Synthesis and Anti-HIV Activity of 1,1′-Dideoxygossypol and Related Compounds

Robert E. Royer,*,† Lorraine M. Deck,‡ Timothy J. Vander Jagt,† Francella J. Martinez,† Ray G. Mills,§ Stephen A. Young,§ and David L. Vander Jagt†

Departments of Biochemistry and Microbiology, University of New Mexico School of Medicine, and Department of Chemistry, University of New Mexico, Albuquerque, New Mexico 87131

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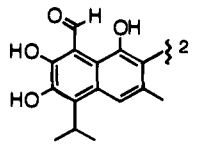
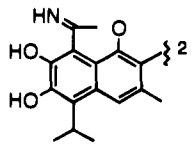
1,1′-Dideoxygossypol (DDG), 1,1′-dideoxygossylic acid (DDGA), 8-deoxyhemigossypol (DHG), and 8-deoxyhemigossylic acid (DHGA) were synthesized and tested for their ability to inhibit the replication of HIV *in vitro*. The EC_{50} for DDGA was <1 μM, and its threshold cytotoxicity was approximately 20 μM. DDG was less effective than DDGA against HIV and showed considerable toxicity at 5 μM. DHGA was ineffective against HIV and had very low cytotoxicity. DHG showed some anti-HIV activity, but the threshold cytotoxicity was 5 μM. The dissociation constants for the binding of the four compounds to human serum albumin were determined by fluorescence quenching titrations, and all four were found to have much lower affinities for albumin than the parent compound gossypol.

Gossypol (**1**) is a yellow pigment from the cotton plant which has several potentially useful biological activities.[1–4] Since it is active against enveloped viruses,[4–6] including HIV,[7,8] it represents a potential starting point for the development of antiviral drugs. Gossypol is marginally toxic, and this toxicity has been associated with the aldehyde groups, stimulating interest in derivatives or analogs in which the aldehyde groups are changed.[9,10] Lin and co-workers tested a group of compounds related to gossypol for anti-HIV activity and cytotoxicity.[11] Their data support the conclusion that the aldehyde groups in such compounds are not necessary for anti-HIV activity but contribute strongly to cytotoxicity. The availability of stable derivatives of gossypol in which only the aldehyde groups are modified, however, has been limited because the phenolic hydroxyl groups in the 1 and 1′ (*peri*) positions complicate the chemistry of functional groups at the 8 and 8′ positions.[12] Our previous work suggested that free *peri* hydroxyl groups might not be important for at least some of the biological activities of gossypol, including its anti-HIV activity.[13–15] Those studies involved compounds in which the *peri* hydroxyls were acylated or incorporated into the functional groups replacing the aldehyde (e.g., gossylic iminolactone, GIL **2**). Those



1 gossypol



2 GIL

compounds were useful prototypes for initial structure–activity relationship considerations but did not provide the desired range of functionality. Therefore, we designed a synthesis for compounds based on the gossypol structure but without the *peri* hydroxyl groups. The syntheses of 1,1′-dideoxygossypol (DDG, **3**), 1,1′-dideoxygossylic acid (DDGA, **4**), 8-deoxyhemigossypol (DHG, **5**), and 8-deoxyhemigossylic acid (DHGA, **6**) are described here. We also report *in vitro* anti-HIV activity, estimates or cytotoxicity, and dissociation constants for binding to serum albumin for these compounds.

Chemical Syntheses. The syntheses of compounds **3** and **4** are outlined in Scheme 1. This synthesis 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 from 1-bromo-2-isopropyl-3,4-dimethoxybenzene (**7**) with ethyl 3-methy-4-oxobut-2-enoate to form **8**. These precursors are readily prepared from commercially available starting materials.[16–18] The double bond of ester **8** is difficult to reduce in good yield, so the ester was saponified to form carboxylic acid **9** which was catalytically hydrogenolyzed and reduced in one step. Carboxylic acid **10** was cyclized with polyphosphoric ester according to a published procedure to form **11**.[19] Published approaches to the synthesis of gossypol have proceeded through tetralone **11** or similar structures by less efficient routes.[19,20] The ketone function of **11** was reduced with sodium borohydride and the intermediate alcohol dehydrated on acidic workup to form **12**. Compound **12** was treated with 1 equiv of bromine in dichloromethane, and a dibromide formed immediately. This intermediate dibromide was not characterized. Upon warming in DMF, it readily dehydrobrominated to form vinyl bromide **13**. Compound **13** was aromatized by stirring with DDQ in benzene to form bromonaphthalene **14**. The most challenging step in the synthesis is the coupling of two molecules of **14** to form **15**. This was accomplished in 55% yield by treating **14** with *n*-butyllithium and coupling with $CoCl_2$ catalyst.[21,22] The carbonyl groups were introduced by formylation of **15** with titanium tetrachloride and dichloromethyl methyl ether.[23,24] The aldehyde groups were converted to carboxylic acids by mild oxidation with sodium hypochlorite.[25] The methyl groups were removed from the phenolic ethers of **16** and **17** with boron tribromide[26,27] to form **3** and **4**. The syntheses of **5** and **6** are outlined

