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<p>(21) International Application Number: PCT/US96/10080</p> <p>(22) International Filing Date: 7 June 1996 (07.06.96)</p> <p>(30) Priority Data: 08/477,939 7 June 1995 (07.06.95) US</p> <p>(60) Parent Application or Grant (63) Related by Continuation US 08/477,939 (CIP) Filed on 7 June 1995 (07.06.95)</p> <p>(71) Applicants (for all designated States except US): THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; Office of Technology Development, Campus Box #4105, 308 Bynum Hall, Chapel Hill, NC 27599-4105 (US). BIOTHECH RESEARCH LABORATORIES [US/US]; 3 Taft Court, Rockville, MD 20850 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): LEE, Kuo-Hsiung [US/US]; 1426 Gray Bluff Trail, Chapel Hill, NC 27514 (US). KASHIWADA, Yoshiki [JP/JP]; 5-14-31, Kamishin'ei-cho, Niigata 950-21 (JP). XIE, Lan [CN/US]; 201 Culbreth Park Drive, Chapel Hill, NC 27516 (US).</p>	<p>COSENTINO, Louis, Mark [US/US]; 6648 Green Ash Drive, Springfield, VA 22152 (US). MANAK, Mark [US/US]; 200 Stanley Place, Laurel, MD 20707 (US). XIE, Jing-Xi [CN/CN]; Institute of Materia Medica, Chinese Academy of Medical Science, 1 Xian Nong Tan Street, Beijing 100050 (CN). CHENG, Yung-Chi [US/US]; 961 Baldwin Road, Woodbridge, CT 06525 (US). KILKUSKIE, Robert, E. [US/US]; 40 Ireta Road, Shrewsbury, MA 01545 (US).</p> <p>(74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, Washington, DC 20005-3934 (US).</p> <p>(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: BROMINATED HEXAHYDROXYBIPHENYL DERIVATIVES</p>		
<p>(57) Abstract</p>		
<p>Derivatives of 4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (DDB) were synthesized and found to have anti-HIV activity. Of the compounds studied, 3,3'-dibromo DDB and 3-bromo-DDB initially exhibited the greatest anti-HIV activity.</p>		

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Brominated Hexahydroxybiphenyl Derivatives

Field of the Invention

The present invention relates to compounds which have been found to be useful in treating viral infections, such as HIV infections.

5

Background of the Invention

Retroviruses

Retroviruses are small, single-stranded positive-sense RNA viruses. A retroviral particle comprises two identical single-stranded positive sense RNA molecules. Their genome contains, among other things, the sequence of the RNA-dependent DNA polymerase, also known as reverse transcriptase. Many molecules of reverse transcriptase are found in close association with the genomic RNA in the mature viral particles. Upon entering a cell, this reverse transcriptase produces a double-stranded DNA copy of the viral genome, which is then inserted into the chromatin of a host cell. Once inserted, the viral sequence is called a provirus. Retroviral integration is directly dependent upon viral proteins. Linear viral DNA termini (the LTRs) are the immediate precursors to the integrated proviral DNA. There is a characteristic duplication of short stretches of the host's DNA at the site of integration.

Progeny viral genomes and mRNAs are transcribed from the inserted proviral sequence by host cell RNA polymerase in response to transcriptional, regulatory signals in the terminal regions of the proviral sequence, the long terminal repeats, or LTRs. The host cell's protein production machinery is used to produce viral proteins, many of which are inactive until processed by virally encoded proteases. Typically, progeny viral particles bud from the cell surface in a non-lytic manner. Retroviral infection does not necessarily interfere with the normal life cycle of an infected cell or organism. However, neither is it always benign with respect to the host organism. While most classes of DNA viruses can be implicated in tumorigenesis, retroviruses are the only taxonomic group of RNA viruses that are oncogenic. Various retroviruses, such as the Human Immunodeficiency Virus (HIV), which is the etiological agent responsible for

acquired immune deficiency syndrome (AIDS) in humans, are also responsible for several very unusual diseases of the immune system of higher animals.

HIV Infection and AIDS

5 Human Immunodeficiency Virus (HIV) is a member of the lentiviruses, a subfamily of retroviruses. Many retroviruses are well-known carcinogens. HIV
10 *per se* is not known to cause cancer in humans or other animals, but it does present a formidable challenge to the host. The viral genome contains many regulatory elements which allow the virus to control its rate of replication in both resting and dividing cells. Most importantly, HIV infects and invades cells of the
15 immune system; it breaks down the body's immune system and renders the patient susceptible to opportunistic infections and neoplasms. The immune defect appears to be progressive and irreversible, with a high mortality rate that approaches 100% over several years.

15 HIV-1 is trophic and cytopathic for T4 lymphocytes, cells of the immune system which express the cell surface differentiation antigen CD4, also known as OKT4, T4 and leu3. The viral tropism is due to the interactions between the viral envelope glycoprotein, gpl20, and the cell-surface CD4 molecules (Dalglish *et al.*, *Nature* 312:763-767 (1984)). These interactions not only mediate the
20 infection of susceptible cells by HIV, but are also responsible for the virus-induced fusion of infected and uninfected T cells. This cell fusion results in the formation of giant multinucleated syncytia, cell death, and progressive depletion of CD4 cells in AIDS patients. These events result in HIV-induced immunosuppression and its subsequent sequelae, opportunistic infections and
25 neoplasms.

25 In addition to CD4+ T cells, the host range of HIV includes cells of the mononuclear phagocytic lineage (Dalglish *et al.*, *supra*), including blood monocytes, tissue macrophages, Langerhans cells of the skin and dendritic reticulum cells within lymph nodes. HIV is also neurotropic, capable of infecting monocytes and macrophages in the central nervous system causing severe
30 neurologic damage. Macrophage/monocytes are a major reservoir of HIV. They

can interact and fuse with CD4-bearing T cells, causing T cell depletion and thus contributing to the pathogenesis of AIDS.

Anti-HIV Drugs

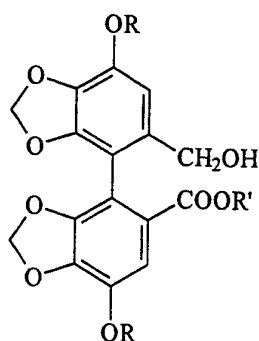
Intensive efforts are currently under way to develop therapies to prevent or intervene in the development of clinical symptoms in HIV-infected individuals. For the most part, efforts have been focused on the use of nucleoside analogue drugs such as AZT (azidothymidine), and on other dideoxynucleoside derivatives such as ddA, ddT, ddI, and ddC. These drugs inhibit the viral enzyme reverse transcriptase, thereby inhibiting *de novo* infection of cells. However, once viral infection has been established within a cell, viral replication utilizes host cell enzymes. Thus, drugs which inhibit only reverse transcriptase tend to have limited effects. While the spread of free virus within the organism can be blocked, the mechanisms of syncytia formation and pathogenesis through direct intercellular spread remain. Accordingly, there is a need to provide new anti-HIV drugs which are not limited to inhibiting reverse transcriptase as their mechanism of action.

In an effort to find new and more effective drugs for treating HIV infections, attention has turned to compounds found in plants or compounds modified from natural products. In particular, tannins have been investigated for their medicinal uses, and several elagitannins exhibit some antitumor and anti-HIV activity (Okuda *et al.*, *Planta Med.* 55:(2):117-122 (1989); Bokesch *et al.*, *J. Nat. Prod.* 56(7):1123-1129 (1993)). Unfortunately, reports of the inhibition of reverse transcriptase of the tannins show that many of these tannins have little or no activity against reverse transcriptase, and there is as yet no reliable means for predicting which of these compounds will be effective (Kakiuchi *et al.*, *J. Nat. Prod.* 48(4):614-621 (1985); Nonaka *et al.*, *J. Nat. Prod.* 53(3):587-595 (1990); Nakashima *et al.*, *Antiviral Res.* 18(1):91-103 (1992); Weaver *et al.*, *Biochem. Pharmacol.* 43 (11):2479-2489 (1992); Kilkuskie *et al.*, *Bioorg. Med. Chem. Lett.* 2(12):1529-1534 (1992)).

Many of the tannin-based compounds are biphenyl derivatives. Historically, biphenyl derivatives have been investigated for their utility in treating a variety of liver diseases. One anti-viral agent has been used for the treatment of hepatitis in China (Liu *et al.*, Hepato-pharmacology of *Fructus Schizandrae* in *Advances in Chinese Medical Materials Research*, edited by Chang *et al.*, pp. 257-267). One compound disclosed therein, 4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (DDB), exhibited some anti-HIV activity, with an ED₅₀ value of 5 microgram/ml and a TI of >20.

Schinsandra chinensis (Chinese name: Wu-Wei-Zi) has long been used in China as an astringent and tonic. In the 1970's it was found to be effective against viral hepatitis, for lowering elevated SGPT levels, and as a protective agent for the liver when injured by chemical agents such as carbon tetrachloride. Further study indicated that schisandrin C, isolated from the seeds of *Schinsandra chinensis*, was an active principle. During the total synthesis of schisandrin C, a new anti-hepatic drug, 4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl was discovered.

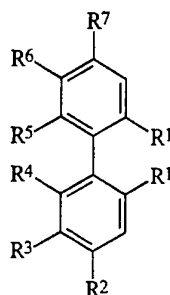
Shi-jie, in U.S. Patent No. 4,868,207, discloses bis(methylenedioxy)biphenyl compounds of the formula



wherein R is an alkyl group having 1 to 6 carbon atoms or a phenyl group, and R' is a hydrogen atom or an alkyl group of 1 to 6 carbon atoms.

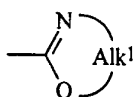
Iwasaki *et al.*, in U.S. Patents Nos. 5,103,023; 5,182,404 and 5,233,057, disclose biphenyl derivatives of the formula:

-5-



wherein R¹ is substituted or unsubstituted aminocarbonyl, aminothiocarbonyl, substituted or unsubstituted lower alkoxy carbonyl, cyano, or a group of the formula:

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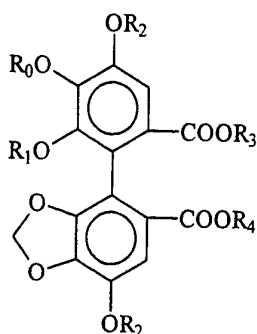
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where one or two of R² to R⁷ are hydrogen, and the remaining groups are the same or different and are lower alkoxy, phenyl(lower) alkoxy or hydroxy, or adjacent two groups thereof combine to form a lower alkylendioxy group, and Alk¹ is a lower alkylene group, or a pharmaceutically acceptable salt thereof. The compounds are taught to be useful for the prophylaxis and treatment of hepatic diseases. Example 19 discloses 5,6,5',6'-dimethylenedioxy-2,2'-biphenylbis(thiocarboxamide).

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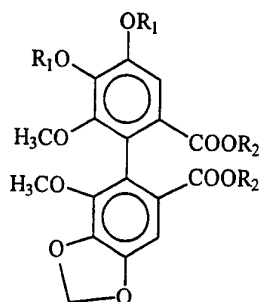
Kimura *et al.*, in U.S. Patents Nos. 4,849,448, 4,996,331 and 5,264,594, disclose biphenyl derivatives which can be used to treat hepatitis. The biphenyl derivatives have the formula:

-6-



where R_0 and R_1 independently stand for lower alkyl, or R_0 and R_1 together represent a group $O=C<$, R_2 is an alkyl group of 1 to 3 carbon atoms, and R_3 and R_4 independently are hydrogen or lower alkyl.

