Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 2439–2441
Tetrahedron Letters

Sequential aldol condensation catalyzed by DERA mutant Ser238Asp and a formal total synthesis of atorvastatin

Junjie Liu, Che-Chang Hsu and Chi-Huey Wong*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, BCC 483A, La Jolla, CA 92037, USA

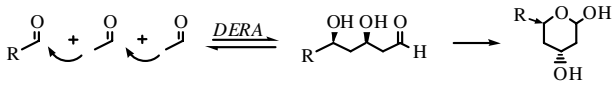
Received 10 December 2003; accepted 13 January 2004

Abstract—A mutant D-2-deoxyribose-5-phosphate aldolase (DERA), Ser238Asp, was used to prepare β-hydroxy-δ-lactol synthons and a key intermediate for atorvastatin synthesis.

2-Deoxyribose-5-phosphate aldolase (DERA, EC 4.1.2.4), a Schiff base forming type I class aldolase, catalyzes the reversible aldol condensation between acetaldehyde and D-glyceraldehyde-3-phosphate (G3P) to form D-2-deoxyribose-5-phosphate (Scheme 1).[1] DERA has been over-expressed in *Escherichia coli* and its structure and catalytic mechanism have been determined at the atomic level.[2] In addition to its natural substrates, DERA accepts a broad range of unnatural substrates, especially different acceptor aldehydes and thus potentially has enormous application in organic synthesis. Recently, a chemo-enzymatic total synthesis of epothilones A and C with DERA catalysis has been reported.[3] The C-2 stereochemistry in acceptor aldehydes was found to play a key role in the DERA catalysis.[3,4]

One type of interesting reaction catalyzed by DERA is the sequential synthesis of β-hydroxy-δ-lactol synthons (Scheme 2).[5] Under optimal conditions, three achiral aldehydes are catalyzed by DERA to afford enantiomerically pure (3*R*,5*R*) dihydroxyl aldehydes. The formation of lactol is expected to shift the reaction to the

Scheme 1. Aldol condensation catalyzed by DERA.



Scheme 2. Sequential aldol reactions catalyzed by DERA.

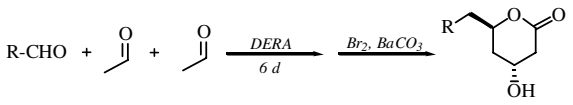
condensation. Several β-hydroxy-δ-lactols were prepared by catalysis of the wild-type DERA.[5] However, the choice of R groups is very limited and only very small or negatively charged groups are accepted. In order to improve the enzyme's tolerance of the R group, the Ser238 residue was mutated to Asp. Such a mutation should still retain the hydrophilic nature of the binding pocket,[4,6] but neutral and positively charged groups should become preferred over negatively charged groups. Indeed, the Ser238Asp mutant shows a 2.5-fold improvement in K_{cat}/K_M compared to that of the wild-type enzyme. Molecular modeling indicates that the C-3 hydroxyl hydrogen forms a hydrogen bond with carboxylate of Asp238, accounting for this increase in reactivity.[6]

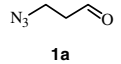
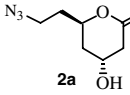
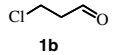
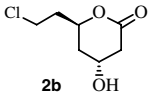
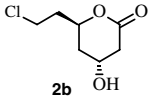
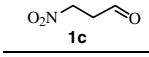
Compared with the wild-type DERA, the mutant Ser238Asp showed a great improvement in catalytic activity toward sequential aldol reactions (Table 1): Incubation of 3-azidopropinaldehyde **1a** and acetaldehyde with wild-type DERA did not afford any product **2a**, while the mutant Ser238Asp gave 35% yield of the sequential aldol condensation product. The mutant also improves the yield of the condensation between 3-chloropropinaldehyde **1b** and acetaldehyde from 25% for the wild-type enzyme to 43% for the mutant. Interestingly, 3-nitropropinaldehyde was not catalyzed by either the

* Corresponding author. Tel.: +1-619-784-2487; fax: +1-619-784-2409; e-mail: wong@scripps.edu

