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Epicatechin B-Ring Conjugates: First Enantioselective Synthesis and Evidence for Their Occurrence in Human Biological Fluids

Fedor Romanov-Michailidis, Florian Viton, René Fumeaux, Antoine Lévèques, Lucas Actis-Goretta, Maarit Rein, Gary Williamson, and Denis Barron*,†

Nestle Research Center, P.O. Box 44, CH-1000 Lausanne 26, Switzerland

Denis.Barron@rd.nestle.com

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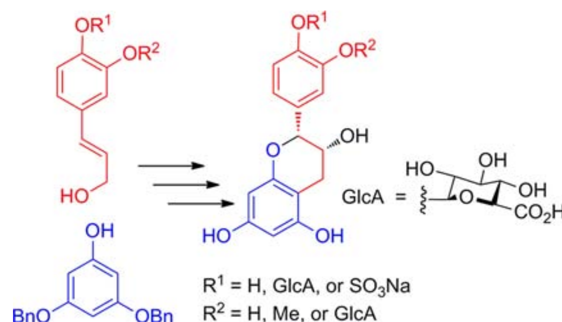
ABSTRACT



Herein, the first enantioselective total synthesis of a number of biologically relevant (−)-epicatechin conjugates is described. The success of this synthesis relied on (i) optimized conditions for the stereospecific cyclization step leading to the catechin C ring; on (ii) efficient conjugation reactions; and on (iii) optimized deprotection sequences. These standard compounds have been subsequently used to elucidate for the first time the pattern of (−)-epicatechin conjugates present in four different human biological fluids following (−)-epicatechin absorption.

Glucuronidated, sulfated, and/or methylated forms of (−)-epicatechin are released in the human bloodstream following consumption of (−)-epicatechin-rich products such as cocoa, red wines, and tea.[1] However, since (−)-epicatechin metabolites are not commercially available, they have often been only partly characterized, and reports studying their biological activities are scarce.[2] Consequently, there is a recurrent need for access to pure analytical standards of (−)-epicatechin conjugates. Over the past years, numerous efforts aimed at developing a scalable (hemi)synthetic approach to produce (−)-epicatechin and (+)-catechin conjugates have been reported. Among these methodologies, direct derivatization[3] and hemisynthetic[4] approaches were prevalent. Unfortunately, these methods were plagued by low chemical yields, poor selectivities, and tedious purification steps. Despite this, few studies making use of synthetic conjugated (epi)catechin standards have been recently published. However, the details of the chemical synthesis involved were not provided,[5,6] except for the preparation of epicatechin glucuronides conjugated at the A-ring.[7]

† Present address: Nestlé Institute of Health Sciences, EPFL innovation square, 1015 Lausanne, Switzerland.

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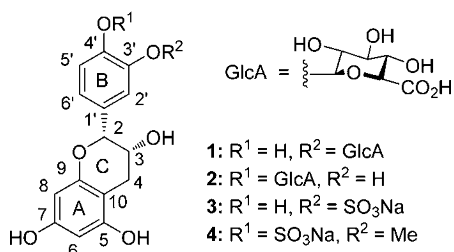

Figure 1. Structures of the synthesized (−)-epicatechin conjugates: 3′-*O*-glucuronide **1**, 4′-*O*-glucuronide **2**, 3′-*O*-sulfonate **3**, 3′-*O*-methyl-4′-*O*-sulfonate **4**.

Herein, we describe the first fully documented enantioselective total synthesis of four biologically relevant (−)-epicatechin B-ring conjugates (Figure 1). These fully characterized molecules were subsequently used as analytical-grade standards to elucidate the distribution pattern of (−)-epicatechin conjugates present in four human biological fluids after (−)-epicatechin consumption. The retrosynthetic analysis scheme leading to the pivotal (−)-epicatechin core **A**, ready for regioselective glucuronide or sulfate conjugation, is displayed in Figure 2.

