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A versatile approach to the regioselective synthesis of diverse (−)-epicatechin-β-D-glucuronides

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ABSTRACT

A versatile new approach is reported for the total synthesis of five glucuronide metabolites of epicatechin, using selective protection/deprotection techniques.

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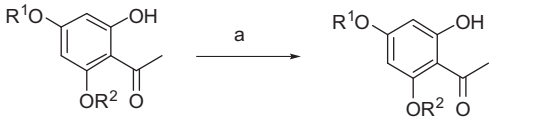
Introduction

The health benefits of flavanols have been widely investigated in recent years.[1] Specifically, the consumption of flavanol-rich foods such as cocoa is known to be associated with improved cardiovascular health.[2] Recent work has shown that many of these effects are mediated by the natural product (−)-epicatechin.[3] (−)-Epicatechin is readily metabolized in humans to give various *O*-methylated, *O*-glucuronidated, and *O*-sulfated metabolites.[4]

Major human metabolites of (−)-epicatechin have been isolated from plasma and urine, and then structurally characterized by NMR.[3a,4a] It is known that (−)-epicatechin is rapidly metabolized after ingestion and that one or more metabolites are likely to be biologically active.[5] Isolation of these species is tedious and leads only to trace quantities of metabolite. It is therefore desirable to develop a synthetic strategy for generating these metabolites in sufficient quantities for further study of their biological activities. We recently reported the synthesis of optically active ^{13}C-labeled (−)-epicatechin.[6] As part of an ongoing program to synthesize putative metabolites of (−)-epicatechin in a stereoselective manner, we report here the first synthesis of five epicatechin glucuronides via a selective protection/deprotection methodology.



1a R^1=H, R^2=Bn
1b R^1=Bn, R^2=H

2a R^1=MOM, R^2=Bn
2b R^1=Bn, R^2=MOM

Scheme 1. Reagents and conditions: (a) methoxymethylbromide, Hünigs base, CH_2Cl_2, 0 °C, 1 h.

Results and discussion

Syntheses of acetophenones **1a**,[7] and **1b**[8] were previously described in the literature. By utilizing the hydrogen bonding between ketone and the adjacent phenol, orthogonally bis-protected acetophenones **2a** and **2b** were synthesized by the reaction of acetophenones **1a** and **1b** with methoxymethyl bromide in dichloromethane as the solvent (Scheme 1). Condensation of acetophenones **2a–c** (**2c** commercially available) and benzaldehydes **3a–d** with sodium hydride in dimethylformamide gave chalcones **4a–e** (Scheme 2). Reduction of chalcones **4a–e** with sodium borohydride and cerium chloride resulted in the decarbonylated product, which was purified by column chromatography. TLC analysis indicated that disappearance of the chalcone was rapid (the reaction appears

