

A reversible photoresponsive activity of a carbonic anhydrase mimic

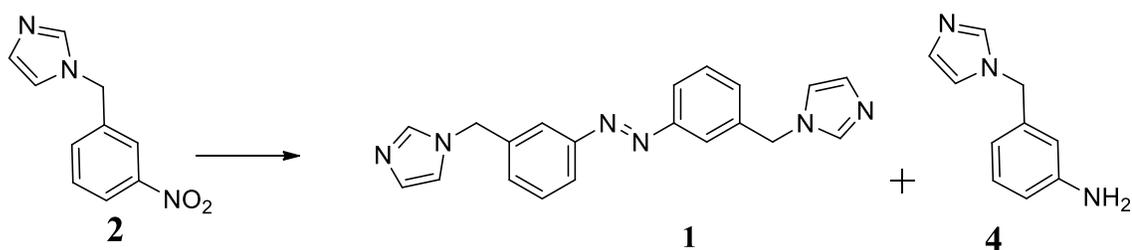
Monochura Saha and Subhajit Bandyopadhyay*

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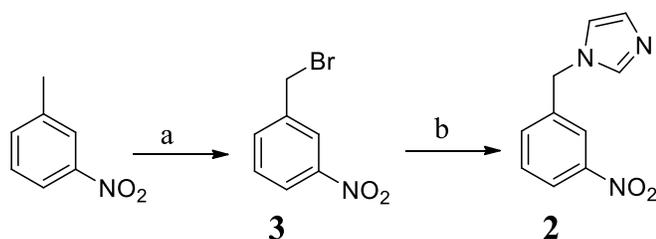
Instrument and reagents

All reactants and reagents were commercially available and were used without further purification unless otherwise indicated. Styrene (99%) and 2-(dimethylamino) ethyl methacrylate (DMAEMA, 98%) were passed through a basic alumina column before polymerization. 2,2'-azobisisobutyronitrile (AIBN, Sigma, 98%) was recrystallized twice from methanol. The chain transfer agent (CTA), 4-cyano-4-(dodecyl sulfanyl thiocarbonyl) sulfanyl pentanoic acid (CDP), was prepared as previously reported.¹Anhydrous *N,N*-dimethylformamide (DMF, Sigma Aldrich, 99.9%) and CDCl₃(Cambridge isotope, 99% D) were used as received. The sodium salt of the commercially available (Sigma Aldrich) Polystyrene-*block*-poly(acrylic acid), DDMAT terminated (PS:PAA 3,000:5,000, PDI ≤1.1) was used as the negative charged polymer. The NMR spectra were recorded with 500 MHz Bruker and 400 MHz Jeol instruments. Chemical shifts are reported in δ values relative to the solvent peaks. The solvents used for the spectroscopy experiments were of the spectroscopic grades and were free from any fluorescent impurities. Double. UV spectra were recorded with an Agilent Cary 60 UV-vis spectrophotometer. The mass spectrometric data were obtained from an AcquityTM Ultra Performance LC-ESI/quadrupole-TOF MS. X-ray data were collected with Mo K α radiation by a Bruker APEX-2 CCD diffractometer. The data were processed using Bruker SAINT package. The structure solution and refinement were performed by SHELX97. FT-IR spectroscopy in the solid state was performed with a Perkin Elmer Spectrum RX1 spectrophotometer using KBr disk method and the far infrared spectra were recorded by using Bruker ALPHA FTIR spectrometer. It was recorded in absorbance mode from the range 200-500 cm⁻¹ with a resolution of 1 cm⁻¹ and was obtained by averaging over 20 scans. All samples were measured in vacuum and at room temperature. Melting point was measured with a Secor, India digital melting point apparatus in melting point tube. pH data were recorded with a PB-11 Sartorius pH Meter calibrated at pH 4, 7 and 10. DLS data were recorded with HORIBA Scientific Nano Partica NANO PARTICLE ANALYZER SZ-100.



Conditions : NaOH, Glucose, EtOH, H₂O, reflux, 24h

Scheme **S1**: Synthesis of the imidazole appended azobenzene compound **1**



Conditions: (a) NBS, CCl₄, AIBN, Reflux, 2h (b) Imidazole, K₂CO₃, CH₃CN, Reflux, 18h

Scheme **S2**: Synthesis of *m*-nitro benzyl imidazole compound **2**

Experimental Section

Synthesis of imidazole-appended azobenzene (1)

To a solution of NaOH (1.94 g, 48.5 mmol) in EtOH/H₂O mixture, compound **2** (0.985 g, 4.85 mmol) was added. A solution of glucose (8.7 g, 48.5 mmol) in hot water was added to the reaction mixture carefully. The mixture was then heated under reflux for 24 h upon which the orange solution turned into red in color. Upon completion of the reaction, the reaction mixture was concentrated under vacuum; the residue was extracted with ethyl acetate, washed with brine, and dried over Na₂SO₄. After the evaporation of the solvent, the crude material was purified by chromatography (100-200) mesh, silica gel deactivated with 5% triethyl amine, using MeOH/ EtOAc(1:19) as eluent upon which compound **1** was obtained as an orange solid (0.356 g, 42 %). Melting point: 163-165 °C; ¹H NMR: (400 MHz, CDCl₃) δ 7.94 (d, *J* = 6.3 Hz, 2H), 7.78 (s, 2H), 7.69 (s, 2H), 7.59 (t, *J* = 6.3 Hz, 2H), 7.34 (d, *J* = 5.6 Hz, 2H), 7.19 (s, 2H), 7.03 (s, 2H), 5.30 (s, 4H). ¹³C NMR (100 MHz, CDCl₃) δ 153.6, 138.2, 130.7, 130.6, 130.5, 130.4, 123.8, 122.2, 120.0, 51.26; IR (KBr): 2982, 2917, 1637, 1585, 1414, 1362, 1121, 1034 cm⁻¹. ESI-MS *m/z* (calc.) [M+H]⁺ = 343.1671, [M+Na] = 365.1491, [M+K] = 381.1230. (obtained) [M+H]⁺ = 343.1678, [M+Na] = 365.1519, [M+K] = 381.1302 are obtained from experimentally.

UV-Vis Data: The *trans* isomer of compound **1** displayed absorption bands at λ_{max} = 230, 319, and 440 nm. The band at λ_{max} = 319 nm (ε = 11,800 M⁻¹cm⁻¹) corresponds to a π-π* transition, and the band at 440 nm is a symmetry forbidden n-π* band (ε = 110 M⁻¹cm⁻¹). For the *cis* **1** λ_{max} 319 (ε = 7040 M⁻¹.cm⁻¹) corresponds to a π-π* transition and a new peak appeared at 440 nm (ε = 400 M⁻¹.cm⁻¹).

XRD-Data: single crystal XRD (CCDC 1846676)

Synthesis of *m*-nitro benzyl imidazole (2)

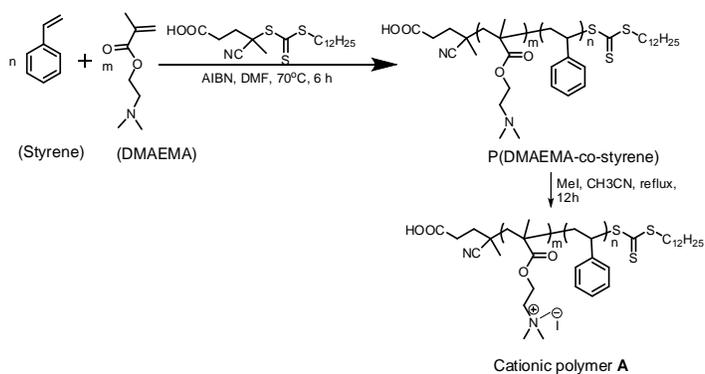
To the anhydrous solution of *m*-nitro benzyl bromide (0.990 g, 4.58 mmol) in acetonitrile, K₂CO₃ (3.9 g, 27.48 mmol) and imidazole (1.24 g, 18.3 mmol) were added. The mixture was refluxed for 15 h under argon. Upon completion of the reaction, the reaction mixture was filtered through a sintered funnel and the solvent was removed under vacuum. The residue was dissolved in EtOAc (200 mL) and the organic phase was washed with a saturated Na₂CO₃ aqueous solution (50 mL), dried over Na₂SO₄, and evaporated to dryness under vacuum. The crude mixture was purified by column chromatography using 100 – 200 mesh silica gel and MeOH: EtOAc as eluent to give the *m*-nitro benzyl imidazole as a yellowish solid (0.760 g, 77 %); **Melting point:** 72-75 °C; **¹H NMR**: (500 MHz, CDCl₃) δ 8.17 (d, *J* = 8.2 Hz, 1H), 8.04 (s, 1H), 7.58 (s, 1H), 7.54 (dd, *J* = 8.2, 7.9 Hz, 1H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.13 (s, 1H), 6.92 (s, 1H), 5.23 (s, 2H); **¹³C NMR** (100 MHz, CDCl₃) δ 143.9, 139.1, 138.2, 133.6, 131.2, 130.9, 124.0, 122.8, 119.9, 50.6; IR (KBr) 2965 (w), 2908 (w), 1615 (m), 1421 (s), 1362 (s), 1025 (s), 907 (m), 739 (m), 617 (w), 558 (w) cm⁻¹; ESI-MS *m/z* (calc.) 204.07 [M+H]⁺, (obtained) 204.14;

Synthesis of *m*-nitro benzyl bromide (3)

To a solution of *m*-nitro toluene (2 gm, 14.59) in CCl₄ (45 mL), NBS (2.85 gm, 16.05 mmol) and AIBN (140 mg, 1.459 mmol) were added and the mixture was refluxed for 2h. The reaction was monitored by TLC. Upon completion, the reaction mixture was filtered and the solvent was evaporated under vacuum. The product was purified by column chromatography by using hexane as eluent and the product was obtained as a white solid (1.92 g, 60 %).

¹H NMR (400 MHz, CDCl₃) δ 8.25 (d, *J* = 2.28 Hz, 1H), 8.15 (dd, *J* = 8 and 2.28 Hz, 1H), 7.72 (d, *J* = 7.64 Hz, 1H), 7.53 (dd, *J* = 8.4, 7.64, 1H), 4.53 (s, 4H); **¹³C NMR** (100 MHz, CDCl₃) δ 148.3, 139.6, 134.9, 129.8, 123.8, 123.2, 31.0

RAFT Copolymerization of DMAEMA and Styrene. In a typical RAFT copolymerization procedure, DMAEMA (440.2 mg, 2.8 mmol), styrene (437.44 mg, 4.2 mmol), CDP (70.6 mg, 0.175 mmol), AIBN (5.75 mg, 0.035 mmol) and DMF (2.4 g) were sealed in a 20 mL glass vial equipped with a magnetic stir bar and purged with dry N₂ for 20 min and the reaction vial was kept in a preheated reactor at 70 °C. The [DMAEMA + Styrene]:[CDP]:[AIBN]ratio was kept at 40:1:0.2, while the concentration ratio of DMAEMA and styrene in the feed was fixed at 40:60, respectively. The polymerization was allowed to continue for 6 h and the resulting solution was quenched by cooling in ice cold water and exposed to air. Approximately 3 mL acetone was added to this solution, and the polymer was precipitated into cold hexanes. The copolymer was re-precipitated four times from acetone/hexanes and dried under high vacuum at room temperature for 18 h. The purified polymer (200 mg, 0.03 mmol) was treated with methyl iodide (2 mL, 0.5 mmol, 20 eq) in acetonitrile under 40 °C heat for 12h. Evaporated the acetonitrile solvent under reduced pressure and purified by ethyl acetate. C H N Analysis of Polymer **A**: C, 53.65; H, 7.25; I, 15.75; N, 3.48; Experimentally Found: C, 44.3; H, 7.14; N, 2.32



Scheme S3: Synthesis of the cationic polymer **A**

Photoisomerization studies:

Photoisomerization experiments were performed with compound **1** (30 μM) in MeOH. The *E* to *Z* photoisomerization of azobenzene was observed by irradiating the sample with 366 nm UV light (8 W, irradiation power 2.3 mW cm^{-2}) under ice-cold conditions (273K). The reverse isomerization (*Z* to *E*) was achieved by exposing the sample with blue LED light (8 W) at 0 $^{\circ}\text{C}$. The isomerization was monitored by studying the change in the UV/Vis absorption spectra and also by ^1H NMR analysis.

