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SYNTHESIS OF MACROCYCLIC PENTA- AND TETRAOXAZOLES AS G-QUADRUPLEX LIGANDS†

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Abstract – The penta- and tetraoxazole telomestatin analogs L2H2-5OTD (**4**) and L2H2-4OTD (**5**) were synthesized as new G-quadruplex ligands in order to evaluate the influence of the size of the planar macrocycle on the G-quadruplex-stabilizing efficacy. These ligands were less potent stabilizers of various G-quadruplex-forming oligonucleotides than L2H2-6OTD (**2a**), which has a hexaoxazole-type macrocycle.

Guanine-rich DNA sequences in telomeric DNA and the promoter regions of some cancer-related genes, such as *c-kit*,[1] *bcl-2,*[2] *c-myc,*[3] and *k-ras*,[4] form characteristic higher-order G-quadruplex structures,[5] which play significant roles in regulation of enzyme functions and/or gene transcription.[6] For example, telomerase activity is inhibited by formation of G-quadruplex structures in telomeric DNA sequences, and dissociation of telomere-related proteins such as TRF2 and Pot1 from 3'-overhang chromosomes induces apoptosis of various cancer cells.[7] Stabilization of G-quadruplex structure is currently considered a promising approach for cancer treatment, and a number of small compounds with G-quadruplex-stabilizing activity (G-quadruplex ligands) have been developed.[8] We have recently synthesized macrocyclic hexaoxazoles (6OTDs, **2**) as G-quadruplex ligands inspired by the natural G-quadruplex ligand telomestatin (TMS) (**1**).[9] Telomestatin (**1**) shows potent telomerase-inhibitory activity by stabilizing telomeric G-quadruplex structure through π-π interaction with the planar G-quartet in an end-stacking mode.[10] Since 6OTD (**2**) has similar size and planarity to **1**, they are expected to interact with G-quadruplex structures in a similar manner to **1**. Recently, we analyzed the interaction

mode of telomeric G-quadruplex with L2H2-6OTD (**3**), which has potent G-quadruplex-stabilizing ability similar to that of TMS, by means of an NMR study, and confirmed that it interacts with the G-quartet in an end-stacking mode through π-π interaction.[11] Other macrocyclic G-quadruplex ligands are also considered to interact similarly with the G-quartet.[12] Although the size of the macrocycle seems likely to be important for the stabilization efficacy, little is known about the structure-activity relationship from the viewpoint of macrocycle size.[12b,12e] In this paper, we describe the synthesis of L2H2-5OTD (**4**) and L2H2-4OTD (**5**), which bear smaller penta- and tetraoxazole macrocycles, respectively, compared to the L2H2-6OTD (**3**) (Figure 1). The G-quadruplex-stabilizing ability of these ligands was evaluated by means of fluorescence resonance energy transfer (FRET) melting assays.

