Tetrahedron Letters 51 (2010) 309–312

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Enzymatic synthesis of (*S*)-glutaric acid monoesters aided by molecular docking

Bo Wang, Ji Liu, Xiaoling Tang, Cheng Cheng, Jiali Gu, Liyan Dai *, Hongwei Yu *

Department of Chemical and Biochemical Engineering, Zhejiang University, Hangzhou 310027, PR China

ARTICLE INFO

Article history:
Received 14 September 2009
Revised 23 October 2009
Accepted 3 November 2009
Available online 10 November 2009

ABSTRACT

An efficient enzymatic method for the synthesis of (S)-3-substituted glutaric acid monoesters which was aided by molecular docking has been described. The reaction was proceeded under mild conditions, and the desired products were afforded with up to 98% ee in the yield of 93%. The results demonstrate that molecular docking is efficient to facilitate selection of substrates in enzymatic reaction.

Statins are a class of pharmaceuticals that inhibit the enzyme hydroxymethylglutaryl CoA reductase (HMG-CoA-R) and are therefore widely used as hypolipidemic agents and agents that lower the level of cholesterol in the blood (hypocholesterollipidemic agents).[1] Recently, statin was reported to be an important part of a new class of triterpene derivatives with anti-HIV activity and some other potential applications.[2] The first generation of the statin are fermentation products including simvastatin, levostatin, and pravastatin; and the second generation of statin are synthetic drugs including atovastatin, fluvastatin, rovovastatin, and pitavastatin. As all these drugs are of optical activity, it is critical to develop an efficient approach to produce optical intermediates which are applied to build them.

(*S*)-3-Substituted glutaric acid monoesters (SGAMs) are skeleton buildings of statins (Fig. 1) and are important intermediates to synthesize statins and their derivative products such as hapalosin,[3] dolastatin,[4] and iostatine. For years, two synthetic routes were mainly applied: One was hydrolysis of dialkyl-3-substituted pentanedioates using hydrolases or esterases.[5] Nevertheless, substrates used for hydrolization were difficult to be dissolved in buffer solution. The other one was alcoholysis of 4-substituted glutaric anhydrides applying pig liver esterase (PLE) and other enzymes.[6] The main drawback of these approaches was that these products were afforded in low ee values (less than 91%), and low yields. Thus they could not be employed in an industrial scale. The aim of this communication is to describe a new approach for the enzymatic asymmetric preparation of 3-substituted glutaric acid monoesters through the esterification reaction with 3-substituted glutaric acid and alcohols (Scheme 1).

Due to applying organic solvents in the esterification reaction, high concentration of substrates is accessible. In addition, compared with those methods in the literatures which hydrolyzed dialkyl-3-substituted pentanedioates in buffer solution, this approach permits the desired products to be isolated, purified, and dried easily, and the organic solvents can be recycled simply by distillation. Therefore, this approach has the potential to be applied in a large scale, which is reported for the first time to the best of our knowledge.

In the enzymatic esterification reaction, water plays a dual-side role. Water surrounding enzyme reduces the rigidity of protein in the enzyme by forming hydrogen bonds, which allows extension of the protein to expose its active section and exhibit activity.[7] Generally, the removal of water from the reaction system is necessary for the formation of ester in a feasible way. Therefore, a reactor unit (Fig. 2) was designed which was applied to remove water in vitro firstly by molecular sieves (4 Å), secondly by anhydrous copper sulfate. This design prevented enzyme activity from being decreased by excess of water produced in the reaction and the direct addition of molecular sieves in the reaction medium.