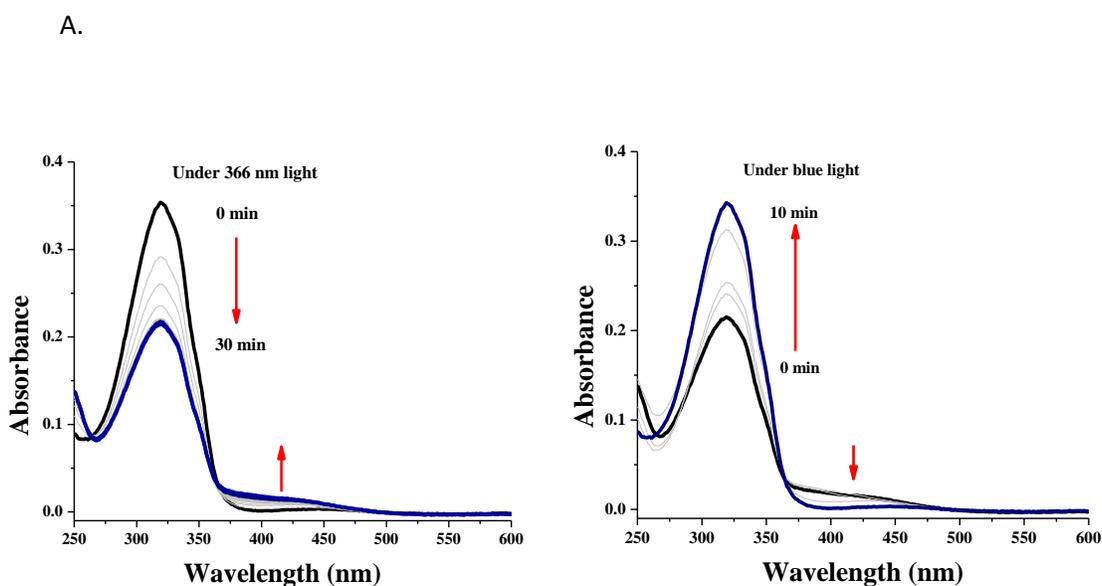


Fig. S1. Changes in electronic absorption spectrum for the (A) *E* to *Z* and (B) *Z* to *E* isomerization of the azobenzene **1** upon exposure to 366 nm UV light and 466 nm blue light respectively in MeOH (30 μM) at 273K.

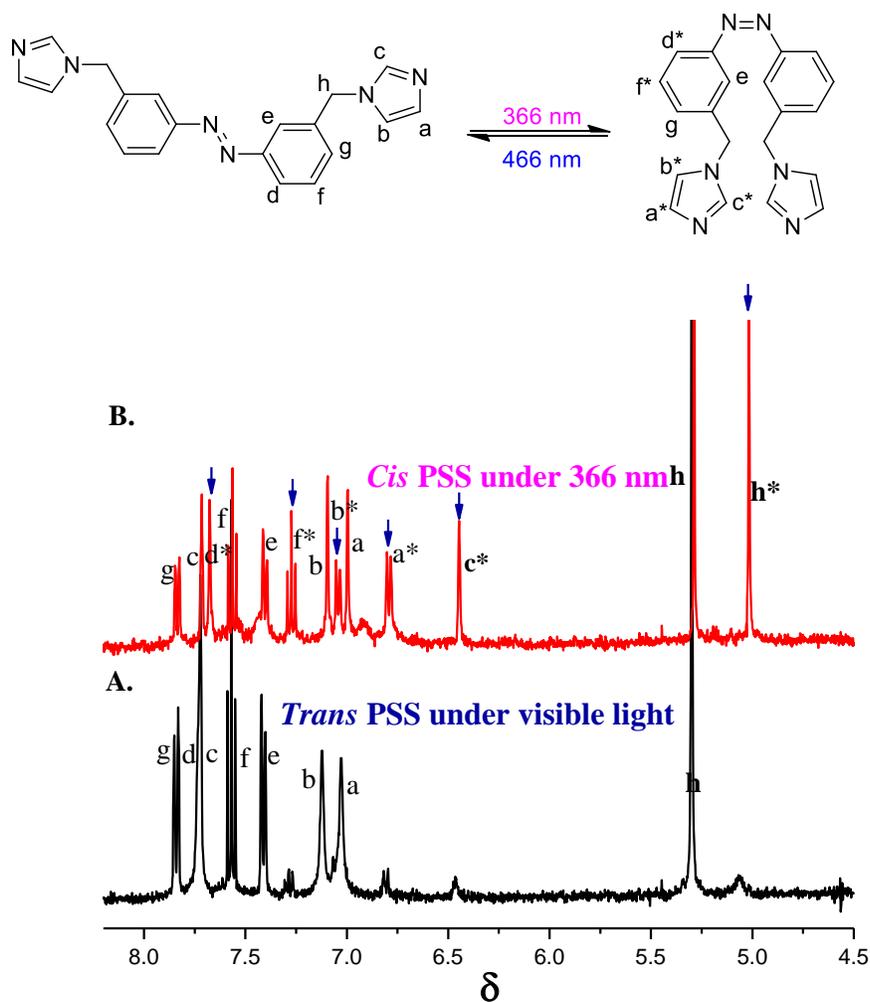


Fig. S2. NMR traces of compound **1** in CD₃OD (10⁻² M) displaying the clear shift in the aromatic and aliphatic protons upon photo isomerization. (A) After radiation of the *trans* form with 366 nm light for 240 min; (B) *trans* form (bottom trace). New peaks are indicating by arrow sign.

General procedure for the hydrolysis

Each assay was performed on 3 mL of samples prepared in 1 mM PBS buffered at pH 7.6 solution with the azobenzene derivative (9 μM) or nothing (as control). The reactions were performed in the darkness at 25 $^{\circ}\text{C}$. The photoirradiated (*Z*)-form of the catalyst was exposed to the 366 nm light periodically for ensuring its retention in the (*Z*)-form. The formation of the 4-nitrophenol (4-NP) was monitored by the appearance of the peak at 400 nm with UV/vis spectroscopy at room temperature. The concentration of the 4-NP species was calculated at individual pH from the molar absorptivity of the species at each of the pH at 400 nm.

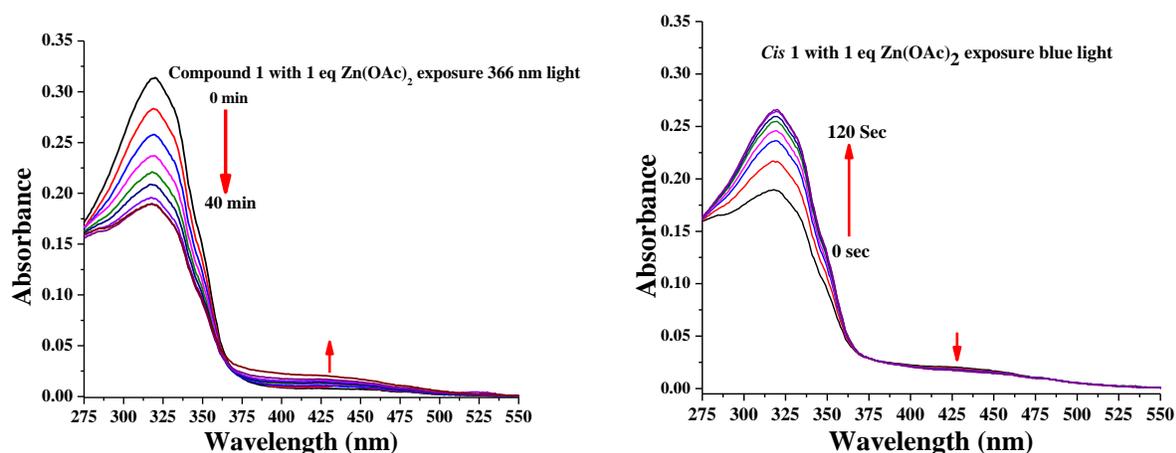


Fig. S3. Changes in electronic absorption spectrum for (A). *E* to *Z* and (B). *Z* to *E* isomerization of azobenzene **1** (30 μM) with $\text{Zn}(\text{OAc})_2$ (30 μM) upon exposure to 366 nm UV light and 466 nm blue light respectively in MeOH solution.

With increasing the $\text{Zn}(\text{OAc})_2$ in the azobenzene solution, it is observed that there was a 10 nm blue shift of the $\pi\text{-}\pi^*$ band from 319 nm to 309 nm. The $[\text{Zn}^{2+}\cdot\mathbf{1}]$ was also studied by NMR spectroscopy.

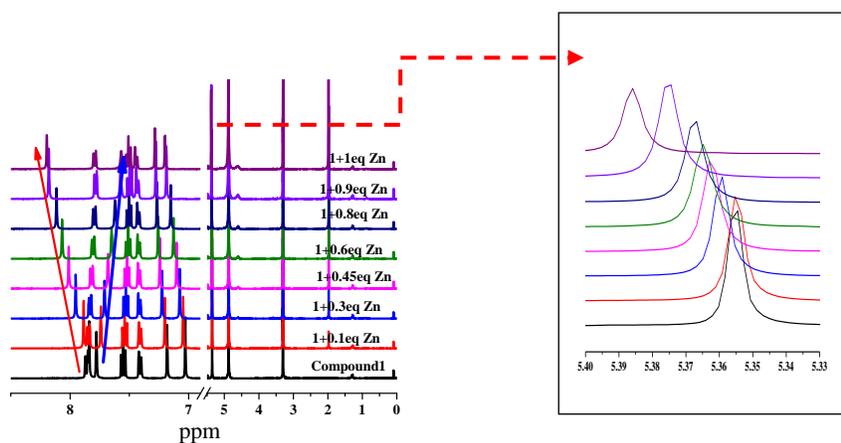


Fig. S4. Systematic NMR titration of *trans* **1** with gradual increase of $\text{Zn}(\text{OAc})_2$ in $\text{MeOD}:\text{D}_2\text{O}(6:1)$ mixture, red arrow for the c^* proton, blue arrow for the a^* proton and black arrows for the h^* proton

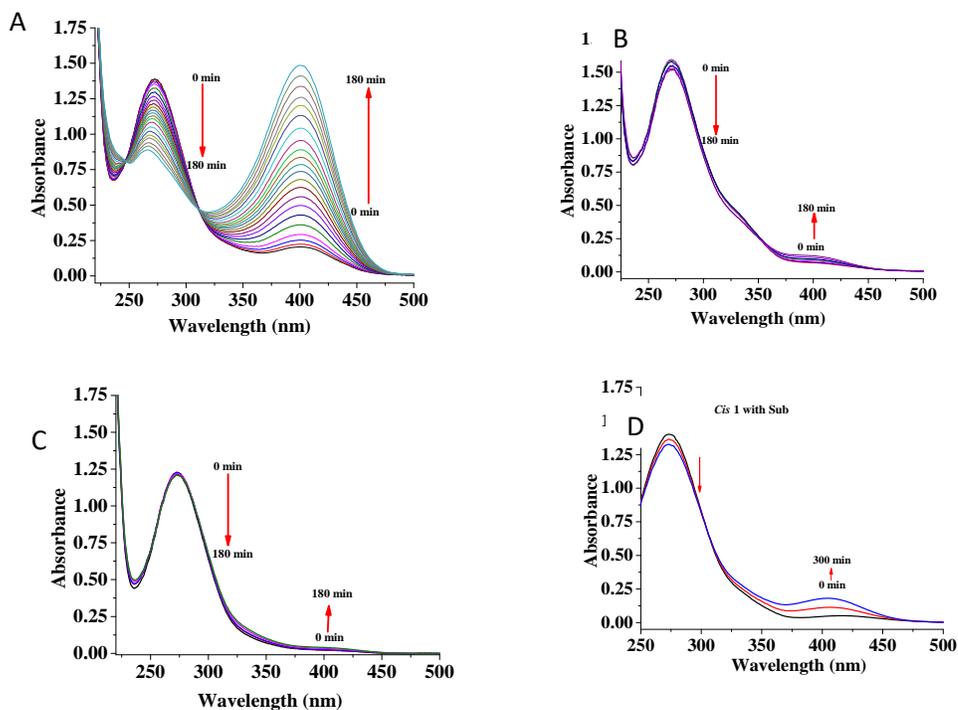


Fig. S5. UV-Vis spectra with the monitoring the hydrolysis of the pNPA substrate with the catalyst in PBS buffered at pH 7.6 (A) With [*Cis* **1** + Zn] ($30 \mu\text{M}$) with the pNPA-substrate ($150 \mu\text{M}$), (B). With [*Trans* **1** + Zn^{2+}] with Substrate (C). Only Zn^{2+} (D). Only *Cis* **1** (No Zn^{2+}).

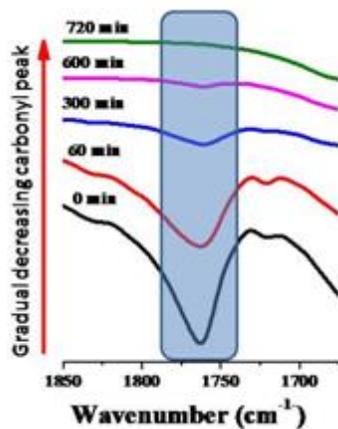


Fig. S6. The disappearance of the carbonyl peak of the pNP substrate upon the catalysis with [*cis* **1**. Zn²⁺] at 25 °C. The IR spectra was recorded with dried aliquots at different time intervals in KBr.

