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DESIGN AND SYNTHESIS OF TELOMESTATIN DERIVATIVES AND THEIR INHIBITORY ACTIVITY OF TELOMERASE

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Abstract – Telomestatin derivatives of **2a-2c**, which have macrocyclic bisamide structure, were designed and synthesized. One of these compounds (**2a**) showed inhibitory activity of telomerase with an IC₅₀ of 2 μM.

Telomestatin (**1**) is a natural product isolated from *Streptomyces anulatus* 3533-SV4 by the screening of Telomeric Repeat Amplification Protocol (TRAP) assay.¹ Telomestatin (**1**) shows potent telomerase inhibitory activity with an IC₅₀ of 5 nM, and it also has been realized to stabilize strongly the telomeric G-quadruplex formation.² Thus, telomestatin (**1**) appears to be an ideal lead compound for the telomere-targeted anticancer agent.^{3,4} Telomestatin (**1**) is also used as a tool for studying biological

effects inducing the formation of G-quadruplex structure in cells.² Herein, we described the synthesis of macrocyclic bisamide compounds (**2a-c**),⁴ which is designed as a new synthetically practical telomerase inhibitor, and evaluation of their telomerase inhibitory activity.

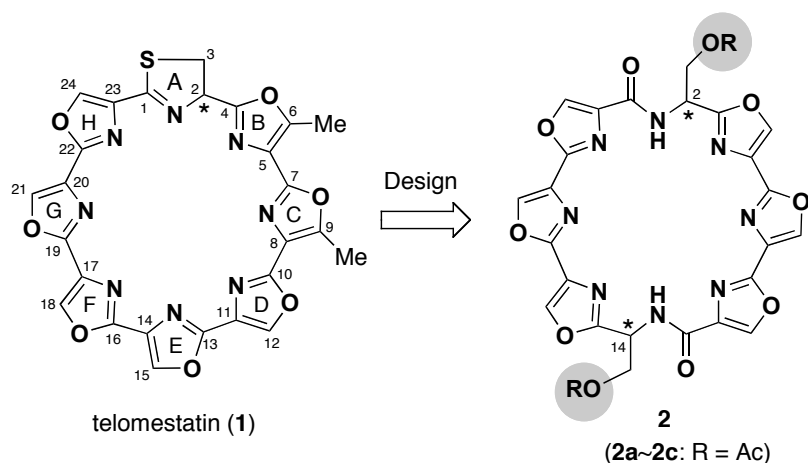
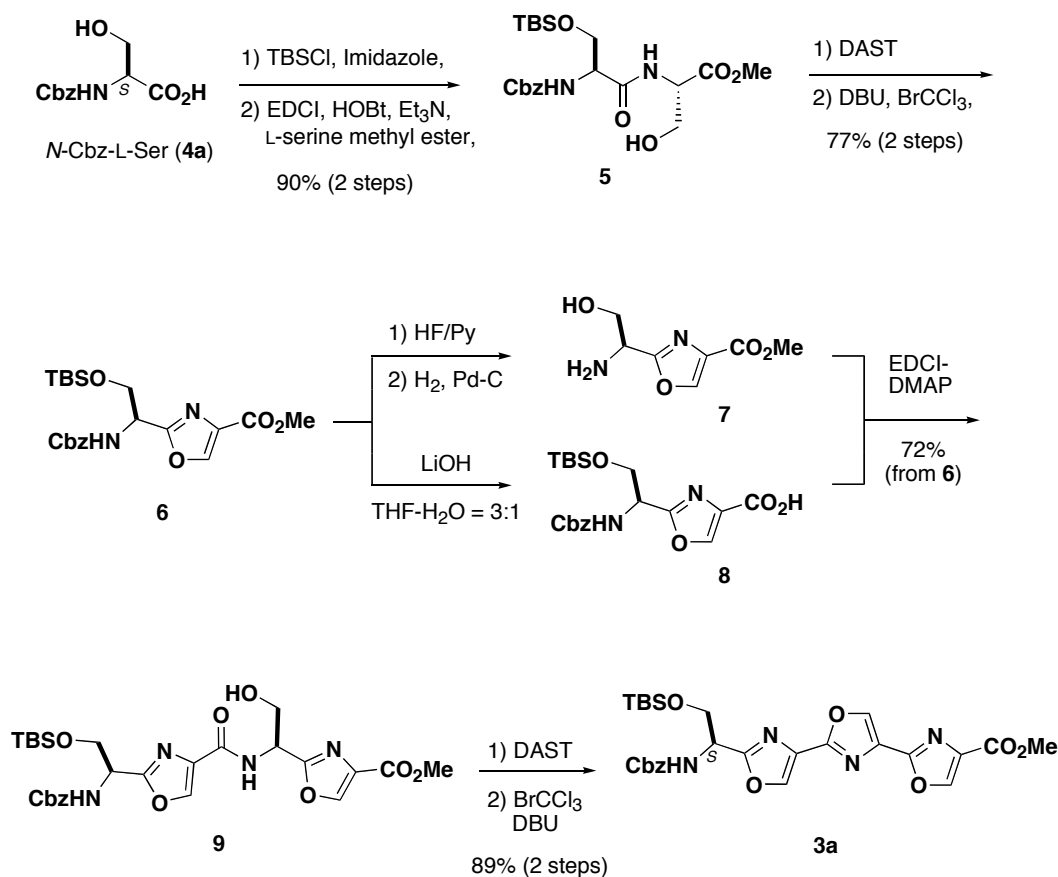


Figure 1. Design of Telomestatin Derivative (**2**).

