

HETEROCYCLES, Vol. 93, No. 1, 2016, pp. 218 - 242. © 2016 The Japan Institute of Heterocyclic Chemistry  
Received, 26th August, 2015, Accepted, 30th September, 2015, Published online, 23rd October, 2015  
DOI: 10.3987/COM-15-S(T)25

## SYNTHESIS OF CHEMICAL-BIOLOGY TOOLS ENABLING *IN VIVO* IMAGING AND ANALYSIS OF EPIGALLOCATECHIN GALLATE

Tomohiro Asakawa,<sup>a</sup> Atsushi Yoshida,<sup>a</sup> Yasuo Hirooka,<sup>a</sup> Takashi Suzuki,<sup>a</sup>  
Kunihiko Itoh,<sup>a</sup> Kosuke Shimizu,<sup>a</sup> Naoto Oku,<sup>a</sup> Takumi Furuta,<sup>a,b</sup> Toshiyuki  
Wakimoto,<sup>a,c</sup> Makoto Inai,<sup>a</sup> and Toshiyuki Kan<sup>a\*</sup>

<sup>a</sup>School of Pharmaceutical Sciences, University of Shizuoka 52-1 Yada, Suruga-ku, Shizuoka, 422-8526, Japan, <sup>b</sup>Present address: Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan, <sup>c</sup>Present address: Faculty of Pharmaceutical Sciences, Hokkaido University, Kita 12-jo Nishi 6-chome, Kita-ku, Sapporo, Japan. E-mail: kant@u-shizuoka-ken.ac.jp

**Abstract** – (–)-Epigallocatechin gallate (EGCg) has multiple bioactivities, and imaging/analytical tools are required for drug development studies. Here we present full details of our synthetic studies aimed at providing building blocks for development of such tools, including a concise synthesis of model compound 5,7-dideoxyEGCg (DOEGCg, **2**) and an asymmetric synthesis of 6-(5-aminopentyl)-5,7-deoxyepigallocatechin gallate (APDOEGCg, **4**), which contains a reactive terminal amino group. To demonstrate its utility, APDOEGCg (**4**) was efficiently converted to a fluorescent probe **53** by linking it to a fluorescein derivative, Tokyo Green, via the amino group. We confirmed that **53** is suitable for *in vivo* imaging studies. We also prepared an immunogen **56** by conjugation of **4** to human serum albumin carrier protein via a glutaraldehyde linker, and we used **56** to raise *anti*-EGCg antiserum in mice. The fluorescent probe and antiserum should be useful tools for biochemical investigations of the localization and target sites of EGCg. APDOEGCg should also be available for developing other novel tools for biochemical studies of catechins.

## INTRODUCTION

(–)-Epigallocatechin gallate (EGCg) (**1**) (Figure. 1), which is a major constituent of green tea extract, has received much attention<sup>1</sup> because of its antitumor,<sup>2</sup> antiviral,<sup>3</sup> and other important bioactivities.<sup>3</sup> Due to these bioactivities, EGCg and its derivatives are considered to be good candidates as lead compounds for

Dedicated to Professor Dr. Lutz F. Tietze, on the occasion of his 75th birthday.

drug development.<sup>4,5</sup> Consequently, there is a requirement for imaging probes and other analytical tools to enable biochemical studies of **1**.

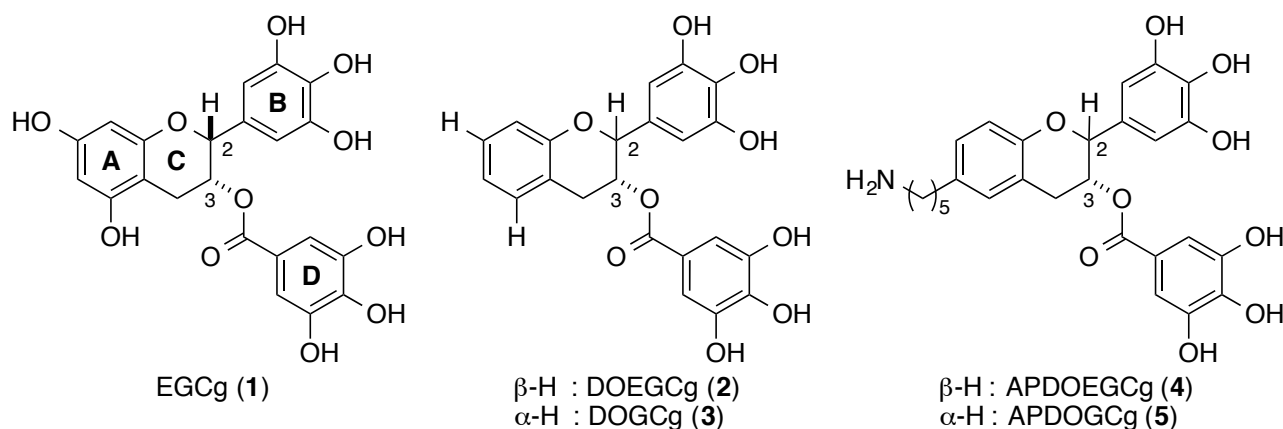
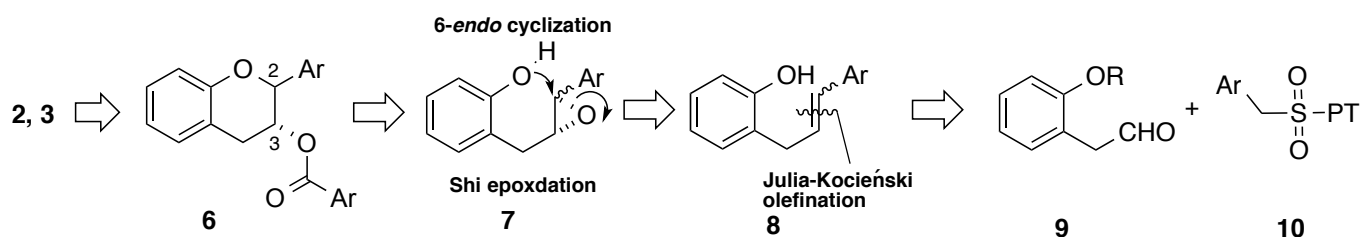


Figure 1. Structures of EGCg (**1**), DOEGCg (**2**), DOGCg (**3**), APDOEGCg (**4**) and APDOGCg (**5**)

So far, there have been few reports aimed at the synthesis of catechin probes.<sup>5,6</sup> Although modification of **1** seems an obvious strategy, direct and selective incorporation of suitable probe moieties into **1** has proved difficult due to the structural instability of **1** and the lack of appropriate tethering functional groups. Therefore, we require a flexible construction method for benzopyran ring structures.

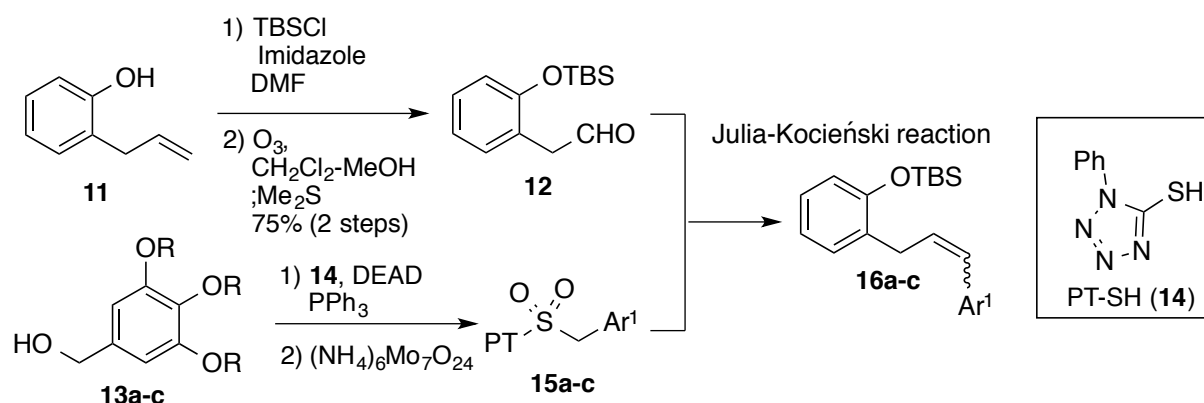
## RESULTS AND DISCUSSION

Our synthetic strategy is illustrated in Scheme 1. Since enantio- and diastereo-specific synthesis of the dihydrobenzofuran ring would be desirable, we focused on the olefin derivative **8** as a key cyclization precursor. We initially focused on 5,7-dideoxyEGCg (DOEGCg, **2**) as a model compound. Incorporation of the galloyl moiety are expected to proceed smoothly, so the key issue in the synthesis of **2** and 5,7-dideoxygalocatechin gallate (DOGCg, **3**) should be the stereoselective construction of the dihydrobenzopyran ring **6**. We anticipated that **6** could be synthesized by 6-*endo*-cyclization of epoxy-phenol **7**, which in turn could be readily obtained by asymmetric epoxidation<sup>7</sup> of **8**. Several selective 6-*endo* cyclization-mediated pyran ring constructions have been reported.<sup>8</sup> Because the reaction should be favored by stabilization of the cation at the reaction site, an electron-rich B-ring group should promote dihydrobenzopyran ring synthesis. The olefin **8** could be prepared by Julia-Kocięński reaction of aldehyde **9** and sulfone **10**.



Scheme 1. Synthetic plan of DOEGCg (**2**) and DOGCg (**3**)

As shown in Scheme 2, condensation of the A- and B-ring was accomplished by means of Julia-Kocięński (JK) reaction<sup>9</sup> between aldehyde **12** and phenyltetrazole (PT)-sulfone **15**. Aldehyde **12** was prepared by ozonolysis of TBS-protected **11**. PT-sulfones **15** were prepared by Mitsunobu reaction of **13** with PT-SH (**14**) and the oxidation of the afforded sulfides, respectively. Upon treatment of **12** and **15a** with LHMDS, JK reaction proceeded smoothly to provide **16** as a single isomer in 95% yield with *Z*-selectivity (Table 1, entry 1). The selectivity and the reactivity depend on the protecting group at the B-ring of **15**, as shown in Table 1; the high *Z*-selectivity of this JK reaction is discussed below. With the TBS-protected PT-sulfone **15b**, the JK reaction gave the corresponding desired olefin in moderate yield with moderate *cis-trans* selectivity (entry 2). On the other hand, the yield and *cis-trans* selectivity of the reaction were decreased in JK reaction using the mesylate **15c**.



Scheme 2. Preparation of Julia-Kocięński reaction units

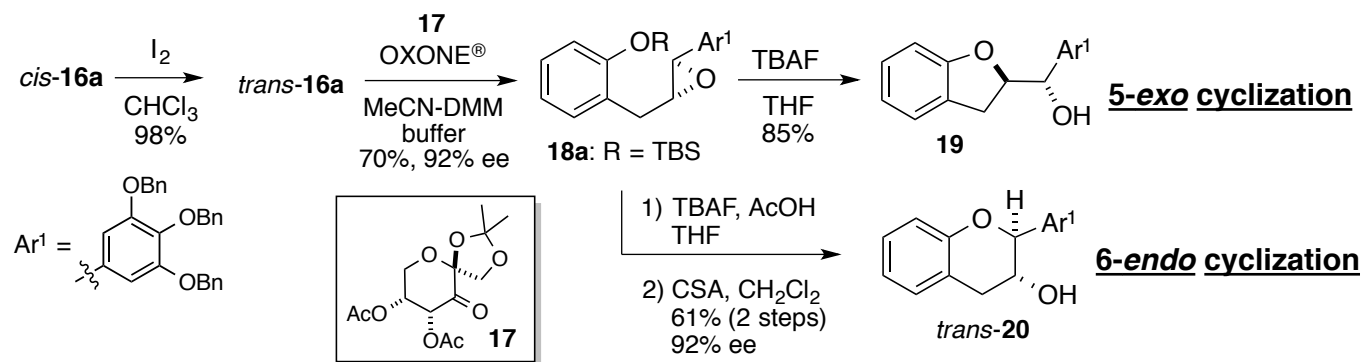
Table 1. Effect of phenolic protecting groups in Julia-Kocięński reaction

entry	PT-sulfone <b>15</b>	product	yield (%) <sup>a</sup>	selectivity ( <i>cis</i> : <i>trans</i> ) <sup>b</sup>
1	<b>15a</b> (R = Bn)	<b>16a</b>	95	30 : 1
2	<b>15b</b> (R = TBS)	<b>16b</b>	75	10 : 1
3	<b>15c</b> (R = Ms)	<b>16c</b>	12	1 : 1

a: isolated yield., b: the ratio was determined by <sup>1</sup>H-NMR.