The reaction conditions were optimized using ethanol to react with 3-OTBS glutaric acid (TBS: *tert*-butyldimethylsilane) as a model reaction. Enzymes such as *Lipozyme TLIM*, *Novozym 435* (lipase from *Candida antarctica* (CALB)), *CRL*, Esterase from *E. coli K12* (Esterase expressed in *Escherichia coli K-12* from genbank accession No. b3412),[8] *Lipase PS 'Amano' SD, Lipase AK 'Amano', Lipase AYS 'Amano'*,and *Lipase AS 'Amano'* were investigated. (The detailed information was provided in Supplementary data, Table S1.) Of them, *Novozym 435* showed the best activity and excellent enantioselectivity, the *Lipozyme TLIM* showed poor activity and excellent enantioselectivity (Table 1), and the other six enzymes showed basically no activity in the reaction (see Supplementary data, Table S1). When ethanol was employed of **3** equivalents to 3-OTBS glutaric acid in the presence of *Novozym 435*, 3-OTBS glutaric acid monoethylester was obtained with 94% ee in 77% yield (Table 1, entry 2).

The reaction conditions were further optimized in the subsequent reactions in terms of reaction temperature, suitable solvents, and amount of *Novozym 435*. The results are summarized in Table 2 and the optimized conditions are: 3-OTBS glutaric acid (1 mmol),

* Corresponding authors. Tel.: +86 571 8795 1873 (H.Y.).
E-mail addresses: dailiyan@zju.edu.cn (L. Dai), yuhongwei@zju.edu.cn (H. Yu).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.11.008

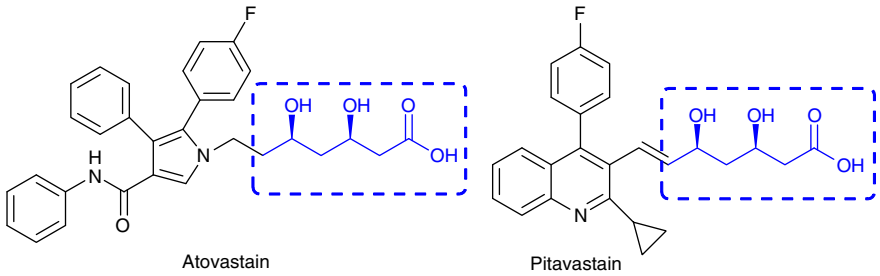
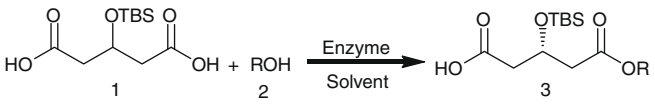


Figure 1. The chemical structures of some statins.



Scheme 1. Reaction of 3-substituted glutaric acid with ROH.

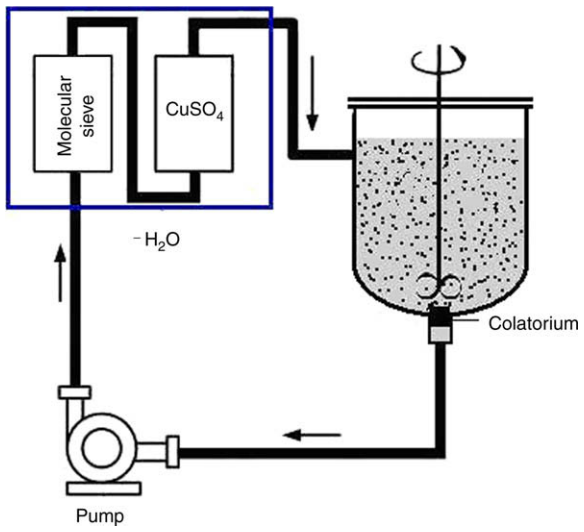
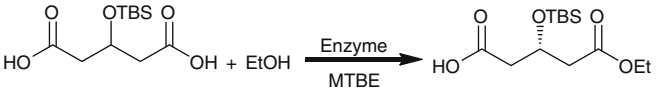


Figure 2. Enzymatic synthetic reaction unit. (The drying unit is in the blue frame.)

Table 1
Screening of enzymes on esterification reaction[a]



Entry	Enzyme	Time (h)	Yield[b] (%)	ee[c] (%)
1	*Lipozyme TLIM*	36	53	91
2	*Novozym 435*[e]	24	77	93

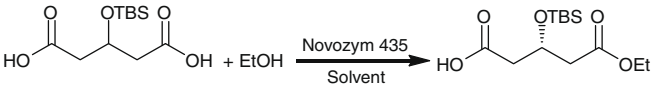
[b,c] Yields and values of ee were determined by HPLC analysis using a chiral AD-H column.

