Irreducible Analogues of Mevaldic Acid Coenzyme A Hemithioacetal as Potential Inhibitors of HMG-CoA Reductase. Synthesis of a Carbon–Sulfur Interchanged Analogue of Mevaldic Acid Pantetheine Hemithioacetal

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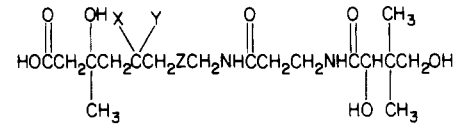
Synthesis of (3,5)-*threo*- and (3,5)-*erythro*-6-[[[[3-(2,4-dihydroxy-3,3-dimethylbutanoyl)amino]propanamido]methyl]thio]-3,5-dihydroxy-3-methylhexanoic acid, *threo*- and *erythro*-**7d**, as well as the corresponding δ-lactones, *cis*- and *trans*-**13a**, is described. The key step in the syntheses is the selective amidomethylation of the sulfhydryl in *cis*- or *trans*-4-hydroxy-6-(mercaptomethyl)-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one, *cis*- or *trans*-**13d**, with *N*-(hydroxymethyl)pantothenamide, **23**. The target compounds are the first in a class being explored as potential inhibitors of HMG-CoA reductase, the key regulated enzyme in cholesterol biosynthesis. They are structurally identical with mevaldic acid pantetheine hemithioacetal, **2b** (a known substrate for the enzyme and an analogue of the enzyme-bound intermediate **2a**), but they are unable to be reduced by the enzyme because the labile C–S bond in **2b** has been replaced with a stable C–C bond in **7d** by interchanging a carbon and a sulfur atom.

The enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase [mevalonate = $NADP^+$ oxidoreductase CoA acylating, EC 1.1.1.34] is generally considered to be the key regulated enzyme in de novo, hepatic sterol biosynthesis.[1] This enzyme catalyzes the irreversible, rate-limiting, two-step reduction of HMG-CoA, **1a**, to mevalonic acid, **3**, and coenzyme A, **4a**, by using 2 equiv of NADPH as the reductant (Scheme I.)[2] The two-step process is believed[3] to proceed via the intermediate mevaldic acid coenzyme A hemithioacetal, **2a**, but the details of the conversion of **2a** to **3** and **4a** are speculative.[4] It is generally accepted that the intermediate **2** is enzyme bound though the hemithioacetal function may well revert to the corresponding aldehyde and CoA-SH (both of which are enzyme bound) prior to transfer of the second hydride from NADPH.

Because of its unique position in the hepatic cholesterogenesis pathway, chemical modulation of this enzyme's activity represents a logical approach to the inhibition of sterol biosynthesis and is being actively explored in many laboratories.[5-7] However, competitive inhibition of the reductase by using structural analogues of the enzyme-bound intermediate hemithioacetal, **2**, has not previously been deliberately examined. Neither has there been any attempt to prepare a stable model of this intermediate for use in exploring the nature of its complex with the enzyme and the stereochemistry of the first reduction step. In attempting to design such an inhibitor/model, two classes of compounds were contemplated: (1) Compounds that could undergo the first reduction step to give an analogue of the intermediate, **2a**, but that are unable to go on through the second reduction step because they lack the labile carbon–sulfur bond in **2a**. (2) Compounds which are already reduced so enter the enzyme as an irreducible analogue of the intermediate. Thus, the key feature sought was replacement of the sulfur atom in **1a** or **2a** with an atom that could no longer act as a leaving group. The obvious candidate is, of course, carbon, a replacement that leads to the models **7a** and **7b** that are ketones rather than the thioester in **1a** and to the models **7c** and **7d** that are secondary alcohols rather than the hemithioacetal in **2a**.

While the former of the two approaches is intuitively more attractive, since it should lead to a model for highly



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a, X,Y = O; Z = CH_2
b, X,Y = O; Z = S
c, X = H; Y = OH; Z = CH_2
d, X = H; Y = OH; Z = S

specific "suicide substrates" that are activated by the first reduction, the literature precedents available argued for

† Taken in part from the Ph.D. dissertation of G.C.F., University of New Mexico, 1982.

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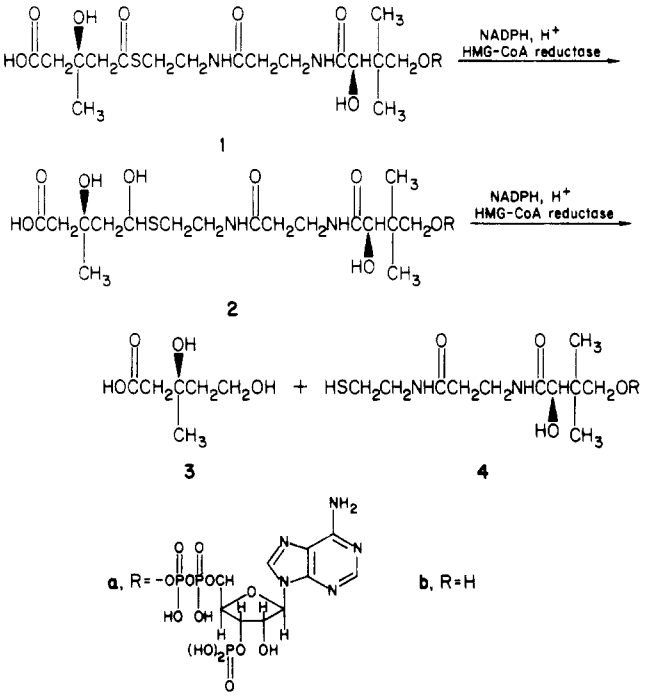
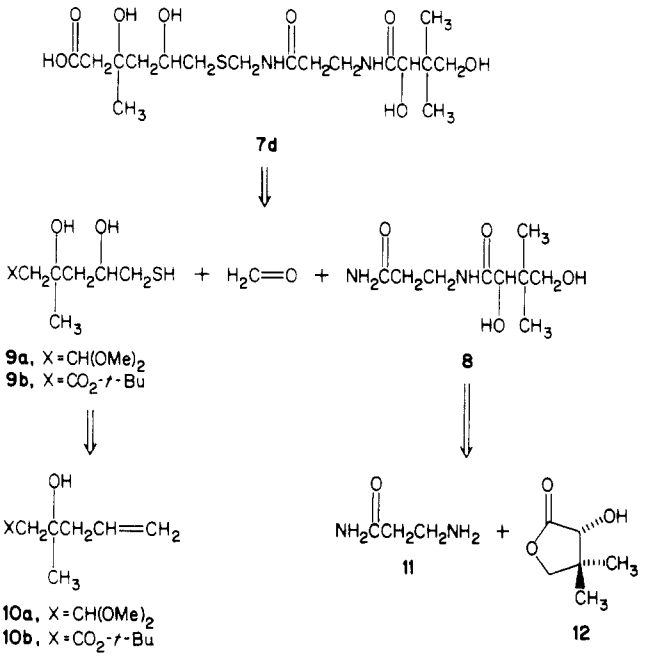
Scheme I



Scheme II



the latter approach. First, work by Lynen and co-workers[3] has shown that mevaldic acid pantetheine hemithioacetal, **2b**, is a substrate for the second reduction step, leading to **3** and **4b**. However, it could not be shown that HMG-*S*-pantetheine, **1b**, is a substrate for the first reduction step.[8] Second, there is, as yet, no evidence that a ketone can be reduced by the HMG-CoA reductase system. Third, there are a number of very potent competitive inhibitors of HMG-CoA reductase which may be considered as secondary alcohol analogues of **2**. Most notable among these is compactin[9] (ML236B),[10,11] and its close relative mevinolin[12] (monacolin K[13]). The inhibitory properties of the many analogues of compactin which have been synthesized strongly support the hypothesis that 3,5-dihydroxyalkanoic acids provide a general class of HMG-CoA reductase inhibitors. Furthermore, analysis of the optimal stereochemistry at carbons 3 and 5 for these inhibitors has shown[14] that the carbon 3 stereochemistry must be that known[15] to be present in the natural intermediate, **2**, while the carbon 5 stereochemistry must be that which is predicted by the Qureshi et al.[16] mechanism for the two-step reduction.

Thus, exploring the inhibitory properties of compounds **7c** and **7d**, which Lynen's work[3] on **2b** suggests will enter the enzyme's active site, will help elucidate the mechanism of inhibition by 3,5-dihydroxyalkanoic acids while enzymatic reduction of **7a** or **7b** to **7c** or **7d** of defined stereochemistry may permit the stereochemical outcome of the first reduction step to be determined. Synthesis of **7d**, one of the compounds required for such studies, in its racemic threo and erythro forms, is described in the remainder of this report. The synthesis of other compounds in the series will be described in future reports.

