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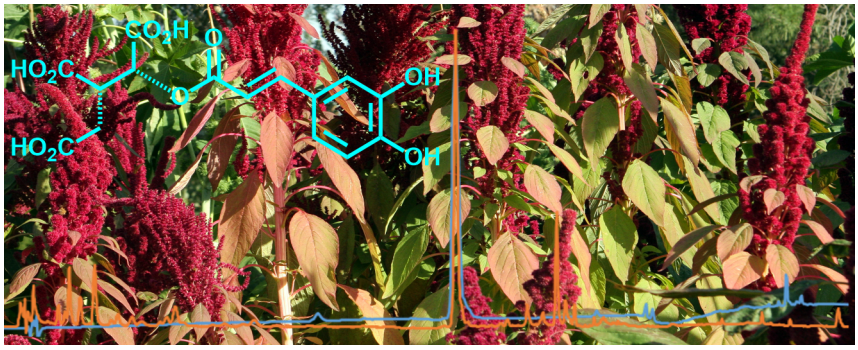

Isolation, Total Synthesis and Quantification of Caffeoylisocitric Acid, a Characteristic Ingredient of the Superfood Amaranth

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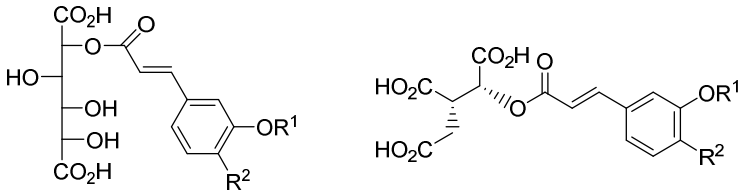
ABSTRACT

Amaranth is regarded as a new "super-vegetable" in western countries, albeit it is consumed for centuries in Africa and Asia. In addition to common carotenoids, flavonoids and polyphenols, caffeoylisocitric esters have been described as amaranth type-specific secondary metabolites. Remarkably, nothing is known on biological effects of this specific polyphenol. Here we detail a concise, diastereoselective synthesis of caffeoylisocitric acid, deuterium-labelling studies and a quantitative determination of the caffeoylisocitric acid content of three different amaranth types.

Keywords: Amaranth; polyphenols; caffeoylisocitric acid; isolation; total synthesis; natural product

1. Introduction

Polyphenols constitute a structurally highly diverse class of plant secondary metabolites, including the hydroxybenzoic acids, hydroxycinnamic acids, flavonoids, stilbenes and lignans as the major families [1]. Polyphenols are an important element of human nutrition ever since. Several beneficial effects of polyphenols such as anti-inflammatory activity, antibacterial or cancer protecting activities as well as neuroprotective effects have been described, though most of them are rapidly metabolized and excreted [2-6]. In particular, polyphenols and glucosides, i.e. their adducts with glucose in traditional fruits and vegetables have been studied intensively in the past [7]. However, in the course of globalization, several uncommon food plants have become quite popular in western countries. Those vegetables constitute a still buried treasure of new, largely uncharacterized phenolic secondary metabolites and other plant natural products with the potential for the development of new healthy plant-based foodstuffs. In addition to African nightshade (*Solanum scabrum*), cowpea (*Vigna unguiculata*) and others, amaranth (*Amaranthus*) is another "super-vegetable" with increasing importance [8]. Amaranth originates from middle- and south America where it is used as a pseudo-grain. In Asia and Africa, the leaves are used as a vegetable. Amaranth leaves provide a broad spectrum of secondary metabolites, such as carotenoids, flavonoids and polyphenols [9]. While esters of hydroxycinnamic acids with glucose and aldaric acids are common in all European traditional vegetables, esters with isocitric acid are characteristic for amaranth plants and the grass *Dactylis glomerate* [10,11].



R^1=H; R^2=OH: caffeoylglucaric acid
R^1=H; R^2=H: coumaroylglucaric acid
R^1=Me; R^2=OH: feruloylglucaric acid

R^1=H; R^2=OH: caffeoylisocitric acid (**1**)
R^1=H; R^2=H: coumaroylisocitric acid
R^1=Me; R^2=OH: feruloylisocitric acid

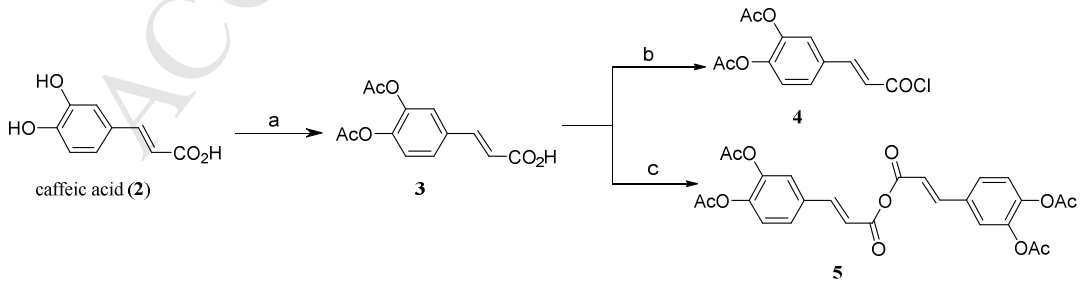
Fig. 1. Structures of *Amaranthus cruentus* caffeic acid esters.

An overview of the natural diversity of hydroxycinnamic acid derivatives in six different amaranth species has been published very recently by Neugart [10]. Although caffeoylisocitric acid **1** (Fig. 1) has been isolated and characterized already in 1987 as a major constituent of *Amaranthus cruentus* cotyledons, nothing is known on beneficial or adverse biological effects of this compound [12]. To date, neither a total synthesis nor a quantitative determination of caffeoylisocitric acid in amaranth leaves has been described.

