Electronic Supplementary Information

for

Synthesis of a poly(Gd(III)-DOTA)-PNA conjugate as a potential MRI contrast agent via Post-Synthetic Click Chemistry Functionalization

Xiaoxiao Wang,[a] Mark Milne,[a] Francisco Martínez,[b] Timothy J. Scholl,[b] Robert Hudson*[a]

[a] Department of Chemistry, and [b]Department of Medical Biophysics and the Robarts Research Institute, The University of Western Ontario, 1151 Richmond St., London, Ontario, N6A 5B7, Canada.

*Corresponding author. Fax: +1-519-661-3022; E-mail: rhudson@uwo.ca

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General experimental protocols:

All chemicals and solvents were used as received unless specified. All solvents were peptide synthesis grade, except water (18.2 MΩ·cm^{-1}). The Fmoc-azide monomer (**Az**) was prepared according to a previously reported method;[1] all reagents for the synthesis of PNA oligomers were commercially available.

Synthesis of PNA Oligomers

PNA synthesis was performed on a 5 μmol scale using an automated ABI 433a peptide synthesizer on PEGA-Lys(Boc)-Fmoc resin (loading 0.113 mmol/g). The quality of PNA synthesis prior to further functionalization was checked by halting the synthesis before N-terminal Fmoc removal. A small portion of the resin was taken for characterization and the oligomer was cleaved/deprotected under standard conditions and analyzed by ESI-TOF MS. Other portions of the resin were taken and manipulated in standard manual peptide synthesis vessels.

General procedure for on-resin click chemistry

Alkynyl-(Gd(III)-DOTA) (30 eq.) in isopropanol (0.5 ml), $CuSO_4 \cdot 5H_2O$ (60 eq.) in H_2O (0.33 ml), sodium ascorbate (120 eq.) in H_2O (0.33 ml), and tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (60 eq.) in H_2O (0.33 ml) were added to the resin in a peptide synthesis vessel. The resin was shaken at room temperature under N_2 overnight. After the reaction was deemed completed by ESI-TOF MS, the solvent was drained and the resin was washed sequentially with DMF, DCM, H_2O, MeOH and finally DCM. Then PNA was cleaved from the resin, purified and characterized following by our previously reported procedure.[2]

The crude poly(Gd(III)-DOTA)-PNA probe was purified by reversed phase HPLC at 50 ^oC on a 250 × 4.6 mm VARIAN C18 column by elution with a gradient of 0-5 min, 1% MeCN, 5-20 min, linear from 1% to 60% MeCN, 20-35 min, linear from 60% to 100% MeCN and 35-40 min, linear from 100% MeCN to 100% H_2O. Both H_2O and MeCN solvents contained 0.05% TFA.

UPLC/Mass Spectroscopy of PNA oligomers

Ultra-performance liquid chromatography (UPLC) was performed using a BEH C18 column (particle size 1.7 μm; 1.0 i.d.×100 mm), high-resolution electrospray ionization mass spectroscopy

(HR-ESI-MS) and diode array UV detectors. The mobile phase was 100% H_2O to 100% MeCN over 5 min by linear gradient followed by 100% MeCN for 2 min at flow rate 0.1 mL/min. Both solvents contained 0.1% HCOOH.

Quantification of oligomers

Concentrations of oligomer solutions were determined by UV-vis spectrophotometry. The absorbance at 258 nm and 260 nm of the samples were measured for poly(rA) and PNA, respectively. An ε_{258} = 9800 $M^{-1}cm^{-1}$ was used as of each nucleobase of poly(rA). The equation below was used for calculating the concentration of nucleobases of poly(rA).

$$c\ (\mu M)=\frac{1000\times A_{258}}{9.8}$$

An ε_{260} = 8800 $M^{-1}cm^{-1}$ was used for each thymine PNA. The equation below was used for calculating the concentration of nucleobases of poly(Gd(III)-DOTA)-PNA.