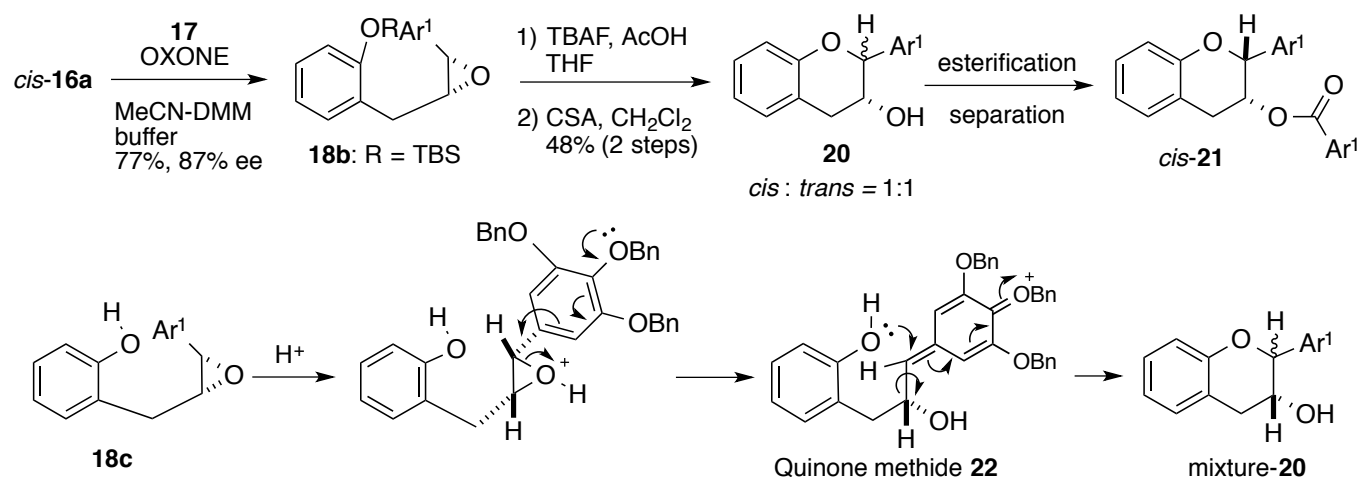
Furthermore, treatment of *cis*-**16a** with I<sub>2</sub> resulted in smooth isomerization to provide *trans*-**16a** predominantly (Scheme 3). Reaction of *trans*-**16a** with a catalytic amount of Shi's reagent **17** and Oxone<sup>®</sup>

gave epoxide **18a**. Treatment of **18a** with TBAF resulted in basic 5-*exo*-cyclization (Baldwin's rule) to give dihydrobenzofuran **19**, so the TBS group was deprotected in the presence of AcOH without cyclization. Upon treatment with CSA, the desired regio- and stereoselective 6-*endo*-cyclization reaction proceeded smoothly with high diastereoselectivity, and subsequent recrystallization gave optically pure *trans*-**20**.



Scheme 3. Stereoselective construction of 2,3-*trans*-dihydrobenzopyran **20**

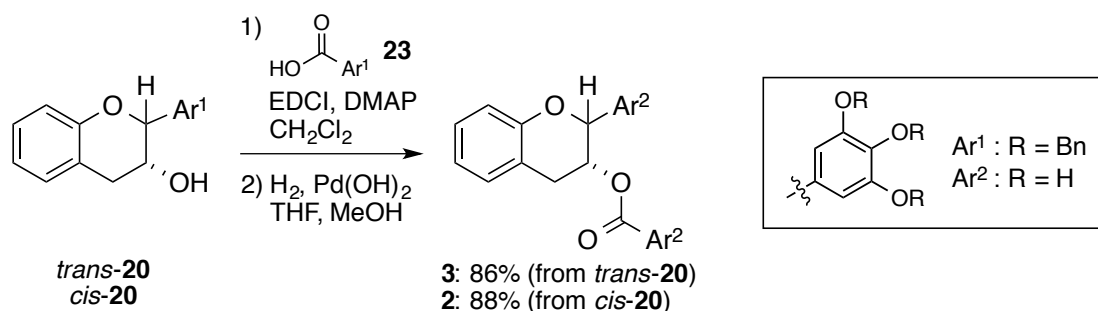
Interestingly, the corresponding epoxide **18b** prepared from *cis*-**16a** was converted into a 1:1 mixture of *trans* and *cis* dihydrobenzopyran **20** under the same conditions (Scheme 4). In comparison with **18a**, acid-mediated epoxide-opening reaction and generation of quinone methide **22** proceeded before the desired  $S_N2$  reaction with the epoxide **18c**. Furthermore, cyclization of the quinone methide intermediate provided both isomers. After esterification of the secondary alcohol with gallic acid, silica gel column chromatography afforded enantiomerically pure *cis*-**21**.



Scheme 4. Construction of dihydropyran ring from *cis*-epoxide

Furthermore, intermolecular cyclization of the quinone methide intermediate provided both isomers. Although conversion to *trans*-**20** from the mixture of **20** was readily accomplished by through the similar quinone methide intermediate, the obtaining EGCg derivative (*cis*-isomer), separation step should be

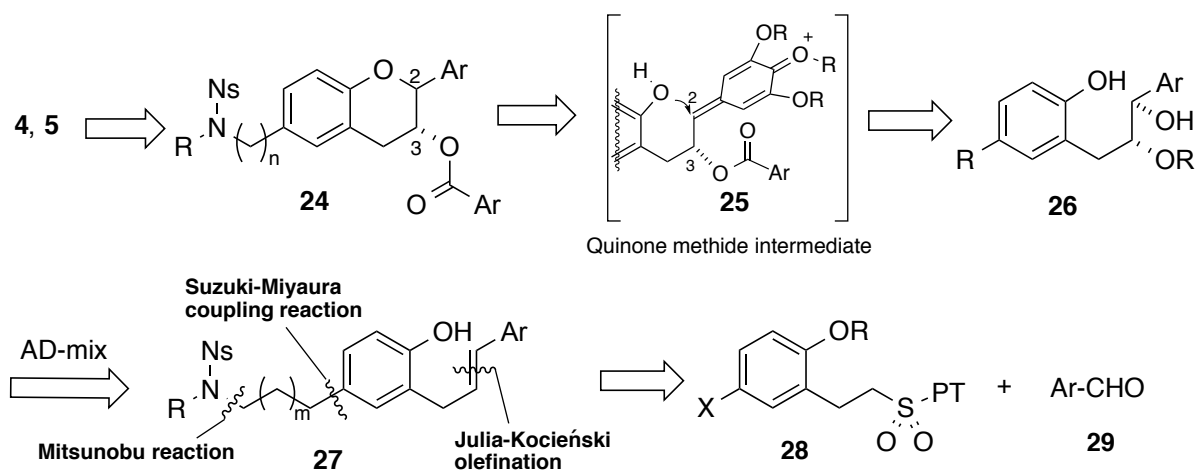
required. After esterification of the secondary alcohol with gallic acid **23**, the separation was accomplished by silica gel column chromatography to afford enantiomerically pure *cis*-**21**. As shown in Scheme 5, efficient syntheses of **3** and **2** were accomplished by cleavage of the benzyl ether under hydrogenation conditions after incorporation of gallic acid **23** and separation. Thus, efficient syntheses of **3** and **2** were accomplished in nine steps from **11**.<sup>10</sup>



Scheme 5. Syntheses of DOEGCg (**2**) and DOGCg (**3**)

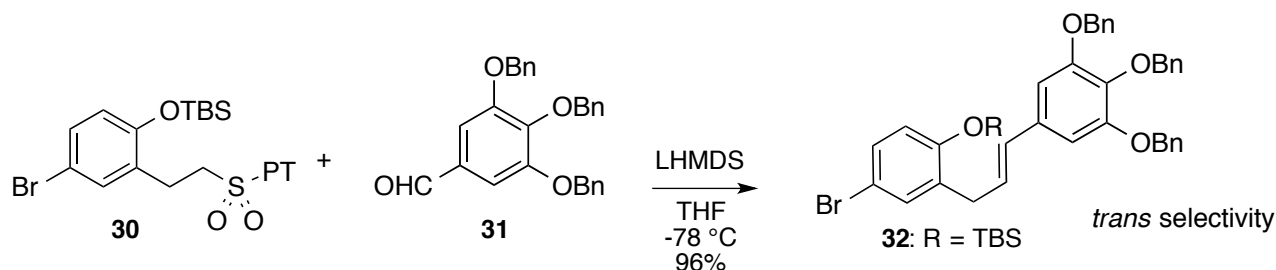
In our previous synthetic investigations, we found that the synthetic derivative **2** possessed more potent anti-influenza infection activity than natural **1**. Inspired by this finding, we next began a synthesis of the EGCg probe precursor **4** (6-(5-aminopentyl)-5,7-deoxyepigallocatechin gallate: APDOEGCg), which contains a linker and a reactive amino group,<sup>11</sup> as shown in Figure 1.

Since direct incorporation of a linker unit into DOEGCg, which possesses a dihydrobenzopyran skeleton, was difficult, we decided to employ cross-coupling reaction of the linker unit into cyclization precursor **27**, which was prepared by condensation reaction of **28** and **29** (Scheme 6). Incorporation of a reactive amino group at the terminal position of the linker was found to be favorable for the Mitsunobu reaction with our Ns-amide (2-nitrobenzenesulfonamide)<sup>12,13</sup> under neutral reaction conditions. Regio- and enantio-selective construction of the *cis* or *trans* dihydrobenzopyran ring would be accomplished by cyclization under acidic conditions from the chiral diol **26** through the cationic quinone methide intermediate **25** with participation of the neighboring hydroxyl group as well as gallate unit.



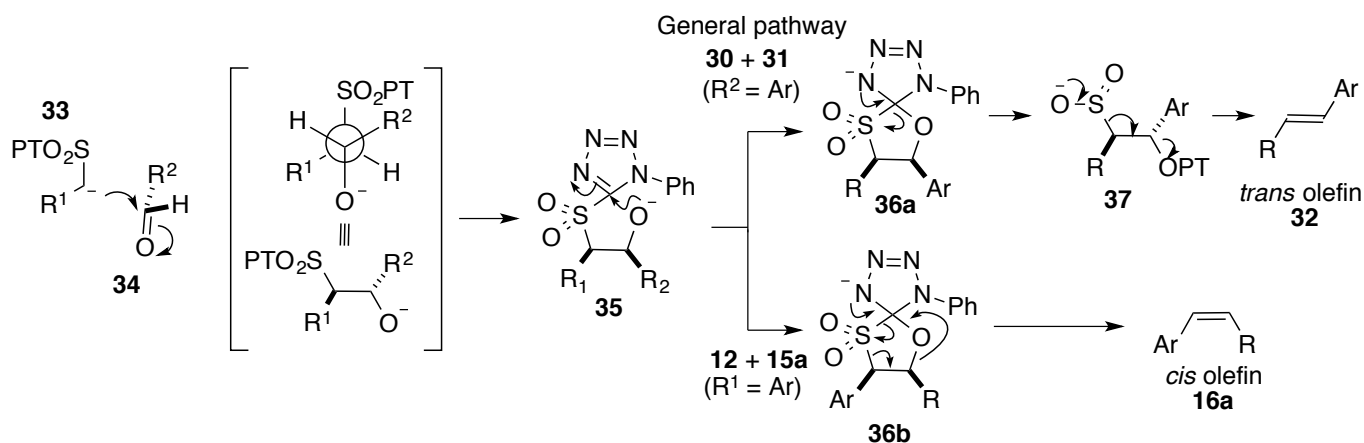
Scheme 6. Synthetic strategy for EGCg probe precursors **4, 5**

As shown in Scheme 7, condensation of the A- and B-ring was accomplished by means of Julia-Kocieński reaction between phenyltetrazole (PT)-sulfone **30** and aldehyde **31** to provide **32** as a single isomer in 87% yield with *E*-selectivity in accordance with the general mechanism of the JK coupling reaction.



Scheme 7. Substrate-controlled *trans*-selective Julia-Kocieński reaction

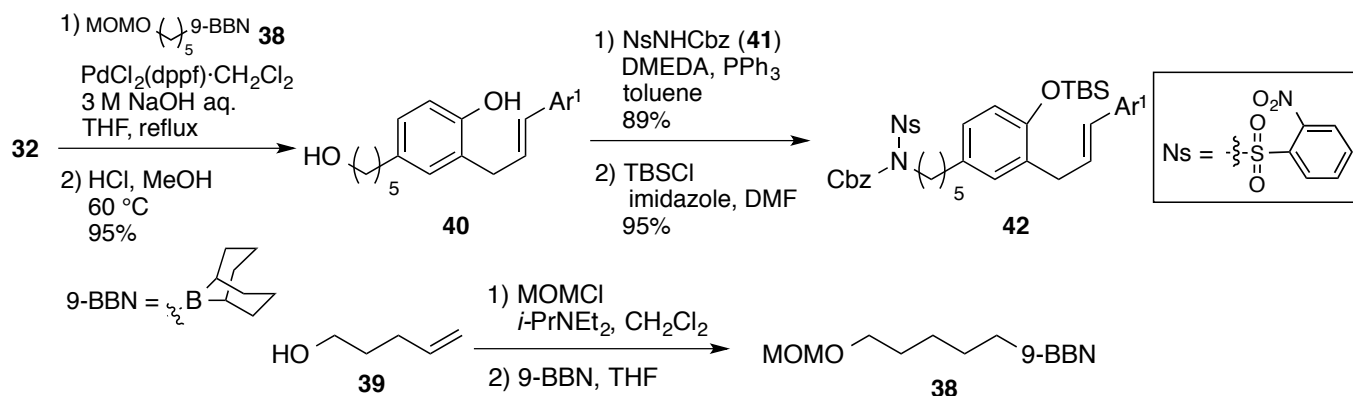
It is noteworthy that the selectivity can be switched simply by changing the combination of sulfone and aldehyde (compare Schemes 2 and 7). Generally, a PT-sulfone affords a *trans* olefin in the Julia-Kocieński reaction, through Smiles rearrangement (**36a**) and antiperiplanar  $\beta$ -elimination (**37**) via extrusion of sulfur dioxide, as shown in Scheme 8. The coupling reaction of electron-rich sulfone **12** and **15a** should proceed through a corresponding intermediate **36b**. However, direct desorption of the phenyl tetrazolone and sulfur dioxide occurred from **36b** to give *cis*-**16a**, presumably because the electron-rich Ar group destabilizes the benzylic anion. Actually, no selectivity was observed in the JK reaction of **15c**, presumably because electron donation from oxygen was restrained by the electron-withdrawing mesyl group.



