The Synthesis and Biological Activity of Pyranonaphthoquione Derivatives from *Streptomyces* sp. and Their Related Substances

Akiko Shimbashi, Ayako Tsuchiya,[1] Masaya Imoto,[1] and Shigeru Nishiyama*

Department of Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522

[1]Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, Hiyoshi 3-14-1, Kohoku-ku, Yokohama 223-8522

Received May 6, 2004; E-mail: nisiyama@chem.keio.ac.jp

The total synthesis of a novel pyranonaphthoquinone (**2**) having Cdc25A phosphatase inhibitory activity is achieved. The key step in this synthetic pathway is the intramolecular Michael addition for construction of the pyran ring system. The inhibitory activity of several derivatives, readily obtained from synthetic intermediates of **2**, is assessed. The resulting data indicates that the pyran ring moiety is not a crucial factor for the activity.

Four pyranonaphthoquinones (**1**–**4**, Fig. 1), isolated from the fermentation broth of *Streptomyces* sp. by the Eli Lilly group,[1] exhibited weak inhibitory activity against Cdc25A phosphatase which plays a pivotal role in the regulation of the G_1/S transition for activating several cell cycle-dependent kinases (CDKs) by dephosphorylating an inhibitory phosphotyrosine and phosphothreonine,[2] and are closely related to the oncogenic properties in human tumors, such as in breast cancer.[3] Because of its significant role in cell proliferation and its correlation with a variety of cancers, Cdc25A is a potential and attractive target for anticancer chemotherapeutic agents. Structurally, the benzoquinone moiety, including the amino substituents, is located at the edge of the tricyclic molecule, whereas those of nanaomycins,[4] eleutherin,[5] and freolicins[6] are inserted between the aromatic and pyran rings. Against such a background, we undertook the assembly of pyranonaphthoquinones **1**–**4** and their related substances, as well as the evaluation of their inhibitory activity against the dual-specificity of the protein phosphatase Cdc25A. We describe herein the synthesis of (±)-**2**, with relatively simple substitutions, and the evaluation of the related derivatives in the Cdc25A enzyme inhibition assay.[7]

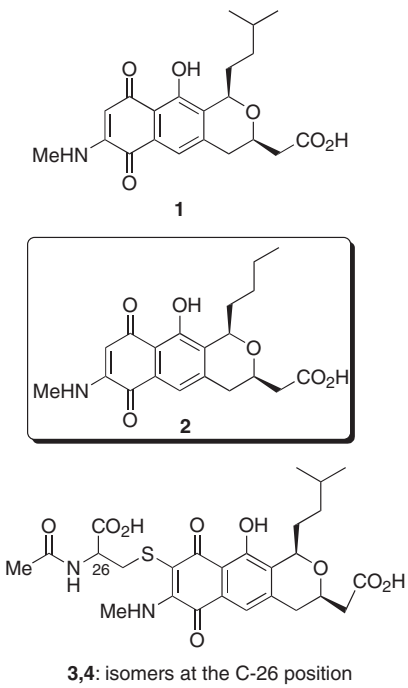


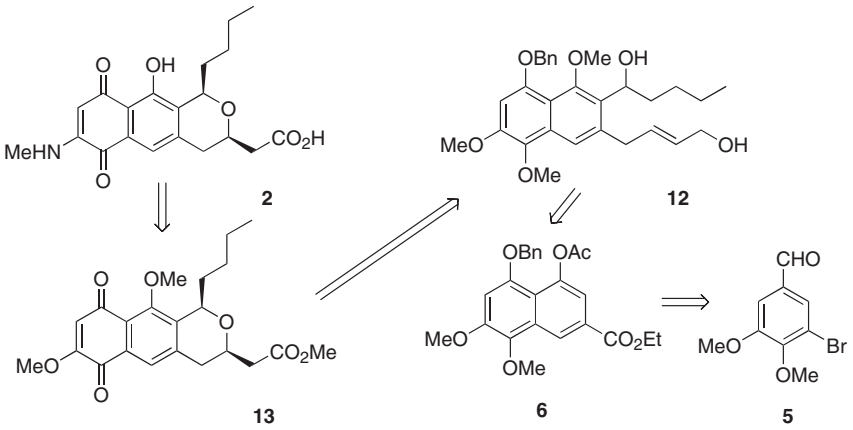
Fig. 1. Structures of Cdc25A phosphatase inhibitors.

