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Design, synthesis, and biological evaluation of chicoric acid analogs as inhibitors of HIV-1 integrase

Trevor T. Charvat,[a,†] Deborah J. Lee,[b,†] W. Edward Robinson[b,c] and A. Richard Chamberlin[a,c,*]

[a]*Department of Chemistry, University of California, Irvine, CA 92697, USA*
[b]*Department of Pathology and Laboratory Medicine, University of California, Irvine, CA 92697, USA*
[c]*Chao Family Comprehensive Cancer Center, UC Irvine Medical Center, Orange, CA 92868, USA*

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Abstract—A series of analogs of the potent HIV-1 integrase (HIV IN) inhibitor chicoric acid (CA) was designed with the intention of ameliorating some of the parent natural product's undesirable properties, in particular its toxicity, instability, and poor membrane permeability. More than 70 analogs were synthesized and assayed for three types of activity: (1) the ability to inhibit 3′-end processing and strand transfer reactions using recombinant HIV IN in vitro, (2) toxicity against the CD4+ lymphoblastoid cell line, MT2, and (3) anti-HIV activity against HIV_{LAI}. CA analogs lacking one of the carboxyl groups of CA and with 3,4,5-trihydroxycinnamoyl sidechains in place of the caffeoyl group of CA exhibited the most potent inhibition of HIV replication and end-processing activity. Galloyl-substituted derivatives also displayed very potent in vitro and in vivo activities, in most cases exceeding the inhibitory effects of CA itself. Conversely, analogous monocarboxy caffeoyl analogs exhibited only modest inhibition, while the corresponding 3,4-dihydroxybenzoyl-substituted compounds were devoid of activity.

1. Introduction

Human immunodeficiency virus (HIV) integrase (IN) is one of three enzymes required by HIV to infect a host cell. The other two, reverse transcriptase and protease, are presently exploited as antiviral targets. Although highly active antiretroviral therapy (HAART) regimens combining three or more RT or protease inhibitors have proven to effectively suppress uncontrolled viral replication, drug resistance and drug-induced side-effects can hinder complete viral suppression. It thus follows that the identification of an inhibitor of HIV IN would add a valuable third component to the antiviral armamentarium. HIV IN is an attractive target because there is no known homolog in human cells, potentially minimizing the side-effects associated with other antiviral agents.

Integrase is an essential viral enzyme which catalyzes the covalent joining of the viral genome to the host chromosome to generate the fully integrated provirus. Integrase first catalyzes the removal of two nucleotides from the 3′-end of each viral long terminal repeat, a reaction termed '3′-end processing.' Next, the free 3′-hydroxyls undergo nucleophilic attack on the host DNA, a reaction termed 'strand transfer.' Both of these reactions can be quantified in vitro using recombinant IN and oligonucleotides homologous to the viral long terminal repeats. Additionally, IN can catalyze the reversal of the integration reaction in vitro, a reaction termed 'disintegration.'

L-Chicoric acid (CA) (**1**) is a potent and selective inhibitor of HIV IN. It exhibits IC_{50} values of 100–500 nM for the end-processing/strand transfer reactions and 100–200 nM for the disintegration reactions, an ED_{50} concentration of 1–2 μM, and a CT_5 value of >200 μM, respectively (Fig. 1).[1] Furthermore, the CA scaffold is easily amenable to analog synthesis in order to determine the structure–activity relationships with HIV IN. Indeed, numerous SAR studies have been conducted and these investigations have revealed several structural characteristics requisite for inhibitory activity and the types of modifications that are tolerated.[1–6]

Keywords: HIV integrase; Chicoric acid; SAR.
* Corresponding author. Tel./fax: +1 949 824 7089; e-mail: archambe@uci.edu
† These authors contributed equally to this work.

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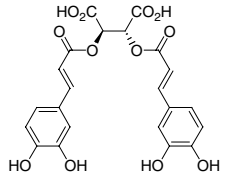
L-Chicoric Acid (CA)



1

Bioactivity
ED_{50} = 4.2 μM
CT_5 = 264 μM
IC_{50} (End Processing) = 100-500 nM
IC_{50} (Disintegration) = 100-200 nM

Figure 1. Structure of L-CA and its bioactivity.

These structure–activity relationship analyses indicate that at least one carboxylic acid moiety and a bisphenol {as caffeoyl (3,4-dihydroxycinnamoyl) or galloyl (3,4,5-trihydroxybenzoyl)} are necessary for anti-HIV activity (Fig. 2). Removal of the carboxylic acids or replacement with either cyclic or acyclic alkyl groups resulted in comparable in vitro HIV IN inhibition, but no antiviral activity.[4] Derivatives of glyceric acid, serine, isoserine, and β-aminoalanine (which contain only one carboxylic acid group) were shown to exhibit anti-HIV IN and antiviral activity comparable to CA. These analogs also revealed that conversion of the diester to the mono- and diamide did not compromise HIV-IN or antiviral inhibition.[1,4] Furthermore, replacing the acid with the corresponding methyl ester resulted in comparable anti-HIV IN activity; however, the antiviral inhibition by these analogs was not examined.[4] The length of the linker unit between the heteroatoms has also been briefly examined. Utilization of lysine in lieu of β-aminoalanine, which increased the linker length from two to four carbons, resulted in a 10-fold less potent compound with comparable toxicity.[1]

Substitution of the biscatechol moiety has also been explored. Converting the biscatechol into the corresponding tetramethyl ether[4] or replacement with thiophene[1] moieties gave completely inactive compounds. Conversion into the tetraacetate, on the other hand, has led to varied results. Pommier and co-workers reported that CA tetraacetate analogs exhibit potent inhibition against HIV IN, but low to moderate antiviral activity.[4] Conversely, Reinke et al. reported that CA tetraacetate displayed significantly decreased HIV IN inhibition relative to CA and little antiviral activity.[1] The antiviral

Required	Tolerated Modifications
1 Acid *Catechol*	Tartaric Acid Stereochemistry Ester linker (Amide OK) Aryl moiety (Galloyl OK)

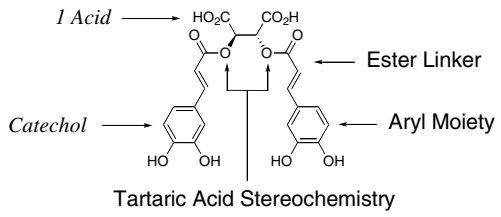


Figure 2. Chicoric acid SAR.

activity exhibited has been attributed to inhibition of viral entry, rather than integrase inhibition, although resistance testing and real-time polymerase chain reaction suggest IN is being inhibited in vivo.[1,7–11] Conversely, replacement of the caffeoyl groups with galloyl moieties led to analogs with enhanced potency, but also increased toxicity.[3,4,12] Finally, activity is insensitive to the stereochemistry of the tartaric acid subunit: (−)-L, (+)-D-, and *meso*-CA all exhibit comparable HIV-IN and antiviral activity.[3,4] Based on these SAR data, there should be considerable latitude in designing CA analogs with improved pharmacological properties, particularly those with modifications of the tartaric acid core region. Identifying surrogates for the catechol groups will be a greater challenge, but the fact that the corresponding galloyl derivatives are active, despite a substantial difference in structure between gallic acid and caffeic acid, is encouraging from that perspective as well.

Although CA and its analogs exhibit potent and selective activity toward HIV IN, the compounds possess several structural characteristics that render them quite poor drug candidates. An analysis of basic medicinal chemistry principles (i.e., Lipinski's 'Rule of 5,'[13] Veber's bioavailability criteria,[14] etc.) reveals that CA is a rather poor drug candidate for several reasons: (1) its limited cell permeability (due to the diacid moiety), (2) the anticipated lability of its two ester linkages toward hydrolytic enzymes, (3) the potential toxicity that is generally associated with catechol groups, of which there are two, and (4) the relatively large number of rotatable bonds that might limit oral bioavailability (according to the rules recently published by Veber) (Fig. 3).[14] On the other hand, the very potent CA is an excellent lead compound for optimization because there are readily apparent potential solutions to each of these problems, respectively: (1) replace the carboxyl groups with precedented surrogates, for example, esters, (2) substitute more robust linkages, such as amides or ethers, for the ester groups, (3) identify catechol surrogates by generating and screening libraries of CA analogs with a variety of heterocyclic rings in place of the catechol groups, and (4) synthesize conformationally constrained analogs (or libraries of analogs) that restrict or prevent rotation around otherwise freely rotating single bonds in the parent compound. Finally, the relatively simple structure of CA, and the resultant synthetic accessibility of analogs, is a further plus as a lead compound. In this paper, we present our progress toward expanding the SAR around the CA scaffold while concurrently increasing the membrane permeability and linker stability of CA.

2. Chemistry

2.1. Synthesis of chicoric acid analogs with increased stability

Synthesis of the diamido CA analogs began with a thionyl chloride-mediated Fisher esterification of the four diamino acids: (DL)-2,3-diaminopropanoic acid (**2**), (DL)-2,4-diaminobutanoic acid (**3**), (DL)-ornithine (**4**), and (L)-lysine (**5**); followed by EDCI mediated biscou-

