Synthesis and Characterization of Fluorescent Oligo(3,4,5-Triethoxycarbonyl-2-Pyrazoline)

Xiangxiang Jia, Yan Li, Longqiang Xiao, Lijian Liu*

Key Laboratory of Biomedical Polymers, Ministry of Education
College of Chemistry and Molecular Sciences, Wuhan University, Wuhan, 430072, China

Supporting information

Supplementary text

The Beer-Lambert law (i.e., Beer's law) states that

$$A=abc. \qquad (1)$$

where A represents absorbance, a and b are constants representing absorptivity and the path length respectively and c represents the concentration.[1] Herein we use the relative peak area (S) to express the real-time concentration of the reagent to some degree. According to Beer's law, the correlation between S and the actual concentration (a-x) of reagents could be stated as expressed below:

$$a-x=rS \qquad (2)$$

where a refers to the original concentration, x refers to the real-time concentration of the product and r refers to a constant.

Characterizations of 3,4,5-triethoxycarbonyl-2-pyrazoline

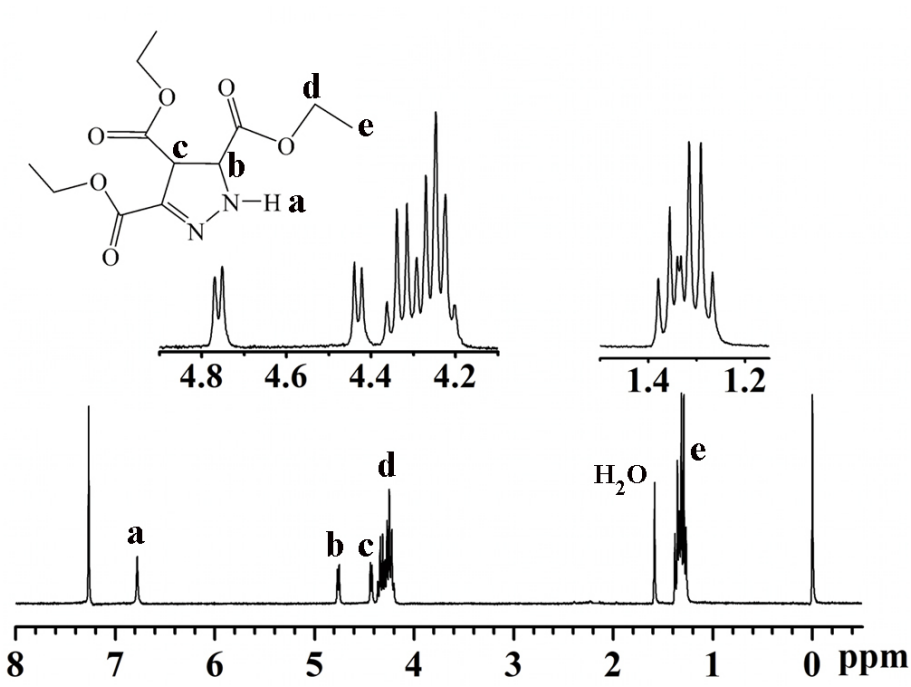

Figure S1. ^{1}H NMR spectrum of 3,4,5-triethoxycarbonyl-2-pyrazoline.

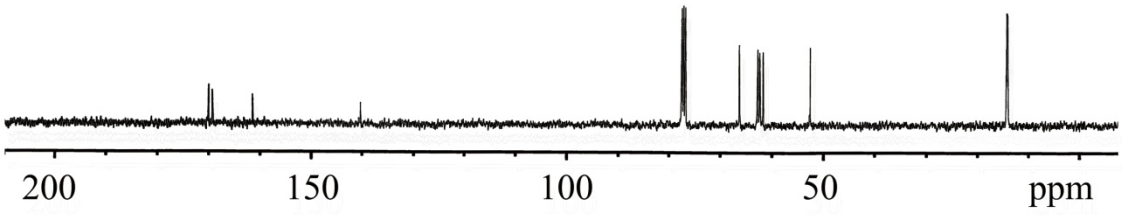

Figure S2. ^{13}C NMR spectrum of 3,4,5-triethoxycarbonyl-2-pyrazoline.

