Assay of HIV-1 Virion Binding to MT-4 Cells. Virion binding to the cell membrane was determined by an indirect immunofluorescence assay using human anti-HIV 1 serum, rabbit anti-human-IG-F(ab')-fluorescein isothiocyanate, and flow cytometry.[44]

Assay of CD4 Expression. MT-4 cells were incubated with the test compound, stained with anti-OKT4A-FITC, and analyzed cytofluorometrically.[6]

Cell Viability Assay in HIV-1-Infected and Uninfected CEM Cells. Cell viability was determined by a tetrazolium (XTT) assay.[45]

Acknowledgment. This investigation was supported by Contract NO1-CM-87268, awarded by the National Cancer Institute, DHHS. The results in CEM cells were obtained under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Rockville, MD. The work of E.D.C. is supported by the AIDS Basic Research Programme of the European Community and grants from the Belgian F. G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek) and the Belgian G.O.A. (Geconcerteerde Onderzoeksacties). D.S. is a fellow of the Janssen Research Foundation. The polymer fractionation and molecular weight distribution studies were sponsored by Contract NO1-CM-87268 from the National Cancer Institute, Developmental Therapeutics Program, Drug Synthesis and Chemistry Branch, Dr. Ven L. Narayanan, Chief. Anti-HIV screening at the NCI was done under the auspices of the Developmental Therapeutics Program and the results provided by Dr. John P. Bader, Special Assistant for AIDS Antiviral Evaluations. M.C. thanks Dr. Edward M. Acton, NCI, for his interest, encouragement, and suggestions.

Registry No. 4, 130246-99-0; 5, 33206-58-5; ATA, 569-58-4; ATPA, 129777-62-4; 2-hydroxybenzenephosphonic acid, 53104-46-4; Salicylic acid, 69-72-7; reverse transcriptase, 9068-38-6; aurintrisulfonic acid, 129608-01-1; sodium 2-hydroxybenzenesulfonate, 51368-26-4.

(44) Schols, D.; Baba, M.; Pauwels, R.; De Clercq, E. *J. Acquired Immune Defic. Syndr.* **1989**, *2*, 10.
(45) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer. Inst.* **1989**, *81*, 577.

Synthesis and Anti-HIV Activities of Low Molecular Weight Aurintricarboxylic Acid Fragments and Related Compounds

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Received April 20, 1990

Several compounds corresponding to fragments of the schematic representation of the polymeric structure of aurintricarboxylic acid (ATA) have been prepared and tested for prevention of the cytopathic effect of HIV-1 and HIV-2 in MT-4 cell culture and HIV-1 in CEM cell culture. Both the triphenylcarbinol **3** as well as the triphenylmethane **5** were found to afford protection against the cytopathogenicity of HIV-2 in MT-4 cells and HIV-1 in CEM cells, but they were inactive against HIV-1 in MT-4 cells. Both substances were also found to inhibit syncytium formation when MOLT-4 cells were cocultured with HIV-2-infected HUT-78 cells, but were inactive in this assay against HIV-1-infected cells. When observed, the activity is generally moderate in degree of protection and requires concentrations in the 10^{-4} molar range. In contrast to ATA, both of these substances were inactive when tested for prevention of the binding of the OKT4A monoclonal antibody to the CD4 receptor and also for inhibition of HIV-1 reverse transcriptase. These substances therefore appear act by a mechanism that is distinct from that of polymeric ATA. Several active and inactive structural analogues of **3** and **5** were also synthesized. The anti-HIV activity in this series seems to depend on the presence of anionic carboxylate groups, since the methyl esters **4**, **6**, and **12** were uniformly inactive. The diphenylmethanes **8**, **14**, **18**, and **19** also reproducibly inhibited the cytopathic effect of HIV-1 in CEM cell culture.

Aurintricarboxylic acid (ATA) is a polymeric, solid substance that forms when a mixture of salicylic acid and formaldehyde is treated with sulfuric acid and sodium nitrite.[1] ATA is known to bind to the nucleotide binding sites of a variety of proteins that normally process nucleotides. Examples include the inhibitory effects of ATA on cell-free DNA[2-4] and RNA[4-8] polymerases, reverse transcriptase,[9,10] aminoacyl-*t*RNA synthetase,[11] ribonucleotide reductases,[12] and ribonucleases.[7,13-17] ATA inhibits cell free protein synthesis by blocking the attachment of mRNA to the ribosome,[18-20] and it also binds to the polynucleotide domains of the dihydroxyvitamin D_3[21] and glucocorticoid receptors.[22]

Recent interest in ATA has resulted from the reports that it inhibits the cytopathic effect of HIV in cell cultures

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(1) Caro, N. *Chem. Ber.* **1892**, *25*, 939.
(2) Seki, S.; Tsutsui, K.; Oda, T. *Biochem. Biophys. Res. Commun.* **1977**, *79*, 179.
(3) Tsutsui, K.; Seki, S.; Tsutsui, K.; Oda, T. *Biochim. Biophys. Acta* **1978**, *517*, 14.
(4) Nakane, H.; Balzarini, J.; De Clercq, E.; Ono, K. *Eur. J. Biochem.* **1988**, *177*, 91.
(5) Blumenthal, T.; Landers, T. A. *Biochem. Biophys. Res. Commun.* **1973**, *55*, 680.
(6) Liao, L.-L.; Horwitz, S. B.; Huang, M.-T.; Grollman, A. P.; Steward, D.; Martin, J. *J. Med. Chem.* **1975**, *18*, 117.
(7) Schulz-Harder, B.; Tata, J. R. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 903.
(8) Iapalucci-Espinoza, S.; Haim, L.; Franze-Fernández, M. *Mol. Cell. Biochem.* **1983**, *55*, 41.
(9) Givens, J. F.; Manly, K. F. *Nucleic Acids Res.* **1976**, *3*, 405.

