ORGANIC LETTERS

1999
Vol. 1, No. 12
2033−2035

Selective Epimerization of Rapamycin via a Retroaldol/Aldol Mechanism Mediated by Titanium Tetraisopropoxide

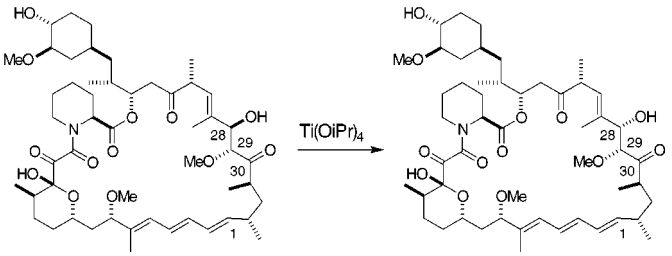
Wu Yang, Cheryl A. Digits, Marcos Hatada, Surinder Narula, Leonard W. Rozamus, Cameran M. Huestis, Jason Wong, David Dalgarno, and Dennis A. Holt*

ARIAD Gene Therapeutics, Inc., 26 Landsdowne Street, Cambridge, Massachusetts 02139-4234

holtd@tiac.net

Received November 1, 1999

ABSTRACT

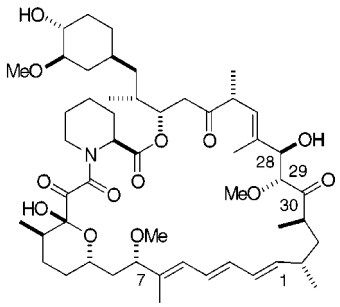


rapamycin (**1**) 28-epirapamycin (**2**)

We describe the efficient and selective epimerization of the immunosuppressant rapamycin to 28-epirapamycin under mild conditions. The mechanism of epimerization involves an equilibrium of the four C28/C29 diastereomers through a two-step retroaldol/aldol (macrocycle ring-opening/ring-closing) sequence. This retroaldol/aldol equilibration is not restricted to rapamycin but is also applicable to acyclic β-hydroxyketones. A potentially useful extension of this method—the use of β-hydroxyketones as enolate synthons for effecting inter- or intramolecular aldol reactions under neutral conditions—is demonstrated.

Rapamycin (**1**), a naturally occurring 31-membered macrolide, was originally isolated from *Streptomyces hygroscopicus* as an antifungal agent.[1] It shares some structural features with the natural product FK506, an immunosuppressant, and as a result also binds to FKBP12 (FK506 binding protein) with high affinity.[2] Like FK506, rapamycin exhibits potent immunosuppressive activity and has completed Phase III clinical evaluation for the prevention of organ transplant rejection.[3]

While exploring alternative Lewis acid catalysts for effecting nucleophilic substitution of the C7 methoxy group of rapamycin,[4,5] we found that treatment of rapamycin alone



rapamycin (**1**)

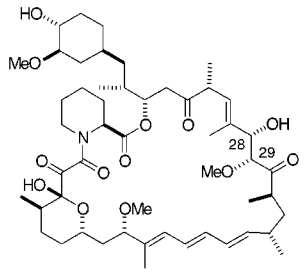
with $Ti(OiPr)_4$ in CH_2Cl_2 at room temperature resulted in the unexpected production of four isomeric compounds. The major reaction product was isolated in 60% yield along with

(1) Vezina, C.; Kudelski, A.; Sehgal, S. N. *J. Antibiot.* **1975**, *28*, 721−726.
(2) Schreiber, S. L.; Liu, J.; Albers, M. W.; Rosen, M. K.; Standaert, R. F.; Wandless, T. J.; Somers, P. K. *Tetrahedron* **1992**, *48*, 2545−2558.
(3) Sehgal, S. N. *Clin. Biochem.* **1998**, *31*, 335−340.
(4) Luengo, J. I.; Konialian-Beck, A.; Rozamus, L. W.; Holt, D. A. *J. Org. Chem.* **1994**, *59*, 6512−6513.

