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# First chemoenzymatic synthesis of immunomodulating macrolactam pimecrolimus

Patrizia Ferraboschi [a,*], Diego Colombo [a], Maria De Mieri [a], Paride Grisenti [b]

[a] Dipartimento di Chimica, Biochimica e Biotecnologie per la Medicina, Università degli Studi di Milano, Via Saldini, 50-20133 Milano, Italy
[b] Poli Industria Chimica SpA, Via Volturno 45/48, Quinto de' Stampi, 20089 Rozzano, Italy

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ABSTRACT

The preparation of pimecrolimus, a synthetic derivative of ascomycin endowed with immunomodulatory activity, requires the selective protection of 24-hydroxy group of the ascomycin, before elaboration of the 32-hydroxy group. The aim was achieved by means of two regioselective *Candida antarctica* lipase-catalyzed steps. The structure of the new key intermediates, 24-, 32-monoacetates, and 24,32-diacetate, was established by means of an unambiguous NMR study.

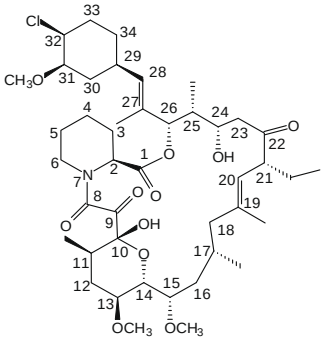


## 1. Introduction

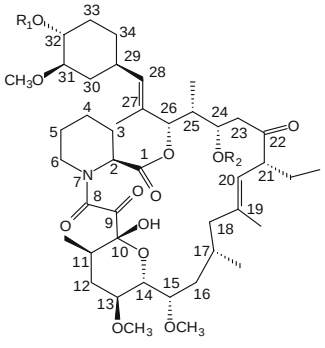
The ascomycin derivative pimecrolimus **1** (32-chloro-32-epiascomycin, present on the market with the trade name of Elidel®) is an immunomodulating 23-membered macrolactam approved for topical treatment of inflammatory skin diseases[1] such as atopic dermatitis.[2] Its pharmacological activity is due to the formation of a complex between the macrolactam and an immunophilin that inhibits calcineurin, the phosphatase involved in T-cell activation and inflammatory cytokines synthesis.

Ascomycin **2**, isolated from fermentation broth of the soil fungus *Streptomyces hygroscopicus*,[3] is converted into pimecrolimus **1** by substitution of 32-hydroxy group with a chlorine with inversion of configuration.

The contemporary presence of two hydroxy groups, at positions 24 and 32, requires, before introduction of the chlorine, the protection of the 24-alcohol, usually carried out by silylation of both hydroxy groups, to give **3**, followed by deprotection at position 32.[4] Large amounts of silylating agent (TBDMSCl[4] or TBDMSOTf[5]) are required and purification by column chromatography is necessary both after the silylation and the deprotection step, affording intermediate **4** in 84% yields.



**1** pimecrolimus



$R_1$=$R_2$=H **2** ascomycin
$R_1$=$R_2$=TBDMS **3**
$R_1$=H; $R_2$= TBDMS **4**

## 2. Results and discussion

### 2.1. Chemoenzymatic approach to the synthesis of pimecrolimus 1

The known selectivity of lipases prompted us to study a chemoenzymatic approach to the preparation of a suitably 24-protected intermediate. A screening among the commercially available lipases, in irreversible transesterification conditions (vinyl acetate in an organic solvent),[6] allowed us to conclude that ascomycin **2** was not substrate for *Pseudomonas fluorescens* (PFL), porcine

* Corresponding author. Tel.: +39 02 50316052; fax: +39 02 50316040.
*E-mail address:* patrizia.ferraboschi@unimi.it (P. Ferraboschi).

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pancreas (PPL), and *Candida cylindracea* (CCL) lipases, whereas *Candida antarctica* lipase (CAL B, Novozym 435) regioselectively afforded 32-monoacetate **5**,[7] as unique product, with a percent conversion depending on the employed solvent (Table 1) (Scheme 1).

