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Lipase Mediated Hydrolysis of Rapamycin 42-Hemisuccinate Benzyl and Methyl Esters

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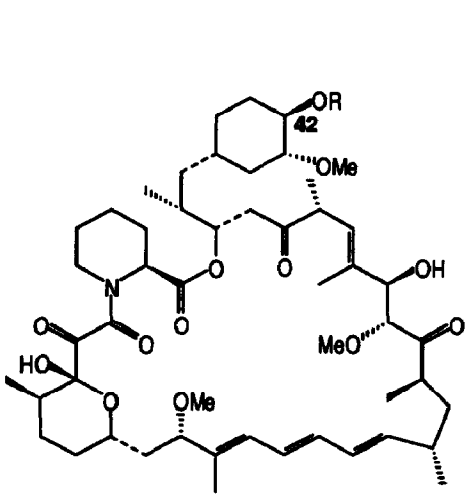
Abstract: Benzyl and methyl esters of rapamycin 42-hemisuccinate were hydrolyzed under very mild conditions to the rapamycin hemisuccinate using lipase from *Pseudomonas sp.* This selective deprotection was performed on a ≥100 mg scale for both esters resulting in 50% isolated yield from the methyl ester and 29% from the benzyl ester of the desired acid.

Rapamycin (**1**) is a naturally occurring 31-membered macrocyclic lactone with potent immunosuppressive properties.[1] Great excitement has been generated in using rapamycin as a therapeutic agent to prevent transplant rejection.[2] Due to its many structural features, rapamycin is sensitive to hydrolysis, elimination, reduction, and oxidation under mild conditions, thus, the preparation of rapamycin derivatives is challenging.[3] We have been exploring methods to conjugate rapamycin for various immunoassay components. In order to prepare immunogens suitable for antibody production we needed an efficient synthesis of rapamycin 42-hemisuccinate. Direct esterfication of the 42-position secondary alcohol with succinic anhydride in the presence of a weak base gave low yields of the desired product **4** (11%).[4] Preparation of rapamycin succinic acid diesters **2** and **3** using methyl succinyl chloride or benzyl succinyl chloride was accomplished with high yields.[5] Attempts to selectively remove the benzyl group by mild reduction (catalytic transfer hydrogenation using either 1,4-cyclohexadiene or ammonium formate and 10% Pd/C)[6] gave the hemisuccinate along with partial reduction of the triene as evidenced by MS and proton NMR. Mild base hydrolysis of the methyl ester was also not successful leading to products of ring degradation.[3]

Under these circumstances we turned to chemo-enzymatic methods. Enzymes have been used very effectively for stereoselective hydrolysis and are also known to carry out regioselective transformations where typical organic reactions fail.[7] Lipase has been used successfully for the mild hydrolysis of methyl esters, including those which have low solubility in water,[8a] and 2-(*N*-morpholino)ethyl esters of protected peptides.[8b] Recently we described the use of lipase for the diastereoselective hydrolysis of steroidal 3-(O-carboxymethyl) oxime methyl esters.[9] Gutman et. al reported the use of lipase for benzyl-alkyl transesterifications.[10] This letter reports the use of lipase from *Pseudomonas sp.* for the enzyme mediated hydrolysis of rapamycin 42-hemisuccinate benzyl (**3**) and methyl (**2**) esters to the free-acid (**4**).

Hydrolysis of Rapamycin Esters The nature of lipase medicated hydrolysis of **2-4** was carried out on a 100 µg of substrate along with 50 µg of lipase (Amano, LPL-80) and 100 µL buffer (sodium phosphate, pH 7.0, and 20% acetonitrile v/v).[11] The mixtures were incubated for 20 hours at room temperature with agitation by rotation. Then the reactions were concentrated in a centrifugal vacuum concentrator and the hydrolysis products were dissolved in MeOH. The distribution of hydrolysis products was determined by HPLC of the methanol extracts (see Table).[12] Larger scale hydrolysis of **2** and **3** were performed by placing

100 - 120 mg of a ester in a 15 mL plastic tube with 80 mg lipase, and 10 mL of buffer. After incubation the hydrolysis products were extracted out with ethyl acetate, dried (Na_2SO_4), and then concentrated under reduced pressure. The hemisuccinate was isolated using semipreparative HPLC.[12-14]



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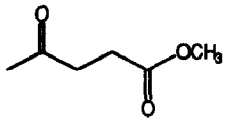
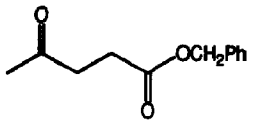
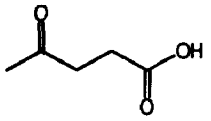
2. 

3. 

4. 

