

*Supporting Information for*

Animal toxicity of hairpin pyrrole-imidazole  
polyamides varies with the turn unit

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Figure S2: Serum concentration of **1-4** after subcutaneous injection.

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Table S1: Liver microsomal stability of **1-4**.

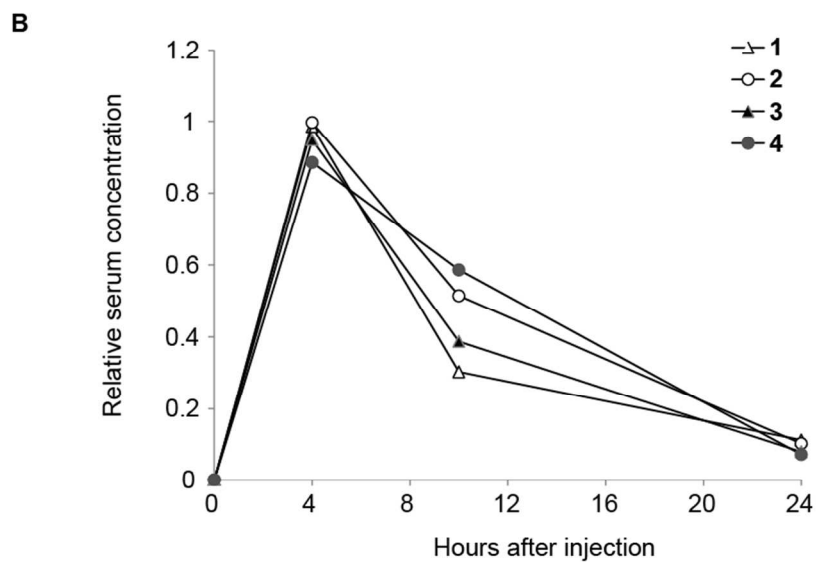
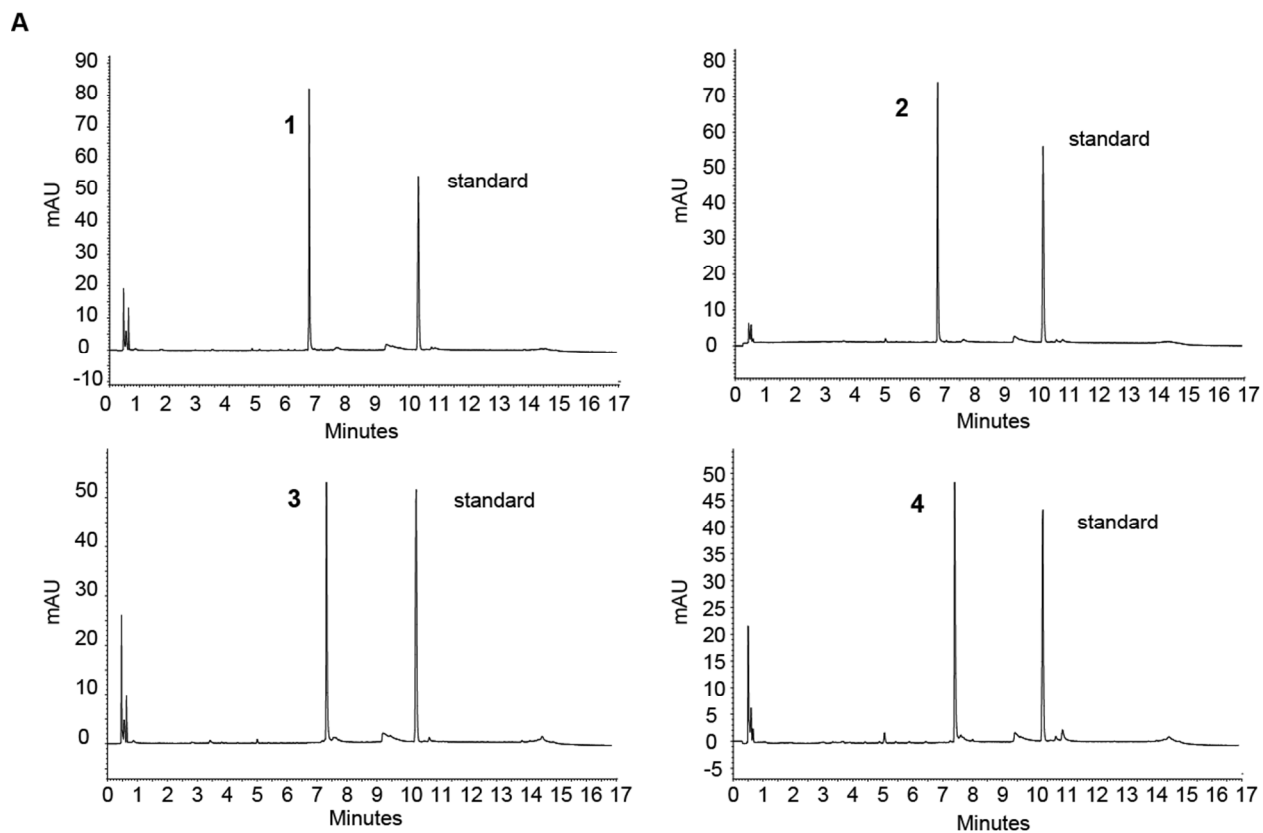
Figure S4: Structure of fluorescein conjugated polyamides **6-9**.

