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PHOSPHINIC ACID INHIBITORS OF 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A REDUCTASE

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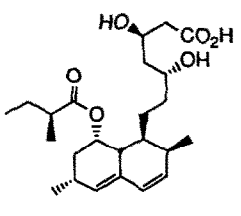
Abstract. Compound **3** was designed as a prototype for a new class of phosphorus-based inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. The synthesis and biochemical evaluation of compound **3** are described.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) is a rate limiting enzyme in cholesterol biosynthesis and the site of action for the cholesterol-lowering drug lovastatin (**1**).[1] HMGR catalyzes the NADPH-dependent reduction of HMG-CoA to mevalonate. The reaction is thought to proceed through an intermediate hemithioacetal arising from delivery of hydride to the HMG-CoA thioester.[2] Lovastatin and compound **2**[3a] are members of a diverse group of potent HMGR inhibitors[3] characterized by a variable lipophilic "anchor," that presumably recognizes a hydrophobic region near the enzyme active site, joined to a conserved 3,5-dihydroxyheptanoic acid tail that most likely occupies the HMG binding site.[4] In explanation of the high potency of these compounds, it is possible that the 5-hydroxymethylene group of **1** and **2** mimics the tetrahedral structure of the enzyme-generated hemithioacetal or a related transition state. Although not a complete description of the behavior of these compounds,[5] this simple hypothesis provides a useful starting point for designing new HMGR inhibitors. Accordingly, we and others have explored alternatives to the 5-hydroxymethylene group, including α,α-difluoroketones[6] and phosphinic acids,[5,7] that could similarly exploit binding interactions germane to hemithioacetal formation. Here we describe the synthesis and preliminary biochemical evaluation of the phosphinic acid analogue, **3**, of compound **2**. While comparing favorably with compound **2** as an inhibitor of purified HMGR, the phosphinate **3** in its underivatized form shows attenuated potency in an hepatic cell culture assay.



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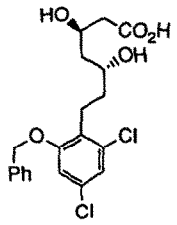

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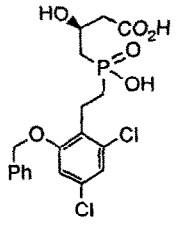
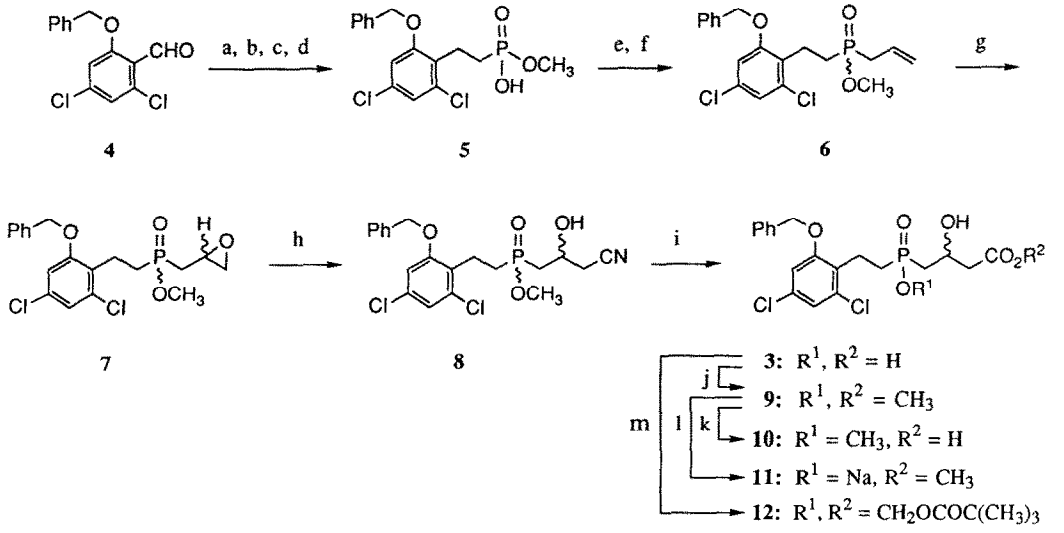


