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Acid Catalyzed Functionalization of Rapamycin

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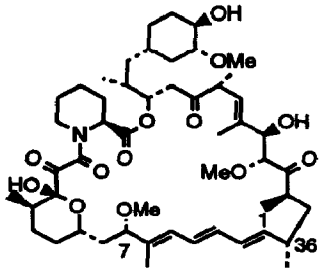
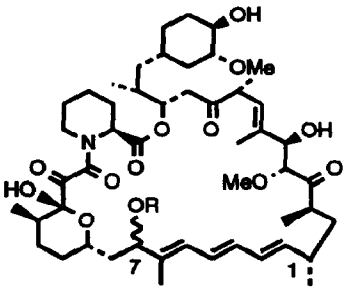
Abstract: Rapamycin rapidly undergoes demethoxylation at C-7 in the presence of Lewis acids ($BF_3 \cdot Et_2O$, $SnCl_4$ etc.) to give a highly stabilized carbocation. This intermediate gives a tetraene or is trapped by nucleophiles to give functionalized trienes. Several examples of the substitution reaction and elaboration of the reaction scheme are reported.

Rapamycin (1), a 31-membered macrocyclic lactone possessing potent immunosuppressive activity, is currently undergoing clinical trials for treatment of transplantation rejection.[1] Investigations into the behavior of rapamycin under basic conditions showed that rapamycin is chemically labile to hydroxide, carbonate, pyridines, and aliphatic amines in protic and aprotic solvents.[2] An earlier publication showed that

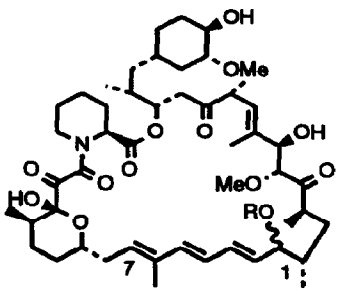


Rapamycin, 1

rapamycin is susceptible to a Lewis acid catalyzed retroaldol reaction.[3] Independent examination in our laboratories of the action of strong Lewis acids on the structural integrity of rapamycin uncovered the potential for Lewis acids to behave as catalysts in the nondestructive functionalization of rapamycin.



2a R = CH_2CH_2OH
2b R = $CH_2CH(OH)CH_2OH$
2c R = $CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$

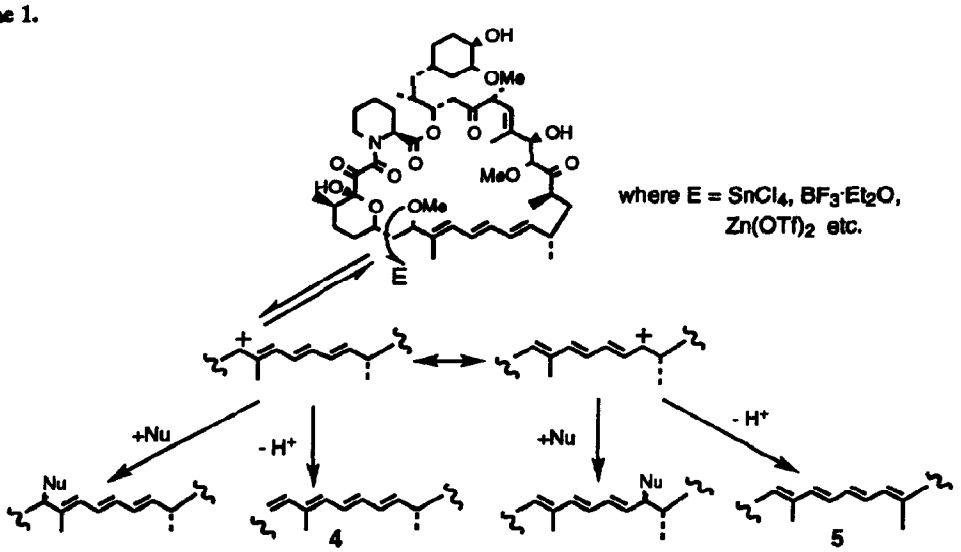


3a R = CH_2CH_2OH
3b R = $CH_2CH(OH)CH_2OH$
3c R = $CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$

