Synthesis of ladderane lipid structures

Bachelor project by: Jan Jacob Kooistra (1629700)
Supervisor: Maria Bastian
Date: 16th of August 2010

ABSTRACT

In recent years, naturally occurring lipids have been found to possess important properties. In particular ladderane lipids have interesting properties because of their ability to form densely packed membranes. In order to study them, synthesis of these compounds is needed, because they can not be obtained from nature in sufficient amounts. In this research, derivates of these natural compounds have been synthesized which can be used for biological screening. The key step in this synthesis is a [2+2] photocycloaddition with different substrates. A ketone has been synthesized which can function as a building block for a ladderane lipid, though the exact structure still needs to be determined. Furthermore a ladderane methyl ester was synthesized. This synthesis was done in several steps using cyclopentene and 2-cyclopenten-1-one as the substrates.

Introduction

Ladderanes are molecules containing multiple fused cyclobutane rings. [1] They are called ladderanes because their structure resembles that of a ladder. [2]

In 2002 ladderane lipids were discovered in *Candidatus* 'Brocadia anammoxidans' and *Candidatus* 'Kuenenia stuttgartiensis'; two strains of anaerobic ammonia oxidation (anammox) bacteria.

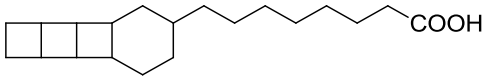

Figure 1: Ladderane lipid [3]

These bacteria convert nitrite and ammonia into nitrogen gas and water in a process called anammox metabolism which takes place in an organelle called the anammoxosome. During this process several toxic intermediates, like hydroxylamine and hydrazine, are formed which diffuse rapidly through a normal biomembrane. This does not appear to happen in the membrane of the anammoxosome due to the presence of ladderane lipids. It was calculated by simulation that these ladderane lipids have a higher density then normal biomembranes (1.5 kg/L and 1.2 kg/L, respectively). This higher density means that the lipids are more densely packed together which in turn means the membrane is less permeable. [4]

Due to the slow growth rate of anammox bacteria it is not feasible to study the exact physical properties of ladderane lipids by collecting them from bacteria. For this reason attempts have been made to synthesize these molecules and derivates of them. One of these syntheses was done by the Corey group. The method Corey employed was a [2+2] photocycloaddition between cyclobutane and 2-cyclopenten-1-one. The resulting product was converted into pentacycloanammoxic acid (Figure 2). [5] [6]

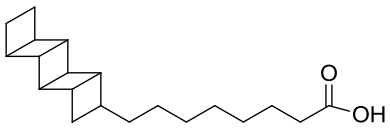

Figure 2: Pentacycloanammoxic acid [7]

The goal of this project was the synthesis of derivates of natural ladderanes like adehyde **1** and **2** (Figure 3), using 2-cyclopenten-1-one/cyclohexene and 2-cyclopenten-1-one/cyclopentene as starting materials respectively, using the method of Corey. The goal was to get a *cis-anti-cis* configuration around the cyclobutane ring which is attached to the cyclohexane or cyclopentane ring. The configuration depends on the flexibility of the ring system.

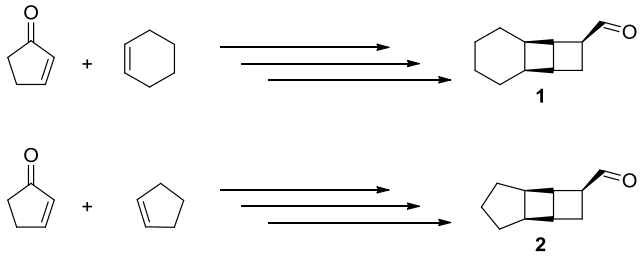

Figure 3: Synthesis of aldehyde **1** and **2**

The planned synthetic scheme for the synthesis of aldehyde **1** is shown in Figure 4. The first step is a [2+2] photocycloaddition between 2-cyclopenten-1-one and cyclohexene forming ketone **3**. α-Diazo-ketone **4** is synthesized by a formylation followed by a Regitz diazo transfer. Methyl ester **5** is synthesized by a Wolff rearrangement followed by the addition of methanol. The product is converted to alcohol **6** by a DIBAL-H reduction. Aldehyde **7** is formed by a Swern oxidation and enantio enrichment to product **1** is done by stirring product **7** in triethylamine for several days during which the product isomerizes. [5]

