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Bioorganic & Medicinal Chemistry Letters 17 (2007) 3095–3098
Bioorganic & Medicinal Chemistry Letters

Concise synthesis of dideoxy-epigallocatechin gallate (DO-EGCG) and evaluation of its anti-influenza virus activity

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Received 2 February 2007; revised 12 March 2007; accepted 13 March 2007
Available online 16 March 2007

This paper is dedicated to the memory of the late Professor Kiyoshi Tanaka, who passed away on December 8, 2004.

Abstract—Dideoxy-epigallocatechin gallate (DO-EGCG) (**2**), a simplified analog of naturally occurring EGCG (**1**), was efficiently prepared by directly introducing a ketone group at C3 and successive reduction to the *sec*-alcohol with 2,3-*cis* stereochemistry. Compound **2** showed potent anti-influenza virus activity, indicating that the hydroxyl substituents on the A-ring are not crucial for anti-influenza virus activity.

(−)-Epigallocatechin gallate (EGCG) (**1**),[1] which is a major constituent of green tea extract, has received special attention for its cancer preventive,[2] antiviral[3], and other important bioactivities.[4] Although **1** is expected to be a promising candidate for drug development, the structure–activity relationship of **1** has not been sufficiently investigated due to the limited availability of EGCG derivatives, which are mainly provided from natural sources. Furthermore, selectively modifying the densely substituted hydroxyl groups of **1** is difficult.

Among the naturally occurring stereoisomeric catechins, including (+)-catechin (2,3-*trans*), compound **1**, which possesses 2,3-*cis* stereochemistry, exhibits the most potential bioactivities.

Therefore, an easy route to structurally diversified EGCG analogs is needed.[5] However, examples of a selective synthesis of **1** and its related derivatives are limited due to the difficulty of selectively constructing 2,3-*cis* stereogenic centers on a C-ring where the chiral center at the C2 position is easily epimerized under basic conditions.[5,6]

Because epigallocatechin derivative **3** should be easily installed by the reduction of the ketone functional group at C3, compound **4** should be a key intermediate.[7] Ketone **4** may be directly prepared from nitrochromene derivative **5**, which possesses a tricyclic system that corresponds to the A, B, and C-rings of the catechin framework. We envisioned that nitrochromene **5** may be constructed by the ring formation between two segments, salicylaldehyde (**6**) and nitroolefin derivative **7**. This divergent synthesis may be applicable to EGCG related derivatives (Scheme 1).

During the course of our investigation, we concisely synthesized dideoxy-epigallocatechin gallate (DO-EGCG) (**2**). Moreover, we found that **2** exhibits potent anti–influenza virus activity, although this analog has simplified structure of **1** without the phenolic hydroxyl groups on the A-ring. Herein, the detailed synthesis of **2** and the preliminary investigation of its anti–influenza virus activity are described (Fig. 1).

Initially a one-pot nitroaldol and dehydration reaction of 3,4,5-tribenzyloxybenzaldehyde (**8**)[5b] with nitromethane under Knoevenagel conditions was performed to afford desired **9** in good yield (Scheme 2).

The successive ring construction between **9** and **6** in the presence of a catalytic amount of DABCO[8] was

Keywords: Epigallocatechin gallate; Nitrochromene derivative; Nef reaction; Anti-influenza virus activity.
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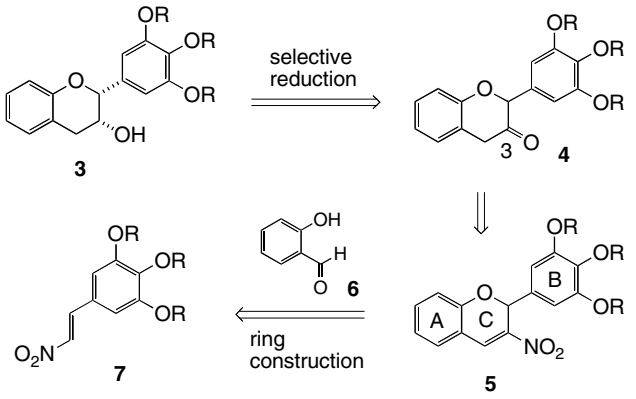
doi:10.1016/j.bmcl.2007.03.041



Scheme 1. Synthetic strategy.

