Synthesis of the Naturally Occurring (–)-1,3,5-Tri-*O*-Caffeoylquinic Acid

Kay M. Brummond,* Jolie E. DeForrest

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260, USA
Fax +1(412)6248611; E-mail: kbrummon@pitt.edu

Received 11 February 2009

Abstract: Ribonuclease H (RNase H) is an essential component to the replication of human immunodeficiency virus (HIV), and only a few inhibitors of this enzyme are known. Millenia Hope Pharmaceuticals Inc. found that (–)-1,3,5-tri-*O*-caffeoylquinic acid is a potent RNase H inhibitor and antiviral agent. A facile route leading to this inhibitor from commercially available (–)-quinic acid is reported within.

Key words: reverse transcriptase, ribonuclease H, (–)-1,3,5-tri-*O*-caffeoylquinic acid, (–)-quinic acid

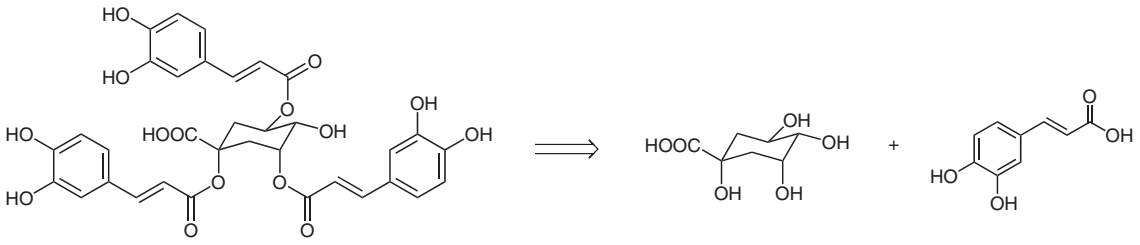
An estimated 33.2 million people worldwide are living with human immunodeficiency virus (HIV). In 2007, 2.5 million people were newly infected with HIV and 2.1 million deaths were attributed to acquired immune deficiency syndrome (AIDS). These epidemic numbers, coupled with studies of the most promising HIV vaccine candidate being halted in 2007, have led to an increased need for therapies that attack HIV via novel mechanisms of action.[1]

Replication of the HIV genome is carried out by HIV-1 reverse transcriptase (RT), an enzyme displaying both DNA polymerase and ribonuclease H (RNase H) activities. While RNase H is essential to viral function, only a few inhibitors have emerged and none are in clinical trials.[2,3]

Recently, Millenia Hope Pharmaceuticals Inc. isolated 1,3,5-tri-*O*-caffeoylquinic acid (**1**, Scheme 1) from cultured plant cells. They subsequently found **1** to be a potent RNase H inhibitor and antiviral agent (IC_{50} = 0.4 μM) using a cellular assay consisting of human peripheral blood mononuclear cells (PBMC) infected with HIV-1.[4] The naturally occurring polyphenol **1** was previously reported by Okuda and co-workers who isolated compound **1** from the fruit of *Xanthium strumarium* L., a fruit that has been used for medicinal purposes in both China and Japan.[5] The intriguing biological activity of 1,3,5-tri-*O*-caffeoylquinic acid, and access to suitable quantities of compound for further biological evaluations and mechanistic studies, motivated the synthesis of this compound. Furthermore, an understanding of the structure–activity relationship (SAR) required for RNase H inhibition and antiviral activity can be gained by analog synthesis.

An obvious synthetic strategy is to assemble 1,3,5-tri-*O*-caffeoylquinic acid (**1**) from (–)-quinic acid (**2**) and caffeic acid (**3**) via three selective esterification reactions, an approach that nature likely uses. However, the lability of the catechol moiety of caffeic acid, the unreactive tertiary alcohol of quinic acid, and the lack of site selectivity in the esterification reactions prohibited this direct approach. Alternatively, through a series of timed protection and deprotection steps it was envisioned that compound **1** can be obtained from commercially available 3,4-(methylenedioxy)cinnamic acid (**3b**) and quinic acid protected as the methyl ester and isopropylidene acetal **4** (Scheme 2).

