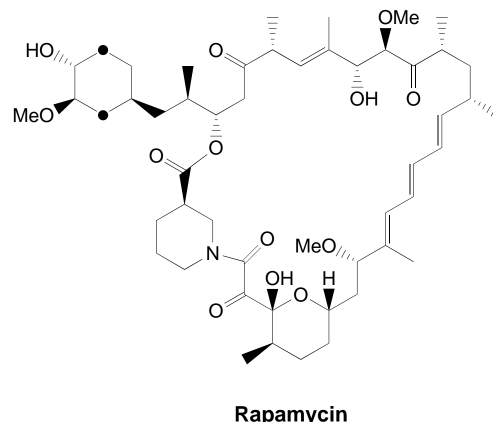
[10] a) A. Müller, F. Peters, M. T. Pope, D. Gatteschi, *Chem. Rev.* **1998**, *98*, 239–271; b) M. T. Pope, *Heteropoly and Isopoly Oxometalates*, Springer, Berlin, **1983**; c) M. T. Pope, A. Müller, *Angew. Chem.* **1991**, *103*, 56; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 34.
[11] a) S. A. Cohen, E. I. Stiefel, *Inorg. Chem.* **1985**, *24*, 4657–4662; b) V. Béreau, E. Cadot, H. Bögge, A. Müller, F. Sécheresse, *Inorg. Chem.* **1999**, *38*, 5803–5808.
[12] D. Coucouvanis, A. Toupadakis, J. D. Lane, S. M. Koo, C. G. Kim, A. Hadjikyriakou, *J. Am. Chem. Soc.* **1991**, *113*, 5271.
[13] a) R. Blessing, *Acta Crystallogr. Sect. A* **1995**, *51*, 33; b) G. M. Sheldrick, a program for the Siemens Area Detector ABSorption correction, University of Göttingen, Germany, **1997**.
[14] G. M. Sheldrick, SHELX-TL version 5.03, Software Package for the Crystal Structure Determination Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA, **1994**.

Origin and True Nature of the Starter Unit for the Rapamycin Polyketide Synthase**

Philip A. S. Lowden, Barrie Wilkinson, Günter A. Böhm, Sandeep Handa, Heinz G. Floss, Peter F. Leadlay, and James Staunton*

Rapamycin is a polyketide macrolide produced by *Streptomyces hygroscopicus* that displays potent immunosuppressant activity.[1] The recognition that such a property could be therapeutically useful has generated great interest in the chemistry[2] and biology[3] of rapamycin, and of the structurally similar immunosuppressants FK 506 and FK 520.

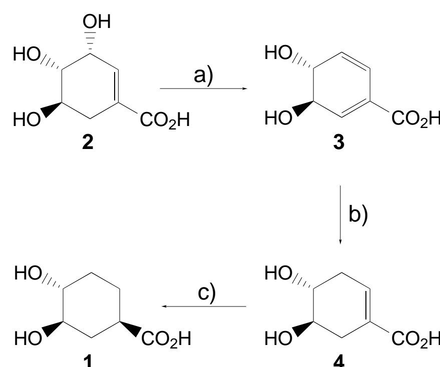
Rapid progress has been made in the manipulation of polyketide biosynthesis,[4] and the entire biosynthetic gene cluster for rapamycin has been sequenced.[5] The rapamycin polyketide synthase (PKS) is a modular system in which 14 chain extension modules are housed in three giant multimodular proteins (RAPS 1–3).[4, 5] The polyketide origin of rapamycin has been verified through feeding studies with labeled precursors.[6] Rapamycin, FK 506, and FK 520 share in common an unusual substituted cyclohexane ring, the origins of which have been traced to shikimic acid for rapamycin[7] and FK 520,[8] and which undergoes O-methylation post-PKS during the biosynthesis of FK 506.[9] The proximal precursor for this unusual starter unit for rapamycin biosynthesis has been shown to be (1*R*,3*R*,4*R*)-3,4-dihydroxycyclohexanecarboxylic acid (**1**, DHCHC).[10] That the saturated molecule **1** is



