On the total synthesis of terpenes containing quaternary stereocenters
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ABSTRACT: Shortly after the discovery of the Mycobacterium tuberculosis specific and abundant lipid 1-tuberculosinyl adenosine (1-TbAd), which is associated with the virulent enzyme Rv3378c, enantiopure synthetic material was desired to facilitate further research. This chapter highlights our efforts focusing on the first asymmetric total synthesis of 1-TbAd. Two routes were explored: a chiral pool synthesis starting from the commercially available natural product sclareolide, and a strategy based on an asymmetric Diels-Alder cycloaddition. The total synthesis was accompanied by computational mechanistic studies into the course of the Diels-Alder reaction, understanding its mechanism of stereoinduction.

This chapter will be published in part:

The DFT studies were performed by I. C. Wan and Prof. Dr. F. M. Bickelhaupt.
CHAPTER 4

4.1 Introduction

Tuberculosis is an infectious disease caused by the bacterium Mycobacterium tuberculosis (Mtb). Although not prevalent in First World countries, it is responsible for a mortality rate exceeding 1.5 million deaths annually, mainly in developing countries.\(^1\) The persistence of Mtb as the world’s most important bacterial pathogen can be attributed to two key factors. First, intracellular survival of the bacterium is granted by successful infection of the endosomal network of phagocytes. In these phagosomes Mtb is able to arrest phagosome maturation by actively inhibiting pH-dependent killing mechanisms and is protected from immune responses during a decades long infection process.\(^2\)

Secondly, its unusually hydrophobic, multi-layered cell wall functions as an additional source of protection.\(^3\) Despite of over a century of active research on Mtb, the precise lipid composition, which makes up for its cell wall, remains unknown. Discovery of new lipids in Mtb can assist understanding Mtb’s survival and virulence. In addition, providing that these lipids are specific for pathogenic Mtb, these compounds could act as *bona fide* chemical markers for infection, as no such tests exists currently.\(^4\)

An analytical chemistry approach allowing rapid profiling of lipid components in Mycobacteria is the recently developed comparative lipidomics platform.\(^5\) The lipidomics platform allows detailed chemotaxonomic analysis of Mtb and already proved to be useful in the revision of the biosynthetic pathway of mycobactin.\(^6\) Recently, we communicated the isolation of a novel halimane-type diterpene nucleoside produced by Mtb using this lipidomics platform.\(^7\) The compound, characterized as 1-tuberculosinyl adenosine (1-TbAd, Figure 1) was found to be an abundant, pathogenic Mtb-specific lipid.\(^8\) The biosynthesis of 1-TbAd requires the virulence-associated enzyme Rv3378c, of which its locus (Rv3377c–Rv3378c) has shown to be essential in phagosome maturation arrest.\(^9\) 1-TbAd is therefore expected to be involved in phagosomal survival of Mtb. Very recently, two closely related compounds, tuberculosinyl-2’-deoxyadenosine (“2’-deoxy 1-TbAd”) and tuberculosinyl 2’-O-acetyladenosine (2’-acetyl TbAd, Figure 1), were discovered.\(^10\) The structures of these analogues were tentatively assigned based on mass spectrometry.

![Figure 1. Tuberculosinyl adenosines from Mycobacterium tuberculosis.](image)

The specificity of 1-TbAd in the pathogenic Mtb makes it a promising chemical marker for tuberculosis infection. It was shown that 1-TbAd is produced *in vivo* in infected BLB/c mice and could be readily detected *ex vivo* in whole-lung homogenates using a
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one-step RP-HPLC-MS method. Next to the detection of 1-TbAd, an unknown isomer with a nearly identical fragmentation pattern was observed which, was shown to be the pseudo-isomer N^6-tuberculosinyl adenosine (N^6-TbAd, Figure 1). This compound is also a specific chemical marker and was postulated to arise from an in vivo Dimroth rearrangement of 1-TbAd. Notably, it was recently shown that 1-TbAd and N^6-TbAd can also be detected in human patient sputum samples.

In this chapter, we report our extensive efforts regarding the enantio- and diastereoselective total synthesis of 1-TbAd and N^6-TbAd. Throughout the course of the investigation several synthetic strategies towards the optically active halimane-skeleton were scrutinized. We mainly focused on the installation of the bicyclic core by means of an asymmetric Diels-Alder cycloaddition. Although many reactions were met with failure, we eventually managed to successfully complete the total synthesis of 1-TbAd (and its congeners, (Z)-1-TbAd, 2'-deoxy 1-TbAd, and ^13C-labelled 1-TbAd) and N^6-TbAd in a stereoselective manner.

4.2 Retrosynthetic analysis

As outlined in the previous chapter, 1-TbAd can be accessed by N-alkylation of adenosine with tuberculosinyl chloride 1, our first retrosynthetic disconnection (Scheme 1). The interesting part of the molecule from a synthetic perspective is the bicyclic core structure of the halimane (tuberculosinyl) skeleton. Containing three contiguous stereogenic centers, one being all-carbon quaternary, two synthetic routes were explored to stereoselectively construct this structure.

At first we envisioned acetate 2 as a suitable precursor to tuberculosinyl chloride 1. We knew from the literature that isomeric acetate 3 could be easily constructed starting from naturally occurring and commercially available (+)-sclareolide (~105 $ / 25 gr), as reported by the George laboratory in 2012. Rearrangement of the sclareolide core by consecutive, stereospecific, Wagner-Meerwein shifts produces acetate 3, having the all-carbon quaternary stereocenter constructed. One intriguing and daunting problem arises however, as a challenging olefin isomerization in 3 has to be performed to access 4. Due to the intrinsic (near) symmetry of 3, regioselective isomerization is regarded provocative. Aside from regioselectivity in the isomerization, another difficult task, namely diastereoselectivity has to be addressed. Despite these foreseen challenges, we were eager to investigate this synthetic route, as rapid access to the halimane core structure was guaranteed, when successful.
Scheme 1. Retrosynthesis of 1-tuberculosinyl adenosine (1-TbAd).

In 2010, the groups of Sorensen\textsuperscript{[13]} and Snider\textsuperscript{[14]} independently reported a synthesis of tuberculosinol (see chapter 3).\textsuperscript{[15]} Both routes relied on a Diels-Alder reaction between 6,6-dimethyl-1-vinylcyclohexene\textsuperscript{6}\textsuperscript{[16]} and a tigloyl based dieneophile\textsuperscript{5} leading to the racemic core (Scheme 2). This Diels-Alder reaction is as such very productive, as it forms the three stereocenters, one of which is quaternary. The reaction, however, is also highly demanding, as neither the diene nor the dienophile is very reactive and the cycloaddition has to occur with \textit{exo} selectivity.

Regarding the diastereoselectivity of the Diels-Alder cyclization, some empirical data was gathered which allowed us to theorize on \textit{exo} selectivity in the [4+2] cycloaddition with 6,6-dimethyl-1-vinylcyclohexene\textsuperscript{6} (Scheme 2). Whereas tiglic aldehyde\textsuperscript{7} gives a high \textit{endo} selectivity (99:1),\textsuperscript{[17]} it is known from work by Danishefsky and co-workers that a bulky tiglic acid derivative\textsuperscript{9} leads to an increased \textit{exo} selectivity.\textsuperscript{[18]} The process can be visualized as depicted in scheme 2 (Danishefsky’s model) and it is the bulkiness of the dieneophile which governs diastereoselectivity since steric hindrance, exerted by the geminal dimethyl in diene\textsuperscript{6}, directs towards an \textit{exo} approach.

In the total synthesis of tuberculosinol by the Sorensen group, the Diels-Alder reaction was performed with the relatively small ethyl tiglate, providing a modest diastereoselectivity of 2:1 in favor of the \textit{exo} diastereoisomer.\textsuperscript{[13]} Increase of the steric
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bulk by using N-tigloyloxazolidinone 12 in the Snider synthesis led to an exo:endo selectivity of ~10:1.\textsuperscript{[14]}

Scheme 2. Exo selectivity explained in the diastereoselective Diels-Alder reaction (Danishefsky’s model).

Although the use of 6,6-dimethyl-1-vinylcyclohexene 6 in diastereoselective Diels-Alder cycloadditions has been reported several times, it has not been used in an enantioselective version.\textsuperscript{[19]} We realized that meeting this challenge would provide a direct entry to the halimane family of diterpenes, and in particular to the members of the TbAd-cluster as discussed above.

4.3 Investigations of the asymmetric synthesis of 1-TbAd

4.3.1 An attempted chiral pool approach to 1-TbAd

The chiral pool approach to 1-TbAd started from commercially available and naturally occurring sclareolide. This molecule is a sesquiterpene isolated from either salvia sclarea\textsuperscript{[20]} or salvia yosgadensis.\textsuperscript{[21]} Sclareolide contains four contiguous stereogenic centers and also the desired amount of carbon atoms required for the chiral bicyclic core of the halimane skeleton. It was recently shown by George and co-workers in their total synthesis of aureol, that the stereocenters in sclareolide could readily be rearranged to set two of the three stereogenic centers present in 1-TbAd’s bicyclic core structure (Scheme 3).\textsuperscript{[12]}

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Scheme 3. Stereospecific synthesis of alkenes 3 and 17 from naturally occurring sclareolide.

Treatment of sclareolide with LiAlH₄ opens the lactone to provide diol 14. After acetylation of the primary alcohol, compound 15 was rearranged via two simultaneous Wagner-Meerwein rearrangements to furnish acetate 3. Since the 1,2-shifts (Wagner-Meerwein shifts) are stereospecific, two of the three desired stereogenic centers were set with known stereochemistry. Acetate 3 was also converted into the silyl protected analogue 17, and both compounds were subjected to a variety of conditions to achieve alkene isomerization (Table 1).[^12]

The isomerization of the unsaturated decalin in both 3 and 17 is intrinsically difficult as a tetra-substituted double bond has to be isomerized to a theoretically less stable trisubstituted double bond. An additional feature which complicates the situation is the close proximity of the geminal dimethyl and quaternary stereocenter to the double bond. If this is not challenging enough, the isomerization has to proceed regioselective and diastereoselective to furnish the desired bicyclic core structure present in 1-TbAd.

As evident from Table 1, a wide variety of different conditions to achieve alkene isomerization were studied. Despite extensive efforts not even a trace of isomerization was observed. We assume that the steric crowding around the double bond impeded any reaction from happening.
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Table 1. Failed attempts in the regio- and diastereoselective alkene isomerization of 3 and 17.

As isomerization could not be realized in one step, we focused our attention on a multi-step sequence to achieve this objective. It was known from the work of Baldwin and Adlington that the double bond in 3 lacking the OAc group could be diastereoselectively epoxidized with ozone. In 1988 Evans communicated the directed reduction of aldol products using tetrabutylammonium triacetoxy borohydride. These reports led us to design a directed epoxide opening with this borohydride reagent (Scheme 4). We also postulated that the addition of BF$_3$•OEt$_2$ to the reaction mixture would facilitate the epoxide opening, and hopefully occur with concurrent dehydration to produce alcohol 20.

We thus epoxidized acetate 3 under ozonolysis condition to give epoxide 18 in 48% yield as a single diastereomer. Although the relative stereochemistry in 18 was not determined, the facial selectivity of epoxidation by ozone was assumed to correspond to that observed by Baldwin and Adlington. The acetate on 15 was removed by treatment with K$_2$CO$_3$ providing alcohol 19. We tested our hypothesis for the directed epoxide opening in several attempts, varying stoichiometry, concentration, and temperature, all leading to the conclusion that no conversion could be achieved. Treatment of acetate 18 with LiAlH$_4$ did lead to epoxide opening, but the desired product 21 was not obtained. Instead 22 was formed, which was considered a dead-end in the synthesis.
Although the chiral pool strategy seemed attractive at first, the failure of the direct alkene isomerization forced us to come up with a more elaborate isomerization sequence. With these efforts also being futile we decided to abandon the chiral pool approach, as we felt the initial benefits of this route were overruled by step count. We therefore focused our efforts on the development of an asymmetric Diels-Alder cyclization to produce the bicyclic core structure.

**4.3.2 Towards an asymmetric catalytic Diels-Alder reaction for the construction of 1-TbAd; DNA catalysis**

At the start of the investigation into the development of a stereoselective Diels-Alder reaction, we were intrigued by the possibility to perform the cycloaddition using DNA-based catalysis, developed in our institute by the Roelfes laboratory.\(^\text{[25]}\) In 2007 the group communicated an asymmetric Diels-Alder reaction with DNA as the chiral ligand.\(^\text{[26]}\) Although the scope of the reaction was limited to the use of cyclopentadiene and \(\alpha,\beta\)-unsaturated 2-acyl methyl-imidazoles (Scheme 5), we did not like to waste this opportunity and in-house expertise.

**Scheme 5. The first asymmetric DNA-based catalytic Diels-Alder reaction by the Roelfes laboratory.**
On the outset of the DNA-catalyzed reactions it was decided to use thiazole tiglate as the dienophile rather than the corresponding methyl-imidazole. From unpublished results by Roelfes laboratory, it was known that α-substitution in the dienophile was not tolerated, as it probably interfered with the methyl substituent on the imidazole.[27] The starting materials for the Diels-Alder reaction had to be synthesized, as none of them was commercially available (Scheme 6). Thiazole tiglate 28 was readily available by reaction of tigloyl chloride 26 with TMS-thiazole 27 (Dondoni’s reagent), according to a procedure by Dondoni and co-workers.[28] The synthesis of diene 6 however was more laborious. At first, 2,2-dimethylcyclohexanone 29 had to be prepared, as the commercial source was expensive (€260 / 1 gr, 92% purity). The most economical way was to alkylate cyclohexanone at -20 °C using KOBu (2.1 eq) and MeI (2.1 eq). Although a mixture of all homologues was obtained, these were to a large extent separable using flash column chromatography. The reaction was found to be selective for the formation of 2,2-dimethylcyclohexanone 29 which we managed to obtain in 49% yield and 90% purity. Reaction with vinyl Grignard furnished alcohol 30 which was subsequently dehydrated to provide the desired ketone. Dehydration with KHSO₄ at 150 °C could be achieved but gave significant polymerization in our hands.[29a,b] The reaction with anhydrous CuSO₄[29a,c] proved to be more reliable, providing diene 6 in 53% yield, although reproducibility could only be assured by using freshly prepared anhydrous CuSO₄.


With the diene and dienophile in hand we first investigated the possibility to achieve a copper-catalyzed Diels-Alder reaction (Scheme 7). To our delight, we found that the [4+2] cycloaddition was possible, as our test reaction using CuBiPySO₄ (30 mol%) in H₂O/MeOH (1:1) provided 50% conversion over three days. In addition we made the surprising observation that the reaction provided the product with a low endo selectivity. This result was somewhat mysterious, but the conversion did indicate that the DNA-based catalytic Diels-Alder reaction might be feasible.
In collaboration with Dr. Almudena García Fernàndez (Roelfes laboratory) we initiated our attempts by subjecting diene 6 and dienophile 28 to standard DNA-based catalysis conditions. The reactions were carried out with salmon testes DNA as the chiral ligand, a DNA-chelating ligand (L1-L7) and Cu(NO₃)₂•3H₂O. As for the reaction medium, a MOPS buffer (pH 6.5) and water, or a mixture with either methanol or 1,4-dioxane, was used. Unfortunately, despite all the efforts, we did not observe any product formation. It is likely that a combination of low solubility of the substrates, the inherent poor reactivity of diene 6, and the relatively high dilution, causes reaction failure. The problem of solubility cannot be solved as we could not maneuver within the confinements of the solvent mixtures allowed within DNA-based catalysis. This inevitably caused us to shift our attention to more conventional asymmetric catalytic Diels-Alder reactions.

