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SYNTHESIS AND BIOLOGICAL EVALUATION OF C-AROMATAXANE DERIVATIVES AS P-GLYCOPROTEIN-MEDIATED MULTI DRUG RESISTANCE REVERSAL AGENTS

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Dedicated to Professor Isao Kuwajima on the occasion of his 77th birthday

Abstract – Synthesis and evaluation of C-aromataxane derivatives as P-glycoprotein-mediated MDR reversal agents have been demonstrated. Several derivatives possessing *N*-benzoylphenylisoserine at the C2 or C14 position of the template **2a** were readily synthesized and were evaluated their affinity for P-glycoprotein. Most of the synthesized derivatives exhibited much lower cytotoxicity in both KB-3-1 cells and MDR KB-G2-cells than paclitaxel (**1**), and it should be noted that the compound (14*R*)-**5a** exhibited high K_m and V_{max}/K_m values, and cytotoxicity of paclitaxel (**1**) in MDR KB-G2 cells was significantly recovered (98% reduction, IC_{50} 30 nM) in the presence of **5a** (5.0 μM). The structural features such as *endo*-cage conformation and the stereochemistry at the C14 position is crucial to exhibit an excellent affinity for P-glycoprotein.

INTRODUCTION

Multidrug resistance (MDR) in human cancer is one of the serious problems in the long-term chemotherapy, which significantly reduces the pharmacological effect of anticancer drugs, such as paclitaxel (**1**) and vincristine.[1] Cancer cells often acquire resistance for antitumor drugs by accretion of target enzymes, decrement of affinity or enhancement of inactive enzyme for drugs and overexpression of P-glycoprotein. Among them, P-glycoprotein-associated MDR is a serious problem because P-glycoprotein extrudes various foreign substrates to outside of cells.[2] In addition, the fact that P-glycoprotein exists not only cancer cells but also normal one makes it difficult to develop novel drugs, selectively targeting P-glycoprotein of cancer cells. Thus, development of MDR reversal agents has been vigorously investigated to solve this critical issue in recent decades. For instance, isolated taxane compounds are usually utilized as a template of MDR reversal agent due to the similar structure for paclitaxel (**1**), and structure activity relationships (SAR) provide the derivatives with lower cytotoxic and higher MDR reversal activity.[3] On the other hand, unnatural synthetic taxane derivatives would also be interested in use of MDR reversal agents.[4] We have previously reported that unnatural nine-membered ether **2a** exhibits a good affinity for P-glycoprotein (Figure 1), because **2a** forms 3D *endo*-cage structure similarly to paclitaxel (**1**), determined by conformational analysis based on MM2 calculation utilizing a Monte Carlo method.[5] The compound **2a** would be a good template to develop novel MDR reversal agents, and further modification of **2a** such as introduction of the side chain of paclitaxel could be effective to increase the affinity for P-glycoprotein. We herein report the synthesis of C-aromataxane derivatives **3**–**6** consisting of the side chain of paclitaxel (**1**) and a new template **2b**, and evaluation of their MDR reversal activity.

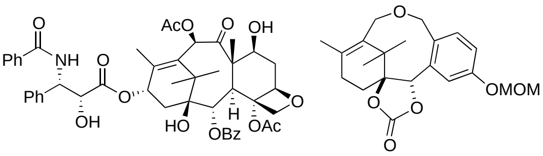


Figure 1. Paclitaxel (**1**), and 3D template for MDR reversal agent **2a**

RESULTS AND DISCUSSION

In order to increase the affinity of **2a** for P-glycoprotein, we planned to introduce the side chain moiety of paclitaxel (**1**) on the template **2a** via condensation of *N*-benzoylphenylisoserine at the C2-hydroxy group or allylic oxidation at the C14 position, followed by the condensation with side chain of paclitaxel (**1**), leading to **3-6**. (Figure 2).

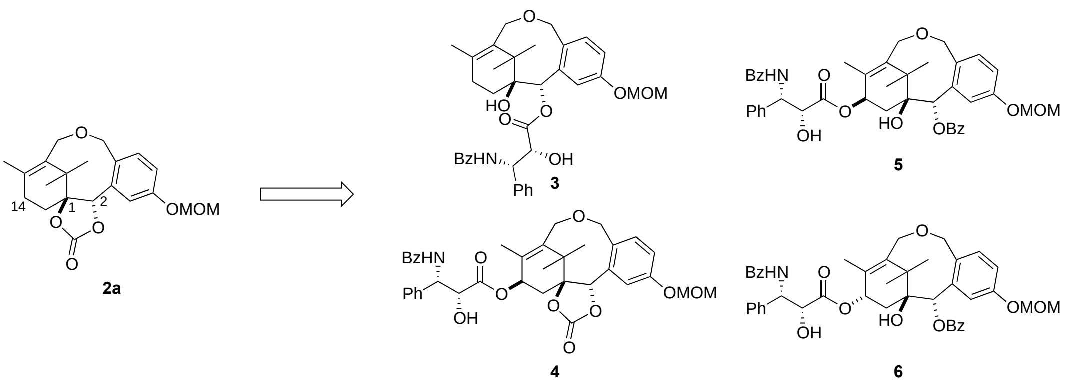
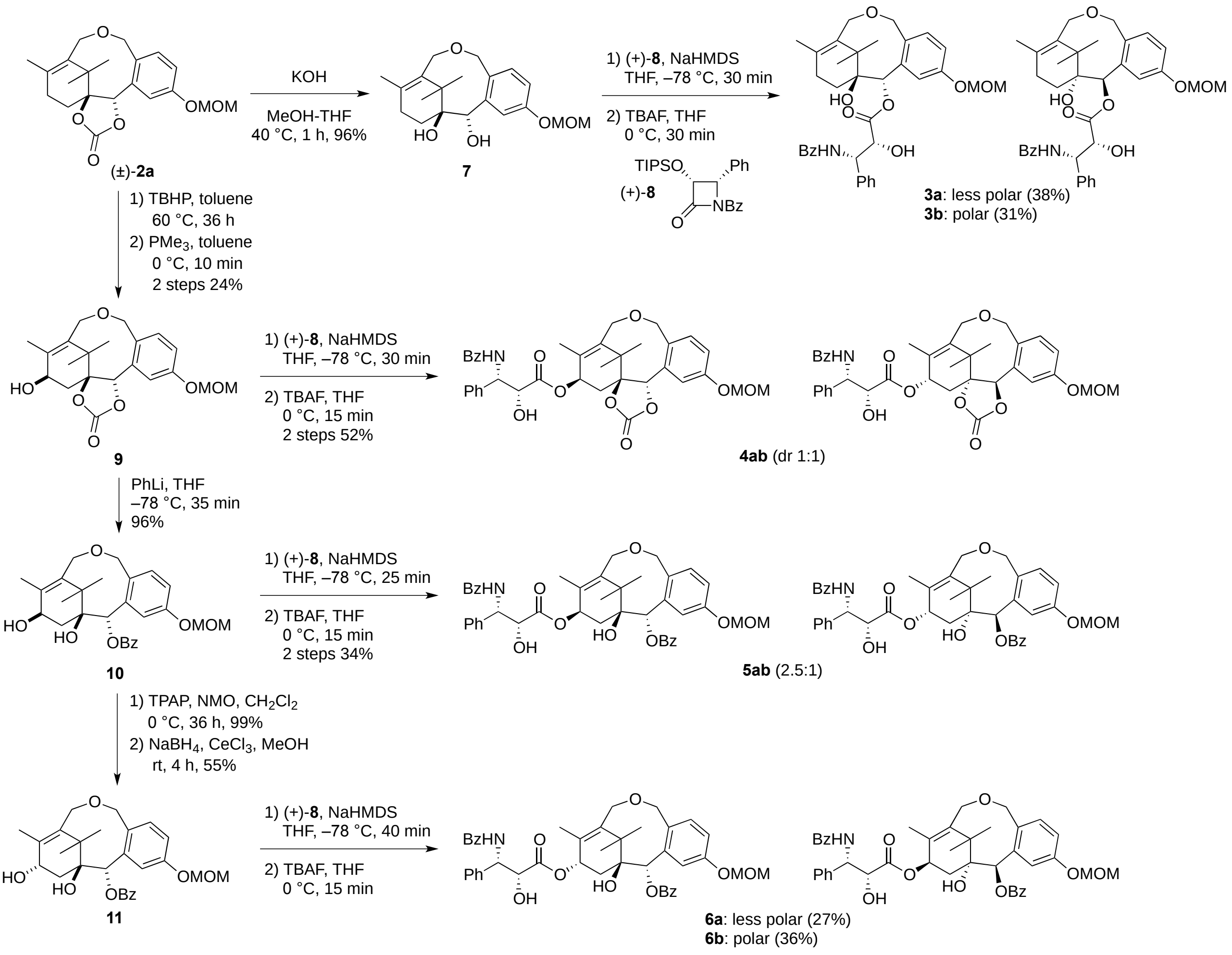


Figure 2. Design of novel C-aromataxane derivatives based on the template **2a**

The synthesis of derivatives **3-6** is summarized in Scheme 1. Toward the synthesis of **3**, a carbonate group in (±)-**2a** was initially removed under basic condition to afford diol **7** in 96% yield. Selective acylation of the secondary alcohol with β-lactam (+)-**8** using NaHMDS at –78 °C,[6] followed by removal of the silyl group provided two diastereomers, which were readily separated by silica gel column chromatography to afford the less polar **3a** (two steps 38%) and the polar **3b** (two steps 31%), respectively.



