Anti-AIDS (Acquired Immune Deficiency Syndrome) Agents. 17. New Brominated Hexahydroxybiphenyl Derivatives as Potent Anti-HIV Agents

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · SEPTEMBER 1995

Impact Factor: 5.45 · DOI: 10.1021/jm00016a002 · Source: PubMed

Articles

Anti-AIDS (Acquired Immune Deficiency Syndrome) Agents. 17.† New Brominated Hexahydroxybiphenyl Derivatives as Potent Anti-HIV Agents

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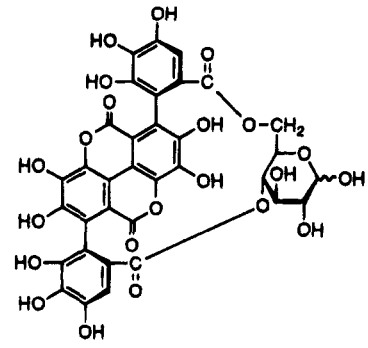
Received March 6, 1995®

Sixteen biphenyl derivatives were synthesized and evaluated for their inhibitory activity against HIV-1 replication in acutely infected H9 cells. 3-Bromo- (**4**) and 3,3′-dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl (**5**) demonstrated potent anti-HIV activity with EC_{50} values of 0.52 and 0.23 μg/mL and therapeutic index values of >190 and >480, respectively. A comparison of the anti-HIV activity of these biphenyl derivatives suggested that the types of substituents on the phenolic hydroxy groups rather than the number of bromine(s) on the aromatic rings are important to the enhanced anti-HIV activity. Compounds **4** and **5** also showed potent inhibitory activity against HIV-1 reverse transcriptase in a template-primer dependent manner. The site of inhibition of HIV could be related to inhibition of this enzyme. Compounds **4** and **5** did not induce virus expression from the chronic HIV-1-infected cell lines ACH-2 and U1. Furthermore, these two agents did not inhibit an increase in virus production from the chronic HIV-1-infected cell lines when the phorbol ester PMA was present.

Our continuing search for new anti-HIV agents from natural products, as well as the modification of active natural products, is aimed at developing more potent anti-HIV agents.[2,3] On the basis of the finding that the ellagitannin punicalin (**1**) demonstrated a relatively potent inhibitory effect against HIV replication in H9 cells,[4] we previously synthesized simpler biphenyl derivatives of ellagic acid and evaluated their anti-HIV activities.[5] Among the biphenyl compounds synthesized, compound **2** exhibited moderate anti-HIV activity with EC_{50} and therapeutic index (TI) values of 7 μg/mL and >14, respectively.[6] In our study of these biphenyl compounds as anti-HIV agents, we suspected 4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl (BBB) (**3**) to be a potential anti-HIV biphenyl compound. BBB was obtained during the total synthesis of schizandrin C[7] and is a known antiviral agent, which is used for the treatment of hepatitis in China.[2,8] Our preliminary evaluation of BBB as an anti-HIV agent demonstrated that it exhibited anti-HIV activity with an EC_{50} value of 5 μg/mL and a TI of >20. This finding prompted our modification of BBB; this paper describes the synthesis of BBB analogs and their anti-HIV activities.

Chemistry

BBB was synthesized according to a literature procedure.[7] As shown in Scheme 1, BBB was treated with



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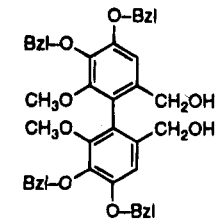

2

Bzl: —CH_2—

1 and 2 mol equiv of bromine in $CHCl_3$ to yield the mono- (**4**) and di- (**5**) bromides, respectively. Compound **5** resisted subsequent hydrolysis with base, probably due to the steric hindrance of the bulky bromine atoms *ortho* to the carboxyl groups. Hydrolysis was achieved by relatively strong conditions (15% KOH–EtOH, refluxed for 4.5 h) to give the dicarboxylic acid **6**. Compound **6** was further treated with acetic anhydride giving an intramolecular anhydride (**8**). Treatment of **8** with alcohol yielded the monoester **9**. The water-soluble trimethylamino carboxylate salt **11** was obtained by treatment of **8** with choline chloride in pyridine. Reaction of **8** with amines furnished monoamides **12** and **13**. Similar treatment of **8** with 1-(2-hydroxyethyl)-pyrrolidine, however, afforded a dicarboxylic acid salt (**14**). In its ^1H NMR spectrum, compound **14** showed the presence of two pyrrolidiniumylethanol moieties [δ 1.95 (8H, m), 3.19 (4H, t, J = 5 Hz), 3.26 (8H, m), 3.75

