Selective Inhibitors of Human Lactate Dehydrogenases and Lactate Dehydrogenase from the Malarial Parasite *Plasmodium falciparum*

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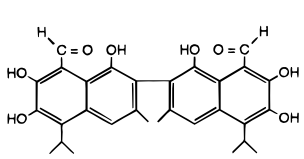
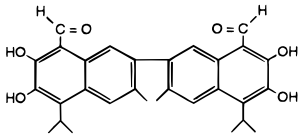
Received June 1, 1998

Derivatives of the sesquiterpene 8-deoxyhemigossylic acid (2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid) were synthesized that contained altered alkyl groups in the 4-position and contained alkyl or aralkyl groups in the 7-position. These substituted dihydroxynaphthoic acids are selective inhibitors of human lactate dehydrogenase-H (LDH-H) and LDH-M and of lactate dehydrogenase from the malarial parasite *Plasmodium falciparum* (pLDH). All inhibitors are competitive with the binding of NADH. Selectivity for LDH-H, LDH-M, or pLDH is strongly dependent upon the groups that are in the 4- and 7-positions of the dihydroxynaphthoic acid backbone. Dissociation constants as low as 50 nM were observed, with selectivity as high as 400-fold.

Gossypol (**1**), a polyphenolic, binaphthyl disesquiterpene found in cottonseed, is an abundant component of cottonseed oil that exhibits a variety of biological activities, including antispermatogenic, anticancer, antiparasitic, and antiviral activity.[1−9] We demonstrated previously that gossypol exhibits antimalarial activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*, with IC_{50} values about 10 μM.[10] Due to the presence of aldehyde functional groups, gossypol is toxic. It has been an open question of whether all of the biological activities of gossypol result from the toxicity associated with the aldehyde functional groups. However, we demonstrated that derivatives of gossypol in which the aldehyde groups were converted into other functional groups retained biological activities, including antimalarial activity[11,12] and antiviral activity.[13] Other derivatives are potent inhibitors of aldose reductase, an enzyme implicated in the etiology of diabetic complications.[9] The synthesis of derivatives of gossypol is limited by the presence of hydroxyl groups at the 1,1′ (peri) positions. These phenolic groups complicate the chemistry of functional groups derived from the aldehydes at the 8,8′-positions. To circumvent this, we recently developed a de novo synthetic scheme for the synthesis of 1,1′-dideoxygossypol (**2**).[14] This versatile synthetic scheme affords the opportunity to deviate from the strict gossypol backbone and to prepare a wide range of gossypol-related compounds.



1 gossypol



2 1,1'-dideoxygossypol

In the present study, we have addressed the question of whether gossypol-related inhibitors can be developed that are selective for lactate dehydrogenase from the malarial parasite *P. falciparum* compared to human lactate dehydrogenases LDH-H ($LDH\text{-}B_4$) and LDH-M ($LDH\text{-}A_4$). Although malarial parasites possess mitochondria, they do not have a functional TCA cycle and depend almost exclusively upon glycolysis for ATP production, which suggests that any of the glycolytic enzymes of *P. falciparum* represent potential drug targets. The amino acid sequence of malarial lactate dehydrogenase (pLDH) revealed many residues at the active site that are unique to pLDH compared to any other known LDH, including human LDH-M and LDH-H.[16] Therefore, pLDH appears to be an attractive target for development of antimalarial drugs.

Previous studies of the antimalarial activities of derivatives of gossypol pointed to the cofactor binding site of lactate dehydrogenase as the target of these compounds.[12] It is not commonly assumed that a cofactor binding site may be a good target for drug development, although there have been recent studies of structure-based drug design targeted at the NAD^+ binding site of glyceraldehyde-3-phosphate dehydrogenase from Trypanosomes.[15] In a preliminary study of the inhibition of pLDH by gossypol-related compounds, derivatives of 8-deoxyhemigossylic acid (**3**) were shown to be promising as selective inhibitors of pLDH.[17] Here we describe the synthesis and inhibitory properties

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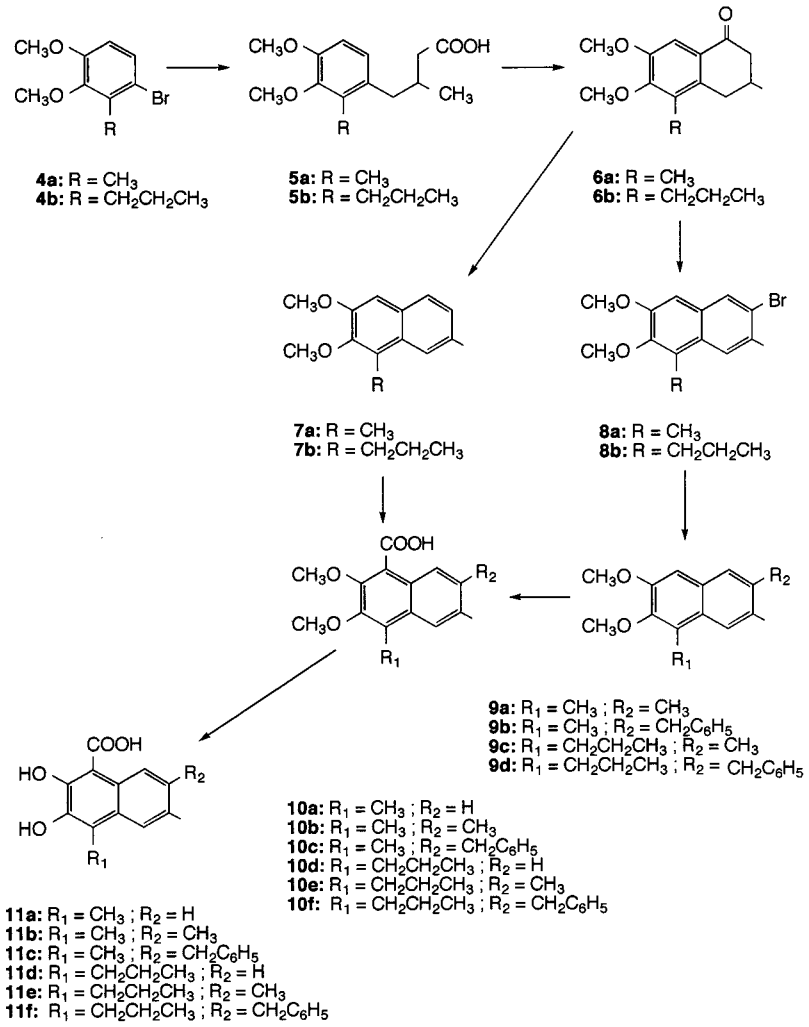
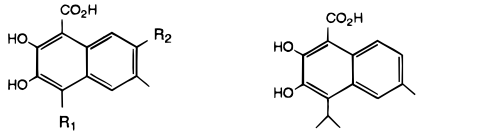
S0022-2623(98)00334-3 CCC: $15.00
Published on Web 08/27/1998

Scheme 1



of a series of compounds structurally related to 8-deoxyhemigossylic acid.



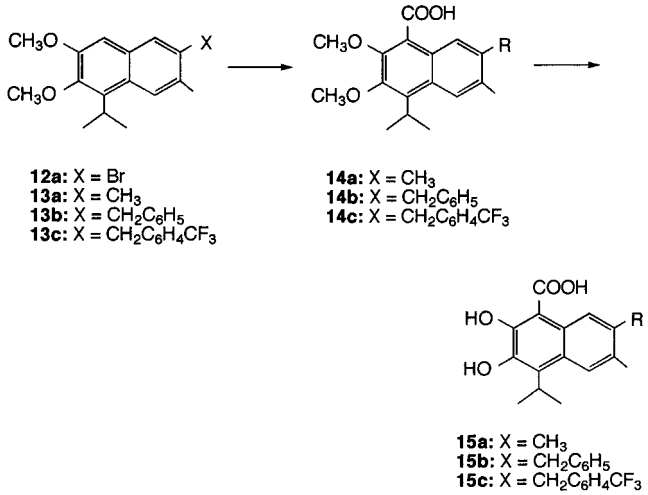
3a 3 8-deoxyhemigossylic acid

Chemical Syntheses

The synthetic schemes were designed to provide a number of compounds that deviate from 8-deoxyhemigossylic acid as follows: (1) the 4-isopropyl group in **3** is replaced by methyl or *n*-propyl (**3a**, R_1) to test the role of alkyl groups at the 4-position and (2) hydrogen, methyl, or benzyl groups are placed at position 7 (**3a**, R_2), which is the coupling position in the formation of disesquiterpenes related to gossypol. The syntheses of compounds in the **3a** series are outlined in Schemes 1 and 2 (compounds **11a**−**f** and **15a**−**c**).

