Structure−Activity Relationships: Analogues of the Dicaffeoylquinic and Dicaffeoyltartaric Acids as Potent Inhibitors of Human Immunodeficiency Virus Type 1 Integrase and Replication†

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Received August 13, 1998

The dicaffeoylquinic acids (DCQAs) and dicaffeoyltartaric acids (DCTAs) are potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase. They also inhibit HIV-1 replication at nontoxic concentrations. Since integrase is an excellent target for anti-HIV therapy, structure−activity relationships were employed to synthesize compounds with: (1) improved potency against HIV-1 integrase, (2) improved anti-HIV effect in tissue culture, and (3) increased selectivity as indicated by low cellular toxicity. Thirty-four analogues of the DCTAs and DCQAs were synthesized and tested for cell toxicity, anti-HIV activity, and inhibition of HIV-1 integrase. Seventeen of the 34 analogues had potent activity against HIV-1 integrase ranging from 0.07 to >10 μM. Seventeen analogues that were synthesized or purchased had no inhibitory activity against integrase at concentrations of 25 μM. Of the biologically active analogues, 7 of the 17 inhibited HIV replication at nontoxic concentrations. The most potent compounds were D-chicoric acid, *meso*-chicoric acid, bis(3,4-dihydroxydihydrocinnamoyl)-L-tartaric acid, digalloyl-L-tartaric acid, bis(3,4-dihydroxybenzoyl)-L-tartaric acid, dicaffeoylglyceric acid, and bis(3,4-dihydroxyphenylacetyl)-L-tartaric acid. Anti-HIV activity of the active compounds in tissue culture ranged from 35 to 0.66 μM. Structure−activity relationships demonstrated that biscatechol moieties were absolutely required for inhibition of integrase, while at least one free carboxyl group was required for anti-HIV activity. These data demonstrate that analogues of the DCTAs and the DCQAs can be synthesized which have improved activity against HIV integrase.

Introduction

The human immunodeficiency virus type 1 (HIV-1) integrase (IN) is absolutely required for stable infection.[1−5] Therefore, it represents a potentially important enzymatic target for antiviral therapy. Despite reports of numerous inhibitors of HIV IN, to date, there has been little success developing inhibitors of IN that block HIV replication at nontoxic concentrations (reviewed in ref 6). One exception has been the dicaffeoylquinic acids (DCQAs) and the dicaffeoyltartaric acids (DCTAs). This class of inhibitors is potently active against HIV IN in biochemical assays and has moderate anti-HIV activity in tissue culture. The lead compound in this series was L-DCTA (L-chicoric acid) (**1**) with a fifty percent inhibitory activity (IC_{50}) against HIV-1 IN of 0.3 μM.[7,8] This same compound blocked HIV-1 infection by 50%, the fifty percent effective dose (ED_{50}), at 4 μM.[7,8] The concentration of this molecule that inhibited cellular replication by 5%, the five percent lethal dose (LD_5), was 222 μM. Therefore, L-DCTA was the most selective and potent of the previously reported inhibitors of HIV-1 IN (reviewed in ref 6).

The utility of the DCQAs and DCTAs as a class was highlighted in several recent publications. It has been shown that these compounds are highly selective for HIV-1 IN when compared to their activity against other HIV proteins and metalloenzymes.[9] Neamati et al. recently used the structure of the DCQAs[10] and the DCTAs[11] to generate a three-part or four-part pharmacophore. When these pharmacophores were used to search a chemical structure database, they returned a high "hit-rate" for inhibitors of HIV-1 IN,[10,11] although none of the compounds identified blocked HIV replication.[10,11]

IN can mediate several in vitro activities including 3′-end processing, strand transfer, and disintegration (reviewed in ref 12). Although a number of investigators have utilized the 3′-end processing and strand transfer reactions to screen compounds for anti-HIV activity,[13−18] the disintegration reaction offers several advantages. First, steady-state kinetic analyses can be performed using the dumbbell substrate in the disintegration reaction.[19,20] Second, disintegration is not simply the reversal of integration but may also measure other properties of IN including single-stranded DNA repair.[21] Third, for the DCTAs and DCQAs, disintegration seems less susceptible to nonspecific inhibition by dicaffeoyl-

† Taken in part from the dissertations of Qi Jia (1996) and Wenfang Miao (1998) submitted to Texas Christian University in partial fulfillment of the requirements of the Ph.D. degree. Preliminary report: Ma, G. X.; Reinecke, M. G.; Jia, Q.; Miao, W.; Robinson, W. E., Jr. Chicoric Acid Analogues as Potential Anti-HIV Integrase Inhibitors. *Abst. Natl. Mtg. Am. Chem. Soc.* **1998**, *215*, MED#28.

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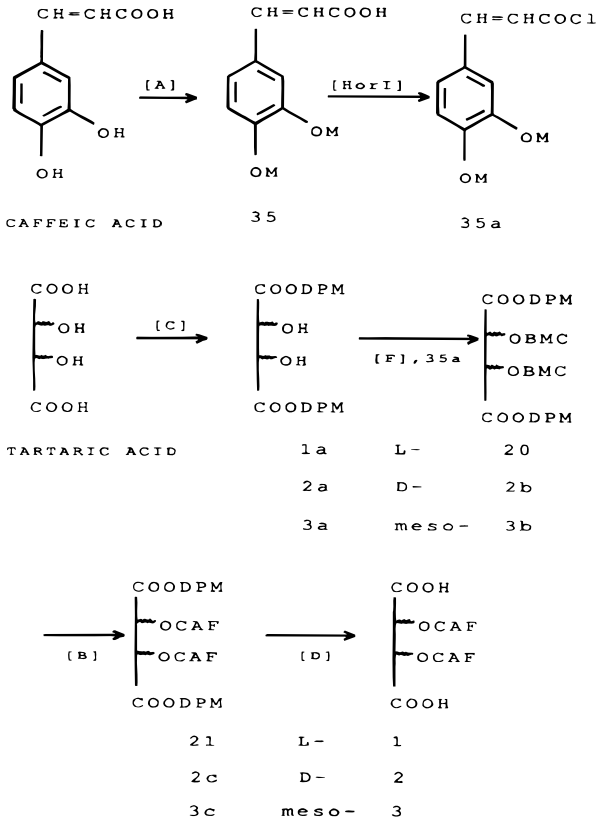
10.1021/jm9804735 CCC: $18.00
Published on Web 01/27/1999

Scheme 1[a]



[a] Abbreviations: OM = *O*-methylcarbonyl = −OCOOMe; DPM = diphenylmethyl = $-CHPh_2$; CAF = caffeoyl = −COCH=CH[3,4-$(OH)_2$-phenyl]; BMC = bis(methoxycarbonylcaffeoyl) = −COCH=CH[3,4-$(OM)_2$-phenyl]. Procedures: [A] ClCOOMe; [B] Na_2CO_3; [C] Ph_2CHN_2; [D] HOAc; [E] RCOCl; [F] RCOCl/R_3N; [G] L-tartaric acid; [H] $(COCl)_2$; [I] $SOCl_2$.

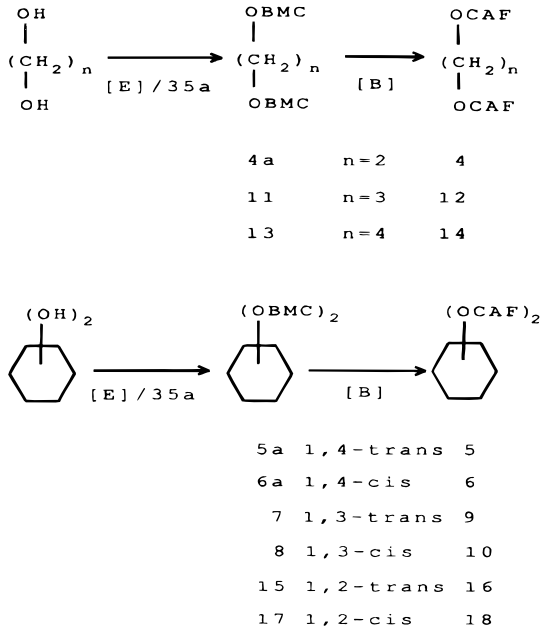
benzoic acid derivatives.[8] And finally, the catalytic core alone, rather than the whole IN molecule, can perform disintegration;[22,23] thus, inhibitors of disintegration are more likely to act against the IN core than other regions of the molecule.[7,8] We have previously shown that the DCTAs and DCQAs act on the catalytic core.[8]

In an effort to develop more potent and selective inhibitors of HIV-1 IN, analogues of the DCTAs and DCQAs were synthesized. The length of the side chains, the spatial arrangement of the phenolic hydroxyl groups, the size and structure of the central molecular core structure, and the requirement of one or more free carboxyl groups were all studied. The effects of these changes were assayed against HIV-1 IN in the disintegration reaction as well as against HIV-1 replication and cell growth in tissue culture.

Results

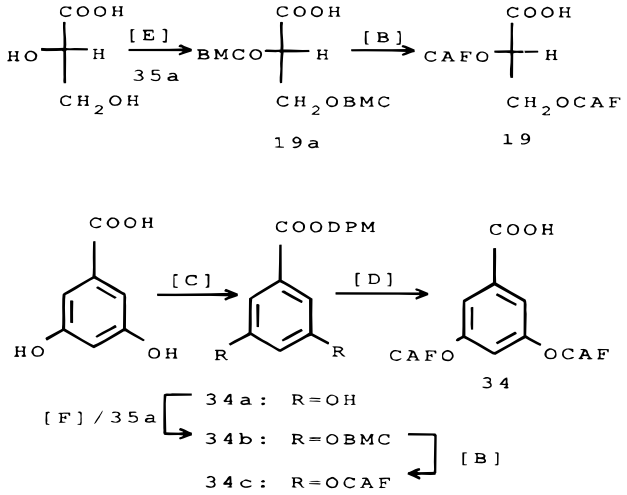
Synthetic Goals and Methodology. Both the DCQAs and the DCTAs are potent and selective inhibitors of both HIV-1 IN and HIV-1 replication in tissue culture.[7−9] Caffeic acid, quinic acid, chlorogenic acid (5-caffeoylquinic acid), and CAPE (caffeic acid phenethyl ester) show no such anti-HIV activity, consistent with the "biscatechol hypothesis",[15,18,24,25] although the last of these is reported to have weak anti-IN activity.[14] Using these compounds as "lead structures", structure−activity relationship (SAR) studies were initiated. To probe the structural features responsible for simultaneous anti-HIV and HIV IN inhibitory activities, a series of DCTA and DCQA analogues was prepared and their biological activities determined.

Scheme 2[a]



[a] See footnote to Scheme 1.

Scheme 3[a]

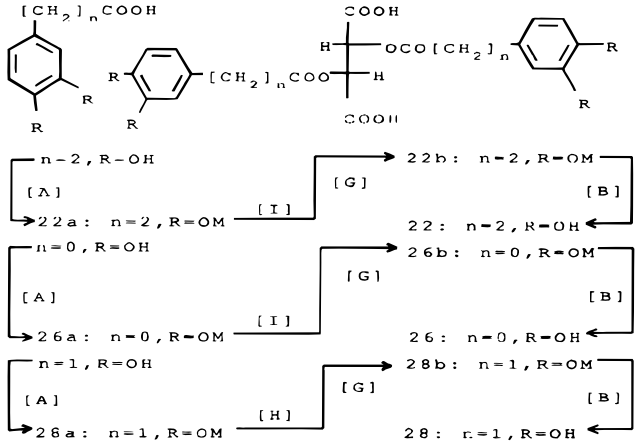


[a] See footnote to Scheme 1.

L- (**1**), D- (**2**)-, and *meso*- (**3**) DCTAs were synthesized by acylation of the bis(diphenylmethyl) tartrates **1a**, **2a**, and **3a** with the protected caffeoyl chloride **35a** to give the fully blocked compounds **20**, **2b**, and **3b**, from which the phenol and carboxyl blocking groups were sequentially removed (**21**, **2c**, and **3c**) (Scheme 1). [This synthesis, without experimental detail, was alluded to in ref 7 but misrepresented in a recent paper (*Synth. Commun.* **1998**, 737−740), as involving condensation of the bisdiphenyl ester (*sic*) of tartaric acid and the 3,4-cyclocarbonate of caffeoyl chloride.]

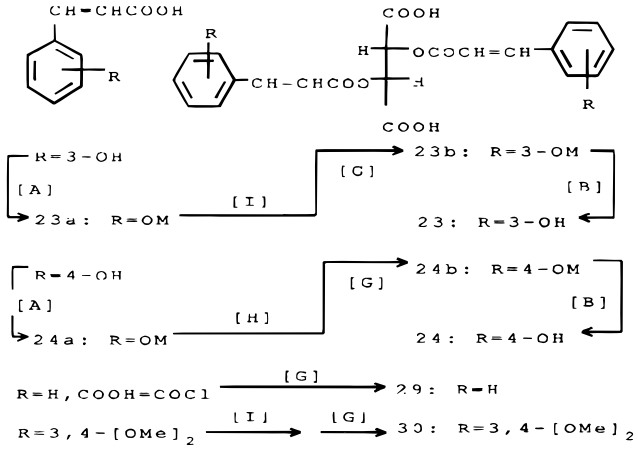
To determine the importance of a free carboxyl group for bioactivity, a series of alkane (**4**, **12**, **14**) and cyclohexane (**5**, **6**, **9**, **10**, **16**, **18**) dicaffeoyl esters lacking this group were prepared by direct acylation of the appropriate diol with **35a** to give the protected compounds **4a**, **5a**, **6a**, **7**, **8**, **11**, **13**, **15**, and **17**, from which

Scheme 4[a]



[a] See footnote to Scheme 1.

