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Design, Synthesis, and Biological Evaluation of Novel Hybrid Dicaffeoyltartaric/Diketo Acid and Tetrazole-Substituted L-Chicoric Acid Analogue Inhibitors of Human Immunodeficiency Virus Type 1 Integrase

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Fourteen analogues of the anti-HIV-1 integrase (IN) inhibitor L-chicoric acid (L-CA) were prepared. Their IC_{50} values for 3′-end processing and strand transfer against recombinant HIV-1 IN were determined in vitro, and their cell toxicities and EC_{50} against HIV-1 were measured in cells (ex vivo). Compounds **1**−**6** are catechol/*β*-diketoacid hybrids, the majority of which exhibit submicromolar potency against 3′-end processing and strand transfer, though only with modest antiviral activities. Compounds **7**−**10** are L-CA/*p*-fluorobenzylpyrroloyl hybrids, several of which were more potent against strand transfer than 3′-end processing, a phenomenon previously attributed to the *β*-diketo acid pharmacophore. Compounds **11**−**14** are tetrazole bioisosteres of L-CA and its analogues, whose in vitro potencies were comparable to L-CA but with enhanced antiviral potency. The trihydroxyphenyl analogue **14** was 30-fold more potent than L-CA at relatively nontoxic concentrations. These data indicate that L-CA analogues are attractive candidates for development into clinically relevant inhibitors of HIV-1 IN.

Introduction

The FDA approval of raltegravir (RGV[a]) and the possible approval of elvitegravir (EVG), two drugs targeting human immunodeficiency virus type 1 (HIV-1) integrase (IN), offer new therapeutic options for HIV-infected patients. Furthermore, these new options are especially vital for patients infected with reverse transcriptase or protease inhibitor-resistant HIV. However, resistance to RGV and EVG has been reported.[1−6] In addition, HIV resistant to RGV is often significantly cross-resistant to EVG and vice versa,[7] indicating that therapeutic failure with RGV or EVG renders both inhibitors ineffectual. Thus, the search for the next generation of IN inhibitors potent against RGV- and EVG-resistant HIV is underway.

HIV IN covalently joins the reverse transcribed viral cDNA genome into the host cellular chromosome. This process is termed integration and is necessary for stable and productive infection of the target cell.[8] Integration involves three steps (reviewed in ref 9 and illustrated in Figure 1): (1) 3′-end processing, (2) strand transfer, and (3) 5′-end joining. Following viral entry and reverse transcription, IN binds conserved cytidine−adenine dinucleotide motifs at the 3′ termini of the viral long terminal repeats (LTRs) in the viral cDNA. Once assembled on the DNA, IN cleaves terminal guanine−cytidine dinucleotides from both 3′ ends, exposing free 3′-hydroxyl groups. Following 3′-end processing, IN, the viral cDNA genome, and several other host and viral cofactors move into the host cell nucleus (reviewed in ref 10). Inside the nucleus, IN mediates a concerted cleavage−ligation reaction, directing the nucleophilic attack of the free 3′-hydroxyls onto opposite strands of the host DNA at a distance five bases apart, yielding a gapped intermediate product. This reaction is termed strand transfer. In the third and final step of integration, 5′-end joining, host cellular DNA repair enzymes fill the gaps flanking the viral genome, producing a fully integrated provirus flanked by five base pair direct repeats.

Among several classes of compounds reported to selectively inhibit integration inside cells (Figure 2), two are of particular interest to this study: dicaffeoyltartaric acids (DCTA), exemplified by L-chicoric acid (L-CA),[11−14] and diketo acids (DKA), exemplified by the *p*-fluorobenzylpyrrole butanoic acid, L-731,988 (FBP).[15] While DCTA IN inhibitors block 3′-end processing and strand transfer at equimolar concentrations,[16] RGV and EVG block 3′-end processing only at concentrations 70-fold higher than those required to inhibit strand transfer.[15] Thus, this latter group of compounds are known as strand transfer inhibitors (STI). Indeed, FBP binds the IN−DNA complex with nearly 1000-fold higher affinity when IN is bound to 3′-end processed substrate DNA than when complexed with blunt-ended DNA.[17]

Mutations that confer resistance to RVG and EVG have been shown to confer cross-resistance to several other STIs

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[a] Abbreviations: CC, column chromatography; cDNA, complementary DNA; CT_{50}, concentration of compound required to inhibit cellular replication by 50% relative to untreated control; CT_5, concentration of compound required to inhibit cellular replication by 5% relative to untreated control; DCC, dicyclohexylcarbodiimide; DCTA, dicaffeoyltartaric acid; DKA, diketo acid; DMAP, 4-dimethylaminopyridine; DPM, diphenylmethyl; EVG, elvitegravir; FBP, 1-[(4-fluorophenyl)methyl]-α,γ-dioxo-1*H*-pyrrole-2-butanoic acid; HIV-1, human immunodeficiency virus type 1; IN, integrase; L-CA, L-chicoric acid; LTR, long terminal repeat; RGV, raltegravir; SAR, structure−activity relationship; STI, strand transfer inhibitor; TBDMS, *tert*-butyldimethylsilyl.

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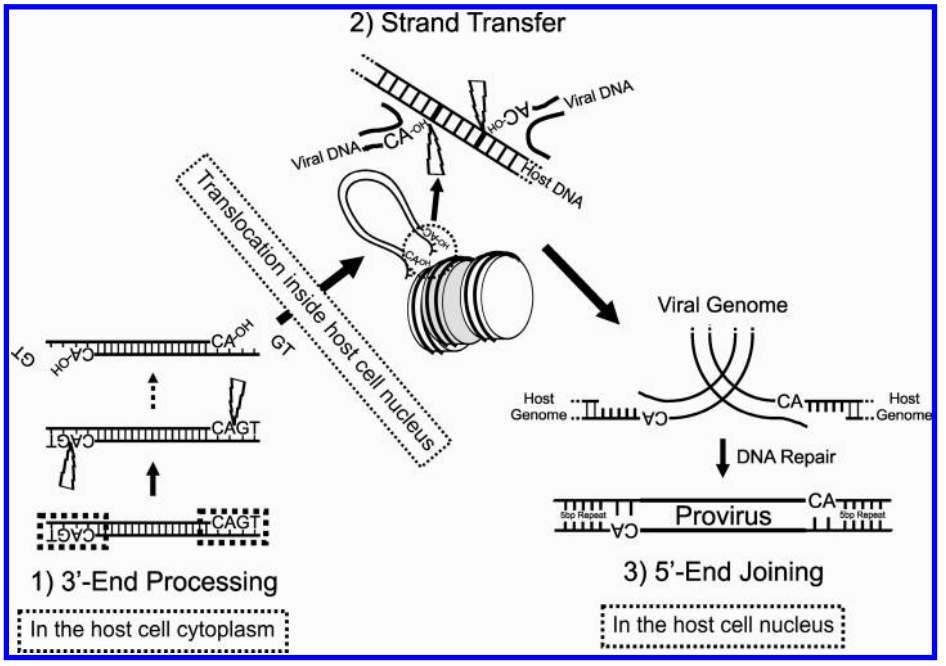


Figure 1. Biochemistry of integration.

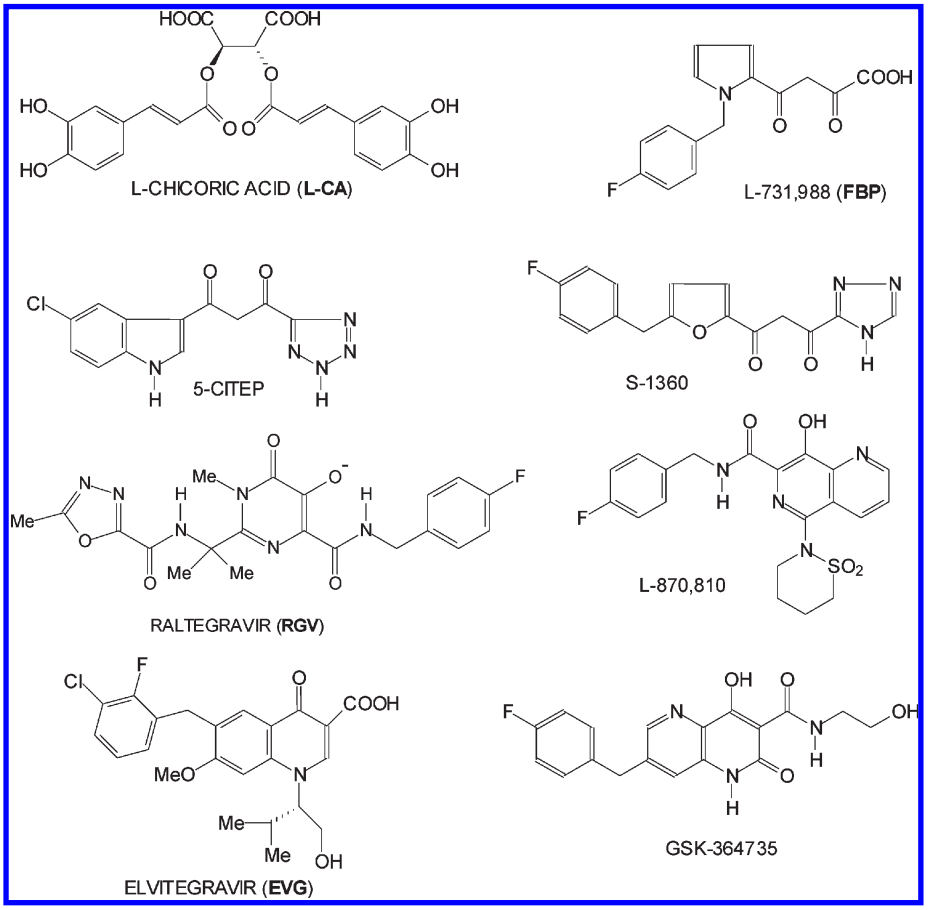


Figure 2. Structures of IN inhibitors.

(Figure 2) including FBP,[15] S-1360,[7,18] L-810,870,[6,7,19,20] and GSK-364735.[7,21] Mutations within IN at amino acids T66, E92, E138, G140, F121, Q148, and N155 are most frequently reported to confer resistance to STI. While cross-resistance among STI is frequently reported, cross-resistance to L-CA, a DCTA, is much less frequent. For example, of the mutations conferring the greatest degrees of resistance to RGV and EVG, including IN T66K, E92Q, G140S, Q148R/K/H, and N155H,[7] only G140S has been reported to confer cross-resistance to L-CA.[13,22] These data suggest that while L-CA binds within the IN active site, the specific inhibitor−IN interactions for L-CA are significantly different than those

for RGV or EVG. The limited cross-resistance of L-CA to EVG- and RGV-resistant variants of HIV makes DCTAs attractive candidates for further development into second generation inhibitors of HIV IN.

In this study, L-CA analogues were designed, synthesized, and biologically characterized with the goals of refining the DCTA pharmacophore, enhancing antiviral potency, and improving cellular membrane permeability. To these ends, three approaches were employed: (1) substitution of the L-CA tartaric acid core with the DKA moiety combined with mono- and dicaffeoyl/catechol substitution of the side chains; (2) substitution of one or both of the L-CA caffeoyl side chains with *p*-fluorobenzylpyrroloyl groups from FBP; (3) substitution of the L-CA tartaric acid core carboxyl groups with bioisosteric tetrazoles and replacement of the catechols by galloyl groups. Biological characterization of each compound included determination of in vitro potency against IN-mediated 3′-end processing and strand transfer catalysis plus determination of ex vivo anti-HIV potency and cellular toxicity.

The first and second approaches were, in essence, a study of DCTA/DKA hybrid inhibitors. While several SAR studies have been reported for DCTAs[23−25] and DKAs,[26−28] no study, to date, has determined if the pharmacophore from one molecule class can be substituted for the other. Compounds **1**−**6** test the hypothesis that the tartaric acid core of DCTAs and the *β*-diketo acid group of DKAs interact similarly with the IN active site and thus can be interchangeably substituted. Compounds **1** and **2** test the simplest combinations of DKA and DCTA motifs, while the remaining compounds test increasingly complex assemblages of catechol-containing and DKA motifs. In addition, compounds **1B**, **3B**, and **6B** are methyl ester analogues of **1**, **3**, and **6** with the aim of improving cellular membrane permeability. A conserved feature of many DKAs includes a halogenated aromatic system adjoining the DKA pharmacophore, typically a fluorobenzyl group, with the exception of 5-CITEP, which contains a chloroindole group (Figure 2). Compounds **7**−**10** explore this conserved DKA feature via hybridization of the tartaric acid core of L-CA with the *N*-(*p*-fluorobenzyl)-2-pyrroloyl side chain of FBP. Assuming that this side chain serves as the pharmacophore for these hybrid compounds, it is possible that these hybrids may exhibit greater potency than either L-CA or FBP. In addition, compounds **7**−**10** test the hypothesis that the aryl side chains of DKAs are essential for the correct orientation of the DKA pharmacophore within the IN active site and may reveal which chemical features are responsible for the strand transfer selective nature of STI. Compounds **11**−**14** test the hypothesis that bioisosteric substitution of the tartaric acid carboxyl with tetrazole groups preserves potency and promotes cellular membrane permeability, resulting in enhanced antiviral potency. We have previously shown that L-CA crosses the membrane quite slowly.[29] Methylation of both the carboxyl and all four phenolic hydroxyl groups significantly improves cellular permeability,[29] indicating that the charged nature of L-CA impairs cellular entry. Thus, substituting the carboxyl moieties with tetrazole groups, bioisosteres known to increase cellular permeability,[30,31] should preserve functionality of the inhibitor while promoting increased permeability across cell membranes. In addition, compound **14** has a trihydroxyphenyl group in place of the dihydroxylphenyl groups of L-CA (Figure 2). A previous SAR study found that the addition of an extra phenolic hydroxyl to the caffeoyl side chains of L-CA significantly increased potency.[23] Combined with potentially improved cellular permeability via tetrazole substitution of the carboxyl, the additional hydroxyl groups on both side chains may further enhance the antiviral activity of the compound. The general designs of the target DCTA hybrid molecules discussed above are shown in Figure 3.

