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Lipase-Catalyzed Regioselective Esterification of Rapamycin: Synthesis of Temsirolimus (CCI-779)

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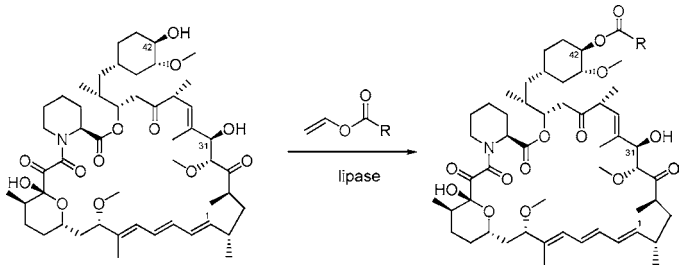
ABSTRACT



A lipase-catalyzed acylation of the immunosuppressant rapamycin with complete regioselectivity is described. The method was successfully applied to the synthesis of 42-hemiesters and temsirolimus (CCI-779), an investigational oncology drug.

Rapamycin **1** is a 31-member macrocyclic polyketide first isolated from *Streptomyces hygroscopicus* NRRL5491 as an antifungal agent[1] and later shown to be a immunosuppressive and antiproliferative agent[2,3] with a mechanism of action different from that of cyclosporine A and FK-506.[4] The compound is currently approved for use in renal transplantation (sirolimus, Rapamune) and shows promise as a coating for coronary stents to prevent restenosis following angioplasty.[5]

The biological activities of rapamycin are dependent on the binding of the left-hand portion of the molecule, from C8 to C31 (so-called "binding domain"), to FKBP12 (FK-506 binding protein) and the subsequent formation of a tertiary complex with mTor (mammalian target-of-rapamycin) protein[6] through the remaining portion of the macrolide (so-called "effector domain"). This unique mode of action, along with its recognized relationship to FK-506, has led to a number of research programs focused on the identification of rapamycin analogues with improved activity. A number of different approaches have been taken, including biological modifications such as precursor-directed biosyntheses[7] and biotransformations,[8] semisynthesis, including four total syntheses,[9] as well as synthetic modifications of different regions through selective manipulation of functional groups.[10] Manipulations of the binding domain such as selective reduction of the C15 ketone of the "tricarbonyl" unit and the C27 ketone,[11] switching the stereochemistry of C31−C33 hydroxyl ketone through a two-step retroaldol/aldol sequence,[12]

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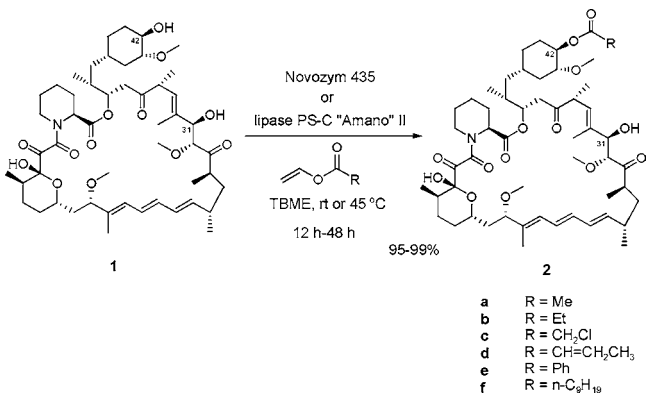
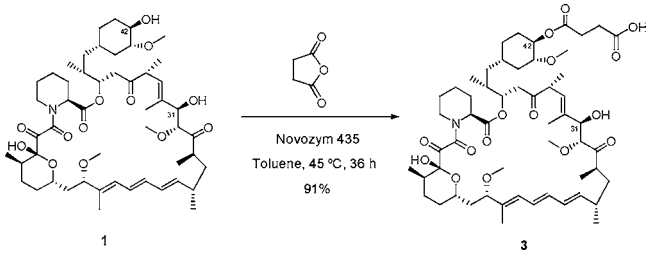
Scheme 1. Lipase-Catalyzed Esterification of Rapamycin 42-OH with Enol Esters



Scheme 2. Synthesis of Rapamycin 42-Hemisuccinate



modification of its effector domain through selective functionalization of the C1−C6 triene subunit via Sharpless's AD reaction,[13] and nucleophilic substitution of C7 methoxy group with different nucleophiles such as alcohols, thiols, and electron-rich aromatic systems[14] often resulted in the loss of its binding affinity and/or immunosuppressive activity. Modification of the cyclohexyl region, particularly in the 42-OH position, however, resulted in the discovery of new derivatives with good activity. One of them, Temsirolimus (CCI-779) (**7**), a 42-ester derivative with 2,2-bis(hydroxymethyl) propionic acid, is in clinical trials as an oncology agent.

