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Synthesis and evaluation of 3D templates based on a taxane skeleton to circumvent P-glycoprotein-associated multidrug resistance of cancer

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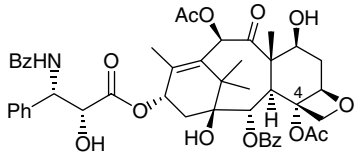
Abstract—We have developed a practical synthetic route to construct C-aromatic taxane derivatives as three-dimension templates by way of intramolecular alkylation. The usefulness of synthesized compounds as the core template was evaluated by using a newly developed screening system for P-glycoprotein.

Multidrug resistance (MDR) in human cancer is the major obstacle to long-term, sustained patient response to chemotherapy. P-glycoprotein (ABCB1 or MDR1) causes MDR in cancer cells by actively extruding the clinically administered chemotherapeutic drugs, such as doxorubicin, vincristine, and paclitaxel.[1] To circumvent P-glycoprotein-associated MDR, a new approach of drug molecular design is needed.[2] The nontaxol-type taxoids have been reported as MDR reversal agents.[3–6] Since several functional groups are attached on the taxane skeleton that has a very tight endo form of a tricyclic system, the skeleton is regarded as a synthetic core template.[7] We have previously reported that a conformationally restricted β-strand mimetic library based on a bicyclic template provided potent and selective inhibitors of serine proteases through combinatorial introduction of substituents.[8] Therefore, the 3D core template could be a useful probe for chemical genomics, when variable substituents can be attached on the template at the various positions.[9] We design core templates **1**, **2**, and **3**, that have different sizes of the B-ring as well as different functional groups attached to the B-ring (Fig. 1).[10] These structures may allow fine tuning by attaching variable substituents on the templates. We herein report the synthesis of new templates of taxane derivatives and evaluation of their MDR reversal activity.



paclitaxel

Conformational analysis based on MM2 calculation using Monte Carlo method suggested that all mimetics possess the endo conformation as paclitaxel (Fig. 2).[11] An *O*-functional group at the C4 or C5 position of **1**–**3** is located at the same position as the C4 acetoxy group in paclitaxel. The tricyclic compounds **1**–**3** could be constructed by way of intramolecular alkylation of **4**–**6**, respectively. The precursors of the alkylation can be prepared by coupling between the A-ring anion **7** and the C-ring aromatic aldehydes **8** or **9** (Fig. 1).

The synthesis of the eight-membered ether skeleton **1** is shown in Scheme 1. The coupling reaction of TBS-protected salicylic aldehyde derivatives **8a**–**c** with a vinyl anion **7** generated from the corresponding hydrazone

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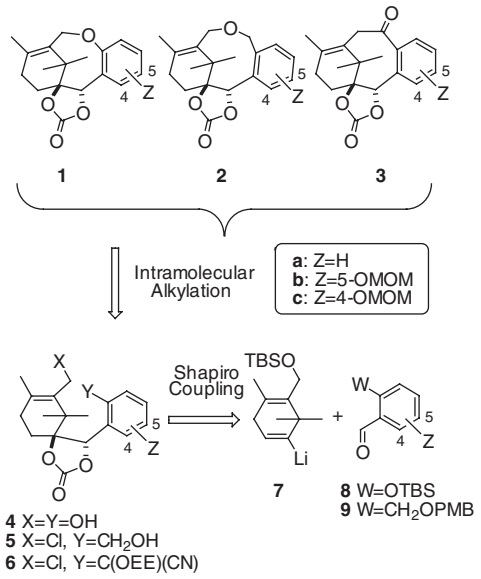


Figure 1. Synthetic strategy for the 3D templates **1**, **2**, and **3**.

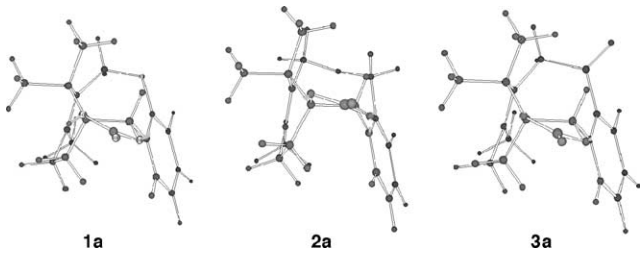
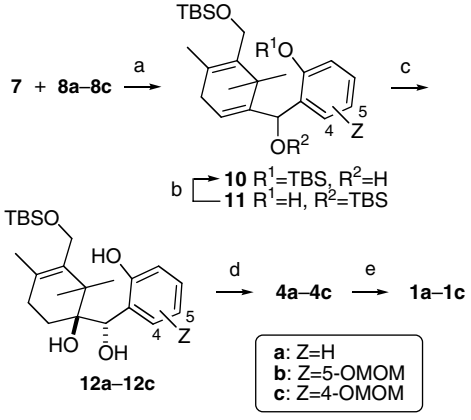


