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 xenoraph tumor models.² Telomestatin I, (Figure 1) a natural product isolated from Streptomyces anulatus 3533-SV4, is the most potent G-quadruplex stabilizer identified and widely used to probe the in vivo significance of G-quadruplex.³ Telomestatin I 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.\textsuperscript{5} The unique structure and significance of biological activity have rendered telomestatin an attractive synthetic target.\textsuperscript{6} The total synthesis of telomestatin was successfully completed in 2006.\textsuperscript{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.\textsuperscript{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 \textit{in vivo}.

![Telomestatin (I)](image_url)

**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.\(^{5a}\) 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 6\(_a\)\(^{6a}\) and the other portion hydrolyzed with LiOH to give carboxylic acid 6\(_b\). (Scheme 2) Coupling of 6\(_a\) and 6\(_b\) 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 \(\mu\)mol/mL) to a solution of DPPA–HOBt (30 \(\mu\)mol/mL) at room temperature. The crude macrolactamization product was purified by flash chromatography was then loaded on a semi-preparative HPLC for further purification. The desired product 9\(_a\) and an epimerization product 9\(_b\) (Scheme 3) were obtained in an overall isolated yield of 20% (9\(_a\) : 9\(_b\) = 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$_2$Cl$_2$ = 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.](image)

Poly-oxazole macrocycles have been demonstrated to selectively bind to G-quadruplex DNA and but not to duplex DNA. 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. These compounds were demonstrated to be effective anion receptors.

![Scheme 3](image)

**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 1H, 75 MHz for 13C) 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$_2$ (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 spectra 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 341-1C 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$\cdot$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$_2$Cl$_2$ 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$_2$SO$_4$, 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$\cdot$H), 4.64 (m, 1H, NHCH(CH$_2$Ophenyl)CO), 4.55 (s, 2H, CH$_2$phenyl), 4.31 (m, 1H, NHCH(CH$_2$OH)CO), 3.95-3.91 (m, 3H, CH$_2$CH$_2$O), 3.77 (s, 3H, CH$_3$O), 3.63 (dd, $J_1$ 5.68 Hz, $J_2$ 9.25 Hz, 1H, CH$_2$OCH$_2$), 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, 127.5, 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$_2$O$_7$Na [M+Na]$^+$ 419.18 found 419.11. HRMS (ESI) calcd. for C$_{19}$H$_{28}$N$_2$O$_7$Na [M+Na]$^+$ 419.1789 found 419.1795.

**Oxazole (6).** To a solution of dipeptide 5 (7.53 g, 19.0 mmol) in CH$_2$Cl$_2$ (57 mL, 3 mL/mmOl) was injected a solution of DAST (3.01 mL, 22.8 mmol) in 10 mL CH$_2$Cl$_2$ 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$_2$Cl$_2$. The organic layer was washed with saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, 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 CH₂Cl₂ (57 mL, 3.0 mL/mmol) was added DBU (8.34 mL, 57 mmol) at 0 °C. To the mixture was added dropwise BrCCl₃ (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 CH₂Cl₂. The organic layer was washed with 3 M HCl, saturated aqueous NaHCO₃, brine, dried over Na₂SO₄, 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. [α]₂⁰ -10.6 (c = 0.1, CHCl₃). ¹H NMR (300 MHz CDCl₃) δ 8.20 (s, 1H, CH=), 7.35-7.20 (m, 5H, phenyl), 5.57 (brd, J 8.46 Hz, 1H, BocNH), 5.13 (m, J 4.31 Hz, 1H, CH), 4.50 (q, 2H, OCH₂phenyl), 3.92-3.90 (m, 4H, CHCH₂O, CH₂O), 3.80 (dd, J 4.41 Hz, 1H, J 9.59 Hz, CHCH₂O), 1.45 (s, 9H, (CH₃)₃). ¹³C NMR (75 MHz CDCl₃) 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 C₁₉H₂₄N₂O₆Na [M+Na]⁺ 399.15 found 399.10. HRMS (ESI) calcd. for C₁₉H₂₄N₂O₆Na [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 H₂O (1.2 mL, 0.2 mL/mmol) was added LiOH•H₂O (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 CH₂Cl₂. The organic layer was washed with brine, dried over Na₂SO₄, 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 CH₂Cl₂ (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 CH₂Cl₂. The organic layer was washed with 3 M HCl, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, 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%). [α]₂⁰ -12.7 (c = 0.1, CHCl₃). ¹H NMR (300 MHz CDCl₃) δ 8.20 (s, 1H, CH=), 8.19 (s, 1H, CH=), 7.76 (brd, J 8.60 Hz, 1H, NH), 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, BocNH), 5.10 (m, 1H, b), 4.60-4.48 (m, 4H, CH₂phenyl), 4.03 (dd, J 4.48 Hz, J 9.69 Hz, 1H, CHCH₂O), 3.93-3.86 (m, 5H, CH₂phenyl, CHCH₂O), 3.82 (dd, J 4.08 Hz, J 9.48 Hz, 1H, CHCH₂O), 1.46 (s, 9H, (CH₃)₃). ¹³C NMR (75 MHz CDCl₃) 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, 6
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 C₃₂H₆₆N₄O₉Na [M+Na]+ 643.24 found 643.15. HRMS (ESI) calcd. for C₃₂H₆₆N₄O₉Na [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•H₂O 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. [α]D²⁰ -10.7 (c = 0.1, CHCl₃). ¹H NMR (300 MHz CDCl₃) δ 88.19 (s, 1H, OCH=), 8.18 (s, 1H, OCH=), 8.17 (s, 1H, OCH=), 8.16 (s, 1H, OCH=), 7.67 (brd, 4H, NH), 7.33-7.18 (m, 20H, phenyl), 5.57 (m, 3H, CH), 5.46 (brd, J 8.38 Hz, 1H, BocNH), 5.07 (m, 1H, CH), 4.61-4.29 (m, 8H, CH₂phenyl), 4.03-3.77 (m, 11H, CH₃, CHCH₂O), 1.44 (s, 9H, (CH₃)₃).

