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Synthesis and Analysis of Chlorogenic Acid Derivatives from Food Processing

by

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Abstract

Chlorogenic acids (CGAs) are known as common secondary plant metabolites and coffee, tea, potatoes as well as many vegetables and fruits are known to display particularly high contents of such esters. Food processing (roasting, cooking, baking, frying, steaming, microwaving, fermenting) of many dietary plants enriches the profile of the CGAs and their derivatives in a given food, which are then available to human consumption and potentially human metabolism. Even the simple exposure of phenolics to hot water at slightly acidic pH results in an astonishing number of transformation products.

These newly formed CGA derivatives contribute to the desired taste, flavor, aroma and color of the foods and beverages. A series of mono-, di- and triacylated chlorogenic acids and derivatives were synthesized in the current project; the chosen cinnamoyl substituents were caffeoyl, feruloyl and dimethoxycinnamoyl. Efficient orthogonal protecting group strategies were developed and employed for the alcohols and carboxylic acid of the quinic acid moiety, and for the phenols of the hydroxycinnamate moiety.

The focus was on generating *γ*-quinide derivatives, compounds which result from CGAs at temperatures routinely employed in food processing. Quinides are among the main contributors to the sensory and organoleptic properties of coffee despite their relatively low concentrations in the final beverage. Little structural information exists in the literature when it comes to hydroxycinnamate-containing compounds despite their ubiquitous presence in nature and human diet. Single-crystal X-ray Diffraction (XRD) data of several compounds in the class, presented in this thesis, provides additional insight about their molecular structures and preferred conformations in solid state.

In food processing, water does not simply act as a solvent and innocent bystander but as a reactive reagent resulting in significant chemical changes of the dietary material. It was documented in the current study that water addition to the olefinic moiety of the cinnamoyl residues of CGAs, caffeoylglucoses, methyl quinates and *γ*-quinides takes place in a regiospecific manner. The water addition derivatives were found not only in compound model brews but also in the coffee beverage and the maté tea brew. Other transformation products were detected to form in hot water at slightly acidic pH from CGAs, including *trans-cis* isomerization

and acyl migration products. Liquid chromatography coupled to powerful mass spectrometry techniques and authentic or synthesized standards were used for the identification and characterization of these chemical transformation products.

A number of novel applications of the synthesized compounds were then documented in co-authored studies, including: influenza virus neuraminidase inhibitory activity; interaction with TRPV1 and TRPA1 receptors allowing rationalization of food off-flavor on a molecular basis; differentiation of prototropic ions in regioisomeric CGAs by electrospray ion mobility MS providing an additional MS-based methodology for isomer analysis and structural elucidation.

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Abbreviations

Ac	Acetyl
All	Allyl
BBA	2,3-Bisacetal
CGA	Chlorogenic Acid
CGAs	Chlorogenic Acids
COMT	Catechol-*O*-methyl Transferase
COSY	Correlation Spectroscopy
CQ	Caffeoylquinate
CQA	Caffeoylquinic Acid
CQL	Caffeoylquinic Acid Lactone / Caffeoylquinide
CQM	Methyl Caffeoylquinate
DAD	Diode Array Detector
DCE	Dichloroethane
DCM	Dichloromethane
di-CQA	Dicaffeoylquinic Acid
DMAP	N,N'-Dimethylaminopyridine
DMP	2,2-Dimethoxypropane
DMSO	Dimethylsulfoxide
DMF	N,N'-Dimethylformamide
DQA	Dimethoxycinnamoylquinic Acid
DQL	Dimethoxycinnamoylquinic Acid Lactone / Dimethoxycinnamoylquinide
DQM	Methyl Dimethoxycinnamoylquinate
EFSA	European Food Safety Authority
ESI	Electrospray Ionisation
EST	Esterase
FQA	Feruloylquinic Acid
FQL	Feruloylquinic acid lactone/ Feruloylquinide
FQM	Methyl Feruloylquinate
GIT	Gastrointestinal Tract
GT	Glucuronyl Transferase

HBV	Hepatitis B Virus
HIV	The Human Immunodeficiency Virus
HMQC	Heteronuclear Multi-Bond Quantum Correlation Spectroscopy
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
ICO	International Coffee Organization
IUPAC	International Union of Pure and Applied Chemistry
LC	Liquid Chromatography
LC-MS^n	Liquid Chromatography Tandem Mass Spectrometry
LD_{50}	Lethal Dose, 50%
MeOH	Methanol
MRM	Multi Reaction Monitoring
MS	Mass Spectrometry
m/z	Mass-to-Charge Ratio
NMR	Nuclear Magnetic Resonance
PTSA	*p*-Toluenesulfonic Acid
p-CoQA	*p*-Coumaroylquinic Acid
QA	Quinic acid
RA	Reductase
RP	Reverse Phase
ST	Sulfate-*O* Transferase
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMB	2,2,3,3-Tetramethoxybutane
TOF	Time of Flight
tri-CQA	Tricaffeoylquinic Acid
Troc	2,2,2,-Trichloroethylformyl
Troc-Cl	2,2,2-Trichloroethylformyl Chloride
UV-VIS	Ultraviolet-Visible
XRD	X-Ray Diffraction

List of Scientific Contributions

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1. **Matei**, **M. F.**; Jaiswal, R.; Kuhnert, N. Investigating the Chemical Changes of Chlorogenic Acids during Coffee and Maté Tea Brewing: Formation of Water-Addition Derivatives. Presented at the 7th World Congress on Polyphenols Applications: ISANH Polyphenols 2013, Bonn, Germany, June 2013.

2. **Matei**, **M. F.**; Jaiswal, R.; Kuhnert, N. LC-MS^n to Discriminate Between Synthetic Chlorogenic Acids Lactones and Cinnamoylshikimate Esters. Presented at the 5th International Conference on Polyphenols and Health (ICPH), Barcelona, Spain, October 17-20, 2011.

3. Kuhnert, N; **Matei**, **M. F.**; Jaiswal, R. Unravelling the Structure of Roasted Coffee Melanoidines. Presented at the 5th International Conference on Polyphenols and Health (ICPH), Barcelona, Spain, October 17-20, 2011.

4. **Matei**, **M. F.**; Jaiswal, R.; Kuhnert, N. LC-MS^n Analysis of Chlorogenic Acids Derivatives Formed During the Brewing of Coffee. Presented at Gesellschaft Deutscher Chemiker (GDCh) Wissenschaftsforum Chemie 2011, Bremen, Germany, September 4-7, 2011.

1. Introduction

1.1. Chlorogenic Acids: Important Hydroxycinnamates

Analytical methods for efficient screening and unambiguous identification of dietary natural products and their chemical transformation products form the root of understanding the impact of diet on human health. Once such analytical methods have been developed and established, dietary materials can be screened for novel compounds, structures can be assigned, novel compounds can be quantified, their dietary burden determined, their biological activity tested, their impact on human health estimated and, after pharmacokinetic evaluation, scientifically sound dietary advice to the consumer can be provided.

The interest in dietary hydroxycinnamates has expanded rapidly in the last 5-10 years. Numerous epidemiological studies have frequently linked the consumption of a diet rich in hydroxycinnamates, in particular chlorogenic acids (CGAs), with numerous beneficial health effects,[1-5] which have been in many cases substantiated by *in vitro*, *in vivo* and human intervention studies. Further interest in this ubiquitous class of compounds is motivated by their attractive sensory and organoleptic properties.[6]

Hydroxycinnamates form a subclass of polyphenolic natural products. They are secondary metabolites, found ubiquitously in plants including most fruits and vegetables relevant to the human diet. Hydroxycinnamic acids can occur in their free form with caffeic **1**, ferulic **5**, sinapic acid **4** and *p*-coumaric acid **2** being the most widespread examples (structures in **Figure 1.1**). Other minor cinnamic acid derivatives such as isoferulic acid **6,** dimethoxycinnamic **7** or trimethoxycinnamic acid **8** have also been reported in plants.[7-10]

Hydroxycinnamates may be conjugated to many molecules, with conjugates to (-)-quinic acid (QA) being to present knowledge the most widespread compounds in the human diet. Other conjugates, of compounds with limited distribution found in only a few species, or frequently found but only as minor components in plants, include: 1. esters of (-)-quinic acid (chlorogenic acids); 2. esters of hydroxyl acids such as, shikimic, tartaric, galactaric, glucaric, gluconic, malic, dihydrocaffeic, hydroxycitric, phenylpyruvic, methoxyaldaric, lactic and tartronic acid; 3. glycosides; 4. amides of biogenic amines and amino acids including aromatic amino acids, glycine, spermidine, spermine, choline and anthranilic acid; 5. esters of carbohydrates and

polyols including monosaccharides, sugar alcohols including glycerol, inositols, and glycosides of anthocyanins, flavanols and diterpenes; 6. esters of lipids including sterols.[11]

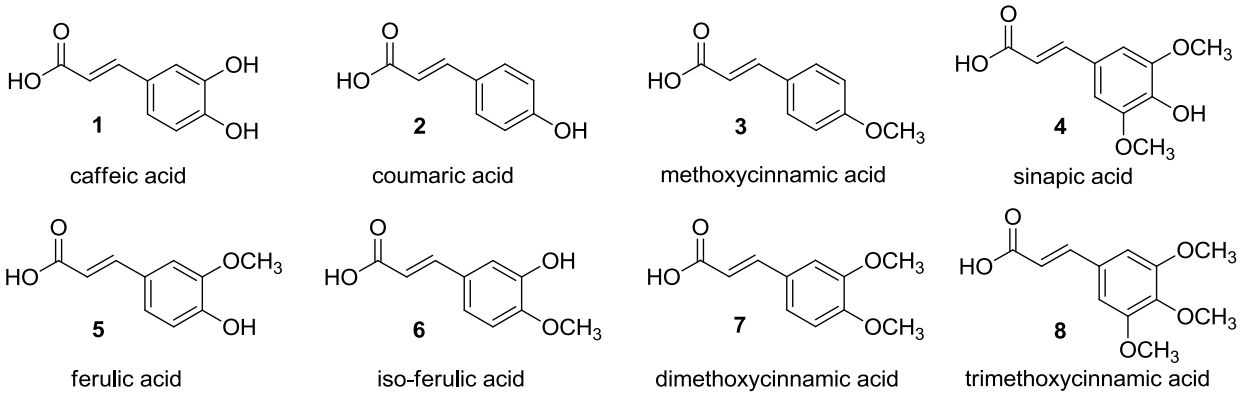

Figure 1.1. Selected structures of typical hydroxycinnamic acids present in the human diet.

Chlorogenic acids form a class of compounds in the comprehensive family of hydroxycinnamates. Chlorogenic acids (CGAs) are classically referred to as a class of esters afforded from *trans*-cinnamic acids and quinic acid, the latter bearing either axial substitution positions (hydroxyl groups on carbons 1 and 3) or equatorial ones (hydroxyls on carbons 4 and 5).[12,13] Most commonly encountered *trans*-cinnamic acids moieties are of caffeic, *p*-coumaric and ferulic acids[13-15] but sinapic acid and dimethoxycinnamic acid are also present in certain plant species.[9,16-18] In the IUPAC system (−)-quinic acid is defined as 1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid, but Eliel and Ramirez recommend 1α,3*R*,4α,5*R*-tetrahydroxycyclohexane carboxylic acid.[19] Representative structures of both mono- and diacylated CGAs are shown in **Figure 1.2** and **Figure 1.3**.

Using the recommended IUPAC nomenclature, the most common CGA is 5-*O*-caffeoylquinic acid (5-CQA) **12**, sometimes referred to as chlorogenic acid or 3-CQA (pre IUPAC and older literature). Throughout the current thesis the IUPAC nomenclature is used. A shorthand notation is used to abbreviate chlorogenic acids. The shorthand has the following format: N-XQA or N,M-diXQL or N,M-diYQM, where N or M denominates the position of acyl substitution and X and Y define an abbreviation for the chemical nature of the substituent (C = caffeoyl, F = feruloyl, D = dimethoxycinnamoyl, *p*Co = *p*-coumaroyl, etc.). QA stands for quinic acid, QL stands for quinic acid lactone, QM stands for methyl quinate whereas e.g., CG stands for

caffeoyl glucose. For example, 1-CQA stands for 1-*O*-caffeoylquinic acid **9** and 3-FQL stands for 3-*O*-feruloylquinic acid lactone (or 3-*O*-feruloyl-1,5-quinide) **43**. For γ-quinide (1,5-quinide) derivatives, the same numbering of the QA moiety is maintained (e.g., C-5 position in a DQA remains as C-5 in a DQL) for simplicity.

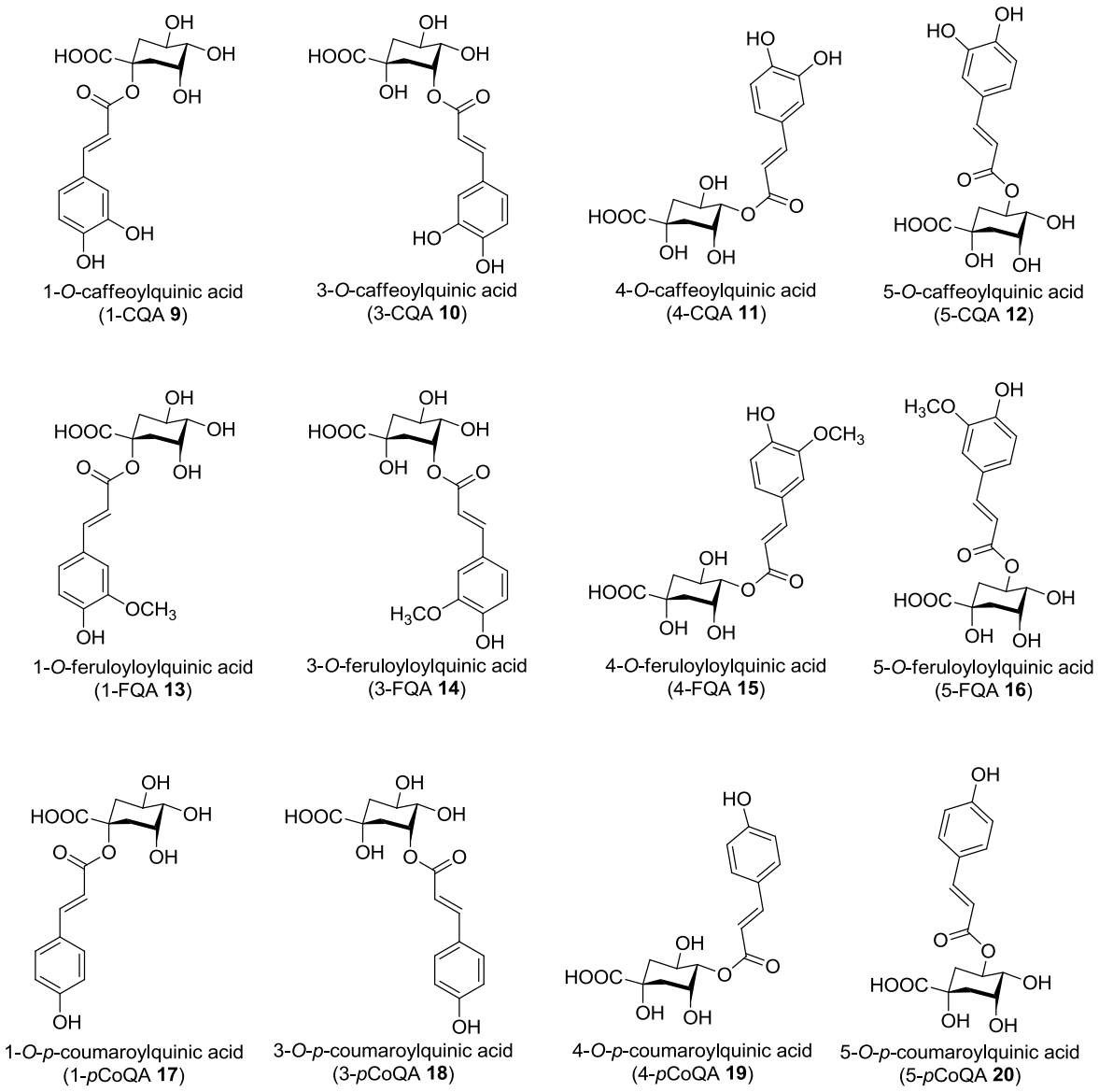

Figure 1.2. Representative monoacylated CGAs.

The CGAs show a variety of biological activities like antioxidant, anti-inflammatory, anti-HIV, anti-HBV, anti-diabetes, radical scavenging, inhibit mutagenesis and carcinogenesis, and are

considered to be beneficial to human health.[20-24] The metabolism of chlorogenic acids in mammals has been poorly studied so far and relatively little is known[25] though their importance as non-nutritive food constituents (*ortho*-diphenyl: antioxidative power[26], radical scavengers[27]) cannot be challenged. It should not come as a surprise that such metabolic studies are still limited since isotopically labeled chlorogenic acids are not readily available; also, the first efficient synthesis of a compound in this class was only published in 2001[28], though analogues of chlorogenic acids were reported earlier by Hemmerle and colleagues.[20]

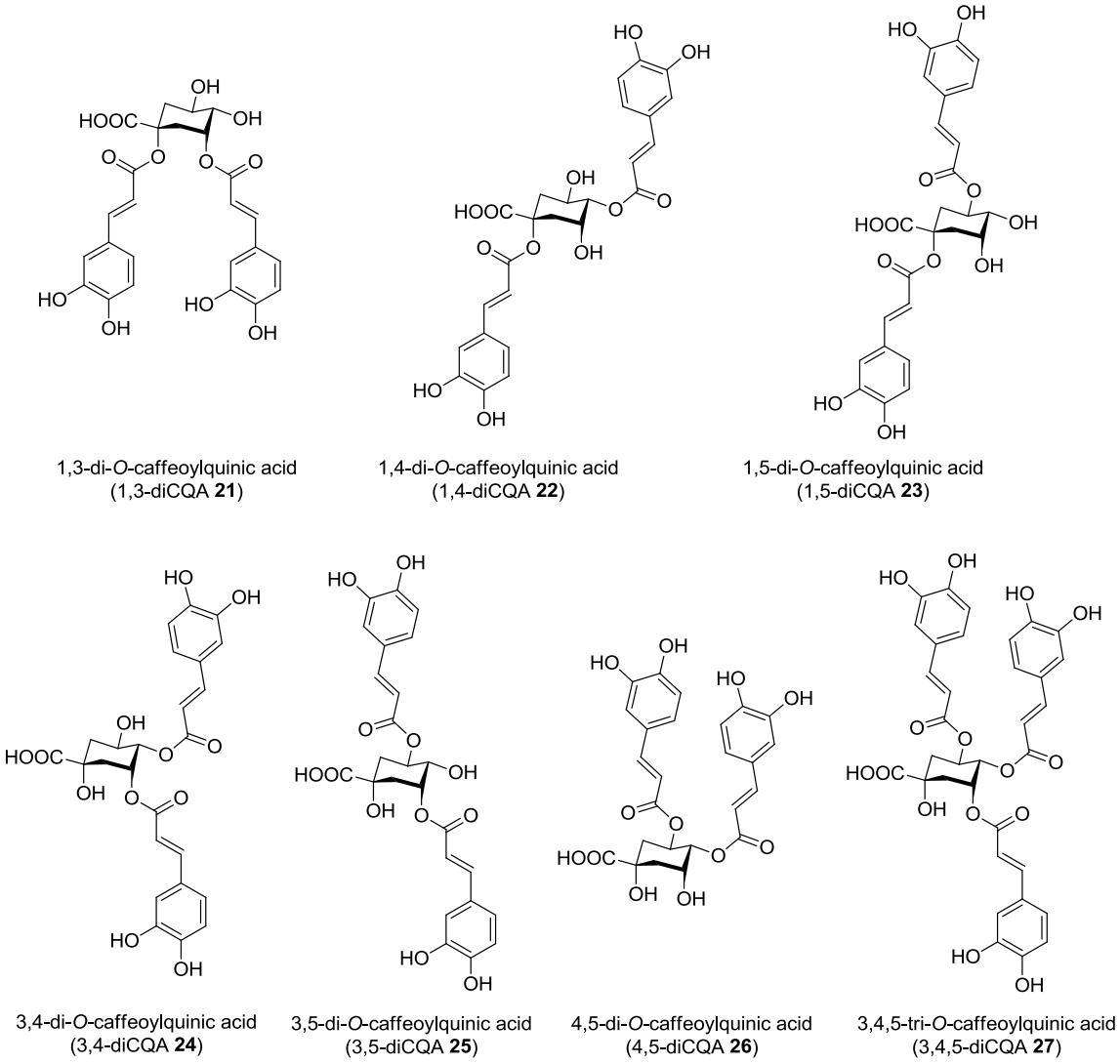

Figure 1.3. Representative non-mixed di- and triacylated CGAs.

A classical subdivision of chlorogenic acids discriminates between compounds of this family based on the identity, number and position of the acyl residues. Clifford divided them into:

a) monoesters of caffeic acid (e.g., caffeoylquinic acids or CQAs, *p*-coumaroylquinic acids or pCoQAs, feruloylquinic acids or FQAs);

b) diesters, triesters and a single tetraester of caffeic acid;

c) mixed diesters of caffeic acid and either ferulic acid (e.g., caffeoylferuloylquinic acids) or sinapic acid (e.g., caffeoylsinapoylquinic acids);

d) mixed esters derived from permutations of one to three caffeic acid moieties with one to two residues of a dicarboxylic acid (e.g., oxalic, succinic, glutaric).[11]

CGAs are common secondary plant metabolites and coffee, tea, potatoes as well as many vegetables and fruits are known to display particularly high content of such esters. Coffee beans and commercial coffee products are usually the main source of CGAs in the human diet and it was reported that esterification never occurs at position 1 of the quinic acid but only at positions 3, 4 and 5 in green coffee.[12] Food processing however can produce the remaining isomer at position 1 and may partially convert *trans* to *cis* isomers among other transformations.

1.2. Chlorogenic Acids and Derivatives: Analysis and Structure Elucidation

While figures for an average dietary intake exist in particular for chlorogenic acids, for other hydroxycinnamate derivatives figures need to be regarded as sketchy. The average daily human intake of CGAs varies depending on the author of the study between 1 g per day per human and 2.5 g per day per human.[4,11] To an extent, the discrepancies between such reported figures are a result of the individual diets and consumer preferences. However, they are inevitably a consequence of the limited knowledge of this important class of compounds as well. Numbers on exact quantities of chlorogenic acids in dietary material are limited to a small number of derivatives and little is known about statistical variances between the CGA content in different plant species and varieties. Moreover, available analytical data is in many cases obsolete, obtained through methods based on derivatisation followed by colorimetry, quantifying only one particular class of CGAs e.g., caffeoyl or feruloyl esters, resulting in a possible underestimation of real CGA content. Secondly, agricultural practice has changed dramatically over the last decade with many new varieties of fruit and vegetables introduced to the market and older varieties consequently disappearing from the market for patent reasons. Therefore, it is likely

that both chlorogenic acid profiles and quantities within these new varieties have changed dramatically in comparison to the published data. With a dietary intake figure of around 2 g per day, combined with bioavailability data suggesting a high level of absorption, hydroxycinnamates must be considered as the most relevant secondary plant metabolites for human diet.

In 2003 the Kuhnert group jointly with the group of Michael Clifford published a novel method for the identification and structure elucidation of regioisomeric chlorogenic acid derivatives (hydroxycinnamate esters of quinic acid). This approach revolutionised chlorogenic acid identification and structure elucidation by introducing a method based on tandem mass spectrometry, allowing assignment of regiochemistry based exclusively on fragment spectra.[12] Prior to 2003 only 40 different chlorogenic acid derivatives were known in the human diet. Since the introduction of the innovative method in 2003 more than 300 novel chlorogenic acid derivatives have been identified in the human diet by other research groups and more than 200 novel derivatives by the Kuhnert group alone. This method has the advantage that CGAs do not need to be isolated but can be identified and their structure elucidated directly from analytical LC-tandem-MS runs, even if present as minor components or as chromatographically close eluting compounds.

Due to the diagnostic differences in the tandem MS fragment spectra, a consistent and predictive structure diagnostic hierarchical key for CGA structure elucidation has been established, which allows reliable determination of CGA regiochemistry even for minor component CGAs from tandem MS data exclusively, superior to structure elucidation methods by Nuclear Magnetic Resonance (NMR). The basis of these differences in tandem MS spectra was rationalized in terms of different hydrogen bonding arrays found in gas phase ions of regioisomeric CGAs inducing distinct pathways for fragmentation. This method was recently extended to the structure elucidation of regioisomeric shikimic acid esters.[17] Furthermore, to illustrate the power of the method, all four regioisomers of feruloylquinic acid (FQA) and isoferuloylquinic acid (iFQA) were synthesized in a co-authored study and then the total of eight regioisomers was resolved by LC/MS/MS (chromatogram shown in **Figure 1.4**).[10]

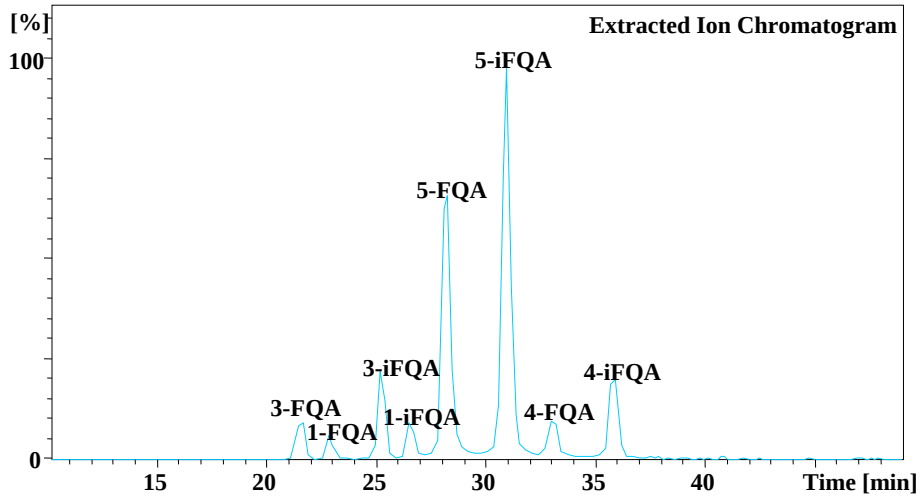

Figure 1.4. HPLC chromatogram of mixtures of regioisomers of FQAs and iFQAs.[10]

All structures of the derivatives could be unambiguously identified to the regioisomeric level. Therefore, it is definitely worth exploring whether such diagnostic differences in tandem MS spectra can as well be observed and hence successfully employed for structure elucidation of structurally related derivatives including other cyclohexenes, lactones, oxidation products or carbohydrate trans-esterification products. The cinnamate chromophore of CGAs is responsible for a typical UV absorption at roughly 320 nm. Kuhnert and co-workers compiled recently a list of published λ_{max} values of selected chlorogenic acids as well as data for molar extinction coefficients (ε), as shown in **Table 1.1**.[33] Such data is especially valuable in HPLC analysis which employs UV detectors such as DAD for quantification purposes.

In the same study the authors observed that the formation of phenolate anions by CGAs in alkaline media was responsible for a bathochromic shift; log E progressively increased with the increasing degree of acyl substitution.[33] UV-VIS spectroscopy is additionally able to discriminate between *cis* and *trans* isomers of CGAs since in the absorption spectra the *cis* isomer typically displays a red-shifted shoulder.[34]

For quantification of hydroxycinnamates and CGAs from dietary sources most of the published information was based on UV-VIS spectroscopy. HPLC-MS techniques have gained popularity recently for quantification purposes. HPLC-MS allows quantification of CGAs with the help of appropriate reference standards. The samples to be analysed by this approach should give MS

spectra with few signals, no co-elution of compounds and no signal overlap. These might appear as demanding conditions to be met at times but for the quantification of the more complex samples, single ion monitoring in tandem MS is an effective approach.

Table 1.1. Characteristic UV-VIS data of selected chlorogenic acids.[33]

CGA	λ_{max} [nm][a]	log E[a]
1-CQA **9**	330	4.26
3-CQA **10**	330	4.22
4-CQA **11**	330	4.26
5-CQA **12**	327	4.29
1-FQA **13**	325	4.27
3-FQA **14**	325	4.28
4-FQA **15**	325	4.29
5-FQA **16**	325	4.29
1-*p*CoQA **17**	375	4.31
3-*p*CoQA **18**	315 (310)[b]	4.30
4-*p*CoQA **19**	315	4.32
5-*p*CoQA **20**	315 (310)[b]	4.31
1,3-diCQA **21**	325	4.50
1,4-diCQA **22**	327	4.53
1,5-diCQA **23**	327	4.50
3,4-diCQA **24**	330	4.53
3,5-diCQA **25**	329	4.55
4,5-diCQA **26**	330	4.52
3,4,5-triCQA **27**	327	4.75

[a]all reported values in ethanol; [b]two reported values in literature.

Before HPLC-MS was introduced as an alternative to the quantification techniques based on UV-VIS spectroscopy, quantification was performed either by HPLC-UV-VIS or by derivatisation followed by UV-VIS spectroscopy. The first approach (HPLC-UV-VIS) has the advantage of being capable of quantifying individual CGAs while the second (derivatisation) was used to calculate the total amount of hydroxycinnamates or CGAs.

In HPLC-UV-VIS the HPLC instrument effects chromatographic separation and the UV-VIS detector coupled to the HPLC system then quantifies the individual chromatographically well

resolved components. Since the maximum absorption of the cinnamate chromophore is at around 320 nm as shown above, the quantification is done at this wavelength value based on the previously obtained calibration curves.[33,35] An important share of the quantification data on total content of hydroxycinnamates and CGAs in various samples was afforded through colour derivatisation reactions. The method is not without limitations and the numbers generated by this manner should be viewed critically.

By this approach, a coloured product which is at the end quantified by UV-VIS spectroscopy is the result of a reaction between a crude dietary extract and a reagent which reacts specifically with a targeted hydroxycinnamate residue. Examples of reagents typically employed to effect this transformation are periodate, molybdate or thiobarbituric acid. But this method can result in either an over- or an under-estimation of the CGAs content. An over-estimation of the CGAs may be due to the presence of additional (poly)phenolic dietary constituents derivatised by this method while CGAs with different substituents than those being targeted (and thus not being detected) would produce an under-estimation of the total content.[33,35,36]

1.3. Chlorogenic Acid Derivatives in Food

Diets particularly rich in chlorogenic acids and therefore relevant to the human health comprise coffee, tea and plants from the *Solanaceae* family including potatoes, tomatoes and eggplants. Small amounts of CGAs (typically below 100 mg/kg, **Table 1.2**) can further be found in citrus fruits (lemon, grapefruit), berry fruits (raspberries, blackberries, strawberries) and *Brassica* vegetables (broccoli, cauliflower, Brussels sprouts). All these plants are being processed by heat treatment prior to consumption. Coffee is roasted and potatoes and eggplants are always, and tomatoes frequently, baked, fried, cooked, steamed or roasted. In this processes the secondary plant metabolites undergo significant chemical changes and new products are formed. Coffee and the above-mentioned plants prompt further investigation for clarifying the chemical fate of hydroxycinnamate derivatives in food processing. For instance, a HPLC-MS measurement on green Robusta coffee beans reveals the presence of around 80 different chlorogenic acids, which upon roasting increases to around 200 derivatives.[16,29,30] This number can be estimated by identifying typical cinnamate absorptions in the UV traces of the chromatogram at 320 nm and by studying extracted ion chromatograms in All MS^n mode indicating fragmentation patterns with fragment ions characteristic to hydroxycinnamate derivatives. Two typical chromatograms

are shown in **Figure 1.5**. A similar observation can be made for cooked tomatoes where the number of 25 hydroxycinnamates present in the untreated plant increases to around 50.[31]

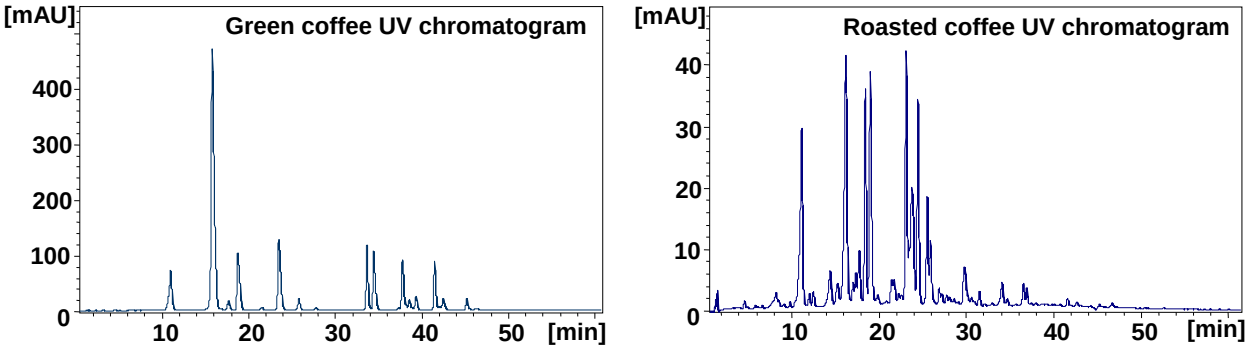

Figure 1.5. UV chromatograms of green and roasted coffee at λ_{max} = 320 nm.

There is a limited number of potential isomers of CGAs produced by plants genuinely as secondary metabolites; in contrast to this, the number of possible regio- and stereoisomers which could be chemically or enzymatically generated by food processing and metabolism has the potential to be large. Food processing (roasting, cooking, baking, frying, steaming, microwaving, fermenting) of many dietary plants can potentially enrich the profile of the CGAs and their derivatives in a given food, which are then available to human consumption and potentially human metabolism.

Among the transformations of CGAs during food processing, hydrolysis can release cinnamic acids from conjugates, which can further be decarboxylated by heat or microorganisms to yield various alkyl and vinyl phenols. Not so much attention has so far received the possibility of formation of novel transformation products triggered by food processing. The first reports on this topic identified the grape reaction product 2-*S*-glutathionylcaftaric acid as well as 2,5-di-*S*-glutathionylcaftaric acid formed from caftaric acid (caffeoyl-tartaric acid) during wine making.[37-39] Adducts generated from the interaction of anthocyanins with vinyl phenol resulted from *p*-coumarate decarboxylation were detected in red wine. During coffee roasting caffeic acid **1** is partially converted to tetrahydroxy-phenylindanes.[4,40,41] As suggested by Maier and co-workers, the resulting hydrolysed quinic acid residue from coffee roasting can be subsequently converted to the full theoretical complement of quinic acid and quinic acid lactone (quinide) diastereomers

(**Figure 1.6**).[42] There are eight possible stereoisomers for quinic acid, out of which four *meso* forms and two pairs of enantiomers.

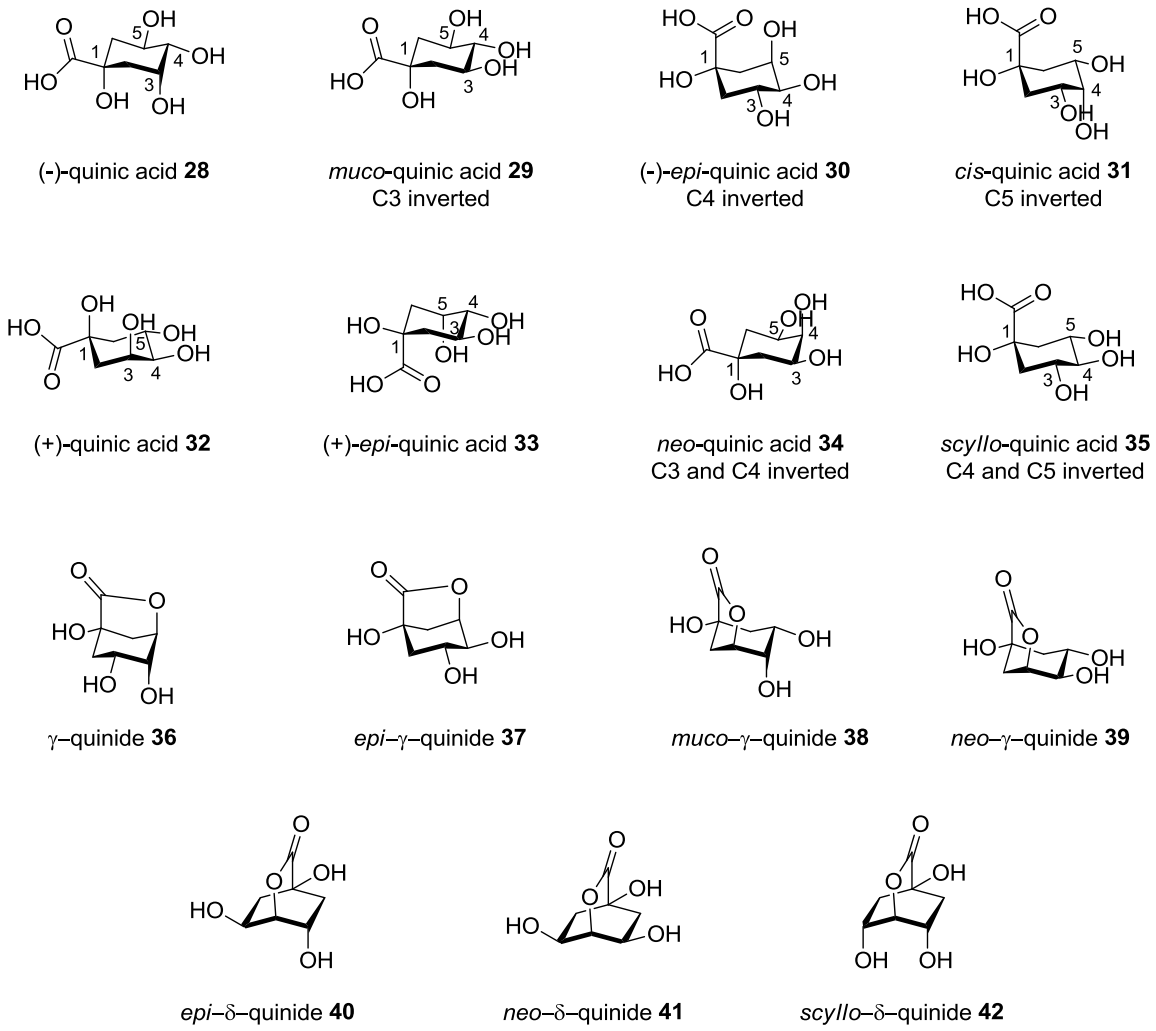

Figure 1.6. Stereoisomers of quinic acid and quinic acid lactones.

Quinides (including 1,5-quinides) can be the products of the intra-molecular water elimination processes from the parent unhydrolysed caffeoylquinic acids and feruloylquinic acids; additional diastereomers of the original acids or of the newly formed lactones might as well be generated.[43,44] The elevated temperatures employed in the roasting process can result in a breakage of the C-C bonds in CGAs and their derivatives.

Roasting times as short as 5 minutes can alter significantly the CGAs profile: the levels of both 3-CQA **10** and 4-CQA **11** were observed to double their original values at the expense of the more abundant 5-CQA **9** whose level decreased substantially. Feruloylquinic acids analogues behaved similarly in the 5-minute roasting experiment. Besides the obvious isomerisation processes, partial hydrolysis of diacylated CQAs to monoacylated CQAs was also observed.[4] Acyl migration was also documented in artichoke, where a similar behaviour as during the roasting of coffee was observed: migration of acyl from C-5 to C-3 positions of the quinic acid moiety to form cynarine (1,3-diCQA) from 1,5-diCQA.[43]

Table 1.2. Occurrence of chlorogenic acids in selected fruits, vegetables and medicinal plants.[45]

	Source	**Total amount of CGAs**	**Reference**
Coffee	Roast coffee	20-675 mg/200 ml	Clifford *et al.*[46]
Tea	Black tea	10-50 g/kg	Cartwright *et al.* and Hara *et al.*[47,48]
Maté	Maté	107-133 mg/200 ml	Clifford *et al.*[11]
Pome fruits	Apple	62-385 mg/kg	Mosel *et al.*, Risch *et al.* and Spanos *et al.*[49-51]
	English apple cider	6-587 mg/l	Marks *et al.*[52]
	Pear	60-280 mg/kg	Wald *et al.* and Igile *et al.*[53,54]
Stone fruits	Black chokeberries	1.8 g/kg	Slimestad *et al.*[55]
	Apricot	1.4 g/kg	Ruiz *et al.*[56]
	Plum	1.5-1.9 g/kg	Nakatani *et al.*[57]
Berry fruits	Blueberries	0.5-2 g/kg	Schuster *et al.*[58]
	Blackcurrants	140 mg/kg	Gao *et al.*[59]
	Blackberries	70 mg/kg	Koeppen *et al.*[60]
	Raspberries	20-30 mg/kg	Koeppen *et al.*[60]
	Strawberries	20-30 mg/kg	Koeppen *et al.*[60]
	Redcurrant	20-30 mg/kg	Koeppen *et al.*[60]
	Gooseberries	20-30 mg/kg	Koeppen *et al.*[60]
Citrus fruits	Oranges	170-250 mg/kg	Risch *et al.* and Winter *et al.*[61,62]
	Grapefruit	27-62 mg/kg	Risch *et al.*[61,63]
	Lemon	55-67 mg/kg	Risch *et al.*[61,63]
Grapes and wines	Grape juice	10-430 mg/l	Spanos *et al.*[51]
	American wine	9-116 mg/l	Okamura *et al.*[64]

Other fruits	Pineapple	3 mg/l	Desimon *et al.*[65]
	Kiwi	11 mg/l	Hernandez *et al.*[66]
	Fig	0.3-5.8 mg/kg	Vallejo *et al.*[67]
Brassica vegetables	Kale	6-120 mg/kg	Winter *et al.* and Brandl *et al.*[62,68]
	Cabbage	104 mg/kg	Winter *et al.* and Brandl *et al.*[62,68]
	Brussels sprouts	37 mg/kg	Winter *et al.* and Brandl *et al.* [62,68]
	Broccoli	60 mg/l	Plumb *et al.*[69]
	Cauliflower	20 mg/kg	Plumb *et al.*[69]
	Radish	240-500 mg/kg	Brandl *et al.*[70]
Chenopodiaceae	Spinach	200 mg/kg	Tadera *et al.* and Winter *et al.*[71,72]
Asteraceae	Lettuce	50-120 mg/kg	Winter *et al.*[72]
	Endive	200-500 mg/kg	Winter *et al.*[72]
	Chicory	20 mg/kg	Winter *et al.*[72]
	Artichoke	9 g/kg	Schutz *et al.*[73]
	Lingularia fischeri	102 g/kg	Shang *et al.*[74]
	Helichrysum monizii	12 g/kg	Gouveia *et al.*[75]
	Helichrysum melaleucum	2-13 g/kg	Gouveia *et al.*[75]
	Helichrysum devium	0.3-4.8 g/kg	Gouveia *et al.*[75]
	Artemesia pallens	130 mg/kg	Niranjan *et al.*[76]
	Baccharis trimera	1.8-38 g/kg	Aboy *et al.*[77]
	Erigeron breviscapus	5.4-12.3 g/kg	Wang *et al.*[78]
	Saussurea laniceps	0.4-10 g/kg	Yi *et al.*[79]
	Helianthus annus	36-41 g/kg	Weisz *et al.*[80]
	Xanthium sps.	1.2-7.3 g/kg	Han *et al.*[81]
	Stevia rebaudiana	361 mg/kg	Karaköse *et al.*[35]
	Pluchea symphytifolia	6.8 g/kg	Scholz *et al.*[82]
Solanaceae	Potato	0.5-1.2 g/kg	Malmberg *et al.*[83]
	Aubergines	600 mg/kg	Malmberg *et al.*[83]
	Tomatoes	10-80 mg/kg	Brandl *et al.*[84]
Apiaceae	Carrot	20-120 mg/kg	Winter *et al.*[62]
	Black carrot	657 mg/kg	Kammerer *et al.*[85]
	Burr parsley	1023 mg/kg	Plazonic *et al.*[86]
Cereals	Barley bran	50 mg/kg	Hernandez *et al.*[66]
	Rice	12 g/kg	Shibuya *et al.*[87]
Anacardiaceae	*Pistacia lentiscus*	46.7 g/kg	Romani *et al.*[88]
Araliaceae	*Acanthopanax senticosus*	0.4-1.2 g/kg	Liu *et al.*[89]
Adoxaceae	*Sambucus nigra*	5.4 g/kg	Banos *et al.*[90]

Caprifoliaceae	*Lonicera caerulea*	180 mg/kg	Palikova *et al.*[91]
Fabaceae	*Onobrychis vicifolia*	0.2-1.6 g/kg	Regos *et al.*[92]
Malpighiaceae	*Byrsonima crossifolia*	36.8 g/kg	Maldini *et al.*[93]
Calophyllaceae	*Caraipa densifolia*	0.5-2.4 g/kg	da Silveira *et al.*[94]
Rhamnaceae	*Ziziphus jujuba*	1.1-68.9 mg/kg	San *et al.*[95]
Rosaceae	*Cratageus monogyna*	5.5 g/kg	Banos *et al.*[90]

Only selected efforts have been made to elucidate the structure of such reaction products. The group of Farah reported on the presence of chlorogenic acid lactones in roasted coffee formed by dehydration of the parent chlorogenic acid,[29,32] whereas the Kuhnert group reported on the presence of alternative shikimic acid dehydration products in roasted maté tea.[17] Nevertheless, a systematic work addressing the fate of hydroxycinnamate derivatives in food processing is lacking at the moment and as clearly seen from the chromatograms above, there are many more unknown structures present in processed food. Being part of the common human diet such derivatives are to humans of foremost importance and relevance and urgent clarification of their structures and properties must be set as a priority.

From the above arguments it becomes obvious that a large proportion of the chlorogenic acids in food are converted by heat treatment into novel structures. Considering the chemical fate of chlorogenic acids at elevated temperatures, a reaction diagram can be developed and a hypothesis on possible reaction pathways introduced. **Figure 1.7** represents such a diagram with examples of one compound per specific transformation.[44] Therefore, it is hereby proposed that at elevated temperatures, neglecting all further chemical species present in the plant, chlorogenic acids (e.g., **14**) can dehydrate to form lactones (**43**) or cyclohexene derivatives (**44**) including shikimic acid derivatives (**45**), can add water (**46**), can undergo epimerization (**47**), can undergo thermal *trans-cis* isomerisation (**48**), oxidation (**49**) or can undergo acyl migration (**16**) (either intra- or inter-molecular with other CGAs or alcohols present in human diet e.g., carbohydrates).

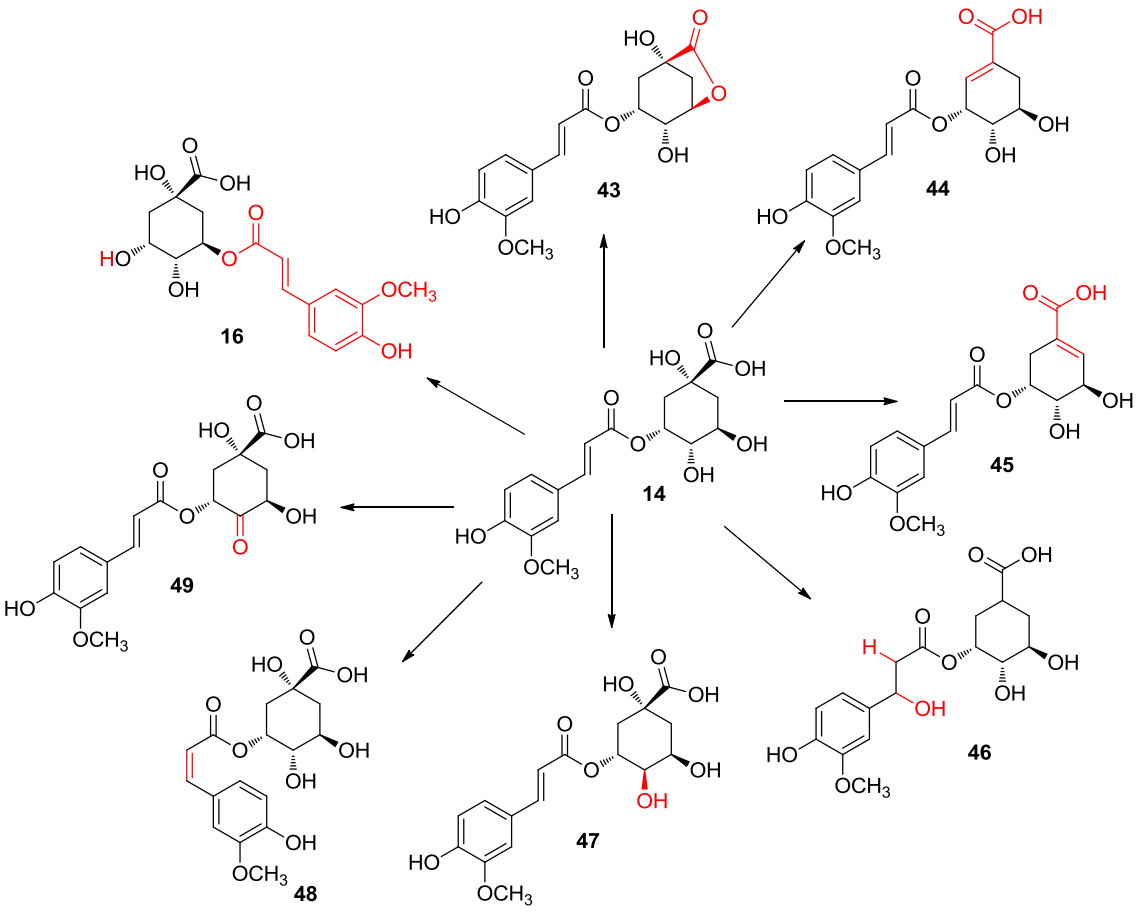

Figure 1.7. Chemical fate of chlorogenic acids at elevated temperatures.[44]

1.4. Chlorogenic Acids and Derivatives: Intake and Bioavailability

The main dietary source of CGAs and their derivatives is coffee for most consumers.[11] Figures of ingested CGAs per serving vary between 20 mg and 675 mg depending on several parameters such as the consumed volume, the concentration of the brew and the type of the roast. As an average figure, 200 mg CGAs per 200 ml serving can be assumed. Simple math indicates that a figure of more than 1 g CGAs per day is a realistic estimation for regular coffee consumers, with an intake of 4-5 servings daily.[11,96] A study performed by Crozier in Glasgow about the CGAs content in espresso coffee samples purchased from 21 coffee shops confirmed the above estimation. The values for the total CGAs dosage per serving varied in a wide range (almost 20-fold), from a minimum of 24 mg per cup to a maximum quantity of 423 mg per cup.[97]

The higher end of this reported interval (423 mg or 1195 μmol per cup) is substantially superior to what other sources of CGAs could offer: 25 mg (72 μmol) per 200 mL serving of cloudy apple juice[98] or 34 mg (96 μmol) per 200 mL serving of apple smoothie.[99] Although there are other rich sources of CGAs (e.g., globe artichoke with 268 mg or 762 μmol per 100 g)[100], they are very rarely consumed in a high enough quantity to contest coffee as the leading source of CGAs for the human diet. With a content of 94-111 mg (270-320 μmol) CGAs per 200 mL cup volume,[101] maté represents an important source in certain populations, particularly in South America, given the daily number of servings for regular consumers. **Table 1.3** summarizes the reported CGAs content in different relevant products.[45]

Coffee represents the dominant source of dietary CGAs and was used in most human studies on CGAs absorption and metabolism. Besides coffee, there are numerous dietary sources of CGAs with varying relevant content. Their compositional data is summarized in **Table 1.2**; however, caution should be exercised when using such data since the composition of the cultivars can be of significant difference.[45]

Table 1.3. CGAs content in different products.[45]

Product	Serving	Dose		Reference
		μmol[a]	mg[a]	
Espresso coffee	27 mL	68	24	Crozier *et al.*[97]
	52 mL	1195	422	Crozier *et al.*[97]
Instant coffee	200 mL	412	146	Stalmach *et al.*[103]
	200 mL	384	136	Stalmach *et al.*[104]
Cloudy apple juice	200 mL	72	25	Kahle *et al.*[98]
Apple smoothie	200 mL	96	34	Hagl *et al.*[99]
Globe artichoke	100 g	762	268	Pandino *et al.*[100]
Maté	200 mL	270-320	107-133	Clifford and Ramìrez-Martìnez[101]

[a]data expressed as mean values (n = 3); in all instances the SE was <10% of the mean value.

In an *in vitro* study employing cultured gastric epithelial cells it was revealed that CQAs, FQAs and caffeoyl quinides (caffeoylquinic acid lactones or CQLs) cross the epithelium by most likely passive diffusion *via* paracellular transport. The authors proposed a facilitated transport

mechanism for the observation that C-4 monoacylated CGAs were transported more rapidly than the CGAs monoacylated at a different position on the QA moiety. Due to their increased hydrophobicity, the diacylated CQAs were observed to cross even more rapidly when compared to the monoacylated CQAs; additionally, for 3,5-diCQA **25** specifically, there was evidence for carrier-mediated efflux.[102]

Such observations are in line with the results of a number of volunteer studies in which it was found that minor components from the CGAs profile of the coffee beverage were detected at higher concentrations in plasma than other CGAs known to be present in comparatively higher amounts in the beverage. An illustrative example was the study by Stalmach *et al.* which looked at maximum plasma concentrations (C_{max}) of different isomers of FQA. It was found that the FQA isomer most abundant in the beverage (5-FQA **16**) peaked at only $C_{max} = 6 \pm 2$ nM, while the values for the less abundant isomers were surprisingly higher, at $C_{max} = 14 \pm 2$ nM (for 4-FQA **15**) and $C_{max} = 16 \pm 2$ nM (for 3-FQA **14**). Stalmach *et al.* identified 21 metabolites (mainly sulfates and glucuronides) in plasma and urine samples of eleven healthy volunteers who were subjected to instant coffee containing thirteen quantified CGAs totaling 412 μmol. **Figure 1.8** reproduces the identified metabolites and the suggested enzymes affecting the metabolism.[103]

The regiomers of CQA were also shown in *in vitro* studies to have different susceptibility to intestinal chlorogenate esterase: 4-CQA **11** was the most resistant, while 5-CQA **12** hydrolysed easier than 3-CQA **10**. Consistent with such *in vitro* results was an *in vivo* study which focused on detection levels of quinic acid and which found the compound in excess of the quantity ingested in free form. The enzymatic hydrolysis by chlorogenate esterase takes place either in the stomach or in the upper gastrointestinal tract (GIT); the study was performed on the ileostomy effluent of volunteers who had servings of coffee, apple juice or apple smoothie in advance.[106] Similar data on maté would be interesting to examine but is currently missing. The observed *in vitro* resistance to chlorogenate esterase of CGAs acylated at C-4 of quinic acid would theoretically result *in vivo* in more elevated levels of such compounds in plasma concentrations.

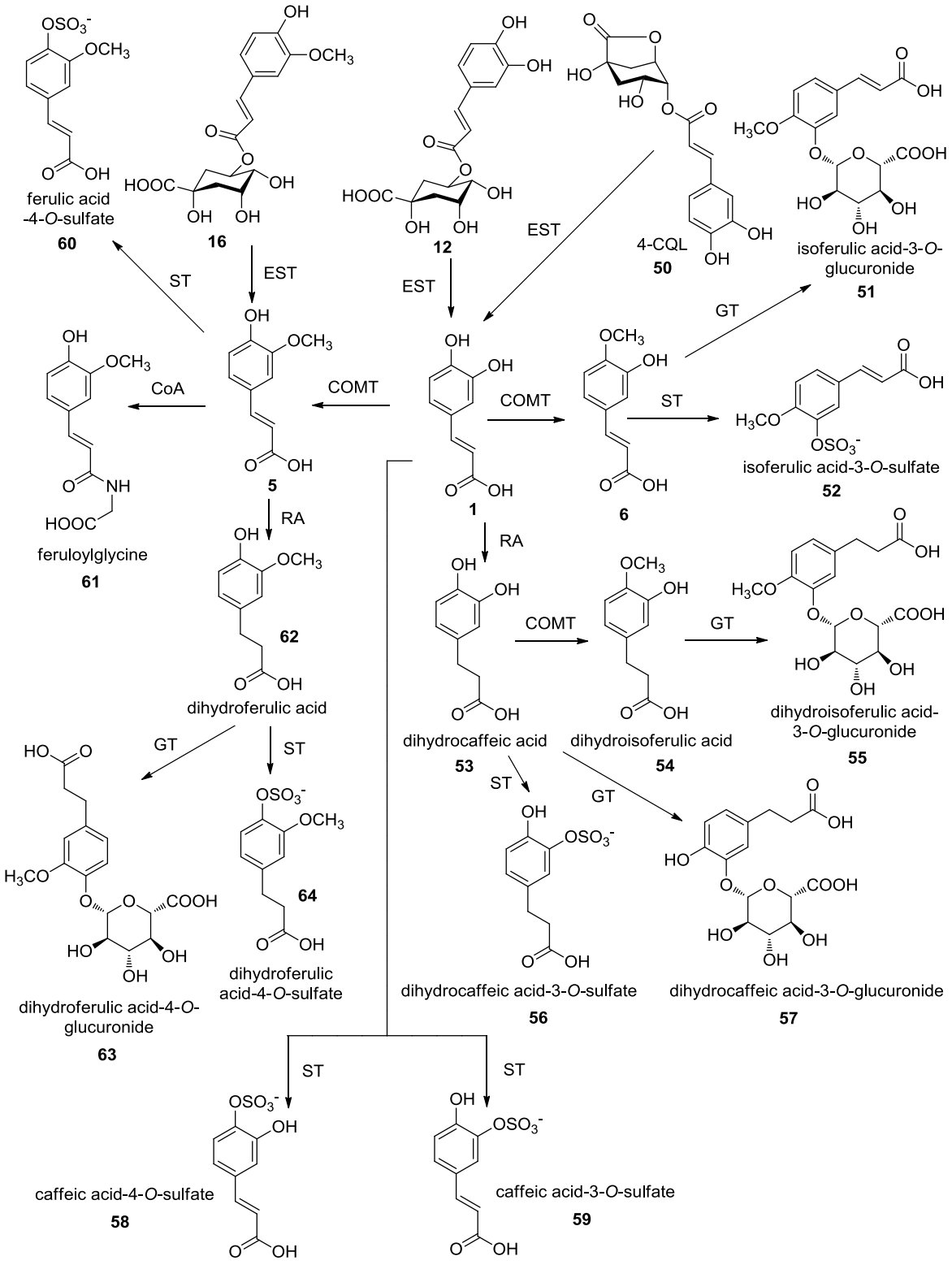

Figure 1.8. CGAs metabolites and enzymes affecting their metabolism: COMT = catechol-*O*-methyl transferase; EST = esterase; RA = reductase; GT = UDP-glucoronyl transferase; ST = sulfate-*O* transferase.[103,105]

Therefore, products with a CGAs profile considerably dissimilar from the one of coffee could generate significantly different plasma profiles of the associated metabolites. Relative to 5-CQA **12**, maté has a comparatively higher content of 3-CQA **10** and diacylated CQAs when compared to coffee and it would be interesting to look at its metabolites plasma profiles.

A number of studies reported on low concentrations in the nM range of CQAs in plasma and low levels in urine after oral intake of coffee[103,107,108], artichoke[109] and 5-CQA **12**[110], implying a limited bioavailability of CGAs. In contrast to these findings, another study reported on concentrations of unmetabolized CQAs in the μM range (C_{max} = 7.7 μM) in the circulatory system after acute ingestion of coffee containing 3395 μmol of CGAs. However, no CGAs were detected in the urine samples collected up to 24 h after ingestion despite the high C_{max}.[111]

The impact of dose on CGAs bioavailability was also investigated. CGAs and their derivatives were looked at in plasma, urine and ileal fluid after coffee consumption by ileostomists, with ingested samples containing 1053, 2219 and 4525 μmole of CGAs.[112] The results of this study by Erk *et al.*[112] were in agreement with the findings by Stalmach *et al.*[104] with a percentage of CGAs in ileal excretion at ~70% of intake, irrespective of the dose. The dose did influence however the amount of conjugated metabolites detected in the ileal fluid which were equivalent to 6.7-8.9 % of the CGAs at higher doses and to 22.3% at the lowest dose. The relative ratio of sulphated metabolites to glucuronides in ileal fluid was also influenced by the dose and while at the lowest dose this was 15.5 times in favor of the first, it decreased to 8.2 at the highest dose.[104,112] Such dose-related changes presumably reflect enzyme saturation, limited transport capacities into and out of the enterocyte in the small intestine and/or differences in GIT transit times. Increasing the CGAs dose resulted in increasing the proportion of glucuronidation and this accounted for the most notable effect of CGAs intake on urinary excretion.[45]

Milk does not appear to meaningfully affect the bioavailability of coffee CGAs, though *in vitro* studies showed that CGAs bind to proteins such as albumin and casein.[113,114] One study found that human consumption of black coffee and black coffee prepared with milk rather than water resulted in urinary excretion of CGAs and metabolites at 68% of intake for the black coffee and at 40% of intake for the black coffee with milk. Though the figures were not considered statistically different, it was concluded that milk may affect negatively the bioavailability of

CGAs in coffee.[115] A different feeding study found no dissimilarity in the pharmacokinetic profiles of plasma CGAs metabolites after consuming black coffee with or without 10% whole milk.[116] Nonetheless, a mixture of sugar and nondairy creamer added to the black coffee lead to lower C_{max} values for caffeic acid **1** and isoferulic acid **6** and resulted in longer T_{max} values for ferulic acid **5** and isoferulic acid **6**.[116] The delayed absorption of the coffee CGAs, which resulted in a longer T_{max} for two of the three metabolites may be due to the added sugar and creamer since sugar[117] and lipids[118] were shown to delay gastric emptying.

1.5. Coffee

The presence of CGAs and their derivatives in coffee has been linked to the positive health effects of the popular beverage; this is one of the main reasons for the extensive scientific attention received by coffee in the past 15 years. CGAs and their derivatives found in coffee have been the object of study in a number of *in vitro* models, in animals and in humans through epidemiological studies.[1-5,40,41] CGAs and especially their derivatives are among the main contributors to the sensory and organoleptic properties of coffee.[6]

With a production figure approximated at eight million metric tonnes per year, coffee represents one of the most valued agricultural commodities for the coffee-producing developing countries in terms of the economic aspects of exports. With an approximate average of 2.3 billion cups consumed worldwide daily, coffee is mankind's third most consumed beverage, following only water and black tea. In a number of countries, among which Germany and the USA, coffee consumption supersedes black tea consumption, thus the beverage being second in consumption after water. In the USA the average daily consumption is over 450 million cups of coffee.[2,44] The coffee beverage is consumed by the human population all over the world (70% - 80%).[119] In certain populations studies report on a genre difference in coffee consumption, which was found significantly higher in males (50.8%) compared to females (32.8%) in the young population (Japan, aged approx. 18-20 years).[120]

The coffee plant is a member of the Rubiaceae family and is cultivated in over 70 countries globally. Its seeds, the coffee beans (nomenclature might be misleading since they are not actual beans), are contained within the fruits of the plant commonly referred to as "cherries" (drupes). Among the numerous major species of coffee, the two most important species economically are

Coffea arabica (Arabica coffee) and *Coffea canephora* (Robusta coffee), accounting for approximately 70% and 30%, respectively of the world production.[4] The next species economically and as popularity is *Coffea liberica* (Liberica coffee) with the variety *Coffea dewevrei* (Excelsa coffee), though on a much smaller scale (International Coffee Organization (ICO), 2014).

Coffee is also the second most traded commodity in the world after crude oil and is the most traded tropical agricultural commodity, with exports worth an estimated US $15.44 billion in 2009/2010. More than 97% of the coffee produced globally originates from the Exporting Members of the ICO (among the over 70 coffee-producing countries); its estimated annual retail value exceeded $70 billion in 2010 with a total coffee sector employment estimated at approximately 26 million people in 52 producing countries (ICO, 2011). Starting with the early 1980s, coffee consumption has increased globally by about 1.2% each year. One of the most significant growths on the major markets took place in Japan, currently the third largest coffee importer in the world (ICO, 2011).

The polyphenolic components in the coffee have been positively linked to the observed beneficial health effects. The concentration and profile of the polyphenols present in green coffee and roasted coffee varies considerably, with different roasting conditions (such as roasting times and temperatures) generating different derivatives of the polyphenolic class. Controlling for such parameters during the roasting process has a direct impact on the antioxidant properties of the resulting beverage. The chemoprotective properties of coffee are a consequence of the coffee constituents that possess antioxidant activity (and their metabolites), the popular drink being a major source of dietary antioxidants. The underlying mechanism for the antioxidant potency of coffee is believed to be a complex one involving different processes such as active oxygen trapping, radical scavenging or transition metal chelation. Besides the CGAs present in coffee, other compounds or classes of compounds were proven to have a contribution towards the antioxidant properties of coffee, among which melanoidins and phenylindanes in roasted coffee or caffeine and caffeoyl-tryptophan.[121,122]

Regular coffee drinking has been positively linked to a series of beneficial health effects such as a reduced risk of rectal cancer, hepatic cirrhosis, cardiovascular disease or diabetes mellitus

among others.[123-132] A meta-analysis study on coffee consumption correlated to the risk of colorectal cancer (12 case-control studies) found a general lower risk for regular drinkers despite the lack of consistency of the discussed results.[123] An inverse association between coffee consumption and risk of colorectal cancer was revealed from the combined results of the 12 studies yet the results of the meta-analysis were considered inconclusive because of a number of reasons: possible avoidance of coffee consumption by the unidentified high-risk individuals, inconsistencies between case-control studies and prospective studies and the lack of control for important covariates in some of the studies.[123] Coffee consumption was also proved beneficial for patients undergoing cervical cancer treatment by radiotherapy; the results of the epidemiological study showed that coffee consumption reduced the risks of severe late radiation injuries.[133]

In a study performed on a middle-aged Norwegian population (aged 20-55 years, median 43.6 years) with a high coffee intake, an inverse relationship between coffee consumption and mortality from liver cirrhosis was found though the underlying mechanisms for the observed negative correlation remained elusive.[124] Norwegians are among the highest per capita coffee consumers in the world and the country has a restrictive alcohol policy in place which is effective at keeping liver cirrhosis mortality rates low.

Another study performed in the USA found that coffee and tea drinking decreased the risk of clinically significant chronic liver disease. The rate of the chronic liver disease for patients who drank more than two cups of coffee per day was less than half the rate of the disease for those who drank under a cup per day. The clinical significance of the protection by coffee and tea against liver injury was limited to individuals at higher risk for liver diseases caused by alcohol intake, diabetes, overweight and high iron saturation.[125] In a Japanese population an association between habitual coffee drinking and reduced risk of hepatocellular carcinoma was found in an epidemiologic study on a high-risk population.[126] Other studies pointed at a reduced risk of developing cirrhosis through excessive alcohol consumption in individuals who were also regular coffee drinkers.[127-129]

It was also found that coffee consumption reduced the risk of cardiovascular and other inflammatory diseases in postmenopausal women by inhibiting inflammation. The protective

effect was consistent with an intake of 1-3 cups daily.[130] Caution was recommended in generalizing the results since there was no control in the study on the method of preparation and the source and type of coffee beans (coffee beans could be of considerable variation and a diversity of noncoffee foods could be added to coffee in different amounts). It was speculated that the percolator and the filter methods were the likely main methods for the beverage preparation by the subjects.[130]

Other study pointed at a possible protective effect by coffee consumption against the development of type 2 diabetes mellitus by inhibition of postprandial hyperglycemia. The relationship between green tea consumption and glucose tolerance status was also looked at in this study performed on middle-aged (46-59 years) Japanese men but no association was observed for green tea.[132] Another study found in a population of subjects of both sexes (aged 40-65 years) a positive association between a reduced risk of type 2 diabetes and consumption of green tea, coffee and total caffeine intake. An association between risk of diabetes and consumption of black or oolong teas was not found.[131]

Clifford and coworkers postulated that coffee consumption (through its CGAs content) modulated glucose uptake, gastrointestinal hormone and insulin secretion in humans. It was concluded that caffeine has a potent biological action to account for the observed differences in plasma glucose, gastrointestinal hormone and insulin profiles and it was proposed that 5-CQA **12** may impart an antagonistic effect on glucose transport.[134] Consequently, it was speculated that certain dietary phenols may have a novel function in humans, namely to attenuate the rates of intestinal glucose absorption and shift to more distal parts of the small intestine the site of glucose absorption.[134]

Besides the high CGAs content in coffee as already mentioned in the current chapter, caffeine is an important component in the beverage with figures varying between 80-200 mg per typical serving (on average 120 mg per 200 ml cup). Caffeine's LD_{50} was found to be 192 mg/kg in rats when given orally, while for humans it is estimated to be between 150-200 mg/kg, depending to a small extent on individual sensitivity.[135] Symptoms of caffeine intoxication (caffeinism) appear if more than 400-500 mg caffeine is ingested at a time. As an important component of coffee, caffeine contributes to the observed positive health effects associated with the beverage, being

the most widely consumed neuroactive compound that is behavior-modifying. Caffeine interacts with the adenosine receptors in the central nervous system being an antagonist of the receptor, which further affects the release of a number of neurotransmitters such as dopamine, acetylcholine, noradrenaline or the γ-aminobutyric acid (GABA)/benzodiazepine system.

Among the positive health effects associated with caffeine consumption, performance improvement, fatigue reduction and alertness were reported.[136] Caffeine was labeled as an active anti-depression compound having been positively associated with prevention of suicidal tendencies. Behavioral modification through coffee-induced neuroactivity was also demonstrated in an epidemiological study where suicidal tendencies were reported to be three to five times lower when compared to the placebo group.[137] Other studies linked caffeine intake to decreased irritability,[138] improved mood,[139] and a series of positive effects such as a subjective increase in the feeling of wellness, motivation, self-confidence and a decrease in social anxiety.[140] Recent recommendations by the European Food Safety Authority (EFSA) include figures for habitual daily caffeine consumption of up to 400 mg for non-pregnant adults and up to 200 mg for pregnant and lactating women. These daily maximum intake figures do not give rise to safety concerns.[141]

1.6. References

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2. Aims and Objectives

In general terms, the purpose of the present thesis was to investigate the chemical fate of hydroxycinnamates at elevated temperatures. To this end, a combination of organic/ organometallic synthesis and analytical techniques (most powerful for our goal: LC-MS^n and NMR) was employed.

Firstly, synthesis of the relevant CGAs derivatives (and sugar conjugates) resulting from food thermal processing was indispensable. The best approach for the synthetic part was to systematically address the derivatives by classes (e.g., lactones *versus* methyl esters; mono- *versus* diacylated CGAs), especially that each class (e.g., lactones, sugar conjugates) bears its own peculiarities from a synthetic point of view. The synthetic part involved development of orthogonal protecting group strategies suitable for each class of derivatives. The synthesized compounds became then standards against which the existence of such derivatives could be probed in different processed food samples.

Additional objectives and landmarks of the project were the development of LC-MS^n methods able to discriminate within regioisomers of the same class and in some cases between compounds belonging to different classes (but of the same *m/z* e.g., a shikimate and a quinide). In order to illustrate the power of such newly-developed methods, series of isomers were assigned from natural sources (e.g., yerba maté leaves) or processed food samples (e.g., brewed coffee). The goal was to afford a convenient way to unambiguously identify such compounds from natural sources, processed food and pharmacokinetic metabolite studies. Eliminating controversy and potential sources of misassignment of isomers was also set as a goal for the project.

Once synthesized, the compounds were made available for further testing and investigation (e.g, Raman studies, ion mobility, single cell patch clamp measurements) in order to correlate certain activities to specific compounds. The testing part involved collaborations with other research groups. The ultimate goal of the entire project was to identify compounds formed in food processing and based on their properties, provide the consumer scientifically sound advice about different processed foods of common daily use.

3. Synthesis of Chlorogenic Acid Derivatives

3.1. General Synthetic Strategy

The chlorogenic acid derivatives, as well as some CGAs, synthesized in the current project were obtained by condensing a protected acid chloride (of the respective hydroxycinnamic acid) with a derivative of quinic acid. The general protecting group strategy involved protecting groups, which were acid-labile, base-labile or labile to metal reducing agents. Firstly, the protected hydroxycinnamic acids derivatives were synthesized and converted into the more reactive acid chlorides; secondly, the quinic acid derivatives were obtained by protecting the reactive alcohol and carboxylic acid moieties of the parent compound in order to prevent undesired side reactions, while leaving unprotected the alcohol moiety/moieties where esterification with the acid chloride was desired; finally, after the condensation of the two generated building blocks, overall deprotection afforded the desired CGAs and derivatives.

Some previous attempts of synthesizing CGAs derivatives by this general strategy have not proven completely successful, with complications in the overall deprotection stage preventing the generation of the final target compound.[1] Particularly for lactones, the deprotection of the phenols (while not modifying the rest of the molecule) of the hydroxycinnamic moieties has proven not to be a straightforward task.[2] In particular, removing one ester protecting group in the presence of other ester moieties is problematic, since it is associated with low yields and byproducts necessitating laborious purification steps. The main challenge of the project was the development of orthogonal protecting group strategies, those that were able to remove selectively one protecting group (or a set of protecting groups if this made the synthetic path shorter) with the aid of specific reagents and conditions, which did not affect other protecting groups. The main hydroxycinnamic acids chosen, based on their occurrence in the targeted natural and processed food samples, were caffeic, ferulic and 3,4-dimethoxycinnamic acids. Orthogonal protecting group strategies were successfully developed and optimized for each set of monoacylated (e.g., 1-*O*-caffeoyl-1,5-quinide **19**, 1-*O*-feruloyl-1,5-quinide **13** and 1-*O*-dimethoxycinnamoyl-1,5-quinide **4**) and diacylated derivatives. Suitable protecting groups needed to be employed for the phenols on the hydroxycinnamoyl moieties and for the alcohols residing on the parent quinic acid residue. Since dimethoxycinnamoyl-containing compounds

needed no protecting group for phenols, such compounds were the easiest to synthesize, their synthetic paths being typically shorter by two steps. In the present project three different protecting groups were attempted for the phenols of the hydroxycinnamoyl residues: acetyl,[3] 2,2,2-trichloroethoxycarbonyl (Troc)[4] and allyl.[5] Acetyl is both acid- and base-labile. Base-removal can be accomplished successfully but it additionally opens lactones, thus introducing an extra step in the synthesis (closing back the lactone). Though very promising in the beginning, acetyl proved to be problematic since its acid-induced deprotection generated many side products from which the desired product could not be purified. Although commonly used for phenols protection, acetyl groups proved to be unreliable since deprotection in either basic or acidic conditions[6] resulted in incomplete or non-selective removal of the acetate groups, with competing hydrolysis of the cinnamate quinic acid ester bonds.[7,8] Nevertheless, synthesis and purification with acetyl could be carried out successfully for 1-*O*-feruloyl-1,5-quinide **4** *via* base-removal, with one extra step for closing back the lactone. An alternative protecting group was searched for which can be removed without affecting the lactone, since in the acetyl-protection synthetic path a non-elegant lactone closed-open-closed sequence is needed. Troc represents a widely employed protecting group in organic synthesis and it is able to protect both aliphatic and aromatic alcohols.[4] Nevertheless, Troc proved successful as a protecting group for the feruloyl hydroxyl but in our hands could not protect both hydroxyls of the caffeoyl moiety, generating a mixture from which the desired product could not be isolated. Consequently, a synthesis with Troc-protected feruloyl could be successful but was not pursued since a universal protecting group (for the purpose of the project, the same group for both caffeoyl and feruloyl) was sought for. Finally, allyl protection[5] proved successful for both caffeoyl and feruloyl, its removal working well (though requiring column chromatography for purification) and not affecting in any way the remaining functionalities in the molecule.

The quinic acid derivatives needed for the esterification steps could be afforded in good to excellent yields by selectively protecting the desired moieties (one carboxylic acid and four alcohols). Whenever referring to yields in the current work, the conventions proposed by Vogel were consistently employed: yields around 100% are referred to as “quantitative”, yields higher than 90% are considered as “excellent”, yields higher than 80% are considered as “very good”, yields higher than 70% are considered as “good”, yields higher than 50% are considered as

"fair", and yields lower than 40% are considered as "poor".[9] Purities of the synthesized compounds (intermediates and final products) were, with some exceptions, typically above 90% as determined by NMR and by LC-MS. The carboxylic acid was either locked in a *γ*-quinide[10] or esterified typically with a short alkyl chain (e.g., methyl).[4] The hydroxyl attached to C1 was Troc-protected;[4] the hydroxyl attached to C-5 was part of the *γ*-quinide;[10] the vicinal *cis*-diols at C-3 and C-4 were jointly protected either with an isopropylidene[10] or a cyclohexylidene acetal;[10-12] an isopropylidene acetal was also used for a joint protection of COOH and the C1 hydroxyl;[10] the vicinal diequatorial *trans*-diols at C-4 and C-5 were jointly protected with a butane 2,3-bisacetal (BBA).[13] The current chapter offers comments mainly on the final products of the syntheses, all other synthesized intermediates, whether novel or not, are described in the **Experimental** section (current chapter) while spectra are shown in the **Appendix**.

3.1.1. Synthesis of 1-*O*-Dimethoxycinnamoyl-1,5-quinide (1-DQL) 4

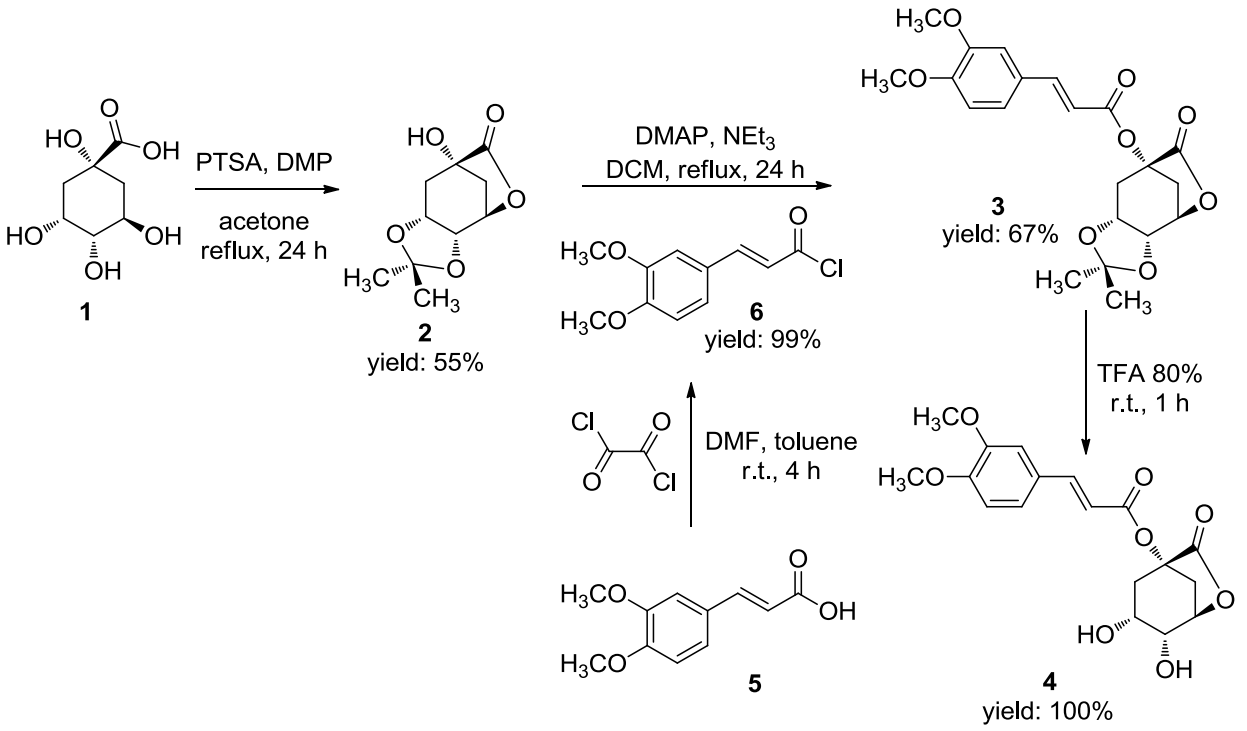


Figure 3.1. Synthetic path to 1-DQL **4**.

1-*O*-dimethoxycinnamoyl-1,5-quinide **4 (**abbreviated 1-DQL from 1-*O*-dimethoxycinnamoyl quinic acid lactone**)** required no protection for a phenol moiety unlike the two structurally-related compounds bearing a caffeoyl (1-CQL **19**) or feruloyl (1-FQL **13**); the reactive moieties (except the hydroxyl at C1) of the quinic acid **1** could be protected in one step.[10] This compound was afforded according to the above scheme (**Figure 3.1**). The nomenclature of CGAs was explained in **Chapter 1**; for *γ*-quinide derivatives, the same numbering of the QA moiety is maintained (e.g., C-5 position in a DQA remains as C-5 in a DQL) for simplicity. Isopropylidene quinide **2** was synthesized under conditions for a thermodynamic acetalization of quinic acid **1** by refluxing the substrate in acetone in the presence of *p*-toluenesulfonic acid (PTSA) and 2,2-dimethoxypropane (DMP).[10] In the original publication, Rohloff et al. proved that the ratio of the desired protected quinide **2** to bisacetonide **23** (**Figure 3.15**) could be altered to preferentially form the first (92:8) under specific conditions (reflux, 2 h).[10] In the current project, isopropylidene quinide **2** was obtained in fair yield (55%) after repeated recrystallizations. The advantages however, are the multiple protections achieved in one step (formally, three alcohols and one carboxylic acid) and the very high purity of the product. Crystals of **2** suitable for single crystal X-Ray Diffraction (XRD) were also generated in the process and are shown in **Figure 3.2**.

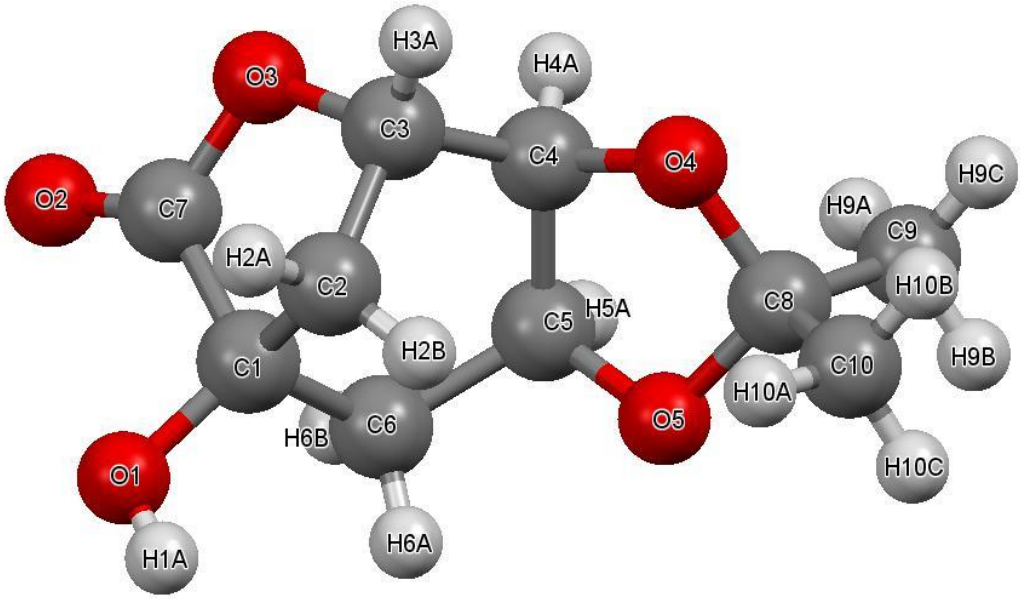


Figure 3.2. X-ray crystal structure of 3,4-*O*-isopropylidene-1,5-quinide **2**.

The acid chloride **6** preparation goes in very high to quantitative yield and should be stored under inert conditions because of its sensitivity to water;[5] all the other intermediates in the above reaction scheme are not water or air sensitive. Product **3** requires column chromatography for purification and was obtained in 67% yield.[5] Crystals of **3** suitable for single crystal XRD were also generated in the process and are shown in **Figure 3.3**. Intermediate **3** was previously synthesized and characterized (as an intermediate in the 1-DQA synthesis)[2] and assignment of all signals was done with the help of 2D NMR techniques (COSY and HMQC); ^{1}H-NMR, ^{13}C-NMR, 2D NMR and analogies to structurally similar compounds were used to assign all the signals in the NMR spectra present in the current chapter. Product **4** was also previously synthesized[18] and peak assignment was done by comparison.

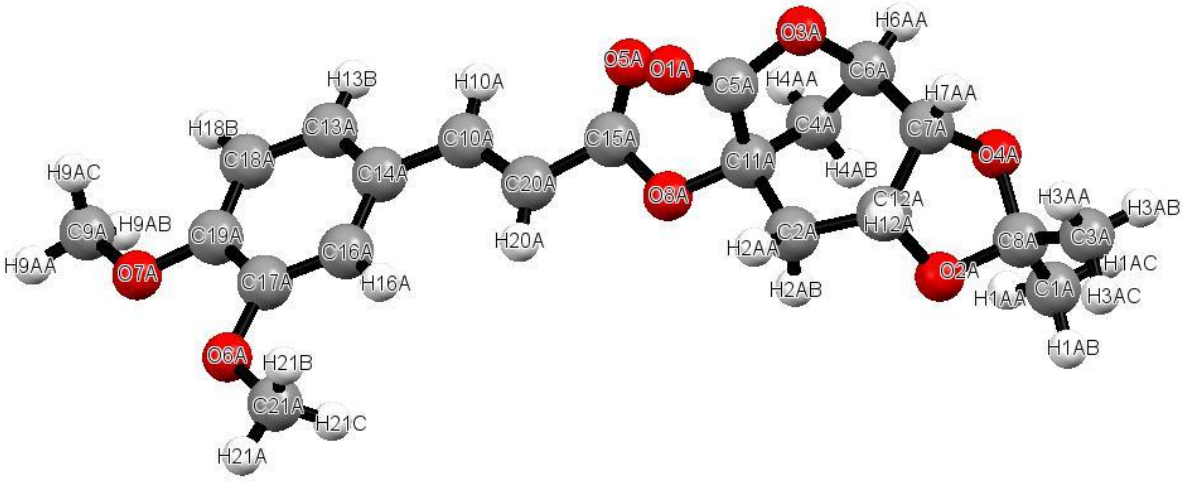


Figure 3.3. X-ray crystal structure of 1-*O*-(3',4'-dimethoxycinnamoyl)-3,4-*O*-isopropylidene-1,5-quinide **3**.

It has often been observed in the purification of the products generated after the esterification step that they co-eluted with their corresponding carboxylic acids (dimethoxycinnamic acid **5** in this case); this was the case even when no flash conditions were used for the chromatographic purification. The reason is the excess acid chloride used in the reaction which gets converted back to the parent carboxylic acid during the aqueous work-up. Therefore, for compound **3** a base extraction was performed prior to the chromatographic purification. Since quinides are sensitive to LiOH (pK_b = -0.63, opened the lactone completely within minutes), the weaker base

$NaHCO_3$ solution (pK_a = 10.33) was used for washing. Product **4** required no purification since all generated side products from the deprotection step are volatile; therefore, water, trifluoroacetic acid (TFA) and the resulting acetone (from the isopropylidene deprotection) can be evaporated under reduced pressure to afford **4** quantitatively.[14]

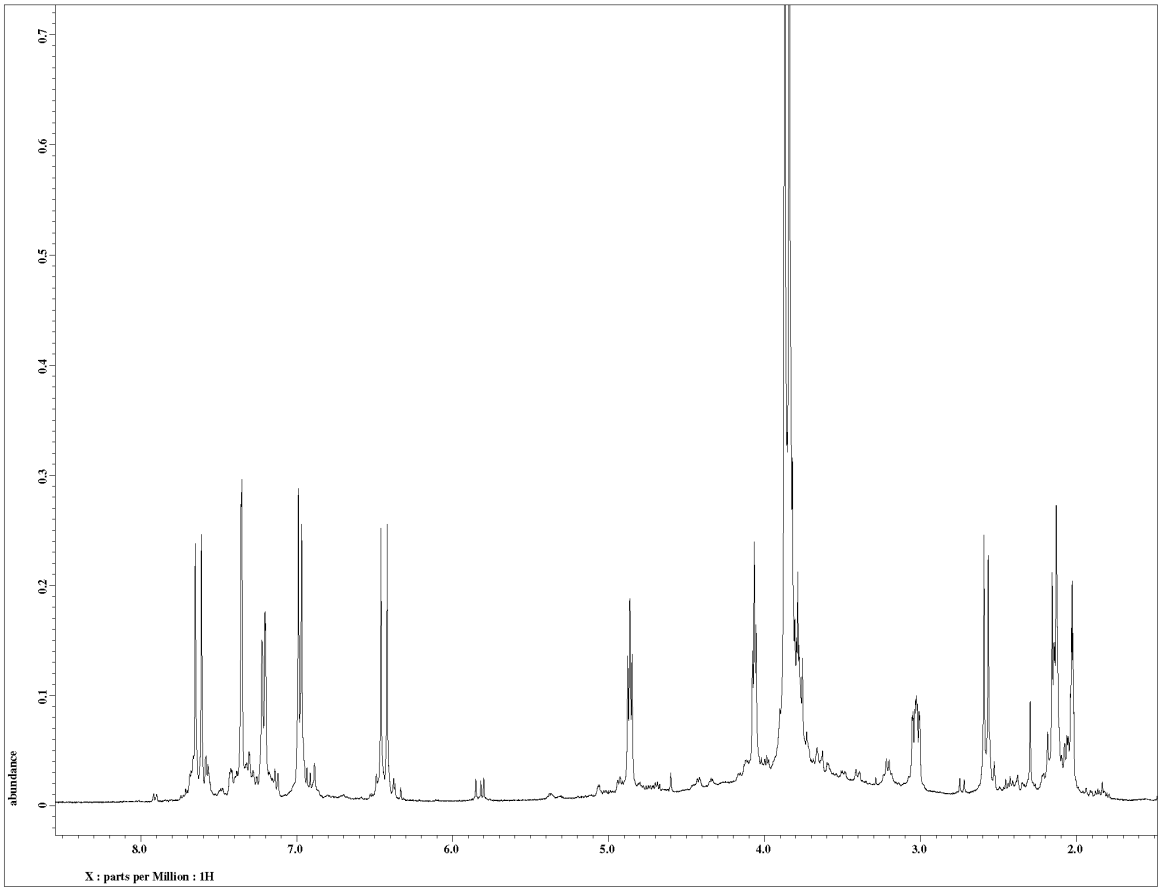


Figure 3.4. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) spectrum of 1-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (1-DQL) **4**.

The spectroscopic data for all compounds in **Figure 3.1** was in full agreement with the structures. The ^{1}H-NMR spectrum (**Figure 3.4**) of the final product **4** showed three signals corresponding to the three aromatic protons on the cinnamoyl residue, each integrating as one proton: a doublet at 7.35 ppm corresponding to the proton on C-2' coupled to the C-6' proton (^4J = 1.8 Hz); a doublet of doublets at 7.21 ppm corresponding to the proton on C-6' coupled to the other two aromatic protons (^3J = 8.2 Hz with the proton on C-5' and ^4J = 1.8 Hz with the proton

on C-2'); and a doublet at 6.97 ppm corresponding to the proton on C-5' coupled to the C-6' proton (^3J = 8.2 Hz). The other two doublets, each integrating as one proton and coupling to each other (^3J = 16.0 Hz), appearing downfield in the ^{1}H-NMR spectrum were the olefinic moiety protons in the cinnamoyl residue at 7.63 ppm (C_{Ar}-CH) and 6.44 ppm (C_{Ar}-CH=C*H*). The 16 Hz coupling constant is typical for a *trans* olefinic geometry. The protons of the two methoxy groups in the molecule resonated as singlets at 3.87 ppm and 3.84 ppm, respectively, each signal integrating as three protons. The C-5, C-4 and C-3 protons of the quinic acid moiety resonated at 4.87 ppm, 4.07 ppm and 3.83 ppm, respectively. The proton at C-4 was split into a doublet of doublets by the two protons attached to the vicinal carbon atoms, while the protons at C-3 and C-5 appeared as multiplets. The acylated 1,5-quinide specific signal (equatorial proton at C-6) came at 3.03 ppm as a doublet of doublets of doublets. The splitting, observed for other structurally similar compounds, is due to coupling to the geminal C-6 proton (^2J = 11.0 Hz), to the proton at C-5 (^3J = 6.0 Hz) and to the equatorial proton at C-2 (^4J = 2.3 Hz). The axial proton at C-6 appeared as a doublet at 2.58 ppm (^2J = 11.0 Hz) while the two protons at C-2 appeared as multiplets between 2.02-2.18 ppm.

As seen here and as a general observation, the protons attached to C-6 in quinide derivatives tended to be downfield shifted compared to the protons on C-2. The coupling constants of the quinide protons were in agreement with the values predicted by the Karplus relationship, which describes the correlation between $^3J_{H\text{-}H}$ coupling constants and dihedral torsion angles, and thus provides information about the spatial orientation between the two protons, in agreement with the XRD structure.[15] The same is true for the other quinic acid derivatives (not only quinides) synthesized in the current project. The $^3J_{H\text{-}H}$ coupling constants between two (pseudo-)equatorial protons were typically found in the interval 4.0-6.0 Hz (e.g., H-5 and equatorial proton H-6). The values for the $^3J_{H\text{-}H}$ coupling constants between two (pseudo-)axial protons were characteristically very large, between 11.0-12.0 Hz (e.g., H-3 and axial proton H-2) suggesting dihedral torsion angles of approximately 150° in compounds diacylated at C-3 and C-4; these values point at a conformation of the cyclohexane ring in the quinic acid moiety not too far from the low-energy chair. However, in isopropylidene- or cyclohexylidene-protected quinide intermediates (e.g., **3** or **52** in **Figure 3.42**) the values for the $^3J_{H\text{-}H}$ coupling constants of such protons decreased, being observed typically in the interval 7.0-8.0 Hz (dihedral torsion angles of

130-140°) because the strain induced by the protective group forces the cyclohexane ring of the quinic acid moiety into a conformation that looks closer to a half-chair, as also suggested by the XRD data. The $^{3}J_{H\text{-}H}$ coupling constants between one (pseudo-)equatorial proton and one (pseudo-)axial proton were typically small (around 3.0 Hz) for isopropylidene- or cyclohexylidene-protected quinide intermediates (e.g., **3, 52**), depending on how far such dihedral angles deviated from 90° (e.g, 2.8 Hz for H-3 and equatorial proton H-2 in **3**). For compounds diacylated at C-3 and C-4 (e.g, **28** in **Figure 3.18**) the values for the $^{3}J_{H\text{-}H}$ coupling constants were around 7.0 Hz and were always observed between the (pseudo-)axial H-3 proton and the (pseudo-)equatorial proton H-2 but never between the (pseudo-)axial proton H-6 and the (pseudo-)equatorial proton H-5.

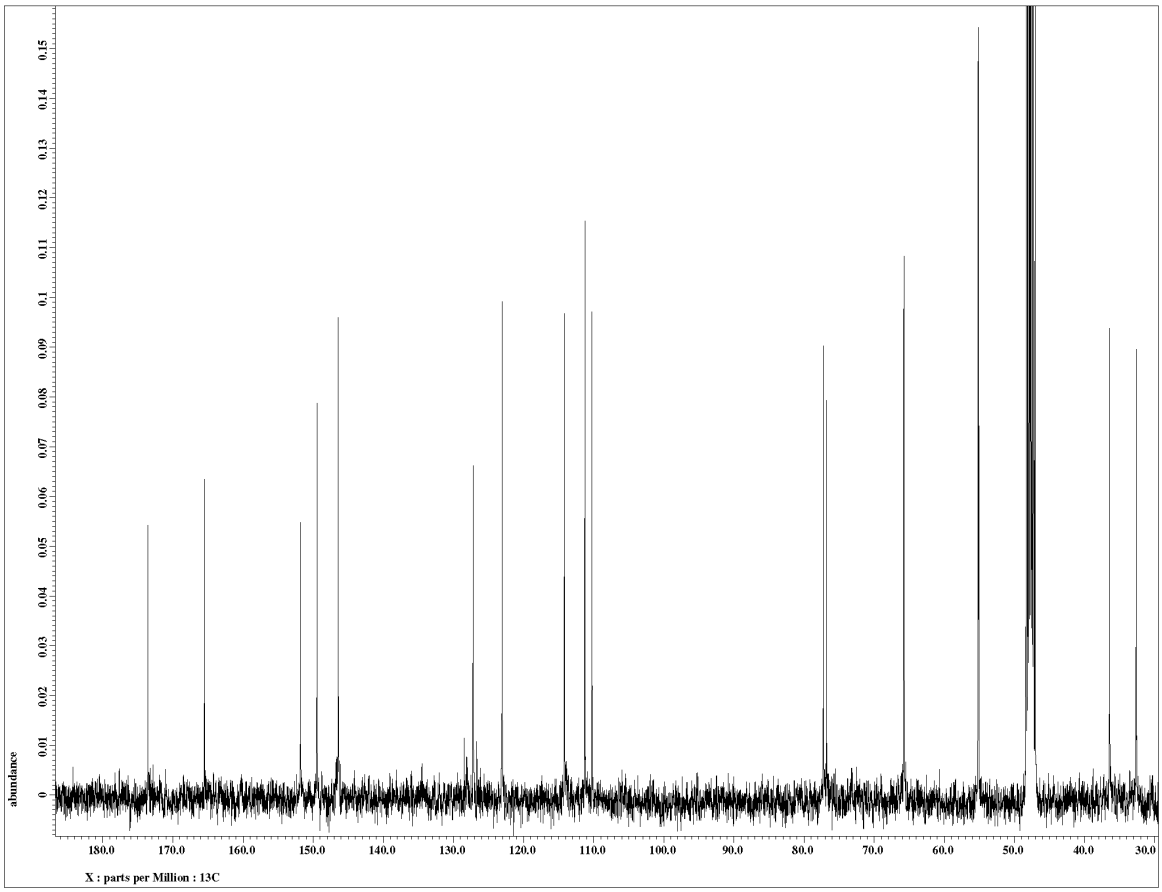


Figure 3.5. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of 1-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (1-DQL) **4**.

The ^{13}C-NMR of **4** (**Figure 3.5**) showed 18 peaks as expected. The ester carbon of the lactone (OC-*C*OO at 173.43 ppm) appeared more downfield than the cinnamoyl ester carbon (CH-*C*OO at 165.48 ppm). The aromatic carbon atoms bearing the electron withdrawing methoxy functionality also came downfield at 151.79 ppm (C_{Ar}-OCH_3) and 149.46 ppm (C_{Ar}-OCH_3), respectively. With one methoxy group in a *para*-position but with another one in a *meta*-position, the aromatic carbon attached to the electron withdrawing group substituted olefin resonated more upfield at 127.29 ppm (C_{Ar}-CH); the remaining of the aromatic carbons appeared at even higher field (C-6' at 122.97 ppm, C-5' at 111.25 ppm and C-2' at 110.26 ppm). The two olefinic carbons resonated at 146.43 ppm (*C*H-C_{Ar}) and 114.03 ppm (C_{Ar}-CH=*C*H), the carbon nucleus closer to the aromatic moiety being more deshielded. The four carbon atoms of the quinic acid moiety bearing heteroatom-containing substituents resonated as following: the C-1 atom bearing the acylated dimethoxycinnamoyl (electron withdrawing) and the electron withdrawing lactone -COOR appeared most downfield of the four carbons at 77.24 ppm; the C-5 atom bearing the electron withdrawing group -OCOR appeared next at 76.81 ppm; the C-3 and C-4 atoms, each bearing a hydroxyl, resonated at 65.72 ppm and 65.77 ppm, respectively. The two methoxy carbons resonated at 55.18 ppm (C_{Ar}-O*C*H_3) and 55.10 ppm (C_{Ar}-O*C*H_3) while the C-2 (36.43 ppm) and C-6 (32.64 ppm) carbons of the quinic acid moiety appeared most upfield in the spectrum of all carbons of the molecule.

3.1.2. Synthesis of 1-*O*-Feruloyl-1,5-quinide (1-FQL) 13 and 1-*O*-Feruloylquinic Acid (1-FQA) 64

1-*O*-feruloyl-1,5-quinide **13** was supposed to be synthesized following a similar reaction scheme as for 1-DQL **4** but a number of complications appeared on the way caused mainly by the protecting group (acetyl) employed for the phenol. The actual synthetic path, by which it was obtained, is shown in **Figure 3.6**. Prior to generating the acid chloride **9** the phenol needed protection and acetyl was tried for this purpose. The procedure reported by Sefkow was employed to synthesize cinnamic acid chloride **9** in two steps.[5] Firstly, ferulic acid **7** and acetic anhydride were reacted in pyridine in the presence of the Steglich catalyst (5% 4-dimethylaminopyridine, DMAP) for the nucleophilic acylation product **8** obtained in high yield (90%). Secondly, the acetate-protected cinnamic acid **8**, dissolved in dichloromethane (DCM), was transformed (95% yield) in the water-sensitive acid chloride **9** in the presence of oxalyl

chloride and dimethylformamide (DMF).[5] The synthesis of 1-FQL **13** was then carried out and intermediate **10** needed column chromatography (yield 61%) as expected even after removal of the side product acetylferulic acid by washing with a $NaHCO_3$ solution. Acetyl and isopropylidene protections were then attempted to be removed in one step with a TFA solution (80-90%, 50 min to 2 h) but the effort and optimization experiments did not lead to the desired product.[14] Although isopropylidene was completely removed, every attempt gave a series of unidentified compounds from which the target product could not be isolated. HCl instead of TFA did not produce better results.

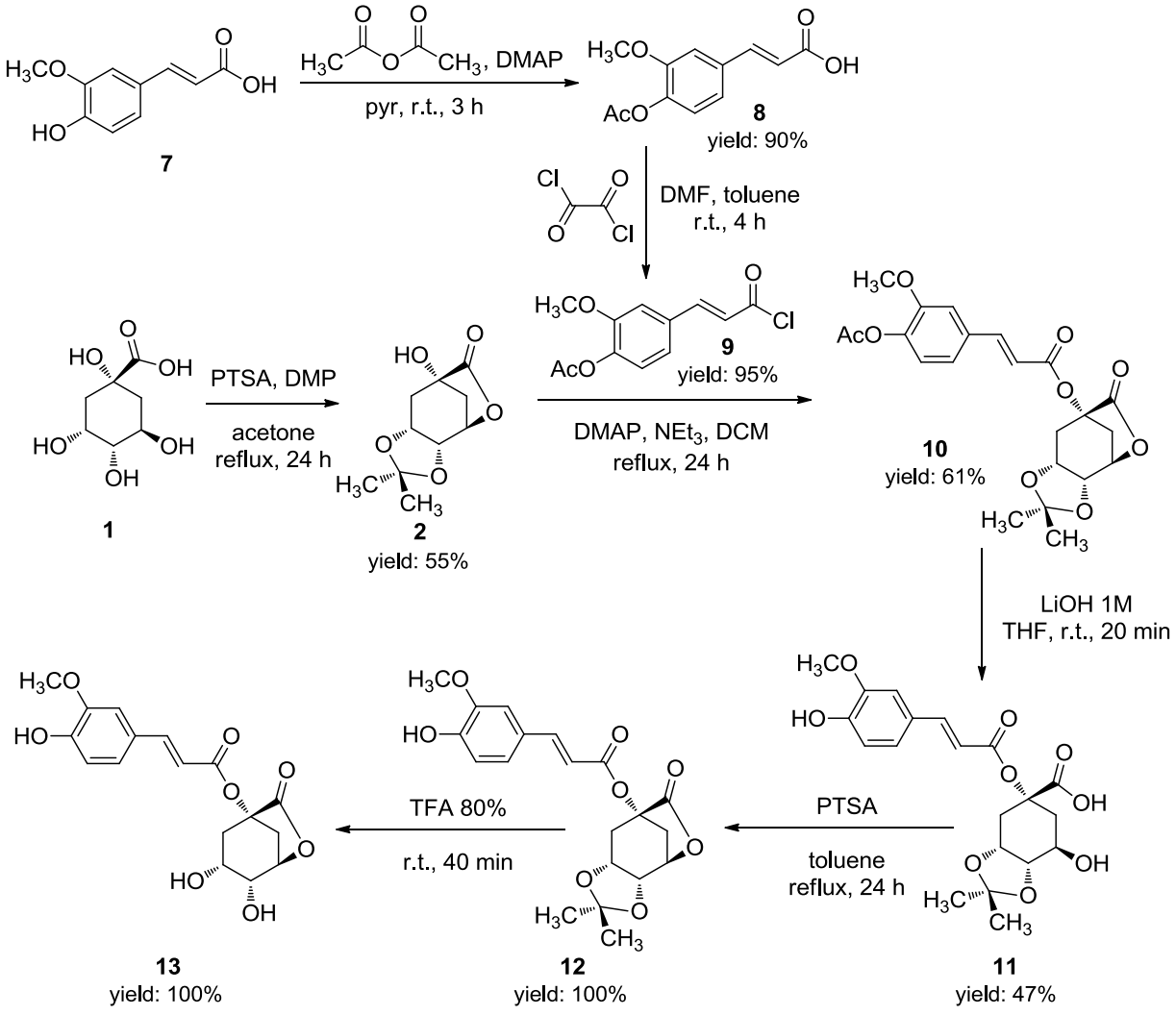


Figure 3.6. Synthetic path to 1-FQL **13**.

Consequently, the acid deprotection was abandoned and the attention was focused on the base deprotection. LiOH effected the desired transformation, did not remove isopropylidene but opened the lactone (yield 47%).[4] After purification by column chromatography the lactone was closed successfully under reflux conditions with PTSA in toluene, which additionally removed the isopropylidene protection partially.[10] The remaining isopropylidene was totally removed in the next step with a TFA 80% solution to give the final product **13** quantitatively. Though not attempted for this particular compound, the allyl protection (instead of acetyl) for the phenolic moiety of the feruloyl is likely to result in better yields and higher purities,[14] judging by the results obtained with it for other feruloyl as well as caffeoyl derivatives synthesized in the current project, as described later in this chapter.

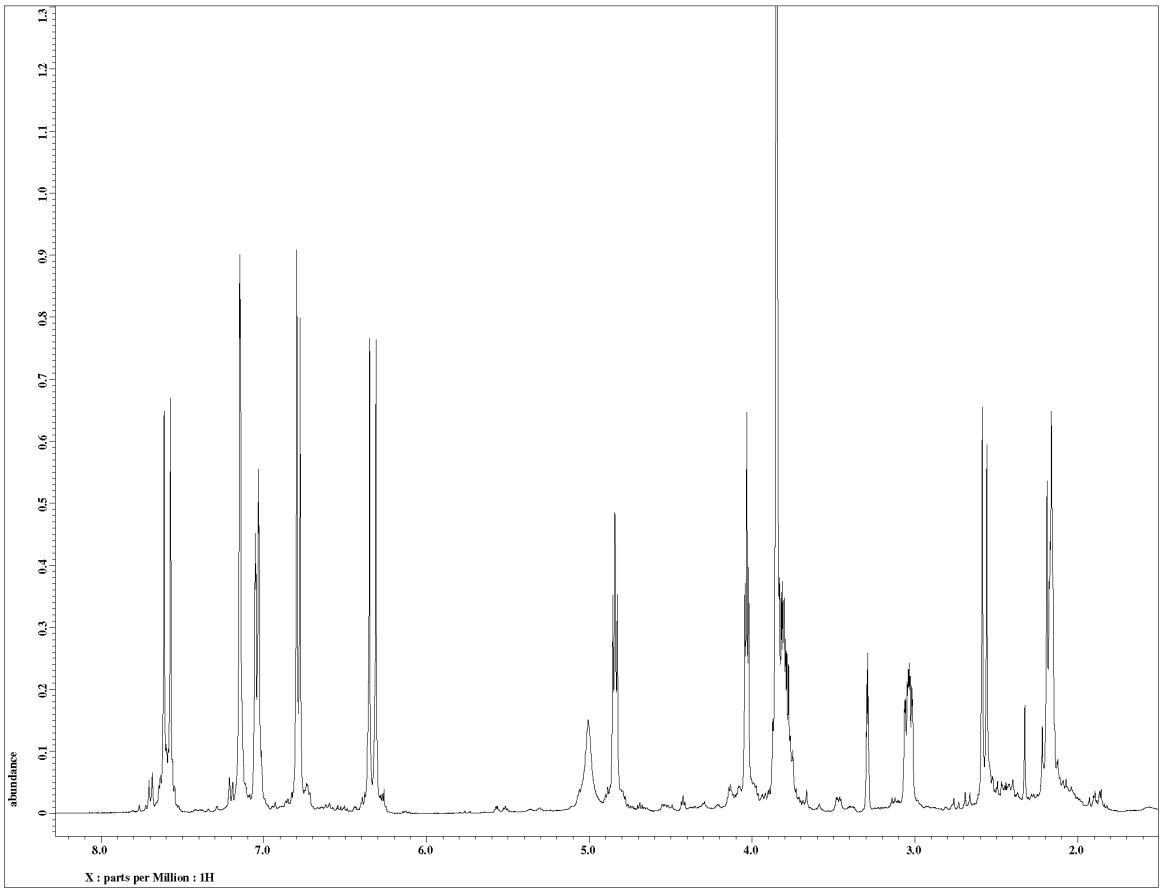


Figure 3.7. ^{1}H-NMR (CD_3OD, 400 MHz) spectrum of 1-*O*-feruloyl-1,5-quinide (1-FQL) **13**.

The spectroscopic data for all compounds in **Figure 3.6** was in full agreement with the structures and assignment was done by analogy to the corresponding dimethoxycinnamic acid-containing compounds in **Figure 3.1**; additionally, intermediate **10** was synthesized and characterized before, as a precursor for 1-FQA **64**.[2] Compounds **11-13** have not been synthesized before. The ^{1}H-NMR spectrum of the final product **13** (**Figure 3.7**) was very similar to the one of 1-DQL **4** (**Figure 3.4**)**,** with the difference that there was only one singlet integrating as three protons at 3.85 ppm for the only methyl group in the molecule compared to two such singlets for 1-DQL **4**, at 3.87 ppm and 3.84 ppm. Even though the spectrum was measured in a different deuterated solvent (CD_3OD for 1-FQL **13** compared to $(CD_3)_2CO$ for 1-DQL **4**) the order of the signals and the splitting patterns were identical while the *J* values were either identical or very close to each other for corresponding protons. The small differences for the ppm values of the corresponding protons in 1-FQL **13** *versus* 1-DQL **4** are attributed to solvent effects on NMR chemical shifts and to the small molecular differences between the two compounds (one methoxy and one phenol for 1-FQL **13** compared to two methoxy groups for 1-DQL **4**).

The ^{13}C-NMR spectrum of 1-FQL **13** (**Figure 3.8**) was very similar to the one of 1-DQL **4** (**Figure 3.5**), with a few differences discussed below, caused by the small molecular differences (CD_3OD was used in both cases) between the compounds. A total of 16 signals were present in the ^{13}C-NMR spectrum of 1-FQL **13**, two signals less than for 1-DQL **4** (instead of expected one signal less) because the C-4 and C-3 carbons showed overlap. 1-FQL **13** showed one signal at 55.20 ppm for the only methoxy group in the molecule compared to two such signals in the case of 1-DQL **4** (55.18 ppm and 55.10 ppm). Additionally, while 1-DQL **4** showed two signals for the two aromatic carbons bearing the methoxy functionality (C_{Ar}-OCH_3) at 151.79 ppm and 149.46 ppm, in the case of 1-FQL **13** one signal "migrated" slightly upfield to 146.90 ppm ($C_{Ar}OH$) while the second remained downfield at 149.55 ppm (C_{Ar}-OCH_3). Another interesting feature of the ^{13}C-NMR spectrum of 1-FQL **13** was that C-4 and C-3 carbons showed overlap (65.74 ppm) while for 1-DQL **4** they could still be observed resolved (65.77 ppm and 65.72 ppm). The remaining signals in the spectrum of 1-FQL **13** appeared in the same order (in terms of chemical shift) as their corresponding peaks in 1-DQL **4**, as detailed above.

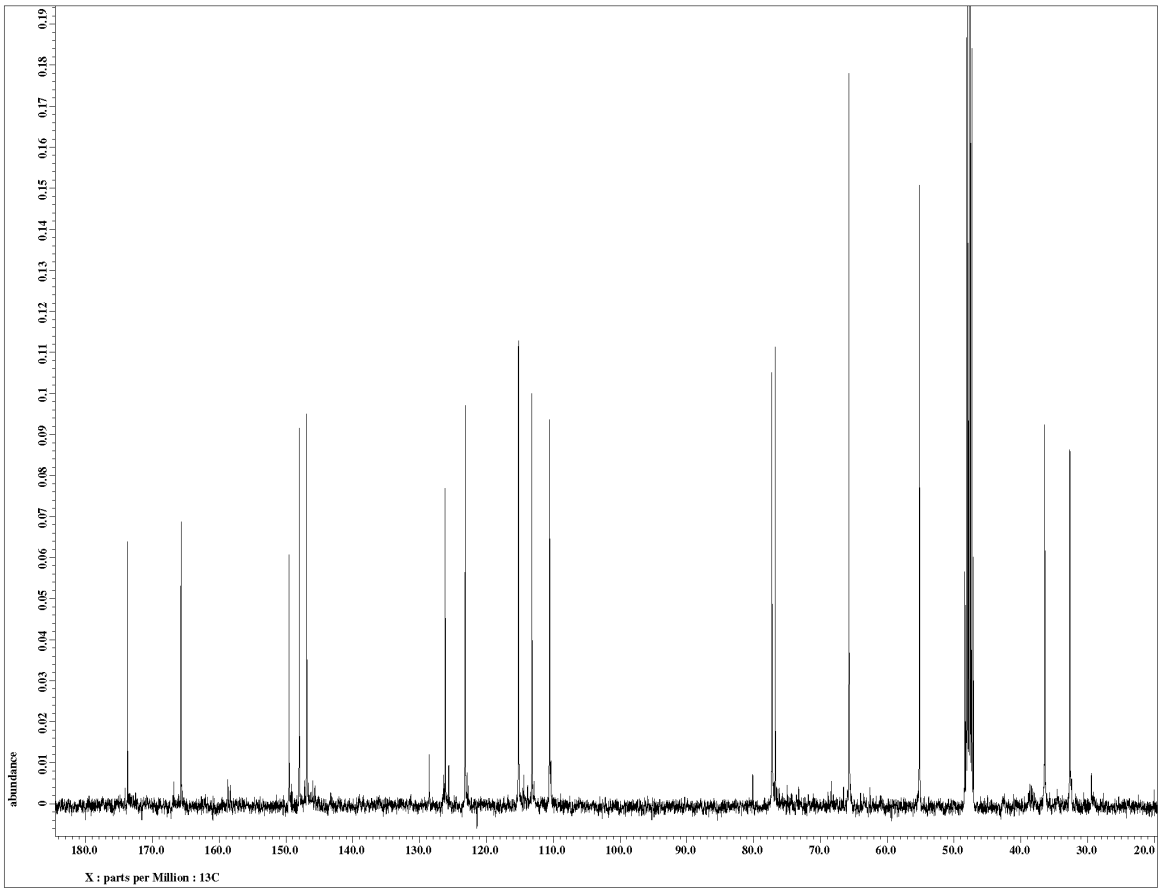


Figure 3.8. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of 1-*O*-feruloyl-1,5-quinide (1-FQL) **13**.

1-FQA **64** (**Figure 3.53**) was synthesized in quantitative yield from 1-FQL **13** by using a 1M LiOH solution.[4] The structure was confirmed by spectroscopic data. The ^{1}H-NMR spectrum of 1-FQA **64** showed a number of notable differences compared to the one of 1-FQL **13**. The C-5 proton shifted by almost 1 ppm upfield resonating at 3.93 ppm in the chlorogenic acid compared to 4.84 ppm in the lactone. The peak appeared as a doublet of doublets of doublets (ddd), being split by the neighboring C-4 proton (^3J = 9.6 Hz) and the two C-6 protons (^3J = 4.6 Hz and ^3J = 11.0 Hz). There were also changes for the C-4 and C-3 protons in the chlorogenic acid compared to the lactone, with the C-4 proton resonating more upfield at 3.44 ppm (in lactone 4.03 ppm) and the C-3 proton resonating more downfield at 4.05 ppm (in lactone 3.80 ppm); the different NMR solvents (D_2O for 1-FQA **64** *versus* CD_3OD for 1-FQL **13**) also account for some of the observed differences in chemical shifts. The four C-2 and C-6 protons came as expected more

upfield in 1-FQA **64**, all between 1.80-2.05 ppm, while in 1-FQL **13** the acylated 1,5-quinide specific signal (equatorial proton at C-6) came at 3.04 ppm while the remaining C-2 and C-6 protons came between 2.14-2.57 ppm.

The couplings between different pairs of protons could also be observed for the hydrogens of the quinic acid moiety, as already shown for the signal splitting of the C-5 proton. The C-4 proton coupled to the two vicinal protons (^3J = 3.2 Hz to the C-3 proton and ^3J = 9.6 Hz to the C-5 proton). The signal of the C-3 proton was split into a ddd by the protons on the two neighboring carbons, in a similar fashion as the signal of the C-5 proton was split. In addition to coupling to the C-4 proton, the C-3 proton also coupled to two vicinal protons on C-2 (^3J = 3.2 Hz to the axial C-2 proton and ^3J = 3.6 Hz to the equatorial C-2 proton). The signal at 1.96 ppm for the axial proton on C-2 showed the geminal coupling (^2J = 14.2 Hz) as well as the coupling to the vicinal C-3 proton (^3J = 3.2 Hz), being a doublet of doublets (dd). The signal at 1.93 ppm for the equatorial C-2 proton was a ddd: geminal coupling ^2J = 14.2 Hz, vicinal coupling ^3J = 3.6 Hz (to the C-3 proton) and "W-coupling" ^4J = 2.8 Hz (to equatorial C-6 proton). Similarly to the C-2 protons, the C-6 protons appeared as a dd and a ddd. The signal at 1.80 ppm for the C-6 axial proton was split by its geminal proton (^2J = 13.3 Hz) and by the vicinal C-5 proton (^3J = 11.0 Hz). The signal at 2.04 ppm for the C-6 equatorial proton (ddd) was split by its geminal proton (^2J = 13.3 Hz), by the vicinal C-5 proton (^3J = 4.6 Hz) and by the C-2 equatorial proton (^3J = 2.8 Hz, "W-coupling").

The ^{13}C-NMR spectrum of 1-FQA **64** showed the expected 17 signals and not 16 signals as it was the case for 1-FQL **13** where the C-4 and C-3 carbons showed overlap. The slightly more deshielded carbon nucleus in the carboxylic acid moiety (compared to the equivalent nucleus in the lactone) appeared at 178.30 ppm in 1-FQA **64** *versus* 173.74 ppm in 1-FQL **13**. The C-5 carbon produced a signal more upfield in the chlorogenic acid (66.45 ppm) compared to 1-FQL **13** where it resonated at 76.77 ppm. While in the lactone C-4 and C-3 gave one signal at 65.74 ppm in the ^{13}C-NMR spectrum, the two carbons were clearly separated in 1-FQA **64**: C-4 at 74.87 ppm and C-3 at 70.06 ppm. It should be mentioned that the ^{13}C-NMR solvents were different for 1-FQA **64** and 1-FQL **13**, as it was also the case for the ^{1}H-NMR spectra measurements of the two compounds.

3.1.3. Synthesis of 1-*O*-Caffeoyl-1,5-quinide (1-CQL) 19

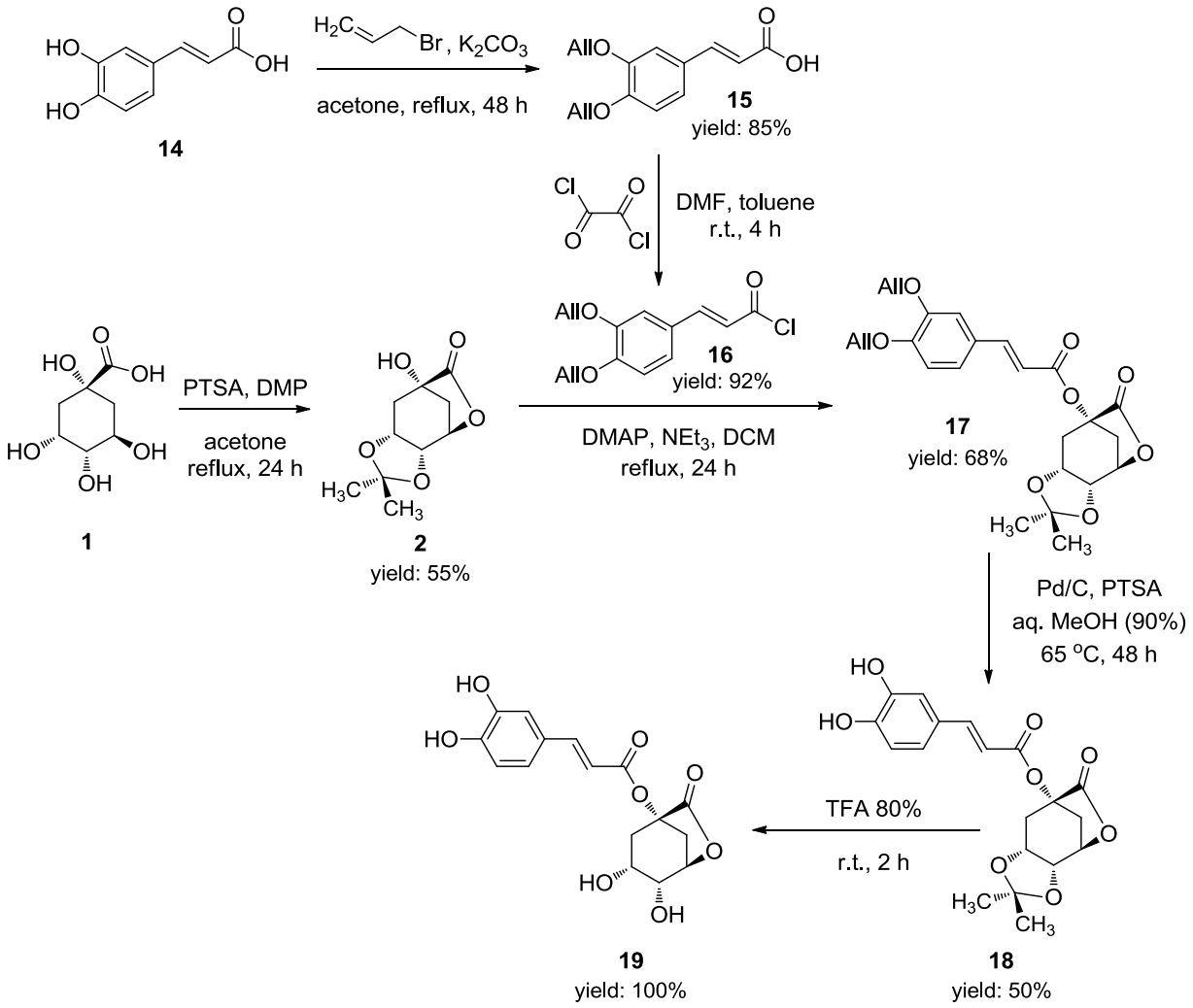


Figure 3.9. Synthetic path to 1-CQL **19**.

1-*O*-caffeoyl-1,5-quinide **19** was successfully synthesized (**Figure 3.9**) employing allyl protection for the aromatic hydroxyls (Williamson ether synthesis).[8,14] The attempted synthesis of 1-CQL **19** using an acetyl protection[3] instead of allyl provided clean NMR spectra for all the intermediates until the esterification step, inclusively. Subsequent deprotection attempts did not generate the title compound (the same challenges as in the total synthesis of 1-FQL **13**) and the acetyl protection was abandoned (intermediate **63** in **Figure 3.53** could not be effectively deprotected to obtain **19**). With the allyl protection every step of the total synthesis was

successful from the first attempt in fair to quantitative yields. The allyl removal step[16,17] gave only a fair yield (50%), being the lowest yield of the entire synthetic scheme. For this step specifically, the reaction was carried in aqueous MeOH 90% at 65 ºC as higher temperatures open the lactone and methylate the carboxylic acid moiety. H_2O is crucial for the effectiveness of the present reaction. This is presumably because *p*-toluenesulfonic acid requires aqueous environment to be efficient in the commencing protonation (of oxygen in the allyl-*O*- moiety) step of the de-allylation mechanism. Compounds **17-19** have not been synthesized before.

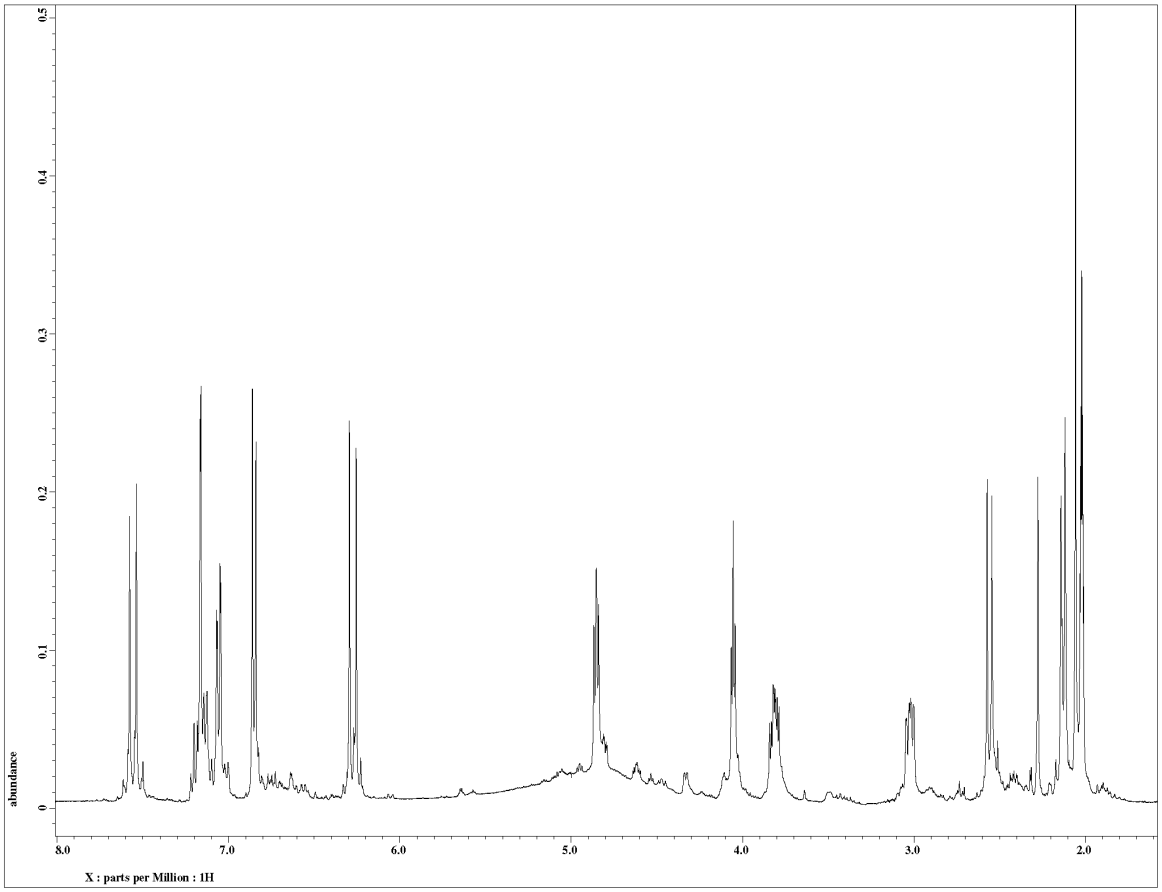


Figure 3.10. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) spectrum of 1-*O*-caffeoyl-1,5-quinide (1-CQL) **19**.