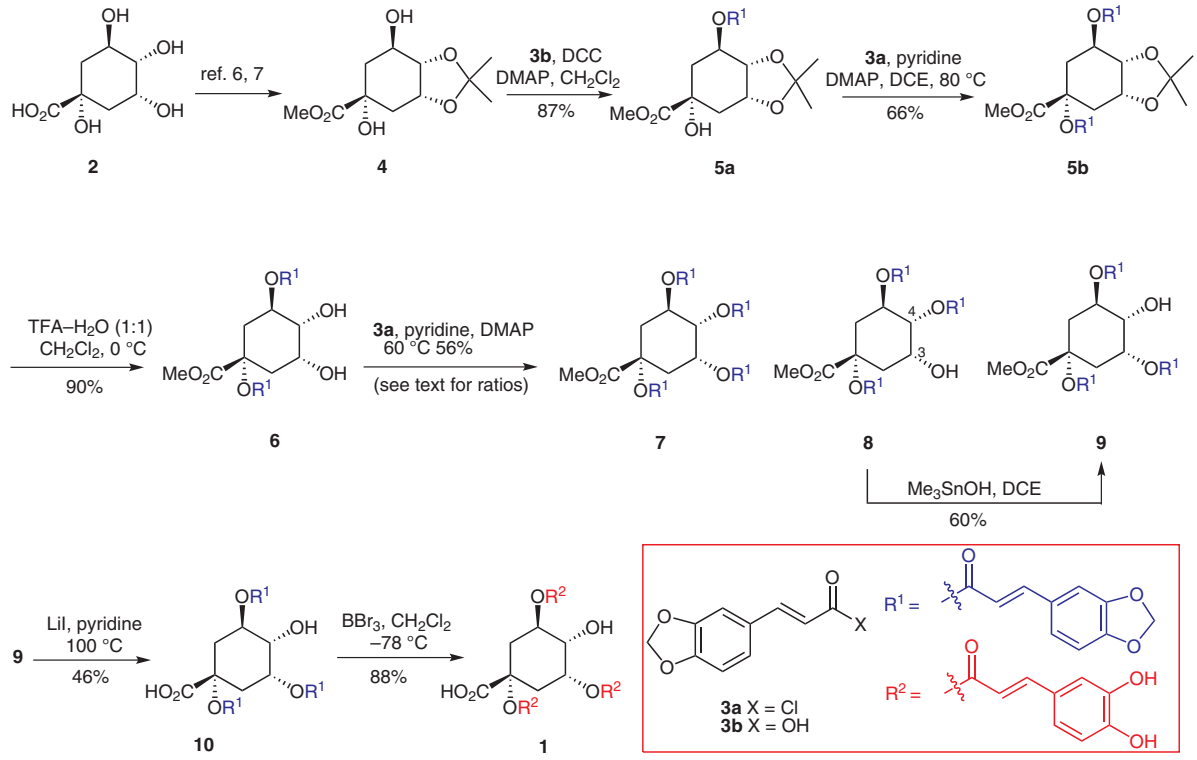
Our synthesis of **1** begins with the formation of diol **4** from (–)-quinic acid (**2**) in 50% yield for two steps using a previously developed protocol.[6,7] Diol **4** is then transformed into the monocaffeoylated compound with 3,4-(methylenedioxy)cinnamic acid (**3b**), *N*,*N*′-dicyclohexylcarbodiimide (DCC), and 4-dimethylaminopyridine (DMAP) at room temperature in 87% yield (Scheme 2). Next, the tertiary alcohol of **5a** was reacted with acid chloride **3a** in refluxing 1,2-dichloroethane (DCE) at 80 °C, and after 48 hours gave a 66% yield of the biscaffeoylated



(–)-1,3,5-tri-*O*-caffeoylquinic acid (**1**) (–)-quinic acid (**2**) caffeic acid (**3**)