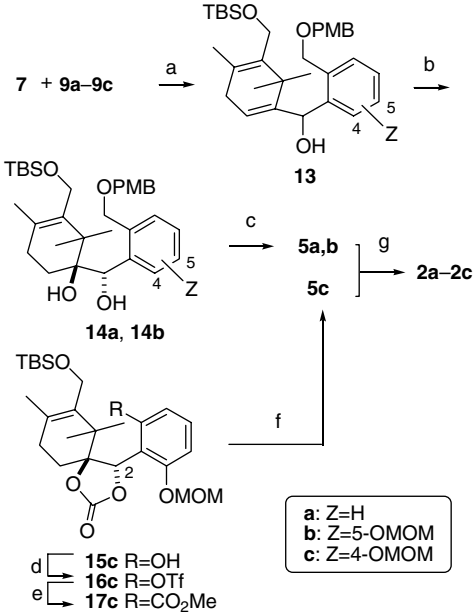
Figure 2. 3D structures of **1a**, **2a**, and **3a** based on MM2 calculation.



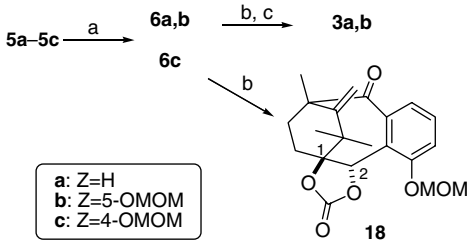
Scheme 1. The synthesis of eight-membered ethers **1**. Reagents and conditions: (a) THF, −78 °C; (b) (i) K_2CO_3, MeOH; (ii) TBSCl, imidazole, CH_2Cl_2, **10a** (89%); **10b** (68%); **10c** (61%); (c) (i) $VO(acac)_2$, *t*-BuOOH, benzene; (ii) $LiAlH_4$, ether, **12a** (91%); **12b** (93%); **12c** (58%); (d) (i) TBSCl, imidazole, CH_2Cl_2; (ii) carbonyldiimidazole, CH_3CN, reflux; (iii) TBAF, THF, **4a** (52%); **4b** (94%); **4c** (61%); (viii) PPh_3, DEAD, THF, RT, **1a** (85%); **1b** (96%); **1c** (65%).

proceeded at −78 °C.[10c,d,12] Concomitant migration of the TBS group in the product **10** provided phenol **11**, which was easily converted to the desired **10**. Hydroxy-directed epoxidation of **10**, followed by reductive opening of the resulting oxirane ring with $LiAlH_4$ provided the triol **12**.[13] Selective protection of the phenolic alcohol with TBSCl, followed by carbonate formation through exposure to carbonyldiimidazole afforded di-TBS ether, which was then desilylated to give diol **4**. The construction of the eight-membered ether was carried out by way of intramolecular Mitsunobu reaction. Treatment of diol **4** with PPh_3 and DEAD provided tricyclic ethers **1a**–**c** in 65–96% yields.[14]

The synthesis of the nine-membered ether skeleton **2** was carried out as summarized in Scheme 2. Aldehyde derivatives **9a**–**c** were coupled to the vinyl anion **7** to give allylic alcohols **13a**–**c**. Subsequent epoxidation, followed by reduction induced partial desilylation and then the primary alcohol was selectively protected with a TBS ether provided diols **14a**,**b**, while **14c** was not obtained since the reductive opening of epoxide had failed. Treatment of **14a**,**b** with carbonyldiimidazole furnished the carbonate, which was desilylated, chlorinated. Then, removal of PMB with DDQ afforded alkylation precursors **5a**,**b**. The **15c**, prepared from **12c** by a similar way to the preparation of **4** except selective deprotection of the TBS group, was converted to triflate **16c**, which underwent carbonylation in methanol using $Pd(OAc)_2$-bis(diphenylphosphono)propane (DPPP) under a CO atmosphere (15 atm) leading to methyl ester **17c**. Then, the desired **5c** was prepared in six steps (66%).