2. Results and discussion

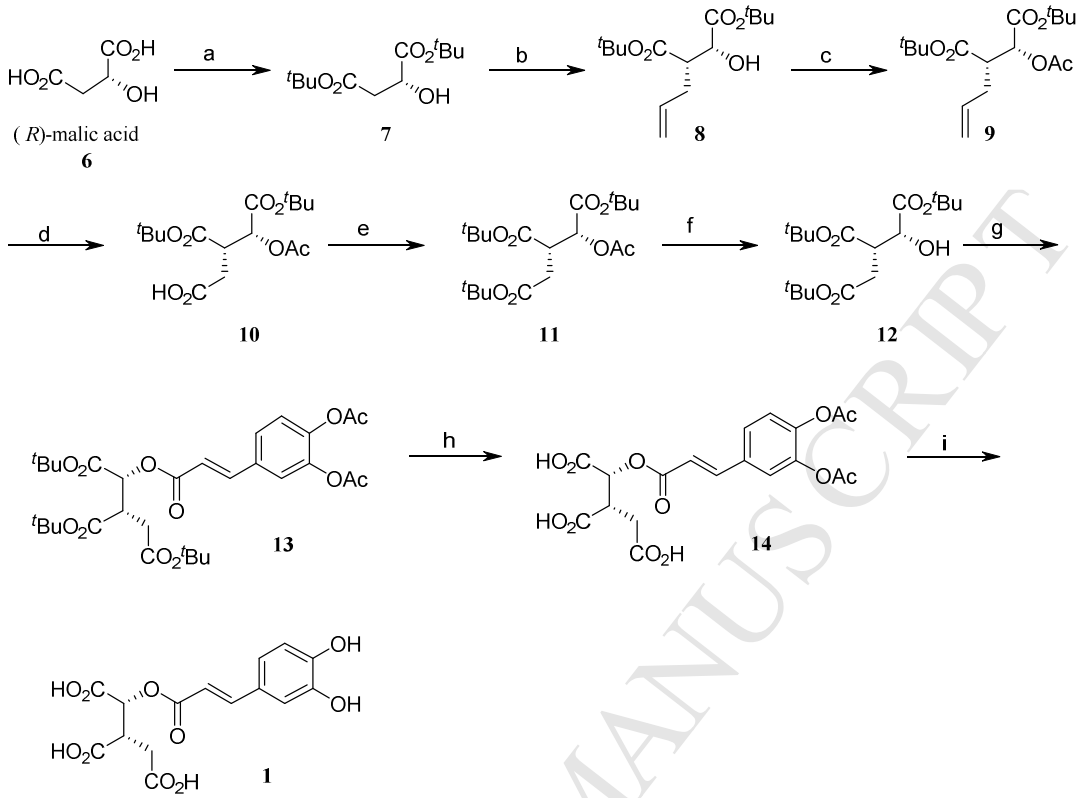
*2.1. Synthesis of caffeoylisocitric acid (**1**)*

Acetyl protected caffeic acid chloride **4** and anhydride **5** were prepared in good yields according to published literature methods [13-15].



Scheme 1. Synthesis of caffeic acid derivatives **4** and **5**. Reagents and conditions: a) Ac_2O, pyridine, DMAP, rt, overnight, 91%; b) $SOCl_2$, DMF, DCM, 60 °C, 4 hours, quantitative; c) Et_3N, triphosgene, EtOAc, 0 °C, 15 minutes, 81%.

Isocitric *tert*-butylester **12** was synthesized with some modifications according to a known procedure [16-18]. In particular, we found the oxidative cleavage of the allylic double bond in diester **9** with $RuCl_3/NaIO_4$ more straightforward compared to an ozonolysis. Acid **10** was obtained in an excellent yield of 96% as a single stereoisomer. Key-step of this chiral-pool synthesis is a highly diastereoselective Fráter-Seebach allylation of the ester enolate, generated with LiHMDS from malic ester **7**. The excellent diastereoselectivity is based on the predominant formation of the (*Z*)-ester enolate followed by addition of allyl bromide to the S*i*-face of the enolate [19,20]. NMR spectroscopic and specific rotation data of the allylation product **8** were identical with reported values [18].



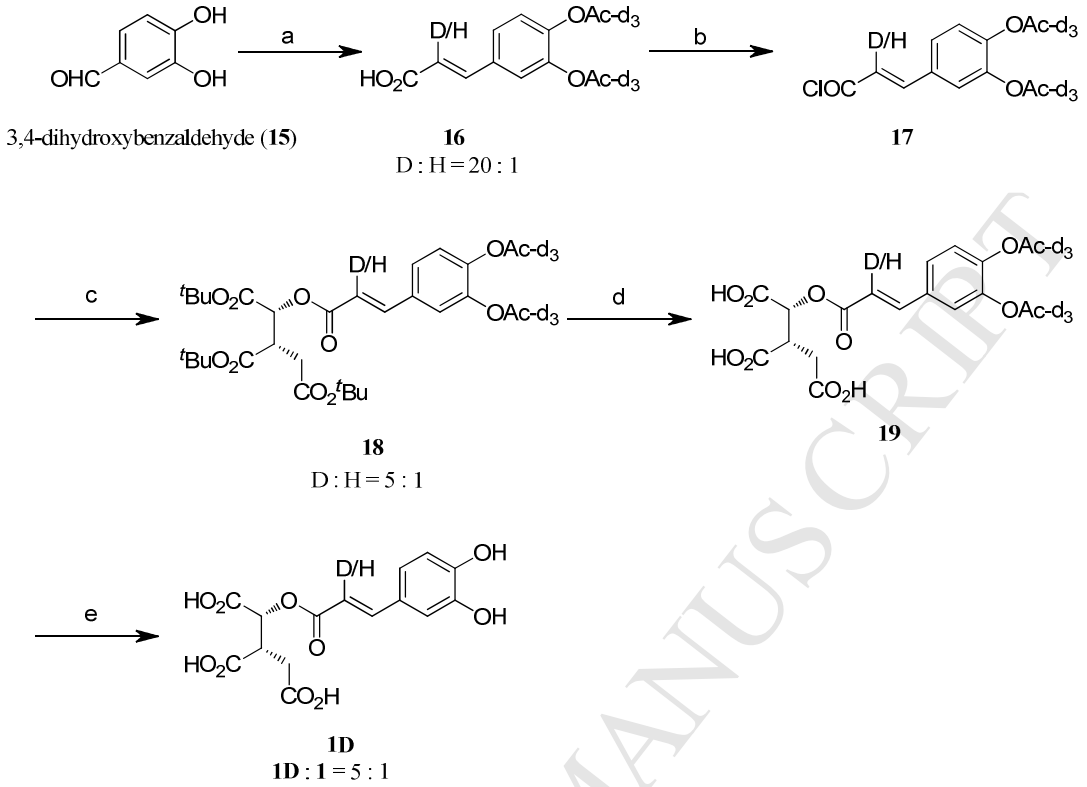
Scheme 2. Synthesis of caffeoylisocitric acid (**1**). Reagents and Conditions: a) *N,N'*-diisopropyl-*O-tert*–butylisourea, DCM, rt, 48 hours, 74%; b) LiHMDS, allyl bromide, THF, -78 °C 1 hour to 0 °C 1 hour, 82%; c) Ac_2O, pyridine, Et_3N, DMAP, THF, rt, 5 hours, 97%; d) $RuCl_3$, $NaIO_4$, MeCN:CCl_4:H_2O=5:5:8, rt, 10 hours, 96%; e) *N,N'*-diisopropyl-*O-tert*–butylisourea, DCM, reflux, 48 hours, 94%; f) K_2CO_3, MeOH, 0 °C, 1 hour, 84%; g) acid chloride **4**, pyridine, rt, 48 hours, 86%; h) TFA, DCM, rt, 48 hours, quantitative; i) 3 M HCl (aq.), acetone, reflux, 5 hours, 61%.

