Supporting Information

A barium based coordination polymer for the activity assay of deoxyribonuclease I

Chan Song,[b,c] Guan-Yao Wang,[a,b] Ya-Ling Wang,[b,d] De-Ming Kong,[b,c] Yong-Jian Wang,[b,d] Yue Li*[a,b] and Wen-Juan Ruan*[a,b]

[a] *Department of Chemistry, Nankai University, Tianjin 300071, China.*

[b] *Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin 300071, China.*

[c] *State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin 300071, China.*

[d] *Key Laboratory of Bioactive Materials (Ministry of Education), College of Life Sciences, Nankai University, Tianjin 300071, China.*

Email: wjruan@nankai.edu.cn; Tel: (+86)-22-23501717

Email: liyue841023@163.com; Tel: (+86)-22-23501717

Experimental details of synthesis and characterizations for 1

1. Materials and methods

All the chemicals used for synthesis were obtained from commercial sources and used as received. H_4L ligand was prepared according to the literature.[1] Elemental analyses (C, H, and N) were performed on a Perkin-Elmer 240C analyzer. IR spectra were measured on a Tensor 27 OPUS (Bruker) FT-IR spectrometer with KBr pellets. Thermogravimetric (TG) analyses were carried out on a Rigaku standard TG-DTA analyzer with a heating rate of 10 °C min^{-1} from ambient temperature to 700 °C, an empty Al_2O_3 crucible was used as reference. The powder X-ray diffraction spectra (PXRD) were recorded on a Rigaku D/Max-2500 diffractometer at 40 kV, 100 mA for a Cu-target tube and a graphite monochromator. Simulation of the PXRD pattern was carried out by the single-crystal data and diffraction-crystal module of the Mercury program version 3.0.[2]

2. Synthesis of $[Ba_2(L)(H_2O)]\cdot(DMF)_{0.5}(H_2O)_3$ (1)

H_4L (25 mg, 0.05 mmol) was dissolved in 2 mL of a mixed solvent of DMF/H_2O (3:1 in v/v) with 1 drop of 2 M HNO_3 in a 10 mL vial. To this solution, $Ba(NO_3)_2$ (39 mg, 0.15 mmol) was added. Then, the vial wassealed, heated at 85°C for 3 days, and colorless block crystals of **1** were obtained. The crystals of **1** were washed with DMF, and dried in air. Yield: 37 mg, 84.9% based on H_4L. Anal. Calcd for $Ba_2C_{25.5}H_{23.5}N_{2.5}O_{14.5}$ (%): C, 35.14; H, 2.72; N, 4.02. Found: C, 34.88; H, 3.01; N, 4.15. The PXRD pattern and IR spectrum of **1** are given in Figs. S1 and S2, respectively.

3. X-ray Data Collection and Structure Determinations

X-ray single-crystal diffraction data for compound **1** was collected on a Rigaku SCX-mini diffractometer at 293(2) K with Mo−Kα radiation (λ = 0.71073 Å) by ω scan mode. The program SAINT[3] was used for integration of the diffraction profiles. The structure was solved by direct method using the SHELXS program of the SHELXTL package and refined by full-matrix least-squares method with SHELXL (semiempirical absorption corrections were applied using SADABS program).[4] Metal atoms in the complex were located from the *E*-maps and other non-hydrogen atoms were located in successive difference Fourier syntheses and refined with anisotropic thermal parameters on F^2. Hydrogen atoms of the ligands were generated theoretically onto the specific atoms and refined isotropically with fixed thermal factors. Hydrogen atoms of the water molecules were added by difference Fourier maps and refined with constrains. Some guest solvent molecules (including DMF and water molecules) were highly disordered and were impossible to be refined using conventional discrete-atom models. To resolve these issues, the contribution of solvent electron density of these solvent molecules was removed by the SQUEEZE routine in PLATON.[5] Detailed crystallographic data is summarized in Table S1. The selected bond lengths and angles are given in Table S2.

