Supporting Information

Bergman Cyclization of Main-Chain Enediyne Polymers for Enhanced DNA Cleavage

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1. Abbreviations

Bergman Cyclization (BC), Thin-layer Chromatography (TLC), Electrospray Ionization Time-of-Flight Mass Spectroscopy (ESI-ToF MS), Differential scanning calorimetry (DSC), Matrix-assisted Laser Desorption / Ionization Time-of-Flight Mass Spectrometry (MALDI-ToF MS), Nuclear magnetic resonance (NMR), Attenuated total reflection (ATR), Infrared (IR), Electrospray Ionization Time-of-Flight Mass Spectrometry (ESI-TOF MS), Fourier transformation (FT), Degree of Polymerization (DP), Tetrahydrofuran (THF), Dichloromethane (DCM), Dimethyl sulfoxide (DMSO), *tert*-Butyloxycarbonyl (Boc), Deoxyribonucleic acid (DNA), Electron paramagnetic resonance (EPR), Ethylenediaminetetraacetic acid (EDTA), Tris and EDTA (TE), 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL)

2. Experimental Part

2.1. Solvents and Materials

Chloroform from VWR, ethyl acetate from Overlack, methanol from Brenntag, and toluene from Roth were purchased in technical grade and distilled at least once prior use. Deuterium chloroform ($CDCl_3$-*d*) was used as NMR deuterium solvents.

Dry solvents were prepared as follows: tetrahydrofuran (THF), from Roth, was predried over potassium hydroxide for several days and refluxed over sodium and benzophenone under inert atmosphere and distilled freshly before use; *n*-hexane, from Roth, was refluxed over concentrated sulfuric acid and oleum to remove olefins and subsequently distilled over sodium and benzophenone under an inert gas atmosphere for several hours; dichloromethane (DCM), from Overlack, was predried over calcium chloride for several days and then refluxed over calcium hydride for several hours. Moisture was removed for the following solvents: diethyl ether, from Overlack, was passed through a column filled with sodium sulfate; dimethyl sulfoxide (DMSO), from Grüssing, was stored over molecular sieves (pore diameters 4Å) for several days; N, N-dimethylformamide (DMF) from Grüssing was stored over calcium hydride for several days before use.

Prop-2-yn-1-amine, Tetrafluoroterephthalic aldehyde and 4-(4-Formylphenoxy)benzaldehyde were purchased from abcr; 4,4'-Biphenyldicarboxaldehyde was purchased from TCI; terephthalaldehyde was purchased from Fluka, *cis*-1,2-dichloroethylene, 4-Hydroxy-TEMPO and Tetrakis(triphenylphosphine)-palladium(0) was purchased from Sigma-Aldrich; All chemicals listed here were used without any purification unless otherwise stated.

2.2. Methods and Instrumentation

TLC was performed on “Merck silica gel 60” plates. Spots on TLC plate were visualized using UV light (254 or 366 nm), oxidizing agent “blue stain”, or potassium permanganate solution. “Blue stain” was prepared as follow: $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (1 g) and $Ce(SO_4)_2\cdot 4H_2O$ (1 g) were dissolved in a mixture of distilled water (90 mL) and concentrated sulfuric acid (6

mL). Potassium permanganate solution was prepared as follow: $KMnO_4$(3 g) and K_2CO_3 (10 g) were dissolved in distilled water (300 ml).

ESI-ToF MS measurements were performed using a Bruker Daltonics microTOF. 0.1 mg of samples were dissolved in HPLC grade methanol. All spectra were obtained by means of direct injection with a flow rate of 180 µL h-1 in the negative mode with an acceleration voltage of 4.5 kV.

DSC measurements were done on a Netzsch DSC 204 F1. Samples pieces with a mass of 5 – 10 mg were placed into aluminum crucibles and were heated under nitrogen atmosphere with a heating rate of 5 $K \cdot min^{-1}$ unless otherwise stated. Evaluation of the measured data was done with Netzsch Proteus Analytic software.

MALDI-ToF MS was done on a Bruker Autoflex III system in the reflection mode. Formation of ions was obtained by laser desorption (smart beam laser at 355, 532, 808, and 1064 ± 6.5 nm; 3 ns pulse width; up to 2500 Hz repetition rate). Ions were accelerated by a voltage of 20 kV, and detected as positive ions. 1,8-dihydroxy-9,10-dihydroanthracen-9-one (Dithranol, 20 $mg \cdot mL^{-1}$ in THF) was used as matrix and sodium iodide (NaI, 20 $mg \cdot mL^{-1}$ in THF) was used as salts for ionizing polymers functionalized with Poly EDY-A (20 $mg \cdot mL^{-1}$ in THF) while applying a volume ratio of 100:20:1.

NMR-spectra were measured on a Varian Gemini 400 spectrometer at 27°C. The ^{1}H-NMR spectra were recorded at 400 MHz or 500 MHz and for ^{13}C-NMR spectroscopy 100 MHz were used. The samples were dissolved in either $CDCl_3$ or DMSO. The NMR spectra were interpreted using MestReNova software (version 9.0.1-13254). Chemical shifts were given in ppm and coupling constants were given in Hz.

ATR-FTIR-spectra were measured on a Bruker Tensor VERTEX 70 spectrometer equipped with a Golden Gate Diamond ATR top-plate. For each measurement 32 background scans and 32 sample scans were averaged. The data were analyzed using Opus 6.5 software.

ESI-TOF-MS measurements were performed on a Bruker Daltonics microTOF via direct injection at a flow rate of 180 µL h^{-1} in positive mode with an acceleration voltage of 4.5 kV. Samples were prepared by dissolving in LC-MS grade methanol with additional sodium iodide salt in acetone. The software Data Analysis (version 4.0) was used for data evaluation

Gel electrophoresis were performed as below: after incubation for specific time, each mixture (10 µL) of polymer containing enediyne and pART7 plasmid in TE buffer (pH = 7.6) was mixed with a 6*loading buffer (2 µL) and subjected to 1% agarose gel electrophoresis at 90 V for 45 min, stained by SYBR® Safe DNA Gel Stain and then the gel was photographed on Intas Science Imaging instrument and analyzed by scanning densitometry.

CW EPR spectra were measured using the Miniscope MS 5000 (Magnettech GmbH, Berlin, and Freiberg Instruments, Freiberg, Germany), the MS 5000 temperature controller (Magnettech GmbH, Berlin, Germany) and Freiberg Instruments software.