Results and Discussion

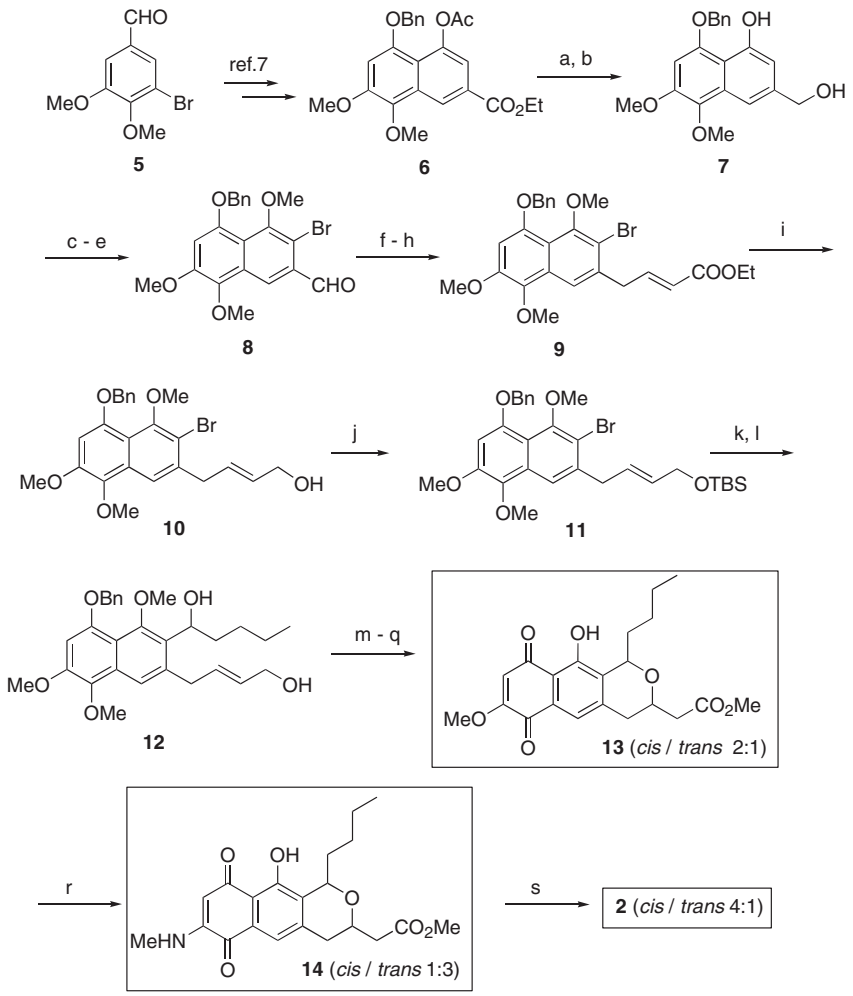
Synthesis of Pyranonaphthoquinone (2). Based on the retrosynthetic analysis of **2** (Scheme 1), the tricyclic compound **13** might be easily converted to the natural substance **2**. The pyran ring moiety of **13** can be constructed from the functionalized derivative **12** by using the intramolecular Michael addition. The naphthalene **6**, which can be obtained from 5-bromoveratraldehyde (**5**), can provide **12**.

In our previous paper,[7] 5-bromoveratraldehyde (**5**) was effectively converted to the intermediate **6** (Scheme 2). Naphthalene **6** was exposed to basic conditions[8] and the successive reduction to give **7**. The regioselective bromination of **7** with $Pyr\cdot HBr_3$[9] was followed by oxidation and methylation to give the aldehyde **8** in 76% yield. The reaction of **8** with dimethylsulfonium methylide[10] gave an epoxide. After isomerization of the epoxide under $ZnBr_2/PhH$ conditions,[11] the resultant aldehyde was subjected to the Horner–Wadsworth–Emmons olefination to furnish the α,β-unsaturated ester **9** in 78% yield from **8**. A reduction with DIBAL produced the corresponding allyl alcohol **10**, which underwent silylation to give **11** in good yield. The coupling reaction of **11** with valeraldehyde via bromine–lithium exchange reaction, and then desilylation, provided **12**. Oxidation to the naphthoquinone, followed by treatment with MnO_2, gave the α,β-unsaturated aldehyde, which was unexpectedly converted into a cyclic aldehyde. The cyclic aldehyde was automatically transformed into a methyl ester, due

Published on the web October 9, 2004; DOI 10.1246/bcsj.77.1925

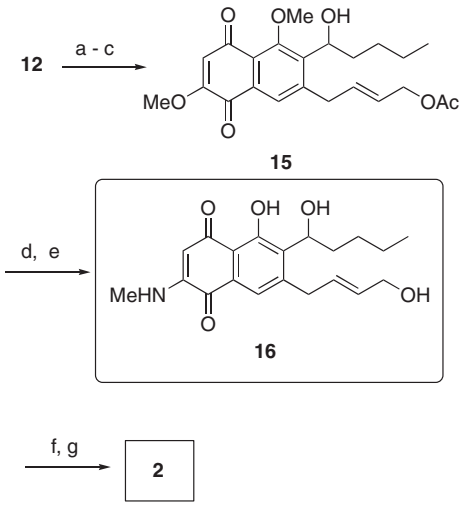


Scheme 1. Retrosynthetic analysis of **2**.



Scheme 2. Reagents and conditions: (a) K_2CO_3, EtOH, rt; (b) $LiAlH_4$, THF, rt, 79% in 2 steps; (c) Pyr·HBr_3, THF, 0 °C; (d) SO_3·Pyr., TEA, DMSO, CH_2Cl_2, rt; (e) MeI, K_2CO_3, DMF, rt, 76% in 3 steps; (f) Me_3SI, NaH, DMSO, THF, 0 °C; (g) $ZnBr_2$, PhH, reflux; (h) $(EtO)_2P(O)CH_2CO_2Et$, NaH, THF, −78 °C, 78% in 3 steps; (i) DIBAL, THF, −78 °C, 93%; (j) TBSCl, Imid., DMF, rt, 96%; (k) valeraldehyde, *n*-BuLi, THF, −78 °C; (l) TBAF, THF, 0 °C, 80% in 2 steps; (m) DDQ, *t*-BuOH, aq. CH_2Cl_2, rt; (n) MnO_2, CH_2Cl_2, 40 °C; (o) PDC, DMF, rt; (p) $TMSCHN_2$, MeOH, rt; (q) BBr_3, CH_2Cl_2, −78 °C to 0 °C, 13% from **12**; (r) $MeNH_2$, MeOH, rt; (s) KOH, H_2O, MeOH, 0 °C to rt, 87%.

to the labile character of the α,β-unsaturated aldehyde. Exposure of the methyl ester to BBr_3 resulted in the selective demethylation at C-6 in 13% yield from **12**.[12] Introduction of an *N*-methyl group with $MeNH_2$[13] provided the methylamine **14**,[14] and the successive hydrolysis with KOH in MeOH quantitatively afforded pyranonaphthoquinone (**2**), contami-



Scheme 3. Reagents and conditions: (a) Ac_2O, Pyr., CH_2Cl_2, rt; (b) DDQ, *t*-BuOH, aq. CH_2Cl_2, 0 °C; (c) BBr_3, CH_2Cl_2, −78 °C, 16% from **12**; (d) $MeNH_2$, THF, 0 °C; (e) K_2CO_3, MeOH, rt, 87% in 2 steps; (f) MnO_2, CH_2Cl_2, 40 °C; (g) PDC, DMF, rt, 19% from **16**; Jones reagent, acetone, −20 °C, 43% from **16**.