Tri-*tert*-butyl protected isocitric derivative **11** was obtained by esterification with a large excess of *N,N'*-diisopropyl-*O-tert*–butylisourea in 94% yield. Removal of the acetoxy protecting group with K_2CO_3 in methanol gave alcohol **12** (84%) required for coupling with a suitably protected caffeic acid derivative.

Direct coupling of isocitric tri-*tert*-butylester **12** and acetyl caffeic acid **3** with *N,N'*-diisopropylcarbodiimide and DMAP in DCM resulted only in low yields of 17%. Also, disappointingly low yields (21%) were observed in the coupling with the acid anhydride **5**, which was prepared in 81% yield by reacting acid **3** with triphosgene in the presence of Et_3N (**Scheme 1**). A very good yield (86%) of ester **13** was indeed obtained with the acid chloride **4** in pyridine. A two-step sequence, consisting of first cleaving the *tert*-butyl esters with TFA, followed by removal of the acetoxy groups with 3 M HCl finally afforded caffeoylisocitric acid (**1**) in 61% yield over the two deprotection steps. Based on all available analytical and spectroscopic data, the synthetic material was identical with the natural substance we isolated from amaranth leaves and literature reports [12].

*2.2. Synthesis of deuterium-labeled caffeoylisocitric acid (**1D**)*

For subsequent biochemical studies we were also interested in a deuterium-labelled derivative of caffeoylisocitric acid (**1D**). Since our synthesis of isocitric acid does not provide any chemical transformation which would allow an economic introduction of a deuterium label, we focused on the synthesis of caffeic acid. In principle, cinnamic acids can be easily prepared by a Perkin reaction of arylaldehydes and acetic anhydride [21]. 3,4-Dihydroxybenzaldehyde (**15**), the starting material for caffeic acid, was first protected as deuterated diacetate. Remarkably, undeuterated acetate groups were found to exchange significantly protons with the deuterium atom of the double bond, introduced in the Perkin condensation. By consequently using deuterated reagents and bases a labelling rate of 20:1 was achieved for caffeic acid (**16**), though with a low chemical yield. However, ester formation of caffeic acid chloride **17** and isocitric acid derivative **12** resulted in some loss (5:1) of the deuterium label, probably caused by the acidic work-up [21].



Scheme 3. Synthesis of deuterium-labelled (*E*)-caffeoylisocitric acid **1D**. Reagents and conditions: (a) Ac_2O-d_6, NaOAc-d_3, 140 °C, 48 hours, 11%; (b) $SOCl_2$, DMF, DCM, 60 °C, 4 hours, quantitative; (c) alcohol **12**, pyridine, rt, 48 hours, 14%; (d) TFA, DCM, rt, 48 hours, quantitative; (e) 3M HCl (aq.), acetone, reflux, 5 hours, 67%.

2.3. Isolation of caffeoylisocitric acid and quantitative determination in amaranth leaves

The content of (*E*)-caffeoylisocitric acid **1** in three amaranth species was determined by LC-UV. High concentrations of caffeoylisocitric acid were found in *A. lividus* L. (1.41 ± 0.20 g/100 g dry weight) and *A. tricolor* L. (red) (1.11 ± 0.04 g/100 g dry weight). Despite the same cultivation conditions, *A. tricolor* L. shows a strong deviation in the caffeoylisocitric acid content (0.0099 ± 0.0002 g/100 g dry weight), while concentrations of other UV active substances were comparable to *A. tricolor* L. (red) and *A. lividus* L. The caffeic acid content in amaranth (*A. cruentus*), cultivated in Kenya, was recently determined in a semi-quantitative approach [10]. Of the total phenolic compounds (0.92-1.42 g/100 g dry weight), caffeic acid derivatives make up the highest percentage with concentrations of 0.57 and 0.92 g/100 g dry weight [9].

3. Conclusion

Our studies have shown unequivocally the relevance of caffeoylisocitric acid as one of the major polyphenols in amaranth leaves. Our synthesis of labelled caffeoylisocitric acid provides the basis for more detailed investigations, which will address the question of beneficial or adverse biological effects and mode of action. Further studies will also include additional amaranth types to clarify the apparently strong variation of caffeoylisocitric acid contents in different amaranth species.

4. Experimental section

4.1. Abbreviations

Cyh, cyclohexane; DCM, dichloromethane; DIC, *N*,*N'*-diisopropylcarbodiimide; DMAP, 4-dimethylaminopyridine; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; DW, dry weight; Et_2O, diethyl ether; EtOAc, ethyl acetate; n-Hex, n-hexane; LiHMDS, lithium

bis(trimethylsilyl)amide; MeCN, acetonitrile; MeOH, methanol; RP, reversed-phase; sat., saturated; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMS, tetramethylsilane.