This result, on the other hand, in agreement with the reported[8] selective lipase-catalyzed 32-acylation of tacrolimus type macrolides,[9] was not suitable for our purposes. However, the very high CAL B regioselectivity toward position 32 suggested us to employ the same enzyme but with a different approach. Starting from 24,32-diacetate **6**, easily prepared from ascomycin **2** by treatment with acetic anhydride and dimethylaminopyridine (DMAP) in pyridine,[10] the selective removal of 32-acetate, by means of a CAL B-catalyzed alcoholysis, with *tert*-butyl methyl ether (TBME) as solvent and *n*-octanol as acyl acceptor, was observed (Scheme 1). Best conditions of alcoholysis, in order to obtain 24-monoacetate **7**,[11] were found after a screening of solvents and acyl acceptors as reported in Table 2.

Evidences about enzymatic reaction outcome were preliminarily obtained by means of a $^1$H NMR analyses comparison with the literature data of ascomycin **2**[12] and 24,32-di-*O*-formyl-ascomycin **8**.[5] In fact $^1$H NMR data of acetates **5–7** are not reported. Ascomycin **2** presents two signals, at 3.40 and 3.92 ppm, assigned to H-32 and H-24,[12] respectively. Whereas the former is overlapped to the $OCH_3$ signals the latter (i.e., H-24 signal) and the H-32 signal (at 4.71 ppm) of 24,32-diformate **8**, are more predictive for our purposes. The presence of a signal at 4.70 ppm, beside the signal at 3.92 ppm, allowed to conclude that the 32-monoacetate **5** was the unique product of CAL B-catalyzed transesterification. In a similar manner we assigned the structure of 24-monoacetate **7** to the product obtained from alcoholysis: the H-32 signal at 4.71 ppm was absent, indicating that the hydroxy group was not engaged in an ester bond; in addition, also the resonance due to the H-24 of 24-alcohol, at 3.92 ppm was absent whereas the region between 5.0 and 5.4 ppm, where usually the H-24 of esterified ascomycin (5.22 ppm for diformate **8**) is present, was modified.

This preliminary assignment was later confirmed through a more complete NMR study as reported below.

Compound **7** was treated with polymer-bound triphenylphosphine (FLUKA) in carbon tetrachloride[13] to afford 32-chloro derivative **9**[14] (40%); removal of 24-acetate was performed under acidic conditions (3 N HCl, 40% yields) to avoid the known degradations of ascomycin family macrocycles in presence of bases,[15–17] affording pimecrolimus **1** in only 14% overall yield (Scheme 2).

Since the presence of 24-acetate seemed to be crucial for the lowering of the yields of two final steps, we planned to prepare, through the same regioselective enzymatic approach, 24-silyl derivative **4**, that is, the key intermediate of traditional syntheses of pimecrolimus **1**[4] (Scheme 3).

Starting from the previously enzymatically obtained 32-monoacetate **5**, by silylation with TBDMSOTf (5 equiv), in presence of 2,6-lutidine, 24-*O*-TBDMS-32-*O*-acetyl derivative **10**[18] was recovered; removal of 32-acetyl group was achieved by means of a CAL B-catalyzed alcoholysis,[19] affording intermediate **4** in good yields (80% from **5**). By these mild conditions, typical of enzyme-catalyzed transformations, problems related to acidic or basic treatments of ascomycin family compounds were avoided. In the

