Synthesize of Ladderane based fatty acids

It has been found that fatty acids incorporating cyclobutane moieties show exceptional properties which make the surviving of the host organism in harsh and toxic environment possible. An example is the presence of linear fused cyclobutane rings, named a ladderane unit, in natural products, such as \textit{Pentacycloanammosic} acid. However, the synthesis of these natural products bearing ladderane structures is challenging. Therefore, after having synthesized successfully precursors in Chapter 2, the ketone bearing fused cycloalkanes are employed in this chapter to synthesize new ladderane fatty acids. The new amphiphilic structures exhibiting three cycloalkane rings and a double bond were obtained employing seven synthetic steps in overall yield of up to 15%.
3.1 Introduction

Several studies have indicated the existence of bacteria which are able to oxidize ammonium (NH$_4^+$) under anaerobic conditions.$^{1,2}$ This was demonstrated experimentally for the first time in The Netherlands in 1995 where in a bioreactor ammonium was converted into nitrogen (N$_2$) gas under anaerobic conditions in presence of nitrite (NO$_2^-$) (Figure 3.1.1a)$^{2,3}$ In 1999, Strous et al. identified the for this process responsible anaerobic ammonium oxidizing (anammox) bacteria as a new member of the planctomycetes.$^4$ Jetten et al. described in 2001 the oxidation of ammonium via anammox bacteria forming toxic intermediates, i.e. hydroxylamine (NH$_2$OH) and hydrazine (N$_2$H$_4$).$^5$ As shown in Figure 3.1.1b, reduction of nitrite to hydroxylamine takes place followed by the formation of hydrazine through the incorporation of ammonium. Finally, nitrogen gas is generated by oxidation of the previously formed hydrazine. The through the anammox bacteria performed anaerobic process described in Figure 3.1.1 is of great interest for wastewater treatment.$^5$ This process gives access to a new strategy to remove nitrogen-containing compounds from wastewater combining two conventional applied technologies, i.e. nitrification and denitrification (Figure 3.1.1c).$^6$ The advantage of the application of anammox bacteria for this purpose is that they perform both steps, nitrification and denitrification. Furthermore, no external electron donor like methanol and no oxygen are required.

\[
\begin{align*}
\text{a} & \quad \text{NH}_4^+ + \text{NO}_2^- & \rightarrow & \quad \text{N}_2 + 2\text{H}_2\text{O} \\
\text{b} & \quad \text{NO}_2^- + 5\text{H}^+ + 4\text{e}^- & \rightarrow & \quad \text{NH}_2\text{OH} + \text{H}_2\text{O} \\
& \quad \text{NH}_2\text{OH} + \text{NH}_3^+ & \rightarrow & \quad \text{N}_2\text{H}_4 + \text{H}_2\text{O} + \text{H}^+ \\
& \quad \text{N}_2\text{H}_4 & \rightarrow & \quad \text{N}_2 + 4\text{H}^+ + 4\text{e}^- \\
\end{align*}
\]

\[\text{Figure 3.1.1:} (\text{a}) \text{ Overall redox reaction during the anammox process. (b) Stepwise process showing} \text{ the generation of toxic intermediates hydrazine (N}_2\text{H}_4\text{) and hydroxylamine (NH}_2\text{OH). (c) Nitrogen cycle.}\]
\[\text{Color code: Black arrow represents anaerobic reactions, gray arrow represents aerobic reactions and hollow arrow represents anaerobic and aerobic reactions. Figure adapted from the in reference 6 and 7 reported data.}\]
The above described anammox catabolism is located at the for bacteria unique organelle, the anammoxosome (Figure 3.1.2). This organelle represents the largest compartment of the bacteria occupying around 60% of the cell. As shown in Figure 3.1.2, the surface of the compartment is enlarged by extensive folding of the membrane. In the anammoxosome the energy metabolism takes place which is necessary for the grow of the anammox and therefore, the function of this organelle can be compared to that of eukaryotic mitochondria. The anammoxosome is surrounded by a cytoplasmic compartment, the riboplasm, in which ribosomes and nucleotides are located. Furthermore, the cell wall typical for Planctomycetes, consisting of an intracytoplasmic and a cytoplasmic membrane, is also present in anammox bacteria. As shown in Figure 3.1.2, between these two membranes the paryphoplasm, the outermost compartment, is located. The function of this compartment is still unknown and the presence of ribonucleic acids is presumed.

Figure 3.1.2: Schematic drawing of the anammox bacteria cell adapted from reference 7.