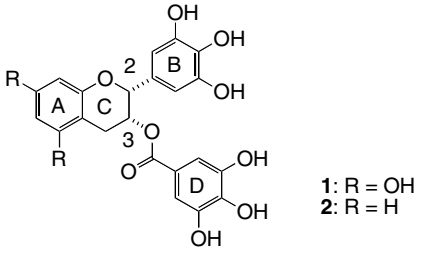


Figure 1. EGCG (**1**) and dideoxy-EGCG (**2**).

achieved via a formal oxy-conjugate addition of the phenolic hydroxyl group of **6** to **9**, and subsequent nitroaldol and condensation reactions gave desired nitrochromene derivative **10** in moderate chemical yield.[9]

Next, we focused on the direct transformation of the nitroolefin moiety to the ketone functional group. Although several Nef-type reactions are available for the transformation,[10] only reductive conditions with $TiCl_3$[11] reliably afforded ketone derivative **11** without the oxime intermediate.[12,13] Ketone **11** was relatively unstable under atmospheric conditions, the product from the $TiCl_3$ reaction was directly subjected without purification to further reduction with L-Selectride to give **12** as a single product with the desired stereochemistry.[14] The relative configuration of **12** was confirmed by the coupling constant for the protons at C2 and C3 on ^1H NMR.[6]

After condensation with 3,4,5-tribenzyloxybenzoic acid (**13**),[15] all of the benzyl groups were removed under catalytic hydrogenation conditions with $Pd(OH)_2$ to afford **2**,[16] which cannot be theoretically obtained through a polyketide biosynthetic pathway.

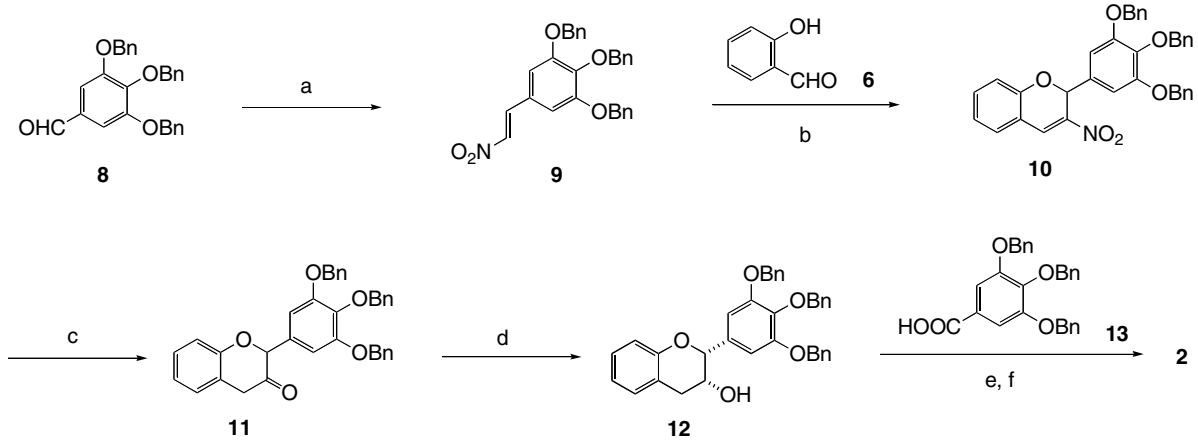
Because EGCG is known to possess anti-influenza virus activity,[3a] we then moved to a preliminary evaluation of the activity of **2**.[17] As shown in Table 1, catechin **2** showed a potent inhibitory effect of the infection of the influenza virus (A/memphis/1/71, H3N2) to MDCK cells with 11.92 μM of IC_{50}, which is three times higher than that of **1** (IC_{50}: 41.25 μM). This result suggests that the hydroxyl groups on the A-ring are unnecessary for the inhibition of the influenza virus infection.

Furthermore, considering this potent bioactivity, further chemical modifications such as introducing a biotin tag and/or photoactivatable groups such as benzophenone might be possible on the A-ring without losing the bioactivities.[18] This is advantageous for further development of EGCG probe molecules.