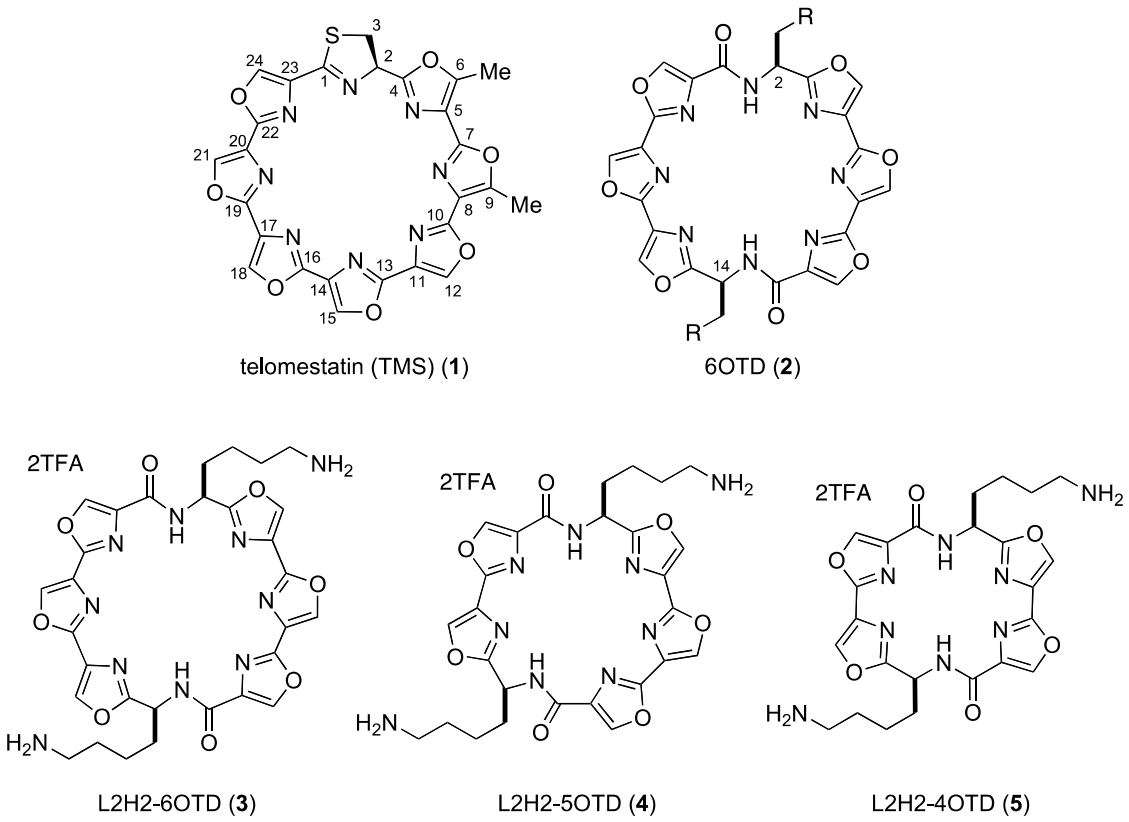
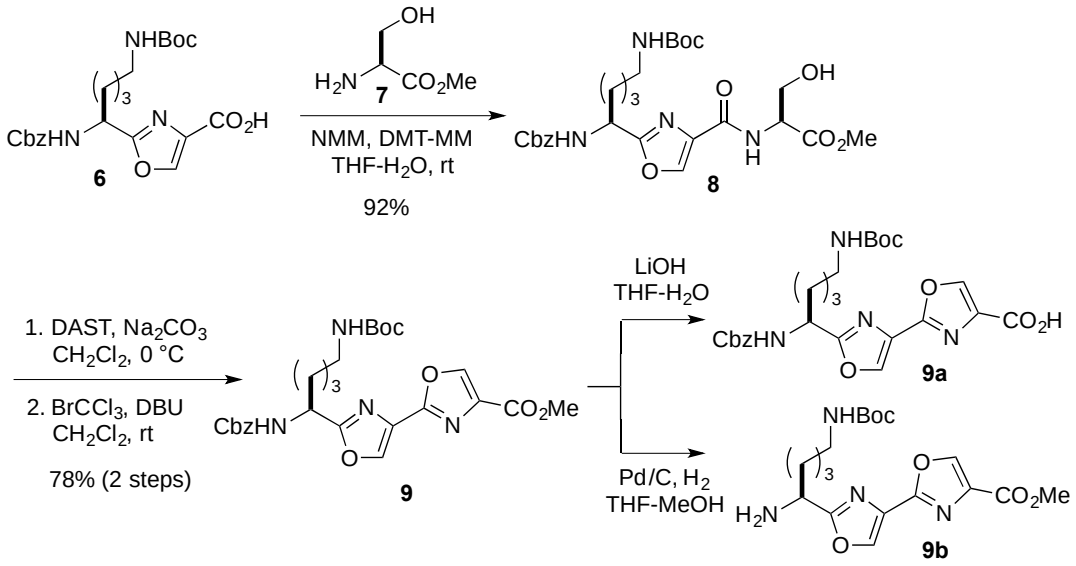


