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PAPER

(*S*)-Stereoisomer of telomestatin as a potent G-quadruplex binder and telomerase inhibitor†

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Total synthesis of the (*S*)-stereoisomer of telomestatin (**1**) was accomplished. (*S*)-Telomestatin exhibited potency four times that of the natural product, (*R*)-telomestatin, which was the most potent telomerase inhibitor previously reported. In the circular dichroism spectral analysis of the complexes possessing randomly structured single-stranded d[TTAGGG]$_4$ oligonucleotide, (*S*)-telomestatin, like (*R*)-telomestatin, induced an antiparallel G-quadruplex structure. The melting temperature (T_m) value of the (*S*)-isomer complex was greater than that of the (*R*)-telomestatin complex. Therefore, it is concluded that the stereochemistry of the thiazoline of telomestatin is important to the binding ability of a G-quadruplex binder, and (*S*)-telomestatin as a G-quadruplex binder is more potent than the natural product.

Introduction

Telomestatin (*R*)-**1**, isolated from *Streptomyces anulatus* 3533-SV4, possesses a unique structure comprising eight continuous heterocyclic rings, such as a thiazoline, two methyloxazoles, and five oxazoles in a macrocycle, and it exhibits extremely potent telomerase inhibitory activity.[1] Telomestatin binds itself to the G-quadruplex basket structure of telomere, composed of repeated (TTAGGG)$_n$ sequences, to stabilize the antiparallel G-quadruplex form,[2] inhibiting the telomerase that elongates telomere sequences.[3] Since most cancer cells rely on telomerase for their survival, it is an attractive target in anticancer drug design.[4] Studies on G-quadruplex binding molecules as telomerase inhibitors revealed that they exhibited potential anticancer activity.[4,5] Recently, we observed that a macrocyclic heptaoxazole comprising an amino group side chain selectively interacted with telomere to induce a conformational change to an antiparallel G-quadruplex structure, and a more planar heptaoxazole derivative exhibits more potent G-quadruplex binding ability than hexaoxazole derivatives.[6] Therefore, it is important that the macrocyclic structure of telomestatin comprises seven continuous oxazole rings. We also reported the total synthesis of telomestatin (*R*)-**1** and determined the absolute configuration of the natural product.[7,8] Next we investigated the effect of the stereocentre of the thiazoline in telomestatin on the binding ability to the G-quadruplex structure of the telomere. We synthesized the (*S*)-stereoisomer of telomestatin, and based on the melting temperature (T_m) values of ss-telo24 G-quadruplex structure and a telomeric repeat-amplification protocol (TRAP) assay, we observed that the enantiomer is more potent than the natural product (Fig. 1). During total synthesis of the (*S*)-stereoisomer, significant epimerization of the modified cysteine residue was observed in the macrolactamization, though the stereogenic center and the activated ester moiety are connected through conjugation by a trisoxazole unit.

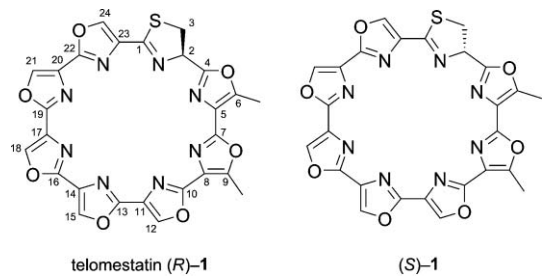

Fig. 1 Structures of telomestatin (*R*)-**1** and its enantiomer (*S*)-**1**.

Results and discussion

In our previous synthesis of (*R*)-**1**,[7] we coupled (*R*)-**2** and (*S*)-**3** using PyBroP[9] to obtain (2*R*,14*S*)-**4** (Scheme 1). Removal