$$c\ (\mu M)=\frac{1000\times A_{260}}{8.8}$$

Temperature dependent UV-vis studies

Thermal denaturation (UV melting experiments) were carried out to measure the T_m values of oligomers in this study using the following ionic conditions: 100 mM NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA, pH 7.0. Samples at 2 μM concentration were heated to 95 ºC, cooled to room temperature over 1-2 hours, and placed at 4 ºC overnight. Denaturation was performed from 15 to 90 ºC at a temperature ramp rate of 0.5 ºC/min. The T_m values are an average of three measurements and are rounded to the nearest 1 ºC. The error in T_m values was ± 0.5 ºC. T_m values were estimated for cooperative transitions by the first derivative method. Temperature dependent UV spectra that lacked upper and lower baselines, lacked sigmoidal shape or were indistinguishable from ssPNA intramolecular melting were deemed not be to cooperative transitions.

General procedure for stoichiometry determination by Job's method

A series solutions containing **(Gd(III)-DOTA)$_4$-PNA** and dA_{10} in the ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 and 100:0, respectively, were prepared with

the concentration of 4 μM at pH 7.0 and100 mM NaCl, 10 mM Na_2HPO_4, 0.1 mM EDTA. The absorbances at 260 nm and 283 nm were plotted against the percentage of **(Gd(III)-DOTA)$_4$-PNA**.

NMRD studies

The *T*1 NMRD profiles of 1 mL samples were acquired with a fast field-cycling NMR relaxometer (SpinMaster FFC2000 1T C/DC, Stelar, s.r.l. Mede, Pavia, Italy). All experiments were acquired with controlled temperatures (25, 38, 60 and 80 °C) and changing the relaxation field in 30 steps, logarithmically distributed from 0.01 to 42.485 MHz (0.23 mT up to 1 T) and using an acquisition field of 16.2 MHz. The quantitative relaxivity (r_1) values were normalized to the reported gadolinium concentration for each sample.

Scheme S1. Synthesis of Gd(III)-DOTA-PNA1 by on-resin CuAAC. a) alkynyl-(Gd(III)-DOTA) (7.5 equiv.), $CuSO_4 \cdot 5H_2O$ (15 equiv.), sodium ascorbate (30 equiv.), tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (15 eq.), isopropanol/H_2O(1:2, v/v), r.t; b) TFA/TES (95:5, v/v).

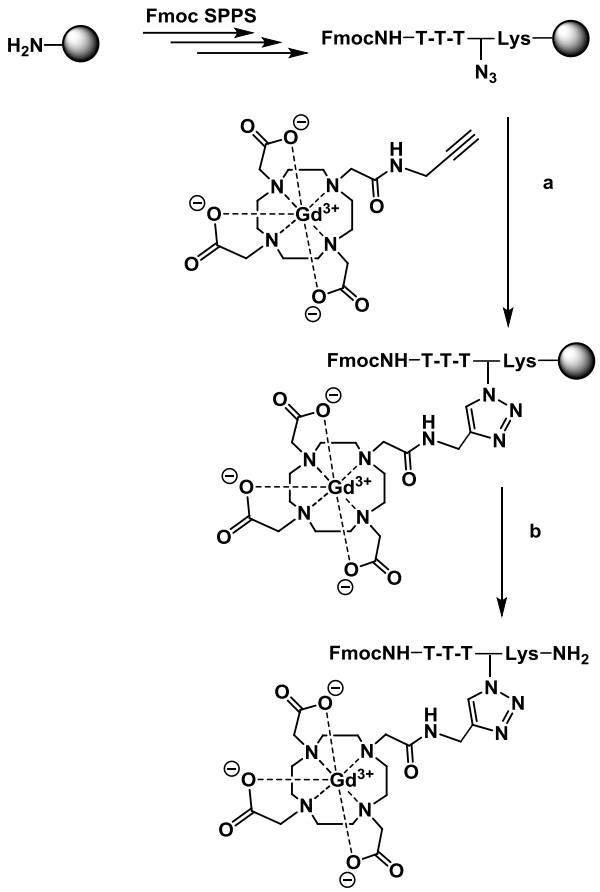


Figure S1. HPLC traces of (a) crude **PNA2**, (b) crude **(Gd(III)-DOTA)$_4$-PNA** and (c) purified **(Gd(III)-DOTA)$_4$-PNA**.

