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Synthesis of Ascomycin and FK 506 Derivatives with Modified Effector Domain

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Abstract

Oxidation of the 22-hydrazone of ascomycin using manganese dioxide generates *in situ* the reactive 22-diazo intermediate, which decomposes to give ascomycin analogues with modified effector region. In dichloromethane several products are obtained, whereas in methanol a cyclopropane derivative is generated as the main product starting from the hydrazone of both ascomycin and FK 506.

Keywords: carbenes and carbenoids; diazo compounds; hydrazones; macrolides

The macrolactam ascomycin (**1a**) and the structurally related compound FK 506 (**1b**) have been the subject of intensive chemical research in efforts to modify and improve their pharmacological profiles [1,2]. The left part of these molecules (binding domain) is responsible for the binding to the cytosolic protein macrophilin, whereas the right part is considered to be the effector domain for biological activity [3]. The high reactivity of the tricarbonyl structural element (C8-C10), with one of the carbonyl groups masked as a hemi-ketal, allows selective chemical derivatisation within the binding domain [4], but limits chemical manipulation at other regions. We report on a simple method for the preparation of ascomycin derivatives with intact binding but modified effector region.

We envisaged to use the oxidative conversion of hydrazones into the corresponding diazo analogues [5] and the high reactivity of the latter [6] to access ascomycin derivatives with a modified carbon skeleton. Recently we have shown that ammonia and methylamine in alcoholic solution react with the tricarbonyl function in the binding domain of ascomycin yielding a mixture of the 9-imine + the 10-amine and the 9-methylimine, respectively [7]. In contrast, starting from unprotected macrolides **1a,b** hydrazine preferentially forms the 22-hydrazone derivatives **2** (not

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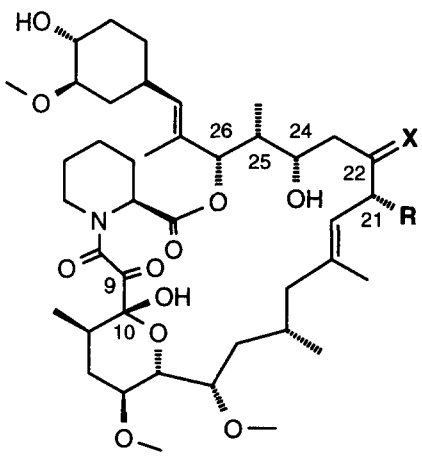
optimised, isolated yields: **2a**, 62 %; **2b**, 56 %). When a large excess of manganese dioxide (16 equiv.) was added at once to a stirred solution of ascomycin hydrazone **2a** in dry dichloromethane at room temperature (at 5 °C the reaction was sluggish and did not come to completion), an exothermic reaction started within a few minutes and evolution of gas was observed. After stirring for one hour, TLC indicated complete conversion of the starting material to a complex mixture of products which was subjected to repeated chromatography on silica gel using various solvent systems as eluents. Most of the products formed could be isolated and their structures elucidated by NMR spectroscopy (compounds **4a-8a**). The major components of the product mixture were the diene **4a** and the cyclopropyl derivative **5a**, whereas **6a** [8], the tetrahydrofuran compounds **7a** and **8a** were produced in minor amounts. Product yields are not specified, because our goal was to isolate pure products, and varying amounts of materials were lost during the intensive chromatographic purification steps.



1a (R = ethyl, X = O), **1b** (R = allyl, X = O)

H_2NNH_2

2a (R = ethyl, X = NNH_2), **2b** (R = allyl, X = NNH_2)

MnO_2

[**3a** (R = ethyl, X = N_2), **3b** (R = allyl, X = N_2)] not isolated

- N_2

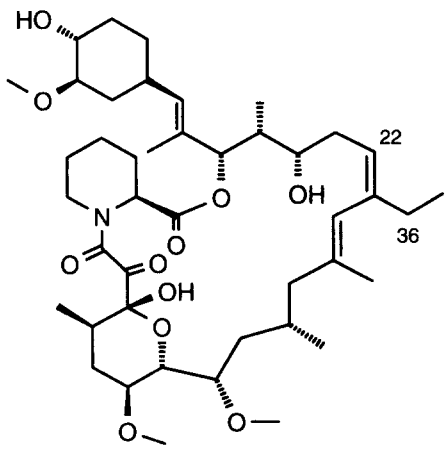
4a-8a, 5b

Characteristic ^1H and ^{13}C NMR signals [ppm] in $CDCl_3$:

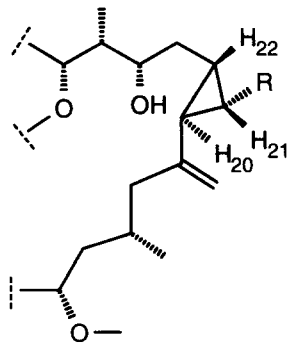
Diene derivative **4a**: 5.40 (s, H-20), 5.61 (dd, H-22, coupled to H-23a at 2.20 with J = 11.0 Hz and H-23b at 1.36 with J = 4.0 Hz); 141.7 (C-19), 135.2 (C-21), 132.6 (C-28), 128.0 (C-29), 121.9 + 121.2 (C-20 + C-22), evidence for *Z*-configuration of the newly generated double bond: NOE from H-22 to H-36a and H-36b;