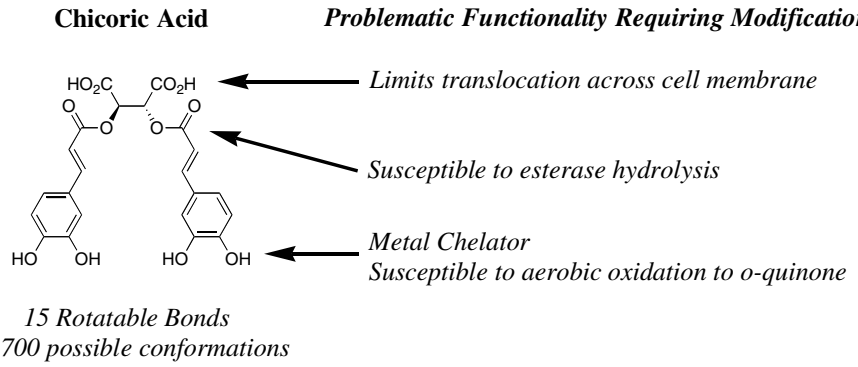
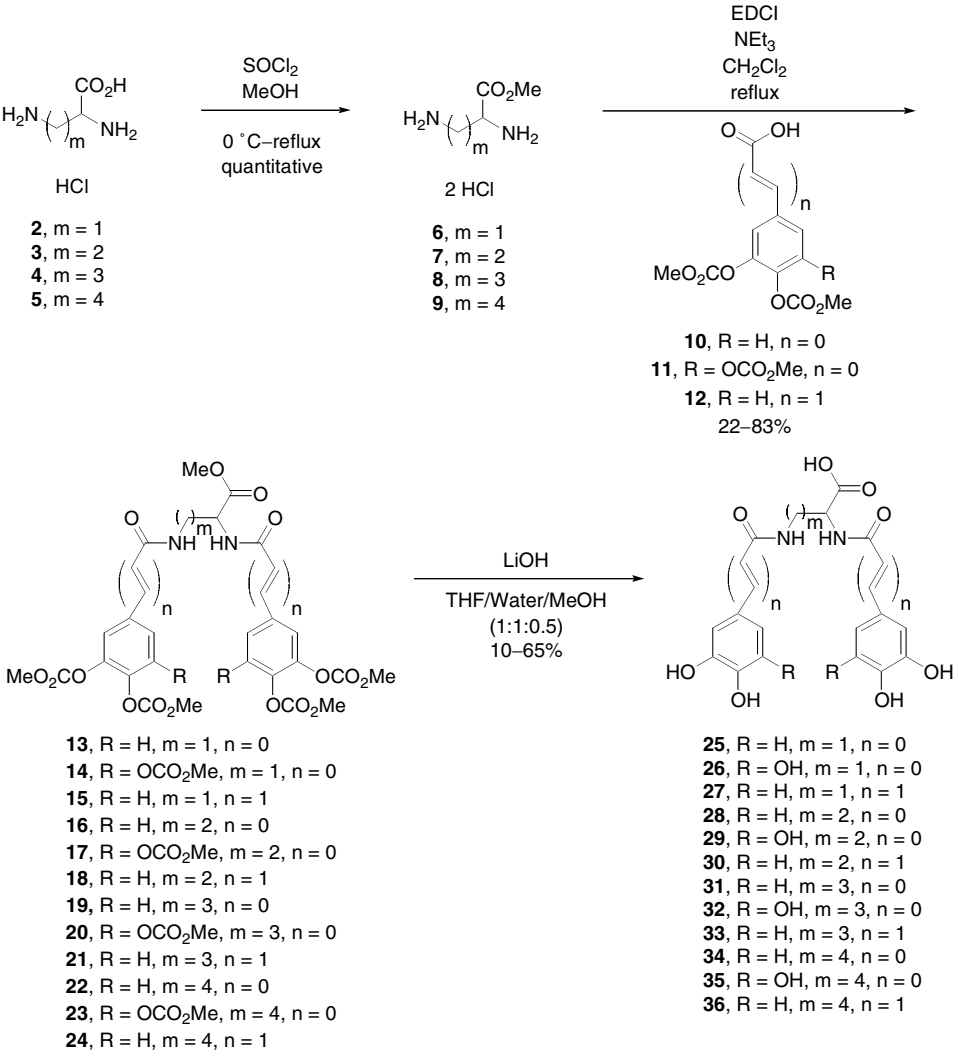


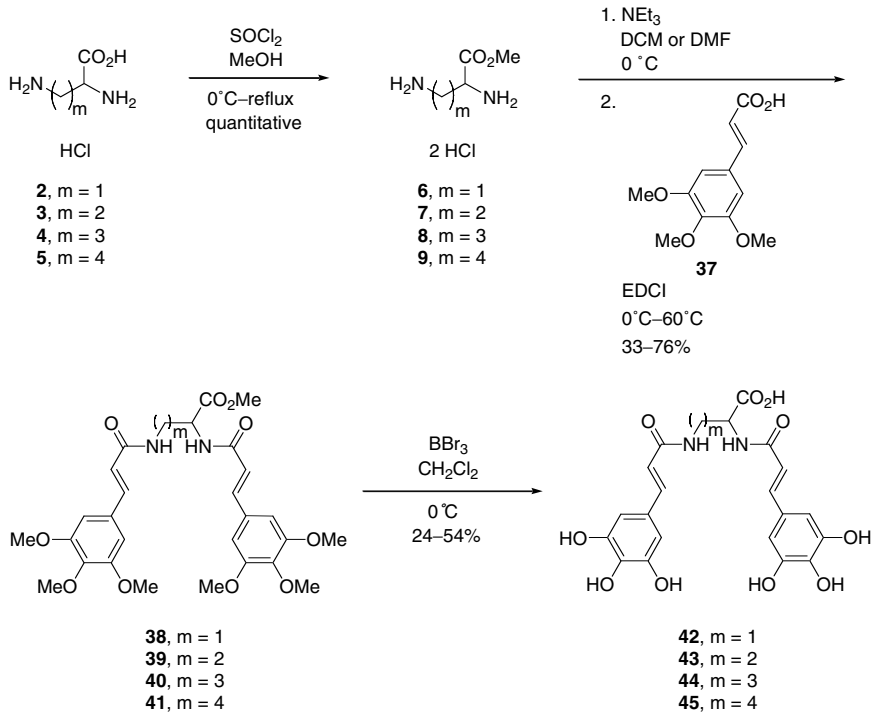
Figure 3. CA's undesirable structural characteristics.

pling to three of the protected aromatic acids: 3,4-dimethoxycarbonyloxybenzoic acid (**10**), 3,4,5-trimethoxycarbonyloxybenzoic acid (**11**), and 3,4-dimethoxycarbonyloxycinnamic acid (**12**)[3] (Scheme 1). The resultant diamido esters were then saponified with lithium hydroxide in a 1:1:0.5 solution of tetrahydrofuran:water:methanol to afford globally deprotected diamido CA analogs **25**–**36**.

The second series of diamido CA analogs synthesized contained the fourth aryl sidechain, 3,4,5-trihydroxycinnamic acid (**37**) (Scheme 2). Synthesis of the CA derivatives was accomplished in a similar manner to the previously assembled analogs, except a permethyl ether protected aromatic acid was employed (the only commercially available trihydroxycinnamic acid derivative is trimethoxycinnamic acid). The four previously em-



Scheme 1. Synthesis of diamido CA analogs.



Scheme 2. Synthesis of trihydroxycinnamoyl CA analogs.

ployed amino acids were esterified, biscoupled to the aromatic acid, and finally globally deprotected with boron tribromide to afford diamido CA analogs **42**–**45**.

2.2. Synthesis of chicoric acid analogs with improved stability and membrane permeability

The first series of potentially cell permeable CA analogs contain a pivaloyloxymethyl ester promoiety attached to the pendant free acid of the amino acid linker. Synthesis of the acyloxymethyl esters began with EDCI biscoupling of diamino ester **6** with the commercially available permethoxyaromatic acids **37** and **46**–**47**, followed by LiOH-mediated hydrolysis of the methyl esters. The resultant carboxylic acids **50**–**52** were deprotonated with triethylamine, followed by addition of excess chloromethyl pivalate to afford the desired acyloxymethyl esters (**53**–**55**) in good yield (Scheme 3).

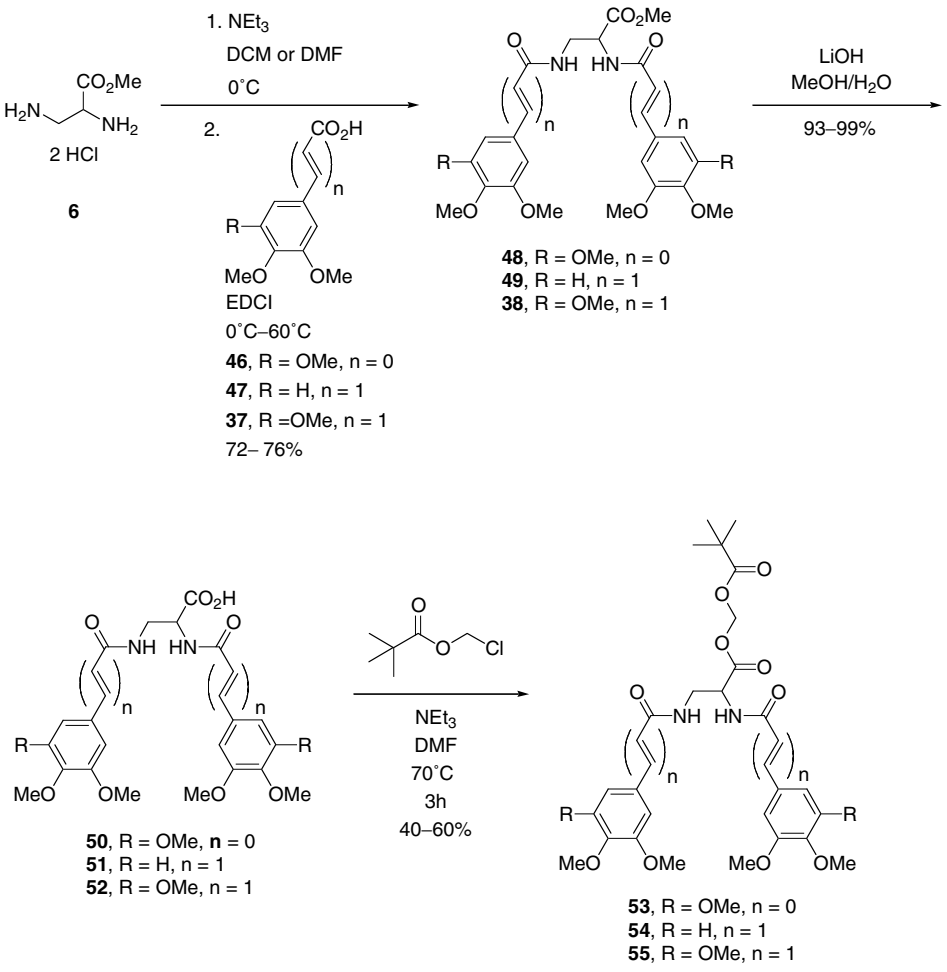
A second series of CA analogs with potential enhanced cell permeation possess a methyl ester promoiety again attached at the carboxylic acid terminus. Employing the previously synthesized permethylcarbonyloxy CA analogs **13**–**24**, the desired methyl ester derivatives were obtained in 15–87% yield following sodium methoxide mediated deprotection of the phenolic methylcarbonates (Scheme 4).

