Cyclic aromatic oligoamides as highly selective receptors for the guanidinium ion

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General Methods

Chemicals were purchased from Aldrich, Acros and used as received. All reactions were performed under argon in absence of light. All chemicals were obtained from commercial suppliers and were used as received unless otherwise noted. CH₂Cl₂ was dried over CaH₂. Unless otherwise specified, all solvents were removed with a rotary vacuum evaporator. Analytical thin layer chromatography (tlc) was conducted on Analtech Uniplate silica gel plates with detection by UV light.

NMR analyses were carried out on Varian INOVA 500 spectrometer (500 MHz) or Varian INOVA 400 spectrometer at 20°C (unless otherwise noted). Tetramethylsilane (TMS) or deuterated solvent DMSO-d₆ was used as the internal standard for ¹H NMR. Chemical shifts are reported in ppm values downfield from tetramethylsilane and J values are reported in Hz. Electrospray mass spectrum (ES-MS) was recorded on a PEAPI III Triple Quad mass spectrometer, and the concentration of the samples was about 1.0 mmol/mL. The diluted solution was electrosprayed at a flow rate of 5×10⁻⁶ L/min with a needle voltage of 4.5 kV. The mobile phase was an aqueous solution of methanol (V/V, 1:1). MALDI experiments were performed using a Bruker Biflex IV matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometer with a matrix of 9-nitroanthracene or dithranol. Mass spectra were acquired in positive reflector mode and using an acceleration voltage of 19 kV. External mass calibration was performed using a standard PEG-2000 mixture. Spectra were obtained by setting the laser power close to the threshold of ionization and generally 300 pulses were acquired and averaged. Solvents were purified and dried according to standard procedures.
II. Experimental

MALDI Binding Experiments. The matrix solution of 9-nitroanthracene (20mM, CHCl₃) was pre-spotted (2µL) onto the steel sample target. Host-guest mixtures (1µL, 1-2mM, 10%MeOH 90%CHCl₃) were directly spotted onto the sample well and drawn back into the capillary tube and spotted again to insure an even matrix-sample mixture. The matrix-sample solution was air-dried on the target at ambient temperature.

¹H NMR experiments in solvents of variable polarity. A stock 10mM solution of 1b and 1.1 eq. guanidinium thiocyanate was prepared in the primary solvent (CDCl₃ or acetone-d₆). To aliquots of the stock solution was added a 9:1 MeOD:MeOH mixture and diluted to equal volumes with the primary solvent to achieve 1mM solutions of 1b of varying MeOD/MeOH concentrations. The same was done to a stock solution of 1b alone.

Synthetic procedures

Scheme 1
Macrocycles 1a-d were prepared as previously described and were characterized by $^1$H NMR and mass spectrometry (ESI and MALDI-TOF).

**Compound 1a** [R= -(CH$_2$CH$_2$O)$_3$OCH$_3$]. Yield 61.0%. $^1$H-NMR (500 MHz, CDCl$_3$): 9.69 (s, Ar, 3H), 9.68 (s, NH, 6H), 9.16 (s, Ar, 3H), 6.70 (s, Ar, 3H), 6.59 (s, Ar, 3H), 4.47 (t, 6H, OCH$_2$), 4.09 (t, 6H, OCH$_2$CH$_2$), 3.98, 3.48, 3.34 (OMe, TgOMe), 3.95, 3.77, 3.64, 3.60, 3.50 (m, t, t, t, t, CH$_2$ of Tg), 3.34 (s, MeO of Tg). MS (ESI) m/e: calcd for C$_{90}$H$_{126}$N$_6$O$_{36}$ (M$^+$): 1866.82, found (M+H)$^+$: 1867.7, (M+Na)$^+$: 1689.7.

**Compound 1b** [R= -CH$_2$CH(CH$_3$)OCH$_2$CH$_2$CH(CH$_3$)$_2$]. Yield 41.8%. $^1$H NMR (CDCl$_3$): δ 9.44 (s, 3H), 9.38 (s, 3H), 9.31 (s, 6H), 6.56 (s, 3H), 6.50 (s, 3H), 4.27 (q, 6H), 4.06 (t, 6H), 4.00 (q, 6H), 3.63 (q, 6H), 3.57 (q, 6H), 1.68 (m, 6H), 1.45 (q, 12H), 1.39 (d, OCH$_3$, 18H), 0.86(d, CH$_3$, 36H). MS (MALDI-TOF) m/z: calcd for C$_{96}$H$_{138}$N$_6$O$_{24}$ (M$^+$): 1758.98, found (M+G)$^+$: 1819.0.