Scheme 8. Plausible mechanism of stereoselectivity of the Julia-Kocieński reaction

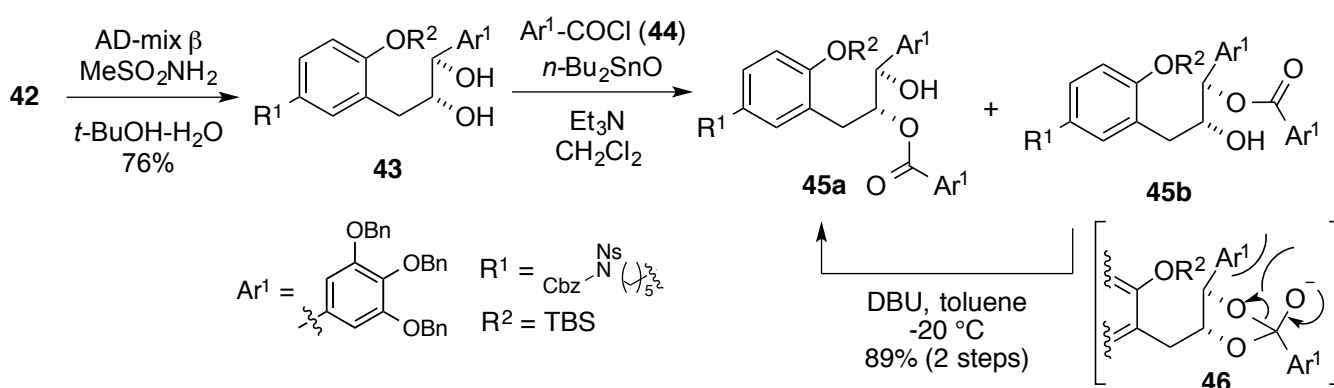
Next, incorporation of a linker group was performed by means of the Suzuki-Miyaura coupling reaction<sup>14</sup> (Scheme 9). After preparation of borate **38** by hydroboration of MOM-protected **39**, coupling reaction of **32** and **38** in the presence of catalytic quantities of PdCl<sub>2</sub>(dppf) and NaOH in THF proceeded smoothly to

give **40** in high yields. Incorporation of the amino group was accomplished by means of the Mitsunobu reaction<sup>15,16</sup> with our *N*-Cbz-*N*-Ns-amide<sup>12</sup> (**41**) (Ns strategy<sup>13</sup>) to afford **42**.



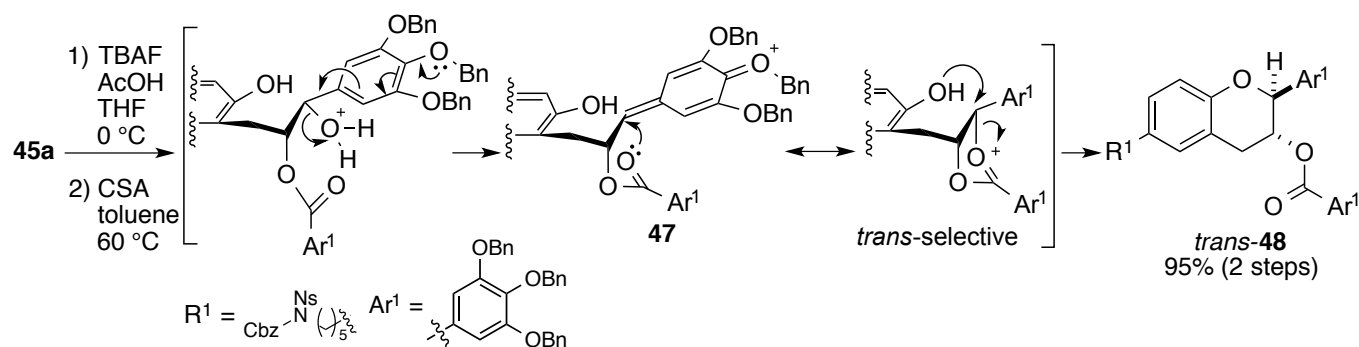
Scheme 9. Incorporation of an amino linker group

Diol **43** was obtained by Sharpless asymmetric dihydroxylation<sup>17</sup> (Scheme 10). Quinone methide-mediated cyclization proceeded smoothly, but provided a 1:1 mixture of diastereomers. Thus, we incorporated a gallate group into the cyclization precursor **43**. Mono-selective acylation of **43** with acyl chloride **44** proceeded smoothly in the presence of *n*-Bu<sub>2</sub>SnO to afford **45a** and **45b** as a 1 : 1 mixture. However, treatment of the mixture with DBU resulted in an interesting migration reaction, affording ester **45a** as a sole product. (Scheme 10) In this reaction, the acyl group of **45b** migrates to the sterically less hindered position.

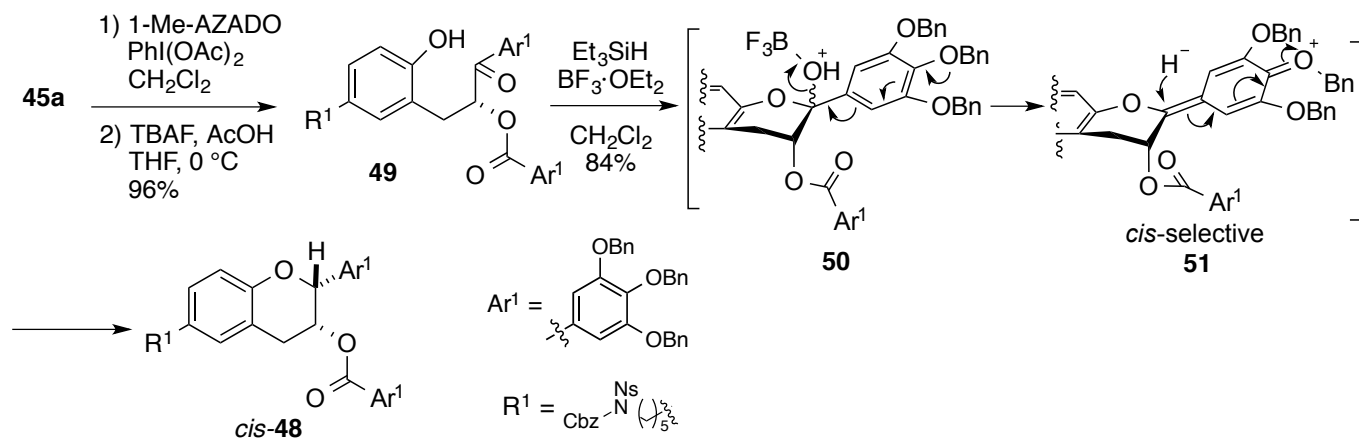
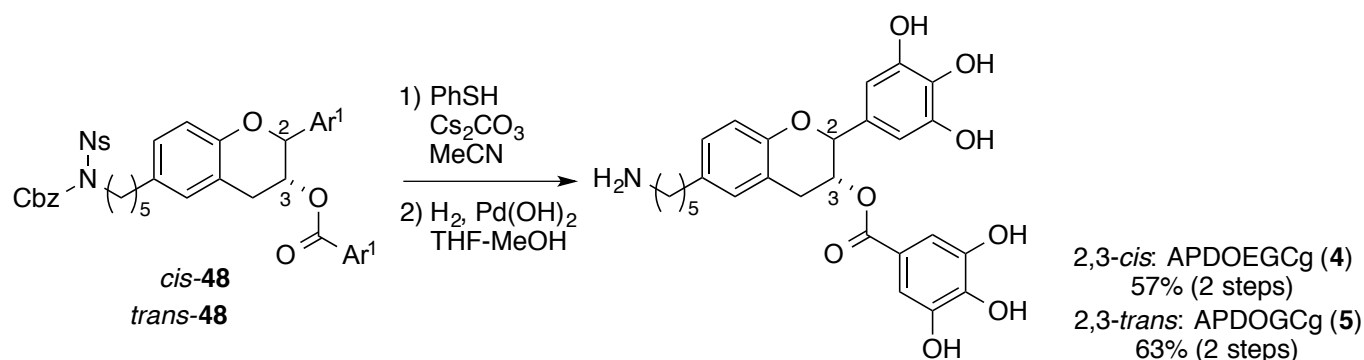


Scheme 10. Asymmetric dihydroxylation and selective esterification

Upon treatment of benzyl alcohol **45a** with CSA, the desired cyclization reaction proceeded smoothly to provide predominantly the *trans*-dihydrobenzofuran ring **48** (Scheme 11). This stereoselectivity is considered to be due to formation of the intermediate **47**, in which the carbonyl group stabilizes the benzylic cation and the phenol group reacts from the  $\beta$ -face.

Scheme 11. *Trans*-selective construction of the benzopyran ring

Based on Kishi's C-glycosidation,<sup>18</sup> hydride reduction of the quinone methide **51** intermediate was expected to provide *cis*-dihydrobenzopyran **48**. Oxidation of the secondary alcohol **45a** was performed by treatment with 1-Me-AZADO to afford the corresponding ketone **49** (Scheme 12). According to Tanaka and Takahashi's procedure,<sup>19</sup> reductive cyclization of **49** with  $\text{Et}_3\text{SiH}$  and  $\text{BF}_3 \cdot \text{OEt}_2$  provided *cis*-dihydrobenzofuran **48**. Because this reaction proceeds via cationic intermediate **51**, the hydride was delivered from the  $\beta$ -face, thus affording exclusively the *cis*-substituted product. Finally, removal of the  $\text{Ns}^{20}$  and benzyl groups of *cis*- and *trans*-**48** provided the desired APDOEGCg (**4**) and APDOGCg (**5**), respectively (Scheme 13).<sup>21</sup>

Scheme 12. *Cis*-selective construction of benzopyran ringScheme 13. Synthesis of probe precursor APDOEGCg (**4**) and APDOGCg (**5**)



With the desired derivatives **4** and **5** in hand, we evaluated their inhibitory activities against influenza virus infection. As shown in Table 2, APDOEGCg (**4**) and APDOGCg (**5**) both potently inhibited the infectivity of the influenza virus A/Memphis/1/71 (H3N2) toward MDCK cells, with IC<sub>50</sub> values of 4.18 and 4.40 μM, respectively. These compounds are more potent than natural **1** and synthetic derivative **2**.<sup>22</sup> Thus, contrary to expectation, introduction of the linker moiety did not result in loss of biological activity, and incorporation of probe moieties and/or tags into **4** via the terminal amino group of the linker might also be possible without loss of activity.

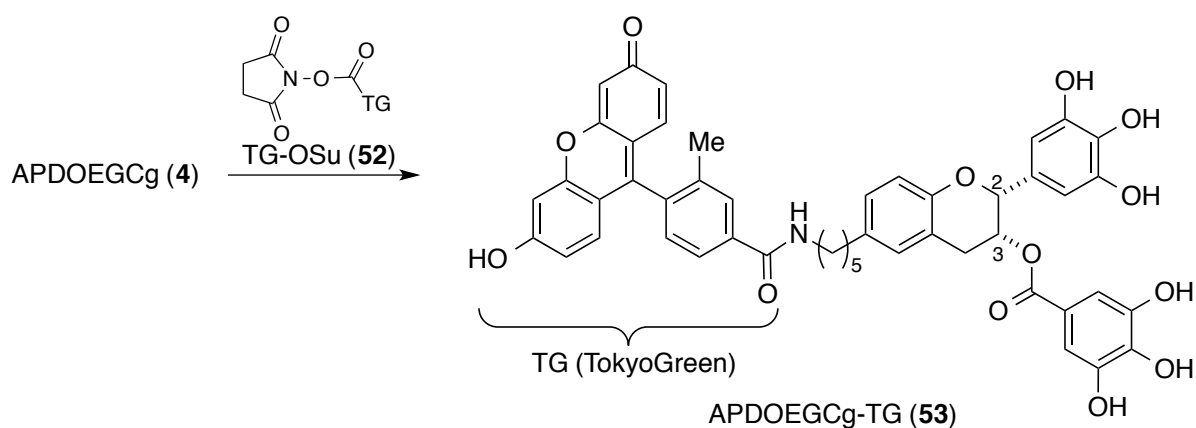
Table 2. Inhibition of influenza A viral infectivity toward MDCK cells

Compound	Complement inhibition IC <sub>50</sub> <sup>a</sup> (μM)
EGCg ( <b>1</b> )	66.3 (± 9.21)
DOEGCg ( <b>2</b> )	9.05 (± 2.26)
APDOEGCg ( <b>4</b> )	4.18 (± 4.29)
APDOGCg ( <b>5</b> )	4.40 (± 2.36)

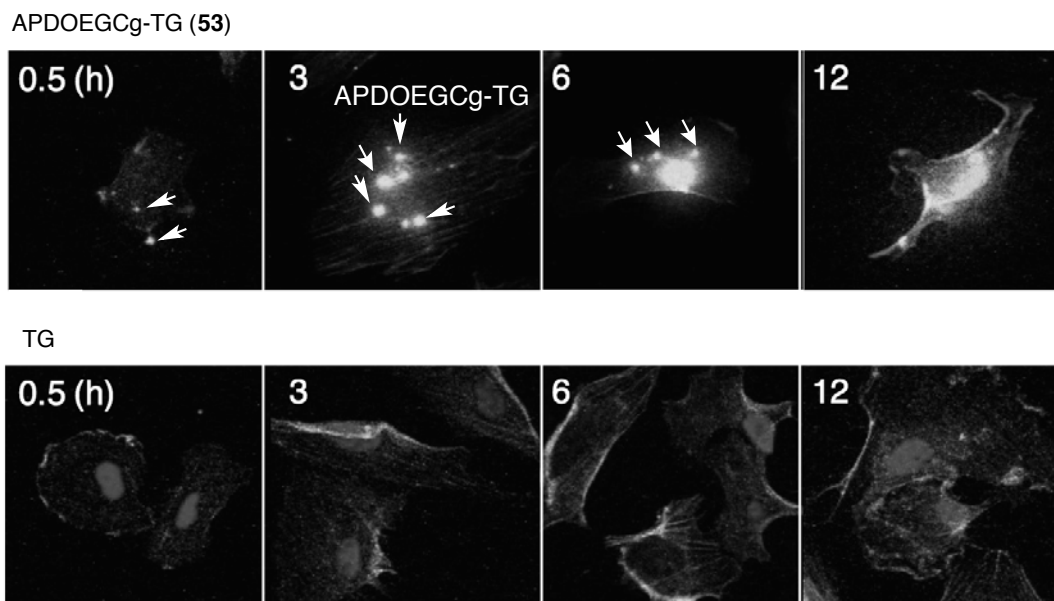
<sup>a</sup> Values are reported as the mean of three experiments, with the standard deviation in parentheses

Encouraged by these results, we turned our attention to the preparation of a fluorescent probe molecule from **4**. The reactive amine group of **4** is advantageous for incorporation of a probe moiety without the need for protection of the phenolic hydroxyl groups. We focused on fluorescein, which is suitable for in vivo imaging under physiological conditions, and selected Tokyo Green (TG) as a well-established photophore.<sup>23</sup> As shown in Scheme 15, reaction of probe precursor **4** and TG activated ester **52** afforded the desired probe **53**.