Regioselective acylation of rapamycin at the 42-OH has proven to be difficult, as there is another secondary hydroxyl group at C31 surrounded by a number of sensitive functionalities. Here we report a lipase-catalyzed synthesis of 42-ester derivatives with various acylating agents with complete regioselectivity and high yields under mild conditions. This discovery provided a practical and efficient method for the synthesis of rapamycin 42-hemisuccinate, 42-hemiadipate, and CCI-779.

The starting point of the present work was to identify an appropriate enzyme catalyst suitable for acylation of rapamycin at the sterically less hindered 42-OH position. Among the wide range of lipases/solvents tested using vinyl acetate as an acyl donor, lipase Novozym 435 (*Candida antarctica* B) and lipase PS "Amano" (*Burkholderia cepacia*), particularly its immobilized form, lipase PS-C "Amano" II (immobilized on ceramic particles), suspended in anhydrous *tert*-butyl methyl ether (TBME) were identified as effective catalysts for rapamycin 42-OH acylation.

The reaction, depicted in Scheme 1, can proceed either at room temperature or, when less reactive enol esters such as vinyl crotonate, vinyl benzoate, and vinyl decanoate are used, at 45 °C. The reaction was completed within 12−48 h in the presence of 25−100% (w/w) enzyme, and nearly quantitative yields of the corresponding 42-esters were obtained after the removal of enzyme. HPLC analysis of the reaction demonstrated that the enzymatic acylation proceeded in an extremely regioselective fashion toward the 42-hydroxyl moiety of rapamycin; no 31-acylation or 31,42-diacylation byproducts were detected. In addition to vinyl esters, anhydrides such as acetic anhydride, propionic anhydride, and isobutyric anhydride also gave exclusively 42-acylated product under the conditions described above, but the reaction proceeded at slower rate.

Rapamycin 42-hemiesters of dicarboxylic acid such as succinic acid, glutaric acid, or adipic acid can serve as precursors for the synthesis of rapamycin−immunogenic protein conjugates that are useful as immunogens for the generation of antibodies for rapamycin as well as for isolating rapamycin binding proteins for immunoassays.[15] These 42-hemiesters are generally obtained by direct esterification of rapamycin with the corresponding anhydrides in the presence of a weak base. This procedure usually gives poor yields of desired 42-hemiesters due to the poor regioselectivity and the instability of rapamycin in the presence of a base. For example, with succinic anhydride and (dimethylamino)-pyridine (DMAP), rapamycin 42-hemisuccinate **3** was obtained in 18% yield after RP-HPLC purification.[16] A group at Abbott reported a two-step chemoenzymatic process[17] in which corresponding benzyl and methyl ester of rapamycin 42-hemisuccinate were hydrolyzed using a lipase from *Pseudomonas* sp. and gave 50% yield of **3** from the methyl ester and 29% from the benzyl ester after HPLC purification. The benzyl and methyl ester of rapamycin 42-hemisuccinate, in turn, were made by reaction of rapamycin with methyl succinyl chloride or benzyl succinyl chloride in the presence of a base.

The lipase-mediated direct acylation using succinic anhydride as an acylating reagent (Scheme 2) offered a simple and efficient route to access rapamycin 42-hemisuccinate **3**. A systematic screening of commercially available lipases showed that only Novozym 435 was able to catalyze the reaction and furnish **3** in good yield (91% isolated yield). Solvent screening revealed that toluene is the most suitable