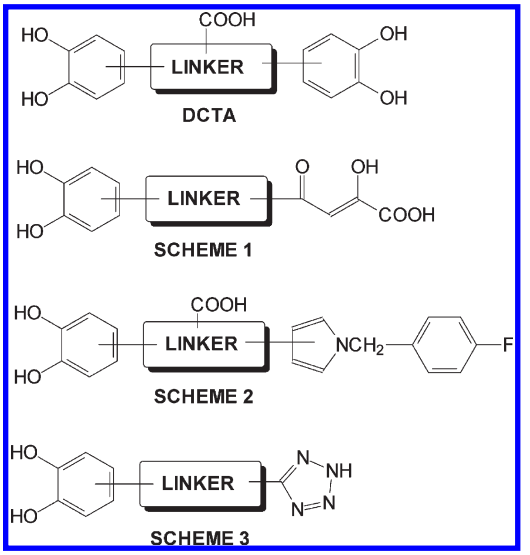


Figure 3. General structures of target DCTA hybrid compounds.

Synthesis

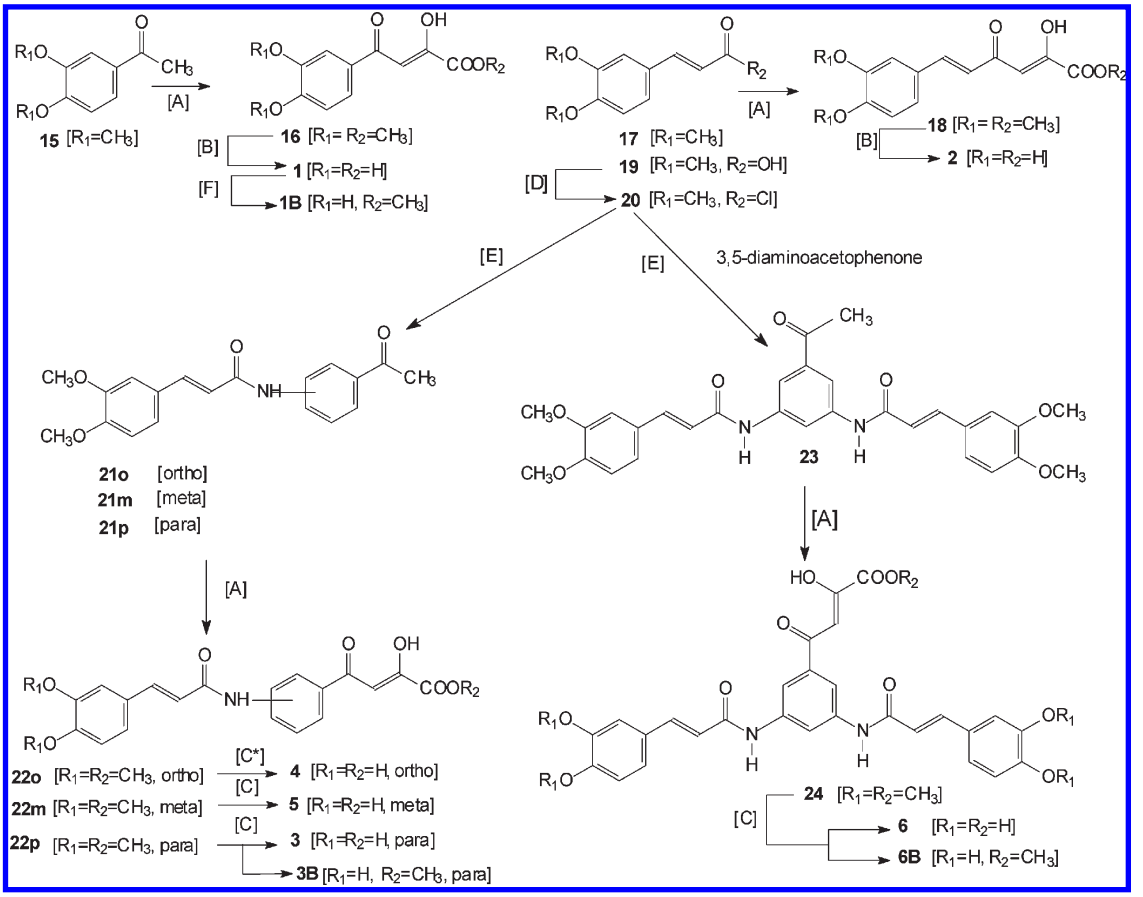
The catechol/DKA hybrids **1**−**6** were prepared as shown in Scheme 1. Compounds **1** and **2** were synthesized from the acetophenone **15** and the methyl ketone **17**, respectively, in a two-step procedure involving the formation of the diketoacid methyl esters **16** and **18** by reaction with dimethyl oxalate in the presence of sodium methoxide followed by a simultaneous demethylation of the ether and ester functions with boron tribromide. Crude **1** was completely converted to its methyl ester **1B** after 3 weeks in methanol.

The acid chloride **20** of 3,4-dimethoxycinnamic acid (**19**) reacted with the *o*-, *m*-, and *p*-isomers of aminoacetophenone to give the *p*- (**21p**), *m*- (**21m**), and *o*- (**21o**) isomers of (3,4-dimethoxycinnamoylamino)acetophenone in 80−90% yield, which reacted with dimethyl oxalate in the presence of sodium *tert*-butoxide to give the diketo acid methyl esters **22p**, **22m**, and **22o** in 86−96% yield. Final demethylation with boron tribromide under a variety of conditions (see Materials and Methods) gave the corresponding deprotected acids **3**, **5**, and **4**, the first as a mixture with its methyl ester **3B**.

Reaction of 3,5-diaminoacetophenone with acid chloride **20** via compounds **23** and **24** gave acid **6** and its methyl ester **6B** as a mixture in three steps similar to the syntheses of the monocaffeoyl analogues **3**−**5**. The mixture was separated by HPLC to give the acid and methyl ester in 30% and 13% yields, respectively.

The *p*-fluorobenzylpyrroloyl/tartaric acid hybrids **7**−**9** were prepared as described in Scheme 2. Treatment of methyl 2-pyrrolecarboxylate with sodium hydride and *p*-fluorobenzyl bromide in DMF gave the *N*-*p*-fluorobenzyl ester **25** which was saponified to the corresponding acid **26** with sodium hydroxide in aqueous methanol. Reaction with 1 equiv of diphenylmethyl (DPM) tartrate[23] using DCC gave both the bis- (**27**) and the mono- (**28**) *N*-(*p*-fluorobenzyl)-2-pyrroloyl diphenylmethyl tartrates, but with 2 equiv of DPM tartrate the monosubstituted compound **28** was obtained in high yield. Removal of the DPM

Scheme 1. Synthesis of Catechol/DKA Hybrids[a]



[a] Procedures: [A] $(COOCH_3)_2$, CH_3ONa or *t*-BuONa; [B] BBr_3, CH_2Cl_2, room temp; [C] BBr_3, CH_2Cl_2, reflux; [C*] BBr_3, CH_2Cl_2, −78 °C; [D] $SOCl_2$; [E] *o*-, *m*-, or *p*-aminoacetophenone or 3,5-diaminoacetophenone, pyridine, toluene; [F] CH_3OH, 3 weeks.

protecting groups by hydrogenolysis in ethanol with Pd/C catalyst gave the bis- and mono-[*N*-(*p*-fluorobenzyl)-2-pyrroloyl]-L-tartaric acids **8** and **7**, respectively. Reaction of **28** with the disilylated caffeic acid **30**[32] formed the disilylated mixed DPM tartrate **29**, which was refluxed with 70% acetic acid to remove both the DPM and silyl protecting groups to give the mixed caffeoyl-*N*-(*p*-fluorobenzyl)-2-pyrroloyltartaric acid derivative **9**. Reaction of **30** with DPM tartrate using the DCC method gave an 85% yield of the DPM ester of TBDMS protected caftaric acid **31**. Removal of the protecting groups gave caftaric acid (**10**) in 96% yield in the first reported synthesis of this natural product found in wine and grapes.[33,34]

Tetrazole analogues of L-CA and related compounds were prepared as shown in Scheme 3. The known[35] acetonide **32** of the diamide of L-tartaric acid was dehydrated to give primarily the dicyano acetonide **33** or the cyanoamide acetonide **36** depending on the equivalents of tosyl chloride used.[36] Reaction of **33** with ammonium azide[37] gave the bis-tetrazole acetonide, which was hydrolyzed to the bis-tetrazole **34** with HCl. Following the same procedures, **36** gave the amide-tetrazole **37**.

Carbomethoxy protected caffeoyl chloride was prepared as described[23] and reacted immediately with the bistetrazole **34** to give the diester **35**. Deprotection with ammonium hydroxide[38] gave the desired dicaffeoyl bis-tetrazole **13**. Similarly, the amide-tetrazole **37** was converted to the protected dicaffeoyl ester **38**, which was hydrolyzed with aqueous sodium carbonate to give the dicaffeoyl tetrazole amide **12**. An analogous sequence was used to prepare the dicaffeoyl ester of the tetrazole analogue of glyceric acid **11** from the known[39] dihydroxytetrazole **42** via the protected dicaffeoyl ester **43**.

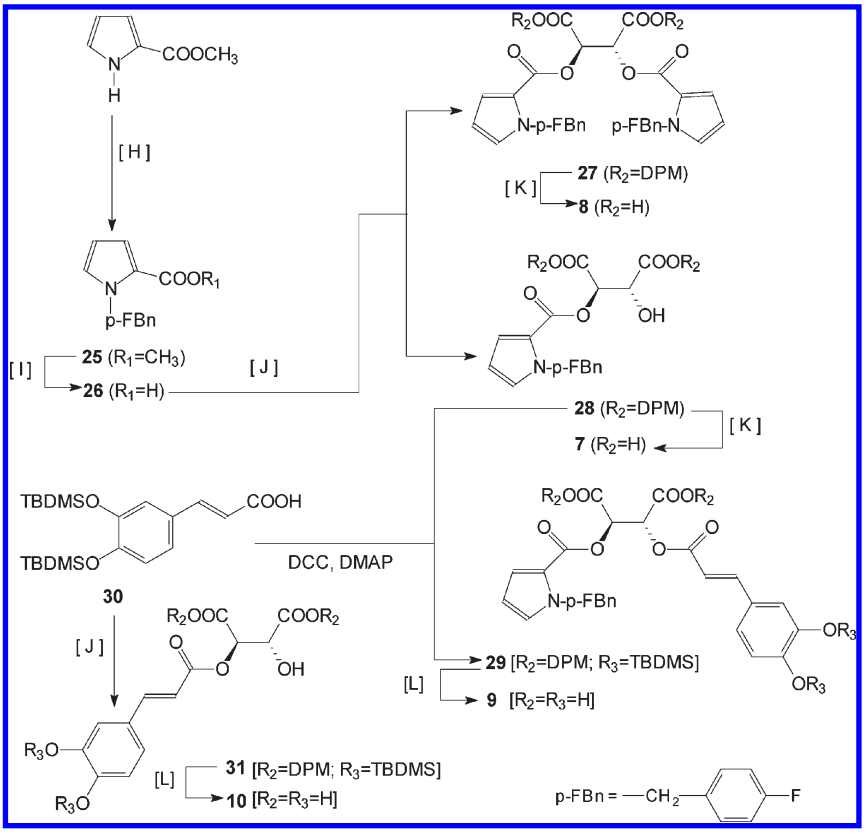
Preparation of the trihydroxycinnamoyl derivative of the bis-tetrazole **34** used the known[40] protected gallaldehyde **39**, which was condensed with malonic acid to give the TBDMS cinnamic acid **40** in good yield. The corresponding acid chloride reacted with bis-tetrazole **34** to give the TBDMS protected bis-tetrazole **41** which was deprotected with tetrabutylammonium fluoride to give **14**, the trihydroxy tetrazole analogue of L-chicoric acid.

Biological Assays

Oligonucleotides. All oligonucleotides were synthesized and desalted by Integrated DNA Technologies (Coralville, IA 52241). Oligonucleotides used for IC_{50} analyses {V1 (5′ ATGTGGAAAATCTCTAGCAGT 3′), V2 (5′ ACTGC-TAGAGATTTTCCACAT 3′), and U5 V1P (5′ ATGTG-GAAAATCTCTAGCA 3′)}[30] were gel purified prior to use.