Details of the biomacromolecule sensing experiments

1. Chemical and reagents

All oligonucleotides (Table S3) were synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). The concentrations of the oligonucleotides were represented as single-stranded concentrations, which were determined by measuring the UV absorbance at 260 nm. Deoxyribonuclease I (DNase I), T4 polynucleotide kinase (T4 PNK), T4 DNA ligase, lysozyme, and sequence-specific endonuclease EcoRI, BamHI and HindIII were purchased from New England Biolabs (Beijing, China). All the chemicals were of analytical grade and used as received.

2. Instrumentation

All fluorescence measurements were carried out on a SHIMADZU RF-5301PC spectrofluorimeter with 1 cm-path-length micro quartz cell (40 μL, Starna Brand, England). The emission spectra were collected from 500 nm to 600 nm at room temperature with an excitation wavelength of 480 nm. Both the excitation and emission slit widths

were set at 10 nm.

3. Fluorescence quenching property of 1 on fluorescein (FAM) labeled ssDNAs

The FAM-labeled ssDNA was dissolved in 1× DNase I buffer (10 mM Tris-HCl, 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$, pH 7.6). The solution was heated at 95 °C for 5 min and gradually cooled to room temperature. Then, compound **1** was added. After the suspension was maintained at 37 °C for 1 h, the fluorescence signal at 518 nm was measured (λ_{ex} = 480 nm).

4. Assay of DNase I activity

For assay of DNase I activity, 50 nM **P** was disolved in 1× DNase I buffer. The solution was heated at 95 °C for 5 min and gradually cooled to room temperature. Then, a given amounts of DNase I was added and incubated at 37 °C for 2 h. Finally, compound **1** (3 mg mL^{-1}, final concentration) was added to a final volume of 100 μL. After the incubation at 37 °C for 1 h, the fluorescence signal at 518 nm was measured (λ_{ex} = 480 nm).

5. Assay of DNase I activity in 1% cell lysate samples

HeLa cells were cultured with modified RPMI-1640 medium (Thermo Fisher) supplemented with 10% fetal calf serum at 37 °C in 5% CO_2 atmosphere. After being washed twice with ice-cold phosphate-buffered saline (PBS), cells were harvested and lysed in lysis buffer (Sangon Biotech). The cell lysate was incubated on ice for 30 min and centrifuged at 4 °C with 12000 g for 10 min. The supernatant was collected, diluted (1:100) and subject to DNase I activity assay.

Table S1 Crystal data and structure refinement for **1**.

compound	**1**
Empirical formula	$Ba_2C_{24}H_{18}N_2O_{13}$
Formula weight	817.06
Temperature (K)	293(2)
Crystal system	triclinic
space group	*P*-1
a (Å)	7.8709(16)
b (Å)	8.8711(18)
c (Å)	19.835(4)
α (°)	100.55(3)
β (°)	92.53(3)
γ (°)	92.59(3)
V (Å^3)	1358.2(5)
Z	2
D_{cal} (g/cm^3)	1.998
μ (mm^{-1})	2.952
θ for data collection	3.14-27.48
Reflections collected	14591
Reflections unique	6224
R_{int}	0.0313
Data / restraints / parameters	6214 / 0 / 371
GOF on F^2	1.076
Final R indices [I>2σ(I)]	R_1 = 0.0301, wR_2 = 0.0684
R indices (all data)	R_1 = 0.0406, wR_2 = 0.0742
Residuals (e Å^{-3})	0.678, -0.787

Table S2 Selected bond lengths (Å) and angles (°) for compound **1**.

