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Regioselective lipase-catalyzed acylation of 41-desmethoxy-rapamycin without vinyl esters

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ABSTRACT

Selective lipase-catalyzed acylation of 41-desmethoxyrapamycin has been achieved with a quaternary carboxylic acid avoiding the use of vinyl ester activation. Among the acyl donors investigated, the novel butanedione-monooxime and the *N*-acetylhydroxamate ester proved to be the most efficient donors, comparable in reactivity to the undesired vinyl ester and allowing selective, preparative acylation on gram scale in excellent yields. These new donors are proposed as sustainable and process-friendly alternatives to the widely used vinyl ester substrate activation in lipase-catalyzed acylations of secondary alcohols.

Rapamycin (*sirolimus*, *Rapamune*), a 31-member polyketide macrolide[1a,b] with immunosuppressive[1c] and antiproliferative properties,[1d] and its analogs have been extensively reviewed.[2] The two most prominent[7] derivatives, *temsirolimus* (CCI-779)[3] and *everolimus* (RAD001)[4] (Fig. 1), are mTOR inhibitors[5] used in cancer therapy.[6,8]

Evidently, as all rapamycin derivatives currently approved (*temsirolimus*, *everolimus*) for human therapy or in clinical trials (Ridaforolimus) are 42-*O*-substituted molecules, the ability to carry out selective modifications at/of the C42-hydroxyl is an important asset.[10] A Wyeth group had reported regioselective lipase-catalyzed acylation of the 42-OH group in Rapamycin via

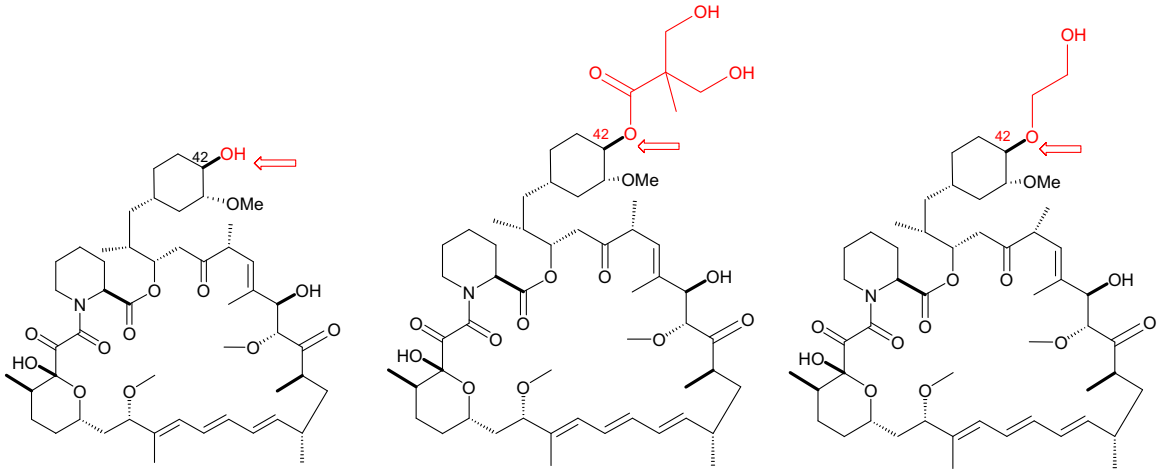

Figure 1.