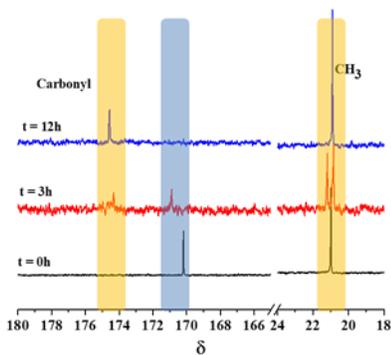


Fig. S7. ¹³C NMR traces of pNPA hydrolysis of pNPA (30 mM) upon hydrolysis with the [*cis*-**1**. Zn²⁺] (0.3 mM) in CD₃OD-D₂O (1:1 v/v) (PBS buffered at pH 7.6).

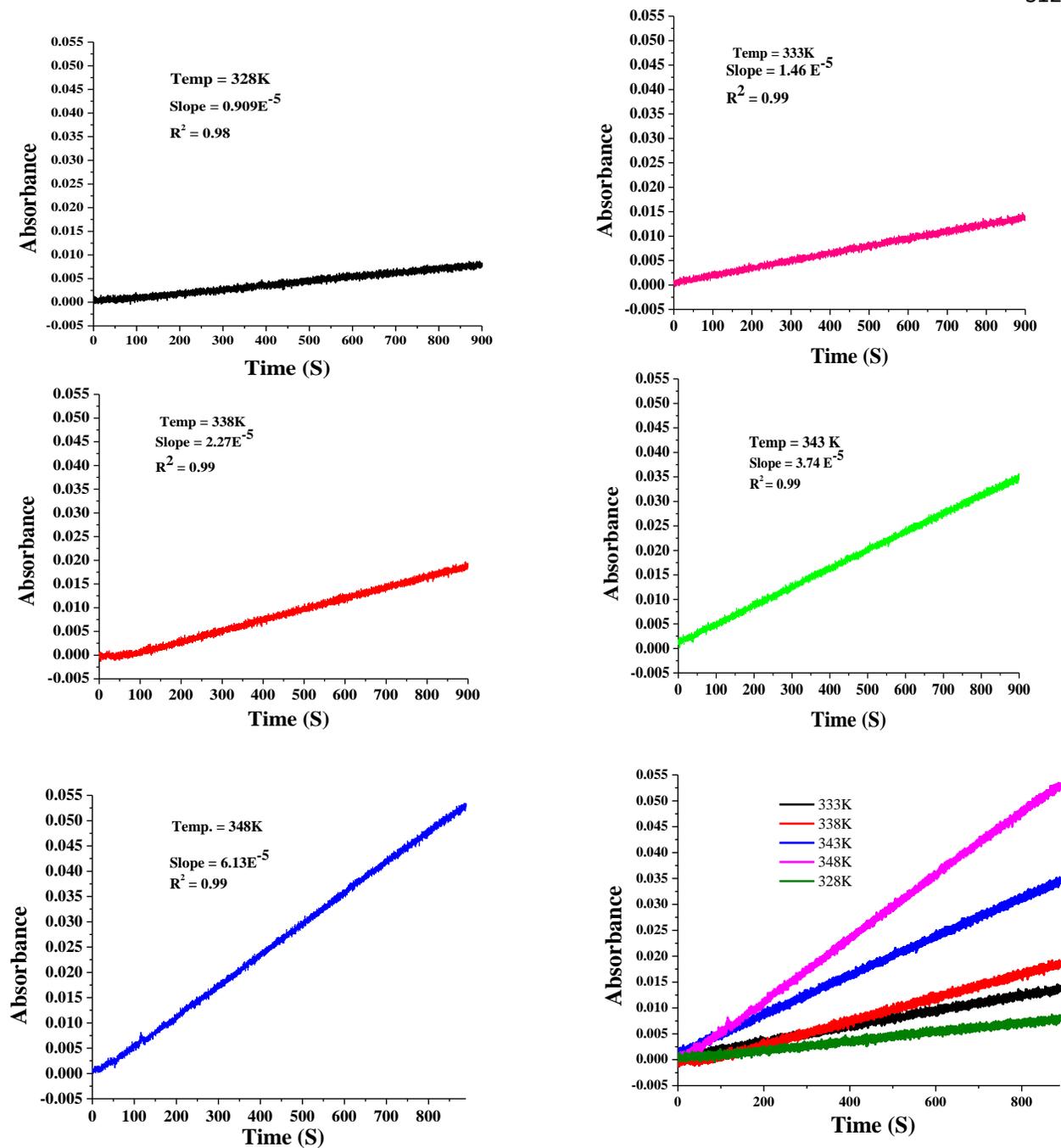


Fig. S8. Thermal isomerization studies of the (*Z*) to (*E*) isomer of the azobenzene **1** at various temperatures.

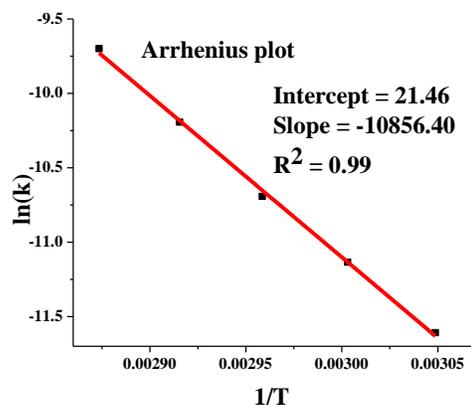


Fig. S9. (A) Arrhenius and (B) Eyring plots for the determination of the activation parameters for the thermal reversal of the (*Z*) to the (*E*) isomer.

Table S1: Thermodynamic parameters for the (*Z*) to (*E*) isomerization of the azobenzene **1**.

Parameters	ΔE_{act} (kcal mol ⁻¹)	ΔH^{\ddagger} (kcal mol ⁻¹)	ΔS^{\ddagger} (cal mol ⁻¹ K ⁻¹)
Values \pm (error)	21.5 \pm (0.5)	21.5 \pm (0.5)	42 \pm (2)

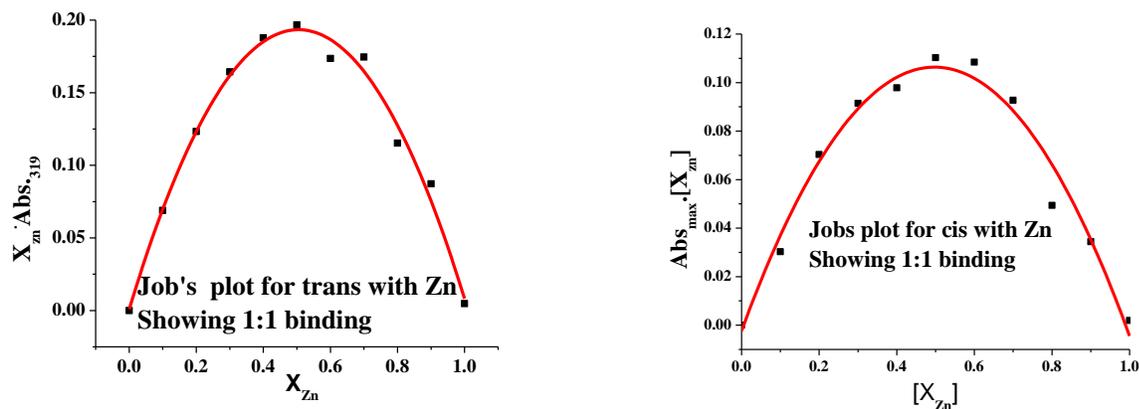


Fig. S10. Job's plots indicating 1:1 complexation of compound **1** with Zn²⁺ ions in methanol media at a total concentration of 0.1 mM of **1** and Zn²⁺.

From the Job's plot the 1:1 association of compound **1** (both *cis* and *trans*) with Zn²⁺ was found.

Table S2: Background reactions: Pseudo first order rate constants (k) for under various conditions in the absence of polymer **A**.

Catalytic conditions* (Without the cationic polymer A)	Rate constant k (min^{-1}) $\times 10^{-4}$
[<i>Cis</i> 1 +Zn ²⁺] ((30 uM)	277
[<i>Trans</i> - 1 .Zn ²⁺] (30 uM)	9.02
[<i>Cis</i> 1 +Zn ²⁺] ((30 uM) (With negative polymer)	130
Only Zn ²⁺ (30 uM)	1.06
<i>Cis</i> - 1 (30 uM, no metal ions)	10.0
<i>Trans</i> - 1 (30 uM, no metal ions)	6.25
Only Substrate in PBS buffer 1 mM	1.06

*General conditions: 1 mM PBS buffer at pH 7.6, 25 °C.

Upon alternating irradiation of 366 and 466 nm light and repeating the sequence of the irradiation in multiple cycles, the corresponding absorbance of **1**, and the peak at 309 nm was used to monitor the switching in cycles.

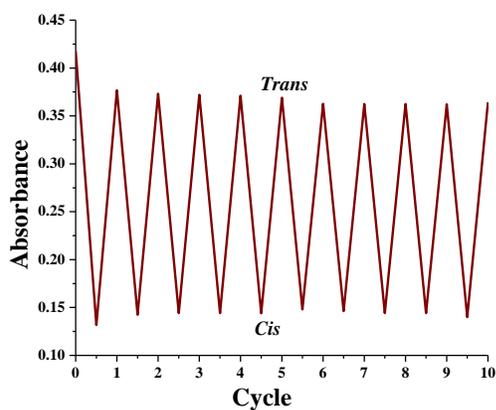


Fig. S11. The changes in the absorbance values of *trans/cis* **1** (3×10^{-5} M) at 309 nm after alternating irradiations at 366 nm (half cycles) and 466 nm (full cycles) over ten complete cycles.