Micropipettes (BLAUBRAND® intraMARK, Wertheim, Germany) were filled with about 10–15 µL of sample solution containing 50 mM TEMPOL and capped with capillary tube sealant (CRITOSEAL® Leica) and placed into the spectrometer. The temperature was fixed at 25 °C or 37 °C with an accuracy of 0.2 °C. For all X-band measurements a magnetic field sweep of 8 mT centered around 338 mT with a scan time of 60 s, modulation amplitude of 0.02 mT, modulation frequency of 100 kHz and a microwave power of 10 mW. Each spectrum is an accumulation of 10 scans.

The samples were directly exposed to light inside the EPR spectrometer using a fiber coupled multi wavelength LED light source (Prizmatix Ltd., Cholon, Israel). The LED 420Z emits light with a peak wavelength of 419.8 nm and a FWHM of 14.74 nm. A 1 m polymer optical fiber with a diameter of 1.5 mm and a NA of 0.5 was used to send the light with a maximum power of 215 mW through the hole for the Mn-standard into the resonator.

2.3. Synthesis

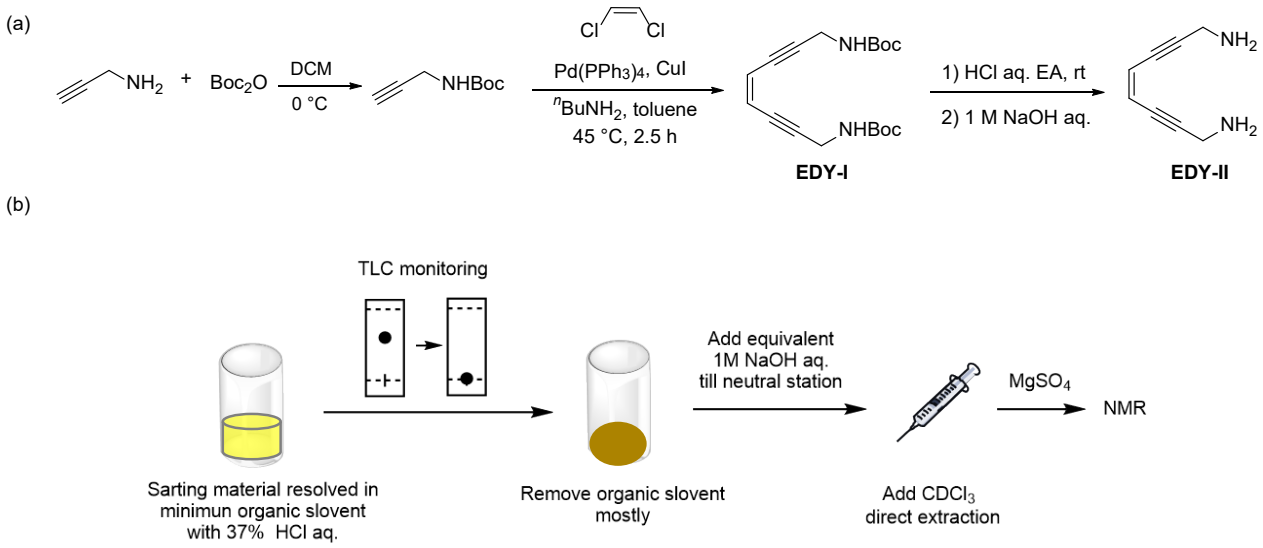
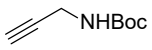


Figure S1 (a) Synthetic route of di-*tert*-butyl octa-4-en-2,6-diyne-1,8-diyl(*Z*)-dicarbamate **EDY-I** and (*Z*)-octa-4-en-2,6-diyne-1,8-diamine **EDY-II**. (b) Designed procedure of handle with **EDY-II** solution

1) Synthesis of di-tert-butyl octa-4-en-2,6-diyne-1,8-diyl(Z)-dicarbamate (EDY-I) and (Z)-octa-4-en-2,6-diyne-1,8-diamine EDY-II.

Di-*tert*-butyl dicarbonate (17.5 g, 80.0 mmol) was added dropwise at 0 °C to a solution of prop-2-yn-1-amine (5.49 mL, 80.0 mmol) in DCM (150 mL). After 1 h of stirring, the solvent was removed in vacuo and the resulting colorless oil was dried under high vacuum overnight to yield a slightly yellow solid (12.4 g, quantitative yield), which was used as such without further purification. The spectral data corresponds to that reported in the literature.[1]

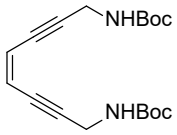


tert-Butyl prop-2-ynylcarbamate

^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm) δ 4.79 (brs, 1H), 3.90 (s, 2H), 2.20 (t, J = 2.5 Hz, 1H),

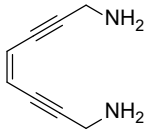
1.43 (s, 9H).

The synthesis of **EDY-I** was realized according to reference[2]. Alkyne (2.2 mol) was added to a mixture of *cis*-1,2-dichloroethylene (1 mol), $Pd(PPh_3)_4$ (0.06 mol), CuI (0.2 mol), *n*-butylamine (5 mol) in toluene at 45 °C and stirring the mixture for 4 h at that temperature. The crude product was purified by flash column chromatography (5% ethyl acetate-dichloromethane). Yield: 60%.



di-*tert*-butyl octa-4-en-2,6-diyne-1,8-diyl(*Z*)-dicarbamate
^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm) δ 5.80 (s, 1H), 4.88 (br, 1H), 4.23-3.94 (m, 2H), 1.46 (s, 9H). ESI-ToF MS: $[M+Na]^+$ calculated for $C_{18}H_{26}N_2O_4$, 357.18; found, 357.20.

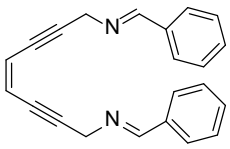
For **EDY-II,** 37% hydrochloric acid solution (99.7 μL, 1.20 mmol) was added to a stirring solution of **EDY-I** (20 mg, 0.06 mmol) in 0.5 mL ethyl acetate. The reaction mixture was stirred for about 30 min at room temperature till complete (monitored by TLC, PE/EA=4/1, the TLC result looks like the above graph). Following by removing the solvent via vacuum directly, equivalent 1 mol/L NaOH aqueous solution (1.2 mL) was added to the "dry" reaction mixture. The mixture was extracted with $CHCl_3$ (5 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO4, filtrated and concentrated via vacuo. The concentration must not be over than 0.25 mol/L. **EDY-II** would stay safe and pure in this solution. Add hexamethylbenzene as reference label to obtain the concentration of enediyne for further reaction and DNA-cleavage test. Yield: 45%.