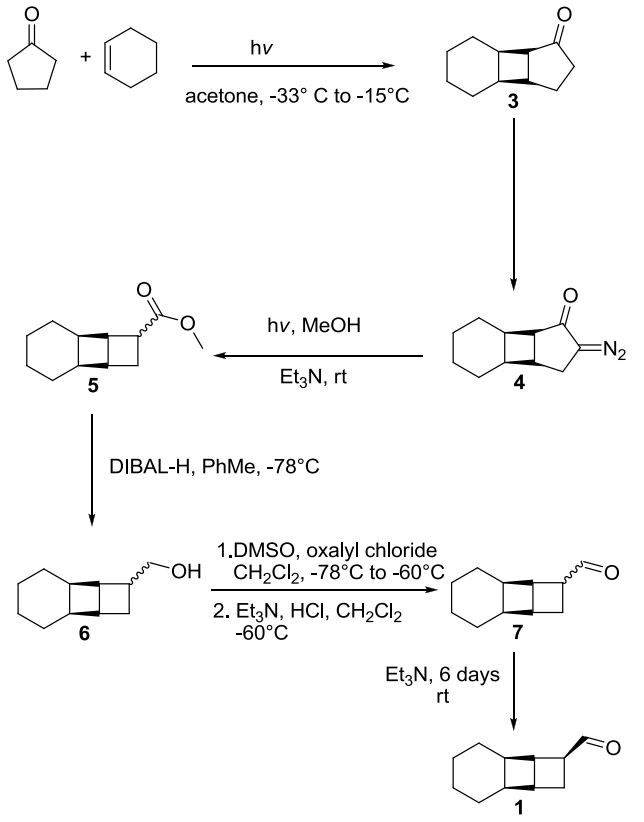

Figure 4: Synthetic scheme of the planned synthesis of aldehyde **1**

The synthesis of aldehyde **2** is analogous to that of aldehyde **1** with the exception that the starting materials are 2-cyclopenten-1-one and cyclopentene.

Ketone **3** and **8** were converted into their hydrazone derivatives by a coupling with 2,4-dinitrophenylhydrazine.[8] This gave the opportunity to perform x-ray crystallography on the compound to determine the exact configuration of the ketone. Only the x-ray image of hydrazone **11** was obtained. Hydrazone **12** was too impure due to the formation of multiple products, so no x-ray measurements could be performed on this compound

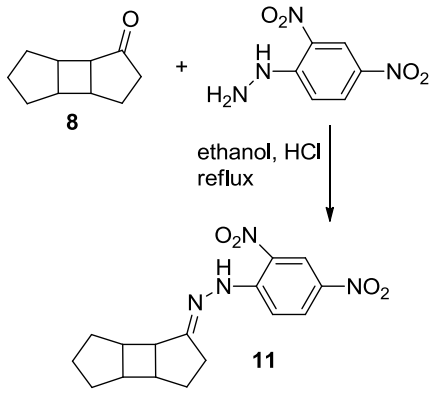

Figure 5: Hydrazone formation of ketone **8**

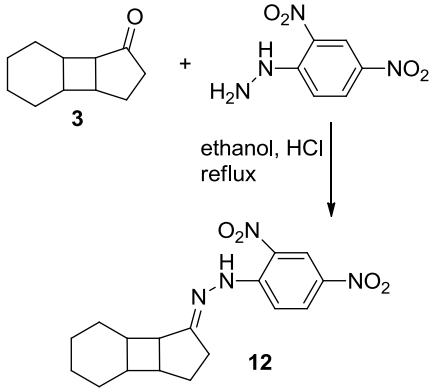

Figure 6: Hydrazone formation of ketone **3**

Characterization of the different compounds was done with ^{1}H NMR, ^{13}C NMR, different types of 2D-NMR, GC/MS and HPLC.

Results and Discussion

Synthesis of α-Diazo-ketone 4

The [2+2] photocycloaddition between cyclohexene and 2-cyclopenten-1-one was conducted in a mixture yield of 51%. The reaction was not run to full conversion because of the formation of an aldehyde degradation product (Figure 7), which is formed by a C-C cleavage of the desired ketone. It was determined that a mixture of products (Figure 8, 9 and 11) was obtained which was not separable by column chromatography.

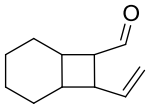

Figure 7: Possible degeneration product of aldehyde **3**

	Equivalents of cyclopentenone	Equivalents of cyclohexene	Run time (hours)	Temperature (°C)	Mixture yield
Variant 1	1	2	8	-33 to -20	51%
Variant 2	1	2	6.5	-33 to -20	40%
Variant 3	1	4	5	-23	57%
Variant 4	1	4	8	-23	39%
Variant 5	1	6	7	-23	47%

Table 1: Variants tried for the synthesis of ketone **3**

To avoid the possibility of dimerization of the starting materials to form products **13, 14** and **15** (Figure 8 and 9**)**, several variants of the reaction were tried with the conditions given in table 1. None showed any significant change in product composition by NMR. When comparing variant one to variant two it can be concluded that a shorter reaction time leads to a lower mixture yield. Comparing variant three to variant two leads to the conclusion more equivalents of cyclohexene, a slightly shorter run time and a slightly lower temperature leads to an increase in mixture yield. When comparing variant four to variant three it can be concluded a longer reaction time leads to a lower mixture yield. More equivalents of cyclohexene and a slightly lower run time lead to a higher yield as can be concluded when variant five is compared to variant four.

GC/MS showed four peaks with the predicted mass of the product (164 g/mol), which also happens to be the mass of the possible dimerization products of the starting materials. For this reason the products of the dimerization reactions (Figure 8 and 9) were synthesized.