Scheme 5[a]



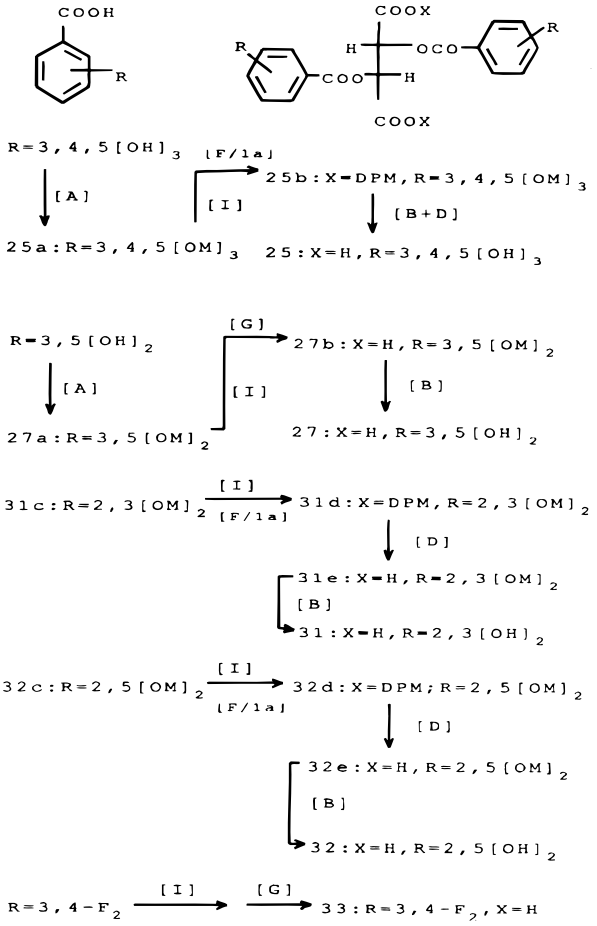
[a] See footnote to Scheme 1.

the phenol blocking groups were removed (Scheme 2). Two compounds with a single carboxyl group, dicaffeoylglyceric acid (**19**) and 3,5-dicaffeoylbenzoic acid (**34**), also were prepared by acylation with **35a** and deprotection via **19a** and **34a**−**c** (Scheme 3).

To determine the effect on bioactivity of the group linking the catechol and tartaric acid moieties of **1**, analogues were prepared with the CH=CH replaced by two (**22**), one (**28**), or zero (**26**) methylene groups. Direct acylation of L-tartaric acid with the acid chlorides of the phenol-protected acids **22a**, **28a**, and **26a** gave **22b**, **28b**, and **26b** from which the phenol blocking groups were removed (Scheme 4).

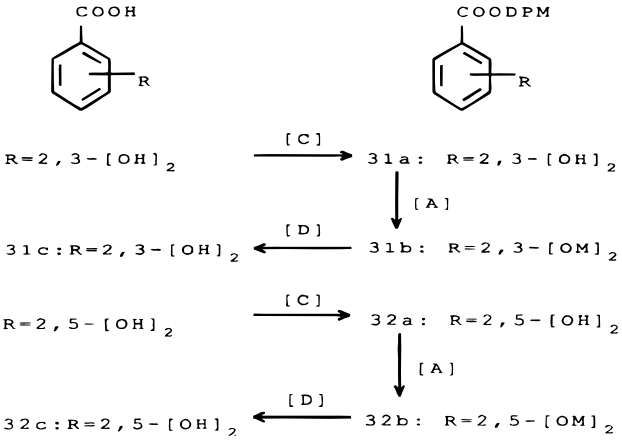
The significance for bioactivity of the number and position of the phenol groups was determined in both the cinnamic acid series (Scheme 5) and benzoic acid series (Scheme 6). Analogues with one (**23**, **24**) or three (**25**) phenolic hydroxyl groups as well as the resorcinol (**27**), 2,3-catechol (**31**), and hydroquinone (**32**) analogues were synthesized by acylation of L-tartaric acid (Scheme 5) or its diester **1a** (Scheme 6) with the acid chlorides of the phenol-protected acids **23a**, **24a**, **25a**, **27a**, **31c**, and **32c** followed by removal of the appropriate blocking groups. The preparation of acids **31c** and **32c** required initial blocking of the carboxyl groups as the diphenylmethyl esters so that the ortho phenol group could be protected (Scheme 7). Finally, several analogues {**29**, **30** (Scheme 5) and **33** (Scheme 6)} lacking phenol groups were made by direct acylation of L-tartaric acid.

Scheme 6[a]



[a] See footnote to Scheme 1.

Scheme 7[a]



[a] See footnote to Scheme 1.

Activity against HIV-1 IN (Initial Screening). Each compound was screened for inhibitory activity against HIV-1 IN in the disintegration assay.[21] The results of this screen are illustrated in Figure 1. The data were quantitated and are presented in Tables 1 and 2. Active analogues were defined as those compounds which inhibited HIV-1 IN by greater than 65% in the disintegration assay at 25 μM. Seventeen analogues met this criterion; they are listed in Table 1. Another 17 new analogues were inactive, as defined by

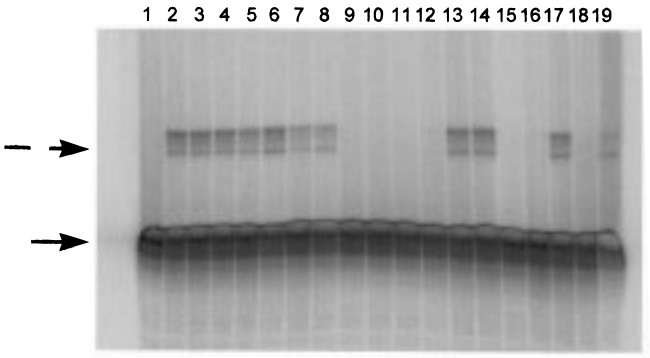


Figure 1. Representative screen of several DCTA and DCQA analogues in the disintegration assay. Compounds were diluted to 500 μM; 1 μL was added to 19 μL of disintegration reaction mixture containing 1.5 pmol of HIV-1 IN and 0.1 pmol of radiolabeled and annealed oligonucleotide primers. Reactions proceeded at 37 °C for 60 min. Products were separated on a 15% denaturing acrylamide gel. Compounds: lane 1, substrate alone; lane 2, substrate and enzyme; lane 3, substrate, enzyme, and 0.7% ethanol; lane 4, quinic acid; lane 5, chlorogenic acid; lane 6, tartaric acid; lane 7, **12**; lane 8, **14**; lane 9, **1**; lane 10, **2**; lane 11, **3**; lane 12, **22**; lane 13, **23**; lane 14, **24**; lane 15, **25**; lane 16, **26**; lane 17, **27**; lane 18, **28**; lane 19, **29**. Dashed long arrow indicates product; solid, short arrow indicates substrate.

less than or equal to 65% inhibition of HIV-1 IN in the disintegration assay at 25 μM (Table 2).

Anti-IN Activities of Biologically Active Compounds. The IC_{50} values for all of the biologically active compounds were determined using the disintegration assay. One representative assay is illustrated in Figure 2. The IC_{50} values for five representative compounds ranging from an inactive compound, chlorogenic acid, to the most potent inhibitor (**25**) are illustrated in Figure 3. The most potent compounds were **2** and **3** which had IC_{50} values of approximately 70−80 nM. The DCTAs, L-DCTA (**1**), D-DCTA (**2**), and *m*-DCTA (**3**), were the most potent group of compounds against HIV-1 IN in the disintegration assay. The least active compound that also demonstrated anti-HIV activity in tissue culture was **22** with an IC_{50} of 2.4 μM. With the exception of **2** and **3**, which were more potent than the lead compounds **1** and the DCQAs, all were of similar potency to **1** and the DCQAs.

Toxicity and Anti-HIV Activities. Next, all of the analogues were tested for cell toxicity and anti-HIV activity in tissue culture. Although the toxicities of these compounds varied widely, from a low of 7.4 μM to a high of 1389 μM, none of the compounds defined as biologically inactive (less than or equal to 65% inhibition of HIV-1 IN at 25 μM) blocked HIV-1 replication in tissue culture (Table 2). The biologically active compounds, on the other hand, contained compounds with anti-HIV activity and those without detectable activity. As shown in Table 1, cell toxicity for these compounds varied from a low of 2.7 μM to a high of 373 μM. Eleven compounds failed to block HIV-1 replication at nontoxic concentrations, while 7 compounds were potent inhibitors of HIV-1 replication (Table 1). The most active compound, **25**, had an ED_{50} of 660 nM. The least active compound, **28**, had an ED_{50} of 35 μM. The other six compounds had ED_{50} values ranging from 1 to 8 μM. One representative cell toxicity and anti-HIV activity experiment for each of the seven new, active compounds is illustrated in Figure 4.

Table 1. Anti-HIV Activity of Biologically Active DCQA and DCTA Analogues[a]

compd	formula wt	LD_5[b] (in μM)	ED_{50}[c] (μM)	% inhibn of IN at 25 μM[d]	IC_{50}[e] (μM)
1	474	264	4.2	96.9	0.18 (0.04)[f−h]
2	**474**	**115**	**6.3**	**100**	**0.07 (0.01)**
3	**474**	**373**	**4.2**	**97.5**	**0.08 (0.01)**
4	386	194	>388	83.4	>10
5	440	170	>341	85.2	1.03 (0.47)
6	440	20.5	>45	90.3	1.01 (0.21)
9	440	9.1	>34.1	80.7	1.8 (0.2)
10	440	36.4	>68.2	73.3	1.63 (0.39)
12	400	117.5	>350	81.2	>10
14	414	121	>242	67.6	>10
16	440	63.6	>136	80.2	>10
18	440	22.7	>90.9	81.7	8.36 (0.36)
19	**430**	**145**	**2.3**	**100**	**0.52 (0.06)**
22	**478**	**>263**	**8.4**	**94.5**	**2.38 (0.67)**
25	**454**	**2.7**	**0.66**	**100**	**0.97 (0.00)**
26	**422**	**90**	**0.95**	**97.2**	**0.43 (0.05)**
28	**450**	**70**	**35**	**100**	**0.88 (0.40)**
31	422	19	>38	100	1.07 (0.29)
34	478	100	>75	NT	6.9 (1.79)[h]
1,5-DCQA	516	145	4	NT	1.6 (0.16)[h]
3,5-DCQA	516	290	2	NT	1.3 (0.46)[g,h]
3,4-DCQA	516	116	12	NT	1.4 (0.6)[h]
4,5-DCQA	516	145	4	NT	0.6 (0.06)[h]
1-MO-3,5-DCQA	602	372	7	NT	0.8 (0.45)[g,h]

[a] Analogues of L-DCTA or the DCQAs were synthesized and screened for activity against HIV IN. Only active analogues (inhibition of enzyme by greater than 65% at 25 μM) are shown. [b] The five percent lethal dose (LD_5) is indicated. This is the concentration of analogue that inhibits growth of cells by 5%. This represents a truly nontoxic dose and is within 1 SD of cell control wells lacking any inhibitor. [c] The fifty percent effective dose (ED_{50}) is the concentration of compound that inhibits HIV-induced cell death by 50%. [d] Each compound was tested for inhibition of HIV IN (whole molecule) in the disintegration reaction[21] at a concentration of 25 μM. Any compound that inhibited the reaction by 65% or greater was selected for further testing. [e] IC_{50} of triplicate reactions performed against wild-type HIV_{NL4-3} IN. Analogues were tested in 0.5 log dilutions from 0.033 to 10 μM; 1.5 pmol of HIS-tagged whole HIV_{NL4-3} IN was tested for inhibitory activity in the disintegration assay using the method of Chow et al.[21] Reactions were allowed to proceed for 60 min at 37 °C. Products were separated on a 15% polyacrylamide sequencing gel and quantitated using a PhosphorImager. Numbers in parentheses are 1 SD. [f] Inhibitory activity for **1** differs slightly from that published by us previously (0.3 μM) but falls within the original standard deviation for the assays. The source of IN in these experiments was different from that published previously. Furthermore, IC_{50} results shown in this table were performed in 0.5 log dilutions rather than 1.0 log dilutions in the reactions reported previously. Either may play a role in the slightly different (50% lower) IC_{50} reported herein. [g] Reference 7. [h] Reference 8. Numbers in **bold** are for new compounds with anti-HIV activity at nontoxic concentrations.

Activities against an L-Chicoric Acid-Resistant Molecular Clone of HIV-1. We have previously described a molecular clone of HIV-1, called $HIV\text{-}1_{NL4-3clone1-D4}$, that is resistant to the antiviral activity of **1**.[26] Resistance results from a single amino acid change in IN. To determine whether analogues of **1** were also inhibiting HIV replication through inhibition of IN, the anti-HIV activity of **22**, **25**, **26**, and **28** was tested against $HIV\text{-}1_{NL4-3clone1-D4}$. The results are illustrated in Table 3. The mutation in IN confers cross-resistance to all four analogues; therefore, all four are acting to inhibit HIV replication, at least in large part, through inhibition of IN.

