LUND INSTITUTE
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Organic Chemistry 2

Structural studies of FR96001M

-A new antibacterial compound produced by *Cyphellopsis anomala* TA96001

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Abstract

FR96001M, (4R, 5R)-4,5-Dihydroxy-5-((S)-1-hydroxy-heptyl)-cyclopent-2-enone, is a new antibacterial compound isolated from *Cyphellopsis anomala* TA96001. Its structure was determined by spectroscopic methods. The absolute structure was determined with a FT-NMR version of Mosher´s method. FR96001 is very similar to the previously known antibacterial pentenomycins, which is produced by *Streptomyces eurythermus.* Derivatives to evaluate structure activity relationship were synthesized. The antibacterial and antifungal activity of the new derivatives were tested on different species of bacteria and fungi. The results indicated that the double bond is essential for the activity, the tertiary hydroxyl group important for selectivity against bacteria and the hydroxyl group in the carbon tail important for the activity. Synthetic routes to pentenomycins were found in the literature and at least one of them could probably be modified and used to produce racemic FR96001.

1. Introduction

The aim of this study was to determine the configuration (both relative and absolute) of a compound called FR96001M. FR96001M, (4R,5R)-4,5-Dihydroxy-5-((S)-1-hydroxy-heptyl)-cyclopent-2-enone, is a new compound. It is produced by and isolated from *Cyphellopsis anomala* TA96001 and probably belongs to the pentenomycins, a class of fungal metabolites. FR96001M shows antibacterial properties. The structure determination was done by spectroscopic methods, mostly NMR. Mosher´s method was used to determine the absolute configuration. Derivatives were made, which were used both for structure activity relationship studies and to the structure determination. The double bond in the molecule was found to be important for the activity. Total synthetic routes to pentenomycin I are available and at least one of them seems possible to modify, which would make it useful for producing (±) FR96001M.

1.1 Natural products

Chemists have always been interested in molecules produced by nature and inspired by the fantasy of nature when it comes to chemical structures. Many synthetic approaches have been developed when trying to synthesize molecules that have been produced naturally. Nature has also for long been screened to find substances that are biologically active and which may be used as potential pharmaceuticals. Fungi have always been of interest to mankind but the interests of fungi grew when Flemming discovered penicillin. That fungus must have some defense against bacteria is quite obvious otherwise would they have no chance to compete with them.

Two quite different but interesting compounds discovered in nature are Taxol and Combretastatin (see *Figure 1.1*.). Both were discovered about 20-30 years ago and were found to have strong antitumor/anticancer properties and are now used or tested for use as drugs.

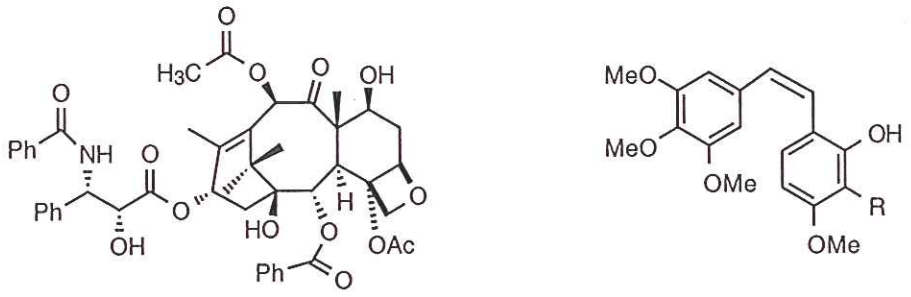

Figure 1.1. The molecule to the right is Taxol. The molecule to the left is Combretastatin (R=OH in Combretastatin A-1 and R=H in Combretastatin A-4).

Taxol is already on the market. Its structure was first reported by Wall and Wani [1]. The first successful total synthesis was reported by Nicolauo *et al* [2]. Taxol is primarily used against breast and ovarian cancer. The Taxol molecule is quite complex and the synthetic routes known today are not commercially applicable. Taxol used to be extracted from the bark from the pacific yew tree, but just very small amount could be gained this way. Today is Taxol made through semi synthetic methods from a similar precursor molecule, which can be extracted from the needles of various *Taxus* species and in a few steps converted to Taxol [3].

Combretastatin is a much simpler but not less potent molecule. It is produced by the African willow tree *Combretum caffrum.* The isolation and structure determination of it was reported 1982 by Pettit et al [4]. The first synthesis was also made by Pettit et al [5]. Pettit was also the one that modified the molecule to an inactive (prodrug) precursor form that could be taken up by the patient [6]. Combretastin is today in clinical trial and thought to become a member of new generation of cytostatic compounds.

1.1.1. FR96001M and pentenomycins

The group at LB-biotechnologie der Universität Kaiserslauten in Germany, lead by professor Timm Anke, found FR96001M, when screening micro fungi for new antibiotics, in the fungous *Cyphellopsis anomala* TA96001. The fungus was isolated from a dead beach tree in Wallhaben, Germany. They fermentated *Cyphellopsis anomala*

TA96001 in a fermentator to produce larger amounts of FR96001M. The fungus was of interest because an other genus of it was known to produce the well known fungal metabolites strobiliurin A, D and F.

Kimio Umino et al [7] discovered the first of the pentenomycins, pentenomycin I. It was found during a screening project for new antibiotics. The structure of pentenomycin I is very similar to the one of FR96001M (see *Figure 1.2.*) although the absolute stereo chemistry of FR96001M seems to be inversed. Pentenomycin I is produced by the fungus *Streptomyces eurythermus*. In the laboratory the fungus is fermented and pentenomycins I is isolated from the fermentation batch by chromatography. The structure of pentenomycin I was confirmed by x-ray crystallography [8]. The presence of the α,β-unsatured ketone was known from NMR and the fact that pentenomycin did show a strong absorption at 216 nm. The diol in the five membered ring was known to be *cis* due to the fact that it formed an acetal with acetone.

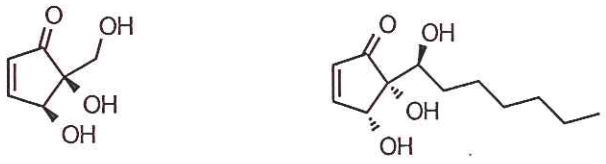

Figure 1.2. The left molecule is pentenomycin I and the right one is FR96001M.

The antibacterial effect of pentenomycin I and a row of derivatives were tested on different bacteria (*S.aureus, B.pertussis and N.gonorrhoeae)* [9]. All of the tested derivatives were esters or acetals made from pentenomycin I. A brominated triacetat, used for the x-ray crystallography, was also tested. Most of the derivatives did not show any large change in activity except for the 2-bromo-4,5,6-triacetyl pentenomycin I and 4,6-dibenzoyl pentenomycin I (see *Figure 1.3.*). Both of them showed an increased antibacterial effect. Pentenomycin I has also been tested on mice. A dose of 50 mg/kg gives the mice an increased protection against *S.aureus* and a dose of 400 mg/kg was not lethal over 24 days.

Figure 1.3. The left molecule is 2-bromo-4,5,6-triacetyl pentenomycin I and the right one is 4,6-dibenzoyl pentenomycin I.

1.2. QSAR-quantitative structure activity relationship and SAR-structure activity relationship

SAR is the name for the methodical investigation of what part of the molecule that is more or less important for the activity of a molecule. SAR is based on the testing of derivatives made from the molecule investigated (same test method as for the original compound). The principle when making derivatives is to try to block different parts of the molecule that could be responsible for the activity leaving the other ones free. Much of the focus when making these derivatives is on so called functional groups (carbonyls, hydroxyl groups, double bonds etc.). These groups can often be blocked quite easily. If the activity decreases much for such a derivative it is much possible that the functional group is important for the activity of the molecule.

QSAR-determinations are more advanced than SAR-determinations. In QSAR is the goal to be able to predict the quantitative behavior of new derivatives. QSAR can be divided into a number of subproblems: 1. Description of the chemical structure, 2. Design of the test series, 3. Testing the biological activity of the compounds in the test serie, 4. Mathematical modeling [10]. The mathematical model developed can then be used to simulate the behavior of new compounds without having to synthesize and test them. The simulation can be an indicator of which novel compounds that should be synthesized and tested and which ones that are not interesting. The model can also be used to compare to

hypothetical derivatives. The model is based on parameters, which are thought to describe the behavior of the molecule.

1.3. Biological testing for antibacterial activity

There are lots of test methods or assays to measure different sorts of biological activity, e.g. antibacterial, antifungal, microtubuli inhibition etc. This section will only cover how to determine the antibacterial activity of a compound.

The two most common and used methods are "Dilution susceptibility test" and "Disk diffusion test" [11]. The two parameters usually used when describing antibacterial activity are MIC (minimal inhibitory concentration) and MLC (minimal lethal concentration). The "Dilution susceptibility test" can be used to determine both of these parameters while "Disk diffusion test" only can be used to determine MIC and needs standardization.

In the "Dilution susceptibility test" a series of tubes or plates containing various concentrations (usually 0,1-128 μm/ml) of the compound to be tested for antibacterial effect are prepared. A well-defined clone of bacteria is then ymped on to the plates or tubes. The plate or tube with the smallest concentration where the bacteria does not grow is equal to the MIC. The MLC is determined by taking bacteria from the different tubes or plates and reincubate them in fresh media. If no colonies show up, the concentration from which the bacteria where taken was lethal to the bacteria and the MLC can be determined.

The "Disk diffusion test" is based on the placement of small paper discs, containing the compound to be tested, on plates. The compound will diffuse from the disc and result in a concentration gradient from the disc. The growing of bacteria on such a plate will result in a "ring" around the disc without any bacteria. The size of the ring will depend on how sensitive the bacteria are to the tested compound and will be proportional to the MIC. Standardization can then by used to translate ring diameters to MIC values, but often the

ring diameter is used as test parameter and there is no need for standardization. By this method many compounds can be tested at the same time by placing more than one disc at the same plate.

1.4. Structure determinations

1.4.1. The principle

The determination of the structure of a previously unknown molecule is a bit like puzzling. One can get a lot of clues from different spectropic methods but these have to be combined to a realistic proposal (see *Figure 1.4.*). Mass spectroscopy and NMR(nuclear magnetic resonance) -spectroscopy are today the two most important methods for organic chemists when determining structures but IR(infra red)-spectroscopy is also important because it gives clues about which functional groups that are present in the molecule.

Structure determination is much about being methodical, precise and to be able to overview and combine all the different type of information gained from the spectroscopic measurements. It is also important not to close any doors when there are many structural possibilities.

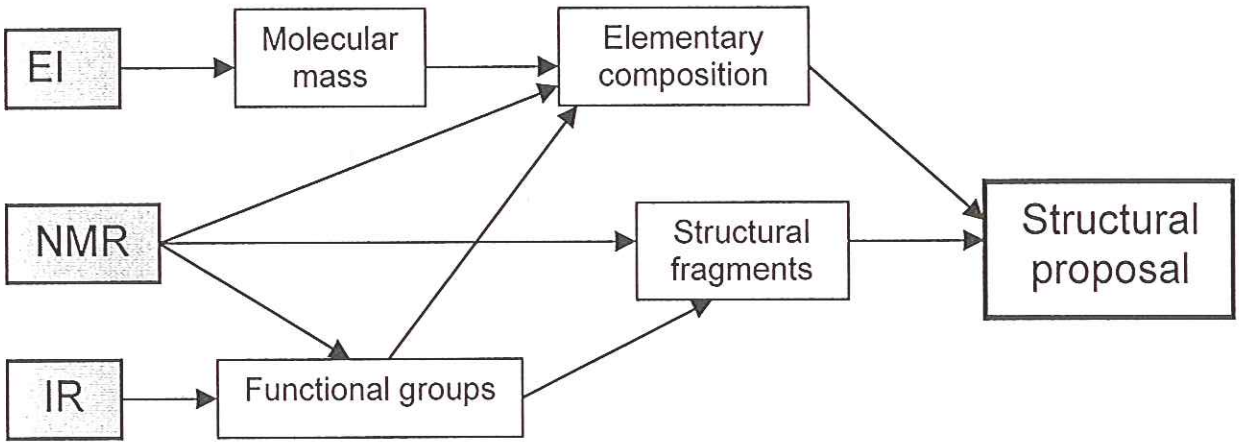

Figure 1.4. Flowchart over the structure determination process. The gray boxes to the left represent the spectroscopic methods used. The boxes in the middle represents information gained from the spectroscopic methods used to propose a structure (the box to the right).