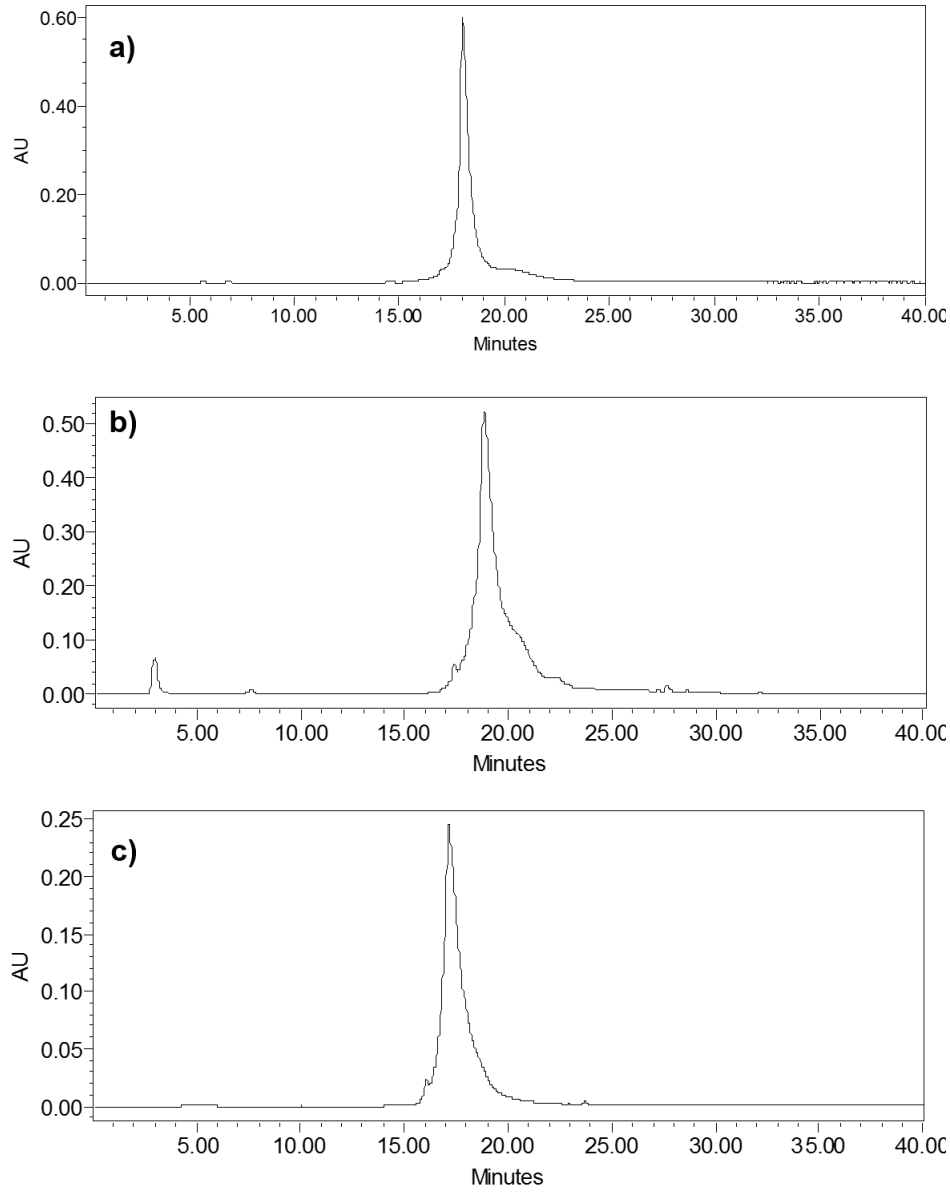
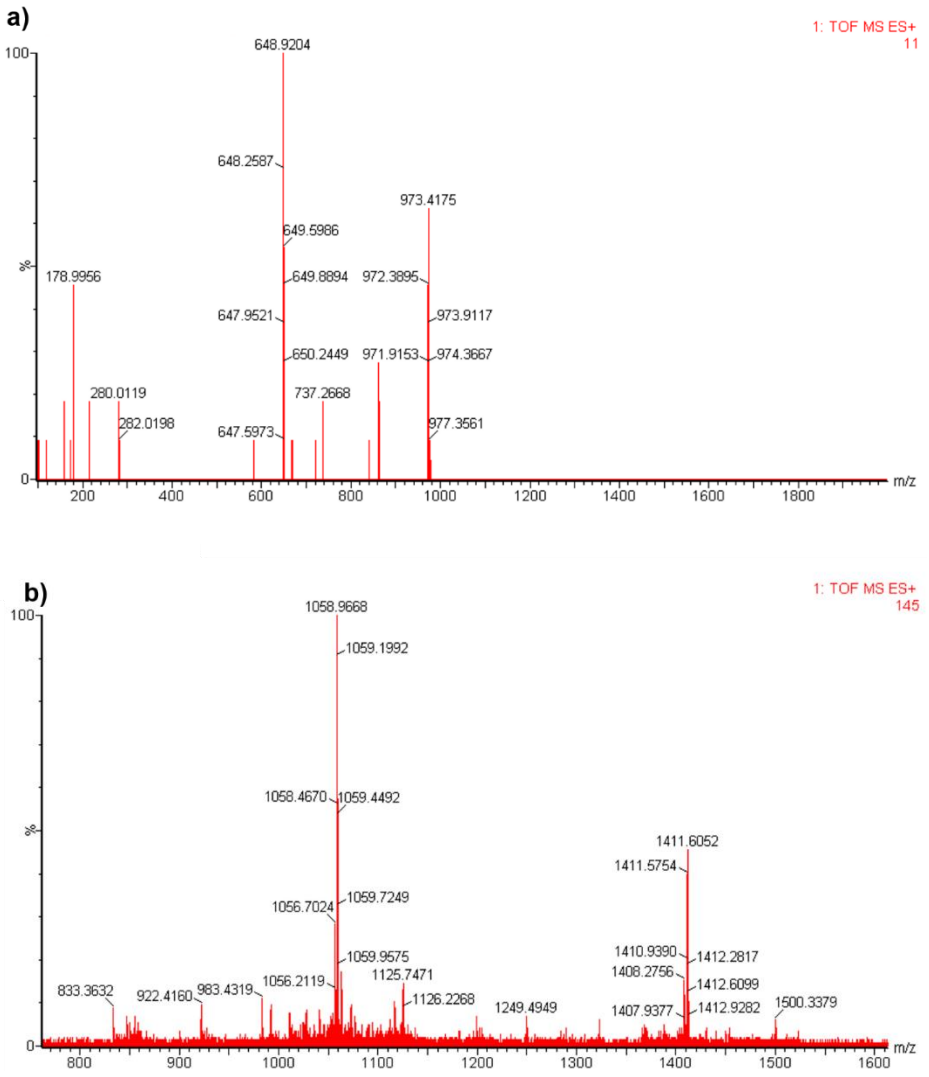