Synthetic Scheme 1 features the incorporation of the carbon atoms for the second ring of the naphthalene system in one step by the reaction of the Grignard reagent formed from 1-bromo-3,4-dimethoxy-2-methylbenzene (**4a**) and 1-bromo-3,4-dimethoxy-2-*n*-propylbenzene (**4b**) with ethyl 3-methyl-4-oxobut-2-enoate. These precursors are readily prepared from commercially available starting materials using a procedure described by Royer et al.[14] Because the esters were difficult to reduce they were saponified, and the acids were hydrogenolyzed and reduced. The resulting carboxylic acids **5a**,**b** were cyclized with polyphosphoric ester to give tetralones **6a**,**b**. The ketone functional groups of **6a**,**b** were reduced with sodium borohydride

Scheme 2



and the intermediate alcohols dehydrated on acidic workup to form alkenes which were dehydrogenated with DDQ to form the naphthalenes **7a**,**b**. Addition of bromine to the alkenes formed dibromides which were immediately dehydrohalogenated with DMF to form vinyl bromides which were dehydrogenated with DDQ to afford the bromonaphthalenes **8a**,**b**. Compounds **8a**,**b** were reacted with *n*-butyllithium and benzaldehyde to form the benzylic alcohols which were hydrogenolyzed with Pd/C in ethanol to form **9b**,**d**. Formation of **9a**,**c** was accomplished by reaction of **8a**,**b** with *n*-butyllithium and methyl iodide. Carbonyl groups were introduced to compounds **7a**,**b**, and **9a**−**d** by formylation with titanium tetrachloride and dichloromethyl methyl ether. Oxidation of the aldehyde groups with sodium hypochlorite formed the carboxylic acids **10a**−**f**. The methyl groups were removed from the phenolic ethers with boron tribromide to form compounds **11a**−**f**.

The syntheses of **15a**−**c** are outlined in Scheme 2. The precursor compound **12** was prepared from 2-isopropylphenol using procedures described by Royer et al.[14] The transformations to form compounds **13a**−**c**, **14a**−**c**, and **15a**−**c** were accomplished using the same procedures used for the corresponding steps in Scheme 1.

Inhibition of pLDH, LDH-H, and LDH-M by Derivatives of 8-Deoxyhemigossylic Acid

The inhibition of pLDH, LDH-H, and LDH-M by 8-deoxyhemigossylic acid (**3**), the reference compound for these studies, is summarized in Table 1. Compound **3** is nonselective in its inhibiton of pLDH and LDH-M, with dissociation constants in the low-micromolar range. By comparison, the dissociation constant for inhibition of LDH-H is 30-fold higher than for LDH-M. The dimer of **3**, namely, 1,1′-dideoxygossylic acid, was shown previously[17] to be nonselective in its inhibition of these three LDH with dissociation constants about 1 μM. Thus the dimerization of **3** only affects inhibition of LDH-H, raising the question of whether only half of the gossypol backbone is involved in the inhibition of pLDH and LDH-M by disesquiterpenes related to gossypol. The inhibition of these three LDHs by **3** is competitive with the binding of NADH, which is also consistent with previous observations.[17]

The first series of derivatives of **3** addressed the question of whether addition of groups at the 7-position has any effect on binding, in view of the fact that this is the coupling position in the gossypol series and in view of the similar inhibitory properties of **3** and its dimer against pLDH and LDH-M. Compounds **15a**,**b** (Table 1) show the effects of methyl or benzyl groups in the 7-position on inhibition of LDH. There is little change in the dissociation constants by introduction of a methyl group. Introduction of a benzyl group results in markedly stronger inhibition of LDH-M and LDH-H but not of pLDH. However, introduction of a substituted benzyl group reverses this pattern; the *p*-(trifluoromethyl)benzyl derivative **15c** (Table 1) is more active against pLDH ($K_i = 0.2\ \mu$M) and less active against both human LDH-M and LDH-H. Compound **15c** thus shows selectivity for pLDH.

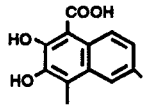
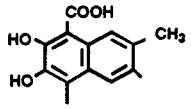
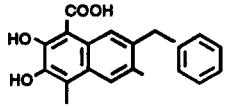
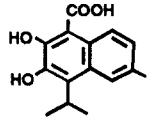
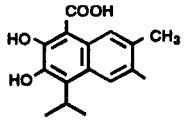
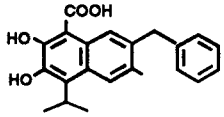
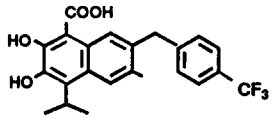
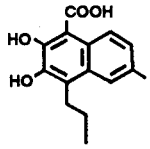
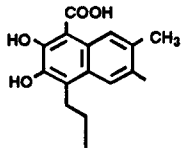
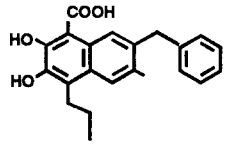
The second series of compounds related to **3** addressed the question of whether modification of the alkyl group in the 4-position affects inhibition of LDH. Two groups of compounds were compared, one with methyl at the 4-position and one with *n*-propyl at the 4-position, both groups of compounds containing hydrogen, methyl, or benzyl at the 7-position. The results are shown in Table 1. For the 4-methyl derivatives **11a**−**c**, (Table 1), there is selectivity for pLDH and LDH-M compared to LDH-H, and affinity increases with the introduction of groups at the 7-position for all three LDHs, especially for LDH-M. For the 4-*n*-propyl derivatives **11d**−**f** (Table 1), the presence of the *n*-propyl group at the 4-position has a marked effect both on affinity and on selectivity. Compound **11e** exhibits 190-fold selectivity for LDH-M compared to LDH-H. The most potent inhibitor of the compounds tested in this study is **11f** which inhibits LDH-M, $K_D = 50$ nM. Thus both the 4-position and the 7-position represent sites for modification of the dihydroxynaphthoic acid backbone in the development of LDH inhibitors.

Discussion

The mechanism of NADH reduction of pyruvate to lactate catalyzed by LDH is thought to involve direct hydride transfer of the pro-R (H_A) C_4-hydrogen from the reduced nicotinamide ring of NADH to the ketone of pyruvate to form L-lactate.[18] In this mechanism, the ordered formation of the LDH−NADH binary complex and LDH−NADH−pyruvate ternary complex is followed by rate-determining closure of a substrate specificity loop to encase the reactants in a desolvated environment before hydride transfer occurs. Hydride transfer is facilitated by the D168/H195 proton donor dyad which transfers a proton to the ketone functional group of pyruvate in concert with hydride transfer, a process that is also facilitated by polarization of the ketone group by R109. R171 acts to anchor the substrate through interaction with the carboxylate group of pyruvate.[19] These catalytic residues are conserved in all LDHs.