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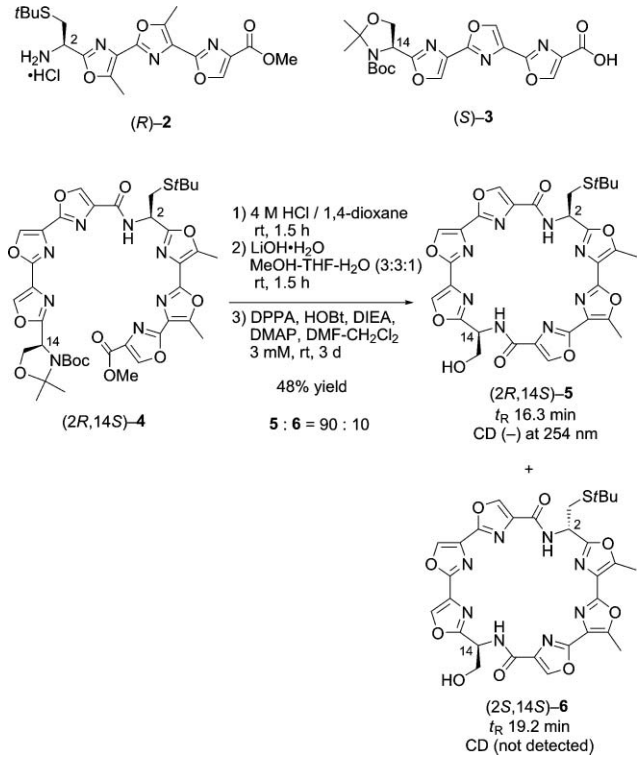
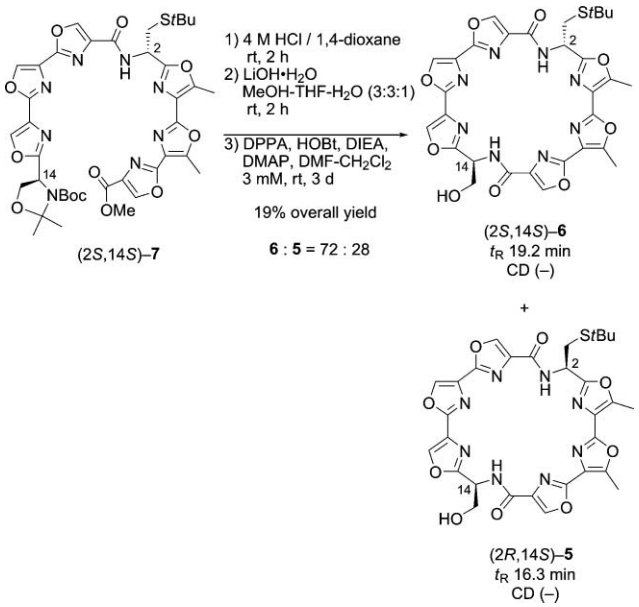
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra of (2*S*,14*S*)–**7**, (2*S*,14*S*)–**6**, (2*S*,14*R*)–**5**, (*S*)–**8**, ^{1}H–^{1}H COSY spectrum of (*S*)–**8**, and ^{1}H NMR spectrum of (*S*)–**1**. See DOI: 10.1039/c0ob00513d



Scheme 1 Macrolactamization of (2*R*,14*S*)-**4** in the synthesis of (*R*)-**1**.

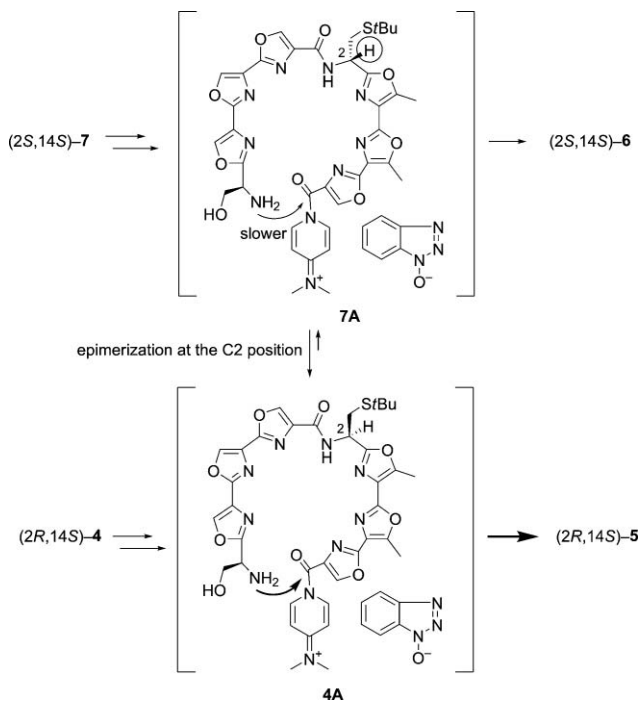
of the acetonide and Boc groups with acid, basic hydrolysis of the methyl ester, and macrolactamization using DPPA,[10] 1-hydroxybenzotriazole (HOBt), *N*,*N*-diisopropylethylamine (DIEA), and 4-(dimethylamino)pyridine (DMAP) proceeded smoothly, leading to the desired (2*R*,14*S*)-**5** in 48% yield. In the reaction, we observed the formation of a small amount of its diastereomer, (2*S*,14*S*)-**6**. The ratio of (2*R*,14*S*)-**5** to (2*S*,14*S*)-**6** was 90 : 10, as determined by high-performance liquid chromatography (HPLC) peak area (UV 214 nm).

