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Synthesis and G-quadruplex stabilizing properties of a series of oxazole-containing macrocycles

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Abstract—The synthesis of 24-membered macrocycles containing four, six, and seven oxazole moieties is described. Selected compounds were evaluated for their ability to specifically bind and stabilize G-quadruplex DNA and for cytotoxic activity. An unexpected oxidative cleavage reaction afforded a macrocyclic imide that was also evaluated for G-quadruplex stabilizing and cytotoxic activity.

Telomeres are the ends of chromosomes consisting of repeated DNA sequences and bound proteins. In humans TTAGGG repeats are present at the ends of telomeres.[1,2] These G-tails can readily adopt the thermodynamically stable G-quadruplex conformation in vitro,[3,4] although it is not known whether G-quadruplexes exist in vivo in human cells. It is known however that deprotection of telomeres due to defects in telomere binding proteins or shortened telomeres can activate DNA damage responses including cell cycle arrest, senescence, and apoptosis.[5–11] Telomerase is an enzyme that maintains telomere length. Telomerase activity has been found in 85–90% of all human tumors, but not in normal cells.[12] Thus, telomerase may play a role in the uncontrolled growth of tumor cells. A number of compounds have been identified that stabilize G-quadruplexes and also inhibit telomerase.[13] Telomestatin (SOT-095) **1**, a natural product isolated from *Streptomyces anulatus* 3533-SV4, is the strongest and most specific inhibitor of telomerase identified to date.[14] The inhibition of telomerase in vitro by telomestatin is presumed to occur by sequestering the telomere template in its G-quadruplex form.[15] Telomestatin is reported to shorten telomere length and induce apoptosis in freshly isolated primary acute leukemia cells.[16] These results suggest that G-quadruplex stabilizers may represent a new class of anticancer agents (Fig. 1).

In this report, the synthesis of a series of macrocyclic compounds that retain the 24-membered ring structure of telomestatin is described. Each compound contains 4–7 oxazole units and at least one valine residue. Compounds were evaluated for their ability to specifically bind and stabilize G-quadruplex DNA and also for cytotoxic activity.

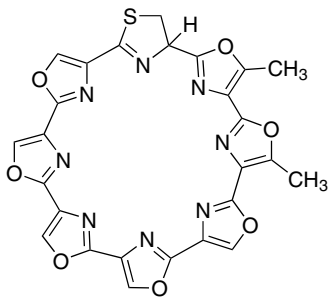

Figure 1. Structure of telomestatin (**1**).

