Manipulation of the Rapamycin Effector Domain. Selective Nucleophilic Substitution of the C_7 Methoxy Group[1]

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Summary: The C_7 methoxy group in rapamycin has been found to be labile toward acidic reagents. Conditions have been developed to replace this group with a number of different nucleophiles, such as alcohols, thiols, and electron-rich aromatic systems. This novel, efficient transformation allows the selective manipulation of the rapamycin effector domain.

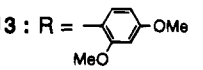
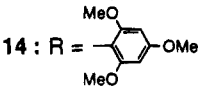
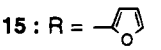
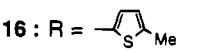
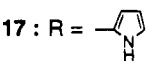
Rapamycin (**1**) is a macrocyclic natural product which is under clinical investigation as an immunosuppressive agent for organ transplantation.[2] Rapamycin suppresses the T-cell response to IL-2, blocking the progression of the cell cycle at the G1 phase. This action is mediated by the intracellular receptor FKBP12 and involves the inhibition of the activation of $p70^{S6k}$ and cyclin-dependent kinases.[3] Recently, the target of the FKBP12–rapamycin complex has been reported as a 289 KDa protein[4] with homology to the yeast DRR/TOR gene products.[5] In analogy with cyclosporin A and FK506, rapamycin is viewed as a ligand with two functional domains: (a) binding domain, centered on the pipecolinyl α-ketoamide, which binds in a hydrophobic cavity of FKBP12, and (b) effector domain, residing within the rest of the macrocyclic tether, which together with FKBP12 interacts with the downstream protein in the T-cell.

We have recently reported[6] the reactivity of rapamycin under Lewis acid catalysis at two different sites of the molecule: the C_{12}–C_{14} "tricarbonyl", which rearranges to a hydroxy ester, and the C_{28}–C_{30} hydroxy ketone, which undergoes retroaldol cleavage. We would like to report a third type of acid-catalyzed reaction of rapamycin, namely the nucleophilic substitution of the C_7 methoxy group, a novel transformation promoted by the neighboring conjugated triene.

Unlike Lewis acids, strong protic acids (*p*-TsOH, HCl, TFA) in MeOH did not effect benzilic acid rearrangement of rapamycin, but instead resulted in epimerization at C_7 (rt, 2:1 mixture of isomers **1**:**2**). Epimerization arises from solvolysis of the C_7 allylic methoxyl, since the corresponding reaction in EtOH provided the C_7 ethyl ethers **3** and, likewise, simply dissolving rapamycin in formic acid at 10 °C gave the C_7 formates **4**. The clean solvolysis of the C_7 methoxy group is quite remarkable, considering the complexity of the rapamycin molecule, and is certainly the result of an intermediate carbonium ion which is highly delocalized by the adjacent triene. This is supported by the facile ionic hydrogenation of the C_7 methoxy group at low temperature with TFA/Et_3SiH in CH_2Cl_2 (−45 °C, 1 h, 4:1 mixture of 7-demethoxyrapamycin (**5**) along with regioisomer **6** in 90% yield), a reaction based on the transfer of a hydride ion from a silane to a carbonium ion.[7] Capture of the carbonium ion has also been observed at C_1 with concomitant triene rearrangement when the hydroxyl nucleophile is internally located such as in the 30-dihydrorapamycins **7** and **8**.[8] In this case demethoxylation and C_1 attack readily took place with TFA (12 equiv) in CH_2Cl_2 at −78 °C to provide the tetrahydropyrans **9** and **10** from **7** and **8**, respectively, in 60% yields (Scheme 1).

The acid-catalyzed nucleophilic substitution of the C_7 methoxy group was found to have a broad scope and could be effected with different types of nucleophiles under a variety of conditions. The more general conditions involved the treatment of a mixture of rapamycin and the appropriate nucleophile with 10% TFA in dichloromethane for several hours at −45 °C. With the exception of the 30-dihydrorapamycins **7**–**8** and Et_3SiH above, the reaction was regioselective, proceeding by nucleophilic attack at C_7 and providing mixtures of readily separable C_7 epimers, in which the natural 7(*S*)

1 : R = ····OMe rapamycin
2 : R = —OMe
3 : R = OEt
4 : R = OCHO
5 : R = H

11 : R = SMe
12 : R = SPh
13 : R = 
14 : R = 
15 : R = 
16 : R = 
17 : R = 
18 : R = $CH_2CH{=}CH_2$
19 : R = CH_2COPh
20 : R = =O

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(2) (a) Sehgal, S. N.; Molnar-Kimber, K.; Ocain, T. D.; Weichman, B. M. *Med. Res. Rev.* **1994**, *14*, 1–22. (b) Rosen, M. K.; Schreiber, S. L. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 384–400. (c) Morris, R. E. *Transpl. Rev.* **1992**, *6*, 39–87. (d) Kahan, B. D.; Chang, J. Y.; Sehgal, S. N. *Transplantation* **1991**, *52*, 185–191.