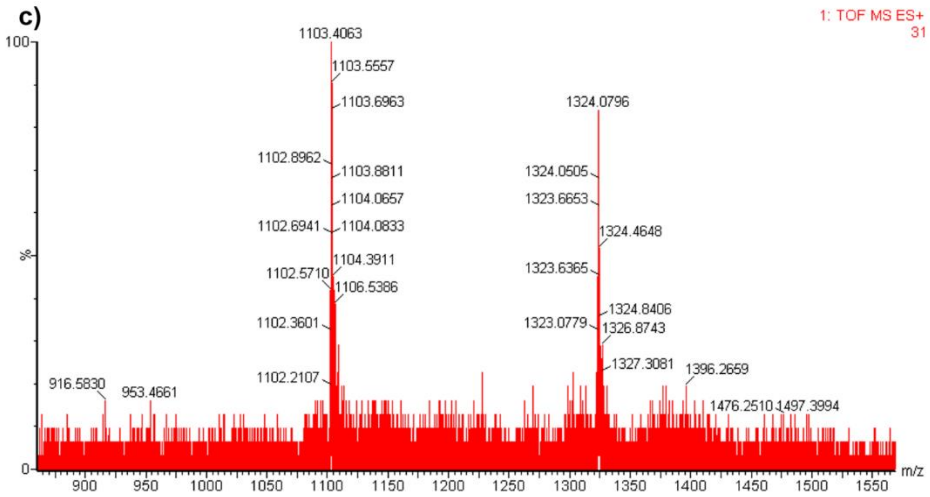


