrates for Norrish-Yang photocyclization leading to 6/4/8-fused triterpenoid analogues. In this reaction, the excitation of the opposing keto groups at C-8 and C-11 would theoretically lead to two possible regioisomeric photoproducts. Irradiation of bryonolic acid-derived substrate 2.16 yielded only the product of pathway a 3.2, while the formation of the second possible product via pathway b (3.1) was not observed under various irradiation conditions. This observation was rationalized based on the allowance of minimal interatomic distance formulated by the Scheffer group. Both conformers of tetraketone 2.8 were taken into consideration for the prediction of Norrish-Yang reactivity of this molecule. While the least-populated conformer was predictably unreactive, the most-populated conformer was envisioned to undergo Norrish-Yang only through pathway a, and the pathway b was anticipated to be intractable for this molecule.
based on the special arrangements of reactive functionalities. Accordingly, only product 3.6 was formed after irradiation of 2.8.

The triketones 2.7 and 2.14 were taken to break the molecular symmetry. Specifically, the C-7, C-8 dione system became the major chromophoric reactive group in these molecules. The conformational analysis using prior accumulated knowledge revealed that the formation of product 3.4 from trikone 2.14 is theoretically possible. However, upon irradiation of 2.14 only the unexpected product of transannular pinacolization 3.5 was formed, but the desired 3.4 was not observed. Thus, the formation of a similar product of pinacolization from lanosterol-derived substrate 2.7 could be predicted. Accordingly, product 3.9 was formed exclusively after irradiation of 2.7.

Despite the common unsaturation at the B/C ring fusion, the structural differences between the parent natural products led to dramatic differences in reactivity of the polyketones derived from these molecules, and, concomitantly, the dramatically different composition of the resultant chemical library. The results outlined here constitute a general approach that could be applied to a wide range of natural product families, allowing access to broad categories of novel and potentially biologically relevant molecules that would be otherwise very difficult to attain.

4.2 Future directions

One of the most promising activities that has recently been discovered among various members of triterpenoid family includes inhibition of inflammation via Nrf2-Keap1 regulatory pathway.1-2 Moreover, Gatbonton-Schwager et al.2 recently showed that
bryonolic acid and oleanolic acid are potent inhibitors of Nrf2-Keap1 interaction. In this report, the inhibition of nitric oxide (NO) production in LPS-induced RAW macrophages was measured using Griess assay. Specifically, treatment with bryonolic acid (BA) reduced nitrite levels, showing an IC₅₀ value of 53.3 ± 3 μM after a 24 h treatment. RAW cells remained viable in BA concentrations as high as 300 μM (gray shaded area), but cytotoxicity was apparent at higher concentrations, as measured by the MTT assay (Figure 4.1.1.).

![Figure 4.1.1. Biological activity of BA, and introduction of CDDO](image)

**Figure 4.1.1.** Biological activity of BA, and introduction of CDDO

Furthermore, oleanolic acid served as a platform for the discovery of the semisynthetic triterpenoid 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO).³⁴ CDDO and its methyl ester derivative, CDDO-Me, may be the strongest known inhibitors of the de novo synthesis of iNOS and COX-2, and have advanced to phase III clinical trials for renal sparing effects in diabetic nephropathy.

The future directions will bifurcate into the synthesis of molecules containing a ring A cyano-enone functionality of CDDO, and evaluation of the bioactivity of the resulting
compounds. Synthesis of the cyano-enone functionality of CDDO and its analogues was previously described by the groups of Honda,4-6 Gribble7-8 and Sporn9-10 (Scheme 4.1.1).

Scheme 4.1.1. Synthesis of analogues of CDDO

The synthesis begins with Jones oxidation of the deprotected parent triterpenoid to form the corresponding 3-oxo compound (step A). The resultant ketone is then converted to the corresponding enolate, followed by reaction with ethyl formate to form the α-formyl derivative (step B). The formyl derivative is then converted to the isoxazole by reaction with hydroxylamine (step C). The opening of the isoxazole ring under strong basic conditions yields the new cyano compound (step D). The compound is subsequently converted to the desired ring A cyano-enone derivative by dehydrogenation with DDQ (step E).

