Facile synthesis of 4″–*O*–alkyl (–)-EGCG derivatives through regioselective deacetylative alkylation

Yujin Seo[1], Mi Kyoung Kim[1], Hyunah Choo[2,3], Youhoon Chong[1]

[1]Department of Integrative Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Hwayang-dong, Gwangjin-gu, Seoul, Korea, [2]Center for Neuro-Medicine, Korea Institute of Science and Technology, Hawolgok-dong, Seoungbuk-gu, Seoul, Korea, [3]Department of Biological Chemistry, Korea University of Science and Technology, Youseong-gu, Daejeon, Korea

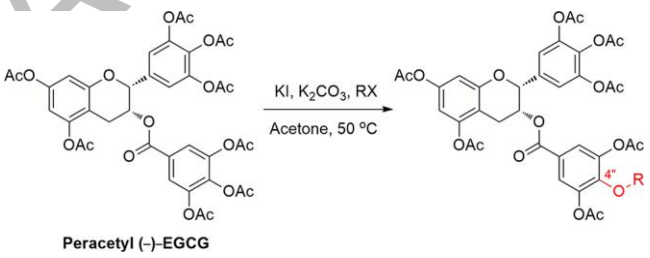
[†]These two authors contributed equally to this work

Corresponding authors: HC, E-mail: hchoo@kist.re.kr
YC, E-mail: chongy@konkuk.ac.kr

Abstract

Therapeutic potential of the D–ring methyl ethers of (–)–epigallocatechin–3–gallate ((–)–EGCG) warrants extensive structure–activity relationship study of various D–ring ethers of (–)–EGCG but, for this purpose, efficient synthetic strategy needs to be developed. In this study, efficient preparation of the 4″–*O*–alkyl–(–)–EGCGs (**4a**–**4e**) was demonstrated by employing KI/K_2CO_3–promoted deacetylative alkylation of peracetyl (–)–EGCG, which could be broadly utilized for preparation of various D–ring alkyl ethers of (–)–EGCG and thereby extensive structure–activity relationship study.

GRAPHICAL ABSTRACT



KEYWORDS: (–)–EGCG, regioselectivity, deacetylative alkylation

INTRODUCTION

(–)–Epigallocatechin–3–gallate ((–)–EGCG, Figure 1), the major polyphenol in green tea, has been reported to possess numerous health–promoting effects.[1] (–)–EGCG is characterized by its 8 phenolic hydroxyl groups on ring A (5–OH and 7–OH), ring B (3′–OH, 4′–OH, and 5′–OH), and ring D (3″–OH, 4″–OH, and 5″–OH), and they are known to play critical roles in the biological activity of (–)–EGCG.[2] On the other hand, D–ring methyl ethers of (–)–EGCG such as (–)–3″–Me–EGCG and (–)–4″–Me–EGCG (Figure 1) also showed potent anti–allergic effects[3] as well as inhibitory activity against matrix metalloproteinases[4], which indicated that an alkyl substitution on the D–ring hydroxyl groups would be beneficial for potentiating the biological activity of (–)–EGCG.

Therefore, for structure–activity relationship study, a synthetic method for the preparation of to the D–ring ethers of (–)–EGCG has been required and, until now, esterification of an appropriately substituted caffeic acid (**2**, Scheme 1) with *O*–pernitrobenzenesulfonyl (Ns)–protected (–)–EGC (**1**, Scheme 1) reported by Aihara *et al*.[5] (Scheme 1) has proved to be most efficient.

However, tedious protection–deprotection steps are associated with this coupling reaction (Scheme 1) and, for facile preparation of various (–)–EGCG derivatives with D–ring ether moiety, development of a more straightforward synthetic methodology has been required. Herein, we report KI/K_2CO_3–promoted deacetylative alkylation at the 4″–

position of the peracetyl (–)–EGCG and application of this novel reactivity to regioselective preparation of 4″–alkyl–(–)–EGCG derivatives.

RESULTS AND DISCUSSION

First, direct alkylation of (–)–EGCG was attempted by using the general polyphenol alkylation conditions (K_2CO_3 and MeI in refluxing acetone) (Scheme 2). The reaction resulted in many different alkylated (–)–EGCG derivatives. After extensive silica gel chromatography, the desired (–)–4″–Me–EGCG (**4a**, Scheme 2) could be isolated but in very low (12%) yield. The structure of **4a** was confirmed by comparison of its ^{1}H and ^{13}C NMR spectra with those reported previously[6], which showed 4.6–, 5.2–, and 1.6–ppm down–field shifts of C1″, C3″, and C4″ carbon peaks, respectively, upon methylation at the 4″–*O* position. Direct alkylation with other alkyl halides (ethyl iodide, propyl iodide, allyl bromide, and benzyl bromide) also provided the corresponding 4″–alkyl–(–)–EGCG derivatives (**4b**–**4e**, Scheme 2) in 10–14% yields.