3

The synthesis of compound **3** and several of its esters is outlined in Scheme 1. Aldehyde **4**, prepared by Reimer-Tiemann formylation of 3,5,-dichlorophenol[8] and subsequent benzylation (K_2CO_3, BnBr, DME),[3a] was converted via its corresponding benzylic bromide[6] to the phosphonic acid **5** in 64% overall yield as shown. The phosphonochloridate derivative of **5** was treated with allylmagnesium bromide to give the allyl phosphinate **6**. Epoxidation of **6** afforded epoxide **7** as a mixture of diastereomers, which was expected to provide access to the required 3-hydroxyacyl side chain via reaction with a suitable acyl anion equivalent. Attempts to effect opening of epoxide **7** with BF_3 etherate in combination with either bromomagnesium divinyl cuprate[9] or vinylmagnesium bromide[10] were unsuccessful, leading instead to the 1,2-bromohydrin. Reaction of epoxide **7** with either vinyllithium - BF_3 etherate[10] or the higher order mixed organocuprate derived from vinyllithium[11] each led to slow decomposition with no observable vinyl group incorporation. In contrast, diethylaluminum cyanide[12] reacted readily with epoxide **7** to provide exclusively the 1,2-cyanohydrin **8** in high yield. What remained was to effect the relatively demanding hydrolysis of the nitrile group (and phosphinyl methyl ester) of compound **8** in the presence of a potentially sensitive β-hydroxyl group. Heating compound **8** with aqueous sodium hydroxide

Scheme 1



a. $NaBH_4$; 100%. b. $P(Ph)_3$, CBr_4, THF; 81%. c. $CH_3PO(OCH_3)_2$, n-BuLi, THF, -78° → 25°.
d. NaOH, H_2O, MeOH; 79%. e. PCl_5, CH_2Cl_2. f. CH_2CHCH_2MgBr, THF, -78°; 78%.
g. m-CPBA, CH_2Cl_2; 94%. h. Et_2AlCN, toluene, 0°; 75%. i. Na_2O_2, H_2O, 55°; 97%.
j. CH_2N_2, Et_2O, CH_2Cl_2; 85%. k. NaOH, H_2O, MeOH, THF; 98%.
l. Me_3SiBr, CH_2Cl_2; NaOH, MeOH; 100%. m. $ClCH_2OCOC(CH_3)_3$, K_2CO_3, DMF, 60°; 45%.

(3% NaOH in 2:1 methanol:water, 70 °C) led to rapid removal of the phosphinyl methyl group followed by much slower hydrolysis of the nitrile. After an extended reaction period the desired product **3** was formed in low yield, contaminated with the corresponding *trans*-acrylate (from elimination of water) and several additional unidentified products. In contrast to hydrolysis with sodium hydroxide, the more nucleophilic, less basic reagent sodium peroxide[13] was far more efficient. Heating a suspension of compound **8** at 55 °C in aqueous sodium peroxide (5 equiv.) caused smooth stepwise hydrolysis over a period of 18-24 hr, resulting initially in removal of the phosphinyl methyl ester, followed by hydrolysis of the nitrile to the carboxamide, and finally the carboxamide to the acid. Following acidification a quantitative yield of the crystalline diacid, **3**, was obtained. This synthesis is expedient, proceeding in 34% overall yield from aldehyde **4**, and is readily extended to analogues of compound **3** possessing other lipophilic groups.

Compound **3** proved to be a potent competitive inhibitor (vs HMG-CoA) of purified recombinant human HMGR,[14] exhibiting an inhibition constant (K_i) of 16 nM. In comparison, a K_i value of 3.0 nM was found for compound **2**.[5] Thus the phosphinoyl analogue **3** compares favorably in enzyme-binding affinity with its more classical 5-hydroxymethylene-containing counterpart, **2**, and is an order of magnitude more potent than the corresponding 6,6-difluoro-5-keto analogue of compound **2**.[6]