The experiments will continue toward the evaluation of the resultant secondary library for inhibition of production of (NO) in LPS-induced RAW cells using Griess assay. The cell viability will be subsequently measured using MTT assay.

4.3 References


Appendix I. Key 2-D NMR correlations of synthesized compounds

Figure AI-1. Key HMBC and COSY correlations of 2.9

Figure AI-2. Key NOESY correlations of 2.9

Figure AI-3. Key HMBC correlations of 2.14
Figure AI-4. Key HMBC and COSY correlations of 2.17

Figure AI-5. Key HMBC and COSY correlations of 2.19

Figure AI-6. Key NOESY correlations of 2.19
Figure AI-7. Key HMBC and COSY correlations of 2.20

Figure AI-8. Key NOESY correlations of 2.20

Figure AI-9. Key HMBC and COSY correlations of 2.21
Figure Al-10. Key HMBC and COSY correlations of 2.22

Figure Al-11. Key NOESY correlations of 2.22

Figure Al-12. Key HMBC and COSY correlations of 2.23
Figure Al-13. Key NOESY correlations of 2.23

Figure Al-14. Key HMBC and COSY correlations of 2.24

Figure Al-15. Key NOESY correlations of 2.24
Figure AI-16. Key HMBC and COSY correlations of 2.25

Figure AI-17. Key NOESY correlations of 2.25

Figure AI-18. Key HMBC correlations of 2.26
Figure AI-19. Key NOESY correlations of 2.26

Figure AI-20. Key HMBC and COSY correlations of 2.27

Figure AI-21. Key NOESY correlations of 2.27
Figure AI-22. Key HMBC and COSY correlations of 2.28

Figure AI-23. Key NOESY correlations of 2.28

Figure AI-24. Key HMBC and COSY correlations of 2.29
Figure AI-25. Key NOESY correlations of 2.29

Figure AI-26. Key HMBC and COSY correlations of 2.31

Figure AI-27. Key NOESY correlations of 2.31
Figure A1-28. Key HMBC and COSY correlations of 2.32

Figure A1-29. Key NOESY correlations of 2.32

Figure A1-30. Key HMBC and COSY correlations of 2.33
Figure AI-31. Key HMBC and COSY correlations of 2.35

Figure AI-32. Key NOESY correlations of 2.35

Figure AI-33. Key HMBC and COSY correlations of 2.36
Figure A1-34. Key NOESY correlations of 2.36

Figure A1-35. Key HMBC and COSY correlations of 2.37

Figure A1-36. Key NOESY correlations of 2.37
Figure AI-37. Key HMBC and COSY correlations of 2.38

Figure AI-38. Key NOESY correlations of 2.38

Figure AI-39. Key HMBC and COSY correlations of 2.41
Figure AI-40. Key NOESY correlations of 2.41

Figure AI-41. Key HMBC and COSY correlations of 3.6

Figure AI-42. Key NOESY correlations of 3.6
Figure AI-43. Key HMBC and COSY correlations of 3.9

Figure AI-44. Key NOESY correlations of 3.9
Appendix II. NMR spectra of synthesized compounds

Figure AII-1. $^1$H NMR spectrum of 2.1
Figure AII-2. $^{13}$C NMR spectrum of 2.1
Figure AII-3. $^1$H NMR spectrum of 2.2
Figure AII-4. $^{13}$C NMR spectrum of 2.2
Figure AII-5. $^1$H NMR spectrum of 2.3
Figure AII-6. $^{13}$C NMR spectrum of 2.3
Figure AII-7. HMQC spectrum of 2.3
Figure AII-8. HMBC spectrum of 2.3
Figure AII-9. $^1$H spectrum of 2.4
Figure AII-10. $^{13}$C spectrum of 2.4
Figure AII-11. COSY spectrum of 2.4
Figure AII-12. HMBC spectrum of 2.4
Figure AII-13. 1H spectrum of 2.5
Figure AII-14. $^{13}$C spectrum of 2.5
Figure AII-15. $^1$H spectrum of 2.6
Figure AII-16. $^{13}$C spectrum of 2.6
Figure AII-17. 1H spectrum of 2.7
Figure AII-18. $^{13}$C spectrum of 2.7
Figure AII-19. $^1$H spectrum of 2.8
Figure AII-20. $^{13}$C spectrum of 2.8