The precursor of ring A of tribenzylated (−)-epicatechin (**A**) is the phloroglucinol derivative **E**, while the cinnamic alcohol derivative **D**, itself prepared from the benzaldehyde derivative **F**, is the precursor of rings B and C. A Friedel−Crafts alkylation of **E** by **D** would lead to diphenol **C**, which could then be transformed into the enantiomerically enriched tetraol **B** following a standard *Sharpless* asymmetric dihydroxylation protocol. Finally, a stereospecific cyclization of **B** would give rise to a catechin derivative, eventually converted to **A** following an inversion of configuration at C3.

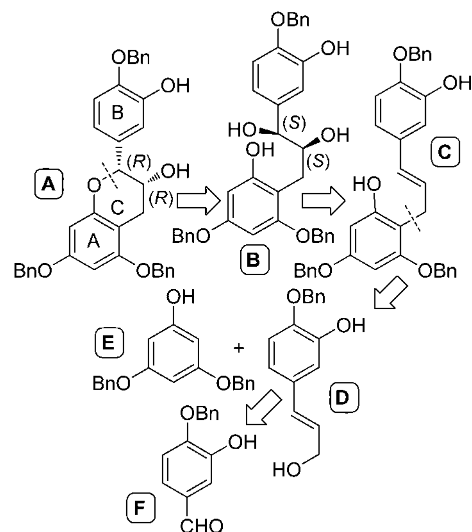

Figure 2. Retrosynthetic analysis.

Starting from enantiomerically pure tetraols **5**−**7** (Figure 3; synthesis details to be found in the Supporting Information), the key step of the synthesis of the epicatechin core **A** was the stereospecific cyclization. The success of the cyclization relied on emphasizing the *kinetic* S_N2 pathway for cyclization by applying milder reaction conditions as compared to the previously reported ones.[8] Namely, (i) triethyl orthoformate was replaced by the less reactive triethyl orthoacetate, (ii) the PPTS catalyst loading was lowered from 60 to 10 mol %, (iii) the reaction temperature was dropped down from 60 to 45 °C, and (iv) the reaction time was shortened from 6 to 4 h. On the other hand, in order to avoid potential base-induced epimerization problems,[9] the classical alkaline methanolysis step (K_2CO_3/MeOH) was replaced by a reductive step involving DIBAL-H

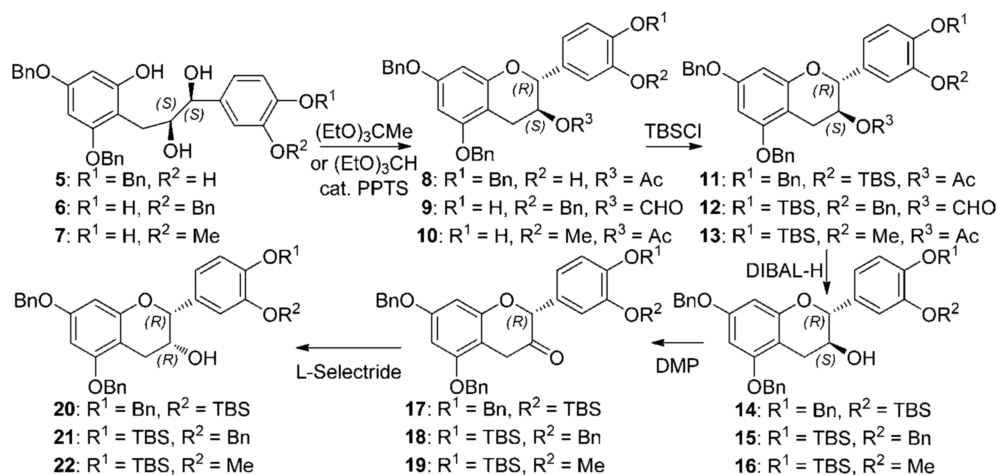

Figure 3. Synthesis of the protected (*R*,*R*)-epicatechin derivatives **20**−**22**.

