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Studies on Selective Reductions of Rapamycin

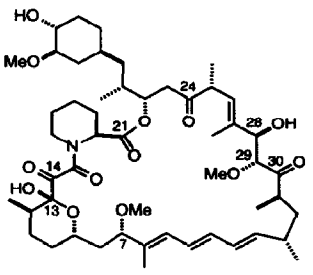
Juan I. Luengo,* Leonard W. Rozamus, and Dennis A. Holt

Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals
P.O. Box 1539, King of Prussia, PA 19406, USA

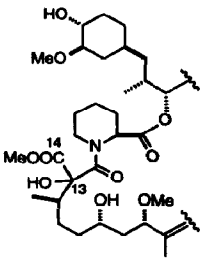
Abstract: The reaction of rapamycin (**1**) with different reductive agents has been studied. As expected, the C_{14} ketone of the "tricarbonyl" unit is the most electrophilic center in the molecule and could be selectively converted to either the alcohol (Zn/AcOH or DIBAL) or to the C_{14} methylene (H_2S, pyridine/MeOH). Under Luche's conditions, the C_{14} carbonyl was protected and reduction took place stereoselectively at both C_{24} and C_{30} ($NaBH_4/CeCl_3$) or exclusively at C_{30} ($NaBH_3CN/CeCl_3$). Selective reaction at C_{30} also took place under Evans conditions with $NaBH(OAc)_3$. These reactions allow the selective manipulation of the rapamycin "effector domain".

Rapamycin (**1**), a naturally occurring macrolide originally isolated from *Streptomyces hygroscopicus*, is a potent immunosuppressive agent which inhibits T-cell proliferation by a novel, not yet fully elucidated, mechanism.[1] This 31-membered macrocyclic lactone has a unique structure, with a complex array of sensitive functionality and numerous sites for chemical instability. Recently, there has been considerable interest in the chemical reactivity of rapamycin to identify the structural requirements for biological activity as well as to provide mechanistic probes for the investigation of its mode of action.[2,3] Rapamycin contains a number of potentially reactive carbonyl groups, namely four ketones (one of them masked as a hemiketal) in addition to the macrocyclic lactone. We decided to investigate the chemical reactivity of these carbonyl groups towards different reducing agents; in this letter some of the results of these studies are reported.

The most electrophilic center in rapamycin is the ketone of the "tricarbonyl" unit (C_{14}). We have previously reported two different reactions which are based on the nucleophilic attack at C_{14} by either MeOH (Lewis acid-catalyzed benzilic acid rearrangement to **2**)[3c] or by cyanide (C_{13}-C_{14} fragmentation to **3**).[4] We have found that this center is also extremely sensitive to reducing agents. Thus, reduction of the C_{14} ketone could be effected with such a mild reducing agent as zinc dust in acetic acid at room temperature to provide **4** in 90% yield (1:1 mixture of C_{14} epimers). This chemoselective dissolving metal reduction in the presence of other potentially reactive groups such as the C_7 or C_{29} methoxyl groups (α to a conjugated triene or a ketone, respectively) is noteworthy. The same reduction took place with DIBAL in THF (-78 °C, 78% yield of **4** as a 7:1 mixture of C_{14} epimers).



1 rapamycin



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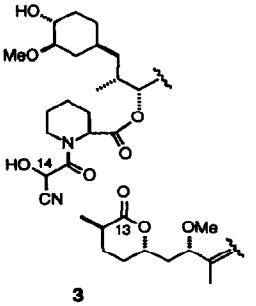

