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Regioselective synthesis of methylated epigallocatechin gallate via nitrobenzenesulfonyl (Ns) protecting group

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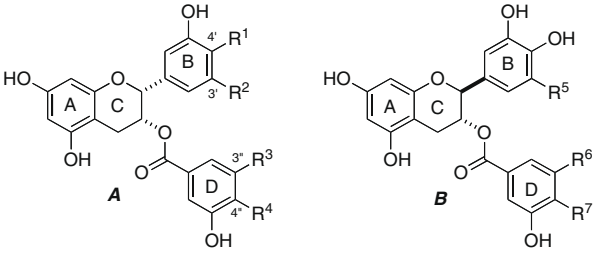
ABSTRACT

Regioselective synthesis of methylated epigallocatechin gallate from epigallocatechin was accomplished using a 2-nitrobenzenesulfonyl (Ns) group as a protecting group for phenols. This methodology provided several methylated catechins, which are naturally scarce catechin derivatives.

Epigallocatechin gallate (EGCG: **1**), which exhibits various biological activities, including cancer prevention, antiviral, or antimicrobial activities, is a major component of catechin derivatives derived from tea.[1] Recently, methylated derivatives, such as (−)-3″-Me-epigallocatechin gallate (3″-Me-EGCG: **2**), have been identified as scarce catechins in natural tea leaves and mammal metabolites of tea catechins.[2a] Because **2** and its regioisomer (4″-Me-EGCG: **3**) exhibit potent inhibitory activities against type I allergic reactions in mice[2a,b] and matrix metalloproteinases,[2c–g] other methylated catechin derivatives are also expected to possess therapeutic potential.

However, due to the availability of **2** and **3** from natural and synthetic sources,[3] investigations of the structure and activity relationship have been limited to these compounds. Considering naturally available catechins (EGC, GC, EC, and C), the ready supply of all possible regio- and stereoisomers of methylated catechins (Fig. 1), should be significant in systematic biological evaluation of these molecules. Hence, we are pursuing the concise synthesis of methylated catechins (**2**–**11**). Although many synthetic investigations of the catechin skeleton have been reported,[1a] including our group,[4] conversion of methylated catechins from natural catechins, which can be represented epigallocatechin (EGC) (**12**)[5] and other derivatives (GC, EC, and C), should be suitable due to an inexpensive and readily available natural source.



A	R^1	R^2	R^3	R^4
(−)-EGCG (**1**) :	OH	OH	OH	OH
(−)-3"-Me-EGCG (**2**) :	OH	OH	OMe	OH
(−)-4"-Me-EGCG (**3**) :	OH	OH	OH	OMe
(−)-3'-Me-EGCG (**4**) :	OH	OMe	OH	OH
(−)-4'-Me-EGCG (**5**) :	OMe	OH	OH	OH
(−)-3"-Me-ECG (**6**) :	OH	H	OMe	OH
(−)-4"-Me-ECG (**7**) :	OH	H	OH	OMe

B	R^5	R^6	R^7
(−)-3"-Me-GCG (**8**) :	OH	OMe	OH
(−)-4"-Me-GCG (**9**) :	OH	OH	OMe

ent-B	R^5	R^6	R^7
(+)-3"-Me-CG (**10**) :	H	OMe	OH
(+)-4"-Me-CG (**11**) :	H	OH	OMe

Figure 1. Structure of (−)-EGCG and methylated derivatives.

