DESIGN, SYNTHESES AND BIOLOGICAL ACTIVITIES OF L-CHICORIC ACID ANALOGUES AS HIV-1 INTEGRASE INHIBITORS

by

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Ac	Acetyl
AIDS	Acquired immune deficiency syndrome
Ar	Aryl
Anal.	Analysis (in elemental analysis data)
br	Broad
t-Bu	*tert*-Butyl
L-CA	L-Chicoric acid
calcd.	Calculated
CAPE	Caffeic acid phenethyl ester
5CITEP	1-(5-Chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)-propenone
CT_5	Five percent cytotoxic dose
d	Doublet (in NMR)
dd	Doublet of doublets (in NMR)
dec.	Decompose
dt	Doublet of triplets (in NMR)
DCTA	Dicaffeoyltartaric acid
DKA	Diketo acid
DMAP	4-Dimethylaminopyridine
DME	1,2-Dimethoxyethane

DMF	*N*,*N*-Dimethylformamide
DMPU	*N*, *N*'-Dimethylpropyleneurea
DMSO-d_6	Deuterated dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ED_{50}	Fifty percent effective dose
EDC	1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide
eq	Equivalent
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
Fig.	Figure
h	Hour
HIV-1	Human immunodeficiency virus type 1
HMPA	Hexamethylphosphoramide
HOAc	Acetic acid
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
IC_{50}	Fifty percent inhibitory activity
IN	Integrase
KHMDS	Potassium bis(trimethylsilyl)amide

LDA	Lithium diisopropylamide
lit.	Literature
m	Multiplet (in NMR)
MCPBA	*m*-Chloroperoxybenzoic acid
min	Minute
mol	Mole (s)
mmol	Millimole
mp	Melting point
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
ppm	Parts per million
PR	Protease
Ret.	Retention (in HPLC)
R_f	Retention factor (in chromatography)
RNA	Ribonucleic acid
rt	Room temperature
RT	Reverse transcriptase
s	Singlet (in NMR)
SAR	Structure-activity relationship
t	Triplet (in NMR)
td	Triplet of doublets (in NMR)

TEA	Triethylamine
TFA	Trifluoro acetic acid
THF	Tetrahydrofuran
TI	Therapeutic index
TLC	Thin layer chromatography
TMS	Trimethylsilyl
XLI or XLII	Notebook number. The same compound prepared at different times or by different methods will have different notebook numbers.

Chapter 1. Introduction

The human immunodeficiency virus type 1 (HIV-1) is the causative agent of the acquired immune deficiency syndrome (AIDS), which has been one of the world's most serious health problems since 1981. By killing or damaging cells of the immune system, HIV progressively destroys the body's ability to withstand infections and certain cancers.

Some key points in the HIV life cycle (Fig. 1-1) include (1) viral attachment to the cell surface; (2) viral penetration of the cell membrane; (3) viral RNA decoding by reverse transcription to the proviral DNA; (4) integration of the proviral DNA into the host genome; (5) synthesis of the viral proteins including the nucleocapsid protein; (6) processing of the viral polypeptide with the HIV protease and the assembly of the viral RNA and proteins into a viral particle which undergoes final maturation and shedding of the mature infectious viral particles. [1]

Looking at the HIV life cycle, three essential enzymes can be distinguished: reverse transcriptase (RT), integrase (IN), and protease (PR). Currently available drugs target RT and PR to interrupt the viral replication cycle. There are now several nucleoside RT inhibitors and non-nucleoside RT inhibitors [2] that have been approved for use in HIV infection as well as some Food and Drug Administration (FDA)-approved agents that act at HIV PR. [3] RT inhibitors, when used in monotherapy, are relatively weak anti-HIV agents whose efficacy is further impaired by toxicity and the emergence of variants of HIV that are resistant to their effects. PR inhibitors alone and in combination with RT inhibitors are potent inhibitors of HIV replication. As with the RT inhibitors, however, resistance to PR inhibitors can occur. [4] In recent years, the advent of this

therapy has made it possible to suppress HIV replication to such an extent that the virus becomes undetectable in many infected persons, leading to a reduction of both morbidity and mortality. [5] However, despite chemotherapy, these treatments fail to eradicate viral replication, which persists at a lower level. In such conditions HIV-1 escapes the therapeutic protocol via generation of resistant mutant strains. The reasons for this evolution are 1) suboptimal regimens due to the high toxicity of the drugs and 2) the low compliance of patients in long-term therapies. [6] To obtain a more effective treatment of AIDS, there is substantial interest in the development of inhibitors targeted at the other viral enzyme, IN. [7-10]

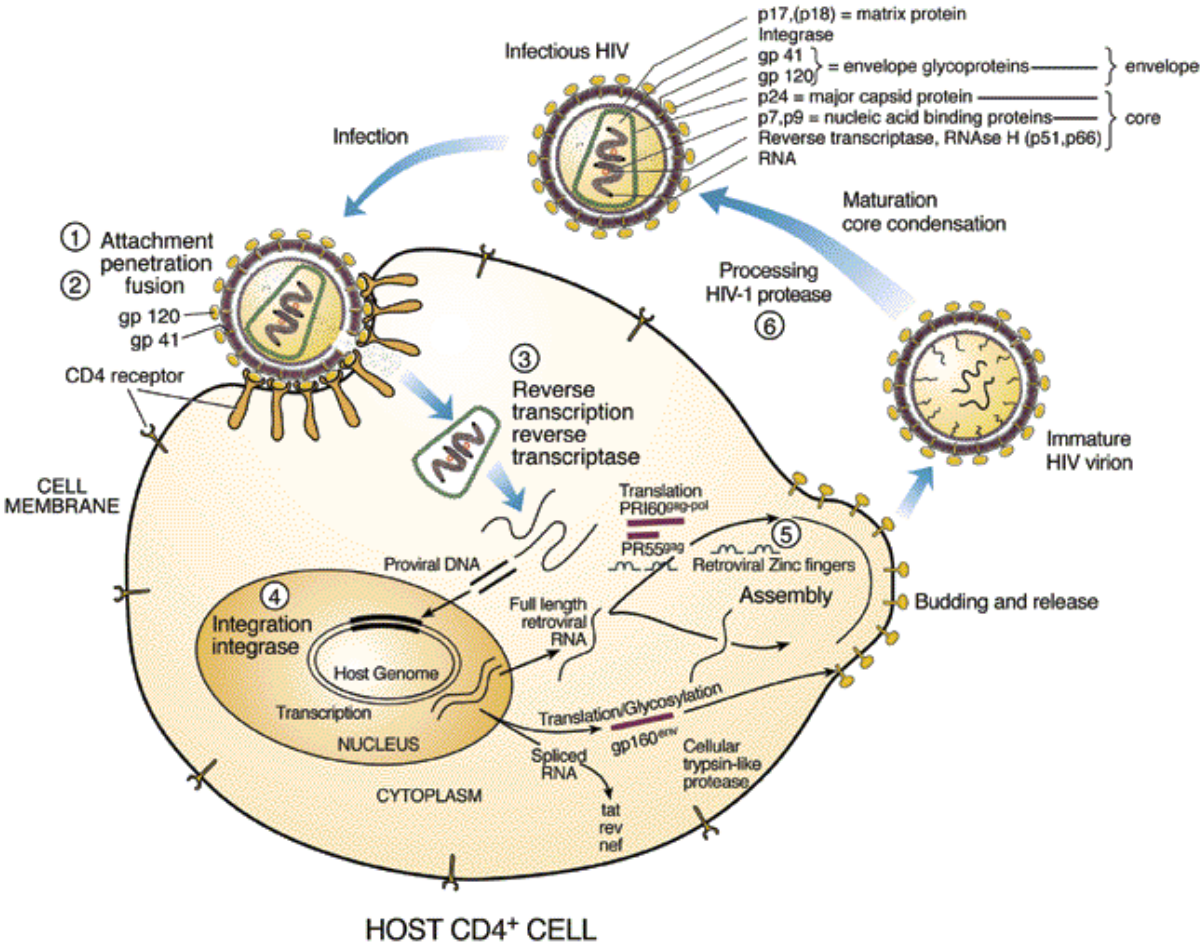


Fig. 1-1 Targets within the different phases of HIV-1 viral replication cycle and infection of a T-cell, as used for anti-HIV cell-based assays [1]

IN catalyzes multiple steps in the integration process, as shown schematically in Fig. 1-2. Integration of the proviral DNA into the host cell's genomic DNA is achieved by IN performing a series of DNA cutting and joining reactions. The first step in the integration process is 3'-processing, in which two nucleotides are removed from each 3'-end of the proviral DNA, leaving recessed CA OHs at the 3' ends (Fig. 1-2A). In a second step, termed "strand transfer", the processed 3' ends are inserted into the target DNA at the site of integration (Fig. 1-2B). The sites of integration on the two target DNA strands are separated by five base pairs. The final step is the repair step, which requires removal of the two unpaired nucleotides at the 5'-ends of the viral DNA, filling the single gaps and finally ligation (Fig. 1-2C). IN is responsible for 3'-processing

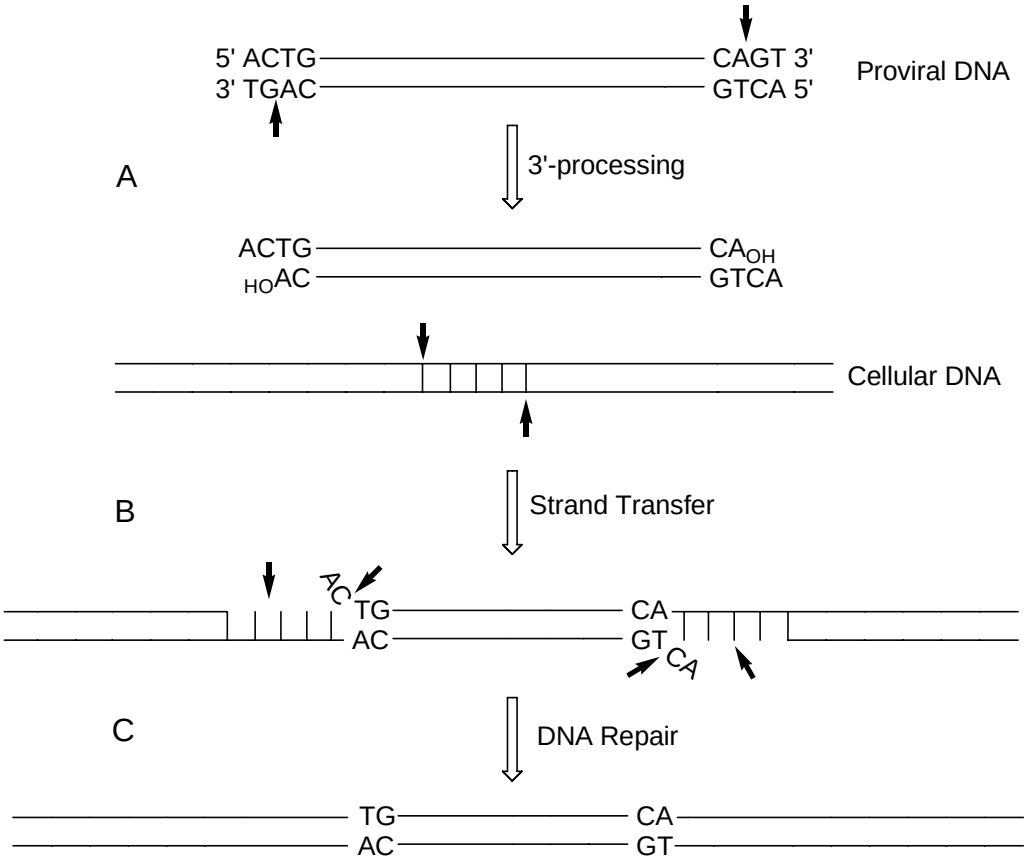
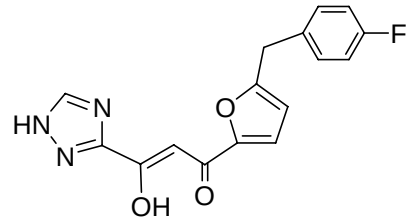


Fig. 1-2 Schematic steps for HIV-1 integration [11,12]

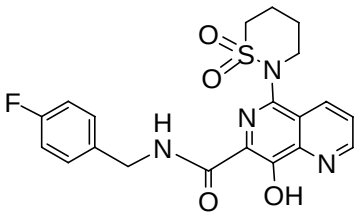
and strand transfer, but the repair steps are likely to be carried out by cellular enzymes. [6,8,11-14]

IN is a very attractive target for developing new anti-HIV drugs because: 1) it plays a vital role in the retroviral replication cycle; 2) it has no known functional homologue in human cells, and so potential IN-selective inhibitors could be relatively nontoxic; 3) synergistic potency against viruses has been found when IN inhibitors are combined with either RT or PR inhibitors in *in vitro* assays. [4,15]

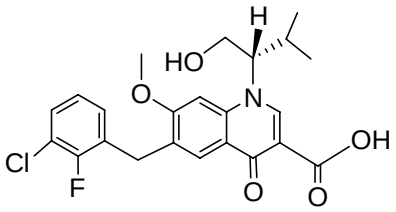
The design of strong and selective HIV-1 IN inhibitors is currently hampered by the fact that the complete three-dimensional bioactive structure of HIV-1 IN is still unknown. Although a wide variety of compounds have been reported as IN inhibitors, no drug active against this enzyme has as yet been approved by the U.S. FDA. Two compounds, Merck's L-870,810 [16,17] and Shionogi/GlaxoSmithKline's S-1360 [18] (Fig. 1-3), were developed in Phase I/II clinical trials as promising anti-IN drugs. [19] However, L-870,810 had to be abandoned after unacceptable liver and kidney cell toxicity was found in dogs given the compound, [20] and S-1360 had to be discontinued because of poor bioavailability. [21] Current promising anti-IN drugs include Merck's MK-0518 in Phase III trials, [22,23] and Gilead's GS 9137 in Phase II trials. [24-26] The early results of both compounds look positive and encouraging. [20,22-25,27] The structure of MK-0518 has not yet been reported. [28]



S-1360



L-870,810

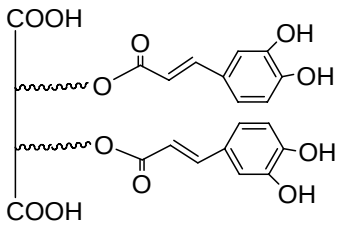


GS 9137

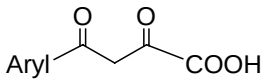
MK-0518

Fig. 1-3 IN inhibitors previously and currently in clinical studies

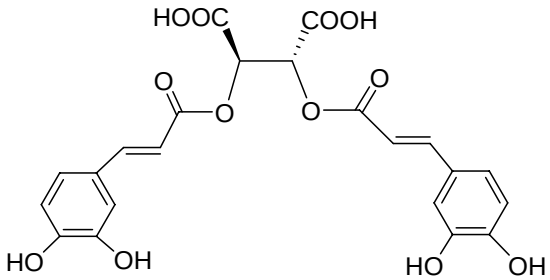
Among numerous reported HIV IN inhibitors, [29-36] to date, there are only two classes of molecule that meet the criteria necessary to be considered lead molecules in the search for clinically useful inhibitors of IN. The first are the dicaffeoyltartaric acids (DCTAs), [37-40] and the second are the aryl diketo acids (DKAs) (Fig. 1-4). [41-43] Both S-1360 and L-870,810 are analogues of DKAs.



DCTAs



DKAs



L-CA

Fig. 1-4 Structures of DCTAs, DKAs and L-CA

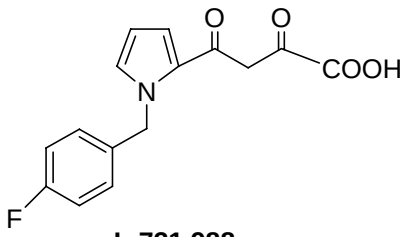
DCTAs inhibit HIV IN in biochemical assays and HIV replication at non-toxic concentrations in tissue culture. [37] The lead compound in the series of DCTAs is L-DCTA (L-

chicoric acid, L-CA) with a fifty percent inhibitory activity (IC_{50}) against HIV-1 IN of 0.18 μM. This same compound blocked HIV-1 infection of MT-2 cells with a fifty percent effective dose (ED_{50}) of 4.2 μM. The concentration of this molecule that inhibited cell growth by 5%, the five percent cytotoxic dose (CT_5), was 264 μM. [37,44] Thus it has a therapeutic index ($TI = CT_5/ED_{50}$) of 63, which indicates this molecule has a good selectivity for HIV over cellular proteins. The DCTAs have been used as part of a pharmacophore that successfully identified a new class of compounds that inhibit HIV IN. [45,46] Indeed, thirty of these molecules inhibited HIV replication below 10 μM and eight below 1 μM. Structure-activity relationship (SAR) studies using L-CA as a lead compound identified an additional three molecules with IC_{50} against HIV IN below 1 μM and twelve with IC_{50} below 10 μM. [38] Our own laboratory has identified numerous L-CA analogues with IC_{50} below 10 μM, twenty-one analogues with IC_{50} below 1 μM, and fully eighteen of these inhibit HIV replication at non-toxic concentrations.[37,39]

The key structural features of L-CA (Fig. 1-4) are two caffeoyl moieties and two free carboxyl groups. Previous SAR studies of L-CA analogues demonstrated that the bis-catechol moieties were absolutely required for inhibition of IN, while at least one free carboxyl group was required for maximal inhibition of HIV IN and for anti-HIV activity in tissue culture. [37,47] Studies also showed that while two caffeoyl moieties were required, attachment of caffeoyl groups to the central linking structure could be achieved via ester, amide or mixed amide/ester linkages. [38,39]

More recently, DKAs have been identified as a new class of potent IN inhibitors. These compounds effectively prevent proviral DNA integration and inhibit HIV-1 replication in cell culture. The first inhibitor L-731,988 is a pyrrole derivative synthesized by the Merck

Company.[41] The activity of L-731,988 is remarkable in that it discriminates between the two catalytic activities of IN----inhibiting strand transfer with an IC_{50} of 100 nM and 3'-processing with an IC_{50} of 5 μM. Previous SAR studies of DKAs included 1) changing aryl rings and their substituents; [41-43,48,49] 2) masking the terminal carboxylic function with a tetrazole ring; [43,48] 3) shortening the dioxobutanoic group into an oxopropanoic moiety; [50] and 4) elongating the dioxobutanoic group into a dioxohexenoic moiety. [11,51] Like the DCTAs, the DKAs require either a free carboxylic acid [41] or one of its bioisosteres, such as a tetrazole ring.[33,43,48]



L-731,988

L-CA and DKAs appear to act in a different manner on the IN protein. L-CA inhibits equally well the 3'-processing, strand transfer, and disintegration [52] (the reverse of strand transfer) reactions. Docking experiments, using program AutoDock version 3.0, suggest that half of the L-CA molecule makes use of the area in front of the two catalytic aspartates (D64 and D116) but without contacting them, and that L-CA interacts with lysines K156 and K159, as well as histidine H67, glutamates E92 and E152, and glutamine Q148 (Fig. 1-5). In terms of van der Waals interactions, most contacts are made with E152 and Q148, followed by K159 and H67. [53] The most recent molecular modeling study on the interaction between HIV IN and L-CA indicates that L-CA contacts D64, cysteine C65, threonine T66, H67, D116, Q148, glycine G149, H51, E152, asparagine N155, and K159 in the HIV IN active site, and that the interactions

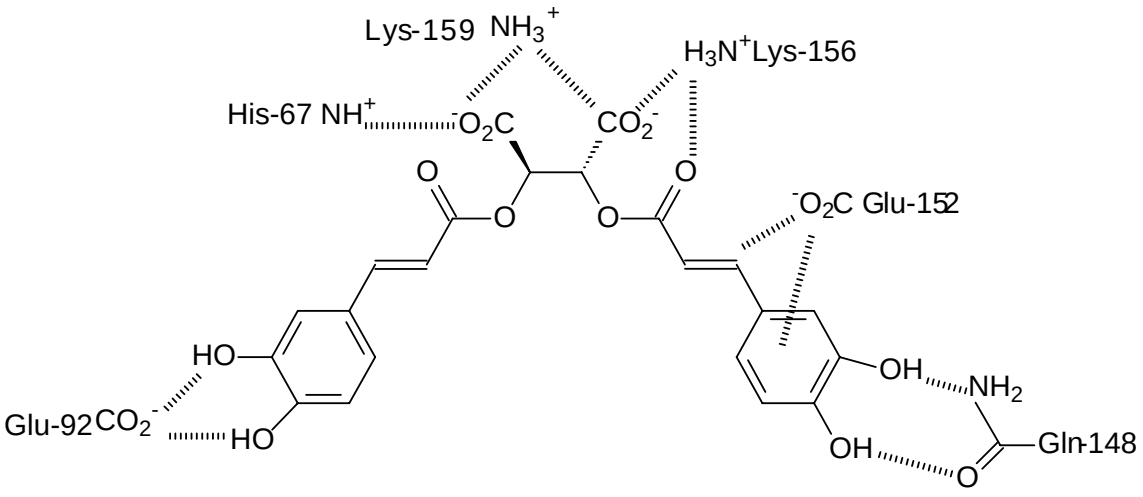
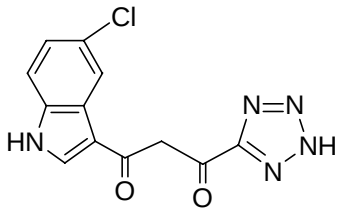


Fig. 1-5 Computer Model of L-chicoric acid in the HIV IN active site [53]

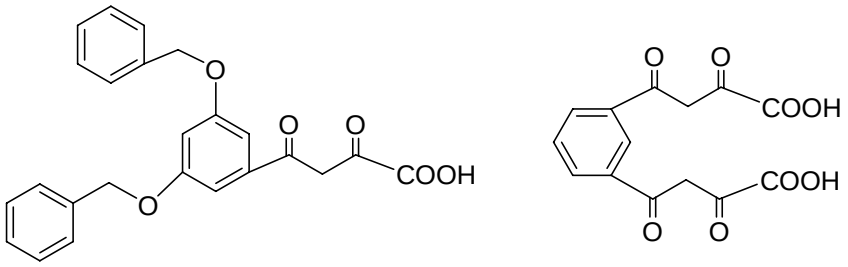
between the so-called conserved DDE motif (D64, D116, and E152) and the caffeoyl groups play a critical role in the inhibitory activity. [54] In the latter case, no specific interactions between functional groups of L-CA and residues were given. In both cases, results are based on the crystal structure of IN complexed with the inhibitor 1-(5-chloroindol-3-yl)-3-hydroxy-3-(2*H*-tetrazol-5-yl)-propenone (5CITEP). [55] As stated by Schames *et al*, [56] the information provided by the crystal structure is questionable since IN is thought to form a large complex with DNA, but this relevant IN-DNA complex structure is unknown. Thus, the actual binding site of IN is not understood; only key residues are known.



5CITEP

Studies indicate that monofunctional DKAs, such as L-731,988 and L-708,906 (Fig. 1-6), preferably or selectively interfere with strand transfer, [41] while bifunctional DKAs, such as the

bis-aryldiketo acid **1** (Fig. 1-6), whose aromatic portion has been modified to bear a second diketo acid side chain, inhibit 3'-processing as well as strand transfer (Table 1-1). [13,43] This suggests that the second acidic function of DKA **1** binds to the enzyme site that catalyzes 3'-processing. [13] Based on the X-ray structure of the IN catalytic core domain complexed with the inhibitor 5CITEP, [55] a model for the binding of the monofunctional DKA analogues to IN was developed (Fig. 1-7) [57] in which the inhibitor coordinates two metals (possibly Mg^{2+}) [8,12] bound at the active site by the conserved DDE motif of HIV-1 IN. The mechanism of action of these inhibitors is therefore likely a consequence of the interaction between the enolic diketo-acid moiety and the metal ion(s) in the IN active site. Docking models of L-708,906 [43] and **1** [58] in the



L-708,906 **1**

Fig. 1-6 Chemical structures of typical DKAs

Table 1-1 Comparison of biological activity (in μM) of typical monofunctional and bifunctional DKAs

Compound	IC_{50} 3'-Processing	IC_{50} Strand transfer
L-731,988 [41]	5	0.1
L-708,906 [41]	>1000	0.42 ± 0.08
1 [43]	7.8 ± 2.2	1.83 ± 0.32

IC_{50}, the 50% inhibitory concentration, is the concentration of compound that inhibits full-length IN in the 3'-processing or strand transfer reaction by 50%.

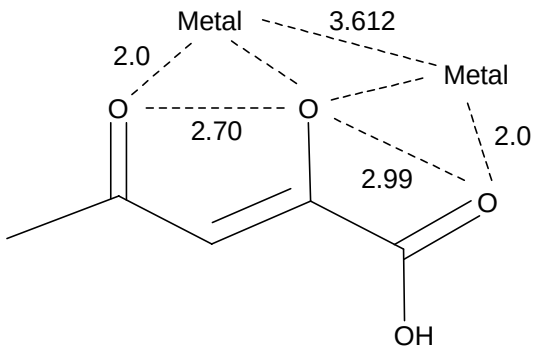


Fig. 1-7 Model for the binding of two divalent metals by DKA inhibitors [57]

IN active site also indicate involvement of the metal ion(s). More recently structural models of the full-length IN and its complex with viral and human DNA were developed based on all available experimental evidence. [59] Using these models, docking studies indicate that the DKA IN inhibitors probably chelate the metal ion(s) in the catalytic site and also prevent the exposure of the nucleophilic hydroxyl group at the 3'-processed end of the viral DNA to human DNA, thus leading to the selective potency in strand transfer inhibition. [59]

The goal of the research described in this dissertation is to synthesize analogues of L-CA and DKAs so as to identify compounds with improved potency against HIV IN, improved anti-HIV activity in tissue culture, and increased selectivity as indicated by low cellular toxicity. These analogues primarily will involve modifications of L-CA, a potent anti-HIV compound at non-toxic concentrations. Specific modifications will involve the catechols as well as the scaffold to which they are attached. Additionally, several L-CA analogues that incorporate the DKA moiety will be synthesized. The IC_{50} of each synthesized molecule against HIV IN will be determined. Second, the CT_5 and the ED_{50} will be determined against HIV and the target cells (MT-2, a CD4+ T lymphoblastoid cell line).

Scaffold Modification

Since L-CA displays potent activity against HIV IN and can inhibit HIV replication with moderate anti-HIV activity, extensive efforts have been made to develop caffeoyl-based HIV IN inhibitors. The common structural features of reported synthetic analogues are caffeic acid esters/amides separated by aliphatic, alicyclic, or aromatic linkers, [37-39] or a glucose ring as a central linker. [36] More recently, dicaffeoyl compounds joined through a functionalized pyrrolidine or furan ring linker were reported (Fig. 1-8). [60] The linkers were designed as functionalized five-membered rings which have hydrogen bond accepting or donating groups in place of the carboxylic acid moieties of L-tartaric acid. Even though these compounds showed moderate HIV IN inhibitory activities, none of them inhibited the replication of HIV in a cell-based assay at nontoxic concentration supporting the important role of the carboxylic acid in L-CA. [60] The goal of our project was the modification of the central scaffold of L-CA using conformationally-restricted analogues to probe the geometry of the HIV IN active site which may lead to more potent IN inhibitors.

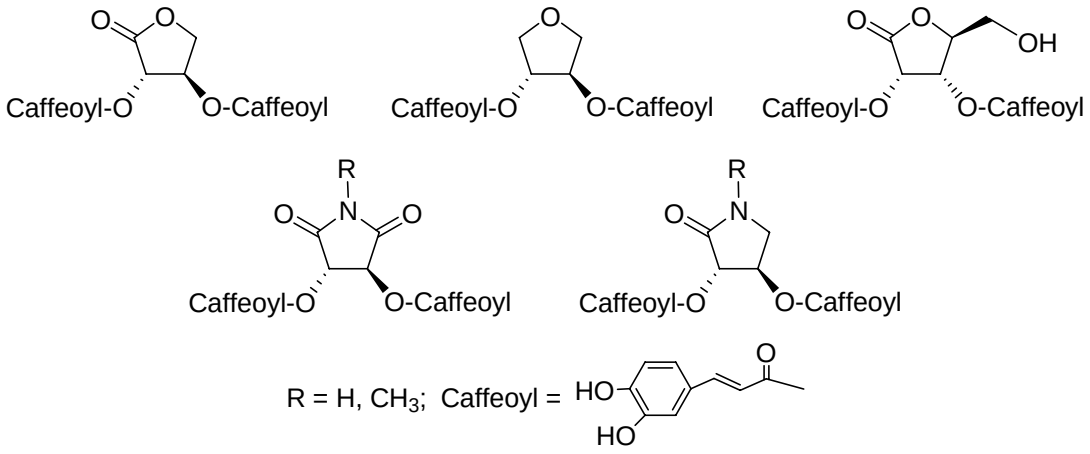


Fig. 1-8 Reported dicaffeoyl compounds joined through a functionalized pyrrolidine or furan ring linker [60]

The use of conformationally restricted molecules as a means to better understand or improve the activity of the parent molecule is a common theme in medicinal chemistry. This approach involves the synthesis of conformationally rigid analogues of flexible drug molecules. The potential pharmacophore becomes locked into various configurations by judicious incorporation of cyclic or unsaturated moieties into the drug molecule. These conformationally rigid analogues are then tested, and the analogue with the optimal activity (or potency) can be used as the prototype for further structural modification. An example is the syntheses of three conformationally rigid chair analogs of 4-(4-hydroxypiperidino)-4'-fluorobutyrophenone (**2** in Fig. 1-9) to determine the effect on receptor binding of having the hydroxyl group in the equatorial (**3**), axial (**4**), or both positions (**5**; as a stable ketal analogue). [61] Moreover, replacing conformationally flexible analogues with rigid analogues can increase binding affinity by simply lowering the entropy of binding. [62]

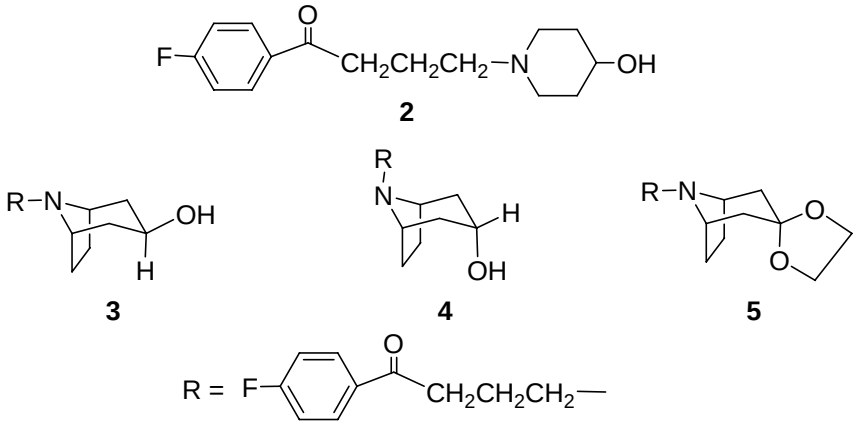


Fig. 1-9 4-(4-hydroxypiperidino)-4'-fluorobutyrophenone and its conformationally rigid chair analogues [61]

Because of the reported importance of the carboxylic acid group, our target compounds will incorporate this functional moiety. Our target compounds are therefore the stereoisomers of

3,4-dicaffeoylcyclopentane-1-carboxylic acid (Fig. 1-10). A conformationally flexible analogue, racemic-(3*R*,4*R*)-dicaffeoyl-adipic acid (**9**), was also synthesized for comparison of its inhibitory activity against HIV IN.

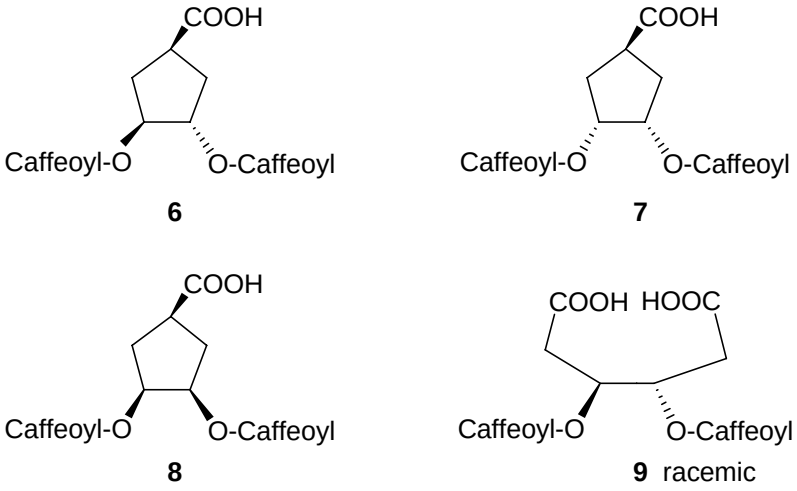
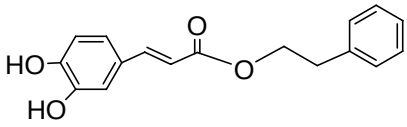


Fig. 1-10 Rigid analogues of L-CA, and *threo*-3,4-dicaffeoyloxyadipic acid

Since L-CA has poor cellular penetration, [44] some methyl esters of the targeted compounds were examined as potential prodrugs which could enter the cell more easily and be converted into an active molecule by a metabolic biotransformation catalyzed by esterases.

Catechol Modification

Catechols are considered to be undesirable as IN inhibitors because, as exemplified by caffeic acid phenethyl ester (CAPE)-like analogues, the catechol moiety can be oxidized *in situ*

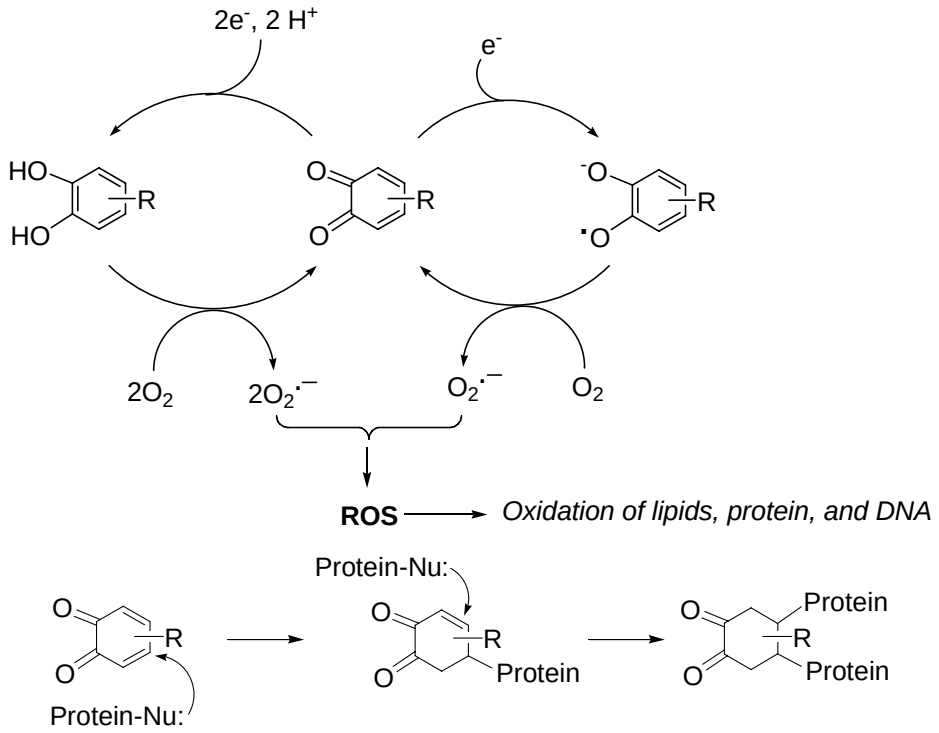


CAPE

to reactive quinone species that subsequently cross-link cellular protein nucleophiles via multiple 1,4-Michael-type additions (Scheme 1-1), leading to toxic side effects. [63-66] Moreover, the

reactive oxygen species (ROS) generated from the redox cycling of *ortho*-quinones can lead to oxidative stress and thus, cellular damage. [67] Thus, modification of the L-CA catechol moiety is desirable to decrease potential cytotoxicity.

Scheme 1-1 Redox cycling of *ortho*-quinones and possible mechanism of cross-linking by catechols [63,67]



To address this problem, one could either 1) replace the catechol moieties with their bioisosteres to reduce the ability of the compounds to form quinone species or 2) increase inhibitory potency relative to parent compounds, thereby reducing the amount of compound required for effective inhibition. The first solution is a common method in design and syntheses of analogues of catechol-containing biological molecules. [68-74] One example is the replacement of one or both catechol groups of AG 538, a potent inhibitor of insulin-like growth factor-1

receptor, with benzoxazolone groups. (Fig. 1-11). [69] Our goal in this part is to target the first solution----replace the catechol moieties with 2-pyridone groups.

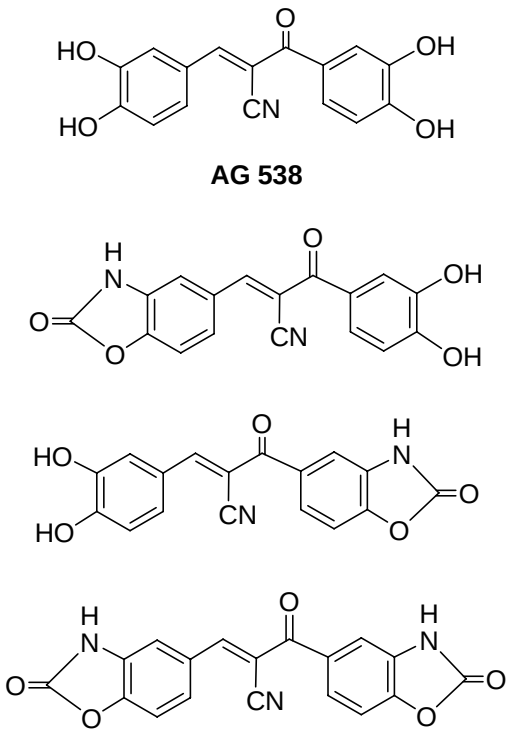


Fig. 1-11 AG 538 and its bioisosteres [69]

A number of biologically active molecules contain 2-pyridone moieties. For example, **10** (Fig. 1-12) is a potent inhibitor of dipeptidyl peptidase-4 (DPP-4), a novel therapeutic target to treat type 2 diabetes, [75] and reisantin B (**11**) has moderate anti-tumor activity. [76]

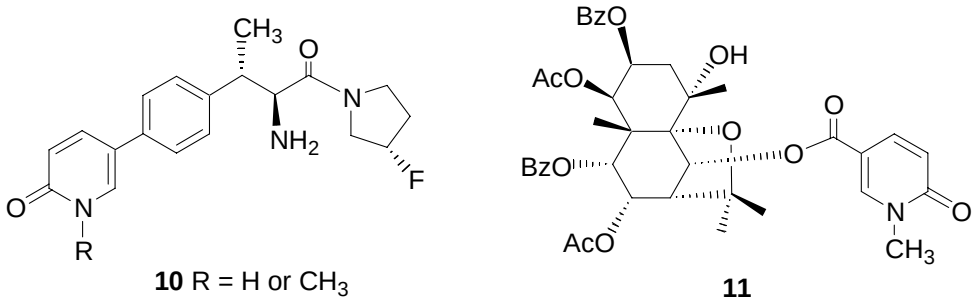
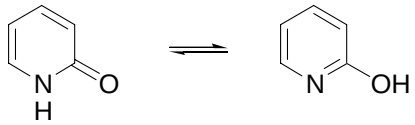


Fig. 1-12 Two biologically active molecules containing the 2-pyridone moieties

2-Pyridone can exist in two tautomeric forms (Fig. 1-13). In the gas phase and in dilute solution in nonpolar solvents the hydroxy tautomer **13** predominates over **12** by about 2:1. [77,78] On the other hand, in water the pyridone tautomer **12** is favored by almost 1000:1.[77,78] Therefore, it can be reasonably expected that once entering the cell, the pyridone tautomer **12** dominates *in vivo*.



12 **13**

Fig. 1-13 Tautomeric equilibrium of 2-pyridone

Both catechols [79,80] and 2-pyridones [81-84] complex metals, such as Mn, Mo, Pd, Pt, and Cu, a property which may be important for their inhibitory activities against HIV replication. Moreover, pyridones are much more resistant to oxidation than catechols. Only with very strong oxidants, such as ceric perchlorate in perchloric acid, is the oxidation of pyridones observed, leading to scission of the pyridine ring. [85] Thus, pyridones are more stable to metabolic oxidation than catechols while their ability to chelate metals is retained. Therefore, 2-pyridones may serve as bioisosteres of catechols.

Previous SAR studies of L-CA analogues demonstrated that one free carboxyl group was enough for inhibition of HIV IN and for anti-HIV activity in tissue culture (L-CA vs. **14** in Fig. 1-14 and Table 1-2), [37,40,47] and that attachment of side chains to the central linking structure could be achieved via ester, amide or mixed amide/ester linkages (**14** vs. **15** vs. **17**).[38,39] Moreover, based on the SAR studies of the caffeoyl-based analogs of L-CA, the amides, unlike

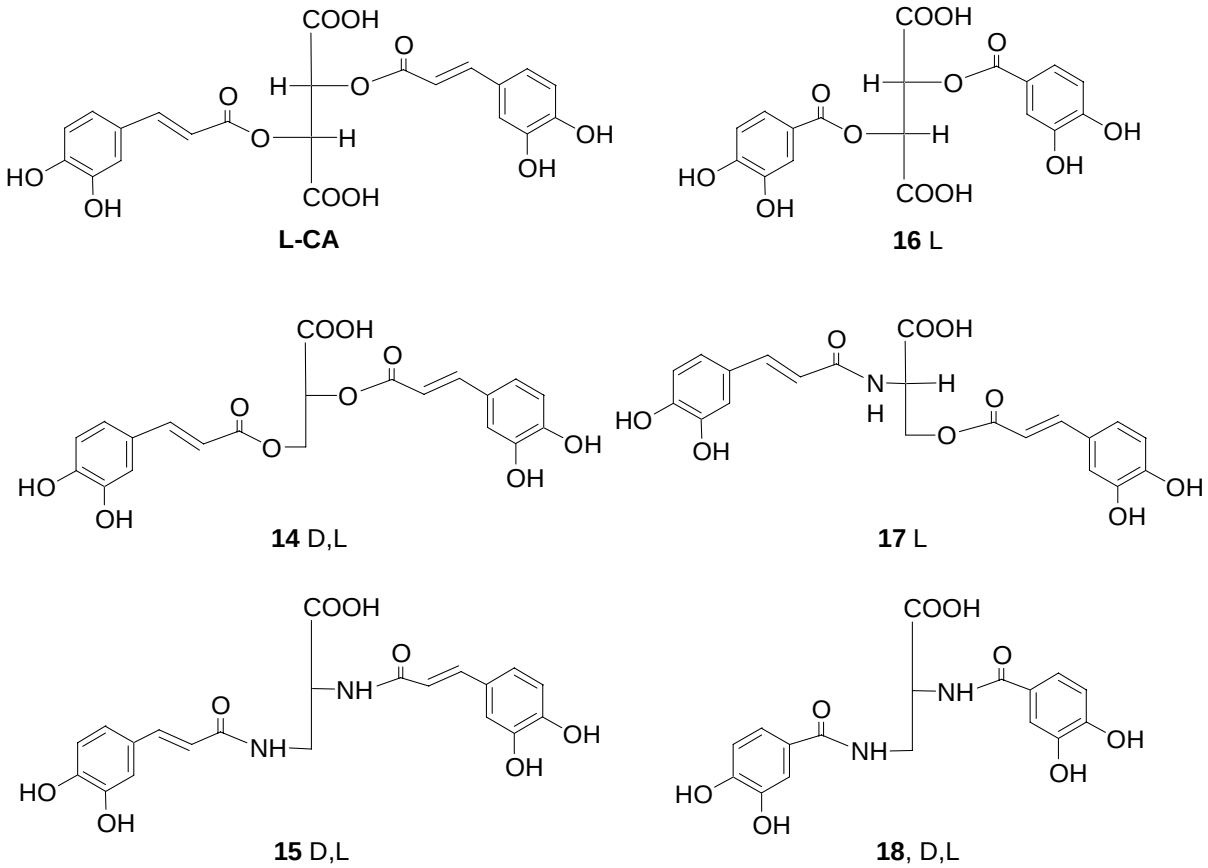


Fig. 1-14 Structures of L-CA and its analogues

Table 1-2 Biological activity (in μM) of L-CA and its analogues

Compound	CT_5	ED_{50}	TI_5	IC_{50} 3'-Processing	IC_{50} Disintegration
L-CA [39]	264	4.2	63	0.53	0.18
14 [37]	145	2.3	63		0.52
15 [86]	30	4.5	6.7	0.81	
16 [37]	90	0.95	94.7		0.43
17 [39]	39	2.5	16	0.33	0.12
18 [86]	39	94	0.4	>>10	

CT_5, the 5% cytotoxic dose, is the concentration of compound that inhibits cell growth by 5%; ED_{50}, the 50% effective dose, is the concentration of compound that inhibits HIV replication by 50%; TI_5, the therapeutic index, is the ratio of the CT_5 to ED_{50} and thus indicates the relative selectivity of the compound for HIV over cellular proteins. Refer to Table 1-1 for definitions of IC_{50}.

the esters, need a longer side chain. [37,86] Specifically, if the amide carbonyl is directly attached to the aromatic ring, the compounds had no activity. For example, without a double bond between the ester carbonyl and the aromatic ring, compound **16** (Fig. 1-14) is only slightly less active against HIV IN while it is even more active against HIV replication than L-CA. However, compound **18**, whose amide carbonyl is directly attached to the aromatic ring, has no activity against either HIV IN or HIV replication (**18** vs. **15**). Based on these results, possible target compounds are listed in Fig. 1-15. The ones actually prepared were selected based on the availability of chemicals and ease of synthesis.