- 5 Matsuoka *et al.*, in U.S. Patents Nos. 4,904,694 and 4,987,240, disclose biphenyl derivatives useful for moderating liver ailments and for treating hepatitis. These compounds have the formula:



where R_1 and R_2 are hydrogen or methyl.

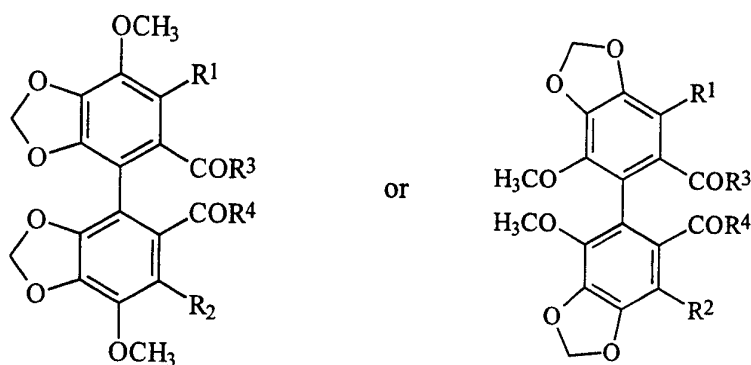
- 10 Hirayama *et al.*, U.S. Patent No. 5,159,069, disclose sulfated tannins which have antiviral activity and reverse transcriptase inhibition which can be used to treat patients infected with viruses such as HIV.

Summary of the Invention

The present invention is intended to overcome one or more deficiencies of the related art. The present invention is also intended to provide compounds which have anti-viral activity and/or anti-retroviral activity, such as anti-HIV activity, *in vitro*, *in situ*, and/or *in vivo*, as well as preparing these compounds.

The present invention produces biphenyl derivatives which can be used to inhibit retroviral growth, replication, binding and/or metabolism, and/or to treat a retroviral infection or related symptoms. The present invention also provides a process for preparing biphenyl derivatives.

The compounds of the present invention have one of the following formula:



wherein R¹ and R² are hydrogen or bromine, with the proviso that at least one of R¹ or R² is bromine;

R³ and R⁴ are independently hydroxy, C₁₋₆ alkoxy, —O(CH₂)₂N⁺(CH₃)C1⁻,
 —NH(CH₂)₂—N $\begin{array}{c} \diagup \\ \diagdown \end{array}$ NH, or —NH(CH₂)₂—N $\begin{array}{c} \diagup \\ \diagdown \end{array}$ • HBr

In particular, compounds having the most potent anti-HIV activity are:

3-bromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl, and

3,3'-dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl.

5 The invention is also directed to a method for treating a subject infected with HIV-1 by administering at least one of the above-noted compounds, optionally in combination with any one or more of the known anti-AIDS therapeutics or an immunostimulant.

10 The treatment methods of the present invention also include administering to a subject infected with HIV-1 a conjugate of a compound as described above with soluble CD4, CD4 derivatives, antibodies specific for CD4, or HIV-coded glycoproteins such as gp120 and gp41, or antibodies thereto.

15 Other features, advantages, embodiments, aspects and objects of the present invention will be clear to those skilled in the areas of relevant art, based upon the description, teaching and guidance presented herein.

Brief Description of the Figures

Figure 1 shows inhibition of HIV reverse transcriptase associated RNase H activity by two compounds of the present invention.

Figure 2 illustrates compounds according to the present invention.

20 Figure 3 depicts Reaction Scheme 1.

Figure 4 illustrates compounds according to the present invention.

Figure 5 depicts Reaction Scheme 2.

Detailed Description of the Preferred Embodiments

The present invention relates to compounds which have been discovered to be useful in inhibiting retroviral infection and/or replication in eukaryotic cells and/or for the treatment of retroviral infections, such as HIV infection, as well as to methods of preparing the compounds.

Derivatives of 4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (3, DDB) were synthesized and evaluated for their inhibitory activity against HIV-1 replication in acutely infected H9 cells. Four compounds demonstrated particularly potent anti-HIV activity with EC₅₀ values of <0.8 and 0.3 µg/ml and therapeutic index values of >100 and >333, respectively. A comparison of the anti-HIV activity of these compounds suggested that the types of substituents on the phenolic hydroxy groups as well as the presence of at least one bromine substituent rather than the number of bromine(s) on the aromatic rings are important to the enhanced anti-HIV activity. The compounds of the present invention also exhibited potent inhibitory activity against HIV-1 reverse transcriptase in a template-primer dependent manner.

The compounds of the present invention have been unexpectedly discovered to have anti-retroviral activity, thus providing suitable compounds and compositions for treating retroviral infections, optionally with additional pharmaceutically active ingredients, such as anti-retroviral, anti-HIV, and/or immuno-stimulating compounds or antiviral antibodies or fragments thereof.

By the term "anti-retroviral activity" or "anti-HIV activity" is intended the ability to inhibit at least one of:

- (1) retroviral attachment to cells;
- (2) viral entry into cells;
- (3) cellular metabolism which permits viral replication;
- (4) inhibition or intercellular spread of the virus;
- (5) synthesis and/or cellular expression of viral antigens;
- (6) activity of virus-coded enzymes (such as reverse transcriptase and protease); and/or

-10-

(7) any known retroviral or HIV pathogenic actions, such as, for example, immunosuppression. Thus, any activity which tends to inhibit any of these mechanisms is "anti-retroviral activity" or "anti-HIV activity."

Synthesis

5 DDB (4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-2,2'-
dimethoxycarbonyl biphenyl (compound **3** in Scheme 1) was synthesized
according to the procedure of Xie *et al.*, *Acta Pharmaceutica sinica* 17:23-27
(1982)). As shown in Scheme 1 (Figure 3), DDB was treated with one and two
10 molar equivalents of bromine in CHCl₃ to yield the mono- (**4**) and di- (**5**)
bromide, respectively. Compound **5** resisted subsequent hydrolysis with base,
probably due to the steric hindrance of the bulky bromine atoms ortho to the
hydroxy groups. Hydrolysis was achieved using relatively strong conditions to
yield the dicarboxylic acid (**6**). Compound **6** was treated with acetic anhydride
to give an intramolecular anhydride (**8**). Treatment of **8** with alcohol yielded the
15 monoester (**9**), while reaction with amines furnished monoamides (**12** and **13**).
The water-soluble trimethylamino carboxylate salt (**11**) was obtained by treating
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, yielded a dicarboxylic acid salt (**14**). In its
20 ¹H NMR spectrum, compound **14** showed the presence of two pyrrolidinium
ethanol moieties [δ 1.95 (8H,m), 3.19 (4H, t, J= 5Hz), 3.26 (8H, m), 3.75 (4H, t,
J=5Hz), along with the signals due to two methoxy [δ 3.9 (6H,s)] and two
methylenedioxy [δ 5.83 and 5.88 (each 2H,s)] groups. The chemical shift [δ 3.75
(4H, t, J=5Hz)] of the hydroxymethyl groups of the pyrrolidinium ethanol
25 moieties suggested that these positions were not esterified. In addition, the IR
spectrum of **14** did not exhibit extra 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 dipyrrolidinium ethanol salt of **6**. Treatment of **6** with 1--
hexanol under acidic conditions yielded the corresponding diester **15**.

Compounds **6** and **9** were treated with appropriate molar equivalents 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 (Kashiwada *et al.*, *Bioorg., Med. Chem. Lett.* 2:234-238 (1992); Kashiwada *et al.*, *J. Med. Chem.* 37:195-200 (1994)) were also prepared by the procedure described above.

DDB has three different groups in each benzyl ring, and a series of isomers can be obtained by changing the relative position of these groups. During the total synthesis of DDB from gallic acid as the starting material, two isomers, 6,6'-dimethoxy-4,5,4',5'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (**114**) and 4,6'-dimethoxy-5,6,4',5'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (**115**) were both obtained in the Ullmann reaction. Additional isomers of DDB, **116** and **117**, were synthesized according to the route shown in Scheme 2 (Figure 5).

The following is a description of the reaction scheme set forth in Scheme 2. The starting material, 2,3,4-trihydroxybenzoic acid, was first esterified to give methyl 2,3,4-trihydroxybenzoate **120**. The monoethylation of the 4-OH group in compound **120** was achieved by Scheline's method. The IR spectra of the product showed the absorption peak of the ester carbonyl group at 1660 cm⁻¹, indicating the presence of internal hydrogen bonding. In its ¹H NMR spectra, a signal was observed at δ10.98 ppm, ascribable to the chelated OH group. These spectroscopic data indicated that monomethylation occurred at the 4-OH group, not at the 2-position. Thus, the structure of the monomethyl ether of compound **120** was assigned as methyl 2,3-dihydroxy-4-methoxybenzoate **121**. Methylenation of compound **121** with CH₂Cl₂ in DMF afforded methyl 2,3-methylenedioxy-4-methoxybenzoate **122** in good yield (19%). The IR absorption bands of the ester carbonyl group in **122** shifted from 1660 cm⁻¹ to 1710- cm⁻¹. Bromination of compound **122** with dioxane-dibromide reagent in ethylene oxide yielded methyl 2,3-methylenedioxy-4-methoxy-5-bromobenzoate **123** in good yield (>80%). The brominated compound **123** was also obtained by reacting compound **122** with dry bromine in CHCl₃. The position of the bromine atom

introduced was determined unequivocally on the basis of a 2D-NOE spectrum. A strong NOE response was observed between the aromatic proton and the methyl protons of the methoxycarbonyl group. Therefore, bromination must have taken place at the 5-position, which was in accordance with the expected orientation for electrophilic aromatic substitution. Ullmann reaction of compound **123** with active copper powder in DMF gave only a very small amount of the biphenyl derivative **117**. This compound gave a molecular ion at m/z 418 as the base peak in its MS spectrum. The IR and ^1H NMR spectra agreed well with the assigned structure. The new compound **117** was named δ -DDB.

Four isomers of DDB (**114-117**) were obtained via different pathways. The dibromination of three of these biphenyl compounds was readily accomplished at room temperature with a molar ratio of bromine to biphenyl of about 2:1. The structures of the dibrominated biphenyl derivatives **118**, **119** and **110**, prepared from **114**, **115** and **116**, respectively, were identified by their ^1H NMR, MS, IR and EA data. However, compound **117** could not be brominated because of the effects of other functional groups in the biphenyl system.

Testing Anti-HIV Activity In Vitro

The following are examples of methods which can be used to screen the compounds of the present invention for determining at least one therapeutic utility and/or mechanism of action as an anti-viral compound, such as an anti-HIV compound, without undue experimentation, based upon the teaching and guidance presented herein.

