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

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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 attention1 because of its antitumor,2 antiviral,3 and other important bioactivities.3 Due to these bioactivities, EGCg and its derivatives are considered to be good candidates as lead compounds for
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)](image)

So far, there have been few reports aimed at the synthesis of catechin probes. 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-dideoxygallocatechin 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 of 8. Several selective 6-endo cyclization-mediated pyran ring constructions have been reported. 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)](image)
As shown in Scheme 2, condensation of the A- and B-ring was accomplished by means of Julia-Kocieński (JK) reaction\(^2\) 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-Kocieński reaction units

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

<table>
<thead>
<tr>
<th>entry</th>
<th>PT-sulfone 15</th>
<th>product</th>
<th>yield (%)(^a)</th>
<th>selectivity (cis : trans)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15a (R = Bn)</td>
<td>16a</td>
<td>95</td>
<td>30 : 1</td>
</tr>
<tr>
<td>2</td>
<td>15b (R = TBS)</td>
<td>16b</td>
<td>75</td>
<td>10 : 1</td>
</tr>
<tr>
<td>3</td>
<td>15c (R = Ms)</td>
<td>16c</td>
<td>12</td>
<td>1 : 1</td>
</tr>
</tbody>
</table>

\(a\): isolated yield., \(b\): the ratio was determined by \(^1\)H-NMR.

Furthermore, treatment of cis-16a with I\(_2\) 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\(^6\)
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](image)

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_{N}2 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](image)

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 11,10

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,11 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)12,13 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. Synthesis 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](image)

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 β-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](image)

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 (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$_2$(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 with our N-Cbz-N-Ns-amide (41) (Ns strategy) to afford 42.

\[ \text{Scheme 9. Incorporation of an amino linker group} \]

Diol 43 was obtained by Sharpless asymmetric dihydroxylation (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\(_2\)SnO to afford 45\(_a\) and 45\(_b\) as a 1:1 mixture. However, treatment of the mixture with DBU resulted in an interesting migration reaction, affording ester 45\(_a\) as a sole product. (Scheme 10) In this reaction, the acyl group of 45\(_b\) migrates to the sterically less hindered position.

\[ \text{Scheme 10. Asymmetric dihydroxylation and selective esterification} \]

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

Scheme 11. \( \text{Trans-selective construction of the benzopyran ring} \)

Scheme 12. \( \text{Cis-selective construction of benzopyran ring} \)

Scheme 13. Synthesis of probe precursor \( \text{APDOEGCg (4)} \) and \( \text{APDOGCg (5)} \)
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$_{50}$ values of 4.18 and 4.40 μM, respectively. These compounds are more potent than natural 1 and synthetic derivative 2.22 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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Complement inhibition IC$_{50}$ a(μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG (1)</td>
<td>66.3 (± 9.21)</td>
</tr>
<tr>
<td>DOEGCg (2)</td>
<td>9.05 (± 2.26)</td>
</tr>
<tr>
<td>APDOEGCg (4)</td>
<td>4.18 (± 4.29)</td>
</tr>
<tr>
<td>APDOGCg (5)</td>
<td>4.40 (± 2.36)</td>
</tr>
</tbody>
</table>

a 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.23 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).24 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.25

![Scheme 14. Synthesis of the fluorescein probe 53 from 4](image)
Next we focused on the generation of EGCg antibodies,\textsuperscript{26, 27} 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,\textsuperscript{28} 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.

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 [\(^1\)H NMR (270 MHz), \(^{13}\)C NMR (68 MHz)] spectra were determined on a JEOL EX-270 instrument and [\(^1\)H NMR (500 MHz), \(^{13}\)CNMR (125 MHz)] spectra were determined on JEOL ECA-500 instrument. Chemical shifts for \(^1\)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 \(^{13}\)C NMR were reported in ppm relative to the centerline of a triplet at 77.0 ppm for 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 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) CH\(_2\)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 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 cm\(^{-1}\); \(^1\)H NMR (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); \(^{13}\)C NMR (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, 127.6, 129.8, 128.6, 128.7, 129.6, 129.9, 130.2, 131.3, 131.5, 132.9, 137.1, 137.7–137.9, 152.5, 153.4; FAB-MS \( m/z \) 643 (M+H\(^+\)). HRMS (FAB) calculated for C\(_{42}\)H\(_{47}\)O\(_4\)Si 643.3244 [(M+H\(^+\))], found 643.3268.