The unique structural features of pLDH that separate it from human LDH as well as from all other known LDHs involve residues at both the cofactor and substrate sites.[16] Residues 98−109 of human LDH-H and LDH-M (^{98}AGVRQQEGESRL) define the substrate specificity loop.[19] This sequence is quite highly conserved in all other known LDHs, except pLDH where not only the sequence differs but there is also a 5-amino acid insert from residues 104 to 108 (^{98}AGFTKAPGKSDKEWNRD) which forms an extended specificity loop. The recent crystal structure of the pLDH−NADH−oxamate ternary complex described a closed loop structure with a cleft at the active site which is not present in other LDHs.[20] Nevertheless, despite these unique structural features of pLDH, this LDH exhibits high specificity for pyruvate.[17] Additional residues that are conserved in other LDHs but differ in pLDH include S163, I250, and T246. In most LDHs, the nitrogen of the carboxamide group of NADH is H-bonded to the oxygen of S163, whereas in pLDH residue 163 is leucine. In addition, I250 normally provides a hydrophobic side chain that stacks against the nicotinamide ring; in pLDH residue 250 is proline. T246, which is adjacent to both the nicotinamide group and the substrate in the

Table 1. Inhibition of Human Lactate Dehydrogenase (LDH-H and LDH-M) and Malarial Parasite *P. falciparum* Lactate Dehydrogenase (pLDH) by Dihydroxynaphthoic Acids

	Inhibitor	Ki (μM) LDH-M	Ki (μM) LDH-H	Ki (μM) pLDH
11a		34	>250	22
11b		4	190	13
11c		0.5	39	8
3		3	91	2
15a		2	78	1
15b		0.2	7	0.7
15c		13	81	0.2[a]
11d		1	49	6
11e		0.1	19	0.1
11f		0.05	1	0.3

[a] From ref 17. All data at pH 7.5, 25 °C.

ternary complex of most LDHs, is replaced by proline in pLDH. All of these unique features of pLDH suggest that the active site of pLDH may be a selective drug target.

The results of the present study demonstrate that substituted dihydroxynaphthoic acids structurally related to 8-deoxyhemigossylic acid (**3**) can be developed that are selective inhibitors of pLDH compared to

human LDH and that these inhibitors appear to be competitive with cofactor binding. Compound **15c** with a *p*-(trifluoromethyl)benzyl moiety at the 7-position of **3** shows 75- and 400-fold selectivity for pLDH over LDH-M and LDH-H, respectively. Surprisingly, however, some of these substituted dihydroxynaphthoic acids are highly selective for LDH-M over LDH-H, despite the high sequence homologies of these two human LDHs. Generally, these inhibitors show higher affinities for LDH-M compared to LDH-H, with selectivities ranging from 5- to 190-fold.

The compounds in Table 1 are competitive inhibitors of cofactor binding. Inhibition with respect to substrate binding is generally mixed but sometimes appears competitive. These kinetic observations raise the question of whether inhibition of LDH by some of the substituted dihydroxynaphthoic acids involves complexation at both the cofactor and substrate binding sites and whether this can be exploited to develop selective dehydrogenase inhibitors. However, the kinetic pattern of competitive inhibition against both cofactor and substrate is problematic. In a recent study of the inhibition of human aldehyde dehydrogenase (ALDH1 and ALDH2) by the isoflavone prunetin, Sheikh and Weiner demonstrated that this inhibitor appears to be competitive against both NAD^+ and aldehyde substrate.[21] However, this was shown to result from allosteric inhibition of ALDH. A detailed study of the binding of substituted dihydroxynaphthoic acids to LDH will be required to determine whether these inhibitors bind to both the cofactor and substrate sites of LDH or to a portion of these two sites.

Experimental Section

Chemical Synthesis. Reagent quality solvents were used without further purification. THF and ether were distilled from calcium hydride. 1-Bromo-3,4-dimethoxy-2-methylbenzene (**4a**) and 1-bromo-3,4-dimethoxy-2-*n*-propylbenzene (**4b**) were synthesized from *o*-cresol and 2-*n*-propylphenol, respectively, according to published procedures.[14] Melting points were determined with a VWR Scientific Electrothermal capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC250 NMR spectrometer in $CDCl_3$, unless otherwise stated. Chemical shifts are in ppm (δ) relative to TMS.

General Procedure for Removing the Methyl Groups from Phenolic Methyl Ethers with Boron Tribromide. The procedure for removal of methyl groups was identical to that described by us previously.[14]

General Procedure for Formylation and Oxidation To Form Carboxylic Acids. A reaction mixture with 1 mmol of the compound to be formylated and 2.6 mmol of dichloromethyl methyl ether in 20 mL of dichloromethane under nitrogen was cooled in an ice bath. Titanium tetrachloride (1.5 mmol) was added slowly with stirring. The mixture was allowed to come to ambient temperature and was stirred for 2 h. The mixture was added with stirring to 100 g of ice containing 10 mL of 6 M HCl, and the organic layer was washed with water and brine and dried over magnesium sulfate. Filtration and evaporation of the solvent gave a crude aldehyde which was dissolved in 15 mL of acetonitrile and cooled in an ice bath. Sodium dihydrogen phosphate (0.2 mmol) and 30% hydrogen peroxide (1.1 mmol) were added followed by 1.4 mmol of sodium chlorite dissolved in 5 mL of water. The reaction mixture was stirred at ambient temperature for 2 h and then poured onto 100 g of ice containing 10 mL of 6 M HCl and extracted with ether. The ether layer was washed with water and brine and dried over magnesium sulfate. Filtration and evaporation of the solvent gave a crude carboxylic acid.

4-(3,4-Dimethoxy-2-methylphenyl)-3-methylbutanoic Acid (5a). Compound **4a** (12.0 g, 51.9 mmol) was added to magnesium turnings (1.26 g, 52.1 mmol) in 100 mL of dry THF in a 250-mL ground glass Erlenmeyer flask equipped with a reflux condenser and magnetic stirrer. The mixture was refluxed with stirring for 1 h, cooled to 0 °C, and added dropwise to 3-methyl-4-oxobut-2-enoate (7.39 g, 52.1 mmol) in 25 mL of dry THF at 0 °C. The mixture was stirred at ambient temperature for 1 h and poured onto 100 g of ice containing 25 mL of 6 M HCl. The organic layer was extracted into ether, washed with water and brine, dried over magnesium sulfate, and filtered. The ether was removed by rotary evaporation to give 12.3 g (41.8 mmol, 80% yield) of ester as a crude oil which was dissolved in 100 mL of ethanol with 4.70 g of KOH. Water, 20 mL, was added, and the mixture was refluxed for 3 h. The mixture was poured onto 100 g of ice containing 25 mL of 6 M HCl and stirred. The white solid was filtered, washed with water, dried, and recrystallized from ethyl acetate to give 10.0 g (37.5 mmol, 90% yield) of white crystalline acid: mp 174−176 °C. ^{1}H NMR: 6.69 (d, 1H), 6.74 (d, 1H), 6.32 (s, 1H), 5.32 (s, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 2.34 (s, 3H), 2.00 (s, 3H), 1.60 (s, 1H). Anal. ($C_{14}H_{18}O_5$) C,H.

The acid (10.0 g, 37.6 mmol) in 100 mL of acetic acid was hydrogenated on a Parr hydrogenator with 0.4 g of 10% palladium on carbon and 60 psi of hydrogen pressure at 60 °C for 20 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was evaporated in a fume hood, and the residual oil was distilled bulb-to-bulb (170 °C, 1 Torr) to give 7.5 g (29.7 mmol, 79% yield) of **5a** as an amber oil which crystallized on standing to form colorless crystals: mp 84−86 °C. ^{1}H NMR: 6.78 (d, 1H), 6.67 (d, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 2.62−2.33 (m, 5H), 2.22 (s, 3H), 0.992 (d, 3H). Anal. ($C_{14}H_{20}O_4$) C,H.