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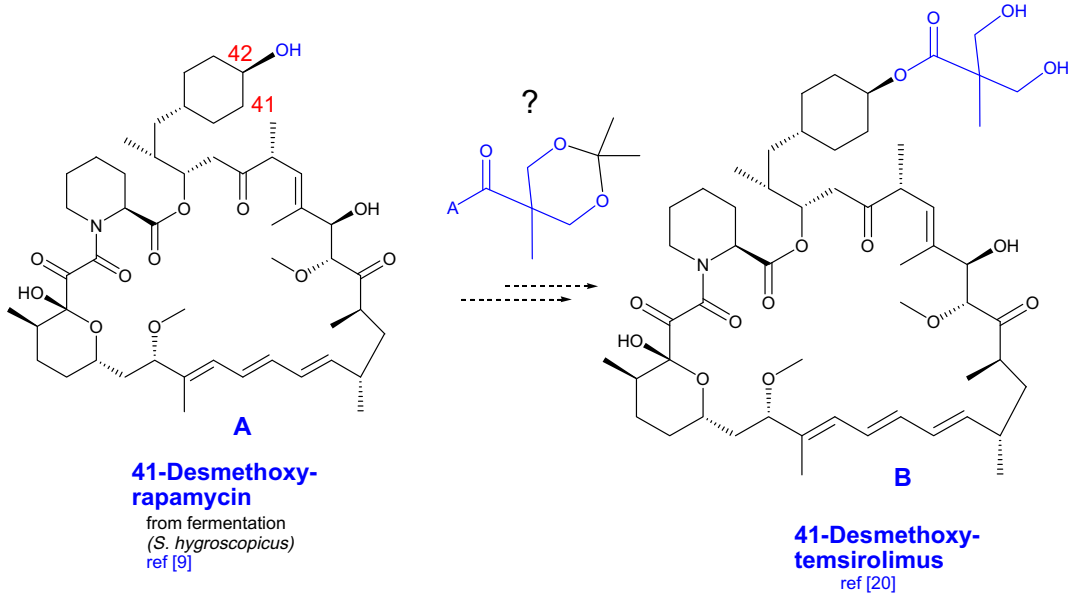
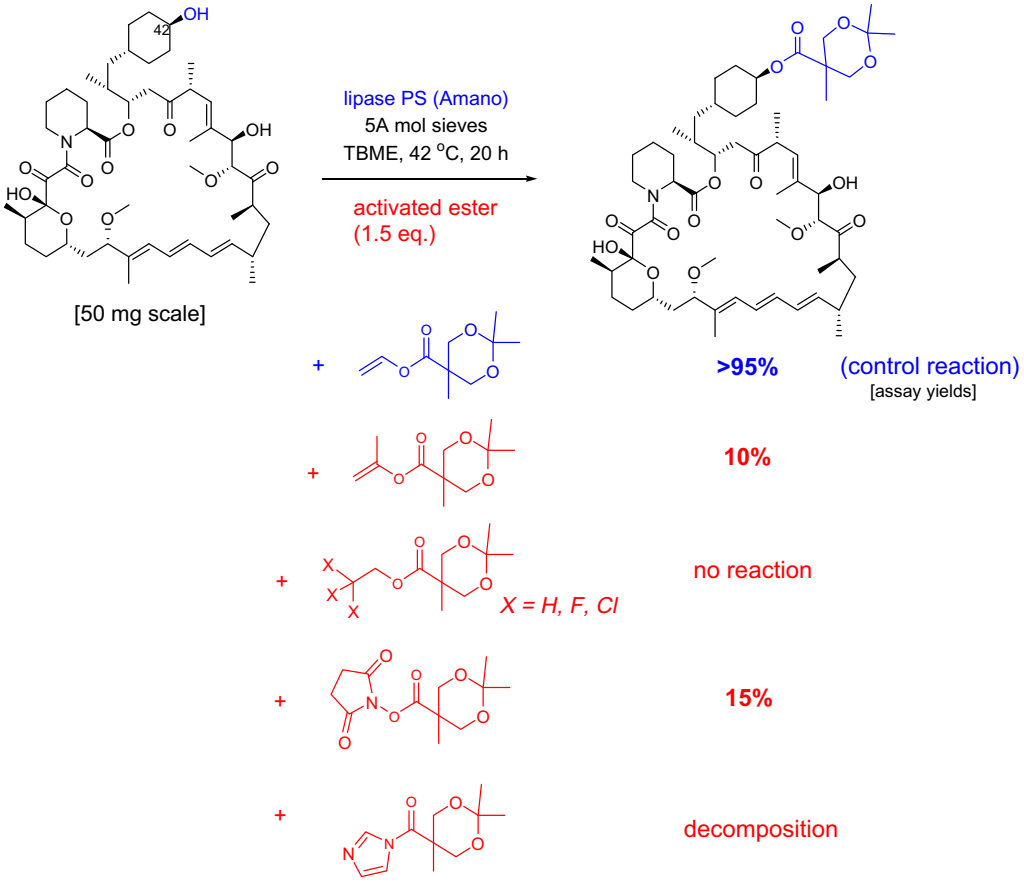


