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Selective Synthesis of a New Ascomycin Rearrangement Product (SDZ ASD732) on a Pilot Plant Scale

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Abstract:

The process for a pilot plant synthesis and the purification of a semisynthetic ascomycin derivative (1, SDZ ASD732) showing interesting biological activities is described. Conditions for the key transformation, a base-induced cascade of rearrangement and epimerization reactions of ascomycin leading to the selective formation of the new ascomycin derivative 1, were developed and successfully upscaled. Rapid screening of important reaction parameters was achieved by using automated parallel synthesis. In addition, a highly efficient purification process was found and implemented in the pilot plant process, resulting in an efficient separation of the semisynthetic ascomycin derivative from its by-products. The purification protocol consisted of preparative silica gel chromatography followed by a crystallisation step. Starting from crystalline ascomycin with a purity of ≥95%, an overall yield of 53% was achieved with a final purity of >98%.

Introduction

The macrolide ascomycin **2**, which was first isolated from *Streptomyces hygroscopicus* fermentation cultures,[1−3] has elicited great interest among medicinal chemists. It has been shown that **2**, upon treatment with nucleophiles or strong bases,[4,5] undergoes a wide variety of rearrangement-, cyclization-, cleavage-, and epimerization reactions. Careful investigation of the reaction conditions allowed us to obtain a clean conversion of ascomycin **2** to its rearrangement product **1** in a one-pot procedure. This ascomycin derivative, (*E*)-(*4S,5R,6S,13S,15S,16R,17S*)-9-ethyl-6,16,20-trihydroxy-4-[(*E*)-2-((*1R,3R,4R*)-4-hydroxy-3-methoxy-cyclohexyl)-1-methyl-vinyl]-15,17-dimethoxy-5,11,13,19-tetramethyl-3-oxa-22-aza-tricyclo[18.6.1.0]-1,22-heptacos-10-ene-2,8,21,27-tetraone **1**, (the structure is given in Figure 1) is a representative of a novel subclass of ascomycin rearrangement products, characterised by a profile of biological activities which is clearly distinct from that of ascomycin (**2**) or FK-506 (**3**) (Figure 1).

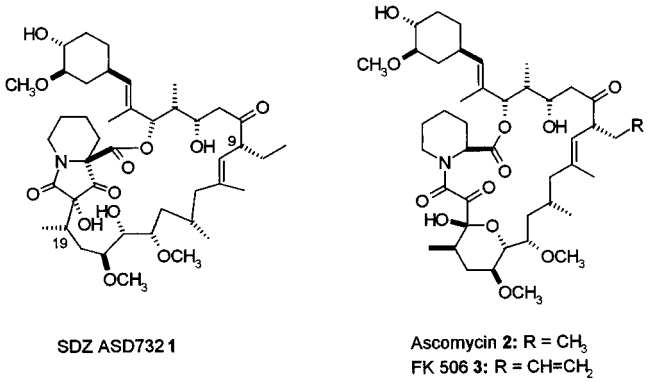

Figure 1.

In contrast to FK-506, **1** does not inhibit T-cell activation in vitro and is not active in a model of immunosuppression in vivo. It has been proposed for topical treatment as well as for oral treatment of allergic contact dermatitis, psoriasis, and atopic dermatitis.[6]

Results and Discussion

Semisynthetic **1** is prepared in a one-step chemical transformation starting from purified ascomycin **2** as summarised in Scheme 1. To achieve a reproducible high yielding process for the conversion of **2** to its rearrangement derivative **1**, a high quality (>95%) of starting material was found to be important. Thus, a highly efficient purification protocol for ascomycin had to be developed and implemented. This is necessary because the fermentation by-products, present in less pure starting material, undergo analogous chemical transformations causing the chromatographic removal of the corresponding impurities from the drug substance **1** to be rather difficult. This results in a lower overall yield of the process. The finally performed process chosen for the manufacturing of hundreds of kilograms of **1** is a combination of a chemical transformation (step 1), followed by silica gel chromatography (step 2) and crystallization (step 3).

