ORGANIC LETTERS

2001
Vol. 3, No. 6
843−846

Synthesis of a 3,4,5-Trimethoxybenzoyl Ester Analogue of Epigallocatechin-3-gallate (EGCG): A Potential Route to the Natural Product Green Tea Catechin, EGCG

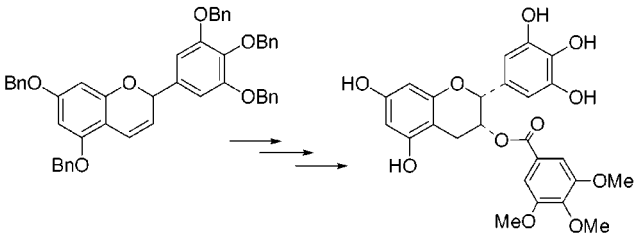
Nurulain T. Zaveri

Pharmaceutical Discovery Division, SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025

nurulain.zaveri@sri.com

Received December 14, 2000

ABSTRACT



The synthesis of a trimethoxybenzoyl ester (D-ring) analogue of epigallocatechin-3-gallate (EGCG) is described. The versatile synthesis route can be used to synthesize A, B, and D ring analogues of EGCG and involves a key cyclization of the chalcone to the 3-flavene. This synthesis provides a possible route to the polyphenolic green tea natural product EGCG.

Green tea and its constituent polyphenols have received increasing attention as potential cancer chemopreventives and are currently undergoing Phase I clinical trials.[1] Green tea consumption has been associated with decreased incidence of breast, pancreatic, colon, esophageal, and lung cancers.[2] The cancer preventive properties of green tea extract are mainly associated with the polyphenolic catechins, the 3-flavanols. There are four catechins in green tea which make up ∼10% of the dry weight of green tea. These are shown in Figure 1 and include (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epicatechin (EC).[3] Several studies have demonstrated that EGCG, the most abundant catechin, is the most potent chemopreventive component of green tea, and inhibition of carcinogenesis by green tea is mediated by EGCG.[4]

Several in vivo experiments have shown the chemopreventive effects of EGCG against all stages of carcinogenesis—initiation, promotion, and progression—in animal models of

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10.1021/ol007000o CCC: $20.00
Published on Web 02/16/2001

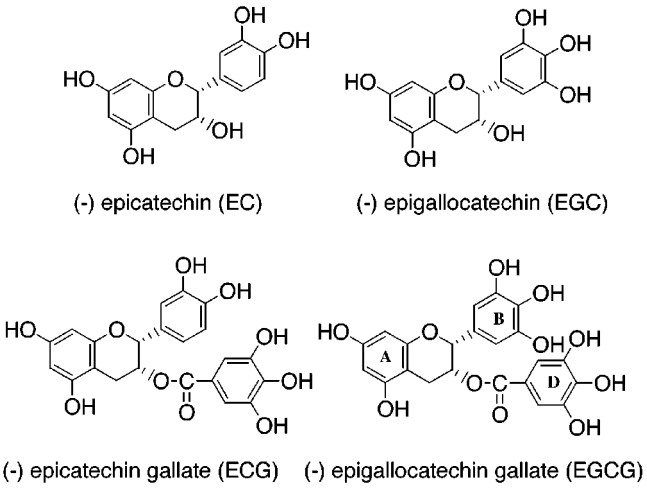

Figure 1. Structures of the four major catechins from green tea.

breast, lung, skin, prostate, and colon cancers.[5] There have been extensive investigations into the possible mechanisms of cancer prevention by EGCG, and recent efforts in defining the molecular mechanisms of green tea and EGCG action have found that EGCG affects several molecular targets and pathways of carcinogenesis.[6] However, there have been no clear structure−activity studies defining what parts of the EGCG molecule are important for its anticarcinogenic action. While many studies have shown that the antitumor activity of EGCG stems from its exceptional antioxidant potential,[7] two recent studies by Valcic et al.[8] have shown that the trihydroxyphenyl B-ring is the principal site of antioxidant reactions, regardless of the presence of the 3-galloyl moiety. However, the 3-galloyl moiety may enhance the antioxidant potential of EGCG.[9]

The synthesis of EGCG has not yet been described in the literature. We have developed a versatile chemical synthesis of EGCG and other tea catechins that allows us to vary different portions of the EGCG molecule to study the structure−function relationship of EGCG. This synthesis affords the 2,3-cis (epicatechin)[10] as well as the 2,3-trans (catechin) racemic analogues of EGCG and is easily amenable to chiral synthesis of 2β,3β-cis EGCG analogues, which have the same configuration as the natural product (−)-EGCG. An enantioselective synthesis of the permethylaryl ether analogues of epicatechin has been reported by Rensburg et al.;[11] however, neither the choice of starting synthons nor the yields of the epicatechin are very practical for the synthesis of epi*gallo*catechins or analogues for a structure−function study of EGCG action.