Pulse Sequence: s2pul
Solvent: Toluene
Temp. 0.2°C / 273.3 K
User: 1-14-07
File: T=0
INOVA-500 “Joe”

Pulse 58.7 degrees
Acq. time 1,800 sec
Width 40000.0 Hz
512 repetitions
CISTORE C13, 150.865641 MHz
DECcouple H1, 999.909218 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 524288
Total time 11 min, 10 sec
Figure AII-21. COSY spectrum of 2.8
Figure AII-22. HMBC spectrum of 2.8
Figure AII-23. $^1$H spectrum of 2.9
Figure AII-24. $^{13}$C spectrum of 2.9
Figure AII-25. HMQC spectrum of 2.9
Figure AII-26. HMBC spectrum of 2.9
Figure AII-27. COSY spectrum of 2.9
Figure AII-28. NOESY spectrum of 2.9
Figure AII-30. 13C spectrum of 2.10

STANDARD CARBON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0°C / 298.1 K
User: 1-14-87
File: C13
INOVA-500 "jco"

Pulse 58.7 degrees
Acq. time 1.300 sec
Width 40000.0 Hz
152 repetitions
OBSERVE C13, 150.8466510 MHz
DECOUPLE H1, 599.9097318 MHz
Power 42 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 131072
Total time 44 min, 41 sec
Figure AII-31. 1H spectrum of 2.11
Figure AII-32. $^{13}$C spectrum of 2.11
Figure AII-33. $^1$H spectrum of 2.12
Figure AII-34. $^{13}$C spectrum of 2.12
Figure AII-35. 1H spectrum of 2.14

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Archive directory: /export/home/va2/vnmrsys/data
Sample directory: va2.08Dec2010

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: VA2_064
INOVA 500 "joe"

Relax, delay 1,000 sec
Pulse 45.0 degrees
Acq. time 3.744 sec
Width 0395.9 Hz
8 repetitions
OBSERVE H1, 199.7-134.786 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 38 sec
Figure AII-36. $^{13}$C spectrum of 2.14
Figure AII-37. HMQC spectrum of 2.14
Figure AII-38. HMBC spectrum of 2.14
Figure AII-39. $^1$H spectrum of 2.15
Figure AII-40. $^{13}$C spectrum of 2.15
Figure AII-41. $^1$H spectrum of 2.16
Figure AII-42. $^{13}$C spectrum of 2.16
Figure AII-43. 1H spectrum of 2.17

STANDARD PROTON PARAMETERS
Pulse Sequences: s2pul
Solvent: CDC13
Temp: 25.0°C / 298.1 K
File: 1H
INOWA-500 "Joe"

