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Synthesis of phosphates and phosphates–acetates hybrids of green tea polyphenol (−)-*epi*gallocatechine-3-gallate (EGCG) and its G ring deoxy analogs as potential anticancer prodrugs

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ABSTRACT

A series of phosphate or phosphate–acetate hybrid modified EGCG or EGCG G ring deoxy analogs were synthesized by a convenient semi-synthesis strategy from the abundant natural compound EGCG.

Green tea, produced from the unfermented dried leaves of the plant *Camellia sinensis*, originated in China and has been consumed by humans for thousands of years. Regular drinking of green tea has been associated with many health benefits.[1,2] Since tea consumption is generally not associated with any toxic effect, the attraction of using green tea extract as potential therapeutic agents is considerable.[3] Polyphenolic catechins constituents are thought to contribute to the biological effects of green tea. A number of catechins have been identified and the major ones are (−)-*epi*catechin (EC), (−)-*epi*catechin gallate (ECG), (−)-*epi*gallocatechin (EGC) and (−)-*epi*gallocatechin gallate (EGCG). EGCG is found to be the most abundant and significant active compound among them.[4,5] It is known that a number of cancer-related proteins are affected by tea polyphenols, in particular by EGCG. However, the exact mechanism of tea-mediated cancer prevention is under active investigations.[6] Several years ago, we proposed that inhibition of proteasome may be a key mechanism in the cancer prevention activity of green tea.[7] Furthermore, it is the gallate ester bond-containing tea polyphenols (e.g., ECG, EGCG) but not the flavan-3-ols (e.g., EC, EGC), which inhibit the proteasomal chymotrypsin-like (β5) activities of the proteasome[8] and are responsible for the cancer prevention activity.

A major challenge in extrapolating the biological activities of green tea polyphenols in vitro to possible effects in vivo is bioavailability. In this respect, it is known that EGCG itself has poor bioavailability.[9] EGCG (Fig. 1) is relatively unstable under neutral or alkaline conditions and could be rapidly degraded, involving deprotonation of hydroxyl groups on the phenol rings. Moreover, the hydroxyl groups of EGCG could be modified through biotransformation reactions, such as methylation, glucuronidation, and sulfate formation, resulting in reduced biological activities in vivo. We have suggested that EGCG peracetate (**1**, Fig. 1) which can be converted to EGCG under cellular conditions by esterases with enhanced bioavailability in vivo can act as a prodrug.[10] Even though it is not an inhibitor of proteasome in cell-free system, **1** is more potent than EGCG at inhibiting the proteasomal chymotrypsin-like activity in human breast cancer MDA-MB-231 cells.[11] More importantly, the enhanced bioactivity also manifested in animal xenograft models.[11,12] This progress has encouraged us to screen other potential EGCG prodrugs in order to enhance and promote more desirable qualities, such as chemical stability, bioavailability and site selectivity.

Phosphate esters of alcohol or phenol functionalities have been used as a prodrug approach. For example, a combretastatin A-4

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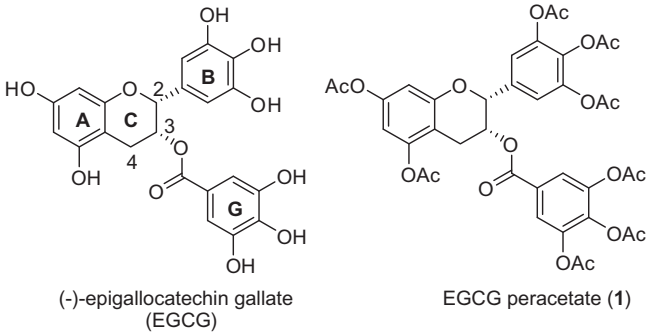
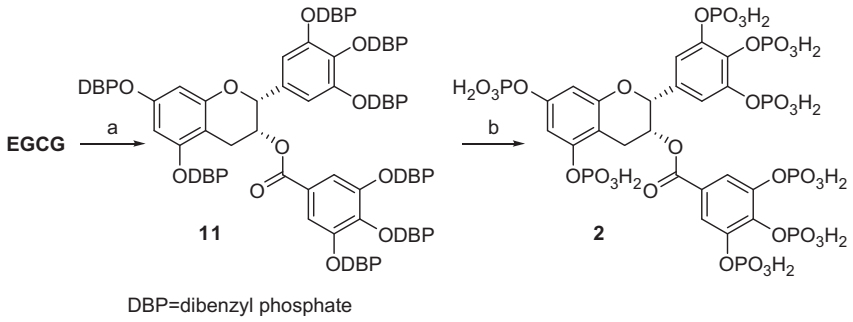


Figure 1. EGCG and EGCG peracetate (**1**).