First, various concentrations of the compounds can be incubated with a chronically HIV-1 infected T cell line, e.g., ACH-2, and a chronically HIV-1 infected monocytic cell line, e.g., UI. These cell lines are useful in predicting if the compounds of the present invention could induce virus expression *in vivo* when given to an individual who is latently infected with HIV and not actively expressing virus. In addition, when these two cell lines are incubated with the phorbol ester, PMA, HIV-1 expression is increased. Since the compounds of the present invention can inhibit virus replication during an acute HIV-1 infection of

H9 cells, it will be of interest to determine if these compounds can also suppress HIV-1 expression from these two chronically infected cell lines when they are stimulated with PMA.

5 Compounds of the present invention can be tested with other cell types (e.g., freshly isolated cells and/or cell lines) which are infected with HIV. Freshly isolated monocyte/macrophages and peripheral blood mononuclear cells (PBMCs) can be infected with a monotropic isolate of HIV-1, Ba-L and/or a laboratory isolate (e.g., IIIB) of HIV-1, respectively. In addition, virus suppression can be evaluated when a biphenyl derivative is added to acutely
10 HIV-1 (IIIB isolate) infected monocytic cell line, U937, and/or the HIV-2 (dl94 isolate) infected T cell line, HUT-78. These studies will determine if the suppressive effect of the biphenyl derivatives are specific to a particular cell phenotype or a virus isolate.

15 Other studies can be used to screen for the mechanism of action of compounds of the present invention by:

(a) Determining if the compound is capable of inactivating HIV-1 by culturing the compounds with HIV-1 for one hour before adding the virus to H9 cells;

20 (b) Determining if the compounds' mechanism of action is competition with HIV for the same receptor (CD4) on the cell surface. This can be tested by adding HIV-1, biphenyl derivative and H9 cells together and then monitoring the amount of virus produced in the presence and absence of the biphenyl derivative;

25 (c) H9 cells can also be pretreated with the compounds to determine if the effect of the drug is on the cells or on the virus;

30 (d) Molecular biology studies, in which DNA and/or RNA levels can be measured in cells that had been treated with various concentrations of the compounds. This is preferred where negative results are obtained from one or more of methods (a)-(c). Both cellular and/or viral regulatory elements can be examined.

The compounds can also be tested in the presence of nucleoside analogs (AZT, ddI, ddC) or other accepted anti-HIV agents, to determine if the biphenyl derivatives are synergistic with any of these currently licensed antiretroviral agents which can ultimately enhance their individual suppressive capability, especially at lower concentrations.

At least one compound of the present invention can be used for treatment of retroviral (e.g., HIV) infection either alone, or in combination with other modes of therapy known in the art. Such modes of therapy can include chemotherapy with drugs, such as, but not limited to, at least one of AZT, ddC, ddA, ddT, ddI, or any other anti-retroviral agents in combination with each other, or associated with a biologically based therapeutic, such as, for example, soluble CD4, antibodies to CD4, and conjugates of CD4 or anti-CD4, or as additionally presented herein.

Because the compounds of the present invention are relatively less toxic or substantially non-toxic to normal cells, their utility is not limited to the treatment of established retroviral infections. For example, a biphenyl derivative according to the present invention can be used in treating blood products, such as those maintained in blood banks. The nation's blood supply is currently tested for antibodies to HIV. However, the test is still imperfect and samples which yield negative tests can still contain HIV virus. Treating the blood and blood products with the compounds of the present invention can add an extra margin of safety by killing any retrovirus that may have gone undetected.

Pharmaceutical Compositions

Pharmaceutical compositions of the present invention can comprise at least one of the compounds of the present invention. Pharmaceutical compositions according to the present invention can also further comprise other anti-viral agents such as, but not limited to, AZT, ddI, 2'- β -fluoro-ddI, ddA, ddG, ddC, 2'- β -fluoro-ddC, d4T, AzddU, phosphonylmethoxyethyl-adenine, or soluble CD4, or immunomodulators, e.g., as presented below. For a review of

therapeutic agents in HIV infections, see, e.g., Mitsuya *et al.*, *FASEB J.* 5:2369-2381 (1991), which reference is hereby incorporated by reference.

Additional suitable antiviral agents for optimal use with at least one compound of the present invention can include, but are not limited to, AL-721
5 (lipid mixture, manufactured by Ethigen Corporation and Matrix Research laboratories; Amphotericin B methyl ester; Ampligen (mismatched RNA) developed by DuPont/HEM Research; anti-AIDS antibody (Nisshon Food); AS-101 (heavy metal based immunostimulant); AZT (azidothymidine/Retrovir/Zidovudine), manufactured by Burroughs Wellcome;
10 Betaseron (β -interferon) manufactured by Triton Biosciences (Shell Oil); butylated hydroxytoluene; Carrosyn (polymannoacetate) Castanospermine; Contracan (stearic acid derivative); Creme Pharmatex (containing benzalkonium chloride) manufactured by Pharmalec; CS-87 (5-unsubstituted derivative of Zidovudine); Cytovene (ganciclovir) manufactured by Syntex Corporation; ddC
15 (dideoxycytidine) manufactured by Hoffmann LaRoche, and other nucleoside analogues; dextran sulfate; D-penicillamine (3-mercapto-D-valine) manufactured by Carter Wallace and Degussa Pharmaceutical; Foscarnet (trisodium phosphonoformate) manufactured by Astra AB; fusidic acid manufactured by Leo Lovens; glycyrrhizin (a constituent of licorice root); HPA-23 (ammonium-21-tungsto-9-antimonate) manufactured by Rhone-Poulenc Santé; human immune
20 virus antiviral developed by Porton Products International; Ornidyl (eflornithine) manufactured by Merrell-Dow; nonoxinol; pentamidine isethionate (PENTAM-300) manufactured by Lypho Med; Peptide T (octapeptide sequence) manufactured by Peninsula Laboratories; Phenytoin (Warner-Lambert);
25 Ribavirin; Rifabutin (ansamycin) manufactured by Adria Laboratories; rsT4 (recombinant soluble T4) manufactured by Biogen, Genentech and Smith Kline Beecham; Trimetrexate manufactured by Warner-Lambert Company; SK-818 (germanium-derived antiviral) manufactured by Sanwa Kagaku; suramin and analogues thereof manufactured by Miles Pharmaceuticals; UA001 manufactured
30 by Ueno Fine Chemicals Industry; Wellferon (α -interferon) manufactured by

Burroughs Wellcome; Zovirex (acyclovir) manufactured by Burroughs Wellcome.

Pharmaceutical compositions of the present invention can also further comprise immunomodulators. Suitable immunomodulators for optional use with at least one compound of the present invention in accordance with the present invention can include, but are not limited to: AEPP (Bropririmine); Ampligen (mismatched RNA)DuPont/HEM Research; anti-human interferon- α -antibody (Advance Biotherapy and Concepts); anti-AIDS antibody (Nisshon Food); A-101 (heavy metal based immunostimulate; ascorbic acid and derivatives thereof; interferon- β ; Carrosyn (polymannoacetate); Ciamexon (Boehringer-Mannheim); cyclosporin; cimetidine; CL-246,738 (American Cyanamide) ; colony stimulating factors, including GM-CSF (Sandoz, Genetics Institute); dinitrochlorobenzene; interferon- β ; inteferon-gamma; glucan; hyperimmune gamma-globulin (Bayer); IMREG-1 (leukocyte dialyzate) and IMREG-2 (IMREG Corp.); immuthiol (sodium diethylthiocarba-mate) (Institut Merieux); interleukin-1 or interleukin-2 (Cetus Corporation; Hoffmann-LaRoche; Immunex); isoprinosine (inosine pranobex); Krestin (Sankyo); LC-9018 (Yakult); lentinan (Ajinomoto/Yamanouchi); LF-1695 (Fournier); methionine-enkaphalin (TNI Pharmaceuticals; Sigma Chemicals); Minophagen C; muramyl tripeptide, MTP-PE (Ciba-Geigy); naltrexone ("Trexan," DuPont); Neutropin; PNA immunomodulator (Nippon Shingaku) ; shosaikoto and ginseng; thymic humoral factor; TP-05 (Thymopentin, Ortho Pharmaceuticals) ; Thymosin factor 5 and Thymosin 1; Thymostimulin; TNF (Tumor necrosis factor) manufactured by Genentech; and vitamin B preparations.

The preferred animal subjects of the present invention are mammals and birds, with mammals being more preferred. By the term "mammal" is meant an individual belonging to the class Mammalia. The invention is particularly useful in the treatment to human patients.

Medicaments are considered to be provided "in combination" with one another if they are provided to the patient concurrently or if the time between the

administration of each medicament is such as to permit an overlap of biological activity.

In one preferred embodiment, at least one compound of the present invention is present in a single pharmaceutical composition.

5 Pharmaceutical compositions for administration according to the present invention can comprise at least one compound according to the present invention in a pharmaceutically acceptable form optionally combined with a pharmaceutically acceptable carrier. These compositions can be administered by any means that achieve their intended purposes. Amounts and regimens for the
10 administration of a biphenyl derivative according to the present invention can be determined readily by those with ordinary skill in the clinical art of treating a retroviral pathology.

For example, administration can be by parenteral, such as subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal, or buccal routes.
15 Alternatively, or concurrently, administration can be by the oral route. The dosage administered depends upon the age, health and weight of the recipient, type of previous or concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

Compositions within the scope of this invention include all compositions
20 comprising at least one compound according to the present invention in an amount effective to achieve its intended purpose. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise about 0.1 to about 100 mg/kg body weight. The preferred dosages comprise about 1 to about 100 mg/kg body weight
25 of the active ingredient. The most preferred dosages comprise about 10 to about 100 mg/kg body weight.

Therapeutic administration can also include prior, concurrent, subsequent or adjunctive administration of at least one additional compound according to the present invention or other therapeutic agent, such as an anti-viral or immune
30 stimulating agent. In such an approach, the dosage of the second drug can be the same as or different from the dosage of the first therapeutic agent. Preferably, the

drugs are administered on alternate days in the recommended amounts of each drug.

Administration of a compound of the present invention can also optionally include previous, concurrent, subsequent or adjunctive therapy using immune system boosters or immunomodulators. In addition to the pharmacologically active compounds, a pharmaceutical composition of the present invention can also contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Preferably, the preparations, particularly those preparations which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees, and capsules, and also preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration by injection or orally, contain from about 0.01 to 99 percent, preferably from about 20 to 75 percent of active compounds, together with the excipient.

Pharmaceutical preparations of the present invention are manufactured in a manner which is itself known, for example, by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. Thus, pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipients, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired or necessary, to obtain tablets or dragee cores.

Suitable excipients are, e.g., fillers such as saccharide, for example, lactose or sucrose, mannitol or sorbitol; cellulose preparations and/or calcium phosphates, such as tricalcium phosphate or calcium hydrogen phosphate; as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. if desired, disintegrating agents can be added such as the above-mentioned starches and also carboxymethyl starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate.

Auxiliaries are, above all, flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethyl cellulose phthalate are used. Dyestuffs or pigments can be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules which can be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils or liquid paraffin. In addition, stabilizers can be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories which consist of a combination of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions can be administered. Suitable lipophilic solvents or

vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides. Aqueous injection suspensions that can contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension can also contain stabilizers.

A pharmaceutical formulation for systemic administration according to the invention can be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation can be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, dragees, pills, tablets, including coated tables, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

Solid dosage forms in addition to those formulated for oral administration include rectal suppositories.