Figure S5: Nuclear localization of **6-9** in the liver.

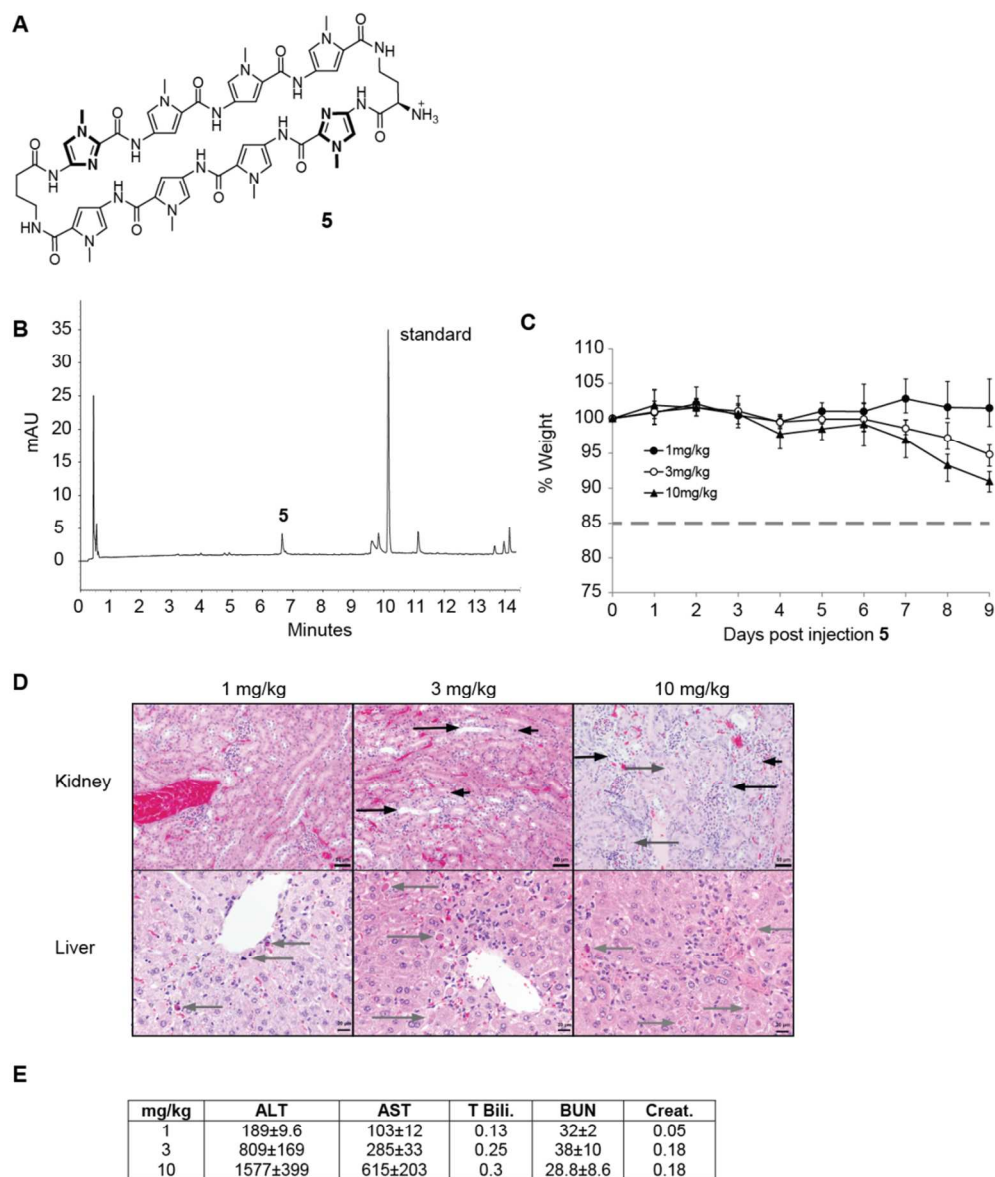
Table S2: MALDI-ToF characterization of polyamides **1-5**.