4-(3,4-Dimethoxy-2-*n*-propylphenyl)-3-methylbutanoic Acid (5b). Compound **4b** (10.0 g, 38.4 mmol) and ethyl bromide (0.6 g, 5.5 mmol) were added to magnesium turnings (1.2 g, 49.4 mmol) in 100 mL of dry THF in a 250-mL ground glass Erlenmyer flask and refluxed for 1 h with stirring. After cooling, the mixture was added dropwise to 3-methyl-4-oxobut-2-enoate (6.90 g, 48.5 mmol) in 25 mL of dry THF at 0 °C. The mixture was stirred at ambient temperature for 1 h and poured onto 100 g of ice containing 25 mL of 6 M HCl. The organic layer was extracted into ether, washed with water and brine, and dried over magnesium sulfate. The ether was evaporated to give 10.6 g (32.9 mmol, 85% yield) of ester as a crude oil which was dissolved in 100 mL of ethanol with 4.50 g of KOH. Water, 20 mL, was added, and the mixture was refluxed for 3 h, then poured onto 100 g of ice and 25 mL of 6 M HCl, and stirred. The semisolid was extracted with ether, washed with water and brine, and dried over magnesium sulfate. Filtration and evaporation of the ether gave 9.20 g (31.2 mmol, 95% yield) of acid as a viscous oil. The acid (9.20 g, 31.2 mmol) in 100 mL of acetic acid was hydrogenated on a Parr hydrogenator with 0.4 g of 10% palladium on carbon and 60 psi of hydrogen pressure at 60 °C for 24 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was evaporated in a fume hood, and the residual oil was distilled bulb-to-bulb (180 °C, 1 Torr) to give 7.46 g (26.6 mmol, 85% yield) of **5b** as a pale-yellow oil. ^{1}H NMR: 6.77 (d, 1H), 6.68 (d, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.74−2.08 (m, 7H), 1.50 (sex, 2H), 0.96 (t, 3H), 0.94 (d, 3H). Anal. ($C_{16}H_{24}O_4$) C,H.

3,4-Dihydro-6,7-dimethoxy-3,5-dimethyl-1(2*H*)-naphthalenone (6a). Compound **5a** (7.0 g, 27.7 mmol) was added to 35 g of polyphosphoric ester in 100 mL of methylene chloride and refluxed for 1 h. The reaction mixture was poured onto ice and stirred to hydrolyze the polyphosphoric ester, and the resulting solid was filtered and recrystallized from methanol to give 5.4 g (23.0 mmol, 83% yield) of **6a** as white plates: mp 106−108 °C. ^{1}H NMR: 7.48 (s, 1H), 3.89 (s, 3H), 3.84 (s, 3H), 2.95−2.28 (m, 5H), 2.22 (s, 3H), 1.18 (d, 3H). Anal. ($C_{14}H_{18}O_3$) C,H.

3,4-Dihydro-6,7-dimethoxy-3-methyl-5-*n*-propyl-1(2*H*)-naphthalenone (6b). Compound **5b** (7.0 g, 24.9 mmol) was

added to 35 g of polyphosphoric ester in 100 mL of methylene chloride and refluxed for 1 h. The reaction mixture was poured onto ice, stirred, and extracted with ether. The ether was evaporated, and the residual oil was chromatographed on silica gel using ethyl acetate/hexane to give 5.63 g (21.4 mmol, 86% yield) of **6b** as white crystals: mp 69−70 °C. ^{1}H NMR: 7.49 (s, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.00−2.23 (m, 7H), 1.50 (mult, 2H), 1.15 (d, 3H), 1.01 (t, 3H). Anal. ($C_{16}H_{22}O_3$) C,H.

2,3-Dimethoxy-1,7-dimethylnaphthalene (7a). Compound **6a** (4.5 g, 19.2 mmol) was dissolved in 20 mL of 2-propanol, sodium borohydride (1.08 g, 28.8 mmol) was added, and the mixture was stirred at reflux for 1 h. The cooled solution was acidified by dropwise addition of 6 M HCl with stirring, then refluxed for 1 h, poured onto ice, and extracted with ether. The ether layer was washed with water and brine and dried over magnesium sulfate. The ether was removed by rotary evaporation, and the residue was distilled bulb-to-bulb (170 °C, 1 Torr) to give 3.31 g (15.2 mmol, 79% yield) of alkene which crystallized on standing. ^{1}H NMR: 6.50 (s, 1H), 6.31 (m, H), 5.81 (mult, 1H), 3.83 (s, 3H), 3.77 (s, 3H), 2.84−2.23 (mult, 3H), 2.19 (s, 3H), 1.10 (d, 3H). Anal. ($C_{14}H_{18}O_2$) C,H.

A mixture of alkene (1.95 g, 8.93 mmol) in 25 mL of benzene and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.03 g, 8.93 mmol) was stirred for 2 h, and the resulting mixture was filtered through a short column of alumina. The solvent was evaporated, and the residual oil was purified by silica gel column chromatography with dichloromethane. Removal of solvent provided 1.66 g of **7a** (7.67 mmol, 86% yield) as colorless crystals: mp 68−69 °C. ^{1}H NMR: 7.63 (s, 1H), 7.59 (d, 1H), 7.21 (d, 1H), 6.99 (s, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 2.56 (s, 3H), 2.49 (s, 3H). Anal. ($C_{14}H_{16}O_2$) C,H.

2,3-Dimethoxy-7-methyl-1-*n*-propylnaphthalene (7b). Compound **6b** (5.00 g, 19.0 mmol) was dissolved in 20 mL of 2-propanol, sodium borohydride (1.08 g, 28.8 mmol) was added, and the reaction mixture was stirred with refluxing for 1 h. After cooling, the mixture was acidified by dropwise addition of 6 M HCl with stirring. The acidified mixture was refluxed for 1 h, poured onto ice, and extracted with ether. The ether extract was washed with water and brine and was dried over magnesium sulfate. The ether was removed by rotary evaporation, and the residue was distilled bulb-to-bulb (190 °C, 1 Torr) to give 4.46 g (18.0 mmol, 95% yield) of an alkene. ^{1}H NMR: 6.51 (s, 1H), 6.31 (dd,1H), 5.80 (dd, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.86−2.35 (m, 5H), 1.50 (m, 2H), 1.10 (d, 3H), 0.99 (t, 3H).

The alkene (4.46 g, 18.0 mmol) was dissolved in 25 mL of benzene, was treated while stirring with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (4.10 g, 18.0 mmol), and was stirred for an additional 2 h. The reaction mixture was filtered through a short column of alumina and washed with benzene. The solvent was evaporated, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 4.24 g (17.4 mmol, 96% yield) of **7b** as buff-colored crystals: mp 48−49 °C. ^{1}H NMR: 7.64 (s, 1H), 7.60 (d, 1H), 7.21 (d, 1H), 7.01 (s, 1H), 3.95 (s, 3H), 3.87 (s, 3H), 3.01 (t, 2H), 2.50 (s, 3H), 1.67 (m, 2H), 1.06 (t, 3H). Anal. ($C_{16}H_{20}O_2$) C,H.

6-Bromo-2,3-dimethoxy-1,7-dimethylnaphthalene (8a). Compound **6a** (4.5 g, 19.2 mmol) was converted into the alkene as described in the synthesis of **7a**. The alkene (1.48 g, 6.78 mmol) was dissolved in 100 mL of dry dichloromethane, and bromine (1.08 g, 6.78 mmol) in 5 mL of dichloromethane was added dropwise with stirring over a 15-min period. The solvent was evaporated, and the residue was taken up in 25 mL of DMF and warmed to 60−70 °C for 1 h. The reaction mixture was poured onto ice and stirred. The solid was filtered, dried, and recrystallized from petroleum ether to give 1.51 g (5.08 mmol, 75% yield) of colorless crystals: mp 75−76 °C. ^{1}H NMR: 6.65 (s, 1H), 6.43 (s, 1H), 3.82 (s, 3H), 3.77 (s, 3H), 3.03−2.62 (m, 3H), 2.18 (s, 3H), 1.11 (d, 3H). Anal. ($C_{14}H_{17}BrO_2$) C,H.