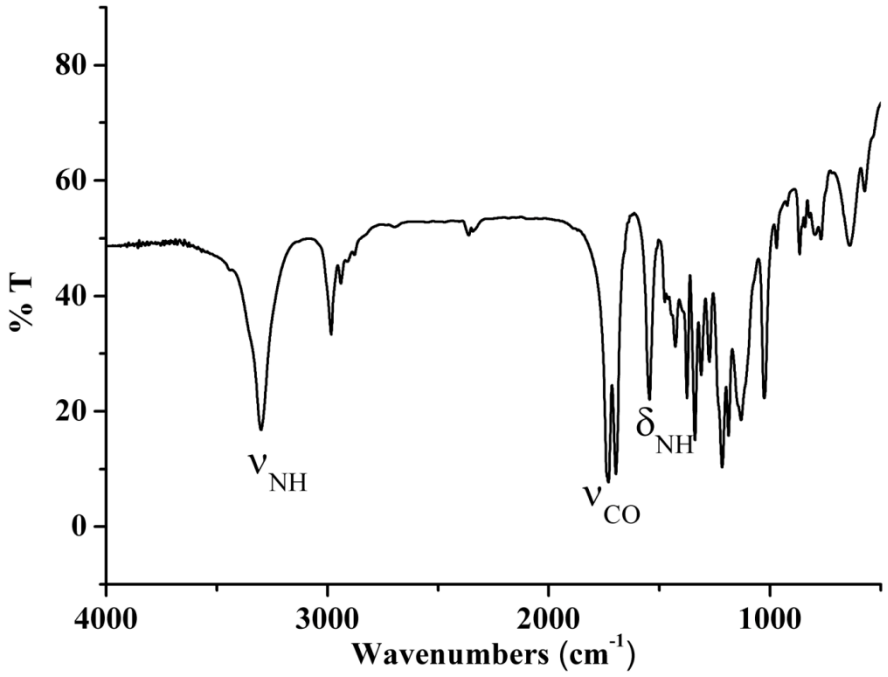

Figure S3. FT-IR spectrum of 3,4,5-triethoxycarbonyl-2-pyrazoline.