![Fig. S1 MALDI-TOF spectrum of compound 1b in the presence of guanidine thiocyanate](image-url)
**Compound 1c** (R=n-C$_8$H$_{17}$). Yield 65.8%. $^1$H NMR (80%DMF-d$_7$-20%CDCl$_3$, 60°C): 10.06 (s, NH, 6H), 9.66 (s, Ar, 3H), 9.09 (s, Ar, 3H), 7.00 (s, Ar, 3H), 6.91 (s, Ar, 3H), 4.47 (t, OCH$_2$, 12H), 4.02 (s, OCH$_3$, 18H), 2.01 (quintet, CH$_2$, 12H), 1.54 (quintet, CH$_2$, 12H), 1.42 (quintet, CH$_2$, 12H), 1.31-1.26 (m, CH$_2$, 36H), 0.86 (m, CH$_3$, 18H). MS (ESI) m/z: calcd for C$_{96}$H$_{138}$N$_6$O$_{18}$ (M$^+$): 1663.0, found (M+H)$^+$: 1664.0, (M+Na)$^+$: 1686.0.

**Compound 1d** (R=CH$_2$CH$_2$COOEt). Yield 81.5%. $^1$H NMR (DMSO-d$_6$): δ 9.58 (s, NH, 6H), 9.54 (s, Ar, 3H), 8.97 (s, Ar, 3H), 6.89 (s, Ar, 3H), 6.87 (s, Ar, 3H), 4.42 (t, ArOCH$_2$, 12H), 4.11 (q, CH$_2$ of COOEt, 12H), 3.96 (s, OMe, 18H), 2.60 (t, CH$_2$ of CH$_2$COOEt, 12H), 2.26 (m, COOCH$_2$CH$_3$, 18H). MS (ESI) m/e: calcd for C$_{84}$H$_{102}$N$_6$O$_{30}$ (M$^+$): 1674.66, found (M+H)$^+$: 1675.3, (M+Na)$^+$: 1697.4.

$^1$H NMR (CDCl$_3$): 9.70 (s, Ar, 3H), 9.50 (s, NH, 6H), 9.24 (s, Ar, 3H), 6.90 (s, Ar, 3H), 6.63 (s, Ar, 3H), 4.37-3.70 (m, OCH$_2$, OMe), 2.37 (t, CH$_2$ of COOEt), 1.27 (t, Me of COOEt, 18H). D$_2$O exchange experiment in 60%DMSO-d$_6$-40%CDCl$_3$ shows the disappearance of the NH signal at 9.55ppm.
III. ESI spectra

**Fig. S2** ESI spectrum of cyclic hexamer 1a [R=-(CH₂CH₂O)₃CH₃].

**Fig. S3** ESI spectrum of 1a [R=-(CH₂CH₂O)₃CH₃] and guanidine thiocyanate (1:1).
**Fig. S3** ESI spectra of (a) 1a [R=-(CH$_2$CH$_2$O)$_3$CH$_3$] alone, (b) 1a and 1 equivalent of each of guanidine thiocyanate, LiCl, NaCl, KCl, RbCl, CsCl, NH$_4$Cl in 80%CHCl$_3$-20%MeOH.

**Fig. S4** ESI spectra of 1c [R=-(CH$_2$)$_7$CH$_3$].
Fig. S5 ESI spectrum of **1c** [R=-(CH₂)₇CH₃] and guanidine thiocyanate (1:1).

Fig. S6 ESI spectrum of **1d** [R=-(CH₂)₃CO₂CH₂CH₃].
**Fig. S7** ESI spectrum of 1d [R=-(CH₂)₃CO₂CH₂CH₃] and guanidine thiocyanate (1:1).

