Concise synthesis of catechin probes enabling analysis and imaging of EGCg†

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A concise synthesis of APDOEGCg (3) was accomplished. Due to the reactivity of its amine group, the compound could be easily converted to the fluorescein probe 21 and immunogen probe 22 efficiently. We then demonstrated the usefulness of the probes for imaging studies and the generation of antibodies.

(–)-Epigallalocatechin gallate (EGCg) (**1**), which is a major constituent of green tea extract,[1] has received special attention for its antitumor,[2] antiviral,[3] and other important bioactivities.[3] Due to these promising bioactivities,[4] EGCg and its derivatives are expected to constitute lead compounds for drug development. Thus, the imaging and/or analysis of the dynamics of **1** by probe molecules will be essential for future drug development.

However, there are few reports on the preparation of catechin probes.[5] Although conversion of **1** to a probe molecule would be an excellent strategy, the direct selective incorporation of a probe unit into **1** has been difficult due to the structural instabilities of **1** and the lack of appropriate tethering functional groups. Over the course of our synthetic investigations into **1**,[6] we found that the synthetic derivative **2** possessed a more potent anti-influenza infection activity than natural **1**.[6a] Inspired by this finding, we began a synthesis of the EGCg probe precursor **3** (6-(5-aminopentyl)-5,7-deoxy-epigallocatechin gallate: APDOEGCg), which contains linkers and reactive amino groups as shown in Fig. 1.[7] In this communication we report on the synthesis of probe precursor **3** and its conversion to catechin probe molecules **21** and **22**.

Our retrosynthetic analysis for **3** is described in Scheme 1. In our preliminary investigation into the incorporation of a linker unit in the DOEGCg derivatives, we attempted a Suzuki–Miyaura coupling[8] of the catechin skeletons with and without a gallate ester, but we could not produce the target compounds. We decided to incorporate the linker unit into a cyclization precursor **7a**, which was prepared by a condensation reaction of **8** and **9**. Incorporation of a reactive amino group at the terminal position of the linker was found to be suitable for

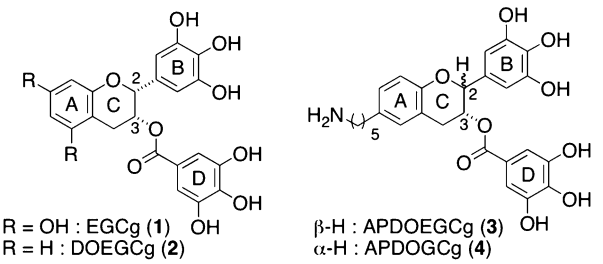
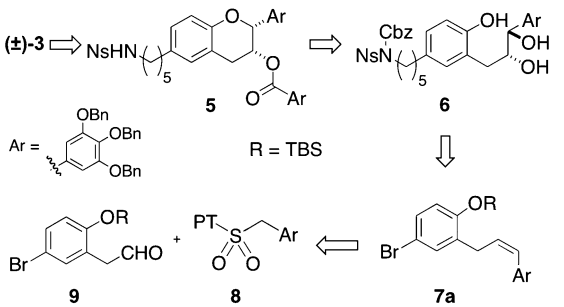


Fig. 1 Structures of EGCg (**1**), DOEGCg (**2**), APDOEGCg (**3**) and APDOGCg (**4**).



Scheme 1 Our synthetic strategy for the EGCg probe precursor **3**.

the Mitsunobu reaction with our Ns amide (2-nitrobenzene-sulfonamide)[9,10] under neutral reaction conditions. The separation of *cis* and *trans* isomers of the dihydrobenzopyran ring was readily accomplished by incorporating the gallate unit,[6b] and the dihydrobenzopyran ring of **5** was constructed by cyclization under acidic conditions from the diol **6** through the cationic intermediate.