† For part 16, see ref 1.
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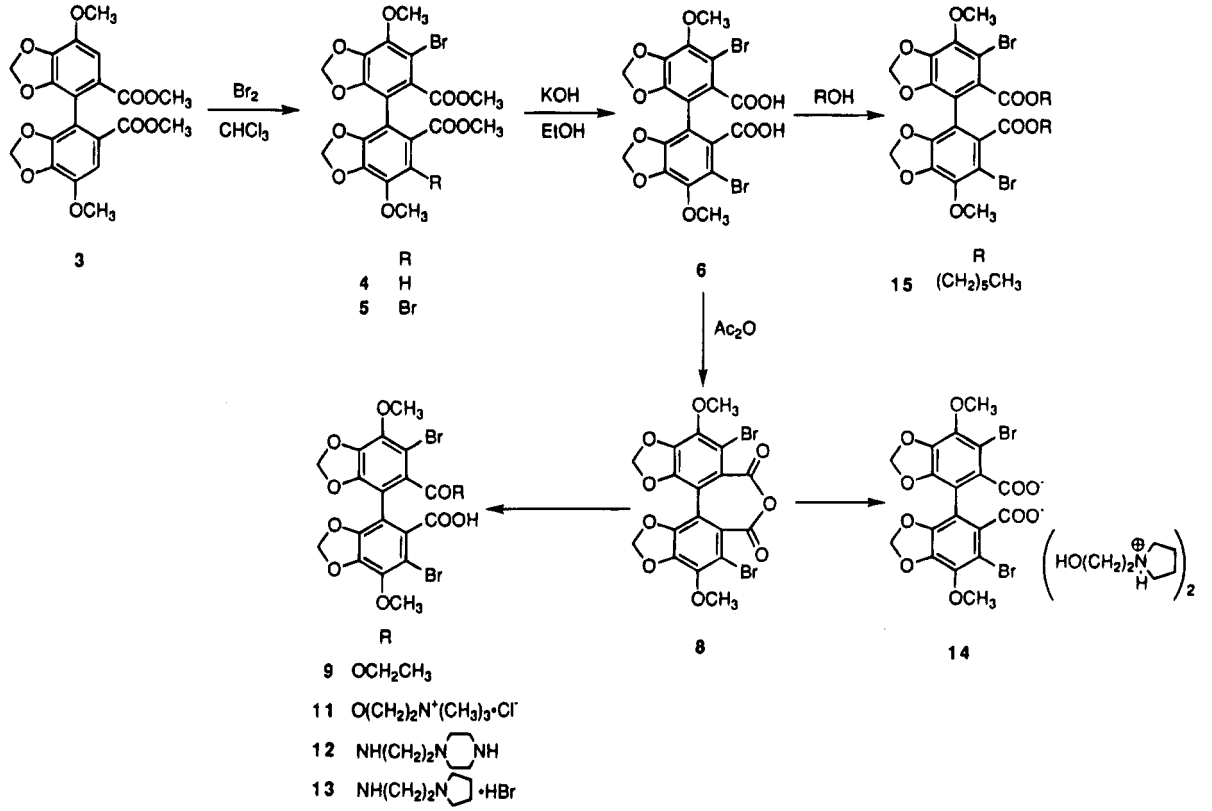
® Abstract published in *Advance ACS Abstracts,* July 15, 1995.