Interestingly, however, analysis of the by–products formed during the direct alkylation of (–)–EGCG revealed that they are inseparable mixtures of multiple–alkylation products with the 4″–alkyl substituents in common. Therefore, we reasoned that, in the (–)–EGCG scaffold, alkylation occurs preferentially at the 4″–*O* position, but the problem is over–alkylation at other phenolic hydroxyl groups. We also reasoned that the 4″–selective

alkylation of (–)–EGCG might be attributed to the characteristic stabilizing resonance structure of the phenolate anion at the 4″–position (Figure 2).

Based on these observations, a new synthetic strategy for regioselective preparation of the 4″–alkyl–(–)–EGCG derivatives could be developed to include global protection of all the phenolic hydroxyl groups of (–)–EGCG followed by selective removal of the protecting group at the 4″–position. Thus, we attempted selective deprotection of the 4″–OAc group from the peracetyl (–)–EGCG[7] (**5**, Scheme 2). Treatment of (–)–EGCG with excess amount of Ac_2O in pyridine provided **5** in excellent yield[7] but, under various deacetylation conditions, selective deacetylation of **5** could not be achieved. Finally, the reaction conditions employed for preparation of 3,5-di-*O*-acetyl-4-*O*-(*para*-methoxybenzyl) methyl gallate from 3,4,5-tri-*O*-acetyl methyl gallate[8] was tested, and treatment of **5** with KI and K_2CO_3 in refluxing acetone provided the corresponding 4″–PMB ether in good yield (70%, data not shown). Prompted by this result, a regioselective deacetylative alkylation was attempted and, in the presence of KI, K_2CO_3, and alkyl halide in refluxing acetone, **5** was shown to nicely convert into 3′,3″,4′,5,5′,5″,7–*O*–heptaacetyl–4″–alkyl–EGCG derivatives (**6a**–**6e**, Scheme 2) in 65–75% yields. Interestingly, both KI and K_2CO_3 were required for successful transformation and, when KI or K_2CO_3 was used alone, the reaction did not proceed at all. After global deacetylation by $NaBH_4$ in MeOH[9], the desired 4″–alkyl–EGCGs (**4a**–**4e**) were obtained (72–85% yield).

CONCLUSION

In summary, regioselective synthesis of the therapeutically important 4″–alkyl–(–)–EGCGs (**4a**–**4e**) was accomplished in good yields by employing KI/K_2CO_3–promoted deacetylative alkylation, which can be broadly utilized for preparation of various D–ring alkyl ethers of (–)–EGCG and thereby extensive structure–activity relationship study.

EXPERIMENTAL

General Procedure For Synthesis Of 4″–Alkyl–(–)–Egcgs

Peracetyl (–)–EGCG[7] (**5**) (0.30 g, 0.38 mmol) was dissolved in acetone (5 mL) and, to this solution, K_2CO_3 (0.09 g, 0.05 mmol), KI (0.13 g, 0.76 mmol), and CH_3I (0.06 mL, 0.76 mmol) were added. After stirring at 50 ^oC for 4 h, the reaction mixture was cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography on silica gel (hexanes:acetone = 1:1) to give the desired product **6a** in 68% yield.

3′,3″,4′,5,5′,5″,7–*O*–Heptaacetyl–4″–methyl–EGCG (**6a**) obtained above was dissolved in MeOH (5 mL), and the resulting solution was treated with $NaBH_4$ (71 mg, 1.88 mmol) and then stirred for 6 h at rt. After addition of 2 M HCl, the reaction mixture was extracted with EtOAc three times. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2:MeOH = 10:1) to give **4a** as a pink solid (72% yield).