Rapamycin

the starter unit of the rapamycin PKS brings into question the function of an apparently catalytically competent enoyl reductase domain (ER) present in the initiation module,[5] the structure of which is also conserved for the PKSs of FK 506[11] and FK 520.[12]

The steps between shikimic acid (**2**) and **1** for FK 520 have been elucidated and are described in Scheme 1.[8, 13] An initial *anti*-1,4-conjugate elimination of water from **2** provides



Scheme 1. Biosynthetic pathway to DHCHC (**1**) in FK 520 and rapamycin. a) Stereospecific 1,4-*anti*-elimination of water; b) reduction of the Δ^1 double bond and isomerization of the remaining double bond from Δ^2 to Δ^1; c) reduction of the final Δ^1 double bond.

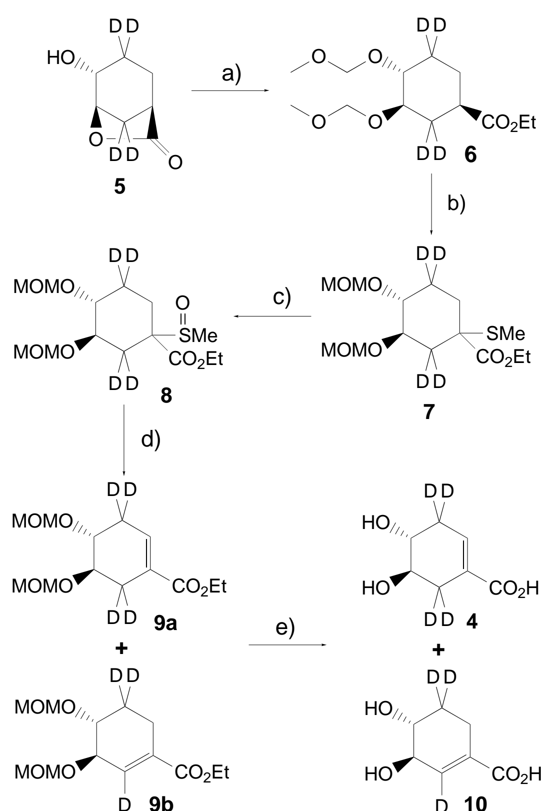
(4*R*,5*R*)-4,5-dihydroxycyclohexa-1,5-dienecarboxylic acid (**3**). The α,β-double bond is then reduced in a *syn* fashion and the system subsequently undergoes a suprafacial 1,3-allylic rearrangement to form 4,5-dihydroxycyclohex-1-enecarboxylic acid (**4**). A final *anti* reduction of the conjugated double bond provides **1**. This pathway is related to that which was elucidated for the cyclohexanecarboxylic acid (CHC) precursor of ω-cyclohexyl fatty acids in *Alicyclobacillus acidocaldarius*[14] and of ansatrienin A in *Streptomyces collinus*.[15] Herein we will attempt to address the question of the identity of the intermediates between shikimic acid and **1** during rapamycin biosynthesis.

[*] Prof. J. Staunton, Dr. P. A. S. Lowden, Dr. B. Wilkinson, Dr. G. A. Böhm
University Chemical Laboratory and
Cambridge Centre for Molecular Recognition
Lensfield Road, Cambridge CB2 1EW (UK)
Fax: (+44) 1223-762018
E-mail: js24@cam.ac.uk
Prof. P. F. Leadlay
Department of Biochemistry and
Cambridge Centre for Molecular Recognition
80 Tennis Court Road, Cambridge CB2 1GA (UK)
Dr. S. Handa, Prof. H. G. Floss
Department of Chemistry, Box 351700
University of Washington
Seattle, WA 98195-1700 (USA)

[**] We thank Dr. Bradley S. Moore for help with the deuterium NMR experiments. This work was supported by grants from The Wellcome Trust (to J.S. and P.F.L.) and from the NIH (AI20264 to H.G.F.).