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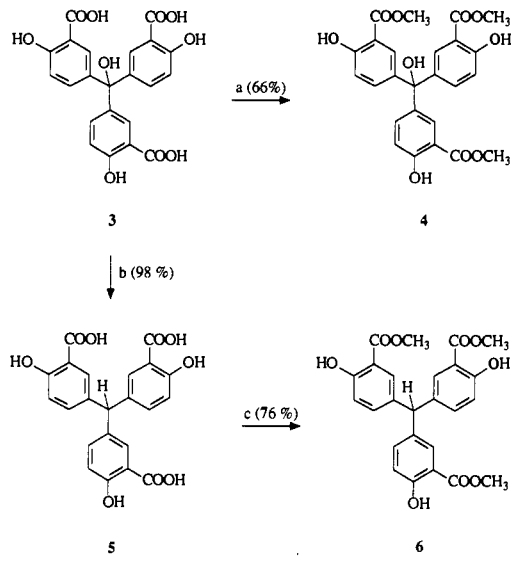
Scheme I

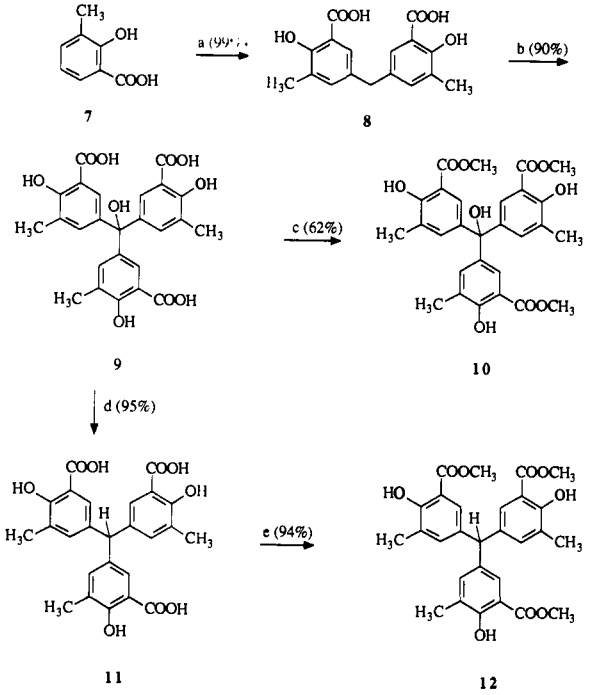


[a] Diazomethane, Et_2O, 5 °C (48 h). [b] H_2, Pd/C (10%), EtOH, room temperature (72 h). [c] Diazomethane, Et_2O, 5 °C (24 h).

and has low cytotoxicity, a high LD50 in mice, and does not inhibit cellular DNA, RNA, and protein synthesis.[23] This activity appears to be due to a specific interaction of ATA with the CD4 receptor as well as gp120.[24] Although ATA inhibits HIV-1 reverse transcriptase in cell-free systems at doses that inhibit HIV-1 replication in cell culture,[10] this effect may not be responsible for the activity, since ATA does not prevent the biosynthesis of macromolecules in cells,[23] presumably due to its failure to penetrate the cell membrane.

ATA is commonly represented in the literature dating back to the 19th century as the triphenylmethane dye structure **1**, but studies done in the 1970s showed rather clearly that ATA is actually a heterogeneous mixture of polymers of the phenol formaldehyde type, which has been represented schematically as structure **2**.[25,26] Aside from an elemental analysis provided in the original paper by Caro,[1] there was little evidence in the literature to support

Scheme II



[a] HCHO, H_2SO_4 (25%), 90 °C (12 h). [b] 3-Methylsalicylic acid, H_2SO_4, $NaNO_2$, room temperature (16 h). [c] Diazomethane, Et_2O, 5 °C (24 h). [d] H_2, Pd/C, EtOH, room temperature (48 h). [e] Diazomethane, Et_2O, 5 °C (24 h).

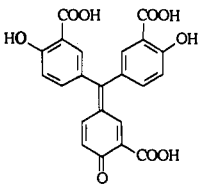
the incorrect structure **1** when a condensed form of it appeared in the procedure reported by Heisig and Lauer.[27] The polymeric nature of ATA was shown by fractionation and molecular weight determination by vapor-phase osmometry.[25] In addition, we recently reported a synthesis of compound **3**, which is the covalent hydrate of **1**.[26] Comparison of the properties of **3** with those of ATA confirmed the polymeric nature of ATA.[26] At elevated concentrations, the triphenylcarbinol **3** was found to afford protection against the cytopathic effect of HIV-1 in CEM cell culture (60–90% in four tests) with little or no accompanying cytotoxicity.[26] Compound **3** appears to be a new lead, distinct from polymeric ATA, for anti-AIDS drug development. The design and synthesis of analogues of **3** was therefore undertaken in order to define some of the structural parameters associated with its anti-HIV activity.

Results

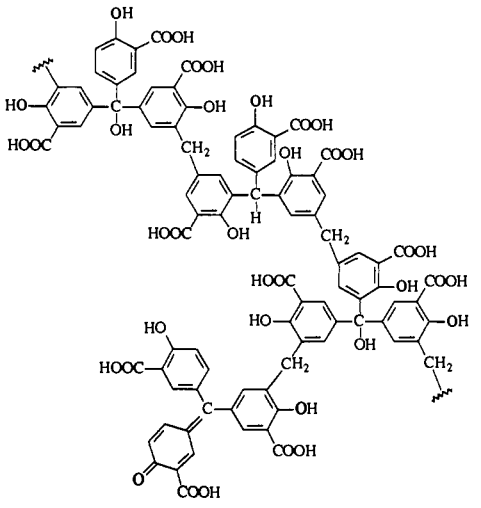
Chemistry. The synthesis of the triphenylcarbinol **3** and its conversion to the trimethyl ester **4** were recently described.[26] The hydrogenolysis of compound **3** to afford the triphenylmethane **5** was accomplished with hydrogen and palladium on charcoal. Treatment of compound **5** with diazomethane gave the trimethyl ester **6** (Scheme I).