The first thing done with an unknown compound is to determine its molecular mass. This is done by mass spectroscopy (see "Mass spectroscopy"). When the molecular mass is known, an adequate guess (typical the number of carbon atoms is said to be the molecular mass divided by 13, and the number of hydrogen atoms two times the number of carbons) about the molecular formula is made and that guess is then bettered with help of the information which be can gained about functional groups and the number of different kind of atoms from IR- and NMR-spectroscopy (see "IR-spectroscopy" and "NMR"). With a realistic molecular formula at hand the index of hydrogen deficiency [12] can be calculated. This is an index that tells the number of eventual double bounds or rings in the molecule. With all this information at hand it is time to start puzzling. Hopefully it is possible to connect a smaller or larger group of carbon atoms and hydrogen atoms from the 2d-NMR (see "NMR") results. Hopefully there are then only a small number of ways to connect the carbon fragments, but none of them should be ignored. Often the scientist's sense for chemistry and the NOESY correlations are enough to determine which proposal that is correct. If there is not evidence enough to be sure that the proposed structure is correct there is always the possibility to do reactions with the molecule where the outcome of the reactions can be predict from the proposed structure. The structure can also be verified or falsified through an unambiguous total synthesis.

1.4.2. Mass spectroscopy

Simply a mass spectrometer can be divided into three parts. The ionizater which ionize the sample, the separator which separates the ionized molecules according to the relation between their mass and charge and the detector which detects the ions from the separator.

For small molecules (less than 1000 Da) ionization is often done by electron bombardment. The bombarding electron rips off another electron when it collides with the molecule and leaves a charged radical. This method is called electron impact. There are also other methods to generate ions. The ions formed through the bombardment are then typical accelerated by an electric field. Molecules with different charge to mass ratio gain different velocity when leaving the electric field according to classical mechanics. There is then a row of methods to determine the mass of the ions. One of the more simple methods simply measures the time it takes for accelerated ions to "fly" a defined length. The time is proportional to the mass to charge ratio and the mass can then be calculated. The detector is typical an electron multiplier which generates a current when an ion hits it.

Mass spectrometry can also be used to gain information of the structure besides mass. This can be done in two ways. Both of the methods are based on the fact that molecules often fall into parts (fragments) when they are ionized. This happens because they sometimes gain enough energy from the impact with the electrons to break bonds in the molecule. The fragmentation is not randomized and it can be predicted and is the same if the experiment is redone. In a spectrum of a molecule, which has fallen apart, there are a lot of peaks. One represents the mass of the whole molecule and the others different fragments. There are databases where this type is of spectra are gathered and these databases can be screened for a match (this is the way urine samples from athletes are analyzed for contents of illegal substances). The fragmentation pattern also gives clues about fragments of the structure, which can be used for the structure determination.

1.4.3. NMR-spectroscopy

NMR-spectroscopy is probably the most used tool for structure determination of organic molecules. Despite that only a few chemists fully understand the math and physics behind NMR. To try to fully explain the theoretics behind NMR is out of scope of this text. The focus will be on how to make conclusions from the spectra.

NMR is based on a property called spin, which is exhibited by electrons and some nucleus (ex. ^{1}H, ^{13}C, ^{19}F and ^{31}P). Spin can be up or down and in the absence of a magnetic field there is no difference in their energy. In a magnetic field however up and down spin show a difference in energy, which is proportional to the strength of the magnetic field (see *Figure 1.5.*)

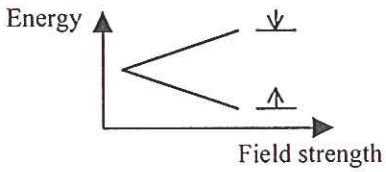

Figure 1.5. The difference in energy between down and up spin in the presence of a magnetic field.

The NMR-spectrometer measures at which energy (wavelength, which in this case is in the radio frequency area) excitation from the lower spin level to the higher spin level takes place. Because this is dependent on the magnetic field and different spectrometers work with different field strengths, the absorbency is reported as absorbed frequency divided with applied frequency. This value is named shift and reported in ppm. By doing this the same compound shows the same shift when measured on different spectrometers. The difference between up and down spin for a specific nucleus does not only depend on the magnetic field but also on the nucleus environment in the molecule. This means that for example hydrogen atoms, which are not equivalent will show different shifts and the value will tell something about the chemical surroundings of that hydrogen atom. The electrons in the bonds between the atoms will have influence on the magnetic field that the atom nucleus senses and this will change the energy required for spin exitation and to

change the spin level. This means for example that the energy needed to change the spin for a ^{13}C-nucleus is less for a carbon in a carbonyl group than one in a carbon chain (note that the energy is inversely proportional to the shift, i.e. a lower energy correspond to a higher shift). This is because of the electronegativity of oxygen, which is responsible for the polarization of the bond and the localization of the electrons closer to the oxygen. Because of this it is possible from the shift to tell much about the chemical surroundings of different atom nucleus. In *Table 1.1.* the shifts of some common groups are listed.

Table 1.1. Typical ppm interval for some common groups [13]

^{1}H-nucleus	Shift (ppm)	^{13}C-nucleus	Shift
Alkyl	0.8-1.7	Alkyl	0-50
α to Ketone	2.1-2.6	Alcohol or ether	50-90
Ether	3.3-3.9	Alkene	100-170
H on same C as a OH	3.3-4.0	Aromatic	100-170
Vinylic	4.6-5.7	Carbonyl	160-215
Aromatic	6.0-9.5		
Aldehyde	9.5-9.6		
Carboxylic	10.0-13.0		

The ppm-scale used in the spectra is quite odd. It is decreasing from left to right, but as mentioned above, this means that the energy increases which is the explanation for the odd scale. Because the energy or field strength in the left part is lower than in the right part, the left in the spectra is said to be downfield and the right to be upfield.

Another phenomenon that is very useful in ^{1}H-NMR is the so-called splitting. This arises because atom nucleuses that are close to each other affect each other so that splitting of peaks in the spectra occurs. This is because the neighbor atom nucleus spin can be either up or down and this affects the field sensed by the nucleus. This phenomenon arises for hydrogen atoms, which are no more than three sigma bonds away. A hydrogen which is neighbored by one hydrogen get split into two peaks (the neighbor is either up or down), a hydrogen which is neighbored by two hydrogen get split into three peaks (both up, one down one up or both down) (also see *Figure 1.6.*). The distance between the split peaks is measured in hertz and is called coupling constant. The coupling constant is the same for neigbouring hydrogen atoms affecting each other. This means that it can be used to tell which hydrogen atoms that are close to each other in the molecule.

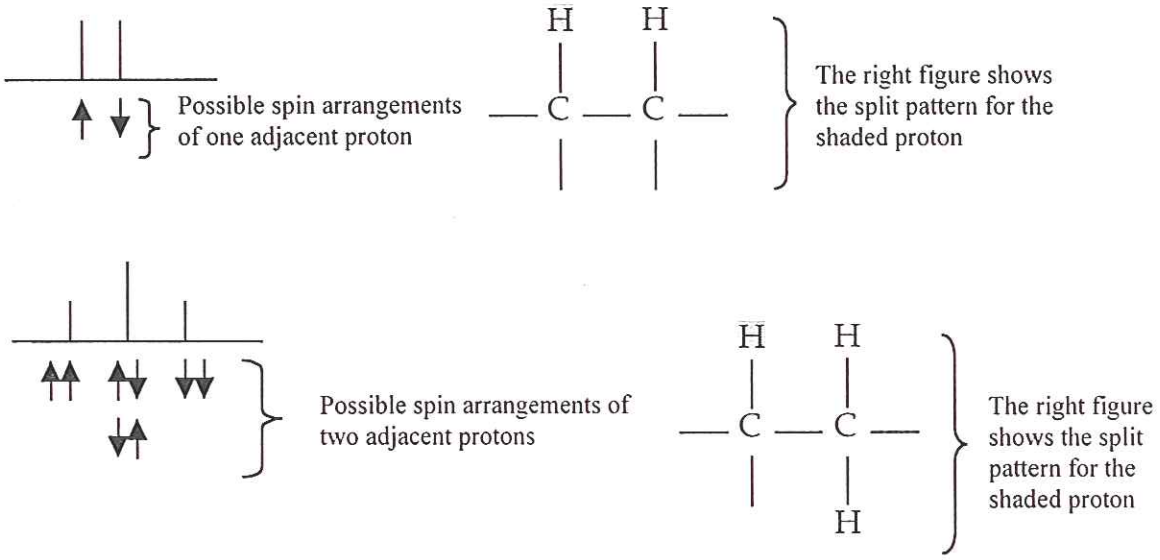

Figure 1.6. The principle behind splitting.

In modern FT-NMR-spectrometers the sample is pulsed so that all nucleus are exitated. Instead of exitating at one frequency at a time the instrument measures the relaxation pattern, which is called a FID (free-induction decay). The FID, which is the time domain, can then be Fourier transformed to get the frequency domain. This method is very fast and it only takes a couple of seconds to collect a FID. Normally many (16-64 for ^{1}H and 500-5000 for ^{13}C) FID: s are taken up and then superimposed on each other before the Fourier transformation. This is done to better the signal to noise ratio. The FT-NMR-spectrometers have other big advantages over the older CW-spectrometers (continuos wave). On FT-NMR-spectrometers it is possible to take up ^{13}C-spectra and 2-dimensionell spectra, which show different relationships between hydrogen and carbon in the molecule. ^{13}C-spectra demands much more sensitive instruments then ^{1}H-spectra, this because of the low abundance (ca. 1%) of ^{13}C. The theories behind 2d-NMR are very complicated. But in short it is based on two different pulses that exitate the sample. The time between the pulses and other parameters are very well defined.

All types of 2d-NMR spectra show different types of correlations between hydrogen and hydrogen or hydrogen and carbon. There are different types of 2d-spectra and some of

the most common are listed and explained below. Most 2d spectra have in common that 1d-^{1}H and/or 1d-^{13}C spectra are on the x- and y-axis. Correlations between different nucleuses are shown as height curves similar to the ones on a map. In COSY and NOESY spectra correlations show up on the diagonal which are of no interest because they show the way nucleus correlates with it self.

COSY (H-H COrrelation SpectroscopY): In a COSY spectrum the 1d-^{1}H-spectrum is on both axis. The spectrum shows the same things that can be told from the coupling constants namely which protons that are close to each other, no more than three σ-bonds away, and interact. This is true but if there are π-bonds between the protons they can interact even when separated with more than three bonds (ex three σ-bonds and a π-bond). With double bonds or aromatic structures in the molecule this very important to be aware of. It is often easier to tell from the COSY spectrum than from the coupling constant which protons that are neighboring and couple.

NOESY (H-H Nuclear Overhauser Effect SpectroscopY): In a NOESY spectrum the 1d-^{1}H-spectrum is on both axis. The correlations show which protons that are close (2-4 angstrom) to each other in space but can be far away from each other in the molecule. These correlations are often used to determine stereo chemical relationships in the molecule, *cis* or trans in a double bond for example. The method is based and named after the nuclear Overhauser effect.

HMQC (Heteronuclear Multiple Quantum Coherence or ^{1}H-^{13}C COSY): In a HMQC spectrum the 1d-^{1}H-spectrum is on one axis and the 1d-^{13}C-spectrum on the other. The correlations shows which proton is situated on which carbon. This is very useful information in structure determinations but it gives no information about the carbons that do not bear any protons, like ketone carbons (of course you can say that you gain the information that the ketone carbon does not bear any hydrogen atoms).

HMBC (Heteronuclear Multiple Bond Connectivity or long-range ^{1}H-^{13}C COSY): In a HMQC spectrum the 1d-^{1}H-spectrum is on one axis and the 1d-^{13}C-spectrum on the

other. The spectrum shows correlations between protons and carbons which are two and three bonds away from each other. This is very useful in structure determinations especially for carbonyl carbons, which are very hard to place correctly in the molecules based on other kinds of spectra. The drawback of the method is that it is possible to "see" both two and three bonds away and is hard to tell which is which, but often the COSY correlations can be used to overcome that. This property of HMBC can also be used in a positive way because it makes it possible to "see" from one side of a ketone to the other.

1.4.4. IR-spectroscopy

The bonds in a molecule are always bending and stretching. This motion, like many other energy depending properties of the molecule, is quantized. The energy needed to change the state of bending or stretching is equal to the energy of photons in the infrared area. Molecules absorb infra red light if the bending and stretching makes the dipole moment of the bond change. The absorption takes place at well-defined wavelengths because the levels are quantized. These wavelengths or more commonly used wave numbers (wave number is the inverse wavelength in cm and proportional to the energy of the photon) correspond to different types of functional groups (se *Table 1.2.*)

Table 1.2. The table shows the characteristic wave number (cm^{-1}) for different types of interesting chemical bonds [14].