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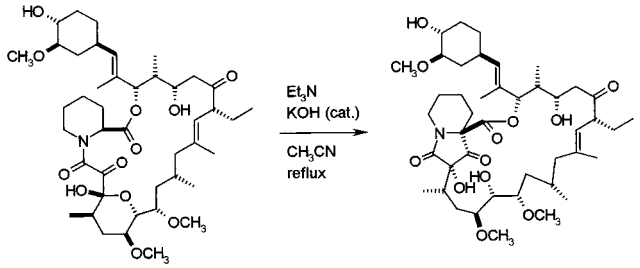
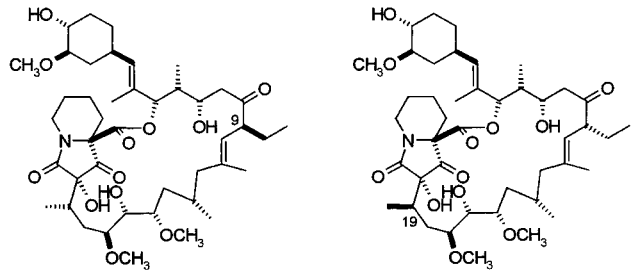
Scheme 1



Optimization of Ascomycin Rearrangement (Step 1). In the original medicinal chemistry synthesis, unprotected ascomycin **1** was treated with an excess of triethylamine (which had been dried over potassium hydroxide in refluxing acetonitrile). Under these reaction conditions the ascomycin derivative **2** was formed in moderate yield. The two most dominant by-products formed were identified as the 9- (**4**) and 19-epimer (**5**) of **1** (Figure 2). These structures were assigned on the basis of spectroscopic data together with X-ray analysis.[7] A mechanistic study of this rearrangement is in progress and will be published elsewhere.[8]



9-epimer **4** 19-epimer **5**

Figure 2.

Preliminary experiments had already shown that $Ca(OH)_2$ and $Ba(OH)_2$ were significantly weaker promotors for the desired transformation, resulting in a lower conversion of starting material **2** to its corresponding rearrangement product **1**. Thus, several experiments were conducted to improve the yield and suppress the formation of isomers **4** and **5**. The effect of various combinations of base was studied using the Anachem SK233, a commercial laboratory automated parallel solution-phase synthesis system.[9] This straightforward experimental setup allowed us to rapidly screen and identify the most critical reaction parameters. The presence of catalytic amounts of potassium hydroxide was found to be crucial. However, addition of larger amounts of KOH resulted in the preferential formation of isomer **4**. Initial experiments were designed to screen a series of tertiary amine bases with different steric and electronic properties. Potassium *tert*-butoxide was investigated as an alternative catalyst to potassium hydroxide. A total of 24 screening experiments, using catalytic amounts (0.1 equiv) of potassium *tert*-butoxide or KOH in combination with 0.5, 1.0, or 5.0 equiv of a tertiary amine base, were conducted. The results with triethylamine, pyridine, and tetramethylpiperidine in combination with KOH or *tert*-BuOK are summarised in Charts 1−4. The use of DBU mainly resulted in the formation of polar decomposition products of **2**.