The ^{1}H-NMR spectrum of the final product **19** (**Figure 3.10**) confirmed the structure and the signals were rather similar to the ones for 1-DQL **4,** with a few differences. There were no singlets integrating as three protons at around 3.85 ppm since no methyls are present in the

molecule. Both ^{1}H-NMR spectra (1-DQL **4** and 1-CQL **19**) were measured in the same deuterated solvent ($(CD_3)_2CO$) and the order of the signals and the splitting were identical while the *J* values were either identical or very close to each other for corresponding protons. The small differences for the ppm values of the corresponding protons in 1-CQL **19** *versus* 1-DQL **4** are attributed to the small molecular differences between the two compounds (two phenols for 1-CQL **19** compared to two methoxy groups for 1-DQL **4**). Notably, the three aromatic protons (6.85-7.17 ppm for 1-CQL **19** *versus* 7.12-7.54 ppm for 1-DQL **4**) as well as the two olefinic α-protons (6.28 ppm for 1-CQL **19** *versus* 6.37 ppm for 1-DQL **4**), resonated more upfield in **19** compared to **4**.

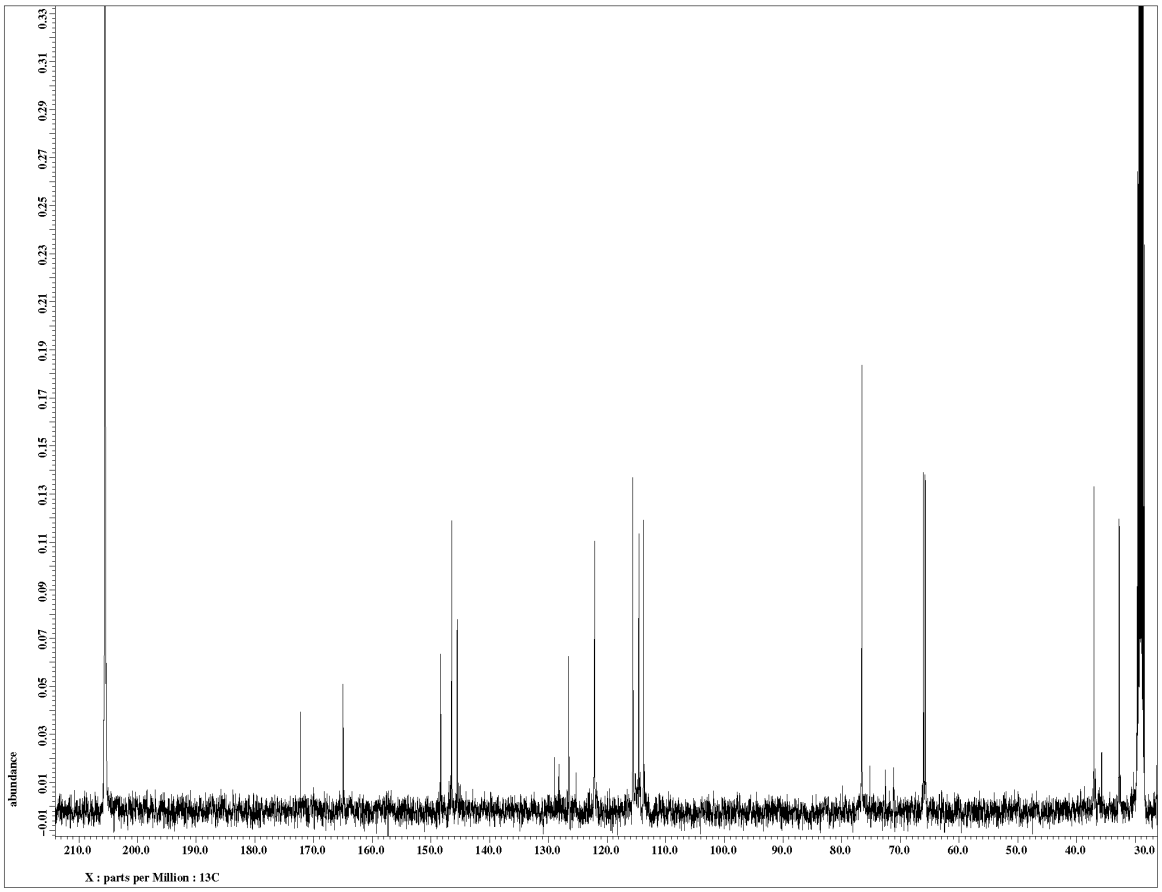


Figure 3.11. ^{13}C-NMR ($(CD_3)_2CO$, 100 MHz) spectrum of 1-*O*-caffeoyl-1,5-quinide (1-CQL) **19**.

The small structural differences between 1-CQL **19** and 1-DQL **4** were more noticeable in the ^{13}C-NMR spectra. A total of 15 signals were present in the ^{13}C-NMR spectrum of 1-CQL **19** (**Figure 3.11**), three signals less than for 1-DQL **4** (instead of expected two signals less), because the C-1 and C-5 carbons showed overlap. 1-CQL **19** showed no signals at around 55.15 ppm since no methoxy groups are present in the molecule, compared to two such signals in the case of 1-DQL **4** (55.18 ppm and 55.10 ppm). Additionally, while 1-DQL **4** showed two signals for the two aromatic carbons bearing the methoxy functionality (C_{Ar}-OCH_3) at 151.79 ppm and 149.46 ppm, in the case of 1-CQL **19** the two corresponding peaks "migrated" slightly upfield to 148.40 ppm ($C_{Ar}OH$) and 145.53 ($C_{Ar}OH$). Another interesting feature of the ^{13}C-NMR spectrum of 1-CQL **19** (measured in $(CD_3)_2CO$) was that the C-1 and C-5 carbons showed overlap at 76.59 ppm while for 1-DQL **4** (measured in CD_3OD) they were observed resolved (77.24 ppm and 76.81 ppm).

3.1.4. Synthesis of Methyl 5-*O*-Caffeoylquinate (5-CQM) 22

In order to achieve the title compound acylated at the C-5 position on the quinic acid moiety, the C-3 and C-4 positions needed protection as well as the carboxylic acid. The hydroxyl at C-1 did not require special protection since the condensation with the acid chloride is sterically favored at C-5 over C-1 when both positions are available (relative nucleophilicity might be an additional reason).[18] The common intermediate 3,4-*O*-isopropylidene-1,5-quinide **2** (synthesized from quinic acid **1** in a 55% yield)[10] was treated with a 21% NaOMe/MeOH solution in order to achieve two goals in one step: obtain the desired methyl ester functionality required for the final product and make available for condensation (with the acid chloride **16**) the C-5 position on the quinic acid moiety after opening the γ-quinide;[19] however, this step proceeded in a relatively low yield of 36%. Subsequently, monoesterification was possible (yield 65%) with the allyl-protected[8,14] caffeoyl chloride **16** in the presence of the much weaker organic base pyridine (pK_a = 5.21, compared to triethylamine pK_a = 10.75) in order to avoid diacylation.[5] This condensation step was followed by allyl-deprotection[16,17] and acidic removal of the isopropylidene group[14] in order to afford the targeted 5-CQM **22** (yield 50%, **Figure 3.12**).[20] Compounds **21** and **22** have not been synthesized before.

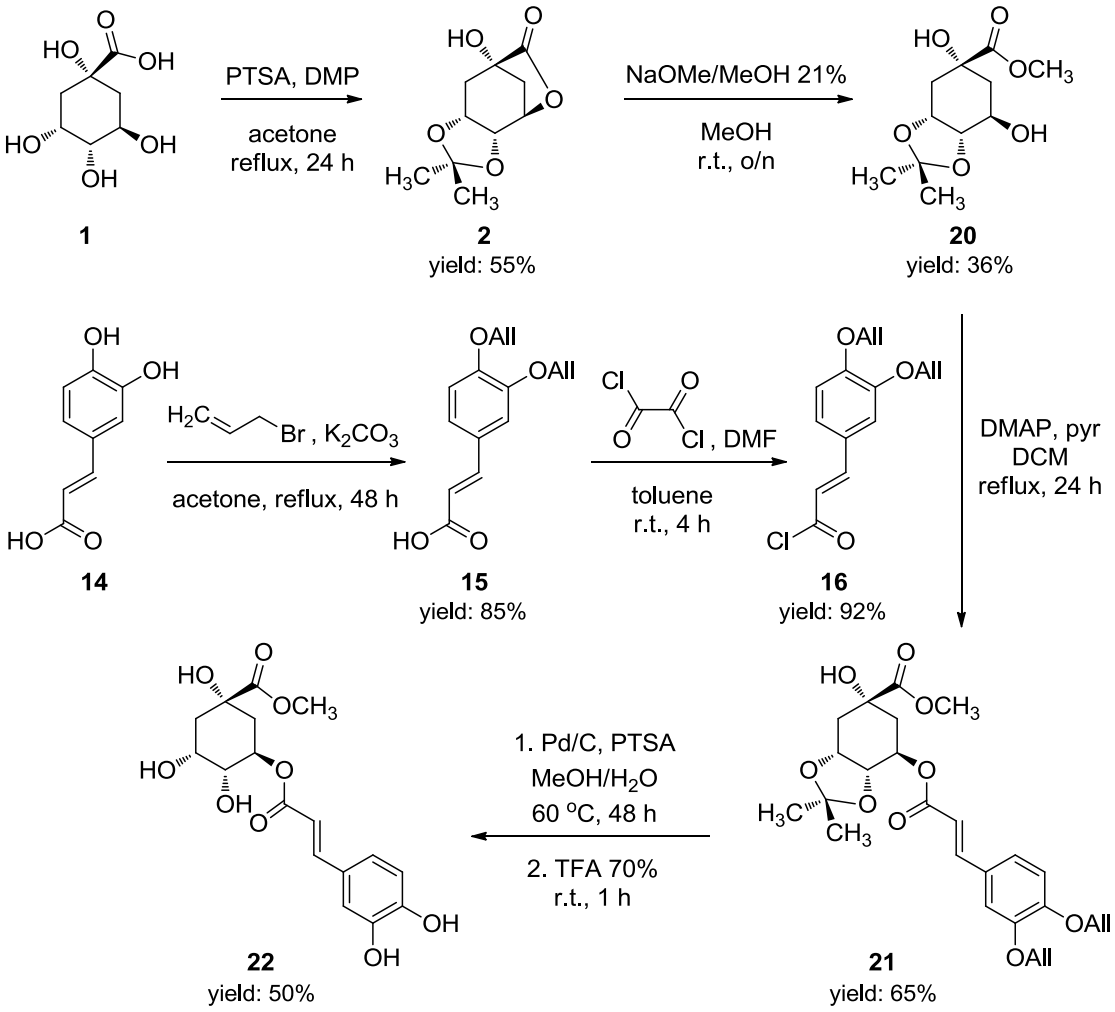


Figure 3.12. Synthetic path to 5-CQM **22**.

In the ^{1}H-NMR spectrum (**Figure 3.13**) of 5-CQM **22** (in $(CD_3)_2CO$) the aromatic and olefinic protons of the caffeoyl moiety resonated similarly to the corresponding protons in the compounds containing a caffeoyl residue, which were previously reported in the current chapter. Acylation at the C-5 position was reflected in the spectrum by a more downfield shift of the C-5 hydrogen at 5.31 ppm (proton at non-acylated C-5 in 1-FQA **64** came at 3.93 ppm). The proton at C-3 appeared at 4.14 ppm (4.05 ppm for 1-FQA **64** in D_2O) while the one at C-4 resonated at 3.71 ppm (3.44 ppm for 1-FQA **64** in D_2O). The methyl ester protons resonated as a singlet integrating as three at 3.65 ppm. The four protons at C-2 and C-6 came between 2.00-2.21 ppm (1.80-2.05 ppm for 1-FQA **64** in $(CD_3)_2CO$).

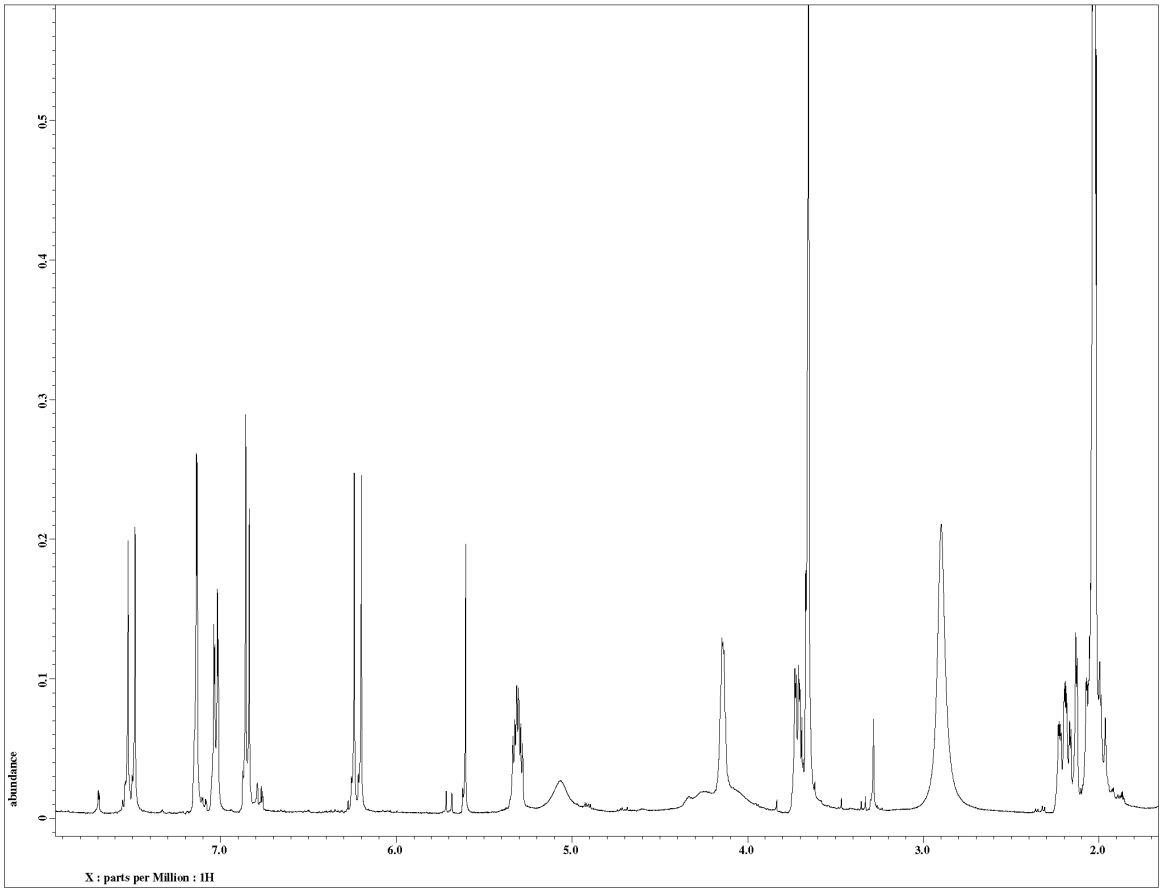


Figure 3.13. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) spectrum of methyl 5-*O*-caffeoylquinate (5-CQM) **22**.

In the ^{13}C-NMR spectrum (in $(CD_3)_2CO$) of 5-CQM **22** (**Figure 3.14**) the carbonyl carbon of the methyl ester resonated at 173.63 ppm while the methyl carbon appeared at 51.74 ppm. The other ester carbon of the molecule appeared at 166.08 ppm (CH-*C*OO). The aromatic carbons of the caffeoyl moiety appeared as for other compounds containing this hydroxycinnamic acid residue between 114.34-147.98 ppm, while the olefinic carbons came at 144.99 ppm (*C*H-C_{Ar}) and 114.91 ppm (C_{Ar}-CH=*C*H). Of the carbon nuclei belonging to the quinic acid moiety, the C-1 nucleus was the most deshielded at 75.06 ppm, followed by the C-4 nucleus at 72.17 ppm. The non-acylated C-3 and the acylated C-5 appeared close to each other at 70.65 ppm and 69.91 ppm, respectively, while C-2 and C-6 showed overlap surprisingly at 37.14 ppm.

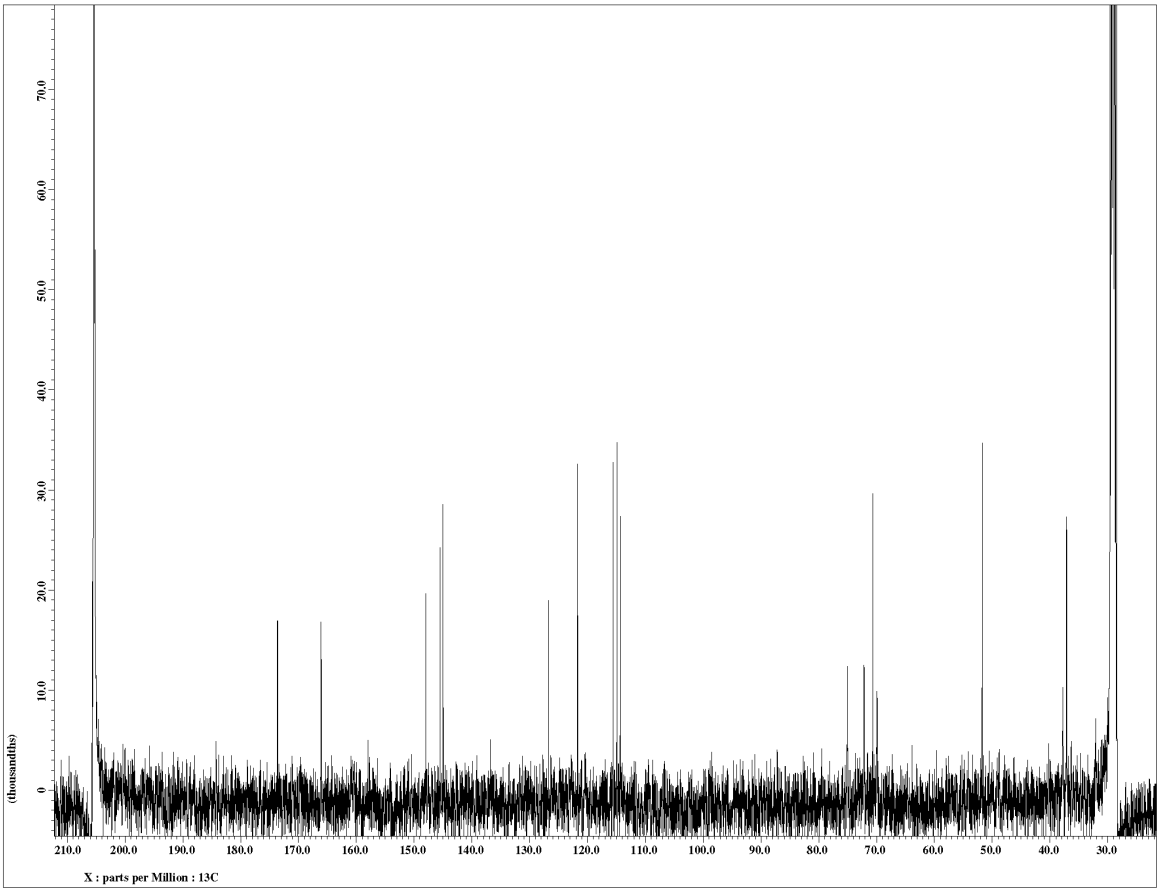


Figure 3.14. ^{13}C-NMR ($(CD_3)_2CO$, 100 MHz) spectrum of methyl 5-*O*-caffeoylquinate (5-CQM) **22**.

3.1.5. Synthesis of 5-*O*-(3',4'-Dimethoxycinnamoyl)-quinic Acid (5-DQA) 25

The synthesis of compound 5-DQA **25** (**Figure 3.15**) involved esterifying bisacetonide **23** with a suitable activated dimethoxycinnamic acid **5** in its acid chloride form **6** (*a priori* generated in 99% yield by using the Vilsmeier reagent oxalyl chloride and DMF), in order to generate **24** (yield 74%).[5] Bisacetonide **23** was obtained as the main product from the kinetic acetalization of quinic acid transformed in its penta-silylated derivative, as reported by Sefkow.[5] Complete deprotection of intermediate **24** required only one hour reaction time with 70% TFA (quantitative yield);[20] in Sefkow's work, the corresponding intermediate of this quinic acid bisacetonide acylated at C-5 with the protected caffeic acid (di-acetyl) required ten days reaction time for complete deprotection with aqueous 1N HCl containing 15% THF.[5]

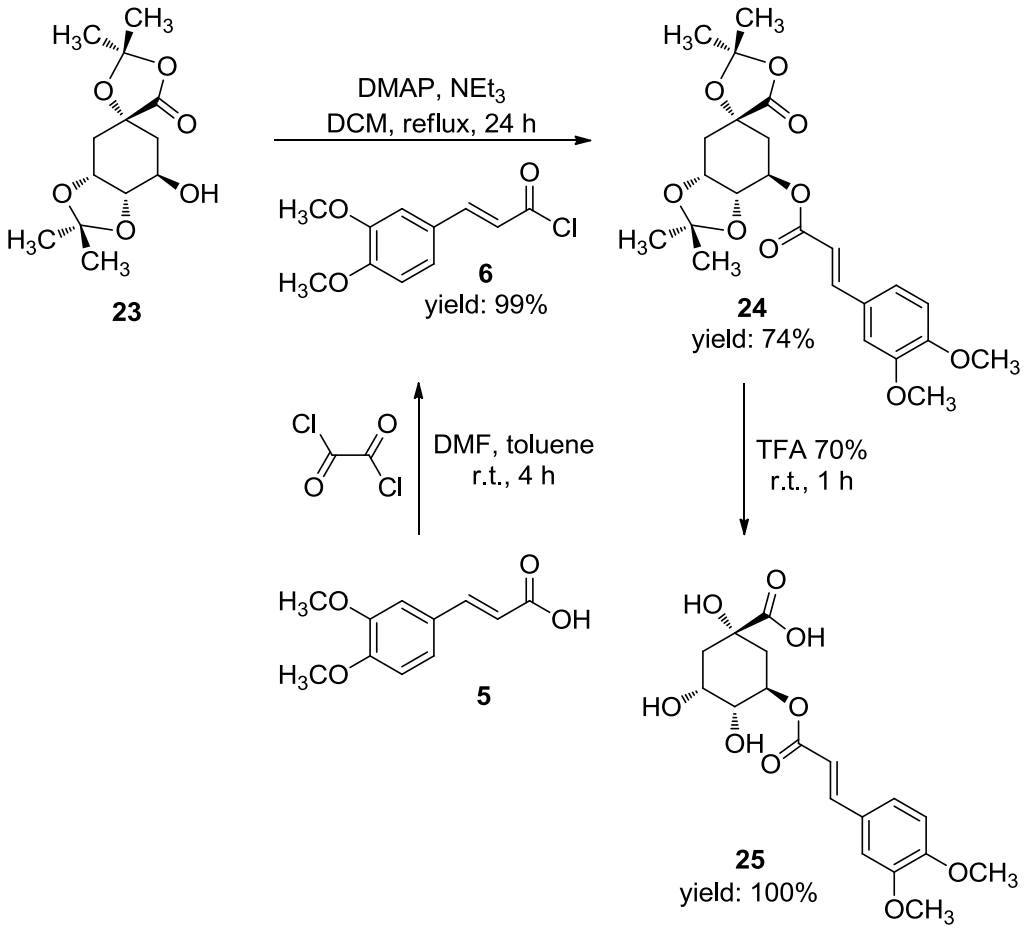


Figure 3.15. Synthetic path to 5-DQA **25**.

In the ^{1}H-NMR spectrum (**Figure 3.16**) of 5-DQA **25** (in CD_3OD) the aromatic and olefinic protons of the dimethoxycinnamoyl moiety resonated similarly to and appeared in the same order (in terms of chemical shift) as the corresponding protons in the caffeoyl moiety of 5-CQM **22**. Thus, the olefinic protons coupling to each other (^{3}J = 15.6 Hz) came as doublets at 7.60 ppm and 6.31 ppm, respectively. The three aromatic protons on the cinnamoyl residue, each integrating as one proton, resonated as following: a doublet at 7.17 ppm corresponding to the proton on C-2' coupled to the C-6' proton (^{4}J = 1.8 Hz); a doublet of doublets at 7.13 ppm corresponding to the proton on C-6' coupled to the other two aromatic protons (^{3}J = 8.7 Hz with the proton on C-5' and ^{4}J = 1.8 Hz with the proton on C-2'); and a doublet at 6.93 ppm corresponding to the proton on C-5' coupled to the C-6' proton (^{3}J = 8.7 Hz).

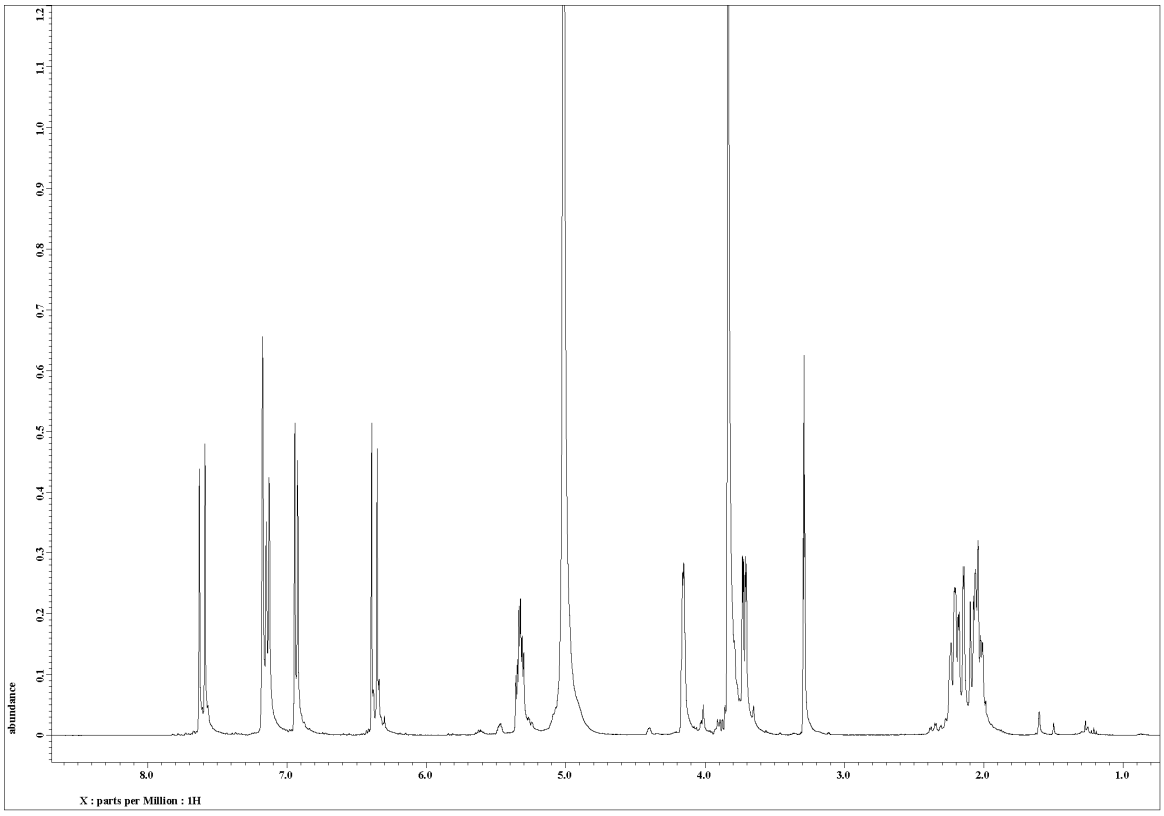


Figure 3.16. ^{1}H-NMR (CD_3OD, 400 MHz) spectrum of 5-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid (5-DQA) **25**.

Acylation at the C-5 position was reflected in the spectrum by a paramagnetic shift of the C-5 hydrogen at 5.31 ppm (proton at non-acylated C-5 in 1-FQA **64** came at 3.93 ppm while the corresponding proton in 5-CQM **22** had an identical chemical shift-5.31 ppm); in general, the experimental data in the current project showed that acylation of an alcohol on quinic acid (or its derivatives) resulted in a downfield (paramagnetic) shift of approximately 1.2-1.4 ppm for the proton attached to the carbon bearing the acylation. The proton at C-3 appeared at 4.15 ppm (4.14 ppm for 5-CQM **22** in $(CD_3)_2CO$) while the one at C-4 resonated at 3.71 ppm (3.71 ppm for 5-CQM **22** in $(CD_3)_2CO$). The two methoxy groups of the cinnamoyl residue gave a singlet integrating as six protons at 3.83 ppm, while in 5-CQM **22** the methyl ester protons produced a signal at 3.65 ppm. The four aliphatic protons at C-2 and C-6 showed overlap as multiplets between 2.06-2.18 ppm.

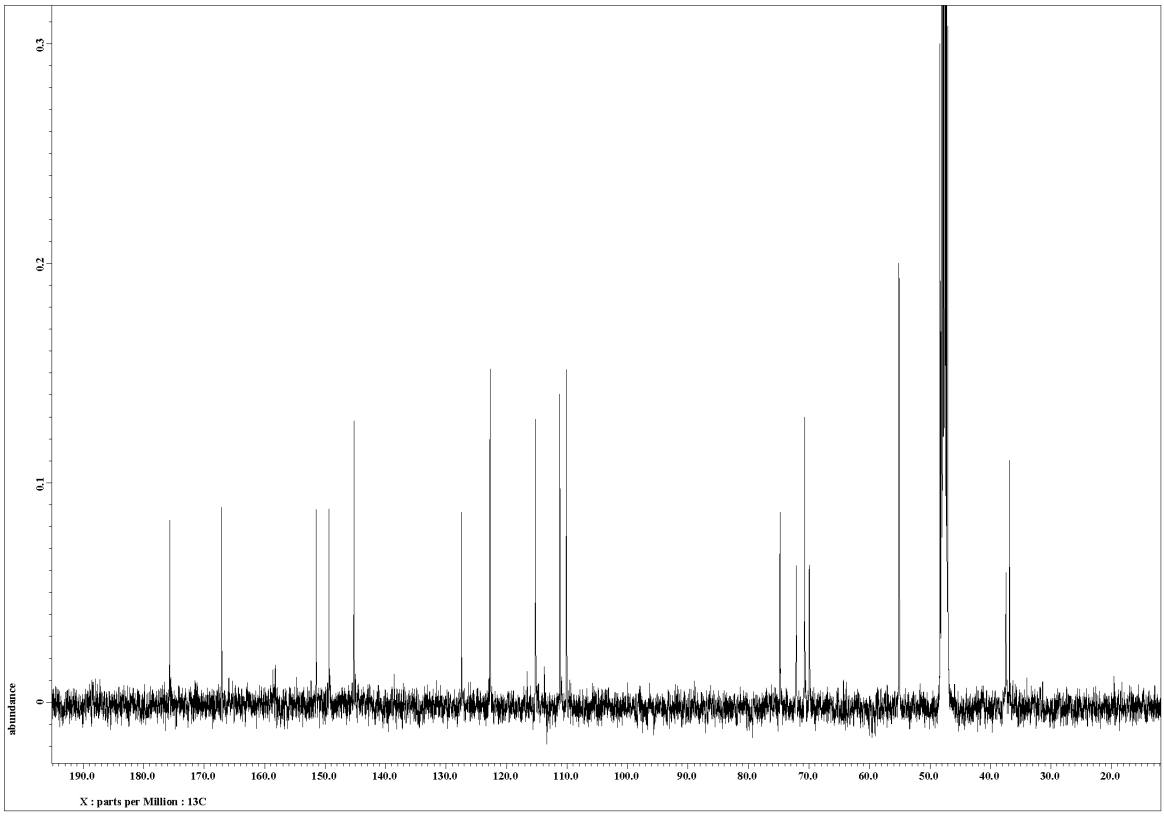


Figure 3.17. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of 5-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid (5-DQA) **25**.

In the ^{13}C-NMR spectrum (in CD_3OD) of 5-DQA **25** (**Figure 3.17**) the carboxylic acid carbon resonated at 175.54 ppm, (for comparison, the carbonyl carbon of the methyl ester in 5-CQM **22** resonated at 173.63 ppm in $(CD_3)_2CO$). When comparing the chemical shift of the carbonyl carbon nucleus in quinic acid *versus* methyl quinate (both spectra measured in D_2O) the small shielding effect of the electron donating short alkyl group in methyl quinate is noticeable in the ^{13}C-NMR spectrum, with the carbonyl carbon atom resonating at 175.99 ppm compared to 177.68 ppm in quinic acid. The other ester carbon of the 5-DQA **25** molecule appeared at 167.11 ppm (CH-*C*OO). The aromatic carbons of the caffeoyl moiety appeared as for other compounds containing this hydroxycinnamic acid residue between 110.08-151.50 ppm, while the olefinic carbons came at 145.23 ppm (*C*H-C_{Ar}) and 115.12 ppm (C_{Ar}-CH=*C*H). The two methoxy carbons produced two signals very close to each other at 55.02 ppm and 55.13 ppm. Of the carbon nuclei belonging to the quinic acid moiety, the C-1 nucleus was the most deshielded at

74.91 ppm, followed by C-4 at 72.14 ppm. The non-acylated C-3 and the acylated C-5 appeared close to each other at 70.71 ppm and 69.93 ppm, respectively, while C-2 and C-6 did not resonate together as one peak as in the case of 5-DQA **25** (37.14 ppm) but gave two distinct signals at 37.21 ppm and 37.43 ppm, respectively.

3.1.6. Synthesis of 3,4-di-*O*-(3',4'-Dimethoxycinnamoyl)-1,5-quinide (3,4-diDQL) 29

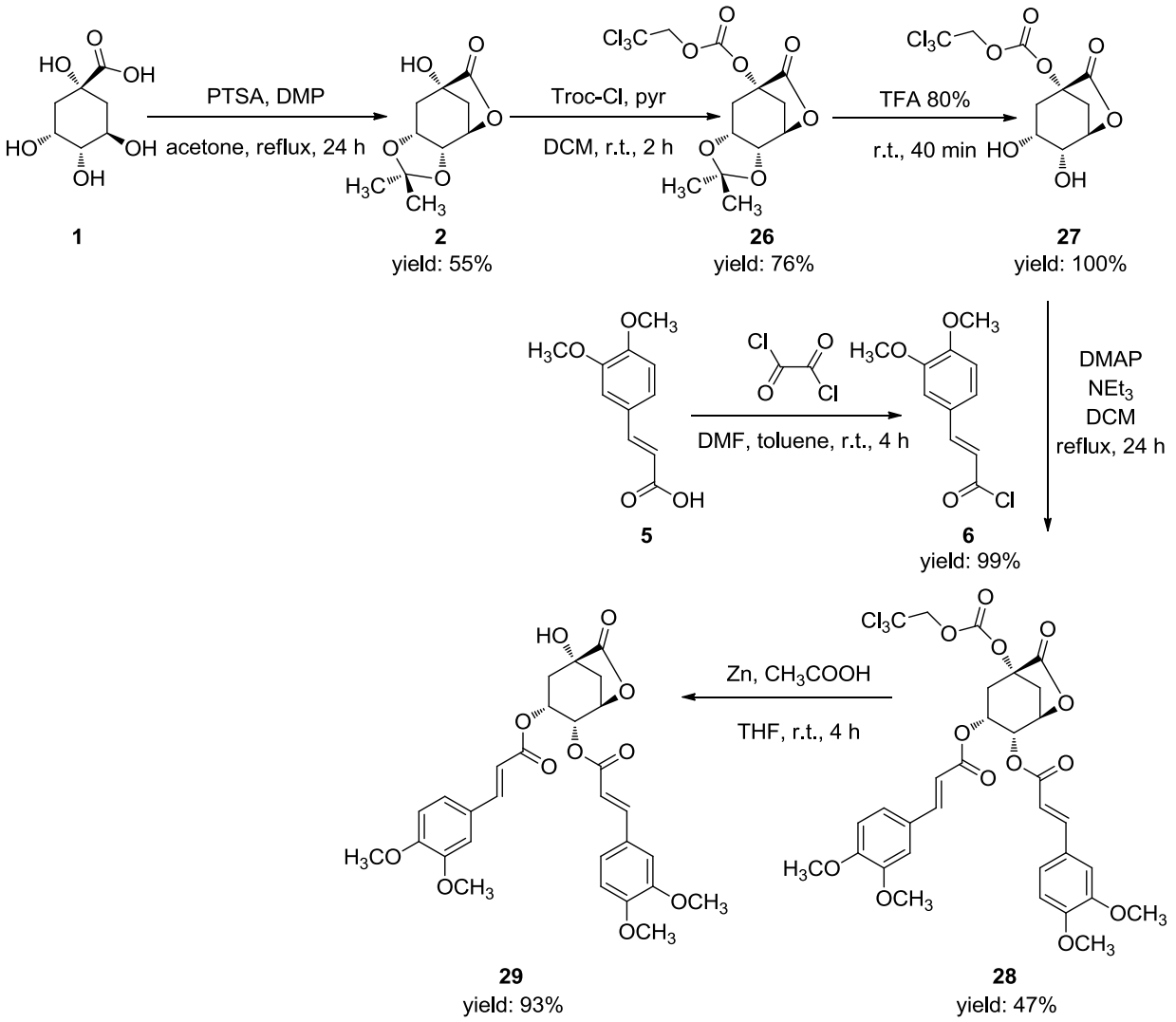


Figure 3.18. Synthetic path to 3,4-diDQL **29**.

In order to synthesize 3,4-diDQL **29**, a quinic acid intermediate in the form of a γ-quinide bearing an adequate protective group at the C-1 alcohol needed to be first synthesized. This

intermediate was 1-*O*-Troc-1,5-quinide **27** obtained in three steps from quinic acid by successively protecting all the alcohols of the molecule, closing the lactone and then deprotecting the desired alcohols at C-3 and C-4 (**Figure 3.18**). The joint protecting group for the hydroxyls at C-3 and C-4 was isopropylidene[10] while for the C-1 alcohol 2,2,2-trichloroethoxycarbonyl (Troc) was employed.[4] 3,4-*O*-isopropylidene-1,5-quinide **2** was synthesized from quinic acid **1** in one step in 55% yield under reflux in acetone, by employing *p*-toluenesulfonic acid monohydrate ($PTSA \cdot H_2O$) and 2,2-dimethoxypropane (DMP),[10] while the Troc protecting group was added by using 2,2,2-trichloroethylchloroformate and pyridine to generate **26** with a 76% yield in DCM.[4] Crystals of **26** suitable for single crystal X-Ray Diffraction (XRD) were also generated in the process and are shown in **Figure 3.19**.

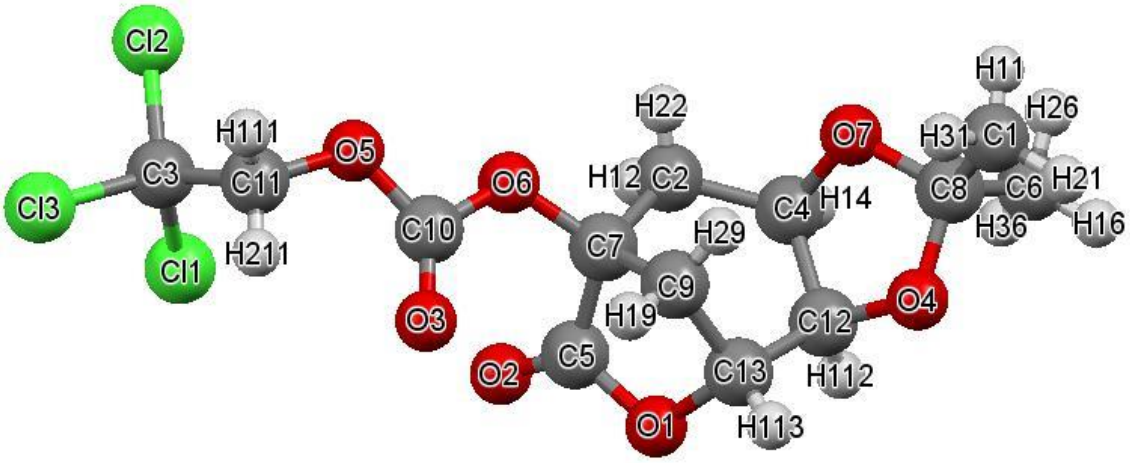


Figure 3.19. X-ray crystal structure of 1-*O*-Troc-3,4-*O*-isopropylidene-1,5-quinide **26**.

The isopropylidene was then removed (quantitative yield) under acidic conditions (TFA 80%) to free the C-3 and C-4 vicinal diol for a subsequent double esterification.[4] The synthesized 1-*O*-Troc-1,5-quinide **27** was then reacted as described earlier in the current chapter with the more reactive acid chloride **6** of dimethoxycinnamic acid **5**, with the later in excess of the stoichiometric requirement of 1:2 molar, to generate **28** in 47% yield.[5] Additionally, reflux and the stronger base NEt_3 (compared to pyridine) were used to ensure diacylation since milder conditions (pyridine, lower temperature) yield a mixture of the two possible monoacylated derivatives.[5] In the last step of the synthetic scheme, Troc deprotection afforded quantitatively

the desired diacylated lactone, the group being mechanistically removed by Zn insertion in acidic conditions (CH_3COOH), which results in elimination (E1cB type) and decarboxylation.[4] Compounds in **Figure 3.18** are not novel.[2]

In the ^{1}H-NMR (in $CDCl_3$) of 3,4-diDQL **29** (**Figure 3.20**), the six aromatic protons of the diacylated compound resonated as following: the two C-6' protons came as dd at 7.06 ppm and 6.92 ppm (^4J = 2.3 Hz, ^3J = 8.2 Hz); the two C-2' protons appeared as doublets at 7.02 ppm and 6.89 ppm (^4J = 2.3 Hz); the two C-5' protons resonated as doublets at 6.81 ppm and 6.70 ppm (^3J = 8.2 Hz). The two olefinic protons at the β-position (C_{Ar}-CH) resonated the most downfield at 7.63 ppm and 7.50 ppm as doublets (^3J = 16.0 Hz), each coupling to the corresponding protons at the α-position (C_{Ar}-CH=C*H*), which appeared more shielded than the six aromatic protons, at 6.35 ppm and 6.16 ppm, respectively. The three following peaks in the spectrum appearing at higher field than the α-protons were the protons at C-4 (5.64 ppm), C-3 (5.24 ppm) and C-5 (4.88 ppm). Acylation at C-4 and C-3 resulted in chemical shifts of the protons attached to these carbons to resonate considerably more downfield; for 1-DQL **4** (spectrum measured in $(CD_3)_2CO$) the protons at the corresponding non-acylated C-4 and C-3 positions produced signals at 4.07 ppm and 3.83 ppm, respectively. For 3,4-diDQL **29**, the proton at C-4 was a dd coupling to the two vicinal protons at C-3 (^3J = 4.6 Hz) and C-5 (^3J = 5.0 Hz); the proton at C-3 was a ddd coupling to the three vicinal protons at C-4 (^3J = 4.6 Hz) and C-2 (to equatorial proton: ^3J = 6.0 Hz; to axial proton: ^3J = 11.5 Hz); the proton at C-5 was a triplet coupling to the vicinal proton at C-4 (^3J = 5.0 Hz) and to the equatorial proton at C-6 (^3J = 5.0 Hz), while coupling to the axial proton at C-6 was not observed (this coupling was not observed for 3,4-diFQL **32** either). The four methyl groups of 3,4-diDQL **29** produced three singlets, out of which one integrated as six protons (3.86 ppm) and two integrated as three protons (3.80 ppm and 3.72 ppm). Between 2.22-2.59 ppm the four protons at C-2 and C-6 resonated. The splitting pattern could be clearly observed for the axial protons at these two positions but not for the equatorial ones, which appeared as multiplets. The axial proton at C-6 resonated at 2.59 ppm and was a doublet being split by its geminal proton (^2J = 11.9 Hz) while the axial proton at C-2 was a dd at 2.22 ppm coupling to its geminal proton (^2J = 11.9 Hz) and to the proton at C-3 (^3J = 11.5 Hz). The equatorial protons appeared as multiplets at 2.50 ppm (equatorial proton at C-6) and 2.36 ppm (equatorial proton at C-2).

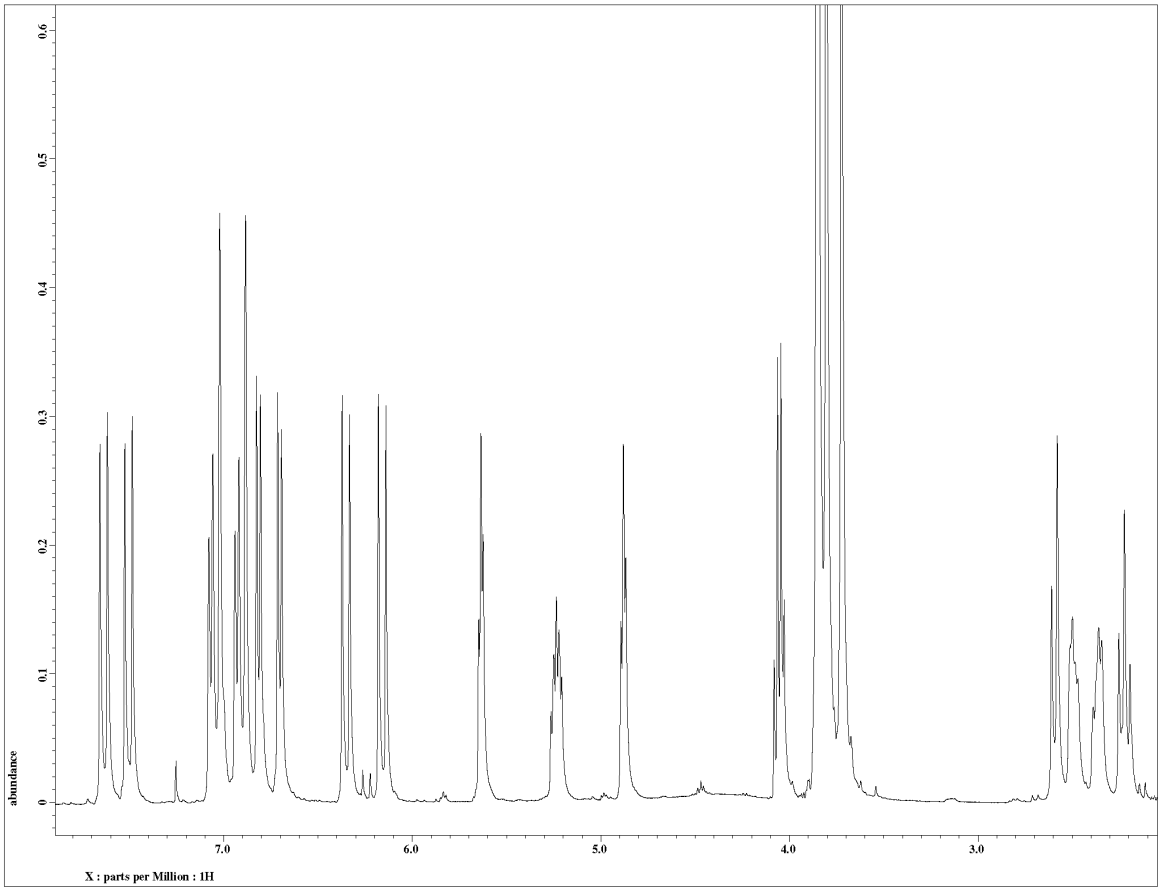


Figure 3.20. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of 3,4-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (3,4-diDQL) **29**.

For the ^{13}C-NMR (in $CDCl_3$) of 3,4-diDQL **29** (**Figure 3.21**) a number of 29 signals is expected but 28 were observed when the spectrum of the synthesized compound was measured in $CDCl_3$ since two aromatic carbons showed overlap. The ester carbon of the lactone (OC-*C*OO at 177.15 ppm) appeared more downfield than the two cinnamoyl ester carbons (CH-*C*OO at 165.78 ppm and 165.63 ppm). The four aromatic carbon atoms bearing the electron withdrawing methoxy functionalities (C_{Ar}-OCH_3) also came downfield at 151.69 ppm, 151.36 ppm, 149.35 ppm and 149.17 ppm. The olefinic carbons adjacent to the aromatic moieties were more shielded (than C_{Ar}-OCH_3) at 146.73 ppm and 146.11 ppm. The two aromatic carbons (C_{Ar}-CH) attached each to the substituted olefin resonated more upfield at 127.07 ppm and 126.93 ppm. The two C-6' aromatic carbons appeared at 123.23 ppm and 122.93 ppm; the C-5' and C-2' aromatic carbons

(expected four peaks, observed three peaks) appeared between 109.85-111.00 ppm. More downfield than this group of carbons but more upfield than the C-6' carbons resonated the two remaining olefinic carbons (C_{Ar}-CH=*C*H) at 114.49 ppm and 114.17 ppm, respectively.

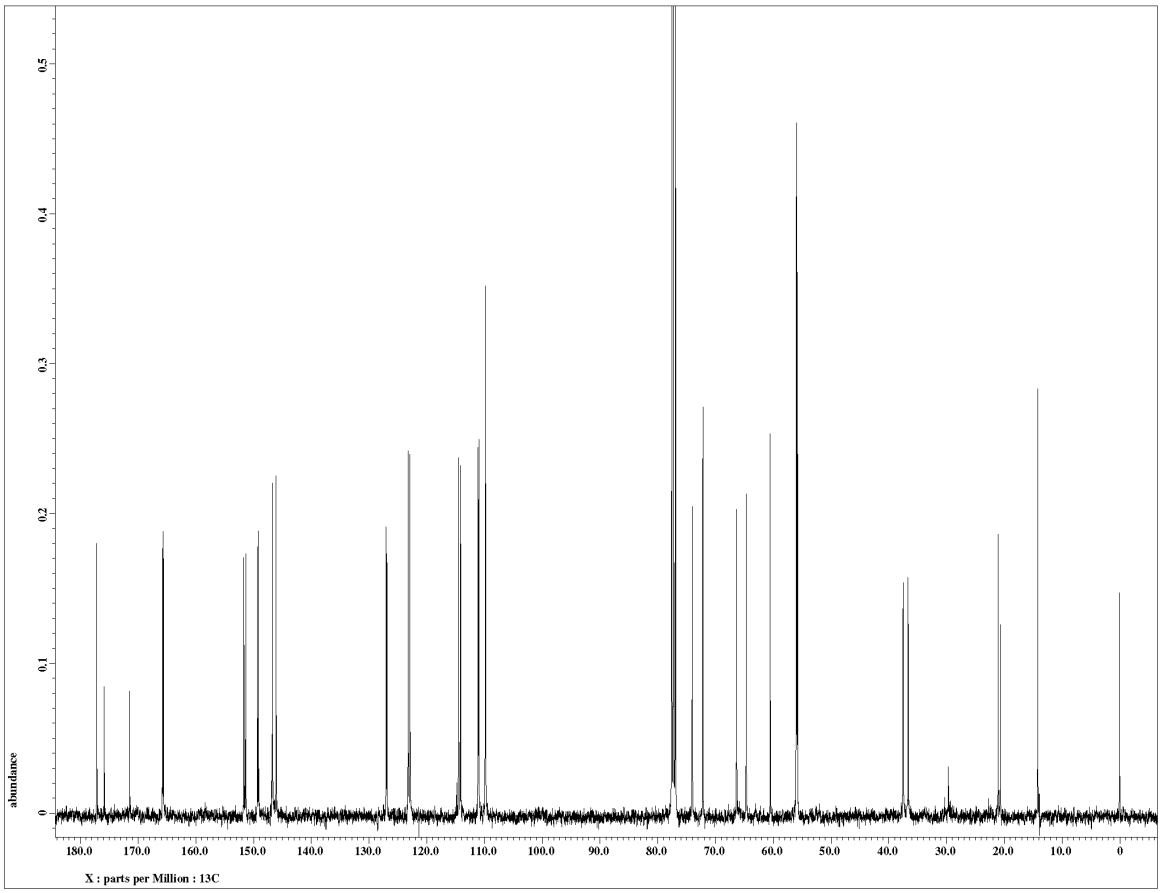


Figure 3.21. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of 3,4-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (3,4-diDQL) **29**.

The four carbon atoms of the quinic acid moiety bearing heteroatom-containing substituents resonated as following: the C-5 atom bearing the electron withdrawing -OCOR appeared at 74.09 ppm; the C-1 atom bearing the electron withdrawing lactone -COOR and -OH appeared at 72.21 ppm; the C-3 and C-4 atoms, each bearing an identical ester, resonated at 66.37 ppm and 64.69 ppm, respectively. The four methoxy carbons resonated at 56.06 ppm, 56.00 ppm, 55.97 ppm and 55.82 ppm. The C-2 (36.66 ppm) and C-6 (37.53 ppm) carbons of the quinic acid moiety gave signals which appeared most upfield in the spectrum of all carbons of the molecule. For

comparison, in the ^{13}C-NMR of 1-DQL **4** (measured in CD_3OD) the C-1 carbon resonated slightly more downfield (77.24 ppm) than the C-5 carbon (76.81 ppm) due to deshielding from the acyl moiety; in 3,4-diDQL **29** the reverse was true since the lack of acylation at C-1 (72.21 ppm) resulted in this carbon to be slightly more shielded than the C-5 carbon (74.09 ppm). This observation was consistent with the data for other molecules not acylated at C-1 synthesized in the current project: for 1,5-quinide **49** (in D_2O) C-1 resonated at 74.75 ppm while C-5 came at 75.56 ppm; for 3,4-*O*-isopropylidene-1,5-quinide **2** (in $CDCl_3$) C-1 resonated at 72.18 ppm while C-5 appeared at 75.94 ppm.

3.1.7. Synthesis of 3,4-di-*O*-Feruloyl-1,5-quinide (3,4-diFQL) 32

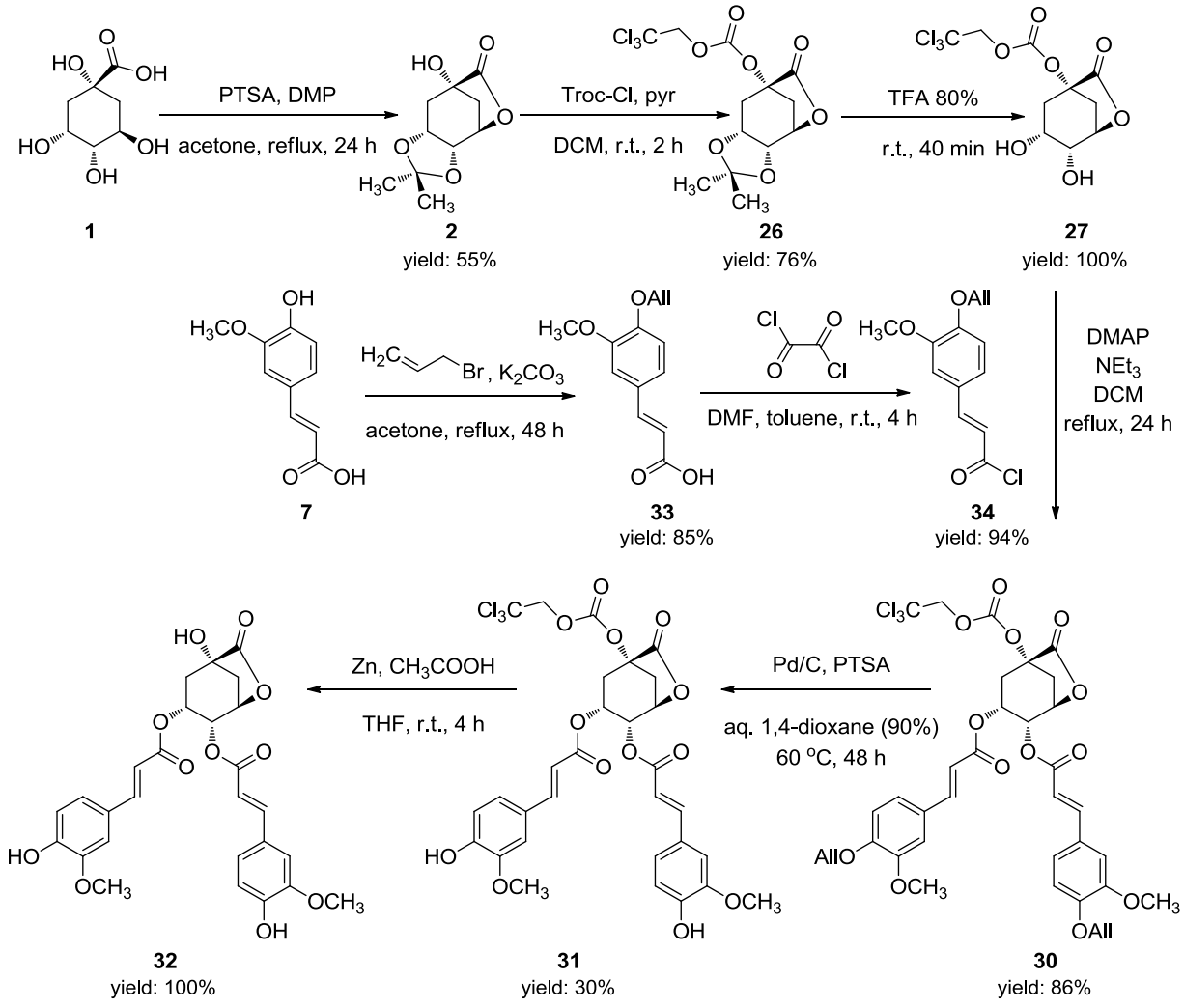


Figure 3.22. Synthetic path to 3,4-diFQL **32**.

3,4-diFQL **32** was synthesized (**Figure 3.22**) according to a similar synthetic scheme as 3,4-diDQL **29** (**Figure 3.20**) but required two additional steps: protecting the phenol on the ferulic acid (by allyl) and deprotecting it on the corresponding moiety in intermediate **30**, respectively, in the penultimate synthetic step. The allylation of ferulic acid **7** was carried out similarly to that of caffeic acid **14**.[8,14] While for the allylation of caffeic acid **14** (two phenols) a number of 3.5 equivalents of allyl bromide were needed for 85% yield, the same yield could be obtained for ferulic acid **7** (one phenol) with 2 equivalents of allyl bromide. The conversion of the allylated acid to its acid chloride went in excellent yield (94%);[5] Jaiswal et al. reported 88% for this step.[8] It is worth noting that while for 3,4-diDQL **29** the diacylation step proceeded in a moderate yield (47%), the equivalent step for 3,4-diFQL **32** produced the desired intermediate **30** in a much higher yield (86%).[5] The final step of the entire synthesis was a Troc deprotection and afforded quantitatively the desired diacylated lactone **32**, by Zn reduction under acidic conditions (CH_3COOH).[4] Compounds **30**-**32** have not been synthesized before.

In the ^{1}H-NMR (in $CDCl_3$) of 3,4-diFQL **32** (**Figure 3.23**), the six aromatic protons resonated as following: the two C-6' protons came as dd at 7.09 ppm and 6.96 ppm (^4J = 1.8 Hz, ^3J = 8.2 Hz); the two C-2' protons appeared as doublets at 7.01 ppm and 6.91 ppm (^4J = 1.8 Hz); the two C-5' protons resonated as doublets at 6.93 ppm and 6.82 ppm (^3J = 8.2 Hz). The two olefinic protons at the β-position (C_{Ar}-CH) resonated the most downfield at 7.65 ppm and 7.54 ppm as doublets (^3J = 16.0 Hz), each coupling to the corresponding protons at the α-position (C_{Ar}-CH=C*H*), which appeared more upfield than the six aromatic protons, at 6.35 ppm and 6.17 ppm, respectively. Two broad signals for phenolic protons could be observed at 5.94 ppm and 5.84 ppm. The three following peaks in the spectrum, appearing more upfield than the α-protons and the hydroxyls protons, were the protons at C-4 (5.67 ppm), C-3 (5.28 ppm) and C-5 (4.94 ppm). Acylation at C-4 and C-3 resulted in chemical shifts of the protons attached to these carbons to resonate considerably more downfield; for 1-FQL **13** (spectrum measured in CD_3OD) the protons at the corresponding non-acylated C-4 and C-3 positions produced signals at 4.03 ppm and ~3.80 ppm, respectively. For 3,4-diFQL **32**, the proton at C-4 was a dd coupling to the two vicinal protons at C-3 (^3J = 4.6 Hz) and C-5 (^3J = 5.0 Hz); the proton at C-3 was a ddd coupling to a total of three vicinal protons, at C-4 (^3J = 4.6 Hz) and C-2 (to equatorial proton: ^3J = 6.9 Hz; to axial proton: ^3J = 11.9 Hz); the proton at C-5 was a dd coupling to the vicinal proton at C-4 (^3J

= 5.0 Hz) and to the equatorial proton at C-6 (^3J = 5.5 Hz), while coupling to the axial proton at C-6 was not observed (this coupling was not observed for 3,4-diDQL **29** either).

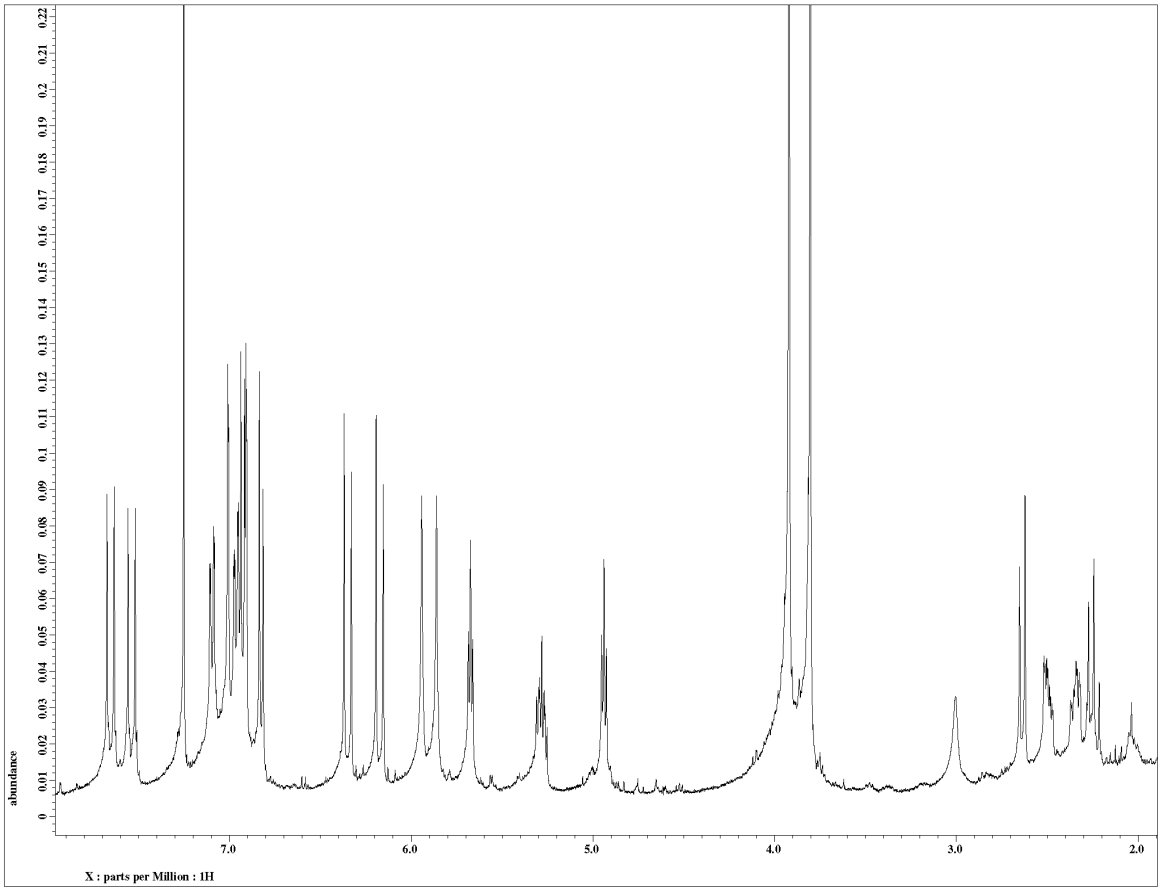


Figure 3.23. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of 3,4-di-*O*-feruloyl-1,5-quinide (3,4-diFQL) **32**.

The two methyl groups of 3,4-diFQL **32** produced two singlets, each integrating as three protons at 3.92 ppm and 3.80 ppm. The proton of the C-1 alcohol could be observed as a broad signal due to fast exchange at 3.00 ppm. The four protons at C-2 and C-6 resonated between 2.24-2.64 ppm and their splitting pattern could be clearly observed. The axial proton at C-6 resonated at 2.64 ppm and was a doublet being split by its geminal proton (^2J = 11.9 Hz) while the axial proton at C-2 was a triplet at 2.24 ppm coupling to its geminal proton (^2J = 11.9 Hz) and to the proton at C-3 (^3J = 11.9 Hz). The equatorial proton at C-6 appeared as a ddd at 2.50 ppm and it coupled to the geminal proton (^2J = 11.9 Hz), to the proton at C-5 (^3J = 5.5 Hz) and to the

equatorial proton at C-2 (^4J = 2.3 Hz, "W-coupling"). The equatorial proton at C-2 came at 2.34 ppm as a ddd, which showed, besides the "W-coupling" to the C-6 equatorial proton (^4J = 2.3 Hz), coupling to the proton at C-3 (^3J = 6.9 Hz) and geminal coupling (^2J = 11.9 Hz).

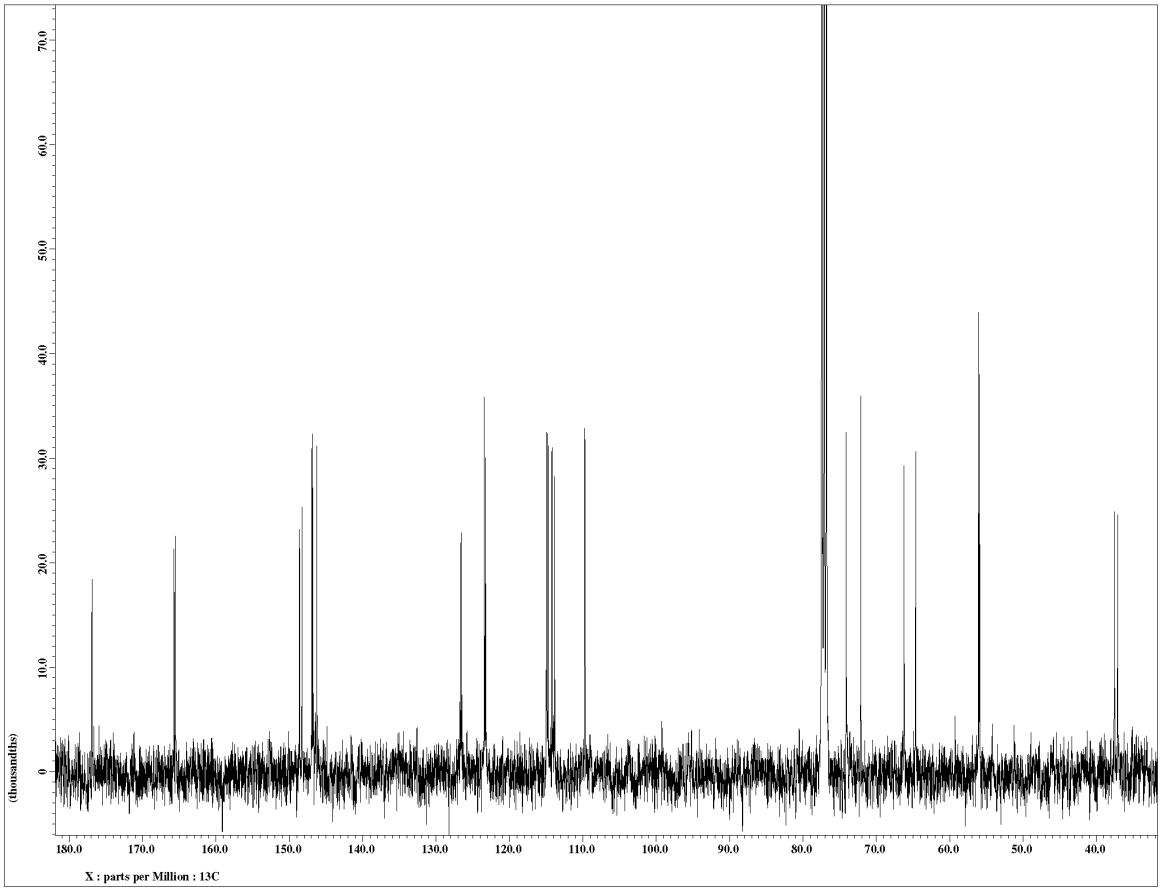


Figure 3.24. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of 3,4-di-*O*-feruloyl-1,5-quinide (3,4-diFQL) **32**.

For the ^{13}C-NMR (in $CDCl_3$) of 3,4-diFQL **32** (**Figure 3.24**) a number of 27 signals is expected and all of them could be observed individually. The ester carbon of the lactone (OC-*C*OO at 176.91 ppm) appeared more downfield than the two cinnamoyl ester carbons (CH-*C*OO at 165.71 ppm and 165.53 ppm). The two aromatic carbon atoms bearing the electron withdrawing methoxy functionalities (C_{Ar}-OCH_3) also came downfield at 148.63 ppm and 148.27 ppm while the two aromatic carbons bearing the phenols came slightly more upfield at 146.80 ppm and 146.26 ppm. The olefinic carbons adjacent to the aromatic moieties (*C*H-C_{Ar}) resonated at

146.96 ppm and 146.92 ppm. The two aromatic carbons (C_{Ar}-CH) attached each to the substituted olefin resonated more upfield at 126.72 ppm and 126.53 ppm. The two C-6' aromatic carbons appeared at 123.44 ppm and 123.27 ppm; the two C-5' aromatic carbons resonated at 114.21 ppm and 113.86 ppm; the two C-2' aromatic carbons appeared at 109.74 ppm and 109.69 ppm. More downfield than the C-5' carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum the two remaining olefinic carbons (C_{Ar}-CH=*C*H) at 114.97 ppm and 114.77 ppm, respectively.

The four carbon atoms of the quinic acid moiety bearing oxygen-containing substituents resonated as following: the C-5 atom bearing the electron withdrawing group -OCOR appeared at 74.12 ppm; the C-1 atom bearing the electron withdrawing lactone -COOR and -OH appeared at 72.13 ppm; the C-3 and C-4 atoms, each bearing an identical ester, resonated at 66.23 ppm and 64.65 ppm, respectively. The two methoxy carbons resonated at 56.09 ppm and 55.94 ppm. The C-2 (37.11 ppm) and C-6 (37.55 ppm) carbons of the quinic acid moiety gave signals, which appeared most upfield in the spectrum of all carbons of the molecule. For comparison, in the ^{13}C-NMR of 1-FQL **13** (measured in CD_3OD) the C-1 carbon resonated slightly more downfield (77.27 ppm) than the C-5 carbon (76.77 ppm) due to deshielding from the acyl moiety; for 3,4-diFQL **32** the reverse was true since the lack of acylation at C-1 (72.13 ppm) resulted in this carbon to be slightly more shielded than the C-5 nucleus (74.12 ppm). The same order of signals in terms of chemical shift was observed for 1-DQL **4** *versus* 3,4-diDQL **29**.

3.1.8. Synthesis of Methyl 3,4-di-*O*-Feruloylquinate (3,4-diFQM) 36

The strategy to make 3,4-diFQM **36** (**Figure 3.25**) was identical as the one for synthesizing 3,4-diFQL **32** (**Figure 3.22**). The key difference in the synthetic scheme discovered accidentally was in the penultimate step when aqueous MeOH (90%) was chosen as the reaction solvent instead of the corresponding 1,4-dioxane solution; additionally, a slightly higher temperature was employed (75 °C instead of 60 °C). The formation of the methyl ester intermediate **35** (yield 48%) from the lactone intermediate **30** was possible because the reaction conditions mimic the Fischer-Speier esterification: methanol becomes a nucleophile for the substrate (the cyclic ester) and the nucleophilic acyl substitution is possible given the acidic conditions (PTSA).[21] By altering the two parameters in the reaction conditions (solvent and temperature) two purposes could be

achieved at once: allyl deprotection of the feruloyl moiety and opening of the *γ*-quinide into a methyl ester of quinic acid. The final step of the synthetic scheme produced 3,4-diFQM **36** quantitatively, the success of the Troc deprotection being confirmed by the ^{1}H-NMR of the product in which the two signals for the diastereotopic protons of the protecting group disappeared and a broad signal for the unprotected C-1 alcohol appeared compared to the precursor's spectrum. Compounds **30**, **35** and **36** have not been synthesized before.

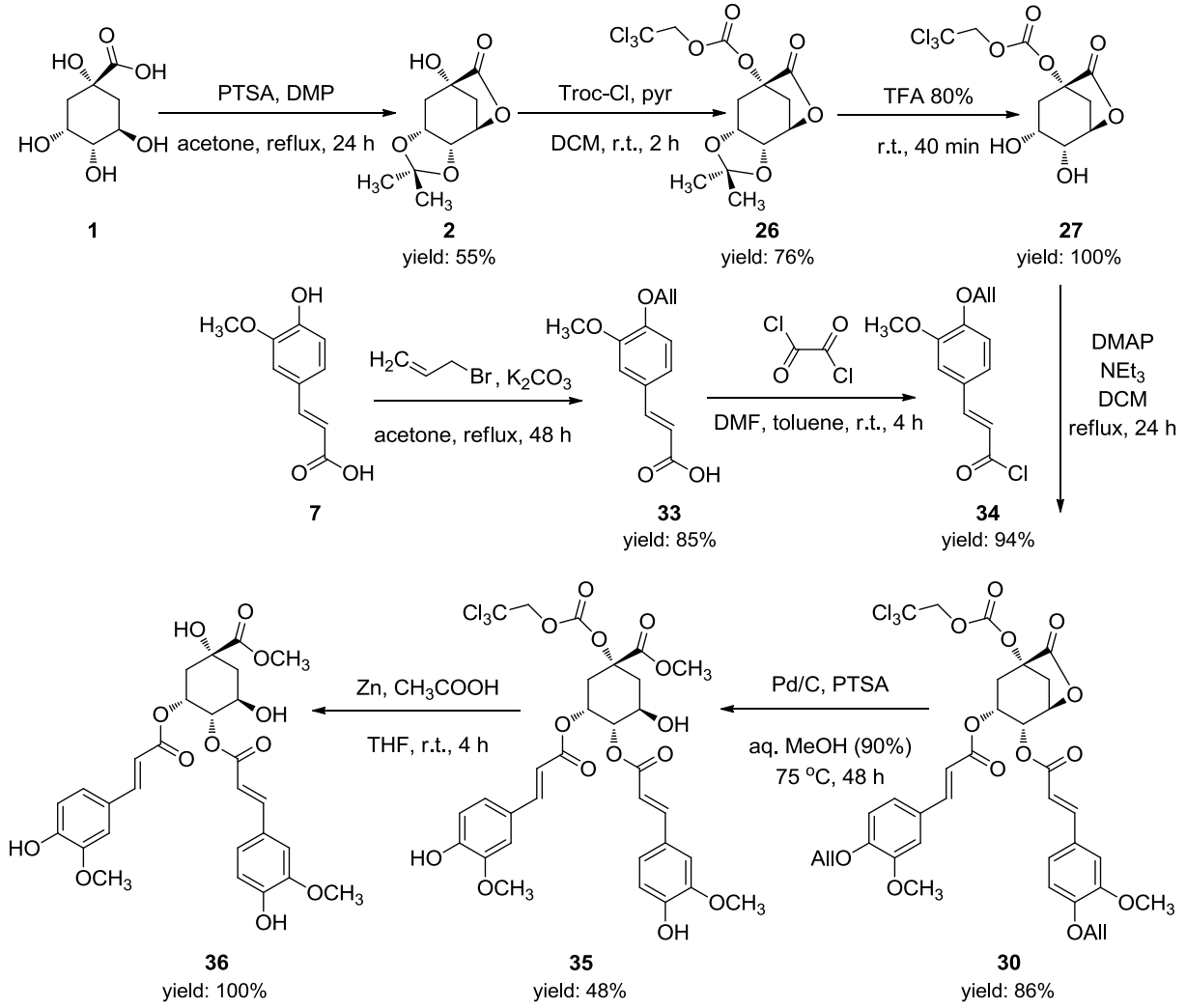


Figure 3.25. Synthetic path to 3,4-diFQM **36**.

The ^{1}H-NMR (in $CDCl_3$) of 3,4-diFQM **36** (**Figure 3.26**) contained a number of differences compared to the one of 3,4-diFQL **32** (also in $CDCl_3$)**,** notably for the protons of the quinic acid moiety, in addition to the presence of a signal for the methyl esters protons. The differences were

not significant for the protons of the cinnamoyl moiety. The six aromatic protons of 3,4-diFQM **36** resonated between 6.86-7.05 ppm, similarly to those of 3,4-diFQL **32**, which appeared between 6.82-7.09 ppm, as detailed above. As expected, the similarity was also observed for the two olefinic protons at the *β*-position (C_{Ar}-CH), which were the most deshielded of all the protons in the molecule at 7.62 ppm (7.65 ppm for 3,4-diFQL **32**) and 7.61 ppm (7.54 ppm for **32**). These protons coupled to the corresponding protons at the *α*-position (C_{Ar}-CH=C*H*), which displayed a paramagnetic shift compared to the six aromatic protons, resonating at 6.31 ppm (6.35 ppm for 3,4-diFQL) and 6.27 ppm (6.17 ppm for 3,4-diFQL), respectively. Only one broad signal integrating as two protons (as opposed to two such signals integrating as one proton each for 3,4-diFQL) was observed for the phenolic protons at 5.90 ppm.

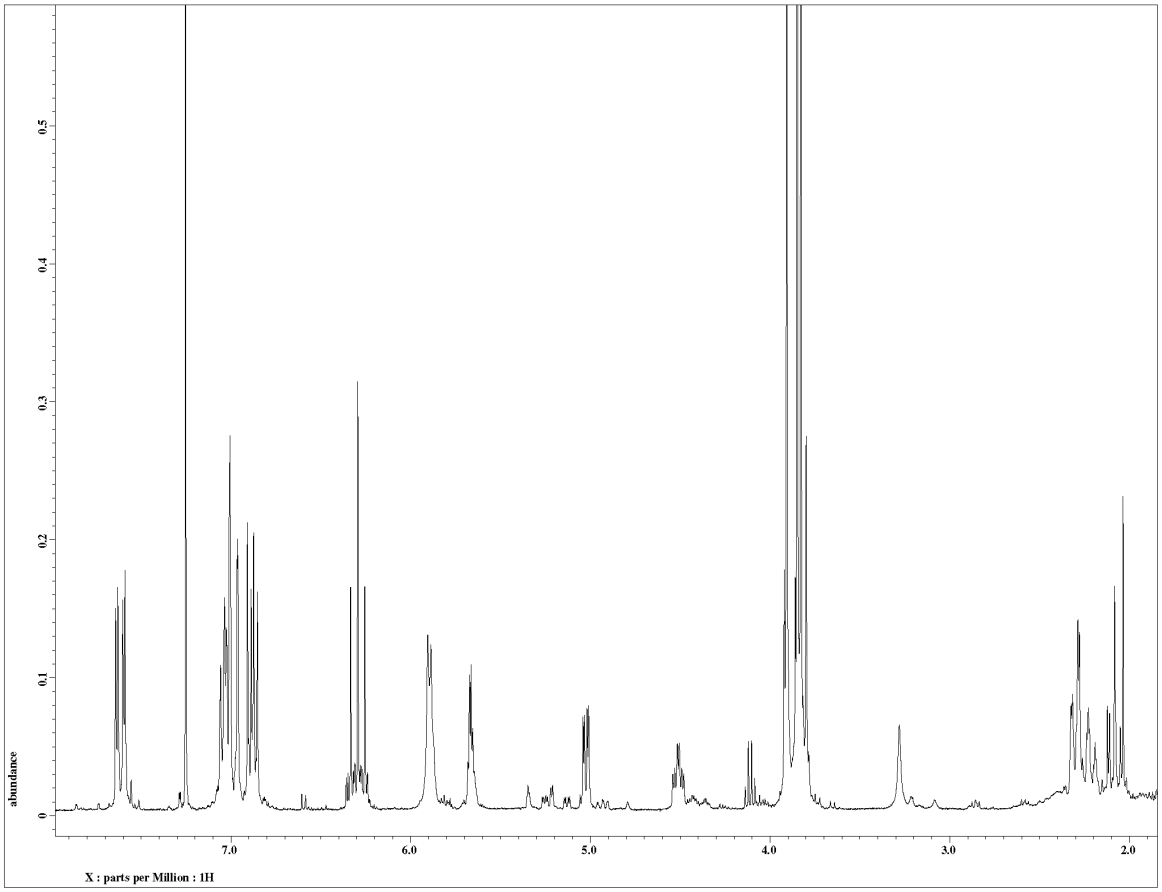


Figure 3.26. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of methyl 3,4-di-*O*-feruloylquinate (3,4-diFQM) **36**.

The differences from 3,4-diFQL **32** were clearly observed for the protons at C-3, C-4 and C-5; the signals appeared in a different order, at different chemical shifts and showed differences in the splitting patterns and coupling constants. For 3,4-diFQM **36** the proton at C-3 appeared as a multiplet at 5.66 ppm displaying a paramagnetic shift compared to the ddd at 5.28 ppm for 3,4-diFQL **32**, the proton at C-4 was a dd at 5.02 ppm displaying a diamagnetic (upfield) shift compared to the dd at 5.67 ppm for 3,4-diFQL **32**, and the proton at C-5 resonated as a ddd at 4.51 ppm displaying a diamagnetic shift compared to the dd at 4.94 ppm for **32**. The distinct singlet integrating as three protons from the methyl ester was observed at 3.85 ppm, in between the two singlets produced by the two methoxy functionalities at 3.91 ppm (3.92 ppm for 3,4-diFQL **32**) and 3.83 ppm (3.80 ppm for 3,4-diFQL **32**). Another notable difference from **32** was that the protons at C-2 and C-6 came mainly as multiplets between 2.11-2.30 ppm while in lactone **36** the paramagnetic shift of this group of protons was obvious (2.24-2.64 ppm) in addition to their individual splitting pattern.

In the ^{13}C-NMR (in $CDCl_3$) of 3,4-diFQM **36** (**Figure 3.27**) a number of 26 signals was observed because on two occasions two peaks showed overlap. The nucleus of the ester carbon in the methyl ester (*C*OOCH$_3$) was the most deshielded at 175.37 ppm; in 3,4-diFQL **32** the corresponding nucleus OC-*C*OO was even more deshielded at 176.91 ppm. The two cinnamoyl ester carbons in 3,4-diFQM **36** (CH-*C*OO) resonated at slightly lower frequency, at 167.00 ppm and 166.41 ppm, and thus at comparative values to their corresponding nuclei in 3,4-diFQL **32** (165.71 ppm and 165.53 ppm). The two aromatic carbons bearing the electron withdrawing methoxy functionalities (*C*$_{Ar}$-OCH$_3$) also came downfield at 148.27 ppm and 148.24 ppm (148.63 ppm and 148.27 ppm for 3,4-diFQL **32**) while the two aromatic carbons bearing the phenols resonated at slightly lower frequencies at 145.89 ppm and 146.26 ppm (146.80 ppm and 146.26 ppm for 3,4-diFQL **32**). The olefinic carbons adjacent to the aromatic moieties (*C*H-C$_{Ar}$) resonated at 146.87 ppm and 146.81 ppm (146.96 ppm and 146.92 ppm for 3,4-diFQL **32**). The two aromatic carbons (*C*$_{Ar}$-CH) attached each to the substituted olefin resonated more upfield at 126.91 ppm and 126.82 ppm (126.72 ppm and 126.53 ppm for 3,4-diFQL). While for 3,4-diFQL **32** the two C-6' aromatic carbons appeared at 123.44 ppm and 123.27 ppm, for 3,4-diFQM **36** they showed overlap (one signal at 123.45 ppm); the two C-5' aromatic carbons resonated at 114.79 ppm (shared signal with C$_{Ar}$-CH=*C*H) and 114.57 ppm (C-5' aromatic carbons for 3,4-

diFQL **32**: 114.21 ppm and 113.86 ppm); the two C-2' aromatic carbons appeared at 109.57 ppm and 109.47 ppm (109.74 ppm and 109.69 ppm for 3,4-diFQL **32**). More downfield than the C-5' carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum the two remaining olefinic carbons (C_{Ar}-CH=*C*H) at 115.07 ppm and 114.79 ppm (shared signal with C-5'), thus at comparative values to their corresponding nuclei in 3,4-diFQL **32**, which came at 114.97 ppm and 114.77 ppm, respectively.

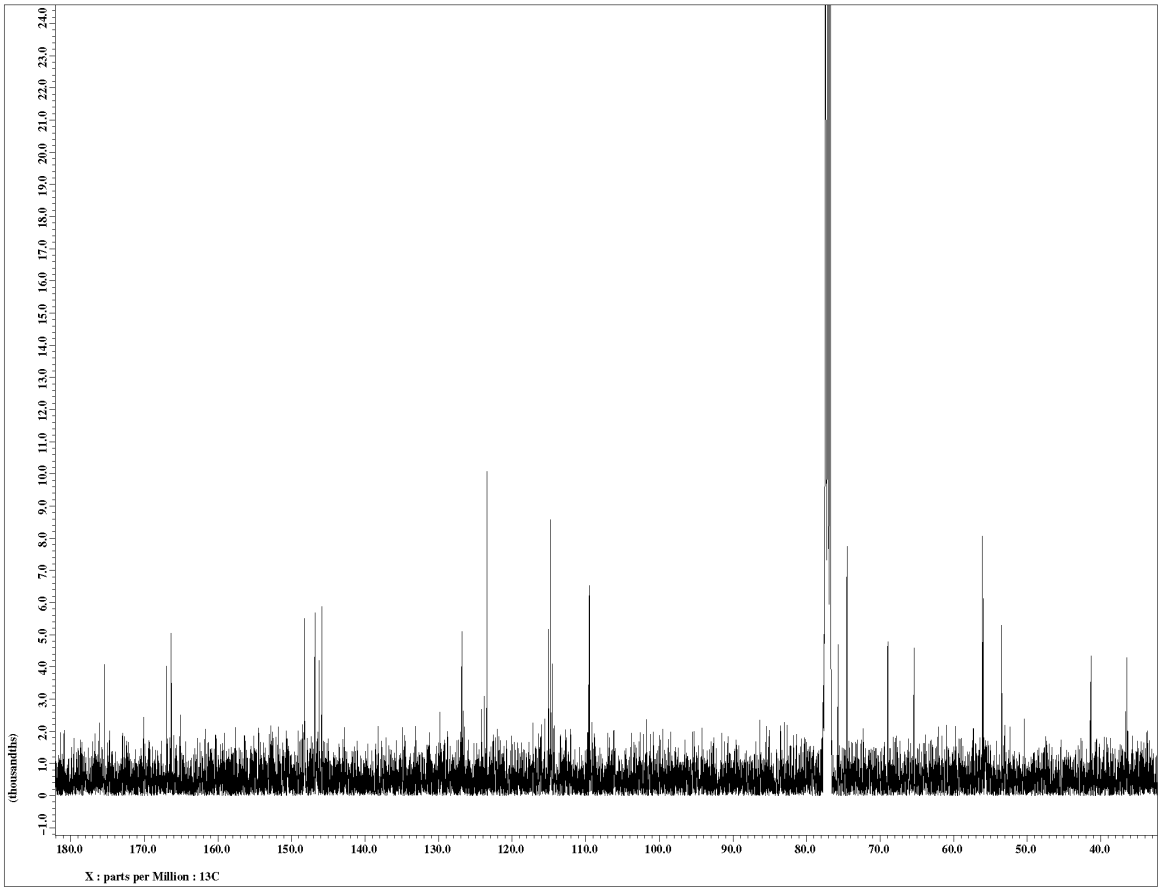


Figure 3.27. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of methyl 3,4-di-*O*-feruloylquinate (3,4-diFQM) **36**.

The four carbon atoms of the quinic acid moiety bearing oxygen-containing substituents showed a few differences from 3,4-diFQL **32** and resonated as following: the C-1 nucleus bearing the electron withdrawing groups -OH and methyl ester -$COOCH_3$ was the most deshielded of the four and appeared at 75.73 ppm (72.13 ppm for 3,4-diFQL); the C-5 carbon bearing the electron

withdrawing -OH appeared at 74.40 ppm (74.12 ppm for 3,4-diFQL); the C-3 and C-4 atoms, each bearing an identical ester, resonated at 68.96 ppm and 65.38 ppm, respectively. The two methoxy carbons resonated at 56.09 ppm and 55.99 ppm (56.09 ppm and 55.94 ppm for 3,4-diFQL) while the methyl ester came at 53.47 ppm. The C-2 (36.46 ppm) and C-6 (41.34 ppm) carbons of the quinic acid moiety gave signals which appeared most upfield in the spectrum of all carbons of the molecule; the two signals resonated closer to each other in the corresponding lactone (C-2 at 37.11 ppm and C-6 at 37.55 ppm) due to inductive effects.

3.1.9. Synthesis of Methyl 3,4-di-*O*-Caffeoylquinate (3,4-diCQM) 39

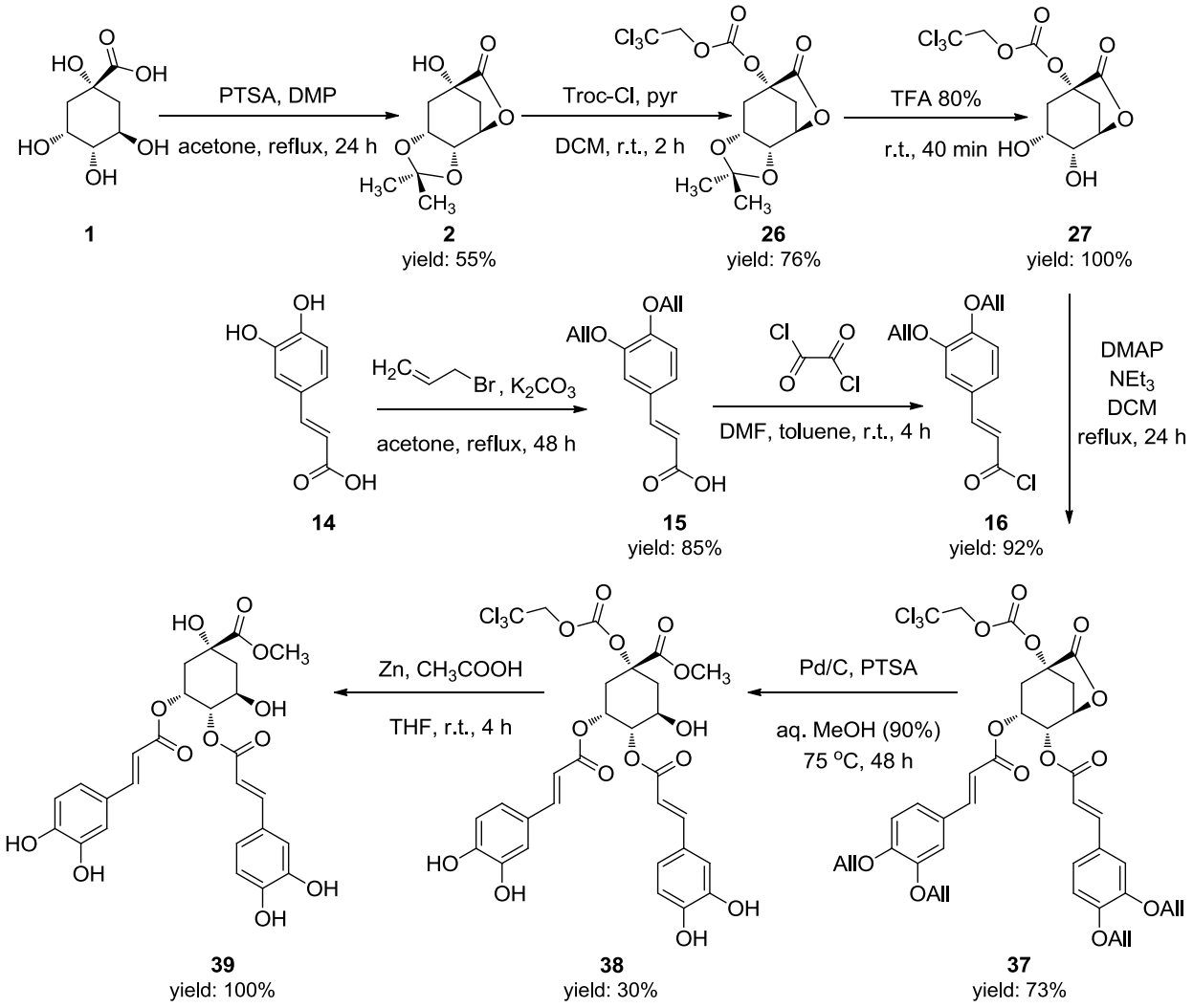


Figure 3.28. Synthetic path to 3,4-diCQM **39**.

The strategy to synthesize 3,4-diCQM **39** (**Figure 3.28**) was identical as the one for 3,4-diFQM **36** (**Figure 3.25**), with the difference that caffeic acid **14** was employed instead of ferulic acid **7** as the starting hydroxycinnamic acid to be allyl-protected[8,14,22,23] and converted into the more reactive acid chloride **16**.[5] In the allyl-deprotection step,[16,17] by altering two parameters in the reaction conditions (solvent and temperature) an additional purpose could be achieved: opening of the *γ*-quinide into a methyl ester of quinic acid. Aqueous MeOH (90%) was chosen as the reaction solvent instead of the corresponding 1,4-dioxane solution; additionally, a slightly higher temperature was employed (75 °C instead of 60 °C) and methyl ester **38** could be generated in 30% yield. As in the case of the 3,4-diFQM **36** synthesis, the formation of the methyl ester intermediate **38** from the lactone intermediate **37** was possible in this synthesis because the reaction conditions mimic the Fischer-Speier esterification: methanol becomes a nucleophile for the substrate (the cyclic ester) and the nucleophilic acyl substitution is facilitated by the acidic conditions (PTSA).[21] The final step of the synthetic scheme produced 3,4-diCQM **39** quantitatively, the success of the Troc deprotection being confirmed by the ^{1}H-NMR of the product in which the two signals for the diastereotopic protons of the protecting group disappeared compared to the precursor's spectrum (the broad signal for the unprotected C-1 alcohol, which could be observed for this step of the 3,4-diFQM **36** synthesis, was not present in this case, nor could signals from the caffeoyl phenols be seen in CD_3OD). Compounds **37-39** have not been synthesized before.

The ^{1}H-NMR (in CD_3OD) of 3,4-diCQM **39** (**Figure 3.29**) was rather similar to the corresponding spectrum of 3,4-diFQM **36** (in $CDCl_3$). The six aromatic protons of 3,4-diCQM **39** resonated between 6.71-7.01 ppm, similarly to those of 3,4-diFQM **36**, which appeared between 6.86-7.05 ppm. As expected, the similarity was also observed for the two olefinic protons at the *β*-position (C_{Ar}-CH), which were the most deshielded of all the protons in the molecule at 7.53 ppm (7.62 ppm for 3,4-diFQM) and 7.52 ppm (7.61 ppm for 3,4-diFQM **36**). These protons coupled to the corresponding protons at the *α*-position (C_{Ar}-CH=C*H*), which displayed a paramagnetic shift compared to the six aromatic protons, resonating at 6.24 ppm (6.31 ppm for 3,4-diFQM **36**) and 6.23 ppm (6.27 ppm for **36**), respectively. No broad signals could be observed for the phenolic protons of 3,4-diCQM **39** when the ^{1}H-NMR spectrum was

measured in CD_3OD; for 3,4-diFQM **36** one broad signal integrating as two protons (phenolic) could be seen at 5.90 ppm in $CDCl_3$.

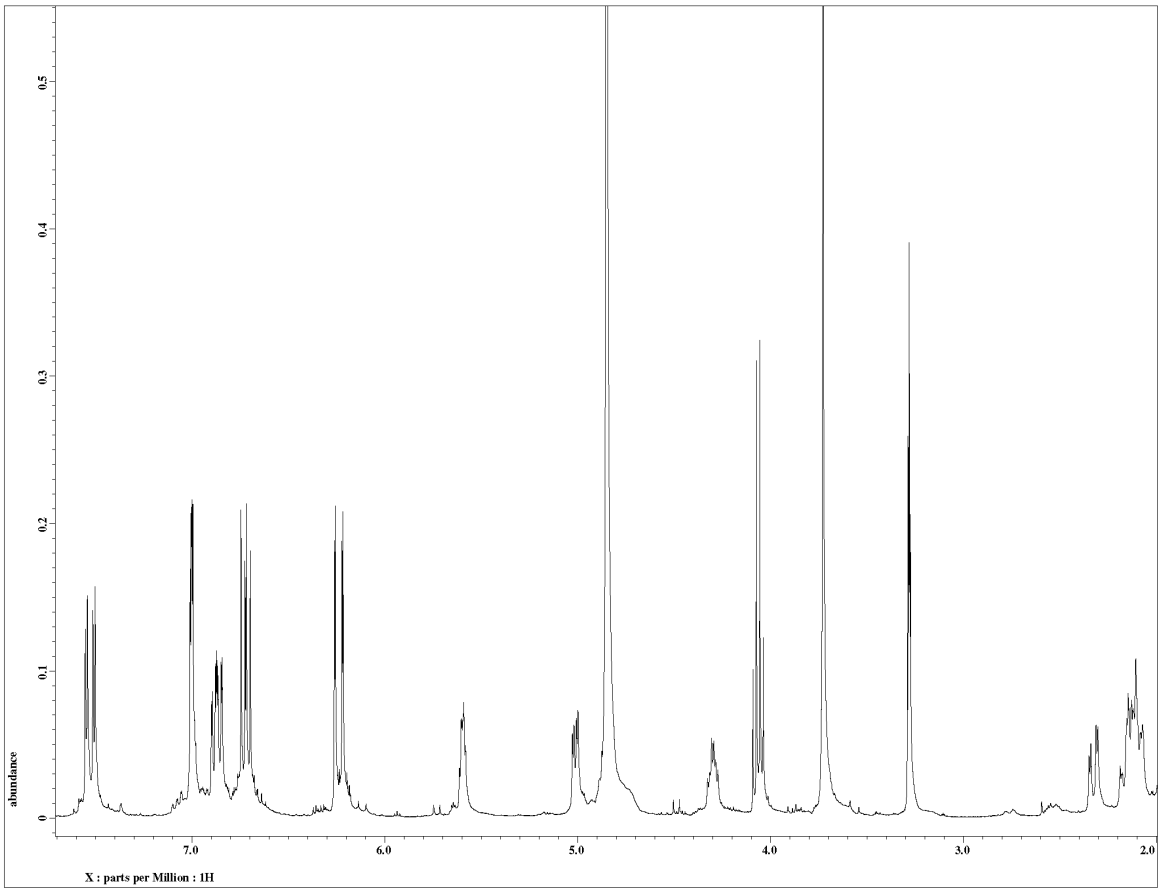


Figure 3.29. ^{1}H-NMR (CD_3OD, 400 MHz) spectrum of methyl 3,4-di-*O*-caffeoylquinate (3,4-diCQM) **39**.

The similarities to 3,4-diFQM **36** could be observed for the protons at C-3, C-4 and C-5; the signals appeared in the same order, at similar chemical shifts and displayed similar splitting patterns and coupling constants. For 3,4-diCQM **39** the proton at C-3 appeared as a multiplet at 5.60 ppm (m at 5.66 ppm for 3,4-diFQM **36**); the proton at C-4 was a dd at 5.01 ppm (dd at 5.02 ppm for 3,4-diFQM **36**); and the proton at C-5 resonated as a multiplet at 4.30 ppm (ddd at 4.51 ppm for 3,4-diFQM **36**). The distinct singlet integrating as three protons from the methyl ester was observed at 3.73 ppm (s at 3.85 ppm for 3,4-diFQM **36**). Since a methoxy functionality is not present in the molecule, the ^{1}H-NMR spectrum of 3,4-diCQM **39** showed two signals less

than the one of 3,4-diFQM **36** in which two singlets integrating as three protons each could be observed at 3.91 ppm and 3.83 ppm from the two methoxy groups on the aromatic moieties. Another similarity to the spectrum of 3,4-diFQM **36** was that the four protons at C-2 and C-6 appeared between 2.06-2.33 ppm for 3,4-diCQM **39** (2.11-2.30 ppm for 3,4-diFQM **36**).

In the ^{13}C-NMR (in CD_3OD) of 3,4-diCQM **39** (**Figure 3.30**) a number of 23 signals was observed (instead of expected 26 signals) because on three occasions two peaks showed overlap. The nucleus of the ester carbon in the methyl ester (*C*OOCH$_3$) was the most deshielded at 174.81 ppm (175.37 ppm for 3,4-diFQM **36** in $CDCl_3$). The two cinnamoyl ester carbons in 3,4-diCQM **39** (CH-*C*OO) resonated at slightly lower frequency, at 167.19 ppm and 167.12 ppm, and thus at comparative values to their corresponding nuclei in 3,4-diFQM **36** (167.00 ppm and 166.41 ppm). The four aromatic carbons bearing the electron withdrawing hydroxyl functionalities (*C*$_{Ar}$-OH) also came downfield at 148.31 ppm, 148.27 ppm and 145.45 (2xC$_{Ar}$-OH); for 3,4-diFQM **36** the two aromatic carbons bearing the phenols resonated at 145.89 ppm and 146.26 ppm (while the two aromatic carbons bearing the electron withdrawing methoxy functionalities came at 148.27 ppm and 148.24 ppm). Another caffeoyl-containing diacylated molecule synthesized in the current project, cynarine **47** (**Figure 3.36**), showed in the ^{13}C-NMR spectrum (measured in $(CD_3)_2CO$) four distinct signals for the four aromatic carbons bearing hydroxyls, at 148.11 ppm, 147.71 ppm, 145.44 ppm and 145.21 ppm.

The olefinic carbons adjacent to the aromatic moieties (*C*H-C$_{Ar}$) resonated for 3,4-diCQM **39** together as one peak at 146.04 ppm (146.87 ppm and 146.81 ppm for 3,4-diFQM **36**). The two aromatic carbons (*C*$_{Ar}$-CH) attached each to the substituted olefin resonated more upfield at 126.42 ppm and 126.34 ppm (126.91 ppm and 126.82 ppm for 3,4-diFQM **36**). For 3,4-diCQM **39** the two C-6' aromatic carbons appeared at 121.92 ppm and 121.81 ppm, while for 3,4-diFQM **36** they showed overlap (one signal at 123.45 ppm); the two C-5' aromatic carbons resonated at 113.84 ppm and 113.74 ppm (C-5' aromatic carbons for 3,4-diFQM **36**: 114.79 ppm and 114.57 ppm); the two C-2' aromatic carbons appeared at 113.66 ppm and 113.53 ppm (109.57 ppm and 109.47 ppm for 3,4-diFQM **36**; 114.82 ppm and 114.75 ppm for cynarine **47**). More downfield than the C-5' carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum the two remaining olefinic carbons (C$_{Ar}$-CH=*C*H) as a shared signal at 115.14 ppm, thus at

comparative values to their corresponding nuclei in 3,4-diFQM **36**, which came at 114.79 ppm and 114.57 ppm, respectively.

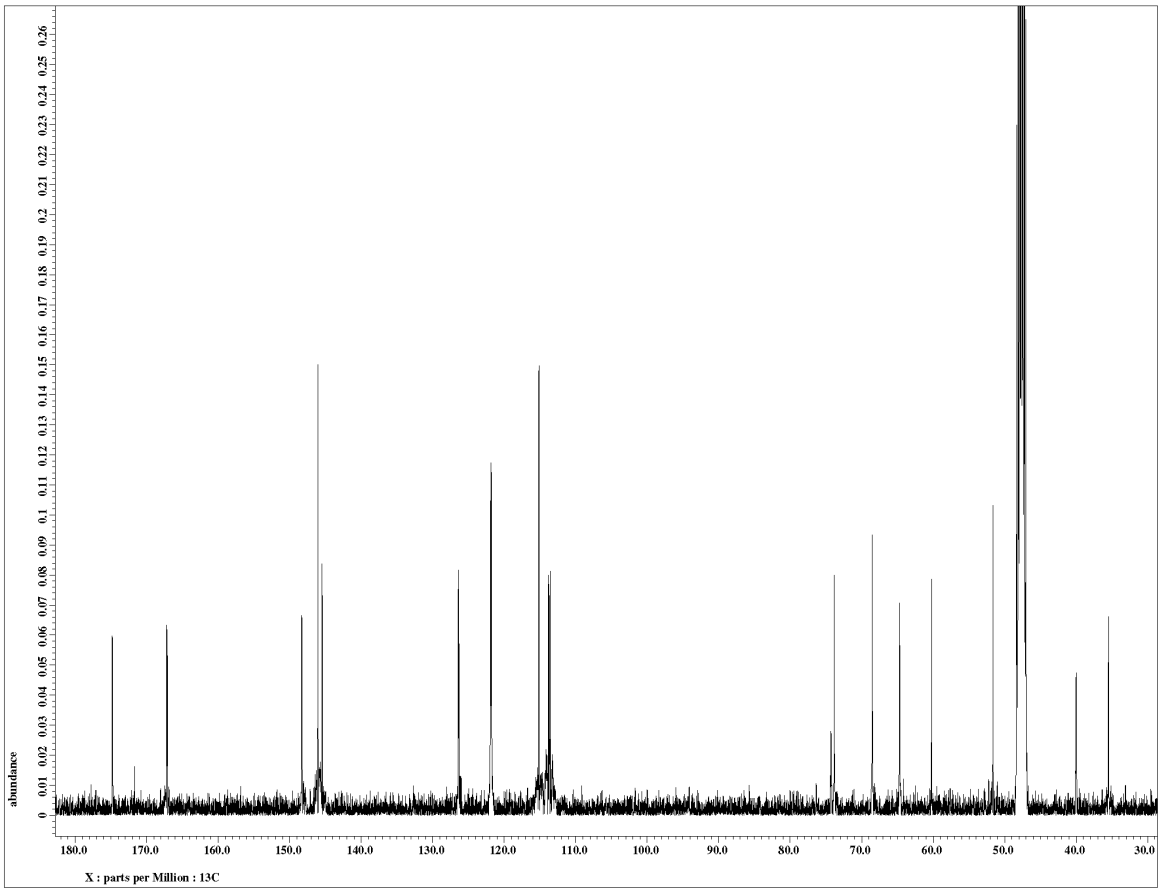


Figure 3.30. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of methyl 3,4-di-*O*-caffeoylquinate (3,4-diCQM) **39**.

The four carbon atoms of the quinic acid moiety bearing oxygen-containing substituents in 3,4-diCQM **39** resonated similarly to their corresponding nuclei in 3,4-diFQM **36**, as following: the C-1 nucleus bearing the electron withdrawing groups -OH and methyl ester -$COOCH_3$ was the most deshielded of the four and appeared at 74.29 ppm (75.73 ppm for 3,4-diFQM **36**); the C-5 carbon bearing the electron withdrawing -OH appeared at 73.85 ppm (74.40 ppm for 3,4-diFQM **36**); the C-3 and C-4 atoms, each bearing an identical ester, resonated at 68.52 ppm (68.96 ppm for 3,4-diFQM **36**) and 64.71 ppm (65.38 ppm for 3,4-diFQM **36**), respectively. The methyl ester carbon came at 51.65 ppm (53.47 ppm for 3,4-diFQM **36**). The C-2 and C-6 carbons of the

quinic acid moiety gave signals which appeared most upfield in the spectrum of all carbons of the molecule, at 35.48 ppm (36.46 ppm for 3,4-diFQM **36**) and 40.01 ppm (41.34 ppm for 3,4-diFQM **36**), respectively.

3.1.10. Synthesis of 1,3-di-*O*-(3',4'-Dimethoxycinnamoyl)-1,5-quinide (1,3-diDQL) 44 and 1,3-di-*O*-(3',4'-Dimethoxycinnamoyl)-quinic Acid (1,3-diDQA) 43

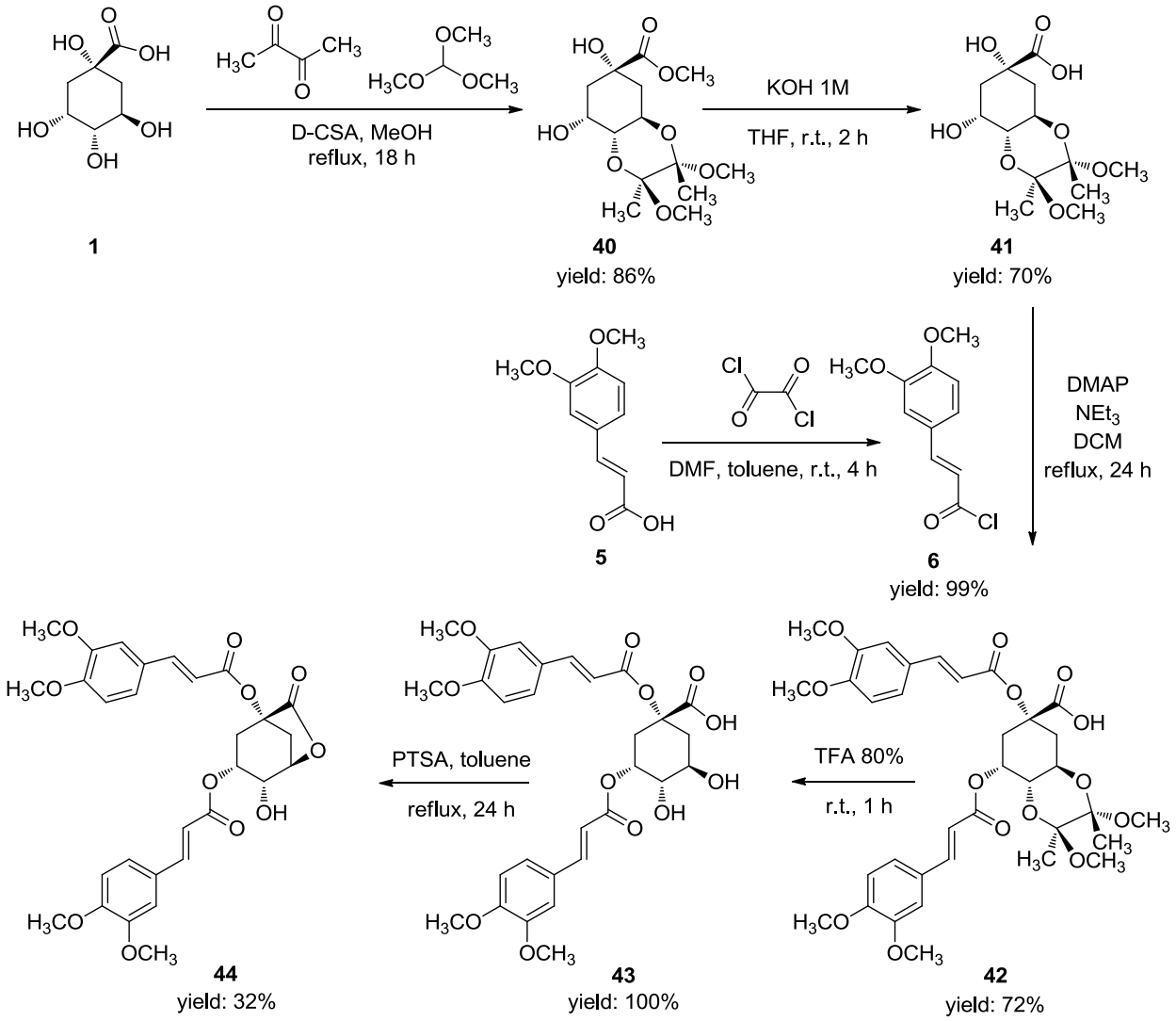


Figure 3.31. Synthetic path to 1,3-diDQL **44** and 1,3-diDQA **43**.

In order to synthesize 1,3-diDQL **44** (**Figure 3.31**), a quinic acid intermediate (**40**) bearing an adequate hydroxyl protecting group at C-4 and C-5 needed to be first synthesized. To this end,

Ley's procedure involving tetramethoxy butane (TMB) protection was used, which also resulted in esterification of the COOH group in the process (yield 86%).[24] De-methylation was performed as a next step with a 1M LiOH solution in THF (yield 70%). The synthesized bis-acetal protected quinic acid **41** was then reacted, as described earlier in the current chapter, with the more reactive acid chloride **6** of dimethoxycinnamic acid, with the later in excess of the stoichiometric requirement of 1:2 molar**.**[5,8] Additionally, reflux and the stronger base NEt_3 (compared to pyridine) were used to ensure diacylation (yield 72%) since milder conditions (pyridine, lower temperature) produce a mixture of the two possible monoacylated derivatives. 1,3-diDQA **43** was obtained quantitatively by removing the BBA-protection in the following step after TFA treatment[8] while 1,3-diDQL **44** could be made from **43** by using PTSA in toluene (yield 32%; a similar lactone-closing procedure was employed in the penultimate step of the synthesis of 1-FQL **13**, **Figure 3.6**). Compound **42** has not been synthesized before while **44** was made available through a different synthetic path,[2] *via* γ-quinide **49** (**Figure 3.39**).

The identity of the product could be established from the spectral data, although the purity was lower in this case. The ^{1}H-NMR of 1,3-diDQL **44** (measured in $(CD_3)_2CO$, **Figure 3.32**) is discussed in comparison to the corresponding spectrum of another dimethoxycinnamoyl-containing diacylated compound synthesized in the current project, namely 3,4-diDQL **29** (measured in $CDCl_3$, **Figure 3.18**). In the ^{1}H-NMR of 1,3-diDQL **44**, the six aromatic protons resonated as following: the two C-2' protons appeared as doublets at 7.35 ppm and 7.31 ppm, each displaying "W-coupling" (^4J = 1.8 Hz) to a C-6' proton (for 3,4-diDQL **29** they were doublets at 7.02 ppm and 6.89 ppm, ^4J = 2.3 Hz); the two C-6' protons came as doublets of doublets at 7.21 ppm and 7.19 ppm, each coupling to a C-2' proton (^4J = 1.8 Hz) and a C-5' proton (^3J = 8.2 Hz) (for 3,4-diDQL **29** they appeared each as a dd at 7.06 ppm and 6.92 ppm, ^4J = 2.3 Hz and ^3J = 8.2 Hz); the two C-5' protons could be observed both as doublets very close to each other (^3J = 8.2 Hz, coupling each to a C-6' proton) at 6.98 ppm (for 3,4-diDQL **29** the signals were better separated at 6.81 ppm and 6.70 ppm, ^3J = 8.2 Hz). The two olefinic protons at the β-position (C_{Ar}-CH) resonated the most downfield at 7.66 ppm and 7.65 ppm (for 3,4-diDQL **29** they appeared at 7.63 ppm and 7.50 ppm as doublets, ^3J = 16.0 Hz), each coupling to the corresponding protons at the α-position (C_{Ar}-CH=C*H*), which appeared more shielded than the

six aromatic protons, at 6.46 ppm and 6.42 ppm (6.35 ppm and 6.16 ppm for 3,4-diDQL **29**), respectively. The 16 Hz coupling constant is typical for a *trans* olefinic geometry.

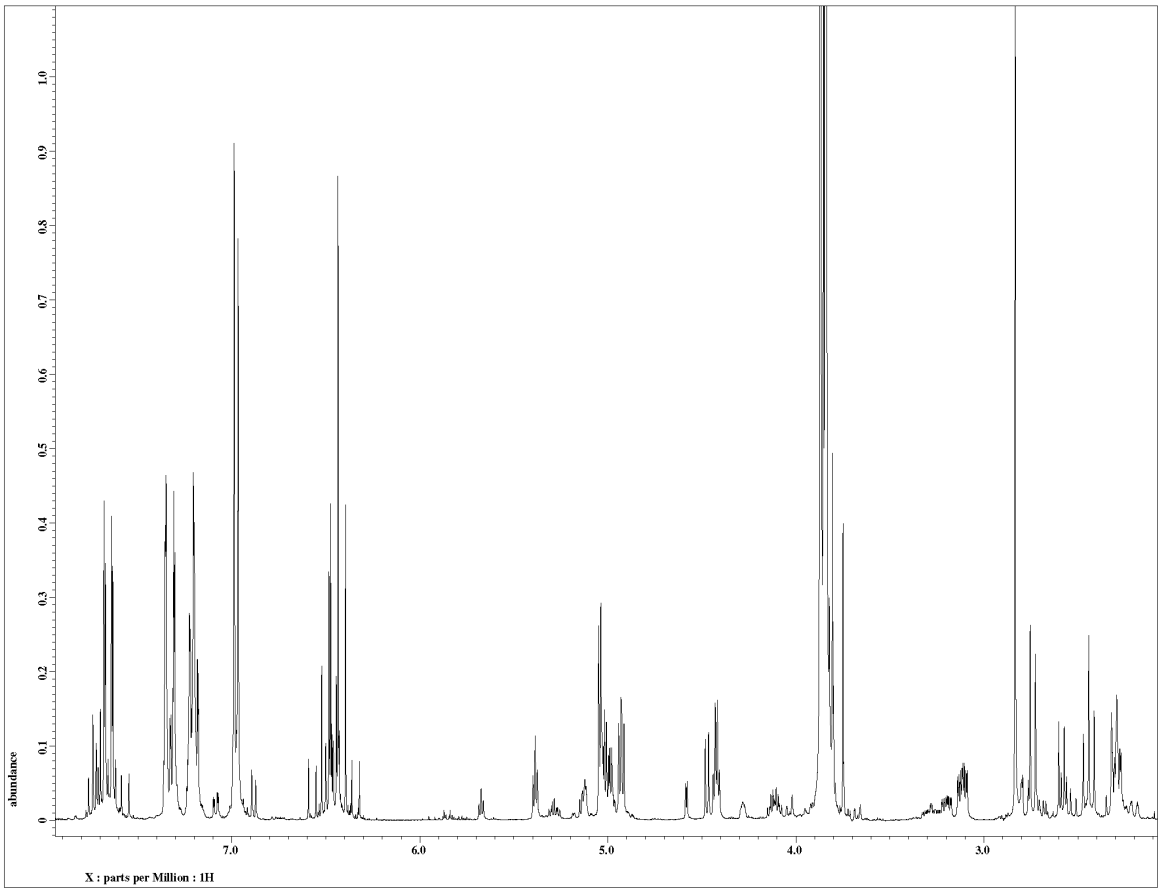


Figure 3.32. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) spectrum of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (1,3-diDQL) **44**.

The three following peaks in the spectrum appearing at higher field than the α-protons were the protons at C-3 (5.01 ppm), C-5 (4.93 ppm) and C-4 (4.42 ppm), with the proton attached to the esterified position (C-3) being the most deshielded of the three; for 3,4-diDQL **29** the order in which the corresponding protons produced signals in the ^{1}H-NMR spectrum was different but the protons attached to the esterified positions were, similarly, more deshielded: the proton at C-4 (5.64 ppm), the proton at C-3 (5.24 ppm) and the proton at C-5 (4.88 ppm). The signal splitting for these three protons was however similar for the two compounds, **44** and **29**. For 1,3-diDQL **44**, the proton at C-3 was a ddd coupling to the three vicinal protons at C-4 (^{3}J = 4.6 Hz) and C-2

(to equatorial proton: ^3J = 4.1 Hz; to axial proton: ^3J = 11.5 Hz); the proton at C-5 was a dd coupling to the vicinal proton at C-4 (^3J = 5.0 Hz) and to the equatorial proton at C-6 (^3J = 6.4 Hz); the proton at C-4 was a dd coupling to the two vicinal protons at C-3 (^3J = 4.6 Hz) and C-5 (^3J = 5.0 Hz).

The four methyl groups of 1,3-diDQL **44** produced three singlets, out of which two integrated as three protons at 3.87 ppm and 3.86 ppm (3.80 ppm and 3.72 ppm for 3,4-diDQL **29**) and one integrated as six protons at 3.84 ppm (3.86 ppm for 3,4-diDQL **29**). Between 2.29-3.11 ppm (2.22-2.59 ppm for 3,4-diDQL **29**) resonated the four protons at C-2 and C-6. The axial proton at C-6 resonated at 2.74 ppm (2.59 ppm for 3,4-diDQL **29**) was a doublet being split by its geminal proton (^2J = 11.5 Hz) while the axial proton at C-2 was a triplet at 2.44 ppm (dd at 2.22 ppm for 3,4-diDQL **29**) coupling to its geminal proton (^2J = 11.5 Hz) and to the proton at C-3 (^3J = 11.5 Hz). For 3,4-diDQL **29**, the C-6 and C-2 equatorial protons appeared as multiplets at 2.50 ppm (equatorial proton at C-6) and 2.36 ppm (equatorial proton at C-2); for 1,3-diDQL **44** the splitting of the acylated 1,5-quinide specific signal (equatorial proton at C-6) as well as of the signal of the equatorial proton at C-2 could be observed. The equatorial proton at C-6 was a ddd at 3.11 ppm coupling to its geminal proton (^2J = 11.5 Hz), to the vicinal proton at C-5 (^3J = 6.4 Hz), and to the equatorial proton at C-2 (^4J = 2.8 Hz, "W-coupling"). The equatorial proton at C-2 was also a ddd and displayed, besides the "W-coupling" to the equatorial proton at C-6 (^4J = 2.8 Hz), coupling to the vicinal proton at C-3 (^3J = 4.1 Hz) and geminal coupling (^2J = 11.5 Hz).

The ^{13}C-NMR (in $(CD_3)_2CO$) of 1,3-diDQL **44** (**Figure 3.33**) revealed the peak from the ester carbon of the lactone (OC-*C*OO at 172.11 ppm) to be the most downfield of the spectrum, as expected (for 3,4-diDQL **29** the corresponding peak came at 177.15 ppm in $CDCl_3$). The two cinnamoyl ester carbons (CH-*C*OO) resonated slightly more upfield at 165.42 ppm and 165.27 ppm (for 3,4-diDQL **29** they appeared at 165.78 ppm and 165.63 ppm). The four aromatic carbon atoms bearing the electron withdrawing methoxy functionalities (C_{Ar}-OCH_3) also came downfield in the spectrum, as three peaks at 151.65 ppm, 151.62 ppm and 149.34 ppm (2xC_{Ar}-OCH_3); these values were very similar to the ones of 3,4-diDQL **29** since the structural differences between the two molecules were not enclosed in these moieties: 151.69 ppm, 151.36 ppm, 149.35 ppm and 149.17 ppm. The olefinic carbons in 1,3-diDQL **44** adjacent to the

aromatic moieties were more shielded (than C_{Ar}-OCH_3) at 146.78 ppm and 146.57 ppm (for 3,4-diDQL **29** they appeared at 146.73 ppm and 146.11 ppm). The two aromatic carbons (C_{Ar}-CH) attached each to the substituted olefin resonated more upfield at 127.07 ppm and 126.97 ppm (127.07 ppm and 126.93 ppm for 3,4-diDQL **29**). The two C-6' aromatic carbons appeared at 123.19 ppm and 123.12 ppm (123.23 ppm and 122.93 ppm for 3,4-diDQL **29**); the C-5' and C-2' aromatic carbons (expected four peaks, observed two peaks) appeared at 111.12 ppm and 109.75 ppm (three peaks instead of expected four, between 109.85-111.00 ppm for 3,4-diDQL **29**). More downfield than this group of carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum the two remaining olefinic carbons (C_{Ar}-CH=*C*H) at 114.47 ppm and 114.21 ppm (114.49 ppm and 114.17 ppm for 3,4-diDQL **29**).

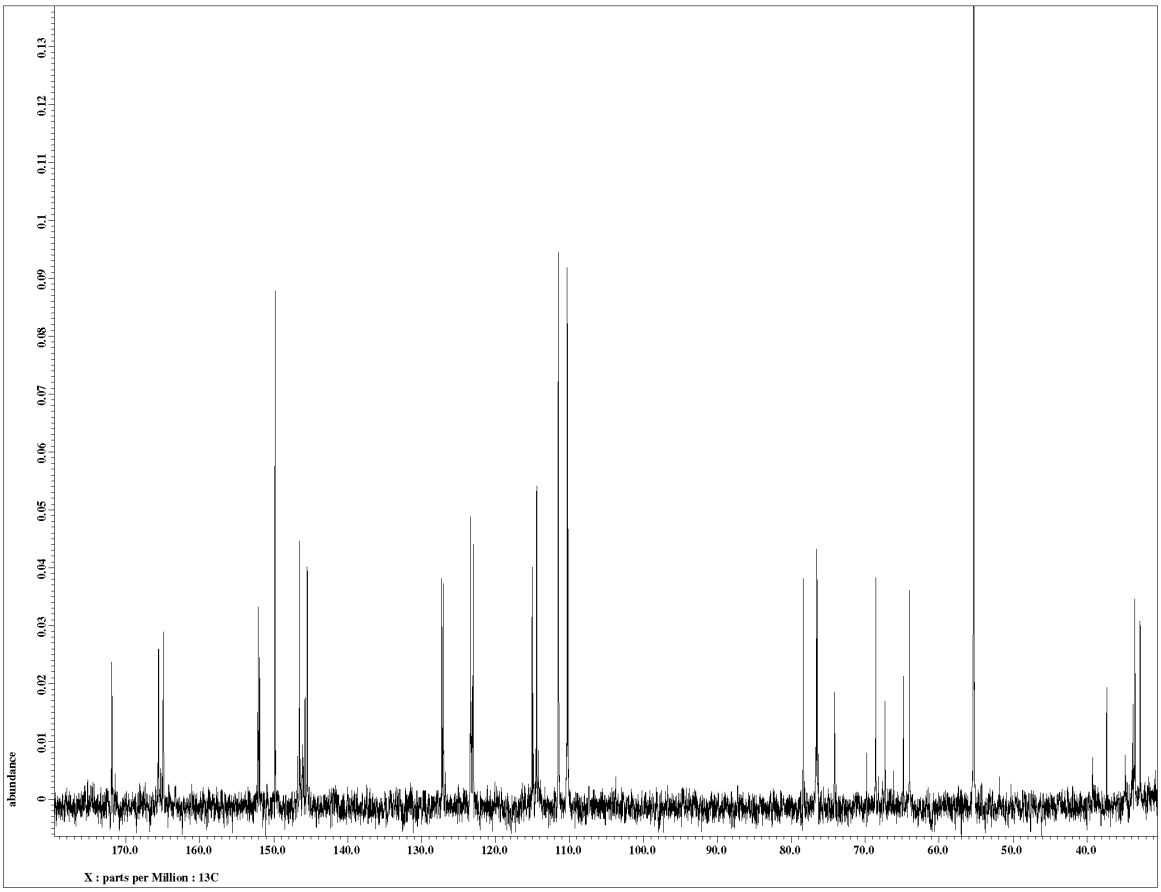


Figure 3.33. ^{13}C-NMR ($(CD_3)_2CO$, 100 MHz) spectrum of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (1,3-diDQL) **44**.