Telomestatin (**1**) has a characteristic macrocyclic ring system, which consists of sequential pentaioxazoles, bismethyloxazoles, and a thiazoline. These structural features of **1** are suggested to bind to G-quartet strongly by overlapping, and this interaction is believed to promote the formation and stabilization of G-quadruplex structure.² To increase this interaction by using the designed telomestatin derivative, installation of additional functional groups, such as carbonyl, hydroxyl, and/or amino groups, together with macrocyclic ring system to **1** would be required. Based upon this concept, we designed macrocyclic bisamide (**2**) as a synthetic derivative of **1**. This derivative has two hydroxyl groups ($R = H$), and variety of functional groups can be introduced to this moiety. Since the chiral centers at C2 and C14 in (**2**) are expected to affect the binding to G-quartet, we synthesized three possible diastereomers of **2**, i.e., **2a-2c**.^{5,6}

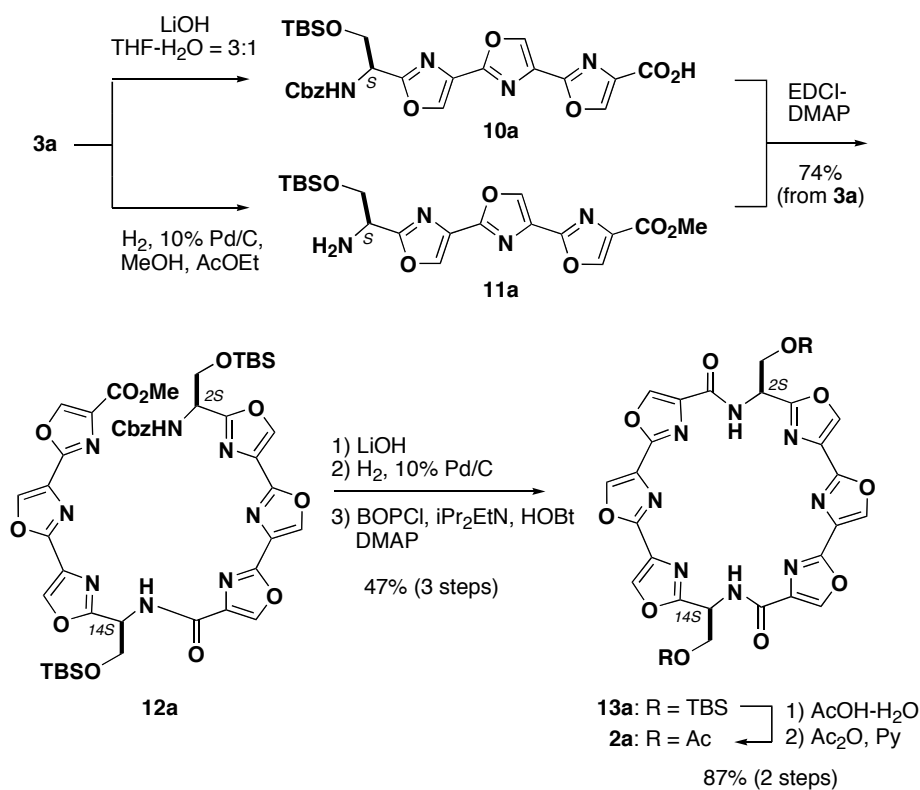
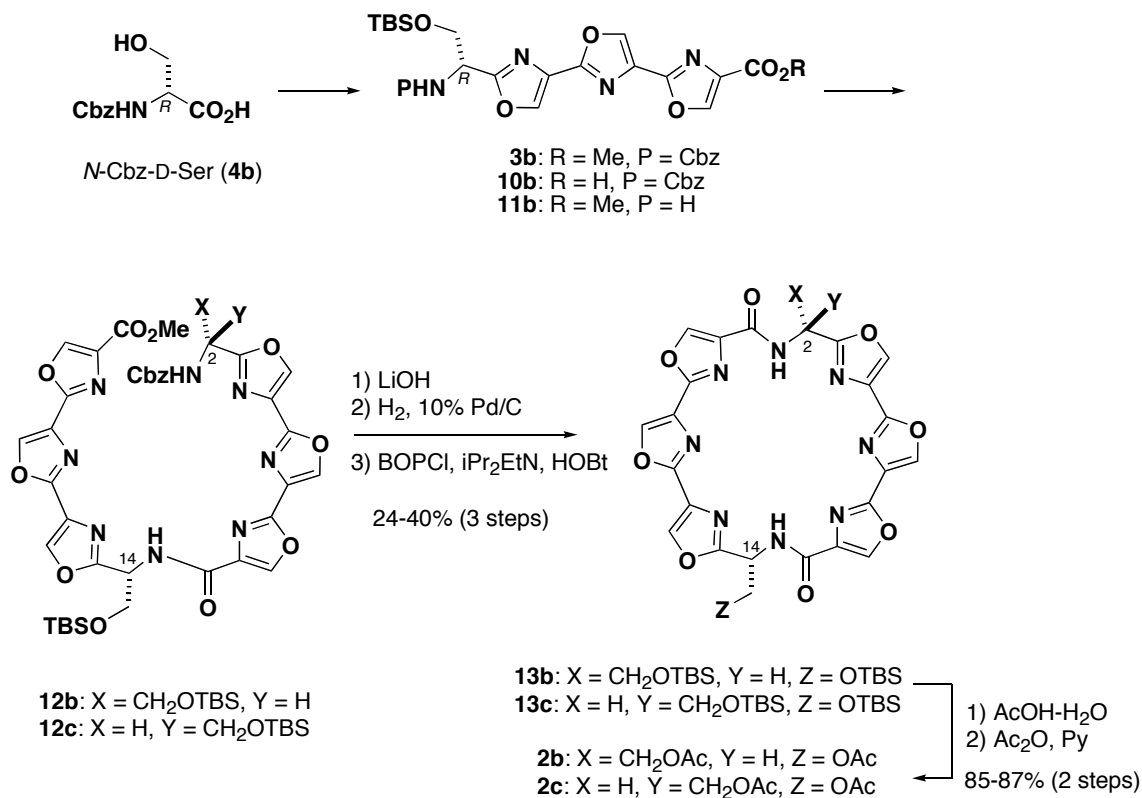
Trioxazole (**3a**) was synthesized from *N*-Cbz-L-serine (**4a**) (Scheme 1). Hydroxyl group of **4a** was protected with TBS ether and resulting carboxylic acid was reacted with L-serine methyl ester in the presence of 1-[3-(diethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxybenzotriazole (HOBt) and triethylamine to give amide (**5**) in 90% yield. Reaction of **5** with diethylaminosulfurtrifluoride (DAST) in CH_2Cl_2 at 0 °C gave oxazoline,⁷ which was subsequent treatment with bromotrichloromethane in the presence of DBU to give oxazole (**6**) in 77% yield from **5**.⁸ Deprotection of the TBS and Cbz groups of **6** gave aminoalcohol (**7**). At the same time, methyl ester of **6** was hydrolyzed with lithium hydroxide to give carboxylic acid (**8**). These two segments, (**7**) and (**8**), were coupled in the presence of EDCI and DMAP to give amide (**9**) in 72% from **6**.