(3) (a) Kuo, C. J.; Chung, J.; Fiorentino, D. F.; Flanagan, W. M.; Blenis, J.; Crabtree, G. R. *Nature* **1992**, *358*, 70–73. (b) Schreiber, S. L.; Albers, M. W.; Brown, E. J. *Acc. Chem. Res.* **1993**, *26*, 412–420.

(4) (a) Brown, E. J.; Albers, M. W.; Shin, T. B.; Ichikawa, K.; Keith, C. T.; Lane, W. S.; Schreiber, S. L. *Nature* **1994**, *369*, 756–758. (b) Sabatini, D. M.; Erdjument-Bromage, H.; Lui, M.; Tempst, P.; Snyder, S. H. *Cell* **1994**, *78*, 35–43.

(5) (a) Cafferkey, R.; Young, P. R.; McLaughlin, M. M.; Bergsma, D. J.; Koltin, Y.; Sathe, G. M.; Faucette, L.; Eng, W.; Johnson, R. K.; Livi, G. P. *Moll. Cell. Biol.* **1993**, *13*, 6012–6023. (b) Kunz, J.; Henriquez, R.; Schneider, U.; Deuter-Reinhard, M.; Movva, N. R.; Hall, M. N. *Cell* **1993**, *73*, 585–596.

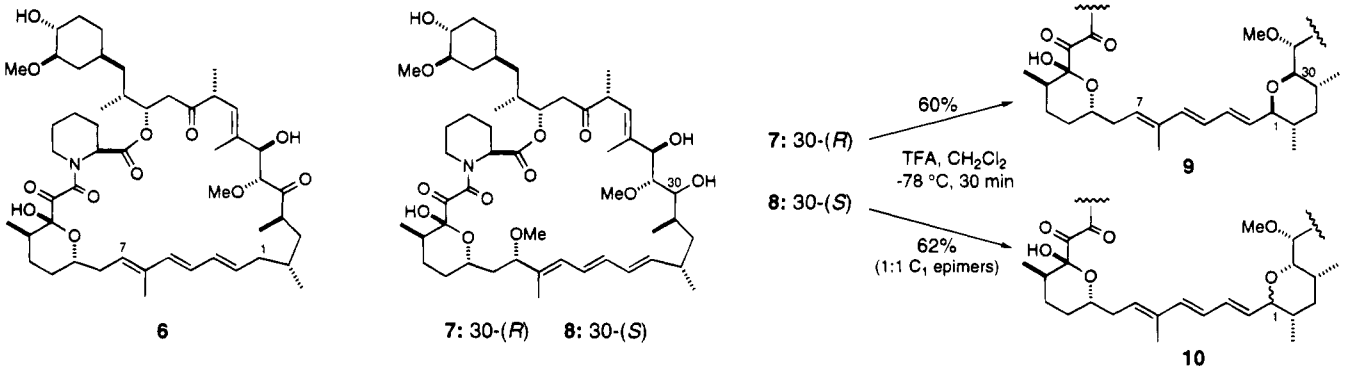
(6) Luengo, J. I.; Konialian, A. L.; Holt, D. A. *Tetrahedron Lett.* **1993**, *34*, 991–994.

(7) Kursanov, D. N.; Parnes, Z. N.; Loim, N. M. *Synthesis* **1974**, 633–651.

(8) Luengo, J. I.; Rozamus, L. W.; Holt, D. A. *Tetrahedron Lett.* **1994**, *35*, 6469–6472.

