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Selective photochemical cleavage of an α-ketoamide in a highly functionalised macrolide ascomycin

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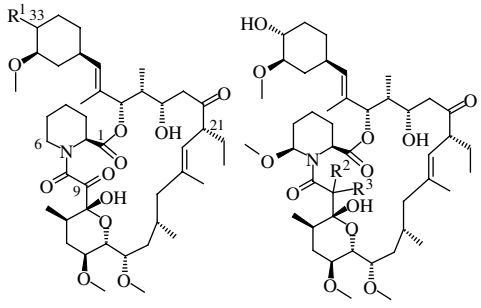
Abstract—A novel photochemical amide cleavage reaction of (6*S*)-methoxyascomycin opening a pathway for the selective cleavage of the pipecolic acid, is described. The scope of this reaction with several analogues carrying suitable protecting groups is examined.

The immunomodulatory macrolactam ascomycin (**1**, Fig. 1) is a fermentation product from *Streptomyces hygroscopicus* var. *ascomyceticus*, originally isolated due to its antifungal activities.[1] Pimecrolimus **2**, (the 33-epichloro derivative of **1**, Elidel,® SDZ ASM 981) has shown high therapeutic efficacy in patients with inflammatory skin diseases and pimecrolimus cream 1% is on the market for the topical treatment of atopic dermatitis.[2] Ascomycin is a highly functionalised molecule containing several free hydroxy groups, an aldol functionality, and a reactive tricarbonyl unit attached to pipecolic acid. As demonstrated by studies with the structurally closely related fermentation product FK506 (tacrolimus), the pipecolic acid moiety of the macrolactam is involved in the binding to its receptor macrophilin.[3] It would, therefore, be of interest to examine the impact of modifications in this region of the molecule on its biological activities. However, because of the lack of functionalities in this part of the molecule, selective modifications, in particular on the amino acid unit, require complex synthetic strategies.[4] We have earlier described the phototransformation of **1** into (6*S*)-methoxy-9-hydroxy ascomycin **3** (MeOH, $\lambda \geqslant 366$ nm, 0–5 °C, 8 h, 75%) and its subsequent selective Cu(II)-catalysed oxidation leading to (6*S*)-methoxyascomycin **4**.[5a,b] Using these reactions we were able to synthesise several analogues of **1** with modified amino acid units.[5c] Recently we have found that photolysis of **4** featuring C(6)–OMe under the same reaction conditions follows a different pathway leading to selective cleavage of the amide bond. This reaction opens a potential strategy for the selective removal of the pipecolic acid and replacement by other amino acids.



1: Ascomycin: $R^1 = (R)$-OH **3**: R^2 = H, R^3 = OH
2: Pimecrolimus: $R^1 = (S)$-Cl; **4**: (R^2, R^3) = O

Figure 1.

The phototransformation of **1** into **3** involves abstraction of the equatorial C(6)–H by the excited C(9)=O leading to a diradical, and its reorganisation to the zwitterion **Z1** (Fig. 2), which is trapped by attack of MeOH on C(6).[5,6] (6*S*)-Methoxyascomycin **4** still possesses an hydrogen atom on C(6) in the equatorial position, and can, in principle, undergo a similar photoreaction, which would initially lead to the intermediate **Z4**. Attack of MeOH on C(6) of **Z4**, as observed with **Z1**, appeared to be unfavourable now because of the resulting steric congestion due to the additional OMe group. We were therefore interested to know the fate of this intermediate, and to this end we looked at the photochemistry of **4**.

Keywords: Phototransformation; Photochemical amide cleavage; Zwitterion.