4.2. General experimental procedures

Optical rotations were measured on a Krüss P-8000T polarimeter. IR spectra were recorded on a Bruker ALPHA FTIR spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance III 600 and Bruker Avance 400 spectrometers operating at 600 and 400 MHz (^{1}H) respectively and 150 and 100 MHz (^{13}C) respectively. Accurate mass determinations were achieved with a Bruker micrOTOF mass spectrometer. The reactions were monitored by TLC carried out on Macherey Nagel silica gel plates (60F-254) or Merk silica gel 60 RP-18 F254 plates using UV light and aqueous solution of $KMnO_4$, K_2CO_3, NaOH and heat as the visualizing agents. HPLC analysis was conducted on a PerfectSil Target ODS-3 HD 5 μm 100×4.6 mm column using an Agilent 1100 instrument. Reagents and solvents were purchased from commercial sources and used without further purification, unless otherwise stated. Allyl bromide was redistilled over $CaCl_2$. TFA was redistilled over P_2O_5. DCM, THF were dried with MB-SPS-800 solvent purification system. Dry Et_2O was obtained by passing through an activated aluminium oxide column. DMF and pyridine were redistilled over CaH_2. Reactions were stirred magnetically under an argon atmosphere unless otherwise stated.

4.3. Plant material

The three amaranth species, *A. tricolor*, *A. tricolor red* and *A. lividus* were cultivated over an 8-week period during April and May 2017 in a greenhouse (Wermelskirchen, Germany). Leaves were harvested before flowering and stored till extraction at -80 °C.

4.4. Extraction and quantification of caffeoylisocitric acid from amaranth

The extraction was based on an optimized procedure, originally developed by Neugart [9]. 20 mg shred and freeze-dried amaranth leaves were extracted with 600 µL methanol/water (60/40, v/v) for 40 minutes under continuous shaking at room-temperature. The mixture was centrifuged afterwards for 10 min at 4 °C and 20,000 ×*g*. The extractions were repeated four times, each with 300 µL methanol/water (60/40, v/v). The solvent from the collected supernatants was completely evaporated under nitrogen. The residue was suspended in 1 mL methanol/water (5/95, v/v). The solution was analyzed by LC-UV at 295 nm for quantitative analysis and ESI(-)-MS/MS for structure elucidation. For quantification the reconstituted extract was mixed (1/1, v/v) with a solution of internal standard (2 µM chlorogenic acid in methanol/water (5/95, v/v)). Accuracy of the linear calibration curve (R^2 = 0.9989) was in a range of 88 % and 104 % over the whole calibration in a range between 1 µM and 30 µM. Quantification was carried out by external calibration in a range between 1 µM and 30 µM. LC-UV analysis was performed using Merck Hitachi liquid chromatography system (Interface D-7000, Diode Array Detector L-7455, Programmable Autosampler LaChrom, Pump L-7100 LaChrom; Hitachi High Technologies, Tokyo, Japan) with a column oven at 40 °C (IGLOO-CIL, ERC-GmbH, Riemerling, Germany) and a 3-line degasser (Jasco DG-2080-53, JASCO Corporation, Tokyo, Japan). Separation was performed on a RP-18 column of the dimensions 150 x 4.6 mm, a particle size of 5 µm and a pore size of 80 Å (C18 XDB Zorbax Eclipse, Agilent Technologies, Santa Clara, USA). The analytes and amaranth extracts (injection volume 10 µL) were separated by a binary gradient at a flow rate of 0.8 mL/min of water containing 5 % acetic acid as solvent A and pure MeCN as solvent B. The following linear gradient was used: 0.0 to 15.0 minutes 5 % B, 15.0 to 25.0 minutes linear to 20 % B, 25.0 to 35.0 minutes linear to 95 % B, 35.0 to 45.0 isocratic 95 % B, 45.0 to 45.1 minutes

returning to initial conditions of 5 % B, and reconditioning between 45.1 and 60.0 minutes. Analytes were detected at 295 nm. LC-MS measurements were performed with an Agilent 1200-LC system (Agilent Technologies, Santa Clara, USA) with an Autosampler (CTC-Pal, CTC Analytics, Zwingen, Swiss Confederation) and a AB Sciex API 3200 QqQ mass spectrometer (AB Sciex, Darmstadt, Germany). Separations were performed on an RP-18 column (100 x 2 mm, particle size 1.8 µm. EC102 Nucleodur C18 Gravity from Macherey-Nagel, Düren, Germany). The analytes and amaranth extracts (injection volume 10 µL) were separated by a binary gradient at a flow rate of 0.3 mL/min of water containing 5 % acetic acid as solvent A and pure MeCN as solvent B. The following gradient was used: 0.0 to 2.0 minutes 5 % B, 2.0 to 8.5 minutes linear to 100 % B, 8.5 to 9.5 minutes isocratic 100 % B, 9.5 to 9.7 minutes returning to initial conditions of 5 % B, and reconditioning between 9.7 and 11.0 minutes. Analytes were detected in ESI (-) ionization mode with a declustering potential of -15 V and a collision energy of -30 V. Product ion spectra were measured with an entrance *m/z* of 353.4 Da and a SCAN range between 100 and 360 Da. The further instrument settings were: Ion Spray voltage: -4500 V, Heater gas temperature: 500 °C, Ion source gas 1: 70, Ion source gas 2: 55, Curtain gas: 20.

4.5. Synthesis

*4.5.1. 3,4-Diacetoxycinnamic acid (**3**)*

Acetic anhydride (2.20 mL, 23.30 mmol, 2.1 eq.) was added dropwise to a solution of *trans*-caffeic acid **2** (2.00 g, 11.10 mmol, 1.0 eq) and DMAP (0.03 g, 0.25 mmol, 0.02 eq.) in pyridine (10.0 mL) over 10 minutes at 0 °C. The reaction mixture was stirred at rt overnight and was then poured onto cold water. The aqueous layer was extracted with EtOAc. The organic layer was washed with 1 M HCl (aq.) and water, dried over Na_2SO_4, filtered and concentrated in vacuo.