[a] Conditions: The reaction was performed by employing ethanol (5 mmol), 3-OTBS glutaric acid (1 mmol), and immobilized enzyme (0.18 g) in methyl *tert*-butyl ether (MTBE) (5 ml) at 30 °C, the enzyme was added at the start after 8 h, one half each.

ethanol (3 mmol) *iso*-octane (5 ml), temperature, 35 °C, and *Novozym 435,* 0.24 g (added at start also after 8 h, each time 0.12 g) (Table 2, entry 7).

CALB has been proven to be a highly stereoselective enzyme for the kinetic resolution of secondary alcohols.[9] The sequence and crystal structure of CALB in two crystal forms were reported by Uppenberg et al.[10] These structures revealed that the enzyme has

Table 2
Optimization of esterification reaction conditions catalyzed by *Novozym 435*[a]



Entry	Solvents	*Novozym 435* (g)	Temp (°C)	Yield/ee (%/%)[b]
1	Ethanol	0.16	35	51/84
2	Ethyl ether	0.16	30	64/80
3	Toluene	0.16	35	59/87
4	MTBEP	0.20	30	71/90
5	*iso*-Octane	0.16	30	73/92
6	*iso*-Octane	0.18	35	76/94
7	*iso*-Octane	0.24	30	84/94
8	*iso*-Octane	0.14	35	54/91
9	THF	0.18	30	30/7
10	*iso*-Propyl ether	0.16	35	72/90

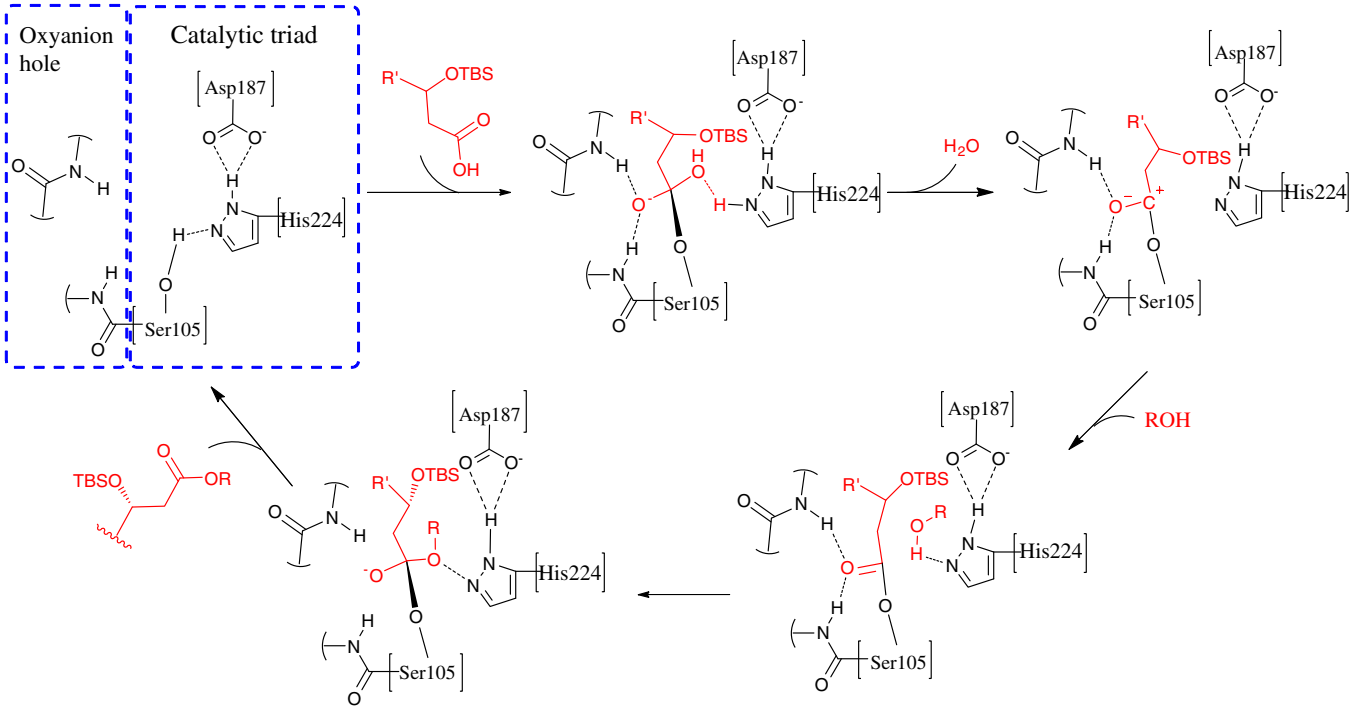
[a] Reaction conditions: The reaction was performed by employing ethanol (3 mmol), 3-OTBS glutaric acid (1 mmol), and *Novozym 435* in *iso*-octane (5 ml). *Novozym 435* was added at the start after 8 h.