Hydroxymethylamide moiety of **9** was converted into oxazole with DAST and subsequent reaction with bromotrichloromethane and DBU to give trioxazole (**3a**) in 89%.



Scheme 1. Synthesis of Trioxazole (**3a**).

Macrocyclic bisamide [(2*S*,14*S*)-**2a**] was synthesized from the trioxazole (**3a**) (Scheme 2). Methyl ester and Cbz groups of **3a** was removed to give carboxylic acid (**10a**) and amine (**11a**), respectively. These two segments were reacted with EDCI in the presence of HOBT and triethylamine to give linear bis-trioxazole (**12a**). After hydrolysis of ester group and deprotection of Cbz group of **12a**, resulting amino acid was subjected to the macrocyclization reaction using *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) and diisopropylethylamine under the high dilution condition (1 mM) in dichloromethane-DMF to give macrocyclic bisamide (**13a**) in 47% yield from **12a**. The TBS ether was exchanged to acetate to give bisamide acetate [(2*S*,14*S*)-**2a**] in 87% yield.

Scheme 2. Synthesis of Bistrioxazole Acetate (**2a**).Scheme 3. Synthesis of Bistrioxazole Acetate (**2b**) and (**2c**).

The macrocyclic bisamide acetate [(2*R*,14*R*)-**2b**] was synthesized from *N*-Cbz-D-serine (**4b**) by the same way as **2a** via **3b** and **12b** (Scheme 3). Meso bisamide acetate (**2c**) was also synthesized from carboxylic acid (**10b**) and amine (**11a**). These two segments were efficiently converted into **2c** via **12c** (Scheme 3).

Designed macrocyclic bisamide compounds (**2a-2c**) in hand, telomerase inhibitory activity of these derivatives were evaluated by TRAP assay. Among them, **2a**, which was derived from *N*-Cbz-L-serine (**4a**), showed telomerase inhibitory activity with an IC₅₀ of 2 μM⁹. On the other hand, **2b** and **2c** did not show inhibitory activity at the concentration of 10 μM.

In conclusion, we have designed and synthesized macrocyclic bisamide type telomestatin derivatives (**2a-2c**). Biological activity of these derivatives was examined, and we found that the only **2a** showed telomerase inhibitory activity with an IC₅₀ of 2 μM. These results suggest that the asymmetric centers in derivatives (**2**) importantly affect the interaction with G-quartet. Additional structural development focusing on functional group at hydroxylmethyl moiety in **2a** are in progress.

EXPERIMENTAL

General: Flash chromatography was performed using Silica gel 60 (spherical, particle size 0.040-0.100 mm; Kanto Co., Inc., Japan). Optical rotations were measured with a JASCO DIP polarimeter 370. ¹H and ¹³C NMR spectra were recorded on JEOL EX300, ECA/ECX400 instruments. Mass spectra were recorded on JEOL JMS-T100LC spectrometer.