**Table 1**
Lipases-catalyzed transesterification of **2** to **5** with vinyl acetate

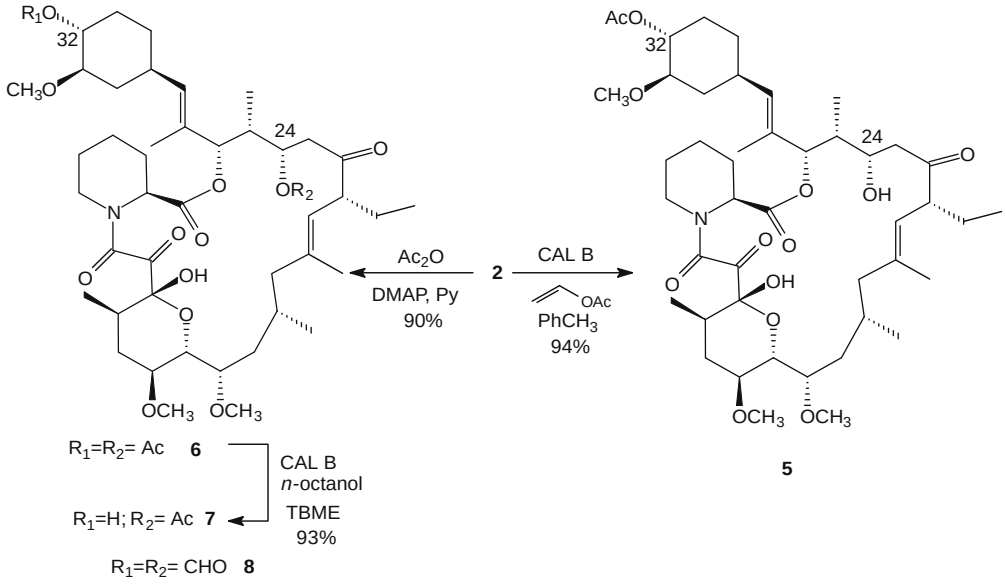
| Lipase | Solvent | Time (h) | Conversion[a] (%) |
|---|---|---|---|
| PFL | Chloroform | 100 | 0 |
| PPL | Toluene | 94 | 0 |
| CCL | Toluene | 92 | 0 |
| CAL B | Acetonitrile | 53 | 30 |
| CAL B | *tert*-Butyl methyl ether | 53 | 10 |
| CAL B | Toluene | 80 | 100 |

[a] From TLC and $^1$H NMR.

**Table 2**
CALB-catalyzed hydrolysis or alcoholysis of **6** to **7**

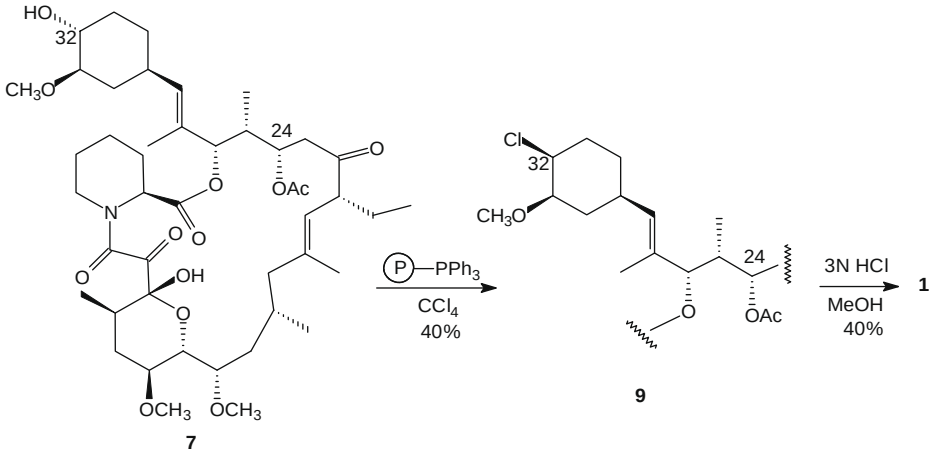
| Solvent | Acyl acceptor | Time (h) | Conversion[a] (%) |
|---|---|---|---|
| Toluene | $H_2O$ | 80 | 0 |
| Toluene | Methanol | 48 | 0 |
| Toluene | Ethanol | 100 | 0 |
| Toluene | *n*-Butanol | 120 | 30 |
| *tert*-Butyl methyl ether | *n*-Octanol | 100 | 100 |

[a] From TLC and $^1$H NMR.