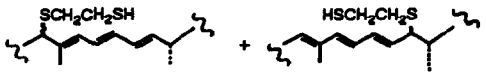
Facile and efficient substitution of the C-7 methoxy functionality takes place in the presence of various Lewis acids and appropriate nucleophiles. Thus, treatment of rapamycin with $HOCH_2CH_2OH$ in tetrahydrofuran in the presence of tin(IV) chloride resulted in formation of products **2a** and **3a** with reasonable yields (30-40% of each after HPLC).[4]

The proposed mechanism of the reaction is illustrated in **Scheme 1** and includes attack on a Lewis acid by the methoxy at C-7 with subsequent formation of a reactive carbocation. Reaction of the triene-stabilized carbenium ion with nucleophiles may occur from either end of the triene system giving C-1 or C-7 substitution products. Alternatively, in the absence of a strong nucleophile, elimination occurs to give a mixture of isomeric tetraenes **4** and **5**.

Scheme 1.



To avoid the well-known retroaldol opening of the rapamycin ring,[3] carboxylate β-elimination, [2] and benzilic acid rearrangement [2] these conditions must be met: 1) the reaction progress must be very carefully monitored (at least for the synthesis of tetraenes **4** and **5**), 2) preliminary dissolution of the Lewis acid in a coordinating solvent is highly desirable to regulate the reactivity of the acid, and 3) the optimal quantity of the acid used may range from 10 mol % to 500 mol % depending on the nature of nucleophile and purity of the reagents. Typically, 1 eq of rapamycin is allowed to react in THF at 0° C with 5-20 eq of the nucleophile in the presence of 50-75 mol % of a Lewis acid for 1-12 hours. Ethers **2b,3b** (from glycerol) and **2c,3c** (from triethyleneglycol monomethyl ether) were prepared in yields of 30-40 % for each isomer.