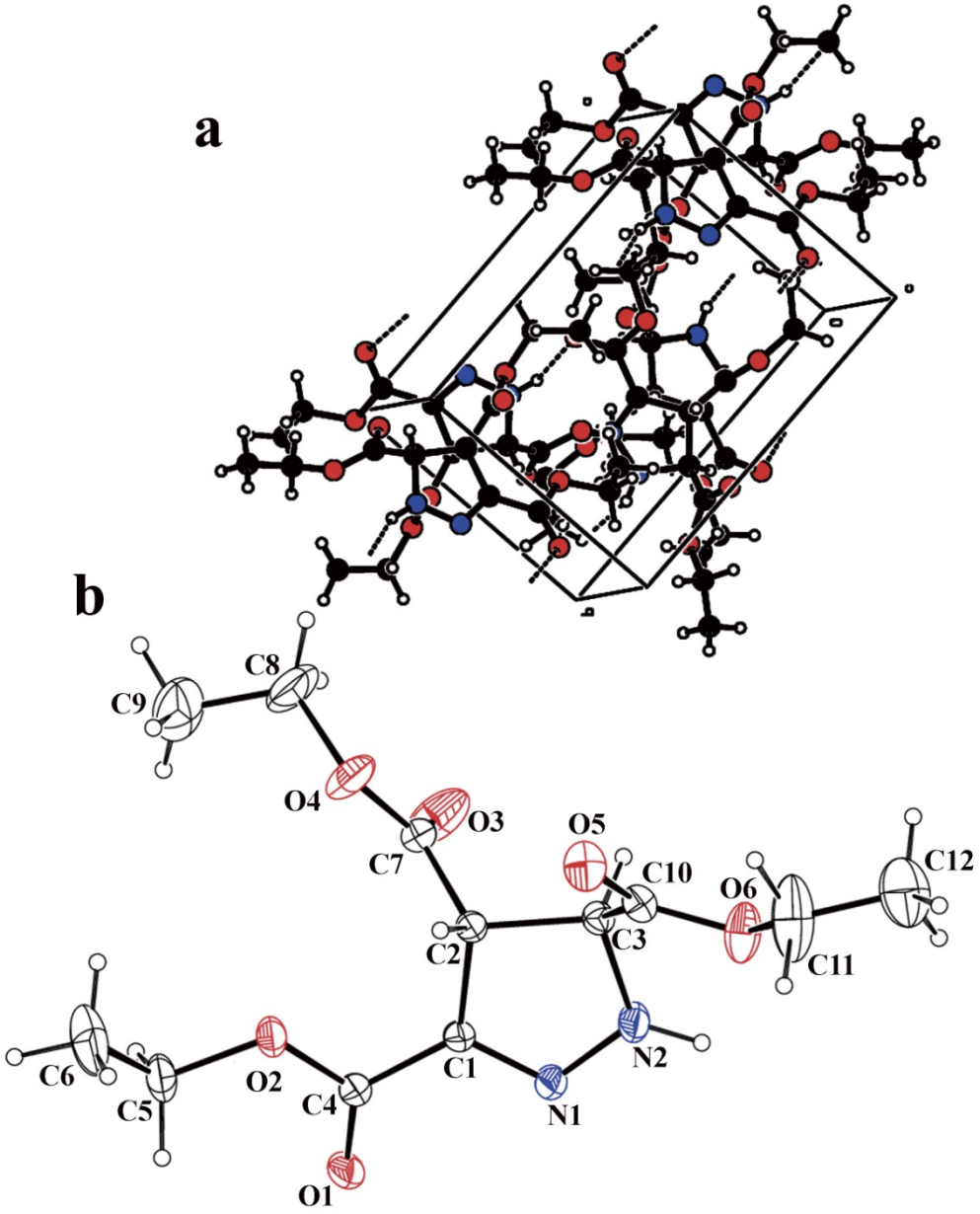

Figure S4. Molecular structure of 3,4,5-triethoxycarbonyl-2-pyrazoline characterized by single-crystal X-ray diffraction.

ON-line FT-IR characterization of the reaction of EDA

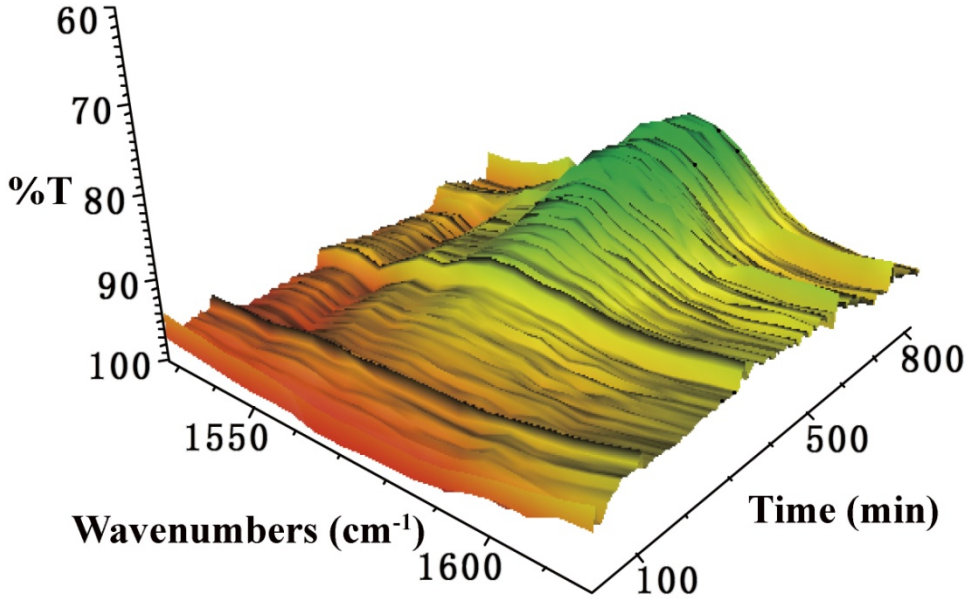

Figure S5. On-line FT-IR spectrum of the reaction synthesizing 3,4,5-triethoxycarbonyl-2-pyrazoline for the band near 1565 cm^{-1}.

DSC analysis of 3,4,5-triethoxycarbonyl-2-pyrazoline and comparisons of NMR and FT-IR spectra of oligomers and monomer

