Supplementary Information

IDB-containing low molecular weight short peptide as efficient DNA cleavage reagent

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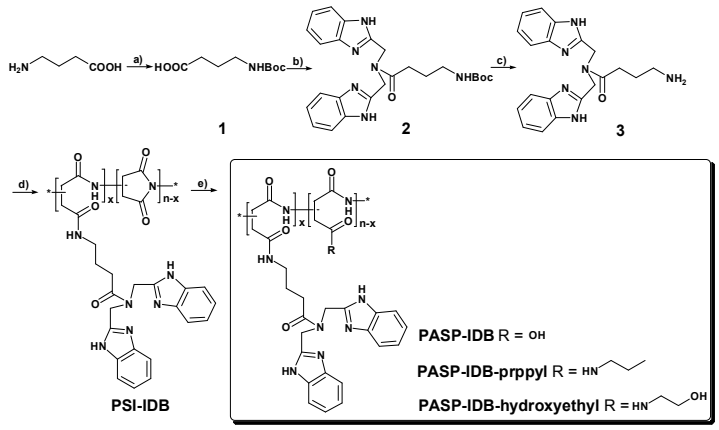
1. The synthesis of the title compound and its control compounds are shown in Scheme S1.



Scheme S1 The synthesis of the title compound and control compounds. Reagents and conditions: (a) $(Boc)_2O$, H_2O, 1,4-dioxane, r.t. (b) IDB, HATU, DIEA (c) HCl, 1,4-dioxane; aqueous Na_2CO_3 (d) PSI, DMF (e) aqueous NaOH, DMF; propylamine, DMF; hydroxylethylamine, DMF.

Synthesis of 4-(tert-butoxycarbonyl)aminobutyric acid (1). The compound of **1** was obtained from 4-aminobutyric acid using standard protection methods. Yield 70%. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 1.43 (s, 9 H), 1.81 (qt, 2 H, J = 7.0 Hz), 2.38 (t, 2 H, J = 7.0 Hz), 3.27-3.09 (m, 2 H), 4.80-4.62 (m, 1 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 25.21, 28.50, 31.53, 39.92, 79.51, 156.32, 178.14.

Compound 2. 1.4 g (1.85 mmol) compound **1** was dissolved in 3 mL DMF, and then 1.06 g (2.78 mmol) HATU and 0.37 mL (3.7 mmol) DIEA was added to the above mentioned reaction with stirring for 5 min at room temperature. 0.96 g (1.85 mmol) compound **IDB** was added to the reaction and monitored by TLC. After stirring for 10 h, the solvent was evaporated under reduced pressure and the crude product was dissolved in EA. The solution was washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and saturated brine (3 × 50 mL). The organic layer was dried with anhydrous Na_2SO_4. After the solvent was removed under vacuum, the residue was purified by column chromatography on silica gel (CH_2Cl_2 : MeOH = 50 : 1, R_f = 0.4) to afford the compound **2** (1.33 g, 1.05 mmol): yield 57%. ESI-MS (m/z): 463.2(M+H)$^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ/ppm: 12.37 (br, 2 H), 7.59 (d, 4 H, J = 20 Hz), 7.21 (d, 4 H, J = 8 Hz), 5.17 (s, 1 H), 4.96 (s, 2 H), 4.89 (s, 2 H), 3.03 (d, 2 H, J = 8 Hz) , 2.38 (t, 2 H, J = 8 Hz), 1.68 (d, 2 H, J = 4 Hz), 1.38 (s, 9 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ/ppm: 175.8, 174.3, 156.4, 152.0, 151.1, 138.5, 137.6, 122.8, 122.6, 115.2, 114.8, 79.2, 77.2, 64.5, 49.0, 47.4, 39.6, 28.4.

Compound 3. A mixture formed by 1,4-Dioxane (4 mL) and hydrochloric acid (1 mL) was added to 25 mL round bottom flask, followed by 0.82 g (1.05 mmol) compound **2**. The solution was stirred at room temperature to generate white solid. After stirring for 4 h, the white solid is obtained by centrifugation. The free base was obtained by neutralization of the reaction mixture with 1 M K_2CO_3 solution followed by extraction with EA. The product was 0.45 g (83%). ESI-MS (m/z): 363.3 (M+H)$^+$.

