A convenient method for synthesis of tetraoxazole peptide macrocycles

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Abstract

G-quadruplex DNA plays important regulatory roles in the maintenance of telomere length and transcriptions inhibition of such oncogenes as *c-myc*, and thus has become an attractive target for the development of anti-cancer therapeutic agents. Poly-oxazole macrocycles are a promising new class of G-quadruplex binding ligands. Herein is described the synthesis of a tetraoxazole peptide macrocycle and an epimerization product. The synthetic unit was prepared by cyclization-oxidation of a diserine with one hydroxyl protected. Two-cycle coupling of the unit gave a linear tetraoxazole amide. The targeted molecule and the epimerization product were obtained after macrocyclization of the linear precursor and following removal of the protecting benzyl groups. With structural similarities to the most potent G-quadruplex stabilizer telomestatin, these two molecules might potentially be used to probe the biological significance of G-quadruplex' *in vivo*.

Keywords: G-quadruplex, poly-oxazole, anticancer drug discovery, macrocyclization, peptide

Introduction

G-quadruplex stabilization by small molecules is a promising strategy to develop anticancer therapeutic drugs.[1] Many small molecules have been reported to bind to and stabilize G-quadruplex and some of them have proven to be efficacious in cancer cell lines and xenoraft tumor models.[2] Telomestatin **1**, (Figure 1) a natural product isolated from *Streptomyces anulatus* 3533-SV4, is the most potent G-quadruplex stabilizer identified[3] and widely used to probe the *in vivo* significance of G-quadruplex.[4] Telomestatin **1** has a unique macrocyclic structure consisting of seven oxazole rings and one thiazoline ring (Figure 1). Molecular modeling studies suggest that the exceptional activity of telomestatin may be attributed to the π-π stacking interaction

between the oxazole rings and the end G-tetrads of G-quadruplex.[5] The unique structure and significance of biological activity have rendered telomestatin an attractive synthetic target.[6] The total synthesis of telomestatin was successfully completed in 2006.[7] However, the synthesis involved many steps and thus telomestatin is still not available in great quantity. Alternatively, some poly-oxazole macrocycles have emerged as a promising new class of anticancer agents that target G-quadruplex DNA.[8] Here, we report the synthesis of a tetraoxazole peptide macrocycle **2a** and an epimerization product **2b**. With structural similarities with telomestatin, these two molecules might potentially be used to probe G-quadruplex biological significance *in vivo*.