Scheme 1 1,3,5-Tri-*O*-caffeoylquinic acid (**1**) from (–)-quinic and caffeic acids (**2**) and (**3**)

SYNLETT 2009, No. 9, pp 1517–1519
Advanced online publication: 18.05.2009
DOI: 10.1055/s-0029-1217183; Art ID: S01809ST



Scheme 2 Synthesis of 1,3,5-tri-*O*-caffeoylquinic acid (**1**) from (–)-quinic acid (**2**)

compound **5b** (79% based upon recovered starting material). Attempts to effect the esterification of the **5a** at 40 °C gave the biscaffeoylated compound **5b** in 11% yield and extending the reaction time to one week at 80 °C resulted in complete consumption of starting material, but gave only a 7% yield of **5b**. Unfortunately, attempts to convert diol **4** directly into compound **5b** were unsuccessful due to the forcing conditions required for esterification of the tertiary alcohol (DCC, DMAP, CH_2Cl_2, 25–40 °C).

Removal of the acetonide protecting group of **5** is accomplished in 90% yield with TFA–H_2O (1:1) at 0 °C to give diol **6** (Scheme 2). Diol **6** is then reacted with acid chloride **3a**, DMAP, and pyridine at 60 °C to give a mixture of esters **7**, **8**, and **9** in 9%, 27%, and 20% yield, respectively. After some experimentation it was found that the compounds **7**, **8**, and **9** could be separated using a Biotage Horizon Flash Collector eluting with an ethyl acetate–toluene solvent system (2–15%). This esterification reaction can also be performed using DCC-mediated coupling conditions; however, desired triester **9** was difficult to separate from the urea byproduct produced during the reaction.

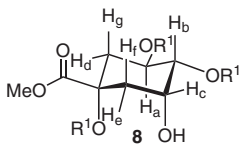
Interestingly, it was found that reacting the undesired 1,4,5-tricaffeoylated isomer **8** with Me_3SnOH in refluxing 1,2-dichloroethane (DCE) resulted in a transposition of the ester from C4 to C3, giving the desired 1,3,5-tricaffeoylated isomer **9** in 60% yield (Scheme 2).[8] This transposition is evidenced in the ^1H NMR by the change in chemical shift of the proton on C4 from δ = 5.21 ppm to δ = 3.93 ppm (Table 1). Importantly, the preferred conformation for the esters **8** and **9** are the ones depicted in Table 1 based upon the respective coupling constants for the C4 protons (H_b in ester **8**: dd, J = 10.0, 3.5 Hz; H_b in ester **9**: ddd, J = 10.0, 7.0, 3.3 Hz, Table 1). The driving force for this tin-mediated transesterification reaction is not clear; however, intramolecular ester migration has been shown to be a facile process for compounds containing adjacent hydroxyl and ester functionalities.[9a–9d]

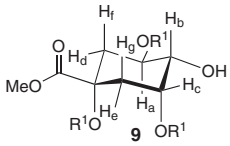
With the desired tricaffeoyl ester **9** in hand, removal of the catechol protecting groups and saponification of the methyl ester was accomplished by first refluxing **9** in pyridine with excess LiI to give the analogous acid **10** in 46% yield, which was purified using silica gel chromatography.[10a–c] The pure carboxylic acid was then reacted with excess BBr_3 at –78 °C to give (–)-1,3,5-tri-*O*-caffeoylquinic acid (**1**) in 88% yield.[11a] A workup procedure utilizing saturated aqueous sodium phosphate was essential, because all other methods gave ester cleavage products.[11b] This compound was 95% pure by ^1H NMR. Attempts to remove the methylene acetal protecting groups first and then to saponify the methyl ester to the acid gave very low yields of the natural product, which is attributed to the instability of the liberated catechol moieties.