Chart 1. **Triethylamine**

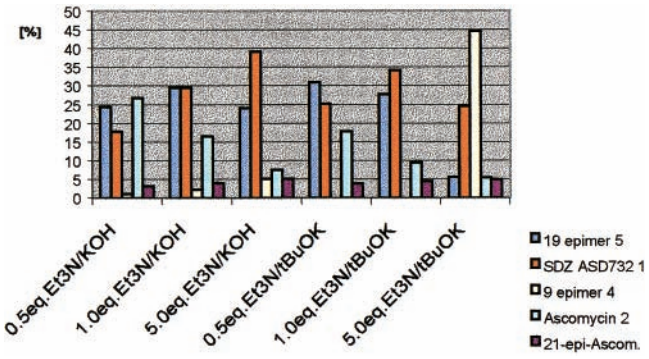


Chart 2. **Pyridine**

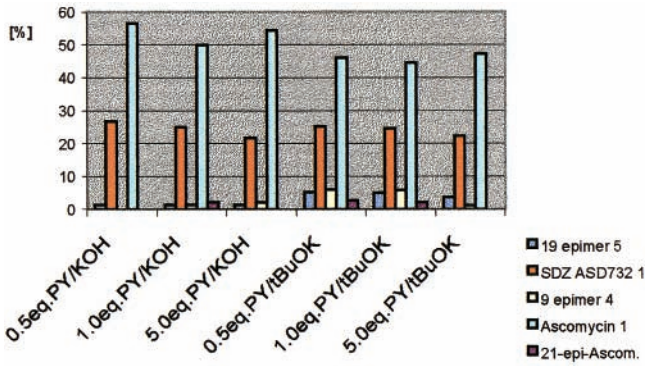


Chart 3. **Tetramethylpiperidine**

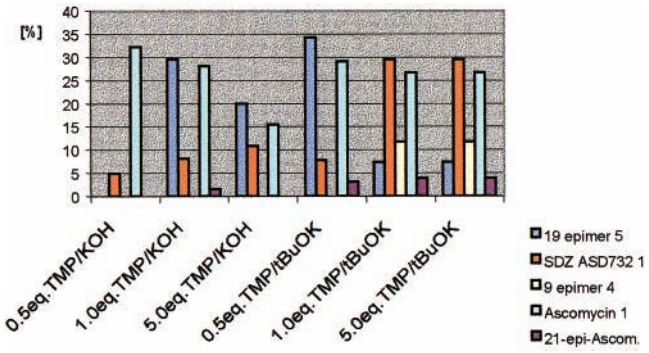
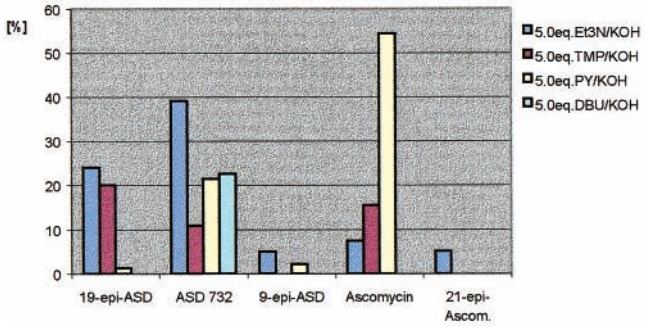


Chart 4. **Summary**



The best results were obtained when using an excess of sterically flexible tertiary amines such as triethylamine in

(7) Baumann, K. Unpublished results
(8) Baumann, K. et al. Manuscript in preparation.
(9) For a review, see: Armitage, M. A.; Smith, E. G.; Veal, K. T. *Org, Process Res. Dev.* **1999**, *3*, 189.

the presence of 0.1 equiv of KOH. After having elaborated the best reaction conditions, scale-up experiments were carried out manually on a multigram scale. According to the previous results from the screening experiments, additional amine bases were investigated in the rearrangement process. Again, the best results were achieved when tertiary amines, for example, triethylamine or tributylamine, were used. A selection of results is summarised in Table 1.

Table 1. **Scale-Up Results for the Synthesis of 1**[a]

entry	base	yield [%]
1	triethylamine	55
2	tri-*n*-butylamine	51
3	*N*-methylmorpholine	30
4	ethyldiisopropylamine	45
5	diisopropylamine	47
6	di-*n*-propylamine	42
7	diazabicyclooctane	32
8	DBU	<5
9	tetramethylguanidine	<5
10	tetramethylpiperidine	13

[a] All experiments were conducted in refluxing acetonitrile for 15 h using 6 equiv of the indicated base and 1.6 mol % of powdered KOH as promotor.

The formation of the ascomycin derivative **1** is a consequence of a cascade of epimerization and isomerization reactions of ascomycin **2** followed by the rearrangement of a hydroxy ketone unit. Under the reaction conditions employed numerous equilibrium isomers of ascomycin derivatives could potentially coexist.[5d] As the reaction mixture is at least partially heterogeneous, their individual reactivity is expected to be strongly influenced by the solvent. The best results were achieved in polar nonprotic solvents such as propionitrile and acetonitrile. Protic solvents led to increasing amounts of polar products derived from decomposition of the highly sensitive ascomycin core. The presence of trace amounts of water also led to a significant reduction of yield this being most likely due to starting material decomposition. In nonpolar solvents low reactivity was observed, probably due to the low solubility of the basic catalysts (Table 2).