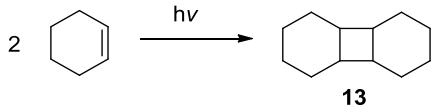

Figure 8: Product of cyclohexene dimerization

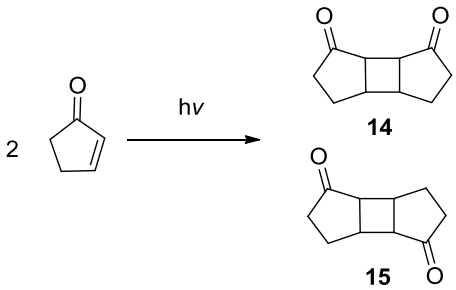

Figure 9: Products of 2-cyclopenten-1-one dimerization

The synthesis of product **13** was not run to full conversion because only a small amount of material was necessary for characterization. GC/MS showed five products with the desired mass. Literature gives the possibility of four different configurations of product **13**. In addition there is also the possibility of different side products such as the ones given in Figure 10. [9] ^{1}H NMR and ^{13}C NMR studies were inconclusive about the exact structures of the products. By comparing the retention times and NMR spectra of the dimerization products **13** to the retention times and NMR spectra of the product mixture obtained by the reaction between cyclohexene and 2-cyclopenten-1-one, the dimerization products **13** could be ruled out as being part of the mixture of the reaction between cyclohexene and 2-cyclopenten-1-one.

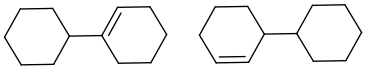

Figure 10: Examples of possible side products from dimerization of cyclohexene

The four different configurations of **13** are formed because of the flexibility of cyclohexane ring in the products. This might also be valid in the case for the product mixture obtained in the synthesis of ketone **3**. The possible product configurations are given in Figure 11. [10]

.

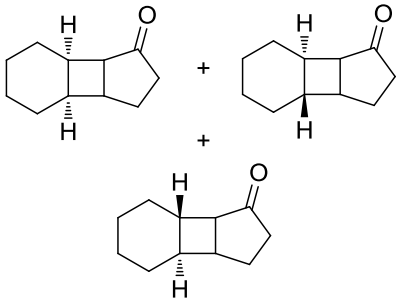

Figure 11: Possible configurations of ketone **3**

The synthesis of product **14** and **15** was not run to full conversion because only a small amount of material was necessary for characterization. Both products were identified by GC/MS and NMR with a ratio of 9:1. It is not clear which isomer is the major or the minor product. It might be possible that one of the diketone products is in the product mixture acquired in the reaction between cyclohexene and 2-cyclopenten-1-one, because one of the retention times of the diketone reaction is close to one found in the product mixture of the reaction between cyclohexene and 2-cyclopenten-1-one. The NMR was inconclusive due to the overlap of peaks in the product mixture.

An attempt was made to separate the four products by preparative HPLC equipped with a UV detector. Unfortunately the compounds could not be detected by this type of detector. For this reason ketone **3** was converted into hydrazone **12** because this compound can be detected with a UV detector. The reaction was conducted in a low yield of (5.6%). This is probably due to the three recrystallizations performed on the compound for purification. An attempt was made to develop a method to separate the hydrazone products by HPLC which might be

used for preparative HPLC. This way the structures of the different products of the reaction between cyclohexene and 2-cyclopenten-1-one can be elucidated. A promising separation was found with a Chiralcel OB-H column using heptane/IPA (95:5) as the solvent system with a flow rate of 0.5mL/min at 40°C. 1 microlitre of a 1mg/mL solution was injected. The compound was dissolved in heptane with a few drops of IPA. Five products were observed as can be seen in Figure 12.

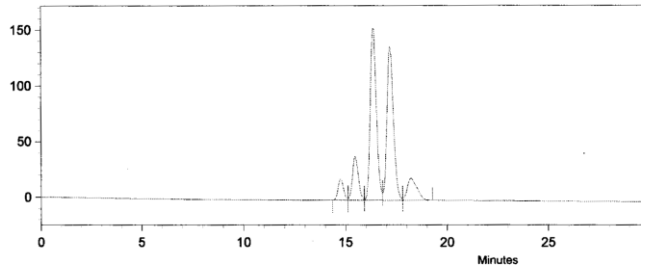

Figure 12: Chromatogram of the separation of hydrazone **12**

To find out if the products mixture might be separable in a later stage of the synthesis of aldehyde **1**, α-diazo-ketone **4** was synthesized in a yield of 35% in a test reaction. ^{1}H NMR and ^{13}C NMR showed the formation of several products which could not be separated by column chromatography. The exact structures of the products could not be elucidated by the performed NMR studies. Because of the continued formation of a product mixture further attempts of the synthesis of aldehyde **1** were abandoned.

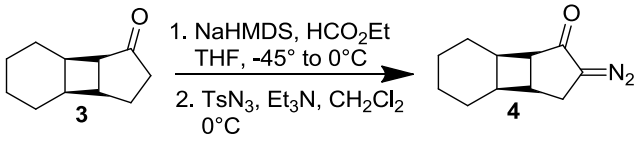

Figure 13: Synthesis of α-diazo-ketone **4**

Because the product mixture was probably formed due to flexibility of the ring system, a smaller ring system was chosen to avoid this problem.