In summary, we have developed a facile route leading to 1,3,5-tri-*O*-caffeoylquinic acid (**1**) from commercially available quinic acid (**2**). The convergent synthetic strategy developed is readily amenable to the syntheses of a variety of biologically interesting analogs which may offer mechanistic insight into the unique activity exhibited by (–)-1,3,5-tri-*O*-caffeoylquinic acid. The biological activities of the synthetic caffeoylquinic acid (**1**) has been evaluated and is in agreement with that reported by Millenia

Hope Pharmaceuticals Inc. In addition, the methyl ester of **1** showed comparable Rnase H inhibition. These results will be published elsewhere.

Table 1 Conformation and *J* Values of Tricaffeoyl Esters **8** and **9**





		Chemical shift (δ, ppm)	Spin multiplicity	*J* Values (Hz)
8	H_a	5.76	ddd	10.0, 10.0, 4.0
8	H_b	5.21	dd	10.0, 3.5
8	H_c	4.51–4.48	m	–
8	H_d	2.81–2.73	m	–
8	H_e	2.81–2.73	m	–
8	H_f	2.44	dd	15.5, 3.5
8	H_g	2.12	dd	13.5, 10.0
9	H_a	5.64	ddd	11.0, 10.0, 4.0
9	H_b	3.93	ddd	10.0, 7.0, 3.3
9	H_c	5.54	q	3.3
9	H_d	3.02	dt	16.0, 3.3
9	H_e	2.74	ddd	13.5, 4.0, 3.3
9	H_f	2.36	dd	16.0, 3.3
9	H_g	2.01	dd	13.5, 11.0

All ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 300, 500, 600, or 900 MHz instrument, with chemical shifts (δ) reported relative to the respective solvent peak $CDCl_3$ (7.27 ppm) or MeOD (3.31 ppm). All NMR spectra were acquired at r.t. unless otherwise stated. The abbreviations used to describe spin multiplicity for all ^{1}H NMR spectra are as follows: s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet, dd = doublet of doublet, dt = doublet of triplet, ddd = doublet of doublet of doublet, etc. All infrared spectra were obtained from a Nicolet Avatar E.S.P 360 FT-IR spectrometer. Mass spectra were obtained on a high-resolution mass spectrometer using electron impact or electrospray ionization. Optical rotations were measured with a 241 Perkin-Elmer polarimeter.

1,3,5-Tri-*O*-caffeoylquinic Acid (1)

A 5 mL, single-necked, round-bottomed flask equipped with a stir bar, rubber septum, and nitrogen line is charged with methylene acetal **10** (6 mg, 8 μmol) and CH_2Cl_2 (0.1 mL). The reaction flask is cooled to –78 °C in a dry ice-acetone bath and BBr_3 (0.12 mL, 1.0 M in CH_2Cl_2, 0.12 mmol) is added dropwise via syringe. Upon addition of BBr_3, the reaction turns orange from colorless. After 2 h of stirring, the reaction mixture is transferred to a separatory funnel containing EtOAc and sat. aq Na_2HPO_4. The aqueous layer is separated from the organic layer and is extracted with EtOAc (4×). The combined organic layers are washed with H_2O (3×), brine (1×), and are dried on $MgSO_4$. The solids are filtered off using gravity filtration, and the filtrate is concentrated under reduced pressure by rotary evaporation to give 5 mg of natural product **1** in 88% crude yield as a foamy brown solid; $[\alpha]_D^{23}$ –19.0 (*c* 0.10, MeOH). ^{1}H NMR (600 MHz, MeOD): δ = 7.64 (d, *J* = 16.2 Hz, 1 H), 7.56 (d, *J* = 15.6 Hz, 1 H), 7.52 (d, *J* = 16.2 Hz, 1 H), 7.08 (d, *J* = 1.8 Hz, 1 H), 6.98 (dd, *J* = 8.4, 1.8 Hz, 1 H), 6.97 (d, *J* = 2.4 Hz, 1 H), 6.85 (d, *J* = 1.8 Hz, 1 H), 6.79 (d, *J* = 8.4 Hz, 1 H), 6.79 (dd, *J* = 8.4, 1.8 Hz, 1 H), 6.65 (d, *J* = 8.4 Hz, 1 H), 6.65 (dd, *J* = 8.4, 2.4 Hz, 1 H), 6.55 (d, *J* = 8.4 Hz, 1 H), 6.34 (d, *J* = 15.6 Hz, 1 H), 6.24 (d, *J* = 16.2 Hz, 1 H), 6.19 (d, *J* = 15.8 Hz, 1 H), 5.58 (td, *J* = 10.2, 4.2 Hz, 1 H), 5.46 (q, *J* = 3.3 Hz, 1 H), 3.99 (dd, *J* = 10.2, 3.3 Hz, 1 H), 2.91 (dt, *J* = 15.9, 3.3 Hz, 1 H), 2.67 (ddd, *J* = 13.2, 4.2, 3.3 Hz, 1 H), 2.42 (dd, *J* = 15.9, 3.3 Hz, 1 H), 2.01 (dd, *J* = 13.2, 10.2 Hz, 1 H). ^{13}C NMR (225 MHz, MeOD): δ = 174.1, 168.8 (2 C), 167.8, 149.7 (2 C), 149.4, 148.0, 147.4 (2 C), 146.9 (2 C), 146.5, 127.8, 127.5 (2 C), 123.1 (2 C), 122.2, 116.6 (3 C), 116.1, 115.5, 115.3 (2 C), 115.1, 115.0, 80.7, 72.9, 72.2, 71.2, 38.3, 32.9. IR (thin film): 3279, 2923, 1733, 1699, 1603, 1260 cm^{-1}. MS *m/z* (%) = 701 (31), 633 (41), 527 (42), 365 (100). ESI$^+$-HRMS: *m/z* calcd for $C_{34}H_{30}O_{15}Na$ [M + Na]$^+$: 701.1482; found: 701.1526.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