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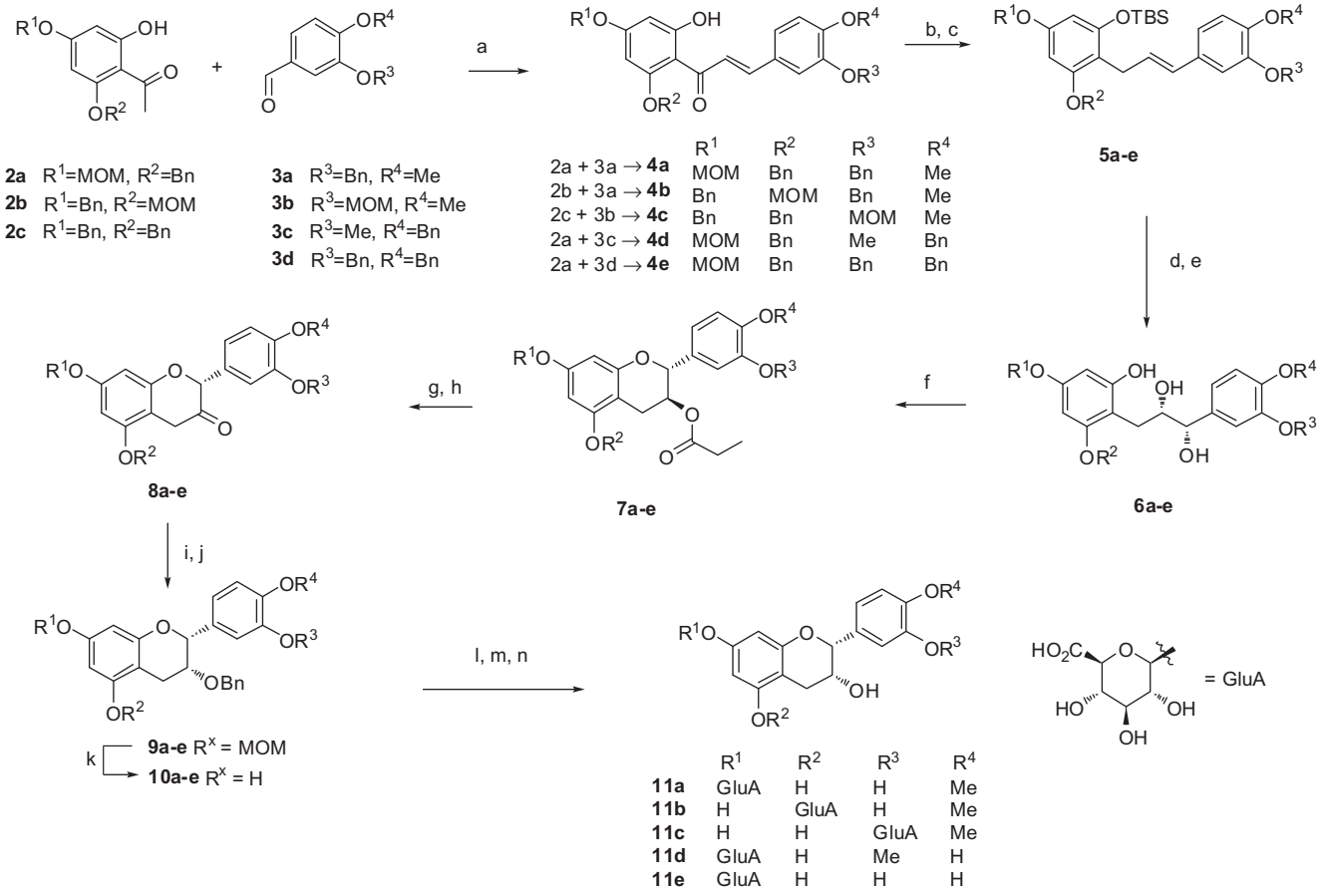

Scheme 2. Reagents and conditions: (a) NaH, DMF, 0 °C to RT, 1 h; (b) $CeCl_3 \cdot 7H_20$, 1:4 EtOH/THF, then $NaBH_4$, −40 to 0 °C, 5 h; (c) TBSCl, imidazole, DMF, 16 h, RT; (d) AD-mix α, $MeSO_2NH_2$, 1:1 *t*-BuOH/water, 0 °C to RT, 16 h; (e) TBAF, AcOH, 0 °C, 1 h; (f) $EtC(OEt)_3$, PPTS, 1,2-dichloroethane, 65 °C, 2 h; (g) 7 N NH_3 in MeOH, 48 h; (h) DMP(wet), CH_2Cl_2, 2 h; (i) $Al(OiPr)_3$, 2:1 toluene/IPA, 100 °C, 1 h; (j) NaH, BnBr, DMF, 2 h; (k) 4 N HCl in dioxane, 1:1 MeOH/CH_2Cl_2, 1 h, (l) 2,3,4-tri-*O*-acetyl-α-D-glucuronic acid methyl ester trichloroacetimidate, 4 Å mol. sieves, CH_2Cl_2, 1 h at RT then cooled to −40 °C, then $BF_3 \cdot Et_2O$, −40 to 0 °C, 2 h; (m) 1 N NaOH, 0 °C to RT, 2 h; (m) H_2 (g), $Pd(OH)_2$, MeOH, 2 h.

complete after 1 h), however high yield requires continuous stirring for an additional 5 h.[6] The remaining free phenol was protected as the *tert*-butyldimethylsilyl (TBS) ether **5a–e** before performing an asymmetric Sharpless *cis*-dihydroxylation using AD-mix-α,[9] and then deprotecting the TBS ether with a 1:1 molar mixture of tetrabutylammonium fluoride:acetic acid to give **6a–e**.[6] It is important to use an equimolar amount of glacial acetic acid to avoid epimerization of the product. Cyclization of compounds **6a–e** in the presence of triethyl orthopropionate and pyridinium *p*-toluenesulfonate in 1,2-dichloroethane gave **7a–e** in good yield after chromatography.[6]

After room temperature deprotection of the ester with 7 N ammonia in methanol, the protected catechin was converted to protected epicatechin by oxidizing the hydroxyl group to the ketone with Dess–Martin periodinane (DMP) in wet dichloromethane **8a–e**[10] and then stereoselectively reducing the ketone with aluminum triisopropoxide and isopropyl alcohol under Meerwein–Ponndorf–Verley reduction conditions.[11] Benzyl protection of the 3-hydroxyl with benzyl bromide and sodium hydride **9a–e**, followed by deprotection of the methoxymethyl (MOM) group with hydrochloric acid, provided the free phenol at the desired position while all other hydroxy groups were protected as the benzyl ethers **10a–e**. Coupling of phenols **10a–e** with commercially available 2,3,4-tri-*O*-acetyl-α-D-glucuronic acid methylester trichloroacetimidate and boron trifluoride etherate provided the fully protected *O*-glucuronide in moderate yield after silica gel chromatography.[12] The *O*-acyl protected glucuronic acid methyl ester was deprotected with 1 N sodium hydroxide at 0 °C then subjected to hydrogenation conditions with Pearlman's catalyst to provide crude **11a–e** (purity ~85% by HPLC).