**(E)**-Olefin 16a (**trans-16a**). To a stirred solution of *cis-16a* (195 mg, 0.303 mmol) in CHCl\(_3\) (2 mL) was added a solution of iodine (65 mg, 0.26 mmol) in 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 Na\(_2\)S\(_2\)O\(_3\) and the resulting mixture was extracted with CHCl\(_3\) three times. The extracts were dried over anhydrous 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 \(^1\)H NMR spectrum.

IR (film) 2920, 1579, 1253, 925 cm\(^{-1}\); \(^1\)H NMR (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); \(^{13}\)C NMR (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 (M+H\(^+\)). HRMS (FAB) calculated for C\(_{42}\)H\(_{46}\)O\(_4\)Si 642.3165 [(M\(^+\))], 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), Bu\(_4\)N\(^+\)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 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]_D^{24} +14 (c 0.99, \text{CHCl}_3); \text{IR (film)} 3030, 2927, 1591, 1253, 1116 \text{ cm}^{-1}; \text{\(^1\)H NMR (CDCl}_3, 500 \text{ 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, \( J = 15.2, 2.0 \) 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); \(^{13}\)C NMR (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,
The residue was taken up in chloroform (2 mL), dried over anhydrous MgSO4, filtered and evaporated under reduced pressure to give a residue. The residue was purified by column chromatography (n-hexane / AcOEt = 3 / 1) to afford 19 (198 mg, 85%) as a yellow oil.

\[ \text{[a]}^24 +0.56 \text{ (c 0.82, CHCl3); IR (film) 3450, 1593, 1112 cm}^{-1}; \text{^1H NMR (CDCl3, 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); \text{^13C NMR (CDCl3, 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; \text{FAB-MS m/z} 544 \text{ (M)^+]}. \text{HRMS (FAB) Calculated for C}_{34}\text{H}_{32}\text{O}_{5} 544.2250 \text{ [(M)^+], Found 544.2258.}
\]

**Dihydrobenzopyran (+)-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 ℃ 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 MgSO4, 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 ℃ for 30 min. To the reaction mixture was added water and the resulting mixture was extracted with CH2Cl2 three times. The extracts were dried over anhydrous MgSO4, 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.

\[ [a]^24 +1.7 \text{ (c 0.84, CHCl3); IR (film) 3032, 1597, 1246, 1132 cm}^{-1}; \text{^1H NMR (CDCl3, 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...} \]
The reaction mixture was 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. [a]$_D$$^{24}$ +41.0 (c 1.06, CHCl$_3$); IR (film) 3032, 2933, 1714, 1591, 1228 cm$^{-1}$; $^1$H NMR (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); $^{13}$C NMR (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, 124.9, 125.7, 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 (M+H$^+$). HRMS (FAB) calculated for C$_{60}$H$_{55}$O$_9$ 967.3846 [(M+H)$^+$]; Found 967.3863.

(−)-DOGCg (−)-3. A suspension of 57 (50 mg, 52 $\mu$mol) and 20% Pd(OH)$_2$-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 Na$_2$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. [α]$_D$$^{24}$ −73.5 (c 1.08, acetone/H$_2$O = 1 : 1); IR (film) 3287, 1693, 1336, 1230 cm$^{-1}$; $^1$H NMR (acetone-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); $^{13}$C NMR (acetone-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 (M+H$^+$). HRMS (FAB) calculated for C$_{22}$H$_{19}$O$_9$ 427.1029 [(M+H)$^+$]; Found 427.1049.

cis-Epoxide (−)-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), Bu$_4$N$^+$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 MgSO₄, 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 yellow 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.