The usefulness of **53** for imaging studies was next assessed using human umbilical vein endothelial cells (HUVECs).<sup>24</sup> After incubation of **53** with HUVECs for 3 h, imaging with a fluorescence microscope showed strong intracellular fluorescence, indicating that **53** would be useful for studies of the dynamics of EGCg (**1**) cellular uptake, intracellular transport, and metabolism.<sup>25</sup>



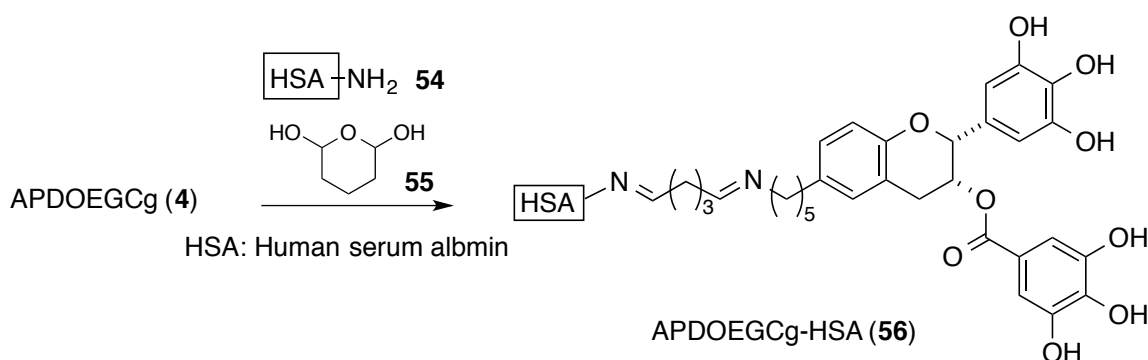
Scheme 14. Synthesis of the fluorescein probe **53** from **4**



Condition: HUVECs, 37 °C, 1  $\mu$ M of APDOEGCg-TG or TG, 5 U/mL of rhodamine/phalloidin; 10  $\mu$ g/mL of DAPI

Figure 2. Fluorescence microscopic images of HUVECs incubated with **53**

Next we focused on the generation of EGCg antibodies,<sup>26,27</sup> which should be useful for immunohistology, as well as for developing enzyme-linked immunosorbent assays (ELISA) with color or fluorescence endpoints for quantitating trace amounts of EGCg in serum. As shown in Scheme 15, conjugation of **4** to carrier protein **54** (HSA: human serum albumin) was performed by using glutaraldehyde (**55**) as a cross-linker,<sup>28</sup> to give the immunogen **56**. A solution of **56** in saline containing Freund's complete adjuvant was injected into mice. After several weeks, the mice were sacrificed, and venous blood was collected. Serum was separated by centrifugation and used as antiserum for subsequent experiments.



Scheme 15. Conjugation of **4** to HSA carrier protein via glutaraldehyde linker

In summary, we have developed a novel synthetic method for bioactive catechin derivatives that contain a reactive terminal amino group, to which a variety of functional moieties can be linked. To demonstrate its utility, APDOEGCg (**4**) was efficiently converted to a fluorescent probe **53** and an immunogen **56** by utilizing its reactive amino group. We confirmed that **53** is suitable for imaging EGCg at the cellular level,

and we used **56** to raise *anti*-EGCg antiserum in mice. The probe and antiserum are expected to be useful tools for biochemical investigations into the localization and target sites of EGCg. APDOEGCg should also be useful for development of other novel biochemical tools for studies of catechins.

## EXPERIMENTAL

**General.** Nuclear magnetic resonance [ $^1\text{H}$  NMR (270 MHz),  $^{13}\text{C}$  NMR (68 MHz)] spectra were determined on a JEOL EX-270 instrument and [ $^1\text{H}$  NMR (500 MHz),  $^{13}\text{C}$  NMR (125 MHz)] spectra were determined on JEOL ECA-500 instrument. Chemical shifts for  $^1\text{H}$  NMR were reported in parts per million downfields from tetramethylsilane ( $\delta$ ) as the internal standard and coupling constants were in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Chemical shifts for  $^{13}\text{C}$  NMR were reported in ppm relative to the centerline of a triplet at 77.0 ppm for  $\text{CDCl}_3$ . High-resolution mass spectra (HRMS) were obtained on a BRUKER DALTONICS micrOTOF (ESI) and JEOL MStation 700 (FAB). FAB mass spectra were obtained with 3-nitrobenzylalcohol as the matrix. Infrared (IR) spectra were recorded on a SHIMADZU IRPrestige-21. Optical rotations were measured on a JASCO P-1030 Polarimeter at RT using the sodium D line. Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60  $F_{254}$ . Preparative TLC separations were made on 7 x 20 cm plates prepared with a 0.25 mm layer of Merck silica gel 60  $F_{254}$ . Compounds were eluted from the adsorbent with 10% MeOH in  $\text{CHCl}_3$ . Column chromatography separations were performed on KANTO CHEMICAL Silica Gel 60 (spherical) 40 - 50 mm, Silica Gel 60 (spherical) 63–210 mm or Silica Gel 60 N (spherical, neutral) 63–210 mm. Reagents and solvents were commercial grades and were used as supplied with the following exceptions. 1)  $\text{CH}_2\text{Cl}_2$ , THF and toluene: dried over molecular sieves 4A. 2) MeOH and acetonitrile: dried over molecular sieves 3A. All reactions sensitive to oxygen and/or moisture were conducted under an argon atmosphere.

**(Z)-Olefin 16a (cis-16a).** To a stirred solution of **12** (2.96 g, 4.79 mmol) in THF (25 mL) was added 1.0 M solution of lithium bis(trimethylsilyl)amide (LHMDS) in THF (9.6 mL, 9.6 mmol) at 0 °C under an argon atmosphere. After 30 min, a solution of **15a** (1.03 g, 3.99 mmol) in 10 mL of THF was added to the reaction mixture at 0 °C. After being stirred for 1 h at room temperature, to the reaction mixture was added 1 N aqueous HCl and the resulting mixture was extracted with EtOAc three times. The extracts were dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 9 / 1) to afford *cis*-**16a** (2.44 g, 95%) as a yellow oil.

IR (film) 2930, 1572, 1259, 927  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  0.20 (s, 6H), 0.96 (s, 9H), 3.50–3.64 (m, 2H), 5.02 (s, 4H), 5.06 (s, 2H), 5.79 (dt,  $J = 14.0, 5.8$  Hz, 1H), 6.45 (d,  $J = 14.0$  Hz, 1H), 6.59 (s, 2H),

6.82 (d,  $J = 7.9$  Hz, 1H), 6.91 (m, 1H), 7.13, (m, 1H), 7.18–7.41 (m, 16H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  –4.1, 18.2, 25.7, 25.8, 29.5, 71.1, 75.2, 105.9, 108.6, 118.5, 121.2, 121.4, 127.1, 127.2, 127.3, 127.4, 127.7, 128.1, 128.4, 128.6, 128.7, 129.6, 129.9, 130.2, 131.3, 131.5, 132.9, 137.1, 137.4, 137.9, 152.5, 153.4; FAB-MS  $m/z$  643 ( $\text{M}+\text{H}$ )<sup>+</sup>. HRMS (FAB) calculated for  $\text{C}_{42}\text{H}_{47}\text{O}_4\text{Si}$  643.3244 [ $\text{M}+\text{H}$ ]<sup>+</sup>, found 643.3268.

**(E)-Olefin 16a (trans-16a).** To a stirred solution of *cis*-**16a** (195 mg, 0.303 mmol) in  $\text{CHCl}_3$  (2 mL) was added a solution of iodine (65 mg, 0.26 mmol) in  $\text{CHCl}_3$  (2 mL) at room temperature under an argon atmosphere. After being stirred at the same temperature for 25 h, to the reaction mixture was added 10% aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  and the resulting mixture was extracted with  $\text{CHCl}_3$  three times. The extracts were dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under reduced pressure to afford *trans*-**16a** (192 mg, 98%) as a yellow oil. The *E/Z* ratio was determined by  $^1\text{H}$  NMR spectrum.

IR (film) 2920, 1579, 1253, 925  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  0.25 (s, 6H), 1.02 (s, 9H), 3.50 (d,  $J = 6.1$  Hz, 2H), 5.03 (s, 2H), 5.08 (s, 4H), 6.23 (td,  $J = 15.8, 6.1$  Hz, 1H), 6.28 (d,  $J = 15.8$  Hz, 1H), 6.65 (s, 2H), 6.82 (d,  $J = 7.9$  Hz, 1H), 6.91 (t,  $J = 7.9$  Hz, 1H), 7.11 (td,  $J = 7.9, 1.6$  Hz, 1H), 7.17 (dd,  $J = 7.9, 1.6$  Hz, 1H), 7.31–7.43 (m, 15H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  –4.1, 18.3, 25.8, 33.6, 71.2, 75.2, 105.9, 118.4, 121.1, 127.2, 127.4, 127.7, 127.8, 128.1, 128.4, 128.6, 130.3, 130.5, 130.7, 133.5, 137.2, 137.8, 137.9, 152.9, 153.4; FAB-MS  $m/z$  643 ( $\text{M}+\text{H}$ )<sup>+</sup>. HRMS (FAB) calculated for  $\text{C}_{42}\text{H}_{46}\text{O}_4\text{Si}$  642.3165 [ $\text{M}$ ]<sup>+</sup>, Found 642.3146.

**Epoxide 18a.** To a stirred solution of *trans*-**16a** (77.0 mg, 0.255 mmol) in a mixture of MeCN (0.9 mL) and dimethoxymethane (1.8 mL) were successively added **17** (100 mg, 0.156 mmol),  $\text{Bu}_4\text{N}^+\text{HSO}_4^-$  (2.4 mg, 7.0 mmol), 4 mL of phosphate buffer (pH = 9.18), OXONE (376 mg, 0.611 mmol) and potassium carbonate (125 mg, 0.90 mmol) at 0 °C. After being stirred at the same temperature for 25 min, to the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 10 / 1) to afford **18a** (71.5 mg, 70%) as a yellow oil.

The enantiomeric excess of **18a** was determined to be 92% *ee* by HPLC analysis on a chiral stationary phase under the conditions described below.

$[\alpha]_{\text{D}}^{24} +14$  ( $c$  0.99,  $\text{CHCl}_3$ ); IR (film) 3030, 2927, 1591, 1253, 1116  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  0.24 (s, 6H), 1.01 (s, 9H), 2.93 (dd,  $J = 14.3, 5.2$  Hz, 1H), 3.04 (dd,  $J = 14.3, 5.2$  Hz, 1H), 3.15 (td,  $J = 5.2, 2.0$  Hz, 1H), 3.58 (d,  $J = 2.0$  Hz, 1H), 5.02 (s, 2H), 5.07 (s, 4H), 6.57 (s, 2H), 6.82 (dd,  $J = 7.9, 1.2$  Hz, 1H), 6.92 (td,  $J = 7.9, 1.2$  Hz, 1H), 7.13 (td,  $J = 7.9, 1.2$  Hz, 1H), 7.31–7.42 (m, 15H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  –4.0, 18.3, 25.8, 33.0, 58.6, 62.2, 71.2, 75.2, 105.0, 118.4, 121.2, 127.4, 127.6, 127.8,

128.1, 128.5, 128.6, 130.7, 133.3, 137.0, 137.8, 153.0, 153.7; FAB-MS  $m/z$  659 (M+H)<sup>+</sup>. HRMS (FAB) calculated for C<sub>42</sub>H<sub>47</sub>O<sub>5</sub>Si 659.3193 [(M+H)<sup>+</sup>], found 659.3167.

Chiral HPLC: Daicel ChiralPak AD-H 0.46 cm f x 25 cm, eluent : 7% *i*-PrOH/*n*-hexane, flow rate : 0.5 mL/min, retention time : 98.7 min (96.3%), 109.7 min (3.7%)

**(+)-(R)-(S)-2,3-Dihydrobenzofuran (+)-19.** To a stirred solution of **18a** (285 mg, 0.432 mmol) in THF (8 mL) was added 1.0 M solution of tetrabutylammonium fluoride (TBAF) in THF (0.53 mL, 0.53 mmol) at room temperature under an argon atmosphere. After being stirred at the same temperature for 3.5 h, to the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were washed with brine, dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 1) to afford **19** (198 mg, 85%) as a yellow oil.