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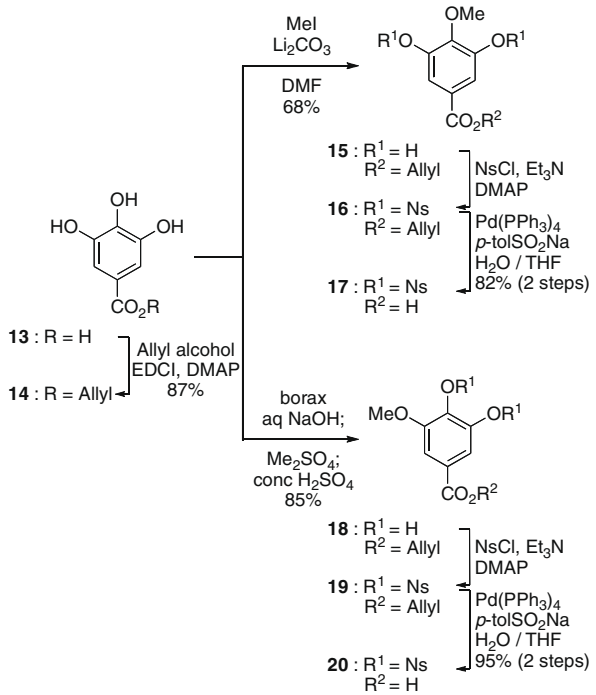
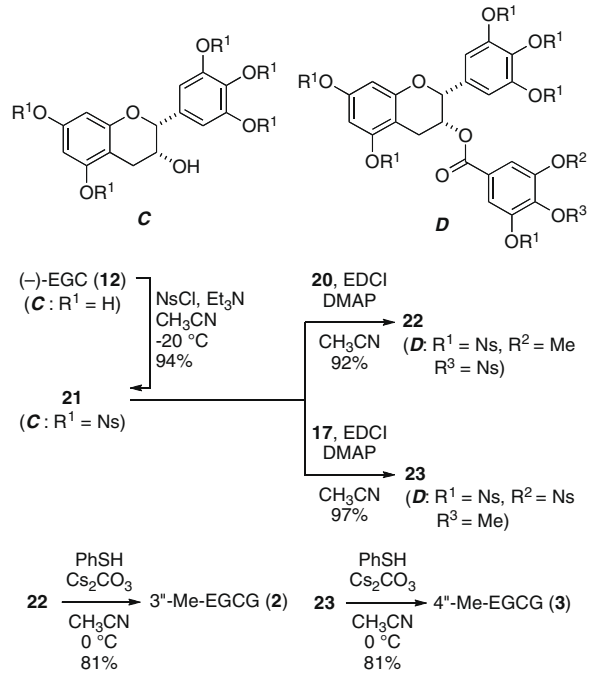


Scheme 1. Synthesis of selective methylated gallic acids.

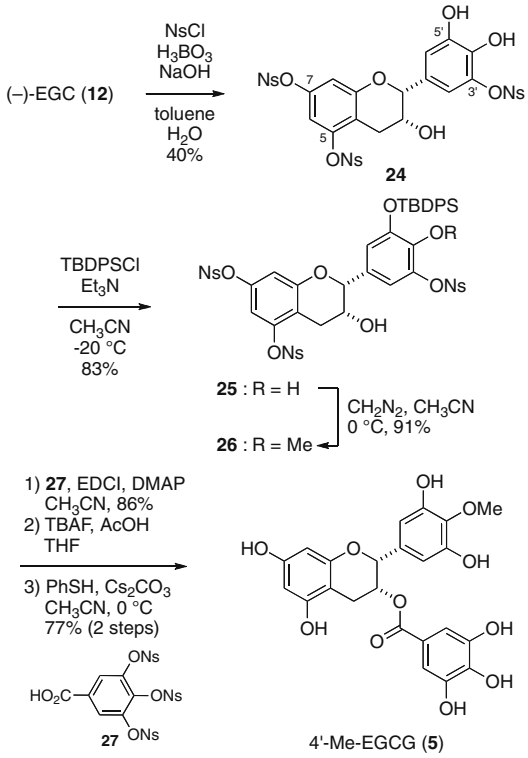
A significant task in the catechin synthesis is selecting a suitable protecting group for phenol. Although benzyl ether has been employed for catechin synthesis due to its readily deprotectable feature under hydrogenolysis conditions, ether formation of phenol is often troublesome, for example, epimerization of the 2-position occurs under basic conditions. Recently, the 2-nitrobenzenesulfonyl (Ns) group has been employed as a novel protecting and activating group for amines.[6] Moreover, the Ns group has been used in the synthesis of nitrogen containing complex natural products. The Ns group should be an ideal protecting group for labile polyphenols because it can be easily deprotected under mild conditions.[6,7] Furthermore, the electron-withdrawing nature of the Ns group should enhance the stability of polyphenol under various conditions. Herein, we report the efficient synthesis of methylated catechins (**2**–**11**) by exploiting the Ns protecting group for phenols.