Generation of Recombinant IN Proteins. Recombinant IN proteins were expressed in BL21 DE3 pLysS *Escherichia coli* (Stratagene, La Jolla, CA 92037) and purified from the included portion following bacterial lysis. Briefly, following culture, induction, and harvest via centrifugation, bacteria were lysed via freeze/thaw and digested with endogenous lysozyme produced by the pLysS plasmid. The viscous mixture was subjected to brief ultrasonic disruption and clarified via centrifugation. The insoluble fraction was washed with 100 mM NaCl and clarified via centrifugation. The remaining insoluble fraction was then solubilized via slow stir in high salt buffer (1 M NaCl)

Scheme 2. Synthesis of *p*-Fluorobenzylpyrroloyl/Tartaric Acid Hybrids **7**−**9**[a]



[a] Procedures: [H] *p*-F-benzyl bromide, NaH, DMF; [I] NaOH, H_2O; [J] DPM tartrate, DCC, DMAP; [K] Pd/C, H_2; [L] 70% HOAc, reflux.

containing 5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Each of the above steps took place at 5 °C under reducing conditions (5 mM βME) in buffer containing 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) at pH 7.5. Histidine-tagged (amino terminal 6-His tag) recombinant IN was purified via Ni^{2+} affinity column chromatography as described previously.[41,42] Purity was confirmed via sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by staining with Coomassie Blue dye. IN resolved to a MW of approximately 32 kDa. Fractions containing highly purified IN (> 95%) were pooled and dialyzed into storage buffer (20 mM HEPES, pH 7.5, 300 mM NaCl, 5 mM CHAPS, 10% glycerol, and 5 mM dithiothreitol) and then stored at −80 °C.

50% Inhibitory Concentration (IC_{50}). The susceptibility of recombinant IN to inhibitors was determined utilizing radiolabeled duplex DNA substrates mimicking the viral LTRs as described previously.[41,43] The potency of each compound against IN-mediated 3′-end processing was determined using ^{32}P-labeled DNA oligonucleotide V1 annealed to DNA oligonucleotide V2 (V1/V2). The potency of each compound against IN-mediated strand transfer, independent of 3′end processing, was determined using ^{32}P-labeled DNA oligonucleotide U5 V1P annealed to DNA oligonucleotide V2 (V1/V2). Representative analyses are shown in Figure 4.

Cells and Viruses. H9 cells, a CD4+ human T-lymphoblastoid cell line, MT-2 cells, a CD4+ human T-lymphotropic virus type-1 (HTLV-1) transformed CD4+ cell line, and HIV_{LAI} were obtained from the AIDS Research and Reference Reagent Program and cultured in RPMI-1640 growth media without antibiotics as described previously.[42]

50% Effective Concentration and Cellular Toxicity Analyses (EC_{50} and CT_{50}/CT_5). The 50% effective concentration and cellular toxicity analyses were performed as described previously.[42,44] The CT5, the concentration of compound at which 95% of cells are viable, is a nontoxic concentration of the compound.

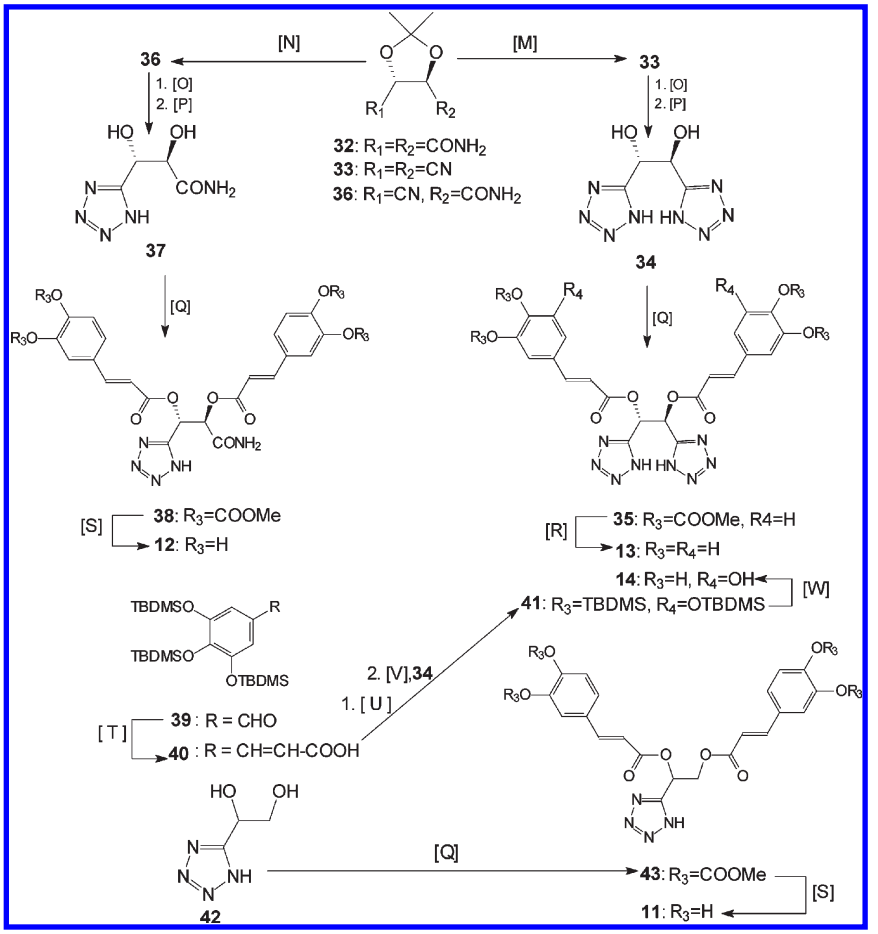
Results

Catechol/DKA Hybrid Compounds Are Potent in Vitro Against 3′-End Processing and Strand Transfer but Not ex Vivo Against HIV at Nontoxic Concentrations. To test the hypothesis that the DCTA/DKA pharmacophores can be interchanged, compounds **1**−**6** containing combinations of the DKA motif and one or more catechol side chains were synthesized and evaluated for biological activity. In vitro, all compounds exhibited low to submicromolar potency against IN-mediated 3′-end processing and strand transfer catalysis. In addition, all of the compounds exhibited near equimolar potency against both enzymatic functions, indicating that despite having the DKA pharmacophore, they were not strand transfer selective inhibitors. The methyl ester DKA variants **1B**, **3B**, and **6B** exhibited in vitro potency comparable to (within 3-fold), yet reduced relative to, that of their free acids (Table 1).

Ex vivo, these hybrid molecules did not exhibit anti-HIV potency but on the other hand exhibited only mild cellular toxicity (Table 1). In addition, all compounds were readily soluble in culture medium at the concentrations employed for ex vivo studies (data not shown).

***p*-Fluorobenzylpyrroloyl/Tartaric Acid Hybrid Compounds Selectively Inhibit Strand Transfer in Vitro and Are Modestly Potent ex Vivo at Nontoxic Concentrations.** To determine the

Scheme 3. Synthesis of Tetrazole Analogues **11**−**14**[a]



[a] Procedures: [M] 5 equiv of TsCl, 5.5 h, 100 °C; [N] 2.2 equiv of TsCl, 3.5 h, 100 °C; [O] DMF, $(NH_4)_2SO_4$, NaN_3, 90 °C; [P] 2 N HCl; [Q] dicarbomethoxycaffeoyl chloride, pyridine, Et_3N; [R] 1 N NH_4OH; [S] 10% Na_2CO_3; [T] pyridine, piperidine (cat.), malonic acid (2 equiv), 85 °C; [U] $SOCl_2$; [V] pyridine, Et_3N; [W] $(n\text{-Bu})_4N^+$, F^-, THF.

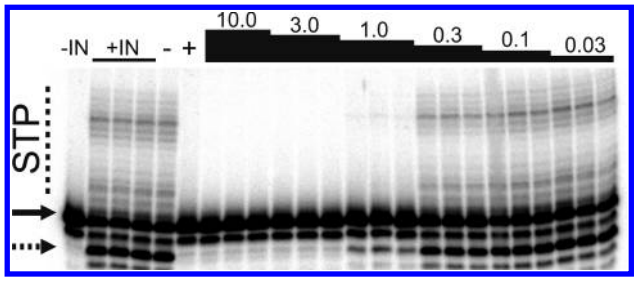


Figure 4. Representative enzyme IC_{50} analysis of compound **14** against recombinant IN from HIV_{NL4-3}. Recombinant reference IN was incubated with radiolabeled blunt-end 3′-end processing and strand transfer oligonucleotide substrate (V1/V2) or in increasing concentrations of compound **14** at 37 °C for 1 h in triplicate. Reactions were terminated, resolved via denaturing polyacrylamide gel electrophoresis, then visualized and quantified via phosphorimager analysis. 3′-End processing was quantified as the percent conversion of blunt-end 21mer substrate (V1/V2) into 19mer 3′-end processed product. IC_{50} values were calculated using CalcuSyn software for Windows. −IN is substrate in buffer. +IN is reaction with IN and no inhibitor. (−) contains 25 μM L-tartaric acid, an inactive compound. (+) contains 25 μM L-CA, a concentration that completely inhibits IN-mediated catalysis. Numbers above triplicates indicate concentrations of compound **14** in μM. Solid arrows indicate V1/V2 substrate, and a dashed arrow indicates 3′-end processed products. STP indicates strand transfer products.

interchangeability of DCTA/DKA side chains, namely, the caffeoyl and *p*-fluorobenzylpyrroloyl groups, respectively, compounds **7**−**9** were synthesized, biologically characterized, and compared to caftaric acid (**10**). In vitro, the mono- and disubstituted *p*-fluorobenzylpyrroloyl variants (**7** and **8**) exhibited relatively modest potency. However, combining both *p*-fluorobenzylpyrroloyl and caffeoyl side chains as in **9** significantly improved the potency of the inhibitor. In addition, **9** exhibited greater potency than either the monocaffeoyl (**10**) or mono-*p*-fluorobenzylpyrroloyl (**7**) substituted variants. The monocaffeoyl compound **10**, while exhibiting modest potency, exhibited significantly less potency than L-CA.[41] Perhaps most interesting was the observation that compounds **7**, **9**, and **10** required greater than 3-fold higher concentrations to inhibit 3′-end processing than to inhibit strand transfer. Thus, the *p*-fluorobenzyl group alone seems to add some selectivity toward inhibition of strand transfer.

While both the mono- and disubstituted *p*-fluorobenzylpyrroloyl variants (**7** and **8**) failed to exhibit anti-HIV potency, both molecules were not cytotoxic; however, the caffeoyl/*p*-fluorobenzylpyrroloyl (**9**) and monocaffeoyl (**10**) analogues exhibited modest anti-HIV activity at nontoxic concentrations.

Tetrazole Substitution of the L-CA Carboxyl Group and Addition of a Third Phenolic Hydroxyl Maintain in Vitro Potency Against 3′-End Processing and Strand Transfer and Enhance the ex Vivo Antiviral Potency at Nontoxic Concentrations. All four tetrazole-substituted L-CA analogues (**11**−**14**) exhibited submicromolar potency against IN in vitro and near

Table 1. Biological Activities of Compounds **1**−**14**[a]

compd	3′- end processing (μM) IC_{50} (SD)[b]	strand transfer (μM) IC_{50} (SD)[b]	anti-HIV_{LAI} activity (μM) EC_{50} (SD)[c]	cell toxicity (μM) CT_{50} (SD)[d]	cell toxicity (μM) CT_5 (SD)[e]
L-CA	0.22(0.01)	0.27(0.01)	0.81(0.13)	124(27.2)	41.0(9.0)
FBP	13.02(0.58)	0.14(0.01)	0.40(0.04)	> 69.3	> 69.3
1	0.47(0.07)	1.13(0.1)	≫67	178	67
1B	2.81(0.13)	1.89(0.25)	≫36	182	36
2	1.10(0.06)	0.81(0.02)	≫36	80	32
3	0.40(0.05)	0.35(0.02)	≫16.9	47.2(2.4)	26.1(2.2)
3B	1.82(0.59)	1.38(0.05)	≫54	39.5(3.4)	12.4(1.3)
4	2.28(0.21)	2.55(0.08)	≫37.5	53.0(1.6)	23.3(2.6)
5	0.11(0.01)	0.36(0.02)	> 34	52.8(7.2)	20.4(3.4)
6	0.12(0.02)	0.13(0.01)	≫51	> 50.7	> 50.7
6B	0.75(0.10)	0.63(0.02)	≫48	> 47.7	> 47.7
7	277(36.3)	47.3(6.1)	≫170	792(233)	60.8(2.9)
8	136(8.7)	74.2(4.4)	≫90.5	374(176)	28.7(4.3)
9	4.05(0.17)	1.27(0.02)	110(20.4)	399(323)	41.8(6.4)
10	19.1(1.7)	6.08(0.02)	80.5(7.1)	325(101)	49.6(7.7)
11	0.14(0.01)	0.12(0.01)	0.85	> 42.5	> 42.5
12	0.21(0.02)	0.27(0.02)	0.42(0.08)	31.9(4.0)	5.7(2.6)
13	0.34(0.02)	0.44(0.02)	0.33(0.02)	422(253)	28.4(8.5)
14	0.39(0.06)	0.21(0.01)	0.06(0.01)	48.9(2.2)	11.3(1.1)

[a] Values are the mean of triplicate assays; values in parentheses are 1 standard deviation (SD). [b] IC_{50}, concentration of compound required to inhibit IN-mediated catalysis 50% relative to untreated control. [c] EC_{50}, concentration of compound required to protect 50% of cells from HIV-induced cytopathic effect. ≫: No detectable activity at any concentration. >: 50% protection was not achieved. [d] CT_{50}, concentration of compound required to inhibit cellular replication by 50% relative to untreated control. [e] CT_5, concentration of compound required to inhibit cellular replication by 5% relative to untreated control. Cells are 95% viable, a nontoxic concentration.

equimolar potency against in vitro 3′-end processing and strand transfer (Table 1).