Keywords: Synthesis; Macrocycles; Oxazoles; G-quadruplex; Cytotoxicity.
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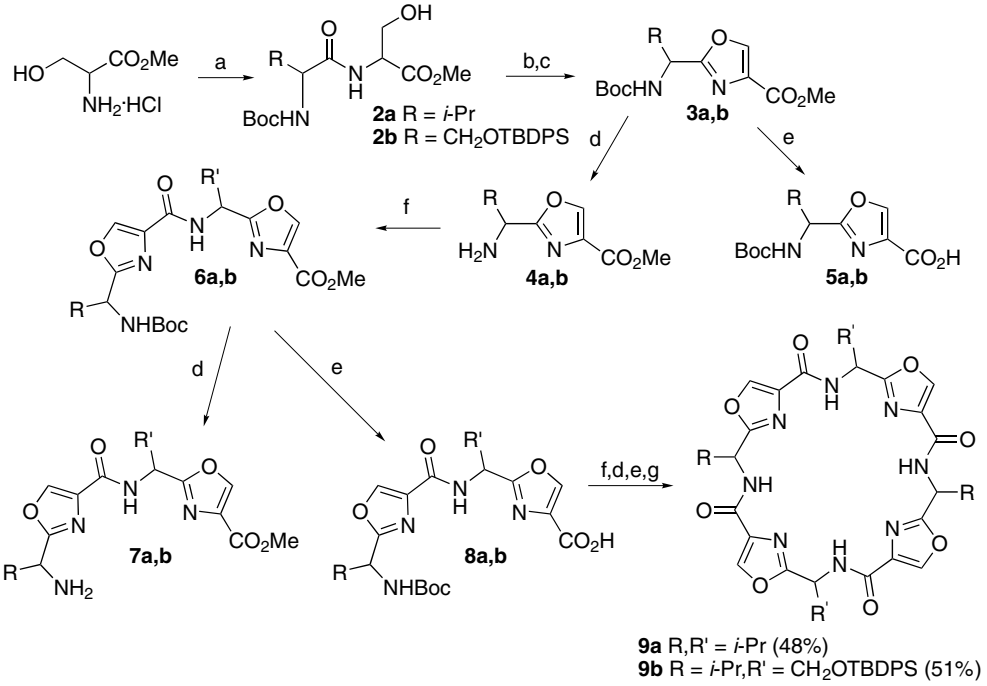
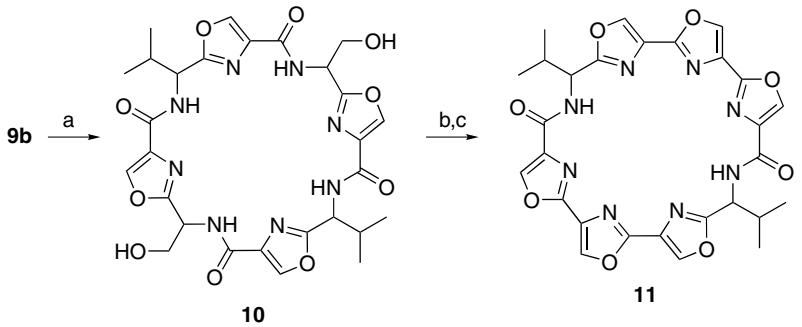
Syntheses of compounds having alternating oxazole and amino acid residues are outlined in Scheme 1. Oxazoles having an amino acid residue and carboxyl group at the 2- and 4-positions, respectively, were prepared using established methodology starting from serine methyl ester and the appropriate N-Boc-protected amino acid.[17,18] The resulting oxazoles **3a**,**b** were then divided into two portions, with one treated with trifluoroacetic acid to remove the Boc group to give amines **4a**,**b** and the other hydrolyzed with LiOH to give carboxylic acids **5a**,**b**. The amines were coupled in very high yield with the carboxylic acids using BOP reagent to give amides **6a**,**b**.[19] Once again the products were divided into two portions with one treated with TFA to give **7a**,**b** and the other with LiOH to form **8a**,**b**. These were again coupled with BOP, deprotected, and then the macrocyclization was performed using BOP under high dilution conditions (<0.01 M solution). The yields of **9a**,**b** were 48% and 51%, respectively.[20]

Macrocycle **9b** was treated with 50% HF in acetonitrile to remove the TBDPS groups (Scheme 2). Diol **10** was then cyclized using DAST and dehydrogenated with $BrCCl_3$ as described above to give macrocyclic hexaoxazole **11** in 28% yield for the two steps.[20]

