Bioorganic & Medicinal Chemistry Letters, Vol. 1, No. 1, pp. 61-64, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

SYNTHESIS OF SUBSTRATE-BASED INHIBITORS OF HMG CoA REDUCTASE

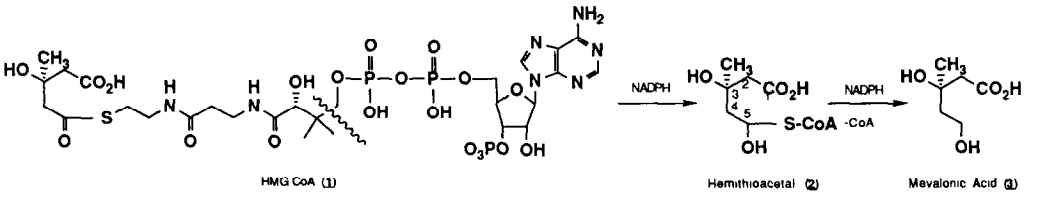
E.M.Gordon*, Jelka Pluscec and C. P. Ciosek, Jr.

Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543-4000

(*Received* 6 *October* 1990)

Abstract: Synthesis of chemically stable sub-structures (5-7) of substrate (1) and putative intermediate hemithioacetal (2) of the enzymatic HMG CoA reductase (HMGR) reaction are described. Methods are reported for the preparation of a modified pantetheine residue (24), and for its subsequent attachment to various hydroxy(methyl)glutaryl mimics, to elaborate a series of potential substrate-based HMGR inhibitors.

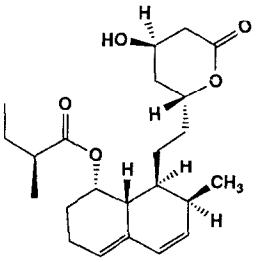
Inhibition of HMGR is becoming a major mechanism of controlling hypercholesterolemia in man. The enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is considered to be the critically regulated point in (hepatic) cholesterol biosynthesis. HMGR catalyzes a two step, irreversible, rate -limiting, reduction of HMG CoA (1) to mevalonic acid (3) and coenzyme A, which proceeds through the intermediacy of hemithioacetal (2). The origin of potent HMG CoA reductase inhibitors can be traced to the discovery of compactin (4) and other "mevinic acids" by Endo and Co-workers [1,2,3]. Abeles studied the mode of interaction of compactin-like inhibitors with HMGR, and proposed that the upper sidechain of compactin likely mimics the 3-hydroxy-3-methylglutaryl portion of substrate, while the decalin group binds to a



HMG CoA (1) Hemithioacetal (2) Mevalonic Acid (3)

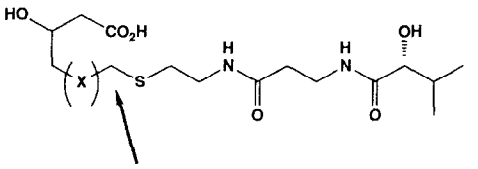
hydrophobic region which neighbors the active-site [4]. The critical C-5 hydroxyl group of inhibitor apparently satisfies the enzyme binding interactions normally engaged by the newly formed hydroxy group of hemithioacetal 2. Though a great deal of work has been devoted to the study of mevinic acids and synthetic 3,5,dihydroxyheptanoic acids, less attention has been directed toward the design of substrate based (1) inhibitors of HMGR [5].

Synthetic inhibitor targets such as 5-7 may be considered chemically stable, non-reducible analogs of substrate 1 and intermediate 2. The introduction of an extra methylene group between sulfur and "X" in substances 5 and 6 is a deliberate design feature intended to create "hyperextended" versions of 1,2. Such a



Compactin (4)

modification simultaneously introduces enhanced chemical stability (over thioester and hemithioacetal) while attempting to mimick the separation of HMG and CoA components that occurs as the enzyme reaction proceeds [6]. In these targets several refinements have been incorporated to facilitate inhibitor design and synthesis: a section of coenzyme A containing the pyrophosphate, sugar, and base, moieties has been deleted (see **1**) to allow for chemical/pharmacological stability; and the C-3 methyl group is not present. The sub-section of coenzyme A selected for use is essentially a desmethylenehydroxy pantetheine. This material was intentionally selected to obviate the previously observed intramolecular decomposition of pantetheine derivatives via loss of pantalolactone. The balance of this letter describes synthetic routes to materials **5-7**.