Ex vivo, all four analogues (**11**−**14**) exhibited submicromolar anti-HIV potency (Table 1). While the disubstituted tetrazole analogue **13** exhibited significantly greater potency than either of the monosubstituted analogue **11** or **12**, the trihydroxphenyl compound **14** was more potent than the dihydroxyphenyl compounds **11**−**13**. Indeed, compound **14** was the most potent anti-HIV compound evaluated herein. All four tetrazole-substituted analogues exhibited anti-HIV potency at nontoxic concentrations with compounds **12**−**14** yielding therapeutic indices (TI = CT_{50}/EC_{50}) of greater than 350.

Discussion

While previous SAR studies have refined the relevant pharmacophore for both DCTAs and DKAs, combining their pharmacophores into a single hybrid molecule is relatively novel.[45,46] For DCTAs, the linker between the core tartaric acid and the catechol can be varied with little affect on antiviral potency. For example, it can be from 0 to 4 carbon atoms and can be either sp^3 or sp^2 hybridized without losing antiviral potency.[23,25] An amide in place of an ester linkage of the caffeoyl groups does not negatively impact antiviral potency.[24,25] However, while the tartaric acid core can be replaced by an aromatic[23,24] or quinic acid ring,[12] at least one carboxyl group must be present to preserve potency.[23,25,47] In addition, modification of the catechols, with the exception of adding a third phenolic hydroxyl group,[23,25] abolishes antiviral activity.[24] Potent inhibitors must contain 3,4-catechol units, as opposed to 2,3-catechol units.[23] With respect to the DKA class of IN inhibitors, having a core β-diketo group adjacent to an acidic moiety, carboxyl or tetrazole/triazole, is a basal requirement for potency.[48] Methyl esterification of the DKA carboxyl group abolishes inhibitor potency,[45,48,49] although a wide array of aromatic and halogen-containing aryl groups may be adjacent to the DKA pharmacophore.[45,50,51]

Compounds **1**−**6** are DKA/DCTA hybrids containing the DKA pharmacophore adjacent to DCTA-like catechol/caffeoyl groups. An SAR study conducted by Maurin et al.[45] exploring related catechol/DKA hybrid molecules appeared after the present studies were completed[52] and reported nearly identical in vitro strand transfer potency for compound **1**. Given the metal-chelating properties of catechols,[53] it is possible that this group may coordinate the bound metal ion within the IN active site. Available structural data[54−57] indicate that only a single metal ion binds the IN active site. However, homology modeling of HIV IN with the orthologous avian sarcoma virus IN, which is shown to bind two metals within its active site,[58] suggests that a second metal ion is bound during catalysis.[59] A recently reported crystal structure of prototype foamy virus IN complexed with 3′-end processed DNA substrate indicates that two metal ions are bound within the enzyme active site prior to strand transfer.[60] Given the presence of two metal chelating groups on compounds **1**−**5** and potentially two metal ions within the IN active site during catalysis, it is plausible that the two metal ions are coordinated simultaneously by the catechol and DKA groups. If this were the case, compounds **1B** and **3B**, in which the DKA carboxyl is esterified, should lose much of their potency because of compromised ability to efficiently coordinate metal. However, compounds **1B** and **3B** exhibited comparable in vitro activity to their carboxyl counterparts, suggesting that methyl esterification does not affect the metal chelating ability of the β-diketo moiety. In contrast, Maurin et al.[45] found that methyl esterification of the DKA carboxyl group (the group studied compounds **1** and **1B**) nearly abolished potency. The choice of divalent metal cation cofactor employed in the in vitro assays reported by Maurin et al.[45] (Mg^{2+}) and herein (Mn^{2+}) presents a possible explanation for this discrepancy. Although either metal ion cofactor facilitates catalysis in vitro,[61] it is believed that Mg^{2+} is the relevant metal ion ex vivo because of its significantly greater prevalence in the cell. The observation that the methyl ester, **1B**, inhibits

IN in the presence of Mn^{2+} but not Mg^{2+} suggests that the esterified DKA moiety may interact differently with the two metal cofactors. Nevertheless, compound **1B**, along with each esterified catechol/DKA variant reported herein, failed to exhibit antiviral activity, reinforcing previous findings that an acidic group, such as carboxyl or tetrazole, is a requisite for anti-HIV potency.[48]

The position of the DKA group with respect to the caffeoyl group around an aromatic core has a significant effect on in vitro potency. Compound **5**, with the DKA and caffeoyl groups meta is 4-fold more potent than the para isomer, **3**, and 22-fold more potent than the ortho isomer, **4**. Compound **6**, containing a second meta caffeoyl group, was equally potent against 3′-end processing as **5**, suggesting that the second caffeoyl group does not alter binding within the active site. However, compound **5** exhibited 3-fold selectivity for 3′-end processing while **6** did not, suggesting that **5** interacts with the active site in a unique manner. In addition, compound **6** is 5-fold more potent than a previously reported analogue containing only a single free carboxyl group on the core aromatic ring[23] and 50-fold more potent than a comparable analogue containing ester-linked caffeoyl side chains.[24] These data suggest that the DKA group interacts with the metals bound within the active site more efficiently than the carboxyl group alone. These data are also consistent with previous findings that analogues with amide-linked caffeoyl side chains are more potent than their ester-linked counterparts,[25] suggesting that the amide linkages are more resistant to hydrolytic cleavage in an aqueous environment.

The next group of DCTA/DKA hybrids includes a panel of compounds containing the tartaric acid core of L-CA (DCTA) combined with the *p*-fluorobenzylpyrroloyl side chains from FBP (DKA). While compounds **7** and **8**, containing one and two *p*-fluorobenzylpyrroloyl side chains, respectively, exhibited significantly less potency in vitro against both 3′-end processing and strand transfer compared to FBP or L-CA, both selectively inhibited strand transfer over 3′-end processing. Indeed, **7**, which more closely resembles FBP, inhibited 3′-end processing at concentrations 6-fold higher than those required to inhibit strand transfer. Given that tartaric acid alone exhibits no potency ($IC_{50} \gg 25\ \mu M$, $EC_{50} > 1000\ \mu M$),[23] it follows that the attached side chains, either *p*-fluorobenzylpyrroloyl (**7** and **8**) or caffeoyl (**10**), are critical for the functionality of this DCTA pharmacophore. The modest in vitro activity of **7** compared to FBP and the lack of anti-HIV activity for the former molecule strongly suggest that the metal chelating activity of the DKA moiety (strong) versus that of compound **7** (relatively weak) is critical for activity. The last and most potent hybrid in this series, compound **9**, suggests that an asymmetric molecule may be better suited for interacting with an asymmetric binding pocket within the IN active site. Considering that **9** is significantly more potent than either **7** or **10**, it is possible that the two side chains, caffeoyl and *p*-fluorobenzylpyrroloyl, interact with the IN active site in a mutually nonexclusive manner. Computer modeling suggests that the *p*-fluorobenzylpyrroloyl side chain occupies a cleft between catalytic residues D64 and E152, extending to Q148,[62] while the caffeoyl side chain extends between H67 and D64, forming hydrogen bonds with E92.[63,64] In this configuration, the tartaric acid core carboxyl groups would be in a suitable position to form salt bridges with K156 and K159, as is hypothesized for L-CA.[63,64] The low micromolar potency of caftaric acid (**10**) further supports the assertion that both DCTA side chains are essential to the potency of the molecule. Indeed, caffeic, chlorogenic, and quinic acid are all completely impotent compounds.[23]

With the exception of compounds **9** and **10**, both exhibiting modest antiviral activity, the majority of these DCTA/DKA hybrids did not protect cells from the cytopathic effects of HIV. However, the vast majority of these molecules exhibited little cytotoxicity, an attribute sometimes ascribed to catechol-containing and metal-chelating compounds.[65] Nevertheless, these hybrid molecules, especially compound **9**, represent an attractive platform on which further SAR study may improve antiviral potency.

The last set of DCTA analogues, compounds **11**−**14**, examines the substitution of a tetrazole in place of the carboxyl group in the tartaric acid core of L-CA. Previously, it has been shown that tetrazoles, as carboxyl group bioisosteres, can be successfully substituted in the DKA pharmacophore; however, this substitution yields a significantly less potent inhibitor.[48] In contrast, tetrazole substitution significantly improved the antiviral properties of L-CA, as compound **13** exhibited a nearly 6-fold increase in anti-HIV potency relative to L-CA (EC_{50} HIV: 2 μM).[16] Interestingly, the in vitro potencies of these four analogues were slightly less than those of L-CA (see above), suggesting that the tetrazole substitution may facilitate cellular entry. In addition, two correlations, one negative and one positive, were observed between the number of tetrazole groups and the observed in vitro and ex vivo potencies. For example, there is an increase in the compound IC_{50} for both 3′-end processing and strand transfer when the number of tetrazole groups is doubled. At the same time, the increasing number of tetrazole groups resulted in decreasing EC_{50} values. This inverse correlation further supports the notion that the additional tetrazole groups improve antiviral potency via nonbiochemical mechanisms, at least those measured in in vitro assays utilizing recombinant IN. The addition of a third phenolic hydroxyl group on both catechols (compound **14**) further enhanced antiviral potency of these tetrazole-substituted analogues, a result consistent with previous SAR studies of DCTA analogues containing galloyl or trihydroxycinnamoyl groups in place of the caffeoyl groups.[23,25] The mild toxicity associated with compounds **11**−**14** is promising for future development as lead molecules. Indeed, given the TI value of 815 for compound **14**, this analogue is an especially attractive candidate for further development into a potentially clinically relevant inhibitor.

As reported herein, these DCTA/DKA hybrid and tetrazole-substituted DCTA analogues represent two significant developments for this long-standing class of IN inhibitors. While the majority of these new hybrid molecules exhibited limited antiviral potency, they also exhibited little cytotoxicity. Furthermore, compounds **1**−**10** were potent against both 3′-end processing and strand transfer, suggesting that these novel inhibitors may be potent against STI-resistant IN. In addition, future study of the strand transfer-selective nature of compound **7**, lacking a DKA pharmacophore, may reveal further insight into the chemical determinants of STI catalytic selectivity. The tetrazole-substituted DCTA analogues, compounds **11**−**14**, enhanced antiviral activity with limited cytotoxicity. Compound **14** exhibited nanomolar potency and a TI of 815, thus approaching potency and toxicity requirements necessary for clinical consideration.

Materials and Methods

Chemistry. Chemical Syntheses. General. Melting points were determined on either a Mel-Temp apparatus or a Bausch & Lomb Optical Co. hot-stage microscope and are uncorrected.

Elemental analyses were performed by M-H-W Laboratories of Phoenix, AZ, or Atlantic Microlab, Inc. of Norcross, GA. HRFABMS experiments were performed by the Washington University Mass Spectrometry Resource of St. Louis, MO, or by the Notre Dame Mass Spectrometry & Proteomics Facility, IN. NMR spectra were obtained on a Varian XL-300 instrument at 300 MHz (^{1}H) or 75 MHz (^{13}C). The chemical shift (δ) is in parts per million (ppm) ($\pm$0.1 ppm), and observed coupling constants (J) are in Hz ($\pm$0.1 Hz). ^{1}H NMR spectra were referenced to TMS = 0 ppm and reported as δ (multiplicity, number of H, J). ^{13}C NMR spectra were proton-decoupled, referenced to one of the internal standards (TMS = 0 ppm, central peak of $CDCl_3$ = 77.00 ppm, central peak of DMSO-d_6 = 39.52 ppm, central peak of acetone-d_6 = 29.84 ppm, or central peak of CD_3OD = 49.00 ppm), and reported as δ (number of C, if more than 1). HPLC used a C18 10 μm, 250 mm $\times$ 4.6 mm analytical column or a C18 10 μm, 250 mm $\times$ 22 mm preparative column eluted with either methanol−water or acetonitrile−water mixtures containing 1% HOAc and UV detection at 254 nm. Purity was maximized by cutting off the shoulders of absorption peaks, leading to lower recovery yields. All tested compounds had purities exceeding 95% as determined by combustion analysis or HPLC.

The following general synthetic procedures refer to Scheme 1. No attempt was made to optimize yields. Although both the keto−enol and the diketo tautomers of the fully blocked diketo acids and the free diketo acids were often observed by ^{1}H NMR ($\geq$90% keto−enol), no attempt was made to record them, so only the peaks for the keto−enol forms are reported except when comparisons are being made to literature values. In those cases the compositions were calculated from the relative areas of the ethylenic proton signal of the keto−enol form and the methylenic protons signal of the diketo form.

Differences in the melting points of compounds **3**, **5**, and **22m** from literature values[44] may also be due to differences in tautomer content and hence crystal form. Significantly, the ^{13}C spectra of these compounds agree with the literature,[44] the slight differences [−2.9, −2.9, and −0.2 ppm, respectively] being uniform in direction and degree as expected when comparing different instruments or standardization procedures.

Procedure A: Condensation of Acetophenone with Dimethyl Oxalate. To a solution of acetophenone (or a vinylogue) and 2 equiv of dimethyl oxalate in THF at room temperature was added 4 equiv of sodium methoxide or sodium *tert*-butoxide. The resulting mixture was stirred at room temperature for 2 h, poured into 2 M HCl, and extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na_2SO_4 and the solvent was removed to give the crude product which was recrystallized if necessary.

Procedure B: Demethylation with Boron Tribromide. To a solution of fully methylated compound (**16** or **18**) in CH_2Cl_2 at room temperature was added 30 equiv of 1 M BBr_3 in CH_2Cl_2. The resulting mixture was stirred overnight at room temperature. After the mixture was cooled to −78 °C, the reaction was quenched by adding H_2O. The resulting mixture was extracted with ethyl acetate three times. The organic extracts were combined, dried over Na_2SO_4, and evaporated with a Rotavapor to give crude product that was purified by HPLC.