The crude product was recrystallized from hot water to obtain acid **3** as a white powder (2.67 g, 10.10 mmol, 91%). IR (ATR) ν_{max} 2551, 1753, 1675, 1629, 1500, 1432, 1375, 1184 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.45 (br. s, 1H), 7.66 (d, *J* = 1.9 Hz, 1H), 7.63 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.57 (d, *J* = 15.8 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 1H), 6.53 (d, *J* = 15.8 Hz, 1H), 2.29 (s, 3H), 2.28 (s, 3H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 168.1, 168.0, 167.3, 143.3, 142.3, 142.1, 133.1, 126.7, 124.0, 122.9, 120.3, 20.3, 20.3. EIMS *m/z* (%) 205.1 $[M\text{-}OAc]^+$ (61), 265.1 $[M+H]^+$ (55), 282.1 $[M+NH_4]^+$ (75), 287.1 $[M+Na]^+$ (27); HREIMS *m/z* 287.0523 $[M+Na]^+$ (calcd for $C_{13}H_{12}NaO_6^+$, 287.0532); R_f= 0.46 (DCM:MeOH=19:1, v/v).

*4.5.2. 3,4-Diacetoxycinnamoyl chloride (**4**)*

Thionyl chloride (1.90 mL, 26.10 mmol, 6.9 eq.) and DMF (2.90 μL) were added to the solution of acid **3** (1.00 g, 3.78 mmol, 1.0 eq.) in DCM (5.0 mL). After refluxing at 60 °C for 7 hours, the solvent and excess thionyl chloride were removed in vacuo. The residue was used directly without any further purification.

*4.5.3. 3,3,4,4'-Tetraacetoxycinnamic anhydride (**5**)*

Et_3N (0.79 mL, 5.68 mmol, 1.0 eq.) were added dropwise at 0 °C to a suspension of acid **3** (1.50 g, 5.68 mmol, 1.0 eq.) in $MgSO_4$-dried EtOAc (110.0 mL). After all solids have dissolved, triphosgene (0.29 g, 0.97 mmol, 0.2 eq.) was added in one portion. The reaction mixture was stirred at 0 °C for 10 minutes and then warmed up to rt over 15 minutes. The reaction mixture was filtered. The filtrate was concentrated in vacuo. The residue was recrystallized from toluene (18.0 mL) to give acid anhydride **5** as white powder (1.17 g, 2.29 mmol, 81%). IR (ATR) ν_{max} 1778, 1760, 1630, 1503, 1366 cm^{-1}; ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.93 (d, *J* = 15.8 Hz, 2H), 7.74-7.71 (m, 4H), 7.38 (d, *J* = 8.3 Hz, 2H), 6.74 (d, *J* = 16.2 Hz, 2H), 2.32 (d, *J* = 1.5 Hz,

12H); ^{13}C NMR (150 MHz, Acetone-d_6) δ 169.3, 169.2, 163.7, 148.1, 146.4, 144.7, 134.2, 128.7, 125.9, 125.2, 119.5, 21.2, 21.1; HREIMS *m/z* 533.1054 $[M+Na]^+$ (calcd for $C_{26}H_{22}NaO_{11}^+$, 533.1060); R_f=0.7 (Cyh:EtOAc=3:1, v/v).

*4.5.4. di-tert-Butyl-(R)-malate (**7**)*

N,*N'*-Diisopropyl-*O*-*tert*-butylisourea (30.00 g, 149.90 mmol, 6.7 eq.) was added to the suspension of (*R*)-malic acid (3.00 g, 22.40 mmol, 1.0 eq.) in DCM (150.0 mL). After stirring at rt for 48 hours, the mixture was filtered through a short pad of celite. The filtrate was concentrated in vacuo and dissolved in EtOAc (100.0 mL). The solution was washed with 1 M HCl (aq.), sat. $NaHCO_3$ (aq.), brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=9:1, v/v) to obtain ester **7** as colorless oil (4.06 g, 16.50 mmol, 74%). $[\alpha]_D^{25}$ +6.2 (c 0.87, $CHCl_3$); IR (ATR) ν_{max} 3500, 2979, 2935, 1727, 1367, 1252, 1144, 1101 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.29 (dd, *J* = 5.9, 4.5 Hz, 1H), 2.71 (dd, *J* = 16.3, 4.5 Hz, 1H), 2.63 (dd, *J* = 16.3, 5.9 Hz, 1H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.8, 169.8, 82.6, 81.3, 67.6, 39.9, 28.1, 28.0; EIMS *m/z* (%) 269.1 $[M+Na]^+$ (52), 515.3 $[2M+Na]^+$ (40); HREIMS *m/z* 269.1359 $[M+Na]^+$ (calcd for $C_{12}H_{22}NaO_5^+$, 269.1365); R_f= 0.51 (Cyh:EtOAc=4:1, v/v).

*4.5.5. di-tert-Butyl-(S)-2-allyl-(R)-3-hydroxy-succinate (**8**)*

1.1 M LiHMDS in THF (27.30 mL, 30.03 mmol, 2.1 eq.) was added dropwise to a solution of ester **7** (3.52 g, 14.30 mmol, 1.0 eq.) and allyl bromide (1.27 mL, 14.70 mmol, 1.0 eq.) in THF (50.0 mL) at -78 °C. The solution was stirred at -78 °C for 1 hour and for another hour at 0 °C. The reaction mixture was then poured onto 1 M HCl (aq.) (191.0 mL). The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4

and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=19:1, v/v) to afford alcohol **8** as colorless oil (3.34 g, 11.70 mmol, 82%). $[\alpha]_D^{25}$ -10.1 (c 0.99, DCM)[18]; IR (ATR) ν_{max} 3518, 2979, 1726, 1367, 1250, 1149 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.81 (dddd, *J* = 17.1, 10.2, 7.6, 6.3 Hz, 1H), 5.18-5.05 (m, 2H), 4.10 (dd, *J* = 7.6, 3.1 Hz, 1H), 3.18 (d, *J* = 7.6 Hz, 1H), 2.79 (ddd, *J* = 7.9, 7.1, 3.1 Hz, 1H), 2.60-2.51 (m, 1H), 2.43-2.33 (m, 1H), 1.49 (s, 9H), 1.43 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.7, 171.4, 135.3, 117.4, 82.6, 81.6, 70.6, 48.9, 32.7, 28.1, 28.0. EIMS *m/z* (%) 309.2 $[M+Na]^+$ (39), 575.4 $[2M+Na]^+$ (60); HREIMS *m/z* 309.1683 $[M+Na]^+$ (calcd for $C_{15}H_{26}NaO_5^+$, 309.1678); R_f= 0.48 (Cyh:EtOAc=9:1, v/v).