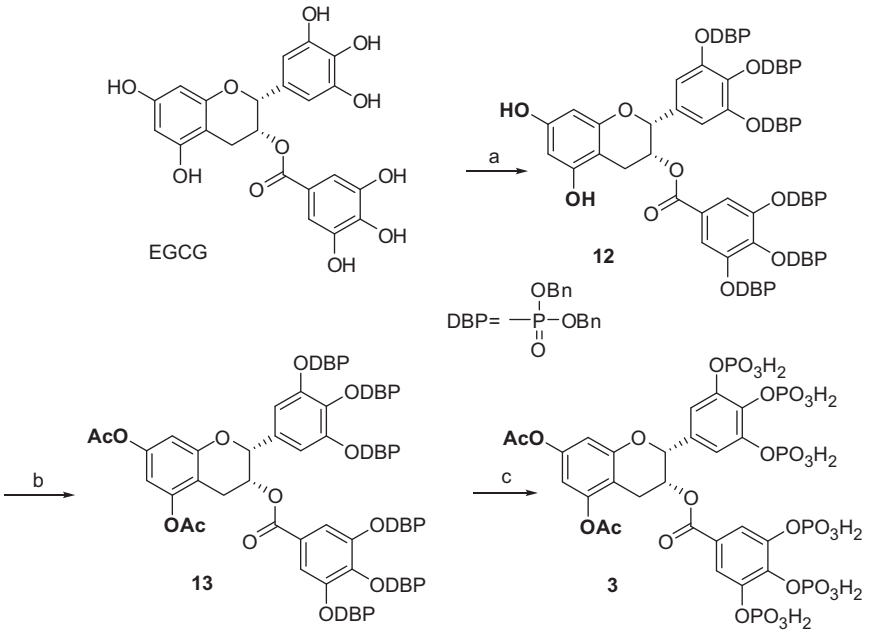
phosphate prodrug is now undergoing phase 1 clinical trial with the potential for combination with other conventional antitumour drugs and radiotherapy.[13–15] The phosphate salt itself is inactive but there is rapid phosphate hydrolysis in vivo to produce combretastatin A-4.[15] Another example is the successful development of Amifostine as the first broad-spectrum cytoprotective agent, on the basis of higher concentration and activity of alkaline phosphatase (which dephosphorylates phosphate esters) in normal cell.[16] An intriguing possibility is to use the cyclic phosphate prodrug approach to direct the parent drug selectively to the liver by taking advantage of the cytochrome P-450 catalyzed oxidation predominantly in hepatocytes.[17–19] Such liver-targeted drug delivery approach has been applied to a collection of nucleosides.[19]

These considerations have prompted us to examine the potential of phosphates or phosphate–acetate hybrids of EGCG and analogs as prodrugs.

