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Marine Natural Products: Synthetic Exercises and Biological Data

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Abstract: In the first part, synthetic experiments will be described that provide information of the structural sub units responsible for the very powerful tumor inhibiting activity of the cephalostatins. These are bis-steroidal pyrazines that have been isolated by Petit and coworkers from cephalodiscus gilchristi in 1988. By comparison of relevant compounds the Δ 14,15 double bond which is very characteristic of these natural products is shown to be very important and it is also demonstrated that structural dissymmetry in the spiroketale moiety is very crucial.
A very efficient route to introduce the Δ 14,15 double bond and techniques for the preparation of nonsymmetric pyrazines are reported accordingly.
In the second part an inspection of the structures of antibacterial compounds from agelas oroides (agelorin) will show that very probably the halogenated spirocyclohexenone substructure of these compounds will be the essential part in these powerful antibiotics. An enantioselective total synthesis of this spiro-compound will be described employing the differentiation of the enantiotopic double bonds of spirocyclohexadieno precursors and a series of investigations proving their remarkable biological activity will be presented.

In the first part of the lecture a report is given on several experiments that were done with the aim to determine those substructures of the cephalostatins - a highly potent group of tumorinhibitors, that was isolated by *Petit* and coworkers from *ceph. gil.* (1) - which are essential for the biological activity.
On the one hand, the structures are so complicated that total synthesis is very probably no practical way to arrive at useful amounts of active material; on the other hand, there are so many similarities to easily available hecogenine, that one is tempted to study the α-aminoketone dimerisation of hecogenine and either before or afterwards add those structural items which are indispensable.

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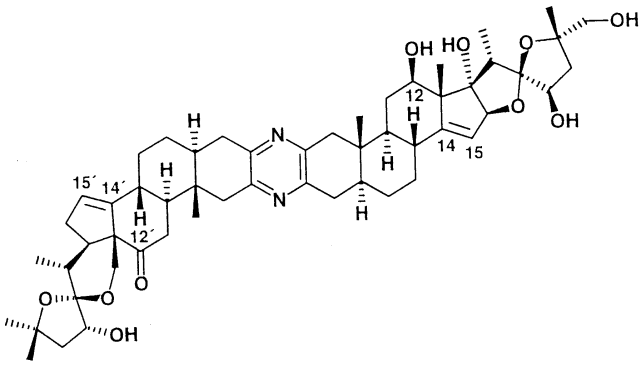
A close study of the different active structures in this group of compounds shows that they all contain the Δ-14,15 double bond, have additional hydroxy groups in the spiroketale system and as cephalostatine I (Scheme 1), which is non symmetric, shows the highest activity, dissymmetry may be an important aspect, too.