† Department of Biochemistry, University of New Mexico School of Medicine.
‡ University of New Mexico.
§ Department of Microbiology, University of New Mexico School of Medicine.
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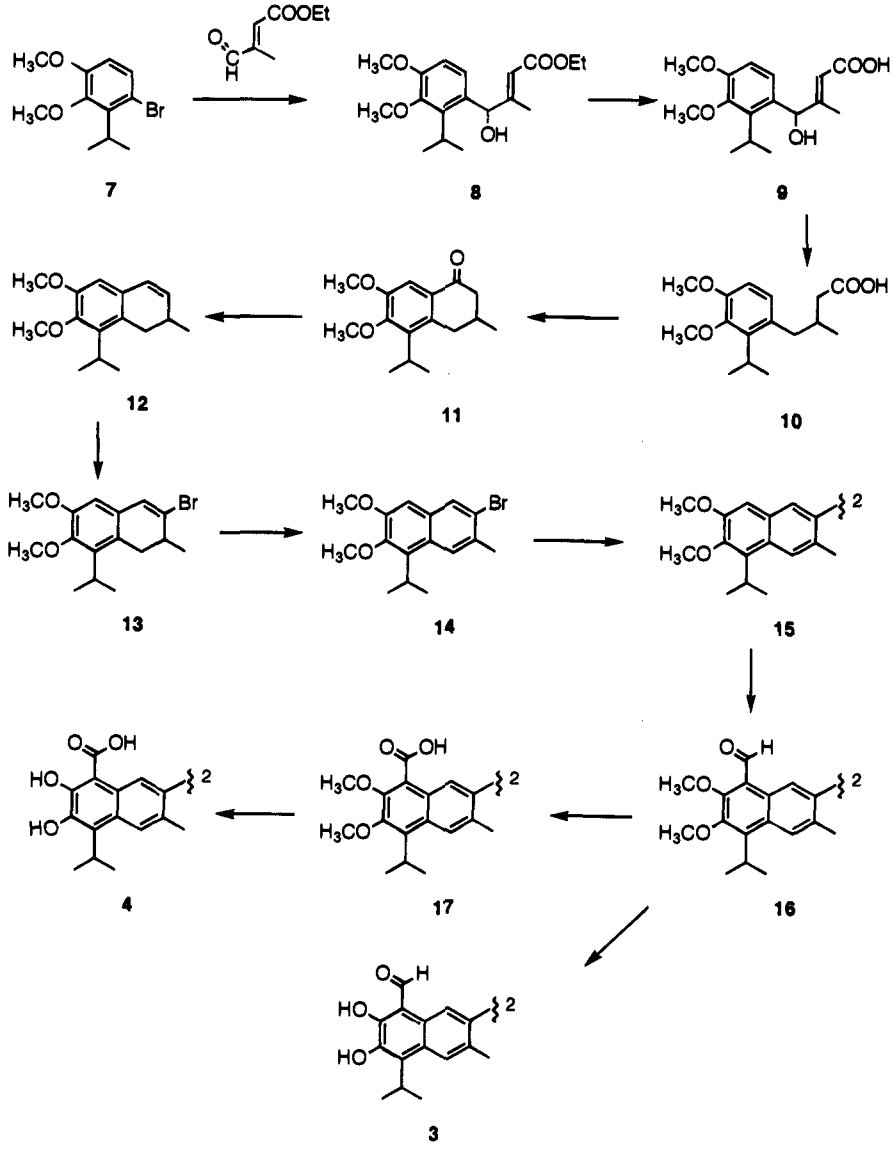
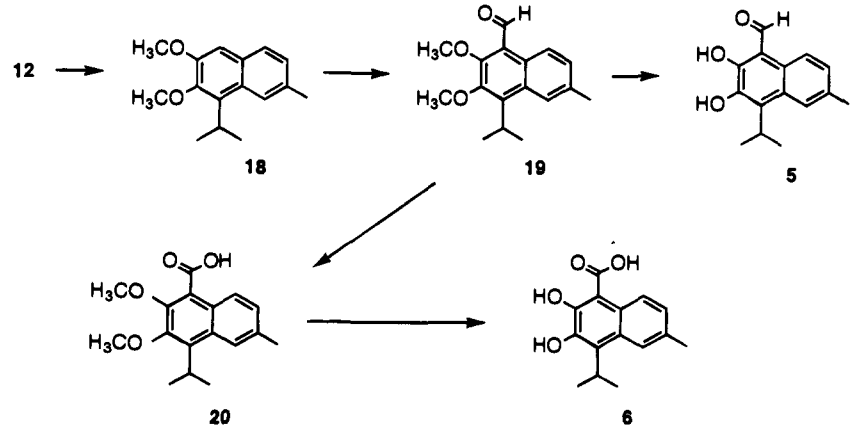
Scheme 1



Scheme 2



in Scheme 2. The transformations were accomplished by the same procedures used for the corresponding steps in Scheme 1.