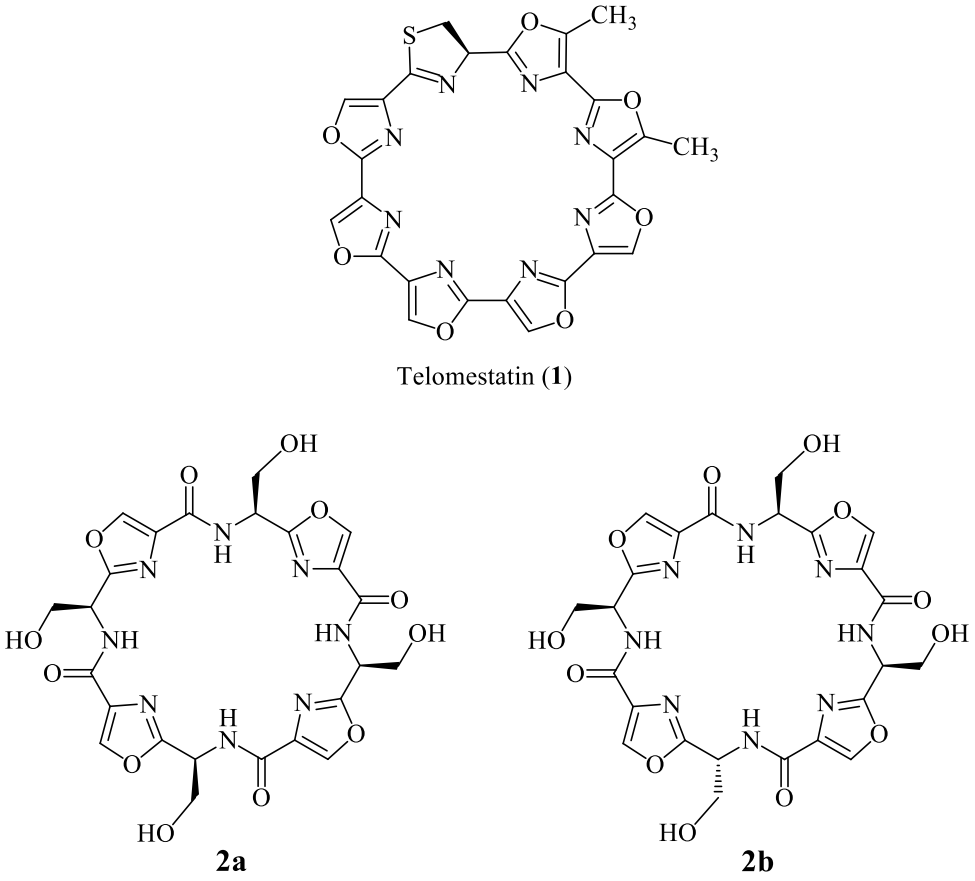
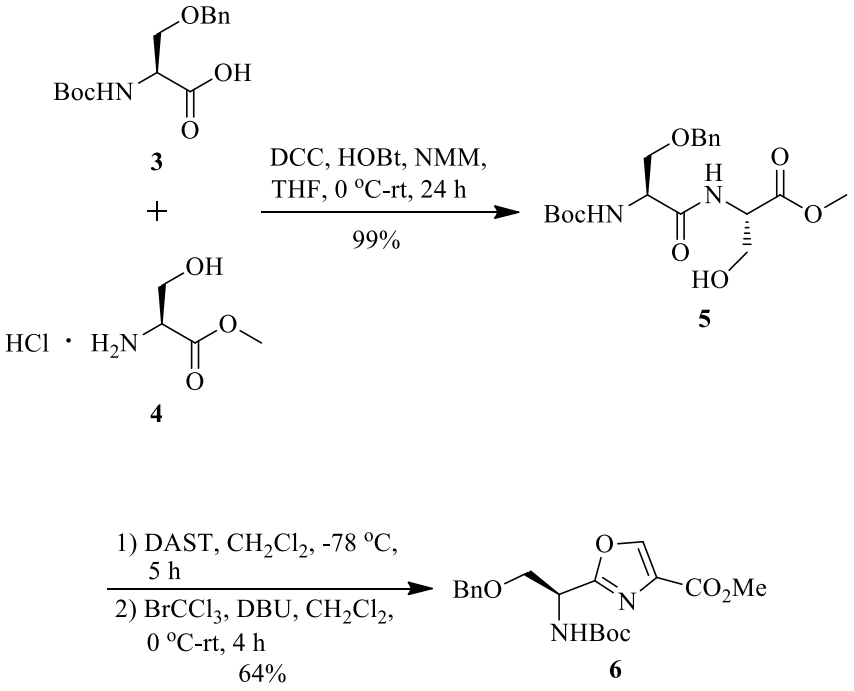


Figure 1. Structure of telomestatin **1** and two tetraoxazole peptide macrocycles **2a** and **2b.**

Results and Discussion

The biosynthesis of telomestatin has not been elucidated. A reasonable hypothesis is that it is constructed from a cyclooctapeptide containing five serine, two threonine and one cysteine. According to this, we selected Boc-L-Serine(Bzl)-OH **3** and H-L-Serine-OMe·HCl **4** as starting materials for the synthesis of **2a**. (Scheme 1).



Scheme 1. Synthesis of 2,4-disubstituted oxazole unit **6**.