[ $\alpha$ ]<sub>D</sub><sup>24</sup> +0.56 (*c* 0.82, CHCl<sub>3</sub>); IR (film) 3450, 1593, 1112 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  2.25 (s, 1H), 2.71 (dd, *J* = 15.8, 9.1 Hz, 1H), 3.10 (dd, *J* = 15.8, 9.1 Hz, 1H), 4.83 (td, *J* = 8.8, 3.7 Hz, 1H), 5.01-5.14 (m, 7H), 6.69 (s, 2H), 6.77 (d, *J* = 7.9 Hz, 1H), 6.84 (t, *J* = 7.9 Hz, 1H), 7.09 (d, *J* = 7.9 Hz, 1H), 7.30-7.44 (m, 16H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz)  $\delta$  29.0, 71.2, 73.6, 75.2, 86.2, 106.0, 109.1, 120.7, 124.9, 127.0, 127.4, 127.8, 128.1, 128.5, 134.6, 137.0, 137.8, 138.0, 152.8, 159.3; FAB-MS  $m/z$  544 (M)<sup>+</sup>. HRMS (FAB) Calculated for C<sub>36</sub>H<sub>32</sub>O<sub>5</sub> 544.2250 [(M)<sup>+</sup>], Found 544.2258.

**Dihydrobenzopyrane (+)-trans-20.** To a stirred solution of **18a** (127 mg, 0.193 mmol) in THF (4.5 mL) were successively added of acetic acid (33 mL, 0.58 mmol) and 1.0 M solution of TBAF in THF (0.23 mL, 0.23 mmol) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 10 min, to the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to afford a crude product (185 mg) as a yellow oil. The crude product was used in the next reaction without further purification.

A solution of the crude product (185 mg) and CSA (45.4 mg, 0.193 mmol) was stirred at 0 °C for 30 min. To the reaction mixture was added water and the resulting mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> three times. The extracts were dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to give a residue. The residue was purified by column chromatography (*n*-hexane / AcOEt = 3 / 1) to afford **20** (63.7 mg, 61% for 2 steps, (*cis* : *trans* = 1 : 30) as a yellow oil. The product (20.3 mg) was recrystallized from AcOEt / *n*-hexane to afford optically pure *trans* isomer **20** (13.7 mg, 67%) as colorless solids.

The enantiomeric excess of **2** was determined to be > 99% *ee* by HPLC analysis on a chiral stationary phase under the conditions described below.

[ $\alpha$ ]<sub>D</sub><sup>24</sup> +1.7 (*c* 0.84, CHCl<sub>3</sub>); IR (film) 3032, 1597, 1246, 1132 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz)  $\delta$  1.63 (s, 1H), 2.89 (dd, *J* = 15.8, 9.1 Hz, 1H), 3.07 (dd, *J* = 15.8, 5.5 Hz, 1H), 3.99 (dq, *J* = 15.8, 5.5 Hz, 1H), 4.65

(d,  $J = 7.9$  Hz, 1H), 5.11–5.16 (m, 6H), 6.73 (s, 2H), 6.91–6.95 (m, 1H), 7.11 (d,  $J = 7.3$  Hz, 1H), 7.16 (t,  $J = 7.3$  Hz, 1H), 7.25–7.44 (m, 16H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  32.9, 68.1, 71.2, 75.2, 81.9, 106.7, 116.4, 120.2, 121.1, 127.5, 127.7, 127.8, 127.9, 128.2, 128.5, 133.0, 133.3, 136.8, 137.7, 138.7, 153.0, 153.9; FAB-MS  $m/z$  544 ( $\text{M}$ )<sup>+</sup>. HRMS (FAB) calculated for  $\text{C}_{36}\text{H}_{32}\text{O}_5$  544.2250 [ $\text{M}$ ]<sup>+</sup>, Found 544.2264. Chiral HPLC: Daicel ChiralCel OD 0.46 cm f x 25 cm, eluent: 10% *i*-PrOH/*n*-hexane, flow rate : 0.5 mL/min, retention time : 77.8 min (>99%)

**Hexakisbenzyl DOGCg (57).** A solution of *trans*-**20** (40.0 mg, 73.4  $\mu\text{mol}$ ), **23** (97.1 mg, 220  $\mu\text{mol}$ ), EDCI (21 mg, 110  $\mu\text{mol}$ ), and dimethylaminopyridine (0.1 mg, 8  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (4 mL) was stirred at room temperature for 3 hours. To the reaction mixture was added saturated aqueous  $\text{NH}_4\text{Cl}$  and the resulting mixture was extracted with  $\text{CH}_2\text{Cl}_2$  three times. The extracts were dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 5 / 1) to afford **57** (72.0 mg, quant) as a yellow oil.

$[\alpha]_{\text{D}}^{24}$   $-41.0$  (*c* 1.06,  $\text{CHCl}_3$ ); IR (film) 3032, 2933, 1714, 1591, 1228  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  2.91 (dd,  $J = 16.4$ , 6.1 Hz, 1H), 3.02 (dd,  $J = 16.4$ , 4.6 Hz, 1H), 4.91–5.13 (m, 12H), 5.21 (d,  $J = 5.5$  Hz, 1H), 5.45 (q,  $J = 5.3$  Hz, 1H), 6.68 (s, 2H), 6.97 (td,  $J = 7.3$ , 1.2 Hz, 1H), 7.01 (d,  $J = 7.3$  Hz, 1H), 7.08 (d,  $J = 7.9$  Hz, 1H), 7.19–7.42 (m, 31H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  28.6, 70.0, 71.1, 71.2, 78.3, 106.0, 109.1, 116.3, 119.1, 121.1, 124.8, 127.5, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.6, 129.9, 133.7, 136.5, 136.8, 137.4, 137.7, 138.4, 152.0, 152.9, 153.5, 165.2; FAB-MS  $m/z$  967 ( $\text{M}+\text{H}$ )<sup>+</sup>. HRMS (FAB) calculated for  $\text{C}_{64}\text{H}_{55}\text{O}_9$  967.3846 [ $\text{M}+\text{H}$ ]<sup>+</sup>; Found 967.3863.

**(–)-DOGCg ((–)-3).** A suspension of **57** (50 mg, 52  $\mu\text{mol}$ ) and 20%  $\text{Pd}(\text{OH})_2\text{-C}$  (5.2 mg) in a mixture of THF (1.5 mL) and MeOH (1.5 mL) was stirred at room temperature for 17 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and evaporated under reduced pressure to give a residue. The residue was dissolved in AcOEt and the resulting solution was washed with water and extracted with EtOAc three times. The extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 1 / 2) to afford **3** (19 mg, 86%) as colorless amorphous solids.

$[\alpha]_{\text{D}}^{24}$   $-73.5$  (*c* 1.08, acetone/ $\text{H}_2\text{O}$  = 1 : 1); IR (film) 3287, 1693, 1336, 1230  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (acetone- $\text{d}_6$ , 500 MHz)  $\delta$  2.79 (dd,  $J = 16.2$ , 5.6 Hz, 1H), 2.93 (dd,  $J = 16.2$ , 4.6 Hz, 1H), 5.11 (d,  $J = 5.3$  Hz, 1H), 5.30 (q,  $J = 5.3$  Hz, 1H), 6.34 (s, 2H), 6.76 (t,  $J = 7.9$  Hz, 2H), 6.97 (d,  $J = 6.6$  Hz, 1H), 6.98 (s, 2H), 7.05 (t,  $J = 7.6$  Hz, 1H), 7.93 (br s, 6H);  $^{13}\text{C}$  NMR (acetone- $\text{d}_6$ , 68 MHz)  $\delta$  52.1, 70.5, 79.0, 106.3, 110.1, 110.2, 117.1, 120.4, 121.7, 121.8, 128.8, 131.0, 131.1, 133.6, 139.2, 146.2, 146.9, 155.0, 166.3; FAB-MS  $m/z$  427 ( $\text{M}+\text{H}$ )<sup>+</sup>. HRMS (FAB) calculated for  $\text{C}_{22}\text{H}_{19}\text{O}_9$  427.1029 [ $\text{M}+\text{H}$ ]<sup>+</sup>; Found 427.1049.

**cis-Epoxyde (–)-18b.** To a stirred solution of *cis*-**16a** (329 mg, 1.09 mmol) in a mixture of MeCN (6 mL) and dimethoxymethane (12 mL) were successively added **17** (700 mg, 1.09 mmol),  $\text{Bu}_4\text{N}^+\text{HSO}_4^-$  (16.7 mg,

0.491 mmol), 12 mL of phosphate buffer (pH = 9.18), OXONE (2.21 g, 3.60 mmol) and potassium carbonate (873 mg, 6.32 mmol) at 0 °C. After being stirred at the same temperature for 1 h, to the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 9 / 1) to afford **18b** (556 mg, 77%) as a orange oil.

The enantiomeric excess of **18b** was determined to be 87% *ee* by HPLC analysis on a chiral stationary phase under the conditions described below.

[α]<sub>D</sub><sup>24</sup> -19.4 (*c* 1.15, CHCl<sub>3</sub>); IR (film) 3032, 2930, 1591, 1259, 1120 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 0.20 (s, 6H), 1.00 (s, 9H), 2.30 (dd, *J* = 14.6, 5.5 Hz, 1H), 2.81 (dd, *J* = 14.6, 5.5 Hz, 1H), 3.44 (td, *J* = 5.5, 1.8 Hz, 1H), 4.00 (d, *J* = 1.8 Hz, 1H), 5.09 (s, 6H), 6.58 (m, 1H), 6.63 (s, 2H), 6.75 (m, 1H), 7.05 (td, *J* = 7.9, 1.2 Hz, 1H), 7.25–7.44 (m, 15H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 68 MHz) δ -4.2, 18.2, 25.8, 28.1, 57.4, 58.4, 62.1, 71.1, 75.1, 106.2, 118.2, 121.0, 127.4, 127.5, 127.8, 128.1, 128.5, 128.6, 130.7, 131.1, 137.0, 137.6, 137.8, 152.7, 153.6; FAB-MS *m/z* 659 (M+H)<sup>+</sup>. HRMS (FAB) calculated for C<sub>42</sub>H<sub>47</sub>O<sub>5</sub>Si 659.3139 [(M+H)<sup>+</sup>], Found 659.3163.

Chiral HPLC: Daicel ChiralPak AD-H 0.46 cm f x 25 cm, eluent: 4% *i*-PrOH/*n*-hexane, flow rate : 0.5 mL/min, retention time : 33.7 min (93.4%), 49.9 min (6.6%)

**Dihydrobenzopyrane 20** (mixture). To a stirred solution of **18b** (250 mg, 0.379 mmol) in THF (7 mL) were successively added acetic acid (109 mL, 1.90 mmol) and 1.0 M solution of TBAF in THF (0.76 mL, 0.76 mmol) at 0 °C under an argon atmosphere. After being stirred at the same temperature for 20 min, to the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to give a crude product **18c** (279 mg) as yellow oil. The crude product was used in the next reaction without further purification.

A solution of the crude product (**18c**, 279 mg) and CSA (98.2 mg, 0.423 mmol) in toluene (8 mL) was stirred at 0 °C for 1 hour. To the reaction mixture was added water and the resulting mixture was extracted with AcOEt three times. The extracts were dried over anhydrous MgSO<sub>4</sub>, filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 4 / 1) to afford **20** (110 mg, 48% for 2 steps, *cis* : *trans* = 1 : 1) as a pale yellow oil.

The enantiomeric excess of *trans* isomer and *cis* isomer were determined to be 72% *ee* and 87% *ee* respectively by HPLC analysis on a chiral stationary phase under the conditions described below.

IR (film) 3448, 2916, 1592, 1234, 1112 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz, a mixture of diastereomers) δ 2.86–2.97 (m, 2H), 3.06 (dd, *J* = 16.4, 5.5 Hz, 1H), 3.23 (dd, *J* = 16.8, 4.0 Hz, 1H), 3.99 (m, 1H, *trans*

3-H), 4.22 (br s, 1H, *cis* 3-H), 4.65 (d,  $J = 8.5$  Hz, 1H, *trans* 2-H), 4.97 (br s, 1H, *cis* 2-H), 5.06–5.14 (m, 12H), 6.73 (s, 2H), 6.81 (s, 2H), 6.91–6.97 (m, 3H), 7.10–7.17 (m, 2H), 7.30–7.43 (m, 35H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz, a mixture of diastereomers)  $\delta$  32.9, 33.2, 66.8, 68.1, 71.2, 71.3, 75.2, 78.1, 81.9, 106.0, 106.8, 116.5, 113.7, 118.8, 120.2, 121.2, 121.4, 127.5, 127.6, 127.7, 127.8, 127.9, 128.2, 128.5, 130.0, 130.3, 133.3, 133.7, 136.8, 137.0, 137.8, 153.0, 153.1, 153.8; FAB-MS :  $m/z$  544 ( $\text{M}$ ) $^+$ .

Chiral HPLC: Daicel ChiralCel OD 0.46 cm f x 25 cm, eluent: 10% *i*-PrOH/*n*-hexane, flow rate : 0.5 mL/min, retention time : *trans* ; 65.5 min (86.2%), 74.2 min (13.8%), *cis* ; 108.2 min (93.9%), 120.6 min (6.1%).

**Hexakisbenzyl DOEGCg (58)**. A solution of **20** (33.5 mg, 61.5  $\mu\text{mol}$ ), **23** (81.3 mg, 184  $\mu\text{mol}$ ), EDCI (29.0 mg, 154  $\mu\text{mol}$ ) and dimethylaminopyridine (0.8 mg, 6  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was stirred at room temperature for 3 h. To the reaction mixture was added saturated aqueous  $\text{NH}_4\text{Cl}$  and the resulting mixture was extracted with  $\text{CH}_2\text{Cl}_2$  three times. The extracts were dried over anhydrous  $\text{MgSO}_4$ , filtered and evaporated under reduced pressure to give a residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 6 / 1) to afford **57** (33.3 mg, 56%) ( $R_f = 0.42$  for *n*-hexane / AcOEt = 3 / 1) and **58** (26.2 mg, 44%) ( $R_f = 0.49$  for *n*-hexane / AcOEt = 3 / 1) as yellow amorphous solids.