Anti-HIV Activity. Inhibition of replication of H9/HIV-III$_B$ in HT46c cells by DDG, DDGA, DHG, and DHGA is shown in Figure 1. In a similar previous study, the EC_{50} for GIL was 0.27 μM compared to 0.6 μM for gossypol and GIL was less cytotoxic than gossypol.[14] Therefore, GIL was used in the present study as the basis for comparison. Concentrations of drug which did not cause detectable changes in cell morphology or loss of monolayer adherence to the culture plate were considered to be below the threshold toxic concentration. The EC_{50}s values and the highest concentrations which showed no cytotoxicity in the test are listed in Table 1. The EC_{50} for GIL and DDGA were

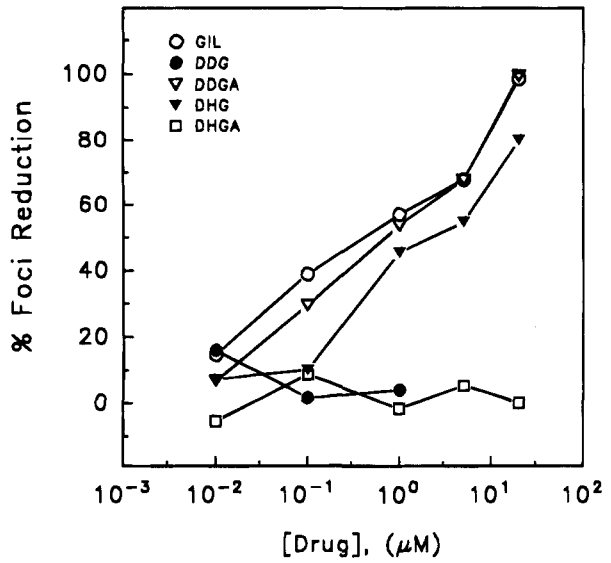


Figure 1. Anti-HIV activity of gossylic iminolactone, 1,1′-dideoxygossypol, 1,1′-dideoxygossylic acid, 8-deoxyhemigossypol, and 8-deoxyhemigossylic acid.

Table 1. EC_{50} Values and Cytotoxic Concentrations for Gossypol-Related Compounds in Anti-HIV Tests in HeLaT^{4+}-Cells

no.	compound	EC_{50} (μM)	TC[a] (μM)
2	gossylic iminolactone	0.4	>20
3	1,1′-dideoxygossypol	*b*	>1
4	1,1′-dideoxygossylic acid	0.7	>5[c]
5	8-deoxyhemigossypol	20	>1
6	8-deoxyhemigossylic acid		>20

[a] The highest concentration used in the test at which no visible changes in cell morphology or monolayer adhesion were detected. [b] Anti-HIV activity was masked by cytotoxicity. [c] Some signs of toxicity occurred at 20 μM.

Table 2. Dissociation Constants (K_ds) for Binding of Gossypol-Related Compounds to Human Serum Albumin

no.	compound	K_d (μM)
1	gossypol	0.09
2	gossylic iminolactone	3.0
3	1,1′-dideoxygossypol	1.2
4	1,1′-dideoxygossylic acid	1.5
5	8-deoxyhemigossypol	0.66
6	8-deoxyhemigossylic acid	5.28

<1 μM. The same reduction in foci (68%) over controls was obtained with GIL and DDGA at 5 μM. No toxicity was noted for GIL or DDGA at 5 μM. Some signs of monolayer destruction were observed with DDGA at 20 μM. DDG, on the other hand, was ineffective at 1 μM and destroyed >80% of the cell monolayer at 5 μM. DHGA had no observable effect on viral replication or cell morphology at 20 μM. DHG, on the other hand, did show some anti-HIV activity but was less active and more cytotoxic than GIL or DDGA.

Binding to HSA. Table 2 lists the dissociation constants (K_ds) for binding of compounds **1**–**6** to human serum albumin. The K_d for gossypol was determined by multiple methods are previously reported.[28] The K_ds for compounds **2**–**6** were determined by fluorescence quenching titrations.

