ORGANIC LETTERS

2006
Vol. 8, No. 6
1013−1016

Bifunctional Inhibitors of Mevalonate Kinase and Mevalonate 5-Diphosphate Decarboxylase

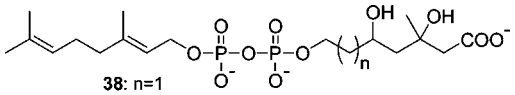
Yongge Qiu and Ding Li*

Department of Biology and Chemistry, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR, P. R. China

bhdingli@cityu.edu.hk

Received October 24, 2005

ABSTRACT



38: n=1
39: n=2
40: n=3

A bifunctional inhibitor of mevalonate kinase and mevalonate 5-diphosphate decarboxylase was synthesized. Both enzymes are in the cholesterol biosynthetic pathway and play an important role in regulating cholesterol biosynthesis. The molecule may become a useful lead compound for further development for treating cardiovascular disease and cancer. This study provides a novel example of a single inhibitor blocking two sequential steps simultaneously in the cholesterol biosynthetic pathway.

In animal cells, the cholesterol biosynthetic pathway contains a unique series of three sequential ATP-dependent enzymes that convert mevalonate to isopentenyl diphosphate (IPP): mevalonate kinase (MVK), phosphomevalonate kinase (PMK), and mevalonate 5-diphosphate decarboxylase (MDD) (Figure 1).[1] These three enzymes catalyze consecutive steps downstream from the HMG-CoA reductase in the mevalonate pathway, and are responsive to cholesterol intake in animals. The pathway has been exploited in the design of cholesterol-lowering drugs treating cardiovascular disease. HMG-CoA reductase catalyzes the main rate-limiting step in the synthesis of cholesterol and nonsteroid isoprenoid derivatives. Inhibition of HMG-CoA reductase by lovastatin and related compounds blocks cholesterol biosynthesis and increases transcription of the LDL receptor gene.[2] A variation on this approach has been suggested for cancer chemotherapy.[3] Extremely high levels of reductase inhibitors, given for a short period could prevent farnesylation of Ras proteins, inhibiting the growth of Ras-dependent tumor cells. Although HMG-CoA reductase is a key regulatory site in the pathway, enzymes and genes further along the pathway are also important in regulation of pathway function. Several investigators have suggested the involvement of MVK and MDD as important regulatory steps in the biosynthesis of cholesterol.[4]

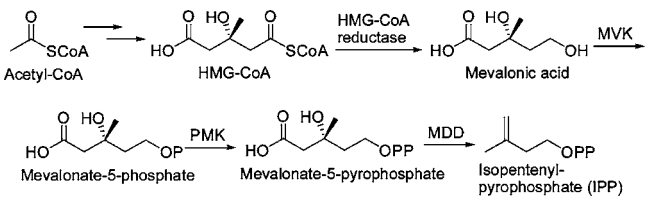


Figure 1. Mevalonate pathway in animal cells.

The X-ray structures of MVK and MDD from various sources have been solved,[5] and both enzymes belong to the GHMP (Galactokinase, Homoserine kinase, Mevalonate

(1) (a) Goldstein, J. L.; Brown, M. S. *Nature* **1990**, *343*, 425. (b) Houten, S. M.; Schneiders, M. S.; Wanders, R. J. A.; Waterham, H. R. *J. Biol. Chem.* **2003**, *278*, 5736. (c) Kaur, M.; Kaul, D. *FASEB J.* **1997**, *11*, A1217.

(2) (a) Brown, M. S.; Goldstein, J. L. *Science* **1986**, *232*, 34. (b) Ma, P. T. S.; Gil, G.; Sudhof, T. C.; Bilheimer, D. W.; Goldstein, J. L.; Brown, M. S. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8370.

(3) Schafer, W. R.; Kim, R.; Sterne, R.; Thorner, J.; Kim, S. H.; Rine, J. *Science* **1989**, *245*, 379.