Oxazole (6): To a solution of **5** (34.2 g, 75.4 mmol) in CH₂Cl₂ (100 mL) was added DAST (13 mL, 98.0 mmol) at 0 °C under N₂, and the mixture was stirred for 10 min. To the reaction mixture was added sat-NaHCO₃, and the organic layer was extracted with CH₂Cl₂. The extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ (75 mL). To the solution was added DBU (24 mL, 147.2 mmol) and BrCCl₃ (14.6 mL, 147.2 mmol) at 0 °C under N₂, and the mixture was stirred at rt for 1.5 h. H₂O was added to the reaction mixture at 0 °C, and organic layer was extracted with CH₂Cl₂. The extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel (hexane-AcOEt = 8:1) to give oxazole (**6**) as a pale yellow oil (24.7 g, 77%). [α]_D²⁵ = -7.4 (c 2.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.19 (s, 1H), 7.36 (m, 5H), 5.77-5.75 (d, *J* = 8.3 Hz, 1H), 5.17-5.07 (m, 3H), 4.11-4.07 (dd, *J* = 3.7, 11.7 Hz, 1H), 3.96-3.92 (m, 4H), 0.79 (s, 9H), -0.03 (s, 3H), -0.06 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 163.2, 161.2, 155.6, 144.0, 135.9, 133.2, 128.3, 128.0, 128.0, 67.0, 64.1, 52.1, 51.3, 25.4, 17.9, -5.8; HRMS (ESI, M+Na) calcd for C₂₁H₃₀N₂O₆SiNa 457.1771, found 457.1758.

Amide (9): To a solution of oxazole (**6**) (122 mg, 0.281 mmol) in THF (2 mL) in a polypropylene tube, was added HF-pyridine complex (0.1 mL) at 0 °C. After stirring at rt for 10 h, sat- NaHCO₃ and solid

NaHCO₃ was added. The mixture was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexane-AcOEt = 1:4) to afford alcohol (91 mg, 99%) as a clear oil. To a solution of the alcohol in MeOH (5 mL) was added 10% Pd/C and the reaction mixture was stirred at rt under an atmosphere of hydrogen gas (balloon). After 8 h, the reaction mixture was filtered through a pad of Celite and eluted with MeOH. The solution was concentrated *in vacuo* to give aminoalcohol (**7**) (40 mg), which was used without further purification. To a solution of oxazole (**6**) (116 mg, 0.266 mmol) in THF-H₂O (3:1, 2.6 mL), was added LiOH (13 mg, 0.532 mmol) at 0 °C. After stirring at rt for 1 h, Dowex[®] 50WX4 ion-exchange resin was added. The reaction mixture was filtered through a cotton with MeOH and the filtrates were concentrated *in vacuo* to give carboxylic acid (**8**) (110 mg), which was used without further purification. To a solution of aminoalcohol (**7**) and carboxylic acid (**8**) in DMF (5 mL) was added DMAP (65 mg, 0.532 mmol) and EDCI (102 mg, 0.532 mmol) at rt. After stirring for 18 h, H₂O was added to the reaction mixture, and the mixture was extracted with AcOEt. The organic layer was washed with H₂O and brine, dried over MgSO₄, and concentrated *in vacuo*. The resulting residue was purified on silica gel (CHCl₃-AcOEt = 1:3) to give amide (**9**) (113 mg, 72%, 3 steps) as a colorless oil. $[\alpha]_D^{25} = -19.4$ (c 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.19 (s, 1H), 8.12 (s, 1H), 7.91-7.88 (d, *J* = 8.6 Hz, 1H), 7.36 (m, 5H), 5.86-5.83 (d, *J* = 8.6 Hz, 1H), 5.51-5.45 (dt, *J* = 4.2, 8.6 Hz, 1H), 5.18-5.09 (m, 2H), 5.03-4.98 (dt, *J* = 3.7, 8.6 Hz, 1H), 4.27-4.24 (m, 1H), 4.05-3.89 (m, 6H), 0.788 (s, 9H), -0.02 (s, 3H), -0.06 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 162.7, 162.4, 161.3, 160.4, 155.8, 144.4, 141.8, 136.0, 135.4, 133.1, 128.5, 128.2, 67.3, 63.9, 63.0, 52.1, 51.2, 48.6, 25.5, 18.1, -5.7; HRMS (ESI, M+Na) calcd for C₂₇H₃₆N₄O₉SiNa 611.2149, found 611.2147.

Trioxazole (**3a**): To a solution of amide (**9**) (550 mg, 0.935 mmol) in CH₂Cl₂ (10 mL) was added DAST (136 μL, 1.03 mmol) at 0 °C under N₂, and the mixture was stirred for 10 min. To the reaction mixture was added sat-NaHCO₃, and organic layer was extracted with CH₂Cl₂. The extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was dissolved with CH₂Cl₂ (9.5 mL), and to the resulting solution was added DBU (0.38 mL, 2.34 mmol) and BrCCl₃ (0.23 mL, 2.34 mmol) at 0 °C, and the mixture was stirred for 4 h at rt. The reaction mixture was cooled at 0 °C, and H₂O was added. The organic layer was extracted with CH₂Cl₂, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was chromatographed on silica gel (hexane-AcOEt = 1:1) to give oxazole (**3a**) as a pale yellow powder (403 mg, 77%, 2 steps). $[\alpha]_D^{25} = -11.2$ (c 1.3, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.42 (s, 1H), 8.32 (s, 2H), 7.36 (m, 5H), 5.81-5.79 (d, *J* = 8.8 Hz, 1H), 5.19-5.10 (m, 3H), 4.15-4.10 (d, *J* = 3.7, 10.1 Hz 1H), 4.00-3.98 (br, 1H), 3.97-3.95 (m, 4H), 0.78 (s, 9H), -0.03 (s, 3H), -0.08 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 164.0, 161.3, 156.0, 155.8, 155.4, 143.9, 143.8, 139.6, 139.4, 139.3, 136.0, 134.3, 130.8, 129.9, 128.5, 128.2, 67.3, 64.3, 52.4, 51.5, 25.5, 18.1, -5.6; HRMS (ESI, M+Na) calcd for C₂₇H₃₂N₄O₈SiNa 591.1887, found 591.1886.