The compounds of the present invention can also be administered in the form of an implant when compounded with a biodegradable slow-release carrier. Alternatively, at least one compound of the present invention can be formulated as a transdermal patch for continuous release of the active ingredient.

Suitable formulations for topical administration include creams, gels, jellies, mucilages, pastes and ointments. Suitable injectable solutions include intravenous subcutaneous and intramuscular injectable solutions. Alternatively, at least one compound may be administered in the form of an infusion solution or as a nasal inhalation or spray.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Examples

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. ¹H NMR spectra were obtained using a Bruker AC-300 instrument with TMS or trimethylsilylpropionate-d₄ (for D₂O solvent) as an internal standard, and chemical shifts are given in δ (ppm). Mass spectra were measured on a VG Trio-1000. Elemental analyses were performed by Atlantic MicroLab Inc, Norcross, GA. TLC was conducted on precoated Kieselgel 60 F₂₅₄ plates (0.20 mm, Merck), and spots were detected by UV illumination. EM Kieselgel 60 (70- 230 mesh ASTM) was used for column chromatography. 0.0982N NaOH aqueous solution was purchased from Aldrich. All new compounds were characterized by ¹H NMR and IR spectral analyses and elemental analyses.

Example 1

Bromination of Biphenyl Compounds

The following biphenyl compounds were employed as starting materials: 4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl, 4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy-2,2'-dimethoxycarbonyl biphenyl, and 4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy-2,2'-dihydroxymethyl biphenyl. A solution of biphenyl (300-500 mg, 0.66-1 mmol) in chloroform (8-10 mL) was added dropwise to bromine (1 or 2 mol equivalent) in chloroform (5-8 mL) at 0-5°C. The temperature was elevated to room temperature, and the mixture was stirred for 3 hours. The reaction mixture was poured into 20% sodium bisulfite aqueous solution (40-50 mL), which was extracted with chloroform. The chloroform layer was washed successively with 5% aqueous sodium bicarbonate, water, and brine, dried over sodium sulfate, and concentrated to dryness. The residue was crystallized from EtOH or purified by silica gel chromatography [hexane-ethyl acetate (7:1 or 4:1)] to yield mono- or dibromide.

3-Bromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (4). Yield: 85%; mp 157 -159°C; IR (KBr) 1720 (CO)cm⁻¹; ¹H NMR(CDCl₃) δ 3.62(s, 3H, 2'-COOCH₃), 3.71 (s, 3H, 2-COOCH₃), 3.97 (s, 3H, 4'-OCH₃), 4.08 (s, 3H, 4-OCH₃), 6.01, 6.04 (m, 4H in total, -OCH₂O-), 7.33 (s, 1H, H-3'). Anal. (C₂₀H₁₇O₁₀Br) C, H.

3,3'-Dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (5). Yield: 98%; mp 227-229°C; IR (KBr) 1710 (CO)⁻¹; ¹H NMR(CDCl₃) δ 6.01, 6.03 (each s, 2H-OCH₂O). Anal. (C₂₀H₁₆O₁₀Br₂) C, H, Br.

2,2'-Dimethoxycarbonyl-3-bromo-4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy biphenyl (16). Yield: 85%; A white amorphous powder, ¹H NMR (CDCl₃) δ 3.51, 3.60, 3.73, 3.74 (each s, 3H, OCH₃), 5.10 - 5.16 (m, 8H in total, PhCH₂O), 7.27 - 7.53 (m, 21H in total, aromatic-H and H-3'). Anal. (C₄₆H₄₁O₁₀Br) C, H.

2,2'-Dimethoxycarbonyl-3,3'-dibromo-4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy biphenyl (17). Yield: 90%; A white amorphous powder, ¹H NMR (CDCl₃) δ 3.61, 3.79 (each s, 6H, OCH₃), 5.10 - 5.12 (m, 8H in total, PhCH₂O), 7.33 - 7.50 (m, 20H in total, aromatic-H). Anal. (C₄₆H₄₀O₁₀Br₂) C, H, Br.

2,2'-Dihydroxymethyl-3-bromo-4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy biphenyl (18). Yield: 76%; A white amorphous powder, ¹H NMR (CDCl₃) δ 3.60, 3.67 (each s, 3H, OCH₃), 4.09, 4.17, 4.25, 4.50 (each d, J=12 Hz, 1H, CH₂OH), 5.09 - 5.16 (m, 8H in total, PhCH₂O), 7.00 (s, 1H, H-3'), 7.28 - 7.55 (m, 20H in total, aromatic-H). Anal. (C₄₄H₄₁O₈Br) C, H.

2,2'-Dihydroxymethyl-3,3'-dibromo-4,4',5,5'-tetrakis(benzyloxy)-6,6'-dimethoxy diphenyl (19). Yield: 78%; A white amorphous powder, ¹H NMR (CDCl₃) δ 3.61 (each s, 3H, OCH₃), 4.50, 4.56 (each d, J= 12 Hz, 1H, CH₂OH), 5.11 (s, 4H, PhCH₂O), 5.13, 5.18 (each d, J=12 Hz, 2H, PhCH₂O), 7.32 - 7.55 (m, 20H in total, aromatic-H). Anal. (C₄₄H₄₀O₈Br) C, H.

Example 2**3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy-2,2'-dicarboxy biphenyl (6)**

A solution of 3 (500 mg, 0.87 mmol) in 40% potassium hydroxide aqueous solution (12 mL) and ethanol (20 mL) was refluxed for 4.5 hours. After cooling, the reaction mixture was neutralized with 37% hydrochloric acid. The resulting white precipitate was collected by filtration, washed with water, and dried to give the dicarboxylic acid (6) Yield: 90%; mp 230°C (decomp); IR (KBr) 3000 - 2500 (COOH), 1690 (CO) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ 4.03 (s, 6H, 4,4' -OCH₃) 5.95, 6.0 (each s, 2H, -OCH₂O-) MS m/z (%) 546 (M) + (24) 548 (M+2) + (48) 550 (M+4) + (23). Anal. ($\text{C}_{18}\text{H}_{12}\text{O}_{10}\text{Br}_2$) C, H, Br.

Example 3**3,3'-Dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dicarboxy biphenyl disodium salt (7)**

A solution of 6 (107.2 mg, 0.1963 mmol) in 0.0892N sodium hydroxide 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 (decomp); IR (KBr) 1585, 1380 (COO⁻) cm^{-1} ; $^1\text{H NMR}$ (D_2O) δ 3.93 (s, 6H, 4,4' -OCH₃), 5.85, 5.90 (each s, 2H, -OCH₂O-); Anal. ($\text{C}_{18}\text{H}_{10}\text{Br}_2\text{Na}_2 \cdot 2\text{H}_2\text{O}$) C, H.

Example 4**3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy biphenyl-2,2'-dicarboxylic anhydride (8)**

A solution of 6 (200 mg, 0.36 mmol) in acetic anhydride (3 mL) was refluxed for 24 hours 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 ethyl acetate 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); $^1\text{H NMR}$ (CDCl_3) δ 4.11 (s, 6H, 4, 4' -OCH₃), 6.06, 6.11 (each s, 2H, -OCH₂O-); Anal. ($\text{C}_{18}\text{H}_{10}\text{Br}_2$)C, H, Br.

Example 5**3,3'-Dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2-ethoxycarbonyl-2'-carboxy biphenyl (9)**

A solution of **8** (150 mg, 0.284 mmol) in ethanol (25 mL) was refluxed for 3 days with stirring. After removal of the solvent by evaporation, the residue was treated with 5% sodium hydroxide aqueous solution (20 mL), washed with ethyl acetate, acidified with 10% aqueous hydrochloric acid, and extracted with ethyl acetate. The ethyl acetate layer was concentrated to dryness to furnish **9** (134 mg) as a white powder. Yield 84%; mp 239°C (decomp.); IR (KBr) 3000-2500 (COOH), 1725 (CO) cm^{-1} ; $^1\text{H NMR}$ (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, 2x- OCH_2O -); Anal. ($\text{C}_{20}\text{H}_{16}\text{O}_{10}\text{Br}_2 \cdot 1.5 \text{H}_2\text{O}$) C, H, Br.

Example 6**3,3'-Dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2-ethoxycarbonyl-2'-carboxy biphenyl monosodium salt (10)**

A solution of **9** (56 mg, 0.0982 mmol) in 0.0982 N sodium hydroxide aqueous solution (1 mL, 0.0982 mmol) was kept standing at room temperature for 5 minutes. Work up as for **7** gave the mono sodium salt (**10**) (52 mg) as a white solid. Yield: 90%; mp 227°C; IR (KBr) 1725 (CO) 1590, 1380 (COO^-) cm^{-1} ; $^1\text{H NMR}$ (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, 2x- OCH_2O -); Anal. ($\text{C}_{20}\text{H}_{15}\text{O}_{10}\text{Br}_2\text{Na}$) C, H, Br.

Example 7**3,3'-Dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2-carboxy-2'-(β -N,N,N-trimethylamino)ethoxycarbonyl biphenyl chloride (11)**

A mixture of **8** (950 mg, 1.8 mmol) and choline 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 methanol gave **11** (1.02 g) as colorless prisms. Yield: 85%; mp 210°C (decomp.); $^1\text{H NMR}$ (DMSO- d_6) δ 3.11(br s, 9H, N- CH_3) 3.40

-25-

(t, $J=6$ Hz, 2H, N-CH₂), 3.82 (m, 2 H, CH₂O), 3.97 (s, 6H, 4, 4' -OCH₃), 5.96, 6.03 (each s, 2H, -OCH₂O-); Anal. (C₂₃H₂₄N₁₀Br₂Cl) C, H.

Example 8

3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy-2-carboxy-2'-(β -N-piperazine) ethylcarbonyl biphenyl (12)

5 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 hours. The reaction mixture was concentrated to dryness to give an off-white solid. Crystallization from methanol gave 12 (693 mg) as an off-white solid.
10 Yield: 99%; mp 184°C (decomp); ¹H NMR (CD₃OD) δ 2.52 (4H, m, 2xNHCH₂ of piperazine), 2.84 (2H, t, $J=6$ Hz, 2'CONHCH₂), 2.88 (6H, m, 3xNCH₂), 3.98 (6H, s, 4, 4' -OCH₃), 5.86, 5.93 (each 2H, s, -OCH₂O-); Anal. (C₂₄H₂₅N₃O₉Br₂•2H₂O) C, H.

Example 9

3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy-2-carboxy-2'-(β -N-pyrrolidinyl)ethylcarbonyl biphenyl Hydrobromide (13)

15 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 hour. The reaction mixture was concentrated to dryness to give an off-white
20 solid. Crystallization from ethanol containing 2% HBr yielded 13 (122 mg) as a white powder. Yield: 58%; mp 188°C (decomp.); IR (KBr) 1710 (CO) 1610, 1590 (amide) cm⁻¹; ¹H NMR (CD₃OD) δ 2.04, 2.52, (each m, 2H, CH₂), 3.16 (t, $J=6$ Hz, 2H, 2' -CONHCH₂), 3.41 (m, 4H, 2xNCH₂), 3.58 (m, 4H, 2xNCH₂) 3.58 (m, 4H, 2xNCH₂ of pyrrolidine), 4.00, 4.11 (each s, 3H, 4,4' -OCH₃), 6.02, 6.08
25 (each s, 2H, -OCH₂O-); Anal. (C₂₄H₂₅N₂O₉Br₃•H₂O) C, H.