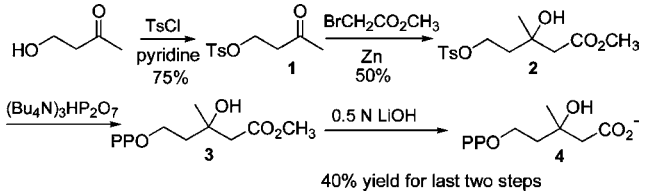
10.1021/ol052573s CCC: $33.50
Published on Web 02/11/2006

kinase, and Phosphomevalonate kinase) superfamily of small-molecule kinases. MVK and MDD share the same fold, catalyze phosphorylation of chemically similar substrates (MDD decarboxylation involves phosphorylation of mevalonate diphosphate), and seem to have evolved from a common ancestor.[5c] Kinases are a ubiquitous group of enzymes central to many biochemical processes.[6] The transfer of the terminal phosphoryl group from one nucleotide to another, or to a small molecule (by enzymes which we term "small molecule kinases"), or to a protein substrate (by protein kinase) is a fundamental process in many aspects of metabolism, gene regulation, and signal transduction.[6]

A variety of substrate analogues of MVK and MDD targeting the mevalonate binding site have been studied,[7] and some have been found to be enzyme inhibitors. In addition, MVK is subject to posttranscriptional regulation via competitive inhibition at the ATP-binding site by prenyl phosphates of varying length, including geranyl diphosphate (GPP), farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP).[8] Because of the potential importance of MVK and MDD in the regulation of cholesterol biosynthesis, and their similar structure and catalyzed reaction, we tried to develop a common inhibitor effective for both enzymes. In the present study, we report organic synthesis and biological characterization of a bifunctional molecule, which can inhibit both MVK and MDD. This molecule may become a lead for further development by the pharmaceutical industry for treating cardiovascular disease and certain forms of cancer.

The substrate of MDD, mevalonate 5-diphosphate, has been obtained previously through hydrolysis of mevalonic lactone followed by reaction with pyrophosphate.[9] The method required protection of the carboxylate group of mevalonate through formation of methyl ester after hydrolysis of mevalonic lactone, and also needed deprotection of the methyl group after reaction with pyrophosphate. Mevalonate lactone is relatively expensive, which prevents large-scale preparation of mevalonate 5-diphosphate. We developed a new synthetic method for the preparation of mevalonate 5-diphosphate as shown in Scheme 1, which facilitated our

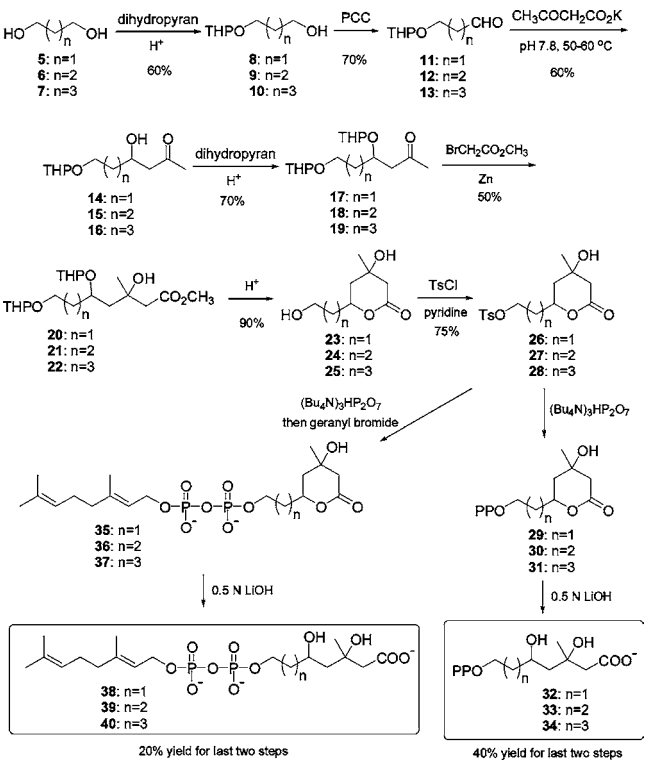
Scheme 1. Organic Synthesis of Mevalonate Diphosphate



further study of MDD. 4-Hydroxy-2-butanone reacted with toluenesulfonyl chloride in the presence of pyridine to give 4-tosyloxy-2-butanone (**1**), which was then reacted with methyl 2-bromoacetate through a Reformatsky reaction yielding methyl 3-hydroxy-3-methyl-5-tosyloxypentanoate (**2**). Mevalonate 5-diphosphate (**4**) was obtained through reaction of compound **2** with pyrophosphate followed by hydrolysis with base.