[a]$_D^{24} = -19.4$ (c 1.15, CHCl₃); IR (film) 3032, 2930, 1591, 1259, 1120 cm$^{-1}$; $^1$H NMR (CDCl₃, 500 MHz) $\delta$ 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); $^{13}$C NMR (CDCl₃, 68 MHz) $\delta$ –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)$^+$. HRMS (FAB) calculated for C$_{42}$H$_{47}$O$_{5}$Si 659.3139 [(M+H)$^+\,\]$], 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 MgSO₄, 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 MgSO₄, 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$^{-1}$; $^1$H NMR (CDCl₃, 500 MHz, a mixture of diastereomers) $\delta$ 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*C NMR (CDCl₃, 68 MHz, a mixture of diastereomers) δ 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 (M⁺).

Chiral HPLC: Daicel ChiralCel OD 0.46 cm 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 (61%).

**Hexakisbenzyl DOEGCg (58).** A solution of **20** (33.5 mg, 61.5 μmol), **23** (81.3 mg, 184 μmol), EDCI (29.0 mg, 154 μmol) and dimethylaminopyridine (0.8 mg, 6 μmol) in CH₂Cl₂ (3 mL) was stirred at room temperature for 3 h. To the reaction mixture was added saturated aqueous NH₄Cl and the resulting mixture was extracted with CH₂Cl₂ three times. The extracts were dried over anhydrous MgSO₄, 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%) (*Rₜ* = 0.42 for *n*-hexane / AcOEt = 3 / 1) and **58** (26.2 mg, 44%) (*Rₜ* = 0.49 for *n*-hexane / AcOEt = 3 / 1) as yellow amorphous solids.

[*α*]D²⁴⁻83.0 (c 1.33, CHCl₃); IR (film) 3032, 2933, 2872, 1714, 1591, 1230 cm⁻¹; *1H* NMR (CDCl₃, 500 MHz) δ 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*C NMR (CDCl₃, 68 MHz) δ 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 (M+H)⁺. HRMS (FAB) calculated for C₆₄H₅₅O₉ 967.3846 [M+H]⁺, found 967.3863.

(–)-DOEGCg ((–)-2). A suspension of **58** (41 mg, 42 mmol) and 20% Pd(OH)₂-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 Na₂SO₄, filtered and evaporated under reduced pressure to afford **2** (18 mg, quant.) as colorless amorphous solids.

[*α*]D²⁴⁻158 (c 1.02, acetone / H₂O = 1 : 1); IR (film) 3286, 1689, 1614, 1336, 1226 cm⁻¹; *1H* NMR (acetone-d₆, 500 MHz) δ 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*C NMR (acetone-d₆, 68 MHz) δ 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 (M+H)⁺. HRMS (FAB) calculated for C₂₂H₁₉O₉ 427.1024 [(M+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 NH₄Cl was added and the mixture was extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na₂SO₄ 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⁻¹; ¹H NMR (CDCl₃, 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);