3. Biology

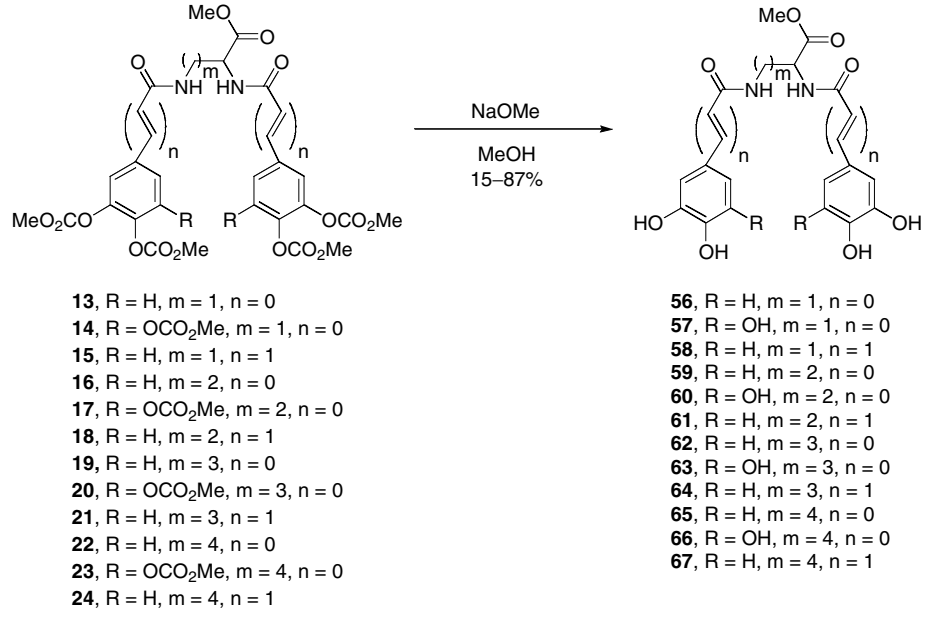
Three biological activities were determined for each CA analog. The first was the ability of each compound to inhibit recombinant HIV IN in vitro in the 3′-end processing and strand transfer reactions (Fig. 4). The second was the toxicity of each analog against the CD4+ lymphoblastoid cell line, MT2 (Fig. 5). The third and final biological activity was the anti-HIV activity of each compound against HIV_{LAI} (Fig. 5). Each assay was performed as described previously.[1,3]

4. Results and discussion

The first of CAs undesirable structural characteristics addressed are the labile ester bonds. The undesired esters were replaced by a significantly more robust diamide-linkage. Based on the biscaffeoyl monoacid CA analogs that exhibit potent in vitro HIV IN inhibition,[1] a small library of amide-linked CA analogs were designed in which both the aromatic ring and linker length between the diamines are varied. Four different aromatic acids (3,4-dihydroxybenzoic acid, gallic acid, caffeic acid, and 3,4,5-trihydroxycinnamic acid) and four different diamines {(DL)-2,3 diaminopropanoic acid, (DL)-2,4-diaminobutyric acid, (DL)-ornithine, and L-lysine} were utilized in which the linker length will be two-to-five carbons. The above four acids were strategically chosen because they contain all the possible permutations of aromatic hydroxyl groups and unsaturation present in the two active aryl substituents (gallic acid and caffeic acid): two or three hydroxy moieties attached to either benzoic or cinnamic acid. The diamines were also carefully chosen based on the HIV inhibition (as mentioned in the CA SAR section) of biscaffeoyl (DL)-2,3 diaminopropanoic acid (linker length equals two) and biscaffeoyl L-lysine (linker length equals five). However, linker lengths of three {(DL)-2,4-diaminobutyric acid} and four {(DL)-ornithine} carbons have not been examined.



Scheme 3. Synthesis of pivaloyloxymethyl ester CA analogs.



Scheme 4. Synthesis of diamido CA methyl ester analogs.

The second of CA's undesirable structural characteristics addressed is the diacid moiety, which leads to its poor cell permeation profile. The obvious method to improve CA's membrane permeability is to remove one carboxylic acid to afford the unsymmetrical monoacid. Numerous monocarboxylic acids have been syn-

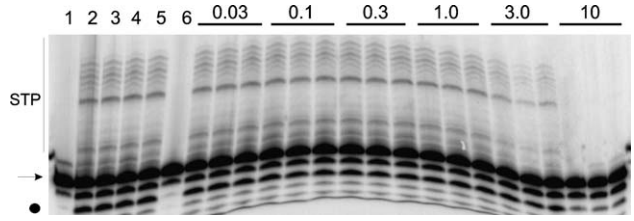


Figure 4. Representative IC_{50} determination. IN was incubated with compound **33** and substrate for 1 h at 37 °C. Substrate was separated from products by denaturing polyacryl amide gel electrophoresis (PAGE). Products and substrate were quantified by phosphorimager analysis and the IC_{50} calculated through the linear portion of the curve using CalcuSyn for Windows. Lane 1, substrate alone; lanes 2–4 IN plus substrate; lane 5, 25 μM L-TA (negative control); lane 6, 25 μM L-CA (positive control); lanes 7–24, IN plus substrate plus compound **33** in triplicate with concentrations in micromolar above the lanes. STP, strand transfer products; arrow, V1/V2 substrate; dot, 3′-end-processing products.

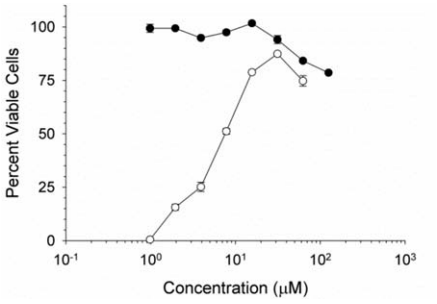


Figure 5. Representative cell toxicity and anti-HIV results. MT-2 cells were incubated in triplicate with 2-fold serial dilutions of compound **33** for 1 h at 37 °C. For cell toxicity determination (closed circles), cells were incubated at 37 °C for three days and then harvested for cell viability determined using Finter's neutral red dye. For anti-HIV activity (open circles), HIV_{LAI} was added at a multiplicity of infection <1 and the cells were incubated for three days at 37 °C. Cells were harvested for cell viability and quantified using Finter's neutral red dye. The percentage of cells protected from HIV-induced death was calculated at each concentration. Points are means of triplicates.

thesized and shown to be potent HIV IN inhibitors in vitro;[1,4] however, the ability of all these analogs to translocate into the cell has not been examined. Our efforts were thus focused on monocarboxylate analogs, but it is possible that they will also be cell impermeable. Thus, we also investigated several pro-drug strategies to promote permeation of the analogs into the cell. Numerous pro-drug strategies have been developed that successfully convert cell impermeable compounds into membrane permeable counterparts. These strategies are generally based on transforming the carboxylic acid to an ester or related derivative that contains either a lipophilic moiety or functional group that is a substrate for a membrane transport system. The pro-drug strategies we evaluated are based on the enhanced lipophilicity method and include methyl and pivaloyloxymethyl esters. Both ester pro-drugs have been shown to be enzymatically cleaved in vivo to reveal the target acid.[19] Furthermore, methylation of the phenols was performed to examine the role the acidic phenols play in the CA analogs poor cell permeation profile.

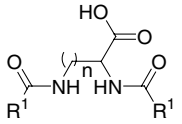
Analysis of the biological activity of the CA analogs reveals positive SAR progress was accomplished (Tables 1 and 2). Seven compounds (the boxed entries) exhibited more potent anti-HIV activity relative to the lead compound, L-CA. Examination of the catecholic sidechains reveals that the free acid CA analogs with 3,4,5-trihydroxycinnamoyl aryl substituents (entries 5, 9, 3, and 17) displayed the strongest antiviral and end-processing activity; for example, Orn-linked derivative **44** (entry 13) demonstrated an antiviral EC_{50} concentration of 1.1 μM and an anti-IN IC_{50} of 380 nM. The gallic acid-coupled analogs (entries 4, 8, 12, and 16) were also potent inhibitors of HIV replication, with EC_{50}s of 2.5–7.6 μM. The caffeoyl-substituted derivatives (with the identical catecholic sidechains as CA), on the other hand, exhibited less potent antiviral and end-processing inhibition relative to CA, leading to anti-HIV EC_{50} concentrations of 4.5 to >62.5 μM (the upper limit of the assay). Finally, all the 3,4-dihydroxy-coupled derivatives were inactive in the anti-HIV assay. The length of the amino acid linker connecting the catecholic sidechains also modulated the activity of the CA analogs. The 2,4-diaminobutyric acid (entries 6–9) and ornithine (entries 10–13) linkers led to significantly more potent end-processing and antiviral activity compared to the corresponding 2,3-diaminopropanoic acid (entries 2–5) and lysine (entries 14–17) derivatives (Fig. 4).

Although the potency of numerous CA analogs improved relative to CA, the cytotoxicity of the amide-linked compounds also increased. The toxicity effects appear unaffected by the diaryl substituent, as all four aryl moieties displayed comparable cytotoxicity levels. Thus, the diamide linker appears responsible for the lethal effects of the compounds (Fig. 5).

The increase in cytotoxicity levels associated with the diamido CA analogs in turn led to reduced therapeutic indices (TIs) relative to CA (TI = 63). The 3,4,5-trihydroxycinnamoyl- and galloyl-coupled analogs were the most selective, leading to TIs generally greater than 10. The most potent and selective inhibitor of HIV was analog **44** (entry 13), exhibiting a TI of 33.6.

Conversion of the pendant free acid of the CA analogs to the corresponding methyl ester (Table 2) generally led to a considerable decrease in both antiviral and end-processing activity. However, the galloyl-coupled esters (entries 20, 26, 30, and 34) retained substantial levels of potency (the 3,4,5-trihydroxycinnamoyl derivatives were not synthesized). Similarly, methylating the catechols (entries 21–23, 27, 31, and 35) abolished all anti-HIV inhibition (due in part to a significantly decreased solubility profile). Thus, simple ester and methyl ether pro-drug strategies appear ineffective as methods to improve the bioactivity of the diamido CA analogs. Finally, the pivaloyloxymethyl ester pro-drug strategy was unable to be evaluated due to the poor aqueous solubility of the resultant esterified CA analogs.