0022-2623/95/1838-3003$09.00/0

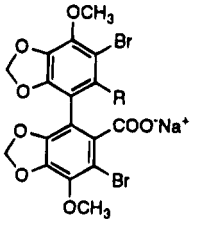
Scheme 1



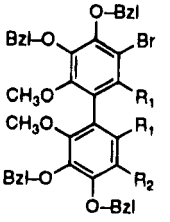
(4H, t, J = 5 Hz)], along with the signals due to two methoxy [δ 3.93 (6H, s)] and two methylenedioxy [δ 5.83 and 5.88 (each 2H, s)] groups. The chemical shift [δ 3.75 (4H, t, J = 5 Hz)] of the hydroxymethyl groups of the pyrrolidiniumylethanol moieties suggested that these positions were not esterified. In addition, the IR spectrum of **14** did not exhibit ester carbonyl absorption around 1700 cm^{-1} but instead showed absorption bands due to a carboxylate (1580 and 1370 cm^{-1}), indicating that **14** is the dipyrrolidiniumylethanol salt of **6**. Treatment of **6** with 1-hexanol under acidic conditions yielded the diester **15**. Compounds **6** and **9** were treated with appropriate molar equivalents of NaOH to furnish di- (**7**) and mono- (**10**) sodium salts. Similar mono- (**16** and **18**) and di- (**17** and **19**) bromo derivatives of previously reported biphenyl compounds[5,9] were also prepared by the procedure described above. The structures of all biphenyl derivatives were confirmed by their spectral data and elemental analyses.

Results and Discussion

The anti-HIV activity of the biphenyl derivatives is shown in Table 1. Among these compounds, 3,3′-dibromo-BBB (**5**) demonstrated potent anti-HIV activity with an EC_{50} value of 0.23 μg/mL. It also exhibited a good therapeutic index of >480. Compound **4**, 3-bromo-BBB, also showed potent anti-HIV activity with EC_{50} and TI values of 0.52 μg/mL and 89. Comparison of the anti-HIV activities of **3**–**5** suggested that the number of bromine atoms on the aromatic rings might enhance anti-HIV activity. Therefore, compounds **16** and **17** were expected to be active, since compounds **4** and **16** and **5** and **17**, respectively, are structurally similar to each other, except for the substituents on the phenolic hydroxy groups. However, compounds **16** and **17** did not show anti-HIV activity. Compounds **18** and **19** also showed no anti-HIV activity, although their parent compound (**2**) does possess anti-HIV activity,[3,5] which is comparable to that of BBB. This result suggested that the substituents on the phenolic hydroxy groups rather than the number of bromine(s) on the aromatic rings are important to the enhanced anti-HIV activity.



R
7 COO^-Na^+
10 $COOC_2H_5$



	R_1	R_2
16	$COOCH_3$	H
17	$COOCH_3$	Br
18	CH_2OH	H
19	CH_2OH	Br

Replacement of the methyl carboxylate groups at C-2 and -2′ of **5** by carboxylic acids (**6** and **9**), sodium carboxylates (**7**), alkylamino carboxylates (**11**), and alkylamino amides (**12** and **13**) all led to a decrease in the anti-HIV activity.

The inhibitory activity of compounds **4** and **5** against HIV-1 recombinant reverse-transcriptase-associated reverse transcriptase (RT) activity was investigated as a mechanism of action study. As shown in Table 2, these compounds exhibited template-primer dependent HIV-1 RT inhibitory activity. This type of inhibitory activity was also observed for other non-nucleoside HIV RT inhibitors.[10–12] The spectra of template-primer dependence are different between **4** and **5**. In the case of **4**, the enzyme was approximately 2 times more sensitive

Table 1. Anti-HIV Activities of Brominated Hexahydroxybiphenyl Derivatives in Acutely Infected H9 Lymphocytes

compd	EC_{50} (μg/mL)[a]	IC_{50} (μg/mL)[b]	TI[c]
3	5	>100	>20
4	0.52	>100	>190
5	0.23	>100	>480
6	>100	>100	1
7	>100	>100	1
8		>100	
9	60	>100	>1.7
10		>100	
11	>100	>100	1
12	30	>100	>3.3
13	>100	>100	1
14	17	>20 but <100	>1.2 but <5.9
15	2	>100	>50
16	>100	>100	1
17	>100	>100	1
18	100	>100	>1
19	>100	>100	1

[a] The EC_{50} values for compounds **1** and **2** are 2.5 and >30 μg/mL, respectively. [b] The IC_{50} values for compounds **1** and **2** are >30 and >140 μg/mL, respectively. [c] The therapeutic index values for compounds **1** and **2** are >12 and >14, respectively.