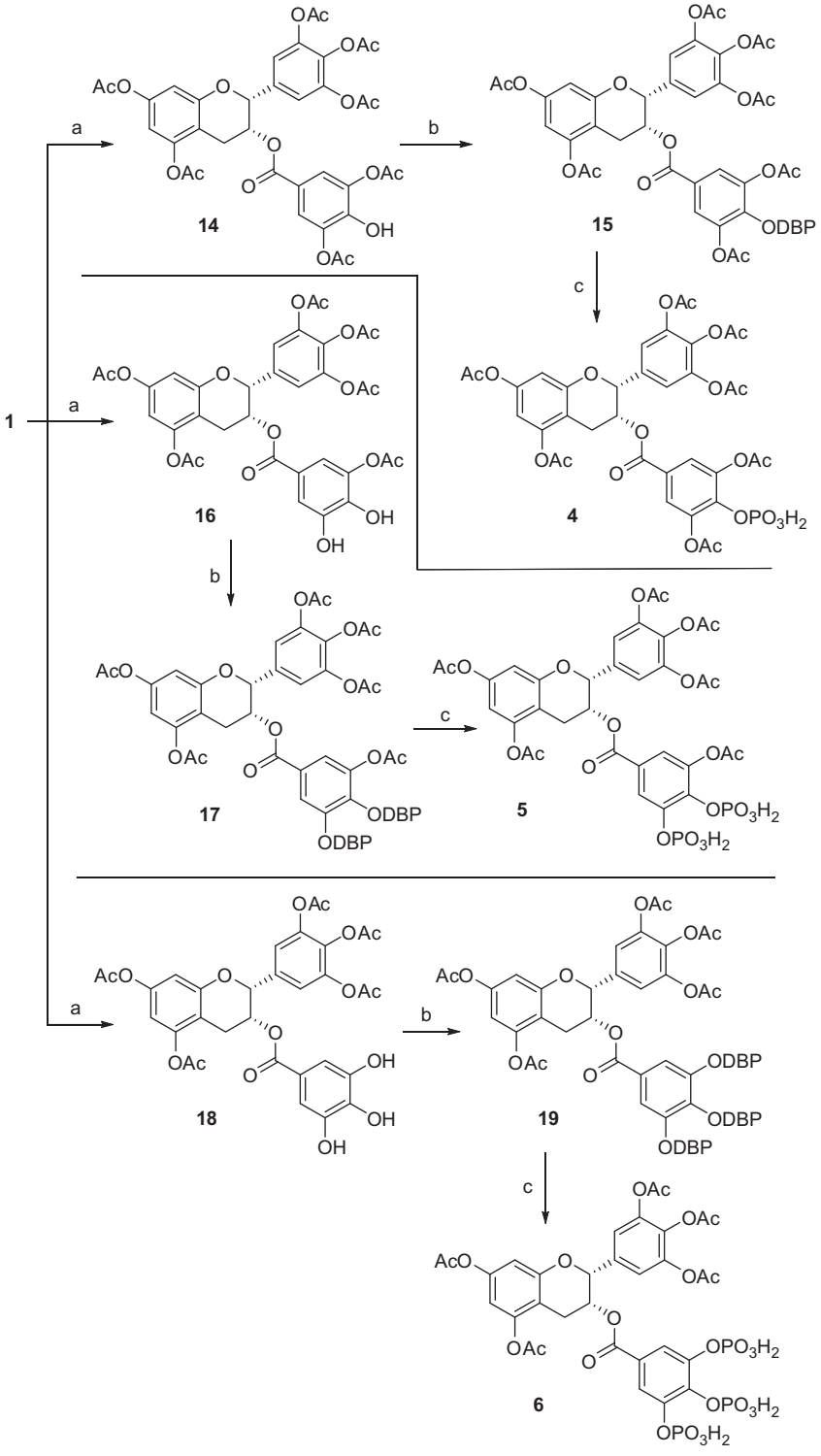
A challenge in EGCG chemistry is to differentiate the reactivity of the eight phenolic OH groups. When EGCG was treated with excess dibenzyl phosphite with carbon tetrachloride (CCl_4) together with DMAP/DIPEA in CH_3CN at −10 °C, EGCG was converted to the corresponding dibenzyl phosphate ester **11** (38% yield) with all eight OH phosphorylated (Scheme 1). The phosphorylation was believed to occur through the intermediate dibenzyl chlorophosphate which was generated in situ from the reaction of dibenzyl phosphite with CCl_4.[20] The ^{31}P NMR of **11** showed the expected six signals in the relative intensity ratios of 1:1:1:2:1:2 for the phosphate groups in A, B and G rings. The ^{1}H NMR of **11** also showed the aromatic protons to be well separated and easily assigned to those in A (δ 6.94 and 6.61), B (7.79) and G (7.52) rings. Their chemical shifts are all shifted downfield about 0.8 ppm compared with the same protons in EGCG due to the electron



Scheme 1. Reagents and conditions: (a) Dibenzyl phosphite (8.5 equiv), CCl_4 (40 equiv), DMAP (0.8 equiv), DIPEA (16 equiv), MeCN, 0 °C, 2 h; (b) Pd/C, H_2, THF/MeOH, 4 h.