Table 2. Anti-HIV Activity of Biologically Inactive DCQA and DCTA Analogues[a]

compd	formula wt	LD_5[b] (in μM)	ED_{50}[c] (in μM)	% inhibn of IN at 25 μM[d]
quinic acid	192	907	>1823	18.7
caffeic acid	180	1389	>1389	0
L-tartaric acid	150	>1000	>1000	0
chlorogenic acid	354	249	>497	59.7
7	672	7.4	>14.8	−1.4
8	672	7.4	>29.6	30.6
11	632	26.9	>55.4	16.8
13	646	12.4	>23.3	6.3
15	672	8.9	>14.8	19.0
17	672	11.9	>44.6	39.7
20	1038	24.1	>48.2	16.7
21	806	16.1	>37.2	22.8
23	442	566	>283	23.7
24	442	114	>227	29.9
27	422	>119	>119	25.9
29	410	>245	>245	7.4
30	530	89	>178	0
32	422	>238	>238	20
33	430	28.9	>59.2	28

[a] Analogues of L-DCTA or the DCQAs were synthesized and screened for activity against HIV IN. Only inactive analogues (inhibition of enzyme by less than 65% at 25 μM) are shown. See Table 1 for active analogues. [b] The five percent lethal dose (LD_5) is indicated. This is the concentration of analogue that inhibits growth of cells by 5%. This represents a truly nontoxic dose and is within 1 SD of cell control wells lacking any inhibitor. [c] The fifty percent effective dose (ED_{50}) is the concentration of compound that inhibits HIV-induced cell death by 50%. [d] Each compound was tested for inhibition of HIV IN ('whole molecule) in the disintegration reaction[21] at a concentration of 25 μM.

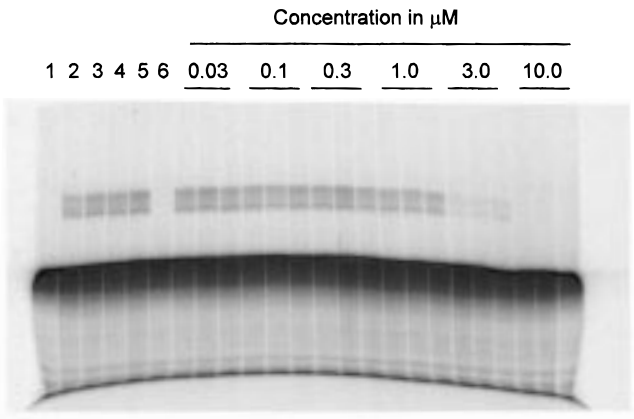


Figure 2. Representative assay for determining IC_{50} of active analogues. **31** was tested in the disintegration assay: lane 1, 0.1 pmol of substrate alone; lanes 2−24, 1.5 pmol of enzyme plus 0.1 pmol of substrate; lanes 2−4, enzyme and substrate alone. The remaining lanes contained in addition to enzyme and substrate: lane 5, 25 μM L-tartaric acid; lane 6, 25 μM **1**; lanes 7−9, 0.03 μM **31**; lanes 10−12, 0.1 μM **31**; lanes 13−15, 0.3 μM **31**; lanes 16−18, 1.0 μM **31**; lanes 19−21, 3.0 μM **31**; lanes 22−24, 10.0 μM **31**. Reactions were allowed to proceed at 37 °C for 60 min.

Discussion

Structure−activity relationships for IN inhibitors have demonstrated that biscatechols are active inhibitors of IN in biochemical assays, if not HIV in tissue culture[7−11,14,15,17,18,24,27−29] (reviewed in ref 6). However, the DCQAs and DCTAs, while biscatechols, are unique in their selectivity against HIV-1 IN and are also potent inhibitors in tissue culture.[7−9] Thus, it is possible to ask which structural components are (1) necessary for inhibition of IN? (2) necessary for inhibition of HIV-1 replication in tissue culture? and (3) selective for inhibition of HIV-1 at nontoxic concentrations?

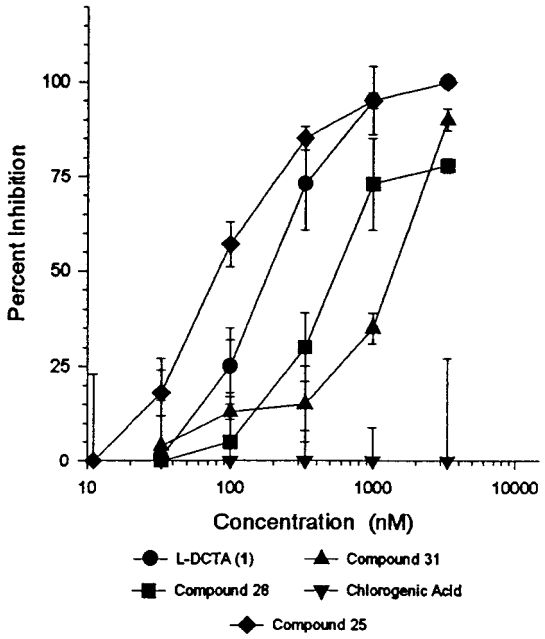


Figure 3. IC_{50} analysis of five representative analogues of the DCTAs and DCQAs in the disintegration assay. **1**, the lead compound, as well as **25**, the most potent inhibitor of IN, **28**, a strong inhibitor, **31**, a weak inhibitor, and chlorogenic acid, an inactive compound, were diluted and tested in the HIV disintegration assay. Each point represents the mean of triplicate assays; error bars are 1 SD.

The first compounds in the series altered the central core by lengthening or shortening the aliphatic central core domain. Compounds **4**, **12**, and **14** vary the length of the core from 2 to 4 carbons but lack the free carboxylic acid(s) of the DCQAs and **1**. All three compounds weakly inhibited HIV-1 IN (IC_{50} > 10 μM) but had no activity in tissue culture. Blocking the phenolic OH on the biscatechols of these compounds (**11** and **13**) resulted in loss of anti-IN activity. Having a cyclohexanediol central core (**5**, **6**, **9**, **10**, **16**, and **18**) increased the potency of the compounds against IN but did not result in compounds that could interfere with HIV-1 replication in tissue culture. Interestingly, several of these compounds, **5**, **6**, **9**, and **10**, were as active against HIV-1 IN as the DCQAs in biochemical assays (IC_{50} of 1−2 μM) but did not inhibit HIV-1 replication. These data suggest that a free carboxylic acid or additional, free hydroxyl groups on the central core may be necessary for uptake of the compounds by the cell or into the virion. Blocking the phenolic groups of the dicaffeoylcyclohexanediols (**7**, **8**, **15**, and **17**) abolished activity.

Previous data on the DCQA[8] had indicated that substitution patterns around the quinic acid core had little effect on anti-HIV-1 activity and IN inhibition. The same was not true with the dicaffeoylcyclohexanediols; although both the 1,4-substitution **(5, 6)** and the 1,3-substitution **(9, 10)** resulted in an IC_{50} of approximately 1−2 μM, the 1,2-substitution **(16, 18)** resulted in compounds with IC_{50} values of greater than 10 μM. Both cis- (**6**, **10**, and **18**) and trans- (**5**, **9**, and **16**) substituted rings were equally potent inhibitors of HIV-1 IN. The same was not true for the D- (**2**) and *meso*- (**3**) isomers of L-DCTA, which inhibited HIV-1 IN at submicromolar

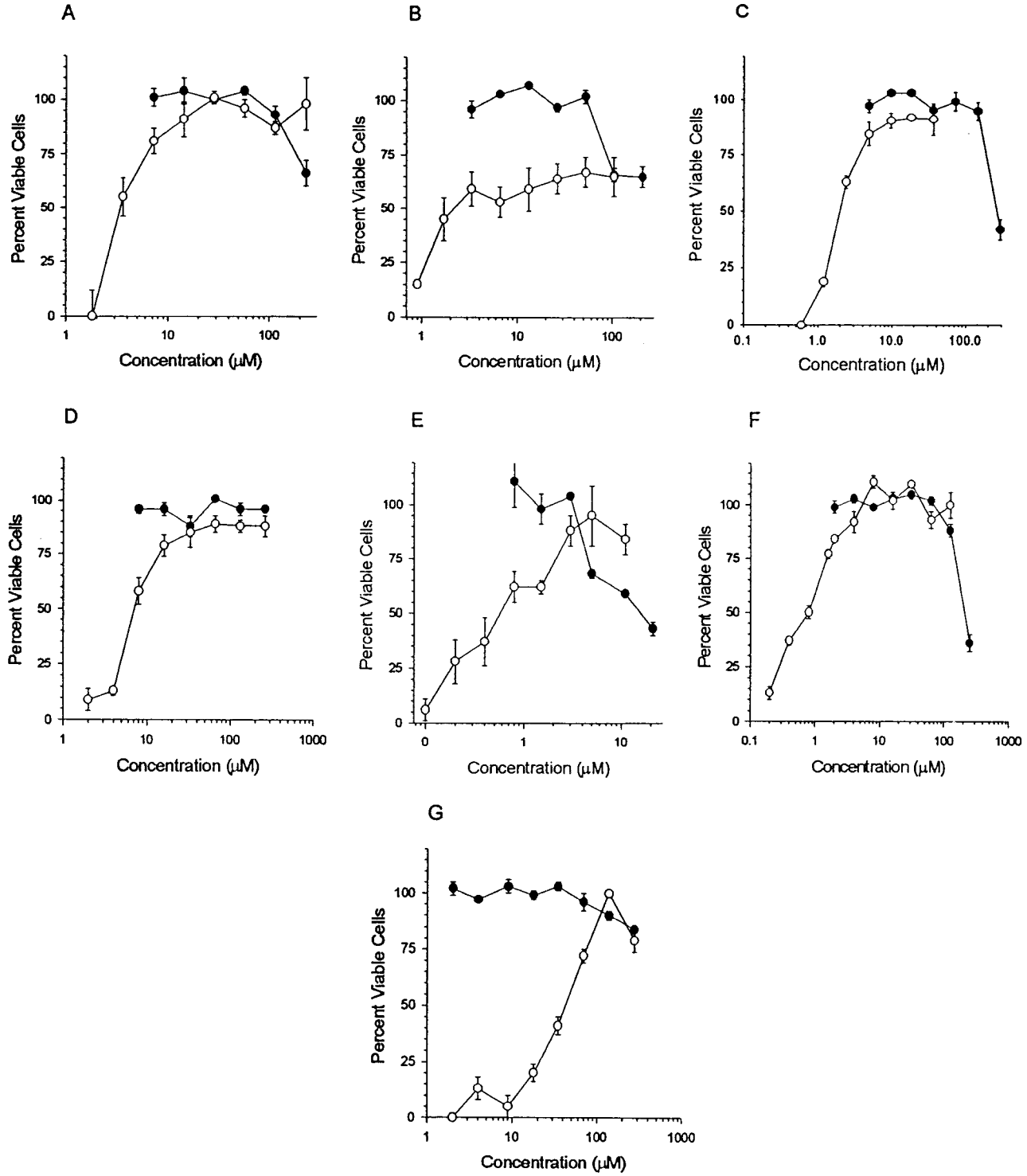


Figure 4. Cell toxicity and anti-HIV effects of seven novel integrase inhibitors. Representative experiments for each novel analogue of the DCTAs and the DCQAs that also inhibited HIV-1 IN are illustrated: (A) **2**; (B) **3**; (C) **19**; (D) **22**; (E) **25**; (F) **26**; (G) **28**. Closed circles are cell toxicity, and open circles are anti-HIV effect. Error bars are 1 SD of assays performed in triplicate.

concentrations with IC_{50} values of 0.07 and 0.08 μM, respectively. L-DCTA (**1**), on the other hand, was 2−3 times less potent. The addition of large blocking groups to the free carboxylic acids on L-DCTA (**21**) or the blocking of both phenolic hydrogens and the free carboxylic acid (**20**) resulted in loss of activity against HIV-1 IN. Although this was expected for **20**, the loss of activity when blocking the L-DCTA carboxyl groups (**21**) was unexpected in view of the HIV IN inhibition by the other biscatechols discussed above. This suggests that the presence of large groups on the central core (such as the diphenylmethylesters on **21**) may interfere with the ability of the compounds to fit in the active site of IN. Only one carboxylic acid was required for both anti-HIV IN activity and antiviral activity for the DCTAs, as **19** was a potent inhibitor of both disintegration and viral replication.