Cyclopropane derivative **5a**: 4.54 (d, J = 1.5 Hz) + 4.46 (d, J = 1.5 Hz, exocyclic methylene group), 0.90 (m, H-22), 0.65 (t, J = 5.2 Hz, H-20), 0.57 (m,H-22, $J_{21/22}$ = 9 Hz); 149.1 (C-19), 135.1 (C-28), 129.9 (C-29), 103.9 (=CH_2), 33.6 (C-23), 32.1 (C-20), 28.9 (C-21), 18.1 (C-22), the ^{13}C signals were assigned by a HMQC spectrum, from the splitting of the signals of the cyclopropyl protons follows: H-20 trans to H-21 and H-22; stereochemistry not determined;

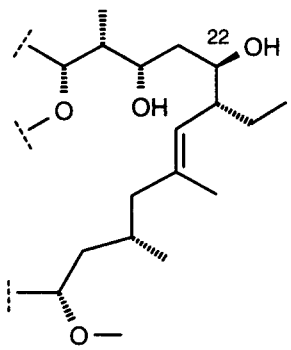
Tetrahydrofuran derivative **7a**: 4.97 (s, H-20). 132.7 (C-19), 126.4 (C-20), 77.2 (C-21), 76.2 (C-24), 40.3 (C-25), 39.5 (C-23), 29.1 (C-22); isomer **8a**: 4.99 (d, H-20), 4.60 (dd, H-21, coupled to H-20 with J = 9.2 Hz and to H-22 at 1.82 with J = 4.4 Hz); 141.2 (C-19), 123.4 (C-20), 71.7 (C-24), 68.3 (C-21), 41.0 (C-22), 39.4 (C-25), 36.2 (C-23), stereochemistry at C-21 and C-22 for both **7a** and **8a** not determined.



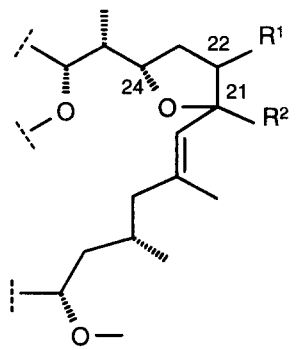
4a



5a, R = ethyl **5b**, R = allyl



6a



7a, R^1 = H, R^2 = Et **8a**, R^1 = Et, R^2 = H

The diene **4a** and the cyclopropane derivative **5a** presumably are products of an isomerisation of the carbenoid and/or carbo-cation species resulting from the decomposition of the diazo intermediate **3a** [6]. The detection of a small amount of the 22(*R*)-dihydro analogue of ascomycin (**6a**) can be explained by reaction of residual, non-decomposed **3a** with water. For the formation of the tetrahydrofuran product **7a** and its isomer **8a**, which probably resulted from a Wagner-Meerwein rearrangement prior to the cyclisation step, a "carbo-cationoid" mechanism should be involved, that is normally favoured in protic solvents [6]. We assume that the 24-hydroxy

function is in such a steric vicinity to the reaction center at C-22 that it can deliver a proton to generate the carbo-cation intermediate which then can undergo several reactions, including rearrangements and intramolecular ring closure to the tetrahydrofuran ring system. The reaction pathways following the decomposition of the diazo intermediate **3a** are more complex than those usually observed for simple diazo compounds in aprotic solvents.

When the reaction was performed in methanol, the cyclopropane **5a** was formed almost exclusively. This finding confirmed the difference in the decomposition of the 22-diazo ascomycin (**3a**) relative to less complex diazo compounds. NMR studies with the macrolides **1a,b** indicate differences in the conformation of the macrocyclic ring in methanol relative to chloroform solution (e.g., for FK 506 the coupling constant $J_{25/26}$ = 2.5 Hz for the major *E*-amide rotamer and 1.5 Hz for the minor *Z*-amide rotamer in $CDCl_3$ versus 6.0 Hz and 5.5 Hz in CD_3OD solution) [9]. Conformational changes might result in an altered environment of the reaction center (C-22) favouring one pathway for the decomposition of the diazo intermediate in methanol producing the cyclopropane **5a**.

In contrast to ascomycin, FK 506 has a second double bond close to the reaction center in its allyl side chain. Therefore we investigated, whether this double bond would participate in the decomposition reactions of the *in situ* generated 22-diazo FK 506 analogue **2c**. Interestingly, treatment of **2b** with excess manganese dioxide in methanol again yielded the corresponding cyclopropyl analogue **5b** as the main product without affecting the allyl side chain.

In summary, the presented method is useful for the preparation of macrolide derivatives modified at the effector region without the necessity of a protection/deprotection protocol. The access to analogues with modified carbon skeleton is particularly important for the establishment of structure-activity relationships.

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