(*Z*)-octa-4-en-2,6-diyne-1,8-diamine
^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm): ^{1}H NMR (500 MHz, cdcl$_3$) δ 5.79 (s, 1H, CH), 3.62 (s, 2H, NCH_2), 1.50 (s, 2H, NH_2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 118.98 (CH), 97.80 (Cquart), 79.83 (Cquart), 31.14 (NCH_2). ESI-ToF MS: $[M+H]^+$ calculated for $C_8H_{10}N_2$, 135.09; found, 135.12.

2) Synthesis of (1E,1'E)-N,N'-((Z)-octa-4-en-2,6-diyne-1,8-diyl)bis(1-phenylmethanimine) (EDY-III)

The **EDY-III** was synthesized according to reference[3]. (*Z*)-octa-4-en-2,6-diyne-1,8-diamine **EDY-II** (0.2 mmol) in 2 ml dichloromethane was added to a stirring solution of benzaldehyde (0.4 mmol) in 4 ml dichloromethane containing molecular sieves. After stirring under room temperature for 3 hours and monitoring the total consumption of benzaldehyde, the mixture was filtered through a celite bed and the solvent was removed under vacuum to obtain crude product **EDY-III**. Further purification was performed by recrystallization in mixture of hexane and ethyl acetate. Yield: 45%.



(1*E*,1'*E*)-*N*,*N*'-((*Z*)-octa-4-en-2,6-diyne-1,8-diyl)bis(1-phenylmethanimine)

^{1}H NMR (400 MHz, $CDCl_3$, δ in ppm) δ 8.63 (s, 1H, NCH), 7.81-7.63 (m, 2H), 7.46-7.33 (m, 3H), 5.97 (s, 1H, CH), 4.71 (s, 2H, NCH_2). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.33 (NCH), 135.88 (CH), 130.88 (CH), 129.71 (CH), 128.97 (CH), 128.58 (CH), 128.23 (CH), 119.44 (CH), 92.33 (Cquart), 85.07 (Cquart), 48.01 (NCH_2). ESI-ToF MS: $[M+H]^+$ calculated for $C_{22}H_{18}N_2$, 311.15; found, 311.16. T_{peak} for BC From DSC (heating rate: 5 K/min): 145.9 °C.

3) General Synthesis of main-chain enediyne polymers

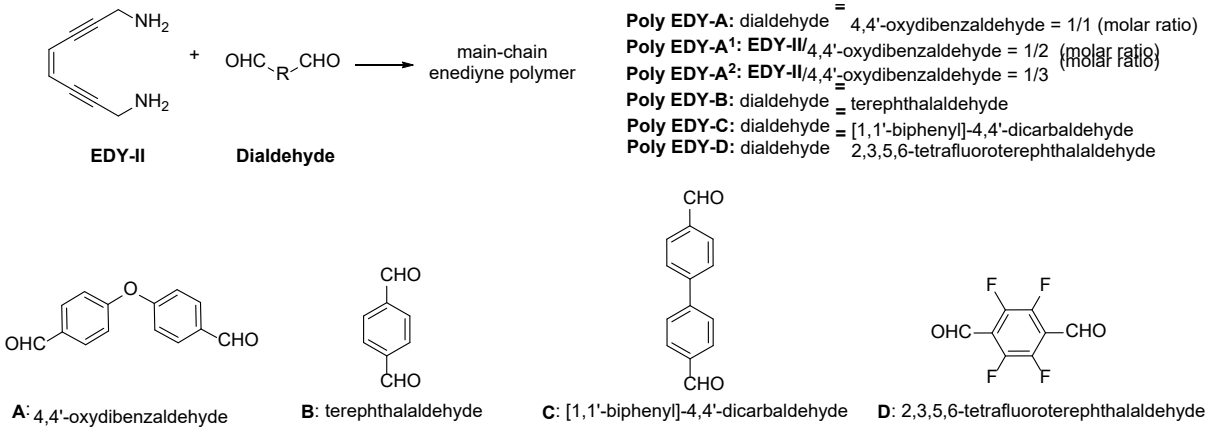
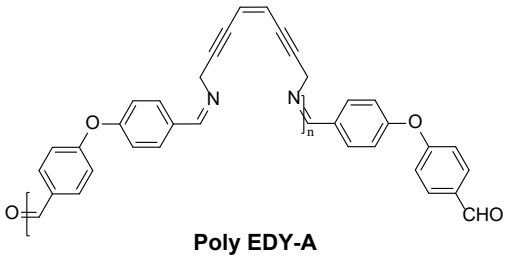


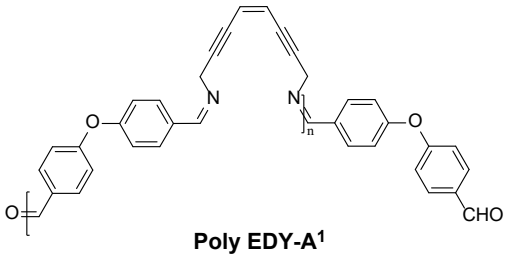
Figure S2 Synthetic route of main-chain enediyne polymers derived from different dialdehydes

To synthesize main-chain enediyne polymer, molar concentration of **EDY-II** in solution needs to be obtained based on the previous description. In a closed system, a solution of **EDY-II** (0.16 mmol) in 1.6 mL $CHCl_3$ was added to a stirring solution of dialdehyde (0.16 mmol) in 3.2 mL isopropanol. The reaction mixture was stirred at room temperature for 16 h (only for **Poly EDY-D**: 4 h) in dark. Following by taking 0.5 mL of reaction mixture, filtration of precipitated particles and removing the solvent via vacuum, NMR was carried out to check the reaction station, together with adding hexamethylbenzene to obtain the concentration of enediyne segment in the solution polymer for further DNA-cleavage tests and EPR measurements.



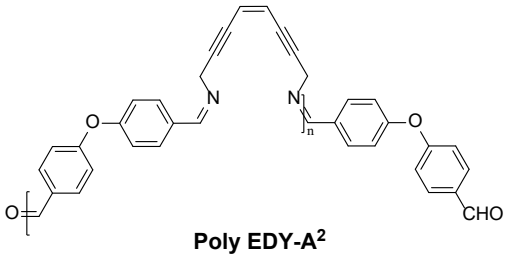
Poly EDY-A

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96-9.94 (m, 0.03H, CHO), 8.62-8.60 (m, 1H, NCH), 7.73-7.72 (m, 1H, CH), 7.63-7.61 (m, 1H, CH), 7.03-7.01 (m, 1H, CH), 6.89-6.88 (m, 1H, CH), 6.00-5.98 (m, 1H, CH), 4.72-4.71 (m, 2H, NCH_2). T_{peak} for BC From DSC (heating rate: 5 K/min): 199.5 °C.