Scheme 1. Molecular structure of Cephalostatine 1

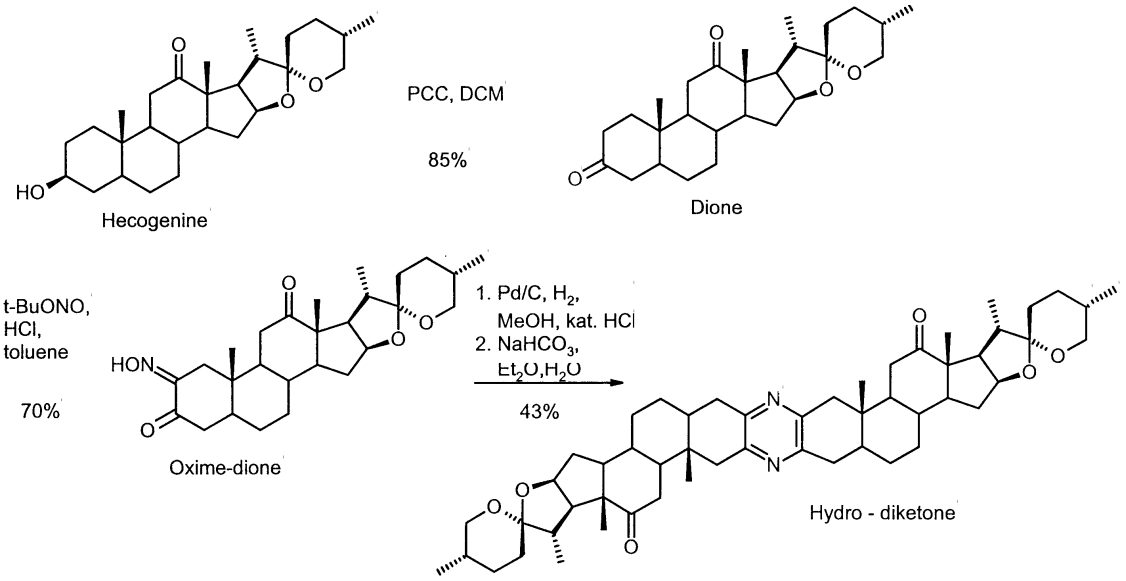


characteristic structural features

- central pyrazine ring
- $\Delta^{14,15}$-$\Delta^{14',15'}$-double bonds
- 12, 12´-functionalization
- hydoxylated spiro-ketal moiety
- asymmetric nature

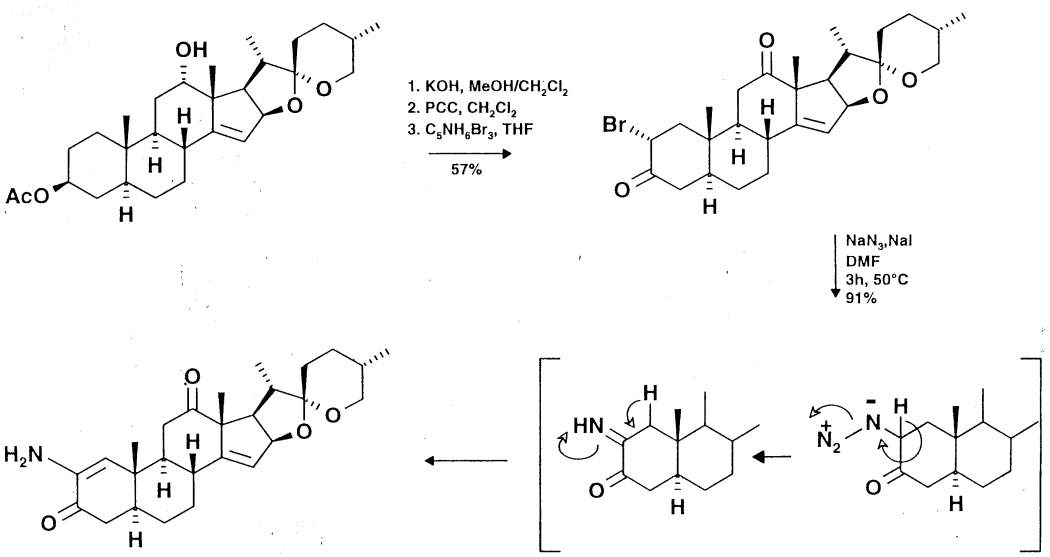
This analysis was confirmed when a bissteroidal pyrazine which was prepared by the well known dimerisation of a hecogenin derived α-aminoketone proved to be absolutely inactive (Scheme 2).