As shown in Scheme 1, we initially investigated the selective incorporation of the methyl group to 3- and 4-OH of allyl gallate (**14**). Gallic acid (**13**) was converted to allyl ester **14** by treating with allyl alcohol and EDCI. Upon treating **14** with Li_2CO_3 and methyl iodide, selective deprotonation of the most acidic 4-OH and alkylation proceeded smoothly to provide **15**. On the other hand, 3-OH selective alkylation was performed utilizing a bridged boronic ester intermediate[8] between the *ortho* phenolic hydroxyl groups. After forming the boronic ester by treating borax and **14** in the presence of NaOH, methylation with Me_2SO_4 and subsequent acidic hydrolysis of the boronic ester provided **18** exclusively. The Ns group into the resultant phenols of **15** and **18** was incorporated by treating with NsCl and Et_3N to give **16** and **19**, respectively. Upon treating **16** and **19** with catalytic amounts of $Pd(PPh_3)_4$ and *p*-$tolSO_2Na$,[9] deprotection of the allyl group proceeded smoothly to provide desired **17** and **20**, respectively.

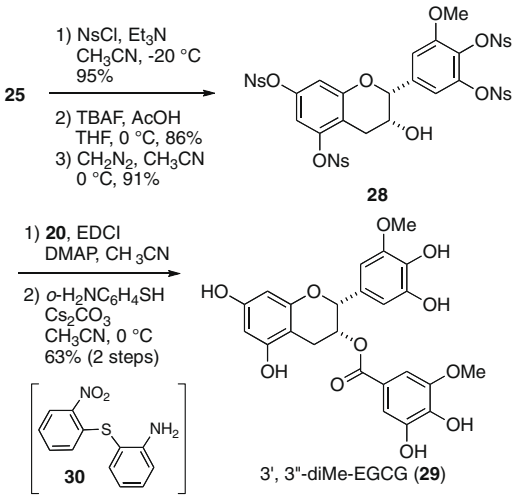
Next, we focused on incorporating gallate derivatives of **17** and **20** into protected epigallocatechin derivatives and deprotection of the Ns group (Scheme 2). Protection of EGC (**12**) with the Ns group was carried out by treating with NsCl and Et_3N to provide **21**. Upon treating **21** with **20** and EDCI, condensation reaction proceeded smoothly to provide desired **22** in high yield. A similar condensation of **21** and **17** provided desired methylated EGCG derivatives **23**. Deprotection of the Ns groups of **22** and **23** was accomplished by treating with thiophenol and cesium carbonate to provide 3″-methylated EGCG (**2**) and 4″-methylated EGCG (**3**), respectively. During this transformation, epimerizations at the 2-position of the benzopyran ring and decomposition of gallate ester were not observed. A similar protocol, which employed natural catechin



Scheme 2. Synthesis of 3″-Me-EGCG (**2**) and 4″-Me-EGCG (**3**).



Scheme 3. Synthesis of 4′-Me-EGCG (**5**).



Scheme 4. Synthesis of 3′, 3″-diMe-EGCG (**29**).

derivatives (GC, EC, and C), provided desired methylated gallate catechin derivatives **4**–**11**.[10]