In this letter, we describe the synthesis of a D-ring analogue of EGCG using our synthesis approach (Scheme 1). With the appropriate choice of starting materials, this synthesis can be used to make A, B, and/or D-ring analogues of EGCG.

O-Benzyl-protected 3,4,5-trihydroxybenzaldehyde **(2)** (synthesized by a known method in 87% overall yield from methyl gallate, **1**)[12] was condensed with 4′,6′-bis(benzyloxy)-2′-hydroxyacetophenone **(4)** in piperidine/EtOH[13] to yield the chalcone **5**. The chalcone was cyclized directly to the 3-flavene **6** with $NaBH_4$ in tetrahydrofuran (THF) and EtOH at 65 °C in a 50% yield.[14] This method of chalcone cyclization directly to the 3-flavene was established in our laboratory after considerable experimentation because chalcone **5** failed to cyclize under several other conditions reported in the literature.[15] This conversion of 2′-hydroxy-

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(14) **Representative Procedure:** The chalcone **5** (1 g, 1.33 mmol) was dissolved in THF (20 mL) and EtOH (10 mL) at room temperature, and $NaBH_4$ (51 mg, 1.33 mmol) was added. The solution was heated to a gentle reflux (65−70 °C) for 16 h, after which TLC (CH_2Cl_2) indicated no starting material. The cooled reaction mixture was evaporated to dryness and dissolved in CH_2Cl_2. The organic solution was washed with water and brine, dried ($MgSO_4$), and evaporated to give a crude yellow solid which was purified via column chromatography using a gradient of 45% to 20% hexanes in CH_2Cl_2 to obtain 0.49 g (50% yield) of **6** as an off-white solid.

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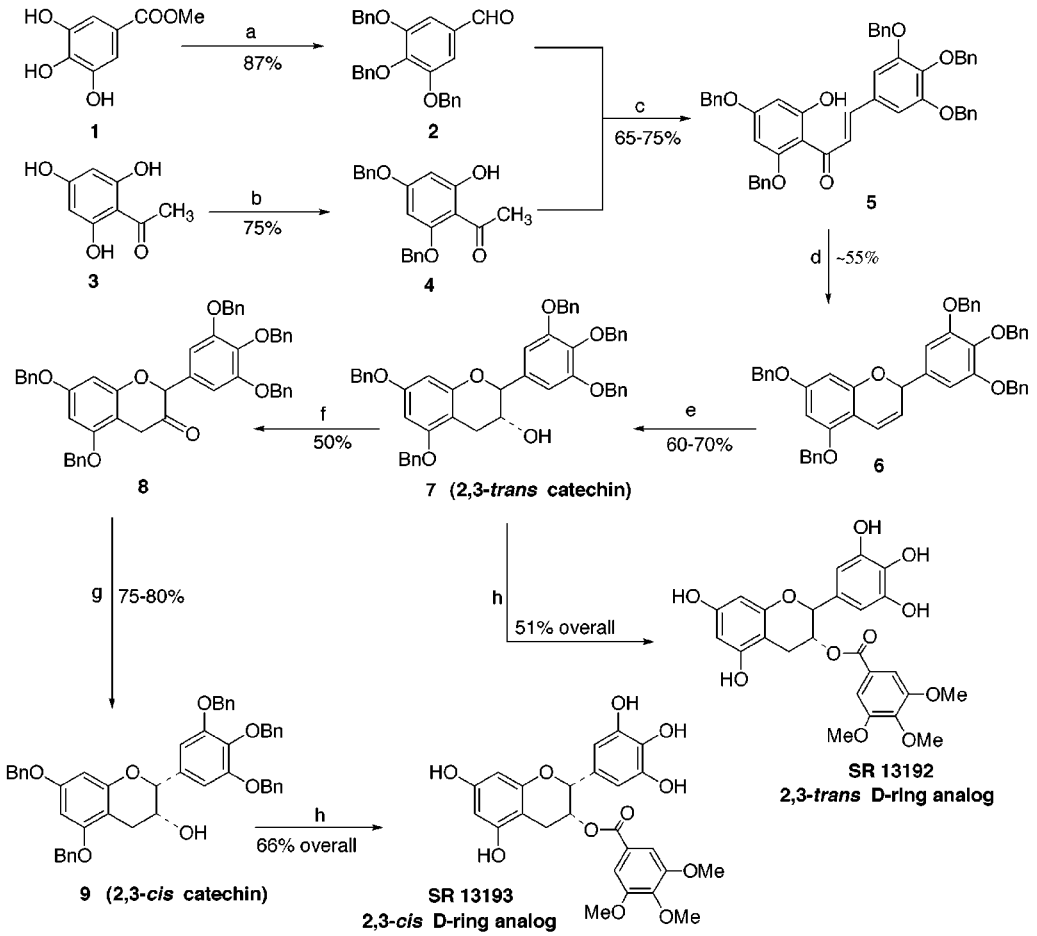
Scheme 1[a]