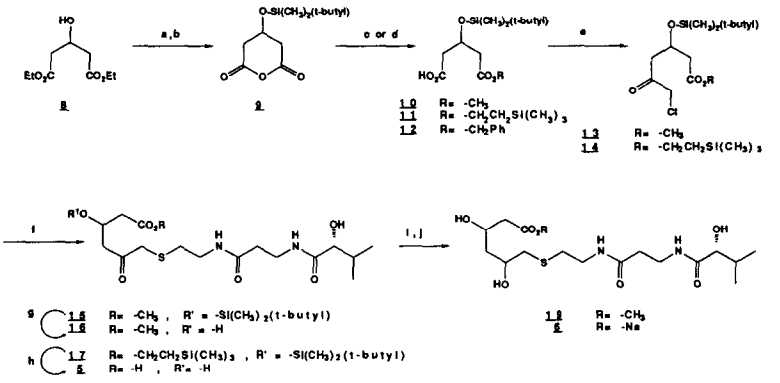


No.	X
5	C=O
6	CH-OH
7	(deleted)

Syntheses of β-ketosulfide **5** and β-hydroxysulfide **6** proceeded via the alkylation of a modified pantetheine mercaptan (**24**) by protected chloroketones (**13,14**). Chloroketone preparation is outlined in **Scheme 1**. After silylation, saponification and dehydration of diethyl 3-hydroxyglutarate (**8**) to glutaric anhydride **9**, monoesterification was smoothly achieved by alcoholysis (**10-12**). The half-ester **10** was treated under a haloketone forming sequence (i) mixed anhydride ii) diazomethane iii)HCl) to provide **13** as a key intermediate (^{13}C NMR (CD_3CN) δ -1.4, 18.5, 26.1, 42.9, 50.6, 52.0, 66.8, 172.2, 173.4 ppm; IR ($CHCl_3$) 1733 cm^{-1} ; CI-MS: m/e 309 (M+1)). The inhibitor component modelled after pantetheine was synthesized as shown in **Scheme 2.** Diazotization of D-valine afforded lactic acid derivative **21** with retention of configuration. Coupling of **21** with β-alanine benzyl ester , followed by deesterification and subsequent coupling with cysteamine, afforded pantetheine analog **24** (mp 104-106°C; ^{13}C NMR ($CDCl_3$) δ 16.3, 19.7, 24.9, 32.5, 35.8, 36.4, 43.2, 76.9, 172.4, 175.0 ppm; $[\alpha]^{25}D$= +32.5° (c= 1.04, CH_3OH); CI-MS : m/e 249 (M+1)). Alkylation of **24** by **13** smoothly produced the protected α-thioketone **15** . Successful desilylation afforded **16**, but attempted saponification of this material failed to afford desired product (**5**). On the other hand, reduction of **15** with lithium tri-t-butoxy aluminum hydride yielded the corresponding alcohol, which was desilylated and saponified to give target **6**. Preparation of trimethylsilylethyl ester **11** from anhydride **9**, led via the aforementioned sequence to chloroketone **14** .

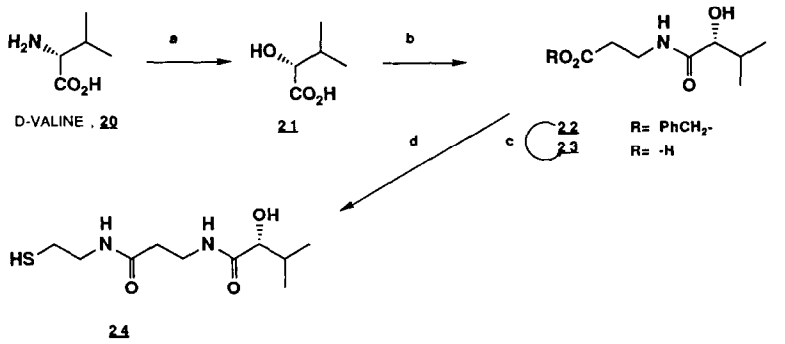
This substance underwent alkylation as described above, and was smoothly deprotected to give the ketosulfide target **5**.

Scheme 1



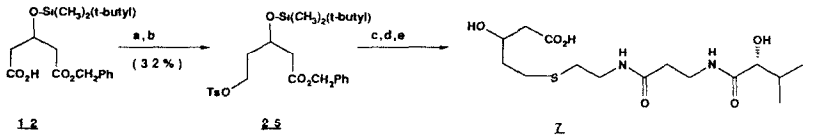
a) t-butyl$(CH_3)_2$Si-Cl ,CH_2Cl_2, imidazole, (100%) , b) i)NaOH, CH_3OH, ii) Ac_2O, heat, (58%), c) trimethylsilylethanol, DMAP, CH_2Cl_2 (93 %), d) CH_3OH, DMAP, CH_2Cl_2 (100%); e) i) isobutyl chloroformate, NMM , ii) CH_2N_2 , iii) HCl, EtOAc (40-50-%), f) compound **24** , Et_3N, DMF (58% , 100%), g) HF, CH_3CN (23%), h) tetrabutylammonium fluoride, HOAc, THF (66%), i) LiAl(t-BuO)$_3$H , THF (31%), j) NaOH, CH_3OH (93%)