Table 1. Inhibition of influenza A virus infection to MDCK cells

Compound	Complement inhibition IC_{50}[a] (μM)
2	11.92 (±4.50)
EGCG (**1**)	41.25 (±15.45)

[a] Values are means of three experiments, standard deviation is given in parentheses.



Scheme 2. Preparation of dideoxy-EGCG (**2**). Reagents and conditions: (a) CH_3NO_2 (3.0 equiv), piperidine (3.0 equiv), AcOH (3.0 equiv), toluene, 80 °C, 63%; (b) salicylaldehyde (**6**) (1.5 equiv), DABCO (10 mol%), CH_2Cl_2, sealed tube, 60 °C, 54%; (c) $TiCl_3$ (5.0 equiv), $AcONH_4$ (2.0 equiv), 1,4-dioxane–50% AcOH (5:1), rt; (d) L-Selectride (2.0 equiv), THF, rt, 38% (2 steps); (e) 3,4,5-tribenzyloxybenzoic acid (**13**) (1.0 equiv), EDCI (1.5 equiv), DMAP (0.1 equiv), CH_2Cl_2, rt, 69%; (f) $Pd(OH)_2$, H_2, THF–MeOH (5:1), rt, 57%.

In conclusion, the synthesis of EGCG analog **2** has been performed via a concise synthetic route with the selective construction of the 2,3-*cis* stereochemistry. The methodology presented here should be easily applicable to further functionalized analogs. Thus, this methodology should promote a detailed structure–activity relationship study. Further preparation of EGCG derivatives, investigation of the anti-influenza virus activity mechanism, and a survey of other bioactivities such as anti-Alzheimer's disease effect[4] are currently underway.

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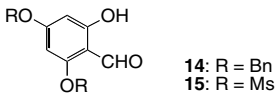
6. Recently an efficient synthesis of EGCG, including the direct formation of 2,3-*cis* stereochemistry by reductive intramolecular etherification, was reported. See: Kitade, M.; Ohno, Y.; Tanaka, H.; Takahashi, T. *Synlett* **2006**, 2827.
7. For examples, see: Ref. 5a,b. In Ref. 5a, a satisfactory yield of 2,3-*cis* derivative was obtained using this strategy, but a small amount of 2,3-*cis* stereochemistry was directly constructed via an intramolecular substitution reaction of the phenolic hydroxyl group and the benzyl bromide moiety.
8. To construct this ring, DABCO is the most effective. Other basic conditions using Et_3N, DBU, CsF, and NaH as well as acidic conditions employing $BF_3 \cdot Et_2O$, $SnCl_4$, and acetic acid were ineffective. These results suggest that the reaction proceeds via Baylis-Hillman type catalytic cycles. For other examples with similar ring formations, see: (a) Yan, M.-C.; Jang, Y.-J.; Yao, C.-F. *Tetrahedron Lett.* **2001**, *42*, 2717; (b) Yao, C.-F.; Jang, Y.-J.; Yan, M.-C. *Tetrahedron Lett.* **2003**, *44*, 3813.
9. Bn and Ms protected hydroxyl groups to, respectively, construct rings with A-ring moieties **14** and **15**, which both possess electron donation and withdrawing groups, were unsuccessful. In both cases, only the starting aldehydes were recovered.



14: R = Bn
15: R = Ms

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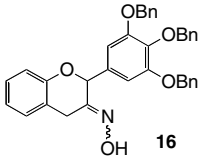
12. The reductive conditions with Zn/AcOH and Pb/AcOH afforded only oxime derivative **16**.