Results and Discussion

A retrosynthetic analysis of **7d** (Scheme II) suggested a convergent synthesis in which the amide **8** of pantothenic acid, formaldehyde, and a synthetic equivalent of 3,5-dihydroxy-6-mercapto-3-methylhexanoic acid, **9**, are coupled through amidomethylation of the thiol.[17,18] Introduction

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(8) Lynen and co-workers (ref 3) interpret the failure of HMG-S-pantetheine, **1b**, to be reduced as implying a very high degree of specificity for HMG-CoA in the first reduction step.

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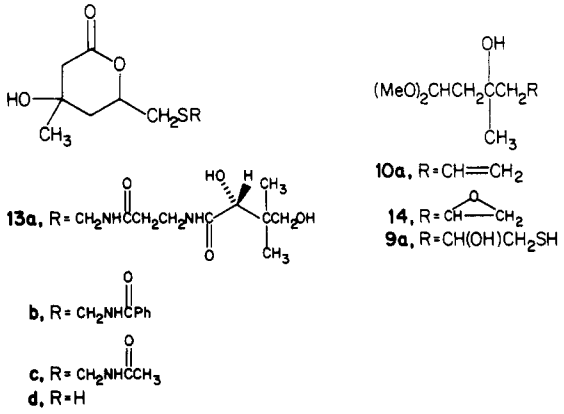
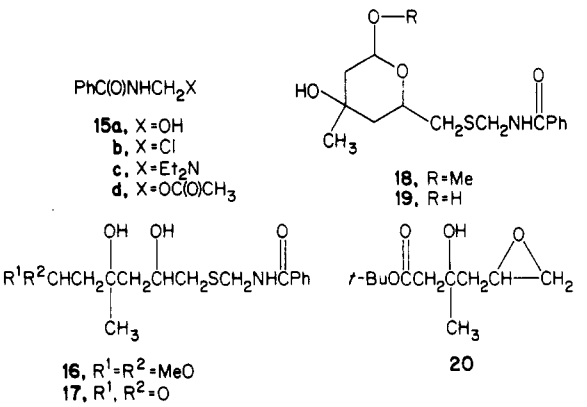
of the eventual carboxylic acid function in either of two masked acid forms, **9a** in which the acid function is represented by an acetal or **9b** in which the acid function is represented by a *tert*-butyl ester, was thought to be straightforward since the precursor, **10a**[19] or **10b**,[6a] respectively, of each intermediate had previously been synthesized, and the required alkene epoxidation and nucleophilic ring opening reactions were well understood. A synthesis of pantothenamide, **8**, from β-alanineamide, **11**, and pantolactone, **12**, has been reported.[20] Once the 5-hydroxy carboxylic acid moiety of **7d** had been prepared, it was expected to display a strong tendency to form the corresponding δ-lactone, **13**, under mildly acidic conditions. This lactone would provide suitable protection for both the secondary alcohol and the carboxylic acid during several of the anticipated steps and so was expected to permit removal of the acetal or *tert*-butyl ester protecting group at an optimal time.



Initially, synthesis of **9a** was explored because the preparation of **10a** and its epoxidation had been worked out as part of another synthetic scheme.[19] However, because of the relative complexity of **7d**, two simpler, less functionalized model compounds, **13b** and **13c**, were synthesized first.

The required 6,6-dimethoxy-1-mercapto-4-methyl-2,4-hexanediol, **9a**, was prepared in good yield from 1,1-dimethoxy-3-methyl-5-hexen-3-ol, **10a**, by epoxidizing the alkene with *m*-CPBA in chloroform solution[19] and opening the resulting epoxide, **14**, with methanolic NaSH with the general method of Baker.[21] Three reagents **15** were investigated for benzamidomethylation of the sulfhydryl in **9a**: *N*-(hydroxymethyl)benzamide, **15a**,[18a,22] *N*-(chloromethyl)benzamide, **15b**,[18c,23] and *N*-[(*N*,*N*-diethylamino)methyl]benzamide, **15c**.[18c,d] While all three reagents led to at least some benzamidomethylation of **9a**, the best results (45% yield of **16**) were obtained by using **15c** and sodium hydroxide in refluxing benzene. [By contrast, the model compound 1-mercapto-2-butanol[24] was most successfully S-benzamidomethylated (85% yield) with *N*-(hydroxymethyl)benzamide.]

Hydrolysis of acetal **16** to give aldehyde **17** was one of the questionable steps in the synthesis since the stability



of the *N*-[(alkylthio)methyl]amide moiety under hydrolytic conditions had not been extensively investigated. However, reports by Veber et al. using simpler systems suggested this moiety should be stable even to reasonably acidic or basic conditions.[18e,f] As hoped, the thiomethyl amide linkage in **16–19** proved to be stable at pH 2 in acetone/water solution for three days, as this extended hydrolysis time was required of complete conversion of the readily formed **18** to the equilibrium mixture of **17** and **19** required for oxidation to the model compound **13b**.

Oxidation of the **17/19** mixture to the lactone **13b** could not be effected by using any of the conditions examined. While the (*n*-alkylthio)methyl group was stable to hydrolysis under acid conditions, it was rapidly destroyed by the mild oxidant silver oxide.[25] It was anticipated that the second alternative, oxidation with peracid,[26] would be complicated by oxidation of both the aldehyde and sulfide functions[27] which would introduce an additional, but uncomplicated, synthetic step: reduction of the newly formed sulfoxide back to the desired sulfide. Repeated attempts at the oxidation of **17/19** with several peracids provided only small amounts of the corresponding sulfoxide.

In a somewhat different approach to **13b**, *N*-bromosuccinimide was examined as the oxidant in an attempted conversion of acetal **14** directly to the corresponding ester. This well documented method[28] appeared to be particularly attractive since it avoided the need to hydrolyze the dimethyl acetal in **14**, and it was not expected to affect the *N*-thiomethyl amide function under the reaction conditions employed.[29] Unfortunately, repeated oxidation attempts with freshly recrystalized NBS failed to provide any of the desired ester.

Because of the difficulties encountered in the attempted oxidation of **16** and the **17/19** mixture, this approach to **13** was set aside and efforts were focused on a synthesis that would avoid any oxidative steps. A review of the literature concerning potential intermediates in such a synthesis revealed the compound, *tert*-butyl 3-hydroxy-

(19) Zbur-Wilson, J.; Morrow, C. J., unpublished results.
(20) (a) Beutel, R. H. Fr. Patent 1 345 845, Dec 13, 1963; *Chem. Abstr.* **1964**, *60*, 10786h. (b) Beutel, R. H. Fr. Patent 1 343 206 Nov 15, 1963; *Chem. Abstr.* **1964**, *60*, 10787i.
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(24) 1-Mercapto-2-butanol was prepared from 1,2-epoxybutane by the general method of Baker.[21].
(25) This was somewhat surprising since silver oxide oxidation in the presence of potentially labile thioethers has been reported. (a) Champaigne, E.; LeSuer, W. M. "Organic Syntheses"; Wiley: New York, 1963; Collect. Vol. 4, 919. (b) Carna, L. D.; Christensen, B. G. *Tetrahedron Lett.* **1973**, 3505. (c) Krebs, A.; Kimling, H. *Tetrahedron Lett.* **1970**, 761.
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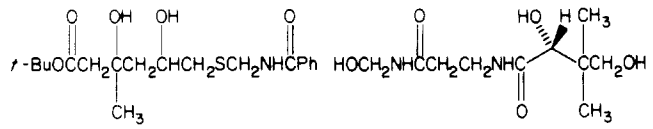
(29) Groebel, W. *Chem. Ber.* **1959**, *92*, 2887.

3-methyl-5-hexenoate, **10b**, which nicely fulfilled the structural requirements. By modifying the synthesis of **10b** reported by Tschesche and Machleidt,[6a] the yield for its preparation from allyl bromide, *tert*-butyl acetoacetate, and zinc was increased to 80%. Treatment of **10b** with 2 equiv of MCPBA in chloroform solution converted it to the corresponding epoxide, **20**, (70% yield after distillation). Treatment of the epoxide with a hydrogen sulfide-saturated solution of sodium hydroxide in methanol, as described by Baker,[21] provided **9b** in 87% yield (52% yield from *tert*-butyl acetoacetate).

Benzamidomethylation of the sulfhydryl in **9b** using **15c** and sodium hydroxide in refluxing benzene provided the desired product, **21**, in only 15% yield. This result was not significantly improved by using **15a** in acidic water/acetone solution. The yield was increased[30] to 36%, however, when *N*-(acetoxymethyl)benzamide,[32] **15d**, was used in acidified ether or acetone.