Discussion

The gossypol derivative GIL was previously found to be more effective than gossypol in inhibiting the replication of HIV *in vitro*, showing that free 1,1′-hydroxyl groups are not necessary for good anti-HIV activity in compounds related to gossypol. The gossypol analog DDGA is approximately as effective as GIL, indicating that the 1,1′-oxygen atoms may be absent entirely. DDG is more toxic than gossypol, suggesting that the toxicity of the aldehyde groups in gossypol itself is moderated by lactol formation with the 1,1′-hydroxyl groups. The ineffectiveness of DHGA as an anti-HIV agent suggests that a simple monomeric structure is not sufficient for good anti-viral activity in compounds related to gossypol, with the possible exception of those with cytotoxic aldehyde groups. Binding of gossypol-like compounds to albumin could reduce their free serum concentration and therefore their effectiveness as a drug. This effect has been noted for gossypol in *in vitro* experiments.[29] However, fluorescence quenching studies of binding of compounds **3**–**6** show that removal of 1,1′-hydroxyl groups moderates the very tight binding of the molecule to albumin. The K_d for DDG is about 13 times that of gossypol.

The range of functional groups which can replace the aldehydes in gossypol analogs is extended in the absence of the *peri* hydroxyl groups. Testing of DDGA has shown that compounds of this type may retain good anti-HIV activity, be less cytotoxic than gossypol, and have a lower affinity for albumin than gossypol. Therefore, DDGA is a lead compounds in the synthesis of a new type of potential anti-HIV agent.

Experimental Section

Chemical Synthesis. Reagent quality solvents were used without further purification. THF and ether were dried over calcium hydride and filtered through scintered glass. The petroleum ether had bp 90–100 °C. Crystallizations were completed in a freezer at −15 °C. 1-Bromo-2-isopropyl-3,4-dimethoxybenzene (**7**) was synthesized by brominating 1,2-dimethoxy-3-isopropylbenzene[16] according to a published procedure[17] at −5–0 °C. Melting points were determined with a VWR Scientific Electrothermal capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Beckman model IR-33 spectrometer in KBr pellets. NMR spectra were recorded on a Bruker AC250 NMR spectrometer in $CDCl_3$. Chemical shifts are in δ units relative to TMS. Signals for isopropyl methyl groups are reported as doublets but at 25 °C some show slight additional splitting due to rotational isomers.

General Procedure for Removing the Methyl Groups from Phenolic Methyl Ethers with Boron Tribromide. A reaction mixture with about 1 mmol of the compound to be demethylated in 15 mL of dichloromethane under nitrogen was cooled in a dry ice/2-propanol or similar bath. Boron tribromide was added with stirring: 1 equiv for each ether group, 1 equiv for each functional group, and an additional 0.5 equiv. The mixture was stirred under a static nitrogen atmosphere for 1 h periods at successively dry ice bath, ice bath, and ambient temperature. The mixture was cooled in an ice bath and poured with vigorous stirring onto 50 g of ice with 2 mL of 6 M HCl. The organic material was extracted with ether and the ether layer washed with water and brine and dried over magnesium sulfate.

Ethyl 4-Hydroxy-4-[3,4-dimethoxy-2-(1-methylethyl)-phenyl]-3-methylbut-2-enoate (8). Compound **7** (10.0 g, 38.6 mmol) and ethyl bromide (0.60 g, 5.5 mmol) were added to magnesium turnings (1.20 g, 49.4 mg atom) in 100 mL of dry THF in a 250 mL round bottom flask. The mixture was refluxed with stirring under a static nitrogen atmosphere for 1 h. It was then cooled to 0 °C and 3-methyl-4-oxobut-2-enoate (6.90 g, 48.5 mmol) in 25 mL of dry THF at 0 °C was added. This mixture was stirred at ambient temperature for 1 h and poured into 100 g of ice and 25 mL of 6 M HCl. The organic

layer was extracted into ether, and the ether was washed with water and brine and dried over $MgSO_4$. The ether was evaporated, and the byproducts were distilled bulb to bulb (120 °C/1 Torr) to leave 9.8 g (30.4 mmol, 79%) of **8** as a residual oil: IR 3440 (alcohol), 1722 (ester), 1655 (alkene) cm^{-1}; ^1H NMR δ 1.31 (t, J = 7.1 Hz, 3 H), 1.37 (d, J = 7.0 Hz, 6 H), 1.99 (s, 3 H), 3.40 (br s, 1 H), 3.75–3.85 (m, 1 H), 3.847 (s, 3 H), 3.853 (s, 3 H), 4.19 (q, J = 7.1 Hz, 2 H), 5.39 (s, 1 H), 6.26 (s, 1 H), 6.75 (d, J = 8.6 Hz, 1 H), 6.94 (d, J = 7.1 Hz, 1 H). Anal. ($C_{18}H_{26}O_5$) C,H.