We had previously demonstrated that two caffeic acid groups were necessary for both potent IN inhibition and anti-HIV activity in tissue culture.[7,8] Several isomers

Table 3. Anti-HIV Activity of L-Chicoric Acid Analogues against an L-Chicoric Acid-Resistant Molecular Clone of HIV, HIV-$1_{NL4-3clone1-D4}$

	ED_{50} (in μM)		
compd	HIV_{NL4-3}[a]	$HIV_{NL4-3clone1-D4}$[b]	fold change
1	0.78	>61.25	>78
22	7.8	31.25	4
25	0.29	31.25	107
26	0.82	>52.5	>64
28	15.6	>62.5	>4

[a] The anti-HIV activity of each compound was tested against the infectious molecular clone of HIV-1, HIV_{NL4-3}. The ED_{50} is expressed as the mean of triplicate infections. Methods were the same as for uncloned HIV although the multiplicity of infection is between 0.1 and 1 for the clones. [b] The anti-HIV activity of each compound was measured against an L-chicoric acid-resistant molecular clone of HIV-1_{NL4-3}, HIV-$1_{NL4-3clone1-D4}$.[26] The results are expressed as the mean of triplicate infections. The multiplicity of infection was the same as for HIV-1_{NL4-3}.

or analogues were also active, however, including gallic acid (3,4,5-trihydroxybenzoyl) (**25**), which was significantly more toxic to dividing cells than were the other compounds (LD_5 of 2.7 μM) indicating that the third phenolic hydroxyl group decreases specificity against IN. The activity of the 3,4-dihydroxyphenylacetyl (**28**), 3,4-dihydroxybenzoyl (**26**), and 3,4-dihydroxydihydrocinnamoyl (**22**) analogues indicates that the length (0, 1, or 2 carbons) or hybridization (sp^2 or sp^3) of the linker group separating the catechol from the central core is not critical. The 3,4-biscatechol moiety was absolutely required, however, since the 3,5- (**27**), 2,3- (**31**), and 2,5- (**32**) dihydroxyphenyl isomers were inactive. This indicates that the anti-IN and anti-HIV activity of the biscatechols is not simply due to their antioxidant or metal-chelating properties. Blocking the phenolic hydroxyls (**30**) or reducing the number of hydroxyls from two to one (**23** and **24**) or none (**29**) also abolished activity as did replacement of the OH with fluorine (**33**).

Without exception, compounds that inhibited HIV-1 IN by less than 65% at 25 μM failed to inhibit HIV-1 replication. Indeed, only two compounds that inhibited HIV-1 IN by more than 90% at 25 μM (**6**) failed to inhibit HIV-1 replication in tissue culture. Therefore, for this class of compounds using the disintegration assay, inhibition of IN by >90% at a concentration of 25 μM would seem to be a reasonable "cutoff" value to screen for inhibitors of HIV-1 IN. We have identified two compounds that are more potent than **1**, the most potent inhibitor of HIV-1 IN reported previously. These two compounds, **2** and **3**, were as active or more active in tissue culture than either **1** or the DCQAs.

One question surrounding the use of this class of compounds as lead molecules in the search for inhibitors of HIV IN has been the mechanism of their anti-HIV activity. Previous work by Mahmood et al.[30] had suggested that one DCQA blocked HIV-1 gp120 from binding to its cellular receptor, CD4. Therefore, it seemed plausible that their mechanism of anti-HIV-1 activity was at the level of virus binding to its receptor. However, recent work has demonstrated that the DCTAs and DCQAs are selective inhibitors of HIV-1 IN.[9] Furthermore, the isolation of an L-DCTA-resistant HIV-1 molecular clone mapped the site of resistance to a single amino acid change in HIV-1 IN.[26] A likely L-DCTA-binding site was identified by cocrystallization of a structurally similar compound and an analogous IN protein from avian sarcoma virus.[31] The close proximity of the mutant amino acid to the likely drug-binding site supports the hypothesis that the anti-HIV-1 activity of this class of compounds, at least in part, is directed toward the IN protein. Studies on the anti-HIV activity of this class of compounds against the drug-resistant clone demonstrates that the four compounds tested (**22, 25, 26,** and **28**) were all less active against the drug-resistant virus. This data demonstrates that, like the parent molecule **1**, all are likely mediating their anti-HIV activity in tissue culture through inhibition of IN.

Several groups have recently identified compounds that are potent inhibitors of HIV IN in vitro and HIV replication in tissue culture.[7,8,32,33] New lead compounds described herein would include both meso- (**3**) and D- (**2**) DCTA, as well as dicaffeoylglyceric acid (**19**), digalloyl-L-tartaric acid (**25**), bis(3,4-dihydroxybenzoyl)-L-tartaric acid (**26**), and bis(3,4-dihydroxyphenylacetyl)-L-tartaric acid (**28**). Improvement in the selectivity of **25** and, likely, cell uptake of **28** may significantly enhance their use as lead compounds in the search for potent and selective inhibitors of HIV-1 IN. These new successes in analogue synthesis, coupled with improved crystallographic information on the IN core,[31,34,35] indicate that, through diligent SAR studies, clinically useful inhibitors of IN may be "just over the horizon".

Experimental Section

Chemical Syntheses. General. All melting points were measured on a Mel-Temp apparatus and are corrected. Elemental analyses were performed by M-H-W laboratories of Phoenix, AZ. MS analyses were obtained on a HP-5989A instrument at 70 eV in the EI mode with GC sampling unless otherwise noted as DIP (direct insertion probe). Selected peaks are reported as *m*/*z* (rel. int.) including all (except isotope peaks) with *m*/*z* >100 and rel. int. >25. HRFABMS were performed at the Washington University Resource for Biomedical and Bio-Organic Mass Spectrometry, St. Louis, MO. NMR spectra were obtained on a Varian XL-300 instrument at 299.936 (^{1}H) or 75.427 (^{13}C) unless otherwise noted in the indicated solvent (D = DMSO-d_6, C = $CDCl_3$, M = CD_3OD) and are reported in order as: ppm downfield from TMS at δ = 0, multiplicity (s, d, dd, m, bs), observed couplings *J* in Hz, and relative number of H's. APT spectra results are expressed as d = C or CH_2, u = CH or CH_3. HPLC used a C-18 10-μm, 250-mm × 4.6-mm analytical column or a C18 10-μm, 250- × 22-mm preparative column eluted with either methanol−water or acetonitrile−water mixtures containing 1% HOAc and UV detection at 254 nm. The following general synthetic procedures refer to both Schemes 1−7 and the preparation of the specific compounds to follow. No attempt was made to optimize yields.

Procedure A, Carboxymethylation of Phenols. A solution of the phenolic acid in 2.2 equiv of 1 N NaOH was cooled to 0 °C, and 3.5 equiv of methyl chloroformate was added dropwise with stirring. The precipitate was collected by filtration, washed with DI water, air-dried, and recrystallized to give the blocked phenolic acid.

Procedure B, Removal of Carboxymethyl Groups. The carboxymethylated phenol was dissolved in THF and hydrolyzed with 2% Na_2CO_3 with enough MeOH for homogeneity at room temperature, under N_2 for 5−7 h. The reaction mixture was acidified to pH = 1−2 with 10 N HCl and extracted with ether, the ether evaporated, and the residue purified by chromatography on silica gel, if necessary.

Procedure C, Formation of Diphenylmethyl Esters. A solution of 1.25 equiv of diphenyldiazomethane per COOH group in chloroform was added to the carboxylic acid in MeOH−$CHCl_3$. The mixture was stirred at room temperature

until the red color disappeared and washed successively with 1 N HCl, saturated $NaHCO_3$, and H_2O. The organic layer was dried with Na_2SO_4 and evaporated on a Rotovap to afford the diphenylmethyl ester which was recrystallized if necessary.

Procedure D, Hydrolysis of Diphenylmethyl Esters. The diphenylmethyl esters were deprotected in refluxing 70% acetic acid under N_2 for 4 h. The solvent was removed by lyophilization and the residue purified by chromatography on Sephadex LH-20.

Procedure E, Direct Acylation of Alcohol Groups. The alcohol was reacted with a slight excess of the acid chloride without solvent in an oil bath at 130−140 °C for 15 min and the residue chromatographed on silica gel.

Procedure F, Solution Acylation of Alcohol Groups. The acyl chloride was reacted with the alcohol in anhydrous benzene with pyridine or triethylamine as a catalyst for 2.5 h at room temperature. The reaction mixture was successively washed with 1 N HCl, saturated $NaHCO_3$, and water and the organic layer evaporated to give the ester which was purified on a silica gel column.

Procedure G, Direct Acylation of L-Tartaric Acid. A modification of Scarpati's method[36] involves heating L-tartaric acid with an excess of an acyl chloride in an oil bath at 135−160 °C for 10−30 min followed by hydrolysis of the intermediate anhydride with 80% HOAc on a steam bath for 30 min. The residue from removal of the solvent below 40 °C under reduced pressure was partitioned between water and ether and the latter dried and evaporated to give a crude product which was purified by chromatography on silica gel or Sephadex LH-20.

Procedure H, Formation of Acyl Chlorides with Oxalyl Chloride. A solution of the acid in excess oxalyl chloride was stirred with a 25-fold excess of oxalyl chloride at room temperature for 1 h, the excess reagent removed on a Rotovap, and the acid chloride used immediately without purification.

Procedure I, Formation of Acyl Chlorides with Thionyl Chloride. A solution of the acid in excess thionyl chloride was heated (NaOH trap) in an oil bath at 80−90 °C until HCl evolution ceased. Removal of the thionyl chloride on a Rotovap gave the acid chloride used immediately without purification.

Specific Syntheses. Dicaffeoyl-L-tartaric Acid (L-Chicoric Acid, L-DCTA) (1). L-Tartaric acid was converted by procedure C to **bis(diphenylmethyl) L-tartrate (1a)** as a white powder, mp = 107−108 °C; ^{1}H NMR (C) 7.24−7.32 (m, 20H), 6.98 (s, 2H), 4.75 (s, 2H); ^{13}C NMR (C) 170.7, 139.0, 138.9, 128.6, 128.5, 128.4, 128.1, 127.6, 126.9, 79.2, 72.3; DIP/MS 315 (2), 183 (33), 167 (100), 165 (43). Reaction of **1a** with **35a** via procedure F gave **bis(diphenylmethyl) bis[bis(methoxycarbonyl)caffeoyl]-L-tartrate (20)**, mp = 62−64 °C; ^{1}H NMR (C) 7.50 (d, 16.0, 2H), 7.10−7.38 (m, 20H), 6.94 (s, 2H), 6.16 (d, 16.0, 2H), 6.04 (s, 2H), 3.93 (s, 6H), 3.92 (s, 6H); ^{13}C NMR (C) 164.8 (×2), 153.1, 152.9, 144.3, 143.9, 142.7, 138.8, 138.7, 133.0, 128.63, 128.57, 128.2 (×2), 127.3, 127.0 (×2), 123.50, 122.6, 117.5, 79.1, 71.1, 56.0 (×2). Removal of the methoxycarbonyl groups by procedure B gave **bis(diphenylmethyl) dicaffeoyl-L-tartrate (21)**, mp = 135−136 °C dec; ^{1}H NMR (M) 7.47 (d, 15.8, 2H), 6.91 (s, 2H), 6.79−7.36 (m, 20H), 6.10 (s, 2H), 6.07 (d, 15.8, 2H); ^{13}C NMR (M) 167.4d, 166.8d, 150.2d, 148.8u, 146.9d, 140.7d (×2), 129.7u (×2), 129.2u (×2), 128.2u, 128.0u, 127.5d, 123.6u, 116.6u, 115.4u, 113.3u, 80.3u, 72.5u. Removal of the diphenylmethyl groups by procedure D gave L-**DCTA (1)**, whose ^{1}H and ^{13}C NMR agreed with the literature.[37]

Dicaffeoyl-D-tartaric Acid (D-Chicoric Acid, D-DCTA) (2). D-Tartaric acid was converted by procedure C to **bis(diphenylmethyl) D-tartrate (2a)** as a white powder, mp = 108−110 °C; ^{1}H NMR (C) 7.28−7.34 (m, 20H), 6.99 (s, 2H), 4.76 (s, 2H); ^{13}C NMR (C) 170.7, 139.0, 138.9, 128.6, 128.5, 128.4, 128.1, 127.6, 126.9, 79.2, 72.3; DIP/MS 315 (2), 183 (37), 167 (100), 165 (47). Reaction of **2a** with **35a** via procedure F gave **bis(diphenylmethyl) bis[bis(methoxycarbonyl)caffeoyl]-D-tartrate (2b)**, mp = 61−63 °C; ^{1}H NMR (C) 7.50 (d, 16.0, 2H), 7.09−7.37 (m, 20H), 6.94 (s, 2H), 6.16 (d, 16.0, 2H), 6.04 (s, 2H), 3.93 (s, 6H), 3.92 (s, 6H); ^{13}C NMR (C) 164.8 (×2), 153.1, 152.9, 144.3, 143.9, 142.7, 138.8, 138.7, 133.0, 128.63, 128.57, 128.2 (×2), 127.3, 127.0 (×2), 123.50, 122.6, 117.5, 79.1, 71.1, 56.0 (×2). Removal of the methoxycarbonyl groups by procedure B gave **bis(diphenylmethyl) dicaffeoyl-D-tartrate (2c)**, mp = 134−136 °C dec; ^{1}H NMR (M) 7.49 (d, 15.8, 2H), 6.90 (s, 2H), 6.80−7.33 (m, 20H), 6.08 (s, 2H), 6.07 (d, 15.8, 2H); ^{13}C NMR (M) 167.4d, 166.8d, 150.0d, 148.7u, 146.8d, 140.6d, 140.5d, 129.6u (×2), 129.1u (×2), 128.1u, 127.9u, 127.4d, 123.6u, 116.5u, 115.3u, 113.2u, 80.4u, 72.5u. Removal of the diphenylmethyl groups by procedure D gave **D-DCTA (2)**, whose ^{1}H and ^{13}C NMR agreed with the literature.[38]