### 4.3.3 Development of an asymmetric catalytic Diels-Alder reaction for the construction of 1-ThAd; Organocatalysis

Our venture into the asymmetric Diels-Alder reaction was continued by investigation of organocatalytic methods. In a pioneering study, the MacMillan laboratory reported the first asymmetric organocatalytic Diels-Alder reaction (Scheme 8) using imidazolidinone based catalyst C1. It was shown that even unreactive dienes such as 32 could successfully be used. Hayashi and co-workers reported an exo selective asymmetric organocatalytic Diels-Alder based on proline based catalyst C2. These two reports caught our attention and led us to investigate the possibility of an organocatalytic entry to the halimane scaffold.
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For our organocatalytic experiments we decided to use Hayashi’s catalyst C2,[31] thiourea-based catalyst C3,[32] MacMillan’s catalyst C1,[30] and chiral aminophosphine oxide C4 (Scheme 9). The latter structure (C4)[33] has not been reported in the literature but was synthesized in our laboratory during investigations on chiral phosphine oxides.[34] Although this compound has never been used as an organocatalyst, we regarded it worthy of trying in our asymmetric Diels-Alder reaction.

We performed the organocatalytic reactions with the aforementioned catalysts to activate tiglic aldehyde 7. The reactions were initially executed at elevated temperatures, but unfortunately to no avail, as no Diels-Alder adduct could be detected. The origin of reaction impediment is likely due to the α-substituent in the dienophile which exerts steric hindrance on the catalyst, prohibiting proper activation of the tiglic aldehyde 7. With no result in hand at this stage we also cut short our organocatalysis investigation and shifted our attention to organometallic asymmetric catalysis.

Scheme 9. Short investigation into organocatalytic Diels-Alder reactions.
4.3.4 Development of an asymmetric catalytic Diels-Alder reaction for the construction of 1-TbAd; Copper-catalyzed approach

In 1999 the Evans laboratory communicated pioneering studies in the field of asymmetric copper-catalyzed Diels-Alder reactions.\(^\text{[35]}\) In their manuscript, they outlined the use of the now famous BOX ligands to achieve asymmetric induction in copper-catalyzed [4+2] cycloaddition reactions (Scheme 10). Interestingly, oxazolidinone based dienophile 38, activated by the Cu-tBu-BOX catalyst, could be successfully coupled even with the unreactive piperylene 37. This report and our in-house expertise regarding copper catalysis prompted us to investigate the asymmetric Diels-Alder under copper-catalyzed conditions.

Scheme 10. Asymmetric copper-catalyzed Diels-Alder reactions reported by Evans and co-workers.

Based on Evans’ report, we synthesized N-tigloyloxazolidinone 12 according to the procedure of Snider.\(^\text{[33]}\) This dienophile, and the tigloylthiazole based dieneophile 28 used in the DNA-based catalysis studies (\textit{vide supra}), were both screened for their usefulness in the copper-catalyzed Diels-Alder reaction with diene 6 (Scheme 11). The dienophiles were subjected to Cu(OTf)\(_2\) (20 mol\%) in combination with chiral Ph-BOX-ligand L9 (30 mol\%). Initial reaction at room temperature proved to be fruitless, and the reaction temperature was therefore elevated. In case of dienophile 12, no conversion was detected even at 100 °C for three hours under microwave conditions. This initial setback however was nullified, as it was found that the thiazole based dienophile 28 did give conversion with copper-catalysis. With this promising result in hand, we decided to set up a ligand screening and evaluate stereoselective induction.
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Scheme 11. Aiming for conversion in the asymmetric copper-catalyzed Diels-Alder reaction.

In our ligand screening, eight BOX-ligands (L9 to L16), four phosphine ligands (L17 to L20) and bis-imine ligand L21 were investigated (Scheme 12). The BOX and phosphine ligands were purchased from commercial sources, whereas the bis-imine ligand L21 was prepared in-house and apparently had survived storage over more than 17 years. 20 mol% of \textit{in situ} formed chiral catalyst was used with dichloroethane as the solvent, at 50 °C overnight. The reaction outcomes were analyzed by chiral HPLC analysis which proved to be troublesome and not always reproducible. It was found that separation of the enantiomers for both the \textit{exo} and the \textit{endo} diastereomer was difficult. Separation could only be achieved with pure heptane as the eluent and placing two chiral columns after each other. The reproducibility problems arose probably from polar impurities present in the crude samples. Since pure heptane, was used these polar impurities can significantly disturb reproducibility in the HPLC analysis. Despite these difficulties we did manage to acquire data indicating the stereochemical induction exerted by the chiral ligands.

At first the BOX ligands were studied. Although full conversion was achieved in most of the cases, ligand L12 proved to be useless. Unfortunately, the diastereoselectivity in most cases was rather poor (see L9-L13). In those cases where the \textit{exo}:\textit{endo} ratio exceeded 2:1, as for ligands L14-L16, the enantioselectivities for the desired \textit{exo} product were determined. Ligand L14 and L16 gave ~25% and ~11% ee respectively, but the most interesting result was obtained with spiro-BOX ligand L15. An acceptable diastereomeric ratio of 4:1 in favor of the desired \textit{exo} isomer was found, together with ~35% ee.

Next we investigated the four phosphine ligands. To our surprise no conversion was obtained except for L20, but the diastereoselectivity was low. As a final entry we used bis-imine ligand L21, for which we found a diastereoselectivity in favor of the \textit{exo} isomer of 5.2:1. Together with this result we found that the \textit{exo} isomer exhibited an enantiomeric excess ~44%. These results matched with that obtained for spiro-BOX ligand L15, so we had two potential lead ligands to follow up the initial screening process.
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Scheme 12. Ligand screening in the asymmetric Diels-Alder reaction.

To function as a promising lead ligand, easy access to related structures is important. Spiro-BOX ligands do not fit this requirement as the synthesis requires multiple steps with a relatively small window for structural diversity. On the other hand, the bis-imine ligands are readily accessible by reaction of a chiral 1,2-diamine and a functionalized benzaldehyde. As many of such reagents are commercially available, a broad scope of chiral bis-imines can be accessed. We therefore set out to construct a variety of bis-imine ligands (L21-L29) and employed those in the asymmetric Diels-Alder reaction (Scheme 13).
Encouraged by the results using bis-amine ligand L21 we investigated seven other bis-imine ligands (L22-L29)\textsuperscript{(36)} which span a wide range of structural variety, both electronically as sterically. None of the ligands showed an improvement in diastereoselectivity or the enantioselectivity (5-26% ee). A solvent screening was performed for reactions involving ligand L29, but also these reactions were to no avail. The results significantly narrowed down our window of opportunities to achieve an asymmetric Diels-Alder using (copper) catalysis, and we therefore had to reconsider our approach.
4.3.5 A chiral auxiliary based Diels-Alder approach towards 1-TbAd

Concurrent with our work on the copper-catalyzed Diels-Alder reactions we explored chiral auxiliaries to achieve stereoinduction. We chose our auxiliaries on the basis of reports by Helmchen, Oppolzer and Evans, who successfully employed pantolactone,$^{[38]}$ camphorsultam,$^{[39]}$ and oxazolidinone$^{[40]}$ auxiliaries in Diels-Alder reactions, respectively. We were particularly interested in how the Evans auxiliary would perform since in their tuberculosinol total synthesis publication Snider and co-workers stated the following:

“The unsubstituted oxazolidinone 12 (number in this chapter) was chosen to minimize steric interactions between the α-methyl group and the oxazolidinone and because asymmetric induction seemed unlikely in an exo Diels-Alder reaction in which a chiral oxazolidinone would be far away from the diene.”$^{[41]}$

We thus synthesized tigloyl based dienophiles 41-43 that were reacted with diene 6 in the presence of Me$_2$AlCl (Scheme 14). The choice for this Lewis acid was based on previous work regarding Lewis Acid activation in Diels-Alder cycloadditions with diene 6. Danishefsky and co-workers reported in their work that TiCl$_4$, and BF$_3$•OEt$_2$ were not compatible with diene 6, which decomposed under the reaction conditions.$^{[18]}$ In unpublished results from the Snider laboratory it was reported that Lewis activation with Et$_2$AlCl or EtAlCl$_2$, in reaction between an isomer of diene 6 and oxazolidinone 12 (Scheme 2), led to poor and no conversion, respectively.$^{[41]}$ In their published synthesis, Me$_2$AlCl was shown to be a suitable Lewis acid,$^{[14]}$ which we therefore used in our chiral auxiliary based reactions. The stoichiometry of the Lewis acid was based on work by the Evans group which showed that the use two equivalents of Lewis acid provides a
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bidentate coordination with the dienophile via chloride abstraction, considerably enhancing both diastereoselectivity and reactivity in the reaction (Scheme 15).\textsuperscript{[42]}

The Diels-Alder reactions with pantoyltiglate 42 and camphorsultam 43 were unproductive, as no conversion of the starting materials was observed. On the other hand, as expected, the reaction with (S)-isopropyl-N-tigloyloxazolidinone 41\textsuperscript{[43]} gave the desired cycloaddition, providing 44 in 55\% yield with a diastereomeric ratio of \(~10\):1.

These results matched with that reported for the achiral variant (Scheme 2).\textsuperscript{[14]}

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme_15.png}
\end{center}

\textbf{Scheme 15. Enhanced reactivity by the used of an excess of Lewis acid.}

\textbf{4.3.6 Removal of the chiral auxiliary and the battle against steric hindrance}

With the desired cycloadduct 44 in hand, we had to determine whether asymmetric induction had taken place. We decided to first remove the chiral auxiliary by converting Diels-Alder adduct 44 to the corresponding alcohol, since this compound had been shown by Sorenson and co-workers to be easily separable using liquid chromatography.

Snider and co-workers had shown the removal of the oxazolidinone auxiliary was trivial for their racemic adduct 13 (Scheme 2), as the oxazolidinone was cleaved with LiBH\textsubscript{4} in 82\% yield. In our case however, both LiBH\textsubscript{4} and NaBH\textsubscript{4} failed to cleave the chiral auxiliary even at elevated temperatures for prolonged reaction times (Scheme 16a). The use of LiAlH\textsubscript{4} did produce 50 but in only a moderate yield of 30-43\%.
The difficulties encountered in the removal of the chiral auxiliary were not surprising. Prior to these reactions, we performed a plethora of experiments on functionalities proximal to the quaternary stereocenter (see Scheme 16 for a selection). In early studies on the cleavage of the thiazole in Diels-Alder adduct 31, N-methylation with MeOTf did not take place (Scheme 16b). Instead, O-methylation was observed, causing the esterification with ethanol to fail. An apparently simple hydrolysis of ethyl ester 11 (or thiazole 31) also proved cumbersome as even after reaction under harsh conditions (10 eq KOH, EtOH/H2O, reflux, 72 h), starting material was retrieved (Scheme 16c). Ethyl ester 11 however is reduced with LiAlH4 to 50 in near quantitative yield (see chapter 3).[13] Accessing the carboxylic acid via oxidation of 50 was also troublesome. For example; RuCl3/NaIO4,[44] CrO3/H2O,[45] and CrO3/H2SO4 (Jones oxidation)[46] failed to produce the carboxylic acid as the reaction stopped in the aldehyde stage (Scheme

\[ \text{Scheme 16. Steric hindrance hampers reactivity at the neopentyl-type position.} \]
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16d). Even Grignard formation of bromide 53 using freshly activated magnesium turnings, and a pinch of iodine, in refluxing THF over the weekend failed and the starting material was quantitatively retrieved.

It was evident from the examples given that the carbonyl functionality is considerably shielded. In particular, reactions involving a tetrahedral intermediate are problematic. An obvious candidate which imposes steric hindrance is the neighboring quaternary stereocenter (= neopentyl type structure). However, we reason another less known contributing factor is present, which can be explained by Newman’s “rule of six”.[47a]

In 1950 Newman analyzed the reaction rates in the Fischer esterification of carboxylic acids. It was found that the rate of reaction for acetic acid and propionic acid with MeOH are equal (Scheme 17). However, a significant rate decrease was observed for butyric acid which does not further decrease for higher homologues. This low reactivity of butyric acid has been observed before, and it was postulated by Smith and McReynolds that a “coiled” conformation of butyric acid might be responsible for the impediment of reaction (Scheme 17).[47b] This proposition together with the empirical data for other carboxylic acids led Newman to postulate the “rule of six” stating: “In reactions involving additions to an unsaturated function, the greater number of atoms in the six position, the greater will be the steric effect”.

Interestingly, in the Diels-Alder adduct, there are actually two “coiled butyric acids” present (in blue and numbered). Moreover, in contrary to butyric acid itself, the coiled structural element is fixed in the decalin structure and might therefore be a significant contributing factor of steric hindrance.

![Scheme 17. Newman’s “rule of six” to explain steric hindrance in the Diels-Alder adducts.](image)
Despite the inherent steric hindrance, we did manage to remove the chiral auxiliary with LiAlH₄, however alcohol 50 was obtained in only a moderate yield up to 43% (Scheme 16a). This outcome was attributed to irreversible attack of the reducing agent onto the more accessible carbonyl group of the chiral auxiliary. It was therefore concluded that auxiliary cleavage had to be performed with a powerful, small nucleophile allowing reversibility in the attack on the auxiliary’s carbonyl group. Lithium ethyl thiolate was therefore considered suitable (Scheme 18). We were delighted to see the reaction worked to great satisfaction as thioester 55 was cleanly produced in 96% yield. Reduction of 55 with LiAlH₄ then produced alcohol 50, this time in 95% yield. Somewhat naively, we also tried to convert thioester 55 directly into aldehyde 8, an intermediate used in the eventual total synthesis (vide infra). Treatment with DIBAL-H and using Fukuyama reduction conditions gave no reaction as we already expected. In spite of the introduction of an additional step to our total synthesis, the drastic increase in overall yield of alcohol 50 outweighed this disadvantage.

**Scheme 18. Successful cleavage of the chiral oxazolidinone in Diels-Alder adduct 44.**

### 4.3.7 Determination of asymmetric induction in the chiral auxiliary aided Diels-Alder cycloaddition

Alcohol 50 allowed to assess the enantioselectivity of the Diels-Alder reaction. Chiral HPLC analysis of racemic alcohol 50 obtained in our racemic 1-TbAd synthesis (see chapter 2) provided the HPLC trace depicted in figure 2, top. The exo and endo isomer are completely separated, as are the enantiomers (17.55 min and 21.15 min) of the exo diastereoisomer. We were excited to see the HPLC trace of our enantioenriched alcohol 50 as we found complete disappearance of the peak at 21.15 min (figure 2, bottom). So 50 had been prepared with an enantiomeric excess exceeding 98% for the exo isomer. The previously discussed assertion from Snider and co-workers, regarding the notion that asymmetric induction with a chiral oxazolidinone being unlikely, had therefore to be reconsidered.

Further confirmation of the excellent stereoselectivity was obtained by comparison of the optical rotation with that reported in the literature. The optical rotation of 50 ([α]₀₂₃
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= + 27.0 (c = 0.2, CHCl₃)) matched with that of the Sorensen laboratory ([α]D²₃ = +28.3 (c = 0.2, CHCl₃)), who obtained enantiopure alcohol 50 by chiral SFC (supercritical fluid chromatography) of the racemic diastereomeric mixture. The enantiomeric excess of the minor endo isomer (15.6 min and 15.9 min) was measured to be ~60% ee.

Figure 2. Chiral HPLC analysis of alcohol 50; racemic (top), asymmetric (bottom).