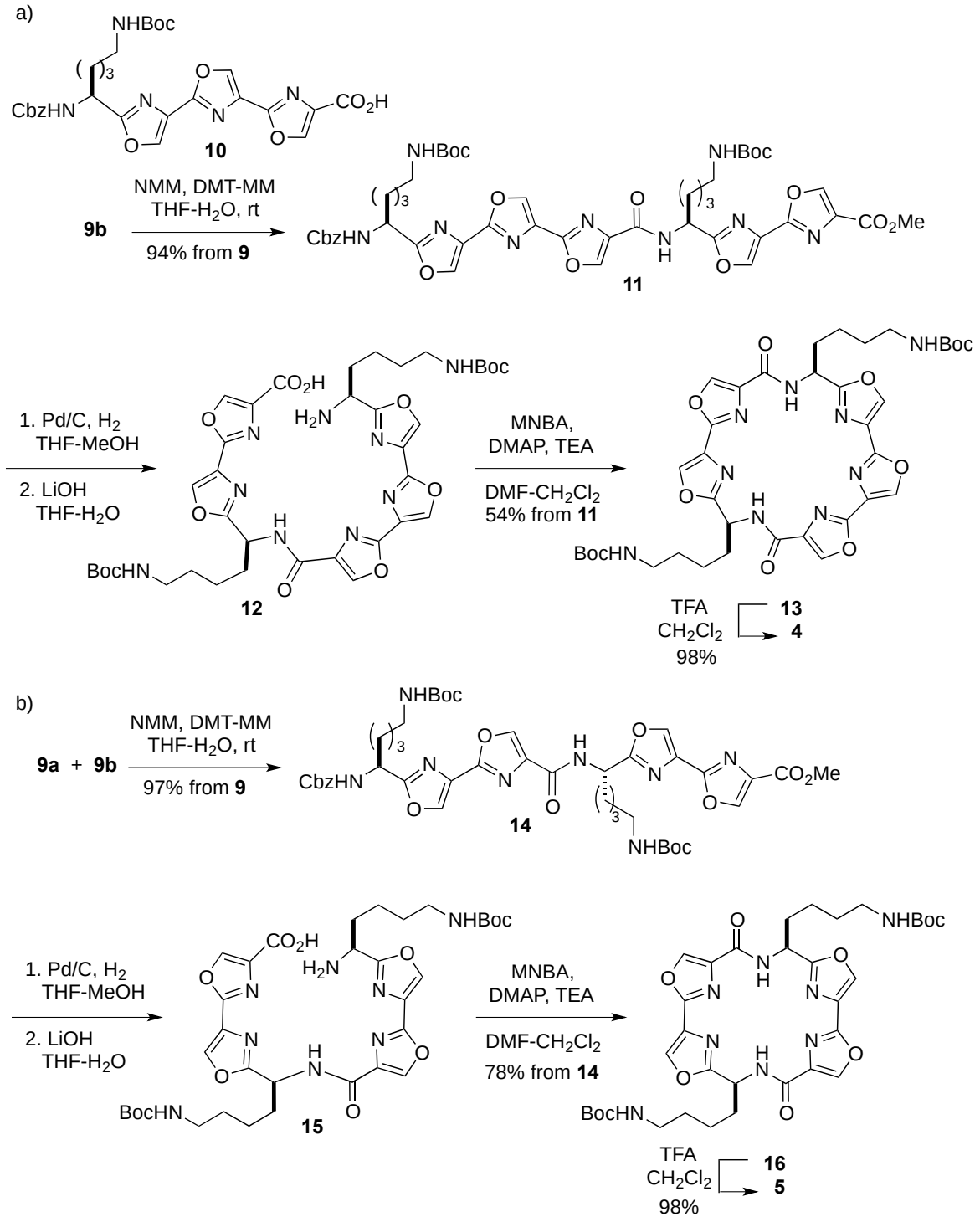
Figure 1. Structures of telomestatin (**1**) and macrocyclic polyoxazole analogs (**2**-**5**)

Synthesis of L2H2-5OTD (**4**) and L2H2-4OTD (**5**) was commenced with preparation of bisoxazoles **9a** and **9b** (Scheme 1). A carboxylic acid-bearing mono-oxazole **6** was reacted with serine methylester (**7**) in the presence of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[13] and *N*-methylmorpholine (NMM) to give β-hydroxy amide **8** in 92% yield. Cyclodehydration of **8** with diethylaminosulfur trifluoride (DAST)[14] followed by oxazole formation with bromotrichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)[15] gave bisoxazole **9** in 78% yield from **8**. Then, carboxylic acid **9a** and amine **9b** were synthesized from **9** by hydrolysis of the methyl ester with lithium hydroxide or deprotection of Cbz group under hydrogen in the presence of Pd/C, respectively.



Scheme 1. Synthesis of bisoxazoles **9a** and **9b**

Synthesis of L2H2-5OTD (**4**) is depicted in Scheme 2a. Bisoxazole amine **9a** was reacted with trioxazole carboxylic acid **10**[9b] in the presence of NMM and DMT-MM to give amide **11** in 94% yield. Deprotection of the Cbz group in **11** followed by hydrolysis of methyl ester gave an amino acid, which was subjected to macrocylization using 2-methyl-6-nitrobenzoic anhydride (MNBA)[16] in the presence of triethylamine (TEA) and 4-dimethylaminopyridine (DMAP) under high dilution conditions (3 mM) in CH_2Cl_2-DMF (2:1) to give **13** in 54% yield from **11**. The Boc group was deprotected with TFA to give L2H2-5OTD (**4**) in 98% yield.[17] Macrocyclic tetraoxazole L2H2-4OTD (**5**) was similarly synthesized from **9a** and **9b** via **14**, **15**, and **16** (Scheme 2b).[18]



Scheme 2. Synthesis of a) L2H2-5OTD (**4**) and b) L2H2-4OTD (**5**)