Both of the latter methods using an acid catalyst were found to cause partial conversion of the intermediate **21** to the lactone **13b**. Complete conversion was effected by treating crude **21** with concentrated formic acid,[33] a methylene chloride solution saturated with HCl,[34] or concentrated trifluoroacetic acid[34] with the last method affording the greatest yield (86%) of **13b**.



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To gain additional insight into amidomethylation of relatively complex thiols with alkyl amides and to develop synthetic methods more directly applicable to the preparation of **7d**, model compound **13c** was prepared.[35] Rather than acetamidomethylate **9b**, the *tert*-butyl ester in **9b** was first cleaved to provide the mercaptomethyl lactone **13d**. Changing the order of these steps prevented two competing side reactions, ester cleavage and sulfhydryl alkylation (by isobutylene), both of which occurred during the acid catalyzed benzamidomethylation of **9b**. The *tert*-butyl ester was cleaved by treating **9b** with trifluoroacetic acid in methylene chloride solution.[36] To avoid sulfhydryl alkylation during the hydrolysis, excess dimethyl sulfide, which acts as an isobutylene scavanger, was added to the reaction mixture.[18f] Improved yields (quantitative) of **13d** were obtained by treating **9b** with concentrated formic acid.[33] Since formic acid acts as both a catalyst for the ester cleavage and an isobutylene scavanger, dimethyl sulfide was not necessary when using this reagent. Once prepared, **13d** was allowed to react with 1.25 equiv of *N*-(hydroxymethyl)acetamide (**15e**) in concentrated formic acid to afford model compound **13c** in 69% yield.

With the amidomethylation studies successfully completed, preparation of D-(−)-pantothenamide, **8**, was initiated by using a slightly modified version[37] of the synthesis reported by Beutel.[20] Thus, 3-aminopropanenitrile,[38] **22**, prepared from liquid ammonia and acrylonitrile, was treated with concentrated sulfuric acid to afford 3-ammoniopropanamide sulfate which, upon treatment with ammonia in methanolic pyridine, provided the free amide **11** in 80% yield. Treatment of **11** with D-(−)-pantolactone, **12**, in methanol gave D-(−)-pantothenamide, **8**, in 93% yield (74% yield from 3-aminopropanenitrile).

The required amidomethylating agent, *N*-(hydroxymethyl)pantothenamide, **23**, was prepared by treatment of **8** with either formalin[22] or paraformaldehyde[39] in the presence of a basic catalyst. The method employing paraformaldehyde resulted in only low yields (26%) partially because chromatographic removal of a bis(hydroxymethylated) byproduct was required. Formalin, on the other hand, provided nearly quantitative yields of **23** once the catalyst concentration was optimized.

With both intermediates **13d** and **23** in hand, the final synthetic step—coupling these compounds to form the *N*-thiomethyl amide function—was investigated. Initial attempts at producing a diastereomeric mixture of **7d** by allowing both diastereomers of **13d** to react with **23** in concentrated formic acid failed. Changing the reaction medium to a tetrahydrofuran/methanol solution and employing hydrochloric acid as the catalyst met with limited success producing **13a** in 50% yield. However, attempts at chromatographically separating this product into its diastereomers failed. Therefore, an alternative approach to diastereomer separation involving chromatographic separation of the **13d** mixture into racemic cis and trans lactones **13d**[40] prior to coupling with **23** was investigated. Not only were these diastereomers easily separated by reverse-phase HPLC, but monitoring the coupling of each diastereomer with **23** by HPLC and ^{13}C NMR was significantly simplified. The acid-catalyzed condensation of the trans lactone, *trans*-**13d**, with *N*-(hydroxymethyl)-pantothenamide, **23**, was studied in a variety of solvent systems. In general, formation of the desired product was favored in protic solvents and when the reaction scale was limited to <0.5 mmol. Increasing the scale led to an increase in byproduct formation. *trans*-**13a** was produced in satisfactory yield (45% after chromatography) by allowing *trans*-**13d** to react with **23** in acidified 50% acetonitrile/isopropyl alcohol solution.

As anticipated, adequate coupling (64%) of the cis lactone, *cis*-**13d**, with **23** occurred when the reaction was performed in acidified 50% acetonitrile/isopropyl alcohol solution and on a reaction scale of <0.5 mmol. However, an increase in the scale to 1.5 mmol resulted in only 8% of the desired product being formed. Instead a byproduct, *N*-(isopropoxymethyl)pantothenamide, resulting from the condensation of **23** with the isopropyl alcohol, predominated. To overcome this problem, the solvent system was

(30) The increased yield was probably due to the improved reactivity reported by Barker and co-workers for *N*-(acetoxymethyl)benzamide relative to *N*-(hydroxymethyl)- or *N*-(methoxymethyl)benzamide. The increase in reactivity is attributed to the better leaving ability of the acetoxy group.[31]

(31) Vail, S. L.; Kullman, R. M. H.; Reeves, W. A.; Barker, R. H. *Text. Res. J.* **1970**, *40*, 355.

(32) Walter, W.; Steffen, M.; Heyns, C. *Chem. Ber.* **1966**, *99*, 3204.

(33) Chandrosekaran, S.; Kluge, A. F.; Edwards, J. A. *J. Org. Chem.* **1977**, *42*, 3972.

(34) Cornforth, D. A.; Opara, A. E.; Read, G. *J. Chem. Soc. C* **1969**, 2799.

(35) Barker and co-workers observed a significant difference in the reactivity of aroylamido- and acylamidomethylating reagents which was attributed to a difference in the stability of the carbonium–immonium ion intermediates generated.[31]

(36) Bryan, D. B.; Hall, R. F.; Holden, K. G.; Huffman, W. F.; Gleason, J. G. *J. Am. Chem. Soc.* **1977**, *99*, 2353.

(37) It was found that improved yields of pantothenamide, **8**, were produced by warming pantolactone, **12**, and 3-aminopropanamide, **11**, in methanol.

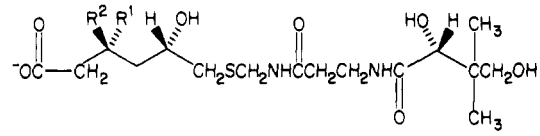
(38) Terent'ev, A. P.; Chursina, K. I.; Kost, A. N. *Zh. Obsh. Khim.* **1950**, *20*, 1073; *Chem. Abstr.* **1951**, *44*, 9349f.

(39) (a) Parris, C. L. U.S. Patent 3 024 282, March 6, 1962; *Chem. Abstr.* **1963**, *57*, 11099g. (b) Haworth, R. D.; Peacock, D. H.; Smith, W. R.; MacGillwary, R. *J. Chem. Soc.* **1952**, 2972.

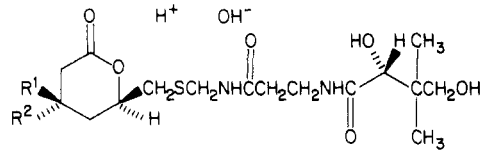
(40) The stereochemistry of the two diastereomers was assigned based on comparison of the ^1H NMR spectra obtained for these isomers with spectra of known compounds having similar structures.[14a] Cis and trans refer to the relationship of the hydroxyl at carbon-3 and the side chain at carbon-5.

modified by increasing the acetonitrile content to 80% and replacing the alcohol with water. In this way, the necessary protic character of the solvent was maintained without introducing an interfering, reactive cosolvent. *cis*-**13a** was prepared in 39% yield, after chromatography, from *cis*-**13d** and **23** in acidified acetonitrile/water solution.

Finally, the corresponding acids, *erythro*- and *threo*-**7d**, were prepared in situ, as their potassium salts, by treating *trans*-**13a** and *cis*-**13a**, respectively, with aqueous potassium carbonate. Both diastereomers of **7d** were found to remain in the acyclic form under conditions ranging from neutral to basic pH. However, in acidic solution lactonization occurred rapidly to regenerate the parent lactones, *trans*- and *cis*-**13a**.



7d, *erythro*, R^1=OH; R^2=CH_3
threo, R^1=CH_3; R^2=OH



13a, *cis*, R^1=OH; R^2=CH_3
trans, R^1=CH_3; R^2=OH

The individual diastereomers of lactone **13a** and carboxylate **7d** were tested for inhibition of rat liver, microsomal, HMG-CoA reductase.[41] All of the compounds demonstrated some degree of inhibition, the details of which will be reported elsewhere.

Experimental Section

A. General Experimental Methods and Instrumentation. General Methods. Elemental analyses were performed by Ruby Ju of the University of New Mexico microanalytical laboratory. All procedures requiring anhydrous conditions employed oven-dried glassware cooled under a nitrogen atmosphere. Magnesium sulfate was used to dry organic extracts. All reactions were stirred magnetically unless otherwise indicated.