Characterization Data For Selected Compounds

5-((2R,3R)-5,7-Diacetoxy-3-((3,5-Diacetoxy-4-Methoxybenzoyl)Oxy)Chroman-2-Yl)Benzene-1,2,3-Triyl Triacetate (6a)

White solid; yield 68%; ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.50 (s, 2H), 7.42 (s, 2H), 6.74 (d, *J* = 2.2 Hz, 1H), 6.61 (d, *J* = 2.2 Hz, 1H), 5.74 (m, 1H), 5.53 (s, 1H), 3.84 (s, 3H), 3.21 (dd, *J* = 16.7 Hz, 4.6 Hz, 1H), 3.08 (dd, *J* = 17.9 Hz, 1.6 Hz, 1H), 2.26 (m, 21H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 168.03, 167.65, 166.99, 166.18, 163.12, 154.41, 149.69, 149.48, 148.31, 143.52, 141.17, 135.42, 134.22, 123. 95, 121.91, 118.52, 109.62, 108.71, 107.28, 75.97, 67.85, 60.09, 25.13, 19.59, 19.30, 19.27, 19.13, 18.66; HRMS (FAB) *m*/*z* Found: 767.1865 $[M+H]^+$. Calcd for $C_{37}H_{35}O_{18}$: 767.1823.

(2R,3R)-5,7-Dihydroxy-2-(3,4,5-Trihydroxyphenyl)Chroman-3-Yl 3,5-Dihydroxy-4-Methoxy Benzoate (4a)

Off-white solid; yield: 72%; ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.32 (br s, 1H), 8.26 (br s, 1H), 8.10 (br s, 1H), 7.81 (br s, 1H), 7.00 (s, 2H), 6.64 (s, 2H), 6.33 (d, *J* = 2.2 Hz, 1H), 6.03 (d, *J* = 2.2 Hz, 1H), 5.56 (m, 1H), 5.08 (s, 1H), 3.82 (s, 3H), 3.04 (dd, *J* = 17.4 Hz, 4.5 Hz, 1H), 2.92 (dd, *J* = 17.4 Hz, 2.1 Hz, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 164.44, 156.40, 156.09, 155.68, 149.73, 144.90, 139.05, 131.75, 129.29, 125.10, 108.54, 105.25, 97.53, 95.07, 94.41, 76.55, 68.29, 59.22, 25.24; HRMS (FAB) *m*/*z* Found: 495.0915 $[M+Na]^+$. Calcd for $C_{23}H_{20}O_{11}Na$: 495.0903.

FUNDING

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (2015R1A2A1A10053461) and by a grant from the Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093824).

SUPPORTING INFORMATION

Full experimental detail, ^{1}H and ^{13}C NMR spectra, high-resolution mass spectrometry data. This material can be found via the "Supplementary Content" section of this article's webpage.

REFERENCES

[1] (a) Stoner, G. D.; Muktar, H. *J. Cell. Biochem. Suppl.* **1995**, *22*, 169. (b) Landau, J. M.; Wanf, Z. Y.; Yang, G. Y.; Ding, W.; Yang, C. S. *Carcinogenesis* **1998**, *19*, 501. (c) Moyers, S. B.; Kumar, N. B. *Nutr. Rev.* **2004**, *62*, 204. (d) Zheng, Y.; Song, H. J.; Kim, C. H.; Kim, H. S.; Kim, E. G.; Sachinidis, A.; Ahn, H. Y. *J. Cardiovasc. Pharmacol.* **2004**, *43*, 200.

[2] (a) Higdon, J. V.; Frei, B. *Crit. Rev. Food Sci. Nutr.* **2003**, *43*, 89. (b) Barras, A.; Mezzetti, A.; Richard, A.; Lazzaroni, S.; Roux, S.; Melnyk, P. Betbeder, D.; Monfilliette-Dupont, N. *Int. J. Pharm.* **2009**, *379*, 270.

[3] (a) Maeda-Yamamoto, M.; Inagaki, N.; Kitaura, J.; Chikumoto, T.; Kawahara, H.; Kawakami, Y.; Sano, M.; Miyase, T.; Tachibana, H.; Nagai, H.; Kawakami, T. *J.*

Immunol. **2004**, *172*, 4486. (b) Suzuki, M.; Yoshino, K.; Maeda-Yamamoto, M.; Miyase, T.; Sano, M. *J. Agric. Food Chem.* **2000**, *48*, 5649.

[4] Maeda-Yamamoto, M.; Suzuki, N.; Sawai, Y.; Miyase, T.; Sano, M.; Ohta, A.; Isemura, M. *J. Agric. Food Chem.* **2003**, *51*, 1858.