Procedure C: Demethylation with Boron Tribromide. To a mixture of fully methylated compound (**22o**, **22m**, or **22p**) in CH_2Cl_2 at room temperature was added 30 equiv of 1 M BBr_3 in CH_2Cl_2. The resulting mixture was stirred at room temperature for 0.5 h followed by reflux overnight. After the mixture was cooled to −78 °C, the reaction was quenched by adding H_2O. The resulting suspension was filtered to give the crude product that was purified by HPLC.

Procedure D: Preparation of 3,4-Dimethoxycinnamoyl Chloride (20). A solution of 3,4-dimethoxycinnamic 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.

Procedure E: Acylation of Amino Groups. 3,4-Dimethoxycinnamic acid chloride (2 equiv) was reacted with the aminoacetophenone in anhydrous toluene with pyridine as a catalyst overnight at room temperature. The reaction mixture was diluted with EtOAc and then successively washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give the crude product which was purified on a silica gel column or by recrystallization from appropriate solvents.

Specific Syntheses. 4-(3,4-Dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic Acid (1) and Its Methyl Ester (1B). 3,4-Dimethoxyacetophenone was converted by procedure A to methyl 4-(3,4-dimethoxyphenyl)-2-hydroxy-4-oxo-2-butenoate (**16**) as yellow crystals (82%) from $CHCl_3$: mp 151−152.5 °C (lit.[66] 150−151 °C); ^{1}H NMR ($CDCl_3$) 7.64 (dd, 1H, J = 8.5, 2.1 Hz), 7.53 (d, 1H, J = 2.1 Hz), 7.04 (s, 1H), 6.93 (d, 1H, J = 8.5 Hz), 3.97 (s, 3H), 3.96 (s, 3H), 3.94 (s, 3H), no detectable signals from the diketo form; ^{13}C NMR ($CDCl_3$) 190.8, 166.7, 162.9, 154.2, 149.3, 128.0, 122.9, 110.4, 109.8, 98.1, 56.2, 56.0, 53.2. Anal. Calcd for $C_{13}H_{14}O_6$: C, 58.63; H, 5.30. Found: C, 58.36; H, 5.26.

Reaction of **16** with BBr_3 via procedure B gave 4-(3,4-dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid (**1**) as a yellow solid (60% after HPLC): mp 198 °C (dec); lit.[45] yellow powder, 180−182 °C (acetone); ^{1}H NMR (DMSO-d_6) 10.24 (br s, 1H), 9.58 (br s, 1H), 7.50 (dd, 1H, J = 8.5, 2.1 Hz), 7.45 (d, 1H J = 2.1 Hz), 6.96 (s, 1H), 6.88 (d, 1H, J = 8.5 Hz). Diketo form (7%): 7.38 (br d, 1H, J = 8.5 Hz), 7.34 (d, 1H, J = 2.1 Hz), 6.84 (d, 1H, J = 8.5 Hz), 4.42 (s, 2H). ^{13}C NMR (DMSO-d_6) 190.7, 167.5, 163.5, 152.2, 145.8, 126.2, 121.9, 115.8, 114.6, 97.6. Anal. Calcd for $C_{10}H_8O_6$: C, 53.56; H, 3.60. Found: C, 53.36; H, 3.85.

A methanol solution of crude **1** was allowed to sit at room temperature for 3 weeks to give the methyl ester which was purified by flash chromatography (EtOAc/hexanes = 1/1, R_f = 0.38) followed by HPLC to give methyl ester **1B** as a yellow solid (52% from **16**): mp 165−168 °C; lit.[45] pale green powder: mp 170−174 °C (acetone); ^{1}H NMR (DMSO-d_6) 10.27 (br s, 1H), 9.62 (br s, 1H), 7.52 (dd, 1H, J = 8.5, 2.3 Hz), 7.46 (d, 1H, J = 2.1 Hz), 6.99 (s, 1H), 6.89 (d, 1H, J = 8.5 Hz), 3.85 (s, 3H). Diketo form (8%): 10.10 (br s, 1H), 9.49 (br s, 1H), 7.37 (dd, 1H, J = 8.5, 2.2 Hz), 7.33 (d, 1H, J = 2.2 Hz), 6.84 (d, 1H, J = 8.4 Hz), 4.46 (s, 2H), 3.77 (s, 3H). ^{13}C NMR (DMSO-d_6) 190.3, 166.0, 162.3, 152.3, 145.7, 125.8, 121.9, 115.6, 114.4, 97.7, 52.9. Anal. Calcd for $C_{11}H_{10}O_6$: C, 55.45; H, 4.23. Found: C, 55.52; H, 4.35.

6-(3,4-Dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoic Acid (2). (*E*)-4-(3,4-Dimethoxyphenyl)-3-buten-2-one (**15**)[67,68] was converted by procedure A to methyl 6-(3,4-dimethoxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoate (**18**) as a red-orange solid (98%): mp 121−123 °C; ^{1}H NMR ($CDCl_3$) 7.70 (d, 1H, J = 15.8 Hz), 7.18 (dd, 1H, J = 8.5, 2.1 Hz), 7.09 (d, 1H, J = 2.1 Hz), 6.90 (d, 1H, J = 8.5 Hz), 6.56 (s, 1H), 6.55 (d, 1H, J = 15.3 Hz), 3.942 (s, 3H), 3.939 (s, 3H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$) 185.9, 172.7, 162.7, 151.9, 149.3, 143.7, 127.3, 123.6, 120.9, 111.1, 109.8, 100.7, 56.0, 55.9, 53.1. Anal. Calcd for $C_{15}H_{16}O_6$: C, 61.62; H, 5.52. Found: C, 61.84; H, 5.47.

Demethylation of **18** by procedure B gave 6-(3,4-dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoic acid (**2**) as a red-yellow solid (38% after HPLC): mp 184 °C (dec); ^{1}H NMR (CD_3OD) 7.65 (d, 1H, J = 15.2 Hz), 7.11 (br s, 1H), 7.04 (br d, 1H, J = 7.0 Hz), 6.80 (d, 1H, J = 7.3 Hz), 6.61 (d, 1H, J = 15.5 Hz), 6.55 (s, 1H); ^{13}C NMR (CD_3OD) 187.1, 175.4, 165.2, 150.4, 147.0, 145.5, 128.1, 123.9, 120.8, 116.7, 115.6, 101.2. Anal. Calcd for $C_{12}H_{10}O_6 \cdot 0.25H_2O$: C, 56.58; H, 4.16. Found: C, 56.95; H, 4.55.

4-(4-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic Acid (3) and Its Methyl Ester (3B). 4-Aminoacetophenone was converted by procedure E to 4-(3,4-dimethoxycinnamoylamino)acetophenone (**21p**) as a white solid (80%, SiO_2 CC): mp 194−196 °C; lit.[46] 199−201 °C; ^{1}H NMR (DMSO-d_6) 10.48 (br s, 1H), 7.96 (d, 2H, J = 9.1 Hz), 7.83 (d, 2H, J = 8.8 Hz), 7.59 (d, 1H, J = 15.5 Hz),

7.24 (d, 1H, J = 1.8 Hz), 7.22 (dd, 1H, J = 8.2, 1.9 Hz), 7.03 (d, 1H, J = 8.2 Hz), 6.73 (d, 1H, J = 15.5 Hz), 3.83 (s, 3H), 3.81 (s, 3H), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6) 196.5, 164.3, 150.6, 148.9, 143.8, 141.2, 131.6, 129.6 (2), 127.3, 122.1, 119.3, 118.4 (2), 111.8, 110.0, 55.6, 55.4, 26.5. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.40; H, 5.75; N, 4.18.

Acetophenone **21p** was converted by procedure A to methyl 4-(4-(3,4-dimethoxycinnamoylamino)phenyl)-2-hydroxy-4-oxo-2-butenoate (**22p**) as a yellow solid (96%) after washing the crude product with 3−5 mL of ethyl acetate: mp 206 °C (dec); lit.[46] 209−212 °C; ^{1}H NMR (DMSO-d_6) 10.61 (br s, 1H), 8.10 (d, 2H, J = 9.1 Hz), 7.90 (d, 2H, J = 8.8 Hz), 7.60 (d, 1H, J = 15.5 Hz), 7.24 (br s, 1H), 7.22 (dd, 1H, J = 7.9, 1.8 Hz), 7.12 (s, 1H), 7.03 (d, 1H, J = 8.2 Hz), 6.73 (d, 1H, J = 15.8 Hz), 3.87 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (DMSO-d_6) 189.6, 167.6, 164.4, 162.1, 150.6, 148.8, 144.9, 141.4, 129.5 (2), 128.6, 127.1, 122.1, 119.0, 118.6 (2), 111.6, 109.9, 97.8, 55.5, 55.3, 53.0. Anal. Calcd for $C_{22}H_{21}NO_7$: C, 64.23; H, 5.14; N, 3.40. Found: C, 64.10; H, 5.07; N, 3.24.

4-(4-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**3**) and its methyl ester (**3B**) were obtained as yellow solids from **22p** by procedure C after the reaction mixture was separated by HPLC. **3** (35%): mp 210 °C (dec); lit.[46] 245−8 °C; ^{1}H NMR (DMSO-d_6) 10.56 (br s, 1H), 9.59 (br s, 1H), 9.29 (br s, 1H), 8.09 (d, 2H, J = 8.8 Hz), 7.88 (d, 2H, J = 8.8 Hz), 7.48 (d, 1H, J = 15.5 Hz), 7.09 (s, 1H), 7.04 (d, 1H, J = 1.8 Hz), 6.95 (dd, 1H, J = 8.2, 1.8 Hz), 6.80 (d, 1H, J = 8.2 Hz), 6.58 (d, 1H, J = 15.5 Hz); ^{13}C NMR (DMSO-d_6) 189.7, 169.0, 164.5, 163.2, 148.0, 145.5, 144.8, 141.9, 129.4 (2), 128.7, 125.8, 121.1, 118.6 (2), 117.6, 115.7, 113.9, 97.5. Anal. Calcd for $C_{19}H_{15}NO_7 \cdot 0.25H_2O$: C, 61.05; H, 4.18; N, 3.75. Found: C, 60.87; H, 4.10; N, 3.62. **3B** (25%): mp 196 °C (dec). ^{1}H NMR (DMSO-d_6) 10.57 (br s, 1H), 9.60 (br s, 1H), 9.29 (br s, 1H), 8.10 (d, 2H, J = 8.8 Hz), 7.89 (d, 2H, J = 8.8 Hz), 7.48 (d, 1H, J = 15.5 Hz), 7.13 (s, 1H), 7.04 (d, 1H, J = 1.5 Hz), 6.95 (dd, 1H, J = 8.2, 1.8 Hz), 6.80 (d, 1H, J = 7.9 Hz), 6.58 (d, 1H, J = 15.5 Hz), 3.87 (s, 3H); ^{13}C NMR (DMSO-d_6) 189.6, 167.6, 164.6, 162.2, 148.0, 145.5, 145.0, 142.0, 129.5 (2), 128.5, 125.8, 121.1, 118.6 (2), 117.6, 115.7, 114.0, 97.8, 53.0. Anal. Calcd for $C_{20}H_{17}NO_7 \cdot 0.75H_2O$: C, 60.53; H, 4.70; N, 3.53. Found: C, 60.71; H, 4.36; N, 3.56.

2-(4-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic Acid (4). 2-Aminoacetophenone was converted by procedure E to 2-(3,4-dimethoxycinnamoylamino)acetophenone (**21o**) as a white solid (91%, SiO_2 CC): mp 142−144 °C; ^{1}H NMR (DMSO-d_6) 11.51 (br s, 1H), 8.54 (br d, 1H, J = 8.5 Hz), 8.03 (br d, 1H, J = 7.9 Hz), 7.64 (br dd, 1H, J = 7.9, 7.6 Hz), 7.58 (d, 1H, J = 15.2 Hz), 7.40 (br s, 1H), 7.25 (br dd, 1H, J = 8.2, 7.3 Hz), 7.22 (br d, 1H, J = 7.9 Hz), 7.00 (d, 1H, J = 8.2 Hz), 6.81 (d, 1H, J = 15.5 Hz), 3.86 (s, 3H), 3.81 (s, 1H), 2.67 (s, 3H); ^{13}C NMR (DMSO-d_6) 202.7, 164.5, 150.7, 149.0, 141.8, 139.5, 134.2, 131.7, 127.2, 124.0, 122.9, 122.8, 120.8, 119.7, 111.5, 110.2, 55.6, 55.5, 28.8. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 69.93; H, 5.68; N, 4.12.

Acetophenone **21o** was converted by procedure A to methyl 4-(2-(3,4-dimethoxycinnamoylamino)phenyl)-2-hydroxy-4-oxo-2-butenoate (**22o**) as a yellow solid (94%) after recrystallization from CH_2Cl_2 and EtOAc: mp 89 °C (dec); ^{1}H NMR ($CDCl_3$) 11.47 (br s, 1H), 8.90 (dd, 1H, J = 8.8, 1.2 Hz), 7.91 (dd, 1H, J = 8.2, 1.5 Hz), 7.71 (d, 1H, J = 15.5 Hz), 7.63 (ddd, 1H, J = 8.8, 7.3, 1.5 Hz), 7.21−7.14 (m, 2H), 7.17 (s, 1H), 7.12 (d, 1H, J = 2.1 Hz), 6.89 (d, 1H, J = 8.5 Hz), 6.47 (d, 1H, J = 15.5 Hz), 3.97 (s, 3H), 3.96 (s, 3H), 3.93 (s, 3H); ^{13}C NMR ($CDCl_3$) 197.6, 164.9, 162.7, 162.4, 151.0, 149.2, 142.8, 141.8, 135.9, 130.3, 127.5, 122.8, 122.6, 121.54, 121.51, 119.4, 111.0, 109.8, 101.0, 56.01, 55.97, 53.4. Anal. Calcd for $C_{22}H_{21}NO_7$: C, 64.23; H, 5.14; N, 3.40. Found: C, 63.90; H, 5.27; N, 3.79.