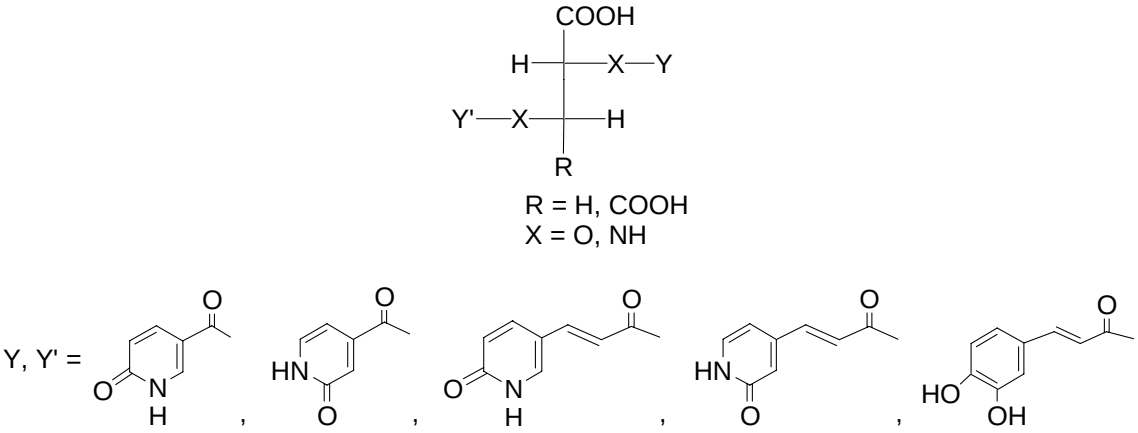
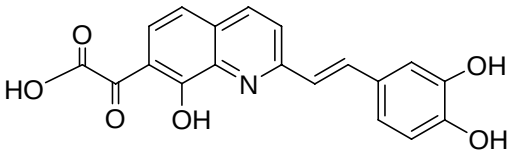


Fig. 1-15 Target L-CA analogues containing 2-pyridone groups

Once again, to achieve better cellular penetration, some methyl esters were also targeted to compare their HIV inhibitory activities with the corresponding acids.

Hybrid DKA/Catechol molecules

One reported example of mixed inhibitors is compound **19**, which is inactive against HIV IN in *in vitro* experiments, but has moderate antiviral activity. [87] The structure of compound **19** incorporates a catechol moiety and a HOOC-CO-C=C(OH)- system, which is similar to the enol



19

form of the α,γ-diketo acid pharmacophore in the DKAs.

Although there are SAR studies on analogues of L-CA [37-39] and DKAs, [11,41-43] until recently, [88] there has been no study on the design and synthesis of compounds containing both caffeoyl and diketo acid functionalities. Therefore, we targeted compounds **20-25** (Fig. 1-16), which are DKA derivatives with caffeoyl residues.

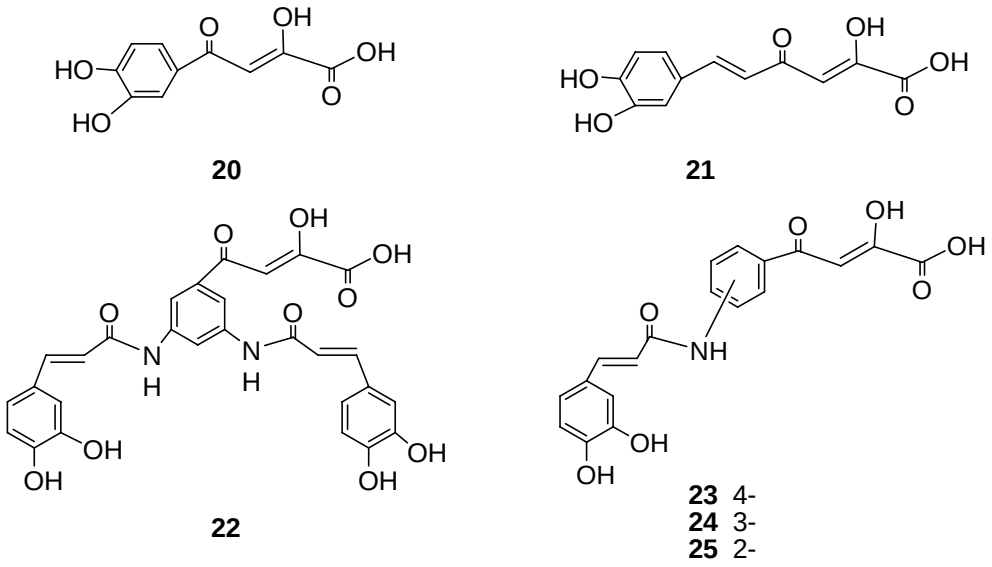
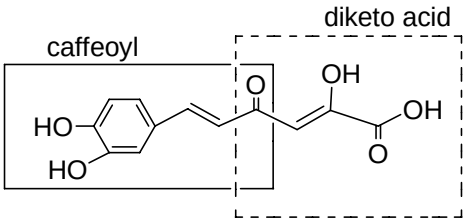


Fig. 1-16 Hybrid DKA/L-CA molecules

Compound **20** is an aryldiketo acid with a catechol side chain. Compound **21**, a dioxohexenoic acid, was targeted based on the partial superimposition of the caffeoyl group and the dioxobutanoic acid moiety (Fig. 1-17).

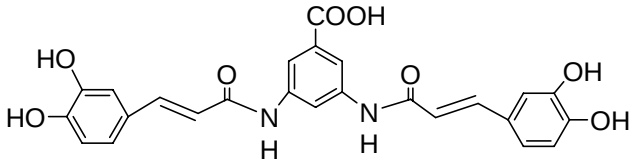
Compounds **22-25** differ from compound **21** in that the DKA group is on a benzene ring, with one or two caffeoylamino groups.



21

Fig. 1-17 Design of compound **21**

Compound **22** was chosen as our target since 3,5-dicaffeoylaminobenzoic acid (**26**) is a potent inhibitor of IN with IC_{50} of 0.73 μM, ED_{50} of 4.6 μM, and CT_5 of 47 μM. [39]



26

In order to test the effect of the number of the caffeoyl groups on the inhibitory activity, isomers **23** and **24** each containing one caffeoyl moiety were targeted as was the ortho isomer **25**.

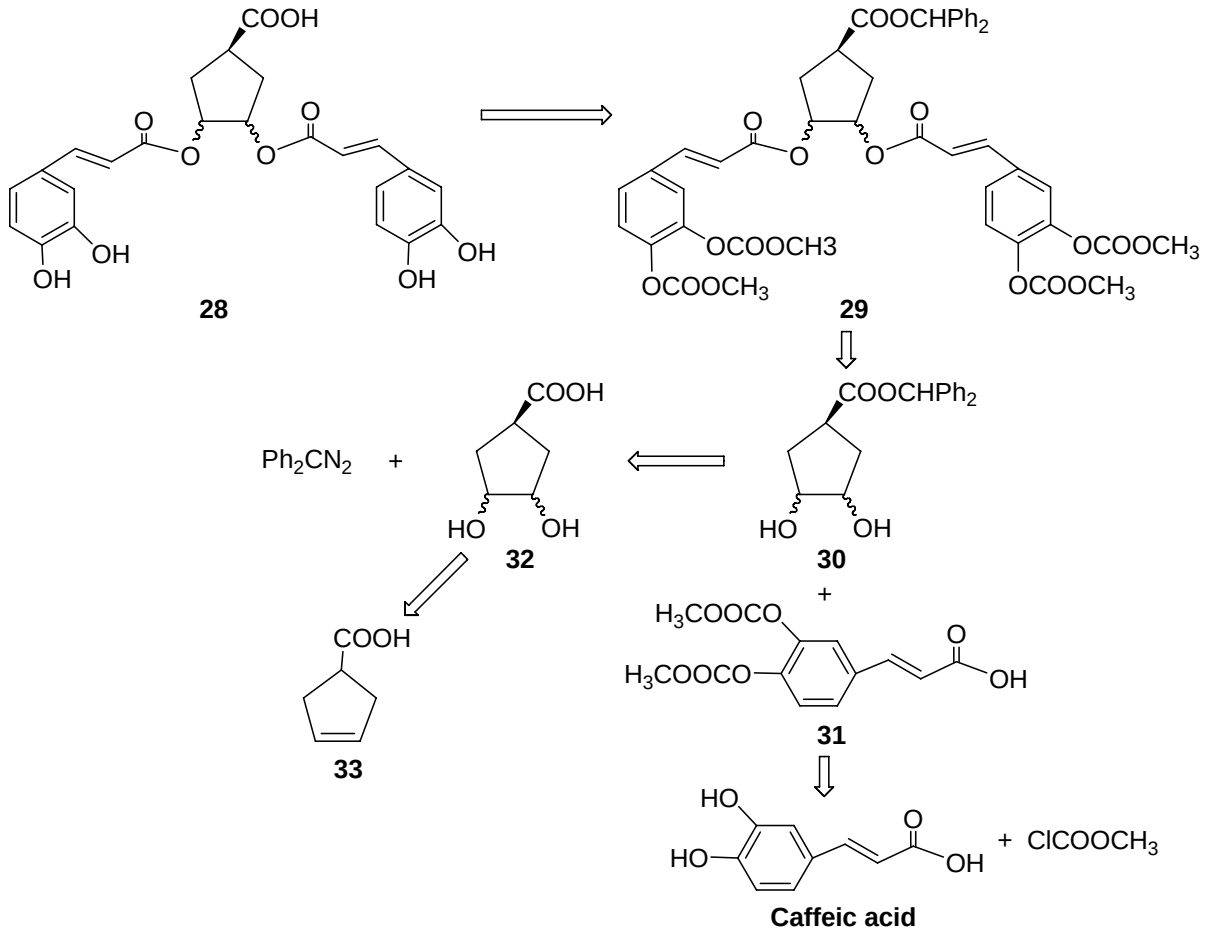
To achieve better cellular penetration, some methyl esters of the targeted compounds were also examined to compare their HIV inhibitory activities with the corresponding acids.

Chapter 2. Syntheses of Rigid Analogues of L-Chicoric Acid

2.1. Introduction

Three stereoisomers of 3,4-dicaffeoyloxycyclopentane-1-carboxylic acid (**28** in Scheme 2-1) were targeted as conformationally rigid analogues of L-CA. The plan was to deblock **29** which would be synthesized from the stereoisomeric diols **30** and acid **31**. The diols **30** would be synthesized by reacting diphenyldiazomethane with the stereoisomeric 3,4-dihydroxycyclopentane-1-carboxylic acids (**32**), which would be obtained from the known [89]

Scheme 2-1 Retrosynthesis of 3,4-dicaffeoyloxycyclopentane-1-carboxylic acids



3-cyclopentene-1-carboxylic acid (**33**). The protected acid **31** would be prepared from commercially available caffeic acid and methyl chloroformate.

2.2. (3*S*,4*R*)-Dicaffeoyloxycyclopentane-*trans*-1-carboxylic acid

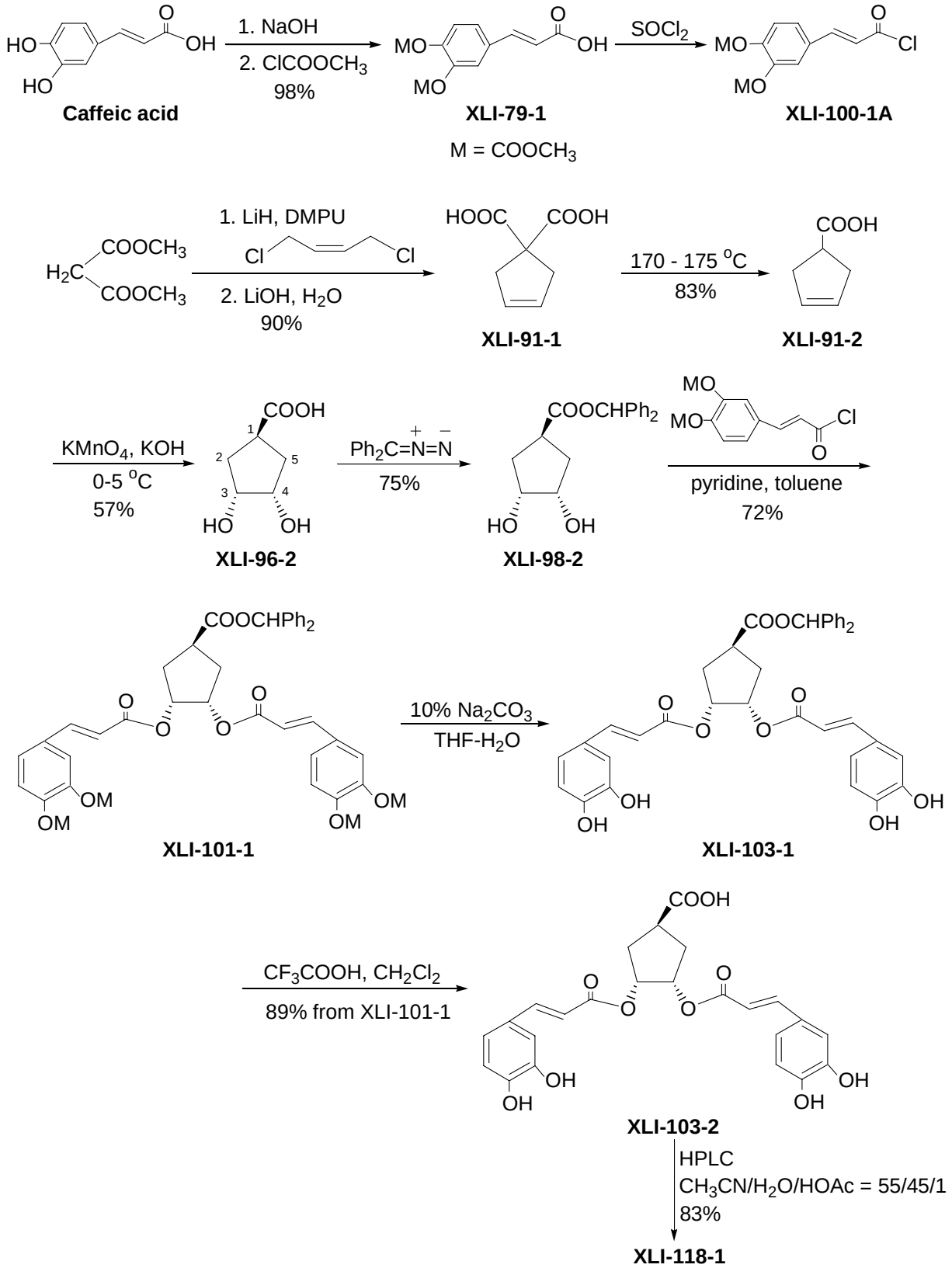
The synthesis of (3*S*,4*R*)-dicaffeoyloxycyclopentane-*trans*-1-carboxylic acid is described in Scheme 2-2. The phenolic hydroxyl groups of caffeic acid were protected as the methyl carbonates to give XLI-79-1 which was converted to its acid chloride as described in the literature. [37] The cyclopentene carboxylic acids XLI-91-1 and XLI-91-2 were prepared by the literature method. [89]

The latter acid, XLI-91-2, was cis-dihydroxylated with $KMnO_4$ to give the known diol acid XLI-96-2 in 57% yield. [90] In order to confirm its configuration by X-ray crystallography, diol XLI-96-2 was recrystallized from a mixture of acetone and hexanes. Interestingly, the crystals obtained were of the acetonide (XLI-96-2A) of XLI-96-2 (Fig. 2-1), but it is clear that the carboxyl group is *trans* to the two *cis*-hydroxy groups. This configuration is probably due to the steric hindrance and/or charge repulsion between the carboxylate and the permanganate anion so that approach to the double bond is mainly opposite to the carboxyl group.

The carboxyl group of XLI-96-2 was blocked with diphenyldiazomethane [91] to give the diphenylmethyl ester XLI-98-2 in 75% yield. The configuration of ester XLI-98-2 was confirmed by X-ray crystallography of its acetonide (XLI-98-4) (Fig. 2-2).

The fully blocked target compound XLI-101-1 was obtained in 72% yield by treating XLI-98-2 with 3,4-dimethoxycarbonylcaffeoyl chloride (XLI-100-1A) and the carboxymethyl groups were removed with 10% aqueous Na_2CO_3 in THF. It was necessary to track the reaction

Scheme 2-2 Synthesis of (3*S*,4*R*)-dicaffeoyloxycyclopentane-*trans*-1-carboxylic acid



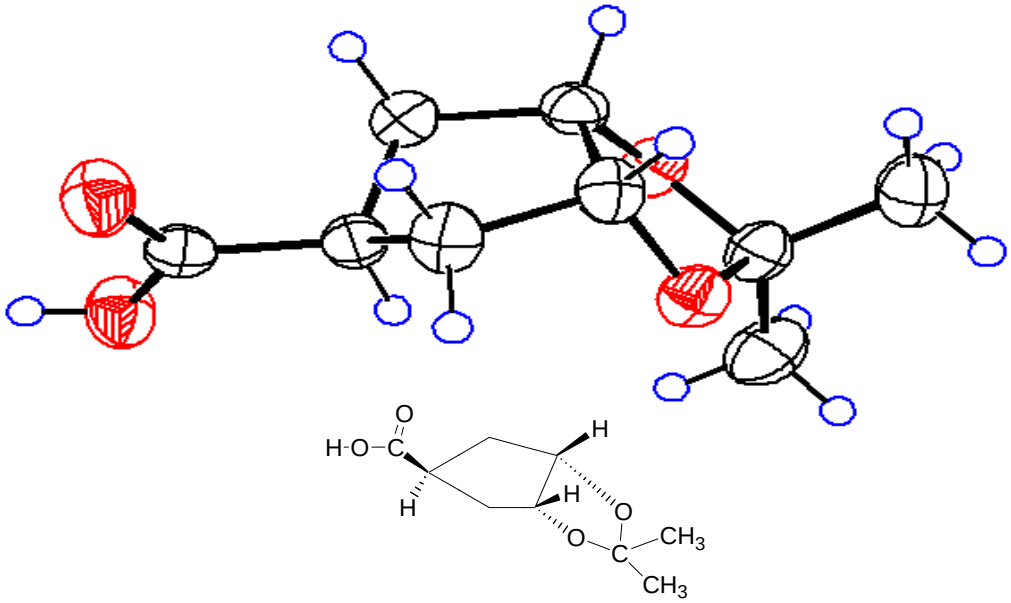


Fig. 2-1 X-ray structure of acetonide (XLI-96-2A) of XLI-96-2

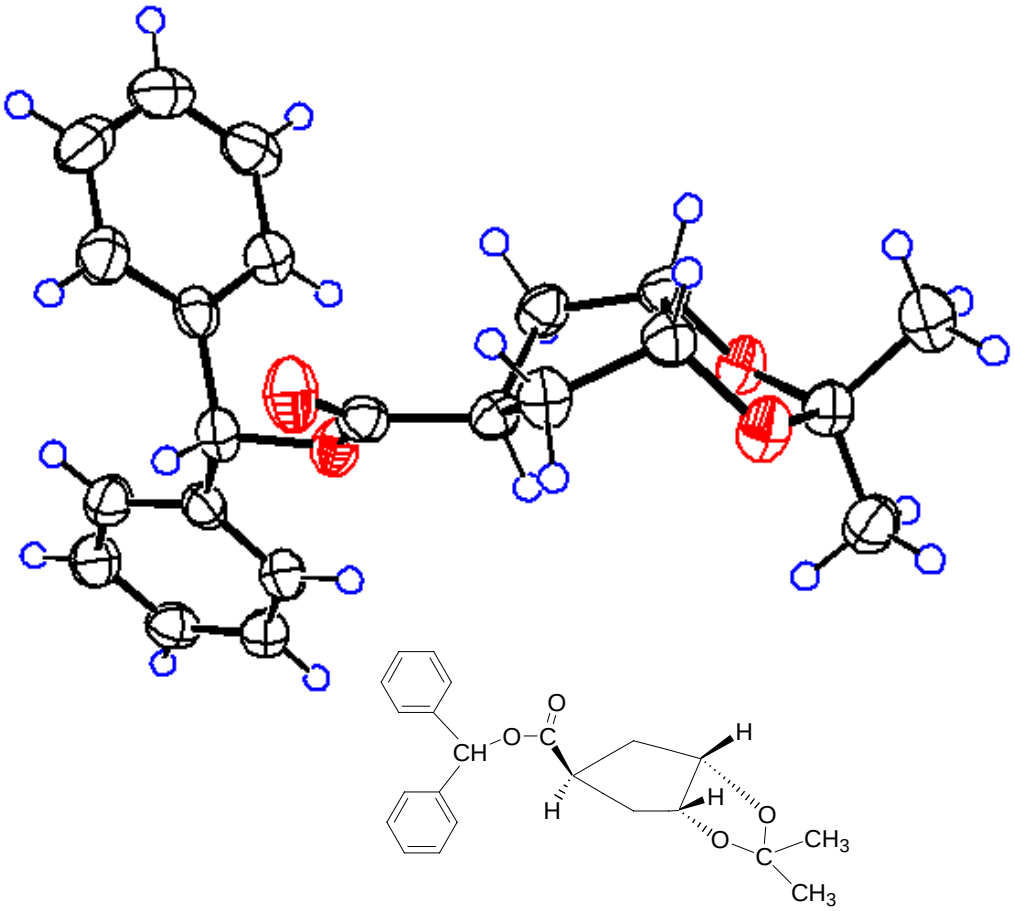


Fig. 2-2 X-ray structure of acetonide (XLI-98-4) of XLI-98-2

with TLC to avoid over-hydrolysis. Phenol XLI-103-1, obtained as a pale yellow solid whose NMR indicated traces of THF, was used in the next step without purification. Cleavage of the diphenylmethyl ester group with TFA in dichloromethane gave the crude target compound XLI-103-2 which was purified by HPLC to give the pure *cis*-*anti* isomer XLI-118-1 for bioassay.

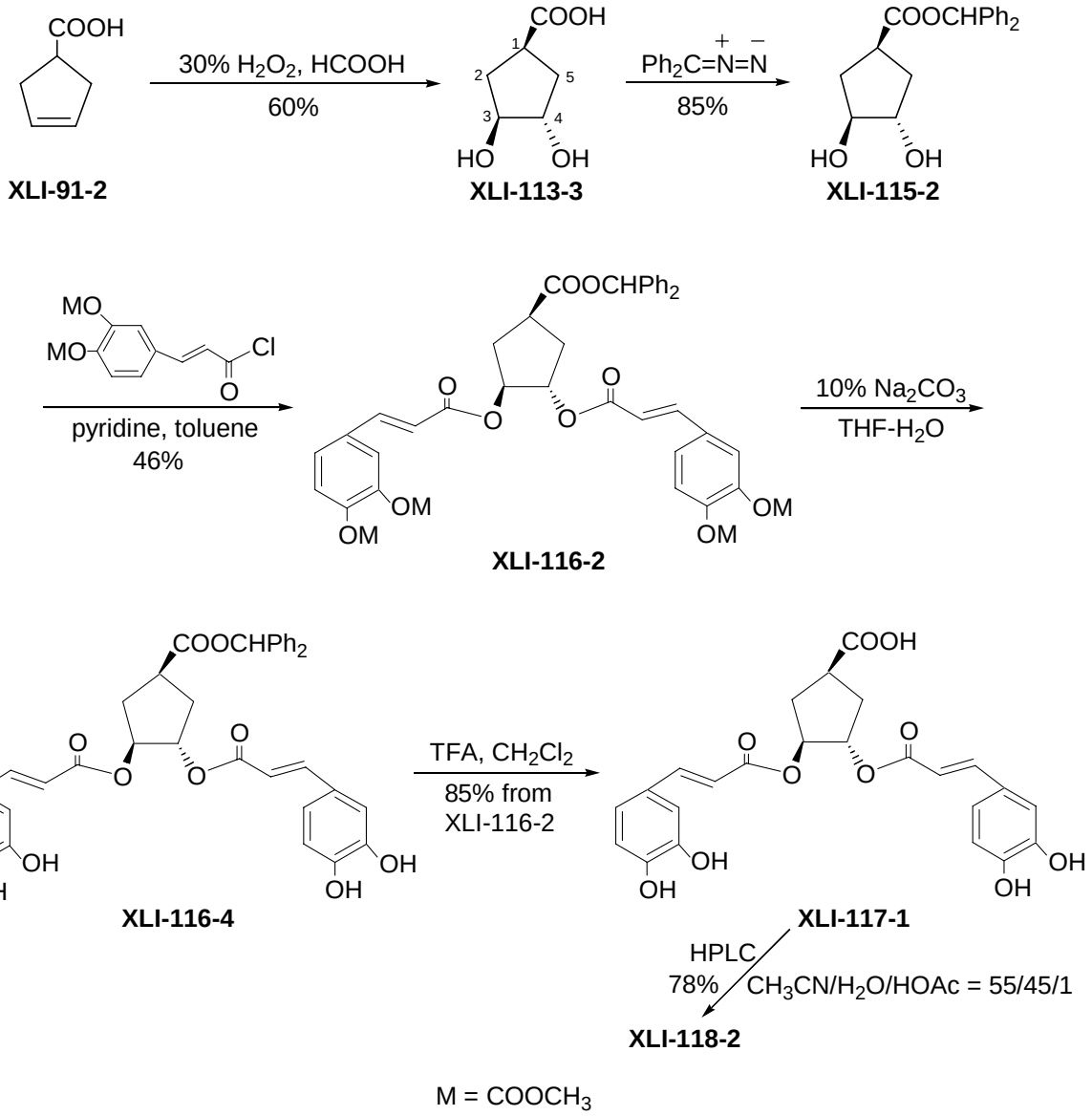
2.3. *Trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylic acid

The synthesis of *trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylic acid is described in Scheme 2-3.

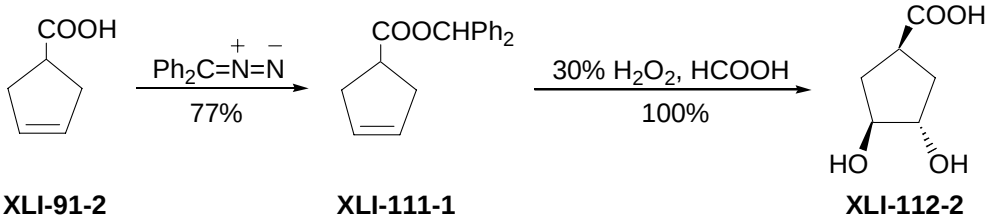
Since oxidation of the alkene XLI-91-2 with *m*-chloroperoxybenzoic acid (MCPBA) led to a reaction mixture from which it was difficult to isolate the product, the carboxyl group was first blocked with diphenyldiazomethane to give the diphenylmethyl ester XLI-111-1 which was then reacted with hydrogen peroxide/formic acid to give the *trans*-diol acid XLI-112-2 directly since the diphenylmethyl ester apparently was hydrolyzed during the reaction (Scheme 2-4). Subsequently this same acid (XLI-113-3) was prepared directly from alkene XLI-91-2 by dihydroxylation with hydrogen peroxide/formic acid in 60% yield after recrystallization from ethyl acetate. The configuration of XLI-113-3 was confirmed by X-ray crystallography (Fig. 2-3) of crystals obtained from acetone and hexanes.

Since it was only slightly soluble in acetonitrile, a mixture of acetone and petroleum ether was used as solvent for blocking acid XLI-113-3 with diphenyldiazomethane. The product diphenylmethyl ester XLI-115-2 was obtained as an orange-yellow gum in 85% yield after purification on a silica gel column.

Scheme 2-3 Synthesis of *trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylic acid



Scheme 2-4 Alternative synthesis of *trans*-3,4-dihydroxycyclopentane-1-carboxylic acid



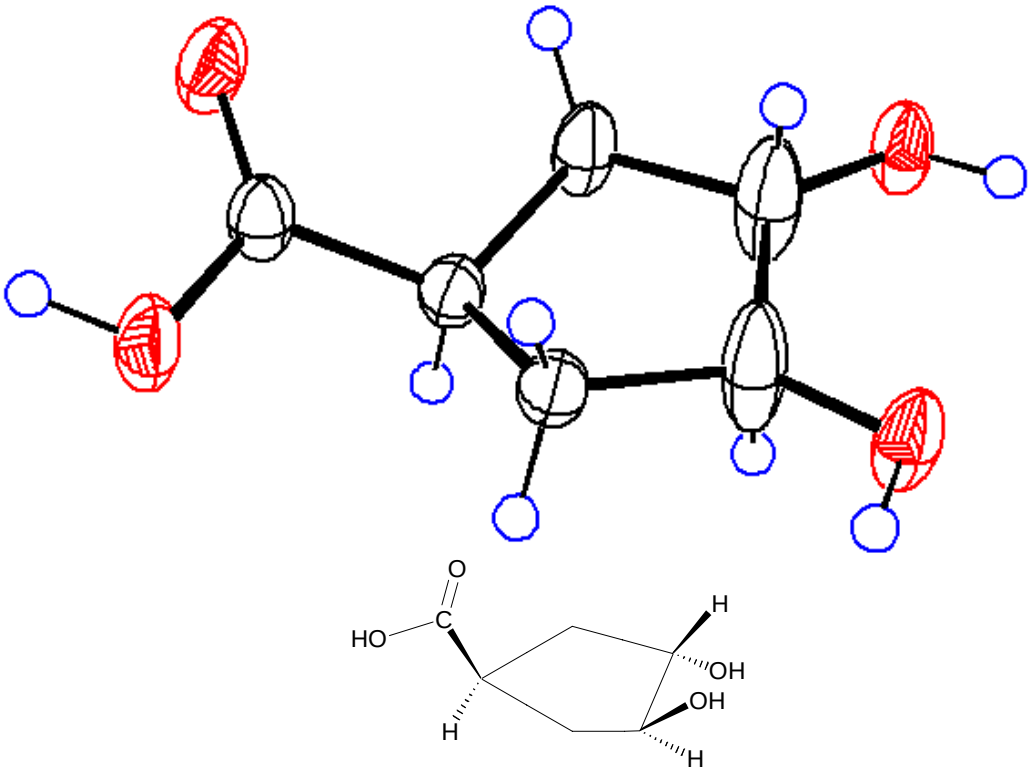


Fig. 2-3 X-ray structure of *trans*-3,4-dihydroxycyclopentane-1-carboxylic acid (XLI-113-3)

The fully blocked compound XLI-116-2 was obtained by treating XLI-115-2 with 3,4-dimethoxycarbonylcaffeoyl chloride in toluene, with pyridine as catalyst. The crude product was purified on a silica gel column to give a pale yellow gum in 46% yield.

Removal of the carboxymethyl groups from the fully blocked XLI-116-2 was achieved with 10% aqueous Na_2CO_3 in THF and tracked by TLC. The crude phenol XLI-116-4 was used in the next step without purification.

Cleavage of diphenylmethyl ester XLI-116-4 with TFA gave the crude product XLI-117-1 which was purified for bioassay by HPLC to give the *trans*-isomer XLI-118-2.

2.4. (3*R*,4*S*)-Dicaffeoyloxycyclopentane-*cis*-1-carboxylic acid

Since the methyl ester of (3*R*,4*S*)-dihydroxycyclopentane-*cis*-1-carboxylic acid can be obtained from the acetonide of methyl (3*S*,4*R*)-dihydroxycyclopentane-*trans*-1-carboxylate by ester enolate kinetic protonation followed by acidic hydrolysis, [92] Scheme 2-5 was the initial approach to the target compound.

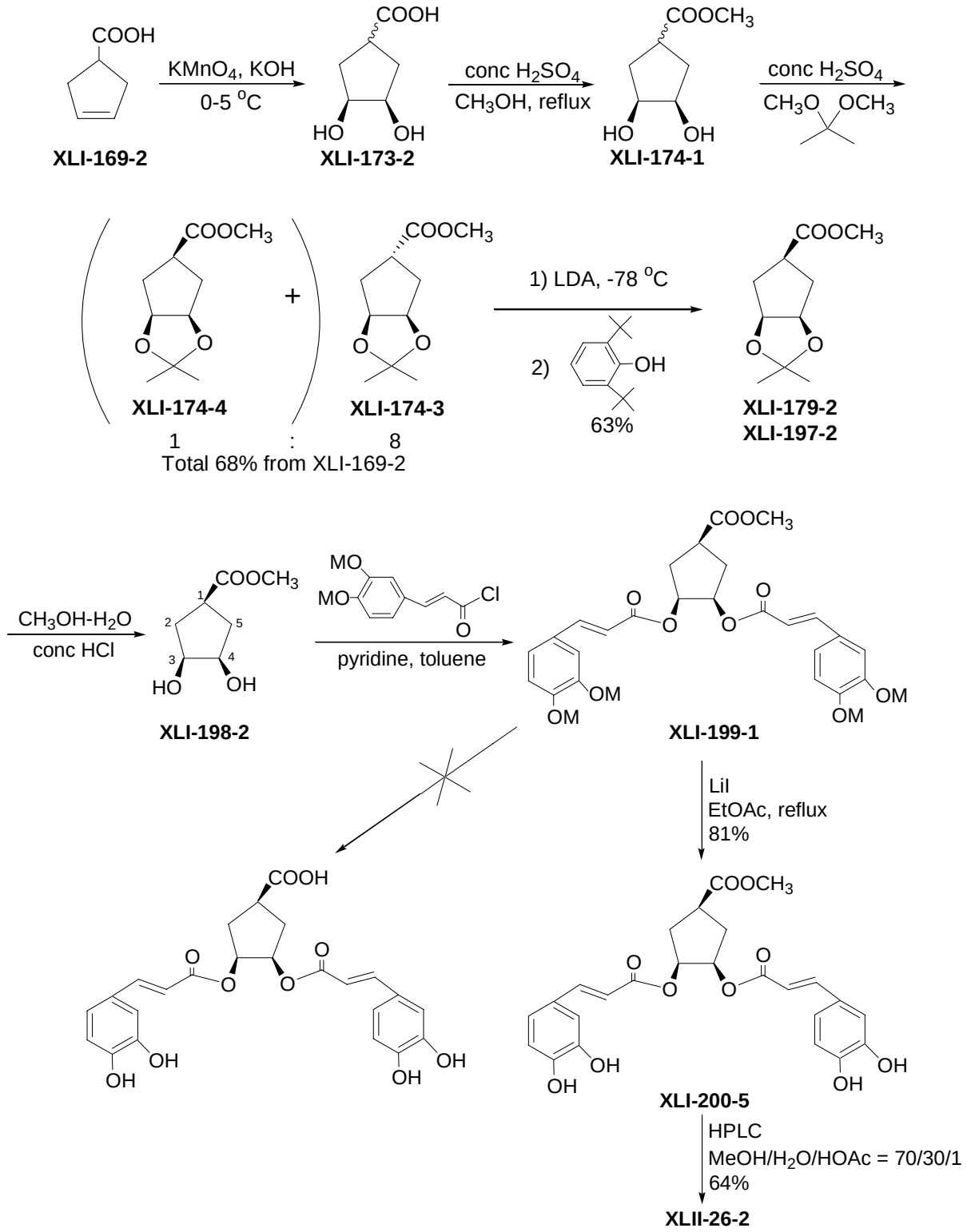
Oxidation of alkene XLI-169-2 with $KMnO_4$ under basic condition gave the crude diol XLI-173-2 which was converted without purification to its methyl ester XLI-174-1 which, also without purification, was reacted with 2,2-dimethoxypropane to give a mixture of acetonides, which were separated on a silica gel column. Both the *trans*-isomer (XLI-174-3) and the *cis*-isomer (XLI-174-4) were obtained (68% total yield) in a ratio of 8 to 1. This prevalence probably is due to preferential approach of the permanganate anion to the double bond of XLI-169-2 on the side opposite to the carboxylate group.

Trans-acetonide XLI-174-3 was deprotonated by LDA to give the enolate, which was kinetically protonated by the bulky proton donor 2,6-di-*tert*-butylphenol. [92] Due to steric hindrance, protonation occurred mainly from the side opposite to the acetonide to give the cis acetonide XLI-179-2 ≡ XLI-197-2 as the major (6:1) product.

The *cis*-diol methyl ester XLI-198-2 was obtained by cleaving the acetonide XLI-197-2 and used in the next step without purification.

The fully blocked target XLI-199-1 was obtained in 42% yield by reacting 3,4-dimethoxycarbonylcaffeoyl chloride with the *cis*-diol XLI-198-2.

Scheme 2-5 Attempt to synthesize (3*R*,4*S*)-dicaffeoyloxycyclopentane-*cis*-1-carboxylic acid

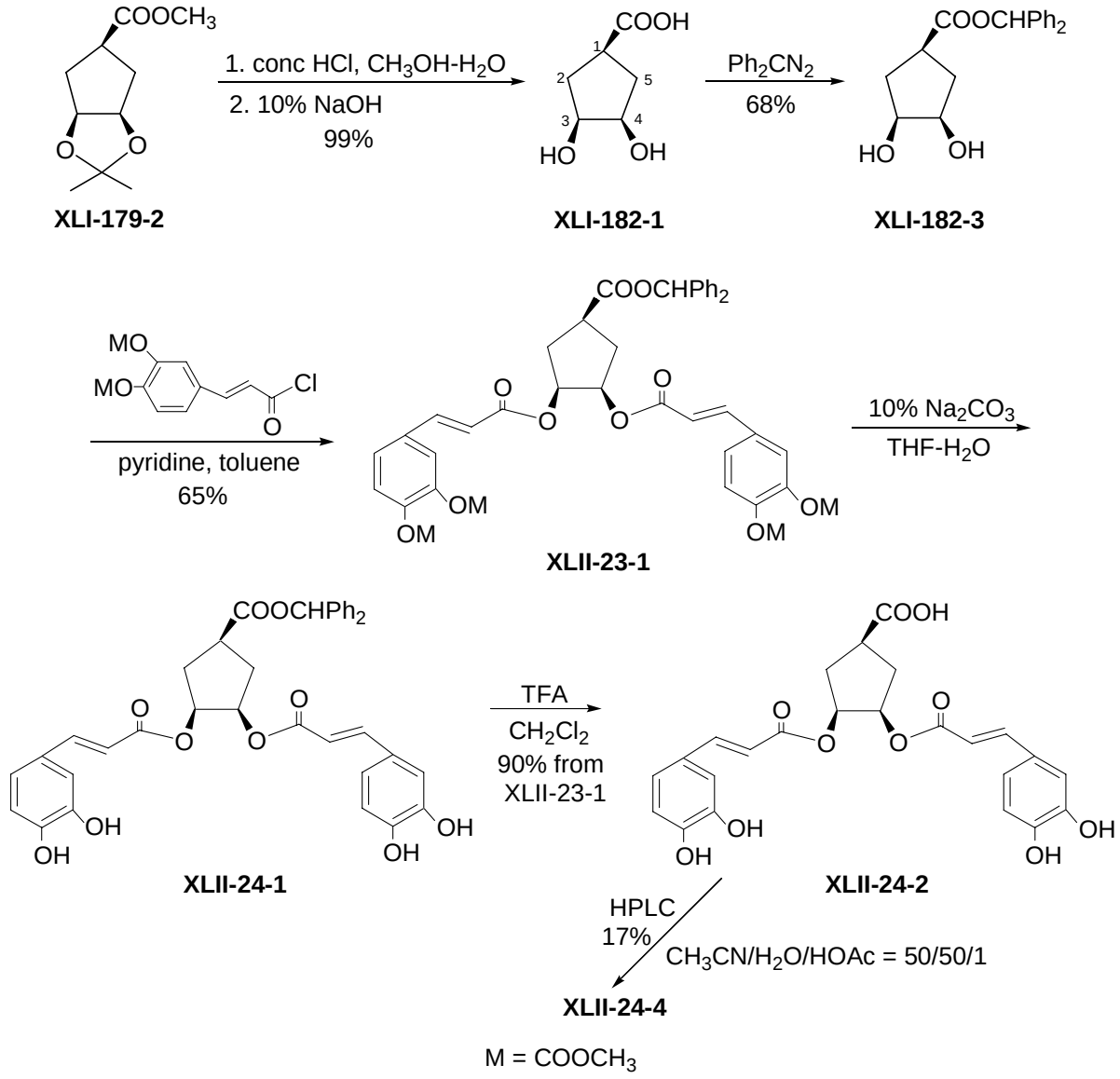


To get the target free acid, simultaneous removal of the carboxymethyl groups and hydrolysis of the methyl ester of XLI-199-1 was attempted. The reagent combinations tried included 10% Na_2CO_3/H_2O in THF, 10% Na_2CO_3/H_2O in THF/MeOH, LiI in refluxing pyridine, and LiI in refluxing AcOEt. In each case, the methyl ester was not hydrolyzed, only the methyl carbonate protecting groups. With LiI in refluxing AcOEt, [93] the all *cis* methyl ester XLI-200-5 was obtained in 81% yield with high purity. Since the methyl ester was also a good target compound to be tested for inhibitory activity, XLI-200-5 was purified by HPLC to give the sample (XLII-26-2) for bioassay. The failure to hydrolyze the methyl ester under any of these conditions suggests it is too sterically hindered to form the usual tetrahedral hydrolysis intermediate or to permit S_N2 attack by a large I^- nucleophile. In order to facilitate removal of the protecting groups, the carboxylic acid XLI-182-1 was therefore protected as its diphenylmethyl, instead of its methyl, ester (Scheme 2-6) since the hydrolysis would be via an S_N1 instead of an S_N2 mechanism (Scheme 2-7).

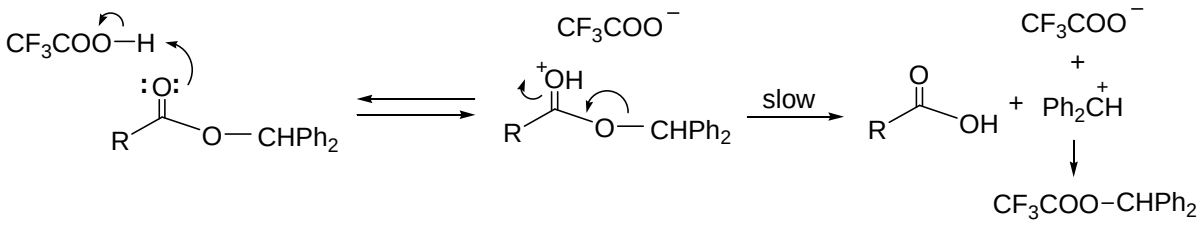
Acetonide XLI-179-2 was deblocked to give acid XLI-182-1 in 99% yield. Protection of the carboxylic group with diphenyldiazomethane gave the pure *cis*-diol diphenylmethyl ester XLI-182-3 as a white solid in 68% yield. This was reacted with 3,4-dimethoxycarbonylcaffeoyl chloride to give the fully blocked *cis*-ester XLII-23-1 as a white solid in 65% yield.

Removal of the carboxymethyl groups with 10% aqueous Na_2CO_3 in THF was unusually slow because the reaction mixture was not homogeneous, but when methanol was used as a co-solvent to homogenize the reaction mixture and shorten the reaction time, some of the caffeoyl groups were hydrolyzed. The crude diphenylmethyl ester XLII-24-1 was cleaved directly with

Scheme 2-6 Synthesis of (3*R*,4*S*)-dicaffeoyloxycyclopentane-*cis*-1-carboxylic acid



Scheme 2-7 Mechanism of the cleavage of diphenylmethyl esters with TFA



TFA in dichloromethane to give the target compound XLII-24-2 in 90% overall yield from the fully blocked compound XLII-23-1. The sample for bioassay (XLII-24-4) was obtained as a white solid by preparative HPLC.