Ba(1)-O(1)	3.121(7)	Ba(2)-O(1)	2.804(6)
Ba(1)-O(2)	3.081(6)	Ba(2)-O11	2.846(8)
Ba(1)-O(2)#2	2.620(5)	Ba(2)-O(6)#8	2.755(7)
Ba(1)-O(3)#3	2.733(6)	Ba(2)-O(10)#8	2.836(5)
Ba(1)-O(7)#4	2.746(5)	Ba(2)-O(7)#4	2.636(6)
Ba(1)-O(8)#4	2.911(6)	Ba(2)-O(3)#9	3.019(6)
Ba(1)-O(8)#5	2.868(6)	Ba(2)-O(4)#9	2.873(6)
Ba(1)-O(9)#6	2.888(6)	Ba(2)-O(9)#6	2.863(6)
Ba(1)-O(10)#7	2.718(5)		
O(1)-Ba(1)-O(2)	41.83(7)	O(1)-Ba(2)-O(11)	67.7(1)
O(1)-Ba(1)-O(2)#2	112.30(8)	O(1)-Ba(2)-O(3)#9	167.66(8)
O(1)-Ba(1)-O(3)#3	91.22(8)	O(1)-Ba(2)-O(4)#9	140.67(9)
O(1)-Ba(1)-O(7)#4	65.85(8)	O(1)-Ba(2)-O(6)#8	82.73(9)
O(1)-Ba(1)-O(8)#5	162.01(8)	O(1)-Ba(2)-O(7)#4	72.10(8)
O(1)-Ba(1)-O(8)#4	111.64(8)	O(1)-Ba(2)-O(9)#6	68.44(8)
O(1)-Ba(1)-O(9)#6	63.95(7)	O(1)-Ba(2)-O(10)#8	119.81(8)
O(1)-Ba(1)-O(10)#7	85.21(8)	O(11)-Ba(2)-O(3)#9	111.7(1)
O(2)-Ba(1)-O(2)#2	75.90(8)	O(11)-Ba(2)-O(4)#9	74.3(1)
O(2)-Ba(1)-O(3)#3	111.05(8)	O(11)-Ba(2)-O(6)#8	63.8(1)
O(2)-Ba(1)-O(7)#4	104.60(8)	O(11)-Ba(2)-O(7)#4	69.1(1)
O(2)-Ba(1)-O(8)#5	152.60(8)	O(11)-Ba(2)-O(9)#6	126.2(1)
O(2)-Ba(1)-O(8)#4	144.58(8)	O(11)-Ba(2)-O(10)#8	167.9(1)
O(2)-Ba(1)-O(9)#6	65.02(7)	O(3)#9-Ba(2)-O(4)#9	43.90(9)
O(2)-Ba(1)-O(10)#7	62.18(8)	O(3)#9-Ba(2)-O(6)#8	108.31(9)
O(2)#2-Ba(1)-O(3)#3	144.31(9)	O(3)#9-Ba(2)-O(7)#4	95.98(8)
O(2)#2-Ba(1)-O(7)#4	129.49(8)	O(3)#9-Ba(2)-O(9)#6	104.80(8)
O(2)#2-Ba(1)-O(8)#5	85.68(8)	O(3)#9-Ba(2)-O(10)#8	63.28(8)
O(2)#2-Ba(1)-O(8)#4	106.19(8)	O(4)#9-Ba(2)-O(6)#8	71.7(1)
O(2)#2-Ba(1)-O(9)#6	68.01(8)	O(4)#9-Ba(2)-O(7)#4	103.57(9)
O(2)#2-Ba(1)-O(10)#7	86.29(8)	O(4)#9-Ba(2)-O(9)#6	148.17(8)
O(3)#3-Ba(1)-O(7)#4	83.97(8)	O(4)#9-Ba(2)-O(10)#8	96.25(8)
O(3)#3-Ba(1)-O(8)#5	73.20(8)	O(6)#8-Ba(2)-O(7)#4	132.11(9)
O(3)#3-Ba(1)-O(8)#4	88.06(8)	O(6)#8-Ba(2)-O(9)#6	136.93(9)
O(3)#3-Ba(1)-O(9)#6	147.46(8)	O(6)#8-Ba(2)-O(10)#8	106.39(9)
O(3)#3-Ba(1)-O(10)#7	68.66(8)	O(7)#4-Ba(2)-O(9)#6	68.99(8)
O(7)#4-Ba(1)-O(8)#5	102.76(8)	O(7)#4-Ba(2)-O(10)#8	121.42(8)
O(7)#4-Ba(1)-O(8)#4	46.10(8)	O(9)#6-Ba(2)-O(10)#8	65.72(7)
O(7)#4-Ba(1)-O(9)#6	67.21(7)		
O(7)#4-Ba(1)-O(10)#7	139.77(8)		
O(8)#5-Ba(1)-O(8)#4	60.07(8)		
O(8)#5-Ba(1)-O(9)#6	126.23(8)		
O(8)#5-Ba(1)-O(10)#7	97.01(8)		
O(8)#4-Ba(1)-O(9)#6	82.70(8)		
O(8)#4-Ba(1)-O(10)#7	151.96(8)		
O(9)#6-Ba(1)-O(10)#7	125.33(7)		