Scheme 2. Synthesis of the Hydro-diketone (symmetrical approach)

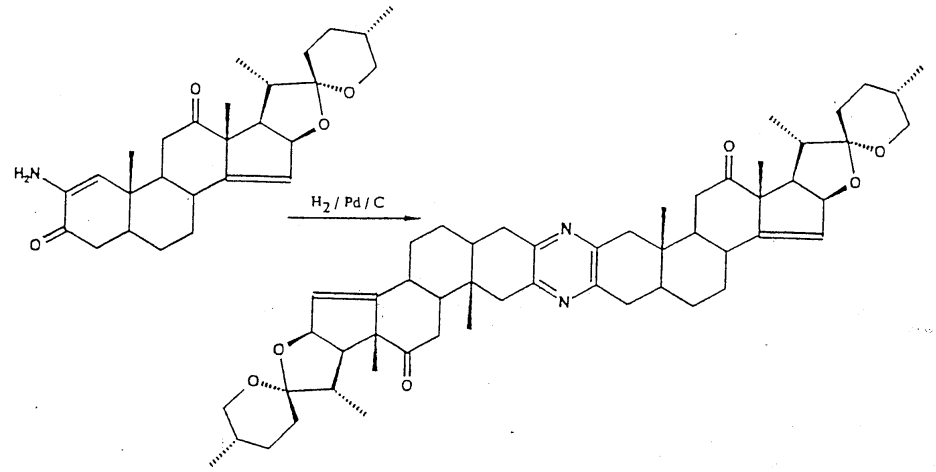


Since the compound was also highly insoluble, we hoped that the introduction of the Δ14,15 double bond might improve the situation. Introduction of this double bond using the improved *Bladon-Welze* (2) technique followed by aminoketone dimerisation led to a symmetric diketone showing much better solubility but only low biologic activity. When dissymmetry was introduced by selective reduction a remarkable rise in the biological activity was observed going along again with remarkably improved solubility. (3) (Scheme 3, 4)

Scheme 3. Synthesis of the enamino-ketone

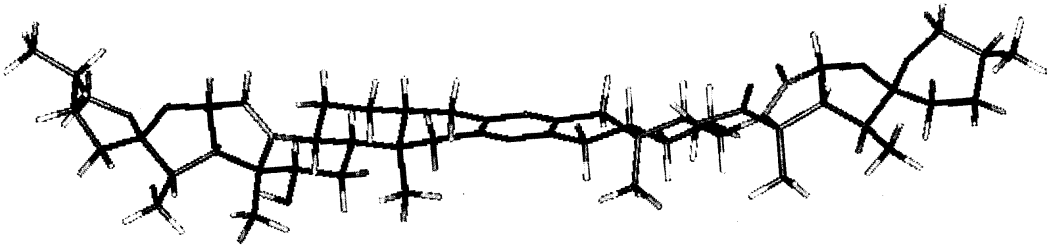


Scheme 4.



Since this solubility effect is very probably due to the double bond this may be explained by the chiral curvature that is typical for these compounds (conformational effect of the Δ14,15 bond, see scheme 5).

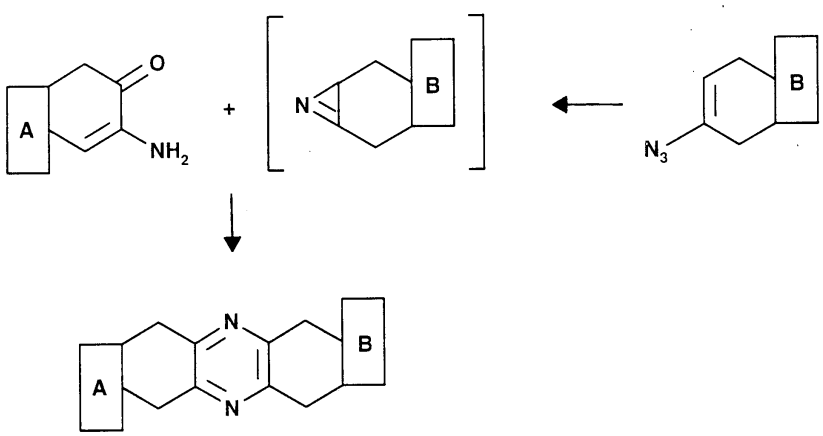
Scheme 5



There is a number of examples in the literature for this solubility phenomenon which with compounds of this type may be explained by the fact that with pure enantiomers the mirror-image is missing which is needed for ideal dense crystal structure packing.(4) Since non-symmetric, 14,15-unsaturated, compounds had the highest biological activity we developed a process for direct non-symmetric pyrazine formation by choosing an azirine as an a-aminoketone equivalent.(5)