Initially, we attempted to synthesize (*S*)-**1** utilizing (*S*)-**2** instead of (*R*)-**2** as the coupling unit. Acylation of (*S*)-**2** with (*S*)-**3** using PyBroP provided (2*S*,14*S*)-**7** in 86% yield. The acetonide and Boc groups were removed by treatment with acid, and methyl ester was hydrolyzed under basic conditions (Scheme 2). However, the macrolactamization did not proceed smoothly, and the product obtained in 3 d was a 72 : 28 mixture of diastereomers (2*S*,14*S*)-**6** and (2*R*,14*S*)-**5** in 19% combined yield. As epimerization was not observed either before or after the cyclization reaction, the epimerization should proceed during the macrolactamization.[11] To identify which stereocentre was epimerized, we measured the HPLC-circular dichroism (CD) of the cyclized products obtained from both (2*R*,14*S*)-**4** and (2*S*,14*S*)-**7** (Schemes 1 and 2). The former products (Scheme 1) included **5** at retention time (t_R) 16.3 min and **6** at t_R 19.2 min in a ratio of 90 : 10 (UV 214 nm). The major product obtained was (2*R*,14*S*)-**5**, which exhibited a (–) CD signal (UV 254 nm).[12] In the HPLC-CD analysis of the products obtained from (2*S*,14*S*)-**7** (Scheme 2), the same peaks were observed in a ratio of 28 : 72 (UV 214 nm) for **5** and **6** described above, and exhibited (–) CD signals, respectively (UV 254 nm). As both peaks for **5** at t_R 16.3 min exhibited (–) CD signals, the stereochemistry of the minor diastereomer **5** obtained from (2*S*,14*S*)-**7** (Scheme 2) was identical with (2*R*,14*S*)-**5** in Scheme 1. As a result, it was found



Scheme 2 Macrolactamization of (2*S*,14*S*)-**7**.

that the significant epimerization occurred at the C2 position during the macrolactamization. In the cyclization of intermediate **7A** produced from (2*S*,14*S*)-**7** (Scheme 3), the reaction proceeded slowly by conformational restriction and then the epimerization at the C2 position where the proton is rather acidic by conjugation with the activated ester formed at the C-terminus through a trisoxazole unit, would proceed leading to (2*R*,14*S*)-**5**. It should be noted that there was a significant difference in the reactivity between the cyclization intermediates **4A** and **7A**, whose single



Scheme 3 Process of the epimerization at the C2 position in the macrolactamization.

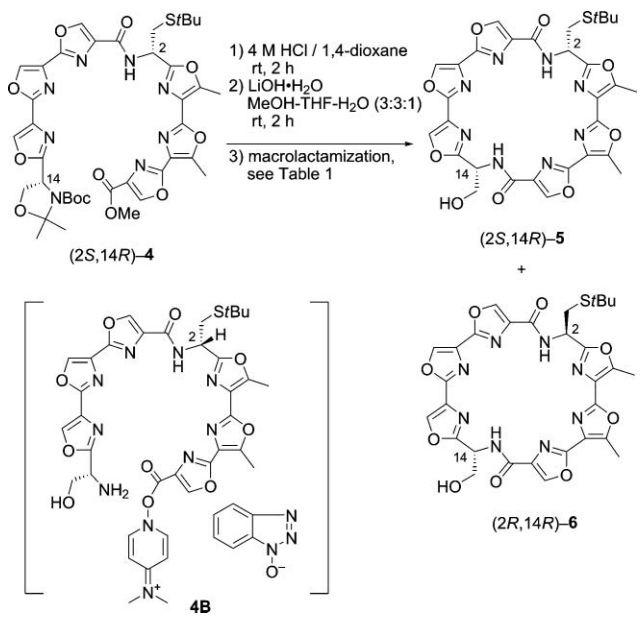
Table 1 Optimization of macrolactamization of (2*S*,14*R*)-**4**[a]

Entry	Reagents	Additive	Concentration	Yield (%)	Ratio of (2*S*,14*R*)-**5** : (2*R*,14*R*)-**6**
1	DPPA–HOBt	DMAP	3 mM	48	90 : 10
2	DPPA–HOBt	DMAP	1 mM	35	88 : 12
3	HATU	DMAP	3 mM	32	86 : 14
4	PyBOP–HOBt	DMAP	3 mM	16	84 : 16
5	DPPA–HOBt	—	3 mM	trace	—
6	DPPA–HOBt	DMAPO	3 mM	68	93 : 7

[a] The reaction was performed in CH_2Cl_2–DMF (1 : 1) at room temperature for 3 d.

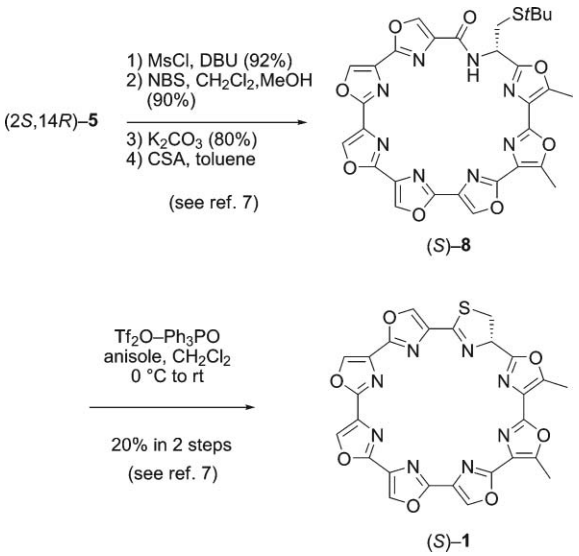
stereocentres are of differing absolute configurations. Therefore, we altered the synthetic route in the synthesis of (*S*)-**1**.