The methylated analogues **9** and **10** were synthesized from 3-methylsalicylic acid as depicted in Scheme II. Treatment of the starting material with formaldehyde under acidic conditions gave the dimethylated methylenedisalicylic acid **8**. Further treatment of intermediate **8** with 3-methylsalicylic acid, sulfuric acid, and sodium nitrite afforded the substituted triphenylcarbinol **9**. As with compound **3**, the triphenylcarbinol structure of **9**, as opposed to a dehydrated quinone methide structure, was supported by fast atom bombardment mass spectrometry run in the negative ion mode, which indicated a negatively

(10) Baba, M.; Schols, D.; Pauwels, R.; Balzarini, J.; De Clercq, E. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1404.
(11) Igarashi, S. J.; Zmean, J. A. *Can. J. Biochem.* **1975**, *53*, 120.
(12) Baumann, H.; Hofmann, R.; Lammers, M.; Schimpff-Weiland, G.; Follmann, H. *Z. Naturforsch.* **1983**, *39C*, 276.
(13) Apirion, D.; Dohnar, D. *Antibiotics (N.Y.)* **1975**, *3*, 327.
(14) Zaug, A. J.; Cech, T. R. *Cell* **1980**, *19*, 331.
(15) González, G.; Haxo, R. S.; Schleich, T. *Biochemistry* **1980**, *19*, 4299.
(16) Ramón, D.; Ferrer, S.; Vicente, E.; Uruburu, F. *FEBS Microbiol. Lett.* **1986**, *36*, 9.
(17) Kumar, A.; Ali, R. *Indian J. Exp. Biol.* **1986**, *24*, 79.
(18) Grollman, A. P.; Stewart, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 719.
(19) Stewart, M. L.; Grollman, A. P.; Huang, M.-T. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 97.
(20) Kreamer, B. L.; Anderson, J.; Liu, D. H. S.; Sparks, M. B.; Richardson, A. *Can. J. Biochem.* **1978**, *56*, 1162.
(21) Mellon, W. S. *Biochem. Pharmacol.* **1984**, *33*, 1047.
(22) Moudgil, V. K.; Caradonna, V. M. *J. Steroid. Biochem.* **1985**, *23*, 125.
(23) Balzarini, J.; Mitsuya, H.; De Clercq, E.; Broder, S. *Biochem. Biophys. Res. Commun.* **1986**, *136*, 64.
(24) Schols, D.; Baba, M.; Pauwels, R.; Desmyter, J.; De Clercq, E. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3322.
(25) González, R.; Blackburn, B. J.; Schleich, T. *Biochim. Biophys. Acta* **1979**, *562*, 534.
(26) Cushman, M.; Kanamathareddy, S. *Tetrahedron* **1990**, *46*, 1491.
(27) Heisig, G. B.; Lauer, W. M. *Org. Synth.* **1928**, *9*, 54.



1



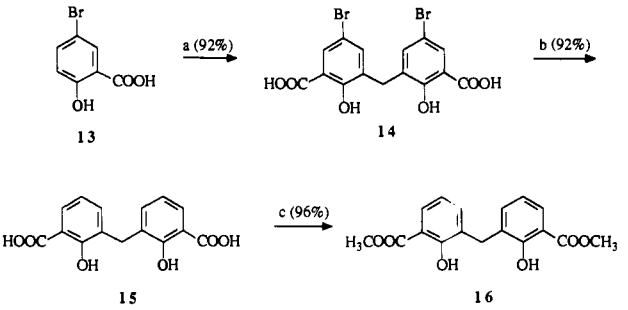
2

charged ion at m/e 481 derived from deprotonation of **9**.[26] Reaction of **9** with diazomethane yielded the triester **10**. The chemical ionization mass spectrum of **10** using ammonia as the ionizing gas was also consistent with the triphenylcarbinol structure. However, in both the FABMS of **9** run in the negative ion mode and the CIMS of **10**, the base peaks corresponded to the dehydrated quinone methide structures, indicating facile dehydration in the mass spectrometer probe. It should be mentioned that the UV spectra of methanol or ethanol solutions of the triphenylcarbinol **3** showed the development of absorbances in the 520–550-nm region of variable intensity (ϵ 2170–10 460), which might be attributed to quinone methide formation. However, the UV spectra of methanol or ethanol solutions of **4**, **9**, and **10** remained transparent in this region. The triphenylcarbinol **9** was also converted to the triphenylmethane **11** by hydrogenolysis, and compound **11** was then transformed into the triester **12** by treatment with diazomethane.

Several diphenylmethane derivatives have also been prepared. Treatment of 5-bromosalicylic acid (**13**) with formaldehyde under acidic conditions afforded the halogenated diphenylmethane **14** (Scheme III). Debromination of **14** was accomplished by hydrogenolysis over palladium on charcoal. Finally, the diacid **15** was converted to the diester **16** on treatment with diazomethane.