Materials. Organic reagents were purchased from Aldrich Chemical Co. unless otherwise indicated. Solvents and commercially available starting materials were generally used without additional purification. Where anhydrous conditions were necessary, either Aldrich Gold Label anhydrous solvents were used, or solvents were dried according to standard procedures.

Instrumentation. ^{1}H NMR spectra were obtained on either a Varian EM-360 spectrometer or a Varian FT-80A spectrometer (79.5 MHz). Peaks are reported as ppm (δ) downfield from Me_4Si (or DSS), which was used as an internal standard. ^{13}C NMR spectra were obtained with a Varian FT-80A spectrometer (20 MHz). Chemical shifts are reported as ppm (δ_c) with respect to Me_4Si, as determined from (1) $CDCl_3$ assuming a chemical shift of 76.9 ppm for $CDCl_3$, or (2) *p*-dioxane assuming a chemical shift of 67.4 ppm for *p*-dioxane in D_2O. High-performance liquid chromatography (HPLC) analyses weree performed by employing a Waters Associates 6000A pump, a U6K injector, and a RCM 100 radical compression module. A Waters Associates R401 differential refractometer and/or a sequential Schoeffel 770 UV detector were used. Columns, solvent systems, and flow rates used are indicated in the experimental procedures. Preparative HPLC was carried out with a Waters Associates Prep LC/System 500. Columns and solvent systems used are indicated in the experimental procedures. Analytical thin-layered chromatography (TLC) was carried out with glass-backed silica gel plates (Fisher Scientific Co.) or polyethylene-backed silica gel plates with fluorescent indicator (Eastman). Visualization was achieved with either 10% ethanolic phosphomolibdic acid (glass-backed silica gel plates) or UV radiation (silica gel with fluorescent indicator).

B. Experimental Procedures. *N*-(Hydroxymethyl)benzamide (15a) was synthesized according to Einhorn et al.[22] and found to give satisfactory ^{1}H and ^{13}C NMR spectra and C, H, N analysis.

***N*-(Chloromethyl)benzamide (15b)** was synthesized according to Bohme et al.[18b] and found to give satisfactory ^{1}H and ^{13}C NMR spectra and C, H, N analysis.

***N*-[(Diethylamino)methyl]benzamide (15c)** was synthesized according to Hellman and Haas.[18c] **15c** (16.27 g, 79%) was obtained: mp 58–60 °C (lit. mp 62–64 °C); ^{1}H and ^{13}C NMR spectra were satisfactory.

***N*-(Acetoxymethyl)benzamide (15d)** was prepared according to Haworth et al.[39b] and found to give satisfactory ^{1}H and ^{13}C NMR spectra.

***N*-(Hydroxymethyl)acetamide (15e)** was prepared according to Walter[32] as white crystals, mp 36–39 °C (lit. mp 55 °C). ^{1}H and ^{13}C NMR spectra were satisfactory.

1-[(Benzamidomethyl)thio]-2-butanol. To 4.32 g (0.03 mol) of *N*-(hydroxymethyl)benzamide, **15b**, and 1.76 g (0.026 mol) of 1-mercapto-2-butanol[24] dissolved in 20 mL H_2O and cooled to 0 °C, was added dropwise with stirring 1 mL of concentrated H_2SO_4. After stirring for 48 h, the solution was extracted with ether (4 × 50 mL) and the combined ether extracts were washed with saturated $NaHCO_3$ solution (2 × 50 mL) and then with 80 mL of H_2O. They were dried and filtered, and the solvent removed in vacuo to afford a clear viscous liquid which upon dissolution in absolute EtOH and concentration by rotary evaporation gave a white solid: mp 85–86 °G; 4.45 g (83%); ^{1}H NMR ($CDCl_3$) δ 7.2–8.1 (m, 6 H, ArH, NH), 4.7 (d, 2 H, NCH_2S), 3.8 (m, 1 H, *CH*OH), 2.8 (m, 3 H, CCH_2S, OH), 1.5 (m, 2 H, CCH_2C), 0.9 (t, 3 H, CH_3). Anal. Calcd for $C_{12}H_{17}O_2NS$: C, 60.22; H, 7.16; N, 5.85. Found: C, 60.38; H, 7.28; N, 5.99.

5,6-Epoxy-1,1-dimethoxy-3-methyl-3-hexanol (14), Diastereomer Mixture.[19] A solution of 4.43 g (0.026 mol) of *m*-chloroperoxybenzoic acid (85%), 3.74 g (0.022 mol) of 1,1-dimethoxy-3-methyl-5-hexene-3-ol (**10a**), and 100 mL of $CHCl_3$ was allowed to stir under nitrogen at room temperature for 64 h. After washing with 10% $Na_2S_2O_3$ solution (2 × 50 mL), 5% Na_2CO_3 solution (2 × 50 mL), and saturated NaCl solution, the mixture was dried and filtered and the $CHCl_3$ removed in vacuo to give a clear oil. Distillation (bp 136–138 °C (0.05 mm)) afforded **14** as a colorless oil: 2.56 g (69%); ^{1}H NMR (CCl_4) δ 4.5 (t, J = 4 Hz, 1 H, 1-H), 3.3 (s, 6 H, CH_3O), 2.3–3.3 (m, 4 H, 5-H, 6-H, and OH), 1.7 (m, 4 H, 2-H and 4-H), 1.2 (s, 3 H, 3-CH_3); ^{13}C NMR (D_2O) δ 102.2 and 102.3 (C-1), 70.1 (C-3), 52.2 and 52.7 (CH_3O), 48.3 (C-2), 45.9 and 46.2 (C-5), 44.9 (C-4), 42.5 and 42.9 (C-6), 26.9 and 27.4 (3-CH_3).

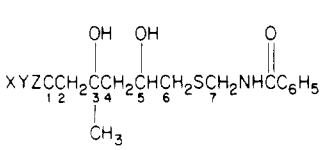
1-Mercapto-6,6-dimethoxy-4-methyl-2,4-hexanediol (9a), diastereomer mixture, was prepared according to the general method of Baker et al.[21] from 1.56 g (0.039 mol) of NaOH, 3.63 g (0.019 mol) of 5,6-epoxy-1,1-dimethoxy-3-methyl-3-hexanol (**14**), and 20 mL of CH_3OH by the procedure described below for **9b**: 3.49 g (81%) of a colorless oil was obtained: HPLC (μ-Porisil, 50% EtOAc–hexanes, flow rate 2 mL/min) R_v 9.0 mL; ^{1}H NMR (see Table I); ^{13}C NMR (see Table II). Anal. Calcd for $C_9H_{20}O_4S$: C, 48.19; H, 8.99. Found: C, 48.91; H, 8.48.

1-[(Benzamidomethyl)thio]-6,6-dimethoxy-4-methyl-2,4-hexanediol (16), Diastereomer Mixture. A solution of 5.57 g (0.027 mol) of *N*-[(diethylamino)methyl]benzamide, **15c**, 6.00 g (0.027 mol) of 6,6-dimethoxy-1-mercapto-4-methyl-2,4-hexanediol, **9a**, 0.03 g (0.00075 mol) of NaOH, and 250 mL of dry C_6H_6 was allowed to reflux under a slow stream of nitrogen for 4 h. After stirring overnight at room temperature, the mixture was washed with saturated $NaHCO_3$ solution (2 × 100 mL), pH 6 phosphate buffer solution (2 × 100 mL), and 100 mL of H_2O. It was then dried and filtered and the solvent removed in vacuo to afford **16**, as a viscous yellow oil: 4.29 g (44.8%); HPLC (μ-Porisil, 50% EtOAc–hexanes, flow rate 2 mL/min) R_v 33.0 mL; ^{1}H NMR (see Table I); ^{13}C NMR (see Table II). Anal. Calcd for $C_{17}H_{27}NO_5S$: C, 57.12; H, 7.61; N, 3.92. Found: C, 57.51; H, 7.66; 3.94.

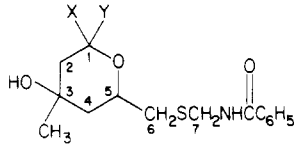
6-[[(Benzamidomethyl)thio]methyl]-2,4-dihydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran (19), Diastereomer

(41) Scallen, T. J.; Martinez-Montano, R., unpublished results.