The cyclization precursor (2*S*,14*R*)-**4** (Scheme 4) was prepared from the coupling of (*S*)-**2** and acid (*R*)-**3**, the same as its enantiomer (2*R*,14*S*)-**4**.[7] After removal of the acetonide and Boc groups and hydrolysis of the methyl ester, the macrolactamization of (2*S*,14*R*)-**4** was investigated (Table 1). Higher dilution conditions (1 mM) were not effective in improving the yield and suppressing the epimerization (entry 2 *vs.* entry 1). The use of HATU[13] or PyBOP[14]–HOBt instead of DPPA–HOBt resulted in a lower yield and considerable epimerization (14–16%) was observed (entries 3 and 4). The reaction did not proceed in the absence of DMAP (entry 5). Interestingly, the addition of 4-(dimethylamino)pyridine oxide (DMAPO) (1 equiv) significantly increased the yield to 68% and suppressed the epimerization to 7% (entry 6). It has been reported that the use of 2-methyl-6-nitrobenzoic anhydride (MNBA)–DMAPO instead of MNBA–DMAP is quite effective in both macrolactonization and peptide coupling.[15] Our results represent that the addition of DMAPO is also effective for activation of the HOBt ester in the macrolactamization *via* formation of the intermediate **4B**. The **4B** is enough active for macrolactamization and the proton at the C2 position in **4B** could be less acidic than that in **4A**.



Scheme 4 Macrolactamization of (2*S*,14*R*)-**4**.

According to our previous report, the seventh oxazole ring was formed from (2*S*,14*R*)-**5** to provide (*S*)-**8**, which underwent thiazoline formation leading to (*S*)-**1** (Tf_2O/Ph_3PO/anisole/CH_2Cl_2) (Scheme 5).[7,16] (*S*)-**1** was isolated by reversed-phase HPLC and its ^{1}H NMR spectrum was measured by LC-NMR in solution right after purification because it formed complexes after concentration, probably due to intermolecular interaction on the basis of π–π stacking of the oxazole rings. The spectral data of (*S*)-**1** were identical to those of the natural product (*R*)-**1**, except for the opposite signal of its CD spectrum (Fig. 2).



Scheme 5 Total synthesis of (*S*)-**1**.

Because synthetic intermediates (*S*)-**8** and (*R*)-**8**[7] as well as telomestatin (*S*)-**1** and (*R*)-**1**[7] were available to us, their inhibitory activities against telomerase were evaluated by a modified TRAP assay with an internal standard.[1,17,18] Interestingly, (*S*)-**1** exhibited potent inhibitory activity with an IC_{50} value of 5 nM while the IC_{50} value of (*R*)-**1** was 20 nM (Table 2). The synthetic intermediate (*S*)-**8** is also equally as potent as the natural product (*R*)-**1**, whereas the potency of (*R*)-**8** decreased to one-fourth of that of (*R*)-**1**. The results are in good agreement with our previous observation that the compounds having seven continuous oxazole rings in a

Table 2 Inhibitory activity of synthetic compounds against telomerase

Compound	(*S*)-**1**	(*R*)-**1**	(*S*)-**8**	(*R*)-**8**
IC_{50} (nM)	5	20	20	80

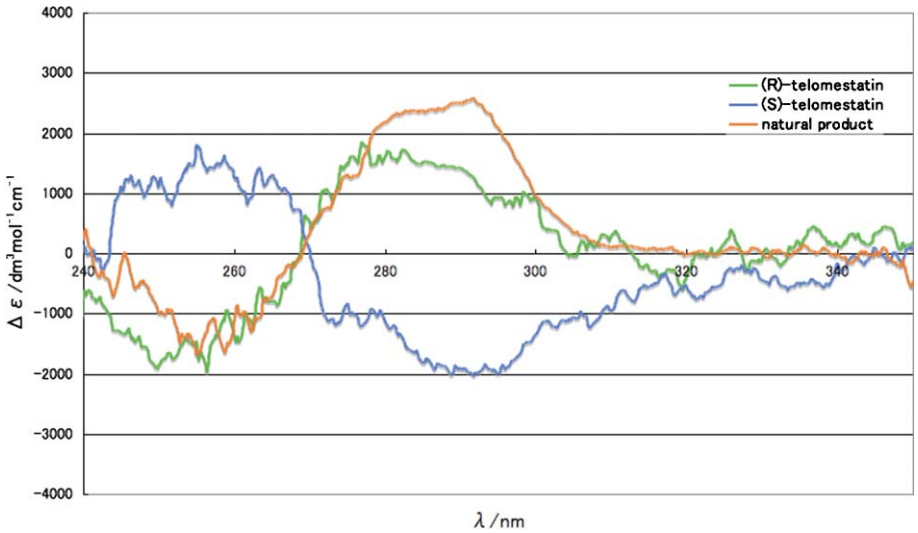


Fig. 2 CD spectra of telomestatins, natural product (orange), synthetic (*R*)-**1** (green), and (*S*)-**1** (blue).

macrocycle possess extremely potent inhibitory activity against telomerase.[6] Interestingly, (*S*)-stereoisomers **1** and **8** are more potent than the corresponding (*R*)-isomers; thus, suggesting that the stereochemistry at the C2 position of telomestatin probably controls the shape of the molecule binding to the G-quadruplex structure of telomere sequences.