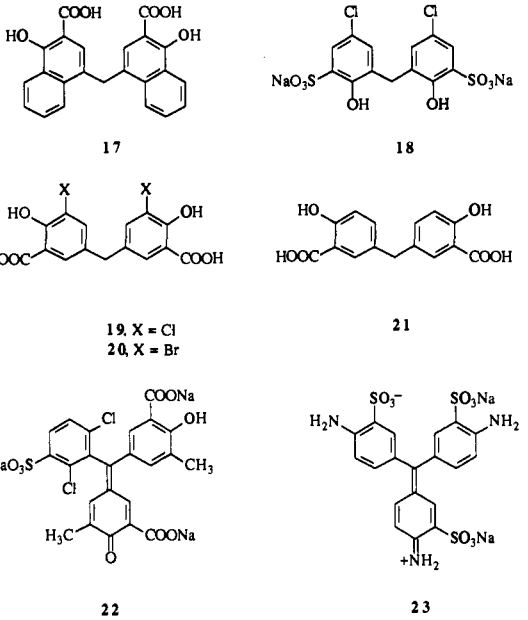
The substituted methylenebis(naphthoic acid) derivative **17** was also obtained in 94% yield on treatment of 1-hydroxy-2-naphthoic acid with formaldehyde and sulfuric acid. The substituted diphenylmethane **18** containing two sulfonic acid groups was obtained by sulfonation of 3,3′-methylenebis(2-hydroxy-5-chlorobenzene).[28] Several substituted diphenylmethanes were also available from previous work on the synthesis of triphenylcarbinol **3**. These included the methylenedisalicylic acid **21** and its dichloro derivative **19**. The dibromomethylenedisalicylic

Scheme III



[a] HCHO, H_2SO_4, −5 to 0 °C (3 h); room temperature (14 h). [b] H_2, Pd/C (10%), KOH in EtOH, room temperature (20 h). [c] Diazomethane, Et_2O, 5 °C (4 h).

acid **20** was obtained from the reaction of 3-bromosalicylic acid[29] with formaldehyde under acidic conditions.



A variety of commercially available triphenylmethane dyes that are structurally related to **3** were also tested. Of these, chrome azurol S (mordant blue 29), compound **22**, proved to be the most interesting.

Biological Evaluation. The biological testing results are listed in Table I. The triphenylcarbinol **3** was inactive in preventing the cytopathic effect of HIV-1 in MT-4 cells. However, it did show activity when tested against HIV-2 in MT-4 cells and, to a lesser extent, against HIV-1 in CEM cells. Compound **3** also prevented syncytium formation when MOLT-4 cells were cocultured with HIV-2-infected HUT-78 cells, but was not active with HIV-1-infected HUT-78 cells. The activity of the triphenylmethane **5** was slightly better than that of **3**. Both of the corresponding trimethyl esters **4** and **6** were inactive against HIV, and both were more cytotoxic than **3** and **5**.

The diphenylmethane **8** was inactive when tested for prevention of cytopathogenicity in MT-4 cells. However, it did give protection against the cytopathic effect of HIV-1 in CEM cells.

The trimethylated triphenylcarbinol **9** was not active in any of the assays. This lack of activity is striking when compared with the activity displayed by **3**. The triphenylmethane **11** was cytotoxic and had low anti-HIV activity. The selectivity of **11** is not as high as that of **3**

(28) Artiss, J. D.; Thibert, R. J.; McIntosh, J. M.; Zak, B. *Microchem. J.* 1981, *26*, 487.

(29) Pudleiner, H.; Laatsch, H. *Synthesis* 1989, 286.

Table I. Anti-HIV Activities of ATA Monomer Analogues[a]

	HIV cytopathogenicity				giant cell assay:[b]	
	HIV-1 MT-4-cells	HIV-2 MT-4-cells	HIV-1 CEM cells	cell viability[c]	HIV-1-infected HUT-78-cells	HIV-2-infected HUT-78-cells
3	>100	15	50.4	248	>100	15
4	>100	>100	>78	66	>100	>100
5	>100	13	32.5	256	>100	15
6	>100	>100	>78	52	>100	>100
8	>100	>100	55.5	>100		
9	>100	>100	>205	>100		
11	80	67	>186	82	>20	>20
12	>100	>100		>100		
14	>100	>100	72.1	>100		
15	>100	>100	>125	>100		
17	75	79	20.0	73	>20	>20
18	>100	>100	122.7	>100	>100	>100
19	>100	>100	117.3	>100		
20	>100	>100		>100		
21	>100	>100	>276	>100		
22			50.2			
ATA	1.1	0.85	2.1	280	4	4

[a] Data are the mean values for at least three experiments. IC_{50} is the 50% inhibitory concentration for cytopathicity of HIV-1 (HTLV-III_B) or HIV-2 (LAV-2_{ROD}) in MT-4 cells or in CEM cells as specified. The viral dose was 100–500-fold higher than that required to infect 50% of the MT-4 cells. [b] IC_{50} for the giant cell formation between HIV-1- or HIV-2-infected HUT-78 cells and uninfected MOLT-4 cells corresponds to the 50% inhibitory concentration that reduced the number of giant cells by 50%. [c] IC_{50} for viability is the 50% cytotoxic concentrations for mock-infected MT-4 cells. Viability was quantified by a tetrazolium (MTT) colorimetric method in 96-well microtrays.

and **5**, since the concentration needed for the low activity observed is close to that producing cytotoxicity in uninfected cells. The trimethyl ester **12** was uniformly inactive, in agreement with the lack of activity displayed by the other trimethyl esters **4** and **6**.

Although the brominated diphenylmethane **14** was inactive in preventing the cytopathic effect of HIV-1 in MT-4 cells, it did prevent HIV-1 cytopathogenicity in CEM cell culture. This profile is similar to that of the dimethylated diphenylmethane **8**, as well as the other diphenylmethanes **18** and **19**. In contrast, the less lipophilic methylenedisalicylic acid **21** was completely inactive in CEM cells. It should also be pointed out that although compounds **3**, **5**, **14**, and **19** did not prevent the cytopathic effect of HIV-1 in MT-4 cells, they did show partial protection against HIV-1 cytopathogenicity in MT-4 cells at the highest concentration tested (100 μg/mL).