Functional group	Wave number (cm^{-1})	Intensity[a]
O-H, N-H	3650-3200	m
C-H	3000-2850	s
C=O	1850-1630	s
C=C	1680-1620	m-w

[a] *s=sharp, m=medium and w=weak*

From IR-spectra it is almost always possible to see if there are any carbonyl, hydroxyl or amine groups present in the molecule. This is because they generate very sharp peaks at wavelengths that do not interfere with other groups. IR-spectra can also be seen as a fingerprint of the molecule because it is very rare that two different molecules have the same IR-spectra. In structure determination, IR is mostly used to see what functional groups that are present in the molecule and to give support to structural proposals.

1.4.5. Determination of absolute structures

The NMR-spectra of two enantiomers are identical and it is impossible to distinguish them by normal NMR measurements. The only way to directly determine the absolute configuration of previously unknown molecule is by an X-ray crystallography or to use the exiton chirality method [15]. X-ray crystallography requires quite big crystals of the compound to be determined and that some sort of heavy atom (typical bromine) is present in the molecule. Often it is hard both to incorporate bromine and to generate crystals good enough. The exiton chirality method demands that there is a chromophore in the molecule, which can be hard to incorporate if not naturally occurring in the molecule. Because of this scientists have developed empirical methods to determine the absolute configuration of organic molecules. Mosher [16] introduced one of the most famous methods in the early seventies. Kahisawa, H et al [17], introduced a modified and more accurate version of Mosher´s method. This method relies on advanced FT-NMR-spectrometers and uses more than one proton signal from the original molecule.

The Mosher methods are used to determine the absolute configuration of a carbon that bears a secondary hydroxyl group. The molecule is reacted with both the R- and S-form of 2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid chloride. This generates a diasteromeric couple of esters, which will show different ^{1}H-shifts. According to the original method it is only the shift of the fluorine atoms or the protons next to the secondary hydroxyl carbon on the smaller side (less hindered) of the molecule that are used to determine the configuration. In the method described by Kahisawa all protons in the alcohol are used. Both methods compares the sign generated when δ_R (shift in hertz of the ester from the *R* acid) from the different protons is subtracted from δ_S (shift in hertz of the ester from the *S* acid). The sign/signs then determine if the carbon bearing the secondary hydroxyl group is S or R. According to Kahisawa the configuration determined from the fluorine nucleus shift is totally unreliable and the result gets more reliable if all the protons in the molecule are used to predict the absolute configuration. If many stereo centers are present in the molecule is it often enough to determine the

absolute configuration for one of them, this because the relative configuration between this stereo center and the others often can be predicted from the NOESY spectrum.

Absolute configuration seems at first glance not to be a very important property to know, this because it has no influence for the physical behavior of the molecule under most circumstances. But this is not true. It has no influence when the molecule interacts with achirale molecules or an ordinary environment but plays a very important role when the molecule interacts with other chiral molecules. This is often the case in biological systems and a horrible example is thalidomide, which is the active substance in Neurosedyne. Neurosedyne was used in Europe and USA during the sixties to treat morning illness in pregnant women. After a couple of years was the use coupled to birth defects. Thalidomide was used in a racemic form and it was later shown that one of the enantiomers was responsible for the birth defects and the other one was effective against morning illness. This shows in a dramatic way the importance of knowing the absolute configuration of a molecule. Another common example is limonene. One of its enantiomers is responsible for the odor of oranges the other for the odor of lemons.

2. Results and discussion

2.1. Structure determination

The structure determinations were done in the way described under "Structure determinations". Professor O.Sterner (Organic Chemistry 2, Lund University, Sweden) gave a proposed structure, which was based on data from NMR, MS and IR. From IR was it quite obvious that there was a carbonyl carbon in the molecule. The conclusion that there were one or many hydroxyl groups present in the molecule could also be drawn from IR. The ^{1}H- and ^{13}C- NMR spectrum indicated the presence of a double bond and at least two carbons connected the oxygen with a single bond (alcohols, esters, ethers etc.). The large peaks in the 0.8-2 ppm interval indicated the presence of an unsubstituted carbon chain. The ^{13}C-NMR confirmed the conclusions and changed the number of carbons connected to oxygen from two to three. From the molecular weight (228 Da) determined from MS and the conclusions above, the molecular formula should be $C_{12}H_{20}O_4$ and the structure was proposed to be **1**.

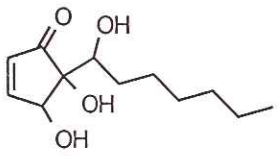

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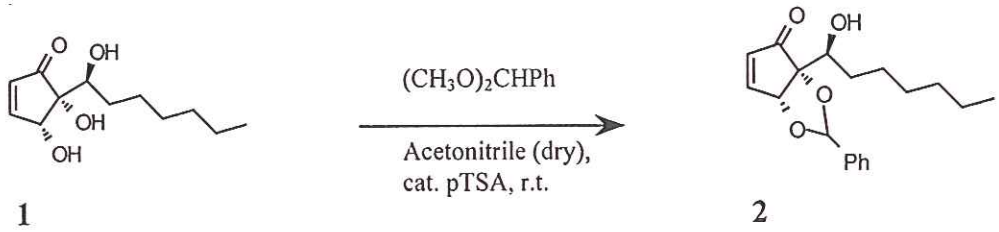
Figure 2.1. The proposed structure of FR96001M

2.1.1. The relative structure

If the proposed structure was right FR96001M should quite easy give some form of acetal with 2,2-dimethoxypropane or dimethoxytoulene. Both reactions were tried and both were successful, but they did not give the same product. The reaction with

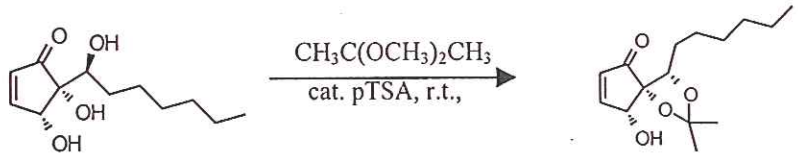
dimethoxytoulene gave acetal **2** connecting the hydroxyl groups in the ring (see *Figure 2.2.*).



1 **2**

Figure 2.2. Acetalization with dimethoxytoulene.

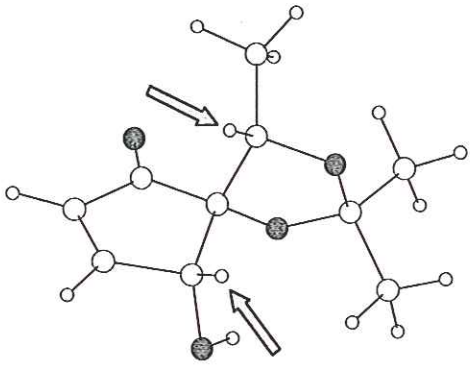
This confirmed that those groups were *cis* to each other because the trans form will not form this type of acetal. This relationship was indicated from the NOESY spectrum of FR96001M but it was not clear. The introduction of a bensyliden group in the molecule introduces a new stereo center and both of the possible diastereomers, **2a** and **2b**, were formed (see "Acetal selectivity and epimerisation") and isolated. The reaction with dimethoxypropane generated acetal **3** connecting the tertiary hydroxyl group and the secondary hydroxyl group in the carbon tail (see *Figure 2.3.*).



1 **3**

Figure 2.3. Acetalization with 2.2-dimethoxypropane

In this molecule the stereo center in the carbon tail is locked to the ring. The NOESY spectrum of the isopropyliden derivative did show an important correlation between two hydrogen atoms (see *Figure 2.4.*). Based on this correlation and the *cis* configuration of the hydroxyl group in the ring could the relationship between the three stereo centers be determined. The outcome of both reactions and the NMR spectra of the products supported the proposed structure. The structure of the earlier mentioned pentenomycin I is very similar to the one of FR96001M (see *Figure 1.2.*). The ^{1}H-NMR spectrum of pentenomycin I was similar to the one of FR96001M.



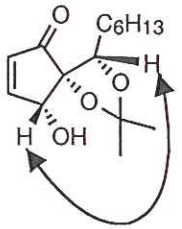


Figure 2.4. Two different representations of the NOESY correlation mentioned in the text above. The arrow/s indicates the protons that correlate.

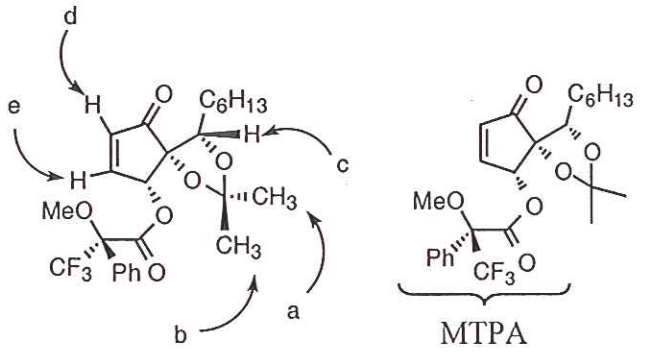
2.1.2. The absolute structure

The small amount of material available made it impossible to make any crystals good enough for X-ray crystallography instead Moshers´s method was tried. Both the *(R)*- and the *(S)*-MTPA esters, **4s** and **4r**, were made from the isopropylidene acetal **3** and the acid chloride. NMR spectra were then collected (^{1}H-NMR and COSY) on a 500 MHz spectrometer. The shifts that differed and were used for the determination are listed in the table below.

Table 2.1. The shift that differed for the two diastereomers and the difference between them

Hydrogen[1]	δ_R	δ_S	δ_S-δ_R (Hz)
a	1.55	1.55	-1.94
b	1.64	1.59	-24.27
c	4.50	4.38	-57.92
d	6.44	6.47	15.80
e	7.38	7.50	63.00

1. See *Figure 2.5.* for an explanation to which proton is which.



4r **4s**

*Picture 2.5. The structure of (R)-3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic acid (4S,5R,6R)-4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4r**) and (S)-3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic acid (4S,5R,6R)-4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4s**). The arrows show the position of the hydrogen used in Table 2.1.*

According to the High-Field FT NMR application of Mosher's method described by Ohtani et al [19] the negative differences should be placed to the left and the positive to the right of a plane defined by the ester group and the proton next to it (see *Figure 2.6.*).

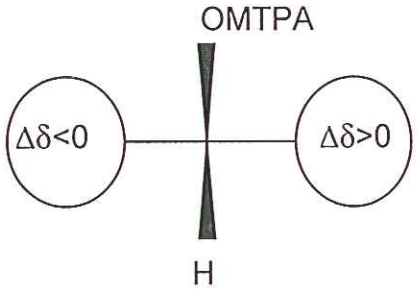
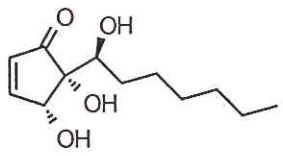


Figure 2.6. Definition of left and right for the Mosher's method

In this case this is easily done and there is no hydrogen atom with a positive sign that has to be placed on the negative side and vice versa. In *Table 2.1.* it is also seen that the size of the difference (Δδ) depends of the distance to the ester group. The difference between the *S* and the *R* forms for hydrogen d is less than for e, which is closer the ester group than d. The same thing is seen for a and b which are on different sides of the plane

defined by the acetal ring. According to this method the carbon in the ring with the secondary hydroxyl group has the *R*-configuration. Because of the *cis* relationship between the two hydroxyl groups in the ring the stereo configuration of the other stereo center in the ring is designated to *R*. The stereo configuration of the stereo center in the carbon tail was earlier correlated to the other two and could now be determined to be *S*. The absolute configuration determined for FR96001M is shown below.



1

Figure 2.7. The structure of (4R,5R)-4,5-Dihydroxy-5-((S)-1-hydroxy-heptyl)-cyclopent-2-enone shown.

This type of determination is totally empirical and there are deviations from the method reported in the literature by, for example, Gustavsson et al [20]. The difference of the molecule investigated by Gustavsson (see *Figure 2.8.*) and the one in this report is the size of the ring, which the secondary hydroxyl group is connected to.

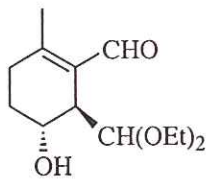


Figure 2.8. The molecule, 5-Hydroxy-2-metyl-6-(diethoxymetyl)-1-cyclohexencarbaldehyde, studied by Gustavson et al.