PASP-IDB. 1.8 g (3.3 mmol) **3** and 128 mg poly(succinimide) (**PSI**) was dissolved in 30 mL DMF, and the reaction was heated at 70°C for 72 h to generate compound **PSI-IDB**. The solvent was removed and 1 M NaOH was added in the reaction with stirring at room temperature. After 6 h, the crude products were dialyzed with distilled water for 48 h and lyophilized to provide the target products 202 mg. ^{1}H NMR (400 MHz, CF_3COOD): δ/ppm: 7.72 (d, 8 H, J = 24 Hz), 5.64 (s, 2 H), 5.35 (s, 2 H), 4.85 (s, 7 H), 3.29 (m, 2 H), 2.96 (s, 14 H), 2.76 (s, 2 H), 1.93 (s, 2 H); ^{13}C NMR (100 MHz, CF_3COOD): δ/ppm: 171.0, 168.8, 128.0, 127.6, 124.1, 123.8, 117.6, 111.1, 46.5, 42.3, 40.2, 33.8, 32.7, 26.8, 20.9.

PASP-IDB-propyl. After the generation of compound **PSI-IDB**, 20 mL propylamine was added to the above reaction with stirring at room temperature for 12 h. The crude products were dialyzed with distilled water for 48 h and lyophilized to provide the target products 159 mg. ^{1}H NMR (400 MHz, CF_3COOD): δ/ppm: 7.69 (d, 8 H, J = 32 Hz), 5.65 (s, 2 H), 5.33 (s, 2 H), 5.09 (s, 15 H), 3.31(s, 28 H), 3.12 (s, 30 H), 2.80 (s, 2 H), 2.01 (s, 2 H), 1.60 (s, 26 H), 0.93 (s, 39 H); ^{13}C NMR (100 MHz, CF_3COOD): δ/ppm: 170.0, 127.3, 127.1, 125.4, 47.8, 39.9, 40.1, 34.0, 33.9, 26.7, 18.7, 18.5, 6.7.

PASP-IDB-hydroxyethyl. The preparation is similar to that of **PASP-IDB-propyl.** (propylamine was replaced by hydroxylethylamine) ^{1}H NMR (400 MHz, CF_3COOD): δ/ppm: 7.75 (d, 8 H, J = 32 Hz), 5.71 (s, 2 H), 5.40 (s, 2 H), 5.14 (s, 7 H), 3.95 (s, 12 H), 3.59 (s, 12 H), 3.38 (s, 2 H), 3.15 (s, 14 H), 2.86 (s, 2 H), 2.07 (s, 2 H); ^{13}C NMR

(100 MHz, CF_3COOD): δ/ppm: 173.5, 131.5, 131.0, 129.3, 77.4, 66.8, 51.7, 46.4, 43.9, 41.2, 37.8, 31.6, 31.1, 22.5.