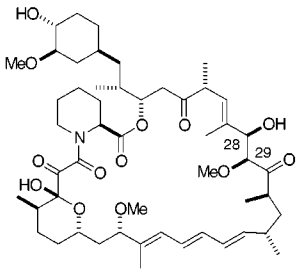
10.1021/ol991209o CCC: $18.00
Published on Web 11/16/1999

minor amounts (1−3%) of each of the other isomers and residual rapamycin. The major product was crystallized from methanol/water, and the structure was determined by X-ray crystallography to be 28-epirapamycin (**2**). The identities of the three minor isomers were shown by extensive NMR analysis to be 29-epirapamycin (**3**), 28,29-bisepirapamycin (**4**), and the known 28-secorapamycin (**5**).[6]

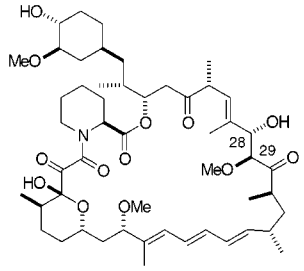
Secorapamycin (**5**) appears within seconds following the addition of $Ti(OiPr)_4$ and remains at a constant level (~3−5%) throughout the course of the reaction. Within the first 2 min of the reaction, more than half of the rapamycin is consumed and roughly equal amounts of the rapamycin diastereomers **2**−**4** are formed (**1**:**2**:**3**:**4** ≈ 2:1:1:1). The amounts of diastereomers **3** and **4** peak at 2 and 4 min, respectively. After 4 min, **3** and **4**, along with rapamycin, decrease steadily while the amount of **2** increases. After 30 min, the ratio of diastereomers effectively arrives at a steady state, which consists of **1**:**2**:**3**:**4** ≈ 1:7:1:1.



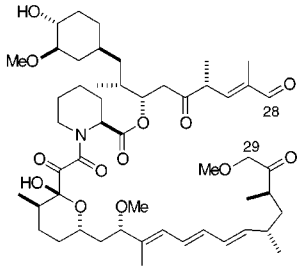
28-epirapamycin (**2**)



29-epirapamycin (**3**)



28,29-bisepirapamycin (**4**)



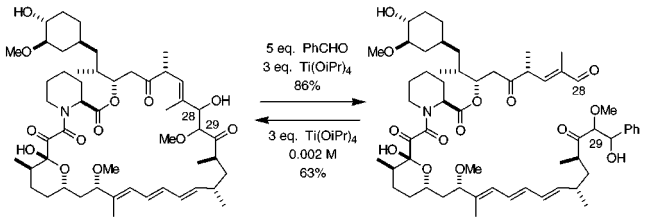
28-secorapamycin (**5**)

Our observations suggest the establishment of an equilibrium between all four C28/C29 diastereomers, with 28-epirapamycin (**2**) predominating as the thermodynamically preferred.[7] Indeed, independent subjection of purified **2**, **3**, or **4** to the same reaction conditions results in a similar distribution of products. A plausible mechanism for this equilibration, consistent with a low steady-state level of secorapamycin, involves a reversible retroaldol/aldol reaction—cleavage of C28−C29 bond with concomitant loss of stereochemical integrity at C28 and C29—followed by reclosure of the macrocycle to provide the mixture of four rapamycin stereoisomers.[8]

Curiously, secorapamycin (**5**) is unreactive under the isomerization conditions, even for extended times, and thus cannot be considered a true intermediate. Presumably, the $Ti(OiPr)_4$-mediated retroaldol produces a ring-opened reactive titanium intermediate (a C29−C30 enolate or its equivalent) which is in equilibrium with the ring-closed rapamycin isomers. This reactive intermediate must be present in small proportions relative to that of the closed macrocycle, and thus only a small amount of secorapamycin is observed following an aqueous quench. The reactive titanium species is apparently not formed directly from secorapamycin under the same conditions but is only produced from the retroaldol of the β-hydroxyketone.[9]