[b] Yields and values of ee were determined by HPLC analysis using a chiral AD-H column.

an α/β-hydrolase-like fold with an active site, serine triad formulated by His224, Asp187, and Ser105 that is accessible from the solvent through a narrow channel.[11]

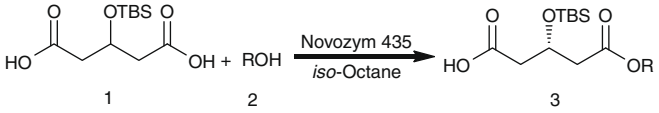
When the molecules of 3-OTBS glutaric acid were fit nicely into the stereospecificity pocket, one of its acid groups was placed at the active site, and its substituent was placed towards the surface of the protein and carboxylic acid reacted with the active site to form an oxyanion hole. The role and the mechanism of CALB are shown in Scheme 2.

In order to predict alcohols which can react with substrate **1** to yield product **3**, and meanwhile to alleviate the experimental workload, the structure-based molecular docking was conducted to facilitate the selection of alcohol candidates. In the molecular docking, a series of alcohols (Table 3) were applied as acyl acceptors. According to the results, the enzyme's enantioselectivity was most likely derived from the different orientation of the carboxyl part in product molecule (Fig. 3a). For instance, the carboxyl group of the *R*-isomer of product **3b** had poor contact with protein atoms (CG2 atom in Ile189 and Val190) and suffered intense electrostatic repulsion from the oxygen atom in Ile189. The detailed information of other product enantiomers was elaborated in Supplementary data and illustrated in Figure S2. The docking results (Table 3) indicated the absence of enzyme activity on bulky alcohol substrates (**2j–l**) and the enantioselectivity in the catalysis of small substrates (**2a–i**) based on the docking free energy difference between product enantiomers.[12] As the active site was accessible from the solvent through a narrow channel, only alcohols with no bulky group could get through and reach the active site to form product **3**. The narrow entrance stopped the bulky molecules of alcohol from passing through, thus no esterification reaction occurred.



Scheme 2. The proposed serine lipase reaction mechanism of enzymatic synthesis of SGAMs (structures of substrate **1**, substrate **2,** and product **3** are in red, R′ = –CH_2COOH).

Table 3
Docking free energy and results of the reactions[a]



Entry		ROH	E_R/E_S[b] (kcal/mol)	Yield/ee[c] (%)	Abs cfg[d]
1	**2a**	Methanol	−21.27/−17.57	81/92	*S*
2	**2b**	Ethanol	−7.23/−13.57	84/94	*S*
3	**2c**	*n*-Propanol	−9.95/−10.14	93/98	*S*
4	**2d**	*i*-Propanol	−16.82/−18.87	89/98	*S*
5	**2e**	*n*-Butanol	−9.85/−10.2	85/97	*S*
6	**2f**	*i*-Butanol	−6.8/−9.05	82/95	*S*
7	**2g**	*t*-Butanol	−12.52/−9.55	81/96	*S*
8	**2h**	*n*-Pentanol	−6.84/−9.6	77/95	*S*
9	**2i**	*i*-Pentanol	−9.05/−15.61	78/94	*S*
10	**2j**[e]	Cyclohexanol	−/−	–	–
11	**2k**[e]	Phenylmethanol	−/−	–	–
12	**2l**[e]	2-Phenylethanol	−/−	–	–