Next, we carried out CD analyses using randomly structured, single-stranded d[TTAGGG]$_4$ 24-mer (ss-telo24) oligonucleotide to investigate the G-quadruplex structure induced by (*S*)-**1** and (*R*)-**1**. As previously reported, ss-telo24 was induced into an antiparallel G-quadruplex structure with (*R*)-**1** on the basis of CD spectra analysis, including 292 nm positive and 262 nm negative signals (Fig. 3(b)).[2c,6] In the presence of (*S*)-**1**, ss-telo24 was again induced into an antiparallel G-quadruplex structure (Fig. 3(a)). Therefore, it was revealed that antiparallel G-quadruplex structure was induced by telomestatin regardless of the stereochemistry of the thiazoline.

The stability of the antiparallel G-quadruplex structure induced by telomestatin was investigated using the T_m values obtained from the CD melting curves at 292 nm molar ellipticity intensity (Fig. 4).[6] The T_m values of the complexes with (*S*)-**1** and (*R*)-**1** were observed to be 62.3 and 56.3 °C, respectively. Thus, (*S*)-**1** stabilizes the telomeric G-quadruplex structure more potently than (*R*)-**1** does, and this is coincident with the results described in the TRAP assay.

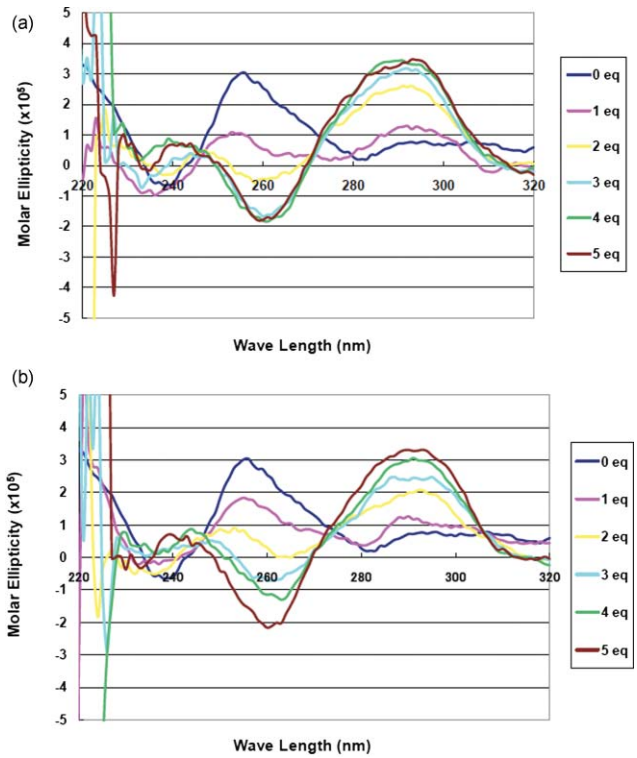


Fig. 3 CD spectra of ss-telo24 (10 μM) in Tris-HCl buffer (50 mM, pH 7.6) with **1** (10–50 μM). (a) (*S*)-**1**; (b) (*R*)-**1**.

Conclusion

We synthesized the (*S*)-stereoisomer of telomestatin by overcoming rare epimerization at the C2 position in the macrolactamization; as compared to the natural product, the (*S*)-isomer exhibited four-fold potent inhibitory activity against telomerase. Comparison of the T_m value of ss-telo24 and changes in the CD spectra in the presence of both enantiomers also suggested that the (*S*)-isomer is a stronger G-quadruplex binder for telomere than the natural product, telomestatin, which is the most potent compound reported to date. Further study of the binding structure of a telomestatin derivative to the G-quadruplex telomere by molecular modeling and by NMR analysis is underway in our laboratory.

Experimental section

General procedures

NMR spectra were recorded on a JEOL Model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Varian 500 MHz NMR

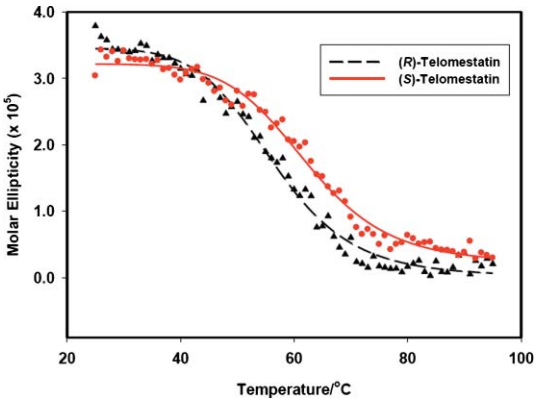


Fig. 4 CD melting curves of ss-telo24 at 292 nm in the presence of 5 equivalents of telomestatin, ● (*S*)-1; ▲ (*R*)-1.