Bis-trioxazole (**12a**): To a solution of trioxazole (**3a**) (119 mg, 0.210 mmol) in MeOH-AcOEt (1:1, 8 mL) was added 10% Pd/C and the reaction mixture was stirred at rt under an atmosphere of hydrogen gas (balloon). After 3 h, the reaction mixture was filtered through a pad of Celite and eluted with MeOH. The solution was concentrated *in vacuo* to give amine (**11a**), which was used without further purification. To a solution of trioxazole (**3a**) (119 mg, 0.211 mmol) in THF-H₂O = 3:1 (3 mL), was added LiOH (17.7 mg, 0.422 mmol) at 0 °C. After stirring at rt for 45 min, Dowex[®] 50WX4 ion-exchange resin was added. The reaction mixture was filtered through a cotton with MeOH, and the filtrates were concentrated *in vacuo* to give carboxylic acid (**10a**), which was used without further purification. To a solution of amine (**11a**) and carboxylic acid (**10a**) in DMF (4 mL) was added EDCI (81 mg, 0.420 mmol), HOBT (29 mg, 0.210 mmol) and Et₃N (59 μ L, 0.420 mmol), and the mixture was stirred at rt. After stirring for 24 h, the reaction was quenched with H₂O and the reaction mixture was extracted with AcOEt. The organic layer was washed with H₂O and brine, dried over MgSO₄, and concentrated *in vacuo*. The resulting residue was purified on silica gel (CHCl₃- AcOEt = 4:1) to give bis-trioxazole (**12a**) (152 mg, 0.156 mmol, 74%, 3 steps). $[\alpha]_D^{25} = +5.5$ (*c* 1.6, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.40 (s, 1H), 8.31-8.26 (m, 5H), 7.85-7.82 (d, *J* = 8.8 Hz, 1H) 7.32 (m, 5H), 5.85-5.82 (d, *J* = 8.8 Hz, 1H), 5.53-5.49 (dt, *J* = 4.6, 8.5 Hz, 1H), 5.16-5.06 (m, 3H), 4.22-4.18 (dd, *J* = 4.4, 10.1 Hz, 1H), 4.11-4.03 (m, 2H), 3.98-3.90 (m, 4H), 0.81 (s, 9H), 0.75 (s, 9H), 0.00 (s, 3H), -0.03 (s, 3H), -0.06 (s, 3H), -0.09 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 163.9, 163.3, 161.1, 159.8, 156.0, 155.9, 155.7, 155.3, 154.4, 143.8, 141.5, 139.6, 139.3, 139.0, 136.6, 135.9, 134.2, 130.8, 130.6, 129.9, 129.8, 128.4, 128.0, 67.1, 64.2, 64.0, 52.3, 51.8, 49.1, 25.5, 18.0, -5.7; HRMS (ESI, M+Na) calcd for C₄₅H₅₄N₈O₁₃Si₂Na 993.3246, found 993.3213

Macrocyclic bisamide (**13a**): A mixture of bis-trioxazole (**12a**) (151 mg, 0.156 mmol) and lithium hydroxide (16 mg, 0.390 mmol) in THF-H₂O (3:1, 6 mL) was stirred at rt for 1.5 h. To the reaction mixture was added Dowex[®] 50WX4 ion-exchange resin. The reaction mixture was filtered through a cotton with MeOH, and the filtrates were concentrated *in vacuo* to give carboxylic acid. The residue was dissolved in MeOH-AcOEt solution (1:1, 20 mL), and 10% Pd/C was added. The mixture was stirred at rt under hydrogen (balloon) for 20 h. The reaction mixture was filtered through a pad of Celite and the filtrates were concentrated *in vacuo*. To a solution of the residue in DMF-CH₂Cl₂ (1:2, 156 mL) was added DMAP (381 mg, 1.56 mmol), HOBT (211 mg, 1.56 mmol), diisopropylethylamine (265 μ L, 1.56 mmol), and BOPCl (397 mg, 1.56 mmol). The resulting mixture was stirred for another 2 days. To the reaction mixture was added H₂O and the organic layer was extracted with AcOEt. The extracts were washed with brine, dried over MgSO₄, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column (CHCl₃:MeOH = 50:1) to give **13a** as an oil (59 mg, 47%). $[\alpha]_D^{25} = +18.7$ (*c* 3.0, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 8.50-8.49 (d, *J* = 7.4 Hz, 2H), 8.21-8.18 (m, 6H), 5.37-5.36 (br, 2H), 4.16-4.13 (dd, *J* = 3.6, 9.6 Hz, 2H), 3.97-3.93 (dd, *J* = 6.9, 9.6 Hz, 2H), 0.786 (s, 18H), -0.0265

(s, 6H), -0.0677 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.7, 159.8, 156.0, 154.6, 140.8, 139.1, 138.4, 136.7, 130.9, 129.7, 64.6, 50.2, 25.6, 18.1, -5.64; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{36}\text{H}_{44}\text{N}_8\text{O}_{10}\text{Si}_2\text{Na}$ 827.2617, found 827.2659.