A cyclopentene is much more planery and less flexible than a cyclohexene. This makes it more accurate to speak in terms of a left and a right side in the case with the cyclopentene. Another problem is also seen in Gustavssons article. One of hydrogen atoms with a negative sign has to be placed on the positive side, which makes that determination more dubious.

It is interesting to compare the structure of FR96001M with the structure of pentenomycin I. This is interesting because they turn plane-polarized light in the same direction and are both biological active and produced by fungi, but seem to have opposite absolute configuration.

2.1.3 Acetal selectivity and epimerisation

Most of the articles regarding acetal selectivity are concerned with carbohydrates and 1,2,4 triols and not with 1,2,3 triols. This makes it hard to find an explanation for the observed selectivity in the literature. The selectivity seen with dimethoxytoulene is the expected. The two hydroxyl groups in the ring are already locked in the confirmation they will have in the acetal and this should promote the reaction. The two diasteromers formed with dimethoxytoulene are not formed in equal amounts. The isomer with the benzene ring close to the five ring, the benzene ring pointing down (**2b**), is formed only in small amounts while the other form **2a** is dominating. The amount of **2b** seems to depend of the amount of reagent used. With an excess of dimethoxytoulene more of **2b** is produced than when a stoichiometric amount is used.

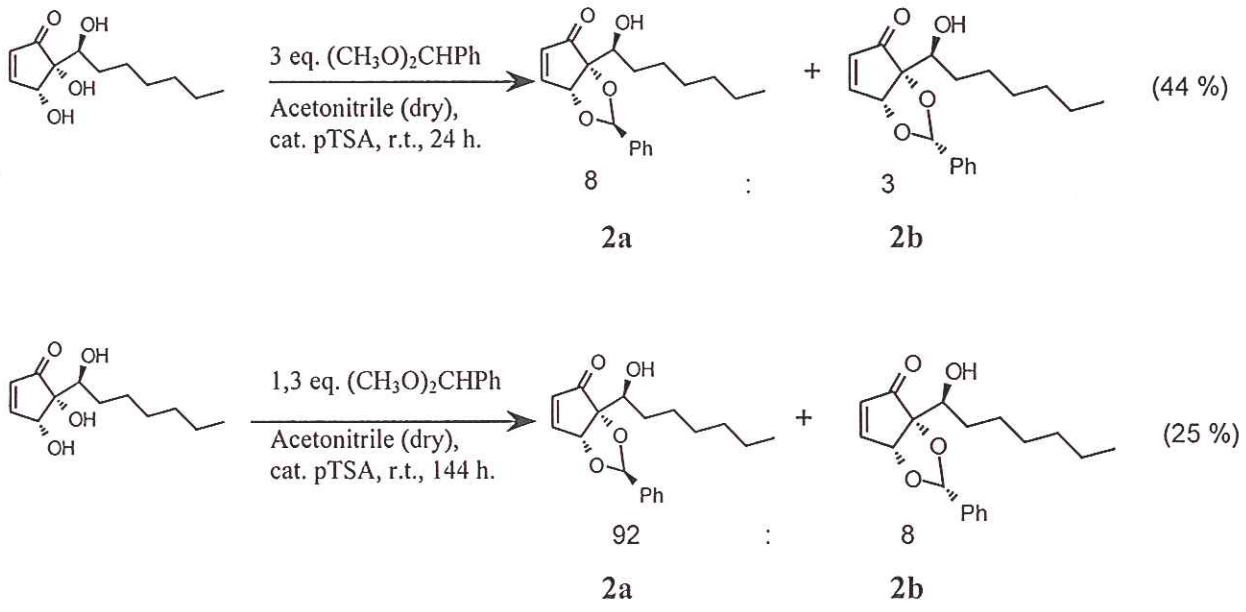
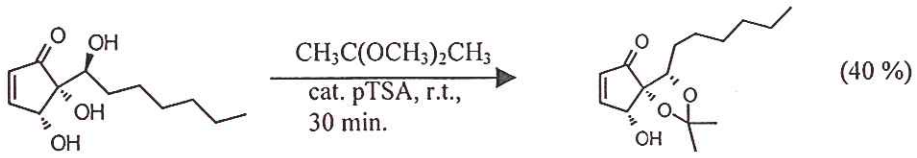


Figure 2.9. The reaction conditions used to form the bensylidene acetal.

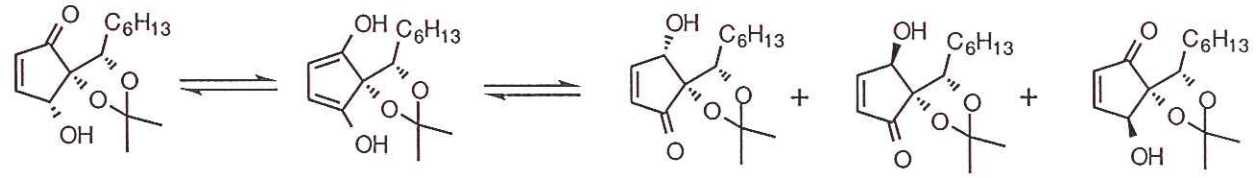
The outcome of the reaction with dimethoxypropane (see *Figure 2.10.*) was more unexpected but positive when it came to the structure determinations. Differences between the two reactions are the amount of reagent used and the reaction time. Excess of dimethoxypropane is used while stoichiometric amount of dimethoxytoulene is used (stoichiometric amount was never tried). The reaction time for the dimethoxypropane reaction is much shorter. Perhaps the different outcomes can be explained in terms of kinetic and thermodynamic products. The slower produced acetal with dimethoxytoulene should then be the thermodynamic acetal and the very fast-formed acetal with dimethoxypropane should then be the kinetic acetal. There is also the possibility of forming the 1,3-acetal. But when building this molecule with molecular models it is quite obvious that the molecule generated is very strained and unlikely to be formed.



3

Figure 2.10. The reaction conditions used to produce the isopropylidene acetal. Notice the difference in reaction time compared with the ones in Figure 2.6.

When **3** was produced an unstable byproduct (**3a**) was also isolated. This product seemed to be the epimer and **3a** spontaneously reformed (after less than an hour in solution could both compound be recognized in a NMR spectrum) to **3** when allowed to stand in solution. This conclusion is based on the different shift (0.1-0.2 ppm) seen for the hydrogen atoms on the same carbon as the secondary hydroxyl groups and the hydrogen in the double bond next to the hydroxyl group. The epimerization could be explained due to an enol like intermediate (see *Figure 2.11.*).



3 **Enol** **3a** **3b** **3c**

Figure 2.11. The epimerization process.

As shown there are three possible products **3a-c**, other than **3**, from the enol. In two of them, **3b** and **3c**, the favorable hydrogen bonding between the hydroxyl proton and the oxygen in the acetal is not possible. In the third, **3a**, this hydrogen bonding is possible. This hydrogen bonding does probably favor this product over **3b** and **3c**. This product **3a** is probably unstable compared to **3** because of steric interactions between the carbon tail and the proton on the carbon in the cyclopentenone ring with the secondary hydroxyl group.

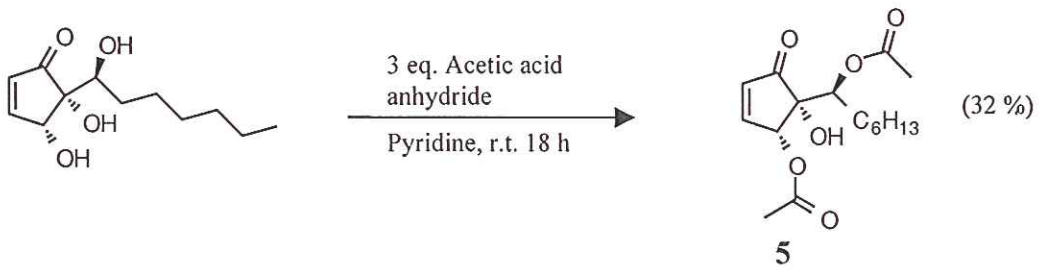
2.2. SAR-Structure activity relationship

2.2.1. Derivatives made for testing

There are five functional groups in the molecule: 3 hydroxyl groups, a double bond and a carbonyl group. The carbonyl is also conjugated with the double bond. There are also two important structural elements: a carbon tail and a five membered ring.

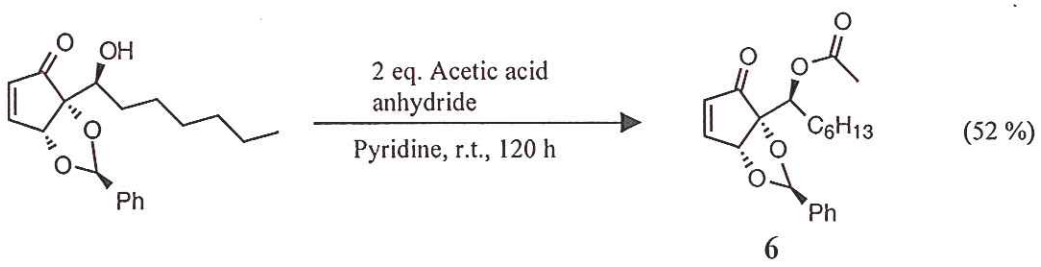
The hydroxyl groups can be blocked or modified in row of combinations. Derivatives where all hydroxyl groups were blocked and where two out of three of the hydroxyl groups, in all possible combinations, were blocked were sent to testing. To just block one of three hydroxyl groups was harder than expected and no derivatives of this type were synthesized in amounts big enough for testing.

The acetals formed for the structure determination were sent to testing. The third hydroxyl group in **2b** was blocked by esterfication with acetic acid anhydride, which gave **5** which also was sent for testing. The two secondary hydroxyl groups of **1** were transformed to esters with acetic acid anhydride leaving the tertiary hydroxyl group free. This diester **6** was also sent to testing.



5

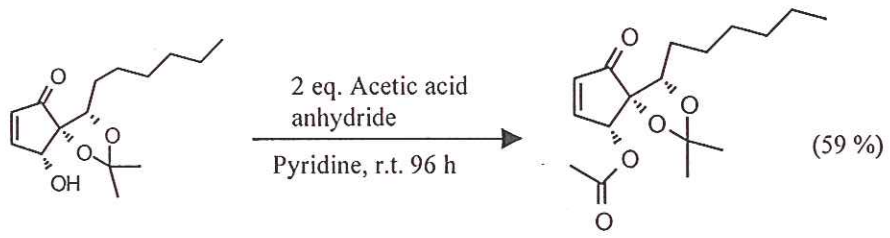
Figure 2.12. The conditions used to produce Acetic acid (S)-1-((2R,3aS,6aR)-4-oxo-2-phenyl-4,6a-dihydro-cyclopenta[1,3]dioxol-3a-yl)-heptyl ester (5).



6

Figure 2.13. The conditions used to produce Acetic acid (S)-1-((1R,2R)-2-acetoxy-1-hydroxy-5-oxo-cyclopent-3-enyl)-heptyl ester (6).

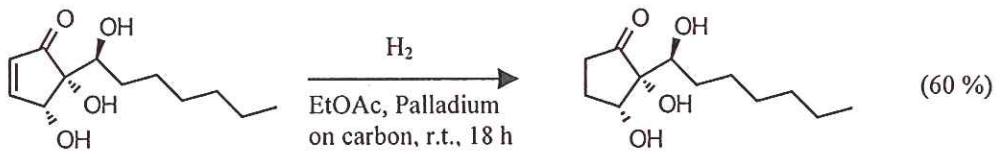
The free hydroxyl group in **3** was also blocked as an ester **7**. Attempts were then made to deprotect the acetal with acid (both 80% acetic acid and 1 M HCl in THF). Nothing happened at all with acetic acid and HCl seemed to deprotect the ester, instead of removing acetal, giving **3** back. Acetal deprotection of **4** was also tried with acetic acid but did not work. Both acetals seem to be quite stable under acidic conditions and the chance that they should be deprotected during the testing should be minimal.



3 7

Figure 2.14. The structure of Acetic acid (4S,5R,6R)-4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (7).

There is a row of methods to effect the double bond. The double bond was hydrogenated with palladium on carbon as catalyst and the product, **8,** was sent to testing.



8

Figure 2.15. (2R,3R)-2,3-Dihydroxy-2-((S)-1-hydroxy-heptyl)-cyclopentanone 8

The carbon tail is hard to modify when doing semisynthesis. It can neither be shortened or lengthen. To be able to make derivatives, from which the dependence of the length of the carbon tail can be studied, a total synthesis route must be available. This is not the case today but there are possibilities (see "***2.3. A proposed synthetic route to (+/-) FR96001M***").