The four carbon atoms of the quinic acid moiety bearing oxygen-containing substituents in **44** resonated as following: the C-1 atom bearing the electron withdrawing lactone -COOR and the acyl appeared at 76.49 ppm (72.21 ppm for 3,4-diDQL **29**); the C-5 atom bearing the electron withdrawing -OCOR appeared at 76.02 ppm (74.09 ppm for 3,4-diDQL **29**); the C-4 atom, bearing an ester, resonated at 68.38 ppm (64.69 ppm for 3,4-diDQL **29**) while the C-3 atom bearing an alcohol resonated at 64.71 ppm (66.37 ppm for 3,4-diDQL **29**). The four methoxy carbons resonated as two peaks at 56.08 ppm ($2xC_{Ar}$-O*C*H_3) and 56.00 ppm ($2xC_{Ar}$-O*C*H_3) in the ^{13}C-NMR of 1,3-diDQL **44** while for 3,4-diDQL **29** they could be observed resolved at 56.06 ppm, 56.00 ppm, 55.97 ppm and 55.82 ppm. The C-2 (33.56 ppm) and C-6 (33.41 ppm) carbons of the quinic acid moiety gave signals which appeared most upfield in the spectrum of all carbons of the molecule (for 3,4-diDQL **29** they appeared at 36.66 ppm and 37.53 ppm, respectively).

1,3-diDQA **43** was obtained as an intermediate in the penultimate step of the synthesis of 1,3-diDQL **44**, being the starting material for the reaction generating lactone **44**. In the ^{1}H-NMR of 1,3-diDQA **43** (in $CDCl_3$, **Figure 3.34**), the two olefinic protons at the *β*-position (C_{Ar}-CH) resonated the most downfield at 7.51 ppm and 7.45 ppm (for 1,3-diDQL **44** they appeared at 7.66 ppm and 7.65 ppm as doublets, ^3J = 16.0 Hz); the two olefinic protons at the *β*-position coupled (^3J = 15.6 Hz) to the corresponding protons at the *α*-position (C_{Ar}-CH=C*H*), which appeared more shielded than the six aromatic protons in the molecule, at 6.19 ppm and 6.07 ppm (6.46 ppm and 6.42 ppm for 1,3-diDQL **44**), respectively. The six aromatic protons resonated as following: the total of four C-2' and C-6' protons appeared as multiplets between 6.60-6.81 ppm and their typical signal splitting pattern observed for other structurally-related molecules (for instance, 1,3-diDQL **44**) could not be easily observed due to signal cluttering; the two C-5' protons could be observed as doublets (^3J = 8.7 Hz, coupling each to a C-6' proton) at 6.51 ppm and 6.39 ppm (two doublets close to each other at 6.98 ppm for 1,3-diDQL **44**, ^3J = 8.2 Hz). At higher field than the *α*-protons were the protons at C-3 (5.44 ppm), C-5 (4.36 ppm) and C-4 (3.81 ppm), with the proton attached to the esterified position (C-3) being the most deshielded of the three; for 1,3-diDQL **44** the order in which the corresponding protons produced signals in the ^{1}H-NMR spectrum was similar, with the proton attached to the esterified position being more deshielded: the proton at C-3 (5.01 ppm), the proton at C-5 (4.93 ppm) and the proton at C-4

(4.42 ppm). The splitting of signals of these three protons, though clearly observed for 1,3-diDQL **44** as described above, could not be readily seen for 1,3-diDQA **43** and all three signals appeared as multiplets. The four methyl groups (C_{Ar}-OCH_3) of 1,3-diDQA **43** produced each a singlet integrating as three protons at 3.77 ppm, 3.74 ppm, 3.67 ppm and 3.59 ppm (3.87 ppm, 3.86 ppm and 3.84 ppm (2xC_{Ar}-OCH_3) for 1,3-diDQL **44**). Between 2.00-2.91 ppm resonated the four protons at C-2 and C-6 in 1,3-diDQA **43**, all as multiplets; they came between 2.29-3.11 ppm for the corresponding quinide **44** and their signal splitting could be observed as described above.

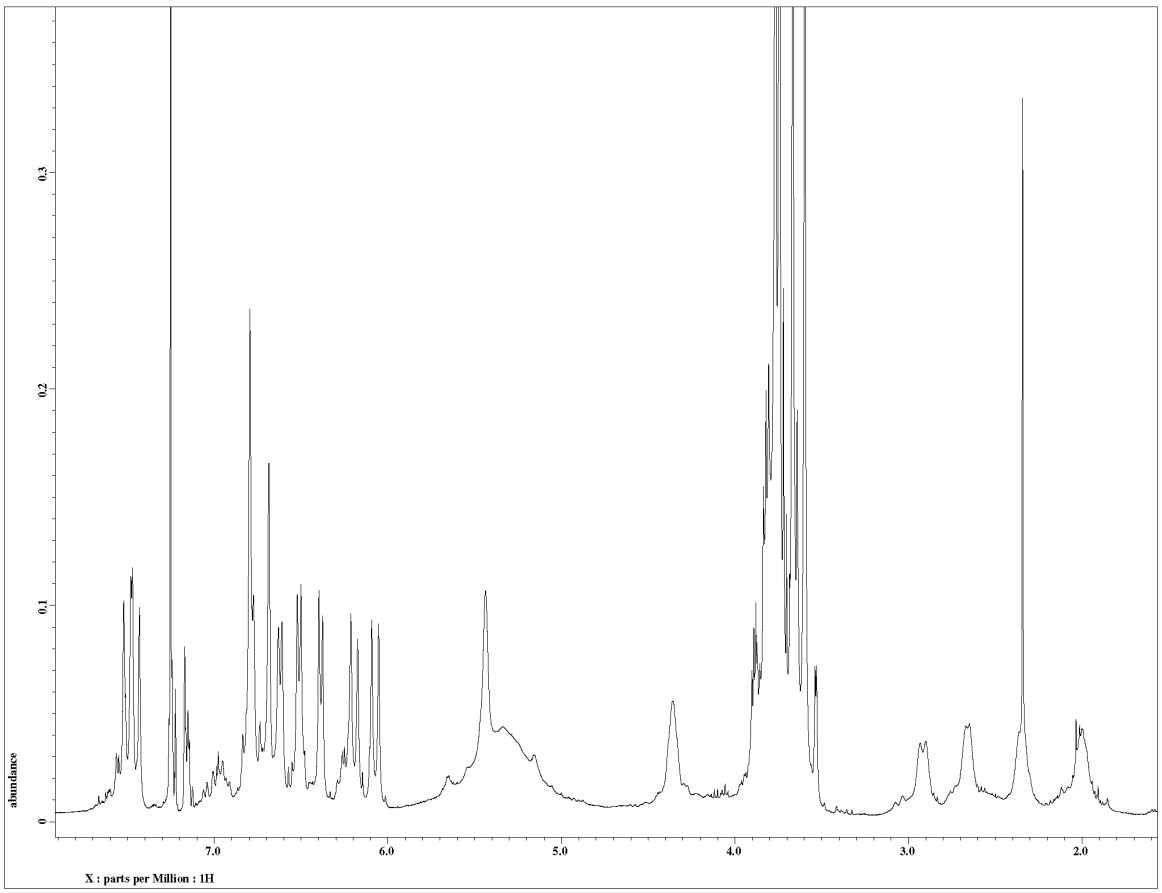


Figure 3.34. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid (1,3-diDQA) **43**.

The ^{13}C-NMR (in $CDCl_3$) of 1,3-diDQA **43** (**Figure 3.35**) revealed the peak from the ester carbon of the carboxylic acid (COOH at 174.17 ppm) to be the most downfield of the spectrum,

as expected (for 1,3-diDQL **44** the corresponding peak from OC-*C*OO came at 172.11 ppm in $(CD_3)_2CO$). The two cinnamoyl ester carbons (CH-*C*OO) resonated slightly more upfield (compared to COOH peak) at 167.36 ppm and 166.29 ppm (for 1,3-diDQL **44** they appeared at 165.42 ppm and 165.27 ppm). The four aromatic carbon atoms bearing the electron withdrawing methoxy functionalities (C_{Ar}-OCH_3) also came downfield in the spectrum at 151.30 ppm, 151.04 ppm, 149.04 ppm, 148.86 ppm; these values were very similar to the ones of 1,3-diDQL **44** since the structural differences between the two molecules were not enclosed in these moieties: 151.65 ppm, 151.62 ppm and 149.34 ppm (2xC_{Ar}-OCH_3). The olefinic carbons in 1,3-diDQA **43** adjacent to the aromatic moieties (*C*H-C_{Ar}) were more shielded (than C_{Ar}-OCH_3) at 146.46 ppm and 145.94 ppm (for 1,3-diDQL **44** they appeared at 146.78 ppm and 146.57 ppm). The two aromatic carbons (C_{Ar}-CH) attached each to the substituted olefin resonated more upfield at 129.12 ppm and 128.31 ppm (127.07 ppm and 126.97 ppm for 1,3-diDQL **44**). The two C-6' aromatic carbons appeared at 122.97 ppm and 122.17 ppm (123.19 ppm and 123.12 ppm for 1,3-diDQL **44**); the two C-5' aromatic carbons resonated at 110.77 ppm and 110.70 ppm; the two C-2' aromatic carbons appeared at 110.31 ppm and 109.88 ppm. For 1,3-diDQL **44** two peaks instead of expected four, at 111.12 ppm and 109.75 ppm, were observed for the C-5' and C-2' aromatic carbons, as detailed above. More downfield than this group of four carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum of 1,3-diDQA **43** the two remaining olefinic carbons (C_{Ar}-CH=*C*H) at 115.18 ppm and 114.89 ppm (114.47 ppm and 114.21 ppm for 1,3-diDQL **44**). The four carbon atoms of the quinic acid moiety bearing oxygen-containing substituents in **43** resonated as following: the C-1 atom bearing the electron withdrawing carboxylic acid -COOH and the acyl appeared at 79.52 ppm (76.49 ppm for 1,3-diDQL **44**); the C-4 atom, bearing an ester, resonated at 74.27 ppm (68.38 ppm for 1,3-diDQL **44**); the C-5 atom bearing the electron withdrawing -OH appeared at 71.38 ppm (76.02 ppm for 1,3-diDQL **44**); the C-3 atom bearing an alcohol resonated at 67.34 ppm (64.71 ppm for 1,3-diDQL **44**); while for lactone **44** the C-5 signal was more deshielded than the C-4 signal, the opposite was true for diacylated CGA **43**. The four methoxy carbons resonated as three peaks at 55.83 ppm, 55.77 ppm (2xC_{Ar}-O*C*H_3) and 55.65 ppm in the ^{13}C-NMR of 1,3-diDQA **43** while for 3,4-diDQL **44** they resonated as two peaks at 56.08 ppm (2xC_{Ar}-O*C*H_3) and 56.00 ppm (2xC_{Ar}-O*C*H_3). The C-2 and C-6 carbons of the quinic acid moiety produced jointly one signal

which appeared most upfield in the spectrum of the molecule at 32.01 ppm (for 1,3-diDQL **44** they appeared resolved at 33.56 ppm and 33.41 ppm, respectively).

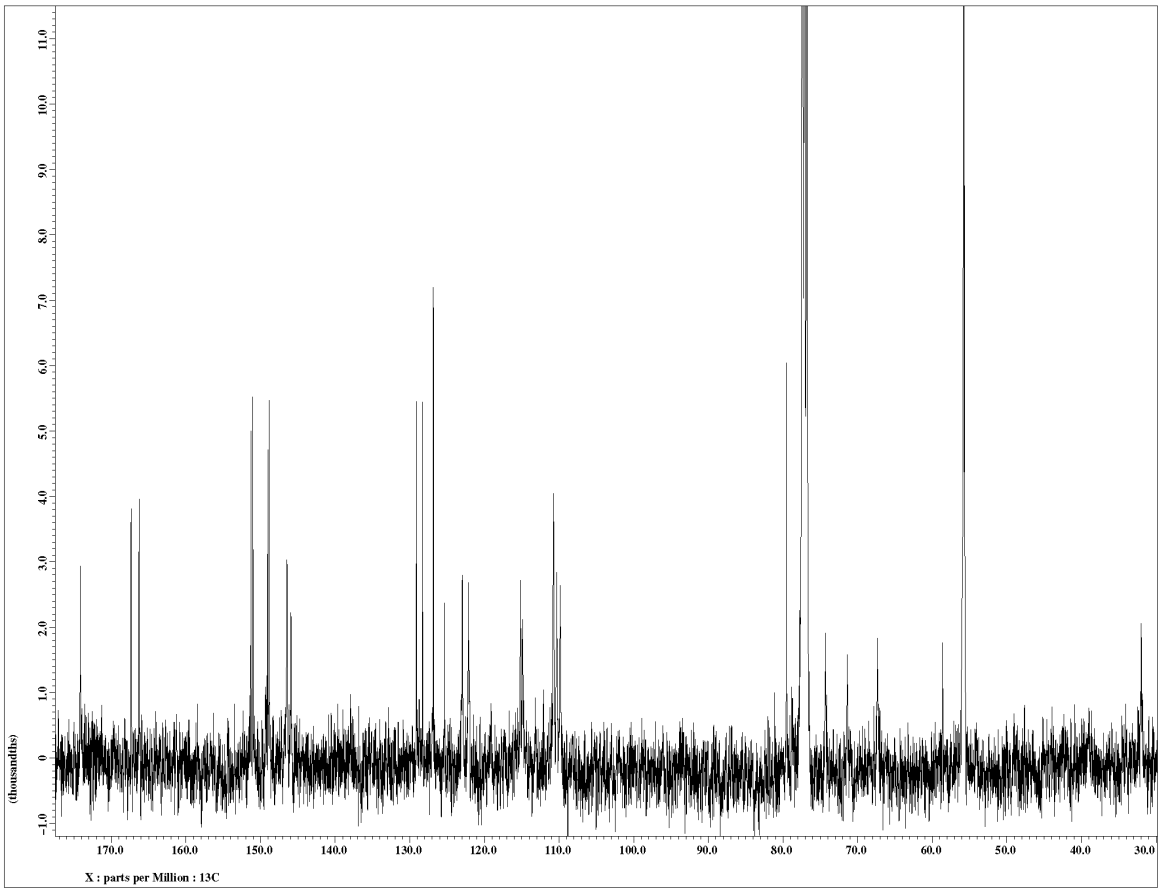


Figure 3.35. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid (1,3-diDQA) **43**.

3.1.11. Synthesis of 1,3-di-*O*-Caffeoylquinic Acid (1,3-diCQA, Cynarine) 47 and Attempted Synthesis of 1,3-di-*O*-Caffeoyl-1,5-quinide (1,3-diCQL) 48

1,3-diCQA **47** could be afforded (**Figure 3.36**) through a similar synthetic scheme as the one for 1,3-diDQA **43** (**Figure 3.31**), but *via* the BBA-protected intermediates **45** (yield 63%) and **46** (yield 31%).[24] Reflux and NEt_3 were used for the diacylation step while allyl-protection was employed for the phenols, as detailed above in the current chapter. The BBA-deprotection could be performed very efficiently to give product **47** quantitatively (the equivalent step in the synthesis of 1,3-diDQL **44** proceeded in quantitative yield also).[8]

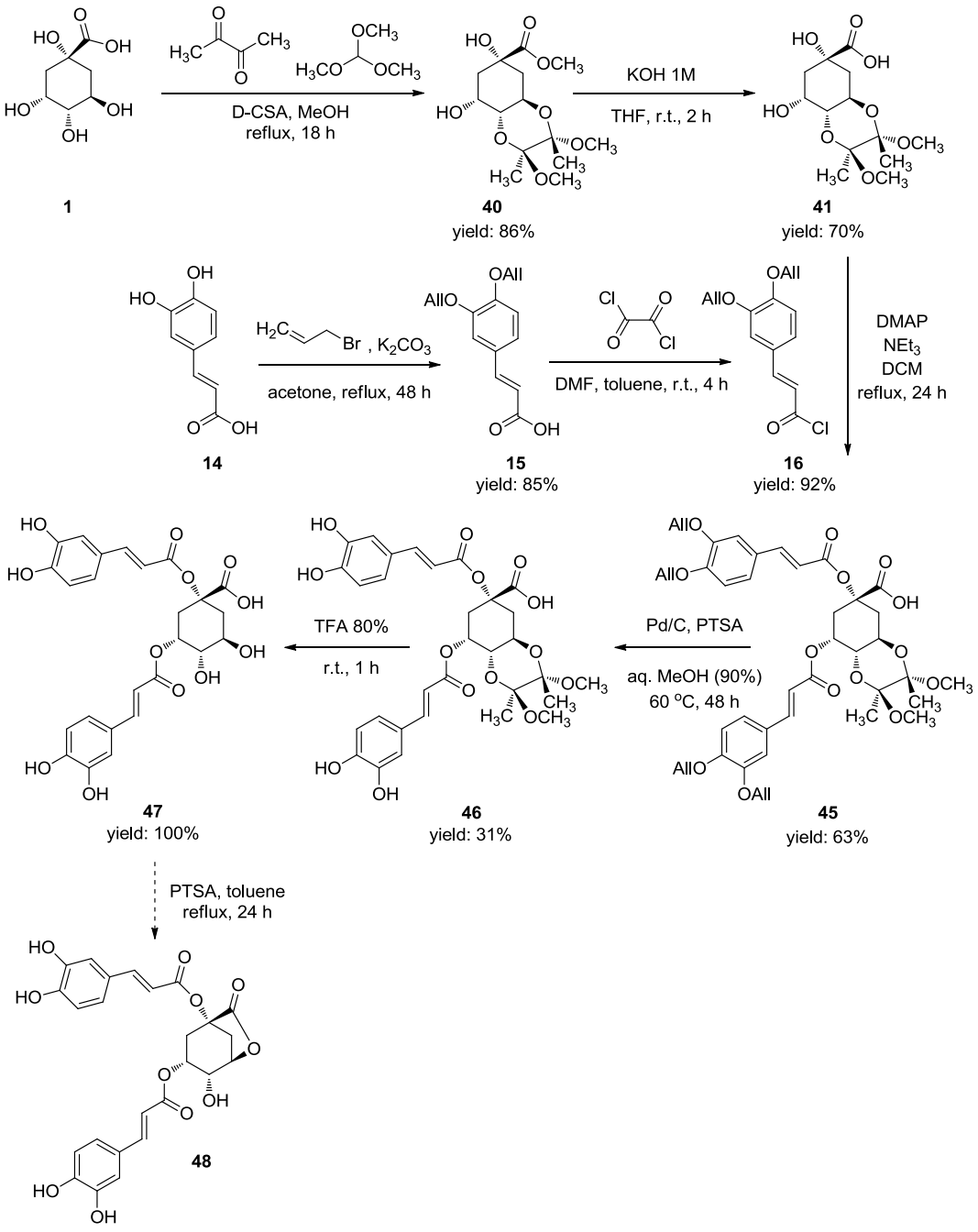


Figure 3.36. Synthetic path to 1,3-diCQA **47** and potentially 1,3-diCQL **48**.

Attempts to synthesize 1,3-diCQL **48** from 1,3-diCQA **47** *via* a similar procedure to the one employed for 1,3-diDQL **44** did not produce the anticipated results and 1,3-diCQL **48** could not be separated. There were solubility issues when the solvent typically used for this step (toluene)

was employed and the alternatives tried (acetone or 1,4-dioxane) did not generate the desired lactone **48** either. One could try to change the order of the last three steps in the reaction scheme and instead of proceeding from **45** with: 1. de-allylation; 2. BBA-deprotection; 3. *γ*-lactonization; one could try: 1. BBA-deprotection; 2. γ-lactonization; 3. de-allylation. Compounds **45** and **46** have not been synthesized before.

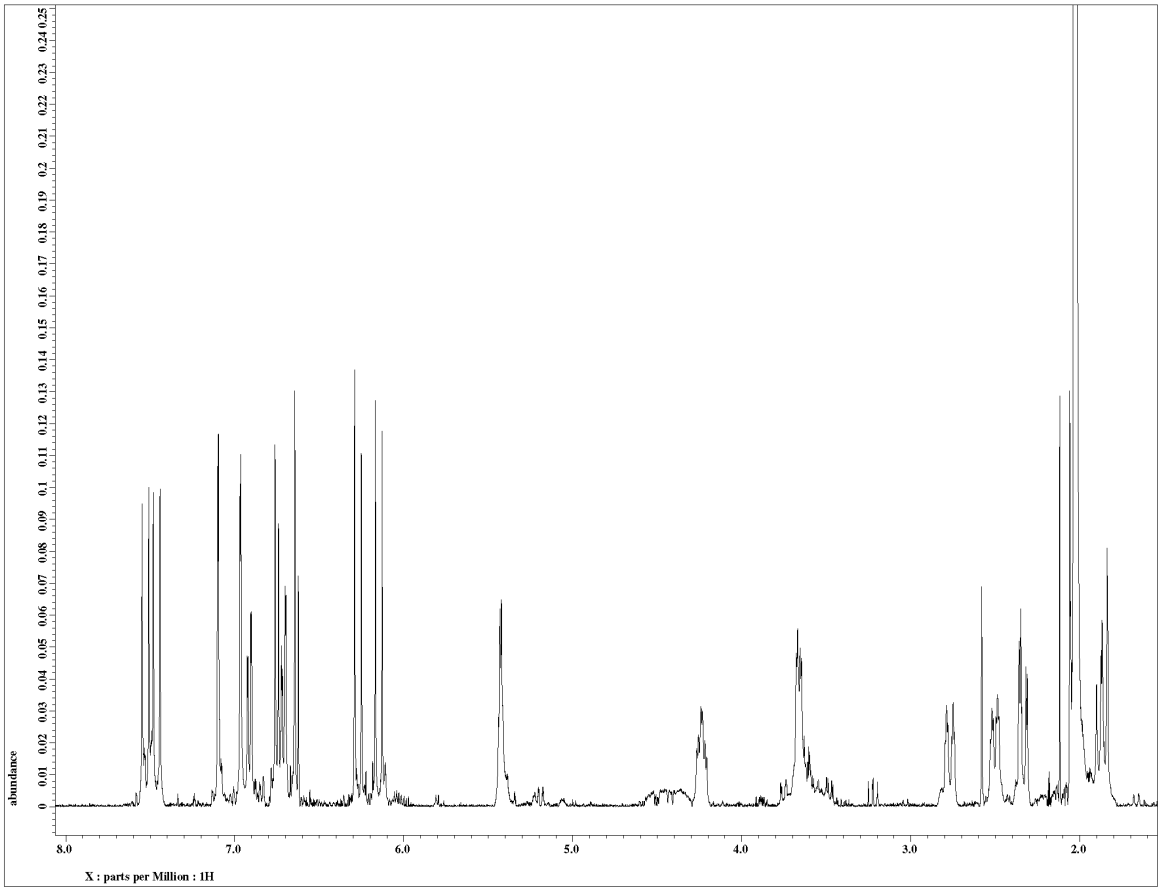


Figure 3.37. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) spectrum of 1,3-di-*O*-caffeoylquinic acid (1,3-diCQA, cynarine) **47**.

In the ^{1}H-NMR (measured in $(CD_3)_2CO$) of 1,3-diCQA **47** (**Figure 3.37**) the signals characteristic to the two cinnamoyl units appeared in the specific order and at similar δ values (6.63-7.53 ppm) as detailed for other diacylated caffeoyl-containing compounds (e.g., 3,4-diCQM **39**). A difference was that the hydroxyl protons (phenolic and alcoholic) could not be observed when the sample (of **47**) was measured in $(CD_3)_2CO$. The signals characteristic to the

quinic acid moiety of 1,3-diCQA **47** resonated similarly to the corresponding ones in 1,3-diDQA **43** (small differences due to NMR solvent choice): the protons attached to C-3, C-5 and C-4 showed a similar relative shielding and they came between 3.66-5.43 ppm (3.81-5.44 ppm for 1,3-diDQA **43** in $CDCl_3$) while the ones attached to the aliphatic C-2 and C-6 carbons resonated most upfield in the spectrum between 1.85-2.77 ppm (2.00-2.91 ppm for 1,3-diDQA **43**).

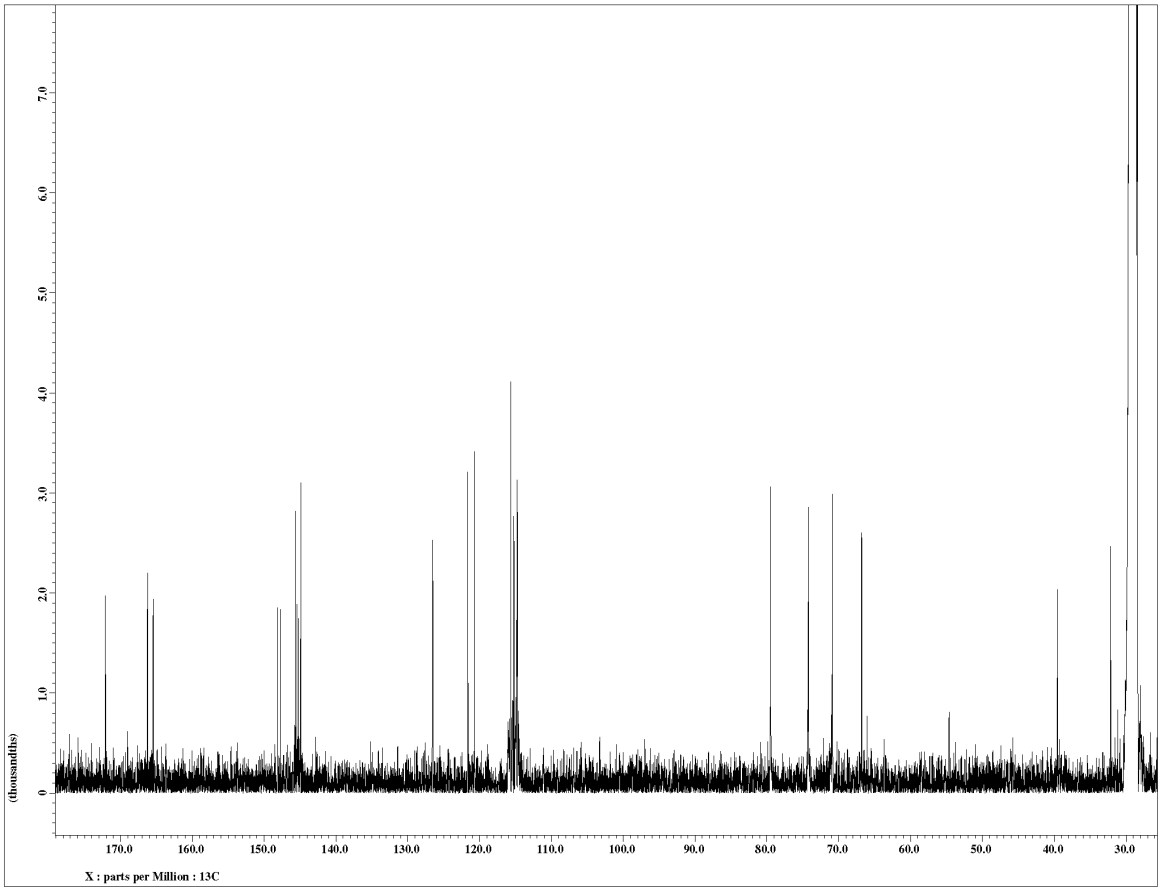


Figure 3.38. ^{13}C-NMR ($(CD_3)_2CO$, 100 MHz) spectrum of 1,3-di-*O*-caffeoylquinic acid (1,3-diCQA, cynarine) **47**.

Due to the structural similarity between 1,3-diCQA **47** and 1,3-diDQA **43**, the differences between the two ^{13}C-NMR spectra were also rather small. The aromatic carbon atoms attached to hydroxyls in 1,3-diCQA **47** (145.21-148.11 ppm) appeared slightly more shielded than the ones attached to methoxy functionalities in 1,3-diDQA **43** (148.86-151.30 ppm). 1,3-diCQA **47** showed no signals at around 55.70 ppm since no methoxy groups are present in the molecule

(**Figure 3.38**), compared to three (expected four) such signals in the case of 1,3-diDQA **43** (55.83 ppm, 55.77 ppm ($2xC_{Ar}$-O*C*H_3), 55.65 ppm). The two aliphatic carbon atoms (C-2 and C-6), which could not be observed resolved (showed overlap at 32.01 ppm) for 1,3-diDQA **43** when the spectrum was measured in $CDCl_3$, were clearly separated in $(CD_3)_2CO$ for 1,3-diCQA **47** (C-6 at 39.54 ppm and C-2 at 32.15 ppm).

3.1.12. Attempted Synthesis of 1,3,4-tri-*O*-Caffeoyl-1,5-quinide (1,3,4-triCQL) 51

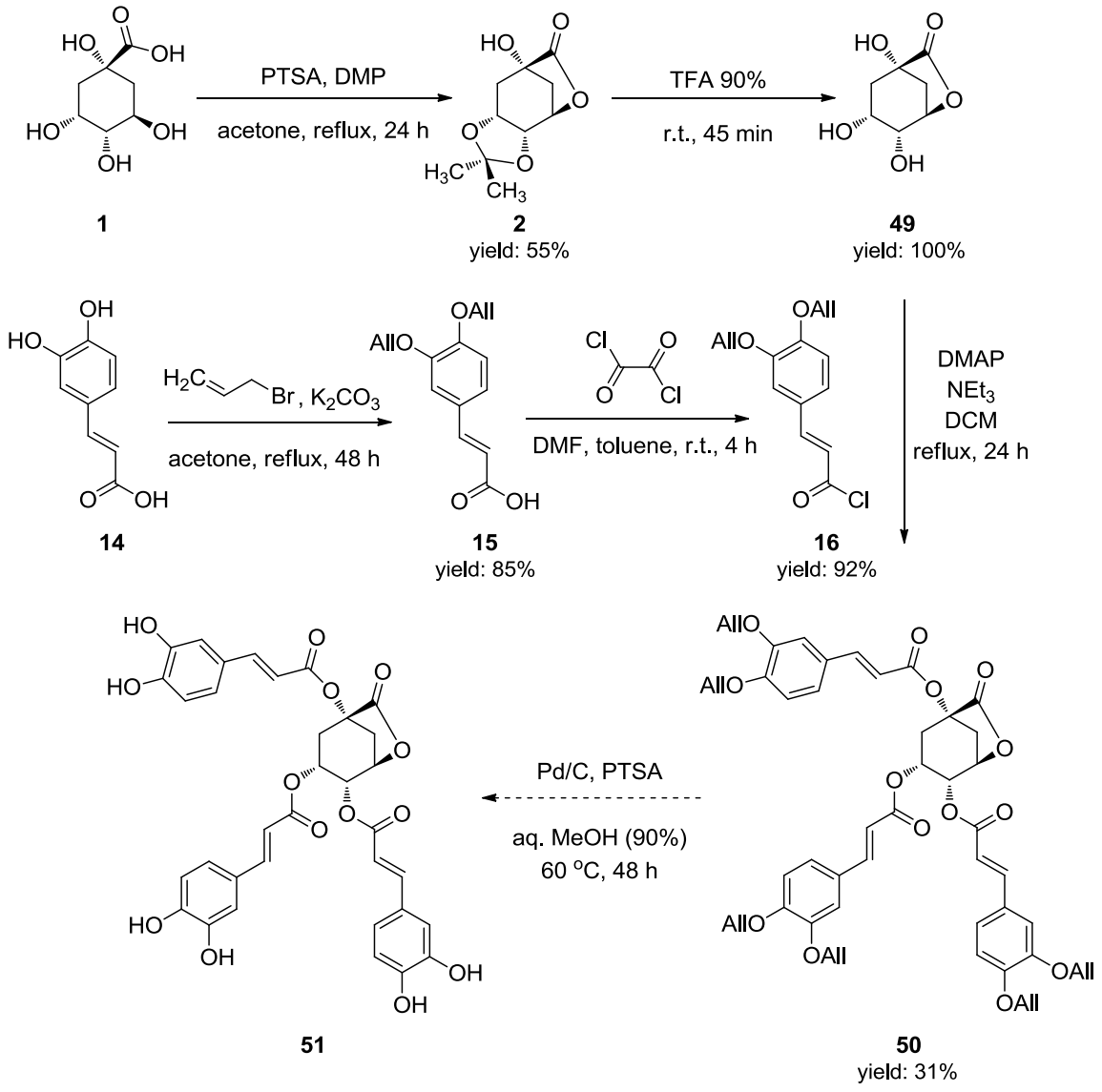


Figure 3.39. Attempted synthetic path to 1,3,4-triCQL **51**.

For the synthesis of 1,3,4-triCQL **51** (**Figure 3.39**) the allyl protection was attempted for the total of six phenols in the targeted final molecule.[22,23] 1,5-Quinide **49** was generated in two steps from quinic acid **1** *via* the intermediate 3,4-*O*-isopropylidene-1,5-quinide **2**;[10] other sources reported on an alternative one-step procedure of affording **49** directly from quinic acid **1** (yield 30%)[2] but attempts to reproduce the reported procedure did not achieve better results than the two-step approach described here. Removal of isopropylidene from **2** to generate **49** went in quantitative yield while 3,4-*O*-isopropylidene-1,5-quinide **2** was obtained in 55% yield from quinic acid, as reported previously in the current chapter. The synthesis went according to the synthetic scheme until the very last step (allyl deprotection), when the final product could not be collected after column chromatography even though crude NMR measurements showed no signals for the allyl groups hinting at a potentially successful procedure. Consequently, the last pure product obtained with the current scheme was 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50** (yield 31%), a novel compound. Due to the nature of the molecule a number of signals in both ^{1}H-NMR and ^{13}C-NMR overlapped when $CDCl_3$ was used as a solvent.

The ^{1}H-NMR spectrum showed six (instead of nine) distinct aromatic protons signals in the interval 6.77-7.08 ppm since three of them integrated as two protons (**Figure 3.40**). The olefinic moiety protons in the cinnamoyl residues produced a total of five signals (instead of expected six) because the area under the most downfield peak at 7.65 ppm (2xC_{Ar}-CH) integrated as two protons. Otherwise, the order of the signals in the downfield part of the spectrum was as with previous compounds: the olefinic protons on the carbons adjacent to the aromatic moieties (C_{Ar}-CH) came more downfield (7.53 ppm and two protons at 7.65 ppm) than the aromatic protons while the more shielded olefinic α-protons resonated individually more upfield (6.16 ppm, 6.29 ppm and 6.35 ppm) than the aromatic ones. The coupling constants were typical for protons belonging to such moieties: ^{4}J = 1.8 Hz and ^{3}J = 8.7 Hz for the aromatic protons and ^{3}J = 15.8 Hz for the olefinic protons.

In an allyl ether group, the five protons give theoretically four distinct signals: three signals coming from the three vinyl protons which reside each in a chemically different environment; and one signal from the two protons on the sp^3-hybridized carbon in the moiety. For 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50** the total of 30 protons in the allyl ether groups resonated

as following: the allyl specific multiplet at 6.03 ppm integrating six protons (6xCH_2=C*H*); the remaining 12 vinyl protons (6xC*H*$_2$=CH) gave another multiplet between 5.21-5.46 ppm but due to significant signal overlapping the two chemically different types of protons in this group could not be easily observed resolved; the 12 protons on the sp^3-hybridized carbons of the allyl ether group resonated as a multiplet at 4.63 ppm (4xC_{Ar}-OCH_2) and as two clearly observable doublets (^3J = 5.3 Hz) at 4.59 ppm (C_{Ar}-OCH_2) and 4.50 ppm (C_{Ar}-OCH_2).

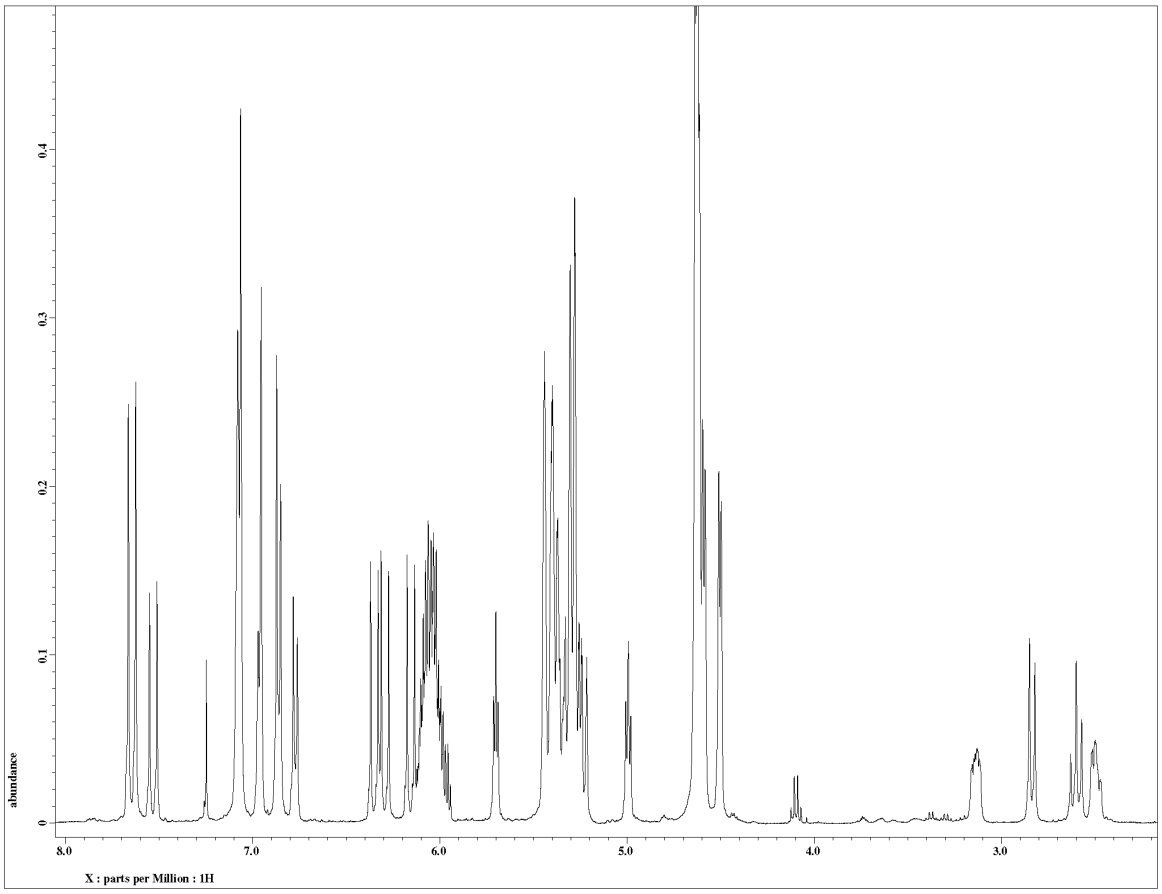


Figure 3.40. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50**.

The C-4, C-3 and C-5 protons of the quinic acid moiety in **50** resonated at 5.70 ppm, as part of the multiplet at 5.21-5.46 ppm (together with the 12 vinyl protons) and at 5.00 ppm, respectively. The same order (in terms of chemical shift) for these three signals was observed for 3,4-diacylated lactones synthesized in the current project when the samples were measured in the

same deuterated solvent ($CDCl_3$): 5.64 ppm (proton at C-4), 5.24 ppm (proton at C-3) and 4.88 ppm (proton at C-5) for 3,4-diDQL **29**; 5.67 ppm (proton at C-4), 5.28 ppm (proton at C-3) and 4.94 ppm (proton at C-5) for 3,4-diFQL **32**. The signal for the C-4 proton in 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50** was a doublet of doublets, being split by the protons on the two vicinal carbons (^3J = 5.0 Hz with the proton on C-5 and ^3J = 6.9 Hz with the proton on C-3). The signal for the C-5 proton was also a doublet of doublets, being split by the protons on the two vicinal carbons (^3J = 5.0 Hz with the proton on C-4 and ^3J = 5.5 Hz with the equatorial proton on C-6). A similar splitting pattern for these quinic acid protons was observed for **29** synthesized in the current project; other authors reported similar results.[1] The acylated 1,5-quinide specific signal (equatorial proton at C-6) came at 3.14 ppm as a doublet of doublets of doublets. The splitting, observed for other structurally similar compounds, is due to coupling to the geminal C-6 proton (^2J = 11.7 Hz), to the proton at C-5 (^3J = 5.5 Hz) and to the equatorial proton at C-2 (^4J = 2.3 Hz). The axial proton at C-6 appeared as a doublet at 2.84 ppm (^2J = 11.7 Hz). The signal for the axial proton on C-2 showed the geminal coupling (^2J = 11.7 Hz) as well as the coupling to the vicinal C-3 proton (^3J = 11.7 Hz), being a triplet (t). The signal for the equatorial C-2 proton was a ddd: geminal coupling ^2J = 11.7 Hz, vicinal coupling ^3J = 6.7 Hz (to the C-3 proton) and "W-coupling" ^4J = 2.3 Hz (to equatorial C-6 proton).

There are 52 carbons in one molecule of 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50;** however, since a number of signals overlapped in the very clean ^{13}C-NMR spectrum of the compound, only 43 peaks could be observed (**Figure 3.41**). The order of the signals was as expected, judging from spectra of similar compounds. The ester carbon of the lactone (OC-*C*OO at 171.61 ppm) appeared more downfield than the three cinnamoyl ester carbons (CH-*C*OO at 165.63 ppm, 165.38 ppm and 165.28 ppm). The aromatic carbon atoms bearing the electron withdrawing allyl ether functionalities (C_{Ar}-OCH_2) also came downfield between 148.56-151.25 ppm as six distinct peaks. The olefinic β-carbons (C_{Ar}-*C*H) resonated more upfield, at 146.94 ppm, 146.74 ppm and 146.07 ppm. The most deshielded carbons of the allyl ether group (the non-terminal vinyl carbons *C*H=CH_2) gave four signals instead of expected six between 132.81-133.06 ppm. The three aromatic carbons (C_{Ar}-CH) attached to the substituted olefin resonated more upfield at 127.25 ppm, 127.11 ppm and 127.06 ppm. The three C-6' aromatic carbons appeared at 123.39 ppm, 123.32 ppm and 122.99 ppm; the C-5' and C-2' aromatic carbons

(expected six peaks, observed five peaks) appeared between 112.60-113.36 ppm. More downfield than this group of carbons but more upfield than the C-6' carbons resonated in the ^{13}C-NMR spectrum the terminal vinyl carbons (*C*H_2=CH) between 117.99-118.17 ppm as three signals instead of expected six, and the three remaining olefinic carbons (C_{Ar}-CH=*C*H) at 114.52 ppm, 114.37 ppm and 114.18 ppm, respectively.

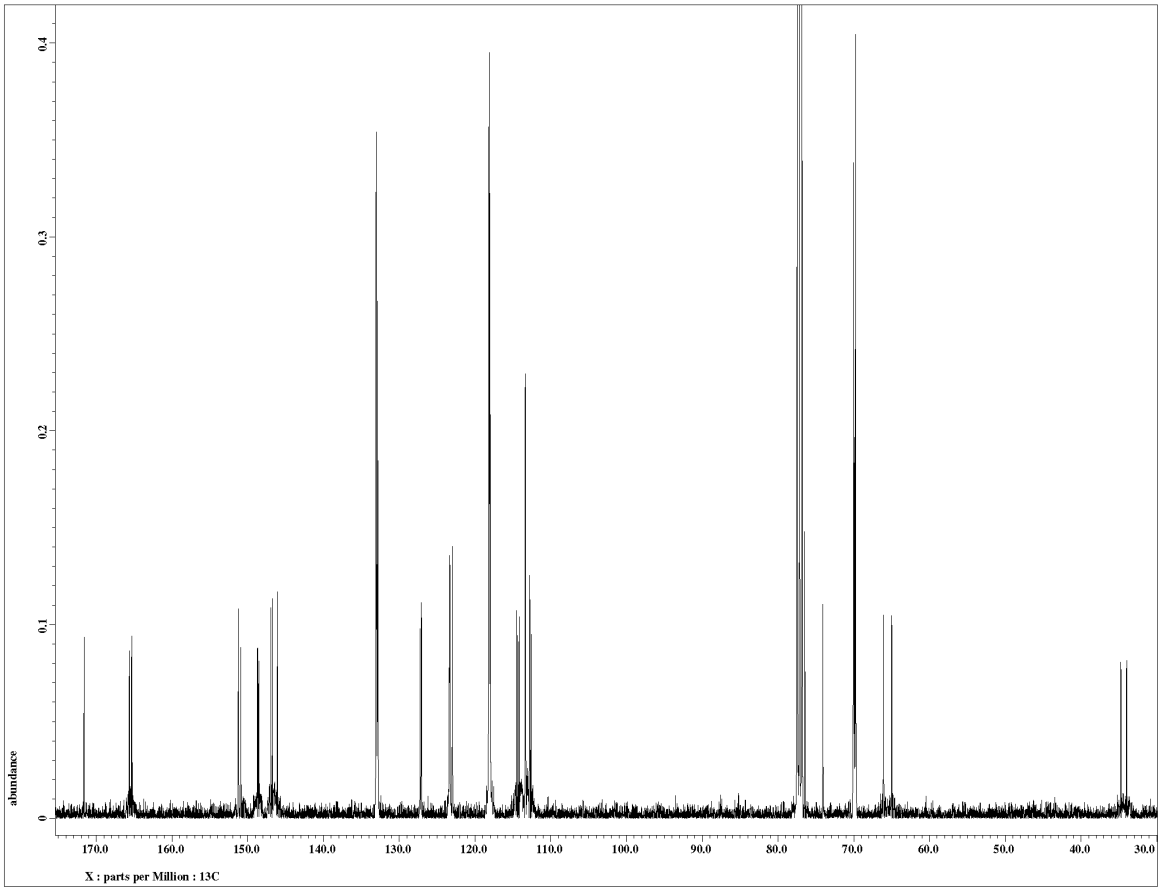


Figure 3.41. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of 1,3,4-tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide **50**.

The four carbon atoms of the quinic acid moiety in **50** bearing oxygen-containing substituents resonated as following: the C-1 atom bearing the acylated allylcaffeoyl (electron withdrawing) and the electron withdrawing lactone -COOR appeared most downfield of the four carbons at 76.83 ppm; the C-5 atom bearing the electron withdrawing -OCOR appeared next at 74.09 ppm; the C-3 and C-4 atoms, each bearing an identical ester, resonated at 66.07 ppm and 64.99 ppm,

respectively. The six sp^3-hybridized carbons of the allyl ether group (C_{Ar}-OCH_2) appeared as three peaks between 69.77-70.04 ppm. The C-2 (33.98 ppm) and C-6 (34.74 ppm) carbons of the quinic acid moiety gave signals which appeared most upfield in the spectrum of all carbons of the molecule.

3.1.13. Synthesis of 5-*epi*-Quinic Acid (*cis*-Quinic Acid) 56 and Methyl 5-*epi*-Quinate (Methyl *cis*-Quinate) 57

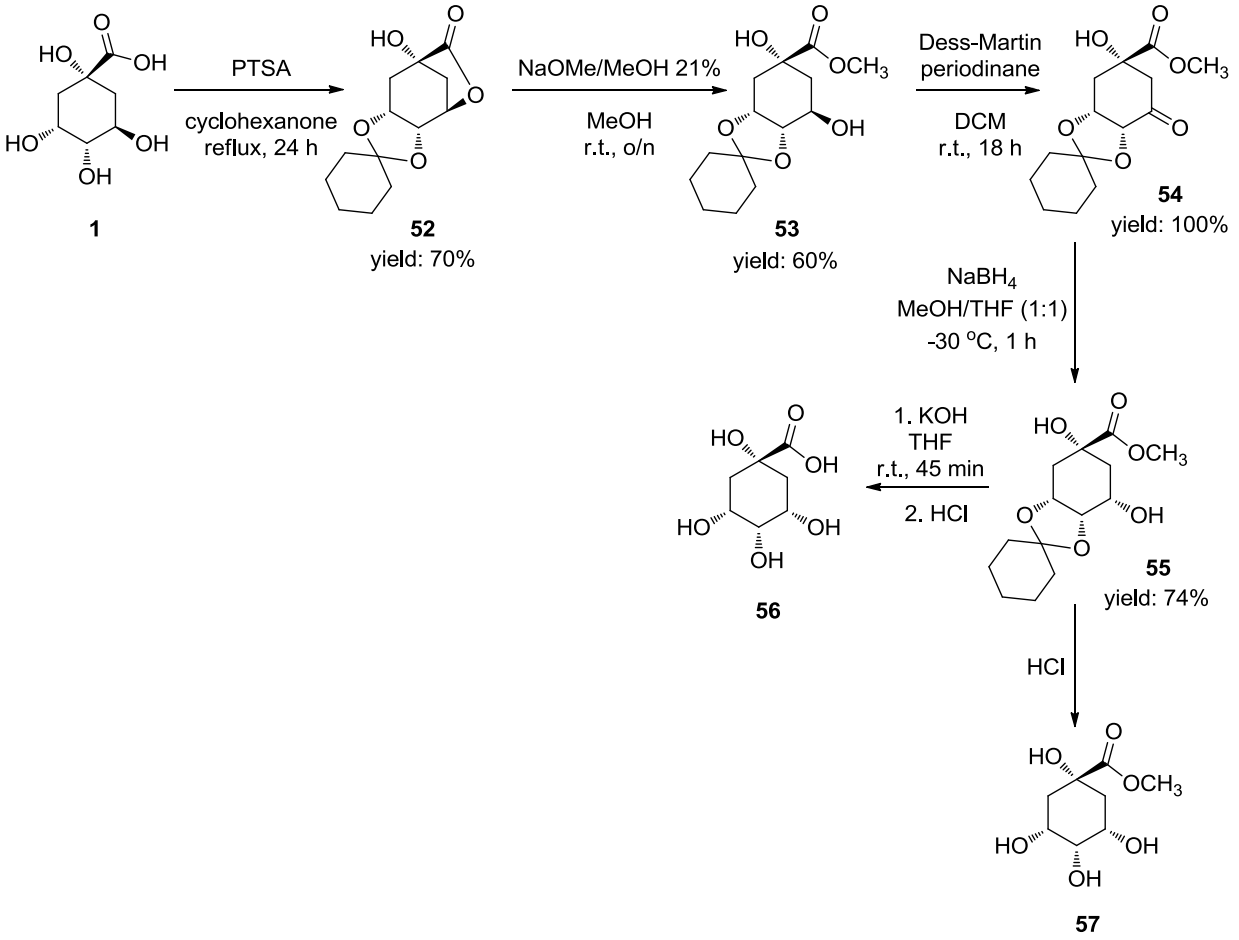


Figure 3.42. Synthetic path to 5-*epi*-quinic acid (*cis*-quinic acid) **56** and methyl 5-*epi*-quinate (methyl *cis*-quinate) **57**.

5-*epi*-Quinic acid **56** was obtained according to the scheme in **Figure 3.42**. A cyclohexylidene acetal instead of an isopropylidene acetal was used for the protection of the *cis*-diol (**52**, yield 70%) in order to avoid undesired loss of the protective group during the following synthetic steps. The cyclohexylidene acetal offers a greater stability compared to the corresponding

isopropylidene acetal towards acid-catalyzed hydrolysis.[11,12] The Dess-Martin oxidation step gave the product in quantitative yield[25] and the subsequent enantioselective reduction of the prochiral ketone produced the 5-*epi*-quinic acid derivative **55** in good yield (74%).[26]

Crystals suitable for single crystal XRD of 3,4-*O*-cyclohexylidene-1,5-quinide **52** (**Figure 3.43**), *cis*-quinic acid **56** (**Figure 3.44**), as well as of methyl *cis*-quinate **57** (**Figure 3.45**) could be generated in the process. Compared to quinic acid **1**, the molecule of 5-*epi*-quinic acid **56** has an additional plane of symmetry going through C-1, C-4, C-7 (COOH) and the oxygen atoms of the hydroxyls attached to C-1 and C-4. Because of the inversion at the C-5 atom, the *cis*-quinic acid **56** molecule belongs to the non-rotational point group C_s (quinic acid belongs to the non-rotational point group C_1); the additional symmetry element in the molecule is reflected in both the ^{1}H-NMR and ^{13}C-NMR spectra of the compound. The compounds in **Figure 3.42**, synthesized by the author of the current project, are also part of a pending co-authored manuscript and were additionally reported, with permission, in another dissertation.[27]

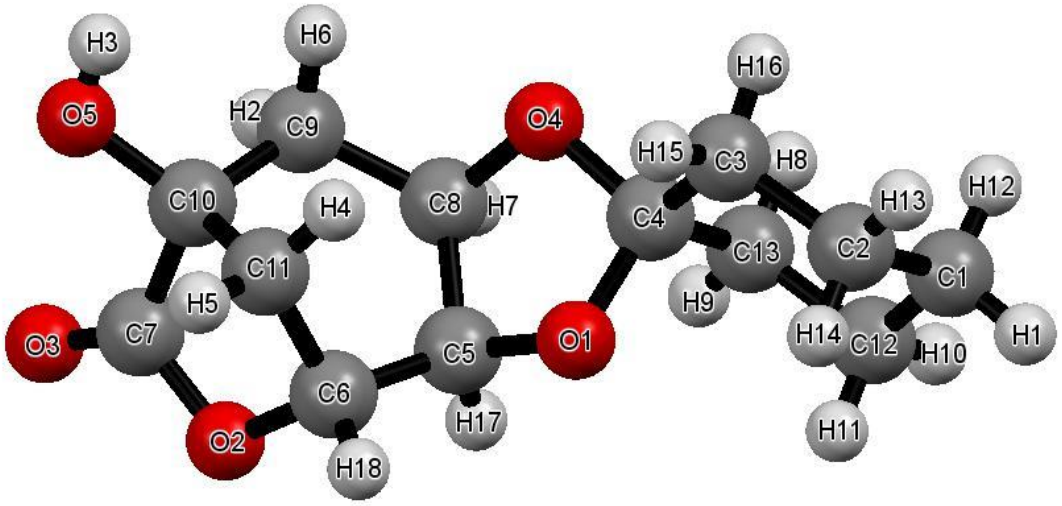


Figure 3.43. X-ray crystal structure of 3,4-*O*-cyclohexylidene-1,5-quinide **52**.

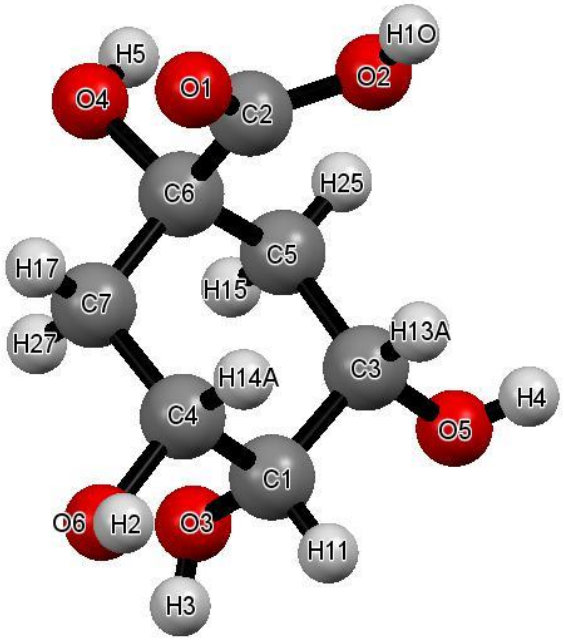


Figure 3.44. X-ray crystal structure of 5-*epi*-quinic acid (*cis*-quinic acid) **56**.

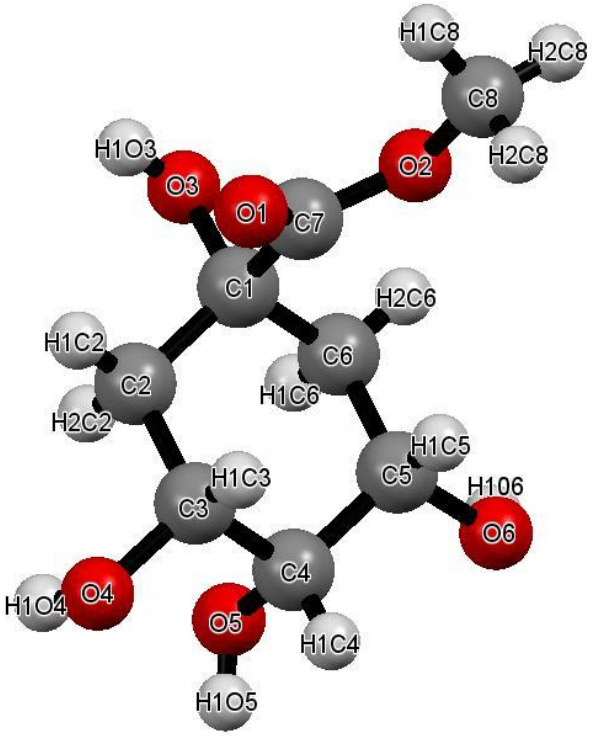


Figure 3.45. X-ray crystal structure of methyl 5-*epi*-quinate (methyl *cis*-quinate) **57**.

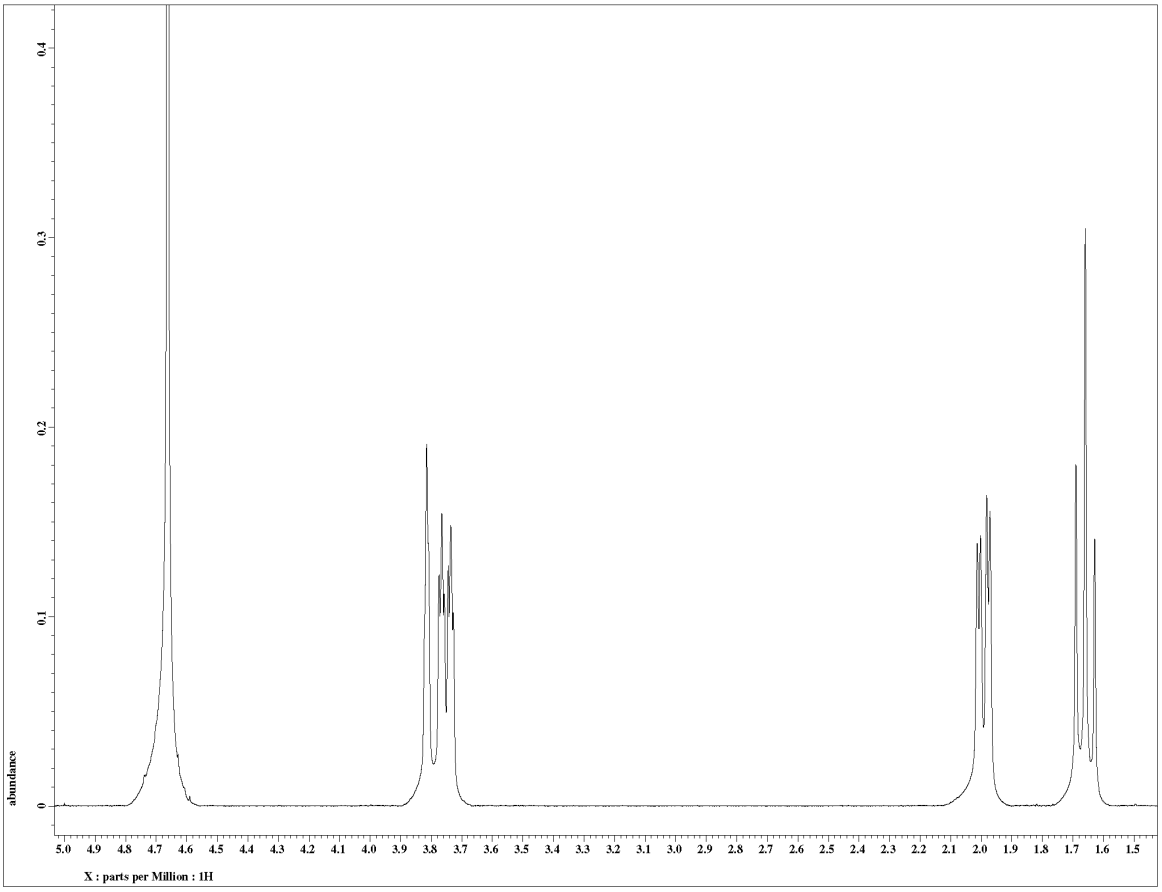


Figure 3.46. ^{1}H-NMR (D_2O, 400 MHz) spectrum of 5-*epi*-quinic acid (*cis*-quinic acid) **56**.

In the ^{1}H-NMR spectrum of **56** (measured in D_2O, **Figure 3.46**) the proton attached to C-4 resonated most downfield at 3.81 ppm while the protons attached to C-5 and C-3 came very close to each other due to symmetry, at 3.77 ppm and 3.74 ppm, respectively. For comparison, the ^{1}H-NMR spectrum (in D_2O) of quinic acid **1** showed the proton attached to C-4 resonating at 3.42 ppm and the protons attached to C-5 and C-3 resonating at 3.91 ppm and 4.03 ppm, respectively. In 5-*epi*-quinic acid **56** the equatorial protons on C-2 and C-6 overlapped as a multiplet at 1.99 ppm while the axial protons on the same carbons overlapped as a dd at 1.66 ppm. In quinic acid **1** the four signals corresponding to the protons on C-2 and C-6 could be clearly distinguished as a ddd at 2.04 ppm (equatorial proton at C-6), a dd at 1.99 ppm (axial proton at C-2), a ddd at 1.93 ppm (equatorial proton at C-2) and a dd at 1.79 ppm (axial proton at

C-6); the observed splitting pattern for these four protons was due to similar couplings as detailed for 1-FQA **13**.

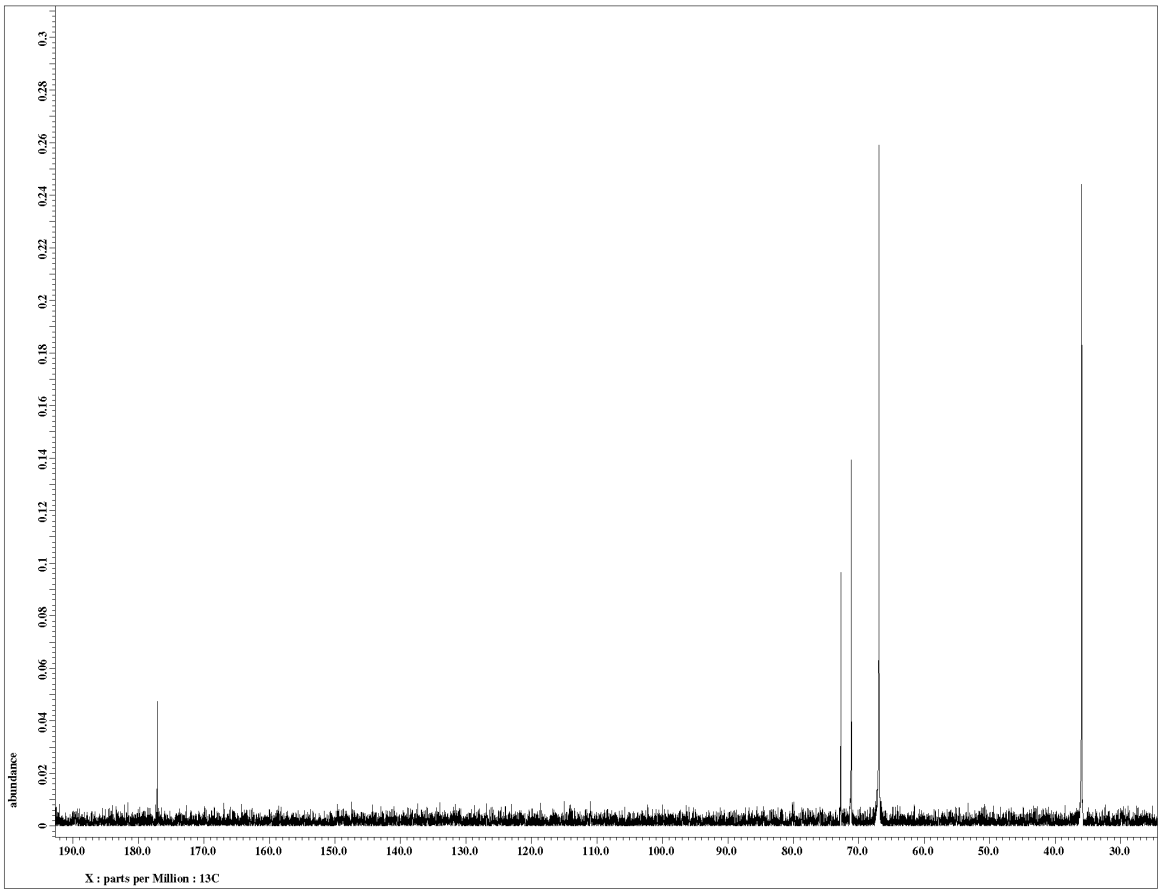


Figure 3.47. ^{13}C-NMR (D_2O, 100 MHz) spectrum of 5-*epi*-quinic acid (*cis*-quinic acid) **56**.

The ^{13}C-NMR spectrum of *cis*-quinic acid **56** (**Figure 3.47**) contained only five peaks as compared to the seven peaks observed for quinic acid **1** (both spectra measured in D_2O). This is because of the above-mentioned symmetry plane of the *cis*-quinic acid **56** molecule: C-2 and C-6 produced one peak at 35.95 ppm while C-3 and C-5 produced one peak at 66.91 ppm. The remaining carbon atoms resonated at 71.15 ppm (C-4), 72.74 ppm (C-1) and 177.14 ppm (COO). The differences are obvious when compared to the spectrum of quinic acid **1** where each carbon atom in the molecule gave one signal as following: 36.60 ppm (C-2), 40.07 ppm (C-6), 66.23 ppm (C-5), 69.87 ppm (C-3), 74.67 ppm (C-4), 75.50 ppm (C-1) and 177.68 ppm (COO).

Similar results were observed for methyl *cis*-quinate **57** when compared to methyl quinate **69** (**Figure 3.53**). Methyl 5-*epi*-quinate (methyl *cis*-quinate) **57** gave six signals in the ^{13}C-NMR spectrum (one peak for C-2 and C-6; one peak for C-3 and C-5) compared to the eight signals of methyl quinate **69** (**Figure 3.49**). Similarly, in the ^{1}H-NMR spectrum of methyl *cis*-quinate **57** (**Figure 3.48**) the equatorial protons at C-2 and C-6 grouped in a multiplet and so did the axial ones on the same carbons (dd), as it was the case for the non-methylated *cis*-derivative **56**.

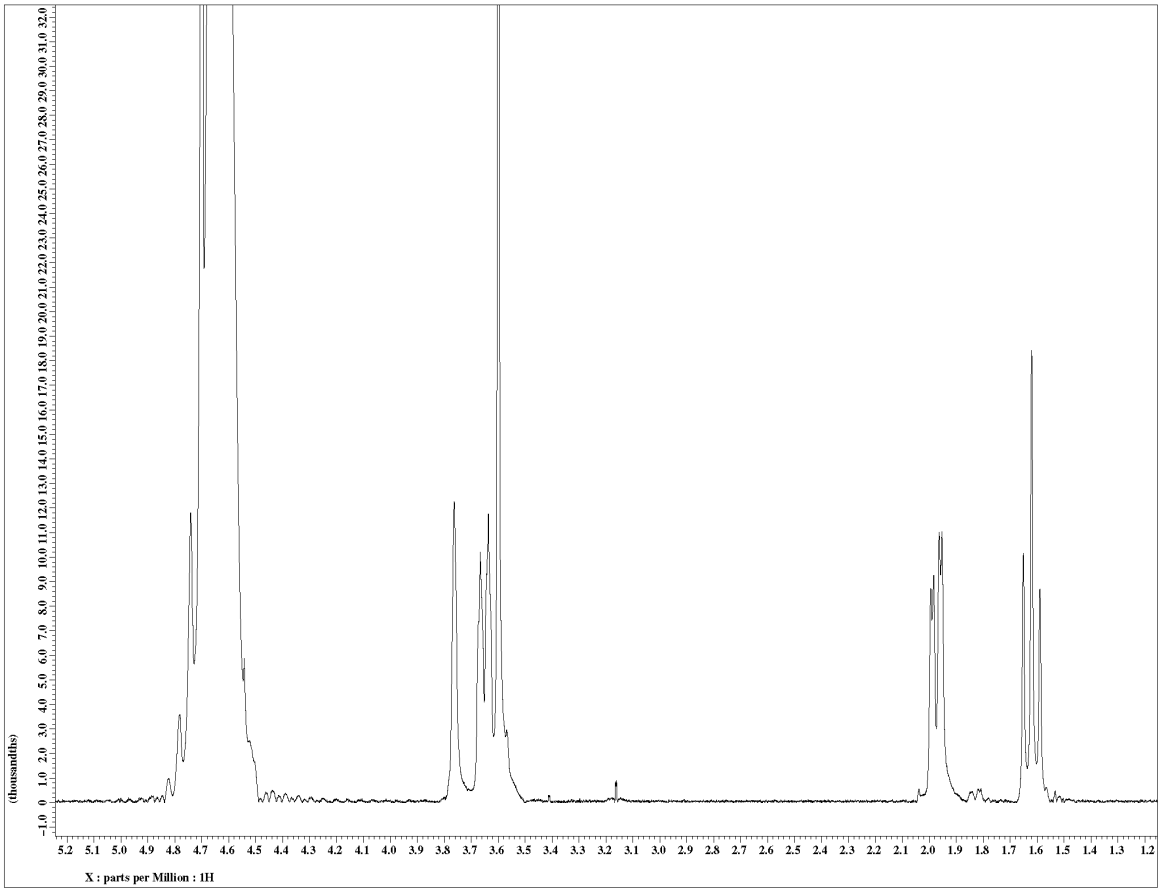


Figure 3.48. ^{1}H-NMR (D_2O, 400 MHz) spectrum of methyl 5-*epi*-quinate (methyl *cis*-quinate) **57**.

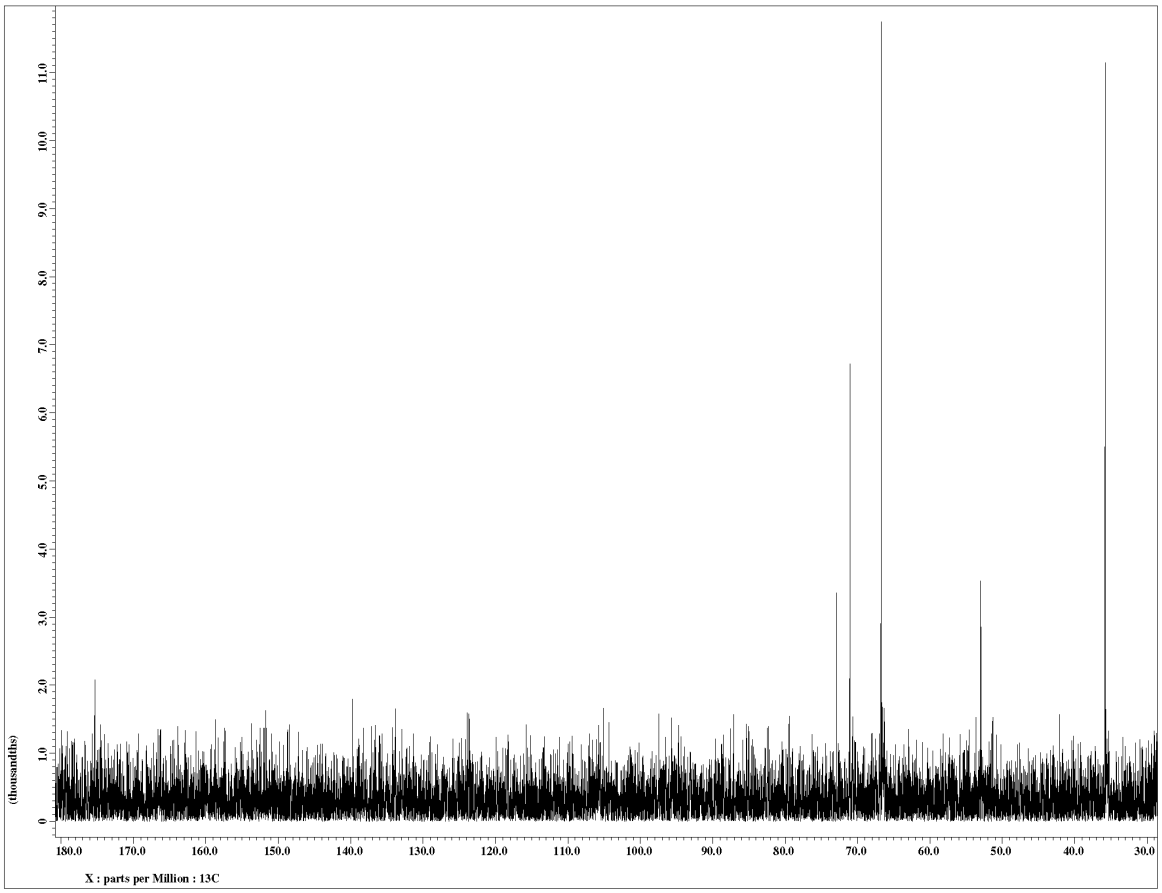


Figure 3.49. ^{13}C-NMR (D_2O, 100 MHz) spectrum of methyl 5-*epi*-quinate (methyl *cis*-quinate) **57**.

Methyl 3-*epi*-quinate (methyl *muco*-quinate) **68** (**Figure 3.53**) was also synthesized[18] in the current project and similarly to methyl 5-*epi*-quinate (methyl *cis*-quinate) **57** it also displays a plane of symmetry going through the same atoms. Consequently, the same peculiarities discussed for methyl *cis*-quinate **57** were present in the ^{1}H-NMR and ^{13}C-NMR spectra (in CD_3OD): the equatorial C-2 and C-6 protons giving one signal together, as well as the axial C-2 and C-6 protons, and one joint ^{13}C-NMR peak for C-3 and C-5, as well as for C-2 and C-6 (**Figure 3.51**). A difference in the ^{1}H-NMR spectrum (**Figure 3.50**) when compared to the ^{1}H-NMR of methyl *cis*-quinate **57** (**Figure 3.48**) was that the protons at C-5, C-3 and C-4 resonated at 3.70 ppm, 3.29 ppm and 3.14 ppm for methyl *muco*-quinate **68**, while for methyl *cis*-quinate **57** they appeared at 3.77 ppm, 3.74 ppm and 3.81 ppm, respectively; thus, while in the *muco*-

epimer **68** the proton at C-4 was the most shielded of the three (as it was also the case for methyl quinate **69**), in the *cis*-epimer **57** this proton was the least shielded of the three.

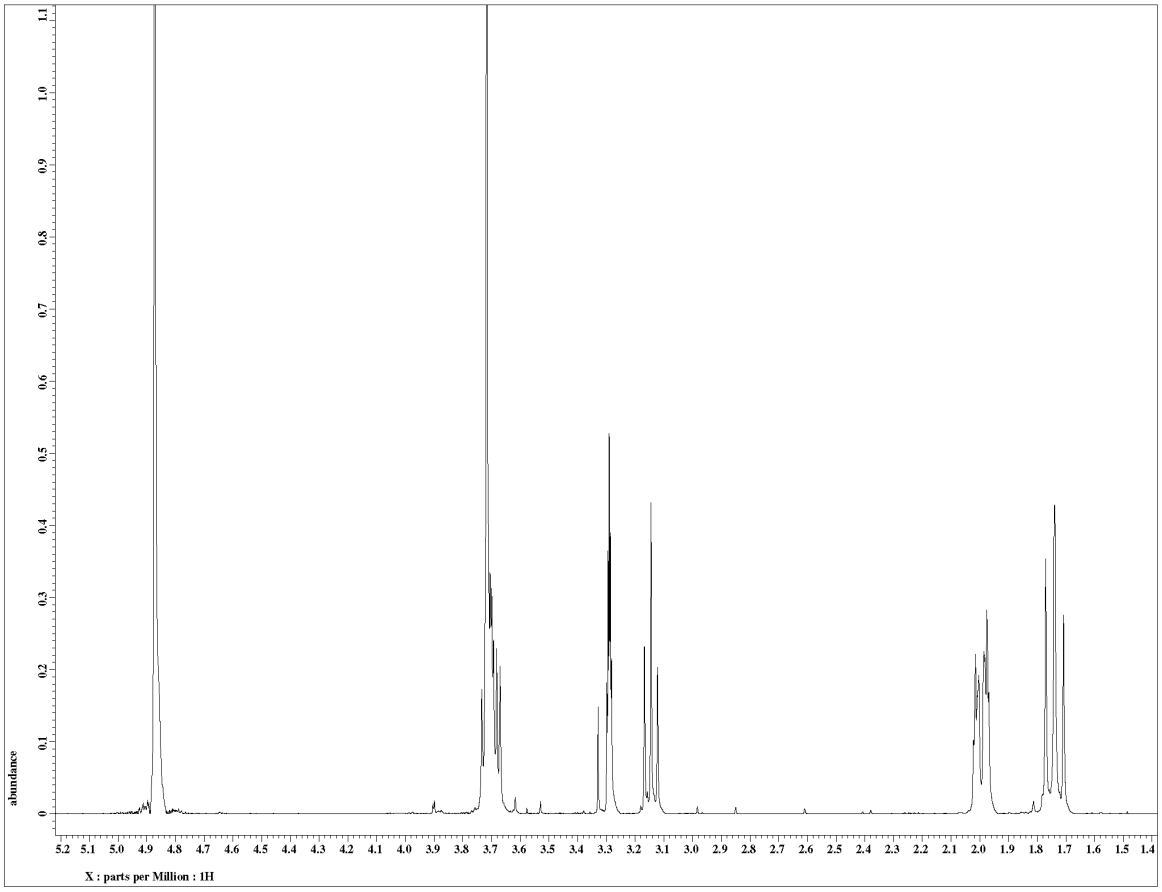


Figure 3.50. ^{1}H-NMR (CD_3OD, 400 MHz) spectrum of methyl 3-*epi*-quinate (methyl *muco*-quinate) **68**.

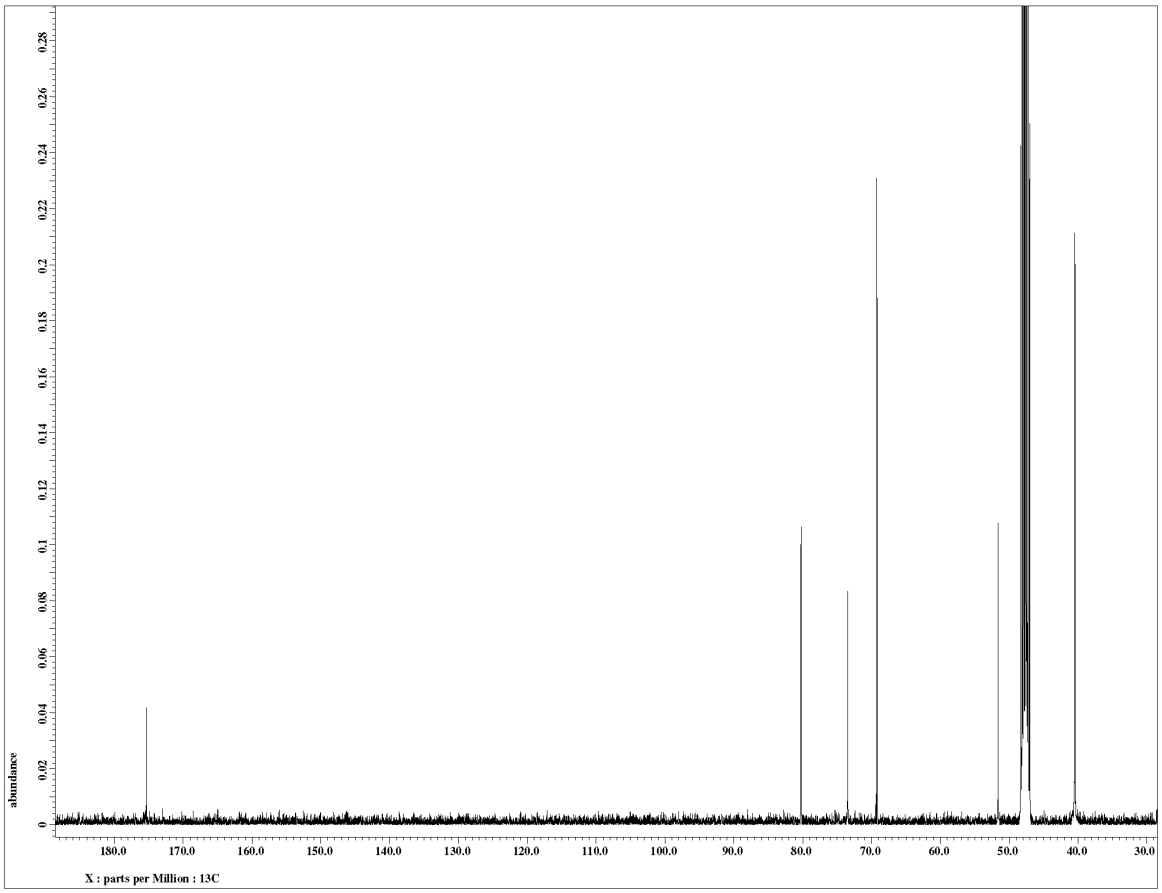


Figure 3.51. ^{13}C-NMR (CD_3OD, 100 MHz) spectrum of methyl 3-*epi*-quinate (methyl *muco*-quinate) **68**.

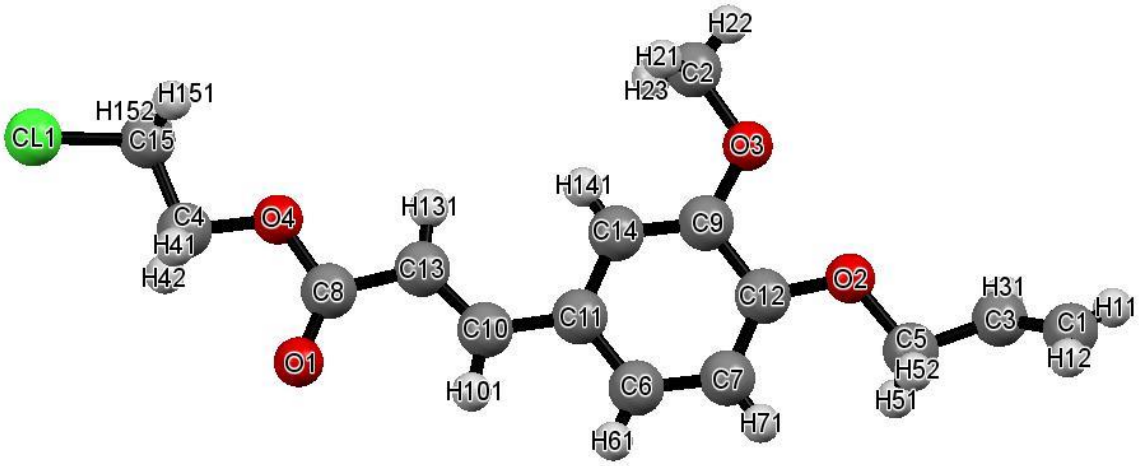


Figure 3.52. X-ray crystal structure of 2'-chloroethyl 4-*O*-allylferulate **74**.

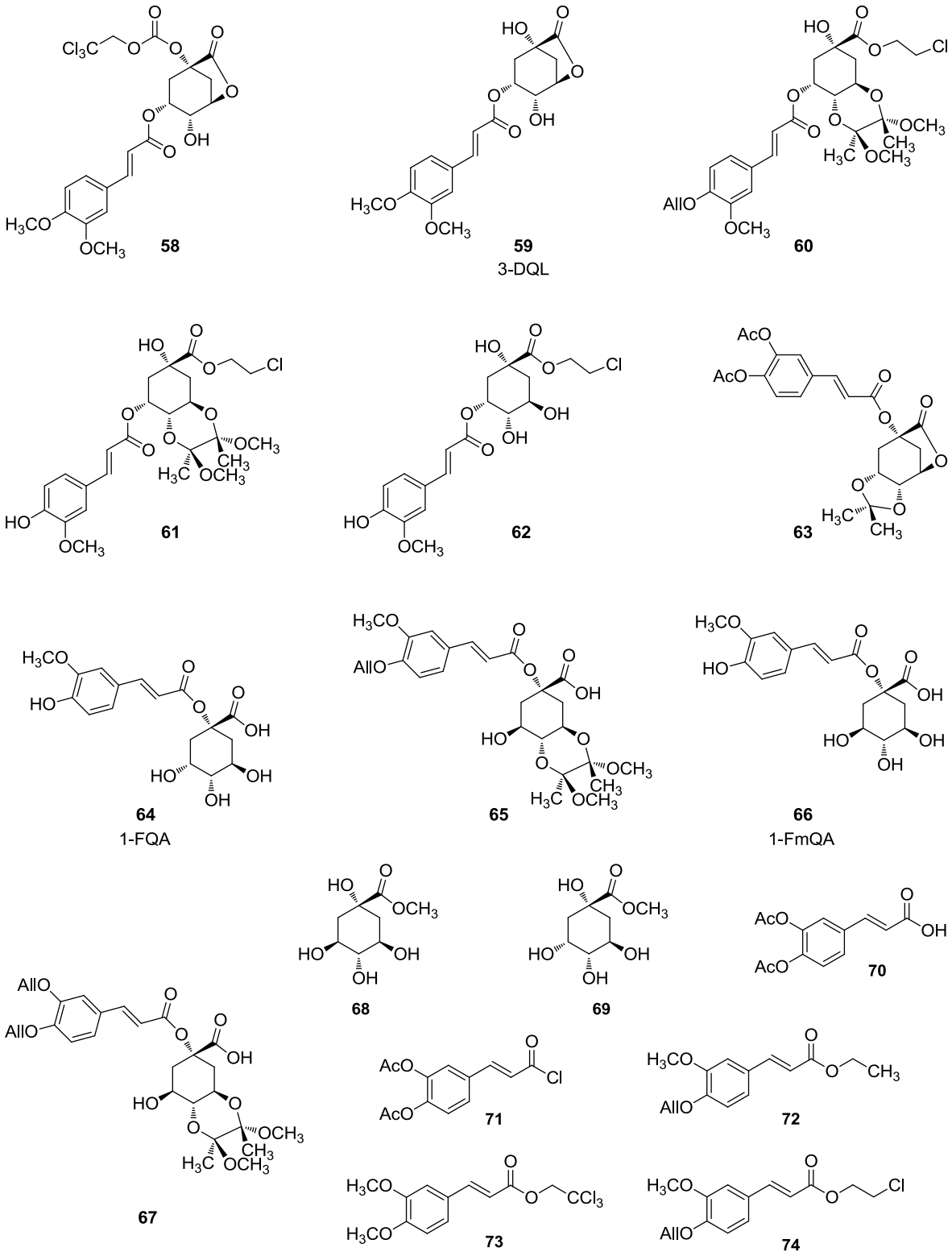


Figure 3.53. Additional synthesized compounds.

Besides the compounds mentioned so far in the current chapter on text or in figures, compounds **58**-**74** (**Figure 3.53**) were also synthesized in the project, some as targeted while others as undesired or side products. Among them, 3-DQL **59**, 1-FQA **64**, 1-FmQA **66** (a *muco*-quinic acid or 3-*epi*-quinic acid derivative) and methyl *muco*-quinate **68** are worth being noted. Compounds **60**-**62**, **65**-**67**, **72**-**74** are novel. As a general rule, most of the various protected QA derivatives (not acylated) and protected hydroxycinnamic acids and hydroxycinnamic acid chlorides shown in the current chapter were previously reported, unless stated otherwise; however, most intermediates and final products appearing after the acylation step (mono-, di- or triacylated) in all the synthetic schemes and in the **Experimental** section are novel (or appear in earlier co-authored publications listed in the current thesis, but were novel at the time of publishing). Crystals of **74** suitable for XRD could be generated (**Figure 3.52**). Compounds **58**-**74** are described in the **Experimental** section of the current chapter.

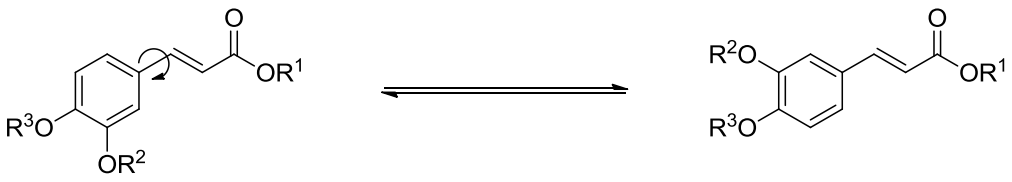
3.2. Discussion of the XRD Structures

In the current project seven structures were successfully resolved by single crystal XRD: 3,4-*O*-isopropylidene-1,5-quinide **2**, 1-*O*-(3',4'-dimethoxycinnamoyl)-3,4-*O*-isopropylidene-1,5-quinide **3**, 1-*O*-Troc-3,4-*O*-isopropylidene-1,5-quinide **26**, 3,4-*O*-cyclohexylidene-1,5-quinide **52**, 5-*epi*-quinic acid (*cis*-quinic acid) **56**, 5-*epi*-quinate (methyl *cis*-quinate) **57** and 2'-chloroethyl 4-*O*-allylferulate **74**. While **2** and **52** were reported previously,[2,27] this is the first time when the crystal structures of **3**, **26**, **56**, **57** and **74** were documented (**56** and **57**, produced by the author of the current project, are also part of a pending co-authored manuscript and were additionally included, with permission, in another dissertation).[27]

Single crystal structures of several quinic acid derivatives have been reported in the literature, in which the quinic acid moiety was found in a perfect chair conformation; when an ester substituent was present it preferred the equatorial position.[8] The two compounds containing an actual quinic acid moiety (not a γ-quinide) analyzed here are the two epimeric structures **56** and **57**, inverted at C-5. They both assume a perfect chair conformation with the carboxylic/carboxylate moiety in the axial position of C-1. Consequently, there are three OH groups in equatorial positions (at C-1, C-3 and C-5) and one OH group in an axial position (at C-4) for **56** and **57**. The four γ-quinide structures (isopropylidene-protected **2**, **3**, **26** and

cyclohexylidene-protected **52**) assumed all a half-chair conformation for the quinic acid part with atoms C-1 to C-5 deviating only slightly from perfect coplanarity and with C-6 out of plane, forced by lactonization. The cyclohexylidene group in **52** is found as expected in a chair conformation. In agreement with the Karplus relationship, the dihedral angles resulting from the XRD structure analyses correspond to the $^{3}J_{HCCH}$ coupling constants.[15] Although not depicted in **Figure 3.3**, two molecules of the same structure were observed in the asymmetric unit of **3**.

Less structural information exists in the literature when it comes to hydroxycinnamate-containing structures despite their ubiquitous presence in nature and human diet. This makes the XRD data of compounds **3** and **74** particularly interesting, especially because they display different conformations around the C_{Ar}-CH=CH bond compared to recently published results about other similar hydroxycinnamate-containing compounds (one of which also synthesized in the current work, **15**).[8] Rotation around the σ-bond in C_{Ar}-CH=CH allows for two theoretical conformations dictated by either a *syn*- or an *anti*-orientation of the aromatic C-3 substituent with respect to the *trans* double bond (**Figure 3.54**). Jaiswal et al. observed that for two caffeic acid derivatives (**15** and 2,2,2-trichloroethyl caffeate, the caffeic acid analogue of **73**) the *anti*-conformation was preferred in the solid state.[8] However, for compounds **3 (**dimethoxycinnamic acid derivative**)** and **74 (**ferulic acid derivative**)**, the *syn*-conformation was observed. Additional crystal structures of similar compounds complemented by molecular mechanics calculations at the MM2 level might help understand better why hydroxycinnamate-containing molecules prefer one of the two conformations.



syn-conformation *anti*-conformation

3 R^1 = isoprop. QL; R^2, R^3 = CH_3
74 R^1 = CH_2CH_2Cl; R^2 = CH_3; R^3 = All

Figure 3.54. *Syn*- and *anti*-conformation of hydroxycinnamate derivatives.

3.3. Conclusions

A series of mono-, di- and triacylated chlorogenic acids and derivatives were synthesized successfully in the current project; the chosen cinnamoyl substituents were caffeoyl, feruloyl and dimethoxycinnamoyl. Efficient orthogonal protecting group strategies were employed for the alcohols and carboxylic acid of the quinic acid moiety, and for the phenols of the hydroxycinnamate moiety. The focus was on generating γ-quinide derivatives, compounds which result from CGAs at temperatures routinely employed in food processing (roasting, cooking, baking, frying, steaming, microwaving, fermenting). Quinides are among the main contributors to the sensory and organoleptic properties of coffee despite their relatively low concentrations in the final beverage. They could also represent potential metabolites or degradation products of naturally occurring CGAs. Hydroxycinnamate esters of methyl quinates are also found in natural sources and representative compounds were synthesized. Additionally, diastereomers of quinic acid and methyl quinate were prepared since such epimers and their hydroxycinnamate esters are expected to form during food processing.[27] X-Ray data of final products or intermediate compounds in the above syntheses provided additional insight about their molecular structures and showed the preferred conformations in the solid state. The synthesized compounds were used for further studies, as detailed in the following chapters or in the annexed co-authored papers. Their synthetic paths represent reliable ways of generating them as reference standards against which their existence can be probed in a variety of unprocessed or processed food sources. Additionally, once synthesized such compounds could be tested for their individual or synergetic effects, which they might possess related to human health, without the need of demanding procedures of extraction and purification from the food source.

3.4. Experimental

Chemicals. All chemicals (analytical grade) and authentic standards of polyphenols were purchased from Sigma-Aldrich or Applichem (Germany) and were used without further purification.

Melting Points. Melting points were determined in open capillaries using a Stuart SMP3 capillary melting point apparatus and are not corrected.

Chromatography. TLC was performed on Macherey-Nagel aluminum-backed plates pre-coated with silica gel 60 (UV_{254}). Column chromatography was carried out on silica gel 60 (0.040-0.063 mm).

NMR. ^{1}H-NMR and ^{13}C-NMR spectra were acquired on a JEOL ECX-400 spectrometer operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR at room temperature in $CDCl_3$, CD_3OD, D_2O, $(CD_3)_2CO$ or $(CD_3)_2SO$ using a 5 mm probe. The chemical shifts (δ) are reported in parts per million and were referenced to the residual solvent peak. The coupling constants (J) are quoted in hertz. The following abbreviations are used: s, singlet; d, doublet; dd, doublet of doublets, ddd, doublet of doublet of doublets; t, triplet; m, multiplet; br, broad signal.

XRD. Crystals were mounted on a Hampton cryoloop in light oil for data collection at 100 K. Indexing and data collection were performed on a Bruker D8 SMART APEX II CCD diffractometer with κ geometry and Mo Kα radiation (graphite monochromator, λ = 0.71073 Å). Data integration was performed using SAINT. Routine Lorentz and polarization corrections were applied. The SHELX package was used for structure solution and refinement. Refinements were full-matrix least-squares against F2 using all data. In the final refinement, all non-hydrogen atoms were refined anisotropically and hydrogen atoms were either found directly and refined isotropically or placed in calculated positions.

3.4.1. Synthesis of Individual Compounds

3,4-*O*-Isopropylidene-1,5-quinide 2: To a solution of 10.00 g (52.04 mmol) of quinic acid in 50 mL acetone, a quantity of 200 mg (1.05 mmol) of *p*-toluenesulfonic acid monohydrate ($PTSA{\cdot}H_2O$) was added, followed by addition of 22.40 mL of 2,2-dimethoxypropane (DMP) to give a white suspension. The reaction was then refluxed for 24 h to give a clear red solution, which was cooled to 50 ºC and neutralized with a solution of NaOEt (71.5 mg) in EtOH (5 mL) to give a yellow clear solution. The solvents were removed under reduced pressure and to the resulting orange viscous liquid a volume of 100 mL of EtOAc was added. The organic phase was washed with 50 mL of H_2O and the aqueous phase was back-extracted with 30 mL EtOAc. The combined organic layers were washed with a half-saturated $NaHCO_3$ solution, dried on Na_2SO_4, filtered and evaporated. The resulting yellow solid was recrystallized successively from a 1:1 n-heptane:EtOAc solution to afford white crystals of (6.13 g, 28.62 mmol, 55%); mp 142 ºC; ^{1}H-

NMR ($CDCl_3$): δ_H 4.71 (dd, 1H, *J* = 2.5, 6.2 Hz, 5-H), 4.48 (ddd, 1H, *J* = 2.8, 6.4, 7.8 Hz, 3-H), 4.29 (ddd, 1H, *J* = 1.4, 2.5, 6.4 Hz, 4-H), 2.96 (br, 1H, OH), 2.63 (d, 1H, *J* = 11.9 Hz, 6-*H*H), 2.39-2.32 (ddd, 1H, *J* = 2.3, 7.8, 14.7 Hz, 2-*H*H), 2.32-2.26 (m, 1H, 6-H*H*), 2.16 (dd, 1H, *J* = 2.8, 14.7 Hz, 2-H*H*), 1.51 (s, 3H, CH_3), 1.31 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 178.95 (COO), 109.88 (CH_3-*C*), 75.94 (C-5), 72.18 (C-1), 71.59 (C-4 and C-3), 38.27 (C-2), 34.35 (C-6), 27.06 (CH_3), 24.38 (CH_3).

1-*O*-(3',4'-Dimethoxycinnamoyl)-3,4-*O*-isopropylidene-1,5-quinide 3: To a solution of 3,4-*O*-isopropylidene-1,5-quinide (1.30 g, 6.07 mmol) in 65 mL DCM, a quantity of 92 mg (0.75 mmol, 15% mol) DMAP was added and a volume of 18 mL of NEt_3. A quantity of 2.06 g (9.10 mmol) 3,4-dimethoxycinnamoyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.66 g (4.10 mmol, 67%) of white solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.64 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.08 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.03 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.29 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 4.80 (dd, 1H, *J* = 2.3, 6.4 Hz, 5-H), 4.55 (ddd, 1H, *J* = 3.2, 6.6, 7.8 Hz, 3-H), 4.33 (m, 1H, 4-H), 3.90 (s, 3H, C_{Ar}-OCH_3), 3.89 (s, 3H, C_{Ar}-OCH_3), 3.10 (m, 1H, 6-*H*H), 2.62 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.51 (ddd, 1H, *J* = 2.8, 7.8, 14.2 Hz, 2-*H*H), 2.42 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-H*H*), 1.52 (s, 3H, CH_3-C), 1.33 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 173.75 (OC-*C*OO), 165.30 (CH-*C*OO), 151.89 (*C*$_{Ar}$-OCH_3), 149.24 (*C*$_{Ar}$-OCH_3), 146.74 (*C*H-C_{Ar}), 127.09 (*C*$_{Ar}$-CH), 122.90 (C_{Ar}H), 114.44 (C_{Ar}-CH=*C*H), 111.12 (C_{Ar}H), 110.04 (C_{Ar}H), 109.74 (CH_3-*C*), 76.13 (C-1), 75.51 (C-5), 72.58 (C-4), 71.28 (C-3), 56.06 (C_{Ar}-O*C*H_3), 55.99 (C_{Ar}-O*C*H_3), 35.78 (C-2), 30.89 (C-6), 27.08 (*C*H_3-C), 24.44 (*C*H_3-C).

1-*O*-(3',4'-Dimethoxycinnamoyl)-1,5-quinide (1-DQL) 4: A quantity of 100 mg of 1-*O*-(3',4'-dimethoxycinnamoyl)-3,4-*O*-isopropylidene-1,5-quinide (0.25 mmol) was suspended in 3.75 mL TFA 80% solution and was stirred for 40 min. The solvents were removed in vacuum to give 90 mg (quantitative yield) of a white solid; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.63 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.35 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.21 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.97 (d, 1H, *J* = 8.2

Hz, $C_{Ar}H$), 6.44 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.87 (m, 1H, 5-H), 4.07 (dd, 1H, *J* = 4.1, 4.6 Hz, 4-H), 3.87 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 3.83 (m, 1H, 3-H), 3.03 (ddd, 1H, *J* = 2.3, 6.0, 11.0 Hz, 6-*H*H), 2.58 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.18-2.02 (m, 2H, 2-*H*H and 2-H*H*); ^{13}C-NMR (CD_3OD): δ_C 173.43 (OC-*C*OO), 165.48 (CH-*C*OO), 151.79 (C_{Ar}-OCH_3), 149.46 (C_{Ar}-OCH_3), 146.43 (*C*H-C_{Ar}), 127.29 (C_{Ar}-CH), 122.97 ($C_{Ar}H$), 114.03 (C_{Ar}-CH=*C*H), 111.25 ($C_{Ar}H$), 110.26 ($C_{Ar}H$), 77.24 (C-1), 76.81 (C-5), 65.77 (C-4), 65.72 (C-3), 55.18 (C_{Ar}-O*C*H_3), 55.10 (C_{Ar}-O*C*H_3), 36.43 (C-2), 32.64 (C-6).

3,4-Dimethoxycinnamoyl chloride 6: 3,4-Dimethoxycinnamic acid (7.00 g, 33.62 mmol) was added to a solution of 170 mL toluene containing 150 µL of DMF. A volume of 6 mL (8.75 mg, 68.96 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for 4 h and the resulting yellow solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under the rotary evaporator to give a yellow solid (7.53 g, 33.23 mmol, 99%); ^{1}H-NMR ($CDCl_3$): δ_H 7.77 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.17 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 7.05 (d, 1H, *J* = 2.3 Hz, $C_{Ar}H$), 6.89 (dd, 1H, *J* = 2.3, 8.2 Hz, $C_{Ar}H$), 6.49 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 3.93 (s, 3H, CH_3), 3.92 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 166.11 (COCl), 152.97 (C_{Ar}-OCH_3), 150.93 (*C*H-C_{Ar}), 149.67 (C_{Ar}-OCH_3), 126.15 (C_{Ar}-CH), 124.89 ($C_{Ar}H$), 119.82 (C_{Ar}-CH=*C*H), 111.31 ($C_{Ar}H$), 110.12 ($C_{Ar}H$), 56.04 (CH_3), 55.99 (CH_3).

3-*O*-Acetylferulic acid 8: To a solution of ferulic acid (9.00 g, 46.35 mmol) and DMAP (283 mg, 2.32 mmol) in 30 mL pyridine was added 7.08 mL (7.65 g, 74.94 mmol) acetic anhydride at 0 ºC. The reaction mixture was stirred for 1 h and then poured onto crushed ice. The aqueous phase was acidified with a HCl 2M solution (pH=2) and extracted with EtOAc/THF (3:1, 3x60 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuum; the crude residue was recrystallized from EtOAc to afford the white solid product (9.85 g, 41.72 mmol, 90%); ^{1}H-NMR (($CD_3)_2SO$): δ_H 12.25 (br, 1H, COOH), 7.54 (dd, 1H, *J* = 1.8, 8.7 Hz, $C_{Ar}H$), 7.51 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.49 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.12 (d, 1H, *J* = 8.7 Hz, $C_{Ar}H$), 6.37 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 3.77 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3-COO); ^{13}C-NMR (($CD_3)_2SO$): δ_C 169.11 (COOH), 168.25 (CH_3-*C*OO), 153.13 (C_{Ar}-OCH_3),

148.53 (*C*H-C_{Ar}), 140.02 (COO*C*$_{Ar}$), 128.33 (*C*$_{Ar}$-CH), 127.86 (C_{Ar}H), 122.53 (C_{Ar}H), 118.21 (C_{Ar}-CH=C*H*), 113.37 (C_{Ar}H), 56.74 (OCH_3), 21.13 (*C*H_3-COO).

3-*O*-Acetylferuloyl chloride 9: 3-*O*-Acetylferulic acid (9.85 g, 41.72 mmol) was added to a solution of 170 mL toluene containing 150 µL of DMF. A volume of 6 mL (8.75 g, 68.96 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for 4 h and the resulting brown solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under the rotary evaporator to give a brown solid (10.09 g, 39.63 mmol, 95%); ^{1}H-NMR ($CDCl_3$): δ_H 7.81 (d, 1H, *J* = 15.5 Hz, C_{Ar}-CH), 7.18 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.11 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.10 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.50 (d, 1H, *J* = 15.5 Hz, C_{Ar}-CH=C*H*), 3.87 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3-COO); ^{13}C-NMR ($CDCl_3$): δ_C 168.95 (CH_3-*C*OO), 166.06 (COCl), 151.97 (*C*$_{Ar}$-OCH_3), 150.17 (*C*H-C_{Ar}), 142.88 (COO*C*$_{Ar}$), 131.54 (*C*$_{Ar}$-CH), 124.47 (C_{Ar}H), 122.60 (C_{Ar}H), 122.55 (C_{Ar}-CH=C*H*), 111.52 (C_{Ar}H), 56.10 (OCH_3), 20.55 (*C*H_3-COO).

1-*O*-(3'-*O*-Acetylferuloyl)-3,4-*O*-isopropylidene-1,5-quinide 10: To a solution of 3,4-*O*-isopropylidene-1,5-quinide (1.00 g, 4.67 mmol) in 50 mL CH_2Cl_2, a quantity of 71 mg (0.58 mmol, 12% mol) DMAP was added and a volume of 14 mL of NEt_3. A quantity of 1.78 g (7.00 mmol) 3-*O*-acetylferuloyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH 2) with a HCl 2M solution and extracted 3 times with CH_2Cl_2 (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.23 g (2.85 mmol, 61%) of white solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.66 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.11 (dd, 1H, *J* = 1.8, 7.8 Hz, C_{Ar}H), 7.08 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.05 (d, 1H, *J* = 7.8 Hz, C_{Ar}H), 6.38 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.81 (dd, 1H, *J* = 2.8, 6.4 Hz, 5-H), 4.56 (ddd, 1H, *J* = 3.2, 6.9, 7.6 Hz, 3-H), 4.34 (m, 1H, 4-H), 3,85 (s, 3H, OCH_3), 3.10 (m, 1H, 6-*H*H), 2.64 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.52 (ddd, 1H, *J* = 2.3, 7.6, 14.2 Hz, 2-*H*H), 2.42 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-H*H*), 2.31 (s, 3H, CH_3-COO), 1.53 (s, 3H, CH_3-C), 1.33 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_c 173.61 (OC-*C*OO), 168.93 (CH_3-*C*OO), 164.97 (CH-*C*OO), 151.55 (*C*$_{Ar}$-OCH_3), 146.08 (*C*H-C_{Ar}), 141.87 (COO*C*$_{Ar}$), 133.02 (*C*$_{Ar}$-CH), 123.18

($C_{Ar}H$), 121.52 ($C_{Ar}H$), 117.03 (C_{Ar}-CH=C*H*), 111.56 ($C_{Ar}H$), 110.02 (CH_3-*C*), 76.43 (C-1), 75.41 (C-5), 72.33 (C-4), 71.12 (C-3), 56.03 (OCH_3), 35.80 (C-2), 30.91 (C-6), 27.11 (*C*H_3-C), 24.22 (*C*H_3-C), 20.74 (*C*H_3-COO).

1-*O*-Feruloyl-3,4-*O*-isopropylidene-quinic acid 11: A quantity of 540 mg (1.16 mmol) of 1-*O*-(3'-*O*-acetylferuloyl)-3,4-*O*-isopropylidene-1,5-quinide was dissolved in 30 mL THF and 50 mL of a solution of 1M LiOH was added. The reaction mixture was stirred at r.t. for 20 min, was then acidified with 2M HCl (pH 2) and extracted with EtOAc (3x40 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed *in vacuo*. The resulting residue was purified by column chromatography (30-40% EtOAc/petroleum ether) to give a pale yellow solid (224 mg, 0.55 mmol, 47%); ^{1}H-NMR ($(CD_3)_2SO$): δ_H 7.48 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.24 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.04 (dd, 1H, *J* = 1.8, 7.8 Hz, $C_{Ar}H$), 6.76 (d, 1H, *J* = 7.8 Hz, $C_{Ar}H$), 6.37 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.29 (m, 1H, 3-H), 3.82 (m, 1H, 5-H), 3.36 (br, 1H, OH), 3.27 (dd, 1H, *J* = 3.2, 7.8 Hz, 4-H), 3.78 (s, 3H, OCH_3), 2.45 (m, 1H, 2-*H*H), 2.28 (dd, 1H, *J* = 5.0, 15.6 Hz, 2-H*H*), 2.12 (m, 1H, 6-*H*H), 1.60 (dd, 1H, *J* = 11.5, 13.3 Hz, 6-H*H*), 1.33 (s, 3H, CH_3-C), 1.20 (s, 3H, CH_3-C); ^{13}C-NMR ($(CD_3)_2SO$): δ_C 173.22 (COOH), 165.86 (CH-*C*OO), 149.96 (*C*$_{Ar}$-OCH_3), 148.49 (*C*H-C_{Ar}), 145.87 ($C_{Ar}OH$), 126.00 (*C*$_{Ar}$-CH), 123.58 ($C_{Ar}H$), 116.08 (C_{Ar}-CH=C*H*), 115.57 ($C_{Ar}H$), 111.57 (CH_3-*C*), 108.15 ($C_{Ar}H$), 80.49 (C-1), 79.08 (C-4), 73.25 (C-3), 67.38 (C-5), 56.19 (OCH_3), 38.65 (C-2), 31.21 (C-6), 28.81 (*C*H_3-C), 26.42 (*C*H_3-C).

1-*O*-Feruloyl-3,4-*O*-isopropylidene-1,5-quinide 12: A quantity of 145 mg (0.36 mmol) of 1-*O*-feruloyl-3,4-*O*-isopropylidene-quinic acid was dissolved in 8 mL toluene and 1.36 mg (0.01 mmol) of PTSA·H_2O was added. The reaction was refluxed for 24 h, was cooled to r.t. and neutralized with solid $NaHCO_3$ (3 mg, 0.03 mmol). Toluene was removed under reduced pressure, the solid residue was dissolved in EtOAc, filtered and the filtrate was concentrated to afford 100 mg (quantitative yield) of the brown powder; ^{1}H-NMR ($CDCl_3$): δ_H 7.64 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.07 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.01 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.91 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.28 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.82 (dd, 1H, *J* = 2.8, 6.4 Hz, 5-H), 4.58 (ddd, 1H, *J* = 3.2, 6.9, 7.6 Hz, 3-H), 4.34 (m, 1H, 4-H), 3.92 (s, 3H, OCH_3), 3.11 (m, 1H, 6-*H*H), 2.64 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.49 (ddd, 1H, *J* = 2.3, 7.6, 14.2 Hz, 2-*H*H), 2.43 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-H*H*), 1.54 (s, 3H, CH_3-C), 1.34 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$):

δ_C 173.96 (OC-*C*OO), 165.42 (CH-*C*OO), 148.66 (*C*$_{Ar}$-OCH_3), 147.04 (*C*H-C_{Ar}), 147.00 ($C_{Ar}OH$), 126.59 (*C*$_{Ar}$-CH), 123.59 ($C_{Ar}H$), 114.99 (C_{Ar}-CH=C*H*), 113.98 ($C_{Ar}H$), 110.05 (CH_3-*C*), 109.68 ($C_{Ar}H$), 76.13 (C-1), 75.58 (C-5), 72.55 (C-4), 71.25 (C-3), 56.06 (OCH_3), 35.74 (C-2), 30.85 (C-6), 27.06 (*C*H_3-C), 24.42 (*C*H_3-C).

1-*O*-Feruloyl-1,5-quinide (1-FQL) 13: A quantity of 100 mg (0.26 mmol) of 1-*O*-feruloyl-3,4-*O*-isopropylidene-1,5-quinide was dissolved in 3.75 mL of TFA 80% solution and stirred for 40 min at r.t. The solvents were then removed in vacuum to give a red solid (90 mg, quantitative yield); mp 90 ºC; ^{1}H-NMR (CD_3OD): δ_H 7.59 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.15 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.04 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 6.79 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.33 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.84 (dd, 1H, *J* = 4.6, 6.4 Hz, 5-H), 4.03 (dd, 1H, *J* = 4.6, 6.7 Hz, 4-H), 3.85 (s, 3H, OCH_3), 3.86-3.74 (m, 1H, 3-H), 3.04 (ddd, 1H, *J* = 2.3, 6.4, 11.0 Hz, 6-*H*H), 2.57 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.20-2.14 (m, 2H, 2-*H*H and 2-H*H*); ^{13}C-NMR (CD_3OD): δ_C 173.74 (OC-*C*OO), 165.72 (CH-*C*OO), 149.55 (*C*$_{Ar}$-OCH_3), 148.03 (*C*H-C_{Ar}), 146.90 ($C_{Ar}OH$), 126.01 (*C*$_{Ar}$-CH), 123.17 ($C_{Ar}H$), 115.20 (C_{Ar}-CH=C*H*), 113.20 ($C_{Ar}H$), 110.55 ($C_{Ar}H$), 77.27 (C-1), 76.77 (C-5), 65.74 (C-4 and C-3), 55.20 (CH_3), 36.42 (C-2), 32.68 (C-6).

3,4-Di-*O*-allylcaffeic acid 15: A mixture of caffeic acid (5.00 g, 27.75 mmol) and anhydrous potassium carbonate (55.30 g, 401 mmol) in acetone (250 mL) was stirred at r.t. for 30 min. To the mixture was added a solution of allyl bromide (11.75 g, 97.13 mmol) in acetone (50 mL) and the entire mixture was refluxed for 48 h. The reaction was cooled to r.t., filtered and the filtrate was dried *in vacuo*. The residue was suspended in ethanol (150 mL) and a NaOH 2M solution (100 mL) was added. The mixture was refluxed for 2 h. The solution was cooled to r.t., poured into a beaker and acidified (pH 2) with conc. HCl. The suspension was stirred at r.t. for 30 min and the solid was filtered off and washed successively with a 1:1 mixture of ethanol/water (200 mL). The solid was dried overnight in vacuum to yield a white powder (6.16 g, 23.59 mmol, 85%); mp 155-157 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.70 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.11 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.09 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.88 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.28 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.07 (m, 2H, 2xCH_2=C*H*), 5.43 (d, 1H, *J* = 16.9 Hz, C*H*H=CH), 5.42 (d, 1H, *J* = 16.9 Hz, C*H*H=CH), 5.31 (d, 1H, *J* = 10.5 Hz, CH*H*=CH), 5.30 (d, 1H, *J* = 10.5 Hz, CH*H*=CH), 4.64 (m, 4H, 2xC_{Ar}-OCH_2); ^{13}C-NMR ($CDCl_3$): δ_C 172.53 (COOH), 151.11 (*C*$_{Ar}$-

OCH_2), 148.56 (C_{Ar}-OCH_2), 147.06 (CH-C_{Ar}), 133.11 (CH=CH_2), 132.89 (CH=CH_2), 127.24 (C_{Ar}-CH), 123.26 (C_{Ar}H), 118.16 (CH_2=CH), 118.09 (CH_2=CH), 114.86 (C_{Ar}-CH=CH), 113.42 (C_{Ar}H), 112.85 (C_{Ar}H), 70.05 (C_{Ar}-OCH$_2$), 69.80 (C_{Ar}-OCH$_2$).

3,4-Di-*O*-allylcaffeoyl chloride 16: 3,4-Di-*O*-allylcaffeic acid (6.16 g, 23.59 mmol) was added to a solution of 100 mL toluene containing 150 μL of dimethylformamide (DMF). A volume of 6.80 mL (9.90 g, 77.98 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for 4 h and the resulting brown solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under rotary evaporator to give a light brown solid (6.05 g, 21.70 mmol, 92%); mp 67-68 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.74 (d, 1H, J = 15.6 Hz, C_{Ar}-CH), 7.14 (dd, 1H, J = 1.8, 8.2 Hz, C_{Ar}H), 7.07 (d, 1H, J = 1.8 Hz, C_{Ar}H), 6.89 (d, 1H, J = 8.2 Hz, C_{Ar}H), 6.45 (d, 1H, J = 15.6 Hz, C_{Ar}-CH=CH), 6.07 (m, 2H, 2xCH_2=CH), 5.44 (d, 1H, J = 16.9 Hz, CHH=CH), 5.43 (d, 1H, J = 16.9 Hz, CHH=CH), 5.32 (d, 1H, J = 10.5 Hz, CHH=CH), 5.31 (d, 1H, J = 10.5 Hz, CHH=CH), 4.65 (m, 4H, 2xC_{Ar}-OCH_2); ^{13}C-NMR ($CDCl_3$): δ_C 166.12 (COCl), 152.33 (C_{Ar}-OCH_2), 150.90 (C_{Ar}-OCH_2), 148.81 (CH-C_{Ar}), 132.90 (CH=CH_2), 132.58 (CH=CH_2), 126.16 (C_{Ar}-CH), 124.68 (C_{Ar}H), 119.85 (C_{Ar}-CH=CH), 118.36 (CH_2=CH), 118.22 (CH_2=CH), 113.33 (C_{Ar}H), 113.19 (C_{Ar}H), 70.10 (C_{Ar}-OCH$_2$), 69.78 (C_{Ar}-OCH$_2$).

1-*O*-(3',4'-Di-*O*-allylcaffeoyl)-3,4-*O*-isopropylidene-1,5-quinide 17: To a solution of 3,4-*O*-isopropylidene-1,5-quinide (717 mg, 3.35 mmol) in 35 mL CH_2Cl_2, a quantity of 123 mg (1.00 mmol, 30% mol) 4-dimethylaminopyridine (DMAP) was added and a volume of 10 mL of NEt_3. A quantity of 1.40 g (5.02 mmol) 3,4-di-*O*-allylcaffeoyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH 2) with a HCl 2M solution and extracted 3 times with CH_2Cl_2 (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.04 g (2.28 mmol, 68%) of a yellow solid; mp 127 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.63 (d, 1H, J = 16.0 Hz, C_{Ar}-CH), 7.06 (dd, 1H, J = 1.8, 8.7 Hz, C_{Ar}H), 7.05 (d, 1H, J = 1.8 Hz, C_{Ar}H), 6.86 (d, 1H, J = 8.7 Hz, C_{Ar}H), 6.26 (d, 1H, J = 16.0 Hz, C_{Ar}-CH=CH), 6.06 (m, 2H, 2xCH_2=CH), 5.45-5.39 (m, 2H, 2xCHH=CH), 5.29 (dd, 2H, J = 1.4,

10.5 Hz, 2xCH*H*=CH), 4.80 (dd, 1H, *J* = 2.3, 6.4 Hz, 5-H), 4.63 (tt, 4H, *J* = 1.4, 5.0 Hz, 2xC_{Ar}-OCH_2), 4.56 (ddd, 1H, *J* = 3.2, 6.9, 7.6 Hz, 3-H), 4.33 (m, 1H, 4-H), 3.09 (m, 1H, 6-*H*H), 2.62 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.51 (ddd, 1H, *J* = 2.3, 7.6, 14.7 Hz, 2-*H*H), 2.41 (dd, 1H, *J* = 3.2, 14.7 Hz, 2-H*H*), 1.53 (s, 3H, CH_3), 1.33 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 173.76 (OC-*C*OO), 165.32 (CH-*C*OO), 151.12 (C_{Ar}-OCH_2), 148.64 (C_{Ar}-OCH_2), 146.71 (*C*H-C_{Ar}), 133.09 (*C*H=CH_2), 132.89 (*C*H=CH_2), 127.21 (C_{Ar}-CH), 123.22 (C_{Ar}H), 118.13 (*C*H_2=CH), 118.07 (*C*H_2=CH), 114.48 (C_{Ar}-CH=*C*H), 113.40 (C_{Ar}H), 112.78 (C_{Ar}H), 110.05 (CH_3-*C*), 76.13 (C-1), 75.51 (C-5), 72.58 (C-4), 71.28 (C-3), 70.04 (C_{Ar}-O*C*H_2), 69.78 (C_{Ar}-O*C*H_2), 35.78 (C-2), 30.86 (C-6), 27.08 (CH_3), 24.35 (CH_3).

1-*O*-Caffeoyl-3,4-*O*-isopropylidene-1,5-quinide 18: To a solution of 963 mg (2.11 mmol) of 1-*O*-(3',4'-di-*O*-allylcaffeoyl)-3,4-*O*-isopropylidene-1,5-quinide in 60 mL of aqueous MeOH (90%), a quantity of 84.3 mg (0.44 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (820 mg) was slowly added at r.t. and it was then heated to 65 ºC for 48 h. The mixture was cooled to r.t., filtered and MeOH removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (40-50% EtOAc/petroleum ether) to give 1-*O*-caffeoyl-3,4-*O*-isopropylidene-1,5-quinide as a white powder (397 mg, 1.05 mmol, 50%); mp 210-212 ºC; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 8.35 (br, 2H, 2xOH), 7.58 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.16 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.06 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.27 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 4.81 (dd, 1H, *J* = 2.3, 6.4 Hz, 5-H), 4.62 (ddd, 1H, *J* = 3.2, 6.9, 7.3 Hz, 3-H), 4.33 (m, 1H, 4-H), 3.08 (m, 1H, 6-*H*H), 2.53 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.44 (ddd, 1H, *J* = 2.3, 7.3, 14.2 Hz, 2-*H*H), 2.31 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-H*H*), 1.48 (s, 3H, CH_3), 1.30 (s, 3H, CH_3); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 173.09 (OC-*C*OO), 164.94 (CH-*C*OO), 148.38 (C_{Ar}OH), 146.67 (*C*H-C_{Ar}), 145.52 (C_{Ar}OH), 126.50 (C_{Ar}-CH), 122.18 (C_{Ar}H), 115.62 (C_{Ar}H), 114.63 (C_{Ar}-CH=*C*H), 113.60 (C_{Ar}H), 109.51 (CH_3-*C*), 75.99 (C-1), 75.24 (C-5), 72.58 (C-4), 71.17 (C-3), 35.74 (C-2), 30.27 (C-6), 26.42 (CH_3), 23.73 (CH_3).

1-*O*-Caffeoyl-1,5-quinide (1-CQL) 19: A quantity of 79 mg (0.21 mmol) of 1-*O*-caffeoyl-3,4-*O*-isopropylidene-1,5-quinide was dissolved in 3.75 mL of TFA 80% solution and stirred for 2 h.

The solvents were then removed in vacuum to give a white solid (70 mg, quantitative yield); mp 135 ºC; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.56 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.17 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.07 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.28 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.86 (dd, 1H, *J* = 4.6, 6.0 Hz, 5-H), 4.06 (dd, 1H, *J* = 4.4, 4.6 Hz, 4-H), 3.82 (m, 1H, 3-H), 3.03 (ddd, 1H, *J* = 2.3, 6.0, 11.0 Hz, 6-*H*H), 2.57 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.16-2.09 (m, 1H, 2-*H*H and 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.27 (OC-*C*OO), 164.97 (CH-*C*OO), 148.40 (C_{Ar}OH), 146.48 (*C*H-C_{Ar}), 145.53 (C_{Ar}OH), 126.51 (*C*$_{Ar}$-CH), 122.12 (C_{Ar}H), 115.59 (C_{Ar}H), 114.59 (C_{Ar}-CH=*C*H), 113.77 (C_{Ar}H), 76.59 (C-1 and C-5), 66.10 (C-4), 65.79 (C-3), 37.04 (C-2), 32.73 (C-6).

Methyl 3,4-*O*-isopropylidenequinate 20: A quantity of 2.33 g (10.41 mmol) of 3,4-*O*-isopropylidene-1,5-quinide was dissolved in MeOH (90 mL) and a 21% solution (6 mg NaOMe) of NaOMe/MeOH was added under stirring. The solution was stirred overnight, was then quenched with glacial acetic acid (7 μL) and the volatile components were removed under vacuum. Water was added (50 mL) to the solid residue, which was then extracted with EtOAc (3x25 mL). The combined organic layers were dried over Na_2SO_4 and the solvents were removed *in vacuo*. The resulting residue was purified by column chromatography (30-40% EtOAc/petroleum ether) to give the solid white product (922 mg, 3.74 mmol, 36%); ^{1}H-NMR ($CDCl_3$): δ_H 4.42 (m, 1H, 3-H), 4.09 (ddd, 1H, *J* = 4.1, 6.9, 11.5 Hz, 5-H), 3.93 (dd, 1H, *J* = 6.4, 6.9 Hz, 4-H), 3.76 (s, 3H, OCH_3), 3.51 (br, 1H, OH), 3.15 (br, 1H, OH), 2.20 (m, 2H, 2-*H*H and 2-H*H*), 2.02 (dd, 1H, *J* = 4.1, 13.3 Hz, 6-*H*H), 1.80 (dd, 1H, *J* = 11.5, 13.3 Hz, 6-H*H*), 1.49 (s, 3H, CH_3-C), 1.32 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 175.66 (COO), 109.27 (CH_3-*C*), 80.11 (C-1), 74.02 (C-4), 73.48 (C-3), 68.10 (C-5), 53.17 (OCH_3), 39.09 (C-6), 34.76 (C-2), 28.27 (*C*H_3-C), 25.78 (*C*H_3-C).

Methyl 3,4-*O*-isopropylidene-5-*O*-(3',4'-di-*O*-allylcaffeoyl)-quinate 21: To a solution of methyl 3,4-*O*-isopropylidenequinate (269 mg, 1.09 mmol) in 16 mL DCM, a quantity of 14 mg (0.11 mmol, 10% mol) DMAP was added and a volume of 3 mL of pyridine. A quantity of 426 mg (1.53 mmol) 3,4-di-*O*-allylcaffeoyl chloride was subsequently added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH 2) with a HCl 2M solution and extracted 3 times with DCM (3x20 mL). The combined organic layers were dried over Na_2SO_4,

filtered and evaporated *in vacuo*. The product was purified by column chromatography (EtOAc/petroleum ether 20-30%) to afford 347 mg (0.71 mmol, 65%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.60 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.03 (dd, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 7.02 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.83 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.31 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.04 (m, 2H, 2xCH_2=C*H*), 5.42 (m, 1H, 5-H), 5.39 (d, 2H, *J* = 17.4 Hz, 2xC*H*H=CH), 5.27 (d, 2H, *J* = 10.5 Hz, 2xCH*H*=CH), 4.60 (m, 2H, C_{Ar}-OCH_2), 4.51 (m, 1H, 4-H), 4.18 (m, 1H, 3-H), 3.75 (s, 3H, OCH_3), 2.30 (m, 2H, 2-*H*H and 2-H*H*), 2.22 (dd, 1H, *J* = 4.1, 13.3 Hz, 6-*H*H), 1.89 (dd, 1H, *J* = 11.5, 13.3 Hz, 6-H*H*), 1.56 (s, 3H, CH_3-C), 1.34 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 174.84 (*C*OOCH_3), 166.36 (CH-*C*OO), 150.72 (OC_{Ar}), 148.54 (OC_{Ar}), 145.19 (*C*H-C_{Ar}), 133.12 (*C*H_2-CH), 132.94 (*C*H_2-CH), 127.52 (*C*$_{Ar}$-CH), 122.85 (C_{Ar}H), 118.09 (*C*H_2=CH), 118.02 (*C*H_2=CH), 115.68 (C_{Ar}-CH=*C*H), 113.37 (C_{Ar}H), 112.62 (C_{Ar}H), 109.65 (CH_3-*C*), 73.95 (C-1 and C-4), 73.69 (C-3), 70.68 (C-5), 69.98 (C_{Ar}-O*C*H_2), 69.75 (C_{Ar}-O*C*H_2), 53.14 (OCH_3), 37.01 (C-6), 34.46 (C-2), 28.10 (*C*H_3-C), 25.93 (*C*H_3-C).