Table S3 The oligonucleotides used in this work.

Oligonucleotides	Oligonucleotide Sequences	Number of bases
3-mer	5′-CCA-FAM-3′	3
6-mer	5′-TAA CCA-FAM-3′	6
12-mer	5′-AGG CAG TAA CCA-FAM-3′	12
24-mer (**P**)	5′-AGG CAG TAA CCA AGG CAG TAA CCA-FAM-3′	24
36-mer	5′-AGG CAG TAA CCA AGG CAG TAA CCA AGG CAG TAA CCA-FAM-3′	36

Table S4 Comparison of different methods for the activity assay of DNase I with respect to detection limit and linear range.

Detection limit (U mL^{-1})	Linear range (U mL^{-1})	Reference
0.04	0.1-10	**This work**
0.01	Not available	6
0.01	0.01-1	7
0.02	0.03-9	8
0.04	0.04-0.5	9
0.1	0.1-1	10
1	5-100	11
1.75	1.75-70	12

Table S5 Analytical results for DNase I activity in 1% cell lysate samples.

Sample	Added (U mL^{-1})	Determined (U mL^{-1})	Recovery (%)
1	0	–	–
2	1	0.98 ± 0.08	98.0 ± 7.8
3	4	4.11 ± 0.04	102.7 ± 1.0

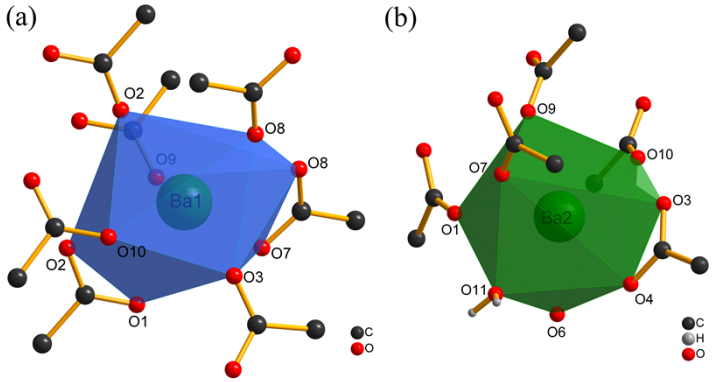


Fig. S1 The coordination geometries of Ba1 (a) and Ba2 (b) centers in compound **1** with coordination polyhedron indicated.

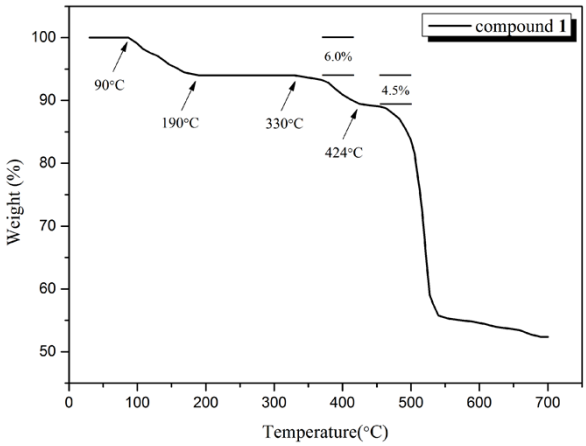


Fig. S2 TGA curve for compound **1**.

