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Selective transformation of ascomycin into 11-*epi*-ascomycin

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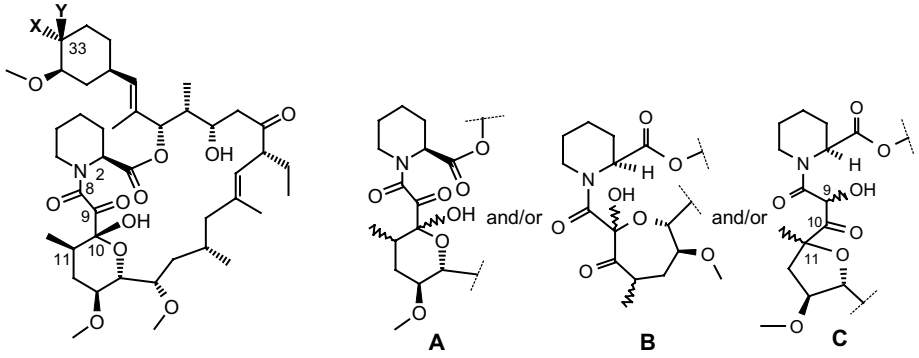
Abstract—Within the binding domain, ascomycin features the unusual pattern of a masked tricarbonyl moiety, which potentially allows for high structural diversity via simple isomerisation events. A cascade of diastereoselective rearrangement reactions at the binding domain, allowing the conversion of ascomycin into 11-*epi*-ascomycin is herein reported.

Ascomycin (**1**, Fig. 1) is a macrolactam isolated from the fermentation broth of *Streptomyces hygroscopicus* var. *ascomyceticus*.[1] Pimecrolimus (**2**, Elidel®, SDZ ASM 981), the 33-*epi*-chloro derivative of ascomycin, which has recently been introduced into the market, heralds major advances in the treatment of inflammatory skin diseases.[2] Ascomycin and related macrolactams feature in the 'binding domain' the unusual pattern of three adjacent carbonyl groups (C8–C10), whereby one carbonyl group (C10) is involved in hemiketal formation.[3] Although the structure shown in Figure 1 is the main isomeric form adopted in organic solution,[4] the close proximity of the tricarbonyl portion to the hydroxyl group at C-14 potentially allows the formation of numerous alternative isomers, that is, liberation and enolisation of the tricarbonyl portion followed by unspecific rehemiketalisations could give rise to the formation of several diastereoisomeric six- and seven-membered hemiketal forms together with a set of diastereoisomeric furano-analogues (**A**, **B**, **C**).[5,6]

Isomerisation and tautomerisation make it difficult to determine the purity of a drug substance. However, for the development of a compound as a drug it is mandatory to prove its purity unambiguously. In case of an ascomycin-derived drug substance, it is essential to



1 ascomycin (X=OH, Y=H)
2 pimecrolimus (X=H, Y=Cl)

Figure 1.