[a] i. BnBr, K_2CO_3, DMF. ii. LAH, THF. iii. PCC, CH_2Cl_2. [b] 2 equiv of BnBr, K_2CO_3, HMPA. [c] piperidine, EtOH, reflux. [d] $NaBH_4$, THF/EtOH. [e] i. BH_3-THF. ii. H_2O_2, NaOH. [f] Dess−Martin periodinane, CH_2Cl_2. [g] L-Selectride, THF, room temp. [h] i. 3,4,5-trimethoxybenzoyl chloride, DMAP, Et_3N. ii. H_2/Pd/C, dioxane.

chalcones to flav-3-enes and its biosynthetic implications have been reported earlier by Clark-Lewis and Skingle.[16]

This cyclization now provides an expeditious route to the flav-3-ene **6**, which was further elaborated to give EGCG analogues. Hydroboration−oxidation of **6** with BH_3/THF followed by H_2O_2/NaOH gave the *trans*-3-flavanol **7** (catechin). The trans stereochemistry of **7** was assigned based on the nuclear magnetic resonance (NMR) coupling constants and nuclear Overhauser effect (NOE) experiments and confirmed by molecular modeling (Insight II, Biosym). The hydroboration−oxidation gave <5% of the *cis*-3-flavanol, as evidenced by the peaks in the NMR spectra assigned to the *cis*-3-flavanol **9**.

The protected trans 3-flavanol **7** was converted to the cis 3-flavanol **9** by a two-step sequence involving oxidation of **7** with the Dess−Martin periodinane[17] followed by selective reduction with L-Selectride[18] to afford exclusively the cis-racemic 3-flavanol **9**. This sequence was recently also described by Kozikowski et al.[19] in their synthesis of proanthocyanidin dimers from catechin. The cis 3-flavanol (protected epigallocatechin) **9** was esterified with 3,4,5-trimethoxybenzoyl chloride and deprotected by hydrogenation to afford the cis D-ring analogue **SR 13193**, which is the trimethoxybenzoyl ester analogue of EGCG. The trans D-ring analogue **SR 13192** was synthesized in the same manner as the cis analogue from the trans 3-flavanol **7**.

Both the cis and trans D-ring analogues inhibited the growth of breast cancer cell lines in vitro, although they were about 2-fold less potent than EGCG itself.[20] Interestingly, the racemic trans analogue **SR 13192** was slightly less potent than the cis analogue **SR 13193** in the growth inhibition assays. The biological activity of these analogues will be reported in detail elsewhere.[21]

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In conclusion, a novel versatile route to the green tea catechins is described, which can be used to design novel analogues of EGCG to study its molecular mechanisms in greater detail.

Acknowledgment. This work was supported by a New Investigator grant from the California Breast Cancer Research Program (2KB-0083).

Supporting Information Available: Spectral characterization of compounds **5**−**9**, **SR 13192**, and **SR 13193**. This material is available free of charge via the Internet at http://pubs.acs.org.

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