$[\alpha]_D^{24} -83.0$  ( $c$  1.33,  $\text{CHCl}_3$ ); IR (film) 3032, 2933, 2872, 1714, 1591, 1230  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.06 (dd,  $J = 17.1, 2.4$  Hz, 1H), 3.39 (dd,  $J = 17.1, 4.3$  Hz, 1H), 4.68–4.98 (m, 12H), 5.12 (br s, 1H), 5.65 (br s, 1H), 6.74 (s, 2H), 6.98–7.36 (m, 36H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 68 MHz)  $\delta$  68.5, 70.7, 75.0, 106.6, 109.0, 116.6, 118.5, 121.3, 124.8, 127.4, 127.7, 127.8, 128.1, 128.2, 128.3, 128.4, 128.5, 130.0, 133.3, 136.4, 136.9, 147.3, 152.4, 152.9, 164.8; FAB-MS  $m/z$  967 ( $\text{M}+\text{H}$ ) $^+$ . HRMS (FAB) calculated for  $\text{C}_{64}\text{H}_{55}\text{O}_9$  967.3846 [ $\text{M}+\text{H}$ ] $^+$ , found 967.3863.

**(-)-DOEGCg ((-)-2)**. A suspension of **58** (41 mg, 42  $\mu\text{mol}$ ) and 20%  $\text{Pd}(\text{OH})_2\text{-C}$  (9 mg) in a mixture of THF (0.8 mL) and MeOH (0.8 mL) was stirred at room temperature for 6 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and evaporated under reduced pressure to give a residue. The residue was dissolved in AcOEt and the resulting solution was washed with water and extracted with AcOEt three times. The extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and evaporated under reduced pressure to afford **2** (18 mg, quant.) as colorless amorphous solids.

$[\alpha]_D^{24} -158$  ( $c$  1.02, acetone /  $\text{H}_2\text{O} = 1 : 1$ ); IR (film) 3286, 1689, 1614, 1336, 1226  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (acetone- $d_6$ , 500 MHz)  $\delta$  2.85 (m, 1H), 3.29 (dd,  $J = 17.5, 4.3$  Hz, 1H), 5.04 (br s, 1H), 5.42 (br s, 1H), 6.85 (s, 2H) 6.71–7.04 (m, 4H), 7.83 (br s, 6H);  $^{13}\text{C}$  NMR (acetone- $d_6$ , 68 MHz)  $\delta$  31.7, 69.3, 78.2, 106.7, 109.9, 117.2, 120.0, 121.5, 121.7, 128.2, 130.5, 130.8, 133.2, 138.8, 145.8, 146.3, 155.6, 166.0; FAB-MS  $m/z$  427 ( $\text{M}+\text{H}$ ) $^+$ . HRMS (FAB) calculated for  $\text{C}_{22}\text{H}_{19}\text{O}_9$  427.1024 [ $\text{M}+\text{H}$ ] $^+$ , found 427.1006.

**trans-Olefin 32**. To a solution of **30** (25.0 g, 47.6 mmol) in THF (250 mL) was added 1.00 M solution of LHMDS in THF (1.56 mL, 1.56 mmol) at  $-78$  °C. After being stirred at  $-78$  °C for 30 min, a THF



solution (50 mL) of **31** (24.2 g, 57.1 mmol) was added to the reaction mixture, which was stirred at –78 °C for 2 h. Then saturated aqueous NH<sub>4</sub>Cl was added and the mixture was extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 4 / 1) to afford **32** (33.1 g, 96%) as a colorless oil.

IR (film) 2928, 1578, 1483, 1255, 1116 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 0.23 (s, 6H), 1.00 (s, 9H), 3.44 (d, *J* = 6.3 Hz, 2H), 5.04 (s, 4H), 5.10 (s, 2H), 6.15 (dt, *J* = 6.3, 16.0 Hz, 1H), 6.27 (d, *J* = 16.0 Hz, 1H), 6.65 (s, 2H), 6.67 (d, *J* = 8.5 Hz, 1H), 7.19–7.43 (m, 17H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ –4.0, 18.4, 25.9, 33.5, 71.3, 75.4, 106.0, 113.5, 118.7, 120.0, 127.5, 127.9, 128.0, 128.2, 128.6, 128.7, 130.0, 131.1, 131.3, 131.6, 133.0, 133.2, 133.3, 137.2, 138.0, 138.0, 152.7, 153.0; MS (ESI) *m/z* 743 (M+Na)<sup>+</sup>. HRMS (ESI) *m/s* calculated for C<sub>42</sub>H<sub>45</sub>BrNaO<sub>4</sub>Si 743.2163 [(M+Na)<sup>+</sup>], found 743.2151.

**Alcohol 40.** Organoborane **38** was prepared by the following procedure: To a solution of MOM-protected **39** (8.10 g, 62.3 mmol) in THF (2 mL) was added 9-BBN dimmer (11.2 g, 37.4 mmol). The mixture was stirred at 50 °C for 30 min to afford a THF solution of **38**.

To a solution of **32** (15.0 g, 20.8 mmol) and **38** in THF (100 mL) were added Pd(dppf)Cl<sub>2</sub>·CH<sub>2</sub>Cl<sub>2</sub> (1.81 mg, 2.08 mmol, 10 mol%) and aqueous 3 M NaOH (22.2 mL). After being heated under reflux for 4 h, the reaction mixture was poured into water, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure to give the crude product. To a solution of the crude product in MeOH (180 mL) was added conc. HCl (10 mL), and the mixture was heated at 60 °C for 12 h. The reaction mixture was concentrated under reduced pressure to give the crude residue. The residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 2) to afford **40** (12.0 g, 95%) as a yellow oil.

IR (film) 3331, 2931, 1581, 1504, 1427, 1114 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 1.37–1.43 (m, 2H), 1.58–1.63 (m, 4H), 2.54 (t, *J* = 7.5 Hz, 2H), 3.52 (d, *J* = 6.3 Hz, 2H), 3.64 (t, *J* = 6.3 Hz, 2H), 5.04 (s, 2H), 5.09 (s, 4H), 6.15 (dt, *J* = 6.3, 16.0 Hz, 1H), 6.37 (d, *J* = 16.0 Hz, 1H), 6.67 (s, 2H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.93–6.95 (m, 2H), 7.23–7.43 (m, 15H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz) δ 25.5, 31.7, 32.7, 34.2, 35.1, 63.1, 71.3, 75.4, 106.0, 115.7, 125.5, 127.6, 127.7, 127.9, 127.9, 128.0, 128.3, 128.6, 128.7, 130.5, 131.1, 133.1, 135.1, 137.2, 137.9, 138.0, 152.1, 153.0; MS (ESI) *m/z* 637 (M+Na)<sup>+</sup>. HRMS (ESI) *m/z* calculated for C<sub>41</sub>H<sub>42</sub>NaO<sub>5</sub> 637.2924 [(M+Na)<sup>+</sup>], found 637.2915.

**Amide 59.** To a solution of **40** (9.51 g, 15.5 mmol) in toluene (50 mL) were added *N*-Cbz-*N*-Ns-amide (**41**) (5.60 g, 16.6 mmol), PPh<sub>3</sub> (5.71 g, 21.8 mmol) and DMEAD (4.40 g, 18.8 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was added saturated aqueous NaCl, and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under

reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 1) to afford **59** (11.4 g, 89%) as a colorless oil.

IR (film) 3523, 2932, 1730, 1581, 1541, 1502, 1367  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.37–1.43 (m, 2H), 1.53–1.62 (m, 2H), 1.75–1.81 (m, 2H), 2.54 (t,  $J = 8.0$  Hz, 2H), 3.46 (d,  $J = 6.3$  Hz, 2H), 3.84 (t,  $J = 7.7$  Hz, 2H), 4.55 (s, 1H), 5.06 (s, 4H), 5.07 (s, 2H), 5.09 (s, 2H), 5.75 (dt,  $J = 7.4, 11.5$  Hz, 1H), 6.51 (d,  $J = 16.0$  Hz, 1H), 6.61 (s, 2H), 6.70 (d,  $J = 8.0$  Hz, 1H), 6.90–6.92 (m, 2H), 7.19–7.46 (m, 21H), 7.65–7.69 (m, 2H), 8.10 (d,  $J = 7.5$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  26.3, 30.1, 31.4, 34.4, 35.0, 48.4, 69.4, 71.3, 75.4, 106.0, 115.7, 124.4, 125.5, 127.5, 127.6, 127.8, 127.9, 128.0, 128.3, 128.6, 128.7, 128.8, 128.8, 128.9, 130.6, 131.2, 131.7, 132.7, 132.9, 133.1, 134.2, 134.3, 134.4, 135.1, 137.2, 137.9, 137.9, 147.8, 152.0, 152.2, 153.0; MS (ESI)  $m/z$  955 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{55}\text{H}_{52}\text{N}_2\text{NaO}_{10}\text{S}$  955.3235 [ $\text{M}+\text{Na}$ ] $^+$ , found 955.3263.

**Olefin 42.** To a solution of **59** (9.51 g, 10.2 mmol) in DMF (10 mL) were added TBSCl (2.30 g, 15.3 mmol) and imidazole (1.02 g, 15.3 mmol) at 0 °C. After being stirred at room temperature for 4 h, the reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ , and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 4 / 1) to afford **42** (10.1 g, 95%) as a colorless oil.

IR (film) 2930, 1736, 1578, 1543, 1498, 1368  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.38–1.44 (m, 2H), 1.57–1.64 (m, 2H), 1.76–1.81 (m, 2H), 2.51 (t,  $J = 8.0$  Hz, 2H), 3.50 (d,  $J = 5.7$  Hz, 2H), 3.84 (t,  $J = 7.5$  Hz, 2H), 5.03 (s, 2H), 5.08 (s, 4H), 5.09 (s, 2H), 5.75 (dt,  $J = 5.7, 16.0$  Hz, 1H), 6.41 (d,  $J = 16.0$  Hz, 1H), 6.66 (s, 2H), 6.73 (d,  $J = 8.6$  Hz, 1H), 6.89 (dd,  $J = 2.3, 8.6$  Hz, 1H), 6.96 (d,  $J = 2.3$  Hz, 1H), 7.19–7.45 (m, 21H), 7.62–7.71 (m, 2H), 8.10 (d,  $J = 6.9$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  -4.0, 18.4, 26.0, 26.4, 30.1, 31.4, 33.9, 35.1, 48.4, 69.3, 71.2, 75.3, 105.8, 118.2, 124.4, 127.0, 127.5, 127.8, 127.9, 128.2, 128.6, 128.6, 128.7, 128.8, 128.9, 130.4, 130.4, 131.7, 132.9, 133.7, 134.3, 134.3, 135.2, 137.3, 138.0, 147.8, 151.4, 151.9, 152.9; MS (ESI)  $m/z$  743 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{61}\text{H}_{66}\text{N}_2\text{NaO}_{10}\text{SSi}$  743.2163 [ $\text{M}+\text{Na}$ ] $^+$ , found 743.2151.

**Diol 43:** To a stirred solution of **42** (3.50 g, 4.06 mmol) in the combined solvent *t*-BuOH / water /  $\text{CH}_2\text{Cl}_2$  (1 / 1 / 1, 90 mL) were added AD-mix- $\beta$  (11.4 g, 8.12 mmol) and  $\text{MeSO}_2\text{NH}_2$  (772 mg, 8.12 mmol) at 0 °C. After being stirred at room temperature for 24 h, the reaction mixture was quenched with saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  at 0 °C, stirred at room temperature for 10 min and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 4 / 1) to afford **43** (2.75 g, 76%) as pale yellow amorphous solids.

$[\alpha]_D^{23} +4.8$  (*c* 1.30,  $\text{CHCl}_3$ ); IR (film) 3537, 2930, 1732, 1591, 1543, 1501, 1368, 1265, 1120  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.37–1.43 (m, 2H), 1.57–1.64 (m, 2H), 1.76–1.81 (m, 2H), 2.52 (d,  $J = 8.0$  Hz, 2H), 2.65 (m, 1H), 2.67 (d,  $J = 6.9$  Hz, 2H), 3.10 (m, 1H), 3.84 (t,  $J = 8.0$  Hz, 2H), 3.86 (m, 1H), 4.43 (m, 1H), 5.03 (s, 2H), 5.09 (s, 2H), 5.10 (s, 4H), 6.41 (d,  $J = 8.0$  Hz, 1H), 6.72 (s, 2H), 6.73 (m, 1H), 6.90–6.91 (m, 2H), 7.19–7.44 (m, 21H), 7.68–7.61 (m, 2H), 8.07 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  –4.0, –3.9, 18.3, 25.9, 26.2, 30.0, 31.2, 34.8, 35.0, 48.4, 69.4, 71.2, 75.2, 76.0, 77.3, 106.5, 118.6, 124.4, 127.7, 127.8, 127.8, 128.0, 128.2, 128.5, 128.6, 128.7, 128.8, 128.9, 130.4, 130.4, 131.7, 132.9, 134.3, 134.4, 135.6, 137.2, 138.0, 147.8, 151.8, 151.9, 152.9; MS (ESI)  $m/z$  1103 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{61}\text{H}_{68}\text{N}_2\text{NaO}_{12}\text{SSi}$  1103.4154 [ $\text{M}+\text{Na}$ ] $^+$ , found 1103.4205.