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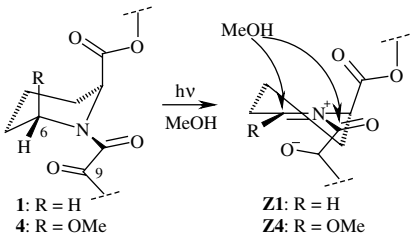
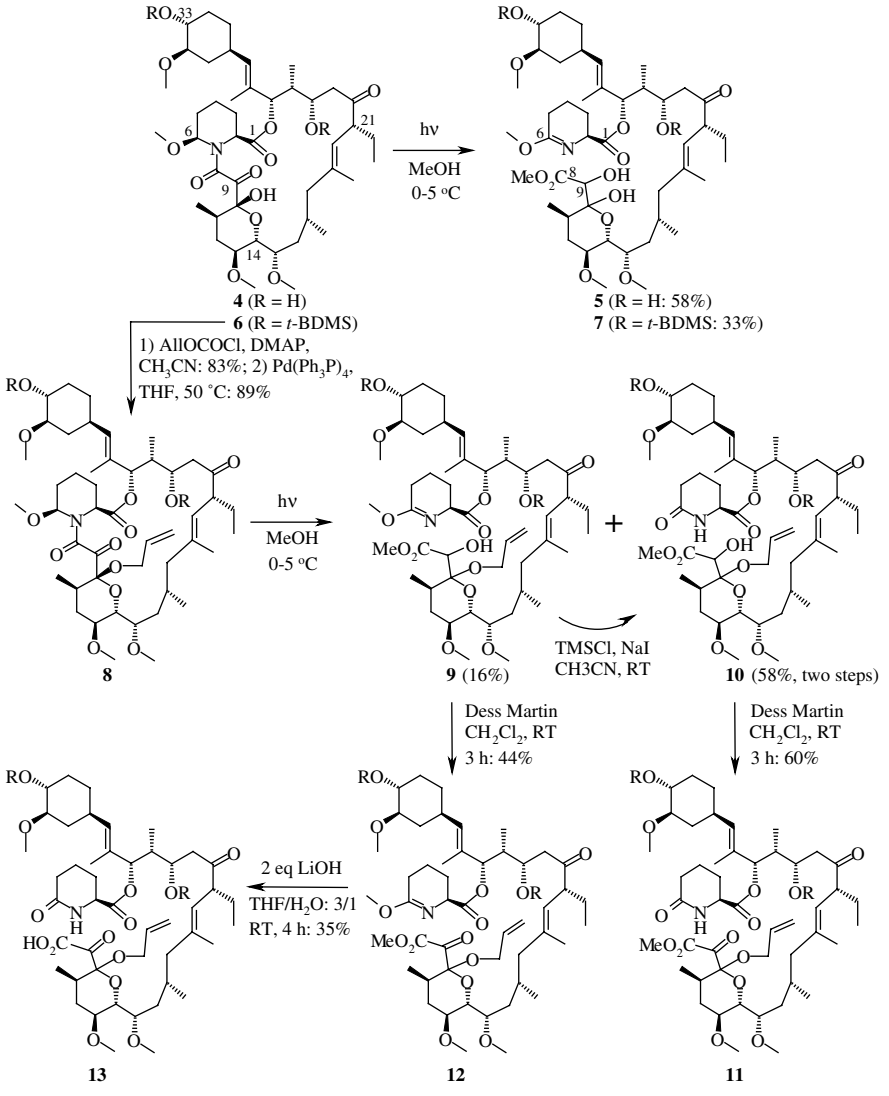


Figure 2. Partial structures of **1** and **4** and the intermediates **Z1** and **Z4** in the photolysis.

Irradiation of **4** in MeOH, as described above, for 4.5 h, followed by separation on preparative HPLC, afforded unreacted **4** (7%) and the amide cleavage product **5** as a mixture of C(9)- and C(10)-isomers in 58% yield (Scheme 1).[7] Through repeated HPLC a pure sample of **5** (27%) consisting of one single isomer could be obtained. Characteristic of **5** are the ^{1}H NMR (500 MHz, $CDCl_3$) signals at δ 4.20 (s, 1H, H–C(9)), 3.81 (s, 3H, CO_2Me), 3.65 (s, 3H, MeO–C(6)), and the ^{13}C NMR (125 MHz, $CDCl_3$) signals at δ 174.2 (C(8)=O), 172.2 (C(1)=O), 164.7 (C(6)=N), 73.3 (C(9)), 52.7 (MeO–C(8)), 52.4 (MeO–C(6)). The NMR spectra of the isomeric mixture **5** showed similar sets of signals for all the isomeric components, thus indicating the same basic structure resulting through amide cleavage for all the isomers. The formation of **5** could be explained on the basis of the postulated intermediate **Z4** (Fig. 2). It appears that attack of MeOH on C(6), or the intramolecular nucleophilic attack by C(9)–O on C(6), are indeed, not the favoured pathways for **Z4**. Instead, it undergoes attack by MeOH on the electrophilic amide carbonyl, leading to the amide cleavage product **5**. Alternatively, the zwitterion **Z4** could undergo cleavage leading to a hydroxy ketene, which is then trapped by MeOH.[6]