[a] Unless otherwise shown, the reaction was performed for 24 h by employing 3-OTBS glutaric acid (1 mmol), alcohol (3 mmol), and *Novozym 435* (0.24 g) in *iso*-octane (5 ml) at 35 °C added at the start after 8 h, 0.12 g each time.
[b] Docking energy of *R*/*S*-isomer (Kcal/mol).
[c] Yields and values of ee were determined by HPLC analysis using a chiral AD-H column.
[d] Absolute configuration was determined compared with the data in the literature.[5b]
[e] Reaction time, 36 h.

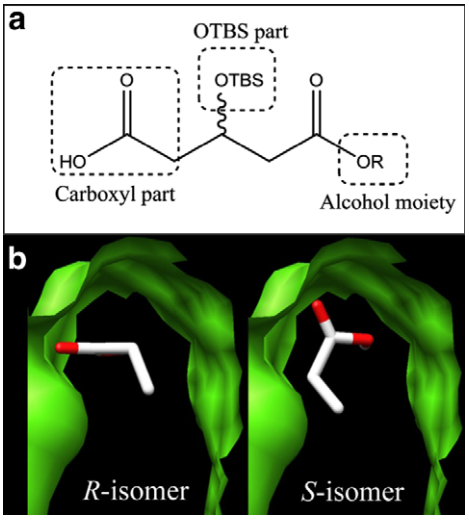


Figure 3. Docking analysis of products (**3a–l**) in the CALB binding pocket. (a) Three major sections of product molecule: carboxyl part, OTBS part, and alcohol moiety; (b) the carboxyl part of product **3b** enantiomer orientates differently in the inner part of CALB binding pocket.

Based on the docking results, the esterification reactions of 3-OTBS glutaric acid with a series of alcohols (**2a–i**) were conducted under the optimized conditions. To our excitement, the experimental results (Table 3, **2a–i**) agreed with the docking results very well. The molecules with bulky group which were predicted as having no activity by molecular docking (Table 3, entry 10–12) did not show activity. It is because the alcohol moiety of products is located at a narrow cavity surrounded by Ile189, Leu278, and Ile285 with the width of less than 5.9 Å (from Ile189 2HD1 atom to Ile 285 3HD1 atom), which is too small for the products (**3j–l**) with big alcohol moiety (benzyl group and cyclohexyl group). Consequently, the esterification of entries 10–12 could not be conducted by CALB. It demonstrated that the docking method based on the structure information of substrates is able to aid the design of experimental work.

In conclusion, molecular docking-aided enzymatic synthesis of (*S*)-3-substituted glutaric acid monoesters has been proven to be an efficient approach. Good substrate prediction, mild reaction conditions, excellent enatioselectivities, and high yields render this

synthetic approach potential for industrial application. We also believe that this approach can be applied in other important synthetic processes.

Acknowledgments

This work was financially supported by Qianjiang Ren Cai Programme. We express our appreciation to Amano Enzyme China Ltd for the generous gifts of the Lipase AS 'Amano', Lipase AYS 'Amano', Lipase PS 'Amano' SD, and Lipase AK 'Amano', and we are grateful to Zhejiang Jiuzhou Pharmaceutical Co., Ltd (China) for the gift of 3-OTBS glutaric acid.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.11.008.