Table 2. Inhibition of HIV-1 Reverse Transcriptase by Compounds **4** and **5**

template-primer	units used[a]	relative rate[b]	IC_{50}(μM)[c] **4**	**5**
RNA Dependent DNA Polymerase-Associated Activity				
poly(rA)-oligo(dT)	1×10^{-3}	100	4.5 ± 1.0	1.9 ± 0.9
poly(rC)-oligo(dG)	1×10^{-3}	36	2.2 ± 0.6	11 ± 0.5
DNA Dependent DNA Polymerase-Associated Activity				
poly(rA)-oligo(dT)	0.05	10	0.056 ± 0.003	0.048 ± 0.004

[a] A unit is defined as the amount of enzyme required to incorporate 1 nmol of [^{3}H]dTMP into the poly(rA)-oligo(dT) template in 1.0 min at 37 °C. [b] All the reaction rates were normalized to the reaction rate obtained using poly(rA)-oligo(dT). [c] The values represent the mean ± standard deviation of three separate determinations.

with poly(rC)-oligo(dG) as template-primer than with poly(rA)-oligo(dT). In contrast, the enzyme was about 6 times more sensitive to **5** with poly(rA)-oligo(dT) as template-primer than with poly(rC)-oligo(dG). Nevarapine, a non-nucleoside RT inhibitor, exerts more inhibitory activity using poly(rC)-oligo(dG) as template than poly(rA)-oligo(dT).[10] Compounds **4** and **5** also demonstrated potent inhibitory effects against HIV-1 reverse-transcriptase-associated DNA polymerase activity using poly(dA)-oligo(dT) as template-primer with IC_{50} values of 0.056 and 0.048 μM, respectively. Comparison of the inhibitory activity against HIV-1 reverse-transcriptase-associated RT and DNA polymerase activities using poly(rA)-oligo(dT) and poly(dA)-oligo(dT), respectively, as template-primers revealed that **4** and **5** possessed about 80 and 40 times more efficacy against DNA polymerase activity, respectively. Both compounds **4** and **5** could also inhibit HIV-RT-associated RNase H activity. This only occurred when poly(rG)-poly(dC) was used as substrate and Mn^{2+} (0.4 mM) was used as the divalent cation. Under these conditions, the two compounds had almost equal potency (Figure 1). When poly(rA)-poly(dT) was used as substrate or Mg^{2+} was used as the divalent cation, no inhibition was observed. Since HIV-1 RT plays an important role in HIV replication, the potent inhibitory activity of **4** and **5** against this enzyme-catalyzed DNA direct DNA synthesis might be related to their anti-HIV activity.

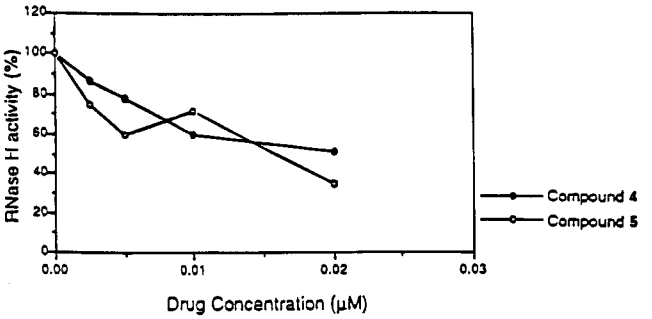


Figure 1. Inhibition of HIV-RT-associated RNase H activity by **4** and **5**.

Table 3. Effect of Compounds **4** and **5** on Chronically Infected U1 Cells

sample identification[a]	p24 (pg/mL) +media	+PMA
U1 cells + **4**		
20	22	10 746
4	11	13 376
0.8	11	8774
0.16	12	7616
0.032	10	7200
0.0064	7	8314
U1 cells + **5**		
20	15	10 131
4	16	10 598
0.8	14	11 482
0.16	13	9850
0.032	15	8320
0.0064	17	7590
U1 cells + AZT		
100	22	9338
10	13	9299
1	12	8218
0.1	18	8070
U1 cells + media	15	7124

[a] Compound concentrations in μg/mL.

Data obtained by using chronically HIV-1-infected cell lines that are cultured with a test drug can give a sense of how these agents may function *in vivo* if given to a latently HIV-infected individual. Therefore, compounds **4** and **5** were separately added for 72 h to the chronically HIV-1-infected T cell line ACH-2 and to the chronically HIV-1-infected promonocytic cell line U1. There was no increase in the induction of virus expression from either cell line. Even when both chronically HIV-1-infected cell lines were cultured in the presence of a known virus inducer such as the phorbol ester PMA (phorbol 12-myristate 13-acetate), there was no alteration in the level of virus expression (Tables 3 and 4). Thus, compounds **4** and **5** did not increase or decrease virus expression from the chronically HIV-infected cells either when cultured alone or in the presence of PMA, respectively.