We were unable to alkylate the reactive species with methyl iodide or methyl triflate; however, we could "trap" the intermediate in a ring-opened form using benzaldehyde as a competing aldol substrate (Scheme 1). The addition of

Scheme 1



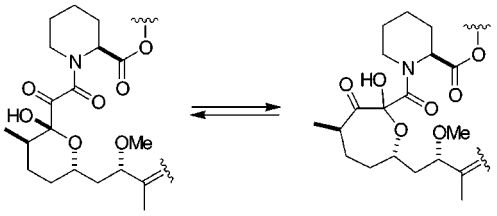
1-4 **6**

1 molar equiv of benzaldehyde at a 20 mM concentration resulted in a 1.4:1 ratio of rapamycin diastereomers (predominantly 28-epi) to benzaldehyde adducts **6** (as a mixture of four diastereomers). The product ratio was shifted to 10:1

(5) Several numbering schemes are in use for rapamycin. We have opted to maintain the scheme previously employed by us (L.W.R.; D.A.H.) and originated by the group responsible for the structure elucidation of rapamycin. Findlay, J. A.; Radics, L. *Can. J. Chem.* **1980**, *58*, 579−590.

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

(7) The oxepane tautomer of **2** is also present in the reaction mixture. This compound is found to the extent of about 1−3% during the first few minutes of reaction and increases to as much as 20% after 60 min and to 80% (with concomitant loss of **2**) following overnight treatment with $Ti(OiPr)_4$. Formation of the oxepane is dependent on $Ti(OiPr)_4$ as **2** is stable in dichloromethane. The oxepane tautomer can be isolated but rapidly equilibrates to a 9:1 mixture of pyran:oxepane in methanol/water. Treatment of FK506 with $Ti(OiPr)_4$ similarly and more rapidly yields its oxepane tautomer which reverts to pyran during chromatography (Wu Yang, unpublished). Hughes has reported the preparation and isolation of the oxepane tautomer of rapamycin: Hughes, P.; Musser, J.; Conklin, M.; Russo, R. *Tetrahedron Lett.* **1992**, *33*, 4739−4742.



pyran tautomer oxepane tautomer

(8) $Ti(OiPr)_4$ has been utilized in macrolide total synthesis to facilitate ring size equilibration through transesterification; Kigoshi, H.; Suenaga, K.; Mutou, T.; Ishigaki, T.; Atsumi, T.; Ishiwata, H.; Sakakura, A.; Ogawa, T.; Ojika, M.; Yamada, K. *J. Org. Chem.* **1996**, *61*, 5326−5351, Paterson, I.; Watson, C.; Yeung, K.-S.; Wallace, P. A.; Ward, R. A. *J. Org. Chem.* **1997**, *62*, 452−453.

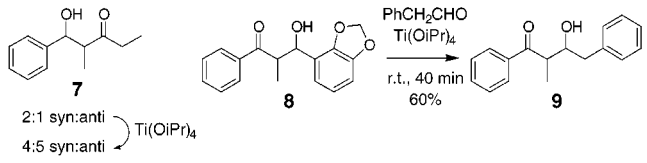
(9) $Ti(OiPr)_4$ has been reported to promote intermolecular aldol condensation of enolizable aldehydes and ketones; however, the reactions are carried out at elevated temperatures and invariably result in dehydrated α,β-unsaturated products. Mahrwald, R.; Schick, H. *Synthesis* **1990**, 592−595.

in favor of the ring-opened adducts (86% isolated yield) by using a 5-fold excess of benzaldehyde.