A solution of **22o** in CH_2Cl_2 was cooled to −78 °C and then treated dropwise with 12 equiv of 1.0 M BBr_3 in CH_2Cl_2. After the resulting mixture was stirred at −78 °C for 3 h, the acetone−dry ice bath was removed and the stirring continued at room temperature for another 6.5 h. The resulting suspension was cooled to −78 °C and the reaction quenched with ice−water. After the acetone−dry ice bath was removed, the mixture was stirred for 20 min and filtered to give an orange-red solid which was dried in a drying pistol to give the crude product, to which was added 2 mL of acetone. The resulting suspension was sonicated for 5 min and filtered to give 2-(4-caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**4**) as a yellow solid (68%): mp 110 °C (dec); ^{1}H NMR (THF-d_8) 11.40 (br s, 1H), 8.91 (br d, 1H, J = 7.6 Hz), 8.02 (br d, 1H, J = 9.2 Hz), 7.59 (br dd partially overlapped by the peak at 7.58 ppm, 1H), 7.58 (d, 1H, J = 15.5 Hz), 7.19 (s, 1H), 7.15 (br dd partially overlapped by the peaks at 7.19 and 7.11 ppm, 1H), 7.11 (d, 1H, J = 1.8 Hz), 6.98 (dd, 1H, J = 8.2, 2.1 Hz), 6.76 (d, 1H, J = 7.9 Hz), 6.50 (d, 1H, J = 15.5 Hz); ^{13}C NMR (THF-d_8) 198.6, 165.5, 165.2, 163.5, 149.1, 146.7, 143.7, 142.9, 135.9, 131.3, 127.6, 123.1, 122.8, 122.0, 121.9, 119.1, 116.0, 115.0, 101.0. Anal. Calcd for $C_{19}H_{15}NO_7 \cdot 1.5H_2O$: C, 57.58; H, 4.58; N, 3.53. Found: C, 57.52; H, 4.34; N, 3.53.

4-(3-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic Acid (5). 3-Aminoacetophenone was converted by procedure E to 3-(3,4-dimethoxycinnamoylamino)acetophenone (**21m**) as a white solid (86%, SiO_2 CC): mp 150−152 °C; lit.[46] 154−5 °C; ^{1}H NMR (DMSO-d_6) 10.36 (br s, 1H), 8.28 (dd, 1H, J = 1.8, 1.8 Hz), 7.98 (ddd, 1H, J = 7.9, 1.2, 0.9 Hz), 7.68 (br dd, 1H, J = 7.6, 1.2 Hz), 7.57 (d, 1H, J = 15.5 Hz), 7.50 (dd, 1H, J = 7.9, 7.9 Hz), 7.23 (d, 1H, J = 1.5 Hz), 7.21 (dd, 1H, J = 8.2, 2.1 Hz), 7.03 (d, 1H, J = 8.2 Hz), 6.71 (d, 1H, J = 15.8 Hz), 3.84 (s, 3H), 3.81 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (DMSO-d_6) 197.6, 164.0, 150.4, 148.8, 140.6, 139.7, 137.3, 129.1, 127.2, 123.5, 123.1, 121.8, 119.4, 118.2, 111.6, 109.9, 55.5, 55.3, 26.7. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.34; H, 5.98; N, 4.43.

Acetophenone **21m** was converted by procedure A to methyl 4-(3-(3,4-dimethoxycinnamoylamino)phenyl)-2-hydroxy-4-oxo-2-butenoate (**22m**) as a yellow solid (89%) after washing the crude product with 3−5 mL of ethyl acetate: mp 148 °C (dec); lit.[46] 167−9 °C; ^{1}H NMR (DMSO-d_6) 10.43 (br s, 1H), 8.41 (br s, 1H), 8.03 (br d, 1H, J = 7.6 Hz), 7.77 (br d, 1H, J = 7.6 Hz), 7.58 (d, 1H, J = 15.8 Hz), 7.54 (dd, 1H, J = 7.9, 7.9 Hz), 7.23 (br s, 1H), 7.22 (br d partially overlapped by the peak at 7.23 ppm, 1H), 7.07 (s, 1H), 7.03 (d, 1H, J = 8.2 Hz), 6.70 (d, 1H, J = 15.5 Hz), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (DMSO-d_6) 190.0, 168.3, 164.1, 162.0, 150.4, 148.8, 140.8, 140.1, 134.8, 129.7, 127.2, 124.3, 122.6, 121.9, 119.3, 117.8, 111.6, 109.9, 98.0, 55.5, 55.3, 53.1. Anal. Calcd for $C_{22}H_{21}NO_7$: C, 64.23; H, 5.14; N, 3.40. Found: C, 64.42; H, 4.94; N, 3.17.

Compound **22m** was converted by procedure C to 4-(3-caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**5**) as a yellow solid (62%) after the crude product was purified by HPLC: mp 137 °C (dec); lit.[46] 196−8 °C; ^{1}H NMR (DMSO-d_6) 10.41 (br s, 1H), 8.43 (br s, 1H), 8.02 (br d, 1H, J = 8.2 Hz), 7.77 (br d, 1H, J = 7.9 Hz), 7.55 (dd, 1H, J = 8.2, 7.9 Hz), 7.48 (d, 1H, J = 15.8 Hz), 7.08 (s, 1H), 7.06 (br s, 1H), 6.96 (br d, 1H, J = 8.2 Hz), 6.82 (d, 1H, J = 8.2 Hz), 6.57 (d, 1H, J = 15.5 Hz); ^{13}C NMR (DMSO-d_6) 190.2, 169.6, 164.4, 163.0, 147.8, 145.5, 141.4, 140.1, 135.1, 129.6, 125.9, 124.2, 122.4, 121.0, 117.8 (2), 115.7, 113.9, 97.8. The peak at 117.8 ppm was resolved into two peaks at 118.7 and 118.6 ppm by changing the solvent to acetone-d_6/DMSO-d_6 = 1/1. Anal. Calcd for $C_{19}H_{15}NO_7$: C, 61.79; H, 4.09; N, 3.79. Found: C, 61.58; H, 3.94; N, 3.70.

4-(3,5-Dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic Acid (6) and Its Methyl Ester (6B). 3,4-Dimethoxycinnamic acid chloride (**20**, 2.6 equiv) was reacted with 3,5-diaminoacetophenone[69] in anhydrous dichloromethane with pyridine as a catalyst overnight at room temperature. The reaction mixture was poured into cold water. The precipitate was collected by filtration, dried in air, and then stirred in chloroform for 4 h. After filtration, 3,5-bis(3,4-dimethoxycinnamoylamino)acetophenone (**23**) was obtained as a light yellow powder (89%): mp 210−212 °C; ^{1}H NMR (DMSO-d_6) 10.47 (br s, 2H), 8.41 (br s, 1H), 8.08 (br s, 2H), 7.58 (d, 2H, J = 15.5 Hz), 7.24 (br s, 2H),

7.22 (br d partially overlapped by the peak at 7.24 ppm, 2H), 7.03 (d, 2H, J = 7.9 Hz), 6.79 (d, 2H, J = 15.5 Hz), 3.85 (s, 6H), 3.82 (s, 6H), 2.58 (s, 3H); ^{13}C NMR (DMSO-d_6) 197.3, 164.1 (2), 150.4 (2), 148.8 (2), 140.6 (2), 140.1 (2), 137.5, 127.3 (2), 121.8 (2), 119.6 (2), 113.8, 113.6 (2), 111.6 (2), 109.9 (2), 55.5 (2), 55.3 (2), 26.6. Anal. Calcd for $C_{30}H_{30}N_2O_7$: C, 67.91; H, 5.70; N, 5.28. Found: C, 68.16; H, 5.67; N, 5.14.

Acetophenone **23** was converted by procedure A (5 h) to methyl 4-(3,5-bis(3,4-dimethoxycinnamoylamino)phenyl)-2-hydroxy-4-oxo-2-butenoate (**24**) as a yellow solid (95%): mp 156 °C (dec); ^{1}H NMR (DMSO-d_6) 10.48 (br s, 2H), 8.46 (br s, 1H), 8.15 (br s, 2H), 7.59 (d, 2H, J = 15.5 Hz), 7.24 (br s, 2H), 7.22 (br d partially overlapped by the peak at 7.24 ppm, 2H), 7.04 (d, 2H, J = 7.9 Hz), 6.99 (s, 1H), 6.75 (d, 2H, J = 15.5 Hz), 3.90 (s, 3H), 3.85 (s, 6H), 3.82 (s, 6H); ^{13}C NMR (DMSO-d_6) 189.8, 167.9, 164.2 (2), 161.9, 150.4 (2), 148.8 (2), 140.8 (2), 140.5 (2), 135.1, 127.2 (2), 121.9 (2), 119.3 (2), 114.5, 112.8 (2), 111.6 (2), 109.9 (2), 97.9, 55.5 (2), 55.3 (2), 53.1. Anal. Calcd for $C_{33}H_{32}N_2O_{10}$: C, 64.28; H, 5.23; N, 4.54. Found: C, 64.21; H, 5.09; N, 4.63.

4-(3,5-Dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**6**) and its methyl ester (**6B**) were obtained as yellow solids from **24** by procedure C (room temperature for 2 h followed by reflux for 22 h) after the reaction mixture was separated by HPLC. **6** (25%): mp 213 °C (dec); ^{1}H NMR (DMSO-d_6) 10.44 (br s, 2H), 9.56 (br s, 2H), 9.29 (br s, 2H), 8.42 (br s, 1H), 8.16 (br s, 2H), 7.47 (d, 2H, J = 15.5 Hz), 7.05 (br s, 2H), 6.99 (s, 1H), 6.95 (br d, 2H, J = 8.2 Hz), 6.81 (d, 2H, J = 8.2 Hz), 6.58 (d, 2H, J = 15.5 Hz); ^{13}C NMR (CD_3OD—trace of DMSO-d_6) 191.3, 171.0, 167.6 (2), 164.8, 149.1 (2), 146.7 (2), 144.0 (2), 141.4 (2), 137.2, 128.1 (2), 122.6 (2), 118.4 (2), 117.1, 116.5 (2), 115.5 (2), 115.3 (2), C-3 not observed but did appear at δ 97.7 in DMSO-d_6. Anal. Calcd for $C_{28}H_{22}N_2O_{10}\cdot 0.9H_2O$: C, 59.77; H, 4.26; N, 4.98. Found: C, 60.04; H, 4.70; N, 4.58. **6B** (30%): mp 182 °C (dec); ^{1}H NMR (DMSO-d_6) 10.44 (br s, 2H), 9.53 (br s, 2H), 9.28 (br s, 2H), 8.42 (br s, 1H), 8.14 (d, 2H, J = 1.8 Hz), 7.46 (d, 2H, J = 15.5 Hz), 7.04 (d, 2H, J = 2.1 Hz), 6.98 (s, 1H), 6.94 (dd, 2H, J = 8.2, 2.1 Hz), 6.80 (d, 2H, J = 8.2 Hz), 6.58 (d, 2H, J = 15.5 Hz), 3.89 (s, 3H); ^{13}C NMR (DMSO-d_6) 189.8, 168.1, 164.3 (2), 161.9, 147.8 (2), 145.5 (2), 141.3 (2), 140.5 (2), 135.1, 125.9 (2), 121.0 (2), 117.9 (2), 115.7, 114.4 (2), 113.8 (2), 112.7 (2), 97.8, 53.1. HRMS $(M + H)^+$ calcd for $C_{29}H_{25}N_2O_{10}$: 561.1509. Found: 561.1502.

Methyl 1-(4-Fluorobenzyl)-1*H*-pyrrole-2-carboxylate (25). To a suspension of 60% sodium hydride (2.4 g, 0.06 mol) in DMF (100 mL) was slowly added methyl 2-pyrrolecarboxylate (6.4 g, 0.05 mol). The mixture was stirred for 30 min, and *p*-fluorobenzyl bromide (10.7 g, 0.055 mol) was added dropwise. The reaction mixture was stirred overnight, poured into 300 mL of water, extracted with ethyl acetate, and dried. Evaporation gave an oil that was distilled (Kugelrhor) to give 11 g (94%) of the product: ^{1}H NMR ($CDCl_3$) δ 7.10−6.88 (m, 6H), 6.19−6.17 (m, 1H), 5.51 (s, 2H), 3.76 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.69, 160.44, 161.41, 133.99, 133.95, 128.85, 128.57, 128.46, 121.76, 118.45, 115.57, 115.28, 108.60, 51.29, 51.03.

1-(4-Fluorobenzyl)-1*H*-pyrrole-2-carboxylic Acid (26). To methanol (15 mL) was added sodium hydroxide (1.1 g, 27 mmol) dissolved in 12.5 mL of water. To this solution was added the pyrrole ester (**25**) (2.3 g, 10 mmol), and the mixture was refluxed for 3 h. The methanol was evaporated, and the aqueous phase was extracted with ether. The aqueous phase was acidified with 3 N HCl to give, after filtration, 1.93 g (88%) of a white solid: mp 122−124 °C; ^{1}H NMR ($CDCl_3$) δ 7.16−6.92 (m, 6H), 6.23−6.21 (m, 1H), 5.50 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 165.99, 163.95, 133.84, 130.26, 128.91, 128.80, 121.23, 120.81, 115.80, 115.52, 109.30, 51.62.