Methyl 5-*O*-caffeoylquinate (5-CQM) 22: To a solution of 262 mg (0.54 mmol) of ester methyl 3,4-*O*-isopropylidene-5-*O*-(3',4'-di-*O*-allylcaffeoyl)-quinate in 60 mL of aqueous MeOH (90%), a quantity of 12 mg (0.06 mmol) of *p*-toluenesulfonic acid monohydrate (PTSA·H_2O) was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (105 mg) was slowly added at r.t. and it was then heated to 65 ºC for 48 h. The mixture was cooled to r.t., filtered and MeOH removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (EtOAc/petroleum ether 40-50%) and the resulting white powder was treated with a 70% TFA solution for 1 h to give the desired methyl 5-*O*-caffeoylquinate (98 mg, 0.27 mmol, 50%); ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.51 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.13 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 7.03 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.84 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.22 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.31 (m, 1H, 5-H), 4.14 (m, 1H, 3-H), 3.71 (m, 1H, 4-H), 3.65 (s, 3H, CH_3), 2.21 (ddd, 1H, *J* = 2.3, 4.1, 12.8 Hz, 6-*H*H), 2.15 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-*H*H), 2.27 (m, 1H, 6-H*H*), 2.00 (m, 1H, 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 173.63 (*C*OOCH_3), 166.08 (CH-*C*OO), 147.98 (OC_{Ar}), 145.51 (OC_{Ar}), 144.99 (*C*H-C_{Ar}), 126.75 (*C*$_{Ar}$-CH), 121.73 (C_{Ar}H), 115.58 (C_{Ar}H),

114.91 (C_{Ar}-CH=*C*H), 114.34 (C_{Ar}H), 75.06 (C-1), 72.17 (C-4), 70.65 (C-3), 69.91 (C-5), 51.74 (CH_3), 37.14 (C-2 and C-6).

5-*O*-(3',4'-Dimethoxycinnamoyl)-quinic acid bisacetonide 24: To a solution of quinic acid bisacetonide (1.00 g, 3.68 mmol) and 4-dimethylaminopyridine (DMAP) (200 mg, 1.63 mmol) in 50 mL dichloromethane (DCM) were added NEt_3 (10 mL) and 3,4-dimethoxycinnamoyl chloride (1.29 g, 5.70 mmol) at r.t. The reaction mixture was refluxed for 24 h, cooled to r.t. and acidified (pH≈3) with 2 M HCl. The layers were separated and the aqueous phase was extracted with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (EtOAc/petroleum ether 30-50%) to give the desired product 1.26 g (2.72 mmol, 74%); ^{1}H-NMR ($CDCl_3$): δ_H 7.61 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.04 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.01 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.82 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.29 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 5.23 (m, 1H, 5-H), 4.49 (dd, 1H, *J* = 4.4, 10.1 Hz, 4-H), 4.18 (m, 1H, 3-H), 3.87 (s, 6H, 2xC_{Ar}-OCH_3), 2.27 (m, 2H, 6-*H*H and 2-*H*H), 2.22 (m, 1H, 2-H*H*), 1.87 (dd, 1H, *J* = 10.9, 13.9 Hz, 6-H*H*), 1.62 (s, 3H, CH_3-C), 1.59 (s, 3H, CH_3-C), 1.52 (s, 3H, CH_3-C), 1.34 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 174.31 (*C*OO-C), 165.62 (CH-*C*OO), 151.34 (OC_{Ar}), 149.22 (OC_{Ar}), 145.41 (*C*H-C_{Ar}), 127.29 (*C*$_{Ar}$-CH), 122.89 (C_{Ar}H), 115.45 (C_{Ar}-CH=*C*H), 111.12 (C_{Ar}H), 110.90 (*C*$_{Ar}$H), 109.61 (CH_3-*C*), 109.52 (CH_3-*C*), 78.19 (C-1), 76.22 (C-4), 72.53 (C-3), 70.00 (C-5), 56.03 (C_{Ar}-O*C*H$_3$), 55.94 (C_{Ar}-O*C*H$_3$), 36.02 (C-6), 34.79 (C-2), 28.72 (CH_3), 28.31 (CH_3), 27.91 (CH_3), 25.62 (CH_3).

5-*O*-(3',4'-Dimethoxycinnamoyl)-quinic acid (5-DQA) 25: 5-*O*-(3',4'-Dimethoxycinnamoyl)-quinic acid bisacetonide (500 mg, 1.08 mmol) was dissolved in a mixture of 20 mL of trifluoroacetic acid (TFA) and water (7:3) at r.t. and was stirred for 1 h. The solvents were removed *in vacuo* to obtain the title compound in quantitative yield; ^{1}H-NMR (CD_3OD): δ_H 7.60 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.17 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.13 (dd, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 6.93 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.31 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 5.31 (ddd, 1H, *J* = 4.8, 9.2, 13.7 Hz, 5-H), 4.15 (m, 1H, 3-H), 3.83 (s, 6H, 2xC_{Ar}-OCH_3), 3.71 (m, 1H, 4-H), 2.18 (m, 2H, 6-*H*H, 2-*H*H), 2.06 (m, 2H, 6-H*H*, 2-H*H*); ^{13}C-NMR (CD_3OD): δ_C 175.54 (COOH), 167.11 (CH-*C*OO), 151.50 (OC_{Ar}), 149.39 (OC_{Ar}), 145.23 (*C*H-C_{Ar}), 127.54 (*C*$_{Ar}$-CH), 122.53

($C_{Ar}H$), 115.12 (C_{Ar}-CH=*C*H), 111.31 ($C_{Ar}H$), 110.08 ($C_{Ar}H$), 74.91 (C-1), 72.14 (C-4), 70.71 (C-3), 69.93 (C-5), 55.13 (C_{Ar}-O*C*H_3), 55.02 (C_{Ar}-O*C*H_3), 37.43 (C-6), 37.21 (C-2).

1-*O*-Troc-3,4-*O*-isopropylidene-1,5-quinide 26: Pyridine (5.66 mL) was added to a solution of 3,4-*O*-isopropylidene-1,5-quinide (4.07 g, 19.00 mmol) in DCM (40 mL). The mixture was then cooled to 0 ºC and a solution of 2,2,2-trichloroethylchloroformate (4.28 g, 20.21 mmol) in 6.50 mL DCM was added drop-wise. After stirring for 2 h at r.t. a volume of 40 mL DCM was added and the mixture was washed with HCl 1M (2x40 mL) followed by water (40 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to 20 mL. Addition of EtOH (40 mL) precipitated the desired white product (5.63 g, 14.44 mmol, 76%); mp 165-166 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 4.81 (d, 1H, *J* = 11.9 Hz, CCl_3-C*H*H), 4.79 (m, 1H, 5-H), 4.71 (d, 1H, *J* = 11.9 Hz, CCl_3-CH*H*), 4.55 (ddd, 1H, *J* = 2.8, 6.4, 7.8 Hz, 3-H), 4.31 (ddd, 1H, *J* = 1.4, 2.3, 6.4 Hz, 4-H), 3.05 (m, 1H, 6-*H*H), 2.65 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.54 (ddd, 1H, *J* = 2.3, 7.8, 14.6 Hz, 2-*H*H), 2.40 (dd, 1H, *J* = 2.8, 14.6 Hz, 2-H*H*), 1.52 (s, 3H, CH_3), 1.32 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 172.30 (OC-*C*OO), 151.55 (O*C*OOCH$_2$), 109.96 (CH_3-*C*), 93.97 (CCl_3), 78.98 (C-1), 77.12 (*C*H_2-CCl_3), 75.22 (C-5), 72.43 (C-4), 71.19 (C-3), 35.37 (C-2), 30.18 (C-6), 27.05 (CH_3), 24.32 (CH_3).

1-*O*-Troc-1,5-quinide 27: A quantity of 1.00 g (2.57 mmol) of 1-*O*-Troc-3,4-*O*-isopropylidene-1,5-quinide and a solution of TFA 80% (21 mL) were cooled to 0 ºC. The acid solution was then added drop-wise at the same temperature to the quinide. The ice bath was removed and the reaction was stirred for 40 min at r.t., then the solvents were removed in vacuum to give 897 mg (quantitative yield) of the white product; mp 123-125 ºC; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 4.90 (m, 3H, CCl_3CH_2 and 5-H), 4.07 (t, 1H, *J* = 4.6 Hz, 4-H), 3.81 (m, 1H, 3-H), 2.98 (m, 1H, 6-*H*H), 2.66 (d, 1H, *J* = 11.4 Hz, 6-H*H*), 2.16 (d, 1H, *J* = 9.2 Hz, 2-*H*H), 2.15 (d, 1H, *J* = 9.2 Hz, 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.12 (OC-*C*OO), 151.21 (O*C*OOCH$_2$), 95.00 (CCl_3), 79.99 (*C*H_2-CCl_3), 77.13 (C-1), 76.60 (C-5), 65.90 (C-4), 65.57 (C-3), 36.63 (C-2), 32.94 (C-6).

1-*O*-Troc-3,4-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide 28: To a solution of 1-*O*-Troc-1,5-quinide (1.03 g, 2.86 mmol) in 50 mL DCM, a quantity of 105 mg (0.86 mmol, 30% mol) DMAP was added and a volume of 15 mL of NEt_3. A quantity of 1.95 g (8.58 mmol) 3,4-dimethoxycinnamoyl chloride was then added and the mixture was refluxed for 24 h. It was then

allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 983 mg (1.35 mmol, 47%) of white solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.67 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.57 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.10 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 7.04 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 7.00 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.94 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 6.86 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.77 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.36 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.19 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.71 (dd, 1H, *J* = 4.6, 5.5 Hz, 4-H), 5.36 (ddd, 1H, *J* = 4.6, 6.9, 11.9 Hz, 3-H), 5.02 (dd, 1H, *J* = 5.5, 6.0 Hz, 5-H), 4.84 (d, 1H, *J* = 11.9 Hz, CCl_3-C*H*H), 4.73 (d, 1H, *J* = 11.9 Hz, CCl_3-CH*H*), 3.91 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.20 (ddd, 1H, *J* = 2.8, 6.0, 11.5 Hz, 6-*H*H), 2.74 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.56 (ddd, 1H, *J* = 2.8, 6.9, 11.5 Hz, 2-*H*H), 2.48 (dd, 1H, *J* = 11.5, 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 170.18 (OC-*C*OO), 165.49 (CH-*C*OO), 165.21 (CH-*C*OO), 151.83 (O*C*OOCH$_2$), 151.52 (*C*$_{Ar}$-OCH$_3$), 151.50 (*C*$_{Ar}$-OCH$_3$), 149.44 (*C*$_{Ar}$-OCH$_3$), 149.26 (*C*$_{Ar}$-OCH$_3$), 146.96 (*C*H-C$_{Ar}$), 146.35 (*C*H-C$_{Ar}$), 127.06 (*C*$_{Ar}$-CH), 126.86 (*C*$_{Ar}$-CH), 123.32 (C$_{Ar}$H), 123.00 (C$_{Ar}$H), 114.24 (C$_{Ar}$-CH=*C*H), 113.91 (C$_{Ar}$-CH=*C*H), 111.15 (C$_{Ar}$H), 111.04 (C$_{Ar}$H), 109.86 (C$_{Ar}$H), 109.73 (C$_{Ar}$H), 94.00 (CCl_3), 78.88 (C-1), 77.35 (*C*H$_2$-CCl_3), 73.87 (C-5), 65.81 (C-3), 64.78 (C-4), 56.12 (CH_3), 56.04 (2xCH_3), 55.88 (CH_3), 33.90 (C-6), 33.83 (C-2).

3,4-Di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (3,4-diDQL) 29: A quantity of 263 mg (0.42 mmol) of 1-*O*-Troc-3,4-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide was suspended in THF (1.75 mL) and an equal volume (1.75 mL) of glacial acetic acid was added, followed by addition of 87 mg (1.51 mmol, 3.6 eq.) of Zn powder. The grey suspension was stirred at r.t. for 4 h and then the solvents were removed under reduced pressure. A volume of 20 mL EtOAc was added to the flask and the new suspension was cooled to 0 ºC and extracted with aqueous HCl 0.5M (2x15 mL) followed by brine (15 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the white solid product (216 mg, 0.39 mmol, 93%); mp 101-103 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.63 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.50 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.06 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 7.02 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 6.92 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.89 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 6.81 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.70 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.35 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.16 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*),

5.64 (dd, 1H, *J* = 4.6, 6.9 Hz, 4-H), 5.24 (ddd, 1H, *J* = 4.6, 5.5, 11.5 Hz, 3-H), 4.88 (dd, 1H, *J* = 4.6, 6.9 Hz, 5-H), 3.86 (s, 6H, 2xCH_3), 3.80 (s, 3H, CH_3), 3.72 (s, 3H, CH_3), 2.59 (d, 1H, *J* = 11.9 Hz, 6-*H*H), 2.50 (m, 1H, 6-H*H*), 2.36 (m, 1H, 2-*H*H), 2.22 (dd, 1H, *J* = 11.5, 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 177.15 (OC-*C*OO), 165.78 (CH-*C*OO), 165.63 (CH-*C*OO), 151.69 (C_{Ar}-OCH_3), 151.36 (C_{Ar}-OCH_3), 149.35 (C_{Ar}-OCH_3), 149.17 (C_{Ar}-OCH_3), 146.73 (*C*H-C_{Ar}), 146.11 (*C*H-C_{Ar}), 127.07 (C_{Ar}-CH), 126.93 (C_{Ar}-CH), 123.23 (C_{Ar}H), 122.93 (C_{Ar}H), 114.49 (C_{Ar}-CH=*C*H), 114.17 (C_{Ar}-CH=*C*H), 111.14 (C_{Ar}H), 111.00 (C_{Ar}H), 109.85 (2xC_{Ar}H), 74.05 (C-5), 72.21 (C-1), 66.37 (C-3), 64.69 (C-4), 56.06 (CH_3), 56.00 (CH_3), 55.97 (CH_3), 55.82 (CH_3), 37.53 (C-6), 36.66 (C-2).

1-*O*-Troc-3,4-di-*O*-(4'-*O*-allylferuloyl)-1,5-quinide 30: To a solution of 1-*O*-Troc-1,5-quinide (800 mg, 2.29 mmol) in 50 mL DCM, a quantity of 112 mg (0.92 mmol, 2x20% mol) DMAP was added and a volume of 14 mL of NEt_3. A quantity of 1.74 g (6.87 mmol) 4-*O*-allylferuloyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.54 g (1.96 mmol, 86%) of a pale yellow solid; mp 85-87 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.65 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH), 7.55 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH), 7.05 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.04 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.95 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.92 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.75 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.35 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH=C*H*), 6.18 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH=C*H*), 6.03 (m, 2H, 2xCH_2=C*H*), 5.68 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 5.43-5.25 (m, 5H, 2xCH_2=CH and 3-H), 5.00 (dd, 1H, *J* = 5.0, 6.0 Hz, 5-H), 4.82 (d, 1H, *J* = 11.9 Hz, CCl_3-C*H*H), 4.72 (d, 1H, *J* = 11.9 Hz, CCl_3-CH*H*), 4.63 (d, 2H, *J* = 5.3 Hz, C_{Ar}-OCH_2), 4.58 (d, 2H, *J* = 5.3 Hz, C_{Ar}-OCH_2), 3.88 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 3.18 (ddd, 1H, *J* = 2.8, 6.0, 11.5 Hz, 6-*H*H), 2.73 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.54 (ddd, 1H, *J* = 2.8, 6.9, 11.9 Hz, 2-*H*H), 2.46 (dd, 1H, *J* = 11.7, 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 170.22 (OC-*C*OO), 165.47 (CH-*C*OO), 165.19 (CH-*C*OO), 151.50 (O*C*OOCH_2), 150.80 (C_{Ar}-OCH_3), 150.46 (C_{Ar}-OCH_3), 149.74 (C_{Ar}-OCH_2), 149.57 (C_{Ar}-OCH_2), 146.92 (*C*H-C_{Ar}), 146.30 (*C*H-C_{Ar}), 132.73 (*C*H=CH_2), 132.64 (*C*H=CH_2), 127.23 (C_{Ar}-CH), 127.02 (C_{Ar}-CH), 123.09 (C_{Ar}H), 122.74 (C_{Ar}H), 118.61 (*C*H_2=CH), 118.50 (*C*H_2=CH), 114.31 (C_{Ar}-CH=*C*H), 113.97 (C_{Ar}-CH=*C*H),

112.88 ($C_{Ar}H$), 112.84 ($C_{Ar}H$), 110.31 ($C_{Ar}H$), 110.16 ($C_{Ar}H$), 94.02 (CCl_3), 78.88 (C-1), 77.06 (CH_2-CCl_3), 73.88 (C-5), 69.81 (C_{Ar}-OCH_2), 69.77 (C_{Ar}-OCH_2), 65.81 (C-3), 64.74 (C-4), 56.07 (CH_3), 55.92 (CH_3), 33.88 (C-6), 33.81 (C-2).

1-*O*-Troc-3,4-di-*O*-feruloyl-1,5-quinide 31: To a solution of 534 mg (0.68 mmol) of 1-*O*-Troc-3,4-di-*O*-(4'-*O*-allylferuloyl)-1,5-quinide in 35 mL of aqueous 1,4-dioxane (90%), a quantity of 25 mg (0.13 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (267 mg) was slowly added at r.t. and it was then heated to 60 ºC for 48 h. The mixture was cooled to r.t., filtered and dioxane removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (30-40% EtOAc/petroleum ether) to give 1-*O*-Troc-3,4-di-*O*-feruloyl-1,5-quinide as a pale yellow powder (143 mg, 0.20 mmol, 30%); mp 88-90 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.65 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.54 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.08 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.00 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.96 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 6.92 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.91 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.82 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.34 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.17 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.02 (br, 1H, OH), 5.94 (br, 1H, OH), 5.70 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 5.35 (ddd, 1H, *J* = 4.6, 6.9, 11.9 Hz, 3-H), 5.02 (dd, 1H, *J* = 5.0, 6.0 Hz, 5-H), 4.84 (d, 1H, *J* = 11.9 Hz, CCl_3-C*H*H), 4.74 (d, 1H, *J* = 11.9 Hz, CCl_3-CH*H*), 3.91 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.19 (ddd, 1H, *J* = 2.8, 6.0, 11.5 Hz, 6-*H*H), 2.74 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.56 (ddd, 1H, *J* = 2.8, 6.9, 11.5 Hz, 2-*H*H), 2.47 (dd, 1H, *J* = 11.5, 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 170.24 (OC-*C*OO), 165.57 (CH-*C*OO), 165.26 (CH-*C*OO), 151.52 (O*C*OOCH$_2$), 148.71 (*C*$_{Ar}$-OCH$_3$), 148.36 (*C*$_{Ar}$-OCH$_3$), 147.14 (*C*H-C_{Ar}), 146.99 (*C*H-C_{Ar}), 146.82 (C_{Ar}-OH), 146.49 (C_{Ar}-OH), 126.64 (*C*$_{Ar}$-CH), 126.46 (*C*$_{Ar}$-CH), 123.51 ($C_{Ar}H$), 123.30 ($C_{Ar}H$), 114.98 (C_{Ar}-CH=*C*H), 114.80 (C_{Ar}-CH=*C*H), 113.94 ($C_{Ar}H$), 113.62 ($C_{Ar}H$), 109.72 ($C_{Ar}H$), 109.72 ($C_{Ar}H$), 94.00 (CCl_3), 78.88 (C-1), 77.13 (CH_2-CCl_3), 73.89 (C-5), 65.79 (C-3), 64.75 (C-4), 56.08 (CH_3), 55.94 (CH_3), 33.90 (C-6), 33.83 (C-2).

3,4-Di-*O*-feruloyl-1,5-quinide (3,4-diFQL) 32: A quantity of 80 mg (0.11 mmol) of 1-*O*-Troc-3,4-di-*O*-feruloyl-1,5-quinide was suspended in THF (0.75 mL) and an equal volume of glacial

acetic acid was added, followed by addition of 54 mg (0.83 mmol, 7.2 eq.) of Zn powder. The grey suspension was stirred at r.t. for 4 h and then the solvents were removed under reduced pressure. A volume of 15 mL EtOAc was added to the flask and the new suspension was cooled to 0 ºC and extracted with aqueous HCl 0.5M (2x10 mL) followed by brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the pale yellow solid product (60 mg, quantitative yield); mp 125-127 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.65 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.54 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.09 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.01 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.96 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.93 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.91 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.82 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.35 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.17 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.94 (br, 1H, OH), 5.86 (br, 1H, OH), 5.67 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 5.28 (ddd, 1H, *J* = 4.6, 6.9, 11.9 Hz, 3-H), 4.94 (dd, 1H, *J* = 5.0, 5.5 Hz, 5-H), 3.92 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 3.00 (br, 1H, OH), 2.64 (d, 1H, *J* = 11.9 Hz, 6-*H*H), 2.50 (ddd, 1H, *J* = 2.3, 5.5, 11.9 Hz, 6-H*H*), 2.34 (ddd, 1H, *J* = 2.3, 6.9, 11.9 Hz, 2-*H*H), 2.24 (t, 1H, *J* = 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 176.91 (OC-*C*OO), 165.71 (CH-*C*OO), 165.53 (CH-*C*OO), 148.63 (C_{Ar}-OCH_3), 148.27 (C_{Ar}-OCH_3), 146.96 (*C*H-C_{Ar}), 146.92 (*C*H-C_{Ar}), 146.80 (C_{Ar}-OH), 146.26 (C_{Ar}-OH), 126.72 (C_{Ar}-CH), 126.53 (C_{Ar}-CH), 123.44 (C_{Ar}H), 123.27 (C_{Ar}H), 114.97 (C_{Ar}-CH=*C*H), 114.77 (C_{Ar}-CH=*C*H), 114.21 (C_{Ar}H), 113.86 (C_{Ar}H), 109.74 (C_{Ar}H), 109.69 (C_{Ar}H), 74.12 (C-5), 72.13 (C-1), 66.23 (C-3), 64.65 (C-4), 56.09 (CH_3), 55.94 (CH_3), 37.55 (C-6), 37.11 (C-2).

4-*O*-Allylferulic acid 33: A mixture of ferulic acid (1.00 g, 5.15 mmol) and anhydrous potassium carbonate (4.27 g, 30.90 mmol) in acetone (50 mL) was stirred at r.t. for 30 min. To the mixture was added a solution of allyl bromide (1.25 g, 10.30 mmol) in acetone (10 mL) and the entire mixture was refluxed for 48 h. The reaction was cooled to r.t., filtered and the filtrate was dried *in vacuo*. The residue was suspended in ethanol (30 mL) and a NaOH 2M solution (20 mL) was added. The mixture was refluxed for 2 h. The solution was cooled to r.t., poured into a beaker and acidified (pH=2) with 3M HCl. The suspension was stirred at r.t. for 30 min and the solid was filtered off and washed successively with a 1:1 mixture of ethanol/water (200 mL). The solid was dried overnight in vacuum to yield a white powder (1.03 g, 4.38 mmol, 85%); ^{1}H-NMR ($CDCl_3$): δ_H 7.72 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.14 (dd, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 7.08 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.87 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.28 (d, 1H, *J* = 16.0 Hz, C_{Ar}-

CH=C*H*), 6.05 (m, 1H, CH_2=C*H*), 5.41 (d, 1H, *J* = 16.9 Hz, C*H*H=CH), 5.31 (d, 1H, *J* = 10.5 Hz, CH*H*=CH), 4.65 (d, 2H, *J* = 5.5 Hz, C_{Ar}-OCH_2), 3.91 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 169.22 (COOH), 149.96 (*C*$_{Ar}$-OCH_3), 149.52 (*C*$_{Ar}$-OCH_2), 144.66 (*C*H-C_{Ar}), 132.81 (*C*H=CH_2), 127.76 (*C*$_{Ar}$-CH), 122.36 (C_{Ar}H), 118.35 (*C*H_2=CH), 116.58 (C_{Ar}-CH=*C*H), 112.94 (C_{Ar}H), 110.11 (C_{Ar}H), 69.73 (C_{Ar}-O*C*H_2), 55.95 (CH_3).

4-*O*-Allylferuloyl chloride 34: 4-*O*-Allylferulic acid (1.03 g, 4.38 mmol) was added to a solution of 20 mL toluene containing 100 µL of DMF. A volume of 2 mL (2.91 g, 22.93 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for 4 h and the resulting yellow solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under the rotary evaporator to give a yellow solid (1.04 g, 4.12 mmol, 94%); ^{1}H-NMR ($CDCl_3$): δ_H 7.76 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.14 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.06 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.89 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.49 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 6.07 (m, 1H, CH_2=C*H*), 5.42 (d, 1H, *J* = 17.4 Hz, C*H*H=CH), 5.32 (d, 1H, *J* = 10.5 Hz, CH*H*=CH), 4.66 (d, 2H, *J* = 5.5 Hz, C_{Ar}-OCH_2), 3.92 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 166.12 (COCl), 151.80 (*C*$_{Ar}$-OCH_3), 150.91 (*C*$_{Ar}$-OCH_2), 149.83 (*C*H-C_{Ar}), 132.45 (*C*H=CH_2), 126.22 (*C*$_{Ar}$-CH), 124.46 (C_{Ar}H), 119.85 (*C*H_2=CH), 118.81 (C_{Ar}-CH=*C*H), 112.85 (C_{Ar}H), 110.61 (C_{Ar}H), 69.84 (C_{Ar}-O*C*H_2), 56.10 (CH_3).

Methyl 1-*O*-Troc-3,4-di-*O*-feruloylquinate 35: To a solution of 450 mg (0.58 mmol) of 1-*O*-Troc-3,4-di-*O*-(4'-*O*-allylferuloyl)-1,5-quinide in 30 mL of aqueous MeOH (90%), a quantity of 23 mg (0.11 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (225 mg) was slowly added at r.t. and it was then heated to 80 ºC for 48 h. The mixture was cooled to r.t., filtered and MeOH removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x25 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (30-40% EtOAc/petroleum ether) to give methyl 1-*O*-Troc-3,4-di-*O*-feruloylquinate as a pale yellow powder (203 mg, 0.28 mmol, 48%); mp 120-121 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.61 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.57 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.05 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.98 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.97 (dd, 1H, *J* = 1.8, 8.2 Hz,

$C_{Ar}H$), 6.92 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.88 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.83 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.27 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.22 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.97 (br, 2H, 2xOH), 5.72 (q, 1H, *J* = 3.7 Hz, 3-H), 5.03 (dd, 1H, *J* = 3.7, 9.4 Hz, 4-H), 4.76 (d, 1H, *J* = 11.9 Hz, CCl_3-C*H*H), 4.53 (d, 1H, *J* = 11.9 Hz, CCl_3-CH*H*), 4.50 (ddd, 1H, *J* = 4.1, 9.4, 10.1 Hz, 5-H), 3.89 (s, 3H, C_{Ar}-OCH_3), 3.81 (s, 3H, $COOCH_3$), 3.78 (s, 3H, C_{Ar}-OCH_3), 2.85 (m, 1H, 2-*H*H), 2.64 (ddd, 1H, *J* = 2.8, 4.1, 14.0 Hz, 6-*H*H), 2.51 (dd, 1H, *J* = 3.2, 15.8 Hz, 2-H*H*), 2.05 (dd, 1H, *J* = 10.1, 14.0 Hz, 6-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 170.54 (*C*$OOCH_3$), 166.88 (CH-*C*OO), 166.16 (CH-*C*OO), 152.33 (O*C*$OOCH_2$), 148.36 (*C*$_{Ar}$-OCH_3), 148.31 (*C*$_{Ar}$-OCH_3), 146.92 (*C*H-C_{Ar}), 146.82 (*C*H-C_{Ar}), 146.39 (C_{Ar}-OH), 145.95 (C_{Ar}-OH), 126.74 (2x*C*$_{Ar}$-CH), 123.53 ($C_{Ar}H$), 123.40 ($C_{Ar}H$), 114.86 (C_{Ar}-CH=*C*H), 114.82 (C_{Ar}-CH=*C*H and $C_{Ar}H$), 114.35 ($C_{Ar}H$), 109.65 ($C_{Ar}H$), 109.49 ($C_{Ar}H$), 94.27 (CCl_3), 82.70 (C-1), 77.11 (*C*H_2-CCl_3), 75.20 (C-5), 67.73 (C-3), 64.98 (C-4), 56.10 (C_{Ar}-O*C*H_3), 55.95 (C_{Ar}-O*C*H_3), 53.34 (COO*C*H_3), 39.31 (C-6), 32.14 (C-2).

Methyl 3,4-di-*O*-feruloylquinate (3,4-diFQM) 36: A quantity of 109 mg (0.15 mmol) of 1-*O*-Troc-3,4-di-*O*-feruloylquinate was suspended in THF (1.00 mL) and an equal volume of glacial acetic acid was added, followed by addition of 70 mg (1.07 mmol, 7.2 eq.) of Zn powder. The grey suspension was stirred at r.t. for 4 h and then the solvents were removed under reduced pressure. A volume of 15 mL EtOAc was added to the flask and the new suspension was cooled to 0 ºC and extracted with aqueous HCl 0.5M (2x10 mL) followed by brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the pale yellow solid product (83 mg, quantitative yield); mp 121-123 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.62 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.61 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.05 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.01 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.00 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.96 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.89 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.86 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.31 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.27 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.90 (br, 2H, 2xOH), 5.66 (m, 1H, 3-H), 5.02 (dd, 1H, *J* = 3.7, 9.9 Hz, 4-H), 4.51 (ddd, 1H, *J* = 4.6, 9.9, 10.3 Hz, 5-H), 3.91 (s, 3H, C_{Ar}-OCH_3), 3.85 (s, 3H, $COOCH_3$), 3.83 (s, 3H, C_{Ar}-OCH_3), 3.28 (br, 1H, OH), 2.30 (dd, 1H, *J* = 3.2, 15.1 Hz, 2-*H*H), 2.29 (m, 1H, 6-*H*H), 2.21 (m, 1H, 2-H*H*), 2.11 (m, 1H, 6-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 175.37 (*C*$OOCH_3$), 167.00 (CH-*C*OO), 166.41 (CH-*C*OO), 148.27 (*C*$_{Ar}$-OCH_3), 148.24 (*C*$_{Ar}$-OCH_3), 146.87 (*C*H-C_{Ar}), 146.81 (*C*H-C_{Ar}), 146.26 (C_{Ar}-OH), 145.89 (C_{Ar}-OH),

126.91 (C_{Ar}-CH), 126.82 (C_{Ar}-CH), 123.45 (2xC_{Ar}H), 115.07 (C_{Ar}-CH=*C*H), 114.79 (C_{Ar}-CH=*C*H and C_{Ar}H), 114.57 (C_{Ar}H), 109.57 (C_{Ar}H), 109.47 (C_{Ar}H), 75.73 (C-1), 74.50 (C-5), 68.96 (C-3), 65.38 (C-4), 56.09 (C_{Ar}-O*C*H$_3$), 55.99 (C_{Ar}-O*C*H$_3$), 53.47 (COO*C*H$_3$), 41.34 (C-6), 36.46 (C-2).

1-*O*-Troc-3,4-di-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide 37: To a solution of 1-*O*-Troc-1,5-quinide (1.77 g, 5.06 mmol) in 100 mL DCM, a quantity of 242 mg (1.98 mmol, 40% mol) DMAP was added and a volume of 20 mL of NEt_3. A quantity of 6.00 g (21.53 mmol) 3,4-di-*O*-allylcaffeoyl chloride was then added and the mixture was refluxed for 72 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 3.08 g (3.69 mmol, 73%) of a pale yellow solid; mp 104-105 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.64 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.55 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.08 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.07 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.98 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.97 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.87 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.79 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.32 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.16 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.13-5.95 (m, 4H, 4xCH_2=C*H*), 5.70 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 5.46-5.22 (m, 9H, 4xCH_2=CH and 3-H), 5.01 (dd, 1H, *J* = 5.0, 6.0 Hz, 5-H), 4.84 (d, 1H, *J* = 11.4 Hz, CCl_3-CH*H*), 4.73 (d, 1H, *J* = 11.4 Hz, CCl_3-C*H*H), 4.66-4.59 (m, 6H, 3xC_{Ar}-OCH_2), 4.51 (d, 2H, *J* = 5.5 Hz, C_{Ar}-OCH_2), 3.20 (ddd, 1H, *J* = 2.8, 6.0, 11.5 Hz, 6-*H*H), 2.73 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.56 (ddd, 1H, *J* = 2.8, 6.9, 11.9 Hz, 2-*H*H), 2.47 (dd, 1H, *J* = 11.5, 11.9 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 170.19 (OC-*C*OO), 165.49 (CH-*C*OO), 165.22 (CH-*C*OO), 151.52 (C_{Ar}-OCH_2), 151.35 (C_{Ar}-OCH_2), 151.01 (O*C*OOCH_2), 148.75 (C_{Ar}-OCH_2), 148.60 (C_{Ar}-OCH_2), 146.86 (*C*H-C_{Ar}), 146.30 (*C*H-C_{Ar}), 133.05 (*C*H=CH_2), 133.00 (*C*H=CH_2), 132.87 (*C*H=CH_2), 132.78 (*C*H=CH_2), 127.22 (C_{Ar}-CH), 126.99 (C_{Ar}-CH), 123.36 (C_{Ar}H), 123.05 (C_{Ar}H), 118.20 (*C*H_2=CH), 118.13 (*C*H_2=CH), 118.11 (*C*H_2=CH), 117.99 (*C*H_2=CH), 113.96 (C_{Ar}-CH=*C*H), 113.41 (C_{Ar}-CH=*C*H), 112.88 (C_{Ar}H), 112.78 (C_{Ar}H), 111.38 (2xC_{Ar}H), 94.00 (CCl_3), 78.88 (C-1), 77.32 (*C*H_2-CCl_3), 73.86 (C-5), 70.10 (C_{Ar}-O*C*H_2), 69.98 (C_{Ar}-O*C*H_2), 69.78 (2xC_{Ar}-O*C*H_2), 65.79 (C-3), 64.78 (C-4), 33.89 (C-6), 33.84 (C-2).

Methyl 1-*O*-Troc-3,4-di-*O*-caffeoylquinate 38: To a solution of 833 mg (1.00 mmol) of 1-*O*-Troc-3,4-di-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide in 50 mL of aqueous MeOH (90%), a quantity of 76 mg (0.40 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (781 mg) was slowly added at r.t. and it was then heated to 80 ºC for 48 h. The mixture was cooled to r.t., filtered and the solvents removed *in vacuo*. The crude product was purified by column chromatography on silica gel (n-heptane/acetone/MeOH = 60/35/5) to give methyl 1-*O*-Troc-3,4-di-*O*-caffeoylquinate as a pale yellow powder (212 mg, 0.30 mmol, 30%); mp 128-130 ºC; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 8.30 (br, 4H, 4xOH), 7.57 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.50 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.12 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 7.11 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 7.02 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.94 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.79 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.23 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.22 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.67 (q, 1H, *J* = 3.7 Hz, 3-H), 5.05 (dd, 1H, *J* = 3.7, 9.6 Hz, 4-H), 4.93 (d, 1H, *J* = 12.4 Hz, CCl_3-C*H*H), 4.63 (d, 1H, *J* = 12.4 Hz, CCl_3-CH*H*), 4.42 (ddd, 1H, *J* = 4.1, 9.6, 10.1 Hz, 5-H), 3.73 (s, 3H, CH_3), 3.11 (br, 1H, OH), 2.73 (ddd, 1H, *J* = 2.8, 3.7, 15.6 Hz, 2-*H*H), 2.63 (dd, 1H, *J* = 3.7, 15.6 Hz, 2-H*H*), 2.54 (ddd, 1H, *J* = 2.8, 4.1, 13.7 Hz, 6-*H*H), 2.04-2.13 (dd, 1H, *J* = 10.1, 13.7 Hz, 6-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 170.08 (*C*OOCH_3), 166.07 (CH-*C*OO), 165.60 (CH-*C*OO), 152.37 (O*C*OOCH_2), 148.08 (C_{Ar}-OH), 148.02 (C_{Ar}-OH), 145.54 (2x*C*H-C_{Ar}), 145.47 (C_{Ar}-OH), 145.33 (C_{Ar}-OH), 126.71 (*C*$_{Ar}$-CH), 126.67 (*C*$_{Ar}$-CH), 121.95 (2xC_{Ar}H), 115.58 (C_{Ar}H), 115.54 (C_{Ar}H), 114.56 (2xC_{Ar}-CH=*C*H), 114.52 (C_{Ar}H), 114.27 (C_{Ar}H), 94.64 (CCl_3), 82.97 (C-1), 76.45 (*C*H_2-CCl_3), 74.70 (C-5), 68.02 (C-3), 64.04 (C-4), 52.45 (CH_3), 39.50 (C-6), 31.94 (C-2).

Methyl 3,4-di-*O*-caffeoylquinate (3,4-diCQM) 39: A quantity of 100 mg (0.14 mmol) of methyl 1-*O*-Troc-3,4-di-*O*-caffeoylquinate was suspended in THF (1.00 mL) and an equal volume of glacial acetic acid was added, followed by addition of 67 mg (1.02 mmol, 7.2 eq.) of Zn powder. The grey suspension was stirred at r.t. for 4 h and then the solvents were removed under reduced pressure. A volume of 20 mL EtOAc was added to the flask and the new suspension was cooled to 0 ºC and extracted with aqueous HCl 0.5M (2x10 mL) followed by brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the pale yellow solid product (75 mg, quantitative yield); mp 131-133 ºC; ^{1}H-NMR (CD_3OD): δ_H 7.53 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.52 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.01 (d, 1H,

J = 2.3 Hz, $C_{Ar}H$), 7.00 (d, 1H, *J* = 2.3 Hz, $C_{Ar}H$), 6.88 (dd, 1H, *J* = 2.3, 8.2 Hz, $C_{Ar}H$), 6.86 (dd, 1H, *J* = 2.3, 8.2 Hz, $C_{Ar}H$), 6.73 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.71 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.24 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.23 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.60 (m, 1H, 3-H), 5.01 (dd, 1H, *J* = 3.7, 8.4 Hz, 4-H), 4.30 (m, 1H, 5-H), 3.73 (s, 3H, CH_3), 2.33 (dd, 1H, *J* = 3.7, 14.7 Hz, 2-*H*H), 2.19-2.06 (m, 3H, 6-*H*H, 2-H*H* and 6-H*H*); ^{13}C-NMR (CD_3OD): δ_C 174.81 (*C*OOCH$_3$), 167.19 (CH-*C*OO), 167.12 (CH-*C*OO), 148.31 (C_{Ar}-OH), 148.27 (C_{Ar}-OH), 146.04 (2x*C*H-C_{Ar}), 145.45 (2xC_{Ar}-OH), 126.42 (*C*$_{Ar}$-CH), 126.34 (*C*$_{Ar}$-CH), 121.92 ($C_{Ar}H$), 121.81 ($C_{Ar}H$), 115.14 (2xC_{Ar}-CH=*C*H), 113.84 ($C_{Ar}H$), 113.74 ($C_{Ar}H$), 113.66 ($C_{Ar}H$), 113.53 ($C_{Ar}H$), 74.29 (C-1), 73.85 (C-5), 68.52 (C-3), 64.71 (C-4), 51.65 (CH_3), 40.01 (C-6), 35.48 (C-2).

Methyl BBA-quinate 40: 2,3-Butadione (20 mL, 228 mmol), trimethylorthoformate (56 mL, 512 mmol) and D-camphorsulfonic acid (1.20 g, 5.17 mmol) were added to a suspension of quinic acid (20.00 g, 104 mmol) in MeOH (160 mL). The mixture was heated to vigorous reflux for 18 h, left to cool to r.t. and treated with $NaHCO_3$ (800 mg, 9.52 mmol). The solvent was removed under reduced pressure to give a paste, which was dissolved in EtOAc. Activated charcoal was added and the mixture was heated to reflux for 2 h then left to cool down to r.t.; the mixture was filtered over a thick pad of silica gel, which was further washed using EtOAc/MeOH (9:1) and the resulting colorless filtrate was evaporated *in vacuo* to give a white solid. The crude product was recrystallized from EtOAc to yield the title compound as white needles (27.40 g, 89.44 mmol, 86%); mp 137-139 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 4.12 (ddd, 1H, *J* = 4.6, 10.1, 12.4 Hz, 5-H), 4.01 (q, 1H, *J* = 2.8 Hz, 3-H), 3.60 (s, 3H, COOCH$_3$), 3.41 (dd, 1H, *J* = 2.8, 10.1 Hz, 4-H), 3.30 (br, 1H, OH), 3.13 (br, 1H, OH), 3.08 (s, 3H, C-OCH$_3$), 3.07 (s, 3H, C-OCH$_3$), 2.00 (m, 1H, 2-*H*H), 1.92 (ddd, 1H, *J* = 2.8, 4.6, 12.4 Hz, 6-*H*H), 1.86 (dd, 1H, *J* = 3.2, 15.1 Hz, 2-H*H*), 1.74 (t, 1H, *J* = 12.4 Hz, 6-H*H*), 1.16 (s, 3H, CH$_3$-C), 1.12 (s, 3H, CH$_3$-C); ^{13}C-NMR ($CDCl_3$): δ_C 174.26 (*C*OOCH$_3$), 100.30 (CH$_3$-*C*), 99.71 (CH$_3$-*C*), 75.73 (C-1), 72.69 (C-4), 69.13 (C-5), 62.38 (C-3), 52.89 (COO*C*H$_3$), 47.87 (2xC-O*C*H$_3$), 38.59 (C-6), 37.33 (C-2), 17.76 (*C*H$_3$-C), 17.61 (*C*H$_3$-C).

BBA-quinic acid 41: A quantity of 2.00 g (6.24 mmol) of methyl BBA-quinate was dissolved in 30 mL THF and a volume of 50 mL KOH 1M was added. The reaction mixture was stirred for 2 h at r.t., then acidified to pH=2 with HCl 2M. The aqueous layer was extracted with EtOAc

(3x40 mL), the combined organic layers were dried over Na_2SO_4, filtered and the solvent was evaporated *in vacuo* to afford the white product (1.33 g, 4.35 mmol, 70%); ^{1}H-NMR ($(CD_3)_2SO$): δ_H 12.60 (br, 1H, COOH), 5.52 (br, 1H, OH), 4.69 (br, 1H, OH), 4.06 (ddd, 1H, *J* = 4.6, 10.1, 12.4 Hz, 5-H), 3.91 (q, 1H, *J* = 2.8 Hz, 3-H), 3.32 (dd, 1H, *J* = 2.8, 10.1 Hz, 4-H), 3.10 (s, 6H, 2xC-OCH_3), 1.89 (dd, 1H, *J* = 4.6, 12.4 Hz, 6-*H*H), 1.88-1.83 (m, 2H, 2-*H*H and 2-H*H*), 1.61 (t, 1H, *J* = 12.4 Hz, 6-H*H*), 1.17 (s, 3H, CH_3-C), 1.13 (s, 3H, CH_3-C); ^{13}C-NMR ($(CD_3)_2SO$): δ_C 175.70 (COOH), 99.83 (CH_3-*C*), 99.38 (CH_3-*C*), 75.48 (C-1), 73.22 (C-4), 69.30 (C-5), 62.96 (C-3), 47.79 (C-O*C*H_3), 47.76 (C-O*C*H_3), 38.73 (C-6), 38.29 (C-2), 18.22 (2x*C*H_3-C).

1,3-Di-*O*-(3',4'-dimethoxycinnamoyl)-BBA-quinic acid 42: To a solution of BBA-quinic acid (1.25 g, 4.07 mmol) in 100 mL DCM, a quantity of 150 mg (1.22 mmol, 30% mol) DMAP was added and a volume of 24 mL of NEt_3. A quantity of 3.23 g (14.26 mmol) 3,4-dimethoxycinnamoyl chloride was then added and the mixture was refluxed for 48 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 2.01 g (2.93 mmol, 72%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.51 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.49 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 6.81-6.70 (m, 4H, 4xC_{Ar}H), 6.53 (d, 1H, *J* = 7.8 Hz, C_{Ar}H), 6.51 (d, 1H, *J* = 7.8 Hz, C_{Ar}H), 6.17 (d, 2H, *J* = 16.0 Hz, 2xC_{Ar}-CH=C*H*), 5.37 (m, 1H, 3-H), 4.41 (m, 1H, 5-H), 3.82 (s, 6H, 2xC_{Ar}-OCH_3), 3.74 (m, 1H, 4-H), 3.69 (s, 3H, C_{Ar}-OCH_3), 3.65 (s, 3H, C_{Ar}-OCH_3), 3.32 (s, 3H, C-OCH_3), 3.21 (s, 3H, C-OCH_3), 2.99 (m, 1H, 2-*H*H), 2.49 (m, 1H, 6-*H*H), 2.24 (m, 1H, 2-H*H*), 1.96 (dd, 1H, *J* = 11.5, 12.4 Hz, 6-H*H*), 1.27 (s, 3H, CH_3-C), 1.25 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 175.55 (COOH), 166.69 (CH-*C*OO), 165.83 (CH-*C*OO), 151.16 (*C*$_{Ar}$-OCH_3), 150.82 (*C*$_{Ar}$-OCH_3), 149.07 (*C*$_{Ar}$-OCH_3), 148.94 (*C*$_{Ar}$-OCH_3), 145.91 (*C*H-C_{Ar}), 144.82 (*C*H-C_{Ar}), 127.39 (*C*$_{Ar}$-CH), 127.04 (*C*$_{Ar}$-CH), 122.71 (C_{Ar}H), 122.14 (C_{Ar}H), 116.44 (C_{Ar}-CH=*C*H), 115.45 (C_{Ar}-CH=*C*H), 110.78 (C_{Ar}H), 110.73 (C_{Ar}H), 110.08 (C_{Ar}H), 109.72 (C_{Ar}H), 100.21 (CH_3-*C*), 99.87 (CH_3-*C*), 79.86 (C-1), 70.96 (C-4), 69.19 (C-5), 63.10 (C-3), 55.94 (C_{Ar}-O*C*H_3), 55.85 (C_{Ar}-O*C*H_3), 55.79 (C_{Ar}-O*C*H_3), 55.71 (C_{Ar}-O*C*H_3), 48.07 (C-O*C*H_3), 48.01 (C-O*C*H_3), 36.83 (C-6), 32.49 (C-2), 17.95 (*C*H_3-C), 17.74 (*C*H_3-C).

1,3-Di-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid (1,3-diDQA) 43: A quantity of 717 mg (1.04 mmol) of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-BBA-quinic acid and a volume of 10 mL TFA 80% solution were cooled to 0 ºC. The acid solution was then added drop-wise at the same temperature to the quinide and all the solids dissolved after a few minutes. The ice bath was removed and the reaction was stirred for 40 min at r.t. The solvents were removed *in vacuo* to afford the title compound in quantitative yield as a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.51 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.45 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 6.81-6.60 (m, 4H, 4xC_{Ar}H), 6.51 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.39 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.19 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 6.07 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 5.60-5.05 (br, 2H, 2xOH), 5.44 (m, 1H, 3-H), 4.36 (m, 1H, 5-H), 3.81 (m, 1H, 4-H), 3.77 (s, 3H, C_{Ar}-OCH_3), 3.74 (s, 3H, C_{Ar}-OCH_3), 3.67 (s, 3H, C_{Ar}-OCH_3), 3.59 (s, 3H, C_{Ar}-OCH_3), 2.91 (m, 1H, 2-*H*H), 2.66 (m, 1H, 6-*H*H), 2.36 (m, 1H, 2-H*H*), 2.00 (m, 1H, 6-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 174.17 (COOH), 167.36 (CH-*C*OO), 166.29 (CH-*C*OO), 151.30 (*C*$_{Ar}$-OCH_3), 151.04 (*C*$_{Ar}$-OCH_3), 149.04 (*C*$_{Ar}$-OCH_3), 148.86 (*C*$_{Ar}$-OCH_3), 146.46 (*C*H-C_{Ar}), 145.94 (*C*H-C_{Ar}), 129.12 (*C*$_{Ar}$-CH), 128.31 (*C*$_{Ar}$-CH), 122.97 (C_{Ar}H), 122.17 (C_{Ar}H), 115.18 (C_{Ar}-CH=*C*H), 114.89 (C_{Ar}-CH=*C*H), 110.77 (C_{Ar}H), 110.70 (C_{Ar}H), 110.31 (C_{Ar}H), 109.88 (C_{Ar}H), 79.52 (C-1), 74.27 (C-4), 71.38 (C-5), 67.34 (C-3), 55.83 (C_{Ar}-O*C*H_3), 55.77 (2xC_{Ar}-O*C*H_3), 55.65 (C_{Ar}-O*C*H_3), 32.01 (C-2 and C-6).

1,3-Di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide (1,3-diDQL) 44: A quantity of 350 mg (0.61 mmol) of 1,3-di-*O*-(3',4'-dimethoxycinnamoyl)-quinic acid was dissolved in 10 mL toluene and 2.34 mg (0.01 mmol) of PTSA·H_2O was added. The reaction was refluxed for 48 h, was cooled to r.t. and neutralized with solid $NaHCO_3$ (3 mg, 0.03 mmol). Toluene was removed under reduced pressure, the solid residue was dissolved in EtOAc and filtered. The filtrate was concentrated *in vacuo* and the crude product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 108 mg (0.20 mmol, 32%) of a white powder; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.66 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.65 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.35 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.31 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.21 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.19 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.98 (d, 2H, *J* = 8.2 Hz, C_{Ar}H), 6.46 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.42 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.01 (ddd, 1H, *J* = 4.1, 4.6, 11.5 Hz, 3-H), 4.93 (dd, 1H, *J* = 5.0, 6.4 Hz, 5-H), 4.42 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 3.87 (s, 3H, C_{Ar}-OCH_3), 3.86 (s, 3H, C_{Ar}-OCH_3), 3.84 (s, 6H, 2xC_{Ar}-OCH_3), 3.11 (ddd, 1H, *J* = 2.8, 6.4, 11.5 Hz,

6-*H*H), 2.74 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.44 (t, 1H, *J* = 11.5 Hz, 2-*H*H), 2.29 (ddd, 1H, *J* = 2.8, 4.1, 11.5 Hz, 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.11 (OC-*C*OO), 165.42 (CH-*C*OO), 165.27 (CH-*C*OO), 151.65 (C_{Ar}-OCH_3), 151.62 (C_{Ar}-OCH_3), 149.34 (2xC_{Ar}-OCH_3), 146.78 (*C*H-C_{Ar}), 146.57 (*C*H-C_{Ar}), 127.07 (C_{Ar}-CH), 126.97 (C_{Ar}-CH), 123.19 (C_{Ar}H), 123.12 (C_{Ar}H), 114.47 (C_{Ar}-CH=*C*H), 114.21 (C_{Ar}-CH=*C*H), 111.12 (2xC_{Ar}H), 109.75 (2xC_{Ar}H), 76.49 (C-1), 76.02 (C-5), 68.38 (C-4), 64.71 (C-3), 56.08 (2xC_{Ar}-O*C*H_3), 56.00 (2xC_{Ar}-O*C*H_3), 33.56 (C-2), 33.41 (C-6).

1,3-Di-*O*-(3',4'-di-*O*-allylcaffeoyl)-BBA-quinic acid 45: To a solution of BBA-quinic acid (1.86 g, 6.08 mmol) in 100 mL DCM, a quantity of 223 mg (1.82 mmol, 30% mol) DMAP was added and a volume of 32 mL of NEt_3. A quantity of 5.08 g (18.23 mmol) 3,4-di-*O*-allylcaffeoyl chloride was then added and the mixture was refluxed for 48 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 3.02 g (3.82 mmol, 63%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.51 (d, 2H, *J* = 16.0 Hz, 2xC_{Ar}-CH), 6.84 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.81 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.74 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.70 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.59 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.55 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.17 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.16 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.10-5.93 (m, 4H, 4xCH_2=C*H*), 5.45-5.22 (m, 9H, 4x*C*H_2=CH and 3-H), 4.61 (m, 1H, 5-H), 4.56 (m, 4H, 2xC_{Ar}-OCH_2), 4.46-4.31 (m, 4H, 2xC_{Ar}-OCH_2), 3.74 (dd, 1H, *J* = 3.7, 10.1 Hz, 4-H), 3.34 (s, 3H, C-OCH_3), 3.25 (s, 3H, C-OCH_3), 2.99 (dt, 1H, *J* = 2.8, 16.0 Hz, 2-*H*H), 2.54 (ddd, 1H, *J* = 2.8, 4.1, 13.3 Hz, 6-*H*H), 2.20 (dd, 1H, *J* = 3.2, 16.0 Hz, 2-H*H*), 1.96 (dd, 1H, *J* = 12.4, 13.3 Hz, 6-H*H*), 1.27 (s, 3H, CH_3-C), 1.25 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 175.11 (COOH), 166.68 (CH-*C*OO), 165.54 (CH-*C*OO), 150.84 (C_{Ar}-OCH_2), 150.41 (C_{Ar}-OCH_2), 148.47 (C_{Ar}-OCH_2), 148.35 (C_{Ar}-OCH_2), 146.05 (*C*H-C_{Ar}), 144.97 (*C*H-C_{Ar}), 133.10 (*C*H=CH_2), 133.06 (*C*H=CH_2), 133.00 (*C*H=CH_2), 132.92 (*C*H=CH_2), 127.45 (C_{Ar}-CH), 127.01 (C_{Ar}-CH), 122.75 (C_{Ar}H), 122.07 (C_{Ar}H), 118.04 (*C*H_2=CH), 117.94 (2x*C*H_2=CH), 117.84 (*C*H_2=CH), 116.33 (C_{Ar}-CH=*C*H), 115.13 (C_{Ar}-CH=*C*H), 113.02 (C_{Ar}H), 112.98 (C_{Ar}H), 112.91 (C_{Ar}H), 112.40 (C_{Ar}H), 100.27 (CH_3-*C*), 99.90 (CH_3-*C*), 79.21 (C-1), 70.97 (C-4), 69.71 (2xC_{Ar}-O*C*H_2), 69.62

(2xC_{Ar}-O*C*H_2), 68.90 (C-5), 62.94 (C-3), 48.09 (2xC-O*C*H_3), 36.68 (C-6), 32.57 (C-2), 17.95 (*C*H_3-C), 17.72 (*C*H_3-C).

1,3-Di-*O*-caffeoyl-BBA-quinic acid 46: To a solution of 575 mg (0.73 mmol) of 1,3-di-*O*-(3',4'-di-*O*-allylcaffeoyl)-BBA-quinic acid in 40 mL of aqueous 1,4-dioxane (90%), a quantity of 45 mg (0.24 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (425 mg) was slowly added at r.t. and it was then heated to 65 ºC for 48 h. The mixture was cooled to r.t., filtered and dioxane removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (30-40% EtOAc/petroleum ether) to give a pale yellow powder (142 mg, 0.22 mmol, 31%); ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.51 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.49 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.07 (d, 1H, *J* = 1.4 Hz, C_{Ar}H), 6.96 (d, 1H, *J* = 1.4 Hz, C_{Ar}H), 6.83 (dd, 1H, *J* = 1.4, 8.2 Hz, C_{Ar}H), 6.73 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.68 (dd, 1H, *J* = 1.4, 8.2 Hz, C_{Ar}H), 6.64 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.23 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.13 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.39 (m, 1H, 3-H), 4.61 (m, 1H, 5-H), 3.74 (dd, 1H, *J* = 3.2, 10.1 Hz, 4-H), 3.31 (s, 3H, C-OCH_3), 3.21 (s, 3H, C-OCH_3), 2.99 (m, 1H, 2-*H*H), 2.43 (m, 1H, 6-*H*H), 2.34 (m, 1H, 2-H*H*), 1.87 (dd, 1H, *J* = 12.4, 12.8 Hz, 6-H*H*), 1.23 (s, 3H, CH_3-C), 1.19 (s, 3H, CH_3-C); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 171.81 (COOH), 166.01 (CH-*C*OO), 165.26 (CH-*C*OO), 148.25 (*C*$_{Ar}$-OH), 147.84 (*C*$_{Ar}$-OH), 145.81 (*C*$_{Ar}$-OH), 145.53 (*C*$_{Ar}$-OH), 145.30 (*C*H-C_{Ar}), 145.26 (*C*H-C_{Ar}), 126.50 (*C*$_{Ar}$-CH), 126.35 (*C*$_{Ar}$-CH), 121.59 (C_{Ar}H), 120.65 (C_{Ar}H), 115.82 (2xC_{Ar}-CH=*C*H), 115.44 (C_{Ar}H), 114.99 (C_{Ar}H), 114.89 (C_{Ar}H), 114.64 (C_{Ar}H), 99.87 (CH_3-*C*), 99.50 (CH_3-*C*), 79.35 (C-1), 71.01 (C-4), 68.84 (C-5), 62.79 (C-3), 47.23 (C-O*C*H_3), 47.18 (C-O*C*H_3), 37.01 (C-6), 32.50 (C-2), 17.34 (*C*H_3-C), 17.23 (*C*H_3-C).

1,3-Di-*O*-caffeoylquinic acid (1,3-diCQA, cynarine) 47: A quantity of 39 mg (0.06 mmol) of 1,3-di-*O*-caffeoyl-BBA-quinic acid and a volume of 5 mL TFA 80% solution were cooled to 0 ºC. The acid solution was then added drop-wise at the same temperature to the quinide and all the solids dissolved after a few minutes. The ice bath was removed and the reaction was stirred for 40 min at r.t. The solvents were removed *in vacuo* to afford the title compound in quantitative yield as a pale yellow powder; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.53 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.46

(d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.10 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 6.97 (d, 1H, *J* = 2.3 Hz, C_{Ar}H), 6.91 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.75 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.71 (dd, 1H, *J* = 2.3, 8.2 Hz, C_{Ar}H), 6.63 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.27 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.15 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 5.42 (dt, 1H, *J* = 3.2, 3.6 Hz, 3-H), 4.24 (m, 1H, 5-H), 3.66 (dd, 1H, *J* = 3.6, 9.2 Hz, 4-H), 2.77 (dt, 1H, *J* = 3.2, 15.6 Hz, 2-*H*H), 2.50 (ddd, 1H, *J* = 3.2, 3.6, 12.8 Hz, 6-*H*H), 2.33 (dd, 1H, *J* = 3.2, 15.6 Hz, 2-H*H*), 1.85 (m, 1H, 6-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.10 (COOH), 166.23 (CH-*C*OO), 165.44 (CH-*C*OO), 148.11 (*C*$_{Ar}$-OH), 147.71 (*C*$_{Ar}$-OH), 145.68 (*C*H-C_{Ar}), 145.44 (*C*$_{Ar}$-OH), 145.21 (*C*$_{Ar}$-OH), 144.90 (*C*H-C_{Ar}), 126.56 (*C*$_{Ar}$-CH), 126.50 (*C*$_{Ar}$-CH), 121.63 (C_{Ar}H), 120.71 (C_{Ar}H), 115.67 (C_{Ar}-CH=*C*H), 115.65 (C_{Ar}-CH=*C*H), 115.27 (C_{Ar}H), 115.17 (C_{Ar}H), 114.82 (C_{Ar}H), 114.75 (C_{Ar}H), 79.49 (C-1), 74.21 (C-4), 70.89 (C-5), 66.81 (C-3), 39.54 (C-6), 32.15 (C-2).

1,5-Quinide 49: A quantity of 500 mg (2.33 mmol) of 3,4-*O*-isopropylidene-1,5-quinide and a solution of TFA 90% (20 mL) were cooled to 0 ºC. The acid solution was then added drop-wise at the same temperature to the quinide. The ice bath was removed and the reaction was stirred for 45 min at r.t., then the solvents were removed in vacuum to give the white product in quantitative yield; mp 184 ºC; ^{1}H-NMR (CD_3OD): δ_H 4.68 (dd, 1H, *J* = 5.0, 6.0 Hz, 5-H), 3.96 (dd, 1H, *J* = 4.6, 5.0 Hz, 4-H), 3.68 (ddd, 1H, *J* = 4.6, 6.9, 11.5 Hz, 3-H), 2.45 (d, 1H, *J* = 11.5 Hz, 6-*H*H), 2.20 (ddd, 1H, *J* = 2.8, 6.0, 11.5 Hz, 6-H*H*), 2.01 (ddd, 1H, *J* = 2.8, 6.9, 11.5 Hz, 2-*H*H), 1.85 (t, 1H, *J* = 11.5 Hz, 2-H*H*); ^{13}C-NMR (D_2O): δ_C 177.69 (COO), 75.56 (C-5), 74.75 (C-1), 69.95 (C-4), 66.30 (C-3), 40.14 (C-2), 36.68 (C-6).

1,3,4-Tri-*O*-(3',4'-di-*O*-allylcaffeoyl)-1,5-quinide 50: To a solution of 1,5-quinide (321 mg, 1.84 mmol) in 80 mL DCM, a quantity of 135 mg (1.10 mmol, 3x20% mol) DMAP and a volume of 20 mL of NEt_3 were added. A quantity of 3.08 g (11.06 mmol) 3,4-di-*O*-allylcaffeoyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 513 mg (0.57 mmol, 31%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.65 (d, 2H, *J* = 15.8 Hz, 2xC_{Ar}-CH), 7.53 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH), 7.08 (d, 2H, *J* = 1.8 Hz, 2xC_{Ar}H), 7.07 (dd, 2H, *J* = 1.8,

8.7 Hz, 2xC_{Ar}H), 6.97 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.96 (d, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 6.86 (d, 2H, *J* = 8.7 Hz, 2xC_{Ar}H), 6.77 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.35 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH=C*H*), 6.29 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH=C*H*), 6.16 (d, 1H, *J* = 15.8 Hz, C_{Ar}-CH=C*H*), 6.03 (m, 6H, 6xCH_2=C*H*), 5.70 (dd, 1H, *J* = 5.0, 6.9 Hz, 4-H), 5.46-5.21 (m, 13H, 6xCH_2=CH and 3-H), 5.00 (dd, 1H, *J* = 5.0, 5.5 Hz, 5-H), 4.63 (m, 8H, *J* = 5.3 Hz, 4xC_{Ar}-OCH_2), 4.59 (d, 2H, *J* = 5.3 Hz, C_{Ar}-OCH_2), 4.50 (d, 2H, *J* = 5.3 Hz, C_{Ar}-OCH_2), 3.14 (ddd, 1H, *J* = 2.3, 5.5, 11.7 Hz, 6-*H*H), 2.84 (d, 1H, *J* = 11.7 Hz, 6-H*H*), 2.60 (t, 1H, *J* = 11.7 Hz, 2-*H*H), 2.49 (ddd, 1H, *J* = 2.3, 6.7, 11.7 Hz, 2-H*H*); ^{13}C-NMR ($CDCl_3$): δ_C 171.61 (OC-*C*OO), 165.63 (CH-*C*OO), 165.38 (CH-*C*OO), 165.28 (CH-*C*OO), 151.25 (C_{Ar}-OCH_2), 151.22 (C_{Ar}-OCH_2), 150.91 (C_{Ar}-OCH_2), 148.72 (C_{Ar}-OCH_2), 148.65 (C_{Ar}-OCH_2), 148.56 (C_{Ar}-OCH_2), 146.94 (*C*H-C_{Ar}), 146.74 (*C*H-C_{Ar}), 146.07 (*C*H-C_{Ar}), 133.06 (2x*C*H=CH_2), 133.00 (*C*H=CH_2), 132.87 (2x*C*H=CH_2), 132.81 (*C*H=CH_2), 127.25 (C_{Ar}-CH), 127.11 (C_{Ar}-CH), 127.06 (C_{Ar}-CH), 123.39 (C_{Ar}), 123.32 (C_{Ar}), 122.99 (C_{Ar}), 118.17 (2x*C*H_2=CH), 118.11 (3x*C*H_2=CH), 117.99 (*C*H_2=CH), 114.52 (C_{Ar}-CH=*C*H), 114.37 (C_{Ar}-CH=*C*H), 114.18 (C_{Ar}-CH=*C*H), 113.36 (2xC_{Ar}H), 113.28 (C_{Ar}H), 112.81 (C_{Ar}H), 112.76 (C_{Ar}H), 112.60 (C_{Ar}H), 76.83 (C-1), 74.09 (C-5), 70.04 (2xC_{Ar}-O*C*H_2), 69.93 (C_{Ar}-O*C*H_2), 69.77 (3xC_{Ar}-O*C*H_2), 66.07 (C-3), 64.99 (C-4), 34.74 (C-6), 33.98 (C-2).

3,4-*O*-Cyclohexylidene-1,5-quinide 52: Quantities of 10.00 g (52.04 mmol) of quinic acid and 200 mg (1.05 mmol) of *p*-toluenesulfonic acid monohydrate (PTSA·H_2O) were added to 100 mL of cyclohexanone to give a white suspension. The reaction was then refluxed for 24 h to give a yellow solution, which was cooled to 50 ºC and neutralized with a solution of NaOEt (71.5 mg) in EtOH (5 mL) to give a yellow clear solution. The solvents were removed under reduced pressure and to the resulting yellow viscous liquid a volume of 100 mL of EtOAc was added. The organic phase was washed with 50 mL of H_2O and the aqueous phase was back-extracted with 30 mL EtOAc. The combined organic layers were washed with a half-saturated $NaHCO_3$ solution, dried on Na_2SO_4, filtered and evaporated. The resulting yellow viscous liquid was recrystallized successively from a 1:1 n-heptane:EtOAc solution to afford white crystals of the title compound (9.26 g, 36.43 mmol, 70%); ^{1}H-NMR ($CDCl_3$): δ_H 4.73 (dd, 1H, *J* = 2.8, 6.0 Hz, 5-H), 4.48 (ddd, 1H, *J* = 3.2, 6.4, 7.8 Hz, 3-H), 4.29 (ddd, 1H, *J* = 1.4, 2.8, 6.4 Hz, 4-H), 2.66 (d, 1H, *J* = 11.9 Hz, 6-*H*H), 2.38-2.31 (ddd, 1H, *J* = 2.3, 7.8, 14.7 Hz, 2-*H*H), 2.31-2.25 (dddd, 1H, *J* = 1.4, 2.3, 6.0, 11.9 Hz, 6-H*H*), 2.17 (dd, 1H, *J* = 3.2, 14.7 Hz, 2-H*H*), 1.73-1.36 (m, 10H,

5xC'H_2); ^{13}C-NMR ($CDCl_3$): δ_C 178.92 (COO), 110.71 (C-1'), 76.07 (C-1), 71.84 (C-5), 71.62 (C-4), 71.17 (C-3), 38.56 (C-6), 36.98 (C-2), 34.46 (C-6'), 33.75 (C-2'), 25.09 (C-4'), 24.04 (C-5'), 23.58 (C-3').

Methyl 3,4-*O*-cyclohexylidene-quinate 53: 3,4-*O*-Cyclohexylidene-1,5-quinide (8.75 g, 34.41 mmol) was dissolved in 100 mL MeOH and a 21% solution NaOMe/MeOH was added (187 mg NaOMe). The clear solution was stirred overnight, the mixture was then quenched with glacial acetic acid (232 µL) and the volatile components were removed under vacuum. The resulting mixture was dissolved in EtOAc and washed 3 times (3x40 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (20-50% EtOAc/petroleum ether) to give the product as a white solid (5.87 g, 20.51 mmol, 60%); ^{1}H-NMR ($CDCl_3$): δ_H 4.45 (m, 1H, 3-H), 4.11 (m, 1H, 5-H), 3.97 (dd, 1H, *J* = 6.0, 6.4 Hz, 4-H), 3.79 (s, 3H, CH_3), 2.25 (m, 2H, 2-*H*H and 2-H*H*), 2.08 (ddd, 1H, *J* = 1.8, 4.1, 13.7 Hz, 6-*H*H), 1.86 (dd, 1H, *J* = 11.0, 13.7 Hz, 6-H*H*), 1.74-1.34 (m, 10H, 5xC'H_2); ^{13}C-NMR ($CDCl_3$): δ_C 175.51 (COO), 110.07 (C-1'), 79.41 (C-1), 74.09 (C-4), 73.12 (C-3), 68.66 (C-5), 53.14 (CH_3), 39.01 (C-6), 38.04 (C-2), 34.91 (C-6'), 34.76 (C-2'), 25.05 (C-4'), 24.07 (C-5'), 23.69 (C-3').

Methyl 3,4-*O*-cyclohexylidene-5-oxoquinate 54: To a suspension of Dess-Martin periodinane (2.96 g, 6.99 mmol) in anhydrous CH_2Cl_2 (65 mL) methyl 3,4-*O*-cyclohexylidene-quinate (1.82 g, 6.36 mmol) was added. The reaction mixture was stirred at r.t. for 18 h, was then diluted with Et_2O (100 mL) and with a 1:1 mixture (v/v) of saturated aqueous $Na_2S_2O_3$ and $NaHCO_3$ solution (100 mL). The mixture was stirred until the solids were dissolved (20 min). The aqueous layer was extracted with Et_2O and the combined organic layers were dried over Na_2SO_4, filtered and concentrated. The product (1.81 g, 6.36 mmol, 100%) was used for the next step without further purification; ^{1}H-NMR ($CDCl_3$): δ_H 4.72 (m, 1H, 3-H), 4.41 (d, 1H, *J* = 5.5 Hz, 4-H), 3.81 (s, 3H, CH_3), 3.76 (br, 1H, OH), 2.89 (d, 1H, *J* = 14.7 Hz, 6-*H*H), 2.80 (dd, 1H, *J* = 2.3, 14.7 Hz, 6-H*H*), 2.55 (m, 2H, 2-*H*H and 2-H*H*), 1.72-1.33 (m, 10H, 5xC'H_2); ^{13}C-NMR ($CDCl_3$): δ_C 204.36 (C-5), 172.92 (COO), 111.66 (C-1'), 78.20 (C-1), 76.87 (C-4), 75.94 (C-3), 53.33 (CH_3), 49.12 (C-2), 36.96 (C-6), 35.21 (C-6'), 34.74 (C-2'), 24.89 (C-4'), 23.90 (C-5'), 23.77 (C-3').

Methyl 3,4-*O*-cyclohexylidene-5-*epi*-quinate 55: Methyl 3,4-*O*-cyclohexylidene-5-oxoquinate (1.53 g, 5.33 mmol) was dissolved in a 1:1 mixture (v/v) MeOH/THF (100 mL) and was cooled to -30 ˚C with an acetone/liquid nitrogen bath. $NaBH_4$ (222 mg, 5.86 mmol) was added and the mixture was stirred at -30 ˚C for 1 h. The solvents were removed in vacuum and the residue was extracted three times with a water/EtOAc mixture. The organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The product (1.13 g, 3.94 mmol, 74%) was obtained after purification by column chromatography (40% EtOAc/petroleum ether); ^{1}H-NMR ($CDCl_3$): δ_H 4.52 (dt, 1H, *J* = 5.0, 6.9 Hz, 3-H), 4.30 (dd, 1H, *J* = 4.1, 6.9 Hz, 4-H), 3.90 (dt, 1H, *J* = 4.1, 10.1 Hz, 5-H), 3.78 (s, 3H, CH_3), 2.18-2.06 (m, 3H, 2-*H*H, 2-H*H* and 6-*H*H), 2.02 (dd, 1H, *J* = 10.1, 14.2 Hz, 6-H*H*), 1.77-1.36 (m, 10H, 5xC'H_2); ^{13}C-NMR ($CDCl_3$): δ_C 175.37 (COO), 110.04 (C-1'), 73.82 (C-1), 73.43 (C-4), 72.51 (C-3), 66.08 (C-5), 53.00 (CH_3), 38.15 (C-6), 36.39 (C-2), 35.61 (C-6'), 33.95 (C-2'), 25.15 (C-4'), 24.07 (C-5'), 23.64 (C-3').

5-*epi*-Quinic acid (*cis*-quinic acid) 56: Crystals of *cis*-quinic acid suitable for single crystal XRD were obtained in an NMR tube containing 3,4-*O*-cyclohexylidene-5-*epi*-quinic acid dissolved in $CDCl_3$ by removal of the acid-labile cyclohexylidene protection promoted by the trace amounts of HCl present in the deuterated solvent; ^{1}H-NMR (D_2O): δ_H 3.81 (m, 1H, 4-H), 3.77 (m, 1H, 5-H), 3.74 (m, 1H, 3-H), 1.99 (m, 2H, 2-*H*H and 6-*H*H), 1.66 (dd, 2H, *J* = 12.1, 12.4 Hz, 2-H*H* and 6-H*H*); ^{13}C-NMR (D_2O): δ_C 177.14 (COO), 72.74 (C-1), 71.15 (C-4), 66.91 (C-3 and C-5), 35.95 (C-2 and C-6).

Methyl 5-*epi*-quinate (methyl *cis*-quinate) 57: Crystals of methyl 5-*epi*-quinate suitable for single crystal XRD were obtained in an NMR tube containing methyl 3,4-*O*-cyclohexylidene-5-*epi*-quinate dissolved in $CDCl_3$, by removal of the acid-labile cyclohexylidene protection promoted by the trace amounts of HCl present in the deuterated solvent; ^{1}H-NMR (D_2O): δ_H 3.76 (m, 1H, 4-H), 3.67 (m, 1H, 5-H), 3.64 (m, 1H, 3-H), 3.60 (s, 3H, CH_3), 1.97 (m, 2H, 2-*H*H and 6-*H*H), 1.62 (dd, 2H, *J* = 12.1, 12.4 Hz, 2-H*H* and 6-H*H*); ^{13}C-NMR (D_2O): δ_C 175.26 (COO), 72.82 (C-1), 71.04 (C-4), 66.71 (C-3 and C-5), 52.99 (CH_3), 35.79 (C-2 and C-6).

1-*O*-Troc-3-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide 58: To a solution of 1-*O*-Troc-1,5-quinide (1.00 g, 2.86 mmol) in 40 mL DCM, a quantity of 53 mg (0.43 mmol, 15% mol) DMAP

was added and a volume of 10 mL of pyridine. A quantity of 648 mg (2.86 mmol) 3,4-dimethoxycinnamoyl chloride was then added and the mixture was stirred at r.t. for 24 h. The mixture was then acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 602 mg (1.12 mmol, 39%) of a pale yellow solid; ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.59 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.30 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.18 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 6.97 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.39 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.91-4.85 (m, 3H, CCl_3-CH_2 and 5-H), 4.07 (m, 1H, 4-H), 3.86 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 3.82 (m, 1H, 3-H), 2.98 (m, 1H, 6-*H*H), 2.66 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.15 (m, 2H, 2-*H*H and 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.22 (OC-*C*OO), 167.28 (CH-*C*OO), 151.68 (O*C*OOCH$_2$), 151.49 (*C*$_{Ar}$-OCH_3), 149.80 (*C*$_{Ar}$-OCH_3), 144.87 (*C*H-C_{Ar}), 127.50 (*C*$_{Ar}$-CH), 122.70 ($C_{Ar}H$), 115.81 (C_{Ar}-CH=*C*H), 111.55 ($C_{Ar}H$), 110.29 ($C_{Ar}H$), 94.57 (CCl_3), 79.49 (C-1), 76.65 (*C*H_2-CCl_3), 76.60 (C-5), 66.11 (C-4), 65.78 (C-3), 55.30 (CH_3), 55.27 (CH_3), 36.54 (C-6), 32.42 (C-2).

3-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide 59: A quantity of 100 mg (0.19 mmol) of 1-*O*-Troc-3-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide was suspended in THF (1.00 mL) and an equal volume (1.00 mL) of glacial acetic acid was added, followed by addition of 44 mg (0.67 mmol, 3.6 eq.) of Zn powder. The grey suspension was stirred at r.t. for 4 h and then the solvents were removed under reduced pressure. A volume of 15 mL EtOAc was added to the flask and the new suspension was cooled to 0 ºC and extracted with aqueous HCl 0.5M (2x10 mL) followed by brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield the white solid product (66 mg, 0.18 mmol, 98%); ^{1}H-NMR ($(CD_3)_2CO$): δ_H 7.63 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.35 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.21 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 6.98 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.44 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.86 (m, 1H, 5-H), 4.58 (br, 1H, OH), 4.27 (br, 1H, OH), 4.06 (m, 1H, 4-H), 3.87 (s, 3H, CH_3), 3.84 (s, 3H, CH_3), 3.82 (m, 1H, 3-H), 3.03 (ddd, 1H, *J* = 2.8, 6.4, 11.0 Hz, 6-*H*H), 2.58 (d, 1H, *J* = 11.0 Hz, 6-H*H*), 2.14 (m, 2H, 2-*H*H and 2-H*H*); ^{13}C-NMR ($(CD_3)_2CO$): δ_C 172.20 (OC-*C*OO), 164.70 (CH-*C*OO), 152.07 (*C*$_{Ar}$-OCH_3), 149.83 (*C*$_{Ar}$-OCH_3), 146.26 (*C*H-C_{Ar}), 127.13 (*C*$_{Ar}$-CH), 123.34 ($C_{Ar}H$), 114.63 (C_{Ar}-CH=*C*H), 111.49 ($C_{Ar}H$), 110.30 ($C_{Ar}H$), 76.66 (C-1 and C-5), 66.11 (C-4), 65.79 (C-3), 55.33 (CH_3), 55.28 (CH_3), 37.05 (C-6), 32.76 (C-2).

2''-chloroethyl 3-*O*-(4'-*O*-allylferuloyl)-BBA-quinate 60: To a solution of BBA-quinic acid (1.79 g, 5.84 mmol) in 100 mL 1,2-dichloroethane, a quantity of 214 mg (1.75 mmol, 30% mol) DMAP was added and a volume of 30 mL of NEt_3. A quantity of 4.43 g (17.53 mmol) 4-*O*-allylferuloyl chloride was then added and the mixture was refluxed for 48 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with 1,2-dichloroethane (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 3.02 g (3.82 mmol, 68%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.62 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.05 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.03 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.31 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.07 (m, 1H, CH_2=C*H*), 5.41 (dd, 1H, *J* = 1.4, 17.4 Hz, C*H*H=CH), 5.30 (dd, 1H, *J* = 1.4, 10.5 Hz, CH*H*=CH), 4.64 (dd, 2H, C_{Ar}-OCH_2), 4.39 (t, 2H, *J* = 6.0 Hz, $COOCH_2$), 4.34 (ddd, 1H, *J* = 4.1, 10.1, 12.4 Hz, 5-H), 4.18 (ddd, 1H, *J* = 2.8, 2.8, 3.2 Hz, 3-H), 3.90 (s, 3H, C_{Ar}-OCH_3), 3.65 (t, 2H, *J* = 6.0 Hz, CH_2Cl), 3.59 (dd, 1H, *J* = 3.2, 10.1 Hz, 4-H), 3.27 (s, 3H, C-OCH_3), 3.26 (s, 3H, C-OCH_3), 2.81 (dt, 1H, *J* = 2.8, 16.0 Hz, 2-*H*H), 2.49 (ddd, 1H, *J* = 2.8, 4.1, 13.7 Hz, 6-*H*H), 2.30 (br, 1H, OH), 2.12 (dd, 1H, *J* = 2.8, 16.0 Hz, 2-H*H*), 1.92 (dd, 1H, *J* = 12.4, 13.7 Hz, 6-H*H*), 1.33 (s, 3H, CH_3-C), 1.30 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 170.93 (*C*$OOCH_2$), 165.99 (CH-*C*OO), 150.40 (*C*$_{Ar}$-OCH_3), 149.60 (*C*$_{Ar}$-OCH_2), 146.20 (*C*H-C_{Ar}), 132.79 (*C*H=CH_2), 127.45 (*C*$_{Ar}$-CH), 123.03 (C_{Ar}H), 118.55 (*C*H_2=CH), 115.08 (C_{Ar}-CH=*C*H), 112.79 (C_{Ar}H), 109.97 (C_{Ar}H), 100.37 (CH_3-*C*), 99.91 (CH_3-*C*), 79.81 (C-1), 72.74 (C-4), 69.81 (C_{Ar}-O*C*H_2), 67.70 (C-5), 64.95 (C-3), 62.14 (COO*C*H_2), 56.06 (C_{Ar}-O*C*H_3), 48.07 (2xC-O*C*H_3), 41.34 (CH_2Cl), 36.48 (C-6), 34.42 (C-2), 17.96 (*C*H_3-C), 17.76 (*C*H_3-C).

2''-chloroethyl 3-*O*-feruloyl-BBA-quinate 61: To a solution of 920 mg (1.57 mmol) of 2''-chloroethyl 3-*O*-(4'-*O*-allylferuloyl)-BBA-quinate in 100 mL of aqueous 1,4-dioxane (90%), a quantity of 33 mg (0.17 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (310 mg) was slowly added at r.t. and it was then heated to 80 ºC for 48 h. The mixture was cooled to r.t., filtered and 1,4-dioxane removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x25 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (30-40% EtOAc/petroleum ether) to give the

title compound as a pale yellow powder (273 mg, 0.50 mmol, 32%); ^{1}H-NMR ($CDCl_3$): δ_H 7.59 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 6.99 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.98 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.87 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.26 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 6.14 (br, 1H, OH), 4.37 (t, 2H, *J* = 6.0 Hz, $COOCH_2$), 4.33 (ddd, 1H, *J* = 4.1, 10.1, 12.4 Hz, 5-H), 4.17 (ddd, 1H, *J* = 2.8, 3.2, 3.2 Hz, 3-H), 3.88 (s, 3H, C_{Ar}-OCH_3), 3.63 (t, 2H, *J* = 6.0 Hz, CH_2Cl), 3.58 (dd, 1H, *J* = 3.2, 10.1 Hz, 4-H), 3.27 (s, 3H, C-OCH_3), 3.24 (s, 3H, C-OCH_3), 2.79 (dt, 1H, *J* = 2.8, 15.6 Hz, 2-*H*H), 2.47 (ddd, 1H, *J* = 2.8, 4.1, 13.3 Hz, 6-*H*H), 2.11 (dd, 1H, *J* = 3.2, 15.6 Hz, 2-H*H*), 1.91 (dd, 1H, *J* = 12.4, 13.3 Hz, 6-H*H*), 1.32 (s, 3H, CH_3-C), 1.29 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 170.97 (*C*$OOCH_2$), 166.07 (CH-*C*OO), 148.34 (*C*$_{Ar}$-OCH_3), 146.92 (*C*H-C_{Ar}), 146.37 (C_{Ar}OH), 126.82 (*C*$_{Ar}$-CH), 123.69 (C_{Ar}H), 114.86 (C_{Ar}-CH=*C*H), 114.65 (C_{Ar}H), 109.36 (C_{Ar}H), 100.35 (CH_3-*C*), 99.91 (CH_3-*C*), 79.76 (C-1), 72.74 (C-4), 67.67 (C-5), 64.96 (C-3), 62.15 (COO*C*H_2), 56.02 (C_{Ar}-O*C*H_3), 48.07 (2xC-O*C*H_3), 41.36 (CH_2Cl), 36.48 (C-6), 34.43 (C-2), 17.95 (*C*H_3-C), 17.75 (*C*H_3-C).

2''-chloroethyl 3-*O*-feruloylquinate 62: 2"-Chloroethyl 3-*O*-feruloyl-BBA-quinate (175 mg, 0.32 mmol) was dissolved in 10 mL of TFA aqueous solution (90%) at 0 °C and the solution was stirred for 45 min at r.t. The solvents were removed *in vacuo* to afford the desired product in quantitative yield as a pale yellow solid; ^{1}H-NMR (CD_3OD): δ_H 7.60 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.17 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.05 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.79 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.35 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.34 (t, 2H, *J* = 6.0 Hz, $COOCH_2$), 4.20 (m, 1H, 5-H), 4.06 (m, 1H, 3-H), 3.87 (s, 3H, C_{Ar}-OCH_3), 3.70 (t, 2H, *J* = 6.0 Hz, CH_2Cl), 3.48 (dd, 1H, *J* = 3.2, 8.2 Hz, 4-H), 2.42 (m, 2H, 2-*H*H and 6-*H*H), 2.28 (dd, 1H, *J* = 3.7, 14.7 Hz, 2-H*H*), 1.89 (dd, 1H, *J* = 9.6, 13.7 Hz, 6-H*H*); ^{13}C-NMR (CD_3OD): δ_C 171.68 (*C*$OOCH_2$), 166.73 (CH-*C*OO), 149.42 (*C*$_{Ar}$-OCH_3), 148.05 (*C*H-C_{Ar}), 146.27 (C_{Ar}OH), 126.35 (*C*$_{Ar}$-CH), 122.96 (C_{Ar}H), 115.12 (C_{Ar}-CH=*C*H), 114.00 (C_{Ar}H), 110.32 (C_{Ar}H), 79.83 (C-1), 74.39 (C-4), 67.99 (C-5), 66.66 (C-3), 64.87 (COO*C*H_2), 55.09 (C_{Ar}-O*C*H_3), 41.07 (CH_2Cl), 38.34 (C-6), 34.47 (C-2).

1-*O*-(3',4'-Di-*O*-acetylcaffeoyl)-3,4-*O*-isopropylidene-1,5-quinide 63: To a solution of 3,4-*O*-isopropylidene-1,5-quinide (773 mg, 3.61 mmol) in 40 mL DCM, a quantity of 66 mg (0.54 mmol, 15% mol) DMAP was added and a volume of 12 mL of NEt_3. A quantity of 1.53 g (5.41 mmol) 3,4-di-*O*-acetylcaffeoyl chloride was then added and the mixture was refluxed for 24 h. It

was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x25 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 927 mg (2.01 mmol, 56%) of white solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.58 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.34 (d, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.31 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.16 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.33 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.75 (dd, 1H, *J* = 2.3, 6.4 Hz, 5-H), 4.51 (ddd, 1H, *J* = 3.2, 6.9, 7.8 Hz, 3-H), 4.27 (m, 1H, 4-H), 3.05 (m, 1H, 6-*H*H), 2.56 (d, 1H, *J* = 11.5 Hz, 6-H*H*), 2.46 (ddd, 1H, *J* = 2.3, 7.8, 14.7 Hz, 2-*H*H), 2.35 (dd, 1H, *J* = 3.2, 14.7 Hz, 2-H*H*), 2.23 (s, 3H, CH_3), 2.22 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 173.45 (OC-*C*OO), 168.06 (CH_3-*C*OO), 167.96 (CH_3-*C*OO), 164.59 (CH-*C*OO), 144.73 (*C*H-C_{Ar}), 143.95 (COO*C*$_{Ar}$), 142.55 (COO*C*$_{Ar}$), 132.79 (*C*$_{Ar}$-CH), 126.65 (C_{Ar}H), 124.08 (C_{Ar}H), 123.02 (C_{Ar}H), 117.98 (C_{Ar}-CH=C*H*), 109.94 (CH_3-*C*), 76.38 (C-1), 75.48 (C-5), 72.49 (C-4), 71.18 (C-3), 35.60 (C-2), 30.64 (C-6), 27.01 (*C*H_3-C), 24.34 (*C*H_3-C), 20.65 (*C*H_3-COO), 20.61 (*C*H_3-COO).

1-*O*-Feruloyl-quinic acid (1-FQA) 64: A quantity of 20 mg (0.06 mmol) of 1-*O*-feruloyl-1,5-quinide was dissolved in 1.00 mL THF and 1.50 mL of a 1M LiOH solution was added. The reaction mixture was stirred at r.t. for 20 min, was then acidified with 2M HCl (pH 2) and extracted with EtOAc (3x5 mL). The combined organic layers were dried over Na_2SO_4 and the solvents removed *in vacuo* to give a light red powder in quantitative yield; ^{1}H-NMR (D_2O): δ_H 7.47 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.09 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.00 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.78 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.21 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.05 (ddd, 1H, *J* = 3.2, 3.2, 3.6 Hz, 3-H), 3.93 (ddd, 1H, *J* = 4.6, 9.6, 11.0 Hz, 5-H), 3.74 (s, 3H, OCH_3), 3.44 (dd, 1H, *J* = 3.2, 9.6 Hz, 4-H), 2.04 (ddd, 1H, *J* = 2.8, 4.6, 13.3 Hz, 6-*H*H), 1.96 (dd, 1H, *J* = 3.2, 14.2 Hz, 2-*H*H), 1.93 (ddd, 1H, *J* = 2.8, 3.6, 14.2 Hz, 2-H*H*), 1.80 (dd, 1H, *J* = 11.0, 13.3 Hz, 6-H*H*); ^{13}C-NMR (D_2O): δ_C 178.30 (COOH), 162.09 (CH-*C*OO), 147.74 (*C*$_{Ar}$-OCH_3), 147.61 (*C*H-C_{Ar}), 146.55 (C_{Ar}OH), 126.94 (*C*$_{Ar}$-CH), 123.35 (C_{Ar}H), 115.61 (C_{Ar}-CH=C*H*), 114.60 (C_{Ar}H), 111.41 (C_{Ar}H), 75.87 (C-1), 74.87 (C-4), 70.06 (C-3), 66.45 (C-5) 55.90 (CH_3), 40.27 (C-6), 36.83 (C-2).

1-*O*-(4'-*O*-Allylferuloyl)-BBA-3-*epi*-quinic acid 65: To a solution of BBA-*muco*-quinic acid (1.00 g, 3.26 mmol) in 50 mL DCM, a quantity of 80 mg (0.65 mmol, 20% mol) DMAP was added and a volume of 10 mL of pyridine. A quantity of 1.24 g (4.89 mmol) 4-*O*-allylferuloyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 324 mg (0.62 mmol, 19%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.60 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.03 (dd, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 7.02 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.84 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.26 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.06 (m, 1H, CH_2=C*H*), 5.40 (m, 1H, C*H*H=CH), 5.30 (m, 1H, CH*H*=CH), 4.63 (m, 2H, C_{Ar}-OCH_2), 4.00 (ddd, 1H, *J* = 4.6, 9.6, 11.5 Hz, 5-H), 3.90 (m, 3H, C_{Ar}-OCH_3), 3.84 (ddd, 1H, *J* = 4.1, 10.1, 12.4 Hz, 3-H), 3.52 (dd, 1H, *J* = 9.6, 10.1 Hz, 4-H), 3.29 (s, 3H, C-OCH_3), 3.22 (s, 3H, C-OCH_3), 2.69 (ddd, 1H, *J* = 2.8, 4.6, 13.7 Hz, 6-*H*H), 2.55 (ddd, 1H, *J* = 2.8, 4.1, 13.7 Hz, 2-*H*H), 2.06 (dd, 1H, *J* = 12.4, 13.7 Hz, 2-H*H*), 1.92 (dd, 1H, *J* = 11.5, 13.7 Hz, 6-H*H*), 1.33 (s, 3H, CH_3-C), 1.29 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 174.47 (COOH), 165.54 (CH-*C*OO), 150.49 (*C*$_{Ar}$-OCH_3), 149.59 (*C*$_{Ar}$-OCH_2), 146.43 (*C*H-C_{Ar}), 132.77 (*C*H=CH_2), 127.27 (*C*$_{Ar}$-CH), 122.95 (C_{Ar}H), 118.56 (*C*H_2=CH), 114.64 (C_{Ar}-CH=*C*H), 112.81 (C_{Ar}H), 110.09 (C_{Ar}H), 99.90 (CH_3-*C*), 99.80 (CH_3-*C*), 79.13 (C-1), 76.23 (C-4), 69.80 (C_{Ar}-O*C*H_2), 66.89 (C-5), 65.07 (C-3), 56.03 (C_{Ar}-O*C*H_3), 48.16 (C-O*C*H_3), 48.04 (C-O*C*H_3), 37.93 (C-6), 34.96 (C-2), 17.81 (2x*C*H_3-C).

1-*O*-Feruloyl-3-*epi*-quinic acid (1-FmQA) 66: To a solution of 1-*O*-(4'-*O*-allylferuloyl)-BBA-3-*epi*-quinic acid (523 mg, 1.00 mmol) and p-TsOH (20 mg, 0.11 mmol) in 30 mL aqueous 1,4-dioxane (90%) was added 10% Pd/C (195 mg) at r.t. The reaction mixture was heated at 65 °C for 48 h, then cooled to r.t., filtered and the solvent was removed under low pressure. The aqueous reaction mixture was extracted with ethyl acetate (3×30 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (EtOAc/petroleum ether 50-70%) to give 1-*O*-feruloyl-BBA-3-*epi*-quinic acid as a pale yellow powder (400 mg, 0.83 mmol, 83%). 1-*O*-feruloyl-BBA-3-*epi*-quinic acid (16 mg, 0.03 mmol) was dissolved in 5 mL of TFA aqueous solution (90%) at 0 °C and the solution was stirred for 1 h at r.t. The solvents were removed *in*

vacuo to afford 1-*O*-feruloyl-3-*epi*-quinic acid in quantitative yield as a pale yellow solid; ^{1}H-NMR (CD_3OD): δ_H 7.57 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.19 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.06 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 6.79 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.36 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 3.87 (s, 3H, C_{Ar}-OCH_3), 3.63 (m, 2H, 5-H and 3-H), 3.23 (dd, 1H, *J* = 7.3, 8.7 Hz, 4-H), 2.57 (m, 2H, 2-*H*H and 6-*H*H), 1.84 (dd, 1H, *J* = 12.4, 13.3 Hz, 2-H*H* and 6-H*H*); ^{13}C-NMR (CD_3OD): δ_C 172.10 (COOH), 165.77 (CH-*C*OO), 149.95 (*C*$_{Ar}$-OCH_3), 148.44 (*C*$_{Ar}$-OH), 146.24 (*C*H-C_{Ar}), 126.92 (*C*$_{Ar}$-CH), 123.95 (C_{Ar}H), 115.69 (C_{Ar}-CH=*C*H), 115.05 (C_{Ar}H), 110.93 (C_{Ar}H), 81.05 (C-1), 79.59 (C-4), 69.52 (C-5 and C-3), 55.97 (C_{Ar}-O*C*H_3), 38.81 (C-6 and C-2).

1-*O*-(3',4'-Di-*O*-allylcaffeoyl)-BBA-3-*epi*-quinic acid 67: To a solution of BBA-*muco*-quinic acid (1.00 g, 3.26 mmol) in 50 mL DCM, a quantity of 80 mg (0.65 mmol, 20% mol) DMAP was added and a volume of 10 mL of pyridine. A quantity of 1.36 g (4.89 mmol) 3,4-di-*O*-allylcaffeoyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The crude product was purified by column chromatography (EtOAc/petroleum ether 30-50%) to afford 313 mg (0.57 mmol, 18%) of a pale yellow solid; ^{1}H-NMR ($CDCl_3$): δ_H 7.58 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.05 (dd, 1H, *J* = 1.8, 8.7 Hz, C_{Ar}H), 7.04 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.85 (d, 1H, *J* = 8.7 Hz, C_{Ar}H), 6.22 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 6.06 (m, 1H, CH_2=C*H*), 5.45 (m, 1H, C*H*H=CH), 5.44 (m, 1H, C*H*H=CH), 5.41 (m, 1H, CH*H*=CH), 5.39 (m, 1H, CH*H*=CH), 4.63 (m, 4H, 2xC_{Ar}-OCH_2), 3.99 (ddd, 1H, *J* = 4.6, 9.6, 11.5 Hz, 5-H), 3.84 (ddd, 1H, *J* = 4.1, 10.1, 12.4 Hz, 3-H), 3.52 (dd, 1H, *J* = 9.6, 10.1 Hz, 4-H), 3.28 (s, 3H, C-OCH_3), 3.22 (s, 3H, C-OCH_3), 2.67 (m, 1H, 6-*H*H), 2.53 (m, 1H, 2-*H*H), 2.04 (dd, 1H, *J* = 12.4, 13.3 Hz, 2-H*H*), 1.91 (dd, 1H, *J* = 11.5, 13.7 Hz, 6-H*H*), 1.33 (s, 3H, CH_3-C), 1.29 (s, 3H, CH_3-C); ^{13}C-NMR ($CDCl_3$): δ_C 174.93 (COOH), 165.53 (CH-*C*OO), 151.04 (*C*$_{Ar}$-OCH_2), 148.59 (*C*$_{Ar}$-OCH_2), 146.61 (*C*H-C_{Ar}), 133.09 (*C*H=CH_2), 132.90 (*C*H=CH_2), 127.16 (*C*$_{Ar}$-CH), 123.25 (C_{Ar}H), 118.14 (*C*H_2=CH), 118.07 (*C*H_2=CH), 114.41 (C_{Ar}-CH=*C*H), 113.35 (C_{Ar}H), 112.64 (C_{Ar}H), 99.90 (CH_3-*C*), 99.81 (CH_3-*C*), 78.90 (C-1), 76.16 (C-4), 70.00 (C_{Ar}-O*C*H_2), 69.78 (C_{Ar}-O*C*H_2), 66.82 (C-5), 65.00 (C-3), 48.17 (C-O*C*H_3), 48.06 (C-O*C*H_3), 37.83 (C-6), 34.90 (C-2), 17.80 (2x*C*H_3-C).

Methyl 3-*epi*-quinate (methyl *muco*-quinate) 68: A quantity of 500 mg of Amberlite IR 120 resin was suspended in 30 mL MeOH and 20 mg of *muco*-quinic acid was added under stirring. The mixture was then refluxed overnight, the resin was filtered and the solvent was evaporated to give the white solid product in quantitative yield; ^{1}H-NMR (CD_3OD): δ_H 3.72 (s, 3H, CH_3), 3.70 (m, 1H, 5-H), 3.29 (m, 1H, 3-H), 3.14 (t, 1H, *J* = 9.2 Hz, 4-H), 2.02-1.96 (m, 2H, 2-*H*H and 6-*H*H), 1.74 (dd, 2H, *J* = 11.9, 12.8 Hz, 2-H*H* and 6-H*H*); ^{13}C-NMR (CD_3OD): δ_C 175.33 (COO), 80.24 (C-1), 73.45 (C-4), 69.21 (C-3 and C-5), 51.61 (CH_3), 40.45 (C-2 and C-6).

Methyl quinate 69: A quantity of 500 mg of Amberlite IR 120 resin was suspended in 30 mL MeOH and 20 mg of quinic acid was added under stirring. The mixture was then refluxed overnight, the resin was filtered and the solvent was evaporated to give the white solid product in quantitative yield; ^{1}H-NMR (D_2O): δ_H 4.02 (q, 1H, *J* = 3.7 Hz, 3-H), 3.92 (m, 1H, 5-H), 3.63 (s, 3H, CH_3), 3.43 (dd, 1H, *J* = 3.7, 9.2 Hz, 4-H), 2.04-1.98 (m, 2H, 2-*H*H and 6-*H*H), 1.91 (m, 1H, 2-H*H*), 1.81 (dd, 1H, *J* = 10.5, 13.2 Hz, 6-H*H*); ^{13}C-NMR (D_2O): δ_C 175.99 (COO), 75.62 (C-1), 74.43 (C-4), 69.61 (C-3), 66.37 (C-5), 53.11 (CH_3), 39.89 (C-2), 36.62 (C-6).

3,4-Di-*O*-acetylcaffeic acid 70: To a solution of caffeic acid (9.00 g, 49.96 mmol) and DMAP (610 mg, 5.00 mmol) in 50 mL pyridine, a volume of 14.15 mL (15.30 g, 149.87 mmol) acetic anhydride was added at 0 ºC. The reaction mixture was stirred for 1 h and then poured onto crushed ice. The aqueous phase was acidified with a HCl 2M solution (pH=2) and extracted with EtOAc/THF (3:1, 3x80 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuum; the crude residue was recrystallized from EtOAc to afford the white solid product (7.86 g, 29.73 mmol, 60%); ^{1}H-NMR ($CDCl_3$): δ_H 7.74 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH), 7.43 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.41 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 7.26 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.57 (d, 1H, *J* = 15.6 Hz, C_{Ar}-CH=C*H*), 2.31 (s, 3H, CH_3), 2.30 (s, 3H, CH_3); ^{13}C-NMR ($CDCl_3$): δ_C 168.04 (CH_3-*C*OO), 167.84 (CH_3-*C*OO), 165.92 (COCl), 148.58 (*C*H-C_{Ar}), 144.95 (COO*C*$_{Ar}$), 142.77 (COO*C*$_{Ar}$), 131.75 (*C*$_{Ar}$-CH), 127.53 (C_{Ar}H), 124.42 (C_{Ar}H), 123.80 (C_{Ar}-CH=C*H*), 123.44 (C_{Ar}H), 20.73 (CH_3), 20.67 (CH_3).

3,4-Di-*O*-acetylcaffeoyl chloride 71: 3,4-Di-*O*-acetylcaffeic acid (2.77 g, 10.48 mmol) was added to a solution of 60 mL toluene containing 120 µL of DMF. A volume of 1.87 mL (2.73 g, 21.50 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t.

for 4 h and the resulting brown solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under the rotary evaporator to give a brown solid (2.84 g, 10.06 mmol, 96%); ^{1}H-NMR ($CDCl_3$): δ_H 7.81 (d, 1H, *J* = 15.5 Hz, C_{Ar}-CH), 7.18 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.11 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 7.10 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.50 (d, 1H, *J* = 15.5 Hz, C_{Ar}-CH=C*H*), 3.87 (s, 3H, OCH_3), 2.32 (s, 3H, CH_3-COO); ^{13}C-NMR ($CDCl_3$): δ_C 168.95 (CH_3-*C*OO), 166.06 (COCl), 151.97 (*C*$_{Ar}$-OCH_3), 150.17 (*C*H-C_{Ar}), 142.88 (COO*C*$_{Ar}$), 131.54 (*C*$_{Ar}$-CH), 124.47 ($C_{Ar}H$), 122.60 ($C_{Ar}H$), 122.55 (C_{Ar}-CH=C*H*), 111.52 ($C_{Ar}H$), 56.10 (OCH_3), 20.55 (*C*H_3-COO).

Ethyl 4-*O*-allylferulate 72: A mixture of ferulic acid (1.00 g, 5.15 mmol) and anhydrous potassium carbonate (4.27 g, 30.90 mmol) in acetone (50 mL) was stirred at r.t. for 30 min. To the mixture was added a solution of allyl bromide (1.25 g, 10.30 mmol) in acetone (10 mL) and the entire mixture was refluxed for 48 h. The reaction was cooled to r.t., filtered and the filtrate was dried *in vacuo*. The residue was suspended in ethanol (30 mL) and a NaOH 2M solution (20 mL) was added. The mixture was refluxed for 2 h. The solution was cooled to r.t., poured into a beaker and acidified (pH=2) with 3M HCl. The suspension was stirred at r.t. for 30 min and the solid was filtered off and washed successively with a 1:1 mixture of ethanol/water (200 mL). The solid was dried overnight in vacuum to yield 4-*O*-allylferulic acid as the main product (1.03 g, 4.38 mmol, 85%) and ethyl 4-*O*-allylferulate as an undesired side product (67 mg, 0.26 mmol, 5%); ^{1}H-NMR ($CDCl_3$): δ_H 7.60 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.08 (dd, 1H, *J* = 1.8, 8.2 Hz, $C_{Ar}H$), 7.06 (d, 1H, *J* = 1.8 Hz, $C_{Ar}H$), 6.86 (d, 1H, *J* = 8.2 Hz, $C_{Ar}H$), 6.33 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.07 (m, 1H, CH_2=C*H*), 5.41 (dd, 1H, *J* = 1.4, 17.4 Hz, C*H*H=CH), 5.30 (dd, 1H, *J* = 1.4, 10.5 Hz, CH*H*=CH), 4.65 (dd, 2H, *J* = 1.4, 5.0 Hz, C_{Ar}-OCH_2), 4.24 (q, 2H, *J* = 7.2 Hz, CH_3-CH_2), 3.90 (s, 3H, OCH_3), 1.32 (t, 3H, *J* = 7.2 Hz, CH_3-CH_2); ^{13}C-NMR ($CDCl_3$): δ_C 167.23 (COO), 150.02 (*C*$_{Ar}$-OCH_3), 149.87 (*C*$_{Ar}$-OCH_2), 144.63 (*C*H-C_{Ar}), 132.92 (*C*H=CH_2), 127.92 (*C*$_{Ar}$-CH), 122.26 ($C_{Ar}H$), 118.43 (*C*H_2=CH), 116.15 (C_{Ar}-CH=*C*H), 112.97 ($C_{Ar}H$), 110.03 ($C_{Ar}H$), 69.93 (C_{Ar}-O*C*H_2), 60.06 (CH_3-*C*H_2), 55.98 (CH_3), 14.18 (*C*H_3-CH_2).

2,2,2-Trichloroethyl 3,4-dimethoxycinnamate 73: To a solution of 1-*O*-Troc-1,5-quinide (1.03 g, 2.86 mmol) in 50 mL DCM, a quantity of 105 mg (0.86 mmol, 30% mol) DMAP was added

and a volume of 15 mL of NEt_3. A quantity of 1.95 g (8.58 mmol) 3,4-dimethoxycinnamoyl chloride was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with DCM (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 983 mg (1.35 mmol, 47%) of the targeted 1-*O*-Troc-3,4-di-*O*-(3',4'-dimethoxycinnamoyl)-1,5-quinide and 137 mg (0.40 mmol, 14%) of the undesired side product 2,2,2-trichloroethyl 3,4-dimethoxycinnamate; ^{1}H-NMR ($CDCl_3$): δ_H 7.76 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.14 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.08 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.88 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.40 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 4.86 (s, 2H, CH_2), 3.92 (s, 6H, 2xCH_3); ^{13}C-NMR ($CDCl_3$): δ_C 165.57 (COO), 151.73 (*C*$_{Ar}$-OCH_3), 149.38 (*C*$_{Ar}$-OCH_3), 147.13 (*C*H-C_{Ar}), 127.03 (*C*$_{Ar}$-CH), 123.36 (C_{Ar}H), 113.88 (C_{Ar}-CH=*C*H), 111.11 (C_{Ar}H), 109.76 (C_{Ar}H), 95.33 (CCl_3), 74.14 (CH_2), 56.09 (CH_3), 56.04 (CH_3).

2-Chloroethyl 4-*O*-allylferulate 74: To a solution of BBA-quinic acid (1.79 g, 5.84 mmol) in 100 mL 1,2-dichloroethane, a quantity of 214 mg (1.75 mmol, 30% mol) DMAP was added and a volume of 30 mL of NEt_3. A quantity of 4.43 g (17.53 mmol) 4-*O*-allylferuloyl chloride was then added and the mixture was heated to 90 °C for 48 h. It was then allowed to cool to r.t., acidified (pH=2) with a HCl 2M solution and extracted 3 times with 1,2-dichloroethane (3x50 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The compound (side product) was obtained in the early fractions after column chromatography (EtOAc/petroleum ether 30-50%) as a white solid (126 mg, 0.53 mmol, 9%), which subsequently crystallized; ^{1}H-NMR ($CDCl_3$): δ_H 7.66 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH), 7.08 (dd, 1H, *J* = 1.8, 8.2 Hz, C_{Ar}H), 7.06 (d, 1H, *J* = 1.8 Hz, C_{Ar}H), 6.86 (d, 1H, *J* = 8.2 Hz, C_{Ar}H), 6.33 (d, 1H, *J* = 16.0 Hz, C_{Ar}-CH=C*H*), 6.06 (m, 1H, CH_2=C*H*), 5.41 (dd, 1H, *J* = 1.4, 17.4 Hz, C*H*H=CH), 5.31 (dd, 1H, *J* = 1.4, 10.5 Hz, CH*H*=CH), 4.65 (dd, 2H, *J* = 1.4, 5.0 Hz, C_{Ar}-OCH_2), 4.45 (dd, 2H, *J* = 5.5, 6.0 Hz, $COOCH_2$), 3.90 (s, 3H, OCH_3), 3.75 (dd, 2H, *J* = 5.5, 6.0 Hz, CH_2Cl).

3.5. References

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4. Investigating the Chemical Changes of Chlorogenic Acids during Coffee Brewing – Conjugate Water Addition to the Olefinic Moiety of Chlorogenic Acids and Their Quinides

4.1. Introduction

Classically, chlorogenic acids are a family of esters formed between quinic acid **51** and certain *trans*-cinnamic acids, most commonly caffeic **49**, *p*-coumaric and ferulic acid **55**,[1-3] and sometimes dimethoxycinnamic, trimethoxycinnamic and sinapic acid.[4-6] In the IUPAC system (−)-quinic acid is defined as 1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid, but Eliel and Ramirez[7] recommend 1α,3*R*,4α,5*R*-tetrahydroxycyclohexane carboxylic acid. Chlorogenic acids are widely distributed in plants,[2,3] but the coffee bean is remarkably rich, containing at least 72 chlorogenic acids that are not acylated at the C1 of the quinic acid moiety.[4-6,8-10]

These have been subdivided into thirteen classes, according to the type of ester substituent (e.g., caffeoyl, feruloyl, sinapoyl, etc.) and the number of ester substituents (monoacylquinic acids, diacylquinic acids or triacylquinic acids).[4-6,8-10] Several pharmacological activities of chlorogenic acids including antioxidant activity,[11] ability to increase hepatic glucose utilization,[12] inhibition of the HIV-1 integrase,[13] antispasmodic activity[14] and inhibition of the mutagenicity of carcinogenic compounds[15] have been reported so far. Coffee is considered the major source of chlorogenic acids in a typical human diet with an estimated 200 mg of total chlorogenic acids present in a 200 mL cup. Coffee, after water and black tea is the third most consumed beverage globally and the second-most traded commodity after crude oil, accounting for exports worth an estimated US$ 15.4 billion in 2010 (International Coffee Organization (ICO), 2011). Its estimated annual retail value exceeded $70 billion[16,17] in 2010 with employment in the coffee sector estimated at about 26 million people worldwide in 52 producing countries (ICO, 2011).

To obtain the popular coffee beverage the coffee cherry undergoes three important processing steps. First the flesh is mechanically removed from the cherry, which after washing and drying yields the green coffee bean. Secondly, the green coffee beans are roasted at temperatures ranging from 180-220 °C for 8 to 15 minutes producing the desired aroma and taste of coffee.[18,19] Finally, the roasted beans are ground into a powder and infused with hot water, at times like in espresso brewing under pressure, to yield after filtration the coffee beverage. At

each of these three processing steps it must be expected that the chemical content of the coffee bean changes. For the first step it is reported that residual enzymatic activity in the harvested beans leads to some chemical changes of coffee components.[20,21]

During roasting a myriad of chemical changes take place producing thousands of novel products from the main phytochemical constituents of the green coffee bean including chlorogenic acids as their main secondary metabolites, carbohydrates and proteins in thermal dehydration reactions and Maillard type reactions. Chemical changes reported for chlorogenic acids include e.g., chlorogenic acid lactones formation through the loss of a water molecule from the quinic acid moiety and formation of an intramolecular ester bond.[22] Furthermore, acyl migration has been reported in model systems forming C1-substituted chlorogenic acids. Along with chlorogenic acids, their lactones also contribute to coffee flavor and, despite their low concentrations, their impact on the final cup quality may be significant. Chlorogenic acids lactones have also been studied for their potential hypoglycemic effects[22] and for their actions at opioid and adenosine brain receptors.[23]

The last processing steps involving the brewing of coffee powder with boiling water has never been investigated although chemical changes of chlorogenic acids can be anticipated at this level as well. In this contribution, the investigation of novel compounds formed from chlorogenic acids during this brewing process is reported. In order to characterize novel chlorogenic acids derivatives LC-MS^n methods developed to characterize hydroxycinnamoyl quinic acids were utilized.[4-6,9-11] The MS fragmentation patterns in tandem MS spectra, UV spectrum, retention time, relative hydrophobicity and synthetic standards have been utilized to develop structure-diagnostic hierarchical keys for the identification of chlorogenic acids and shikimates. In the present study, these methods were applied to the qualitative profiling of novel chlorogenic acids derivatives formed during coffee brewing.

4.2. Materials and Methods

All the chemicals (Analytical grade) were purchased from Sigma-Aldrich (Bremen, Germany). Ground coffee (Robusta) was purchased from a supermarket in Bremen (Germany).

Brewing of Coffee. Ground coffee (3 g) was infused in 100 mL of hot water and stirred for 10 min. The prepared brew was cooled to room temperature, filtered through a membrane filter and directly used for LC-MS.

Brewing of CGAs and Derivatives. Commercially available chlorogenic acids standards together with synthesized chlorogenic acids derivatives (each sample 500 µg) were infused in 3 mL of hot water each and stirred for 5 h under reflux. The solvent was removed under low pressure and the samples were dissolved in MeOH and used for LC-MS^n.

LC-MS^n. The 1100 series LC equipment (Agillent, Bremen, Germany) comprised a binary pump, an auto sampler with a 100 μL loop and a DAD detector with a light-pipe flow cell (recording at 320 and 254 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany) operating in full scan, auto MS^n mode to obtain fragmentation. As necessary, MS^2, MS^3 and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a parent ion at *m/z* 335, 353, 367, 371, 515 and 533. Tandem mass spectra were acquired in Auto-MS^n mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 V, starting at 30% and ending at 200%. MS operating conditions (negative mode) had been optimized using 5-caffeoylquinic acid **30** with a capillary temperature of 365 ^{o}C, a dry gas flow rate of 10 L/min, and a nebulizer pressure of 10 psi.

LC-TOF-MS. High resolution LC-MS experiments were carried out using the same HPLC equipped with a MicrOTOF Focus mass spectrometer (Bruker Daltonics, Bremen, Germany) fitted with an ESI ion source. Internal calibration was achieved with 10 mL of a 0.1 M sodium formate solution injected through a six port valve prior to each chromatographic run. Calibration was carried out using the enhanced quadratic mode and the mass error was below 5 ppm.

HPLC. Separation was achieved on a 250 mm x 3 mm i.d. column containing C18-amide 5 μm, with a 5 mm x 3 mm i.d. guard column of the same material (Varian, Darmstadt, Germany). Alternatively, separation was also achieved on a 150 mm x 3 mm i.d. column containing diphenyl 5 μm, with a 5 mm x 3 mm i.d. guard column of the same material (Varian, Darmstadt, Germany). The data presented in this paper was with few exceptions generated with the C18-

amide column. Solvent A was water/formic acid (1000:0.005 v/v) and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. The gradient profile was linear from 10-70% B in 60 min followed by 10 min isocratic, and a return to 10% B at 90 min and 10 min isocratic to re-equilibrate.

Preliminary Assessment of Data. All data for the chlorogenic acids presented in this paper use the recommended IUPAC numbering system;[1] the same numbering system was adopted for water addition products of chlorogenic acids, their *cis*-isomers and their acyl-migration isomers**.**

Synthesis of 1-*O*-Caffeoylquinic Acid Lactone 31.

3,4-*O*-Isopropylidene-1,5-quinide 52: To a solution of 10.00 g (52.04 mmol) of quinic acid in 50 mL acetone, a quantity of 200 mg (1.05 mmol) of *p*-toluenesulfonic acid monohydrate (PTSA·H_2O) was added, followed by addition of 22.4 mL of 2,2-dimethoxypropane (DMP) to give a white suspension. The reaction was then refluxed for 24 h to give a clear red solution which was cooled to 50 ºC and neutralized with a solution of NaOEt (71.5 mg) in EtOH (5 mL) to give a yellow clear solution. The solvents were removed under reduced pressure and to the resulting orange viscous liquid a volume of 100 mL of EtOAc was added. The organic phase was washed with 50 mL of H_2O and the aqueous phase was back-extracted with 30 mL EtOAc. The combined organic layers were washed with a half-saturated $NaHCO_3$ solution, dried on Na_2SO_4, filtered and evaporated. The resulting yellow solid was recrystallized successively from a 1:1 n-heptane:EtOAc solution to afford white crystals of **52** (6.13 g, 28.62 mmol, 55%); mp 142 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 4.71 (dd, 1H, *J* = 2.5, 6.2 Hz), 4.48 (td, 1H, *J* = 2.8, 7.3 Hz), 4.29 (ddd, 1H, *J* = 1.4, 2.3, 6.4 Hz), 2.96 (br, 1H), 2.63 (d, 1H, *J* = 11.9 Hz), 2.39-2.32 (ddd, 1H, *J* = 2.3, 7.8, 14.7 Hz), 2.32-2.26 (m, 1H), 2.16 (dd, 1H, *J* = 2.8, 14.7 Hz), 1.51 (s, 3H), 1.31 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ_C 178.95, 109.88, 75.94, 72.18, 71.59, 71.59, 38.27, 34.35, 27.06, 24.38.

3,4-Di-*O*-allylcaffeic acid 50: A mixture of caffeic acid (5 g, 27.75 mmol) and anhydrous potassium carbonate (55.3 g, 401 mmol) in acetone (250 mL) was stirred at room temperature for 30 min. To the mixture was added a solution of allyl bromide (6.23 g, 51.5 mmol) in acetone (50 mL) and the entire mixture was refluxed for 48 h. The reaction was cooled to r.t., filtered and the filtrate was dried *in vacuo*. The residue was suspended in ethanol (150 mL) and a NaOH 2M solution (100 mL) was added. The mixture was refluxed for 2 h. The solution was cooled to r.t., poured into a beaker and acidified (pH 2) with conc. HCl. The suspension was stirred at r.t. for

30 min and the solid was filtered off and washed successively with a 1:1 mixture of ethanol/water (200 mL). The solid was dried overnight in vacuum to yield a white powder of **50** (6.16 g, 23.59 mmol, 85%); mp 155-157 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.70 (d, 1H, *J* = 16.0 Hz), 7.10 (s, 1H), 7.09 (d, 1H, *J* = 7.8 Hz), 6.88 (d, 1H, *J* = 7.8 Hz), 6.28 (d, 1H, *J* = 16.0 Hz), 6.07 (m, 2H), 5.47-5.39 (m, 2H), 5.30 (dd, 2H, *J* = 1.4, 10.5 Hz), 4.64 (m, 4H). ^{13}C-NMR ($CDCl_3$): δ_C 172.53, 151.11, 148.56, 147.06, 133.11, 132.89, 127.24, 123.26, 118.16, 118.09, 114.86, 113.42, 112.85, 70.05, 69.80.

3,4-Di-*O*-allylcaffeoyl chloride 53: 3,4-di-*O*-allylcaffeic acid **50** (6.16 g, 23.59 mmol) was added to a solution of 100 mL toluene containing 150 μL of dimethylformamide (DMF). A volume of 6.8 mL (9.90 g, 77.98 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for overnight and the resulting brown solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under rotary evaporator to give a light brown solid of **53** (6.05 g, 21.70 mmol, 92%); mp 67-68 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.74 (d, 1H, *J* = 15.6 Hz), 7.14 (dd, 1H, *J* = 1.8, 8.7 Hz), 7.07 (d, 1H, *J* = 1.8 Hz), 6.89 (d, 1H, *J* = 8.7 Hz), 6.45 (d, 1H, *J* = 15.6 Hz), 6.07 (m, 2H), 5.47-5.40 (m, 2H), 5.31 (dd, 2H, *J* = 1.4, 10.5 Hz), 4.65 (m, 4H). ^{13}C-NMR ($CDCl_3$): δ_C 172.63, 151.11, 148.66, 147.06, 133.11, 132.89, 127.24, 123.26, 118.16, 118.09, 115.00, 113.42, 112.85, 70.05, 69.80.

1-*O*-(3',4'-Diallylcaffeoyl)-3,4-*O*-isopropylidene-1,5-quinide 54: To a solution of isopropylidene quinide **52** (717 mg, 3.35 mmol) in 35 mL CH_2Cl_2, a quantity of 123 mg (1.00 mmol, 20% mol) 4-dimethylaminopyridine (DMAP) was added and a volume of 10 mL of NEt_3. A quantity of 1.40 g (5.02 mmol) 3,4-di-*O*-allylcaffeoyl chloride, **53** was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH 2) with a HCl 2M solution and extracted 3 times with CH_2Cl_2 (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.04 g (2.28 mmol, 68%) of a yellow solid of **54**; mp 127 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.63 (d, 1H, *J* = 16.0 Hz), 7.06 (dd, 1H, *J* = 1.8, 8.7 Hz), 7.05 (d, 1H, *J* = 1.8 Hz), 6.86 (d, 1H, *J* = 8.7 Hz), 6.26 (d, 1H, *J* = 16.0 Hz), 6.06 (m, 2H), 5.45-5.39 (m, 2H), 5.29 (dd, 2H, *J* = 1.4, 10.5 Hz), 4.80 (dd, 1H, *J* = 2.3, 6.4 Hz), 4.63 (tt, 4H, *J* = 1.4, 5.0 Hz), 4.56 (td, 1H, *J* = 3.2, 7.3 Hz), 4.33 (m, 1H), 3.09 (m, 1H), 2.62 (d, 1H, *J*

= 11.5 Hz), 2.51 (ddd, 1H, *J* = 2.3, 7.8, 14.2 Hz), 2.41 (dd, 1H, *J* = 3.2, 14.7 Hz), 1.53 (s, 3H), 1.33 (s, 3H). ^{13}C-NMR ($CDCl_3$): δ_C 173.76, 165.32, 151.12, 148.64, 146.71, 133.09, 132.89, 127.21, 123.22, 118.13, 118.07, 114.48, 113.40, 112.78, 110.05, 76.13, 75.51, 72.58, 71.28, 70.04, 69.78, 35.78, 30.86, 27.08, 24.35.

1-*O*-Caffeoyl-3,4-*O*-isopropylidene-1,5-quinide 55: To a solution of 963 mg (2.11 mmol) of 1-*O*-(3',4'-diallylcaffeoyl)-3,4-*O*-isopropylidene-1,5-quinide **54** in 60 mL of aqueous MeOH (90%), a quantity of 84.3 mg (0.44 mmol) of PTSA·H_2O was added. The reaction mixture was put under a nitrogen atmosphere, Pd/C (820 mg) was slowly added at r.t. and it was then heated to 65 ºC for 48 h. The mixture was cooled to r.t., filtered and MeOH removed *in vacuo*. The aqueous reaction mixture was extracted with EtOAc (3x40 mL). The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under low pressure. The crude product was purified by column chromatography on silica gel (40-50% EtOAc/petroleum ether) to give 1-*O*-caffeoyl-3,4-*O*-isopropylidene-1,5-quinide **55** as a white powder (397 mg, 1.05 mmol, 50%); mp 210-212 ºC; ^{1}H-NMR (acetone-D6): δ_H 8.35 (br, 2H), 7.58 (d, 1H, *J* = 15.6 Hz), 7.16 (d, 1H, *J* = 1.8 Hz), 7.06 (dd, 1H, *J* = 1.8, 8.2 Hz), 6.85 (d, 1H, *J* = 8.2 Hz), 6.27 (d, 1H, *J* = 15.6 Hz), 4,81 (dd, 1H, *J* = 2.3, 6.4 Hz), 4.62 (td, 1H, *J* = 3.2, 7.8 Hz), 4.33 (m, 1H), 3.08 (m, 1H), 2.53 (d, 1H, *J* = 11.5 Hz), 2.44 (ddd, 1H, *J* = 2.3, 7.8, 14.2 Hz), 2.31 (dd, 1H, *J* = 3.2, 14.2 Hz), 1.48 (s, 3H), 1.30 (s, 3H). ^{13}C-NMR (acetone-D6): δ_C 173.09, 164.94, 148.38, 146.67, 145.52, 126.50, 122.18, 115.62, 114.63, 113.60, 109.51, 75.99, 75.24, 72.58, 71.17, 35.74, 30.27, 26.42, 23.73.

1-*O*-Caffeoyl-1,5-quinide (1-CQL) 31: A quantity of 79 mg (0.21 mmol) of 1-*O*-caffeoyl-3,4-*O*-isopropylidene-1,5-quinide **55** was dissolved in 3.75 mL of TFA 80% solution and stirred for 2 h. The solvents were then removed in vacuum to give a white solid of **31** (70 mg, quantitative yield); mp 135 ºC; ^{1}H-NMR (acetone-D6): δ_H 7.56 (d, 1H, *J* = 16.0 Hz), 7.17 (d, 1H, *J* = 1.8 Hz), 7.07 (dd, 1H, *J* = 1.8, 8.2 Hz), 6.85 (d, 1H, *J* = 8.2 Hz), 6.28 (d, 1H, *J* = 16.0 Hz), 4.86 (dd, 1H, *J* = 4.6, 6.0 Hz), 4.06 (t, 1H, *J* = 4.6 Hz), 3.82 (m, 1H), 3.03 (ddd, 1H, *J* = 2.3, 6.4, 11.0 Hz), 2.57 (d, 1H, *J* = 11.0 Hz), 2.15-2.05 (m, 2H). ^{13}C-NMR (acetone-D6): δ_C 172.27, 164.97, 148.40, 146.48, 145.53, 126.51, 122.12, 115.59, 114.59, 113.77, 76.59 (2C), 66.10, 65.79, 37.04, 32.73.

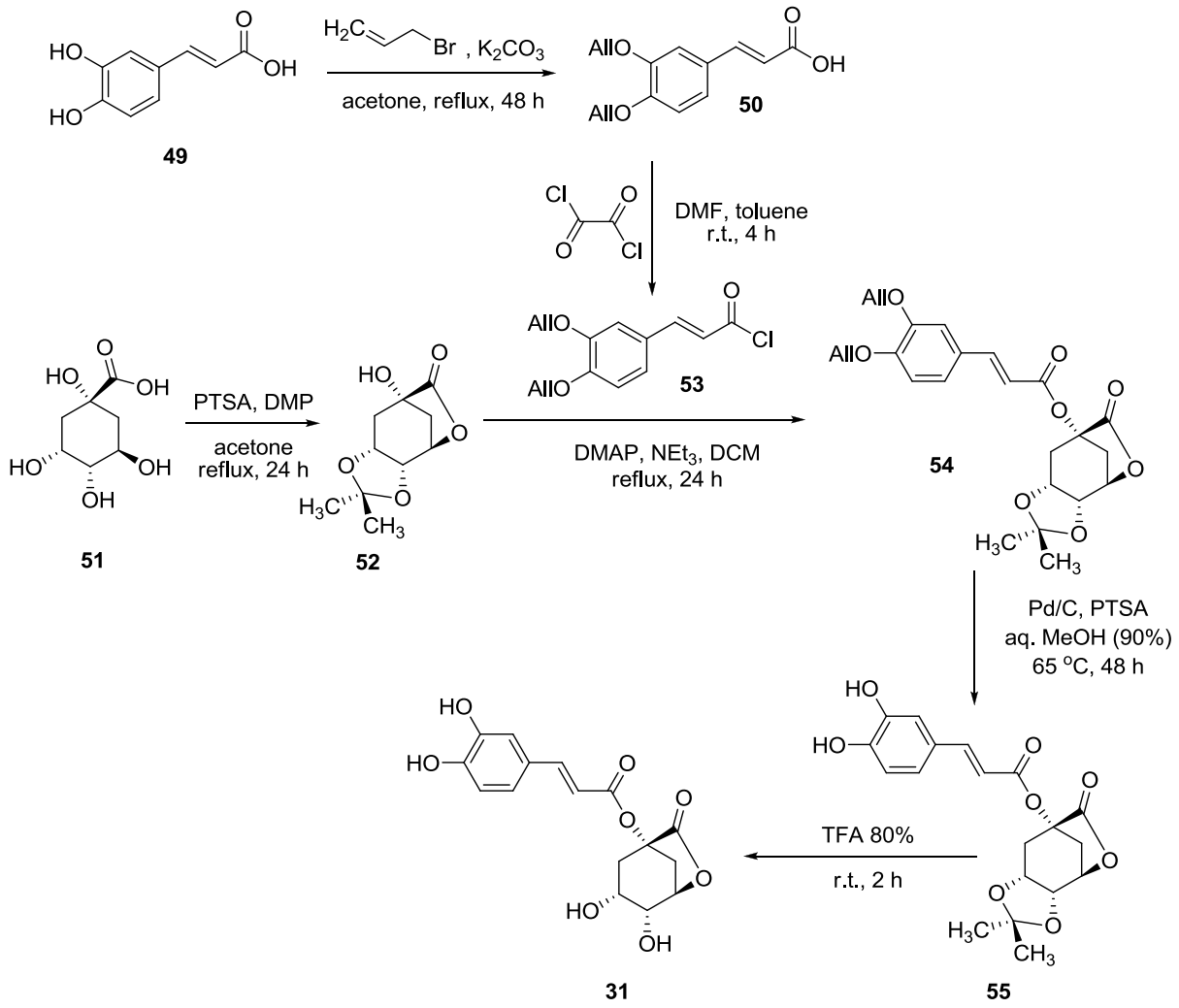

Figure 4.1. Synthetic path to 1-*O*-caffeoyl-1,5-quinide **31**.