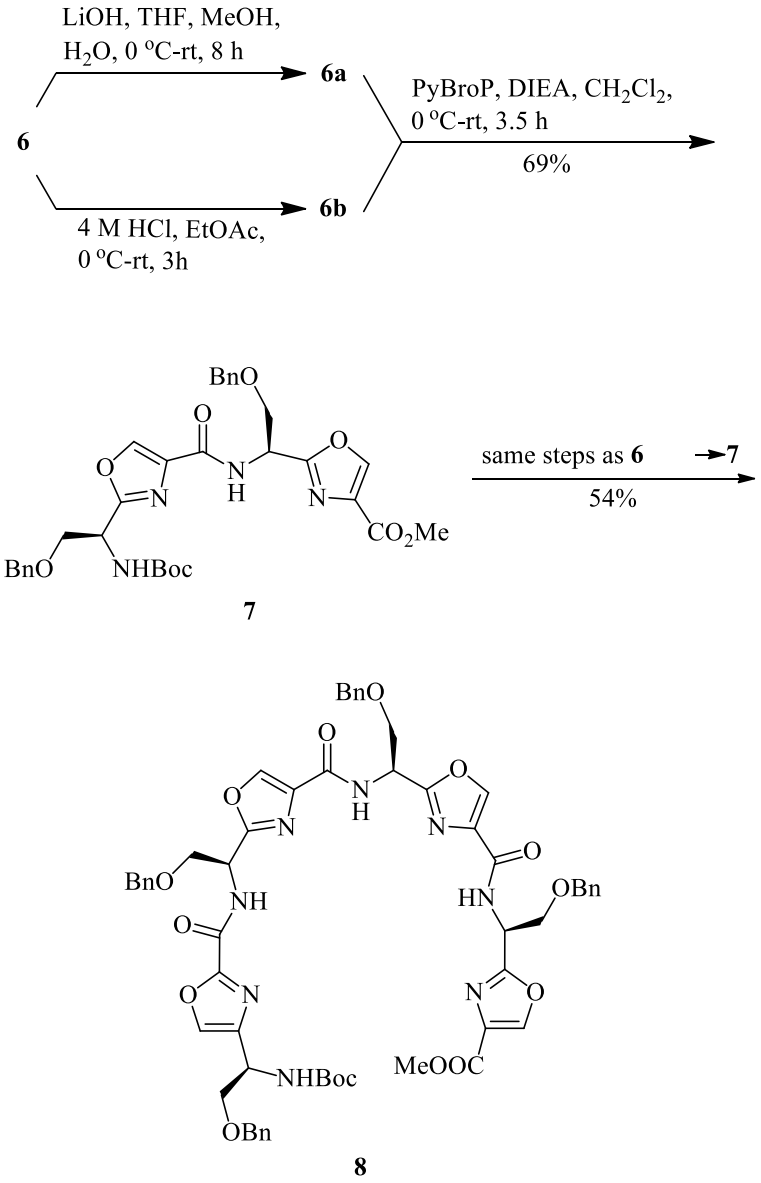
Dipeptide **5** was readily synthesized by condensation of **3** and **4** using the DCC[9] –HOBt[10] method in 99% yield.[6a] Cyclodehydration of **5** using DAST,[11] followed by treatment with $BrCCl_3$–DBU[12] afforded 2,5-disubstituted oxazole **6**,[6a] to be used as the repeating unit to construct the linear tetraoxazole amide **8** (Scheme 2).

The resulting 2,5-disubstituted oxazole **6** was then divided into two portions, with one portion treated with 4 M HCl in ethyl acetate to remove the Boc group to give ammonium chloride **6a**[6a] and the other portion hydrolyzed with LiOH to give carboxylic acid **6b**. (Scheme 2) Coupling of **6a** and **6b** using PyBroP[13]–DIEA[14] gave a di-oxazole amide **7** in 69% yield. Then, the same protocol was used again to treat **7**. These two-cycle couplings afforded the linear tetraoxazole amide **8**.

The precursor for macrocyclization was prepared by successive use of the hydrolysis and acidolysis treatments on **8** to remove both the methyl and Boc group (Scheme 3).

We tried both HATU[15] and DPPA[16] –HOBt as coupling reagents for macrolactamization of **8**, but only the latter in the presence of DMAP[17] gave the desired product in a satisfactory yield. (Scheme 3) The reaction was performed by adding a solution of the linear precursor (10 μmol/mL) to a solution of DPPA-HOBt (30 μmol/mL) at room temperature. The crude macrocyclization product was purified by flash chromatography was then loaded on a semi-preparative HPLC for further purification. The desired product **9a** and an epimerization product **9b** (Scheme 3) were obtained in an overall isolated yield of 20% (**9a** : **9b** = 3 : 1). This is easy to understand since racemization is inevitable in the long reaction time for hydrolysis, acidolysis