Keywords: Ascomycin; Epimerisation; Rearrangement; Binding domain; Tricarbonyl.
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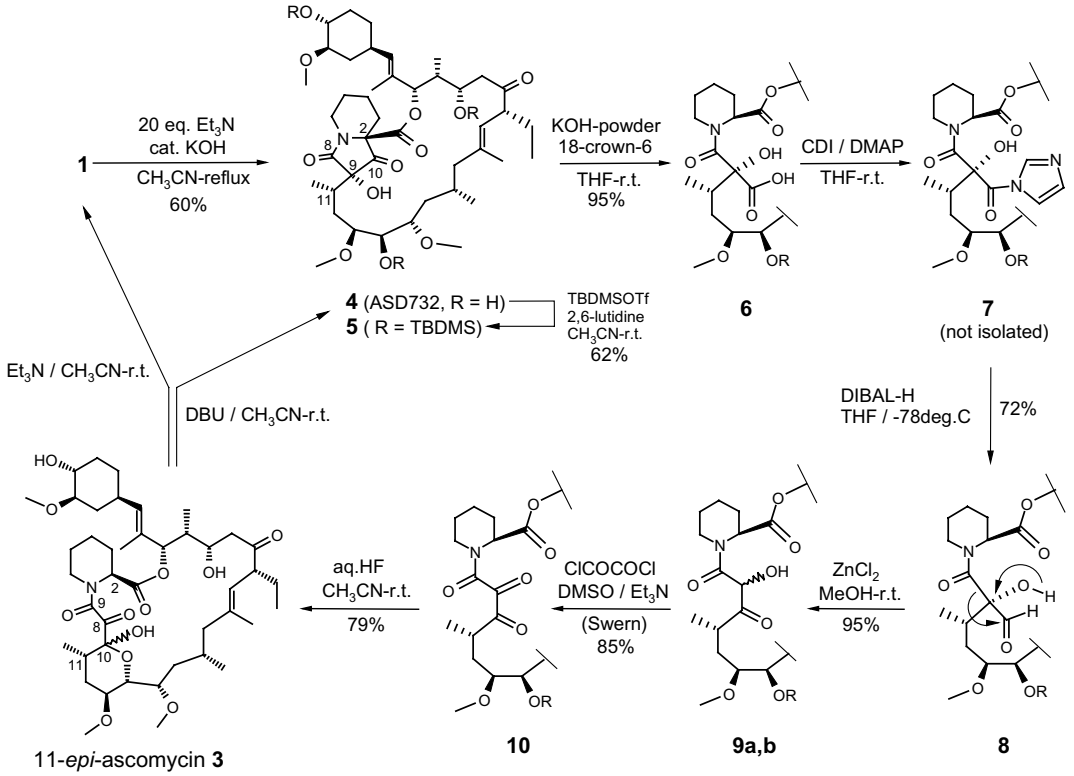
differentiate unmistakably between 'real by-products' and inherent drug substance related equilibrium compounds. To achieve this goal, either the isolation or the synthesis of each potential equilibrium- and by-product and/or the development of suitable powerful analytical tools, which allow their clean discrimination and assignment, even in complex mixtures (such as in galenical formulations), is mandatory. In previous communications we have reported on the selective conversion of ascomycin into a seven-membered hemiketal form **B**[6] and into four diastereoisomeric furano isomers **C**.[7] In an extension of this work, we describe here a concise synthesis of 11-*epi*-ascomycin (Scheme 1).

For the synthesis of 11-*epi*-ascomycin **3** we focused on a ring expansion strategy, starting from 2(*R*),10(*S*),11(*S*)-cyclo-ascomycin **4** (ASD 732). The latter is readily available in a single step from unprotected ascomycin applying excess triethylamine in the presence of catalytic amounts of powdered potassium hydroxide in refluxing acetonitrile.[8] ASD 732 already possesses the required *S*-configuration at C11, but also has a branched (rearranged) C8–C10 chain with an additional ring element. Thus, for the conversion of ASD 732 **4** into 11-*epi*-ascomycin **3**, a reassembling of the C8 to C11 skeleton into a linear carbon chain is required. Anticipating that an acyloin rearrangement (i.e., **8** → **9a**,**b** in Scheme 1) might be productive for this purpose, we aimed at the conversion of ASD 732 **4** into the α-hydroxy aldehyde **8**. The synthesis of **8** started with the protection of the 14-, 24- and 33-hydroxy groups of ASD 732 **4**. Thus, reaction with *tert*-butyldimethylsilyl triflate (TBDMSOTf, 4 equiv) in the presence of 2,6-lutidine (15 equiv) in acetonitrile solution at room temperature (15 min) provided after a chromatographic purification 14,24,33-tris-OTBDMS-ASD 732 **5** in reasonable yield (62%). Treatment of **5** with an excess of powdered potassium hydroxide (2 equiv) in presence of 18-crown-6 (8 equiv) in tetrahydrofuran at room temperature accomplished, in an irreversible and stereoselective fashion, cleavage of the pyrrolidine-dione ring (*retro*-ester condensation) to yield the α-hydroxy acid **6** almost quantitatively. Next, production of the desired α-hydroxy aldehyde **8** required a chemoselective reduction of the carboxyl function in the presence of other sensitive functionalities (i.e., the 22-carbonyl group and the ester and amide functionalities). This could be achieved by conversion of the α-hydroxy acid into the corresponding imidazolide **7** (3 equiv 1,1-carbonyldiimidazole, 3 equiv DMAP, THF—rt), followed by the action of diisobutylaluminiumhydride (DIBAL-H, 8 equiv) in THF at 5 °C, to give after chromatographic purification, the desired α-hydroxy aldehyde **8** (72% from **6**). Having accomplished the synthesis of the key intermediate **8**, its anticipated propensity to undergo the expected ring enlargement to the basic skeleton of 11-*epi*-ascomycin could be explored. Gratifyingly, the first experiment was successful. Thus, exposure of **8** to zinc chloride (10 equiv) in methanol (rt; 40 min) provided in a regioselective manner (no 10-dihydro derivatives are formed), the tris-OTBDMS-protected 9(*R*)- and 9(*S*),11(*S*)-9-dihydro-ascomycin derivatives **9a**,**b** almost quantitatively. Swern oxidation of the diastereoisomeric mixture **9a**,**b** gave the characteristically yellow-coloured 14,24,33-tris-



Scheme 1.

