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Novel analogues of ascomycin with modifications in the amino acid unit through photochemistry: the synthesis of 5,6-dehydroascomycin, SDZ ASQ 871

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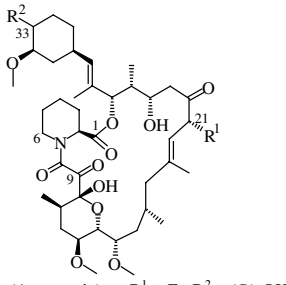
Abstract—Irradiation of ascomycin **1a** and its derivatives in MeOH, EtOH and propanol resulted in alkoxylation of the pipecolic acid moiety in the ε-position with concomitant reduction in the tricarbonyl region leading to 6-alkoxy-9-hydroxy derivatives in high stereoselectivities and good yields. The products, after reoxidation of the C(9)-OH, afforded the 6-alkoxy analogues of the parent compounds. Elimination of MeOH from the photoproducts, followed by oxidation gave the corresponding 5,6-dehydro amino acid analogues. Similarly, starting from the proline analogue **7** modifications in the pyrrolidine moiety could be achieved.

The macrolactam ascomycin[1] (**1a**, Fig. 1) is a fermentation product from *Streptomyces hygroscopicus* var. *ascomyceticus*, originally isolated due to its antifungal activities. Preclinical and clinical studies with two ascomycin derivatives, SDZ 281-240[2] and pimecrolimus[3] (**1b**, SDZ ASM 981, 33-epichloroascomycin, Elidel®) revealed that this substance class has a high therapeutic potential for the treatment of inflammatory skin diseases, such as atopic dermatitis and psoriasis. These results stimulated the search for novel ascomycin derivatives.[4] We have earlier described the photochemical transformation of **1a** into **2a** (MeOH, $\lambda = 366$ nm, 0–5 °C, 8 h, 75%, Scheme 1).[5] The reaction is easy to carry out, is highly stereoselective and gave good yields. Further, the photoproduct **2a** features a versatile functionality in the amino acid region, which is not accessible through routine chemistry. With a view to developing short synthetic sequences, without involving protecting groups, for the preparation of derivatives with modified amino acids, we examined the scope of this photoreaction and down-stream chemistry of the products.



1a (Ascomycin): R^1 = Et, R^2 = (*R*)-OH
1b (Pimecrolimus): R^1 = Et, R^2 = (*S*)-Cl

Figure 1.

The new substrates studied were pimecrolimus **1b**, the 21-methyl analogue **1c**, and the 21-allyl analogue **1d**, all containing the pipecolic acid moiety and the proline analogue of ascomycin **7** (Schemes 1 and 2). Irradiation of **1b–d** in methanol, as described above, resulted in the (6*S*)-methoxy-9-hydroxy derivatives **2b–d** (70–75%), and the open chain lactones **4b–d** (13–15%) as the only products (Scheme 1).[6] Irradiation of the proline analogue **7** in methanol under the same conditions gave rise to both the (5*R*)-and (5*S*)-methoxy derivatives **8** (8%) and **9** (24%). The lower stereoselectivity observed in the reaction of **7**, as opposed to that with the pipecolic acid analogues **1a–d**, is probably due to the higher flexibility of the five-membered enaminium intermediate **Z** ($n = 0$, Scheme 1).[5,7] It may be noted that β-attack by MeOH is still the more favoured pathway.

Irradiation of **1a** in other alcohols was also studied. Upon irradiation of **1a** in ethanol and *n*-propanol, the