4.3.8 The stereoselective synthesis of 1-TbAd and N⁶-TbAd

With this exquisite result in hand we were now eager to elucidate the absolute stereochemical outcome of the Diels-Alder reaction. Up to this point we had performed the asymmetric Diels-Alder reactions on a small scale (0.4 mmol = ~50 mg of diene 6). Scaling up of the reaction proved to be facile as we ultimately managed to perform the reaction starting from 40 mmol (~5.4 g) of diene 6. No complications in the scale-up were met as equal results in terms of yield and stereoselectivity were obtained compared to the small scale reactions.
A welcoming feature which arose from the scale-up was that we managed to obtain multi-grams of crystalline material. This provided us with a crystal suitable for X-ray crystallography, revealing the absolute stereochemistry of Diels-Alder adduct 44 and consequently that of tuberculosinol. Remarkably, despite the interest in tuberculosinol and related compounds, the absolute stereochemistry of tuberculosinol had not been unequivocally established. It had been tentatively assigned\textsuperscript{[49]} by comparing the sign of the optical rotation of tuberculosinol with that of neopolypodatetraene A, which has the same bicyclic core structure with known absolute configuration.\textsuperscript{[50]} This is not fully convincing, since small differences in structure can lead to a different sign of rotation. In addition, tuberculosinol is formed by the GGPP cyclase Rv3377c,\textsuperscript{[15]} whereas neopolypodatetraene A is formed by a squalene cyclase.\textsuperscript{[50]} The X-crystal structure of 44 closed the debate as the absolute stereochemistry was found to correspond to that in figure 3.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{X-ray crystal structure (ORTEP) of Diels-Alder adduct 44. Atom color code: Carbon = grey; Hydrogen = white; Oxygen = red; Nitrogen = blue.}
\end{figure}

4.3.9 Completion of the stereoselective synthesis of 1-TbAd and N\textsuperscript{6}-TbAd

Finally, we could complete our total synthesis of the tuberculosinyl adenosines. As shortly discussed within the introduction of this chapter (see for details chapter 3), both 1-TbAd and N\textsuperscript{6}-TbAd were shown to be potential chemical markers for tuberculosis.\textsuperscript{[8,11]} Additionally, 1-TbAd is produced by the virulence-associated enzyme Rv3378c, of which its locus (Rv3377c–Rv3378c) has shown to be essential in phagosome maturation arrest.\textsuperscript{[9]} 1-TbAd is therefore expected to be involved in phagosomal survival of \textit{Mtb}, although the exact role it plays remains to be investigated. To facilitate further studies of the TbAd molecules, reference material is essential, and we therefore set out to produce multi-grams of the natural product.

At this stage of our synthesis, we had several grams of Diels-Alder adduct 44 in hand which was subjected to removal of the chiral auxiliary and reduction of the
Asymmetric Total Synthesis of 1-Tuberculosinyl Adenosine

corresponding thioester 55, providing alcohol 50 in 91% yield over the two steps (Scheme 19). In earlier efforts to racemic TbAd, we found the Sorensen[13] and Snider[14] routes reproducible and high yielding. We therefore decided to pursue the route along these lines.

A Ley-Griffith oxidation of alcohol 50 provided aldehyde 8 which subsequently was condensed with acetone to furnish enone 56, in 70% yield over the two steps. The enone was then efficiently reduced by Wilkinson's catalyst in combination with triethylsilane in 90% yield. Installation of the double bond was achieved by a Horner-Wadsworth-Emmons olefination with triethyl phosphonoacetate to produce the desired enolate 58 in a 90:10 E:Z mixture. This mixture was reduced with DIBAL-H, which after purification afforded pure, naturally occurring, tubercullosinol in 82% yield over the two steps.

Scheme 19. Asymmetric total synthesis of the tubercullosinyl adenosines, 1-TbAd and N6-TbAd.

To complete the synthesis of the tubercullosinyl adenosines, tubercullosinol was converted into tubercullosinyl chloride 1, using a Corey-Kim chlorination, in near quantitative yield.[21] 1 proved to be unstable on silica, but purification was efficiently achieved by straightforward precipitation of the residual NCS and succinimide.
Addition of pentane to the reaction mixture and subsequent filtration afforded, after removal of the volatiles, near pure 1. We were now only one step removed from finalizing our total synthesis of 1-TbAd, which required alkylation of adenosine with tuberculosinyl chloride 1. In our racemic total synthesis this step was performed in a poor 17% yield (Chapter 3). After a thorough study of the alkylation of adenosine with 1, we eventually managed to produce 1-TbAd in 76% yield with a procedure involving NaI (Finkelstein conditions) in DMF (0.5 M in 1).[52]

In addition to the synthesis of naturally occurring 1-TbAd, we also used this alkylation procedure to construct the derivatives (Z)-1-TbAd, 2’-deoxy 1-TbAd[10] and 13C-labelled 1-TbAd (see the experimental section). The latter compound, made from adenosine which was fully 13C-labelled in the ribose, was prepared to facilitate the development of 1-TbAd as a chemical marker for tuberculosis. Isotope-labelled chemical markers are important as internal standards for quantification with HPLC-MS.[53]

In order to verify its chemical structure, as proposed by Lau et al.,[10] 2’-deoxy 1-TbAd was produced, by reacting tuberculosinyl chloride with 2’-deoxy adenosine. CID/MS-analysis of synthetic 2’-deoxy 1-TbAd produced MS spectra closely matching that of the proposed natural product. This, and the fact that 2’-deoxy adenosine is an abundant nucleoside, provides strong evidence that 2’-deoxy 1-TbAd is a Mycobacterium tuberculosis produced natural product.

The finishing touch of our synthesis was the construction of N6-TbAd. It is known that 1-alkyl adenosines rearrange to N6-alkyl adenosines under nucleophilic conditions via a so-called Dimroth rearrangement.[54] Treatment of 1-TbAd with 60% aqueous Me2NH brought about this rearrangement to N6-TbAd quantitatively, concluding our total synthesis of the naturally occurring tuberculosinyl adenosines.

4.4 On the mechanism and enantioselectivity of the asymmetric Diels-Alder reaction

This Diels-Alder cycloaddition is the first of its kind to produce the halimane core structure with both high diastereo- and enantioselectivity. As previously mentioned, in 2010 the Snider laboratory discarded the use of a tigloyl based chiral oxazolidinone to achieve such stereoinduction.[14] Intrigued by this discrepancy, we decided to systematically investigate the stereoselective nature of the reaction. The aforementioned statement from the Snider laboratory was based on the mechanistic model proposed by Danishefsky (Figure 4a), explaining exo-selectivity in the Diels-Alder reaction with diene 6.[18] A steric clash between the diene’s geminal dimethyl group and the bulky dienophile disfavors the endo approach, hence providing the exo-Diels-Alder adduct selectively. This model, however, only addresses the dienophile in its s-trans conformation. Although this model predicts the observed exo-selectivity, it ignores a possible contribution of the s-cis conformation of the dienophile.[55] In a combined experimental and computational study by Houk, Gouverneur et al., it was shown that Evans chiral auxiliary-based dienophiles provide efficient chiral induction and endo or exo-selectivity depending on the substitution at the β-position of the...
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dienophile and the diene. The reaction takes place from the \(s\)-\(cis\) conformation of the dienophile as expected as the diene is not substituted at the \(\alpha\)-position, like in the current tigloyl-based dienophile.

![Figure 4](image_url)

**Figure 4.** Models for asymmetric induction in the Diels-Alder cycloaddition between diene 6 and dienophile 41.

In figure 4b, a model for the [4+2] cycloaddition between diene 6 and dienophile 41, in its \(s\)-\(trans\) conformation, is presented. As in Danishefsky’s model,[18] the \(endo\) approach is disfavored for reasons previously mentioned. The \(exo\) approach on the \(Si\)-face of the dienophile seems disfavored, as the isopropyl group on the chiral oxazolidinone clashes with the vinyl substituent of the diene. The \(Re\)-face approach on the other hand seems plausible, as no obvious steric interactions shield this face from reaction. However, this facial approach would provide the Diels-Alder adduct 44 with a stereochemistry opposite to that observed experimentally.

Considering the \(s\)-\(cis\) conformer of dienophile 41 (Figure 4c), the \(endo\) approach is again disfavored, this time because of a clash between the dimethylaluminum chelate...
and the geminal dimethyl functionality. The Re-face appears unapproachable due to a steric interaction between the isopropyl group and the geminal dimethyl group in diene 6. A Si-face approach does not result in such steric clash and provides Diels-Alder adduct 44 with the experimentally obtained stereochemistry. This model therefore seems to be a better picture of the course of the reaction, despite the expectation that the s-cis conformation of the dienophile is unfavorable compared to the s-trans conformation. To get a complete picture of the reaction we studied the reaction through in silico studies.

Calculations were carried out using the ADF program suite \(^{(57)}\) at the BP86\(^{(58)}\)/TZ2P level of theory. The geometries of all stationary points were optimized in the gas phase and verified to be proper local minima or transition states through vibrational analyses. The gas-phase harmonic frequencies were used for the enthalpic and entropic corrections to the free energies at 233.15 K (-40 °C).

First, it was determined whether the s-cis conformer is energetically feasible by calculating the s-trans/s-cis equilibrium for Me\(_2\)Al-complexed dienophile 41 (Figure 5). We found the s-trans 41b conformer is favored over the s-cis 41a conformer by 0.2 kcal/mol on the Gibbs free energy surface at 233.15 K, providing a ratio of 6 : 4 respectively at -40 °C. With a rotational barrier of \(\Delta G^\ddagger = 3.2\) kcal/mol, however, the conformers are readily interconvertible and, due to the Curtin-Hammet principle,\(^{(59)}\) the observed product can be reached via the s-cis conformer.\(^{(55)}\)

**Figure 5.** Intramolecular distances (between the chiral oxazolidinone hydrogen atom and the \(\beta\)-hydrogen atoms) and dihedral angle of s-cis conformer 41a and s-trans conformer 41b obtained from the optimized geometries.

The energy profile for the entire reaction pathway was calculated next (Figure 6). On the basis of our calculations, and according to the models in figure 4, the endo approach for both the s-trans and s-cis conformers can be ruled out. Attack of the s-trans conformer from the exo Si-face, and attack of the s-cis conformer from the exo Re-face are prohibited as well as the computations showed significant steric clash for initial bond formation (see experimental section). The two models in which calculations were carried out were the Re-face approach and the Si-face approach for the s-trans and s-cis conformers respectively (both exo attack).
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The reaction of the s-trans conformer (Figure 6, red pathway) has a considerably higher activation energy (ΔG‡ = 19.4 kcal/mol) for the initial and enantio-determining step, than the reaction of the s-cis conformer (blue path, ΔG‡ = 15.5 kcal/mol). This convincingly explains the reaction outcome.

Figure 6. DFT BP86/TZ2P calculated reaction coordinate for the Diels-Alder cycloaddition between diene 6 and dienophile 41. For these studies, the s-cis exo Si-face approach was chosen as the reference point. IRC analysis were performed on both transition states on the s-cis pathway (blue lines) and are connected with solid lines. The dashed lines connect the structures in which IRC was not performed, but was found to exist on the potential energy surface. (Relative electronic energies are given in kcal/mol, and relative Gibbs free energies are given in kcal/mol in parentheses). The geometry of diene 6, aluminum-complexed s-cis conformer of the dienophile 41a) aluminum-complexed s-cis conformer of the dienophile (41b), and the transition state between 41a and 41b corresponding to the rotation along the C(2)-C(3) bond (41c) were optimized separately. The energies of the three states 6+41a, 6+41b and 6+41c are the sum of the corresponding diene and aluminum-complexed dienophile fragments (See Supporting Information).

The Diels-Alder reaction was found to proceed stepwise rather than concerted. After the first bond formation, the cationic intermediate is in an energy well with a relative energy of 12.4 kcal/mol, before proceeding to form the second bond through a saddle point at 14.8 kcal/mol. From this point, the reaction is thermodynamically downhill to form the Me₂Al complexed Diels-Alder adduct 44f with a relative ΔG = -1.3 kcal/mol. The differences in activation energies between the two pathways can be rationalized by using the activation strain model. In this model, the electronic activation energy (ΔE‡) is the sum of strain energy (ΔE‡strain), which is associated with the deformation of the starting materials to the geometry they acquire in the TS, and the interaction energy.
(ΔE‡\text{int}), which is associated with the favorable electronic interactions between the deformed starting materials, \(\text{i.e.}\),

\[
\Delta E^\ddagger = \Delta E^\ddagger\text{strain} + \Delta E^\ddagger\text{int}
\]

As outlined in Table 2, the difference in ΔE‡ between TS 44b and TS 44h is mainly due to strain, not due to orbital interaction. Upon inspection on the geometry of the dienophile at the transition state, the dihedral angle O(1)-C(2)-C(3)-C(4) is deviating 17.2° and 18.2° from planarity for TS 44b and TS 44h respectively, while the corresponding dihedral angle of the \textit{s-cis} and \textit{s-trans} conformers are deviating 29.8° and 43.0° from planarity respectively (Figure 5).

<table>
<thead>
<tr>
<th>Transition states</th>
<th>ΔE^\ddagger</th>
<th>ΔE^\ddagger\text{strain}</th>
<th>ΔE^\ddagger\text{int}</th>
<th>ΔH^\ddagger</th>
<th>TΔS^\ddagger</th>
<th>ΔG^\ddagger</th>
</tr>
</thead>
<tbody>
<tr>
<td>44b</td>
<td>3.4</td>
<td>16.5</td>
<td>-13.1</td>
<td>4.2</td>
<td>-11.4</td>
<td>15.6</td>
</tr>
<tr>
<td>44h</td>
<td>7.7</td>
<td>23.4</td>
<td>-15.7</td>
<td>8.4</td>
<td>-11.3</td>
<td>19.7</td>
</tr>
<tr>
<td>44d</td>
<td>-0.2</td>
<td>79.1</td>
<td>-79.3</td>
<td>2.2</td>
<td>-12.6</td>
<td>14.8</td>
</tr>
<tr>
<td>44j</td>
<td>3.9</td>
<td>75.6</td>
<td>-71.6</td>
<td>5.8</td>
<td>-13.9</td>
<td>19.7</td>
</tr>
</tbody>
</table>

**Table 2.** Activation strain analysis (in kcal/mol) for the four transition states shown in figure 5.

The larger deviation from planarity, of the dihedral angle O(1)-C(2)-C(3)-C(4), in the \textit{s-trans} conformer might be attributed to a steric interaction between the hydrogen atom at the stereocenter of the oxazolidinone scaffold, and that of the hydrogen atom at C(4) (Figure 5). The distance between these two hydrogen atoms is 2.07 Å for the \textit{s-trans} conformer, while the closest distance between the hydrogen atom at the oxazolidinone and the hydrogens on the \(\alpha\)-methyl group in the \textit{s-cis} conformer is 2.17 Å. Since both distances are less than the sum of the van der Waals radii of two hydrogen atoms (2.4 Å), the steric interactions between the hydrogen atoms in both cases are non-negligible, and the steric interaction in the \textit{s-cis} conformer is indeed less severe than that in the \textit{s-trans} conformer. The \textit{s-cis} conformer can thus retain a relatively planar geometry without experiencing severe steric interactions. The larger difference of the dihedral angle between the ground state and the transition state of the \textit{s-trans} conformer (44h) compared to that of the \textit{s-cis} conformer (44b) could explain the difference in activation strain.

While ΔE^\ddagger\text{int} plays a larger role in determining the ΔE‡ of the second transition state, this difference is argued to be less important due to the fact that enantioselectivity is determined at the first transition state.