¹³C NMR (CDCl₃, 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)⁺. HRMS (ESI) m/z calculated for C₄₂H₄₅BrNaO₄Si 743.2163 [(M+Na)⁺], 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)Cl₂.CH₂Cl₂ (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 Na₂SO₄, 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⁻¹; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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)⁺. HRMS (ESI) m/z calculated for C₄₁H₄₅BrNaO₅ 637.2924 [(M+Na)⁺], 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), PPh₃ (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 CH₂Cl₂. The organic layer was dried over anhydrous Na₂SO₄ 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) 2930, 1730, 1581, 1541, 1502, 1367 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 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); ¹³C NMR (CDCl₃, 125 MHz) δ 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, 128.9, 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 (M+Na)⁺. HRMS (ESI) m/z calculated for C₅₅H₅₂N₂NaO₁₀S 955.3235 [(M+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 NH₄Cl, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na₂SO₄, 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) 2932, 1736, 1578, 1543, 1498, 1368 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 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); ¹³C NMR (CDCl₃, 125 MHz) δ –4.0, 18.4, 24.0, 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, 128.7, 128.8, 128.9, 130.4, 130.4, 131.7, 132.9, 133.7, 134.3, 134.3, 135.1, 135.2, 137.3, 138.0, 147.8, 151.4, 151.9, 152.9; MS (ESI) m/z 743 (M+Na)⁺. HRMS (ESI) m/z calculated for C₆₁H₆₆N₂NaO₁₀SSi 743.2163 [(M+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 / CH₂Cl₂ (1 / 1 / 1, 90 mL) were added AD-mix-β (11.4 g, 8.12 mmol) and MeSO₂NH₂ (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 Na₂S₂O₃ 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 Na₂SO₄, 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.
[$\alpha$]$b^{23} +4.8 (c 1.30, CHCl$_3$); IR (film) 3537, 2930, 1732, 1591, 1543, 1501, 1368, 1265, 1120 cm$^{-1}$; $^1$H NMR (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); $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$ –4.0, –3.9, 18.3, 25.9, 26.2, 30.0, 31.2, 34.9, 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, 128.7, 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 (M+$Na)^+$. HRMS (ESI) $m/z$ calculated for C$_{61}$H$_{68}$N$_2$NaO$_{12}$SSi 1103.4154 [(M+$Na)^+], found 1103.4205.

**Ester 45a.** To a solution of 43 (6.78 g, 6.27 mmol) in CH$_2$Cl$_2$ (250 mL) was added Bu$_2$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 Et$_3$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 NH$_4$Cl, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was purifed by silica gel column chromatography (n-hexane / 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 HCl, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (n-hexane / CH$_2$Cl$_2$ = 2 / 1 to 1 / 2) to afford 45a (8.40 g, 89%) as colorless amorphous solids.
Dihydrobenzopyran 18a. To a solution of 37 (100 mg, 66.5 µmol) in THF (1 mL) were added AcOH (12.0 µL, 200 µmol) and 1 M TBAF (133 µL, 133 µmol) in THF at 0 °C. After being stirred at 0 °C for 30 min, the reaction mixture was quenched with saturated aqueous NH₄Cl, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. To a mixture of the crude product in toluene (2.0 mL) were added CSA (15.4 mg, 100 µmol), and the mixture was heated at 60 °C for 12 h. The organic layer was washed with water, dried over anhydrous Na₂SO₄, 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. 

\[ \alpha_D^{23} -23 \] (c 1.5, CHCl₃); IR (film) 2927, 1734, 1726, 1591, 1543 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 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 – 7.4 (dt, J = 1.7, 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); ¹³C NMR (CDCl₃, 125 MHz) δ 165.2, 153.0, 152.5, 151.8, 151.9, 147.8, 142.7, 138.5, 137.8, 137.5, 136.9, 136.9, 136.6, 135.2, 134.4, 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 (M+Na⁺). HRMS (ESI) m/z calculated for C₈₃H₇₄N₂NaO₁₅S 1393.4702 [(M+Na)⁺], found 1393.4733.

Ketone 49. To a solution of 45a (8.40 g, 5.59 mmol) in CH₂Cl₂ (80 mL) were added 1-Me-AZADO (192 mg, 1.12 mmol) and PhI(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 Na₂S₂O₅ 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 Na₂SO₄, 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 NH₄Cl, and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na₂SO₄, 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 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 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,
The organic layer was extracted with AcOEt. The organic layer was 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.

Dihydrobenzopyran 18b. To a solution of 40 (7.31 g, 5.37 mmol) in CH$_2$Cl$_2$ (200 mL) were added Et$_3$SiH (1.74 mL, 10.7 mmol) and BF$_3$·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 Na$_2$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.

[α]$_{D}^{23}$ −1.9 (c 1.3, CHCl$_3$), IR (film) 3064, 2927, 1732, 1713, 1593, 1537 cm$^{-1}$; $^1$H NMR (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); $^{13}$C NMR (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, 127.9, 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 (M+Na)$^+$. HRMS (ESI) $m/z$ calculated for C$_{83}$H$_{76}$NaO$_{16}$S 1393.4702 [(M+Na)$^+]$, found 1393.4769.