Table I. ^{1}H NMR Spectral Data in $CDCl_3$ for 6-[[(Benzamidomethyl)thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one, 13b, Its Precursors, and Related Compounds



9a, X = H; Y = Z = OMe
9b, X, Y = O; Z = $OCMe_3$
16, X = H; Y = Z = OMe
21, X, Y = O; Z = $OCMe_3$



13b,c,d, X, Y = O
18, X = H; Y = OMe
19, X = H; Y = OH

	chemical shifts, δ[a]										
no.[b]	1-H	2-H	3-CH_3	4-H	5-H	6-H	7-H	NH	Ar-H	Y,Z	OH
9a	4.7 (t)	1.4–2.0 (m)	1.3 (s)	1.4–2.0 (m)	3.6–4.2 (m)	2.3–2.8 (m)				3.4 (2 s)	4.0–4.5
9b[c]		2.2–2.9 (m)	1.3 (s)	1.5–1.9 (m)	4.0 (m)	2.2–2.9 (m)				1.5 (s)	4.2
13b		2.6 (ABq)	1.3 (s)	1.8–2.3 (m)	4.4, 4.9 (2 m)	2.9 (m)	4.5–4.8 (m)	8.1 (m)	7.3–8.0 (m)		3.6
13c[d]		2.6 (ABq)	1.4 (2 s)	1.8–2.3 (2 m)	4.5, 4.8 (2 m)	3.0 (d) J = 5	4.4 (d) J = 5	7.4 (t)	*d*		4.8
13d[e]		2.5–2.8 (m)	1.4 (s)	1.9–2.4 (m)	4.4, 4.8 (2 m)	2.9 (d)					2.8
16	5.1–5.4 (m)	1.1–2.0 (m)	1.3 (s)	1.1–2.0 (m)	4.0–4.5 (m)	2.8 (d)	4.5–4.8 (m)	8.3 (m)	7.3–8.0 (m)	3.3 (2 s)	4.0–4.5
18	3.6–4.9 (m)	1.4–1.9 (m)	12, 1.4 (2 s)	1.4–1.9 (m)	3.6–4.9 (m)	2.6–3.0 (m)	4.6 (d) J = 6	7.2–8.0 (m)	7.2–8.0 (m)	3.2, 3.5 (2 s)	3.6–4.9
19	5.1, 5.9 (2 m)	1.4–1.9 (m)	1.2 (s)	1.4–1.9 (m)	3.7–4.9 (m)	2.6–3.0 (m)	4.6 (d) J = 6	7.2–8.0	7.2–8.0 (m)		3.7–4.9
21		2.5 (ABq)	1.3 (s)	1.6–2.1 (m)	4.0–4.5 (m)	2.8 (d) J = 5	4.6 (d) J = 6	8.2 (t) J = 6	7.2–8.1 (m)	1.4 (s)	4.8

[a] Spectral data was obtained with a Varian EM 360 NMR spectrometer. [b] Each spectrum is of a mixture of diastereomers. [c] The sulfhydryl proton appears as a multiplet in the region δ 1.5–1.9. [d] The methyl hydrogens in $CH_3C(O)$ appear as a singlet at δ 2.2. [e] The sulfhydryl proton appears as a triplet at δ 1.7.

Mixture. A solution of 1.5 g (0.005 mol) of **16**, 5 mL of aqueous acetone, and 4 drops of concentrated H_2SO_4 was allowed to stir under nitrogen at room temperature for 7 days. After adjusting the pH to 7, the solution was extracted with ether (3 × 100 mL), the combined extracts were dried and filtered, and the solvent removed in vacuo to give **19** as a yellow semisolid: 0.76 g (58%); TLC (silica, 85% EtOAc–hexanes) R_f 0.20 and 0.28; ^{1}H NMR (see Table I), ^{13}C NMR (see Table II).

6-[[(Benzamidomethyl)thio]methyl]-4-hydroxy-2-methoxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran (18), diastereomer mixture, was prepared by modifying the procedure described for **19**. Thus, from 100 mg (0.3 mmol) of **16**, 2 mL of 50% CH_3OH–acetone, and 1 drop of concentrated H_2SO_4 allowed to stir for 44 h was produced 80 mg (90%) **18**: ^{1}H NMR (see Table I); ^{13}C NMR (see Table II).

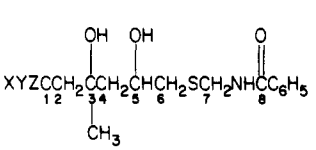
***tert*-Butyl 3-hydroxy-3-methyl-5-hexenoate (10b)** was prepared by using the method of Tschesche and Machleidt.[6a] The yield was improved by employing an alternative method of workup. Thus, in a 500-mL three-neck round-bottom flask equipped with a reflux condenser and mechanical stirrer was placed 30.0 g (0.46 mol) of anhydrous Zn turnings and 66 mL of anhydrous ether. To this was added dropwise with stirring a mixture of 52.1 g (0.33 mol) of *tert*-butyl acetoacetate and 51.8 (0.43 mol) of allyl bromide dissolved in 130 mL of 75% THF–ether (anhydrous). (Reflux was initiated by warming the Zn suspension with a heat gun and maintained by careful control of the addition rate.) After addition was complete, the mixture was allowed to stir overnight under nitrogen, decanted from unreacted Zn, poured into 200 mL of ice water, carefully acidified (dropwise addition of 4 N H_2SO_4), saturated with $(NH_4)_2SO_4$, filtered, and extracted with ether (5 × 100 mL). The combined ether extracts were washed with 1 N NaOH solution (2 × 150 mL) and saturated NaCl solution, then were dried, and filtered and the ether removed in vacuo to afford **10b**, as a light yellow oil: 64.4 g (78%). The ^{1}H and ^{13}C NMR spectra confirmed the structure.

***tert*-Butyl 5,6-Epoxy-3-hydroxy-3-methylhexanoate (20), Diastereomer Mixture.** To a stirred solution of 36.3 g (0.18 mol) of *tert*-butyl 3-hydroxy-3-methyl-5-hexenoate, **10b**, and 700 mL of $CHCl_3$ was added 73.6 g (2 equiv) of 85% *m*-chloroperoxybenzoic acid. The solution was allowed to stir at room temperature under nitrogen for 24 h, and the precipitate formed (*m*-chlorobenzoic acid) was then removed by filtration. The filtrate was washed with 10% $Na_2S_2O_3$ solution (2 × 150 mL), 5% Na_2CO_3 solution (2 × 150 mL), and 100 mL saturated NaCl solution, then was dried, and filtered, and the solvent removed in vacuo to afford **20**, as a yellow oil: 27.2 g (70%); ^{1}H NMR ($CDCl_3$) δ 4.0 (s, 1 H, OH), 2.9–3.3 (m, 1 H, 5-H), 2.8 (t, 1 H, 6-H), 2.5 (m, 3 H, 6-H and 2-H), 1.6–2.1 (m, 2 H, 4-H), 1.5 (s, 9 H, $C(CH_3)_3$), 1.3 (s, 3 H, 3-CH_3); ^{13}C NMR ($CDCl_3$) δ_c 171.8 and 172.0 (C-1), 81.2 ($C(CH_3)_3$), 70.3 and 70.4 (C-3), 48.4 (C-4), 46.1 and 46.2 (C-5), 44.2 and 44.4 (C-6), 27.8 and 27.9 ($C(CH_3)_3$), 27.3 and 27.5 (3-CH_3). Anal. Calcd for $C_{11}H_{20}O_4$: C, 61.09; H, 9.34. Found: C, 60.88; H, 9.40.

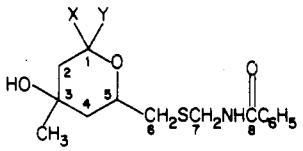
***tert*-Butyl 3,5-Dihydroxy-6-mercapto-3-methylhexanoate (9b), Diastereomer Mixture.** When the general method of Baker et al.[21] was followed, a well-stirred solution of 2.06 g (0.052 mol) of NaOH in 100 mL of CH_3OH was saturated with H_2S by passing in the gas at −10 to 0 °C during 2 h. While a slow stream of H_2S was maintained, 5.55 g (0.026 mol) of *tert*-butyl 5,6-epoxy-3-hydroxy-3-methylhexanoate, **20**, dissolved in 10 mL of CH_3OH, was added dropwise to the cold solution over a period of 0.75 h. The solution was allowed to stir for 17 h at room temperature, then was diluted with 100 mL of ice water, and cooled to 0 °C, and 5 N H_2SO_4 was added dropwise with stirring until pH 6.3. Five 50-mL portions of $CHCl_3$ were used to extract the solution. The combined extracts were washed with saturated $NaHCO_3$ solution (50 mL) and saturated NaCl solution, then was dried, and filtered, and the chloroform was removed in vacuo to afford **9b** as a colorless oil: 5.60 g (87%); ^{1}H NMR (see Table I); ^{13}C NMR (see Table II). Anal. Calcd for $C_{11}H_{25}O_4S$: C, 52.77; H, 8.88. Found: C, 53.09; H, 8.89.