The dependence of the ring is also hard to investigate. Most of the other functionalities are coupled to the ring and this makes it difficult to alter the ring size without affecting the groups attached to it. But the hydrogenation of the double bond will also affect the configuration of the ring. The ring can be said to be an essential part of the molecule, which cannot be even slightly modified without ending up with a totally different molecule.

Treatment of FR96001M with 100 % formic acid generated at least three different products. Only one of them, **9**, was generated in amounts big enough to be isolated and investigated with NMR. This product, **9**, seemed to be FR96001M esterfied on the secondary hydroxyl group in the carbon tail.

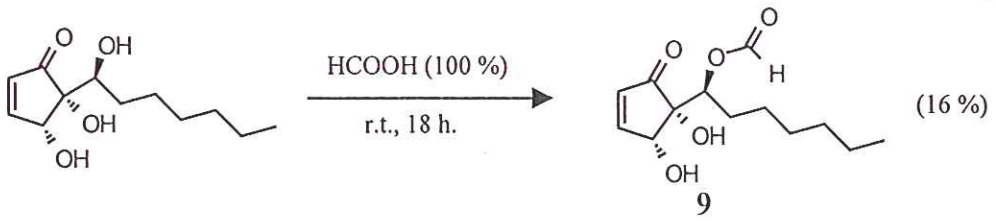


Figure 2.16. The conditions used to produce Formic acid (S)-1-((1S,2R)-1,2-dihydroxy-5-oxo-cyclopentyl)-heptyl ester (9).

2.2.2. Unsuccessful reactions.

Attempts were made to selectively [21] reduce the ketone with a stoichiometric amount of $NaBH_4$ in the presence of $CeCl_3$ in methanol without reducing the double bond. This attempt was not successful and the NMR spectrum of the product showed that the double bond was gone. Attempts were also made to reduce the ketone in **3**. This was also unsuccessful.

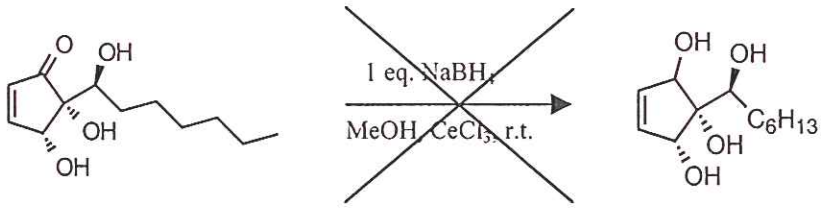


Figure 2.17. The unsuccessful selective reduction.

Date et al reported during their work with pentenomycin I [22] that bromination α to the ketone did increase the antibacterial potency of the compound. Due to this fact the same was tried with FR96001M. FR96001M dissolved in acetic acid was added to a solution of bromine in CCl_4. The reaction did not go clean and at least three products were generated in low yields. Two of them could be isolated with chromatography. The outcome of the reaction could not be explained from just ^{1}H-NMR, but the NMR spectra of these two products did show that the double bond was gone and that they were quite similar. This could be explained if bromine had reacted with the double instead of reacting with the

enol form of the keton and the two products could then be the diastereomers. In the third product the double bond was intact after the reaction but the proton α to keton had altered shift from 6.3 to 5.7 ppm. The sample was unclean and it was hard to do a correct assignment of the NMR-peaks even with a COSY spectrum.

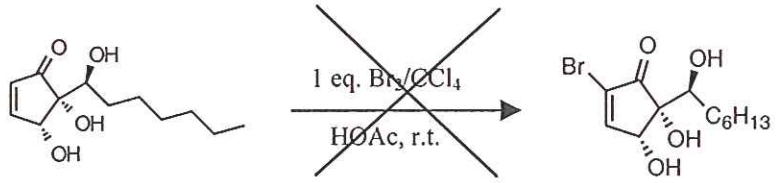


Figure 2.18. The conditions used for the unsuccessful attempt to brominate *α to the ketone.*

Epoxidation of the double bond was also tried, both with m-MCPA and hydrogen peroxide. Neither of the reactions was successful. With m-MCPA nothing happened and the starting material was recovered. With hydrogen peroxide the starting material disappeared but no product could be detected. No traces of any product were found in the water phase either, which could have been the case if the epoxide had been opened generating a water soluble product.

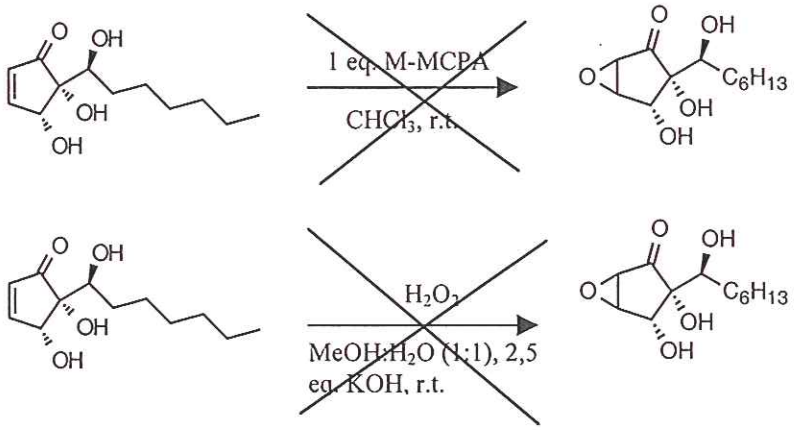


Figure 2.19. The unsuccessfully tried epoxidations.

A Michael addition with $NHCH_3OH$ was tried but this reaction was not successful.

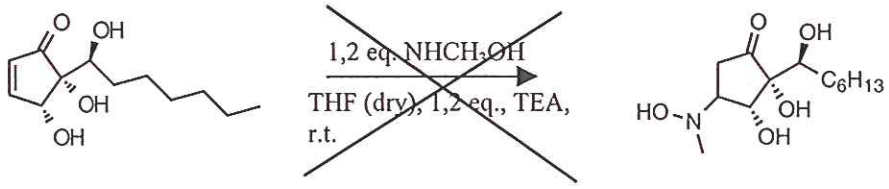


Figure 2.20. The unsuccessful Michael addition.

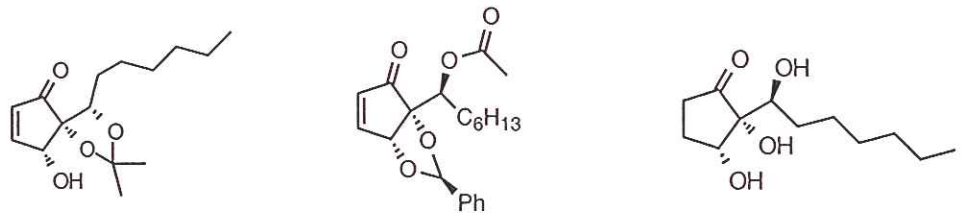
2.2.3. The antibacterial activity of the derivatives

The synthesized derivatives were tested in Kaiserslauten, Germany, by the Prof. Anke group. They were tested on both bacteria and fungi with a disk diffusion test (paper disc method). Tests for cytotoxity were also done on a human cell-line. The results are shown below in *Table 2.2.*

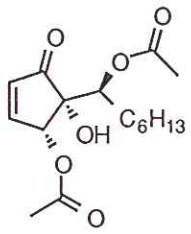
Table 2.2. Antimicrobial activity of derivatives from the paper disc method (20 µg/disc, results in millimeter). The first four organisms are fungi and the last four bacteria (last one is G- and the other G+).

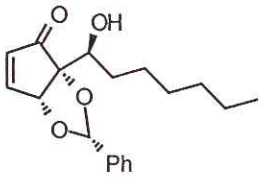
Substance	Pn	Tü	HA3	Pvar	99	63	Sl	Ed
3	11	8d	12	7	17	10	11d	0
6	0	0	10	0	15	14	11d	0
8	0	0	0	0	10	7	0	0
5	7	10	0	7	11	11/15i	10	0
2b	17/23i	21	13	15/28i	25	20/27i	20	0
FR96001M	0	0	8	0	25	11	0	8

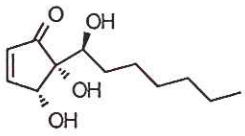
Pn: *Penicillium notatum;* Tü: *Mucor miehei;* HA3: *Nematospora coryli,* Pvar: *Paecilomyces variotii;* 99: *Bacillus brevis;* 63: *B.subtilis;* Sl: *Micrococcus luteus,* Ed: *Enterobacter disolvens*
d:diffuse; i: incomplete



3 **6** **8**







5 **2b** **FR96001M**

A few conclusions can be drawn from these data but they should be verified. The double bond seems to be very important for the activity. The activity is almost lost when the double bond is hydrogenated. The same was seen from the cytotoxity test where all the derivatives except **8** did show similar IC_{50}-value against L1210 cells. Those IC_{50}-value was 5-10 μg/ml but for **8** was it 10-25 μg/ml, i.e. the cytotoxity of **8** was lower. One reason for the lower activity could be that the conformation of the ring changes then the double bond is reduced. The double bond makes the ring take a flat conformation. With the double bond lost the conformation is more envelope like. It is interesting to note that FR96001M is selective against bacteria over fungi. This selectivity is lost for most of the derivatives except **6**. Of the active derivatives **6** is the only one with the tertiary hydroxyl group free. From this one can assume that this hydroxyl group is important for the selectivity. By comparing **5** with **2b** and **3**and **6** with FR96001M it can be assumed that the hydroxyl group in the carbon tail is important for the activity.

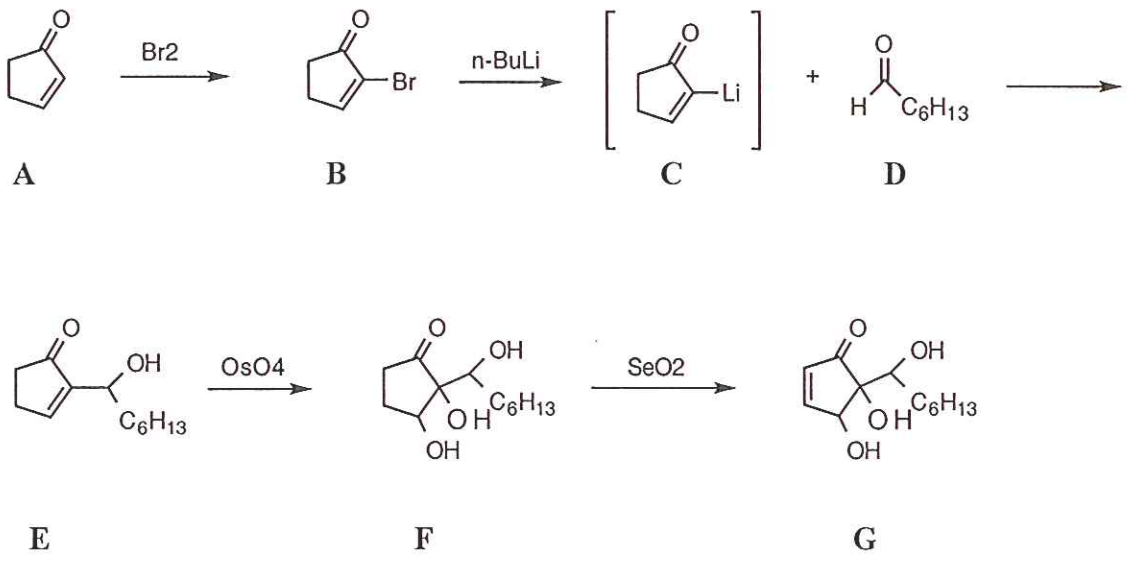
2.3. A proposed synthetic route to (+/-) FR96001M

Several synthetic routes to pentenomycin I have been reported. Most of them are based on carbohydrate [23] and therefore not applicable to FR96001M. This because there is no possibility to affect the length of the carbon tail generated in these syntheses.

One of the reported synthesis [24] toward pentenomycins could probably be modified and used to synthesize FR96001M. This route will however not yield enantiomerically pure product but the racemate. The synthesis is schematically shown in *Scheme 2.1* without showing the necessary protecting groups.

The first step from **A** to **B** to passes over the enol form of **A** which acts as a nucleophile on Br_2. The addition of *n*-BuLi changes Br to Li in a halide metal exchange. The formed lithium compound is very nucleophilic and will attack the aldehyde **D** *in situ* to give alcohol **E**. **E** is then converted to **F** by dihydroxylation with OsO_4, which then is dehydrogenated by SeO_2 to yield **G**.