Table 1. Inhibitory Activity of Cdc25A Phosphatase

Compound	IC_{50}/μM
Pyranonaphthoquinone (**2**)[a)]	>50
13	>50
14	>50
16	13
Na_3VO_4[b)]	0.005

a) 15.5% Inhibition at 10 μM concentration was reported in Ref. 1. b) Positive control.

nated by the corresponding *trans*-isomer of substituents in the benzopyran moiety (*cis*:*trans* = 4/1).[15]

To accomplish the efficient synthesis of **2**, we planned to construct the pyran ring moiety in the final stage, as shown in Scheme 3. At first, the primary allyl alcohol of **12** was selectively acetylated, followed by the oxidation and selective demethylation to give *p*-quinone **15** in 16% yield from **12**. Introduction of a methylamino group and then basic treatment provided **16**, which upon oxidation in two steps gave **2**. Their spectroscopic data was superimposable with those of the reported data, which indicated the *cis* substitution in the pyran moiety based on the NOE experiments.[1,16]

Biological Evaluation. The synthetic intermediates **13**, **14**, and **16** were evaluated as inhibitors of the Cdc25A phosphatase, using 3-*O*-methylfluorescein phosphate (OMFP) as the substrate. Their inhibitory activities are shown in Table 1. Compound **16** showed a stronger activity than the natural substance **2** and its derivatives **13**, **14**. This result suggested that the pyran ring system is not necessary for the inhibitory activity.

In conclusion, we have accomplished a total synthesis of (±)-pyranonaphthoquinone (**2**) using the intramolecular Michael addition for construction of the pyran ring system. This synthetic route can be utilized for the synthesis of optically active derivatives and other pyranonaphthoquinones such as chloroquinocin.[17] In addition, it was revealed that the pyran ring system was not crucial for the expression of Cdc25A inhibition. The syntheses of related substances and more detailed biological investigations are in progress.

Experimental

General. IR spectra were recorded on a JASCO Model A-202 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were obtained on JEOL JNM EX-270 and JEOL JNM GX-400 spectrometers in $CDCl_3$ using tetramethylsilane as an internal standard, unless otherwise stated. High-resolution mass spectra were obtained on a Hitachi M-80 B GC–MS spectrometer operating at an ionization energy of 70 eV. Melting points were measured on a Yanaco MP-S3 and were uncorrected. Silica-gel column chromatography was carried out using Kanto Chemical silica 60N (sherical, neutral, 63–210 μm). Preparative and analytical thin-layer chromatography (TLC) were carried out on silica-gel plates (Kieselgel 60 F_{254}, E. Merck AG, Germany). The reaction was monitored by UV (254 nm) light and/or stained with 5% phosphomolybdic acid in ethanol as a developing agent, followed in the latter case by heating on an electric plate. Work-up procedure: a mixture was partitioned between EtOAc or $CHCl_3$ and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and then evaporated.

8-Benzyloxy-3-hydroxymethyl-5,6-dimethoxy-1-naphthol (7): A mixture of **6** (3.76 g, 8.9 mmol) and K_2CO_3 (12.7 g, 92 mmol) in EtOH (80 mL) was stirred at room temperature for 18 h. After evaporation, the work-up afforded a crude naphthol. A mixture of the naphthol and $LiAlH_4$ (0.70 g, 19 mmol) in THF (40 mL) was stirred at room temperature for 30 min. After the addition of saturated aq. NH_4Cl at 0 °C, the mixture was worked up and recrystallized from hexane–EtOAc to afford **7** (2.39 g, 79%) as a yellow crystal: mp 131–132 °C; IR (disk) 3386, 2941, 2844, 1635, 1616 cm^{-1}; ^{1}H NMR δ 3.89 (s, 3H), 3.94 (s, 3H), 4.74 (s, 2H), 5.24 (s, 2H), 6.66 (s, 1H), 6.72 (s, 1H), 7.39–7.50 (complex, 6H), 9.25 (s, 1H); ^{13}C NMR δ 57.1, 61.0, 65.5, 72.1, 72.3, 96.5, 107.4, 109.6, 110.6, 127.9, 128.8, 129.0, 131.5, 134.9, 137.6, 137.7, 141.1, 148.0, 151.8, 154.7. HRMS calcd for $C_{20}H_{20}O_5$ (M^+) 340.1311, found *m*/*z* 340.1305. Anal. Calcd for $C_{20}H_{20}O_5$: C, 70.57; H, 5.92%. Found: C, 70.48; H, 5.79%.