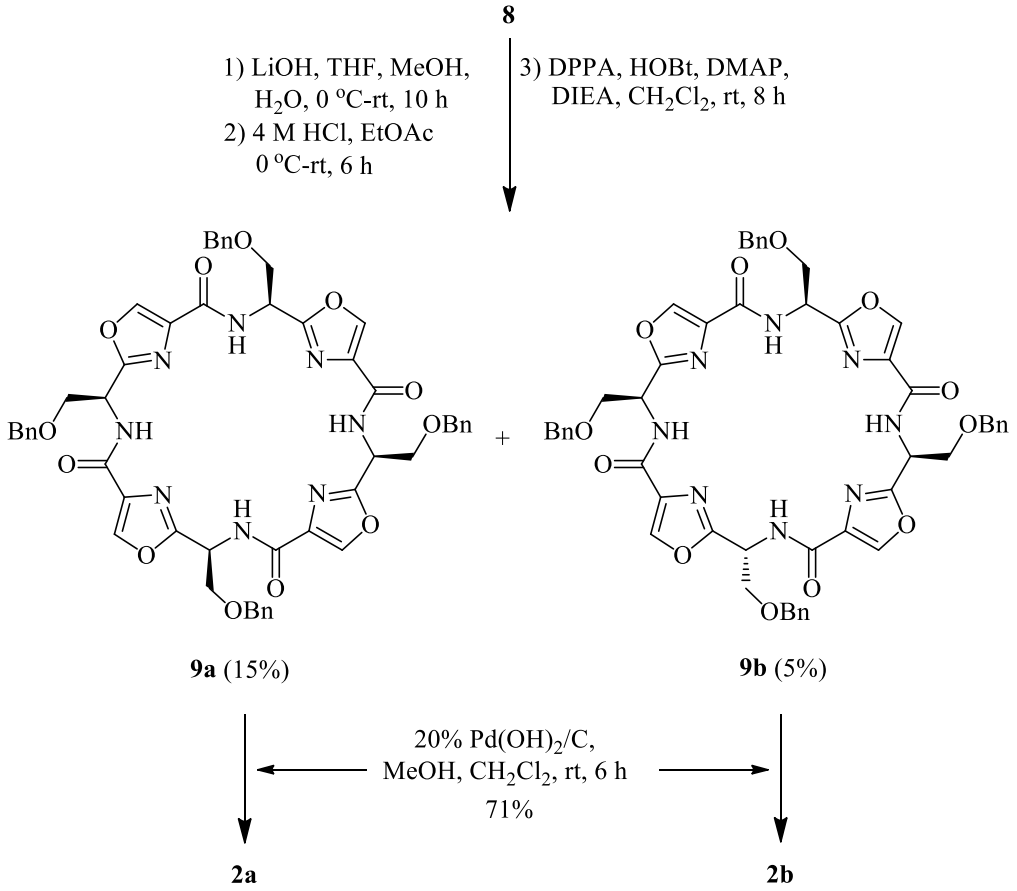
and macrolactamization. The two products were then treated by 20 wt% $Pd(OH)_2/C$ in a solution of MeOH : CH_2Cl_2 = 1 : 1 respectively to remove the protecting benzyl groups. The target molecule **2a** and a stereomer **2b**, corresponding to **9a** and **9b** respectively, were obtained in a same yield (71%).



Scheme 2. Synthesis of linear tetraoxazole amide **8**.

Poly-oxazole macrocycles have been demonstrated to selectively bind to G-quadruplex DNA and but not to duplex DNA.[8] It remains to be seen whether compounds **2a** and **2b** could be useful in this regard. Very recently, a family of azole-modified cyclic peptides which have a core

similar to that in **2a** were reported.[18] These compounds were demonstrated to be effective anion receptors.



Scheme 3. Synthesis of tetraoxazole peptide macrocycles **2a** and **2b.**

Conclusions

In conclusion, the convergent synthesis of a tetraoxazole peptide macrocycle **2a** and an epimerization product **2b** has been demonstrated: two-cycle couplings of the oxazole-containing synthetic unit **6** were employed, followed by macrocyclization and removal of the protecting groups. These two molecules might potentially be used to probe G-quadruplex biological significance *in vivo*.

Experimental Section

General. NMR spectra were recorded on Varian 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C) or a Bruker 400 MHz NMR instrument in the indicated solvent. Chemical shifts are reported in 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) when the internal standard is not indicated. Multiplicities are reported by using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; *J*, coupling constants in Hertz. ESI-MS spectra were obtained using a Finnigan LCQ Deca XP Plus mass spectrometer. HRMS (ESI) spectra were obtained using Bruker Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (APEX IV). IR **s**pectra were recorded on AVATAR 330FT-IR THERMO NICOLET spectrophotometer. Only the strongest and/or structurally important IR absorption data is reported, given in cm^{-1}. Optical rotations were measured with a Perkin-Elmer 341-LC polarimeter. CD spectra were recorded on a Jasco J-810 spectropolarimeter. Spectra were baseline-corrected and the signal contributions of the buffer were subtracted. Semi-preparative reversed-phase HPLC (UV 215 nm) was performed on a Waters 600 system with 2996 Photodiode Array Detector. The column used was Waters Symmetry Prep C18 Column, 7 μm, 78x300 mm.