Scheme 2. The synthesis of nine-membered ethers **2**. Reagents and conditions: (a) THF, −78 °C, **13a** (83%); **13b** (53%); **13c** (64%); (b) (i) $VO(acac)_2$, *t*-BuOOH, benzene; (ii) $LiAlH_4$, ether, reflux; (iii) TBSCl, imidazole, CH_2Cl_2, **14a** (88%); **14b** (72%); (c) (i) carbonyldiimidazole, CH_3CN, reflux; (ii) TBAF, THF; (iii) MsCl, LiCl, NEt_3, THF, CH_2Cl_2; (iv) DDQ, CH_2Cl_2, H_2O, **5a** (69%); **5b** (66%); (d) Tf_2O, NEt_3, CH_2Cl_2, 85%; (e) CO (15 atm), $Pd(OAc)_2$, DPPP, NEt_3, MeOH, THF, 80 °C, quant.; (f) (i) $LiAlH_4$, ether; (ii) Ac_2O, NEt_3, CH_2Cl_2; (iii) carbonyldiimidazole, CH_3CN, reflux (iv) TBAF, AcOH, THF; (v) MsCl, LiCl, NEt_3, CH_2Cl_2, THF; (vi) K_2CO_3, MeOH, **5c** (66%); (g) NaH, PhH, DC-18-crown-6, RT, **2a** (66%); **2b** (92%); **2c** (64%).



Scheme 3. The synthesis of eight-membered ketones **3**. Reagents and conditions: (a) (i) TPAP, NMO, CH_2Cl_2, **a** (67%); **b** (91%); **c** (47%); (ii) TMSCN then 0.5 M HCl aq, THF; (iii) EVE, CSA, CH_2Cl_2, two steps **6a** (75%); **6b** (84%); **6c** (79%); (b) (i) $LiN(TMS)_2$, THF, 50 °C; (ii) CSA, MeOH; (iii) NaOH aq, ether, three steps **a** (56%); **b** (79%); **18** (35%); (c) carbonyldiimidazole, CH_3CN, reflux, **3a** (65%); **3b** (52%).

Intramolecular *O*-alkylation of **5a**–**c** was performed at room temperature using NaH in the presence of 2 equiv of 18-crown-6[15] to afford nine-membered ethers **2a**–**c** in good yields.[16,17]

The construction of the eight-membered ketones **3** by way of an intramolecular alkylation of the protected cyanohydrin ethers **6** is outlined in Scheme 3.[10a,18]

Oxidation of the alcohols **5a**–**c** to the aldehyde, followed by three-step formation of the protected cyanohydrin provided **6a**–**c**. Alkylation of **6a**,**b** was accomplished in a refluxing THF solution of lithium hexamethyldisilazide, and the resulting cyclized products were sequentially treated with acid and base. As a carbonate group was deprotected under the cyclization conditions using excess amount of base, the resulting diols were reprotected as carbonate to afford **3a**,**b**.[19] Alkylation of **6c**, however, did not give a desired **3c** but **18** via S_N2' alkylation without losing a carbonate group.[13,20]

The synthesized compounds with the 3D core template were evaluated by using a newly developed screening system for P-glycoprotein.[21,22] For the evaluation, the cDNA of human P-glycoprotein was overexpressed in

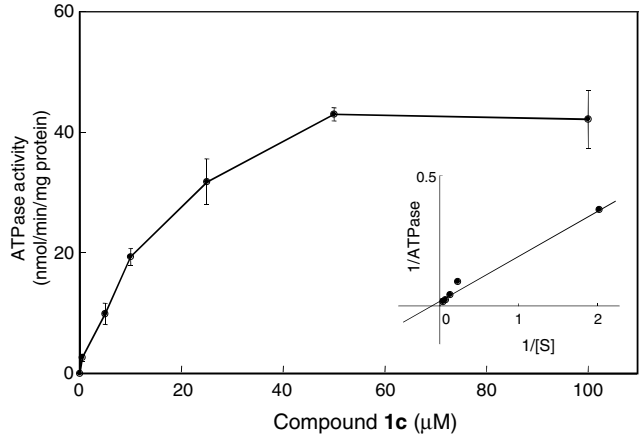


Figure 3. Relationship between the ATPase activity of P-glycoprotein (Val-185) and the concentration of compound **1c**. The inset demonstrates the Lineweaver–Burk plot of the experimental data.