2. The NMR spectra of compounds are shown in Figs. S1-S4.

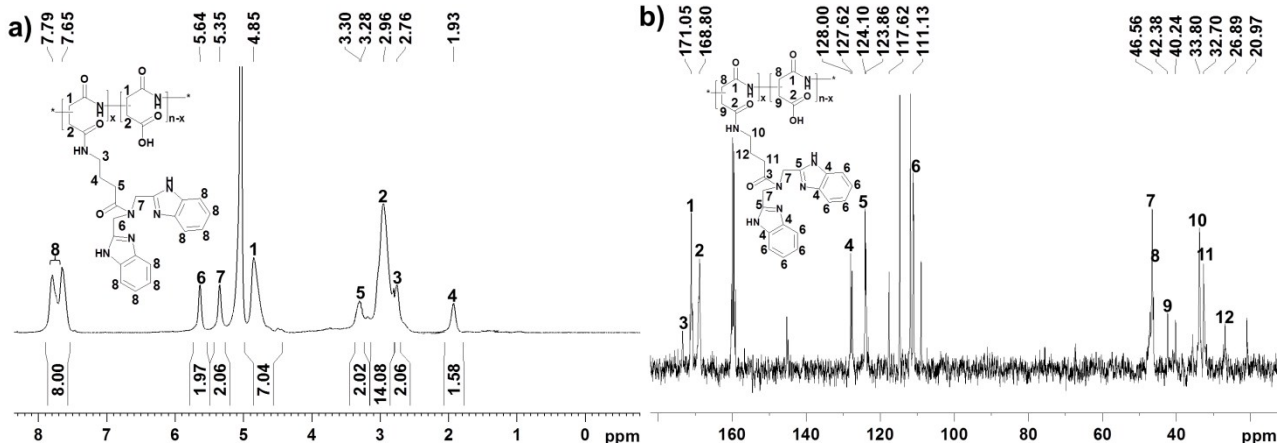


Fig. S1 ^1H NMR (a) and ^{13}C NMR (b) spectra of PASP-IDB (DS = 14.2%) (in CF_3COOD).