Polyamide	5'- TTGC <b>TGTTCT</b> GCAA -3'	
	$T_m / ^\circ\text{C}$	$\Delta T_m / ^\circ\text{C}$
—	61.8 ( $\pm 0.5$ )	—
<b>1</b>	74.1 ( $\pm 0.3$ )	12.3
<b>2</b>	75.1 ( $\pm 0.4$ )	13.3
<b>3</b>	70.1 ( $\pm 0.2$ )	8.3
<b>4</b>	74.9 ( $\pm 0.2$ )	13.2

**Fig. S1.** DNA thermal stabilization analysis of compounds **1-4**.



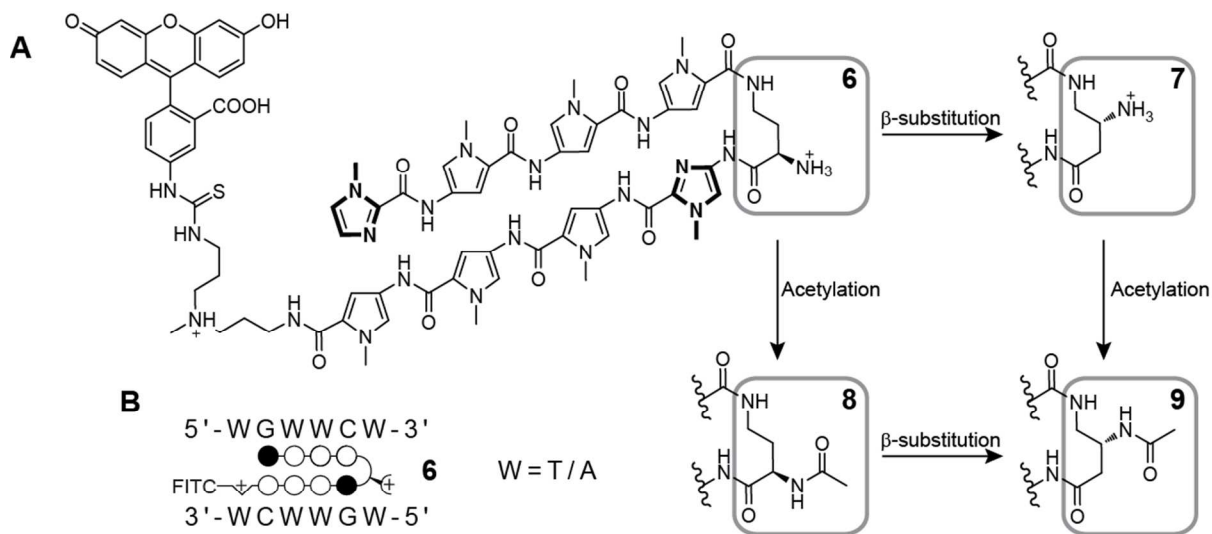
**Fig S2. (A)** Analytical HPLC traces of compounds 1-4 in the serum 4 hr after injection. **(B)** Relative serum levels of compounds 1-4 at 4 hr, 10 hr, and 24 hr after a single subcutaneous injection of each compound at 10 mg/kg.