The dinaphthylmethane **17** was the only compound that showed antiviral activity against HIV-1 and HIV-2 in MT-4 cells as well as in CEM cell culture. However, it is toxic and the activity is seen at concentrations that almost coincide with the cytotoxic concentration. It was also the most potent of the ATA monomer analogues tested for prevention of the cytopathic effect of HIV-1 in CEM cells.

The commercially available dye chrome azurol S (**22**) was also tested for prevention of the cytopathic effect of HIV-1 in CEM cells and it displayed slight but reproducible reproducible antiviral activity.

In addition to the results listed in Table I, all of the compounds prepared in this study were tested for inhibition of HIV-1 reverse transcriptase, since it is known that ATA inhibits that enzyme.[10] Without exception, all of these substances lacked any activity against HIV-1 reverse transcriptase. This result is in contrast to the reported ability of a related triphenylmethane dye, fuchsin acid (**23**), to inhibit HIV-1 reverse transcriptase.[10]

The triphenylcarbinol **3** and the triphenylmethane **5** were also tested for prevention of the binding of the OKT4A monoclonal antibody to the CD4 receptor, since ATA is known to block that binding.[24] It was found that **3** and **5** were also inactive in that assay.

Discussion

One of the unanswered questions about ATA is its chemical composition. The structure **2** previously offered may only be considered as a schematic representation at best.[25] Eventually, the complex, heterogeneous mixture of polymers composing ATA will have to be fractionated and broken down into simpler mixtures or even individual components in order to allow further structure elucidation. Comparison of the spectral data of the ATA partial structures **3**, **5**, **15**, and **21** with those of the ATA components should eventually aid in defining the true chemical nature of ATA.

In view of the fact that the long-accepted triphenylmethane dye structure **1** of ATA has turned out to be incorrect, it seems legitimate to question how many of the other triphenylmethane dyes structures misrepresent the true chemical compositions of these substances. It seems somewhat puzzling that ATA and the triphenylmethane dye fuchsin acid (**23**) inhibit HIV-1 reverse transcriptase,[10] but compound **3** does not. On the other hand, the present results showing the failure of the triphenylcarbinol **3** to inhibit HIV-1 reverse transcriptase do agree with the conclusions of other workers "that aurintricarboxylic acid in the form of the commonly accepted structure (**1**) is ineffective as an inhibitor of protein nucleic acid interactions".[25]

Inspection of the results in Table I indicates that some of the compounds consisting of small ATA fragments and their analogues do have moderate anti-HIV activity. However, the anti-HIV effect of these materials seems to be structurally nonspecific, and these low molecular weight monomer analogues are markedly less potent than unfractionated, polymeric ATA. It is clear that carboxylate groups are necessary for antiviral activity, as the methyl esters **4** and **6** of the active compounds **3** and **5** were inactive. As documented in the accompanying paper, polymeric ATA analogues in which the carboxylic acid groups are replaced by sulfonic acid and phosphonic acid moieties retain significant anti-HIV activity. Since the triphenylcarbinol **3** and the triphenylmethane **5** display similar activities, the hydroxyl group on the central carbon in **3** does not appear to play a role in the anti-HIV activity. The introduction of methyl groups on the aromatic rings to afford **9** and **11** decreased antiviral potency. Compound **17** suggests that the third salicylic acid moiety may not be absolutely required for activity.

The data in Table I indicate that compounds 3 and 5 are more active against HIV-2 than HIV-1. Several other antiviral agents are more active against HIV-2 than HIV-1, including pentosan sulfate,[30] dextran sulfate,[30] soluble CD4,[31] glycosylation inhibitors (i.e. castanospermine),[32] and low molecular weight (i.e. 1000–2000) sulfated polysaccharides.[33] On the other hand, TIBO derivatives[34] and HEPT derivatives[35] are active against HIV-1 and inactive against HIV-2.

The results presented in this study indicate that whereas ATA prevents the binding of the OKT4A monoclonal antibody to the CD4 receptor[24] and inhibits HIV-1 reverse transcriptase,[10] both 3 and 5 do not. It therefore appears that the substances prepared in this study that prevent the cytopathic affect of HIV in cell cultures are not acting by the same mechanism(s) of action as ATA, but are instead acting by an as yet unidentified mechanism. In this sense, the low molecular weight ATA monomer analogues prepared in this study provide a new lead, distinct from polymeric ATA, for further anti-HIV drug development.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Chemagnetics A-200 spectrometer. IR spectra were recorded on a Beckman IR-33 spectrophotometer. Low-resolution chemical-ionization mass spectra (CIMS) were determined on a Finnegan 4000 spectrometer using 2-methylpropane as the reagent gas. Low-resolution fast atom bombardment mass spectra (FABMS) were obtained on a Kratos MS50 spectrometer. Microanalyses were performed by the Purdue Microanalytical Laboratory. The results in CEM cells were obtained under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Rockville, MD. Studies on the inhibition of HIV-1 reverse transcriptase were performed at Merck Sharp and Dohme Research Laboratories, West Point, PA. All of the other biological tests were done at the Rega Institute for Medical Research, Katholiecke Universiteit, Leuven, Belgium.

3,3′,3″-Tricarboxy-4,4′,4″-trihydroxytriphenylmethane (5). A solution of the triphenylcarbinol 3 (0.660 g, 1.5 mmol) in absolute ethanol (150 mL) was shaken under hydrogen atmosphere at 80 psi in the presence of palladium on charcoal (10%) for 72 h. The catalyst was filtered off and the solvent removed under reduced pressure to give the product 5 (0.605 g, 98%): mp 280–282 °C dec; IR (KBr) 3600–2800, 1670, 1610, 1590, 1485, 1440, 1285, 1180, 1070 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.65 (d, 3 H, J = 2 Hz), 7.28 (dd, 3 H, J = 8 and 2 Hz), 6.91 (d, 3 H, J = 8 Hz), 5.51 (s, 1 H); FABMS (positive ion mode) m/e (relative intensity) 425 (MH$^+$, 100).