Stereospecifically deuterated (−)-(6*R*)-[6-$^2H_1$]- and (−)-(6*S*)-[6-$^2H_1$]-shikimic acids[16] (see **2**) were fed separately (to provide a final concentration of 1 mM) to growing cultures of *S. hygroscopicus* (ATCC 29253) after 24 h. After a total of four days growth the mycelia were harvested, extracted with methanol, and the rapamycin was purified by reversed-phase HPLC. Analysis by ^{2}H NMR spectroscopy, liquid chromatography – electrospray mass spectrometry, and high-resolution mass spectrometry confirmed that the pro-6_R proton is stereospecifically lost during the biosynthesis of rapamycin; the specific incorporation of **2** was measured at 25 %. Thus, the conversion of **2** into **3** proceeds through an *anti*-1,4-conjugate elimination of water with loss of the pro-6_R proton, as do the analogous reactions during FK 520[13] and CHC biosynthesis.[16] In a similar manner, incorporation of the cross-conjugated diene **3**[15] was confirmed (79 % specific incorporation). These data clearly indicate the intermediacy of these two species during rapamycin biosynthesis, which most probably mirrors the process leading to FK 520. The specific incorporation levels that we observed are striking. In isolation, the very high specific incorporation of saturated **1** reported previously (44 %) strongly implicated this molecule as the specific starter unit for the rapamycin PKS; as a control we repeated the feeding of **1** in parallel to those above and obtained a value of 52 %. However, the striking data for **2** and **3** prompted us to question the true intermediacy of **1**, and further to investigate whether **4**, the unsaturated biosynthetic precursor of **1**,[8] could be the true starter unit for the rapamycin PKS.

To examine this question we developed a stereocontrolled route to **4** (Scheme 2) that utilized an intermediate from our chiral synthesis of [$^2H_4$]-**1**.[10] Thus, the hydroxylactone **5** was readily converted into the bis-MOM-protected ethyl ester **6** in two steps. This material was then added to LDA and the lithium enolate thus formed quenched with dimethyl sulfide. The resultant thioether **7** was partially purified before treating with sodium periodate to provide sulfoxide **8**. This compound was then heated in refluxing toluene to provide a 2:1 mixture of the regioisomers **9a** and **9b**. This route takes advantage of a primary isotope effect to direct elimination in favor of the desired compound **9a** (2:1 versus 1:1.3 using non-deuterated **8**). The mixture of **9a** and **9b** was not separable but, provided **4** and **10** after deprotection. It was possible to use this mixture to test the intermediacy of **4** as a result of the differing deuterium content of the two molecules. On feeding this material to *S. hygroscopicus* we were gratified to observe selective incorporation of the [$^2H_4$]-**4** species with a remarkable 87 % specific incorporation. This immediately indicated that **4** may indeed be the true starter unit for the rapamycin PKS. To verify the intermediacy of **4**, and by implication show that **1** is incorporated advantitiously into rapamycin as a result of some broad substrate tolerance in the PKS initiation module, we performed a series of competition feeding experiments utilizing the labeled [$^2H_4$]-**1** and -**4** in conjunction with their unlabeled, racemic equivalents, which were prepared following literature procedures.[15] Each precursor was fed as the enantiomerically enriched, labeled form (1 mM) along with varying concentrations of the other precursor in its racemic, unlabeled form (Table 1). The data obtained clearly indicated that **4** is indeed the preferred starter unit, even when fed in the presence of a fivefold excess of **1**. Conversely, the ability of **1** to act as the starter unit was significantly inhibited in the presence of unlabeled **4**, even when present at a concentration of only 0.25 mM. It is further noteworthy that the absolute levels of rapamycin were not significantly affected by the feeding conditions, which indicates that the remarkably high levels of specific incorporation were not an experimental artefact. Furthermore, these high specific incorporations indicate no limitation in the substrate penetrating the cell membrane and progressing to the site of biosynthesis. These data also lend support to the notion that the precursors of **4** are formed de novo in the free acid form before activation immediately prior to the loading of the PKS.