Then we turned our attention to selectively incorporating a methyl group at the B-ring (Scheme 3). Because to our knowledge, (−)-EGCG derivatives methylated at the B-ring have yet to be reported, a SAR study of these compounds should be significant. However, there are few reports on selectively modifying the five phenolic hydroxyl groups of EGC. We found that a bridged boronic ester intermediate effectively distinguishes the hydroxyl groups at the A-ring and B-ring. Treating **12** with NsCl and H_3BO_3 in the presence of NaOH[11] causes the regioselective sulfonylation to proceed to afford predominantly 3′,5,7-Ns-EGCG (**24**). Next, selective incorporation of the TBDPS group at the less hindered hydroxyl group (5′-OH) was accomplished by treating with TBDPSCl and Et_3N to give **25**. The 4′-methylated EGCG derivatives were prepared after methylation of **25** by diazomethane, condensation of **26** with Ns-protected gallic acid **27**,[12] and stepwise deprotection of TBDPS and Ns group to yield 4′-Me-epigallocatechin gallate (4′-Me-EGCG: **5**).

As shown in Scheme 4 and 3′,3″-diMe-EGCG (**29**) was also synthesized from **25**. Protection of **25** with a Ns group, deprotection of the TBDPS group, and incorporation of a methyl group afforded **28**. Although condensation of **28** and **27**, and deprotection of Ns group readily provided 3′-Me-EGCG (**4**), a modified preparation with double methylated derivatives was demonstrated. After condensation **28** and **20**, deprotection of the Ns group was accomplished using 2-aminothiophenol instead of thiophenol to afford 3′,3″-diMe-EGCG (**29**).[13] The advantage of this method is 2-aminothiophenol is odorless compared to thiophenol. Furthermore, taking advantage of the amino group, the by-product, 2-(2-nitrophenylthio)aniline (**30**), can be removed by washing the ethereal layer with a 1 M HCl solution. Additionally, combining the selective methylation strategy on the B- and D-rings of EGCG should yield several types of double methylated EGCG derivatives. Furthermore, this regioselective modification of EGCG should be applicable for alkylation as well as acylation; thus, employing this synthetic strategy with the other natural catechins (GC, EC, and C) suggests the possibility of constructing a diverse catechin library.

Table 1
Inhibitory effects on MMPs

Catechins	IC_{50} (μM)		
	r-MT1-MMP	r-MMP-7	r-MMP-2
(−)-EGCG (**1**)	6.8	21.5	9.6
(−)-3″-Me-EGCG (**2**)	1.7	20	8.7
(−)-4″-Me-EGCG (**3**)	12.5	72	18.7
(−)-3″-Me-ECG (**6**)	15.6	>100	88
(−)-4″-Me-ECG (**7**)	3.7	>100	34
(−)-3″-Me-GCG (**8**)	3.1	20	5.4
(−)-4″-Me-GCG (**9**)	10.5	32	19
(+)-3″-Me-CG (**10**)	52	>100	28
(+)-4″-Me-CG (**11**)	>100	>100	22

With a variety of methylated catechin derivatives in hand, a preliminary biological investigation tested the inhibition of matrix metalloproteinases. As shown in Table 1, inhibitory activities were examined in a recombinant matrix metalloproteinases (r-MMP-2 and r-MMP-7) and a recombinant membrane-type 1 metalloprotease (r-MT1-MMP)[14,15] where catalytic domains were expressed in *Escherichia coli*. Monomethylated catechins (**2**, **3**, **6**–**10**) exhibited inhibitory activity against r-MMPs as shown in Table 1. The IC_{50} values against r-MMPs varied according to the position of methyl substitution. Among gallocatechin type derivatives, 3″-Me analogs (**2**, **8**) displayed more potent inhibitory activity to r-MT1-MMP and r-MMP-2 than 4″-Me analogs (**3**, **9**). In the case of r-MMP-7, methylation decreased the inhibitory activity. The weaker inhibitory activities of **6**, **7**, **10** and **11** against r-MMP-7 suggest that the number of hydroxyl group is important for intensity and specificity.

In summary, we have developed an efficient synthetic method to prepare methylated catechin derivatives utilizing the Ns protecting group for phenol. Furthermore, the present synthetic strategy for regioselective alkylation at the B-ring and gallate group should readily provide additional derivatives. Further exploitation, including probing the biological activities, is under investigation in our laboratory.

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Supplementary data

Supplementary data (general experimental procedures and characterization for all new compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.111.

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