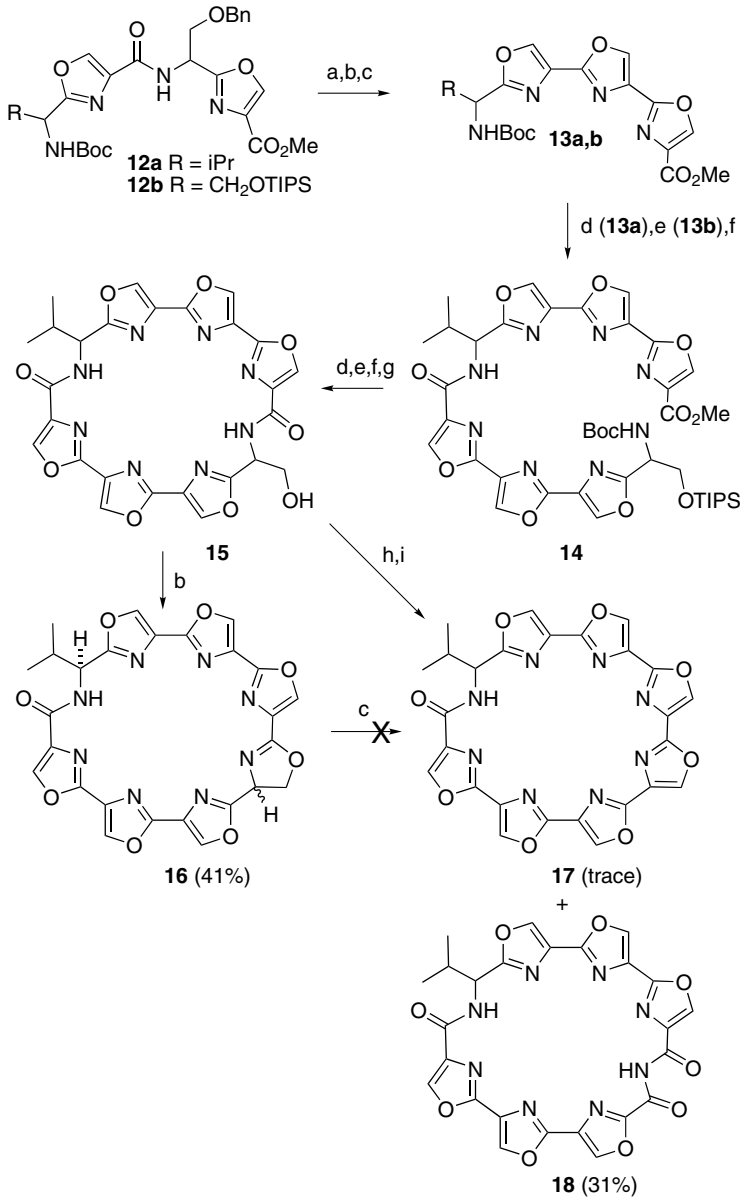
The synthesis of macrocycles containing six and seven oxazole units is shown in Scheme 3. Amides **12a**,**b** (prepared using the procedure outlined in Scheme 1) were converted into teroxazoles **13a**,**b** by hydrogenolysis of the benzyl-protecting group, cyclization of the resulting alcohol with DAST, and aromatization with $BrCCl_3$. Treatment of **13a** with TFA yielded an amine. Hydrolysis of **13b** with LiOH afforded a carboxylic acid that was then coupled with the amine using BOP reagent to give amide **14**. The Boc group was removed with TFA and the methyl ester hydrolyzed with LiOH to afford an amino acid that was macrocyclized in 67% yield using BOP reagent under high dilution conditions. Removal



Scheme 1. Reagents: (a) RCH(NHBoc)CO_2H, EDC, HOBt, Et_3N; (b) DAST, K_2CO_3; (c) $BrCCl_3$, DBU; (d) TFA, CH_2Cl_2; (e) LiOH, MeOH/THF; (f) BOP reagent, Et_3N, CH_3CN; (g) BOP reagent, DIPEA, DMF, <0.01 M.



Scheme 2. Reagents: (a) 50% HF/CH_3CN; (b) DAST, K_2CO_3; (c) $BrCCl_3$, DBU.



Scheme 3. Reagents and conditions: (a) H_2, $Pd(OH)_2/C$, EtOH; (b) DAST, K_2CO_3, CH_2Cl_2, −78 °C to rt; (c) $BrCCl_3$, DBU, CH_2Cl_2, 0 °C; (d) 50% TFA/CH_2Cl_2; (e) LiOH, MeOH/THF; (f) BOP reagent, Et_3N, DMF; (g) HCl, MeOH, CH_2Cl_2; (h) DMP, CH_2Cl_2, Δ; (i) PPh_3, I_2, Et_3N.