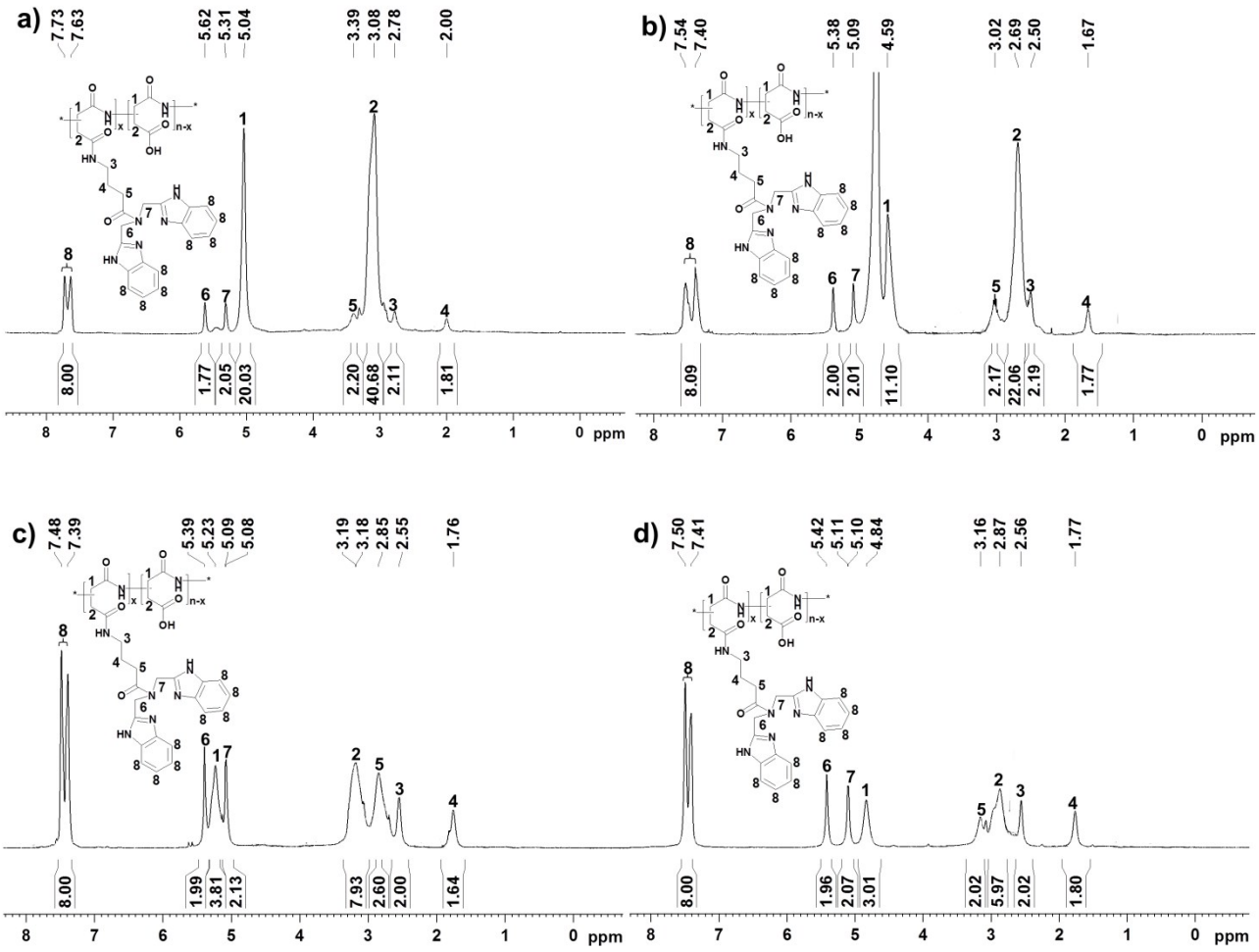


Fig. S2 ^1H NMR spectra of PASP-IDB (DS = 5.0, 9.1, 26.2 and 33.3% for (a), (b), (c) and (d), respectively.) (in CF_3COOD).

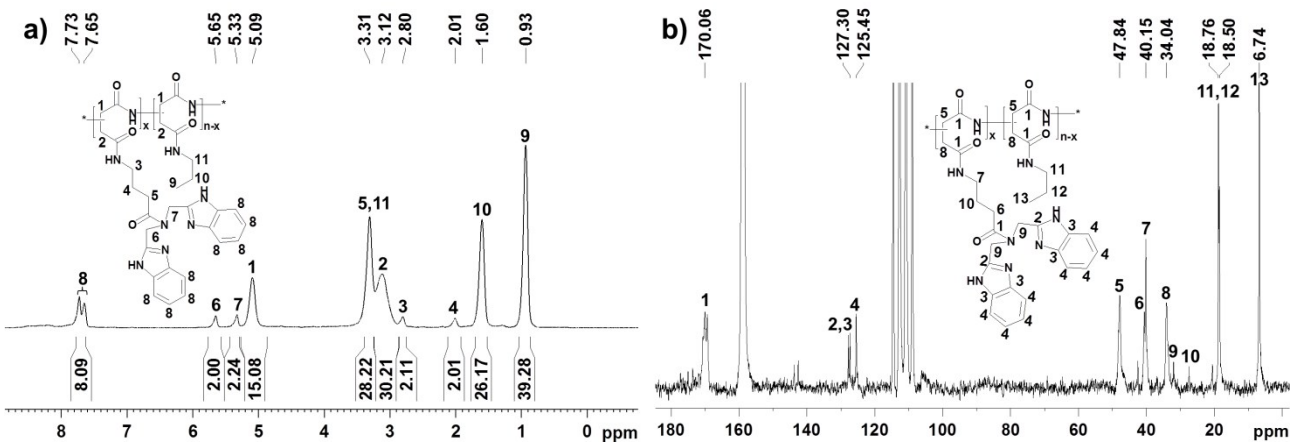


Fig. S3 ^{1}H NMR (a) and ^{13}C NMR (b) spectra of PASP-IDB-propyl (in D_2O+5%CF_3COOD).