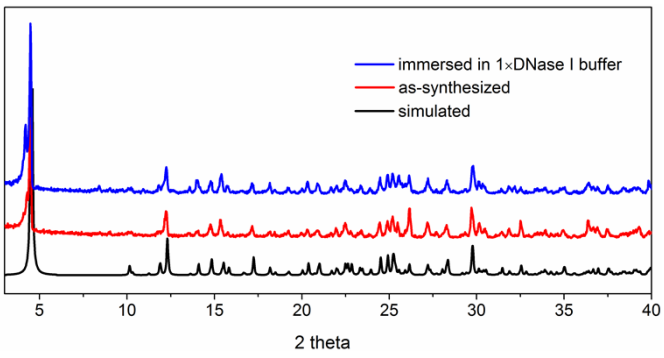


Fig. S3 PXRD pattern of compound **1**.

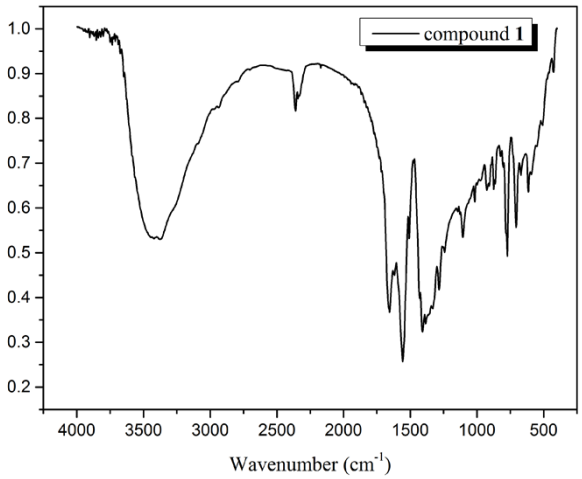
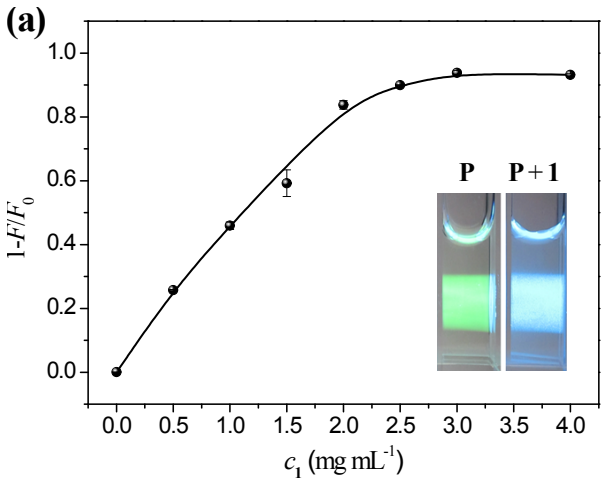
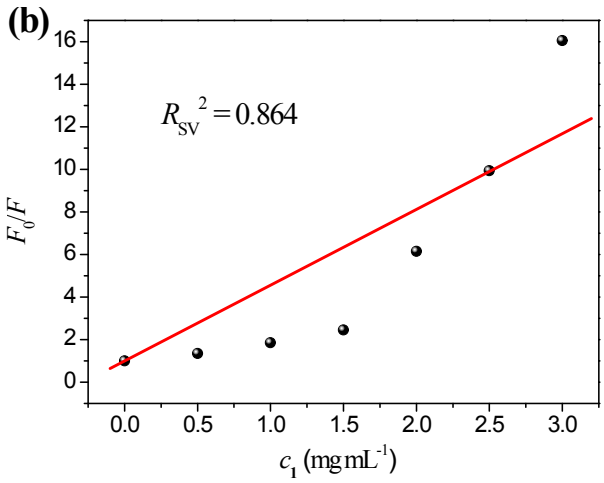


Fig. S4 IR spectrum of compound **1**.