As shown in Scheme 2,[11] condensations of the A- and B-ring were accomplished by the Julia–Kocieński reaction[12] between a phenyltetrazole (PT)-sulfone **8** and an aldehyde **9** to provide **7a** as a single isomer in 87% yield. The observed *Z*-selective reactivity[13] was similar to that reported in a previous synthesis.[6b] The subsequent incorporation of a linker group into **7a** was performed by the Suzuki–Miyaura coupling reaction.[8] After hydroboration of MOM-protected 4-pentenol **10** with 9-BBN, alkyl borate **11** was subjected to cross-coupling reactions without purification. Upon treatment of a mixture of borate **11** and **7a** with catalytic quantities of $PdCl_2$(dppf) and NaOH in THF, the coupling reaction proceeded smoothly to give **12** in high yields. After simultaneous deprotection of the MOM and TBS ether, incorporation of the amino group into **13** was accomplished by the Mitsunobu reaction[14,15] using Cbz and

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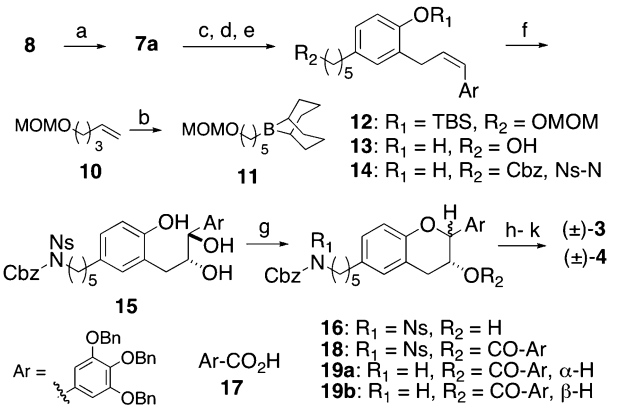


Scheme 2 Synthesis of EGCg probe precursors **3**. *Reagents and conditions:* (a) LHMDS, **9**, THF, 0 °C, 87%; (b) 9-BBN, THF, 50 °C; (c) **11**, $PdCl_2(dppf)\cdot CH_2Cl_2$, 3 M NaOH aq., THF, reflux; (d) conc. HCl, MeOH, 60 °C, 89%; (e) NsNHCbz, DMEAD, PPh_3, toluene, 72%; (f) OsO_4, NMO, acetone/H_2O, 72%; (g) $TsOH\cdot H_2O$, toluene, 60 °C, 89%; (h) EDCI, DMAP, toluene, 95%; (i) PhSH, Cs_2CO_3, MeCN, 92%; (j) Separation; (k) $Pd(OH)_2$, H_2, THF/MeOH, 89%. TBS = *tert*-butyldimethylsilyl, PT = phenyltetrazole; MOM = methoxymethyl, Cbz = carboxybenzyl; Ns = *o*-nitrobenzenesulfonyl, Bn = benzyl, LHMDS = lithiumhexamethyl disilazane, 9-BBN = 9-borabicyclo[3.3.1]nonane, dppf = 1,1′-bis (diphenylphosphino)-ferrocene, DMEAD = di-2-methoxyethyl-azodicarboxylate, Ts = *p*-toluenesulfonyl, EDCI = 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide, DMAP = 4-dimethylaminopyridine.

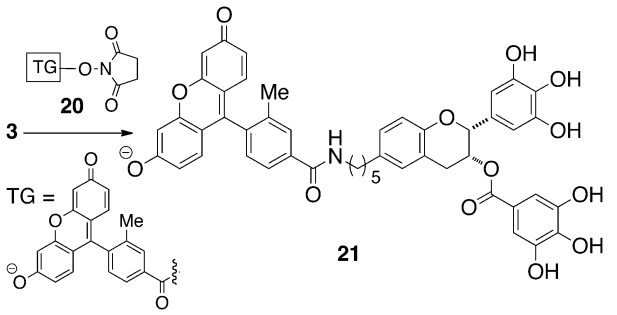
Ns-amide[9] (Ns strategy).[10] A racemic mixture of **3** and **4** was prepared by dihydroxylation of the *cis*-olefin **14** with OsO_4 and NMO to give the diol **15**.[16] Upon treatment of **15** with TsOH, the regioselective cyclization reaction proceeded smoothly to provide the desired dihydrobenzopyran **16** as a 1 : 1 mixture.[17] After incorporation of the gallate derivative **17**, deprotection of the Ns groups of **18** was performed by treatment with a thiol and base.[18] Separation of **19a** and **19b**[19] was readily accomplished by silica gel column chromatography. Finally, deprotection of all benzyl and Cbz groups under hydrogenolysis conditions afforded **3** and **4** from **19b** and **19a**, respectively.