Synthesis of 1-*O*-Feruloylquinic Acid Lactone 32.

3-*O*-Acetylferulic acid 57: To a solution of ferulic acid (9.00 g, 46.35 mmol) and DMAP (283 mg, 2.32 mmol) in 30 mL pyridine was added 7.08 mL (7.65 mg, 74.94 mmol) acetic anhydride at 0 ºC. The reaction mixture was stirred for 1 h and then poured onto crushed ice. The aqueous phase was acidified with a HCl 2M solution (pH 2) and extracted with EtOAc/THF (3:1, 3x60 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated in vacuum; the crude residue was recrystallized from EtOAc to afford the white solid product **57** (9.85 g, 41.72 mmol, 90%); mp 196-197 ºC; ^{1}H-NMR (DMSO-D6): δ_H 12.25 (br, 1H), 7.54 (dd, 1H, *J* = 1.8, 8.7 Hz), 7.51 (d, 1H, *J* = 1.8 Hz), 7.49 (d, 1H, *J* = 16.0 Hz), 7.12 (d, 1H, *J* = 8.7 Hz),

6.37 (d, 1H, *J* = 16.0 Hz), 3.77 (s, 3H), 2.32 (s, 3H). ^{13}C-NMR (DMSO-D6): δ_C 169.11, 168.25, 153.13, 143.53, 140.02, 128.33, 127.86, 122.53, 118.21, 113.37, 56.74, 21.13.

3-*O*-Acetylferuloyl chloride 58: 3-*O*-acetylferulic acid (9.85 g, 41.72 mmol) was added to a solution of 170 mL toluene containing 150 μL of DMF. A volume of 6 mL (8.75 g, 68.96 mmol) oxalyl chloride was added drop-wise at 0 ºC. The reaction mixture was stirred at r.t. for 4 h and the resulting brown solution was transferred slowly to a new round bottom flask (dark color viscous residues remaining on the bottom of the reaction vessel). The toluene and the unreacted oxalyl chloride were removed under the rotary evaporator to give a brown solid of **58** (10.09 g, 39.63 mmol, 95%); mp 121-122 ºC; ^{1}H-NMR ($CDCl_3$): δ_H 7.81 (d, 1H, *J* = 15.5 Hz), 7.18 (dd, 1H, *J* = 1.8, 8.2 Hz), 7.11 (d, 1H, *J* = 1.8 Hz), 7.10 (d, 1H, *J* = 8.2 Hz), 6.50 (d, 1H, *J* = 15.5 Hz), 3.87 (s, 3H), 2.32 (s, 3H). ^{13}C-NMR ($CDCl_3$): δ_C 168.95, 166.06, 151.97, 150.17, 142.88, 131.54, 124.47, 122.60, 122.55, 111.52, 56.10, 20.55.

1-*O*-(3'-*O*-Acetylferuloyl)-3,4-*O*-isopropylidene-1,5-quinide 59: To a solution of isopropylidene quinide, **52** (1000 mg, 4.67 mmol) in 50 mL CH_2Cl_2, a quantity of 71 mg (0.58 mmol, 15% mol) DMAP was added and a volume of 14 mL of NEt_3. A quantity of 1.78 g (7.00 mmol) 3-*O*-acetylferuloyl chloride **58** was then added and the mixture was refluxed for 24 h. It was then allowed to cool to r.t., acidified (pH 2) with a HCl 2M solution and extracted 3 times with CH_2Cl_2 (3x30 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated *in vacuo*. The product was purified by column chromatography (20-30% EtOAc/petroleum ether) to afford 1.23 g (2.85 mmol, 61%) of white solid **59**. ^{1}H-NMR ($CDCl_3$): δ_H 7.66 (d, 1H, *J* = 16.0 Hz), 7.11 (dd, 1H, *J* = 1.8, 7.8 Hz), 7.08 (d, 1H, *J* = 1.8 Hz), 7.05 (d, 1H, *J* = 7.8 Hz), 6.38 (d, 1H, *J* = 16.0 Hz), 4.81 (dd, 1H, *J* = 2.3, 6.4 Hz), 4.56 (td, 1H, *J* = 3.2, 6.9 Hz), 4.34 (m, 1H), 3,85 (s, 3H), 3.10 (m, 1H), 2.64 (d, 1H, *J* = 11.5 Hz), 2.52 (ddd, 1H, *J* = 2.3, 7.8, 14.7 Hz), 2.42 (dd, 1H, *J* = 3.2, 14.2 Hz), 2.31 (s, 3H), 1.53 (s, 3H), 1.33 (s, 3H). ^{13}C-NMR ($CDCl_3$): δ_C 173.61, 168.93, 164.97, 151.55, 146.08, 141.87, 133.02, 123.18, 121.52, 117.03, 111.56, 110.02, 76.43, 75.41, 72.33, 71.12, 56.03, 35.80, 30.91, 27.11, 24.22, 20.74.

1-*O*-Feruloyl-3,4-*O*-isopropylidene-quinic acid 60: A quantity of 540 mg (1.16 mmol) of 1-*O*-(3'-*O*-acetylferuloyl)-3,4-*O*-isopropylidene-1,5-quinide **59** was dissolved in 30 mL THF and 50 mL of a solution of 1M LiOH was added. The reaction mixture was stirred at r.t. for 20 min, was then acidified with 2M HCl (pH 2) and extracted with EtOAc (3x40 mL). The combined organic

layers were dried over Na_2SO_4 and the solvents removed *in vacuo*. The resulting residue was purified by column chromatography (30-40% EtOAc/petroleum ether) to give a pale yellow solid (224 mg, 0.55 mmol, 47%); ^{1}H-NMR ($(CD_3)_2SO$): δ_H 7.48 (d, 1H, *J* = 16.0 Hz), 7.24 (d, 1H, *J* = 1.8 Hz), 7.04 (dd, 1H, *J* = 1.8, 7.8 Hz), 6.76 (d, 1H, *J* = 7.8 Hz), 6.37 (d, 1H, *J* = 16.0 Hz), 4.29 (m, 1H), 3.82 (m, 1H), 3.36 (br, 1H), 3.27 (dd, 1H, *J* = 3.2, 7.8 Hz), 3.78 (s, 3H), 2.45 (m, 1H), 2.28 (dd, 1H, *J* = 5.0, 15.6 Hz), 2.12 (m, 1H), 1.60 (dd, 1H, *J* = 11.5, 13.3 Hz), 1.33 (s, 3H), 1.20 (s, 3H); ^{13}C-NMR ($(CD_3)_2SO$): δ_C 173.22, 165.86, 149.96, 148.49, 145.87, 126.00, 123.58, 116.08, 115.57, 111.57, 108.15, 80.49, 79.08, 73.25, 67.38, 56.19, 38.65, 31.21, 28.81, 26.42.

1-*O*-Feruloyl-3,4-*O*-isopropylidene-1,5-quinide 61: A quantity of 145 mg (0.36 mmol) of 1-*O*-feruloyl-3,4-*O*-isopropylidene-quinic acid was dissolved in 8 mL toluene and 1.36 mg (0.01 mmol) of PTSA·H_2O was added. The reaction was refluxed for 24 h, was cooled to r.t. and neutralized with solid $NaHCO_3$ (3 mg, 0.03 mmol). Toluene was removed under reduced pressure, the solid residue was dissolved in EtOAc, filtered and the filtrate was concentrated to afford 100 mg (quantitative yield) of the brown powder; ^{1}H-NMR ($CDCl_3$): δ_H 7.64 (d, 1H, *J* = 16.0 Hz), 7.07 (dd, 1H, *J* = 1.8, 8.2 Hz), 7.01 (d, 1H, *J* = 1.8 Hz), 6.91 (d, 1H, *J* = 8.2 Hz), 6.28 (d, 1H, *J* = 16.0 Hz), 4.82 (dd, 1H, *J* = 2.8, 6.4 Hz), 4.58 (ddd, 1H, *J* = 3.2, 6.9, 7.6 Hz), 4.34 (m, 1H), 3.92 (s, 3H), 3.11 (m, 1H), 2.64 (d, 1H, *J* = 11.5 Hz), 2.49 (ddd, 1H, *J* = 2.3, 7.6, 14.2 Hz), 2.43 (dd, 1H, *J* = 3.2, 14.2 Hz), 1.54 (s, 3H), 1.34 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ_C 173.96, 165.42, 148.66, 147.04, 147.00, 126.59, 123.59, 114.99, 113.98, 110.05, 109.68, 76.13, 75.58, 72.55, 71.25, 56.06, 35.74, 30.85, 27.06, 24.42.

1-*O*-Feruloyl-1,5-quinide (1-FQL) 32: A quantity of 100 mg (0.26 mmol) of 1-*O*-feruloyl-3,4-*O*-isopropylidene-1,5-quinide **61** was dissolved in 3.75 mL of TFA 80% solution and stirred for 40 min. The solvents were then removed in vacuum to give a red solid (90 mg, quantitative yield); mp 90 ºC; ^{1}H-NMR (CD_3-OD): δ_H 7.59 (d, 1H, *J* = 16.0 Hz), 7.15 (d, 1H, *J* = 1.8 Hz), 7.04 (dd, 1H, *J* = 1.8, 8.2 Hz), 6.79 (d, 1H, *J* = 8.2 Hz), 6.33 (d, 1H, *J* = 16.0 Hz), 4.84 (dd, 1H, *J* = 4.6, 6.4 Hz), 4.03 (t, 1H, *J* = 4.6 Hz), 3.85 (s, 3H), 3.86-3.74 (m, 1H), 3.04 (m, 1H), 2.57 (d, 1H, *J* = 11.0 Hz), 2.20-2.14 (m, 2H). ^{13}C-NMR (CD_3-OD): δ_C 173.74, 165.72, 149.55, 148.03, 146.90, 126.01, 123.17, 115.20, 113.20, 110.55, 77.27, 76.77, 65.74, 65.74, 55.20, 36.42, 32.68.

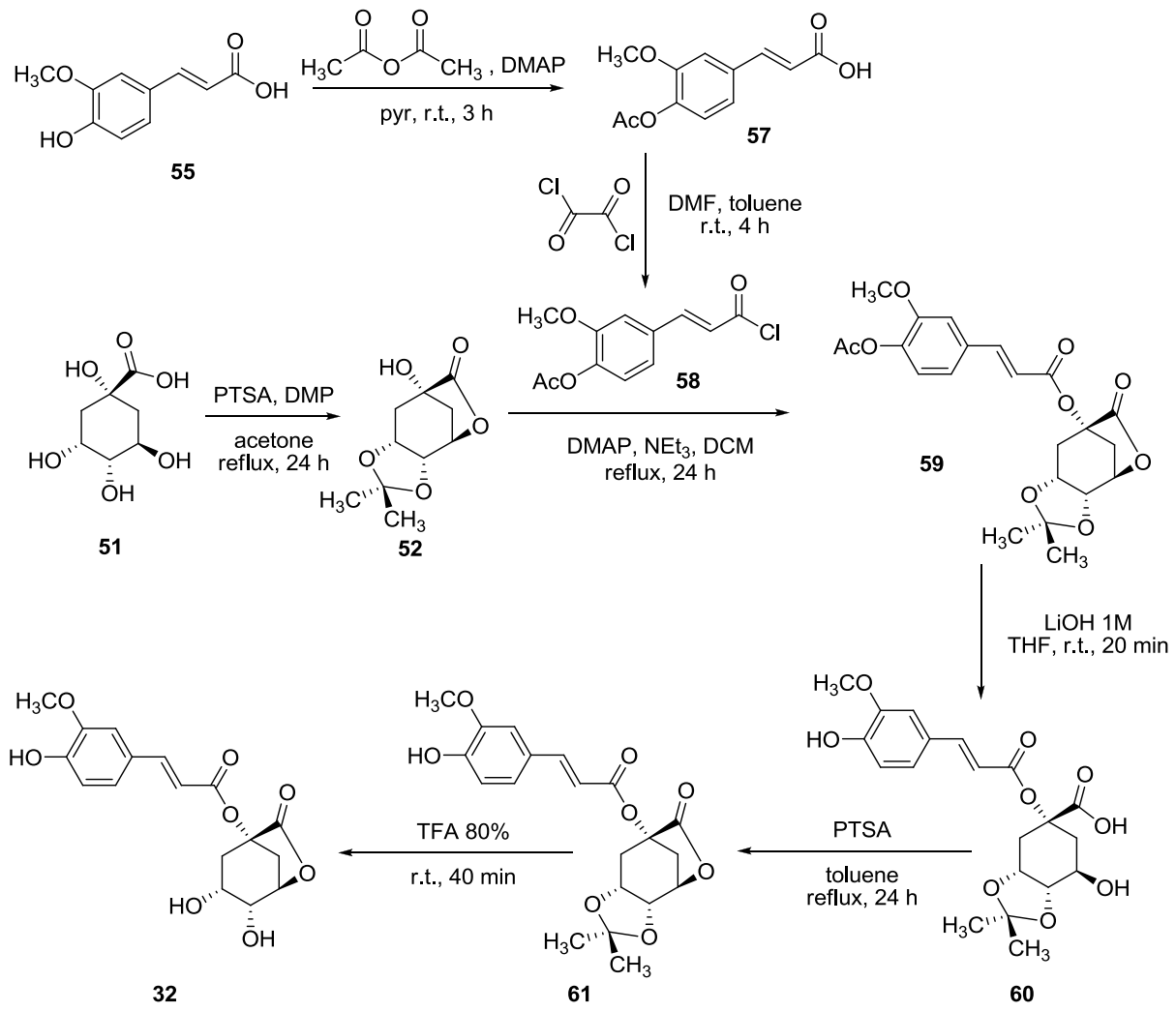

Figure 4.2. Synthetic path to 1-*O*-feruloyl-1,5-quinide **32**.

4.3. Results and Discussion

Food processing changes dramatically the chemical composition of a food. It is well established that new products arising from food processing are responsible for desirable sensory and organoleptic properties of food; it is less established but highly probable that these products are responsible for health benefits of numerous foods. Therefore, elucidating structures of processing products and their mechanism of formation constitutes an important area of food analysis. Coffee processing consists of four steps, removal of flesh, drying, roasting and brewing. While the first three have been investigated in some detail the last step has received no attention from a chemical composition perspective. During analysis of roasted coffee it was noted that samples

obtained from methanol extraction if compared to samples obtained from hot water extraction displayed notable differences in their chemical profile. Hot water extracts contained a significant larger number of chromatographically resolvable caffeoyl and dicaffeoylquinic acid derivatives along with a series of further previously unidentified components clearly produced in the brewing process.

Hence, it was decided that the products formed during coffee brewing from chlorogenic acid derivatives needed investigation in more detail. Surprisingly, hot water is not just a simple solvent in food chemistry, but can on occasions act as a reactive reagent, as shown here and in previous work on tea fermentation, where water was shown to be the key reagent in thearubigin formation.[24] For this study a total of three mono- and three diacylated chlorogenic acids, all purchased as reference standards, were used individually as model systems along with established food processing products in roasted coffee 1-*O*-caffeoyl-1,5-quinide **31** and 1-*O*-feruloyl-1,5-quinide **32**, obtained by total synthesis; for the selective syntheses[25-27] of 1-*O*-caffeoyl-1,5-quinide **31** and 1-*O*-feruloyl-1,5-quinide **32** methods reported in literature were followed with minor modifications. The chlorogenic acids and their derivatives tested for water addition at their cinnamoyl residue are shown in **Figure 4.3**. Additionally, four different commercial roasted Robusta coffee samples were prepared by brewing of coffee powder with boiling water and the compounds identified in the model systems were compared to those observed in the real brew.

For the synthetic part, it should be mentioned that the allyl substituent for the phenolic OH is the preferred protecting group over acetyl protection; syntheses with both protecting groups were presented in this work. Acid-removal of the acetyl protecting group seemed to generate a multitude of unidentified side-products which made purification of the desired product rather tedious or even impossible. Base-removal of the acetyl protection opens the 1,5-lactone, thus generating an additional step (closing the lactone) in the synthesis; in addition, after the deprotection step, the compounds were still of higher purity when the allyl-protection was used. 1-*O*-caffeoyl-1,5-quinide **31** was synthesized using allyl-protection for caffeic acid phenols, while 1-*O*-feruloyl-1,5-quinide **32** was made available using acetyl-protection. To generate 1-*O*-caffeoyl-1,5-quinide **31** the commercially available quinic acid **51** and caffeic acid **49** were used as starting material.

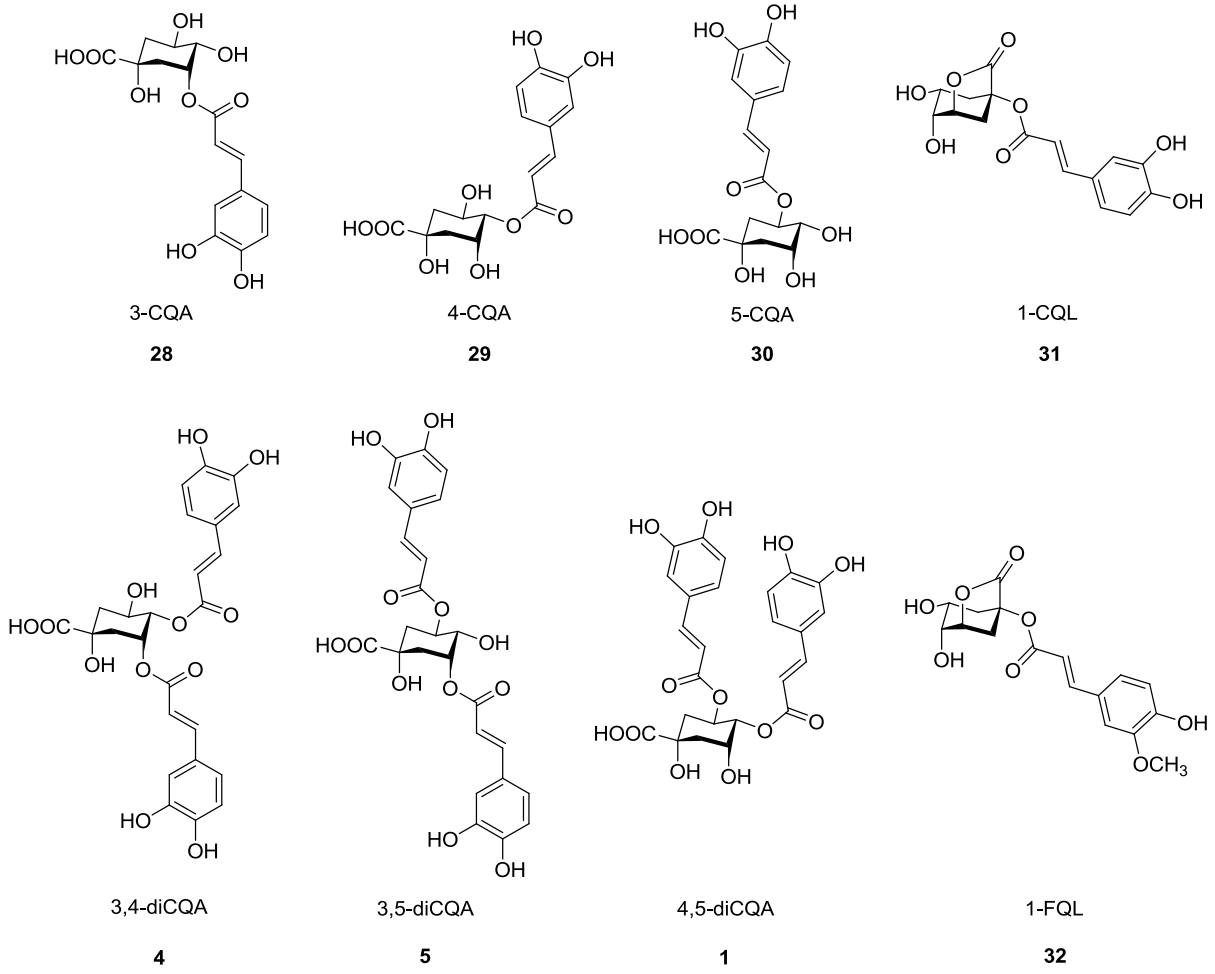

Figure 4.3. Chlorogenic acids and derivatives tested for water addition at the cinnamoyl residue.

After selective protection of the reactive moieties, an esterification step generated the main intermediate in good yield. The desired product was then obtained after the final two deprotections. At temperatures higher than 70 °C in the allyl-deprotection step, the isopropylidene protection is also removed making the synthesis shorter by one step; however, the yield of the product obtained after purification by column chromatography was not satisfactory and an additional step in the synthesis was preferred, with better yields and higher purities.

4.3.1. Investigation of Model Compounds

Firstly, three monocaffeoylquinic acids, 3-*O*-caffeoylquinic acid **28**, 4-*O*-caffeoylquinic acid **29** and 5-*O*-caffeoylquinic acid **30**, were subjected to model brewing conditions using 5 h of hot water (100 ˚C) treatment followed by HPLC-MS analysis. The HPLC-chromatograms showed between four and twelve distinct peaks corresponding to the products formed. The main focus was on the water addition products, a reaction mechanism leading to water addition and elimination being proposed in **Figure 4.4**.

The products observed can be categorized into three types of chlorogenic acids derivatives (observed not only for mono- but also for diacylated compounds): firstly, hydroxydihydrocaffeoylquinic acids arising through conjugate addition of water to the olefinic cinnamoyl moiety (**Figures 4.5** and **4.6**); secondly, acyl-migration products, including a selection of different caffeoylquinic acid regioisomers (**Figure 4.7**); and finally, *trans*-*cis* isomerization (*cis*-caffeoylquinic acids) products, presumably obtained by reversible *β*-elimination of water from hydroxydihydrocaffeoylquinic acids (**Figure 4.8**). The last two classes of derivatives have been analyzed and identified on previous occasions using authentic reference compounds in conjunction with LC-MS/MS.[28,29] In this study they were assigned based on retention times and tandem MS data and listed whenever observed (**Table 4.1**). Further unidentified minor products were observed in the chromatograms.

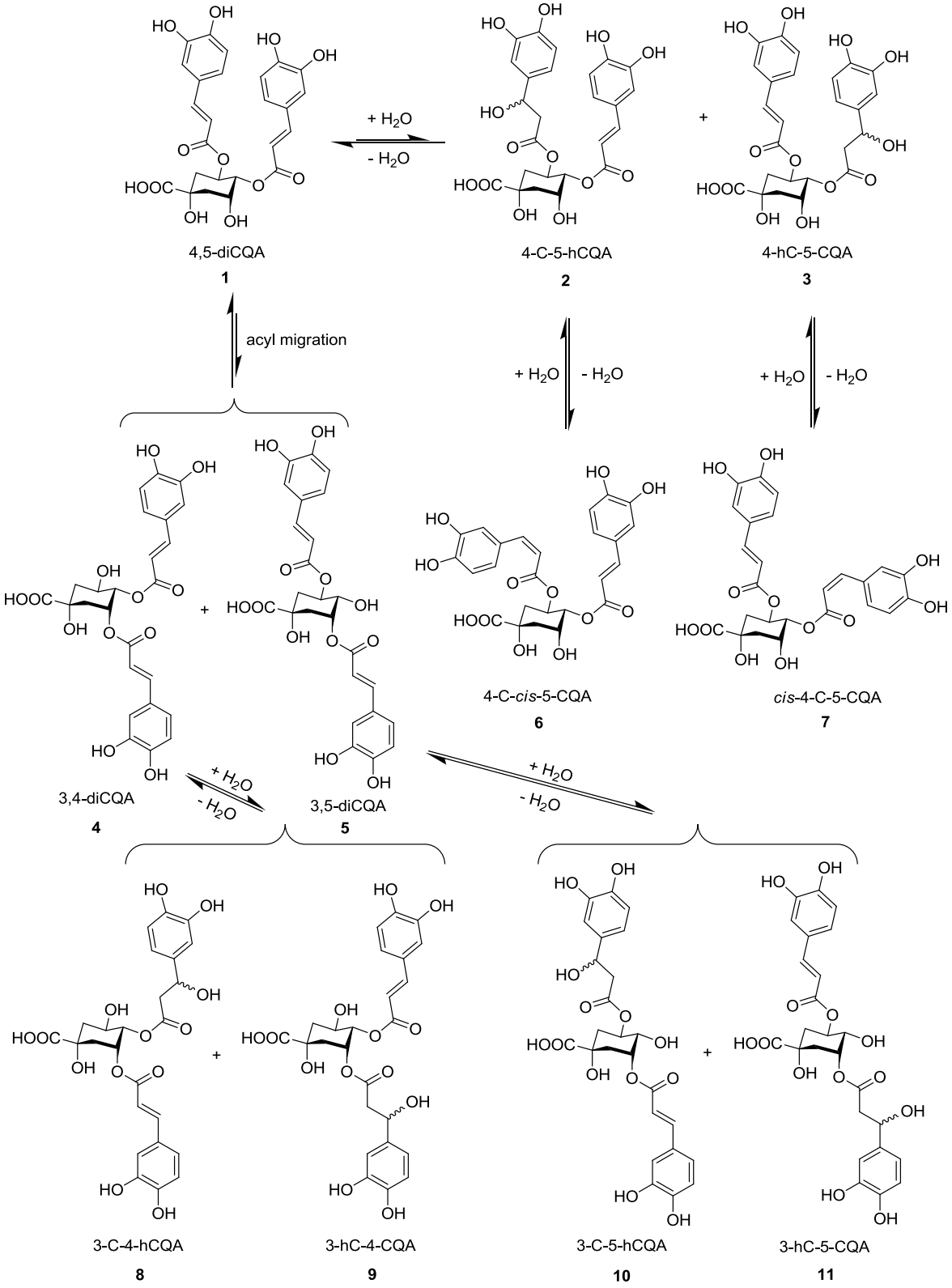

Figure 4.4. Formation of chlorogenic acid derivatives during the brewing of coffee (**Tables 4.1** and **4.2**).

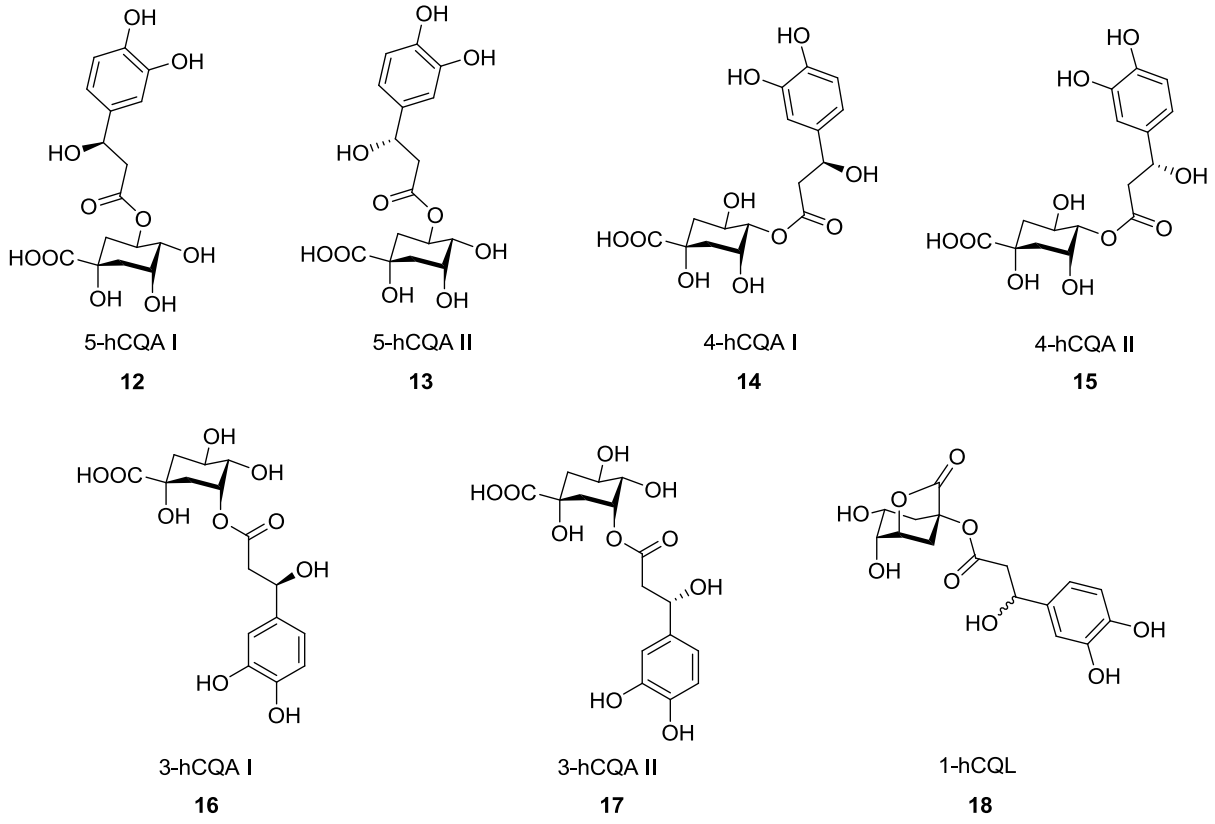

Figure 4.5. Monoacylated water addition derivatives of chlorogenic acids and lactones formed during brewing (**Tables 4.1** and **4.2**).

For the class of diacylated hydroxydihydrocaffeoylquinic acids, a detailed individual tandem MS study was conducted for each novel derivative reported. The substrates tested successfully for water addition at the C=C of the cinnamoyl moiety of chlorogenic acids (hydroxydihydrocaffeoylquinic acids are the products of this process) were: 3,4-di-*O*-caffeoylquinic acid **4**, 3,5-di-*O*-caffeoylquinic acid **5** and 4,5-di-*O*-caffeoylquinic acid **1**. All novel diacylated hydroxydihydrocaffeoylquinic acids observed in the present work are shown in **Figure 4.6**. **Table 4.2** shows complete negative ion MS^4 data for detected hydroxydihydrocaffeoylquinic acids (mono- and diacylated) and hydroxydihydrocaffeoylquinide. **Table 4.3** shows high resolution mass (MS-TOF) data of hydroxycinnamates observed during the brewing of coffee.

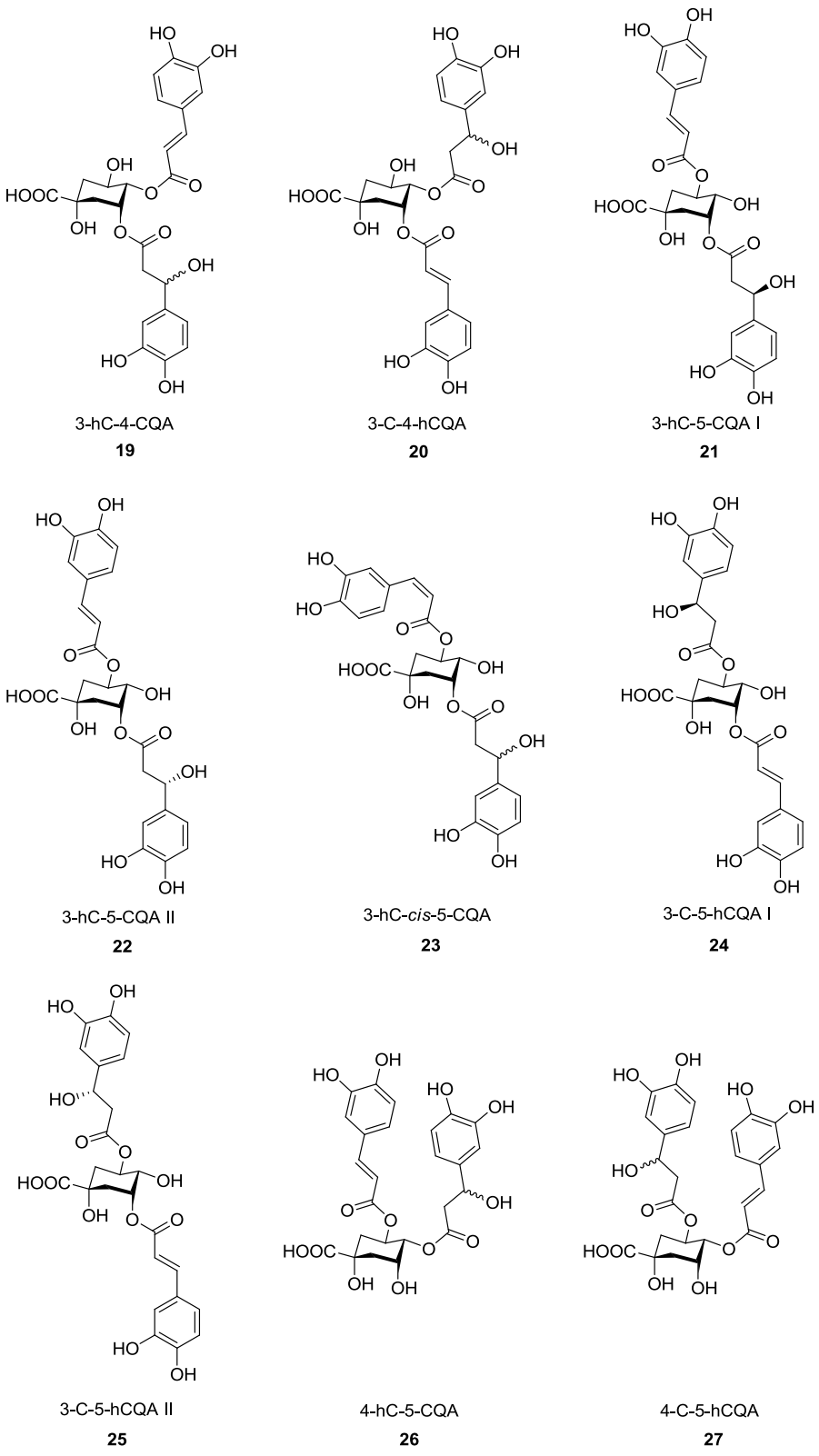

Figure 4.6. Diacylated water addition derivatives of chlorogenic acids formed during brewing (**Tables 4.1** and **4.2**).

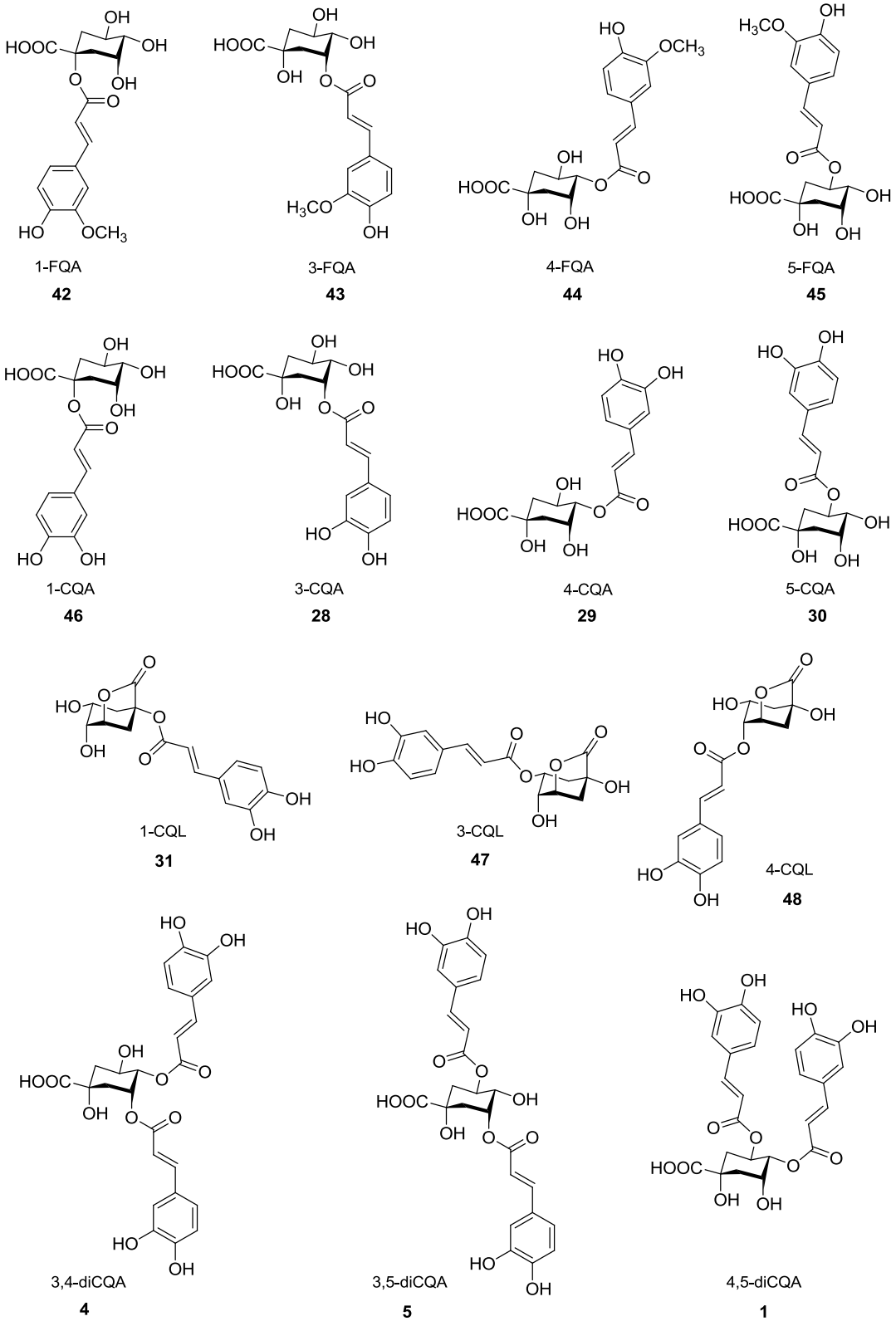

Figure 4.7. Acyl migration isomers of chlorogenic acids and lactones formed during brewing.

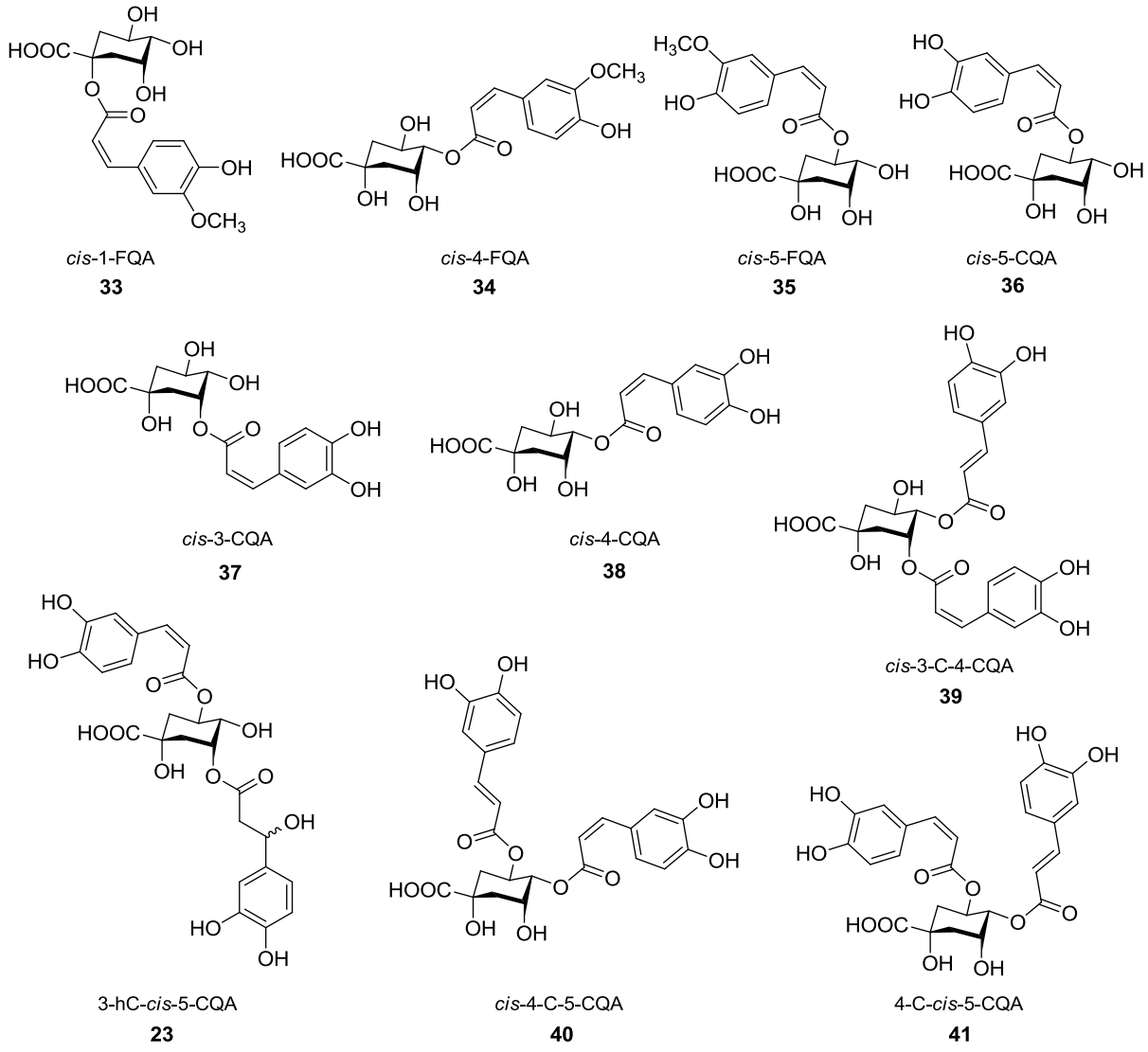

Figure 4.8. *Cis*-isomers of chlorogenic acids formed during brewing.

Table 4.1. Retention times of chlorogenic acids and their derivatives.

Starting‡	Product name‡	RT [min]	*m/z* [M−H]
5-CQA	4-CQA	20.6	353
	5-CQA	20.1	353
	cis-5-CQA	23.0	353
	5-hCQA I	7.3	371
	5-hCQA II	7.9	371
4-CQA	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	cis-3-CQA	11.9	353
	cis-4-CQA	16.5	353
	cis-5-CQA	23.0	353
	4-hCQA I	6.9	371
	4-hCQA II	7.9	371
3-CQA	3-CQA	13.1	353
	4-CQA	20.6	353
	cis-3-CQA	11.9	353
	3-hCQA I + II	5.6	371
3,4-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-3,4-diCQA I	35.9	515
	cis-3,4-diCQA II	38.5	515
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	3-hC-4-CQA	27.9	533
	3-C-4-hCQA	27.4	533
3,5-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-3,5-diCQA I	36.1	515
	cis-3,5-diCQA II	38.5	515
	3-C-5-hCQAI	24.1	533
	3-hC-5-CQA I	27.6	533
	3-hC-*cis*-5-CQA	28.4	533
	3-hC-5-CQA II	30.9	533
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
4,5-diCQA	3,4-diCQA	36.7	515
	3,5-diCQA	37.4	515
	4,5-diCQA	41.4	515
	cis-4,5-diCQA I	39.5	515
	cis-4,5-diCQA II	45.8	515
	3-CQA	13.1	353
	4-CQA	20.6	353
	5-CQA	20.1	353
	4-hC-5-CQA	30.4	533
	3-hC-5-CQA II	30.9	533
	3-C-5-hCQA II	31.8	533
1-CQL (diphenyl column)	1-CQL	31.1	335
	3-CQL	31.1	353
	4-CQL	26.7	353
	1-CQA	28.2	353
	3-CQA	10.9	353
	4-CQA	13.1	353
	5-CQA	20.6	353
	1-hCQL	20.1	353
1-FQL (diphenyl column)	1-FQA	18.7	367
	3-FQA	19.2	367
	4-FQA	26.7	367
	5-FQA	27.1	367
	cis-1-FQA	16.1	367
	cis-4-FQA	24.1	367
	cis-5-FQA	29.9	367
caffeic acid	-	-	-
ferulic acid	-	-	-

‡C = caffeoyl; F = feruloyl; hC = 3-hydroxydihydro caffeoyl; QA = quinic acid; QL = quinic acid lactone.

‡C = caffeoyl; F = feruloyl; hC = 3-hydroxydihydro caffeoyl; QA = quinic acid; QL = quinic acid lactone.

Table 4.2. Negative ion MS^4 data for detected hydroxydihydrocaffeoylquinic acids and hydroxydihydrocaffeoylquinide.

		MS^1	MS^2					MS^3			MS^4		
		Parent ion	Base peak	Secondary peaks		*β*-OH peak		Base peak	Secondary peaks		Base peak	Secondary peaks	
No.	**Cpd.‡**	***m/z***	***m/z***	***m/z***	**Int.**	***m/z***	**Int.**	***m/z***	***m/z***	**Int.**	***m/z***	***m/z***	**Int.**
1.	5-hCQA	370.9	190.5	352.7	86	233.4	0.2	126.5	172.5	60			
						(MS^2)			110.6	18			
									92.7	79			
									84.8	55			
2.	4-hCQA	371.0	352.7	190.5	8	233.4	1.7	172.5	190.5	35	110.6	92.7	83
				178.5	13	(MS^2)			178.8	52			
				172.5	94				134.5	6			
				134.6	16								
3.	3-hCQA	370.9	352.7	190.5	30	232.5	1.5	190.5	178.8	44	126.5	172.4	27
				178.6	16	(MS^2)			172.8	13		85.1	37
				172.6	16				134.6	11			
				134.6	66								
4.	1-hCQL (diphenyl column)	352.9	334.8	172.6	8	214.6	0.1	160.6	178.6	6	132.7		
				160.6	6	(MS^2)			172.8	50			
				136.6	11				132.7	8			
5.	3-hC-4-CQA	533.0	514.9	352.8	95	394.8	6.3	352.7	334.8	12	172.5	191.0	47
				462.5	9	(MS^2)			298.7	2		178.9	93
				370.7	27	232.6	2.0		254.6	6		134.5	12
				334.8	46	(MS^2)			191.0	6			
				298.7	4				178.9	15			
				254.6	6				172.5	21			
				191.0	5								
				178.9	8								
				172.5	18								
6.	3-C-4-hCQA	533.0	370.8	514.8	72	394.8	1.6	352.8	190.6	10	172.5	190.6	23
				462.5	45	(MS^2)			178.9	14		178.9	54
				352.8	95	232.5	1.2		172.5	69			
				334.9	34	(MS^2)			134.5	15			
				190.9	7	232.5	3.3						
				178.8	9	(MS^3)							
				172.5	33								
7.	3-C-5-hCQA	533.1	352.7	514.8	13	394.5	1.1	190.5	334.7	13	85.0	126.8	37
				462.4	62	(MS^2)			178.8	68			
				396.8	15				172.8	20			
				371.2	37				134.5	12			
				335.1	12								
				190.5	16								
				178.8	8								
				173.0	8								
8.	3-hC-5-CQA	533.1	370.7	514.9	2	394.8	0.4	352.7	190.5	30	190.4	178.8	44
				462.5	45	(MS^2)			178.8	21		172.8	23
				396.5	12	232.5	0.1		172.8	25		160.5	6
				352.7	11	(MS^2)			134.5	64		134.5	18
				335.1	2	232.5	1.7						
				190.5	3	(MS^3)							
				178.8	1								
				173.0	2								
9.	4-C-5-hCQA (diphenyl column)	532.9	352.9	514.9	90	394.8	5.4	172.6	334.8	29	154.5	110.8	71
				462.6	27	(MS^2)			190.6	38		92.8	55
				396.6	6	232.8	1.4		178.6	63			
				370.8	16	(MS^2)			134.6	11			
				335.0	33								
				190.6	5								
				178.8	8								
				172.6	16								
10.	4-hC-5-CQA	533.0	370.8	514.8	3	394.8	0.5	352.7	190.5	13	172.5	190.5	81
				462.5	24	(MS^2)			178.5	8		178.5	53
				396.6	9	232.5	0.3		172.5	58		134.5	10
				352.7	24	(MS^2)			134.5	18			
				334.9	2	232.5	0.9						
				190.9	2	(MS^3)							
				178.8	2								
				172.5	9								

‡C = caffeoyl; F = feruloyl; hC = 3-hydroxydihydrocaffeoyl; QA = quinic acid; QL = quinic acid lactone.

Table 4.3. High resolution mass (MS-TOF) data of hydroxycinnamates observed during the brewing of coffee.

No.	CGA‡	Molecular formula	Theoretical *m/z* [M−H]	Experimental *m/z* [M−H]	Error (ppm)
1	3-CQA	$C_{16}H_{18}O_9$	353.0878	353.0864	3.9
2	4-CQA	$C_{16}H_{18}O_9$	353.0878	353.0894	-4.5
3	5-CQA	$C_{16}H_{18}O_9$	353.0878	353.0888	-2.9
4	*cis*-3-CQA	$C_{16}H_{18}O_9$	353.0878	353.0884	-1.7
5	*cis*-4-CQA	$C_{16}H_{18}O_9$	353.0878	353.0881	-0.8
6	*cis*-5-CQA	$C_{16}H_{18}O_9$	353.0878	353.0893	-4.3
7	3-hCQA	$C_{16}H_{20}O_{10}$	371.0984	371.0987	-1.0
8	4-hCQA	$C_{16}H_{20}O_{10}$	371.0984	371.0984	-3.8
9	5-hCQA	$C_{16}H_{20}O_{10}$	371.0984	371.0978	1.5
10	3,4-diCQA	$C_{25}H_{24}O_{12}$	515.1195	515.1173	4.3
11	3,5-diCQA	$C_{25}H_{24}O_{12}$	515.1195	515.1177	3.4
12	4,5-diCQA	$C_{25}H_{24}O_{12}$	515.1195	515.1170	4.9
13	*cis*-3,4-diCQA I	$C_{25}H_{24}O_{12}$	515.1195	515.1169	5.0
14	*cis*-4,5-diCQA I	$C_{25}H_{24}O_{12}$	515.1195	515.1180	2.9
15	*cis*-4,5-diCQA II	$C_{25}H_{24}O_{12}$	515.1195	515.1169	5.0
16	3-hC-4-CQA	$C_{25}H_{26}O_{13}$	533.1301	533.1314	-2.5
17	3-C-4-hCQA	$C_{25}H_{26}O_{13}$	533.1301	533.1275	4.9
18	3-C-5-hCQA	$C_{25}H_{26}O_{13}$	533.1301	533.1278	4.3
19	3-hC-5-CQA	$C_{25}H_{26}O_{13}$	533.1301	533.1277	4.4
20	4-C-5-hCQA	$C_{25}H_{26}O_{13}$	533.1301	533.1276	4.6
21	4-hC-5-CQA	$C_{25}H_{26}O_{13}$	533.1301	533.1329	-4.2

‡C = caffeoyl; F = feruloyl; hC = 3-hydroxydihydrocaffeoyl; QA = quinic acid; QL = quinic acid lactone.

4.3.2. Tandem MS Characterization of Monoacylated 3'-Hydroxydihydrocaffeoylquinic Acids (M_r = 372)

Conjugate water addition to the olefinic cinnamoyl moiety of monoacylated chlorogenic acids was observed for three caffeoyl-containing substrates (3-*O*-caffeoylquinic acid **28**, 4-*O*-caffeoylquinic acid **29** and 5-*O*-caffeoylquinic acid **30**). For each of the three investigated monoacylated chlorogenic acids two corresponding hydroxydihydrocaffeoylquinic acids resulting from water addition could be distinguished as two chromatographically resolved peaks, appearing as pseudomolecular ions in the negative ion mode at *m/z* 371 ($C_{16}H_{19}O_{10}$) (**Figures 4.9** and **4.10**); for 3-*O*-caffeoylquinic acid **28** they virtually co-eluted but an early shoulder in the chromatographic peak can be observed (**Figure 4.11**). The compounds produce in MS^2 a base peak at *m/z* 353 ($C_{16}H_{17}O_9$) from the precursor ion at *m/z* 371 corresponding to loss of water. The

MS^3 fragmentation patterns of the precursor ion at *m/z* 353 ($[M - H^+ - H_2O]^-$) of hydroxydihydrocaffeoylquinic acids are similar or even identical to the MS^2 fragmentation of the corresponding chlorogenic acids, based on the structural identity of the precursor ions, and allow therefore unambiguous assignment of acyl regiochemistry.

Equally, the MS^4 fragment spectra of hydroxydihydrocaffeoylquinic acids are similar to the MS^3 spectra of the corresponding chlorogenic acids, as expected. In all six cases the hydroxyl moiety was confirmed by the MS^n fragmentation to be located at the *β*-position in the dihydrocinnamoyl residue and on no occasion could an *α*-hydroxyl be detected (**Table 4.2**). This assignment of regiochemistry is based on a characteristic fragment ion at *m/z* 233 ($C_9H_{13}O_7$) showing a neutral loss of 138 Da corresponding to $C_7H_6O_3$. The fragmentation mechanism observed here can be classified as a retro-aldol type fragmentation, indicative of the regiochemistry of water addition. It was previously shown for malate esters of quinic acid that this fragmentation pathway can be used to unambiguously establish alcohol regiochemistry in chlorogenic acids chemistry.[30]

This finding implies that water addition to the double bond of the cinnamoyl residue of chlorogenic acids takes place in a regiospecific manner. The result is in contrast to the finding by Dawidowicz et al.[31] who attributed their observations to the presence of both *β*- and *α*-hydroxylated 5-*O*-caffeoylquinic acid, in a similar experiment; a more recent publication by the same group is in agreement with the findings presented in the current work.[29] In our work the two very closely eluting peaks with pseudomolecular ions at *m/z* 371 observed after brewing of 5-*O*-caffeoylquinic acid **30** are clearly the two diastereoisomers of 5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid, **12** and **13** as confirmed by the characteristic retro-aldol fragment ion in the fragment spectra (**Figure 4.9** and **Table 4.2**).

The same is true for 3-*O*-caffeoylquinic acid **28** and 4-*O*-caffeoylquinic acid **29** (2 diastereoisomers for each) but in the case of 3-*O*-caffeoylquinic acid **28** they were virtually co-eluting and could chromatographically not be resolved well with the method used. It should be mentioned that tandem MS is isomer blind with respect to stereochemistry but since the water molecule is added regiospecifically to the cinnamoyl residue, the two chromatographic peaks observed for each *β*-hydroxylated chlorogenic acid giving identical tandem MS data could only be the two diastereoisomers.

5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid I **12** and 5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid II **13** were identified by their *m/z* 371 ($[M - H^+]^-$) parent ion and they both produced the MS^2 base peak at *m/z* 191 ($[quinic\ acid - H^+]^-$) and a secondary peak at *m/z* 353 ($[M - H_2O - H^+]^-$) (**Figure 4.9**). 5-acylation was confirmed by the low intensity MS^2 secondary peak at *m/z* 179 ($[caffeic\ acid - H^+]^-$), the MS^3 base peak at *m/z* 85 and the MS^3 secondary peak at *m/z* 173 ($[quinic\ acid - H_2O - H^+]^-$), as detailed in previous studies.[5,9] The presence of the hydroxyl group at the *β*-position was confirmed by the very low intensity MS^2 peak at *m/z* 233 (**Table 4.2**). A non-regiospecific water addition to the originally *trans* double bond of the cinnamoyl residue should have generated an additional MS^2 fragment at either *m/z* 249 (quinic acid moiety) or *m/z* 123 (caffeoyl moiety), neither being detected. This observation is consistent with the findings for all the mono- and diacylated chlorogenic acids tested for water addition in the present study; the specific MS^2 fragment always points towards the hydroxyl at the *β*-position and never at *α*-position.

4-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid I **14** and 4-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid II **15** were preliminarily identified by their *m/z* 371 and produced the MS^2 base peak at *m/z* 353 ($[M - H_2O - H^+]^-$) and secondary peaks at *m/z* 191 ($[quinic\ acid - H^+]^-$), 179 ($[caffeic\ acid - H^+]^-$), 173 (most intense, $[quinic\ acid - H_2O - H^+]^-$) and 135 ($[caffeic\ acid - CO_2 - H^+]^-$) (**Figure 4.10**). The MS^3 spectrum of *m/z* 353 revealed the base peak at *m/z* 173 and secondary peaks at *m/z* 191, 179 and 135 (low intensity), specific to acylation at C4 of quinic acid. The MS^4 spectrum produced the base peak at *m/z* 85 and secondary peaks at *m/z* 111 and *m/z* 93 (**Table 4.2**), fragments whose structure was proposed before.[9] The water addition specific fragment, though of very low intensity, appeared as expected at *m/z* 233 in the MS^2 spectrum.

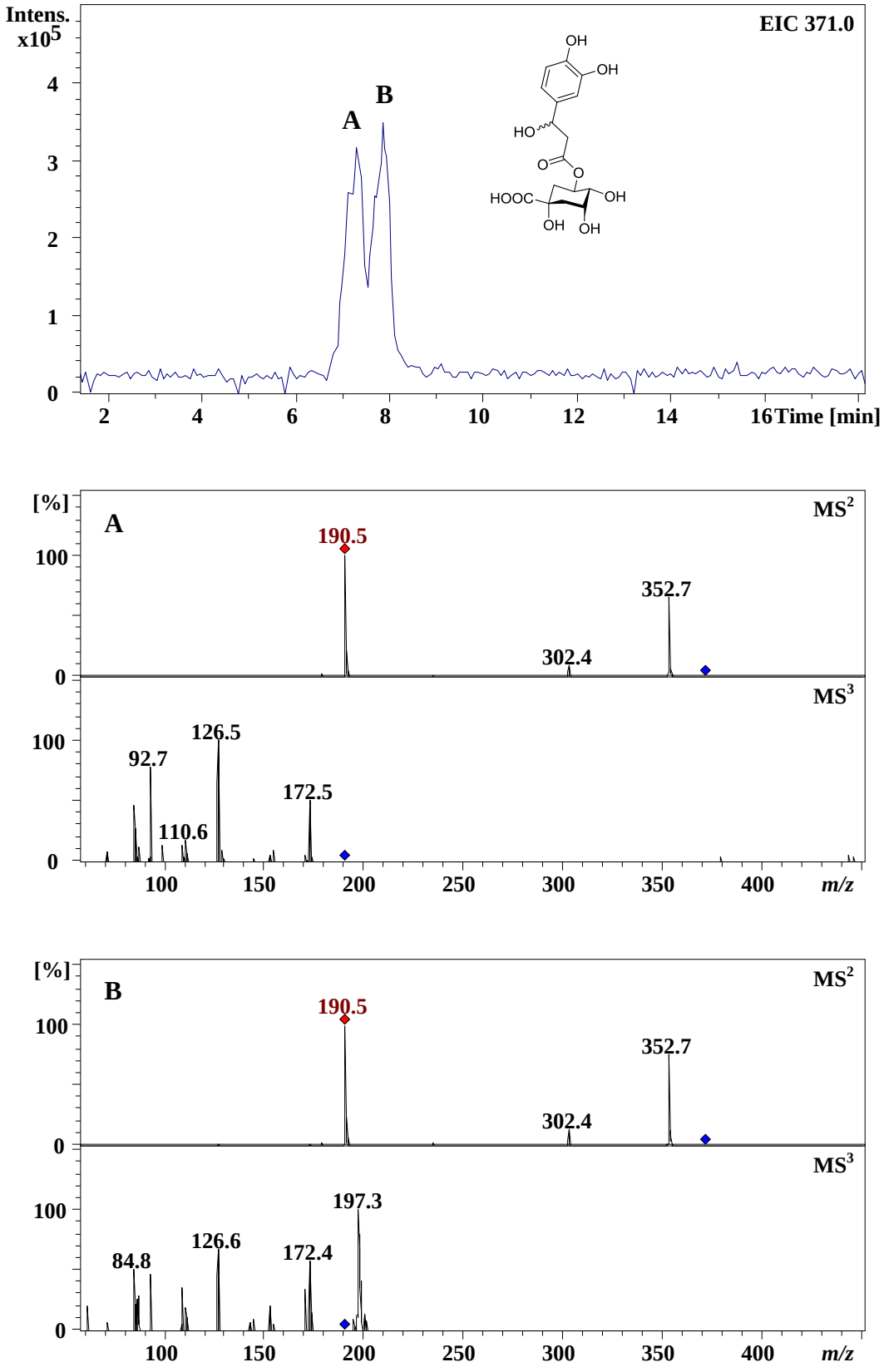

Figure 4.9. EIC of ion at *m/z* 371 showing two diastereomeric water addition compounds, **12** and **13**; tandem MS spectra in negative ion mode of **12** and **13** with precursor ions at *m/z* 371.

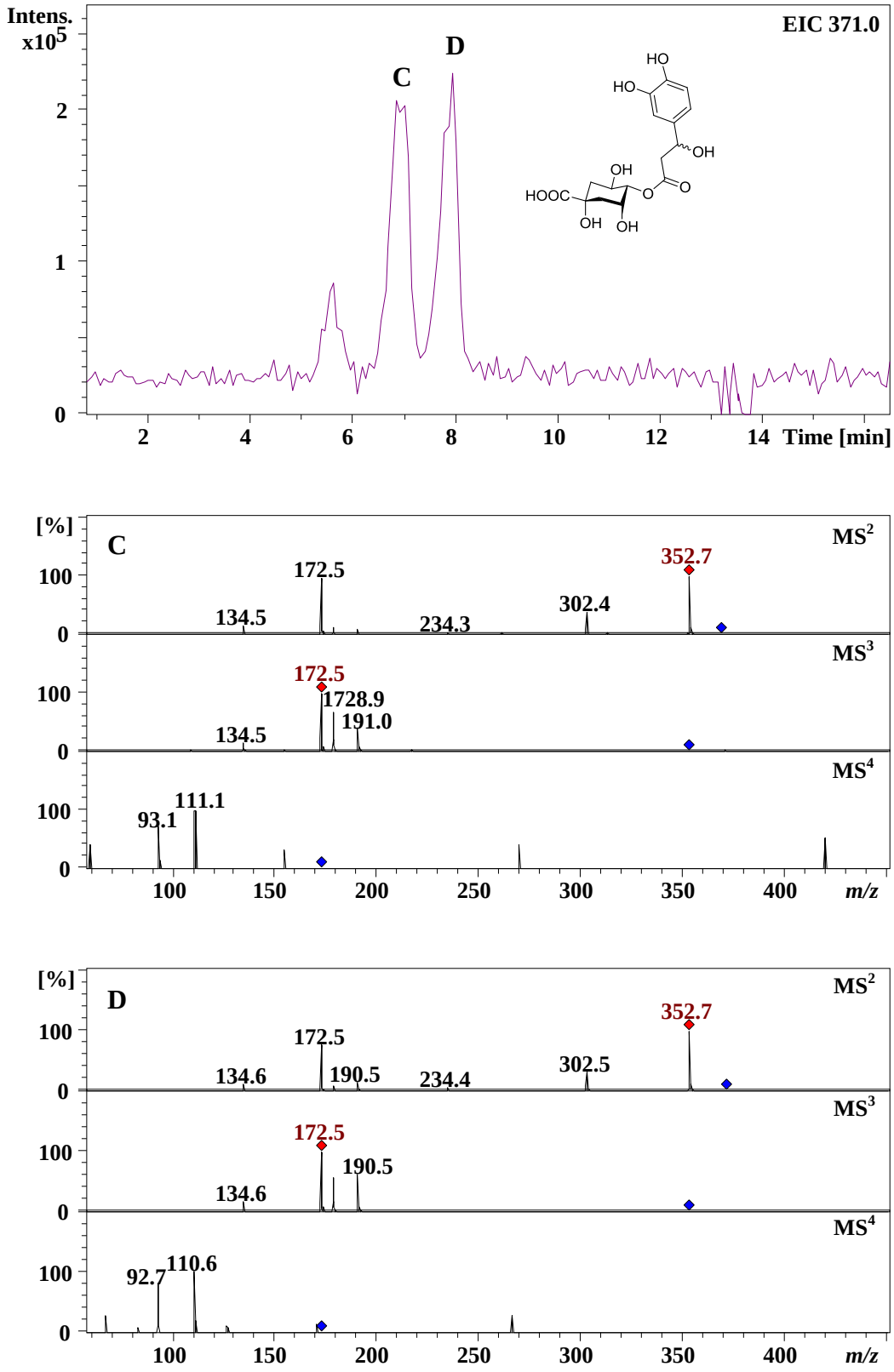

Figure 4.10. EIC of ion at *m/z* 371 showing two diastereomeric water addition compounds, **14** and **15**; tandem MS spectra in negative ion mode of **14** and **15** with precursor ions at *m/z* 371.

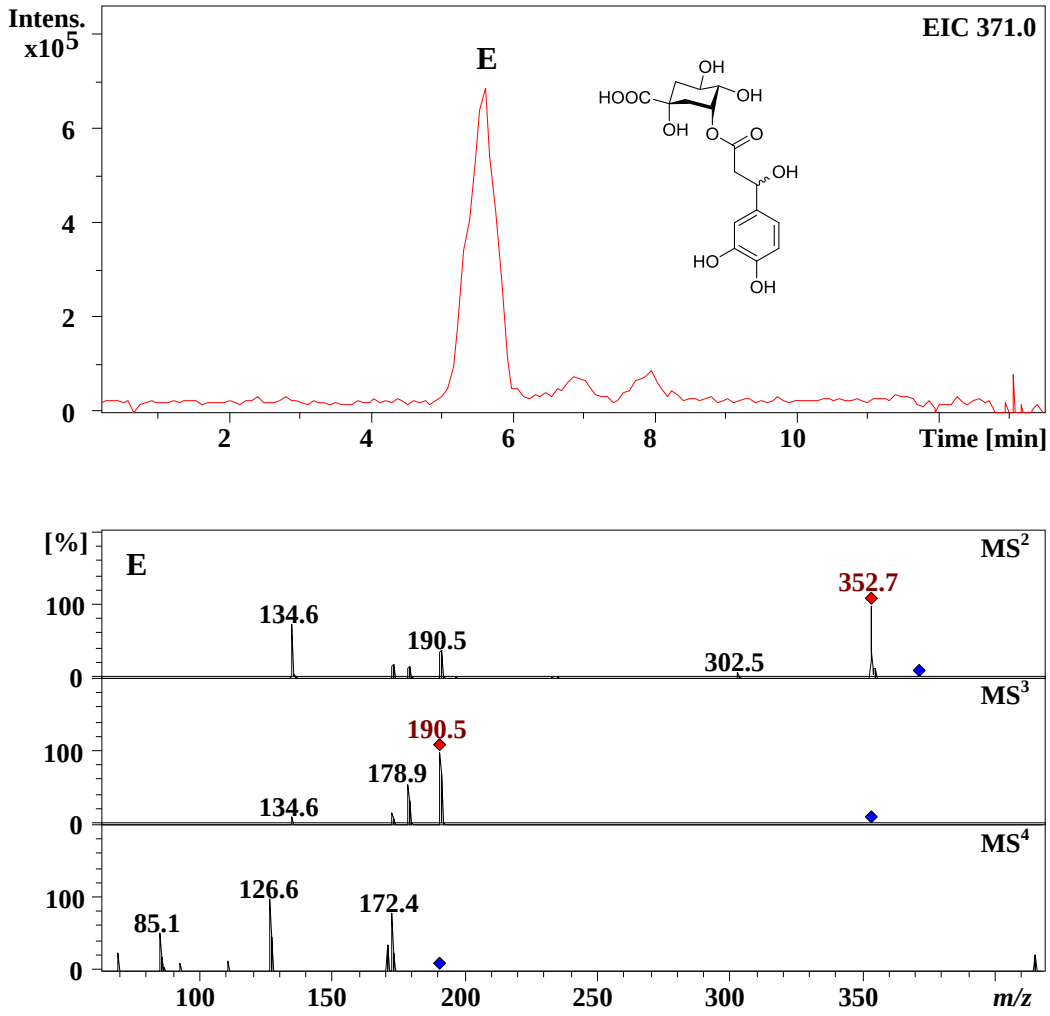

Figure 4.11. EIC of ion at *m/z* 371 showing two diastereomeric water addition compounds co-eluting, **16** and **17**; tandem MS spectra in negative ion mode of **16** and **17** with precursor ion at *m/z* 371.

3-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid I **16** and 3-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid II **17** were preliminarily identified by their *m/z* 371 and produced the MS^2 base peak at *m/z* 353 ($[M - H_2O - H^+]^-$) and secondary peaks at *m/z* 191 ([quinic acid – $H^+]^-$), 179 ([caffeic acid – $H^+]^-$), 173 ([quinic acid – H_2O – $H^+]^-$), 135 ([caffeic acid – CO_2 – $H^+]^-$) (**Figure 4.11**). The MS^3 spectrum of *m/z* 353 revealed the base peak at *m/z* 191 and secondary peaks at *m/z* 179, 173 and 135, with the last two of low intensity, specific to acylation at C3 of quinic acid. The water addition specific fragment, though of very low intensity, appeared as expected at *m/z* 233 in the MS^2 spectrum (**Table 4.2**). It should be noted that assignment of acyl regiochemistry in these cases is only possible in a targeted MS^3 experiment of the precursor fragment ion at *m/z* 353,

since only this ion is structurally identical to the parent ion in the chlorogenic acid reference mass spectra.

4.3.3. Characterization of Monoacylated 3'-Hydroxydihydrocaffeoylquinic Acid Lactone (M_r = 354)

1-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid lactone **18** (**Figure 4.5**) or 1-*O*-(3'-hydroxydihydrocaffeoyl)-1,5-quinide **18** was observed as a derivative of the parent 1-*O*-caffeoyl-1,5-quinide **31**, which was made available through total synthesis. The corresponding water addition derivative containing a feruloyl moiety was not observed for the synthetic 1-*O*-feruloyl-1,5-quinide **32** substrate, but it cannot be concluded at the moment (and it is unlikely) that water addition to the C=C of the cinnamoyl residue is peculiar to caffeoyl-containing substrates only.

1-*O*-(3'-hydroxydihydrocaffeoyl)-1,5-quinide **18** was preliminarily identified by its *m/z* 353 ([M – H^+]$^-$) parent ion. Compound **18** can be easily mistaken as a caffeoylquinic acid because of the identical MS *m/z* value (formally 1-*O*-caffeoylquinic acid - H_2O + H_2O, only that the water elimination and addition take place at different moieties within the molecule), but tandem MS can easily discriminate between 1-*O*-caffeoylquinic acid[10] and **18**. 1-*O*-(3'-hydroxydihydrocaffeoyl)-1,5-quinide **18** produced the MS^2 base peak at *m/z* 335 ([M – H_2O – H^+]$^-$) and secondary peaks with different intensities at *m/z* 173 ([quinic acid – H_2O – H^+]$^-$), 161, 137 and 111 (**Figure 4.12**). The MS^3 spectrum produced the base peak at *m/z* 161 and secondary peaks with different intensities at *m/z* 179, 173, 155, 133, 111 and 93. The structure of these tandem MS fragments was previously proposed.[32] In the MS^4 spectrum the base and only peak was observed at *m/z* 133. The specific fragment confirming the *β*-hydroxyl position was detected in MS^2 at *m/z* 215 (**Table 4.2**).

An *α*-hydroxyl would be expected to generate fragments at most likely *m/z* 231 or *m/z* 123 but neither was detected. It is interesting to note that compound **18** constitutes an isomer of caffeoylquinic acid that could on other occasions easily be mistaken for a diastereoisomer of caffeoylquinic acid observed in roasted coffee.

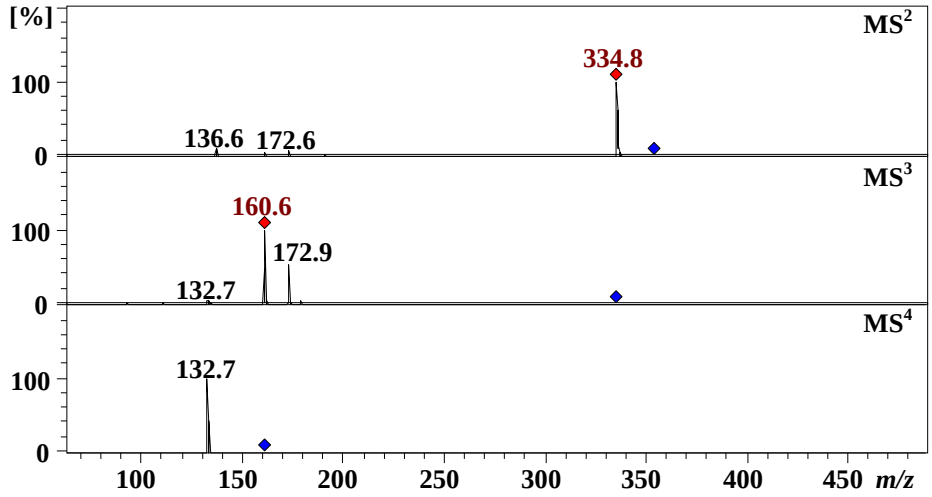

Figure 4.12. Tandem MS spectra in negative ion mode of **18** with precursor ion at *m/z* 353.

4.3.4. Characterization of Diacylated Caffeoyl-3'-hydroxydihydrocaffeoylquinic Acids (M_r = 534)

When homo dicaffeoylquinic acids (3,4-di-*O*-caffeoylquinic acid **4**, 3,5-di-*O*-caffeoylquinic acid **5** and 4,5-di-*O*-caffeoylquinic acid **1**) were subjected to model coffee brewing conditions the chromatograms revealed the formation of six to eighteen novel products. The products included similarly to the monocaffeoyl derivatives, products of acyl migration, *trans-cis* isomerization and conjugate water addition. The conjugate addition of water was always observed only for one of the two caffeoyl moieties showing pseudomolecular ions at *m/z* 533 in the negative ion mode. Compounds showing conjugate water addition to both cinnamoyl moieties with expected pseudomolecular ions at *m/z* 551 were on no occasion observed. Interestingly, for some dicaffeoylquinic acids (3,5-di-*O*-caffeoylquinic acid **5** and 4,5-di-*O*-caffeoylquinic acid **1**), both acyl migration and water addition to one acyl moiety were observed in the corresponding product. The order in which the two processes took place could not be established, though it is suspected that acyl migration should be the faster process of the two. Acyl migration was observed for all tested mono- and diacylated substrates but it was only for the diacylated substrates that both acyl migration and water addition could be detected in a product in the current study. Other work confirms that the two processes take place simultaneously for monoacylated substrates as well and conjugate water addition to an acyl-migration monoacylated

product was reported.[29] Products arising from acyl migration and *trans-cis* isomerization are summarized in **Table 4.1**. The main analytical challenge for the monohydroxylated dicaffeoylquinic acids structure elucidation constitutes the correct assignment of acyl regiochemistry. In theory regiospecific monohydration of a dicaffeoylquinic acid derivative may result in the formation of four isomeric products. For example, 3,4-di-*O*-caffeoylquinic acid **4** may produce one pair of diastereoisomeric monohydroxylated dicaffeoylquinic acids with water being regiospecifically added to the caffeoyl moiety attached to the C4 of quinic acid **51** and a second pair of diastereoisomeric monohydroxylated dicaffeoyl derivatives with water being regiospecifically added to the caffeoyl moiety of the C3 of quinic acid **51**. As suggested earlier the observation of facile acyl migration under the reaction conditions might complicate the observed product profile with a total of six regioisomeric dicaffeoylquinic acids derivatives able to form in theory a total of 24 isomeric monohydroxylated dicaffeoylquinic acids derivatives. In order to assign the regiochemistry of the caffeoyl moiety on the quinic acid **51**, multi reaction monitoring (MRM) MS^3 experiments for the transitions *m/z* 533 → 353 and *m/z* 533 → 515 → 353 for all the diacylated water addition derivatives were performed (**Figures 4.13-4.15**). Loss of the hydroxydihydrocaffeoyl moiety (180 Da) yields a fragment ion at *m/z* 353, whose MS spectrum reveals the identity of the caffeoyl regiochemistry.

For the water addition products of 3,4-di-*O*-caffeoylquinic acid **4**, the EIC reveals the presence of only two peaks showing a pseudomolecular ion at *m/z* 533 in the negative ion mode. The tandem mass spectra of both isomeric compounds show significant differences (**Table 4.2**, **Figure 4.13**). The MS^2 spectra of both derivatives show fragment ions with characteristic intensity differences at *m/z* 515 ($[M - H^+ - H_2O]^-$), 463 ($[M - H^+ - C_7H_6O_3]^-$), 371 ($[M - H^+ - C_9H_8O_3]^-$), 353 ($[M - H^+ - C_9H_{10}O_4]^-$), 335 ($[M - H^+ - C_9H_{12}O_5]^-$) and 173 ($[M - H^+ - C_{18}H_{22}O_7]^-$). The fragment ion at 515 yields an ion of 3,4-di-*O*-caffeoylquinic acid **4** and provides therefore no further information on hydroxylation regiochemistry. Similarly, the ion at 173 is indicative of 4-acylation, however reveals no further regiochemical information of the water addition. The fragment ions at 371 and 353 arise from a neutral loss of caffeic acid **49** and hydroxydihydrocaffeic acid, respectively. Further targeted MS^3 of both of these ions provide regiochemical information, on which side chain is hydroxylated. For the peak eluting at 27.4 min the MS^3 spectrum of *m/z* 371 results in a base peak at *m/z* 353 ($[M - H^+ - H_2O]^-$) and a further fragment ion at 173, indicative of 4-acylation of the hydroxydihydrocaffeic acid side chain. This

regiochemical assignment is further supported by an MS^4 experiment of *m/z* 533 to 371 to 353, which shows a base peak at *m/z* 173 and a targeted MS^3 experiment of 353 showing fragment ions consistent with a 3-acylation of the caffeoyl substituent. Therefore, the compound eluting at 27.4 min is assigned as one diastereoisomer of **20**. The second isomer eluting at 27.8 min shows in a targeted MS^3 experiment of *m/z* 533 to 353 a base peak at *m/z* 173 indicative of a 4-acyl regiochemistry of the caffeoyl substituent. A targeted MS^3 experiment on the fragment ion at *m/z* 371 was not possible due to its low intensity. Therefore, the compound eluting at 27.8 min must be assigned as one diastereoisomer of **19**.

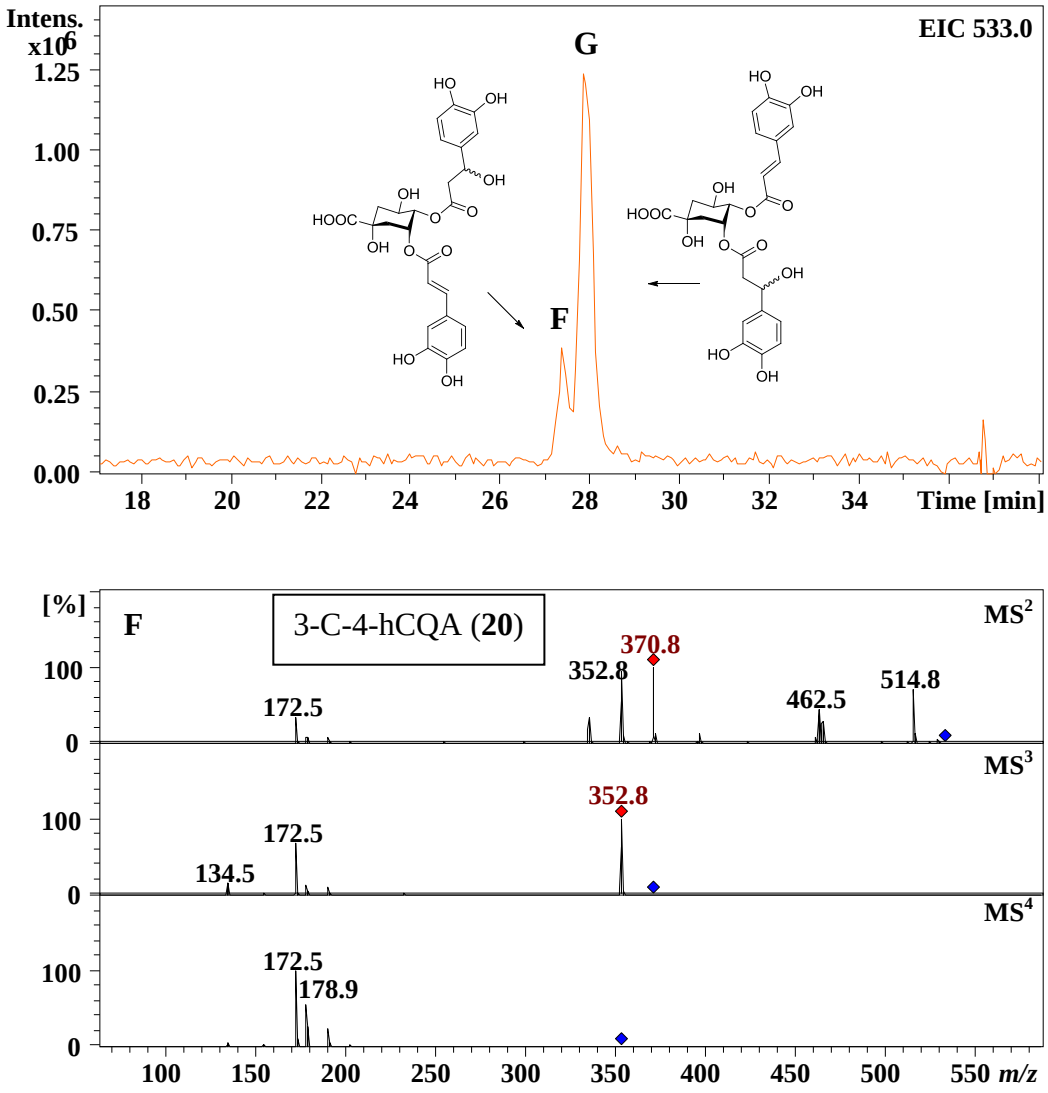

Figure 4.13. Continued.

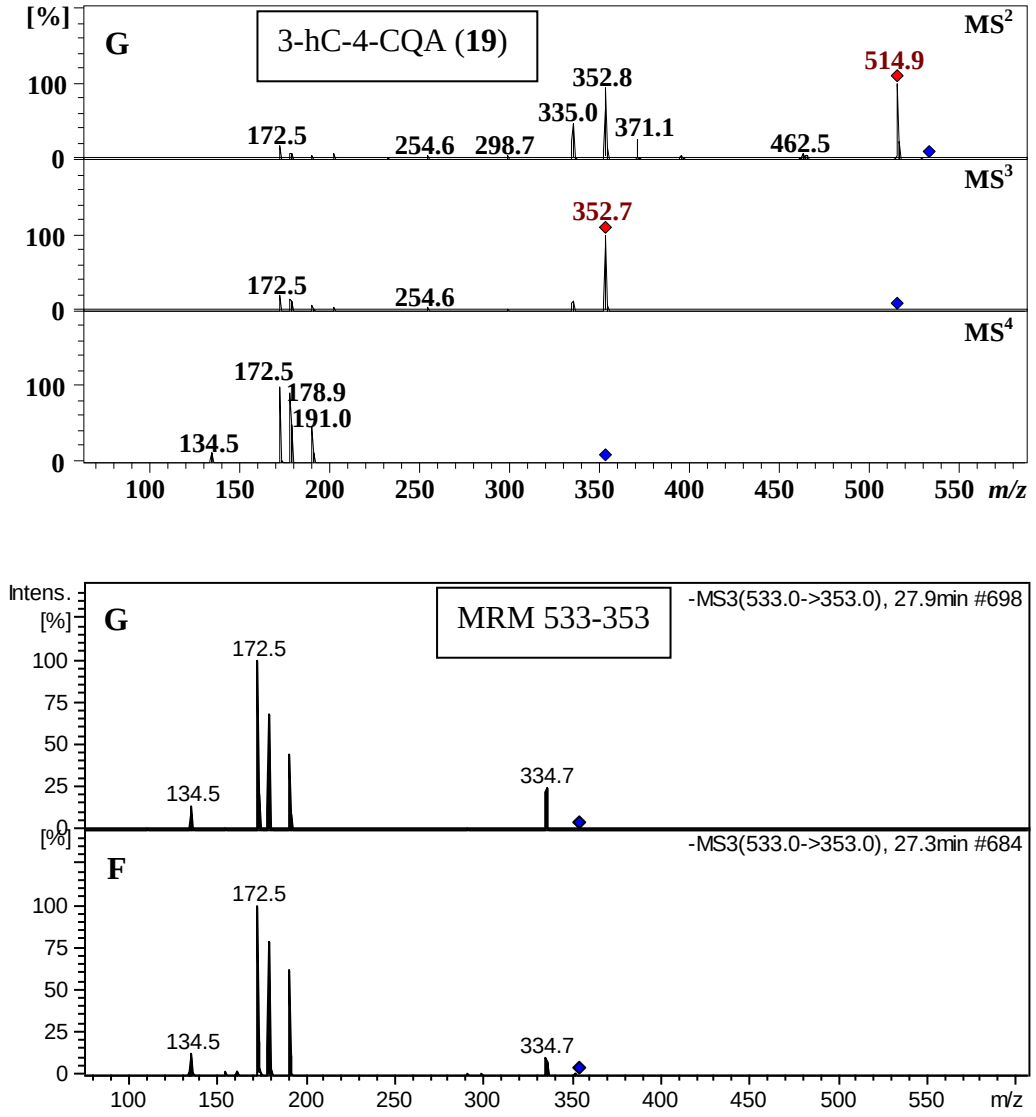

Figure 4.13. EIC of ion at *m/z* 533; MS^n spectra of **20** and **19** with precursor ions at *m/z* 533; MRM spectra in MS^3 of **19** and **20** with fragmentation of precursor ions at *m/z* 353.

The model brewing of 4,5-di-*O*-caffeoylquinic acid **1** resulted in the formation of six (water addition) compounds with peaks of pseudomolecular ions at *m/z* 533 in the negative ion mode. Applying the same method and arguments as above the compound eluting at 30.4 min was assigned as one diastereoisomer of **26** and the compound eluting at 30.9 min was assigned as one diastereoisomer of **27** (**Table 4.2** and **Figure 4.14**). Two other compounds observed showed identical retention times and tandem MS spectra as the previously assigned **19** and **20**, indicating that acyl migration from the C5 to the C3 position occurred during the model brewing.

However, it is unclear whether acyl migration occurred prior to conjugate water addition or after. The remaining two products giving pseudomolecular ions at *m/z* 533 were identified using the same reasoning as mentioned above, the compound eluting at 30.8 min being assigned as one diastereoisomer of **10** while the one eluting at 31.9 min as one diastereoisomer of **11**.

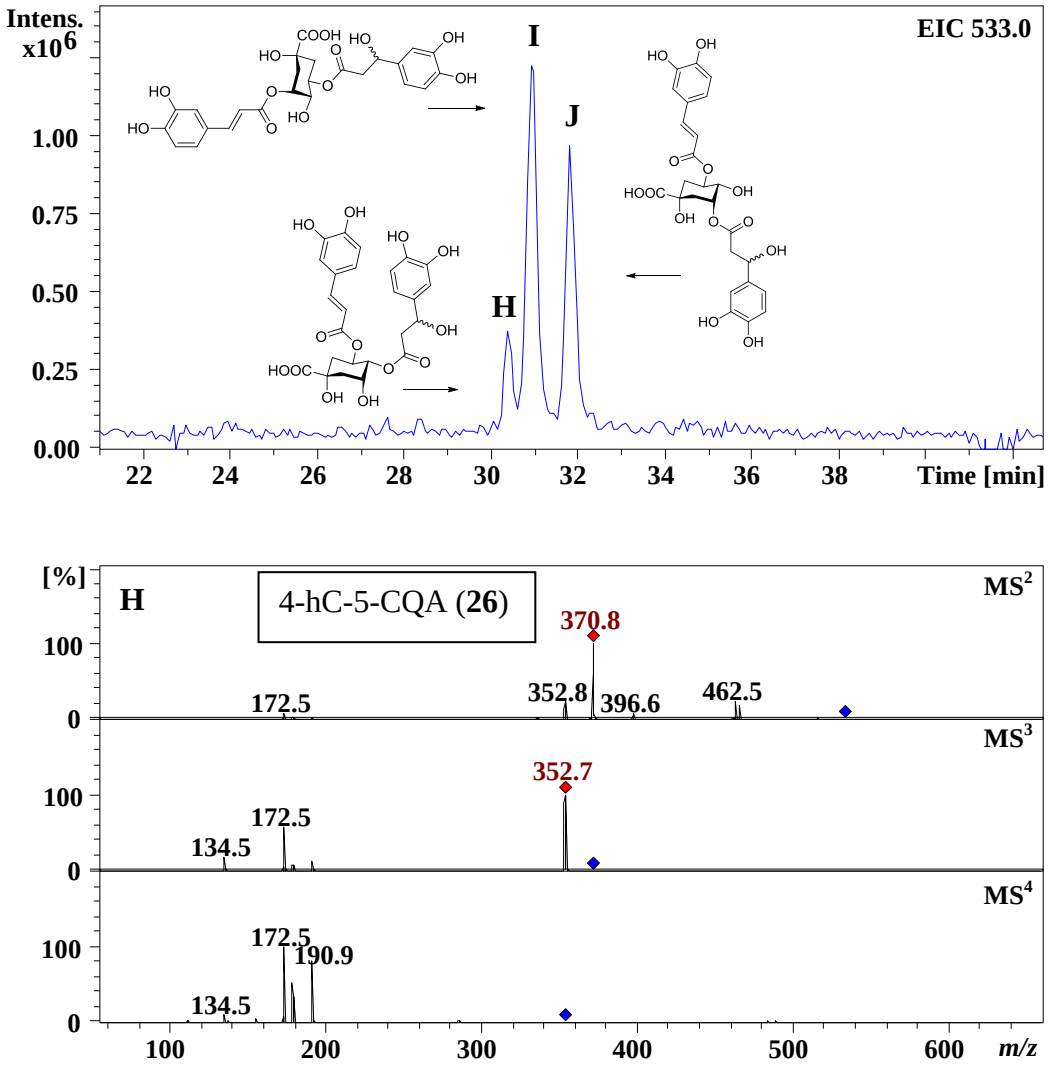

Figure 4.14. Continued.

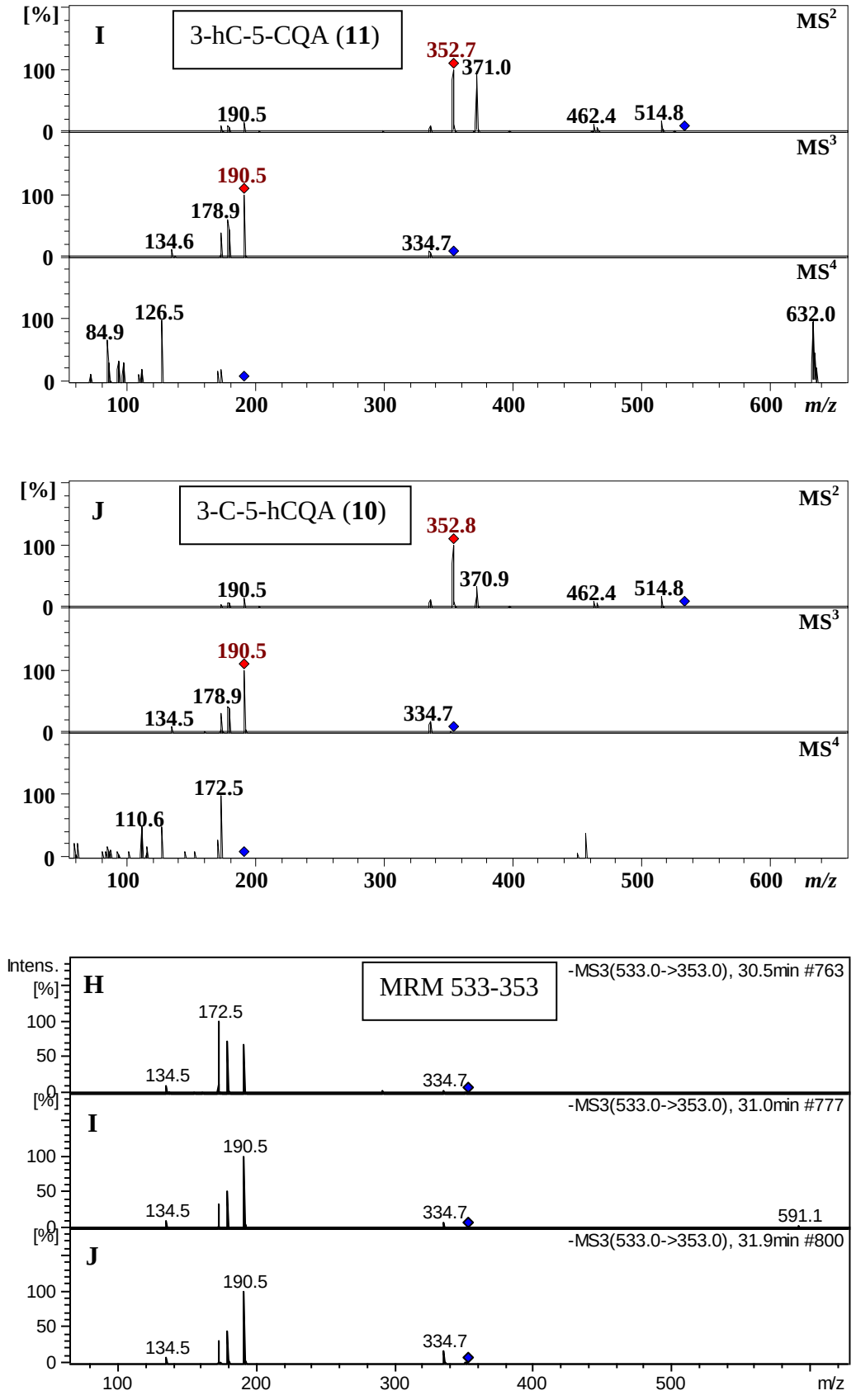

Figure 4.14. EIC of ion at *m/z* 533; MS^n spectra of **26**, **11** and **10** with precursor ions at *m/z* 533; MRM spectra in MS^3 of **26**, **11** and **10** with fragmentation of precursor ions at *m/z* 353.

The model brewing of 3,5-di-*O*-caffeoylquinic acid **5** resulted in the formation of two compounds with peaks of pseudomolecular ions at *m/z* 533 in the negative ion mode. The first compound eluting at 24.1 min showed in MS^2 a base peak at *m/z* 371 accompanied by further fragment ions at *m/z* 515, 463, 397, 353 and 191. A targeted MS^3 experiment on the precursor ion at *m/z* 371 resulted in a base peak at *m/z* 353 accompanied by fragment ions at *m/z* 191 and 135. These fragments are consistent with a 5-acyl regiochemistry of the hydroxydihydrocaffeic acid substituent. A targeted MS^3 experiment of the precursor ion at *m/z* 353 confirmed the 3-regiochemistry of the caffeoyl substituent. Therefore, the compound eluting at 24.1 min was assigned as one diastereoisomer of **10**. The second compound eluting at 27.6 min showed in a targeted MS^3 experiment of the precursor ion of 353 a fragment spectrum identical to the MS^2 spectrum of 5-*O*-caffeoylquinic acid **30** and must therefore be assigned as one diastereoisomer of **11** (**Table 4.2** and **Figure 4.15**). Using this current method it remains unclear whether conjugate water addition to dicaffeoylquinic acids is highly stereoselective, since only one chromatographically resolved signal was observed for each pair of diastereoisomers, or whether both pairs of diastereoisomers are present and chromatographically not resolved.

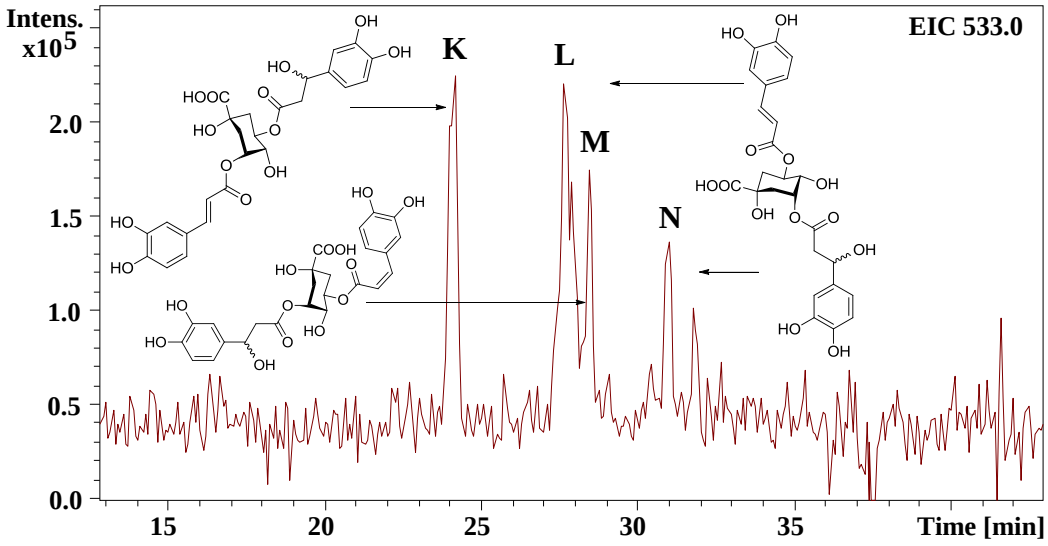

Figure 4.15. Continued.

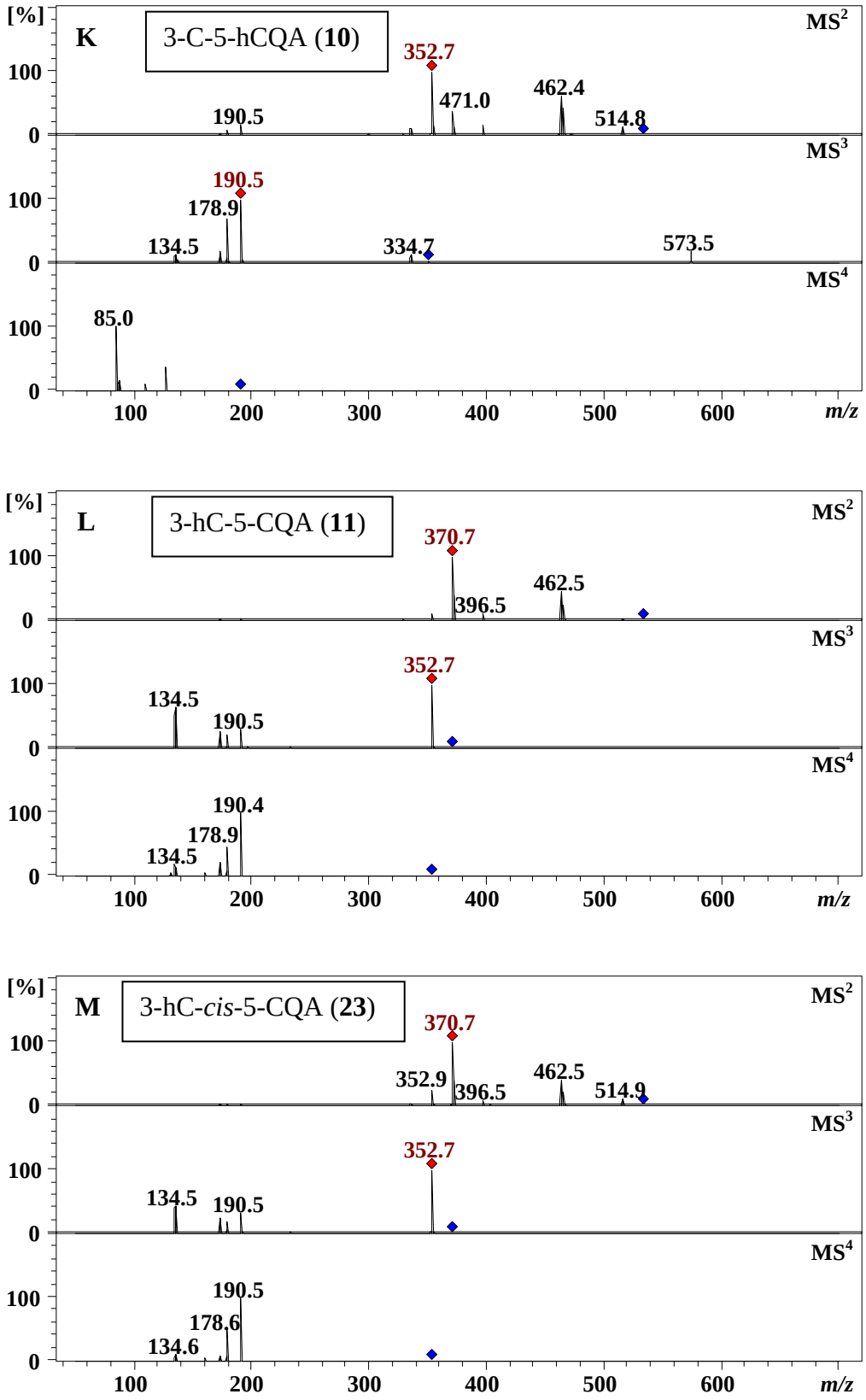

Figure 4.15. Continued.

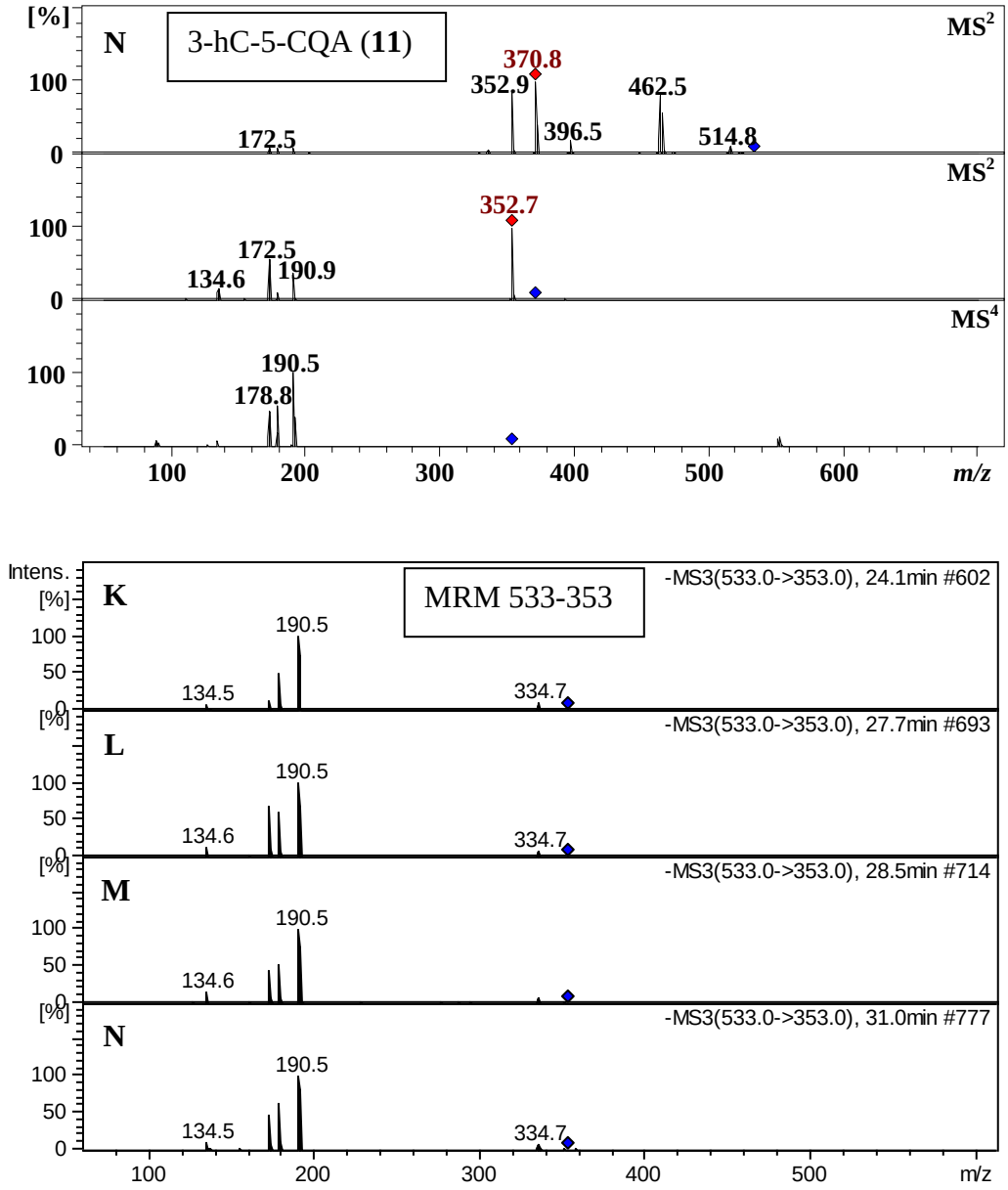

Figure 4.15. EIC of ion at *m/z* 533; MS^n spectra of **10**, **11** (two epimers) and **23** with precursor ions at *m/z* 533; MRM spectra in MS^3 of **10**, **11** (two epimers) and **23** with fragmentation of precursor ions at *m/z* 353.

4.3.5. Identification of Hydroxydihydrocaffeic Acids in Brewed Coffee

With the analytical data of the new derivatives in hand, four real Robusta coffee brews were next analyzed in order to establish whether conjugate water addition takes also place during coffee brewing. The extracted ion chromatograms of each of the four coffee brews revealed three to

four chromatographic peaks with pseudomolecular ions at *m/z* 371 and four to six peaks at *m/z* 533. Molecular formulas of all water addition products (compounds **12**-**27**, **Figures 4.5** and **4.6**) were confirmed using high resolution mass spectrometry. Using retention times and tandem MS data the hydroxydihydrocaffeic acid derivatives (compounds **12**-**17**, **19**-**22** and **24**-**27**, **Figures 4.5** and **4.6**) as well as the *cis*-derivatives (compounds **36**-**41**, **Figure 4.8**) observed in coffee were assigned to regiochemical level. It has to be noted that the coffee beverage is slightly acidic with pH values for the beverages determined at 5.5; this value represents the low end of the pH interval generally recommended to be safe for beverages, with respect to dental erosion of tooth enamel.[33] It has been reported by Dawidowicz that the thermal decomposition of 5-*O*-caffeoylquinic acid **30** in aqueous solvent is strongly pH-dependant.[29]

Using relative peak areas of the EICs of (3'-hydroxydihydrocaffeoyl)-quinic acids if compared to relative peak areas of monocaffeoyl and dicaffeoylquinic acids it can be tentatively estimated the percent of chlorogenic acids transformed into their hydroxylated derivatives. In the case of monoacylated chlorogenic acids up to 1.5-2% of the chlorogenic acids are transformed into their hydroxylated derivatives, while for the diacylated chlorogenic acids up to 4-4.5% of them suffer this transformation. Extrapolating to the total chlorogenic acids content in a typical 200 ml cup of coffee it can be tentatively estimated that per cup of coffee up to 8-9 mg of hydroxydihydrocaffeic acids are consumed.

4.4 Conclusions

In conclusion, it was shown that in model coffee brewing systems mono- and dicaffeoylquinic acids are highly reactive forming acyl migration products, *cis*-caffeoylquinic acids and hydroxydihydrocaffeic acid derivatives. The regiochemistry of the latter compounds was elucidated using advanced tandem MS techniques. It could also be shown that water does not simply act as a solvent and innocent bystander in food processing but acts as a reactive reagent resulting in significant chemical changes of the dietary material. The observation of conjugate addition reactions to the chlorogenic olefinic moiety suggests that as well nucleophilic thiol and amine functionalities in peptides could undergo this reaction pathway contributing to many structures in the coffee melanoidin fraction.[34] Compounds identified in the present study might contribute to organoleptic properties and reported health effects of the coffee beverage but further investigations need to be carried out.

4.5. References

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5. LC-MS^n Study of the Chemical Transformations of Hydroxycinnamates during Yerba Maté (*Ilex paraguariensis*) Tea Brewing

5.1. Introduction

Hydroxycinnamates are a class of natural phenolics and esters formed between alcohols (glucose, glycerol, tartaric acid, malic acid, sterol, shikimic acid, quinic acid etc.) and certain hydroxycinnamic acids (e.g., caffeic, ferulic, *p*-coumaric, dimethoxycinnamic and sinapic acids) (**Figure 5.1**).[1-6] Hydroxycinnamates are present in fruits, vegetables, beverages, spices and grains, which form an important part of human diet (**Figure 5.1**).[1,2] An average intake of hydroxycinnamates is about 4 g per person per day. During food processing e.g., roasting of green coffee, brewing of coffee, fermentation of green tea, fermentation of grapes for wine making, fermentation of cocoa beans, boiling of artichoke, cooking of vegetables and drying of foods, these hydroxycinnamates undergo various chemical transformations e.g., acyl migration, oxidation, reduction, hydrolysis, hydration, cyclization, dehydration, *cis-trans* isomerization, epimerization, Maillard reaction, caramelization and polymerization.[1,2,7-10] These chemical transformation products contribute to the desired taste, flavor, aroma and color of the foods and beverages. Hydroxycinnamates have shown several fascinating biological activities: antioxidant activity, ability to increase hepatic glucose utilization, inhibition of the HIV-1 integrase, antispasmodic activity and inhibition of the mutagenicity of carcinogenic compounds have been reported so far.[11-15]

Yerba maté (*Ilex paraguariensis*) is a popular drink consumed in South American countries, which is prepared from the infusion of dried maté leaves in hot water. Recently, the group of Kuhnert reported 70 hydroxycinnamates including chlorogenic acids, shikimates and caffeoylglucoses in yerba maté samples.[4] The study and follow-up of chemical transformations during food processing in the absence of suitable analytical techniques, methods and authentic standards has proven to be challenging. Jaiswal et al. and Matei et al. reported the chemical transformations of hydroxycinnamates during the roasting and brewing of coffee, respectively.[8,10] Interestingly, the exposure of phenolics to hot water at slightly acidic pH resulted in an astonishing number of transformation products. Liquid chromatography coupled to mass spectrometry techniques and authentic standards were used for the identification and characterization of these chemical transformation products.[8,10] In this chapter, advanced LC-MS^n methods, model brewing and synthetic authentic standards of

caffeoylglucoses were used to study chemical transformations of hydroxycinnamates during the brewing process of yerba maté tea.

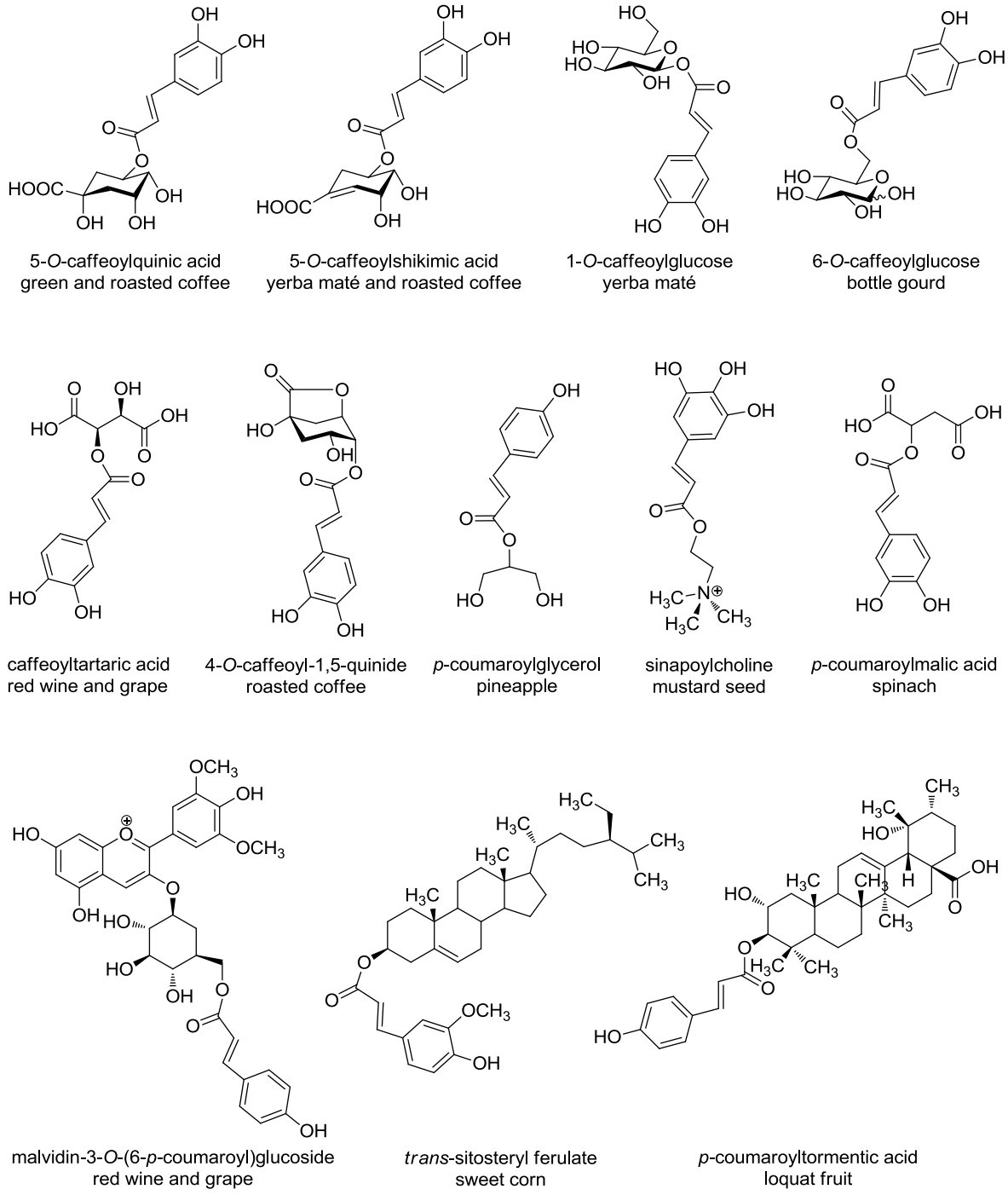

Figure 5.1. Hydroxycinnamates present in foods and beverages.

5.2. Materials and Methods

All the chemicals (Analytical grade) were purchased from Sigma-Aldrich (Bremen, Germany). Green dry yerba maté leaves (Argentinian origin) were purchased from a supermarket in Bremen (Germany).

Brewing of Yerba Maté. Green dry yerba maté leaves (3 g) were infused in 100 mL of hot water and stirred for 4 h. The prepared brew was cooled to room temperature, filtered through a membrane filter and directly used for LC-MS.

Synthesis of Caffeoylglucoses. 3-*O*-Caffeoylglucoses (α- and β-anomers) **5** and **6**, 6-*O*-caffeoylglucoses (α- and β-anomers) **9** and **10** and a mixture of all ten regioisomers of caffeoylglucoses (α- and β-anomers) were synthesized as described by Jaiswal et al. and Alakolanga et al..[6,16]

Brewing of Caffeoylglucoses. Synthetic caffeoylglucoses standards (each sample 500 μg) were infused in 3 mL of hot water each and stirred for 4 h under reflux. The prepared samples were then cooled to room temperature, filtered through a membrane filter and directly used for LC-MS experiments.

LC-MS^n. The 1100 series LC equipment (Agilent, Bremen, Germany) comprised a binary pump, an auto sampler with a 100 μL loop and a DAD detector with a light-pipe flow cell (recording at 320 and 254 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics HCT Ultra, Bremen, Germany) operating in full scan, auto MS^n mode to obtain fragmentation. As necessary, MS^2, MS^3 and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a parent ion at *m/z* 341, 353, 359, 371, 385, 515, 533, 547 and 551. Tandem mass spectra were acquired in auto-MS^n mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 Volt, starting at 30% and ending at 200%. MS operating conditions (negative mode) had been optimized using 3-*O*-caffeoylglucoses (**5** and **6**) and 6-*O*-caffeoylglucoses (**9** and **10**) with a capillary temperature of 365 ^{o}C, a dry gas flow rate of 10 L/min, and a nebulizer pressure of 10 psi.

HPLC Coupled to MS. Separation was achieved on a 250 mm x 3 mm i.d. column containing C18-amide 5 μm, with a 5 mm x 3 mm i.d. guard column of the same material (Varian RP-C18A, Darmstadt, Germany). Solvent A was water/formic acid (1000:0.005 v/v)

and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. The gradient profile was linear from 10-70% B in 60 min followed by 10 min isocratic, and a return to 10% B at 90 min and 10 min isocratic to re-equilibrate.[6]

5.3. Results and Discussion

All data for the hydroxycinnamates, methyl caffeoylquinate, chlorogenic acids and caffeoylglucoses presented in this paper use the recommended IUPAC numbering system; the same numbering system was adopted for water addition products of chlorogenic acids, their *cis*-isomers and acyl migration isomers**.**

5.3.1. Investigation of Model Compounds

Firstly, two caffeoylglucoses, 3-*O*-caffeoylglucoses **5** and **6** (α- and β-anomers, respectively) and 6-*O*-caffeoylglucoses **9** and **10** (α- and β-anomers, respectively) were subjected to model brewing conditions using 4 h of hot water (100 ˚C) treatment followed by HPLC-MS^n analysis, as described by Matei et al..[10] The HPLC-chromatograms showed between 13 and 14 distinct peaks corresponding to the products formed. The main focus was on the water addition products, with examples of compounds resulting from water addition and elimination as well as acyl migration being proposed in **Figure 5.2**. The products observed can be categorized into three types of hydroxycinnamate derivatives: firstly, hydroxydihydrocaffeoylglucoses arising through conjugate addition of water to the olefinic cinnamoyl moiety (**Figure 5.2**); secondly, acyl migration products, including a selection of different caffeoylglucose regioisomers (**1**-**10**); and finally, *trans*-*cis* isomerization (*cis*-caffeoylglucoses) products, presumably obtained by reversible β-elimination of water from hydroxydihydrocaffeoylglucoses (not observed and not shown in **Figure 5.2**).[10,17] Similar classes of derivatives based on quinic acid have been analyzed and identified in nature and during the brewing of coffee by LC-MS^n.[10,18] In this study, the detected compounds were assigned based on retention times and tandem MS data.

5.3.2. Tandem MS Characterization of Hydroxydihydrocaffeoylglucoses (M_r = 360)

A conjugate water addition to the olefinic cinnamoyl moiety of caffeoylglucoses was observed for 3-*O*-caffeoylglucoses (**5** and **6**) and 6-*O*-caffeoylglucoses (**9** and **10**). For each of the investigated caffeoylglucoses four corresponding hydroxydihydrocaffeoylglucoses (α/β pairs and diastereomer pairs at new stereogenic center, **11**-**14** and **15**-**18**) resulting from water

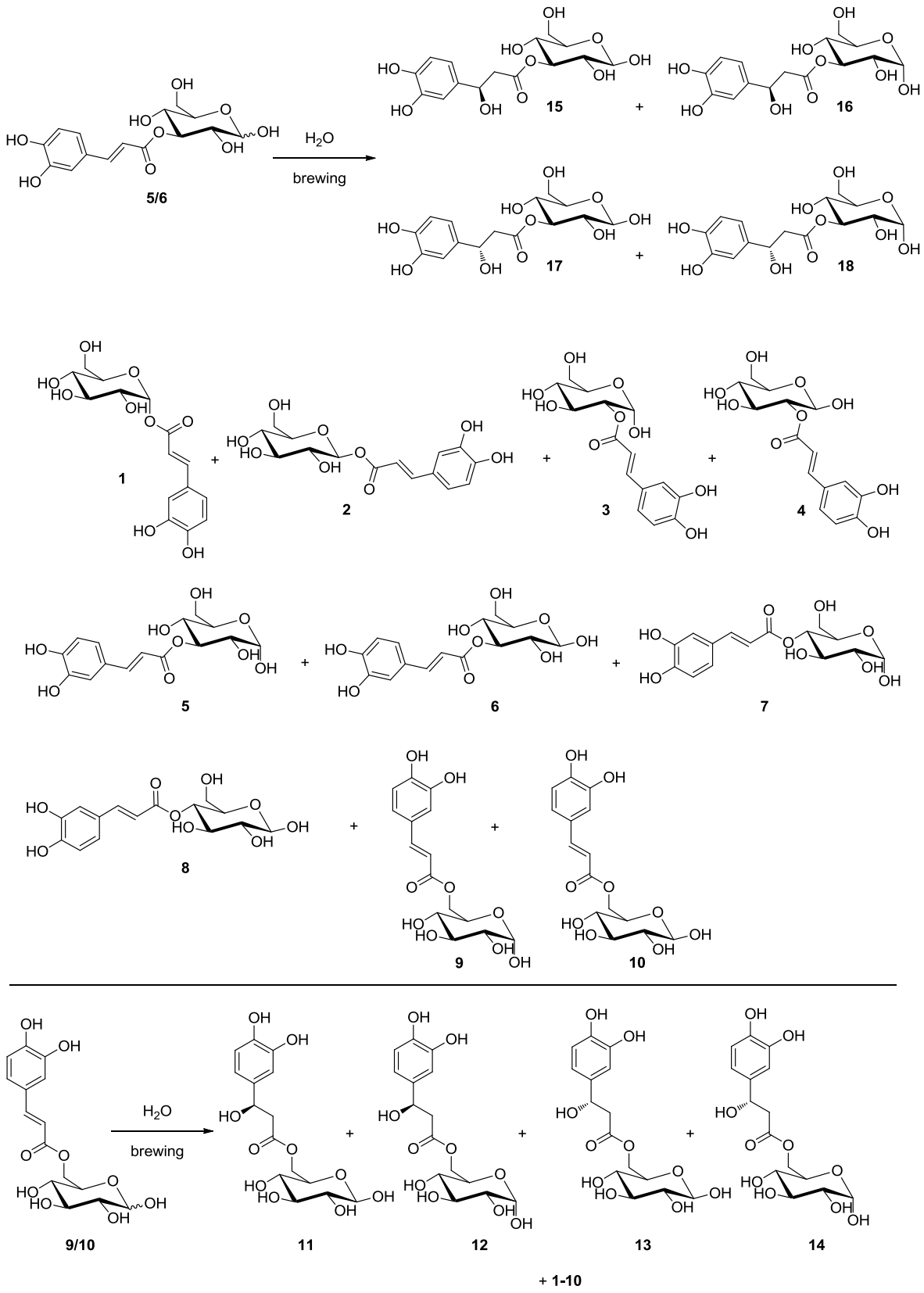

Figure 5.2. Possible chemical transformations of caffeoylglucoses during the brewing process.

addition could be distinguished as four (for 3-*O*-caffeoylglucoses) and three (for 6-*O*-caffeoylglucoses) chromatographically resolved peaks, respectively, appearing as pseudomolecular ions in the negative ion mode at *m/z* 359 ($[M - H^+]^-$) (**Figures 5.3 and 5.4**). The MS^3 fragmentation patterns (MRM) of the precursor ions at *m/z* 341 ($[M - H^+ - H_2O]^-$, $C_{15}H_{17}O_9$) of hydroxydihydrocaffeoylglucoses were similar or even identical to the MS^2 fragmentation of the corresponding caffeoylglucoses, based on the structural identity of the precursor ions, and allowed therefore unambiguous assignment of acyl regiochemistry using the previously published hierarchical key.[6] In all of the cases the hydroxyl moiety was confirmed by the MS^n fragmentation to be located at the *β*-position in the dihydrocinnamoyl residue and on no occasion could an *α*-hydroxyl be detected. This assignment of regiochemistry is based on a characteristic retro-aldol type fragment ion at *m/z* 221 ($C_9H_{13}O_7$) showing a neutral loss of 138 Da ($C_7H_6O_3$) from the parent ion at *m/z* 359, indicative of the regiochemistry of water addition. Kuhnert and co-workers showed for malate esters of quinic acid and chlorogenic acids that this fragmentation pathway can be used to unambiguously establish alcohol regiochemistry in chlorogenic acids chemistry.[10,19]

This finding implies that water addition to the double bond of the cinnamoyl residue of caffeoylglucoses takes place in a regiospecific manner, which has also been observed for chlorogenic acids and their water addition derivatives.[10] In the present chapter, the very closely eluting peaks with pseudomolecular ions at *m/z* 359 observed after brewing of 3-*O*-caffeoylglucoses (**5** and **6**) are clearly the four diastereoisomers of 3-*O*-(3'-hydroxydihydrocaffeoyl)-glucoses **15**-**18** (**Figure 5.4**), as confirmed by the characteristic retro-aldol fragment ion in the fragment spectra. The same is true for 6-*O*-caffeoylglucoses (**9** and **10**). In the case of *α*- and *β*-anomers, the two expected products for each anomer could not be observed. Either they were virtually co-eluting and could chromatographically not be resolved or they did not form at all. The tandem MS is isomer-blind with respect to stereochemistry but since the water molecule is added regiospecifically to the cinnamoyl residue, the three or four chromatographic peaks observed for each *β*-hydroxylated caffeoylglucoses giving identical tandem MS data could only be the theoretical diastereoisomers.