The synthesis of **E** from **A** in a similar way using hexanal instead of heptanal was reported by Kusuda et al [25]. To control the stereo selectivty in the addition of **C** to **D** seems hard to do. The stereo chemistry between **E** and **F** should probably be possible to control using the hydroxyl group as directing group for the hydroxylation (e.g. Sharpless dihydroxylation)



Scheme 2.1. A proposed synthetic route (without the necessary protecting groups) to FR96001M.

3. Experimental

3.1. General procedures

Unless not mentioned the chemicals used were not pretreated, but of p.a. quality and bought from commercial suppliers. TLC was done on "Merck DC-Alufolien Kiselgel 60 F_{254}" SiO_2 plates. The spots were visualized with UV-light and with anisaldehyde/sulfuric acid/ethanol followed by heating with a heat gun. NMR spectra were recorded with the sample dissolved in $CDCl_3$ on a Bruker ARX-500, Bruker DRX-400 or a Bruker DRX-300. For the ^{1}H-spectra the chloroform signal was set to 7.27 ppm and for the ^{13}C-spectra to 77.0 ppm.

3.2. Reactions of FR96001M *and with derivatives made from* FR96001M

4,5-Dihydroxy-5-(1-hydroxy-heptyl)-cyclopent-2-enone (FR96001M, 1)

1H-NMR (500 MHz):δ 0.85 (t, J=6.91 Hz, 3H), 1.20-1.40 (m, 8H), 1.50-1.60 (m, 2H), 3.74 (d, J=10.1 Hz, 1H), 4.71 (dd, J=2.13, 1.32 Hz, 1H), 6.26 (dd, J=6.07, 1.30 Hz, 1H), 7.61 (dd, J=6.06, 2.34 Hz, 1H); TLC R_f=0.40 (4:1 EtOAc/petroleum ether); $[\alpha]_D^{20^\circ C}$=-3.90° (c=2.41, $CHCl_3$); EIMS *m/z* (% rel int) 228 (11%), 227 (100%), 113 (12%).

3a-(1-Hydroxy-heptyl)-2-phenyl-3a,6a-dihydro-cyclopenta-1,3-dioxol-4-one (2a)

To a solution of FR96001M (19,1 mg) in dry acetonitrile (5 ml) a catalytic amount of pTSA and dimethoxytoulene (15 µl, 1,3 eq.) were added. After stirring for 144 h. under nitrogen the reaction was quenched by addition of saturated $NaHCO_3$-solution (10ml). The water phase was extracted with CH_2Cl_2 (3×15 ml). The combined organic phases were dried with $MgSO_4$, concentrated in vacuo and the residue was purified with

flashchromatography (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 6,5 mg (25 %) of acetal **2a** (major isomer) and only traces of **2b** (minor isomer) (**2a**:**2b** 1:0.08). 1H-NMR (300 MHz):δ 0.89(t, J=6.70 Hz, 3H), 1.25-1.40 (m, 8H), 1.50-1.60 (m, 2H), 1.90 (d, J=5.91 Hz, 1H), 4.21 (dt, J=5.83, 2.06 Hz, 1H), 5.39 (d, J=2.33 Hz, 1H), 5.65 (s, 1H), 6.69 (dd, J=6.10, 0.66 Hz, 1H), 7.40-7.43 (m, 3H), 7.45-7.52 (m, 2H), 7.60 (dd, J=6.10, 2.37 Hz, 1H); TLC R_f=0.47 (1:1 EtOAc/petroleum ether).

3a-(1-Hydroxy-heptyl)-2-phenyl-3a,6a-dihydro-cyclopenta-1,3-dioxol-4-one (**2a-b**)

To a solution of FR96001M (19,7 mg) in dry acetonitrile (5 ml) a catalytic amount of pTSA and dimethoxytoulene (15 µl, 1,2 eq.) were added. After 3,5 resp. 5 h. more dimethoxytoulene (15 µl each time) was added. After stirring for 24 h. under nitrogen the reaction was quenched by addition of saturated $NaHCO_3$-solution (10ml). The water phase was extracted with CH_2Cl_2 (3×15 ml). The combined organic phases were dried with $MgSO_4$, concentrated in vacuo and the residue purified with flashchromatography (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 8,8 mg (32 %) of acetal **2a** (major isomer, impure) and 3,3 mg (12 %) of acetal **2b** (minor isomer, pure).
2a: 1H-NMR (300 MHz):δ 0.89 (t, J=6.70 Hz, 3H), 1.25-1.40 (m, 8H), 1.50-1.60 (m, 2H), 1.90 (d, J=5.91 Hz, 1H), 4.21 (dt, J=5.83, 2.06 Hz, 1H), 5.39 (d, J=2.33 Hz, 1H), 5.65 (s, 1H), 6.69 (dd, J=6.10, 0.66 Hz, 1H), 7.40-7.43 (m, 3H), 7.45-7.52 (m, 2H), 7.60 (dd, J=6.10, 2.37 Hz, 1H); TLC R_f=0.47 (1:1 EtOAc/petroleum ether).
2b: 1H-NMR (300 MHz):δ 0.91 (t, J=6.60 Hz, 3H), 1.25-1.40 (m, 8H), 1.75-1.80 (m, 2H), 1.99 (d, J=5.93 Hz, 1H), 4.21 (dt, J=5.89, 2.23 Hz, 1H), 5.37 (dd, J=2.38, 0.53 Hz, 1H), 5.64 (s, 1H), 6.11 (dd, J=6.01, 0.62 Hz, 1H), 6.35 (s, 1H), 7.31-7.36 (m, 5H), 7.53 (dd, J=6.01, 2.36 Hz, 1H); TLC R_f=0.55 (1:1 EtOAc/petroleum ether); $[\alpha]_D^{20^\circ C}$=+124,7° (c=0.17, $CHCl_3$); EIMS *m/z* (% rel int) 339,1569 (M^+, 100, $C_{19}H_{24}O_4Na$ requires 339,1572), 329 (14%).

4-hexyl-9-hydroxy-2,2dimethyl-1,3-dioxa-spiro[4.4]non-7-en-6-one (**3**)

To a solution of FR96001M (18,3 mg) in 2,2-dimethoxypropane (5 ml) a catalytic amount of pTSA was added. After stirring for 30 min. under nitrogen the reaction was quenched by addition of saturated $NaHCO_3$-solution (10 ml). The water phase was extracted with CH_2Cl_2 (2×10 ml). The combined organic phases were dried with $MgSO_4$, concentrated in vacuo and the residue purified with flashchromatography (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 8,7 mg (40 %) of acetal **3** and traces oh the epimer **3a**.

3:1H-NMR (500 MHz):δ 0.87 (t, *J*=7.02 Hz, 3H), 1.15-1.35 (m, 8H), 1.49 (s, 3H), 1.60 (s, 3H), 1.70-1.80 (m, 2H), 2.93 (d, *J*=7.4 Hz, 1H), 4.23 (dd, *J*=9.13, 4.13 Hz, 1H), 4.47(ddd, *J*=7.37, 1.42, 0.65 Hz, 1H), 6.27(dd, *J*=6.22, 1.31 Hz, 1H), 7.58 (dd, *J*=6.23, 2.28 Hz, 1H); TLC R_f=0,65 (4:1 EtOAc/petroleum ether); $[\alpha]_D^{20^\circ C}$=+16,6° (c=0.47, $CHCl_3$); EIMS *m/z* (% rel int) 268.1674 (M^+, 15, $C_{15}H_{24}O_4$ requires 268.1675), 253 (87%), 211 (76%), 210 (89%), 127 (92%), 86 (100%), 84 (100%).

3a:1H-NMR (400 MHz):δ 0.87 (t, *J*=6.69 Hz, 3H), 1.20-1.35 (m, 8H), 1.45-1.47 (m, 2H), 1.48 (s, 3H), 1.67 (s, 3H), 4.11 (dd, *J*=9.44, 3.46 Hz, 1H), 4.61 (dd, *J*=2.63, 1.06 Hz, 1H), 6.28 (dd, *J*=6.30, 1.20 Hz, 1H), 7.50 (dd, *J*=6.30, 2.59 Hz, 1H); TLC R_f=0,72 (4:1 EtOAc/petroleum ether).

[2R] 3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic-acid 4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4r**)

To a solution of **1** (4,1 mg) in dry CH_2Cl_2 (1 ml) a catalytic amount of DMAP, triethylamine (21 µl) and *R*-MTPA-chloride (8,5 µl) were added in the mentioned order. After stirring for 2 h. under nitrogen the solvent was removed in vacuo. The residue was flashchromatographed (12:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,0 mg (68 %) of ester **6r.** 1H-NMR (500 MHz):δ 0.88 (t, *J*=6.67 Hz, 3H), 1.20-1.32 (m, 8H), 1.50-1.57 (m, 2H), 1.55 (s, 3H), 1.64 (s, 3H), 3.65 (s, 3H), 4.50 (dd, *J*=9.70, 2.96 Hz, 1H), 5.76 (dd, *J*=2.88, 0.92 Hz, 1H), 6.44 (dd, *J*=6.31, 1.00 Hz, 1H), 7.37 (dd,

J=6.35, 2.92 Hz, 1H), 7.38-7.43 (m, 3H), 7.66 (d, *J*=6.52 Hz, 2H); TLC R_f=0,43 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20^\circ C}$=+17,3° (c=0.19, $CHCl_3$).

[2S] 3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic-acid 4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4s**)

To a solution of **1** (4,4 mg) in dry CH_2Cl_2 (1 ml) a catalytic amount of DMAP, triethylamine (23 µl) and *S*-MTPA-chloride (9,2 µl) were added in the mentioned order. After stirring for 2 h. under nitrogen the solvent was removed in vacuo. The residue was flashchromatographed (7:1 Hexane:dietheyleter+1% triethylamine) which gave 2,4 mg (30 %) of ester **6s.** 1H-NMR (500 MHz):δ 0.88 (t, *J*=7.02 Hz, 3H), 1.20-1.32 (m, 8H), 1.50-1.58 (m, 2H), 1.54 (s, 3H), 1.59 (s, 3H), 3.50 (s, 3H), 4.38 (dd, *J*=10.39, 2.82 Hz, 1H), 5.76 (dd, *J*=2.04, 0.96 Hz, 1H), 6.47 (dd, *J*=6.33, 0.96 Hz, 1H), 7.37-7.43 (m, 3H), 7.50 (dd, *J*=6.32, 2.87Hz, 1H), 7.64(d, *J*=7.89 Hz, 2H); TLC R_f=0,40 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20^\circ C}$=+50,0° (c=0.06, $CHCl_3$).

Acetic acid 1-(4-oxo-2-phenyl-4,6a-dihydro-cyclopenta[1.3]dioxol-3a-yl)-heptyl ester (**5**)

To a solution of **2a** (9 mg) in dry pyridine (1 ml) acetic acid anhydride (5 µl, 1 eq.) was added. After 48 h another 5 µl aceticanhydride was added and after totally 120 h of stirring under nitrogen the reaction was quenched by repetitive addition of ethanol and removement of solvent in vacuo. The residue was flashchromatographed (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,3 mg (52 %) of ester **5**. 1H-NMR (300 MHz):δ 0.88 (t, *J*=6.79 Hz, 3H), 1.25-1.35 (m, 8H), 1.67-1.74 (m, 2H), 2.02 (s, 3H), 5.35 (d, *J*=2.23 Hz, 1H), 5.50 (dd, *J*=9.71, 3.04 Hz, 1H), 6.11 (s, 1H), 6.66 (dd, *J*=6.12, 0.66 Hz, 1H), 7.40-7.45 (m, 3H), 7.47-7.50 (m, 2H), 7.56 (dd, *J*=6.10, 2.35 Hz, 1H); TLC R_f=0,52 (1:1 EtOAc/Petr.Eter); EIMS *m/z* (% rel int) 381,1669 (M^+, 100, $C_{21}H_{26}O_5Na$ requires 381,1678), 329 (11%).