Dicaffeoyl-*meso*-tartaric Acid (*meso*-Chicoric Acid, *meso*-DCTA) (3). *meso*-Tartaric acid was converted by procedure C to **bis(diphenylmethyl) *meso*-tartrate (3a)** as a white powder, mp = 108−109 °C; ^{1}H NMR (C) 7.18−7.27 (m, 20H), 6.82 (s, 2H), 4.72 (s,2H); ^{13}C NMR (C) 170.2, 139.0, 138.7, 128.52, 128.45, 128.23, 128.18, 128.0, 127.2, 79.4, 73.1; DIP/MS 315 (2), 183 (37), 167 (100), 165 (47); DIP/MS 315 (2), 183 (35), 167 (100), 165 (48). Reaction of **3a** with **35a** via procedure F gave **bis(diphenylmethyl) bis[bis(methoxycarbonyl)-caffeoyl]-*meso*-tartrate (3b)**, mp = 72−74 °C; ^{1}H NMR (C) 7.59 (d, 16.0, 2H), 7.21−7.38 (m, 20H), 6.88 (s, 2H), 6.29 (d, 16.0, 2H), 6.02 (s, 2H), 3.91 (s, 6H), 3.90 (s, 6H); ^{13}C NMR (C) 164.9, 164.8, 153.0, 152.9, 144.6, 143.9, 142.7, 139.01, 138.98, 133.01, 128.6, 128.5, 128.27, 128.1, 127.3, 127.1 (×2), 123.5, 122.6, 117.8, 79.6, 71.6, 55.9 (×2). Removal of the methoxycarbonyl groups by procedure B gave **bis(diphenylmethyl) dicaffeoyl-*meso*-tartrate (3c)**, mp = 142−144 °C dec; ^{1}H NMR (M) 7.55 (d, 16.0, 2H), 6.82 (s, 2H), 6.77−7.25 (m, 20H), 6.20 (d, 16.0, 2H), 6.01 (s, 2H); ^{13}C NMR (M) 167.4d, 166.8d, 150.1d, 148.9u, 146.8d, 140.8 (×2)d, 129.6u, 129.5u, 129.2u, 129.1u, 128.2u, 128.1u, 127.3d, 123.5u, 116.5u, 115.4u, 113.4u, 80.8u, 72.8u. Removal of the diphenylmethyl groups by procedure D gave ***meso*-DCTA (3)** whose ^{1}H and ^{13}C NMR agreed with the literature except that C-1 and C-2 of the tartaric acid portion were 3.5 ppm upfield from that of an alleged *meso*-chicoric acid from *Equisetum arvense*.[39] Since the latter was isolated by chromatography with 1% NH_3 in MeOH, it may have been the ammonium salt. Dissociation of a carboxyl group deshields both the carbonyl and α-carbon resonances by 3−4 ppm.[38] This hypothesis was confirmed since the ^{13}C NMR of the ammonium salt of **3** matched that of the "*meso*-chicoric acid" isolated from *Equisetum* which therefore was of the corresponding ammonium salt.

1,2-Dicaffeoylethanediol (4). Ethylene glycol reacted with **35a** by procedure E to give **1,2-bis[bis(methoxycarbonyl)-caffeoyl]ethanediol (4a)**, mp = 88−90 °C; ^{1}H NMR (C) 7.66 (d, 16.0, 2H), 7.48 (d, 1.9, 2H), 7.45 (dd, 1.9, 8.4, 2H), 7.32 (d, 8.4, 2H), 6.44 (d, 16.1, 2H), 4.48 (s, 4H), 3.91 (s, 12H); ^{13}C NMR (C) 166.2, 153.07, 152.92, 143.6, 143.2, 142.7, 133.4, 126.7, 123.5, 122.4, 119.1, 62.5, 55.9 (×2); DIP/MS 618 (1), 233 (61), 189 (100), 145 (26), 117 (32). Hydrolysis of **4a** by procedure B gave **4**, mp = 239−240 °C; ^{1}H NMR (M) 7.57 (d, 15.9, 2H), 7.04 (d, 1.9, 2H), 6.93 (dd, 2.0, 8.2, 2H), 6.75 (d, 8.2, 2H), 6.28 (d, 16.0, 2H), 4.43 (s, 4H); ^{13}C NMR (M) 169.1, 150.4, 147.6, 147.1, 127.3, 123.2, 116.6, 115.0, 114.4, 63.5. Anal. ($C_{20}H_{18}O_8$) C, H.

1,4-*trans*- and 1,4-*cis*-Dicaffeoylcyclohexanediols (5 and 6). A mixture of *cis*- and *trans*-1,4-cyclohexanediols (Aldrich C10,120-6) reacted with **35a** by procedure E to give a stereoisomeric mixture of 1,4-bis[bis(methoxycarbonyl)caffeoyl]cyclohexanediols which was separated with benzene:acetone on a silica gel column. The first isomer to elute was identified as the 1,4-*trans*-isomer because the diaxial carbinol protons are, as expected,[40] at higher field (4.98 ppm) than those of the axial−equatorial carbinol protons (5.02 ppm) of the *cis*-isomer. **1,4-*trans*-Bis[bis(methoxycarbonyl)caffeoyl]cyclohexanediol (5a),** mp = 174−177 °C; ^{1}H NMR (C) 7.62 (d, 16.0, 2H), 7.47 (s, 2H), 7.43 (d, 8.5, 2H), 7.32 (d, 8.5, 2H), 6.40 (d, 16.0, 2H), 4.98 (bs, 2H), 3.92 (s, 12H), 2.08 (bd, 4H), 1.67 (m, 4H); ^{13}C NMR (C) 165.9, 153.1, 152.9, 143.5, 142.7, 142.4, 133.5, 126.6, 123.5, 122.3, 120.0, 71.2, 55.9 (×2), 28.0. The second isomer to elute was **1,4-*cis*-bis[bis(methoxycarbonyl)caffeoyl]cyclohexanediol (6a),** mp = 60−64 °C; ^{1}H

NMR (C) 7.64 (d, 16.0, 2H), 7.48 (d, 1.9, 2H), 7.44 (dd, 1.9, 8.5, 2H), 7.32 (d, 8.5, 2H), 6.43 (d, 16.0, 2H), 5.02 (m, 2H), 3.91 (s, 12H), 1.90−2.10 (m, 4H), 1.67−1.85 (m, 4H); ^{13}C NMR (C) 165.8, 153.1, 153.0, 143.5, 142.7, 142.4, 133.6, 126.6, 123.5, 122.3, 120.1, 70.4, 55.9 (×2), 27.4. Hydrolysis of **5a** by procedure B gave **5,** mp = 294−296 °C; ^{1}H NMR (M) 7.53 (d, 15.8, 2H), 6.98 (d, 2.0, 2H), 6.84 (dd, 8.1, 2.0, 2H), 6.65 (d, 8.1, 2H), 6.12 (d, 15.8, 2H), 4.92 (bs, 2H), 2.07 (m, 4H), 1.76 (m, 4H); ^{13}C NMR (M) 169.5, 156.4, 149.5, 148.2, 124.3, 123.8, 117.0, 113.3, 112.3, 72.1, 29.2. Anal. ($C_{24}H_{24}O_8$) C, H. Hydrolysis of **6a** by procedure B gave **6,** mp = 262 °C; ^{1}H NMR (M) 7.56 (d, 15.8, 2H), 7.04 (s, 2H), 6.91 (d, 8.1, 2H), 6.73 (d, 8.1, 2H), 6.22 (d, 15.8, 2H), 4.96 (bs, 2H), 1.92 (m, 4H), 1.84 (m, 4H); ^{13}C NMR (M) 169.0, 153.0, 148.2, 147.5, 126.0, 123.4, 116.8, 114.3, 114.0, 71.3, 28.5. Anal. ($C_{24}H_{24}O_8$) C, H.

1,3-*trans*- and 1,3-*cis*-Dicaffeoylcyclohexanediols (9 and 10). A mixture of *cis*- and *trans*-1,3-cyclohexanediols (Aldrich C10,110-9) reacted with **35a** by procedure E to give a stereoisomeric mixture of 1,3-bis[bis(methoxycarbonyl)caffeoyl]cyclohexanediols which was separated with benzene: acetone on a silica gel column. The first isomer to elute was identified as the 1,3-*trans*-isomer because the axial−equatorial carbinol protons are at lower field (5.28 ppm) than those of the diaxial carbinol protons (4.93 ppm) of the *cis*-isomer (cf. **5a** and **6a**). **1,3-*trans*-Bis[bis(methoxycarbonyl)caffeoyl]-cyclohexanediol (7),** mp = 56−57 °C; ^{1}H NMR (C) 7.62 (d, 16.0, 2H), 7.47 (d, 1.9, 2H), 7.44 (dd, 2.0, 8.5, 2H), 7.32 (d, 8.4, 2H), 6.41 (d, 16.0, 2H), 5.28 (bs, 2H), 3.93 (s, 6H), 3.92 (s, 6H), 1.99 (m, 2H), 1.68−1.90 (m, 6H); ^{13}C NMR (C) 165.7, 153.1, 153.0, 143.6, 142.7, 142.5, 133.6, 126.6, 123.5, 122.3, 120.0, 70.3, 55.9 (×2), 35.7, 30.3, 19.3; DIP/MS 672 (1), 279 (29), 252 (33), 238 (26), 235 (53), 221 (25), 208 (54), 194 (25), 191 (40), 189 (92), 81 (100). The second isomer to elute was **1,3-cis-bis-[bis(methoxycarbonyl)caffeoyl]cyclohexanediol (8)**, mp = 59−61 °C; ^{1}H NMR (C) 7.61 (d, 15.9, 2H), 7.45 (d, 1.9, 2H), 7.40 (dd, 1.9, 8.5, 2H), 7.30 (d, 8.4, 2H), 6.37 (d, 16.0, 2H), 4.93 (m, 2H), 3.91 (s, 12H), 2.38 (d, 11.8, 1H), 1.60−2.06 (m, 5H), 1.26−1.48 (m, 2H); ^{13}C NMR (C) 165.6, 153.1, 152.9, 143.6, 142.7, 142.5, 133.5, 126.5, 123.5, 122.3, 119.9, 70.8, 55.9 (×2), 37.1, 30.8, 20.0; DIP/MS 672 (1), 538 (29), 252 (38), 235 (34), 221 (26), 208 (78), 194 (26), 191 (40), 189 (100). Hydrolysis of **7** by procedure B gave **9**, mp = 105−106 °C; ^{1}H NMR (M) 7.55 (d, 15.8, 2H), 7.03 (s, 2H) 6.93 (d, 8.2, 2H), 6.75 (d, 8.2, 2H), 6.24 (d, 15.8, 2H), 5.21 (m, 2H), 1.68−1.98 (m, 8H); ^{13}C NMR (M) 168.7, 151.0, 147.4, 147.2, 127.0, 123.2, 116.6, 114.8, 114.7, 71.3, 36.8, 31.3, 20.5. Anal. ($C_{24}H_{24}O_8 \cdot 0.5H_2O$) C, H. Hydrolysis of **8** by procedure B gave **10**, mp = 108 °C; ^{1}H NMR (M) 7.53 (d, 15.7, 2H), 7.00 (s, 2H) 6.85 (d, 7.9, 2H), 6.69 (d, 7.9, 2H), 6.18 (d, 15.7, 2H), 4.93 (bs, 2H), 2.29 (m, 1H), 1.67−1.96 (m, 5H) 1.38 (m, 2H); ^{13}C NMR (M) 168.8, 152.4, 148.0, 147.5, 126.3, 123.2, 116.7, 114.5, 114.1, 71.6, 37.8, 31.8, 20.6. Anal. ($C_{24}H_{24}O_8$) C, H.

1,3-Dicaffeoylpropanediol (12). 1,3-Propanediol reacted with **35a** by procedure E to give **1,3-bis[bis(methoxycarbonyl)caffeoyl]propanediol (11)**, gum; ^{1}H NMR (C) 7.63 (d, 2H, 16.1), 7.47 (d, 2H, 1.9), 7.41 (d, 2H, 2.0, 8.5), 7.30 (d, 2H, 8.4), 6.41 (d, 2H, 16.1),4.35 (t, 4H, 6.2), 3.92 (s, 6H), 3.91 (s, 6H), 2.12 (t, 2H, 6.2); ^{13}C NMR (C) 166.3, 153.1, 152.9, 143.6, 142.8, 142.7, 133.4, 126.6, 123.5, 122.3, 119.4, 61.4, 55.9 (×2), 28.1. Hydrolysis of **11** by procedure B gave **12**, mp = 210−212 °C; ^{1}H NMR (D) 7.47 (d, 15.9, 2H), 7.02 (d, 1.6, 2H), 6.92 (dd, 1.7, 8.2, 2H), 6.67 (d, 8.1, 2H), 6.22 (d, 15.9, 2H), 4.21 (t, 6.3, 4H), 2.01 (t, 6.3, 2H); ^{13}C NMR (D) 166.6, 151.0, 146.7, 145.7, 124.0, 121.4, 116.0, 114.7, 112.3, 60.6, 27.8. Anal. ($C_{21}H_{20}O_8$) C, H.