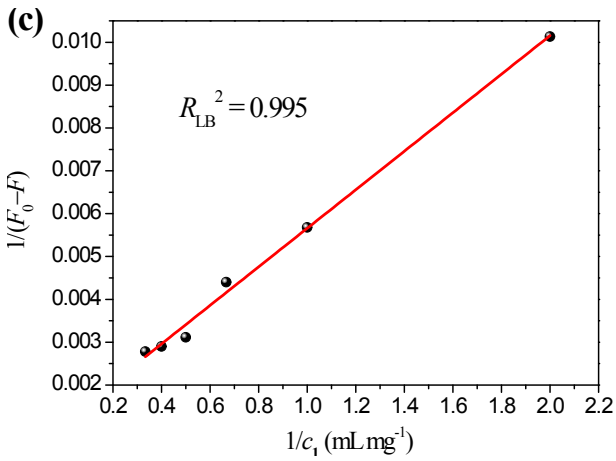


Fig. S5 (a) Quenching efficiency ($1-F/F_0$) of different concentrations of compound **1** on the fluorescence emission of **P**. Inset: the photographs showing the original fluorescence of the solution of **P** and its decreased fluorescence after the addition of compound **1**. (b) Stern−Volmer and (c) Lineweaver−Burk plots of the quenching process for **P** by compound **1**. [**P**] = 50 nM, λ_{em} = 518 nm.

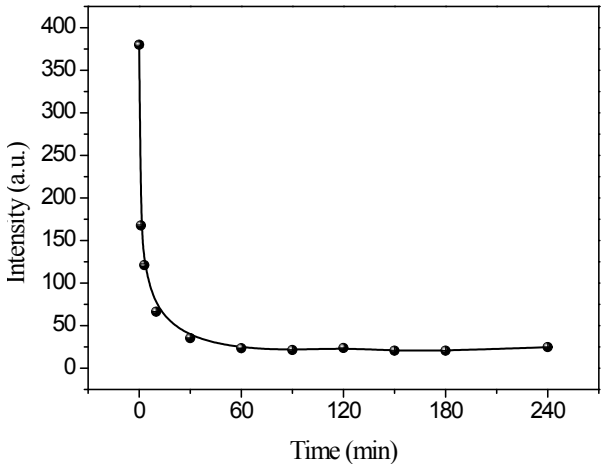
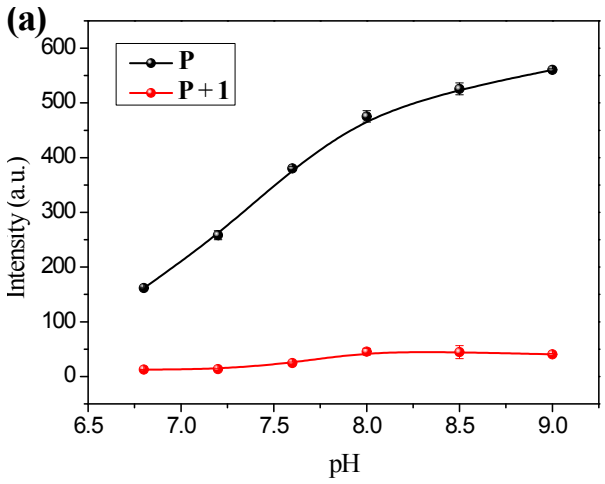


Fig. S6 The temporal change of the fluorescence intensity of **P** at 518 nm after the addtion of compound **1**. [**P**] = 50 nM, [**1**] = 3 mg mL^{-1}, incubated at 37 °C.