Acid-catalyzed coupling of these compounds with en-amino-ketones did provide pyrazines directly in good yield (Scheme 6, 7)

Scheme 6. Our approach



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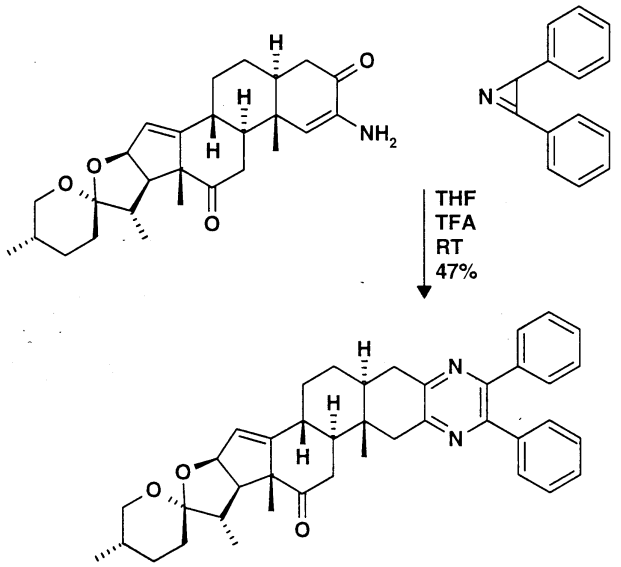
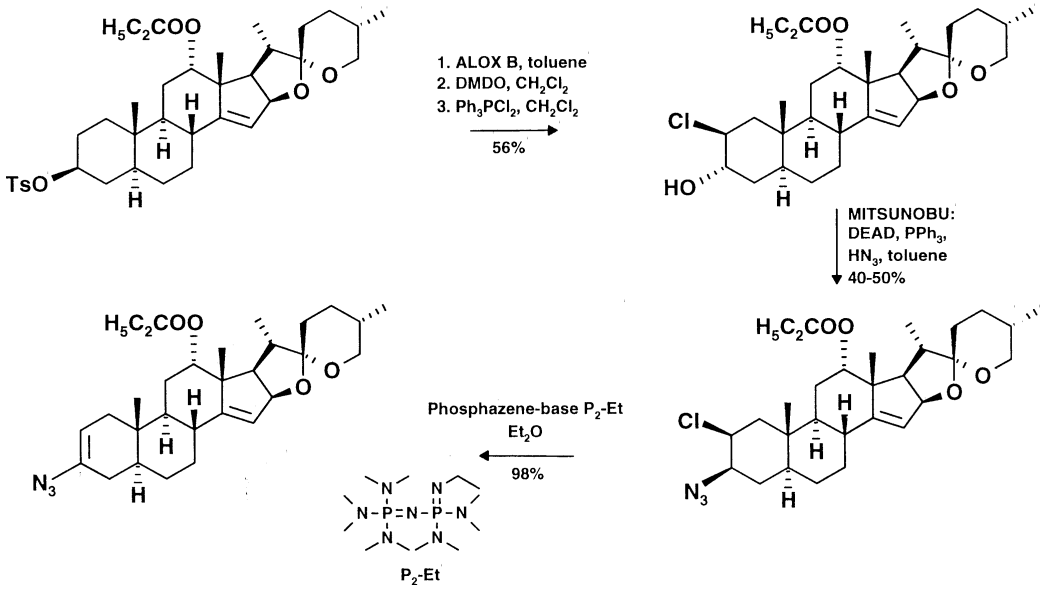
Scheme 7. First result