Three bisubstrate analogues (compounds **38**, **39**, and **40**) were synthesized through three parallel syntheses as shown in Scheme 2. Three corresponding analogues (compounds

Scheme 2. Organic Synthesis of Bisubstrate Analogues



32, **33**, and **34**) without a geranyl group were also synthesized as shown in Scheme 2 for comparative study. The monoprotected diols (**8**, **9**, and **10**) were oxidized with PCC,

(4) (a) Iglesias, J.; Gonzalezpacanowska, D.; Marco, C.; Garciaperegrin, E. *Biochem. J.* **1989**, *260*, 333. (b) Gonzalezpacanowska, D.; Marco, C.; Garciamartinez, J.; Garciaperegrin, E. *Biochim. Biophys. Acta* **1985**, *833*, 449. (c) Mitchell, E. D.; Avigan, J. *Circulation* **1979**, *60*, 31. (d) Tanaka, R. D.; Schafer, B. L.; Lee, L. Y.; Freudenberger, J. S.; Mosley, S. T. *J. Biol. Chem.* **1990**, *265*, 2391. (e) Jabalquinto, A. M.; Cardemil, E. *Arch. Biochem. Biophys.* **1981**, *210*, 132. (f) Gonzalezpacanowska, D.; Marco, C.; Garciamartinez, J.; Linares, A.; Garciaperegrin, E. *Nutr. Rep. Int.* **1985**, *31*, 121. (g) Gonzalez-Pacanowska, D.; Marco, C.; Garcia-Martinez, J.; Garcia-Peregrin, E. *Biochim. Biophys. Acta* **1986**, *875*, 605. (h) Tanaka, R. D.; Lee, L. Y.; Schafer, B. L.; Kratunis, V. J.; Mohler, W. A.; Robinson, G. W.; Mosley, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 2872. (i) Jabalquinto, A. M.; Cardemil, E. *Lipids* **1980**, *15*, 196.

(5) (a) Fu, Z. J.; Wang, M.; Potter, D.; Miziorko, H. M.; Kim, J. J. P. *J. Biol. Chem.* **2002**, *277*, 18134. (b) Yang, D.; Shipman, L. W.; Roessner, C. A.; Scott, A. I.; Sacchettini, J. C. *J. Biol. Chem.* **2002**, *277*, 9462. (c) Bonanno, J. B.; Edo, C.; Eswar, N.; Pieper, U.; Romanowski, M. J.; Ilyin, V.; Gerchman, S. E.; Kycia, H.; Studier, F. W.; Sali, A.; Burley, S. K. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 12896.

(6) Matte, A.; Tari, L. W.; Delbaere, L. T. J. *Structure* **1998**, *6*, 413.

(7) (a) Nave, J. F.; d'Orchymont, H.; Ducep, J. B.; Piriou, F.; Jung, M. J. *Biochem. J.* **1985**, *227*, 247. (b) Reardon, J. E.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4717. (c) Wilde, J.; Eggerer, H. *Eur. J. Biochem.* **1994**, *221*, 463. (d) Wilde, J.; Eggerer, H. *Liebigs Ann. Recl.* **1997**, 581. (e) Dhe-Paganon, S.; Magrath, J.; Abeles, R. H. *Biochemistry* **1994**, *33*, 13355. (f) Vlattas, I.; Dellureficio, J.; Ku, E.; Bohacek, R.; Zhang, X. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2091.

(8) (a) Hinson, D. D.; Chambliss, K. L.; Toth, M. J.; Tanaka, R. D.; Gibson, K. M. *J. Lipid Res.* **1997**, *38*, 2216. (b) Dorsey, J. K.; Porter, J. W. *J. Biol. Chem.* **1968**, *243*, 4667.

(9) Reardon, J. E.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4717.

followed by a regioselective aldol condensation[10] giving organic compounds with β-ketol structure (**14**, **15**, and **16**). The protection with dihydropyran followed by a Reformatsky reaction generated compounds **20**, **21**, and **22**. The deprotection accompanied by self-formation of lactone (**23**, **24**, and **25**), followed by tosylation, gave key intermediate compounds **26**, **27**, and **28**. Compounds **32**, **33**, and **34** were synthesized through the reaction of compounds **26**, **27**, and **28** with pyrophosphate followed by hydrolysis of lactone. The bisubstrate analogues (compounds **38**, **39**, and **40**) were also obtained from intermediate compounds **26**, **27**, and **28** through a one-pot reaction with pyrophosphate and geranyl bromide, followed by hydrolysis of lactone. This reaction followed a previously reported method for syntheses of isoprenoid conjugates of nucleoside 5′-diphosphates[11] with significant improvement, which made the desired product in a one-pot reaction from geranyl bromide, pyrophosphate, and tosylates of mevalonate derivatives instead of the stepwise reactions used previously.