Inasmuch as **6** is also a β-hydroxyketone, potentially capable of undergoing retroaldol reaction to regenerate the same reactive secorapamycin−titanium intermediate from which it was formed, we examined its reactivity under conditions that would favor macrocyclization. Indeed, when **6** was treated with $Ti(OiPr)_4$ at high dilution (2 mM), intramolecular cyclization again prevailed to give predominantly 28-epirapamycin in high yield (63% of combined ring-closed rapamycin diastereomers at 70% conversion, Scheme 1). These results highlight the potential utility of the β-hydroxyketone moiety as an enolate synthon—masked with a sacrificial aldehyde—for effecting aldol reactions under very mild, neutral conditions where the target aldehyde may be delivered either in excess or intramolecularly as in a macrocyclization. In comparison, the title step of the total synthesis of rapamycin carried out by Hayward and Danishefsky[10] features a titanium-mediated aldol macrocyclization to form the C28−C29 bond. After many unsuccessful attempts, conditions employing $Ti(OiPr)Cl_3/NEt_3$ finally provided a 33% yield of macrocyclized products (as a 1:2.3 mixture of natural and 28,29-bisepi diastereomers[11]) along with recovered seco starting material (over 20%).

Equilibrations of aldolates by metals such as Li, Zn(II), Mg(II), and Ba(II) have been reported;[12−14] however, the conditions are typically strongly basic which often leads to retroaldol products and which may be incompatible with many complex natural products. To demonstrate that the mild conditions for this retroaldol/aldol method may be more broadly applicable for the equilibration of β-hydroxyketones as well as for the generation of new β-hydroxyketones by inclusion of a second aldehyde substrate, we examined two simple acyclic systems. First, treatment of a 2:1 mixture (syn/anti) of 1-hydroxy-1-phenyl-2-methyl-3-pentanone (**7**) with $Ti(OiPr)_4$ resulted in a 4:5 (syn/anti) mixture, slightly favoring the thermodynamically more stable anti isomer.[15] Second, treatment of β-hydroxyketone **8** with $Ti(OiPr)_4$ and an excess of enolizable phenylacetaldehyde afforded aldol product **9** in 60% yield (Scheme 2).

Scheme 2



In summary, we have described the efficient and selective epimerization of the natural product immunosuppressant rapamycin to 28-epirapamycin under extremely mild and neutral conditions. The structures of the novel 28-epirapamycin and the minor 29-epi and 28,29-bisepi diastereomers were confirmed through NMR spectroscopic and X-ray crystallographic analyses. The mechanism of epimerization appears to involve the establishment of an equilibrium of the four C28/C29 diastereomers through a two-step retroaldol/aldol (macrocycle ring-opening/ring-closing) sequence. We have shown that this retroaldol/aldol equilibration is not restricted to the rapamycin system but is also applicable to acyclic β-hydroxyketones. Additionally, we have demonstrated a potentially useful extension of this method—the use of β-hydroxyketones as enolate synthons for effecting inter- or intramolecular aldol reactions under neutral conditions.

Supporting Information Available: Experimental details for the synthesis and spectroscopic data for all new compounds. X-ray crystal structural information on **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL991209O

(10) Hayward, C. M.; Yohannes, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 9345−9346.

(11) The ^1H NMR of the 28,29-bisepirapamycin (**4**) we isolated was found to be consistent with that previously reported from the total synthesis. Hayward, C. M. Ph.D. Dissertation, Yale University, 1994.

(12) Still, W. C.; Hauck, P.; Kempf, D. *Tetrahedron Lett.* **1987**, *28*, 2817−2820.

(13) Swiss, K. A.; Choi, W. B.; Liotta, D. C.; Abdel-Magid, A. F.; Maryanoff, C. A. *J. Org. Chem.* **1991**, *56*, 5978−5980.

(14) Heathcock, C. H.; Buse, C. T.; Kleschick, W. A.; Pirrung, M. C.; Sohn, J. E.; Lampe, J. *J. Org. Chem.* **1980**, *45*, 1066−1081.

(15) House, H. O.; Crumrine, D. S.; Teranishi, A. Y.; Olmstead, H. D. *J. Am. Chem. Soc.* **1973**, *95*, 3310−3324.