Synthesis of methyl ester 10

The [2+2] photocycloaddition between cyclopentene and 2-cyclopenten-1-one was conducted in a low yield of 17%. This could be caused by the volatility of the compound and because the reaction was not run to full conversion due to the possible degredation of the product to an aldehyde. GC/MS showed the formation of only one product with the predicted mass of 150 g/mol. The exact configuration and structure of the ketone could not be determined by 2D-NMR (COSY, NOSY, HSQC, HMBC) because several couplings were missing. For this reason ketone **8**, was converted into hydrazone **11** in a yield of 76%.

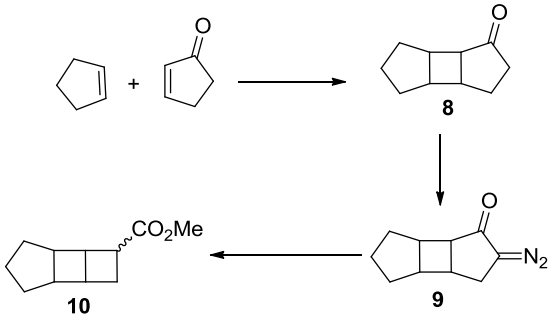

Figure 14: Synthetic scheme of the synthesis of methyl ester **10**

By X-ray crystallography of the hydrazone it was confirmed that the ketone possesses a *cis,anti,cis* configuration around the cyclobutane moiety, as can be seen in Figure 15.

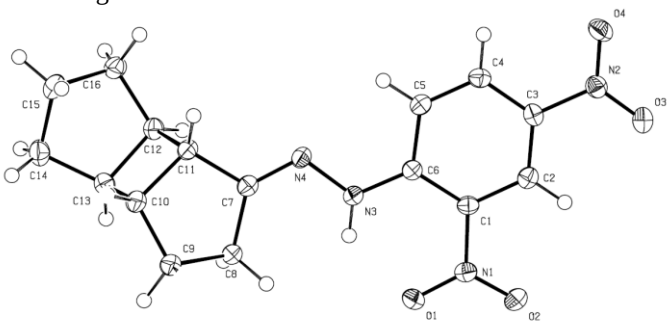

Figure 15: X-ray structure of hydrazone **11**

The conversion of the product to α-diazo ketone **9** was performed in a yield of 0.37 g (31%). An unwanted side product was formed in a yield of 0.44 g. It was later found out that the tosyl chloride, which was used to synthesize the necessary tosyl azide, was contaminated. This might explain the formation of the side product. The synthesis of methyl ester **10** was done in a moderate yield of 44%. Though a TLC of the reaction mixture showed complete conversion, it was shown after concentrating, that there was still some of the starting material remaining. The problem is probably the fact a large scale setup of a fixed size was used for the photo reaction, while the reaction itself was small scale. A smaller setup might improve the yield. GC/MS and NMR studies showed the formation of two isomers of the product in a 3:1 ratio. More NMR studies need to be done to figure which of the isomers are the major and the minor product.

Conclusions and Outlook

The synthesis of ketone **3** was performed with a mixture yield of 57%. The reaction yielded four products of which one is probably a dimerization product of 2-cyclopenten-1-one and the other three are different configurations of the desired product. The products could not be separated by column chromatography. The products might be separated

by preparative HPLC after which the precise structures can be determined by NMR.

The ketone was converted into hydrazone **12** in a yield of 5.6%. The product mixture this yielded was separated by analytical HPLC. In the future the used method can be employed to separate the different products to elucidate their structures by NMR. The ketone was also converted into α-diazo-ketone **4** in a yield of 35%.

Ketone **8** was synthesized in a yield of 17%. Proof of its *cis,anti,cis* configuration was achieved by NMR studies and X-ray of the corresponding hydrazone which was synthesized in a 75% yield. The conversion of ketone **8** into the α-diazo ketone **9** was performed in a yield of 31%. The synthesis of methyl ester **10** was achieved in a yield of 44%.

In the future methyl ester **10** can be converted into an alcohol and after that into an aldehyde. In addition more extensive NMR studies need to be performed on methyl ester **10** to elude the exact structure. Furthermore, all of the performed reactions need to be optimized.

Experimental

General remarks: Unless stated otherwise all reaction setups were dried and flushed with nitrogen gas before use and all reactions were preformed under a nitrogen atmosphere. THF was distilled before use from benzophenone/sodium. All products were stored under a nitrogen atmosphere at 4°C in the dark.

All photoreactions were performed with a medium pressure mercury UV lamp in Pyrex glass. The solvents used for these reactions were of spectroscopic grade and purchased from Merck. GC/MS experiments were performed on a HP 6890/5973 GC/MSD using a HP1 column and helium as the carrier gas. NMR spectra were recorded on a Varian Gemini 200 (^{1}H: 200 MHz; ^{13}C: 50 MHz), Varian Mercury Plus 400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) and Varian Unity Plus 500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) instruments. The chemical shifts are reported in parts per million on a δ scale with the residual undeuterated solvent as the point of reference. HPLC was performed on a Shimadzu LC-10Avp equipped with a UV detector using a mixture of heptane/IPA as the eluent.