Example 10

3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy-2,2'-dicarboxy biphenyl di-1-(2-hydroxyethyl)pyrrolidinium salts (14)

30 A mixture of 6 (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 (D₂O) δ 1.95 (m, 8H, 4xCH₂ of pyrrolidine) 3.19 (t, J=5 Hz, 4H, 2xNCH₂), 3.26 (m, 8H, NCH₂ of pyrrolidine), 3.75 (t, J=5, Hz, 4H, CH₂O), 3.93 (s, 6H, 4,4' -OCH₃), 5.83, 5.88 (each s, 2H, OCH₂O). Anal. (C₃₀H₃₈N₂O₁₂Br₂) C, H.

Example 11

3,3'-Dibromo-4,4'-dimethoxy-5,6,5'6'-bimethylenedioxy-2,2'-dihexanoxycarbonyl biphenyl (15)

To a solution of **6** (273 mg, 0.5 mmol) in 1-hexanol (7 mL), thionyl chloride (2 mL) was added 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-ethyl acetate (4:1) gave **15** (134 mg) as a white powder. Yield: 37%, mp 115 - 117°C; IR (KBr) 2960, 2930, 2860 (CH₂), 1730 (CO) cm⁻¹; ¹H NMR (CDCl₃) δ 0.88 (t, J=6.5 Hz, 6H, 2xCH₃), 1.25(m,12H, 6xCH₂), 1.47(m, 4H, 2xCH₂), 4.07 (s, 6H, 4,4' -OCH₃), 4.07-4.14 (m, 4H, 2xCH₂O), 5.99, 6.01 (each s, 2H, OCH₂O). Anal. (C₃₀H₃₆O₁₀Br₂): C, H, Br.

Example 12

Total Synthesis of DDB Derivatives from Gallic Acid

Methyl 2,3,4-trihydroxybenzoate (120)

Six grams (94 mmol) of 2,3,4-trihydroxybenzoic acid in 200 mL of methanol was refluxed in the presence of molecule sieves and 2 mL of sulfuric acid for five days. After the usual workup, 16.92 grams of methyl ester **111** was obtained in a 98% yield, m.p. 150-152°C (recrystallized from ethyl acetate).

IR cm⁻¹ 3345 (br, s, OH), 1645 (chelated ester C=O) and 1260 (ester C-O); ¹H NMR δppm 3.92 (s, 3H, OCH₃), 5.46 and 5.79 (each 2, 1H, OH exchangeable with D₂O), 10.98 (s, 1H, chelated OH), 6.51 and 7.35 (each d, J=9Hz, 1H, ArH). Anal (C₈H₈O₅) C, H.

Methyl 2, 3-dihydroxy-4-methoxybenzoate (121)

Compound **120** (18.4 g, 0.1 mol) in 5% aqueous borax solution was treated with 47 mL of methyl sulfate and 100 mL of 20% sodium hydroxide solution at 30°C. The mixture was stirred for five hours at room temperature. Then the mixture was cooled in an ice-bath and neutralized to pH 2 with hydroxyl chloride (37%). A precipitate occurred and was filtered and washed with water until the filtrate was neutral. The dried product **121** weighed 16.19g (yield 86%) and was recrystallized from dilute aqueous ethanol as needles. m.p. 100-101°C. IR cm^{-1} 3330 and 3420 (OH), 1660 (chelated ester C=O) and 1255 (ester C-O); ^1H NMR δ ppm 3.94 and 3.95 (each s, 3H, OCH_3), 5.51 (s, 1H, OH), 6.51 and 7.42 (each d, $J=9\text{Hz}$, 1H, ArH), 10.84 (s, 1H, chelated OH). Anal. ($\text{C}_9\text{H}_{10}\text{O}_5$) C, H.

Methyl 2,3-methylenedioxy-4-methoxybenzoate (122)

Compound **121** (16.12 g, 0.081 mol) was dissolved in 500 mL of DMF. Then 100 mL of methylene chloride and 26 g (0.464 mol) of potassium carbonate were added. The mixture was heated to 105°C with vigorous stirring for 6 hours. The potassium carbonate was filtered and the DMF was removed *in vacuo*. The residue was poured into ice-water and let stand overnight. The precipitated product **122** was filtered and washed with water until neutral. The dried gray white solid weighed 15.60 g (yield 91%) and was recrystallized from methanol as brilliant long needles, m.p. 122-124°C. IR cm^{-1} 1710 (ester C=O); ^1H NMR δ ppm 3.90 (s, 3H, OCH_3), 3.95 (s, 3H, ArOCH_3), 6.11 (s, 2H, OCH_2O), 6.57 and 7.45 (each d, $J=9\text{Hz}$, 1H, ArH). Anal. ($\text{C}_{10}\text{H}_{10}\text{O}_5$)C, H.

Methyl 2,3-methylenedioxy-4-methoxy-5-bromobenzoate (123)

Compound **122** (5.56 g, 25.6 mmol) was dissolved in anhydrous chloroform. Bromine (4.5 g, 28.5 mmol) was added dropwise over 2 hours at 5 - 8°C. Then the mixture was maintained for an additional 2 hours at the same temperature. After the usual workup, 6.9 g of white solid **123** was obtained (88% yield). m.p. 165-6°C (crystallized from ethyl acetate). IR cm^{-1} 1710 (s, C=O),

1425, 1400 (s, C-O); MS m/z (%): 288 (M^+ , 100), 290 ($M+2$, 96); $^1\text{H NMR } \delta$ ppm 3.91 (s, 3H, OCH_3), 4.14 (s, 3 H, ArOCH_3), 6.10 (s, 2H, OCH_2O), 7.65 (s, 1H, 6 -ArH); Anal. ($\text{C}_{10}\text{H}_9\text{O}_5\text{Br}$) C, H, Br.

Methyl 2,3-methylenedioxy-4-methoxy-5-iodobenzoate (124)

5 A mixture of dry silver trifluoroacetate (442 mg, 2 mmol) and compound
122 (420 mg, 2 mmol) was placed in a reaction flask. Then a solution of iodine
(506 mg, 2 mmol) in anhydrous methylene chlorate (8 mL) was added under
reflux over a period of 2 hours. To this reaction mixture was added more silver
trifluoroacetate (40 mg) and iodine (59 mg) and reflux was continued until the
10 starting material had disappeared on thin layer chromatography. The mixture was
then filtered and the filtrate was concentrated. The resulting yellowish solid was
crystallized from DMF as short needles (190 mg, yield 28%). m.p. 162-4°C. IR
 cm^{-1} 1706 (ester $\text{C}=\text{O}$), 1390 (C-O); $^1\text{H NMR } \delta$ ppm 3.90 (s, 3H, OCH_3) 4.13 (s,
3H, ArOCH_3), 6.10 (s, 2H, OCH_2O), 7.88 (s, 1H, ArH). Anal. ($\text{C}_{10}\text{H}_9\text{O}_5\text{I}$) C, H,
15 I calcd. 37.90; found 37.80.

Methyl 2,3-methylenedioxy-4-methoxy-5- and -6-nitrobenzoate (125 and 126)

To a stirred suspension of compound 122 (442 mg, 2 mmol) in acetic
anhydride (5 mL), concentrated nitric acid (1 mL) was added dropwise over a
period of 20 minutes. The reaction temperature was maintained at 10 - 22°C.
20 The reaction mixture was stirred for an additional 30 minutes and then was
poured into ice water. A yellowish solid (437 mg) was collected. Total yield was
86%. It was separated by silica gel column chromatography to afford the 5-nitro
compound 125 and 6-nitro compound 126 (ratio 1:10).

25 **125:** m.p. 162-4°C $^1\text{H NMR } \delta$ ppm 3.93 (s, 3H, OCH_3), 4.19 (s, 3H, ArOCH_3),
6.23 (s, 2H, OCH_2O), 8.11 (s, 1H, 6 -ArH). Anal. ($\text{C}_{10}\text{H}_9\text{NO}_7$) C, H, N.

126: m.p. 145-7°C (yellowish needles from ethyl acetate); IR cm^{-1} 1735 (s,
 $\text{C}=\text{O}$); MS m/z (%): 255 (M^+ , 100); $^1\text{H NMR } (\text{CD}_3\text{COCD}_3) \delta$ ppm 3.86 (s, 3H,
 OCH_3), 4.04 (s, 3H, ArOCH_3), 6.31 (s, 2H, OCH_2O), 7.48 (s, 1H, 5-ArH). Anal.
($\text{C}_{10}\text{H}_9\text{NO}_7$) C, H, N.

Methyl 2,3-methylenedioxy-4-methoxy-6- and 5-aminobenzoate (127 and 128)

A solution of **126** (225 mg, 1 mmol) in ethanol (10 ml) and ethyl acetate (5 mL) was placed in a reaction bottle and 10% palladium carbon catalyst (54 mg) was added. Hydrogen was added under vigorous stirring at room temperature and 1 atmosphere pressure for 4 hours. Then the catalyst was filtered and solvent was removed *in vacuo*. The amino compound **128** (148 mg) was thus obtained in a 93% yield. m.p. 163-4°C. IR cm^{-1} 3450, 3320 (NH), 1665 (chelated ester C=O); MS m/z (%): 225 (M^+ , 100); $^1\text{H NMR}$ (CD_3COCD_3) δ ppm 3.77 (s, 3H, OCH_3), 3.82 (s, 3H, ArOCH_3), 5.88 (s, 3H, OCH_2O), 6.02 (s, 1H, 5-ArH), 6.21 (br. s, 1H, NH exchangeable with D_2O), 10.72 (s, 1H, chelated NH). Anal. ($\text{C}_{10}\text{H}_{11}\text{O}_5\text{N}$) C, H, N.

According to the above procedure, **129** was obtained from **125** in a 84% yield. m.p. 139-40°C $^1\text{H NMR}$ δ ppm 3.67 (br. s, 2H, NH_2), 3.89 (s, 3H, OCH_3), 4.09 (s, 3H, ArOCH_3), 5.99 (s, 2H, OCH_2O), 6.78 (s, 1H, 6-ArH). Anal. ($\text{C}_{10}\text{H}_{11}\text{O}_5\text{N}\cdot 1/2\text{H}_2\text{O}$) C, H.

Methyl 2,3-methylenedioxy-4-methoxy-6-iodobenzoate (128)

A suspension of compound **127** (400 mg, 1.78 mmol) in 20% sulfuric acid (2 mL) was cooled to 0-5°C and then a solution of sodium nitrite (127 mg, 1.78 mmol) in water (4 mL) was added. After the diazonium salt was formed, a cooled solution of potassium iodide (332 mg, 2 mmol) in H_2O (2 mL) was added with evolution of a gas. Copper powder (340 mg) was added and the reaction mixture was stirred for 20 minutes, then heated on a water bath to 80 - 90°C for 1 hour. Nitrogen evolved vigorously. When the reaction subsided, the mixture was extracted with chloroform. A deep red oil (2.43 g) was obtained and was purified by silica gel column chromatography (eluent hexane:chloroform = 4:1) giving 150 mg of **128** (25% yield). m.p. 146-7°C (white solid). IR cm^{-1} 1705 (C=O), 1285 and 1265 (s, C-O); MS m/z (%): 336 (M^+ , 100); $^1\text{H NMR}$ δ ppm 3.93 (s, 6H, 2x OCH_3), 6.07 (s, 2H, OCH_2O), 7.11 (s, 1H, ArH). Anal. ($\text{C}_{10}\text{H}_9\text{O}_5\text{I}$) C, H.