Experimental Section

General Experimental Procedures. All melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 1320 spectrometer. ^{1}H NMR spectra were obtained using a Bruker AC-300 instrument with TMS or (trimethylsilyl)propionate-d_4 (for D_2O solvent) as an internal standard, and chemical shifts are given in δ (ppm). Mass spectra were measured on a VG Trio-1000 instrument. Elemental analyses were performed by Atlantic MicroLab Inc., Norcross, GA. TLC was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck), and spots were detected by UV illumination. EM Kieselgel 60 (70-230 mesh ASTM) was used

Table 4. Effect of Compounds **4** and **5** on Chronically Infected ACH-2 Cells

sample identification[a]	p24 (pg/mL) +media	p24 (pg/mL) +PMA
ACH-2 cells + **4**		
20	344	46 208
4	148	28 634
0.8	134	24 832
0.16	156	26 957
0.032	139	23 373
0.0064	124	25 830
ACH-2 cells + **5**		
20	169	31 590
4	141	35 635
0.8	135	30 426
0.16	142	32 768
0.032	122	29 173
0.0064	123	27 699
ACH-2 cells + AZT		
100	289	19 814
10	167	30 758
1	125	31 808
0.1	155	31 360
ACH-2 cells + media	143	31 325

[a] Compound concentrations in μg/mL.

for column chromatography. NaOH (0.0982 N) aqueous solution was purchased from Aldrich. All new compounds were characterized by ^{1}H NMR and IR spectral analyses and elemental analyses.

Bromination of 4,4′-Dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl, 4,4′,5,5′-Tetrakis(benzyloxy)-6,6′-dimethoxy-2,2′-bis(methoxycarbonyl)biphenyl, and 4,4′,5,5′-Tetrakis(benzyloxy)-6,6′-dimethoxy-2,2′-bis(hydroxymethyl)biphenyl. A solution of biphenyl (300−500 mg, 0.66−1 mmol) in $CHCl_3$ (8−10 mL) was added dropwise to bromine (1 or 2 mol equiv) in $CHCl_3$ (5−8 mL) at 0−5 °C. The temperature was elevated to room temperature, and the mixture was stirred for 3 h. The reaction mixture was poured into 20% sodium bisulfite aqueous solution (40−50 mL), which was extracted with $CHCl_3$. The $CHCl_3$ layer was washed successively with 5% aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated to dryness. The residue was crystallized from EtOH or purified by silica gel chromatography [hexane−EtOAc (7:1 or 4:1)] to yield mono- or dibromide.