¹³C NMR (75 MHz CDCl₃) δ 162.6, 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 C₅₈H₇₀N₈O₁₅Na [M+Na]+ 1131.41 found 1131.36. HRMS (ESI) calcd. for C₅₈H₇₀N₈O₁₅Na [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 H₂O (0.27 mL, 3 mL/mmol) was added LiOH•H₂O (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 CH₂Cl₂. The organic layer was washed with brine, dried over Na₂SO₄, 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 CH₂Cl₂ (35 mL) was added dropwise a solution of the above residue in 10 mL CH₂Cl₂ 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 CH₂Cl₂. The organic layer was washed with 3 M HCl, saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, 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 H₂O : MeCN : CF₃COOH = 52 : 48 : 0.5 to afford macrolactam 9a (tr = 62.8 min, 13.2 mg, 13.5 μmmol, 15%) and 9b (tr = 52.3 min 4.4 mg, 4.5 μmmol, 5%), each as a white powder.

**9a.** [α]D²⁰ -1.8 (c = 0.1, CHCl₃). ¹H NMR (300 MHz CDCl₃) δ 8.26 (s, 4H, CH=), 8.05 (brd, J 9.56 Hz, 4H, NH), 7.28-7.14 (m, 20H, phenyl), 5.73 (m, 4H, CH), 4.59-4.44 (m, 8H, OCH₂phenyl), 3.94 (dd, J 3.89 Hz, J 9.06 Hz, 4H, CHCH₂O), 3.82 (dd, J 3.70 Hz, J 9.48 Hz,
4H, CHCH₂O). ¹³C NMR (75 MHz CDCl₃) 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 C₅₂H₆₈N₈O₁₂Na [M+Na]⁺ 999.33 found 999.35. HRMS (ESI) calcd. for C₃₂H₄₈N₈O₁₂Na [M+Na]⁺ 999.3284 found 999.3294.

9b. [α]₀⁺²⁰ –15.3 (c = 0.1, CHCl₃). ¹H NMR (300 MHz CDCl₃) 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 9.61 Hz, 1H, NH), 7.30-7.07 (m, 20H, phenyl), 5.81-5.58 (m, 4H, CH), 4.63-4.38 (m, 8H, CH₂phenyl), 4.09-3.75 (m, 8H, CHCH₂O). ¹³C NMR (75 MHz CDCl₃) 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 C₅₂H₆₈N₈O₁₂Na [M+Na]⁺ 999.33 found 999.36. HRMS (ESI) HRMS (ESI) calcd. for C₅₂H₆₈N₈O₁₂Na [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 CH₂Cl₂ (2.5 mL, 125 mM/μmol) and MeOH (2.5 mL, 125 mM/μmol) at rt. The reaction vessel was purged with H₂ 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 H₂O : CH₃CN : CF₃COOH = 88 : 12 : 0.1 to afford 2a (tₘₜ = 15.5 min, 2.24 mg, 3.64 μmmol, 71%) and as a white powder. [α]₀⁺²⁰ –6.6 (c = 0.1, H₂O). CD (H₂O, c = 1.0 x 10⁻⁵ M) Δε 82,000. ¹H NMR (400 MHz D₂O) δ 8.44 (s, 4H, CH=), 5.52 (t, J 5.57 Hz, 4H, CH), 4.16 (dq, J 5.49 Hz, J 6.13 Hz, J 11.33 Hz, 8H, CH₂), 3.37 (s, c, partially exchanged with D₂O). ¹³C NMR (100 MHz D₂O) 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 C₂₄H₂₅N₈O₁₂ [M+H]⁺ 617.16 found 617.14; calcd. for C₂₄H₂₅N₈O₁₂Na [M+Na]⁺ 639.14 found 639.20. HRMS (ESI) calcd. for C₂₄H₂₅N₈O₁₂Na [M+Na]⁺ 639.1406 found 639.1400; calcd. for C₂₄H₂₅N₈O₁₂K [M+K]⁺ 655.1145 found 655.1138.

2b. (tₘₜ = 8.7 min, 1.79 mg, 2.90 μmmol, 71%) as a white powder was synthesized by using the same protocol as above. [α]₀⁺²⁰ –15.4 (c = 0.1, H₂O). ¹H NMR (400 MHz H₂O) 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, CH₂), 3.37 (s, c, partially exchanged with D₂O). ¹³C NMR (100 MHz D₂O) 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, 133.5, 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 C₂₄H₂₅N₈O₁₂ [M+H]⁺ 617.16 found 617.09; calcd. for C₂₄H₂₅N₈O₁₂Na [M+Na]⁺ 639.14 found 639.24. HRMS (ESI) C₂₄H₂₅N₈O₁₂ [M+H]⁺ 617.1586 found 617.1580; calcd. for calcd. for C₂₄H₂₅N₈O₁₂Na [M+Na]⁺ 639.1406 found 639.1394; calcd. for C₂₄H₂₅N₈O₁₂K [M+K]⁺ 655.1145 found 655.1136.
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References and Notes


14. DIEA = N,N-diisopropylethylamine.


17. DMAP = 4-dimethylaminopyridine.