of the TIPS groups with HCl in MeOH/CH_2Cl_2 gave macrocyclic hexaoxazole **15** in 70% yield. A portion of **15** was treated with DAST to give dihydroheptaoxazole **16** in 41% yield. Treatment with $BrCCl_3$/DBU however failed to give heptaoxazole **17**. A number of other reagents were evaluated for the formation of **17** including NiO_2, MnO_2, NBS, and DDQ, but all were equally unsuccessful. Instead, **15** was oxidized with Dess–Martin periodinane (DMP) to a purported aldehyde which was then treated with triphenylphosphine and iodine[21] in the presence of triethylamine to give traces of heptaoxazole **17** along with imide **18** in 31% yield from **15**.[20] It was determined that **18** formed during the Dess–Martin oxidation of alcohol **15**, which was performed at reflux in CH_2Cl_2 due to solubility problems. There is some precedent for such a transformation. Cabarrocas et al. reported oxidative cleavage of several β-hydroxyamides during oxidation with iodoxybenzoic acid (IBX) at 75 °C in DMSO to give similar imide products.[22] DMP can contain varying amounts of hydrolysis products including IBX and acetoxyIBX, and it is possible that such impurities could be responsible for the oxidative cleavage observed in the present study.[23] A detailed investigation of this is currently underway in our laboratory and will be reported separately.

The tetraoxazole macrocycle **9a**, as well as the hexaoxazole macrocycles **11** and **18**, were evaluated for their abilities to bind and thermally stabilize both duplex and G-quadruplex DNA. Toward this end we monitored the UV absorbance of the DNA as a function of temperature in the absence and presence of saturating amounts

of macrocycle. The melting of duplex DNA is associated with a hyperchromic shift at 260 nm, while the melting of quadruplex DNA is associated with a hypochromic shift at 295 nm. Thus, the temperature-dependent absorption of duplex and quadruplex DNA was monitored at 260 and 295 nm, respectively. Salmon testes DNA (ST-DNA) was used as a representative model for duplex DNA, while the quadruplex structure formed by an oligomer, d(TTAGGG)$_4$, containing four consecutive repeats of the 5′-TTAGGG-3′ sequence that is multiply repeated in human telomeric DNA was used as a representative model for G-quadruplex DNA. All the UV melting studies were conducted at pH 7.5 in the presence of 50 mM potassium ion.

Figure 2 shows the UV melting profiles (depicted in their first-derivative forms) of ST-DNA and d(TTAGGG)$_4$ in the absence and presence of **11**. The transition temperatures (T_{tran}) corresponding to the maxima or minima of these first-derivative melting profiles are listed in Table 1, as are the corresponding T_{tran} values derived from UV melting profiles (not shown) conducted in the presence of **9a** and **18**. Note that the presence of neither **9a**, **11**, nor **18** alters the T_{tran} value (i.e., the thermal stability) of ST-DNA, with any observed changes in T_{tran} being within the experimental uncertainty. This observation is consistent with little or no duplex DNA binding on the part of either macrocycle. Control isothermal titration