Pure (>95% by HPLC) **11a–e**[14] was obtained by reverse phase semi-preparative HPLC (Column: Sunfire prep C18 OBO 5 μM, 30 × 75 mm by Waters Corp.) using a gradient of 0% to 20% ACN/Water with 0.1% TFA modifier.

The optical purity of the final compounds was not determined, although a high degree of stereoselectivity is inferred, based upon similar methodology used for the synthesis of ^{13}C-labled (−)-epicatechin,[6] as well as the synthesis of (+)-myristinin A,[13] in which the absolute stereochemistry at C-2 was determined to be S. The *cis* -configuration at C-2 and C-3 after stereoselective reduction of the ketone was supported by the fact that the resultant C-2 proton exists as a singlet in the ^{1}H NMR spectrum ($J_{2,3}$ <1 Hz), as reported previously.[6,11] Some loss of stereochemical purity, (possibly due to diminished asymmetric induction in the Sharpless *cis*-dihydroxylation step), was revealed after the optically pure glucuronide was attached to the epicatechin core, thus forming diastereomers, which were separable by reverse phase HPLC. Analysis of the minor isomers by NMR showed nearly identical spectra, with the C-2 proton appearing as a singlet. The anomeric proton was determined to be β based upon the coupling constant (J = ~7.7 Hz).[12]

Synthesis of optically pure glucuronide metabolites of epicatechin as well as its in-depth analytical and stereochemical evaluation will be the subject of a future publication.

Conclusion

We have developed efficient methods to prepare epicatechin-β-D-glucuronides in a stereo- and enantio-specific manner. These methods have been utilized in the first total syntheses of 4′-*O*-methyl-(−)-epicatechin-7-β-D-glucuronide (**11a**), 4′-*O*-methyl-(−)-epicate chin-5-β-D-glucuronide (**11b**), 4′-*O*-methyl-(−)-epicatechin-3′-β-D-glucuronide (**11c**), 3′-*O*-methyl-(−)-epicatechin-7-β-D-glucuronide (**11d**), and (−)-epicatechin-7-β-D-glucuronide (**11e**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.01.054. These data include MOL files and InChiKeys of the most important compounds described in this article.