*4.5.6. di-tert-Butyl-(R)-2-acetoxy-(S)-3-allyl-succinate (**9**)*

Et_3N (1.75 mL, 12.57 mmol, 1.2 eq.), DMAP (0.15 g, 1.30 mmol, 0.1 eq.) and acetic anhydride (1.19 mL, 12.57 mmol, 1.2 eq.) were added to the solution of alcohol **8** (3.00 g, 10.50 mmol, 1.0 eq.) in THF (90.0 mL) at 0 °C. The reaction mixture was stirred at rt for 5 hours and then poured onto 1 M HCl (aq.) (105.0 mL). The organic layer was washed with sat. $NaHCO_3$ (aq.) and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=4:1, v/v) to afford ester **9** as colorless oil (3.35 g, 10.20 mmol, 97%). $[\alpha]_D^{25}$ +6.9 (c 0.80, Et_2O); IR (ATR) ν_{max} 2979, 2934, 1734, 1643, 1369, 1222, 1149, 1045, 845 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 5.76 (ddt, *J* = 17.0, 9.9, 6.9 Hz, 1H), 5.10-5.04 (m, 3H), 2.96 (m, 1H), 2.52-2.40 (m, 1H), 2.31-2.19 (m, 1H), 2.10 (s, 3H), 1.46 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.9, 167.5, 134.5, 117.6, 82.5, 81.2, 72.3, 47.0, 32.2, 28.0, 28.0, 20.5; EIMS *m/z* (%) 351.2 $[M+Na]^+$ (7), 679.4 $[2M+Na]^+$ (10); HREIMS *m/z* 351.1779 $[M+Na]^+$ (calcd for $C_{17}H_{28}NaO_6^+$, 351.1784); R_f= 0.65 (n-Hex:Et_2O=4:1, v/v).

*4.5.7. tert-Butyl-(R)-2-acetoxy-(S)-3-tert-butoxycarbonyl-pentanedioic acid (**10**)*

$RuCl_3 \cdot 2H_2O$ (11.50 mg, 0.05 mmol, 0.04 eq.) and $NaIO_4$ (1.12 g, 5.23 mmol, 4.1 eq.) were added to the solution of alkene **9** (0.42 g, 1.27 mmol, 1.0 eq.) in CCl_4: MeCN: H_2O (9.0 mL, 5:5:8, v/v/v). The reaction mixture was stirred at rt for 10 hours and was then diluted with DCM (10.0 mL). The organic layer was extracted with DCM (10.0 mL). The extract was washed with water, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=4:1, 0.5% AcOH, v/v) to afford acid **10** as a white solid (0.42 g, 1.22 mmol, 96%). $[\alpha]_D^{25}$ +35.1 (c 1.07, DCM); IR (ATR) ν_{max} 2980, 1732, 1369, 1220, 1148 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.20 (d, *J* = 3.4 Hz, 1H), 3.38 (dt, *J* = 9.8, 4.5, 3.5 Hz, 1H), 2.80 (dd, *J* = 17.1, 9.9 Hz, 1H), 2.54 (dd, *J* = 17.2, 4.6 Hz, 1H), 2.12 (s, 3H), 1.47 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 176.7, 169.9, 168.9, 166.5, 83.2, 82.4, 71.9, 43.5, 32.1, 28.0, 27.9, 20.5; EIMS *m/z* (%) 347.2 $[M+H]^+$ (10), 364.2 $[M+NH_4]^+$ (87), 369.2 $[M+Na]^+$ (33). HREIMS *m/z* 369.1520 $[M+Na]^+$ (calcd for $C_{16}H_{26}NaO_8^+$, 369.1525); R_f= 0.30 (Cyh:EtOAc=4:1, 1% AcOH, v/v).

*4.5.8. di-tert-Butyl-(R)-2-acetoxy-(S)-3-tert-butoxycarbonyl-pentanedioic ester (**11**)*

N,N'-diisopropyl-*O-tert*-butylisourea (1.68 g, 8.40 mmol, 10.0 eq) was added to a solution of acid **10** (0.29 g, 0.84 mmol, 1.0 eq.) in DCM (18.0 mL). The reaction mixture was refluxed for 48 hours and then filtered through a short pad of celite. The filtrate was concentrated in vacuo. The residue was purified by column chromatography (Cyh:EtOAC=4:1, v/v) to obtain ester **11** as colorless syrup (0.32 g, 0.78 mmol, 94%). $[\alpha]_D^{25}$ +15.6 (c 0.32, Et_2O); IR (ATR) ν_{max} 2979, 1730, 1368, 1222, 1144 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.15 (d, *J* = 3.5 Hz, 1H), 3.36 (ddd, *J* = 9.6, 5.1, 3.3 Hz, 1H), 2.67 (dd, *J* = 16.7, 9.6 Hz, 1H), 2.38 (dd, *J* = 16.8, 5.2 Hz, 1H), 2.11 (s,

3H), 1.49 (s, 9H), 1.48 (s, 9H), 1.47 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.9, 169.1, 166.7, 82.8, 81.9, 80.9, 72.1, 43.7, 33.7, 28.0, 28.0, 28.0, 20.5; EIMS *m/z* (%) 420.3 $[M+NH_4]^+$ (24); HREIMS *m/z* 425.2145 $[M+Na]^+$ (calcd for $C_{20}H_{34}NaO_8^+$, 425.2151); R_f= 0.74 (Cyh:EtOAc=4:1, v/v).