3-Bromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl (4): yield 85%; mp 157−159 °C; IR (KBr) 1720 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 3H, 2′-$COOCH_3$), 3.71 (s, 3H, 2-$COOCH_3$), 3.97 (s, 3H, 4′-OCH_3), 4.08 (s, 3H, 4-OCH_3), 6.01, 6.04 (m, 4H in total, -OCH_2O-), 7.33 (s, 1H, H-3′). Anal. ($C_{20}H_{17}O_{10}Br$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(methoxycarbonyl)biphenyl (5): yield 98%; mp 227−229 °C; IR (KBr) 1710 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.71 (s, 6H, 2,2′-$COOCH_3$), 4.08 (s, 6H, 4,4′-OCH_3), 6.01, 6.03 (each s, 2H, -OCH_2O). Anal. ($C_{20}H_{16}O_{10}Br_2$) C, H, Br.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-dicarboxybiphenyl (6). A solution of **5** (500 mg, 0.87 mmol) in 40% KOH aqueous solution (12 mL) and EtOH (20 mL) was refluxed for 4.5 h. After cooling, the reaction mixture was neutralized with 37% HCl. The resulting white precipitate was collected by filtration, washed with water, and dried to give the dicarboxylic acid **6**: yield 90%; mp 230 °C dec; IR (KBr) 3000−2500 (COOH), 1690 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 6H, 4,4′-OCH_3), 5.95, 6.03 (each s, 2H, -OCH_2O-); MS (%) *m/z* 546 $(M)^+$ (24), 548 $(M + 2)^+$ (48), 550 $(M + 4)^+$ (23). Anal. ($C_{18}H_{12}O_{10}Br_2$) C, H, Br.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-dicarboxybiphenyl Disodium Salt (7). A solution of **6** (107.2 mg, 0.1963 mmol) in 0.0892 N NaOH aqueous solution (4 mL, 0.3928 mmol) was kept standing at room temperature for 5 min. The mixture was concentrated *in vacuo* to yield the disodium salt **7** (114 mg) as a white solid: yield 98%; mp 153 °C dec; IR (KBr) 1585, 1380 (COO^-) cm^{-1}; ^{1}H NMR (D_2O) δ 3.93 (s, 6H, 4,4′-OCH_3), 5.85, 5.90 (each s, 2H, -OCH_2O-). Anal. ($C_{18}H_{10}O_{10}Br_2Na_2{\cdot}2H_2O$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)biphenyl-2,2′-dicarboxylic Anhydride (8). A solution of **6** (200 mg, 0.36 mmol) in Ac_2O (3 mL) was refluxed for 24 h with stirring. The mixture was concentrated *in vacuo*, and the residue was poured into water. The resulting off-white solid was collected by filtration and washed with water. Crystallization from EtOAc afforded **8** (179 mg) as a white solid: yield 93%; mp 258 °C; IR 1800, 1770 (CO-O-CO) cm^{-1}; MS (%) *m/z* 528 $(M)^+$ (31), 530 $(M + 2)^+$ (59), 532 $(M + 4)^+$ (29); ^{1}H NMR ($CDCl_3$) δ 4.11 (s, 6H, 4,4′-OCH_3), 6.06, 6.11 (each s, 2H, -OCH_2O-). Anal. ($C_{18}H_{10}O_9Br_2$) C, H, Br.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2-(ethoxycarbonyl)-2′-carboxydiphenyl (9). A solution of **8** (150 mg, 0.284 mmol) in EtOH (25 mL) was refluxed for 3 days with stirring. After removal of the solvent by evaporation, the residue was treated with 5% NaOH aqueous solution (20 mL), washed with EtOAc, acidified with 10% aqueous HCl, and extracted with EtOAc. The EtOAc layer was concentrated to dryness to furnish **9** (134 mg) as a white powder: yield 84%; mp 239 °C dec; IR (KBr) 3000−2500 (COOH), 1725 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 7 Hz, 3H, CH_3), 3.99, 4.00 (each s, 3H, 4,4′-OCH_3), 3.99−4.06 (m, 2H, OCH_2), 6.00−6.12 (m, 4H, 2 -OCH_2O-). Anal. ($C_{20}H_{16}O_{10}Br_2{\cdot}1.5H_2O$) C, H, Br.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2-(ethoxycarbonyl)-2′-carboxybiphenyl Monosodium Salt (10). A solution of **9** (56 mg, 0.0982 mmol) in 0.0982 N NaOH aqueous solution (1 mL, 0.0982 mmol) was kept standing at room temperature for 5 min. Workup as for **7** gave the monosodium salt **10** (52 mg) as a white solid: yield 90%; mp 227 °C; IR (KBr) 1725 (CO) 1590, 1380 (COO^-) cm^{-1}; ^{1}H NMR (D_2O) δ 0.92 (t, J = 7 Hz, 3H, CH_3), 3.93, 3.94 (each s, 3H, 4,4′-OCH_3), 4.09 (q, J = 7 Hz, 2H, OCH_2), 5.91 (m, 4H, 2 OCH_2O-). Anal. ($C_{20}H_{15}O_{10}Br_2Na$) C, H, Br.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2-carboxy-2′-[[β-(*N,N,N*-trimethylamino)ethoxy]-carbonyl]biphenyl Chloride (11). A mixture of **8** (950 mg, 1.8 mmol) and chlorine chloride (250 mg, 1.8 mmol) in pyridine (3 mL) and DMF (2 mL) was stirred at room temperature for 5 days. The reaction mixture was concentrated to dryness to give an off-white solid. Crystallization from MeOH gave **11** (1.02 g) as colorless prisms: yield 85%; mp 210 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.11 (br s, 9H, N-CH_3), 3.40 (t, J = 6 Hz, 2H, N-CH_2), 3.82 (m, 2H, CH_2O), 3.97 (s, 6H, 4,4′-OCH_3), 5.96, 6.03 (each s, 2H, -OCH_2O-). Anal. ($C_{23}H_{24}NO_{10}Br_2Cl$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2-carboxy-2′-[(β-*N*-piperazinylethyl)carbamoyl]-biphenyl (12). A mixture of **8** (528 mg, 1.1 mmol) and 1-(2-aminoethyl)piperazine (0.15 mL, 1.1 mmol) in benzene (25 mL) was stirred at room temperature for 24 h. The reaction mixture was concentrated to dryness to give an off-white solid. Crystallization from MeOH gave **12** (693 mg) as an off-white solid: yield 99%; mp 184 °C dec; ^{1}H NMR (CD_3OD) δ 2.52 (4H, m, 2 $NHCH_2$ of piperazine), 2.84 (2H, t, J = 6 Hz, 2′-$CONHCH_2$), 2.88 (6H, m, 3 NCH_2), 3.98 (6H, s, 4,4′-OCH_3), 5.86, 5.93 (each 2H, s, -OCH_2O-). Anal. ($C_{24}H_{25}N_3O_9Br_2{\cdot}2H_2O$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2-carboxy-2′-[(β-*N*-pyrrolidinylethyl)carbamoyl]-biphenyl Hydrobromide (13). A mixture of **8** (150 mg, 0.284 mmol) and 1-(2-aminoethyl)piperazine (0.3 mL, 2.3 mmol) in benzene (10 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated to dryness to give an off-white solid. Crystallization from EtOH containing 2% HBr yielded **13** (122 mg) as a white powder: yield 58%; mp 188 °C dec; IR (KBr) 1710 (CO), 1610, 1590 (amide) cm^{-1}; ^{1}H NMR (CD_3OD) δ 2.04, 2.52 (each m, 2H, CH_2), 3.16 (t, J = 6 Hz, 2H, 2′-$CONHCH_2$), 3.41 (m, 4H, 2 NCH_2), 3.58 (m, 4H, 2 NCH_2), 3.58 (m, 4H, 2 NCH_2 of pyrrolidine), 4.00, 4.11 (each s, 3H, 4,4′-OCH_3), 6.02, 6.08 (each s, 2H, -OCH_2O-). Anal. ($C_{24}H_{25}N_2O_9Br_3{\cdot}H_2O$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-dicarboxybiphenyl Bis[1-(2-Hydroxyethyl)-pyrrolidinium Salt] (14). A mixture of **8** (528 mg, 1 mmol)