Acknowledgment

We gratefully acknowledge Dr. Michael Parniak and National Institutes of Health (NIH) grant U19073975 for support of this work and Dr. Rieko Ishima for help with NMR experiments.

References

(1) http://www.unaids.org
(2) Klumpp, K.; Mirzadegan, T. *Curr. Pharm. Des.* **2006**, *12*, 1909.
(3) Tramontano, E. *Mini-Rev. Med. Chem.* **2006**, *6*, 727.
(4) Baroudy, B.; Archambault, J. US 2006005096, **2006**.
(5) Agata, I.; Goto, S.; Hatano, T.; Nishibe, S.; Okuda, T. *Phytochemistry* **1993**, *33*, 508.
(6) Wang, Z.-X.; Shi, Y. *J. Org. Chem.* **1997**, *62*, 8622.
(7) Sánchez-Abella, L.; Fernández, S.; Armesto, N.; Ferrero, M.; Gotor, V. *J. Org. Chem.* **2006**, *71*, 5396.
(8) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem. Int. Ed.* **2005**, *44*, 1378.
(9) (a) Khandelwal, Y.; Rajeshwari, K.; Rajagopalan, R.; Swamy, L.; Dohadwalla, A. N.; de Souza, N. J.; Rupp, R. H. *J. Med. Chem.* **1988**, *31*, 1872. (b) Meek, J. L.; Davidson, F.; Hobbs, F. W. Jr. *J. Am. Chem. Soc.* **1988**, *110*, 2317. (c) Yu, S.-H.; Chung, S.-K. *Tetrahedron: Asymmetry* **2004**, *15*, 581. (d) Lal, B.; Gangopadhyay, A. K.; Gidwani, R. M.; Rajagopalan, R.; Ghate, A. V. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2006**, *45*, 232.
(10) (a) Elsinger, F.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1960**, *43*, 113. (b) Cox, B.; Morley, N.; Procopiou, P. A.; Sharratt, P. J.; Watson, N. S. *Tetrahedron Lett.* **2000**, *41*, 7547. (c) MacKay, J. A.; Bishop, R. L.; Rawal, V. H. *Org. Lett.* **2005**, *7*, 3421.
(11) (a) McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, *24*, 2289. (b) Eicher, T.; Ott, M.; Speicher, A. *Synthesis* **1996**, 755.