5-Benzyloxy-3-bromo-4,7,8-trimethoxynaphthalene-2-carbaldehyde (8): A mixture of **7** (2.42 g, 7.1 mmol) and Pyr·HBr_3 (2.52 g, 7.9 mmol) in THF (50 mL) was stirred at 0 °C for 1 h. After the addition of saturated aq. $Na_2S_2O_3$, the work-up gave a crude bromodiol. A mixture of the crude product, Et_3N (5.9 mL, 43 mmol), and SO_3·Pyr (3.38 g, 21 mmol) in CH_2Cl_2 (20 mL)–DMSO (18 mL) was stirred at room temperature for 20 min. After the addition of saturated aq. NH_4Cl at 0 °C, the work-up afforded a crude aldehyde. A mixture of the aldehyde, K_2CO_3 (3.93 g, 28 mmol), and MeI (0.88 mL, 14 mmol) in DMF (30 mL) was stirred at room temperature for 1 h. The work-up and purification by silica-gel column chromatography (hexane–EtOAc = 1:1) afforded **8** (2.32 g, 76%) as a yellow plate: mp 129–130 °C (hexane/EtOAc); IR (disk) 2943, 2848, 1687, 1612, 1577 cm^{-1}; ^{1}H NMR δ 3.80 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 5.20 (s, 2H), 6.91 (s, 1H), 7.38 (d, 1H, J = 7.2 Hz), 7.43 (t, 2H, J = 7.2 Hz), 7.56 (d, 2H, J = 7.2 Hz), 8.50 (s, 1H), 10.50 (s, 1H); ^{13}C NMR δ 56.9, 61.5, 61.9, 62.0, 62.1, 72.8, 100.5, 103.3, 113.8, 119.6, 121.4, 127.7, 128.1, 128.6, 129.9, 131.5, 136.3, 138.9, 149.0, 151.0, 154.1, 192.2. HRMS calcd for $C_{21}H_{19}{}^{79}BrO_5$ (M^+) 430.0416, found *m*/*z* 430.0345.

Anal. Calcd for $C_{21}H_{19}BrO_5 \cdot 0.2H_2O$: C, 58.00; H, 4.50%. Found: C, 57.78; H, 4.44%.

Ethyl (2*E*)-4-(5-Benzyloxy-3-bromo-4,7,8-trimethoxy-2-naphthyl)but-2-enoate (9): A suspension of NaH (517 mg, 60% dispersion in mineral oil, 13 mmol) in DMSO (5 mL) was stirred at room temperature for 2 h, then diluted with THF (50 mL). A solution of trimethylsulfonium iodide (2.75 g, 13 mmol) in DMSO (10 mL) was slowly added at 0 °C, and the mixture was stirred below 5 °C. After 40 min, a solution of **8** (1.89 g, 4.4 mmol) in DMSO (25 mL) was added at 0 °C over 10 min. After 45 min, saturated aq. NH_4Cl was slowly added. The work-up afforded a crude oxirane. $ZnBr_2$ (2.68 g, 12 mmol) in PhH (60 mL) was refluxed for 40 min. After the addition of a solution of the crude oxirane in PhH (40 mL), the mixture was heated at the same temperature for 20 min. The work-up gave a crude product. To a solution of triethyl phosphonoacetate (4.1 mL, 21 mmol) in THF (40 mL) was added NaH (816 mg, 60% dispersion in mineral oil, 20 mmol) at 0 °C. After 1.5 h, a solution of the above mixture in THF (40 mL) was added at −78 °C. After 25 min, the reaction was quenched by the addition of saturated aq. NH_4Cl at 0 °C and worked up. Purification by silica-gel column chromatography (hexane–EtOAc = 3:1) afforded **9** (1.77 g, 78%) as a pale yellow oil: IR (film) 2937, 2846, 1716, 1614, 1591, 1573 cm^{-1}; ^1H NMR δ 1.27 (t, 3H, $J = 7.0$ Hz), 3.77 (s, 3H), 3.82 (d, 2H, $J = 6.2$ Hz), 3.90 (s, 3H), 3.95 (s, 3H), 4.18 (q, 2H, $J = 7.0$ Hz), 5.18 (s, 2H), 5.81 (d, 1H, $J = 16$ Hz), 7.18 (dt, 1H, $J = 6.2$, 16 Hz), 7.36 (d, 1H, $J = 7.2$ Hz), 7.42 (t, 2H, $J = 7.2$ Hz), 7.56 (d, 2H, $J = 7.2$ Hz), 7.74 (s, 1H); ^{13}C NMR δ 14.3, 39.5, 56.8, 60.2, 61.1, 61.8, 72.5, 100.1, 116.1, 116.3, 118.4, 122.7, 127.6, 127.9, 128.4, 130.3, 130.5, 136.4, 136.5, 136.7, 140.1, 145.7, 148.4, 151.0, 153.7, 166.2. HRMS calcd for $C_{26}H_{27}{}^{79}BrO_6$ (M^+) 514.0991, found m/z 514.0966. Anal. Calcd for $C_{26}H_{27}BrO_6$: C, 60.59; H, 5.28%. Found: C, 60.63; H, 5.33%.

(2*E*)-4-(5-Benzyloxy-3-bromo-4,7,8-trimethoxy-2-naphthyl)-but-2-en-1-ol (10): To a solution of **9** (147 mg, 0.29 mmol) in THF (3 mL) was added DIBAL (1.5 mL of a 1 M solution in toluene, 1.5 mmol) at −78 °C. After 35 min, 4 M HCl was slowly added, the work-up and purification by silica-gel column chromatography (hexane–EtOAc = 1:1) afforded **10** (125 mg, 93%) as a pale yellow plate: mp 104–105 °C (hexane/EtOAc); IR (disk) 3521, 2902, 2860, 1614, 1589, 1574 cm^{-1}; ^1H NMR δ 1.82 (brs, 1H), 3.66 (d, 2H, $J = 6.2$ Hz), 3.77 (s, 3H), 3.90 (s, 3H), 3.94 (s, 3H), 4.13 (q, 2H, $J = 5.4$ Hz), 5.17 (s, 2H), 5.75 (dt, 1H, $J = 5.4$, 16 Hz), 5.95 (dt, 1H, $J = 6.2$, 16 Hz), 6.74 (s, 1H), 7.37 (d, 1H, $J = 7.2$ Hz), 7.42 (t, 2H, $J = 7.2$ Hz), 7.56 (d, 2H, $J = 7.2$ Hz), 7.73 (s, 1H); ^{13}C NMR δ 39.6, 56.8, 61.1, 61.7, 63.4, 63.5, 72.5, 99.8, 115.8, 116.6, 117.7, 127.6, 127.8, 128.3, 129.5, 129.6, 130.5, 131.0, 131.1, 136.7, 138.5, 148.2, 151.0, 153.3. HRMS calcd for $C_{24}H_{25}{}^{79}BrO_5$ (M^+) 472.0885, found m/z 472.0893. Anal. Calcd for $C_{24}H_{25}BrO_5$: C, 60.90; H, 5.32%. Found: C, 60.95; H, 5.63%.