***tert*-Butyl 6-[(Benzamidomethyl)thio]-3,5-dihydroxy-3-methylhexanoate (21), Diastereomer Mixture.** To a solution of 2.25 g (0.009 mol) of *tert*-butyl 3,5-dihydroxy-6-mercapto-3-methylhexanoate, **9b**, were added 25 mL of anhydrous ether and concentrated H_2SO_4 (5 drops) dropwise with stirring followed by 1.74 g (0.009 mol) of *N*-(acetoxymethyl)benzamide, **15d**, dissolved

Table II. ^{13}C NMR Spectral Data in $CDCl_3$ for 6-[[(Benzamidomethyl)thio]methyl]-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one, 13b, Its Precursors, and Related Compounds



9a, X=H; Y=Z=OMe
9b, X, Y=O; Z=$OCMe_3$
16, X=H; Y=Z=OMe
21, X, Y=O; Z=$OCMe_3$



13b,c,d, X, Y=O
18, X=H; Y=OMe
19, X=H; Y=OH

	chemical shifts, δ[a]											
no.[b]	C-1	C-2	C-3	3-CH_3	C-4	C-5	C-6	C-7	C-8	Ar-C	Y	Z
9a	102.1	44.4–46.2	71.7	26.1	44.4–46.2	69.4	31.7				52.9	53.9
	102.5		71.8	28.3			31.9					
9b	171.5	45.4	71.5	26.0	45.1	67.5	31.8					81.1, 81.3
	171.6	47.3		27.8		69.6	32.0					27.8,28.1
13b	170.7	43.7	67.7	28.8	40.3	77.1	37.1	43.4	167.5	127.1–133.4		
	171.0	44.6	68.4	29.6	41.4	77.6						
13c[c]	170.8	43.6	67.4	28.7	40.0	76.6	36.7	42.3	170.5	*c*		
		44.4	68.1	29.3	41.2	76.7	36.8	42.5				
13d	170.5	43.4	67.3	28.4	38.9	76.9	28.5					
	170.6	44.3	67.9	29.1	40.4	77.5	30.4					
16	102.3	46.2	71.7	27.5	45.1	68.9	40.6	43.1	166.7	128.1–134.2	52.9	53.9
	102.8	47.4	71.8	28.9	45.2	69.0	40.8	43.2	166.8			
18	92.8	42.8–43.3	67.9	28.7	42.8–43.3	64.7	38.0	42.8–43.3	167.1	126.9–133.9	54.7	
			68.8	29.6			38.1				56.1	
19	92.7	42.1–43.6	68.7	30.3	42.1–43.6	65.1	37.6	42.1–43.6	167.3	126.9–133.8		
	92.8		69.3	30.9								
21	171.5	46.6	71.6	25.8	42.9	68.8	40.1	44.7	166.8	126.9–133.9		81.1, 81,3
	171.6	47.2	71.8	27.8	43.0		40.4	45.1				27.8, 28.1

[a] Spectral data was obtained with a Varian FT-80A NMR spectrometer. Numbers separated by hyphens show range within which the indicated absorption was observed if specific assignments were not possible because of multiple absorptions within the range. [b] Each spectrum is of a mixture of diastereomers. [c] The methyl carbon in $CH_3C(O)$ appears at δ 22.5.

in 25 mL of anhydrous ether. After stirring for 3 h under nitrogen, the solution was diluted with 25 mL of ether and extracted with saturated $NaHCO_3$ solution. The combined extracts were lyophilized, the resulting residue was sonicated in 25 mL of dry acetone and filtered, and the acetone was removed in vacuo to afford a colorless oil which was purified by preparative HPLC (Waters Assoc. Prep Pak-500, 60% EtOAc–hexanes.) to give **21**: 0.6 g (17%); HPLC (Waters Assoc. Radial-Pak A LC cartridge, 60% EtOAc–hexanes, flow rate 5 mL/min) R_v 11.5 mL; ^1H NMR (see Table I); ^{13}C NMR (see Table II).

trans-6-[[(Benzamidomethyl)thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (230 mg, *trans*-**13b**) and a mixture of *cis*- and *trans*-**13b** (310 mg) were also isolated from the crude product (combined yield of products 1.13 g, 37%) and characterized, as below.

6-[[(Benzamidomethyl)thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (13b), Diastereomer Mixture. To a stirred solution of 5.0 g (0.02 mol) of *tert*-butyl 3,5-dihydroxy-6-mercapto-3-methylhexanoate, **9b**, in 100 mL of acetone and 5 drops of concentrated H_2SO_4 was added dropwise with stirring 3.87 g (0.02 mol) of *N*-(acetoxymethyl)-benzamide, **15d**, dissolved in 25 mL of acetone. After stirring for an additional 1.5 h, the solution was concentrated by rotary evaporation, and the resulting crude product was allowed to stir for 1.5 h under nitrogen in 150 mL of CH_2Cl_2 containing 10 mL of trifluoroacetic acid. The organic phase was washed with saturated $NaHCO_3$ solution (100 mL), then dried, and filtered and the solvent removed in vacuo to afford crude **13b**. Purification by preparative HPLC (Waters Assoc. Prep Pak-500, 60% EtOAc–hexanes.) afforded **13b**, as a colorless oil: 1.7 g (28%); HPLC (Waters Assoc. Radial-Pak A LC cartridge, 60% EtOAc–hexanes, flow rate 5 mL/min) R_v 30 mL; ^1H NMR (see Table I); ^{13}C NMR (see Table II). Anal. Calcd for $C_{15}H_{19}NO_4S$: C, 58.23; H, 6.20; N, 4.53. Found: C, 57.56; H, 6.63; N, 4.09.

6-[[(Acetamidomethyl)thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (13c), Diastereomer Mixture. A solution of 5.0 g (0.02 mol) of *tert*-butyl 3,5-dihydroxy-6-mercapto-3-methylhexanoate, **9b**, and 50 mL of HCOOH (97%) was allowed to stir at room temperature under nitrogen for 1.5 h. *N*-(Hydroxymethyl)acetamide, **15e**, (1.42 g, 0.016 mol) was then added and stirring was continued for 2 h. The sclution was concentrated by rotary evaporation and the resulting oil was dissolved in $CHCl_3$, allowed to stir in the presence of K_2CO_3, filtered, and then extracted with H_2O. The combined extracts were lyophilized, the resulting residue was dissolved in $CHCl_3$ and dried, and the solvent was removed to afford **13c** as a colorless oil: 2.74 g (69.2%); HPLC (Waters Assoc. Radial-Pak A LC cartridge (C_{18}), 92% H_2O–CH_3OH, flow rate 5 mL/min) R_v 20.5 and 31.0 mL; ^1H NMR (see Table I); ^{13}C NMR (see Table II). Anal. Calcd for $C_{10}H_{17}NO_4S$: C, 48.57, H, 6.94; N, 5.66. Found: C, 48.20; H, 7.05; N, 5.06.

4-Hydroxy-6-(mercaptomethyl)-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (13d), Diastereomer Mixture. Method A.[18f] In a 50-mL round-bottom flask was placed 1.27 g (5.1 mmol) of *tert*-butyl 3,5-dihydroxy-6-mercapto-3-methylhexanoate, **9b**, 5 mL (10 equiv) of trifluoracetic acid (TFA) [Eastman], and 3 mL each of CH_2Cl_2 and dimethyl sulfide. The mixture was allowed to stir under nitrogen for 2 h, after which time the solvent (and TFA) was removed in vacuo. The resulting crude product was dissolved in H_2O and extracted with $CHCl_3$, the combined $CHCl_3$ extracts were dried and filtered, and the $CHCl_3$ evaporated to afford **13d**: 540 mg (65%); TLC (silica, 98% EtOAc–CH_3OH) R_f 0.40; ^1H NMR (see Table I), ^{13}C NMR (see Table II).

Method B.[42] In a 250-mL round-bottom flask was placed 13.6 g (0.54 mol) of **9b** and 150 mL (80 equiv) of HCOOH (97%). The mixture was allowed to stir at room temperature under nitrogen for 3 h. Concentration of the product afforded a colorless oil which was dissolved in 150 mL of $CHCl_3$, allowed to stir for 0.5 h in the presence of K_2CO_3, and filtered. The solvent was removed in vacuo to give **13d**: 10.0 g (96%); TLC (silica, 98% EtOAc–CH_3OH) R_f 0.40; HPLC (Waters Assoc. Radial-Pak A LC (C_{18}) cartridge, 96% H_2O–CH_3OH, flow rate 5 mL/min) R_v 27 and 44 mL, ^1H NMR (see Table I); ^{13}C NMR (see Table II).