To the best of our knowledge, this computational study is the first to shine light on the mechanism of Diels-Alder reactions with tigloyl (that means, \(\alpha,\beta\)-disubstituted) chiral oxazolidinones. Although the Curtin-Hammett principle in Diels-Alder reactions has been described before\(^{[55]}\) for the use of tigloyl based dienophiles this concept is novel.
4.5 Conclusion

In summary, 1-TbAd and N6-TbAd have been prepared as pure stereoisomers in 10 and 11 steps respectively, starting from diene 6. Installation of the three chiral centers in tuberculosinol was efficiently achieved (exo:endo = 10:1, >98% ee for the exo isomer) employing a chiral oxazolidinone auxiliary aided Diels-Alder reaction. The synthesis has been scaled to produce 2.4 g of 1-TbAd in 21% overall yield. In addition, the family members N6-TbAd and 2′-deoxy 1-TbAd have been prepared, confirming the structure of the latter. The synthetic material was shared with the Moody laboratory who is currently investigating the virulence-promoting effects of 1-TbAd, and its use as a chemical marker for tuberculosis.

The enantioselective nature of the Diels-Alder reaction was also investigated. In-silico studies showed the Diels-Alder cycloaddition proceeds according to the Curtin-Hammet principle in which the thermodynamically less stable s-cis conformer of the dienophile reacts to form Diels-Alder adduct 44, because this conformer needs less deformation in the transition state which results in a lower reaction barrier. Additionally, the reaction was also found to follow a step-wise mechanism rather than concerted.

4.6 Discussion and outlook

As evident from the this chapter, a thorough investigation into the asymmetric total synthesis of 1-TbAd was performed, focusing mainly on the development of an asymmetric catalytic Diels-Alder reaction. Unfortunately, we did not accomplish this objective, as we cut short our investigation, since we found excellent stereoselectivities with the chiral auxiliary approach. We realized that our sources for achieving asymmetric induction using dienophile 28 slowly dried up, so we started to browse the literature for other methods to achieve our goal.

In 2006 the Corey laboratory applied oxazaborolidinium catalyst C5 in asymmetric Diels-Alder reactions between tiglic aldehyde 7 and several unreactive dienes (Scheme 20).\[61\] If further research is aspired, regarding the development of an asymmetric catalytic Diels-Alder reaction with diene 6, the Corey procedure is definitely worth a study. An especially attractive feature is that tiglic aldehyde can be used as a substrate which would allow the presented synthetic route (Scheme 19) to be cut short by three steps, as the cycloaddition directly produces aldehyde 8. Although the three steps to form aldehyde 8 out of Diels-Alder adduct 44 are all high yielding, the sequence can be regarded inefficient from both a step-count as a redox-economy point of view.\[62\]
The key-step of the TbAd total synthesis is clearly the asymmetric Diels-Alder reaction, which resembles a first asymmetric [4+2] cycloaddition based entry to the halimane bicyclic core structure. This has opened the opportunity for the stereoselective total synthesis of other natural products bearing such scaffold (Figure 7). Relatively straightforward asymmetric syntheses of neopolypodatetraene A,[50] mamanuthaquinone,[63] and a halimane diterpenoid from Plectranthus ornatus[64] are now within reach. This also holds for natural products with an oxidized halimane structure as present in the agelasimines A and B[65] and in purino-diterpene.[66] From a synthetic perspective however, cacospiongionolide[67] and akaterpin are of significant interest.[68] Both compounds possess the halimane skeleton but also additional stereochemical intricacies that are synthetically challenging. Interestingly, akaterpin contains another halimane type moiety within its overall structure which is envisioned to be accessible using a Diels-Alder reaction based on the investigations described within this chapter.
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Figure 7. Natural products envisioned to be accessible using the developed asymmetric Diels-Alder cycloaddition.

4.7 Experimental section

General remarks:
All reactions were performed using oven-dried glassware under an atmosphere of nitrogen (unless otherwise specified) by standard Schlenk techniques, using dry solvents. Reaction temperature refers to the temperature of the oil bath/cooling bath. Solvents were taken from an MBraun solvent purification system (SPS-800). All other reagents were purchased from Sigma-Aldrich, Acros, TCI Europe or Fluorochem and used without further purification unless noted otherwise.

TLC analysis was performed on Merck silica gel 60/Kieselguhr F254, 0.25 mm. Compounds were visualized using either Seebach’s stain (a mixture of phosphomolybdic acid (25 g), cerium (IV) sulfate (7.5 g), H₂O (500 mL) and H₂SO₄ (25 mL)), a KMnO₄ stain (K₂CO₃ (40 g), KMnO₄ (6 g), H₂O (600 mL) and 10% NaOH (5 mL)), or elemental iodine.

Flash chromatography was performed using SiliCycle silica gel type SiliaFlash P60 (230 – 400 mesh) as obtained from Screening Devices or with automated column chromatography using a Reveleris flash purification system purchased from Grace Davison Discovery Sciences.

¹H- and ¹³C-NMR spectra were recorded on a Varian AMX400 or a Varian 400-MR (400 and 100.59 MHz, respectively) using CDCl₃ or DMSO-d₆ as solvent, unless stated otherwise. Chemical shift values are reported in ppm with the solvent resonance as the internal standard (CDCl₃: δ 7.26 for ¹H, δ 77.16 for ¹³C, MeOH-d₄ δ 3.31 for ¹H, δ 49.0 for ¹³C). Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, ddp = double double pentet, td = triple doublet, t = triplet, q = quartet, b = broad, m = multiplet), coupling constants J (Hz), and integration.
CHAPTER 4

GC-MS measurements were performed with an HP 6890 series gas chromatography system equipped with a HP 5973 mass sensitive detector. GC measurements were made using a Shimadzu GC 2014 gas chromatograph system bearing an AT5 column (Grace Alltech) and FID detection. Enantiomeric excesses were determined by chiral HPLC analysis using a Shimadzu LC-10ADVP HPLC instrument equipped with a Shimadzu SPD-M10AVP diode-array detector. Integration at three different wavelengths (254, 225, 190 nm) was performed and the reported enantiomeric excess is an average of the three integrations. Retention times (tR) are given in min.

High resolution mass spectra (HRMS) were recorded on a Thermo Scientific LTQ Orbitrap XL. Optical rotations were measured on a Schmidt+Haensch polarimeter (Polartronic MH8) with a 10 cm cell (c given in g/mL) at ambient temperature (±23 °C). Melting points were recorded on a Stuart SMP 11 apparatus.

Experimental procedures and data:

(1R,2R,8aS)-1-(2-hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (14):

To a suspension of lithium aluminium hydride (4.55 g, 120 mmol, 1 eq) in dry THF (48 mL) was slowly added a solution of sclareolide (20 g, 80 mmol) in dry THF (200 mL) at 0 °C. After addition the reaction was stirred at rt for 1 h after which TLC analysis (pentane : ether = 1:1) indicated complete conversion of the starting material. The reaction was quenched at 0 °C using EtOAc (40 mL) where after a saturated aqueous solution of Rochelle salt (480 mL) was added. The mixture was stirred for 1 h at rt after which the phases were separated. The aqueous phase was extracted with CH₂Cl₂ (3x200 mL) and the combined organic phases were washed with 1 M HCl (120 mL), water and brine. The organic phase was dried over MgSO₄, filtered and concentrated under reduced pressure affording (1R,2R,8aS)-1-(2-hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol 14 (19.9 g, 78 mmol, 98 % yield) as a white solid.

1H-NMR (400 MHz, CDCl₃) δ 3.74 (br s, 2H), 3.69 (dt, J = 10.0, 4.5 Hz, 1H), 3.39 – 3.33 (m, 1H), 1.83 (dt, J = 12.5, 3.0 Hz, 1H), 1.61 – 1.03 (m, 11H), 1.11 (s, 3H), 0.87 (m, 2H), 0.80 (s, 3H), 0.72 (s, 6H).
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$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 72.8, 63.9, 59.3, 56.0, 44.0, 41.9, 39.3, 38.9, 33.4, 33.2, 27.8, 24.5, 21.5, 20.4, 18.4, 15.3.

The analytical data are in agreement with: K. K. W. Kuan, H. P. Pepper, W. M. Bloch, J. H. George, Org. Lett. 2012, 14, 4710.

2-((1R,2R,8aS)-2-hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)ethyl acetate (15):
To a solution of 14 (9.0 g, 35.4 mmol) in dry CH$_2$Cl$_2$ (80 mL) were added acetic anhydride (5.0 mL, 53 mmol, 1.5 eq) and pyridine (5.7 mL, 71 mmol, 2 eq). The reaction mixture was stirred overnight where after it was quenched using an saturated aqueous NH$_4$Cl solution (100 mL). The phases were separated and the aqueous phase was extracted with CH$_2$Cl$_2$ (2x100 mL). The combined organic phases were washed with a saturated aqueous NH$_4$Cl solution, water and brine. The organic phase was dried over MgSO$_4$, filtered and concentrated under reduced pressure. Flash column chromatography employing pentane : ether (1:1) resulted in the isolation of 2-((1R,2R,8aS)-2-hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)ethyl acetate. Pyridine impurities were still visible in the $^1$H-NMR spectrum. The oil was therefore dissolved in Et$_2$O (200 mL) and washed with a saturated aqueous CuSO$_4$ solution (3x100 mL) and once with brine (100 mL). The organic phase was dried over MgSO$_4$, filtered and concentrated under reduced pressure affording pure 2-((1R,2R,8aS)-2-hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl)ethyl acetate 15 (9.4 g, 31.7 mmol, 90% yield) as a slightly yellow, viscous oil.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 4.16 – 4.08 (m, 2H), 2.05 (s, 3H), 1.89 (dt, $J$ = 12.0, 3.0 Hz, 1H), 1.76 – 1.09 (m, 12H), 1.16 (s, 3H) 0.92 (d, $J$ = 12.0 Hz, 2H), 0.87 (s, 3H), 0.79 (s, 6H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 171.1, 73.5, 66.6, 58.0, 56.0, 44.4, 41.9, 39.6, 38.7, 33.4, 33.3, 24.5, 23.9, 21.5, 21.1, 20.5, 18.4, 15.3.

The analytical data are in agreement with: K. K. W. Kuan, H. P. Pepper, W. M. Bloch, J. H. George, Org. Lett. 2012, 14, 4710.
(1R,2S)-1,2,5,5-tetramethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-1-yl)ethyl acetate (3):

To a solution of 15 (9.4 g, 32 mmol) in dry CH$_2$Cl$_2$ (150 mL) was added at rt trifluoroborate ether complex (15.7 ml, 127 mmol, 4 eq.). Shortly after addition the solution turned bright yellow. The reaction was stirred overnight where after the reaction was diluted with H$_2$O (100 mL) and CH$_2$Cl$_2$ (100 mL). The phases were separated and the aqueous layer was extracted with CH$_2$Cl$_2$ (3x100 mL). The combined organic phases were dried over MgSO$_4$, filtered and concentrated under reduced pressure. Flash column chromatography employing pentane : ether (95:5) afforded 2-((1R,2S)-1,2,5,5-tetramethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-1-yl)ethyl acetate 3 (7.5 g, 27 mmol, 85% yield) as a yellowish oil.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 4.06 – 4.01 (m, 1H), 3.87 – 3.83 (m, 1H), 2.06 – 1.27 (m, 13H), 2.02 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.88 (d, $J$ = 7.0 Hz, 3H), 0.84 (s, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 171.4, 137.5, 131.9, 62.3, 40.0, 39.9, 34.6, 34.6, 34.5, 29.2, 27.8, 27.3, 26.0, 25.2, 21.3, 21.2, 20.1, 16.3.

The analytical data are in agreement with: K. K. W. Kuan, H. P. Pepper, W. M. Bloch, J. H. George, Org. Lett. 2012, 14, 4710.

2-((1R,2S)-1,2,5,5-tetramethyl-1,2,3,4,5,6,7,8-octahydronaphthalen-1-yl)ethanol (16):

To a suspension of lithium aluminum hydride (1.8 g, 47.4 mmol, 3 eq) in dry THF (15 mL) was slowly added a solution of 3 (4.4 g, 16 mmol) in dry THF (30 mL) at 0 °C. After addition, the reaction was stirred at rt for 1 h after which TLC analysis (pentane : ether = 7:3) indicated complete conversion of the starting material. The reaction was quenched carefully with a saturated aqueous solution of Rochelle salt (100 mL). The mixture was stirred for 1 h at rt after which the phases were separated. The aqueous phase was extracted with Et$_2$O (3x75 mL) and the combined organic phases were washed with 1 M HCl (100 mL), water and brine. The organic phase was dried over MgSO$_4$, filtered and concentrated under reduced pressure affording 2-((1R,2S)-1,2,5,5-
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tetramethyl-1,2,3,4,5,6,7,8-octahydropyrido[1,2-a]pyrimidine (1) as a yellowish oil.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 3.63 – 3.59 (m, 1H), 3.51 – 3.47 (m, 1H), 2.04 – 1.30 (m, 14H), 0.98 (s, 3H), 0.95 (s, 3H), 0.88 (d, $J = 7.0$ Hz, 3H), 0.84 (s, 3H).

$^{13}$C-NMR (150 MHz, CDCl$_3$) δ 137.1, 132.6, 60.1, 39.9, 38.9, 34.6, 34.5, 29.1, 27.7, 27.2, 26.1, 25.1, 21.2, 19.9, 16.3.


(1R,2R,8aS)-1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2,5,5,8a-tetramethyldecahydropyridin-2-ol (17):

To a solution of imidazole (0.25 g, 3.7 mmol, 1.1 eq) in dry CH$_2$Cl$_2$ (10 mL) was added tert-butyldichlorodiphenylsilane (1.06 ml, 4.06 mmol, 1.2 eq). During addition the mixture became cloudy. The mixture was stirred for 10 min after which 16 (0.8 g, 3.4 mmol) in dry CH$_2$Cl$_2$ (10 mL) was added. The reaction was stirred overnight where after water (20 mL) was added to the reaction mixture. The phases were separated and the aqueous phase was extracted two times with CH$_2$Cl$_2$ (2x30 mL). The organic phases were combined, dried over MgSO$_4$, filtered and concentrated under reduced pressure. Flash column chromatography employing pentane : ether (9:1) afforded (1R,2R,8aS)-1-(2-((tert-butyldiphenylsilyl)oxy)ethyl)-2,5,5,8a-tetramethyldecahydropyridin-2-ol 17 (1.5 g, 3.2 mmol, 94% yield) as a very viscous transparent oil.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 7.71 – 7.65 (m, 4H), 7.45 – 7.34 (m, 6H), 3.68 – 3.57 (m, 1H), 3.45 (tt, $J = 9.2$, 4.3 Hz, 1H), 1.98 – 1.79 (m, 3H), 1.72 (tt, $J = 9.5$, 5.1 Hz, 2H), 1.64 – 1.12 (m, 10H), 1.05 (s, 9H), 0.94 (s, 3H), 0.87 (s, 3H), 0.79 (s, 3H).

$^{13}$C-NMR (150 MHz, CDCl$_3$) δ 135.63, 135.60, 129.47, 127.57, 61.11, 39.89, 39.80, 38.76, 34.51, 34.37, 29.10, 27.71, 27.20, 26.92, 26.52, 25.78, 25.03, 21.06, 19.92, 19.15, 16.33, 7.12.
CHAPTER 4

((1R,2S,4aS,8aS)-1,2,5,5-tetramethyloctahydro-4a,8a-epoxynaphthalen-1-yl)ethyl acetate (18):
To a solution of 3 (1.0 g, 3.6 mmol) in dry CH$_2$Cl$_2$ (40 mL) and MeOH (4 mL), O$_3$ was bubbled through for 1 h at -78 °C. The reaction mixture turned bright blue. After this time, O$_2$ was passed through the reaction vessel for 5 min to remove residual O$_3$. To the solution was added Me$_2$S (2 mL, 27 mmol) at -78 °C where after the reaction was allowed to warm to rt over 3 h. The reaction mixture was concentrated under reduced pressure and purified using flash chromatography (pentane : ether = 9:1). Pure 2-((1R,2S,4aS,8aS)-1,2,5,5-tetramethyloctahydro-4a,8a-epoxynaphthalen-1-yl)ethyl acetate 18 (514 mg, 1.75 mmol, 48% yield) was obtained as a slightly yellow oil.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 4.23 – 4.13 (m, 1H), 4.13 – 4.02 (m, 1H), 2.05 (d, $J = 0.9$ Hz, 3H), 1.95 – 1.82 (m, 2H), 1.82 – 1.61 (m, 4H), 1.52 – 1.39 (m, 1H), 1.39 – 1.25 (m, 4H), 1.15 – 1.02 (m, 2H), 1.00 (d, $J = 0.9$ Hz, 3H), 0.97 (s, 3H), 0.87 (d, $J = 0.9$ Hz, 3H), 0.84 – 0.79 (m, 3H).