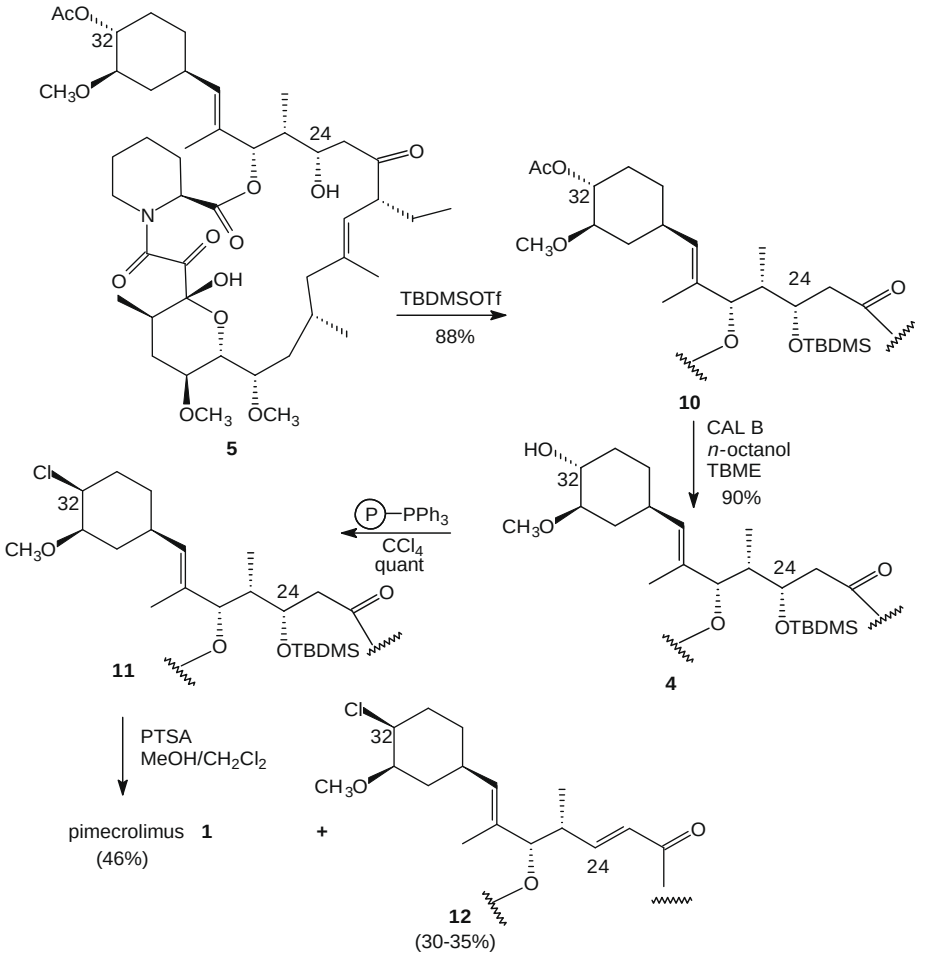



**Scheme 1.** Regioselective *Candida antarctica* lipase B-catalyzed transformations of ascomycin **2** and its diacetate **6**.

biocatalyzed alcoholysis step it is possible to recycle the same enzyme sample used in the previous 32-acylation step, without significant loss of activity; in fact, if fresh CAL B is used, a comparable time is required to achieve the same conversion percent of **10** to **4** (about quantitative). Again, in order to introduce the chlorine at position 32, polymer bound triphenyl phosphine[13] was used, leading to **11**. We observed that when, after a 60–70% transformation, the polymer is removed and replaced with fresh reagent a nearly complete conversion is achieved and the 32-chloro derivative **11** can be used in the next step without any further purification.[20] PTSA in dichloromethane/methanol (1/1) at 25–30 °C, among the tested conditions (hydrochloric acid in methanol, PTSA in tetrahydrofuran/water) gave best results for 24-hydroxy group deprotection;[21] pimecrolimus **1**[22] was recovered in 46% yield, after separation, by column chromatography, from a less polar compound (30–35%), identified as 23-dehydropimecrolimus




**Scheme 2.** Final steps of the synthesis of pimecrolimus **1** from monoacetate **7**.




**Scheme 3.** Chemoenzymatic synthesis of pimecrolimus **1** from monoacetate **5**.

**12**.[23,24] The presence of this compound is common to other deprotection methods, for example, hydrofluoric acid in acetonitrile,[4] in variable elevated amounts.