***trans*-4-Hydroxy-6-(mercaptomethyl)-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one** (*trans*-**13d**) was isolated from the

(42) Bohme, H. *Org. Synth.* **1940**, *20*, 70.

diastereomer mixture of **13d** by preparative HPLC (Waters Assoc. Prep Pak-500/C_{18} column, 85% H_2O–MeOH) and characterized by the following: HPLC (Waters Assoc. Radial-Pak A LC (C_{18}) cartridge, 96% H_2O–CH_3OH, flow rate 5 mL/min) R_v 45 mL; ^1H NMR ($CDCl_3$) δ 4.6–5.1 (m, 1 H, 6-H), 3.4 (s, 1 H, OH), 2.4–3.1 (m, 4 H, 3-H and $HSCH_2$), 1.6–2.4 (m, 3 H, 5-H and HS), 1.3 (s, 3 H, 4-CH_3); ^{13}C NMR ($CDCl_3$) δ_c 170.6 (C-2), 76.5 (C-6), 67.5 (C-4), 43.6 (C-3), 39.0 (C-5), 28.8 and 29.3 (4-CH_3 and $HSCH_2$). Anal. Calcd for $C_7H_{12}O_3S$: C, 47.71; H, 6.88. Found: 47.53; H, 6.97.

***cis*-4-Hydroxy-6-(mercaptomethyl)-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (*cis*-13d)** was isolated from the diastereomer mixture of **13d** by the procedure described for *trans*-**13d** and characterized by the following: HPLC (Waters Assoc. Radial-Pak A LC (C_{18}) cartridge, 96% H_2O–CH_3OH, flow rate 5 mL/ min) R_v 28 mL; ^1H NMR ($CDCl_3$) δ 4.1–4.7 (m, 1 H, 6-H), 3.1 (s, 1 H, OH), 2.5–3.0 (m, 4 H, 3-H and $HSCH_2$), 1.8–2.3 (m, 2 H, 5-H), 1.7 (t, 1 H, HS), 1.4 (s, 3 H, 4-CH_3); ^{13}C NMR ($CDCl_3$) δ_c 170.7 (C-2), 77.8 (C-6), 68.4 (C-4), 41.7 (C-3), 40.8 (C-5), 29.0 (4-CH_3 and $HSCH_2$). Anal. Calcd for $C_7H_{12}O_3S$: C, 47.71; H, 6.88. Found: C, 47.34; H, 6.89.

3-Aminopropanenitrile (22) was prepared by a modification of the Terent'ev[38] method. To 800 mL of liquid NH_3 contained within an insulated 1000-mL three-neck round-bottom flask was added dropwise with stirring 95 g (1.79 mol) of propenenitrile over the course of 1 h. The mixture was allowed to stir until all NH_3 had evaporated (24 h). Vacuum distillation of the crude product afforded two fractions (50–55 and 106–111 °C (0.1 mm)), which upon ^1H NMR analysis was revealed to be **22** (12.5 g, 12%) and *bis*(2-cyanoethyl)amine.

3-Aminopropanamide (11) was synthesized from 3-aminopropanenitrile, **22**, in 78–80% yield according to Beutel:[20] mp (sulfate) 182–186 °C (lit. mp 182–186 °C); it was also characterized by ^1H and ^{13}C NMR.

***N*-(2-Carbamoylethyl)-2,4-dihydroxy-3,3-dimethylbutyramide (Pantothenamide) (8).** A solution of 6 g (0.068 mol, 1.1 equiv) of 3-aminopropanamide, **11**, 7.7 g (0.062 mol) of pantolactone, **12** [Sigma] and 150 mL of CH_3OH was allowed to stir under nitrogen at 40 °C for 10 h. The solution was concentrated by rotary evaporation and diluted with 50 mL of absolute EtOH and additional EtOAc was then added to the cloud point. Refrigeration for several days and filtration of the resulting precipitate afforded **8**: 12.5 g (93%); mp 99–101 °C (lit. mp 114–116 °C); HPLC (Waters Assoc. Radial-Pak A LC cartridge (C_{18}), 96% H_2O–CH_3OH, flow rate 2 mL/min) R_v 15 mL; ^1H NMR[43] (D_2O) δ 4.0 (s, 1 H, 3-H), 3.3–3.7 (m, 4 H, 1-H and 5-H), 2.5 (t, J = 6 Hz, 2 H, 6-H), 0.9 (s, 6 H, 2-CH_3); ^{13}C NMR[43] (D_2O) δ_c 175.8 and 177.5 (C-4 and C-7), 76.9 (C-3), 69.4 (C-1), 39.5 (C-5), 35.4 and 36.1 (C-2 and C-6), 20.2 and 21.3 (2-CH_3). Anal. Calcd for $C_9H_{18}N_2O_4$: C, 49.52; H, 8.33; N, 12.84. Found: C, 48.98; H, 8.41; N, 12.98.

***N*-[2-[*N*-(Hydroxymethyl)carbamoyl]ethyl]-2,4-dihydroxy-3,3-dimethylbutyramide [*N*-(Hydroxymethyl)pantothenamide] (23).** To 4.0 g (0.018 mol) of pantothenamide **8** and 0.4 g (0.003 mol) of K_2CO_3 was added 2.0 g (0.0038 mol, 1.25 equiv) of formalin (34%) [Baker]. The solution was heated at 60 °C for 10 min, then was diluted with 100 mL of CH_3OH, saturated with CO_2 (g), and filtered and the CH_3OH was removed in vacuo to afford **23**, as a colorless oil: 4.2 g (93%); HPLC (Waters Assoc. Radial-Pak A LC cartridge (C_{18}), 96% H_2O–CH_3OH, flow rate 2.0 mL/min) R_v 18 mL; ^1H NMR (D_2O)[43] δ 4.6 (s, 2 H, 8-H), 3.9 (s, 1 H, 3-H), 3.2–3.6 (m, 4 H, 1-H and 5-H), 2.4 (t, J = 7 Hz, 2 H, 6-H), 0.7 (s, 6 H, $C(CH_3)_2$); ^{13}C NMR (D_2O)[43] δ_c 175.3 and 175.8 (C-4 and C-7), 76.8 (C-3), 69.3 (C-1), 63.6 (C-8), 39.5 (C-5), 36.0 and 36.1 (C-2 and C-6), 20.1 and 21.3 ($C(CH_3)_2$). Anal. Calcd for $C_{10}H_{20}N_2O_5$: C, 48.37; H, 8.14; N, 11.29. Found: C, 48.10; H, 8.23; N, 11.22.

***trans*-6-[[[[3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino]propanamido]methyl]thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (*trans*-13a).** To a solution of 200 mg (1.1 mmol) of *trans*-4-hydroxy-6-(mercaptomethyl)-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one, *trans*-**13d**, in 7 mL of 50% CH_3CN–*i*-PrOH containing 1 drop of concentrated HCl was added dropwise over a 45-min period with stirring 280 mg (1.1 mmol) of *N*-(hydroxymethyl)pantothenamide, **23**, dissolved in 7 mL of 50% CH_3CN–*i*-PrOH. After stirring for 0.5 h, the solution was concentrated by rotary evaporation and pure *trans*-**13a** was provided by HPLC:[44] 201 mg (45%); HPLC (Waters Assoc. Radial-Pak A LC (C_{18}) cartridge, 80% H_2O–CH_3OH, flow rate 2 mL/min) R_v 21.5 mL; ^1H NMR[43] (D_2O) δ 4.5–5.1 (m, 1 H, 10-H), 4.4 (s, 2 H, 8-H), 3.9 (s, 1 H, 3-H), 3.2–3.7 (m, 4 H, 1-H and 5-H), 2.9 (d, J = 5 Hz, 2 H, 9-H), 2.7 (s, 2 H, 13-H), 2.5 (t, J = 6 Hz, 2 H, 6-H), 1.8–2.1 (m, 2 H, 11-H), 1.3 (s, 3 H, 12-CH_3), 0.9 (s, 6 H, 2-CH_3); ^{13}C NMR[43] (D_2O) δ_c 174.7, 175.9, and 175.2 (C-4, C-7 and C-14), 78.7 (C-10), 76.5 (C-3), 69.2 (C-1), 68.7 (C-12), 43.4 (C-13), 42.4 (C-8), 39.5 and 39.6 (C-11 and C-5), 36.1 (C-2, C-6, and C-9), 28.9 (12-CH_3), 20.0 and 21.4 (2-CH_3). Anal. Calcd for $C_{17}H_{30}N_2O_7S$: C, 50.23; H, 7.45; N, 6.89. Found: C, 50.33; H, 7.71; N, 7.40.