3,3′,3″-Tricarbomethoxy-4,4′,4″-trihydroxytriphenylmethane (6). A solution of diazomethane (prepared from 0.618 g of nitrosomethylurea in 20 mL of ether) was added to a suspension of compound 5 (0.424 g, 1 mmol) in ether (20 mL), and the resulting mixture was allowed to stand at 5 °C for 24 h. A few drops of acetic acid were then added to decompose unreacted diazomethane. The solvent was evaporated under reduced pressure and the residue was subjected to column chromatography over silica gel (60–200 mesh, 20 g) using 1% ethyl acetate in dichloromethane to give the product 6 (0.355 g, 76%). The analytical sample was recrystallized from hexane–dichloromethane (2:1): mp 201–202 °C; IR (KBr) 3400–3060, 2960, 1675, 1610, 1585, 1480, 1435, 1335, 1290, 1260, 1200, 1180, 960, 825, 780 cm^{-1}; ^{1}H NMR (200 MHz, chloroform-d) δ 10.72 (s, 3 H, exchangeable with D_2O), 7.52 (d, 3 H, J = 2 Hz), 7.18 (dd, 3 H, J = 8 and 2 Hz), 6.92 (d, 3 H, J = 8 Hz), 5.38 (s, 1 H), 3.89 (s, 9 H); ^{13}C NMR (chloroform-d) δ 170.33, 160.31, 136.41, 134.10, 129.88, 117.85, 112.18, 53.98, 52.27; CIMS (isobutane ionizing gas) m/e (relative intensity) 467 (MH$^+$, 100). Anal. ($C_{25}H_{22}O_9$) C, H.

3,3′-Dicarboxy-4,4′-dihydroxy-5,5′-dimethyldiphenylmethane (8). A mixture of 3-methylsalicylic acid (7, 7.6 g, 0.05 mol), dilute sulfuric acid (25%, 100 mL), and formaldehyde solution (37%, 4.05 mL) was heated at 90 °C with stirring. After 4 h, additional formaldehyde solution (2 mL) was added to the reaction mixture. The reaction mixture was then heated at 90 °C for a total period of 12 h, cooled to room temperature, and poured onto crushed ice (100 g). The white precipitate was filtered to give the product 8 (7.82 g, 99%). The analytical sample was recrystallized from ethanol–chloroform (1:1): mp 296–298 °C; IR (KBr) 3500–2830, 1675, 1620, 1470, 1445, 1300, 1230, 1200, 1145, 900, 790 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.52 (s, 2 H), 7.14 (s, 2 H), 3.77 (s, 2 H), 2.19 (s, 6 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 317 (MH$^+$, 100).

3,3′,3″-Tricarboxy-4,4′,4″-trihydroxy-5,5′,5″-trimethyltriphenylcarbinol (9). Powdered sodium nitrite (2.07 g, 0.03 mol) was added to concentrated sulfuric acid (25 mL). A intimate mixture of 3-methylsalicylic acid (1.52 g, 0.01 mol) and compound 8 (3.16 g, 0.01 mol) was added in small portions over a period of 20 min. The dark red reaction mixture was left at room temperature for 16 h and then poured onto crushed ice (100 g). The precipitate was filtered and dried to give the product 9 as a dark red powder (4.32 g, 90%): mp >310 °C dec; IR (KBr) 3400, 3100–2800, 1670, 1585, 1420, 1375, 1300, 1190, 1025, 890, 785 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.50 (s, 3 H), 7.24 (s, 3 H), 2.17 (s, 9 H); ^{13}C NMR (DMSO-d_6) δ 171.35, 157.57, 136.09, 134.47, 125.40, 123.54, 109.39, 77.99, 14.34; FABMS (positive ion mode) m/e (relative intensity) 465 (MH$^+$ – H_2O, 100), 449 (49); FABMS (negative ion mode) m/e (relative intensity) 481 (M – H$^+$, 10), 463 (M – H$^+$ – H_2O, 100).

4,4′,4″-Trihydroxy-3,3′,3″-tricarbomethoxy-5,5′,5″-trimethyltriphenylcarbinol (10). A solution of diazomethane (prepared from 1.545 g of nitrosomethylurea in 25 mL of ether) was added to a solution of 9 (0.964 g, 2 mmol) in methanol (20 mL). The resulting solution was kept at 5 °C for 48 h. A few drops of acetic acid were added to decompose the unreacted diazomethane. The solvent was evaporated and the residue was purified by flash column chromatography over silica gel (200–400 mesh, 20 g) using dichloromethane–hexane (2:1) to afford the product 10 (0.650 g, 62%). The analytical sample was recrystallized from dichloromethane: mp 249–250 °C; IR (KBr) 3420, 3180, 2945, 1680, 1610, 1520, 1440, 1340, 1280, 1195, 1125, 780 cm^{-1}; ^{1}H NMR (200 MHz, chloroform-d) δ 11.04 (s, 3 H, exchangeable with D_2O), 7.75 (s, 3 H), 7.28 (s, 3 H), 3.90 (s, 9 H), 2.22 (s, 9 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 525 (MH$^+$, 8), 507 (MH$^+$ – H_2O, 100).