8-Benzyloxy-2-bromo-3-[(2*E*)-4-(*t*-butyldimethylsiloxy)-2-butenyl]-1,5,6-trimethoxynaphthalene (11): A mixture of **10** (125 mg, 0.26 mmol), imidazole (180 mg, 2.6 mmol), and TBSCl (215 mg, 1.4 mmol) in DMF (3 mL) was stirred at 0 °C for 15 min. The work-up and purification by silica-gel column chromatography (hexane–EtOAc = 3:1) afforded **11** (149 mg, 96%) as a yellow oil: IR (film) 2931, 2894, 2854, 1614, 1591, 1576 cm^{-1}; ^1H NMR δ 0.07 (s, 6H), 0.90 (s, 9H), 3.65 (d, 2H, $J = 6.8$ Hz), 3.76 (s, 3H), 3.89 (s, 3H), 3.94 (s, 3H), 4.19 (q, 2H, $J = 5.2$ Hz), 5.18 (s, 2H), 5.67 (dt, 1H, $J = 5.2$, 15 Hz), 5.93 (dt, 1H, $J = 6.8$, 15 Hz), 6.74 (s, 1H), 7.36 (d, 1H, $J = 7.2$ Hz), 7.42 (t, 2H, $J = 7.2$ Hz), 7.56 (d, 2H, $J = 7.2$ Hz), 7.73 (s, 1H); ^{13}C NMR δ −5.0, 18.5, 25.7, 26.0, 39.6, 56.9, 61.3, 61.7, 63.7, 72.6, 99.9, 115.9, 116.8, 117.7, 127.6, 127.7, 127.9, 128.5, 130.6, 131.6, 135.3, 136.8, 137.0, 139.0, 148.2, 151.1. HRMS calcd for $C_{30}H_{39}{}^{79}BrO_5Si$ (M^+) 586.1750, found m/z 586.1758. Anal. Calcd for $C_{30}H_{39}BrO_5Si$: C, 61.32; H, 6.69%. Found: C, 61.21; H, 6.60%.

(2*E*)-4-(5-Benzyloxy-3-(1-hydroxypentyl)-4,7,8-trimethoxy-2-naphthyl)but-2-en-1-ol (12): To a solution of **11** (1.06 g, 1.8 mmol) in THF (30 mL) were added *n*-BuLi (3.4 mL of a 1.57 M solution in hexane, 5.3 mmol) and valeraldehyde (1.0 mL, 9.4 mmol) at −78 °C all at once. After the addition of saturated aq. $NaHCO_3$, the work-up gave a crude product. To a solution of the crude product in THF (20 mL) was added TBAF (9 mL of a 1 M solution in THF, 9 mmol) at 0 °C. Stirring was continued at room temperature for 1.5 h. After the addition of H_2O at 0 °C, the work-up and purification by silica-gel column chromatography (hexane–EtOAc = 3:1) gave **12** (693 mg, 80%) as a yellow oil: IR (film) 3408, 2933, 2858, 1620, 1574 cm^{-1}; ^1H NMR δ 0.92 (t, 3H, $J = 6.2$ Hz), 1.37–1.39 (complex, 2H), 1.63–1.80 (complex, 2H), 1.92–2.04 (complex, 2H), 3.57 (d, 2H, $J = 5.4$ Hz), 3.80 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 4.09 (d, 2H, $J = 5.6$ Hz), 4.99 (m, 1H), 5.11 (d, 1H, $J = 11$ Hz), 5.19 (d, 1H, $J = 11$ Hz), 5.64 (dt, 1H, $J = 5.6$, 15 Hz), 5.91 (dt, 1H, $J = 5.4$, 15 Hz), 6.73 (s, 1H), 7.36 (d, 1H, $J = 7.2$ Hz), 7.41 (t, 2H, $J = 7.2$ Hz), 7.53 (d, 2H, $J = 7.2$ Hz), 7.65 (s, 1H); ^{13}C NMR δ 14.2, 22.8, 29.2, 36.6, 39.0, 50.3, 56.9, 61.1, 63.4, 64.0, 71.1, 72.9, 100.3, 113.7, 115.1, 118.2, 127.4, 127.8, 128.4, 130.7, 131.0, 131.2, 136.8, 136.9, 137.0, 147.9, 151.1, 154.8, 163.6. HRMS calcd for $C_{29}H_{36}O_6$ (M^+) 480.2512, found m/z 480.2493. Anal. Calcd for $C_{29}H_{36}O_6 \cdot 0.2H_2O$: C, 71.94; H, 7.58%. Found: C, 71.78; H, 7.60%.