The anammoxosome exhibits a membrane known as anammoxosome membrane. Various studies have shown that this membrane incorporates several highly interesting lipids. In 2002 Sinninghe Damsté et al. reported the discovery of fatty acids carrying three to five linear fused cyclobutane rings (Figure 3.1.3). Due to the three-dimensional structure of this acids they are known as [3]- and [5]-ladderanes. A variety of the most common compositions of the ladderane-containing lipids are shown in Figure 3.1.3. Furthermore, it was observed that in addition to the ester bonds typical for bacteria and eukaryotes also ether bonds are present to connect the aliphatic alcohol to the glycerol backbone. Those connections, which are mainly found in Archaea, have been found so far only in a few bacteria, such as Aquifex and Thermotoga. It needs to be noted that both genera are found in locations with high temperatures. Also, Sinninghe Damsté et al. demonstrated in 2002 the low permeability of the anammoxsome membrane for small molecules at temperatures between 0 and 92 °C as well as pH values between 4 to 9 via staining experiments. Additionally, molecular modeling of
ladderane-containing membranes resulted in calculated density values of up to 50% higher than for conventional membranes. The low permeability, high temperature- and pH tolerance of this unique membrane facilitate the anammox catabolism with its toxic intermediates.

![Variable composition of Ladderane lipids.](image)

**Figure 3.1.3:** Variable composition of Ladderane lipids.

The unique structure, the interesting properties and the low availability of ladderane lipids resulted in growing interest in the synthesis of the natural products shown in Figure 3.1.3. The first total synthesis of pentacycloanammoxic acid 1 was reported by Mascitti and Corey in 2004 (Scheme 3.1.1). The 13-step synthesis contains three photo induced reactions leading to the five linear fused cyclobutane rings of the acid 1. As shown in Scheme 3.1.1, a [2+2] photocycloaddition between the commercial available cyclopentenone 3 and the in two steps accessible alkene 2 yielded the intermediate 4. Subsequently, the carbonyl group of 4 was protected and the resulting dimethyl acetal 5 was used for a second photo reaction enabling the formation of two additional cyclobutene rings. To obtain the fifth cyclobutene moiety a ring contraction of the cyclopentenone 6 was performed in three steps. As shown in Scheme 3.1.1, formylation in the α-position followed by a Regitz diazo-transfer yielded an α-diazo ketone 7 which was employed in a photo-induced Wolff rearrangement resulting in derivative 8 exhibiting five cyclobutane rings. Reduction of methyl ester 8 with DIBAL-H (diisobutylaluminiumhydride) and subsequent Swern oxidation gave access to the aldehyde 9. Finally, by applying a Wittig olefination and reduction of the obtained olefin resulted in the desired natural ladderane lipid 1. However, do to the low yield (6%) during the photochemical N₂ extrusion only a moderate overall yield of less than 1% for 1 could be reached. Hence, Mascitti and Corey published in 2006 a modified pathway and obtained the Pentacycloanammoxic acid 1 with an overall yield of 5% employing 18 synthetic steps.
Scheme 3.1.1: First total synthesis of Pentacycloanammoxic acid 1 by Mascitti and Corey in 2004.19
3.2 Results and Discussion

In this chapter we describe the syntheses of ladderane molecules 1a and 1d (Scheme 3.2.1) based on the in nature discovered [5]- and [3]-ladderane fatty acids. As shown in Scheme 3.2.1, these derivatives are carrying three linear fused cycloalkane rings with a cyclobutane or cyclopentane moiety in terminal position of the hydrophilic tail. Hence, the in Chapter 2 synthesized cyclobutane derivatives 10a and 10d (Scheme 3.2.1) are used as starting materials. To obtain fatty acids 1a and 1d we followed the synthetic pathway described by Mascitti and Corey in 2006. In the following past, the total synthesis of 1a and 1d carrying each a mono unsaturated C11 chain and three linear fused cycloalkane rings is described.

Scheme 3.2.1: Molecular structure of starting materials 10a and 10d as well as of the desired ladderane derivatives 1a (n=1) and 1d (n=2).

To obtain a trisubstituted cyclobutene moiety (ring A in 1a and 1d, Scheme 3.2.1) in the terminal position of fatty acid 1 we chose to perform a Wolff rearrangement. As shown in Scheme 3.2.2, to obtain the required α-diazo ketones 12a and 12d for the rearrangement reaction, the cyclopentenone moiety (ring A) of 10a and 10d needs to be activated in the α-position by a formylation reaction using ethyl formate (EF) resulting in α-hydroxymethylene ketones 11a and 11d, respectively. The subsequent Regitz diazo-transfer reaction gives access to the desired starting materials 12a and 12d for the Wolff rearrangement (Scheme 3.2.2).