Scheme 1. Synthesis of C-aromataxane derivatives **3**–**6**

The synthesis of **4** and **5** was performed as follows: face-selective allylic oxidation of **2a** at the C14 position with TBHP,[7] followed by reduction of the resulting peroxide furnished the alcohol **9** in 24% overall yield. Acylation of the resulting alcohol at the C14 position was performed according to the synthesis of **3**, and removal of the silyl group with TBAF afforded **4ab** as a 1:1 mixture of diastereomers. To prepare the benzoyl group at the C2 position, regioselective cleavage of the cyclic carbonate **9** with PhLi was performed to afford benzoate **10** in 96% yield. According to the synthesis of **4**, coupling of **10** with β-lactam (+)-**8** was performed to provide an inseparable 2.5:1 mixture of **5ab** in 52% combined yield. In addition, the compound **6a** and **6b** were also prepared as follows: oxidation of the alcohol **10** at the C14 position, followed by stereoselective reduction of the resulting ketone from the convex face[8] afforded the diastereomeric alcohol **11**, whose stereochemistry was confirmed by nOe observation between H14 and C16 methyl group (Figure 3). Condensation of **11** with (+)-**8** provided separable diastereomers **6a** (27%) and **6b** (36%), respectively.

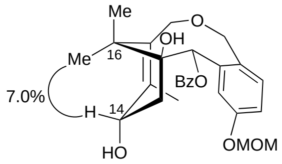


Figure 3. nOe observation of **11**

It is known that P-glycoprotein consumes ATP to extrude the substrates to the outside of cells, thus we measured the compound-stimulated ATPase activity of P-glycoprotein by using plasma membranes from P-glycoprotein-overexpressing Sf9 cells to evaluate the affinity of the synthesized compounds **3-6** for P-glycoprotein.[9] All derivatives showed saturation kinetics in the relationship between the compound-stimulated ATPase activity and the compound concentration, suggesting Michaelis-Menten kinetics. Thus, K_m and V_{max} values were calculated from Lineweaver-Burk plots of (compound concentration) x (compound-stimulated ATPase activity)$^{-1}$ vs (compound concentration); these are summarized in Table 1. The values of K_m and V_{max} for each derivative are lower than those of the parent **2a**, and the value of V_{max}/K_m for each derivative is higher than that of the parent **2a**. In particular, **5ab** showed the highest V_{max}/K_m value, suggesting that **5ab** is the best substrate for P-glycoprotein among the synthetic derivatives shown in Table 1. Most of the derivatives exhibited more than one-twentieth weaker cytotoxicity against both KB-3-1 cells and MDR KB-G2 cells than paclitaxel did, except for **6a** and **6b** (IC_{50} 8.0 μM and 8.5 μM against MDR KB-G2 cells, respectively).

Table 1. ATPase assay and cytotoxicity of the synthesized compounds **3**–**6**

Entry	Compound	K_m [mM]	V_{max} [mM]	V_{max}/K_m	IC_{50} [mM][a] (KB-3-1)	IC_{50} [mM][b] (KB-G2)
1	**2a**	4	41	10	>50	>50
2	**3a**	0.78	18	21	33	31
3	**3b**	1.3	26	8.6	32	23
4	**4ab**	1.5	33	22	33	41
5	**5ab**	0.38	26	69	15	17
6	**6a**	0.5	18	36	55	8.0
7	**6b**	0.8	25	32	21	8.5
8	Paclitaxel (**1**)	0.70	35	50	0.0021	0.93
9	Verapamil	2	65	30	-	-

[a] The IC_{50} value was measured against KB-3-1 cells. [b] The IC_{50} value was measured against KB-G2 cells that have resistance to paclitaxel and vinblastine.

Based on the above results, we chose four derivatives **3a**, **3b**, **4ab** and **5ab** as a candidate for P-glycoprotein-mediated MDR reversal agent, and evaluated their activities to reverse P-glycoprotein-mediated MDR by using MDR KB-G2 cells (Figure 4). It was found that cytotoxicity of paclitaxel (**1**) against MDR KB-G2 cells was enhanced by addition of the derivatives in a dose-dependant manner, therefore the derivatives could be a substrate for P-glycoprotein.

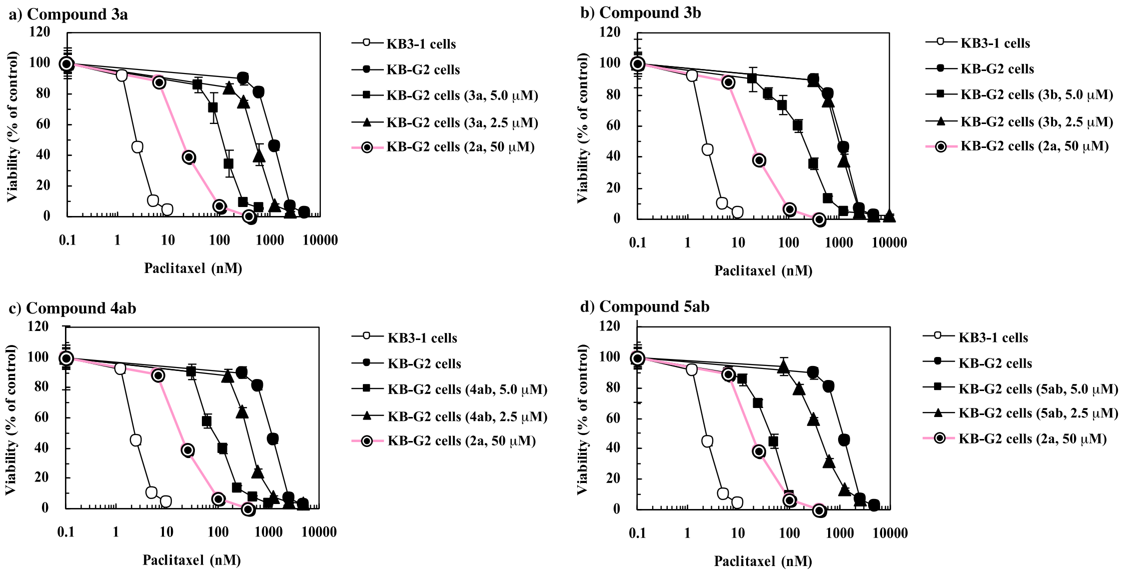
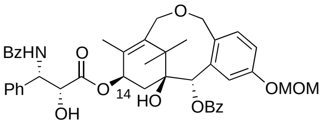


Figure 4. The effect of the derivatives as a MDR reversal agent on the cytotoxicity of paclitaxel against MDR KB-G2 cells over expressing P-glycoprotein. a) **3a**, b) **3b**, c) **4ab**, d) **5ab**

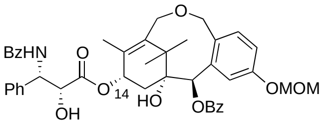
In particular, the sensitivity of MDR KB-G2 cells against paclitaxel (**1**) was significantly recovered in the presence of 5.0 μM **5ab**, where IC_{50} value was diminished from 0.93 μM to 0.041 μM. In addition, it was found that the other derivatives **3a**, **3b** and **4ab** also reversed the cytotoxicity of paclitaxel (**1**) against MDR KB-G2 cells. According to the above results, it should be noted that the derivative that possesses a similar structure to paclitaxel (**1**) would be suitable for the substrate for P-glycoprotein and be dominantly extruded to the outside of cells instead of paclitaxel (**1**).