Table 2. **Solvent Effects**[a]

entry	solvent	yield [%]
1	acetonitrile	65
2	DMF	22
3	ethanol	5
4	toluene	28
5	propionitrile	51

[a] Ascomycin **2** was treated with triethylamine (6 equiv) and powdered KOH (2 × 1.6 mol %) at 80 °C for 15 h in the apropriate solvent.

Yields of up to 65% of the desired ascomycin derivative **2** were obtained when a solution of ascomycin **1** was treated with triethylamine, as a weak base, and adding a catalytic amount (1.6 mol %) of a 1 N potassium hydroxide solution in ethanol in refluxing acetonitrile. Addition of a second portion of the catalyst after 5 h was necessary to drive the reaction to completion. It is thought that catalyst deactivation occurs due to the consumption of KOH by side reactions leading to decomposition products. Importantly, sequential addition of the catalyst significantly minimises the formation of the 9-epimer **4.** A typical HPLC chromatogram of crude **1** (the conditions are given in the Experimental Section) is shown in Figure 3. The assay for **1** is only 44.9%, and the values for the major (critical) impurities are as follows: 19-epimer **5**, (5.4 area %); 9-epimer **4**, (10.9 area %); and ascomycin **2**, (2.8 area %). The presence of the by-products X1 and X4 is not critical, because both compounds can easily be removed during chromatography or crystallisation.

Optimization of Silica Gel Chromatography (Step 2). Crude **1** was purified in the next process step by silica gel chromatography.[10] Many crystallisation experiments were performed to investigate the feasibility of omitting this time-consuming purification step; however, in all experiments no significant decrease of the most critical by-product, the 9-epimer **4,** was observed. Interestingly, the amount of 19-epimer **5** could be decreased from about 8 to 1% by using a mixture of *tert*-butyl-methyl-ether (TBME) and ethyl acetate (ESTP) in one step with practically no loss of drug substance (Table 3). Since the 19-epimer **5** (around 10%) tends to crystallise during chromatography, a prerequisite for chromatography is that the content of this compound in the mixture must be limited in the feed to <10%. As back-up strategy for those cases where higher amounts were present, prior removal via crystallisation was studied. Good solubilities for **2**, **4**, and **5** in the solvent selected for the chromatographic process had therefore to be taken into consideration.

Ethyl acetate/hexane/acetone = 6.3/2.0/7.9 (m/m/m) was evaluated as a suitable mobile phase which demonstrated good solubilities for **2**, **4**, and **5** as well as good discrimination properties on silica gel for these three key compounds. In addition, a high capacity for the stationary phase was observed. Thus, 0.36 kg of crude **1** could be injected in a single run on a preparative column containing 25 kg of silica gel. Using an in-house developed on-line HPLC-apparatus, an on-line performed tailor-made fractionation of the eluate was accomplished. Work-up of the main fraction delivered purified **2** with 97% yield.

Final Crystallisation (Step 3). It is easy to design the chromatography fractionation in such a way that the evaporation residue of chromatographed **1** meets the requirement of >98% purity. Nevertheless, the compound was finally crystallised in order to obtain a product with a desired particle size. This was accomplished as follows: The evaporation residue of the main fraction from the chromatography was dissolved in ethyl methyl ketone and seeded in two portions with a suspension containing 15% crystals of **1** (based on the initial amount of **1**) in the same solvent. During this final purification step, the amount of 9-epimer **4** was somewhat decreased to 0.05% and the amount of ascomycin to 0.04%, respectively. The by-products X2 and X3 in Figure 3, being ascomycin derivatives, are not removed during the crystal-

(10) The purification of ascomycin derivative **1** with SMB was published elsewhere: Kuesters, E.; Heuer, C.; Wieckhusen, D. *J. Chromatogr. A* **2000**, *874 (2),* 155.