Preparation of *N*-Boc-Ser(Bzl)-Ser-OMe (5). To a solution of *N*-Boc-L-Ser(Bzl)-OH **3** (5.91 g, 20.0 mmol) in THF (60 mL, 3 mL/mmol) was added H-Ser-L-OMe•HCl **4** (3.11 g, 20.0 mmol), HOBt (2.97 g, 22.0 mmol) and NMM (2.4 mL, 22.0 mmol) at rt and the mixture was cooled to 0 °C. To the mixture was added DCC (4.54 g, 22.0 mmol) at the same temperature and stirred at room temperature. After being stirred at the same temperature for 24 h, the mixture was filtered and the filtrate was concentrated in vacuo. To the resulted residue, 60 mL CH_2Cl_2 was added and the undissolved solid was removed by filtration. The solution was then washed with 1 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with 50% EtOAc in PE to afford *N*-Boc-Ser(Bzl)-Ser-OMe **5** (7.85 g, 19.8 mmol, 99%) as a colorless oil. $[\alpha]_D^{20}$ -18.6 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35-7.30 (m, 5H, phenyl), 5.43 (brd, *J* 5.42 Hz, 1H, BocN*H*), 4.64 (m, 1H, NHCH(CH_2Ophenyl)CO, 4.55 (s, 2H, CH_2phenyl), 4.31 (m, 1H, NHCH(CH_2OH)CO), 3.95-3.91 (m, 3H, CHCH_2O), 3.77 (s, 3H, CH_3O), 3.63 (dd, J_1 5.68 Hz, J_2 9.25 Hz, 1H, CHCH_2OCH_2), 1.45 (s, 9H, $(CH_3)_3$). ^{13}C NMR (75 MHz $CDCl_3$) δ 170.7, 170.5, 155.6, 137.2, 128.5, 128.0, 127.8, 80.7, 73.5, 69.8, 62.8, 55.0, 54.4, 52.7, 28.2. FT-IR (neat) 3314, 2953, 1664, 1522, 1367, 1165, 1026, 743 cm^{-1}. MS (ESI) calcd. for $C_{19}H_{28}N_2O_7Na$ $[M+Na]^+$ 419.18 found 419.11. HRMS (ESI) calcd. for $C_{19}H_{28}N_2O_7Na$ $[M+Na]^+$ 419.1789 found 419.1795.

Oxazole (6). To a solution of dipeptide **5** (7.53 g, 19.0 mmol) in CH_2Cl_2 (57 mL, 3 mL/mmol) was injected a solution of DAST (3.01 mL, 22.8 mmol) in 10 mL CH_2Cl_2 at -78 °C under N_2. After being stirred at the same temperature for 5 h, the mixture was poured into saturated aqueous $NaHCO_3$ at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was used for the next reaction without further purification.

To a solution of oxazoline in CH_2Cl_2 (57 mL, 3.0 mL/mmol) was added DBU (8.34 mL, 57 mmol) at 0 °C. To the mixture was added dropwise $BrCCl_3$ (5.62 mL, 57 mmol) at the same temperature. After being stirred at rt for 4 h, the mixture was poured into 3 M HCl at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with 3 M HCl, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on flash silica gel with 25% EtOAc in PE to afford oxazole **6** (4.58 g, 12.2 mmol, 64% in 2 steps) as a white powder. $[\alpha]_D^{20}$ -10.6 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.20 (s, 1H, CH=), 7.35-7.20 (m, 5H, phenyl), 5.57 (brd, *J* 8.46 Hz, 1H, BocN*H*), 5.13 (m, *J* 4.31 Hz, 1H, CH), 4.50 (q, 2H, O$\underline{CH_2}$phenyl), 3.92-3.90 (m, 4H, CH$\underline{CH_2}$O, CH_3O), 3.80 (dd, *J* 4.41 Hz, 1H, *J* 9.59 Hz, CH$\underline{CH_2}$O), 1.45 (s, 9H, $(CH_3)_3$). ^{13}C NMR (75 MHz $CDCl_3$) δ 163.6, 161.4, 155.1, 144.1, 137.3, 133.3, 128.4, 127.8, 127.6, 80.4, 73.2, 70.4, 52.2, 49.3, 28.2. FT-IR (neat) 3354, 2978, 1715, 1585, 1513, 1454, 1367, 1323, 1251, 1166, 1111, 885, 741 cm^{-1}. MS (ESI) calcd. for $C_{19}H_{24}N_2O_6Na$ $[M+Na]^+$ 399.15 found 399.10. HRMS (ESI) calcd. for $C_{19}H_{24}N_2O_6Na$ $[M+Na]^+$ 399.1527 found 399.1529.

Di-oxazole (7) and tetra-oxazole (8). To a solution of oxazole **6** (2.26 g, 6.00 mmol) in MeOH (12.0 mL, 2.0 mL/mmol), THF (12.0 mL, 2.0 mL/mmol) and H_2O (1.2 mL, 0.2 mL/mmol) was added $LiOH•H_2O$ (252 mg, 6.00 mmol) at 0 °C. The mixture was allowed to warm to rt over 2 h. After being stirred at the same temperature for another 6 h, the mixture was acidified with 3 M HCl at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The crude acid **6a** was used for next reaction without further purification.