instrument in the indicated solvent. Chemical shifts are reported in units parts per million (ppm) relative to the residual proton solvent signal for internal tetramethylsilane (7.25 ppm for ^{1}H) for solutions in $CDCl_3$. NMR spectral data are reported as follows: chloroform (7.26 ppm for ^{1}H) or chloroform-*d* (77.1 ppm for ^{13}C), dichloromethane (5.30 ppm for ^{1}H) or dichloromethane-d_2 (53.8 ppm for ^{13}C), DMSO (2.50 ppm for ^{1}H) or DMSO-d_6 (39.5 ppm for ^{13}C), CH_3CN (1.93 ppm for ^{1}H) when internal standard is not indicated. Multiplicities are reported by 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 a Perkin Elmer Spectrum One FT-IR spectrophotometer. Only the strongest and/or structurally important absorption is reported as the IR data given in cm^{-1}. Optical rotations were measured with a JASCO P-1020 polarimeter in a 0.3 cm ϕ cell with a pathlength of 5 cm. The $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} and the concentrations are given in 100 cm^{-3}. 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, cerium sulfate or 10% ethanolic phosphomolybdic acid. Merck silica gel 60 (0.063–0.200 mm) was used for column chromatography. Mass spectra were obtained on Waters LCT Premier™ XE with leucine enkephalin (SIGMA) as an external standard. Preparative reversed-phase HPLC (UV 254 nm) was performed on a Gilson 215 system with Senshu Pak PEGASIL-ODS, 5 μm, 20 × 250 mm eluted with CH_3CN–H_2O–CF_3CO_2H (70 : 30 : 0.1). CD spectra were recorded on a JASCO-810 spectropolarimeter using a quartz cell of 1 mm optical path length and an instrument scanning speed of 100 nm min^{-1} with a response time of 1 s, and over a wavelength range of 200–320 nm. The ss-telo24 nucleotide was purchased from Sigma Genosys and was dissolved in doubly-distilled water to be used without further purification.

General procedure for the synthesis of bis-trisoxazole amide

To a solution of the HCl salt of the amine **2**[7] in CH_2Cl_2 (10 mL $mmol^{-1}$) was added a solution of the trisoxazole carboxylic acid **3**[7] in DMF (10 mL $mmol^{-1}$) and DIEA (9 equiv.) at room temperature. To the reaction mixture was added PyBroP (1.5 equiv.) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was poured into 1 M HCl at 0 °C and the aqueous layer was extracted with ethyl acetate. The combined organic layer was washed with 3 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel eluted with 0–30% ethyl acetate in $CHCl_3$ to give bis-trisoxazole amide **4** or **7**.

Bis-trisoxazole amide (2*S*,14*S*)-7. Pale yellow oil. $[\alpha]_D^{18}$ −50.0 (*c* 1.08, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 8.91 (s, 1H), 8.86 (s, 1H), 8.84 (s, 1H), 8.74 (d, *J* = 8.22 Hz, 1H), 8.73 (s, 1H), 5.38 (dt, *J* = 8.22, 7.25 Hz, 1H), 5.16 (dd, *J* = 6.77, 2.90 Hz, 1H), 4.31 (dd, *J* = 9.18, 6.77 Hz, 1H), 4.09 (dd, *J* = 9.18, 2.90 Hz, 1H), 3.87 (s, 3H), 3.29 (dd, *J* = 13.5, 7.25 Hz, 1H), 3.23 (dd, *J* = 13.5, 7.25 Hz, 1H), 2.74 (s, 3H), 2.68 (s, 3H), 1.67 (s, 3H), 1.55 (s, 3H), 1.33 (brs, 18H). ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 164.1, 160.9, 160.5, 159.0, 155.5, 155.3, 154.0, 153.9, 150.4, 150.3, 144.4, 142.1, 140.4, 140.3, 136.2, 133.0, 129.7, 128.8, 124.5, 123.6, 93.8, 78.7, 66.4, 54.2, 51.3, 47.4, 42.0, 30.4, 30.2, 27.5, 10.9, 10.8. FT-IR (neat) 3302, 3139, 2976, 1708, 1658, 1367, 1160, 1098, 757, 725 cm^{-1}. HRMS (ESI-TOF) calcd. for $C_{39}H_{44}N_8O_{12}SNa$ $[M+Na]^+$ 871.2692 found 871.2699.