Figure S2. ESI-MS spectra of (a) **PNA1** calculated mass for $C_{79}H_{104}GdN_{25}O_{24}$: 973.5574 $[M+2H]^{2+}$; (b) **PNA2**, calculated mass for $C_{176}H_{240}N_{70}O_{56}$: 1411.7679 $[M+3H]^{3+}$, 1059.0779 $[M+4H]^{4+}$; (c) Fmoc**(Gd(III)-DOTA)$_4$-PNA)**, calculated mass for $C_{252}H_{352}Gd_4N_{90}O_{84}$: 1324.0326 $[M+5H]^{5+}$, 1103.5285 $[M+6H]^{6+}$ (d) **(Gd(III)-DOTA)$_4$-PNA)**, calculated mass for $C_{237}H_{342}Gd_4N_{90}O_{82}$: 914.2765 [$[M+7H]^{7+}$, 711.3279 $[M+9H]^{9+}$.

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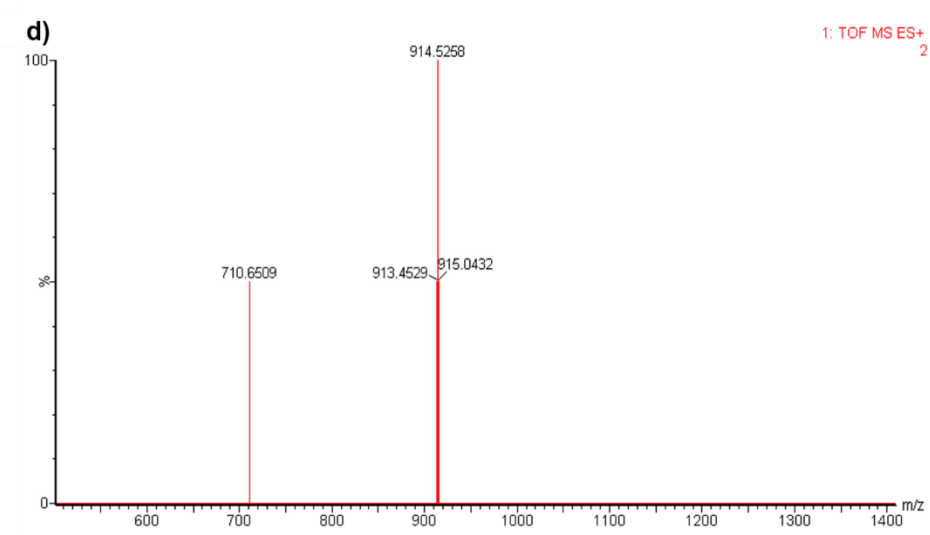
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Figure S3. First derivative plot of the temperature dependent UV spectra of **[(Gd(III)-DOTA)$_4$-PNA]$_2$:poly(rA)** at 260 nm at a concentration of 2 μM. conditions: 100 mM NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, pH 7, 25 ^oC.