$^{13}$C-NMR (150 MHz, CDCl$_3$) δ 170.84, 69.90, 68.35, 61.52, 38.65, 37.99, 35.85, 35.03, 32.82, 27.14, 26.62, 26.27, 24.72, 24.18, 20.98, 17.47, 17.31, 16.29.

2-((1R,2S,4aS,8aS)-1,2,5,5-tetramethyloctahydro-4a,8a-epoxynaphthalen-1-yl)ethanol (19):
To a solution of 18 (308 mg, 1.05 mmol) in MeOH/H$_2$O (2:1, 2 mL) was added potassium carbonate (289 mg, 2.1 mmol, 2 eq). The reaction was stirred for 5 h after which TLC analysis (pentane : ether = 9:1) indicated complete conversion. The reaction mixture was diluted with Et$_2$O and the phases were separated. The aqueous phase was extracted three times with Et$_2$O after which the combined organic layers were washed with brine. The organic phase was dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to afford 2-((1R,2S,4aS,8aS)-1,2,5,5-tetramethyloctahydro-4a,8a-epoxynaphthalen-1-yl)ethanol 19 (246 mg, 0.98 mmol, 93% yield) as a colorless oil.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 3.72 (ddd, $J = 10.4, 9.5, 6.1$ Hz, 1H), 3.63 (td, $J = 10.1, 4.9$ Hz, 1H), 1.92 – 1.80 (m, 3H), 1.80 – 1.54 (m, 4H), 1.47 – 1.35 (m, 1H), 1.35 – 1.21
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\((3aR,4S,6aS,10aR)-3a,4,7,7\text{-tetramethyldecahydro-2H-naphtho}[8a,1-b]\text{-furan-6a-ol (22)}:\)  
To a suspension of lithium aluminum hydride (32 mg, 0.85 mmol) in dry THF (2 mL) was added a solution of 18 (50 mg, 0.17 mmol) in dry THF (3 mL). The reaction was stirred at rt for 30 min after which TLC (pentane : ether = 8:2) indicated complete conversion. The reaction was carefully quenched with a saturated aqueous NH\(_4\)Cl solution. The phases were separated and the aqueous layer was extracted with CH\(_2\)Cl\(_2\) (2x5 mL). The combined organic phases were dried over MgSO\(_4\), filtered and concentrated under reduced pressure affording exclusively (3a\(R\),4\(S\),6a\(S\),10a\(R\))-3a,4,7,7-tetramethyldecahydro-2H-naphtho[8a,1-b]furan-6a-ol 22 (42 mg, 0.17 mmol, 98% yield).

\(^1\text{H-NMR (400 MHz, CDCl}_3\) \(\delta\) 3.79 (q, \(J = 8.5\) Hz, 1H), 3.68 (ddd, \(J = 10.3, 8.7, 3.3\) Hz, 1H), 1.91 – 1.81 (m, 1H), 1.79 – 1.68 (m, 2H), 1.68 – 1.57 (m, 2H), 1.57 – 1.49 (m, 2H), 1.49 – 1.39 (m, 2H), 1.39 – 1.24 (m, 3H), 1.10 (s, 3H), 0.94 (s, 3H), 0.86 – 0.81 (m, 6H).

\(^{13}\text{C-NMR (150 MHz, CDCl}_3\) \(\delta\) 70.15, 68.95, 59.75, 40.42, 38.74, 38.12, 35.20, 32.95, 27.25, 26.79, 26.43, 24.91, 24.31, 17.69, 17.48, 16.52.

\((E)-2\text{-methyl-1-(thiazol-2-yl)but-2-en-1-one (28)}:\)  
To magnetically stirred 2-(trimethylsilyl)thiazole 27 (1.85 ml, 12.7 mmol) was slowly* (dropwise over 5 min!) added \((E)-2\text{-methylbut-2-enoyl chloride 26 (1.54 mL, 12.7 mmol). The reaction was stirred overnight after which it was diluted with dry THF (175 mL). To the resulting light yellow solution was added tetrabutylammonium fluoride (TBAF, 12.7 mL, 12.7 mmol, 1 M in THF) which resulted in a color change to bright orange. The reaction was stirred overnight where after it was concentrated under reduced pressure. Flash column chromatography using pentane : ether (96:4) furnished
(E)-2-methyl-1-(thiazol-2-yl)but-2-en-1-one 28 (1.86 g, 11.0 mmol, 88% yield) as a light yellow oil.

* Approximately one minute after addition of the tigloyl chloride 26 the reaction is exothermic! When performing this reaction on multi-gram scale, cooling with an ice-bath might be necessary!

** Thiazolium tiglate 28 is unstable at room temperature and decomposes over time. Even at 4 °C the product is not entirely stable (the oil darkens significantly over time). Therefore 28 can best be stored at -20 °C at which thiazolium tiglate 28 is a crystalline solid.

\[ \text{L-H-NMR (400 MHz, CDCl}_3\text{) } \delta 7.96 (d, J = 3.1 \text{ Hz}, 1\text{H}), 7.77 – 7.69 (m, 1\text{H}), 7.60 (d, J = 3.1 \text{ Hz}, 1\text{H}), 2.00 – 1.98 (m, 6\text{H}). \]

\[ \text{C-H-NMR (150 MHz, CDCl}_3\text{) } \delta 199.27, 167.74, 145.00, 144.38, 125.41, 116.50, 55.60, 40.76, 36.25, 34.36, 31.47, 29.77, 29.40, 28.65, 21.78, 16.39, 9.72. \]

\[(1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyrene-1-yl](thiazol-2-yl)methanone (31):

To a solution of L3 (30 mol%) in dry dichloroethane (1 mL) was added Cu(OTf)\(_2\) (20 mol%). The copper complex was allowed to form over 3 h. To the in situ generated copper catalyst were added 6,6-dimethyl-1-vinylcyclohex-1-ene 6 (10 mg, 0.073 mmol) and (E)-2-methyl-1-(thiazol-2-yl)but-2-en-1-one (18.4 mg, 0.11 mmol) in a total volume of 1 mL dry dichloroethane. The reaction was stirred at 50 °C overnight where after the reaction mixture was concentrated and purified by flash column chromatography to afford \((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyrene-1-yl](thiazol-2-yl)methanone 31 as a diastereomeric mixture of 2:1. The absolute stereochemistry remained unknown.

\[ \text{H-NMR (400 MHz, CDCl}_3\text{) } \delta 7.95 (d, J = 3.1 \text{ Hz}, 1\text{H}), 7.56 (d, J = 3.3 \text{ Hz}, 1\text{H}), 5.56 (dt, J = 5.8, 2.2 \text{ Hz}, 1\text{H}), 3.86 – 3.72 (m, 1\text{H}), 3.15 – 2.94 (m, 2\text{H}), 2.11 (dt, J = 18.1, 5.6 \text{ Hz}, 1\text{H}), 1.96 (td, J = 5.5, 2.1 \text{ Hz}, 1\text{H}), 1.82 – 1.76 (m, 1\text{H}), 1.46 – 1.35 (m, 2\text{H}), 1.32 – 1.13 (m, 2\text{H}), 1.11 (s, 3\text{H}), 1.08 (s, 3\text{H}), 1.02 (s, 3\text{H}), 0.68 (d, J = 6.7 \text{ Hz}, 3\text{H}). \]

\[ \text{C-NMR (150 MHz, CDCl}_3\text{) } \delta 199.27, 167.74, 145.00, 144.38, 125.41, 116.50, 55.60, 40.76, 36.25, 34.36, 31.47, 29.77, 29.40, 28.65, 21.78, 16.39, 9.72. \]
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General procedure for the synthesis of the diimine chiral ligands L21-L30:
To a solution of (1S,2S)-cyclohexane-1,2-diamine (75 mg, 0.66 mmol) in EtOH (2 mL) was added a solution/suspension of aromatic aldehyde (2 eq) in EtOH (2-3 mL). The reactions were refluxed overnight. The resulting diimines were crystallized from various mixtures of ethylacetate:pentane, ethanol, or methanol.

The bisimine ligands L21-L30 prepared and obtained in accordance to:

(5)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl (E)-2-methylbut-2-enoate (42):
To a cooled solution (-10 °C) of (E)-2-methylbut-2-enoic acid 25 (10 g, 100 mmol), pantolactone (14.3 g, 110 mmol, 1.1 eq), and DCC (21.7 g, 105 mmol, 1.05 eq) in dry CH2Cl2 (250 mL), N,N-dimethylpyridin-4-amine (1.22 g, 10 mmol, 10 mol%) in dry CH2Cl2 (40 mL), was added portionwise. The solution was allowed to warm to rt and was stirred overnight. The resulting suspension was filtered through a path of Celite and the residue was washed with DCM. The filtrate was washed with two portions of a saturated aqueous solution of NaHCO3 (500 mL) and once with brine (200 mL). The organic phase was dried over Na2SO4, concentrated under reduced pressure and purified performing flash chromatography (eluent 35% ether in pentane) to isolate (R,E)-4,4-dimethyl-2-oxotetrahydrofuran-3-yl 2-methylbut-2-enoate 42 (11 g, 52 mmol, 52% yield) as a slightly yellow oil.

1H-NMR (400 MHz, CDCl3) δ 7.01 – 6.91 (m, 1H), 5.40 (s, 1H), 4.02 (s, 2H), 1.84 (s, 3H), 1.79 (d, J = 7.0 Hz, 3H), 1.18 (s, 3H), 1.10 (s, 3H).

13C-NMR (150 MHz, CDCl3) δ 172.75, 166.55, 139.66, 127.50, 76.22, 74.99, 40.41, 23.05, 19.99, 14.58, 12.09.
(E)-1-((3aS,6R,7aR)-8,8-dimethyl-2,2-dioxidotetrahydro-3H-3a,6-methanobenzo[c]isothiazol-1(4H)-yl)-2-methylbut-2-en-1-one (43):
To a -78 °C cooled solution of (3aS,6R,7aR)-8,8-dimethylhexahydro-1H-3a,6-methanobenzo[c]isothiazole 2,2-dioxide 60 (490 mg, 2.28 mmol) in dry THF (10 mL) was added nBuLi (1.1 mL, 2.5 M solution in hexane, 2.75 mmol, 1.2 eq). The reaction was allowed to warm-up to 0 °C and was stirred at this temperature for 30 min where after the mixture was re-cooled to -78 °C and (E)-2-methylbut-2-enoyl chloride 26 (1.51 ml, 13.8 mmol, 6 eq) was added. The reaction was allowed to warm up to room temperature and was stirred overnight where after it was carefully quenched using aqueous HCl (30 mL, 1 M). After phase separation, the aqueous layer was extracted with ether. The combined organic layers were washed with a saturated aqueous solution of NaHCO₃, dried using Na₂SO₄, filtered and concentrated under reduced pressure. The resulting yellow oil was cooled down to 4 °C which resulted in crystallization of the product. The residual oil was removed where after the crystals were washed with cold (-20 °C) ether until the ether layer stayed clear and transparent. (E)-1-((3aS,6R,7aR)-8,8-dimethyl-2,2-dioxidohexahydro-1H-3a,6-methanobenzo[c]isothiazol-1-yl)-2-methylbut-2-en-1-one 43 (400 mg, 1.35 mmol, 59% yield) was obtained as a white solid.

1H-NMR (400 MHz, CDCl₃) δ 6.37 (dq, J = 8.4, 6.9, 5.5, 1.5 Hz, 1H), 4.05 (dd, J = 7.7, 4.5 Hz, 1H), 3.45 (q, J = 27.7, 13.4 Hz, 2H), 2.03 (dd, J = 13.4, 7.7 Hz, 1H), 1.97 – 1.87 (m, 4H), 1.87 (s, 3H), 1.82 (d, J = 6.9 Hz, 3H), 1.47 – 1.32 (m, 2H), 1.23 (s, 3H), 0.99 (s, 3H).

13C-NMR (150 MHz, CDCl₃) δ 139.99, 137.70, 65.53, 53.69, 48.00, 45.36, 38.37, 33.35, 26.68, 21.45, 20.05, 14.74, 14.23, 12.81, 11.82.

(4aR,5R,6S)-5-(bromomethyl)-1,1,5,6-tetramethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene (53):
To a solution of 1-bromopyrroolidine-2,5-dione (592 mg, 3.33 mmol) in dry THF (15 mL) was added slowly a solution of triphenylphosphate (873 mg, 3.33 mmol) in dry THF (15 mL). The reaction is exothermic, and upon addition of the PPh₃ a bright yellow solution formed which quickly turned into a suspension by precipitation of a white
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125 solid. This mixture was allowed to stir for 15 min resulting in an orange color. To the suspension was added a solution of ((1R,25,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydonaphthalen-1-yl)methanol (247 mg, 1.11 mmol) in dry THF (7.5 mL). The reaction was stirred over the weekend at 50 °C. GC-MS indicated complete conversion. The reaction mixture was diluted with pentane which resulted in the precipitation of PPh₃. After gravitational filtration, more PPh₃ crystallized so another filtration was performed. The elute was concentrated under reduced pressure which resulted in an oil. The oil was diluted with pentane which resulted, again, in the precipitation of PPh₃. The suspension was flushed over a SiO₂ column resulting in a slightly yellow oil. Flash column chromatography employing pentane as the eluent afforded (4aR,5R,6S)-5-(bromomethyl)-1,1,5,6-tetramethyl-1,2,3,4,4a,5,6,7-octahydonaphthalene 53 (280 mg, 0.98 mmol, 59% yield) as a colorless oil.

1H-NMR (400 MHz, CDCl₃) δ 5.45 (dt, J = 5.7, 2.0 Hz, 1H), 3.53 (d, J = 10.5 Hz, 1H), 3.35 (d, J = 10.5 Hz, 1H), 2.50 – 2.44 (m, 1H), 2.16 – 2.02 (m, 1H), 1.96 – 1.83 (m, 1H), 1.79 – 1.66 (m, 2H), 1.66 – 1.55 (m, 2H), 1.41 (dt, J = 13.0, 3.3, 1.8 Hz, 1H), 1.32 – 1.12 (m, 2H), 1.07 (s, 3H), 1.03 (s, 3H), 0.84 (d, J = 6.3 Hz, 3H), 0.73 (s, 3H).

13C-NMR (150 MHz, CDCl₃) δ 145.67, 116.11, 77.33, 42.75, 40.92, 39.47, 38.72, 36.28, 33.24, 31.29, 29.86, 29.08, 27.32, 22.07, 14.69, 13.52.