With a good candidate **5ab** for P-glycoprotein-mediated MDR reversal agent in hand, we next evaluated MDR reversal activity of the diastereomers **5a** and **5b**. The mixture of **5a** and **5b** was separated by preparative thin-layer chromatography, and the stereochemistry at the C14 position was determined by circular dichroism spectroscopy.[10,11] As a result of ATPase assay, (14*R*)-**5a** showed greater K_m and V_{max}/K_m values than (14*S*)-**5b** without showing cytotoxicity to KB-G2 cells (IC$_{50}$ >100 μM) although (14*S*)-**5b** exhibited against KB-G2 cells (IC$_{50}$ 23 μM) (Table 2). In particular, cytotoxicity of paclitaxel (**1**) against MDR KB-G2 cells was considerably enhanced (IC$_{50}$ 30 nM) in the presence of 5.0 μM **5a**, thereby (14*R*)-**5a** exhibited significant MDR reversal activity (Figure 5). The conformation of **5a** would be important to be a substrate for a P-glycoprotein; stereochemistry of the C1 and C2 positions in **5a** are identical to that in paclitaxel, thus stereochemistry at the C14 position would be crucial to be dominantly extruded to the outside of cells by P-glycoprotein.

Table 2. ATPase assay and cytotoxicity of the diastereomers **5a** and **5b**



(14*R*)-**5a**



(14*S*)-**5**b

Entry	Compound	K_m [mM]	V_{max} [mM]	V_{max}/K_m	IC$_{50}$ [mM] (KB-G2)
1	**5ab**	0.38	26	69	17
2	(14*R*)-**5a**	0.12	38.6	321	>100
3	(14*S*)-**5b**	0.36	43.7	121	23

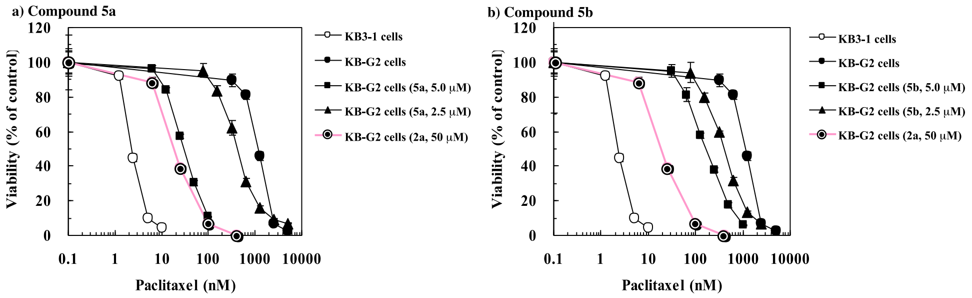
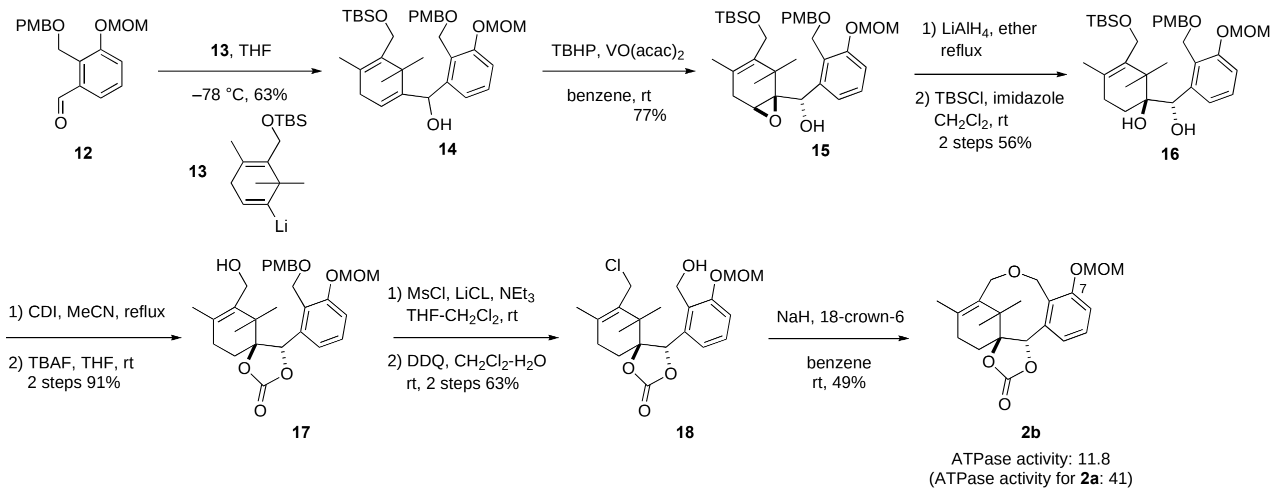


Figure 5. The effect of the derivatives as a MDR reversal agent on the cytotoxicity of paclitaxel against MDR KB-G2 cells over expressing P-glycoprotein. a) **5a**, b) **5b**

In order to investigate the effect of the substituent on the C-ring, we next synthesized **2b** which possesses a methoxymethyloxy (OMOM) group at the C7 position[12] and evaluated the affinity for P-glycoprotein

(Scheme 2). Coupling of aldehyde **12** with a vinyl anion **13**[13] proceeded at –78 °C to afford alcohol **14**. Stereoselective epoxidation of **14**, followed by reductive opening of the resulting epoxide **15** and selective protection of the primary alcohol with a TBS group provided diol **16** in moderate yield. After carbonate formation of **16** with 1,1-carbonyldiimidazole and removal of the TBS group, chlorination of the resulting alcohol **17** and subsequent removal of the PMB group with DDQ[14] provided cyclization precursor **18**. Intramolecular *O*-alkylation of **18** with NaH in the presence of 18-crown-6 proceeded at room temperature to afford the desired nine-membered ether **2b** in 49% yield. The structure of **2b** was determined by ^{1}H and ^{13}C NMR spectra. The affinity of **2b** for P-glycoprotein was evaluated. Although it was found that the compound **2b** exhibited similarly low cytotoxicity as **2a** (data shown in supporting information), ATPase activity of P-glycoprotein in the presence of 100 μM **2b** (11.8 nmol min^{-1} mg protein^{-1}) was lower than that in the presence of **2a** (41 nmol min^{-1} mg protein^{-1}). Thus, the C5 position on the C-ring should be better than the C7 position for introduction of the substituents toward higher affinity for P-glycoprotein.



Scheme 2. Synthesis and evaluation of **2b** possessing the substituent at the C7 position

In summary, we have demonstrated the synthesis of C-aromataxane derivatives based on the parent **2a** and evaluated their affinity for P-glycoprotein. The synthesis of derivatives **3–6** was readily performed by introduction of *N*-benzoylphenylisoserine to the C2 or C14 position by way of the modified Ojima method. According to P-glycoprotein assay, it was found that introduction of the *N*-benzoylphenylisoserine group to the parent **2a** was effective to improve the affinity for P-glycoprotein. In particular, excellent K_m and V_{max}/K_m of (14*R*)-**5a** values were observed without cytotoxicity to KB-G2 cells (IC$_{50}$ >100 μM). It should be noted that (14*R*)-**5a** exhibited significant MDR reversal activity as cytotoxicity of paclitaxel (**1**) was considerably recovered (IC$_{50}$ 30 nM) in the presence of (14*R*)-**5a** at 5.0 μM. This observation suggested that both similar conformation to paclitaxel and stereochemistry at the

C14 position are crucial to exhibit a good MDR reversal activity and to be extruded to the outside of cells instead of paclitaxel. Substituent effect in the affinity for P-glycoprotein was observed, and ATPase activity in the presence of **2a** was greater than that of the new template **2b** which possesses a OMOM group at the C7 position on the C-ring. The above observations would be useful to develop MDR reversal agents for the cancer therapy and further investigation for the synthesis and the evaluation of C-aromataxane derivatives is underway in our laboratory.

EXPERIMENTAL

General Techniques: NMR spectra were recorded on a JEOL Model EX-270 (270 MHz for ^{1}H, 67.8 MHz for ^{13}C) or a JEOL Model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) instrument in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to chloroform (7.26 ppm for ^{1}H) or $CDCl_3$ (77.1 ppm for ^{13}C), and methanol (3.30 ppm for ^{1}H) or CD_3OD (49.8 ppm for ^{13}C), CD_2Cl_2 (53.8 ppm for ^{13}C) when internal standard is not indicated. Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz. IR spectra were recorded on Perkin Elmer Spectrum One FT-IR spectrophotometer. Only the strongest and/or structurally important peaks are reported as the IR data given in cm^{-1}. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by *p*-anisaldehyde solution. Merck silica gel or Chlomatorex NH-silica gel was used for column chromatography. Gel permeation chromatography (GPC) for qualitative and quantitative analysis were performed on a Japan Analytical Industry Model LC 605 (recycling preparative HPLC), on a Japan Analytical Industry Model RI-5 refractive index detector and on a Japan Analytical Industry Model 310 ultra violet detector with polystyrene gel column (JAIGEL-1H, 20 mm x 600 mm), using chloroform as a solvent (3.5 mL/min). High-resolution mass spectra were measured with JEOL MS-AX500 (FAB) or P. E. Biosystems TK-3500 Biospectrometry Workstation (ESI-TOF) instruments.