2.5. *Threo*-3,4-dicaffeoyloxyadipic acid

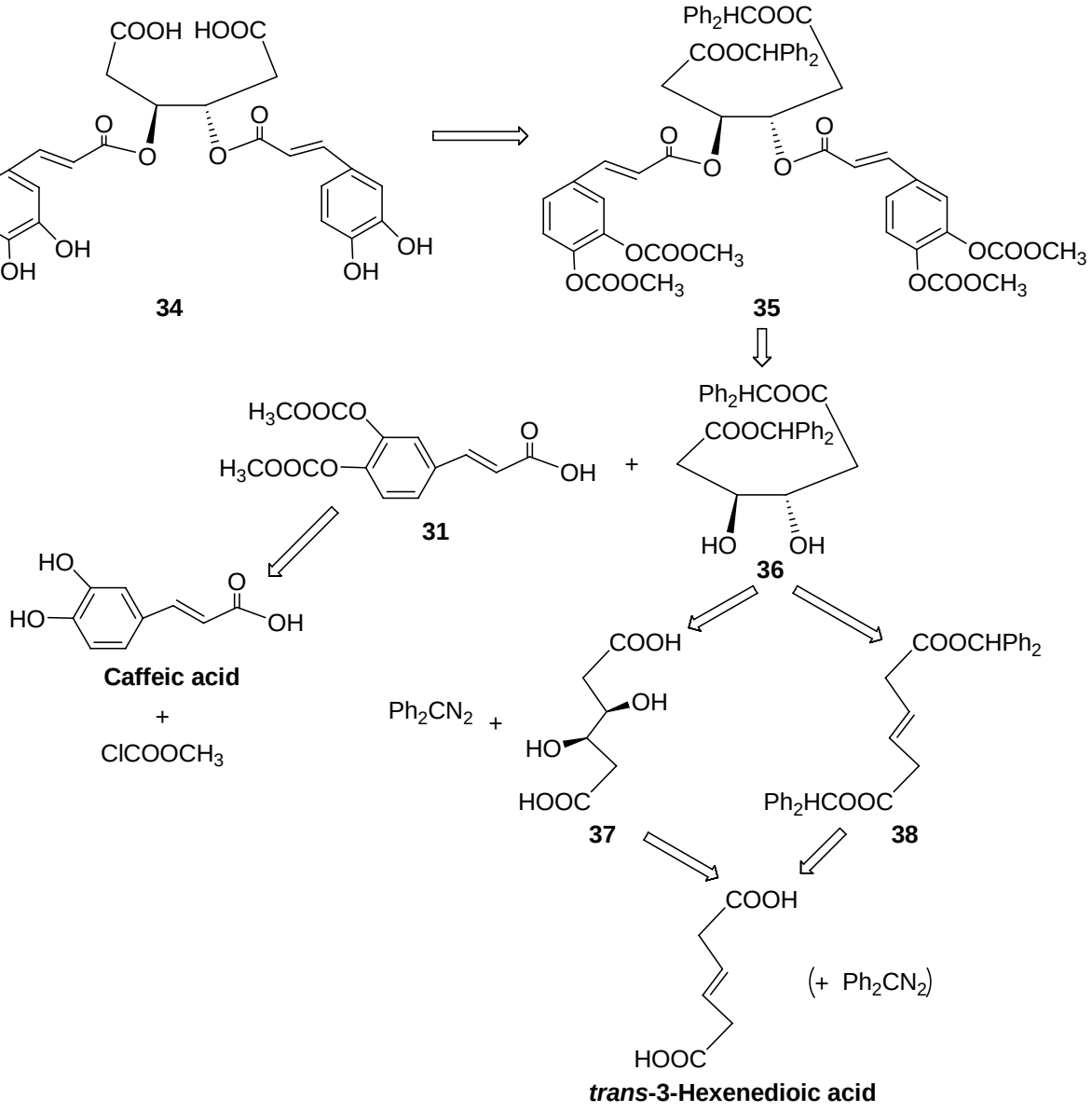
Threo-3,4-dicaffeoyloxyadipic acid (**7**), a conformationally flexible analogue of L-CA and the rigid analogues discussed above, was also synthesized and its inhibitory activity against HIV IN tested for comparison.

Starting from commercially available *trans*-3-hexenedioic acid, there are two routes (Scheme 2-8) to the target compound. In the first route, the double bond of *trans*-3-hexenedioic acid is dihydroxylated to give intermediate **37**, whose carboxyl groups are blocked as the diphenylmethyl esters. In the second route, via the diphenylmethyl ester **38**, these two steps are reversed.

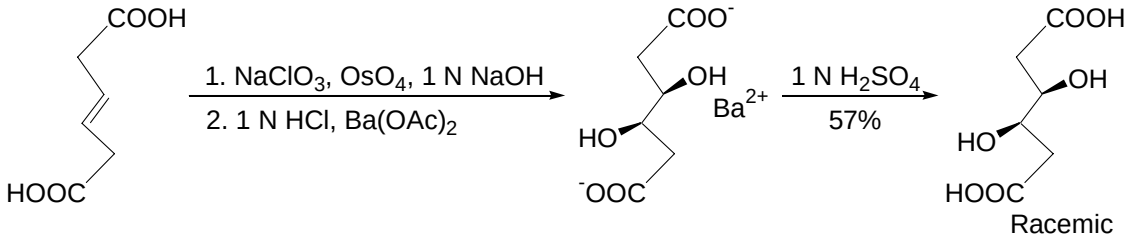
Following the first route, preparation of *threo*-3,4-dihydroxyadipic acid (**37**) [94-97] was attempted using a similar method as described in Scheme 2-9, [94] since this was closer than other methods [95] to the commercially available *trans*-3-hexenedioic acid. [97] Oxidation of *trans*-3-hexenedioic acid with $KMnO_4$ under basic condition only gave dilactone XLI-177-2 (Scheme 2-10), presumably due to insufficient pH control during the acidic workup.

To avoid formation of the dilactone, the second route to *threo*-3,4-dicaffeoyloxyadipic acid was followed as described in Scheme 2-11. The carboxylic groups of *trans*-3-hexenedioic acid were protected as their diphenylmethyl esters XLI-187-2 ≡ XLI-193-1, and the oxidation studied with different reagent combinations including 1) $KMnO_4$ under basic condition at 0 ^{o}C

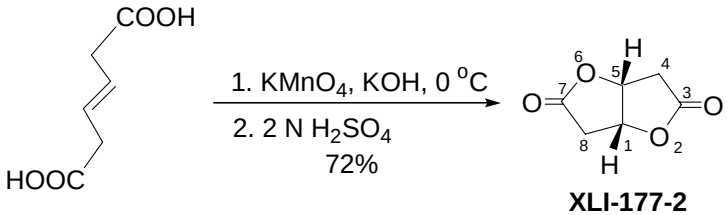
Scheme 2-8 Retrosynthesis of *threo*-3,4-dicaffeoyloxyadipic acid



Scheme 2-9 One [94] of the reported routes to *threo*-3,4-dihydroxyadipic acid

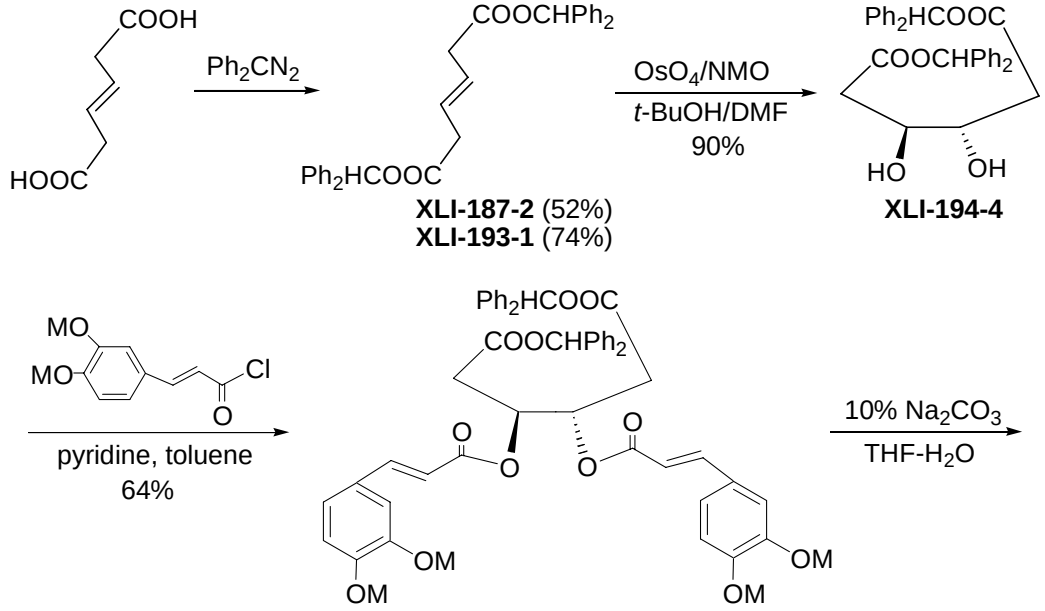


Scheme 2-10 Oxidation of *trans*-3-hexenedioic acid with basic $KMnO_4$ followed by acidic workup giving *threo*-3,4-dihydroxyadipic-*γ, γ'*-dilactone



XLI-177-2

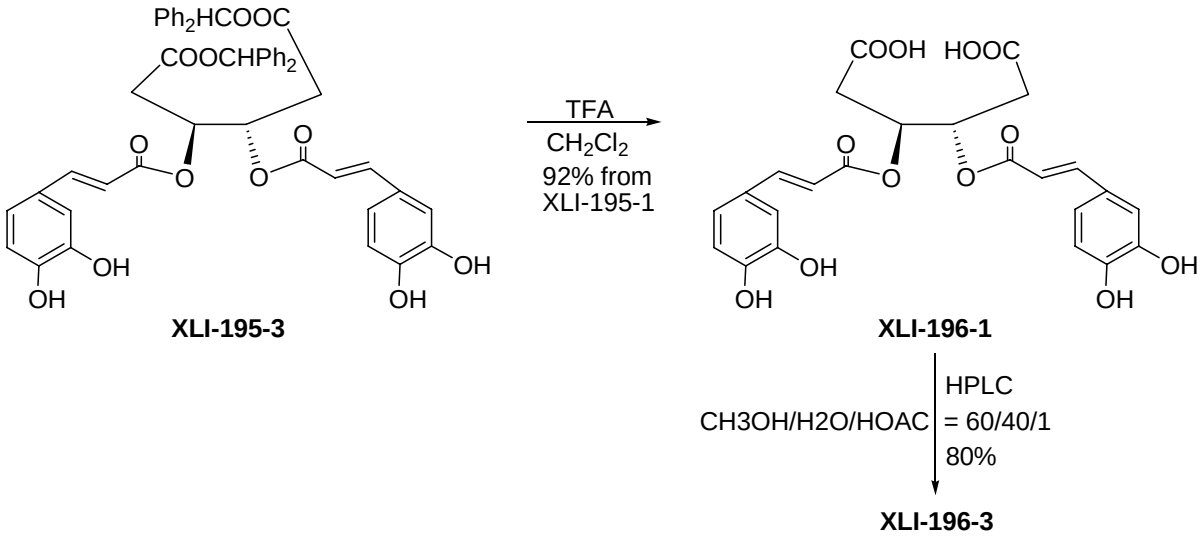
Scheme 2-11 Synthesis of *threo*-3,4-dicaffeoyloxyadipic acid



XLI-187-2 (52%)
XLI-193-1 (74%)

XLI-194-4

XLI-195-1



XLI-195-3

XLI-196-1

XLI-196-3

M = $COOCH_3$

with a phase transfer catalyst, [98] 2) $KMnO_4$ in organic solvents with 18-crown-6 [99] and 3) *N*-methylmorpholine *N*-oxide (NMO) with OsO_4 as catalyst. [100] Under the first two conditions, only the starting material was recovered, but with the third method, chromarography of the crude product gave pure diol XLI-194-4 as a white solid in 90% yield. The configuration was confirmed by X-ray crystallography (Fig. 2-4).

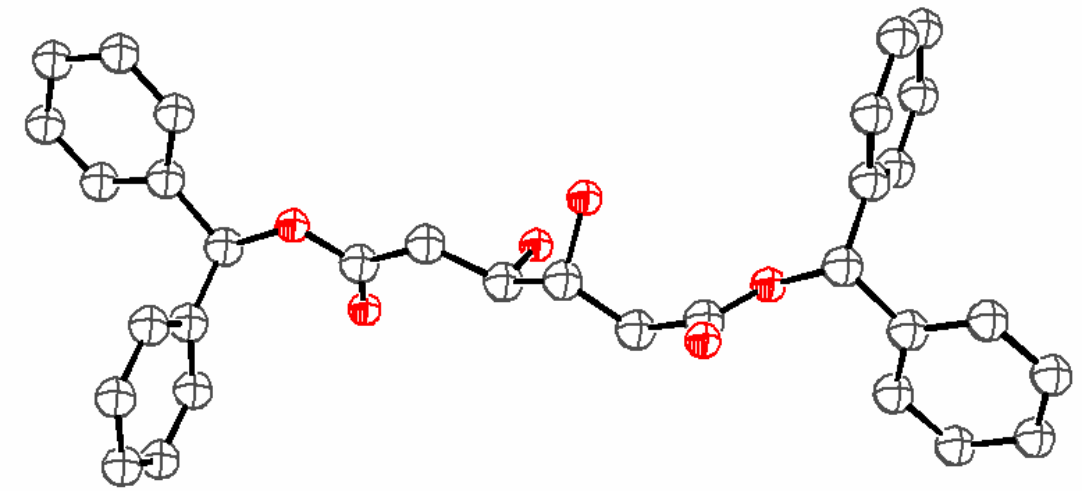


Fig. 2-4 X-ray structure of bis(diphenylmethyl) *threo*-3,4-dihydroxyadipate (XLI-194-4)

The fully blocked compound XLI-195-1 was synthesized from 3,4-dimethoxycarbonylcaffeoyl chloride with diol XLI-194-4 in 64% yield.

Removal of the carboxymethyl groups with 10% aqueous Na_2CO_3 in THF was tracked with TLC to avoid over-hydrolysis to give the phenol XLI-195-3 which was cleaved without purification using TFA in dichloromethane. The crude product XLI-196-1 was purified for bioassay by HPLC to give the desired product XLI-196-3 as a white solid.

2.6. Bioassay results

Compounds were tested for their activities *in vitro* against HIV IN, and *in vivo* against HIV replication. The former is reported as an IC_{50} value----the fifty percent inhibitory concentration or the concentration of compound that inhibits IN activity by fifty percent, and the latter as an ED_{50} value----the fifty percent effective dose or the concentration of compound that inhibits HIV replication by fifty percent. Table 2-1 illustrates the cell toxicity (CT_5 or CT_{50}), antiviral activity (ED_{50}), and anti-IN activities (IC_{50}) of rigid analogues of L-CA.

Table 2-1 Anti-HIV activities (in μM) of rigid analogues of L-CA

	Compound	CT_{50}	CT_5	ED_{50}	IC_{50} 3'-Processing	TI_5	TI_{50}
cis-anti	XLI-118-1	111	102	>106	0.52	< 1	~1
trans	XLI-118-2	193	173	>181	1.17	< 1	~1
cis-syn	XLII-24-4	444	44	>53	3.8	< 1	~8
cis-syn ester	XLII-26-2	149	26	>40	>>10	< 0.65	~3.7
Open-chain	XLI-196-3	199	41	42	0.59	1	4.7
	L-CA[39,40]	333	264	4.2	0.53	63	79

CT_{50}, the 50% cytotoxic dose, is the concentration of compound that inhibits cell growth by 50%; TI_{50}, the therapeutic index, is the ratio of the CT_{50} to ED_{50} and thus indicates the relative selectivity of the compound for HIV over cellular proteins. >> indicates no observed activity at the maximum dose tested. Linearity of dose response curves in all tests was > 0.95 and in almost all cases was > 0.98. Refer to Tables 1-1 and 1-2 for definitions of other terms.

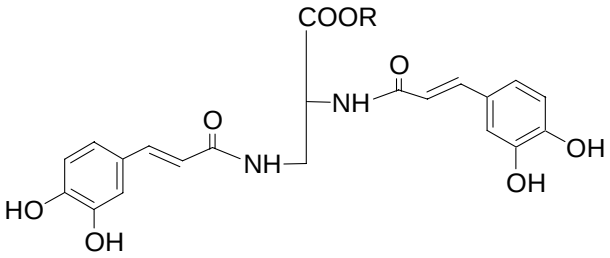
Rigid analogues XLI-118-1, XLI-118-2 and XLII-24-4 and the open-chain analogue XLI-196-3 show good to moderate anti-HIV IN activity with IC_{50} = 0.52-3.8 μM, with the *cis-anti* isomer XLI-118-1 and the open-chain analogue XLI-196-3 being the most active. This suggests

that the preferred conformation of the two caffeoyl groups is on the opposite, rather than on the same, side of the carboxyl group in the active site.

Neither the rigid analogues nor the open-chain analogue have significant anti-HIV replication activity perhaps due to poor cell permeability.

All of these compounds tested are less active than L-CA against HIV replication. A possible reason could be that in L-CA the caffeoyl group is at the α position, while it is at the β position for the open-chain analogue XLI-196-3 and at the γ position for the rigid analogues. Apparently, the closer the carboxyl group is to the caffeoyl group, the more active is the compound against HIV replication resulting in the activity sequence L-CA > open-chain analogue > rigid analogues. However, the distance between the carboxyl group and the caffeoyl group seems to have no effect on the anti-HIV IN activity, which is comparable for the *cis-anti* isomer XLI-118-1, the open-chain analogue XLI-196-3 and L-CA.

The methyl ester XLII-26-2 of the *cis-syn* isomer is less active than the free acid XLII-24-4 against HIV IN. This is consistent with Sotriffer's model (Fig. 1-5) [53] regarding the importance of the interactions between the free carboxyl group (s) of L-chicoric acid and the basic amino acids of the HIV IN active site. Moreover, the ester is more cytotoxic than the acid. These results are also consistent with the methyl ester **13** being less active than the corresponding acid **12** against either HIV IN or HIV replication. [86]



12 R = H (D,L)
13 R = CH_3 (D, L)

Compound	CT_5	ED_{50}	TI_5	IC_{50} 3'-Processing
12 [86]	30	4.5	6.7	0.81
13 [86]	49	58	0.8	1.95

2.7. Experimental

Chromatography Pre-coated silica gel on polyester sheets with 254 nm fluorescence indicator (Aldrich # Z12,278-5) were used for thin layer chromatography (TLC). The spots were detected by UV at 254 nm or with anisaldehyde TLC stain and heat. The R_f value was calculated based on the same eluate solvent as in silica gel flash chromatography unless specified otherwise. Merck silica gel grade 9385 60 Å and 230-400 mesh was used for low-pressure flash chromatography under nitrogen pressure. The size of the column used was either 2 cm x 20 cm, 3 cm x 25 cm, or 5 cm x 25 cm, and the ratio of silica gel to substrate was between 30:1 to 50:1 (wt./wt.) depending on the substrate weight, how many components were present and the difference in R_f values between the desired and the undesired components. Chromatography solvents were C.P. grade and used without purification unless otherwise noted.

Spectroscopy 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) (± 0.1 ppm) and

observed coupling constants (J) in Hz (± 0.1 Hz). ^{1}H NMR spectra were referenced to TMS = 0 ppm and reported as δ (m, J, #H). ^{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 CD_3COCD_3 = 29.84 ppm, or central peak of CD_3OD = 49.00 ppm, and reported as δ [m (if not s), J (if not s), #C (if more than 1)].

HPLC All HPLC analyses were performed with a Biocad Sprint (Series 314) HPLC system with UV detector set at 254 nm. The analytical column (250 x 4.6 mm I.D., CV = 4.155 mL, series number 00010692.1) and preparative column (250 X 22.0 mm I.D., CV = 95.033 mL, series number 00120008.1) were filled with Econosil C18 10 μ (Alltech) as stationary phase. The flow rates of analytical and preparative columns were 1.5 mL/min and 15 mL/min respectively. The purity of all compounds isolated by preparative HPLC was verified by analytical HPLC under identical solvent conditions. The methanol and acetonitrile for HPLC were HPLC grade solvents from Aldrich. Purities were maximized by cutting off the shoulders of absorption peaks leading to lower recovery yields.

General Melting points were determined on either a Melt-Temp apparatus or a Bausch & Lomb Optical Co. hot-stage microscope and were not corrected. Toluene was distilled (108.5 ^{o}C) from CaH_2 and stored over anhydrous $CaCl_2$. THF was distilled (65 ^{o}C) from sodium and kept over anhydrous $CaCl_2$. Conditions for removal of organic solvent with a Rotavapor are < 40 ^{o}C and < 20 mmHg vacuum. Conditions for removal of solvent by lyophilization are 0 ^{o}C and < 0.5 mmHg.

Elemental analyses were done by Atlantic Microlab, Inc. of Norcross, Georgia or M-H-W Laboratories of Phoenix, Arizona, and high-resolution mass spectrometry by the Washington University Mass Spectrometry Resource of St. Louis, Missouri.

The sources of chemicals used are listed below. Chemicals were used as received unless specified otherwise.

Compound	Source	Cat. No.
Dimethyl malonate	Aldrich	13,644-1
N, *N'*-Dimethylpropyleneurea (DMPU)	Aldrich	25,156-9
Lithium hydride, powder	Aldrich	20,104-9
cis-1,4-Dichloro-2-butene	Aldrich	19,5707
Lithium hydroxide monohydrate	Aldrich	40,297-4
Potassium permanganate	Mallinckrodt	7056
Sodium bisulfite	Fisher	S-654
3,4-Dihydroxycinnamic acid	Aldrich	D11,080-9
Methyl chloroformate	Aldrich	M3,530-4
Thionyl chloride	Aldrich	32,053-6
Pyridine	Aldrich	27,097-0
2,2-Dimethoxypropane	Aldrich	D13,680-8
Lithium diisopropylamide (LDA) (2.0 *M* solution in heptane/THF/ethylbenzene)	Aldrich	36,179-8
2,6-Di-*tert*-butylphenol	Aldrich	D4,840-0
Lithium iodide	Aldrich	21,821-9
Trifluoroacetic acid	Aldrich	T6,220-0

trans-3-Hexenedioic acid (*trans*-β-Hydromuconic acid)	Aldrich	H1,785-6
N-Methylmorpholine N-oxide (NMO)	Aldrich	22,428-6
2-Methyl-2-propanol (*tert*-Butyl alcohol)	Aldrich	36,053-8
Osmium tetroxide	Aldrich	20,103-0
Benzophenone hydrazone	Aldrich	B960-2
Mercuric oxide (yellow)	Mallinckrodt	1428
Ethyl ether (anhydrous)	Mallinckrodt	1155
Magnesium sulfate (anhydrous)	Aldrich	24,697-2
Sodium sulfate (anhydrous)	Aldrich	23,931-3
Hexanes	Pharmco-AAPER	359000000
Ethyl acetate	Pharmco-AAPER	330000000
Methanol	Pharmco	339000ACS
Methanol, HPLC	Sigma-Aldrich	64,637-7
Acetonitrile, HPLC	Acros	61001-0040
Acetone	AAPER	06A16607
Dichloromethane	Pharmco	313000DIS
Chloroform	Pharmco	309000DIS
Acetic acid, glacial	EM	AX0073-9
Sodium hydroxide	Sigma-Aldrich	22,146-5
Sodium bicarbonate	Sigma-Aldrich	23,652-7
Potassium hydroxide	Aldrich	22,147-3
Tetrahydrofuran (THF)	Sigma-Aldrich	36,058-9
Hydrochloric acid	VWR	VW3110-3

Sulfuric acid	VWR	VW6840-3
Sodium chloride	Aldrich	22,351-4
Petroleum ether (bp 40-60 ^{o}C)	Pharmco	366000ACS
Ethyl ether	Pharmco	373000ACS
Toluene	Pharmco	347000HPLC
Sodium carbonate	Aldrich	23,095-2

2.7.1. (3*S*,4*R*)-Dicaffeoyloxycyclopentane-*trans*-1-carboxylic acid

3,4-Dimethoxycarbonylcaffeic acid [37] (XLI-79-1 ≡ XLI-147-3 ≡ XLI-204-1 ≡ XLII-51-1) A solution of 18.32 g (0.10 mol) of 3,4-dihydroxycinnamic acid in 227 mL (0.227 mol) of 1 M NaOH was cooled to 0 ^{o}C and 27.5 mL (0.356 mol) of methyl chloroformate was added dropwise with stirring. The reaction lasted for 2 h. The precipitate was collected by filtration, washed with water, and air dried to give 29.41 g (98%) of XLI-79-1 as an off-white solid, mp 141-143 ^{o}C (lit. [37] mp 142-143 ^{o}C). XLI-79-1 was used in the next reaction without purification. XLI-147-3, XLI-204-1, and XLII-51-1 were made by exactly the same procedure. ^{1}H NMR (DMSO-d_6) 7.85 (d, J = 1.8 Hz, 1H), 7.70 (dd, J = 1.8, 8.5 Hz, 1H), 7.60 (d, J = 16.1 Hz, 1H), 7.49 (d, J = 8.5 Hz, 1H), 6.60 (d, J = 16.1 Hz, 1H), 3.87 (s, 3H), 3.86 (s, 3H); ^{13}C NMR (DMSO-d_6) 167.2, 152.5, 152.3, 143.0, 142.2, 141.8, 133.6, 127.2, 123.7, 122.6, 120.8, 56.0 (2). Lit. [37] ^{1}H NMR (CD_3OD, 300 MHz) 7.63 (d, J = 16.0 Hz, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 6.48 (d, J = 16.0 Hz, 1H), 3.870 (s, 3H), 3.866 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) 169.8, 154.6, 154.4, 145.0, 144.2, 144.0, 135.1, 127.8, 124.7, 123.5, 121.1, 56.5 (2).

3,4-Dimethoxycarbonylcaffeoyl chloride [37] **(XLI-100-1A)** A mixture of 3.55 g (12.0 mmol) of XLI-79-1 in 50 mL of thionyl chloride was refluxed for 2 h. Extra thionyl chloride was removed with a Rotavapor to give acid chloride XLI-100-1A as a pale yellow solid, which was used immediately in the esterification reaction.

3-Cyclopentene-1,1-dicarboxylic acid [89] **(XLI-91-1 ≡ XLI-169-1)** A dry, 1-L, two-necked, round-bottomed flask, equipped with a Teflon-covered magnetic stirring bar, was charged under a current of nitrogen with 33.00 g (0.25 mol) of dimethyl malonate, 50 mL of dry *N*, *N*'-dimethylpropyleneurea (DMPU), and 450 mL of dry THF. The resulting solution was cooled by means of an ice bath and treated with 5.00 g (0.63 mol) of lithium hydride powder in one portion. The nitrogen flow was discontinued, and the flask was capped with rubber septa and connected to a Nujol-filled bubbler by means of a syringe needle. After 15 min, the cooling bath was removed and stirring was continued until hydrogen evolution was complete (*ca*. 2 h), whereupon 28.4 mL (0.27 mol) of *cis*-1,4-dichloro-2-butene was rapidly added by syringe. The mixture was heated by means of an oil bath at 40-45 ^{o}C for 24 h. After the mixture was cooled to 20 ^{o}C, 50 mL of water was added dropwise followed by 31.50 g (0.75 mol) of solid lithium hydroxide monohydrate. After the reaction mixture was stirred at 20 ^{o}C for an additional 24 h, it was treated with 350 mL of water, stirred for 10 min, and then transferred to a 2-L separatory funnel. Neutral material was removed by extraction with five 500-mL portions of ethyl acetate, each of which was back-washed with 30 mL of aqueous saturated sodium chloride solution. The combined aqueous phases were then acidified with 160 mL of 6 M hydrochloric acid, and extracted three times with 500-mL portions of ethyl acetate. The ethyl acetate extracts were

combined, washed three times with 100 mL of 3 M hydrochloric acid and twice with 50 mL of saturated sodium chloride solution, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. After removal of traces of solvent under high vacuum, 35.07 g (90%) of diacid XLI-91-1 was obtained as an off-white solid, mp 163-165 ^{o}C (lit. [89] mp 163-165 ^{o}C). XLI-169-1 (35.20 g, 90%) was made by the same procedure from 33.00 g (0.25 mol) of dimethyl malonate. ^{1}H NMR [$CDCl_3$-CD_3COCD_3 (1:1)] 8.81 (br s, 2H), 5.62 (s, 2H), 3.06 (s, 4H); ^{13}C NMR [$CDCl_3$-CD_3COCD_3 (1:1)] 176.1, 127.9 (2), 58.8, 41.1 (2). Lit. [89] ^{1}H NMR [$CDCl_3$-CD_3COCD_3 (8:2), 300 MHz] 10.90 (br s, 2H), 5.63 (br s, 2H), 3.08 (br s, 4H); ^{13}C NMR [$CDCl_3$-CD_3COCD_3 (8:2), 75 MHz] 178.5, 133.1, 64.3, 46.5.

3-Cyclopentene-1-carboxylic acid [89] (XLI-91-2 ≡ XLI-169-2) A 250-mL, one-necked, round-bottomed flask was charged with 35.07 g of XLI-91-1 and then fitted with a reflux condenser capped with a rubber septum and connected to a Nujol-filled bubbler by means of a syringe needle. The contents of the flask were heated in an oil bath at 170-175 ^{o}C until carbon dioxide evolution was complete (ca. 2 h) and then allowed to cool to room temperature. The resulting oil was transferred to a 50-mL, one-necked, round-bottomed flask and vacuum distilled without fractionation to provide 20.93 g (83% or 75% overall from dimethyl malonate) of XLI-91-2 as a clear, colorless oil, bp 83-84 ^{o}C (2 mmHg) (lit. [89] bp 88 ^{o}C (2 mmHg)). XLI-169-2 (20.88 g, 83%) was made by the same procedure from 35.20 g of crude XLI-169-1. ^{1}H NMR ($CDCl_3$) 11.74 (br s, 1H), 5.66 (s, 2H), 3.16 (tt, J = 8.1, 8.1 Hz, 1H), 2.68 (d, J = 8.0 Hz, 4H); ^{13}C NMR ($CDCl_3$) 183.1, 129.0 (2), 41.5, 36.3 (2). Lit.[89] ^{1}H NMR ($CDCl_3$) 11.96 (br s, 1H), 5.68 (br s, 2H), 3.12-3.25 (m, 1H), 2.70 (m, 4H); ^{13}C NMR ($CDCl_3$) 182.5, 129.2 (2), 41.5, 36.2 (2).

(3*S*,4*R*)-Dihydroxycyclopentane-*trans*-1-carboxylic acid (XLI-96-2) A solution of 1.10 g (9.83 mmol) of XLI-91-2 in 106 mL of H_2O containing 2.73 g (48.8 mmol) of KOH was cooled to 0 ^oC with an ice bath and treated with 1.76 g (11.1 mmol) of $KMnO_4$ (finely powdered) in one portion. The mixture was stirred vigorously for 10 min and then treated with 4.43 g (52.7 mmol) of $NaHSO_3$ in one portion. After the mixture was stirred for 10 min at 0 ^oC, the ice bath was removed and the stirring was continued for another 10 min. The suspension was vacuum filtered and the yellow filtrate was neutralized with 2 N H_2SO_4 to pH = 7. The colorless solution was concentrated with a Rotavapor at about 60 ^oC to about 30 mL and then acidified with 2 N H_2SO_4 to pH = 0. The resulting solution was evaporated to dryness with a Rotavapor and an aspirator pump to give a white solid, which was extracted with 2 x 50 mL hot ethyl acetate. After removal of the solvent, 1.55 g of crude product was obtained as a white solid, which was recrystalized from ethyl acetate to give 0.81 g (57%; lit. [90] 50%) of XLI-96-2 as white crystals, mp 97-99 ^oC (lit. [90] mp 99-101 ^oC). ^1H NMR (CD_3COCD_3) 4.68-4.67 (m, 2H), 2.93 (tt, J = 12.0, 6.0 Hz, 1H), 2.01 (dd, J = 14.1, 6.1 Hz, 2H), 1.70 (ddd, J = 12.4, 12.4, 1.7 Hz, 2H); ^{13}C NMR (CD_3COCD_3) 176.2, 80.8 (2), 41.3, 37.6 (2).

Crystals (XLI-96-2A) of **acetonide of XLI-96-2** for X-ray were obtained by recrystallizing XLI-96-2 from acetone and hexanes. mp 120-121.5 ^oC. ^1H NMR ($CDCl_3$) 4.63 (d, J = 4.8 Hz, 2H), 2.98 (tt, J = 12.2, 6.1 Hz, 1H), 2.10 (dd, J = 14.3, 6.0 Hz, 2H), 1.67 (ddd, J = 12.6, 12.6, 3.7 Hz, 2H), 1.38 (s, 3H), 1.22 (s, 3H); ^{13}C NMR ($CDCl_3$) 179.9, 108.1, 79.0 (2), 39.7, 35.8 (2), 25.0, 22.7. Anal. Calcd for $C_9H_{14}O_4 \cdot 0.15H_2O$: C, 57.22; H, 7.63. Found: C, 57.19; H, 7.73.

Diphenyldiazomethane [91] **(XLI-25-2)** A mixture of 6.50 g (33.2 mmol) of benzophenone hydrazone, 7.50 g of anhydrous sodium sulfate, 100 mL of anhydrous ethyl ether, 2.5 mL of ethanol saturated with KOH, and 17.50 g of yellow HgO was shaken for 2 h in a pressure bottle at room temperature. The reaction mixture was vacuum filtered and the solvent was removed from the filtrate with a Rotavapor at room temperature. The resulting dark red oil was dissolved in petroleum ether and again filtered. Removal of the solvent from the filtrate with a Rotavapor at room temperature gave an oil. Freezing this oil in a refrigerator gave 6.25 g (97%; lit. [91] 89%) of XLI-25-2 as dark red crystals, mp 29-31 ^{o}C (lit. [91] mp 29-32 ^{o}C).

Diphenylmethyl (3*S*,4*R*)-dihydroxy-cyclopentane-*trans*-1-carboxylate (XLI-98-2) To a solution of 0.53 g (3.6 mmol) of XLI-96-2 in 15 mL of acetone was added a solution of 0.91 g (4.7 mmol) of diphenyldiazomethane in 10 mL of petroleum ether. The mixture was stirred overnight at room temperature. The solvent was evaporated to afford a residue, which was washed with petroleum ether and then recrystallized from ethyl ether and petroleum ether (v/v = 1/1) to give 0.84 g (75%) of XLI-98-2 as a white solid, mp 65-67 ^{o}C. ^{1}H NMR ($CDCl_3$) 7.34-7.26 (m, 10H), 6.85 (s, 1H), 4.18 (ddd, J = 8.2, 4.1, 4.1 Hz, 2H), 3.29 (tt, J = 9.4, 6.7 Hz, 1H), 2.17-1.99 (m, 4H); ^{13}C NMR ($CDCl_3$) 175.2, 140.1 (2), 128.5 (4), 127.9 (2), 126.9 (4), 77.1, 73.4 (2), 39.4, 34.4 (2). Anal. Calcd for $C_{19}H_{20}O_4 \cdot 0.25H_2O$: C, 72.02; H, 6.52. Found: C, 72.33; H, 6.43.

Crystals (XLI-98-4) of **acetonide of XLI-98-2** for X-ray were obtained by recrystallizing XLI-98-2 from acetone and water. mp 89-91 ^{o}C. ^{1}H NMR ($CDCl_3$) 7.39-7.23 (m, 10H), 6.88 (s, 1H), 4.69-4.64 (m, 2H), 3.16 (tt, J = 12.0, 6.0 Hz, 1H), 2.19 (dd, J = 14.3, 6.0 Hz, 2H), 1.79-1.66

(m, 2H), 1.45 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$) 173.7, 140.2 (2), 128.5 (4), 127.9 (2), 127.0 (4), 109.0, 80.0 (2), 76.9, 41.0, 36.9 (2), 26.1, 23.7. Anal. Calcd for $C_{22}H_{24}O_4$: C, 74.98; H, 6.86. Found: C, 74.99; H, 6.74.

Diphenylmethyl (3*S*,4*R*)-bis(3,4-dimethoxycarbonylcaffeoyloxy)cyclopentane-*trans*-1-carboxylate (XLI-101-1) To a solution of acid chloride XLI-100-1A in 50 mL of toluene was added 30 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated slowly with a solution of 0.55 g (1.8 mmol) of diol XLI-98-2 in 30 mL of toluene. The reaction proceeded overnight at room temperature, and then the mixture was washed successively with 3 x 80 mL 5% HCl, 5% $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.42) to give 1.10 g (72%) of pure XLI-101-1 as a white solid, mp 51-53 ^{o}C. ^{1}H NMR ($CDCl_3$) 7.60 (d, *J* = 15.9 Hz, 2H), 7.45-7.27 (m, 16H), 6.90 (s, 1H), 6.38 (d, *J* = 16.0 Hz, 2H), 5.49-5.45 (m, 2H), 3.91 (s, 12H), 3.44-3.33 (m, 1H), 2.43-2.17 (m, 4H); ^{13}C NMR ($CDCl_3$) 173.8, 165.5 (2), 153.0 (2), 152.9 (2), 143.6 (2), 143.1 (2), 142.7 (2), 139.9 (2), 133.3 (2), 128.6 (4), 128.0 (2), 127.0 (4), 126.8 (2), 123.5 (2), 122.2 (2), 119.1 (2), 77.4, 74.0 (2), 55.9 (4), 39.0, 32.1 (2). Anal. Calcd for $C_{45}H_{40}O_{18}$: C, 62.21; H, 4.64. Found: C, 62.24; H, 4.86.

Diphenylmethyl (3*S*,4*R*)-dicaffeoyloxycyclopentane-*trans*-1-carboxylate (XLI-103-1) To a solution of 0.20 g (0.23 mmol) of XLI-101-1 in 12 mL of THF was added 28 mL of 10% aqueous Na_2CO_3 solution. The mixture was stirred under N_2 at room temperature for 19 h. Excess Na_2CO_3 was neutralized with 8 mL of acetic acid. The mixture was extracted with 2 x 30

mL of dichloromethane. The combined organic layers were successively washed with 10% $NaHCO_3$ and saturated NaCl, and dried over Na_2SO_4. The solvent was evaporated with a Rotavapor to give 0.17 g of XLI-103-1 as a pale yellow solid containing some THF by NMR. ^{1}H NMR (CD_3COCD_3) 7.57 (d, *J* = 15.9 Hz, 2H), 7.47-6.82 (m, 17H), 6.29 (d, *J* = 15.9 Hz, 2H), 5.46-5.44 (m, 2H), 3.54-3.41 (m, 1H), 2.42-2.22 (m, 4H); ^{13}C NMR (CD_3COCD_3) 174.6, 166.7 (2), 148.9 (2), 146.3 (2), 146.2 (2), 141.6 (2), 129.4 (4), 128.6 (2), 127.7 (4), 127.5 (2), 122.8 (2), 116.3 (2), 115.3 (2), 115.1 (2), 77.9, 74.4 (2), 39.8, 32.9 (2).

(3*S*,4*R*)-Dicaffeoyloxycyclopentane-*trans*-1-carboxylic acid (XLI-118-1) To a solution of the above, crude XLI-103-1 in 50 mL of CH_2Cl_2 under N_2 in an ice bath was added dropwise 0.72 mL of TFA. After 5 h, the solution was evaporated with a Rotovapor. The crude product was stirred with 50 mL of CH_2Cl_2 overnight and then filtered to give 112.4 mg (89% from 0.20 g of XLI-101-1) of XLI-103-2 as a pale yellow solid. A pure sample for bioassay was obtained by purifying 60 mg of XLI-103-2 by HPLC with CH_3CN/H_2O/HOAc (v/v/v = 55/45/1) to give 50 mg (83% recovery) of XLI-118-1 (Ret. time = 5.00 min) as a white solid, mp 168-170 ^{o}C. ^{1}H NMR (CD_3COCD_3) 7.56 (d, *J* = 15.8 Hz, 2H), 7.16 (d, *J* = 2.1 Hz, 2H), 7.00 (dd, *J* = 8.2, 2.1 Hz, 2H), 6.83 (d, *J* = 8.2 Hz, 2H), 6.28 (d, *J* = 15.8 Hz, 2H), 5.44 (ddd, *J* = 8.2, 4.1, 4.1 Hz, 2H), 3.29 (tt, *J* = 9.7, 6.4 Hz, 1H), 2.43-2.24 (m, 2H), 2.21 (ddd, *J* = 14.4, 10.0, 5.0 Hz, 2H); ^{13}C NMR (CD_3COCD_3) 176.7, 166.7 (2), 148.8 (2), 146.3 (2), 146.2 (2), 127.5 (2), 122.8 (2), 116.3 (2), 115.3 (2), 115.1 (2), 74.4 (2), 39.2, 33.0 (2). Anal. Calcd for $C_{24}H_{22}O_{10}\cdot 0.25H_2O$: C, 60.68; H, 4.78. Found: C, 60.71; H, 5.05.

2.7.2. *Trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylic acid

Diphenylmethyl 3-cyclopentene-1-carboxylate (XLI-111-1) To a solution of 4.12 g (36.8 mmol) of XLI-91-2 in 10 mL of acetone was added a solution of 8.56 g (44.1 mmol) of diphenyldiazomethane in 30 mL of petroleum ether. The mixture was stirred overnight at room temperature. The solvent was evaporated with a Rotovapor to afford a red residue which was washed with petroleum ether, purified on a silica gel column eluted with ethyl acetate/hexane (v/v = 1/7, R_f = 0.56), and then recrystallized with petroleum ether to give 7.91 g (77%) of XLI-111-1 as a white solid, mp 50-52 ^oC. ^1H NMR ($CDCl_3$) 7.40-7.22 (m, 10H), 6.90 (s, 1H), 5.65 (s, 2H), 3.22 (tt, *J* = 8.0, 8.0 Hz, 1H), 2.68 (d, *J* = 8.1 Hz, 4H); ^{13}C NMR ($CDCl_3$) 174.9, 140.4 (2), 128.9 (2), 128.5 (4), 127.8 (2), 127.0 (4), 76.8, 41.7, 36.2 (2). Anal. Calcd for $C_{19}H_{18}O_2$: C, 81.99; H, 6.52. Found: C, 81.88; H, 6.48.

***Trans*-3,4-dihydroxycyclopentane-1-carboxylic acid (XLI-112-2) from XLI-111-1** To a solution of 0.13 g (1.1 mmol) of 30% H_2O_2 in 1 mL of formic acid was added 0.21 g (0.74 mmol) of XLI-111-1. The mixture was stirred for 2 h at 65-70 ^oC. Most of formic acid and water were removed by lyophilization, and the resulting residue was heated at 65-70 ^oC for 45 min with 4 mL of 20% sodium hydroxide. After cooling, the mixture was neutralized with 12 mL of 10% hydrochloric acid, and extracted with 10 mL × 2 of ethyl acetate. The aqueous layer was lyophilized to almost dryness, and the resulting residue was extracted with 10 mL × 2 of acetone. The combined acetone solution was evaporated with a Rotovapor to give 0.12 g (100%) of crude XLI-112-2 as a pale yellow gum, which was identified as the title compound by NMR (see XLI-113-3 for NMR of pure sample.). No purification was done on XLI-112-2.

***Trans*-3,4-dihydroxycyclopentane-1-carboxylic acid (XLI-113-3) from XLI-91-2** To a solution of 6.39 g (56.4 mmol) of 30% H_2O_2 in 54 mL of formic acid was added 5.06 g (45.2 mmol) of XLI-91-2. The mixture was stirred for 2 h at 65-70 ^oC. Most of formic acid and water were removed with a Rotovapor, and the resulting residue was heated at 65-70 ^oC for 45 min with 30 mL of 20% sodium hydroxide. After cooling, the mixture was neutralized with 10% hydrochloric acid and evaporated to dryness with a Rotovapor. The resulting solid was extracted with acetone twice. The combined organic solution was evaporated with a Rotovapor and the residue was purified by recrystallization from ethyl acetate to give 3.97 g (60%) of XLI-113-3 as a white solid. The crystals for X-ray were obtained from acetone and hexanes. mp 115.5-117.5 ^oC. ^1H NMR (CD_3COCD_3) 4.08-3.94 (m, 2H), 3.03-2.95 (m, 1H), 2.34-2.12 (m, 2H), 1.89-1.77 (m, 2H); ^{13}C NMR (CD_3COCD_3) 177.9, 78.92, 78.87, 40.4, 36.3, 36.2. Anal. Calcd for $C_6H_{10}O_4$: C, 49.31; H, 6.90. Found: C, 49.26; H, 7.04.

Diphenylmethyl *trans*-3,4-dihydroxycyclopentane-1-carboxylate (XLI-115-2) To a solution of 0.65 g (4.5 mmol) of XLI-113-3 in 15 mL of acetone was added 1.04 g (5.34 mmol) of diphenyldiazomethane in 10 mL of petroleum ether. The mixture was stirred overnight at room temperature. The solvent was evaporated to afford a red residue, which was washed with petroleum ether and then passed through a silica gel column with ethyl ether and petroleum ether (v/v = 1/5). After the less polar components were washed out, the column was washed with methanol to give 1.18 g (85%) of XLI-115-2 as an orange-yellow gum. ^1H NMR (CD_3COCD_3) 7.42-7.21 (m, 10H), 6.87 (s, 1H), 4.29 (br s, 1H), 4.07-4.03 (m, 2H), 3.48 (br s, 1H), 3.23-3.12 (m, 1H), 2.41-2.20 (m, 2H), 1.96-1.84 (m, 2H); ^{13}C NMR (CD_3COCD_3) 175.4, 141.6 (2), 129.2

(4), 128.4 (2), 127.5 (4), 78.6 (2), 77.4, 40.5, 36.1, 35.7.