and 1-(2-hydroxyethyl)pyrrolidine (0.2 mL) in pyridine (2 mL) and DMF (2 mL) was stirred at room temperature for 3 days. The reaction mixture was concentrated to dryness, and the residue was washed with acetone to yield **14** (363 mg) as a white solid: yield 49%; mp 149–151 °C; IR (KBr) 1580, 1370 (COO^-) cm^{-1}; ^1H NMR (D_2O) δ 1.95 (m, 8H, 4 CH_2 of pyrrolidine), 3.19 (t, $J = 5$ Hz, 4H, 2 NCH_2), 3.26 (m, 8H, NCH_2 of pyrrolidine), 3.75 (t, $J = 5$ Hz, 4H, CH_2O), 3.93 (s, 6H, 4,4′-OCH_3), 5.83, 5.88 (each s, 2H, OCH_2O). Anal. ($C_{30}H_{38}N_2O_{12}Br_2$) C, H.

3,3′-Dibromo-4,4′-dimethoxy-5,6,5′,6′-bis(methylenedioxy)-2,2′-bis(hexanoxycarbonyl)biphenyl (15). To a solution of **6** (273 mg, 0.5 mmol) in 1-hexanol (7 mL) was added thionyl chloride (2 mL) dropwise, and the mixture was refluxed for 3 days. The reaction mixture was concentrated to dryness, and the residue was subjected to silica gel chromatography. Elution with cyclohexane–EtOAc (4:1) gave **15** (134 mg) as a white powder: yield 37%; mp 115–117 °C; IR (KBr) 2960, 2930, 2860 (CH_2), 1730 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, $J = 6.5$ Hz, 6H, 2 CH_3), 1.25 (m, 12H, 6 CH_2), 1.47 (m, 4H, 2 CH_2), 4.07 (s, 6H, 4,4′-OCH_3), 4.07–4.14 (m, 4H, 2 CH_2O), 5.99, 6.01 (each s, 2H, OCH_2O). Anal. ($C_{30}H_{36}O_{10}Br_2$) C, H, Br.

2,2′-Bis(methoxycarbonyl)-3-bromo-4,4′,5,5′-tetrakis(benzyloxy)-6,6′-dimethoxybiphenyl (16): yield 85%; white amorphous powder; ^1H NMR ($CDCl_3$) δ 3.51, 3.60, 3.73, 3.74 (each s, 3H, OCH_3), 5.10–5.16 (m, 8H in total, $PhCH_2O$), 7.27–7.53 (m, 21H in total, aromatic H and H-3′). Anal. ($C_{46}H_{41}O_{10}Br$) C, H.