6-*O*-(3'-Hydroxydihydrocaffeoyl)-glucose **11**, 6-*O*-(3'-hydroxydihydrocaffeoyl)-glucose **12** and 6-*O*-(3'-hydroxydihydrocaffeoyl)-glucoses **13/14** were identified by their *m/z* 359 ($[M - H^+]^-$) parent ion and they produced the MS^2 base peak at *m/z* 135 ($[\text{caffeic acid} - CO_2 - H^+]^-$)

and secondary peaks at *m/z* 341 ([M – H_2O – H^+]$^-$), *m/z* 323 ([caffeoylglucose – H_2O – H^+]$^-$), *m/z* 299 ([M – H_2O – $C_2H_4O_2$ – H^+]$^-$), *m/z* 269 ([M – H_2O – $C_3H_6O_3$ – H^+]$^-$) and *m/z* 239 ([M – H_2O – $C_4H_8O_4$ – H^+]$^-$) (**Figure 5.3**). 6-Acylation was confirmed by the MRM experiment of the MS^2 secondary ion at *m/z* 341 ([caffeoylglucoses – H^+]$^-$), which produced the MS^3 base peak at *m/z* 281 ([caffeoylglucoses – $C_2H_4O_2$ – H^+]$^-$) and the MS^3 secondary peaks at *m/z* 251 ([caffeoylglucoses – $C_3H_6O_3$ – H^+]$^-$), *m/z* 221 ([caffeoylglucoses – $C_4H_8O_4$ – H^+]$^-$), both obtained through ring fission fragmentation, *m/z* 179 ([caffeic acid – H^+]$^-$) and *m/z* 323 ([caffeoylglucoses – H_2O – H^+]$^-$), as detailed in another study.[6]

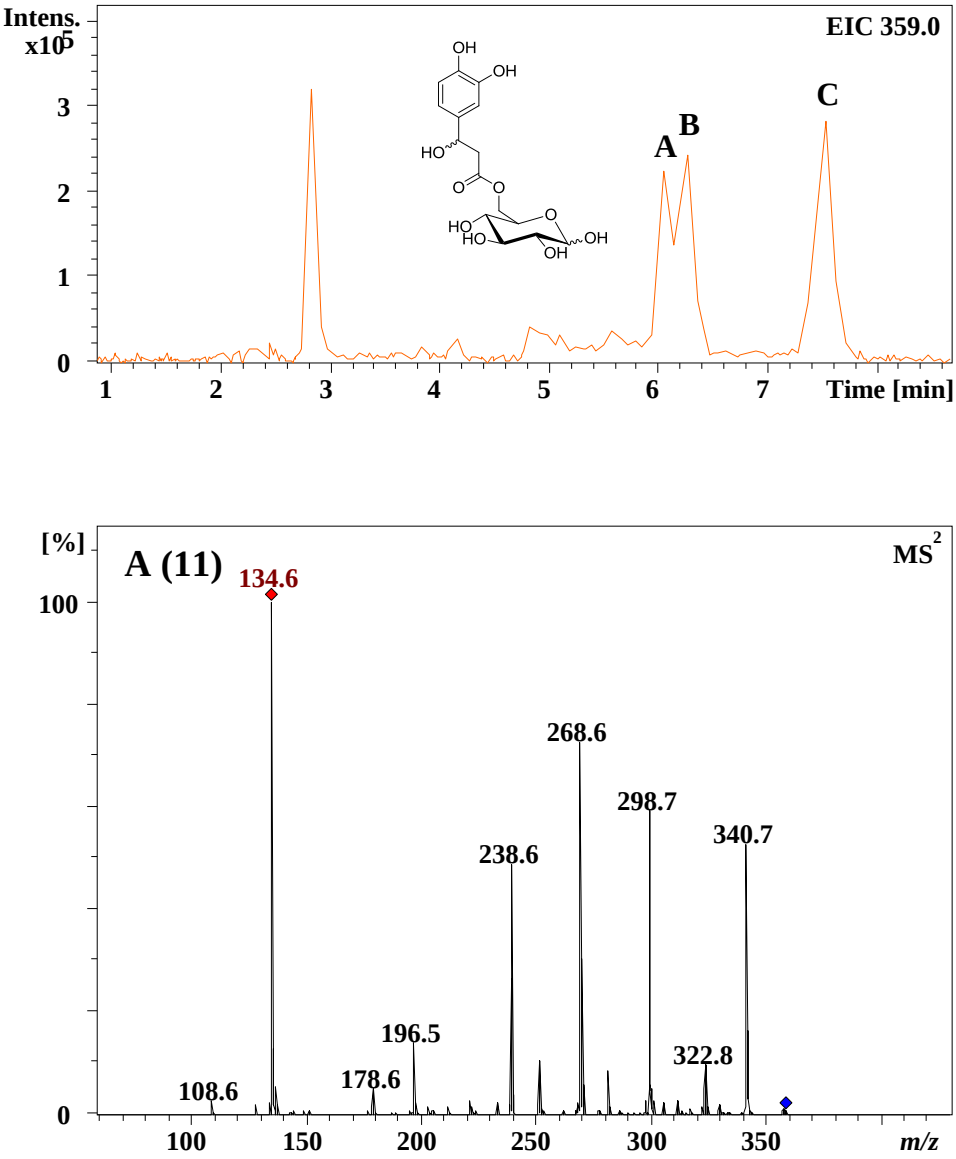

Figure 5.3. Continued.

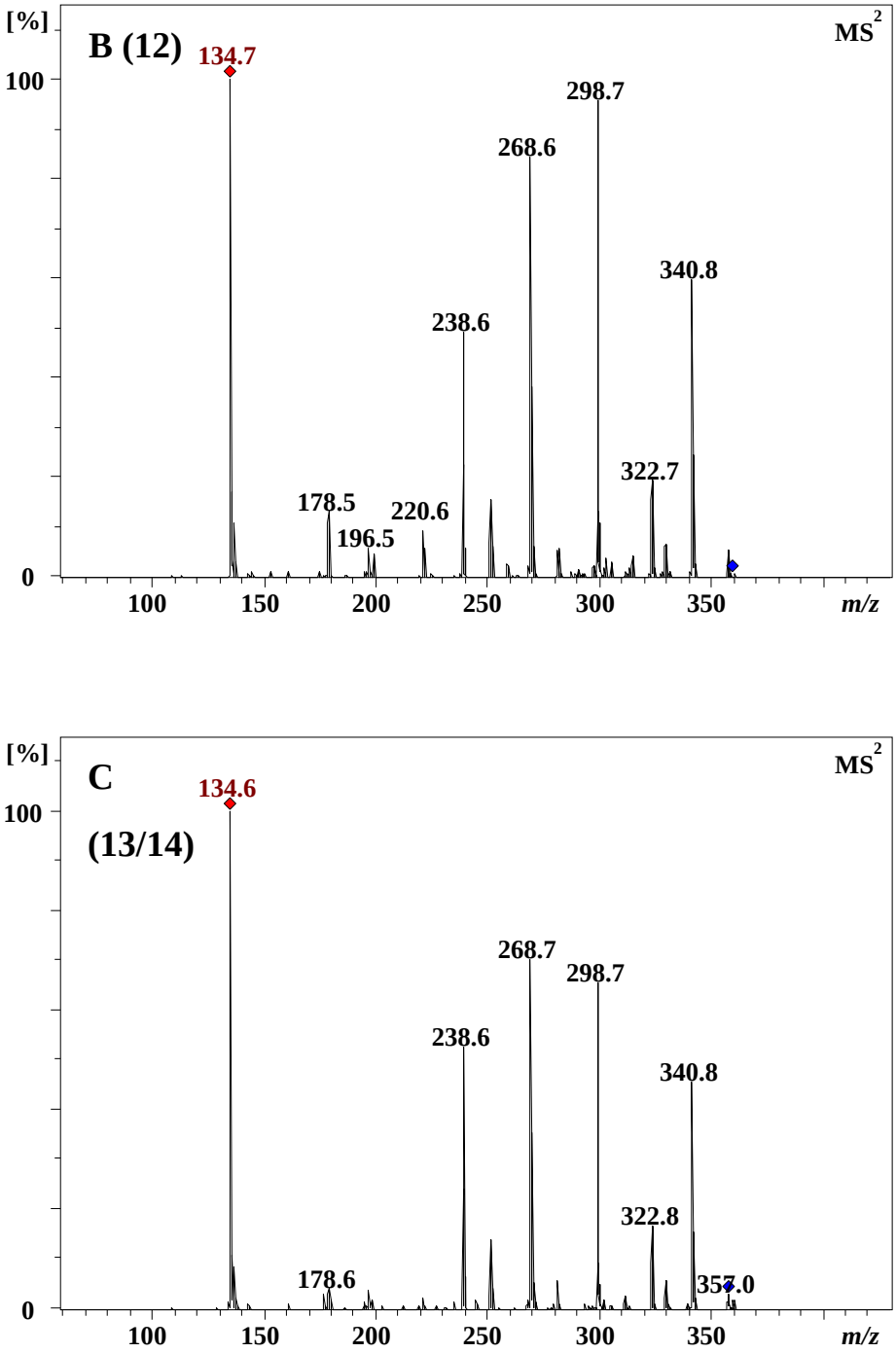

Figure 5.3. Extracted ion chromatogram and MS^2 spectra of 6-*O*-(3'-hydroxydihydrocaffeoyl)-glucoses **11-13/14** at *m/z* 359 in negative ion mode from model brew.

The presence of the hydroxyl group at the *β*-position was confirmed by the very low intensity MS^2 peak at *m/z* 221. A non-regiospecific water addition to the originally *trans* double bond

of the cinnamoyl residue should have generated an additional MS^2 fragment at either *m/z* 237 (glucose moiety) or *m/z* 123 (caffeoyl moiety), neither being detected. This observation is consistent with the findings for all the mono- and diacylated chlorogenic acids tested for water addition in the previous chapter; the specific MS^2 fragment always points towards the hydroxyl at the *β*-position and never at *α*-position. Moreover, acyl migration products at *m/z* 341 were observed, in particular 1-*O*-caffeoylglucoses **1/2**, 2-*O*-caffeoylglucoses **3/4** and 4-*O*-caffeoylglucoses **7/8**, and could be identified based on their retention times and specific fragmentation matching the previously published hierarchical key for caffeoylglucose isomers assignment.[6]

3-*O*-(3'-Hydroxydihydrocaffeoyl)-glucoses **15-18** were detected and identified by their pseudomolecular ion at *m/z* 359. 3-*O*-(3'-Hydroxydihydrocaffeoyl)-glucose **15** produced the MS^2 base peak at *m/z* 135 ([caffeic acid – CO_2 – H^+]$^-$), by the loss of the glucosyl unit, CO_2 and H_2O, and the following secondary peaks: *m/z* 329 ([M – CH_4O_2 – H^+]$^-$) by the loss of CH_2O and H_2O; *m/z* 299 ([M – $C_2H_4O_2$ – H^+]$^-$) by the loss of $C_2H_4O_2$; *m/z* 197 ([M – glucosyl – H^+]$^-$) by the loss of the glucosyl unit (**Figure 5.4**). 3-*O*-(3'-Hydroxydihydrocaffeoyl)-glucoses **16** produced the MS^2 base peak at *m/z* 135 ([caffeic acid – CO_2 – H^+]$^-$) by the loss of the glucosyl unit, CO_2 and H_2O and the following secondary peaks: *m/z* 341 ([caffeoylglucose – H^+]$^-$) by the loss of H_2O; *m/z* 323 ([caffeoylglucose – H_2O – H^+]$^-$) by the loss of two H_2O; *m/z* 299 ([M – $C_2H_4O_2$ – H^+]$^-$) by the loss of $C_2H_4O_2$; *m/z* 269 ([M – $C_3H_6O_3$ – H^+]$^-$) by the loss of $C_3H_6O_3$; *m/z* 251 ([M – $C_3H_8O_4$ – H^+]$^-$) by the loss of $C_3H_6O_3$ and H_2O; *m/z* 239 ([M – $C_4H_8O_4$ – H^+]$^-$) by the loss of $C_4H_8O_4$; *m/z* 221 ([M – $C_4H_{10}O_5$ – H^+]$^-$) by the loss of $C_4H_8O_4$ and H_2O; *m/z* 197 ([M – glucosyl – H^+]$^-$) by the loss of the glucosyl unit; *m/z* 179 ([caffeic acid – H^+]$^-$) by the loss of the glucosyl unit and H_2O; *m/z* 153 ([M – glucosyl – CO_2 – H^+]$^-$) by the loss of the glucosyl unit and CO_2 (**Figure 5.4**).[6]

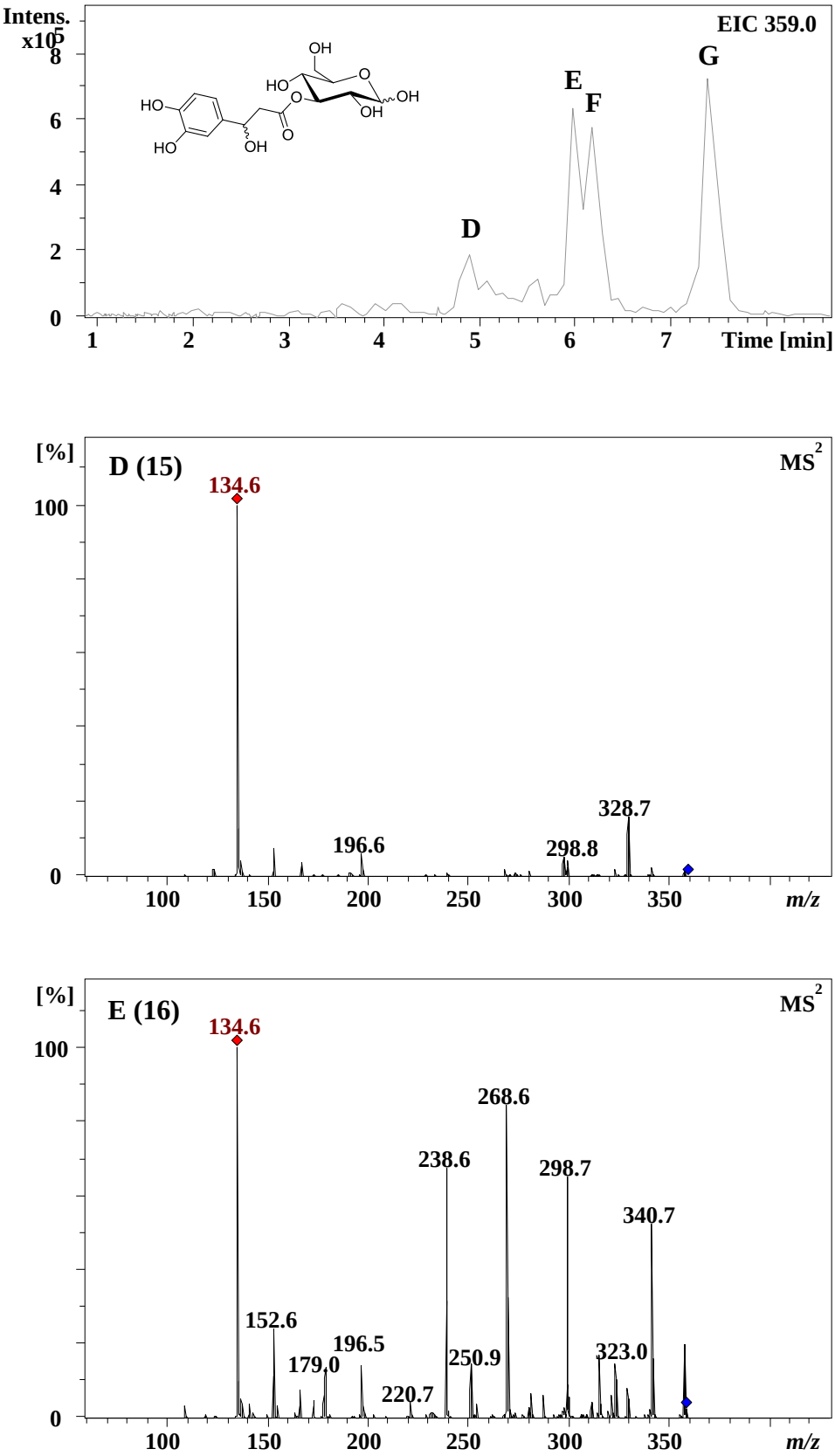

Figure 5.4. Continued.

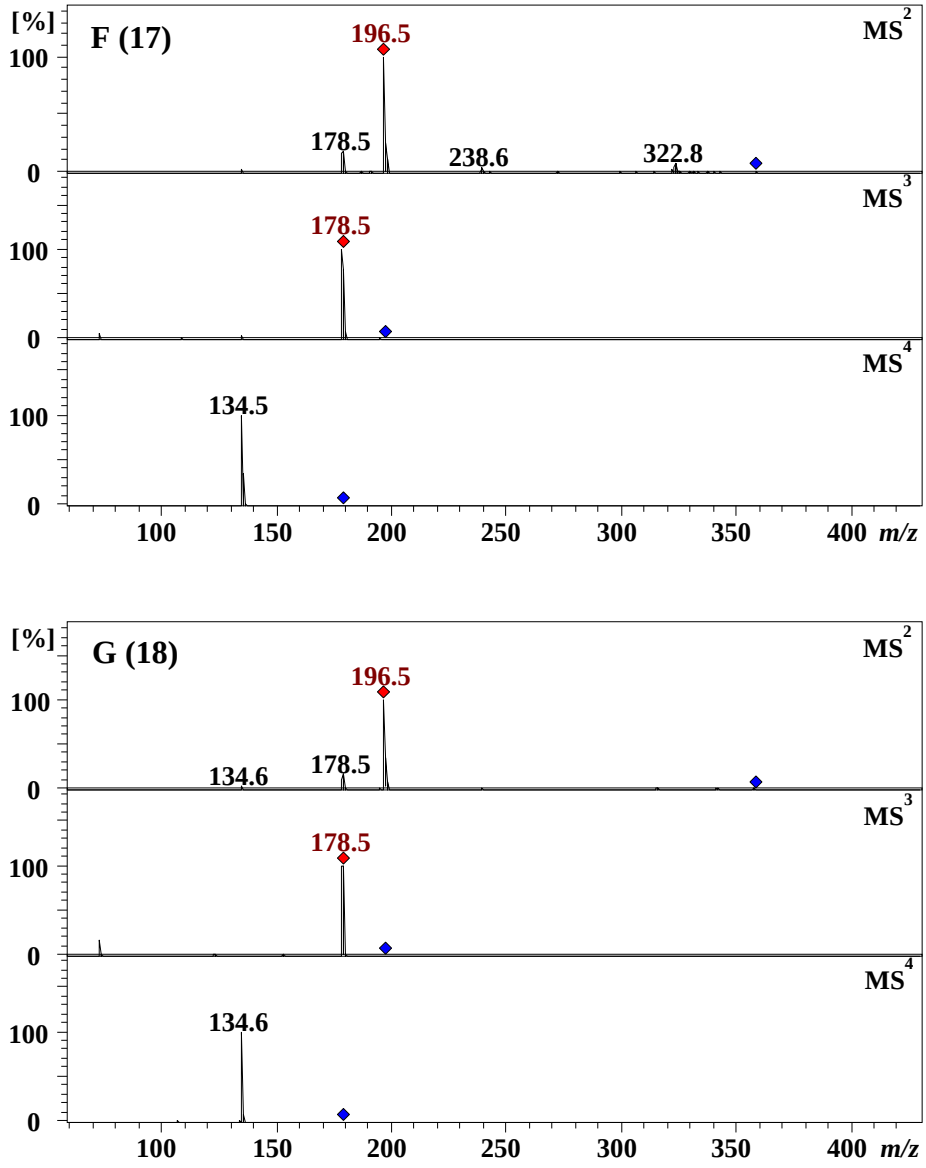

Figure 5.4. Extracted ion chromatogram and MS^2 spectra of 3-*O*-(3'-hydroxydihydrocaffeoyl)-glucoses **15-18** at *m/z* 359 in negative ion mode from model brew.

3-*O*-(3'-Hydroxydihydrocaffeoyl)-glucose **17** and 3-*O*-(3'-hydroxydihydrocaffeoyl)-glucose **18** produced the MS^2 base peak at *m/z* 197 ([caffeic acid + H_2O – H^+]$^-$) by the loss of the glucosyl unit (162 Da) and the following secondary ions: *m/z* 323 ([caffeoylglucose – H_2O – H^+]$^-$) by the loss of two water molecules; *m/z* 239 ([M – $C_4H_8O_4$ – H^+]$^-$) by the loss of $C_4H_8O_4$; *m/z* 179 ([caffeic acid – H^+]$^-$) by the loss of the glucosyl unit and H_2O; *m/z* 135 ([caffeic acid – CO_2 – H^+]$^-$) by the loss of the glucosyl unit, CO_2 and H_2O. They produced the MS^3 base peak at *m/z* 179 ([caffeic acid – H^+]$^-$) by the loss of H_2O and the MS^4 base peak at

m/z 135 ([caffeic acid – CO_2 – H^+]$^-$) by the loss of CO_2 and H_2O. The water addition specific fragments are shown in MS^2 spectra at *m/z* 197 and *m/z* 239 in the MS^2 spectrum.

All the water addition products (**11-18**) and acyl migration products (**1-10**) for 3-*O*-caffeoylglucose and 6-*O*-caffeoylglucose were detected in brewed maté. The pH of the maté brew was slightly acidic (pH 6.3).

5.3.3. Tandem MS Characterization of Methyl 3'-Hydroxydihydrocaffeoylquinate (M_r = 386) in Brewed Maté

Three peaks were detected at *m/z* 385 in the extracted ion chromatogram of brewed maté and were tentatively assigned as methyl 3'-hydroxydihydrocaffeoylquinates **19-21**. They produced the MS^2 base peak at *m/z* 161 ([caffeic acid – H_2O – H^+]$^-$) by the loss of a methyl quinate residue (205 Da) and H_2O; the secondary peaks were observed as following: *m/z* 349 ([methyl caffeoylquinate – H_2O – H^+]$^-$) by the loss of H_2O; *m/z* 179 ([caffeic acid – H^+]$^-$) by the loss of a methyl quinate residue (205 Da); *m/z* 133 ([caffeic acid – H_2O – CO – H^+]$^-$) by the loss of CO and the methyl quinate residue. They produced the MS^3 base peak at *m/z* 133 ([caffeic acid – H_2O – CO – H^+]$^-$) by the loss of CO (**Figure 5.5**). These isomers produced an MS^2 base peak similar to the one produced by methyl 3-*O*-caffeoylquinate and were tentatively assigned as methyl 3-*O*-(3'-hydroxydihydrocaffeoyl)-quinates.[20,21] Since only two theoretically possible *β*-hydroxylated water addition products exist, the third isomer observed here might be a quinic acid diastereomer of 3-*O*-(3'-hydroxydihydrocaffeoyl)-quinate.

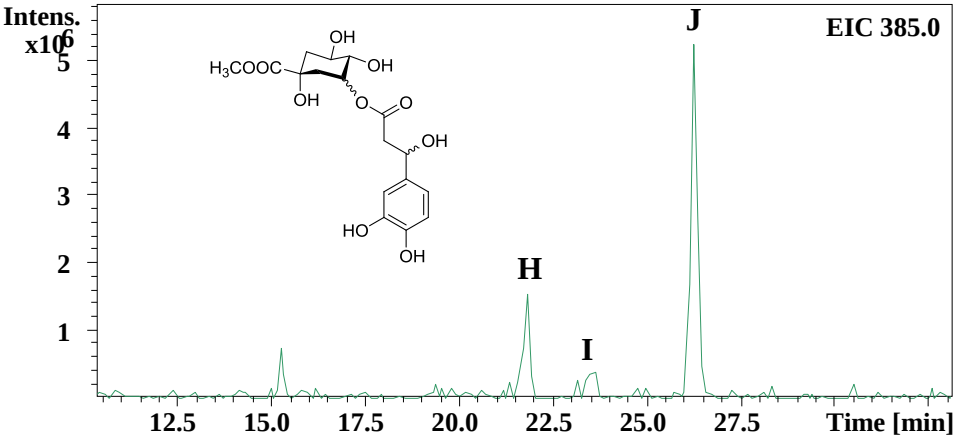

Figure 5.5. Continued.

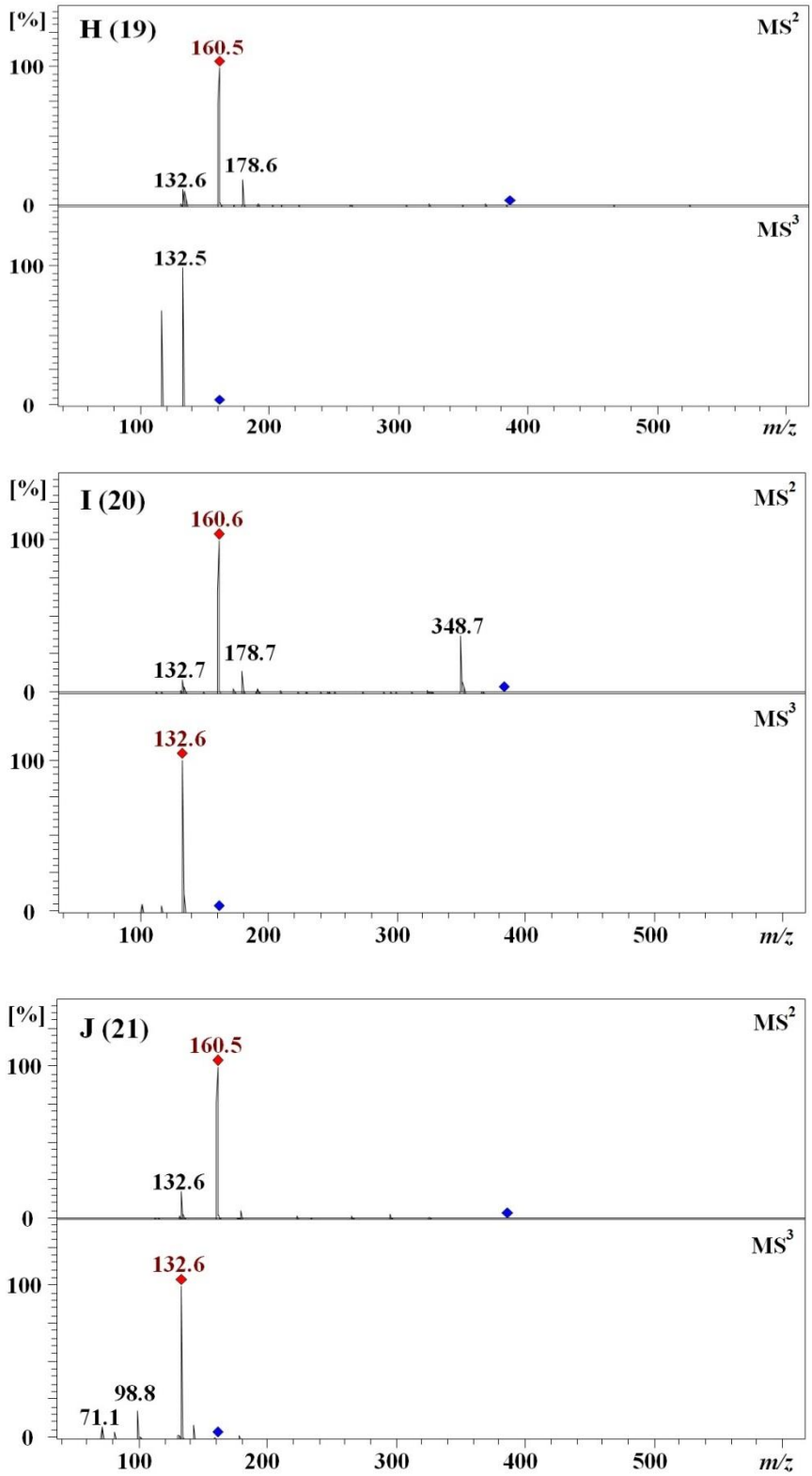

Figure 5.5. Extracted ion chromatogram and MS^2 spectra of methyl 3'-hydroxydihydrocaffeoylquinate isomers **19-21** at *m/z* 359 in negative ion mode.

5.3.4. Tandem MS Characterization of Monoacylated 3'-Hydroxydihydrocaffeoylquinic acids (M_r = 372) and Diacylated 3'-Hydroxydihydrocaffeoyl-caffeoylquinic Acids (M_r = 534) in Brewed Maté

Six monoacylated 3'-hydroxydihydrocaffeoylquinic acids **22**-**27** and six diacylated 3'-hydroxydihydrocaffeoylquinic acids **28-33** were detected in the extracted ion chromatogram and the total ion chromatogram, which produced pseudomolecular ions at *m/z* 371 and 533, respectively. These hydroxylated chlorogenic acids were identified as 3-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **22**, 3-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **23**, 4-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **24**, 4-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **25**, 5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **26**, 5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **27**, 3-*O*-caffeoyl-5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **28**, 3-*O*-(3'-hydroxydihydrocaffeoyl)-5-*O*-caffeoylquinic acid **29**, 3-*O*-caffeoyl-4-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **30**, 3-*O*-(3'-hydroxydihydrocaffeoyl)-4-*O*-caffeoylquinic acid **31**, 4-*O*-caffeoyl-5-*O*-(3'-hydroxydihydrocaffeoyl)-quinic acid **32** and 4-*O*-(3'-hydroxydihydrocaffeoyl)-5-*O*-caffeoylquinic acid **33**. HPLC chromatograms recorded at 320 nm (λ_{max}) of brewed maté showing chromatographic peaks of compounds **22**-**33** are shown in **Figure 5.7**. Detailed MS spectra of **22**-**33** were reported previously and are not discussed here.[10,22]

5.4. Conclusions

In this study, advanced HPLC-ESI-MS^n and HPLC-ESI-HRMS methods were used for the identification and characterization of hydroxycinnamates and their derivatives formed during the brewing process of yerba maté. It was shown that during brewing of yerba maté caffeoylglucoses, caffeoylquinic acid, dicaffeoylquinic acid and methyl caffeoylquinate undergo chemical transformations to form acyl migration products and hydroxydihydrocinnamic acid derivatives (*cis* isomers are also expected to form). Particularly, the focus was on the hydroxylation of the hydroxycinnamates cinnamoyl substituent by conjugate addition of water to form 3-hydroxydihydrocinnamic acid derivatives using a series of model compounds. The regiochemistry of these transformed products was elucidated using targeted tandem MS techniques and authentic standards. All ten theoretical regioisomers of caffeoylglucoses were detected for both water addition experiments while *cis* isomers were not detected/resolved with the current applied HPLC method.

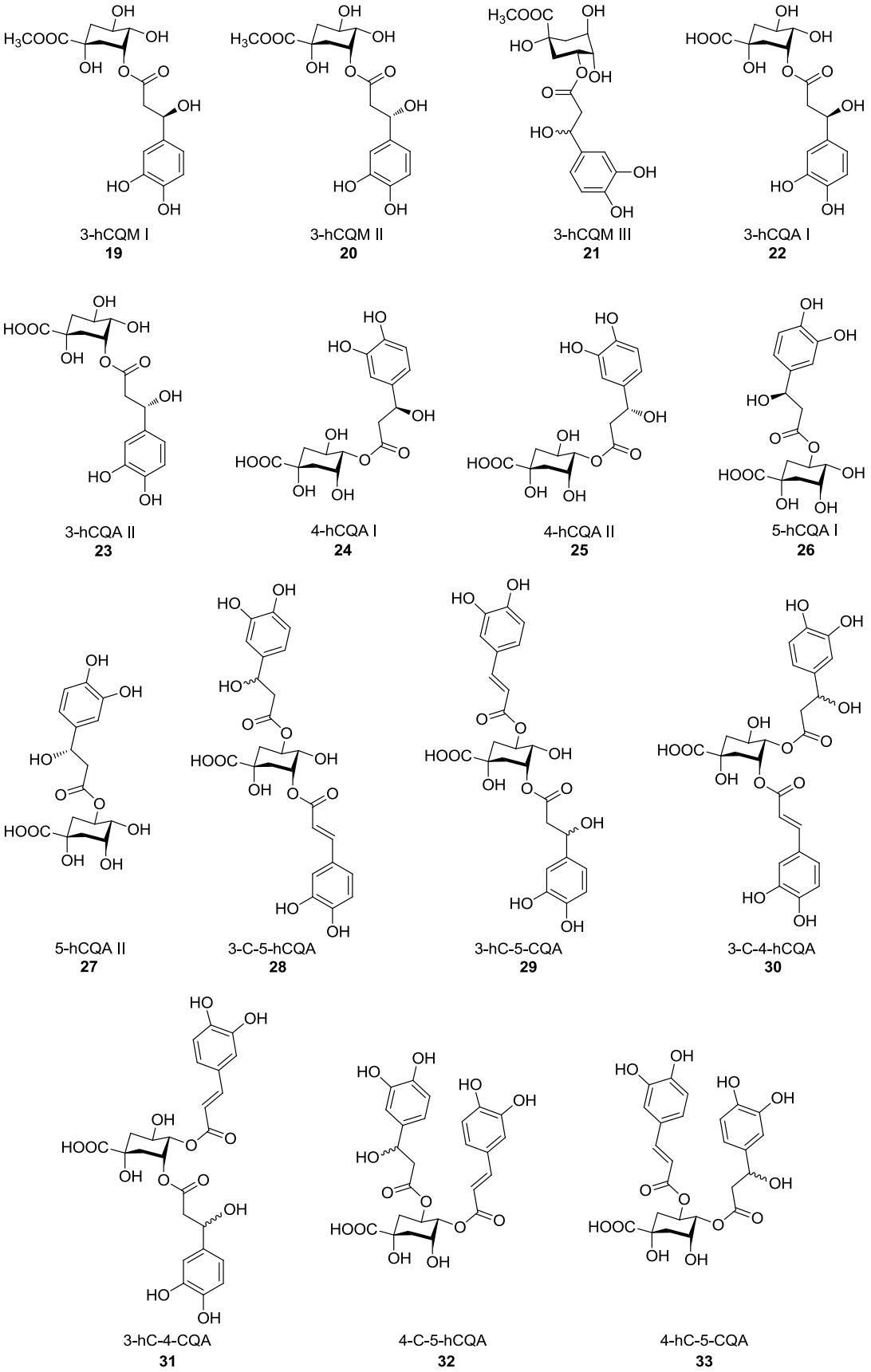

Figure 5.6. Mono- and diacylated water addition derivatives of chlorogenic acids and methyl quinate formed during brewing.

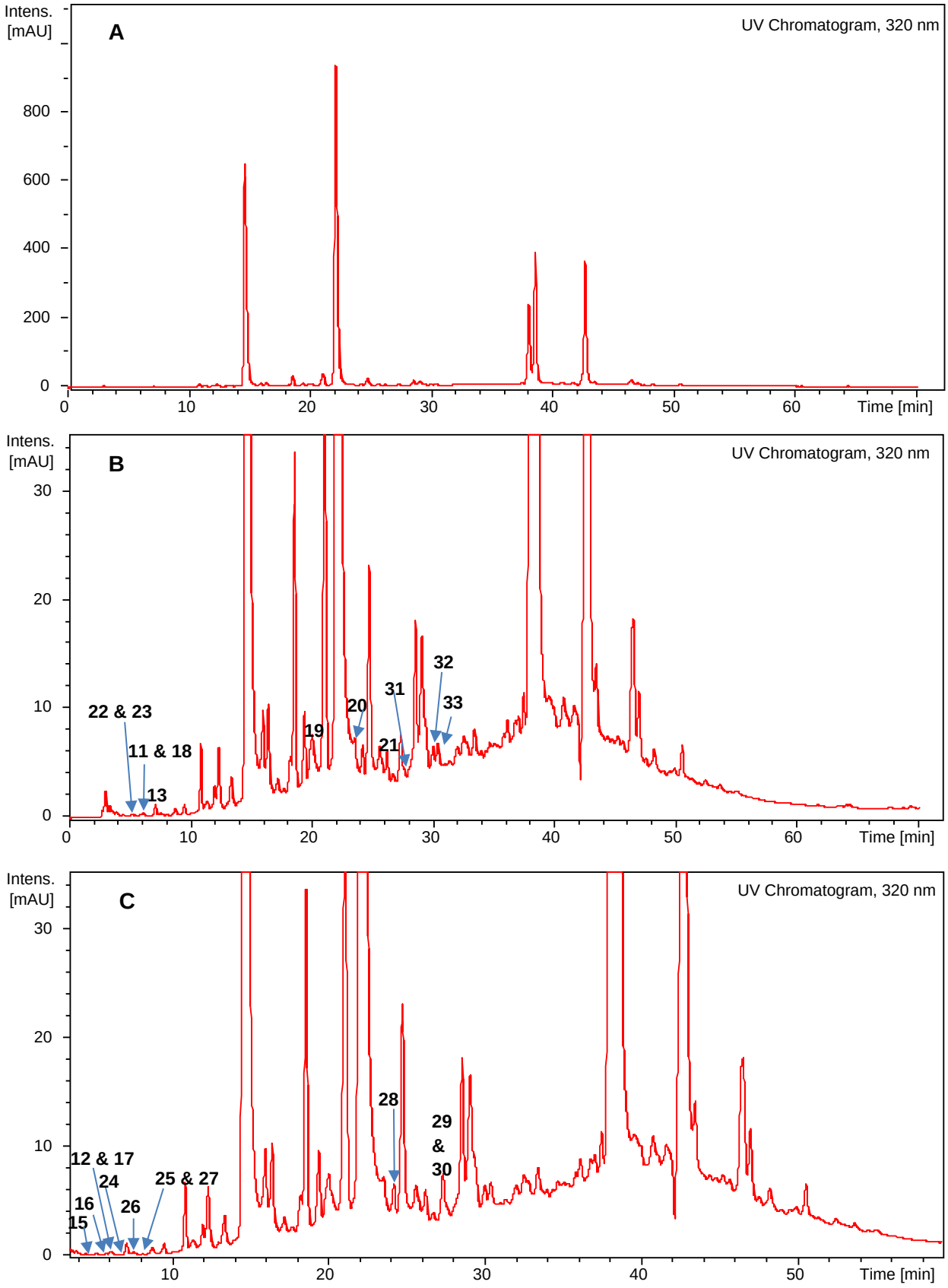

Figure 5.7. HPLC chromatograms recorded at 320 nm (λ_{max}) of brewed maté: A) full view; B) and C) chromatographic peaks of compounds **22**-**33**.

5.5. References

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Appendix A

Journal Articles

The following journal articles were removed from the online version of this dissertation for copyright reasons:

1. Kuhnert, N.; Yassin, G. H.; Jaiswal, R.; **Matei**, **M. F.**; Grün, C. Differentiation of prototropic ions in regioisomeric caffeoyl quinic acids by electrospray ion mobility mass spectrometry. *Rapid Commun. Mass Spectrom*. **2015**, *29*, 675-680. DOI: 10.1002/rcm.7151

2. Jaiswal, R.; **Matei**, **M. F.**; Glembockyte, V.; Patras, M.; Kuhnert, N. Hierarchical Key for the LC-MS^n Identification of All Ten Regio- and Stereoisomers of Caffeoyl Glucose. *J. Agric. Food Chem.* **2014**, *62*, 9252-9265. DOI: 10.1021/jf501210s

3. Deshpande, S.; Jaiswal, R.; **Matei**, **M. F.**; Kuhnert, N. Investigation of Acyl Migration in Mono- and Dicaffeoylquinic Acids under Aqueous Basic, Aqueous Acidic, and Dry Roasting Conditions. *J. Agric. Food Chem.* **2014**, *62*, 9160-9170. DOI: 10.1021/jf5017384

4. Jaiswal, R.; **Matei**, **M. F.**; Subedi, P.; Kuhnert, N. Does roasted coffee contain chlorogenic acid lactones or/and cinnamoylshikimate esters? *Food Res. Int.* **2013**, *61*, 214-227. DOI: 10.1016/j.foodres.2013.09.040

5. **Matei**, **M. F.**; Jaiswal, R.; Kuhnert, N. Investigating the Chemical Changes of Chlorogenic Acids during Coffee Brewing: Conjugate Addition of Water to the Olefinic Moiety of Chlorogenic Acids and Their Quinides. *J. Agric. Food Chem.* **2012**, *60*, 12105-12115. DOI: 10.1021/jf3028599

6. Jaiswal, R.; **Matei**, **M. F.**; Golon, A.; Witt, M.; Kuhnert, N. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct.* **2012**, *3*, 976-984. DOI: 10.1039/C2FO10260A

7. Eravuchira, P. J.; El-Abassy, R. M.; Deshpande, S.; **Matei**, **M. F.**; Mishra, S.; Tandon, P.; Kuhnert, N.; Materny, A. Raman spectroscopic characterization of different regioisomers of monoacyl and diacyl chlorogenic acid. *Vib. Spectrosc.* **2012**, *61*, 10-16. DOI: 10.1016/j.vibspec.2012.02.009

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9. Jaiswal, R.; **Matei**, **M. F.**; Ullrich, F.; Kuhnert, N. How to distinguish between cinnamoylshikimate esters and chlorogenic acid lactones by liquid chromatography–tandem mass spectrometry. *J. Mass Spectrom*. **2011**, *46*, 933-942. DOI: 10.1002/jms.1972

10. Jaiswal, R.; **Matei**, **M. F.**; Ullrich, F.; Kuhnert, N. How to distinguish between feruloyl quinic acids and isoferuloyl quinic acids by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom*. **2010**, *24*, 1575-1582. DOI: 10.1002/rcm.4537

Appendix B

Manuscripts

The following manuscripts were removed from the online version of this dissertation for copyright reasons:

1. Karar, M. G. E.; **Matei**, **M. F.**; Jaiswal, R.; Illenberger, S.; Kuhnert, N. Neuraminidase inhibition of dietary chlorogenic acids and derivatives – potential antivirals from dietary sources. *Food Funct.* **2016**, *7*, 2052-2059. DOI: 10.1039/C5FO01412C

2. Kreir, M.; **Matei**, **M. F.**; Wyss, R.; Pick, H.; Karaköse, H.; Vogel, H.; Kuhnert, N.; Winterhalter, M. Rationalizing off-flavors by investigating the activation of TRPA1 and TRPV1 ion channels by steviol glycosides and caffeoyl quinic acid lactones using whole cell patch clamp measurements, manuscript in preparation.

Appendix C

Book Chapters

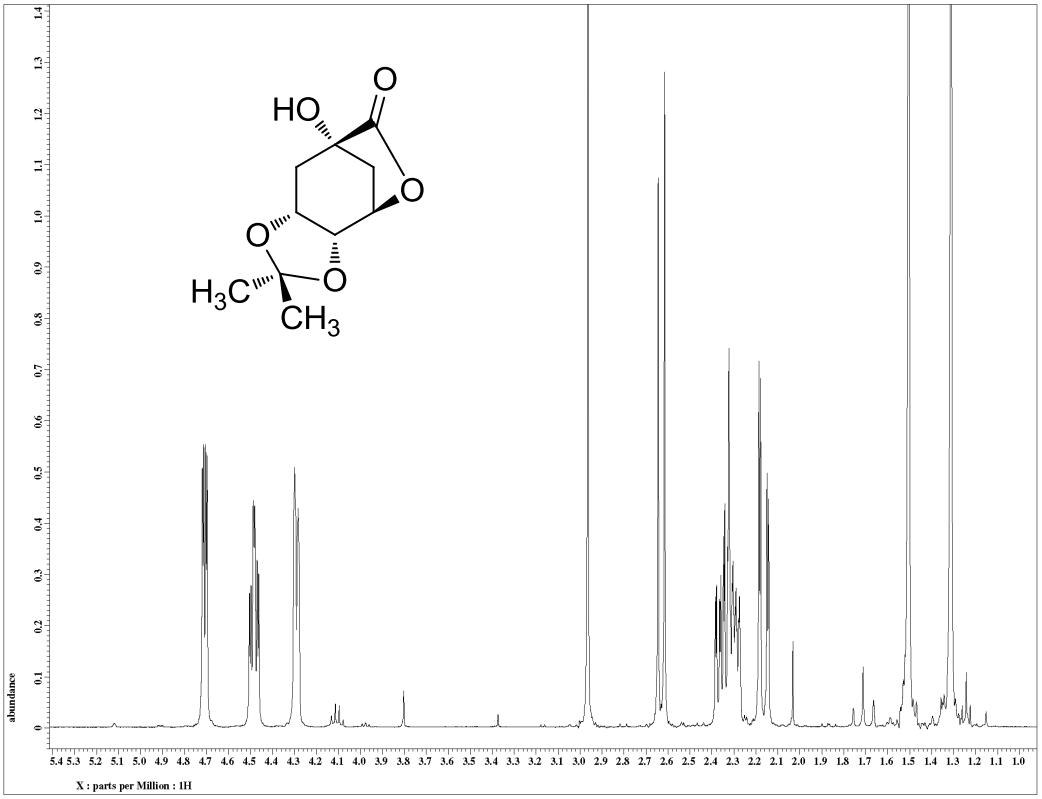
The following book chapters were removed from the online version of this dissertation for copyright reasons:

1. Jaiswal, R.; **Matei**, **M. F.**; Deshpande, S.; Kuhnert, N. Identification and characterization of the hydroxycinnamates of six Galium species from the Rubiaceae family. In *Handbook of Chemical and Biological Plant Analytical Methods*, 1st ed.; Hostettmann, K., Chen, S., Marston, A., Stuppner, H., Eds.; John Wiley & Sons: Chichester, U.K., 2013; Vol. 2, pp 505-524.

2. **Matei**, **M. F.**; Lee, S.-H.; Kuhnert, N. Chlorogenic Acids in Coffee, In *Chemistry of Coffee*, 1st ed.; Farah, A., Ed.; Royal Society of Chemistry: Cambridge, U.K., 2016, submitted.

Appendix D

Spectra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CH3
abundance
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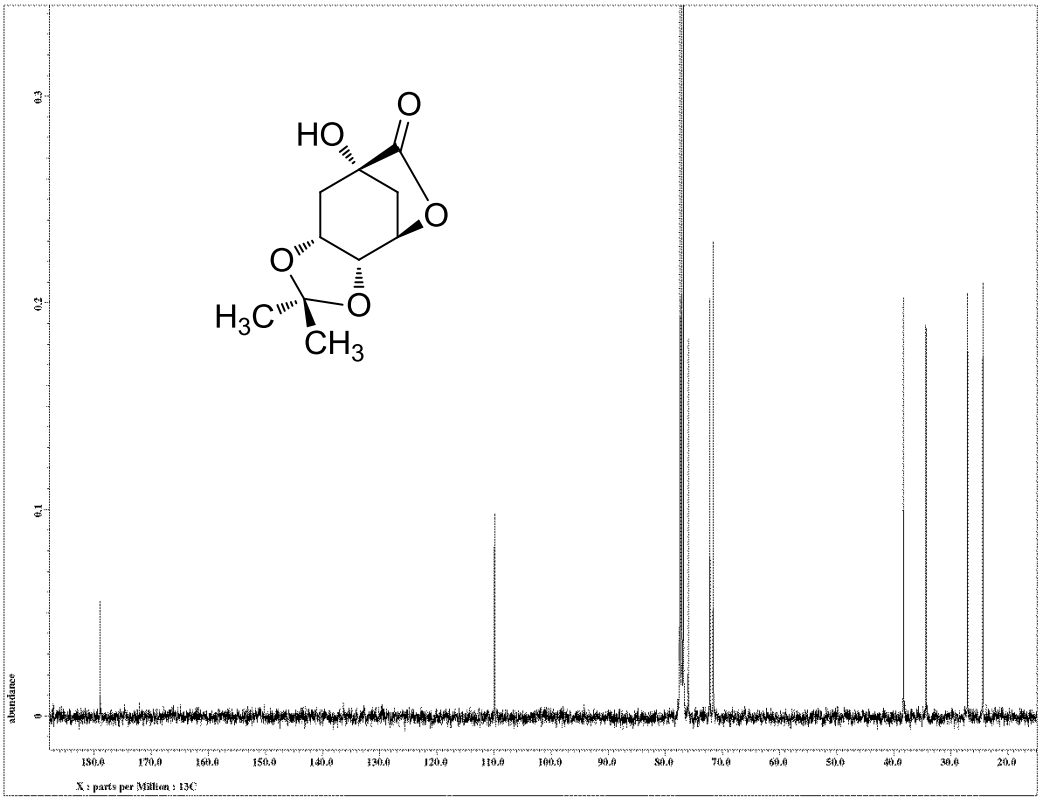
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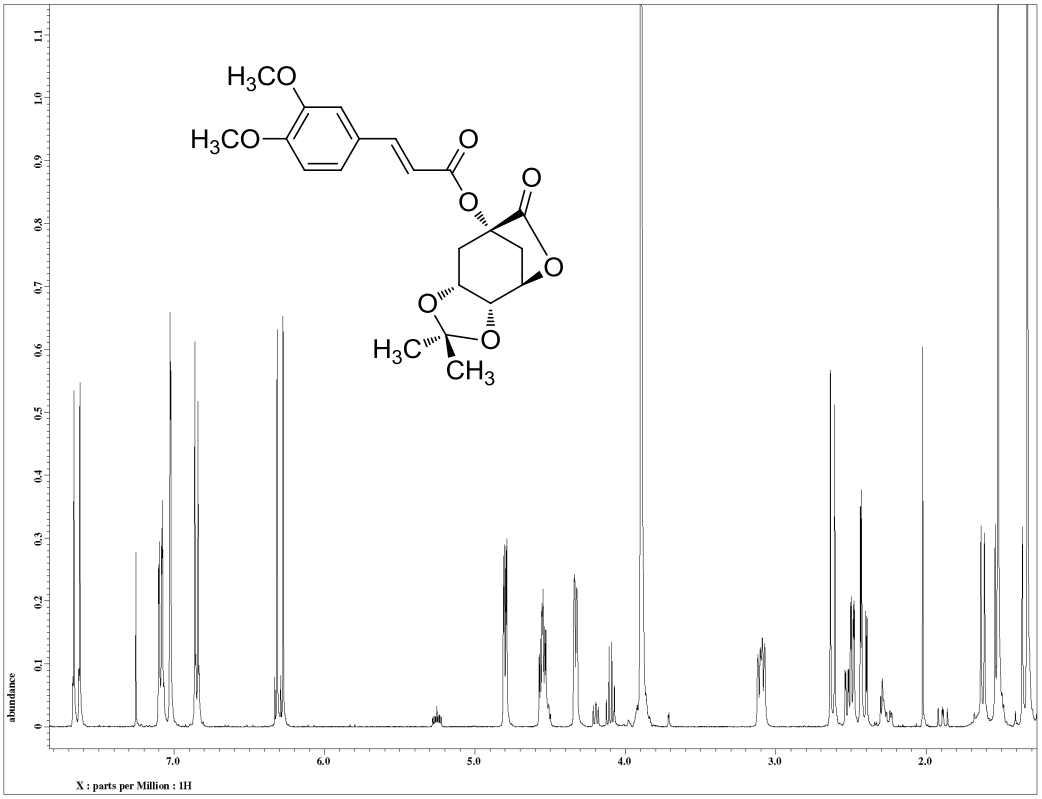
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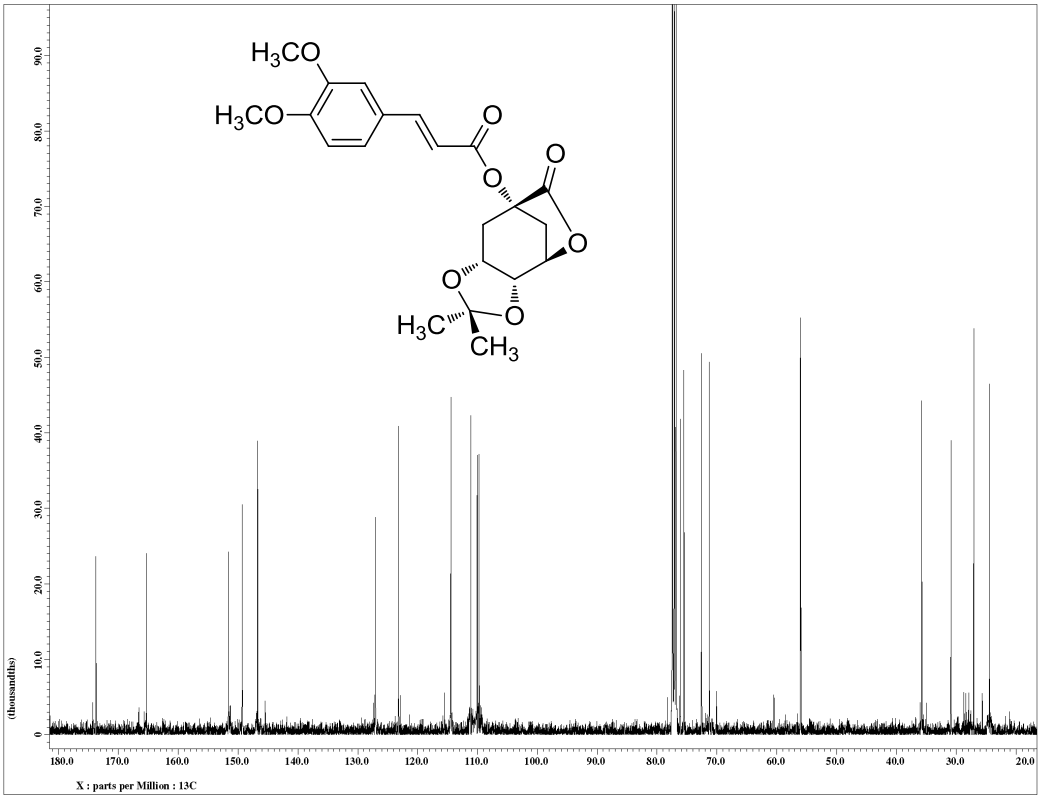
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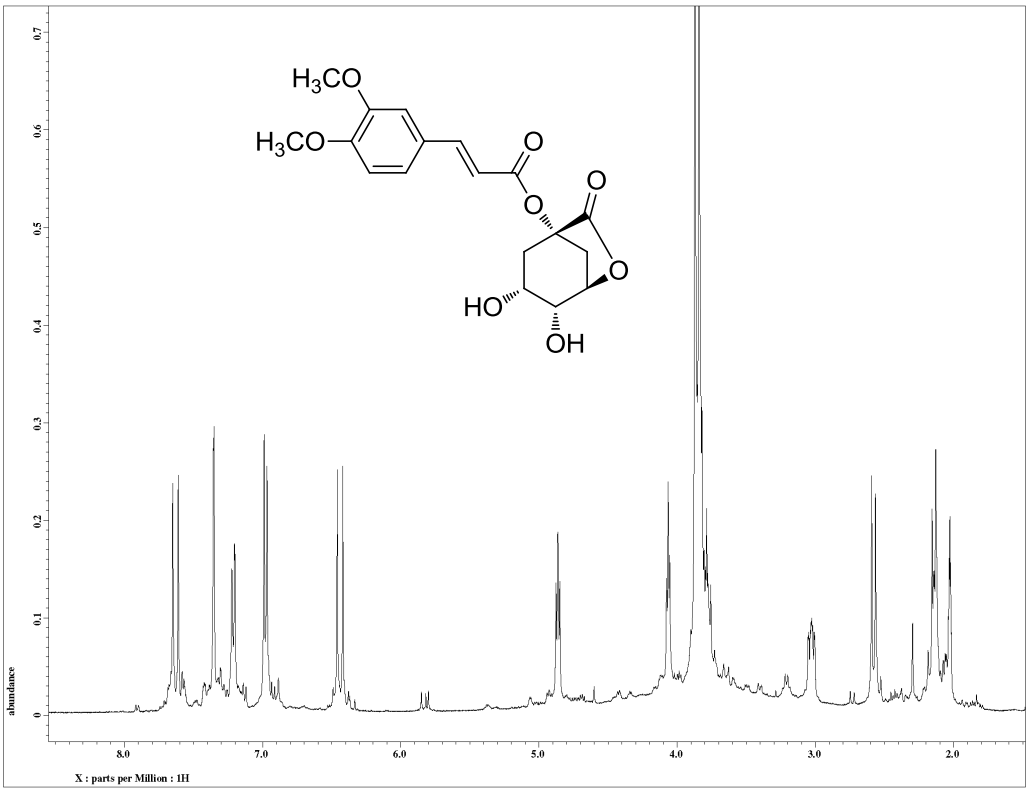
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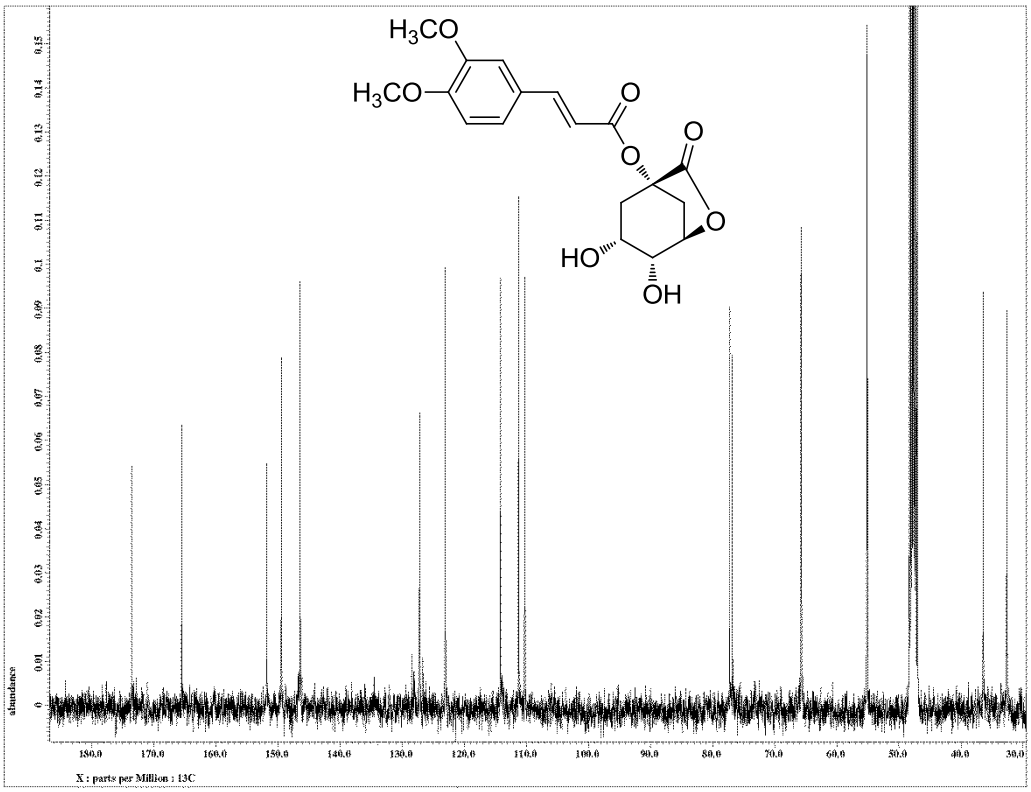
X : parts per Million : 1H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0.07
0.08
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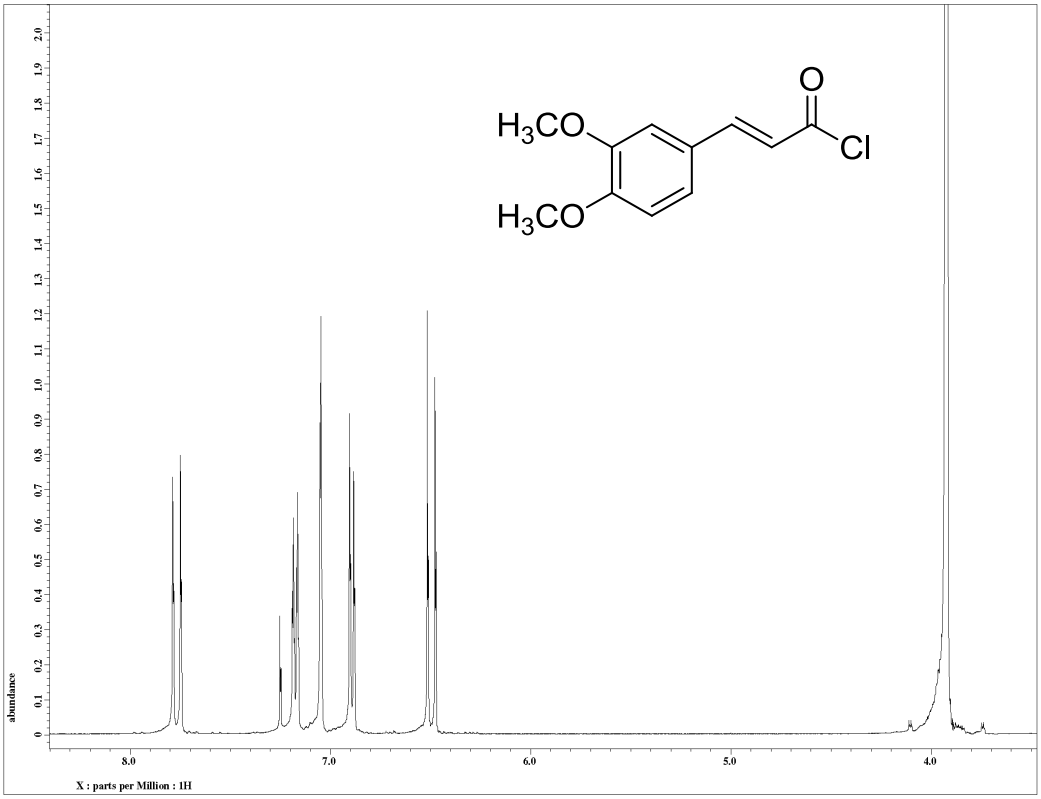
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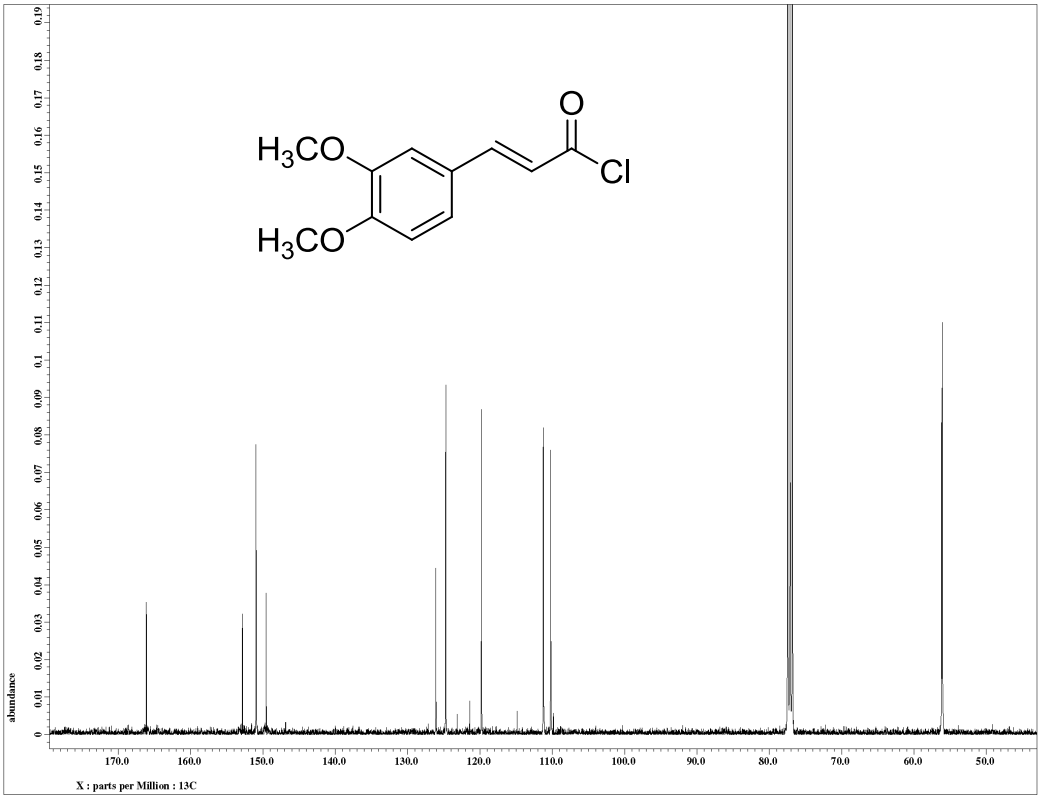
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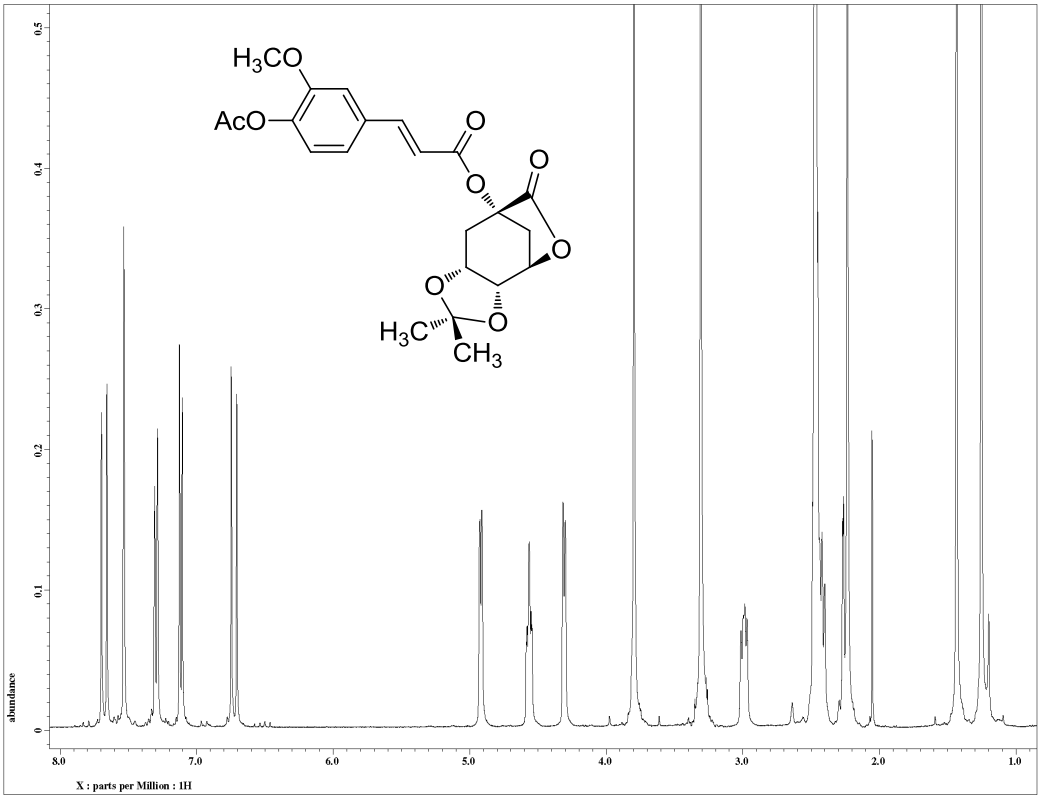
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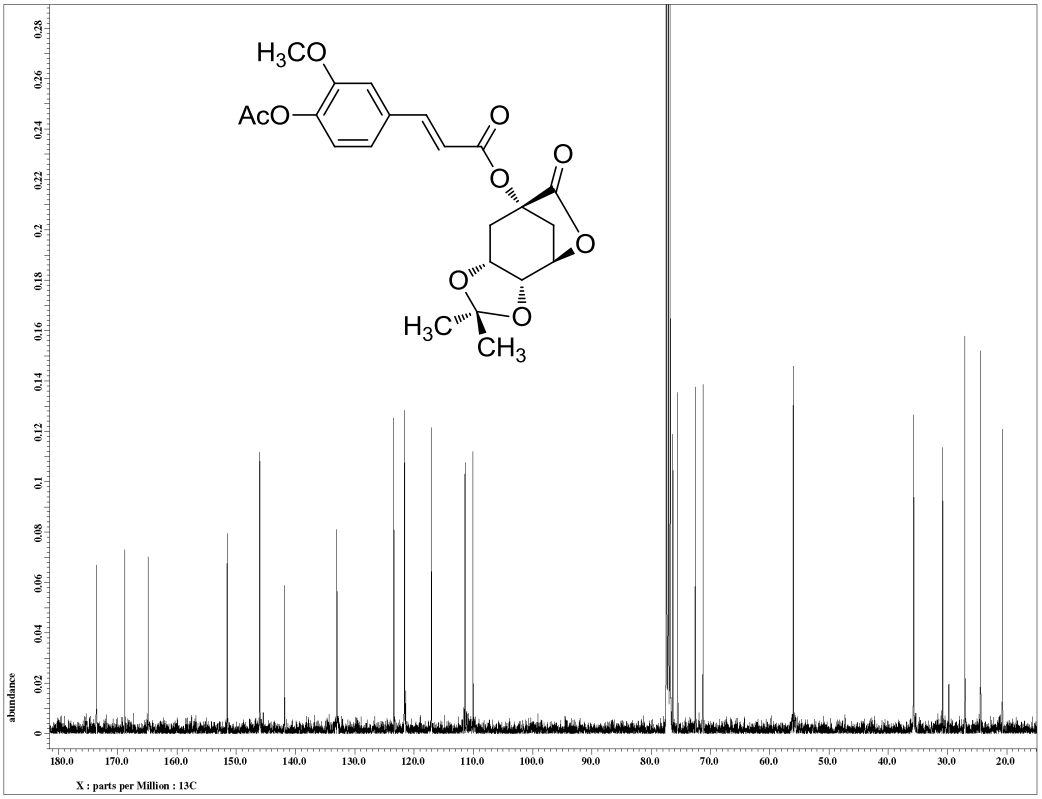
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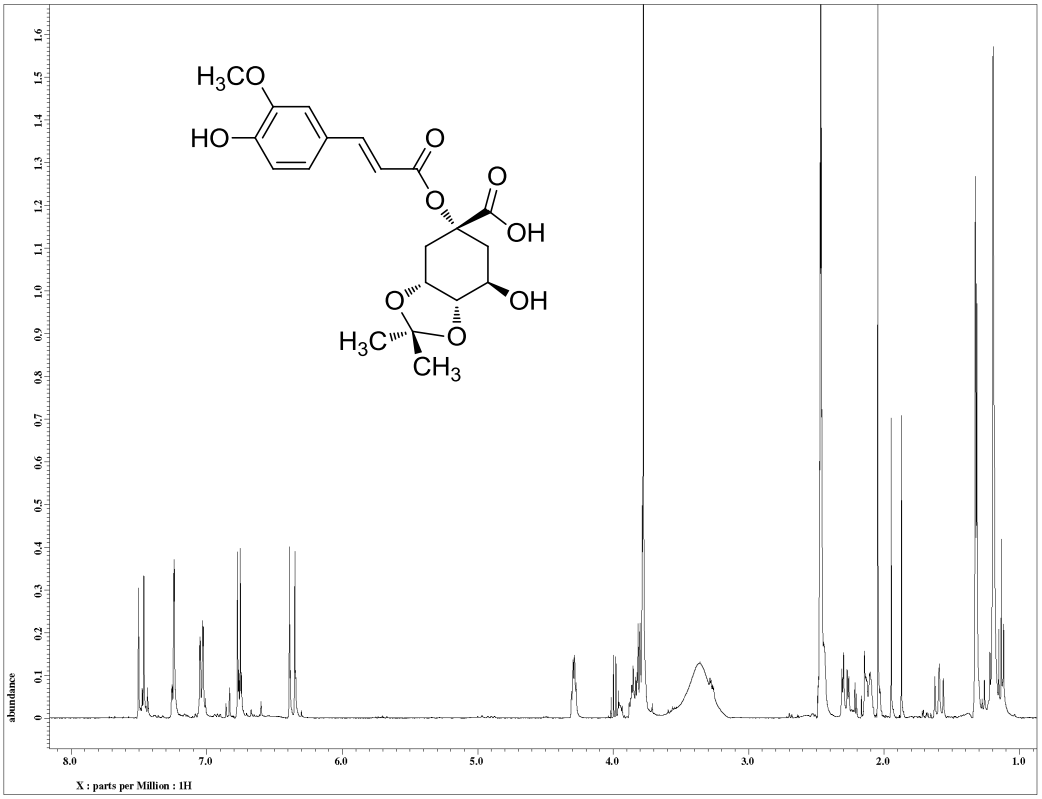
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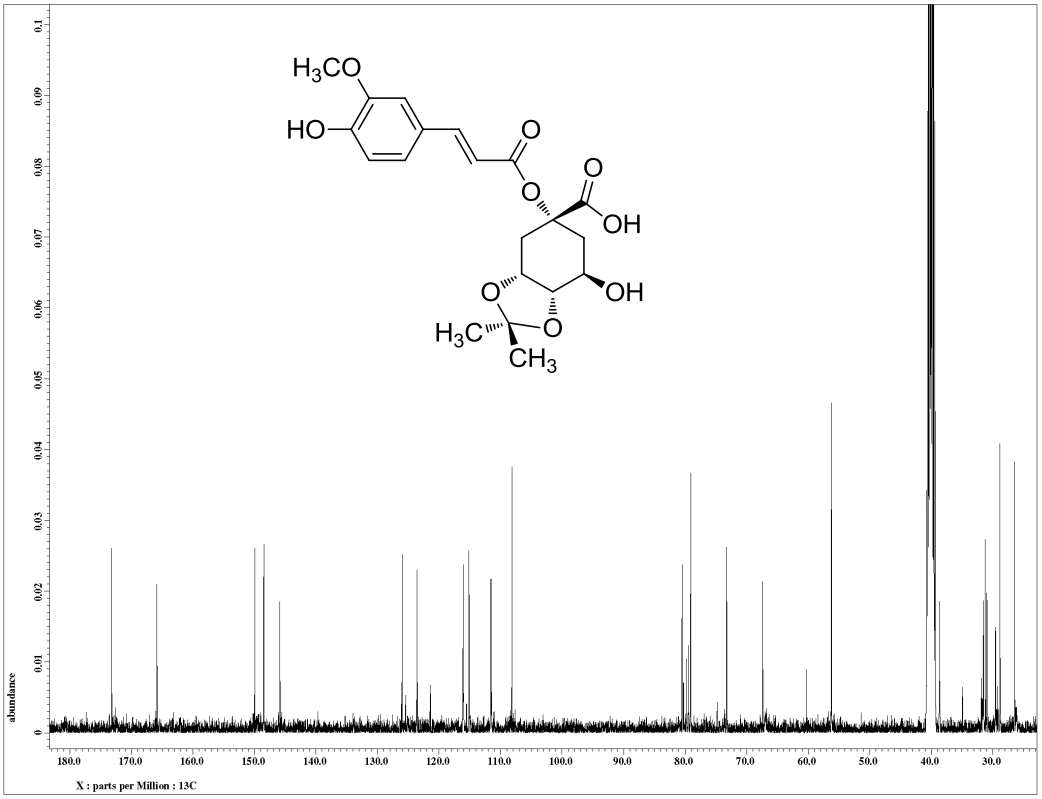
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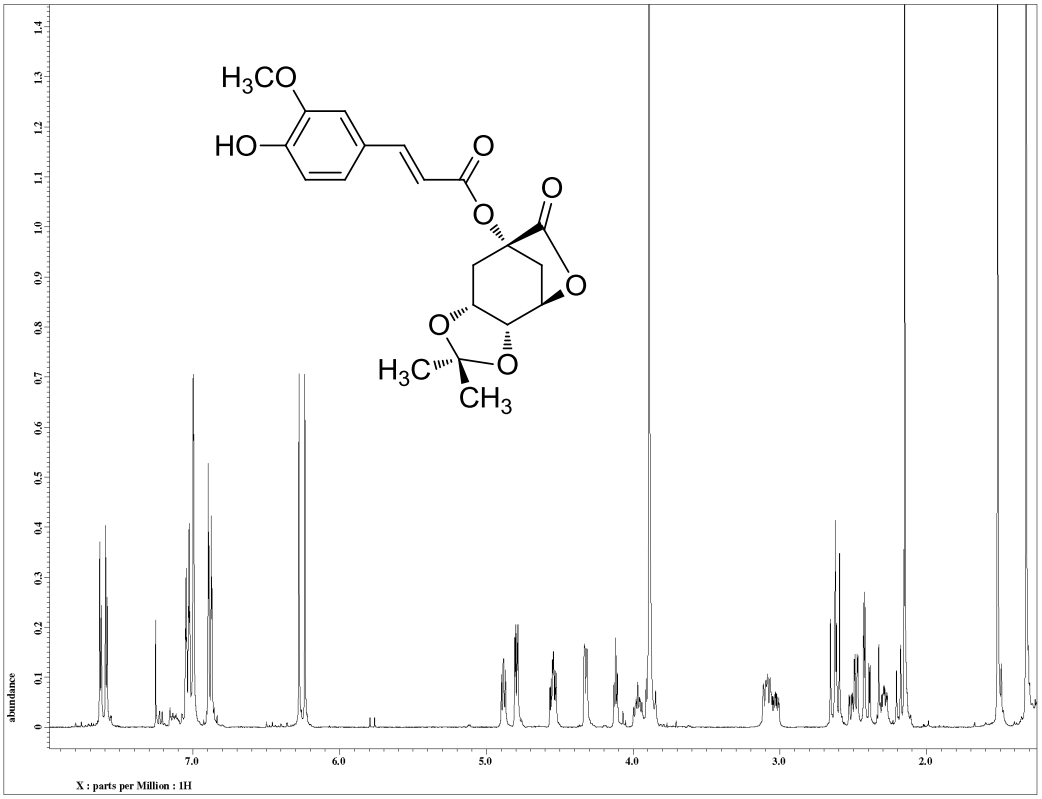
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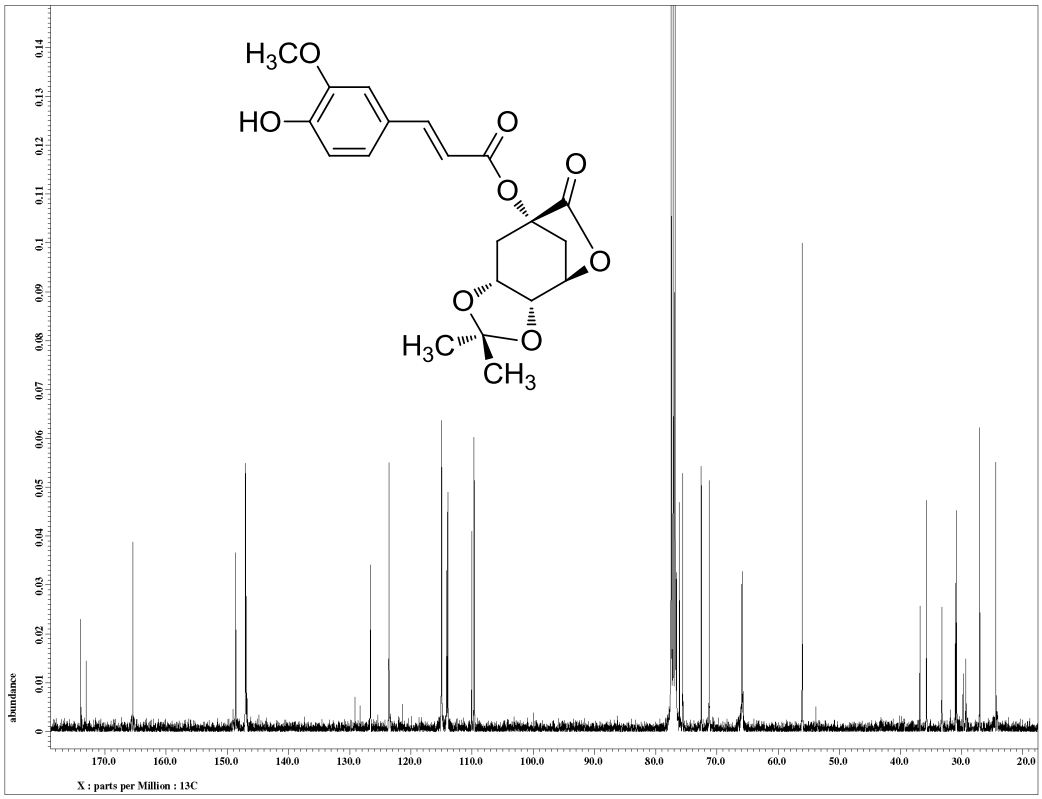
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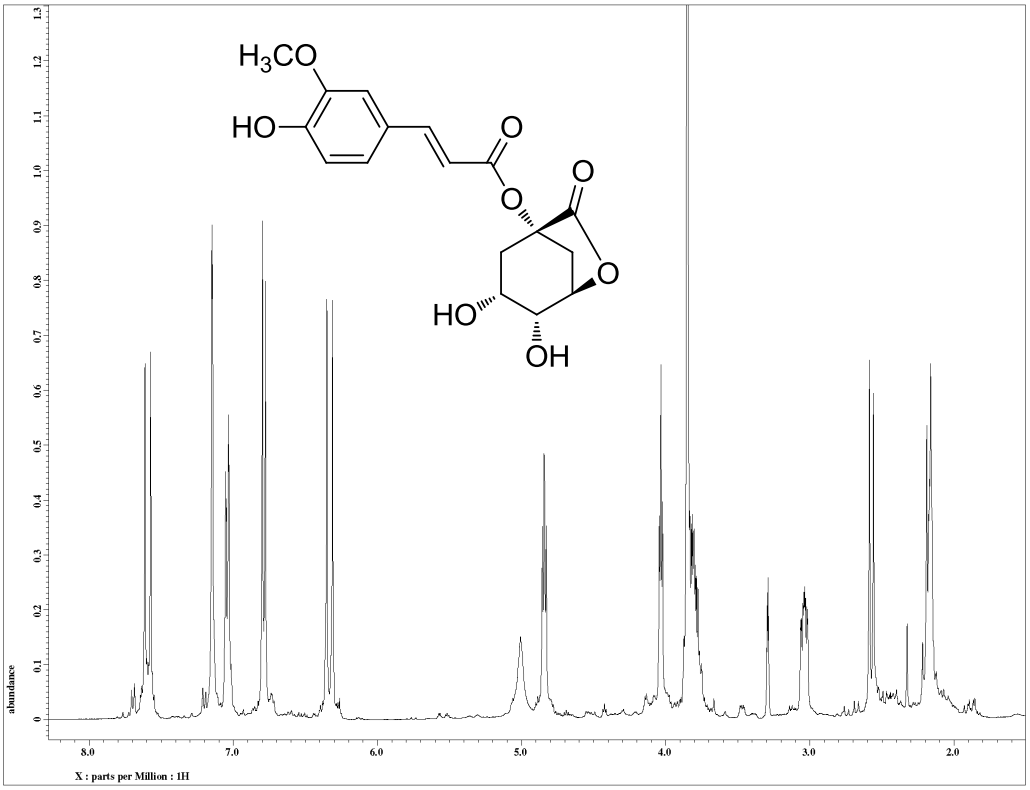
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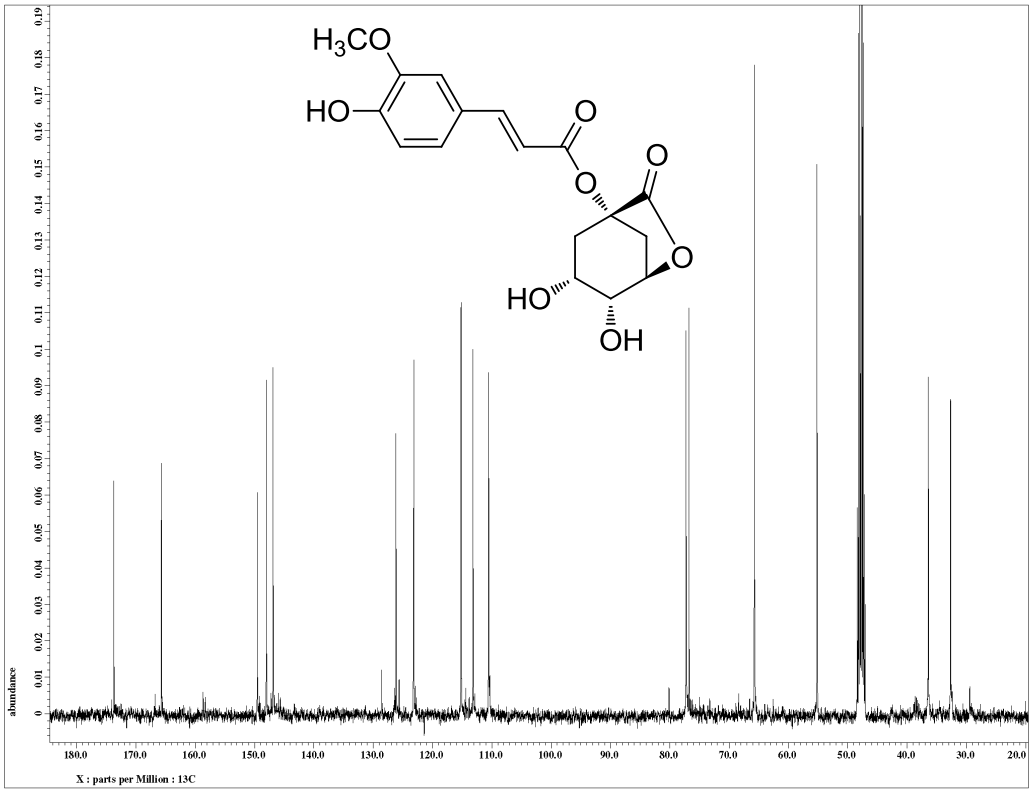
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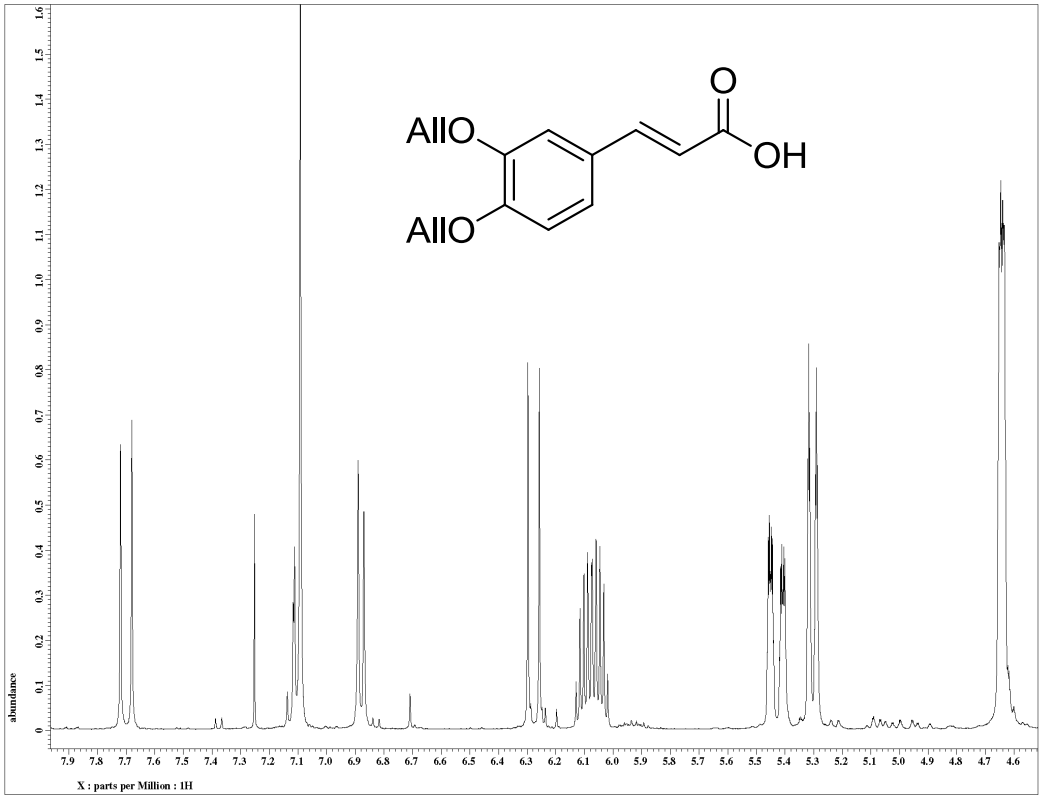
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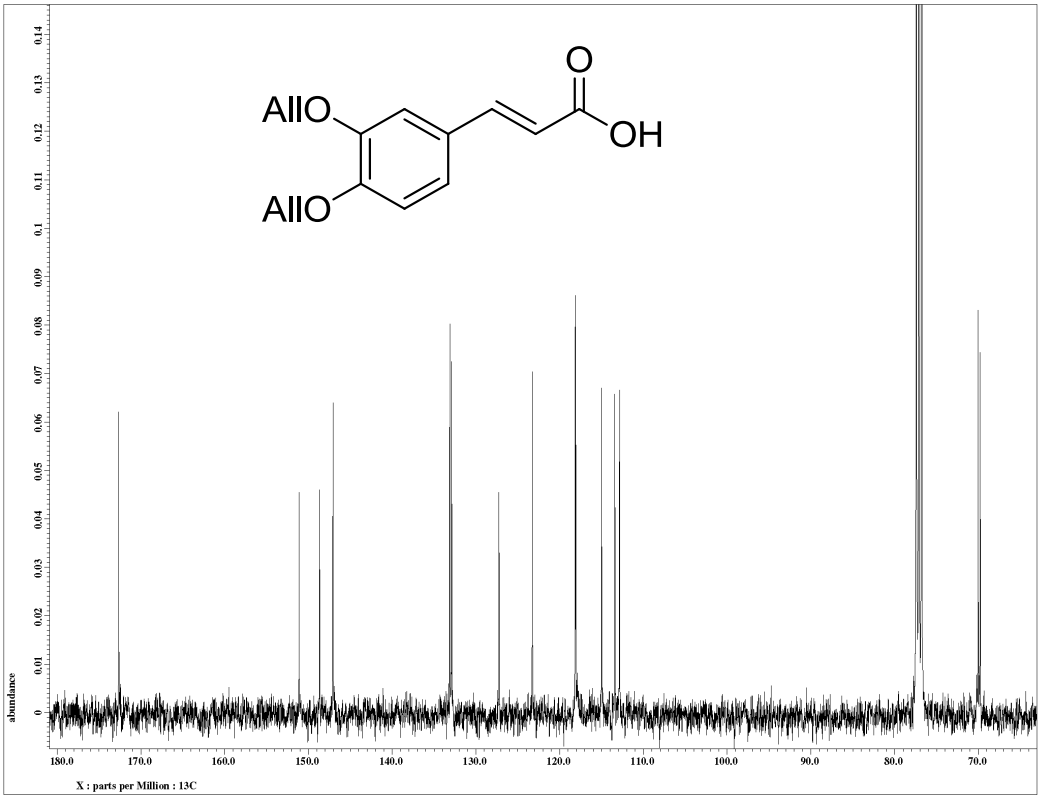
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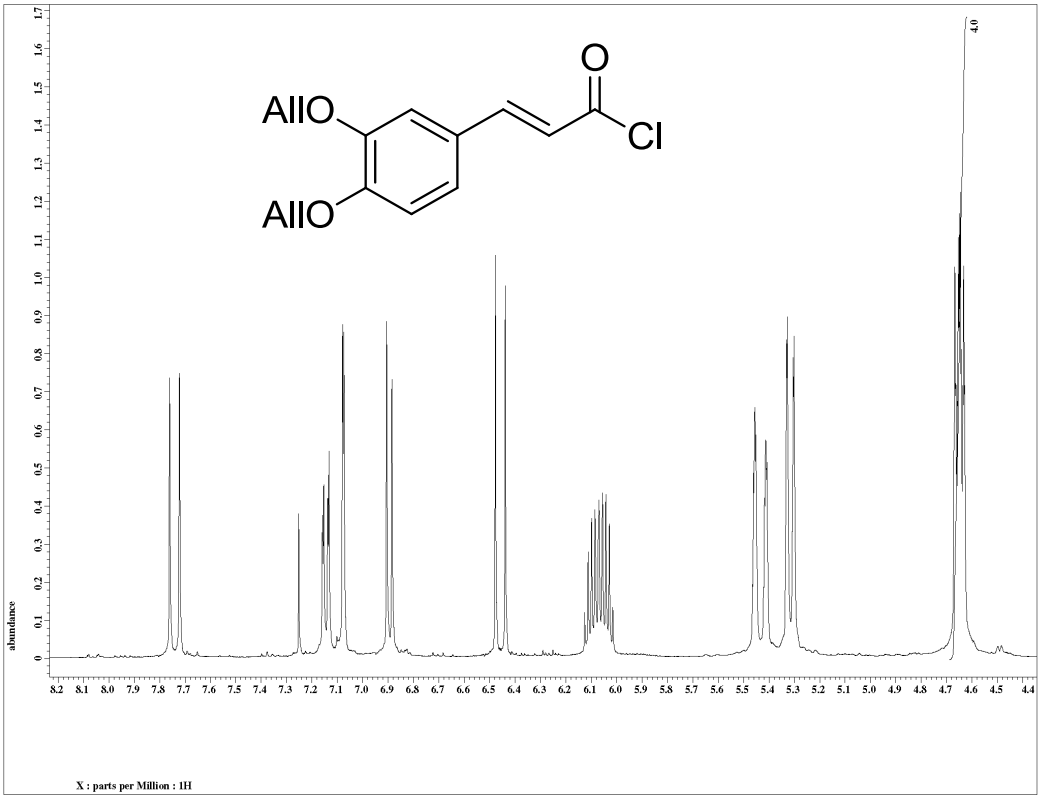
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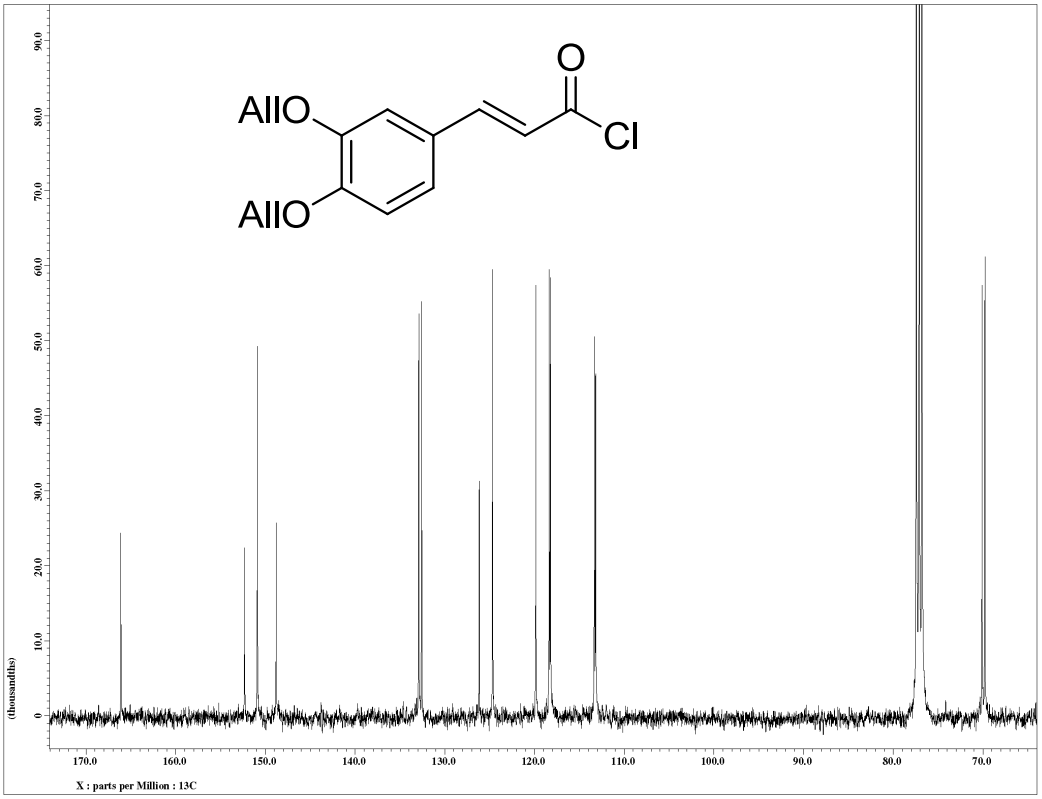
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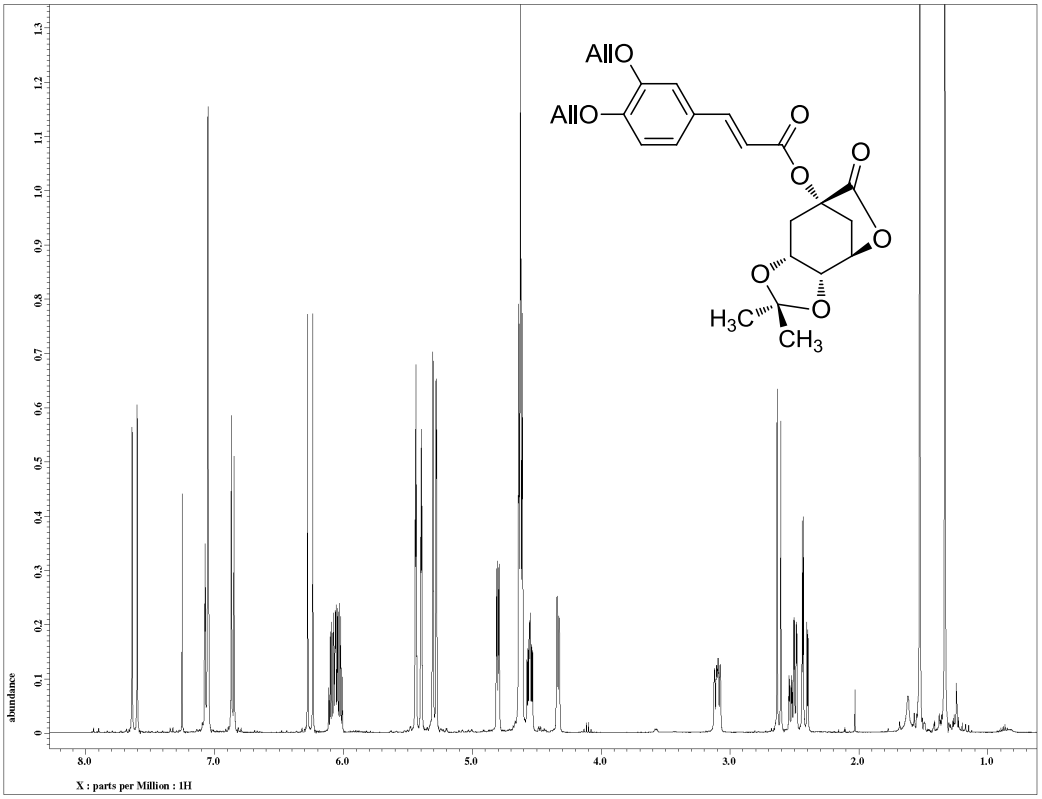
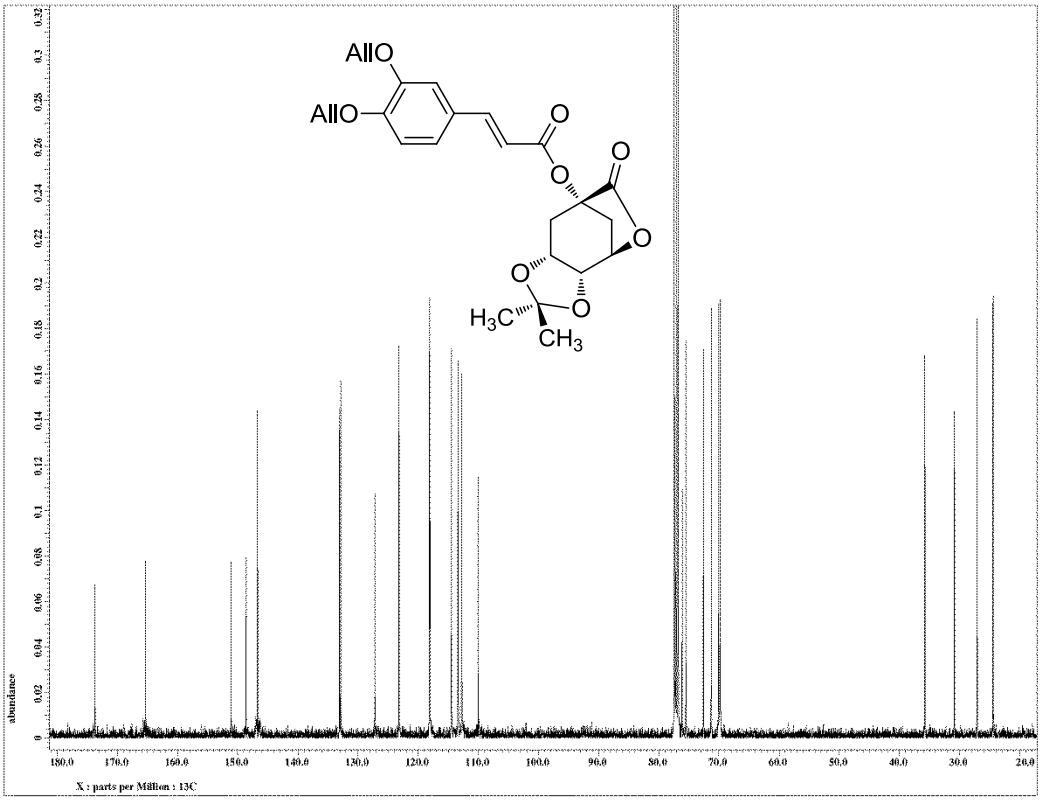
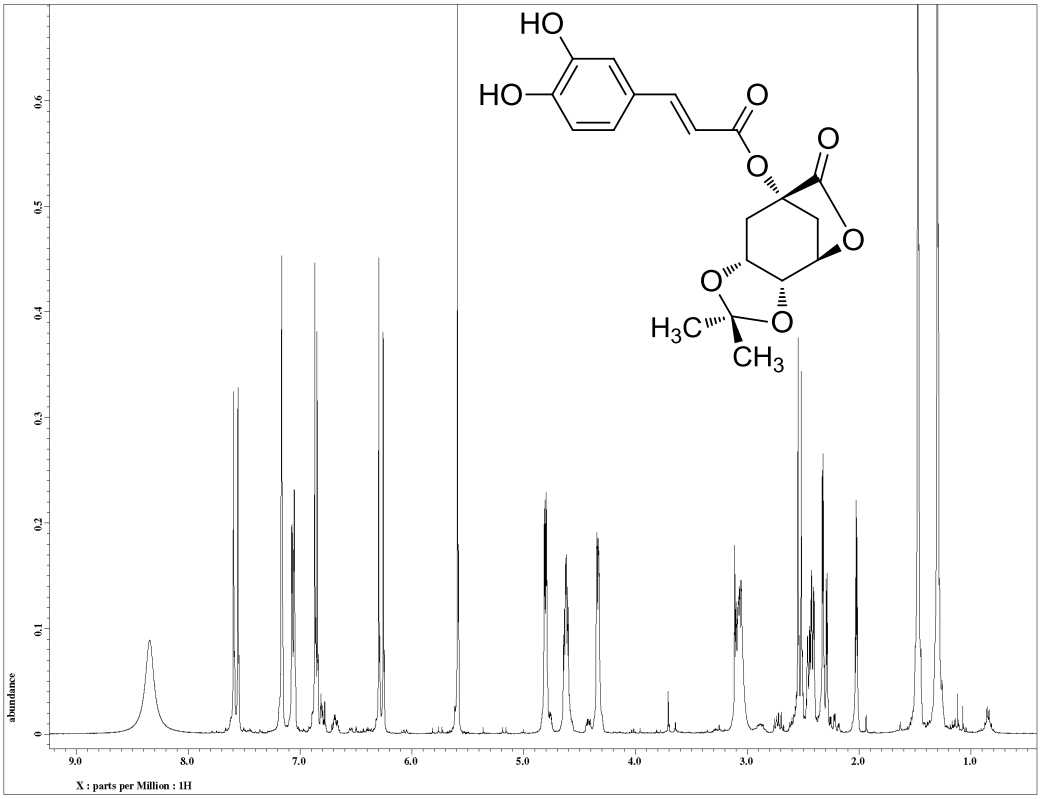
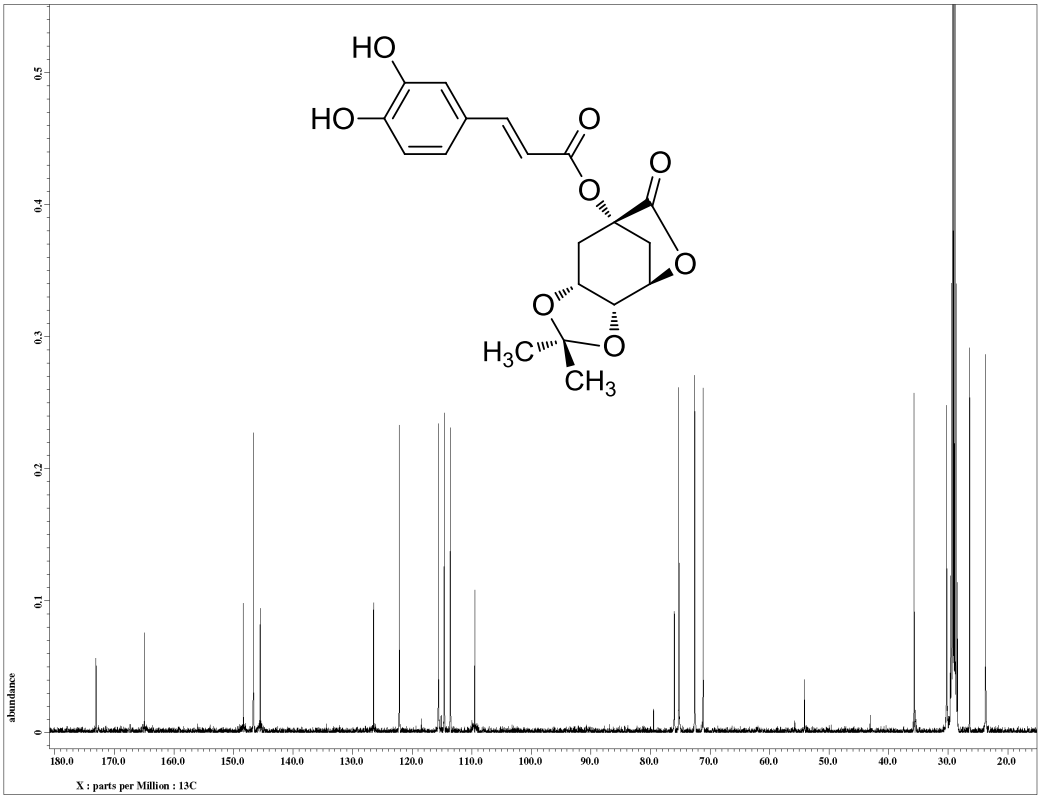
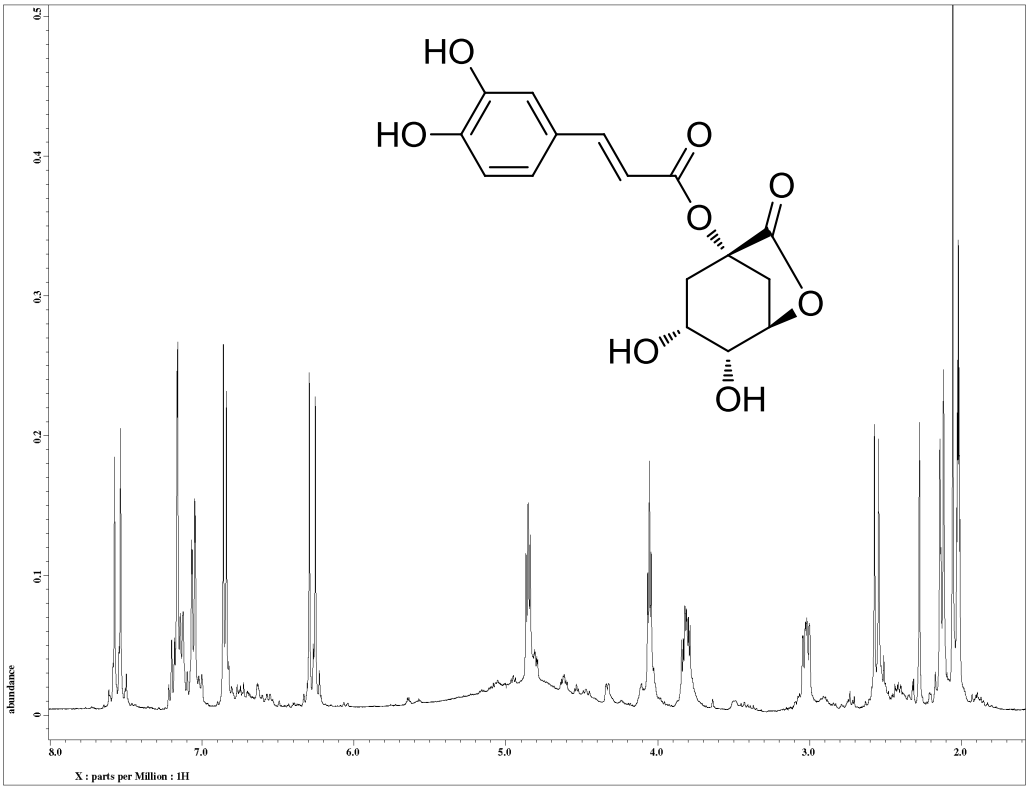
(thousandths)
X : parts per Million : 13C

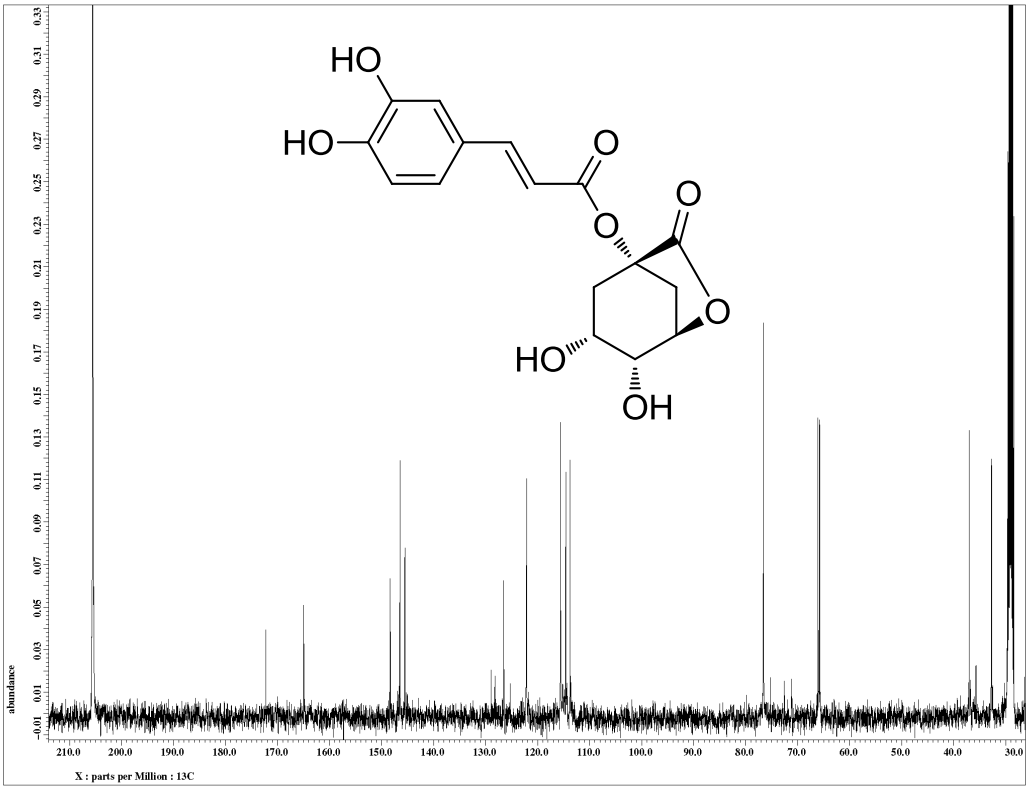

AllO
AllO
O
O
O
O
O
O
H3C
O
CH3
abundance
X : parts per Million : 1H

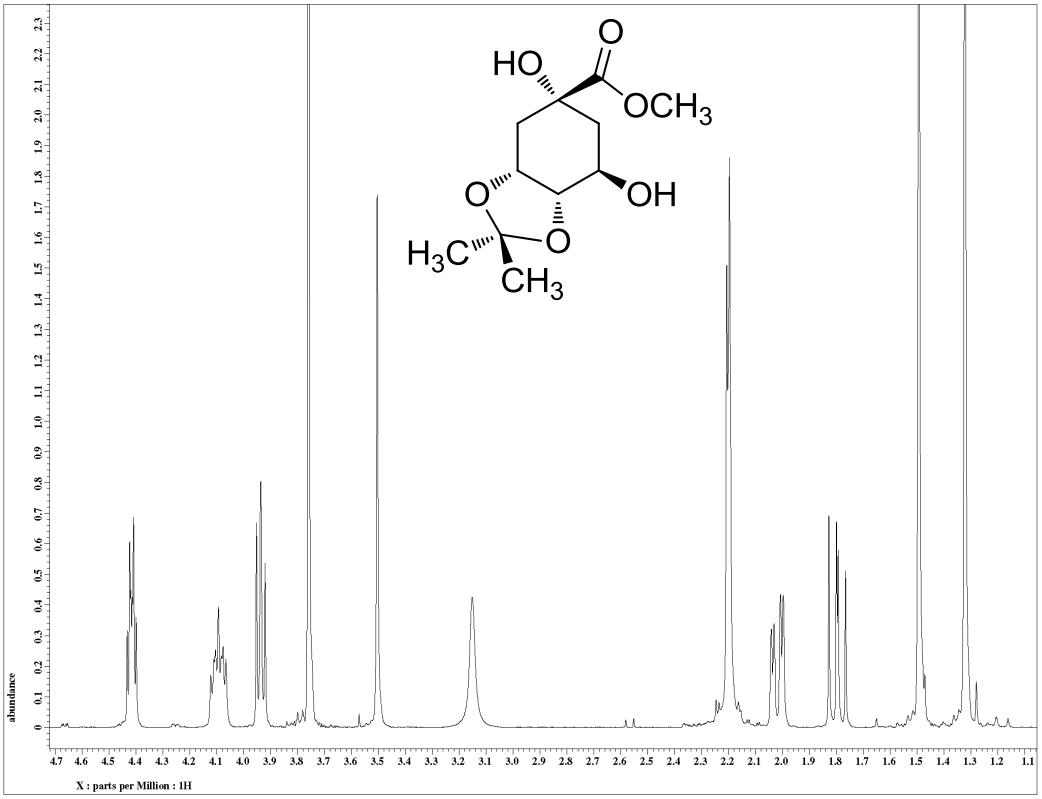

AllO
AllO
O
O
O
O
O
O
H3C
O
CH3
abundance
X : parts per Million : 13C

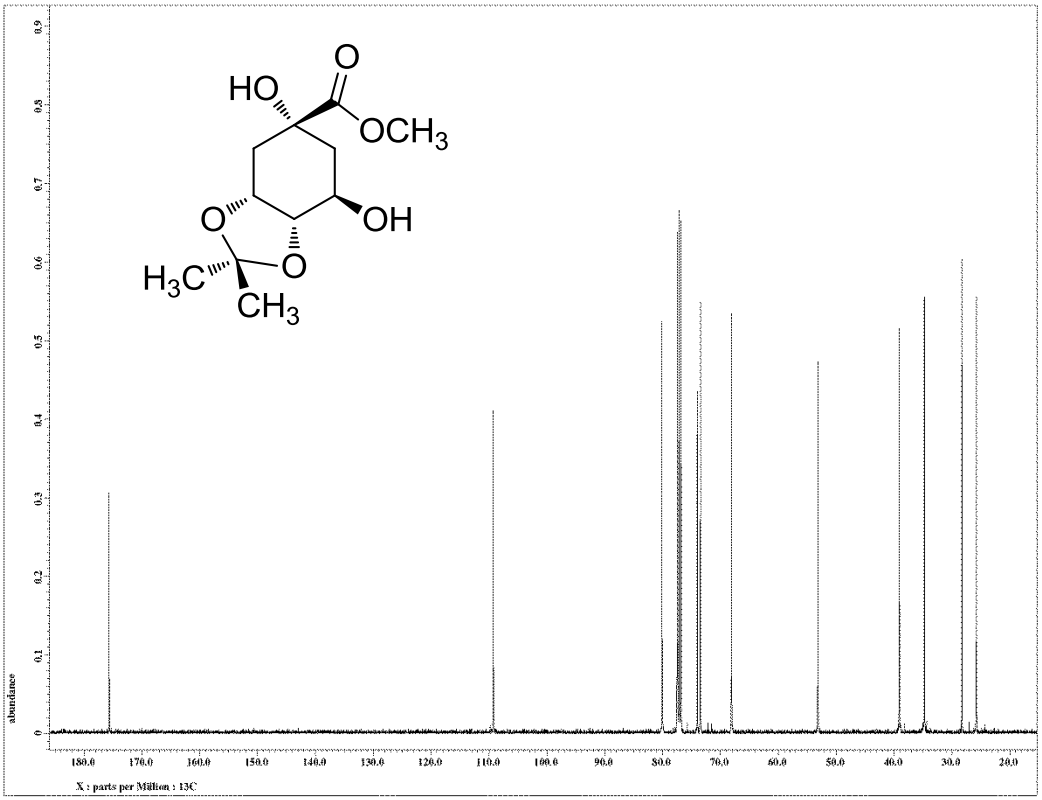

HO
HO
O
O
O
O
O
O
H3C
CH3
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : 1H

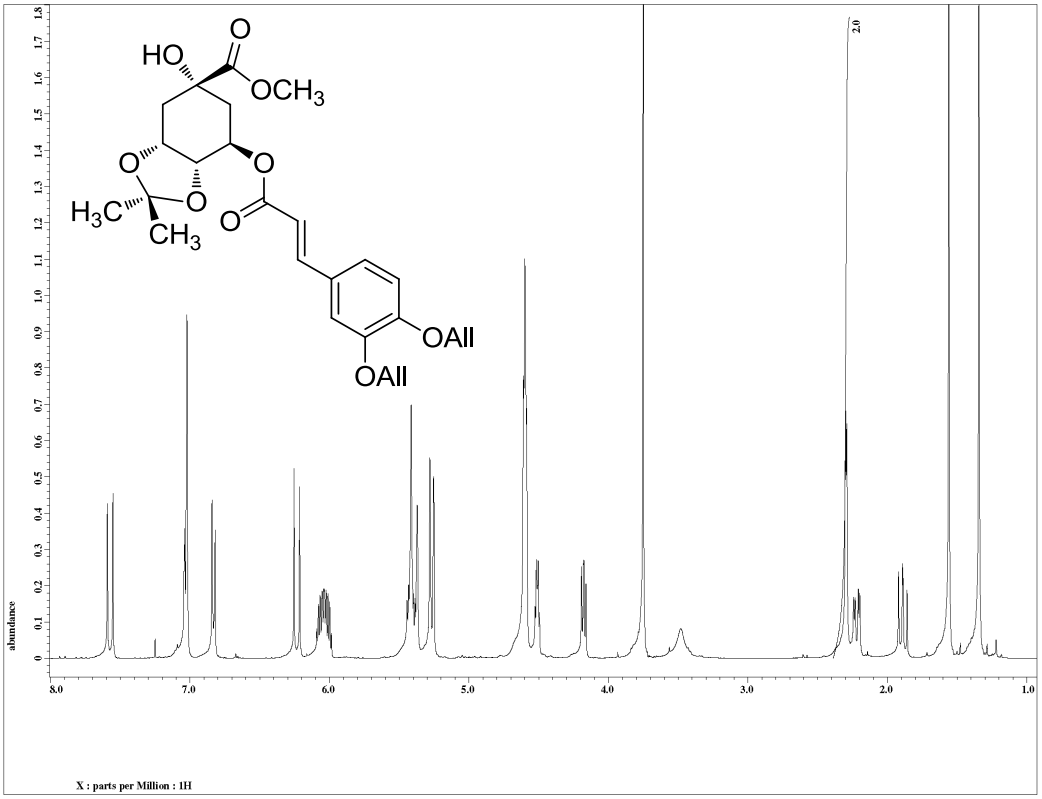

HO
HO
O
O
O
O
O
O
H3C
CH3
abundance
0
0.1
0.2
0.3
0.4
0.5
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
X : parts per Million : 13C

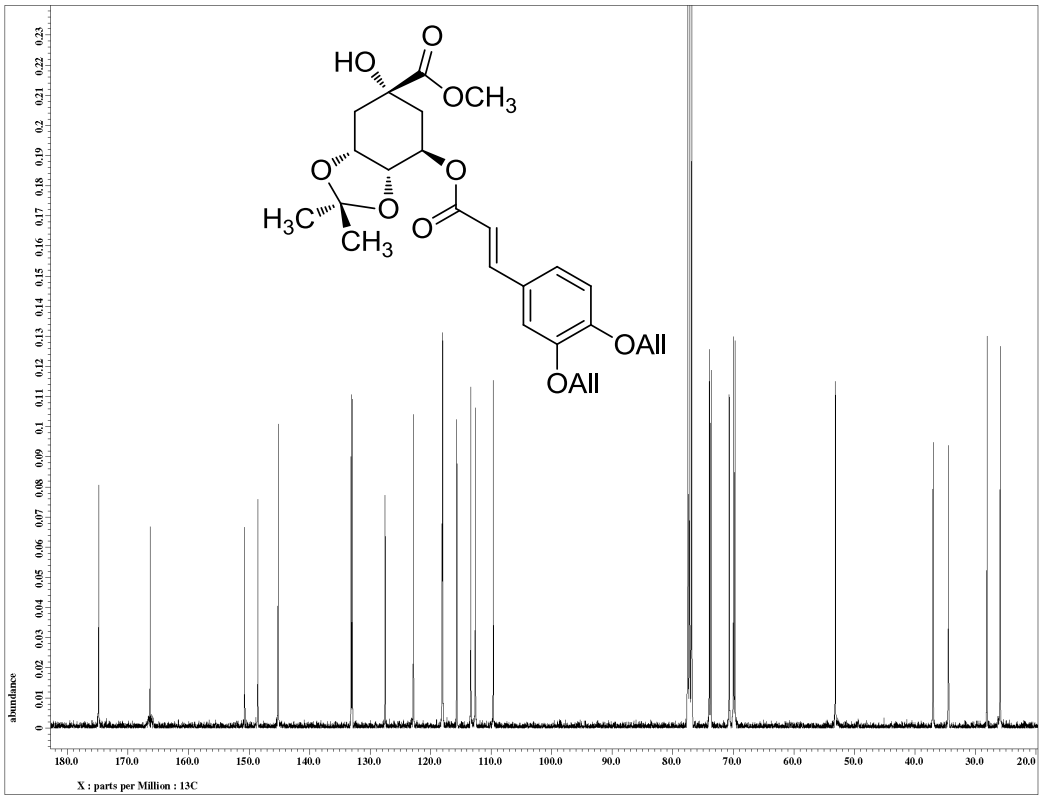

HO
HO
O
O
O
O
HO
OH
abundance
X : parts per Million : 1H

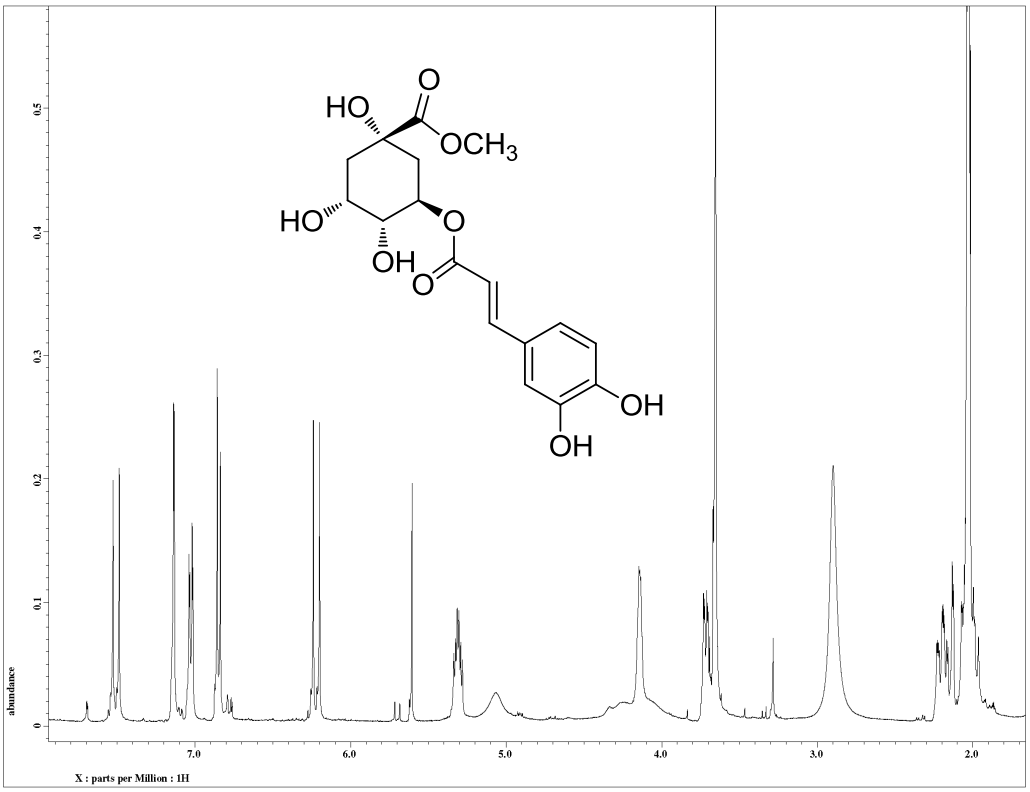

HO
HO
O
O
O
O
HO
OH
abundance
X : parts per Million : 13C

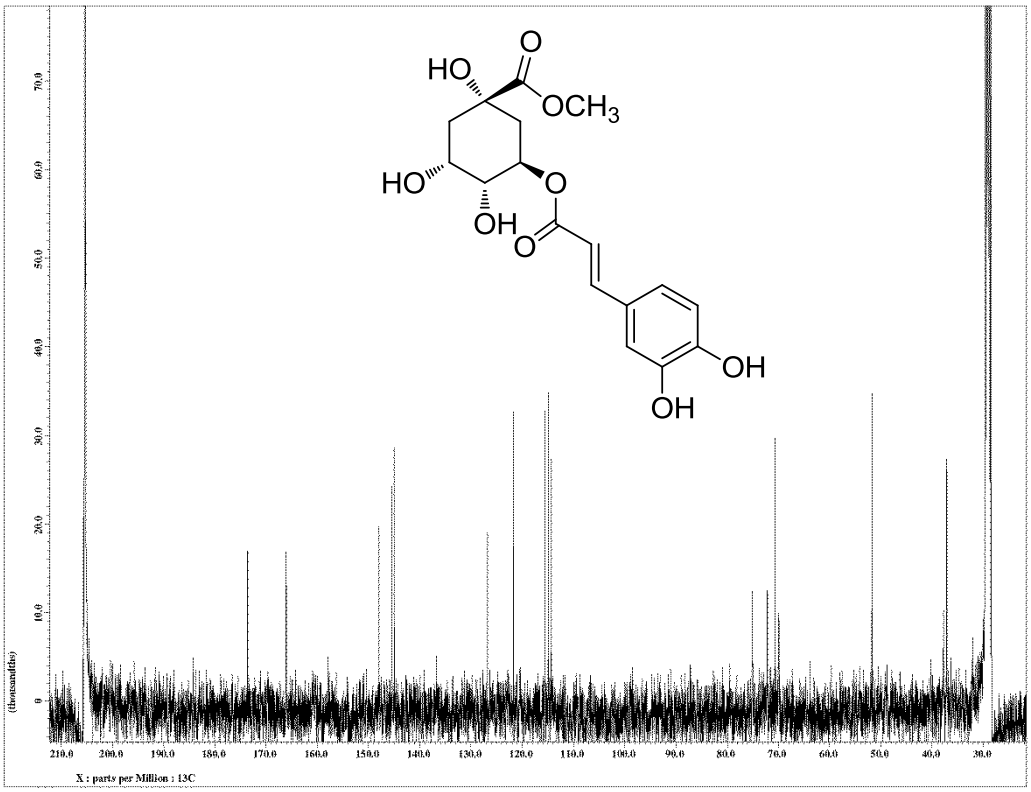

HO
O
OCH3
O
OH
H3C
O
CH3
abundance
X : parts per Million : 1H

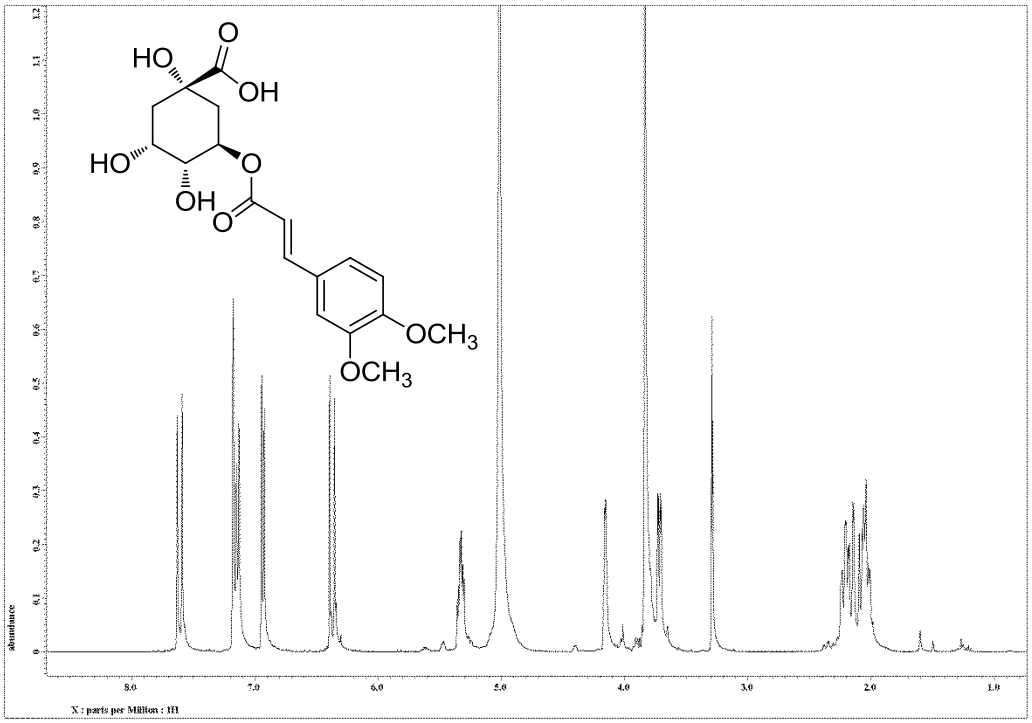

HO
O
OCH3
O
OH
H3C
O
CH3
abundance
X : parts per Million : 13C

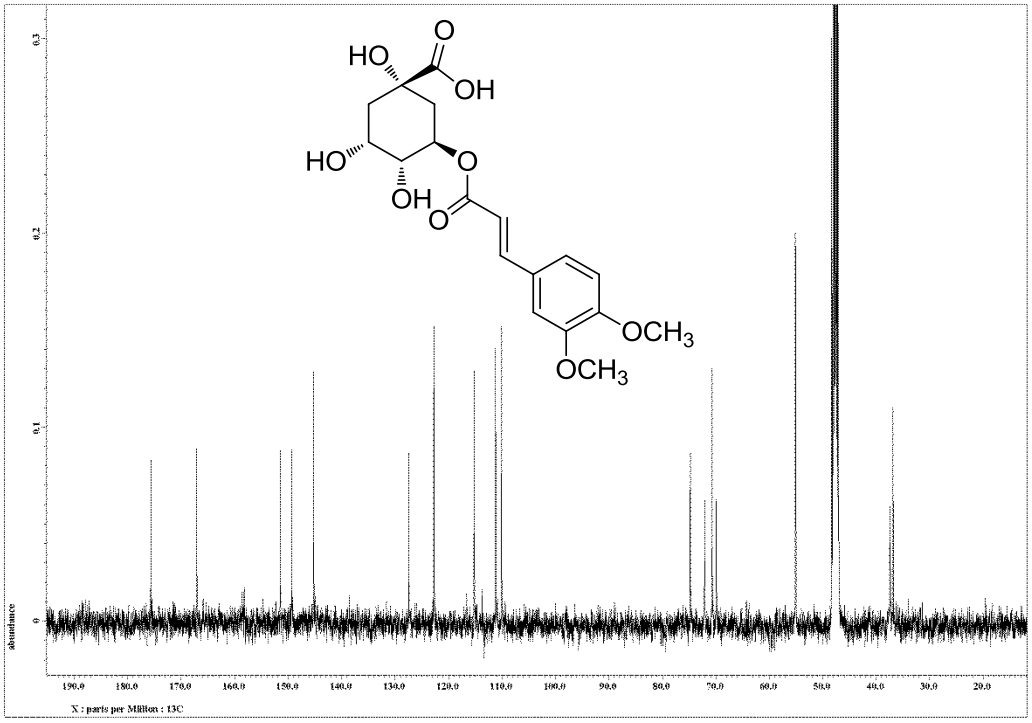

HO
O
OCH3
O
O
O
H3C
O
CH3
O
OAll
OAll
2.0
abundance
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : 1H