Table 1. P-glycoprotein assay for the synthesized templates

Entry	Compound	K_m [μM]	V_{max} [μM]	V_{max}/K_m	IC_{50} [μM]
1	**1a**	40	23	0.56	>50 (>50)
2	**1b**	11	35	3.2	>50 (>50)
3	**1c**	28	40	1.4	>50 (>50)
4	**2a**	20	23	1.2	>50 (>50)
5	**2b**	4	41	10	>50 (>50)
6	**2c**	8	45	6	45 (>40)
7	**3a**	50	48	0.96	>50 (>50)
8	**3b**	12	47	3.9	>50 (>50)
9	**18**	35	39	1.1	>50 (>50)
10	Paclitaxel	0.7	35	50	0.60 (0.0021)
11	Verapamil	2	65	30	—

IC_{50} was measured against KB-G2 cells that have resistance with paclitaxel and vinblastin. The value against KB-3-1 cells is given in parentheses.

Sf9 cells and drug-induced ATPase activity of P-glycoprotein was measured by using the plasma membrane fraction of Sf9 cells. Figure 3 depicts the relationship between the ATPase activity of P-glycoprotein and the concentration of compound **1c**, demonstrating a typical Michaelis–Menten-type kinetics with a K_m value of 28 μM and a V_{max} of 40 nmol/min/mg protein. As indicated by the K_m value, the affinity of this compound **1c** to P-glycoprotein is one order of magnitude lower than that of paclitaxel (K_m = 0.7 μM).

The results of the P-glycoprotein assay for the synthesized templates are summarized in Table 1. The K_m values of all of the synthesized compounds were greater than those of paclitaxel and verapamil, typical substrates for P-glycoprotein. A significant difference was noticed between the OMOM substituent at the C5 position, at the C4 position, and non-substituent with respect to V_{max}/K_m (**1b** > **1c** > **1a**, **2b** > **2c** > **2a**, **3b** > **3a**). For the purpose to circumvent P-glycoprotein-associated drug resistance, an *O*-functional group can be introduced at the C5 position better than at the C4 position on the aromatic ring. Interestingly, the nine-membered cyclic ether **2b** shows better affinity to P-glycoprotein than taxane skeleton **3b** and eight-membered cyclic ether **1b**. All template compounds, except

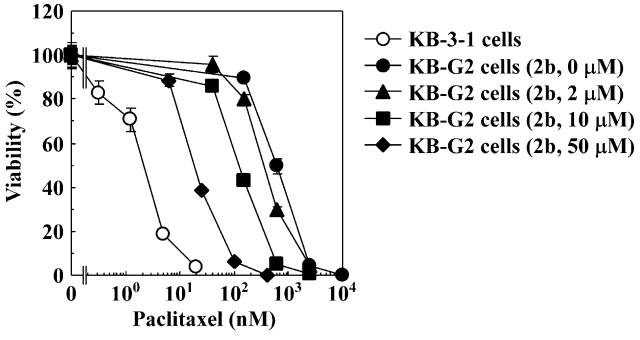


Figure 4. The effect of **2b** as a MDR reversal agent on the cytotoxicity of paclitaxel against MDR KB-G2 cells overexpressing P-glycoprotein (Val-185). The concentration-dependent effects are compared with the sensitivity of KB-3-1 (parental control) cells.

2c, did not exhibit detectable cytotoxicity in either MDR KB-G2 cells or KB-3-1 cells at concentrations of up to 50 μM.

The reversal activity of compound **2b** was further evaluated in MDR KB-G2 cells. Figure 4 demonstrates that compound **2b** sensitized MDR KB-G2 cells to paclitaxel in a dose-dependent manner. In the presence of compound **2b** at the concentration of 50 μM, the IC_{50} value of MDR KB-G2 cells was 0.018×10^3 nM, which is about 30 times lower than the value observed with paclitaxel per se ($IC_{50} = 0.60 \times 10^3$ nM).

In summary, we have herein demonstrated that the synthesis and P-glycoprotein assay for the novel 3D templates based on the taxane skeleton. The affinity to P-glycoprotein of the synthetic templates suggested that introduction of the *O*-functional group would enhance the affinity to P-glycoprotein. Further study for MDR reversal agents by optimization of those template compounds is underway in our laboratory.