Overall yields (29%) of pimecrolimus **1** from ascomycin **2**, were comparable to these observed when we prepared intermediate **4** through the reported bissilylation–monodesilylation process.[4]

### 2.2. NMR study of acetates 5–7

To ascertain the structure of **5**–**7** a more accurate NMR study[25] was done and, through 1D and 2D COSY, HSQC, and HMBC experiments, it was possible to unambiguously establish the acetyl positions in these compounds. The experiments were carried out in $Pyd_5$ at 323 K. In fact using these experimental conditions the spectra showed a good spread of the proton resonances and, especially, of all the methyl groups. Two sets of signals are identified, since the macrolactam system exists as a mixture of two rotamers in a 60/40 ratio, as in the case of ascomycin **2**.[12] The study started with 24,32-diacetate **6** and was focused on the signals of ascomycin moiety carrying the acetyl groups. As an entry point for the study of **6**, the well resolved characteristic C-25-linked methyl doublet at 1.12 and 1.11 ppm (major and minor rotamers) was selected. Starting from that signal, H-25 (2.18 and 2.42 ppm, major and minor rotamers), H-24 (5.50 and 5.65 ppm, major and minor rotamers), and H-26 (5.47 and 5.52 ppm, major and minor rotamers) were assigned by COSY. Long range HMBC correlations between H-24 and a carbonyl $^{13}C$ resonance (169.5 ppm) and between this carbonyl and a methyl singlet at 2.05 and 2.07 ppm (major and minor rotamers) confirmed the presence of an acetoxy group linked at C-24. From H-26 it was also possible to assign, by COSY, H-28 (5.40 and 5.51 ppm, major and minor rotamers) because of an allylic coupling between the two protons and, consequently, H-29 (2.42 ppm, major and minor rotamers), H-30a (1.20, major and minor rotamers), H30b (2.15 ppm, major and minor rotamers), H-31 (3.34 ppm, major and minor rotamers), and H-32 (4.97 ppm, major and minor rotamers). The identities of H-31 and H-32 were proved, also in this case, by heteronuclear experiments. In particular, the resonances assigned to H-31 and to a methoxyl (3.36 and 3.35 ppm singlets, major and minor rotamers) were shown to be correlated to the same carbon (C-31, 80.5 ppm) by means of HSQC and HMBC, respectively. Also H-32 was shown to be linked to a carbon carrying an acetyl group from the presence of HMBC cross peaks between H-32 and a carbonyl at 169.7 ppm, and between the same carbonyl and an acetyl methyl singlet resonance at 2.02 ppm (major and minor rotamers).

Using the same NMR strategy, the identity of 32- and 24-monoacetates **5**, and **7** was then confirmed.[26]

## 3. Conclusion

Through a chemoenzymatic approach the synthesis of pimecrolimus **1**, from ascomycin **2**, was realized with satisfactory yields, considering the very high value of the final product; moreover during the regioselective CAL B-catalyzed steps, performed in mild conditions, only the desired products are obtained in absence of rearrangements or degradation processes very frequently observed in the case of sensitive molecules as ascomycin and related compounds, containing a large number of functionalities.[5,15–17] The same sample of immobilized CAL B can be easily recycled for either transesterification or alcoholysis, without loss of activity. Use of polymer-bound triphenyl phosphine, for the introduction of chlorine at position 32, in addition to high yields, allows a simple recovery of reagent that can be regenerated by treating with trichlorosilane.[13] Key intermediate **4**, obtained through two regioselective lipase-catalyzed steps, can be useful not only for the preparation of pimecrolimus but also for the preparation of other 32-substituted derivatives of ascomycin.[21,27]

In order to unambiguously ascertain the outcome of lipase-catalyzed transformations a careful high field NMR study, through 1D and 2D experiments, was performed. This investigation has lead to the complete assignment of proton resonances of both rotamers of monoacetates **5**,**7** and diacetate **7**, in the moiety directly involved in the enzymatic transesterification and alcoholysis.