4-Hydroxyl-4-[3,4-dimethoxy-2-(1-methylethyl)phenyl]-3-methylbut-2-enoic Acid (9). Compound **8** (9.00 g, 27.9 mmol) was dissolved in 100 mL of ethanol with 3 g of KOH, and the mixture was refluxed for 1 h. It was then poured onto 100 g of ice with 25 mL of 6 M HCl and stirred for 24 h. The white solid was filtered, washed with water, dried, and recrystallized from ether/petroleum ether to give 7.38 g (25.1 mmol, 90%) of **9** as white plates: mp 154–155 °C; IR 3411 (OH), 1694 (carbonyl), 1644 (alkene) cm^{-1}; ^1H NMR δ 1.38 (d, J = 7.0 Hz, 6 H), 2.00 (s, 3 H), 3.44 (br s, 1 H), 3.85 (s, 6 H), 5.41 (s, 1 H), 6.33 (s, 1 H), 6.75 (d, J = 8.6 Hz, 1 H), 6.92 (d, J = 7.1 Hz, 1 H). Anal. ($C_{16}H_{22}O_5$) C,H.

4-[3,4-Dimethoxy-2-(1-methylethyl)phenyl]-3-methylbutanoic Acid (10). Compound **9** (7.00 g, 23.8 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 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 5.95 g (21.2 mmol, 89%) of **10** as an amber oil which crystallized on standing to form nearly colorless microcrystals: mp 100–101 °C (lit.[18] mp not reported); IR 1710 (carbonyl) cm^{-1}; ^1H NMR δ 0.982 (d, J = 6.1 Hz, 3 H), 1.33 (d, J = 7.1 Hz, 6 H), 2.0–2.7 (m, 5 H), 3.14 (sept, J = 7.1 Hz, 1 H), 3.82 (s, 3 H), 3.85 (s, 3 H), 6.62 (d, J = 8.0 Hz, 1 H), 6.75 (d, J = 8.0 Hz, 1 H). Anal. ($C_{16}H_{24}O_4$) C,H.

3,4-Dihydro-6,7-dimethoxy-3-methyl-5-(1-methylethyl)-1(2*H*)-naphthalenone (11). Compound **10** (5.90 g, 21.0 mmol) was added to 6 g of polyphosphoric ester[30] in 15 mL of dichloromethane, and the mixture was refluxed for 1 h. The reaction mixture was poured over ice, stirred to hydrolyze the polyphosphoric ester, and extracted with ether. The ether was evaporated and the residue crystallized from a concentrated methanol solution to give 4.43 g (16.9 mmol, 80%) of 11 as white plates: mp 99–100 °C (lit.[19] mp 98–99 °C); IR 1680 (ketone) cm^{-1}; ^1H NMR δ 1.16 (d, J = 6.1 Hz, 3 H), 1.34 (d, J = 7.0 Hz, 6 H), 2.1–3.1 (m, 5 H), 3.35 (sept, J = 7.0 Hz, 1 H), 3.885 (s, 3 H), 3.895 (s, 3 H), 7.52 (s, 1 H). Anal. ($C_{16}H_{22}O_3$) C,H.

1,2-Dihydro-6,7-dimethoxy-2-methyl-8-(1-methylethyl)naphthalene (12). Compound 11 (4.00 g, 15.2 mmol) was dissolved in 20 mL of 2-propanol, $NaBH_4$ (0.57 g, 15 mmol) was added, and the reaction mixture was stirred and refluxed for 1 h. It was then cooled and acidified by the dropwise addition of 6 M HCl with stirring. The acidified mixture was refluxed for 1 h and then poured onto ice and extracted with ether. The ether layer was washed with water and brine and dried over $MgSO_4$. Petroleum ether was added, the solution was concentrated to remove most of the ether, and the product was crystallized to give 3.40 g (13.8 mmol, 91%) of **12** as colorless prisms: mp 56–57 °C; ^1H NMR δ 1.10 (d, J = 7 Hz, 3 H), 1.34 (d, J = 7 Hz, 6 H), 2.46 (m, 2 H), 2.92 (m, 1 H), 3.49 (m, 1 H), 3.80 (s, 3 H), 3.83 (s, 3H), 5.81 (m, 1 H), 6.31 (m, 1 H), 6.50 (s, 1 H). Anal. ($C_{16}H_{22}O_2$) C,H.

3-Bromo-1,2-dihydro-6,7-dimethoxy-2-methyl-8-(1-methylethyl)naphthalene (13). Compound **12** (3.00 g, 12.2 mmol) was dissolved in 20 mL of dry dichloromethane, and bromine (1.95 g, 12.2 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. The DMF solution was warmed to 50–60 °C for 1 h. The reaction mixture was poured onto ice and stirred. The solid product was filtered, dried, and recrystallized from a concentrated petroleum ether solution to give 3.60 g (11.1 mmol, 91%) of **13** as nearly colorless, microcrystalline plates: mp 71–72 °C; ^1H NMR δ 1.09 (d, J = 6.95 Hz, 3 H), 1.32 (d, J = 7.2 Hz, 6 H), 2.4–3.2 (m, 3 H), 3.49 (m, 1 H), 3.80 (s, 3 H), 3.82 (s, 3 H), 6.43 (s, 1 H), 6.64 (s, 1 H). Anal. ($C_{16}H_{21}BrO_2$) C,H.