Acetic acid 1-(2-acetox-1-hydroxy-5-oxo-cyclopent-3-enyl)-heptyl ester (6)

To a solution of FR96001M (11,6 mg) in dry pyridine (0,5 ml) aceticanhydride (16 µl, 3eq.) was added. After stirring for 18 h under nitrogen the reaction was quenched by repetitive addition of ethanol and removement of solvent in vacuo. The residue was flashchromatographed (2:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,0 mg (32 %) of ester **6**. 1H-NMR (300 MHz):δ 0.87 (t, *J*=6.80, 3H), 1.20-1.35 (m, 8H), 1.65-1.75 (m, 2H), 2.07 (s, 3H), 2.16 (s, 3H), 2.73 (s, 1H), 5.10 (dd, *J*=9.45, 3.81 Hz, 1H), 5.80 (dd, *J*=2.85, 1.14 Hz, 1H), 6.45 (dd, *J*=6.21, 1.14 Hz, 1H), 7.56 (dd, *J*=6.17, 2.84 Hz, 1H); TLC R_f=0,37 (1:1 EtOAc/Petr.Eter); EIMS *m/z* (% rel int) 335,1456 (M^+, 100, $C_{16}H_{24}O_6Na$ requires 335,1471), 325 (10%), 303 (9%), 265 (18%), 245 (30%), 243 (29%).

Acetic acid 4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (7)

To a solution of **3** (13,3 mg) in dry pyridine (1 ml) aceticanhydride (5 µl, 1,1 eq.) was added. After 24 h. was another 5 µl aceticanhydride added and after 96 h. of stirring under nitrogen the reaction was quenched by repetitive addition of ethanol and removement of solvent in vacuo. The residue was flashchromatographed (8:1 petroleum ether/EtOAc+1% triethylamine) which gave 9,0 mg (59 %) of ester **7**. 1H-NMR (500 MHz):δ 0.87 (t, *J*=6.27 Hz, 3H), 1.20-1.35 (m, 8H), 1.48-1.53 (m, 2H), 1.53 (s, 3H), 1.56 (s, 3H), 2.16 (s, 3H), 4.40 (dd, *J*=9.48, 3.23 Hz, 1H), 5.66 (dd, *J*=2.75, 1.34 Hz, 1H), 6.42 (dd, *J*=6.36, 1.36 Hz, 1H), 7.48 (dd, *J*=6.31, 2.77 Hz, 1H); TLC R_f=0,84 (4:1 EtOAc/Petr.Eter); $[\alpha]_D^{20^\circ C}$=-4,30° (c=0.12, $CHCl_3$).

2,3-dihydroxy-2-(1-hydroxy-heptyl)-cyclopentaone (8)

A flask with a solution of FR96001M (10,2 mg) in EtOAc (2 ml) was flushed with nitrogen and a catalytic amount of palladium on carbon (10 %) was added. The flask then was filled with hydrogen and the mixture stirred overnight. The reaction mixture was then filtered through celite and concentrated in vacuo, which gave 6,2 mg (60 %) of **8**. 1H-NMR (300 MHz):δ 0.88 (t, 6.70 Hz, 3H), 1.20-1.35 (m, 8H), 1.39-1.47 (m, 2H),

2.02-2.11 (m, 1H), 2.23-2.37 (m, 2H), 2.40-2.52 (m, 1H), 3.64 (t, *J*=6.33 Hz, 1H), 4.41 (t, 4.06 Hz, 1H) ; TLC R_f=0,33 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20^\circ C}$=-30,0° (c=0.31, $CHCl_3$); EIMS *m/z* (% rel int) 253,1415 (M^+, 44, $C_{12}H_{22}O_4Na$ requires 253,1416), 251 (23%), 217 (96%), 201 (27%), 195 (26%). 187 (32%).

Formic acid (S)-1-((1S,2R)-1,2-dihydroxy-5-oxo-cyclopent-3-enyl)-heptyl ester (**9**)

A solution of FR96001M (8,6 mg) in formic acid (2 ml) was allowed to react overnight. The reaction was when neutralized by the addition of saturated $NaHCO_3$-solution. The solution was then extracted with diethylether (3×10 ml). The combined organic phases were dried ($MgSO_4$), concentrated in vacuo and the residue was purified with flashchromatography (heptane:EtOAc 4:1 to 1.1) which gave 1.5 mg of **9** (15,5 %). 1H-NMR (400 MHz):δ 0.88 (t, *J*=7.05 Hz, 3H), 1.40-1.20 (m, 8H), 1.69-1.63 (m, 2H), 2.87-2.78 (m, 1H), 4.85 (s, 1H), 5.29 (dd, *J*=10.82, 2.82 Hz, 1H), 6.32 (dd, *J*=6.07, 1.32 Hz, 1H), 7.65 (dd, *J*=6.08, 2.34 Hz, 1H), 8.02 (s, 1H); TLC R_f=0,55 (4:1 EtOAc/Petr.Eter); $[\alpha]_D^{20^\circ C}$=-6.14° (c=0.077, $CHCl_3$).

4. Future work

The small amount of FR96001M available has been a problem throughout the whole project. When reactions did not succeed the first time it was not always possible to do a second try. This limitation did force through a priority order for which derivatives should be synthesized. A total synthesis to FR96001M could be the solution to this problem and make it possible to succeed with some of the reactions tried unsuccessfully in this study and to make other new derivatives.

Future work should be focused on generating larger amounts of FR96001M, which would make it possible to synthesize new derivatives. New derivatives should first be concerned with modifying hydroxyl groups. This to see if the speculations made about selectivity and activity could be verified. New derivatives could perhaps make a QSAR study possible generating more information about the mechanism behind the biological activity of FR96001M.

If possible the absolute structure of FR96001M should be determined with X-ray crystallography.

It would also be interesting to test FR96001M for other sort of biological activity, like its ability to interact with microtubuli.

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Structure studies of FR96001M

-A new antibacterial substance produced by *Cyphellopsis anomala* TA96001

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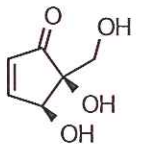
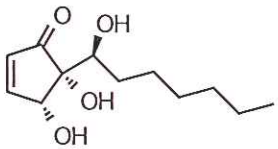
Abstract: FR96001M, (4R, 5R)-4,5-Dihydroxy-5-((S)-1-hydroxy-heptyl)-cyclopent-2-enone, is a new antibacterial compound isolated from *Cyphellopsis anomala* TA96001. Its structure was determined by spectroscopic methods. The absolute structure was determined with a FT-NMR version of Mosher´s method. FR96001M is very similar to the previously known antibacterial pentenomycin I, which is produced by *Streptomyces eurythermus*. Derivatives to evalulate structure activity relationship were synthesized. The antibacterial and antifungal activity of derivatives was tested on different species of bacteria and fungi. The results indicated that the double bond is essential for the activity, the tertiary hydroxyl group important for selectivity against bacteria and the hydroxyl group in the carbon tail important for the activity.

I wish to report a new substance, FR96001M. FR96001M is produced by micro fungi *Cyphellopsis anomala* TA96001 and shows antibacterial properties. The structure of FR96001M, (4R, 5R)-4,5-Dihydroxy-5-((S)-1-hydroxy-heptyl)-cyclopent-2-enone, was determined with NMR-spectroscopic methods. The absolute stereo chemistry was determined with the modified version [1] of Mosher´s method [2]. Derivatives were, in order to investigate structure activity relationship, made. The double bond was found to be important for the activity. In literature was the pentenomycins I found. Its structure is similar to FR96001M.

Kimio Umino et al [3] discovered the pentenomycin I. Pentenomycin I is antibacterial and was found during a screening project for new antibiotics in the seventies. The structure of Pentenomycin I (**1**) is similar to the one of FR96001M (**2**) although the absolute stereo chemistry of **2** seems to be inverse. **1** is produced by *Streptomyces eurythermus*. The antibacterial effect of **1** and a row of derivatives where tested on different bacteria (*S.aureus, B.pertussis and N.gonorrhoeae)* [4].

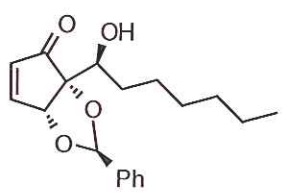


1

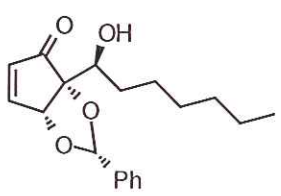


2

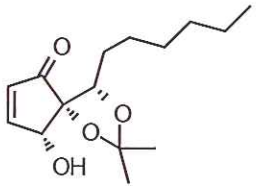
The structure of FR96001M was confirmed by acetalisation with dimethoxypropane and dimethoxytoluene. Both reactions were successfully, but did not give the same type of acetale. The reaction with dimethoxytoluene gave acetale **3a** (major diastereomer) and **3b** (minor diastereomer) and the one with dimethoxypropane gave acetale **4**. NMR studies on **3a** confirmed the *cis* relationship between the two hydroxyl groups in the ring. This relationship was indicated from the NOESY spectrum of FR96001M but not clear. In **4** is the stereo center in the carbon tail locked to the ring. The NOESY spectrum of **4** did show a correlation between the hydrogen atoms next to the both secondary hydroxyl groups and due to this was the relative configuration determined.



3a



3b



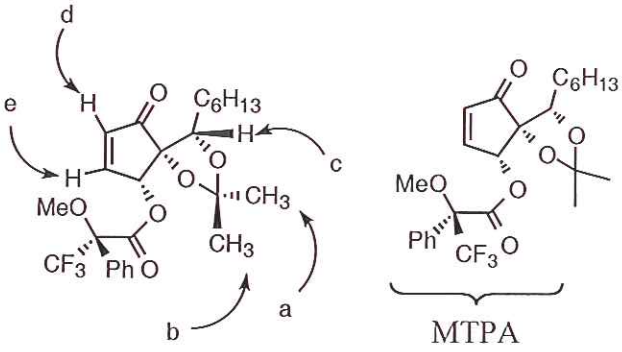
4

The small amount of material available made it impossible to make any crystals good enough for X-ray crystallography. Instead was a modified [1] Moshers's method [2] used to determine the absolute configuration. Both the *(R)*- and the *(S)*-MTPA esters, **5s** and **5r**, were made from **4** and the MTPA-chloride. NMR spectra were then collected (^{1}H-NMR and COSY) on a 500 MHz spectrometer. The shifts that differed and were used for the determination are listed in the table below (**Table 1**). The configuration of the carbon bearing the ester group was determined to be *R*. Due to the *cis* relationship between the two hydroxyl groups in the ring was the other stereo center in the ring determined to be *R*. From the mentioned NOESY correlation from **4** could the configuration of the stereo center in the carbon tail be determined to be *S*.

Table 1. *The shift that differed for the two diastereomers and the difference between them*

Hydrogen[1]	δ_R	δ_S	δ_S-δ_R (Hz)
a	1.55	1.55	-1.94
b	1.64	1.59	-24.27
c	4.50	4.38	-57.92
d	6.44	6.47	15.80
e	7.38	7.50	63.00

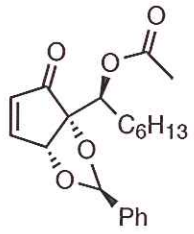
1. See *Figure 1.* for an explanation to which proton is which.



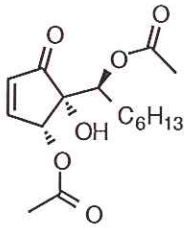
4r **4s**

*Figure 1. (to the right) The structure of (R)-3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic acid (4S,5R,6R)-4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4r**) and (S)-3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic acid (4S,5R,6R)-4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**4s**). The arrows show the position of the hydrogen used in* **Table 1.**

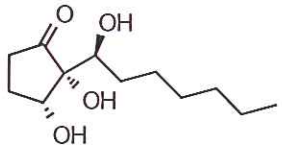
The antibacterial activity of the two acetals **3b** and **4** generated during the structure determination were tested (see **Table 2**). Other derivatives **6,7** and **8** were also synthesized and tested. The focus when choosing which derivatives to make was on the hydroxyl groups. The hydroxyl groups were interesting due to the possibility for them to be involved in hydrogen bonding with a receptor.



6



7



8

Table 2. Antimicrobial activity of derivatives from a paper disc test (20 µg/disc, figures in millimeter). The first four microorganisms are fungus and last four bacteria.

Substance	Pn	Tu	HA3	Pvar	99	63	Sl	Ed
4	11	8d	12	7	17	10	11d	0
7	0	0	10	0	15	14	11d	0
8	0	0	0	0	10	7	0	0
6	7	10	0	7	11	11/15i	10	0
3b	17/23i	21	13	15/28i	25	20/27i	20	0
FR96001M	0	0	8	0	25	11	0	8

Pn: *Penicillium notatum;* Tü: *Mucor miehei;* HA3: *Nematospora coryli,* Pvar: *Paecilomyces variotii;* 99: *Bacillus brevis;* 63: *B.subtilis;* Sl: *Micrococcus luteus,* Ed: *Enterobacter disolvens*
d:diffuse; i: incomplete

From **Table 2.** could the conclusion that the double bond is important to the activity be drawn. The other conclusions are more speculative. But it seems like that the tertiary hydroxyl group is important for the selectivity against bacteria and that the hydroxyl group in the carbon tail is important for the activity.