6,6-dimethyl-1-vinylcyclohex-1-ene (6):
To a solution of cyclohexanone (9.91 ml, 96 mmol) and iodomethane (12.5 ml, 201 mmol) in dry THF (500 mL), cooled to -20 °C, was added dropwise, using a dropping funnel, a solution of KOtBu (22.6 g, 201 mmol) in dry THF (125 mL). Upon addition, a white precipitate, formed and the reaction turned yellow. After addition, the mixture was stirred for 60 min at this temperature after which GC-MS indicated complete conversion of the cyclohexanone. The reaction mixture was quenched with an aqueous saturated NH₄Cl solution (200 mL). Quenching resulted in significant, but not complete, disappearance of the white precipitate, which was completely removed by filtration of the quenched mixture over a glass filter. The phases were separated and the aqueous phase was extracted twice with Et₂O. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated under reduced pressure. Flash column chromatography of the resulting oil using pentane : ether (4:1) afforded 2,2-dimethylcyclohexanone 29 (18.3 g, 145 mmol, 49% yield) ~90% pure. In order to obtain the desired 90% purity, the individual column fractions were analyzed with GC-MS.
Note: The reaction was performed 3x on this scale. The extractions were performed on the combined reaction mixtures. The yield represents that of the combined reactions.

To a stirred solution of vinylmagnesium bromide (174 mL, 174 mmol) at 0 °C was added dropwise a solution of 2,2-dimethylcyclohexanone 29 (18.3 g, 145 mmol) in dry THF (50 mL) over 30 min. The reaction was allowed to warm up to room temperature and stirred for 2 h. The reaction mixture was carefully quenched with an aqueous saturated NH₄Cl solution (75 mL). After phase separation the aqueous layer was extracted with Et₂O (3x25 mL). The organic layers were combined and dried with sodium sulfate, filtered and concentrated under reduced pressure. The resulting yellowish oil (27.3 g, >100% yield) was used in the subsequent dehydration reaction.

Crude alcohol 30 was dissolved in benzene (300 mL). To the stirred solution, anhydrous CuSO₄ (50 g, 313 mmol, 2.2 eq) was added. The suspension was refluxed under Dean-Stark conditions overnight. The reaction mixture was cooled to rt and thereafter filtered over Celite and flushed with pentane. The filtrate was concentrated under reduced pressure and the residual oil was subjected to flash column chromatography employing pentane as the eluent. Pure 6,6-dimethyl-1-vinylcyclohex-1-ene 6 (10.5 g, 77 mmol, 53% yield) was obtained as a colorless oil.

\begin{align*}
^{1}H-NMR (400 MHz, CDCl₃) & δ 6.32 (dd, J = 17.2, 10.9 Hz, 1H), 5.78 (t, J = 3.9 Hz, 1H), \\
& 5.28 (d, J = 17.2 Hz, 1H), 4.92 (d, J = 10.9 Hz, 1H), 2.05 (q, J = 5.7 Hz, 2H), 1.61 (dt, J = 12.0, 5.9 Hz, 2H), 1.53 – 1.46 (m, 2H), 1.07 (s, 6H).
\end{align*}

\begin{align*}
^{13}C-NMR (101 MHz, CDCl₃) & δ 144.74, 137.20, 123.20, 112.82, 39.55, 33.33, 28.50, 26.33, 19.30.
\end{align*}

Note: Diene 6 is rather volatile and therefore concentration, after column chromatography, was performed very carefully. As a result the NMR spectra of diene 6 contain benzene and traces of pentane.


\begin{align*}
\text{(E)-2-methylbut-2-enoyl chloride (26):}
\end{align*}

To vigorously stirred thionyl chloride (27.2 ml, 375 mmol) in a 250 mL 3-neck round bottom flask (open flask!) was added portion wise (E)-2-methylbut-2-enoic acid 25 (25 g, 250 mmol). Each portion was added after significant gas evolution (HCl, SO₂)

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ceased. After addition of all the acid, the reaction mixture (open flask!) was heated to 40 °C until no gas evolution was observed. The 3-neck round bottom flask was equipped with a reflux condenser, and the reaction was refluxed for 1 h. After this time no gas evolution was observed. The excess of thionyl chloride was removed by concentration under reduced pressure. The product was subsequently distilled in the rotavapor to afford (E)-2-methylbut-2-enoyl chloride 26 (29.6 g, 250 mmol, quantitative) as a clear liquid.

Note: The reaction is exothermic with vigorous evolution of HCl/SO₂ gas.

(S,E)-4-isopropyl-3-(2-methylbut-2-enoyl)oxazolidin-2-one (41):
To a cooled solution (-60 °C), of (S)-4-isopropyl oxazolidin-2-one 61 (25.0 g, 194 mmol) in dry THF (500 mL) was added dropwise n-butyllithium (133 ml, 213 mmol) by the aid of a dropping funnel. Upon addition, the reaction mixture became turbid. After addition, the reaction was allowed to warm-up to 0 °C and was stirred at this temperature for 30 min where after the mixture was re-cooled to -60 °C and (E)-2-methylbut-2-enoyl chloride 26 (29.6 g, 250 mmol) was added dropwise by the aid of a syringe pump over 20 min. Upon addition the reaction mixture turned yellow/orange. After addition of acid chloride 26 the reaction was allowed to warm up to rt, and the reaction became transparent. The reaction was stirred overnight where after it was carefully quenched using aqueous HCl (1 M). After phase separation, the aqueous layer was extracted with ether. The combined organic layers were washed with a saturated aqueous solution of NaHCO₃ and dried using Na₂SO₄, filtered and concentrated under reduced pressure. A solid formed which was dissolved in CH₂Cl₂ (15 mL) and subjected to flash column chromatography employing pentane : ether (3:2) as the eluent to afford pure (S,E)-4-isopropyl-3-(2-methylbut-2-enoyl)oxazolidin-2-one 41 (25 g, 118 mmol, 61% yield) as a waxy solid.

1H-NMR (400 MHz, CDCl₃) δ 6.20 (q, J = 7.0 Hz, 1H), 4.51 (dt, J = 9.0, 4.9 Hz, 1H), 4.31 (t, J = 8.9 Hz, 1H), 4.17 (dd, J = 8.9, 5.5 Hz, 1H), 2.36 (dq, J = 13.8, 6.9 Hz, 1H), 1.90 (s, 3H), 1.80 (d, J = 7.0 Hz, 3H), 0.91 (t, J = 6.8 Hz, 6H).

13C-NMR (101 MHz, CDCl₃) δ 171.92, 153.80, 134.69, 131.92, 63.52, 58.39, 28.36, 17.97, 15.14, 14.20, 13.48.


(S)-4-isopropyl-3-((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbonyl)oxazolidin-2-one (44):

To a stirred solution of dienophile 41 (9.13 g, 43.2 mmol, 1.1 eq) in dry (CH$_2$)$_2$Cl$_2$ (250 mL) at -40 °C under N$_2$ was added Me$_2$AlCl (86.5 mL, 1 M in hexanes, 86.5 mmol, 2.2 eq) over 15 min, by the aid of a dropping funnel. The yellow reaction mixture was stirred for 20 min, and diene 6 (5.35 g, 39.3 mmol) in dry (CH$_2$)$_2$Cl$_2$ (90 mL) was added dropwise over 15 min by the aid of a dropping funnel. The reaction was then allowed to warm to rt and was stirred for 36 h at this temperature. GC-MS indicated complete conversion of the diene.

The reaction mixture was cooled to 0 °C and carefully quenched by dropwise addition of aqueous 1 M HCl (50 mL). After phase separation, the aqueous layer was extracted with CH$_2$Cl$_2$ (3x20 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. Flash column chromatography with pentane : ether (6:1) provided (S)-4-isopropyl-3-((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbonyl)oxazolidin-2-one 44 as a white appearing crystalline solid (8.0 g, 19.6 mmol, 59% yield, exo:endo = 10:1).

Note: the reaction temperature was monitored inside the flask. Excessively low temperatures lead to crystallization of the dienophile.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 5.47 (d, $J = 5.7$ Hz, 1H), 4.54 (d, $J = 8.2$ Hz, 1H), 4.33 – 4.13 (m, 2H), 3.34 (d, $J = 12.8$ Hz, 1H), 3.15 (tt, $J = 12.5$, 6.5 Hz, 1H), 2.39 – 2.30 (m, 1H), 1.95 (dt, $J = 17.5$, 5.5 Hz, 1H), 1.79 – 1.63 (m, 2H), 1.54 (tt, $J = 7.0$, 3.1 Hz, 1H), 1.43 – 1.29 (m, 2H), 1.28 – 1.13 (m, 2H), 1.06 (s, 3H), 1.03 (s, 3H), 1.01 (s, 3H), 0.90 (t, $J = 6.3$ Hz, 6H), 0.78 (d, $J = 6.8$ Hz, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 178.00, 153.03, 144.80, 116.12, 62.88, 61.16, 53.54, 40.94, 37.57, 36.39, 31.25, 29.71, 29.53, 28.93, 28.31, 22.21, 18.54, 16.48, 14.53, 12.43.

HRMS (ESI+): Calculated mass [M+H]$^+$ C$_{21}$H$_{34}$NO$_3$ = 348.2533; found: 348.2536. Calculated mass [M+Na]$^+$ C$_{21}$H$_{33}$NO$_3$Na$^+$ = 370.2353; found: 370.2355.

Melting point: 96 °C
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Optical rotation: $[\alpha]_D^{23} = +57.4$ (c = 0.0135, CHCl$_3$).

(1R,2S,8aR)-S-ethyl-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbothioate (55):
To a solution of ethanethiol (8.3 mL, 115 mmol, 5.9 eq) in dry THF (200 mL), cooled to 0 °C, was added dropwise n-butyllithium (57.6 mL, 92 mmol, 4.7 eq). Upon addition, a white precipitate formed. After addition, the milk white, now viscous, suspension was allowed to warm to rt. Diels-Alder adduct 44 (8.0 g, 19.6 mmol) in dry THF (50 mL) was added and the reaction was stirred for 7 h. Full conversion was observed by TLC and GC-MS analysis. The reaction was diluted with Et$_2$O and quenched by addition of a saturated aqueous NH$_4$Cl. The white precipitate dissolved. The phases were separated, and the aqueous phase was extracted with Et$_2$O. The combined organic phases were dried over MgSO$_4$, filtered and concentrated under reduced pressure to afford a yellow oil. Upon standing, the oxazolidinone chiral auxiliary crystallized. Pentane was added to wash the crystals, which became transparent in color, and the organic layer turned yellowish. The suspension was cooled to -20 °C where after the cold suspension was filtered over a sintered glass filter (pore size 3). The residue was rinsed with cold pentane (20 °C) to provide the chiral auxiliary as needle shaped transparent crystals (2.2 g). The filtrate was concentrated under reduced pressure to afford crude (1R,2S,8aR)-S-ethyl 1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbothioate 55 (7.2 g, 25.7 mmol >100% yield) as a yellowish oil. The product was considered sufficiently pure to be used in the next step.

Alternatively flash column chromatography can be performed: The oil/crystal mixture was dissolved in a minimum amount of ether and loaded on the column. Elution using pentane : ether (98:2) as the eluent afforded pure (1R,2S,8aR)-S-ethyl 1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbothioate 55 (5.06 g, 18.0 mmol, 96% yield) as a yellowish oil. (The 96% yield was obtained for a reaction starting from 6.5 g of Diels-Alder adduct 3).

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 5.46 (d, $J = 5.3$ Hz, 1H), 2.88 (q, $J = 7.4$ Hz, 2H), 2.79 (d, $J = 12.9$ Hz, 1H), 2.02 – 1.86 (m, 2H), 1.75 (ddt, $J = 13.4, 11.5, 3.6$ Hz, 1H), 1.53 – 1.46 (m, 3H), 1.44 – 1.36 (m, 1H), 1.25 (t, $J = 7.4$ Hz, 3H), 1.21 – 1.10 (m, 2H), 1.08 (s, 3H), 1.05 (s, 3H), 0.98 (s, 3H), 0.79 (d, $J = 6.5$ Hz, 3H).
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$^{13}$C-NMR (101 MHz, CDCl$_3$) δ 207.85, 144.94, 116.00, 56.71, 43.49, 40.58, 36.77, 36.04, 31.41, 29.68, 29.44, 28.22, 23.14, 21.68, 15.87, 14.83, 9.61.

HRMS (ESI+): Calculated mass [M+H]$^+$ C$_{17}$H$_{29}$O$^+$ = 281.1934; found: 281.1932.

Optical rotation: [α]$_D^{23}$ = +8.7 (c = 0.0184, CHCl$_3$).

((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyridin-1-yl)methanol (50):
To a cooled (0 °C) solution of crude thioester 55 (7.2 g, 25.7 mmol) in dry THF (150 mL) was added portionwise lithium aluminum hydride (4.9 g, 128 mmol, 5 eq). After addition, the reaction was allowed to warm-up to rt where after the reaction was heated to 40 °C and stirred for 2 h. GC-MS and TLC analysis indicated complete conversion of the starting material. The reaction mixture was cooled to 0 °C, diluted with ether and carefully quenched using a saturated aqueous Rochelle salt. After addition, the quenched reaction mixture was stirred for 30 min. After phase separation, the aqueous layer was extracted three times with ether where after the combined organic layers were washed brine. The organic phase was then dried over MgSO$_4$, filtered and concentrated under reduced pressure to yield ((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyridin-1-yl)methanol 50 (6.2 g, 30 mmol, quantitative yield) as yellow oil. The material was deemed pure enough to be used in the next step without purification.

Alternatively flash column chromatography can be performed: purification of the obtained yellow oil, using pentane : ether (9:1) as the eluent, afforded pure ((1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyridin-1-yl)methanol 50 (4.7 g, 21.1 mmol, 91% yield) as a yellowish oil. (The 91% yield reflects that obtained over the past 2 steps, starting from Diels–Alder adduct 44. With 96% isolated yield for the oxazolidinone cleavage this corresponds to 95% isolated yield for the reduction of thioester 55 to alcohol 50).

Chiral HPLC analysis of alcohol 50 showed an enantiomeric excess exceeding 98% for the exo isomer. Also the endo isomer proved to be enantiomerically enriched, possessing an enantiomeric excess of ~60%.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 5.43 (d, $J$ = 5.2 Hz, 1H), 3.48 (d, $J$ = 11.4 Hz, 1H), 3.39 (d, $J$ = 11.3 Hz, 1H), 2.36 (d, $J$ = 12.9 Hz, 1H), 1.91 – 1.81 (m, 1H), 1.81 – 1.71 (m,
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2H), 1.70 – 1.63 (m, 2H), 1.61 – 1.51 (m, 2H), 1.38 (s, 1H), 1.28 – 1.14 (m, 2H), 1.05 (s, 3H), 1.00 (s, 3H), 0.86 (d, $J = 6.5$ Hz, 3H), 0.51 (s, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 146.12, 116.11, 65.78, 41.07, 39.33, 38.05, 36.27, 31.93, 31.33, 29.86, 28.86, 27.74, 22.26, 15.09, 11.63.

HRMS (ESI+): Calculated mass [M+H]$^+$ $C_{15}H_{27}O^+$ = 223.2056; found: 223.2059.

Chiral HPLC analysis on a Chiracel AD-H column, $n$-Heptane : $i$-PrOH = 98 : 2, 40 °C, flow = 0.5 mL/min, UV detection at 190 nm, 210 nm and 254 nm, retention times (min): 15.7 (endo minor), 15.1 (endo major), 17.7 (exo major), and 21.1 (exo minor, not detected for chiral alcohol 50).


(1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbaldehyde (8):

To a solution of alcohol 50 (4.7 g, 21.1 mmol) in dry CH$_2$Cl$_2$ (80 mL) were added TPAP (371 mg, 1.06 mmol, 5 mol%), 4-methylmorpholine-N-4-oxide (or its monohydrate) (3.22 g, 27.5 mmol, 1.3 eq) and 3 Å molecular sieves. The reaction was stirred at rt for 2 h after which TLC and GC-MS analysis indicated complete conversion of the starting material. The reaction mixture was diluted using pentane which resulted in precipitation of the TPAP. The mixture was filtered over Celite to remove the TPAP. Removal of NMO and reduced analogue were removed by flash column chromatography using pentane: ether (95:5) as the eluent to afford pure (1R,2S,8aR)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-1-carbaldehyde 8 (4.43 g, 20.1 mmol, 95% yield) as a yellowish oil which crystallized upon standing.