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14. *Analytical data for* ***11a****:* MS m/z = 481.3 [M^++1]. ^{1}H NMR (500 MHz, d6-acetone): δ 7.07 (d, 1H, J = 2.0 Hz), 6.95 (dd, 1H, J = 8.4, 2.0 Hz), 6.91 (d, 1H, J = 8.4 Hz), 6.22 (d, 1H, J = 2.4 Hz), 6.18 (d, 1H, J = 2.4 Hz), 5.02 (d, 1H, J = 7.7 Hz), 4.94 (s, 1H), 4.27–4.23 (m, 1H), 4.07 (d, 1H, J = 9.4 Hz), 3.83 (s, 3H), 3.70 (dd, 1H, J = 9.4, 9.1 Hz), 3.60 (t, 1H, J = 9.1 Hz), 3.49 (dd, 1H, J = 9.1, 7.7 Hz), 2.90 (dd, 1H, J = 16.9, 4.5 Hz), 2.77 (dd, 1H, J = 16.9, 3.0 Hz). ^{13}C NMR (125 MHz, d6-acetone): δ 170.3, 157.9, 157.4, 157.0, 147.7, 147.0, 133.4, 119.0, 115.1, 111.9, 102.8, 101.9, 97.5, 96.8, 79.5, 77.1, 76.0, 74.3, 72.6, 66.7, 56.3, 29.1.
Analytical data for ***11b****:* MS m/z = 481.3 [M^++1]. ^{1}H NMR (500 MHz, d6-acetone): δ 7.06 (d, 1H, J = 1.9 Hz), 6.94 (dd, 1H, J = 8.4, 1.9 Hz), 6.91 (d, 1H, J = 8.4 Hz), 6.30 (d, 1H, J = 2.2 Hz), 6.09 (d, 1H, J = 2.2 Hz), 5.01 (d, 1H, J = 7.4 Hz), 4.91 (s, 1H), 4.23–4.20 (m, 1H), 4.07 (d, 1H, J = 9.6 Hz), 3.83 (s, 3H), 3.73 (dd, 1H, J = 9.6, 9.0 Hz), 3.61 (t, 1H, J = 9.0 Hz), 3.57 (dd, 1H, J = 9.0, 7.4 Hz), 2.91 (dd, 1H, J = 17.1, 3.1 Hz), 2.85 (dd, 1H, J = 17.1, 4.4 Hz). ^{13}C NMR (125 MHz, d6-acetone): δ 170.3, 157.9, 157.6, 156.7, 147.7, 147.0, 133.4, 118.9, 115.0, 112.0, 102.4, 102.0, 98.2, 96.5, 79.4, 77.2, 76.1, 74.4, 72.6, 66.7, 56.3, 29.0.
Analytical data for ***11c****:* MS m/z = 481.3 [M^++1]. ^{1}H NMR (500 MHz, D_2O): δ 7.18 (d, 1H, J = 1.7 Hz), 7.07 (dd, 1H, J = 8.6,1.7 Hz), 7.00 (d, 1H, J = 8.6 Hz), 6.06 (m, 0.79H, partial deuterium exchange), 6.02 (m, 0.42H, partial deuterium exchange), 5.08 (d, 1H, J = 7.7 Hz), 4.3 (m, 1H), 4.03 (d, 1H, J = 9.6 Hz), 3.83 (s, 3H), 3.69–3.56 (m, 3H), 2.81 (dd, 1H, J = 16.9, 4.5 Hz), 2.66 (dd, 1H, J = 16.9, 2.5 Hz). ^{13}C NMR (125 MHz): δ 155.6, 155.2, 155.1, 148.3, 145.0, 131.2, 121.9, 114.4, 112.5, 112.5, 100.4, 99.6, 96.0, 95.4, 77.7, 75.2, 72.6, 71.3, 65.5, 55.8, 27.3.
Analytical data for ***11d****:* MS m/z = 481.3 [M^++1]. ^{1}H NMR (500 MHz, d6-acetone): δ 7.19 (d, J = 1.3 Hz, 1H), 6.97 (dd, 1H, J = 8.5, 1.3 Hz), 6.81 (dd, 1H, J = 8.5, 1.3 Hz), 6.24 (d, J = 1.9 Hz, 1H), 6.17 (d, J = 1.9 Hz, 1H), 4.99 (d, 1H, J = 7.7 Hz), 4.96 (s, 1H), 4.27–4.22 (m, 1H), 4.07 (d, 1H, J = 9.3 Hz), 3.84 (s, 3H), 3.70 (t, 1H, J = 9.3 Hz), 5.58 (t, 1H, J = 9.3 Hz), 3.48 (dd, 1H, J = 9.3, 7.7 Hz), 2.91 (dd, 1H, J = 16.9, 4.5 Hz), 2.80 (dd, 1H, J = 16.7, 2.2 Hz). ^{13}C NMR (125 MHz, d6-acetone): δ 170.3, 158.0, 157.6, 157.1, 148.0, 147.0, 132.0, 120.7, 115.2, 112.0, 102.9, 102.1, 97.6, 97.1, 79.8, 77.2, 76.1, 74.4, 72.7, 66.8, 56.3, 29.3.
Analytical data for ***11e****:* MS m/z = 467.3 [M^++1]. ^{1}H NMR (500 MHz, d6-acetone): δ 7.06 (d, 1H, J = 1.6 Hz), 6.84 (dd, 1H, J = 8.1, 1.6 Hz), 6.79 (d, 1H, J = 8.1 Hz), 6.22 (d, 1H, J = 2.4 Hz), 6.16 (d, 1H, J = 2.4 Hz), 5.01 (d, 1H, J = 7.7 Hz), 4.90 (s, 1H), 4.25–4.21 (m, 1H), 4.07 (d, 1H, J = 9.7 Hz), 3.71 (t, 1H, J = 9.0 Hz), 3.60 (t, 1H, J = 9.0 Hz), 3.49 (dd, 1H, J = 9.0, 7.7 Hz), 2.90 (dd, 1H, J = 16.7, 4.6 Hz), 2.77 (dd, 1H, J = 16.7, 3.0 Hz). ^{13}C NMR (125 MHz, d6-acetone): δ 170.2, 158.0, 157.5, 157.1, 145.4, 145.4, 132.1, 119.5, 115.5, 115.4, 102.9, 102.0, 97.5, 97.0, 79.6, 77.2, 76.1, 74.4, 72.7, 66.8, 29.1.