6-Bromo-2,3-dimethoxy-7-methyl-1-(1-methylethyl)naphthalene (14). Compound **13** (3.50 g, 10.8 mmol) was dissolved in 25 mL of benzene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.45 g, 10.8 mmol) was added slowly with stirring, and stirring was continued for 2 h. The reaction mixture was filtered through a short column of silica gel and eluted with dichloromethane. The solvent was evaporated, and the product was crystallized from petroleum ether to give 3.21 g (9.93 mmol, 92%) of **14** as colorless crystals: mp 62–63 °C; ^1H NMR δ 1.51 (d, J = 7.2 Hz, 6 H), 2.56 (s, 3 H), 3.90 (s, 3 H), 3.96 (s, 3 H), 3.97 (m, 1 H), 6.94 (s, 1 H), 7.92 (s, 1 H), 7.96 (s, 1 H). Anal. ($C_{16}H_{19}BrO_2$) C,H.

6,6′,7,7′-Tetramethoxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-2,2′-binaphthalene (15). Compound **14** (3.00 g, 9.28 mmol) in 25 mL of dry ether was cooled to 0 °C under nitrogen. *n*-Butyllithium (9.50 mmol) was added as a solution in hexane. The mixture was stirred for 15 min at 0 °C, and then finely powdered $CoCl_2$ (1.23 g, 9.50 mmol) and bromobenzene (1.46 g, 9.30 mmol) were added. The mixture was stirred at ambient temperature under nitrogen for 24 h. Dichloromethane (100 mL) was added, and the organic layer was washed with water and brine. Methanol (100 mL) was added, and the mixture was concentrated to incipient crystallization and allowed to cool. The first three crops of white, microcrystalline **15** weighed 1.23 g (2.53 mmol, 53%) in total: mp 215–216 °C; ^1H NMR δ 1.57 (d, J = 7.2 Hz, 12 H), 2.22 (s, 6 H), 3.91 (s, 6 H), 3.95 (s, 6 H), 3.95 (m, 2 H), 7.01 (s, 2 H), 7.51 (s, 2 H), 8.00 (s, 2 H). Anal. ($C_{32}H_{38}O_4$) C,H.

1,1′-Dideoxygossypol Tetramethyl Ether (16, 6,6′,7,7′-Tetramethoxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)[2,2′-binaphthalene]-8,8′-dicarboxaldehyde). A reaction mixture consisting of **15** (1.10 g, 2.26 mmol) and dichloromethyl methyl ether (1.30 g, 11.3 mmol) in 50 mL of dry dichloromethane under nitrogen was cooled in an ice bath. Titanium tetrachloride (1.20 g, 6.33 mmol) was added dropwise with stirring. The mixture was allowed to come to ambient temperature and stirred for 2 h. It was then poured onto 100 g of ice with 10 mL of 6 M HCl and stirred. The organic material was extracted with methylene chloride, and the organic layer was washed with water and brine and dried over magnesium sulfate. The solvent was evaporated, and the product was crystallized from acetone to give 1.02 g (1.89 mmol, 84%) of light yellow **16** as microcrystalline prisms: mp 269–270 °C; IR 1675 (aldehyde) cm^{-1}; ^1H NMR δ 1.53 (d, J = 7.2 Hz, 12 H), 2.20 (s, 6 H), 3.90 (s, 6 H), 3.99 (s, 6 H), 4.03 (m, 2 H), 8.02 (s, 2 H), 9.01 (s, 2 H), 10.68 (s, 2 H). Anal. ($C_{34}H_{38}O_6$) C,H.

1,1′-Dideoxygossypol (3, 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)[2,2′-binaphthalene]-8,8′-dicarboxaldehyde). Compound **16** (0.500 g, 0.921 mmol) was demethylated by the standard procedure described above. Petroleum ether was added to the ether extract, the solution was concentrated, and the product was crystallized to give 0.370 g (0.760 mmol, 83%) of **3** as a light brown, microcrystalline powder: mp 269–270 °C; IR 3540, 3320 (phenols), 1630 (aldehyde) cm^{-1}; ^1H NMR δ 1.57 (d, J = 7.0 Hz, 12 H), 2.25 (s, 6 H), 3.96 (sept, J = 7.0 Hz, 2 H), 6.22 (s, 2 H), 8.05 (s, 2 H), 8.13 (s, 2 H), 10.64 (s, 2 H), 13.78 (s, 2 H). Anal. ($C_{30}H_{30}O_6$) C,H.