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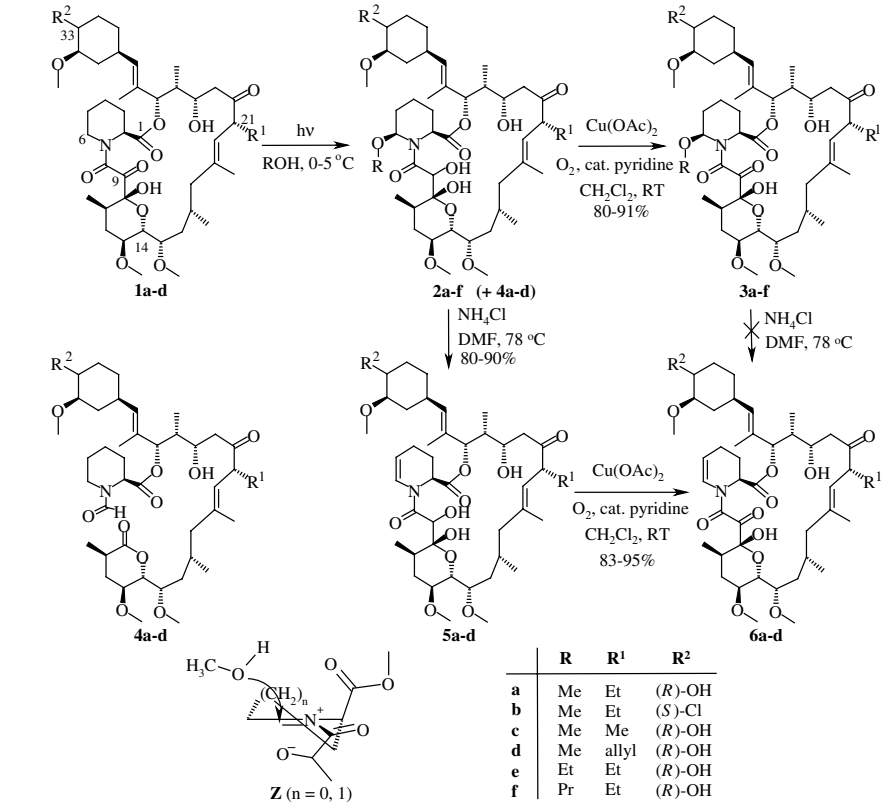
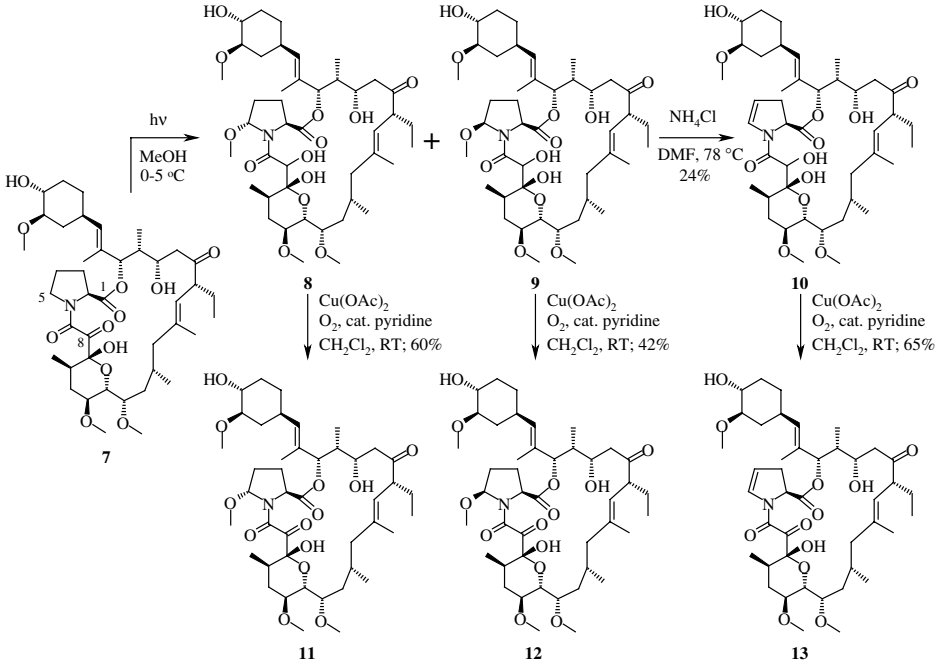


	R	R1	R2
a	Me	Et	(R)-OH
b	Me	Et	(S)-Cl
c	Me	Me	(R)-OH
d	Me	allyl	(R)-OH
e	Et	Et	(R)-OH
f	Pr	Et	(R)-OH

Scheme 1. Modifications in the pipecolic acid moiety of ascomycin derivatives.



Scheme 2. Modifications in the proline moiety of the proline analogue of ascomycin **7**.

(6*S*)-ethoxy derivative **2e** (59%) and the (6*S*)-propoxy derivative **2f** (10%), respectively, were formed. Irradiations in higher alcohols such as *n*-butanol and *iso*-butanol led to complex mixtures, which were not pursued further. The lower yields of the alkoxy products with higher alcohols, is probably due to their lower nucleophilicity, which allows unspecific side reactions to predominate.