1,2-Diol 7

To a solution of the 9-membered ether **2a**[5] (49.7 mg, 0.133 mmol) in THF (0.5 mL) was added KOH (0.800 M in MeOH, 5.0 mL) at 0 °C. After being stirred at 40 °C for 1 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (35% EtOAc in hexane) to afford the 1,2-diol **7** (44.5 mg, 0.128 mmol, 96%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 2.4 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 6.84 (dd, J = 2.4, 8.2 Hz, 1H), 5.22 (d, J = 6.8 Hz, 1H), 5.22 (s, 1H), 5.14 (d, J = 6.8 Hz, 1H), 4.69 (d, J = 13.0 Hz, 1H) 4.47 (d, J = 13.0 Hz, 1H), 4.31 (s, 2H), 3.47 (s, 3H), 3.13 (s, 1H, OH), 2.64 (s, 1H, OH), 2.30 (m, 1H), 1.97 (m, 1H), 1.67 (m, 1H), 1.49 (s, 3H), 1.10 (m, 1H), 1.01 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 155.8, 142.5, 139.7, 132.8, 132.2, 130.7, 114.9, 114.2, 94.4, 78.7, 69.6, 67.6, 64.9, 56.0, 40.8, 29.5, 28.3, 26.2, 21.5, 20.3; FT-IR (Neat) 3402, 2952, 1606, 1493, 1461, 1375, 1234, 1153, 1018, 802 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{20}H_{29}O_5$ $[M+H]^+$ 349.2015, found 349.2015.

(1*R**, 2*R**, 14*S**)-Carbonate 9

To a solution of the 9-membered ether **2a** (260 mg, 0.695 mmol) in toluene (7.0 mL) was added *t*-butyl hydroperoxide (5.0 mL, 70 wt % in water) at 0 ˚C. After being stirred at 60 ˚C for 36 h, the reaction mixture was quenched with brine at 0 ˚C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by short-pass column chromatography on silica gel (35~40% EtOAc in hexane) to afford the hydroperoxide (78.3 mg), and the above product was subsequently used for next reaction.

To a solution of the hydroperoxide (78.3 mg) in dry toluene (4.5 mL) was added trimethylphosphine (0.33 mL, 1.00 M in toluene, 0.33 mmol, 1.7 equiv) at 0 ˚C under argon. After being stirred at 0 ˚C for 15 min, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel (2% MeOH in $CHCl_3$) to afford the carbonate **9** (60.5 mg, 0.155 mmol, 24% in 2 steps) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 2.9 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 6.95 (dd, J = 2.9, 8.2 Hz, 1H), 6.23 (s, 1H), 5.24 (d, J = 6.8 Hz, 1H), 5.11 (d, J = 6.8 Hz, 1H), 4.78 (d, J = 15.0 Hz, 1H), 4.50 (d, J = 15.0 Hz, 1H), 4.36 (d, J = 13.0 Hz, 1H), 4.27 (d, J = 13.0 Hz, 1H), 3.47 (s, 3H), 2.65-2.75 (m, 2H), 2.22 (dd, J = 3.9, 13.5 Hz, 1H), 1.78 (d, J = 4.4 Hz, 1H, OH), 1.55 (s, 3H), 1.32 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 156.1, 153.6, 139.6, 135.4, 133.5, 131.8, 130.7, 116.5, 114.3, 94.5, 93.0, 77.6, 71.2, 68.3, 66.5, 56.2, 40.0, 34.7, 30.6, 20.7, 18.3; FT-IR (Neat) 3399, 2927, 1801, 1609, 1502, 1436, 1262, 1208, 1153, 1039, 804, 755 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{21}H_{27}O_7$ $[M+H]^+$ 391.1757, found 391.1755.

Benzoate 10

To a solution of the carbonate **9** (9.6 mg, 25 μmol) in dry THF (1.0 mL) was added PhLi (0.98 M in ether, 0.13 mL, 0.12 mmol, 5.0 equiv) at –78 ˚C under argon. After being stirred at –78 ˚C for 35 min, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 ˚C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel (5% MeOH in $CHCl_3$) to afford benzoate **10** (11.1 mg, 23.7 μmol, 96%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.47 (t, *J* = 7.3 Hz, 2H) , 7.29 (d, *J* = 8.7 Hz, 1H), 7.27 (d, *J* = 2.4 Hz, 1H), 6.87 (dd, *J* = 2.4, 8.7 Hz, 1H), 6.64 (s, 1H), 5.12 (s, 2H), 4.85 (d, *J* = 13.5 Hz, 1H), 4.57 (d, *J* = 13.5 Hz, 1H), 4.46 (d, *J* = 12.6 Hz, 1H), 4.35 (d, *J* = 12.6 Hz, 1H), 3.40 (s, 3H), 3.15 (dd, *J* = 4.8, 9.7 Hz 1H), 2.86 (dd, *J* = 9.7, 14.5 Hz 1H), 2.66 (brs, 1H, OH), 2.12 (dd, *J* = 4.8, 14.5 Hz 1H), 1.62 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H, e); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 165.7, 156.2, 139.8, 139.5, 135.9, 133.5, 132.8, 132.6, 129.9, 129.7, 128.7, 115.4, 114.1, 94.6, 80.9, 73.1, 69.3, 68.0, 65.1, 55.9, 41.6, 38.3, 31.2, 21.6, 18.1; FT-IR (Neat) 3471, 2947, 1716, 1607, 1499, 1452, 1275, 1153, 1071, 1026, 713 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{27}H_{33}O_7$ $[M+H]^+$ 469.2226, found 469.2226.

(1*R**, 2*R**, 14*R**)-Diol 11

To a solution of the allylic alcohol **10** (7.2 mg, 15.4 μmol) and NMO (9.0 mg, 77 μmol, 5.0 equiv.) in CH_2Cl_2 (2.0 mL) was added a catalytic amount of TPAP at 0 ˚C under argon. After being stirred for 36 h, the reaction mixture was diluted with hexane (15 mL) and EtOAc (15 mL) and stirred with Florisil® at room temperature for 4 h. The suspension was filtered through a pad of Celite® and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel (40-50% EtOAc in hexane) to afford the ketone (7.1 mg, 15 μmol, 99%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 7.3 Hz, 2H), 7.61 (t, *J* = 7.3 Hz, 1H), 7.48 (t, *J* = 7.3 Hz, 2H), 7.24 (d, *J* = 8.2 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 6.80 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.73 (s, 1H), 5.15 (d, *J* = 7.3 Hz, 1H), 5.04 (d, *J* = 7.3 Hz, 1H), 5.01 (d, *J* = 13.5 Hz, 1H), 4.64 (d, *J* = 13.5 Hz, 1H), 4.62 (d, *J* = 13.1 Hz, 1H), 4.44 (d, *J* = 13.1 Hz, 1H), 3.38 (s, 3H), 3.27 (d, *J* = 18.9 Hz, 1H), 2.87 (d, *J* = 18.9 Hz, 1H), 2.80 (brs, 1H, OH), 1.62 (s, 3H), 1.23 (s, 3H), 1.17 (s, 3H, d); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 197.7, 165.9, 156.9, 153.0, 140.6, 138.8, 133.7, 132.9, 130.7, 129.8, 129.6, 128.7, 115.8, 114.2, 94.4, 78.2, 73.0, 69.4, 65.5, 55.8, 43.9, 43.1, 31.3, 21.2, 12.1; FT-IR (Neat) 3504, 2989, 1718, 1671, 1607, 1498, 1451, 1359, 1269, 1215, 1151, 757 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{27}H_{31}O_7$ $[M+H]^+$ 467.2070, found 467.2078.