5,5′,5″-Tricarboxy-4,4′,4″-trihydroxy-3,3′,3″-trimethyltriphenylmethane (11). Triphenylcarbinol 9 (1.00 g, 2.07 mmol) was dissolved in absolute ethanol (100 mL) and the catalyst Pd–C (10%, 0.3 g) was added. The contents were stirred under hydrogen atmosphere at 80 psi for 48 h. The catalyst was filtered off and the solvent was removed under reduced pressure to give 0.92 g (95%) of the triphenylmethane 11, which was recrystallized from chloroform–methanol: mp >300 °C dec; IR (KBr) 3400 (br), 3100 (br), 1660, 1605, 1470, 1430, 1265, 1180, 1125, 1005, 880, 785 cm^{-1}; ^{1}H NMR (200 MHz, methanol-d_4) δ 7.44 (bs, 3 H), 7.13 (bs, 3 H), 5.34 (s, 1 H), 2.19 (s, 9 H); FABMS m/e (negative ion mode) 465 (M – H$^+$, 47), 275 (35), 185 (100).

4,4′,4″-Trihydroxy-3,3′,3″-tricarbomethoxy-3,3′,3″-trimethyltriphenylmethane (12). An ethereal solution of diazomethane (20 mL, prepared from nitrosomethylurea, 1.03 g) was added to a cooled suspension of the tricarboxylic acid 11 (0.466 g, 1.00 mmol) in Et_2O (20 mL) and the mixture was kept at 5 °C for 24 h. The solid that separated was filtered off (0.435 g), the

(30) Witvrouw, M.; Baba, M.; Balzarini, J.; Pauwels, R.; De Clercq, E. *J. Acquired Immune Defic. Syndr.* **1990**, *3*, 343.
(31) Looney, D. J.; Hayashi, S.; Nicklas, M.; Redfield, R. R.; Broder, S.; Wong-Staal, F.; Mitsuya, H. *J. Acquired Immune Defic. Syndr.* **1990**, *3*, 649.
(32) De Clercq, E. Plenary lecture presented at the VI International Conference on AIDS, San Francisco, June 20–24, 1990.
(33) De Clercq, E., personal communication.
(34) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; Breslin, H. J.; Raeymaeckers, A.; Gelder, J. V.; Woestenborghs, R.; Heykants, J.; Schellekens, K.; Janssen, M. A. C.; De Clercq, E.; Janssen, P. A. J. *Nature* **1990**, *343*, 470.
(35) Baba, M.; Tanaka, H.; De Clercq, E.; Pauwels, R.; Balzarini, J.; Schols, D.; Nakashima, H.; Perno, C.-F.; Walker, R. T.; Miyasaka, T. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 1375.

solvent was removed, and the residue was chromatographed over silica gel (60–200 mesh, 15 g) to give additional product (0.04 g), resulting in a total amount of 0.475 g (94%) of the trimethyl ester 12. The analytical sample was recrystallized from dichloromethane: mp 249–250 °C; IR (KBr) 3440, 1670, 1605, 1430, 1340, 1260, 1220, 1190, 1120, 780 cm^{-1}; ^{1}H NMR (200 MHz, chloroform-d) δ 7.36 (s, 3 H), 7.03 (s, 3 H), 5.26 (s, 1 H), 3.88 (s, 9 H), 2.22 (s, 9 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 509 (MH^+, 100), 477 ($MH^+ - CH_3OH$, 40), 343 (25), 79 (86). Anal. ($C_{28}H_{28}O_9$) C, H.

5,5′-Dibromo-3,3′-dicarboxy-2,2′-dihydroxydiphenylmethane (14). A stirred suspension of 5-bromosalicylic acid (4.34 g, 0.02 mmol) in water (2 mL) was cooled to −5 °C and concentrated sulfuric acid (80 mL) was added dropwise while the temperature was maintained between −5 and 0 °C. A mixture of 38% aqueous formaldehyde (10 mL) and methanol (10 mL) was added to the reaction mixture over a period of 20 min. Stirring was continued for 2 h at 0 °C. The reaction mixture was left at room temperature for 14 h and then poured into crushed ice. The white precipitate was filtered, washed with water, and dried to give the product 14 (4.13 g, 93%). The analytical sample was recrystallized by dissolving the solid in a minimum amount of methanol and then adding chloroform: mp 290–291 °C dec; IR (KBr) 3400–2800, 1655, 1600, 1445, 1425, 1285, 1230, 1170, 870, 855, 790, 675 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.90 (d, 2 H, J = 2 Hz), 7.56 (d, 2 H, J = 2 Hz), 4.02 (s, 2 H); CIMS m/e (relative intensity) 449 ($MH^+ + 4$, 50), 447 ($MH^+ + 2$, 100), 445 (MH^+, 56), 431 (39), 429 (78), 427 (41), 231 (43), 217 (25). Anal. ($C_{15}H_{10}Br_2O_6$) C, H.

3,3′-Dicarboxy-2,2′-dihydroxydiphenylmethane (15). Compound 14 (4.46 g, 0.01 mmol) was dissolved in 10% alcoholic potassium hydroxide (150 mL). Palladium on charcoal (10%, 0.5 g) was added and the solution was hydrogenated at room temperature and atmospheric pressure for 20 h. The catalyst was filtered off, the solvent was evaporated, and water (100 mL) was added to the residue. The alkaline solution was cooled in an ice bath and neutralized with dilute hydrochloric acid. The precipitate was filtered, washed with water, and dried to give the product 15 (2.62 g, 91%). The analytical sample was recrystallized from methanol–chloroform: mp 284–286 °C; IR (KBr) 3400–2840, 1650, 1605, 1440, 1420, 1280, 1230, 1175, 1065, 880, 740 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.70 (dd, 2 H, J = 7 and 2 Hz), 7.26 (dd, 2 H, J = 7 and 2 Hz), 6.72 (t, 2 H, J = 8 Hz), 3.95 (s, 2 H); CIMS m/e (relative intensity) 289 (MH^+, 80), 271 ($MH^+ - H_2O$, 100), 151 (8), 139 (7). Anal. ($C_{15}H_{12}O_6$) C, H.