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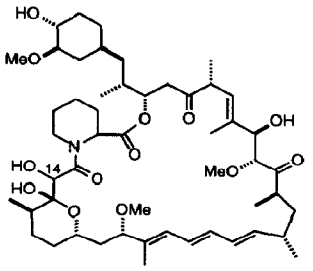
Over thirty years ago, Mayer reported a selective reduction of α-diketones to monoketones with hydrogen sulfide and amines.[5a] Application of this procedure to rapamycin (H_2S, pyridine/methanol, r.t., 1 d) resulted in an efficient conversion to the 14-desoxo derivative **5** in 79% yield. The reaction results from the enhanced electrophilicity at C_{14} and presumably proceeds by reduction of a C_{14} *gem*-dithiol intermediate, in analogy to the reaction of α-sulfenylated ketones with thiolates.[5b]



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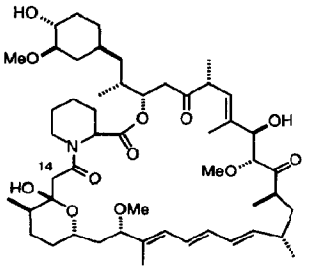

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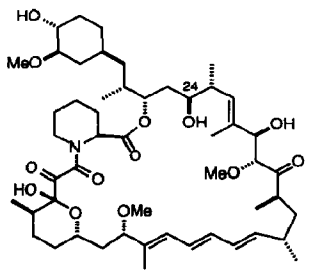
Structural studies of the complex of rapamycin with its protein target, FKBP, show that the C_{14} carbonyl is an important element for FKBP affinity.[6] From the above experiments, however, it would seem that reductions of the other two ketones at C_{24} and C_{30}, which are located in the "effector domain" of the molecule, without affecting the C_{14} carbonyl will be difficult. We therefore became interested in Luche's report[7] on the selective reduction of ketones in the presence of aldehydes with $NaBH_4/CeCl_3$, a selectivity based on the *in situ* Ce^{3+}-catalyzed hydration of the aldehyde. We have found an analogous protective effect at C_{14} in rapamycin. Thus, whereas rapamycin reacted very slowly with $NaBH_4$ in MeOH at -78 °C, immediate reduction occurred in the presence of $CeCl_3 \cdot 7H_2O$. With 0.4 equiv of $NaBH_4$, competing stereoselective reactions at C_{24} and C_{30} took place to give **6** and **7**, respectively. With 1.9 equiv of hydride, compound **8**, the product of reduction at both C_{24} and C_{30}, was isolated in 75% yield. No reaction could be detected at C_{14} even after use of a large excess (6 equiv) of $NaBH_4$, confirming the Ce^{3+}-induced protection of the C_{14} ketone. Interestingly, $CeCl_3$ by itself in methanol catalyzes the benzilic acid rearrangement[8] of rapamycin, quantitatively providing the corresponding rearranged methyl esters **2** after several hours at room temperature.



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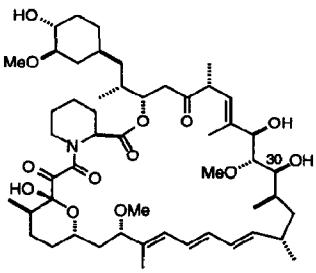

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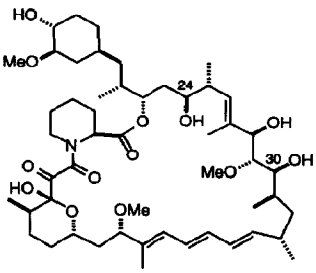