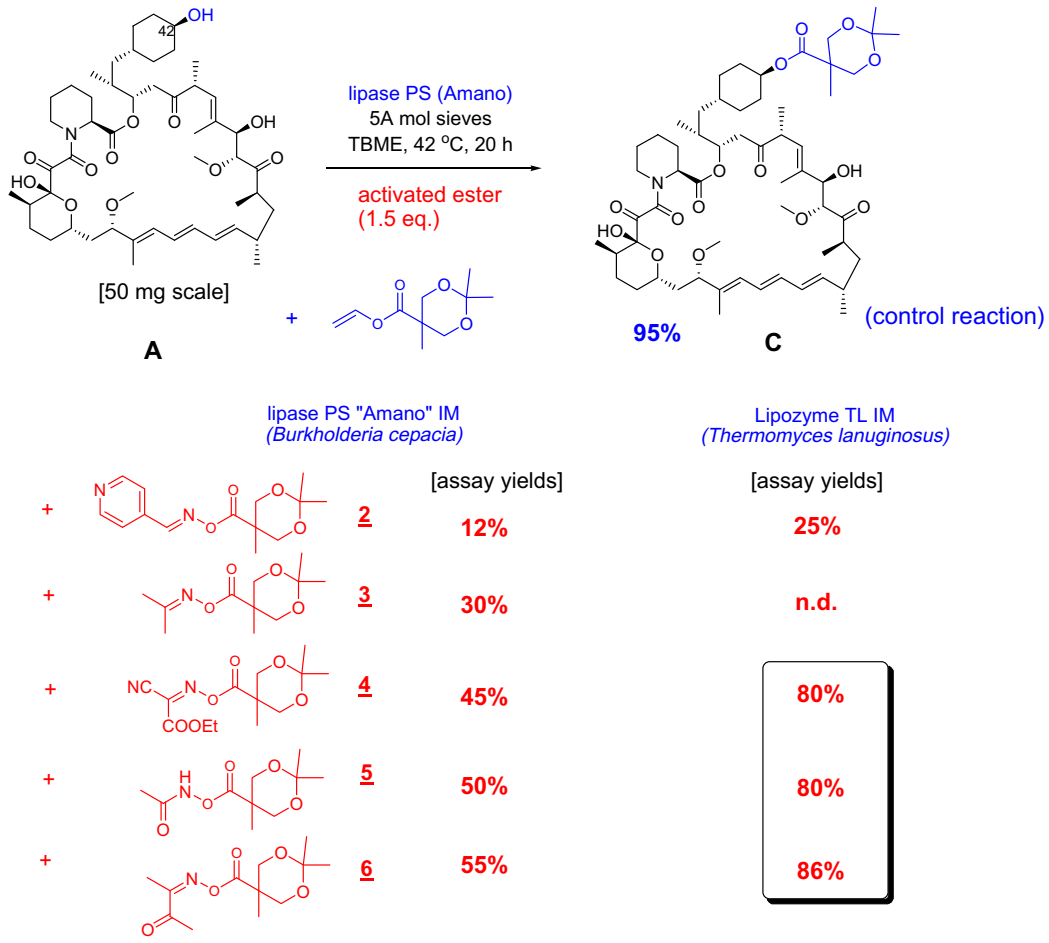
Figure 2.

the Maillard/Wong enol ester activation method[11] for a number of different carboxylic acids, enabling the efficient conversion of rapamycin into *temsirolimus*[12] (compare Fig. 1).[13]

A serious drawback of this vinyl ester methodology lies in the stoichiometric generation of acetaldehyde.[14] The low boiling point (21 °C) and flash point (−40 °C) of acetaldehyde and the explosive properties of acetaldehyde-air mixtures[15] present significant process obstacles. Also, synthesis of hindered vinyl ester donors typically requires the use of excess vinyl acetate at reflux temperature and its subsequent distillative removal,[16,17] another safety hazard due to the potential of vinyl acetate to undergo exothermic polymerization in the gas phase[18a] and the polymerization hazard of



Scheme 1.



Scheme 2.

vinyl compounds in general.[18b] Published reports on scale-up of selective vinyl ester-mediated acylations are therefore very scarce.[19]

Our investigation focused on finding a benign and scalable acyl donor for selective acylation of 41-desmethoxy-rapamycin **A** to the recently disclosed, biologically active rapamycin derivative 41-desmethoxy *temsirolimus*[20] **B** (Fig. 2[9,20]).

We initially evaluated a number of obvious vinyl ester substitutes, such as the corresponding isopropenyl ester[21] **2**, the trihaloethyl esters[22] **3**/**4**, and a few other activated ester derivatives depicted in Scheme 1.

Disappointingly, all of them were significantly inferior to the vinyl ester control reaction under the same conditions.[12] Surprisingly, there was a large rate difference between the vinyl and the isopropenyl ester reactions (entries 1 and 2).[23]