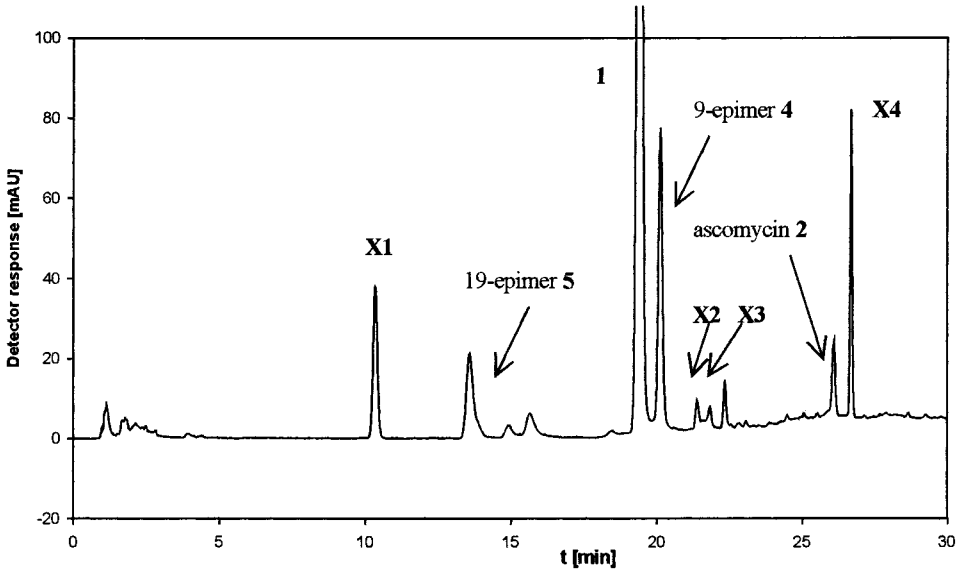


Figure 3. **HPLC analysis of crude 1 after chemical-mediated cyclization of ascomycin. For chromatographic conditions, see Experimental Section.**

Table 3. **Solvent Selection for the Removal of 19-Epimer 5 through Crystallisation (Initial Amount: 7.1%)**

solvent	ethyl acetate	acetone	TBME/ethyl acetate = 1/1	TBME	THF	methanol	propanol
concentration (**2**/solv. = g/ml)	1:10	1:13	1:13	1:34	1:4	1:4	1:4
temp (crystallization)	−10 °C	−10 °C	−10 °C	0 °C	0 °C	0 °C	0 °C
yield of **2**	> 99%	> 99%	> 99%	92%	> 99%	99%	97%
remaining 19-epimer **5**	2.1%	7.8%	0.8%	1.0%	4.7%	8.3%	3.6%

lisation and the previous performed purification procedure. Their amount in **1** corresponded to that observed in the ascomycin raw material obtained from the fermentation process. It is not necessary to remove these two compounds, because their occurrence and amounts can be limited during the work-up of the fermentation process. Nevertheless, as a backup variant, it was possible to remove them during the chromatographic process; however, lower yields result.

Conclusions

Starting from purified ascomycin of high quality, a highly efficient one-step process to the rather complex rearrangement product SDZ ASD732 was developed. In fact, due to the achieved selectivity of the chemical transformation step, which resulted in significantly less by-product formation, a remarkable simplification of the purification step was achieved. All isomers, which are formed in minor amounts, can be effectively removed simply by silica gel chromatography followed by crystallisation. The process was successfully scaled up to multikilogram batches in the pilot plant environment.

Experimental Section:

Materials. Solvents and reagents were obtained from commercial sources and used without further purification unless otherwise indicated. Ascomycin was purchased from Biochemie Kundl and purified prior to use.

Analytics. High-performance liquid chromatography (Figure 3) was performed using a Hewlett-Packard 1100 system with UV-detection was used. The analytical separations were performed on a YMC ODS AM silica gel (5 μm) analytical column (250 mm × 3 mm i.d.). A water/acetonitrile gradient was used in the following manner:

solvent A: water/acetonitrile = 9:1 (V/V)
solvent B: water/acetonitrile = 2:8 (V/V)

time (min)	solvent A (%)	solvent B (%)
0	50	50
15.0	50	50
25.0	0	100
30.0	0	100

The flow rate was kept at 1.0 mL/min, and the column temperature, at 70 °C. UV-detection was performed at 205 nm, and the injected volume was 10 μL (concentration: 10 mg/10 mL acetonitrile).