1,4-Dicaffeoylbutanediol (14). 1,4-Butanediol reacted with **35a** by procedure E gave **1,4-bis[bis(methoxycarbonyl)caffeoyl]butanediol (13),** mp = 143−144 °C; ^{1}H NMR (C) 7.62 (d, 16.0, 2H), 7.47 (d, 1.9, 2H), 7.42 (dd, 8.5, 1.9, 2H), 7.31 (d, 8.4, 2H), 6.41 (d, 15.9, 2H), 4.27 (t, 5.0, 4H), 3.91 (s, 6H), 3.90 (s, 6H), 1.84 (bs, 4H); ^{13}C NMR (C) 166.5, 153.1, 152.9, 143.4, 142.7, 142.6, 135.5, 126.6, 123.5, 122.3, 119.6, 64.2, 55.9 (×2), 25.4. Hydrolysis of **13** by procedure B gave **14**, mp = 241−242 °C; ^{1}H NMR (D) 7.45 (d, 15.7, 2H), 6.99 (d, 2.0, 2H), 6.89 (dd, 2.0, 8.2, 2H), 6.62 (d, 8.1, 2H), 6.16 (d, 15.8, 2H), 4.14 (bs, 4H), 1.73 (bs, 4H); ^{13}C NMR (D) 166.8, 152.9, 147.2, 145.8, 122.8, 121.9, 115.6, 113.5, 111.4, 63.0, 25.0. Anal. ($C_{22}H_{22}O_8 \cdot 0.8H_2O$) C, H.

1,2-*trans*-Dicaffeoylcyclohexanediol (16). 1,2-*trans*-Cyclohexanediol reacted with **35a** by procedure E to give **1,2-*trans*-bis[bis(methoxycarbonyl)caffeoyl]cyclohexanediol (15)**, mp = 46−47 °C; ^{1}H NMR (C) 7.58 (d, 16.0, 2H), 7.43 (d, 2.0, 2H), 7.39 (dd, 2.0, 8.5, 2H), 7.29 (d, 8.5, 2H), 6.34 (d, 15.9, 2H), 5.02 (m, 2H), 3.91 (s, 6H), 3.90 (s, 6H), 2.16 (bd, 2H), 1.78 (m, 2H), 1.42−1.53 (m, 4H); ^{13}C NMR (C) 165.8, 153.1, 152.9, 143.6, 142.8, 142.6, 133.5, 126.7, 123.4, 122.3, 119.6, 74.1, 55.9 (×2), 30.3, 23.5. Hydrolysis of **15** by procedure B gave **16**, mp = 99 °C; ^{1}H NMR (D) 7.44 (d, 16.0, 2H), 7.01 (s, 2H), 6.96 (d, 8.4, 2H), 6.73 (d, 8.4, 2H), 6.19 (d, 15.8, 2H), 4.91 (d, 6.4, 2H), 2.01 (bd, 10.9, 2H), 1.46 (m, 2H), 1.41−1.47 (m, 4H); ^{13}C NMR (D) 165.8, 148.3, 145.4, 145.3, 125.3, 121.2, 115.6, 114.8, 113.7, 73.0, 29.8, 22.9. Anal. ($C_{24}H_{24}O_8$) C, H.

1,2-*cis*-Dicaffeoylcyclohexanediol (18). 1,2-*cis*-Cyclohexanediol reacted with **35a** by procedure E to give **1,2-*cis*-bis-[bis(methoxycarbonyl)caffeoyl]cyclohexanediol (17)**, mp = 60.5−63 °C; ^{1}H NMR (C) 7.61 (d, 16.0, 2H), 7.45 (d, 1.9, 2H), 7.41 (dd, 8.5, 1.9, 2H), 7.33 (d, 9.1, 2H), 6.41 (d, 15.9, 2H), 5.21 (d, 7.0, 2H), 3.91 (s, 12H), 1.97 (m, 2H), 1.73 (m, 4H), 1.52 (m, 2H); ^{13}C NMR (C) 165.7, 153.1, 152.9, 143.6, 142.7, 142.7, 133.6, 126.7, 123.5, 122.3, 119.9, 71.5, 55.9 (×2), 27.8, 21.8. Hydrolysis of **17** by procedure B gave **18**, mp = 105−106 °C; ^{1}H NMR (D) 7.47 (d, 16.0, 2H), 7.06 (s, 2H) 6.99 (dd, 8.2, ca. 2, 2H), 6.76 (d, 8.1, 2H), 6.27 (d, 16.0, 2H), 5.10 (d, 6.4, 2H), 1.86 (m, 2H), 1.69 (m, 4H) 1.47 (m, 2H); ^{13}C NMR (D) 165.8, 148.3, 145.4, 145.1, 125.4, 121.4, 115.6, 114.7, 114.0, 70.4, 27.3, 21.3. Anal. ($C_{24}H_{24}O_8 \cdot 0.33H_2O$) C, H.

Dicaffeoyl-L-glyceric Acid (19). Calcium L-glycerate dihydrate (Aldrich 37241-2) was dried to constant weight and reacted with **35a** by procedure E to give **bis[bis(methoxycarbonyl)caffeoyl]glyceric acid (19a)**, gum; ^{1}H NMR (C) 7.73 (d, 16.0, 1H), 7.66 (d, 16.0, 1H), 7.3−7.5 (m, 6H), 6.51 (d, 16.0, 1H), 6.44 (d, 16.0, 1H), 5.56 (m, 1H), 4.6−4.8 (m, 2H), 3.92 (s, 12H); ^{13}C NMR (C) 171.9, 165.9, 165.4, 153.1, 153.0, 152.9 (×2), 144.6 (×2), 143.83 (×2), 143.79, 142.7, 133.2, 133.1, 126.90, 126.85, 123.5 (×2), 122.6, 122.5, 118.5, 117.9, 70.2, 62.9, 56.0 (×4). Hydrolysis of **19a** by procedure B but with a reaction time of only 10 min gave **19**, mp = 234−236 °C; ^{1}H NMR (D) 7.54 (d, 15.9, 1H), 7.51 (d, 15.9, 1H), 7.0−7.1 (m, 4H), 6.78 (dd, 1.6, 8.1, 2H), 6.37 (d, 15.9, 1H), 6.29 (d, 15.9, 1H), 5.36 (m, 1H), 4.56 (m, 2H); ^{13}C NMR (D) 168.5, 166.0, 165.6, 148.6, 148.5, 146.4, 146.0 (×2), 145.5, 125.2 (×2), 121.5, 121.4, 115.7 (×2), 115.0, 114.9, 113.0, 112.8, 70.2, 62.4. Anal. ($C_{21}H_{18}O_{10}$) C, H.

Bis(3,4-dihydroxydihydrocinnamoyl)-L-tartaric Acid (22). 3,4-Dihydroxydihydrocinnamic acid was reacted by procedure A to give a mixture of **3,4-dimethoxycarbonyldihydrocinnamic acid (22a)** and its anhydride which were separated by silica gel column chromatography and the latter hydrolyzed by 80% HOAC solution on a steam bath to give **22a**, gum; ^{1}H NMR (C) 7.16 (d, 7.2, 1H), 7.14 (s, 1H), 7.10 (d, 7.2, 1H), 3.90 (s, 6H), 2.97 (t, 8.0, 2H), 2.69 (t, 8.1, 2H); ^{13}C NMR (C) 177.5, 153.4 (×2), 142.2, 140.8, 139.4, 126.7, 123.0, 122.9, 55.8 (×2), 35.0, 29.9. Reaction of **22a** by procedure I gave the acid chloride which was subjected to procedure G to give **bis(3,4-dimethoxycarbonyldihydrocinnamoyl)-L-tartaric acid (22b)**, gum; ^{1}H NMR (C) 7.18 (d, 8.1, 2H), 7.10 (dd, 8.1, 1.9, 2H), 7.09 (d, 1.9, 2H), 5.55 (s, 2H), 3.92 (s, 6H), 3.91 (s, 6H), 2.98 (m, 4H), 2.81 (m, 4H); ^{13}C NMR (C) 171.0, 167.1, 154.7, 153.7, 142.0, 140.5, 139.3, 127.0, 123.2, 123.0, 70.3, 56.1, 56.0, 34.6, 30.1. Deprotection of **22b** by procedure B and preparative HPLC gave **22** as a gum which on analytical HPLC gave a single peak with a retention time of 2.5 min (70% MeOH−H_2O + 1% HOAc) or 3.1 min (45% MeCN−H_2O + 1% HOAc); ^{1}H NMR (D) 6.66 (bs, 2H), 6.54 (d, 8.0, 2H), 6.35 (bd, 7.5, 2H), 5.38 (s, 2H), 2.75 (m, 4H), 2.49 (m, 4H); ^{13}C NMR (D) 172.2, 170.8, 145.5, 143.5, 131.4, 118.5, 115.6, 115.5, 74.8, 35.3, 29.5; HRFABMS calcd *m/z* for $C_{22}H_{22}O_{12}Na$ 501.1009, found 501.1006.

Bis(3-hydroxycinnamoyl)-L-tartaric Acid (23). 3-Hydroxycinnamic acid was reacted by procedure A to give **3-methoxycarbonylcinnamic acid (23a)**, mp = 158−160 °C; ^{1}HNMR (C) 7.75 (d, 16.0, 1H), 7.44 (m, 2H), 7.38 (s, 1H), 7.24 (m, 1H), 6.46 (d, 16.0, 1H), 3.93 (s, 3H); ^{13}C NMR (C) 171.3, 151.5, 145.5, 135.8, 130.2, 130.1, 126.1, 123.2, 120.5, 118.7, 55.6. Procedure I converted **23a** to the acid chloride which reacted by procedure G to give **bis(3-methoxycarbonylcinnamoyl)-L-tartaric acid (23b)**, mp = 108−110 °C: ^{1}H NMR (D) 7.7−7.8 (m, 6H), 7.49 (t, 7.6, 1H), 7.32 (1H, d, 6.6), 6.79 (d, 16.2, 1H), 5.66 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (D) 167.6, 165.0, 153.4, 151.1, 144.4, 135.4, 130.1, 126.4, 123.4, 120.9, 118.4, 71.2, 55.4. Deprotection of **23b** by procedure B and preparative HPLC gave **23,** mp = 115−116 °C; ^{1}H NMR (D) 7.57 (d, 15.9, 2H), 7.20 (t, 7.5, 2H), 7.12 (d, 7.1, 2H), 7.05 (1H, s, 2H), 6.84 (d, 7.5, 2H), 6.53 (d, 15.9, 2H), 5.57 (s, 2H); ^{13}C NMR (D) 168.4, 165.3, 157.7, 145.1, 135.0, 129.8, 119.1, 117.7, 117.5, 114.7, 72.0. Anal. ($C_{22}H_{18}O_{10}$·0.5H_2O) C, H.

Bis(4-hydroxycinnamoyl)-L-tartaric Acid (24). 4-Hydroxycinnamic acid was reacted by procedure A to give **4-methoxycarbonylcinnamic acid (24a),** mp = 201−203 °C; ^{1}H NMR (D) 7.78 (d, 8.6, 2H), 7.62 (d, 16.1, 1H), 7.30 (d, 8.6, 2H), 6.55 (d, 16.1, 1H), 3.85 (s, 3H); ^{13}C NMR (D) 167.5, 153.3, 152.0, 142.8, 132.3, 129.6, 121.8, 119.6, 55.6. Procedure H converted **24a** to the acid chloride which reacted by procedure G to give **bis(4-methoxycarbonylcinnamoyl)-L-tartaric acid (24b)**, mp = 157−160 °C; ^{1}H NMR (D) 7.84 (d, 8.6, 2H), 7.72 (d, 16.0, 1H), 7.31 (d, 8.6, 2H), 6.73 (d, 16.0, 1H), 5.68 (s, 1H), 3.85 (s, 3H); ^{13}C NMR (D) 168.0, 165.2, 153.2, 152.2, 144.3, 131.8, 129.9, 121.8, 117.7, 71.7, 55.5. Deprotection of **24b** by procedure B and preparative HPLC gave **24**, mp = 137−140 °C; ^{1}H NMR (M) 7.73 (d, 15.9, 2H), 7.51 (d, 8.7, 2H), 6.82 (d, 8.7, 2H), 6.44 (d, 15.9, 2H), 5.81 (s, 2H); ^{13}C NMR (M) 169.6, 167.7, 161.6, 148.1, 131.5, 127.0, 116.9, 113.7, 72.5. Anal. ($C_{22}H_{18}O_{10}$) C, H.