5,5'-Dimethoxy-3,4,3',4'-bismethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (ω -DDB)(116)

Method A (Ullmann reaction):

5 A mixture of compound **128** (1g, 2.85 mmol), activated copper powder (1g) an anhydrous DMF (3.5 mL) was heated to reflux for 2.5 hours under vigorous stirring. After cooling to 100°C, it was poured into ice water and the precipitate was collected and chromatographed on alumina. Elution with hexane, then hexane-chloroform afforded ω -DDB **116** (280 mg, 44% yield). m.p. 253-5°C. IR cm^{-1} 1730 (C=O), 1175 and 1150 (s, C-O); MS $m/z(\%)$: 418 (M^+ , 100);
10 $^1\text{H NMR } \delta$ ppm 3.68 (s, 6H, 2xOCH₃), 3.92 (s, 6H, 2xOCH₃), 6.13 (s, 4H, 2xOCH₂O), 6.36 (s, 2H, 2xArH). Anal. (C₂₀H₁₈O₁₀) C, H.

Method B:

5 Copper (II) sulfate (1.25 g) was dissolved in 5 mL of water containing 2.5 mL ammonium hydroxide (conc.). The mixture was cooled in an ice-bath to
15 below 10°C and solution of 0.4 g hydroxylamine chloride in 2 mL water and 1 mL sodium hydroxide (6N) was added. The diazonium salt of compound **127** (225 mg), prepared according to the above method given above in the synthesis of **128**, was slowly added into the copper (II) solution and remained at <10°C with vigorous stirring. The blue color faded gradually. The mixture was heated
20 to 80 - 90°C for a few minutes, then cooled at room temperature and neutralized with hydrochloric acid to pH 2 - 3. After the usual work up, 86 mg of pure compound **116** was obtained (41% yield).

2,2'-Dimethoxy-3,4,3',4'-bismethylenedioxy-5,5'-dimethoxycarbonyl biphenyl (δ -DDB)(117)

25 Method A (Ullmann reaction):

Compound **117** was obtained from **123** in a 10% yield using method A described in the previous example. m.p. 195-200°C. IR cm^{-1} 1700 (s, ester C=O), 1430, 1270 and 1210 (s, C-O); MS $m/z(\%)$: 418 (M^+ , 100); $^1\text{H NMR } \delta$ ppm 3.90 and 4.00 (each s, 3H, OCH₃), 6.13 (s, 4H, OCH₂O), 7.30 (s, 2H, ArH). Anal. (C₂₀H₁₈O₁₀), C, H.

30 Method B:

Compound 117 was obtained in a 40% yield from 129 using method B as described in the previous example.

Example 13

Bromination of biphenyl derivatives

5 A biphenyl compound in chloroform was dropped slowly into two molar equivalents of bromine at 0-5°C. The mixture was stirred at room temperature for 3 hours and poured with stirring into ice-water containing sodium disulfide until the orange-red color faded completely. The organic phase was separated and washed successively with aqueous sodium bicarbonate, water and brine, then
10 dried over sodium sulfate. The organic solvent was removed *in vacuo*. The crude produce was purified by crystallization.

3,3'-Dibromo-6,6'-dimethoxy-4,5,4',5'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (118) Obtained in a 87% yield from 123. Crystals from chloroform and methanol (2:1); m.p. 181-2°C; MS m/z(%): 574 (M⁺, 48), 576 (M+2, 100), 578 (M+4, 51); ¹HNMR δ ppm 3.67 (s, 6H, 2,2' -OCH₃), 3.90 (s, 6H, 6, 6' -OCH₃), 6.12 (s, 4H, 2xOCH₂O), Anal. (C₂₀H₁₆O₁₀Br₂) C, H.

3,3'-Dibromo-4,6'-dimethoxy-5,6,4'5'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (119) Obtained in a 82% yield from 115. Crystals from chloroform and methanol (2:1). m.p. 166-9°C. MS m/z(%): 574 (M⁺, 45), 576 (M+2, 90), 578 (M+4, 45), 59 (100); ¹HNMR δ ppm 3.68 and 3.69 (each s, 3H, 2,2'OCH₃), 3.91 (s, 3H, 4-OCH₃), 4.09 (s, 3H, 6'-OCH₃), 5.98-6.08 (m, 4H, 2xOCH₂O). Anal. (C₂₀H₁₆O₁₀Br₂) C, H.

6,6'-Dibromo-5,5'-dimethoxy-3,4,3'4'-bimethylenedioxy-2,2'-dimethoxycarbonyl biphenyl (110) Obtained in 98% yield from 116. m.p. 187-8°C (crystals from ethyl acetate). MS m/z(%): 574 (M⁺, 50) 576 (M+2, 100), 578 (M+4, 50); ¹H NMR δ ppm 3.69 (s, 6H, 2, 2' OCH₃), 4.14 (s, 6H, 5, 5' -OCH₃), 6.10 and 6.13 (each s, 4H, 2xOCH₂O). Anal. (C₂₀H₁₆O₁₀Br₂•1/2H₂O) C, H.

ELEMENTAL ANALYSES

		Calculated			Found		
		C	H	Br	C	H	Br
4	$C_{20}H_{17}O_{10}Br$	48.31	3.45		47.97	3.38	
5	$C_{20}H_{16}O_{10}Br$	41.69	2.80	27.74	41.66	2.83	27.67
6	$C_{18}H_{12}O_{10}Br_2$	39.45	2.81	29.16	39.39	2.24	29.07
5	7 $C_{18}H_{10}O_{10}Br_2Na_2 \cdot 2H_2O$	34.42	2.25		34.15	2.23	
8	$C_{18}H_{10}O_9Br_2$	40.79	1.89	30.15	40.80	1.93	30.09
9	$C_{20}H_{16}O_{10}Br_2 \cdot 1.5H_2O$	39.83	3.18		39.83	2.78	
10	$C_{20}H_{15}O_{10}Br_2Na$	40.16	2.53		39.90	2.54	
11	$C_{23}H_{24}NO_{10}Br_2Cl$	41.25	3.61		40.96	3.88	
10	12 $C_{24}H_{25}N_3O_9Br_2 \cdot 2H_2O$	41.46	4.20		41.12	4.42	
13	$C_{24}H_{25}N_2O_9Br_3 \cdot 0.5H_2O$	39.21	3.70		39.24	3.56	
14	$C_{30}H_{38}N_2O_{12}Br_2$	46.29	4.92		46.11	5.25	
15	$C_{30}H_{36}O_{10}Br_2$	50.30	5.06	22.31	50.53	5.11	22.14
16	$C_{46}H_{41}O_{10}Br$	66.27	4.96		66.10	5.02	
15	17 $C_{46}H_{40}O_{10}Br_2$	60.54	4.42	17.51	60.17	4.49	17.66
18	$C_{44}H_{41}O_8Br$	67.95	5.31		68.17	5.37	
19	$C_{44}H_{40}O_8Br_2$	61.70	4.71		61.66	4.47	

ELEMENTAL ANALYSES (continued)

		Calculated			Found			
		C	H	N	C	H	N	
61	$C_{20}H_{18}O_{10}$	57.42	4.34		57.21	4.59		
71	$C_{20}H_{18}O_{10}$	57.42	4.34		57.32	4.34		
81	$C_{20}H_{16}O_{10}Br_2$	41.82	2.81		41.48	2.94		
5	91	$C_{20}H_{16}O_{10}Br_2$	41.82	2.81		41.78	2.91	
	110	$C_{20}H_{16}O_{10}Br_2 \cdot 1/2H_2O$	41.12	2.92		41.27	2.79	
	111	$C_8H_8O_5$	52.16	4.38		51.99	4.34	
	112	$C_9H_{10}O_5$	54.53	5.09		54.51	5.09	
	113	$C_{10}H_{10}O_5$	57.13	4.80		57.01	4.80	
10	114	$C_{10}H_9O_5Br$	41.67	3.15		41.65	3.13	
	116	$C_{10}H_9O_7N$	47.05	3.56		47.21	3.59	
	117	$C_{10}H_9O_7N$	47.05	3.56	5.49	47.22	3.57	5.52
	118	$C_{10}H_{11}O_5N$	53.32	4.93	5.49	53.49	5.03	5.42
	119	$C_{10}H_9O_5I$	35.72	2.70	6.22	35.86	2.79	5.84
15	120	$C_{20}H_{11}O_5N \cdot 1/2H_2O$	47.61	5.60		47.30	5.43	

Example 14
Pharmacological Activity

HIV Growth Inhibition Assay.

20 The H9 T cell line was maintained in continuous culture with complete medium (RPMI 1640 and 10% fetal calf serum) pharmacological activity at 5% CO₂ and 37°C and was used in experiments only when in log phase of growth. The cells were incubated with HIV-1 (IIIB isolate, TCID₅₀10⁴IU/mL, at a multiplicity of infection of 0.1 - 0.01 IU/cell) for 1 hour at 37°C and 5% CO₂.

25 The cells then were washed thoroughly to remove unabsorbed virions and resuspended at 4 X 10⁵ 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 four 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 is used to tag any p24 "captured" onto the microliter well surface. Peroxidase conjugated goat anti-rabbit serum is then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples is then revealed by addition of substrate. The cut-off for the p24 ELISA assay is 12.5 pg/mL. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. The effective (EC₅₀) and inhibitory (IC₅₀) concentrations (for anti-HIV activity and cytotoxicity, respectively) were determined.

Preparation of HIV-1 Reverse Transcriptase.

HIV-1 reverse transcriptase was purified using *Escherichia coli* J-M 109 containing PKRT 2 kindly provided by W.C. Summers (Yale University).

Enzyme Assay.

All reactions were developed in a total volume of 50µl containing 50 mM Tris HCl pH 7.8, 2mM MgCl₂, 100µg/mL nuclease-free BSA, 1 mM dithiothreitol, 0.5 O.D.₂₆₀ unit/mL of template-primer, and 330nM of [³H]dNTP according to Cheng *et al.*

Chronically HIV-infected cell line.

HIV-1 chronically infected T cell line, ACH-2, and HIV-1 chronically infected promonocytic cell line, U1¹⁶ were continuously maintained in RPMI1640 with 10% fetal calf serum (FCS). For experiments, the cell lines were only used in log-phase of growth. Cells (1 X 10⁶ 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% CO₂, an aliquot of the cell-free supernatants was collected and analyzed for p24 antigen by ELISA.