**Ester 45a.** To a solution of **43** (6.78 g, 6.27 mmol) in  $\text{CH}_2\text{Cl}_2$  (250 mL) was added  $\text{Bu}_2\text{SnO}$  (1.56 g, 6.27 mmol). After being stirred at room temperature for 1 h, 3,4,5-trisbenzylgalloyl chloride (**44**) (3.45 g, 7.42 mmol) and  $\text{Et}_3\text{N}$  (1.34 mL, 9.41 mmol) were added and the reaction mixture was stirred at room temperature for 10 h. The resulting mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ , and extracted with  $\text{AcOEt}$ . The organic layer was washed with saturated aqueous  $\text{NaCl}$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane /  $\text{AcOEt} = 3 / 1$ ) to afford **45a** / **45b** = 2 / 1 as yellow amorphous solids. To a mixture of the **45a** and **45b** in toluene (200 mL) was added DBU (5.00 mL, 33.4 mmol, 5.3 equiv), and the mixture was stirred at  $-20$  °C for 48 h. The reaction mixture was quenched with aqueous 2 M  $\text{HCl}$ , and extracted with  $\text{AcOEt}$ . The organic layer was washed with saturated aqueous  $\text{NaCl}$ , dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane /  $\text{CH}_2\text{Cl}_2 = 2 / 1$  to 1 / 2) to afford **45a** (8.40 g, 89%) as colorless amorphous solids.

$[\alpha]_D^{23} -1.9$  (*c* 1.25,  $\text{CHCl}_3$ ); IR (film) 3501, 2930, 1732, 1714, 1591, 1543, 1504, 1429, 1371, 1265, 1116  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  0.23 (s, 3H), 0.25 (s, 3H), 1.02 (s, 9H), 1.30–1.36 (m, 2H), 1.49–1.55 (m, 2H), 1.71–1.77 (m, 2H), 2.44 (t,  $J = 7.5$  Hz, 2H), 2.93 (dd,  $J = 8.0, 13.8$  Hz, 1H), 2.97 (d,  $J = 4.6$  Hz, 1H), 3.14 (dd,  $J = 6.9, 13.8$  Hz, 1H), 3.82 (t,  $J = 8.0$  Hz, 2H), 4.68 (t,  $J = 4.6$  Hz, 2H), 4.84 (d,  $J = 11.5$  Hz, 2H), 4.92 (d,  $J = 11.5$  Hz, 2H), 4.95 (s, 2H), 4.97 (s, 2H), 5.06 (s, 2H), 5.07 (s, 4H), 5.55 (m, 1H), 6.66 (s, 2H), 6.73 (d,  $J = 8.6$  Hz, 1H), 6.88 (dd,  $J = 2.3, 8.6$  Hz, 1H), 7.01 (d,  $J = 2.3$  Hz, 1H), 7.15–7.43 (m, 35H), 7.59–7.66 (m, 2H), 8.03 (d,  $J = 6.9$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  –3.9, –3.8, 18.5, 26.1, 26.2, 29.9, 31.1, 31.8, 34.9, 48.4, 69.4, 71.2, 71.4, 75.2, 106.3, 109.3, 118.8, 124.4, 125.5, 127.0, 127.6, 127.8, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.6, 128.6, 128.8, 128.9, 131.6, 131.7, 132.9, 134.2, 134.3, 135.3, 136.4, 136.7, 137.1, 137.6, 138.0, 142.6, 147.8, 151.9, 152.6, 152.8, 165.5, 174.5; MS (ESI)  $m/z$  1525 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{89}\text{H}_{90}\text{N}_2\text{NaO}_{16}\text{SSi}$  1525.5673 [ $\text{M}+\text{Na}$ ] $^+$ , found 1525.5655.

**Dihydrobenzopyran 18a.** To a solution of **37** (100 mg, 66.5  $\mu\text{mol}$ ) in THF (1 mL) were added AcOH (12.0  $\mu\text{L}$ , 200  $\mu\text{mol}$ ) and 1 M TBAF (133  $\mu\text{L}$ , 133  $\mu\text{mol}$ ) in THF at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ , and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. To a mixture of the crude product in toluene (2.0 mL) were added CSA (15.4 mg, 100  $\mu\text{mol}$ ), and the mixture was heated at 60 °C for 12 h. The organic layer was washed with water, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 2) to afford **18a** (87.3 mg, 95%) as colorless amorphous solids.

$[\alpha]_{\text{D}}^{23}$   $-23$  (*c* 1.5,  $\text{CHCl}_3$ ); IR (film) 2927, 1734, 1726, 1591, 1543  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  8.11 (d, *J* = 6.9 Hz, 1H), 7.70–7.67 (dd, *J* = 1.7, 8.0 Hz, 1H), 7.65–7.62 (dt, *J* = 1.2, 8.0 Hz, 1H), 7.46–7.42 (dt, *J* = 1.2, 7.5 Hz, 1H), 7.37–7.20 (m, 35H), 7.02 (dd, *J* = 1.7, 8.6 Hz, 1H), 6.93 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 1.7 Hz, 1H), 6.72 (s, 2H), 5.46 (dt, *J* = 5.2, 6.3 Hz, 1H), 5.14 (d, *J* = 6.3 Hz, 1H), 5.11 (s, 2H), 5.04–4.98 (m, 12H), 3.86 (t, *J* = 8.0 Hz, 2H), 3.10 (dd, *J* = 5.2, 16.6 Hz, 1H), 2.95 (dd, *J* = 6.9, 16.6 Hz, 1H), 2.57 (t, *J* = 8.0 Hz, 2H), 1.83–1.76 (m, 2H), 1.68–1.61 (m, 2H), 1.46–1.40 (m, 2H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  165.2, 153.0, 152.5, 151.9, 151.8, 147.8, 142.7, 138.5, 137.8, 137.5, 136.9, 136.6, 135.2, 134.4, 134.3, 134.3, 133.9, 132.9, 131.7, 129.7, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 125.0, 124.4, 118.9, 116.3, 109.1, 106.3, 78.6, 75.3, 75.2, 71.3, 71.2, 70.3, 69.4, 48.4, 35.0, 31.4, 30.0, 29.5, 26.3; MS (ESI) *m/z* 1393 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI) *m/z* calculated for  $\text{C}_{83}\text{H}_{74}\text{N}_2\text{NaO}_{15}\text{S}$  1393.4702 [ $\text{M}+\text{Na}$ ] $^+$ , found 1393.4733.

**Ketone 49.** To a solution of **45a** (8.40 g, 5.59 mmol) in  $\text{CH}_2\text{Cl}_2$  (80 mL) were added 1-Me-AZADO (192 mg, 1.12 mmol) and  $\text{PhI}(\text{OAc})_2$  (3.74 g, 11.2 mmol). After being stirred at room temperature for 5 h, the reaction mixture was quenched with saturated aqueous  $\text{Na}_2\text{S}_2\text{O}_3$  at 0 °C, stirred at room temperature for 10 min and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. To a mixture of the crude product in THF (10 mL) were added AcOH (1.01 mL, 16.8 mmol) and TBAF (11.2 mL, 11.2 mmol, 2.0 equiv) at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$ , and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 2) to afford **49** (7.31 g, 96%) as a colorless amorphous. IR (film) 3441, 2933, 1724, 1589, 1543, 1501, 1429, 1369, 1115  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.36–1.42 (m, 2H), 1.55–1.61 (m, 2H), 1.81–1.75 (m, 2H), 2.51 (m, 2H), 2.97 (dd, *J* = 5.1, 13.8 Hz, 1H), 3.16 (m, 1H), 3.26 (dd, *J* = 9.2, 13.8 Hz, 1H), 3.84 (t, *J* = 7.5 Hz, 2H), 4.76 (brs, 1H), 4.84 (d, *J* = 11.5 Hz, 2H), 4.81 (d, *J* = 11.5 Hz, 2H), 4.88 (d, *J* = 2.9 Hz, 2H), 5.00 (s, 2H), 5.08 (s, 2H), 5.09 (s, 4H), 5.27 (m,

1H), 6.64 (s, 2H), 6.78 (d,  $J = 9.2$  Hz, 1H), 6.85 (brs, 1H), 6.94 (m, 1H), 6.95 (m, 1H), 7.15–7.43 (m, 35H), 7.59–7.66 (m, 2H), 8.04 (d,  $J = 7.5$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  26.2, 30.0, 31.2, 31.8, 34.8, 48.4, 69.4, 71.3, 71.4, 73.2, 75.2, 75.2, 77.9, 106.3, 109.4, 116.4, 121.9, 124.4, 124.9, 127.6, 127.8, 127.9, 128, 128.2, 128.3, 128.5, 128.6, 128.7, 128.8, 128.9, 131.7, 132.8, 134.2, 134.3, 134.4, 134.8, 135.8, 136.7, 137.0, 137.5, 137.8, 137.8, 138.0, 143.0, 147.8, 152.0, 152.7, 152.9, 153.0, 166.3; MS (ESI)  $m/z$  1411 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{83}\text{H}_{76}\text{N}_2\text{NaO}_{16}\text{S}$  1411.4808 [ $\text{M}+\text{Na}$ ] $^+$ , found 1411.4850

**Dihydrobenzopyran 18b.** To a solution of **40** (7.31 g, 5.37 mmol) in  $\text{CH}_2\text{Cl}_2$  (200 mL) were added  $\text{Et}_3\text{SiH}$  (1.74 mL, 10.7 mmol) and  $\text{BF}_3 \cdot \text{OEt}_2$  (2.41 mL, 16.1 mmol) at 0 °C. After being stirred at 0 °C for 5 hours, the reaction mixture was treated with water and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (*n*-hexane / AcOEt = 3 / 1) to afford *cis*-**48** (6.16 g, 84%) as colorless amorphous solids.

$[\alpha]_{\text{D}}^{23}$   $-1.9$  ( $c$  1.3,  $\text{CHCl}_3$ ), IR (film) 3064, 2927, 1732, 1713, 1593, 1537  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.40–1.47 (m, 2H), 1.61–1.68 (m, 2H), 1.77–1.84 (m, 2H), 2.58 (t,  $J = 8.0$  Hz, 2H), 3.05 (dd,  $J = 2.9, 17.8$  Hz, 1H), 3.40 (dd,  $J = 4.6, 17.8$  Hz, 1H), 3.86 (t,  $J = 7.5$  Hz, 2H), 4.67 (d,  $J = 11.5$  Hz, 2H), 4.80 (d,  $J = 11.5$  Hz, 2H), 4.90 (s, 2H), 4.94–5.04 (m, 18H), 5.09 (m, 1H), 5.11 (s, 2H), 5.65 (m, 1H), 6.74 (s, 2H), 6.95 (d,  $J = 1.7$  Hz, 1H), 7.00 (d,  $J = 8.6$  Hz, 1H), 7.03 (dd,  $J = 1.7, 8.6$  Hz, 1H), 7.18–7.37 (m, 35H), 7.43–7.47 (m, 1H), 7.33–7.66 (dd,  $J = 1.2, 8.0$  Hz, 1H), 7.68–7.72 (dd,  $J = 1.2, 8.0$  Hz, 1H), 8.11 (d,  $J = 8.0$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  26.3, 30.0, 31.3, 35.1, 48.4, 68.9, 69.4, 71.1, 71.2, 75.1, 75.2, 77.9, 106.7, 109.1, 116.6, 118.3, 124.4, 125.0, 127.6, 127.8, 127.8, 127.9, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 128.9, 129.8, 131.7, 133.0, 133.6, 134.3, 134.3, 134.4, 135.4, 136.5, 137.0, 137.6, 137.9, 138.4, 142.7, 147.8, 151.9, 152.4, 153.0, 165.0; MS (ESI)  $m/z$  1393 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{83}\text{H}_{74}\text{N}_2\text{NaO}_{15}\text{S}$  1393.4702 [ $\text{M}+\text{Na}$ ] $^+$ , found 1393.4769.

**60:** To a solution of **48b** (3.03 g, 2.21 mmol) in MeCN (15 mL) were added PhSH (674  $\mu\text{L}$ , 6.62 mmol) and  $\text{Cs}_2\text{CO}_3$  (1.78 g, 5.46 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The crude residue was purified by column chromatography (*n*-hexane / AcOEt = 5 / 1  $\rightarrow$  4 / 1) to afford **60** (1.26 g, 48%) as colorless amorphous solids.

IR (film) 3417, 3030, 2927, 1732, 1712, 1591, 1543  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.37 (m, 2H), 1.53 (m, 2H), 1.63 (m, 2H), 2.57 (t,  $J = 7.5$  Hz, 2H), 3.05 (dd,  $J = 4.6, 16.6$  Hz, 1H), 3.19 (q,  $J = 6.9$  Hz, 2H), 3.38 (dd,  $J = 4.6, 16.6$  Hz, 1H), 4.68 (d,  $J = 11.5$  Hz, 2H), 4.81 (d,  $J = 11.5$  Hz, 2H), 4.90 (s, 2H), 4.95–5.0 (m, 6H), 5.10 (s, 2H), 5.65 (m, 1H), 6.74 (s, 2H), 6.94 (m, 1H), 6.99–7.01 (d,  $J = 8.0$  Hz, 1H),

7.02–7.04 (brd,  $J = 8.0$  Hz, 1H), 7.18–7.37 (m, 35H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  26.5, 30.0, 31.3, 31.4, 35.1, 41.1, 66.7, 68.8, 71.1, 71.3, 75.1, 75.2, 77.9, 106.7, 109.1, 116.6, 118.0, 125.0, 127.6, 127.9, 127.9, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 129.8, 133.5, 135.4, 136.5, 136.7, 137.5, 137.9, 138.5, 142.8, 152.5, 152.9, 156.5, 165.0; MS (ESI)  $m/z$  1208 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{77}\text{H}_{71}\text{NNaO}_{11}$  1208.4919 [ $\text{M}+\text{Na}$ ] $^+$ , found 1208.4954.

**APDOEGCg (4).** A suspension of **60** (100 mg, 84.3  $\mu\text{mol}$ ) and 20%  $\text{Pd}(\text{OH})_2$  (30 mg) in THF / MeOH (1 / 1, 4.0 mL) was stirred under hydrogen atmosphere at room temperature for 5 h. The reaction mixture was filtered through a bed of Celite and the solvent was removed under reduced pressure to afford **4** (38 mg, 89%) as colorless amorphous solids.