[5] Aihara, Y.; Yoshida, A.; Furuta, T.; Wakimoto, T.; Akizawa, T.; Konishi, M.; Kan, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4171.

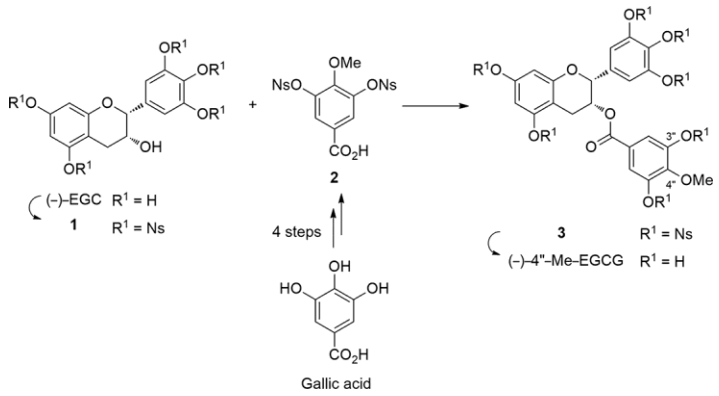
[6] Wan, S. B.; Dou, Q. P.; Chan, T. H. *Tetrahedron* **2006**, *62*, 5897.

[7] Lam, W. H.; Kazi, A.; Kuhn, D. J.; Chow, L. M. C.; Chan, A. S. C.; Dou, Q. P.; Chan, T. H. *Bioorg. Med. Chem. Lett.* **2004**, *12*, 5587.

[8] Kuboki, A.; Yamamoto, T.; Taira, M.; Arishige, T.; Konishi, R.; Hamabata, M.; Shirahama, M.; Hiramatsu, T.; Kuyama, K.; Ohira, S. *Tetrahedron Lett.* **2008**, *49*, 2558.

[9] Kohri, T.; Nanjo, F.; Suzuki, M.; Seto, R.; Matsumoto, N.; Yamakawa, M.; Hojo, H.; Hara, Y.; Desai, D.; Amin, S.; Conaway, C. C.; Chung, F. –L. *J. Agric. Food Chem.* **2001**, *49*, 1042.

Scheme 1. Known synthetic protocol for preparation of (–)–4″–Me–EGCG[5]



Scheme 2. Deacetylative alkylation in comparison to direct alkylation for preparation of 4″–alkyl–(–)–EGCGs (**4a**–**4e**)

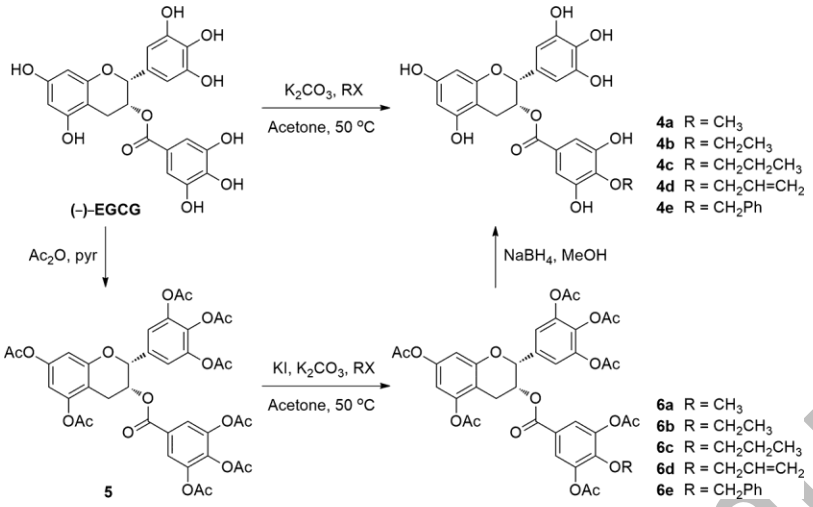
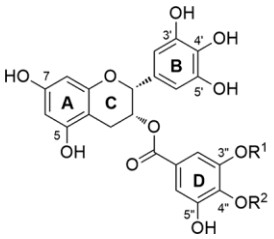


Figure 1. Structures of the indicated compounds



(–)-EGCG $R^1 = R^2 = H$

(–)-3"-Me-EGCG $R^1 = Me$, $R^2 = H$

(–)-4"-Me-EGCG $R^1 = H$, $R^2 = Me$

Figure 2. Resonance–stabilization of the 4″–O phenolate anion of (–)–EGCG