5 **Results**

The anti-HIV activities of the biphenyl derivatives are shown in Table IA. Among these compounds, 3,3'-dibromo DDE (5) demonstrated potent anti-HIV activity with an EC₅₀ of 0.23 µg/mL and TI of >480. Comparison of the anti-HIV activities of compounds suggested that the relative position and types of substituents on the phenolic hydroxy groups rather than the numbers of bromine atoms on the aromatic rings are the most important. The 2- (or 2'-) methoxycarbonyl and 4- (or 4'-) methoxy groups on the biphenyl ring as found in compounds 111 and 115 composed an essential anti-HIV structural feature. Activity could then be enhanced greatly by a bromine introduced at the 3- or 3'-position, as found in compounds 4, 5, and 119. Table IA shows this structure-activity relationship with an order of activity of 5>4>119>111>115 (based on Figure 4). The other five compounds have no or less anti-HIV activity, regardless of the presence of bromine in the molecule. Therefore, the replacement of the methyl carboxylate groups at C-2 and C-2' or the methoxy groups at C-4 and C-4' by other groups led to a decrease in the anti-HIV activity.

Preliminary mechanism of action studies with compounds 4 and 5 have shown template-primer HIV-1 reverse transcriptase inhibitory activity. This type of inhibitory activity has also been observed for other non-nucleoside HIV reverse transcriptase inhibitors. Potent inhibitory effects were also found with 4 and 5 against HIV-1 reverse transcriptase associated DNA polymerase and RNase II activities under certain conditions.

The structures of all of the biphenyl derivatives described above were confirmed by their spectral data and elemental analyses.

The anti-HIV activity of the biphenyl derivatives described above is shown in Table I. Among these compounds, 3,3'-dibromo DDB, compound 5,

demonstrated potent anti-HIV activity with an EC_{50} value of 0.23 $\mu\text{g/mL}$. It also exhibited a good therapeutic index of >480 . Compound **4**, 3-bromo DDB, also showed potent anti-HIV activity, with EC_{50} and TI values of 0.52 $\mu\text{g/mL}$ and 190, respectively. Comparison of the anti-HIV activities of compounds **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** showed no anti-HIV activity in the tests conducted. Compounds **18** and **19** likewise showed no anti-HIV activity to date, although their parent compound (**2**) does possess anti-HIV activity, which is comparable to that of DDB.

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

Table I Anti-HIV Activities of Brominated Hexahydroxybiphenyl Derivatives in Acutely Infected H9 Lymphocytes			
Compound Number	IC ₅₀ (μg/ml) ^a	EC ₅₀ (μg/ml)	Therapeutic Index ^b
3	>100	5	>20
4	>100	0.52	>190
5	>100	0.23	>480
6	>100	>100	1
7	>100	>100	1
8	>100	-	-
9	>100	60	>1.7
10	>100	-	-
11	>100	>100	1
12	>100	30	>3.3
13	>100	>100	1
14	>20 but <100	17	>1.2 but <5.9
15	>100	2	>50
16	>100	>100	1
17	>100	>100	1
18	>100	100	>1
19	>100	>100	1
111	>100	5	>20
4	100	0.52	190
5	>100	0.23	>480
114	>100	65	>1.2
115	>100	7.5	>13
116	>100	70	>1.4
117	1.8	no inhibition	-
118	>100	90	>1.1
119	>100	2.1	>48
110	45	no inhibition	-

^a IC₅₀ refers to the concentration of drug that causes a 50 % reduction in total cell number. Drugs that have IC₅₀ values > 100 μg/ml can not be tested at higher concentrations for a more exact IC₅₀ value due to the effect of the solvent, DMSO.

^b Therapeutic Index (TI) is a ratio of the IC₅₀ value/EC₅₀ value. Therefore, when the IC₅₀ value is > 100 μg/ml (refer to a above), the TI value must also be reported as greater than.

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 II, these compounds exhibited template-primer dependent HIV-1 RT inhibitory activity. This type of inhibitory activity was also observed with other nonnucleoside HIV

RT inhibitors. 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 with Poly(rC)- olig(dG) as template-primer than with Poly(rA)-olig(dT). In contrast, the enzyme was about 6 times more sensitive to 5 with Poly(rA)-olig(dT) as template-primer than with Poly(rC)-olig(dG). Nevarapine, a non-nucleoside RT inhibitor, exerts more inhibitory activity using Poly(rC)-olig(dg) than with Poly(rA)-olig(dT). Compounds 4 and 5 also demonstrated potent inhibitory effects against HIV-1 reverse-transcriptase-associated DNA polymerase activity using Poly(dA)-olig(dT) as template primer with IC₅₀ values of 0.056 and 0.048 μM, respectively. Comparison of the inhibitory activity against HIV-1 reverse transcriptase-associated reverse transcriptase and DNA polymerase activities using Poly(rA)oligo(dt) and Poly(dA)-oligo(dT), respectively, as template-primer revealed that compounds 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, as shown in Figure 1. When Poly(rA)poly(dT) was used as a 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 compounds 4 and 5 against this enzyme catalyzed DNA-directed DNA synthesis might be related to their anti-HIV activity.

Data obtained by using chronically HIV-1 infected cell lines that are cultured with a test drug can give a sense of how these compounds may function *in vivo* if given to a latently HIV- infected individual. Therefore, compounds 4 and 5 were separately added for 72 hours to the chronically HIV-1 infected T cell line, ACH-2, and to the chronically HIV-1 infected promonocytic cell line, UI. 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, as shown in Tables III and IV. 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.

5

Template-primer	Units used ^a	Relative rate ^b IC ₅₀ (μ M) ^c		
		4	5	
RNA-dependent DNA polymerase associated activity				
poly(rA)-olig(dT) ₁₀	1 X 10 ⁻³	100	4.5 \pm 1.0	1.9 \pm 0.9
poly(rC)-olig(dG) ₁₂₋₁₈	1 x 10 ⁻³	36	2.2 \pm 0.6	11 \pm 0.5
DNA-dependent DNA polymerase associated activity				
poly(rA)-olig(dT) ₁₀	0.05	10	0.056 \pm 0.003	0.048 \pm 0.004

10

^a A unit is defined as the amount of enzyme required to incorporate 1 nmol of [³H]dTTP into the poly(rA)-oligo(dT)₁₀ template in 1.0 min at 37°C.

^b All the reaction rates were normalized to the reaction rate obtained using poly(rA)-olig(dT)₁₀.

15

^c The values represent the mean \pm standard deviation of three separate determinations.

Table III Effect of Compounds 4 and 5 on Chronically Infected U1 Cells		
Sample Identification*	p24 pg/mL	
	+Media	+PMA
U1 cells + 4		
20	22	10,746
4	11	13,376
0.8	11	8,774
0.16	12	7,616
0.032	10	7,200
0.0064	7	8,314
U1 cells + 5		
20	15	10,131
4	16	10,598
0.8	14	11,482
0.16	13	9,850
0.032	15	8,320
0.0064	17	7,590
U1 cells + AZT		
100	22	9,338
10	13	9,299
1	12	8,218
0.1	18	8,070
U1 cells + media	15	7,124

* Compound concentrations in $\mu\text{g/mL}$.

Table IV Effect of Compounds 4 and 5 on Chronically Infected ACH-2 Cells		
Sample Identification*	p24 pg/mL	
	+Media	+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

* Compound concentrations in $\mu\text{g/mL}$.

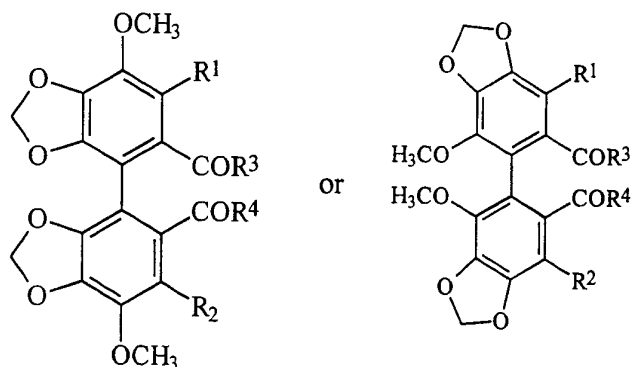
5 All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are hereby incorporated in their entirety by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

10 Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.



15 The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based upon the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purposes of description and not of
20 limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one or ordinary skill in the art.

What Is Claimed Is:

1. A compound of the formula:



- 5 wherein R^1 and R^2 are selected from the group consisting of hydrogen and bromine, with the proviso that R^1 and R^2 cannot both be hydrogen;

R^3 and R^4 are independently hydroxy, C_{1-6} alkoxy, $-O(CH_2)_2N^+(CH_3)Cl^-$,
 $-NH(CH_2)_2-N$  NH, or $-NH(CH_2)_2-N$  $\cdot HBr$

2. A compound according to claim 1, wherein R^1 and R^2 are both bromine.
- 10 3. A compound according to claim 1, wherein R^3 and R^4 are both methoxy.
4. A compound according to claim 1, which is
 3-bromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl
 biphenyl, or
 15 3,3'-dibromo-4,4'-dimethoxy-5,6,5',6'-bimethylenedioxy-2,2'-dimethoxycarbonyl
 biphenyl.

5. A pharmaceutical composition comprising at least one compound according to claim 1, or a pharmaceutically acceptable ester, ether, sulfate, carbonate, glucuronide or salt thereof, and a pharmaceutically acceptable carrier.

5 6. A pharmaceutical composition according to claim 5, wherein R³ and R⁴ are both methoxy.

7. A pharmaceutical composition according to claim 6, further comprising at least one drug selected from the group consisting of anti-viral agents and immunostimulating agents.

10 8. A pharmaceutical composition according to claim 7, wherein said antiviral agent is selected from the group consisting of gamma globulin, amantadine, guanidine, hydroxybenzimidazole, interferon- α , interferon- β , interferon-gamma, thiosemicarbazones, methisazone, rifampin, ribvirin, pyrimidine analogues, purine analogues, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, and ganciclovir.

15 9. A method for inhibiting a retroviral infection in cells or tissues of an animal comprising administering an effective retroviral inhibiting amount of a pharmaceutical composition according to claim 5.

20 10. The method of claim 9, wherein said composition is administered to provide said compound in an amount ranging from about 1 to about 100 mg/kg body weight.

11. The method of claim 10, wherein said composition is administered to provide said compound in an amount ranging from about 10 to about 100 mg/kg body weight.

12. The method of claim 9, wherein said animal is selected from the group consisting of mammals and birds.

13. The method of claim 12, wherein said mammal is a human.

5 14. A method for treating a patient suffering from a retroviral related pathology, comprising administering to said patient a retroviral inhibiting effective amount of a pharmaceutical composition according to claim 5.