Diphenylmethyl *trans*-3,4-bis(3,4-dimethoxycarbonylcaffeoyloxy)cyclopentane-1-carboxylate (XLI-116-2) To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 12.05 g (0.041 mol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 80 mL of toluene was added 30 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated with a solution of 2.12 g (6.79 mmol) of XLI-115-2 in 20 mL of toluene. After the reaction proceeded overnight at room temperature, the mixture was successively washed with 3 x 100 mL each of 5% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to give a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 0.9/1) to give 2.71 g (46%) of XLI-116-2 (R_f = 0.55 when EtOAc/hexane = 1/1) as a white solid, mp 44-46 ^oC. ^1H NMR ($CDCl_3$) 7.63 (d, *J* = 16.1 Hz, 1H), 7.53 (d, *J* = 15.8 Hz, 1H), 7.47-7.21 (m, 16H), 6.89 (s, 1H), 6.38 (d, *J* = 16.1 Hz, 1H), 6.22 (d, *J* = 16.1 Hz, 1H), 5.38-5.34 (m, 1H), 5.31-5.27 (m, 1H), 3.931 (s, 3H), 3.928 (s, 3H), 3.923 (s, 6H), 3.26 (tt, *J* = 8.1, 8.1 Hz, 1H), 2.68-2.54 (m, 2H), 2.23-2.15 (m, 2H); ^{13}C NMR ($CDCl_3$) 173.3, 165.3 (2), 153.1 (2), 152.9 (2), 143.70, 143.66, 143.3, 143.2, 142.7, 142.6, 140.03, 139.95, 133.34, 133.29, 128.6 (4), 128.0 (2), 127.0 (2), 126.9 (2), 126.75, 126.72, 123.5, 123.4, 122.42, 122.35, 119.1, 119.0, 78.2, 77.9, 77.3, 56.0 (4), 40.7, 33.9, 33.5. Anal. Calcd for $C_{45}H_{40}O_{18}$: C, 62.21; H, 4.64. Found: C, 62.51; H, 4.77.

Diphenylmethyl *trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylate (XLI-116-4) To a solution of 0.17 g (0.20 mmol) of XLI-116-2 in 10 mL of THF was added 22 mL of 10% aqueous Na_2CO_3 solution. The mixture was stirred under N_2 at room temperature for 24 h.

Excess Na_2CO_3 was neutralized with 6.5 mL of acetic acid. The mixture was extracted with 2 x 30 mL of dichloromethane. The combined organic layer was successively washed with 10% $NaHCO_3$ and saturated NaCl, and dried over Na_2SO_4. The solvent was evaporated with a Rotovapor to give XLI-116-4 as a pale yellow solid containing some THF by NMR, which was used in the next step without purification. ^{1}H NMR (CD_3COCD_3) 7.61 (d, *J* = 15.9 Hz, 1H), 7.53 (d, *J* = 15.9 Hz, 1H), 7.45-6.86 (m, 16H), 6.89 (s, 1H), 6.30 (d, *J* = 15.8 Hz, 1H), 6.17 (d, *J* = 15.9 Hz, 1H), 5.32-5.25 (m, 2H), 3.42-3.28 (m, 1H), 2.65-2.50 (m, 2H), 2.20-1.97 (m, 2H); ^{13}C NMR (CD_3COCD_3) 174.0, 166.7, 166.6, 149.09, 149.06, 146.5 (2), 146.40, 146.36, 141.7, 141.6, 129.4 (4), 128.6 (2), 127.6 (4), 127.4 (2), 122.8, 122.7, 116.3 (2), 115.25, 115.21, 115.1, 115.0, 78.6, 78.4, 77.9, 41.3, 34.5, 34.2.

***Trans*-3,4-dicaffeoyloxycyclopentane-1-carboxylic acid (XLI-118-2)** To a solution of the above, crude XLI-116-4 in 20 mL of CH_2Cl_2 under N_2 in an ice bath was added dropwise 0.62 mL of TFA. After 5 h, the solution was evaporated with a Rotovapor. The crude product was sonicated with 20 mL of CH_2Cl_2 for 5 min and then filtered to give 78.3 mg (85% from 0.17 g of XLI-116-2) of XLI-117-1 as a pale yellow solid. A pure sample for bioassay was obtained by purifying 50 mg of XLI-117-1 by HPLC with CH_3CN/H_2O/HOAc (v/v/v = 55/45/1) as eluting solvent to give 39 mg (78% recovery) of XLI-118-2 (Ret. time = 7.71 min) as a white solid, mp 197-199 ^{o}C (dec.). ^{1}H NMR (CD_3COCD_3) 7.60 (d, *J* = 15.8 Hz, 1H), 7.58 (d, *J* = 15.8 Hz, 1H), 7.19 (d, *J* = 2.1 Hz, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 7.08 (dd, *J* = 2.1, 8.2 Hz, 1H), 7.06 (dd, *J* = 2.1, 8.2 Hz, 1H), 6.88 (br d, *J* = 8.2 Hz, 2H), 6.31 (d, *J* = 15.8 Hz, 1H), 6.28 (d, *J* = 15.8 Hz, 1H), 5.31-5.28 (m, 1H), 5.26-5.22 (m, 1H), 3.20 (tt, *J* = 8.2, 8.2 Hz, 1H), 2.64-2.46 (m, 2H), 2.14-2.05

(m, 2H); ^{13}C NMR (CD_3COCD_3) 175.9, 166.7, 166.6, 148.9 (2), 146.4 (2), 146.3 (2), 127.5 (2), 122.7 (2), 116.4 (2), 115.3 (2), 115.2 (2), 78.6, 78.5, 40.6, 34.5, 34.2. Anal. Calcd for $C_{24}H_{22}O_{10}\cdot 0.25H_2O$: C, 60.68; H, 4.78. Found: C, 60.42; H, 5.09.

2.7.3. (3*R*,4*S*)-Dicaffeoyloxycyclopentane-cis-1-carboxylic acid and its methyl ester

(3*R*,4*S*)-Dihydroxycyclopentane-*trans*/*cis*-1-carboxylic acid (XLI-173-2) A solution of 3.06 g (27.3 mmol) of XLI-169-2 in 325 mL of H_2O containing 8.35 g (0.15 mol) of KOH was cooled to 0 ^oC with an ice bath, and treated with 5.39 g (34.1 mmol) of $KMnO_4$ (finely powdered) in one portion. The mixture was stirred vigorously for 10 min and then treated with 13.56 g (0.16 mol) of $NaHSO_3$ in one portion. After the mixture was stirred for 10 min at 0 ^oC, the ice bath was removed and the stirring was continued for another 10 min. The suspension was vacuum filtered and the yellow filtrate was neutralized with 2 N H_2SO_4 to pH = 7 (colorless, ca. 400 mL). The solution was concentrated with a Rotavapor at about 60 ^oC to about 60 mL and then acidified with 2 N H_2SO_4 to pH = 0. The resulting solution was dried with a Rotavapor to give a white solid, which was extracted with 2 x 100 mL hot ethyl acetate. After removal of the solvent, 4.23 g of crude product (XLI-173-2) was obtained as a white residue which was used in the next step without purification.

Acetonide of methyl (3*R*,4*S*)-dihydroxy-cyclopentane-*trans*/*cis*-1-carboxylate (XLI-174-3 and XLI-174-4) A mixture of 4.23 g (29.0 mmol) of XLI-173-2, 20 drops of conc H_2SO_4 and 50 mL of methanol was refluxed overnight. The resulting solution was neutralized with excess $NaHCO_3$, filtered, and concentrated with a Rotavapor to give 4.78 g of XLI-174-1 as a

residue, to which was added 5.68 g (54.6 mmol) of 2,2-dimethoxypropane and 35 mL of acetone. The mixture was acidified with conc H_2SO_4 and refluxed overnight. Most of the acetone was removed with a Rotavapor to give a residue, which was dissolved in 30 mL of ethyl ether, and successively washed with saturated aqueous $NaHCO_3$ and H_2O. The organic layer was dried over $MgSO_4$, filtered and concentrated with a Rotavapor to give a yellow solution which was separated on a silica gel column eluted with ethyl ether and hexanes (v/v = 1/4) to give the *cis*-isomer XLI-174-4 (0.41 g, 7%, R_f = 0.20) and the *trans*-isomer XLI-174-3 (3.31 g, 61%, R_f = 0.35) as colorless liquids. The combined yield was 68% from 3.06 g of XLI-169-2.

XLI-174-3: ^{1}H NMR ($CDCl_3$) 4.61 (dd, *J* = 4.0, 1.3 Hz, 2H), 3.61 (s, 3H), 2.96 (tt, *J* = 12.3, 6.2 Hz, 1H), 2.06 (ddd, *J* = 14.1, 6.2, 0.9 Hz, 2H), 1.70-1.60 (m, 2H), 1.37 (s, 3H), 1.21 (s, 3H); lit. [92] ^{1}H NMR ($CDCl_3$) 4.62 (half an AA'XX' pattern, 2H), 3.63 (s, 3H), 2.98 (tt, *J* = 12.0, 6.0 Hz, 1H), 2.08 (ddd, *J* = 14.0, 6.0, 1.1 Hz, 2H), 1.68 (br t, *J* = 12-14 Hz, 2H), 1.39 (s, 3H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$) 175.5, 109.2, 80.2 (2), 51.9, 40.8, 37.2 (2), 26.3, 23.9.

XLI-174-4: ^{1}H NMR ($CDCl_3$) 4.56 (dd, *J* = 3.1, 1.8 Hz, 2H), 3.62 (s, 3H), 2.74 (tt, *J* = 8.3, 3.1 Hz, 1H), 2.41 (ddd, *J* = 14.1, 3.1, 1.3 Hz, 2H), 1.84-1.75 (m, 2H), 1.30 (s, 3H), 1.19 (s, 3H); lit. [92] ^{1}H NMR ($CDCl_3$) 4.61 (half an AA'XX' pattern, 2H), 3.67 (s, 3H), 2.79 (tt, *J* = 8.1, 3.2 Hz, 1H), 2.45 (dd, *J* = 14.0, 3.2 Hz, 2H), 1.85 (m, 2H), 1.35 (s, 3H), 1.24 (s, 3H); ^{13}C NMR ($CDCl_3$) 174.6, 110.5, 80.7 (2), 51.9, 42.5, 35.1 (2), 26.1, 24.2.

Ester enolate kinetic protonation (XLI-197-2 ≡ XLI-179-2) To a stirred solution of 12.0 mL (24.0 mmol) of 2 M LDA in 20 mL of THF at –78 ^{o}C was added dropwise a solution of 3.20 g (16.0 mmol) of XLI-174-3 in 30 mL of THF. Stirring was continued for 30 min at –78 ^{o}C

and then the resulting solution was transferred via canula to a stirred solution of 14.86 g (72.0 mmol) of 2,6-di-*tert*-butylphenol in 40 mL of THF at –78 ^{o}C. The mixture was stirred 5 min at -78 ^{o}C, warmed to ambient temperature, and concentrated with a Rotavapor. The resulting residue was diluted with 80 mL of EtOAc, washed sequentially with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4. Removal of solvent with a Rotavapor gave a residue, which was separated on a silica gel column with hexanes eluting the phenol and ethyl ether eluting the mixture of esters, XLI-197-1, which was further separated on a silica gel column with ethyl ether and hexanes (v/v = 1/4) to give the cis isomer XLI-197-2 (2.03 g, 63%, R_f = 0.20) and the trans isomer XLI-197-3 (0.34 g, 11%, R_f = 0.35). The NMR data of XLI-197-2 and XLI-197-3 were identical to those of XLI-174-3 and XLI-174-4.

Methyl (3*R*,4*S*)-dihydroxycyclopentane-*cis*-1-carboxylate (XLI-198-2) A mixture of 1.30 g (6.50 mmol) of acetonide XLI-197-2 in 30 mL of CH_3OH-H_2O (v/v = 1/1) containing 10 drops of conc HCl was stirred overnight at room temperature. The resulting solution was neutralized with excess $NaHCO_3$, the methanol was removed with a Rotavapor and the water by lyophilization to give a white solid, which was extracted with methanol. The methanol solution was evaporated to give 1.39 g of white solid (XLI-198-1). NMR indicated that XLI-198-1 was a mixture of (3*R*,4*S*)-dihydroxycyclopentane-cis-1-carboxylic acid and its methyl ester. A solution of XLI-198-1, 5 drops of conc H_2SO_4 and 40 mL of methanol was refluxed overnight and the resulting solution neutralized with excess $NaHCO_3$, filtered, and concentrated with a Rotavapor to give a white solid, which was extracted with methanol. The methanol solution was evaporated to give 1.04 g (100% from XLI-197-2) of crude XLI-198-2 as a white solid containing some

impurities by NMR, which was used in the next step without purification. ^{1}H NMR ($CDCl_3$) 3.98-3.93 (m, 2H), 3.63 (s, 3H), 3.32 (br s, 2H), 2.76-2.67 (m, 1H), 2.16-2.06 (m, 2H), 1.95-1.86 (m, 2H); ^{13}C NMR ($CDCl_3$) 177.7, 73.6 (2), 52.4, 38.8, 34.6 (2).

Methyl (3*R*,4*S*)-bis(3,4-dimethoxycarbonylcaffeoyloxy)cyclopentane-*cis*-1-carboxylate (XLI-199-1) To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 2.60 g (8.78 mmol) of 3, 4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 40 mL of toluene was added 14 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated with a mixture of 0.34 g (2.1 mmol) of XLI-198-2 in 10 mL of toluene. After the reaction proceeded overnight at room temperature, it was successively washed three times each with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent was evaporated with a Rotovapor to give 1.87 g of yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 0.8/1, R_f = 0.26) to give 0.64 g (42%) of XLI-199-1 as a white gum. ^{1}H NMR ($CDCl_3$) 7.61 (d, *J* = 16.3 Hz, 2H), 7.46 (br s, 2H), 7.39 (dd, *J* = 8.4, 1.3 Hz, 2H), 7.30 (d, *J* = 8.4 Hz, 2H), 6.38 (d, *J* = 16.3 Hz, 2H), 5.34 (dd, *J* = 4.6, 4.6 Hz, 2H), 3.91 (s, 12H), 3.73 (s, 3H), 2.95 (tt, *J* = 8.4, 8.4 Hz, 1H), 2.46-2.28 (m, 4H); ^{13}C NMR ($CDCl_3$) 174.8, 165.4 (2), 152.9 (2), 152.8 (2), 143.5 (2), 143.1 (2), 142.5 (2), 133.2 (2), 126.7 (2), 123.4 (2), 122.1 (2), 119.0 (2), 73.4 (2), 55.8 (4), 52.1, 37.7, 31.6 (2).

Methyl (3*R*,4*S*)-dicaffeoyloxycyclopentane-*cis*-1-carboxylate (XLII-26-2) A solution of 55.0 mg (7.68 x 10^{-2} mmol) of XLI-199-1, 8 mL of ethyl acetate and 0.41 g (3.1 mmol) of LiI was refluxed for 3 h. Filtration gave an orange-yellow solid, which was dissolved in 4 mL of

H_2O, and then acidified to pH = 2-3 with 6 M HCl. The resulting yellow precipitate was filtered to give 30.0 mg (81%) of XLI-200-5 as a pale yellow solid. In order to get a pure sample for bioassay, 30.0 mg of XLI-200-5 was purified by HPLC with MeOH, H_2O and HOAc (v/v/v = 70/30/1) as eluting solvents to give 19.2 mg (64% recovery) of XLII-26-2 (Ret. time = 5.53 min) as a white solid, mp 92-94 ^oC. XLI-200-5: ^1H NMR (CD_3OD) 7.51 (d, *J* = 15.8 Hz, 2H), 7.03 (d, *J* = 1.8 Hz, 2H), 6.88 (dd, *J* = 8.2, 1.8 Hz), 6.74 (d, *J* = 8.2 Hz, 2H), 6.22 (d, *J* = 16.1 Hz, 2H), 5.31 (dd, *J* = 4.7, 4.7 Hz, 2H), 3.72 (s, 3H), 3.07-2.96 (m, 1H), 2.44-2.34 (m, 2H), 2.30-2.22 (m, 2H); ^{13}C NMR (CD_3OD) 177.0, 168.4 (2), 149.7 (2), 147.5 (2), 146.8 (2), 127.6 (2), 123.3 (2), 116.5 (2), 115.0 (2), 114.8 (2), 74.8 (2), 52.7, 39.2, 32.8 (2). Anal. Calcd for $C_{25}H_{24}O_{10}\cdot H_2O$: C, 59.76; H, 5.22. Found: C, 59.99; H, 5.16.

(3*R*,4*S*)-Dihydroxy-*cis*-1-carboxylic acid (XLI-182-1) A mixture of 1.31 g (65.5 mmol) of acetonide XLI-179-2 in 60 mL of CH_3OH-H_2O (v/v=1/1) containing 2 mL of conc HCl was stirred overnight at RT. The resulting solution was basified with 10% NaOH to pH = 13-14. The mixture was stirred overnight at RT, and then acidified with conc HCl to pH = 2-3. After the mixture was lyophilized to dryness, it was extracted with ethyl acetate. The combined organic solution was evaporated to give 0.95 g (99%) of XLI-182-1 as a white gum, which was used in the next step without purification. ^1H NMR (CD_3COCD_3) 4.64-4.59 (m, 2H), 2.83 (tt, *J* = 8.4, 4.4 Hz, 1H), 2.32-2.26 (m, 2H), 1.94 (dddd, *J* = 12.8, 8.4, 4.4, 1.8 Hz, 2H); ^{13}C NMR (CD_3COCD_3) 175.4, 81.5 (2), 42.9, 35.6 (2).

Diphenylmethyl (3*R*,4*S*)-dihydroxy-*cis*-1-carboxylate (XLI-182-3) To a solution of 0.95 g (6.5 mmol) of XLI-182-1 in 15 mL of acetone was added a solution of 1.52 g (7.84 mmol)

of diphenyldiazomethane in 10 mL of petroleum ether. The mixture was stirred overnight at room temperature, and then vacuum filtered. The solvent was evaporated to afford a pink solid, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1.5/1, R_f = 0.16) to give 1.38 g (68%) of XLI-182-3 as a white solid, mp 75-77 ^oC. ^1H NMR ($CDCl_3$) 7.33-7.20 (m, 10H), 6.85 (s, 1H). 3.96-3.90 (m, 2H), 3.46 (br s, 2H), 2.82 (tt, *J* = 9.2, 7.3 Hz, 1H), 2.18-2.08 (m, 2H), 2.00-1.92 (m, 2H); ^{13}C NMR ($CDCl_3$) 175.9, 140.3 (2), 128.8 (4), 128.2 (2), 127.3 (4), 77.7, 73.6 (2), 39.2, 34.6. Anal. Calcd for $C_{19}H_{20}O_4 \cdot 0.25H_2O$: C, 72.02; H, 6.52. Found: C, 72.38; H, 6.45.

Diphenylmethyl (3*R*,4*S*)-bis(3,4-dimethoxycarbonylcaffeoyloxy)cyclopentane-*cis*-1-carboxylate (XLII-23-1) To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 1.70 g (5.74 mmol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 30 mL of toluene was added 12 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated with a solution of 0.30 g (0.96 mmol) of XLI-182-3 in 10 mL of toluene. After reacting overnight at room temperature, the mixture was successively washed with three portions each of 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent was evaporated with a Rotovapor to give 2.34 g of a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.47) to give 0.54 g (65%) of XLII-23-1 as a white solid, mp 55-57 ^oC. ^1H NMR ($CDCl_3$) 7.53 (d, *J* = 16.1 Hz, 2H), 7.38-7.20 (m, 16H), 6.91 (s, 1H), 6.26 (d, *J* = 16.1 Hz, 2H), 5.36 (dd, *J* = 4.7, 4.7 Hz, 2H), 3.90 (s, 12H), 3.08 (tt, *J* = 8.1, 8.1 Hz, 1H), 2.45-2.40 (m, 4H); ^{13}C NMR ($CDCl_3$) 173.2, 165.4 (2), 152.9 (2), 152.7 (2), 143.5 (2), 143.0 (2), 142.4 (2), 139.9

(2), 133.2 (2), 128.4 (4), 127.8 (2), 126.7 (6), 123.3 (2), 122.2 (2), 118.9 (2), 77.3, 73.4 (2), 55.8 (4), 38.3, 31.3 (2). Anal. Calcd for $C_{45}H_{40}O_{18}$: C, 62.21; H, 4.64. Found: C, 62.57; H, 4.85.

Diphenylmethyl (3*R*,4*S*)-dicaffeoyloxycyclopentane-*cis*-1-carboxylate (XLII-24-1) To a solution of 0.15 g (0.17 mmol) of XLII-23-1 in 10 mL of THF was added 20 mL of 10% aqueous Na_2CO_3. The mixture was stirred under N_2 at room temperature for 13 h, acidified with 6 mL of AcOH, and then extracted with three portions of CH_2Cl_2. The combined organic solution was washed with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated with a Rotovapor. Traces of AcOH were removed by lyophilization to give 0.11 g of XLII-24-1 as a yellow residue, which was used in the next step without purification. ^{1}H NMR (CD_3OD) 7.44 (d, *J* = 16.1 Hz, 2H), 7.36-7.19 (m, 16H), 6.87 (s, 1H), 6.10 (d, *J* = 15.8 Hz, 2H), 5.35-5.29 (m, 2H), 3.16 (tt, *J* = 9.4, 6.5 Hz, 1H), 2.48-2.30 (m, 4H); ^{13}C NMR (CD_3OD) 175.2, 168.4 (2), 149.7 (2), 147.5 (2), 146.8 (2), 141.7 (2), 129.6 (4), 129.0 (2), 128.0 (4), 127.6 (2), 123.4 (2), 116.4 (2), 115.1 (2), 114.7 (2), 78.9, 74.9 (2), 39.8, 32.8 (2).

(3*R*,4*S*)-Dicaffeoyloxycyclopentane-*cis*-1-carboxylic acid (XLII-24-4) To a solution of 0.11 g of XLII-24-1 in 30 mL of CH_2Cl_2 in an ice bath was added dropwise 0.52 mL of TFA. After 4.5 h at 0 ^{o}C under N_2, the solvents were removed with a Rotovapor, and the residue was washed with CH_2Cl_2 to leave 73.1 mg (90% from XLII-23-1) of XLII-24-2 as a pale yellow solid. A pure sample for bioassay was obtained by HPLC with CH_3CN, H_2O and HOAc (v/v/v = 50/50/1) to give 12.4 mg (17% recovery) of XLII-24-4 (Ret. time = 4.45 min) as a white solid, mp 142-144 ^{o}C. ^{1}H NMR (CD_3OD) 7.52 (d, *J* = 15.8 Hz, 2H), 7.02 (d, *J* = 2.1 Hz, 2H), 6.87 (dd, *J* = 8.2, 1.8 Hz, 2H), 6.72 (d, *J* = 8.2 Hz, 2H), 6.22 (d, *J* = 15.8 Hz, 2H), 5.34-5.28 (m, 2H), 2.96

(tt, J = 8.4, 8.4 Hz, 1H), 2.44-2.35 (m, 2H), 2.31-2.22 (m, 2H); ^{13}C NMR (CD_3OD) 179.1, 168.5 (2), 149.7 (2), 147.4 (2), 146.8 (2), 127.7 (2), 123.4 (2), 116.5 (2), 115.0 (2), 114.9 (2), 74.9 (2), 39.7, 33.1 (2). Anal. Calcd for $C_{24}H_{22}O_{10}\cdot H_2O$: C, 59.02; H, 4.95. Found: C, 58.91; H, 4.74.

2.7.4. *Threo*-3,4-dicaffeoyloxyadipic acid

***Threo*-3,4-dihydroxyadipic-*γ, γ'*-dilactone (XLI-177-2)** A solution of 0.72 g (5.0 mmol) of *trans*-3-hexenedioic acid and 2.73 g (48.8 mmol) of KOH in 106 mL of H_2O was cooled to 0 ^{o}C in an ice bath and treated with 0.88 g (5.6 mmol) of finely powdered $KMnO_4$ in one portion. The mixture was stirred vigorously for 12 min and then treated with 2.22 g (26.3 mmol) of $NaHSO_3$ in one portion. The reaction was stirred for 10 min at 0 ^{o}C, the ice bath removed and the stirring continued for another 10 min. The suspension was vacuum filtered and the yellow filtrate neutralized with 2 N H_2SO_4 to pH = 7. The solution was concentrated to about 30 mL with a Rotavapor and then acidified with 2 N H_2SO_4 to pH = 2. The resulting solution was dried with a Rotovapor to give a white solid, which was extracted with ethyl acetate. After removal of solvent with a Rotovapor, 0.67 g of crude product was obtained as a white solid, which was recrystallized from ethyl acetate to give 0.51 g (72%) of XLI-177-2 as white crystals, mp 129-130 ^{o}C (lit. mp 125-126 ^{o}C, [95] 129-131 ^{o}C [94], 132 ^{o}C [101]). ^{1}H NMR (CD_3COCD_3) 5.39 (dd, J = 5.6, 0.9 Hz, 2H), 3.12 (ddd, J = 18.8, 4.4, 0.9 Hz, 2H), 2.79 (d, J = 18.8 Hz, 2H); lit. [101] (CD_3COCD_3) 5.38 (m, 2H), 3.16 (dd, J = 18.0, 4.5 Hz, 2H), 2.76 (d, J = 18.0 Hz, 2H); ^{13}C NMR (CD_3COCD_3) 174.6 (2), 79.9 (2), 35.6 (2).

Bis(diphenylmethyl) *trans*-3-hexenedioate (XLI-187-2 ≡ XLI-193-1) To a solution of 0.50 g (3.5 mmol) of *trans*-3-hexenedioic acid in 20 mL of acetone was added a solution of 1.62

g (8.35 mmol) of diphenyldiazomethane in 10 mL of petroleum ether. The mixture was stirred overnight at room temperature. Removal of the solvent afforded a pink solid, which was washed with petroleum ether to give 0.94 g of pale yellow solid, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/3, R_f = 0.49) to give 0.86 g (52%) of XLI-187-2 as a white solid, mp 108-110 ^oC. ^1H NMR ($CDCl_3$) 7.38-7.23 (m, 20H), 6.88 (s, 2H), 5.74 (tt, *J* = 3.7, 1.8 Hz 2H), 3.20 (dd, *J* = 4.0, 1.8 Hz, 4H); ^{13}C NMR ($CDCl_3$) 170.7 (2), 140.3 (4), 128.8 (8), 128.2 (4), 127.3 (8), 126.2 (2), 77.3 (2), 38.3 (2). XLI-193-1 (74%), a crude product without purification on silica gel, was prepared in three-fold scale following the same procedures. Anal. Calcd for $C_{32}H_{28}O_4 \cdot 0.5H_2O$: C, 79.15; H, 6.02. Found: C, 79.05; H, 6.05.

Bis(diphenylmethyl) *threo*-3,4-dihydroxyadipate (XLI-194-4) To a solution of 2.38 g (5.00 mmol) of XLI-193-1 and 1.17 g (10.0 mmol) of *N*-methylmorpholine *N*-oxide (NMO) in a mixture of 25 mL of *tert*-BuOH and 25 mL of DMF was added 7.6 mL of *tert*-BuOH containing 0.38 g (1.5 mmol) of OsO_4 and the reaction was followed by the TLC (hexanes/EtOAc = 3/1) until disappearance of the starting material (about 1 h). The reaction was quenched by addition of a saturated aqueous $NaHSO_3$ solution, diluted with 50 mL of H_2O, and extracted with 3 x 50 mL of AcOEt. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated with a Rotovapor to give a pale yellow residue, which was separated on silica gel with ethyl acetate and hexanes (v/v = 1/2, R_f = 0.32) as eluting solvents to give 2.30 g (90%) of XLI-194-4 as a white solid, mp 89-91 ^oC. ^1H NMR ($CDCl_3$) 7.33-7.23 (m, 20H), 6.89 (s, 2H). 4.00-3.93 (m, 2H), 3.12-3.09 (m, 2H), 2.80-2.60 (m, 4H); ^{13}C NMR ($CDCl_3$) 171.6 (2), 139.8 (2), 139.7 (2), 128.6 (4), 128.5 (4), 128.1 (2), 128.0 (2), 127.1 (4), 127.0 (4), 77.4 (2), 69.7 (2),

38.2 (2). Anal. Calcd for $C_{32}H_{30}O_6$: C, 75.28; H, 5.92. Found: C, 75.02; H, 5.92.

Bis(diphenylmethyl) *threo*-3,4-bis(3,4-dimethoxycarbonylcaffeoyloxy)adipate (XLI-195-1) To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 1.78 g (6.01 mmol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 40 mL of toluene was added 10 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated with a mixture of 0.51 g (1.0 mmol) of XLI-194-4 in 10 mL of toluene. After reacting overnight at room temperature, the mixture was successively washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to give 2.50 g of a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 0.9/1, R_f = 0.40) to give 0.69 g (64%) of XLI-195-1 as a white solid, mp 72-74 ^oC. ^1H NMR ($CDCl_3$) 7.52 (d, J = 15.8 Hz, 2H), 7.39-7.17 (m, 26H), 6.87 (s, 2H), 6.24 (d, J = 16.3 Hz, 2H), 5.76 (t, J = 5.5 Hz, 2H), 3.92 (s, 6H), 3.91 (s, 6H), 2.83 (d, J = 6.2 Hz, 4H); ^{13}C NMR ($CDCl_3$) 168.5 (2), 165.2 (2), 153.0 (2), 152.9 (2), 143.7 (2), 142.6 (2), 139.58 (2), 139.55 (4), 133.2 (2), 128.40 (4), 128.39 (4), 127.9 (4), 127.1 (4), 127.0 (4), 126.7 (2), 123.4 (2), 122.3 (2), 118.4 (2), 77.5 (2), 70.0 (2), 55.9 (4), 36.2 (2). Anal. Calcd for $C_{58}H_{50}O_{20}$: C, 65.29; H, 4.72. Found: C, 65.37; H, 4.96.

Bis(diphenylmethyl) *threo*-3,4-dicaffeoyloxyadipate (XLI-195-3) To a solution of 0.20 g (0.19 mmol) of XLI-195-1 in 10 mL of THF was added 22 mL of 10% aqueous Na_2CO_3 solution. After 16 h at room temperature under N_2, the resulting solution was neutralized with 6 mL acetic acid and extracted with CH_2Cl_2. The organic layer was washed with 5% $NaHCO_3$ and dried over Na_2SO_4. The solvent was removed with a Rotovapor to give 0.17 g of XLI-195-3 as a

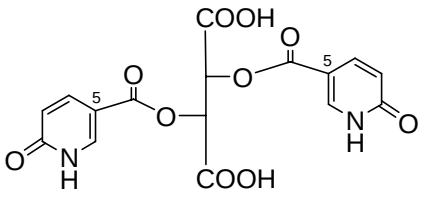
yellow residue containing some THF by NMR. ^{1}H NMR ($CDCl_3$) 7.46 (d, *J* = 15.8 Hz, 2H), 7.34-6.75 (m, 28H), 6.06 (d, *J* = 15.8 Hz, 2H), 5.76 (br s, 2H), 2.83 (br s, 4H); ^{13}C NMR ($CDCl_3$) 169.2 (2), 166.5 (2), 147.1 (2), 146.6 (2), 144.3 (2), 139.5 (4), 128.4 (8), 127.9 (4), 127.1 (4), 127.0 (4), 126.8 (2), 122.4 (2), 115.2 (2), 114.4 (2), 113.7 (2), 77.8 (2), 69.8 (2), 36.3 (2).

***Threo*-3,4-dicaffeoyloxyadipic acid (XLI-196-3)** To a solution of 0.17 g of XLI-195-3 in 12 mL of CH_2Cl_2 in an ice bath was added dropwise 1.20 mL of TFA. After 4 h at 0 ^{o}C under N_2, the CH_2Cl_2 and extra TFA were removed with a Rotovapor to give crude product, which was washed with CH_2Cl_2 and then filtered to give 87.0 mg (92% from XLI-195-1) of XLI-196-1 as a pale yellow solid. In order to get a pure sample for bioassay, 44.0 mg of XLI-196-1 was purified by HPLC with MeOH, H_2O and HOAc (v/v/v = 60/40/1) to give 35.4 mg (80% recovery) of XLI-196-3 (Ret. time = 3.96 min) as a white solid, mp 190-192 ^{o}C. XLI-196-3: ^{1}H NMR (CD_3OD) 7.58 (d, *J* = 16.1 Hz, 2H), 7.05 (d, *J* = 2.1 Hz, 2H), 6.95 (dd, *J* = 8.2, 2.1 Hz, 2H), 6.78 (d, *J* = 8.2 Hz, 2H), 6.29 (d, *J* = 15.8 Hz, 2H), 5.66 (br s, 2H), 2.71 (br s, 4H); ^{13}C NMR (CD_3OD) 174.1 (2), 168.2 (2), 149.8 (2), 147.8 (2), 146.8 (2), 127.7 (2), 123.2 (2), 116.5 (2), 115.2 (2), 114.5 (2), 71.7 (2), 37.1 (2). Anal. Calcd for $C_{24}H_{22}O_{12}$: C, 57.36; H, 4.42. Found: C, 57.07; H, 4.43.

Chapter 3. Syntheses of 2-Pyridone Analogues of L-Chicoric Acid

3.1. Introduction

For the reasons discussed in Chapter 1 (page 13-18), the following 2-pyridone analogues of L-CA were synthesized. Compounds **39**, **41** and **43** are esters, while compounds **40**, **42** and **44** are amides. For compounds **41** and **43**, only one caffeoyl group of L-CA was replaced by a 2-pyridone group, but for the other compounds, both caffeoyl groups were replaced. For compounds **39**-**41**, the carbonyl groups were attached directly to the 5-position of the 2-pyridone rings. Compounds **42** and **43** were synthesized to be compared with compounds **40** and **41**, respectively, to determine if a double bond between the carbonyl group and the 2-pyridone ring



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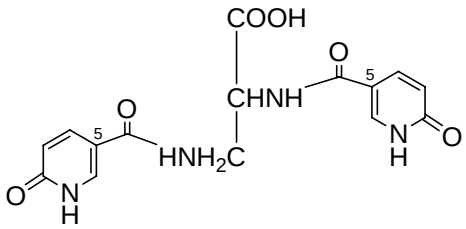

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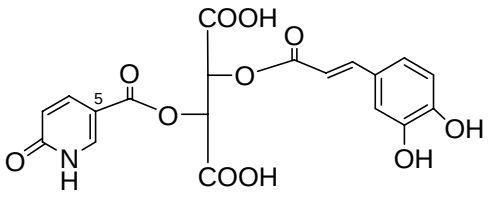

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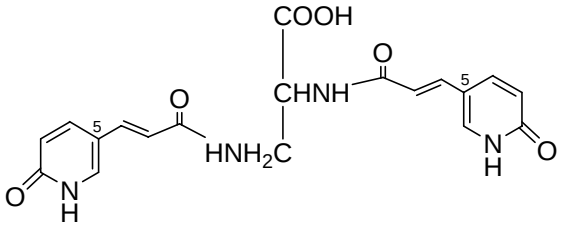

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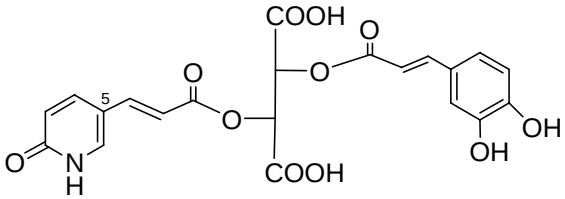

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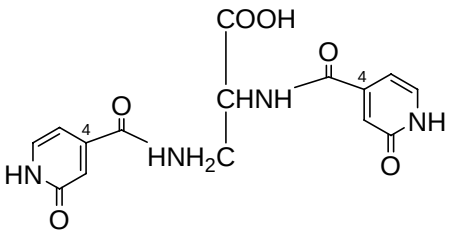

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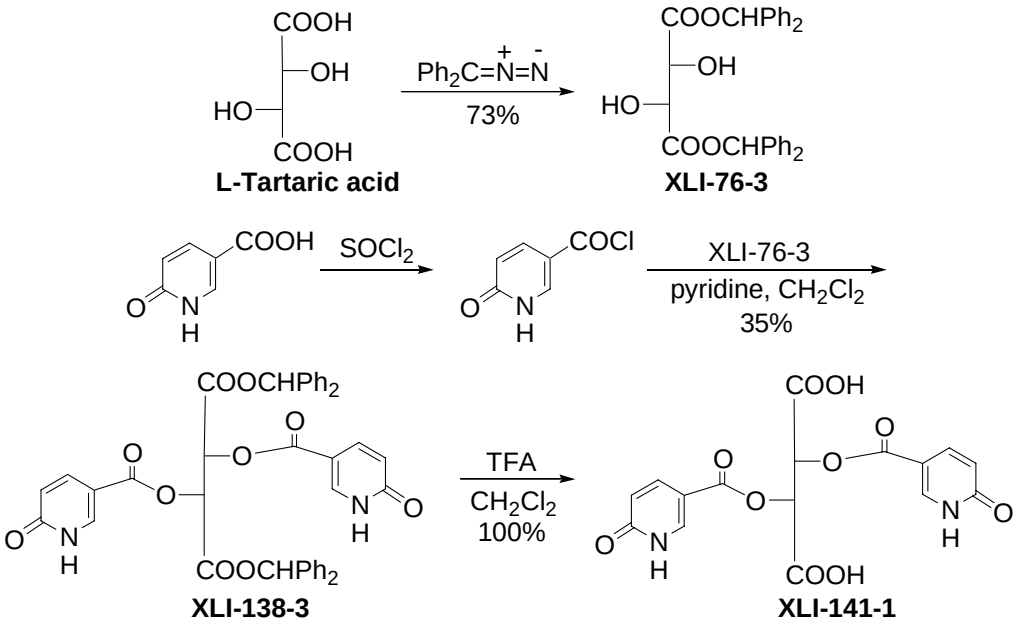
affected the bioactivity. For compound **44**, the carbonyl groups were attached directly to the 4-position of the 2-pyridone rings to be compared with compound **40** to determine if this change had an effect on the biological activity.

3.2. 2,3-Bis(6-hydroxynicotinyl)-L-tartaric acid

As in the literature [37] the carboxyl groups of L-tartaric acid were protected as the diphenylmethyl ester XLI-76-3 (Scheme 3-1) except acetone was used as a solvent rather than methanol-chloroform and the excess diphenyldiazomethane was removed with petroleum ether instead of 10% HCl. 6-Hydroxynicotinoyl chloride prepared from 6-hydroxynicotinic acid with thionyl chloride was reacted with the ester XLI-76-3 in dichloromethane/pyridine to give the blocked compound XLI-138-3 in 35% yield. Hydrolysis of diphenylmethyl ester XLI-138-3 with TFA in dichloromethane at 0 ^{o}C gave the desired product XLI-141-1 in 100% yield. Analytical HPLC of XLI-141-1 showed only one peak.

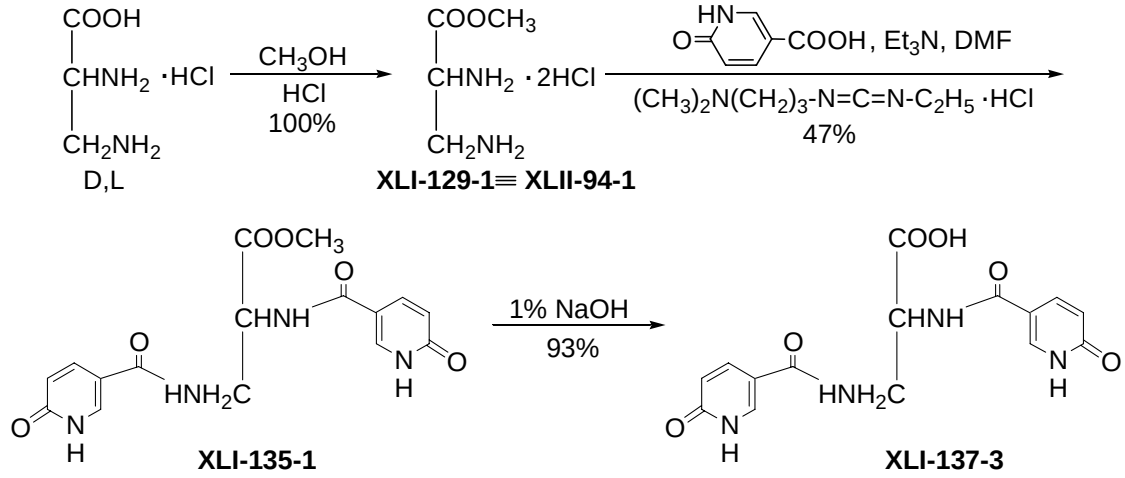
Scheme 3-1 Synthesis of 2,3-bis(6-hydroxynicotinyl)-L-tartaric acid



3.3. *N*,*N*-Bis(6-hydroxynicotinyl)-D,L-2,3-diaminopropionic acid

The carboxyl group of D,L-2,3-diaminopropionic acid was protected as its methyl ester XLI-129-1 ≡ XLII-94-1 in 100% yield (Scheme 3-2). This product was coupled with 6-hydroxynicotinic acid using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) to give the methyl ester XLI-135-1 in 47% yield. Hydrolysis of XLI-135-1 with 1% aqueous NaOH solution followed by acidic workup gave the final product XLI-137-3 in 93% yield. Analytical HPLC of XLI-137-3 showed only one peak.

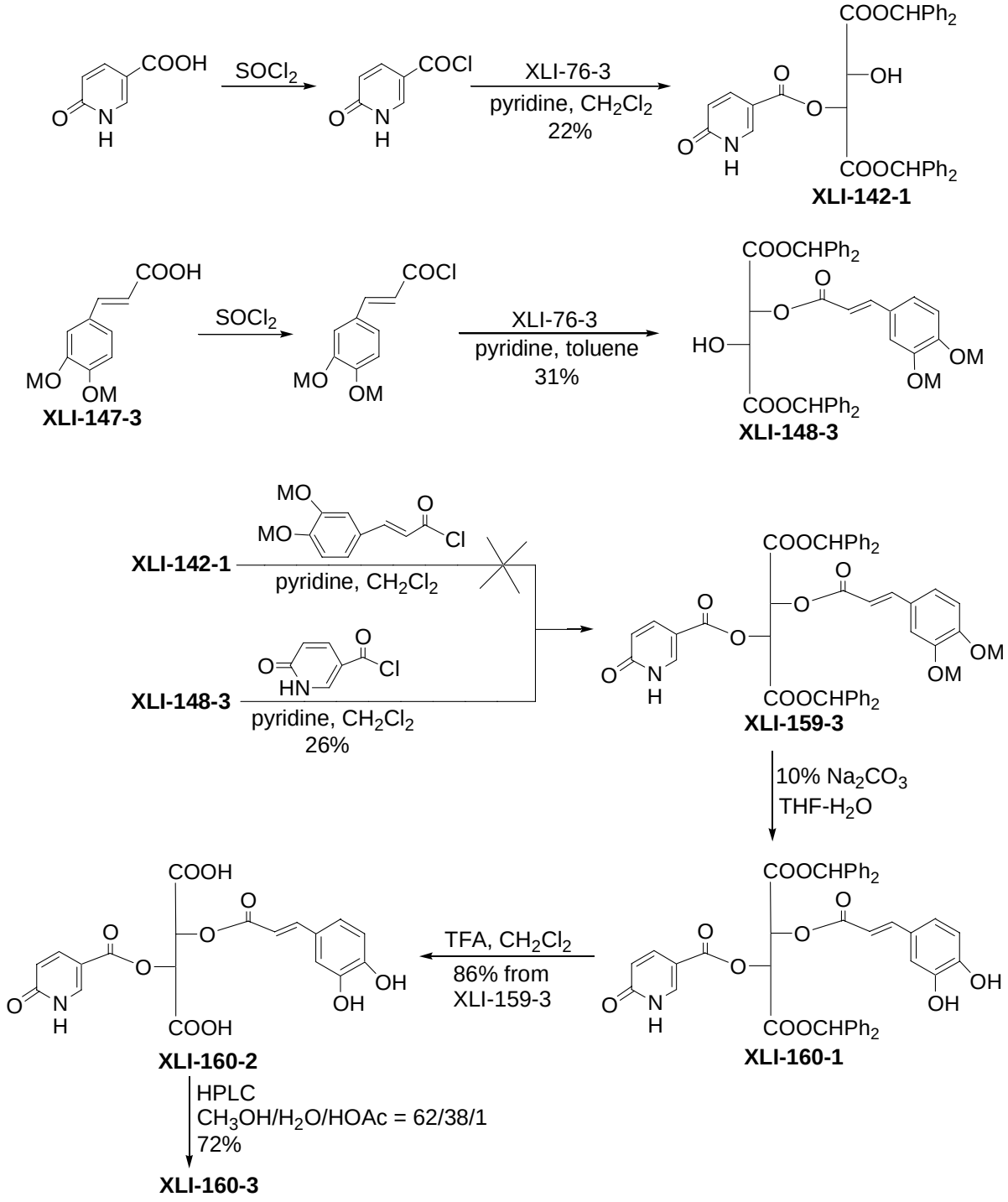
Scheme 3-2 Synthesis of *N*,*N*-bis(6-hydroxynicotinyl)-D,L-2,3-diaminopropionic acid



3.4. 2-Caffeoyl-3-(6-hydroxynicotinyl)-L-tartaric acid

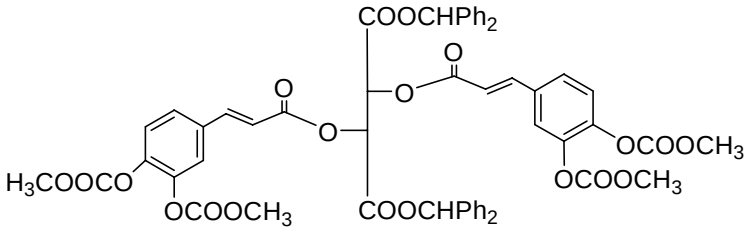
The synthesis of 2-caffeoyl-3-(6-hydroxynicotinyl)-L-tartaric acid (XLI-160-2) is summarized in Scheme 3-3. Two routes were considered, via the mononicotinoyl ester XLI-142-1 or via the monocaffeoyl ester XLI-148-3. The first intermediate XLI-142-1 was obtained in 22% yield by reaction of 6-hydroxynicotinoyl chloride with diol XLI-76-3 in ratio of 1.3 to 1. TLC of the crude product showed the presence of some bis-product (XLI-138-3 in Scheme 3-1) as well as some starting diol XlI-76-3. Reaction of XLI-142-1 with 3,4-dimethoxycarbonyl-

Scheme 3-3 Synthesis of 2-caffeoyl-3-(6-hydroxynicotinyl)-L-tartaric acid



caffeoyl chloride similar to the procedures used for the synthesis of XLI-101-1 and XLI-116-2 (See Chapter 2.) gave a mixture containing none of the desired mixed ester XLI-159-3 as determined by NMR.