In contrast to its potent activity with isolated HMGR, compound **3** showed relatively low potency ($IC_{50} > 1$ μM) as an inhibitor of cholesterol biosynthesis in HepG2 cells (a human hepatoma cell line).[15] The large discrepancy (2-3 orders of magnitude) between activities in the cell-free and cell-based assays suggested that compound **3**, as a doubly charged phosphinate-carboxylate, has low cell membrane permeability.[16] To examine this possibility we prepared a series of ester derivatives of compound **3** (Scheme 1). Each of the esters **9-12** were weak inhibitors of isolated HMGR, with K_i values in the micromolar range or greater. Nevertheless, methyl carboxylate **11** and pivaloyloxymethyl diester **12** were markedly more effective than diacid **3** in the HepG2 cell assay, with approximate IC_{50} values of 0.1 μM. Esters **11** and **12** evidently serve as lipophilic "prodrugs" which permeate HepG2 cells more readily than diacid **3** and are efficiently deprotected by intracellular esterases. Methyl phosphinates **9** and **10**, however, exhibited only weak activity in the HepG2 cell assay, comparable to that of diacid **3**, suggesting that while carboxylic esterase activity is abundant within these cells, hydrolysis of these phosphinyl methyl esters is more difficult to achieve (as is the case with nonenzymatic hydrolysis). An alternative explanation for the weak activity of **9** and **10**, that the methyl phosphinates are efficiently deprotected but poorly taken up by HepG2 cells, is unlikely in light of the potency of the esters **11** and **12**.

We have subsequently prepared analogues of **3** which possess various substituents in place of the 2-benzyloxy-3,5-dichlorophenethyl group.[5,17] Although some of these related phosphinic acids are more potent than compound **3**, they are generally somewhat weaker inhibitors of recombinant HMGR than the corresponding hydroxymethylene-containing compounds. This apparent intrinsic disadvantage of the phosphinic acids could be offset by their enhanced selectivity for hepatic cells, as has been reported by Karanewski *et al.*[7] A second potential disadvantage is suggested by the relatively low potency of compound **3** in the HepG2 cell assay. The improvement in activity in HepG2 cells achieved by esterification of compound **3** suggests that a prodrug strategy might aid in realizing the full potential of these compounds *in vivo*.

Acknowledgement. We gratefully acknowledge the assistance of Pieter Groot and Keith Suckling in establishing the HepG2 cell assay.

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[15]HepG2 cells were seeded in 24-well plates in DMEM, 10% FCS. At confluence, medium was replaced with serum-free medium, inhibitor was added in DMSO (final concentration 1.6%) and cells incubated for 1 hr. [^{14}C]-acetate (1 μCi/24 nmol) was added to a final concentration of 100 μM, and cells incubated for 90 min. Lipids were extracted in chloroform:methanol, resuspended in hexane and lipid classes separated by sequential elution on silica Bond-Elut columns with hexane:diethyl ether mixtures of increasing polarity.

[16]Lovastatin and other substituted 3,5-dihydroxyheptanoic acid inhibitors generally exhibit activity in cultured hepatocyte assays that is comparable to their activity in cell-free assays. For some of these compounds substantial differences in activity are observed in nonhepatic tissues, due to differences in cellular uptake. For examples, see: (a) Kaneko, I.; Hazama-Shimada, Y.; Endo, A. *Eur. J. Biochem.* **1978**, *87*, 313. (b) Brown, M. S.; Faust, J. R.; Goldstein, J. L.; Kaneko, I.; Endo, A. *J Biol. Chem.* **1978**, *253*, 1121. (c) Tsujita, Y.; Kuroda, M.; Shimada, Y.; Tanzawa, K.; Arai, M.; Kaneko, I.; Tanaka, M.; Masuda, H.; Tarumi, C.; Watanabe, Y.; Fujii, S. *Bioch. Bioph. Acta* **1986**, *877*, 50. (d) Mosely, S. T.; Kalinowski, S. S.; Schafer, B. L.; Tanaka, R. D. *J. Lipid Res.* **1989**, *30*, 1411. (e) Sinensky, M.; Beck, L. A.; Leonard, S.; Evans, R. *J. Biol. Chem.* **1990**, *265*, 19937.

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