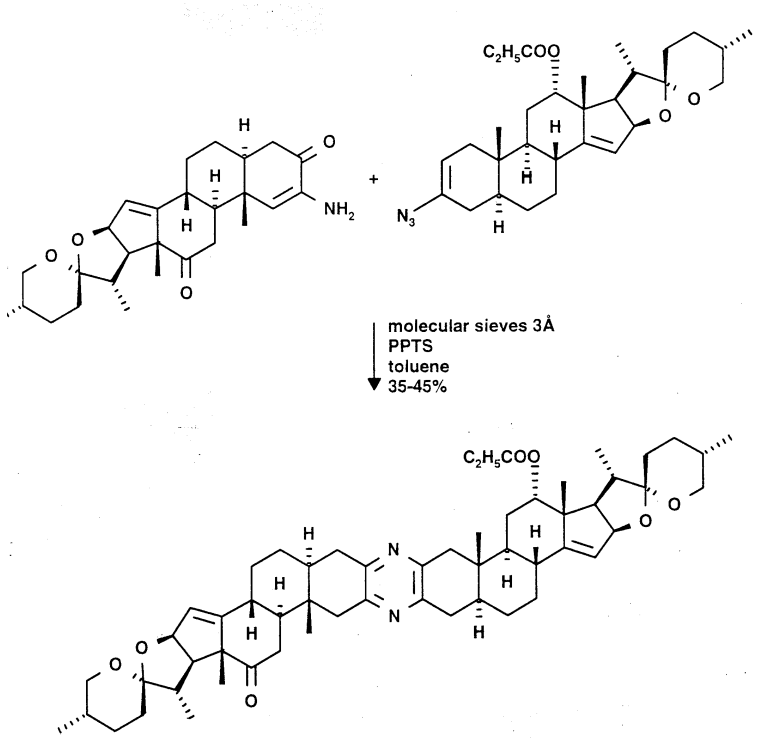


As azirines annellated to six-membered rings are highly reactive and cannot be isolated, we generated them in situ from vinylazides in a thermal process and succeefully captured the corresponding azirines with an enaminoketone (see Scheme 8, 9).