Digalloyl-L-tartaric Acid (25). Gallic acid (3,4,5-trihydroxybenzoic acid) was reacted by procedure A to give **3,4,5-trimethoxycarbonylbenzoic acid (25a),** mp = 143−144 °C; ^{1}H NMR (C) 7.99 (s, 2H), 3.94 (s, 6H), 3.93 (s, 3H); ^{13}C NMR (C) 169.1, 152.6, 151.6, 143.8, 139.1, 127.8, 122.6, 56.4, 56.2. Procedure I converted **25a** to the acid chloride which reacted with bis(diphenylmethyl) L-tartrate **(1a)** according to procedure F to give **bis(diphenylmethyl) bis(3,4,5-trimethoxycarbonylbenzoyl)-L-tartrate (25b)**, gum; ^{1}H NMR (C) 7.62 (s, 4H), 7.30−7.36 (m, 20H), 6.96 (s, 2H), 6.08 (s, 2H), 3.94 (s, 6H), 3.92 (s, 12H); ^{13}C NMR (C) 164.0, 162.5, 152.4, 151.5, 143.6, 138.4, 138.1, 128.6, 128.54, 128.50, 128.3, 128.2, 127.5, 126.8, 126.5, 122.3, 79.3, 71.6, 56.4, 56.1. Sequential deprotection of **25b** by procedures D and B and preparative HPLC gave **25** as a gum which on analytical HPLC gave a single dominant peak with a retention time of 1.7 min (10% MeOH−water + 1% HOAc) or 4.8 min (10% MeCN−H_2O + 1% HOAc); ^{1}H NMR (D) 7.01 (s, 4H), 5.70 (s, 2H); ^{13}C NMR (D) 167.3, 164.8, 117.8, 108.8, 145.5, 139.1, 70.9; HRFABMS calcd *m/z* for $C_{18}H_{14}O_{14}Na$ 477.0281, found 477.0276.

Bis(3,4-dihydroxybenzoyl)-L-tartaric Acid (26). 3,4-Dihydroxybenzoic acid (Aldrich 10,980-0) was reacted by procedure A to give **3,4-dimethoxycarbonylbenzoic acid (26a)** as an off-white solid, mp = 168−169 °C; ^{1}H NMR (C + M) 8.00 (dd, 8.6, 2.2, 1H), 7.99 (s, 1H), 7.39 (d, 8.6, 1H), 3.93 (s, 6H); ^{13}C NMR (C + M) 167.0, 153.3, 153.0, 146.1, 142.3, 129.7, 128.8, 125.0, 123.1, 56.1 (×2). Procedure I converted **26a** to the acid chloride which reacted by procedure G to give **bis(3,4-dimethoxycarbonylbenzoyl)-L-tartaric acid (26b)**, gum; ^{1}H NMR (C + M) 8.04 (dd, 8.4, 1.9, 2H), 8.07 (bs, 2H), 7.46 (d, 8.4, 2H), 6.00 (s, 2H), 5.78 (bs, 2H), 3.93 (s, 3H), 3.92 (s, 3H); ^{13}C NMR (C + M) 167.5, 163.5, 152.7, 152.4, 146.3, 142.0, 128.5, 127.4, 124.6, 123.0, 71.8, 55.6 (×2). Deprotection of **26b** by procedure B and preparative HPLC gave **26** as a white solid, mp = 175−176 °C; ^{1}H NMR (D) 9.97 (s, 2H), 9.56 (s, 2H), 7.41 (d, 2.5, 2H), 7.39 (d, 8.1, 2H), 6.87 (dd, 8.1, 2.5, 1H), 5.73 (d, 2.7, 2H); ^{13}C NMR (D) 167.4, 164.5, 151.1, 145.1, 122.3, 119.1, 116.4, 115.4, 71.0. Anal. ($C_{18}H_{14}O_{12}$·1.5H_2O) C, H.

Bis(3,5-dihydroxybenzoyl)-L-tartaric Acid (27). 3,5-Dihydroxybenzoic acid was reacted by procedure A to give **3,5-dimethoxycarbonylbenzoic acid (27a)**, mp = 155−156 °C; ^{1}H NMR (C) 7.84 (d, 2.3, 2H), 7.38 (d, 2.3, 1H), 3.94 (s, 6H); ^{13}C NMR (C) 169.8, 153.5, 151.4, 131.6, 120.5, 119.8, 55.8. Procedure I converted **27a** to the acid chloride which reacted by procedure G to give **bis(3,5-dimethoxycarbonylbenzoyl)-L-tartaric acid (27b)**, gum; ^{1}H NMR (D) 7.79 (d, 2.1, 4H), 7.67 (m, 2H), 5.89 (s, 2H), 3.86 (s, 12H); ^{13}C NMR (D) 166.8, 162.9, 152.9, 151.3, 130.8, 120.8, 120.0, 71.8, 55.7. Deprotection of **27b** by procedure B and preparative HPLC gave **27**, mp = 193−195 °C; ^{1}H NMR (D) 9.78 (s, 4H), 6.87 (s, 4H), 6.50 (s, 2H), 5.77 (s, 2H). ^{13}C NMR (D) 167.0, 164.7, 158.5, 130.0, 107.8, 107.3, 71.2. Anal. ($C_{18}H_{14}O_{12}$·3H_2O) C, H.

Bis(3,4-dihydroxyphenylacetyl)-L-tartaric Acid (28). 3,4-Dihydroxyphenylacetic acid was reacted by procedure A to give **3,4-dimethoxycarbonylphenylacetic acid (28a)**, oil; ^{1}H NMR (C) 7.28−7.17 (m, 3H), 3.89 (s, 6H), 3.63 (s, 2H); ^{13}C NMR (C) 175.7, 153.2 (×2), 142.1, 141.5, 132.5, 127.9, 124.0, 123.0, 55.8 (×2), 40.1. Reaction of **28a** by procedure H gave the acid chloride which was subjected to procedure G to give **bis(3,4-dimethoxycarbonylphenylacetyl)-L-tartaric acid (28b)**, gum; ^{1}H NMR (M) 7.35−7.20 (m, 3H), 5.72 (s, H-2), 3.85 (s, 6H), 3.80 (s, 2H); ^{13}C NMR (M) 171.3, 168.9, 154.74, 154.69, 143.6, 143.0, 134.2, 129.1, 125.3, 124.1, 72.7, 56.4 (×2), 40.3. Deprotection of **28b** by procedure B and preparative HPLC gave **28** as a gum. Anal. ($C_{20}H_{18}O_{12}$·H_2O) Calcd: C, 51.3; H, 4.3. Found: C, 51.5; H, 4.8. Analytical HPLC gave a single major peak with a retention time of 2.72 min (10:1 MeCN:H_2O + 1% HOAc) or 1.76 min (5:1 MeOH:H_2O); ^{1}H NMR (M) 6.72−6.68 (m, 4H), 6.59 (dd, 8.0, 1.9, 2H), 5.65 (s, 2H), 3.59 (s, 4H); ^{13}C NMR (M) 172.6, 169.1, 146.2, 145.5, 126.2, 121.9, 117.6, 116.3, 72.4, 40.6.

Dicinnamoyl-L-tartaric Acid (29). L-Tartaric Acid was treated with cinnamoyl chloride according to procedure G to give **29**, mp = 90−94 °C; ^{1}H and ^{13}C NMR values agree with literature;[41] ^{1}H NMR (D) 7.80 (bs, 4H), 7.77 (d, 16.1, 2H), 7.47 (m, 6H), 6.78 (d, 16.1, 2H), 5.79 (s, 2H); ^{13}C NMR (D) 167.5, 165.3, 146.4, 133.7, 130.9, 129.0, 128.7, 116.7, 71.1; DIP/MS 392 (10) (M − H_2O), 148 (32), 147 (38), 131 (100), 103 (60). Anal. ($C_{22}H_{18}O_8$·1.25H_2O) C, H.

Bis(3,4-dimethoxycinnamoyl)-L-tartaric Acid (30). The acid chloride of 3,4-dimethoxycinnamic acid prepared via procedure I was reacted with L-tartaric acid according to procedure G to give **30**, mp = 204−206 °C; ^{1}H NMR (M) 7.70 (d, 15.9, 2H), 7.15 (m, 4H), 6.93 (d, 8.2, 2H), 5.86 (s, 2H), 3.83 (s, 6H), 3.82 (s, 6H); ^{13}C NMR (M) 170.3, 167.6, 152.9, 150.6, 147.8, 128.4, 124.4, 115.1, 112.5, 111.3, 72.9, 56.4 (×2); DIP-MS: 512 (1) (M − H_2O), 209 (13), 208 (100), 193 (24), 191 (34). Anal. ($C_{26}H_{26}O_{12}$) C, H.

Bis(2,3-dihydroxybenzoyl)-L-tartaric Acid (31). 2,3-Dihydroxybenzoic acid treated by procedure C gave **diphenylmethyl 2,3-dihydroxybenzoate (31a)**, gum; ^{1}H NMR (C) 10.81 (s, 1H), 7.57 (dd, 8.1, 1.7, 1H), 7.31−7.44 (m, 11H), 7.11 (s, 1H), 6.84 (t, 8.0, 1H), 5.68 (s, 1H); ^{13}C NMR (C) 169.4, 149.1, 145.1, 139.5, 128.7, 128.3, 127.1, 120.6, 120.0, 119.3, 112.5, 78.1. Application of procedure A to **31a** gave **diphenylmethyl 2,3-dimethoxycarbonylbenzoate (31b)**, mp = 88−89 °C; ^{1}H NMR (C) 8.00 (dd, 8.0, 1.7, 1H), 7.50 (dd, 8.0, 1.7, 1H), 7.25−7.40 (m, 11H), 7.10 (s, 1H), 3.90 (s, 3H), 3.60 (s, 3H); ^{13}C NMR (C) 163.1, 153.2, 152.7, 143.9, 142.7, 139.6, 129.5, 128.6, 128.1, 127.5, 127.4, 126.5, 125.3, 78.3, 56.0, 55.7. Hydrolysis of **31b** according to procedure D gave **2,3-dimethoxycarbonylbenzoic acid (31c)**, mp = 107−110 °C; ^{1}H NMR (C) 8.00 (dd, 7.9, 1.7, 1H), 7.77 (dd, 7.9, 1.7, 1H), 7.38 (t, 8.0, 1H), 3.92 (s, 6H); ^{13}C NMR (C) 169.1, 153.1, 152.7, 143.8, 143.1, 129.8, 128.2, 126.5, 124.1, 56.0 (×2). Procedure I converted **31c** to the acid chloride which was reacted with **1a** by procedure F to give **bis(diphenylmethyl) bis(2,3-dimethoxycarbonylbenzoyl)-L-tartrate (31d)**, mp = 187−189 °C; ^{1}H NMR (C) 7.52 (dd, 7.9, 1.4, 2H), 7.48 (dd, 1H, 7.8, 1.4), 6.9−7.4 (m, 22H), 6.88 (s, 2H), 6.11 (s, 2H), 3.93 (s, 6H), 3.80 (s, 6H); ^{13}C NMR (C) 164.2 161.8 153.1 152.4 143.8 143.3 138.64 138.60 129.5 128.5 128.4 128.3 128.2 128.1 127.2 127.0 126.3 126.3 123.0 79.5 71.5 56.0 (×2).

Hydrolysis of **31d** by procedure D gave **bis(2,3-dimethoxycarbonylbenzoyl)-L-tartaric acid (31e)**, gum; ^{1}H NMR (C) 9.91 (s, 2H), 7.98 (dd, 7.9, 1.4, 2H), 7.50 (dd, 7.9, 1.4, 2H), 7.36 (t, 7.9, 2H), 5.94 (s, 2H), 3.90 (s, 6H), 3.81 (s, 6H); ^{13}C NMR (C) 169.9, 162.2, 153.1, 152.7, 143.8, 143.0, 129.7, 128.3, 126.8, 123.3, 71.3, 56.1, 56.0. Deprotection of **31e** by procedure B gave **bis(2,3-dihydroxybenzoyl)-L-tartaric acid (31)**, mp = 202 °C; ^{1}H NMR (D) 7.26 (dd, 7.9, 1.3, 2H), 7.08 (dd, 7.9, 1.3, 2H), 6.81 (t, 7.9, 2H), 5.86 (s, 2H); ^{13}C NMR (D) 167.2, 167.1, 149.1, 146.2, 121.0, 119.7, 119.1, 112.8, 71.8. Anal. ($C_{18}H_{14}O_{12}$) C, H.