0022-3263/94/1959-6512$04.50/0

Scheme 1



configuration predominated.[9] Thus, reaction with thiols such as methanethiol or thiophenol (TFA/CH_2Cl_2, −45 °C, 4 h) gave the corresponding thioethers **11** and **12** in 75−80% yields (1.2:1 and 1.5:1 ratios of 7(*S*):7(*R*) epimers, respectively). Reaction with electron-rich aromatic compounds, such as 1,3-dimethoxybenzene, 1,3,5-trimethoxybenzene, furan, and 2-methylthiophene, took place under the same conditions to give **13**−**16** in >90% yields.[10] The Friedel−Crafts alkylations could also be effected with other Lewis acids ($TiCl_4$ and BF_3·OEt_2 at −78 °C), though generally in lower yields than with TFA. Reaction with the acid-sensitive pyrrole could only be carried out with milder Lewis acids such as $ZnCl_2$[11] (CH_2Cl_2, 0 °C, 8 h, 41% of **17**). This catalyst also worked well for the more labile *C*-centered nucleophiles allyltrimethylsilane and 1-phenyl-1-((trimethylsilyl)oxy)ethylene (**18**−**19**, $ZnCl_2$/CH_2Cl_2 at 0 °C).[12] The stability of the highly conjugated C_7 carbocation would make the abstraction of a C_7 hydride a relatively facile process. Indeed, we have found that rapamycin can be very readily oxidized at C_7 with DDQ[13] (CH_2Cl_2 0 °C, 2 min, 65% yield) to the corresponding 7-demethoxy-7-oxorapamycin (**20**).[4a]

In summary, the acid-catalyzed nucleophilic substitution of the C_7 methoxy group of rapamycin is a general reaction which allows the introduction of a number of different substituents at that center. This unique transformation allows the selective manipulation of the rapamycin effector domain[14] and should provide invaluable tools for the study of the downstream target of the FKBP12−rapamycin complex.[15]

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Supplementary Material Available: Experimental procedures and characterization data (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(9) Separation of the C_7 epimers could be easily achieved by silica gel flash chromatography; almost invariably, the natural 7(*S*)-epimer turned out to be less polar than its 7(*R*)-isomer. The regiochemistry of the addition was determined by ^1H NMR spectroscopic analysis of the different trienyl regions or by the distinct UV spectra of the C_7 and C_1 adducts (MeOH, λ_{max} 267, 277, 289 and 260, 270, 280 nm, respectively). Assignment of the C_7 configuration was based on the diagnostic ^{13}C NMR resonances of the C_{14} and C_{30} ketone carbonyls ($CDCl_3$, trans-amide rotamers): δ 191−193, 215−217 for the 7(*S*)-epimers; 195.5−196.5, 210−212 for the 7(*R*)-epimers. In addition, both C_7 epimers showed very different ^1H NMR spectra from each other, but strikingly similar within each epimeric series, an indication of different macrocycle conformations for each of the two epimers. Some of the more distinct protons in the major trans-amide rotamers as follows: (a) for the 7(*S*)-epimers ($CDCl_3$) δ 4.2 (d, *J* = 6 Hz, H-28), 3.7 (d, *J* = 6 Hz, H-29), 2.7 (dd, *J* = 17, 6.5 Hz, H-23), 2.6 (dd, *J* = 17, 6 Hz, H-23); (b) for the 7(*R*)-epimers ($CDCl_3$) δ 4.3 (d, *J* = 3.5 Hz, H-28), 4.1 (d, *J* = 3.5 Hz, H-29), 2.7 (dd, *J* = 17.5, 3 Hz, H-23), 2.35 (dd, *J* = 17.5, 8 Hz, H-23).

(10) The reaction with 1,3,5-trimethoxybenzene was unique since it was the only case where the unnatural 7(*R*)-epimer **14** was exclusively obtained. Subsequent studies of this reaction showed that this is due to a reversible Friedel−Crafts alkylation. Thus, at −78 °C a 4:1 mixture of 7(*S*):7(*R*) epimers **14** was initially formed; as the temperature was raised to −40 °C, the 7(*S*)-epimer was converted to the 7(*R*)-isomer, a conversion that is accelerated by a large excess of TFA. Eventually, after 4 h at −40 °C, (7*R*)-**14** is obtained in quantitative yield.

(11) (a) Alonso, F.; Yus, M. *Tetrahedron* **1991**, *47*, 313−316. (b) Groebel, B. T.; Konz, E.; Millauer, H.; Pistorius, R. *Synthesis* **1979**, 605−607.

(12) Alonso, F.; Yus, M. *Tetrahedron* **1991**, *47*, 9119−9124.

(13) Braude, E. A.; Jackman, L. M.; Linstead, R. P.; Lowe, G. *J. Chem. Soc.* **1960**, 3123−3132 and 3133−3138.

(14) Examination of the FKBP12−rapamycin crystal structure shows that the C_7 methoxyl makes little contact with FKBP12 and that, pointing directly away from the binding pocket, it is placed in a key position to interact with the downstream target of the FKBP12−rapamycin complex. See: Van Duyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433−7434.

(15) Results of biological evaluation of the C_7 rapamycin derivatives will be reported elsewhere.