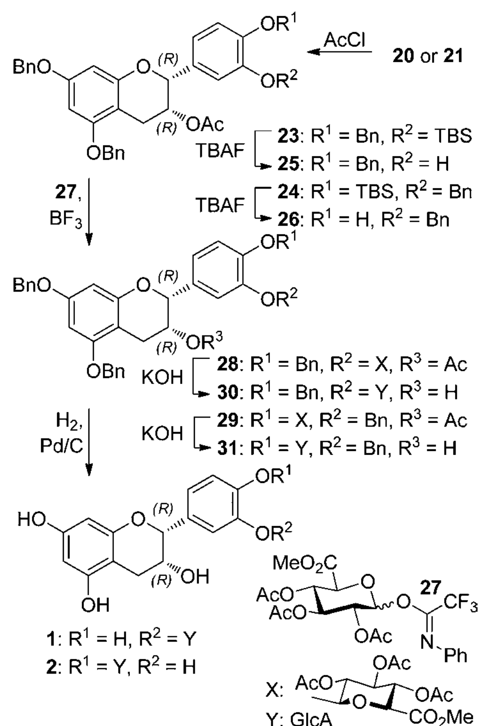

Figure 4. Syntheses of (−)-epicatechin 3′- and 4′-*O*-*β*-D-glucuronides.

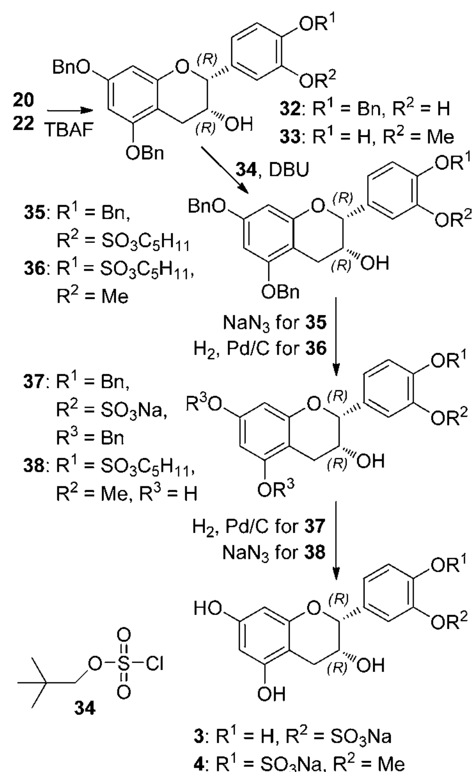

Figure 5. Syntheses of (−)-epicatechin 3′-sulfate and 3′-methyl-4′-sulfate.

as a reagent. Thus tetraols **5**−**7** were first cyclized cleanly to the corresponding 3-acylcatechins **8**−**10** with complete *trans*-selectivity (>98% inversion according to ^{1}H NMR analysis of the crude reaction mixture). Subsequent TBS-protection of the sole bare hydroxyl group, followed by DIBAL-promoted deacetylation afforded the desired (*R*,*S*)-catechin skeletons **14**−**16** in excellent yields. These were then subjected to a two-step C3-inversion protocol (DMP-oxidation followed by diastereoselective L-selectride reduction), yielding epicatechin derivatives **20**−**22**.

En route to the synthesis of (−)-epicatechin 3′- and 4′-glucuronides, compounds **20** and **21** were acetylated at carbon C3 to compounds **23** and **24** (Figure 4). Removal of the TBS protecting groups afforded compounds **25** and **26**, ready for regiospecific *O*-glucuronidation at positions 3′ and 4′, respectively. Thus, **25** and **26** were coupled with glucuronic acid donor **27**, under BF_3•OEt_2 catalysis. Mild alkaline hydrolysis, followed by hydrogenolysis over $Pd(OH)_2/C$, yielded the desired glucuronides **1** and **2**.

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Regiospecific *O*-sulfonations were carried out on protected scaffolds **20** and **22** (Figure 5). Neopentyl chlorosulfate (**34**) was chosen as the optimal sulfonating reagent.[10] In the preparation of compound **3** from compound **35**, the neopentyl protecting group was first removed with NaN_3, followed by hydrogenative debenzylation over $Pd(OH)_2/C$.

However, partial cleavage of the free sulfate moiety took place during hydrogenolysis, and compound **3** was isolated in only small amounts (9% of **3** from **35** over two steps). Thus, in the synthesis of 3′-*O*-methyl-(−)-epicatechin 4′-*O*-sulfonate (**4**) from compound **36**, we adopted a reverse deprotection sequence (first hydrogenation, and then treatment with NaN_3). This markedly increased the isolated yield (66% of **4** from **36** over two steps).