Synthesis of ketone 3 (variant 1)

Anhydrous acetone (150 mL) was degassed for one hour, by bubbling N_2 through the solvent, and placed in a Pyrex photo reactor. The solvent was cooled down to -33°C and 3.7 mL (37 mmol) of cyclohexene and 1.6 mL (19 mmol) of 2-cyclopenten-1-one were added. While being stirred, the solution was irradiated with a medium pressure mercury UV lamp. After six hours the temperature was raised to -20°C and after another two hours the reaction was stopped as ^{1}H NMR indicated the degradation of the product into an aldehyde. The solution was concentrated and purified by column over silica with pentane/ether (30:1) as the eluent which yielded a mixture yield of 1.6 g (9.8 mmol) 51% of a colorless liquid.

Synthesis of ketone 3 (variant 2)

Synthesis was done analogues to variant 1 with the difference that the temperature was raised to -20°C after four-and-a-half hours and the reaction was stopped after another two hours. The reaction yielded a mixture yield of 1.2 g (7.6 mmol) 40% of a colorless liquid.

Synthesis of ketone 3 (variant 3)

Synthesis was done analogues to variant 1 with the difference that the reaction temperature was -23°C for five hours. In addition 7.4 mL (74 mmol) of cyclohexene and 1.6 mL (19 mmol) 2-cyclopenten-1-one were used as starting materials. This yielded a mixture yield of 3.0 g (10.8 mmol) 57% of a colorless liquid.

Synthesis of ketone 3 (variant 4)

Synthesis was done analogues to variant 1 with the difference that the reaction temperature was -23°C for eight hours. In addition 37 mL (370 mmol) of cyclohexene and 8 mL (95 mmol) of 2-cyclopenten-1-one were used as starting materials. This yielded a mixture yield of 6.1 g (37 mmol) 39% of a colorless liquid.

Synthesis of ketone 3 (variant 5)

Synthesis was done analogues to variant 1 with the difference that the reaction temperature was -23°C for seven hours. In addition 16 mL (160 mmol) of cyclohexene and 2.5 mL (30 mmol) 2-cyclopenten-1-one were used as starting materials. This yielded a mixture yield of 1.2 g (7.6 mmol) 47% of a colorless liquid.

Characterization of the product mixtures: ^{1}H NMR (500 MHz, $CDCl_3$, δ [ppm]): 3.10 (m);, 2.96 (m), 2.85 (m), 2.78 (m), 2.71 (m), 2.62 (m), 2.53 (m), 2.47 (m), 2.43 (m), 2.37 (m), 2.23 (m) 2.08 (m), 1.97 (m), 1.86 (m), 1.75 (t, j=14.5Hz), 1.65 (m), 1.56 (m), 1.51 (m), 1.42 (m). Individual protons could not be assigned because all the peaks are broad multiplets falling in the same region (1.42-3.10 ppm)
^{13}C NMR (125 MHz, $CDCl_3$, δ [ppm]): 222.7 (carbonyl carbon), 53.9, 51.5, 50.2, 48.2, 47.2, 45.4, 45.3, 45.3, 44.1, 41.3, 40.7, 40.1, 39.2, 38.5, 37.2, 36.4, 34.7, 34.0, 33.9, 31.1, 31.0, 28.6, 28.2, 27.4, 26.6, 26.3, 26.3, 26.1, 26.0, 25.8, 25.8, 24.7, 22.2, 22.1 22.0, 21.8, 21.7, 21.2, 21.0, 19.7.

For the last two variants a GC/MS was taken:
GC/MS: *m/z* = 164.1, 149.1, 135.0, 121.0, 108.1, 696.1, 91.0, 82.1, 77.1, 67.0, 55.1, 41.0, 28.1. Four peaks were visible with the same mass spectrum with a relative peak intensity of 10:58:12:20