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$ 9.38 (s, 1H), 5.52 – 5.47 (m, 1H), 2.50 (d, $J = 13.1$ Hz, 1H), 2.00 – 1.91 (m, 1H), 1.90 – 1.80 (m, 1H), 1.80 – 1.69 (m, 1H), 1.57 – 1.49 (m, 2H), 1.45 – 1.37 (m, 1H), 1.36 – 1.28 (m, 1H), 1.27 – 1.14 (m, 2H), 1.08 (s, 3H), 1.04 (s, 3H), 0.80 (s, 3H), 0.78 (d, $J = 6.5$ Hz, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) $\delta$ 207.16, 143.92, 116.56, 52.26, 40.61, 38.15, 36.28, 32.65, 30.47, 29.66, 29.13, 28.68, 21.77, 16.14, 7.49.

Melting point: 53 °C


\((E)-4-((1S,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)but-3-en-2-one \(56\):

A solution of NaHMDS (30 mL, 2 M in THF, 30 mmol, 3 eq) diluted to a 1 M solution with dry THF (30 mL) was cooled to \(-78\) °C. The orange solution became slightly turbid and viscous, however stirring was assured. Acetone (4.4 ml, 60.3 mmol, 3 eq) was added dropwise to the turbid solution which became clear upon addition. The mixture was stirred at this temperature for 20 min after which a solution of aldehyde \(8\) (4.43 g, 20.1 mmol) in dry THF (125 mL) was added dropwise over 20 min by the aid of a dropping funnel. After addition the reaction was taken out of the cooling bath and allowed to warm-up to rt (by the aid of a water bath at rt). The reaction was stirred for 2 h after which TLC and GC-MS indicated complete consumption of the aldehyde \(8\).

The reaction was diluted with ether and quenched with a saturated NaHCO₃. The phases were separated, and the organic phase was washed with distilled water and brine. The combined aqueous layers were back-extracted once with Et₂O and the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. Flash column chromatography employing pentane : ether (95:5) afforded \((E)-4-((1S,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)but-3-en-2-one 56\) as an amorphous solid (3.4 g, 13.1 mmol, 65% yield).

Note: The aldol condensation was also performed on a 3.5 g scale which resulted in a slightly higher isolated yield of 74%. It is very important to monitor the reaction and not let it run overnight. This gives over-condensation and therefore lower isolated yield.

\(^1\)H-NMR (400 MHz, CDCl₃) δ 6.60 (d, \(J = 16.2\) Hz, 1H), 6.02 (d, \(J = 16.3\) Hz, 1H), 5.49 (d, \(J = 5.8\) Hz, 1H), 2.26 (s, 3H), 2.13 (d, \(J = 13.1\) Hz, 1H), 1.93 (dt, \(J = 17.8, 4.7\) Hz, 1H), 1.80 – 1.68 (m, 1H), 1.62 – 1.34 (m, 5H), 1.17 (dd, \(J = 12.6, 5.1\) Hz, 1H), 1.07 (s, 3H), 1.00 (s, 3H), 0.96 – 0.89 (m, 1H), 0.78 (s, 3H), 0.72 (d, \(J = 6.8\) Hz, 3H).
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\[ ^{13} \text{C-NMR (101 MHz, CDCl}_3] \delta 198.72, 158.19, 144.73, 129.58, 116.47, 43.67, 43.00, 40.77, 36.65, 36.22, 31.12, 29.67, 29.28, 28.27, 27.43, 21.96, 16.38, 10.28. \]

HRMS (ESI+): Calculated mass [M+H]^+ C_{18}H_{29}O = 261.2213; found: 261.2215. Calculated mass [M+Na]^+ C_{21}H_{33}NO_{3}Na^+ = 283.2032; found: 283.2034.

Melting point: 72 °C


4-((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)butan-2-one (57):

To a solution of α,β-unsaturated ketone 56 (3.4 g, 13.1 mmol) in dry (CH\(_2\)Cl\(_2\)) (150 mL) were added Wilkinson’s catalyst (909 mg, 0.98 mmol, 7.5 mol%) and triethylsilane (10.4 mL, 65.5 mmol, 5 eq). The reaction mixture was heated to reflux and stirred for 90 minutes after which TLC indicated complete conversion of the starting material. The reaction was cooled to rt where after it was quenched with an aqueous solution of HCl (6 M, 100 mL). The mixture was stirred for 30 minutes after which the phases were separated, and the organic layer was washed with water, followed by a saturated aqueous solution of NaHCO\(_3\) and brine. The organic layer was dried over MgSO\(_4\), filtered and concentrated under reduced pressure. Flash column chromatography employing pentane : ether (95:5) afforded pure 4-((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)butan-2-one 57 (3.1 g, 11.7 mmol, 90% yield) as a slightly yellow oil.

\[ ^{1} \text{H-NMR (400 MHz, CDCl}_3] \delta 5.41 (s, 1H), 2.37 – 2.28 (m, 2H), 2.14 (s, 3H), 1.99 (d, J = 12.5 Hz, 1H), 1.88 – 1.60 (m, 4H), 1.60 – 1.32 (m, 6H), 1.17 (td, J = 13.0, 4.4 Hz, 1H), 1.04 (s, 3H), 0.98 (s, 3H), 0.78 (d, J = 6.8 Hz, 3H), 0.63 (s, 3H). \]

\[ ^{13} \text{C-NMR (101 MHz, CDCl}_3] \delta 209.58, 145.91, 116.30, 40.95, 40.12, 37.77, 36.71, 36.18, 33.59, 31.61, 30.15, 30.07, 29.84, 29.04, 27.57, 22.26, 16.04, 15.11. \]

Ethyl-3-methyl-5-(((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyrranonaphthalen-1-yl)pent-2-enoate (58):
ethyl 2-(diethoxyphosphoryl)acetate (6.96 mL, 35.1 mmol, 3 eq) in dry THF (50 mL) was added dropwise to a suspension of sodium hydride (1.4 g, 60% in oil, 35.1 mmol, 3 eq) in dry THF (50 mL) at 0 °C by the aid of a dropping funnel. The suspension turned into a clear solution and was stirred for 20 min after which ketone 57 (3.1 g, 11.7 mmol) in dry THF (100 mL) was added dropwise over 10 minutes by the aid of a dropping funnel. The cooling bath was removed and the mixture was allowed to warm to rt. The reaction vessel was then sealed under N₂ and placed in an oil bath at 80 °C and was allowed to stir for 6 h. TLC and GC-MS indicated full conversion of the starting material.

The reaction was cooled to rt and quenched with saturated aqueous NH₄Cl. The reaction mixture was diluted with ether, and the phases were separated. The aqueous phase was extracted with ether, were after the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to afford a yellow oil.

Flash column chromatography employing pentane : ether (95:5) afforded crude ethyl 3-methyl-5-(((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydropyrranonaphthalen-1-yl)pent-2-enoate 58 (4.0 gr, 12.0 mmol, 103%) as an E:Z mixture of ~9:1.

Note: The sodium hydride was pre-washed three times with pentane to remove the oil.

**¹H-NMR** (400 MHz, CDCl₃) δ 5.67 (s, 1H), 5.45 – 5.40 (m, 1H), 4.13 (q, J = 7.1 Hz, 2H), 2.17 (s, 3H), 2.13 (d, J = 13.1 Hz, 1H), 2.03 (dt, J = 10.1, 4.4 Hz, 2H), 1.90 – 1.67 (m, 4H), 1.65 – 1.44 (m, 5H), 1.44 – 1.34 (m, 2H), 1.26 (d, J = 6.9 Hz, 2H), 1.22 – 1.14 (m, 1H), 1.05 (s, 3H), 0.99 (s, 3H), 0.81 (d, J = 6.7 Hz, 3H), 0.63 (s, 3H).

**¹³C-NMR** (101 MHz, CDCl₃) δ 166.93, 161.30, 145.98, 116.29, 115.33, 59.52, 40.99, 39.97, 37.19, 36.19, 34.73, 34.60, 33.50, 31.70, 29.87, 29.10, 27.55, 22.33, 19.21, 16.25, 15.18, 14.47.

(E)-3-methyl-5-((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)pent-2-en-1-ol (Tuberculosinol):

To a solution of crude enoate 58 (4.0 g, 12.0 mmol) in dry CH₂Cl₂ (75 mL) cooled to -78 °C was added dropwise DIBAL-H (36.0 mL, 1.0 M in CH₂Cl₂, 36.0 mmol) over 15 min by the aid of a dropping funnel. The cooling bath was removed and the reaction mixture was allowed to slowly warm up to rt. TLC and GC-MS indicated complete conversion of the starting material after 1 h of reaction time.

The reaction mixture was carefully quenched using MeOH (10 mL) where after saturated aqueous Rochelle salt (75 mL) was added at 0 °C. The mixture was stirred for 30 min while warming up to rt where after the phases were separated. The aqueous phase was extracted three times with CH₂Cl₂ and the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to afford a yellowish oil. Flash column chromatography was executed using pentane : ether (4:1) as the eluent, affording pure tuberculosinol (2.33 g, 8.0 mmol, 68% yield) as a colorless oil.

A mixture of (E)-tuberculosinol and (Z)-tuberculosinol was also obtained (0.9 g). This was subjected to an additional chromatographic purification, affording 460 mg of (E)-tuberculosinol, giving a combined yield of 82%.

Also (Z)-tuberculosinol (0.4 g, 1.4 mmol, 11%) was isolated as a colorless oil.

Analytical data for (E)-tuberculosinol:

1H-NMR (400 MHz, CDCl₃) δ 5.43 – 5.33 (m, 2H), 4.08 (d, J = 6.8 Hz, 2H), 2.57 (s, 1H), 2.13 (d, J = 12.6 Hz, 1H), 1.92 – 1.85 (m, 2H), 1.81 – 1.67 (m, 3H), 1.65 (s, 3H), 1.59 – 1.28 (m, 6H), 1.28 – 1.10 (m, 1H), 1.02 (s, 3H), 0.97 (s, 3H), 0.78 (d, J = 6.8 Hz, 3H), 0.59 (s, 3H).

13C-NMR (101 MHz, CDCl₃) δ 145.99, 140.10, 123.11, 116.13, 59.09, 40.94, 39.81, 36.92, 36.05, 34.97, 33.38, 32.76, 31.65, 29.78, 29.00, 27.44, 22.27, 16.46, 16.18, 15.10.

HRMS (ESI+): Calculated mass [M+Na⁺]⁺ C₁₇H₂₉OSNa⁺ = 313.2502; found: 313.2500.

Optical rotation: [α]D²⁵ = +40.1 (c = 0.024, EtOH) and [α]D²¹ = +39.5 (c = 0.013, CHCl₃).
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Analytical data for (Z)-tuberculosinol:

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.46 – 5.42 (m, 1H), 5.39 (t, \(J = 6.9\) Hz, 1H), 4.13 (d, \(J = 7.0\) Hz, 2H), 2.21 (d, \(J = 13.2\) Hz, 1H), 1.99 (ddp, \(J = 17.4, 12.6, 5.3\) Hz, 3H), 1.91 – 1.79 (m, 1H), 1.76 (s, 3H), 1.67 – 1.47 (m, 4H), 1.47 – 1.15 (m, 6H), 1.07 (s, 3H), 1.02 (s, 3H), 0.85 (d, \(J = 6.7\) Hz, 3H), 0.62 (s, 3H).

\(^{13}\)C-NMR (101 MHz, CDCl\(_3\)) \(\delta\) 145.82, 139.86, 123.89, 116.02, 58.67, 40.86, 39.69, 37.05, 35.97, 35.25, 33.24, 31.56, 29.70, 28.95, 27.48, 25.36, 23.59, 22.23, 16.03, 15.12.


(4aS,5R,6S)-5-((E)-5-chloro-3-methylpent-3-en-1-yl)-1,1,5,6-tetramethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene (1):

To a suspension of 1-chloropyrrolidine-2,5-dione (1.0 g, 7.61 mmol, 1.3 eq) in dry CH\(_2\)Cl\(_2\) (15 mL), cooled to \(\sim -20^\circ\)C, was added dropwise dimethyl sulfide (0.65 mL, 8.78 mmol, 1.5 eq) in dry CH\(_2\)Cl\(_2\) (5.0 mL). The milk white suspension was allowed to warm to 0 °C for 15 min after which the temperature was lowered to \(-40^\circ\)C. Tuberculosinol (1.7 g, 5.85 mmol) in dry CH\(_2\)Cl\(_2\) (20 mL) was added slowly by the aid of a syringe pump over 15 min. After addition, the cooling bath was removed, and the reaction was allowed to warm-up to rt. The reaction was allowed to stir at this temperature for 2 h after which TLC and GC-MS analysis indicated complete conversion of the tuberculosinol. The reaction mixture was concentrated under reduced pressure and treated with pentane upon which succinimid e oiled out. The mixture was decanted and filtered and the elute was concentrated under reduced pressure affording nearly pure (4aS,5R,6S)-5-((E)-5-chloro-3-methylpent-3-en-1-yl)-1,1,5,6-tetramethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene 1 (1.85 gr, 6.0 mmol, 103% yield) as a yellow oil contaminated only with DMSO formed during the reaction.

Analytical data for (E)-tuberculosinyl chloride 1:

\(^1\)H-NMR (400 MHz, CDCl\(_3\)) \(\delta\) 5.51 – 5.41 (m, 2H), 4.09 (d, \(J = 7.9\) Hz, 2H), 2.16 (d, \(J = 12.8\) Hz, 1H), 1.96 (dt, \(J = 10.4, 4.6\) Hz, 2H), 1.83 (ddd, \(J = 17.7, 14.5, 3.5\) Hz, 2H), 1.75 (s, 3H), 1.72 (s, 1H), 1.63 – 1.25 (m, 7H), 1.20 (td, \(J = 12.9, 4.8\) Hz, 1H), 1.06 (s, 3H), 1.01 (s, 3H), 0.82 (d, \(J = 6.8\) Hz, 3H), 0.63 (s, 3H).
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\[ \text{C-NMR (101 MHz, CDCl}_3\text{)} \delta 146.11, 144.03, 119.92, 116.27, 41.34, 41.05, 39.93, 37.11, 36.20, 34.82, 33.51, 32.88, 31.75, 29.90, 29.13, 27.56, 22.37, 16.46, 16.28, 15.22. \]

HRMS (APCI): Calculated mass [M-Cl\(^+\)]\(^{+}\) C\(_{20}\)H\(_{30}\) = 273.2582; found: 273.2576.

**Analytical data for (Z)-tuberculosinyl chloride:**

\[ \text{H-NMR (400 MHz, CDCl}_3\text{)} \delta 5.46 – 5.42 (m, 1H), 5.39 (t, J = 6.9 Hz, 1H), 4.13 (d, J = 7.0 Hz, 2H), 2.21 (d, J = 13.2 Hz, 1H), 1.99 (ddp, J = 17.4, 12.6, 5.3 Hz, 3H), 1.91 – 1.79 (m, 1H), 1.76 (s, 3H), 1.67 – 1.47 (m, 4H), 1.47 – 1.15 (m, 6H), 1.07 (s, 3H), 1.02 (s, 3H), 0.85 (d, J = 6.7 Hz, 3H), 0.62 (s, 3H). \]

\[ \text{C-NMR (101 MHz, CDCl}_3\text{)} \delta 145.82, 139.86, 123.89, 116.02, 58.67, 40.86, 39.69, 37.05, 35.97, 35.25, 33.24, 31.56, 29.70, 28.95, 27.48, 25.36, 23.59, 22.23, 16.03, 15.12. \]

6-amino-9-((2R,3R,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1-(E)-3-methyl-5-((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydonaphthalen-1-yl)pent-2-en-1-yl)-9H-purin-1-ium (1-TbAd):

To a solution (0.5 M) of nearly pure tuberculosinyl chloride 1 (1.8 g, 5.8 mmol) in peptide grade DMF (11.6 mL) was added sodium iodide (1.05 g, 7.0 mmol) and adenosine (1.87 g, 7.0 mmol). The suspension was stirred in the dark at rt overnight, forming a dark turbid solution. The reaction mixture was concentrated under reduced pressure and subsequently purified using flash column chromatography (15% MeOH in CH\(_2\)Cl\(_2\)) affording 1-TbAd (2.4 g, 4.43 mmol, 76% yield).