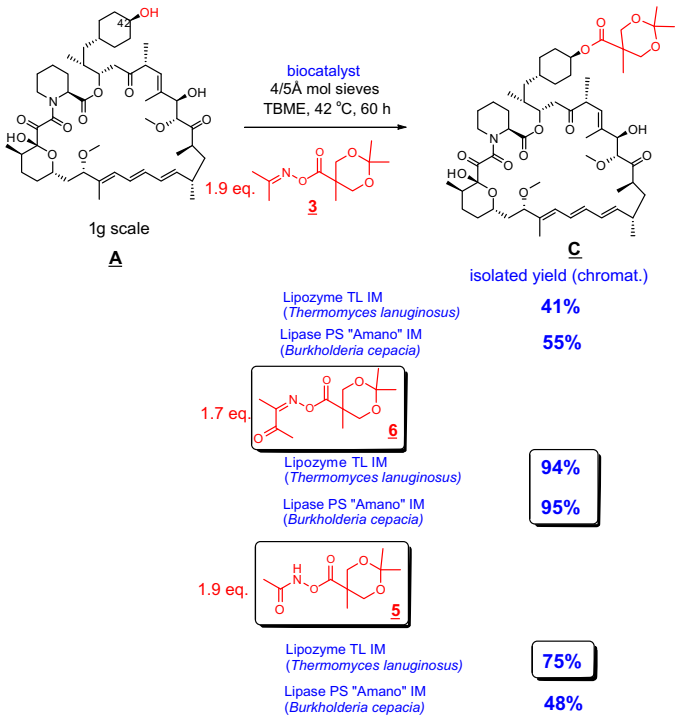
Since some initial reactivity was seen with the *N*-hydoxysuccinmidyl ester (entry 4), we decided to expand this structural motive into leaving groups that would not be basic enough to cause decomposition of the sensitive des methoxyrapamycin starting material, such as seen with the imidazolide (entry 5) and a number of nucleophilic catalysts also tried in conjunction with simple alkyl and a number of different haloalkyl esters (data not shown)[†].

The acetoneoxime active ester method[24] came to mind, especially since this active ester had been used in a selective, lipase-catalyzed acylation of a primary hydroxyl group (nucleoside derivative) carried out on pilot plant scale by a Schering–Plough group a few years ago.[25] Surprisingly, the intriguing reports of Schowen et al.,[26] Pratt et al.,[27] and Demuth et al.[28] on *N*-peptidyl-*O*-acylhydroxylamines, inhibitors of several classes of proteases (via irreversible acylation of a SER-hydroxy group in the active center of the enzyme), had been known for some time without triggering any curiosity in the potential use of this activation principle for lipase-catalyzed acylations.[29] An initial screen with the acetone oxime ester **3** and a number of additional oximates (**2**, **4**, **6**) as well as the *N*-acetylhydroxamate ester **5** with two selected, immobilized lipases was conducted (Scheme 2).

The oxime esters **4** and **6** showed promising activity surpassing that of the known acetone oxime ester (**3**). More efficient initial conversions were observed with the *T. lanuginosus* lipase compared to the *B. cepacia* lipase, the benchmark enzyme from the earlier vinyl ester study.[12] Remarkably, the hydroxamate **5** also showed acyl donor activity better than the acetone oxime ester **3**. Little is known on the impact of oxime ester structure on acylation efficiency in lipase-catalyzed reactions,[30] whereas *N*-acylhydroxamates such as **6** have not previously been evaluated as acyl donors in enzyme-catalyzed reactions.[29]

The two oxime esters **3** and **6** as well as the hydroxamate ester **5** were investigated further in a proof-of-concept study with immobilized *T. lanuginosus* and *B. cepacia* lipases in preparative gram-scale experiments (Scheme 3). The butanedione(mono)oxime ester **6** and the *N*-acetylhydroxamate ester **5** emerged as the most promising candidates for further development. Interestingly, for the

[†] Rapamycin is rather labile to base; prolonged heating in the presence of a weak base, even at low concentration, leads to decomposition, the major side-product being the lactone ring-opened product (i.e., "seco-rapamycin").



Scheme 3.

butanedione monoxime ester, we observed practically no difference in acylation efficiency between the two lipases tested, whereas the *N*-acetylhydroxamate ester appeared significantly more sensitive to lipase origin. We consider the hydroxamate ester to be an especially promising lead, as the *N*-acyl group can be readily modified (e.g., from acetyl to halo- or trihaloacetyl, or to a chiral acyl group, for applications in stereoselective synthesis) to adjust acylating properties.[31] Certainly both these novel acylating agents are much more amenable to scale-up and comparatively non-toxic[32] compared with the corresponding vinyl ester and its leaving group acetaldehyde.

As a conclusion, we have identified two promising alternatives for vinyl ester-activated, sterically hindered carboxylic acids in lipase-catalyzed acylations. From a process point of view, both the butanedione monooxime ester and the *N*-acetylhydroxamate ester are much more desirable candidates for scale-up development and we expect them to be useful for other applications in natural product synthesis as well.

Acknowledgments

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Supplementary data

Supplementary data (experimentals for acylation screen and preparative, gram scale acylation experiments) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.08.020.

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