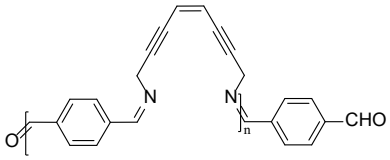
Poly EDY-A[1]

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.91-9.90 (m, 0.24H, CHO), 8.61-8.56 (m, 1H, NCH), 7.75-7.73 (m, 1H, CH), 7.70-7.68 (m, 1H, CH), 7.08-7.03 (m, 1H, CH), 6.99-6.97 (m, 1H, CH), 5.96-5.95 (m, 1H, CH), 4.69-4.67 (m, 2H, NCH_2). T_{peak} for BC From DSC (heating rate: 5 K/min): 197.1 °C.



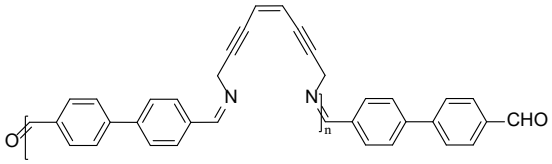
Poly EDY-A[2]

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88-9.87 (m, 0.24H, CHO), 8.61-8.58 (m, 1H, NCH), 7.76-7.73 (m, 1H, CH), 7.71-7.68 (m, 1H, CH), 7.05-7.02 (m, 2H, CH), 5.95-5.94 (m, 1H, CH), 4.70-4.67 (m, 2H, NCH_2). T_{peak} for BC From DSC (heating rate: 5 K/min): 172.8 °C.



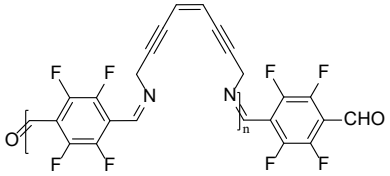
Poly EDY-B

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.06-10.02 (m, 0.02H, CHO), 8.63-8.51 (m, 1H, NCH), 7.87-7.45 (m, 2H, CH), 5.97-96 (m, 1H, CH), 4.74-4.68 (m, 2H, CH). T_{peak} for BC From DSC (heating rate: 5 K/min): 152.3 °C.



Poly EDY-C

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.07-10.04 (m, 0.04H, CHO), 8.72-8.58 (m, 1H, NCH), 7.82-7.30 (m, 4H, CH), 6.02-5.80 (m, 1H, CH), 4.77-4.74 (m, 2H, CH). T_{peak} for BC From DSC (heating rate: 5 K/min): 148.9 °C.



Poly EDY-D

^{1}H NMR (500 MHz, $CDCl_3$) δ 10.10-10.06 (m, 0.02H, CHO), 8.78-8.47 (m, 1H, NCH),

5.90-5.88 (m, 1H, CH), 4.77-4.64 (m, 2H, CH). T_{peak} for BC From DSC (heating rate: 5 K/min): 176.9 °C.

2.4. DNA-cleavage related test

1) DNA-cleavage test

Freshly prepared EDY-containning compounds were dissolved in DMSO (12 μL) at concentrations of 200 mM. The concentration of enediyne segment is calculated by adding hexamethylbenzene as reference label. Each EDY solution was added to a solution of supercoiled plasmid pART7 (1.8 μg μL-1, 8 μL) in TE buffer (pH 7.6, 100 μL). Negative samples consisted of a solution of pART7 (1.8 μg μL-1, 8 μL) in TE buffer (pH 7.6) separately incubated with or without 12 μL DMSO. All the systems maintained in 120 μL in total volume and were incubated at 37 ℃. After incubation for specific time, each mixture (10 μL) was mixed with a 6*loading buffer (2 μL) and subjected to 1% agarose gel electrophoresis at 90 V for 45 min, stained by ethidium bromide and then the gel was photographed on a UV transilluminator (FR-200A) and analyzed by scanning densitometry

2) Hydrolysis experiment to verify the source of cleavage activity

Based on the condition of DNA-cleavage test, an equivalent volume of pure TE buffer except the plasmid Part7 in TE buffer was added to the DMSO solution of EDY-containning compounds. The mixture was incubated at 37 ℃. After 8 h, from NMR, DP slightly decreased but no obvious diamino EDY signals appeared, which gives the conclusion of that EDY in polymer chain is responsible for DNA-cleavage performance, rather than small molecule EDY dissociated after hydrolysis.

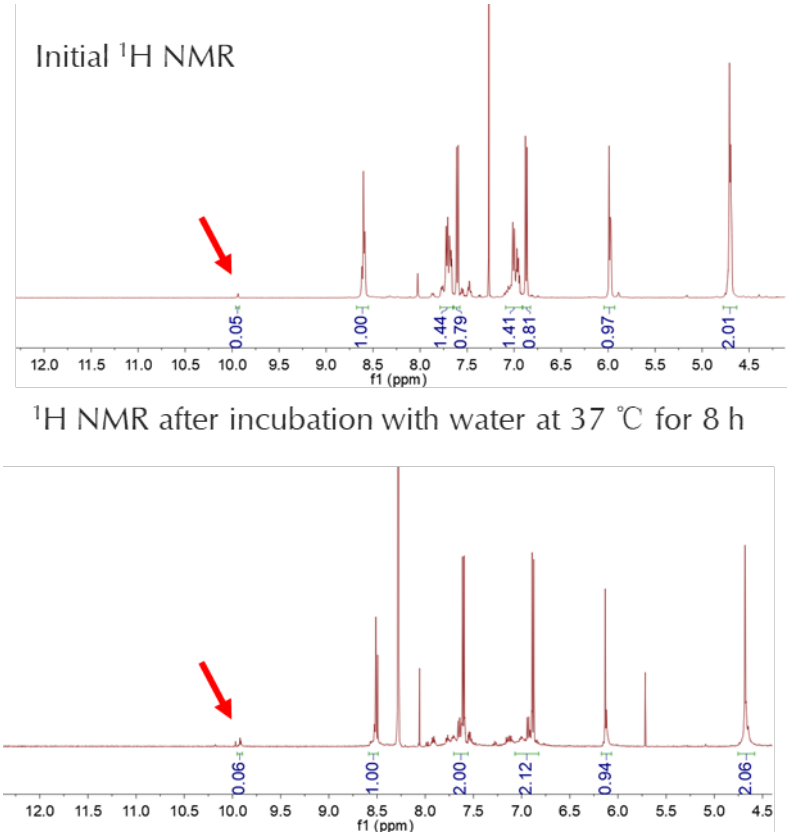


Figure S3 NMR comparison between before and after hydrolysis experiment of main-chain enediyne polymers

2.5. Concentration control of EDY-II and EPR test

1) Concentration control of EDY-II

Under >0.25 mol/L concentration, brown solids spontaneously appear in solution of diamino enediyne **EDY-II** in chloroform.