Methyl 2-((1*R,3*R**)-1-Butyl-3,4-dihydro-10-hydroxy-7-methoxy-6,9-dioxobenzo[*g*]-1*H*-isochromen-3-yl)acetate (13):** To a solution of **12** (120 mg, 0.25 mmol) in CH_2Cl_2 (1 mL) was added a suspension of DDQ (171 mg, 0.75 mmol) in *t*-BuOH (1 mL)–H_2O (1 mL) at 0 °C. After 15 min, saturated aq. $NaHCO_3$ was added, and the work-up afforded a crude naphthoquinone. A mixture of the crude product and MnO_2 (872 mg, 10 mmol) in CH_2Cl_2 (5 mL) was heated at 40 °C. After 3.5 h, precipitates were filtered through a Celite pad and washed with $CHCl_3$–MeOH, then the filtrate and washings were then evaporated to give a residue, which was passed through a silica-gel column (hexane–EtOAc = 1:1) to give a crude product. A mixture of the crude aldehyde and PDC (1.19 g, 3.2 mmol) in DMF (5 mL) was stirred at room temperature for 21 h. The work-up gave a crude product. A mixture of the crude product and $TMSCHN_2$ (0.4 mL of a 2 M solution in hexane, 0.8 mmol) in MeOH (1 mL) was stirred at room temperature for 2 h. The work-up afforded a crude naphthopyran. A mixture of the naphthopyran and BBr_3 (0.1 mL of a 1 M solution in CH_2Cl_2, 0.1 mmol) in CH_2Cl_2 (1 mL) was stirred at −78 °C for 20 min. The work-up and purification by preparative TLC (hexane–EtOAc = 1:1) afforded **13** (12.6 mg, 13%) as a yellow oil: ^1H NMR δ 0.92 (t, 3H, $J = 6.8$ Hz), 1.25–1.58 (complex, 4H), 1.74–1.76 (m, 1H), 1.92–1.98 (m, 1H), 2.58–2.78 (complex, 4H), 3.74 (s, 3H), 3.91 (s, 3H), 3.91–3.97 (m, 1H), 4.38 (m, 1H), 6.06 (s, 1H), 7.41 (s, 1H), 12.6 (s, 1H). HRMS calcd for $C_{21}H_{24}O_7$ (M^+) 388.1522, found m/z 388.1532.

Methyl 2-((1*R,3*R**)-1-Butyl-3,4-dihydro-10-hydroxy-7-(methylamino)-6,9-dioxobenzo[*g*]-1*H*-isochromen-3-yl)acetate (14):** To a solution of **13** (3.9 mg, 10 μmol) in THF (1 mL) was added $MeNH_2$ (20 μL, 40% MeOH solution, 0.3 mmol) at 0 °C.

After 30 min, the solvent was evaporated to afford crude **14**: ^{1}H NMR (C_3D_6O) δ 0.82 (t, 3H, $J = 6.8$ Hz), 1.18–1.82 (complex, 5H), 2.16 (m, 1H), 2.47–2.93 (complex, 4H), 2.86 (d, 3H, $J = 5.4$ Hz), 3.55 (s, 3H), 3.83 (m, 1H), 4.26 (m, 1H), 5.43 (s, 1H), 7.17 (s, 1H), 7.17 (m, 1H), 13.8 (s, 1H). HRMS calcd for $C_{21}H_{25}NO_6$ (M^+) 387.1682, found m/z 387.1658.

2-[(1*R,3*R**)-1-Butyl-3,4-dihydro-10-hydroxy-7-(methylamino)-6,9-dioxobenzo[*g*]-1*H*-isochromen-3-yl]acetic Acid (2):** A mixture of **14** (1.3 mg, 3.4 μmol) and KOH (1 mg, 17 μmol) in H_2O (0.3 mL) in MeOH (1 mL) was kept for 24 h. A solution of 1 M HCl was slowly added, and the work-up afforded **2** (1.9 mg, 87%): IR (film) 3355, 2924, 1710, 1612 cm^{-1}; ^{1}H NMR δ 0.87 (t, 3H, $J = 6.8$ Hz), 1.25–1.39 (complex, 4H), 2.15 (m, 1H), 2.78 (complex, 4H), 2.95 (d, 3H, $J = 5.2$ Hz), 3.98 (m, 1H), 5.14 (m, 1H), 5.59 (s, 1H), 6.10 (m, 1H), 7.34 (s, 1H), 13.64 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 14.3, 23.3, 27.8, 34.4, 36.0, 40.8, 70.6, 75.1, 99.0, 113.3, 119.5, 129.3, 135.7, 142.7, 151.4, 159.1, 172.1, 181.6, 189.7. HRMS calcd for $C_{20}H_{23}NO_6$ (M^+) 373.1525, found m/z 373.1549. *trans*-isomer: ^{1}H NMR δ 0.94 (t, 3H, $J = 6.8$ Hz), 1.25–1.39 (complex, 4H), 2.33 (m, 1H), 2.82 (complex, 4H), 2.95 (d, 3H, $J = 5.2$ Hz), 4.40 (m, 1H), 5.05 (m, 1H), 5.59 (s, 1H), 6.10 (m, 1H), 7.34 (s, 1H), 13.50 (s, 1H); ^{13}C NMR (CD_3COCD_3) δ 14.3, 23.0, 27.4, 28.9, 32.6, 34.1, 41.1, 72.8, 79.2, 99.0, 114.8, 119.7, 129.1, 136.8, 140.4, 155.7, 164.7, 172.1, 182.7, 189.7.