Experimental: Unless not mentioned the chemicals used were not pretreated, but of p.a. quality and bought from commercial suppliers. TLC was done on "Merck DC-Alufolien Kiselgel 60 F_{254}" SiO_2 plates. The spots were visualized with UV-light and with anisaldehyde/sulfuric acid/ethanol followed by heating with a heat gun. NMR spectra were recorded with the sample dissolved in $CDCl_3$ on a Bruker ARX-500, Bruker DRX-400 or a Bruker DRX-300. For the ^{1}H-spectra the chloroform signal was set to 7.27 ppm and for the ^{13}C-spectra to 77.0 ppm.

4,5-Dihydroxy-5-(1-hydroxy-heptyl)-cyclopent-2-enone (FR96001M, **2**)

1H-NMR (500 MHz):δ 0.85 (t, J=6.91 Hz, 3H), 1.20-1.40 (m, 8H), 1.50-1.60 (m, 2H), 3.74 (d, J=10.1 Hz, 1H), 4.71 (dd, J=2.13, 1.32 Hz, 1H), 6.26 (dd, J=6.07, 1.30 Hz, 1H), 7.61 (dd, J=6.06, 2.34 Hz, 1H); TLC R_f=0.40 (4:1 EtOAc/petroleum ether); $[\alpha]_D^{20°C}$=-3.90° (c=2.41, $CHCl_3$); EIMS *m/z* (% rel int) 228 (11%), 227 (100%), 113 (12%).

3a-(1-Hydroxy-heptyl)-2-phenyl-3a,6a-dihydro-cyclopenta-1,3-dioxol-4-one (**3a-b**)

To a solution of FR96001M (19,7 mg) in dry acetonitrile (5 ml) a catalytic amount of pTSA and dimethoxytoulene (15 µl, 1,2 eq.) were added. After 3,5 resp. 5 h. more dimethoxytoulene (15 µl each time) was added. After stirring for 24 h. under nitrogen the reaction was quenched by addition of saturated $NaHCO_3$-solution (10ml). The water phase was extracted with CH_2Cl_2 (3×15 ml). The combined organic phases were dried with $MgSO_4$, concentrated in vacuo and the residue purified with flashchromatography (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 8,8 mg (32 %) of acetal **3a** (major isomer, impure) and 3,3 mg (12 %) of acetal **3b** (minor isomer, pure).

3a: 1H-NMR (300 MHz):δ 0.89 (t, *J*=6.70 Hz, 3H), 1.25-1.40 (m, 8H), 1.50-1.60 (m, 2H), 1.90 (d, *J*=5.91 Hz, 1H), 4.21 (dt, *J*=5.83, 2.06 Hz, 1H), 5.39 (d, *J*=2.33 Hz, 1H), 5.65 (s, 1H), 6.69 (dd, *J*=6.10, 0.66 Hz, 1H), 7.40-7.43 (m, 3H), 7.45-7.52 (m, 2H), 7.60 (dd, *J*=6.10, 2.37 Hz, 1H); TLC R_f=0.47 (1:1 EtOAc/petroleum ether).
3b: 1H-NMR (300 MHz):δ 0.91 (t, *J*=6.60 Hz, 3H), 1.25-1.40 (m, 8H), 1.75-1.80 (m, 2H), 1.99 (d, *J*=5.93 Hz, 1H), 4.21 (dt, *J*=5.89, 2.23 Hz, 1H), 5.37 (dd, *J*=2.38, 0.53 Hz, 1H), 5.64 (s, 1H), 6.11 (dd, *J*=6.01, 0.62 Hz, 1H), 6.35 (s, 1H), 7.31-7.36 (m, 5H), 7.53 (dd, *J*=6.01, 2.36 Hz, 1H); TLC R_f=0.55 (1:1 EtOAc/petroleum ether); $[\alpha]_D^{20°C}$ =+124,7° (c=0.17, $CHCl_3$); EIMS *m/z* (% rel int) 339,1569 (M^+, 100, $C_{19}H_{24}O_4Na$ requires 339,1572), 329 (14%).

4-hexyl-9-hydroxy-2,2dimethyl-1,3-dioxa-spiro[4.4]non-7-en-6-one (**4**)

To a solution of FR96001M (18,3 mg) in 2,2-dimethoxypropane (5 ml) a catalytic amount of pTSA was added. After stirring for 30 min. under nitrogen the reaction was quenched by addition of saturated $NaHCO_3$-solution (10 ml). The water phase was extracted with CH_2Cl_2 (2×10 ml). The combined organic phases were dried with $MgSO_4$, concentrated in vacuo and the residue purified with flashchromatography (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 8,7 mg (40 %) of acetal **4**.
4:1H-NMR (500 MHz):δ 0.87 (t, *J*=7.02 Hz, 3H), 1.15-1.35 (m, 8H), 1.49 (s, 3H), 1.60 (s, 3H), 1.70-1.80 (m, 2H), 2.93 (d, *J*=7.4 Hz, 1H), 4.23 (dd, *J*=9.13, 4.13 Hz, 1H), 4.47(ddd, *J*=7.37, 1.42, 0.65 Hz, 1H), 6.27(dd, *J*=6.22, 1.31 Hz, 1H), 7.58 (dd, *J*=6.23, 2.28 Hz, 1H); TLC R_f=0,65 (4:1 EtOAc/petroleum ether); $[\alpha]_D^{20°C}$ =+16,6° (c=0.47, $CHCl_3$); EIMS *m/z* (% rel int) 268.1674 (M^+, 15, $C_{15}H_{24}O_4$ requires 268.1675), 253 (87%), 211 (76%), 210 (89%), 127 (92%), 86 (100%), 84 (100%).

[2R] 3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic-acid 4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**5r**)

To a solution of **4** (4,1 mg) in dry CH_2Cl_2 (1 ml) a catalytic amount of DMAP, triethylamine (21 µl) and *R*-MTPA-chloride (8,5 µl) were added in the mentioned order. After stirring for 2 h. under nitrogen the solvent was removed in vacuo. The residue was flashchromatographed (12:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,0 mg (68 %) of ester **5r**. 1H-NMR (500 MHz):δ 0.88 (t, *J*=6.67 Hz, 3H), 1.20-1.32 (m, 8H), 1.50-1.57 (m, 2H), 1.55 (s, 3H), 1.64 (s, 3H), 3.65 (s, 3H), 4.50 (dd, *J*=9.70, 2.96 Hz, 1H), 5.76 (dd, *J*=2.88, 0.92 Hz, 1H), 6.44 (dd, *J*=6.31, 1.00 Hz, 1H), 7.37 (dd, *J*=6.35, 2.92 Hz, 1H), 7.38-7.43 (m, 3H), 7.66 (d, *J*=6.52 Hz, 2H); TLC R_f=0,43 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20°C}$ =+17,3° (c=0.19, $CHCl_3$).

[2S] 3,3,3-Trifluoro-2-methoxy-2-phenyl-propionic-acid 4-hexyl-2,2-dimethyl-9-oxo-1,3-dioxa-spiro[4.4]non-7-en-6-yl ester (**5s**)

To a solution of **4** (4,4 mg) in dry CH_2Cl_2 (1 ml) a catalytic amount of DMAP, triethylamine (23 µl) and *S*-MTPA-chloride (9,2 µl) were added in the mentioned order. After stirring for 2 h. under nitrogen the solvent was removed in vacuo. The residue was flashchromatographed (7:1 Hexane:dietheyleter+1% triethylamine) which gave 2,4 mg (30 %) of ester **5s**. 1H-NMR (500 MHz):δ 0.88 (t, *J*=7.02 Hz, 3H), 1.20-1.32 (m, 8H), 1.50-1.58 (m, 2H), 1.54 (s, 3H), 1.59 (s, 3H), 3.50 (s, 3H), 4.38 (dd, *J*=10.39, 2.82 Hz, 1H), 5.76 (dd, *J*=2.04, 0.96 Hz, 1H), 6.47 (dd, *J*=6.33, 0.96 Hz, 1H), 7.37-7.43 (m, 3H), 7.50 (dd, *J*=6.32, 2.87Hz, 1H), 7.64(d, *J*=7.89 Hz, 2H); TLC R_f=0,40 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20°C}$ =+50,0° (c=0.06, $CHCl_3$).

Acetic acid 1-(4-oxo-2-phenyl-4,6a-dihydro-cyclopenta[1.3]dioxol-3a-yl)-heptyl ester (**6**)

To a solution of **3a** (9 mg) in dry pyridine (1 ml) acetic acid anhydride (5 µl, 1 eq.) was added. After 48 h another 5 µl aceticanhydride was added and after totally 120 h of stirring under nitrogen the reaction was quenched by repetitive addition of ethanol and removement of solvent in vacuo. The residue was flashchromatographed (4:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,3 mg (52 %) of ester **6**. 1H-NMR (300 MHz):δ 0.88 (t, *J*=6.79 Hz, 3H), 1.25-1.35 (m, 8H), 1.67-1.74 (m, 2H), 2.02 (s, 3H), 5.35 (d, *J*=2.23 Hz, 1H), 5.50 (dd, *J*=9.71, 3.04 Hz, 1H), 6.11 (s, 1H), 6.66 (dd, *J*=6.12, 0.66 Hz, 1H), 7.40-7.45 (m, 3H), 7.47-7.50 (m, 2H), 7.56 (dd, *J*=6.10, 2.35 Hz, 1H); TLC R_f=0,52 (1:1 EtOAc/Petr.Eter); EIMS *m/z* (% rel int) 381,1669 (M^+, 100, $C_{21}H_{26}O_5Na$ requires 381,1678), 329 (11%).

Acetic acid 1-(2-acetox-1-hydroxy-5-oxo-cyclopent-3-enyl)-heptyl ester (**7**)

To a solution of **2** (11,6 mg) in dry pyridine (0,5 ml) aceticanhydride (16 µl, 3eq.) was added. After stirring for 18 h under nitrogen the reaction was quenched by repetitive addition of ethanol and removement of solvent in vacuo. The residue was flashchromatographed (2:1 petroleum ether/EtOAc+1% triethylamine) which gave 5,0 mg (32 %) of ester **7**. 1H-NMR (300

MHz):δ 0.87 (t, *J*=6.80, 3H), 1.20-1.35 (m, 8H), 1.65-1.75 (m, 2H), 2.07 (s, 3H), 2.16 (s, 3H), 2.73 (s, 1H), 5.10 (dd, *J*=9.45, 3.81 Hz, 1H), 5.80 (dd, *J*=2.85, 1.14 Hz, 1H), 6.45 (dd, *J*=6.21, 1.14 Hz, 1H), 7.56 (dd, *J*=6.17, 2.84 Hz, 1H); TLC R_f=0,37 (1:1 EtOAc/Petr.Eter); EIMS *m/z* (% rel int) 335,1456 (M^+, 100, $C_{16}H_{24}O_6Na$ requires 335,1471), 325 (10%), 303 (9%), 265 (18%), 245 (30%), 243 (29%).

2,3-dihydroxy-2-(1-hydroxy-heptyl)-cyclopentaone (**8**)

A flask with a solution of **2** (10,2 mg) in EtOAc (2 ml) was flushed with nitrogen and a catalytic amount of palladium on carbon (10 %) was added. The flask then was filled with hydrogen and the mixture stirred overnight. The reaction mixture was then filtered through celite and concentrated in vacuo, which gave 6,2 mg (60 %) of **8**. 1H-NMR (300 MHz):δ 0.88 (t, 6.70 Hz, 3H), 1.20-1.35 (m, 8H), 1.39-1.47 (m, 2H), 2.02-2.11 (m, 1H), 2.23-2.37 (m, 2H), 2.40-2.52 (m, 1H), 3.64 (t, *J*=6.33 Hz, 1H), 4.41 (t, 4.06 Hz, 1H) ; TLC R_f=0,33 (4:1 petroleum ether/EtOAc); $[\alpha]_D^{20^\circ C}$ =-30,0° (c=0.31, $CHCl_3$); EIMS *m/z* (% rel int) 253,1415 (M^+, 44, $C_{12}H_{22}O_4Na$ requires 253,1416), 251 (23%), 217 (96%), 201 (27%), 195 (26%). 187 (32%).

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