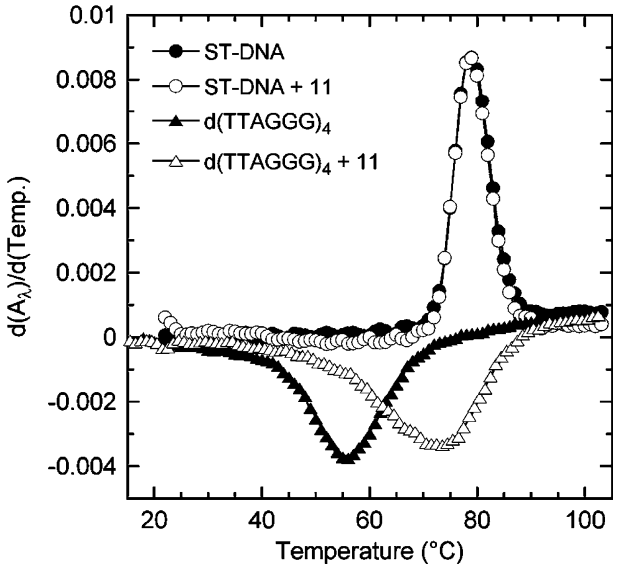


Figure 2. First derivatives of the UV melting profiles of ST-DNA and d(TTAGGG)$_4$ in the absence and presence of **11**. Profiles were acquired on an AVIV model 14DS spectrophotometer using quartz cuvettes with a 1 cm pathlength. The temperature was raised in 0.5 °C increments and the samples were allowed to equilibrate for 2 min at each temperature setting, whereupon absorbances were recorded over a period of 6 s and averaged. When present, ST-DNA was used at a base pair concentration of 15 μM, while d(TTAGGG)$_4$ was used at a strand concentration of 4 μM. Macrocyclic ligands were used at a concentration of 15 μM in the ST-DNA experiments and 8 μM in the d(TTAGGG)$_4$ experiments. Solution conditions were 10 mM EPPS (pH 7.5), 50 mM KCl, and 0.1 mM EDTA. In the ST-DNA experiments, the wavelength (λ) was 260 nm, while being 295 nm in the d(TTAGGG)$_4$ experiments.

Table 1. Transition temperatures for the melting of ST-DNA and d(TTAGGG)$_4$ in the absence and presence of oxazole-containing macrocyclic ligands

Sample	No. of oxazoles	T_{tran}[a] (°C)
ST-DNA	NA[b]	78.5
ST-DNA + **9a**	4	78.5
ST-DNA + **11**	6	78.9
ST-DNA + **18**	6	78.5
d(TTAGGG)$_4$	NA[b]	56.0
d(TTAGGG)$_4$ + **9a**	4	56.0
d(TTAGGG)$_4$ + **11**	6	73.5
d(TTAGGG)$_4$ + **18**	6	62.5

[a] DNA transition temperatures (T_{tran}) reflect the maxima or minima of the first derivatives of UV melting profiles acquired as described in the legend to Figure 2. The uncertainty in the T_{tran} values is ±0.5 °C.
[b] NA, not applicable.

calorimetry (ITC) experiments (not shown) in which **9a**, **11**, and **18** were sequentially titrated into solutions of ST-DNA confirmed this observation.

Not only does the presence of **9a** fail to alter the thermal stability of ST-DNA, it also fails to alter thermal stability of d(TTAGGG)$_4$. Thus, the tetraoxazole macrocycle exhibits little or no binding to either duplex or G-quadruplex DNA.

In striking contrast to the behavior of **9a**, **11** and **18** increase the T_{tran} value of d(TTAGGG)$_4$ by 17.5 and 6.5 °C, respectively. Thus, both hexaoxazoles bind and thermally stabilize G-quadruplex DNA, with the extent of thermal enhancement being greater for **11**. ITC experiments are currently underway to assess whether this differential extent of thermal enhancement correlates with a corresponding difference in binding affinity. Viewed as a whole, our UV melting data indicate that hexaoxazole macrocycles bind G-quadruplex DNA with a high degree of specificity, with the G-quadruplex binding behavior being lost upon reduction of the oxazole number from six to four. Results from G-quadruplex binding studies for dihydroheptaoxazole **16** and other heptaoxazoles will be reported separately. Additional evidence in support of the importance of six oxazoles within the macrocyclic structure for specific G-quadruplex binding was provided by preliminary computational studies of **9a**, **11**, and **18** (Table 2). Hexaoxazoles **11** and **18** have E_{dock} values that are approximately 2-fold more favorable than that of tetraoxazole **9a**. While these E_{dock} values were determined for a capping interaction of the macrocycle with the quadruplex, the nature of the actual interactions between the macrocycles and quadruplex (intercalation vs capping) remains to be determined.

Select macrocycles were evaluated for cytotoxic activity. IC_{50} values (μM) for **11**, **16**, and **18** were: 0.4, 0.21, and 0.8, respectively, for the human lymphoblastoma RPMI 8402, and 0.5, 1.5, and 1.1 for the murine leukemia P388.