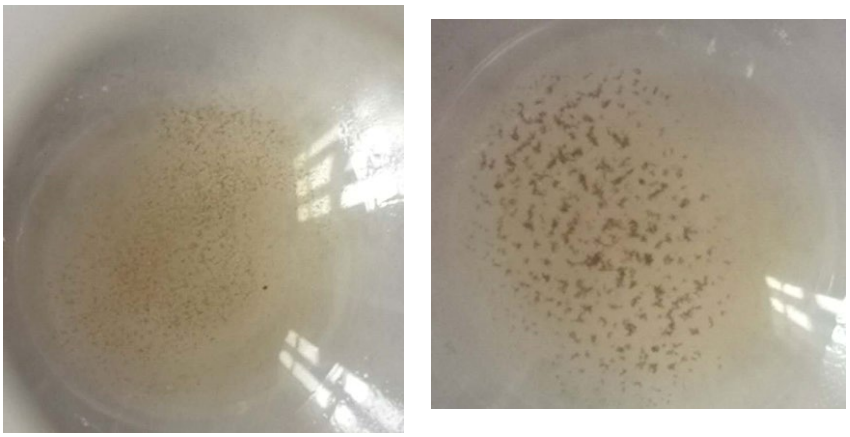


Figure S4 Brown solids spontaneously appear and grow up

2) EPR test of generated particles

Once generated, solid samples were placed in EPR tubes immediately (T=30 °C, air atmosphere). Spinning concentration was recorded every 30 min. Without heating, the radical species grew gradually and kept stable after reaching to peak.

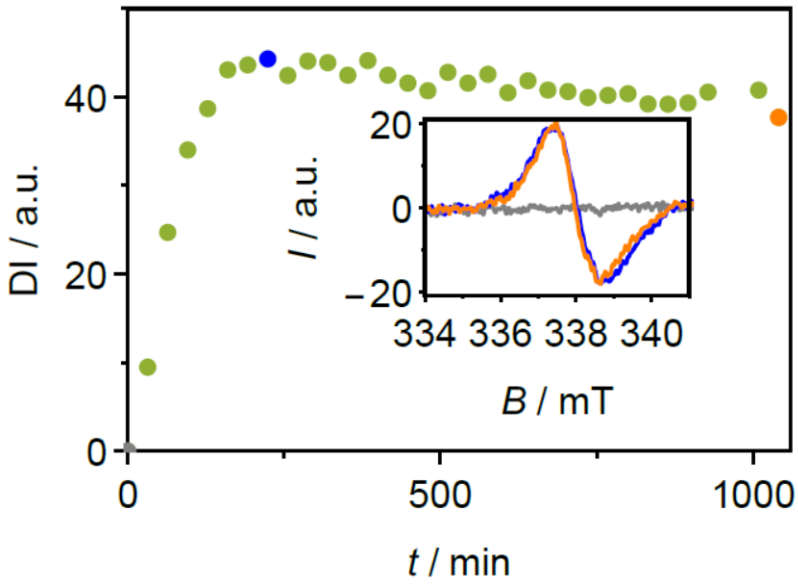
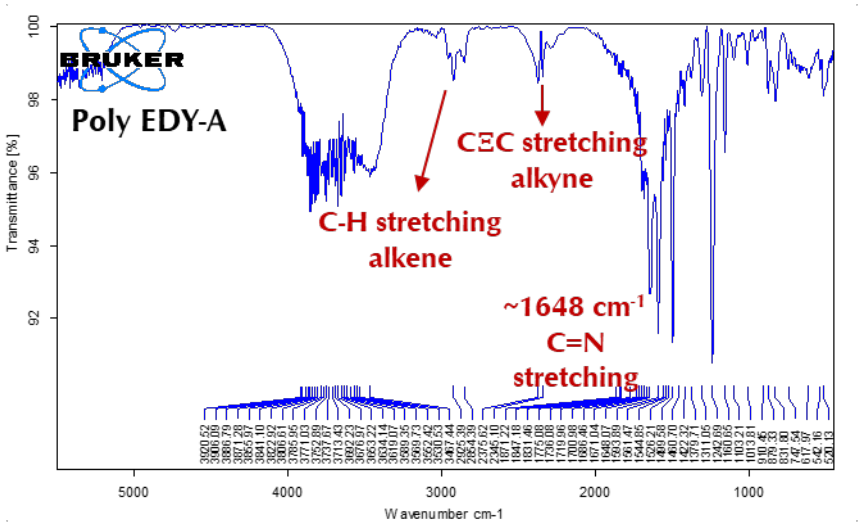
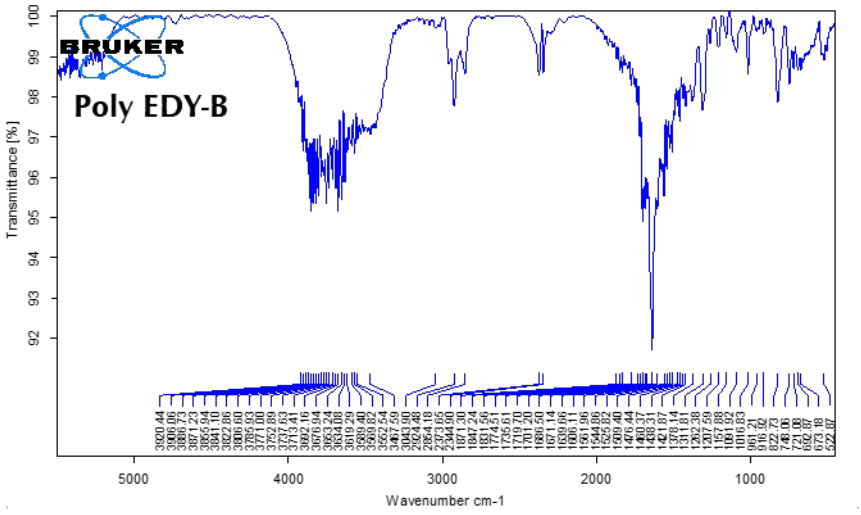
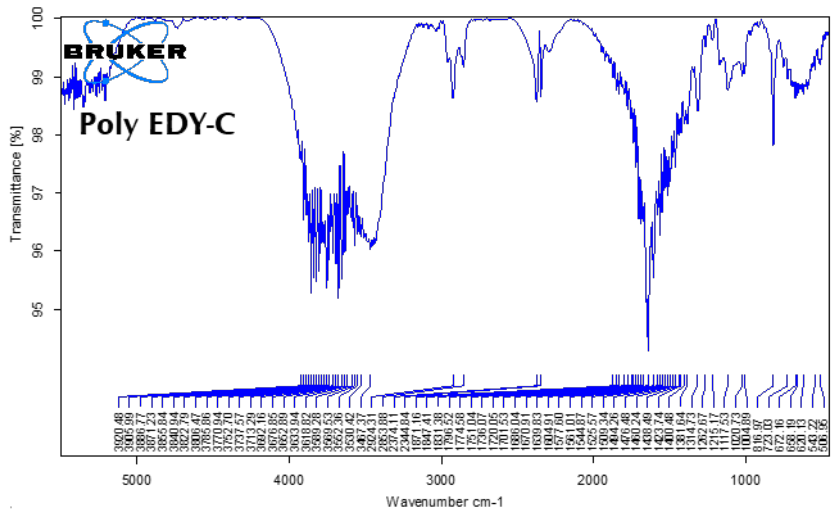