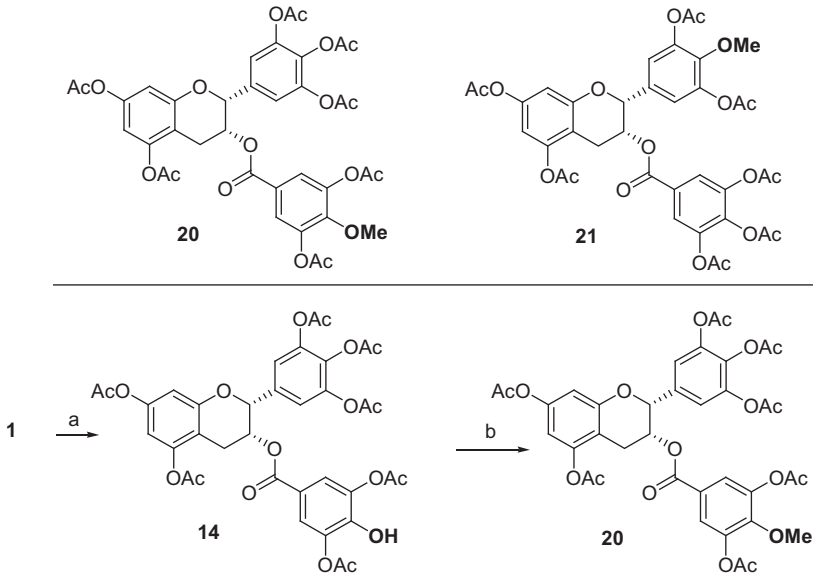
Scheme 2. Reagents and conditions: (a) Dibenzyl phosphate (6 equiv), CCl_4 (30 equiv), DMAP (0.6 equiv), DIPEA (12 equiv), MeCN, 0 °C, 1 h; (b) DMAP (2.1 equiv), Ac_2O (2.1 equiv), MeCN, 5 min; (c) Pd/C, H_2, THF/MeOH, 4 h.



Scheme 3. Reagents and conditions: (a) $NaHCO_3$, MeCN, reflux, 2.5 h or Li_2CO_3, MeCN, reflux, 4 h or Lipase, *n*-butanol, THF, 24 h; (b) dibenzyl phosphite, CCl_4, DMAP, DIPEA, MeCN, 0 °C, 1 h; (c) Pd/C, H_2, THF/MeOH, 4 h.

withdrawing effect of the phosphate groups. These assignments are useful in deducing the structures of subsequent compounds. Removal of the benzyl groups by catalytic hydrogenation over Pd/C afforded EGCG octaphosphate **2** (75% yield).

When the same reaction of EGCG was carried out with only 6 equiv of dibenzyl phosphite, EGCG hexa-dibenzyl phosphate **12** was obtained as the major product in 45% yield. The assignment of structure **12** was based on the following considerations. In the ^{31}P NMR of **12**, there were only four signals in the ratio of 1:2:1:2 consistent with the structure. Equally instructive is the ^{1}H NMR: the A ring protons at 5.98 and 5.97 ppm are nearly the same chemical shifts of the A ring protons of EGCG at 6.08 and 6.05 ppm; whereas the B ring protons at 7.71 ppm and G ring protons at 7.50 ppm are similar to those of compound **11** at 7.79



Scheme 4. Reagents and conditions: (a) $NaHCO_3$, MeCN, reflux, 2.5 h or Li_2CO_3, MeCN, reflux, 4 h or Lipase, *n*-butanol, THF, 24 h; (b) (i)TBDMSCl, Im, DMF; (ii) MeI, KF, DMF, rt.