The inhibitory effect of the above synthetic substrate analogues on the activities of MVK was investigated by using Dixon plot,[12] and the results are summarized in Table 1.

Table 1. IC_{50} Values of Synthetic Compounds for Rat MVK and MDD

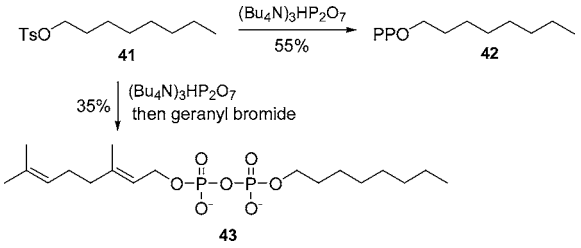
compd	IC_{50} for rat MVK (μM)	IC_{50} for rat MDD (μM)
4	180	
32	275	215
33	300	185
34	225	200
GPP	5.0	65
38	8.0	2.6
39	3.8	2.0
39a	2.5	1.9
39b	7.5	2.0
40	3.9	6.5
42	450	600
43	5.5	600

Mevalonate-5-diphosphate (**4**) and its derivatives (**32**, **33**, and **34**) showed weak inhibitory activity against rat MVK. A IC_{50} value of 5.0 μM was obtained for geranyl diphosphate on MVK, which is comparable with that previously reported on rat liver MVK.[13] A considerably lower K_i value of 104−116 nM has been reported for geranyl diphosphate on human MVK.[14] It should be mentioned that measured IC_{50} and K_i values are dependent on assay conditions, and should be used for comparison only when the same assay conditions are used. It is also possible that enzymes from two sources have slightly different structures, and rat MVK is less susceptible to feedback inhibition. P′-Geranyl 3,5,8-trihydroxy-3-methyloctanate 8-diphosphate (**39**) and P′-geranyl 3,5,9-trihydroxy-3-methylnonanate 9-diphosphate (**40**) showed a slightly better inhibitory effect while P′-geranyl 3,5,7-trihydroxy-3-methylheptanate 7-diphosphate (**38**) showed a slightly less inhibitory effect than GPP on MVK. This result showed that the bisubstrate analogue, compound **39**, is a much better inhibitor than the analogue without the geranyl group (**33**). Compounds **38**, **39**, and **40** all exhibit competitive inhibition with respect to both ATP and mevalonate, while compounds **32**, **33**, and **34** show competitive inhibition on mevalonate only.

The inhibitory effect of the above synthetic substrate analogues on the activities of MDD was also investigated and the results are shown in Table 1. Mevalonate-5-diphosphate derivatives (**32**, **33**, and **34**) and GPP showed weak inhibitory activity against rat MDD, while bisubstrate analogues **38**, **39**, and **40** showed a much stronger inhibitory effect than GPP. The result showed that the bisubstrate analogue, compound **39**, is a much better inhibitor than the analogue without a geranyl group (**33**). Compounds **38**, **39**, and **40** all exhibit competitive inhibition with respect to both ATP and mevalonate, while compounds **32**, **33**, and **34** show competitive inhibition on mevalonate-5-diphosphate only.

Two other organic molecules, compounds **42** and **43**, were also synthesized as reference molecules, using the same reactions for comparison, as shown in Scheme 3. Octanoyl-

Scheme 3. Organic Synthesis of Control Compounds



1-diphosphate (**42**) shows much weaker competitive inhibition on both MVK and MDD than compounds **38**, **39**, and **40** (Table 1). P′-Geranyl octanate 8-diphosphate (**43**) exhibits rather weak competitive inhibition on MDD, while comparable inhibition with that of GPP on MVK. This control experiment indicates simple lipophilic chains bearing a pyrophosphate moiety are not good inhibitors.