Figure S5 Records of spinning concentration of generated solid particles at room temperature

3. IR spectra of main-chain enediyne polymers







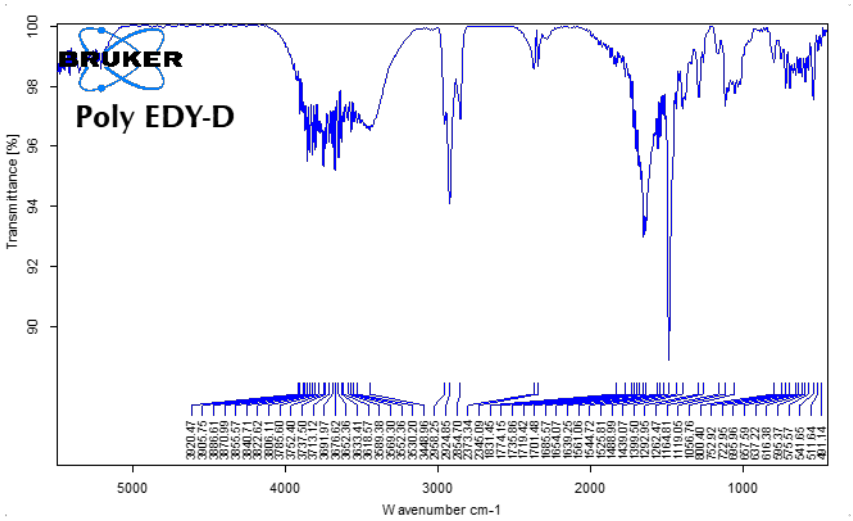


Figure S6 IR spectra of **Poly EDY-A/B/C/D**

4. NMR Spectra of Model Compounds and Polymers

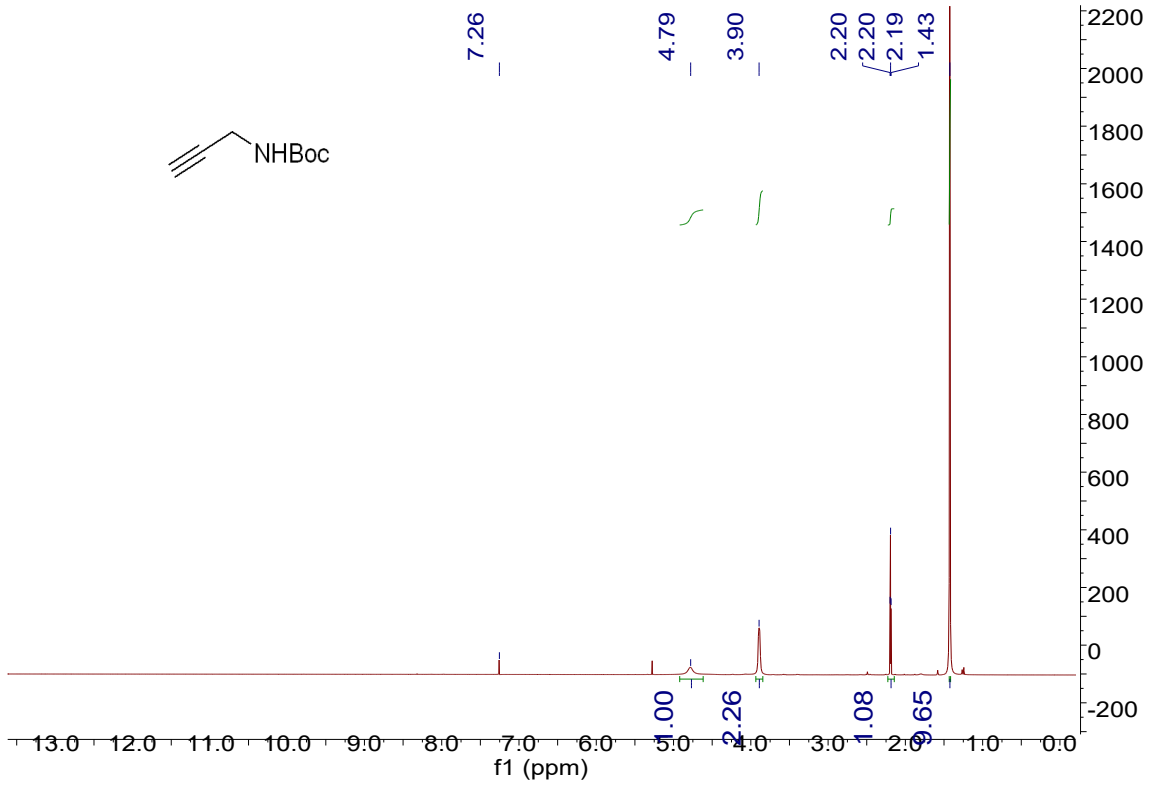


Figure S7 ^{1}H NMR spectrum of ***tert*-Butyl prop-2-ynylcarbamate**

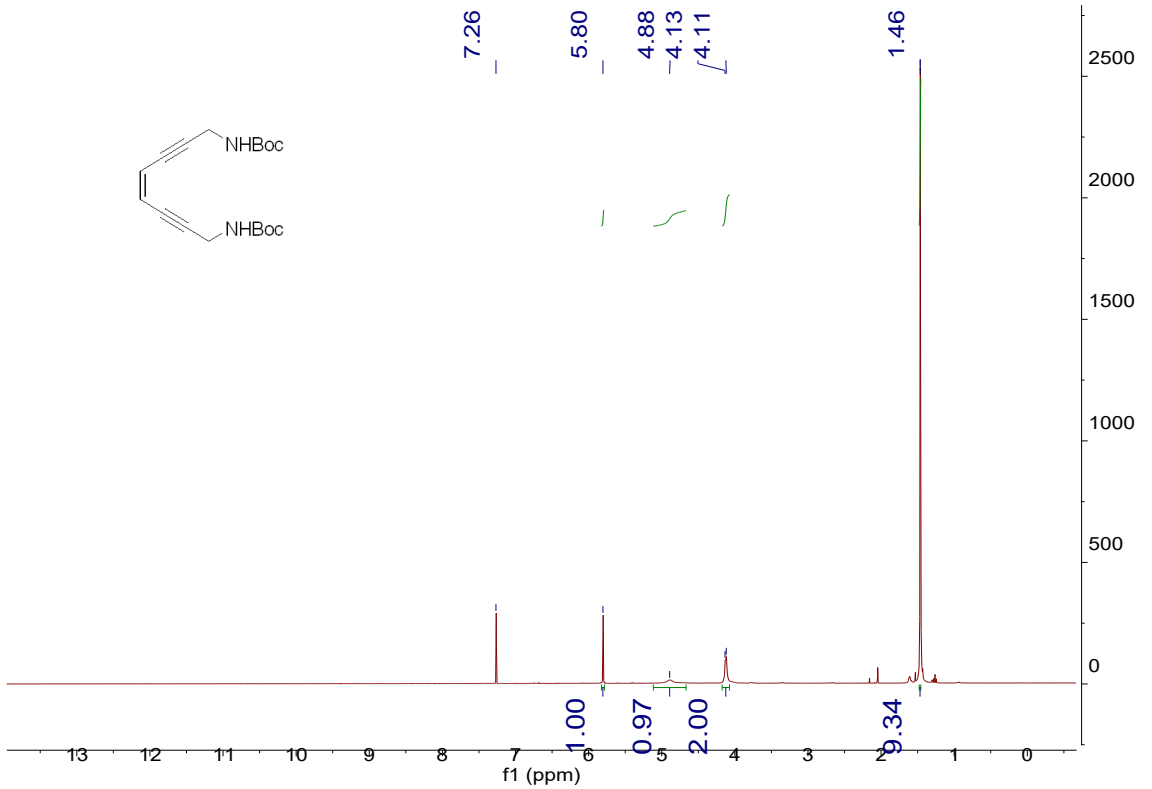


Figure S8 ^{1}H NMR spectrum of **EDY-I: di-*tert*-butyl octa-4-en-2,6-diyne-1,8-diyl(*Z*)-dicarbamate**