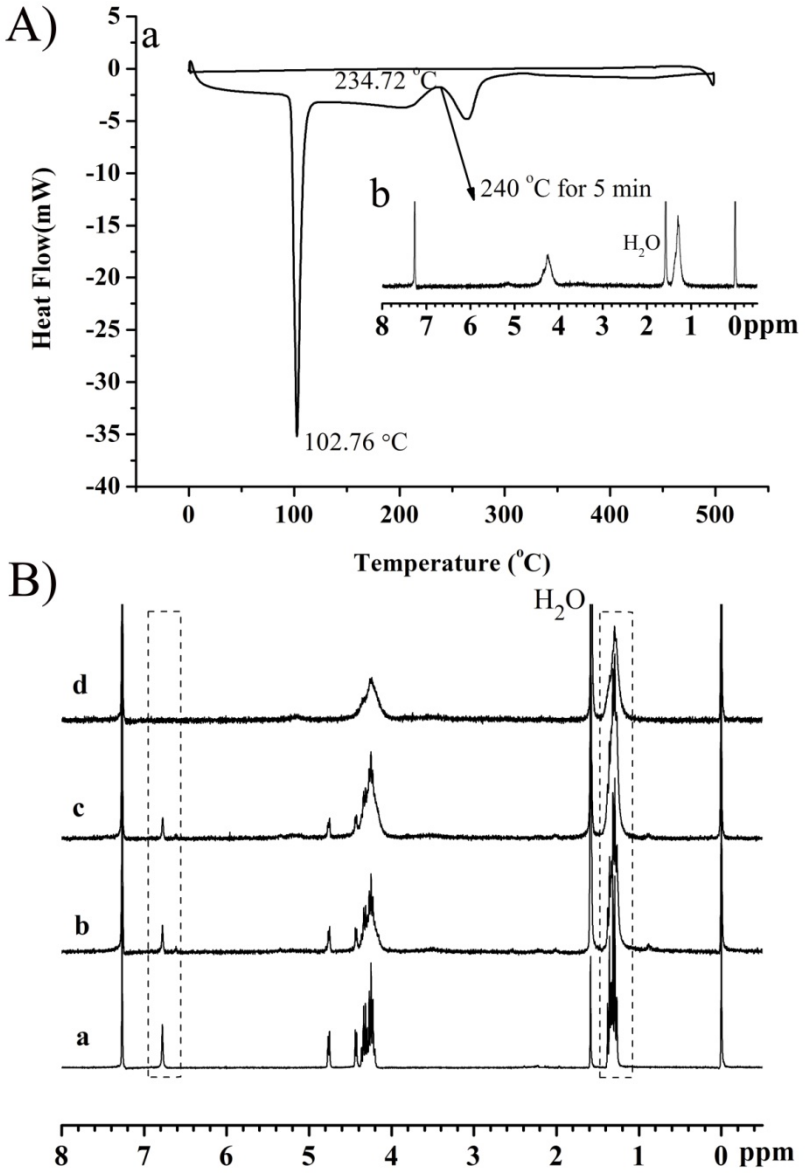

Figure S6. A) DSC curve of 3,4,5-triethoxycarbonyl-2-pyrazoline (a) and the corresponding ^{1}H NMR spectrum of the sample heated from -10 to 240 ºC and hold for 5 min (b). B) ^{1}H NMR spectra of samples after DSC detection at different temperature for different isothermal time (a: 2-pyrazoline with I_{NH}/I_{CH_3}=0.0955; b: the sample heated from -10 to 240 ºC and then cooled to 0 ºC at a rate of 15 ºC/min, I_{NH}/I_{CH_3}=0.0538; c: the sample heated from -10 to 210 ºC, hold for 15 min and then cooled to 0 ºC at the same rate, I_{NH}/I_{CH_3}=0.0384; d: the sample heated from -10 to 240 ºC, hold for 5 min and then cooled to 0 ºC at the same rate). I_{CH_3}: the nuclear magnetic resonance integration value of CH_3 in all. I_{NH}: the nuclear magnetic resonance integration value of NH marked by rectangles.