Acknowledgements

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14. Selected spectral data of **1a**: IR (neat) 2927, 1801, 1605, 1485, 1458, 1289, 1179, 1036, 978, 750 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.92 (s, 3H), 1.22 (s, 3H), 1.42–1.60 (m, 1H), 1.55 (s, 3H), 1.91–2.04 (m, 1H), 2.20–2.31 (m, 1H), 2.40–2.55 (m, 1H), 4.66 (d, 1H, J = 11.2 Hz), 4.73 (d, 1H, J = 11.2 Hz), 5.77 (s, 1H), 7.08–7.42 (m, 4H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 20.0 (CH_3), 20.7 (CH_3), 23.2 (CH_2), 24.1 (CH_3), 28.5 (CH_2), 39.1 (C), 72.0 (CH_2), 78.3 (CH), 92.3 (C), 124.0 (CH), 124.3 (CH), 124.6 (CH), 124.6 (C), 127.2 (C), 130.2 (CH), 132.0 (C), 143.1 (C), 153.7 (C).
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16. Selected spectral data of **2a**: IR (neat) 2923, 1801, 1464, 1376, 1292, 1205, 1179, 1044, 757 (cm^{-1}); ^1H NMR (270 MHz, $CDCl_3$) δ 1.11 (s, 3H), 1.14 (s, 3H), 1.54 (s, 3H), 1.20–1.45 (m, 2H), 1.95–2.25 (m, 2H), 4.21 (d, 1H, J = 12.5 Hz), 4.30 (d, 1H, J = 12.5 Hz), 4.50 (d, 1H, J = 15.2 Hz), 4.95 (d, 1H, J = 15.2 Hz), 6.32 (s, 1H), 7.17 (dd, 1H, J = 3.96, 4.95 Hz), 7.22–7.32 (m, 2H), 7.59 (ddd, 1H, J = 1.98, 3.96, 4.95 Hz); ^{13}C NMR (99.6 MHz, $CDCl_3$, δ) 20.2 (CH_3), 20.3 (CH_3), 23.9 (CH_3), 27.2 (CH_2), 28.1 (CH_2), 39.8 (C), 66.5 (CH_2), 72.3 (CH_2), 77.8 (CH), 91.6 (C), 126.1 (CH), 126.9 (CH), 128.5 (CH), 128.7 (CH), 130.7 (C), 132.4 (C), 138.5 (C), 139.5 (C), 154.1 (C).
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19. Selected spectral data of **3a**: IR (neat) 2926, 1810, 1694, 1599, 1462, 1377, 1281, 1204, 1039, 748 (cm^{-1}); ^1H NMR (270 MHz, $CDCl_3$) δ 0.99 (s, 3H), 1.25 (s, 3H), 1.34 (s, 3H), 1.38–1.52 (m, 1H), 1.95–2.10 (m, 1H), 2.23–2.32 (m, 1H), 2.34–2.57 (m, 1H), 3.45 (d, 1H, J = 16.5 Hz), 3.61 (d, 1H, J = 16.5 Hz), 5.48 (s, 1H), 7.18 (br d, 1H, J = 7.92 Hz), 7.34 (dt, 1H, J = 1.65, 7.26 Hz), 7.39–7.49 (m, 2H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 21.0 (CH_3), 21.4 (CH_3), 22.9 (CH_2), 24.5 (CH_3), 28.4 (CH_2), 40.4 (C), 48.0 (CH_2), 79.6 (CH), 92.6 (C), 123.5 (CH), 125.8 (CH), 127.4 (C), 128.7 (CH), 129.5 (CH), 131.3 (C), 138.8 (C), 140.4 (C), 153.5 (C), 207.8 (C).
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22. The cDNA of P-glycoprotein was expressed in Sf9 cells using the pFASTBAC1 vector and recombinant baculoviruses. The plasma membrane fraction of Sf9 cells was prepared by sucrose-density gradient ultracentrifugation. The ATPase activity of the isolated Sf9 cell membranes was determined by measuring inorganic phosphate liberation. The Sf9 cell membranes (2 μg of protein) were suspended in 10 μl of the incubation medium containing 50 mM Tris-Mes (pH 6.8), 2 mM EGTA, 2 mM dithiothreitol, 50 mM potassium chloride, 5 mM sodium azide, 2 mM ouabain. This medium was mixed with 10 μl of a test compound solution (0–1.2 mM) and then pre-incubated at 37 °C for 3 min. The ATPase reaction was started by adding 10 μl of 4 mM ATP solution to the reaction mixture (30 μl) and the incubation was maintained at 37 °C for 30 min. The reaction was then stopped by the addition of 20 μl of 5% trichloroacetic acid, and liberated inorganic phosphate was measured in a Multiskan JX system (Dainippon Pharmaceuticals Co., Osaka, Japan).