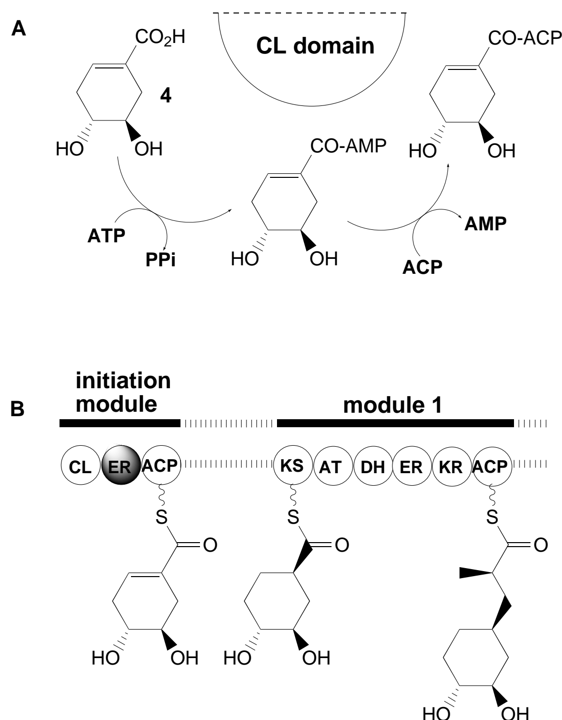
Scheme 2. Synthesis of the labeled precursor **4**. a) 1. KOH, EtOH, 2. CH_3OCH_2Cl, *i*Pr_2NEt (99 %); b) 1. LDA, 2. MeSSMe; c) $NaIO_4$, MeOH (70 %); d) Δ/toluene (67 %); e) 1. HCl, MeOH, 2. KOH, H_2O (90 %). LDA = lithium diisopropylamide, MOM = methoxymethyl.

Table 1. Results of competition feeding experiments between compounds **1** and **4**. The *m*/*z* 936.5 peak corresponds to the sodium ion adduct of rapamycin, and *m*/*z* 940.5 to the equivalent $^2H_4$-labeled isomer. The concentration of the unlabeled precursor refers to that for the enantiomer that corresponds to rapamycin.

$^2H_4$-labeled precursor [mM]	Unlabeled precursor [mM]	Intensity of *m*/*z* 936.5 peak [%]	Intensity of *m*/*z* 940.5 peak [%]
1 (1)	**4** (0.05)	50	50
1 (1)	**4** (0.25)	81	19
1 (1)	**4** (0.5)	89	11
4 (1)	**1** (0.5)	2	98
4 (1)	**1** (1)	9	91
4 (1)	**1** (5)	23	77

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001

The data in this report, in conjunction with the N-terminal structure of the rapamycin PKS, allow us to identify **4** as the true starter unit for the rapamycin PKS. The differing stereospecificity of the enoyl reductions during the biosynthesis of **1** for FK520, and by extrapolation for rapamycin, is consistent with the use of the initiation module ER domain.[17] The differences in stereochemical detail between the biosynthesis of **1**,[8] and the biosynthesis of CHC[14, 15] suggest that precursors of **4** occur as the free acids.[18] By analogy to non-ribosomal peptide synthesis[19] we suggest (Scheme 3) that the



Scheme 3. A) Proposed pathway for CL-catalyzed activation and subsequent attachment of **4** to the rapamycin PKS; B) translocation of ACP-bound **4** involves reduction of the Δ^1 bond by the initiation module ER domain prior to chain elongation on module one of RAPS1, the N-terminus of which is shown with a linear arrangement of the predicted catalytically active domains. AMP = adenosine monophosphate; Ppi = inorganic phosphate; CL = carboxylic acid ligase; ER = enoyl reductase; ACP = acyl carrier protein; KS = β-ketoacyl synthase; AT = acyl transferase; DH = dehydratase; KR = β-ketoacyl reductase.

CL domain catalyses formation of an AMP-activated form of **4**, which is subsequently transferred to the ACP domain. Reduction by the ER domain is followed by transfer to the KS1 domain of RAPS1 to initiate chain elongation. The observation that **1** can also be directly incorporated points to a broad substrate specificity in the CL domain which may allow for its use for the production of analogues of rapamycin by incorporation of a variety of alicyclic starter acids.