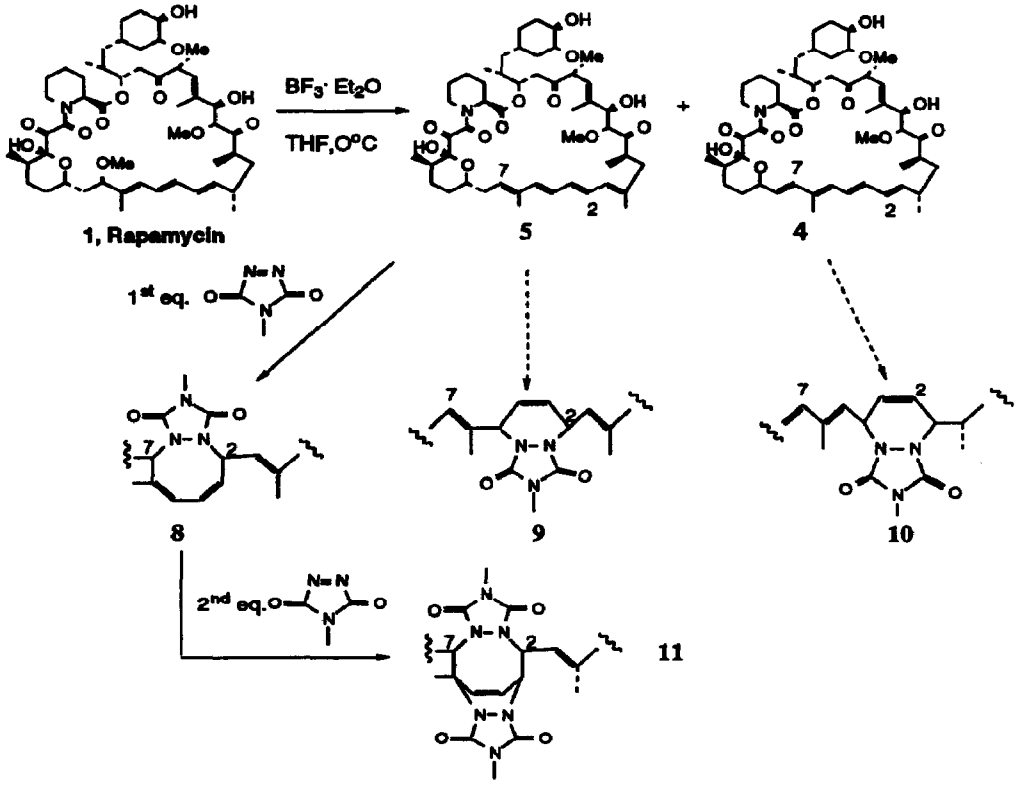


6 **7**

The approximate ratio of diastereomers in any given regioisomer was determined by ^1H NMR to be ~ 60:40. No assignment to any particular absolute configuration has been made. When nonpolar solvents were used, lower concentrations of acid were necessary to avoid decomposition. Using 1-5 mol % of $Zn(OTf)_2$ in dichloromethane in the presence of 1,2-ethanedithiol, **6** and **7** were isolated in 10-15% yield.

The elimination pathway was confirmed by carrying out the reaction in the absence of nucleophile. For the purpose of generalization, BF_3-etherate was used as the Lewis acid. The products **4** and **5**, isolated in 25-30 % yield, were a mixture of both possible regioisomers (from 1,2- and 1,8-elimination). The tetraene products were unstable and decomposition occurred before full analytical workup was possible.[5] To obtain added evidence that the tetraene products were formed, the mixture was subjected to the action of a powerful dienophile, N-methyltriazolinedione (See Scheme 2).

Scheme 2.



The reaction was initially carried out using one equivalent of N-methyltriazolinedione. A single addition product was obtained in which N-methyltriazolinedione added across the 2- and 7-positions of the tetraene **5** to give a diazacyclooctadiene ring **8** in what is formally a [6+2]-cycloaddition.[6] No products were obtained in which the dienophile added in a [4+2] fashion (i.e. **9** and **10**) and no products were obtained from

cycloadditions to tetraene 4.[7] To confirm this rather unusual result, the reaction was repeated using 2 equivalents of N-methyltriazolinedione in an effort to react diazacyclooctadiene 8 with a second equivalent of dienophile. Again, a single product was obtained in which the initially formed diazacyclooctadiene 8 reacts with the dienophile to give the tetra-azabicyclo[4.2.2]decaene 11.[8]

The triene substitution products as well as the Diels-Alder addition products were tested *in vitro* for their ability to inhibit T cell proliferation. Although all compounds synthesized in this paper were active in the IL-1 induced proliferation assay, they were all significantly less active than rapamycin. The perturbation of the triene region has been shown to profoundly effect the immunosuppressive activity implicating this region in rapamycin - effector binding protein interactions.[7]

With the discovery of the Lewis acid catalyzed substitution and elimination reactions, the behavior of rapamycin under acidic conditions is being elucidated. In addition, new cycloaddition reactions involving the reactive and unstable tetraene and N-methyltriazolinedione were reported. The very interesting chemistry associated with this complex natural product continues to be explored.

References and Notes.