Table 1. Biological analysis—amide-linked CA analogs with increased stability



Entry	Compound	n	R^1	Cell Toxicity (μM)	Anti-HIV (μM)	TI	Anti-IN 3'-Processing	
				CT_5	EC_{50}		10 μM Screen	IC_{50} (μM)
1	**1 (CA)**	-	-	264	4.2	62.9	NA[a]	0.53
2	**25**	1	BA[b]	39	94	0.4	2%	NA[a]
3	**27**	1	CA[c]	30	4.5	6.7	100%	0.81
4	**26**	1	GA[d]	32	3	10.7	100%	0.38
5	**42**	1	TA[e]	30	1.6	18.8	NA[a]	NA[a]
6	**28**	2	BA[b]	75	>62.5	-	0%	NA[a]
7	**30**	2	CA[c]	39	67.2	0.6	93%	2.3
8	**29**	2	GA[d]	56	2.7	20.7	100%	0.54
9	**43**	2	TA[e]	29	1.7	17.1	NA[a]	NA[a]
10	**31**	3	BA[b]	17	>62.5	-	0%	NA[a]
11	**33**	3	CA[c]	38	7.2	5.3	90%	2.9
12	**32**	3	GA[d]	29	2.5	11.6	95%	0.39
13	**44**	3	TA[e]	37	1.1	33.6	NA[a]	0.38
14	**34**	4	BA[b]	104	>62.5	-	0%	NA[a]
15	**36**	4	CA[c]	51	>62.5	-	78%	NA[a]
16	**35**	4	GA[d]	38	7.6	5.0	100%	2.25
17	**45**	4	TA[e]	39	1.7	22.9	NA[a]	0.81

[a]Experiment not performed.
[b]3,4-Dihydroxybenzoyl.
[c]Caffeoyl.
[d]Galloyl.
[e]3,4,5-Trihydroxycinnamoyl.

5. Conclusions

The CA analogs with 3,4,5-trihydroxycinnamoyl aryl sidechains exhibited the most potent inhibition of HIV replication and end-processing activity. The galloyl-substituted derivatives also displayed very potent in vitro and in vivo activity, in most examples exceeding the inhibitory effects of CA. Conversely, the caffeoyl-coupled analogs led to modest inhibition, while the 3,4-dihydroxybenzoyl-substituted compounds were devoid of activity.

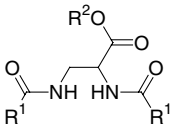
The linker length of the amino acid bridge also played a significant role in the bioactivity of the CA analogs. Interestingly, the γ-aminoalanine and ornithine linkers, with three and four carbons, respectively, led to potent inhibition of HIV replication and end-processing, while the β-aminoalanine- (two carbons) and lysine- (five carbons) coupled derivatives exhibited diminished in vitro and in vivo activity. Finally, initial attempts to mask the problematic carboxylic acid and catecholic functionality were unsuccessful, as the methyl ester and permethyl ether CA analogs failed to improve the bioactivity of the parent compounds.

6. Experimental

6.1. General information

Nuclear magnetic resonance spectra were recorded on a Bruker DRX 500 (500 MHz), GN 500 (500 MHz), or a

Table 2. Biological analysis—amide-linked CA analogs with improved stability and membrane permeability



Entry	Compound	*n*	R^1	R^2	Cell toxicity (μM)	Anti-HIV (μM)	TI	Anti-IN 3′-processing	
					CT_{50}	EC_{50}		10 μM Screen	IC_{50} (μM)
18	**66**	1	BA[c]	Me	29	>62.5	—	0%	NA[a]
19	**68**	1	CA[d]	Me	49	58	0.8	98%	1.95
20	**67**	1	GA[e]	Me	15	10	1.5	100%	0.89
21	**55**	1	OMe-GA[f]	Me	Insol[b]	—	—	—	—
22	**56**	1	OMe-CA[g]	Me	Insol[b]	—	—	—	—
23	**38**	1	OMe-TA[h]	Me	Insol[b]	—	—	—	—
24	**69**	2	BA[c]	Me	86	>62.5	—	0%	NA[a]
25	**71**	2	CA[d]	Me	66	>62.5	—	52%	NA[a]
26	**70**	2	GA[e]	Me	69	19.4	3.6	100%	0.44
27	**39**	2	OMe-TA[h]	Me	282	≫62.5	—	NA[a]	NA[a]
28	**72**	3	BA[c]	Me	37	>62.5	—	0%	NA[a]
29	**74**	3	CA[d]	Me	33	>62.5	—	99%	NA[a]
30	**73**	3	GA[e]	Me	42	10	4.2	96%	0.74
31	**40**	3	OMe-TA[h]	Me	56	≫31.3	—	NA[a]	NA[a]
32	**75**	4	BA[c]	Me	70	>62.5	—	0%	NA[a]
33	**77**	4	CA[d]	Me	68	>62.5	—	55%	NA[a]
34	**76**	4	GA[e]	Me	62	35.9	1.7	100%	2.4
35	**41**	4	OMe-TA[h]	Me	104	≫31.25	—	NA[a]	NA[a]
36	**63**	1	OMe-GA[f]	AM[i]	Insol[b]	—	—	—	—
37	**64**	1	OMe-CA[g]	AM[i]	Insol[b]	—	—	—	—
38	**65**	1	OMe-TA[h]	AM[i]	Insol[b]	—	—	—	—

[a] Experiment not performed.
[b] Compound was insoluble under assay conditions.
[c] 3,4-Dihydroxybenzoyl.
[d] Caffeoyl.
[e] Galloyl.
[f] Galloyl 3,4,5-trimethylether.
[g] Caffeoyl 3,4-dimethylether.
[h] 3,4,5-Trimethoxycinnamoyl.
[i] Pivaloyloxymethyl.

Bruker DRX 400 (400 MHz) spectrometer. Chemical shifts are reported in ppm (δ) referenced to $CHCl_3$ (7.24 ppm) or CH_3OH (3.31 ppm) for ^1H NMR and $CDCl_3$ (77.0 ppm) or CD_3OD (49.15 ppm) for ^{13}C NMR. Coupling constant, J_{HH}, values are given in hertz. Data are reported as follows: chemical shift, multiplicity (app, apparent; par obsc, partially obscured; br, broad; s, singlet; d, doublet; t, triplet; q, quartet; qn, quintet; and m, multiplet), coupling constant, and integration. Infrared spectra (IR) were taken with a Perkin and Elmer Model 1600 Series FTIR spectrophotometer. Optical rotations were obtained with a JASCO DIP-360 digital polarimeter. Melting points (mp) were obtained from a Laboratory Devices Mel-Temp melting-point apparatus and are reported uncorrected. High resolution mass spectrometry was obtained from the Departmental Mass Spectrometry facility. Thin-layer chromatography (TLC) was performed on 0.25 mm Merck pre-coated silica gel plates (60 F-254), and silica gel chromatography was performed using ICN 200–400 mesh silica gel. Rotary evaporation was used to remove solvents. Inert atmosphere operations were conducted under nitrogen passed through a Drierite tube in oven- or flame-dried glassware. Anhydrous tetrahydrofuran (THF), methylene chloride (CH_2Cl_2), diethyl ether (Et_2O), methanol (MeOH), ethanol (EtOH), dimethylformamide (DMF), diisopropylamine, and triethylamine were filtered through two columns of activated basic alumina under Ar(g) and transferred under Ar(g) in a solvent purification system designed and manufactured by J. C. Meyer. All reagents were used as purchased from Aldrich, Lancaster, or Acros; unless stated otherwise. The term 'shaken' when used below refers to agitation of the reaction mixture on a standard orbital shaker.

6.2. Inhibition of integrase

Integrase inhibition assays were performed as described previously.[1,3,9,10,12,15] Briefly, the V1 oligonucleotide was 5′-end labeled using γ-[^{32}P]ATP and T4 polynucleotide kinase. The labeled oligonucleotide was annealed to its complementary V2 oligonucleotide.[16] In a final volume of 20 μl, 1 μl of each compound was incubated with 1.5 pmol of recombinant HIV IN purified from *Escherichia coli* and 2 pmol of V1/V2 in a reaction buffer of 20 mM 4-(2-hydroxyeth-

yl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 10 mM dithiothreitol, 0.05% Nonidet P-40, and 10 mM $MnCl_2$ at 37 °C for 1 h. Reactions were then stopped by addition of EDTA to a final concentration of 18 mM. Products were separated from substrate by electrophoresis through a denaturing 15% polyacrylamide gel and quantified using a Storm phosphorimager (Molecular Dynamics) and ImageQuant software. Analogs were first screened for activity at 10 μM; any compound that inhibited HIV IN by 50% or greater at that concentration was selected for calculation of the fifty percent inhibitory concentration (IC_{50}). In triplicate reactions, with inhibitor concentrations ranging from 30 nM to 10 μM, active inhibitors were studied. Following quantification, the IC_{50} was calculated through the linear range of the log dose–response curve using CalcuSyn for Windows software (Biosoft).

6.3. Cell toxicity and anti-HIV activities of CA analogs

Cell toxicity and anti-HIV activities of each CA analog were determined as described previously.[1,3,9,10,12,15,17,18] Each analog was dissolved in H_2O, 25% EtOH, or 33% dimethylsulfoxide plus 33% EtOH, depending on its solubility profile. In triplicate wells of a 96-well plate, each analog was diluted in 2 fold serial dilutions. Next, approximately 5×10^5 MT2 cells were added and the plates were incubated for 72 h at 37 °C. Cells were transferred to poly-L-lysine coated 96-well plates, stained with Finter's Neutral red dye, and the viability was determined as the A_{540}. The CT_5 was calculated through the linear range of the dose response curve using CalcuSyn for Windows. The CT_5, a dose where 95% of the cells are viable, was chosen because it is a truly non-toxic dose. Since HIV is an obligate intracellular parasite, any anti-HIV activity at the CT_5 is due to an effect of the analog on viral, rather than cellular, proteins.

Once the CT_5 was determined, each analog was tested for anti-HIV activity. Each analog was 2 fold serially diluted in triplicate. Next, 5×10^5 MT2 cells were added to each well and the cells were incubated for 1 h at 37 °C. Next, approximately 5×10^5 infectious particles of HIV_{LAI} were added to each well and the cells were incubated for approximately 72 h at 37 °C. Cells were harvested, stained for viability using Finter's neutral red, and viability calculated as described for cell toxicity assays. The fifty percent effective concentration (EC_{50}) was calculated based on protection from HIV-induced cytopathic effect. Percent protection was relative to cell controls with no virus added (100% viable) and virus control infections in which no analog was added to cells plus virus (0% viable). The range between these two controls was between 0.25 and 0.35 A_{540}. The therapeutic index, or TI, was calculated as the ratio of CT_5 to EC_{50}. Both MT2 cells and H9 cells infected with HIV_{LAI} were cultured in RPMI-1640 media supplemented with 25 mM HEPES, 12.5% fetal bovine serum, and 2 mM L-glutamine. All cells and virus were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. HIV_{LAI} was clarified of cells by low-speed centrifugation followed by filtration through 0.45 μm cellulose acetate filters.