Bis(diphenylmethyl) 2,3-Bis(*N*-(*p*-fluorobenzyl)-2-pyrroloyl)-L-tartrate (27) and Bis(diphenylmethyl) 2-(*N*-(*p*-Fluorobenzyl)-2-pyrroloyl)-L-tartrate (28). To a solution of dichloromethane (50 mL) containing the pyrrole acid (**26**) (0.69 g, 3.1 mmol) and DPM tartrate (1.5 g, 3.1 mmol) were added DCC (0.64 g, 3.1 mmol) and 40 mg of DMAP. The mixture was stirred overnight, filtered, and flash chromatographed (dichloromethane/petroleum ether, 60:40 to 80:20). There was obtained 0.18 g (7%) of **27** as a foam and 1.07 g (51%) of **28** as a foam. **27**: : ^{1}H NMR ($CDCl_3$) δ 7.31−6.83 (m, 32H), 6.12−6.10 (m, 2H), 6.04 (s, 2H), 5.40 (dd, 2H, J = 23.5, 15.2 Hz); ^{13}C NMR ($CDCl_3$) δ 165.39, 163.75, 160.50, 159.07, 139.00, 138.88, 133.51, 133.46, 129.49, 128.93, 128.82, 128.47, 128.23, 128.06, 127.82, 127.57, 127.02, 127.00, 126.52, 120.52, 120.49, 115.63, 115.35, 109.21, 78.98, 70.80, 51.17. **28**: ^{1}H NMR ($CDCl_3$) δ 7.37−6.82 (m, 28H), 6.16−6.13 (m, 1H), 5.75 (d, 1H, J = 2.64 Hz), 5.27 (m, 2H), 4.97 (d, 1H, J = 7.48 Hz), 3.07 (d, 1H, J = 7.48 Hz); ^{13}C NMR ($CDCl_3$) δ 170.04, 166.02, 163.70, 160.43, 159.06, 139.28, 138.67, 138.61, 133.57, 133.53, 129.58, 128.63, 128.66, 128.56, 128.47, 128.41, 128.33, 128.09, 128.01, 127.93, 127.22, 127.18, 127.09, 126.91, 126.53, 120.53, 120.38, 115.63, 115.34, 109.19, 79.42, 78.82, 72.84, 70.79, 51.18.

The reaction was repeated with **26** (1.1 g, 5 mmol), DPM tartrate (4.8 g, 10 mmol), DCC (1.1 g, 5.5 mmol), 80 mg of DMAP in 75 mL dichloromethane to give 2.81 g (82%) of **28**.

2,3-Bis(*N*-(*p*-fluorobenzyl)-2-pyrroloyl)-L-tartaric Acid (8). To a solution of dipyrroloyl DPM tartrate (**27**) (0.18 g, 0.2 mmol) in absolute ethanol (15 mL) was added 30 mg of 10% Pd/C. The mixture was stirred under a blanket of hydrogen overnight. The mixture was filtered through Celite, and the solvent was evaporated. The residue was dissolved in ethyl acetate and extracted with aqueous sodium bicarbonate. The aqueous phase was acidified with 3 N HCl, extracted with ethyl acetate, and dried. Evaporation gave 0.11 g (99%) of a clear gum: ^{1}H NMR ($CDCl_3$) δ 8.60 (br s, 2H), 7.12−6.84 (m, 12H), 6.14−6.12 (m, 2H), 5.86 (s, 2H), 5.43 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 170.99, 163.94, 160.68), 159.27, 133.55, 133.50, 130.08, 129.07, 128.96, 120.66, 120.39, 115.77, 115.48, 109.40, 70.19, 51.46. HRMS $(M + H)^+$ calcd for $C_{28}H_{23}O_8N_2F_2$: 553.142 25. Found: 553.144 50.

2-(*N*-(*p*-Fluorobenzyl)-2-pyrroloyl)-L-tartaric Acid (7). (A) To a solution of pyrroloyl DPM tartrate (**28**) (0.66 g, 0.97 mmol) in absolute ethanol (30 mL) was added 70 mg of 10% Pd/C. The mixture was stirred under a blanket of hydrogen overnight. The mixture was filtered through Celite, and the solvent was evaporated. The residue was dissolved in ethyl acetate and extracted with aqueous sodium bicarbonate. The aqueous phase was acidified with 3 N HCl, extracted with ethyl acetate, and dried. Evaporation gave 0.32 g (94%) of a white solid: mp 169−171 °C; ^{1}H NMR (DMSO-d_6) δ 7.34−7.05 (m, 6H), 6.22−6.19 (m, 1H), 5.84 (br s, 1H), 5.49 (s, 2H), 5.33 (d, 1H, J = 2.6 Hz), 4.60 (d, 1H, J = 2.35 Hz); ^{13}C NMR (DMSO-d_6) δ 172.07, 168.60, 163.17, 159.91, 159.38, 134.82, 130.53, 129.22, 129.11, 120.22, 119.64, 115.39, 115.10, 108.67, 73.07, 70.22, 50.17. HRMS $(M + H)^+$ calcd for $C_{16}H_{15}O_7NF$: 352.083 23. Found: 352.084 80.

Bis(diphenylmethyl) 2-(3,4-Di(*tert*-butyldimethylsilyloxy)cinnamoyl)-3-(*N*-(*p*-fluorobenzyl)-2-pyrroloyl)-L-tartrate (29). To a solution of the disilylcaffeic acid **30**[32] (0.51 g, 1.25 mmol) and pyrroloyl DPM tartrate **28** (0.82 g, 1.2 mmol) in dichloromethane (30 mL) were added DCC (0.27 g, 1.3 mmol) and 70 mg of DMAP. The mixture was stirred overnight, refluxed for 4 h, filtered, and evaporated to dryness. Flash chromatography (petroleum ether/dichloromethane, 60:40 to 40:60) gave 0.86 g (67%) of the product as a foam: ^{1}H NMR ($CDCl_3$) δ 7.48 (d, 1H, J = 15.83 Hz), 7.38−6.83 (m, 31H), 6.15−6.13 (m, 1H), 6.06 (s, 2H), 5.32 (s, 2H), 1.02 (s, 9H), 1.01 (S, 9H), 0.26 (s, 6H), 0.23 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 165.82, 165.29, 160.59, 159.13, 149.88, 147.20, 146.71, 139.03, 138.98, 138.89, 133.58, 129.59), 128.98, 128.88, 128.57, 128.54, 128.29, 128.18, 128.13, 127.87, 127.76, 127.31, 127.15, 127.08, 127.01, 126.57, 122.77, 121.12, 120.75, 120.44, 115.66, 115.37, 113.69, 109.25, 79.08, 79.01, 71.10, 70.68, 51.25, 25.94, 25.92, 18.58, 18.49, −3.97, −4.02.

2-Caffeoyl-3-(*N*-(*p*-fluorobenzyl)-2-pyrroloyl)-L-tartaric Acid (9). A suspension of **29** (0.86 g, 0.8 mmol) in 35 mL of 70% acetic acid was refluxed for 10 h. The solvents were evaporated, taken up in ethyl acetate, and extracted with aqueous sodium bicarbonate. The aqueous layer was acidified with concentrated HCl, extracted with ethyl acetate, and dried. Evaporation gave 0.45 g of a gum which was flash chromatographed (dichloromethane/methanol/acetic

acid, 95:4:1) to give 0.32 g (78%) of a thick gum. ^{1}H NMR (CD_3OD) δ 7.61 (d, 1H, J = 15.83 Hz), 7.24−6.76 (m, 9H), 6.32 (d, 1H, J = 15.83 Hz), 6.20 (m, 1H), 5.80 (s, 2H), 5.55 (s, 2H); ^{13}C NMR (CD_3OD) δ 169.82, 167.76. 164.83, 162.40, 161.00, 149.99, 148.51, 146.89, 135.91, 135.88, 131.45, 130.35, 130.26, 127.54, 123.30, 121.89, 121.21, 116.54, 116.33, 116.12, 115.26, 113.72, 110.04, 72.59, 72.30, 52.05. HRMS $(M + H)^+$ calcd for $C_{25}H_{21}O_{10}NF$: 514.1137. Found: 514.1144.

Bis(diphenylmethyl) 2-(3,4-Di(*tert*-butyldimethylsilyloxy)cinnamoyl-3-hydroxy-L-tartrate (31). To a solution of the disilylcaffeic acid (**30**)[32] (2.25 g, 5.5 mmol) and DPM tartrate (6.6 g, 13.75 mmol) in dichloromethane (120 mL) were added DCC (1.25 g, 6.1 mmol), DMAP (0.34 g, 2.75 mmol), and 120 mg of dry tosic acid. The mixture was stirred overnight, filtered, and evaporated to dryness. Flash chromatography (petroleum ether/ethyl acetate, 95:5 to 80:20) gave, along with recovered DPM tartrate, 4.1 g (85%) of **31** as a foam: ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 1H, J = 15.83), 7.37−6.94 (m, 24H), 6.83 (d, 1H, J = 8.80 Hz), 6.03 (d, 1H, J = 16.27), 5.79 (d, 1H, J = 2.20 Hz), 5.01 (dd, 1H, J = 2.20 and 7.48 Hz), 3.22 (d, 1H, J = 7.48 Hz), 1.01 (S, 9H), 1.00 (s, 9H), 0.25 (s, 6H), 0.23 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.00, 166.00, 165.71, 149.89, 147.22, 146.58, 139.26, 139.16, 138.68, 128.69, 128.58, 128.49, 128.42, 128.27, 128.31, 128.08, 127.71, 127.34, 127.19, 127.15, 127.12, 122.81, 121.11, 120.53, 113.67, 79.52, 78.92, 73.06, 70.73, 25.94, 25.90, 18.55, 18.48, −4.02.

Caftaric Acid (10). A suspension of **31** (1.42 g, 1.6 mmol) in 70 mL of 70% acetic acid was refluxed for 10 h and lyophilized to dryness. The residue was dissolved in ethyl acetate and extracted with aqueous sodium bicarbonate. The aqueous layer was acidified with HCl to ~pH 2, extracted with ethyl acetate, dried, and evaporated to give 0.48 (96%) of **10** as a thick gum: ^{1}H NMR (CD_3OD) δ 7.68 (d, 1H, J = 15.83 Hz), 7.08 (d, 1H, J = 2.20 Hz), 6.98 (dd, 1H, J = 2.20, 8.36 Hz), 6.79 (d, 1H, J = 8.36 Hz), 6.33 (d, 1 h, J = 15.83 Hz), 5.56 (d, 1H, J = 2.20 Hz), 4.79 (d, 1H, J = 2.20 Hz); ^{13}C NMR (CD_3OD) δ 173.54, 170.38, 167.82, 149.67, 148.09, 146.67, 127.47, 123.09, 116.32, 114.95, 113.78, 74.54, 71.49.

(4*R*,5*R*)-2,2-Dimethyl-4,5-dicyano-1,3-dioxolane (33). A solution of the dicarbamoyldioxolane **32**[35] (8.6 g, 46.2 mmol) and *p*-toluenesulfonyl chloride (35.25 g, 185 mmol) in 100 mL of pyridine was heated at 100 °C for 3.5 h. The oil bath was turned off, and the reaction mixture was allowed to slowly cool overnight. The solvent was evaporated in vacuo, and water was added. The aqueous phase was extracted with 5 × 100 mL of ether. The ether was washed with 10% KOH and brine, then dried over $MgSO_4$. The ether was filtered and decolorized with carbon. Evaporation of the solvent gave a tan solid. The ^{1}H NMR showed the presence of two compounds, which was confirmed by TLC (petroleum ether/ethyl acetate, 60:40). Flash chromatography gave 2.73 g (46%) of **33**: mp 155−157 °C (lit.[35] 162 °C); ^{1}H NMR ($CDCl_3$) 5.13 (s, 2H), 1.62 (s, 6H); ^{13}C NMR ($CDCl_3$) 117.32, 115.38, 67.37, 26.25.

Also isolated was 1.18 g (15%) of (4*R*,5*R*)-2,2-dimethyl-4-carbamoyl-5-cyano-1,3-dioxolane (**36**). Recrystallization from water gave a white solid: mp 150−151 °C; ^{1}H NMR ($CDCl_3$) δ 6.43 (s, 1H), 5.81 (s, 1H), 5.09 (d, 1H, J = 3.96 Hz), 4.83 (d, 1H, J = 3.96 Hz), 1.62 (s, 3H), 1.54 (s, 3H); ^{13}C NMR ($CDCl_3$) 170.52, 117.54, 78.80, 26.64, 25.59. When the reaction was repeated for 5.5 h with a 5:1 molar ratio of *p*-toluenesulfonyl chloride to **32**, the only product was **33** by TLC, while a 2.2:1 molar ratio for 3.5 h gave a 64% yield of **36** along with only 12% of **33**.