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2. Steffan, R. J.; Kearney, R. M.; Hu, D. C.; Failli, A. A.; Skotnicki, J. S.; Schiksnis, R. A.; Mattes, J. F.; Chan, K. W. Caufield, C. E. *Tetrahedron Lett.*, **1993**, *34*, 3699; Yohannes, D.; Myers, C. D.; Danishefsky, S. J. *Tetrahedron Lett.*, **1993**, *34*, 2075; Yohannes, D.; Danishefsky, S. J. *Tetrahedron Lett.*, **1992**, *33*, 7469.
3. Luengo, J. I.; Konialian, A. L.; Holt, D. A. *Tetrahedron Lett.*, **1993**, *34*, 991. It was shown in our laboratories that the action of the very weak Lewis acid, $Ru(PPh_3)_2Cl_2$, also catalyzed the retroaldol reaction - C. E. Caufield, D. C. Hu, unpublished results.
4. Spectral data follows for **3a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.04-6.36 (m, 4 H, vinylic), 5.25 (m, 1 H, C-1 H), 4.02 (m, 1 H, C-9 H), 3.42 (m, 2 H, C-7 -O*CH*$_2$CH$_2$OH), 3.28 (s, 3 H, C-42 *CH*$_3$O-), 3.26 (m, 2 H, C-7 -OCH$_2$*CH*$_2$*O*H), 3.12 (s, 3 H, C-31 *CH*$_3$O-); MS (neg. FAB) m/e 943 (M-). 2D COSY experiments show an olefinic-aliphatic H1 to H36 correlation which indicates unrearranged triene. Spectral data follows for **3a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.04-6.52 (m, 4 H, vinylic), 5.62 (m, 1 H, C-2 H), 4.03 (m, 1 H, C-9 H), 3.43 (m, 2 H, C-7 -O*CH*$_2$CH$_2$OH), 3.29 (s, 3 H, C-42 *CH*$_3$O-), 3.27 (m, 2 H, C-7 -OCH$_2$*CH*$_2$*O*H), 3.17 (s, 3 H, C-31 *CH*$_3$O-); MS (neg. FAB) m/e 943 (M-). 2D COSY experiments show an aliphatic-aliphatic H1 to H36 correlation which suggests rearranged triene. For both isomers, in the ^{1}H NMR spectra under D_2O exchange conditions, the two OCH_3 resonances are doubled in a ratio of 60:40 which indicate possible diastereomers.
5. The tetraene products **4** and **5**, when separated, were stored under vacuum at 0° C overnight. Significant decomposition had occurred which prevented extensive characterization. Although **4** and **5** are written as being all *trans*, there is no spectral data to support this assignment. Spectral data follows for **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02-6.39 (m, 4H, vinylic), 5.2-5.5 (m, 3 H, vinylic), 3.38 (s, 3 H, C-42 *CH*$_3$O-), 3.10 (s, 3 H, C-31 *CH*$_3$O-); MS (neg. FAB) 881 (M-). Spectral data follows for **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93-6.39 (m, 4H, vinylic), 5.3 - 5.42 (m, 2 H, vinylic), 3.38 (s, 3 H, C-42 *CH*$_3$O-), 3.10 (s, 3 H, C-31 *CH*$_3$O); MS (neg. FAB) 881 (M-). Significant decomposition occurred when running ^{13}C NMR experiments leading to significant line broadening which prevented accurate interpretation of the spectra (especially in the olefinic region).
6. The reaction is formally a [6+2] cycloaddition and is disallowed. However, sufficient precedent exists in which N-phenyltriazolinedione reacts in a stepwise allowed fashion: see Adam, W.; DeLucchi, O.; Erden, I. *J. Am. Chem. Soc.*, **1980**, *102*, 4806; Jensen, F.; Foote, C. S. *J. Am. Chem. Soc.*, **1987**, *109*, 6376. Spectral data follows for **8**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.46 (dd, 1 H, J = 11.2, 14.3 Hz, C-4 H), 5.78 (d, 1 H, J = 11.2 Hz, C-5 H), 5.50 (dd, 1 H, J = 6.59, 14.9 Hz, C-3 H), 5.45 (bs, 1 H, C-1 H), 4.80 (bs, 1 H, C-2 H), 4.32 (bt, 1 H, J = 6.0 Hz, C-7-H), 3.28 (s, 3 H, C-42 *CH*$_3$O-), 3.14 (s, 3 H, C-31 *CH*$_3$O-), 2.90 (s, 3 H, *CH*$_3$N(C=O)); MS (neg. FAB) m/e 994.6 (M-); Other evidence for this structural assignment include 2D-COSY experiments which correlate C-7 H and C-9 H with C-8 Hs and 3 CH_3 singlets with correlation to resonances near δ 5.0 and the presence of 4 CH_3 doublets.
7. Ocain, T. D.; Longhi, D.; Steffan, R. J.; Caccese, R. G.; Sehgal, S. N. *Biochem. Biophys. Res. Commun.*, **1993**, *192*, 1340.
8. Spectral data follows for **11**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.8 (d, 1 H, J = 10.8 Hz, C-5 H), 5..6 (bd, 1 H, J = 10.8 Hz, C-4 H), 5.45 (bs, 1 H, C-1 H), 3.31 (s, 3 H, C-42 *CH*$_3$O-), 3.15 (s, 3 H, C-31 *CH*$_3$O-), 2.86 (s, 3 H, *CH*$_3$N(C=O)), 2.84 (s, 3 H, *CH*$_3$N(C=O)); MS (neg.FAB) m/e 1107 (M-). Other evidence for this structural assignment include the presence of 4 CH_3 singlets and 4 CH_3 doublets.

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