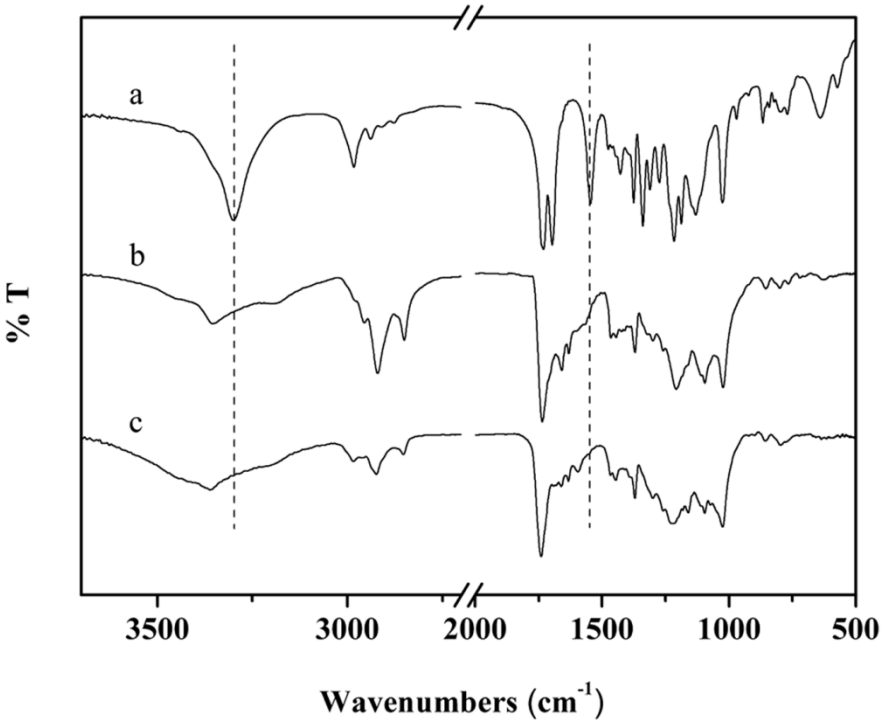

Figure S7. FT-IR spectra of samples after DSC detection at different temperatures for different isothermal time (a: 2-pyrazoline; b: the sample heated from -10 to 240 ºC and then cooled to 0 ºC at a rate of 15 ºC/min; c: the sample heated from -10 to 240 ºC, hold for 5 min and then cooled to 0 ºC at the same rate).

Characterizations of the obtained oligomers

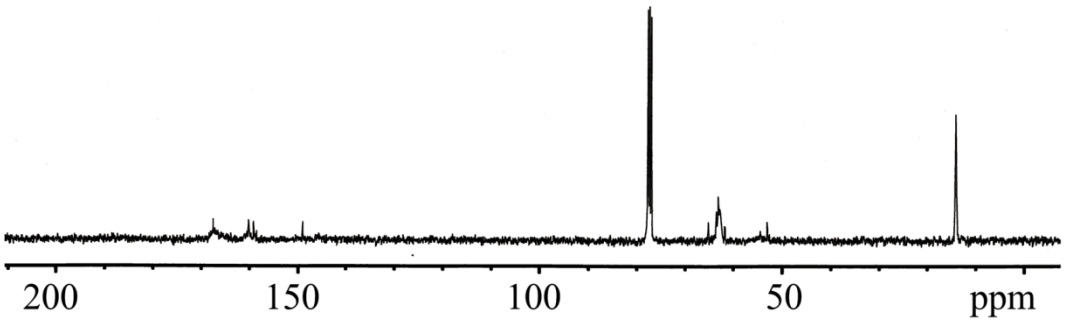

Figure S8. ^{13}C NMR spectrum of the obtained oligomer.

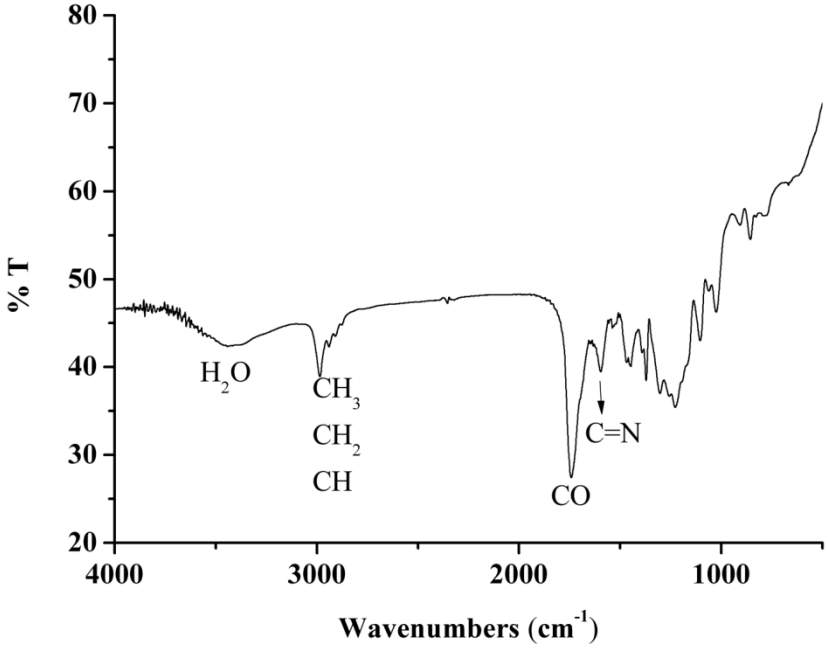

Figure S9. FT-IR spectrum of the obtained oligomer.