The vinylic bromide (1.51 g, 5.08 mmol) was dissolved in 25 mL of benzene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (1.14 g, 5.02 mmol) was added with stirring, and stirring was continued for 2 h. The reaction mixture was filtered through a short column of alumina and eluted with benzene. The solvent was evaporated, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 1.24 g (4.21 mmol, 83% yield) of **8a** as buff-colored crystals: mp 96−97 °C. ^{1}H NMR: 7.89 (s, 1H), 7.67 (s, 1H), 6.89 (s, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 2.54 (s, 6H). Anal. ($C_{14}H_{15}BrO_2$) C,H.

6-Bromo-2,3-dimethoxy-7-methyl-1-*n*-propylnaphthalene (8b). Compound **6b** (3.5 g, 13.4 mmol) was reduced with sodium borohydride as described in the synthesis of **7b**. The alkene (2.5 g, 10.1 mmol) was dissolved in 100 mL of dry dichloromethane, and bromine (1.62 g, 10.1 mmol) in 5 mL of dichloromethane was added dropwise with stirring over a period of 15 min. The solvent was evaporated, and the residue was taken up in 15 mL of DMF and warmed to 60−70 °C for 1 h. The reaction mixture was poured onto ice and stirred, after which the solid was filtered, dried, and recrystallized from petroleum ether to give 2.46 g (7.56 mmol, 75% yield) of white crystals. The vinylic bromide (1.23 g, 3.78 mmol) was dissolved in 25 mL of benzene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (857 mg, 3.78 mmol) was added slowly with stirring, and stirring was continued for 2 h. The reaction mixture was filtered through a short column of alumina and eluted with benzene. The solvent was evaporated, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 1.04 g (3.21 mmol, 85% yield) of **8b** as colorless crystals: mp 64−66 °C. ^{1}H NMR: 7.90 (s, 1H), 7.69 (s, 1H), 6.91 (s, 1H), 3.94 (s, 3H), 3.87 (s, 3H), 2.99 (t, 2H), 2.54 (s, 3H), 1.66 (m, 2H), 1.05 (t, 3H). Anal. ($C_{16}H_{19}BrO_2$) C,H.

2,3-Dimethoxy-1,6,7-trimethylnaphthalene (9a). Compound **8a** (646 mg, 2.19 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (2.50 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then methyl iodide (0.38 g, 3.0 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h. The reaction mixture was poured onto 10 g of ice containing 2 mL of HCl, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether was evaporated to give an oil which was purified by silica gel column chromatography using dichloromethane to give 426 mg (1.84 mmol, 84% yield) of **9a** as buff crystals: mp 59−60 °C. ^{1}H NMR: 7.59 (s, 1H), 7.44 (s, 1H), 6.93 (s, 1H), 3.93 (s, 3H), 3.83 (s, 3H), 2.56 (s, 3H), 2.41 (s, 3H), 2.38 (s, 3H). Anal. ($C_{15}H_{18}O_2$) C,H.

6-Benzyl-2,3-dimethoxy-1,7-dimethylnaphthalene (9b). Compound **8a** (570 mg, 1.93 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (2.2 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then benzaldehyde (265 mg, 2.5 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h. The reaction mixture was poured onto ice containing HCl and stirred for 1 h. The mixture was filtered to give a buff solid: mp 174−175 °C. ^{1}H NMR: 7.85 (s, 1H), 7.60 (s, 1H), 7.34−7.25 (m, 5H), 7.03 (s, 1H), 6.09 (s, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 2.55 (s, 3H), 2.34 (s, 3H), 2.28 (br s, 1H, disappears upon shaking with D_2O).

The alcohol was dissolved in ethanol and hydrogenated on a Parr hydrogenator with 10% palladium on carbon and 60 psi of hydrogen pressure at room temperature for 2 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was removed, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 522 mg (1.70 mmol, 88% yield) of **9b** as white crystals: mp 85−86 °C. ^{1}H NMR: 7.63 (s, 1H), 7.41 (s, 1H), 7.30−7.14 (m, 5H), 6.95 (s, 1H), 4.11 (s, 2H), 3.93 (s, 3H), 3.84 (s, 3H), 2.56 (s, 3H), 2.36 (s, 3H). Anal. ($C_{21}H_{22}O_2$) C,H.

2,3-Dimethoxy-6,7-dimethyl-1-*n*-propylnaphthalene (9c). Compound **8b** (700 mg, 2.16 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (3.0 mmol)

was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then methyl iodide (0.38 g, 3 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h. The reaction mixture was acidified, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether was evaporated to give an oil which was purified by silica gel column chromatography to give 446 mg (1.73 mmol, 80% yield) of **9c** as a colorless oil. ^{1}H NMR: 7.89 (s, 1H), 7.44 (s, 1H), 6.93 (s, 1H), 3.92 (s, 3H), 3.86 (s, 3H), 3.01 (t, 2H), 2.40 (s, 3H), 2.37 (s, 3H), 1.67 (m, 2H), 1.05 (t, 3H). Anal. ($C_{17}H_{22}O_2$) C,H.

6-Benzyl-2,3-dimethoxy-7-methyl-1-*n*-propylnaphthalene (9d). Compound **8b** (800 mg, 2.47 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (3.0 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then benzaldehyde (424 mg, 4 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h. The reaction mixture was acidified, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether was evaporated to give a semisolid which was dissolved in ethanol and hydrogenated on a Parr hydrogenator with 10% palladium on carbon and 60 psi of hydrogen pressure at room temperature for 2 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was evaporated, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 727 mg (2.17 mmol, 88% yield) of **9d** as a white crystalline solid: mp 83−85 °C. ^{1}H NMR: 7.64 (s, 1H), 7.39 (s, 1H), 7.14−7.29 (m, 5H), 6.95 (s, 1H), 4.09 (s, 2H), 3.91 (s, 3H), 3.86 (s, 3H), 3.01 (t, 2H), 2.36 (s, 3H), 1.68 (m, 2H), 1.05 (t, 3H). Anal. ($C_{23}H_{26}O_2$) C,H.

2,3-Dimethoxy-4,6-dimethyl-1-naphthoic Acid (10a). Compound **7a** (1.38 g, 6.38 mmol) was formylated by the general procedure for formylation to give 1.51 g (6.18 mmol, 97% yield) of solid aldehyde. ^{1}H NMR: 10.74 (s, 1H), 9.15 (d, 1H), 7.72 (s, 1H), 7.40 (d, 1H), 4.04 (s, 3H), 3.87 (s, 3H), 2.62 (s, 3H), 2.50 (s, 3H).

Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography using dichloromethane to give 1.18 g (4.53 mmol, 72% yield) of **10a** as buff-colored crystals. ^{1}H NMR: 8.07 (d, 1H), 7.70 (s, 1H), 7.35 (d, 1H), 4.06 (s, 3H), 3.89 (s, 3H), 2.60 (s, 3H), 2.51 (s, 3H). Anal. ($C_{15}H_{16}O_4$) C,H.

2,3-Dimethoxy-4,6,7-trimethyl-1-naphthoic Acid (10b). Compound **9a** (317 mg, 1.38 mmol) was formylated by the general procedure for formylation to give 318 mg (1.23 mmol, 89% yield) of solid aldehyde. ^{1}H NMR: 10.75 (s, 1H), 9.05 (s, 1H), 7.67 (s, 1H), 4.05 (s, 3H), 3.88 (s, 3H), 2.63 (s, 3H), 2.48 (s, 3H), 2.44 (s, 3H).

Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography using dichloromethane to give 306 mg (1.12 mmol, 81% yield) of **10b** as white crystals: mp 138−140 °C. ^{1}H NMR: 8.08 (s, 1H), 7.68 (s, 1H), 4.07 (s, 3H), 3.89 (s, 3H), 2.61 (s, 3H), 2.44 (s, 6H). Anal. ($C_{16}H_{18}O_4$) C,H.