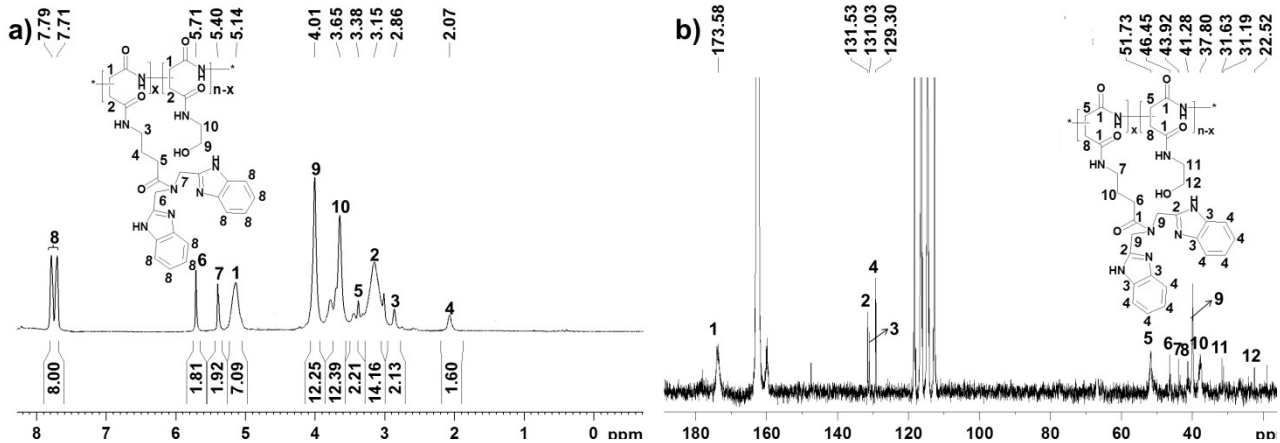
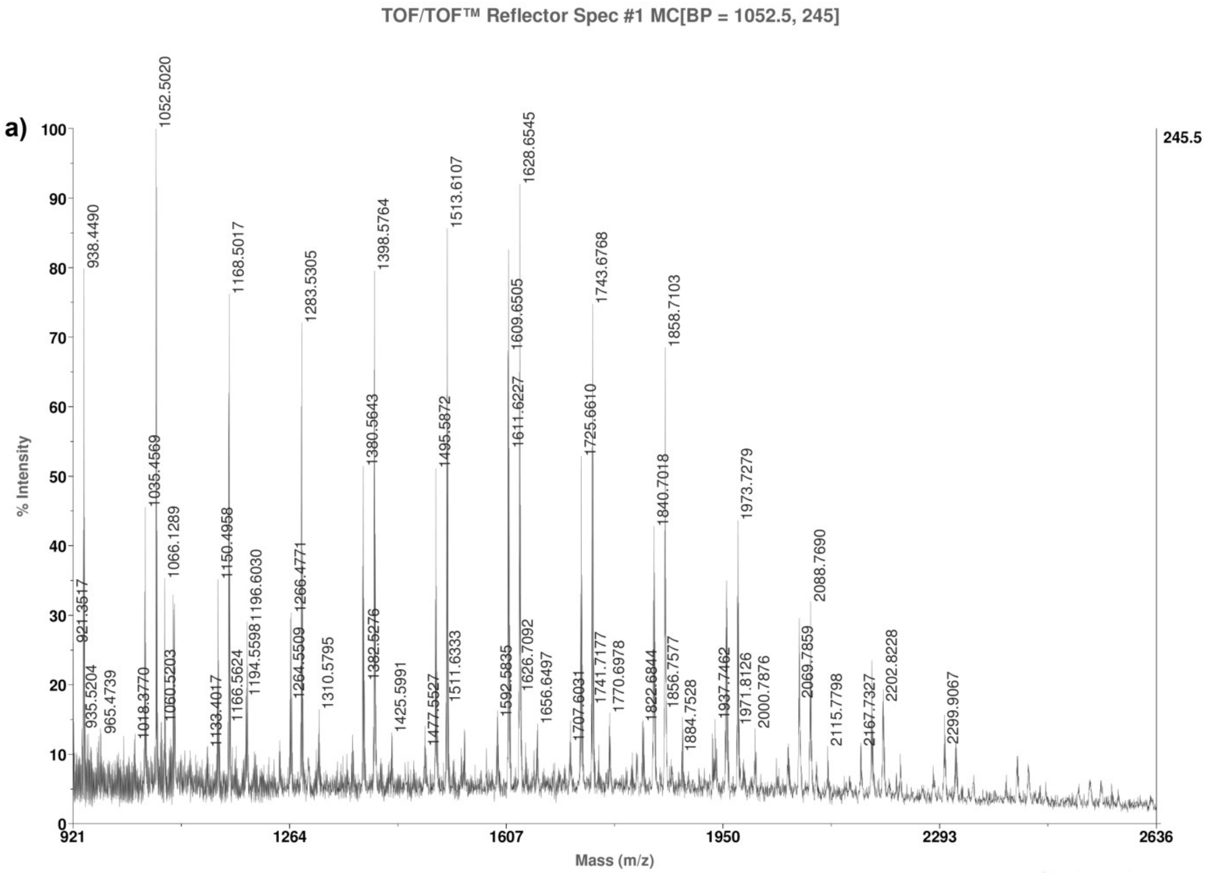


Fig. S4 ^{1}H NMR (a) and ^{13}C NMR (b) spectra of PASP-IDB-hydroxyethyl (in CF_3COOD).