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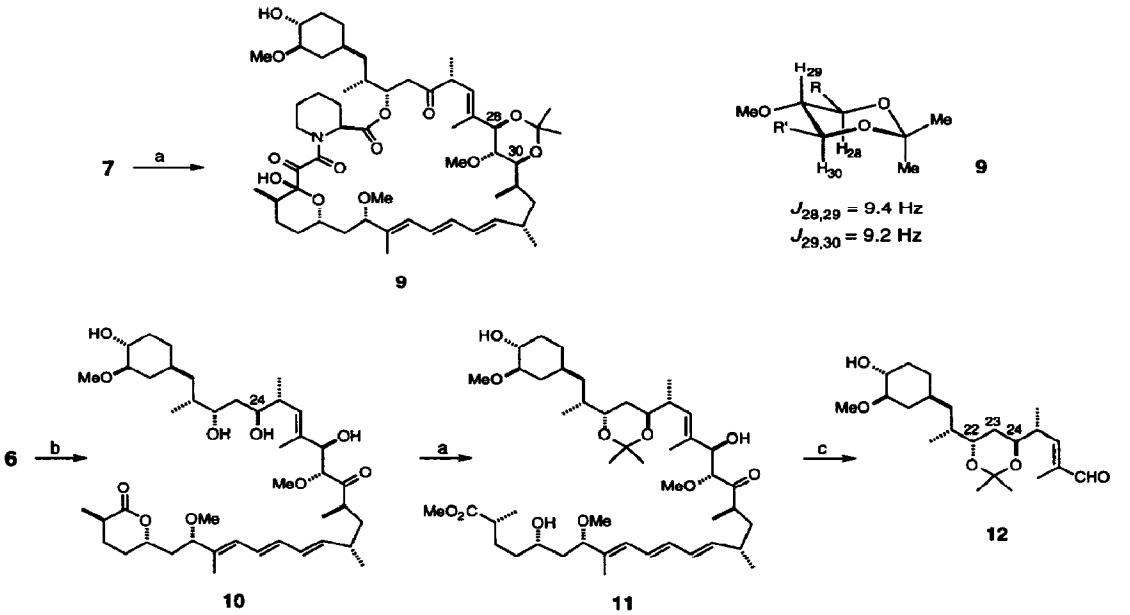
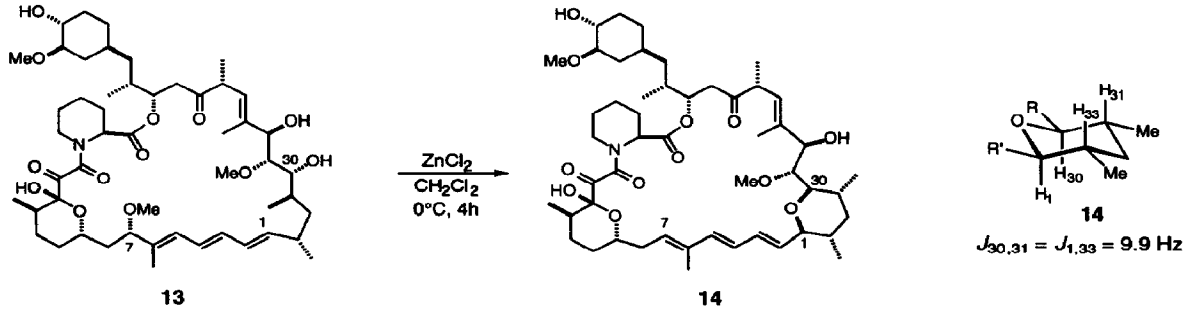
The assignment of the 30-(*S*) stereochemistry in **7** was based on spectroscopic analysis of the C_{28}-C_{30} acetonide **9**. The vicinal ^1H NMR coupling constants $J_{28,29}$ and $J_{29,30}$ were 9.2-9.4 Hz, consistent with a trans-diaxial relationship for H_{28}, H_{29} and H_{30}. A similar type of cyclic derivative was used for the assignment of the C_{24} alcohol, although in this case the 1,3-diol had to be revealed first. Thus, cyanide-catalyzed double cleavage[9] of **6** provided **10**, which was converted to acetonide **11**; subsequent retroaldol fragmentation with zinc chloride[3c] produced aldehyde **12**. The four vicinal ^1H NMR coupling constants among both C_{22} protons and their neighbors (6.2-9.5 Hz) confirmed the stereochemistry at C_{24} as (*S*).[10]



a) 2,2-dimethoxypropane, Dowex/H+. b) $(n\text{-Bu})_4N^+CN^-$, THF, H_2O, r.t., 15 min. c) $ZnCl_2$, THF, 55 °C, 1 h.