**Fig. S8** ESI spectra of (a) 1d [R=-(CH₂)₃CO₂CH₂CH₃] alone, (b) 1d and 1 equivalent of each of guanidine thiocyanate, LiCl, NaCl, KCl, RbCl, CsCl, NH₄Cl in 80%CHCl₃-20%MeOH.
IV. MALDI Spectra

Fig. S9. MALDI spectrum of a) 1a alone, b) 1a and guanidinium HCl (G) after 5mins. mixing time, c) 1a and guanidinium HCl (G) after 1hour of mixing time.
MALDI (time dependence)

Fig. S10. MALDI spectrum of 1a and guanidinium HCl, LiCl, NaCl, KCl, RbCl, CsCl, and NH₄Cl after: a) 5mins of mixing time b) 1 hour mixing time.
MALDI Competition Experiment

Fig. S11. MALDI spectrum after one hour of mixing of 1a with a) 1 eq. guanidinium tetraphenylborate (G) b) 1 eq. octylguanidinium tetraphenylborate (OG) c) 1 eq. each of guanidinium tetraphenylborate (G) and octylguanidinium tetraphenylborate (OG).
V. $^1$H NMR spectra

Fig. S12 Partial $^1$H NMR spectra in acetone-d$_6$ (2mM, 500MHz, 10°C): (a) free guanidine thiocyanate; (b) free compound 1b; (c) 1b : guanidium in 1:1 molar ratio
NMR Spectra (CDCl₃, MeOH/CD₃OD)

Fig. S13. Increasing equivalents of MeOH/CD₃OD in CDCl₃. Partial ¹H NMR spectra of 1b and 1eq. guanidinium thiocyanate in CDCl₃ and varying concentration of MeOH/CD₃OD (1b:1mM, 400MHz, 293K).
Fig S14. Increasing equivalents of CH$_3$OH/CD$_3$OD in CDCl$_3$. Partial $^1$H NMR spectra of 1b (alone) in CDCl$_3$ and varying concentration of MeOH/CD$_3$OD (1b: 1mM, 400MHz, 293K).
NMR Spectra (acetone-d$_6$ with variable MeOH/OD)

![NMR Spectra Diagram](image)

**Fig. S15.** Partial $^1$H NMR spectra of guanidinium thiocycante in acetone-d$_6$ and varying ratios of CH$_3$OH/CD$_3$OD (1b: 2 mM, 400 MHz, 293 K).
**Fig. S16.** Partial $^1$H NMR spectra of $\text{1b}$ and 1eq. guanidinium thiocyanate in acetone-\text{d}_6 and varying concentration of CH$_3$OH/CD$_3$OD ($\text{1b}$:1 mM, 400 MHz, 293 K).
Fig. S17. Partial $^1$H NMR spectra of 1b (alone) in acetone-d$_6$ and varying ratios of CH$_3$OH/ CD$_3$OD (1b:1 mM, 400 MHz, 293 K).
Tables of chemical shifts

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<th>(Ha) ppm</th>
<th>(amide NH) ppm</th>
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**Table S1.** Cyclic hexamer 1b interior aromatic protons Ha and Hb and amide proton NH with varying concentration of MeOH/OD in d$_6$-acetone in the presence of 1 eq. guanidinium thiocyanate.

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<thead>
<tr>
<th>% MeOH</th>
<th>(Hb) ppm</th>
<th>(Ha) ppm</th>
<th>(amide NH) ppm</th>
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</table>

**Table S2.** Cyclic hexamer 1b interior aromatic protons Ha and Hb and amide proton NH with varying concentration of MeOH/OD in d$_6$-acetone.
Fig. S18. Cyclic hexamer 1b with varying MeOH/OD concentrations. Plot of the chemical shift of proton b in the presence of guanidinium thiocyanate (red) and in the absence of guanidinium thiocyanate (black).
Fig. S19. Cyclic hexamer 1b with varying MeOH/OD concentrations. Plot of proton Ha in the presence of guanidinium thiocyanate (red) and in the absence of guanidinium thiocyanate (black).
Fig S20. Cyclic hexamer 1b with varying MeOH/OD concentrations. Plot of amide proton NH in the presence of guanidinium thiocyanate (red) and in the absence of guanidinium thiocyanate (black).
VI. Computer Modeling

Energy-minimized (AM1) structure of the six-residue macrocycle (side chain = CH₃): (a) top view; (b) side view; (c) with a guanidinium ion docked in the cavity.