3. MALDI-TOF MS spectra are shown in Fig. S5.

MALDI-TOF MS was carried out on a Bruker Autoflex operating in reflected mode. 2-(4-Hydroxyphenylazo)benzoic acid (HABA) was used as matrix, and NaCl or KCl was used as cationizing agent. Samples were dissolved in MeOH-H_2O (1:1) at a concentration of 1.0 $\mu g \cdot mL^{-1}$. HABA was dissolved in dioxane at a concentration of 0.05 M. Sample (20 μL) and matrix (80 μL) solutions were mixed, and then 80 μL of 0.02 M NaCl or KCl was added. Finally, 1 μL of the resulting mixture was placed on the MALDI plate.


TOF/TOF™ Reflector Spec #1 MC[BP = 1052.5, 245]
a)
% Intensity
Mass (m/z)
245.5
1052.5020
938.4490
1168.5017
1283.5305
1398.5764
1513.6107
1628.6545
1609.6505
1743.6768
1858.7103
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2088.7690

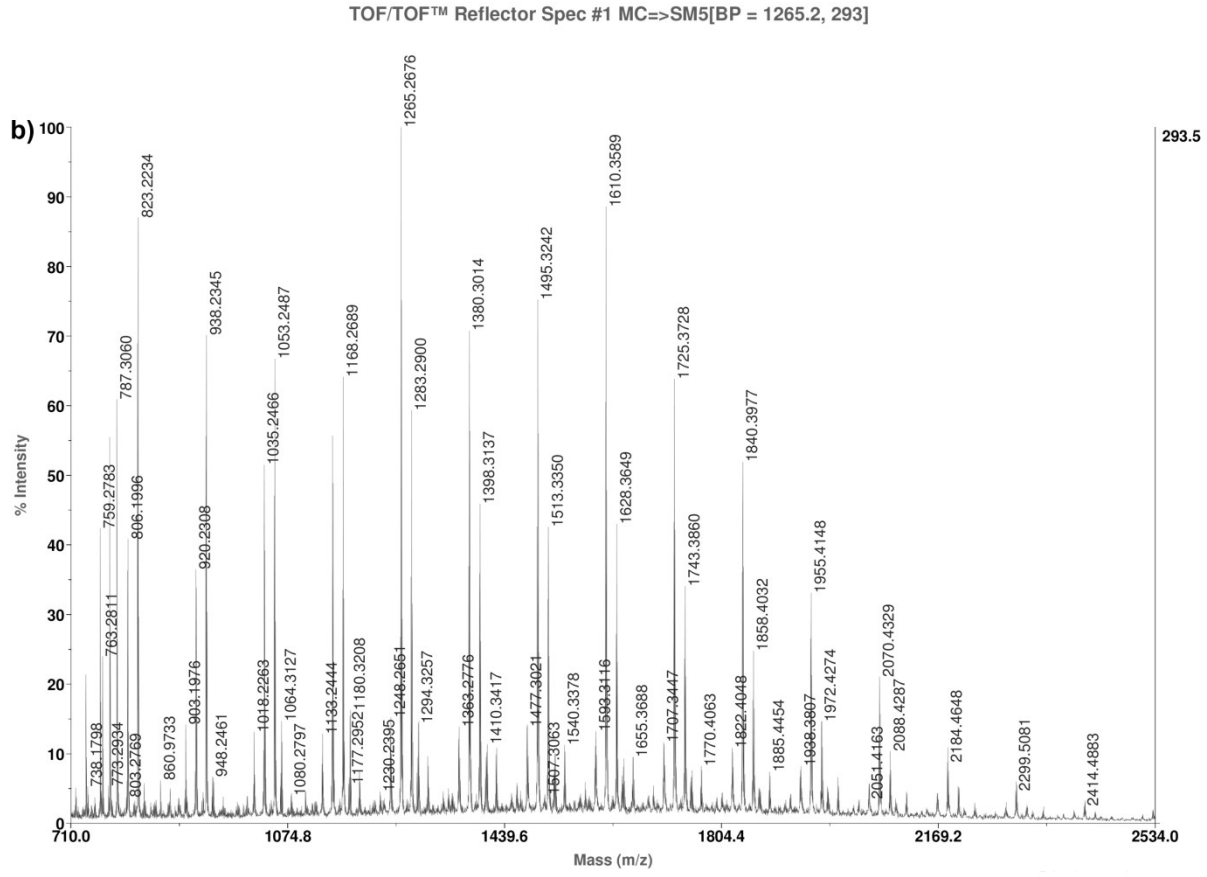
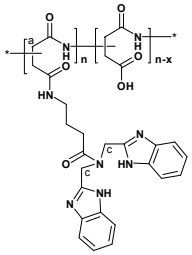