Therefore, the blocked ester XLI-159-3 was prepared via intermediate XLI-148-3. When a solution of bis(diphenylmethyl) L-tartrate (XLI-76-3) in toluene was added to a suspension of 1.3 equivalents of 3,4-dimethoxycarbonylcaffeoyl chloride in toluene and pyridine, the known [37] symmetrical bis(diphenylmethyl) bis(3,4-dimethoxycarbonylcaffeoyl)-L-tartrate (**45**) might be



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expected to be the major product since the acid chloride was in excess during the addition. When the order of addition was reversed using 1.1 equivalents of acid chloride, the desired mono-caffeoyl ester XLI-148-3 was expected to be the predominant product. However, for both methods, TLC showed that the crude products were a mixture of the di-substituted compound (**45**), the starting diol (XLI-76-3) and the desired mono-caffeoyl compound (XLI-148-3). Therefore, the crude products were combined and purified on a silica gel column to give XLI-148-3 in 31% overall yield. Reaction of XLI-148-3 with 6-hydroxynicotinoyl chloride in the presence of pyridine gave the fully-blocked product XLI-159-3 in 26% yield.

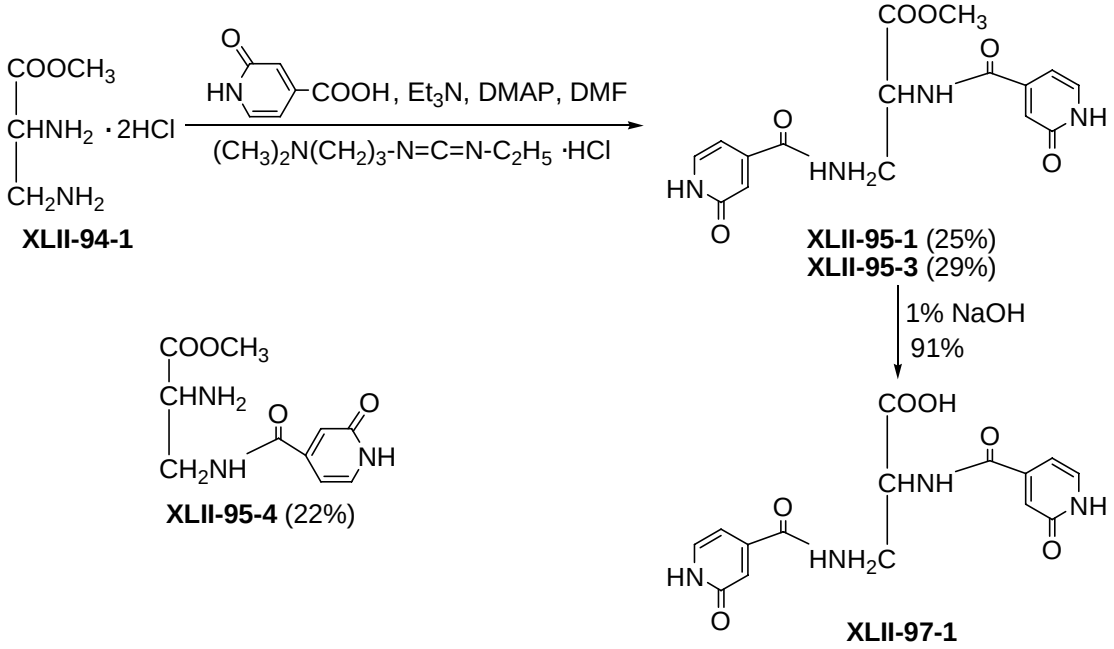
Removal of the carboxymethyl groups from XLI-159-3 with 10% Na_2CO_3 in THF gave crude XLI-160-1, which was used in the next step without purification.

Cleavage of the diphenylmethyl ester XLI-160-1 with TFA in dichloromethane at 0 ^{o}C gave the target product XLI-160-2. A sample for bioassay was purified by HPLC.

3.5. *N*,*N*-Bis(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionic acid

Methyl D,L-2,3-diaminopropionate (XLII-94-1, see Scheme 3-2) was coupled with 2-hydroxyisonicotinic acid using EDC as coupling agent and 4-dimethylaminopyridine (DMAP) as a catalyst to give the target compound XLII-95-1 ≡ XLII-95-3 in 25-29% yield. The monosubstituted compound (XLII-95-4) was a by-product. A possible reason for the low yields and the formation of XLII-95-4 might be the low solubility of 2-hydroxyisonicotinic acid in DMF. Since sufficient target diamide XLII-95-1 ≡ XLII-95-3 for further reaction was obtained, no attempt to improve the yield was undertaken. Saponification of the methyl ester XLII-95-3 with 1% NaOH gave the desired acid XLII-97-1 in 91% yield.

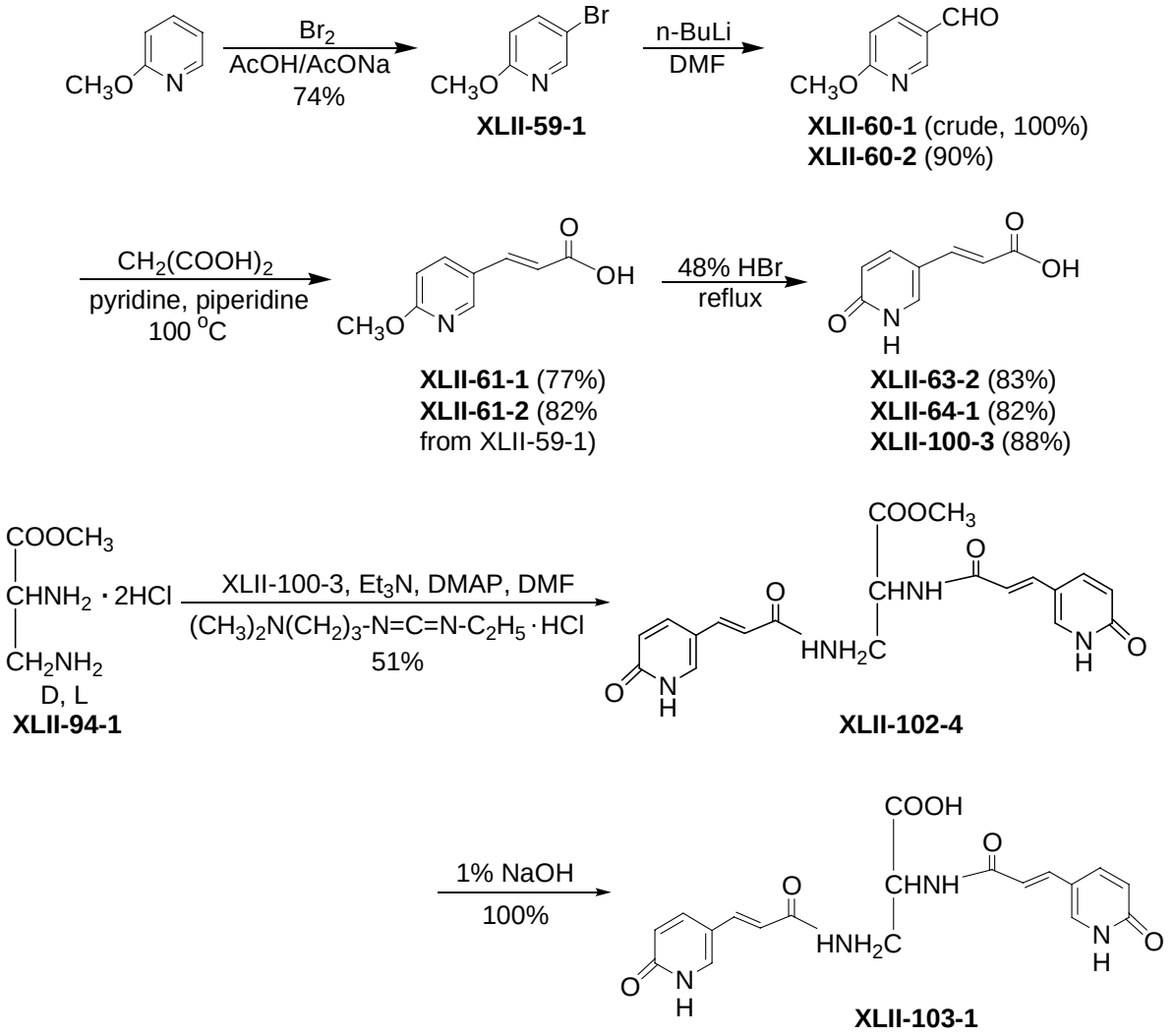
Scheme 3-4 Synthesis of *N*,*N*-bis(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionic acid



3.6. *N*,*N*-Bis[3-(2-hydroxy-5-pyridyl)acrylyl]-D,L-2,3-diaminopropionic acid

The synthesis of *N*,*N*-bis[3-(2-hydroxy-5-pyridyl)acrylyl]-D,L-2,3-diaminopropionic acid is described in Scheme 3-5.

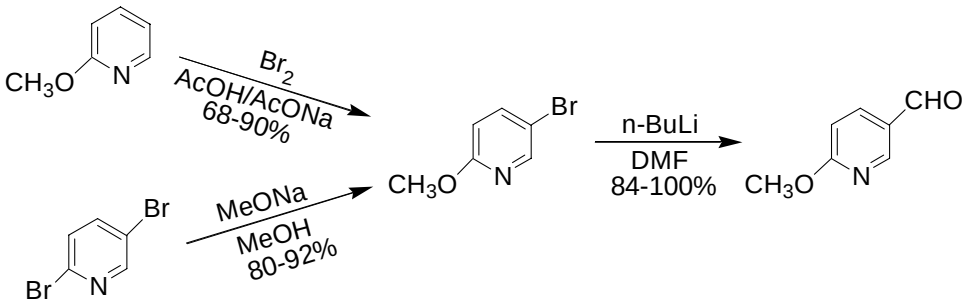
Scheme 3-5 Synthesis of *N*,*N*-bis[3-(2-hydroxy-5-pyridyl)acrylyl]--D,L-2,3-diaminopropionic acid



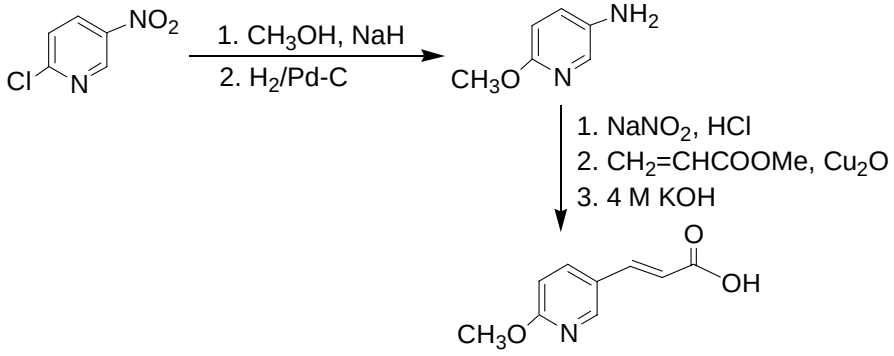
Although 6-methoxy-3-pyridinecarboxaldehyde is commercially available, it was very expensive ($205.00/5 g from Aldrich) and was therefore synthesized. Two reported methods [102-106] are shown in Scheme 3-6. Considering that the reported overall yields were similar and 2-

methoxypyridine ($21.20/25 g from Aldrich) was much cheaper than 2,5-dibromopyridine ($52.90/10 g from Aldrich), the former was chosen as the starting material. Bromination of 2-methoxypyridine gave 5-bromo-2-methoxypyridine XLII-59-1, which was converted to 6-methoxy-3-pyridinecarboxaldehyde XLII-60-1≡XLII-60-2 via metal-halogen exchange and reaction with DMF. [102] Reaction of the aldehyde with malonic acid gave the acrylic acid XLI-61-1≡ XLI-61-2 in *ca.* 80% yield. This route is superior to the reported method [107-109] (Scheme 3-7) based on the overall yield (61% vs. 26%), numbers of steps (3 vs. 4), the simplicity of the workup (no chromatography necessary), and the cost of the starting material ($21.20/25 g vs. $80.10/25 g from Aldrich).

Scheme 3-6 Reported syntheses of 6-methoxy-3-pyridinecarboxaldehyde [102-106]



Scheme 3-7 Reported synthesis of 3-(6-methoxy-3-pyridyl)acrylic acid [107]



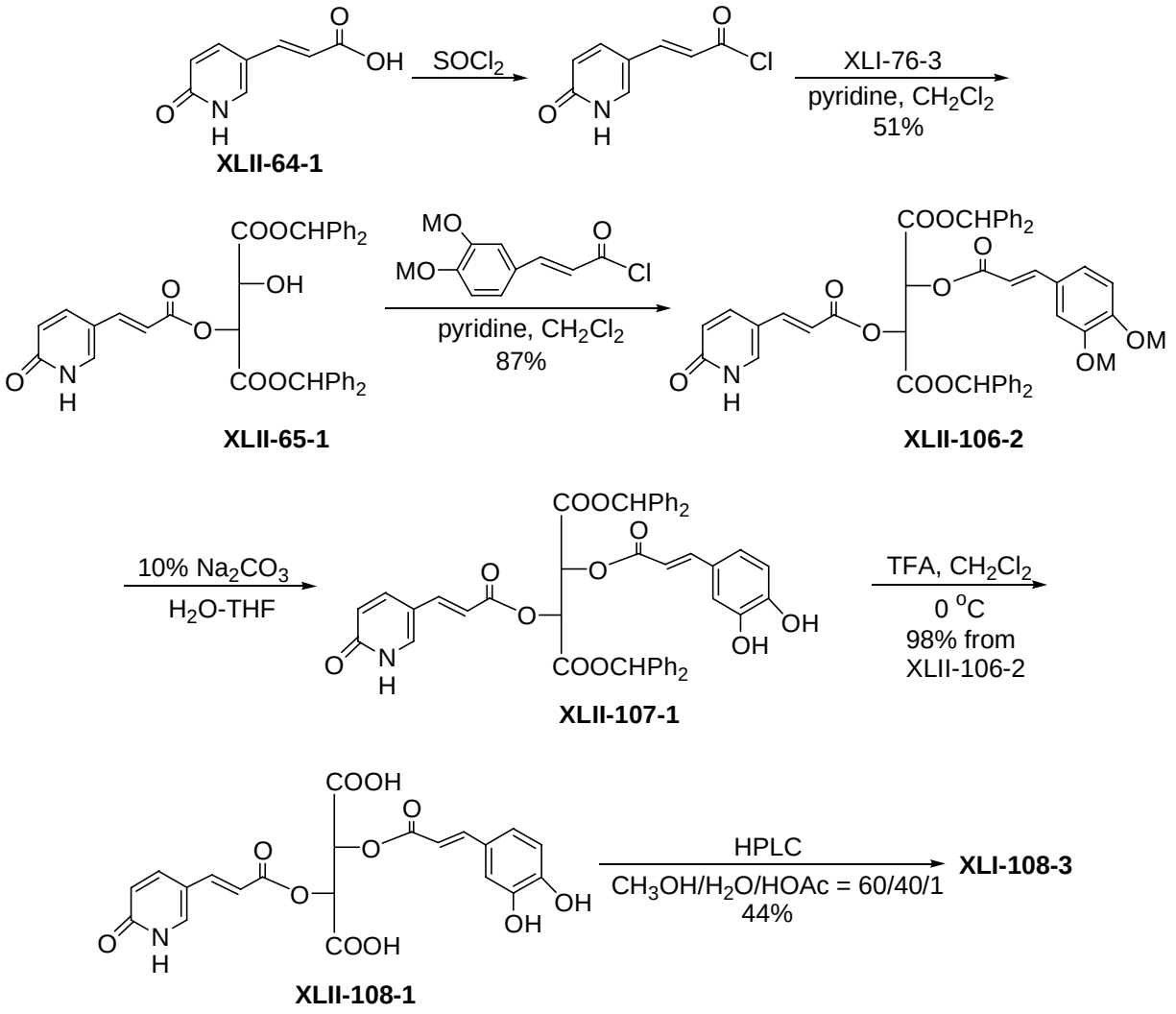
Concerned that selective removal of the methoxy group later in the synthesis might prove difficult, it was decided to remove it at the acrylic acid stage. While BBr_3 gave only recovered starting material, 48% aqueous HBr [102,104] gave 3-(2-hydroxy-5-pyridyl)acrylic acid XLII-63-2≡XLII-64-1≡XLI-100-3 in 82-88% yield with very high purity even without purification.

Coupling of the acrylic acid XLII-100-3 with methyl D,L-2,3-diaminopropionate (XLII-94-1) using EDC and DMAP gave the methyl ester diamide XLII-102-4 in 51% yield. Saponification of methyl ester XLII-102-4 gave the target compound XLII-103-1 which was insoluble in water and most organic solvents including DMSO and methanol. Therefore, acid XLII-103-1 was converted to its sodium salt with 1% NaOH to obtain ^{1}H and ^{13}C NMR spectra both of which showed two 3-(2-hydroxy-5-pyridyl)acrylyl moieties as well as the propionate residue as expected.

3.7. 2-Caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartaric acid

The synthesis of 2-caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartaric acid (XLII-108-3) is described in Scheme 3-8. Reaction of 3-(6-hydroxy-3-pyridyl)acrylic acid XLII-64-1 with thionyl chloride gave the acid chloride which was coupled with diphenylmethyl L-tartrate (XLI-76-3) to give the mono-substituted compound (XLII-65-1). Reaction with 3,4-dimethoxycarbonylcaffeoyl chloride gave the fully blocked compound XLII-106-2 in 87% yield. Removal of the carboxymethyl groups was tracked by TLC to avoid hydrolysis of other ester linkages, and the product XLII-107-1 cleaved with TFA to give crude XLI-108-1 in 98% yield, which was purified by HPLC to give XLII-108-3 for bioassay.

Scheme 3-8 Synthesis of 2-caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartaric acid



3.8. Bioassay results

Bioassays revealed that XLII-108-3, which has both a caffeoyl group and a 3-(2-hydroxy-5-pyridyl)acrylyl moiety joined through L-tartaric acid, had moderate anti-HIV IN and anti-HIV activity, but none of the other pyridone analogues showed significant inhibition against either HIV replication or HIV IN (Table 3-1). The fact that XLI-108-3 is active while XLI-160-3 is inactive indicates that the double bond between the 2-pyridone ring and the carbonyl group in

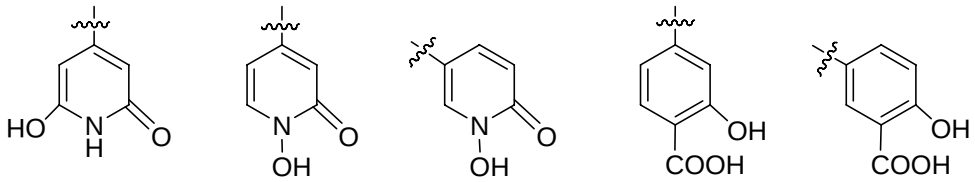
Table 3-1 Anti-HIV activities (in μM) of 2-pyridone analogues of L-CA [a]

Compound	CT_{50}	CT_5	ED_{50}	IC_{50} 3'-Processing	TI_5	TI_{50}
XLI-135-1				>>10		
XLI-137-3	193	86	>57.8	>>10	≈ 1	< 3.3
XLI-141-1	194	64	>73	>>10	< 1	< 2.7
XLI-160-3	>173		144	10		≈ 1
XLII-95-1	178	74	>>146	>>10	< 1	< 1.2
XLII-97-1	> 181		> 181			≈ 1
XLII-102-4	191	61	>>66	>>10	< 1	< 2.9
XLII-103-1	>>126	>>126	>>63	>>10	≈ 2	≈ 2
XLII-108-3	150	68	8.5	3.9	8	17.6
16 [37]		90	0.95	0.43 [b]	94.7	
L-CA [39,40]	333	264	4.2	0.53	63	79

[a] Refer to Tables 1-1, 1-2, and 2-1 for definitions of terms; [b] IC_{50} for the disintegration step

XLI-108-3 may play an important role in its activity. Comparison of these results with L-CA and its analogue **16** (Fig. 1-14) lacking the double bond between the carbonyl group and the catechol ring, supports our previous conclusion based on SAR, [37] that catechols are required for anti-HIV and anti-HIV IN activity. It also suggests that the role of the catechols is more than metal chelation. As indicated by Sotriffer's model (Fig. 1-5) [53] of L-chicoric acid in the HIV IN active site discussed in Chapter 1, the interactions between the free phenolic hydroxyl groups and Gln-148 and Glu-92 in the IN active site may play a more important role than metal chelation for L-CA's potent activity. Apparently, a simple 2-pyridone ring cannot function as a bioisostere of the

catechol system. Other bioisosteres of catechols that might be tried are shown below. [110]



3.9. Experimental

General: Materials and methods are the same as described in Section 2.7.

The sources of additional chemicals used are listed below.

Compound	Source	Cat. No.
6-Hydroxynicotinic acid	Aldrich	12,875-9
2,3-Diaminopropionic acid monohydrochloride	Aldrich	21,963-0
1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC)	Aldrich	16,146-2
2-Hydroxyisonicotinic acid	Matrix Scientific	011249
4-Dimethylaminopyridine (DMAP)	Aldrich	33,245-3
Triethylamine (TEA)	Aldrich	47,128-3
2-Methoxypyridine	Aldrich	M2,540-6
Sodium acetate	Aldrich	24,124-5
Acetic acid glacial	Mallinckrodt	2504
Butyllithium, 10.0*M* solution in hexanes	Aldrich	23,071-5
Malonic acid	Aldrich	M129-6
Piperidine	Aldrich	10,409-4
N,*N*-Dimethylformamide, anhydrous (DMF)	Sigma-Aldrich	22,705-6
Hydrobromic acid, 48%	Aldrich	24,426-0

Other general chemical solvents and reagents are the same as described in Section 2.7.

3.9.1. 2,3-Bis(6-hydroxynicotinyl)-L-tartaric acid

Bis(diphenylmethyl) L-tartrate (XLI-76-3) To a solution of 9.70 g (64.7 mmol) of L-tartaric acid in 40 mL of acetone was added a solution of 27.60 g (142.3 mmol) of diphenyldiazomethane in 40 mL of acetone. The mixture was stirred overnight at room temperature, and then vacuum filtered. The filtrate was evaporated to afford a residue, which was washed with petroleum ether and then recrystallized from water and acetone to give 22.80 g (73%) of XLI-76-3 as a white solid, mp 110-111.5 ^{o}C (lit. [37] mp 107-108 ^{o}C). NMR data match those in the literature. [37]

Bis(diphenylmethyl) 2,3-bis(6-hydroxynicotinyl)-L-tartrate (XLI-138-3) A suspension of 4.00 g (28.8 mmol) of 6-hydroxynicotinic acid in 40 mL of thionyl chloride was refluxed for 1 h to give a yellow solution. Extra thionyl chloride was removed with a Rotavapor to give a pale yellow solid. To a suspension of the pale yellow solid in 120 mL of CH_2Cl_2 was added 10 mL of pyridine. The resulting suspension was stirred for 10 min at room temperature and then treated with a solution of 2.30 g (4.77 mmol) of XLI-76-3 in 20 mL of CH_2Cl_2. The mixture was stirred overnight at room temperature, and then filtered to give a yellow solution, which was successively washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4. The solvent was evaporated to give a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 4/1) to give 1.21 g (35%) of XLI-138-3 (R_f = 0.16) as a white powder, mp 125-127 ^{o}C. ^{1}H NMR (DMSO-d_6) 12.08 (br s, 2H), 7.69 (d, J = 2.3 Hz, 2H), 7.54 (dd, J = 9.7, 2.6 Hz, 2H), 7.49-7.02 (m, 20H), 6.92 (s,

2H), 6.32 (d, J = 9.7 Hz, 2H), 6.27 (s, 2H); ^{13}C NMR (DMSO-d_6) 164.4 (2), 162.2 (2), 162.0 (2), 141.2 (2), 139.3 (2), 139.1 (2), 138.6 (2), 128.4 (4), 128.2 (4), 127.9 (2), 127.6 (2), 126.4 (8), 119.5 (2), 106.3 (2), 78.1 (2), 70.7 (2). Anal. Calcd for $C_{42}H_{32}N_2O_{10}$: C, 69.61; H, 4.45; N, 3.87. Found: C, 69.39; H, 4.43; N, 3.83.

2,3-Bis(6-hydroxynicotinyl)-L-tartaric acid (XLI-141-1) To a solution of 0.30 g (0.41 mmol) of XLI-138-3 in 30 mL of CH_2Cl_2 in an ice bath was added dropwise 2.60 mL (33.8 mmol) of TFA. After 5.5 h, CH_2Cl_2 and extra TFA were removed with a Rotavapor to give the crude product, which was washed with 30 mL of CH_2Cl_2 and then filtered to give 0.16 g (100%) of XLI-141-1 as a white powder, mp 207-209 ^{o}C. Analytical HPLC with H_2O/CH_3CN/AcOH (v/v/v = 85/15/1) as eluting solvents showed only one peak. Since XLI-141-1 has poor solubility in methanol, acetonitrile, water and acetic acid, no preparative HPLC was done. ^{1}H NMR (DMSO-d_6) 12.23 (br s, 2H), 8.09 (d, J = 2.3 Hz, 2H), 7.80 (dd, J = 9.6, 2.5 Hz, 2H), 6.45 (d, J = 9.7 Hz, 2H), 5.71 (s, 2H); ^{13}C NMR (DMSO-d_6) 167.1 (2), 162.6 (2), 162.2 (2), 141.4 (2), 138.8 (2), 119.9 (2), 106.7 (2), 71.0 (2). Anal. Calcd for $C_{16}H_{12}N_2O_{10}$: C, 48.97; H, 3.08; N, 7.14. Found: C, 48.98; H, 3.19; N, 6.86.

3.9.2. *N*,*N*-Bis(6-hydroxynicotinyl)-D,L-2,3-diaminopropionic acid

Methyl D,L-2,3-diaminopropionate dihydrochloride (XLI-129-1 ≡ XLII-94-1) [111] A mixture of 1.50 g (10.7 mmol) of D,L-2,3-diaminopropionic acid monohydrochloride in 150 mL of methanol in a three-neck flask was saturated with gaseous HCl generated by adding conc HCl to conc H_2SO_4. The reaction was stirred for 10 h at room temperature and the solvent removed with a Rotavapor and lyophilization to give 2.06 g (100%; lit. [111] 100%) of XLI-129-1 as a white

powder, mp 166-168 °C (dec.). XLII-94-1 (0.98 g, 100%) was prepared from 0.72 g (5.12 mmol) of D,L-2,3-diaminopropionic acid monohydrochloride by the same procedure. ^{1}H NMR (CD_3OD) 4.51 (dd, *J* = 7.9, 5.6 Hz, 1H), 3.94 (s, 3H), 3.57 (dd, *J* = 13.8, 7.9 Hz, 1H), 3.49 (dd, *J* = 13.5, 5.6 Hz, 1H); ^{13}C NMR (CD_3OD) 168.0, 54.6, 51.1, 39.5. Lit. [39] ^{1}H NMR (CD_3OD) 4.51 (br s, 1H), 3.95 (s, 3H), 3.52 (br s, 2H); ^{13}C NMR (CD_3OD) 168.0, 55.2, 51.4, 39.9.

Methyl *N*,*N*-bis(6-hydroxynicotinyl)-D,L-2,3-diaminopropionate (XLI-135-1) To a solution of 0.50 g (2.6 mmol) of XLI-129-1 and 1.09 g (7.84 mmol) of 6-hydroxynicotinic acid in 40 mL of DMF was added 1.2 mL of TEA. After the resulting mixture was stirred for 15 min at room temperature, it was treated with 1.51 g (7.88 mmol) of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride in one portion. Reaction overnight at room temperature gave a suspension, which was filtered to give a white solid and a yellow solution. The solution was lyophilized to almost dryness to give a yellow residue, which was separated on a silica gel column eluted with chloroform and methanol (v/v = 5/1) to give 0.44 g (47%) of XLI-135-1 (R_f = 0.32 for v/v = 4/1) as a white solid, mp 175-177 °C. ^{1}H NMR (DMSO-d_6) 11.97 (br s, 2H), 8.61 (d, *J* = 7.0 Hz, 1H), 8.44 (dd, *J* = 5.2, 5.2 Hz, 1H), 8.03 (br s, 1H), 7.95 (br s, 1H), 7.86-7.80 (m, 2 H), 6.37 (d, *J* = 9.7 Hz, 1H), 6.36 (d, *J* = 9.7 Hz, 1H), 4.55-4.51 (m, 1H), 3.75-3.69 (m, 1H), 3.63 (s, 3H), 3.58-3.49 (m, 1H); ^{13}C NMR (DMSO-d_6) 170.8, 164.2, 163.7, 162.1 (2), 138.9 (2), 137.7, 137.3, 119.1, 119.0, 112.0, 111.5, 52.4, 51.9, 39.9. Anal. Calcd for $C_{16}H_{16}N_4O_6 \cdot H_2O$: C, 50.79; H, 4.80; N, 14.81. Found: C, 50.65; H, 4.64; N, 14.65.

***N*,*N*-Bis(6-hydroxynicotinyl)-D,L-2,3-diaminopropionic acid (XLI-137-3)** To a solution of 0.36 g (9.0 mmol) of NaOH in 36 mL of H_2O was added 0.16 g (0.44 mmol) of XLI-

135-1. The resulting solution was stirred for 5 h at room temperature, and then acidified to pH = 0-1 with 10% HCl. The mixture was lyophilized to give a white solid, to which was added 10 mL of H_2O. The resulting suspension was filtered to give 0.14 g (93%) of XLI-137-3 as a white powder, mp 265-268 ^oC. Analytical HPLC with H_2O/CH_3CN/AcOH (v/v/v = 75/251) as eluting solvents showed only one peak. Since XLI-137-3 has poor solubility in methanol, acetonitrile, water and acetic acid, no preparative HPLC was done. ^1H NMR (DMSO-d_6) 12.05 (br s, 2H), 8.52 (d, J = 7.8 Hz, 1H), 8.43 (dd, J = 5.6, 5.6 Hz, 1H), 8.02 (d, J = 2.2 Hz, 1H), 7.95 (d, J = 2.4 Hz, 1H), 7.86-7.80 (m, 2H), 6.37 (d, J = 9.7 Hz, 1H), 6.35 (d, J = 9.4 Hz, 1H), 4.54-4.48 (m, 1H), 3.76-3.70 (m, 1H), 3.56-3.49 (m, 1H); ^{13}C NMR (DMSO-d_6) 171.7, 164.1, 163.6, 162.15, 162.12, 138.9 (2), 137.5, 137.2, 119.02, 119.00, 112.1, 111.8, 52.5, C3 apparently was overlapped by the peaks of DMSO-d_6 (approximately 39.9 ppm based on the ^{13}C NMR of XLI-135-1). Anal. Calcd for $C_{15}H_{14}N_4O_6$: C, 52.03; H, 4.07; N, 16.18. Found: C, 52.26; H, 4.20; N, 16.00.

3.9.3. 2-Caffeoyl-3-(6-hydroxynicotinyl)-L-tartaric acid

Bis(diphenylmethyl) 2-(6-hydroxynicotinyl)-L-tartrate (XLI-142-1) A suspension of 1.00 g (7.19 mmol) of 6-hydroxynicotinic acid in 20 mL of thionyl chloride was refluxed for 1 h to give a yellow solution. Extra thionyl chloride was removed with a Rotavapor to give a pale yellow solid, to which was added successively 40 mL of CH_2Cl_2 and 6 mL of pyridine. The resulting suspension was stirred for 10 min at RT and then treated with a solution of 2.92 g (6.06 mmol) of XLI-76-3 in 20 mL of CH_2Cl_2. The mixture was stirred overnight at room temperature, and then filtered to give a yellow solution, which was washed successively with 10% HCl, 5%

$NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated with a Rotovapor to give a residue, whose TLC showed the presence of bis(diphenylmethyl) 2,3-bis(6-hydroxynicotinyl)-L-tartrate (XLI-138-3) and starting diol XLI-76-3. Separation on a silica gel column eluted with ethyl acetate and hexanes (v/v = 2.5/1) gave 0.80 g (22%) of XLI-142-1 (R_f = 0.47) as a white solid, mp 90-92 ^oC. ^1H NMR ($CDCl_3$) 7.99 (d, *J* = 2.3 Hz, 1H), 7.68 (dd, *J* = 9.6, 2.4 Hz, 1H), 7.33-6.99 (m, 20H), 6.97 (s, 1H), 6.95 (s, 1H), 6.42 (d, *J* = 9.6 Hz, 1H), 5.80 (d, *J* = 2.2 Hz, 1H), 5.10 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) 169.9, 165.7, 164.9, 162.4, 140.8, 140.7, 139.1, 139.0, 138.7, 138.6, 128.6 (4), 128.52 (2), 128.48 (2), 128.3 (2), 128.2, 128.1, 127.3 (2), 127.2 (2), 127.1 (2), 127.0 (2), 119.4, 109.6, 79.2 (2), 73.8, 70.8. Anal. Calcd for $C_{36}H_{29}NO_8 \cdot 0.25H_2O$: C, 71.10; H, 4.89; N, 2.30. Found: C, 71.00; H, 5.03; N, 2.32.

Bis(diphenylmethyl) 2-(3,4-dimethoxycarbonylcaffeoyl)-L-tartrate (XLI-148-3)

Method 1: To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 1.10 g (3.72 mmol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 30 mL of toluene was added 5 mL of pyridine. After the resulting suspension was stirred for 10 min at room temperature, it was treated with a solution of 1.38 g (2.86 mmol) of XLI-76-3 in 20 mL of toluene. The mixture was stirred overnight at room temperature, and then washed successively with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give residue XLI-147-2.

Method 2: To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride (made from 0.47 g (1.6 mmol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 25

mL of toluene was added 3 mL of pyridine. The resulting mixture was added to a solution of 0.70 g (1.5 mmol) of XLI-76-3 in 15 mL of toluene and 2 mL of pyridine. The resulting mixture was stirred overnight at room temperature, and then washed successively with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give residue XLI-148-2.

For both methods, TLC showed that the di-substituted compound bis(diphenylmethyl) bis(3,4-dimethoxycarbonylcaffeoyl)-L-tartrate (**45**) as well as some starting diol (XLI-76-3) was present in the crude product to the same extent. Therefore, crude products XLI-147-2 and XLI-148-2 were combined and then separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 0.75/1) to give 1.02 g (31% from 2.08 g of XLI-76-3) of XLI-148-3 (R_f = 0.38) as a white solid, mp 63-65 ^oC. ^1H NMR ($CDCl_3$) 7.44 (d, *J* = 16.0 Hz, 1H), 7.34-7.09 (m, 23H), 6.97 (s, 2H), 6.12 (d, *J* = 16.1 Hz, 1H), 5.76 (d, *J* = 2.3 Hz, 1H), 5.02 (dd, *J* = 7.3, 2.4 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 3.32 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) 169.9, 165.7, 164.8, 153.0, 152.9, 144.0, 143.9, 142.7, 139.2, 139.1, 138.7, 138.6, 133.1, 128.7 (2), 128.63 (2), 128.56 (2), 128.5 (2), 128.4 (2), 128.2, 128.1, 127.3 (2), 127.2 (2), 127.12 (2), 127.08 (2), 126.7, 123.5, 122.5, 117.7, 79.4, 79.0, 73.4, 70.6, 55.9 (2). Anal. Calcd for $C_{43}H_{36}O_{13}$: C, 67.89; H, 4.77. Found: C, 67.97; H, 4.96.

Bis(diphenylmethyl) 2-(3,4-dimethoxycarbonylcaffeoyl)-3-(6-hydroxynicotinyl)-L-tartrate (XLI-159-3) A mixture of 0.20 g (1.4 mmol) of 6-hydroxynicotinic acid in 10 mL of thionyl chloride was refluxed for 1 h. Extra thionyl chloride was removed with a Rotovapor to give a pale yellow solid, to which was added successively 20 mL of CH_2Cl_2 and 3 mL of

pyridine. The resulting mixture was stirred for 10 min at room temperature and then treated with a solution of 0.25 g (0.33 mmol) of XLI-148-3 in 20 mL of CH_2Cl_2. The mixture was stirred overnight at room temperature, and then washed successively with 10% HCl, 5% $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give a residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1.5/1) to give 74.8 mg (26%) of XLI-159-3 (R_f = 0.35 when v/v = 1.8/1) as a white solid, mp 72-75 ^oC. ^1H NMR ($CDCl_3$) 7.88 (d, *J* = 2.3 Hz, 1H), 7.76 (dd, *J* = 9.6, 2.5 Hz, 1H), 7.50 (d, *J* = 16.0 Hz, 1H), 7.40-7.05 (m, 23H), 6.94 (s, 2H), 6.50 (d, *J* = 9.7 Hz, 1H), 6.17 (d, *J* = 15.9 Hz, 1H), 6.06 (s, 2H), 3.93 (s, 6H); ^{13}C NMR ($CDCl_3$) 165.1, 164.8, 164.7, 164.6, 162.4, 153.1, 152.9, 144.5, 144.0, 142.7, 140.7, 140.5, 138.74, 138.70, 138.6, 133.0, 128.7 (2), 128.6 (4), 128.5 (2), 128.4, 128.32, 128.27, 128.2, 127.4 (2), 127.3 (2), 127.1, 127.04 (2), 126.99 (2), 126.9, 123.5, 122.6, 119.6, 117.5, 109.2, 79.3, 79.0, 71.3, 71.0, 56.0 (2). Anal. Calcd for $C_{49}H_{39}NO_{15}\cdot 2H_2O$: C, 64.10; H, 4.72; N, 1.53. Found: C, 63.99; H, 4.62; N, 1.41.

Bis(diphenylmethyl) 2-caffeoyl-3-(6-hydroxynicotinyl)-L-tartrate (XLI-160-1) To a solution of 67.8 mg (0.077 mmol) of XLI-159-3 in 5 mL of THF was added 5 mL of 10% aqueous Na_2CO_3 solution. The mixture was stirred under N_2 at room temperature for 16 h. Extra Na_2CO_3 was neutralized with 2 mL of acetic acid. The resulting mixture was diluted with 20 mL of H_2O and then extracted with dichloromethane. The combined organic extracts were successively washed with 5% $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO and the solvent evaporated to give 56.2 mg of XLI-160-1 as a pale yellow solid, which was used in the next step without purification. ^1H NMR ($CDCl_3$) 7.93 (br s, 1H), 7.80 (br d, *J* =

10.1 Hz, 1H), 7.42 (d, *J* = 16.2 Hz, 1H), 7.36-6.99 (m, 23H), 6.94 (s, 1H), 6.92 (s, 1H), 6.59 (d, *J* = 8.8 Hz, 1H), 6.09 (br s, 1H), 6.05 (br s, 1H), 5.93 (d, *J* = 15.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) 165.84, 165.76, 165.2, 164.7, 162.2, 147.5, 144.4, 141.1, 140.6, 138.6(2), 138.5, 138.4, 128.7 (2), 128.6 (4), 128.54 (2), 128.47, 128.3 (2), 128.2, 127.5, 127.3, 127.21 (2), 127.18, 127.1 (2), 126.9 (2), 126.6, 122.2, 119.4, 115.7, 112.6, 109.7, 79.6, 79.4, 70.4, 70.1.

2-Caffeoyl-3-(6-hydroxynicotinyl)-L-tartaric acid (XLI-160-3) To a solution of XLI-160-1 in 10 mL of CH_2Cl_2 in an ice bath was added dropwise 0.48 mL of TFA. After 4 h under N_2, the CH_2Cl_2 and extra TFA were removed with a Rotavapor to give crude product, which was sonicated in 10 mL of CH_2Cl_2 and then filtered to give 28.8 mg (86% from XLI-159-3) of XLI-160-2 as a pale yellow solid. The sample for bioassay was obtained by purifying 28.8 mg of XLI-160-2 by HPLC eluted with CH_3OH, H_2O, HOAc (v/v/v = 62/38/1) to give 20.8 mg (72% recovery) of XLI-160-3 (Ret. time = 3.63 min) as a white solid, mp 190 ^{o}C (dec.). ^{1}H NMR (CD_3OD) 8.26 (d, *J* = 2.3 Hz, 1H), 8.03 (dd, *J* = 9.6, 2.2 Hz, 1H), 7.65 (d, *J* = 15.8 Hz, 1H), 7.08 (br s, 1H), 6.99 (br d, *J* = 8.2 Hz, 1H), 6.79 (d, *J* = 8.1 Hz, 1H), 6.56 (d, *J* = 9.7 Hz, 1H), 6.37 (d, *J* = 15.8 Hz, 1H), 5.88 (d, *J* = 2.7 Hz, 1H), 5.83 (d, *J* = 2.6 Hz, 1H); ^{13}C NMR (CD_3OD) 169.5, 169.1, 167.6, 165.6, 164.3, 150.0, 148.7, 146.9, 142.2, 141.7, 127.5, 123.4, 120.7, 116.5, 115.3, 113.5, 110.6, 72.9, 72.3. HRMS: $(M+H)^+$ calcd for $C_{19}H_{16}NO_{11}$ 434.0723, found 434.0706.

3.9.4. *N*,*N*-Bis(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionic acid

Methyl *N*,*N*-bis(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionate (XLII-95-1 ≡ XLII-95-3) To a suspension of 0.10 g (0.52 mmol) of XLII-94-1 and 0.30 g (2.2 mmol) of 2-hydroxyisonicotinic acid in 20 mL of DMF was added 0.5 mL of triethylamine. After the reaction

was stirred for 15 min at RT, it was treated with 0.44 g (2.3 mmol) of EDC and 12 mg (0.098 mmol) of DMAP. After reacting overnight at room temperature, the suspension was filtered to give 0.11 g of XLII-94-2 (Et_3NHCl) as a white solid and a yellow filtrate. The filtrate was lyophilized to give 1.26 g of XLII-94-3 as a pale yellow residue, which was separated on a silica gel column eluted with ethyl acetate and methanol (v/v = 2/1) to give 48 mg (25%) of XLII-95-1 (R_f = 0.32) as a white solid, mp 248 ^oC (dec.). ^1H NMR (DMSO-d_6) 9.15 (d, J = 7.0 Hz, 1H), 9.07 (dd, J = 6.0, 6.0 Hz, 1H), 7.49 (d, J = 6.5 Hz, 1H), 7.47 (d, J = 6.7 Hz, 1H), 6.84 (d, J = 1.5 Hz, 1H), 6.72 (d, J = 0.9 Hz, 1H), 6.56 (dd, J = 6.7, 1.5 Hz, 1H), 6.48 (dd, J = 6.7, 1.8 Hz, 1H), 4.59-4.53 (m, 1H), 3.88-3.67 (m, 2H), 3.64 (s, 3H); ^{13}C NMR (DMSO-d_6) 170.2, 165.0, 164.9, 162.3 (2), 145.6, 145.0, 136.0 (2), 118.7, 118.3, 102.9, 102.8, 52.6, 52.1, C3 apparently was overlapped by the peaks of DMSO-d_6 (approximately 39.9 ppm based on the ^{13}C NMR of XLI-135-1); ^{13}C NMR (CD_3OD) 171.5 (3), 168.3, 167.5, 148.8, 148.4, 136.9 (2), 119.9, 119.7, 106.4 (2), 54.4, 53.2, 41.6. Anal. Calcd for $C_{16}H_{16}N_4O_6$: C, 53.33; H, 4.48; N, 15.55. Found: C, 53.53; H, 4.38; N, 15.56. Following the same procedure on a two-fold scale gave 0.11 g (29%) of XLII-95-3 (R_f = 0.32) and 54 mg (22%) of methyl *N*3-(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionate (XLII-95-4) (R_f = 0.23) as white solids. By ^1H and ^{13}C NMR, the latter was contaminated by triethylammonium chloride. XLII-95-4: ^1H NMR (DMSO-d_6) 9.04 (dd, J = 5.7, 5.7 Hz, 1H), 7.49 (d, J = 6.7 Hz, 1H), 6.80 (d, J = 1.2 Hz, 1H), 6.52 (dd, J = 6.7, 1.5 Hz, 1H), 4.56 (dd, J = 5.7, 5.7 Hz, 1H), 3.76-3.61 (m, 2H), 3.69 (s, 3H), 3.06 [q, J = 7.3 Hz, $(CH_3C\underline{H_2})_3NHCl$], 1.21 [t, J = 7.3 Hz, $(C\underline{H_3}CH_2)_3NHCl$)]; ^{13}C NMR (DMSO-d_6) 169.5, 165.5, 162.5, 145.6, 136.0, 118.8, 103.1, 52.8, 52.2, C3 apparently was overlapped by the peaks of

DMSO-d_6 (approximately 39.9 ppm based on the ^{13}C NMR of XLI-135-1), 45.3 [$(CH_3\underline{C}H_2)_3NHCl$], 8.5 [$(\underline{C}H_3CH_2)_3NHCl$].