Bis-trisoxazole amide (2*S*,14*R*)-4. Yellow solid. mp (ethyl acetate) 175–177 °C. $[\alpha]_D^{20}$ +20.6 (*c* 1.29, $CHCl_3$). ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 8.91 (s, 1H), 8.85 (s, 1H), 8.82 (s, 1H), 8.75 (d, *J* = 7.24 Hz, 1H), 8.73 (s, 1H), 5.37 (dt, *J* = 7.73, 7.24 Hz, 1H), 5.15 (dd, *J* = 6.52, 2.41 Hz, 1H), 4.31 (dd, *J* = 9.42, 6.52 Hz, 1H), 4.08 (dd, *J* = 9.42, 2.41 Hz, 1H), 3.86 (s, 3H), 3.29 (dd, *J* = 13.5, 7.73 Hz, 1H), 3.23 (dd, *J* = 13.5, 7.73 Hz, 1H), 2.73 (s, 3H), 2.67 (s, 3H), 1.66 (s, 3H), 1.54 (s, 3H), 1.32 (brs, 18H). ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 164.1, 160.9, 160.5, 159.1, 155.5, 155.3, 154.0, 153.9, 150.4, 150.3, 144.4, 142.1, 140.4, 140.2, 136.2, 133.0, 129.7, 128.8, 124.5, 123.6, 93.8, 78.7, 66.4, 54.2, 51.3, 47.4, 42.1, 30.4, 30.2, 27.5, 10.9, 10.8. FT-IR (solid) 3304, 3137, 2975, 1705, 1657, 1365, 1154, 1097, 755, 724 cm^{-1}. HRMS (ESI-TOF) calcd. for $C_{39}H_{44}N_8O_{12}SNa$ $[M+Na]^+$ 871.2692 found 871.2675. Anal. calcd. for $C_{39}H_{44}N_8O_{12}S$: C, 55.18; H, 5.22; N, 13.20, found: C, 55.32; H, 5.42; N, 13.08.

General procedure for the macrocyclization

To a solution of bis-trisoxazole amide **4** or **7** in 1,4-dioxane (10 mL $mmol^{-1}$) was added 4 M HCl/1,4-dioxane (60 mL $mmol^{-1}$) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated *in vacuo*. The residue was used for the next reaction without further purification.

To a solution of the residue in MeOH (50 mL $mmol^{-1}$), THF (50 mL $mmol^{-1}$) and H_2O (16 mL $mmol^{-1}$) was added $LiOH{\cdot}H_2O$ (0.1 M) at 0 °C, and then warmed up to room temperature. After being stirred at the same temperature for 2 h, the reaction mixture was quenched with 4 M HCl/1,4-dioxane at 0 °C, and concentrated *in vacuo*. The residue was dried under reduced pressure for 12 h and used for the next reaction without further purification.

To a solution of a condensing agent (10 equiv., DPPA/HOBT, HATU or PyBOP/HOBt), DIEA (15 equiv.) and an additive (1 equiv., DMAP or DMAPO) in CH_2Cl_2 (160 mL $mmol^{-1}$) and DMF (80 mL $mmol^{-1}$) was added dropwise a solution of the above residue in DMF (80 mL $mmol^{-1}$) at room temperature under argon. After being stirred at room temperature for 3 d, the reaction mixture was poured into 3 M HCl at 0 °C and the aqueous layer

was extracted with ethyl acetate. The combined organic layer was washed with 3 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by column chromatography on silica gel eluted with 0–5% MeOH in $CHCl_3$ to give macrocyclic lactams **5** and **6**.

Macrocyclic lactam (2*S*,14*S*)-6. White solid. mp (CH_2Cl_2) >300 °C (decomposed). $[\alpha]_D^{24}$ −36.1 (*c* 0.205, $CHCl_3$). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.49 (d, J = 4.83 Hz, 1H), 8.41 (d, J = 6.28 Hz, 1H), 8.32 (s, 1H), 8.28 (s, 1H), 8.25 (s, 1H), 8.23 (s, 1H), 5.41 (m, 1H), 5.33 (m, 1H), 4.27 (m, 1H), 4.00 (m, 1H), 3.33 (dd, J = 13.0, 5.32 Hz, 1H), 3.17 (dd, J = 13.0, 3.38 Hz, 1H), 2.73 (s, 3H), 2.68 (s, 3H), 1.21 (s, 9H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 162.7, 161.6, 161.2, 160.0, 156.7, 156.6, 155.7, 155.1, 151.7, 151.3, 141.3, 141.1, 140.7, 139.2, 137.4, 136.9, 131.5, 130.1, 126.1, 125.1, 65.2, 52.8, 49.1, 42.8, 32.5, 31.0, 12.0. FT-IR (neat) 3370, 3104, 2925, 1666, 1588, 1515, 1166, 1105, 1071, 916, 726, 620 cm^{-1}. HRMS (ESI-TOF) calcd. for $C_{30}H_{28}N_8O_9SNa$ $[M+Na]^+$ 699.1592 found 699.1608.