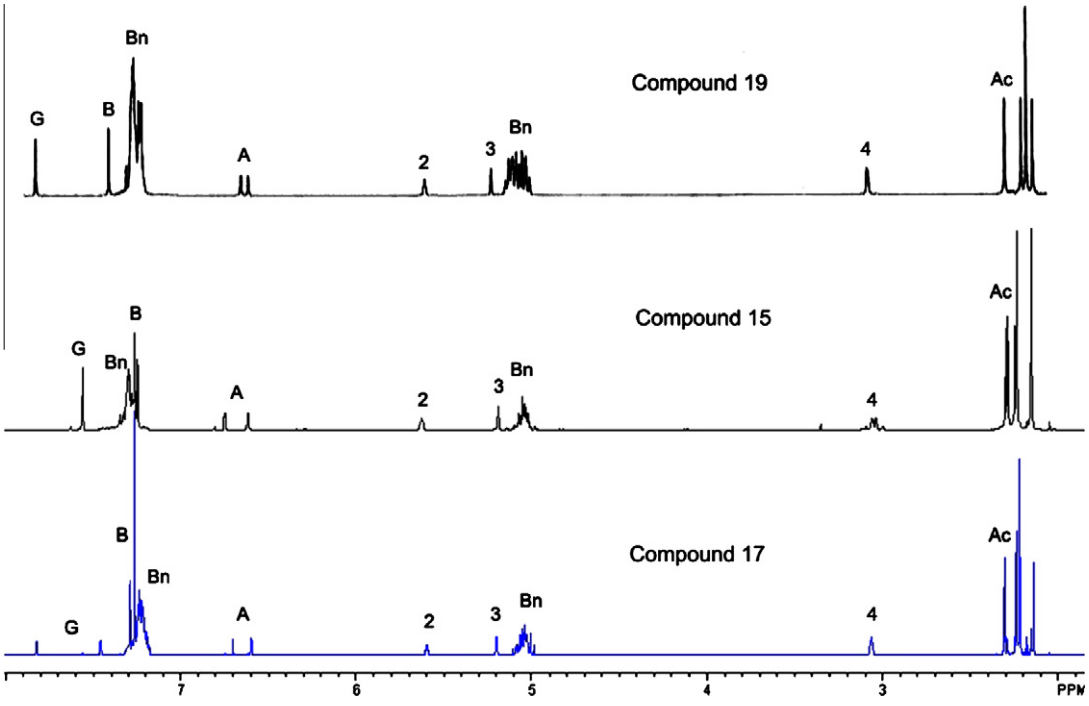


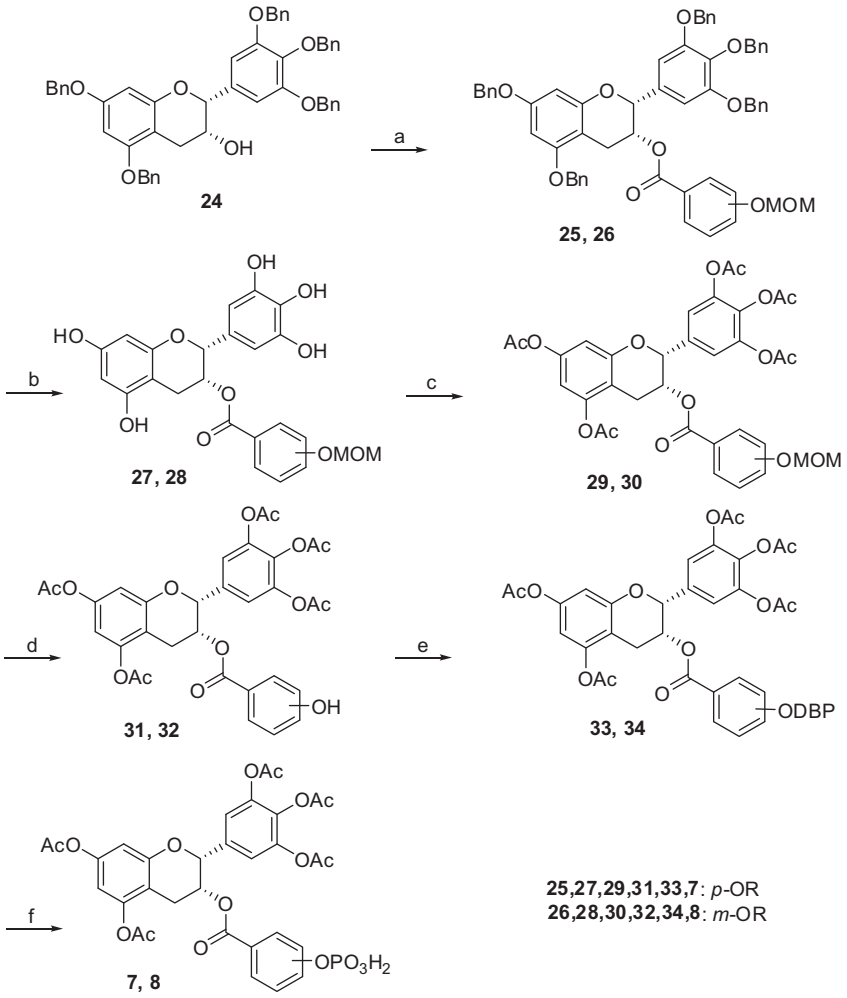
Figure 2. HNMR spectra of compounds **19**, **15**, **17**.