***N,N*-Bis(2-hydroxyisonicotinyl)-D,L-2,3-diaminopropionic acid (XLII-97-1)** A mixture of 40 mg (0.11 mmol) of XLII-95-3 and 10 mL of 1% (2.5 mmol) NaOH was stirred for 4 h at room temperature, and then acidified to pH = 1-2 by adding 6 M HCl dropwise. The resulting solution was lyophilized to give a white solid, to which was added 1 mL of H_2O. The resulting suspension was filtered to give 35 mg (91%) of XLII-97-1 as a white solid, mp 258 ^{o}C (dec.). ^{1}H NMR (DMSO-d_6) 8.86 (d, J = 7.9 Hz, 1H), 8.77 (dd, J = 5.7, 5.7 Hz, 1H), 7.48 (d, J = 6.4 Hz, 1H), 7.46 (d, J = 6.7 Hz, 1H), 6.77 (d, J = 1.8 Hz, 1H), 6.66 (d, J = 1.5 Hz, 1H), 6.45 (dd, J = 6.7, 1.5 Hz, 1H), 6.41 (dd, J = 6.7, 1.8 Hz, 1H), 4.59-4.52 (m, 1H), 3.77-3.70 (m, 1H), 3.66-3.56 (m, 1H); ^{13}C NMR (DMSO-d_6) 171.1, 165.2, 164.8, 162.3 (2), 145.9, 145.4, 135.93, 135.89, 118.5, 118.2, 102.8 (2), 52.2, C3 apparently was overlapped by the peaks of DMSO-d_6 (approximately 39.9 ppm based on the ^{13}C NMR of XLI-135-1). Anal. Calcd for $C_{15}H_{14}N_4O_6\cdot 0.25H_2O$: C, 51.36; H, 4.17; N, 15.97. Found: C, 51.33; H, 4.05; N, 15.96.

3.9.5. *N,N*-Bis[3-(2-hydroxy-5-pyridyl)acrylyl]-D,L-2,3-diaminopropionic acid

5-Bromo-2-methoxypyridine (XLII-59-1) [102] A solution of 41.30 g (0.26 mol) of bromine in 45 mL of glacial acetic acid was added dropwise into a mechanically stirred suspension of 20.00 g (0.18 mol) of 2-methoxypyridine and 16.00 g (0.20 mol) of sodium acetate in 90 mL of glacial acetic acid at 10 ^{o}C. The suspension was stirred at room temperature for 12 h and then concentrated with a Rotavapor to 1/3 of its original volume, poured onto ice, and made basic (pH *ca*. 8) with 5 M NaOH before extraction with ether (4x). The combined organic

extracts were dried over Na_2SO_4, and the solvent was removed with a Rotavapor. The crude product was distilled under reduced pressure (47-50 ^oC, 0.5 mmHg) to give 25.65 g (74%; lit. 68%, [102] 90% [106]) of XLII-59-1 as a colorless liquid. ^1H NMR ($CDCl_3$) 8.19 (d, J = 2.2 Hz, 1H), 7.61 (dd, J = 8.8, 2.2 Hz, 1H), 6.64 (d, J = 8.8 Hz, 1H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$) 162.9, 147.5, 141.0, 112.6, 111.7, 53.7. The NMR data match those in the literature. [103]

6-Methoxy-3-pyridinecarboxaldehyde (XLII-60-1 ≡ XLII-60-2) [102] To a solution of 2.00 g (10.6 mmol) of XLII-59-1 in 20 mL of dry tetrahydrofuran at -78 ^oC was added dropwise 1.12 mL of 10 M (11.2 mmol) butyllithium in hexanes. The resulting suspension was stirred at -78 ^oC for 90 min, 1.56 mL (21.3 mmol) of DMF was then added dropwise, and stirring continued for a further 90 min. The mixture was allowed to warm to room temperature and then poured into aqueous saturated $NaHCO_3$ and extracted with ether (3x). The combined organic extracts were dried over Na_2SO_4, and the solvent was removed with a Rotavapor to give 1.88 g of crude product as a pale yellow solid, which was sublimed to give 1.31 g (90 %; lit. 84-100% [102-106]) of XLII-60-2 as a white solid, mp 51-52 ^oC (lit. mp 51-52 ^oC [104]). ^1H NMR ($CDCl_3$) 9.96 (s, 1H), 8.64 (d, J = 2.1 Hz, 1H), 8.07 (dd, J = 8.8, 2.4 Hz, 1H), 6.85 (d, J = 8.8 Hz, 1H), 4.04 (s, 3H); ^{13}C NMR ($CDCl_3$) 189.5, 167.7, 152.9, 137.4, 126.7, 112.1, 54.3. The NMR data match those in the literature. [103,106] Starting from 16.72 g (88.94 mmol) of XLII-59-1 and following the above procedure, 12.68 g of crude product XLII-60-1 was obtained as a pale yellow solid in quantitative yield, which was used in the next step without purification by sublimation.

3-(6-Methoxy-3-pyridyl)acrylic acid (XLII-61-1≡ XLII-61-2) A mixture of 0.50 g (3.7 mmol) of XLII-60-2, 0.57 g (5.5 mmol) of malonic acid, 0.3 mL of piperidine, and 14 mL of

pyridine was stirred at 100 °C for 3 h. The reaction mixture was concentrated in vacuo, 2.5 mL of water added to the residue and the resulting precipitate collected by filtration to give 0.50 g (77%) of XLII-61-1 as a white solid, mp 189-191 °C (lit. [107] mp 235-240 °C). ^{1}H NMR (DMSO-d_6) 8.44 (d, J = 2.4 Hz, 1H), 8.11 (dd, J = 8.8, 2.4 Hz, 1H), 7.57 (d, J = 16.1 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.51 (d, J = 16.1 Hz, 1H), 3.89 (s, 3H); ^{13}C NMR 167.5, 164.5, 148.4, 140.4, 137.2, 123.9, 118.2, 111.0, 53.4. Lit. [107] ^{1}H NMR (DMSO-d_6, 60 MHz) 7.53 (d, J = 16 Hz, 1H), 6.54 (d, J = 16 Hz, 1H), 3.89 (s, 3H) (no further peaks reported); no ^{13}C NMR data was reported. Starting from 12.68 g of XLII-60-1 and following the above procedure, 13.05 g (82% from 16.72 g of XLII-59-1) of XLII-61-2 was obtained as a pale yellow solid.

3-(2-Hydroxy-5-pyridyl)acrylic acid (XLII-63-2≡ XLII-64-1≡ XLII-100-3) A solution of 0.20 g (1.1 mmol) of XLII-61-2 in 5 mL of 48% aqueous HBr was refluxed for 1 h, the reaction mixture cooled to room temperature and the resulting precipitate collected by filtration to give 0.15 g (83%) of XLII-63-2 as a white solid, mp 246 °C (dec.). XLII-64-1 (2.27 g, 82%) and XLII-100-3 (3.33 g, 88%) were prepared from 3.02 g (16.9 mmol) and 4.10 (22.9 mmol) of XLII-61-2, respectively, by the same procedure. ^{1}H NMR (DMSO-d_6) 7.90 (dd, J = 9.7, 2.4 Hz, 1H), 7.81 (d, J = 2.4 Hz, 1H), 7.45 (d, J = 16.1 Hz, 1H), 6.39 (d, J = 9.7 Hz, 1H), 6.23 (d, J = 15.8 Hz, 1H); ^{13}C NMR (DMSO-d_6) 167.8, 162.1, 140.2, 138.7, 137.7, 120.4, 114.9, 113.3. Anal. Calcd for $C_8H_7NO_3$: C, 58.18; H, 4.27; N, 8.48. Found: C, 58.33; H, 4.04; N, 8.27.

Methyl *N*,*N*-bis[3-(2-hydroxy-5-pyridyl)acrylyl]-D,L-2,3-diaminopropionate (XLII-102-4) To a mixture of 0.20 g (1.1 mmol) of XLII-94-1 and 0.87 g (5.3 mmol) of XLII-100-3 in 20 mL of DMF was added 1.2 mL of TEA. After the resulting mixture was stirred for 15 min at

room temperature, it was treated with 1.06 g (5.55 mmol) of EDC and 30 mg (0.25 mmol) of DMAP. Reaction overnight at room temperature gave a suspension, which was filtered to give 0.55 g of Et_3NHCl (XLII-102-1-1) as a white solid and a yellow filtrate. The filtrate was lyophilized to give 3.25 g of pale yellow residue, which was separated on a silica gel column eluted with ethyl acetate and methanol (4/1 to 2/1) to give 0.22 g (51%) of XLII-102-4 (R_f = 0.32 when EtOAc/MeOH = 2/1) as a white solid, mp 262 ^oC (dec.). ^1H NMR (DMSO-d_6) 11.90 (br s, 2H), 8.35 (d, J = 7.9 Hz, 1H), 8.10 (dd, J = 5.4, 5.4 Hz, 1H), 7.72-7.67 (m, 4H), 7.29 (br d, J = 15.2 Hz, 1H), 6.42 (d, J = 9.4 Hz, 1H), 6.40 (d, J = 9.4 Hz, 1H), 6.38 (d, J = 15.8 Hz, 1H), 6.30 (d, J = 15.8 Hz, 1H), 4.50 (m, 1H), 3.66-3.58 (m, 1H), 3.63 (s, 3H), 3.50-3.41 (m, 1H); ^{13}C NMR (DMSO-d_6) 170.9, 165.7, 165.2, 162.0, 137.8, 137.7, 137.2 (2), 135.8, 135.3, 120.6 (2), 117.5, 117.2, 113.4 (2), 52.2, 52.0, C3 apparently was overlapped by the peaks of DMSO-d_6 (approximately 39.9 ppm based on ^{13}C NMR of XLI-135-1). Anal. Calcd for $C_{20}H_{20}N_4O_6 \cdot 0.25H_2O$: C, 57.62; H, 4.96; N, 13.44. Found: C, 57.94; H, 4.94; N, 13.01.

***N*,*N*-Bis[3-(2-hydroxy-5-pyridyl)acrylyl]-D,L-2,3-diaminopropionic acid (XLII-103-1)** A mixture of 35.0 mg (0.085 mmol) of XLII-102-4 and 10 mL of 1% (2.5 mmol) NaOH was stirred overnight at room temperature, acidified to pH = 2-3 by adding conc HCl dropwise and the white precipitate collected by filtration to give 34 mg (100%) of XLII-103-1 as a white solid, mp 255 ^oC (dec.). Preparation of sodium salt of XLII-103-1 for NMR: To 20 mg of XLII-103-1 was added 1% NaOH dropwise until XLII-103-1 was dissolved completely; the resulting solution was lyophilized to give a white solid, which was dissolved in D_2O for NMR. ^1H NMR (D_2O) 7.81 (dd, J = 9.5, 2.5 Hz, 1H), 7.76 (dd, J = 9.4, 2.6 Hz, 1H), 7.59 (d, J = 2.9 Hz, 1H),

7.58 (d, J = 2.6 Hz, 1H), 7.21 (d, J = 15.8 Hz, 1H), 7.19 (d, J = 15.5 Hz, 1H), 6.53 (d, J = 9.4 Hz, 1H), 6.51 (d, J = 9.4 Hz, 1H), 6.42 (d, J = 15.8 Hz, 1H), 6.30 (d, J = 15.8 Hz, 1H), 4.62 (dd, J = 8.6, 4.5 Hz, 1H), 3.81 (dd, J = 14.1, 4.4 Hz, 1H), 3.69 (dd, J = 13.9, 8.6 Hz, 1H); ^{13}C NMR (D_2O) 188.7, 179.0, 171.7, 171.0, 169.3, 142.7 (2), 142.1, 142.0, 140.0, 139.9, 121.0 (2), 120.4, 120.2, 119.8, 119.7, 58.0, 44.3. HRMS: $(M+H)^+$ calcd for $C_{19}H_{19}N_4O_6$ 399.1305, found 399.1310.

3.9.6. 2-Caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartaric acid

Diphenylmethyl 2-[3-(2-hydroxy-5-pyridyl)acrylyl]-3-hydroxy-L-tartrate (XLII-65-1) A mixture of 1.35 g (8.18 mmol) of XLII-64-1 and 45 mL of thionyl chloride was refluxed for 2.5 h. Extra thionyl chloride was removed with a Rotavapor and the resulting acid chloride treated with 80 mL of CH_2Cl_2 and 20 mL of pyridine. After the mixture was stirred at RT for 15 min, it was further treated with a solution of 0.66 g (1.4 mmol) of XLI-76-3 in 15 mL of CH_2Cl_2. The resulting mixture was stirred overnight at RT, and then sequentially washed with 10% HCl, 5% $NaHCO_3$ and saturated NaCl. The organic extract was dried over Na_2SO_4, and the solvent removed with a Rotavapor to give 1.41 g of crude product as a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (EtOAc/hexanes = 4/1) to give 0.44 g (51%) of XLII-65-1 (R_f = 0.23) as a white solid, mp 123-126 ^{o}C. ^{1}H NMR (CD_3OD) 7.74 (dd, J = 9.7, 2.6 Hz, 1H), 7.54 (d, J = 2.4 Hz, 1H), 7.41 (d, J = 16.1 Hz, 1H), 7.35-7.08 (m, 20H), 6.905 (s, 1H), 6.901 (s, 1H), 6.54 (d, J = 9.7 Hz, 1H), 6.08 (d, J = 15.8 Hz, 1H), 5.82 (d, J = 2.6 Hz, 1H), 5.05 (d, J = 2.6 Hz, 1H); ^{13}C NMR (CD_3OD) 170.9, 167.6, 167.0, 165.1, 142.8, 141.0, 140.9 (2), 140.8, 139.8, 139.2, 129.6 (2), 129.51 (2), 129.46 (2), 129.4 (2), 129.03,

129.00, 128.97, 128.9, 128.1 (4), 128.0 (2), 127.9 (2), 121.5, 116.3, 115.0, 80.0, 79.6, 75.0, 71.8. Anal. Calcd for $C_{38}H_{31}NO_8$: C, 72.49; H, 4.96; N, 2.22. Found: C, 72.19; H, 5.10; N, 2.29.

Diphenylmethyl 2-(3,4-dimethoxycarbonylcaffeoyl)-3-[3-(2-hydroxy-5- pyridyl)-acrylyl]-L-tartrate (XLII-106-2) To a solution of 3, 4-dimethoxycarbonylcaffeoyl chloride (made from 0.19 g (0.64 mmol) of 3, 4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A) in 30 mL of dichloromethane was added 2 mL of pyridine. After the resulting solution was stirred for 10 min at room temperature, it was treated with a solution of 0.10 g (0.16 mmol) of XLII-65-1 in 3 mL of dichloromethane. After reacting overnight at room temperature, the mixture was washed successively with three portions each of 5% HCl, 5% $NaHCO_3$ and saturated NaCl. The organic layer was dried over Na_2SO_4 and the solvent evaporated to give a yellow residue, which was separated on a silica gel column eluted with ethyl acetate and hexanes (v/v = 2/1) to give 0.125 g (87%) of XLII-106-2 (R_f = 0.12) as a white solid, mp 101-103 ^oC. ^1H NMR (CD_3COCD_3) 7.89 (dd, *J* = 9.7, 2.1 Hz, 1H), 7.84-7.10 (m, 26H), 6.97 (br s, 2H), 6.54 (d, *J* = 9.7 Hz, 1H), 6.41 (d, *J* = 15.8 Hz, 1H), 6.27 (s, 2H), 6.13 (d, *J* = 15.8 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (CD_3COCD_3) 166.1, 165.8, 165.7, 165.6, 163.3, 153.8, 153.6, 145.2, 145.0, 143.8, 143.3, 140.6 (2), 140.5 (2), 140.0, 138.5, 134.0, 129.4 (8), 128.9 (3), 128.8, 128.2, 127.8 (2), 127.7 (2), 127.63 (2), 127.61 (2), 124.6, 123.8, 121.9, 118.5, 114.4, 113.2, 79.6, 79.5, 72.1, 71.7, 56.4 (2). Anal. Calcd for $C_{51}H_{41}NO_{15}\cdot H_2O$: C, 66.16; H, 4.68; N, 1.51. Found: C, 66.27; H, 4.46; N, 1.34.

Diphenylmethyl 2-caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartrate (XLII-107-1) To a solution of 113 mg (0.12 mmol) of XLII-106-2 in 12 mL of THF was added 8 mL of 10%

aqueous Na_2CO_3 solution. The mixture was stirred under N_2 at room temperature for 12 h. Extra Na_2CO_3 was neutralized by adding 3 mL of acetic acid. After the resulting mixture was diluted with 20 mL of water, it was extracted with 3 x 30 mL of dichloromethane. The organic layers were combined, twice washed with 5% $NaHCO_3$, and dried over Na_2SO_4. After the solvent was evaporated, 103 mg of XLII-107-1 was obtained as a yellow residue, which was used in the next step without purification. ^{1}H NMR (CD_3COCD_3) 7.92 (br d, *J* = 10.0 Hz, 1H), 7.57 (d, *J* = 15.8 Hz, 1H), 7.51-7.10 (m, 24H), 7.04 (d, *J* = 7.9 Hz, 1H), 6.95 (br s, 2H), 6.60 (d, *J* = 9.7 Hz, 1H), 6.24 (s, 2H), 6.17 (d, *J* = 15.8 Hz, 1H), 6.15 (d, *J* = 15.5 Hz, 1H); ^{13}C NMR (CD_3COCD_3) 166.3, 166.1, 165.92, 165.88, 163.7, 149.3, 148.0, 146.3, 143.0, 140.62, 140.59, 140.5, 140.4, 139.8, 138.9, 129.3 (8), 128.9 (2), 128.8 (2), 127.7 (2), 127.6 (4), 127.1, 123.1, 121.7, 116.3, 115.2, 114.8, 113.6, 113.4, 79.5, 79.4, 71.84, 71.76.

2-Caffeoyl-3-[3-(2-hydroxy-5-pyridyl)acrylyl]-L-tartaric acid (XLII-108-3) To a mixture of 103 mg of XLII-107-1 in 8 mL of CH_2Cl_2 in an ice bath was added 0.75 mL (9.7 mmol) of TFA dropwise. After 5 h under N_2, the CH_2Cl_2 and extra TFA were removed with a Rotovapor and the residue stirred in 10 mL of CH_2Cl_2 and filtered to give 56 mg (98% from 113 mg of XLII-106-2) of XLI-108-1 as a pale yellow solid. A pure sample for bioassay was obtained by purifying 56 mg of XLII-108-1 by HPLC with CH_3OH, H_2O and HOAc (v/v/v = 60/40/1) as eluting solvents to give 24.7 mg (44% recovery) of XLII-108-3 (Ret. time = 3.26 min) as a white solid, mp 191 ^{o}C (dec.). ^{1}H NMR (CD_3OD) 7.98 (dd, *J* = 9.6, 2.2 Hz, 1H), 7.75 (d, *J* = 1.9 Hz, 1H), 7.64 (d, *J* = 16.0 Hz, 1H), 7.62 (d, *J* = 16.0 Hz, 1H), 7.07 (d, *J* = 1.9 Hz, 1H), 6.98 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.79 (d, *J* = 8.3 Hz, 1H), 6.58 (d, *J* = 9.6 Hz, 1H), 6.44 (d, *J* = 15.7 Hz, 1H),

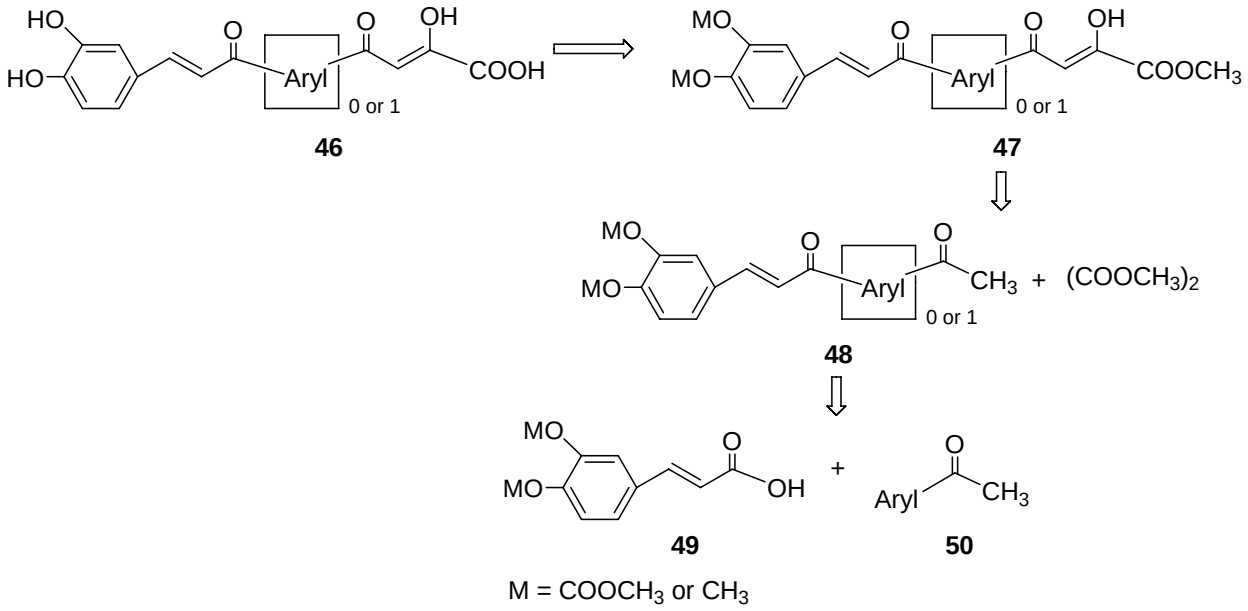
6.35 (d, J = 15.7 Hz, 1H), 5.81 (br s, 2H); ^{13}C NMR (CD_3OD) 169.7, 169.6, 167.6, 167.1, 165.4, 150.0, 148.6, 146.9, 143.0, 140.0, 139.5, 127.5, 123.4, 121.6, 116.7, 116.5, 115.2 (2), 113.6, 72.6, 72.5. Anal. Calcd for $C_{21}H_{17}NO_{11}\cdot H_2O$: C, 52.84; H, 4.01; N, 2.93. Found: C, 53.19; H, 3.93; N, 2.76.

Chapter 4. Syntheses of Hybrid Diketo Acid/Catechol Molecules

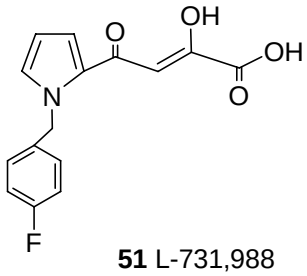
4.1. Introduction

The so-called β-diketo acid integrase inhibitors (DKAs) are predominantly 4-aryl-(or 4-styryl-)2-hydroxy-4-oxo-2-butenoic acids. For the reasons discussed in Chapter 1, the syntheses of several hybrid diketo acid/catechol molecules were undertaken. The general synthetic strategy is described in Scheme 4-1. The target molecules **46** are to be obtained from the fully blocked compounds **47**, which will be synthesized from the methyl ketones **48** and dimethyl oxalate in the presence of base. Those methyl ketones **48** which are not commercially available may be synthesized by coupling of the acid chloride of acids **49** with the commercially available methyl ketones **50**. The phenolic hydroxyl groups will be protected as methoxycarbonyl or methoxy groups.

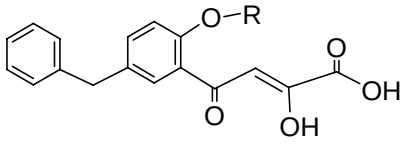
Scheme 4-1 General synthetic strategy for the hybrid diketo acid/catechol molecules



The three known diketo acids (**51**-**53**) were also synthesized for testing of their biological activities.



51 L-731,988

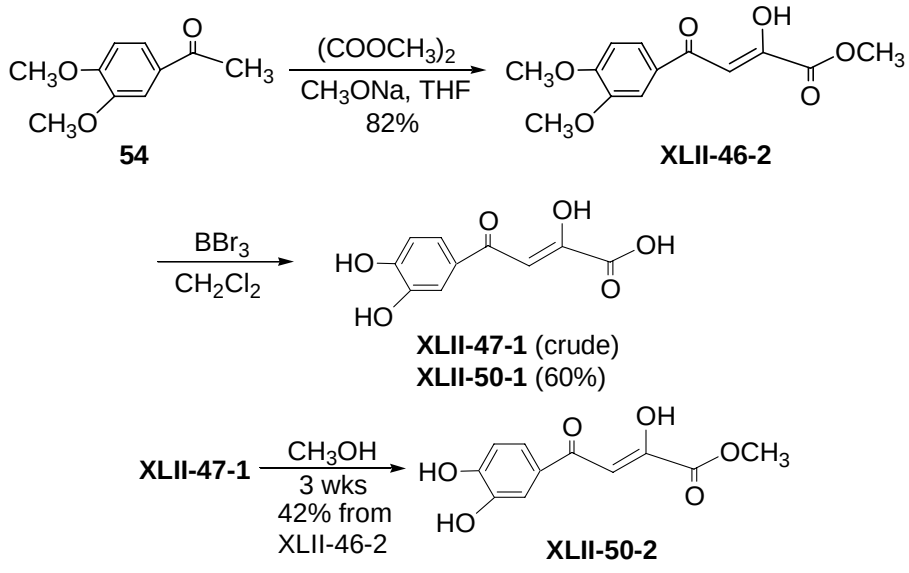


52, R = -$CH(CH_3)_2$
53, R = -CH_2CH_3

4.2. 4-(3,4-Dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid

The synthesis of 4-(3,4-dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid started with the deprotonation of 3,4-dimethoxyacetophenone (**54**) by sodium methoxide followed by reaction with dimethyl oxalate to give the desired diketo acid methyl ester XLII-46-2 in 82% yield (Scheme 4-2). The methoxy groups and methyl ester were simultaneously removed with boron tribromide. The pure acid XLII-50-1 was obtained in 60% yield after purification by HPLC. After three weeks in methanol the crude product XLII-47-1 was completely converted to

Scheme 4-2 Synthesis of 4-(3,4-dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid



54 **XLII-46-2**

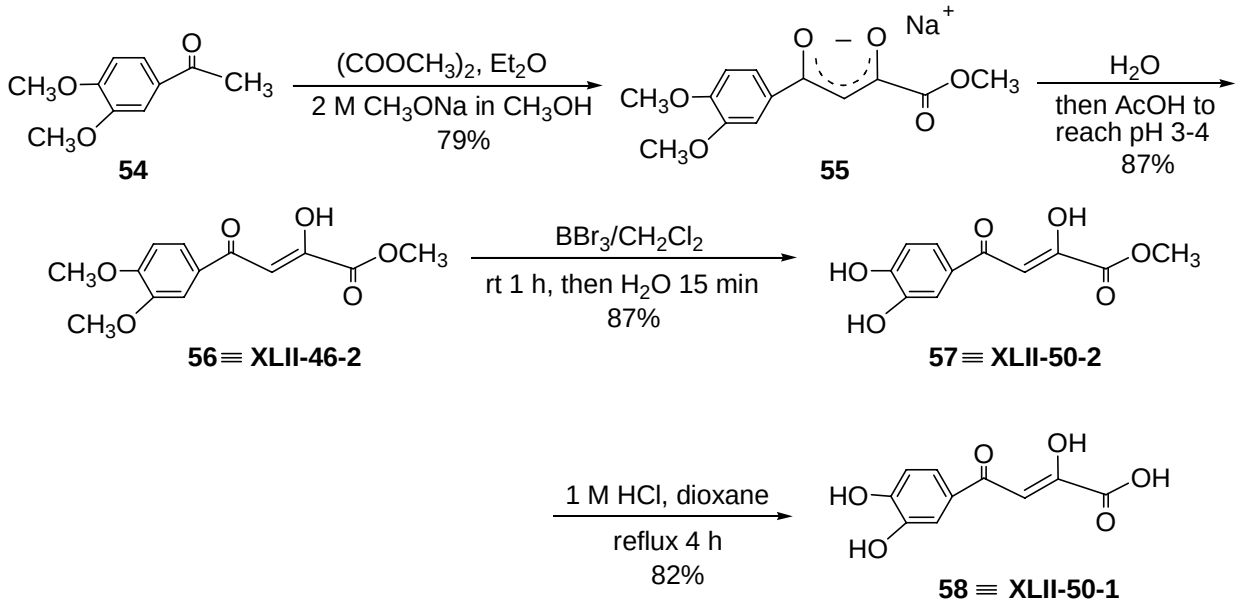
XLII-47-1 (crude)
XLII-50-1 (60%)

XLII-50-2

its methyl ester as determined by NMR. Purification by flash chromatography followed by HPLC gave the pure methyl ester XLII-50-2 in 42% yield for testing against HIV IN.

After this synthesis was completed, a similar synthesis (Scheme 4-3) appeared in the literature. [88,112] It also started with acetophenone **54**, but the intermediate sodium ketoenolate (**55**) was isolated before conversion to the ester **56** ≡ XLII-46-2. Also, the order of deblocking the methoxy groups was reversed, that is, the two ether functions were demethylated with boron tribromide to first give the methyl ester, which was then hydrolyzed using 1 M HCl in refluxing dioxane. Although our yield of XLII-46-2 was higher than that in the literature (82% vs. 69%), our isolated yields of XLII-50-1 and XLII-50-2 were lower because ultra purification by HPLC led to low recovery yields. The methyl ester XLII-50-2 and acid XLII-50-1 are identical to compounds **57** and **58**, respectively, by ^{1}H and ^{13}C NMR (see Experimental section).

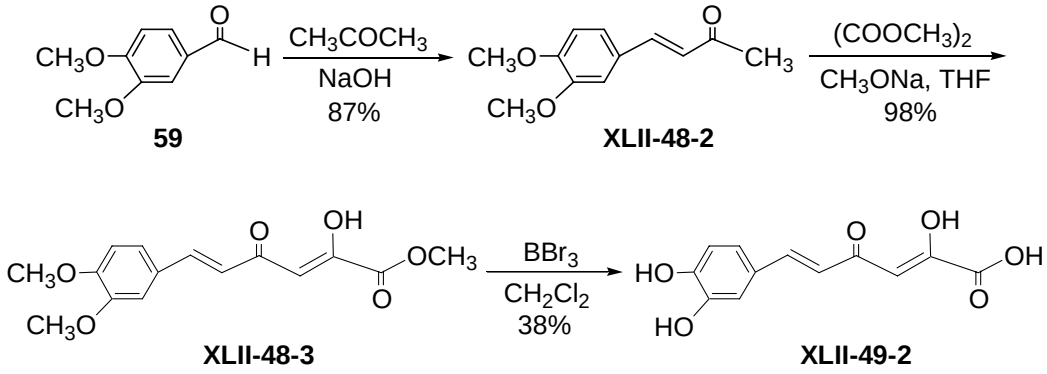
Scheme 4-3 Reported [88,112] synthesis of 4-(3,4-dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid



4.3. 6-(3,4-Dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoic acid

The known [113,114] methyl ketone XLII-48-2 was obtained in 87% yield by condensation of 3,4-dimethoxybenzaldehyde (**59**) and acetone with sodium hydroxide (Scheme 4-4). Deprotonation with sodium methoxide followed by reaction with dimethyl oxalate gave the desired methyl ester XLII-48-3 in 98% yield. The methyl ether and ester groups were cleaved by reaction with boron tribromide. Pure diketo acid XLII-49-2 was obtained in 38% yield after purification by HPLC.

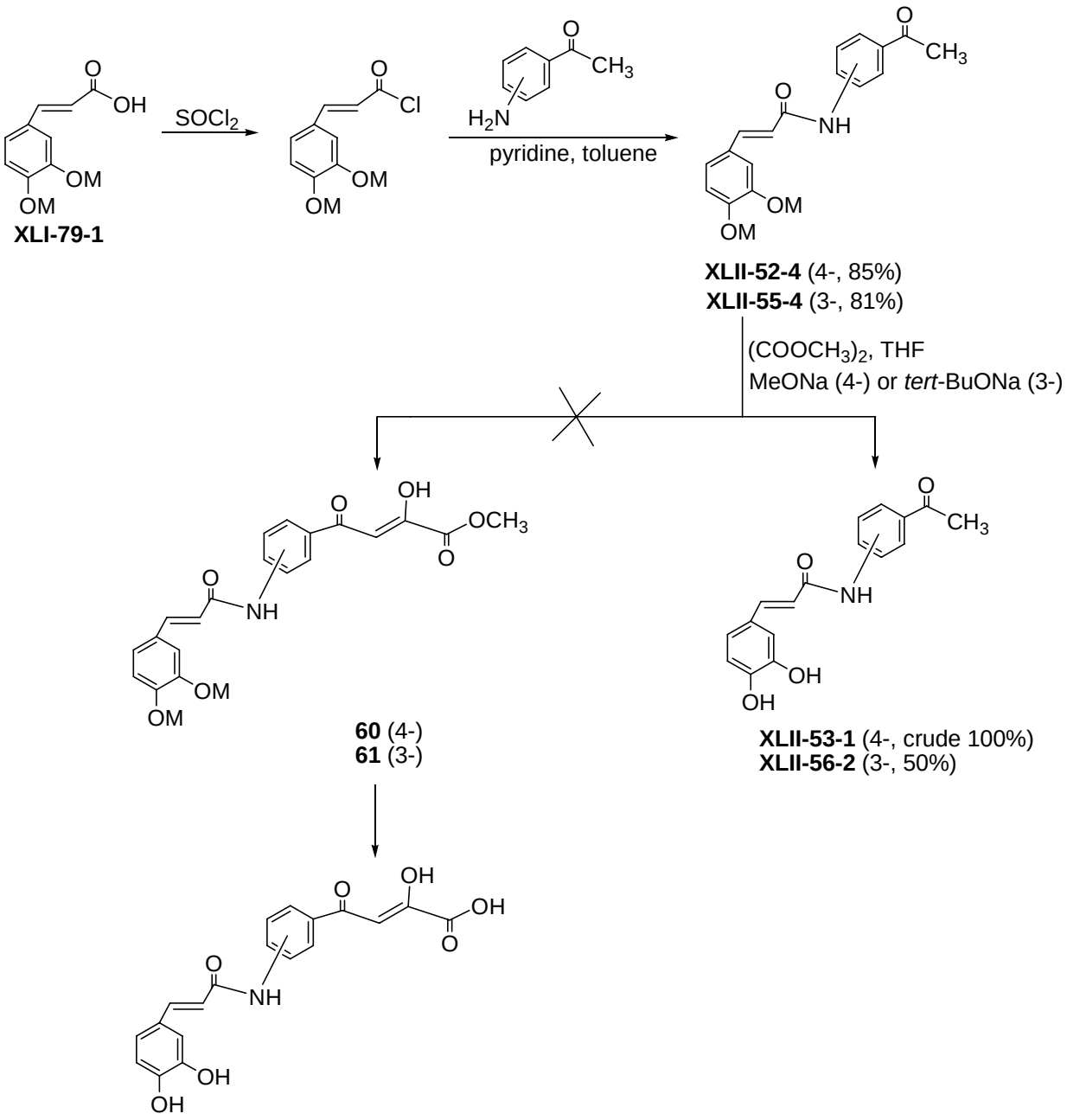
Scheme 4-4 Synthesis of 6-(3,4-dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-butenoic acid



4.4. 4-(x-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acids (x = 4, 3 or 2)

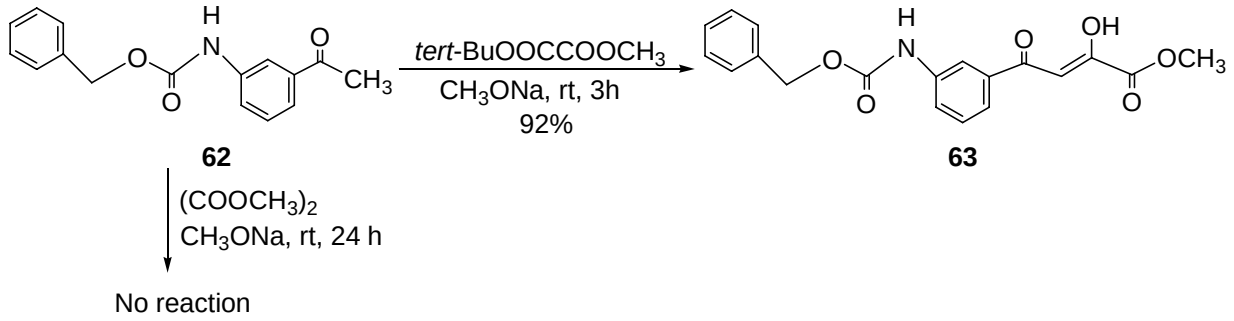
Starting from protected caffeic acid XLI-79-1 (Chapter 2), the procedure shown in Scheme 4-5 was attempted first. 3,4-Dimethoxycarbonylcaffeoyl chloride reacted with the commercially available 4- and 3- isomers of aminoacetophenone to give amides XLII-52-4 and XLII-55-4 in 80% yield. Following the same procedure used for the syntheses of methyl esters XLII-46-2 and XLII-48-3 (Scheme 4-2 and 4-4), the attempted preparation of methyl ester **60**

Scheme 4-5 Attempt to synthesize 4-(x-caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acids (x = 4 or 3)



from methyl ketone XLII-52-4 only gave catechol XLII-53-1. A similar compound **62** (Scheme 4-6) was also reported [115] to be unreactive with methyl oxalate and sodium methoxide, but the target diketo ester (**63**) was obtained in high yield using *tert*-butyl methyl oxalate and sodium

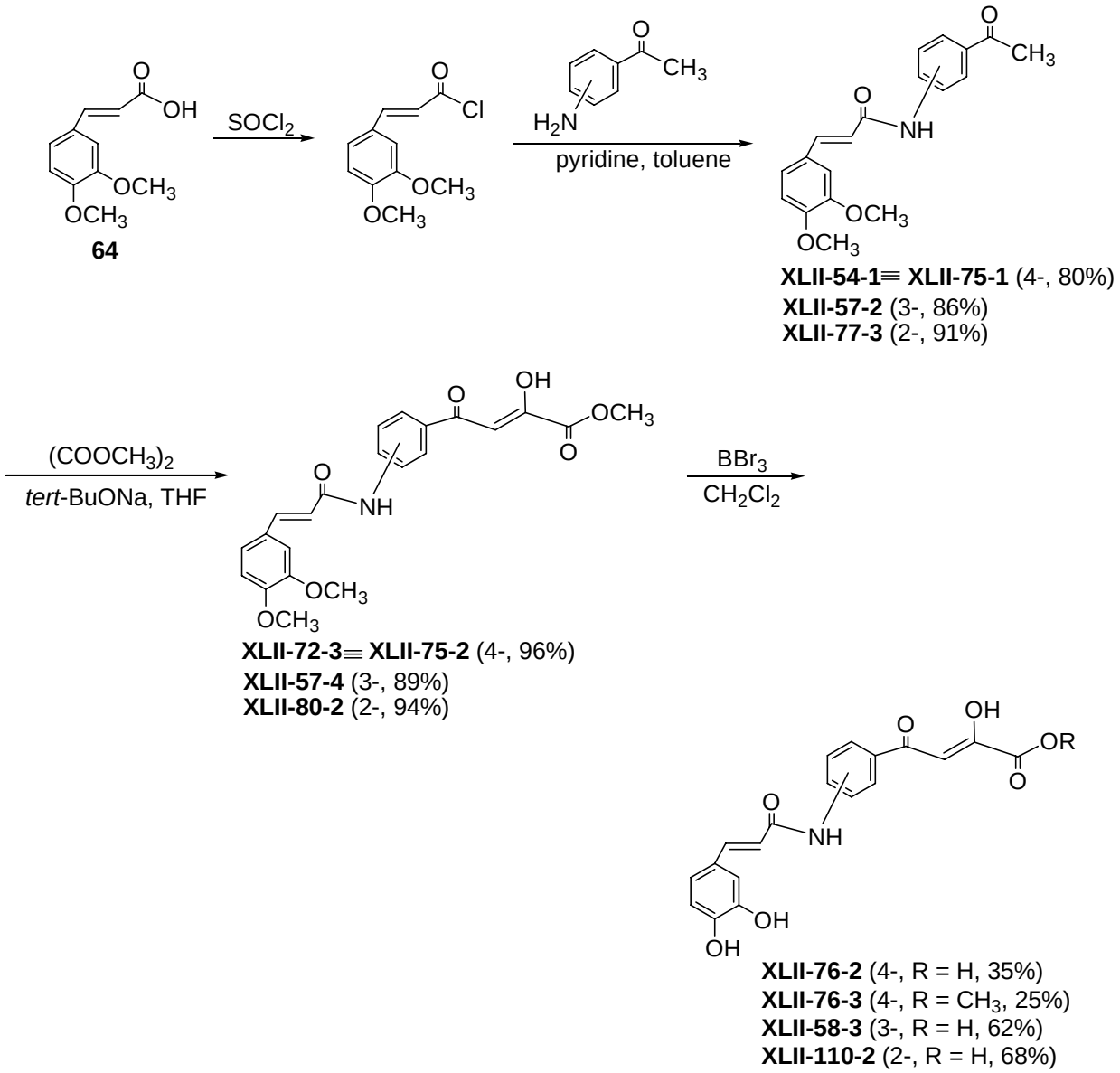
Scheme 4-6 Reported [115] coupling of an aryl methyl ketone with tert-butyl methyl oxalate or dimethyl oxalate in the presence of sodium methoxide



methoxide. It was speculated [115] that sodium methoxide transesterified the *tert*-butyl methyl oxalate to generate dimethyl oxalate and sodium *tert*-butoxide which is a better base than sodium methoxide. Therefore, in the attempt to synthesize diketo acid methyl ester **61** from methyl ketone XLII-55-4, sodium *tert*-butoxide was used as a base instead of sodium methoxide. However, the catechol XLII-56-2 was obtained instead of the desired methyl ester **61**. Apparently the base removes the methoxycarbonyl blocking groups to generate the weakly basic phenoxide instead of the strongly basic enolate. To avoid this problem, 3,4-dimethoxycarbonylcaffeic acid was replaced by 3,4-dimethoxycinnamic acid (**64**). The syntheses of the isomers of 4-caffeoylaminophenyl-2-hydroxy-4-oxo-2-butenoic acid are described in Scheme 4-7.

3,4-Dimethoxycinnamic acid reacted with thionyl chloride to give its acid chloride, which reacted with the three isomers of aminoacetophenone to give the 4- (XLII-54-1≡ XLII-75-1), 3- (XLII-57-2), and 2- (XLII-77-3) isomers of (3,4-dimethoxycinnamylamino)acetophenone in 80-90% yield. These acetophenones reacted with dimethyl oxalate in the presence of sodium *tert*-butoxide to give the diketo acid methyl esters in 86-96% yield.

Scheme 4-7 Syntheses of 4-(x-caffeoylaminophenyl)-2-hydroxy -4-oxo-2-butenoic acids (x = 4, 3 or 2)



For the final demethylation with boron tribromide in dichloromethane several reaction conditions were attempted including a long reaction time (22-62 h) at room temperature and 0.5h at room temperature followed by heating at reflux overnight. [30] For the *para*-isomer (XLII-72-3 ≡ XLII-75-2), a mixture of acid (XLII-76-2) and its methyl ester (XLII-76-3) was obtained under both conditions; for the *meta*-isomer (XLII-57-4), the acid (XLII-58-3) was obtained as the

predominant product under both conditions (> 95%) by NMR; for the *ortho*-isomer (XLII-80-2), a complex mixture of unidentified products was obtained under both conditions. The desired ortho-compound XLII-110-2 was finally obtained from the reaction of XLII-80-2 with boron tribromide at -78 ^{o}C for 3 h followed by room temperature for 6.5 h.