Scheme 3.2.2: Synthesis of α-diazo ketones 12a and 12d, precursors for the Wolff rearrangement.

In order to optimize the formylation reaction, different reaction conditions were tested. In this regard, cyclohexanone 13 was used as a model substrate to obtain the corresponding activated ketone 14 (Scheme 3.2.3). The utilization of the commercial available organic bases like Et₃N, n-BuLi (n-butyllithium), NaHMDS (sodium hexamethyldisilazide), KHMDS (potassium hexamethyldisilazide), NaOMe and freshly prepared LDA (lithium diisopropylamide) resulted
in isolated yields of up to 75% of 14 (Scheme 3.2.3). However, using same conditions and ethyl formate (EF) for the transformation of ketone 10a resulted only in moderate yields of 11a ranging from 25 % to 41% (Table 3.2.1, entries 1 to 4). In order to increase the yield of the formylation reaction we tested the application of freshly synthesized 2,2,2-trifluoroethyl formate (TFEF) instead of EF. This reagent was already employed successfully by Zayia in 1999.21 However, as shown in Table 3.2.1 (entry 5) the application of TFEF resulted in a comparable yield of 11a as observed previously employing EF (entry 4). Using ketone 10d as starting compound and EF as reagent in the presence of LDA gave the corresponding α-hydroxymethylene ketone 11d in a yield of 45%.

![Scheme 3.2.3: Model reaction used to optimize the formylation reaction.](image)

Table 3.2.1: Reaction conditions used for the formylation of ketone 10a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Formate</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NaHMDS</td>
<td>EF</td>
<td>-45 to 0</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>n-BuLi</td>
<td>EF</td>
<td>-45 to 0</td>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>LDA</td>
<td>EF</td>
<td>-78 to -45</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>LDA</td>
<td>EF</td>
<td>-78 to -45</td>
<td>8</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>LDA</td>
<td>TFEF</td>
<td>-78 to -45</td>
<td>8</td>
<td>40</td>
</tr>
</tbody>
</table>

All reactions were performed employing the general procedure (see Experimental part, 3.4.2). *Yields are given for α-hydroxymethylene ketone 11a.

As shown in scheme 3.2.2, the obtained α-hydroxymethylene derivatives 11a and 11d were further transformed in a Regitz diazo-transfer reaction. The tosylazide necessary for this diazotation was synthesized accordingly to the procedure described by Ghosh et al. in 2003.22 The corresponding α-diazoketones 12a and 12d were obtained in isolated yields of 89% and 83%, respectively. Both synthetic steps, i.e. formylation and the diazo-transfer reaction, were performed subsequently avoiding the purification of the intermediates 11a and 11d resulting in comparable overall yields of up to 33% and 35% for 12a and 12d, respectively.

![Scheme 3.2.4: Wolff rearrangement of 12a and 12d.](image)
After successful synthesis of α-diazoketones 12a and 12d a Wolff rearrangement was performed to give access to 15a and 15d exhibiting a trisubstituted cyclobutene moiety (ring A, Scheme 3.2.4). Methyl esters 15a and 15d were obtained reaching yields of 77% and 75%, respectively. It needs to be noted that the rearrangement resulted in two isomers, i.e. the endo- and exo-isomer (stereo center marked in Scheme 3.2.4). 1H-NMR studies revealed that compounds 15a and 15d were obtained in a ratio of 3 to 1 of their corresponding endo- and exo-isomers. Nevertheless, as shown in Scheme 3.2.5, we utilized methyl esters 15a and 15d as isomer mixture for subsequent reactions to obtain aldehydes 17a and 17d, respectively. First, 15a and 15d were reduced applying DIBAL-H (Diisobutylaluminium hydride) resulting in alcohols 16a and 16d, respectively (Scheme 3.2.5). Employing a Swern oxidation of the corresponding alcohol the desired aldehyde 17a or 17d was obtained reaching isolated yields of up to 86% and 68%, respectively, over two steps (Scheme 3.2.5).

