described. More particularly, the present disclosure, in one or more embodiments, is directed to a method for the production of an enzymatically acylated chemical substrate (simvastatin or a simvastatin precursor), wherein a thiol scavenger is utilized to prevent inhibition of the acyltransferase enzyme. *See* Figure 1 (illustrating an exemplary and generalized LovD acyltransferase reaction wherein thiol byproduct is sequestered).

[0014a] In one aspect, there is provided a method of making a statin compound, comprising contacting a LovD acyltransferase substrate with a LovD acyltransferase in the presence of a thioester donor and a precipitating agent under conditions which yield a statin compound, wherein the reaction product is precipitated.

[0015] In one or more embodiments, the method comprises the steps of combining a LovD acyltransferase enzyme in a reaction medium with (i) a thiol scavenger and/or (ii) a precipitating agent, a substrate comprising a free hydroxyl moiety, and a thioester comprising an acyl moiety. The acyltransferase mediates the donation of an acyl moiety from the thioester to the free hydroxyl moiety, thereby producing the target compound. While not intending to be bound by any theory of operation, in embodiments wherein a thiol scavenger is utilized, the thiol scavenger is believed to sequester (bind or otherwise inactivate) the resulting thiol byproduct. The thiol byproduct may act as an enzyme inhibitor of LovD, thereby reducing enzyme activity and conversion of the substrate to product. Enzyme inhibition may be a result of competitive binding of the inhibitor to the enzyme. Also, again while not intending to be bound by any theory of operation, in embodiments wherein a precipitating agent is utilized, it is believed that removal of product from the reaction medium by precipitation results in a favorable shifting of the reaction equilibrium. Removal of thiol byproduct may also provide a favorable shifting of the equilibrium.

[0016] Embodiments described herein also include methods for generating simvastatin with a minimum of chemical steps using an improved LovD acyltransferase reaction. Advantageously, the improved acyltransferase reaction can optionally be combined with additional chemical steps, such as in "one-pot" chemical synthesis methods, to provide a method of producing simvastatin from lovastatin without isolation and purification of the monacolin J intermediate. For example, lovastatin may be hydrolyzed to monacolin J and the crude reaction product used as the substrate for the enzyme mediated acylation reaction. The present disclosure also provides methods and materials designed to take advantage of the improved LovD enzymatic process, such as that by which lovastatin is made, in order to produce related compounds such as the pravastatin derivative huvastatin.

[0017] Those of skill in the art will understand that the disclosure provided herein allows artisans to produce a wide variety of embodiments. In one exemplary embodiment, simvastatin is produced by combining together monacolin J or a monacolin J derivative (preferably monacolin J hydroxy acid), a thioester that donates an acyl moiety to the C8 hydroxyl group of monacolin J (or derivative) in the presence of a LovD acyltransferase, (i) a thiol scavenger (comprising, for example, activated charcoal) and/or (ii) a precipitating agent (such as ammonium hydroxide (NH_4OH)), and a LovD acyltransferase. The LovD acyltransferase functions to mediate the transfer of an acyl group from the thioester to regioselectively acylate the C8 hydroxyl group of monacolin J (or derivative), thereby producing simvastatin. The resulting byproduct thiol is sequestered (bound or deactivated by the thiol scavenger) when thiol scavenger is present. Reaction product is precipitated when a precipitating agent is present. Advantageously, reaction rate is noticeably or markedly improved.

[0017a] In one aspect, there is provided a method of making simvastatin hydroxy acid ammonium salt comprising contacting monacolin J or lovastatin with a LovD acyltransferase in the presence of an alpha-dimethylbutyryl thioester donor and ammonium hydroxide under conditions which yield simvastatin hydroxy acid ammonium salt, wherein the reaction product is precipitated.

[0018] A related embodiment provides a method of making simvastatin, comprising the steps of combining together lovastatin, a thioester that donates an acyl moiety to the C8 hydroxyl group of monacolin J in the presence of a LovD acyltransferase, (i) a thiol scavenger (comprising, for example, activated charcoal) and/or (ii) a precipitating agent (such as ammonium hydroxide (NH_4OH)) and a LovD acyltransferase. In this embodiment of the method, the LovD acyltransferase is allowed to hydrolyze lovastatin into monacolin J prior to the transfer of an acyl group from the thioester via a regioselective acylation of the C8 hydroxyl group of monacolin J using the acyltransferase, thereby providing simvastatin. The resulting byproduct thiol is sequestered (bound or deactivated by the thiol scavenger) when thiol scavenger is present. Reaction product is precipitated when a precipitating agent is present. Advantageously, reaction rate is improved and high conversions achieved.

[0019] The methods and materials described herein that are used to make simvastatin can be adapted to produce other compounds including those that are structurally similar to simvastatin, for example huvastatin. In this context, a method of making huvastatin is provided that comprises the steps of combining together hydrolyzed pravastatin tetra-ol, a thioester that donates an acyl moiety to the C8 hydroxyl group of hydrolyzed pravastatin tetra-ol in the presence of a LovD acyltransferase, (i) a thiol scavenger (comprising, for example, activated charcoal) and/or (ii) a precipitating agent (such as NH_4OH), and a