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.01.110

Table 1. Sequential aldol reactions catalyzed by mutant Ser238Asp



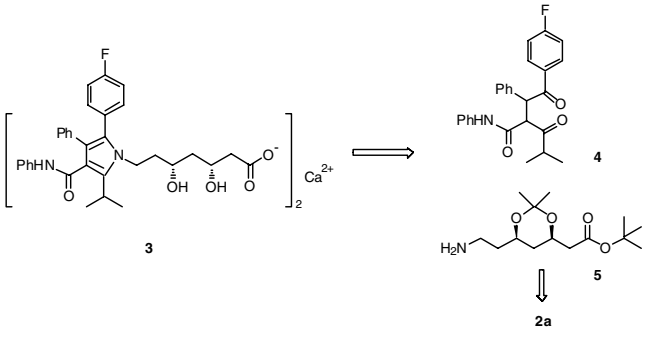
R–CHO	Wild type (yield %)	Ser238Asp (yield %)
 **1a**	N.R.[b]	 **2a** (35)[a]
 **1b**	 **2b** (25)[a]	 **2b** (43)[a]
 **1c**	N.R.[b]	N.R.[b]

[a] For two steps.
[b] N.R.: no reaction.

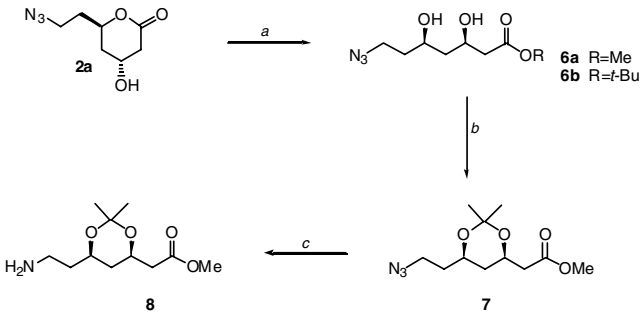
wild-type or the mutant enzymes, presumably because of the negative charge on the oxygen atom of the nitro group, which is less favored by the mutant than the wild-type enzyme.

The sequential aldol reaction product **2a** could easily be transformed to molecules such as **5**, a key chiral component of atorvastatin **3** (Lipitor®, Sortis®), which is an inhibitor of HMG-CoA reductase. Previous syntheses of atorvastatin involve a Paal–Knorr pyrrole formation reaction, which couples aromatic diketone **4** with chiral ester **5** (Scheme 3). Several different approaches to the key intermediate **5** or its analogues have been reported.[7]

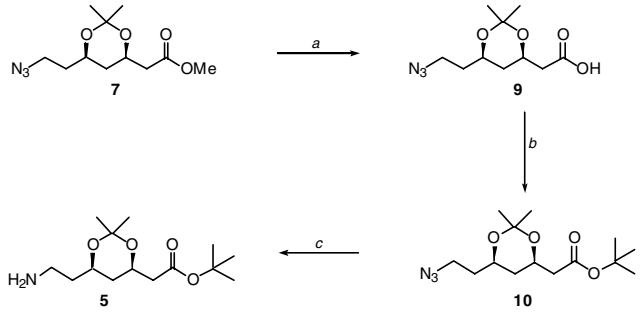
In our research, the lactone ring of **2a** was first opened under basic conditions to afford methyl or *tert*-butyl esters **6a** and **6b** (Scheme 4). Methyl ester **6a** was then protected with 2,2-dimethoxypropane to afford the acetonide **7**. Ph_3P was employed to reduce the azido group to the primary amine in high yield. However, we found that *tert*-butyl ester **6b** also became methyl ester **7** when **6b** was treated with 2,2-dimethylpropane under acid conditions. The *tert*-butyl ester amine **5** could be prepared from methyl ester **7** in a three-step procedure (Scheme 5).[10] The methyl ester was first hydrolyzed under basic conditions.[8] Esterification of **9** with Boc_2O afforded *tert*-butyl ester **10**,[9] and **5** was synthesized after



Scheme 3.



Scheme 4. Reagents and conditions: (a) MeONa, MeOH, 83%; *t*-BuOK, *t*-BuOH, 72%; (b) camphorsulfonic acid, 2,2-dimethoxypropane, 76%; (c) Ph_3P, 3d, 88%.



Scheme 5. Reagents and conditions: (a) LiOH, MeOH–H_2O, 83%; (b) Boc_2O, DMAP, 86%; (c) Ph_3P, 3d, 72%.

reduction of **10** with Ph_3P. Following previously described methodology,[7d] atorvastatin can be synthesized from **5**.

In conclusion, we examined the mutant DERA Ser238Asp's activity toward different substrates and found that the substrate specificity of the mutant enzyme had opened up to tolerate more unnatural substrates. A new way to prepare *tert*-butyl[(4*R*,6*R*)-6-aminoethyl-2,2-dimethyl-1,3-dioxn-4-yl]acetate, a key chiral intermediate in the synthesis of atorvastatin, was developed from the product of enzymatic catalysis **2a**. Compared to the previous syntheses, the new chemoenzymatic route described here is much shorter and more efficient.