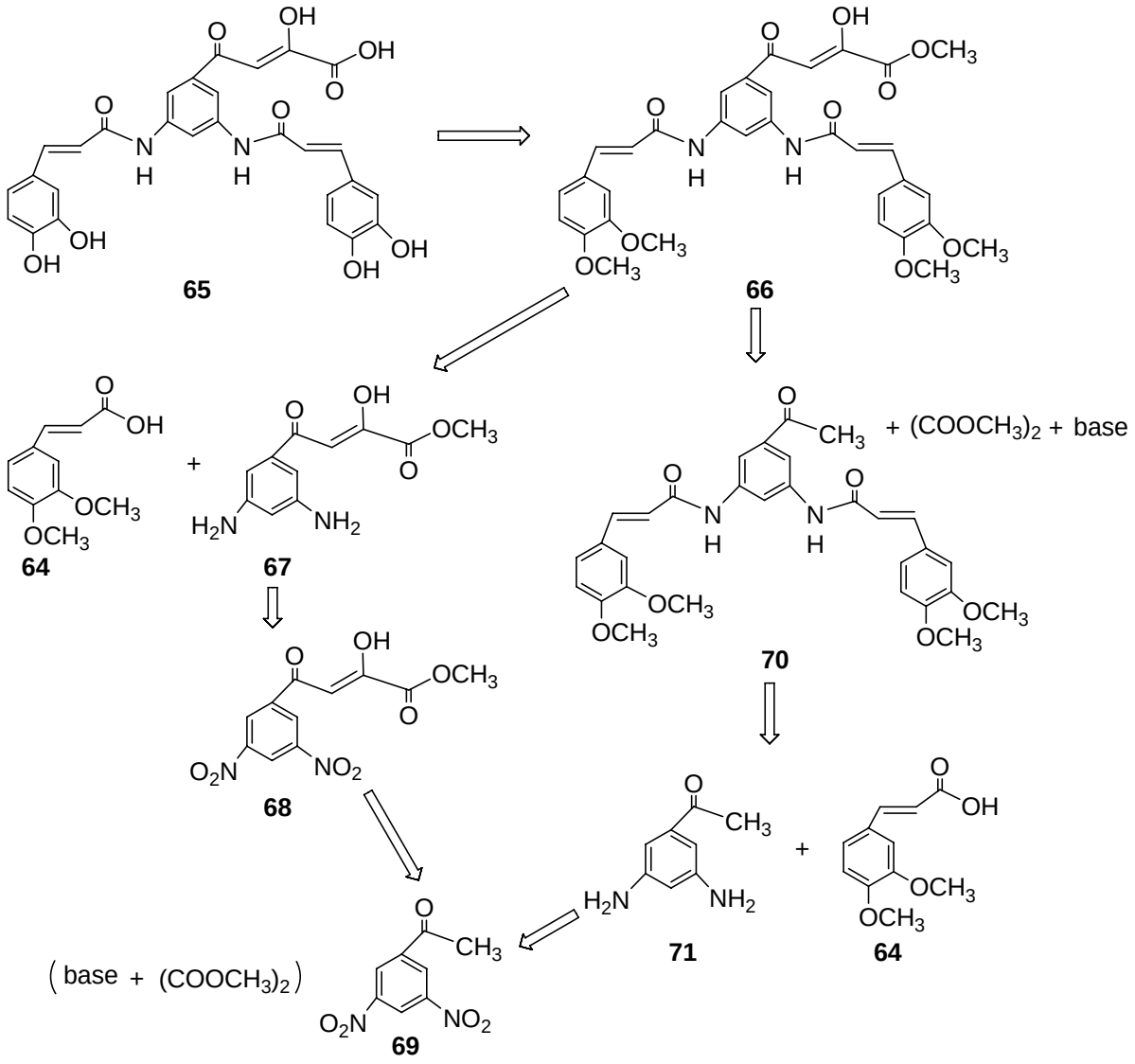
4.5. 4-(3,5-Dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid and its methyl ester

Starting from the known [116-119] 3,5-dinitroacetophenone (**69**), 4-(3,5-dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**65**) could be obtained by two routes (Scheme 4-8). In the first route, the acetophenone **69** would be converted to methyl ester **68**, whose nitro groups would be reduced to amino groups. The resulting diamine **67** would react with the acid chloride of commercially available 3,4-dimethoxycinnamic acid (**64**) to give the fully blocked diamide **66**, which would be demethylated to the desired compound **65**. In the second method, the nitro groups of the acetophenone **69** would be reduced to amino groups to give diamine **71**, which would be reacted with the acid chloride of acid **64** to give diamide **70**, which upon reaction with dimethyl oxalate and base would give the diketo acid methyl ester **66**.

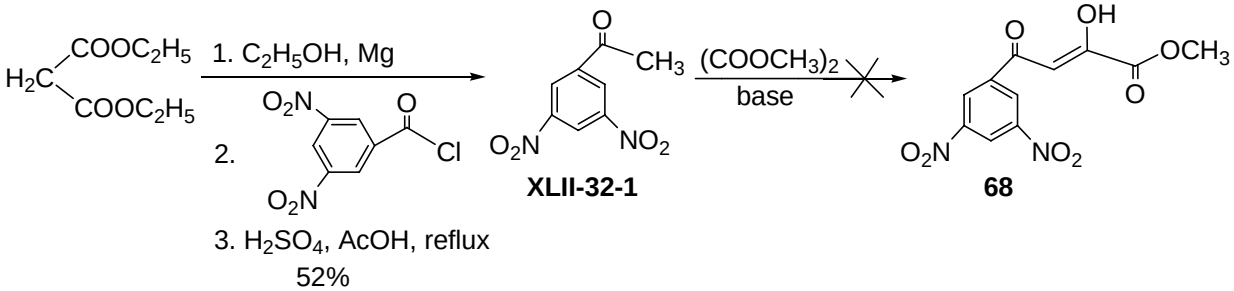
The first route was initially attempted (Scheme 4-9). Although 3,5-dinitroacetophenone was commercially available, it was relatively expensive ($44.90/g from Aldrich) and was therefore prepared from diethyl malonate and 3,5-dinitrobenzoyl chloride in 52% yield according to the literature procedure. [118-120]

Although *p*-nitroacetophenone **72** is reported [115] to react with dimethyl oxalate or *tert*-butyl methyl oxalate in the presence of sodium methoxide to give methyl 4-(4-nitrophenyl)-2-

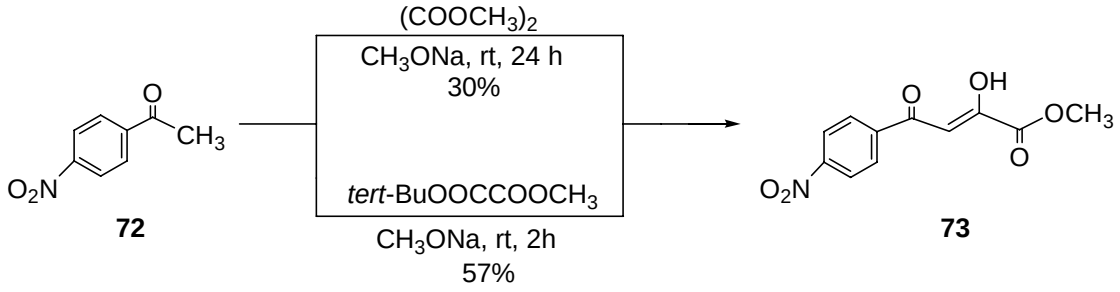
Scheme 4-8 Retrosynthesis of 4-(3,5-dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid



Scheme 4-9 Attempt to synthesize 4-(3,5-dicaffeoylaminophenyl) -2-hydroxy-4-oxo-2-butenoic acid



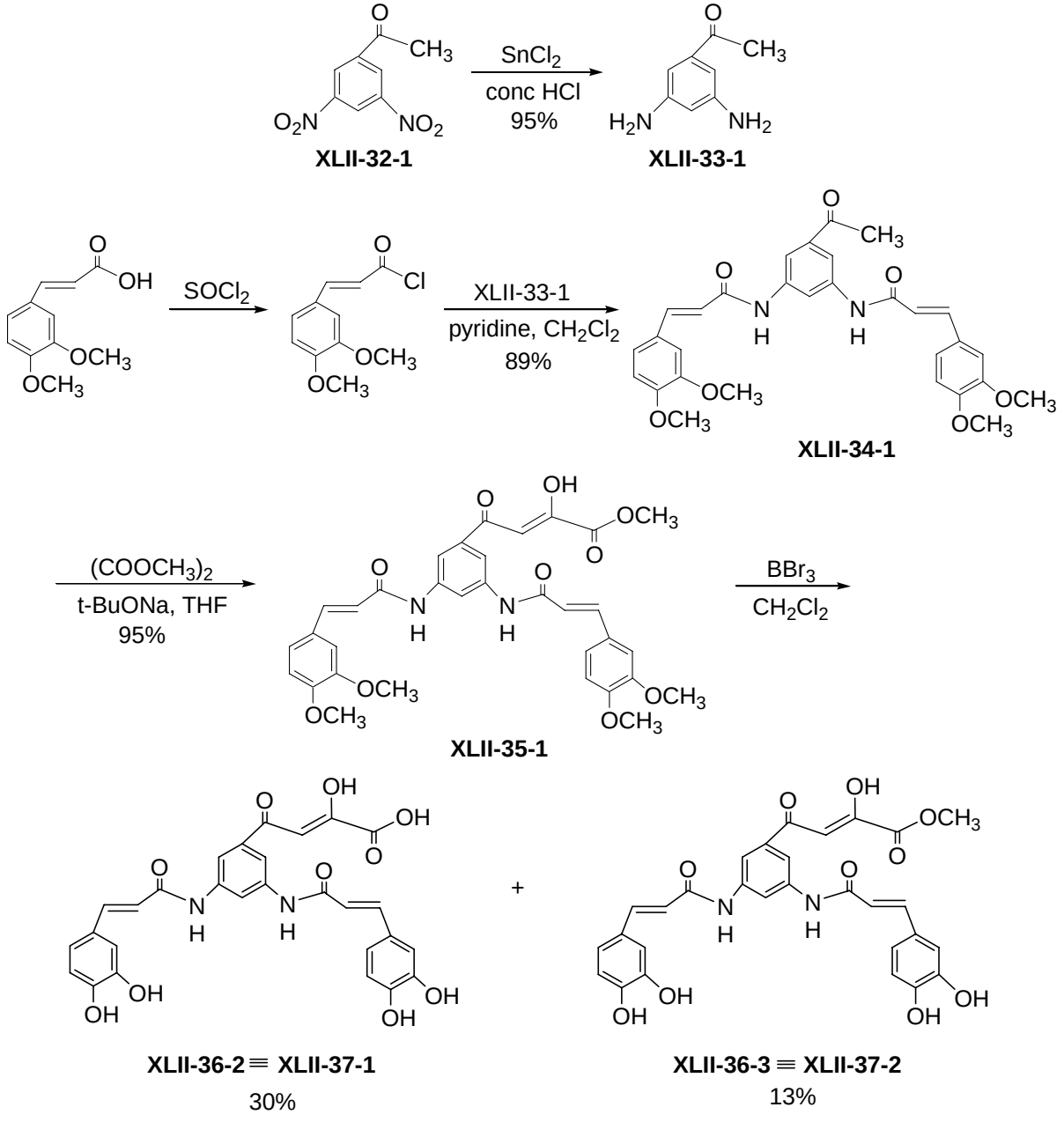
Scheme 4-10 Reaction of 3-nitroacetophenone with oxalic acid diesters in the presence of sodium methoxide [115]



hydroxy-4-oxo-2-butenoate (**73**, Scheme 4-10), there was no reaction between 3,5-dinitroacetophenone (XLII-32-1) and dimethyl oxalate under similar conditions, and only the starting methyl ketone XLII-32-1 was recovered in 95% yield.

The target compound 4-(3,5-dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (**65**) was finally synthesized via the synthetic route described in Scheme 4-11. The nitro groups of XLII-32-1 were reduced with tin (II) chloride in concentrated hydrochloric acid [120] to give the known [116,117] diamine XLII-33-1 in 95% yield. 3,4-Dimethoxycinnamic acid reacted with thionyl chloride to give the acid chloride, which was reacted with diamine XLII-33-1 to give diamide XLII-34-1 in 89% yield. Methyl ketone XLII-34-1 reacted with dimethyl oxalate in the presence of sodium *tert*-butoxide to give the methyl ester XLII-35-1 in 95% yield. Demethylation was achieved with boron tribromide in dichloromethane. The crude product, a mixture of 4-(3,5-dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLII-36-2 ≡ XLII-37-1) and its methyl ester (XLII-36-3 ≡ XLII-37-2) by NMR, was separated by HPLC to give the acid and methyl ester in 30% and 13% yield, respectively.

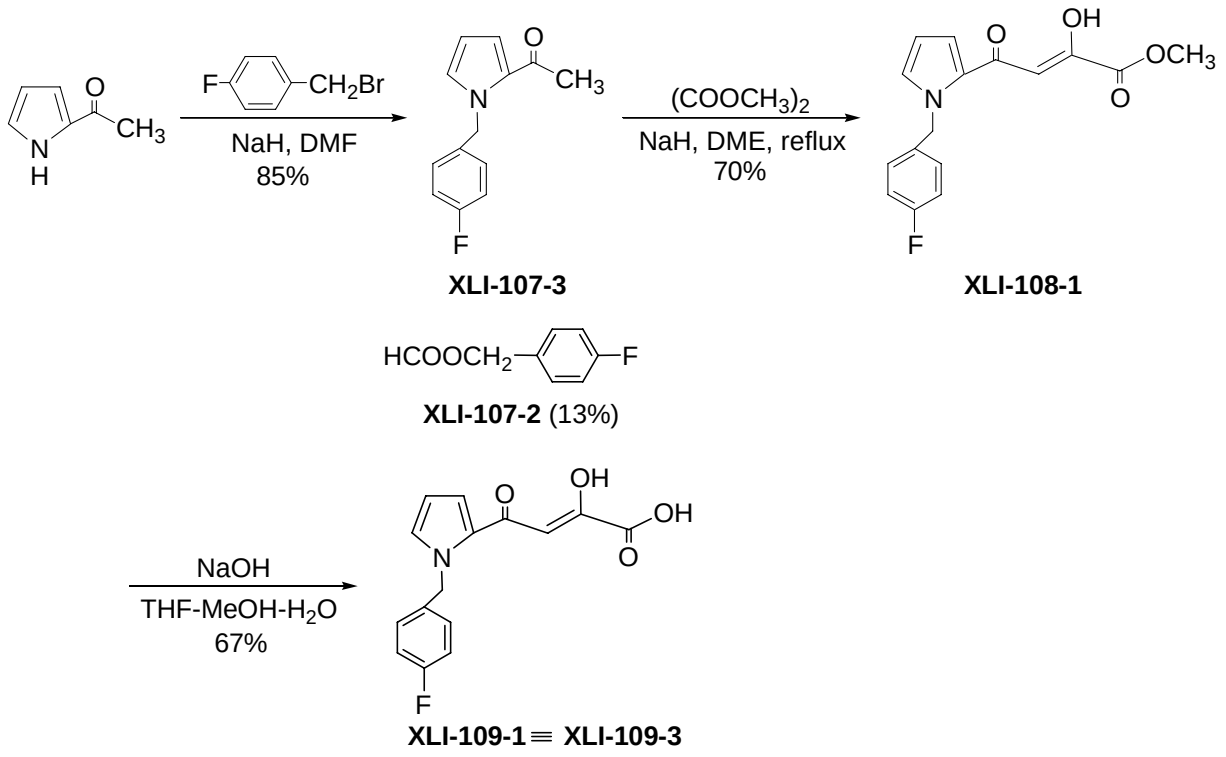
Scheme 4-11 Synthesis of 4-(3,5-dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid and its methyl ester



4.6. 4-[1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoic acid

The known [121] 4-[1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoic acid (L-731,988) (XLI-109-1) was synthesized according to the literature (Scheme 4-12).

Scheme 4-12 Synthesis of 4-[1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoic acid



XLI-107-3 **XLI-108-1**

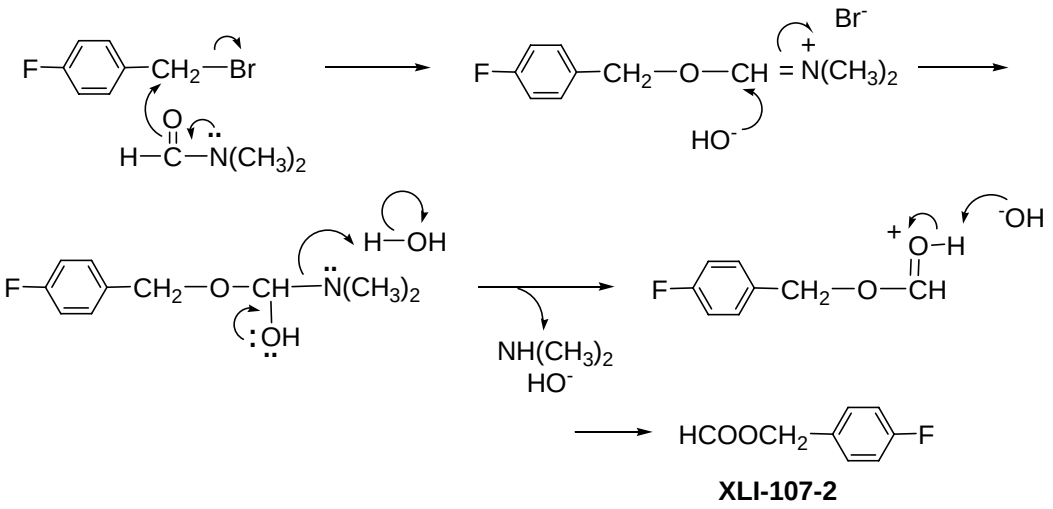
XLI-107-2 (13%)

XLI-109-1 ≡ **XLI-109-3**

2-Acetylpyrrole was treated with sodium hydride followed by 4-fluorobenzyl bromide to give methyl ketone XLI-107-3 in 85% yield after purification on a silica gel column. 4-Fluorobenzyl formate (XLI-107-2) was obtained as a by-product in 13% yield presumably by the reaction of 4-fluorobenzyl bromide and DMF as postulated in Scheme 4-13. The base for the hydrolysis of the imine presumably came from the workup. The ^{1}H NMR of XLI-107-2 showed the formyl group as a singlet at 8.09 ppm, the two-proton benzyl methylene singlet at 5.14 ppm, and the para-substituted doublets which were para- or meta- coupled with the fluorine to give the doublets of doublets at 7.34 ppm and 7.03 ppm, respectively. The ^{13}C NMR of XLI-107-2 showed one carbonyl peak (162.6 ppm), one aromatic carbon ipso to the fluorine with J = 247.3 Hz, two identical aromatic carbons ortho to the fluorine with J = 21.8 Hz, two identical aromatic

carbons meta to the fluorine with J = 8.3 Hz, one aromatic carbon para to the fluorine (J not observed.), and the benzyl methylene carbon at 64.7 ppm.

Scheme 4-13 Mechanism of the formation of 4-fluorobenzyl formate



XLI-107-2

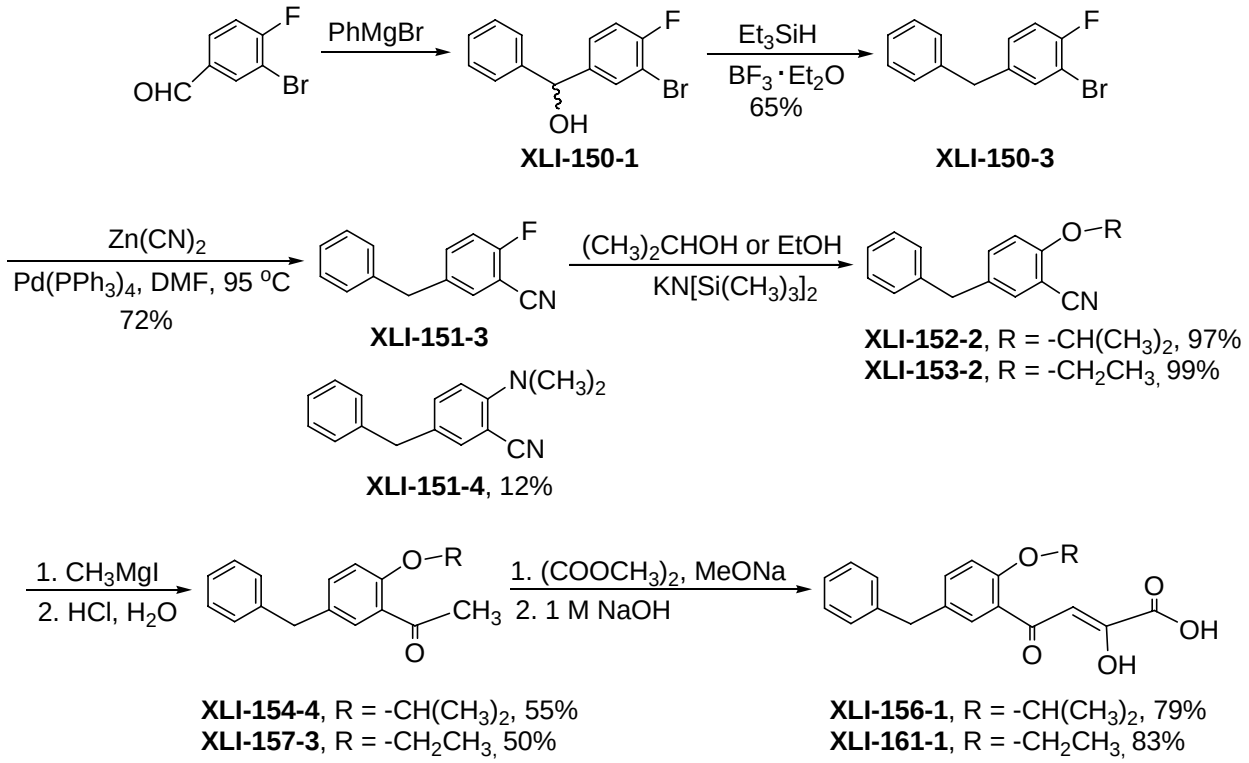
Methyl ketone XLI-107-3 was reacted with dimethyl oxalate in the presence of sodium hydride to give methyl ester XLI-108-1 in 70% yield after recrystallization. Methyl ester XLI-108-1 was hydrolyzed to give acid XLI-109-1 ≡ XLI-109-3 in 67% yield after recrystallization.

4.7. 4-(5-Benzyl-2-isopropoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid and 4-(5-benzyl-2-ethoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid

Following the synthetic route in the literature [42] (Scheme 4-14), 3-bromo-4-fluorobenzaldehyde was treated with phenylmagnesium bromide to give crude alcohol XLI-150-1 which was used in the next step without purification. Alcohol XLI-150-1 was reduced with triethylsilane and boron trifluoride to give XLI-150-3 in 65% yield. Reaction of XLI-150-3 with zinc cyanide and a palladium catalyst gave XLI-151-3 in 72% yield. A by-product was identified as 5-benzyl-2-(*N*,*N*-dimethylamino)benzonitrile (XLI-151-4) from its NMR spectra. The ^{1}H

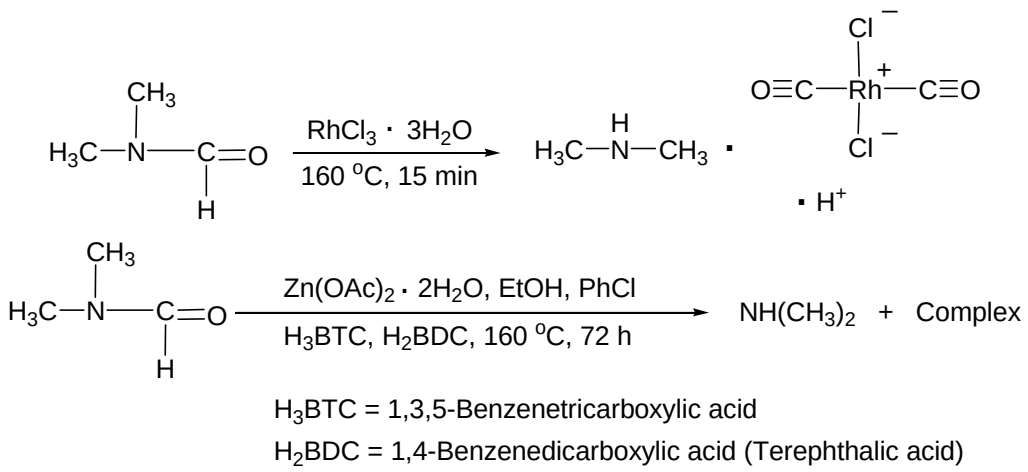
NMR of XLI-151-4 showed eight aromatic protons, one two-proton benzyl methylene singlet, and one six-proton *N*-methyl singlet. Its ^{13}C NMR showed ten aromatic peaks for two phenyl rings, one cyano peak at 119.5 ppm, one peak for two methyl groups at 43.0 ppm, and one benzyl methylene peak at 40.4 ppm.

Scheme 4-14 Synthesis of 4-(5-benzyl-2-isopropoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid and 4-(5-benzyl-2-ethoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid

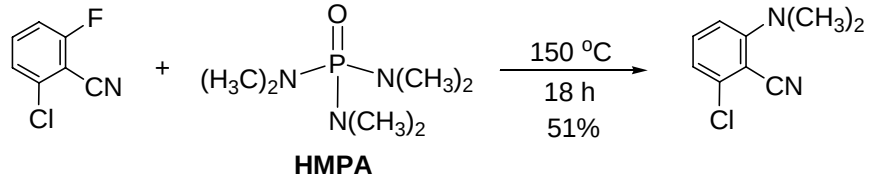


This by-product probably arises by nucleophilic substitution of XLI-151-3 by dimethylamine which might be generated from DMF in the presence of metals. Two examples of such metal-catalyzed decomposition of DMF are given in Scheme 4-15. [122,123] A similar reaction of 6-chloro-2-fluorobenzonitrile with hexamethylphosphoramide (HMPA) at elevated temperature has been reported (Scheme 4-16). [124]

Scheme 4-15 Two examples [122,123] of generation of dimethylamine from DMF



Scheme 4-16 A reported reaction of an activated aryl dihalide with HMPA [124]



HMPA

The fluorine of XLI-151-3 was replaced by an isopropoxy or ethoxy group using potassium bis(trimethylsilyl)amide (KHMDS) as a non-nucleophilic base to give XLI-152-2 and XLI-153-2 in 97% and 99% yield, respectively. Nitriles XLI-152-2 and XLI-153-2 were treated with methylmagnesium iodide to give methyl ketones XLI-154-4 and XLI-157-3 in 55% and 50% yield, respectively. These in turn were reacted with dimethyl oxalate and sodium methoxide to give the diketo acid methyl esters, which were hydrolyzed directly to give acids XLI-156-1 and XLI-161-1 in 79% and 83% yield, respectively. The samples for bioassay were purified by recrystallization or HPLC. The x-ray structure of XLI-156-1 is given in Fig. 4-1.

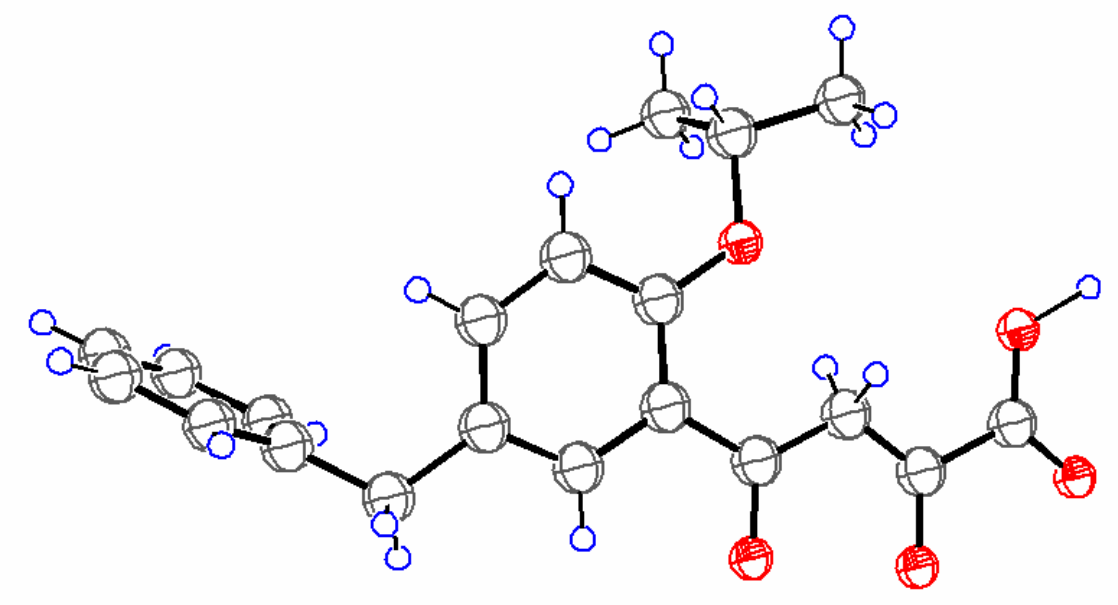


Fig. 4-1 X-ray structure of XLI-156-1

4.8. Bioassay results

Bioassays (Table 4-1) showed that all the diketo acid/catechol hybrids tested are potent IN inhibitors with the meta-isomer XLII-58-3 being the most active (IC_{50} = 0.08 μM), even more than L-CA and the simple diketo acids XLI-109-1 and XLI-156-1. The high potency of XLII-58-3 is consistent with the conclusion that compounds with a meta- orientation of the substituent and the diketo acid side chain are more active than those with other substitution patterns. [42]

For the inhibitory activity against HIV replication in cell culture, the meta dicaffeoyl compound XLII-36-2 and the para-isomer XLII-76-2 showed moderate activity with TI_5 = 5.4 and 2.8, respectively, but both of them are less active than either L-CA or the simple diketo acids. The other compounds have no significant anti-HIV activity with TI < 1. Compared with 3,5-dicaffeoylaminobenzoic acid (**26**, Chapter 1, page 19), the DKA/catechol hybrid XLII-36-2 was three-fold more active against HIV IN while it was less active against HIV replication.

Table 4-1 Anti-HIV activities (in μM) of diketo acid/catechol hybrids

Compound	CT_{50}	CT_5	ED_{50}	IC_{50} 3'-Processing	TI_5	TI_{50}
XLII-49-2	80	32	> 36	0.96	< 1	< 2.2
XLII-50-1	178	67	>> 67	0.69	< 1	< 2.7
58 ≡ XLII-50-1 [88]				3.9, 1.1 [a]		
XLII-50-2	182	36	>> 36	> 10	< 1	< 5
57 ≡ XLII-50-2 [88]				> 100, 84.4 [a]		
XLII-36-2	>>51	>>51	9.4	0.23	> 5.4	> 5.4
XLII-36-3	>>48	>>48	>>48	0.78	≈ 1	≈ 1
XLII-58-3	49.5	24	>34	0.08	< 1	< 1.5
XLII-76-2	47	28	10	0.45	2.8	4.7
XLII-76-3	40	13	>>54	2.5	< 1	< 1
XLII-110-2 [b]				0.9		
XLI-109-1	> 42	35	0.42 ± 0.15	0.14	83	> 100
XLI-109-1 [41]			1.0	0.05 [a]		
XLI-156-1	79		< 0.19	0.55		> 416
XLI-156-1 [42]				< 0.10 [a]		
XLI-161-1			0.086	0.43		
XLI-161-1 [42]				< 0.10 [a]		
L-CA [39,40]	333	264	4.2	0.53	63	79
26 [39]		47	4.6	0.73	10	

[a] IC_{50} for the strand transfer step; [b] Remaining results are pending;
Refer to Tables 1-1, 1-2, and 2-1 for definitions of terms.

The anti-HIV IN activities of the diketo acids XLII-49-2 and XLII-50-1 containing a simple catechol group are comparable to those of compounds containing a caffeoyl group, but the fact that both XLII-49-2 and XLII-50-1 are inactive while two caffeoyl-containing compounds (XLII-36-2 and XLII-76-2) are active against HIV replication suggests that a caffeoyl group is preferred to a simple catechol group.

The fact that meta-isomer XLII-58-3 (one catechol) is even more potent an inhibitor of IN than XLII-36-2 (two catechols) and that the inhibitory activity of para-isomer XLII-76-2 (one catechol) against HIV replication is comparable to that of XLII-36-2 indicates that for the diketo acid/catechol hybrids, one catechol group is sufficient.

Methyl esters XLII-50-2, XLII-36-3, and XLII-76-3 are generally less active than their corresponding acids against either HIV replication or HIV IN, which illustrates again that blocking the carboxylic acid decreases potency and was consistent with the literature result [88] that the methyl ester **57**≡ XLII-50-2 was less active than the acid **58** ≡ XLII-50-1. However, caffeoyl-containing XLII-36-3 and XLII-76-3 are still potent inhibitors of HIV IN, which indicates that the carboxylic group is not the important pharmacophore for the diketo acid/caffeoyl hybrids.

The biological activities of XLII-50-1, XLII-50-2, XLI-109-1, XLI-156-1 and XLI-161-1 are comparable to those in the literature [42,88,121] taking into account variations in the bioassay systems.

4.9. Experimental

NMR Spectra: Although both the ketoenol and the diketo forms of the fully blocked diketo acids and the free diketo acids were often observed by ^{1}H NMR (≥ 90% ketoenol), no attempt was made to record them, so only the peaks for the ketoenol 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 ketoenol form and the methylenic protons signal of the diketo form.

General: Materials and methods are the same as described in Section 2.7. The sources of additional chemicals used are listed below.

Compound	Source	Cat. No.
2-Acetylpyrrole	Aldrich	24,735-9
Sodium hydride, 60% dispersion in mineral oil	Aldrich	45,291-2
4-Fluorobenzyl bromide	Aldrich	20,953-8
1,2-Dimethoxyethane (DME)	Aldrich	25,952-7
Dimethyl oxalate	Aldrich	13,562-3
3-Bromo-4-fluorobenzaldehyde	Aldrich	33,954-7
Phenylmagnesium bromide, 3.0*M* solution in diethyl ether	Aldrich	17,156-5
Zinc cyanide	Aldrich	25,649-8
Tetrakis (triphenylphosphine) palladium (0)	Aldrich	21,666-6
Potassium bis(trimethylsilyl)amide, 0.5*M* solution in toluene	Aldrich	27,730-4
Ammonium chloride	Aldrich	21,333-0
Methylmagnesium iodide, 3.0*M* solution in diethyl ether	Aldrich	25,436-3

Sodium methoxide	Aldrich	16,499-2
3',4'-Dimethoxyacetophenone	TCI America	D1878
Boron tribromide, 1.0*M* solution in dichloromethane	Aldrich	21,122-2
3,4-Dimethoxybenzaldehyde	Aldrich	14,375-8
Magnesium, turnings	Aldrich	20,090-5
Ethyl alcohol (absolute)	AAPER	00D13QA
Isopropanol	AAPER	06B13UIPA
Carbon tetrachloride, anhydrous	Aldrich	28,911-6
Dimethyl malonate	Aldrich	24,104-0
Diethyl malonate	Aldrich	D9,775-4
3,5-Dinitrobenzoyl chloride	Aldrich	15,627-2
Diethyl oxalacetate, sodium salt, 95%	Aldrich	17,126-3
Tin (II) chloride dihydrate	Aldrich	24,352-3
3,4-Dimethoxycinnamic acid	Aldrich	D13,380-9
Sodium *tert*-butoxide	Aldrich	35,927-0
4'-Aminoacetophenone	Aldrich	A3,800-2
3'-Aminoacetophenone	Aldrich	13,935-1
2'-Aminoacetophenone	Aldrich	A3,780-4

Other general chemical solvents and reagents are the same as described in Section 2.7.

4.9.1. 4-(3,4-Dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid

Methyl 4-(3,4-dimethoxyphenyl)-2-hydroxy-4-oxo-2-butenoate (XLII-46-2) To a solution of 3.05 g (16.9 mmol) of 3,4-dimethoxyacetophenone and 3.62 g (30.7 mmol) of dimethyl oxalate in 150 mL of THF at room temperature was added 2.45 g (42.2 mmol) of

sodium methoxide. The resulting mixture was stirred at room temperature for 2 h, and then poured into 80 mL of 2 M HCl solution. The resulting mixture was extracted with ethyl acetate three times. The organic extracts were combined, washed with brine, and dried over $MgSO_4$. After removal of solvent, 4.42 g of crude product was obtained as a yellow solid, which was recrystallized from chloroform to give 3.68 g (82%) of XLII-46-2 as a yellow solid, mp 151-152.5 ^oC (lit. [112] 150-151 ^oC). ^1H NMR ($CDCl_3$) 7.64 (dd, J = 8.5, 2.1 Hz, 1H), 7.53 (d, J = 2.1 Hz, 1H), 7.04 (s, 1H), 6.93 (d, J = 8.5 Hz, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.94 (s, 3H), no detectable signals from the diketo form; lit. [112] ^1H NMR (DMSO-d_6) 7.77 (dd, J = 8.6, 2.2 Hz, 1H), 7.52 (d, J = 2.2 Hz, 1H), 7.11 (s, 1H), 7.10 (d, J = 8.6 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), detectable signals of the diketo form: 7.63 (br d, J = 8.7 Hz, 1H), 7.42 (br s, 1H), 7.08 (d, J = 8.7 Hz, 1H), 4.56 (s, 2H), 3.81 (s, 3H), 3.77 (s, 3H); ^{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; lit. [112] ^{13}C NMR (DMSO-d_6) 190.5, 166.7, 162.3, 154.2, 148.9, 127.1, 123.2, 111.3, 110.0, 98.1, 55.9, 55.6, 53.0. Anal. Calcd for $C_{13}H_{14}O_6$: C, 58.63; H, 5.30. Found: C, 58.36; H, 5.26.

4-(3,4-Dihydroxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLII-50-1) and its methyl ester (XLII-50-2) To a solution of 0.15 g (0.56 mmol) of XLII-46-2 in 10 mL of CH_2Cl_2 was added 17.0 mL of 1 M (17.0 mmol) BBr_3 in CH_2Cl_2. The resulting mixture was stirred overnight at room temperature. After the mixture was cooled to –78 ^oC, the reaction was quenched by adding 50 mL of 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 0.21 g (> 100%) of crude product as a red-yellow solid, half of which was purified by

HPLC (CH_3CN/H_2O/HOAc = 40/60/1) to give 39.3 mg (60%) of XLII-50-1 (Ret. time = 4.23 min) as a yellow solid, mp 198 °C (dec.) [lit. [88] 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, J = 8.5, 2.1 Hz, 1H), 7.45 (d, J = 2.1 Hz, 1H), 6.96 (s, 1H), 6.88 (d, J = 8.5 Hz, 1H), detectable signals of the diketo form (7%): 7.38 (br d, J = 8.5 Hz, 1H), 7.34 (d, J = 2.1 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 4.42 (s, 2H); ^{1}H NMR data of the ketoenol form matched literature [88] values ± 0.03 ppm, reported [88] detectable signals of the diketo form (7%): 10.21 (br s, 1H), 9.55 (br s, 1H), 7.34 (d, 1H), 7.31 (s, 1H), 6.81 (d, J = 8.6 Hz, 1H), 4.39 (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; ^{13}C NMR data matched literature [88] values ± 0.1 ppm. Anal. Calcd for $C_{10}H_8O_6$: C, 53.56; H, 3.60. Found: C, 53.36; H, 3.85.

Following exactly the same procedure on a two-fold scale gave 0.32 g (> 100%) of crude product as a red-yellow solid whose NMR was identical to that above. A methanol solution after three weeks gave the methyl ester which was purified by flash chromatography (EtOAc/hexanes = 1/1, R_f = 0.38) to give 0.14 g (52% from XLII-46-2) of XLII-47-2 as a yellow solid. 70.0 mg of XLII-47-2 was further purified by HPLC (MeOH/H_2O/HOAc = 70/30/1) to give 56.2 mg (80% recovery) of XLII-50-2 (Ret. time = 4.69 min) as a yellow solid, mp 165-168 °C [lit. [88] 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, J = 8.5, 2.3 Hz, 1H), 7.46 (d, J = 2.1 Hz, 1H), 6.99 (s, 1H), 6.89 (d, J = 8.5 Hz, 1H), 3.85 (s, 3H), detectable signals of the diketo form (8%): 10.10 (br s, 1H), 9.49 (br s, 1H), 7.37 (dd, J = 8.5, 2.2 Hz, 1H), 7.33 (d, J = 2.2 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 4.46 (s, 2H), 3.77 (s, 3H); ^{1}H NMR data of the ketoenol form matched literature [88] values ± 0.05 ppm, reported [88]

detectable signals of the diketo form (8%): 10.26 (br s, 1H), 9.57 (br s, 1H), 7.35 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.30 (d, *J* = 2.0 Hz, 1H), 6.84 (d, *J* = 8.1 Hz, 1H), 4.43 (s, 2H), 3.75 (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; ^{13}C NMR data matched literature [88] values ± 0.2 ppm. Anal. Calcd for $C_{11}H_{10}O_6$: C, 55.45; H, 4.23. Found: C, 55.52; H, 4.35.

4.9.2. 6-(3,4-Dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoic acid

(*E*)-4-(3,4-Dimethoxyphenyl)-3-buten-2-one [114] (XLII-48-2) To 6.10 g (36.8 mmol) of 3,4-dimethoxybenzaldehyde dissolved in 115 mL of ethanol and 115 mL of H_2O were slowly added from a dropping funnel 13.0 mL (0.18 mol) of acetone and then 45.0 mL of 10% (0.11 mol) aqueous NaOH solution. After the resulting mixture was stirred at room temperature for 75 min, it was neutralized with 2 M HCl to pH = 7, and extracted with $CHCl_3$. The organic extracts were combined, washed with brine, dried over Na_2SO_4, and evaporated to give crude product as a yellow residue, which was crystallized from diethyl ether and hexanes (v/v = 1/1) to give 6.61 g (87%; lit. 52% [113], 96% [114]) of XLII-48-2 as a yellow solid, mp 83-85 ^{o}C (lit. mp 83.5-84.0 ^{o}C [113], 85 ^{o}C [114]). ^{1}H NMR ($CDCl_3$) 7.47 (d, *J* = 16.1 Hz, 1H), 7.13 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.08 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 8.2 Hz, 1H), 6.61 (d, *J* = 16.1 Hz, 1H), 3.92 (s, 6H), 2.37 (s, 3H); lit. [113] ^{1}H NMR ($CDCl_3$) 7.50 (d, *J* = 15 Hz, 1H), 7.30-6.75 (m, 3H), 6.60 (d, *J* = 15 Hz, 1H), 3.92 (s, 6H), 2.36 (s, 3H); ^{13}C NMR ($CDCl_3$) 198.3, 151.3, 149.2, 143.5, 127.3, 125.2, 123.0, 111.1, 109.6, 56.0, 55.9, 27.3.

Methyl 6-(3,4-dimethoxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoate (XLII-48-3) To a solution of 1.61 g (7.82 mmol) of XLII-48-2 and 2.05 g (17.4 mmol) of dimethyl oxalate in

60 mL of THF at room temperature was added 1.64 g (30.4 mmol) of CH_3ONa. The resulting mixture was stirred at room temperature for 2 h, and then poured into 60 mL of 2 M HCl solution. The resulting mixture was extracted three times with ethyl acetate. The organic extracts were combined, washed with brine, and dried over Na_2SO_4. After removal of solvent, 2.23 g (98%) of XLII-48-3 was obtained as a red-orange solid, mp 121-123 ^oC. ^1H NMR ($CDCl_3$) 7.70 (d, J = 15.8 Hz, 1H), 7.18 (dd, J = 8.5, 2.1 Hz, 1H), 7.09 (d, J = 2.1 Hz, 1H), 6.90 (d, J = 8.5 Hz, 1H), 6.56 (s, 1H), 6.55 (d, J = 15.3 Hz, 1H), 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.

6-(3,4-Dihydroxyphenyl)-(5*E*)-ene-2-hydroxy-4-oxo-2-hexenoic acid (XLII-49-2) To a solution of 0.40 g (1.4 mmol) of XLII-48-3 in 15 mL of CH_2Cl_2 was added 40.0 mL of 1 M (40.0 mmol) BBr_3 in CH_2Cl_2. The resulting mixture was stirred overnight at room temperature. After the mixture was cooled to –78 ^oC, the reaction was quenched by adding 100 mL of H_2O. The resulting mixture was extracted three times with ethyl acetate. The organic extracts were combined, dried over Na_2SO_4 and evaporated with a Rotavapor to give 0.27 g (79%) of crude product as a red-yellow solid, half of which was purified by HPLC (CH_3CN/H_2O/HOAc = 40/60/1) to give 64.2 mg (38%) of XLII-49-2 (Ret. time = 4.42 min) as a red-yellow solid, mp 184 ^oC (dec.). ^1H NMR (CD_3OD) 7.65 (d, J = 15.2 Hz, 1H), 7.11 (br s, 1H), 7.04 (br d, J = 7.0 Hz, 1H), 6.80 (d, J = 7.3 Hz, 1H), 6.61 (d, J = 15.5 Hz, 1H), 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 $C_{12}H_{10}O_6 \cdot 0.25\ H_2O$: C, 56.58; H, 4.16. Found: C, 56.95; H, 4.55.