7-Benzyl-2,3-dimethoxy-4,6-dimethyl-1-naphthoic Acid (10c). Compound **9c** (250 mg, 0.816 mmol) was formylated by the general procedure for formylation to give 250 mg (0.748 mmol, 92% yield) of solid aldehyde. ^{1}H NMR: 10.74 (s, 1H), 9.16 (s, 1H), 7.66 (s, 1H), 7.18−7.10 (m, 5H), 4.13 (s, 2H), 4.02 (s, 3H), 3.84 (s, 3H), 2.59 (s, 3H), 2.31 (s, 3H).

Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography using dichloromethane to give 207 mg (0.591 mmol, 79% yield) of **10c** as white crystals: mp 179−180 °C. ^{1}H NMR (DMSO-d_6): 8.14 (s, 1H), 7.70 (s, 1H), 7.13−7.25 (m, 5H), 4.16 (s, 2H), 4.07 (s, 3H), 3.89 (s, 3H), 2.61 (s, 3H), 2.35 (s, 3H). Anal. ($C_{22}H_{22}O_4$) C,H.

2,3-Dimethoxy-6-methyl-4-*n*-propyl-1-naphthoic Acid (10d). Compound **7b** (1.38 g, 5.63 mmol) was formylated by the general procedure for formylation to give 1.51 g (5.52 mmol, 98% yield) of solid aldehyde. Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography using dichloromethane to give 1.18 g (4.08 mmol, 72% yield) of **10d** as off-white crystals: mp 123−125 °C. ^{1}H NMR: 8.10 (d, 1H), 7.71 (s, 1H), 7.33 (d, 1H), 4.06 (s, 3H), 3.94 (s, 3H), 3.06 (t, 2H), 2.52 (s, 3H), 1.70 (m, 2H), 1.09 (t, 3H). Anal. ($C_{17}H_{20}O_4$) C,H.

2,3-Dimethoxy-6,7-dimethyl-4-*n*-propyl-1-naphthoic Acid (10e). Compound **9c** (317 mg, 1.22 mmol) was formylated by the general procedure for formylation to give 318 mg (1.11 mmol, 91% yield) of solid aldehyde. Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography to give 306 mg (1.01 mmol, 80% yield) of **10e** as buff-colored crystals: mp 144−145 °C. ^{1}H NMR (DMSO-d_6): 7.92 (s, 1H), 7.68 (s, 1H), 4.05 (s, 3H), 3.90 (s, 3H), 3.05 (t, 2H), 2.43 (s, 6H), 1.68 (m, 2H), 1.08 (t, 3H). Anal. ($C_{18}H_{22}O_4$) C,H.

7-Benzyl-2,3-dimethoxy-6-methyl-4-*n*-propyl-1-naphthoic Acid (10f). Compound **9d** (250 mg, 0.75 mmol) was formylated by the general procedure for formylation to give 250 mg (0.69 mmol, 92% yield) of solid aldehyde. Oxidation by the general procedure for oxidation gave 207 mg (0.55 mmol, 73% yield) of **10f** as off-white crystals: mp 143−145 °C. ^{1}H NMR: 8.03 (s, 1H), 7.70 (s, 1H), 7.27−7.10 (m, 5H), 4.15 (s, 2H), 4.05 (s, 3H), 3.93 (s, 3H), 3.05 (t, 2H), 2.34 (s, 3H), 1.69 (m, 2H), 1.08 (t, 3H). Anal. ($C_{24}H_{26}O_4$) C,H.

2,3-Dihydroxy-4,6-dimethyl-1-naphthoic Acid (11a). Compound **10a** (500 mg, 1.92 mmol) was demethylated by the general procedure for demethylation.[14] The solvent was evaporated, and the product was recrystallized from ether/petroleum ether to give 350 mg (1.51 mmol, 79% yield) of **11a** as an off-white solid: mp 196−197 °C. ^{1}H NMR (DMSO-d_6): 8.58 (d, 1H), 7.60 (s, 1H), 7.20 (d, 1H), 2.44 (s, 3H), 2.40 (s, 3H). Anal. ($C_{13}H_{12}O_4$) C,H.

2,3-Dihydroxy-4,6,7-trimethyl-1-naphthoic Acid (11b). Compound **10b** (400 mg, 1.46 mmol) was demethylated,[14] and the product was purified as above to give 270 mg (1.10 mmol, 75% yield) of **11b** as a tan solid: mp 162−163 °C. ^{1}H NMR (DMSO-d_6): 8.43 (s, 1H), 7.60 (s, 1H), 2.44 (s, 3H), 2.34 (s, 6H). Anal. ($C_{14}H_{14}O_4$) C,H.

7-Benzyl-2,3-dihydroxy-4,6-dimethyl-1-naphthoic Acid (11c). Compound **10c** (634 mg, 1.81 mmol) was demethylated,[14] and the product was purified as above to give 420 mg (1.30 mmol, 72% yield) of **11c** as a white solid: mp 179−180 °C. ^{1}H NMR (DMSO-d_6): 8.49 (s, 1H), 7.62 (s, 1H), 7.10−7.28 (m, 5H), 4.06 (s, 2H), 2.52 (s, 3H), 2.45 (s, 3H). Anal. ($C_{20}H_{18}O_4$) C,H.

2,3-Dihydroxy-6-methyl-4-*n*-propyl-1-naphthoic Acid (11d). Compound **10d** (600 mg, 2.08 mmol) was demethylated,[14] and the product was purified as above to give 390 mg (1.50 mmol, 72% yield) of **11d** as a white solid: mp 160−161 °C. ^{1}H NMR (DMSO-d_6): 8.57 (d, 1H), 7.64 (s, 1H), 7.20 (d, 1H), 2.97 (t, 2H), 2.42 (s, 3H), 1.55 (m, 2H), 0.98 (t, 3H). Anal. ($C_{15}H_{16}O_4$) C,H.

2,3-Dihydroxy-6,7-dimethyl-4-*n*-propyl-1-naphthoic Acid (11e). Compound **10e** (550 mg, 1.82 mmol) was demethylated,[14] and the product was purified as above to give 380 mg (1.27 mmol, 70% yield) of **11e** as a white solid: mp 171−172 °C. ^{1}H NMR: 8.43 (s, 1H), 7.60 (s, 1H), 2.96 (t, 2H), 2.33 (s, 3H), 2.31 (s, 3H), 1.53 (m, 2H), 0.96 (t, 3H). Anal. ($C_{16}H_{18}O_4$) C,H.

7-Benzyl-2,3-dihydroxy-6-methyl-4-*n*-propyl-1-naphthoic Acid (11f). Compound **10f** (634 mg, 1.67 mmol) was demethylated,[14] and the product was purified as above to give 417 mg (1.19 mmol, 71% yield) of **11f** as a white solid: mp 183−184 °C. ^{1}H NMR (DMSO-d_6): 8.63 (s, 1H), 7.61 (s, 1H), 7.27−7.12 (m, 5H), 4.01 (s, 2H), 2.97 (t, 2H), 2.28 (s, 3H), 1.57 (m, 2H), 0.99 (t, 3H). Anal. ($C_{22}H_{22}O_4$) C,H.

2,3-Dimethoxy-6,7-dimethyl-1-(1-methylethyl)naphthalene (13a). Compound **12a**[14] (1.40 g, 4.33 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (6.0 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then methyl iodide (1.14 g, 8.0 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h and then

acidified, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether was evaporated to give an oil which was purified by silica gel column chromatography using dichloromethane to give 892 mg (3.46 mmol, 80% yield) of **13a** as white crystals: mp 75−77 °C. ^{1}H NMR: 7.85 (s, 1H), 7.44 (s, 1H), 6.93 (s, 1H), 3.89 (m, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 2.39 (s, 3H), 2.35 (s, 3H), 1.51 (d, 6H). Anal. ($C_{17}H_{22}O_2$) C,H.