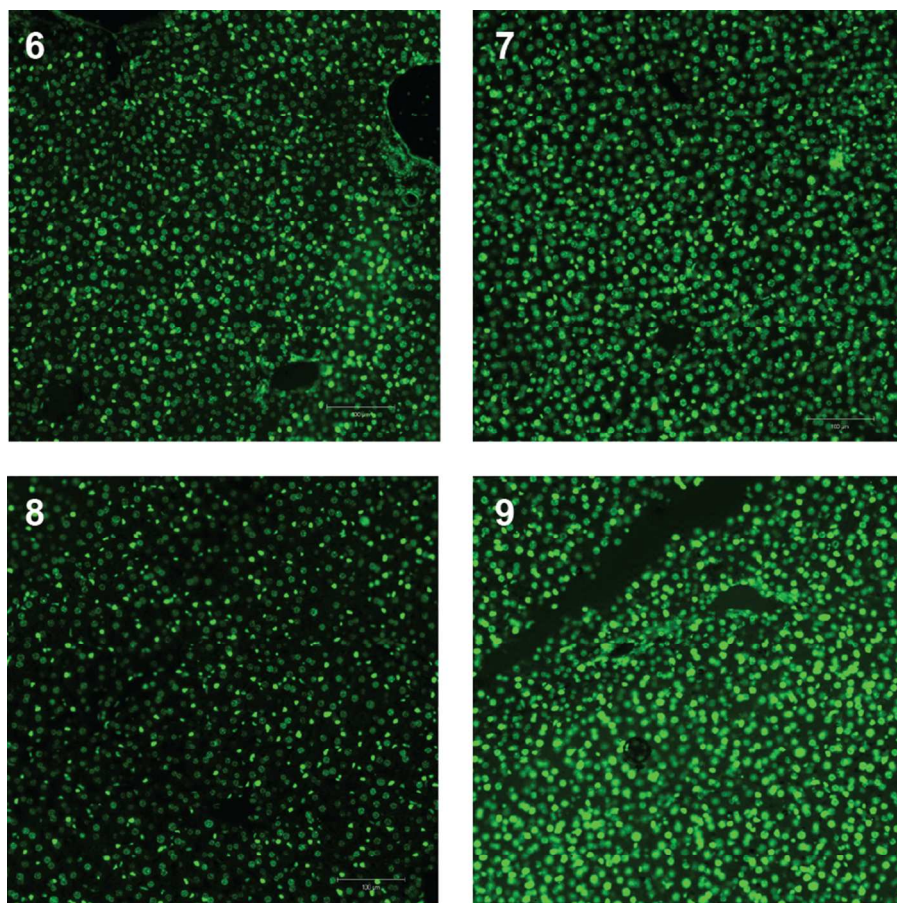
**Fig S3.** Characterization of a cyclic polyamide targeting the sequence 5'-WGWWCW-3'. **(A)** Chemical structure of compound 5. **(B)** Serum circulation of 5 4 hr after SC injection. **(C)** Changes in animal weights after a single SC injection of 5 at the indicated concentrations. **(D)** Kidney and liver histology of sacrificed animals after 9 days of monitoring. **(E)** Serum chemistry of animals treated with 5. **Liver:** long gray arrow=hepatocellular apoptosis/necrosis, arrowheads=outline area of bridging hepatocellular necrosis/apoptosis. **Kidney:** short gray arrow=tubular epithelial karyomegaly, long gray arrow=tubular epithelial apoptosis/necrosis, short black arrow=tubular epithelial mitoses, long black arrow=tubular epithelial attenuation

	Test conc (μM)	Test species	Mean remaining parent with NADPH (%)	Mean remaining parent NADPH-free (%)
Verapamil high metabolism control	1	Human	4.2%	100%
	1	Mouse	1.1%	100%
Warfarin low metabolism control	1	Human	100%	100%
	1	Mouse	100%	100%
<b>1</b>	1	Human	96.9%	92.3%
	1	Mouse	95.2%	96.8%
<b>2</b>	1	Human	91.9%	100%
	1	Mouse	92.4%	100%
<b>3</b>	1	Human	95.3%	94.9%
	1	Mouse	97.3%	100%
<b>4</b>	1	Human	3.0%	3.8%
	1	Mouse	4.0%	4.9%

**Table S1.** Microsomal stability analysis of **1-4** in the presence and absence of NADPH. Samples were incubated for 1 hr at 37 °C with 1 mg/ml of human or mouse microsomes.



**Figure S4.** Chemical structures. **(A)** Structures of polyamides **6-9**. The compounds only vary by the amino substitution on the  $\gamma$ -turn unit. **(B)** The preferred DNA binding sequence of the polyamide core. Polyamide **6** is shown bound to the sequence 5'-WGWWCW-3'. Closed circles represent imidazole units and open circles represent pyrrole units.



**Figure S5.** Nuclear localization of compounds **6-9** in the liver 24 hr after SC injection

Compound	Chemical Formula	Calculated Mass		Observed Mass
<b>1</b>	C <sub>65</sub> H <sub>76</sub> N <sub>22</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	1357.44	1357.86
<b>2</b>	C <sub>65</sub> H <sub>76</sub> N <sub>22</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	1357.44	1357.69
<b>3</b>	C <sub>67</sub> H <sub>78</sub> N <sub>22</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	1399.48	1399.91
<b>4</b>	C <sub>67</sub> H <sub>78</sub> N <sub>22</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	1399.48	1399.36
<b>5</b>	C <sub>54</sub> H <sub>61</sub> N <sub>21</sub> O <sub>10</sub>	[M+Na] <sup>+</sup>	1186.5	1186.6

**Table S2.** MALDI-ToF analysis of compounds.