Since compound **39** includes two stereo centers, further effort was made to separate diastereomers. Lipase was used to resolve the racemic mixtures without success. The cyclic intramolecular esters, which are resistant to hydrolysis by lipase, are quite stable. The mixture of four diastereomers was separated by silica gel column into two spots **39a** and **39b** on TLC. Spot **39a** includes two diastereomers, (3*S*),(5*R*)-P′-geranyl 3,5,8-trihydroxy-3-methyloctanate 8-diphosphate and (3*R*),(5*S*)-P′-geranyl 3,5,8-trihydroxy-3-methyloctanate

(10) Kourouli, T.; Kefalas, P.; Ragoussis, N.; Ragoussis, V. *J. Org. Chem.* **2002**, *67*, 4615.
(11) Ryu, Y. H.; Scott, A. I. *Org. Lett.* **2003**, *5*, 4713.
(12) (a) Dixon, M. *Biochem. J.* **1953**, *55*, 170. (b) Burlingham, B. T.; Widlanski, T. S. *J. Chem. Educ.* **2003**, *80*, 214.
(13) (a) Dorsey, J. K.; Porter, J. W. *J. Biol. Chem.* **1968**, *243*, 4667. (b) Tanaka, R. D.; Schafer, B. L.; Lee, L. Y.; Freudenberger, J. S.; Mosley, S. T. *J. Biol. Chem.* **1990**, *265*, 2391.
(14) Hinson, D. D.; Chambliss, K. L.; Toth, M. J.; Tanaka, R. D.; Gibson, K. M. *J. Lipid Res.* **1997**, *38*, 2216.

8-diphosphate. Spot **39b** includes two other diastereomers, (3*S*),(5*S*)-P′-geranyl 3,5,8-trihydroxy-3-methyloctanate 8-diphosphate, and (3*R*),(5*R*)-P′-geranyl 3,5,8-trihydroxy-3-methyloctanate 8-diphosphate. It appears that **39a** and **39b** have similar competitive inhibition on both MVK and MDD (Table 1). **39a** is slightly better than **39b** on inhibition of both MVK and MDD.

Compound **39** showed the strongest inhibition for both MVK and MDD, indicating that a 3-carbon bridge is the most suitable chain length between the mevalonate part and geranyl diphosphate for both enzymes. It may reflect the natural distance between ATP and mevalonate in the active sites of MVK and MDD. It should be mentioned that drug combination has been used in the clinical treatment of various diseases,[15] and sequential blocking of two enzymes in a metabolic or biosynthetic pathway is one strategy for enhancing treatment. Because it is difficult to inhibit an enzyme completely (particularly with a reversible inhibitor), inhibition of two consecutive enzymes in a pathway by using a combined drug treatment can block the pathway much more effectively than a single inhibitory drug therapy. Reversible enzyme inhibition, however, is hyperbolic in nature and requires a large excess of the inhibitory drugs, which may have toxic side effects. Normally, two separate drugs are given to patients when using a drug combination treatment. In contrast, our bisubstrate analogue compound **39** provides a novel example of a single inhibitor carrying out the sequential blocking of MVK and MDD in the biosynthesis of cholesterol. Although some bifunctional inhibitors have been previously reported for inhibition of other enzymes,[16] they were designed and synthesized with a different strategy. Two enzymes are usually significantly different, and two known inhibitors are linked together through a covalent chemical bond. Our strategy takes advantage of similar active-site structures of two consecutive enzymes involved in the biosynthesis of cholesterol, which provides a new rational approach for drug design. More and more enzymes are grouped into superfamilies, and members of the same superfamily normally have similar peptide folding; therefore, our approach may have general utility in drug design. Compound **39** may be a useful lead compound for further pharmaceutical development. Compound **39** is composed of a hydrophilic part (mevalonate) and a hydrophobic part (geranyl group), which is structurally similar to fatty acids and may facilitate penetration of lipid membranes to reach the target enzymes therefore increasing its effectiveness.

Acknowledgment. The work described in this paper was substantially supported by a grant from the City University of Hong Kong (Project No. 7001793).

Supporting Information Available: Experimental procedures and spectroscopic data for all new compounds, and assay methods for MVK and MDD. This material is available free of charge via the Internet at http://pubs.acs.org.

OL052573S

(15) Silverman, R. B. *The organic chemistry of drug design and drug action*, 2nd ed.; Elsevier Academic Press: Boston, MA, 2004.

(16) Jaulent, A. M.; Leatherbarrow, R. J. *Protein Eng. Des. Sel.* **2004**, *17*, 681.