Fig. S5 The MALDFI-TOF spectra of the a) PASP and b) PASP-IDB.

4. Degree of substitution (DS) of PASP-IDB

The degrees of substitution (DS) of PASP-IDB were calculated by the amount ratio of Hc/4 to Ha. The DS of PSI-IDB, PASP-IDB-propyl and PASP-IDB-hydroxyethyl are the same with PASP-IDB due to the preparation from the same intermediate PSI-IDB.



Samples	DS (%)
PASP-IDB	5.0, 9.1, 14.2, 26.2 and 33.3
PSI-IDB	7.1
PASP-IDB-propyl	7.1
PASP-IDB-hydroxyethyl	7.1

5. Agrose gel electrophoresis experiments

Electrophoresis experiments were performed with plasmid pUC18 DNA. In a typical experiment, supercoiled pUC18 DNA (5 μL, 0.05 $\mu g \cdot \mu L^{-1}$) in Tris-HCl buffer (40 mM, pH 7.4) was treated with different concentration catalyst, followed by dilution with the Tris-HCl buffer to a total volume of 80 μL. The samples were then incubated at different temperature and time intervals, and quenched with loading buffer containing 0.5 M EDTA, and loaded on a 1% agarose gel containing 1.0 $\mu g \cdot mL^{-1}$ ethidium bromide (EB). Electrophoresis apparatus was using a Biomeans Stack II Electrophoresis system, PPSV-010. Electrophoresis was carried out at 85 V for 1 h in TAE buffer, and bands were visualized by UV light and photographed, recorded on an Olympus Grab-IT 2.0 Annotating Image Computer System.

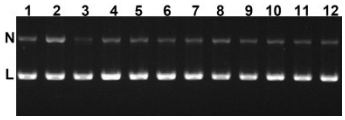


Fig. S6 Time dependence of plasmid pUC18 DNA (0.05 $\mu g \cdot \mu L^{-1}$) cleavage by IDB (0.12 μM) in 40 mM pH 7.4 Tris-HCl buffer at 37°C. Lane 1: control; Lanes 2-12: 0.5, 1, 2, 4, 6, 8, 12, 14, 16, 20 and 24 h, respectively. N: nicked DNA; L: linear DNA; S: supercoiled DNA.

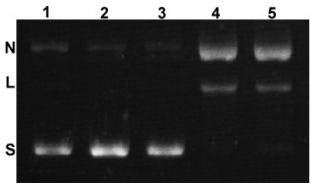


Fig. S7 Agrose gel electrophoresis of plasmid pUC18 DNA cleavage in 40 mM pH 7.4 Tris-HCl buffer at 37°C for 12 h. Lane 1: DNA control; Lane 2: EDTA (1 mM); Lane 3: IDB (0.12 μM); Lane 4: PASP-IDB (0.12 μM); Lane 5: PASP-IDB (0.12 μM) + EDTA (1 mM). N: nicked DNA; L: linear DNA; S: supercoiled DNA.