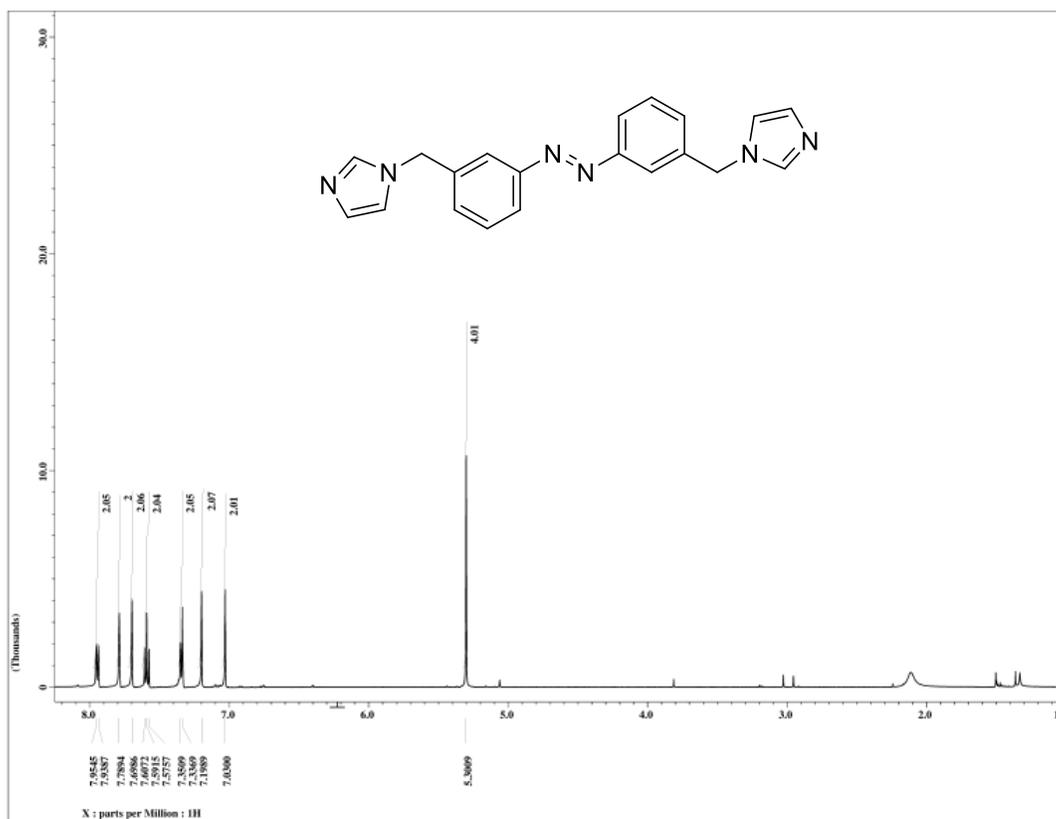


Fig. S12. ¹H NMR spectrum of compound 1

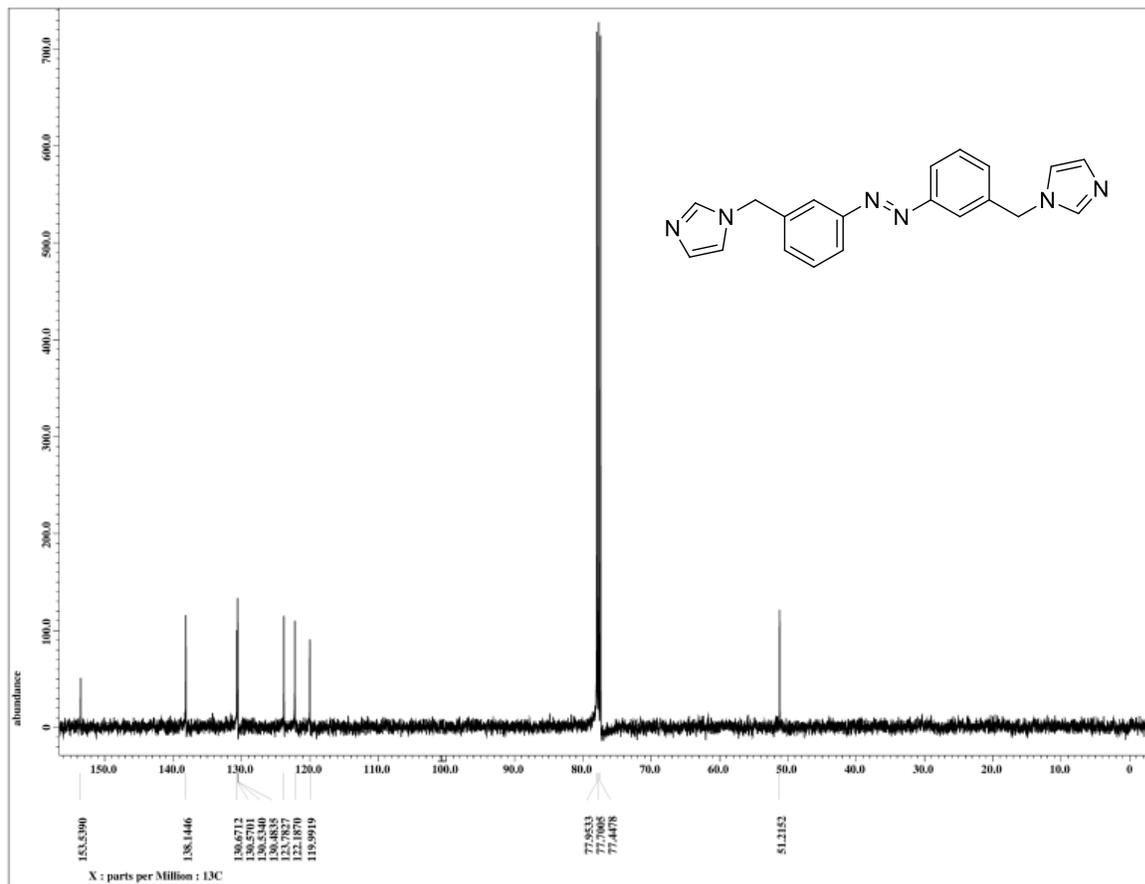


Fig. S13. ^{13}C NMR spectrum of compound 1

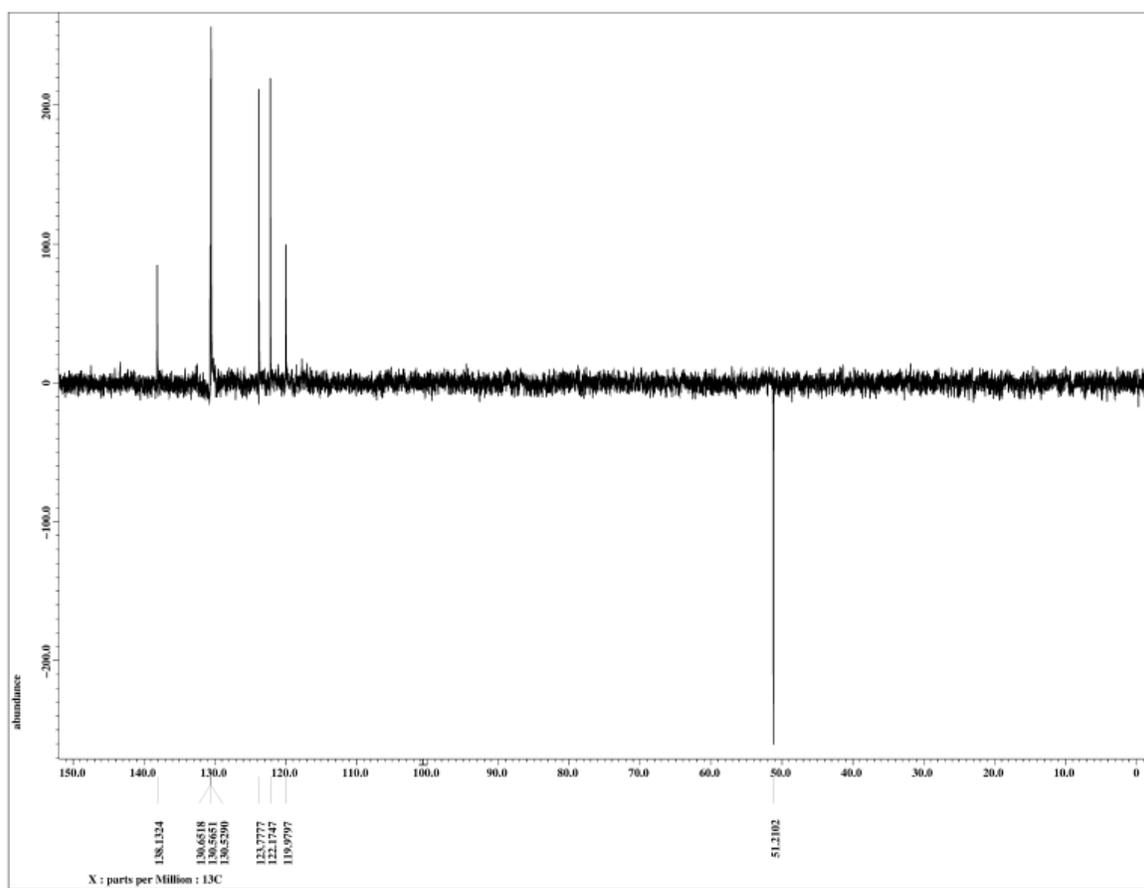


Fig. S14. DEPT spectrum of compound 1

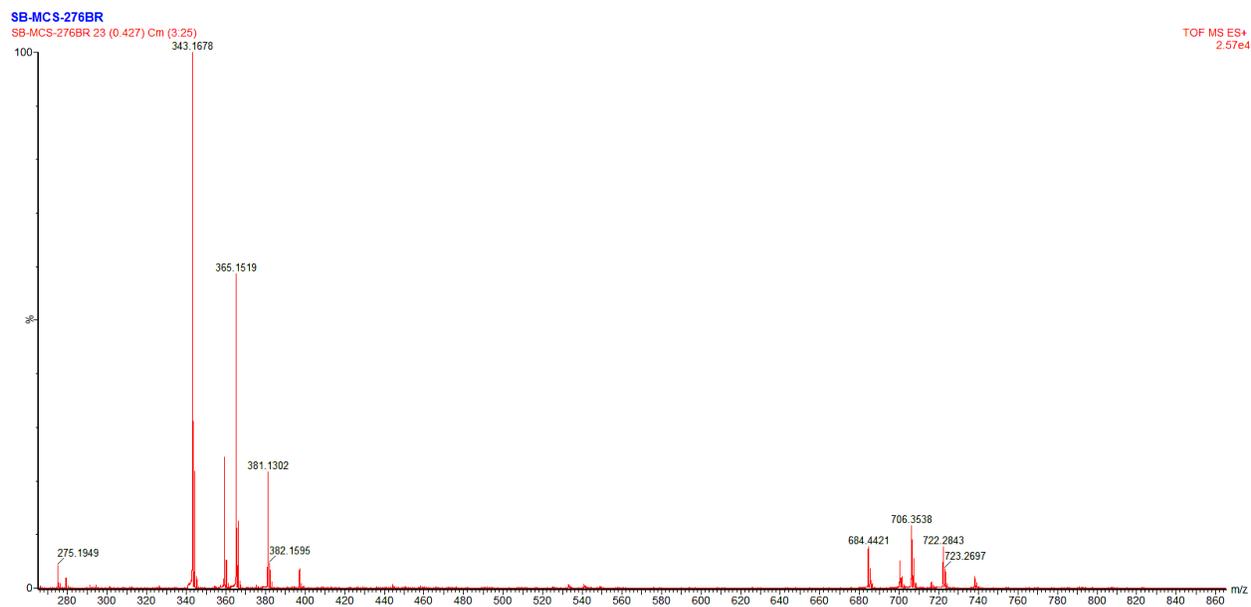


Fig. S15. ESI MS of compound 1, $[M+H]^+ = 343.1678$, $[M+Na] = 365.1519$, $[M+K] = 381.1302$ are obtained from experimentally.