The reaction can also be performed in dimethylacetamide as the solvent. Starting from tuberculosinyl chloride 1 (0.4 gr, 1.29 mmol), 1-TbAd (560 mg, 1.04 mmol, 79% yield) was obtained.

**Note:** 1-TbAd proved to be difficult to separate from unreacted adenosine due to tailing of the 1-TbAd. It is recommended to use a long column (30 cm) and analyze individual fractions by \(^1\text{H-NMR analysis} \) to determine the presence of free adenosine. Visualization of the adenosine on a TLC plate proved to be difficult.
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Analytical data for naturally occurring 1-TbAd:
$^1$H-NMR (400 MHz, CD$_3$OD) δ 8.62 (s, 1H), 8.49 (s, 1H), 6.08 (d, $J = 5.2$ Hz, 1H), 5.51 – 5.42 (m, 2H), 4.91 (d, $J = 6.6$ Hz, 2H), 4.62 (t, $J = 5.1$ Hz, 2H), 4.58 (s, 1H), 4.38 – 4.31 (m, 2H), 4.15 (q, $J = 3.3$ Hz, 1H), 3.87 (dd, $J = 12.3$, 2.9 Hz, 1H), 3.77 (dd, $J = 12.2$, 3.4 Hz, 1H), 2.23 (d, $J = 12.1$ Hz, 2H), 2.12 – 2.04 (m, 2H), 1.89 (s, 3H), 1.87 – 1.82 (m, 1H), 1.65 – 1.47 (m, 5H), 1.47 – 1.36 (m, 3H), 1.21 (dd, $J = 12.7$, 5.7 Hz, 1H), 1.06 (s, 3H), 1.01 (s, 3H), 0.85 (d, $J = 6.7$ Hz, 3H), 0.66 (s, 3H).

$^{13}$C-NMR (101 MHz, CD$_3$OD) δ 152.52, 147.78, 147.58, 147.31, 147.08, 143.74, 121.49, 117.34, 115.89, 90.43, 87.41, 76.35, 71.79, 62.62, 49.39, 42.00, 41.06, 38.05, 36.94, 35.87, 34.52, 33.88, 32.62, 30.29, 29.49, 28.53, 23.18, 17.35, 16.58, 15.54.

HRMS (ESI+): Calculated mass [M]$^+$ C$_{30}$H$_{46}$N$_5$O$_4^+$ = 540.3544; found: 540.3542.

Analytical data for non-natural $(Z)$-1-TbAd constructed from $(Z)$-tuberculosinyl chloride:
$^1$H-NMR (400 MHz, CD$_3$OD) δ 8.56 (2x s, 2H), 8.41 (2x s, 1H), 6.09 – 6.02 (m, 1H), 5.44 (dt, $J = 23.8$, 6.5 Hz, 2H), 4.88 (d, $J = 6.6$ Hz, 2H), 4.61 (t, $J = 4.6$ Hz, 1H), 4.33 (t, $J = 4.3$ Hz, 1H), 4.14 (d, $J = 3.1$ Hz, 1H), 3.91 – 3.82 (m, 1H), 3.76 (dd, $J = 12.8$, 2.5 Hz, 1H), 2.32 – 2.14 (m, 2H), 2.07 (t, $J = 8.2$ Hz, 1H), 1.88 (s, 3H), 1.85 – 1.71 (m, 3H), 1.65 – 1.47 (m, 5H), 1.43 (dd, $J = 14.6$, 8.6 Hz, 2H), 1.22 (dd, $J = 12.4$, 6.2 Hz, 1H), 1.06 (s, 3H), 1.02 (2x s, 3H), 0.86 (2x d, $J = 6.8$ Hz, 3H), 0.66 (2x s, $J = 10.9$ Hz, 3H).

$^{13}$C-NMR (101 MHz, CD$_3$OD) δ 152.74, 152.49, 147.80, 147.58, 147.31, 147.08, 146.99, 143.57, 143.41, 121.58, 121.49, 117.24, 117.15, 116.46, 115.98, 90.36, 87.36, 76.22, 71.73, 62.60, 42.00, 41.94, 40.96, 38.17, 37.97, 36.93, 36.88, 35.79, 34.44, 34.33, 33.83, 32.56, 30.29, 29.49, 28.70, 28.46, 26.81, 23.94, 23.19, 23.12, 17.46, 16.64, 16.58, 15.84, 15.56.

HRMS (ESI+): Calculated mass [M]$^+$ C$_{30}$H$_{46}$N$_5$O$_4^+$ = 540.3544; found: 540.3542.

Note: The appearance of additional signals in the NMR spectra of $(Z)$-1-TbAd, compared to that of naturally occurring $(E)$-1-TbAd, is attributed to rotamers.

$^{13}$C-labelled 1-TbAd:
To a solution (0.5 M) of nearly pure tuberculosinyl chloride 1 (142 mg, 0.459 mmol, 1.25 eq) in peptide grade DMF (0.92 mL) was added sodium iodide (69 mg, 0.459 mmol, 1.25 eq) and [1',2',3',4',5'-13C$_5$] adenosine (100 mg, 0.367 mmol). The suspension was stirred in the dark at rt overnight, forming a dark turbid solution. The reaction mixture was concentrated under reduced pressure where after purified using flash column chromatography (15% MeOH in CH$_2$Cl$_2$) affording [1',2',3',4',5'-13C$_5$] 1-TbAd (100 mg, 0.18 mmol, 57% yield) as an off-white solid.
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$^1$H-NMR (400 MHz, CD$_3$OD) $\delta$ 8.56 (s, 1H), 8.42 (s, 1H), 6.26 (s, 1H), 5.84 (s, 1H), 5.52 – 5.40 (m, 2H), 4.58 (s, 1H), 4.51 (s, 1H), 4.42 (s, 1H), 4.33 (s, 1H), 4.14 (s, 1H), 4.04 (d, $J = 11.4$ Hz, 1H), 3.94 (d, $J = 9.7$ Hz, 1H), 3.77 – 3.63 (m, 1H), 3.59 (d, $J = 12.0$ Hz, 1H), 2.22 (d, $J = 13.3$ Hz, 2H), 2.07 (t, $J = 8.3$ Hz, 2H), 1.88 (s, 3H), 1.86 – 1.70 (m, 3H), 1.65 – 1.47 (m, 4H), 1.47 – 1.35 (m, 2H), 1.21 (td, $J = 12.4$, 5.5 Hz, 1H), 1.06 (s, 3H), 1.01 (s, 3H), 0.84 (d, $J = 6.7$ Hz, 3H), 0.65 (s, 3H).

$^1$C-NMR (101 MHz, CD$_3$OD) $\delta$ 152.56, 147.83, 147.32, 147.09, 147.03, 143.56, 121.55, 117.27, 116.00, 90.40 (dd, $J = 42.0$, 3.7 Hz), 87.37 (t, $J = 41.7$, 38.5 Hz), 76.21 (dd, $J = 42.0$, 37.8 Hz), 71.72 (td, $J = 38.1$, 3.8 Hz), 62.60 (d, $J = 41.7$ Hz), 49.85, 41.96, 40.99, 38.00, 36.90, 35.82, 34.47, 33.84, 32.58, 30.27, 29.48, 28.48, 23.14, 17.42, 16.57, 15.54.

HRMS (ESI+): Calculated mass [M+H]$^+$ $C_{25}$$^{(13)}$C$_{46}$N$_2$O$_4$ = 545.3712; found: 545.3702

(2R,3S,4R,5R)-2-(hydroxymethyl)-5-(6-(((E)-3-methyl-5-((1R,2S,8aS)-1,2,5,5-tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-yl)pent-2-en-1-yl)amino)-9H-purin-9-yl)tetrahydrofuran-3,4-diol ($N^6$-TbAd):

A solution of 1-TbAd (550 mg, 1.02 mmol) in 60% Me$_2$NH in water (5.5 mL) was stirred for 90 min. NMR analysis indicated complete conversion of the 1-TbAd. The reaction mixture was concentrated under reduced pressure and subsequently subjected to flash column chromatography, with 15% MeOH in CH$_2$Cl$_2$, to afford $N^6$-TbAd as a white solid (550 mg, 1.02 mmol, quantitative yield).

Note: The rearrangement could be performed with similar results using Et$_2$NH or iPr$_2$NEt (2M) in MeOH.

$^1$H-NMR (400 MHz, CD$_3$OD) $\delta$ 8.25 (s, 1H), 8.23 (s, 1H), 5.95 (d, $J = 6.4$ Hz, 1H), 5.51 – 5.44 (m, 1H), 5.41 (t, $J = 6.5$ Hz, 1H), 4.74 (t, $J = 5.6$ Hz, 1H), 4.35 – 4.29 (m, 1H), 4.20 (s, 1H), 4.17 (s, 1H), 3.89 (dd, $J = 12.6$, 2.1 Hz, 1H), 3.74 (dd, $J = 12.9$, 2.3 Hz, 1H), 2.24 (d, $J = 15.9$ Hz, 1H), 2.07 – 1.93 (m, 3H), 1.91 – 1.82 (m, 2H), 1.80 (s, 3H), 1.65 – 1.49 (m, 4H), 1.49 – 1.36 (m, 3H), 1.21 (td, $J = 11.9$, 7.3 Hz, 2H), 1.06 (s, 3H), 1.02 (s, 3H), 0.85 (d, $J = 6.6$ Hz, 3H), 0.65 (s, 3H).
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$^1$H-NMR (400 MHz, CDCl$_3$) δ 8.19 (s, 1H), 7.81 (s, 1H), 5.86 (bs, 1H), 5.82 (d, $J = 7.2$ Hz, 1H), 5.44 (d, $J = 4.7$ Hz, 1H), 5.36 (d, $J = 7.3$ Hz, 1H), 5.00 (s, 1H), 4.47 (d, $J = 4.9$ Hz, 1H), 4.33 (s, 1H), 4.19 (bs, 2H), 3.94 (d, $J = 12.9$ Hz, 1H), 3.76 (d, $J = 12.9$ Hz, 1H), 3.25 (s, 2H), 2.49 (bs, 2H), 2.16 (d, $J = 10.4$ Hz, 1H), 2.01 – 1.90 (m, 2H), 1.85 (d, $J = 23.8$ Hz, 2H), 1.76 (s, 3H), 1.72 (s, 1H), 1.64 – 1.28 (m, 6H), 1.21 (tt, $J = 13.0$, 6.2 Hz, 2H), 1.06 (s, 3H), 1.01 (s, 3H), 0.82 (d, $J = 6.6$ Hz, 3H), 0.63 (s, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) δ 154.59, 152.44, 147.00, 146.09, 141.73, 140.05, 120.70, 118.96, 116.23, 91.08, 87.56, 73.89, 72.46, 63.15, 41.00, 39.89, 38.87, 37.04, 36.17, 35.00, 33.47, 32.80, 30.14, 29.87, 29.12, 27.51, 22.33, 16.83, 16.26, 15.22.

HRMS (ESI+): Calculated mass [M+H]$^+$ C$_{30}$H$_{46}$N$_{5}$O$_{4}$ + = 540.3544; found: 540.3542


6-amino-9-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-1-((E)-3-methyl-5-((1R,2S,8aS)-1,2,5,6,7,8,8a-octahydonaphthalen-1-yl)pent-2-en-1-yl)-9H-purin-1-ium (2’-deoxy-1-TbAd):
To a solution (0.5 M) of nearly pure tuberculosinyl chloride 1 (300 mg, 0.97 mmol) in peptide grade DMA (2 mL) was added sodium iodide (175 mg, 1.17 mmol, 1.2 eq) and 2’-deoxy-adenosine monohydrate (314 mg, 1.17 mmol, 1.2 eq). The suspension was stirred in the dark at rt overnight, forming a dark turbid solution. The reaction mixture was concentrated under reduced pressure where after purified using flash column chromatography (15% MeOH in CH$_2$Cl$_2$) affording 2’-deoxy-1-TbAd (190 mg, 0.36 mmol, 37% yield) as a white solid.

Also a slightly impure fraction of 2’-deoxy-1-TbAd (150 mg, 0.29 mmol, 31%) was obtained. The impurity, based on NMR analysis, remained unresolved.

$^1$H-NMR (400 MHz, CDCl$_3$) δ 8.54 (s, 1H), 8.44 (s, 1H), 6.46 (t, $J = 6.5$ Hz, 1H), 5.46 (s, 2H), 4.90 (d, $J = 6.5$ Hz, 2H), 4.57 (s, 1H), 4.09 – 4.01 (m, 1H), 3.77 (qd, $J = 12.1$, 3.5 Hz, 2H), 2.76 (dt, $J = 13.0$, 6.3 Hz, 1H), 2.56 – 2.44 (m, 1H), 2.21 (d, $J = 12.5$ Hz, 2H), 2.11 – 1.99 (m, 2H), 1.89 (s, 3H), 1.86 – 1.70 (m, 3H), 1.54 (d, $J = 12.3$ Hz, 4H),
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1.42 (dd, $J = 17.1, 8.8$ Hz, 2H), 1.19 (td, $J = 12.3, 5.8$ Hz, 1H), 1.05 (s, 3H), 1.00 (s, 3H), 0.83 (d, $J = 6.6$ Hz, 3H), 0.64 (s, 3H).

$^{13}$C-NMR (101 MHz, CDCl$_3$) δ 152.47, 147.65, 147.05, 146.99, 146.95, 143.45, 121.40, 117.24, 116.06, 89.53, 86.33, 72.30, 62.96, 49.85, 41.95, 41.76, 40.96, 37.98, 36.89, 35.79, 34.45, 33.86, 32.59, 30.32, 29.50, 28.47, 23.14, 17.46, 16.62, 15.59.

HRMS (ESI+): Calculated mass [M]$^+$ C$_{30}$H$_{46}$N$_5$O$_3$ = 524.3595; found: 524.3588.

Reported MS spectrum of 2’-deoxy 1-TbAd:

Taken from: Emerg. Microbes Infect. 2015, 4, e6 (doi: 10.1038/emi.2015.6)

Complete CID-MS spectrum of synthetic 2’-deoxy 1-TbAd:
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Computational models:

*Aluminum complexed s-cis pathway, first transition state (44b)*

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[Diagram of s-cis pathway]
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*Aluminum complexed s-trans pathway, first transition state (44h)*

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[Diagram of s-trans pathway]
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The disfavored approaches for the Diels-Alder reaction
The reaction partners were brought together but not optimized. From the different approaches the steric interactions are clearly visible. The carbons in the diene and dienophile involved in initial bond formation are highlighted.
4.8 References


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[27] PhD dissertation of Rik Megens, *DNA-Based Asymmetric Catalysis as a Synthetic Tool*, University of Groningen, 2012, see page 87.


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[41] PhD dissertation of N. Maugel, Palladium-Catalyzed Alkylation, Arylation and Dehydrogenation of Unactivated C-H bonds. Syntheses of the Tetracyclic Aminoquinone Moiety of Marmycin A, (+/-)-Nosyberkol (Isotuberculosinol, Revised Structure of Edaxadiene), and (+/-)-Tuberculosinol, Brandeis University, 2011.


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