Finally, a isomerization of 17a and 17d was performed to give access to the desired exo-isomer as main product (Figure 3.2.1a). Hence, isomer mixture of 17a and 17d was stirred in triethylamine at room temperature. The isomerization process was monitored by 1H-NMR spectroscopy. As shown in Figure 3.2.1b, employing a reaction time of six days the isomerization resulted in a final endo/exo ratio of 1 to 6 for compound 17a. A similar result was obtained for the isomerization of aldehyde 17d (data not shown).
After having successfully synthesized ladderane aldehydes 17a and 17d we performed an elongation at the terminal position to obtain the final ladderane fatty acids 1a and 1d. As shown in Scheme 3.2.6c, for this purpose we have chosen to employ the Wittig reaction using the aliphatic phosphonium salt 19. But prior to the final synthetic step, the Wittig salt 19 was first synthesized from 10-bromodecanoic acid 18 (Scheme 3.2.6.a). Using solvent free conditions, bromide 18 was transform to salt 19 in melted PPh₃ at 100 °C in quantitative yield. Also, before transforming 17a and 17b to their corresponding fatty acids 1a and 1d, respectively, we decided to use the obtained salt 19 to perform a Wittig reaction employing valeraldehyde 20 as a model compound (Scheme 3.2.6b). In this study, it was found that due to the low solubility of 19 an excess of base was needed and longer deprotonation times were necessary to form the more soluble ylide. Furthermore, in order to optimize the reaction conditions of the Wittig reaction, several bases were used to obtain the olefin 21 (Scheme 3.2.6b, Table 3.2.2, entries 1-5). While n-BuLi and LiHMDS gave the desired product 21 in only low yields (Table 3.2.2, entries 1 and 3), using NaHMDS resulted in a moderate yield (Table 3.2.2, entry 2). Nevertheless, best results were achieved employing LDA as base (Table 3.2.2, entries 4 and 5). However, it needs to be emphasized that fresh preparation of LDA is essential to obtain olefin 21 in a high yield reaching 82% (Table 3.2.2, entry 5).

After optimization of the reaction conditions, aldehydes 17a and 17d were used in a Wittig reaction with salt 19 to provide the corresponding ladderane fatty acids 1a and 1d, respectively (Scheme 3.2.6c). As shown in Table 3.2.2 (entry 6 and 7), the amphiphilic compounds 1a and
1d were obtained in a yield of 62% and 53%, respectively, using LDA as base. The crude product, composed of a Z:E mixture with a ratio of 1:2 and 1:3 for 1a and 1d, respectively, was purified by column chromatography yielding the pure E-isomer. Both products 1a (E) and 1d (E) were analyzed using 1H-, 13C-NMR and exact mass (Data shown in experimental section 3.4.2). No further modification of 1a and 1d were performed due to shortage of material. However, already at this point the optimized synthetic pathway for fatty acids containing ladderane moieties gives access to several derivatives of the naturally occurring acid. Hence, further studies are of high interest analyzing the direct influence of ring-size and unsaturation on the packing behavior of ladderane fatty acids. Therefore, the synthesis of phospholipids containing these fatty acids would allow aggregation studies using analytical methods like cryo-TEM (cryogenic transmission electron microscopy) and DSC (differential scanning calorimetry).

![Scheme 3.2.6](image)

**Scheme 3.2.6:** (a) Synthesis of the Wittig salt 19. (b) Test reaction with valeraldehyde 20. (c) Synthesis of the desired ladderane derivatives 1a and 1d.

**Table 3.2.2:** Reaction conditions applied during the Wittig reaction using 20, 17a and 17d as starting material.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Product</th>
<th>Base (equiv)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>21</td>
<td>BuLi</td>
<td>4</td>
<td>23*</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>21</td>
<td>NaHMDS</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>21</td>
<td>LiHMDS</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>21</td>
<td>LDA</td>
<td>2</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>21</td>
<td>LDA**</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>17a</td>
<td>1a</td>
<td>LDA**</td>
<td>2</td>
<td>62</td>
</tr>
<tr>
<td>7</td>
<td>17d</td>
<td>1d</td>
<td>LDA**</td>
<td>2</td>
<td>53</td>
</tr>
</tbody>
</table>

All reactions were performed employing the general procedure (see experimental part 3.4.2) in THF at temperatures between -78 °C and 0 °C. * Including small amount of unknown side product; reaction was performed using temperatures between -78 °C and room temperature. ** Fresh prepared LDA.
3.3. Conclusion