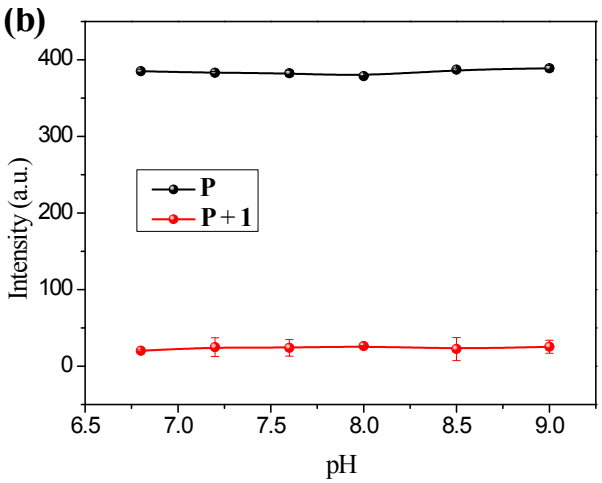


Fig. S7 The fluorescence intensity of the solution of **P** with different original pH values (given as the abscissa of the graphs) at 518 nm with and without addition of **1**. (a) None or (b) 10 mM Tris-HCl buffer (pH 7.6) was added before fluorescence measurement. [**P**] = 50 nM, [**1**] = 3 mg mL^{-1}.

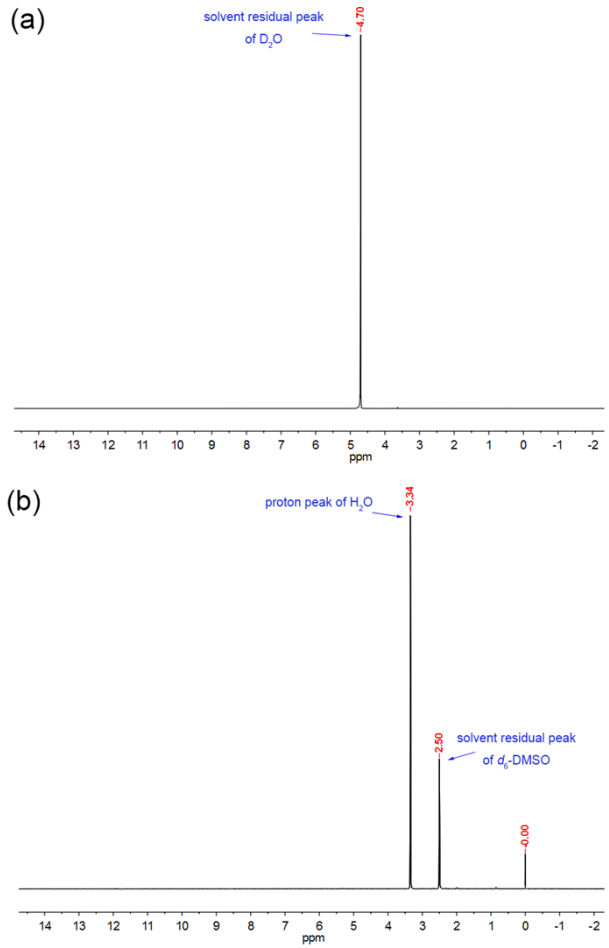


Fig. S8 ^{1}H NMR spectra of the (a) D_2O filtrate and (b) d_6-DMSO elution of the D_2O suspension of **1**. Compound **1** (1.5 mg) was dispersed in the D_2O solution (0.5 mL) containing 10 mM Tris-HCl (pH 7.6), 2.5 mM $MgCl_2$ and 0.5 mM $CaCl_2$. After the immersion process, the suspension was filtrated, and the D_2O filtrate was subject to ^{1}H NMR analysis. The separated solid was washed with d_6-DMSO to elute potential adsorbed species, and the elution was also analyzed with ^{1}H NMR.