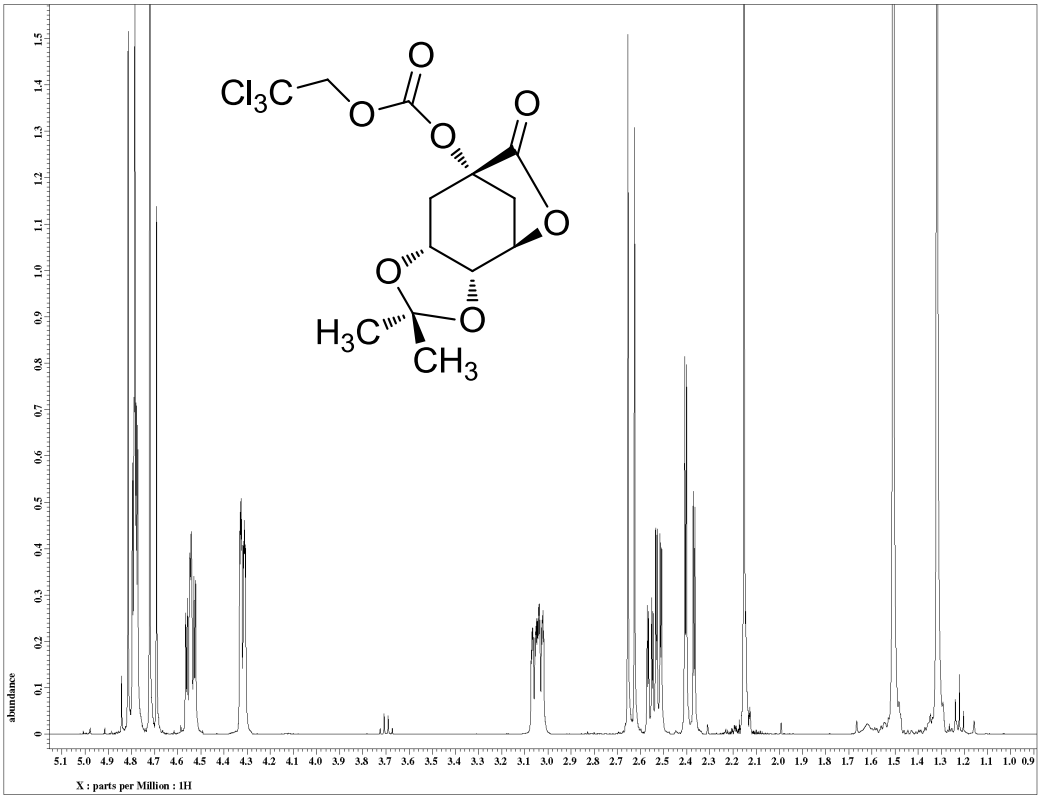

HO
O
OCH3
O
O
O
H3C
O
CH3
O
OAll
OAll
abundance
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
X : parts per Million : 13C

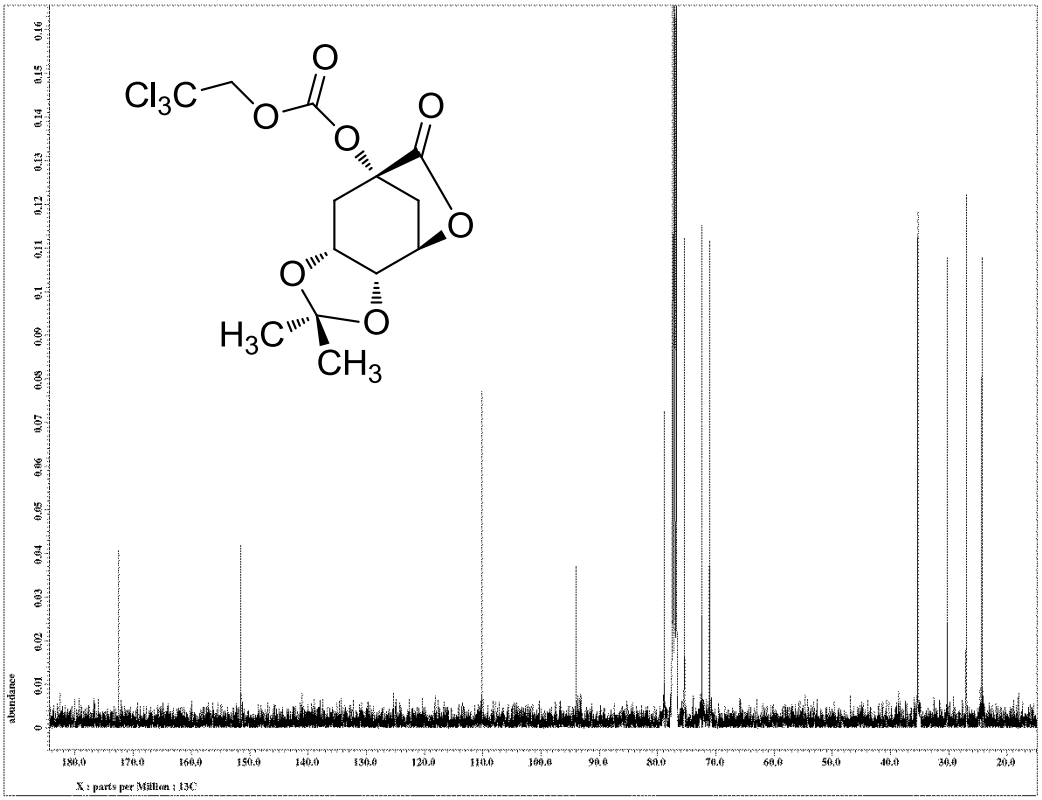

HO
O
OCH3
HO
O
OH
O
OH
OH
abundance
0
0.1
0.2
0.3
0.4
0.5
7.0
6.0
5.0
4.0
3.0
2.0
X : parts per Million : 1H

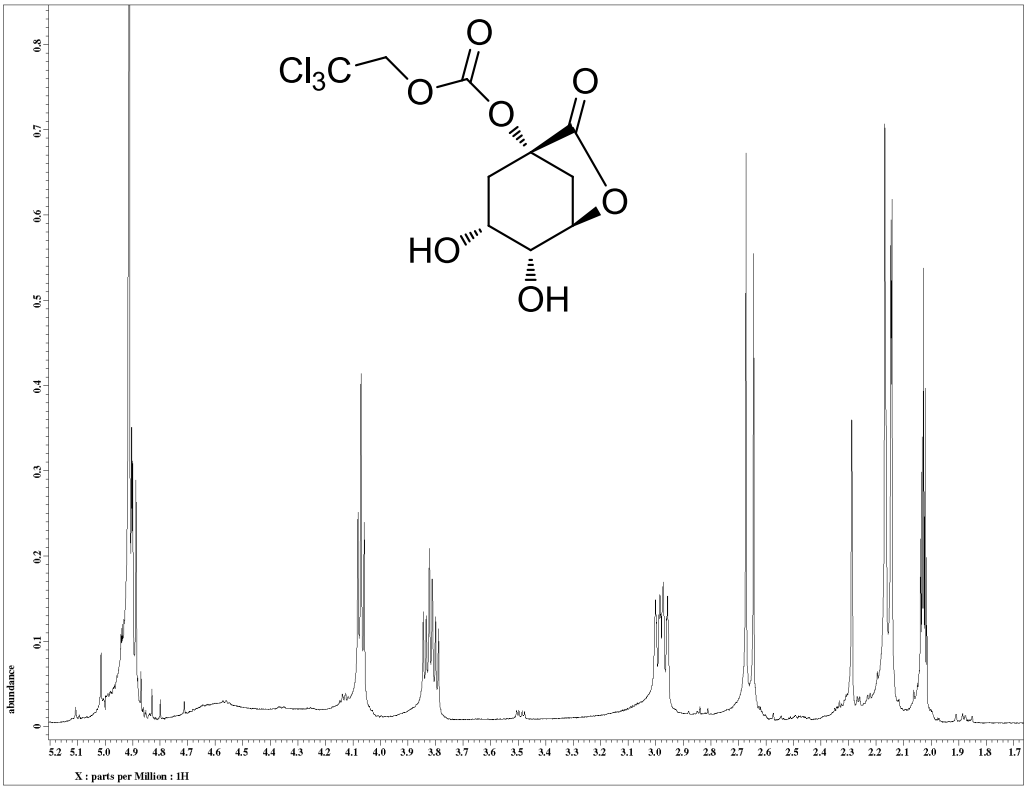

HO
O
OCH3
HO
O
OH
O
OH
OH
(thousands)
0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
X : parts per Million : 13C

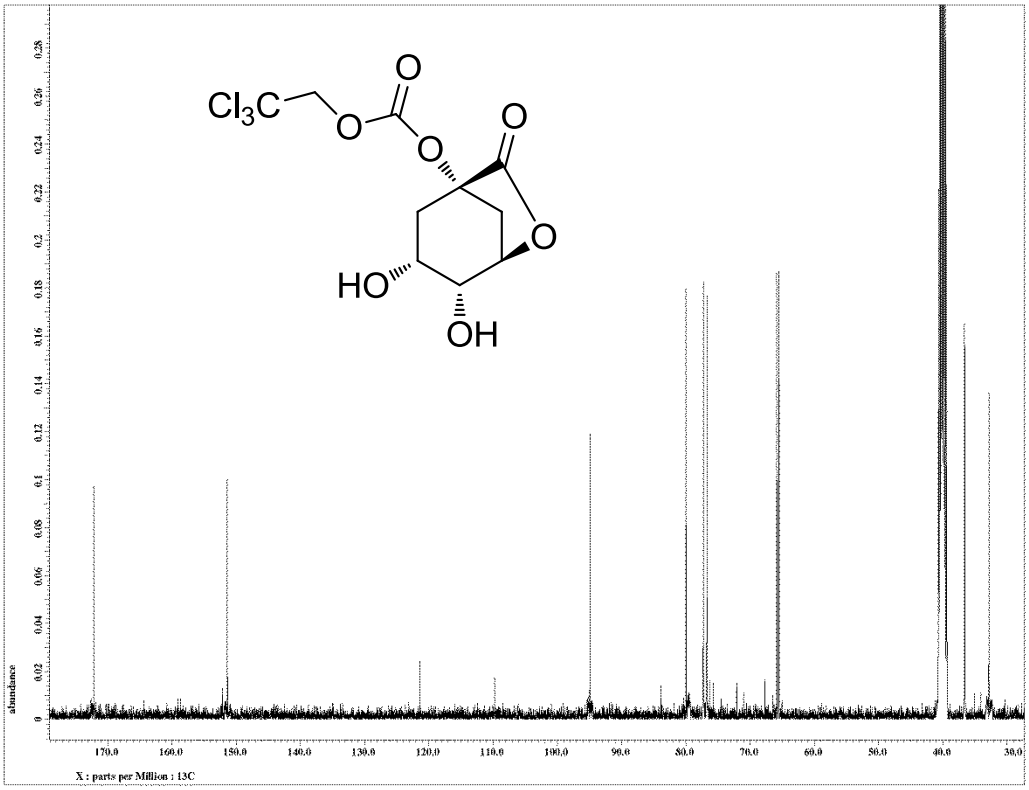

HO
O
OH
HO
O
OH
O
OCH3
OCH3
absorbance
X : parts per Million : 1H

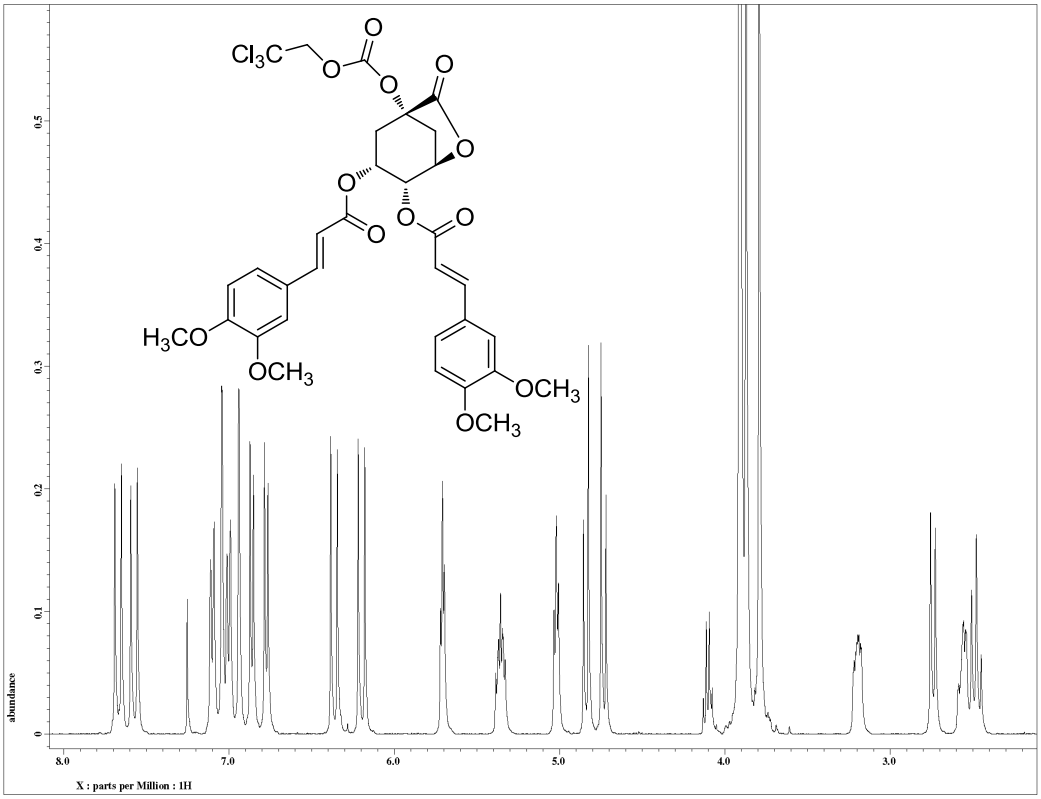

HO
O
OH
HO
O
OH
O
OCH3
OCH3
absorbance
X : parts per Million : 13C

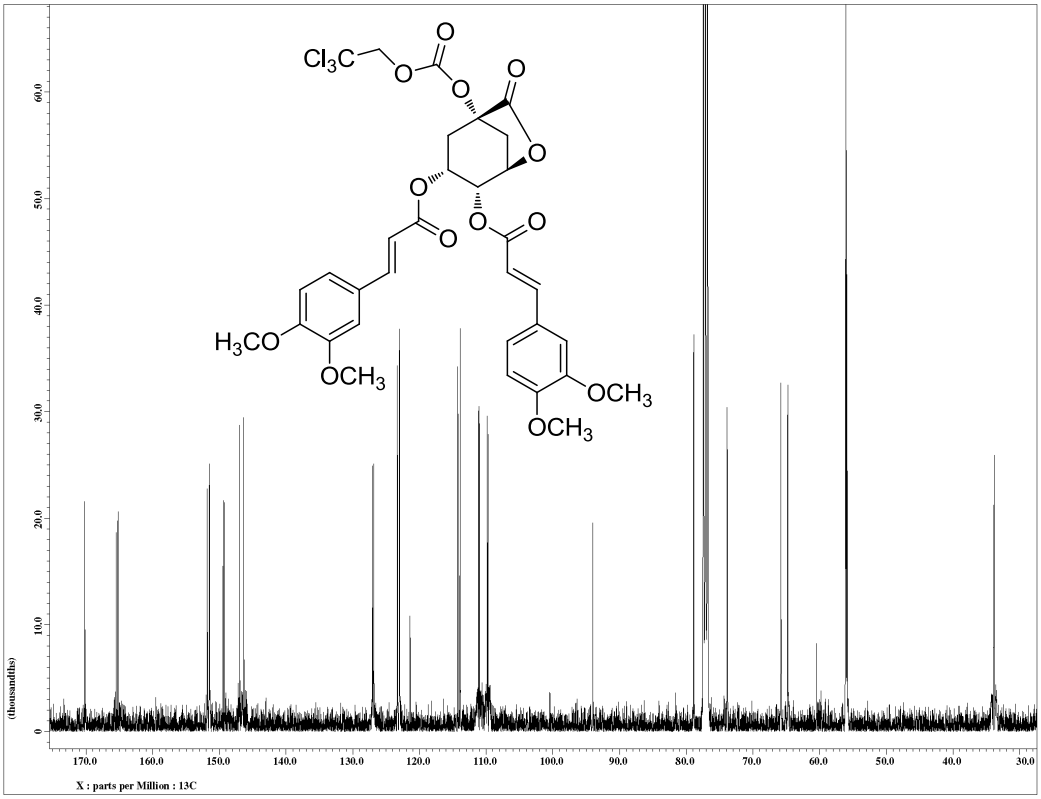

abundance
X : parts per Million : 1H

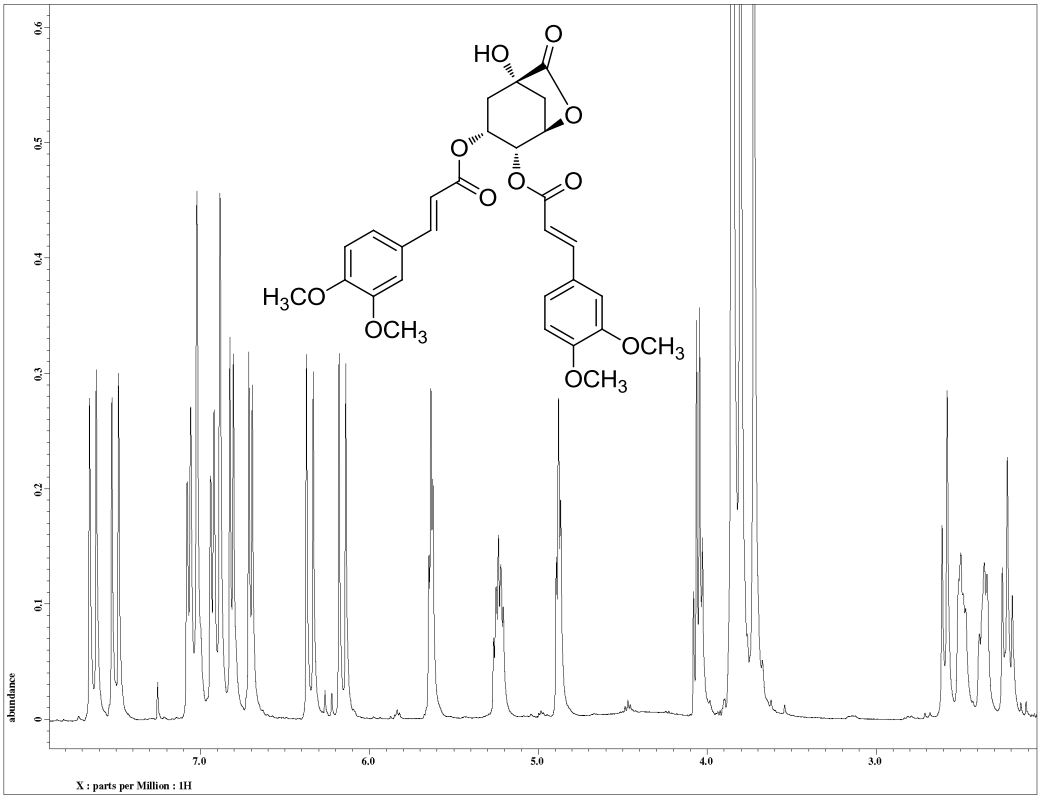

abundance
X : parts per Million : 13C

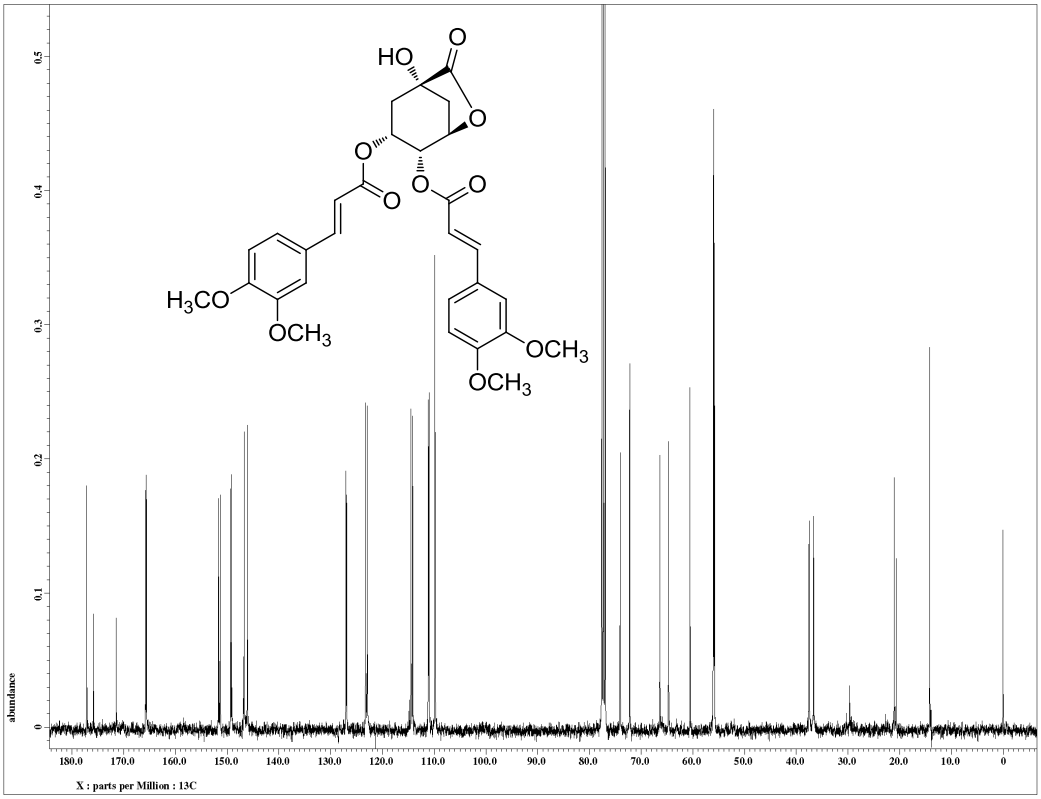

Cl3C
O
O
O
O
O
HO
OH
abundance
X : parts per Million : 1H

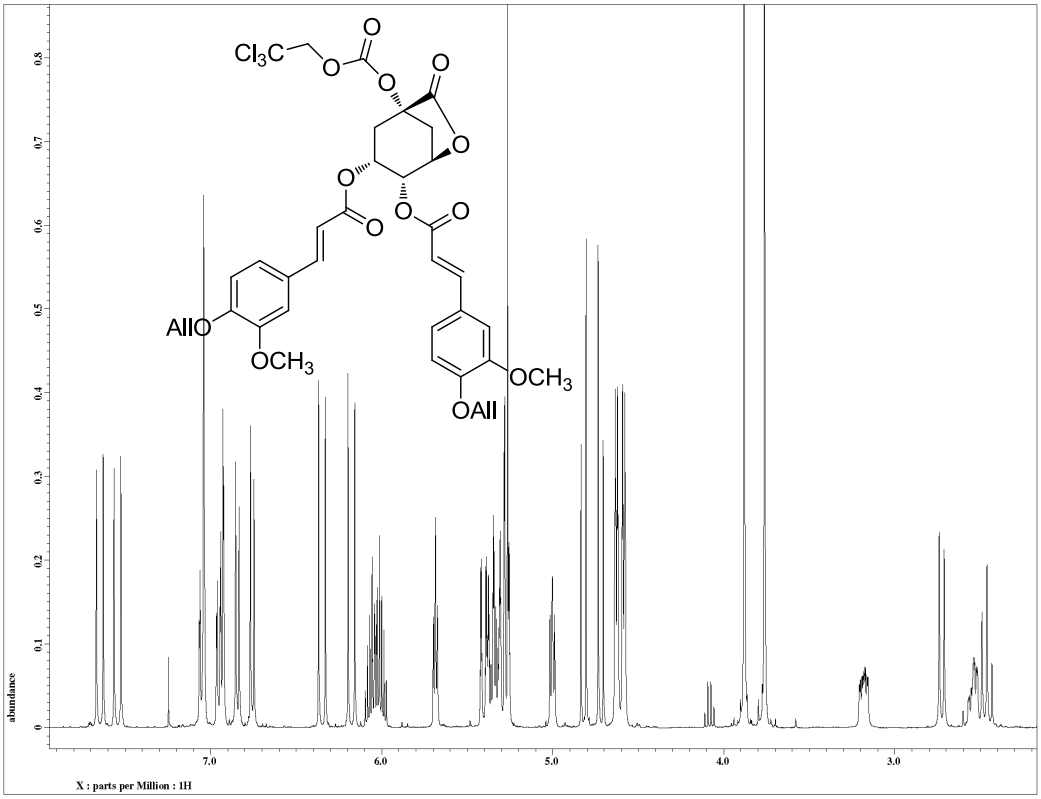

Cl3C
O
O
O
O
O
HO
OH
abundance
X : parts per Million : 13C

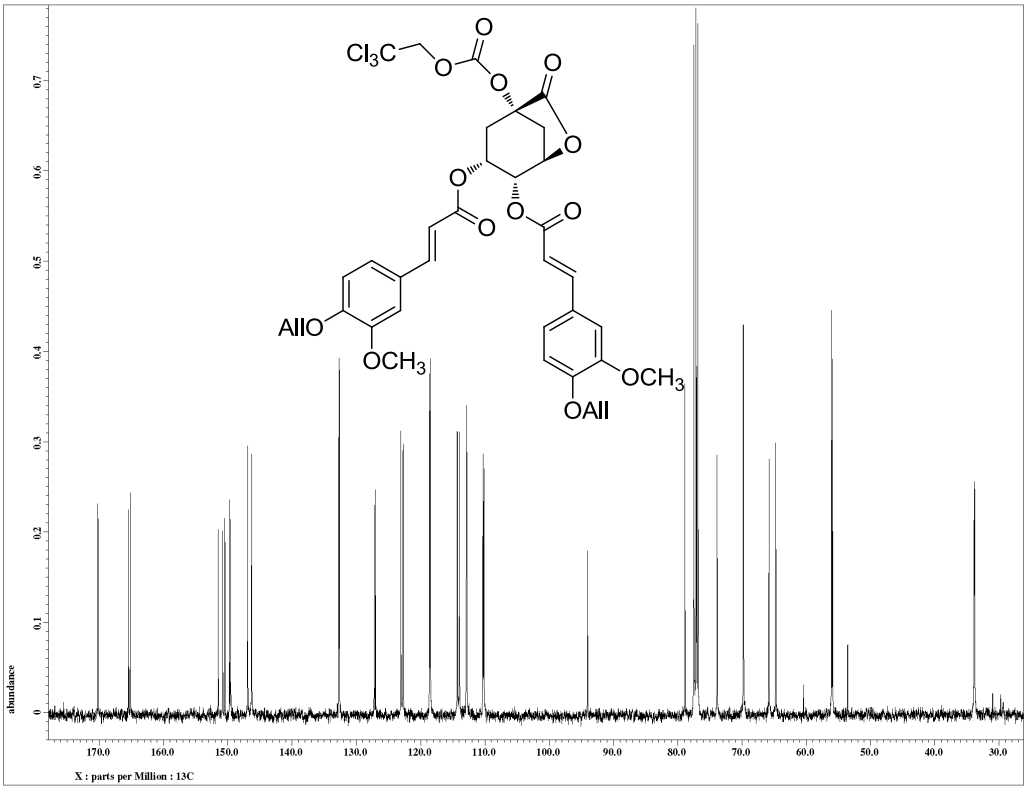

abundance
X : parts per Million : 1H

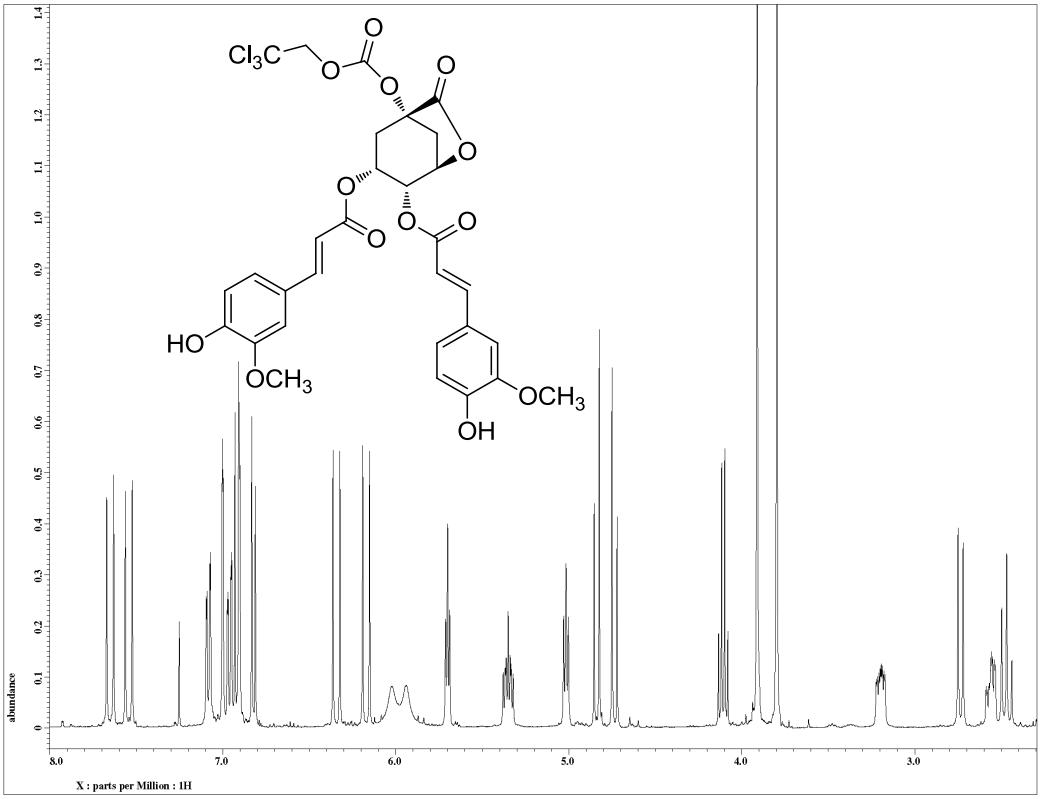

(thousandths)
X : parts per Million : 13C

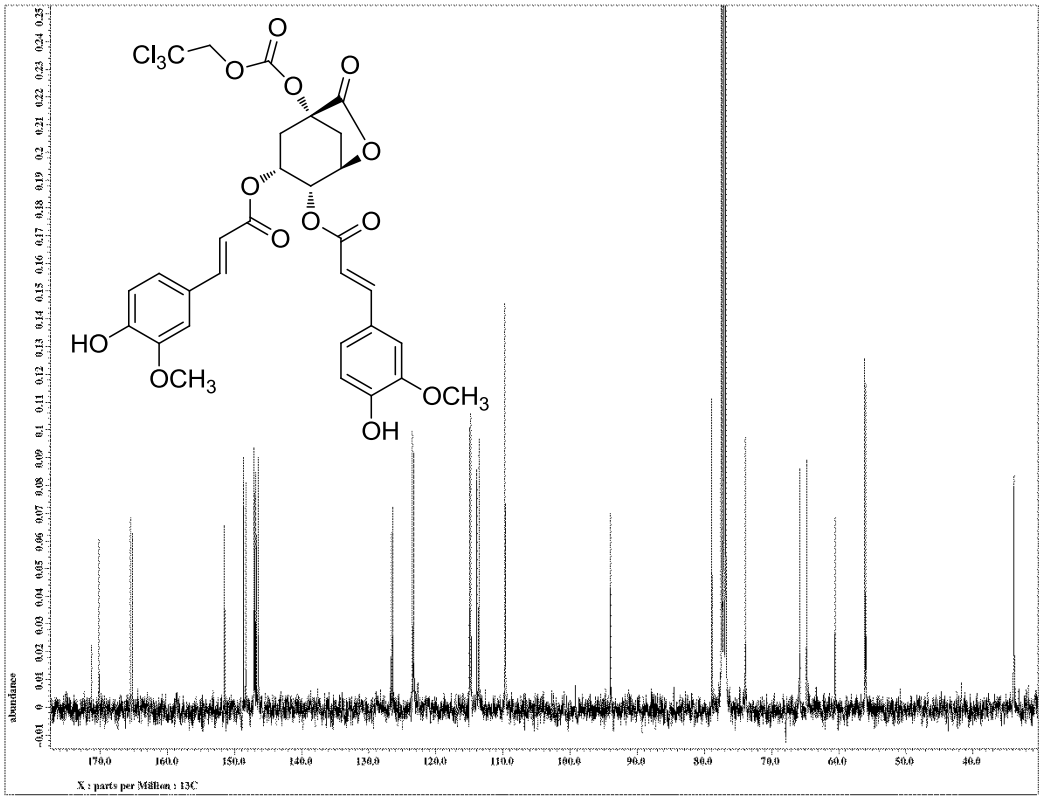

HO
O
O
O
O
O
O
H3CO
OCH3
OCH3
OCH3
abundance
0.6
0.5
0.4
0.3
0.2
0.1
0
7.0
6.0
5.0
4.0
3.0
X : parts per Million : 1H

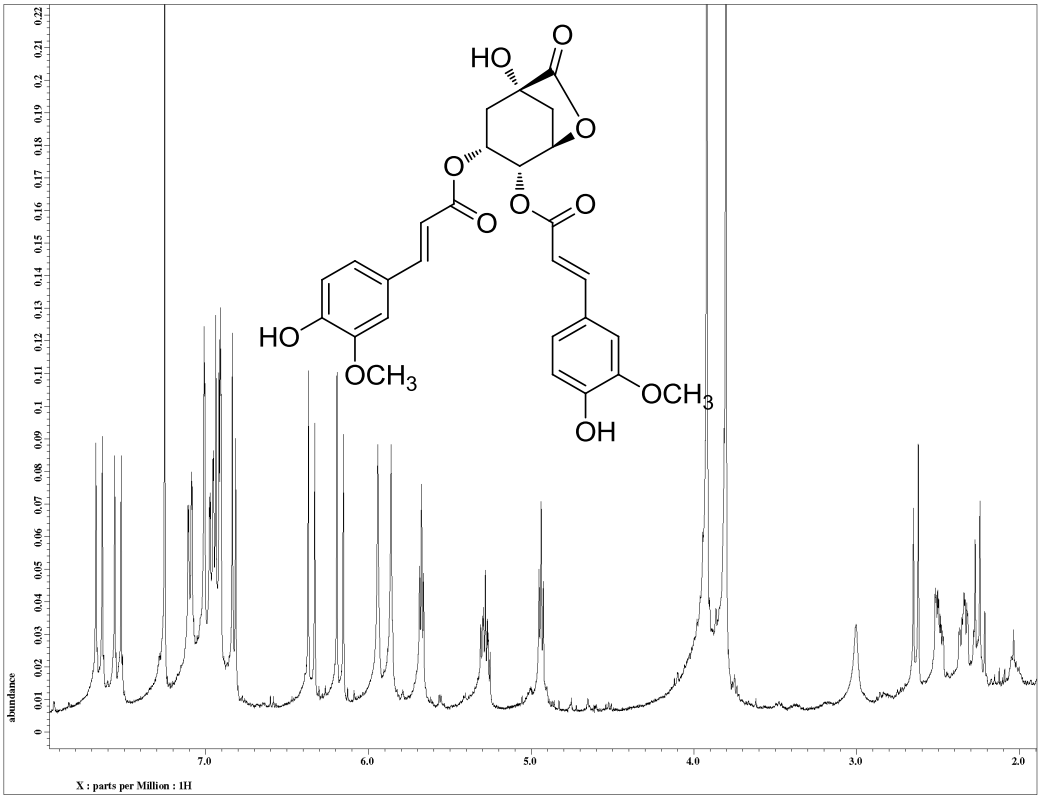

HO
O
O
O
O
O
O
H3CO
OCH3
OCH3
OCH3
abundance
0.5
0.4
0.3
0.2
0.1
0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0
X : parts per Million : 13C

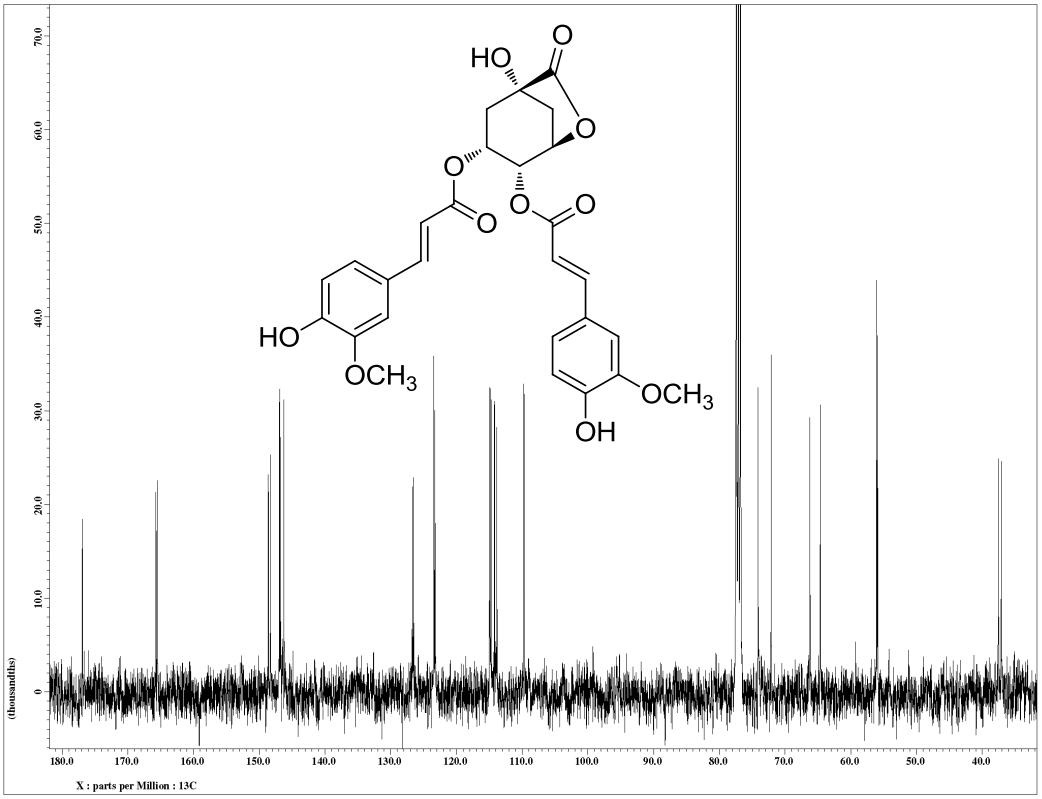

abundance
X : parts per Million : 1H

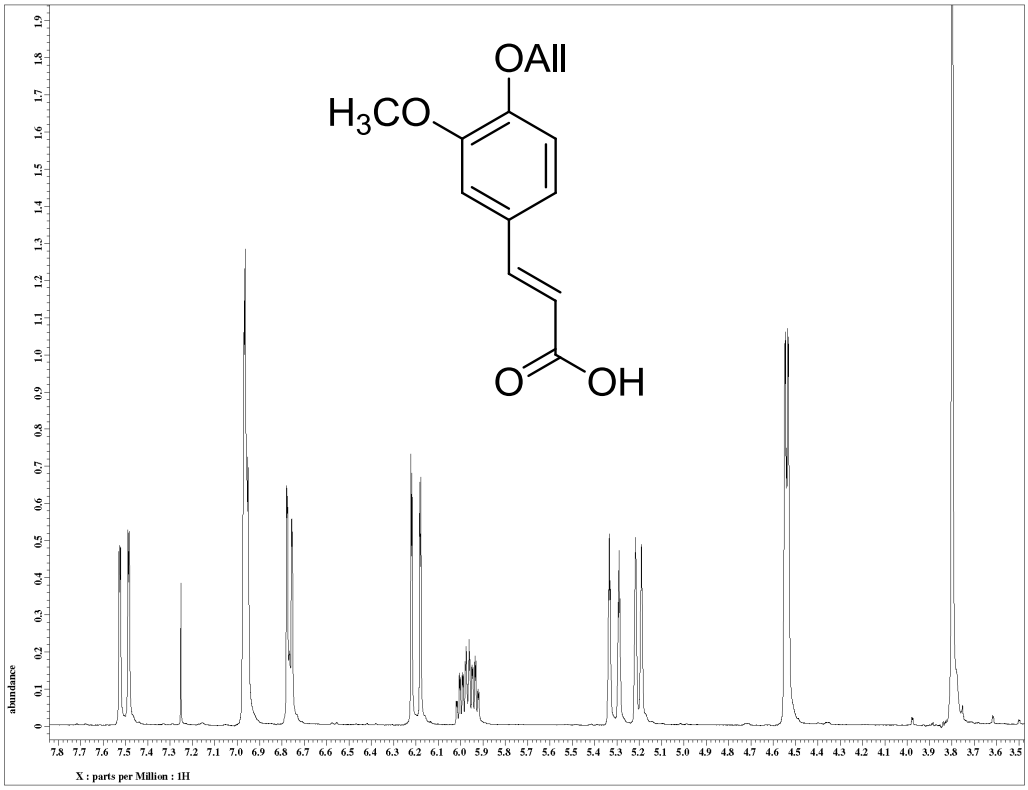

abundance
X : parts per Million : 13C

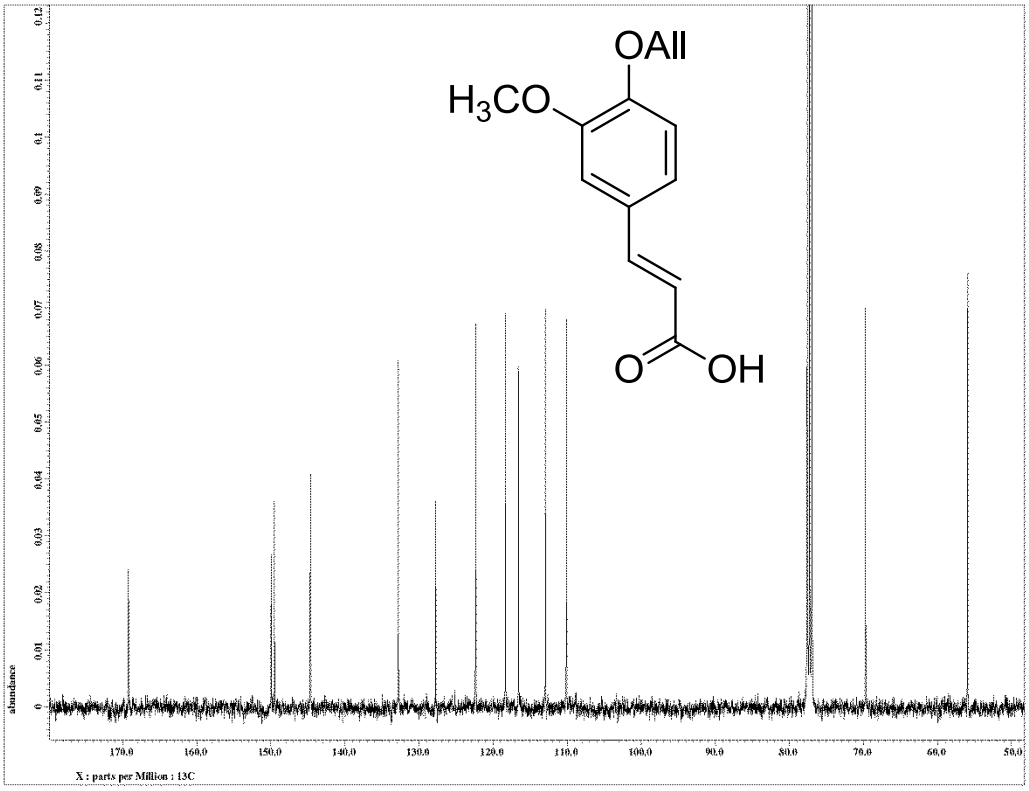

abundance
X : parts per Million : 1H

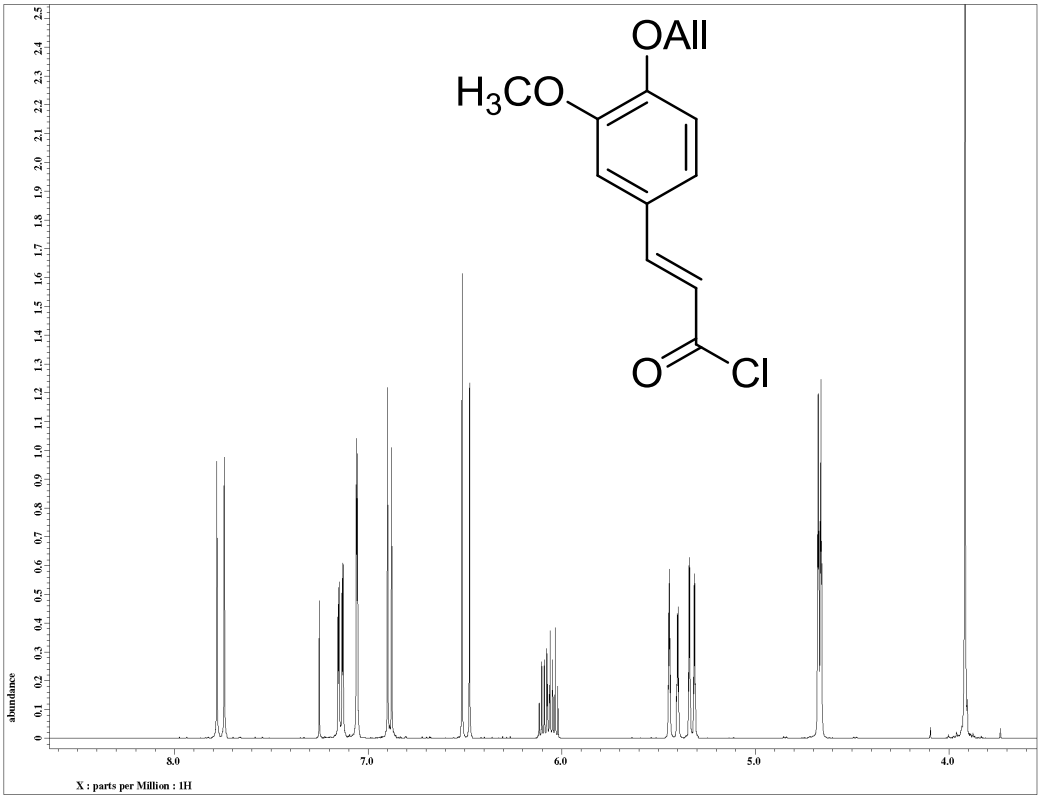

abundance
X : parts per Million : 13C

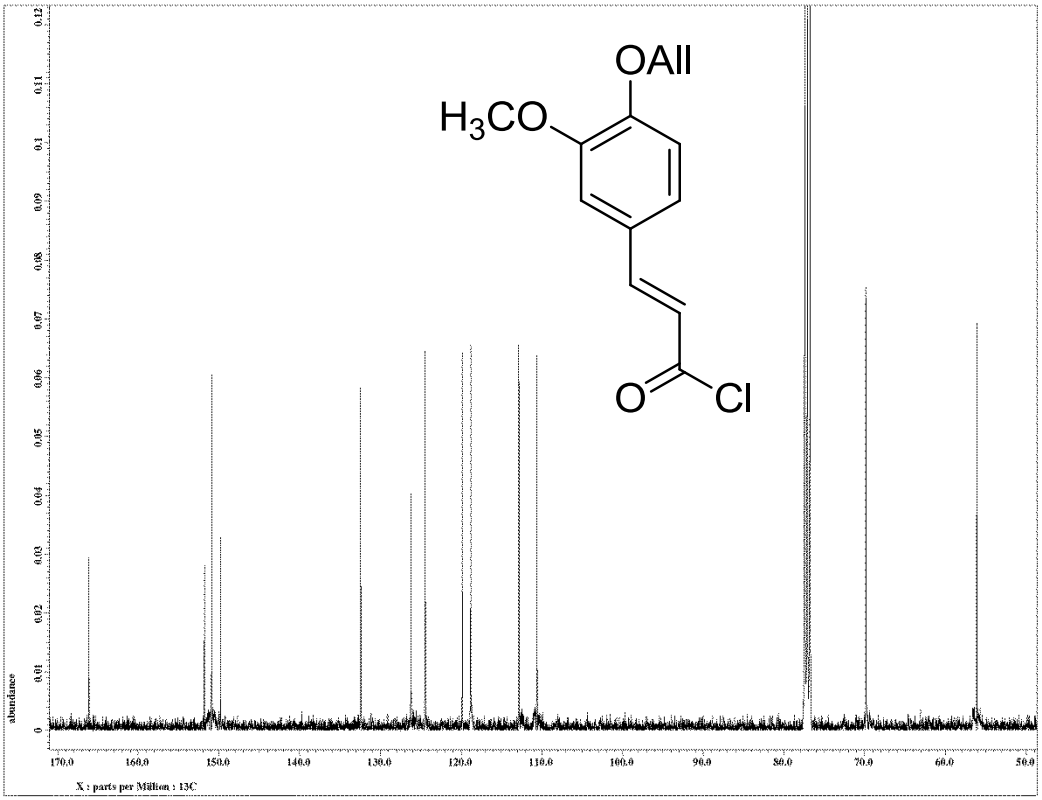

HO
O
O
O
O
O
O
HO
OCH3
OCH3
OH
abundance
X : parts per Million : 1H

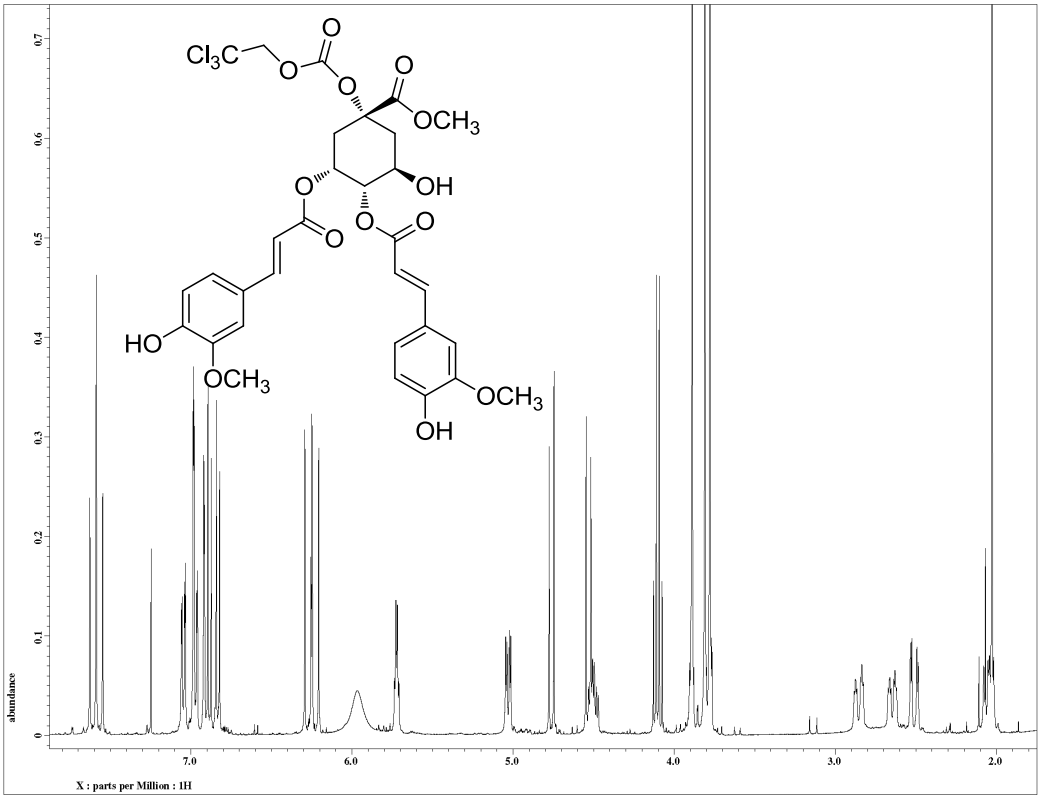

HO
O
O
O
O
O
O
HO
OCH3
OCH3
OH
(thousandths)
X : parts per Million : 13C

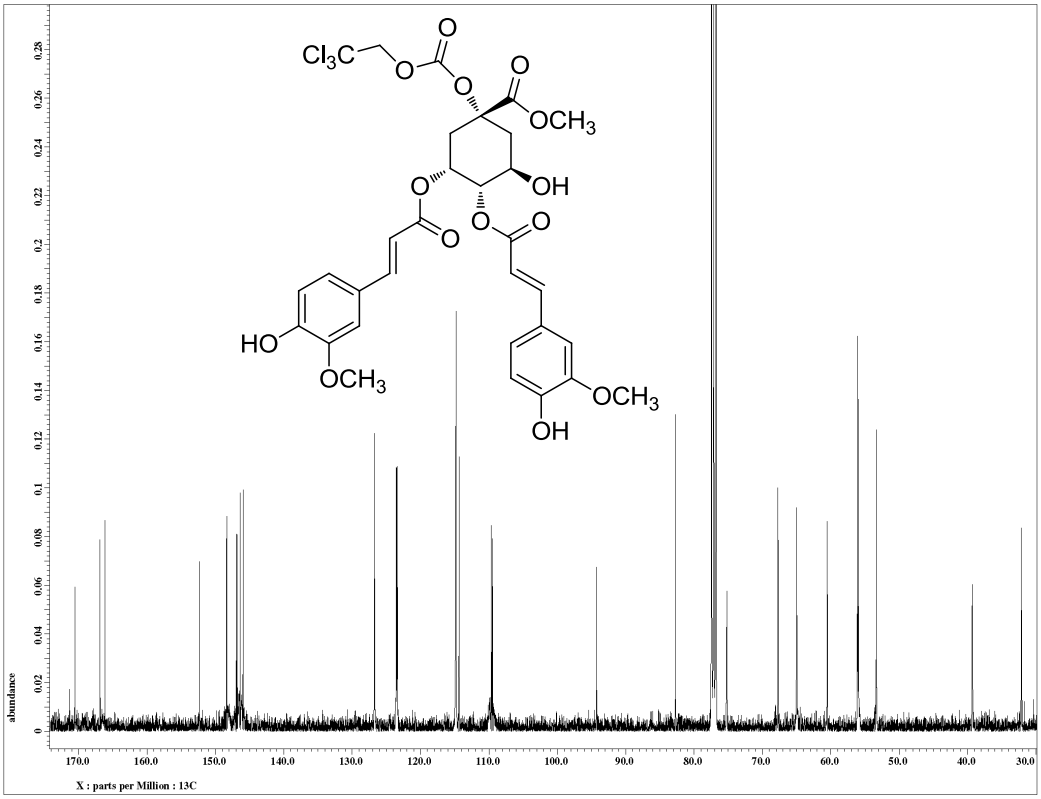

OAll
H3CO
O
OH
abundance
X : parts per Million : 1H

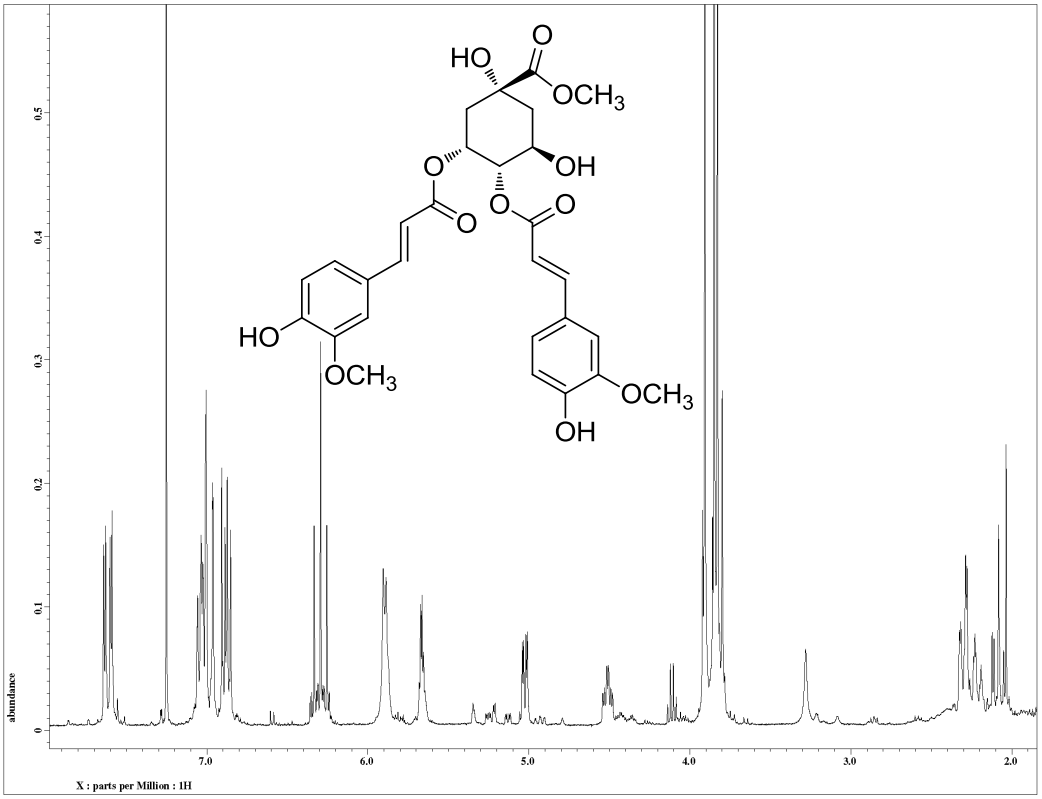

OAll
H3CO
O
OH
abundance
X : parts per Million : 13C

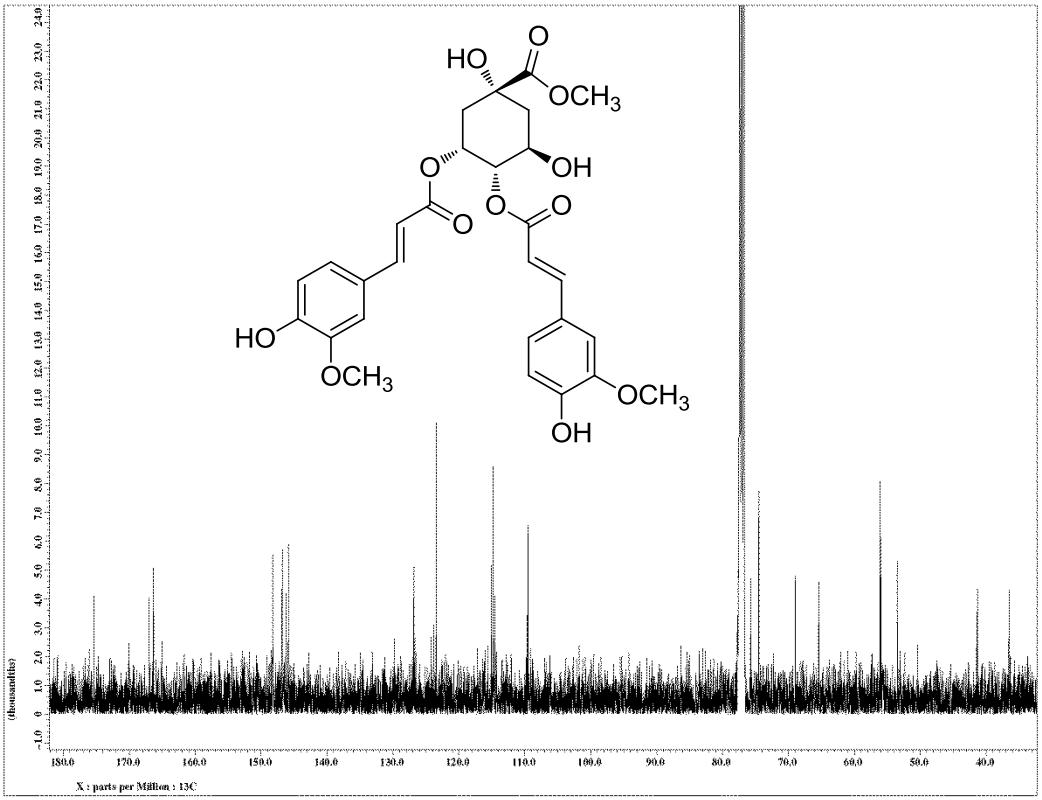

OAll
H3CO
O
Cl
abundance
X : parts per Million : 1H

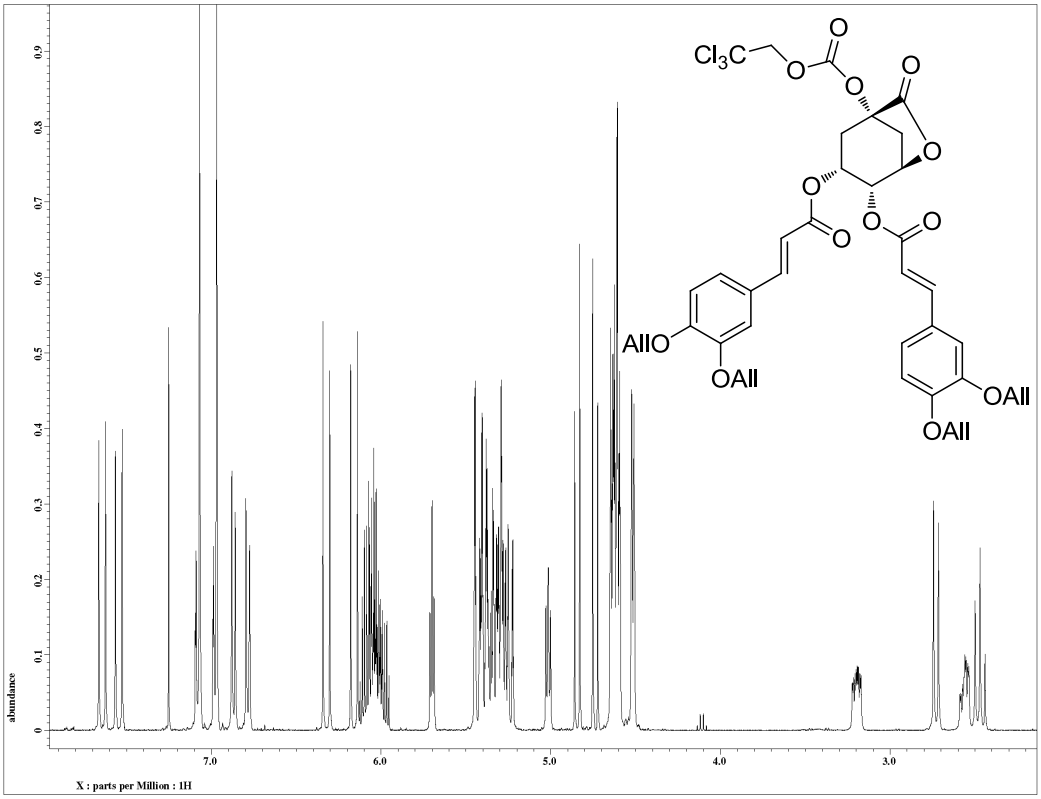

OAll
H3CO
O
Cl
abundance
X : parts per Million : 13C

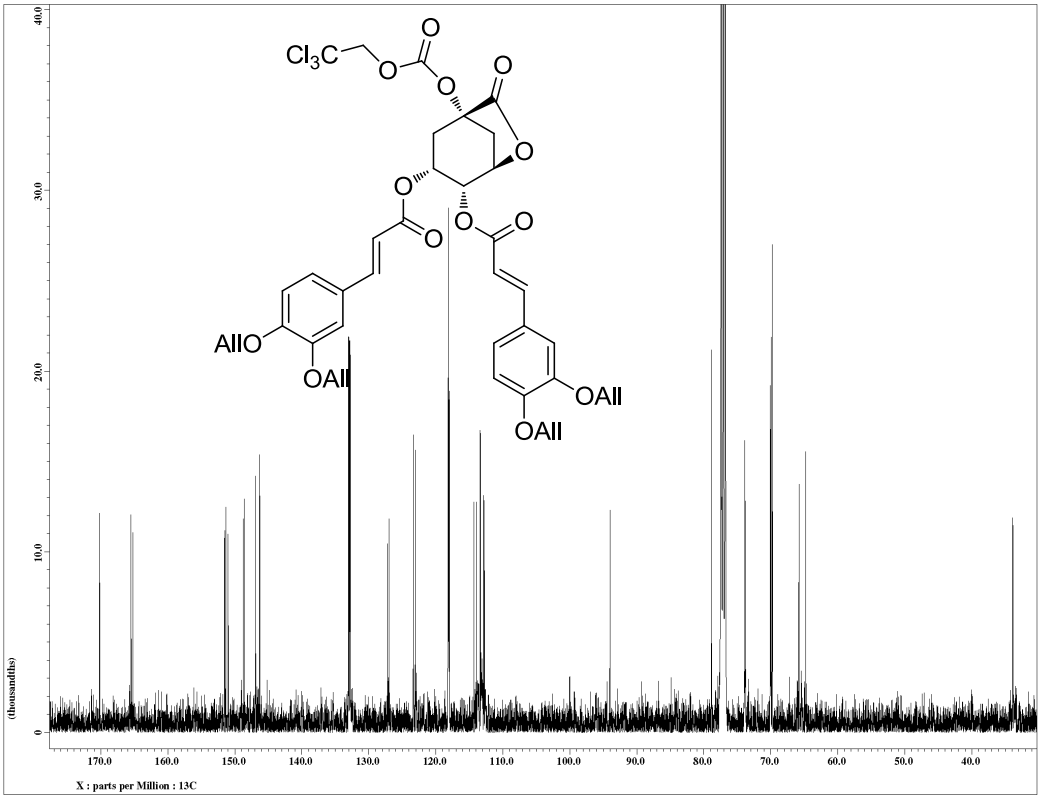

abundance
X : parts per Million : 1H

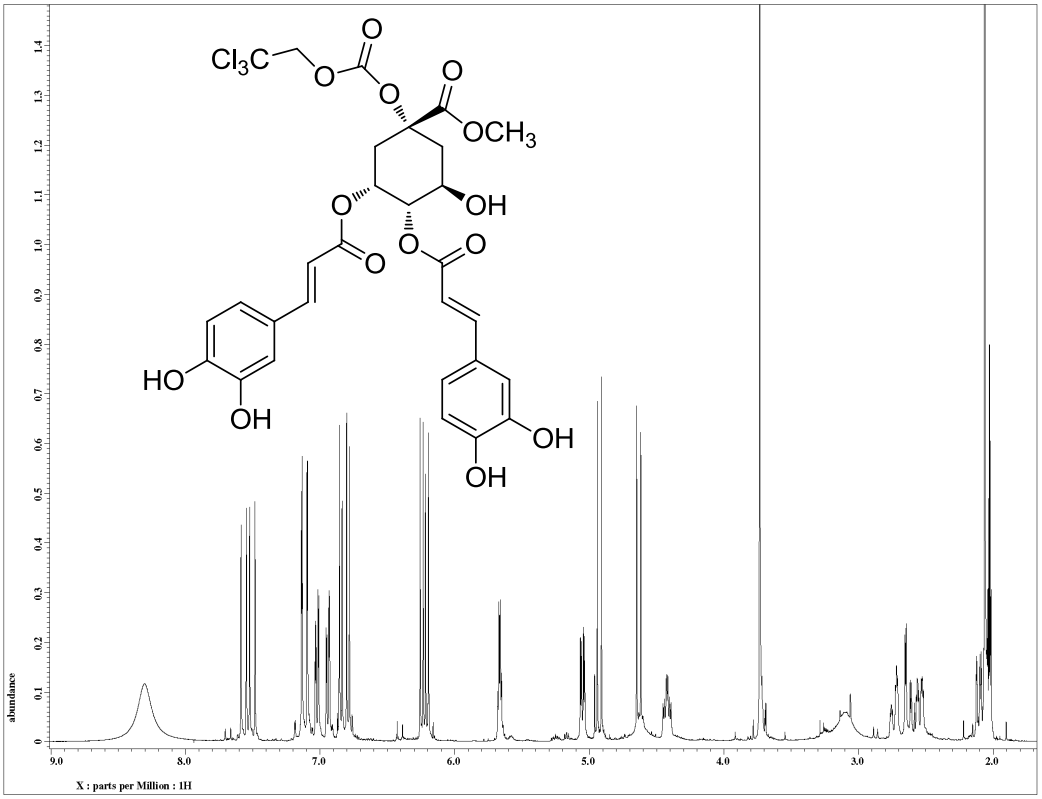

abundance
X : parts per Million : 13C

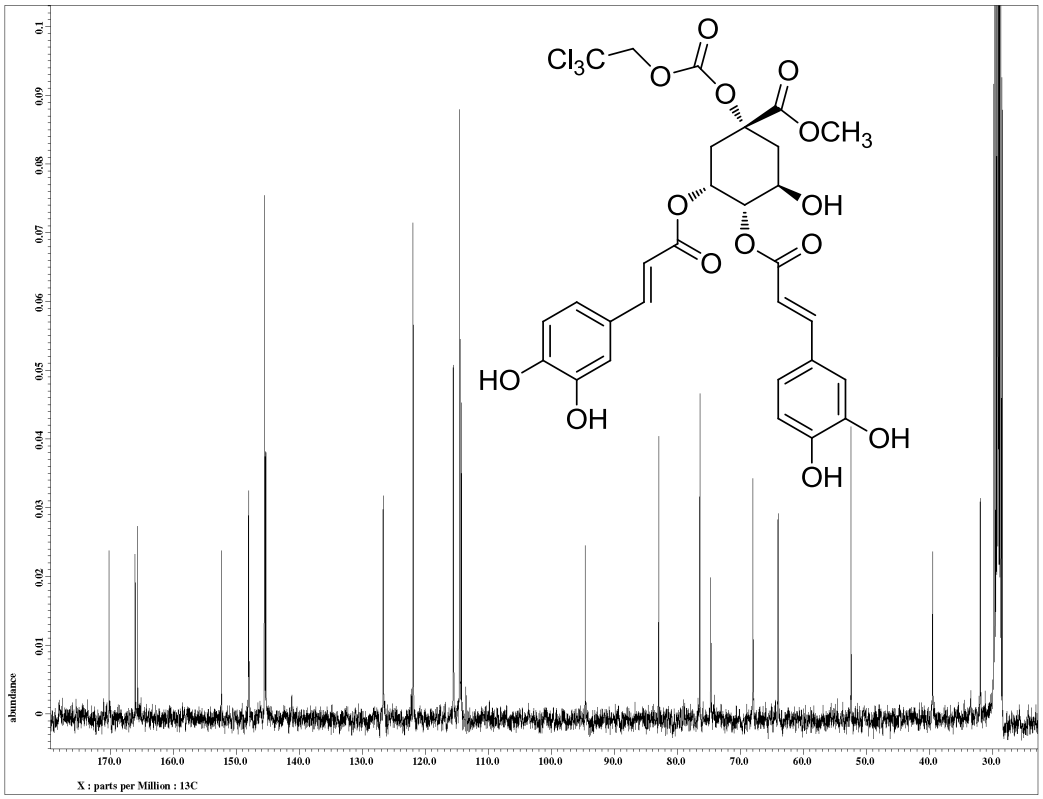

HO
O
OCH3
OH
O
O
O
O
HO
OCH3
OCH3
OH
abundance
0
0.1
0.2
0.3
0.4
0.5
7.0
6.0
5.0
4.0
3.0
2.0
X : parts per Million : 1H

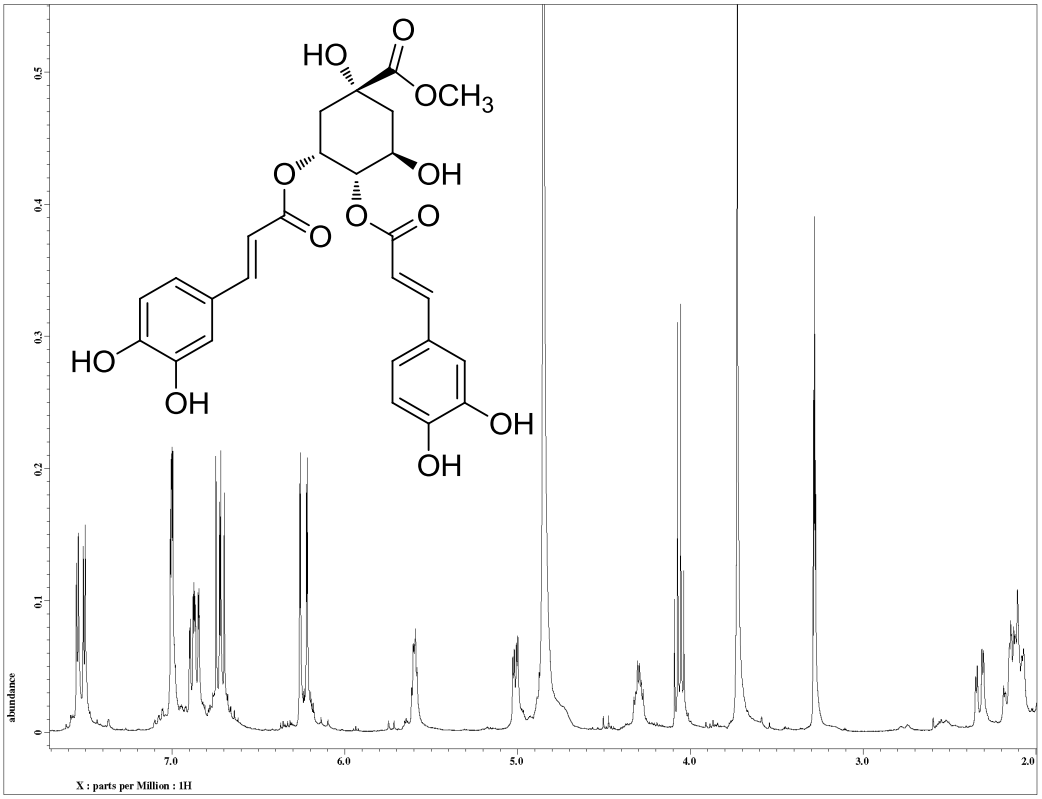

HO
O
OCH3
OH
O
O
O
O
HO
OCH3
OCH3
OH
(thousandths)
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
X : parts per Million : 13C

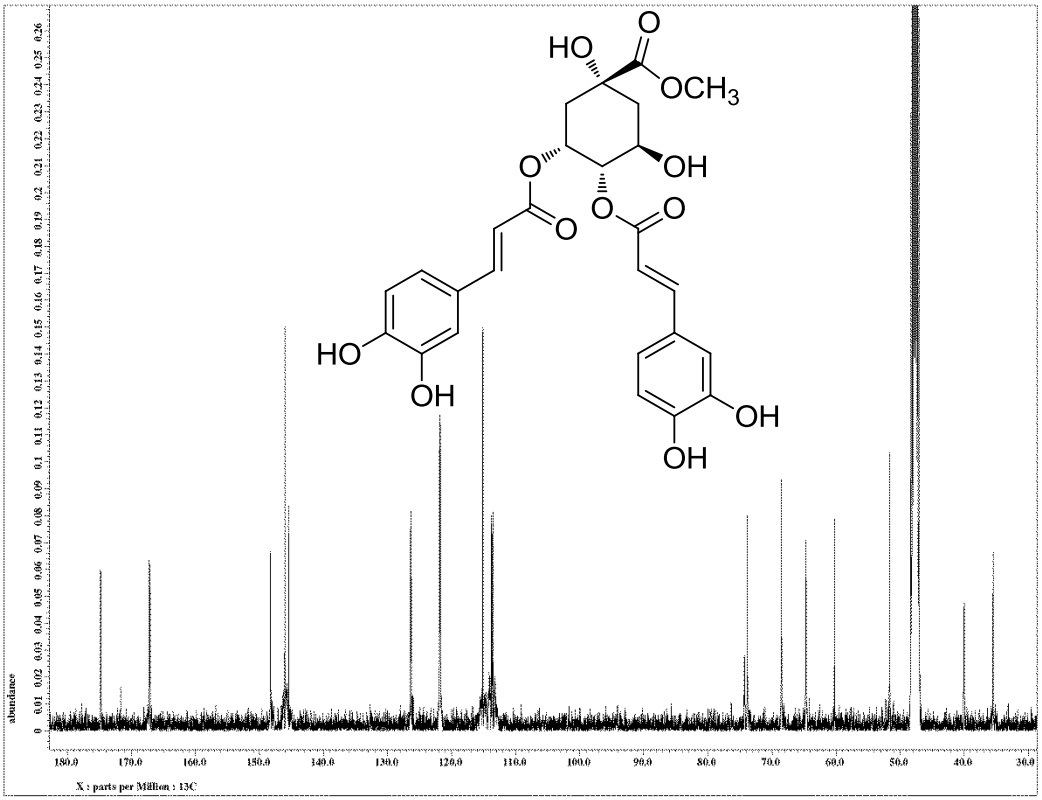

abundance
X : parts per Million : 1H
AllO
OAll
OAll
OAll

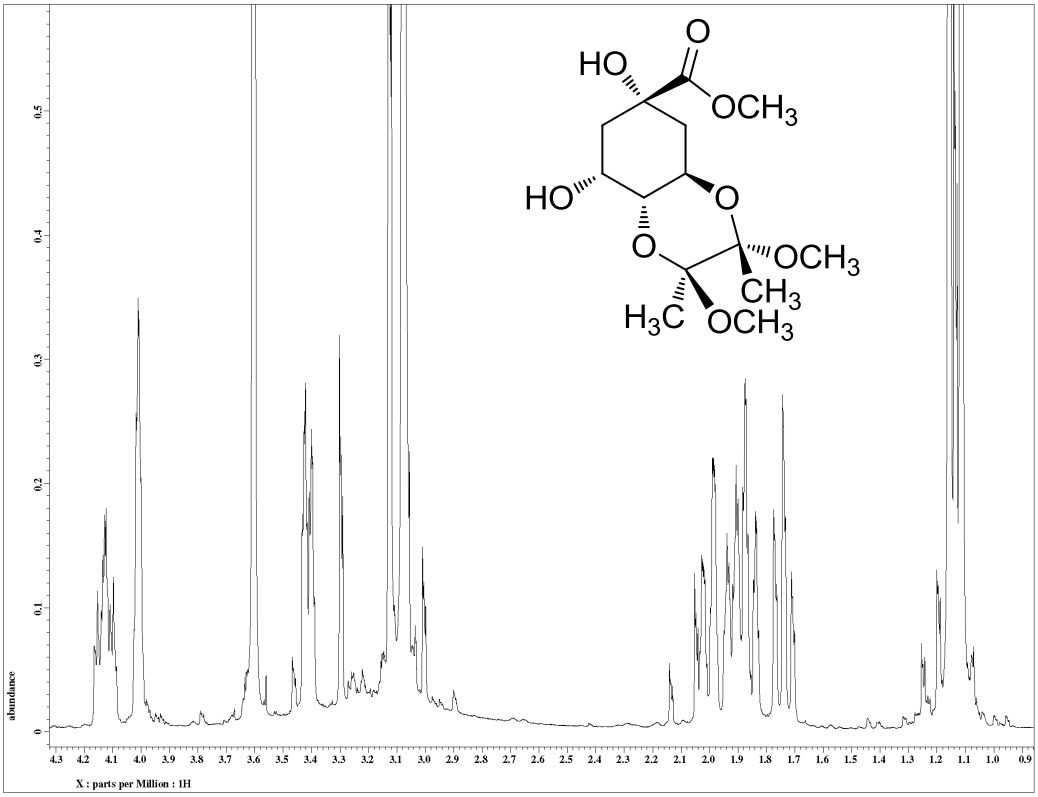

(thousandths)
X : parts per Million : 13C
AllO
OAll
OAll
OAll

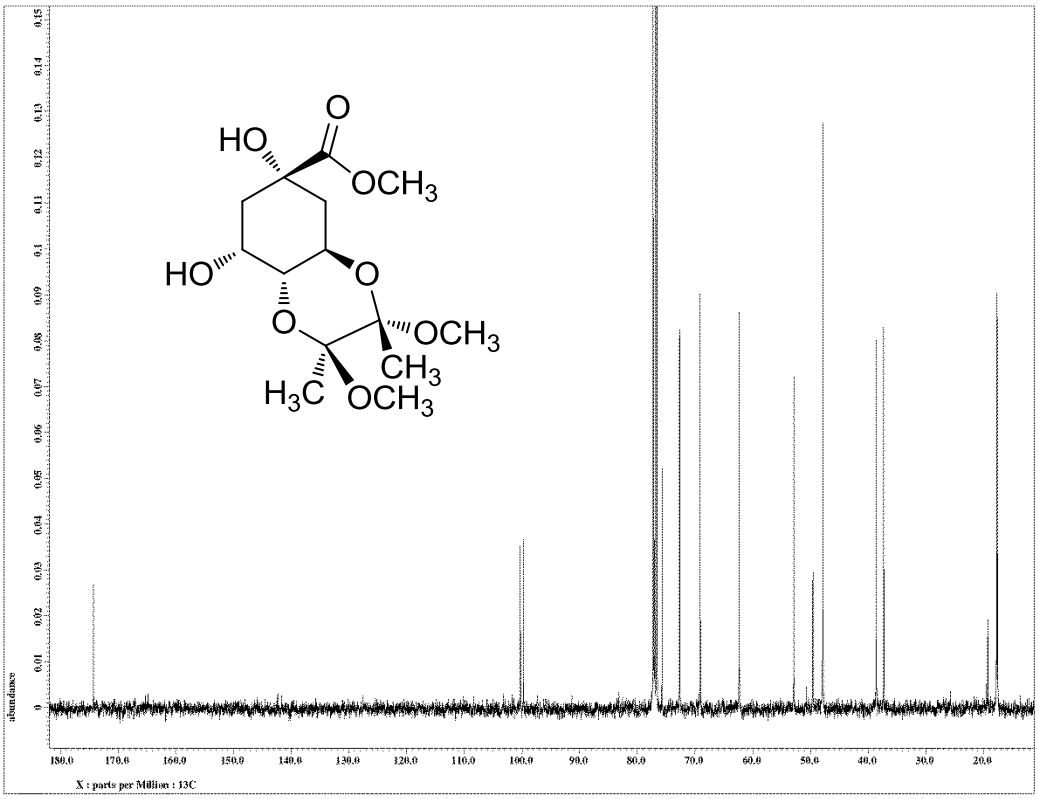

abundance
X : parts per Million : 1H

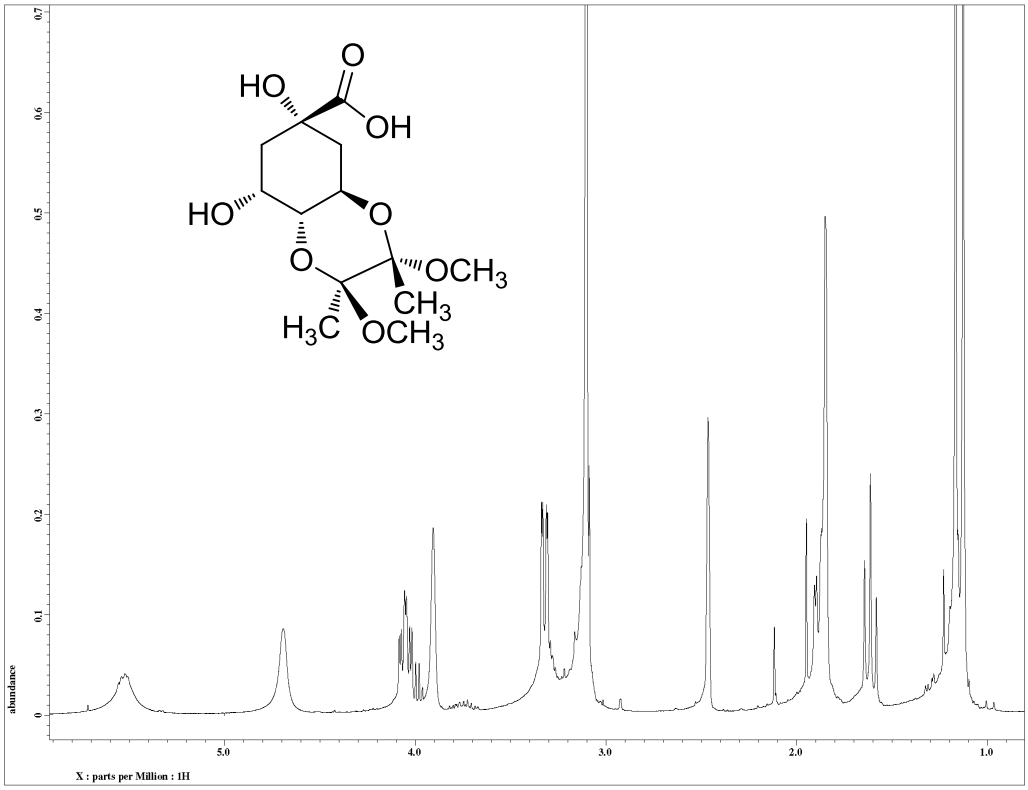

abundance
X : parts per Million : 13C

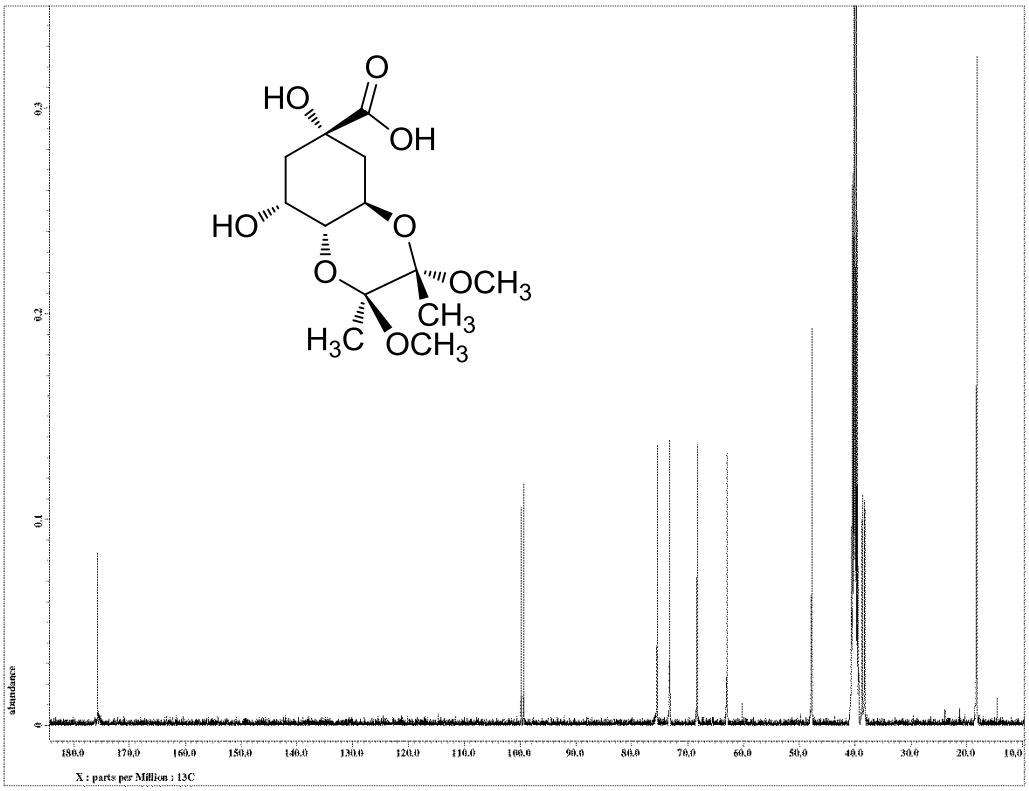

HO
O
OCH3
OH
O
O
O
O
HO
OH
OH
OH
abundance
X : parts per Million : 1H

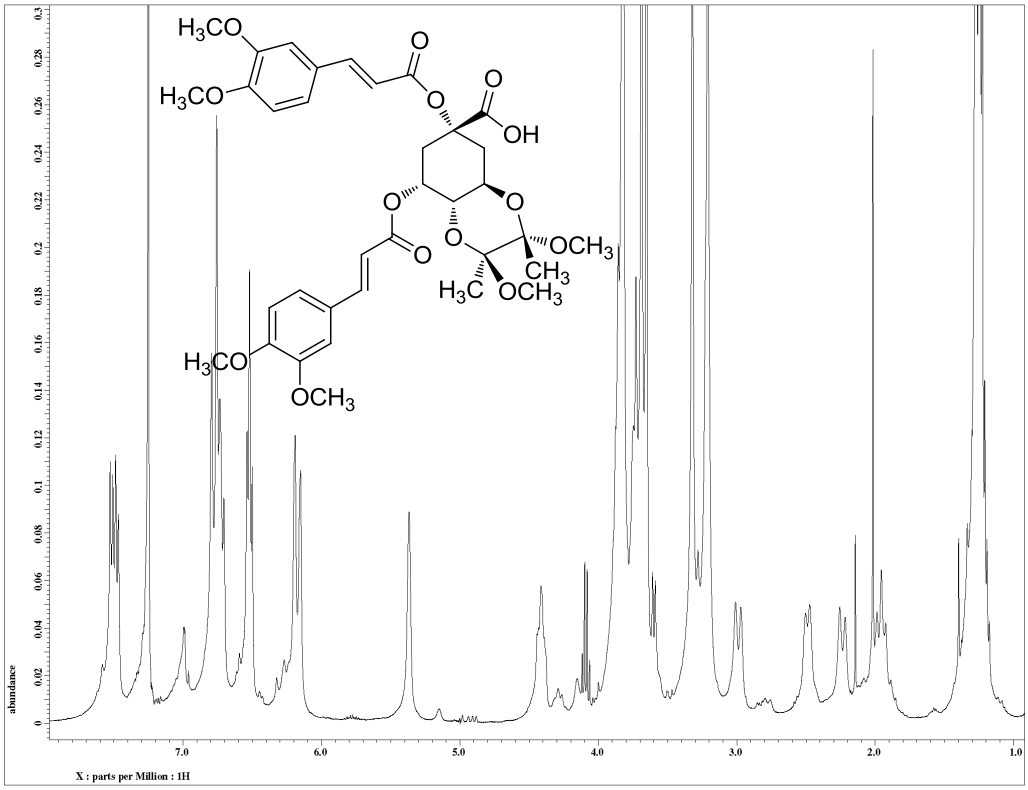

HO
O
OCH3
OH
O
O
O
O
HO
OH
OH
OH
abundance
X : parts per Million : 13C

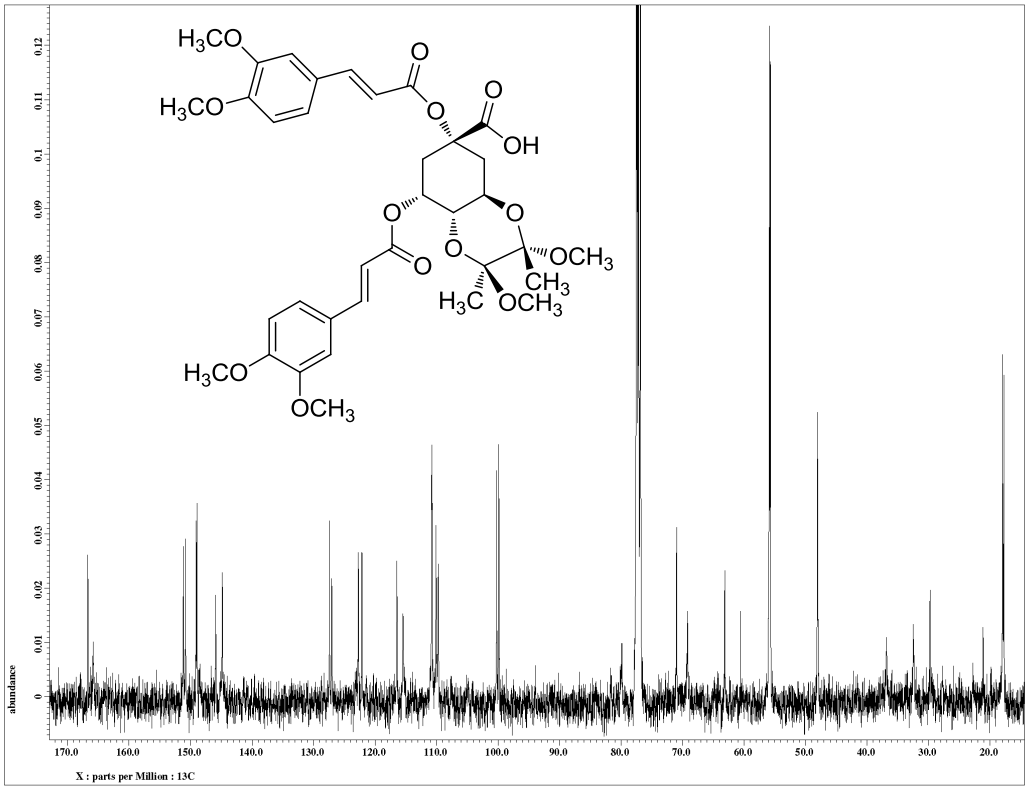

HO
O
OCH3
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 1H

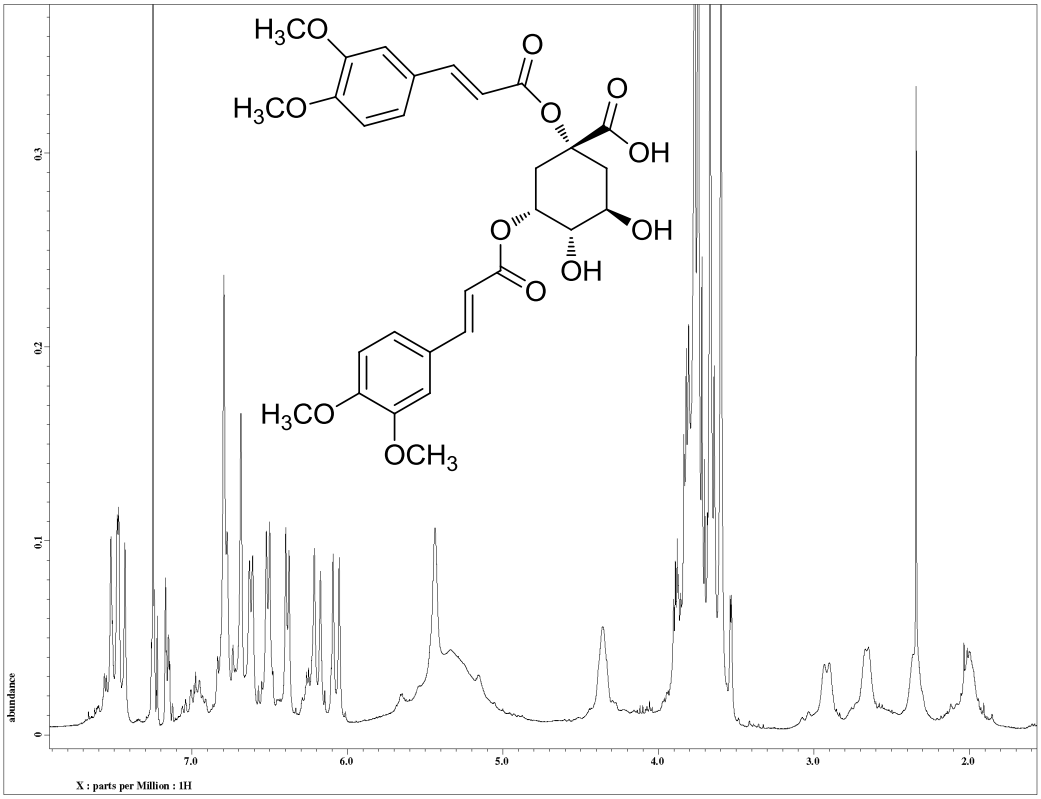

HO
O
OCH3
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 13C

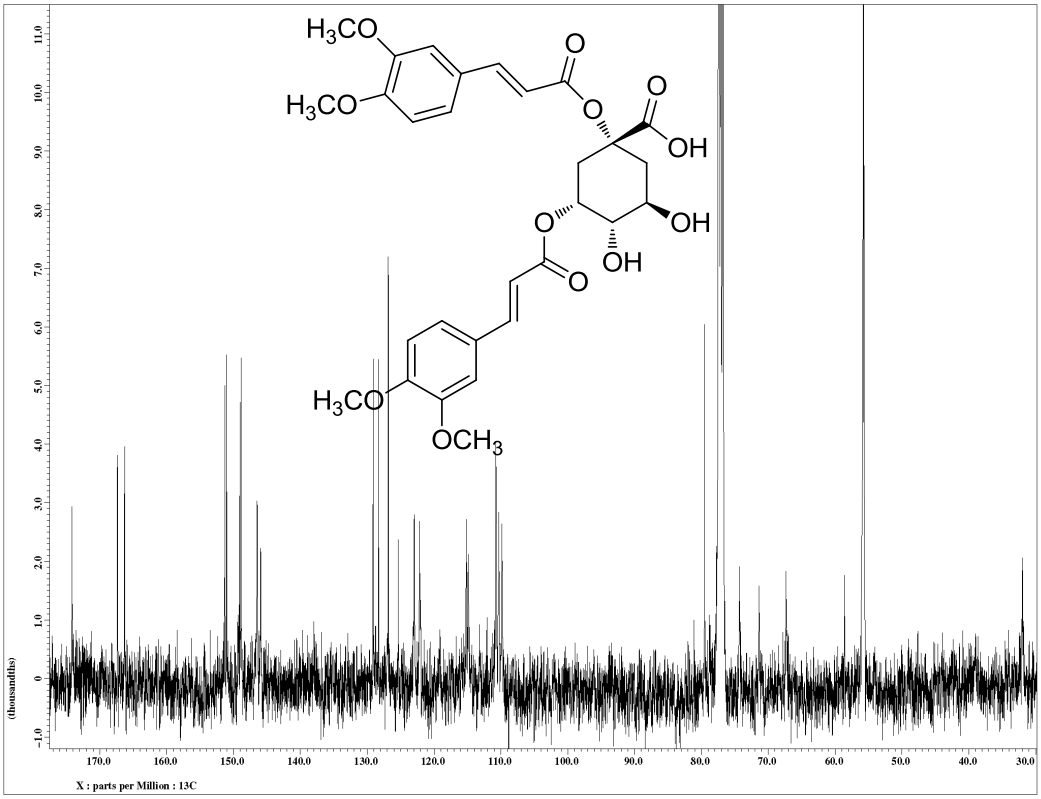

HO
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
5.0
4.0
3.0
2.0
1.0
X : parts per Million : 1H

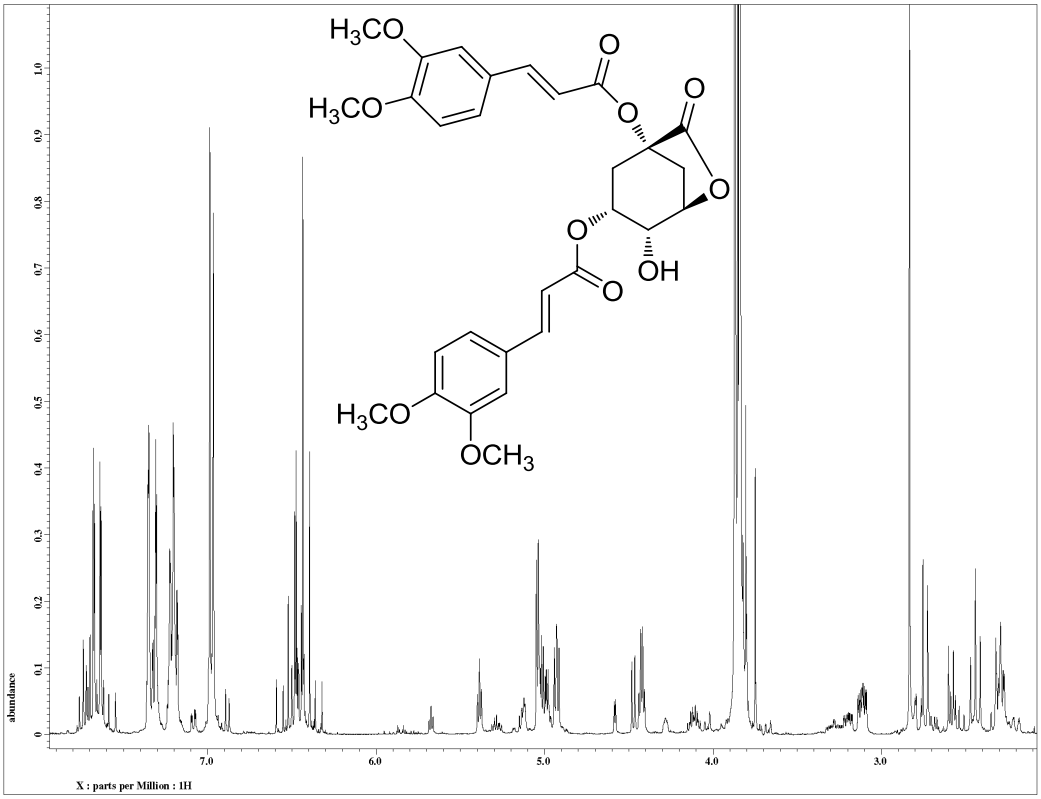

HO
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 13C

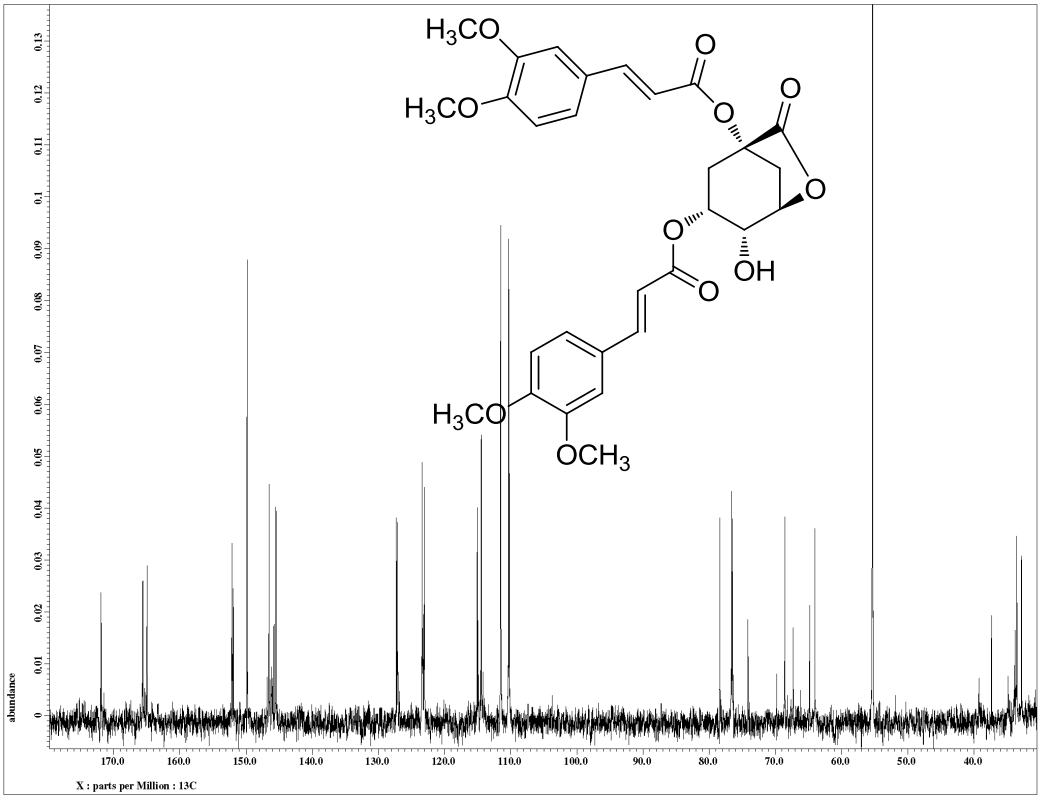

abundance
X : parts per Million : 1H

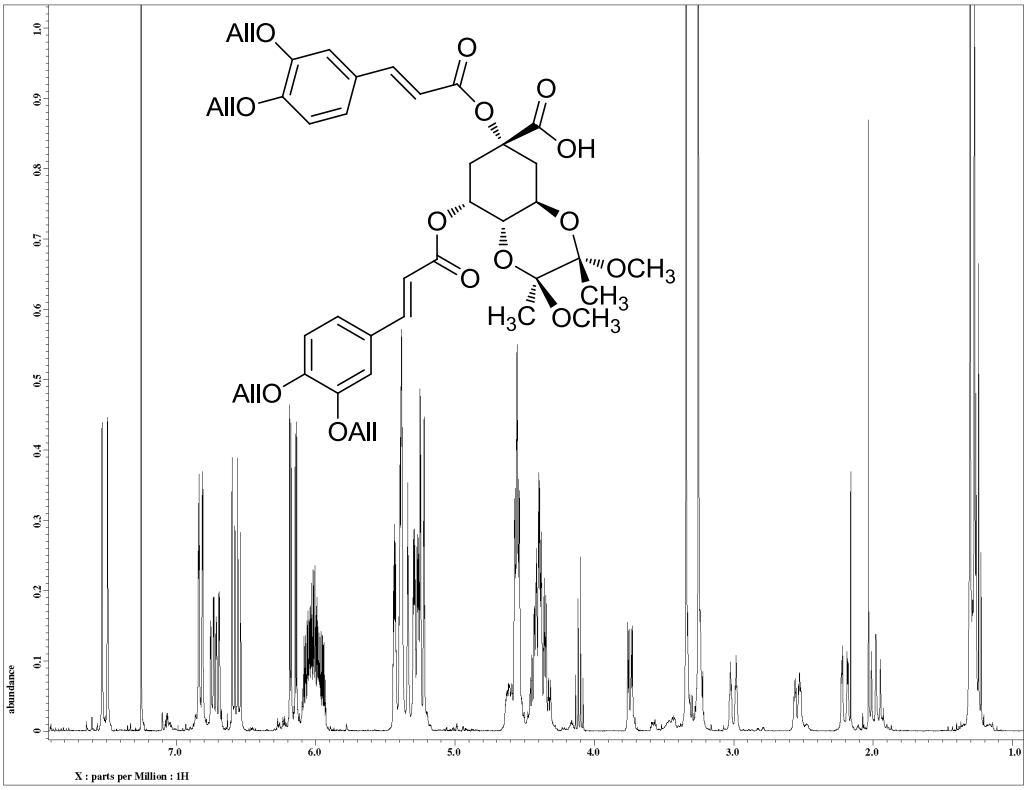

abundance
X : parts per Million : 13C

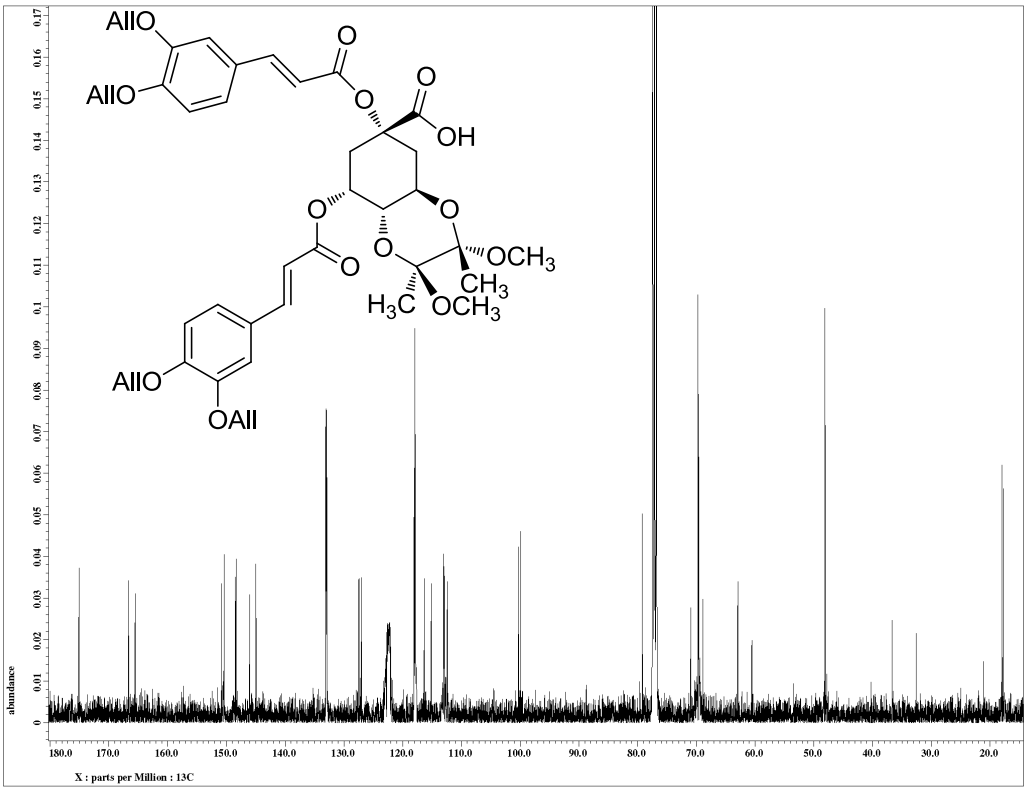

H3CO
H3CO
O
O
O
OH
OH
OH
O
O
H3CO
OCH3
abundance
0
0.1
0.2
0.3
7.0
6.0
5.0
4.0
3.0
2.0
X : parts per Million : 1H

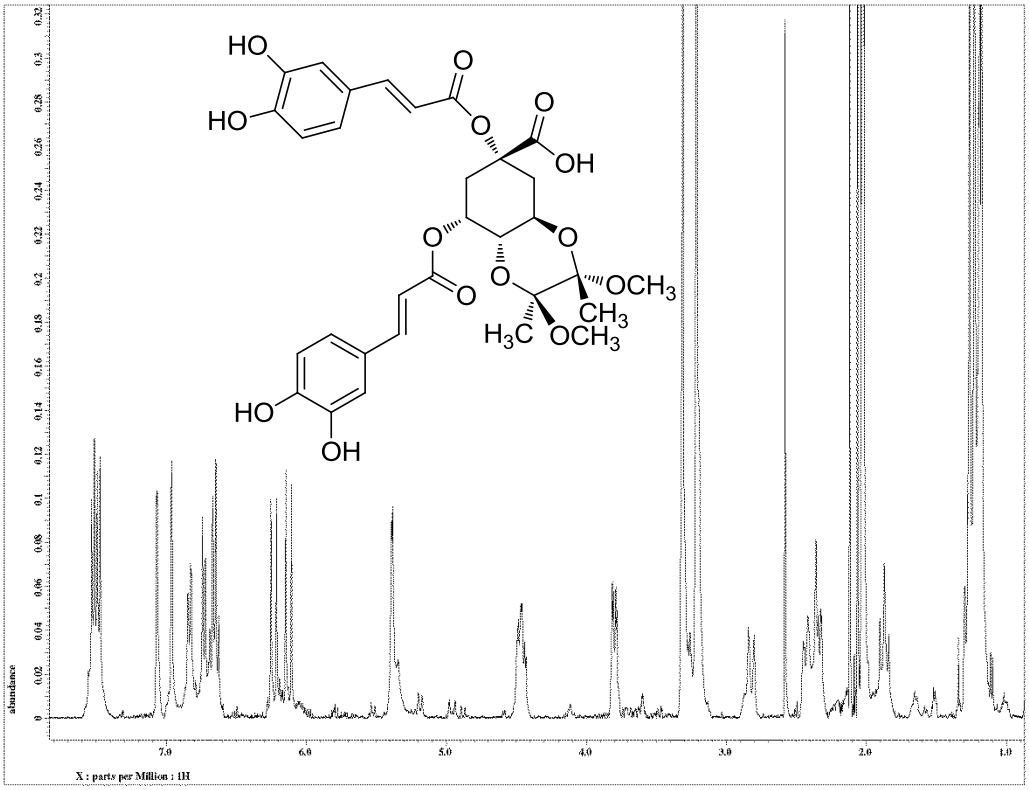

H3CO
H3CO
O
O
O
OH
OH
OH
O
O
H3CO
OCH3
(thousandths)
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
-1.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
X : parts per Million : 13C

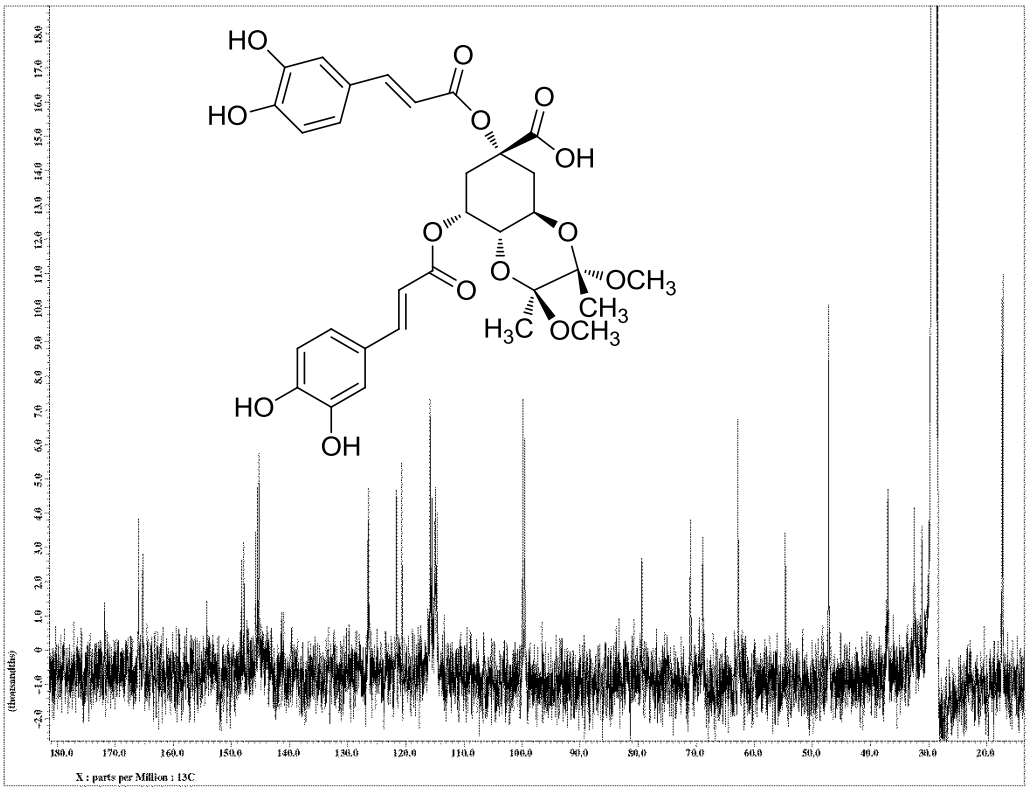

H3CO
H3CO
O
O
O
O
O
OH
O
H3CO
OCH3
abundance
X : parts per Million : 1H

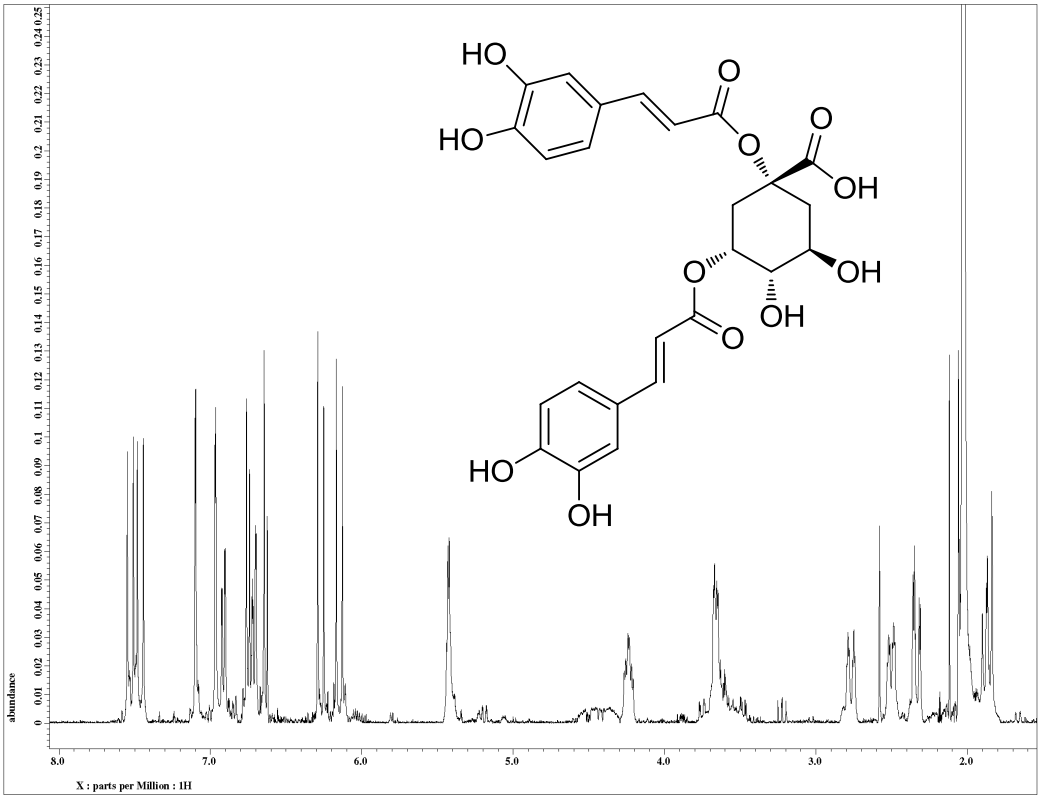

H3CO
H3CO
O
O
O
O
O
OH
O
H3CO
OCH3
abundance
X : parts per Million : 13C

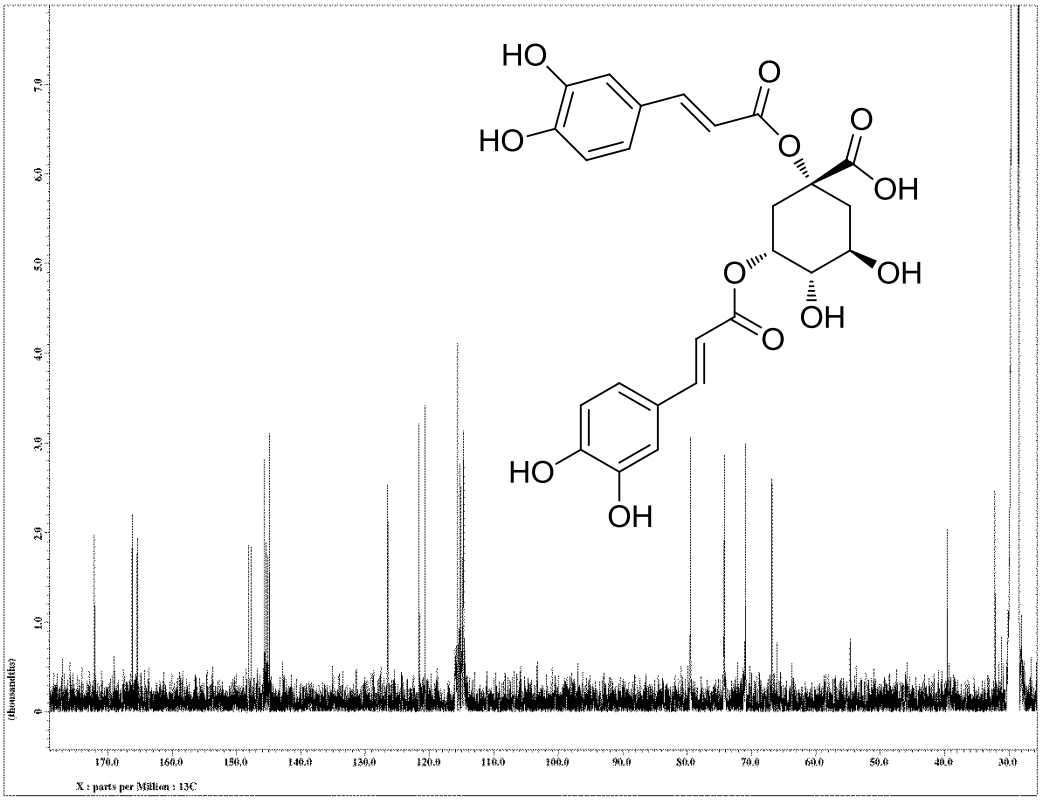

AllO
AllO
O
O
O
OH
O
O
O
O
OCH3
CH3
H3C
OCH3
AllO
OAll
abundance
X : parts per Million : 1H

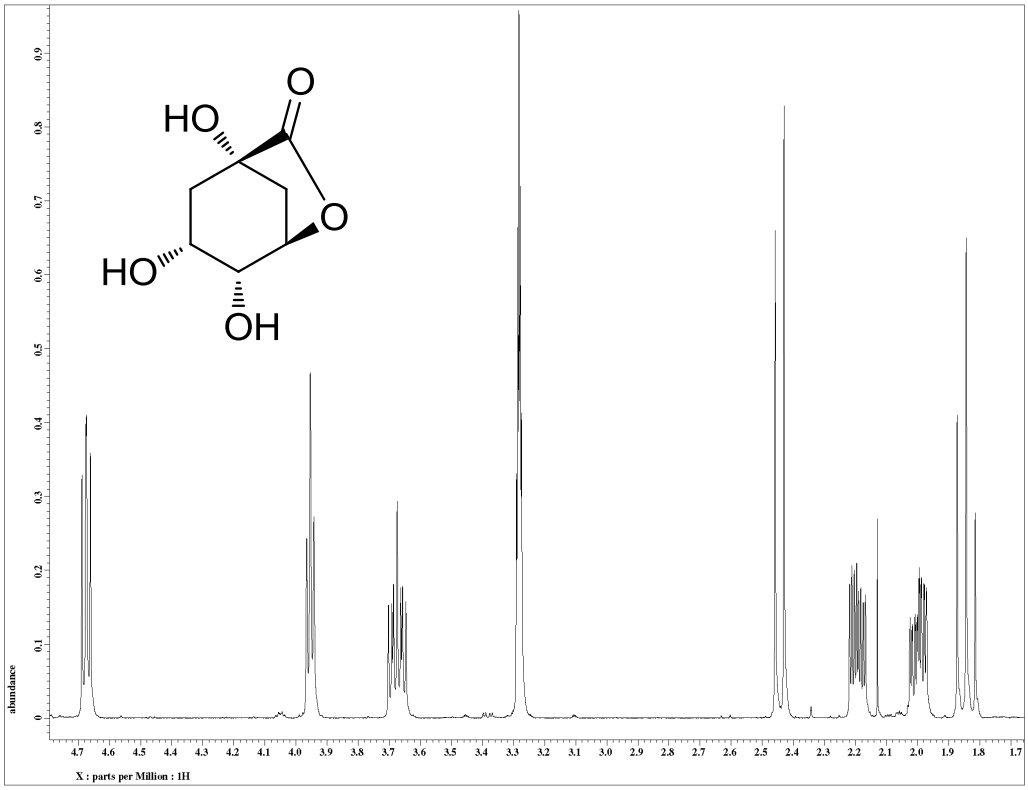

AllO
AllO
O
O
O
OH
O
O
O
O
OCH3
CH3
H3C
OCH3
AllO
OAll
abundance
X : parts per Million : 13C