Synthesis of α-diazo ketone 4

To a solution of 10 mL of a 1M NaHMDS solution in THF (1.2 equiv) at -45°C was added over 20 minutes a solution of 1.42 g (8.6 mmol) of ketone **3** in 13 ml of THF. The resulting solution was stirred for one hour while the temperature was allowed to rise to -30°C. Freshly distilled ethyl formate (2.1 mL (3 equiv)) was added in one portion. The reaction was stirred for one-and-a-half hours after which TLC showed full consumption of the starting ketone. During this time the temperature was allowed to rise to 0°C. Diethyl ether (22mL) was added before the reaction was quenched by the dropwise addition of 16.5 mL of a 1M HCl solution until the pH was 5. The two phases were separated and the aqueous layer was extracted three times with 25 mL diethyl ether. The combined organic fractions were washed with brine and dried over Na_2SO_4. The solution was filtered and concentrated under reduced pressure to yield a brown oil.
The brown oil (0.53 g (4 mmol)) was dissolved in 7.5 mL of DCM at 0°C. To this solution 0.92 g (1.2 equiv) of freshly made tosyl azide in 2 mL of DCM was added followed by 2.4 mL (2 equiv.) of Et_3N. The solution was stirred for 30 minutes and concentrated under reduced pressure at a temperature of 35°C. Purification was performed by doing a column over silica gel with pentane/ether (8:2) as the eluent. This yielded 0.27 g (1.4 mmol) of a yellow solid which became an oil upon standing. The total yield over two steps was 35%. ^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 4.08 (m), 3.48 (q, J=6.8Hz), 3.27 (dd, J=14Hz, J=5.5Hz), 3.17 (dd, J=13.4Hz, J=7.4Hz, 1H), 3.09 (m,), 3.04-2.94 (m), 2.94-2.87 (m, 0H), 2.84 (m), 2.75 (m), 2.62 (d, J =7.0Hz), 2.40 (m), 1.99 (m), 1.76 (m), 1.54 (m), 1.38 (m), 1.25 (m), 1.13 (m). Protons could not be assigned because of the formation of a product mixture and the fact all peaks are broad mutiplets overlapping in the area between 1.13 and 4.08 ppm.
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): 202.7, 201.3, 165.3, 135.3, 54.7, 53.3, 52.7, 50.6, 48.8, 48.2, 47.7, 46.8, 45.5, 44.8, 40.7, 38.1, 37.2, 35.9, 34.8, 34.5, 34.1, 33.6, 33.1, 31.1, 30.8, 29.8, 29.3, 28.8, 28.0, 27.5, 26.4, 26.2, 26.1, 25.8, 25.8, 23.0, 22.8, 22.1, 22.0, 21.60 21.5, 21.1, 20.7.

Synthesis of p-tosyl azide (used in the synthesis of α-diazo ketone 4)

p-Tosyl chloride (1.15 g (5.8 mmol)) was dissolved in 36 mL of an ice cooled mixture of acetone/water (1:1). 0.39 g (6.0 mmol) of sodium azide was added to the solution. The resulting solution was stirred for 2 hours at 0°C. The acetone was evaporated under reduced pressure (35°C, 150 mbar). The layers were separated and the aqueous layer was extracted with diethyl ether. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. This yielded 1.12 g (5.7 mmol) of a colorless liquid (quant.).

Synthesis of hydrazone 12

Ketone **3** (0.88 g (5.5 mmol)) was dissolved in 75 mL of ethanol. To this 1.7 g (1.5 equiv) of 2,4-dinitrophenylhydrazine was added. The mixture was heated untill reflux and was then allowed to cool slightly. After addition of 2 mL of concentrated HCl the mixture was refluxed for two minutes. The solution was set aside to wait for crystallization. After filtration the crystals were washed with ethanol followed by three recrystallizations in ethanol. This yielded 0.20 g (0.58 mmol, 5.6%) of a orange solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 10.80 (m), 9.12 (1H), 8.27 (1H), 7.29 (1H), 3.72 (q, J=4Hz), 3.50 (m), 3.36 (m), 3.16 (m) 2.98 (m), 2.91(m), 2.80 (m), 2.29 (m), 2.06 (m), 1.90 (m), 1.72 (m), 1.59 (m), 1.32 (m), 1.25 (m).
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): 173.7, 171.5, 171.1, 147.6, 135.1, 130.1, 123.9, 116.6, 116.4, 44.4, 35.8, 34.9, 34.1, 33.4, 32.3, 29.4, 28.5, 28.3, 27.7, 26.8, 26.0, 22.8, 22.7, 22.3, 21.8.

Dimerization of 2-cyclopenten-1-one (14 and 15)

Anhydrous acetone (150 mL) was degassed for one hour, by bubbling N_2 through the solvent, and placed in a Pyrex photo reactor. The solvent was cooled down to -23°C and 2 mL (23 mmol) of 2-cyclopenten-1-one was added. While being stirred, the solution was irradiated with a medium pressure mercury UV lamp. After six hours the reaction was stopped as ^{1}H NMR indicated the degradation of the product into an aldehyde. After concentrating the solution and standing overnight, white crystals were filtered off from the colorless liquid. The crystals were not further purified. The yield was 0.2 g (1.2 mmol, 5%).
^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 2.80 (m, 2H), 2.66 (m, 2H), 2.57 (m, 2H), 2.34 (m, 2H), 2.20 (m, 2H), 2.05 (m, 2H). Impurities: 2.46 (d, J=5.2Hz).
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): major product:
219.7, 49.5, 47.4, 36.2, 28.1; minor product: 218.5, 45.0, 41.2, 37.5, 27.4.
GC/MS: *m/z* = 164.0, 128.9, 136.0, 128.9, 122.0, 115.0, 108.0, 94.0, 82.0, 73.9, 66.0, 54.0, 45.9, 39.0, 27.0. Two different isomers were obtained with the same mass spectrum and a relative peak intensity of 9:1.