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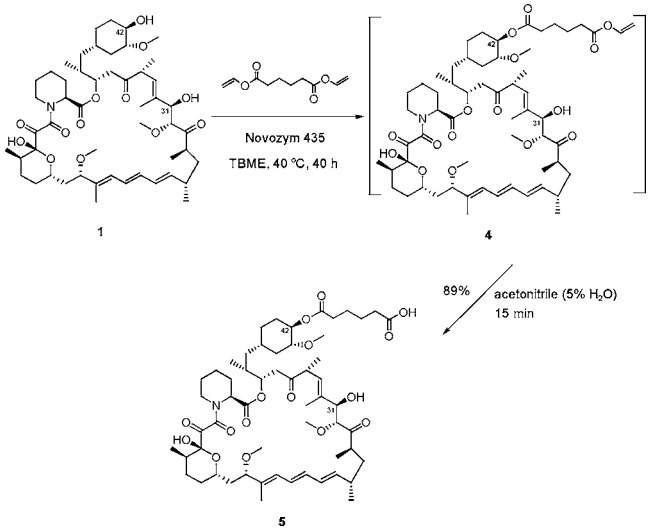
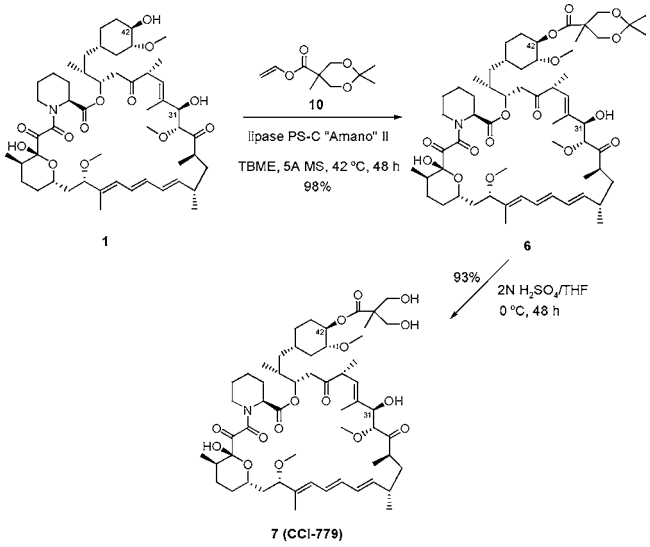
Scheme 3. Synthesis of Rapamycin 42-Hemiadipate



Scheme 4. Synthesis of CCI-779 with Ketal-Protected Vinyl Ester



medium for this reaction in regard to the rate of conversion. Once again, the lipase demonstrated excellent regioselectivity toward 42-hydroxyl group.

When similar reactions were carried out with other dicarboxylic anhydrides such as glutaric anhydride, diglycolic anhydride, and 3-methylglutaric anhydride, the conversion was low; the yields of the corresponding 42-hemiesters were 10−20% even with prolonged reaction times. The results indicated that these six-membered cyclic anhydrides are poor substrates for Novozym 435 lipase.

Divinyl adipate, a bifunctional acylating reagent that had been used previously to prepare a paclitaxel derivative via an enzyme-catalyzed two-step strategy,[18] was found to be a good acyl donor to synthesize 42-hemiadipate **5**. The synthesis of **5** via enzymatic transesterification/hydrolysis catalyzed by the same lipase was performed in a one-pot, two-step procedure as illustrated in Scheme 3. In the first step, rapamycin was reacted with divinyl adipate in anhydrous TBME in the presence of Novozym 435 to give intermediate **4**. Subsequent hydrolysis of the terminal vinyl ester group by adding wet acetonitrile (containing 5% H_2O) to the reaction mixture furnished rapamycin 42-adipic acid with 89% isolated yield. In this step, the lipase-catalyzed hydrolysis proceeded quickly, and the cleavage of the vinyl ester was completed within a few minutes.

A remarkable application of this enzymatic acylation approach was further demonstrated through the synthesis of temsirolimus (CCI-779) **7**, a compound currently under development as an mTor inhibitor for a number of tumor types. In a previously reported regioselective synthesis of CCI-779, both 31- and 42-OH in rapamycin were silylated, and the 42-OH was then selectively unmasked by mild acid hydrolysis. An isopropylidene ketal-protected 2,2-bis(hydroxymethyl) propionic acid side chain was then introduced via a 2,4,6-trichlorobenzyl mixed anhydride.[19] By employing a chemoenzymatic approach, as shown in Scheme 4, we were able to derive CCI-779 from a lipase-catalyzed acylation with ketal-protected vinyl ester **10** and subsequent acid-catalyzed deprotection of resulting intermediate **6** with excellent yield. The procedure offers a simple and efficient method to synthesize CCI-779 without the need of extra steps of protection−deprotection.