6.4. Preparatory HPLC purification

The target compound was purified via reverse-phase preparatory HPLC utilizing a Column Engineering Matrix C_{18} column (5 μm, 20 × 50 mm, 0.1% TFA/H_2O (eluent A):0.08% TFA/MeCN (eluent B)).

6.5. Analytical HPLC analysis

The purity of the target compound was analyzed via reverse-phase HPLC utilizing a Column Engineering Matrix C_{18} column (5 μm, 4.6 × 50 mm, 95:5–5:95 0.1% TFA/H_2O (eluent A):0.08% TFA/MeCN (eluent B) over 10 min).

6.6. (DL)-2,3-Diaminopropionic acid methyl ester dihydrochloride (6)

To a dry 250 mL three-necked round-bottomed flask fitted with a drying tube was added (DL)-2,3 diaminopropanoic acid hydrochloride (2.00 g, 14.23 mmol) and 77 mL methanol. The heterogeneous solution was cooled to 0 °C, and thionyl chloride (7.8 mL, 106.8 mmol) was added dropwise over 15 min. The reaction mixture quickly became homogeneous and was stirred for 20 min at 0 °C. The reaction mixture was subsequently fitted with a condenser and heated at reflux for 5 h. The resultant solution was concentrated in vacuo, the crude product was diluted with methanol, and the solution was concentrated again to remove any residual acid. The crude solid was then placed on the high vacuum pump overnight to afford 2.71 g (100%) of **6** as a white solid, which was used directly in the subsequent reactions (e.g., 6.7, 6.8, and 6.9).

6.7. (DL)-2,4-Diaminobutyric acid methyl ester dihydrochloride (7)

Following the procedure for **6**, diamine **7** (384 mg) was produced in 99% yield from (DL)-2,4-diaminobutyric acid dihydrochloride (362 mg, 1.90 mmol) and used directly in the subsequent reactions (6.10, 6.11, and 6.12).

6.8. (DL)-2,5-diaminopentanoic acid methyl ester dihydrochloride (8)

Following the procedure for **6**, diamine **8** (2.58 g) was generated in 100% yield from (DL)-2,5-diaminopentanoic acid hydrochloride (2.00 g, 11.86 mmol) and used directly in the subsequent reactions (6.13, 6.14, and 6.15).

6.9. L-2,6-Diaminohexanoic acid methyl ester dihydrochloride (9)

Following the procedure for **6**, diamine **9** (2.50 g) was produced in 98% yield from (L)-2,5-diaminohexanoic acid hydrochloride (2.00 g, 10.95 mmol) and used directly in the subsequent reactions (6.16, 6.17, and 6.18).

6.10. (DL)-2,3-Bis(3,4-dimethoxycarbonyloxybenzoylamino)propionic acid (13)

A 100 mL three-necked flask was charged with diamine **6** (142 mg, 0.741 mmol), triethylamine (0.204 mL, 1.48 mmol), and methylene chloride (3.7 mL). The resultant homogeneous solution was cooled to 0 °C, and 3,4-dimethoxycarbonyloxybenzoic acid (500 mg, 1.85 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (377 mg, 1.93 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then heated at reflux for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL); followed by drying with $MgSO_4$ and concentration in vacuo to generate the crude diamide (409 mg, 66% yield), which was used directly in the subsequent reactions.

6.11. (DL)-2,3-Bis(3,4,5-trimethoxycarbonyloxybenzoylamino)propionic acid (14)

Following the procedure for **13**, diamine **6** (151 mg, 0.793 mmol) and 3,4,5-trimethoxycarbonyloxybenzoic acid (600 mg, 1.74 mmol) were converted to diamide **14** (252 mg, 41% yield), which was used directly in the subsequent reactions.

6.12. (DL)-2,3-Bis[3-(3,4-dimethoxycarbonyloxyphenyl)acryloylamino]propionic acid (15)

Following the procedure for **13**, diamine **6** (129 mg, 0.676 mmol) and 3-(3,4-dimethoxycarbonyloxyphenyl)acrylic acid (500 mg, 1.69 mmol) were converted to diamide **15** (416 mg, 38% yield), which was used directly in the subsequent reactions.

6.13. (DL)-2,4-Bis(3,4-dimethoxycarbonyloxybenzoylamino)butyric acid (16)

Following the procedure for **13**, diamine **7** (177 mg, 0.863 mmol) and 3,4-dimethoxycarbonyloxybenzoic acid (583 mg, 2.16 mmol) were converted to diamide **16** (313 mg, 49% yield), which was used directly in the subsequent reactions.

6.14. (DL)-2,4-Bis(3,4,5-trimethoxycarbonyloxybenzoylamino)butyric acid (17)

Following the procedure for **13**, diamine **7** (144 mg, 0.699 mmol) and 3,4,5-trimethoxycarbonyloxybenzoic acid (575 mg, 1.67 mmol) were converted to diamide **17** (308 mg, 56% yield), which was used directly in the subsequent reactions.

6.15. (DL)-2,4-Bis[3-(3,4-dimethoxycarbonyloxyphenyl)acryloylamino]butyric acid (18)

Following the procedure for **13**, diamine **7** (166 mg, 0.811 mmol) and 3-(3,4-dimethoxycarbonyloxyphenyl)acrylic acid (600 mg, 2.03 mmol) were converted to diamide **18** (439 mg, 79% yield), which was used directly in the subsequent reactions.

6.16. (DL)-2,5-Bis(3,4-dimethoxycarbonyloxybenzoylamino)pentanoic acid (19)

Following the procedure for **13**, diamine **8** (195 mg, 0.889 mmol) and 3,4-dimethoxycarbonyloxybenzoic acid (600 mg, 2.22 mmol) were converted to diamide **19** (480 mg, 83% yield), which was used directly in the subsequent reactions.

6.17. (DL)-2,5-Bis(3,4,5-trimethoxycarbonyloxybenzoylamino)pentanoic acid (20)

Following the procedure for **13**, diamine **8** (153 mg, 0.699 mmol) and 3,4,5-trimethoxycarbonyloxybenzoic acid (575 mg, 1.67 mmol) were converted to diamide **20** (156 mg, 28% yield), which was used directly in the subsequent reactions.

6.18. (DL)-2,5-Bis[3-(3,4-dimethoxycarbonyloxyphenyl)acryloylamino]pentanoic acid (21)

Following the procedure for **13**, diamine **8** (178 mg, 0.811 mmol) and 3-(3,4-dimethoxycarbonyloxyphenyl)acrylic acid (600 mg, 2.03 mmol) were converted to diamide **21** (383 mg, 67% yield), which was used directly in the subsequent reactions.

6.19. L-2,6-Bis(3,4-dimethoxycarbonyloxybenzoylamino)hexanoic acid (22)

Following the procedure for **13**, diamine **9** (173 mg, 0.742 mmol) and 3,4-dimethoxycarbonyloxybenzoic acid (500 mg, 1.85 mmol) were converted to diamide **22** (370 mg, 75% yield), which was used directly in the subsequent reactions.

6.20. L-2,6-Bis(3,4,5-trimethoxycarbonyloxybenzoylamino)hexanoic acid (23)

Following the procedure for **13**, diamine **9** (129 mg, 0.552 mmol) and 3,4,5-trimethoxycarbonyloxybenzoic acid (475 mg, 1.38 mmol) were converted to diamide **23** (250 mg, 56% yield), which was used directly in the subsequent reactions.

6.21. L-2,6-Bis[3-(3,4-dimethoxycarbonyloxyphenyl)acryloylamino]hexanoic acid (24)

Following the procedure for **13**, diamine **9** (157 mg, 0.676 mmol) and 3-(3,4-dimethoxycarbonyloxyphenyl)acrylic acid (500 mg, 1.69 mmol) were converted to diamide **24** (419 mg, 87% yield), which was used directly in the subsequent reactions.

6.22. (DL)-2,3-Bis(3,4-dihydroxybenzoylamino)propionic acid (25)

To crude diamide **13** (75 mg, 0.121 mmol) in 0.85 mL THF and 0.43 mL MeOH was added LiOH (0.84 mL,

1.0 M) dropwise. The resultant solution was shaken for 2.5 h, quenched with 2.0 mL of 10% HCl, lyophilized, and purified via preparatory HPLC (98–45%, eluent A) to afford 18 mg (40%) of acid **25**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.31 (d, J = 2.2, 1H), 7.27 (d, J = 2.2, 1H), 7.25 (dd, J = 8.3, 2.2, 1H), 7.19 (dd, J = 8.3, 2.2, 1H), 6.81 (d, J = 8.3, 1H), 6.79 (d, J = 8.3, 1H), 4.73 (dd, J = 6.6, 5.3, 1H), 3.85 (app d, J = 5.2, 2H); HRMS (CI/methanol) m/z calcd for $C_{17}H_{16}N_2O_8Na$ $(M+Na)^+$ 399.0804, found 399.0812; Analytical HPLC t_R = 3.65 min.

6.23. (DL)-2,3-Bis(3,4,5-trihydroxybenzoylamino)propionic acid (26)

Following the procedure for **25**, crude diamide **14** (40 mg, 0.0519 mmol) was hydrolyzed to afford 4 mg (10%) of acid **26**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.89 (s, 2H), 6.84 (s, 2H), 4.70 (dd, J = 6.7, 5.3, 1H), 3.81–3.85 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{17}H_{15}N_2O_{10}Na_2$ $(M + 2Na–H)^+$ 453.0522, found 453.0526; Analytical HPLC t_R = 3.23 min.

6.24. (DL)-2,3-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]propionic acid (27)

Following the procedure for **25**, crude diamide **15** (75 mg, 0.111 mmol) was hydrolyzed to afford 9 mg (20%) of acid **27**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (d, J = 15.7, 1H), 7.41 (d, J = 15.7, 1H), 7.02 (d, J = 2.1, 1H), 7.00 (d, J = 2.1, 1H), 6.88–6.94 (m, 2H), 6.77 (d, J = 8.1, 1H), 6.76 (d, J = 8.1, 1H), 6.45 (d, J = 15.7, 1H), 6.37 (d, J = 15.7, 1H), 4.70 (dd, J = 7.5, 4.8, 1H), 3.84 (dd, J = 13.8, 4.7, 1H), 3.68 (dd, J = 13.8, 7.5, 1H); HRMS (CI/methanol) m/z calcd for $C_{21}H_{20}N_2O_8Na$ $(M+Na)^+$ 451.1117, found 451.1115; Analytical HPLC t_R = 4.29 min.