Bis(2,5-dihydroxybenzoyl)-L-tartaric Acid (32). 2,5-Dihydroxybenzoic acid treated by procedure C gave **diphenylmethyl 2,5-dihydroxybenzoate (32a)**, mp = 140−141 °C; ^{1}H NMR (C) 10.30 (s, 1H), 7.47 (d, 3.1, 1H), 7.30−7.39 (m, 10H), 7.09 (s, 1H), 7.00 (dd, 8.9, 3.1, 1H), 6.87 (d, 8.9, 1H); ^{13}C NMR (C) 168.8, 156.1, 147.7, 139.5, 128.7, 128.3, 127.1, 124.3, 118.6, 114.7, 112.3, 78.0. Application of procedure A to **32a** gave **diphenylmethyl 2,5-dimethoxycarbonylbenzoate (32b)**, gum; ^{1}H NMR (C) 7.90 (d, 2.9, 1H), 7.45−7.25 (m, 11H), 7.21 (d, 8.8, 1H), 7.10 (s, 1H), 3.92 (s, 3H), 3.62 (s, 3H); ^{13}C NMR (C) 162.7, 153.7 (×2), 148.6, 148.3, 139.5, 128.5, 128.1, 127.4, 126.7, 126.5, 124.6, 124.4, 78.3, 55.7, 55.5. Hydrolysis of **32b** according to procedure D gave **2,5-dimethoxycarbonylbenzoic acid (32c)**, mp = 150−152 °C; ^{1}H NMR (C) 7.95 (d, 3.0, 1H), 7.46 (dd, 8.7, 3.0, 1H), 7.25 (d, 8.7, 1H), 3.94 (s, 6H); ^{13}C NMR (C) 168.5, 153.73, 153.68, 148.8, 148.6, 127.5, 125.0, 124.7, 123.1, 55.8, 55.7. Procedure I converted **32c** to the acid chloride which was reacted with **1a** by procedure F to give **bis(diphenylmethyl) bis(2,5-dimethoxycarbonylbenzoyl)-L-tartrate (32d)**, gum; ^{1}H NMR (C) 7.51 (d, 2.9, 2H), 7.43 (dd, 8.8, 2.9, 2H), 7.20 (d, 2H, 8.8), 7.00−7.36 (m, 20H), 6.88 (s, 2H), 6.14 (s, 2H), 3.89 (s, 6H), 3.76 (s, 6H); ^{13}C NMR (C) 164.0, 161.7, 153.5, 153.4, 148.8, 148.4, 138.9, 128.6, 128.52, 128.46, 128.2, 128.1, 127.6, 127.3, 127.2, 126.9, 124.52, 124.47, 79.4, 71.4, 55.7, 55.6. Hydrolysis of **32d** by procedure D gave **bis(2,5-dimethoxycarbonylbenzoyl)-L-tartaric acid (32e)**, gum; ^{1}H NMR (D) 7.84 (d, 2H, 2.9), 7.72 (dd, 8.8, 3.0, 2H), 7.53 (d, 8.8, 2H), 5.87 (s, 2H), 3.87 (s, 6H), 3.79 (s, 6H); ^{13}C NMR (D) 166.7, 161.7, 153.3, 153.0, 148.4, 148.2, 128.5, 125.6, 124.0, 122.5, 71.6. Deprotection of **32e** by procedure B gave **bis(2,5-dihydroxybenzoyl)-L-tartaric acid (32)**, mp = 138.5−140 °C; ^{1}H NMR (D) 9.35 (s, 2H), 7.18 (d, 3.0, 2H), 7.03 (dd, 9.0, 3.0, 2H), 6.86 (d, 9.0, 2H), 5.85 (s, 2H); ^{13}C NMR (D) 167.0 (×2), (in 20% CD_3OD splits into 167.2/166.6) 153.1, 149.5, 124.4, 118.4, 114.3, 111.9, 71.5. Anal. ($C_{18}H_{14}O_{12}$) C, H.

Bis(3,4-difluorobenzoyl)-L-tartaric Acid (33). The acid chloride of 3,4-difluorobenzoic acid prepared via procedure I was reacted with L-tartaric acid according to procedure G to give **33**, mp 200−202 °C; ^{1}H NMR (M) 7.95 (m, 4H), 7.45 (ddd, ca. 8.8 each, 2H), 5.98 (s, 2H); ^{13}C NMR (M) 168.9, 164.7, 155.2 (dd, 255.4, 12.8), 151.4 (dd, 249.0, 13.2), 128.4 (dd, 7.8, 3.6), 127.6 (m), 120.0 (d, 18.6), 119.0 (d, 18.4), 73.4. Anal. ($C_{26}H_{26}O_{12}$) C, H.

3,5-Dicaffeoylbenzoic Acid (34). 3,5-Dihydroxybenzoic acid treated by procedure C gave **diphenylmethyl 3,5-dihydroxybenzoate (34a)**, mp = 129−131 °C; ^{1}H NMR (M) 7.25−7.42 (m, 10H), 7.12 (d, 2, 2H), 7.00 (s, 1H), 6.58 (s, 1H); ^{13}C NMR (M) 167.0, 159.8, 141.8, 133.1, 129.5, 128.9, 127.8, 108.9, 108.6, 78.9; DIP/MS 320 (19), 167 (100), 152 (31). Reaction of **34a** and **35a** according to procedure F gave **diphenylmethyl 3,5-bis(3,4-dimethoxycarbonylcaffeoyl)-benzoate (34b)**, mp = 62−64 °C; ^{1}H NMR (C) 7.84 (d, 2.2, 2H), 7.82 (d, 2.2, 2H), 7.25−7.53 (m, 17H), 7.13 (s, 1H), 6.58 (d, 16.0, 2H), 3.93 (s, 6H), 3.92 (s, 6H); ^{13}C NMR (C) 164.2. 163.9, 153.0, 152.9, 151.1, 145.1, 144.1, 142.8, 139.8, 133.0, 132.4, 128.6, 128.1, 127.2, 127.0, 123.7, 122.6, 120.5, 120.4, 118.1, 78.1, 56.0 (×2); DIP/MS 437 (13), 436 (47), 253 (32), 167 (73), 166 (100), 165 (79). Hydrolysis of **34b** according to procedure B gave **diphenylmethyl 3,5-dicaffeoylbenzoate (34c)**, mp = 179−181 °C dec; ^{1}H NMR (D) 7.87 (d, 2.1, 2H), 7.79 (d, 15.7, 2H), 7.22−7.59 (m, 13H), 7.14 (dd, 8.4, 1.7, 2H), 7.11 (s, 1H), 6.86 (d, 8.0, 2H), 6.55 (d, 15.8, 2H); ^{13}C NMR (D) 164.9, 163.4, 151.3, 149.7, 148.0, 145.9, 140.2, 131.4, 128.6, 127.9, 126.53, 126.46, 125.0, 122.2, 120.1, 115.8, 114.9, 112.0, 77.7. Hydrolysis of **34c** by procedure D gave **3,5-dicaffeoylbenzoic acid (34)**, mp = 170−172 °C; ^{1}H NMR (D) 9.82 (bs, 2H), 9.33 (bs, 2H), 7.76 (d, 15.7, 2H), 7.67 (d, 2.2, 2H), 7.44 (t, 2.2, 1H), 7.19 (d, 1.9, 2H), 7.14 (dd, 8.3, 2.0, 2H), 6.84 (d, 8.1), 6.53 (d, 15.9, 2H); ^{13}C NMR (D) 165.8, 164.9, 151.0, 149.0, 147.7, 145.6, 132.8, 125.2, 122.1, 120.4, 120.0, 115.8, 115.1, 112.3. Anal. ($C_{25}H_{18}O_{10}\cdot 1.6H_2O$) C, H.

3,4-Dimethoxycarbonylcaffeic Acid (35). Caffeic acid was treated according to procedure A to give **35**, mp =142−143 °C (lit.[43] mp 141.5−143 °C); ^{1}H NMR (M) 7.63 (d, 16.0, 1H), 7.58 (d, 2.0, 1H), 7.53 (d, 8.4, 1H), 7.34 (d, 8.5, 1H), 6.48 (d, 16.0, 1H), 3.870 (s, 3H), 3.866 (s, 3H); ^{13}C NMR (M) 169.8, 154.6, 154.4, 145.0, 144.2, 144.0, 135.1, 127.8, 124.7, 123.5, 121.1, 56.5 (×2); DIP/MS 296 (75), 252 (100), 221 (79), 193 (55). The acid chloride was made according to procedure H or I to give **3,4-dimethoxycarbonylcaffeoyl chloride (35a)**, MS 314 (22), 235 (100), 191 (80), which was used immediately.

Biological Assays. 1. Cells and Virus. MT-2 cells are a CD4$^+$ T-lymphoblastoid cell line that is completely lysed by T-cell tropic isolates of HIV-1. HIV_{NL4-3} and $HIV_{NL4-3clone1-D4}$ were obtained as described.[26] Plasmids were transfected into HeLa cells with Lipofectin (Gibco/BRL); the cells were washed and then cultured with H9 cells, a CD4$^+$ T-lymphoblastoid cell line that supports chronic production of HIV. Virus was obtained when the culture became 100% positive for HIV antigens by immunofluorescence. HIV_{LAI} is the LAI isolate of HIV-1. HIV_{LAI} was grown in H9 cells. Cells were grown in RPMI-1640 containing 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and supplemented with 11.5% fetal bovine serum and 2 mM L-glutamine. All HIVs were obtained from H9 cell culture supernatant clarified of cells by low-speed centrifugation followed by filtration through 0.45-μm nitrocellulose filters.

2. Cell Toxicity Assays. Cell toxicity assay was performed as reported, previously.[7,8,44] Briefly, lyophilized compounds were solubilized in either H_2O or 95% ethanol, diluted 1:5 in growth medium, filter-sterilized, and further 2-fold serially diluted from 1:10 to 1:1280 in triplicate wells of a microtiter plate. To each 50 μL of diluted compound, 50 μL of growth medium was added followed by 100 μL of MT-2 cell suspension (2×10^5 cells). Cells were incubated with drug for 48 or 72 h at 37 °C and then harvested for cell viability in a neutral red dye assay as described previously.[32] The lethal dose was defined as 5% inhibition of MT-2 cell growth in 48 h (LD_5). Several of the compounds were not available in sufficient quantity or demonstrated a solubility profile that precluded determination of a true LD_5. For these compounds, cell toxicity is defined as greater than the maximum concentration of compound tested. The LD_5 is a better measure of toxicity than LD_{50} as 5% inhibition of cell growth is within 1 SD of the cell controls. Thus, this is a truly nontoxic concentration of compound.

3. Anti-HIV Assay. Anti-HIV assays were performed as described.[7,8,44] On the basis of cell toxicity data, compounds were diluted in growth medium such that a final 1:4 dilution of the sample would result in a concentration of sample equal to the LD_5. The compounds were then 2-fold serially diluted in triplicate. To each 50 μL of diluted compound, 50 μL of HIV_{LAI} was added, and the virus−compound mixture was incubated for 1 h at 37 °C. Next, 100 μL of MT-2 cell suspension (2×10^5 cells) was added to each well, and cells were incubated for 72 h at 37 °C. Final multiplicity of infection (MOI) was 1−5. Cells were harvested to quantitate cytopathic effect using a neutral red dye assay as described.[44] The antiviral concentration reported is the concentration of sample necessary to protect 50% of MT-2 cells from viral-induced cell death; this is referred to as the ED_{50}. Inhibition of virus-induced cell death has correlated well with virus replication as measured by synthesis of antigens, reverse transcriptase release, and production of infectious progeny virions.[45]

4. Disintegration Assay. The disintegration activities of IN in the presence and absence of inhibitors was assayed in vitro as modified from Chow et al.[21] The following oligonucleotides (GenoSys, Inc.) were used as DNA substrates: T1 (16-

mer), 5′-CAGCAACGCAAGCTTG-3′; T3 (30-mer), 5′-GTCGACCTGCAGCCCAAGCTTGCGTTGCTG-3′; V2 (21-mer), 5′-ACTGCTAGAGATTTTCCACAT-3′; V1/T2 (33-mer), 5′-ATGTGGAAAATCTCTAGCAGGCTGCAGGTCGAC-3′.

The oligonucleotides were gel-purified. Oligonucleotide T1 was labeled at the 5′-end using T4 polynucleotide kinase and [γ-^{32}P]ATP (3000 Ci/mmol; Amersham). The substrate for assaying disintegration activity, the Y-oligomer, was prepared by annealing the labeled T1 strand with oligonucleotides T3, V2, and V1/T2.[21] In a 20-μL volume, the DNA substrate (0.1 pmol) was incubated with 1.5 pmol of recombinant IN for 60 min at 37 °C in a buffer containing a final concentration of 20 mM HEPES (pH 7.5), 10 mM DTT, 0.05% Nonidet P-40, and 10 mM $MnCl_2$. To each 19 μL of reaction mixture, 1 μL of inhibitor at various concentrations in solvent or solvent alone was added. The reaction was stopped by the addition of EDTA to a final 18 mM concentration. Reaction products were heated at 90 °C for 3 min before analysis by electrophoresis on a 15% polyacrylamide gel with 7 M urea in Tris−borate−EDTA buffer. All reactions were performed at enzyme excess, and reactions were stopped within the linear range of the reaction.[21] Although it has been suggested by one group that the inhibitory effects of biscatechols are metal ion-dependent,[46,47] all reactions were performed in the presence of $MnCl_2$ rather than $MgCl_2$. Our findings, in collaboration with our collaborator Prof. Samson Chow, indicate that the DCTAs and DCQAs inhibit HIV IN whether Mg^{2+} or Mn^{2+} is the source of divalent cation, and recent work from our groups suggests that metal ion is not required at all (Zhu et al., unpublished results). These data are more consistent with the inhibitory activity of biscatechols against avian sarcoma virus IN.[46] All compounds were first tested at 25 μM. For active compounds, IC_{50} analysis was determined from a median effect plot using CalcuSyn software (Biosoft, Cambridge, U.K.) on 0.5 log dilutions of inhibitor in triplicate experiments.

Acknowledgment. This work was supported in part by grants from the National Institutes of Health (1RO1AI-41360, W.E.R.) and a NIH training grant (5T32-GM07311, P.J.K.). The authors are indebted to S. Chow for his gift of the HIV-1 IN expression vector and his advice on the purification of biologically active HIV-1 IN.

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JM9804735