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13. The one-pot transformation of the nitrochromene derivative to the ketone derivative, which includes two steps ($NaBH_4$ reduction and $CrCl_2$ reduction in 10% aqueous HCl), has been reported. However, further selective reduction to the 2,3-*cis* catechin derivative has not been investigated. See: Rao, T. S.; Trivedi, G. K. *Indian J. Chem.* **1985**, *24B*, 1159.
14. *Experimental procedure for* **12**: $TiCl_3$ (872 μL, 1.35 mmol) was added to a solution of **10** (154 mg, 0.266 mmol) and $AcONH_4$ (43 mg, 0.54 mmol) in dioxane –50% AcOH (5:1, 2.5 mL) under an Ar atmosphere. The mixture was stirred for 17 h at room temperature. Then H_2O was added to the reaction mixture, extracted with EtOAc, dried over anhydrous $MgSO_4$, and evaporated to afford a mixture (166 mg) containing **11** as a yellow oil. The mixture (166 mg) in THF (4 mL) was cooled at −78 °C and L-Selectride (1.0 M solution in THF, 540 μL, 0.54 mmol) was added at −78 °C under an Ar atmosphere. The mixture was allowed to warm to room temperature and stirred for 24 h. Then satd. $NaHCO_3$ aq was added to the reaction mixture, extracted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by chromatography on a silica gel column (*n*-hexane–EtOAc, 3:1) to afford **12** (55 mg, 38%, 2 steps) as a yellow oil. Spectral data of **12**: ^1H NMR (500 MHz, $CDCl_3$): δ 2.95 (dd, $J = 1.8$, 16.4 Hz, 1H), 3.23 (dd, $J = 4.3$, 16.4 Hz, 1H), 4.22 (br s, 1H), 4.97 (s, 1H), 5.07 (s, 2H), 5.14 (s, 4H), 6.82 (s, 2H), 6.9–7.5 (m, 19H); MS (FAB) *m/z* 545 $(M+H)^+$; HRMS calcd for $C_{36}H_{33}O_5$ $(M+H)^+$ 545.2328. found 545.2337.
15. Nakazono, M.; Ma, L.; Zaitsu, K. *Tetrahedron Lett.* **2002**, *43*, 8185.
16. Spectral data of **2**: ^1H NMR (270 MHz, acetone-d_6): δ 2.97 (d, $J = 17.8$ Hz, 1H), 3.41 (dd, $J = 4.0$, 17.8 Hz, 1H), 5.16 (s, 1H), 5.56 (br s, 1H), 6.63 (s, 2H), 6.98 (s, 2H), 6.8–7.2 (m, 4H); MS (FAB) *m/z* 427 $(M+H)^+$; HRMS calcd for $C_{22}H_{19}O_9$ $(M+H)^+$ 427.1029. found 427.1054.
17. *A general procedure for the Inhibition of the influenza virus infection by the analog of catechin*. Each sample (1 mg/ml) in a serum free medium (SFM) (hybridoma-SFM complete DPM, Invitrogen Corp. NY, USA) was serially diluted twofold with SFM. Seventy-five microliters of each sample dilution was mixed with 75 μl of an influenza virus A/Memphis/1/71 (H3N2) suspension (100 FFU) in SFM and then incubated at 4 °C for 1 h. Confluent monolayers of Madine-Darby canine

kidney (MDCK) cells in 96-well microplates (Corning Costar Corporation, Cambridge, MA) were inoculated with 100 μl of the mixture at room temperature. After 1 h at 34 °C, the inoculum was removed from each plate, the monolayers were washed three times with PBS, and incubated for 16 h at 34 °C in 100 μl SFM. The monolayers in each well were washed three times with PBS, fixed with 50 μl of methanol at room temperature for 5 min, and washed three more times with PBS. Infectious foci of cells were detected by focus-forming assay as previously described (a,b) using an Anti-NP monoclonal antibody and horseradish peroxidase–conjugated goat anti-mouse immunoglobulin G plus M (IgG + M) antibody. Infectious cells were defined as the mean of three counts of blue-stained cells within one well. The virus infection was determined as focus-forming units (FFU). A concentration that caused 50% inhibition of FFU was determined by plotting the percentage inhibition against the concentration of each sample. (a) Suzuki, T.; Takahashi, T.; Guo, C.-T.; Hidari, IP. J. K.; Miyamoto, D.; Goto, H.; Kawaoka, Y.; Suzuki, Y. *J. Virol.* **2005**, *79*, 11705; (b) Aoki, C.; Hidari, IP. J. K.; Itonori, S.; Yamada, A.; Takahashi, N.; Kasama, T.; Hasebe, F.; Islam, M. A.; Hatano, K.; Matsuoka, K.; Taki, T.; Guo, C.-T.; Takahashi, T.; Sakano, Y.; Suzuki, T.; Miyamoto, D.; Sugita, M.; Terunuma, D.; Morita, K.; Suzuki, Y. *J. Biochem.* **2006**, *139*, 607.

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