References and notes

1. Lim, K. M. WO 03/087112 A1, 2003, 10, 23.
2. (a) Mayux, J. F.; Bousseau, A.; Pauwels, R.; Huest, T.; Henin, Y.; Dereu, N.; Evans, M.; Soler, F.; Poujade, C.; De Clercq, E.; Le Pecq, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3564–3568; (b) Sun, I. C.; Chen, C. H.; Kashiwada, Y.; Wu, J. H.; Wang, H. K.; Lee, K. H. *J. Med. Chem.* **2002**, *45*, 4271–4275.
3. Stratmann, K.; Burgoyne, D. L.; Moore, R. E.; Patterson, G. M.; Smith, C. D. *J. Org. Chem.* **1994**, *59*, 7219–7226.
4. (a) Shiori, T.; Hayashi, K.; Hamada, Y. *Tetrahedron* **1993**, *49*, 1913–1924; (b) Okuno, T.; Ohmori, S.; Nishiyama, S.; Yamamura, S.; Nakamura, K.; Houk, K. N. *Tetrahedron* **1996**, *52*, 14723–14734.
5. a Enzo, S.; Patrizia, F.; Paride, G. e-EROS Encyclopedia of Reagents for Organic Synthesis, Conference, General Review, 21–24, 2001.; (b) Egholm, J. E.; Helge, H. B.; Riise, M. A.; Thorleif, A. *J. Mol. Catal. B* **2003**, *21*, 55–58; (c) Christoph, A. S.; Wolfgang, E.; Markus, G.; Peter, S.; Wolfgang, W.; Ernst, K.; Adelbert, B. *J. Org. Chem.* **2002**, *67*, 2257–2262; (d) Julieta, M. B.; Francois, L. G. *Synth. Commun.* **1990**, *20*, 315–319; (e) Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Fehlhaber, H.-W.; Jendralla, H.; Kesseler, K.; Saric, R.; Schüssler, H.; Teetz, V.; Weber, M.; Wess, G. *Tetrahedron Lett.* **1988**, *29*, 2563–2565; (f) Heathcock, C. H.; Hadley, C. R.; Rosen, T.; Theisen, P. D.; Hecker, S. J. *J. Med. Chem.* **1987**, *30*, 1858–1873; (g) Gopalan, A. S.; Sih, C. J. *Tetrahedron Lett.* **1984**, *25*, 5235–5238; (h) Santaniello, E.; Chiari, M.; Ferraboschi, P.; Trave, S. *J. Org. Chem.* **1988**, *53*, 1567–1569.
6. (a) Kimiko, H.; Jun-ichi, K.; Yasuhiro, M.; Tadao, K.; Haruhisa, S. *Tetrahedron Lett.* **1996**, *37*, 2275–2278; (b) Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1866–1875; (c) Barbour, R. H.; Robins, D. J. *J. Chem. Soc., Perkin Trans. 1* **1985**, *11*, 2745–2748.
7. (a) Gayot, S.; Santarelli, X.; Coulon, D. *J. Biotechnol.* **2003**, *2*, 101–117; (b) Laane, C.; Boeren, S.; Vos, K. *Biotechnol. Bioeng.* **1986**, *30*, 81–87.
8. Wang, B.; Tang, X. L.; Ren, G. F.; Liu, J.; Yu, H. W. *Biochem. Eng. J.* **2009**, *46*, 345–349.
9. Frykman, H.; Ohmer, N.; Norin, T.; Hult, K. *Tetrahedron Lett.* **1993**, *34*, 1367–1370.
10. Uppenberg, J.; Hansen, M. T.; Patkar, S.; Jones, T. A. *Structure* **1994**, *2*, 293–308.
11. (a) Uppenberg, J.; Ohmer, N.; Norin, M.; Hult, K.; Kleywegt, G. J.; Patkar, S.; Waagen, V.; Anthomen, T.; Jones, T. A. *Biochemistry* **1995**, *34*, 16838–16851; (b) Magnusson, A. O.; Rotticci-Mulder, J. C.; Santagostino, A.; Hult, K. *ChemBioChem* **2005**, *6*, 1051–1056.
12. Hermann, J. C.; Ghanem, E.; Li, Y.; Raushel CF, M. J.; Irwin, J.; Shoichet, B. K. *J. Am. Chem. Soc.* **2006**, *128*, 15882–15891.