Scheme 8. Synthesis of vinyl azide

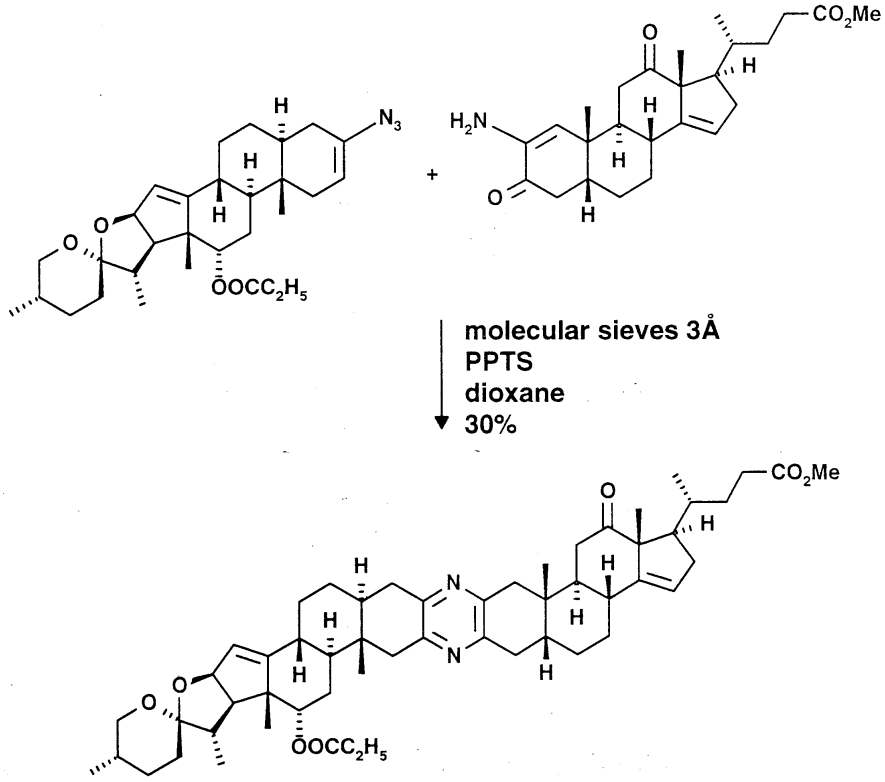


Scheme 9. The asymmetric coupling reaction

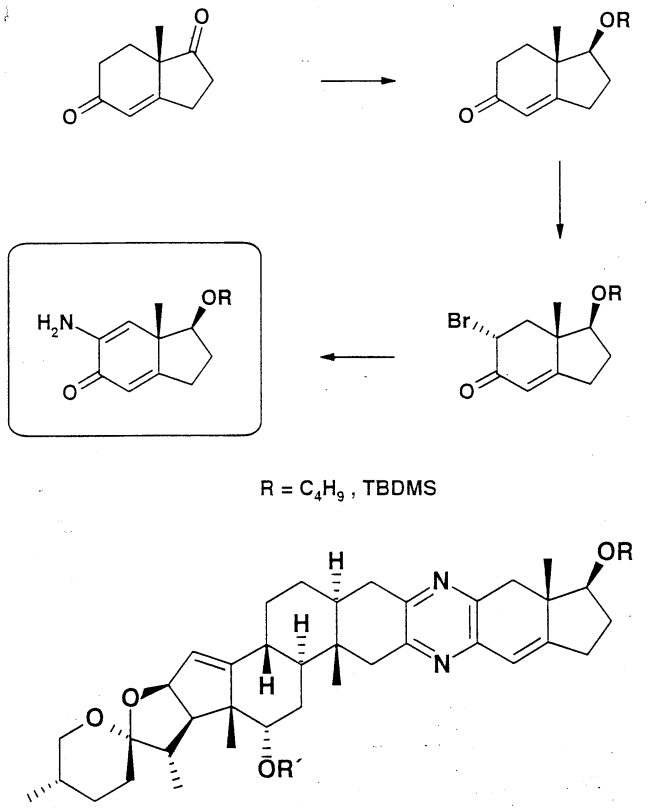


With this direct method available, a number of nonsymmetric unsaturated compounds were prepared which proved the importance of the Δ14, 15 double bond in a non-symmetric molecule (see Scheme 9, 10, 11)

Scheme 10. Coupling reaction with a steroidal A/B-cis-system

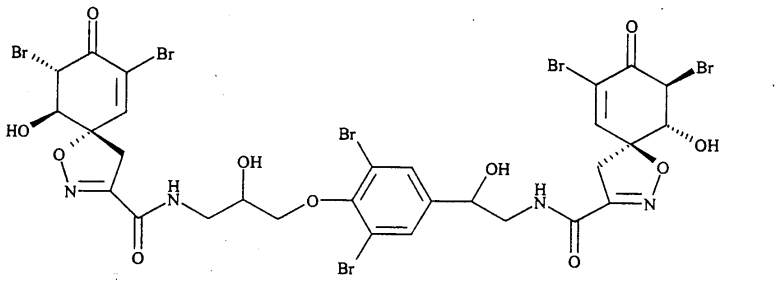


Scheme 11. Synthesis of bicyclic enamino-enones

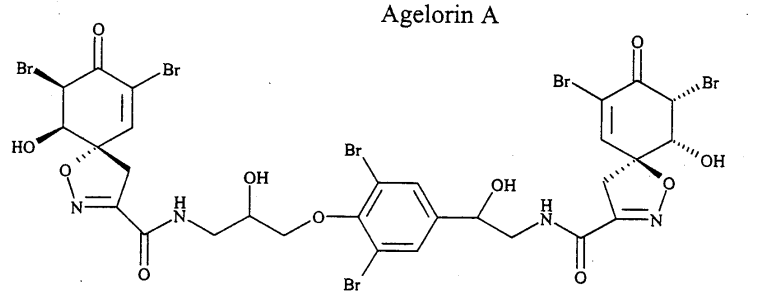


In the second part of the lecture, the structures of the agelorins are discussed and in this case the halogenated cyclohexadienone-substructure of these compounds clearly emerges as the most important part of the molecules (see Scheme 12)