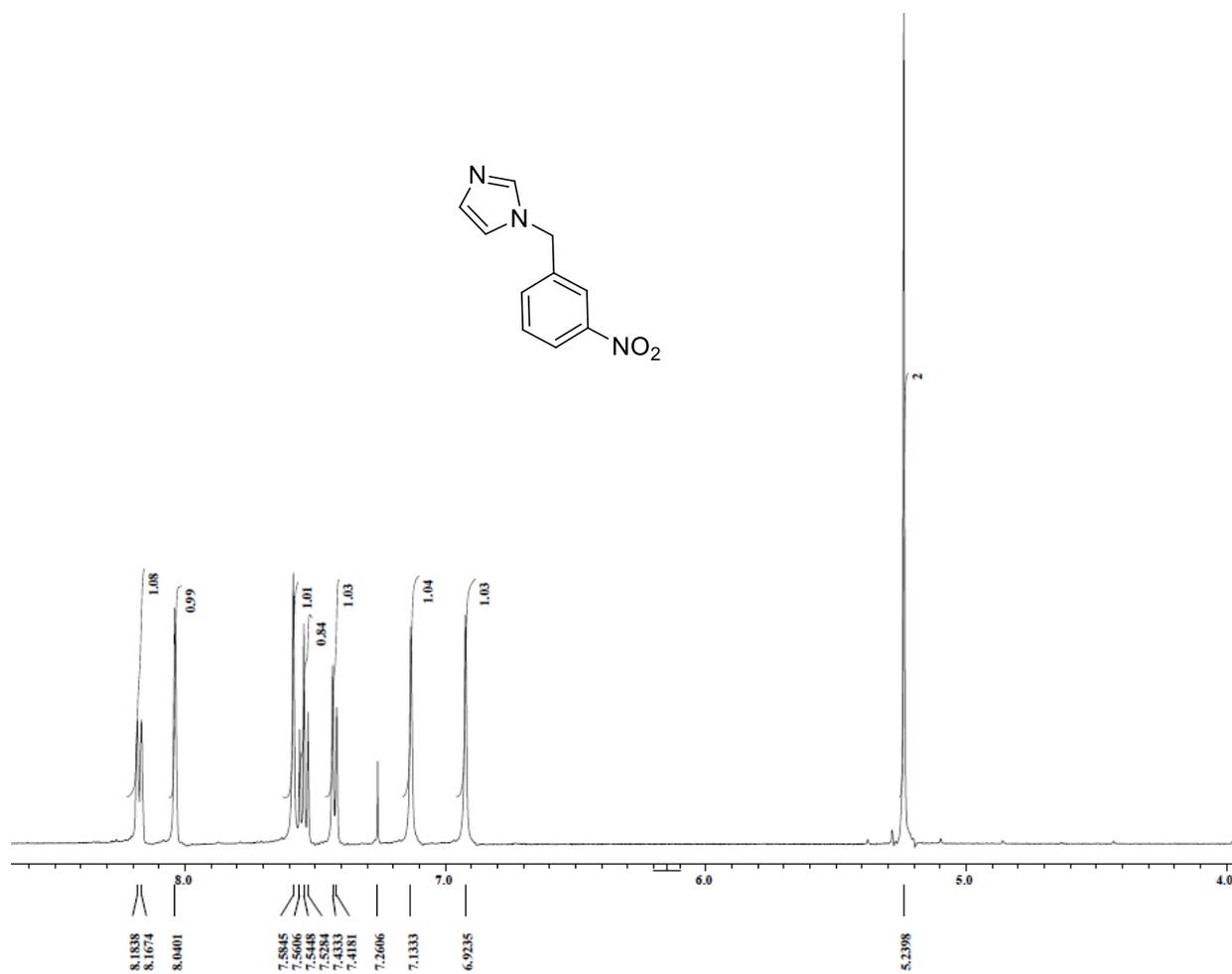


Fig. S16. ¹H NMR spectrum of compound 2

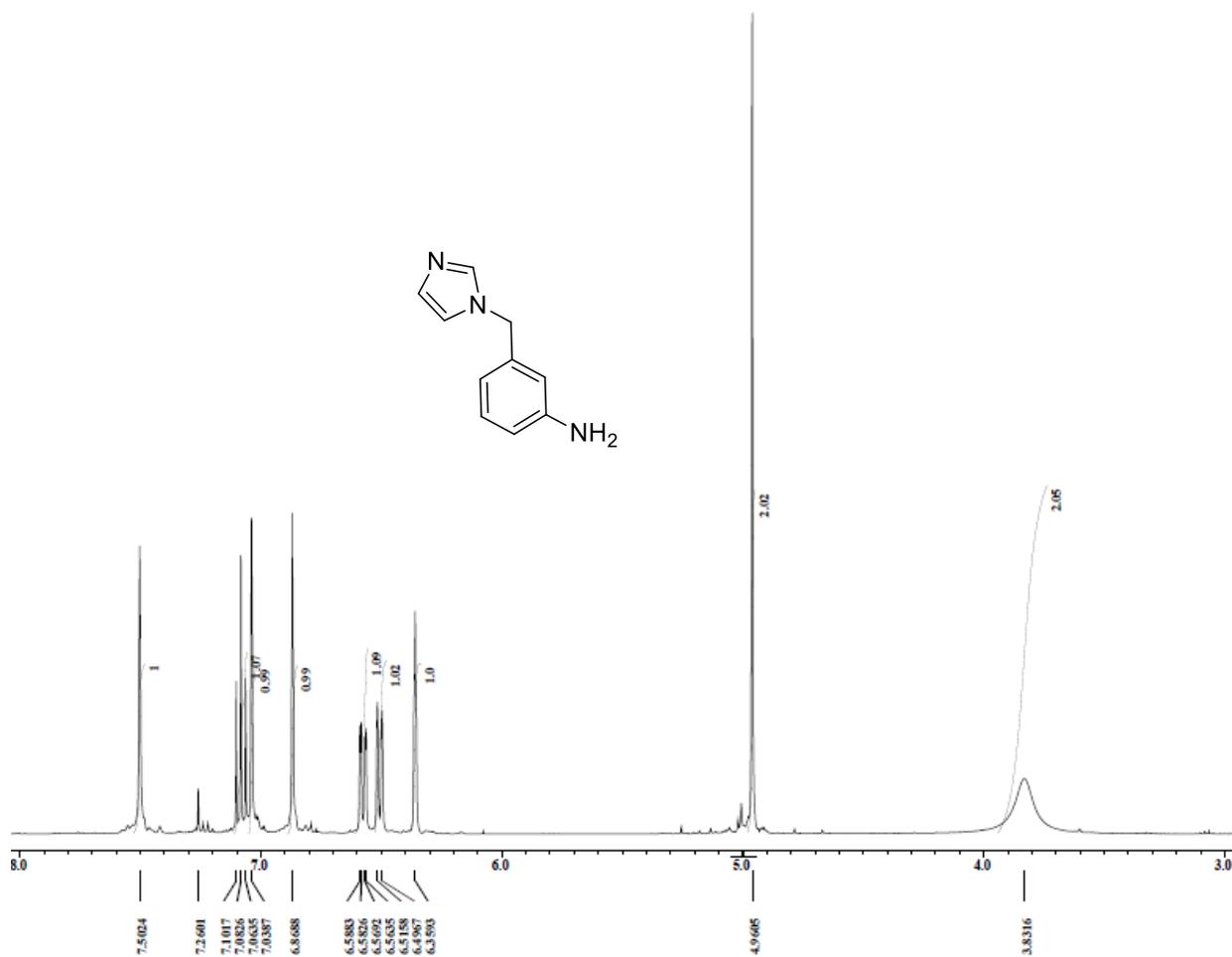


Fig. 17. ¹H NMR spectrum of Compound 4

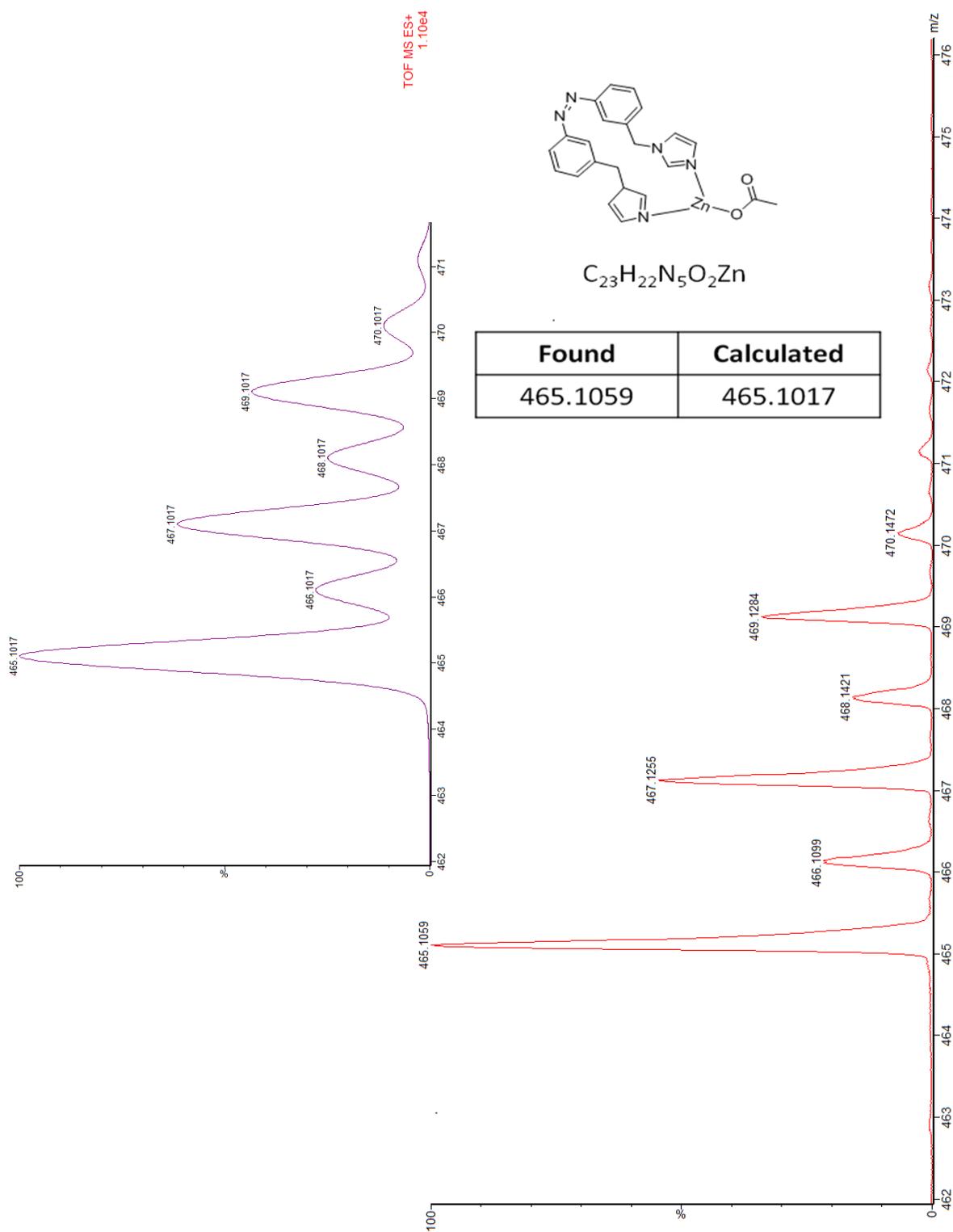


Fig S18. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of [Zn²⁺. cis-1]

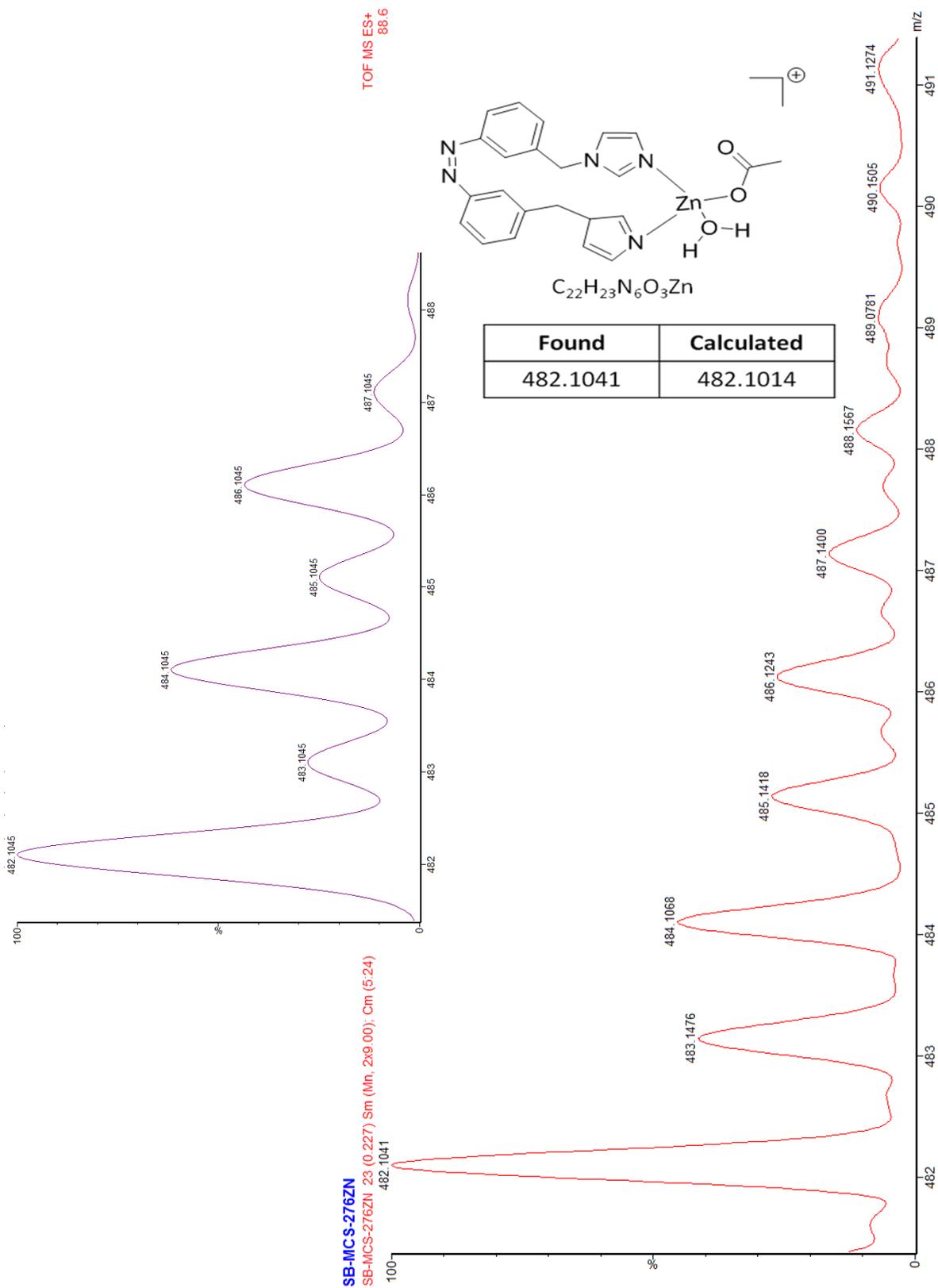
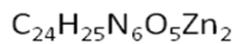
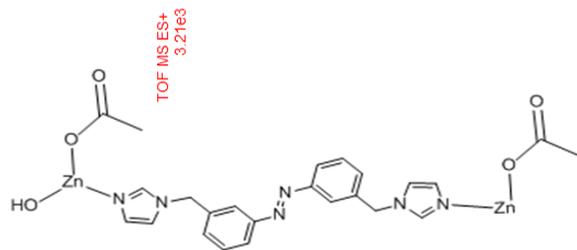


Fig. S19. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of $[Zn^{2+} \cdot cis-1]$