Pulse 75.7 degrees
Acq. time: 3.500 sec
Width: 8000.0 Hz
8 repetitions
OBSERVE H1, 599.9967214 MHz
DATA PROCESSING
FT size: 65536
Total time: 0 min, 28 sec
Figure AII-44. $^{13}$C spectrum of 2.17
Figure AII-45. HMQC spectrum of 2.17
Figure AII-46. HMBC spectrum of 2.17
Figure AII-47. COSY spectrum of 2.17
Figure AII-48. $^1$H spectrum of 2.18
Figure AII-49. $^{13}$C spectrum of 2.18
Figure AII-50. $^1$H spectrum of 2.19
Figure AII-51. $^{13}$C spectrum of 2.19
Figure AII-52. HMQC spectrum of 2.19
Figure AII-53. HMBC spectrum of 2.19
Figure AII-54. COSY spectrum of 2.19
Figure AII-55. NOESY spectrum of 2.19
Figure AII-56. $^1$H spectrum of 2.20
Figure AII-57. $^{13}$C spectrum of 2.20
Figure AII-58. HMQC spectrum of 2.20
Figure AII-59. HMBC spectrum of 2.20
Figure AII-60. COSY spectrum of 2.20
Figure AII-61. NOESY spectrum of 2.20
Figure AII-62. $^1$H spectrum of 2.21
Figure AII-63. $^{13}$C spectrum of 2.21
Figure AII-64. HMQC spectrum of 2.21
Figure AII-65. HMBC spectrum of 2.21
Figure AII-66. COSY spectrum of 2.21
Figure AII-67. $^1$H spectrum of 2.22
Figure AII-68. $^{13}$C spectrum of 2.22
Figure AII-69. HMQC spectrum of 2.22
Figure AII-70. HMBC spectrum of 2.22
Figure AII-71. COSY spectrum of 2.22
Figure AII-72. NOESY spectrum of 2.22
Figure AII-73. $^1$H spectrum of 2.23
Figure AII-74. $^{13}$C spectrum of 2.23
Figure AII-75. HMQC spectrum of 2.23
Figure AII-76. HMBC spectrum of 2.23
Figure AII-77. COSY spectrum of 2.23
Figure AII-78. NOESY spectrum of 2.23
Figure AII-79. $^1$H spectrum of 2.24
Figure AII-80. $^{13}$C spectrum of 2.24
Figure AII-81. HMQC spectrum of 2.24
Figure AII-82. HMBC spectrum of 2.24
Figure AII-83. COSY spectrum of 2.24
Figure AII-84. NOESY spectrum of 2.24
Figure AII-85. $^1$H spectrum of 2.25
Figure AII-86. 13C spectrum of 2.25

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
User: 1-14-87
File: C13
INNVA-500 "joe"

Pulse 56.7 degrees
Acq. time 1.300 sec
Width 40000.0 Hz
512 repetitions
OBSERVE C13, 150.846422 MHz
DECOUPLE H1, 599.997618 MHz
Power 42 dB continuously on
WALTZ-16 modulated
DATA PROCESSING
Line-broadening 0.5 Hz
FT size 524288
Total time 1 hr, 29 min, 23 sec
Figure AII-87. HMQC spectrum of 2.25
Figure AII-88. HMBC spectrum of 2.25
Figure AII-89. COSY spectrum of 2.25
Figure AII-90. NOESY spectrum of 2.25
Figure AII-91. $^1$H spectrum of 2.26
Figure AII-92. $^{13}$C spectrum of 2.26
Figure AII-93. HMQC spectrum of 2.26
Figure AII-94. HMBC spectrum of 2.26
Figure AII-95. COSY spectrum of 2.26
Figure AII-96. NOESY spectrum of 2.26
Figure AII-97. 1H spectrum of 2.27

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Sample directory: vai2_18Apr2011
File: PROTON

Pulse Sequence: spul
Solvent: CDCl3
Ambient temperature
INOVA-400 "Chen400"

Relax, delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.744 sec
Width 395.9 Hz
8 repetitions