and 7.52, respectively. Acetylation of **12** gave the diacetate **13** (92% yield) which on hydrogenation gave the phosphate–acetate hybrid **3** (85% yield) (Scheme 2).

These results suggest that of the eight OH groups in EGCG, the two OH groups in the A-ring are less reactive toward phosphorylation under the reaction conditions. When less than 6 equiv of dibenzyl phosphite was used to see if selectivity among the remaining six OH groups could be achieved, a complex mixture of products was found.

In order to see if phosphorylation can be selectively introduced into the B or G ring, we examined the controlled hydrolysis of EGCG peracetate **1**. By stopping the reaction at the appropriate time and phosphorylate the intermediates, it was possible to obtain EGCG heptaacetate monophosphate **4** (23% yield) and hexaacetate di-phosphate **5** (18% yield) after phosphorylation and hydrogenolysis. If we prolonged the hydrolysis time properly, EGCG pentaacetate triphosphate **6** (11% yield) was obtained as well (Scheme 3).

The assignment of the structures of **4**, **5**, **6**, is based on the following argument. Firstly, our group had previously synthesized both 4″-*O*-methyl-EGCG heptaacetate **20** and 4′-*O*-methyl-EGCG heptaacetate **21**.[21] In the sequential hydrolysis of **1**, it was possible to methylate the first intermediate to give a EGCG heptaacetate monomethyl ether, identical to 4″-*O*-methyl-EGCG heptaacetate



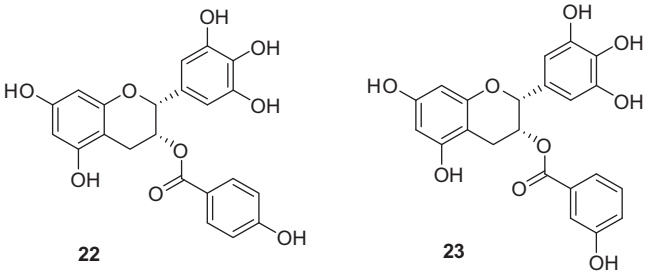
Scheme 5. Reagents and conditions: (a) 3-(Methoxymethoxy)benzoic acid or 4-(methoxymethoxy)benzoic acid, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 48 h; (b) Pd/C, H_2, EtOAc, 4 h; (c) Ac_2O, DMAP, MeCN, 1 h; (d) TMSBr, CH_2Cl_2, rt 4 h; (e) dibenzylphosphite, CCl_4, DMAP, DIPEA, MeCN, 2 h; (f) Pd/C, H_2, EtOAc, 4 h.

20 but distinctly different from **21** according to their NMR spectra. It follows therefore that the first EGCG heptaacetate monophosphate must have structure **4** (Scheme 4).