In this chapter, new ladderane fatty acid derivatives 1a and 1d have been successfully synthesized. Amphiphiles 1a and 1d were obtained in seven synthetic steps starting from the corresponding ketones 10a and 10d (see synthesis in Chapter 2.2) in an overall yield of 15% and 10%, respectively. Essential for the success was the optimization of two key steps, i.e. formylation reaction of the ketones 10a and 10d and the Wittig reaction to transform the corresponding aldehydes 17a and 17d to the desired products 1a and 1d, respectively. A further key step in this synthetic pathway is the application of an isomerization reaction of the aldehydes 17a and 17d. It needs to be emphasized that employing Wolff rearrangement yielded both aldehydes as mixture of isomers resulting mainly in an undesired endo-product. However, employing subsequently a thermal isomerization under basic conditions both aldehydes 17a and 17d were obtained mainly as exo-isomer.

Having in mind that ladderane structures allow bacteria to survive in an environment of high temperatures and of a broad range of pH value, the obtained compounds 1a and 1d will be of use for future studies allowing access to more stable artificial membranes.
3.4 Experimental Section

3.4.1 Materials and Methods

All chemicals and reagents were purchased from commercial suppliers (Acros and Sigma-Aldrich) and used without further purification. Dry solvents were taken from an MBraun solvent purification system (SPS-800). Thin layer chromatographic (TLC) analysis was performed on Merck silicagel 60/Kieselguhr F254, 0.25 mm TLC plates and visualized by UV and staining with Seebach’s reagent. Column chromatography was performed using silica gel (P60, 230 – 400 mesh).

$^1$H-NMR-, $^{13}$C-NMR-, heteronuclear single-quantum correlation- (HSQC) spectra, Nuclear Overhauser effect spectroscopy (NOESY) and correlation spectroscopy (COESY) were recorded on a Agilent 400 (400 MHz for $^1$H-NMR, 100.59 MHz for $^{13}$C-NMR) using CDCl$_3$ (CDCl$_3$: δ 7.26 for $^1$H-NMR, δ 77.16 for $^{13}$C-NMR). Data are reported as follows: chemical shifts, multiplicity (s= singlet, d= doublet, t= triplet, q= quartet, br= broad, m= multiplet), coupling constants $J$ (Hz) and integration.

High resolution mass spectrometry (HRMS) was carried out on a LTQ ORBITRAP XL spectrometer (Thermo Scientific) employing electrospray ionization (ESI) in positive ion mode (EI’) and negative ion mode (EI).
3.4.2 Synthesis and Characterization

**LDA (Lithium diisopropylamide):**

Diisopropylamine (1 equiv) was added slowly to a solution of $n$-BuLi (1.6 M in hexane; 1 equiv.) in Et$_2$O (1 mL/mmol) at -40 °C. After additional stirring for 30 min at this temperature the LDA solution was used without further purification.

**Tosyl azide:**

A solution of NaN$_3$ (1.2 equiv) in water (1.2 mL/mmol) was added at 0 °C to a solution of tosyl chloride (1 equiv) in acetone (1.2 mL/mmol). The mixture was stirred for 3 h at this temperature and subsequently diluted with water (4 mL/mmol). Finally, the organic phase was separated and the aqueous solution extracted twice with Et$_2$O (2x 2 mL/mmol), the combined organic extracts were dried over MgSO$_4$ and carefully (<30 °C) concentrated. The tosyl azide was used without further purification in the next step.

[16 mmol scale, full conversion, colorless oil]. $^1$H-NMR (CDCl$_3$, 400 MHz): δ (ppm) = 7.84 (d, J= 7.2 Hz, 2H); 7.41 (d, J= 8 Hz, 2H); 2.48 (s, 3H). $^{13}$C-NMR (CDCl$_3$, 100 MHz): δ (ppm) = 130.41 (2C; CH); 127.68 (2C, CH); 21.91 (1C, CH3). IR (film, cm$^{-1}$): 2122, 1368, 1164, 1086, 813, 746, 659, 591, 539.

**10-(bromotriphenylphosphoranyl)decanoic acid (19):** Triphenyl phosphine (1 equiv) was melted and the 10-bromodecanoic acid 18 (1 equiv) was added. The resulting reaction mixture was heated for 16 h at 100 °C. Subsequently, the melt was cooled down to room temperature and washed with pentane. The obtained solid was used without further purification. $^1$H-NMR (CDCl$_3$, 400 MHz): δ (ppm) = 7.81 (m, 9H); 7.71 (m, 6H); 3.65 (t, J=11.2 Hz, 2H); 2.36 (t, J=7.2 Hz, 2H); 1.59 (m, 6H); 1.23 (m, 8H). $^{31}$P-NMR (CDCl$_3$, 162 MHz): δ (ppm) = 24.37.