3,3′-Dicarbomethoxy-2,2′-dihydroxydiphenylmethane (16). A cooled solution of diazomethane (prepared from 0.9 g of nitrosomethyl urea in 20 mL of ether) was added to a suspension of 15 (0.5 g, 1.74 mmol) in Et_2O (20 mL). The solution was stored in a refrigerator for 4 h. The solvent was evaporated and the residue was crystallized to give the product 16 (0.52 g, 96%): mp 125–126 °C; IR (KBr) 3400, 3080, 1660, 1605, 1430, 1285, 1190, 1140, 990, 740 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.72 (dd, 2 H, J = 8 and 2 Hz), 7.34 (dd, 2 H, J = 8 and 2 Hz), 6.84 (t, 2 H, J = 8 Hz), 4.00 (s, 2 H), 3.94 (s, 6 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 317 (MH^+, 100), 285 ($MH^+ - CH_3OH$, 35), 157 (93).

4,4′-Methylenebis(1-hydroxy-2-naphthoic acid) (17). A mixture of 1-hydroxy-2-naphthoic acid (1.88 g, 10 mmol), sulfuric acid (25 % aqueous, 100 mL), and formaldehyde (37% aqueous, 1.0 mL) was heated at 90 °C for 6 h, left at room temperature for 12 h, and then poured over crushed ice. The precipitate of 17 (1.82 g, 94%) was filtered and dried. An analytical sample was recrystallized by dissolving the material (0.2 g) in hot ethanol (250 mL), decolorizing with charcoal, and filtering the hot solution: mp 290–93 °C dec; IR (KBr) 3420, 3080, 1665, 1645, 1590, 1520, 1465, 1300, 1260, 1100, 915, 770 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.45–8.41 (m, 2 H), 7.97–7.93 (m, 2 H), 7.66–7.51 (m 4 H), 7.37 (s, 2 H), 4.61 (s, 2 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 389 (MH^+, 9), 345 (49), 157 (100). Anal. ($C_{23}H_{16}O_6$) C, H.

3,3′-Dibromo-5,5′-dicarboxy-4,4′-dihydroxydiphenylmethane (20). 3-Bromosalicylic acid[29] (0.434 g, 2.00 mmol) was dissolved in methanol (2.5 mL) and water (1.5 mL) was added. The reaction mixture was cooled to 0 °C. Concentrated H_2SO_4 (10 mL) was added through an addition flask in about 10 min. After 1 h, formaldehyde (37%, 0.5 mL) was added, and the mixture was left standing at room temperature for 14 h and then poured over crushed ice (25 g). The white precipitate of 20 (0.432 g, 97%) was filtered and dried. The analytical sample was recrystallized from chloroform–methanol (3:1): mp >300 °C dec; IR (KBr) 3400, 3060, 1670, 1610, 1440, 1235, 1165, 785, 690 cm^{-1}; ^{1}H NMR (200 MHz, acetone-d_6) δ 7.75 (s, 2 H), 7.62 (s, 2 H), 3.92 (s, 2 H); CIMS (isobutane ionizing gas) m/e (relative intensity) 449 ($MH^+ + 4$, 37), 447 ($MH^+ + 2$, 79), 445 (MH^+, 44), 431 (42), 429 (100), 427 (50), 231 (37).

Anti-HIV Assays. Anti-HIV activity in MT-4 cells was quantitated by a tetrazolium-based colorimetric method, as described previously.[36] For giant cell formation, MOLT-4 cells were cultured with an equal number of HUT-78/HTLV-III$_B$ cells or HUT-78/LAV-2$_{ROD}$ cells in microtiter tray wells containing various concentrations of the test compunds. After a 24-h cocultivation period, the number of giant cells was recorded microscopically and analyzed by laser flow cytometry, as described previously.[37] Cell viability in CEM cells was determined by a tetrazolium (XTT) assay.[38].

Acknowledgment. This investigation was supported by Contract NO1-CM-67699, awarded by the National Cancer Institute, DHHS. The work of E.D.C. is supported by the AIDS Basic Research Programme of the European Community and grants from the Belgian F.G.W.O. (Fonds voor Geneeskundig Wetenschappelijk Onderzoek), the Belgian G.O.A. (Geconcerteerde Onderzoeksacties), and the Janssen Research Foundation. D.S. is a fellow of the Janssen Research Foundation. The polymer fractionation and molecular weight distribution studies were sponsored by Contract NO1-CM-87268 from the National Cancer Institute, Development Therapeutics Program, Drug Synthesis and Chemistry Branch, Dr. Ven L. Narayanan, Chief. Anti-HIV screening at the NCI was done under the auspices of the Developmental Therapeutics Program and the results provided by Dr. John P. Bader, Special Assistant for AIDS Antiviral Evaluations. M.C. thanks Drs. Edward M. Acton and Rudiger D. Haugwitz, NCI, for their interest, encouragement, and suggestions.

Registry No. 3, 639-56-5; 4, 128626-48-2; 5, 129749-38-8; 6, 129749-39-9; 7, 83-40-9; 8, 2581-36-4; 9, 130883-72-6; 10, 129749-41-3; 11, 129749-43-5; 12, 130883-73-7; 13, 89-55-4; 14, 35232-55-4; 15, 5578-67-6; 16, 129749-42-4; 17, 1175-41-3; 18, 33206-58-5; 19, 128626-47-1; 20, 130883-74-8; 21, 122-25-8; 22, 130883-75-9; ATA, 4431-00-9; 1-hydroxy-2-naphthoic acid, 86-48-6; 3-bromosalicylic acid, 3883-95-2.

(36) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol Methods* **1988**, *20*, 309.
(37) Schols, D.; Pauwels, R.; Baba, M.; Desmyter, J.; De Clercq, E. *J. Gen. Virol.* **1989**, *70*, 2397.
(38) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. *J. Natl. Cancer. Inst.* **1989**, *81*, 577.