OTBDMS-11-*epi*-ascomycin **10**. Finally, conventional removal of the TBDMS-protecting groups (5% aq HF–acetonitrile) completed the conversion of ascomycin into 11-*epi*-ascomycin **3** via ASD 732 **4**.

In contrast to ascomycin, which exists in $CDCl_3$ solution as a mixture of six- and seven-membered hemiketal forms (ratio ~ 15:1), 11-*epi*-ascomycin adopts exclusively two diastereoisomeric [10(*S*) and 10(*R*)] six-membered hemiketal forms in the ratio 4:1. Whether the 10(*R*) or the 10(*S*) isomer represents the major constituent could not be determined as no diagnostically relevant NOEs could be observed. As deduced from two-dimensional NMR experiments, the major isomer exists as a mixture of rotamers with respect to the geometry (*E* vs *Z* = 1:1) at the amide bond, whereas for the minor isomer >90% of the *trans*-amide configuration is observed.[9,10] Notably, 11-*epi*-ascomycin is stable towards acidic reaction conditions (e.g., the conditions used for its deprotection, **10** → **3** in Scheme 1), whereas under weakly basic conditions (triethylamine in acetonitrile solution at rt) a rapid and complete conversion into ascomycin is observed. In contrast, applying the same reaction conditions to ascomycin, no formation of 11-*epi*-ascomycin is detectable. Upon storage of ascomycin in protic or aprotic organic solutions or aqueous-organic solutions, no formation of 11-*epi*-ascomycin could be detected as well. Surprisingly, upon treatment of 11-*epi*-ascomycin with the strong base DBU in acetonitrile solution at room temperature, ASD 732 **4** is rapidly formed as the sole product together with only minor amounts of ascomycin. As the mechanism of the conversion of ascomycin itself into ASD 732, which requires epimerisation, rearrangement and ring formation, is not yet fully understood, the smooth conversion of 11-*epi*-ascomycin into ASD 732 **4** gives a first hint that this peculiar transformation might proceed via a C11-epimerisation as the first step. More detailed mechanistic investigations of this reaction are ongoing and will be reported in due course.

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10. ^{13}C NMR ($CDCl_3$): δ (*cis*/*trans*-major isomer/*trans*-minor isomer, ppm): 169.99/170.26/168.65 (C1); +/52.44/52.66 (C2); 26.79/27.53/26.72 (C3); 20.83/22.01/21.06 (C4); 23.92/24.67/24.85 (C5); 40.31/44.70/44.09 (C6); 167.10*/167.13*/164.44 (C8); 199.01/193.65/200.36 (C9); 99.10/98.93/99.80 (C10); 29.28/34.88/32.39 (C11); 28.99/++/++ (C12); 71.10/70.96/n.d. (C13); 72.91/72.91/75.10 (C14); 80.61/76.32/76.95 (C15); 36.51/32.61/32.33 (C16); 27.32*/26.81*/26.47 (C17); 50.00/49.01/48.64 (C18); 138.85/139.26/140.39 (C19); 124.45/123.59/123.38 (C20); 55.18/54.38/55.42 (C21); 214.10/213.32/214.67 (C22); 44.59/44.59/43.51 (C23); 69.96/70.18/69.63 (C24); 40.16*/39.98*/40.77 (C25); 78.10/77.77/78.70 (C26); 131.98/132.33/131.96 (C27); 14.78/14.49/14.78 (C28); 129.31/130.95/128.83 (C29); 35.32/35.46/35.32 (C30); 35.25/35.02/34.62 (C31); 84.74*/84.57*/84.61 (C32); 73.95/73.95/73.98 (C33); 31.60/31.60/31.72 (C34); ++/++/++ (C35); 25.33/25.08/25.33 (C36); 11.97*/11.89*/12.09 (C37); +/+/+ (13-OMe); 57.50/57.81/57.57 (15-OMe); +/+/+ (32-OMe); +++/15.61/15.76 (11-Me); 21.22/20.46/19.56 (17-Me); +++/16.44/+++ (19-Me); 10.31*/10.22*/10.38 (25-Me). (*) Opposite *E*/*Z*-assignment possible; (+) one of seven signals between 56.58 and 56.96; (++) one of the five signals at 31.14, 31.09, 30.95, 30.79 and 30.73; (+++) one of three signals at 16.17, 16.14 and 16.08.