(2*E*)-4-(3-(1-Hydroxypentyl)-4,7-dimethoxy-5,8-dioxo(2-naphthyl)(but-2-enyl Acetate (15): A mixture of **12** (111 mg, 0.23 mmol), pyridine (36 μL, 0.46 mmol), and Ac_2O (15 μL, 0.16 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 2 days. The work-up afforded a crude secondary alcohol. A mixture of the crude secondary alcohol in CH_2Cl_2 (5 mL) and DDQ (109 mg, 0.48 mmol) in *t*-BuOH (2 mL)–H_2O (2 mL) was stirred at 0 °C for 10 min. After the addition of saturated aq. $NaHCO_3$, the mixture was worked up to afford a crude naphthoquinone. To a solution of BBr_3 (0.20 mL of a 1 M solution in CH_2Cl_2, 0.20 mmol) in CH_2Cl_2 (3 mL) was added a solution of the crude naphthoquinone in CH_2Cl_2 (5 mL) at −78 °C. After 20 min, the work-up and purification by preparative TLC (hexane–EtOAc = 1:2) afforded **15** (13.8 mg, 16%) as a yellow oil and the naphthoquinone (25.8 mg, 29%). **15**: IR (film) 3534, 2956, 1739, 1683, 1625 cm^{-1}; ^{1}H NMR δ 0.90 (t, 3H, $J = 6.8$ Hz), 1.33–1.42 (complex, 4H), 1.50–1.76 (complex, 2H), 2.05 (s, 3H), 3.50 (d, 2H, $J = 5.8$ Hz), 3.92 (s, 3H), 4.52 (d, 2H, $J = 6.2$ Hz), 4.86 (m, 1H), 5.59 (dt, 1H, $J = 5.8$, 15 Hz), 5.87 (dt, 1H, $J = 6.2$, 15 Hz), 6.07 (s, 1H), 7.48 (s, 1H), 13.11 (s, 1H); ^{13}C NMR δ 14.1, 21.0, 22.7, 28.6, 36.3, 36.4, 49.2, 49.3, 56.7, 64.4, 70.6, 109.3, 121.7, 126.8, 129.0, 131.6, 138.4, 144.4, 159.5, 161.0, 190.7, 206.5. HRMS calcd for $C_{22}H_{26}O_7$ (M^+ + H) 403.1757, found m/z 403.1732. Anal. Calcd for $C_{22}H_{26}O_7 \cdot 0.2H_2O$: C, 65.08; H, 6.55%. Found: C, 65.10; H, 6.45%.

7-[(2*E*)-4-Hydroxybut-2-enyl]-5-hydroxy-6-(1-hydroxypentyl)-2-(methylamino)-1,4-naphthoquinone (16): A mixture of **15** (3.9 mg, 10 μmol) and $MeNH_2$ (20 μL, 40% MeOH solution, 0.28 mmol) in THF (1 mL) was stirred at 0 °C for 30 min. The solvents were then evaporated to afford a crude (aminomethyl)-quinone. A mixture of the crude product and K_2CO_3 (20 mg, 0.14 mmol) in MeOH (1 mL) was stirred at room temperature for 40 min. The work-up and purification by preparative TLC (EtOAc) afforded **16** (3.1 mg, 87%) as a yellow oil: IR (film) 3357, 2929, 1614 cm^{-1}; ^{1}H NMR δ 0.90 (t, 3H, $J = 6.8$ Hz), 1.25–1.39 (complex, 4H), 1.49–1.78 (complex, 2H), 2.94 (d, 3H, $J = 5.4$ Hz), 3.43 (complex, 2H), 4.11 (d, 2H, $J = 5.2$ Hz), 4.84 (m, 1H), 5.57 (s, 1H), 5.65 (dt, 1H, $J = 5.2$, 15 Hz), 5.81 (dt, 1H, $J = 5.9$, 15 Hz), 6.17 (s, 1H), 7.38 (s, 1H), 14.07 (s, 1H); ^{13}C NMR δ 14.2, 21.5, 22.7, 28.6, 29.3, 36.2, 36.3, 49.3, 49.4, 63.2, 70.9, 99.2, 121.0, 128.6, 131.8, 139.0, 149.6, 159.7, 188.8, 208.0. HRMS calcd for $C_{20}H_{25}NO_5$ (M^+ + H) 360.1811, found m/z 360.1800.

2-[(1*R,3*R**)-1-Butyl-3,4-dihydro-10-hydroxy-7-(methylamino)-6,9-dioxobenzo[*g*]-1*H*-isochromen-3-yl]acetic Acid (2):** *PDC oxidation.* A mixture of **16** (9.9 mg, 28 μmol) and MnO_2 (106 mg, 1.2 mmol) in CH_2Cl_2 (3 mL) was heated at 40 °C for 2.5 h. The work-up gave a crude product. A mixture of the crude product (4.4 mg, 12 μmol) and PDC (215 mg, 0.57 mmol) in DMF (2 mL) was stirred at room temperature for 3 h. The work-up afforded a crude pyranonaphthoquinone **2** (1.9 mg, 19% from **16**).

Jones oxidation. A mixture of the crude aldehyde and the Jones reagent (ca. 1 M, excess amount) in acetone (0.7 mL) was stirred at −20 °C for 15 min. The work-up and purification by preparative TLC (chloroform–methanol = 5:1) afforded pyranonaphthoquinone **2** (2.3 mg, 43% from **16**).

Enzymatic Assay of Cdc25A Phosphatase Inhibition. *Protein purification. Esherichia coli* BL21 (DE-3) cells were transformed with a plasmid encoding GST fusion of cdc25A. Cultures were grown at 37 °C to an A_{600} of 0.8, and isopropyl 1-thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.1 mM. After growing for an additional 4 h at 37 °C, the cells were collected by centrifugation. Cell pellets were suspended in lysis buffer (MT-PBS (150 mM NaCl, 20 mM Na_2HPO_4, 4 mM NaH_2PO_4), 0.1% tritonX-100, 10 μg/mL leupeptin, 1 mM phenylmethanesulfonyl fluoride) and lysed by sonication. GST-cdc25A was precipitated with GSH-agarose beads (sigma, St. Louis, MO.), washed four times with MT-PBS, and eluted by elution buffer (50 mM Tris–HCl (pH 8.0), 20 mM reduced glutathione). The amount of GST-cdc25A was estimated by comparison with a BSA standard after SDS-PAGE and Coomasie blue staining.