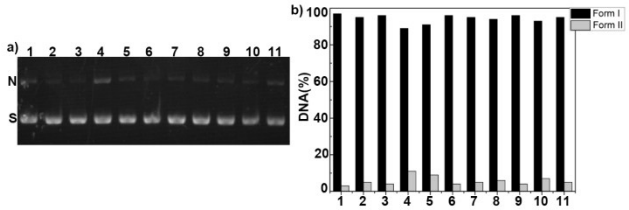


Fig. S8 (a) Concentration dependence of plasmid pUC18 DNA (0.05 $\mu g \cdot u L^{-1}$) by PASP in 40 mM pH 7.4 Tris-HCl buffer at 37°C for 12 h. Lane 1: DNA control; Lane 2–11: 1.0, 4.7, 9.5, 19.0, 38.1, 76.0, 95.1, 114.1, 133.1 and 190.1 × 10^{-3} μM, respectively. (b) Quantitation of % various DNA forms per lane. N: nicked DNA; S: supercoiled DNA.

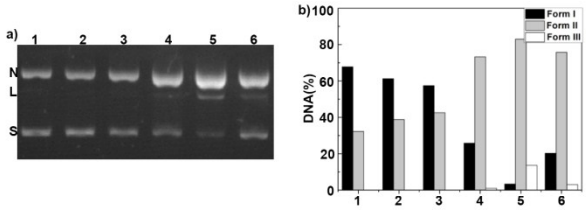


Fig. S9 (a) pUC18 plasmid DNA cleavage by PASP-IDB with different degrees of substitution in 40 mM pH 7.4 Tris-HCl buffer at 37°C for 12 h. Lane 1: DNA control; Lane 2: 5.0%; Lane 3: 9.1%; Lane 4: 14.2%; Lane 5: 26.2%; Lane 6: 33.3%. (b) Quantitation of % various DNA forms per lane. N: nicked DNA; L: linear DNA; S: supercoild DNA.

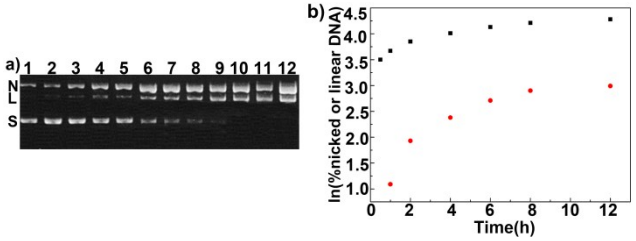


Fig. S10 The plot of ln(% nicked or linear DNA) vs reaction time..

6. UV-Vis absorption spectra

UV-Vis absorption spectra as a convenient technology are usually used to study the interaction of molecules with DNA. Absorption spectra of PASP-IDB (DS = 14.2%) in the presence of ct-DNA are shown in Fig. S10. Fig. S10 shows that PASP-IDB had the characteristic absorption peak at 276 nm and 281 nm. With increasing amounts of ct-DNA added to the solution containing a fixed concentration of PASP-IDB (0.45 mM), an obvious hyperchromicity effect and blue shift (1 nm) was observed. Generally, red shift and hypochromic effect were observed in the absorption spectra of molecules if they intercalated into DNA.[1] The obvious hyperchromicity

effect and blue shift indicated strong interaction between the PASP-IDB and ct-DNA, and the intercalation binding of PASP-IDB to ct-DNA was excluded. The possible binding mode of PASP-IDB to ct-DNA may be electrostatic attraction.

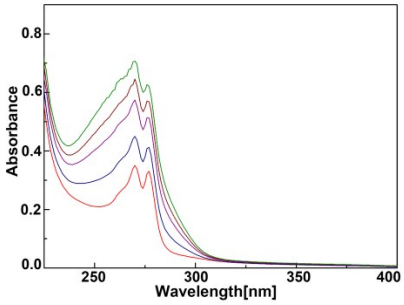


Fig. S11 Absorption spectra of 0.45 mM PASP-IDB (DS = 14.2%) in the absence and presence of increasing amounts of ct-DNA in 40 mM pH 7.4 Tris-HCl buffer. [DNA] = 0 – 6.8 μM from bottom to top curves.

References

1. E. C. Long and J. K. Barton, *Acc. Chem. Res.*, 1990, **23**, 273.