1,1′-Dideoxygossylic Acid Tetramethyl Ether (17, 6,6′,7,7′-Tetramethoxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)[2,2′-binaphthalene]-8,8′-dicarboxylic acid). Finely powdered **16** (0.500 g, 0.921 mmol) as a slurry in 20 mL of acetonitrile was cooled in an ice bath, and 60 mg of NaH_2PO_4 and 200 μL of 30% H_2O_2 were added. Then 0.287 g (3.17 mmol) of sodium chlorite dissolved in 2 mL of water was added. The reaction mixture was stirred at ambient temperature for 24 h and then poured onto 40 g of ice with 10 mL of 6 M HCl. The product was filtered off, take up in 10 mL of acetone, and chilled. The acetone solution was filtered, petroleum ether was added, and the solution was concentrated to incipient crystallization. The first crop of cream-colored **17**, in the form of microcrystalline plates, weighed 0.455 g (0.792 mmol, 86%)

but retained acetone. The analytical sample was prepared by dissolving the material in ether and removing ether rapidly under a high vacuum: mp 258–259 °C; IR 1695 (carbonyl) cm^{-1}; ^1H NMR δ 1.55 (d, J = 7.1 Hz, 12 H), 2.45 (s, 6 H), 3.91 (s, 6 H), 3.95 (buried sept), 4.00 (s, 6 H), 8.04 (s, 2 H), 8.11 (s, 2 H). Anal. ($C_{34}H_{38}O_8$) C,H.

1,1′-Dideoxygossylic Acid (4, 6,6′,7,7′-Tetrahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)[2,2′-binaphthalene]-8,8′-dicarboxylic acid). Compound **17** (0.400 g, 0.696 mmol) was demethylated by the standard procedure described above. The solvent was evaporated, and the product was recrystallized from ether/petroleum ether to give 260 mg (0.501 mmol, 72%) of yellow **4** as a reasonably pure microcrystalline powder. The analytical sample was prepared by recrystallization from concentrated chloroform solution at −15 °C over a period of several days: decomposes above 225 °C; IR 3545 (phenol), 1640 (carbonyl) cm^{-1}; ^1H NMR δ 2.25 (d, J = 7.2 Hz, 12 H), 2.26 (s, 6 H), 3.95 (sept, J = 7.2 Hz, 2 H), 6.28 (br s, 2 H), 7.45 (br s, 2 H), 8.02 (s, 2 H), 8.63 (s, 2 H). Anal. ($C_{30}H_{30}O_8$) C,H.

2,3-Dimethoxy-7-methyl-1-(1-methylethyl)naphthalene (18). Compound **12** (3.00 g, 12.2 mmol) was dissolved in 25 mL of benzene, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (2.80 g, 12.2 mmol) was added slowly with stirring, and stirring was continued for 3 h. The reaction mixture was filtered through a short column of alumina which was washed with dichloromethane. The solvent was evaporated from the colorless eluant, and the residual product was crystallized from petroleum ether to give 2.83 g (11.6 mmol, 95%) of **18** as colorless, microcrystalline plates: mp 62–63 °C; ^1H NMR δ 1.51 (d, J = 7.2 Hz, 6 H), 2.50 (s, 3 H), 3.88 (s, 3 H), 3.90 (sept, J = 7.2 Hz, 1 H), 3.94 (s, 3 H), 7.01 (s, 1 H), 7.20 (d, J = 8.3 Hz, 1 H), 7.60 (d, J = 8.3 Hz, 1 H), 7.88 (s, 1 H). Anal. ($C_{16}H_{20}O_2$) C,H.

8-Deoxyhemigossypol Dimethyl Ether (19, 2,3-Dimethoxy-6-methyl-4-(1-methylethyl)-1-naphthaldehyde). A reaction mixture consisting of **18** (2.5 g, 10.2 mmol) and dichloromethyl methyl ether (3 g, 2.4 mL, 26.5 mmol) in 20 mL of dichloromethane under nitrogen was cooled in an ice bath. Titanium tetrachloride (1.5 mL, 14 mmol) was added slowly with stirring. The mixture was allowed to come to ambient temperature and stirred for 2 h. Then it was stirred into 100 g of ice with 10 mL of 6 M HCl, and the organic material was extracted with ether. The ether layer was washed with water and brine and dried over magnesium sulfate. Petroleum ether was added, the solution was concentrated, and the product was crystallized to give 2.28 g (8.36 mmol, 82%) of **19** as light yellow crystals: mp 103–104 °C; IR 1685 (aldehyde) cm^{-1}; ^1H NMR δ 1.52 (d, J = 7.2 Hz, 6 H), 2.50 (s, 3 H), 3.91 (s, 3 H), 3.99 (sept, J = 7.2 Hz, 1 H), 4.02 (s, 3 H), 7.38 (d, J = 8.7 Hz, 1 H), 7.93 (br s, 1 H), 9.16 (d, J = 8.7 Hz, 1 H), 10.75 (s, 1 H). Anal. ($C_{17}H_{20}O_3$) C,H.