60: To a solution of 48b (3.03 g, 2.21 mmol) in MeCN (15 mL) were added PhSH (674 µL, 6.62 mmol) and Cs$_2$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 NH$_4$Cl and extracted with AcOEt. The organic layer was washed with saturated aqueous NaCl, dried over anhydrous Na$_2$SO$_4$ and concentrated under reduced pressure. The crude residue was purified by column chromatography (n-hexane / AcOEt = 5 / 1 → 4 / 1) to afford 60 (1.26 g, 48%) as colorless amorphous solids.

IR (film) 3417, 3030, 2927, 1732, 1712, 1591, 1543 cm$^{-1}$; $^1$H NMR (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), 4.95–5.00 (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),
3. The reaction mixture was purified by preparative HPLC (Cholester Waters 10 x 250, 20% to 60% MeCN in water, 0.1% TFA, 30 minute ramp, flow rate: 3.5 mL/min) to afford 4 (200 µL) were added to the reaction mixture. After being 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. APDOEGCg (4). A suspension of 60 (100 mg, 84.3 µmol) and 20% Pd(OH)2 (30 mg) in THF / MeOH (1:1, 4.0 mL) was stirred 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, 2932, 2858, 1701, 1610, 1498, 1448, 1340, 1219, 1037 cm\(^{-1}\); \(^1\)H NMR (CD\(_2\)OD, 500 MHz) \(\delta\) 3.31–3.29 (m, 2H), 2.50 (m, 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 (m, 1H), 6.90 (s, 2H), 6.94 (dd, \(J = 1.7, 8.6\) Hz, 1H); \(^13\)C NMR (CD\(_3\)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 (M+H\(^+\)). HRMS (ESI) \(m/z\) calculated for C\(_{77}\)H\(_{17}\)NNaO\(_{11}\) 1208.4919 [(M+Na\(^+\)], found 1208.4954.

APDOEGCg-TokyoGreen conjugate 53. To a solution of TokyoGreen (30.0 mg, 86.7 µmol) in DMF (200 µL) were added 4 (19.9 mg, 173 µmol) and EDCI (16.6 mg, 86.7 µmol) at 0 °C. After being stirred at room temperature for 1 h, added 4 (22.1 mg, 43.3 µmol) was added to the reaction mixture. After being stirred at room temperature for 4 h, the reaction mixture was purified by preparative HPLC (Cholester Waters 10 x 250, 20% to 60% MeCN in water, 0.1% TFA, 30 minute ramp, 1 = 280 nm, flow rate; 3.5 mL/min) to afford 53 (25.1 mg, 29.9 µmol, 69%, RT = 18.9 min) as a colorless film.

IR (film) 3406, 2920, 1732, 1712, 1680, 1639, 1587, 1205 cm\(^{-1}\); \(^1\)H NMR (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), 6.93 (dd, \(J = 1.7, 8.6\) Hz, 1H), 7.34 (d, \(J = 0.8\) Hz, 1H), 7.82 (d, \(J = 8.0\) Hz, 1H), 7.90 (s, 1H), 8.03 (s, 1H), 8.71 (s, 1H), 8.92 (s, 1H), 9.17 (s, 2H); \(^13\)C NMR (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, 132.5, 134.8, 138.5, 142.7, 145.0, 145.4, 152.5, 156.1, 166.2; MS (ESI) \(m/z\) 862 (M+Na\(^+\)). HRMS (ESI) \(m/z\) calculated for C\(_{48}\)H\(_{41}\)NNaO\(_{13}\) 862.2470 [(M+Na\(^+\)], found 862.2485.

APDOEGCg-carrier protein conjugate 56. Conjugation of APDOEGCg to carrier protein using glutaraldehyde was performed as described previously.\(^{28}\)
(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.

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