The phototransformation of **4** into **5** features the cleavage of the strongest (amide) bond, with retention of the tricarbonyl backbone, in the unprotected multi-



Scheme 1. Photochemical amide cleavage reactions of ascomycin derivatives and their further transformations.

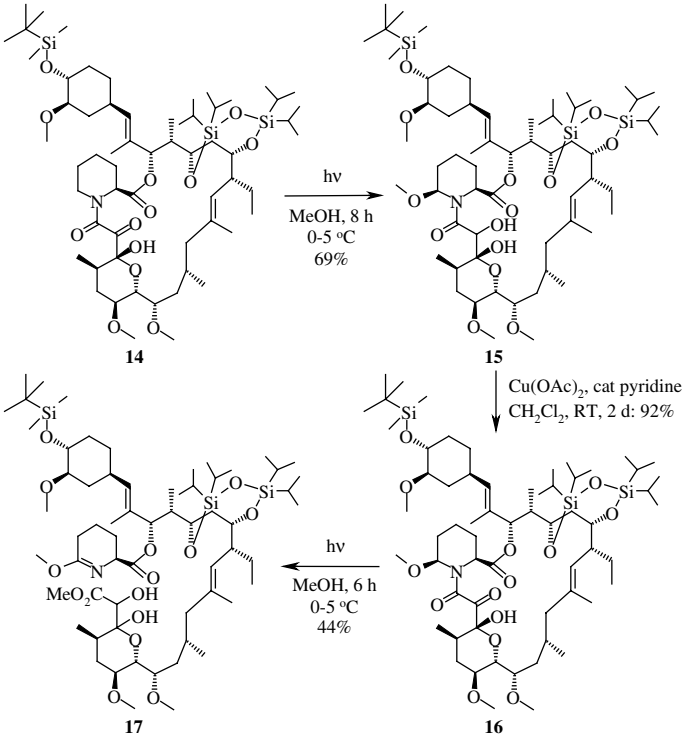
functional molecule ascomycin under simple reaction conditions and hence is very attractive. To date there are no methods available for selective cleavage of the amide bond in ascomycin even after introducing suitable protecting groups, especially because of the sensitive tricarbonyl moiety. Previously modifications in the amino acid region have involved cleavage of the C(1)–C(9) unit followed by reconstruction of the tricarbonyl and other units.[4,8] With a view to exploring the scope and potential of this photo amide cleavage reaction for developing a shorter semi-synthetic approach, we looked at the photoreaction of the protected ascomycins **6** and **8**. Compound **6** was prepared from **4** through silylation (**4**, 3 equiv *t*-BDMSCl, 3.5 equiv imidazole, DMF, 75 °C, 70%). The allyl protected derivative **8** was prepared from **6** through allyloxycarbonylation of C(10)–OH (**6**, 4.4 equiv allyloxycarbonyl chloride, 10 equiv DMAP, CH_3CN, rt, 4 h, 83%), followed by its decarboxylation[9] (0.02 equiv $Pd(Ph_3P)_4$, THF, 50 °C, 5 min, 89%) in a good overall yield.

Irradiation of **6** and **8** in MeOH, as described before, resulted in total consumption of the starting materials giving the amide cleavage products **7** and **9**, respectively, as mixtures of isomers. Single isomers of **7** and **9** could be isolated in 33% and 16% yields, respectively, after chromatography on SiO_2 pretreated with 5% $NaHCO_3$ and which were susequently characterised. The ^1H NMR spectrum of crude **9** showed the presence of varying amounts of its hydrolysis product **10**. In fact, the crude photolysate mixture could be transformed into **10** (58%) by treatment[10] with TMSCl/NaI/CH_3CN at rt for 28 h, and purification by chromatography, thus giving an experimental protocol leading to the isolation of stable single products in good yields.