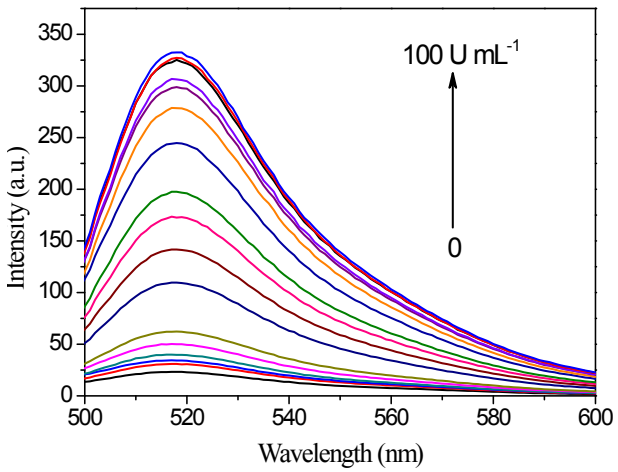


Fig. S9 The fluorescence spectra of the solution of **P** after the addtiion of different amount of DNase I and compound **1**. [**P**] = 50 nM, [**1**] = 3 mg mL^{-1}, the activities of DNase I are (arrow direction) 0, 0.1, 0.2, 0.5, 1, 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80 and 100 U mL^{-1}. All experiments were performed in triplicate.

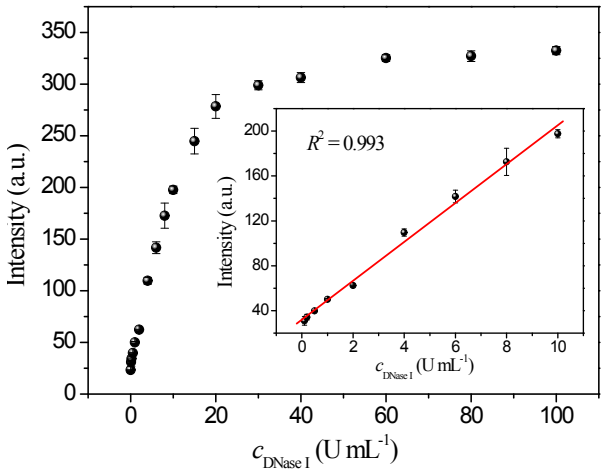


Fig. S10 DNase I activity dependent change of the fluorescence intensity at 518 nm. The insert shows the linear fluorescence response in the DNase I activity range of 0.1−10 U mL^{-1}. [**P**] = 50 nM, [**1**] = 3 mg mL^{-1}, all experiments were performed in triplicate.

Reference

1 (a) D. Hu and R. Kluger, *Biochemistry*, 2008, **47**, 12551; (b) G.-Y. Wang, L.-L. Yang, Y. Li, H. Song, W.-J. Ruan, Z. Chang and X.-H. Bu, *Dalton Trans.*, 2013, **42**, 12865; (c) G.-Y. Wang, C. Song, D.-M. Kong, W.-J. Ruan, Z. Chang and Y. Li, *J. Mater. Chem. A*, 2014, **2**, 2213.

2 C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Crystallogr.*, 2006, **39**, 453.

3 *SAINT Software Reference Manual*, Bruker AXS, Madison, WI, 1998.

4 G. M. Sheldrick, SHELXTL NT Version 5.1. *Program for Solution and Refinement of Crystal Structures*, University of Göttingen, Göttingen, Germany, 1997.

5 A. L. Spek, J. Appl. Crystallogr., 2003, **36**, 7.

6 W. Zhao, M. M. Ali, S. D. Aguirre, M. A. Brook and Y. Li, *Anal. Chem.*, 2008, **80**, 8431.

7 S. Sato, K. Fujita, M. Kanazawa, K. Mukumoto, K. Ohtsuka and S. Takenaka, *Anal. Chim. Acta*, 2009, **645**, 30.

8 P. Gong, B. Shi, P. Zhang, D. Hu, M. Zheng, C. Zheng, D. Gao and L. Cai, *Nanoscale*, 2012, **4**, 2454.

9 X. Su, C. Zhang, X. Zhu, S. Fang, R. Weng, X. Xiao and M. Zhao, *Anal. Chem.*, 2013, **85**, 9939.

10 S. Sato, K. Fujita, M. Kanazawa, K. Mukumoto, K. Ohtsuka, M. Waki and S. Takenaka, *Anal. Biochem.*, 2008, **381**, 233.

11 S.-K. Sun, B.-B. Wang and X.-P. Yan, *Analyst*, 2013, **138**, 2592.

12 Z. Zhou, C. Zhu, J. Ren and S. Dong, *Anal. Chim. Acta*, 2012, **740**, 88.