2,2′-Bis(methoxycarbonyl)-3,3′-dibromo-4,4′,5,5′-tetrakis(benzyloxy)-6,6′-dimethoxybiphenyl (17): yield 90%; white amorphous powder; ^1H NMR ($CDCl_3$) δ 3.61, 3.79 (each s, 6H, OCH_3), 5.10–5.12 (m, 8H in total, $PhCH_2O$), 7.33–7.50 (m, 20H in total, aromatic H). Anal. ($C_{46}H_{40}O_{10}Br_2$) C, H, Br.

2,2′-Bis(hydroxymethyl)-3-bromo-4,4′,5,5′-tetrakis(benzyloxy)-6,6′-dimethoxybiphenyl (18): yield 76%; white amorphous powder; ^1H NMR ($CDCl_3$) δ 3.60, 3.67 (each s, 3H, OCH_3), 4.09, 4.17, 4.25, 4.50 (each d, $J = 12$ Hz, 1H, CH_2OH), 5.09–5.16 (m, 8H in total, $PhCH_2O$), 7.00 (s, 1H, H-3′), 7.28–7.55 (m, 20H in total, aromatic H). Anal. ($C_{44}H_{41}O_8Br$) C, H.

2,2′-Bis(hydroxymethyl)-3,3′-dibromo-4,4′,5,5′-tetrakis(benzyloxy)-6,6′-dimethoxybiphenyl (19): yield 78%; white amorphous powder; ^1H NMR ($CDCl_3$) δ 3.61 (each s, 3H, OCH_3), 4.50, 4.56 (each d, $J = 12$ Hz, 1H, CH_2OH), 5.11 (s, 4H, $PhCH_2O$), 5.13, 5.18 (each d, $J = 12$ Hz, 2H, $PhCH_2O$), 7.32–7.55 (m, 20H in total, aromatic H). Anal. ($C_{44}H_{40}O_8Br$) C, H.

HIV Growth Inhibition Assay. The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) at 5% CO_2 and 37 °C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1–0.01 IU/cell) for 1 h at 37 °C and 5% CO_2. The cells then were washed thoroughly to remove unabsorbed virions and resuspended at 4×10^5 cells/mL in complete medium. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compound (diluted in the culture medium). After a 4 day incubation at 37 °C, cell density of uninfected cultures was determined by counting cells in a Coulter counter to assess toxicity of the test compound. A p24 antigen ELISA assay was used to determine the level of virus released in the medium of the HIV-infected cultures. The p24 antigen assay uses an HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 was used to tag any p24 "captured" onto the microtiter well surface. Peroxidase-conjugated goat anti-rabbit serum was then used to tag HIV-1 p24 specific rabbit antibodies that had complexed with captured p24. The presence of p24 in test samples was then revealed by addition of substrate. The cutoff for the p24 ELISA assay was 12.5 pg/mL. p24 in the culture medium was quantitiated against a standard curve containing known amounts of p24. The effective (EC_{50}) and inhibitory (IC_{50}) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

Preparation of HIV-1 Reverse Transcriptase. HIV-1 RT was purified using *Escherichia coli* JM 109 containing pKRT 2 kindly provided by W. C. Summers (Yale University).[13]

Enzyme Assay. All reactions were developed in a total volume of 50 μL containing 50 mM Tris HCl pH 7.8, 2 mM $MgCl_2$, 100 μg/mL nuclease-free BSA, 1 mM dithiothreitol, 0.5 $O.D._{260}$ unit/mL template-primer, and 330 nM [^{3}H]dNTP according to Cheng *et al.*[14]

Chronically HIV-Infected Cell Line. The HIV-1 chronically infected T cell line ACH-2[15] and the HIV-1 chronically infected promonocytic cell line U1[16] were continuously maintained in RPMI 1640 with 10% fetal calf serum (FCS). For experiments, the cell lines were only used in log phase of growth. Cells (1×10^6 cells/well) and either various concentrations of **4**, **5**, AZT, or media alone were added to 24-well plates in the presence or absence of PMA (10^{-8} M). After 72 hours at 37 °C and 5% CO_2, an aliquot of the cell-free supernatants was collected and analyzed for p24 antigen by ELISA.

Acknowledgment. This investigation was supported by Grant AI-33066 from the National Institute of Allergies and Infectious Disease awarded to K. H. Lee.

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JM9501700