To a solution of the above ketone (7.30 mg, 15.6 μmol) in MeOH (3.0 mL) and THF (0.50 mL) was added $CeCl_3$ (400 mg, large excess) at 0 ˚C under argon. After being stirred at 0 ˚C for 15 min, the mixture was treated with $NaBH_4$ (7.49 mg, 0.198 mmol, 10 equiv) in eight portions for 30 min at room temperature. The reaction mixture was quenched with saturated aqueous NH_4Cl at 0 ˚C, and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous

$NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (35% EtOAc in hexane) to afford the diol **11** (4.00 mg, 8.54 μmol, 55 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, J = 7.3 Hz, 2H), 7.53 (t, J = 7.3 Hz, 1H), 7.41 (t, J = 7.3 Hz, 2H), 7.29 (d, J = 8.2 Hz, 1H), 7.19 (d, J = 2.4 Hz, 1H), 6.85 (dd, J = 2.4, 8.2 Hz, 1H), 6.58 (s, 1H), 5.09 (d, J = 7.3 Hz, 1H), 5.06 (d, J = 7.3 Hz, 1H), 4.78 (d, J = 13.5 Hz, 1H), 4.51 (d, J = 13.5 Hz, 1H), 4.36 (d, J = 12.6 Hz, 1H), 4.27 (d, J = 12.6 Hz, 1H), 4.08 (m, 1H), 3.36 (s, 3H), 2.56 (dd, J = 10.6, 15.5 Hz, 1H), 2.56 (brs, 1H, OH), 2.39 (dd, J = 2.9, 15.5 Hz, 1H), 1.55 (s, 3H), 1.11 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 165.7, 155.8, 142.9, 141.4, 133.6, 133.3, 131.6, 129.9, 129.7, 128.7, 116.1, 113.3, 94.3, 76.9, 73.5, 67.9, 67.1, 65.4, 56.1, 42.2, 40.1, 29.8, 26.8, 21.8, 15.2; FT-IR (Neat) 3546, 2928, 1719, 1605, 1498, 1451, 1272, 1153, 1069, 1025, 753, 711 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{27}H_{33}O_7$ $[M+H]^+$ 469.2226, found 469.2227.

General Procedure for acylation with *N*-benzoyl-β-phenylisoserine side chain.

To a mixture of the alcohol and β-lactam (+)-**8** in THF was added sodium hexamethyldisilazide (1.0 M in THF) at –78 ˚C under argon. After being stirred at –78 ˚C, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 ˚C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$. After removal of the solvent in vacuo, the crude mixture of diastereomers was used for the next reaction without further purification.

To a solution of the mixture in THF was added TBAF (1.0 M in THF) at 0 ˚C under argon. After being stirred at 0 ˚C, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ at 0 ˚C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was purified by preparative TLC or column chromatography on silica gel (eluted with EtOAc in hexane) to afford the coupling product.

3a and 3b

Condition: 1) substrate **7** (25.0 mg, 71.8 μmol), sodium hexamethyldisilazide (180 μL, 0.178 mmol, 2.50 equiv), β-lactam (+)-**8** (54.1 mg, 0.128 mmol, 1.78 equiv), THF (1.0 mL), –78 °C, 30 min; 2) TBAF (0.72 mL, 0.72 mmol, 10.0 equiv), THF (2.0 mL), 0 °C, 30 min; Purification: preparative TLC (50% EtOAc in hexane). The absolute configuration of two diastereomers was not determined.

3a (less polar diastereomer) 38 % (8.5 mg, 14 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, J = 7.3 Hz, 2H), 7.56 (d, J = 7.3 Hz, 2H), 7.53 (t, J = 7.3 Hz, 1H), 7.45 (t, J = 7.3 Hz, 2H), 7.41 (t, J = 7.3 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.23 (d, J = 8.2 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 6.96 (d, *J* = 9.7 Hz, 1H), 6.85 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.42 (s, 1H), 6.05 (dd, *J* = 1.5, 9.7 Hz, 1H), 5.18 (d, *J* = 6.8 Hz, 1H), 5.14 (d, *J* = 6.8 Hz, 1H), 4.83 (d, *J* = 13.0 Hz, 1H), 4.66 (d, *J* = 1.5 Hz, 1H, NH), 4.51 (d, *J* = 13.0 Hz, 1H), 4.33 (s, 2H), 3.45 (s, 3H), 3.26 (brs, 1H, OH), 3.05 (brs, 1H, OH), 2.39 (m, 1H), 1.93-2.09 (m, 2H), 1.55 (s, 3H), 1.19 (m, 1H), 1.05 (s, 3H), 0.96 (s, 3H, e); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.3, 167.8, 155.8, 140.2, 139.5, 138.7, 133.8, 132.6, 132.2, 132.1, 131.0, 128.9, 128.7, 128.2, 127.3, 15.0, 113.9, 94.5, 75.2, 72.9, 68.1, 65.2, 56.0, 54.5, 41.8, 29.8, 29.4, 28.1, 26.6, 21.3, 20.5; FT-IR (Neat) 3420, 2955, 1743, 1652, 1521, 1488, 1237, 1152, 1077, 1020, 700 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{36}H_{42}NO_8$ $[M+H]^+$ 616.2910, found 616.2908.

3b (polar diastereomer) 31% (6.9 mg, 11 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (d, *J* = 7.3 Hz, 2H), 7.46 (t, *J* = 7.3 Hz, 1H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.35 (t, *J* = 7.3 Hz, 4H), 7.31 (t, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 8.2 Hz, 1H), 7.11 (d, *J* = 2.4 Hz, 1H), 6.96 (d, *J* = 8.2 Hz, 1H), 6.72 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.47 (s, 1H, f), 5.62 (dd, *J* = 2.4, 8.2 Hz, 1H), 5.08 (d, *J* = 6.8 Hz, 1H), 4.95 (d, *J* = 6.8 Hz, 1H), 4.81 (d, *J* = 13.5 Hz, 1H), 4.72 (d, *J* = 2.4 Hz, 1H, NH), 4.48 (d, *J* = 13.5 Hz, 1H), 4.31 (d, *J* = 13.0 Hz, 1H), 4.28 (d, *J* = 13.0 Hz, 1H), 3.66 (brs, 1H, OH), 3.37 (s, 3H), 2.44 (brs, 1H, OH), 2.26 (m, 1H), 2.00 (m, 1H), 1.83 (m, 1H), 1.49 (s, 3H), 1.15 (m, 1H), 1.03 (s, 3H), 1.00 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.0, 167.3, 155.8, 139.4, 139.1,138.6, 134.1, 132.1, 132.0, 131.7, 130.7, 128.8, 128.5, 128.1, 127.2, 127.0, 115.3, 113.6, 94.1, 75.0, 73.8, 68.4, 65.0, 55.9, 55.7, 41.3, 29.8, 29.3, 27.9, 27.0, 21.3, 20.1; FT-IR (Neat) 3374, 2962, 1733, 1646, 1521, 1489, 1216, 1152, 1076, 1027, 943, 755 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{36}H_{42}NO_8$ $[M+H]^+$ 616.2910, found 616.2917.

4ab

Conditions: 1) Substrate **9** (7.6 mg, 20 μmol), sodium hexamethyldisilazide (49 μL, 49 μmol, 2.5 equiv), β-lactam (+)-**8** (14.8 mg, 35.0 μmol, 1.79 equiv), THF (0.50 mL), –78 °C, 30 min; 2) TBAF (0.11 mL, 0.11 mmol, 10 equiv), THF (1.00 mL), 0 °C, 15 min; Purification: preparative TLC (50% EtOAc in hexane); 4.4 mg, 6.7 μmol, 52% in 2 steps.