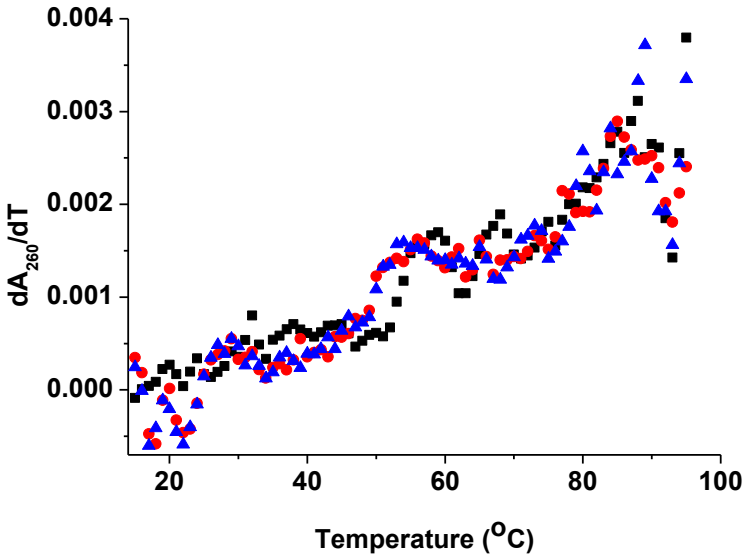
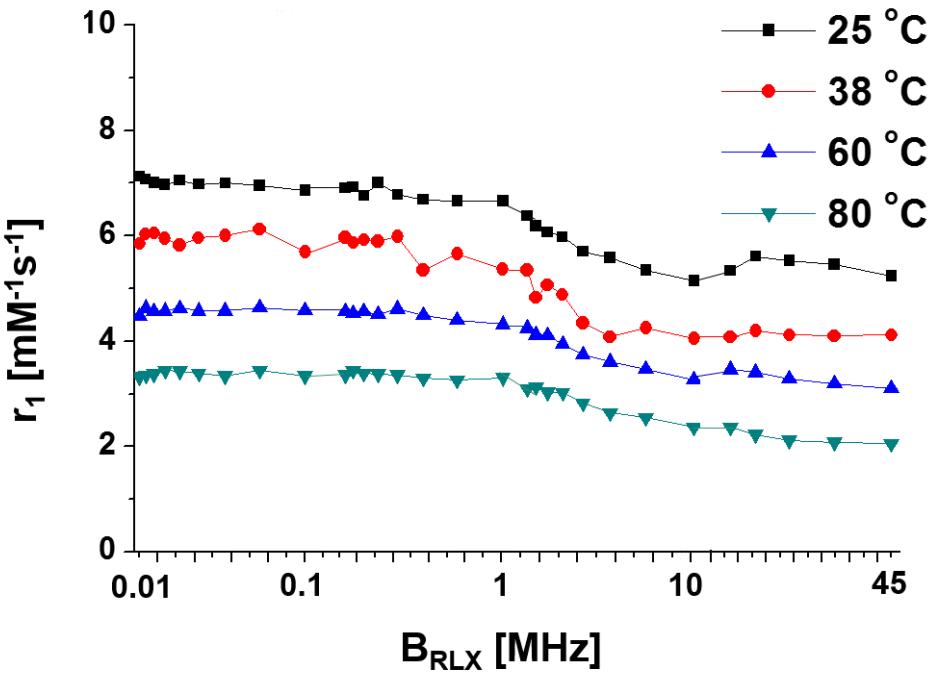


Figure S4. NMRD profiles for **(Gd(III)-DOTA)$_4$-PNA** at 25, 38, 60 and 80 ºC. conditions: 100 mM NaCl, 10 mM NaH_2PO_4, 0.1 mM EDTA, 6 M urea, pH 7, 25 ºC.



References:

1. A. H. St Amant, C. Engbers, and R. H. E. Hudson, *Artif. DNA. PNA XNA*, 2013, **4**, 4–10.
2. R. H. Hudson, Y. Liu, and F. Wojciechowski, *Can. J. Chem.*, 2007, **85**, 302–312.