OBSERVE 1H, 399.2434710 MHz
DATA PROCESSING
FT size 65536
Total time 0 min. 38 sec
Figure AII-98. $^{13}$C spectrum of 2.27
Figure AII-99. HMQC spectrum of 2.27
Figure AII-100. HMBC spectrum of 2.27
Figure AII-101. COSY spectrum of 2.27
Figure AII-102. NOESY spectrum of 2.27
Figure AII-104. 13C spectrum of 2.28
Figure AII-105. HMQC spectrum of 2.28
Figure AII-106. HMBC spectrum of 2.28
Figure AII-107. COSY spectrum of 2.28
Figure AII-108. NOESY spectrum of 2.28
Figure AII-109. 1H spectrum of 2.29
Figure AII-110. 13C spectrum of 2.29
Figure AII-111. HMQC spectrum of 2.29
Figure AII-112. HMBC spectrum of 2.29
Figure AII-113. COSY spectrum of 2.29
Figure AII-114. NOESY spectrum of 2.29
Figure AII-115. 1H spectrum of 2.31
Figure AII-116. 13C spectrum of 2.31
Figure AII-117. HMQC spectrum of 2.31
Figure AII-118. HMBC spectrum of 2.31
Figure AII-119. COSY spectrum of 2.31
Figure AII-120. NOESY spectrum of 2.31
Figure AII-121. $^1$H spectrum of 2.32

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Soltvent: CDCl3
Temp.: 25.0 °C / 298.1 K
File: H1
INNOVA 500 "joe"

Pulse 75.7 degrees
Acq. time 3.300 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 599.9067200 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 56 sec
Figure AII-122. $^{13}$C spectrum of 2.32
Figure AII-123. HMQC spectrum of 2.32
Figure AII-124. HMBC spectrum of 2.32
Figure AII-125. COSY spectrum of 2.32
Figure AII-126. NOESY spectrum of 2.32
Figure AII-127. $^1$H spectrum of 2.33
STANDARD CARBON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0°C/298.1 K
User: 1-14-87
File: C13
INOVVA-500 "jvo"

Pulse 58.7 degrees
Acq. time 1.300 sec
Width 40000.0 Hz
1216 repetitions

Observe C13, 150.8466412 MHz
Decouple H1, 599.96097318 MHz
Power 42 dB
Continuous on

WALTZ-16 modulated

DATA PROCESSING
Line broadening 0.5 Hz
FT size 131072
Total time 44 min, 41 sec
Figure AII-129. HMQC spectrum of 2.33
Figure AII-130. HMBC spectrum of 2.33
Figure AII-131. $^1$H spectrum of 2.35
Figure AII-132. $^{13}$C spectrum of 2.35
Figure AII-133. HMQC spectrum of 2.35
Figure AII-134. HMBC spectrum of 2.35
Figure AII-135. COSY spectrum of 2.35
Figure AII-136. NOESY spectrum of 2.35
Figure AII-137. $^1$H spectrum of 2.36

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature: INOVA-400 °C/chem400°
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.744 sec
Width 0.395.9 Hz
# repetitions
OBSERVE H1, 399.7434706 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 0 sec
Figure AII-138. $^{13}$C spectrum of 2.36
Figure AII-139. HMQC spectrum of 2.36
Figure AII-140. HMBC spectrum of 2.36
Figure AII-141. COSY spectrum of 2.36
Figure AII-142. NOESY spectrum of 2.36
Figure AII-143. $^1$H spectrum of 2.37

Pulse Sequence: cdpul
Solvent: CDCl3
Ambient temperature
FMR: VA2500
INOVA-500 "Joe"

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.744 sec
Width 6995.0 Hz
8 repetitions
RESERVE: H1, 398.74234712 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 38 sec
Figure AII-144. $^{13}$C spectrum of 2.37
Figure AII-145. HMQC spectrum of 2.37
Figure AII-146. HMBC spectrum of 2.37
Figure AII-147. COSY spectrum of 2.37
Figure AII-148. NOESY spectrum of 2.37
Figure AII-149. $^1$H spectrum of 2.38

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0 °C / 298.1 K
File: H1
INova-500 "Joe"