^{1}H NMR (400 MHz, $CDCl_3$, a mixture of 1:1 diastereomers)

δ 7.77 (d, *J* = 7.3 Hz, 2H), 7.68 (d, *J* = 7.3 Hz, 2H), 7.24-7.67 (m, 19H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.10 (d, *J* = 8.2 Hz, 1H), 6.96 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.92 (dd, *J* = 2.4, 8.2 Hz, 1H), 6.90 (d, *J* = 9.2 Hz, 1H, m), 6.86 (d, *J* = 9.2 Hz, 1H, m), 6.24 (s, 1H), 6.19 (s, 1H), 5.69 (dd, *J* = 2.9, 9.2 Hz, 1H), 5.58 (dd, *J* = 1.9, 9.2 Hz, 1H), 5.21 (m, 2H), 5.17 (d, *J* = 7.3 Hz, 1H), 5.13 (d, *J* = 6.8 Hz, 1H), 4.82 (d, *J* = 15.0 Hz, 1H), 4.78 (d, *J* = 15.0 Hz, 1H), 4.56 (brs, 2H, NH), 4.51 (d, *J* = 15.0 Hz, 1H), 4.48 (d, *J* = 15.0 Hz, 1H), 4.37 (d, *J* = 13.0 Hz, 1H), 4.36 (d, *J* = 13.0 Hz, 1H), 4.28 (d, *J* = 13.0 Hz, 1H), 4.25 (d, *J* = 13.0 Hz, 1H), 3.85 (dd, *J* = 4.83, 9.67 Hz, 1H), 3.80 (dd, *J* = 4.8, 9.7 Hz, 1H), 3.45 (s, 3H), 3.42 (s, 3H), 3.28 (brs, 1H, OH), 3.21 (brs, 1H, OH), 2.90 (dd, *J* = 9.7, 15.0 Hz, 1H), 2.74 (dd, *J* = 9.7, 15.0 Hz, 1H), 2.11 (dd, *J* = 4.8, 15.0 Hz, 1H), 2.06 (dd, *J* = 4.8, 15.0 Hz, 1H), 1.56 (s, 3H), 1.56 (s, 3H), 1.33 (s, 3H), 1.28 (s, 3H), 1.16 (s, 3H), 1.14 (s, 3H); HRMS (ESI-TOF) calcd for $C_{37}H_{40}NO_{10}$ $[M+H]^+$ 658.2652, found 658.2654.

5a and 5b

Condition: 1) Substrate **10** (33.0 mg, 70.4 μmol), sodium hexamethyldisilazide (0.18 mL, 0.18 mmol, 2.6 equiv), β-lactam (+)-**8** (57.3 mg, 0.135 mmol, 1.92 equiv.), THF (3.00 mL, 42.6 mL/mmol), –78 °C, 30 min; 2) TBAF (46 μL, 46 μmol, 1.1 equiv.), THF (2.0 mL), 0 °C, 10 min; Purification: column chromatography on silica gel (40-50% EtOAc in hexane)

5a (less polar diastereomer): 35% (18.0 mg, 24.5 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.3 Hz, 2H), 7.79 (d, *J* = 7.3 Hz, 2H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.53 (t, *J* = 7.3 Hz, 1H), 7.45 (t, *J* = 7.3 Hz, 2H), 7.43 (t, *J* = 7.3 Hz, 2H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.38 (d, *J* = 2.9 Hz, 1H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.29 (t, *J* = 7.3 Hz, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 6.94 (d, *J* = 9.2 Hz, 1H), 6.86 (dd, *J* = 2.9, 8.7 Hz, 1H), 6.62 (s, 1H), 5.69 (dd, *J* = 1.9, 9.2 Hz, 1H), 5.23 (d, *J* = 6.8 Hz, 1H), 5.09 (d, *J* = 6.8 Hz, 1H), 4.85 (d, *J* = 13.5 Hz, 1H), 4.59 (d, *J* = 13.5 Hz, 1H), 4.58 (d, *J* = 1.9 Hz, 1H, NH), 4.48 (d, *J* = 12.6 Hz, 1H), 4.36 (d, *J* = 12.6 Hz, 1H), 4.33 (dd, *J* = 4.8, 9.7 Hz, 1H), 3.38 (s, 3H, k), 3.25 (brs, 1H, OH), 2.90 (dd, *J* = 9.7, 14.5 Hz, 1H), 2.02 (dd, *J* = 4.8, 14.5 Hz, 1H), 1.63 (s, 3H), 1.26 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 172.9, 166.8, 165.3, 156.7, 140.1, 140.0, 138.9, 134.7, 134.3, 133.4, 132.7, 132.5, 131.8, 129.9, 129.6, 128.7, 128.6, 127.9, 127.1, 126.9, 115.8, 113.7, 94.7, 80.2, 74.2, 73.5, 72.7, 68.2, 64.8, 55.7, 54.9, 53.9, 41.5, 35.2, 30.5, 21.3, 17.7; FT-IR (Solid) 3430, 2929, 1732, 1651, 1604, 1487, 1452, 1273, 1152, 1070, 967, 753, 712 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{43}H_{46}NO_{10}$ $[M+H]^+$ 736.3122, found 736.3121.

5b (polar diastereomer): 36% (14.0 mg, 25.3 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 7.3 Hz, 2H), 7.63 (d, *J* = 7.3 Hz, 2H), 7.53 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.3 Hz, 1H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.33 (d, *J* = 6.8 Hz, 2H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.26 (t, *J* = 6.8 Hz, 2H), 7.21 (t, *J* = 6.8 Hz, 1H), 7.17 (d, *J* = 8.7 Hz, 1H), 6.87 (d, *J* = 9.2 Hz, 1H), 6.75 (dd, *J* = 2.4, 8.7 Hz, 1H), 6.55 (s, 1H), 5.54 (dd, *J* = 1.9, 9.2 Hz, 1H, q), 5.12 (d, *J* = 7.3 Hz, 1H), 4.99 (d, *J* = 7.3 Hz, 1H), 4.79 (d, *J* = 13.5 Hz, 1H), 4.50 (d, *J* = 13.5 Hz, 1H), 4.49 (d, *J* = 1.9 Hz, 1H, NH), 4.38 (d, *J* = 12.6 Hz, 1H), 4.28 (d, *J* = 12.6 Hz, 1H), 4.26 (dd, *J* = 4.8, 10.1 Hz, 1H), 3.24 (s, 3H), 3.15 (brs, 1H, OH), 2.99 (dd, *J* = 10.1, 15.0 Hz, 1H), 2.63 (brs, 1H, OH), 1.91 (dd, *J* = 4.8, 15.0 Hz, 1H), 1.55 (s, 3H), 1.12 (s, 3H), 0.93 (s, 3H); ^{13}C NMR (100 MHz, CD_2Cl_2) δ 172.7, 166.5, 165.4, 156.7, 139.9, 139.9, 139.0, 134.8, 134.2, 133.5, 132.6, 132.3, 131.8, 129.9, 129.7, 128.6, 127.9, 127.0, 126.8, 115.9, 113.5, 94.6, 80.3, 74.3, 73.8, 72.8, 68.3, 64.9, 55.6, 54.9, 41.5, 35.8, 30.2, 21.2, 17.6; FT-IR

(Solid) 3430, 2957, 1725, 1655, 1604, 1486, 1451, 1271, 1108, 1071, 1026, 754, 711 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{43}H_{46}NO_{10}$ [M+H]$^+$ 736.3122, found 736.3120.

6a and 6b

Condition : 1) substrate **11** (6.6 mg, 14 μmol), sodium hexamethyldisilazide (0.15 mL, 0.15 mmol, 10 equiv), β-lactam (+)-**8** (23 mg, 55 μmol, 3.8 equiv), THF (1.5 mL), –78 °C, 30 min; 2) TBAF (43 μL, 43 μmol, 5.0 equiv), THF (2.0 mL), 0 °C, 15 min; Purification : preparative TLC (50% EtOAc in hexane). The absolute configuration of two diastereomers was not determined.

6a (less polar diastereomer): 27% (2.8 mg, 3.8 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, J = 7.3 Hz, 2H), 7.85 (d, J = 7.3 Hz, 2H), 7.52-7.61 (m, 5H), 7.49 (t, J = 7.3 Hz, 2H), 7.47 (t, J = 7.3 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.31 (t, J = 2.9 Hz, 1H), 7.28 (d, J = 8.2 Hz, 1H), 6.99 (d, J = 9.7 Hz, 1H), 6.90 (dd, J = 2.4, 8.2 Hz, 1H), 6.59 (s, 1H), 6.05 (dd, J = 1.45, 9.7 Hz, 1H), 5.64 (dd, J = 3.4, 11.1 Hz, 1H), 4.89 (d, J = 13.0 Hz, 1H), 4.84 (d, J = 6.8 Hz, 1H), 4.68 (d, J = 6.8 Hz, 1H), 4.68 (d, J = 1.5 Hz, 1H, NH), 4.59 (d, J = 13.0 Hz, 1H), 4.41 (d, J = 12.6 Hz, 1H), 4.29 (d, J = 12.6 Hz, 1H), 3.31 (s, 1H, OH), 3.11 (s, 3H), 2.73 (dd, J = 3.4, 15.5 Hz, 1H), 2.67 (s, 1H, OH), 2.41 (dd, J = 11.1, 15.5 Hz, 1H), 1.63 (s, 3H), 1.03 (s, 3H), 0.91 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.8, 166.8, 165.7, 156.4, 140.7, 139.0, 137.6, 134.4, 133.4, 132.9, 132.8, 132.7, 131.9, 130.1, 129.9, 128.8, 128.7, 127.8, 127.3, 127.1, 117.7, 115.7, 95.3, 76.3, 73.7, 72.9, 71.1, 68.0, 64.8, 55.9, 54.3, 42.2, 35.5, 29.8, 26.1, 22.0, 14.6; FT-IR (Solid) 3429, 2927, 1726, 1661, 1520, 1488, 1452, 1271, 1101, 1073, 1026, 758, 713 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{43}H_{46}NO_{10}$ [M+H]$^+$ 736.3122, found 736.3123.