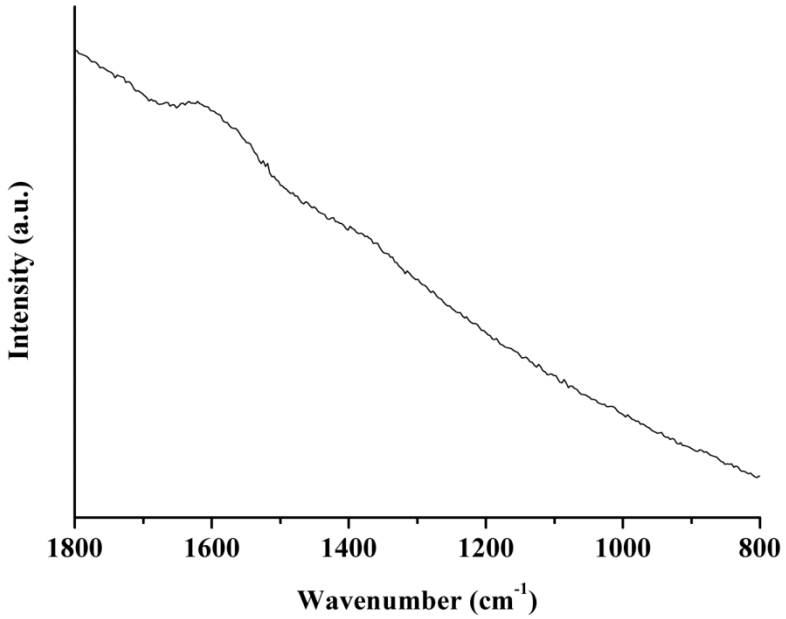

Figure S10. Raman spectrum of the obtained oligomer.

MALDI-TOF-MS analysis of the obtained oligomer catalyzed by $Rh_2(OAc)_4$

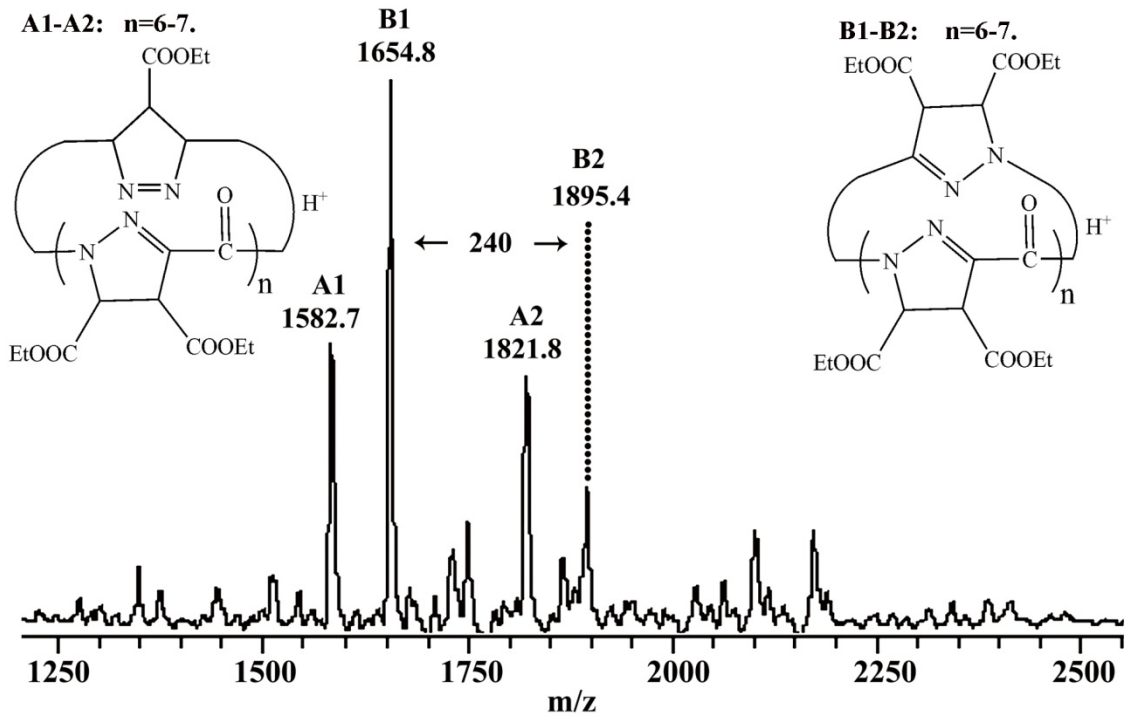

Figure S11. MALDI-TOF-MS spectrum of the oligomer catalyzed by $Rh_2(OAc)_4$.