 Pulse: 75.7 degrees
 Acq. time 3.500 sec
 Width 8000.0 Hz
 16 repetitions
 OBSERVE: H1, 599.006/7239 MHz
 DATA PROCESSING
 FT size 65536
 Total time 0 min, 56 sec
Figure AII-150. $^{13}$C spectrum of 2.38
Figure AII-151. HMQC spectrum of 2.38
Figure AII-152. HMBC spectrum of 2.38
Figure AII-153. COSY spectrum of 2.38
Figure AII-154. NOESY spectrum of 2.38
Figure AII-155. 1H spectrum of 2.39
Figure AII-156. 13C spectrum of 2.39
Figure AII-157. HMQC spectrum of 2.39
Figure AII-158. HMBC spectrum of 2.39
Figure AII-159. COSY spectrum of 2.39
Figure AII-160. NOESY spectrum of 2.39
Figure AII-161. $^1$H spectrum of 2.41

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Temp: 25.0°C / 298.1 K
INOVA-600 "chem600"

Pulse 75.7 degrees
Acq. time 3.500 sec
Width 8000.0 Hz
8 repetitions
OBSERVE H1, 599.9067223 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 28 sec
Figure AII-162. $^{13}$C spectrum of 2.41
Figure AII-163. HMQC spectrum of 2.41
Figure AII-164. HMBC spectrum of 2.41
Figure AII-165. COSY spectrum of 2.41
Figure AII-166. NOESY spectrum of 2.41
SUDDEN PROTON PARAMETERS
Pulse Sequence: 2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Files: H1
INOVA-500 "Joe"

Pulse 75.7 degrees
Acq. time 3.500 sec
Width 8000.0 Hz
8 repetitions

OBSERVE: H1, 599.9007222 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 28 sec

Figure AII-167. \( ^1H \) spectrum of 3.2
Figure AII-168. $^{13}$C spectrum of 3.2
Figure AII-169. HMQC spectrum of 3.2
Figure AII-170. HMBC spectrum of 3.2
Figure AII-171. COSY spectrum of 3.2
Figure AII-172. NOESY spectrum of 3.2
Figure AII-173. $^1$H spectrum of 3.3
Figure AII-174. $^{13}$C spectrum of 3.3
Figure AII-175. HMQC spectrum of 3.3
Figure AII-176. HMBC spectrum of 3.3
Figure AII-177. COSY spectrum of 3.3
Figure AII-178. NOESY spectrum of 3.3
Figure AII-179. $^1$H spectrum of 3.5
Figure AII-180. $^{13}$C spectrum of 3.5
Figure AII-181. HMQC spectrum of 3.5
Figure AII-182. HMBC spectrum of 3.5
Figure AII-183. COSY spectrum of 3.5
Figure AII-184. NOESY spectrum of 3.5
Figure AII-185. $^1$H spectrum of 3.6

STANDARD PROTON PARAMETERS

Pulse Sequence: $^{1}$Hpol
Solvent: CDCl3
Temp: 25.0 °C / 298.1 K
INOVA-600 "chem600"

Pulse: 75.2 degrees
Acq. time 3.000 sec
Width 8000.0 Hz
8 repetitions
OFFSET: H1, 599.900/7241 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 28 sec
Figure AII-186. $^{13}$C spectrum of 3.6
Figure AII-187. HMQC spectrum of 3.6
Figure AII-188. HMBC spectrum of 3.6
Figure AII-189. COSY spectrum of 3.6
Figure AII-190. NOESY spectrum of 3.6
Figure AII-191. $^1$H spectrum of 3.9
Figure AII-192. $^{13}$C spectrum of 3.9

Pulse Sequence: s2pul
Solvent: CDCl$_3$
Temp: 25.0 C / 298.1 K
Users: 1-14-07
INOVA-600 "chem600"

Pulse 50.7 degrees
Acq. time 1.000 sec
Width 60000.0 Hz
31458 repetitions

OBSEERVE C13, 150.8406407 MHz
DECOUPLE H1, 599.9997318 MHz
Power 42 dB continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 524288
Total time 11 hr. 26 min. 30 sec.
Figure AII-193. HMQC spectrum of 3.9
Figure AII-194. HMBC spectrum of 3.9
Figure AII-195. COSY spectrum of 3.9
Figure AII-196. NOESY spectrum of 3.9
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