Found	Calculated
605.0487	605.0469

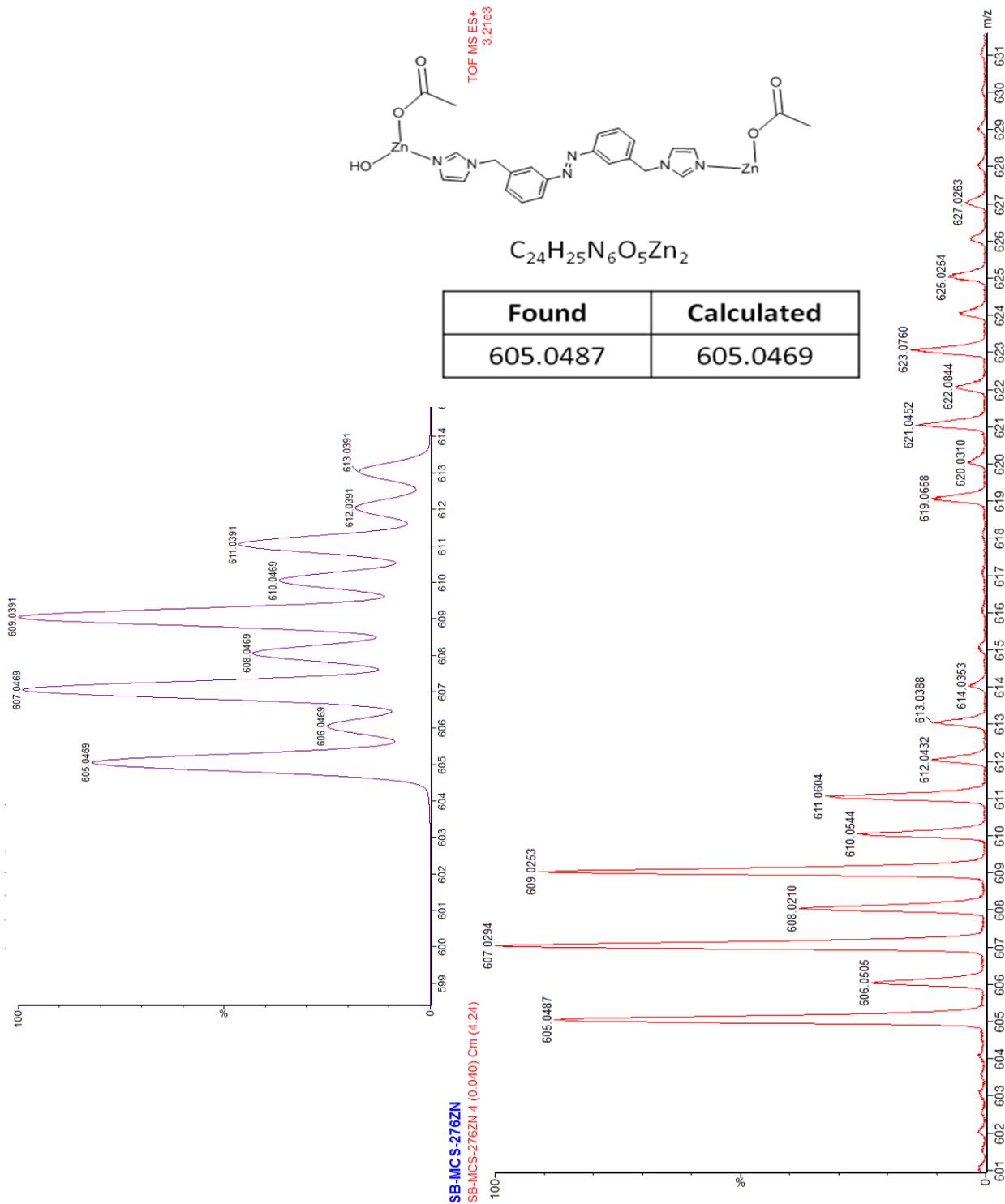


Fig. S20. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of $[\text{Zn}^{2+} \cdot \text{trans-1}]$

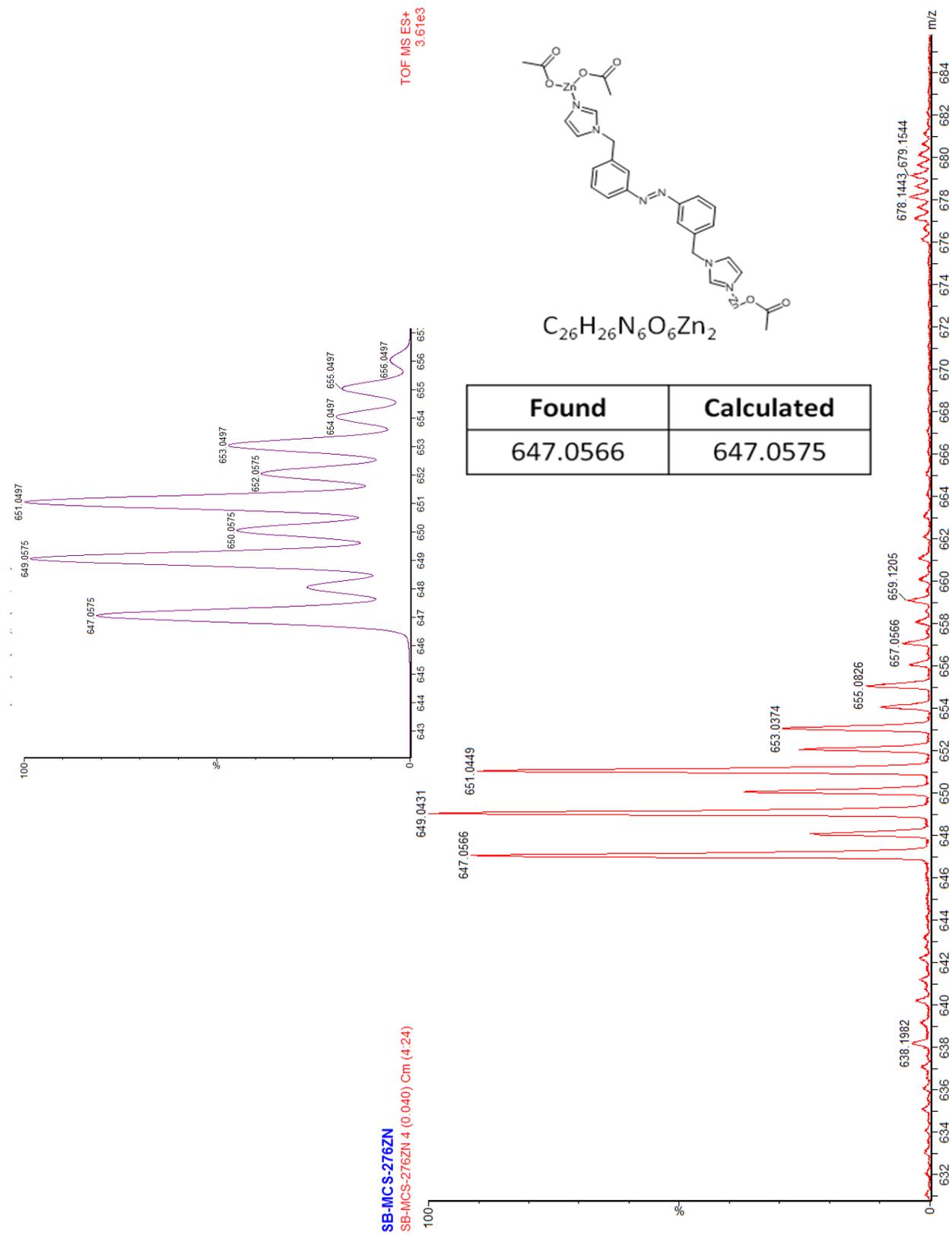


Fig. S21. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of $[Zn^{2+} \cdot trans-1]$

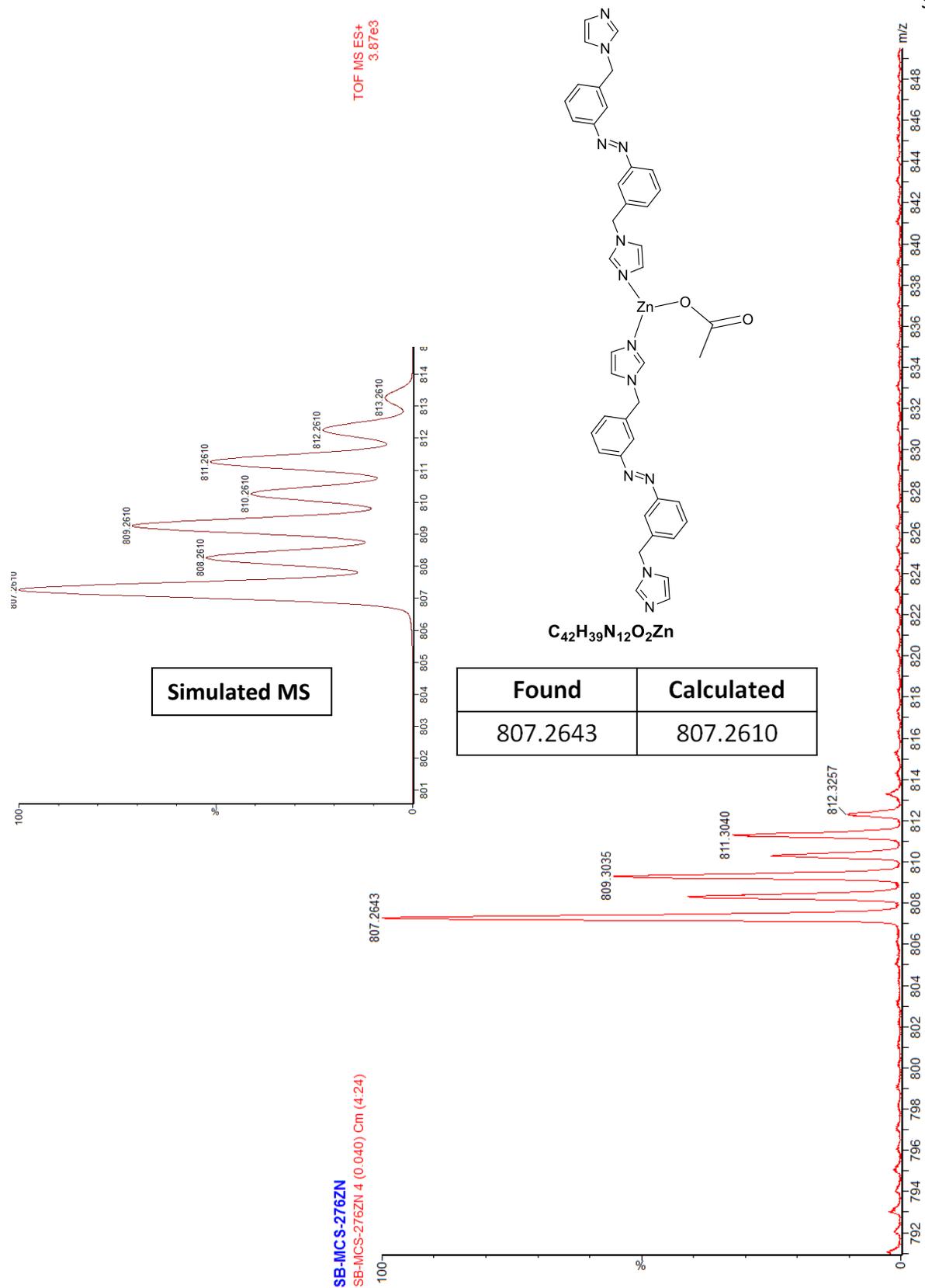


Fig. S22. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of $[Zn^{2+} \cdot trans-1]$

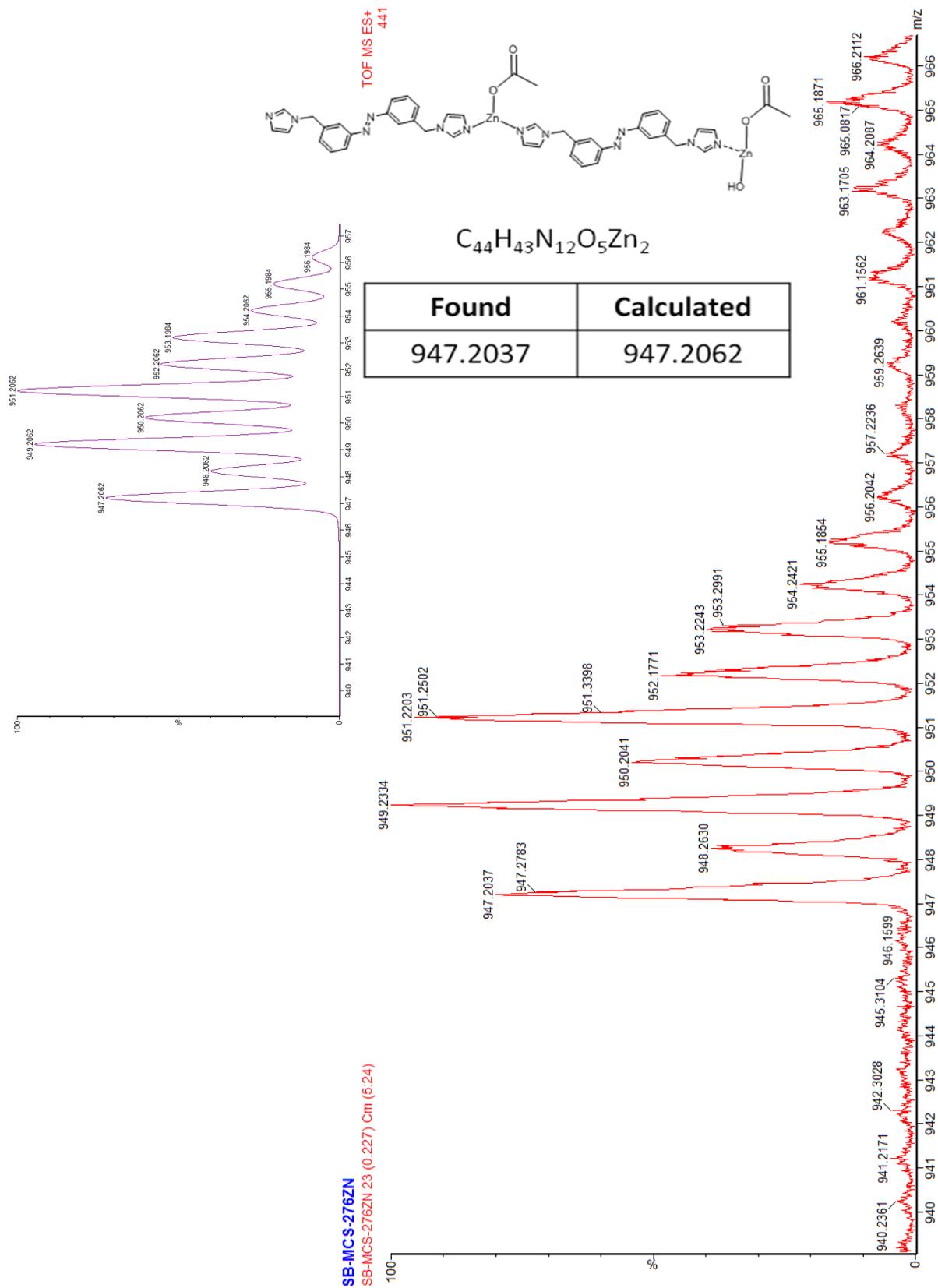


Fig. S23. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of [Zn²⁺ . *trans*-1]