A solution of the ketone 10a or 10d (1 equiv) in anhydrous THF (1.5 mL/mmol) was added over 20 min dropwise to a THF solution of the base (1M in THF, 1.2 equiv) at -45 °C. After warming the reaction mixture slowly (30 min) to -30 °C fresh distilled ethyl formate (3 equiv) was added in one portion. The reaction mixture was allowed to warm up during 2 h to 0 °C to complete the reaction. The mixture was diluted with Et$_2$O (2 mL/mmol) and quenched at 0 °C with an aqueous HCl solution (1M, until pH 5-6). The layers were separated and the aqueous layer was extracted three times with Et$_2$O (3x 2 mL/mmol), the combined organic extracts were washed with brine (1 mL/mmol), dried over MgSO$_4$ and concentrated under reduced pressure. The α-hydroxymethylene ketones 11a or 11d were used without further purification in the next step.
Chapter 3

n=1: 8-(hydroxymethylene)tricyclo[4.3.0.0^{2,5}]nonan-7-one (11a): [0.6 mmol scale, 41% yield, yellowish oil]
n=2: 9-(hydroxymethylene)tricyclo[5.3.0.0^{2,6}]nonan-8-one (11d): [2.7 mmol scale, 45% yield, yellowish oil]

A solution of fresh prepared tosyl azide (1 equiv) in anhydrous CH$_2$Cl$_2$ (0.5 mL/mmol) was added at 0 °C to a solution of the α-hydroxymethylene ketone 11a or 11d (1 equiv) in anhydrous CH$_2$Cl$_2$ (1.5 mL/mmol). After adding of Et$_3$N (2 equiv) the reaction mixture was stirred at this temperature for 30 min, concentrated under reduced pressure and purified by flash chromatography over silica gel (pentane/Et$_2$O : 8/2).

n=1: 8-diazotricyclo[4.3.0.0^{2,5}]nonan-7-one (12a): [0.3 mmol scale, 89% yield, yellow oil that solidifies upon standing]. 1H NMR (400 MHz, CDCl$_3$); δ (ppm): 3.40 (dd, J= 13.6 Hz, J= 8.8 Hz, 1H); 3.06 (d, J= 6.1 Hz H); 2.93 (m, 1H); 2.89 (d, J= 13.6 Hz, 1H); 2.83 (m, 1H); 2.59 (m, 1H); 2.52 (m, 2H); 2.19 (m, 1H); 2.01 (m, 1H). Measured NMR data are corresponding to those reported in the literature.20

n=2: 9-diazotricyclo[5.3.0.0^{2,6}]nonan-8-one (12d): [1.5 mmol scale, 83% yield, yellow oil that solidifies upon standing]. 1H NMR (400 MHz, CDCl$_3$); δ (ppm): 3.27 (m, 1H); 2.90 (d, J= 13.6 Hz, 1 H); 2.74 (m, 1H); 2.52 (m, 1H); 2.44 (m, 1H); 2.23 (m, 1H); 1.84 (m, 1H); 1.68 (m, 3H); 1.51 (m, 2H). 13C NMR (100 MHz, CDCl$_3$); δ (ppm): 203.1 (1C, C=O); 147.11 (1C, C=N); 50.83 (1C, CH$_2$); 45.74 (1C, CH$_2$); 42.92 (1C, CH$_2$); 35.41 (1C, CH$_2$); 33.16 (1C, CH); 32.85 (1C, CH); 31.37 (1C, CH); 24.98 (1C, CH). IR (film, cm$^{-1}$): 1939; 2857; 2068; 1655; 1459; 1340; 1317; 1291; 1277; 1246; 1223.