Dimerization of cyclohexene (13)

Anhydrous acetone (150 mL) was degassed for one hour, by bubbling N_2 through the solvent, and placed in a Pyrex photo reactor. The solvent was cooled down to -23°C and cyclohexene (5 mL (50 mmol)) was added. While being stirred, the solution was irradiated with a medium pressure mercury UV lamp for four hours. The solution was concentrated under reduced pressure. This yielded 110 mg (0.7 mmol, 3%) of a colorless liquid.
^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 5.67 (m), 4.84 (d, *J*=14.6Hz), 4.75 (m), 4.73 (s), 2.60 (q, J=8.4 Hz), 2.43 (m),

2.29 (s), 2.26 (s, J=6.8Hz), 1.94 (m), 1.67 (m), 1.29 (m), 1.04 (m). The different products were not isolable and because of peak overlap, proton assignment could not be achieved. ^{13}C was performed but the data is not reported because of the many peaks which can not be assigned by any reasonable means.
GC/MS: *m/z* = 164.0, 149.1, 135.1, 121.1, 107.1, 95.0, 82.1, 77.0, 67.1, 55.1, 50.0, 41.1, 28.1. Five peaks with the same mass spectrum were visible with a relative peak intensity of 7:11:4:2:34

Synthesis of ketone 8

Anhydrous acetone (150 mL) was degassed for one hour, by bubbling N_2 through the solvent, and placed in a Pyrex photo reactor. The solvent was cooled down to -23°C and cyclopentene (12.8 mL (152 mmol)) and 2-cyclopenten-1-one (6.4 mL (76 mmol)) were added. While being stirred, the solution got irradiated with a medium pressure mercury UV lamp. After eight hours the reaction was stopped as ^{1}H NMR indicated the degradation of the product into an aldehyde. The solution was concentrated and purified by column over silica with pentane/ether (30:1) as the eluent which yielded 1.44 g (1.2 mmol) 17% of a colorless liquid.
^{1}H NMR (500 MHz, $CDCl_3$, δ [ppm]): 2.70 (m, 1H), 2.47 (m, 2H), 2.26 (m, 1H), 2.20 (ddd, J=8.8Hz, J=3.6Hz, J=1.8 Hz, 1H), 2.09 (d, J=6.5 Hz, 1H), 2.01 (m, 1H), 1.89 (m, 1H), 1.70-1.80 (m, 3H), 1.67 (dd, J=12.6, 5.6 Hz, 1H), 1.50 (m, 2H).
^{13}C NMR (125 MHz, $CDCl_3$, δ [ppm]): 222.5 49.0, 43.3, 40.6, 38.8, 36.4, 33.3, 33.2, 28.4, 24.8.
GC/MS: *m/z* =150.0, 135.0, 122.0, 115.0, 108.0, 93.0, 83.0, 75.1, 68.0, 60.9, 53.0, 46.1, 39.1, 28.1.

Synthesis of α-diazo ketone 9

To a solution of 13 mL of a 1M NaHMDS solution in THF (1.2 equiv) at -45°C was added over 20 minutes a solution of 1.0 g (6.7 mmol) of ketone **3** in 10 mL of THF. The resulting solution was stirred for one hour while the temperature was allowed to rise to -30°C. Freshly distilled ethyl formate (1.65 mL (3 equiv)) was added in one portion. The reaction was stirred for two hours after which TLC showed full consumption of the starting ketone. During this time the temperature was allowed to rise to 0°C. Diethyl ether (17mL) was added before the reaction was quenched by the dropwise addition of 13 mL of a 1M HCl solution until the pH was 6. The two phases were separated and the aqueous layer was extracted three times with 20 mL of diethyl ether. The combined organic fractions were washed with brine and dried over Na_2SO_4. The solution was filtered and concentrated under reduced pressure to yield a brown/orange oil.
The brown/orange oil (0.69 g (3.9 mmol)) was dissolved in 6 mL of DCM at 0°C. To this solution 0.8 g (1.2 equiv.) of freshly prepared tosyl azide in 1 mL of DCM was added, followed by 1.2 mL (2 equiv.) of Et_3N. The solution was stirred for 30 minutes and concentrated under reduced pressure at a temperature of 35°C. Purification was performed by a column over silica gel with pentane/ether (8:2) as the eluent. This yielded 0.37 g (2.1 mmol) of a yellow liquid. The total yield over two steps was 31%. Also an unwanted side product was formed in the second step with a yield of 0.44 g. ^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 3.28 (dd, 1H, J=14.4 Hz, J=9.2 Hz), 2.89 (d, 1H, J=3.4 Hz), 2.73 (m, 1H), 2.52 (m, 1H), 2.44 (m, 1H), 2.24 (m, 1H), 1.83 (m, 1H), 1.74 (m, 2H), 1.67 (m, 1H), 1.60-1.41 (m, 2H).
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): 202.8, 50.7, 45.6, 42.8, 35.2, 33.0, 32.7, 31.2, 24.8

Synthesis of p-tosyl azide (used in the synthesis of α-diazo ketone 9)

p-Tosyl chloride (1.15 g (5.8 mmol)) was dissolved in 36 mL of an ice cooled mixture of acetone/water (1:1). 0.39 g (6.0 mmol) of sodium azide was added to the solution. The resulting solution was stirred for 2 hours at °C. The acetone was evaporated under reduced pressure (35°C, 150 mbar). The layers were separated and the aqueous layer was extracted with diethyl ether. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. This yielded 0.81 g (4.1 mmol) of a colorless liquid (71%).