The G-quadruplex-stabilizing ability of L2H2-5OTD (**4**) and L2H2-4OTD (**5**) was examined and compared with that of L2H2-6OTD (**3**) by means of FRET melting assay.[19] In this assay, ligand-induced stabilization of a folded G-quadruplex is evaluated in terms of the increment of the

melting temperature, ΔT_m. The ΔT_m values of the G-quadruplex-forming DNA sequences of telo21, *bcl-2*, *c-kit*, *c-myc*, and *k-ras* (0.2 μM) were measured in the presence of **3**, **4** and **5** (2 μM), and the results are summarized in Table 1. In the case of telo21, the ΔT_m values were found to be 20.7, 14.9 and 5.1 °C in the presence of **3**, **4** and **5**, respectively. The G-quadruplex-forming sequences of *bcl-2*, *c-kit*, *c-myc*, and *k-ras* showed similar trends. Thus, the size of the macrocyclic structure is clearly significant for polyoxazole G-quadruplex ligands, and the stabilizing ability of these ligands decreases as the size of the structure is reduced.

Table 1. ΔT_m (ºC) values in FRET melting assay[a-c)]

Ligand	ΔT_m (°C)				
	telo21[d)]	bcl-2[d)]	c-kit[d)]	c-myc[d)]	k-ras[d)]
3	20.7	18.1	22.1	21.7	16.1
4	14.9	11.5	15.8	11.0	8.5
5	5.1	2.4	7.6	2.3	1.3

[a)]The FRET melting assays were conducted with DNA (0.2 μM) and ligands (2.0 μM) in the presence of K^+ (60 mM). [b)]ΔT_m values were obtained in two independent experiments each performed in duplicate. [c)]The G4-forming DNA sequences used in FRET melting assay are shown in Table 2. [d)]T_m values of telo21, *bcl-2*, *c-kit*, *c-myc*, and *k-ras* in the absence of ligands were found to be 62.4 °C, 69.7 °C, 63.6 °C, 77.4 °C, and 64.9 °C, respectively.

Table 2. G4-forming DNA sequences used in FRET melting assay

Oligomer name	Sequences
telo21	GGGTTAGGGTTAGGGTTAGGG
bcl-2	GGGCGCGGGAGGAAGGGGGCGGG
c-kit	GGGAGGGCGCTGGGAGGAGGG
c-myc	GAGGGTGGGGAGGGTGGGGAAG
k-ras	AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG

In conclusion, we have synthesized novel G-quadruplex ligands L2H2-5OTD (**4**) and L2H2-4OTD (**5**) bearing pentaoxazole and tetraoxazole core structures, respectively. Examination of the effect of the size of macrocyclic structure on G-quadruplex-stabilizing activity revealed that hexaoxazole structure is optima among these three sizes, and the stabilizing ability decreases with decreasing size of the macrocycle.

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†We would like to dedicate this paper to Professor Dr. Isao Kuwajima on the occasion of his 77th birthday.

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17 Spectral data for L2H2-5OTD (**4**): $[\alpha]_D^{25}$ -6.5 (*c* 1.9, MeOH); ^{1}H NMR (400 MHz, DMSO- d_6) δ 9.12 (s, 1H), 9.07 (s, 1H), 9.06 (s, 1H), 8.90 (s, 1H), 8.89 (s, 1H), 8.81 (d, J = 6.0 Hz, 1H), 8.65 (d, J = 6.0 Hz, 1H), 7.75 (brs, 4H), 5.35 (m, 2H), 2.74 (m, 4H), 2.16 (m, 2H), 1.96 (m, 2H), 1.57 (m, 4H), 1.40 (m, 2H), 1.13 (m, 2H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 164.3, 164.2, 158.9, 158.7, 155.2, 154.3, 154.2, 142.5, 142.3, 141.8, 140.7, 139.9, 136.4, 135.7, 129.3, 128.5, 128.0, 48.1, 48.0, 38.6 (2 carbons), 32.6, 32.2, 26.7 (2 carbons), 20.8, 20.6 ppm; HRMS (ESI, M+H) cacld for $C_{27}H_{30}N_9O_7$ 592.2268, found 592.2234.

18 Spectral data for L2H2-4OTD (**5**): $[\alpha]_D^{25}$ -10.2 (*c* 1.5, MeOH); ^{1}H NMR (400 MHz, DMSO- d_6) δ 9.02 (s, 1H), 9.00 (s, 1H), 8.85 (s, 2H), 8.76 (s, 2H), 7.84 (brs, 4H), 5.29 (m, 2H), 2.80 (m, 4H), 2.06 (m, 4H), 1.60 (m, 4H), 1.43 (m, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 165.4, 159.9, 154.7, 142.8, 141.1, 136.5, 128.8, 46.2, 38.6, 30.9, 26.5, 22.3 ppm; HRMS (ESI, M+H) cacld for $C_{24}H_{29}N_8O_6$ 525.2210, found 525.2236.

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