Macrocyclic bisamide acetate (**2a**): Macrocyclic bis-amide (**13a**) (17 mg, 0.0221 mmol) was dissolved in AcOH-MeOH (4:1, 2.5 mL). After stirring for 40 h at rt, the mixture was concentrated *in vacuo* to give diol. To a solution of the diol in pyridine (0.5 mL) was added Ac_2O (0.5 mL) at rt for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified on silica gel (CHCl_3 -MeOH = 50:1) to give macrocyclic bis-amide acetate (**2a**) (13 mg, 87%, 2 steps). $[\alpha]_D^{25} = +42.3$ (*c* 0.1, CHCl_3 -MeOH = 3:1); ^1H NMR (400 MHz, DMSO *d*-6) δ 9.16-9.15 (br, 4H), 8.98 (s, 2H), 8.35-8.33 (d, $J = 5.9$ Hz), 5.68 (br, 2H), 4.66-4.64 (d, $J = 9.2$ Hz, 2H), 4.50-4.47 (d, $J = 10.1$ Hz, 2H), 1.94 (s, 6H); ^{13}C NMR (100 MHz, DMSO *d*-6) δ 170.1, 161.8, 159.0, 155.5, 154.6, 142.8, 142.4, 141.3, 135.8, 129.8, 128.6, 63.1, 47.5, 20.4; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{28}\text{H}_{20}\text{N}_8\text{O}_{12}\text{Na}$ 683.1098, found 683.1148.

Trioxazole (**3b**): $[\alpha]_D^{25} = +9.9$ (*c* 0.8, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.43 (s, 1H), 8.32 (s, 2H), 7.36 (m, 5H), 5.79-5.76 (d, $J = 8.4$ Hz, 1H), 5.20-5.10 (m, 3H), 4.13-4.10 (m, 1H), 4.00-3.95 (m, 4H), 0.79 (s, 9H), -0.02 (s, 3H), -0.06 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 164.0, 161.3, 156.0, 155.8, 155.4, 143.9, 143.8, 139.6, 139.4, 139.3, 136.0, 134.4, 130.8, 129.9, 128.5, 128.2, 67.3, 64.3, 52.5, 51.5, 25.6, 18.1, -5.7; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{27}\text{H}_{32}\text{N}_4\text{O}_8\text{SiNa}$ 591.1887, found 591.1867.

Bis-trioxazole (**12b**): $[\alpha]_D^{25} = -7.0$ (*c* 0.9, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.42 (s, 1H), 8.34-8.28 (m, 5H), 7.86-7.83 (d, $J = 8.8$ Hz, 1H), 7.37 (m, 5H), 5.80-5.77 (d, $J = 8.4$ Hz, 1H), 5.58-5.52 (dt, $J = 4.6$, 8.6 Hz, 1H), 5.20-5.10 (m, 3H), 4.26-4.21 (dd, $J = 4.4$, 10.1 Hz, 1H), 4.15-4.06 (m, 2H), 4.01-3.95 (m, 4H), 0.84 (s, 9H), 0.79 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H), -0.02 (s, 3H), -0.05 (s, 3H); ^{13}C NMR (75 MHz, CDCl_3) δ 164.0, 163.4, 161.3, 159.9, 156.1, 156.0, 155.8, 155.4, 154.5, 143.9, 141.6, 139.6, 139.4, 139.1, 136.7, 136.0, 134.4, 130.9, 130.8, 130.0, 129.9, 128.5, 128.2, 67.3, 64.3, 64.1, 52.2, 51.5, 49.2, 25.6, 18.1, -5.6; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{45}\text{H}_{54}\text{N}_8\text{O}_{13}\text{Si}_2\text{Na}$ 993.3246, found 993.3216.

Macrocyclic bisamide (**13b**): $[\alpha]_D^{25} = -18.8$ (*c* 1.5, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.52-8.50 (d, $J = 7.3$ Hz, 2H), 8.22 (s, 2H), 8.19 (s, 2H), 8.18 (s, 2H), 5.40-5.35 (dd, $J = 6.4$, 11.4 Hz, 2H), 4.17-4.13 (dd, $J = 4.1$, 9.6 Hz, 2H), 3.98-3.93 (dd, $J = 6.9$, 9.9 Hz, 2H), 0.80 (s, 18H), -0.02 (s, 6H), -0.06 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.9, 160.0, 156.2, 154.8, 141.0, 139.3, 138.5, 137.0, 131.1, 129.9, 64.6, 50.4, 25.8, 18.3, -5.4; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{36}\text{H}_{44}\text{N}_8\text{O}_{10}\text{Si}_2\text{Na}$ 827.2617, found 827.2585.