4.9.3. 4-(x-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acids (x = 4, 3 or 2)

4-(3,4-Dimethoxycarbonylcaffeoylamino)acetophenone (XLII-52-4) To a solution of 3,4-dimethoxycarbonylcaffeoyl chloride [made from 2.76 g (9.32 mmol) of 3,4-dimethoxycarbonylcaffeic acid by the same procedure as XLI-100-1A (see Chapter 2, section 2.7.1.)] in 40 mL of toluene was added 12 mL of pyridine. After the resulting mixture was stirred for 10 min at room temperature, it was treated with a solution of 0.68 g (5.0 mmol) of 4'-aminoacetophenone in 2 mL of pyridine and 5 mL of toluene. After the mixture was stirred overnight at room temperature, it turned into a brown residue (2.11 g), which stuck to the flask, and a yellow solution. The yellow solution was diluted with 60 mL of EtOAc, washed successively with 10% HCl, 5% $NaHCO_3$, and saturated NaCl. The organic layer was dried over Na_2SO_4. The solvent was evaporated to give 0.62 g of yellow residue. The brown and yellow residues were combined and purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.30) to give 1.77 g (85%) of XLII-52-4 as a white solid, mp 173-174 ^oC. ^1H NMR (DMSO-d_6) 10.63 (br s, 1H), 7.98 (d, J = 8.5 Hz, 2H), 7.85 (d, J = 8.8 Hz, 2H), 7.76 (d, J = 1.8 Hz, 1H), 7.66 (dd, J = 8.8, 2.1 Hz, 1H), 7.65 (d, J = 15.5 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 6.87 (d, J = 15.5 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (DMSO-d_6) 196.6, 163.7, 152.6, 152.5, 143.5, 142.8, 142.3, 139.0, 134.0, 131.9, 129.6 (2), 126.7, 124.0, 123.5, 122.4, 118.6 (2), 56.1 (2), 26.5. Anal. Calcd for $C_{21}H_{19}NO_8$: C, 61.02; H, 4.63; N, 3.39. Found: C, 61.09; H, 4.61; N, 3.34.

3-(3,4-Dimethoxycarbonylcaffeoylamino)acetophenone (XLII-55-4) This compound was synthesized from 3'-aminoacetophenone by the same procedure as XLII-52-4 with exactly the same amounts of reagents. The brown (1.87 g) and yellow residues (1.51 g) were combined and purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.35) to give 1.69 g (81%) of XLII-55-4 as a white solid, mp 144-145 ^{o}C. ^{1}H NMR (DMSO-d_6) 10.51 (br s, 1H), 8.28 (br d, *J* = 1.5 Hz, 1H), 7.98 (ddd, *J* = 7.9, 1.2, 0.9 Hz, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.70 (br dd, *J* = 7.6, 0.9 Hz, 1H), 7.65 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.63 (d, *J* = 15.8 Hz, 1H), 7.52 (d, *J* = 8.5 Hz, 1H), 7.51 (dd, *J* = 7.6, 7.6 Hz, 1H), 6.84 (d, *J* = 15.8 Hz, 1H), 3.88 (s, 3H), 3.87 (s, 3H), 2.59 (s, 3H); ^{13}C NMR (DMSO-d_6) 197.5, 163.4, 152.5, 152.4, 142.6, 142.2, 139.4, 138.5, 137.3, 133.9, 129.2, 126.5, 123.8, 123.6, 123.5 (2), 122.2, 118.3, 56.0 (2), 26.7. The peak at 123.5 ppm was resolved into two peaks at 123.8 and 123.7 ppm by changing the solvent to CD_3OD/DMSO-d_6 = 1/2. Anal. Calcd for $C_{21}H_{19}NO_8 \cdot 0.25H_2O$: C, 60.36; H, 4.70; N, 3.35. Found: C, 60.33; H, 4.69; N, 3.30.

4-Caffeoylaminoacetophenone (XLII-53-1) To a solution of 0.20 g (0.48 mmol) of XLII-52-4 and 0.12 g (1.0 mmol) of dimethyl oxalate in 30 mL of THF at room temperature was added 0.12 g (2.2 mmol) of sodium methoxide. The resulting mixture was stirred at room temperature for 4.5 h, and then poured into 40 mL of 2 M HCl solution. The resulting mixture was extracted three times with ethyl acetate. The organic extracts were combined and dried over Na_2SO_4. Removal of solvent with a Rotavapor gave 0.18 g (100%) of XLII-53-1 as a yellow solid, mp 213 ^{o}C (dec.). ^{1}H NMR (DMSO-d_6) 10.45 (br s, 1H), 7.97 (d, *J* = 8.5 Hz, 2H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 15.5 Hz, 1H), 7.06 (br s, 1H), 6.96 (br d, *J* = 8.5 Hz, 1H), 6.82 (d, *J*

= 7.9 Hz, 1H), 6.60 (d, *J* = 15.5 Hz, 1H), 2.55 (s, 3H); ^{13}C NMR (DMSO-d_6) 196.6, 164.6, 148.1, 145.7, 143.9, 141.8, 131.6, 129.6 (2), 126.1, 121.2, 118.4 (2), 118.0, 115.9, 114.1, 26.5.

3-Caffeoylaminoacetophenone (XLII-56-2) To a solution of 0.30 g (0.73 mmol) of XLII-55-4 and 0.20 g (1.7 mmol) of dimethyl oxalate in 30 mL of THF at room temperature was added 0.28 g (2.9 mmol) of sodium *tert*-butoxide. The resulting mixture was stirred at room temperature for 3 h, and then poured into 60 mL of 2 M HCl solution. The resulting mixture was extracted three times with ethyl acetate. The organic extracts were combined and dried over Na_2SO_4. Removal of the solvent gave 0.42 g of crude product as a yellow residue, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.19) to give 0.11 g (50%) of XLII-56-2 as a yellow solid, mp 204 ^{o}C (dec.). ^{1}H NMR (CD_3OD) 8.30 (dd, *J* = 2.1, 1.5 Hz, 1H), 7.90 (ddd, *J* = 7.9, 1.2, 0.9 Hz, 1H), 7.71 (ddd, *J* = 7.9, 1.5, 0.9 Hz, 1H), 7.54 (d, *J* = 15.5 Hz, 1H), 7.45 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.06 (d, *J* = 2.1 Hz, 1H), 6.96 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.55 (d, *J* = 15.5 Hz, 1H), 2.60 (s, 3H); ^{13}C NMR (CD_3OD) 200.1, 167.5, 149.2, 146.8, 143.9, 140.8, 139.0, 130.2, 128.1, 125.7, 124.9, 122.5, 120.7, 118.4, 116.5, 115.2, 26.8.

4-(3,4-dimethoxycinnamylamino)acetophenone (XLII-54-1 ≡ XLII-75-1) A mixture of 2.00 g (9.62 mmol) of 3,4-dimethoxycinnamic acid and 20 mL of thionyl chloride was refluxed for 1.5 h. The extra thionyl chloride was removed with a Rotavapor to give a yellow solid, which was dissolved in 80 mL of toluene. To the resulting solution was added 12 mL of pyridine followed by a solution of 0.68 g (5.0 mmol) of 4'-aminoacetophenone in 2 mL of pyridine and 5 mL of toluene. The mixture was stirred overnight at room temperature, diluted with 150 mL of

EtOAc, and then washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl sequentially. The organic solution was dried over Na_2SO_4 and the solvent evaporated to give the crude product as a pale yellow solid which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/1, R_f = 0.27) to give 1.31 g (80%) of XLII-54-1≡XLII-75-1 as a white solid, mp 194-196 ^oC. ^1H NMR (DMSO) 10.48 (br s, 1H), 7.96 (d, *J* = 9.1 Hz, 2H), 7.83 (d, *J* = 8.8 Hz, 2H), 7.59 (d, *J* = 15.5 Hz, 1H), 7.24 (d, *J* = 1.8 Hz, 1H), 7.22 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 15.5 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 2.54 (s, 3H); ^{13}C NMR (DMSO) 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.

3-(3,4-Dimethoxycinnamylamino)acetophenone (XLII-57-2) This compound was prepared as described for XLII-54-1 on a two-fold scale using 3-aminoacetophenone. The crude product was recrystallized from ethyl acetate to give 2.81 g (86%) of XLII-57-2 as a white solid, mp 150-152 ^oC. ^1H NMR (DMSO-d_6) 10.36 (br s, 1H), 8.28 (dd, *J* = 1.8, 1.8 Hz, 1H), 7.98 (ddd, *J* = 7.9, 1.2, 0.9 Hz, 1H), 7.68 (br dd, *J* = 7.6, 1.2 Hz, 1H), 7.57 (d, *J* = 15.5 Hz, 1H), 7.50 (dd, *J* = 7.9, 7.9 Hz, 1H), 7.23 (d, *J* = 1.5 Hz, 1H), 7.21 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.03 (d, *J* = 8.2 Hz, 1H), 6.71 (d, *J* = 15.8 Hz, 1H), 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.

2-(3,4-Dimethoxycinnamylamino)acetophenone (XLII-77-3) This compound was prepared as described for XLII-54-1 on a two-fold scale using 2-aminoacetophenone. The crude product was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/2, R_f = 0.22) to give 2.97 g (91%) of XLII-77-3 as a white solid, mp 142-144 ^{o}C. ^{1}H NMR (DMSO-d_6) 11.51 (br s, 1H), 8.54 (br d, *J* = 8.5 Hz, 1H), 8.03 (br d, *J* = 7.9 Hz, 1H), 7.64 (br dd, *J* = 7.9, 7.6 Hz, 1H), 7.58 (d, *J* = 15.2 Hz, 1H), 7.40 (br s, 1H), 7.25 (br dd, *J* = 8.2, 7.3 Hz, 1H), 7.22 (br d, *J* = 7.9 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.81 (d, *J* = 15.5 Hz, 1H), 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.

Methyl 4-[x-(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (x = 4, 3 or 2) (XLII-72-3 ≡ XLII-75-2, XLII-57-4, and XLII-80-2) To a solution of the appropriate acetophenone (XLII-54-1 ≡ XLII-75-1, XLII-57-2, or XLII-77-3) and dimethyl oxalate in THF at room temperature was added sodium *tert*-butoxide. The resulting mixture was stirred at room temperature for 2 h, and then poured into 2 M HCl. The resulting mixture was extracted with ethyl acetate for three times. The organic extracts were combined, washed with brine, and dried over Na_2SO_4. Removal of solvent gave the crude products as yellow solids. Specific amounts are given in the following Table.

Reactant	Wt. (g)	$(COOMe)_2$ (g)	THF (mL)	*t*-BuONa (g)	2M HCl (mL)	Product	Wt. (g)	Yield (%)
XLII-54-1	0.11	0.08	20	0.13	20	XLII-72-3	0.14	93
XLII-75-1	0.55	0.40	40	0.65	100	XLII-75-2	0.67	96
XLII-57-2	0.50	0.37	30	0.59	50	XLII-57-4	0.56	89
XLII-77-3	0.50	0.37	30	0.59	50	XLII-79-1	0.60	86
XLII-77-3	1.33	0.99	50	1.57	60	XLII-80-2	1.58	94

Methyl 4-[4-(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (XLII-72-3 ≡ XLII-75-2) The pure product was obtained as a yellow solid by washing the crude product with 3-5 mL of ethyl acetate, mp 206 ^{o}C (dec.). ^{1}H NMR (DMSO-d_6) 10.61 (br s, 1H), 8.10 (d, J = 9.1 Hz, 2H), 7.90 (d, J = 8.8 Hz, 2H), 7.60 (d, J = 15.5 Hz, 1H), 7.24 (br s, 1H), 7.22 (dd, J = 7.9, 1.8 Hz, 1H), 7.12 (s, 1H), 7.03 (d, J = 8.2 Hz, 1H), 6.73 (d, J = 15.8 Hz, 1H), 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.

Methyl 4-[3-(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (XLII-57-4) The pure product was obtained as a yellow solid by washing the crude product with 3-5 mL of ethyl acetate, mp 148 ^{o}C (dec.). ^{1}H NMR (DMSO-d_6) 10.43 (br s, 1H), 8.41 (br s, 1H), 8.03 (br d, J = 7.6 Hz, 1H), 7.77 (br d, J = 7.6 Hz, 1H), 7.58 (d, J = 15.8 Hz, 1H), 7.54 (dd, J = 7.9, 7.9 Hz, 1H), 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, J = 8.2 Hz, 1H), 6.70 (d, J = 15.5 Hz, 1H), 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.

Methyl 4-[2-(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (XLII-80-2) The pure product XLII-80-2 was obtained by recrystallization from CH_2Cl_2 and EtOAc, mp 89 ^oC (dec.). ^1H NMR ($CDCl_3$) 11.47 (br s, 1H), 8.90 (dd, *J* = 8.8, 1.2 Hz, 1H), 7.91 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.71 (d, *J* = 15.5 Hz, 1H), 7.63 (ddd, *J* = 8.8, 7.3, 1.5 Hz, 1H), 7.21-7.14 (m, 2H), 7.17 (s, 1H), 7.12 (d, *J* = 2.1 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.47 (d, *J* = 15.5 Hz, 1H), 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.

4-(x-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acids (x = 4 or 3) (XLII-76-2 and XLII-58-3) and the methyl ester of the 4-isomer (XLII-76-3) To a mixture of methyl 4-[x-(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (x = 4 or 3) (XLII-72-3 ≡ XLII-75-2 or XLII-57-4) in CH_2Cl_2 at room temperature was added 1 M BBr_3 in CH_2Cl_2. The resulting mixture was stirred as described in the Table below. After the mixture was cooled to –78 ^oC, the reaction was quenched by adding H_2O. The resulting suspension was filtered to give the crude product as a brown solid. Specific amounts are given in the following Table.

Reactant	Wt. (mg)	CH_2Cl_2 (mL)	1 M BBr_3 (mL)	Condition	Crude Product	Wt. (mg)	Components
XLII-72-3	80	50	5.7	rt, 0.5 h Reflux, ON	XLII-74-1	76	Acid and ester
XLII-75-2	250	100	18.3	rt, 22 h	XLII-76-1	200	Acid and ester
XLII-57-4	100	20	7.2	rt, 62 h	XLII-58-1	85	Acid
XLII-57-4	100	20	7.2	rt, 0.5 h Reflux, ON	XLII-58-2	86	Acid

rt = room temperature; ON = overnight

4-(4-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLII-76-2) and its methyl ester (XLII-76-3) A 60 mg portion of crude product (XLII-76-1) was separated by HPLC (CH_3CN/H_2O/AcOH = 65/35/1) to give 23.5 mg (35%) of XLII-76-2 (Ret. time = 3.92 min) and 17.3 mg (25%) of XLII-76-3 (Ret. time = 6.53 min) as yellow solids.

XLII-76-2: mp 210 ^oC (dec.). ^1H NMR (DMSO-d_6) 10.56 (br s, 1H), 9.59 (br s, 1H), 9.29 (br s, 1H), 8.09 (d, J = 8.8 Hz, 2H), 7.88 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 15.5 Hz, 1H), 7.09 (s, 1H), 7.04 (d, J = 1.8 Hz, 1H), 6.95 (dd, J = 8.2, 1.8 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 15.5 Hz, 1H). ^{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.

XLII-76-3: mp 196 ^oC (dec.). ^1H NMR (DMSO-d_6) 10.57 (br s, 1H), 9.60 (br s, 1H), 9.29 (br s, 1H), 8.10 (d, J = 8.8 Hz, 2H), 7.89 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 15.5 Hz, 1H), 7.13 (s, 1H), 7.04 (d, J = 1.5 Hz, 1H), 6.95 (dd, J = 8.2, 1.8 Hz, 1H), 6.80 (d, J = 7.9 Hz, 1H), 6.58 (d, J = 15.5 Hz, 1H), 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.

4-(3-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLII-58-3) A 34 mg portion of crude product (XLII-58-2) was separated by HPLC (CH_3CN/H_2O/AcOH = 80/20/1) to give 22 mg (62%) of XLII-58-3 (Ret. time = 3.37 min) as a yellow solid, mp 137 ^oC (dec.). ^1H NMR (DMSO-d_6) 10.41 (br s, 1H), 8.43 (br s, 1H), 8.02 (br d, J = 8.2 Hz, 1H), 7.77 (br d, J = 7.9 Hz, 1H), 7.55 (dd, J = 8.2, 7.9 Hz, 1H), 7.48 (d, J = 15.8 Hz, 1H), 7.08 (s, 1H), 7.06 (br s, 1H), 6.96 (br d, J = 8.2 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.57 (d, J = 15.5 Hz, 1H); ^{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 CD_3COCD_3/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.

2-(4-Caffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLII-110-2) A solution of 0.21 g (0.51 mmol) of XLII-80-2 in 12 mL of CH_2Cl_2 was cooled to -78 ^oC and then treated dropwise with 6.0 mL of 1.0 M (6.0 mmol) BBr_3 in CH_2Cl_2. After the resulting mixture was stirred at -78 ^oC 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 ^oC and the reaction quenched with 30 mL of iced H_2O. 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 0.16 g (84%) of crude product, to which was added 2 mL of acetone. The resulting suspension was sonicated for 5 min and filtered to give 0.13 g (68%) of XLII-110-2 as a yellow solid, mp 110 ^oC (dec.). ^1H NMR (THF-d_8) 11.40 (br s, 1H), 8.91 (br d, J = 7.6 Hz, 1H), 8.02 (br d, J = 9.2 Hz, 1H), 7.59 (br dd partially overlapped by the peak at 7.58 ppm, 1H), 7.58 (d, J =

15.5 Hz, 1H), 7.19 (s, 1H), 7.15 (br dd partially overlapped by the peaks at 7.19 and 7.11 ppm, 1H), 7.11 (d, *J* = 1.8 Hz, 1H), 6.98 (dd, *J* = 8.2, 2.1 Hz, 1H), 6.76 (d, *J* = 7.9 Hz, 1H), 6.50 (d, *J* = 15.5 Hz, 1H); ^{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.

4.9.4. 4-(3,5-Dicaffeoylaminophenyl)-2-hydroxy-4-oxo-2-butenoic acid and its methyl ester

3,5-Dinitroacetophenone [120] **(XLII-32-1)** To a stirred mixture of 6.07 g (0.25 mol) of magnesium turnings, 60 mL of absolute ethanol, and 2 mL of carbon tetrachloride was added 20 mL of chloroform. The mixture was heated under reflux for 2 h. To the mixture was then added a solution containing 36.80 g (0.23 mol) of diethyl malonate in 35 mL of chloroform. The mixture was heated under reflux for 3 h and then evaporated to dryness under reduced pressure. The oily residue was dissolved in 45 mL of chloroform and to this solution was added a solution containing 34.60 g (0.15 mol) of 3,5-dinitrobenzoyl chloride in 55 mL of chloroform. The reaction mixture was stirred at room temperature for 4 h and then cooled in an ice bath to 5 ^{o}C. To this solution was slowly added 110 mL of 2 M sulfuric acid. The chloroform layer was separated, and the aqueous layer was extracted three more times with chloroform. The chloroform extracts were combined and concentrated to dryness. To the oily residue was added 15 mL of water, 85 mL of glacial acetic acid, and 7 mL of concentrated sulfuric acid and the mixture was heated under reflux for 7 h. Most of the solvent was removed with a Rotavapor to give a tan residue, which was poured into 800 mL of iced water. The precipitate was collected by

filtration and recrystallized from carbon tetrachloride--ethanol to give 16.52 g (52%; lit. 70% [118]) of XLII-32-1 as a white solid, mp 80-82 °C (lit. 80-82 °C, [118] 81-83 °C [119]). ^{1}H NMR ($CDCl_3$) 9.22 (t, *J* = 2.2 Hz, 1H), 9.10 (d, *J* = 2.2 Hz, 2H), 2.85 (s, 3H); ^{13}C NMR ($CDCl_3$) 193.6, 148.9, 139.7 (2), 128.0 (2), 122.3, 26.9.

3,5-Diaminoacetophenone (XLII-33-1) To a solution of 35.20 g (0.16 mol) of tin (II) chloride dihydrate in 100 mL of conc HCl was added 5.00 g (24.0 mmol) of XLII-32-1. The mixture was stirred for 6 h at room temperature, and then cooled to 0 °C with an ice bath. The mixture was made strongly basic (pH > 13) with cold 50% aqueous sodium hydroxide. The resulting dark suspension was filtered, and the filtrate extracted three times with ethyl acetate. The organic extracts were combined, dried over sodium sulfate, filtered, and evaporated to give 2.32 g (65%) of XLII-33-1 as a pale yellow solid, mp 130-132 °C (lit. [116] 133-134 °C). ^{1}H NMR (CD_3COCD_3) 6.59 (d, *J* = 2.2 Hz, 2H), 6.23 (t, *J* = 2.2 Hz, 1H), 2.40 (s, 3H); ^{13}C NMR (CD_3COCD_3) 198.7, 150.1 (2), 139.8 (2), 105.4, 104.7 (2), 26.7.

3,5-Bis(3,4-dimethoxycinnamylamino)acetophenone (XLII-34-1) A mixture of 2.88 g (13.9 mmol) of 3,4-dimethoxycinnamic acid and 30 mL of thionyl chloride was refluxed for 2 h, and the extra thionyl chloride removed with a Rotavapor to give a yellow solid, which was dissolved in 50 mL of dichloromethane. To the resulting solution was added 5 mL of pyridine followed by a solution of 0.80 g (5.3 mmol) of XLII-33-1 in 4 mL of pyridine and 10 mL of dichloromethane. The mixture was stirred overnight at room temperature, and then poured into 120 mL of cold water. The precipitate was collected by filtration, dried in air, and then stirred in 100 mL of chloroform for 4 h. After filtration, 2.53 g (89%) of XLII-34-1 was obtained as a light

yellow powder, 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, J = 15.5 Hz, 2H), 7.24 (br s, 2H), 7.22 (br d partially overlapped by the peak at 7.24 ppm, 2H), 7.03 (d, J = 7.9 Hz, 2H), 6.79 (d, J = 15.5 Hz, 2H), 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.

Methyl 4-[3,5-bis(3,4-dimethoxycinnamylamino)phenyl]-2-hydroxy-4-oxo-2-butenoate (XLII-35-1) To a solution of 0.53 g (1.0 mmol) of XLII-34-1 and 0.24 g (2.0 mmol) of dimethyl oxalate in 60 mL of THF at room temperature was added 0.38 g (4.0 mmol) of sodium *tert*-butoxide. The resulting mixture was stirred at room temperature for 5 h, and then poured into 50 mL of 2 M aqueous HCl solution. The resulting mixture was extracted three times with ethyl acetate. The organic extracts were combined, washed with brine, and dried over Na_2SO_4. Removal of solvent gave 0.59 g (95%) of XLII-35-1 as a yellow solid, 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, J = 15.5 Hz, 2H), 7.24 (br s, 2H), 7.22 (br d partially overlapped by the peak at 7.24 ppm, 2H), 7.04 (d, J = 7.9 Hz, 2H), 6.99 (s, 1H), 6.75 (d, J = 15.5 Hz, 2H), 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 (XLII-36-2 ≡ XLII-37-1) and its methyl ester (XLII-36-3 ≡ XLII-37-2) To a mixture of 0.20 g (0.32 mmol) of XLII-35-1 in 60 mL of CH_2Cl_2 was added 16.0 mL of 1 M (16.0 mmol) BBr_3 in CH_2Cl_2. The resulting mixture was stirred at room temperature for 2 h, and then heated at reflux for 22 h. After the mixture was cooled to –78 ^oC, the reaction was quenched by adding 25 mL of H_2O. The resulting suspension was filtered to give 0.16 g of crude product as a brown solid, 60 mg of which was separated by HPLC (MeOH/H_2O/HOAc = 80/20/1) to give 16.5 mg (25%) of XLII-36-2 (Ret. Time = 3.98 min) and 7.7 mg (11%) of XLII-36-3 (Ret. Time = 5.89 min) as yellow solids. Another 85 mg of crude product was separated in the same way to give 28.2 mg (30%) of XLII-37-1 and 12.6 mg (13%) of XLII-37-2.

XLII-36-2 ≡ XLII-37-1: mp 213 ^oC (dec.); ^1H 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, J = 15.5 Hz, 2H), 7.05 (br s, 2H), 6.99 (s, 1H), 6.95 (br d, J = 8.2 Hz, 2H), 6.81 (d, J = 8.2 Hz, 2H), 6.58 (d, J = 15.5 Hz, 2H); ^{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 didn't show up, but it appeared 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.

XLII-36-3 ≡ XLII-37-2: mp 182 ^oC (dec.); ^1H 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, J = 1.8 Hz, 2H), 7.46 (d, J = 15.5 Hz, 2H), 7.04 (d, J = 2.1 Hz, 2H), 6.98 (s, 1H), 6.94 (dd, J = 8.2, 2.1 Hz, 2H), 6.80 (d, J = 8.2 Hz, 2H), 6.58 (d, J = 15.5 Hz, 2H), 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.

4.9.5. 4-[1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoic acid [121]

4-[1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl]ethanone (XLI-107-3) A solution of 1.09 g (0.01 mol) of 2-acetylpyrrole in 20 mL of DMF was treated with 0.48 g (0.012 mol, 60% dispersion in oil) of sodium hydride followed by 1.73 g (0.012 mol) of 4-fluorobenzyl bromide and stirred overnight at room temperature. The solution was poured into 300 mL of saturated $NaHCO_3$ and extracted three times with EtOAc. The combined organic layers were washed with $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated to give a clear yellow oil, which was purified on a silica gel column eluted with ethyl acetate and hexane (v/v = 1/5.5) to give 1.85 g (85%) of desired product XLI-107-3 (R_f = 0.36) and 0.24 g (13%) of a by-product XLI-107-2 (R_f = 0.49) as yellow oils.

XLI-107-3: ^{1}H NMR ($CDCl_3$) 7.10-7.05 (m, 2H), 6.98-6.95 (m, 3H), 6.93-6.87 (m, 1H), 6.18-6.15 (m, 1H), 5.50 (s, 2H), 2.37 (s, 3H); lit. [121] ^{1}H NMR ($CDCl_3$) 7.1 (m, 2H), 7.0 (m, 3H), 6.9 (m, 1H), 6.2 (m, 1H), 5.52 (s, 2H), 2.4 (s, 3H); ^{13}C NMR ($CDCl_3$) 188.2, 161.9 (d, J = 245.9 Hz), 133.9 (d, J = 3.1 Hz), 130.1, 130.0, 128.7 (d, J = 8.3 Hz, 2C), 120.4, 115.2 (d, J = 21.4 Hz, 2C), 108.5, 51.6, 27.0.

XLI-107-2: ^{1}H NMR ($CDCl_3$) 8.09 (s, 1H), 7.34 (dd, J = 8.7, 5.3 Hz, 2H), 7.03 (dd, J = 8.7, 8.7 Hz, 2H), 5.14 (s, 2H); ^{13}C NMR ($CDCl_3$) 162.6 (d, J = 247.3 Hz), 160.5, 131.1, 130.3 (d,

J = 8.3 Hz, 2C), 115.4 (d, *J* = 21.8 Hz, 2C), 64.7.

Methyl 4-[1-(4-fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoate (XLI-108-1) A solution of 1.07 g (4.94 mmol) of XLI-107-3 in 10 mL of DME was treated with 0.30 g (7.5 mmol, 60% dispersion in oil) of sodium hydride followed by 0.71 g (6.0 mmol) of dimethyl oxalate and a drop of methanol. The solution was heated to reflux overnight, the reaction mixture poured into 150 mL of saturated $NaHCO_3$ and extracted three times with EtOAc. The combined organic layers were washed with $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The crude product was crystallized with diethyl ether to give 1.05 g (70%) of XLI-108-1 as yellow-orange crystals, mp 104.5-106 ^{o}C (lit. [121] no reported mp). ^{1}H NMR ($CDCl_3$) 7.15 (dd, *J* = 4.1, 1.5 Hz, 1H), 7.10 (dd, *J* = 8.5, 5.6 Hz, 2H), 7.01-6.95 (m, 3H), 6.84 (s, 1H), 6.28 (dd, *J* = 4.1, 2.3 Hz, 1H), 5.59 (s, 2H), 3.89 (s, 3H); lit. [121] ^{1}H NMR ($CDCl_3$) 7.15 (dd, *J* = 4.21, 1.65 Hz, 1H), 7.10 (m, 2H), 7.0 (m, 3H), 6.84 (s, 1H), 6.28 (dd, *J* = 4.11, 2.57 Hz, 1H), 5.6 (s, 2H), 3.9 (s, 3H); ^{13}C NMR ($CDCl_3$) 184.3, 163.1, 162.2 (d, *J* = 245.9 Hz), 160.3, 133.5 (d, *J* = 2.9 Hz), 132.6, 129.3, 128.8 (d, *J* = 8.3 Hz, 2C), 121.4, 115.6 (d, *J* = 21.6 Hz, 2C), 110.1, 101.8, 53.0, 52.2.

4-[1-(4-Fluorobenzyl)-1*H*-pyrrol-2-yl]-2-hydroxy-4-oxo-2-butenoic acid (XLI-109-1 ≡ XLI-109-3) A solution of 0.19 g (0.63 mmol) of XLI-108-1 was dissolved in 6 mL of THF/MeOH (1:1) and treated with 3 mL of 1 M (3 mmol) NaOH and stirred overnight. The reaction mixture was diluted with 15 mL of H_2O, washed with diethyl ether, acidified to pH = 2 with 1 M HCl and then extracted three times with EtOAc. The organic layers were combined, washed with 1 M HCl, dried over Na_2SO_4, filtered and evaporated to dryness. The crude product was recrystallized from $CHCl_3$ to give 0.12 g (67%) of XLI-109-3 as yellow crystals, mp 155-

157 °C (dec.) [lit. [121] 172 °C (dec.)]. Sample (XLI-109-1; 46 mg, 30%) for bioassay was obtained by the same procedure from 0.16 g of XLI-108-1. ^{1}H NMR ($CDCl_3$) 7.20 (dd, *J* = 4.2, 1.5 Hz, 1H), 7.13-7.06 (m, 3H), 7.00 (dd, *J* = 8.7, 8.7 Hz, 2H), 6.92 (s, 1H), 6.32 (dd, *J* = 4.2, 2.7 Hz, 1H), 5.59 (s, 2H); lit. [121] ^{1}H NMR ($CDCl_3$) 7.2 (dd, *J* = 4.21, 1.65 Hz, 1H), 7.09 (m, 3H), 7.0 (m, 2H), 6.86 (s, 1H), 6.3 (dd, *J* = 4.21, 2.56 Hz, 1H), 5.58 (s, 2H); ^{13}C NMR (CD_3COCD_3) 185.5, 163.6, 162.9 (d, *J* = 243.6 Hz), 162.3, 135.6 (d, *J* = 3.5 Hz), 134.6, 129.85, 129.84 (d, *J* = 8.1 Hz, 2C), 122.6, 116.0 (d, *J* = 21.9 Hz, 2C), 110.9, 101.9, 52.6.

4.9.6. 4-(5-Benzyl-2-isopropoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid and 4-(5-benzyl-2-ethoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid

1-Benzyl-3-bromo-4-fluorobenzene (XLI-150-3) To a cold (0 °C) solution of 5.00 g (24.6 mmol) of 3-bromo-4-fluorobenzaldehyde in 60 mL of THF under an atmosphere of nitrogen was added 10 mL of a solution of 3 M phenylmagnesium bromide (0.03 mol) in diethyl ether. The resulting solution was stirred at room temperature for 5.5 h, and treated with 10% HCl. The resulting mixture was diluted with 60 mL of ethyl acetate, and then washed with 10% HCl. The organic extract was dried over sodium sulfate, filtered, and concentrated with a Rotavapor to provide 8.30 g of crude **(3-bromo-4-fluorophenyl)phenylmethanol (XLI-150-1)** which was used directly.

To a cold (0 °C) solution of XLI-150-1 and 20.10 g (0.17 mol) of triethylsilane in 80 mL of dichloromethane, 6 mL of boron trifluoride diethyl etherate was added dropwise. The resulting mixture was stirred at 0 °C for 2 h, and neutralized with 5% sodium bicarbonate. The organic extract was washed with brine, dried over sodium sulfate, filtered, and concentrated with a

Rotavapor to give a yellow liquid, which was purified on a silica gel column eluted with hexanes to give 4.24 g (65%) of XLI-150-3 (R_f = 0.45) as a colorless liquid. ^{1}H NMR ($CDCl_3$) 7.33-6.92 (m, 8H), 3.85 (s, 2H); lit. [42] ^{1}H NMR ($CDCl_3$) 7.4-6.9 (m, 8H), 3.93 (s, 2H); ^{13}C NMR ($CDCl_3$) 157.6 (d, *J* = 245.7 Hz), 140.0, 138.5 (d, *J* = 3.9 Hz), 133.6, 129.2 (d, *J* = 7.1 Hz), 128.8 (2), 128.6 (2), 126.4, 116.2 (d, *J* = 22.5 Hz), 108.8 (d, *J* = 21.2 Hz), 40.7.

5-Benzyl-2-fluorobenzonitrile (XLI-151-3) A mixture of 3.29 g (12.4 mmol) of XLI-150-3 and 5.00 g (42.6 mmol) of zinc cyanide in 25 mL of anhydrous DMF was purged with a steady stream of nitrogen for 45 min and then 1.60 g of tetrakis (triphenylphosphine) palladium (0) was added. The resulting mixture was stirred at 95 ^{o}C for 2 days under an atmosphere of nitrogen. The resulting mixture was diluted with 40 mL of ethyl acetate, washed successively with water, aqueous HCl, and brine. The organic extract was dried over sodium sulfate, filtered, and concentrated with a Rotavapor to give a yellow liquid, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/40) to give 1.88 g (72%) of XLI-151-3 (R_f = 0.30 with petroleum ether/Et_2O = 10/1) as a colorless liquid and 0.36 g (12%) of a by-product identified as 5-benzyl-2-(N,N-dimethylamino)benzonitrile (XLI-151-4, R_f = 0.19 with petroleum ether/Et_2O = 10/1) as a yellow liquid.

XLI-151-3: ^{1}H NMR ($CDCl_3$) 7.40-7.02 (m, 8H), 3.93 (s, 2H); lit. [42] ^{1}H NMR ($CDCl_3$) 7.45-7.10 (m, 8H), 3.97 (s, 2H); ^{13}C NMR ($CDCl_3$) 161.7 (d, *J* = 257.6 Hz), 139.3, 138.4 (d, *J* = 3.9 Hz), 135.5 (d, *J* = 8.2 Hz), 133.3, 128.9 (4), 126.8, 116.4 (d, *J* = 19.6 Hz), 114.0, 101.2 (d, *J* = 14.7 Hz), 40.6.

XLI-151-4: ^{1}H NMR ($CDCl_3$) 7.28-7.11 (m, 7H), 6.78 (d, *J* = 8.7 Hz, 1H), 3.83 (s, 2H),

2.94 (s, 6H); ^{13}C NMR ($CDCl_3$) 153.8, 140.3, 134.6, 134.1, 132.3, 128.7 (2), 128.6 (2), 126.3, 119.5, 117.1, 101.6, 43.0 (2), 40.4.

5-Benzyl-2-isopropoxybenzonitrile (XLI-152-2) and 5-benzyl-2-ethoxybenzonitrile (XLI-153-2) General procedure: To a mixture of 0.90 g (4.3 mmol) of XLI-151-3 and an appropriate amount of alcohol (cf. Table) in 40 mL of THF at room temperature was added a solution of 18 mL of 0.5 M (9.0 mmol) of potassium bis(trimethylsilyl)amide in toluene. The resulting mixture was stirred at room temperature for 2 days under an atmosphere of nitrogen. The resulting mixture was diluted with 40 mL of ethyl acetate and washed successively with 1% aqueous NH_4Cl, and brine. The organic extract was dried over sodium sulfate, filtered, and concentrated with a Rotavapor to give the crude product as a yellow liquid, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/9). The pure products were obtained as yellow liquids.

Alcohol	Amount	Product	Weight	Yield	R_f*
$(CH_3)_2CHOH$	0.47 g (7.8 mmol)	XLI-152-2	1.04 g	97%	0.40
C_2H_5OH	0.39 g (8.5 mmol)	XLI-153-2	1.00 g	99%	0.27

* ethyl acetate and hexanes (v/v = 1/9)

XLI-152-2: ^{1}H NMR ($CDCl_3$) 7.31-7.17 (m, 6H), 7.13 (d, J = 6.9 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 4.57 (heptet, J = 6.1 Hz, 1H), 3.87 (s, 2H), 1.35 (d, J = 6.2 Hz, 6H); lit. [42] ^{1}H NMR ($CDCl_3$) 7.4-7.2 (m, 6H), 7.14 (d, J = 7.1 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 4.59 (heptet, J = 6.1 Hz, 1H), 3.91 (s, 2H), 1.38 (d, J = 6.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) 158.4, 140.1, 134.7, 133.7, 133.6, 128.8 (2), 128.7 (2), 126.5, 116.8, 114.0, 102.9, 71.9, 40.5, 21.8 (2).

XLI-153-2: ^{1}H NMR ($CDCl_3$) 7.31-7.16 (m, 6H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 4.06 (q, *J* = 7.0 Hz, 2H), 3.87 (s, 2H), 1.42 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 159.1, 140.1, 134.8, 133.7 (2), 128.8 (2), 128.7 (2), 126.5, 116.6, 112.4, 101.9, 64.7, 40.5, 14.5.

5-Benzyl-2-isopropoxyacetophenone (XLI-154-4) and 5-benzyl-2-ethoxyacetophenone (XLI-157-3) General procedure: To a solution of the appropriate nitrile in 18 mL of toluene was added a solution of 3 M methylmagnesium iodide in ether. The resulting mixture was heated at 80 ^{o}C overnight under an atmosphere of nitrogen. The resulting mixture was treated with 20 mL of 10% HCl for 10 min, and then extracted three times with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated with a Rotavapor to give a yellow residue, which was purified on a silica gel column eluted with ethyl acetate and hexanes (v/v = 1/10). The pure products were obtained as white solids.

Nitrile	Weight	3 M CH_3MgI	Product	Weight	Yield	R_f*
XLI-152-2	0.68 g	2.0 mL	XLI-154-4	0.40 g	55%	0.36
XLI-153-2	0.67 g	2.2 mL	XLI-157-3	0.36 g	50%	0.31

* ethyl acetate and hexanes (v/v = 1/10)

XLI-154-4: mp 65-68 ^{o}C (lit. [42] no reported mp). ^{1}H NMR ($CDCl_3$) 7.59 (d, *J* = 2.3 Hz, 1H), 7.28-7.14 (m, 6H), 6.83 (d, *J* = 8.5 Hz, 1H), 4.61 (heptet, *J* = 6.0 Hz, 1H), 3.89 (s, 2H), 2.60 (s, 3H), 1.35 (d, *J* = 6.1 Hz, 6H); lit. [42] 7.59 (d, *J* = 2 Hz, 1H), 7.3-7.1 (m, 6H), 6.85 (d, *J* = 8.5 Hz, 1H), 4.62 (heptet, *J* = 6.1 Hz, 1H), 3.92 (s, 2H), 2.51 (s, 3H), 1.38 (d, *J* = 6.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) 200.2, 155.8, 141.0, 133.8, 132.9, 130.6, 129.0, 128.8 (2), 128.5 (2), 126.1, 113.7,

70.6, 40.8, 32.1, 22.1 (2).

XLI-157-3: mp 56-59 ^{o}C (lit. [42] no reported mp). ^{1}H NMR ($CDCl_3$) 7.61 (d, *J* = 2.5 Hz, 1H), 7.29-7.12 (m, 6H), 6.79 (d, *J* = 8.5 Hz, 1H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.87 (s, 2H), 2.59 (s, 3H), 1.40 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 199.7, 156.9, 141.0, 134.0, 133.1, 130.5, 128.7 (2), 128.5 (2), 128.0, 126.1, 112.6, 64.1, 40.8, 32.0, 14.7.

4-(5-Benzyl-2-isopropoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLI-156-1) and 4-(5-benzyl-2-ethoxyphenyl)-2-hydroxy-4-oxo-2-butenoic acid (XLI-161-1) General procedure: To a solution of the appropriate ketone and dimethyl oxalate in 12 mL of THF at room temperature was added sodium methoxide. The resulting mixture was stirred at room temperature for 2 h under an atmosphere of nitrogen. The resulting mixture was treated with 1M aqueous NaOH and stirred at room temperature for 1 h. The product solution was diluted with 25 mL of ethyl acetate, washed successively with 10% aqueous HCl and brine. The organic extract was dried over sodium sulfate, filtered, and concentrated with a Rotavapor to give the crude products as yellow solids.

Ketone	Weight	$(COOCH_3)_2$	CH_3ONa	1 M NaOH	Product	Weight	Yield
XLI-154-4	0.33 g	0.39 g	0.18 g	6.0 mL	XLI-156-1	0.33 g	79%
XLI-157-3	0.27 g	0.35 g	0.16 g	5.6 mL	XLI-161-1	0.29 g	83%

XLI-156-1: Yellow solid; sample for bioassay was purified by recrystallization from ethyl acetate and pentane; mp 106-107 ^{o}C (lit. [42] no reported mp). ^{1}H NMR ($CDCl_3$) 7.81 (d, *J* = 2.3 Hz, 1H), 7.64 (s, 1H), 7.32-7.17 (m, 6H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.68 (heptet, *J* = 6.2 Hz,

1H), 3.95 (s, 2H), 1.42 (d, J = 5.9 Hz, 6H); lit. [42] ^{1}H NMR ($CDCl_3$) 7.8-6.8 (m, 9H), 4.62 (heptet, J = 6.1 Hz, 1H), 3.95 (s, 2H), 1.43 (d, J = 6.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) 188.8, 169.6, 164.8, 156.7, 140.7, 135.5, 133.4, 130.9, 128.8 (2), 128.6 (2), 126.3, 123.8, 114.2, 102.7, 71.5, 40.8, 22.0 (2).

XLI-161-1: Yellow solid; sample for bioassay was purified by HPLC eluted with CH_3CN/H_2O/HOAc (v/v/v = 75/25/1, Ret. time = 4.76 min); mp 101-102 ^{o}C (lit. [42] no reported mp). ^{1}H NMR ($CDCl_3$) 7.80 (d, J = 2.4 Hz, 1H), 7.58 (s, 1H), 7.33-7.16 (m, 6H), 6.90 (d, J = 8.6 Hz, 1H), 4.14 (q, J = 7.0 Hz, 2H), 3.95 (s, 2H), 1.51 (t, J = 7.0 Hz, 3H); lit. [42] ^{1}H NMR ($CDCl_3$) 7.84 (s, 1H), 7.64 (s, 1H), 7.35-7.15 (m, 6H), 6.93 (d, J = 8.4 Hz, 1H), 4.20 (q, J = 7.0 Hz, 2H), 4.00 (s, 2H), 1.52 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) 188.8, 171.5, 164.5, 157.6, 140.7, 135.5, 133.6, 130.8, 128.8 (2), 128.6 (2), 126.3, 123.3, 113.0, 102.6, 64.7, 40.9, 14.6.

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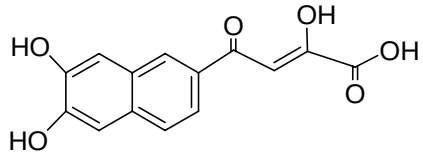

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ABSTRACT

DESIGN, SYNTHESES AND BIOLOGICAL ACTIVITIES OF
L-CHICORIC ACID ANALOGUES AS HIV-1 INTEGRASE INHIBITORS

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The human immunodeficiency virus type 1 (HIV-1) causes the acquired immune deficiency syndrome (AIDS). Three essential enzymes are necessary for HIV-1 replication: reverse transcriptase (RT), integrase (IN), and protease (PR). Current drugs target RT and PR, but there is substantial interest in inhibitors targeted at IN, which catalyzes the integration of the proviral DNA into the host cell's DNA.

The only two reported classes of compounds considered lead molecules for clinically useful HIV IN inhibitors are the dicaffeoyltartaric acids, of which L-chicoric acid (L-CA) is the lead compound, and the second are the aryl diketo acids (DKAs). These compounds prevent proviral DNA integration and inhibit HIV-1 replication at non-toxic concentrations in cell culture**.**

Our research focused on: 1) scaffold modification of L-CA; 2) catechol modification of L-CA; and 3) synthesis of hybrid DKA/catechol molecules.

In the first part, three conformationally restricted cyclopentane analogues of L-CA were synthesized and tested against HIV IN, and HIV replication. Several showed good anti-HIV IN activity, with the isomer having caffeoyl groups on the opposite side of the ring from the

carboxyl group being the most active, thus suggesting this preferred conformation in the IN active site. Neither the rigid analogues nor a synthesized open-chain analogue had significant anti-HIV replication activity.

In the second part, six 2-pyridone analogues of L-CA were synthesized, but only one had moderate anti-HIV IN and anti-HIV activity supporting the conclusion that catechols are required for anti-HIV and anti-HIV IN activity and that simple 2-pyridones are not bioisosteres of catechols.

In the third part, six synthesized DKA/catechol hybrids were potent IN inhibitors with the compound with the diketo acid and catechol moiety in a *meta*-orientation being the most active, even more so than L-CA and some DKA drug candidates. Against HIV replication, only two compounds showed moderate activity, but less so than either L-CA or simple DKAs. The results suggest that caffeoyl are preferred to simple catechol groups and one caffeoyl group is sufficient for inhibition of either HIV IN or HIV replication.

Methyl esters in all the above series were less active than their corresponding acids in both assays.