Synthesis of methyl ester 10

Anhydrous MeOH (150 mL) was degassed for one hour with N_2, and placed into a Pyrex photo reactor. 0.19 g (1.3) mmol of α-diazo ketone **9** was added followed by 0.20 mL (1 equiv.) of Et_3N. The mixture was irradiated for one hour with a medium pressure mercury UV lamp at room temperature. At this point TLC showed full consumption of the starting material. The solution was concentrated and purified by column over silica gel using pentane/ether (15:1) as the eluent. This yielded a colorless liquid with a yield of 0.103 g (0.57 mmol, 44%).^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 3.64 (s, 3H), 3.61 (s, 1H (3*1/3H)), 3.40 (m, 1H), 3.09 (m, 1/3H), 2.62 (m), 2.50 (m), 2.46 (m), 2.42 (m), 2.39 (dd, J=11.2Hz, J=3.6Hz), 2.33-2.38 (m), 2.25 (m), 2.19 (m), (2.07 (m), 2.04 (m), 1.68 (m), 1.49 (m). Because of overlap of the peaks of the major and minor isomer, not all protons could be assigned.
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): major-isomer: 174.9 (C_{quart},, ester carbon), 51.5, 46.7, 42.5, 40.7, 39.2, 36.4, 33.6, 33.3, 28.9, 25.6. Minor-isomer: 174.9 (C_{quart}, ester carbon), 51.9, 45.9, 35.6, 43.5, 43.3, 27.6, 33.4, 33.3, 30.4, 25.5. GC/MS: *m/z* = 180.1, 165.0, 149.0, 136.9, 131.0, 126.0, 121.0, 116.0, 111.0, 105.0, 100.0, 91.0, 85.0, 79.0, 74.0, 67.0, 59.0, 53.0, 46.0, 41.1, 32.0, 27.1. Two peaks with the same mass spectrum were obtained.

Synthesis of hydrazone 11

Ketone **8** (0.5 g (3.3 mmol)) was dissolved in 45 mL of ethanol. To this solution 0.79 g (1.2 equiv.) of 2,4-dinitrophenylhydrazine was added. The mixture was heated until reflux and was then allowed to cool slightly. After addition of 1.2 mL of concentrated HCl the mixture was refluxed for two minutes. The solution was set aside to wait

for crystallization. After filtration, the crystals were washed with ethanol followed by recrystallization in ethanol. This yielded 0.82 g (2.5 mmol, 76%) of a orange solid. ^{1}H NMR (400 MHz, $CDCl_3$, δ [ppm]): 10.91 (s, 0.3H,), 10.77 (s, 0.7H), 9.11 (d, 1H, J=4Hz), 8.27 (m, 1H) 7.92 (d, 1H, J=20Hz), 2.97 (m, 1H) 2.74 (m, 2H), 2.44 (m, 3H), 1.92 (m, 5H), 1.64 (m, 3H).
^{13}C NMR (100 MHz, $CDCl_3$, δ [ppm]): Major isomer: 172.4, 145.4, 137.5, 130.1, 124.1, 116.5, 116.4, 43.0, 41.9, 41.4, 40.5, 33.3, 33.2, 32.6, 29.9, 25.2. Minor isomer: 172.4, 145.4, 137.5, 130.1, 124.1, 116.5, 116.4, 47.2, 43.1, 42.7, 39.9, 33.3, 33.1, 30.9, 26.7, 25.1.

References

[1] Miller, M. A.; Schulman, J. M. *J. Mol. Struc. (Theochem),* **1988**, *163*, 133-141.
[2] Nouri, D.H.; Tantillo, D.J. *Current Organic Chemistry*, **2006,** *10***,** 2055-2074.
[3] Jaeschke, A. et al. *Organic Geochemistry*, **2008**, *39*, 1735-1741.
[4] Damsté, J.S. et al. *Nature*, **2002**, *419*, 708-711.
[5] Mascitti, V.; Corey, E. J. *J. Am. Chem. Soc.* **2006**, *128*, 3118-3119.
[6] Mascitti, V.; Corey, E. J. *J. Am. Chem. Soc.* **2004**, *126*, 15664-15665.
[7] Mascitti, V.; Corey, E.J., *Tetrahedron Letters,* **2006**, 47, 5879–5882.
[8] Allen, C.F.H. *J. Am. Chem. So.* **1930**, *52*, 2955–2959.
[9] Salomon, R.G. et al. *J. Am. Chem. Soc.* **1974**, *96*, 1145-1152.
[10] Grota, J. et al. *Synthesis,* **2005**, *14*, 2321-2326.