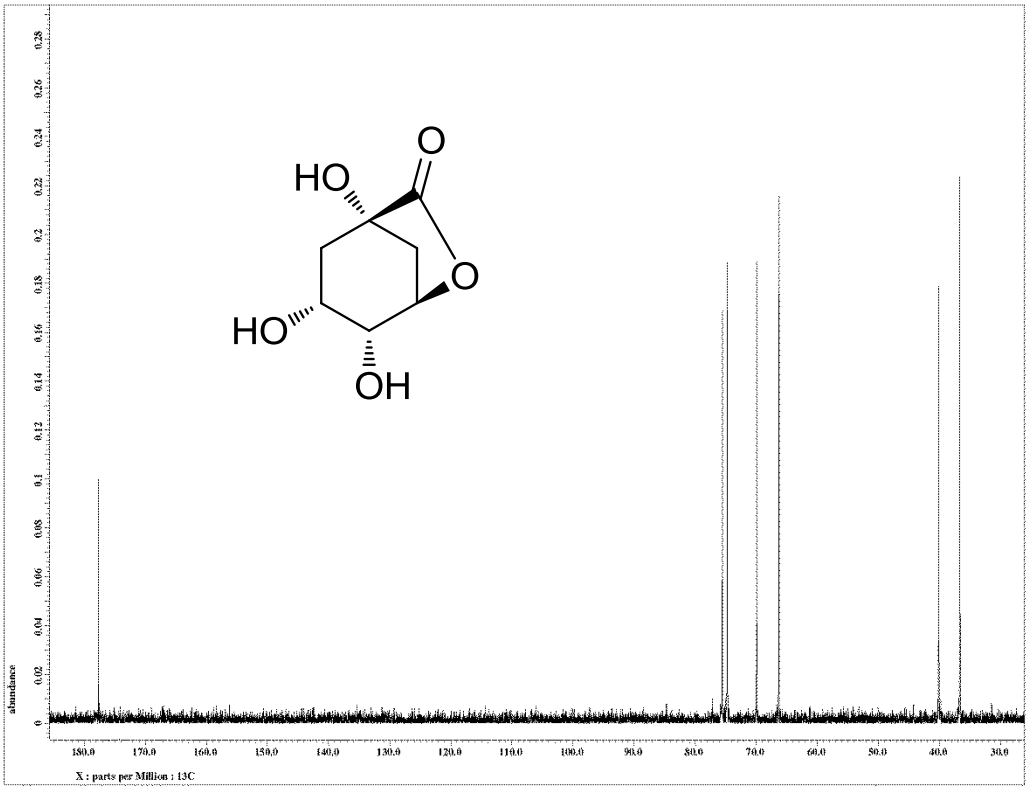

HO
HO
O
O
O
OH
O
O
O
O
O
OCH3
CH3
H3C
OCH3
HO
OH
abundance
X : parts per Million : 1H

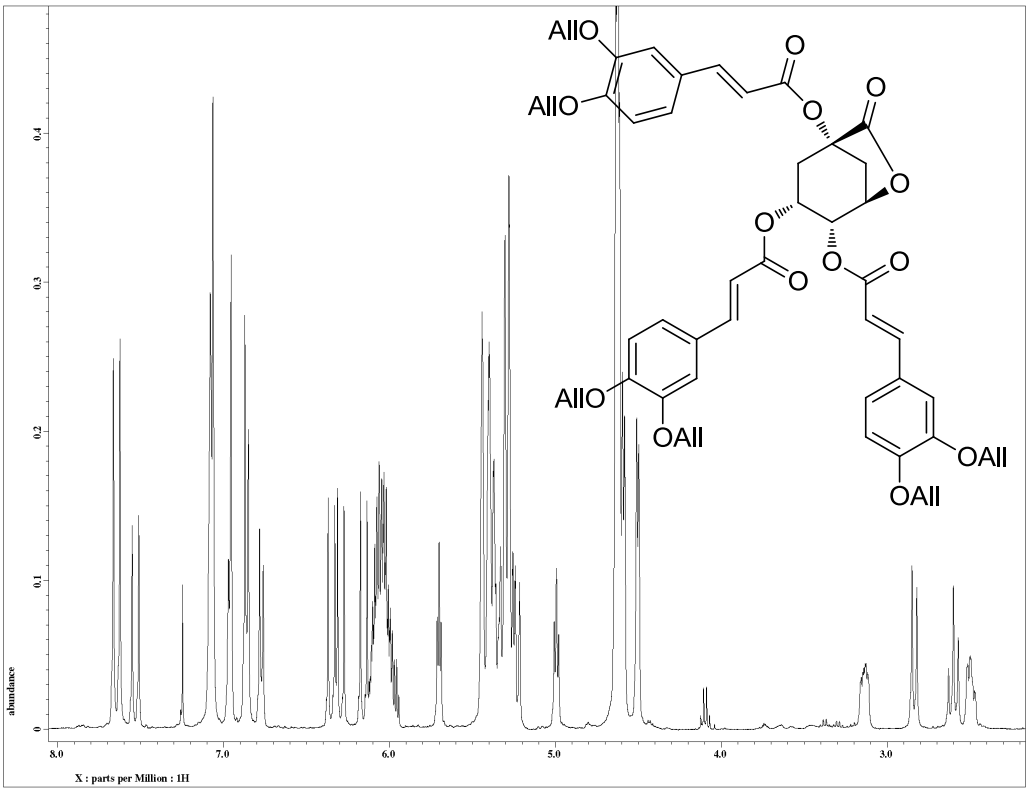

HO
HO
O
O
O
OH
O
O
O
O
O
OCH3
CH3
H3C
OCH3
HO
OH
(thousandths)
X : parts per Million : 13C

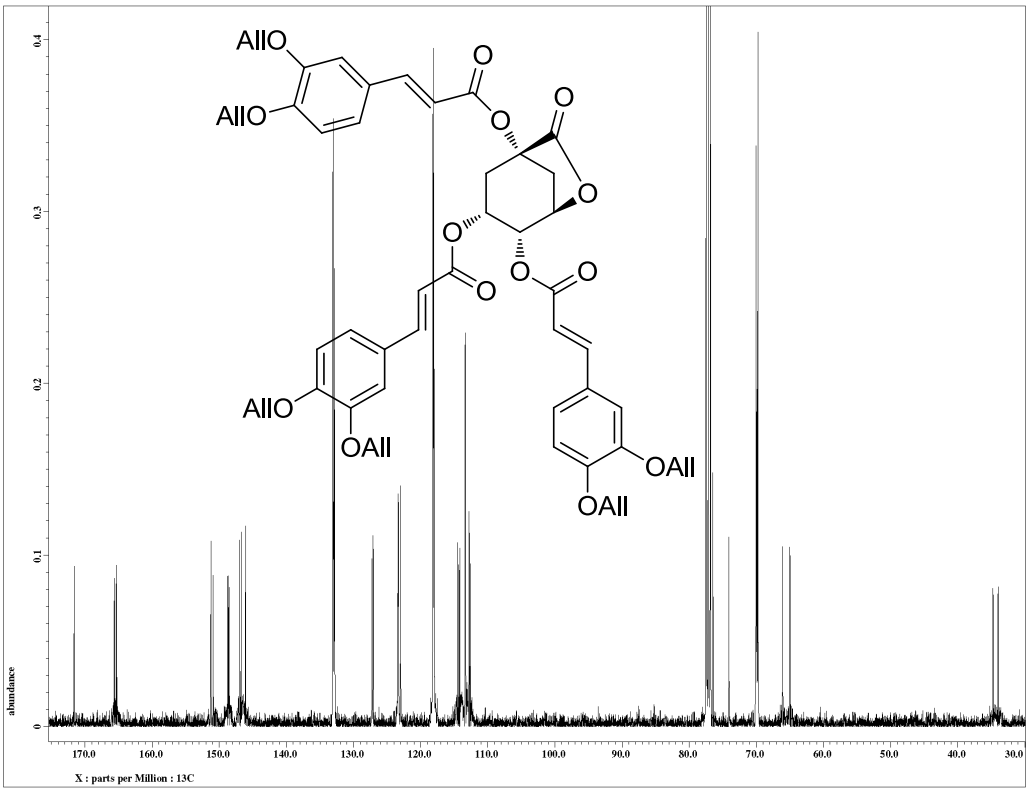

HO
HO
O
O
O
OH
O
OH
OH
O
O
HO
OH
abundance
0 0.01 0.02 0.03 0.04 0.05 0.06 0.07 0.08 0.09 0.1 0.11 0.12 0.13 0.14 0.15 0.16 0.17 0.18 0.19 0.2 0.21 0.22 0.23 0.24 0.25
8.0 7.0 6.0 5.0 4.0 3.0 2.0
X : parts per Million : 1H

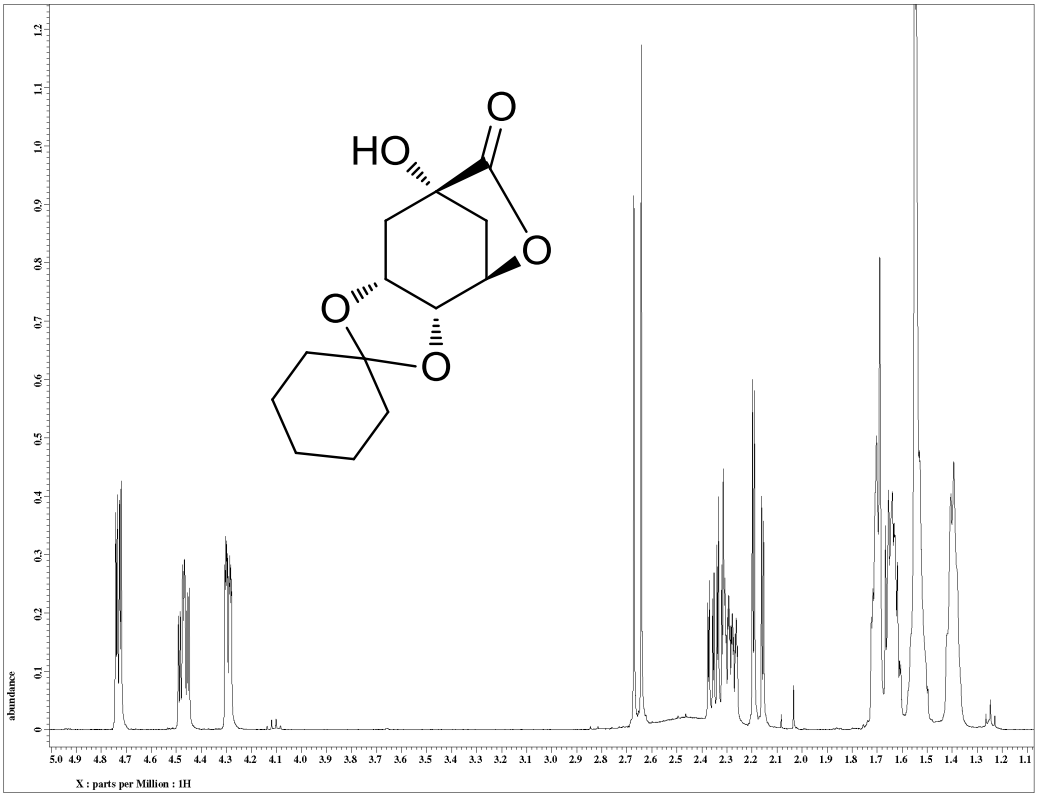

HO
HO
O
O
O
OH
O
OH
OH
O
O
HO
OH
(thousandths)
0 1.0 2.0 3.0 4.0 5.0 6.0 7.0
170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0
X : parts per Million : 13C

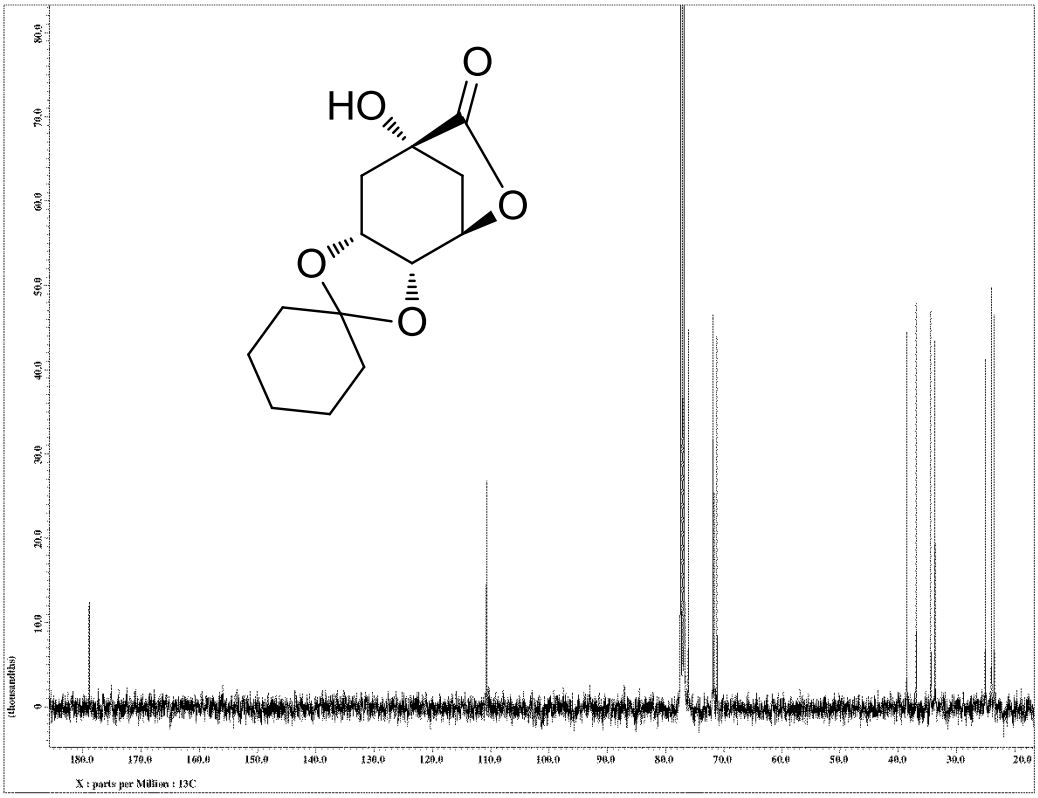

HO
O
O
HO
OH
abundance
X : parts per Million : 1H

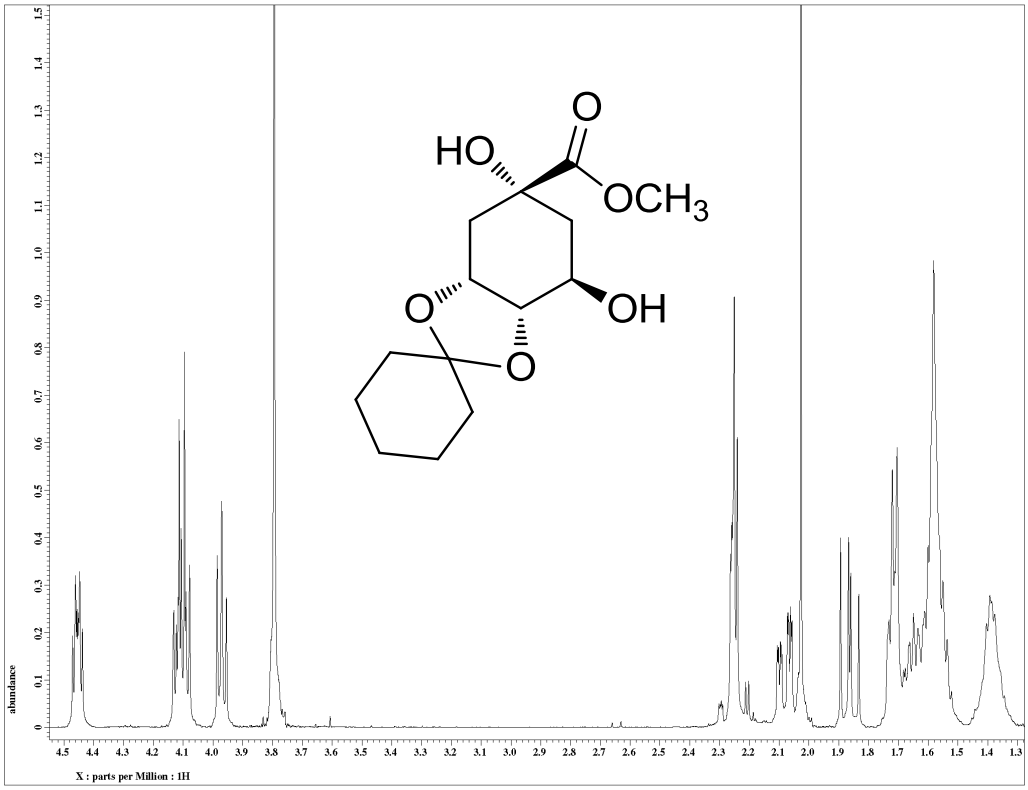

HO
O
O
HO
OH
abundance
X : parts per Million : 13C

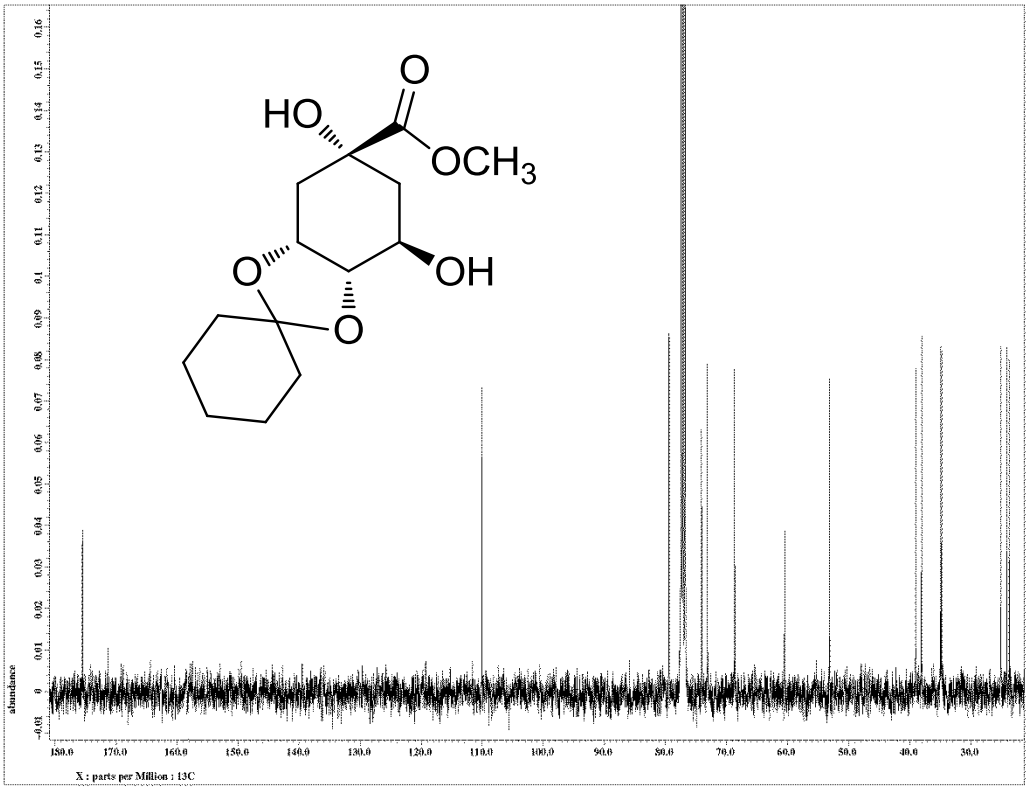

AllO
AllO
O
O
O
O
O
O
O
O
AllO
OAll
OAll
OAll
abundance
0
0.1
0.2
0.3
0.4
8.0
7.0
6.0
5.0
4.0
3.0
X : parts per Million : 1H

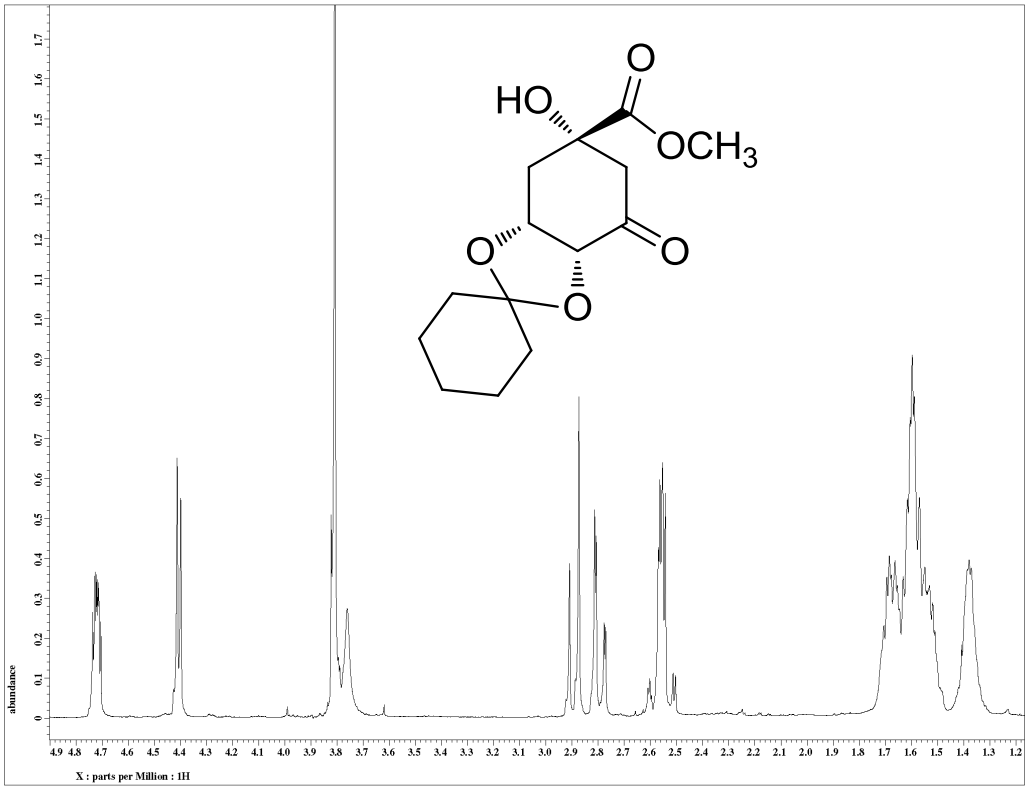

AllO
AllO
O
O
O
O
O
O
O
O
AllO
OAll
OAll
OAll
abundance
0
0.1
0.2
0.3
0.4
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
X : parts per Million : 13C

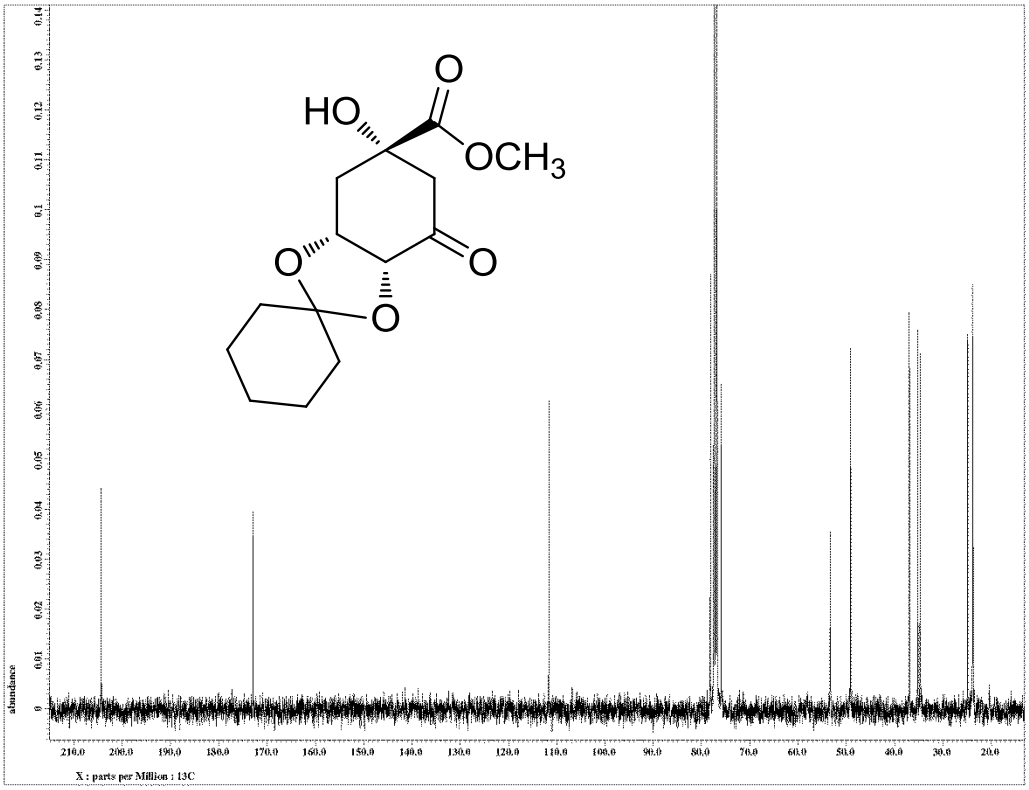

HO
O
O
O
O
abundance
X : parts per Million : 1H

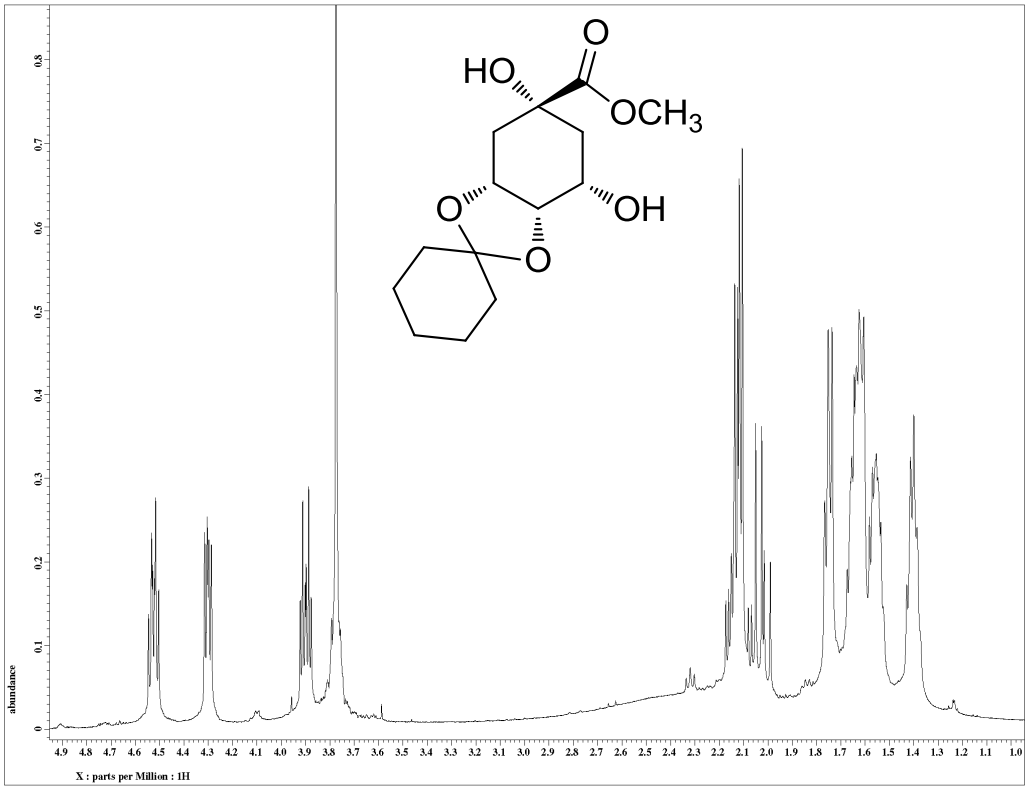

HO
O
O
O
O
(thousandths)
X : parts per Million : 13C

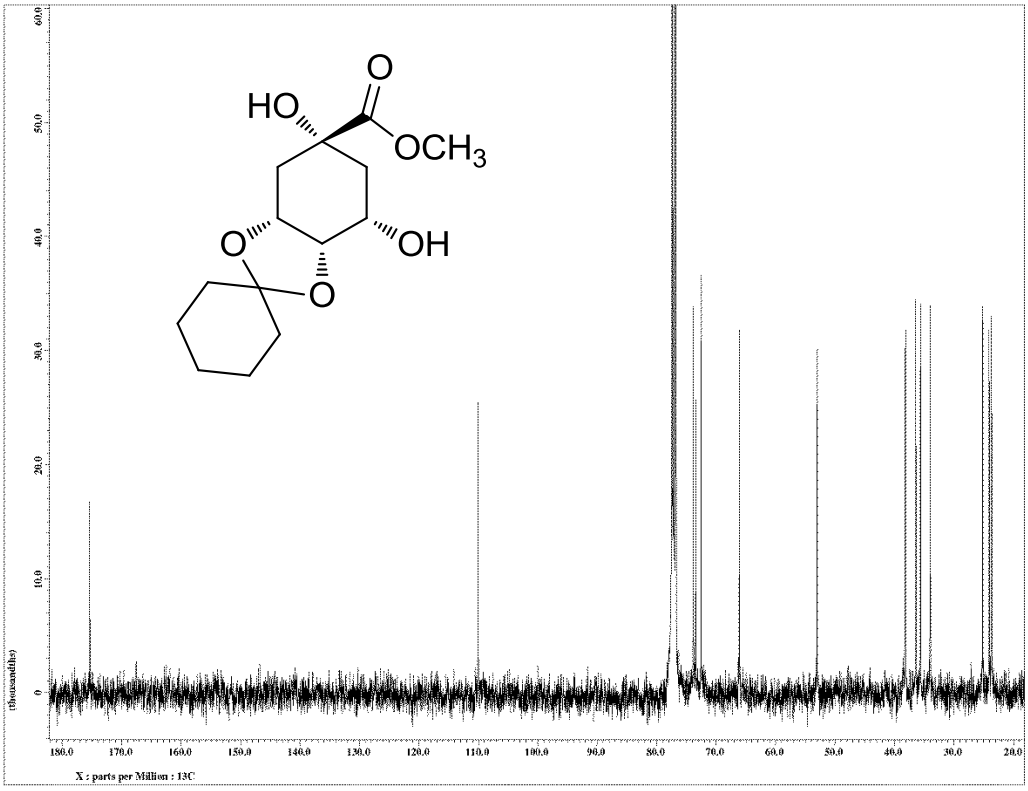

HO
O
OCH3
O
OH
O
abundance
X : parts per Million : 1H

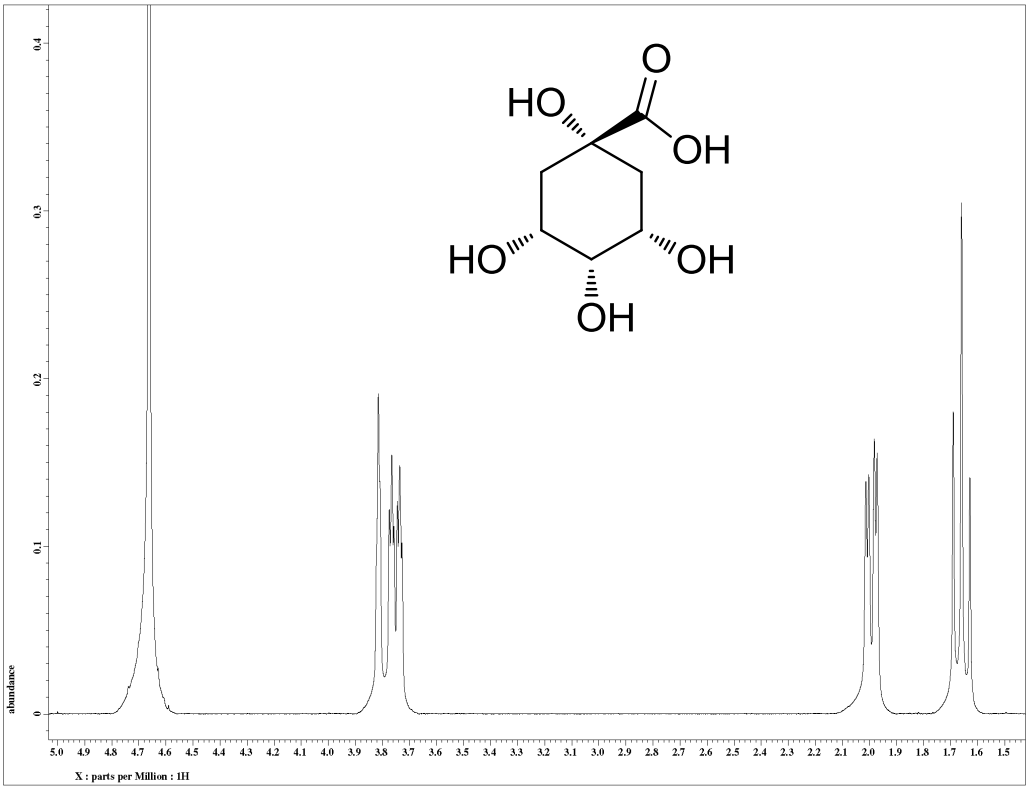

HO
O
OCH3
O
OH
O
abundance
X : parts per Million : 13C

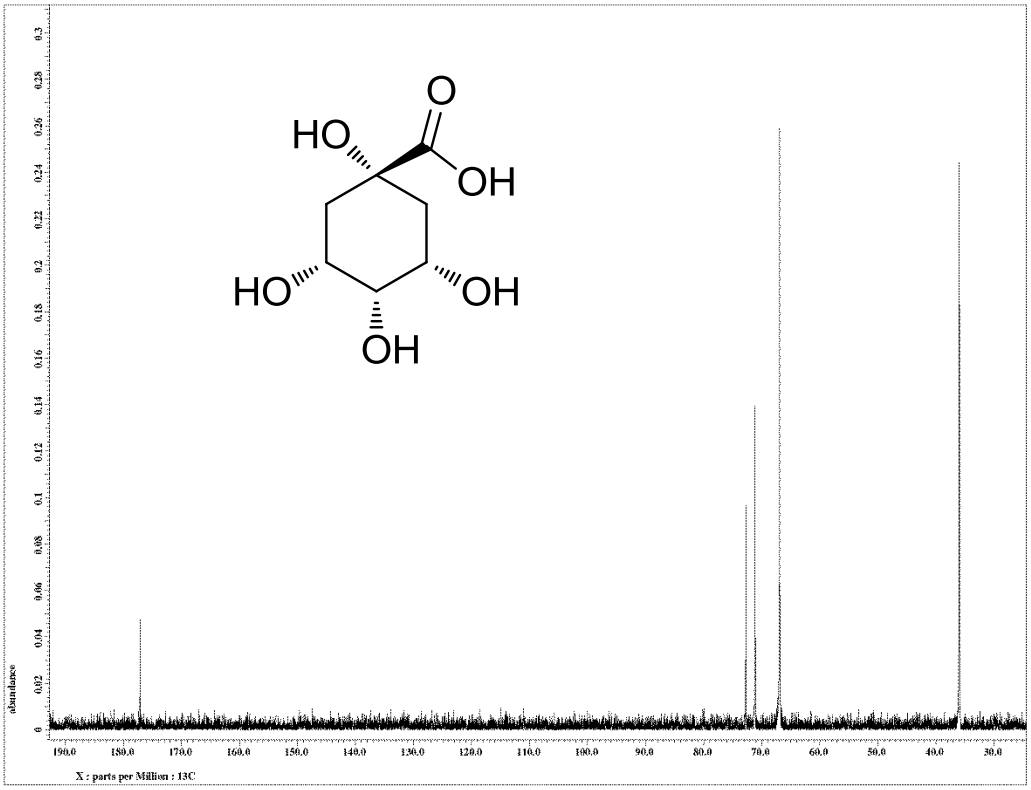

HO
O
OCH3
O
O
O
abundance
X : parts per Million : 1H

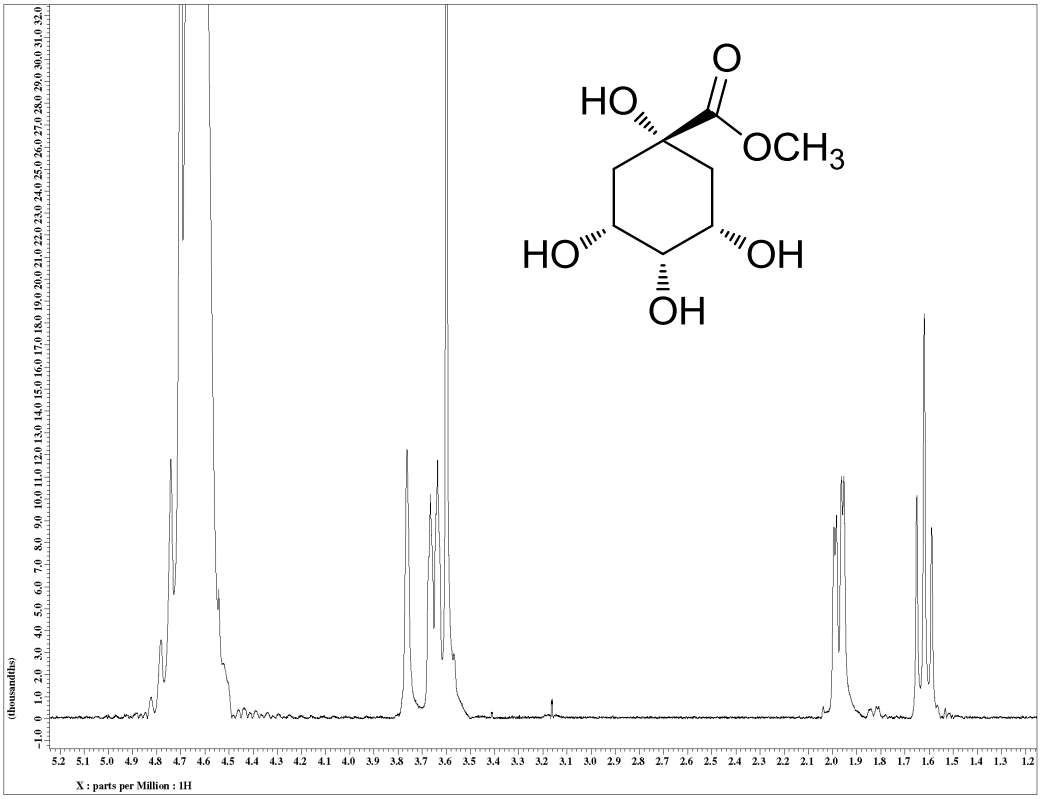

HO
O
OCH3
O
O
O
abundance
X : parts per Million : 13C

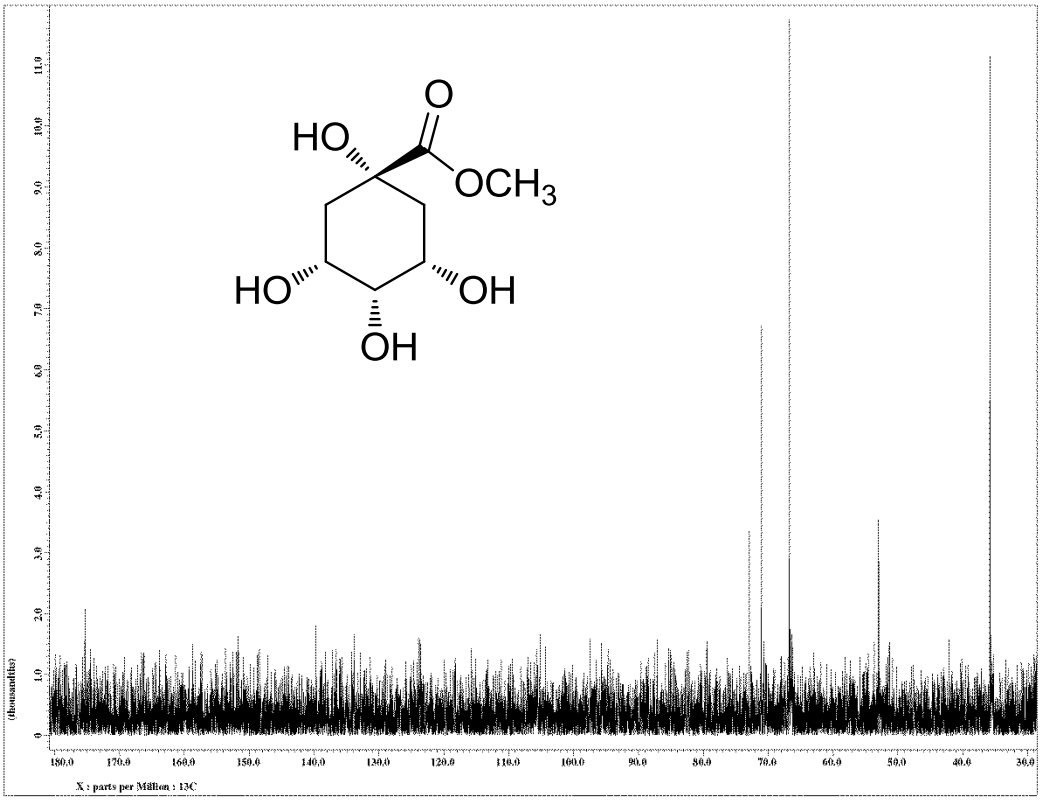

HO
O
OCH3
O
OH
O
abundance
X : parts per Million : 1H

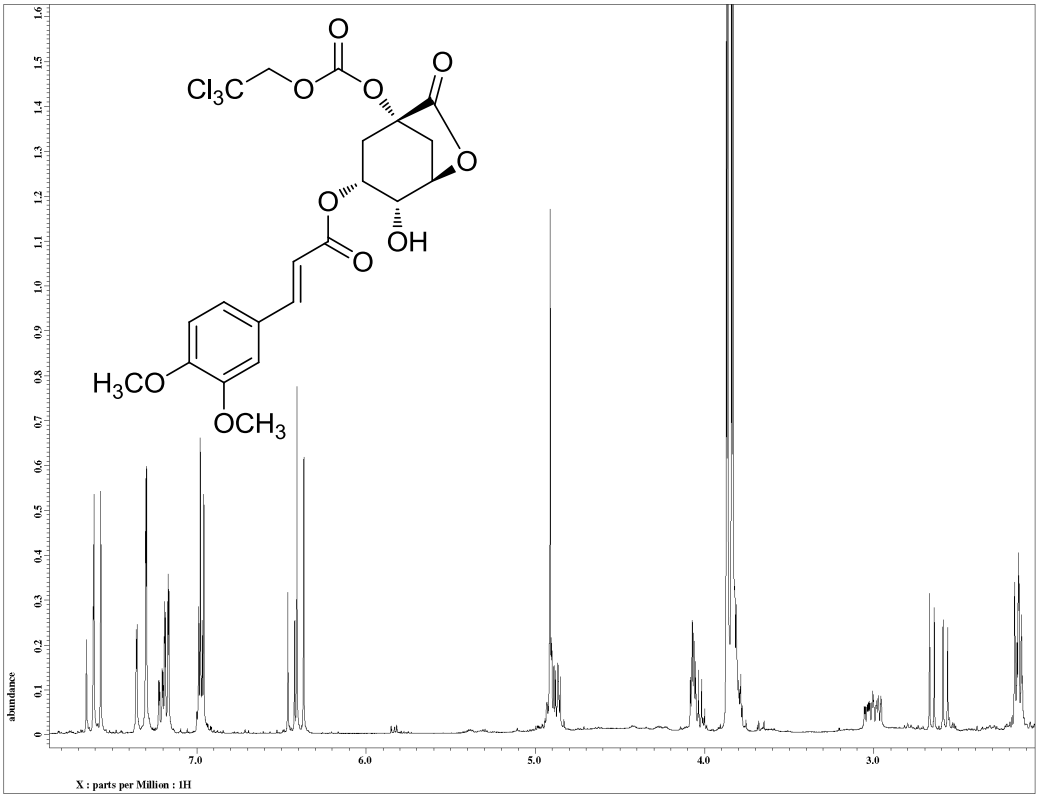

HO
O
OCH3
O
OH
O
X : parts per Million : 13C

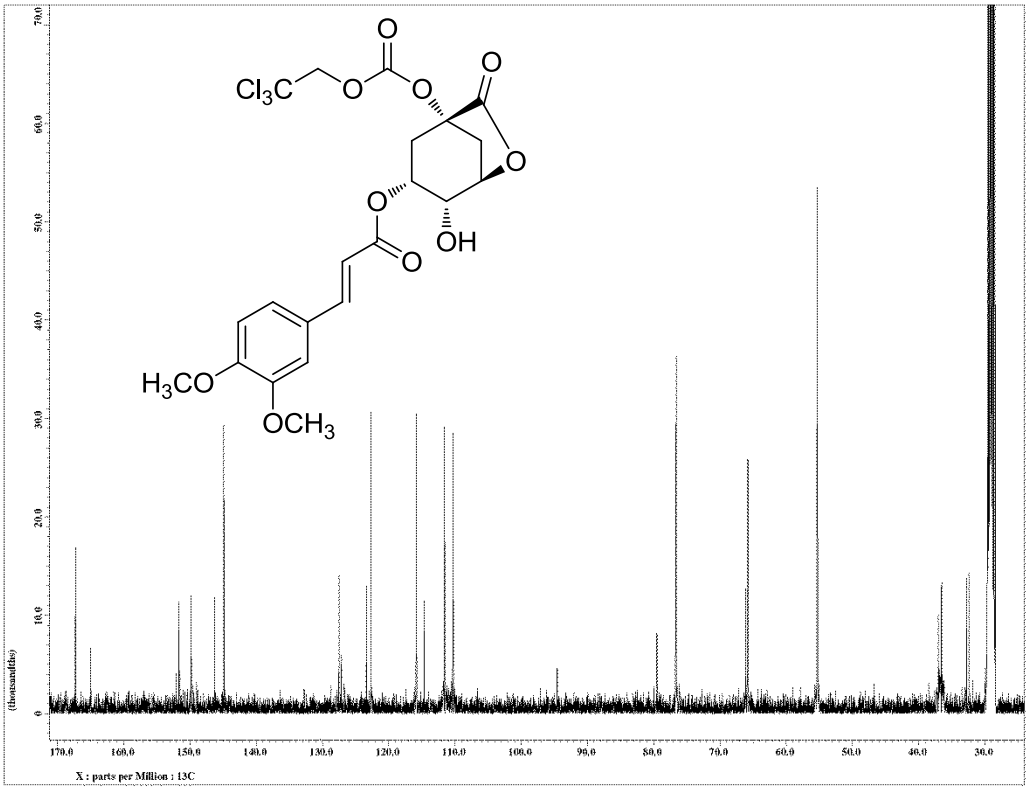

O
HO
OH
HO
OH
OH
abundance
X : parts per Million : 1H

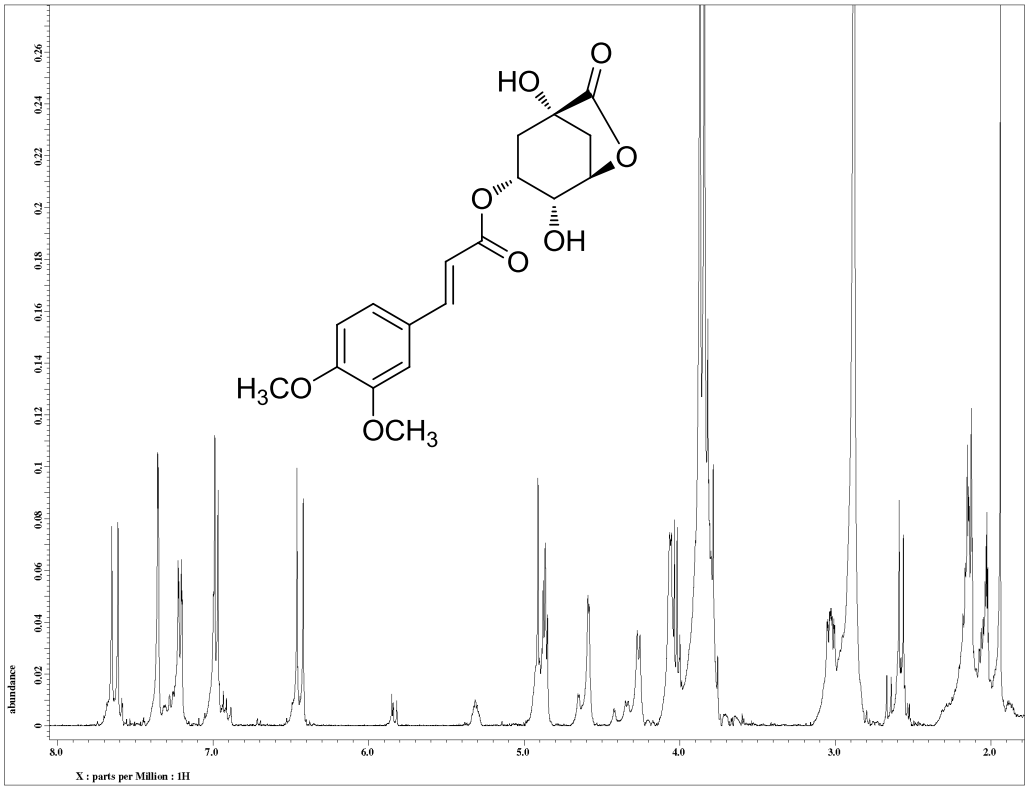

O
HO
OH
HO
OH
OH
abundance
X : parts per Million : 13C

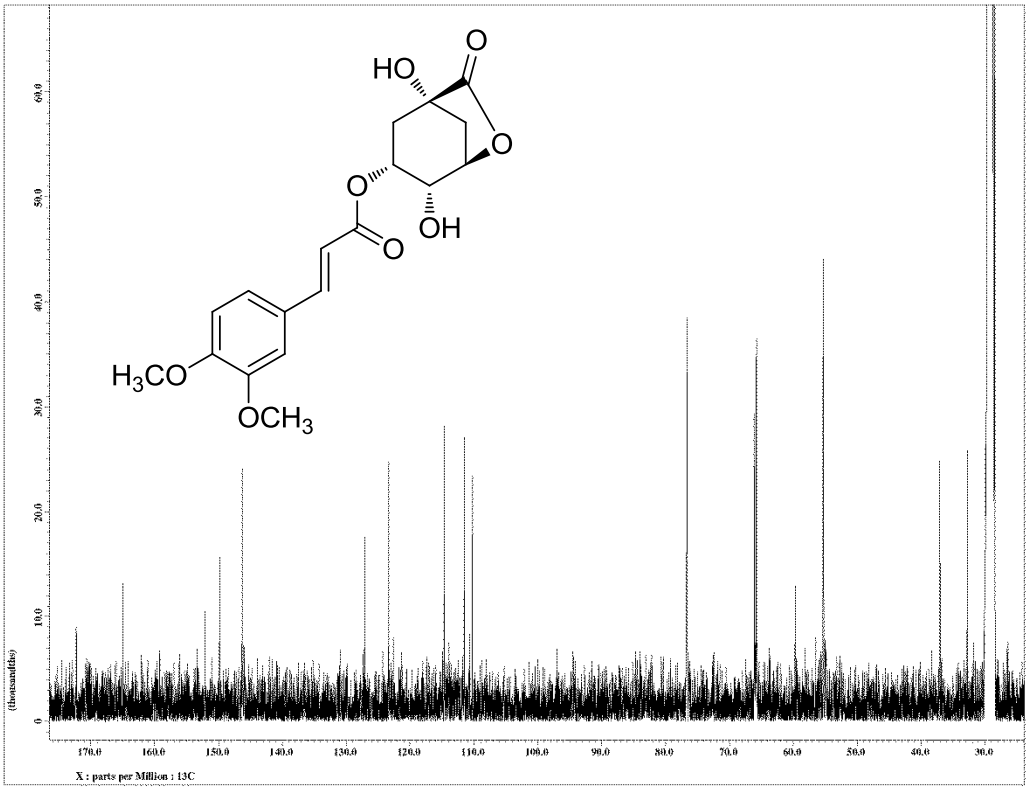

HO
O
OCH3
HO
OH
OH
(thousandths)
X : parts per Million : 1H

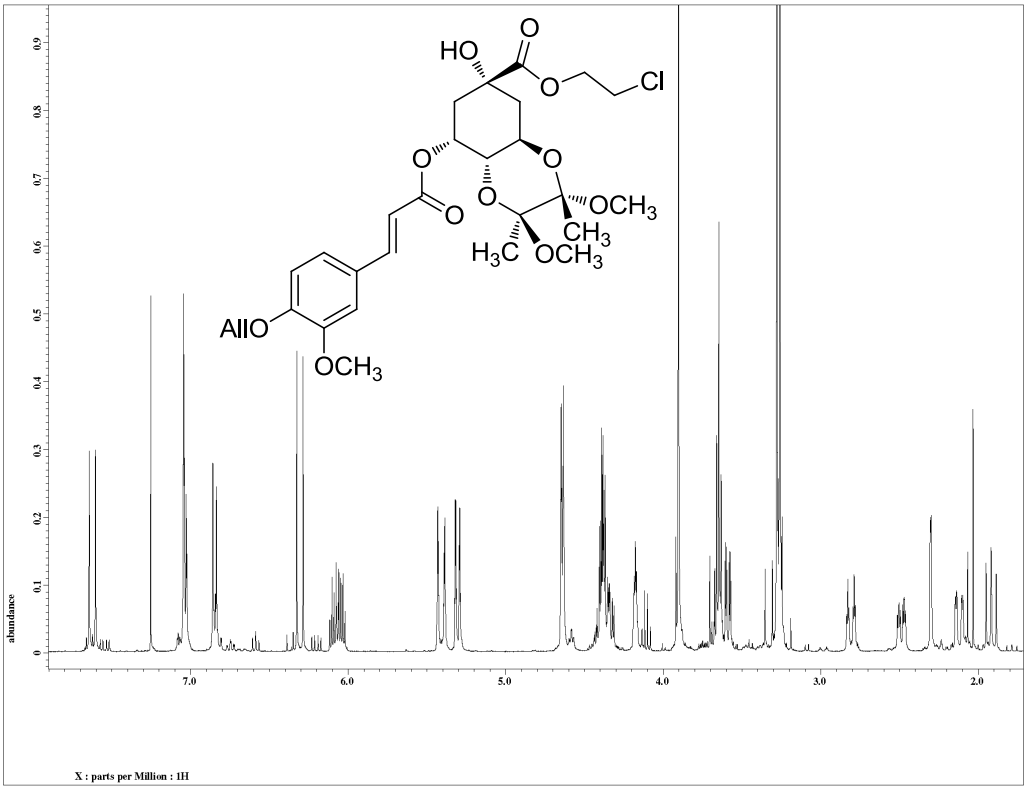

HO
O
OCH3
HO
OH
OH
(thousandths)
X : parts per Million : 13C

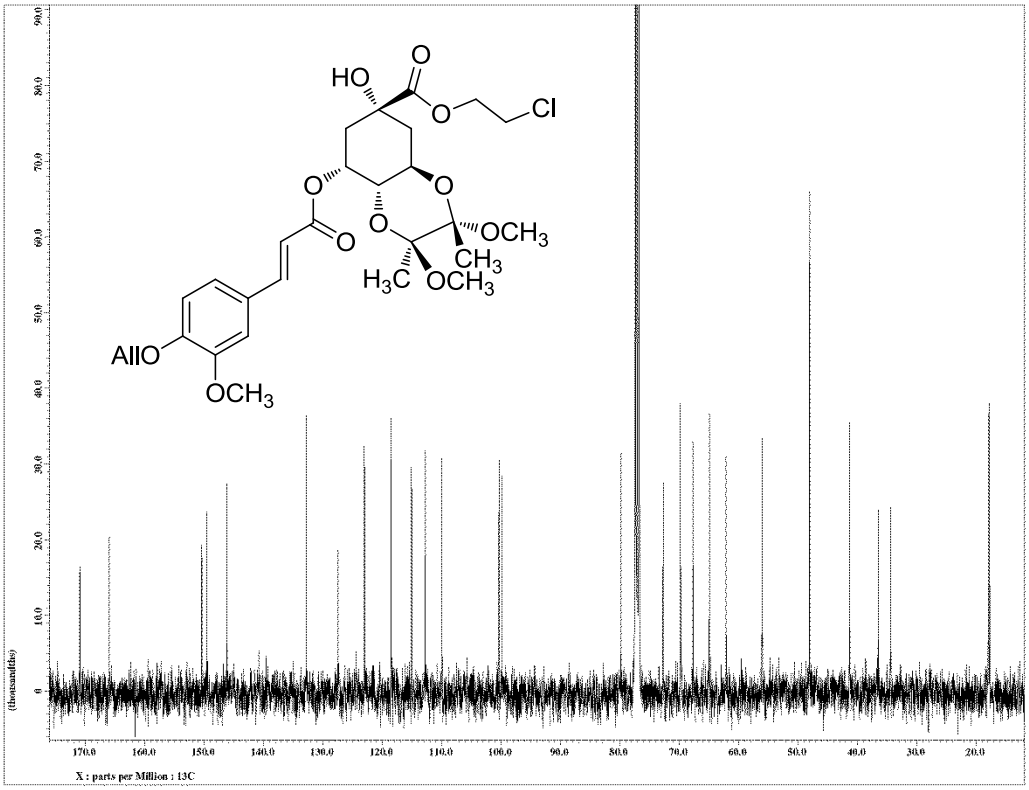

abundance
X : parts per Million : 1H

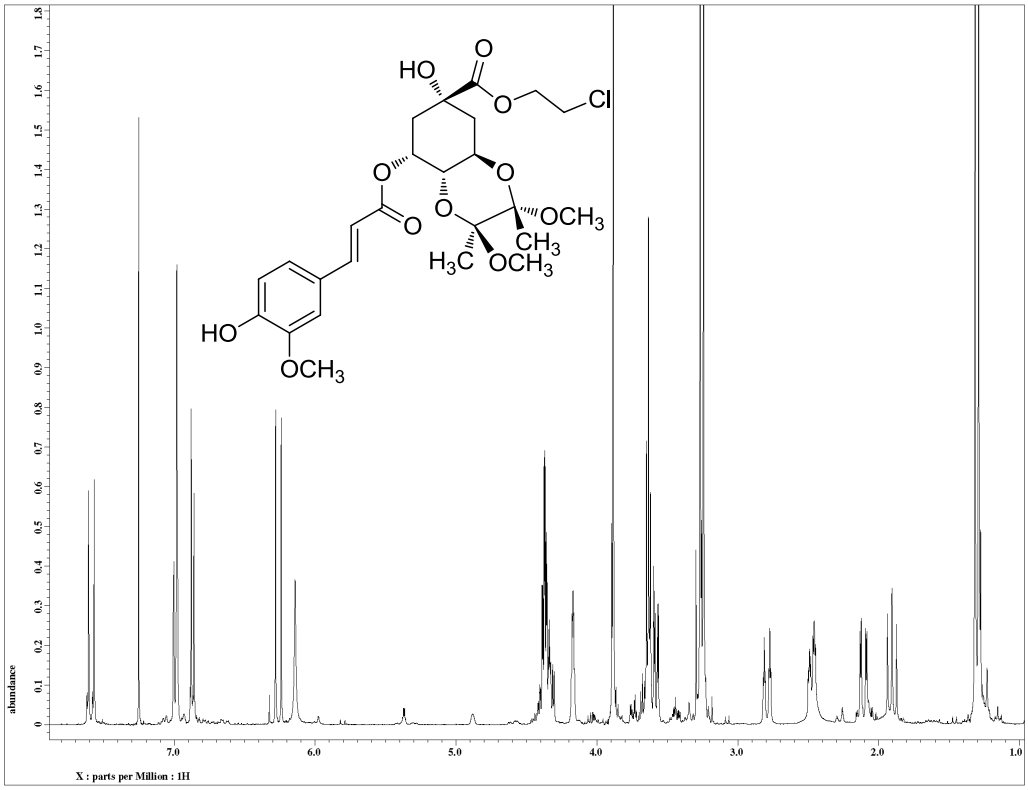

(thousands)
X : parts per Million : 13C

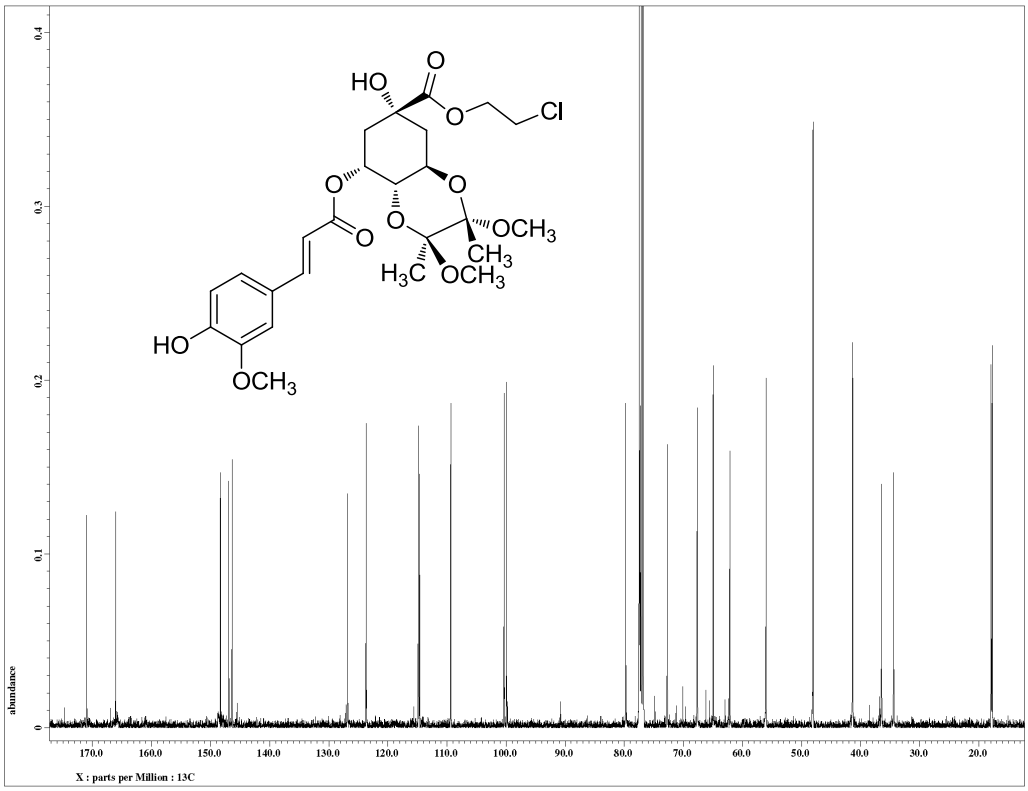

HO
O
O
O
OH
O
H3CO
OCH3
abundance
X : parts per Million : 1H

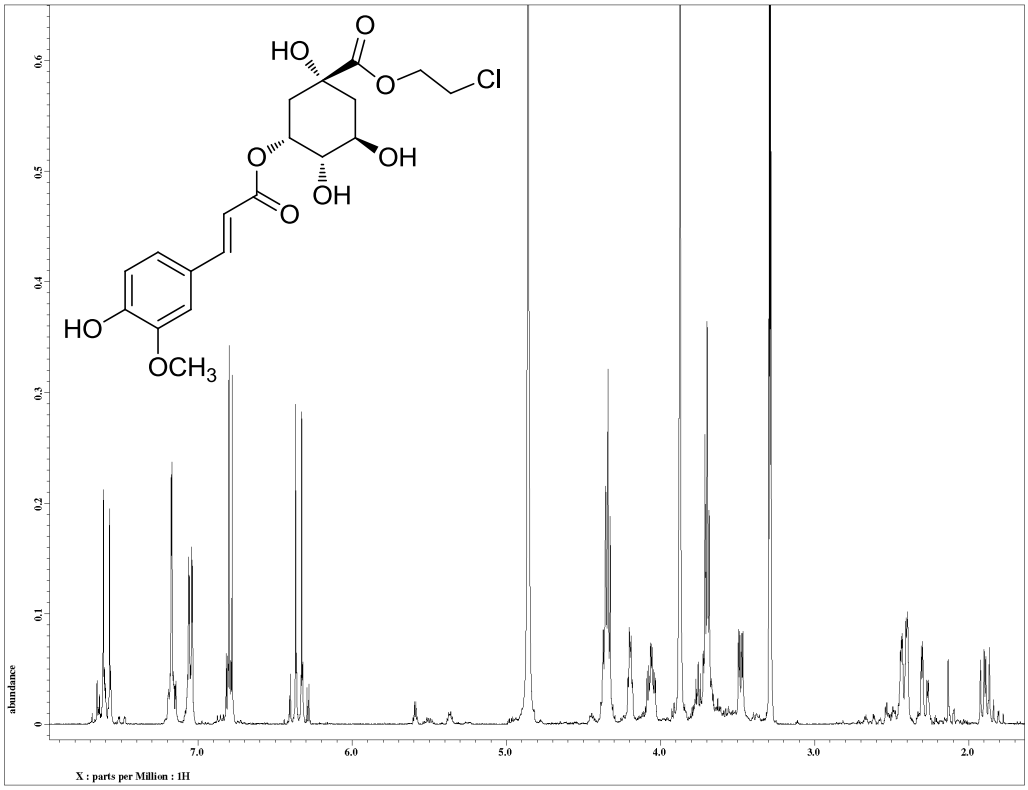

HO
O
O
O
OH
O
H3CO
OCH3
(thousands)
X : parts per Million : 13C

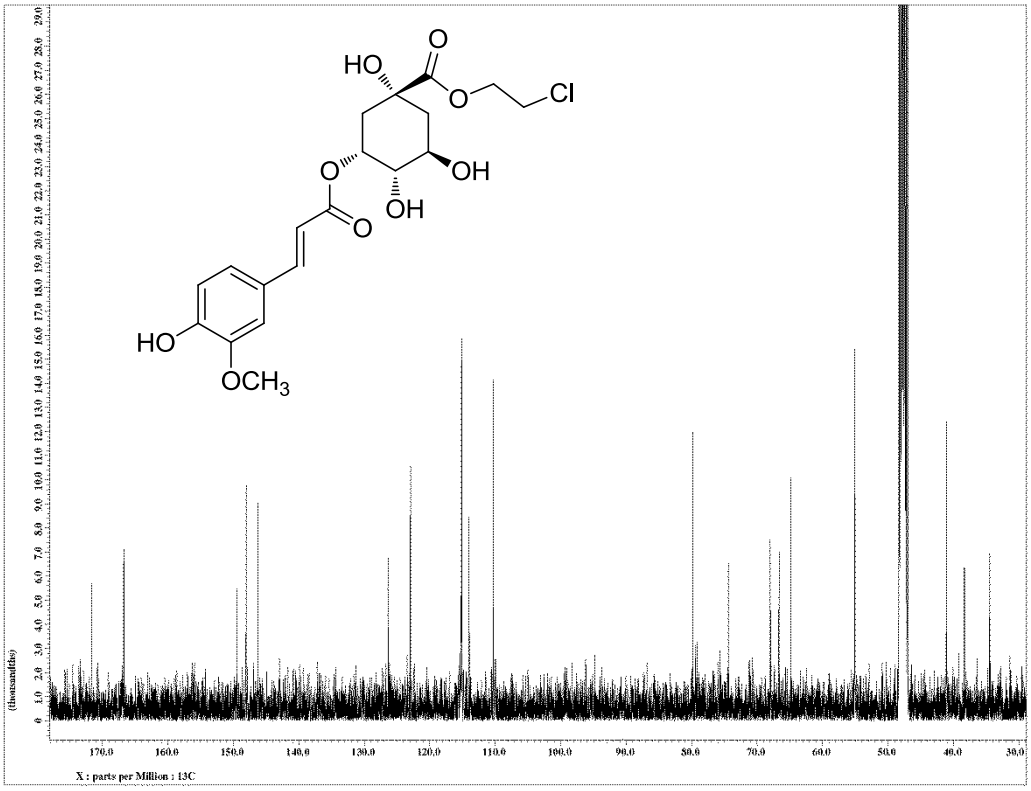

HO
O
O
Cl
O
O
O
O
OCH3
CH3
H3C
OCH3
AllO
OCH3
abundance
X : parts per Million : 1H

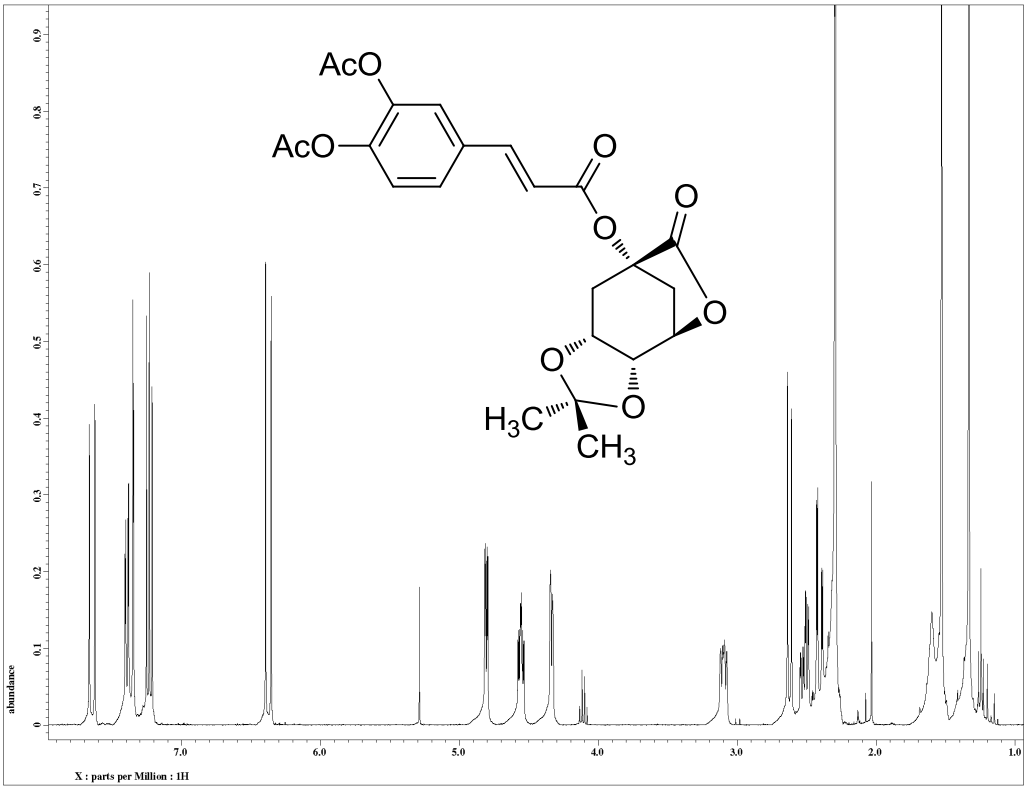

HO
O
O
Cl
O
O
O
O
OCH3
CH3
H3C
OCH3
AllO
OCH3
(thousands)
X : parts per Million : 13C

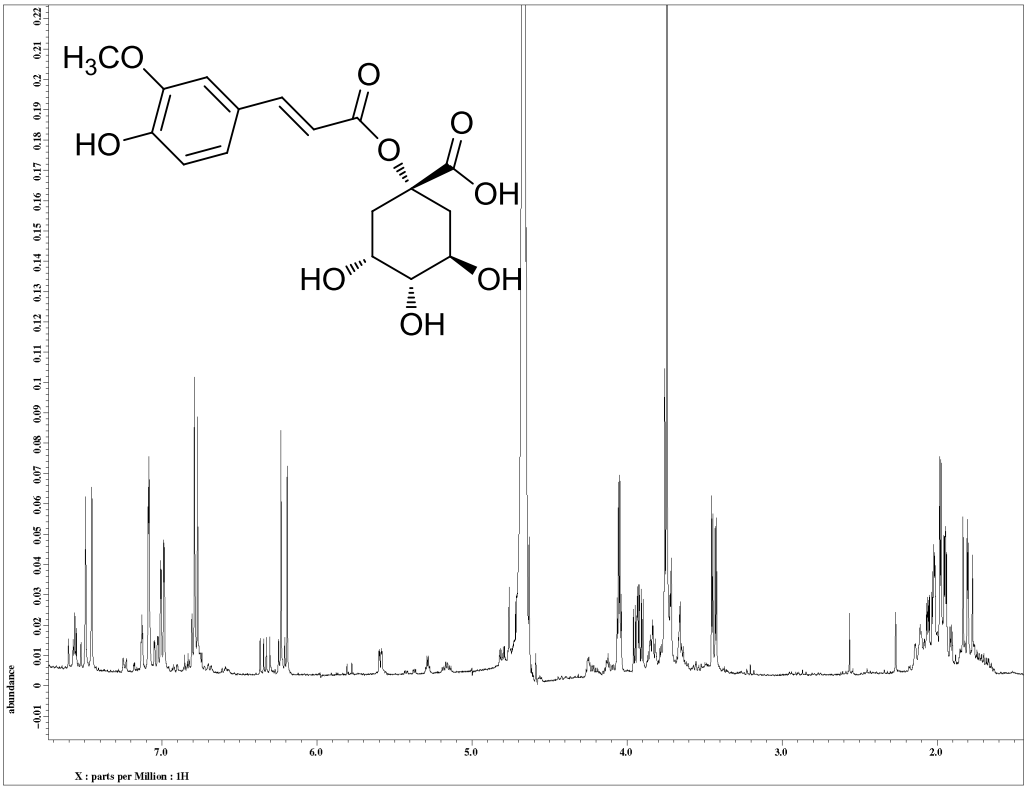

HO
O
O
Cl
O
O
O
O
OCH3
CH3
H3C
OCH3
HO
OCH3
abundance
X : parts per Million : 1H

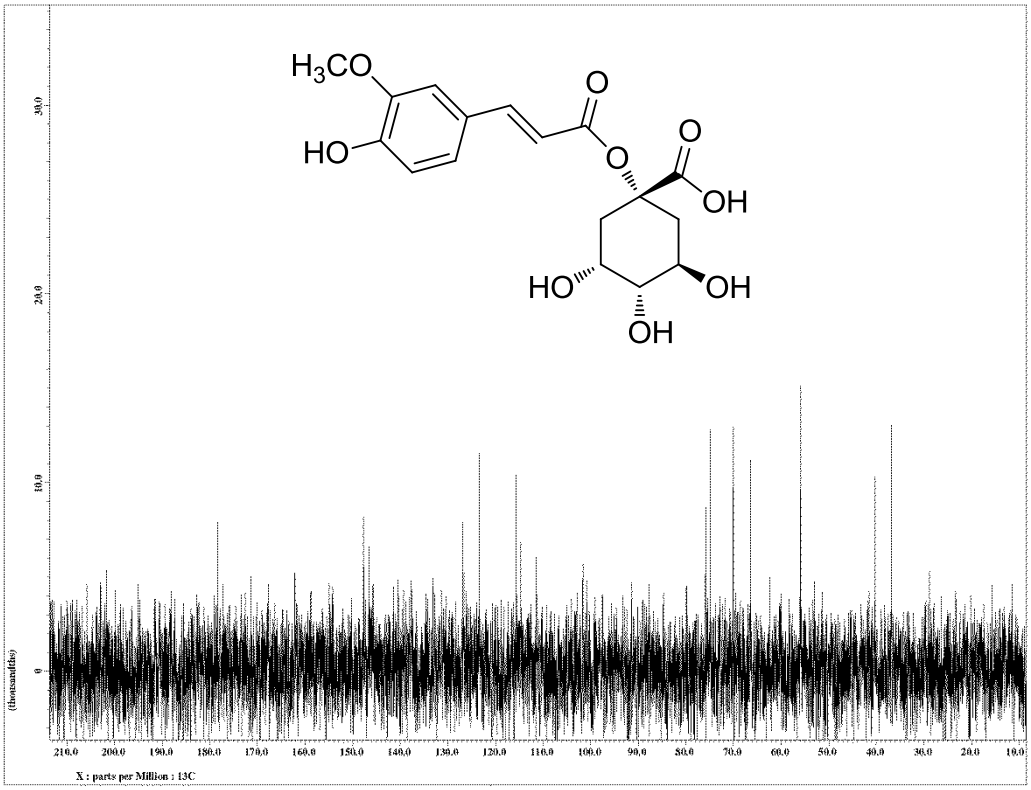

HO
O
O
Cl
O
O
O
O
OCH3
CH3
H3C
OCH3
HO
OCH3
abundance
X : parts per Million : 13C

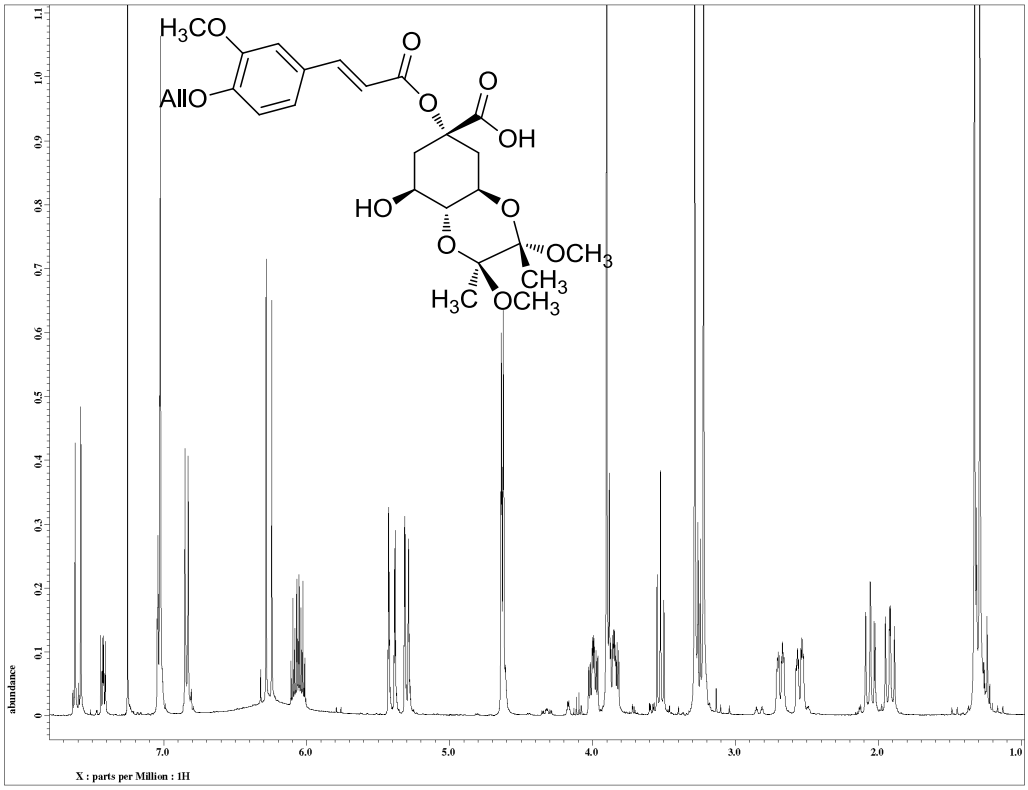

HO
O
O
Cl
O
OH
OH
O
HO
OCH3
abundance
X : parts per Million : 1H

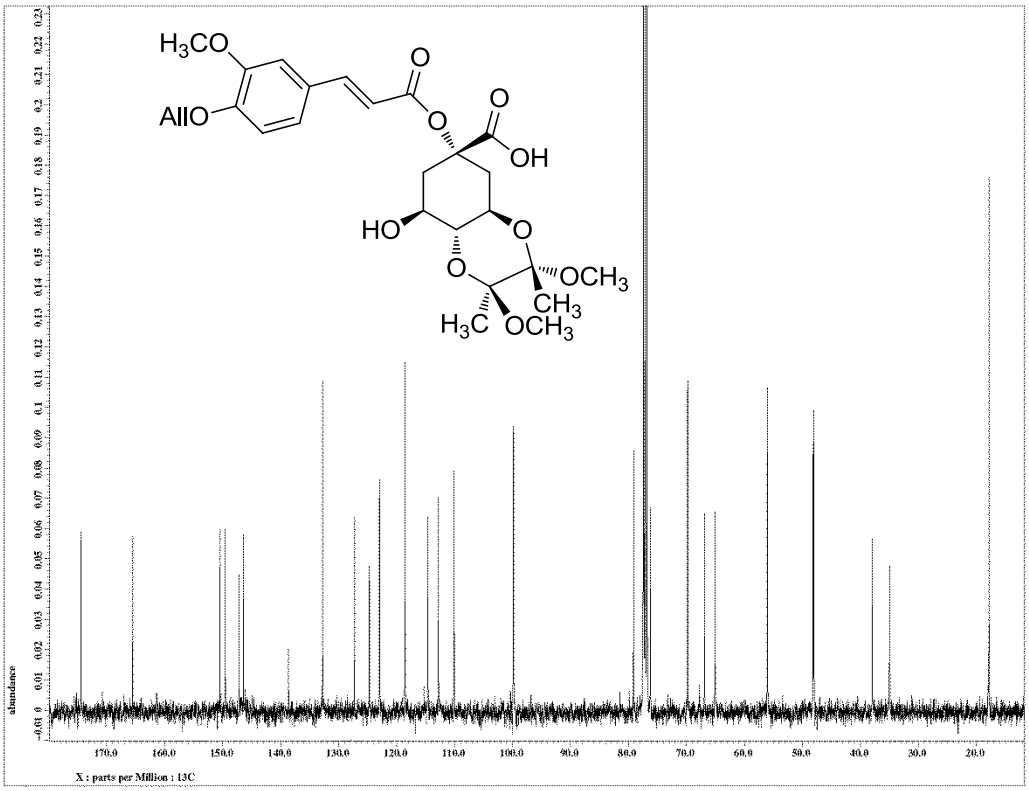

HO
O
O
Cl
O
OH
OH
O
HO
OCH3
(thousandths)
X : parts per Million : 13C

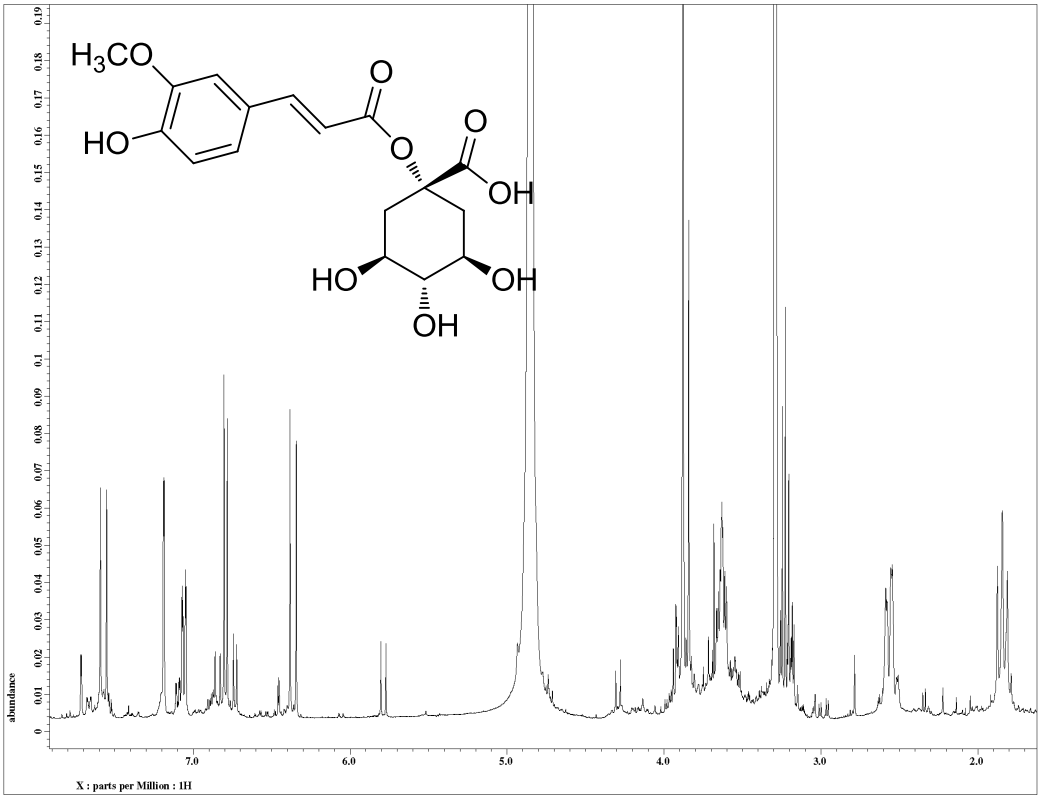

AcO
AcO
O
O
O
O
O
O
H3C
CH3
abundance
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
7.0
6.0
5.0
4.0
3.0
2.0
1.0
X : parts per Million : 1H

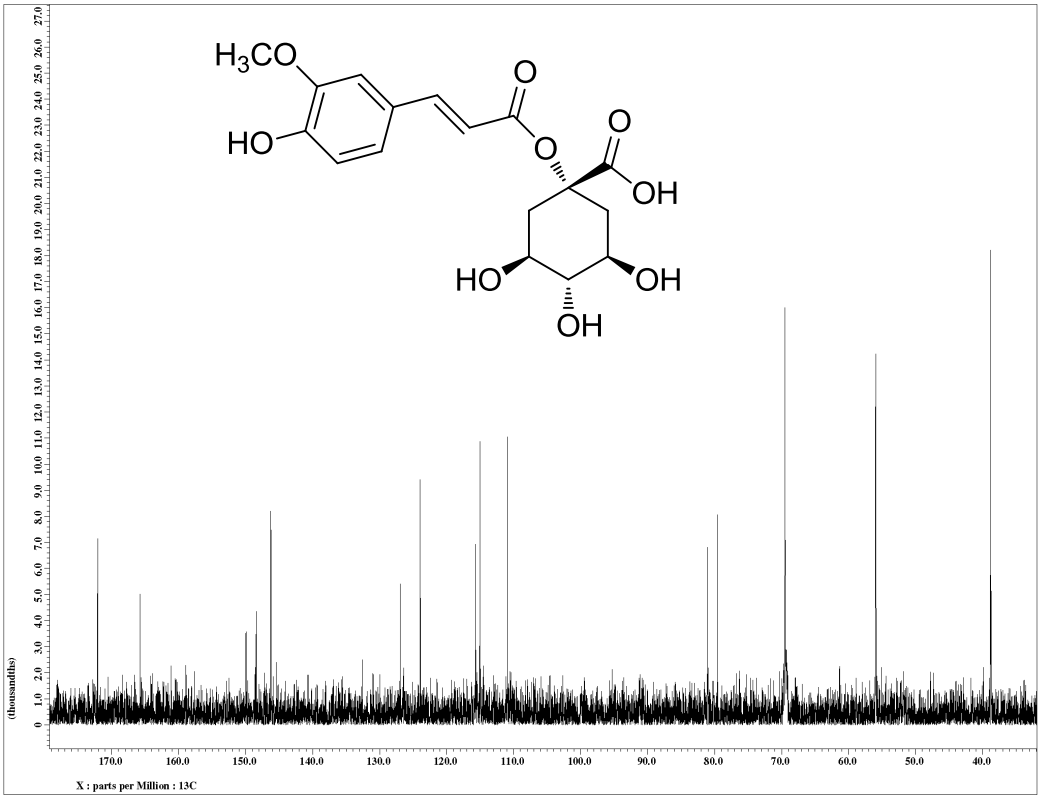

H3CO
HO
O
O
O
OH
HO
OH
OH
abundance
X : parts per Million : 1H

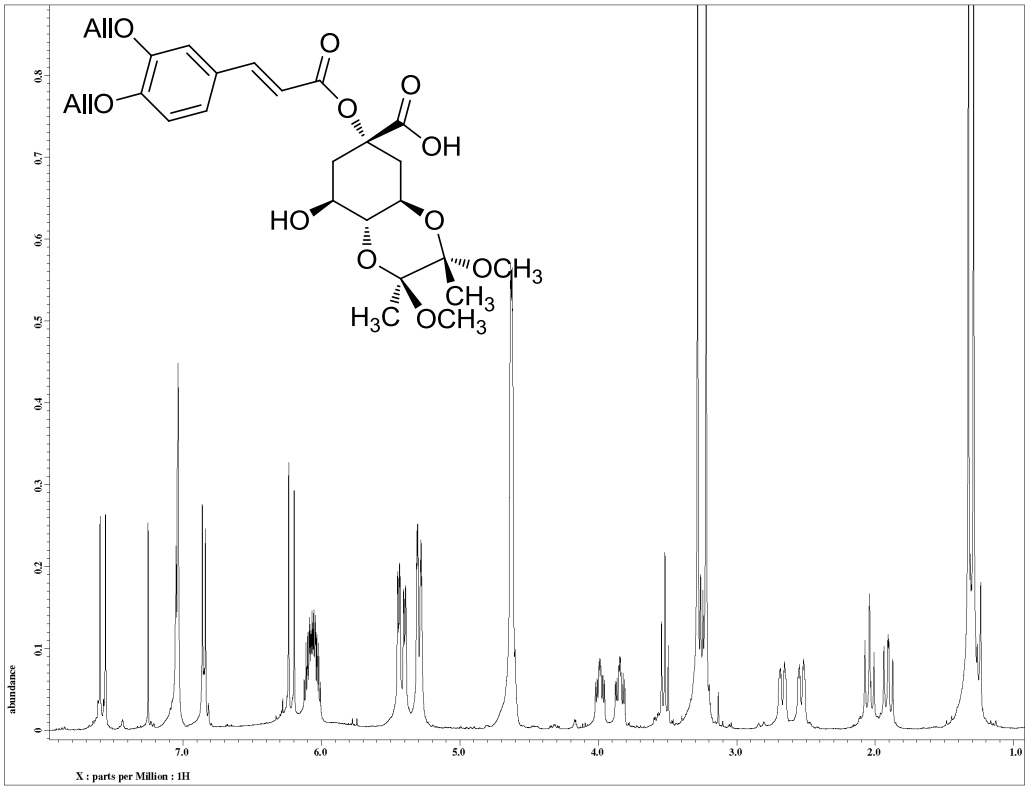

H3CO
HO
O
O
O
OH
HO
OH
OH
(thousandths)
X : parts per Million : 13C

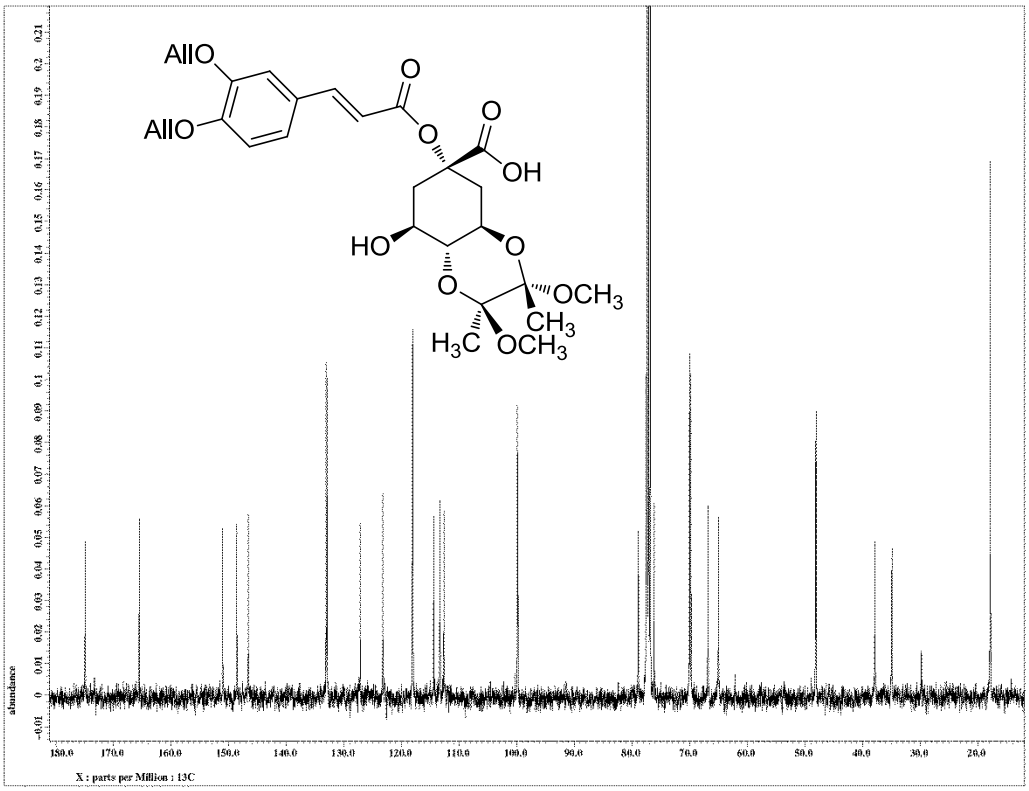

H3CO
AllO
O
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 1H

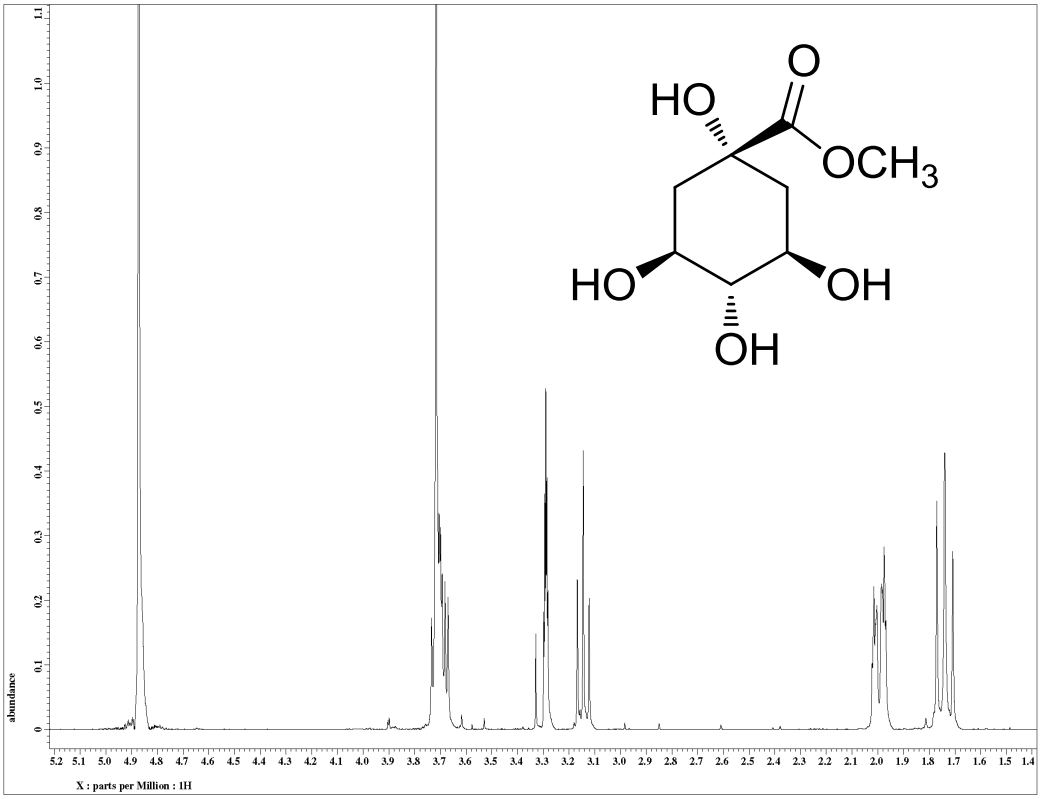

H3CO
AllO
O
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 13C

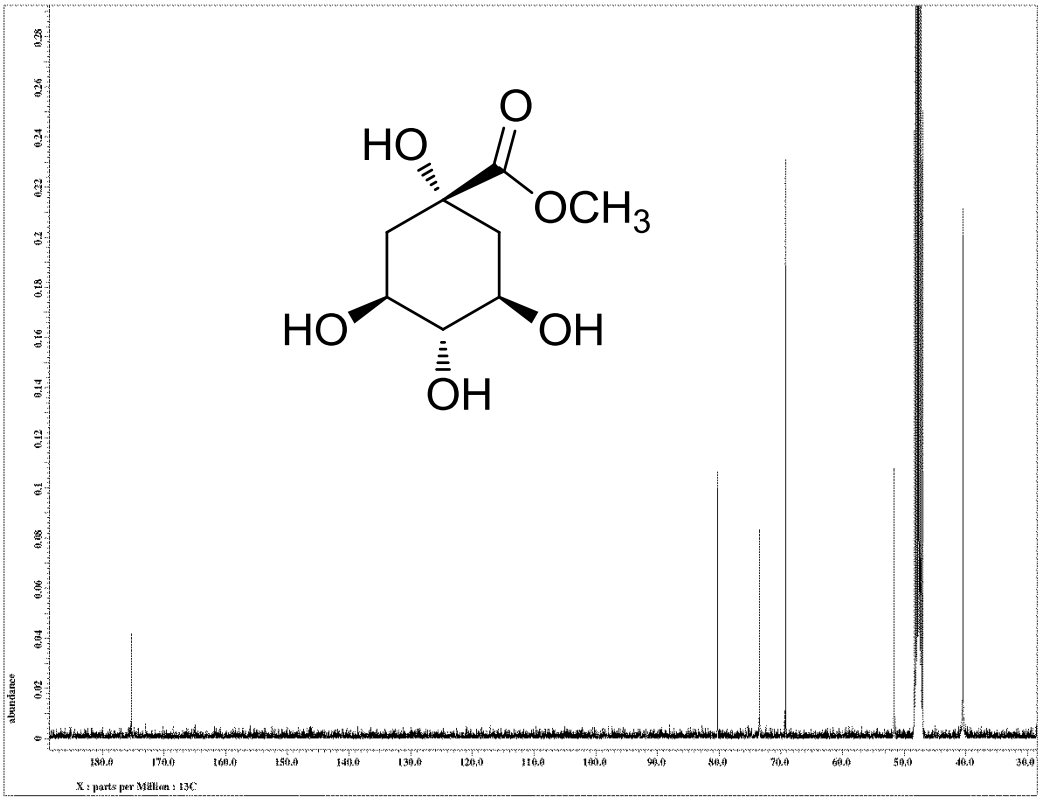

H3CO
HO
O
O
O
OH
HO
OH
OH
abundance
X : parts per Million : 1H

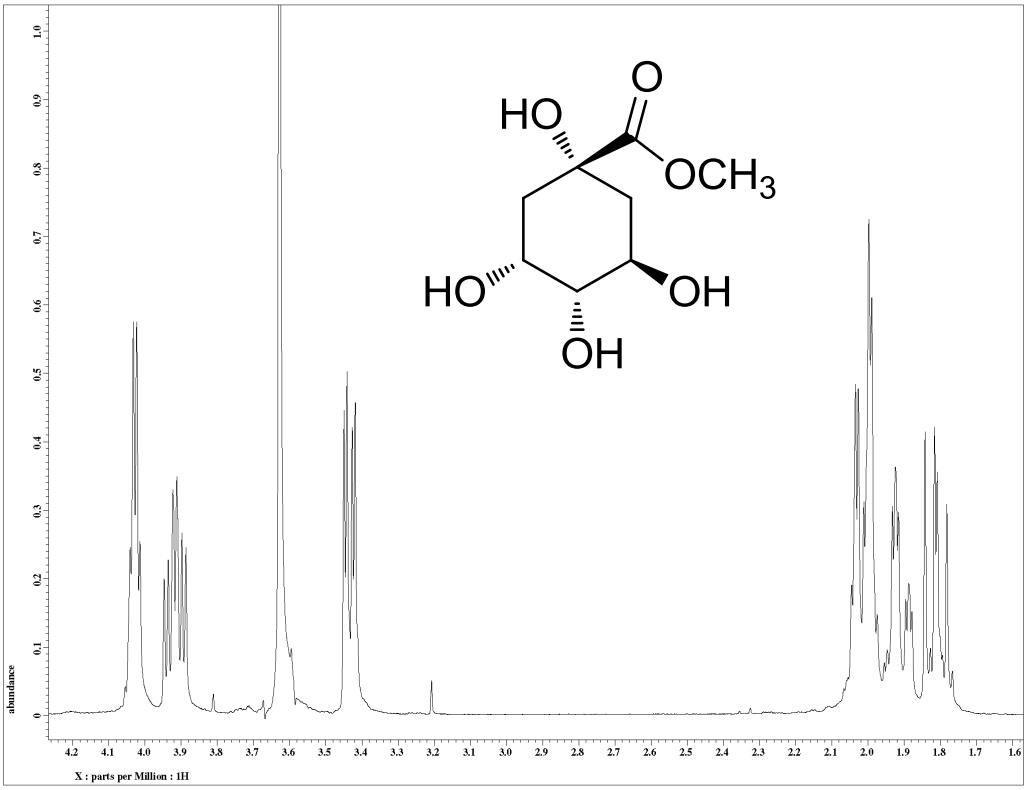

H3CO
HO
O
O
O
OH
HO
OH
OH
(thousandths)
X : parts per Million : 13C

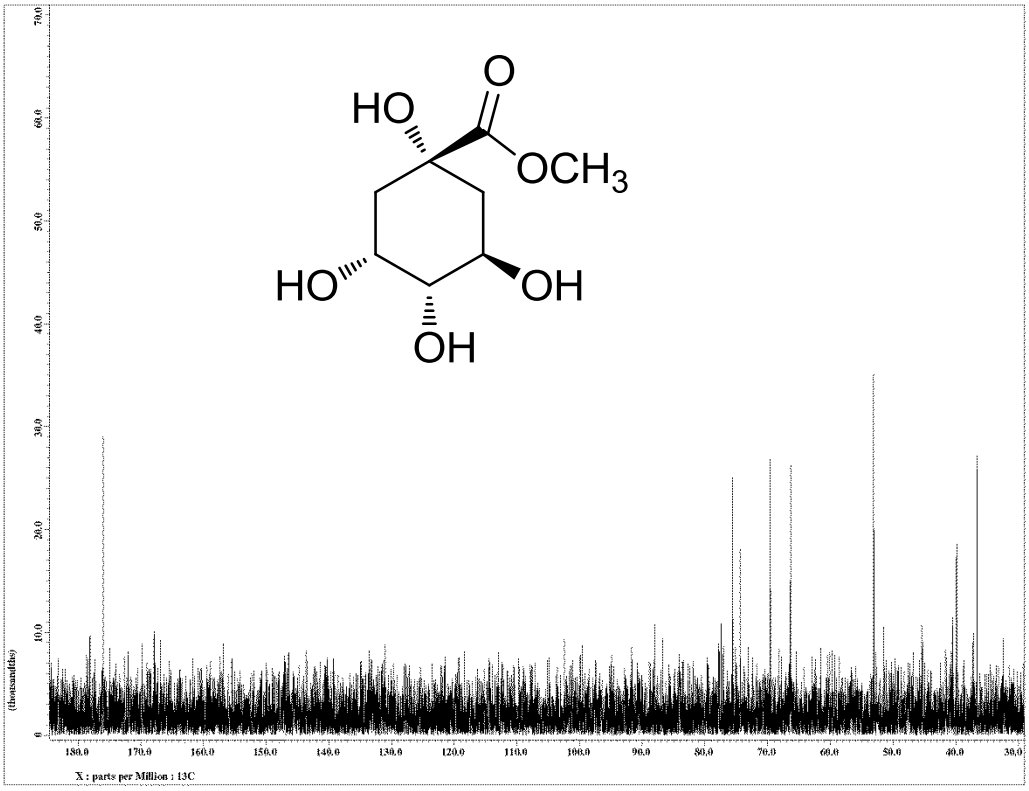

AllO
AllO
O
O
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 1H

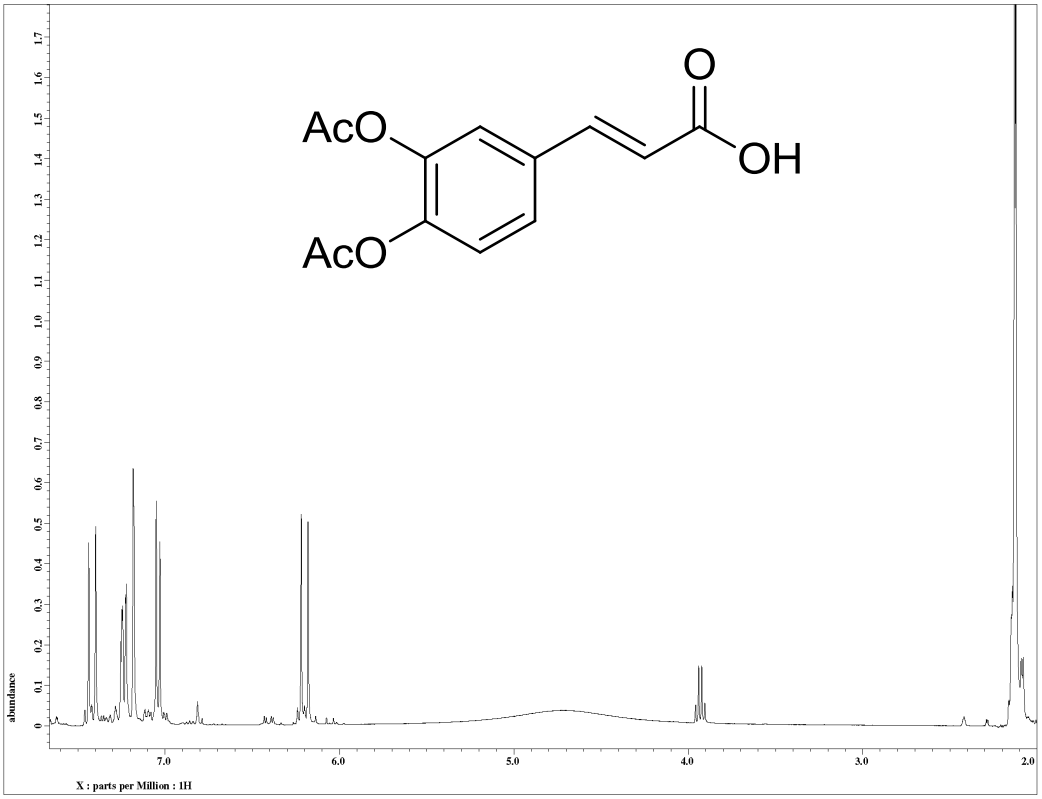

AllO
AllO
O
O
O
OH
HO
O
O
OCH3
CH3
H3C
OCH3
abundance
X : parts per Million : 13C

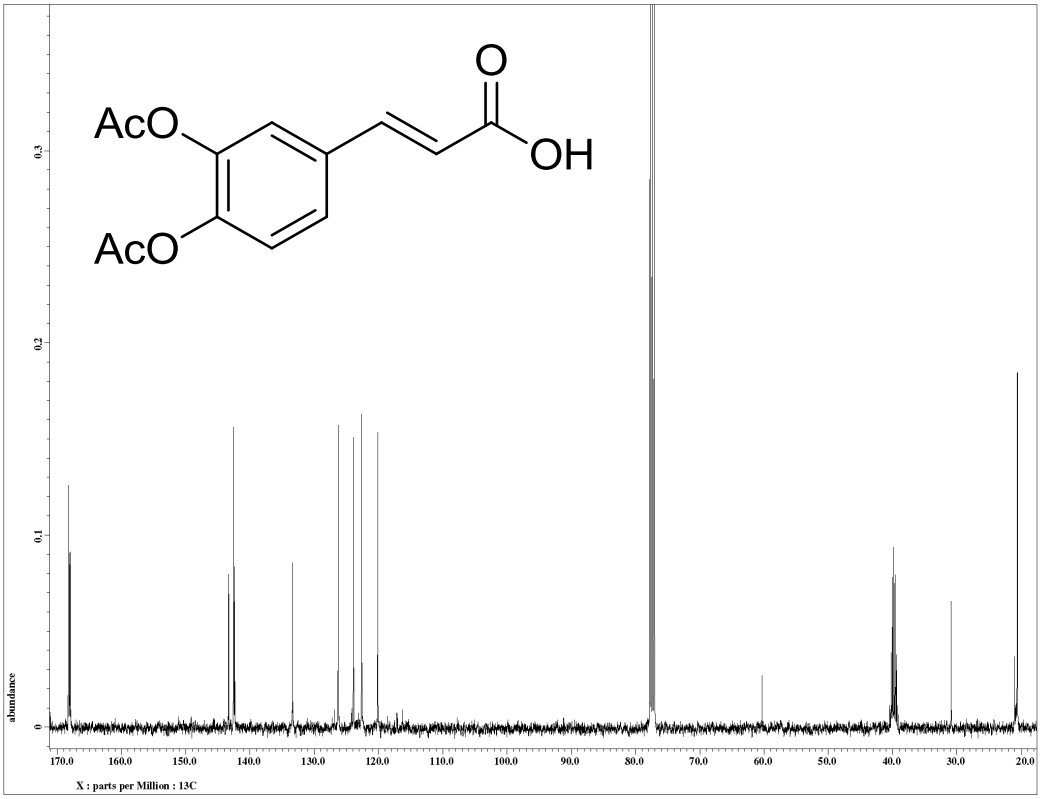

HO
O
OCH3
HO
OH
OH
abundance
X : parts per Million : 1H

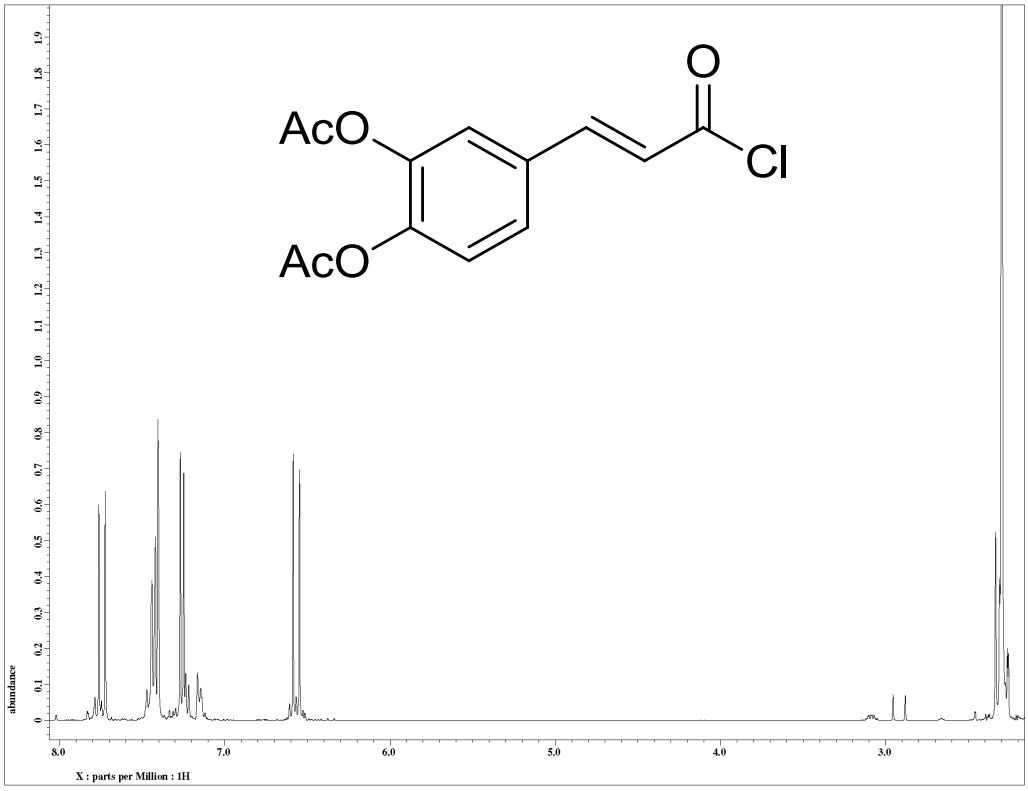

HO
O
OCH3
HO
OH
OH
abundance
X : parts per Million : 13C

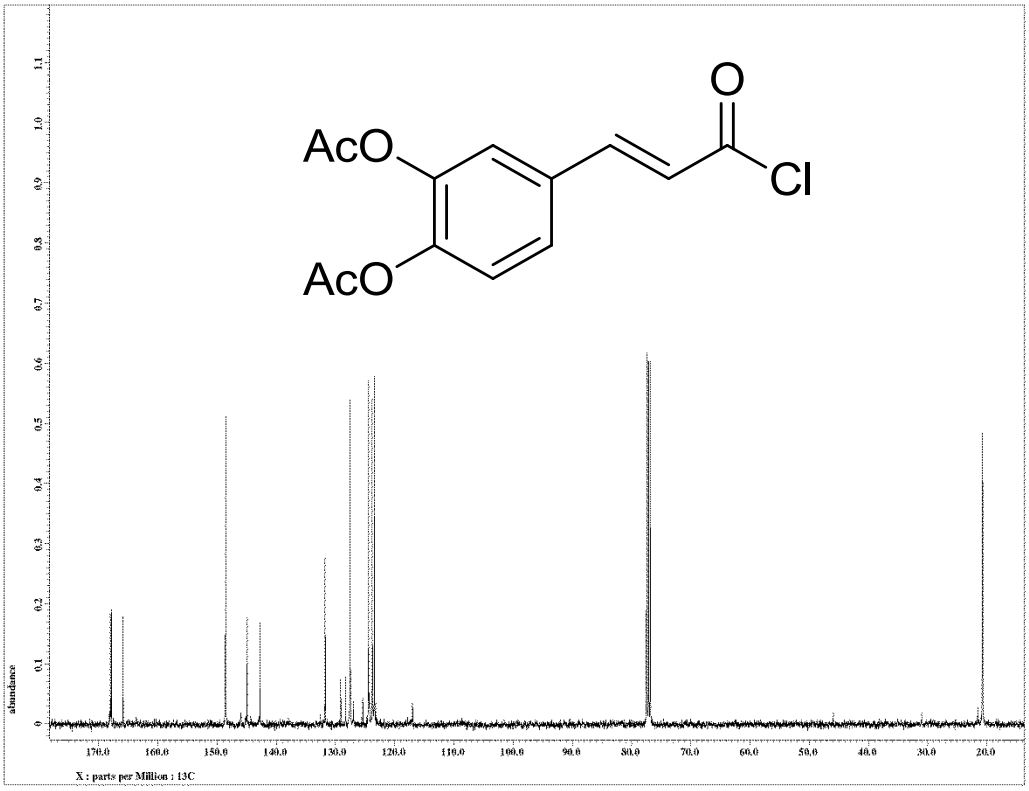

HO
O
OCH3
HO
OH
OH
abundance
X : parts per Million : 1H

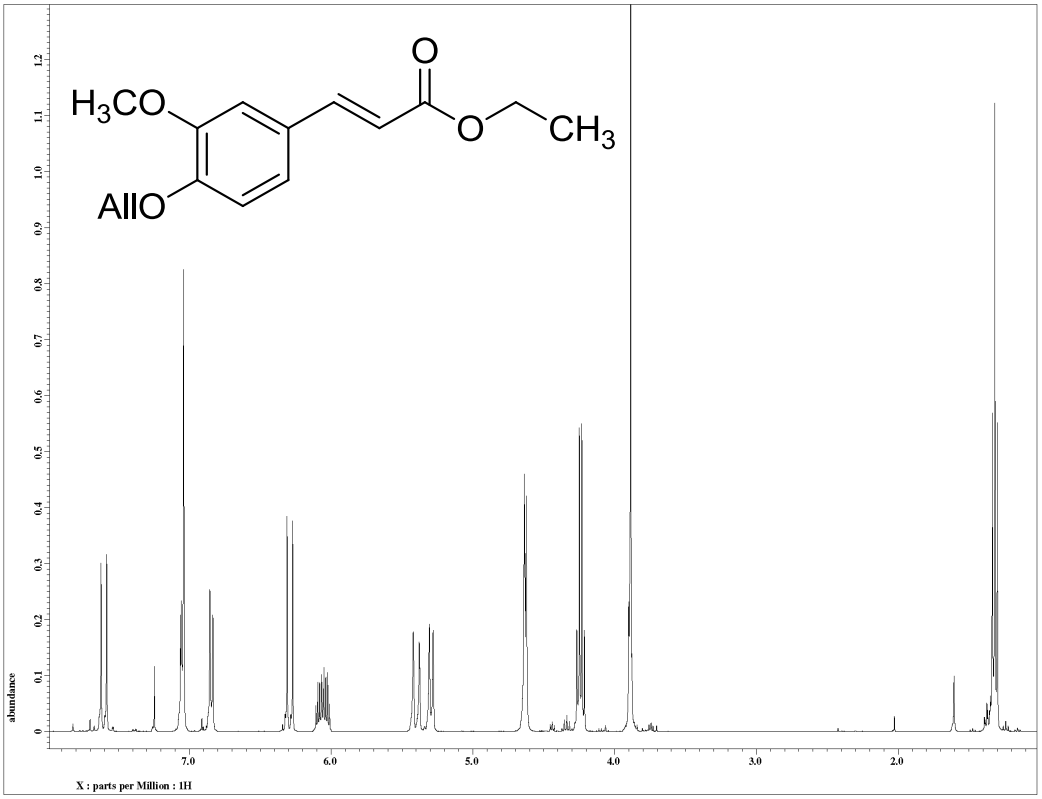

HO
O
OCH3
HO
OH
OH
(thousands)
X : parts per Million : 13C

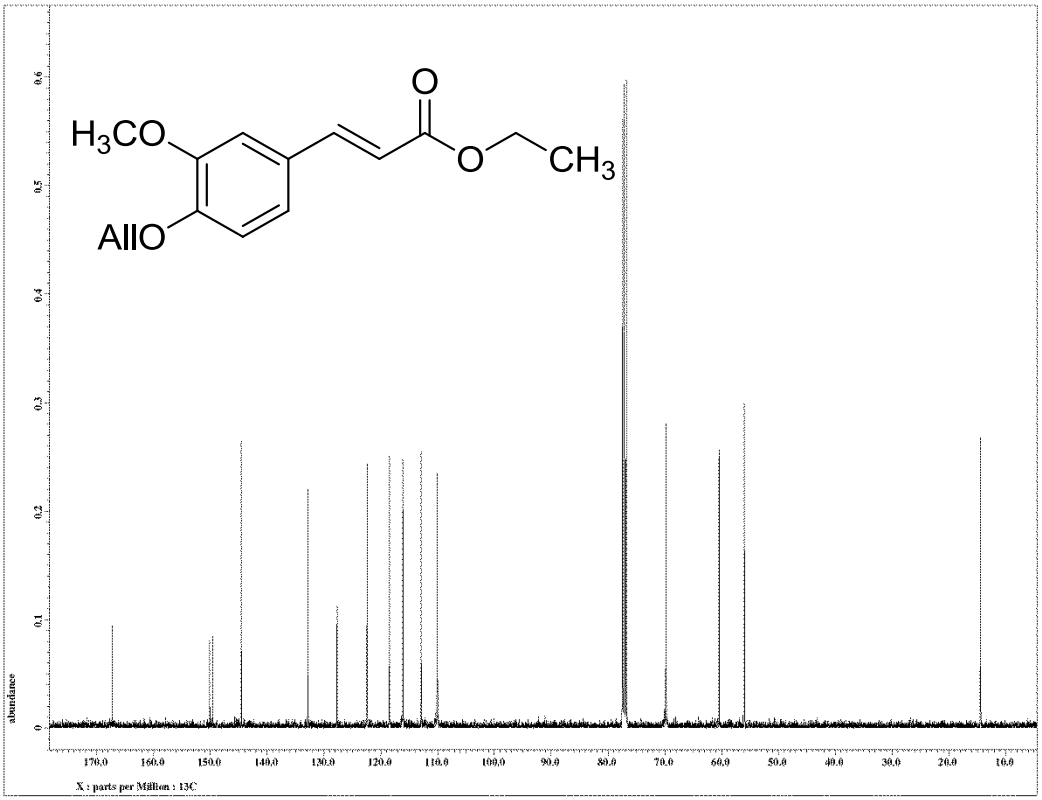

AcO
AcO
O
OH
abundance
X : parts per Million : 1H

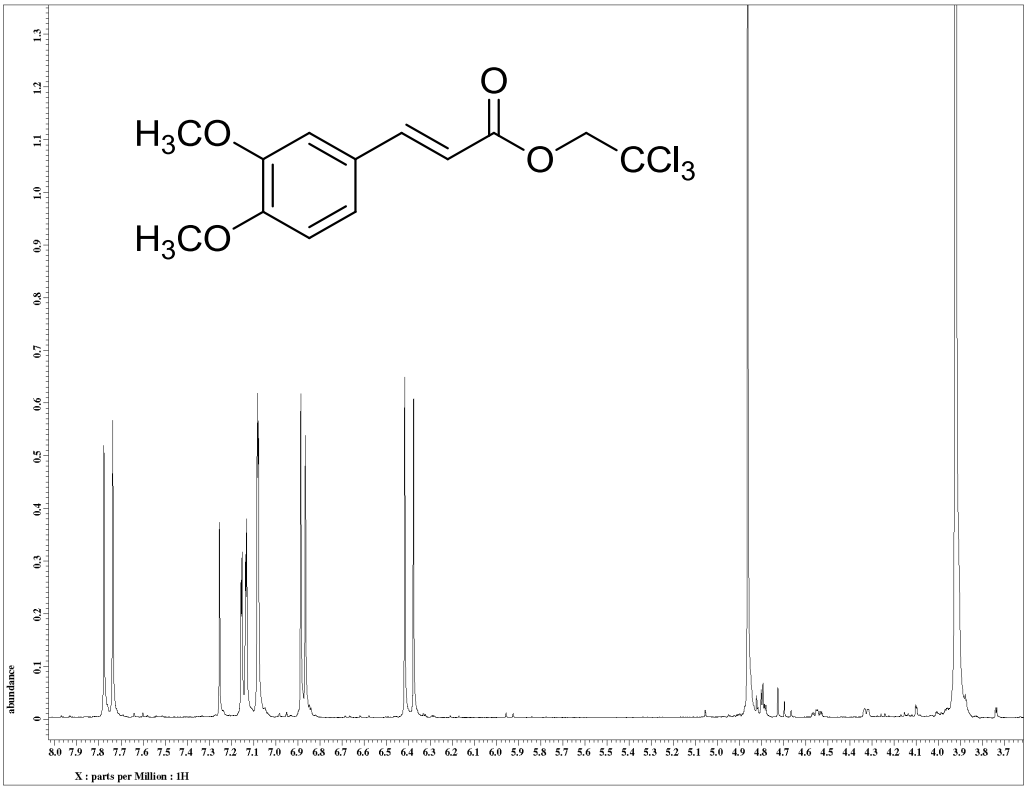

AcO
AcO
O
OH
abundance
X : parts per Million : 13C

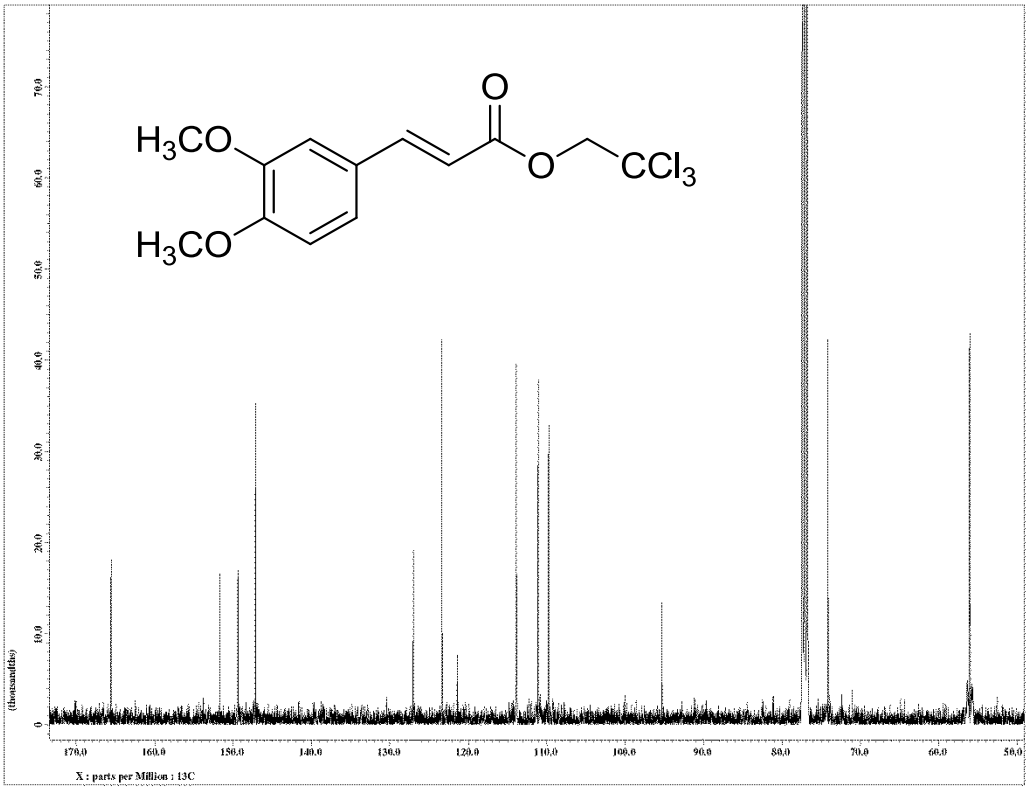

O
AcO
Cl
AcO
abundance
8.0
7.0
6.0
5.0
4.0
3.0
X : parts per Million : 1H

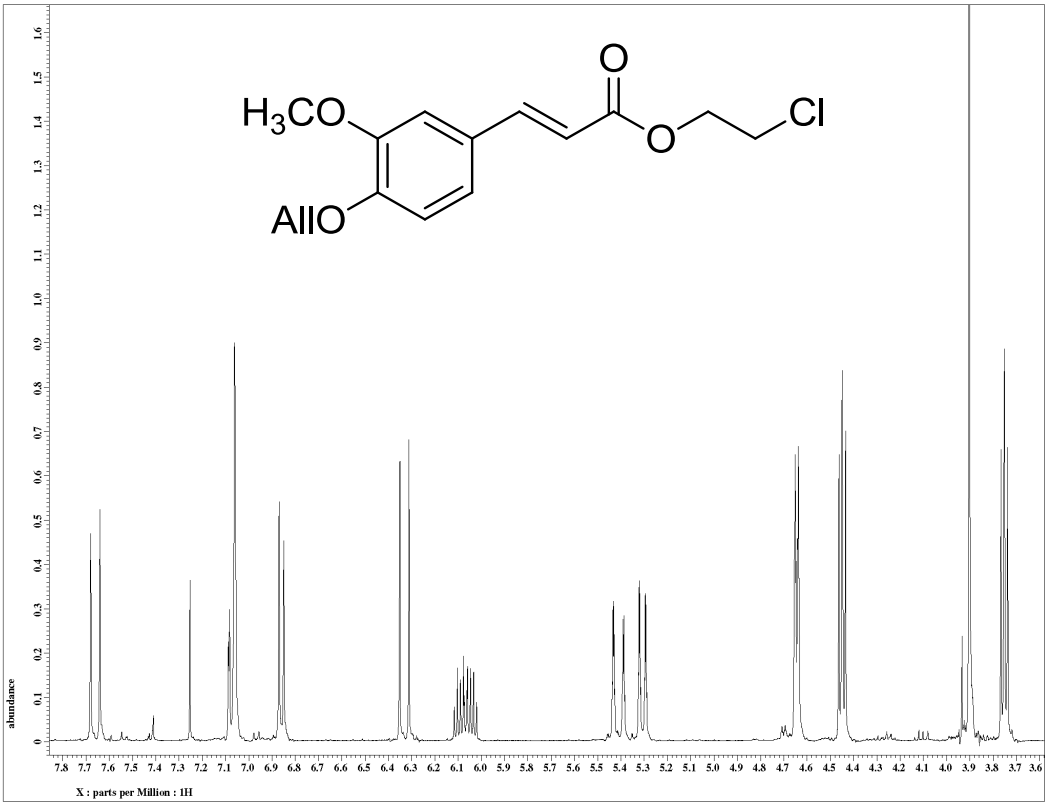

O
AcO
Cl
AcO
abundance
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
X : parts per Million : 13C


O
H3CO
O
CH3
AllO
abundance
7.0
6.0
5.0
4.0
3.0
2.0
X : parts per Million : 1H


O
H3CO
O
CH3
AllO
abundance
X : parts per Million : 13C


O
H3CO
H3CO
O
CCl3
abundance
X : parts per Million : 1H


O
H3CO
H3CO
O
CCl3
(thousands)
X : parts per Million : 13C


O
H3CO
Cl
O
AllO
abundance
X : parts per Million : 1H