6.25. (DL)-2,4-Bis(3,4-dihydroxybenzoylamino)butyric acid (28)

Following the procedure for **25**, crude diamide **16** (70 mg, 0.110 mmol) was hydrolzyed to afford 9 mg (21%) of acid **28**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (d, J = 2.2, 1H), 7.32 (dd, J = 8.3, 2.2, 1H), 7.29 (d, J = 2.2, 1H), 7.21 (dd, J = 8.3, 2.2, 1H), 6.83 (d, J = 8.3, 1H), 6.80 (d, J = 8.3, 1H), 4.68 (dd, J = 8.9, 4.9,1H), 3.60–3.68 (m, 1H), 3.34–3.40 (m, 1H), 2.21–2.30 (m, 1H), 2.06–2.16 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{18}H_{18}N_2O_8Na$ $(M+Na)^+$ 413.0961, found 413.0974; Analytical HPLC t_R = 3.64 min.

6.26. (DL)-2,4-Bis(3,4,5-trihydroxybenzoylamino)butyric acid (29)

Following the procedure for **25**, crude diamide **17** (75 mg, 0.0957 mmol) was hydrolyzed to afford 5 mg (12%) of acid **29**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.95 (s, 2H), 6.87 (s, 2H), 4.67 (dd, J = 8.9, 5.0, 1H), 3.60–3.70 (m, 2H), 2.20–2.28 (m, 1H), 2.05–2.15 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{18}H_{18}N_2O_{10}Na$ $(M+Na)^+$ 445.0859, found 445.0852; Analytical HPLC t_R = 3.19 min.

6.27. (DL)-2,4-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]butyric acid (30)

Following the procedure for **25**, crude diamide **18** (75 mg, 0.109 mmol) was hydrolyzed to afford 20 mg (42%) of acid **30**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.44 (d, J = 15.7, 1H), 7.40 (d, J = 15.7, 1H), 7.03 (d, J = 2.0, 1H), 7.01 (d, J = 2.0, 1H), 6.93 (dd, J = 8.2, 2.0, 1H), 6.90 (dd, J = 8.2, 2.0, 1H), 6.77 (d, J = 8.2, 1H), 6.76 (d, J = 8.2, 1H), 6.48 (d, J = 15.7, 1H), 6.36 (d, J = 15.7, 1H), 4.59 (dd, J = 9.1, 4.7, 1H), 3.46–3.55 (m, 1H), 3.27–3.37 (m, 1H), 2.14–2.24 (m, 1H), 1.93–2.01 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{22}H_{22}N_2O_8Na$ $(M+Na)^+$465.1274, found 465.1275; Analytical HPLC t_R = 4.38 min.

6.28. (DL)-2,5-Bis(3,4-dihydroxybenzoylamino)pentanoic acid (31)

Following the procedure for **25**, crude diamide **19** (75 mg, 0.115 mmol) was hydrolyzed to afford 24 mg (52%) of acid **31**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.31 (d, J = 2.1, 1H), 7.27 (d, J = 2.2, 1H), 7.27 (dd, J = 8.3, 2.2, 1H), 7.19 (dd, J = 8.3, 2.2, 1H), 6.81 (d, J = 8.3, 1H), 6.79 (d, J = 8.3, 1H), 4.58 (dd, J = 9.2, 4.9, 1H), 3.39 (t, J = 7.0, 2H), 2.0–2.1 (m, 1H), 1.84–1.94 (m, 1H), 1.69–1.83 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{19}H_{20}N_2O_8Na$ $(M+Na)^+$ 427.1117, found 427.1120; Analytical HPLC t_R = 3.70 min.

6.29. (DL)-2,5-Bis(3,4,5-trihydroxybenzoylamino)pentanoic acid (32)

Following the procedure for **25**, crude diamide **20** (75 mg, 0.0940 mmol) was hydrolyzed to afford 10 mg (24%) of acid **32**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.89 (s, 2H), 6.84 (s, 2H), 4.56 (dd, J = 9.1, 5.0, 1H), 3.38 (t, J = 6.8, 2H), 1.99–2.08 (m, 1H), 1.81–1.91 (m, 1H), 1.64–1.78 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{19}H_{20}N_2O_{10}Na$ $(M+Na)^+$ 459.1016, found 459.1001; Analytical HPLC t_R = 3.22 min.

6.30. (DL)-2,5-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]pentanoic acid (33)

Following the procedure for **25**, crude diamide **21** (75 mg, 0.107 mmol) was hydrolyzed to afford 24 mg (49%) of acid **33**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (d, J = 15.9, 1H), 7.38 (d, J = 16.0, 1H), 7.02 (d, J = 2.0, 1H), 7.00 (d, J = 2.0, 1H), 6.91 (dd, J = 8.4, 2.0, 1H), 6.89 (dd, J = 8.6, 2.0, 1H), 6.77 (d, J = 8.2, 1H), 6.76 (d, J = 8.2, 1H), 6.47 (d, J = 15.7, 1H), 6.35 (d, J = 15.7, 1H), 4.54 (dd, J = 8.8, 4.8, 1H), 3.34 (t, J = 6.3, 2H), 1.94–2.03 (m, 1H), 1.75–1.84 (m, 1H), 1.65–1.74 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{23}H_{24}N_2O_8Na$ $(M+Na)^+$ 479.1530, found 479.1421; Analytical HPLC t_R = 4.39 min.

6.31. L-2,6-Bis(3,4-dihydroxybenzoylamino)hexanoic acid (34)

Following the procedure for **25**, crude diamide **22** (75 mg, 0.13 mmol) was hydrolyzed to afford 30 mg

(64%) of acid **34**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (d, J = 2.2, 1H), 7.26 (d, J = 2.1, 1H), 7.25 (dd, J = 8.3, 2.2, 1H), 7.16 (dd, J = 8.3, 2.2, 1H), 6.79 (d, J = 8.3, 1H), 6.77 (d, J = 8.3, 1H), 4.55 (dd, J = 9.4, 4.9, 1H), 3.35 (app dt, J = 7.2, 2.1, 2H), 1.96–2.05 (m, 1H), 1.85–1.93 (m, 1H), 1.61–1.72 (m, 2H), 1.46–1.59 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{20}H_{22}N_2O_8Na$ $(M+Na)^+$ 441.1274, found 441.1261; Analytical HPLC t_R = 3.87 min.

6.32. L-2,6-Bis(3,4,5-trihydroxybenzoylamino)hexanoic acid (35)

Following the procedure for **25**, crude diamide **23** (75 mg, 0.0924 mmol) was hydrolyzed to afford 32 mg (65%) of acid **35**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.89 (s, 2H), 6.83 (s, 2H), 4.54 (dd, J=9.0, 5.0, 1H), 3.30–3.36 (m, 2H), 1.95–2.04 (m, 1H), 1.82–1.90 (m, 1H), 1.60–1.70 (m, 2H), 1.46–1.57 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{20}H_{22}N_2O_{10}Na$ $(M+Na)^+$ 473.1172, found 473.1165; Analytical HPLC t_R = 3.42 min.

6.33. L-2,6-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]hexanoic acid (36)

Following the procedure for **25**, crude diamide **24** (75 mg, 0.105 mmol) was hydrolyzed to afford 11 mg (26%) of acid **36**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (d, J = 15.7, 1H), 7.38 (d, J = 15.7, 1H), 7.02 (d, J = 2.0, 1H), 6.99 (d, J = 2.0, 1H), 6.90 (dd, J = 8.3, 2.2, 1H), 6.87 (dd, J = 8.2, 2.0, 1H), 6.76 (d, J = 8.2, 1H), 6.75 (d, J = 8.2, 1H), 6.48 (d, J = 15.7, 1H), 6.35 (d, J = 15.7, 1H), 4.51 (dd, J = 9.0, 4.8, 1H), 3.30–3.35 (m, 2H), 1.91–1.99 (m, 1H), 1.77–1.84 (m, 1H), 1.57–1.67 (m, 2H) 1.46–1.55 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{24}H_{26}N_2O_8Na$ $(M+Na)^+$ 493.1587, found 493.1576; Analytical HPLC t_R = 4.55 min.

6.34. (DL)-2,3-Bis[3-(3,4,5-trimethoxyphenyl)acryloylamino]propionic acid methyl ester (38)

A 100 mL three-necked flask was charged with diamine **6** (532 mg, 2.79 mmol), triethylamine (0.777 mL, 5.57 mmol), and methylene chloride (30 mL). The resultant homogeneous solution was cooled to 0 °C and 3,4,5-trimethoxycinnamic acid (1.46 g, 6.13 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (1.22 g, 6.41 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then heated at reflux for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL); followed by drying with $MgSO_4$ and concentration in vacuo. The resultant diamide was subsequently recrystallized from EtOAc/hexanes to produce 1.18g of diamide **38** (76%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, J = 6.4, 1H), 7.49 (d, J = 6.4, 1H), 7.21 (d, J = 6.9, 1H), 6.64–6.69 (m, 5H), 6.39 (d, J = 15.6, 1H), 6.31 (d, J = 15.6, 1H), 4.80 (dd, J = 12.3, 5.6, 1H), 3.82–3.86 (m, 14H), 3.81 (s, 6H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 167.5, 166.6, 153.6, 142.3, 142.1, 140.0, 130.2, 130.2, 119.3, 119.3, 105.3, 105.3, 61.1, 56.3, 56.3, 54.1, 53.1, 42.2; HRMS (CI/methanol) m/z calcd for $C_{28}H_{34}N_2O_{10}Na$ $(M+Na)^+$ 581.2111, found 581.2101.

6.35. (DL)-2,3-Bis[3-(3,4,5-trihydroxyphenyl)acryloylamino]propionic acid (42)

To a solution of permethylated ester **38** (50 mg, 0.090 mmol) in 0.55 mL methylene chloride at 0 °C was added boron tribromide (1.3 mL, 1.0 M) dropwise. The resultant solution was stirred for 5 h 30 min at 0 °C and then quenched with 1.0 mL of 4 M NaOH and 0.5 mL MeOH. The methylene chloride was subsequently removed in vacuo, followed by preparatory HPLC (95–60% eluent A) purification to provide 19 mg (46%) acid **42**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 (d, J = 15.7, 1H), 7.32 (d, J = 15.7, 1H), 6.59 (s, 2H), 6.57 (s, 2H), 6.41 (d, J = 15.7, 1H), 6.34 (d, J = 15.7, 1H), 4.69 (dd, J = 7.5, 4.7, 1H), 3.84 (dd, J = 13.9, 4.8, 1H), 3.67 (dd, J = 13.8, 7.5, 1H); HRMS (CI/methanol) m/z calcd for $C_{21}H_{20}N_2O_{10}Na$ $(M+Na)^+$ 483.1016, found 483.1000; Analytical HPLC t_R = 3.75 min.