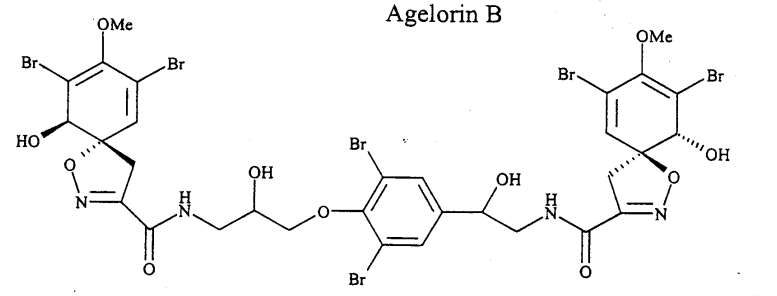
Scheme 12.



Agelorin A



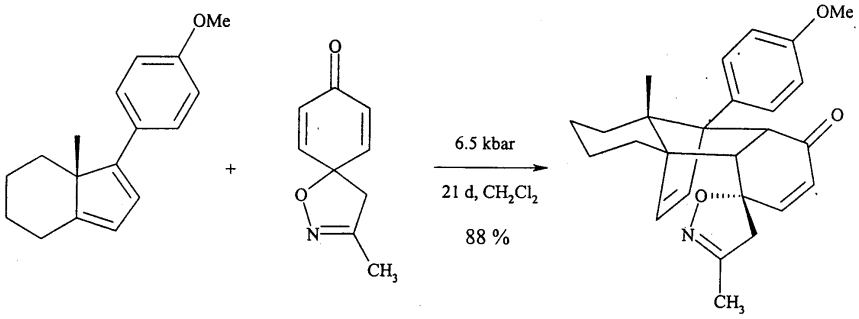
Agelorin B



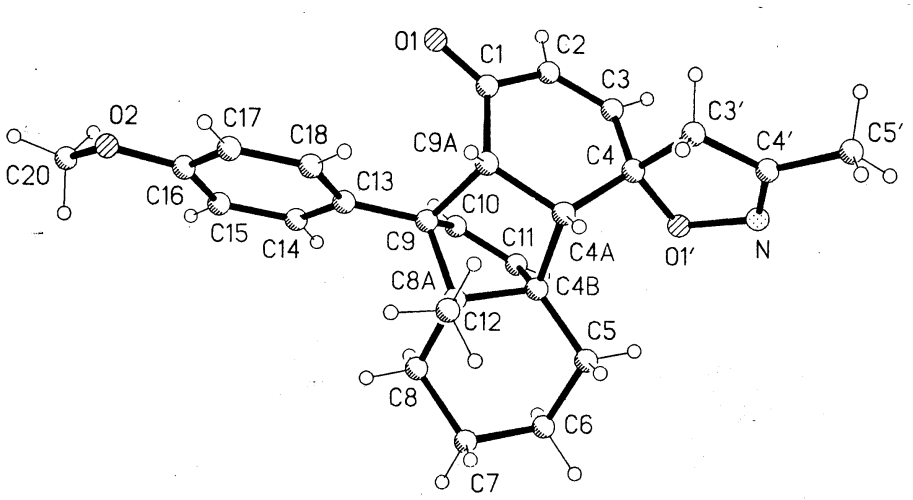
11-*epi*-Fistularin-3

Since the differentiation of enantiotopic double bonds in spirocyclohexadienones is a well established procedure in our group, we applied this process to a corresponding spiroisoxazole (see Scheme 13) and proved the structure of the crucial *Diels-Alder* cycloadduct by an X-ray investigation (see Scheme 14):

Scheme 13.