Incubation of the succinic acid diesters **2** and **3** with lipase resulted in the desired hemisuccinate (**4**) and rapamycin (**1**). The table below outlines the percent yield of hydrolysis products which reflects the regioselectivity of lipase between the different ester moieties. Lipase had greatest regio preference for the terminal methyl ester of substrate **2**. Once the terminal ester group is hydrolyzed the produced monoester **4** is not a substrate for lipase. This is probably due to the greater polar nature of the hemisuccinate and it has been reported that the enzyme has the greatest affinity for lipid-like substrates.[15] It is interesting that lipase was also able to hydrolyze the succinate next to the bulky rapamycin part of diester **2** and **3**. The 42-position is part of a cyclohexane ring which is an appendage off the macrocyclic system. X-ray data on rapamycin has revealed that the 42-position hydroxy group is situated the greatest distance from the parent ring.[1]

	Yield of Hydrolysis Products (%)*	
Substrate	**42-Hemisuccinate**	**Rapamycin**
2	68	32
3	42	58
4	>99	<1

* Percentages determined by HPLC of incubation extract (see experimental)

In summary we found lipase to be effective for the regioselective deprotection of rapamycin hemisuccinate under mild conditions. This method allowed us to prepare desired rapamycin haptens with good

yield. Additionally we want to note that to our knowledge this is the first reported use of lipase in the selective deprotection of benzyl esters in the presence of other easily reducible functionalities, therefore can be considered an equivalent of selective reduction. Currently we are pursuing the utility of this method for the preparation of rapamycin haptens with appended monoesters.

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5. Rapamycin, 42-hemisuccinate methyl ester (**2**): Rapamycin (1 g, 1.1 mmol) was dissolved in 5 mL of anhydrous CH_2Cl_2. Anhydrous pyridine (1.25 equiv.) was added, followed by the dropwise addition of a solution of methyl succinyl chloride (1.25 equiv.) in 5 mL CH_2Cl_2 over 40 min. The reaction mixture was stirred for 1 h, then directly chromatographed on silica gel column eluting with 50:50 ethyl acetate:cyclohexane to give 1.1 g (98%) of the desired ester. MS [FAB with KI]: m/z @ 1066 $[M + K]^+$; HPLC retention 23.1 min; ^1H NMR ($CDCl_3$) was consistent for proposed structure.
Rapamycin 42-hemisuccinate benzyl ester (**3**): Rapamycin (2g, 2.2 mmol) was dissolved in 10 mL of anhydrous CH_2Cl_2. Anhydrous pyridine (1.5 equiv.) was added, followed by the dropwise addition of a solution of benzyl succinyl chloride (1.5 equiv.) in 5 mL CH_2Cl_2 over 40 min. The reaction mixture was stirred for 2 h, then directly chromatographed on silica gel column eluting with 60:40 ethyl acetate:cyclohexane to give 1.58 g (65%) of the desired ester. MS [FAB with KI]: m/z @ 1142 $[M + K]^+$; HPLC retention 29.0 min; ^1H NMR ($CDCl_3$) identical to published spectra.[4]

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11. Hydrolysis was slow in the absence of a surfactant which is presumed to provide a hydrophilic interface required by lipase to facilitate efficient catalytic activity. Originally we used Triton X-100, however, it was difficult to separate product from the detergent. Acetonitrile was found to be a suitable substitute. Smith, G. B.; Bhupathy, M.; Dezeny, G. C.; Douglas, A. W.; Lander, R. J. *J. Org. Chem.* **1992**, *57*, 4544, Rubingh, D. M.; Bauer, M. in "Mixed Surfactant Systems" Holland, P. M. and Rubingh, D. M. Eds.; ACS Symposium Series 501; American Chemical Society: Washington, DC, 1992; Chapter 12, and the references therein.

12. HPLC was performed using a YMC ODS-AQ (4.6 × 250 mm) reversed phase column. Elution was with a linear gradient of 74:26 MeOH:50 mM NH_4OAc to 95:5 MeOH:50 mM NH_4OAc in 30 min with a flow rate of 1 mL/min. Column temperature was 45 °C and detection was with a photo diode array detector. Retention times for rapamycin (**1**) is 18.0 min and 13.9 min for 42-hemisuccinate (**4**). Semipreparative HPLC was carried out on a 9.8 × 150 mm column (Custom L. C., ODS, 3 μ) using the same mobile phase and gradient with a flow rate of 2 mL/min. Fractions were collected and concentrated under reduced pressure.

13. Hydrolysis of methyl ester (**2**): Starting with 120 mg gave 65 mg of hemisuccinate (**4**) which gave correct MS and NMR compared to fully characterized material.[4]

14. Hydrolysis of benzyl ester (**3**): Starting with 100 mg gave 32 mg of hemisuccinate (**4**) which gave correct MS and ^1H NMR compared to fully characterized material.[4]

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