Macrocyclic bisamide acetate (**2b**): $[\alpha]_D^{25} = -58.1$ (*c* 0.2, CHCl_3 -MeOH = 3:1); ^1H NMR (400 MHz, DMSO *d*-6) δ 9.17-9.15 (br, 4H), 8.98 (s, 2H), 8.35-8.33 (d, $J = 6.9$ Hz, 2H), 5.68 (br, 2H), 4.67-4.63 (dd, $J = 3.7$, 11.5 Hz, 2H), 4.50-4.46 (m, 2H), 1.94 (s, 6H); ^{13}C NMR (100 MHz, DMSO *d*-6) δ 169.7, 161.7, 158.8, 155.3, 154.4, 142.3, 141.9, 140.8, 135.7, 129.6, 128.5, 63.0, 47.2, 20.1; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{28}\text{H}_{20}\text{N}_8\text{O}_{12}\text{Na}$ 683.1098, found 683.1106.

Bis-trioxazole (**12c**): $[\alpha]_D^{25} = +28.3$ (c 2.2, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 8.41 (s, 1H), 8.33-8.29 (m, 5H), 7.85-7.82 (d, $J = 8.7$ Hz, 1H), 7.35 (m, 5H), 5.81-5.79 (d, $J = 8.7$ Hz, 1H), 5.56-5.51 (m, 1H), 5.17-5.09 (m, 3H), 4.23-4.19 (m, 1H), 4.13-4.05 (m, 2H), 3.99-3.95 (m, 1H), 3.92 (s, 3H), 0.814 (s, 9H), 0.766 (s, 9H), 0.0125 (s, 3H), -0.015 (s, 3H), -0.0414 (s, 3H), -0.0769 (s, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.9, 163.4, 161.2, 159.8, 156.1, 155.9, 155.8, 155.3, 154.4, 143.8, 141.5, 139.6, 139.3, 139.0, 136.6, 136.0, 134.3, 130.9, 130.7, 129.9, 129.8, 128.4, 128.2, 128.1, 67.2, 64.2, 64.0, 52.3, 51.6, 49.2, 25.5, 18.1, -5.61; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{45}\text{H}_{54}\text{N}_8\text{O}_{13}\text{Si}_2\text{Na}$ 993.3246, found 993.3229.

Macrocyclic bisamide (**13c**): ^1H NMR (400 MHz, CDCl_3) δ 8.52-8.51 (d, $J = 7.3$ Hz, 2H), 8.23 (s, 2H), 8.20 (s, 2H), 8.19 (s, 2H), 5.41-5.36 (dd, $J = 6.9, 11.5$ Hz, 2H), 4.18-4.14 (dd, $J = 4.1, 9.6$ Hz, 2H), 3.98-3.94 (dd, $J = 6.8, 10.0$ Hz, 2H), 0.80 (s, 18H), -0.01 (s, 6H), -0.051 (s, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.8, 159.9, 156.1, 154.6, 140.8, 139.1, 138.3, 136.8, 130.9, 129.7, 64.4, 50.2, 25.7, 18.1, -5.59; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{36}\text{H}_{44}\text{N}_8\text{O}_{10}\text{Si}_2\text{Na}$ 827.2617, found 827.2640.

Macrocyclic bisamide acetate (**2c**): ^1H NMR (400 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD} = 3 : 1$) δ 8.27 (s, 2H), 8.23 (s, 2H), 8.20 (s, 2H), 5.46-5.44 (dd, $J = 3.2, 7.3$ Hz, 2H), 4.50-4.46 (m, 2H), 4.38-4.34 (m, 2H), 1.83 (s, 6H); ^{13}C NMR (100 MHz, $\text{CDCl}_3\text{-CD}_3\text{OD} = 3 : 1$) δ 170.7, 161.4, 160.1, 155.9, 154.6, 141.7, 140.3, 139.1, 135.9, 130.1, 129.2, 63.4, 47.3, 20.0; HRMS (ESI, $\text{M}+\text{Na}$) calcd for $\text{C}_{28}\text{H}_{20}\text{N}_8\text{O}_{12}\text{Na}$ 683.1098, found 683.1123.

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REFERENCES (AND NOTES)

1. a) K. Shin-ya, K. Wierzba, K. Matsuo, T. Ohtani, Y. Yamada, K. Furihata, Y. Hayakawa, and H. Seto, *J. Am. Chem. Soc.*, 2001, **123**, 1262. b) K. Shin-ya, *Biosci. Biotechnol. Biochem.*, 2005, **69**, 867.
2. Studies of telomestatin (**1**), see: a) M. Kim, H. Vankayalapati, K. Shin-ya, K. Wierzba, and L. H. Hurley, *J. Am. Chem. Soc.*, 2002, **124**, 2098. b) L. Zhang, K. Tamura, K. Shin-ya, and H. Takahashi, *Biochem. Biophys. Acta*, 2006, **1763**, 39. c) R. De Armond, S. Wood, D. Sun, L. H. Hurley, and S. W. Ebbinghaus, *Biochemistry*, 2005, **44**, 16341. d) H. Tahara, K. Shin-ya, H. Seimiya, H. Yamada, T. Tsuruo, and T. Ide, *Oncogene*, 2006, **25**, 1955. e) N. Binz, T. Shalaby, P. Rivera, K. Shin-ya, and M. A. Grotzer, *Eur. J. Cancer*, 2005, **41**, 2873. f) D. Sun, K. Kuo, J. J. Rusche, and L. H. Hurley, *Nucleic Acids Res.*, 2005, **33**, 6070. g) E. M. Rezler, J. Seenisamy, S. Bashyam, M. Y. Kim, E. White, W. D. Wilson, and L. H. Hurley, *J. Am. Chem. Soc.*, 2005, **127**,