## Acknowledgments

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## References and notes

1. (a) Bornhövd, E. C.; Burgdorf, W. H. C.; Wollenberg, A. *Curr. Opin. Invest. Drugs* **2002**, *3*, 708–712; (b) Gupta, A. K.; Chow, M. *J. Eur. Acad. Dermatol.* **2003**, *17*, 493–503; (c) Stuetz, A.; Baumann, K.; Grassberger, M.; Wolff, K.; Meingassner, J. G. *Int. Arch. Allergy Immunol.* **2006**, *141*, 199–212; (d) Wollina, U.; Hansel, G.; Koch, A.; Abdel-Naser, M. B. *Expert Opin. Pharmacother.* **2006**, *7*, 1967–1975; (e) Day, I.; Lin, A. N. *J. Cutan. Med. Surg.* **2008**, *12*, 17–26.
2. Hebert, A. A. *Clin. Ther.* **2006**, *28*, 1972–1982.
3. Arai, T.; Koyama, Y.; Suenaga, T.; Honda, H. *J. Antibiot. Ser. A.* **1962**, *15*, 231–232.
4. Baumann, K.; Emmer, G. E.P. 0, 427, 680, A1, 1991.
5. Baumann, K.; Bacher, M.; Damont, A.; Högenauer, K.; Steck, A. *Tetrahedron* **2003**, *59*, 10075–10087.
6. (a) Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B. *Tetrahedron Lett.* **1987**, *28*, 953–954; (b) Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200–7205.
7. *32-O-Acetyl-ascomycin* **5**. To a solution of ascomycin (0.1 g; 0.126 mmol) in toluene (8 mL) vinyl acetate (0.473 g, 5.5 mmol) and lipase from *Candida antarctica* (CAL B, Novozym 435, FLUKA, 0.140 g, 2 U/mg) were added. The reaction progress was monitored by TLC (hexane/acetone 65/35). After stirring at 40 °C for 80 h the enzyme was filtered off and the recovered filtrate was concentrated under reduced pressure. The residue was crystallized from acetone/water (0.1 g; 94%). Differential Scanning Calorimetry (DSC) endothermic peak of fusion 134.25 °C; $[\alpha]_D^{20}$ −74.2 (*c* 0.5 $CHCl_3$); IR $\nu_{max}$ 3484.245, 2935.287, 1735.331, 1649.741, 1450.039, 1372.278 $cm^{-1}$; MS (ESI+) (*m*/*z*) 856.4 $[M+Na]^+$.
8. Petuch, B. R.; Chen, S.-S. T.; Arison, B. H. E.P. Patent 0, 464, 895, A2, 1992. The 32-acetylation was performed with acetic anhydride in benzene and the yields were in the range of 20–30%.
9. Tacrolimus is the 21-allyl analogue of ascomycin.
10. *24,32-Di-O-acetyl-ascomycin* **6**. To a stirred solution of ascomycin (1 g; 1.26 mmol) in pyridine (12.5 mL), kept at 0 °C, DMAP (0.680 g) and acetic anhydride (0.570 g, 5.6 mmol) were added. After 1.5 h, under stirring at 0 °C, the reaction mixture was diluted with water and extracted with ethyl acetate (3 × 25 mL); collected organic phases were washed with 0.5 N HCl (5 × 10 mL), dried over $Na_2SO_4$, and evaporated at reduced pressure. The residue was crystallized from acetone/water (0.985 g; 90%). A sample was purified for analytical purposes by silica gel column chromatography (hexane/acetone 8/2 as eluant). DSC endothermic peak of fusion 234.10 °C; $[\alpha]_D^{20}$ −100.0 (*c* 0.5 $CHCl_3$); IR $\nu_{max}$ 3462.749, 2935.824, 1734.403, 1650.739, 1449.091, 1371.079 $cm^{-1}$; MS (ESI+) (*m*/*z*) 898.4 $[M+Na]^+$.
11. *24-O-Acetyl-ascomycin* **7**. To a solution of 24,32-di-*O*-acetyl-ascomycin, **6** (0.5 g; 0.57 mmol) in TBME (25 mL), *n*-octanol (4.5 equiv; 0.371 g), and lipase from *Candida antarctica* (CAL B, Novozym 435, 1.1 g) were added. The reaction progress was monitored by TLC (hexane/acetone 65/35). After stirring at 40 °C for 100 h the enzyme was filtered off and the recovered filtrate was concentrated under reduced pressure. The residue was crystallized from acetone/water (0.44 g; 93%). A sample was purified for analytical purposes by column chromatography on silica gel (hexane/acetone 7/3 as eluant). DSC endothermic peak of fusion 134.68 °C; $[\alpha]_D^{20}$ −102.7 (*c* 0.5 $CHCl_3$); IR $\nu_{max}$ 3491.528, 2935.860, 1744.728, 1710.227, 1652.310, 1448.662, 1371.335 $cm^{-1}$; MS (ESI+) (*m*/*z*) 856.4 $[M+Na]^+$.
12. Or, Y. S.; Clark, R. F.; Xie, Q.; McAlpine, J.; Whittern, D. N.; Henry, R.; Luly, J. R. *Tetrahedron* **1993**, *49*, 8771–8786.
13. Regen, S. L.; Lee, D. P. *J. Org. Chem* **1975**, *40*, 1669–1670.
14. *24-O-Acetyl-pimecrolimus* **9**. DSC endothermic peak of fusion 231.67 °C; $[\alpha]_D^{20}$ −75.2 (*c* 0.5 $CHCl_3$); $^1H$ NMR ($CDCl_3$) selected data of major rotamer $\delta$ (ppm) 2.06 (s, $CH_3CO$), 4.50–4.60 (m, H-2 and H-32), 5.22 (m, H-24). IR $\nu_{max}$ 3464.941, 2934.360, 1738.993, 1650.366, 1450.424, 1371.557 $cm^{-1}$; MS (ESI+) (*m*/*z*) 874.3 $[M+Na]^+$.
15. Zimmer, R.; Grassberger, M. A.; Baumann, K.; Schulz, G.; Haidl, E. *Tetrahedron* **1994**, *50*, 13655–13670.