Procedure for the Ascomycin Rearrangement Step. Crystallised ascomycin (31.8 kg (98.1%) and 19.3 kg (97.0%)) was dissolved in 529 l toluene at 70 °C jacket temperature in a 630 L stainless steel reaction vessel and the solvent was distilled at 60 °C jacket temperature to effect azeotropic water removal. The residue was redissolved in 427 L of acetonitrile, and 37.96 kg of triethylamine and 56.5 g of potassium hydroxide (90%) were added. The suspension was refluxed at 74 °C internal temperature (corresponding to a jacket temperature of about 100 °C), for 7 h. Afterwards, the suspension was cooled to an internal temperature of 50 °C, and a second portion of 56.5 g of potassium hydroxide (90%) was added. The suspension was again refluxed at 74

°C internal temperature for 5 h, and finally the solvents were removed by distillation at a jacket temperature of 60 °C/ process vacuum. The residue was dissolved in 136 L of ethyl methyl ketone at 60 °C internal temperature, and 80.0 L heptane was added. On cooling to room temperature (20−25 °C) the product precipitated within 2.5 h. The suspension was filtered via a centrifuge (80 cm i.d.) equipped with a Meraklon filter plate. The product was washed with 34 L of ethyl methyl ketone/heptane = 1/4 (V/V) and dried at 60 °C/< 20 mbar. Yield: 33.6 kg (assay: 78.9%, purity: 82.5%, **4**: 11.8%).

Silica Gel Chromatography. A suspension of 24.6 kg of silica gel (Kromasil KR100-10-SIL) in 60 L of 2-propanol was transferred into a Prochrom HPLC-DAC-column (30 cm i.d.) and compressed. The required final bed height was achieved by pumping more 2-propanol through the column at 50 bar for 45 min followed by ethyl acetate at the same pressure within 30 min. The column was then equilibrated with the mobile phase until a constant UV-signal was obtained. The mobile phase consisted of ethyl acetate/hexane/acetone = 63.0/20.1/7.9 (m/m/m). Crude product from step 1, 0.354 kg, was dissolved in 1.1 L of ethyl acetate at 40 °C, and the solution was filtered. The feed solution was then injected onto the column and the product chromatographed with the above-mentioned mobile phase at a flow rate of 2 L/min which corresponded to a back pressure of 48 bar. In addition, the elution profile was monitored using an on-line HPLC-system, which analyzes the eluate by-pass in intervals of 1 min. A total of 190 L ofl mobile phase was used for complete elution. Fractions, complying with the specifications, were collected, and the solvents were removed by distillation at a jacket temperature of 50 °C. Those fractions not fulfilling the specifications were subjected to re-chromatography. Yield: 0.272 kg (97.4%, **4**: < 0.1%). The residue obtained was dissolved in the 10-fold amount of acetone by weight and stored in solution.

Final Crystallization. The evaporation residue (38.6 kg) from the chromatography step was dissolved in 181.2 L ofmethyl ethyl ketone at 70 °C internal temperature, and the hot solution was filtered (0.45 μm) and transferred to a preheated (70 °C jacket temperature) reaction vessel. The seed crystals were prepared as follows: to a saturated solution of 200 g of prepurified and micronized final product in 1.8 L of ethyl methyl ketone a further 400 g of prepurified and micronized final product and 1.8 l L of ethyl methyl ketone were added. The seed crystals thus obtained (as a suspension) were added, in two portions, into the reaction vessel within 15 min. The internal temperature was adjusted to −10 °C within 10 h, and the suspension was stirred for a further 12 h. The suspension was then filtered via a centrifuge (60 cm i.d.) containing a Meraklon filter plate. The product was washed with 19 L of ethyl methyl ketone (−10 °C), and the wet product was dried at 70 °C/< 20 mbar. Yield: 36.6 kg fulfilling the required specifications.

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