*4.5.9. di-tert-Butyl-(S)-3-tert-butoxycarbonyl-(R)-2-hydroxy-pentanedioate (**12**)*

K_2CO_3 (0.14 g, 1.04 mmol, 1.5 eq.) was added to the solution of ester **11** (0.28 g, 0.70 mmol, 1.0 eq.) in MeOH (6.0 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 hour and then poured onto sat. NH_4Cl solution (10.0 mL). The aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=9:1, v/v) to obtain alcohol **12** as colorless oil (0.21 g, 0.58 mmol, 84%). $[\alpha]_D^{25}$ +7.3 (c 0.67, DCM)[18]; IR (ATR) ν_{max} 3500, 2979, 1726, 1367, 1250, 1144 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (s, 1H), 3.27 (ddd, *J*=8.4, 6.2, 2.8 Hz, 1H), 3.14 (br. s, 1H), 2.72 (dd, *J*=16.7, 8.4 Hz, 1H), 2.48 (dd, *J*=16.8, 6.2 Hz, 1H), 1.49 (s, 9H), 1.45 (s, 9H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 171.0, 170.2, 83.1, 81.7, 80.8, 70.8, 45.8, 33.9, 28.1, 28.0; EIMS *m/z* (%) 383.1 $[M+Na]^+$ (27), 743.4 $[2M+Na]^+$ (84). HREIMS *m/z* 383.2040 $[M+Na]^+$ (calcd for $C_{18}H_{32}NaO_7^+$, 383.2046); R_f=0.77 (Cyh:EtOAc=3:1, v/v).

*4.5.10. 2-O-(3,4-Diacetoxycaffeoyl)-tri-tert-butyl isocitrate (**13**)*

Pyridine (1.3 mL) and acid chloride **4** (0.18 g, 0.62 mmol, 1.5 eq.) were added to a solution of alcohol **12** (0.15 g, 0.42 mmol, 1.0 eq.) and DMAP (7.60 mg, 0.06 mmol) in DCM (4.2 mL). After stirring at rt overnight, the reaction mixture was diluted with DCM, washed with 1 M HCl (aq.) and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by

column chromatography (Cyh:EtOAc=4:1, v/v) to obtain ester **13** as colorless oil (0.22 g, 0.36 mmol, 86%). $[\alpha]_D^{25}$ -6.3 (c 0.42, DCM); IR (ATR) ν_{max} 2978, 2928, 1723, 1639, 1504, 1367, 1201, 1141, 1109 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.66 (d, *J* = 16.0 Hz, 1H), 7.39 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.35 (d, *J* = 2.0 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 6.41 (d, *J* = 16.0 Hz, 1H), 5.27 (d, *J* = 3.4 Hz, 1H), 3.43 (ddd, *J* = 9.6, 5.0, 3.6 Hz, 1H), 2.73 (d, *J* = 16.7, 9.7 Hz, 1H), 2.44 (d, *J* = 16.7, 5.0 Hz, 1H), 2.30 (s, 3H), 2.29 (s, 3H), 1.48 (s, 9H), 1.47 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.7, 169.4, 168.1, 168.0, 166.8, 165.5, 144.2, 143.9, 142.6, 133.2, 126.6, 124.1, 123.0, 118.4, 83.1, 82.1, 81.1, 72.4, 44.0, 34.0, 28.2, 28.2, 20.8, 20.7; EIMS *m/z* (%) 624.3 $[M+NH_4]^+$ (100); HREIMS *m/z* 629.2568 $[M+Na]^+$ (calcd for $C_{31}H_{42}NaO_{12}^+$, 629.2574); R_f= 0.20 (Cyh:EtOAc=4:1, v/v).

*4.5.11. (E)-Caffeoylisocitric acid (**1**)*

Fresh distilled TFA (0.62 mL, 8.10 mmol, 23.8 eq.) was added slowly to the solution of ester **13** (0.21 g, 0.34 mmol, 1.0 eq.) in DCM (5.0 mL). The reaction mixture was stirred at rt for 48 hours. Then it was concentrated in vacuo to dryness, redissolved in acetone, reconcentrated and dried to afford a white powder (0.15 g, quantitative). This white powder (0.075 g, 0.17 mmol, 1.0 eq.) was dissolved in acetone (11.0 mL). 3 M HCl (aq.) (3.40 mL, 60.0 eq.) was then added. The reaction mixture was stirred at 75 °C for 5 h. After cooling down the reaction mixture was diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified chromatographically by a RP-C18 column (water: MeCN=19:1, 1% AcOH, v/v) to afford acid **1** as brown oil (0.037 g, 0.10 mmol, 61%). $[\alpha]_D^{25}$ 11.4 (c 0.46, DCM); IR (ATR) ν_{max} 2980, 1694, 1596, 1146 cm^{-1}. ^1H NMR (600 MHz, MeOD-d_4) δ 7.61 (d, *J* = 15.8 Hz, 1H), 7.07 (d, *J* = 1.8 Hz, 1H), 6.97 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 6.32 (d, *J* = 15.8 Hz, 1H), 5.47 (d, *J* = 3.36 Hz, 1H), 3.57 (s, 1 H), 2.82 (dd, *J* = 17.0, 9.0 Hz,

1H), 2.60 (dd, *J* = 17.0, 5.07 Hz, 1H); ^{13}C NMR (150 MHz, MeOD-d_4) δ 174.9, 173.8, 168.0, 149.8, 148.1, 146.8, 127.7, 123.3, 116.5, 115.3, 114.1, 73.1, 44.4, 33.4; EIMS *m/z* (%) 353.1 [M-H]$^-$ (33), 191.0 [M-caffeoyl]$^-$ (21), 173.0 [M-caffeoyl-H_2O]$^-$ (39), 111.0 [M-caffeoyl-2H_2O-CO_2]$^-$ (19); HREIMS *m/z* 353.0513 [M-H]$^-$ (calcd for $C_{15}H_{13}O_{10}^-$, 353.0509); R_f= 0.27 (RP, water:MeCN=19:1, 1% AcOH, v/v).