It should be noted here that all the derivatives studied so far, except **8**, bear a free OH on C(10) of ascomycin.[5] In solution these hemiketal structures, featuring a dicarbonyl chromophore, exist in equilibrium with the corresponding tricarbonyl structures. Hence, one cannot be sure which chromophore is actually involved in the photoreaction. The successful photoreaction of **8**, featuring a locked dicarbonyl chromophore, clearly demonstrates that these reactions can, indeed, also proceed efficiently through the dicarbonyl chromophore even at the longer wavelengths (>366 nm) employed in the reaction, which are necessary for avoiding photoreaction at other sites.[5] This is a crucial finding as this allows for protection of the sensitive tricarbonyl moiety in the form of the dicarbonyl chromophore with retention of the photoreactivity.

The α-hydroxyesters **9** and **10** could be independently oxidised (Dess Martin, pyridine, 4 Å MS, CH_2Cl_2, rt, 3 h) leading to the ketoesters **12** (44%) and **11** (60%), respectively. Saponification (THF/H_2O; 3:1, 2 equiv of LiOH, rt, 4 h) of the ester **12** afforded the required acid **13** (35%) with concomitant hydrolysis of the imine-ether occurring either during the reaction or during acidic workup.

The above results have established the viability of this approach for a semi-synthetic strategy leading to analogues of **1** in which pipecolic acid is replaced by other amino acids. However, attempts to cleave the secondary ester in **13** resulted in formation of several products arising through ß-elimination of the 24-silyloxy moiety and subsequent reactions. To circumvent this problem from the beginning, we studied the photochemistry of the suitably protected derivative **14**[8] (Scheme 2), which



Scheme 2. Photoreactions of protected ascomycin derivatives.

was prepared from **1** through monosilylation of 33-OH, Evans' reduction of C(22)=O (1.1 equiv $Me_4N^+(OAc)_3HB^-$, CH_3CN/AcOH (100:35), −5 to 3 °C, 12 h, 70%), followed by silylation (3.0 equiv $(Cl^iPr_2Si)_2O$, 3.0 equiv imidazole, DMF, rt, 3 d, 70%).

Irradiation of **14** in MeOH for 8 h, followed by chromatography, afforded **15** (69%), which after oxidation with Cu(II) acetate gave the 6-methoxy derivative **16** (92%), which is ready for the photochemical amide cleavage. Indeed, irradiation of **16** in MeOH for 6 h followed by chromatography led to the amide cleavage product **17** (44%). This establishes a crucial reaction in the context of the semi-synthetic strategy.

In summary, we have shown that (6*S*)-methoxyascomycin and several of its protected derivatives undergo a novel amide cleavage reaction upon irradiation in useful yields. The selective cleavage of an amide bond in an unprotected multifunctional molecule such as ascomycin is a remarkable finding. The applicability of this reaction for developing a semi-synthetic strategy leading to analogues of **1** modified in the amino acid region will be further explored.