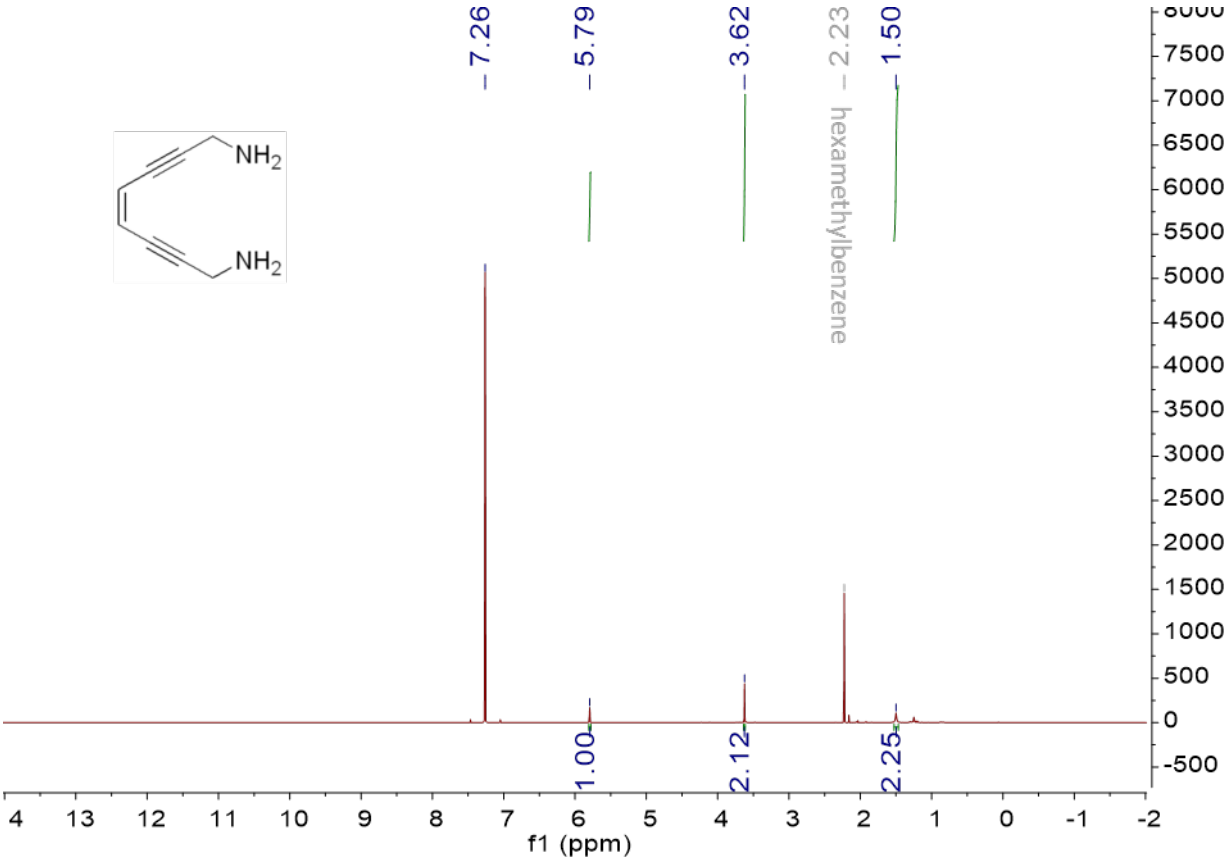


Figure S9 ^{1}H NMR spectrum of **EDY-II**

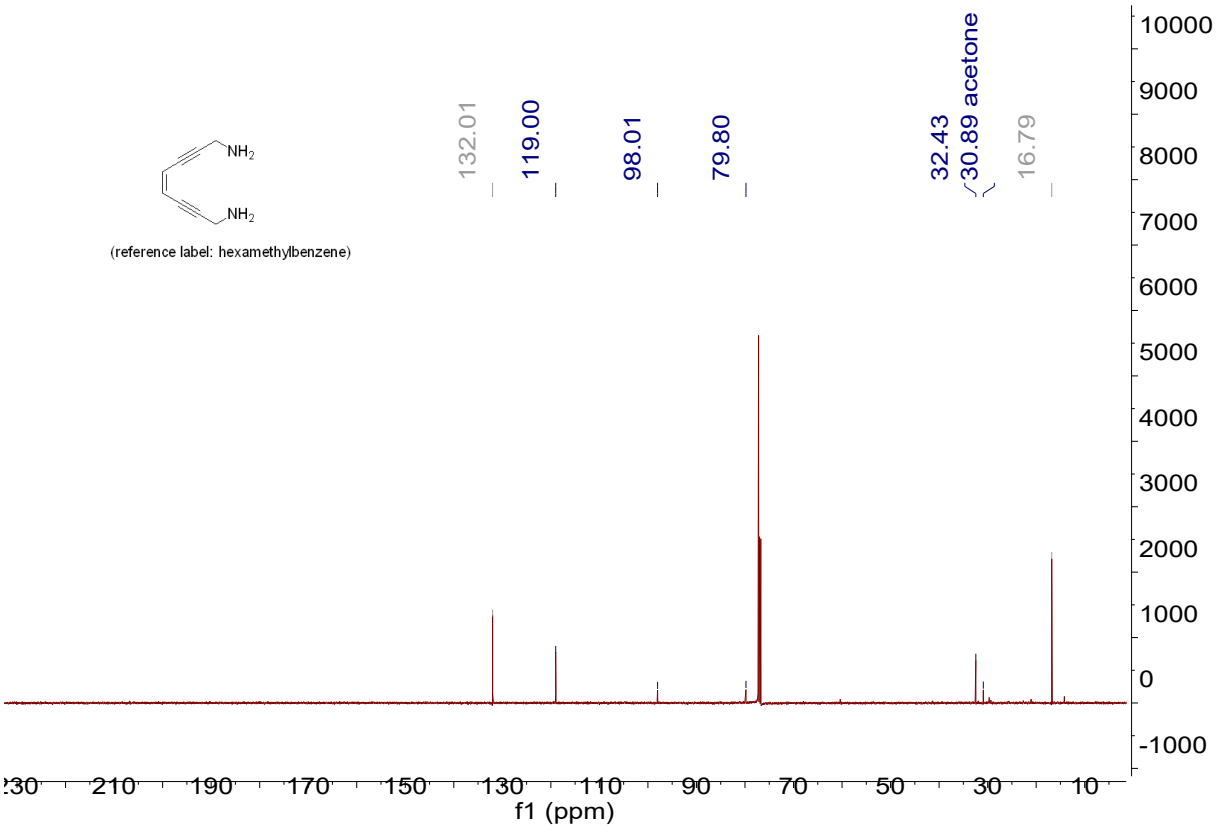