6-Benzyl-2,3-dimethoxy-7-methyl-1-(1-methylethyl)-naphthalene (13b). Compound **12** (1.14 g, 3.53 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (4.5 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then benzaldehyde (1.06 g, 5.0 mmol) was added. The mixture was stirred at ambient temperature under nitrogen fo 1 h. The reaction mixture was acidified, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether layer was evaporated to give a white solid: mp 163−165 °C. ^{1}H NMR: 7.84 (s, 1H), 7.25−7.34 (m, 6H), 7.03 (s, 1H), 6.08 (s, 1H), 3.93 (s, 3H), 3.91 (m, 1H), 3.88 (s, 3H), 2.34 (s, 3H), 2.25 (br s, 1H, disappeared upon shaking with D_2O), 1.50 (d, 6H).

The alcohol was dissolved in ethanol and hydrogenated on a Parr hydrogenator with 10% palladium on carbon and 60 psi of hydrogen pressure at room temperature for 2 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was removed to afford a pale-yellow solid that was recrystallized from ethyl acetate to give 1.04 g (3.11 mmol, 88% yield) of **13b** as a white crystalline solid: mp 159−161 °C. ^{1}H NMR: 7.88 (s, 1H), 7.40 (s, 1H), 7.32−7.15 (m, 5H), 6.96 (s, 1H), 4.09 (s, 2H), 3.93 (s, 3H), 3.87 (s, 3H), 2.37 (s, 3H), 1.50 (d, 6H). Anal. ($C_{23}H_{26}O_2$) C,H.

6-[*p*-(Trifluoromethyl)benzyl]-2,3-dimethoxy-7-methyl-1-(1-methylethyl)naphthalene (13c). Compound **12** (1.00 g, 3.09 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (4 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then *p*-(trifluoromethyl)benzaldehyde (0.87 g, 5.0 mmol) was added. The mixture was stirred at ambient temperature under nitrogen for 1 h. The reaction mixture was acidified, and the organic layer was separated, washed with water and brine, and dried over magnesium sulfate. After filtration, the ether layer was evaporated to give a semisolid which was dissolved in ethanol and hydrogenated on a Parr hydrogenator with 10% palladium on carbon and 60 psi of hydrogen pressure at room temperature for 2 h. The reaction mixture was vacuum-filtered through Celite, and the Celite was washed with ether. The solvent was removed, and the residual oil was purified by silica gel column chromatography using dichloromethane to give 1.09 g (2.71 mmol, 88% yield) of **13c** as a pale-yellow oil. ^{1}H NMR: 7.90 (s, 1H), 7.55 (d, 2H), 7.26 (d, 2H), 6.98 (s, 1H). 4.15 (s, 2H), 3.93 (s, 3H), 3.88 (s, 3H), 3.48 (m, 1H), 2.04 (s, 3H), 1.50 (d, 6H). Anal. ($C_{24}H_{25}O_2F_3$) C,H.

2,3-Dimethoxy-6,7-dimethyl-4-(1-methylethyl)-1-naphthoic Acid (14a). Compound **13a** (550 mg, 2.12 mmol) was formylated by the general procedure for formylation to give 506 mg (1.77 mmol, 83% yield) of solid aldehyde. Oxidation by the general procedure for oxidation gave a solid that was purified by silica gel column chromatography using dichloromethane to give 450 mg (1.49 mmol, 84% yield) of **14a** as white crystals: mp 191−193 °C. ^{1}H NMR: 7.96 (s, 1H), 7.93 (s, 1H), 4.05 (s, 3H), 3.95 (m, 1H), 3.93 (s, 3H), 2.44 (br s, 6H), 1.52 (d, 6H). Anal. ($C_{18}H_{22}O_4$) C,H.

7-Benzyl-2,3-dimethoxy-6-methyl-4-(1-methylethyl)-naphthoic Acid (14b). Compound **13b** (500 mg, 1.49 mmol) was formylated by the general procedure for formylation to give 446 mg (1.23 mmol, 82% yield) of solid aldehyde. ^{1}H NMR: 10.75 (s, 1H), 9.16 (s, 1H), 7.93 (s, 1H), 7.32−7.13 (m, 5H), 4.16 (s, 2H), 4.02 (s, 3H), 3.95 (m, 1H), 3.92 (s, 3H), 2.35 (s, 3H), 1.52 (d, 6H).

Oxidation by the general procedure described for oxidation gave a solid which was purified by silica gel column chromatography using dichloromethane to give 391 mg (1.03 mmol, 84% yield) of **14b** as a buff-colored crystalline solid: mp 187−188 °C. ^{1}H NMR: 8.00 (s, 1H), 7.94 (s, 1H), 7.24−7.11 (m, 5H), 4.15 (s, 2H), 4.04 (s, 3H), 3.95 (m, 1H), 3.93 (s, 3H), 2.34 (s, 3H), 1.52 (d, 6H). Anal. ($C_{24}H_{26}O_4$) C,H.

2,3-Dimethoxy-6-methyl-4-(1-methylethyl)-7-[*p*-(trifluoromethyl)benzyl]-1-naphthoic Acid (14c). Compound **13c** (600 mg, 1.49 mmol) was formylated by the general procedure for formylation to give 526 mg (1.22 mmol, 82% yield) of solid aldehyde. ^{1}H NMR: 10.74 (s, 1H), 9.18 (s, 1H), 7.97 (s, 1H), 7.49 (d, 2H), 7.22 (d, 2H), 4.19 (s, 2H), 4.02 (s, 3H), 3.90 (m, 1H), 3.91 (s, 3H), 2.30 (s, 3H), 1.50 (d, 6H).

Oxidation by the general procedure for oxidation gave a solid which was purified by silica gel column chromatography to give 458 mg (1.02 mmol, 84% yield) of **14c** as white crystals: mp 177−178 °C. ^{1}H NMR (DMSO-d_6): 8.05 (s, 1H), 7.96 (s, 1H), 7.48 (d, 2H), 7.23 (d, 2H), 4.19 (s, 2H), 4.04 (s, 3H), 3.93 (s, 3H), 2.30 (s, 3H), 1.52 (d, 6H). Anal. ($C_{25}H_{25}O_4F_3$) C,H.

2,3-Dihydroxy-6,7-dimethyl-4-(1-methylethyl)-1-naphthoic Acid (15a). Compound **14a** (450 mg, 1.49 mmol) was demethylated,[14] and the product was recrystallized from ether/petroleum ether to give 302 mg (1.01 mmol, 68% yield) of **15a** as a white solid: mp 192−193 °C. ^{1}H NMR (DMSO-d_6): 8.47 (s, 1H), 7.82 (s, 1H), 3.86 (m, 1H), 2.33 (s, 3H), 2.32 (s, 3H), 1.41 (d, 6H). Anal. ($C_{16}H_{18}O_4$) C,H.

7-Benzyl-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic Acid (15b). Compound **14b** (652 mg, 1.72 mmol) was demethylated,[14] and the product was recrystallized from ether/petroleum ether to give 416 mg (1.19 mmol, 69% yield) of **15b** as a buff-colored solid: mp 151−152 °C. ^{1}H NMR: 8.61 (s, 1H), 7.89 (s, 1H), 7.25−7−15 (m, 5H), 6.25 (s, 1H), 4.15 (s, 2H), 3.90 (m, 1H), 2.36 (s, 3H), 1.51 (d, 6H). Anal. ($C_{22}H_{22}O_4$) C,H.

7-[*p*-(Trifluoromethyl)benzyl]-2,3-dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic Acid (15c). Compound **14c** (300 mg, 0.672 mmol) was demethylated,[14] and the product was recrystallized from ether/petroleum ether to give 196 mg (0.47 mmol, 70% yield) of **15c** as a white solid: mp 216−218 °C. ^{1}H NMR (DMSO-d_6): 8.60 (s, 1H), 7.86 (s, 1H), 7.82 (d, 2H), 7.20 (d, 2H), 4.12 (s, 2H), 3.87 (m, 1H), 2.27 (s, 3H), 1.41 (d, 6H). Anal. ($C_{23}H_{21}F_3O_4$) C,H.