Et$_3$N (1 equiv) was added to an anhydrous and degassed solution of α-diazo ketone 12a or 12d (1 equiv) in MeOH (10 mL/mmol). The reaction mixture was placed in a photo reactor and irradiated at room temperature for 1 h. After full conversion the mixture was concentrated under reduced pressure and purified by flash chromatography over silica gel (pentane/CH$_2$Cl$_2$ : 6/4).

n=1: methyl tricyclo[4.2.0.0^{2,5}]octane-3-carboxylate (15a): [6.2 mmol scale, 77% yield, colorless oil]. 1H NMR (400 MHz, CDCl$_3$); δ (pppm): major (endo): 3.66 (s, 3H); 3.59 (dt, J= 11.6 Hz, J= 8 Hz, 1 H); 3.04 (m, 1H); 2.76 (m, 1H); 2.90 (m, 1H); 2.65 (m, 1H); 2.40-2.62 (m, 3H); 2.35 (ddd, J= 12.8 Hz, J= 7.6 Hz, J= 3.2 Hz, 1H); 1.87-1.99 (m, 2H). 1H NMR (400 MHz, CDCl$_3$); δ (ppm): minor (exo): 3.67 (s, 3H); 3.06 (m, 1 H); 2.89 (m, 1H); 2.80 (m, 1H); 2.76 (m, 2H); 2.40-2.62 (m, 3H); 2.17 (ddd, J= 12.4 Hz, J= 8.7 Hz, J= 1.6 Hz, 1H); 1.87-1.99 (m, 2H). Measured NMR data are corresponding to those reported in the literature.20

n=2: methyl tricyclo[4.3.0.0^{2,5}]nonane-3-carboxylate (15d): [5.7 mmol scale, 75% yield, colorless oil]. 1H NMR (400 MHz, CDCl$_3$); δ (ppm): major (endo): 3.70 (s, 3H); 3.47 (dt, J= 11.2 Hz, J= 8 Hz, 1H); 2.69 (m, 2H); 2.57 (t, J= 6 Hz, 1H); 2.45-2.54 (m, 1H); 2.39-2.45 (m, 1H); 2.10 (dt, J=7.6 Hz, J=3.6 Hz, 1H); 1.74 (m, 1H); 1.45-1.69 (m, 5H). 1H NMR (400 MHz, CDCl$_3$); δ (ppm): minor (exo): 3.67 (s, 3H); 3.15 (t, J=6.8 Hz, 1 H); 2.39-2.52 (m, 3H); 2.32

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Synthesis of Ladderane-based fatty acids

DIBAL-H (5 equiv, 0.5 M in toluene) was added dropwise at -78 °C to a solution of the diastereomeric methyl esters 15a or 15d (1 equiv) in toluene (5 mL/ mmol). The reaction mixture was stirred additionally for 30 min at this temperature. Subsequently, an aqueous HCl (1 M) solution was added dropwise to quench the reaction (pH 5) followed by a mixture of pentane/Et₂O (1/1). After warming up to room temperature the mixture was stirred for 30 min. The layers were separated and the organic layer was washed with water (0.5 mL/mmol) and brine (0.5 mL/mmol), dried over MgSO₄ and concentrated under reduced pressure. The crude alcohol was used without further purification in the next step.

**n=1:** tricyclo[4.2.0.0²,5]octan-3-ylmethanol (16a): [1.2 mmol scale, 95% yield, colorless oil]  
**n=2:** tricyclo[4.3.0.0²,5]nonan-3-ylmethanol (16d): [1.2 mmol scale, 93% yield, colorless oil]

DMSO (6 equiv) was added as solution in CH₂Cl₂ (0.5 mL/mmol) at -78 °C to a solution of oxalyl chloride (3 equiv) in CH₂Cl₂ (2 mL/mmol) and the mixture stirred for 30 min at this temperature. To this mixture a solution of the crude alcohol 16a or 16d (1 equiv) in CH₂Cl₂ (0.5 mL/mmol) was added dropwise and the temperature was allowed to increase over 30 min to -60 °C. After Et₃N (9 equiv) was added the reaction mixture was stirred for additional 30 min at -60 °C before being warmed to room temperature. An aqueous HCl (1 M) solution was added followed by CH₂Cl₂ (0.5 mL/mmol). The layers were separated and the aqueous layer was extracted with CH₂Cl₂ (2x 0.5 mL/mmol), the combined organic extracts were washed with brine (0.5 mL/mmol), dried over MgSO₄ and concentrated under reduced pressure. The residue was filtered over silica gel (pentane/Et₂O: 8/2). This mixture of exo and endo aldehyde was dissolved in degassed Et₃N and stirred at room temperature for 6 days. After removing of the triethylamine under reduced pressure the aldehyde was used without further purification in the next step.

**n=1:** tricyclo[4.2.0.0²,5]octane-3-carbaldehyde (17a): [1 mmol scale, 90% yield, colorless oil]  
**n=2:** tricyclo[4.3.0.0²,5]nonane-3-carbaldehyde (17d): [1 mmol scale, 73% yield, colorless oil].