6b (polar diastereomer): 15% (1.2 mg, 2.1 μmol)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 7.3 Hz, 2H), 7.74 (d, J = 7.3 Hz, 2H), 7.59 (t, J = 7.3 Hz, 1H), 7.38-7.50 (m, 9H), 7.34 (t, J = 7.3 Hz, 1H), 7.33 (t, J = 8.2 Hz, 1H), 7.07 (d, J = 2.4 Hz, 1H), 6.94 (d, J = 9.2 Hz, 1H), 6.89 (dd, J = 2.4, 8.2 Hz, 1H), 6.64 (s, 1H), 5.44 (dd, J = 1.9, 9.2 Hz, 1H), 5.37 (dd, J = 2.9, 10.6 Hz, 1H), 4.93 (d, J = 6.8 Hz, 1H), 4.85 (d, J = 14.0 Hz, 1H), 4.75 (d, J = 6.77 Hz, 1H), 4.66 (d, J = 1.93 Hz, 1H, NH), 4.59 (d, J = 14.0 Hz, 1H), 4.42 (d, J = 13.0 Hz, 1H), 4.33 (d, J = 13.0 Hz, 1H), 3.35 (brs, 1H, OH), 3.19 (s, 3H), 2.69 (brs, 1H, OH), 2.66 (dd, J = 10.6, 15.5 Hz, 1H), 2.40 (dd, J = 2.9, 15.5 Hz, 1H), 1.63 (s, 3H), 1.18 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.6, 166.7, 165.7, 155.8, 140.4, 139.1, 137.6, 134.2, 133.7, 133.6, 132.8, 132.7, 131.8, 129.8, 129.7, 128.9, 128.7, 128.1, 127.2, 127.1, 116.7, 115.1, 94.9, 76.5, 73.3, 73.0, 71.9, 68.4, 65.2, 56.1, 55.1, 42.2, 37.0, 29.8, 26.7, 21.7, 15.5; FT-IR (Solid) 3436, 2926, 1726, 1661, 1515, 1486, 1273, 1111, 1071, 1026, 755, 712 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{43}H_{46}NO_{10}$ [M+H]$^+$ 736.3122, found 736.3118.

Alcohol **14**

To a solution of vinyllithium **13** in dry THF (11 mL) was added a solution of benzaldehyde **12** (300 mg, 0.948 mmol) in dry THF (6.6 mL) via cannula at −78 °C under argon. After being stirred at −78 °C for 4.5 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at −78 °C under argon atmosphere. The organic layer was separated and the aqueous layer was extracted twice with Et_2O. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the resulting residue was purified by flash column chromatography on silica gel (10% EtOAc in hexane) to afford the allylic alcohol **14** (381 mg, 6.54 mmol, 63%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.7 Hz, 2H), 7.13 (d, *J* = 4.6 Hz, 1H), 7.12 (d, *J* = 4.6 Hz, 1H), 7.03 (t, *J* = 4.6 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.00 (t, *J* = 3.2 Hz, 1H), 5.58 (brs, 1H), 5.18 (s, 2H), 4.99 (d, *J* = 10.5 Hz, 1H), 4.66 (d, *J* = 10.5 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 11.4 Hz, 1H), 4.18 (d, *J* = 11.0 Hz, 1H), 4.13 (d, *J* = 11.0 Hz, 1H), 3.79 (s, 3H), 3.47 (s, 3H), 3.38 (d, *J* = 2.7 Hz, 1H), 2.78 (d, *J* = 3.2 Hz, 2H), 1.78 (s, 3H), 1.22 (s, 3H), 0.88 (s, 9H), 0.77 (3H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 155.9, 145.6, 143.4, 135.5, 129.9, 129.7, 129.5, 129.3, 129.2, 124.6, 121.7, 121.3, 113.96, 113.85, 95.2, 72.4, 68.9, 62.4, 58.9, 56.2, 55.3, 38.2, 33.5, 28.3, 26.0, 25.9, 19.2, 18.4, –5.3, –5.4; IR (Neat) 3449, 2955, 2930, 2856, 1612, 1585, 1514, 1466, 1250, 837 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{34}H_{50}O_6SiNa$ $[M+Na]^+$ 605.3248, found 605.3269.

Epoxide **15**

To a solution of the allylic alcohol **14** (300 mg, 0.52 mmol) in dry benzene (5.2 mL) was added TBHP (~5.5 M in decane solution, 0.18 mL, 0.98 mmol, 1.9 equiv) and $VO(acac)_2$ (6.84 mg, 26 μmol, 0.05 equiv) at 0 °C under argon. After being stirred at room temperature for 1.5 h, the reaction mixture was quenched with 10% aqueous $Na_2S_2O_3$ at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo and the resulting residue was purified by column chromatography on silica gel (25% EtOAc in hexane) to afford the epoxide **15** (239 mg, 0.399 mmol, 77%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.8 Hz, 2H), 7.23 (t, *J* = 8.0 Hz, 1H), 7.05 (dd, *J* = 0.7, 8.0 Hz, 1H), 7.02 (dd, *J* = 0.7, 8.0 Hz, 1H), 6.86 (d, *J* = 8.8 Hz, 2H), 5.56 (s, 1H), 5.20 (d, *J* = 6.6 Hz, 1H), 5.14 (d, *J* = 6.6 Hz, 1H), 4.81 (d, *J* = 10.6 Hz, 1H), 4.78 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 11.3 Hz, 1H), 4.51 (d, *J* = 11.3 Hz, 1H), 4.10 (d, *J* = 11.4 Hz, 1H), 4.06 (d, *J* = 11.4 Hz, 1H), 3.79 (s, 3H), 3.46 (s, 3H), 3.40 (s, 1H), 2.88 (brs, 1H), 2.51 (s, 2H), 1.69 (s, 3H), 1.30 (s, 3H), 0.89 (s, 3H), 0.88 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 156.0, 142.2, 134.0, 129.4, 129.0, 125.5, 125.1, 120.8, 114.2, 113.6, 94.9, 72.2, 66.3, 66.0, 62.2, 58.9, 57.7, 56.2, 55.3, 38.6, 31.8, 26.0, 22.2, 19.8, 18.4, –5.3, –5.4; IR (Neat) 3472, 2953, 2930, 1614, 1587, 1513, 1470, 1250, 837 cm^{-1}; HRMS (FAB) calcd for $C_{34}H_{50}O_7SiNa$ $[M+Na]^+$ 621.3223, found 621.3225.

Diol **16**

To a solution of epoxide **15** (500 mg, 0.84 mmol) in dry Et_2O (8.4 mL) was added a solution of $LiAlH_4$ (ca. 0.35 M solution in dry Et_2O, 29 mL, 10 mmol, 12.0 equiv) dropwise at 0 °C under argon. After being stirred at reflux for 5 h, the reaction mixture was diluted with Et_2O, quenched with saturated aqueous Na_2SO_4 and dissolved with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the resulting crude mixture of the diol **16** and desilylated product was used for the next reaction without further purification.

To a solution of the above crude mixture in dry CH_2Cl_2 (8.4 mL) was added imidazole (171 mg, 2.5 mmol, 3.0 equiv) and TBSCl (139 mg, 0.92 mmol, 1.1 equiv) at 0 °C under argon. After being stirred at room temperature for 1.5 h, the reaction mixture was quenched with saturated aqueous NH_4Cl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The filtrate was concentrated in vacuo and the resulting residue was purified by column chromatography on silica gel (20% EtOAc in hexane) to afford the diol **16** (367 mg, 0.611 mmol, 73%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24-7.26 (m, 2H), 7.25 (d, J = 8.2 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 8.2 Hz, 2H), 5.15 (s, 2H), 4.84 (brs, 3H), 4.49 (s, 2H), 4.17 (s, 2H), 3.78 (s, 3H), 3.45 (s, 3H), 1.85(m, 1H), 1.65 (s, 3H), 1.30-1.58 (m, 2H), 1.27 (m, 1H), 1.23 (s, 3H), 1.20 (s, 3H), 0.92 (s, 9H), 0.10 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 144.3, 135.8, 131.2, 129.5, 129.1, 125.0, 113.7, 95.0, 72.1, 59.3, 56.20, 56.17, 55.3, 43.8, 29.3, 28.0, 26.1, 24.1, 19.4, 18.4, –5.2; IR (Neat) 3443, 2955, 2930, 1612, 1585, 1514, 1464, 1250, 837, 773 cm^{-1}; HRMS (FAB) calcd for $C_{34}H_{52}O_7SiNa$ $[M+Na]^+$ 623.3380, found 621.3416.