To another solution of oxazole **6** (2.26 g, 6.00 mmol) in EtOAc (10 mL) was added 4 M HCl in EtOAc (40 mL) at 0 °C. After being stirred at the same temperature for 3 h, the solvent was removed under reduced pressure. The crude ammonium hydrochloride **6b** was used for the next reaction without further purification.

To a solution of the resulted crude **6a** in CH_2Cl_2 (60 mL, 10 mL/mmol) was added the **6b**, DIEA (4.96 mL, 30.0 mmol) and the mixture was cooled to 0 °C. To the mixture was added PyBroP (4.20 g, 9.00 mmol) at the same temperature. The mixture was allowed to warm up to rt over 2 h. After being stirred at the same temperature for another 1.5 h, the mixture was poured into 1 M HCl at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with 3 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on flash silica gel with 50% PE in EtOAc to afford di-oxazole **7** (2.57 g, 4.14 mmol, 69%). $[\alpha]_D^{20}$ -12.7 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.20 (s, 1H, CH=), 8.19 (s, 1H, CH=), 7.76 (brd, *J* 8.60 Hz, 1H, N*H*), 7.35-7.21 (m, 10H, phenyl), 5.60 (dt, *J* 4.32 Hz, *J* 8.69 Hz, 1H, CH), 5.53 (brd, *J* 7.45 Hz, 1H, BocN*H*), 5.10 (m, 1H, b), 4.60-4.48 (m, 4H, $\underline{CH_2}$phenyl), 4.03 (dd, *J* 4.48 Hz, *J* 9.69 Hz, 1H, CH$\underline{CH_2}$O), 3.93-3.86 (m, 5H, $\underline{CH_2}$phenyl, CH$\underline{CH_2}$O), 3.82 (dd, *J* 4.08 Hz, *J* 9.48 Hz, 1H, CH$\underline{CH_2}$O), 1.46 (s, 9H, $(CH_3)_3$). ^{13}C NMR (75 MHz $CDCl_3$) δ 162.6, 162.5, 161.3, 160.3, 155.2, 144.2, 142.0, 137.1, 135.4, 133.4, 128.4, 127.9, 127.7, 127.6, 80.5, 73.3, 73.2, 70.1,

69.7, 52.2, 49.4, 47.3, 28.2. FT-IR (neat) 3308, 2978, 2873, 1715, 1597, 1508, 1454, 1366, 1323, 1250, 1166, 1108, 863, 738 cm^{-1}. MS (ESI) calcd. for $C_{32}H_{36}N_4O_9Na$ $[M+Na]^+$ 643.24 found 643.15. HRMS (ESI) calcd. for $C_{32}H_{36}N_4O_9Na$ $[M+Na]^+$ 643.2374 found 643.2371.

The same saponification, acidolysis, coupling treatments (same ratios of reactants, reagents, concentrations of reaction solutions and reaction time) and same work-up protocols as above were used to prepare tetra-oxazole **8** from di-oxazole **7** with the exception that 1.5 equiv., instead of 1.0 equiv. $LiOH•H_2O$ was used for saponification and an eluant of 25%, instead of 50%, PE in EtOAc was used for separation and purification of tetra-oxazole **8** (1.20 g, 1.08 mmol, 54%) by column chromatography. $[\alpha]_D^{20}$ -10.7 (*c* = 0.1, $CHCl_3$). ^1H NMR (300 MHz $CDCl_3$) δ8.19 (s, 1H, OCH=), 8.18 (s, 1H, OCH=), 8.17 (s, 1H, OCH=), 8.16 (s, 1H, OCH=), 7.67 (brd, 4H, N*H*), 7.33-7.18 (m, 20H, phenyl), 5.57 (m, 3H, CH), 5.46 (brd, *J* 8.38 Hz, 1H, BocN*H*), 5.07 (m, 1H, CH), 4.61-4.29 (m, 8H, $\underline{CH_2}$phenyl), 4.03-3.77 (m, 11H, CH_3, CH$\underline{CH_2}$O), 1.44 (s, 9H, $(CH_3)_3$). ^{13}C NMR (75 MHz $CDCl_3$) δ162.6, 162.4, 161.54, 161.51, 161.3, 160.1, 160.0, 155.1, 144.1, 141.94, 141.88, 141.79, 127.77, 127.6, 127.5, 80.3, 73.14, 73.07, 70.0, 69.7, 69.51, 69.47, 65.5, 52.0, 49.3, 47.26, 47.21, 28.2. FT-IR (neat) 3269, 2978, 1718, 1669, 1600, 1521, 1454, 1366, 1323, 1207, 1167, 1108, 869, 740 cm^{-1}. MS (ESI) calcd. for $C_{58}H_{60}N_8O_{15}Na$ $[M+Na]^+$ 1131.41 found 1131.36. HRMS (ESI) calcd. for $C_{58}H_{60}N_8O_{15}Na$ $[M+Na]^+$ 1131.4070 found 1131.4091.