Cdc25A assay in vitro. The activity of the GST-cdc25A was measured in a 96-well microtiter plate using the substrate OMFP (sigma), which is readily metabolized to the fluorescent O-methyl fluorescein. The inhibitors were resuspended in methanol, and all reactions including controls were performed at a final concentration of 1% methanol. Approximately 30 μg of purified GST-cdc25A was preincubated at 25 °C for 15 min in reaction buffer consisting of 50 mM Tris–HCl (pH 7.5) and various concentrations of inhibitors, the reaction was started by the addition of 25 μM OMFP. The fluorescence emission from the product was measured over a 30 min reaction period with a maltiwell plate reader (Fluoroskan Ascent FL: Labsystem). The excitation wavelength was 355 nm, and emission was selected using a 538 nm filter. All experiments were done in triplicate. The reaction was linear over the time used in the experiments and was directly proportional to both the enzyme and substrate concentration.

This work was supported by a Grant-in-Aid for the 21st Century COE program "Keio Life Conjugated Chemistry", as well as Scientific Research C from the Ministry of Education, Culture, Sports, Science and Technology.

References

1 P. Kulanthaivel, T. J. Perun, Jr., M. D. Belvo, R. J. Strobel, D. C. Paul, and D. C. Williams, *J. Antibiot.*, **52**, 256 (1999).

2 a) S. Jinno, K. Suto, A. Nagata, M. Igarashi, Y. Kanaoka,

H. Nojima, and H. Okayama, *EMBO J.*, **13**, 1549 (1994). b) I. Hoffmann, G. Draetta, and E. Karsenti, *EMBO J.*, **13**, 4302 (1994).

3 a) K. Galaktionov, A. K. Lee, J. Eckstein, G. Draetta, J. Meckler, M. Loda, and D. Beach, *Science*, **269**, 1575 (1995). b) K. Galaktionov, X. Chen, and D. Beach, *Nature*, **382**, 511 (1996). c) D. Gasparotto, R. Maestro, S. Piccinin, T. Vukosavljievic, L. Barzan, S. Sulfaro, and M. Boiocchi, *Cancer Res.*, **57**, 2366 (1997). d) W. G. Wu, Y. H. Fan, B. L. Kemp, G. Walsh, and L. Mao, *Cancer Res.*, **58**, 4082 (1998). e) D. Dixon, T. Moyana, and M. J. King, *Exp. Cell Res.*, **240**, 236 (1998). f) M. G. Cangi, B. Cukor, P. Soung, S. Signoretti, G. Moreira, M. Ranashinge, B. Cady, M. Pagano, and M. Loda, *J. Clin. Invest.*, **106**, 753 (2000).

4 a) S. Omura, H. Tanaka, Y. Koyama, R. Oiwa, M. Katagiri, J. Awaya, T. Nagai, and T. Hata, *J. Antibiot.*, **27**, 363 (1974). b) H. Tanaka, Y. Koyama, J. Awaya, H. Marumo, R. Oiwa, M. Katagiri, T. Nagai, and S. Omura, *J. Antibiot.*, **28**, 860 (1975). c) H. Tanaka, Y. Koyama, T. Nagai, H. Marumo, and S. Omura, *J. Antibiot.*, **28**, 868 (1975).

5 H. Schmid and A. Ebnöther, *Helv. Chim. Acta*, **34**, 1041 (1951).

6 a) J. C. Van Meter, M. Dann, and N. Bohonos, "Antibacterial Agents Annual-1960," Plenum Press, New York, N. Y. (1961), p. 77. b) G. A. Ellestad, M. P. Kunstmann, H. A. Whaley, and E. L. Patterson, *J. Am. Chem. Soc.*, **90**, 1325 (1968).

7 Preliminary report see: a) A. Shimbashi, Y. Ishikawa, and S. Nishiyama, *Tetrahedron Lett.*, **45**, 939 (2004). b) M. Iinuma, T. Tanaka, and S. Matsuura, *Chem. Pharm. Bull.*, **32**, 2296 (1984).

8 The combined yield in two steps (K_2CO_3/EtOH, then $LiAlH_4$/THF) was better than a one-step procedure using only $LiAlH_4$.

9 W. P. Reeves and R. M. King, *Synth. Commun.*, **23**, 855 (1993).

10 a) E. J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1353 (1965). b) S. Kulasegaram and R. J. Kulawiec, *J. Org. Chem.*, **62**, 6547 (1997).

11 R. L. Settine, G. L. Parks, and G. L. K. Hunter, *J. Org. Chem.*, **29**, 616 (1964).

12 Under BBr_3 conditions, two alkyl substituents of the dihydropyran moiety adopt thermodynamically the more favorable *trans*-form than the corresponding *cis*-form. Examples of stability of the *trans*-form by thermodynamic control: a) M. F. Semmehak and A. Zask, *J. Am. Chem. Soc.*, **105**, 2034 (1983). b) A. D. Webb and T. M. Harris, *Tetrahedron Lett.*, **18**, 2069 (1977). c) T.-T. Li and R. H. Ellison, *J. Am. Chem. Soc.*, **100**, 6263 (1978). d) T. Masquelin, U. Hengartner, and J. Streith, *Synthesis*, **1995**, 780.

13 a) X. L. Tao, J.-F. Cheng, S. Nishiyama, and S. Yamamura, *Tetrahedron*, **50**, 2017 (1994). b) The *cis*- and *trans*-isomers were formed in a ratio of ca. 1:3. The diastereomeric mixture was inseparable.

14 Because the diastereomeric mixture was inseparable, crude **14** was used for Cdc25A phosphatase inhibition assay.

15 A 1:3 ratio of the *cis*- and *trans*-isomers after treatment with $MeNH_2$, changed to ca. 4:1 by KOH, which might induce isomerization through successive β-elimination and Michael addition reactions.

16 No contamination with the *trans*-isomer was monitored by ODS-W TLC (0.5% aq. NH_4OAc–MeCN = 3/2).

17 H. He, H. Y. Yang, S. W. Luckman, D. M. Roll, and G. T. Carter, *J. Antibiot.*, **55**, 1072 (2002).