In summary, macrocycles containing four, six, and seven oxazole moieties have been synthesized. Compounds having at least six oxazole rings bind selectively to

Table 2. Calculated docking energies of macrocyclic ligands with a G-quadruplex

Macrocycle	No. of oxazoles	E_{dock}[a] (kcal/mol)
9a	4	−4.1
11	6	−8.4
18	6	−9.1
16	7	−9.2
17	7	−9.8
(*R*)-Telomestatin	7	−9.7

[a] Docking was performed using the Lamarckian Genetic Algorithm[24] in Autodock 3.0.5. $E_{dock} = E_{VDW} + E_{elec} + E_{int}$ with more negative values signifying a stronger binding energy. An X-ray crystal structure of a parallel G-quadruplex derived from human telomeric DNA (1KF1.pdb) was used as the receptor for these studies.[3] Macrocyclic ligands were constructed and refined using Sybyl (Tripos, Inc.). Gasteiger–Hückel charges were used on the ligands[25] and Kollman charges were used on the G-quadruplex.[26]

G-quadruplex DNA but not to duplex DNA. These compounds also exhibit moderate levels of cytotoxic activity against human lymphoblastoma and murine leukemia.

Acknowledgments

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20. The structures of all compounds were elucidated using ^{1}H (200 MHz) and ^{13}C NMR (50 MHz) in $CDCl_3$. Results are reported as ppm downfield from internal TMS. Compound **9a**: ^{1}H NMR δ 8.15 (s, 4H), 7.27 (d, 4H, $J = 9.4$), 5.26 (m, 4H), 2.34 (m, 4H), 1.08 (d, 12H, $J = 6.6$), 0.98 (d, 12H, $J = 6.6$); ^{13}C NMR δ 163.8, 159.8, 141.6, 135.8, 51.7, 32.9, 19.0, 18.7; HRMS ($M+Li^+$) calcd for $C_{32}H_{40}N_8O_8$ (Li), 671.3129; found, 671.3148.
Compound **11**: ^{1}H NMR δ 8.51 (d, 2H, $J = 8$), 8.20 (m, 6H), 5.30 (dd, 2H, $J = 8.5$), 2.40 (m, 2H), 1.04 (d, 6H, $J = 7$), 0.98 (d, 6H, $J = 7$); ^{13}C NMR δ 163.7, 159.1, 155.3, 153.8, 140.0, 138.2, 137.6, 136.2, 130.2, 128.8, 52.3, 33.2, 17.7, 17.5; HRMS ($M+H^+$) calcd for $C_{28}H_{24}N_8O_8(H^+)$, 601.1795; found, 601.1808.
Compound **16**: ^{1}H NMR δ 8.66 (d, 1H, $J = 9$), 8.21, 8.18, 8.16, 8.15, 8.04 (s, 6H), 5.74 (dd, 1H, $J = 5,9$), 5.33 (dd, 1H, $J = 5,9$), 4.74–4.58 (m, 2H), 2.38 (heptet, 1H, $J = 7$), 1.07 (d, 3H, $J = 7$), 1.00 (d, 3H, $J = 7$); ^{13}C NMR δ 163.8, 159.8, 159.1, 159.0, 155.0, 154.9, 153.9, 140.2, 139.5, 138.4, 137.7, 137.6, 137.0, 136.3, 130.4, 130.3, 130.0, 129.1, 128.7, 70.3, 62.5, 52.1, 33.0, 17.6, 17.4; HRMS ($M+Li^+$) calcd for $C_{26}H_{18}N_8O_8$(Li), 577.1408; found, 577.1389.
Compound **18**: ^{1}H NMR δ 11.17 (s, 1H), 8.50 (d, 1H, $J = 8$), 8.47, 8.45 (s, 2H), 8.27, 8.26, 8.25, 8.245 (s, 4H), 5.33 (dd, 1H, $J = 8,4$), 2.42 (m, 1H), 1.04 (d, 3H, $J = 7$), 0.93 (d, 3H, $J = 7$); ^{13}C NMR δ 163.5, 158.9, 156.7, 155.6, 154.3, 153.4, 142.9, 141.5, 140.2, 138.6, 138.4, 138.0, 136.3, 135.2, 130.5, 130.2, 130.0, 128.7, 52.3, 32.9, 17.6, 17.0; HRMS ($M+H^+$) calcd for $C_{25}H_{16}N_8O_9(H^+)$, 573.1118; found, 573.1091.
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