16. Koch, G.; Jeck, R.; Hartmann, O.; Küsters, E. *Org. Process Res. Dev.* **2001**, *5*, 211–215.
17. Baumann, K.; Bacher, M.; Steck, A.; Wagner, T. *Tetrahedron* **2004**, *60*, 5965–5981.
18. *24-O-tert-Butyldimethylsilyl, 32-O-acetyl-ascomycin* **10**. DSC endothermic peak of fusion 236.43 °C; $[\alpha]_D^{20}$ −81.4 (*c* 0.5 $CHCl_3$); $^1H$ NMR ($CDCl_3$) selected data of major rotamer $\delta$ (ppm) 0.05,(s, $CH_3Si$), 0.06 (s, $CH_3Si$), 0.89 (s, $(CH_3)_3C$), 2.10 (s, $CH_3CO$), 4.08 (m, H-24), 4.71 (m, H-32). IR $\nu_{max}$ 3462.948, 2934.450, 1739.236, 1649.937, 1450.323, 1371.477 $cm^{-1}$; MS (ESI+) (*m*/*z*) 970.5 $[M+Na]^+$.
19. *24-O-tert-Butyldimethylsilyl-ascomycin* **4**. Removal of 32-acetate was realized in the same conditions utilized for preparation of 24-*O*-acetyl-ascomycin **7** from corresponding 24,32-diacetate **6**. Chemical-physical data of **4** are in agreement with those reported in literature (Ref. 5).
20. The same transformation was successfully (73%) realized also with dichlorotriphenylphosphorane (Grassberger, M.; Horvath, A. WO 040111 A2, 2006) not applicable, on the contrary, in the case of 24-monoacetate **7** for the formation of a complex mixture.
21. PTSA is reported (Ok, H. O.; Szumiloski, J. L., Beattie, T. R.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **1997**, 7, 2199–2204) to selectively remove the 32-silyl group from 24,32-disilyl derivative. In this case, we observed that lower temperatures (10–15 °C instead of 25–30 °C) and shorter times (20 h instead of 72 h) were required.
22. *Pimecrolimus* **1**. To a solution of compound **11** (1.23 g, 1.35 mmol) in dichloromethane/methanol (1/1, 11 mL) PTSA was added (0.100 g, 0.53 mmol). The mixture was kept, under stirring, at 20–25 °C for 72 h, monitoring the reaction progress by TLC (hexane/acetone 8/2). A sodium hydrogen carbonate (0.04 g) aqueous solution (6 mL) was added; the organic phase was washed with brine and water, dried over sodium sulfate. After solvent evaporation at reduced pressure crude pimecrolimus, as a foam, was recovered. Silica gel column chromatography (hexane/acetone 8/2) afforded pure pimecrolimus that was crystallized from ethyl acetate/cyclohexane/water (0.5 g, 46%). DSC endothermic peak of fusion 142.58 °C; $[\alpha]_D^{20}$ −52.0 (*c* 0.5 $CHCl_3$) IR $\nu_{max}$ 3458.61, 2937.83, 1738.37, 1690.26 1634.72, 1445.60, 1445.63, 1382.72 $cm^{-1}$; MS (ESI+) (*m*/*z*) 832.5 $[M+Na]^+$. $^1H$ and $^{13}C$ NMR data were in agreement with the reported ones (Dosenbach, C.; Grassberger, M.; Hartmann, O.; Horvath, A,; Mutz, J.-P.; Penn, G.; Pfeffer, S.; Wieckhusen, D. WO 01458, 1999).