(1*R*,2*R*)-Dihydroxy-1,2-bis(5-tetrazoyl)ethane (34). To 60 mL of DMF was added the dicyanodioxolane **33** (2.73 g, 18 mmol), ammonium sulfate (4.75 g, 36 mmol), and sodium azide (4.68 g, 72 mmol). The mixture was heated at 90 °C for 8 h. The DMF was removed in vacuo, and the residue was dissolved in water. The water was acidified with concentrated HCl to approximately pH 2. Upon concentration of the water, crystallization occurred. Filtration and air-drying gave 3.2 g (77%) of (4*R*,5*R*)-2,2-dimethyl-4,5-bis(5-tetrazolyl)-1,3-dioxolane which upon recrystallization from water gave white needles: mp 215−216 °C; ^{1}H (DMSO-d_6) δ 5.85 (s, 2H), 1.51 (s, 6H); ^{13}C (DMSO-d_6) δ 154.80, 112.59, 72.17, 26.32. The above compound (3 g, 12.6 mmol) was added to 125 mL of 2 N HCl and the mixture heated briefly to about 50 °C to bring about solution and then stirred at room temperature for 24 h. Filtration of the white solid gave 2.34 g (94%) of **34** which upon recrystallization from ethanol gave a white solid: mp 320 °C dec; ^{1}H NMR (DMSO-d_6) δ 6.52 (br s, 2H), 5.34 (d, 2H, J = 3.1 Hz); ^{13}C NMR (DMSO-d_6, 5 s delay) δ 156.73, 67.67. Anal. Calcd for $C_4H_6N_8O_2$: C, 24.25; H, 3.05; N, 56.55. Found: C, 24.41; H, 3.30; N, 56.44.

(1*R*,2*R*)-Dihydroxy-1-carbamoyl-2-(5-tetrazolyl)ethane (37). To 40 mL of DMF was added the cyanoamide dioxolane **36** (1.95 g, 11.46 mmol), ammonium sulfate (1.52 g, 11.46 mmol), and sodium azide (1.5 g, 22.92 mmol). The mixture was heated to 95 °C for 4 h. The solvent was evaporated in vacuo and the residue taken up in water. The mixture was acidified and exhaustively extracted with ethyl acetate. Drying and evaporation gave 2.34 g (96%) of (4*R*,5*R*)-2,2-dimethyl-4-carbamoyl-5-(5-tetrazolyl)-1,3-dioxolane as a viscous syrup. ^{1}H NMR (DMSO-d_6) δ 7.60 (d, 2H, J = 10.3 Hz), 5.47 (d, 1H, J = 6.7 Hz), 4.77 (d, 1H, J = 6.7 Hz), 1.48 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 170.44, 112.23, 78.35, 70.78, 26.41, 26.00. The tetrazole carbon was hidden in the baseline. The above compound (1.77 g, 8.3 mmol) and 30 mL of 2 N HCl were subjected to the same procedure used to prepare **34** to give **37** as a white solid, 0.46 g (33%): mp 202−203 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.42 (s, 1H), 7.33 (s, 1H), 6.20 (br s, 1H), 5.61 (br s, 1H), 5.35 (d, 1H, J = 2.10 Hz), 4.13 (d, 1H, J = 2.34 Hz); ^{13}C (DMSO-d_6) δ 173.19, 73.89, 66.39.

(1*R*,2*R*)-Dicaffeoyloxy-1,2-bis(5-tetrazolyl)ethane (13). To 50 mL of dry pyridine containing 2.5 mL of triethylamine was added the dihydroxy-bis-tetrazole **34** (0.99 g, 11 mmol). To this mixture was added dropwise freshly prepared 3,4-dimethoxycarbonylcaffeoyl chloride (3.46 g, 11 mmol)[23] dissolved in 25 mL of dry benzene. The mixture was allowed to stir overnight. The solvent was evaporated, and the residue was taken up in water. The mixture was acidified and extracted with ethyl acetate. Drying and evaporation gave 3.4 g (95%) of (1*R*,2*R*)-di-3,4-dimethoxycarbonylcaffeoyloxy-1,2-bis(5-tetrazolyl)ethane (**35**) as a tan foam. This material was dissolved in 45.6 mL of 1 N NH_4OH and stirred for 3.5 h. The mixture was acidified with 1 N HCl to give a waxy solid that was extracted with ethyl acetate, dried, and evaporated to give 2.2 g (92%) of a tan foam. The foam was recrystallized from MeOH−H_2O to give **13** as a pale yellow solid: mp 243−245 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.85 (s, 2H), 9.37 (s, 2H), 7.56 (d, 2H, J = 15.83 Hz), 7.06 (d, 2H, J = 2.20 Hz), 7.03 (dd, 2H, J = 8.25, 2.20 Hz), 6.86 (s, 2H), 6.77 (d, 2H, J = 8.35 Hz), 6.30 (d, 2H, J = 15.83 Hz); ^{13}C (DMSO-d_6) δ 165.10, 149.05, 147.57, 145.66, 125.14, 121.93, 115.82, 115.12, 111.87, 65.82. The C=N carbon is hidden in the baseline. Anal. Calcd for $C_{22}H_{18}N_8O_8 \cdot 1.5H_2O$: C, 48.09; H, 3.85; N, 20.40. Found: C, 48.26; H, 3.62; N, 20.24. HRMS $(M + H)^+$ calcd for $C_{22}H_{19}O_8N_8(MH^+)$: 523.1320. Found: 523.1333.

(1*R*,2*R*)-Dicaffeoyloxy-1-carbamoyl-2-(5-tetrazolyl)ethane) (12). To 30 mL of dry pyridine containing 1.5 mL of triethylamine was added 519 mg (3 mmol) of **37**. To this mixture was added dropwise freshly prepared 3,4-dimethoxycarbonylcaffeoyl chloride[23] (1.95 g, 6.6 mmol) dissolved in 25 mL of dry benzene. The mixture was allowed to stir overnight. The solvent was evaporated and the residue taken up in water. The mixture was acidified and extracted with a large volume of ethyl acetate. Drying and evaporation gave 2.17 g (99%) of **38** as a tan powder, which, without further purification, was dissolved in 40 mL of 10% sodium carbonate and stirred overnight. The mixture was acidified and extracted with ethyl acetate. Drying and evaporation gave 1.2 g (82%) of a tan foam. Recrystallization from acetonitrile−chloroform gave **12** as a tan foam: mp 185 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.79 (s, 1H), 9.70 (s, 1H), 9.22 (s, 2H), 7.88 (s, 1H), 7.62 (d, 1H, J = 15.2 Hz), 7.59 (s, 1H), 7.47 (d, 1H, J = 15.5 Hz), 7.05 (m, 4H), 6.80 (m, 3H), 6.34(d, 1H,

J = 16.1 Hz), 6.27(d, 1H, J = 16.1 Hz), 5.60 (d, 1H, J = 2.3 Hz); ^{13}C (DMSO-d_6) δ 167.1, 165.4, 149.0, 148.8, 147.4, 146.5, 145.71(2), 145.67, 125.4, 125.2, 121.9, 121.8, 115.9, 115.1, 114.9, 112.8, 112.2, 72.6, 66.3. The C=N carbon is hidden in the baseline. HRMS $(M + H)^+$ calcd for $C_{22}H_{20}O_9N_5(MH^+)$: 498.1256. Found: 498.1261.

(*R*,*S*)-5(1,2-Dicaffeoyloxy-eth-1-yl)tetrazole (11). To a solution of 696 mg (5.35 mmol) of dihydroxyethyltetrazole **42**[39] in 40 mL of dry pyridine containing 2 mL of triethylamine was added dropwise freshly prepared 3,4 dimethoxycarbonylcaffeoyl chloride[23] (3.68 g, 11.7 mmol) in 30 mL of dry benzene. The mixture was stirred overnight, the solvent evaporated, and the residue taken up in water which was acidified and extracted with ethyl acetate. Drying, decolorization, and evaporation gave 3.4 g (92%) of **43** as a tan foam that, without further purification, was dissolved in 50 mL of 10% sodium carbonate and stirred overnight. The dark solution was acidified and extracted with a large volume of ethyl acetate to give 1.3 g of an insoluble dark brown solid in addition to the ethyl acetate phase which was dried, decolorized, and evaporated to give 1.1 g of a yellow foam which after flash chromatography (petroleum ether/ethyl acetate, 70:30, to ethyl acetate, 100%) gave 0.6 g of **11** as a pale yellow foam: ^{1}H NMR (DMSO-d_6) δ 9.74 (s, 1H), 9.68 (s, 1H), 9.22 (s, 1H), 9.19 (s, 1H), 7.60 (d, 1H, J = 15.83 Hz), 7.47 (d, 1H, J = 15.83 Hz), 7.06 (m, 4H), 6.78 (d, 1H, J = 7.91 Hz), 6.76 (d, 1H, J = 7.92 Hz), 6.51 (m, 1H), 6.37 (d, 1H, J = 15.83 Hz), 6.25 (d, 1H, J = 15.83 Hz), 4.70 (m, 2H); ^{13}C (DMSO-d_6) δ 166.06, 165.50, 148.91, 148.69, 147.11, 146.21, 145.65, 145.61, 125.30(2), 121.86, 121.65, 115.81, 115.07, 114.98(2), 112.94, 112.47, 64.50, 63.11. The C=N bond is hidden in the baseline. Anal. Calcd. for $C_{21}H_{18}N_4O_8 \cdot 2H_2O$: C, 51.43; H, 4.52; N, 11.43. Found: C, 51.85; H, 4.18; N, 11.55. HRMS $(M + H)^+$ calcd for $C_{21}H_{19}O_8N_4(MH^+)$: 455.120 27. Found: 455.119 70. A solution of the above ethyl acetate insoluble residue in ammonium hydroxide solution was acidified after 3.5 h, extracted with ethyl acetate and the resulting solution, dried, decolorized, and evaporated to give 0.5 g of a brown foam with a proton NMR identical to that of **11**. Dissolution in methanol, slow evaporation and cooling gave a gum, a methanol solution of which was allowed to evaporate over several days to give a tan powder: mp 192−194 °C effervescent and dec. Total weight of product from both portions was 1.1 g (49%).

3,4,5-Tris(*tert*-butyldimethylsilyloxy)cinnamic Acid (40). To 15 mL of pyridine containing 3 drops of piperidine was added 3,4,5-tris(*tert*-butyldimethylsilyloxybenzaldehyde (**39**)[40] (6 g, 12.07 mmol) and malonic acid (2.5 g, 24.10 mmol). The mixture was heated at 85 °C for 6 h, cooled to room temperature, and allowed to stir overnight. The mixture was added to 150 mL of 3 N HCl and stirred until the resulting oil crystallized. The aqueous phase was decanted, extracted with ether, and combined with the solid. The ethereal organic phase was washed with water and dried with $MgSO_4$. Evaporation of the ether gave 4.2 g (70%) of a solid which was recrystallized from 95% ethanol: white crystals, mp 175−176 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (d, 1H, J = 15.83 Hz), 6.72 (s, 2H), 6.20 (d, 1H, J = 16.27 Hz), 0.99 (s, 9H), 0.95 (s, 18H), 0.23 (s, 12H), 0.14 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 172.58, 148.99(2), 147.38, 141.53, 126.25, 114.84, 114.52(2), 26.16, 26.13, 18.81, 18.47, −3.61, −3.91.

(1*R*,2*R*)-Bis(3,4,5-tris(*tert*-butyldimethylsilyloxy)cinnamoyloxy)-1,2-bis(5-tetrazolyl)ethane (41). To 40 mL of dry pyridine containing 2 mL of triethylamine was added the dihydroxy-bis-tetrazole **34** (0.67 g, 3.4 mmol). To this mixture was added dropwise 3,4,5-tri-*tert*-butyldimethylsilyloxycinnamoyl chloride (3.79 g, 6.8 mmol) (freshly prepared from thionyl chloride) dissolved in 15 mL of dry benzene. The mixture was allowed to stir overnight, the solvent evaporated, and the residue diluted with 3 N HCl, extracted with ethyl acetate, and dried with $MgSO_4$. Evaporation gave a light red-orange foam that was flash chromatographed (petroleum ether/EtOAc, 98:2 to 60:40) to give 1.7 g (27%) of a light orange foam that was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.60 (d, 2H, J = 15.83 Hz), 7.16 (s, 2H), 6.69 (s, 4H), 6.14 (d, 2H, J = 16.27 Hz), 0.97 (s, 9H), 0.92 (s, 18H), 0.20 (s, 12H), 0.11 (s, 6H); ^{13}C NMR 400 MHz ($CDCl_3$) δ 166.28. 149.03, 148.83(2), 142.03, 125.56, 114.63(2), 112.14, 63.59, 26.14, 26.10, 18.76, 18.47, −3.60, −3.92.

(1*R*,2*R*)-Di(3,4,5-trihydroxycinnamoyloxy)-1,2-bis(5-tetrazolyl)ethane (14). To the silyloxy-bis-tetrazole **41** (1.7 g, 1.37 mmol) in 40 mL of THF was added dropwise tetrabutylammonium fluoride (2.37 g, 9 mmol) in 10 mL of THF. The dark reaction mixture was stirred for 2 h at room temperature, quenched with 10 mL of 10% HCL and 40 mL of water, extracted with ethyl acetate, and dried with $MgSO_4$. Evaporation gave a foam that was flash chromatographed (acetone/petroleum ether, 60:40, to acetone, 100%). The product, 0.37 g (49%), was obtained as a light yellow glass. Recrystallization from methanol−water gave a tan powder: mp 228−230 °C dec with gas evolution; ^{1}H NMR (DMSO-d_6) δ 9.20 (br s, 4H), 8.94 (br s, 2H), 7.47 (d, 2H, J = 15.83 Hz), 6.83 (s, 2H), 6.62 (4H), 6.21 (d, 2H, J = 15.83 Hz); ^{13}C NMR (DMSO-d_6) δ 164.96, 147.96, 146.18(2), 137.17, 123.96, 111.84, 107.98(2), 65.90. HRMS $(M + H)^+$ calcd for $C_{22}H_{19}O_8N_8$: 555.1219. Found: 555.1212.

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