Acknowledgements

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References and notes

1. (a) Arai, T.; Koyoma, Y.; Suenaga, T.; Honda, H. *J. Antibiot. Ser. A* **1962**, *15*, 231–232; (b) Hatanaka, H.; Iwami, M.; Kino, T.; Goto, T.; Okuhara, M. *J. Antibiot.* **1988**, *41*, 1586–1591; (c) Hatanaka, H.; Kino, T.; Miyata, S.; Inamura, N.; Kuroda, A.; Goto, T.; Tanaka, H.; Okuhara, M. *J. Antibiot.* **1988**, *41*, 1592–1601; (d) Morisaki, M.; Arai, T. *J. Antibiot.* **1992**, *45*, 126–128.
2. (a) Meingassner, J.; Grassberger, M.; Fahrngruber, H.; Moore, H. D.; Schuurman, H.; Stütz, A. *Br. J. Dermatol.* **1997**, *137*, 568–576; (b) Grassberger, M.; Baumruker, T.; Enz, A.; Hiestand, P.; Hultsch, T.; Kalthoff, F.; Schuler, W.; Schulz, M.; Werner, F.-J.; Winiski, A.; Wolff, B.; Zenke, G. *Br. J. Dermatol.* **1999**, *141*, 264–273; (c) Stütz, A.; Grassberger, M.; Meingassner, J. G. *Semin. Cutaneous Med. Surg.* **2001**, *20*, 233–241; (d) Hebert, A.; Warken, K. A.; Cherill, R. *Semin. Cutaneous Med. Surg.* **2001**, *20*, 260–267; (e) Wellington, K.; Jarvis, B. *Drugs* **2002**, *62*, 817–840; (f) Grassberger, M.; Meingassner, J.; Stütz, A.; Rappersberger, K.; Wolff, K. Ascomycins. In *Psoriasis*; Roenigk, H. H., Maibach, H. I., Eds.; 3rd ed.; Marcel Dekker: New York, Basel, Hong Kong, 1998; pp 769–779, Chapter 66; (g) Rappersberger, K.; Komar, M.; Ebelin, M. E.; Scott, G.; Burtin, P.; Greig, G.; Kehren, J.; Chibout, S. D.; Holter, W.; Richter, L.; Oberbauer, R.; Cordier, A.; Stütz, A.; Wolff, K. *J. Invest. Dermatol.* **2002**, *119*, 876–888; (h) Kapp, A.; Papp, K.; Bingham, A.; Fölster-Holst, R.; Ortonne, J. P.; Potter, P.; Gulliver, W.; Paul, C.; Molloy, S.; Barbier, N.; Hultsch, T.; de Prost, Y. *J. Allergy Clin. Immunol.* **2002**, *110*, 277–284; (i) Meurer, M.; Fölster-Holst, R.; Wozel, G.; Weidinger, G.; Jünger, M.; Bräutigam, M. *Dermatology* **2002**, *205*, 271–277; (j) Wahn, U.; Bos, J. D.; Goodfield, M.; Caputo, R.; Papp, K.; Manjra, A.; Dobozy, A.; Paul, C.; Molloy, S.; Graeber, M.; Cherill, R.; DeProst, Y. *Pediatrics* **2002**, *110*, e2; (k) Ho, V.; Gupta, A.; Kaufmann, R.; Todd, G.; Vanachlocha, F.; Takaoka, R.; Fölster-Holst, R.; Potter, P.; Marshall, K.; Thurston, M.; Bush, C.; Cherill, R. *J. Pediatr.* **2003**, *142*, 155–162.
3. VanDuyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *252*, 839–842.
4. (a) Askin, D.; Reamer, R. A.; Joe, D.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1990**, *55*, 5448–5450; (b) Askin, D.; Joe, D.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1990**, *55*, 5451–5454.
5. (a) Bulusu, A. R. C. M.; Haidl, E. H.; Schulz, G.; Waldstaetten, P.; Grassberger, M. *Indian J. Chem.* **1999**, *38B*, 1159–1164; (b) Bulusu, A. *EP 0626385A*; Sandoz Ltd, 1994 (c) Bulusu, A. R. C. M.; Waldstätten, P.; Schulz, G.; Grassberger, M. *Tetrahedron Lett.* **2004**, *45*, 2523.
6. (a) Chesta, C. A.; Whitten, D. G. *J. Am. Chem. Soc.* **1992**, *114*, 2188–2197; (b) Aoyama, H.; Hasegawa, T.; Watabe, M.; Shiraishi, H.; Omote, Y. *J. Org. Chem.* **1978**, *43*, 419–422; (c) Aoyama, H.; Sakamoto, M.; Kuwabara, K.; Yoshida, K.; Omote, Y. *J. Am. Chem. Soc.* **1983**, *105*, 1958–1964; (d) Aoyama, H.; Hasegawa, T.; Omote, Y. *J. Am. Chem. Soc.* **1979**, *101*, 5343–5347.
7. All compounds were fully characterised using ^1H NMR (250, 400 or 500 MHz), ^{13}C NMR (100 or 125 MHz) and mass spectral data and elemental analysis. The ^1H and ^{13}C-signal assignments for representative compounds were made through C–H correlation spectra and ^1H-decoupling experiments.
8. Horvath, A. Ph.D. Thesis, University of Vienna, **1993**.
9. Guibe, F.; Saint M'Leux, Y. *Tetrahedron Lett.* **1981**, *22*, 3591–3594.
10. Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247–1251.