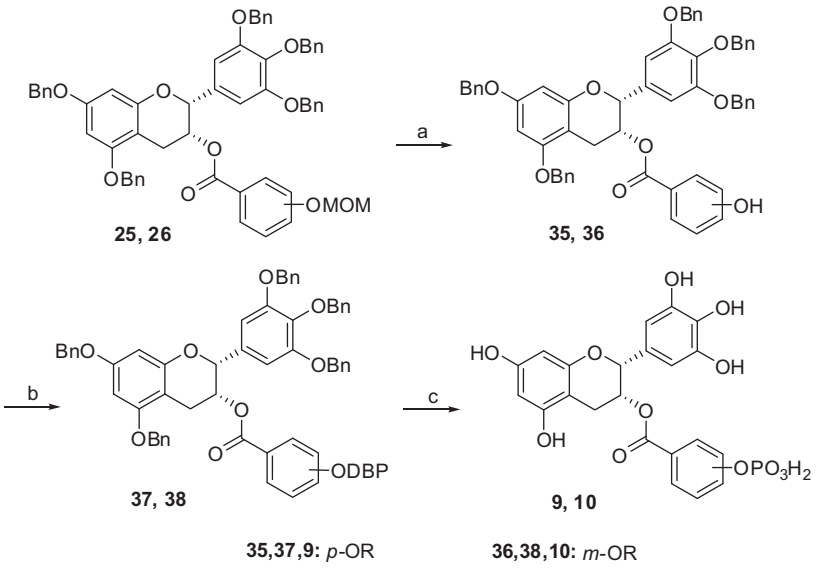
Secondly, as shown in Figure 2, in the spectrum of **15**, we can see that there are five singlets at about 2.3 ppm, which are attributed to five different acetate methyl hydrogens. Two of these five singlets are about twice as high as the others, and should be the two B ring *meta* acetates and the two G ring *meta* acetates. In the spectrum of **17**, there are five singlets at similar positions, but only one singlet has twice the intensity of the others, so we can conclude that the second hydrolyzed acetate should be on either the B ring or the G ring *meta* position. Thirdly, the two aromatic hydrogens of G ring of **15** are equivalent and they show up as a singlet at 7.56 ppm. In **17**, the two aromatic protons of G ring show as two distinct singlets at 7.82 ppm and at 7.46 ppm suggesting that they are no longer equivalent. On the other hand, the two aromatic protons of B ring remain as a singlet. We conclude therefore that the second hydrolyzed acetate occurred on G ring. In the spectrum of **19**, only four acetate singlets remain at around 2.3 ppm and the G ring aromatic hydrogens become equivalent again as a singlet so the third hydrolyzed acetate also occurred on G ring. Finally, the ^{31}P NMR of **15** showed only one signal, whereas that of **17** and **19** showed the expected two signals with relative intensity ratios of 1:1 and 2:1, respectively. These results are consistent with the above structural assignment.

It is reasonable to expect the 4″-acetate to be hydrolyzed first among all the acetates in **1** because this is the only one where there is an electron-withdrawing carbonyl group in the *p*-position. Once this acetate is hydrolyzed, the generated phenoxide anion would have a neighboring group effect in facilitating the hydrolysis of its adjacent acetates, leading to the hydrolysis of 3″- and 5″-acetates.

Previously, we had synthesized (2*R*,3*R*)-*epi*gallocatechin-3-*O*-(4-hydroxybenzoate), compound **22**, which occurs naturally in the plant *Cistus salvifolius*.[22] Compound **22**, together with its regioisomer (2*R*,3*R*)-*epi*gallocatechin-3-*O*-(3-hydroxybenzoate) (**23**) was found to be an inhibitor of proteasome and has the cytotoxicity comparable to EGCG. We thus want to prepare their phosphate derivatives as well.



We started with the benzylated EGC compound **24** which was readily prepared semi-synthetically from EGCG.[23] Compound **24** was benzoated with MOM protected 3- or 4-hydroxylbenzoic acid to give **25** (71%) or **26** (69%), respectively. The benzyl groups of **25**/



Scheme 6. Reagents and conditions: (a) TMSBr, CH_2Cl_2, rt 4 h; (b) dibenzylphosphite, CCl_4, DMAP, DIPEA, MeCN, 2 h; (c) Pd/C, H_2, EtOAc, 4 h.

26 were removed by catalytic hydrogenolysis and followed by acetylation to give **29**/**30** (91%/90%). The MOM group of **29**/**30** was then removed under acidic condition to give compound **31** (93%) or **32** (93%), after phosphorylation followed by catalytic hydrogenolysis, compounds **7** (92%) and **8** (90%) were obtained (Scheme 5).

As shown in Scheme 6, when the MOM group was removed from **25**/**26**, **35** (87%) and **36** (88%) were obtained. Also, the following phosphorylation and catalytic hydrogenolysis gave the corresponding phosphate **9** (80%) and **10** (82%), respectively.

In conclusion, we have therefore prepared regioselectively several phosphate and phosphate–acetate hybrid derivatives of EGCG and analogs. The bioactivity studies on these compounds are being evaluated and will be reported elsewhere.

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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.2011.08.061.

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