Earlier studies have shown that derivatives of **1a** with intact C(9) = O functionality have a higher affinity to macrophilin-12, the cytosolic receptor for compounds of this type.[4] Hence, the C(9)-OH in the derivatives **2a**–**f** was oxidised using the reported procedure[5] ($Cu(OAc)_2$, cat. pyridine, CH_2Cl_2, O_2, rt) to give the (6*S*)-alkoxy analogues **3a**–**f**, respectively, in high yields (80–91%). Interestingly, the ^1H NMR data ($CDCl_3$, rt) of the analogues **3a**,**e** and **3f** showed the existence of two species: one with a $^2C_5$ conformation for the amino acid, and the second with a nonchair conformation in the ratios 2:1 (**3a**, R = Me), 3:2 (**3e**, R = Et), 3:2 (**3f**, R = Pr). This is at variance to the observation of two amide rotamers, both featuring $^2C_5$-chair conformations for the pipecolic acid moiety in the ^1H NMR ($CDCl_3$, rt) of ascomycin **1a**. It appears that the bulky 6-alkoxy substituents destabilise the chair conformation, presumably due to 1,3-diaxial interactions with the C(1)-ester group. Finally, the (5*R*)- and (5*S*)-methoxy proline analogues **11** (60%) and **12** (42%) were obtained starting from **8** and **9**, respectively, as described above.

The 6-methoxy **3a** and the 6-ethoxy **3e** ascomycins were found to have a lower affinity to macrophilin-12 by a factor of 100 and 15,000, respectively, relative to ascomycin **1a**. It appears that the reduced binding of these alkoxy analogues is a result of the increased size of the new substituent, and/or the change in the conformation of the pipecolic acid ring.

We therefore envisaged transforming the photoproducts **2a**–**d** to the corresponding enamides, which would be closer bioisosters of **1a** with potential for a better macrophilin-12 affinity. Thus, heating the derivatives **2a**–**d** with NH_4Cl in DMF at 78 °C under reduced pressure in a rotary evaporator to ensure removal of the MeOH formed in the reaction, afforded the enamides **5a**–**d** (80–90%).[8] Again, oxidation of the C(9)-OH of **5a**–**d**, as described above, using Cu(II) acetate, afforded the ascomycin analogues **6a**–**d** (83–95%) containing the 5,6-dehydropipecolic acid unit. Similarly, the (5*S*)-methoxy proline derivative **9** upon elimination of methanol resulted in **10** (24%), which after oxidation led to the analogue **13** (65%) featuring the unsaturated proline unit. It is noteworthy that elimination of MeOH is possible only for the derivatives of type **2** featuring C(9)-OH, and, not for type **3** where the C(9)-OH is oxidised. Thus, **3a** upon treatment with NH_4Cl/DMF, as above, was recovered (95%) unchanged. A similar behaviour was observed with (6*R*)-methoxy derivatives reported earlier[5] as well, indicating that the configuration of the MeO-bearing carbon is not responsible for this behaviour. It appears that the highly electronegative C(9) = O in the compounds of type **3** would disfavour the formation of a cationic site adjacent to the amide nitrogen, hence disfavouring the acid catalysed elimination reaction. It is also likely that stereoelectronic effects resulting from a change in the conformation (**2** vs **3**) or the conformational flexibility also play a role in this reaction.

The ^1H NMR spectrum of 5,6-dehydroascomycin **6a** showed the presence of two amide rotamers in a ratio of 2:3. The coupling constants of the pipecolic acid ring protons indicate a half-chair conformation for the amino acid moiety, with the ester group in the axial position for both rotamers. Thus, the introduction of the double bond resulted only in a slight flattening of the chair as compared to the parent compound ascomycin **1a**. Indeed, **6a** was found to bind to macrophilin with the same affinity as that of **1a**.

In summary, using the photoreaction of ascomycin we were able to synthesise several analogues with modifications in the amino acid in excellent yields and in a few steps without using any protecting groups. The 5,6-dehydroascomycin **6a**, because of its excellent binding affinity, was of particular interest, and could be prepared on a 100 g scale in three steps in an overall yield of 64%. The enamide **6a** was also used for preparing metabolically-stable-radiolabelled ascomycin through tritiation of the enamide double bond.[9]

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