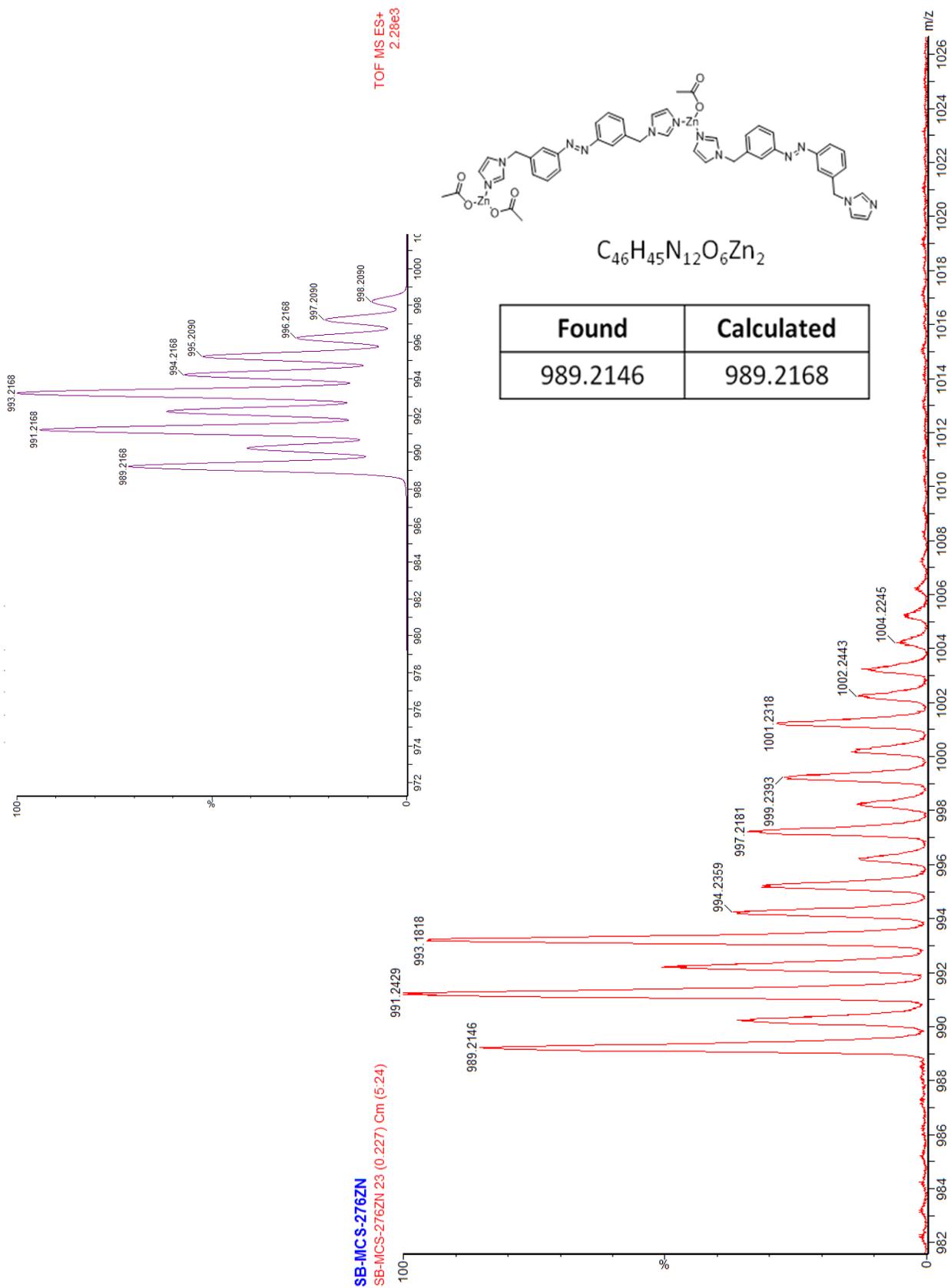


Fig. S24. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of [Zn²⁺ · *trans*-1]

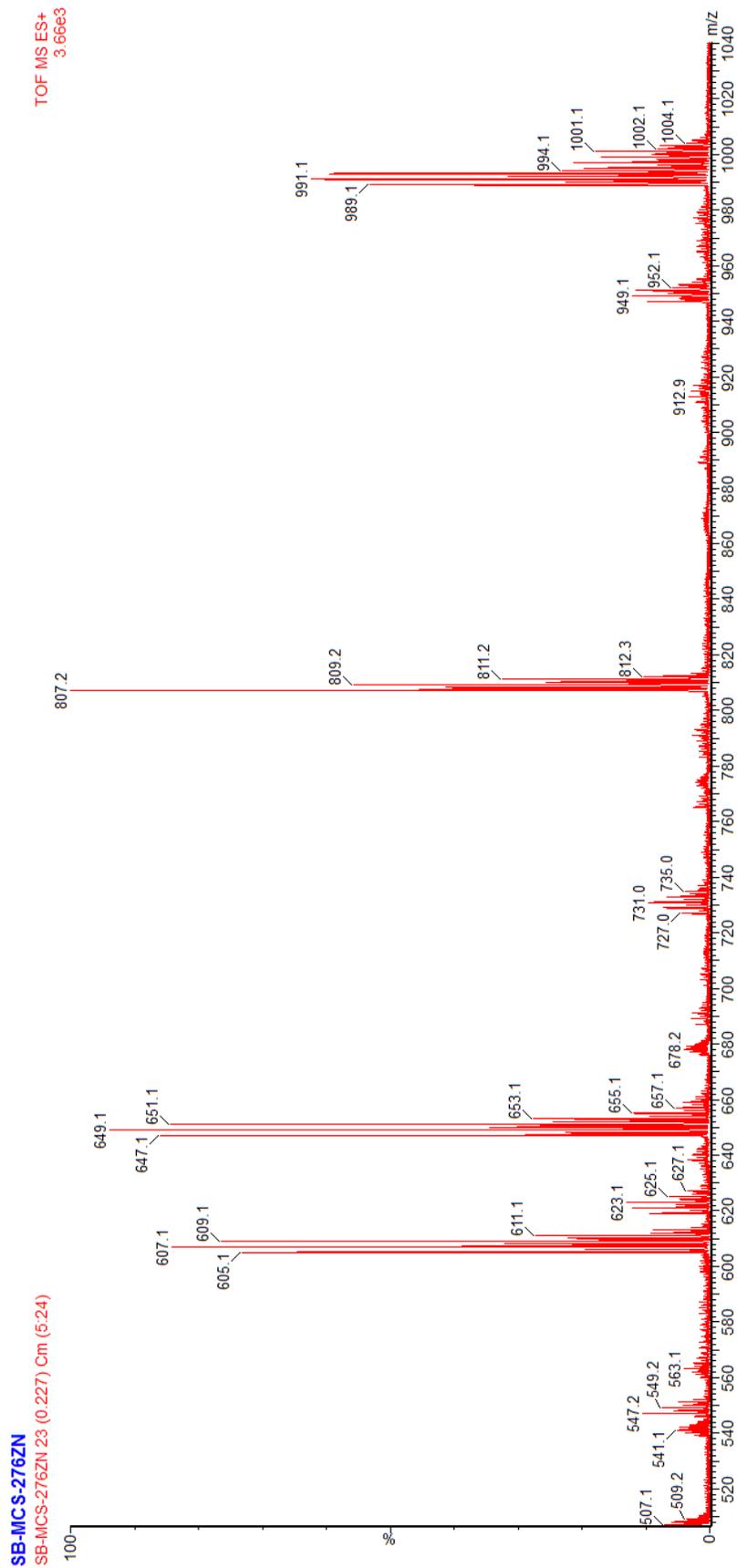


Fig. S25. Calculated (top) and HRMS (ESI/Q-TOF) spectrum of $[Zn^{2+} \cdot trans-1]$

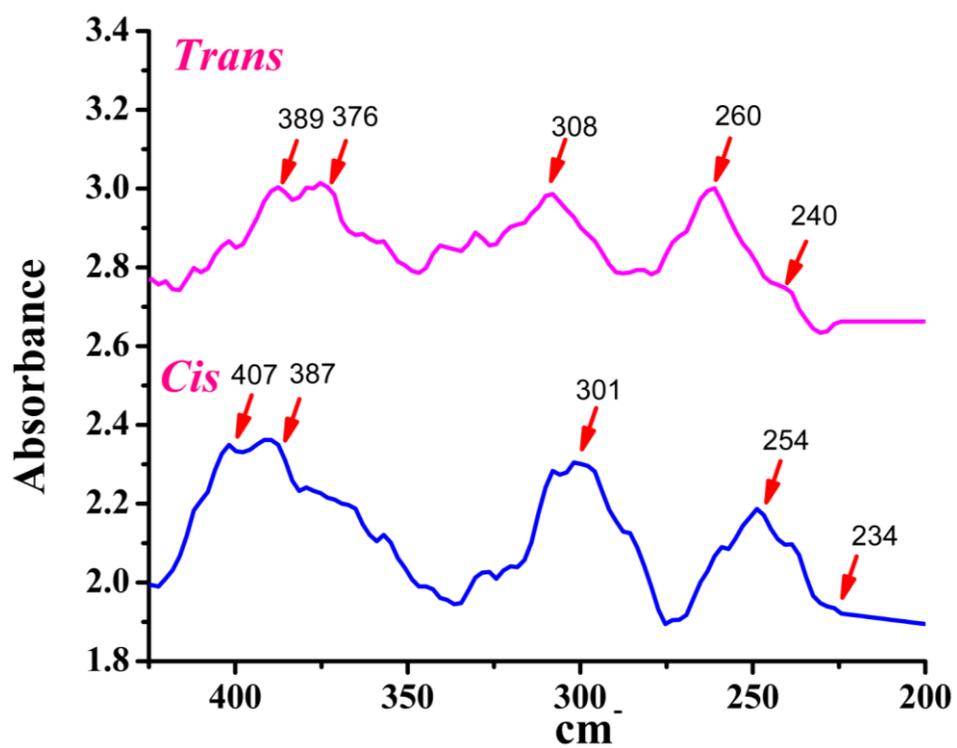


Fig. S26. Far-IR spectra of cis and trans complex of compound 1 with Zn(OAc)₂

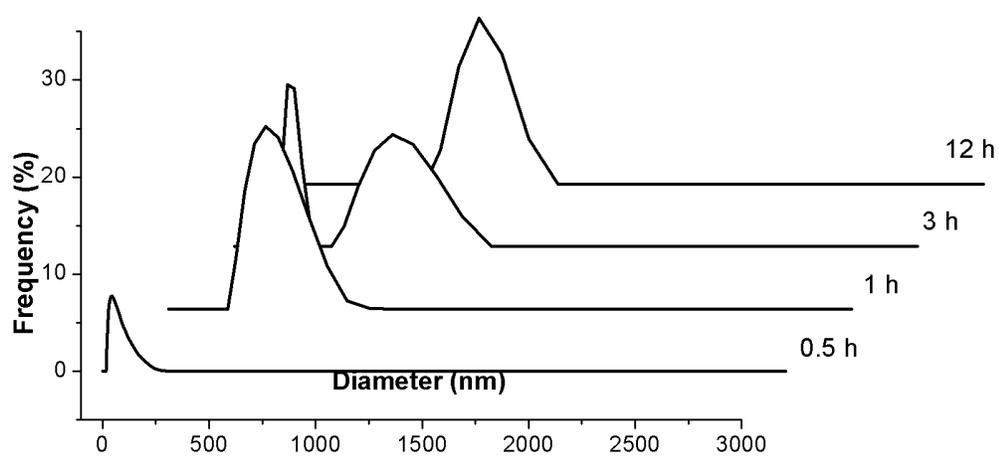


Fig. S27. Time depended DLS Studies of [Zn²⁺ . trans-1].