Cell culture experiments of the monomer and oligomer

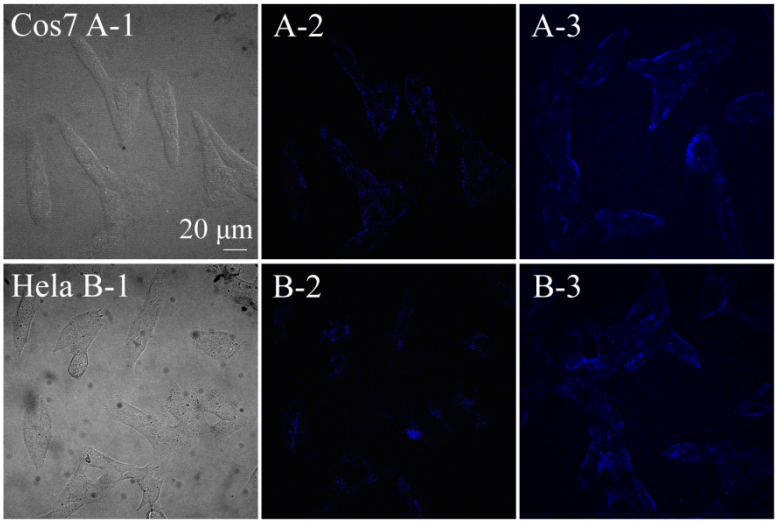

Figure S12. Bright field and confocal fluorescence micrographs at a laser excitation of 405 nm. Line A and B are Cos7 and Hela cells respectively. A-1, A-2, B-1, B-2 are bright field and corresponding fluorescence images of 3,4,5-triethoxycarbonyl- 2-pyrazoline. A-3 and B-3 are fluorescence images of the oligomer catalyzed by $Sn(Oct)_2$.

DSC curves of the oligomers

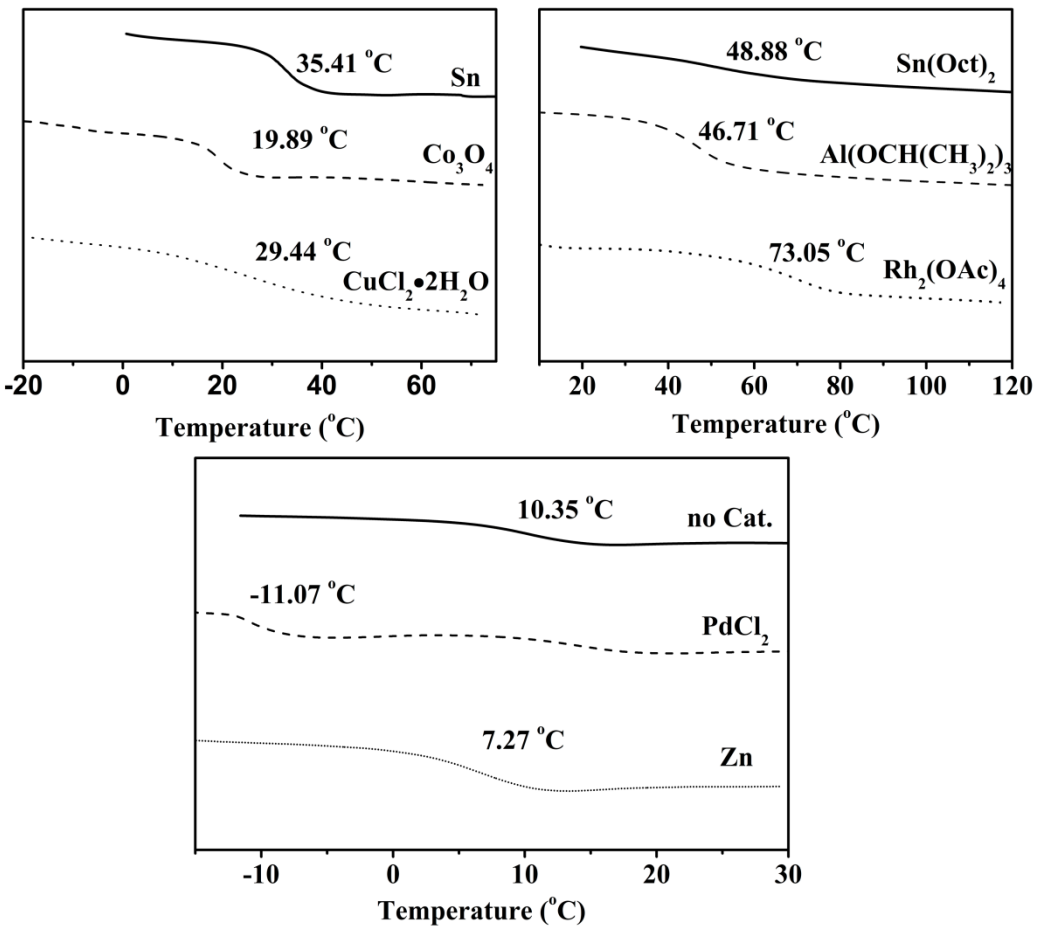

Figure S13. DSC curves of the oligomers with different catalysts.