Purification of Recombinant Parasite Lactate Dehydrogenase. Recombinant pLDH was produced in *Escherichia coli*, similar to the procedure of Bzik et al.,[16] and was purified by Cibacron blue affinity chromatography and chromatofocusing as descibed previously.[17] Human LDH-H_4 and LDH-M_4 were from Sigma.

Enzyme Assays and Kinetics. LDH activity in the direction of NADH reduction of pyruvate to L-lactate was determined spectrophotometrically in pH 7.5 Tris buffer, 100 mM, containing 10 mM pyruvate and 1 mM NADH, 25 °C, 340 nm, $\epsilon = 6.2$ mM^{-1} cm^{-1}. Michaelis constants were determined by nonlinear regression analysis of initial rate data using the Enzfitter program (Elsevier-Biosoft). K_i values were determined from double-reciprocal plots by linear regression analysis, as described previously.[17]

Acknowledgment. This work was supported by Grant GM52576 from the Minority Biomedical Sciences Support (MBRS) program, NIH, and by SBIR Contract DAMD17-94C4037 from the Department of Defense.

References

(1) Wu, D. An Overview of the Clinical Pharmacology and Therapeutic Potential of Gossypol as a Male Contraceptive Agent and in Gynaecological Disease. *Drugs*, **1989**, *38*, 333−341.
(2) Benz, C. C.; Keniry, M. A.; Ford, J. M.; Townsend, A. J.; Cox, F. W.; Palayoor, S.; Matlin, S. A.; Hait, W. N.; Cowan, K. H. Biochemical Correlates of the Antitumor and Antimitochondrial Properties of Gossypol Enantiomers. *Mol. Pharmacol.* **1990**, *37*, 840−847.
(3) Gonzalez-Garza, M. T.; Said-Fernandez, S. Entamoeba histolytica: Potent in Vitro Antiamoebic Effect of Gossypol. *Exp. Parasitol.* **1988**, *66*, 253−255.

(4) Montamat, E. E.; Burgos, C.; Gerez de Burgos, N. M.; Rovai, L. E.; Blanco, A.; Segura, E. L. Inhibitory Activity of Gossypol on Enzymes and Growth of *Trypanosoma cruzi*. *Science* **1982**, *218*, 288−289.
(5) Eid, J. E.; Veno, H.; Wang, C. C.; Donelson, J. E. Gossypol-induced Death of African Trypanosomes. *Exp. Parasitol.* **1989**, *66*, 140−142.
(6) Rikihisa, Y.; Lin, Y. C. Taenia taeniaeformis: Inhibition of Metacestode Development in the Rat by Gossypol. *Exp. Parasitol.* **1989**, *61*, 127−133.
(7) Wichmann, K.; Vaheri, A.; Luukkainen, T. Inhibiting Herpes Simplex Virus Type 2 Infection in Human Epithelial Cells by Gossypol, a Potent Spermicidal and Contraceptive Agent. *Am. J. Obstet. Gynecol.* **1982**, *142*, 593−594.
(8) Lin, T.-S.; Schinazi, R.; Griffith, B. P.; August, E. M.; Eriksson, B. F. H.; Zheng, D.-K.; Huang, L.; Prusoff, W. H. Selective Inhibition of Human Immunodeficiency Virus Type 1 Replication by the (−) but Not the (+) Enantiomer of Gossypol. *Antimicrob. Agents Chemother.* **1989**, *33*, 2149−2151.
(9) Deck, L. M.; Vander Jagt, D. L.; Royer, R. E. Gossypol and Derivatives: A New Class of Aldose Reductase Inhibitors. *J. Med. Chem.* **1991**, *34*, 3301−3305.
(10) Heidrich, J. E.; Hunsaker, L. A.; Vander Jagt, D. L. Gossypol, an Antifertility Agent, Exhibits Antimalarial Activity in vitro. *IRCS Med. Sci.* **1983**, *11*, 304.
(11) Vander Jagt, D. L.; Baack, B. R.; Campos, N. M.; Hunsaker, L. A.; Royer, R. E. A Derivative of Gossypol Retains Antimalarial Activity. *IRCS Med. Sci.* **1984**, *12*, 845−846.
(12) Royer, R. E.; Deck, L. M.; Campos, N. M.; Hunsaker, L. A.; Vander Jagt, D. L. Biologically Active Derivatives of Gossypol: Synthesis and Antimalarial Activities of Peri-Acylated Gossylic Nitriles. *J. Med. Chem.* **1986**, *29*, 1799−1801.
(13) Royer, R. E.; Mills, R. G.; Young, S. A.; Vander Jagt, D. L. Comparison of the Antiviral Activities of 3′-Azido-3′-Deoxythymidine (AZT) and Gossylic Iminolactone (GIL) Against Clinical Isolates of HIV-1. *Pharmacol. Res.* **1995**, *31*, 49−52.
(14) Royer, R. E.; Deck, L. M.; Vander Jagt, T. J.; Martinez, F. J.; Mills, R. G.; Young, S. A.; Vander Jagt, D. L. Synthesis and Anti-HIV Activity of 1,1′-Dideoxygossypol and Related Compounds. *J. Med. Chem.* **1995**, *38*, 2427−2432.
(15) Verlinde, C. L. M. J.; Callens, M.; Van Calenbergh, S.; Van Aerschot, A.; Herdewijn, P.; Hannaert, V.; Michels, P. A. M.; Opperdoes, F. R.; Hol, W. G. J. Selective Inhibition of Trypanosomal Glyceraldehyde-3-phosphate Dehydrogenase by Protein Structure-Based Design: Toward New Drugs for the Treatment of Sleeping Sickness. *J. Med. Chem.* **1994**, *37*, 3605−3613.
(16) Bzik, D. J.; Fox, B. A.; Gonyer, K. Expression of Plasmodium falciparum Lactate Dehydrogenase in *Escherichia coli*. *Mol. Biochem. Parasitol.* **1993**, *59*, 155−166.
(17) Gomez, M. S.; Piper, R. C.; Hunsaker, L. A.; Royer, R. E.; Deck, L. M.; Makler, M. T.; Vander Jagt, D. L. Substrate and Cofactor Specificity and Selective Inhibition of Lactate Dehydrogenase from the Malarial Parasite P. falciparum. *Mol. Biochem. Parasitol.* **1997**, *90*, 235−246.
(18) Westheimer, F. H. In *Pyridine Nucleotide Coenzymes. Chemical, Biochemical, and Medical Aspects Part A; Coenzyes and Cofactors II*; Dolphin, D., Poulson, R., Avramovic, O., Eds.; J. Wiley and Sons: New York, 1987; pp 253−322.
(19) Dunn, C. R.; Wilks, H. M.; Halsall, D. J.; Atkinson, T.; Clarke, A. R.; Muirhead, H.; Holbrook, J. J. Design and Synthesis of New Enzymes Based on the Lactate Dehydrogenase Framework. *Philos. Trans. R. Soc. London B* **1991**, *332*, 177−184.
(20) Dunn, C. R.; Banfield, M. J.; Barker, J. J.; Higham, C. W.; Moreton, K. M.; Turgut-Balik, D.; Brady, R. L.; Holbrook, J. J. The Structure of Lactate Dehydrogenase from Plasmodium falciparum Reveals a New Target for Anti-malarial Design. *Nature Struct. Biol.* **1996**, *3*, 912−915.
(21) Sheikh, S.; Weiner, H. Allosteric Inhibition of Human Liver Aldehyde Dehydrogenase by the Isoflavone Prunetin. *Biochem. Pharmacol.* **1997**, *53*, 471−478.

JM980334N