15. The method of claim 14, wherein said retroviral related pathology is an HIV infection.

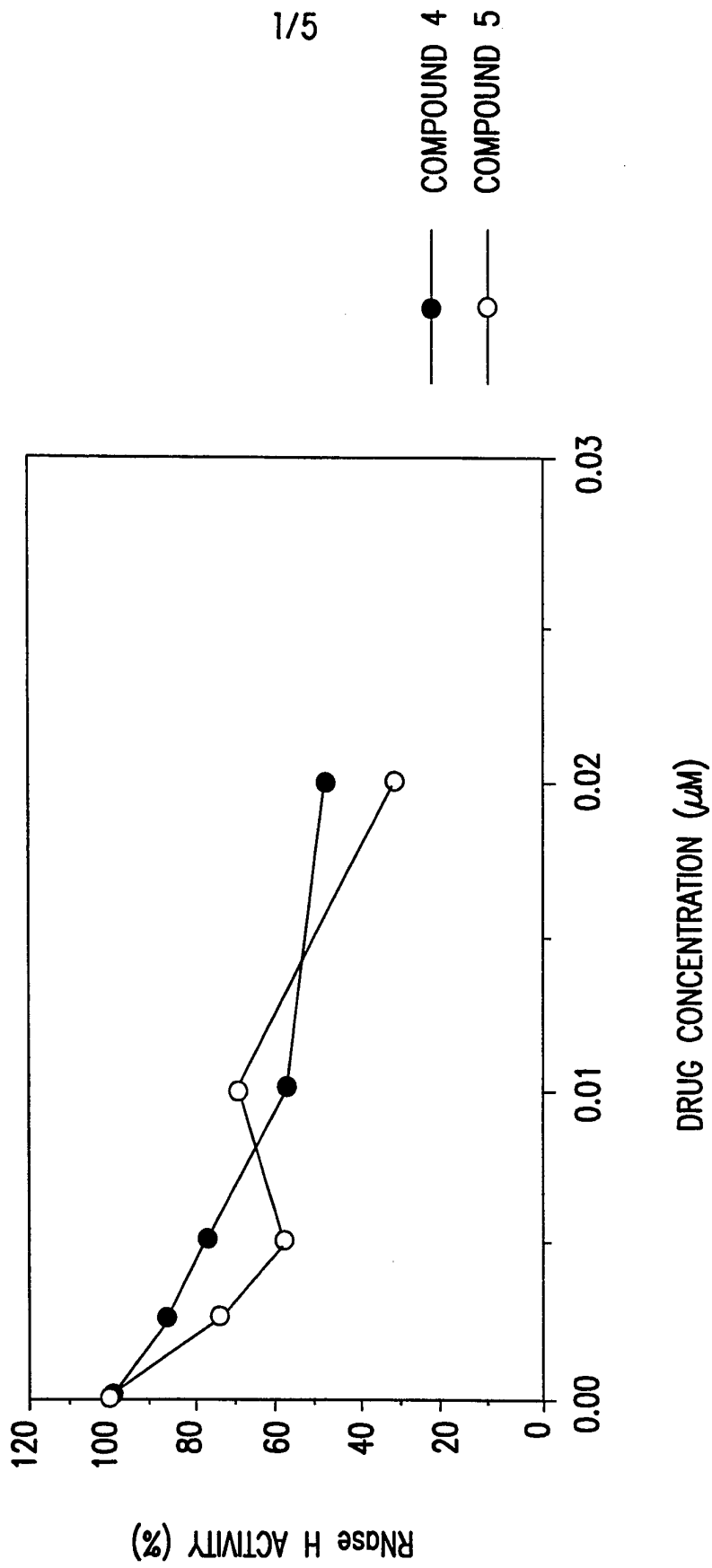


FIG.1

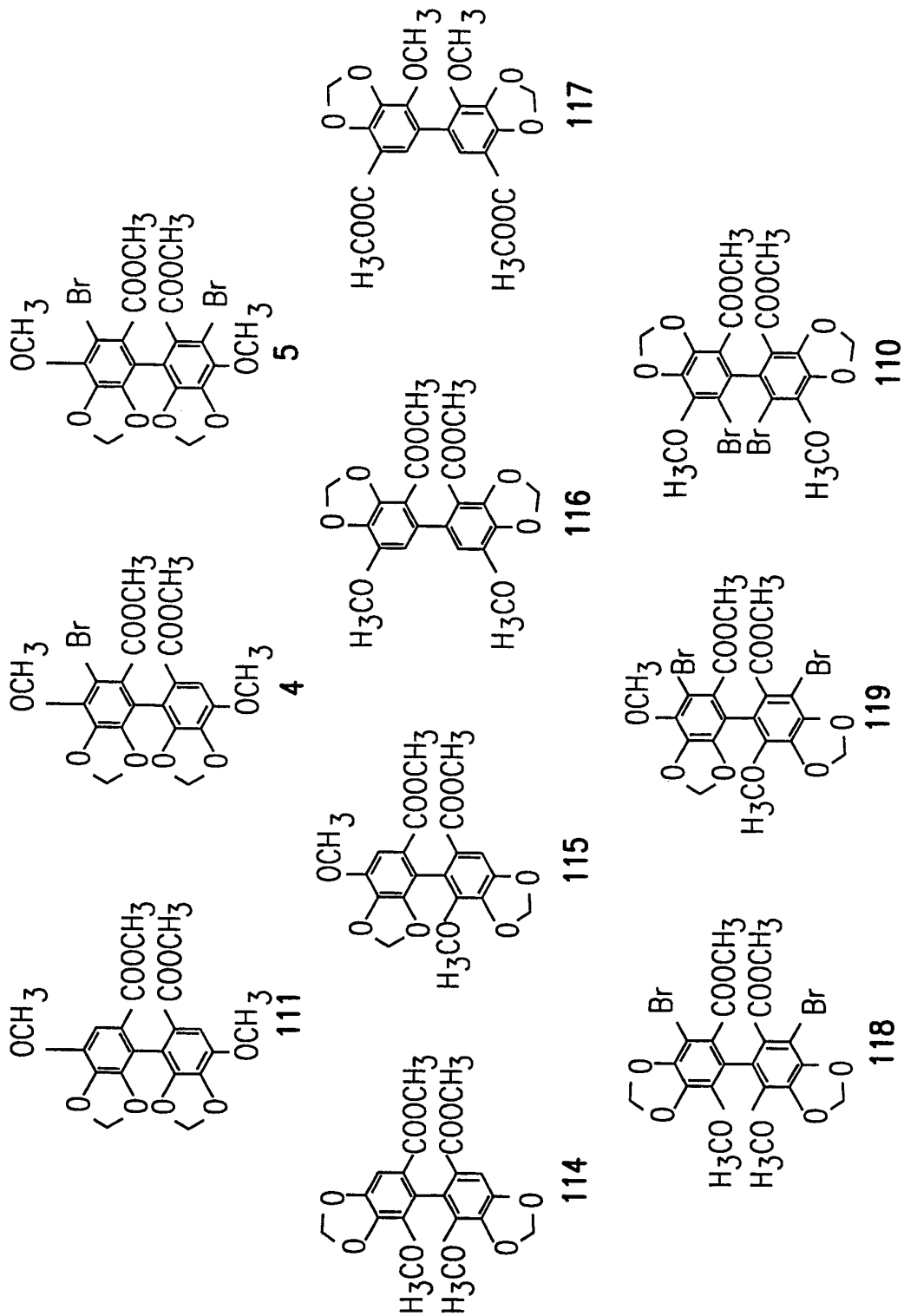
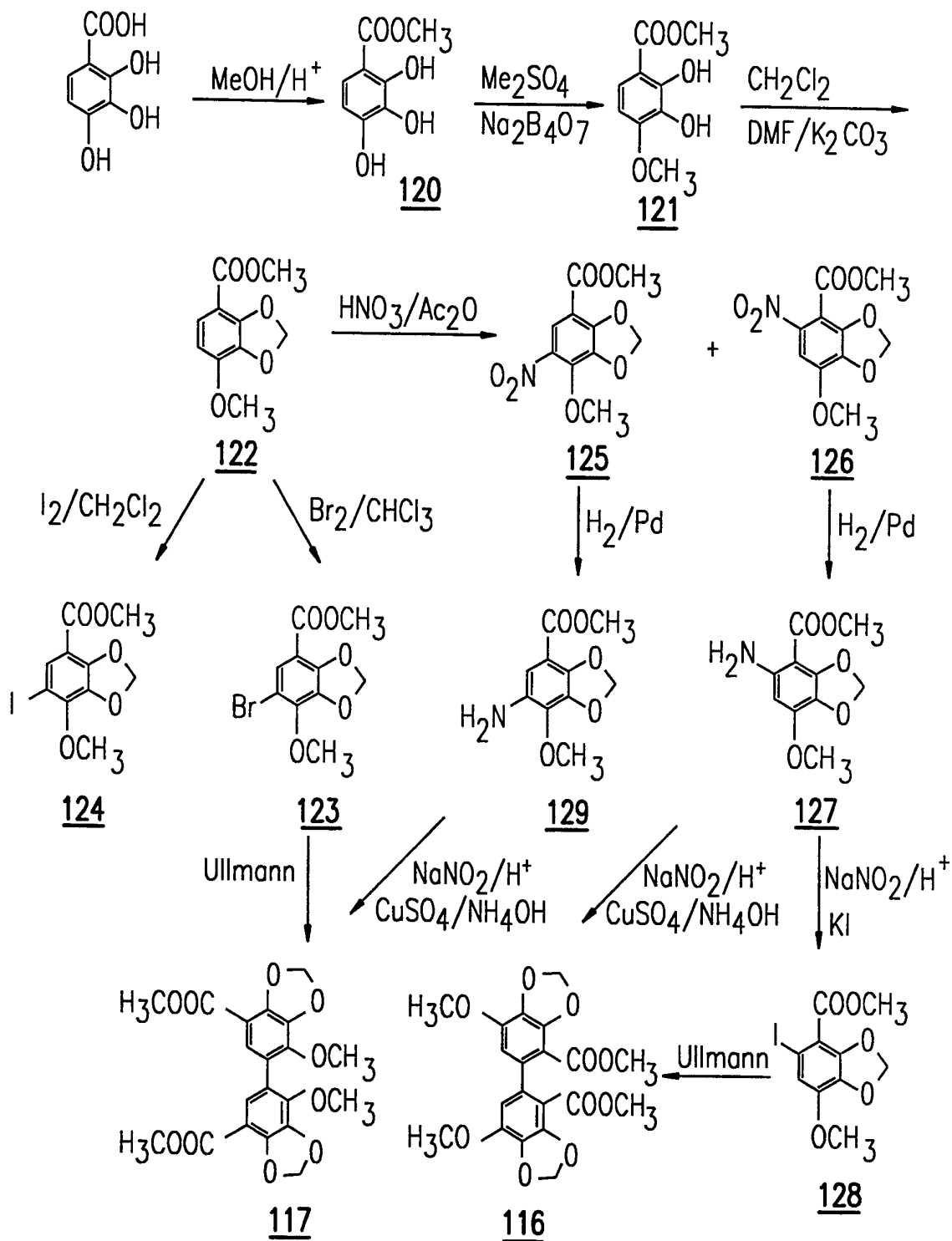


FIG.4

5/5



SCHEME 2

FIG.5

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10080

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/36, 31/40, 31/495; C07D 317/62, 317/64, 317/66, 405/14
US CL :514/253, 422, 464, 466; 544/377; 548/526; 549/435

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/253, 422, 464, 466; 544/377; 548/526; 549/435

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chem. abstr., Vol. 108, 1988, page 664, column 2, abstract No. 108: 204530f, Mingguang et al. 'Synthesis and stability of [³ -H]biphenyl diester.' Yuanzineng Kexue Jishu 1987, 21(2), 212-15 (Ch).	1-6

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 JULY 1996

Date of mailing of the international search report

25 SEP 1996

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