Enzyme−solvent screening showed that the most effective lipase to accomplish this transformation was lipase PS−C "Amano" II suspended in anhydrous TBME. Surprisingly, Novozym 435, a lipase well-known for its ability to accommodate substrates with varying sizes and complexities, and which had shown good activity in acylating rapamycin 42-OH as described above, showed no activity toward ester **10**. Under the optimized conditions, the enzymatic step was carried out with a 1.5 molar excess of enol ester **10** at 42−43 °C in the presence of molecular sieves, and >98% conversion of rapamycin was achieved with 100 wt % lipase after 48 h. Maintaining a low amount of moisture by adding molecular sieves to the reaction mixture was found to be important for achieving high product yield, as it can minimize the formation of seco-rapamycin, a lactone ring-opened product. Experiments showed that 5 Å molecular sieves were particularly suitable for this reaction.

To investigate whether different protecting groups other than the acetonide in ester **10** could be accepted by the lipase and thus eliminate the need for acid-catalyzed deprotection, the cyclic methylboronate **12** was prepared and tested as a substrate to lipase. Enzyme screening revealed that both lipase PS-D "Amano" I and lipase PS-C "Amano" II could effectively catalyze the synthesis of boronate-protected CCI-779 **8** under the conditions shown in Scheme 5. However, the acylation process stopped after reaching about 80% conversion; apparently, the enzyme lost activity at the later

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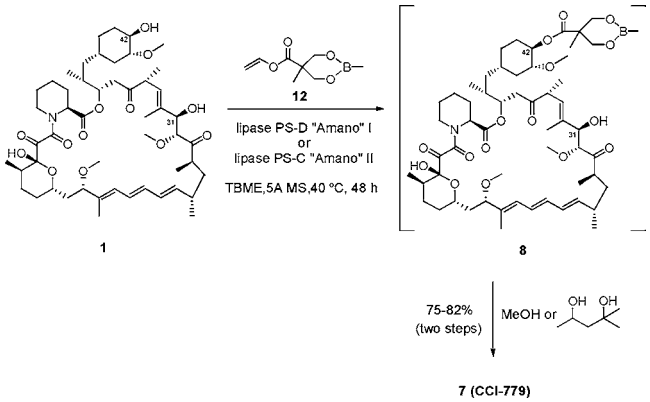
Scheme 5. Synthesis of CCI-779 with Methylboronate-Protected Vinyl Ester



stages of the reaction. The exact cause of this deactivation was not clear, as this was the first example in which a cyclic boronate derivative had been used as a substrate. Changing the temperature and solvent media and increasing the amount of lipase or ester **12** failed to drive the reaction to completion. Nevertheless, by employing the cyclic methylboronate as the protecting group, CCI-779 could be isolated in about 80% yield together with 16−20% recovered rapamycin, simply by adding alcoholic solvents[20] such as methanol, ethanol, or 2-methylpentane-2, 5-diol[21] to the reaction mixture.

Further evaluation showed that other cyclic boronate-protected vinyl esters such as butyl boronate gave low conversion, as the reaction went rather sluggish; a phenyl boronate-protected vinyl ester gave no reaction under similar conditions. Interestingly, the enzymatic acylation did not take place with the unprotected vinyl ester **11**.

The synthesis of vinyl esters **10** and **12** (Scheme 6) is

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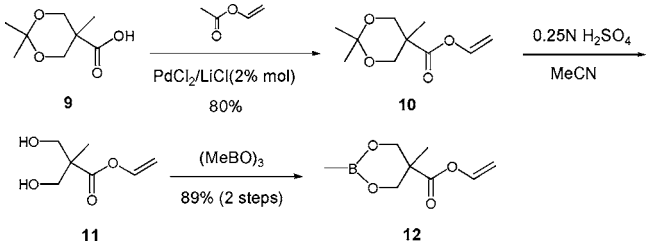
Scheme 6. Synthesis of Vinyl Ester **10** and **12**



straightforward. Ketal-protected **10** was prepared from **9**[22] through a vinyl-exchange reaction with vinyl acetate and $PdCl_2$−LiCl as a catalyst.[23] Deprotection with 0.25 N H_2SO_4 and subsequent treatment with trimethylboroxine gave *O*-methylboranediyl-protected **12** in good overall yield.

In summary, we established for the first time that rapamycin can be enzymatically derivatized with various acylating agents at the 42-hydroxyl position with complete regioselectivity. The synthetic utility of this reaction was demonstrated by the synthesis of 42-hemiesters, intermediates for rapamycin conjugates, and the synthesis of CCI-779, an investigational oncology drug, with excellent yields.

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Supporting Information Available: Experimental procedures for **3**, **5**−**8**, and **10**−**12**; NMR (^1H, ^{13}C) spectra for **3** and **5**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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