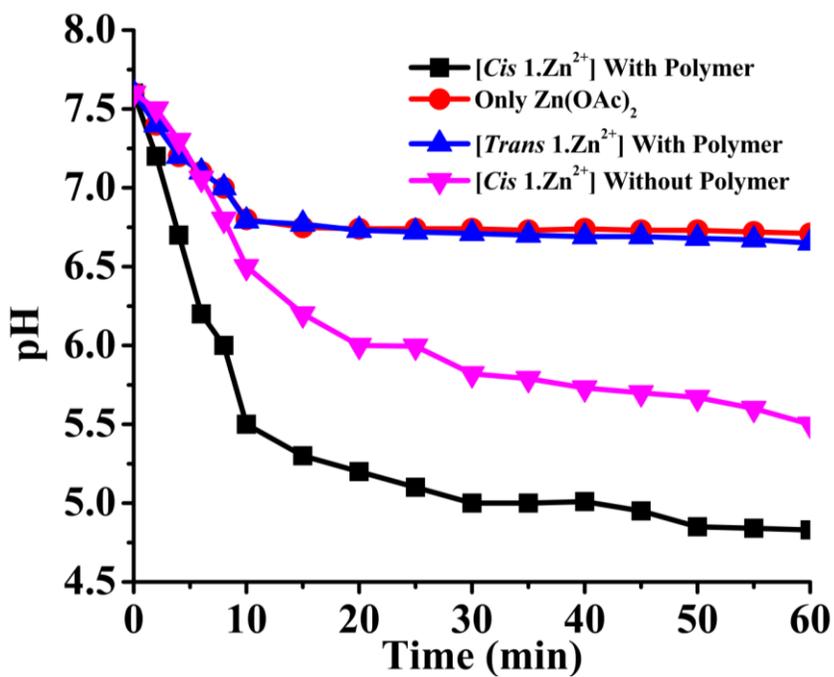


Fig.S28 Change of the pH upon CO₂ hydration

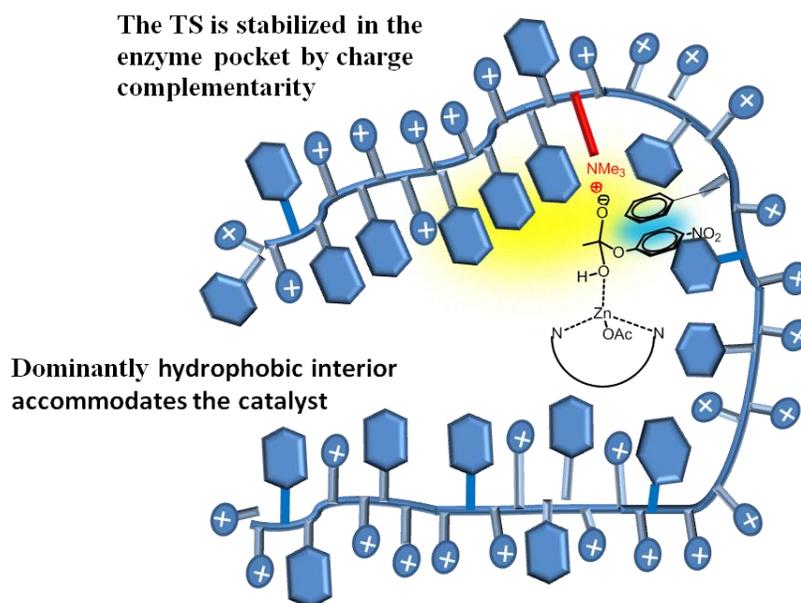


Fig.S29: A schematic cartoon representation of stability of transition state with cationic polymer via various non covalent interactions including Coulombic, π - π , and π -cation interactions.

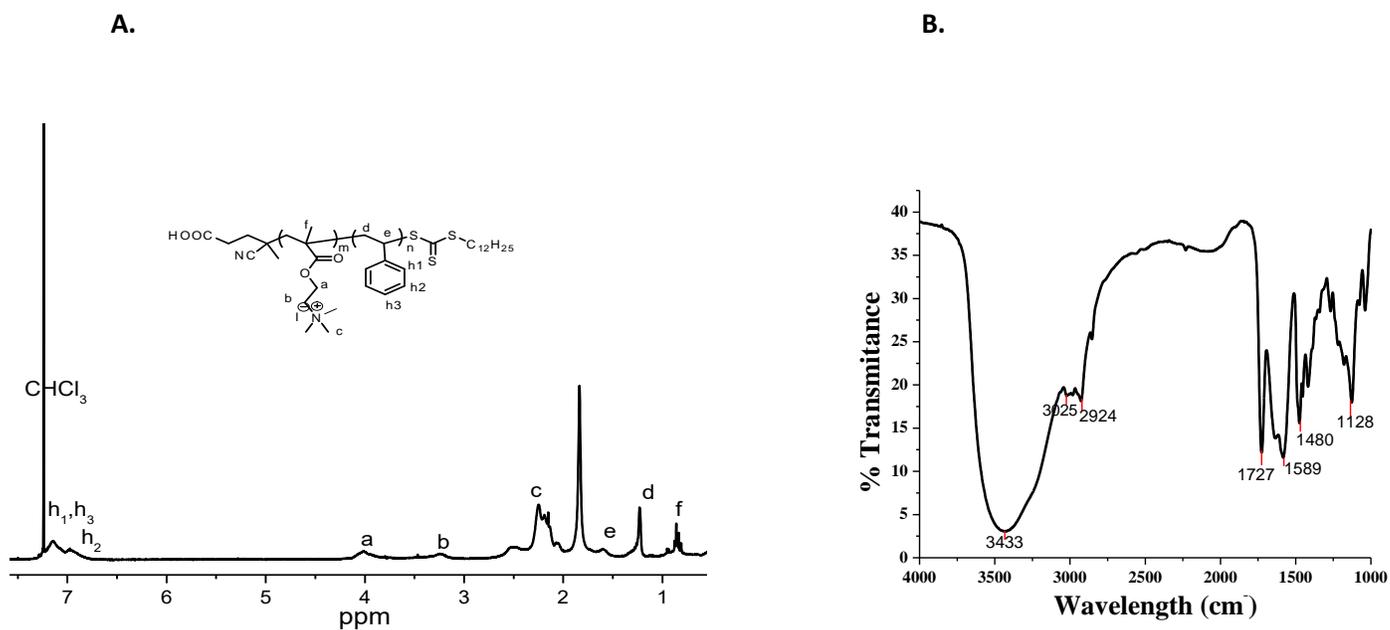


Fig. S30. (A). ^1H NMR spectra of the cationic polymer **A** in CDCl_3 : DMSO-D_6 (4:1, v/v).^[1] **(B).** FTIR of cationic polymer **A**

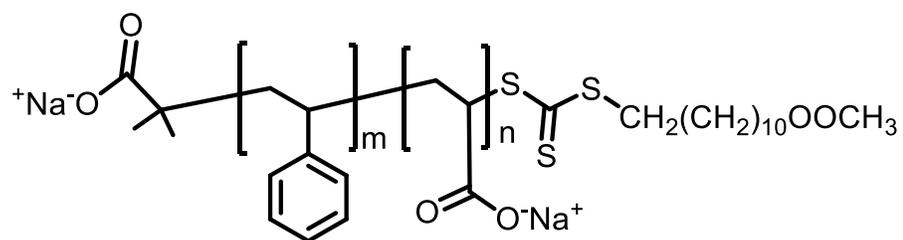


Fig. S31. Structure of the anionic polymer.

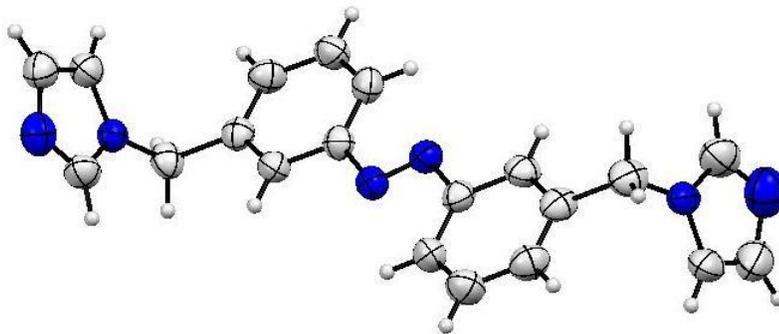


Fig. S32. Single crystal XRD structure of *trans*-1. The ellipsoids are shown at the 50% probability level.

Table S3: Crystallographic Details of Compound 1

Empirical formula	C ₂₀ H ₁₈ N ₆
Formula weight	342.15
Temperature/ <i>K</i>	293(2)
Crystal System	Orthorhombic
Space group	P21cn
<i>a</i> /Å	21.6141(19)
<i>b</i> /Å	5.8209(6)
<i>c</i> /Å	14.1510(13)
α /°	90.00
β /°	90.00
γ /°	90.00
Volume//Å ³	1780.4(3)
<i>Z</i>	4
ρ_{cal} cmg/mm ³	1.299
μ /mm ⁻¹	0.100
F(000)	696
2 θ range for data collection	5.76 to 50.02°
Index ranges	-25 ≤ <i>h</i> ≤ 25, -6 ≤ <i>k</i> ≤ 6, -16 ≤ <i>l</i> ≤ 16
Reflections collected	5860
Independent reflections	2956[R(int) = 0.0758]
Data/restraints/parameters	2956/1/289
Goodness-of-fit on F ²	0.825
Final R indexes[all data]	R1 = 0.0716, wR2 = 0.1495

Largest diff. peak/hole / e Å ⁻³	0.40/-0.15
Flack parameter	-1(3)

Selected Bond length for compound 1:

Atom	Atom	Length/ Å
N1	C14	1.351 (4)
N1	C16	1.466 (4)
N1	C2	1.364 (4)
N2	N4	1.247 (3)
N2	C5	1.434 (4)
N3	C15	1.376 (5)
N3	C3	1.469 (5)
N3	C4	1.345 (5)
N4	C10	1.439 (4)
C5	C8	1.384 (4)
C5	C13	1.387 (5)
C6	C7	1.379 (5)
C6	C12	1.382 (5)
C6	C16	1.514 (5)
C7	C10	1.392 (5)
C8	C9	1.407 (5)
C9	C17	1.385 (5)
C9	C3	1.510 (5)
C10	C19	1.384 (5)
N11	C14	1.313 (5)
N11	C11	1.374 (5)
C12	C18	1.392 (5)
C13	C1	1.389 (5)
C15	C1A	1.333 (6)
C17	C1	1.380 (6)
C18	C19	1.380 (5)
C2	C11	1.340 (5)
C4	N6	1.315 (6)
N6	C1A	1.390 (6)

Selected Bond Angle of Compound 1:

Atom	Atom	Atom	Angle/°
C14	N1	C16	126.4 (3)
C14	N1	C2	106.3 (3)
C2	N1	C16	127.2 (3)
N4	N2	C5	115.1 (2)
C15	N3	C3	125.9 (4)
C4	N3	C15	106.5 (4)
C4	N3	C3	127.5 (3)
N2	N4	C10	115.0 (2)
C8	C5	N2	114.9 (3)
C8	C5	C13	121.2 (3)
C13	C5	N2	123.9 (3)
C7	C6	C12	119.1 (3)
C7	C6	C16	119.8 (3)
C12	C6	C16	121.1 (3)
C6	C7	C10	120.8 (3)
C5	C8	C9	119.5 (3)
C8	C9	C3	120.3 (3)
C17	C9	C8	119.0 (3)
C17	C9	C3	120.8 (3)
C7	C10	N4	115.6 (3)
C19	C10	N4	124.5 (3)
C19	C10	C7	119.9 (3)
C14	N11	C11	103.7 (3)
C6	C12	C18	120.4 (3)
C5	C13	C1	118.9 (3)
N11	C14	N1	112.6 (3)
C1A	C15	N3	106.4 (4)
N1	C16	C6	111.1 (3)
C1	C17	C9	111.1 (3)
C19	C18	C12	120.4 (3)
C17	C1	C13	120.6 (3)
C11	C2	N1	106.0 (3)
N3	C3	C9	111.3 (3)
N6	C4	N3	112.5 (4)
C2	C11	N11	111.4 (3)
C4	N6	C1A	104.0 (4)
C18	C19	C10	119.4 (3)
C15	C1A	N6	110.6 (4)

References:

1. B. Saha, U. Haldar, and P. De, *Rapid Commun.* 2016, **37**, 1015–1020.