MS (EI⁺) (m/z): found 137.096 [M+H]⁺, calculated 137.096 [M+H]⁺.

**n=2:** tricyclo[4.3.0.0²,5]nonane-3-carbaldehyde (17d): [1 mmol scale, 73% yield, colorless oil].

MS (EI⁺) (m/z): found 151.112 [M+H]⁺, calculated 151.112 [M+H]⁺.
A solution of fresh prepared LDA (0.15 M in THF, 4.16 equiv) was added at -78 °C to a suspension of the Wittig salt 19 (2 equiv) in THF (5 mL/ mmol). The reaction mixture was warmed over 2 h to room temperature before being cooled again to -78°C. After dropwise adding of the aldehyde (1 equiv) as a solution in THF (1 mL/ mmol) the reaction mixture was stirred for 1 h at this temperature before being warmed over 4 h to room temperature. The resulting reaction mixture was quenched with HCl (1 M, aqueous solution) and diluted with ether (0.5 mL/ mmol). The layers were separated and the aqueous layer was extracted with ether (2*0.5 mL/ mmol), the combined organic extracts were dried over MgSO4 and concentrated under reduced pressure. The crude product was obtained as mixture of E and Z isomers, which were purified and separated by flash chromatography over silica gel (pentane/ether: 95/5).

n=1: (E)-11-(tricyclo[4.2.0.02,5]octan-3-yl)undec-10-enoic acid (1a): [0.5 mmol scale, 62% yield, colorless oil]. 1H NMR (400 MHz, CDCl3); δ (ppm): 5.38 (m, 1H); 5.24 (m, 1H); 2.9 (m, 1H); 2.74 (m, 1H); 2.37-2.70 (m, 4H); 2.34 (t, J= 7.6 Hz, 2H); 2.28 (dd, J= 12.8 Hz, J= 4 Hz, 1H); 2.04 (m, 1H); 2.01 (s, 2H); 1.91 (m, 3H); 1.62 (m, 2H); 1.27 (m, 10H). 13C NMR (100 MHz, CDCl3); δ (ppm): 178.95 (1C, CO); 131.52 (1C, C=C); 130.76 (1C, C=C); 53.78 (1C, CH); 42.50 (1C, CH); 40.38 (1C, CH2); 38.73 (1C, CH); 38.35 (1C, CH); 30.02 (1C, CH2); 29.56 (1C, CH2); 29.45 (1C, CH2); 29.33 (1C, CH2); 29.18 (1C, CH2); 28.58 (1C, CH2); 25.99 (1C, CH2); 25.54 (1C, CH2); 24.83 (1C, CH2); 12.07 (1C, CH). MS (EI+) (m/z): found 291.232 [M+H]+, calculated 291.232 [M+H]+.

n=2: (E)-11-(tricyclo[4.3.0.02,5]nonan-3-yl)undec-10-enoic acid (1d): [0.5 mmol scale, 53% yield, colorless oil]. 1H NMR (400 MHz, CDCl3); δ (ppm): 5.43 (dt, J= 10.8 Hz, J= 7.6 Hz, 1H); 5.26 (d, J= 11.2 Hz, 1H); 2.9 (m, 1H); 2.52 (dd, J= 12.8 Hz, J= 7.2 Hz, J=3.2 Hz, 1H); 2.39 (m, 2H); 2.34 (t, J= 7.6 Hz, 2H); 2.21 (m, 2H); 2.04 (m, 1H); 1.97 (s, 2H); 1.89 (m, 2H); 1.74 (m, 2H); 1.52-1.67 (m, 4H); 1.23-1.35 (m, 10H). 13C NMR (100 MHz, CDCl3); δ (ppm): 178.89 (1C, CO); 130.31 (1C, C=C); 129.84 (1C, C=C); 49.32 (1C, CH); 44.76 (1C, CH); 41.13 (1C, CH2); 39.79 (1C, CH); 33.72 (1C, CH); 33.13 (1C, CH2); 32.75 (1C, CH2); 32.02 (1C, CH2); 29.08 (1C, CH2); 28.62 (1C, CH2); 28.50 (1C, CH2); 28.32 (1C, CH2); 28.17 (1C, CH2); 27.79 (1C, CH2); 24.71 (1C, CH2); 11.20 (1C, CH). MS (EI+) (m/z): found 305.247 [M+H]+, calculated 305.248 [M+H]+.
3.5 References