6.36. (DL)-2,4-Bis[3-(3,4,5-trihydroxyphenyl)acryloylamino]butyric acid (43)

A 100 mL three-necked flask was charged with diamine **7** (206 mg, 1.00 mmol), triethylamine (0.280 mL, 2.01 mmol), and methylene chloride (5 mL). The resultant homogeneous solution was cooled to 0 °C and 3,4,5-trimethoxycinnamic acid (598 mg, 2.51 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (512 mg, 2.61 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then heated at reflux for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL), followed by drying with $MgSO_4$ and concentration in vacuo to generate the crude diamide (478 mg) in 83% yield.

To a solution of the crude diamide (50 mg, 0.087 mmol) in 0.53 mL methylene chloride at 0 °C was added boron tribromide (1.2 mL, 1.0 M) dropwise. The resultant solution was stirred for 5 h 30 min at 0 °C and then quenched with 1.0 mL of 4 M NaOH and 0.5 mL MeOH. The methylene chloride was subsequently removed in vacuo, followed by preparatory HPLC (95–60% eluent A) purification to provide 10 mg (24%) acid **43**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.37 (d, J = 15.7, 1H), 7.32 (d, J = 15.7, 1H), 6.61 (s, 2H), 6.58 (s, 2H), 6.45 (d, J = 15.6, 1H), 6.33 (d, J = 15.6, 1H), 4.58 (dd, J = 9.2, 4.8, 1H), 3.47–3.56 (m, 1H), 3.26–3.38 (m, 1H), 2.15–2.25 (m, 1H), 1.93–2.03 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{22}H_{22}N_2O_{10}Na$ $(M+Na)^+$ 497.1172, found 497.1162; Analytical HPLC t_R = 3.75 min.

6.37. (DL)-2,5-Bis[3-(3,4,5-trihydroxyphenyl)acryloylamino]pentanoic acid (44)

A 100 mL three-necked flask was charged with diamine **8** (220 mg, 1.00 mmol), triethylamine (0.280 mL, 2.01 mmol), and methylene chloride (5 mL). The resultant homogeneous solution was cooled to 0 °C and 3,4,5-trimethoxycinnamic acid (598 mg, 2.51 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (512 mg, 2.61 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then heated at reflux for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL), followed by drying with $MgSO_4$ and concentration in vacuo to generate the crude diamide (197 mg) in 33% yield.

To a solution of the crude diamide (20 mg, 0.034 mmol) in 0.20 mL methylene chloride at 0 °C was added boron tribromide (0.48 mL, 1.0 M) dropwise. The resultant solution was stirred for 5 h 30 min at 0 °C and then quenched with 0.5 mL of 4 M NaOH and 0.3 mL MeOH. The methylene chloride was subsequently removed in vacuo, followed by preparatory HPLC (95–60% eluent A) purification to provide 9 mg (54%) acid **44**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.34 (d, J = 15.9, 1H), 7.31 (d, J = 15.9, 1H), 6.59 (s, 2H), 6.57 (s, 2H), 6.44 (d, J = 15.6, 1H), 6.32 (d, J = 15.6, 1H), 4.52 (dd, J = 8.8, 4.9, 1H), 3.30–3.35 (m, 2H), 1.93–2.01 (m, 1H), 1.76–1.84 (m, 1H), 1.64–1.75 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{23}H_{24}N_2O_{10}Na$ $(M+Na)^+$ 511.1329, found 511.1330; Analytical HPLC t_R = 3.86 min.

6.38. L-2,6-Bis[3-(3,4,5-trihydroxyphenyl)acryloylamino]hexanoic acid (45)

A 100 mL three-necked flask was charged with diamine **9** (196 mg, 0.840 mmol), triethylamine (0.234 mL, 1.68 mmol), and methylene chloride (4.2 mL). The resultant homogeneous solution was cooled to 0 °C and 3,4,5-trimethoxycinnamic acid (500 mg, 2.10 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (428 mg, 2.19 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then heated at reflux for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL) followed by drying with $MgSO_4$ and concentration in vacuo to generate the crude diamide (448 mg) in 89% yield.

To a solution of the crude diamide (50 mg, 0.083 mmol) in 0.50 mL methylene chloride at 0 °C was added boron tribromide (1.2 mL, 1.0 M) dropwise. The resultant solution was stirred for 5 h 30 min at 0 °C and then quenched with 1.0 mL of 4 M NaOH and 0.5 mL MeOH. The methylene chloride was subsequently removed in vacuo, followed by preparatory HPLC (95–60% eluent A) purification, to provide 20 mg (48%) acid **45**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.34 (d, J = 15.6, 1H), 7.30 (d, J = 15.6, 1H), 6.59 (s, 2H), 6.57 (s, 2H), 6.44 (d, J = 15.6, 1H), 6.32 (d, J = 15.6, 1H), 4.50 (dd, J = 8.9, 4.9, 1H), 3.28–3.32 (m, 2H), 1.90–1.99 (m, 1H), 1.74–1.83 (m, 1H), 1.57–1.66 (m, 2H), 1.44–1.55 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{24}H_{26}N_2O_{10}Na$ $(M+Na)^+$ 525.1485, found 525.1495; Analytical HPLC t_R = 4.00 min.

6.39. (DL)-2,3-Bis(3,4,5-trimethoxybenzoylamino)propionic acid methyl ester (48)

A 100 mL three-necked flask was charged with diamine **6** (532 mg, 2.79 mmol), triethylamine (0.777 mL, 5.57 mmol), and DMF (30 mL). The resultant homogeneous solution was cooled to 0 °C and acid **46** (1.30 g, 6.13 mmol) was added. The reaction mixture was stirred for 10 min, followed by the addition of EDCI (1.22 g, 6.41 mmol). After stirring for 10 min at 0 °C, the reaction mixture was warmed to rt and then placed in a 60 °C oil bath and stirred for 48 h. The resultant solution was concentrated in vacuo, partitioned between EtOAc and 10% HCl, and extracted with EtOAc (4× 40 mL). The combined organics were washed with saturated $NaHCO_3$ (2× 40 mL), 10% HCl (1× 40 mL), and brine (1× 40 mL), followed by drying with $MgSO_4$ and concentration in vacuo. The resultant diamide was subsequently recrystallized from EtOAc/hexanes to produce 1.03 g ester **48** (73%) as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J = 6.5, 1H), 7.26 (t, J = 5.8, 1H), 7.09 (s, 2H), 7.02 (s, 2H), 4.76–4.82 (m, 1H), 3.86–3.93 (m, 2H), 3.87 (s, 6H), 3.85, (s, 3H), 3.84 (s, 3H), 3.83, (s, 6H), 3.75, (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8, 169.1, 167.8, 153.4, 153.4, 141.5, 141.5, 129.0, 128.6, 104.8, 104.8, 61.1, 61.1, 56.4, 56.4, 54.9, 53.1, 42.7; HRMS (CI/MeOH) m/z calcd for $C_{24}H_{30}N_2O_{10}Na$ $(M+Na)^+$ 507.1979, found 507.1970.

6.40. (DL)-2,3-Bis[3-(3,4-dimethoxyphenyl)acryloylamino]propionic acid methyl ester (49)

Following the procedure for **38**, diamide **49** (990 mg) was generated in 71% yield from acid **47** (1.28 g, 6.13 mmol), and diamine **6** (532 mg, 2.79 mmol), which was used directly in the subsequent reactions.

6.41. (DL)-2,3-Bis(3,4,5-trimethoxybenzoylamino)propionic acid (50)

Ester **48** (500 mg, 0.988 mmol) was dissolved in methylene chloride, followed by concentration in vacuo until almost all the solvent was removed (the solid ester is insoluble in methanol). The resultant solution was dissolved in methanol (5.0 mL) and water (2.0 mL), followed by the addition of LiOH (2.96 mL, 1.0 M). After stirring overnight (8 h), the methanol was removed in vacuo, followed by acidification with 10% HCl and placement at 4 °C for 5 d. The resultant white solid was filtered and then washed with cold water to afford 480 mg (99%) of acid **50**, which was used directly in the subsequent reactions.

6.42. (DL)-2,3-Bis[3-(3,4-dimethoxyphenyl)acryloylamino]propionic acid (51)

Ester **49** (500 mg, 1.00 mmol) was dissolved in methylene chloride, followed by concentration in vacuo until almost all the solvent was removed (the solid ester is insoluble in methanol). The resultant solution was dissolved in methanol (2.0 mL), THF (6.0 mL), and water (2.0 mL), followed by the addition of LiOH (3.01 mL, 1.0 M). After stirring overnight (8 h), the methanol was removed in vacuo, followed by acidification with 10% HCl and placement at 4 °C for 5 d. The resultant white solid was filtered and then washed with cold water to afford 482 mg (99%) of acid **51**, which was used directly in the subsequent reactions.

6.43. (DL)-2,3-Bis[3-(3,4,5-trimethoxyphenyl)acryloylamino]propionic acid (52)

Following the procedure for acid **50**, ester **38** (500 mg, 0.896 mmol) was converted into acid **52** (454 mg) in 93% yield, which was used directly in the subsequent reactions.

6.44. (DL)-2,3-Bis(3,4-dihydroxybenzoylamino)propionic acid methyl ester (56)

To crude diamide **13** (75 mg, 0.121 mmol) was added 1.0 M NaOMe (0.51 mL). The resultant solution was shaken for 30 min, neutralized with 10% HCl, and purified via preparatory HPLC (95–45%, eluent A) to afford 41 mg (87%) of ester **56**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (d, J = 2.2, 1H), 7.27 (d, J = 2.2, 1H), 7.25 (dd, J = 8.3, 2.2, 1H), 7.19 (dd, J = 8.3, 2.2, 1H), 6.82 (d, J = 8.3, 1H), 6.79 (d, J = 8.3, 1H), 4.72 (dd, J = 6.9, 4.9, 1H), 3.79–3.88 (m, 2H), 3.76 (s, 3H); HRMS (CI/methanol) m/z calcd for $C_{18}H_{18}N_2O_8Na$ $(M+Na)^+$ 413.0961, found 413.0961; Analytical HPLC t_R = 3.97 min.