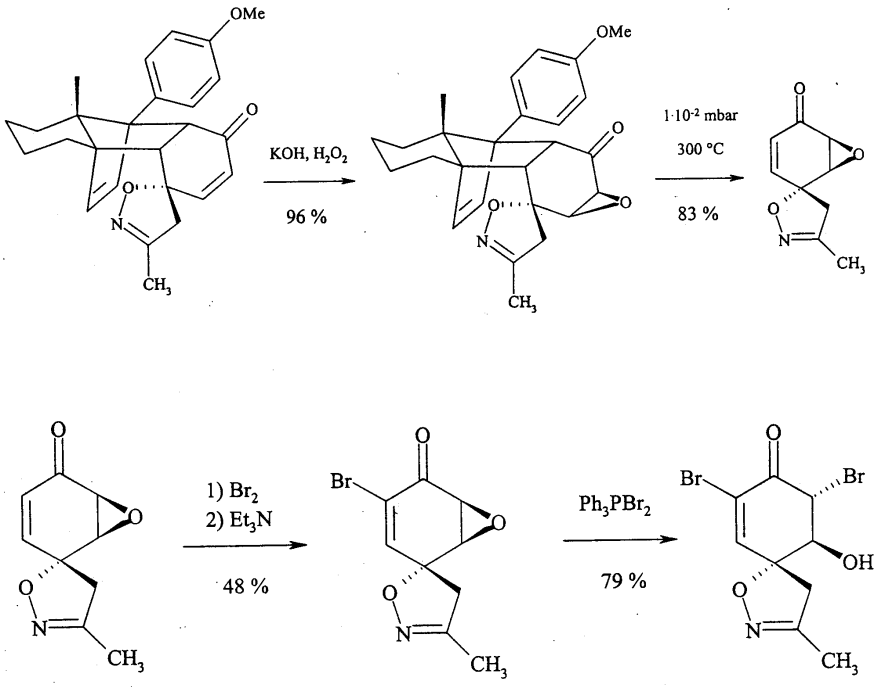


Scheme 14.



Diastereoselective epoxidation followed by a thermal retro-*Diels-Alder* process then did provide the enantiomerically pure cyclohexadienones (Scheme 15).

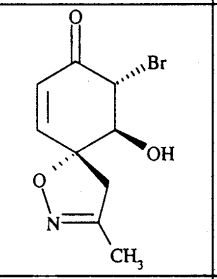
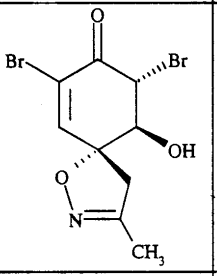
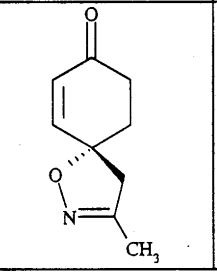
Scheme 15.



Subsequent bromination in the presence of an amine led to the corresponding vinylbromide. Subsequent regioselective opening of the epoxide with bromide anion did then introduce the second halogen atom to generate the agelorin chromophore.

With these enantiopure compounds at hand, a study of their biological activity was undertaken which proved that independent of their special substiution pattern, all spirocyclohexanones prepared in this project showed antibiotic as well as cytotoxic activity, thus confirming the idea that these substructures are indeed the crucial element in the ageloring antibiotics.

Table 1: Biological Activity of agelorin analogs – Wirkung gegen schleimbildendes Adenocarcinom (HM02)

	TGI ($\mu mol \cdot l^{-1}$)
	< 0.01
	0.04
	< 0.01

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