With the desired EGCg derivatives in hand, we evaluated the inhibitory activities of **3** and **4** against influenza virus infection. As shown in Table 1, APDOEGCg (**3**) and APDOGCg (**4**) showed potent inhibition of the infectivity of the influenza virus A/Memphis/1/71 (H3N2) toward MDCK cells, yielding IC_{50} values of 4.18 and 4.40 μM, respectively.[11] These values were higher than those of the natural **1** and synthetic **2**.[20] The biological activity of a molecule usually decreases when a probe unit and/or linker group is attached to

Table 1 Inhibition of influenza A viral infectivity toward MDCK cells

Compound	Complement inhibition IC_{50}[a]/μM
EGCg (**1**)	66.3 (±9.21)
DOEGCg (**2**)	9.05 (±2.26)
APDOEGCg (**3**)	4.18 (±4.29)
APDOGCg (**4**)	4.40 (±2.36)

[a] Values are reported as the mean of three experiments, and the standard deviation is given in parentheses.



Scheme 3 Conversion of **3** to the fluorescein probe **21**.

the original compound. Contrary to expectations, the sustained activities of **3** and **4** demonstrated that incorporation of probe units and/or tags into **3** and **4** *via* terminal amino groups did not result in the loss of biological activity.[5d]

Encouraged by these results, we turned our attention to the preparation of the probe molecules from precursor **3**. Reactive amine **3** possessed an advantage for the incorporation of a probe unit furnished without the need for protection of the phenolic hydroxy groups. A preliminary study of probe molecule preparation was demonstrated by the synthesis of the fluorescein probe, which permitted *in vivo* imaging under physiological conditions. Among several fluorescein variants, we selected a reliable photophore, Tokyogreen (TG).[21] As shown in Scheme 3, a condensation of the probe precursor **3** and TG activated ester **20**[11] provided the fluorescein probe **21**.[11]

Once the desired probe **21**[22] had been synthesized, its usefulness for imaging studies was assessed using HUVECs (human umbilical vein endothelial cells).[23] After incubation of **21** with HUVECs for 3 h, the fluorescence of **21** was imaged under a fluorescence microscope. As shown in Fig. 2, the strong fluorescence observed in the cells indicated that the fluorescence probe **21** will be useful for elucidating the dynamics of EGCg (**1**) cellular uptake, intracellular transport, and metabolism. Our group is currently undertaking further fluorescence imaging studies.

Next we focused on the generation of antibodies specific to EGCg, which would be useful for immunological detection of subcellular and tissue localization. Furthermore, enzyme-linked immunosorbent assays (ELISA) with color or fluorescence endpoints would be useful for quantitating trace amounts of EGCg in serum. The probe precursor **3** was conjugated to a hapten, which enabled the generation of EGCg antibodies.[24,25] As shown in Scheme 4, conjugation of **3** to the carrier protein

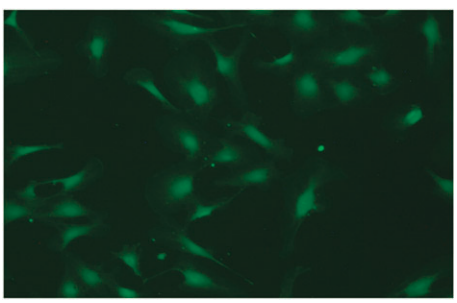
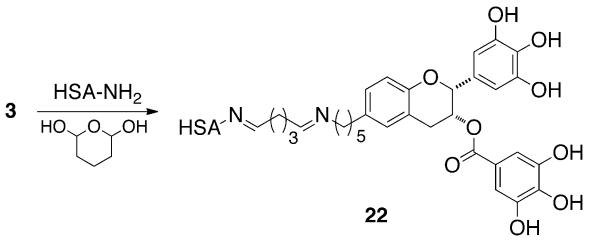


Fig. 2 Fluorescence microscopy image of HUVECs incubated with **21**.



Scheme 4 Conjugation of **3** to the HSA carrier protein.

(HSA: human serum albumin) was performed by using glutaraldehyde as a cross-linker[26] to give **22**. The immunogen **22** was mixed in a saline solution with Freund's complete adjuvant and was injected into mice. After several weeks, the mice were sacrificed, and the venous blood was collected. Sera were separated by centrifugation and used for subsequent experiments.[11]

In summary, we have developed a novel EGCg derivative that includes terminal amino groups without loss of activity. The introduced amino groups were useful for the development of a variety of probe molecules. The fluorescein probes and antibodies show promise for visualizing localization on the cellular and organ scale, respectively. These probe molecules are promising tools for investigations into the localization and target sites of EGCg.

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