IR (film) 3228, 2931, 1701, 1610, 1498, 1448, 1340, 1219, 1037  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  1.37–1.33 (m, 2H), 1.66–1.57 (m, 4H), 2.51 (t,  $J = 7.5$  Hz, 2H), 2.85 (t,  $J = 7.5$  Hz, 2H), 2.90–2.85 (d,  $J = 2.3$  Hz, 1H), 3.31–3.29 (d,  $J = 4.0$  Hz, 0.5H (0.5H was overlap on solvent peak)), 5.01 (s, 1H), 5.49 (m, 1H), 6.51 (s, 2H), 6.81 (d,  $J = 8.6$  Hz, 1H), 6.88 (m, 1H), 6.90 (s, 2H), 6.94 (dd,  $J = 1.7, 8.6$  Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125 MHz)  $\delta$  25.6, 27.1, 30.8, 30.9, 34.4, 39.4, 68.8, 77.4, 105.5, 108.9, 116.0, 118.5, 120.1, 127.2, 129.3, 129.4, 132.5, 134.6, 138.5, 145.0, 145.1, 145.3, 152.6, 166.2; MS (ESI)  $m/z$  512 ( $\text{M}+\text{H}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{27}\text{H}_{30}\text{NO}_9$  512.1915 [ $\text{M}+\text{H}$ ] $^+$ , found 512.1936.

**APDOEGCg-TokyoGreen conjugate 53.** To a solution of TokyoGreen (30.0 mg, 86.7  $\mu\text{mol}$ ) in DMF (200  $\mu\text{L}$ ) were added *N*-hydroxysuccinimide (19.9 mg, 173  $\mu\text{mol}$ ) and EDCI (16.6 mg, 86.7  $\mu\text{mol}$ ) at 0  $^\circ\text{C}$ . After being stirred at room temperature for 1 h, added **4** (22.1 mg, 43.3  $\mu\text{mol}$ ) was added to the reaction mixture. After being stirred at room temperature for 4 h, the reaction mixture was purified by preparative HPLC (Cholest Water 10 x 250, 20% to 60% MeCN in water, 0.1% TFA, 30 minute ramp,  $l = 280$  nm, flow rate; 3.5 mL/min) to afford **53** (25.1 mg, 29.9  $\mu\text{mol}$ , 69%, RT = 18.9 min) as a colorless film.

IR (film) 3406, 2920, 1732, 1712, 1680, 1639, 1587, 1205  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 500 MHz)  $\delta$  1.32–1.37 (m, 2H), 1.52–1.58 (m, 4H), 2.23–2.31 (m, 2H), 2.50 (m, 2H), 2.82 (d,  $J = 16.0$  Hz, 1H), 3.35 (t,  $J = 6.9$  Hz, 2H), 4.44 (td,  $J = 5.7, 47.0$  Hz, 2H), 4.56 (t,  $J = 6.9$  Hz, 2H), 5.03 (m, 1H), 5.36 (m, 1H), 6.39 (s, 2H), 6.59 (brs, 1H), 6.75 (s, 2H), 6.79–6.81 (m, 3H), 6.91 (m, 1H), 6.95 (d,  $J = 8.6$  Hz, 1H), 7.34 (d,  $J = 8.0$  Hz, 1H), 7.82 (d,  $J = 8.0$  Hz, 1H), 7.90 (s, 1H), 8.03 (brs, 1H), 8.57 (t,  $J = 5.7$  Hz, 1H), 8.71 (s, 1H), 8.92 (s, 1H), 9.17 (s, 2H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ , 125 MHz)  $\delta$  26.0, 29.0, 30.7, 30.7, 30.8, 31.1, 34.5, 38.8, 68.8, 77.4, 79.7, 81.0, 105.5, 108.9, 114.4, 115.9, 118.5, 120.0, 126.0, 127.2, 129.3, 129.3, 132.5, 134.8, 138.5, 142.7, 145.0, 145.4, 152.5, 161.1, 166.2; MS (ESI)  $m/z$  862 ( $\text{M}+\text{Na}$ ) $^+$ . HRMS (ESI)  $m/z$  calculated for  $\text{C}_{48}\text{H}_{41}\text{NNaO}_{13}$  862.2470 [ $\text{M}+\text{Na}$ ] $^+$ , found 862.2485.

**APDOEGCg-carrier protein conjugate 56.** Conjugation of APDOEGCg to carrier protein using glutaraldehyde was performed as described previously.<sup>28</sup>

**4** (0.6 mg) was incubated with human serum albumin (HSA) (1.0 mg) in phosphate-buffered saline (PBS) in the presence of 0.025% glutaraldehyde at room temperature for 1 hour. Then the reaction mixture was dialyzed against PBS for 24 h at 4 °C. The degree of substitution was determined by comparing absorbance at 280 nm of the product and equal amount of unconjugated carrier protein.

#### *Immunization of mice*

Female BALB/c mice (6–8 weeks old) (Japan SLC, Hamamatsu, Japan) were immunized intraperitoneally and subcutaneously with APDOEGCg-HSA conjugate (50 µg protein/mouse) in saline with Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA). After 2 and 4 weeks, the same immunization procedure using incomplete adjuvant (Difco Laboratories) was repeated. After 6 weeks, the mice received a booster intravenous injection of 30 µg protein of APDOEGCg-HSA in saline without adjuvant. Three days after the final immunization, the mice were sacrificed and venous blood was collected.

#### ACKNOWLEDGEMENTS

This work was financially supported by the Uehara Memorial Foundation (Y.H.), MEXT/JSPS KAKENHI Grant Numbers 23390007 and 24790017, Grants-in-Aid for Scientific Research on Priority Areas 12045232 and 24105530 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, and a grant for Platform for Drug Discovery, Informatics, and Structural Life Science from the Ministry of Education, Culture, Sports, Science and Technology.

#### REFERENCES

1. Recent review on catechin: (a) S. Das, J. Tanwar, S. Hameed, and Z. Fatima, *J. Biochem. Pharmacol. Res.*, 2014, **2**, 164; (b) D. G. Nagle, D. Ferreira, and Y.-D. Zhou, *Phytochemistry*, 2006, **67**, 1849; (c) M. Friedman, *Mol. Nutr. Food Res.*, 2007, **51**, 116; (d) D. S. Wheeler and W. J. Wheeler, *Drug Dev. Res.*, 2004, **61**, 45.
2. (a) E. C. Yiannakopoulou, *Pharmacol.*, 2014, **94**, 245; (b) C. S. Yang, X. Wang, G. Lu, and S. C. Picinich, *Nat. Rev. Cancer*, 2009, **9**, 429.
3. J. M. Song and B. L. Seong, *Expert Rev. Anti. Infect. Ther.*, 2007, **5**, 497.
4. (a) F. Thielecke and M. Boschmann, *Phytochemistry*, 2009, **70**, 11; (b) C. Cabrera, R. Artacho, and R. Giménez, *J. Am. Coll. Nutr.*, 2006, **25**, 79.
5. A recent SAR study of catechin: (a) S. B. Wan, K. R. Landis-Piwowar, D. J. Kuhn, D. Chen, Q. P. Dou, and T. K. Chan, *Bioorg. Med. Chem.*, 2005, **13**, 2177; (b) Y.-H. Moon, J.-H. Lee, J.-S. Ahn, S.-H. Nam, D.-K. Oh, D.-H. Park, H.-J. Chung, S. Kang, D. F. Day, and D. Kim, *J. Agric. Food Chem.*, 2006, **54**, 1230; (c) M. Dell'Agli, S. Bellosta, L. Rizzi, G. V. Galli, M. Canavesi, F. Rota, R. Parente, E. Bosisio, and S. Romeo, *Cell. Mol. Life Sci.*, 2005, **62**, 2896.

6. A chemical modification of natural catechin and these evaluation of biological activities: (a) K. Fukuhara, I. Nakanishi, E. Sugiyama, M. Kimura, T. Shimada, S. Urano, K. Yamaguchi, and N. Miyata, *J. Am. Chem. Soc.*, 2002, **124**, 5952; (b) W. Hakamata, I. Nakanishi, Y. Maeda, T. Shimizu, H. Higuchi, Y. Nakamura, S. Saito, S. Urano, T. Oku, T. Ozawa, N. Ikota, N. Miyata, H. Okuda, and K. Fukuhara, *J. Am. Chem. Soc.*, 2006, **128**, 6524; (c) K. Fukuhara, A. Ohno, I. Nakanishi, K. Imai, A. Nakamura, K. Anzai, N. Miyata, and H. Okuda, *Tetrahedron Lett.*, 2009, **50**, 6989; (d) K. Fukuhara, I. Nakanishi, K. Ohkubo, Y. Obara, A. Tada, K. Imai, A. Ohno, A. Nakamura, T. Ozawa, S. Urano, S. Saito, S. Fukuzumi, K. Anzai, N. Miyata, and H. Okuda, *Chem. Commun.*, 2009, 6180.
7. X. Y. Wu, X. She, and Y. Shi, *J. Am. Chem. Soc.*, 2002, **124**, 8792.
8. (a) G. Matsuo, K. Kawamura, N. Hori, H. Matsukura, and T. Nakata, *J. Am. Chem. Soc.*, 2004, **126**, 14374. For the disubstituted epoxide, see: (b) T. Oka, K. Fujiwara, and A. Murai, *Tetrahedron*, 1996, **52**, 12091; (c) K. C. Nicolou, C. V. C. Prasad, P. K. Somers, and C.-K. Hwang, *J. Am. Chem. Soc.*, 1989, **111**, 5330; (d) A. C. Jain, P. Arya, and K. N. Nayyar, *Indian J. Chem.*, 1983, **22B**, 1116.
9. (a) Z-selective Julia olefination: M.-E. Lebrun, P. L. Marquand, and C. Berthelette, *J. Org. Chem.*, 2006, **71**, 2009; (b) a review of modified Julia reaction: P. R. Blakemore, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2563; (c) P. R. Blakemore, W. J. Cole, P. J. Kocięński, and A. Morley, *Synlett*, 1998, 26.
10. Y. Hirooka, M. Nitta, T. Furuta, and T. Kan, *Synlett*, 2008, 3234.
11. Our investigation of amide bond-mediated conjugation of ligand molecules and probe units: (a) T. Kan, Y. Kita, Y. Morohashi, Y. Tominari, S. Hosoda, H. Natsugari, T. Tomita, T. Iwatsubo, and T. Fukuyama, *Org. Lett.*, 2007, **9**, 2055; (b) T. Kan, Y. Tominari, Y. Morohashi, H. Natsugari, T. Tomita, T. Iwatsubo, and T. Fukuyama, *Chem. Commun.*, 2003, 2244.
12. T. Fukuyama, M. Cheung, and T. Kan, *Synlett*, 1999, 1301.
13. A reviews of Ns-strategy: (a) T. Kan and T. Fukuyama, *Chem. Commun.*, 2004, 353; (b) T. Kan and T. Fukuyama, *J. Syn. Org. Chem. Jpn.*, 2001, **59**, 779.
14. A review of Suzuki-Miyaura coupling reaction: N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457.
15. A review of Mitsunobu reactions: (a) O. Mitsunobu, *Synthesis*, 1981, 1; (b) D. L. Hughes, *Org. React.*, 1992, **42**, 335.
16. A DMEAD mediated Mitsunobu reactions: T. Sugimura and K. Hagiya, *Chem. Lett.*, 2007, **36**, 566.
17. H. Kolb, M. S. VanNiewenheze, and K. B. Sharpless, *Chem. Rev.*, 1994, **94**, 2483.
18. M. D. Lewis, J. K. Cha, and Y. Kishi, *J. Am. Chem. Soc.*, 1982, **104**, 4976.
19. (a) H. Tanaka, H. Miyoshi, Y.-C. Chuang, Y. Ando, and T. Takahashi, *Angew. Chem. Int. Ed.*, 2007, **46**, 5934; (b) M. Kitada, Y. Ohno, H. Tanaka, and T. Takahashi, *Synlett*, 2006, 2827.



20. (a) W. Kurosawa, T. Kan, and T. Fukuyama, *Org. Synth., Vol X, Wiley, New York*, 2002, 482; (b) T. Fukuyama, C.-K. Jow, and M. Cheung, *Tetrahedron Lett.*, 1995, **36**, 6373.
21. A. Yoshida, Y. Hirooka, Y. Sugata, M. Nitta, T. Manabe, S. Ido, K. Murakami, R. K. Saha, T. Suzuki, M. Ohshima, A. Yoshida, K. Itoh, K. Shimizu, N. Oku, T. Furuta, T. Asakawa, T. Wakimoto, and T. Kan, *Chem. Commun.*, 2011, **47**, 1794.
22. A similar enhancement of anti-influenza activity by incorporation of fatty acid into EGCg: S. Mori, S. Miyake, T. Kobe, T. Nakaya, S. D. Fuller, N. Kato, and K. Kaihatsu, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4249.
23. Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 4888.
24. S. Yamakawa, T. Asai, T. Uchida, M. Matsukawa, T. Akizawa, and N. Oku, *Cancer Lett.*, 2004, **210**, 47.
25. S. Piyaviriyakul, K. Shimizu, T. Asakawa, T. Kan, P. Siripong, and N. Oku, *Biol. Pharm. Bull.*, 2011, **34**, 396.
26. T. Kuzuhara, D. Kise, Y. Shirakawa, K. Sasada, M. Suganuma, and H. Fujiki, *Biol. Pharm. Bull.*, 2008, **31**, 816.
27. Y. Kawai, H. Tanaka, K. Murota, M. Naito, and J. Terao, *Biochem. Biophys. Res. Commun.*, 2008, **374**, 527.
28. K. Mera, M. Nagai, J. W. Brock, Y. Fujiwara, T. Murata, T. Maruyama, J. W. Baynes, M. Otagiri, and R. Nagai, *J. Immunol. Methods*, 2008, **334**, 82.