Macrocyclic lactam (2*S*,14*R*)-5. White solid. mp (CH_2Cl_2) >300 °C (decomposed). $[\alpha]_D^{27}$ +7.03 (*c* 0.730, $CHCl_3$). ^{1}H NMR (400 MHz, CD_2Cl_2) δ 8.49 (d, J = 6.28 Hz, 1H), 8.44 (d, J = 7.25 Hz, 1H), 8.29 (s, 1H), 8.27 (s, 1H), 8.25 (s, 1H), 8.22 (s, 1H), 5.39–5.47 (m, 2H), 4.17 (m, 1H), 4.00 (m, 1H), 3.24 (dd, J = 13.0, 5.80 Hz, 1H), 3.16 (dd, J = 13.0, 4.35 Hz, 1H), 2.73 (s, 3H), 2.68 (s, 3H), 1.24 (s, 9H). ^{13}C NMR (100 MHz, CD_2Cl_2) δ 163.0, 161.5, 161.2, 159.8, 156.7, 156.6, 155.7, 155.1, 151.6, 151.2, 141.2, 140.5, 139.2, 137.2, 136.8, 131.4, 130.0, 126.0, 125.1, 65.3, 51.8, 48.6, 42.9, 33.0, 31.0, 12.0. FT-IR (solid) 3362, 3120, 2938, 1665, 1581, 1499, 1180, 1100, 1065, 914, 768, 726, 582 cm^{-1}. HRMS (ESI-TOF) calcd. for $C_{30}H_{28}N_8O_9SNa$ $[M+Na]^+$ 699.1592 found 699.1577.

Total synthesis of the enantiomer of telomestatin (*S*)-1. The synthesis was performed according to the procedure previously reported.[7]

Cyclic heptaoxazole (*S*)-8. White solid. mp (CH_2Cl_2) >300 °C (decomposed). $[\alpha]_D^{22}$ −13.2 (*c* 0.370, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 × 2 (each s, 2H), 8.16 (s, 2H), 8.13 (s, 1H), 7.80 (brs, 1H), 5.25 (brs, 1H), 3.23 (dd, J = 13.5, 4.35 Hz, 1H), 3.07 (brd, J = 11.1 Hz, 1H), 2.63 (s, 3H), 2.61 (s, 3H), 1.14 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.4, 159.9, 156.6, 156.4, 156.2, 155.8, 155.2, 154.9, 150.7, 149.0, 141.8, 139.2, 138.5, 137.6, 137.0, 130.5, 129.7, 129.5, 125.3, 124.5, 70.7, 42.8, 32.3, 30.8, 11.9, 11.6. FT-IR (neat) 3408, 3123, 2926, 1673, 1588, 1494, 1365, 1176, 1087, 918, 753, 716 cm^{-1}. HRMS (ESI-TOF) calcd. for $C_{30}H_{24}N_8O_8SNa$ $[M+Na]^+$ 679.1330 found 679.1316.

Enantiomer of telomestatin (*S*)-(1). ^{1}H NMR (500 MHz, CD_3CN:D_2O = 1 : 1) δ 8.57 (s, 1H), 8.49 (s, 1H), 8.41 (s, 3H), 5.85 (t, J = 10.0 Hz, 1H), 4.14 (dd, J = 10.0, 10.0 Hz, 1H), 3.68 (dd, J = 10.0, 10.0 Hz, 1H), 2.63 (s, 3H), 2.61 (s, 3H). HRMS (ESI-TOF) calcd. for $C_{26}H_{14}N_8O_7SNa$ $[M+Na]^+$ 605.0598 found 605.0583.

TRAP assay. The oligonucleotides, Ts (5′-AATCCGTC-GAGCAGAGTT-3′) and CX primer (5′-CCCTTACCCTTA-CCCTAACCCTAA-3′), were synthesized by Sigma Genosys. The TRAP reaction was carried out in two steps (elongation and amplification). Elongation reactions were carried out at 30 °C for 30 min in final volume of 40 μL containing 1 × 10^4 cells of extract from namalva cells. The reaction buffer contained 20 mM Tris·HCl (pH 8.3), 63 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.005% Tween 20, 0.01 mg mL^{-1} T4 gene 32 protein and 0.1 mg mL^{-1} BSA supplemented with 50 μM dNTP and 0.3 μM Ts primer. Reactions were stopped by heating for 2 min at 94 °C, and the samples were placed on ice. The amplification step was done on ice by adding to the samples 5 μL of PCR solution containing 0.3 μM CX primer and 1.4 units *Taq* polymerase. The PCRs were then performed for amplification as follows: 94 °C for 2 min and 28 cycles consisting 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s. Telomerase extension products were analyzed by polyacrylamide gel electrophoresis in 1X Tris-borate-EDTA buffer and the 10% acrylamide gels were scanned with a Typhoon (GE Healthcare Bio-Sciences). The extension products were quantified by using ImageQuant 5.2 software and normalized to the signal of the extension products in control solvent.

CD experiments.[6] The CD titration experiment was performed with a modification of the reported procedure.[2c] The ss-telo24 oligonucleotide was dissolved in Tris-buffer (50 mM, pH 7.6) and the solution was heated to 94 °C for 5 min, then slowly cooled to 25 °C. (*S*)-**1** and (*R*)-**1** were diluted with water from a 10 mM stock solution to a concentration of 1 mM, respectively. The solution was titrated into the oligonucleotide samples at 1 mol equiv up to 5 mol equiv (the 10 mM stock solutions of **1** were prepared in DMSO). The final DNA concentration was 10 μM, and the CD spectra are representative of three averaged scans taken at 25 °C. The CD melting curves of the ss-telo24 G-quadruplex were determined from measurements of the CD intensity at 292 nm. The heating rate was 2.0 °C min^{-1}.

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