Allylic alcohol **17**

To a solution of the diol **16** (100 mg, 0.166 mmol) in dry MeCN (3.3 mL) was added CDI (541 mg, 3.3 mmol, 20 equiv) at 0 °C under argon. After being stirred at reflux for 2.5 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo, and the resulting crude carbonate was used for next reaction without further purification.

To a solution of the crude carbonate in dry THF (1.7 mL) was added TBAF (1.0 M in THF solution, 2.5 mL, 2.5 mmol, 15 equiv) at 0 °C under argon. After being stirred at room temperature for 3 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo, and the resulting residue was purified by column chromatography on silica gel (50% EtOAc in hexane) to afford the allylic alcohol **17** (77.7 mg, 0.15 mmol, 91%) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (t, *J* = 8.0 Hz, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 8.0 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.10 (s, 1H), 5.17 (s, 2H), 4.77 (d, *J* = 11.2 Hz, 1H), 4.46 (d, *J* = 11.2 Hz, 1H), 4.40 (d, *J* = 11.2 Hz, 2H), 4.18 (d, *J* = 11.8 Hz, 1H), 4.13 (d, *J* = 11.8 Hz, 1H), 3.80 (s, 3H), 3.46 (s, 3H), 2.01 (m, 1H), 1.75 (m, 1H), 1.67 (s, 3H), 1.53 (m, 2H), 1.26 (s, 3H), 1.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.2, 155.7, 155.1, 136.8, 134.3, 133.2, 130.0, 129.4, 129.3, 125.2, 121.1, 114.9, 113.7, 94.9, 90.1, 78.3, 71.8, 61.0, 58.8, 56.3, 55.3, 42.7, 28.9, 24.9, 22.8, 22.6, 19.3; IR (Neat) 3501, 2957, 2934, 2905, 1790, 1612, 1587, 1514, 1464, 1250, 750 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{29}H_{36}O_8Na$ $[M+Na]^+$ 535.2302, found 535.2286.

Chloride **18**

To a solution of the allylic alcohol **17** (170 mg, 0.33 mmol) and LiCl (28 mg, 0.66 mmol, 2.00 equiv) in dry THF (1.0 mL) and dry CH_2Cl_2 (5.0 mL) was added triethylamine (0.463 mL, 3.3 mmol, 10.0 equiv) and MsCl (64.2 μL, 0.83 mmol, 2.5 equiv) at 0 °C under argon. After being stirred at room temperature for 1 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo, and the resulting residue was used for next reaction without further purification.

To a solution of the crude PMB ether in CH_2Cl_2 (3 mL) and H_2O (176 μL) was added DDQ (113 mg, 0.50 mmol, 1.50 equiv) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, and dried over Na_2SO_4. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (8% EtOAc in toluene) to afford the benzyl alcohol **14** (93 mg, 0.23 mmol, 63% in 2 steps) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (t, 1H, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 5.95 (s, 1H), 5.25 (s, 2H), 4.71 (d, *J* = 12.4 Hz, 1H), 4.63 (d, *J* = 12.4 Hz, 1H), 4.26 (d, *J* = 12.4 Hz, 1H), 4.16 (d, *J* = 12.4 Hz, 1H), 3.51 (s, 3H), 2.06 (m, 1H), 1.83 (m, 1H), 1.74 (s, 3H), 1.69 (m, 1H), 1.57

(m, 1H), 1.41 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 154.9, 137.0, 135.4, 131.2, 129.3, 128.5, 121.5, 115.4, 115.2, 95.3, 89.6, 56.5, 55.5, 43.2, 41.1, 28.9, 24.8, 23.6, 21.7, 19.3; IR (Neat) 3410, 2974, 2914, 1798, 1589, 1466, 1256, 750 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{21}H_{27}O_6ClNa$ $[M+Na]^+$ 433.1388, found 433.1373.

Nine-membered ether **2b**

To a suspension of NaH (43.9 mg, 1.83 mmol, 25 equiv) in dry benzene (10 mL, 137 mL/mmol) was added 18-crown-6-ether (96.5 mg, 0.365 mmol, 5.0 equiv) at room temperature. To the mixture was added a solution of the benzylic alcohol **18** (30.0 mg, 73.0 μmol) in dry benzene (2.5 mL) dropwise at room temperature over a period of 30 min. After being stirred at room temperature for 24 h, the reaction mixture was quenched with 1 M aqueous HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. The filtrate was concentrated in vacuo, and the resulting residue was purified by flash column chromatography on silica gel (25% EtOAc in hexane) to afford the nine-membered ether **2b** (13.4 mg, 35.8 mmol, 49%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 2H), 7.12 (d, J = 7.8 Hz, 1H), 6.22 (s, 1H), 5.23 (s, 2H), 5.00 (d, J = 14.8 Hz, 1H), 4.46 (d, J = 14.8 Hz, 1H), 4.34 (d, J = 12.9 Hz, 1H), 4.28 (d, J = 12.9 Hz, 1H), 3.48 (s, 3H), 2.24 (m, 1H), 2.05 (m, 2H), 1.54 (s, 3H), 1.18 (s, 3H), 1.14 (s, 3H), 0.89 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 153.8, 140.1, 133.3, 130.1, 127.7, 127.4, 119.1, 114.1, 94.3, 91.4, 77.7, 66.0, 64.8, 56.2, 39.4, 28.1, 27.5, 23.5, 21.0, 20.4; IR (Neat) 2932, 2853, 1800, 1585, 1461, 1288, 1256, 754 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{21}H_{26}O_6Na$ $[M+Na]^+$ 397.1622, found 397.1614.

Procedure for MTT assay

The cytotoxicities of compounds against KB-3-1 and MDR KB-G2 cells were examined according to the following procedure. In brief, the cells were seeded into 96-well culture plates at 2×10^3 cells/well and pre-cultured for 24 h. After the pre-culture, the cells were cultured with test compounds for 72 h. In the experiments to examine the activities of the compounds to reverse P-glycoprotein-mediated MDR, MDR KB-G2 cells were cultured with various concentrations of them in the presence of paclitaxel. After a 72 h-incubation with the compounds, the cells were treated with 500 μg/mL MTT reagent for 4 h, and then 10% (w/v) SDS in PBS (100 μL/well) was added to solubilize MTT-formazan. After overnight incubation, absorbances at 570 nm and 630 nm were measured using a Multiskan JX system (Dainippon Pharmaceuticals Co., Osaka, Japan) as test and reference wavelengths, respectively.

Procedure for ATPase assay

The effects of the compounds on the ATPase activities of plasma membrane fractions prepared from the cells were measured according to the following procedure with plasma membrane fractions prepared from P-glycoprotein-expressing Sf9 cells.[9] In brief, cell membranes (1 μg of protein per well) were incubated with compounds in 96-well plates with 40 μL of the incubation medium [50 mM Tris-Mes (pH 6.8), 2 mM EGTA, 2 mM dithiothreitol, 50 mM potassium chloride, 5 mM sodium azide, 2 mM ouabain, and 2 mM ATP/Mg] at 37 °C for 30 min. The reaction was stopped by the addition of 20 μL of 5% trichloroacetic acid followed by 42 μL of solution A [2 M hydrochloric acid : 0.1 M sodium molybdate = 4 : 3] and 18 μL of solution B [0.084% (w/v) malachite green in 1% (w/ v) polyvinyl alcohol]. Thereafter, 120 μL of solution C [7.8% (v/v) sulfuric acid] was added to the mixture in the well plates. One hour after incubation at room temperature, the absorbance of the reaction mixture in each well was measured at a wavelength of 630 nm using a Multiskan JX system (Dainippon Pharmaceuticals Co., Osaka, Japan). The amount of liberated phosphate was quantified on the basis of the calibration line established with inorganic phosphate standards. K_m and V_{max} values for test compounds were calculated from the Lineweaver-Burk plots of (compound concentration) × (compound-stimulated ATPase activity)$^{-1}$ vs (compound concentration).

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