Macrolactams (9a) and (9b). To a solution of tetra-oxazole **8** (100 mg, 0.09 mmol) in MeOH (6.3 mL, 70 mL/mmol), THF (6.3 mL, 70 mL/mmol) and H_2O (0.27 mL, 3 mL/mmol) was added $LiOH•H_2O$ (37.8 mg, 0.9 mmol) at 0 °C. The mixture was allowed to warm up to rt over 2 h. After being stirred at the same temperature for another 8 h, the mixture was acidified with 3 M HCl at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo.

The above crude acid was taken up in 5 mL EtOAc. To the mixture was added 4 M HCl in EtOAc (20 mL) at 0 °C. After being stirred at the same temperature for 6 h, the solvent was removed under reduced pressure. The residue was used for the next reaction without further purification.

To a solution of DPPA (2.7 mmol), HOBt (2.7 mmol), DIEA (3.6 mmol) and DMAP (0.09 mmol) in CH_2Cl_2 (35 mL) was added dropwise a solution of the above residue in 10 mL CH_2Cl_2 over 4 h at rt. After being stirred at the same temperature for another 4 h, the mixture was poured into 1 M HCl at 0 °C. The aqueous layer was extracted twice with CH_2Cl_2. The organic layer was washed with 3 M HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo. The residue was purified by column chromatography on flash silica gel with 50% EtOAc in PE, and by semi-preparative HPLC eluting with H_2O : MeCN : CF_3COOH = 52 : 48 : 0.5 to afford macrolactam **9a** (t_R = 62.8 min, 13.2 mg, 13.5 μmmol, 15%) and **9b** (t_R = 52.3 min 4.4 mg, 4.5 μmmol, 5%), each as a white powder.

9a. $[\alpha]_D^{20}$ –1.8 (*c* = 0.1, $CHCl_3$). ^1H NMR (300 MHz $CDCl_3$) δ 8.26 (s, 4H, CH=), 8.05 (brd, *J* 9.56 Hz, 4H, N*H*), 7.28-7.14 (m, 20H, phenyl, 5.73 (m, 4H, CH), 4.59-4.44 (m, 8H, O$\underline{CH_2}$phenyl), 3.94 (dd, *J* 3.89 Hz, *J* 9.06 Hz, 4H, CH$\underline{CH_2}$O), 3.82 (dd, *J* 3.70 Hz, *J* 9.48 Hz,

4H, CH$\underline{CH_2}$O). ^{13}C NMR (75 MHz $CDCl_3$) δ 161.9, 159.9, 142.5, 137.0, 135.7, 128.4, 127.9, 127.7, 73.2, 69.9, 47.4. FT-IR (neat) 3400, 2920, 1676, 1599, 1512, 1454, 1364, 1204, 1103, 873, 735 cm^{-1}. MS (ESI) calcd. for $C_{52}H_{48}N_8O_{12}Na$ $[M+Na]^+$ 999.33 found 999.35. HRMS (ESI) calcd. for $C_{52}H_{48}N_8O_{12}Na$ $[M+Na]^+$ 999.3284 found 999.3294.

9b. $[\alpha]_D^{20}$ –15.3 (*c* = 0.1, $CHCl_3$). ^{1}H NMR (300 MHz $CDCl_3$) δ 8.27 (s, 1H, CH=), 8.26 (s,1H,CH=), 8.23 (s, 1H, CH=), 8.22 (s, 1H, CH=), 7.95 (d, *J* 9.26 Hz, 1H, NH), 7.76 (d, *J* 9.21 Hz, 1H, NH), 7.72 (d, *J* 8.16 Hz, 1H, NH), 7.57 (d, *J* 9.61Hz, 1H, NH), 7.30-7.07 (m, 20H, phenyl), 5.81-5.58 (m, 4H, CH), 4.63-4.38 (m, 8H, $\underline{CH_2}$phenyl), 4.09-3.75 (m, 8H, CH$\underline{CH_2}$O). 13C NMR (75 MHz $CDCl_3$) δ 162.6, 162.1, 162.0, 161.7, 160.1, 159.9, 159.8, 142.6, 142.5, 142.3, 141.7, 137.2, 136.85, 136.80, 135.84, 135.81, 135.77, 135.3, 135.3, 128.6, 128.48, 128.45, 128.43, 128.2, 128.1, 127.91, 127.86, 127.76, 127.72, 127.64, 127.59, 73.4, 73.3, 73.2, 73.1, 70.3, 70.13, 70.08, 69.6, 47.6, 47.3, 47.2, 45.7. FT-IR (neat) 3407, 3045, 1676, 1598, 1507, 1454, 1363, 1202, 1104, 869, 736 cm^{-1}. MS (ESI) calcd. for $C_{52}H_{48}N_8O_{12}Na$ $[M+Na]^+$ 999.33 found 999.36. HRMS (ESI) HRMS (ESI) calcd. for $C_{52}H_{48}N_8O_{12}Na$ $[M+Na]^+$ 999.3284 found 999.3298.