9439. h) T. Lemarteleur, D. Gomez, R. Paterski, E. Mandine, P. Mailliet, and J. F. Riou, *Biochem. Biophys. Res. Commun.*, 2004, **323**, 802. i) D. Gomez, R. Paterski, T. Lemarteleur, K. Shin-ya, J. L. Mergny, and J. F. Riou, *J. Biol. Chem.*, 2004, **279**, 41487. j) M. Sumi, T. Tauchi, G. Sashida, A. Nakajima, A. Gotoh, K. Shin-ya, J. H. Ohyashiki, and K. Ohyashiki, *Int. J. Oncol.*, 2004, **24**, 1481. k) M. A. Shammash, R. J. Shmookler. Reis, C. Li, H. Koley, L. H. Hurley, K. C. Anderson, and N. C. Munshi, *Clin. Cancer Res.*, 2004, **10**, 770. l) D. Gomez, T. Lemarteleur, L. Lacroix, P. Mailliet, J. L. Mergny, and J. F. Riou, *Nucleic Acids Res.*, 2004, **32**, 371. m) F. Rosu, V. Gabelica, K. Shin-ya, and E. De Pauw, *Chem. Commun.*, 2003, **7**, 2702. n) D. Gomez, N. Aouali, A. Renaud, C. Douarre, K. Shin-ya, J. Tazi, S. Martines, C. Trentesaux, H. Morjani, and J. F. Riou, *Cancer Res.*, 2003, **63**, 6149. o) T. Tauchi, K. Shin-ya, G. Sashida, M. Sumi, A. Nakajima, T. Shimamoto, J. H. Ohyashiki, and K. Ohyashiki, *Oncogene*, 2003, **22**, 5338. p) M. Y. Kim, M. Gleason-Guzman, E. Izbicak, D. Nishioka, and L. H. Hurley, *Cancer Res.*, 2003, **63**, 3247. q) A. Nakajima, T. Tauchi, G. Sashida, M. Sumi, K. Abe, K. Yamamoto, J. H. Ohyashiki, and K. Ohyashiki, *Leukemia*, 2003, **17**, 560.
3. For recent reviews, see: a) S. Neidle and G. Parkinson, *Nature Reviews Drug Discovery*, 2002, **1**, 383. b) L. R. Kelland, *Eur. J. Cancer*, 2005, **41**, 971. c) S. Neidle and M. A. Read, *Biopolymers*, 2001, **56**, 195.
4. Representative G-quadruplex interactive reagents: a) T. S. Dexheimer, D. Sun, and L. H. Hurley, *J. Am. Chem. Soc.*, 2006, **128**, 5404; b) C. Douarre, D. Gomez, H. Morjani, J. M. Zahm, M. F. Odonohue, L. Eddabra. P. Mailliet, J. F. Riou, and C. Trentesaux, *Nucleic Acids Res.*, 2005, **33**, 2192; c) W. Tuntiwechapikul, T. Taka, M. Bethencourt, L. Makonkawkeyoon, and T. R. Lee, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4120; d) A. M. Burger, F. Dai, C. M. Schultes, A. P. Reszka, M. J. Moore, J. A. Double, and S. Neidle, *Cancer Res.*, 2006, **65**, 1489.
5. Synthetic studies on telomestatin, see: a) N. Endoh, K. Tsuboi, R. Kim, Y. Yonezawa, and C. Shin, *Heterocycles*, 2003, **60**, 1567. b) S. K. Chattopadhyay, S. Biswas, and B. K. Pal, *Synthesis*, 2006, 1289. c) J. M. Atkins, and E. Vedejs, *Org. Lett.*, 2005, **7**, 3351. d) J. Deeley and G. Pattenden, *Chem. Commun.*, 2005, 797. e) E. S. Baker, J. T. Lee, J. L. Sessler, and M. T. Bowers, *J. Am. Chem. Soc.*, 2006, **128**, 2641.
6. Quite recently, Rice and co-workers reported similar type of telomestatin derivatives of **2**. G. S. Minhas, D. S. Pilch, J. E. Kerrigan, E. J. LaVoie, and J. E. Rice, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3891.
7. a) G. Burrell, J. M. Evans, G. E. Jones, and G. Stemp, *Tetrahedron Lett.*, 1990, **31**, 3649. b) A. J. Phillips, Y. Uto, P. Wipf, M. J. Reno, and D. R. Williams, *Org. Lett.*, 2000, **2**, 1165.
8. D. R. Williams, D. J. Lowder, Y. G. Gu, and D. A. Brooks, *Tetrahedron Lett.*, 1997, **38**, 331.
9. Inhibitory effects against telomerase were estimated by using TeloChaser assay kit.