Cytotoxicity experiment

The cell toxicity of the monomer and the oligomer product was examined by MTT assay. The Cos7 cells and Hela cells were seeded in the 96-well plate with a density of 5×10^4 cells per well and cultured 24 h in 100 μL DMEM containing 10% FBS respectively. After coincubation with the materials to be detected for 48 h, the medium was replaced with 200 mL of fresh medium. Then 20 μL of MTT (5 mg/mL) solution was added in and further incubated at 37 °C for 4 h. After that, the medium was removed and 150 μL DMSO was added in. A microplate reader (BIO-RAD 550) was used to measure the absorbance of the medium at 570 nm. The relative cell viability was calculated as followed:

Relative cell viability (%) = $(OD570_{(sample)}/OD570_{(control)}) \times 100$

where $OD570_{(sample)}$ was obtained in the presence of the product, $OD570_{(control)}$ was obtained in the absence of the product. Data were shown as mean ± standard deviation (SD) based on triplicate independent experiments.

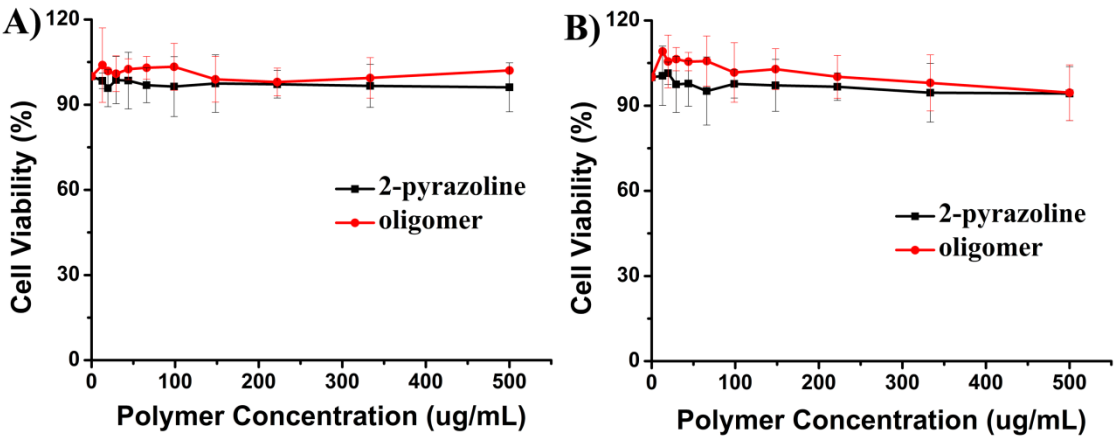

Figure S14. The cytotoxicity of the 2-pyrazoline and oligomer catalyzed by $Sn(Oct)_2$ with Cos7 (A) and Hela (B) cells.

Quantum yields and fluorescence lifetime of the oligomers

The quantum yields are calculated using the following formula:

$$Q = Q_R \frac{I}{I_R} \frac{OD_R n^2}{OD\ n_R^2}$$

with quinine sulfate as the standards for the measurement where Q is the quantum yield, I is the integrated fluorescence intensity, OD is the optical density and n is the refractive index. The subscript R represents the standard fluorophore. The quantum yield of quinine sulfate in 0.1 M H_2SO_4 at 22 ^oC is 0.58 with the excitation of 350 nm.

Table S1. The quantum yields and fluorescence lifetime of oligomers with different catalysts

Run	Catalyst	Quantum yields (%)	Fluorescence lifetime		
			τ_1 (ns) (A%)	τ_2 (ns) (A%)	χ^2
1	No Cat.	16	1.67 (53.54%)	6.41 (46.46%)	1.291
2	$PdCl_2$	22	2.22 (62.16%)	7.74 (37.84%)	1.421
3	$Sn(Oct)_2$	22	2.04 (58.29%)	5.72 (41.71%)	1.255
4	Sn	15	1.90 (60.79%)	7.39 (39.21%)	1.076
5	$Al(OCH(CH_3)_2)_3$	26	2.40 (57.90%)	8.59 (42.10%)	1.503
6	$Rh_2(OAc)_4$	15	2.55 (39.88%)	11.43 (60.12%)	1.233
7	Co_3O_4	13	1.82 (45.81%)	11.07 (54.19%)	1.292
8	Zn	15	1.58 (58.70%)	6.66 (41.30%)	1.316

References

1. K. Fuwa and B. L. Valle, *Anal. Chem.*, 1963, **35**, 942-946.