Chemoselective reduction at the C_{30} ketone could be achieved with $NaBH_3CN$ and AcOH in THF in the presence of $CeCl_3.7H_2O$ (r.t., 4 h) to provide a 9:1 mixture of **7** along with its 30-(*R*) epimer **13** in 51% yield. The stereoselectivity of the reduction at C_{30} can be reversed by the assistance of the 28-hydroxyl group under Evans' conditions[11] ($NaBH(OAc)_3$, THF -20 °C, 1 d) to produce the anti 30-(*R*) epimer **13** as the major component (55% of 2.7:1 **13**:**7**). We have found compound **13** to be relatively labile upon standing at room temperature, slowly being converted into **14**. This transformation, an intramolecular attack at C_1 by the C_{30} hydroxyl group with concommitant displacement of the 7-methoxy group and triene rearrangement, was highly stereoselective (exclusive formation of the 1-(*S*) epimer)[12] and could be accelerated in the presence of Lewis acids ($ZnCl_2$ in CH_2Cl_2). Studies on the scope of this remarkable reaction will be reported elsewhere.



In summary, we have explored the reactivity of rapamycin towards reducing agents and investigated different routes for the selective manipulation of both binding and effector domains in rapamycin. The compounds herein described should provide valuable tools for subsequent elaboration of this fascinating molecule.

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8. This rearrangement has been observed in ref 3c with other Lewis acids (zinc halides, $MgCl_2$, $Cu(OAc)_2$).
9. See ref 4 above. We have found the cyanide-catalyzed cleavage to be ideally suited for spectroscopic characterization of rapamycin derivatives, especially with those compounds such as **6-8** which consisted of equimolecular mixtures of cis-trans amide rotamers in solution.
10. The ^1H NMR spectrum ($CDCl_3$) of **12** showed H_{22}, H_{23a}, H_{23b}, H_{24}, at δ 3.48, 1.64, 1.52, 3.70, respectively, with $J_{22,23a} = J_{23b,24} = 9.3$ Hz, $J_{22,23b} = J_{23a,24} = 6.3$ Hz and $J_{23a,23b} = 12.5$ Hz; these values are consistent with a *trans*-4,6-disubstituted-1,3-dioxane. See: a) Burkert, U. Tetrahedron **1979**, *35*, 691-695. b) Pihlaja, K.; Kellie, G. M.; Riddell, F. G. *J. Chem. Soc., Perkin Trans 2* **1972**, 252-256.
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12. ^1H NMR of **14** (400 MHz, $CDCl_3$, 5:1 mixture of trans:cis amide rotamers; data for the *trans*-rotamer) δ 6.61 (d, J = 15.4 Hz, H_5), 6.44 (dd, J = 15.1, 10.5 Hz, H_3), 6.09 (dd, J = 15.4, 10.5 Hz, H_4), 6.02 (t, J = 7.2 Hz, H_7), 5.63 (d, J = 9.2 Hz, H_{26}), 5.52 (dd, J = 15.1, 8.8 Hz, H_2), 5.38-5.35 (m, H_{22}), 5.32 (d, J = 4.7 Hz, H_{20}), 4.41 (br s, H_{28}), 4.13-4.09 (m, H_9), 3.77 (s, OH), 3.48 (s, 3H), 3.38 (s, 3H), 3.15 (dd, J = 9.9, 8.8 Hz, H_1), 3.01 (dd, J = 9.9, 1.9 Hz, H_{30}), 2.75 (dd, J = 18.2, 1.5 Hz, H_{23}), 2.59 (d, J = 18.2, 9.7 Hz, H_5), 1.73 (s, 3H), 1.68 (s, 3H), 1.24 (d, J = 6.9 Hz, 3H), 0.86 (d, J = 6.9 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 0.76 (d, J = 6.7 Hz, 3H), 0.71 (d, J = 6.7 Hz, 3H).

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