The synthesized compounds were subsequently used as analytical-grade standards to elucidate the pattern of (−)-epicatechin conjugates present in human biological samples. Blood, urine, intestinal fluids, and bile samples were collected after human volunteers receiced 50 mg of (−)-epicatechin.[11] (−)-Epicatechin metabolites were extracted (see Supporting Information) and analyzed by UPLC-MS/MS on reversed-phase. The UPLC-MS/MS analysis demonstrated the presence of nine conjugates

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Table 1. Comparative UPLC and MS Data for Authentic Standards of (−)-Epicatechin Glucuronides/Sulfates, and (−)-Epicatechin Conjugates Present in Human Biological Fluids

epicatechin metabolites	peak t_R	exact mass	found elemental composition	MS/MS main fragment
UPLC peak				
(1) EC-*O*-glucuronide	1.70	465.1051	$C_{21}H_{21}O_{12}$	289.0719
(2) EC-*O*-sulfonate	1.84	369.0286	$C_{15}H_{13}O_9S$	289.0722
(3) EC-*O*-sulfonate	2.38	369.0292	$C_{15}H_{13}O_9S$	289.0729
(4) EC-*O*-glucuronide	2.41	465.1051	$C_{21}H_{21}O_{12}$	289.0719
(5) *O*-Methyl-EC-*O*-sulfonate	2.53	383.0444	$C_{16}H_{15}O_9S$	303.0872
(6) *O*-Methyl-EC-*O*-sulfonate	3.36	383.0445	$C_{16}H_{15}O_9S$	303.0865
(7) *O*-Methyl-EC-*O*-sulfonate	3.61	383.0446	$C_{16}H_{15}O_9S$	303.0874
(8) *O*-Methyl-EC-*O*-sulfonate	4.08	383.0445	$C_{16}H_{15}O_9S$	303.0862
(9) *O*-Methyl-EC-*O*-sulfonate	4.43	383.0443	$C_{16}H_{15}O_9S$	303.0869
synthetic standards				
(−)-epicatechin-4′-*O*-glucuronide	1.68	465.1035	$C_{21}H_{21}O_{12}$	289.0712
(−)-epicatechin-3′-*O*-sulfonate	2.42	369.0289	$C_{15}H_{13}O_9S$	289.0719
(−)-epicatechin-3′-*O*-glucuronide	2.45	465.1017	$C_{21}H_{21}O_{12}$	289.0702
3′-*O*-methyl-(−)-epicatechin-4′-*O*-sulfonate	2.58	383.0445	$C_{16}H_{15}O_9S$	303.0872

(Table 1), which were two (−)-epicatechin *O*-glucuronides, two (−)-epicatechin *O*-sulfonates, and five *O*-methyl-(−)-epicatechin-*O*-sulfonates. Comparison with the LC retention times and MS data of our authentic synthetic standards allowed us to unambiguously assign four out of the nine detected conjugates (Table 1).

Table 2. Occurrence of Conjugates **1**−**4** in Human Biological Fluids

compd	intestinal fluid	plasma	bile	urine
1	+	+++	+++	+++
2	+	+	+	+
3	+++	+++	+++	++
4	+	+	+	+

Thus peak 1 was (−)-epicatechin 4′-*O*-*β*-D-glucuronide, peak 3 was (−)-epicatechin 3′-*O*-sulfonate, peak 4 was (−)-epicatechin 3′-*O*-*β*-D-glucuronide, and peak 5 was 3′-*O*-methyl-(−)-epicatechin-4′-*O*-sulfonate.

These molecules were shown to be present in human intestinal fluid, plasma, bile, and urine following exposure to (−)-epicatechin (Table 2). Among these four conjugates (−)-epicatechin 3′-*O*-sulfonate and (−)-epicatechin 3′-*O*-*β*-D-glucuronide were shown to be the major circulating forms in biological samples. Further details concerning the quantification and the biological activity of these conjugates will be reported in a separate paper.

Supporting Information Available. Detailed experimental procedures and complete spectroscopic characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.