6.45. (DL)-2,3-Bis(3,4,5-trihydroxybenzoylamino)propionic acid methyl ester (57)

To crude diamide **14** (40 mg, 0.0519 mmol) was added 0.50 M NaOMe (0.66 mL). The resultant solution was shaken for 1 h, neutralized with 10% HCl, and purified via preparatory HPLC (95–55%, eluent A) to afford 6 mg (15%) of ester **57**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.89 (s, 2H), 6.83 (s, 2H), 4.68 (dd, J = 6.5, 5.0, 1H), 3.79–3.85, (m, 2H), 3.76 (s, 3H); HRMS (CI/methanol) m/z calcd for $C_{18}H_{18}N_2O_{10}Na$ $(M+Na)^+$ 445.0859, found 445.0841; Analytical HPLC t_R = 3.57 min.

6.46. (DL)-2,3-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]propionic acid methyl ester (58)

Following the procedure for **56**, crude diamide **14** (75 mg, 0.111 mmol) was hydrolyzed to afford 26 mg (53%) of ester **58**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (d, J = 15.7, 1H), 7.40 (d, J = 15.7, 1H), 7.02 (d, J = 2.0, 1H), 7.00 (d, J = 2.0, 1H), 6.93 (dd, J = 8.5, 2.0, 1H), 6.91 (dd, J = 8.4, 2.0, 1H), 6.77 (d, J = 8.2, 1H), 6.76 (d, J = 8.2, 1H), 6.45 (d, J = 15.7, 1H), 6.36 (d, J = 15.7, 1H), 4.69 (dd, J = 6.7, 5.3, 1H), 3.80 (dd, J = 13.9, 5.3, 1H), 3.76 (s, 3H), 3.68 (dd, J = 13.8, 6.8, 1H); HRMS (CI/methanol) m/z calcd for $C_{22}H_{22}N_2O_8Na$ $(M+Na)^+$ 465.1274, found 465.1255; Analytical HPLC t_R = 4.69 min.

6.47. (DL)-2,4-Bis(3,4-dihydroxybenzoylamino)butyric acid methyl ester (59)

Following the procedure for **57**, crude diamide **16** (75 mg, 0.118 mmol) was hydrolyzed to afford 10 mg (21%) of ester **59**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (d, J = 2.2, 1H), 7.31 (dd, J = 8.3, 2.2, 1H), 7.29 (d, J = 2.2, 1H), 7.21 (dd, J = 8.3, 2.2, 1H), 6.83 (d, J = 8.3, 1H), 6.79 (d, J = 8.3, 1H), 4.71 (dd, J = 8.6, 4.9, 1H), 3.58–3.66 (m, 1H), 3.68 (s, 3H), 3.30–3.36 (m, 1H), 2.18–2.26 (m, 1H), 2.07–2.16 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{19}H_{20}N_2O_8Na$ $(M+Na)^+$ 427.1117, found 427.1102; Analytical HPLC t_R = 4.04 min.

6.48. (DL)-2,4-Bis(3,4,5-trihydroxybenzoylamino)butyric acid methyl ester (60)

Following the procedure for **56**, crude diamide **17** (75 mg, 0.0957 mmol) was hydrolyzed to afford 29 mg (70%) of ester **60**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.95 (s, 2H), 6.86 (s, 2H), 4.69–472 (m, 1H), 3.65–3.72 (m, 2H), 2.16–2.24 (m, 1H), 2.06–2.14 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{23}H_{24}N_2O_8Na$ $(M+Na)^+$ 479.1430, found 479.1409; Analytical HPLC t_R = 3.57 min.

6.49. (DL)-2,4-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]butyric acid methyl ester (61)

Following the procedure for **56**, crude diamide **18** (75 mg, 0.109 mmol) was hydrolyzed to afford 14 mg (28%) of ester **61**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.42 (d, J = 15.7, 1H), 7.40 (d, J = 15.7, 1H), 7.03 (d, J = 2.0, 1H), 7.00 (d, J = 2.0, 1H), 6.93 (dd, J = 8.2, 2.0, 1H), 6.91 (dd, J = 8.3, 2.0, 1H), 6.77 (d, J = 8.2, 1H), 6.76 (d, J = 8.1, 1H), 6.46 (d, J = 15.7, 1H), 6.36 (d, J = 15.7, 1H), 4.61 (dd, J = 9.0, 4.8, 1H), 3.74 (s, 3H), 3.70–3.77 (m, 1H), 3.43–3.51 (m, 1H), 2.10–2.20 (m, 1H), 1.93–2.01 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{23}H_{24}N_2O_8Na$ $(M+Na)^+$ 479.1430, found 479.1409; Analytical HPLC t_R = 4.69 min.

6.50. (DL)-2,5-Bis(3,4-dihydroxybenzoylamino)pentanoic acid methyl ester (62)

Following the procedure for **57**, crude diamide **19** (75 mg, 0.115 mmol) was hydrolyzed to afford 32 mg (66%) of ester **62**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.31 (d, J = 2.1, 1H), 7.27 (d, J = 2.1, 1H), 7.27 (dd, J = 8.2, 2.2, 1H), 7.20 (dd, J = 8.3, 2.2, 1H), 6.81 (d, J = 8.4, 1H), 6.79 (d, J = 8.5, 1H), 4.59 (dd, J = 9.31, 5.1, 1H), 3.73 (s, 3H), 3.38 (t, J = 7.0, 2H), 1.96–2.0 (m, 1H), 1.83–2.01 (m, 2H), 1.65–1.80 (m, 1H); HRMS (CI/methanol) m/z calcd for $C_{20}H_{22}N_2O_8Na$ $(M+Na)^+$ 441.1274, found 441.1265; Analytical HPLC t_R = 4.16 min.

6.51. (DL)-2,5-Bis(3,4,5-trihydroxybenzoylamino)pentanoic acid methyl ester (63)

Following the procedure for **57**, crude diamide **20** (75 mg, 0.0940 mmol) was hydrolyzed to afford 10 mg (24%) of ester **63**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.89 (s, 2H), 6.84 (s, 2H), 4.57 (dd, J = 9.2, 5.0, 1H), 3.73 (s, 3H), 3.37 (t, J = 7.0, 2H), 1.94–2.03 (m, 1H), 1.82–1.91 (m, 1H), 1.64–1.78 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{20}H_{22}N_2O_{10}Na$ $(M+Na)^+$ 473.1172, found 473.1174; Analytical HPLC t_R = 3.62 min.

6.52. (DL)-2,5-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]pentanoic acid methyl ester (64)

Following the procedure for **57**, crude diamide **21** (75 mg, 0.107 mmol) was hydrolyzed to afford 29 mg (58%) of ester **64**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (d, J = 15.7, 1H), 7.39 (d, J = 15.6, 1H), 7.02 (d, J = 2.0, 1H), 7.00 (d, J = 2.0, 1H), 6.91 (dd, J = 8.2, 2.0, 1H), 6.89 (dd, J = 8.3, 2.0, 1H), 6.77 (d, J = 8.2, 1H), 6.75 (d, J = 8.2, 1H), 6.45 (d, J = 15.7, 1H), 6.36 (d, J = 15.8, 1H), 4.54 (dd, J = 8.9, 5.1, 1H), 3.74 (s, 3H), 3.33 (t, J = 7.0, 2H), 1.90–1.97 (m, 1H), 1.74–1.83 (m, 1H), 1.60–1.72 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{24}H_{26}N_2O_8Na$ $(M+Na)^+$ 493.1587, found 493.1583; Analytical HPLC t_R = 4.92 min.

6.53. L-2,6-Bis(3,4-dihydroxybenzoylamino)hexanoic acid methyl ester (65)

Following the procedure for **57**, crude diamide **22** (75 mg, 0.113 mmol) was hydrolyzed to afford 26 mg (53%) of ester **65**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.30 (d, J = 2.1, 1H), 7.26 (d, J = 2.2, 1H), 7.25 (dd, J = 8.3, 2.2, 1H), 7.17 (dd, J = 8.3, 2.2, 1H), 6.79 (d, J = 8.2, 1H), 6.77 (d, J = 8.2, 1H), 4.55 (dd, J = 9.4, 5.2, 1H), 3.72 (s, 3H), 3.34 (dt, J = 6.8, 1.9, 2H), 1.92–2.01 (m, 1H), 1.83–1.91 (m, 1H), 1.59–1.71 (m, 2H), 1.44–1.56 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{21}H_{25}N_2O_8$ $(M+H)^+$ 433.1611, found 433.1613; Analytical HPLC t_R = 4.32 min.

6.54. L-2,6-Bis(3,4,5-trihydroxybenzoylamino)hexanoic acid methyl ester (66)

Following the procedure for **57**, crude diamide **23** (75 mg, 0.0924 mmol) was hydrolyzed to afford 7 mg (16%) of ester **66**: ^{1}H NMR (500 MHz, CD_3OD) δ 6.88 (s, 2H), 6.83 (s, 2H), 4.53 (dd, J = 9.2, 5.3, 1H), 3.72 (s, 3H), 3.28–3.36 (m, 2H), 1.92–2.00 (m, 1H), 1.81–1.88 (m, 1H), 1.58–1.70 (m, 2H), 1.43–1.56 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{21}H_{24}N_2O_{10}Na$ $(M+Na)^+$ 487.1329, found 487.1328; Analytical HPLC t_R = 3.85 min.

6.55. L-2,6-Bis[3-(3,4-dihydroxyphenyl)acryloylamino]hexanoic acid methyl ester (67)

Following the procedure for **57**, crude diamide **24** (72 mg, 0.101 mmol) was hydrolyzed to afford 27 mg (55%) of ester **67**: ^{1}H NMR (500 MHz, CD_3OD) δ 7.41 (d, J = 15.4, 1H), 7.38 (d, J = 15.3, 1H), 7.01 (d, J = 2.0, 1H), 6.99 (d, J = 2.0, 1H), 6.90 (dd, J = 8.2, 2.0, 1H), 6.87 (dd, J = 8.2, 2.0, 1H), 6.76 (d, J = 8.2, 1H), 6.75 (d, J = 8.2, 1H), 6.45 (d, J = 15.6, 1H), 6.35 (d, J = 15.6, 1H), 4.51 (dd, J = 9.0, 5.1, 1H), 3.73 (s, 3H), 3.29–3.35 (m, 2H), 1.85–1.95 (m, 1H), 1.74–1.82 (m, 1H), 1.56–1.65 (m, 2H) 1.43–1.52 (m, 2H); HRMS (CI/methanol) m/z calcd for $C_{25}H_{28}N_2O_8Na$ $(M+Na)^+$ 507.1743, found 507.1722; Analytical HPLC t_R = 5.15 min.

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