8-Deoxyhemigossypol (5, 2,3-Dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthaldehyde). Compound **19** (1 g, 3.7 mmol) was demethylated by the standard procedure described above. The solvent was evaporated, and the product was recrystallized from methanol to give **5** (0.65 g, 72%) as fine, gold-colored needles: mp 170–171 °C; IR 3520 (phenol), 1640 (aldehyde) cm^{-1}; ^1H NMR δ 1.47 (d, J = 7.2 Hz, 6 H), 2.47 (s, 3 H), 3.85 (sept, J = 7.2 Hz, 1 H), 6.13 (s, 1 H), 7.27 (d, J = 8.6 Hz, 1 H), 7.86 (br s, 1 H), 8.15 (d, J = 8.6 Hz, 1 H), 10.65 (s, 1 H), 13.64 (s, 1 H). Anal. ($C_{15}H_{16}O_3$) C,H.

8-Deoxyhemigossylic Acid Dimethyl Ether (20, 2,3-Dimethoxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid). Compound **19** (1.0 g, 3.67 mmol) in 25 mL of acetonitrile was cooled in an ice bath, and 120 mg of NaH_2PO_4 and 400 μL of 30% H_2O_2 were added. Then sodium chlorite (0.500 g, 5.53 mmol) dissolved in 5 mL of water was added. The reaction mixture was stirred at ambient temperature for 2 h and then poured onto 100 g of ice with 10 mL of 6 M HCl and extracted with ether. The ether layer was washed with water and brine and dried over magnesium sulfate. The solvent was allowed to evaporate, and the residual solid was crystallized from petroleum ether to give 0.889 g (3.08 mmol, 84%) of light yellow **20** as a microcrystalline powder: mp 159–160 °C; IR 3210 (−OH), 1730 (carbonyl) cm^{-1}; ^1H NMR δ 1.53 (d, J = 7.2 Hz, 6 H), 2.53 (s, 3 H), 3.94 (s, 3 H), 3.94 (s, 3 H), 3.95 (buried sept), 4.05 (s, 3 H), 7.37 (d, J = 8.7 Hz, 1 H), 7.95 (s, 1 H), 8.19 (d, J = 8.7 Hz, 1 H), 11.0 (br s, 1 H). Anal. ($C_{17}H_{20}O_4$) C,H.

8-Deoxyhemigossylic Acid (6, 2,3-Dihydroxy-6-methyl-4-(1-methylethyl)-1-naphthoic acid). Compound **20** (0.500 g, 1.73 mmol) was demethylated by the standard procedure described above. The solvent was evaporated, and the product was recrystallized from ether/petroleum ether to give 0.284 g (1.09 mmol, 63%) of brown **6** as microcrystalline needles: mp 173–174 °C dec; IR 3540 (phenol), 1630 (carbonyl) cm^{-1}; ^1H NMR δ 1.52 (d, J = 7.0 Hz, 6 H), 2.51 (s, 3 H), 3.91 (sept, J = 7.0 Hz, 1 H), 6.29 (s, 1 H), 7.31 (d, J = 8.9 Hz, 1 H), 7.91 (s, 1 H), 8.74 (d, J = 8.9 Hz, 1 H), 12.8 (s, 1 H). Anal. ($C_{15}H_{16}O_4$) C,H.

Anti-HIV Testing. The anti-HIV assay used for this work was based on a published procedure.[31] It used HT46c ($HeLaT4^+$) cells as host cells for the H9/HIV-III_B strain of the HIV-1 virus. The cells were seeded into 6-well plates, 5×10^4 cells/well, in RPMI 1640 with 10% FBS and allowed to attach overnight. After treatment of the wells with DEAE-dextran, stock virus was added at a concentration that produced 100–200 foci/well on day 3 in positive control wells. Test compounds were added, and plates were incubated for 3 days at 37 °C, 5% CO_2. Monolayers were fixed with methanol and wells analyzed with a focal immunoassay (FIA). The primary reagent used in the FIA was a polyclonal antibody preparation of sera from AIDS patients. The secondary reagent was a goat anti-human IgG antibody tagged with horseradish peroxidase. The substrate was 3-amino-9-ethylcarbazole in sodium acetate buffer with hydrogen peroxide. Each compound was tested at concentrations of 0.01, 0.1, 1.0, 5.0, and 20 μM. GIL was tested as a standard for comparison.

Fluorescence Quenching Titrations. These experiments were carried out at 25 °C in 2 mL volumes of 0.02 M potassium phosphate buffer (pH 7.4) containing 0.1 M sodium chloride. Aliquots of a 1 mM stock solution of test compound in DMSO were added to a 10 μM solution of human serum albumin in the buffer. Fluorescence was measured with a Perkin Elmer model LS 50 luminescence spectrometer. The K_ds were determined by plotting the reciprocal of free ligand concentration versus the reciprocal of fractional quenching. This plot provides $-1/K_d$ as the x-intercept. Details of the calculations were published previously.[32]

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