Macrocycles (2a) and (2b). To $Pd(OH)_2/C$ (50 mg, 20 wt%) was added a solution of **9a** (5.0 mg, 5.12 μmol) in CH_2Cl_2 (2.5 mL, 125 mL/mmol) and MeOH (2.5 mL, 125 mL/mmol) at rt. The reaction vessel was purged with H_2 three times. After being stirred for 3 h, the mixture was filtered through a pad of Celite and the filtrate was concentrated in vacuo. The residue was purified by semi-preparative HPLC eluted with H_2O : CH_3CN : CF_3COOH = 88 : 12 : 0.1 to afford **2a** (t_R= 15.5 min, 2.24 mg, 3.64 μmmol, 71%) and as a white powder. $[\alpha]_D^{20}$ – 6.6 (*c* = 0.1, H_2O). CD (H_2O, *c* = 1.0 x 10^{-5} M) Δε 82,000. ^{1}H NMR (400 MHz D_2O) δ 8.44 (s, 4H, CH=), 5.52 (t, *J* 5.57 Hz, 4H, $\underline{CH}$), 4.16 (dq, *J* 5.49 Hz, *J* 6.13 Hz, *J* 11.33 Hz, 8H, CH_2), 3.37 (s, c, partially exchanged with D_2O). ^{13}C NMR (100 MHz D_2O) δ 162.2, 161.6, 143.7, 134.4, 61.1, 48.5. FT-IR (neat) 3390, 2973, 2360, 1676, 1600, 1520, 1400, 1200, 1140, 833, 740 cm^{-1}. MS (ESI) calcd. for $C_{24}H_{25}N_8O_{12}$ $[M+H]^+$ 617.16 found 617.14; calcd. for $C_{24}H_{24}N_8O_{12}Na$ [M+Na]+ 639.14 found 639.20. HRMS (ESI) calcd. for $C_{24}H_{24}N_8O_{12}Na$ $[M+Na]^+$ 639.1406 found 639.1400; calcd. for $C_{24}H_{24}N_8O_{12}K$ $[M+K]^+$ 655.1145 found 655.1138.

2b. (t_R= 8.7 min, 1.79 mg, 2.90 μmmol, 71%) as a white powder was synthesized by using the same protocol as above. $[\alpha]_D^{20}$ – 15.4 (*c* = 0.1, H_2O). ^{1}H NMR (400 MHz H_2O) δ 8.51 (s, 1H, CH=), 8.49 (s, 1H, CH=), 8.48 (s, 1H, CH=), 8.45 (s, 1H, CH=), 5.57 (t, *J* 5.22 Hz, 1H, CH), 5.52-5.47 (m, 3H, CH), 4.19-4.08 (m, 8H, CH_2), 3.37 (s, c, partilly exchanged with D_2O). ^{13}C NMR (100 MHz D_2O) δ 162.4, 162.3, 162.21, 162.18, 162.0, 161.9, 161.83, 161.77, 144.6, 143.62, 143.58, 143.1, 134.64, 134.60, 134.5, 134.3, 62.2, 62.0, 61.3, 61.2, 49.4, 49.21, 49.18, 48.6. FT-IR (neat) 3361, 2935, 2395, 1664, 1598, 1519, 1443, 1204, 1120, 830, 726 cm^{-1}. MS (ESI) calcd. for $_{24}H_{25}N_8O_{12}$ $[M+H]^+$ 617.16 found 617.09; calcd. for $C_{24}H_{24}N_8O_{12}Na$ $[M+Na]^+$ 639.14 found 639.24. HRMS (ESI) $C_{24}H_{25}N_8O_{12}$ $[M+H]^+$ 617.1586 found 617.1580; calcd. for calcd. for $C_{24}H_{24}N_8O_{12}Na$ $[M+Na]^+$ 639.1406 found 639.1394; calcd. for $C_{24}H_{24}N_8O_{12}K$ $[M+K]^+$ 655.1145 found 655.1136.

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