23. Treatment of 24-silyl ascomycin with PTSA in benzene, at reflux, is reported to afford $\Delta^{23}$-ascomycin: Wiedeman, P. E.; Fesik, S. W.; Petros, A. M.; Nettesheim, D. G.; Mollison, K. W.; Lane, B. C.; Or, Y. S.; Luly, J. R. *J. Med. Chem.* **1999**, *42*, 4456–4461.
24. The presence of a multiplet at 4.59–4.61 ppm (H-2 and H-32) and two double doublets at 6.21 (*J* = 15.7 Hz, *J* = 1.7 Hz, H-23) and 6.86 (*J* = 15.7 Hz, *J* = 5.3 Hz, H-24) ppm, respectively, in $^1H$ NMR spectrum, allowed to assign to the main side product of deprotection step the structure of $\Delta^{23}$-pimecrolimus **12**; an additional confirmation derived from MS (ESI+) spectrum for the presence of a peak at 814.4 *m*/*z* $[M+Na]^+$; pimecrolimus (exact mass 809.4) spectrum shows the main peak at 832.4 *m*/*z*.
25. $^1H$ NMR analysis was performed at 500 MHz with a Bruker FT-NMR AVANCE™ DRX500 spectrometer using a 5 mm z-PFG (pulsed field gradient) broadband reverse probe, and $^{13}C$ NMR spectra were collected at 125.76 MHz at 323 K. The signals were unambiguously assigned by 2D COSY HSQC and HMBC experiments (standard BRUKER pulse program). Chemical shifts are reported as $\delta$ (ppm) relative to residual pyridine fixed at 7.19 (higher field signal) for $^1H$ NMR spectra and relative to $Pyd_5$ fixed at 123.0 ppm (higher field signal, central line) for $^{13}C$ NMR spectra.
26. Selected NMR chemical shifts, as $\delta$ (ppm), of **5** and **7** (major and minor rotamer resonances are reported in the order). Compound **5**: 25-$CH_3$ doublet (1.20 and 1.18), H-25 (2.10 and 2.24), H-24 (4.47 and 4.55), H-26 (5.78 and 5.81), H-28 (5.43 and 5.42), H-29 (2.40, major and minor rotamers), H-30a (1.18, major and minor rotamers), H-30b (2.15, major and minor rotamers), H-31 (3.33, major and minor rotamers), C-31 (80.4), 31-$OCH_3$ singlet (3.36 and 3.35), H-32 (4.94, major and minor rotamers), CO (32-acetyl) (169.8), $CH_3$ (32-acetyl) singlet (2.03 and 2.02). Compound **7**: 25-$CH_3$ doublet (1.13 and 1.11), H-25 (2.19 and 2.42), H-24 (5.50 and 5.65), CO (24-acetyl) (169.5), $CH_3$ (24-acetyl) singlet (2.05 and 2.07), H-26 (5.48 and 5.52), H-28 (5.44 and 5.45), H-29 (2.44, major and minor rotamers), H-30a (1.18, major and minor rotamers), H-30b (2.15, major and minor rotamers), H-31 (3.21, major and minor rotamers), C-31 (80.7), 31-$OCH_3$ singlet (3.46 and 3.44), H-32 (3.68, major and minor rotamers).
27. Hersperger, R.; Schuler, W.; Zenke, G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 227–232.