References and notes

1. Gijsen, H. J. M.; Qiao, L.; Fitz, W.; Wong, C.-H. *Chem. Rev.* **1996**, *96*, 443–473.
2. Heine, A.; Desantis, G.; Lutz, J. G.; Mitchell, M.; Wong, C.-H.; Wilson, I. A. *Science* **2001**, *294*, 369–374.
3. Liu, J.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2002**, *41*, 1404–1407.
4. Liu, J.; Desantis, G.; Wong, C.-H. *Can. J. Chem.* **2002**, *80*, 643–645.
5. (a) Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 8422–8423; (b) Gijsen, H. J. M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 7585–7591.

6. Desantis, G.; Liu, J.; Clark, D. P.; Heine, A.; Wilson, I. A.; Wong, C.-H. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 43–52.
7. (a) Jendralla, H.; Baader, E.; Bartmann, W.; Beck, G.; Bergmann, A.; Granzer, E.; Kerekjarto, B. v.; Kesseler, K.; Krause, R.; Schubert, W.; Wess, G. *J. Med. Chem.* **1990**, *33*, 61–70; (b) Roth, B. D.; Blankley, C. J.; Chucholowski, A. W.; Ferguson, E.; Hoefle, M. L.; Ortwine, D. F.; Newton, R. S.; Sekerke, C. S.; Sliskovic, D. R.; Stratton, C. D.; Wilson, M. W. *J. Med. Chem.* **1991**, *34*, 357–366; (c) Brower, P. L.; Butler, D. E.; Deering, C. F.; Le, T. V.; Millar, A.; Nanninga, T. N.; Roth, B. D. *Tetrahedron Lett.* **1992**, *33*, 2279–2282; (d) Baumann, K. L.; Butler, D. E.; Deering, C. F.; Mennen, K. E.; Millar, A.; Nanninga, T. N.; Palmer, C. W.; Roth, B. D. *Tetrahedron Lett.* **1992**, *33*, 2283–2284; (e) Rádl, S. *Synth. Commun.* **2003**, *33*, 2275–2283.
8. Corey, E. J.; Székely, C. S.; Shiner, C. S. *Tetrahedron Lett.* **1977**, *18*, 3529–3532.
9. Takeda, K.; Akiyama, A.; Nakamura, H.; Takizawa, S.-i.; Mizuno, Y.; Takayanagi, H.; Harigaya, Y. *Synthesis* **1994**, 1063–1066.
10. Selected NMR data: **7** ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.32 (m, 1H), 4.01 (m, 1H), 3.69 (s, 3H), 3.41 (m, 2H), 2.56 (dd, $J = 7.0$, 15.8 Hz, 1H), 2.39 (dd, $J = 6.6$, 15.4 Hz, 1H), 1.70 (m, 2H), 1.60 (dt, 1H, $J = 2.6$, 14.8 Hz), 1.46 (s, 3H), 1.37 (s, 3H), 1.22 (m, 1H); **8** ^{1}H NMR (600 MHz, CD_3OD) δ 4.29 (m, 1H), 4.00 (m, 1H), 3.58 (s, 3H), 2.92 (m, 2H), 2.37 (s, 1H), 2.36 (d, $J = 1.7$ Hz, 1H), 1.71 (m, 1H), 1.64 (m, 1H), 1.52 (dt, $J = 2.1$, 12.7 Hz, 1H), 1.39 (s, 3H), 1.24 (s, 3H), 1.20 (m, 1H); **10** ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.27 (m, 1H), 3.99 (m, 1H), 3.39 (m, 2H), 2.43 (dd, $J = 7.0$, 15.4 Hz, 1H), 2.31 (dd, $J = 6.2$, 14.9 Hz, 1H), 1.70 (m, 2H), 1.58 (dt, $J = 2.7$, 12.7 Hz, 1H), 1.46 (s, 3H), 1.45 (s, 9H), 1.37 (s, 3H), 1.22 (m, 1H); **5** ^{1}H NMR (600 MHz, CD_3OD) δ 4.33 (m, 1H), 4.09 (m, 1H), 3.03 (m, 2H), 2.38 (dd, $J = 4.8$, 15.4 Hz, 1H), 2.31 (dd, $J = 7.9$, 14.9 Hz, 1H), 1.74 (m, 2H), 1.60 (dt, $J = 2.6$, 12.7 Hz, 1H), 1.48 (s, 3H), 1.45 (s, 9H), 1.33 (s, 3H), 1.21 (m, 1H).