Received: August 21, 2000 [Z 15667]

[1] a) R. R. Martel, J. Klicius, S. Galet, *Can. J. Physiol. Pharmacol.* **1977**, *55*, 48–51; b) D. Latta, *Hum. Exp. Toxicol.* **2000**, *19*, 229.
[2] A. B. Smith, S. M. Condon, J. A. McCauley, *Acc. Chem. Res.* **1998**, *31*, 31–46, and references therein.
[3] F. J. Dumont, Q. X. Su, *Life Sci.* **1995**, *58*, 373–395, and references therein.
[4] J. Staunton, B. Wilkinson, *Chem. Rev.* **1997**, *97*, 2611–2629.
[5] T. Schwecke, J. F. Aparicio, I. Molnár, A. König, L.-E. Khaw, S. F. Haydock, M. Oliynyk, P. Caffrey, J. Cortés, J. B. Lester, G. A. Böhm, J. Staunton, P. F. Leadlay, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7839–7843.
[6] N. L. Paiva, A. L. Demain, M. F. Roberts, *J. Nat. Prod.* **1991**, *54*, 167–177.
[7] N. L. Pavia, M. F. Roberts, A. L. Demain, *J. Ind. Microbiol.* **1993**, *12*, 423–428.
[8] K. K. Wallace, K. A. Reynolds, K. Koch, H. A. I. McArthur, M. S. Brown, R. G. Wax, B. S. Moore, *J. Am. Chem. Soc.* **1994**, *116*, 11600–11601.
[9] A. Shafiee, H. Motamedi, T. Chen, *Eur. J. Biochem.* **1994**, *225*, 755–764.
[10] P. A. S. Lowden, G. A. Böhm, J. Staunton, P. F. Leadlay, *Angew. Chem.* **1996**, *108*, 2395–2397; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2249–2251.
[11] a) H. Motamedi, S. J. Cai, A. Shafiee, K. O. Elliston, *Eur. J. Biochem.* **1997**, *244*, 74–80; b) H. Motamedi, A. Shafie, *Eur. J. Biochem.* **1998**, *256*, 528–534.
[12] K. Wu, L. Chung, W. P. Revill, L. Katz, C. D. Reeves, *Gene* **2000**, *251*, 81–90.
[13] K. A. Reynolds, K. K. Wallace, S. Handa, M. S. Brown, H. A. I. McArthur, H. G. Floss, *J. Antibiot.* **1997**, *50*, 701–703.
[14] B. S. Moore, K. Poralla, H. G. Floss, *J. Am. Chem. Soc.* **1993**, *115*, 5267–5274.
[15] B. S. Moore, H. Cho, R. Casati, E. Kennedy, K. A. Reynolds, U. Mocek, J. M. Beale, H. G. Floss, *J. Am. Chem. Soc.* **1993**, *115*, 5254–5266.
[16] S. Handa, H. G. Floss, *Chem. Commun.* **1997**, 153–154.
[17] H. G. Floss, *Nat. Prod. Rep.* **1997**, *14*, 433–452, and references therein.
[18] K. A. Reynolds, K. A. Holland, *Chem. Soc. Rev.* **1997**, *26*, 337–343, and references therein.
[19] H. von Döhren, U. Keller, J. Vater, R. Zocher, *Chem. Rev.* **1997**, *97*, 2675–2705.

A Novel Approach for the One-Pot Preparation of α-Amino Amides by Pd-Catalyzed Double Carbohydroamination**

Yong-Shou Lin and Howard Alper*

Developing new synthetic methods for the preparation of amino acids and their derivatives has attracted much attention due to their applications to the fine chemical, agrochemical, and pharmaceutical business sectors.[1] Although a variety of elegant routes has been discovered for the synthesis of amino acids, amidocarbonylation (Wakamatsu reaction) is the only method involving a transition metal complex catalyzed three-component reaction of an aldehyde, an amide, and carbon monoxide.[2] Domino reactions, which include amidocarbony-

[*] H. Alper, Y.-S. Lin
Center for Catalysis Research and Innovation
Department of Chemistry, University of Ottawa
10 Marie Curie, Ottawa, ON, K1N 6N5 (Canada)
Fax: (+1) 613-562-5871
E-mail: halper@uottawa.ca

[**] We are grateful to the Natural Sciences and Engineering Research Council of Canada for support of this research.

Supporting information for this article is available on the WWW under http://www.angewandte.com or from the author.

 © WILEY-VCH Verlag GmbH, D-69451 Weinheim, 2001 1433-7851/01/4004-0779 $ 17.50+.50/0