***cis*-6-[[[[3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino]propanamido]methyl]thio]methyl]-4-hydroxy-4-methyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (*cis*-13a).** A solution of 235 mg (1.3 mmol) of *cis*-**13d**, 435 mg (1.7 mmol) of **23**, 20 mL of 80% CH_3CN–H_2O, and 1 drop of concentrated HCl was allowed to stir at room temperature for 1 h and then was concentrated by rotary evaporation, and pure *cis*-**13a** was obtained by HPLC:[44] 204 mg (39%); HPLC (Waters Assoc. Radial-Pak A LC (C_{18}) cartridge, 80% H_2O–CH_3OH, flow rate 1.5 mL/min) R_v 16.3; ^1H NMR[43] (D_2O) δ 4.2–4.6 (m, 1 H, 10-H), 4.4 (s, 2 H, 8-H), 4.0 (s, H, 3-H), 3.3–3.8 (m, 4 H, 1-H and 5-H), 3.0 (d, J = 6 Hz, 2 H, 9-H), 2.7 (ABq, 2 H, 13-H), 2.5 (t, J = 7 Hz, 2 H, 6-H), 1.8–2.3 (m, 2 H, 11-H), 1.4 (s, 3 H, 12-CH_3), 0.9 (s, 6 H, 2-CH_3); ^{13}C NMR[43] (D_2O) δ_c 174.6, 175.7 and 175.9 (C-4, C-7 and C-14); 78.4 (C-10), 76.5 (C-3), 69.5 (C-1), 69.2 (C-12), 44.5 (C-13), 42.2 (C-8), 41.6 (C-11), 39.5 (C-5), 36.1 (C-2 and C-6), 35.7 (C-9), 28.7 (12-CH_3), 20.0 and 21.4 (2-CH_3). Anal. Calcd for $C_{17}H_{30}N_2O_7S$: C, 50.23; H, 7.45; N, 6.89. Found: C, 49.88; H, 7.59; N, 6.88.

(3,5)-*erythro*-6-[[[3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino]propanamido]methyl]thio]-3,5-dihydroxy-3-methylhexanoic acid sodium salt (*erythro*-7d) was prepared in situ by allowing *trans*-**13a** to stir in pH 10 Na_2CO_3/$NaHCO_3$ buffer solution for 10 h at room temperature. Dropwise addition of 1 N HCl to pH 7 then afforded *erythro*-**7d** in neutral solution. *erythro*-**7d** was analyzed by HPLC (Waters Assoc. Radial-Pak A LC C_{18}) cartridge, 80% H_2O–MeOH, flow rate 2 mL/min) and was found to elute at the solvent front. It reverted to *trans*-**13a** upon acidification of the solution.

(3,5)-*threo*-6-[[[3-[(2,4-Dihydroxy-3,3-dimethylbutanoyl)amino]propanamido]methyl]thio]-3,5-dihydroxy-3-methylhexanoic acid sodium salt (threo-7d) was prepared from *cis*-**13a** and analyzed by the procedure described for *erythro*-**7d**. It reverted to *cis*-**13a** upon acidification.

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Registry No. 7d, 96228-26-1; D-(−)-**8**, 7757-97-3; *erythro*-**9a**, 96228-27-2; *threo*-**9a**, 96228-28-3; *erythro*-**9b**, 96228-29-4; *threo*-**9b**, 96228-30-7; **10a**, 96228-31-8; **10b**, 87137-59-5; **11**, 4726-85-6; D-(−)-**12**, 599-04-2; **13a**, 96228-32-9; *trans*-**13b**, 96228-33-0; *cis*-**13b**, 96228-34-1; *trans*-**13c**, 96228-35-2; *cis*-**13c**, 96228-36-3; *trans*-**13d**, 88407-17-4; *cis*-**13d**, 96228-37-4; **14** (isomer 1), 96228-38-5; **14** (isomer 2), 96228-39-6; **15b**, 38792-42-6; **15c**, 38221-33-9; **15d**,

(43) For the purpose of spectral assignments, the carbons in this compound are numbered beginning at the terminal (hydroxylated) methylene of the butyramide moiety. Nitrogen atoms in the chain are *not* numbered.

(44) This separation was performed with the analytical HPLC described earlier with two columns (Water Assoc. Radial-Pak A LC (C_{18}) cartridge and μ-Bondapak-C_{18}) linked together in a series. When 80% H_2O–CH_3OH and a flow rate of 2.0–2.8 mL/min was used, 80 mg of crude product could be injected at a time.

28482-69-1; **15e**, 625-51-4; **16** (isomer 1), 96228-40-9; **16** (isomer 2), 96228-41-0; **18**, 96228-42-1; **19**, 96245-28-2; **20** (isomer 1), 88407-15-2; **20** (isomer 2), 88407-16-3; **21** (isomer 1), 96228-43-2; **21** (isomer 2), 96228-44-3; **22**, 151-18-8; (*R*)-**23**, 96228-45-4; 1-mercapto-2-butanol, 96228-46-5; 1-[(benzamidomethyl)thio]-2-butanol, 96228-47-6; *tert*-butyl acetoacetate, 1694-31-1; propenenitrile, 107-13-1; bis-(2-cyanoethyl)amine, 111-94-4; EC 1.1.1.34, 9028-35-7.

Conceptual Basis of the Selective Activation of Bis(dialkylamino)methoxyphosphines by Weak Acids and Its Application toward the Preparation of Deoxynucleoside Phosphoramidites in Situ

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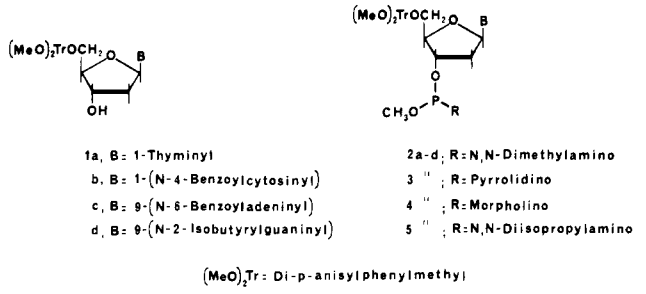
The concept behind the selective activation of bis(dialkylamino)methoxyphosphines is experimentally examined and subsequently used in the preparation of deoxynucleoside phosphoramidites in situ. These intermediates are most efficiently prepared from the reaction of suitably protected deoxynucleosides with dipyrrolidinomethoxyphosphine in the presence of 4,5-dichloroimidazole using 1-methyl-2-pyrrolidinone as solvent. The purity and efficiency of these monomers are evaluated during the solid-phase synthesis of a 22-unit oligomer.

A few years ago, Letsinger and co-workers described the use of a highly reactive 2,2,2-trichloroethyl phosphorochloridite[1,2] as a means of rapidly generating an internucleotidic link. The efficiency of the "phosphite triester" approach was quickly recognized[3-9] and readily adapted to solid-phase oligonucleotide synthesis.[10-19]

Improvements to the methodology were accomplished with the development of the deoxynucleoside phosphoramidites **2a–d** as a new class of monomer units for synthesis.[20] The latter displayed excellent stability toward hydrolysis and produced consistent results during manual solid-phase DNA synthesis.[21-23] However, with the advent of fully automated DNA synthesis, the stability of **2a–d** with prolonged standing in acetonitrile solutions has been questioned. Caruthers[24] and others[25] remedied this problem by using the stable deoxynucleoside phosphoramidite derivatives such as **4a–d** and **5a–d** which can be purified by silica gel chromatography.



1a, B = 1-Thyminyl
b, B = 1-(N-4-Benzoylcytosinyl)
c, B = 9-(N-6-Benzoyladeninyl)
d, B = 9-(N-2-Isobutyrylguaninyl)

2a-d; R = N,N-Dimethylamino
3 " ; R = Pyrrolidino
4 " ; R = Morpholino
5 " ; R = N,N-Diisopropylamino

$(MeO)_2Tr$: Di-p-anisylphenylmethyl

Considering the effort required for large-scale preparation and purification of these key intermediates, a simplification of the procedure was desirable. We briefly reported[26] that the selective activation of dipyrrolidinomethoxyphosphine by 4,5-dichloroimidazole represented a facile and economical approach to the preparation of deoxynucleosides phosphoramidites in situ. Because of our interest in the *stepwise* automated preparation of the latter and their immediate use in a fully automated system for solid-phase DNA synthesis, our investigations were concerned with the highest selectivity of formation of these intermediates within the shortest period of time (less than 10 min) required for such an application.[27]

In this report we wish to describe the conceptual basis involved in the selective activation of bis(dialkylamino)-methoxyphosphines by weak acids. We will also report a detailed study of the reaction conditions (solvent, activator,

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(27) Upon completion of this manuscript, Caruthers and co-workers (Caruthers, M. H.; et al. *Nucleic Acids Res.* **1984**, *12*, 4051) reported a catalytic activation of bis(dialkylamino)phosphines which yielded deoxynucleoside phosphoramidites with high selectivity although some 20 min were required for the formation of these intermediates.

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