*4.5.12. 3,4-Diacetoxy-d_6-cinnamic acid-d_1 (**16**)*

3,4-Dihydroxybenzaldehyde (**15**) (0.35 g, 2.51 mmol, 1.0 eq.) and sodium acetate-d_6 (0.34 g, 3.97 mmol, 1.6 eq.) were refluxed in acetic anhydride-d_6 (2.5 mL, 25.0 mmol, 10.0 eq.) for 48 hours. The reaction mixture was then diluted with EtOAc and treated with a solution of Na_2CO_3 (0.10 g) in deuterium oxide (10.0 mL). The aqueous layer was acidified with a few drops of 12 M HCl (aq.) and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in vacuum. The residue was purified by column chromatography (Cyh: EtOAc=4:1, 1% AcOH, v/v) to obtain **16** as white powder (0.073 g, 0.27 mmol, 11%). IR (ATR) ν_{max} 2544, 1759, 1668, 1629, 1501 cm^{-1}; ^{1}H NMR (600 MHz, Acetone-d_6) δ 7.65 (s, 1H), 7.60 (m, 2H), 7.31 (d, *J*= 8.2 Hz, 1H), 6.53 (d, *J*= 16.0Hz, 0.05H); ^{13}C NMR (150 MHz, Acetone-d_6) δ 169.3, 169.2, 168.2, 145.6, 144.6, 144.3, 134.8, 127.9, 125.7, 124.5; EIMS *m/z* (%) 272.1 [M+H]$^+$ (24), 289.1 [M+NH_4]$^+$ (49), 294.1 [M+Na]$^+$ (40); HREIMS *m/z* 294.0965 [M+Na]$^+$ (calcd for $C_{13}H_5D_7NaO_6^+$, 294.0971); Rf= 0.26 (DCM:MeOH=24:1, v/v).

*4.5.13. 3,4-Diacetoxy-d_6-cinnamoyl-d_1 chloride (**17**)*

Thionyl chloride (0.13 mL, 1.74 mmol, 6.9 eq.) and DMF (1.0 μL) were added to the solution of acid **16** (0.069 g, 0.25 mmol, 1.0 eq.) in DCM (1.0 mL). After refluxing at 60 °C for 7 hours, the

solvent and excess thionyl chloride were removed in vacuo. The residue was used directly without any further purification.

*4.5.14. 2-O-(3,4-Diacetoxy-d_6-cinnamoyl-d_1)-tri-tert-butyl isocitrate (**18**)*

Pyridine (0.5 mL) and acid chloride **17** (0.073 g, 0.25 mmol, 1.5 eq.) were added to a solution of alcohol **12** (0.061 g, 0.17 mmol, 1.0 eq.) and DMAP (0.003 g, 0.03 mmol) in DCM (2.1 mL). After stirring at rt overnight, the reaction mixture was diluted with DCM, washed with 1 M HCl (aq.) and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (Cyh:EtOAc=4:1, v/v) to obtain ester **18** as colorless oil (0.015 mg, 0.02 mmol, 14%). IR (ATR) ν_{max} 2979, 2932, 1723, 1504, 1368, 1210, 1143 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.66 (s, 1H), 7.39 (dd, *J*= 8.4, 2.0 Hz, 1H), 7.35 (d, *J*= 2.0 Hz, 1H), 7.23 (d, *J*= 8.4 Hz, 1H), 6.41 (d, *J*= 16.0 Hz, 0.18H), 5.27 (d, *J*= 3.5 Hz, 1H), 3.44 (ddd, *J*= 9.8, 4.9, 3.4 Hz, 1H), 2.73 (dd, *J*= 16.7, 9.7 Hz, 1H), 2.44 (dd, *J*= 16.8, 5.0 Hz, 1H), 1.48 (s, 9H), 1.48 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.5, 169.2, 168.0, 167.9, 166.7, 165.3, 144.0, 143.7, 142.5, 133.1, 126.5, 123.9, 122.9, 118.2, 82.9, 81.9, 81.0, 72.3, 43.9, 33.8, 28.1, 28.0; EIMS *m/z* (%) 631.4 $[M+NH_4]^+$ (100); HRMS (ESI) *m/z* 636.3008 $[M+Na]^+$ (calcd for $C_{31}H_{35}D_7NaO_{12}^+$, 636.3013); Rf= 0.20 (Cyh:EtOAc=4:1, v/v).

*4.5.15. (E)-Caffeoylisocitric acid-d_1 (**1D**)*

Fresh distilled TFA (0.02 mL, 0.26 mmol, 6.5 eq.) was added slowly to the solution of ester **18** (0.022 g, 0.04 mmol, 1.0 eq.) in DCM (1.0 mL). The reaction mixture was stirred at rt for 48 hours. Then it was concentrated in vacuo to dryness, redissolved in acetone, reconcentrated and dried to afford a white powder. This white powder (0.016 g, 0.04 mmol, 1.0 eq.) was dissolved in acetone (1.5 mL). 3 M HCl (aq.) (0.5 mL, 1.5 mmol, 37.5 eq.) was then added. The reaction

mixture was stirred at 75 °C for 5 h. After cooling down the reaction mixture was diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified chromatographically by a RP-C18 column (water: MeCN=19:1, 1% AcOH, v/v) to obtain acid **1D** as brown oil (0.0084 g, 0.02 mmol, 67%). ^{1}H NMR (600 MHz, MeOD-d_4) δ 7.63 (s, 1H), 7.09 (d, *J*=1.9 Hz, 1H), 7.00 (dd, *J*=8.2, 2.0 Hz, 1H), 6.81 (d, *J*=8.1 Hz, 1H), 6.35 (d, *J*=15.8 Hz, 0.2H), 5.49-5.48 (m, 1H), 3.59-3.58 (m, 1H), 2.85 (dd, *J*=17.0, 9.0 Hz, 1H), 2.62 (dd, *J*=17.0, 4.9 Hz, 1H); ^{13}C NMR (150 MHz, MeOD-d_4) δ 175.0, 168.0, 149.8, 148.0, 146.8, 127.7, 123.3, 116.5, 115.3, 114.1, 73.7, 44.4, 33.4; EIMS *m/z* (%) 354.3 $[M-H]^-$ (3); HRMS (ESI) *m/z* 378.0542 $[M+Na]^+$ (calcd for $C_{15}H_{13}DNaO_{10}^+$, 378.0547); Rf= 0.27 (RP, water:MeCN=19:1, 1% AcOH, v/v).

Conflicts of interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at

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