Scheme 2



a)HCl, $NaNO_2$, HOAc (98%) ; b)B- Ala-Obz hydrochloride, DCC, HBT, diisopropylethylamine,THF (47%);
c) H_2,10% Pd/C, 95% EtOH (100 %); d) cysteamine hydrochloride, diisopropylethylamine, DCC, HBT, THF (69%);

Thioether **7** is a chemically stable, isosteric (non-hyperextended) analog of **2** or **4**. Synthesis of **7** was accomplished by alkylation of thiol **24** with protected tosylate **25**. The latter derives from borane reduction of half ester **12**, followed by tosylation. Desilylation and saponification of the adduct afforded thioether **7** (mp 165-168 °C; FAB-MS : m/e 387 (M+H), 385 (M-H), 409 (M+Na); R_f = 0.66 EM silica gel (n-BuOH:HOAc:



a) borane, THF, b) tosyl chloride, pyridine, c) compound **26**, DMSO, K_2CO_3 (73%), d) HF, CH_3CN (100%), e) 1N NaOH, CH_3OH, HP-20 Resin

H_2O, 3:1:1). Tested in a rat hepatic microsomal HMGR assay system[9], materials **5** and **6** described above were inactive at 300 μM, and **7** inhibited enzyme activity 24% at 100μM . The reasons for lack of expected activity are the subject of a continuing investigation.

Acknowledgement: We thank the Squibb Institute Analytical Department for assistance in obtaining spectral and microanalytical data.

References

1. Endo, A.,Kuroda, M., and Tsuijita, Y., *J. Antibiot.*, **29** , 1346-48 (1976).
2. Endo, A., *J. Med. Chem.*, **28**, 401-405 (1985).
3. Endo, A., *Pharmac. Ther.*, **31**, 257-267 (1987).
4. Nakamura, C.E., and Abeles, R.H., *Biochem.*, **24**, 1364-1376 (1985).
5. Fischer, G.C., Turakhia, R.H., and Morrow, C.J., *J. Org. Chem.*, **50**, 2011-2019 (1985). Tschesche,R.T., Machleidt,H., *Liebigs Ann. Chem* , **631**, 61 (1960). Wilson, W.K., Baca,S.B., Barber, Y.J., Scallen, T.J., Morrow, C.J., *J. Org. Chem.*, **48**, 3960 (1983). Cozzi, P., Carganico, G., Orsini, G., *J. Med. Chem.*, **26**, 1764 (1983). Nguyen, T.G., Aigner, H. and Eggerer, H., *FEBS Lett* , **128**, 145 (1981). Nguyen, T.G., Gerbing, K. and Eggerer, H., *Z. Physiol.Chem.*, **365**, 1 (1984). Fischer, G.C., Ph.D. Dissertation, U. of New Mexico (1982). Turakhia, R.H., Ph.D. Dissertation, U. o New Mexico (1983).
6. We have previously explored the design of other "hyperextended" substrate and reaction intermediates in the study of various proteolytic enzyme inhibitors. See, Gordon, E.M., Godfrey, J.D.,Pluscec, Jelka , Natarajan, S. and Von Langen, D., *Biochem. Biophys. Res. Commun.*, **126**, 419-426 (1985). Gordon, E.M., Natarajan, S., Pluscec, Jelka, Weller, H.N., Godfrey, J.D., Rom, M.B., Sabo, E.F., Engebrecht J., and Cushman, D.W.; *Biochem. Biophys. Res. Commun.*, **124**, 148-155 (1984). Godfrey, J.D., Gordon, E.M., Von Langen, D., Engebrecht, J., and Pluscec, Jelka, *J. Org. Chem.*, **51**,3073 (1986). Gordon, E.M., Godfrey, J.D., Delaney, N.G., Asaad, M., Von Langen, D. and Cushman, D.W. , *J.Med.Chem.*, **31**, 2199-2211 (1988).
7. All materials gave satisfactory IR, NMR and MS spectra, as well as combustion analyses.
8. HP-20-AG is the analytical grade of a macroreticular polystyrene-divinylbenzene copolymer available from Mitsubishi Chemical Industries, Ltd.
9. Edwards, P.A., Lemongello, D., and Fogelman, A.M., J. Lipid Res., **20**, 40-46(1979).