Figure S10 ^{13}C NMR spectrum of **EDY-II**

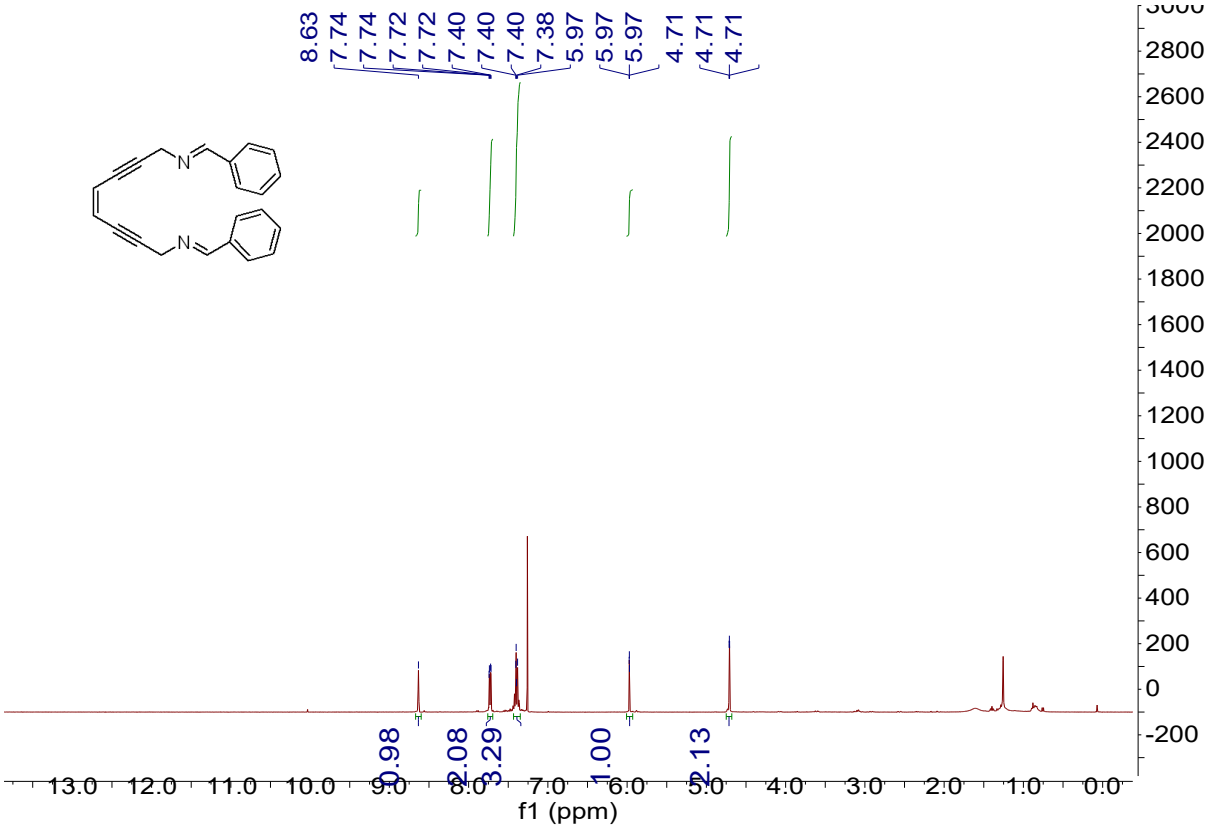


Figure S11 ^{1}H NMR spectrum of **EDY-III**

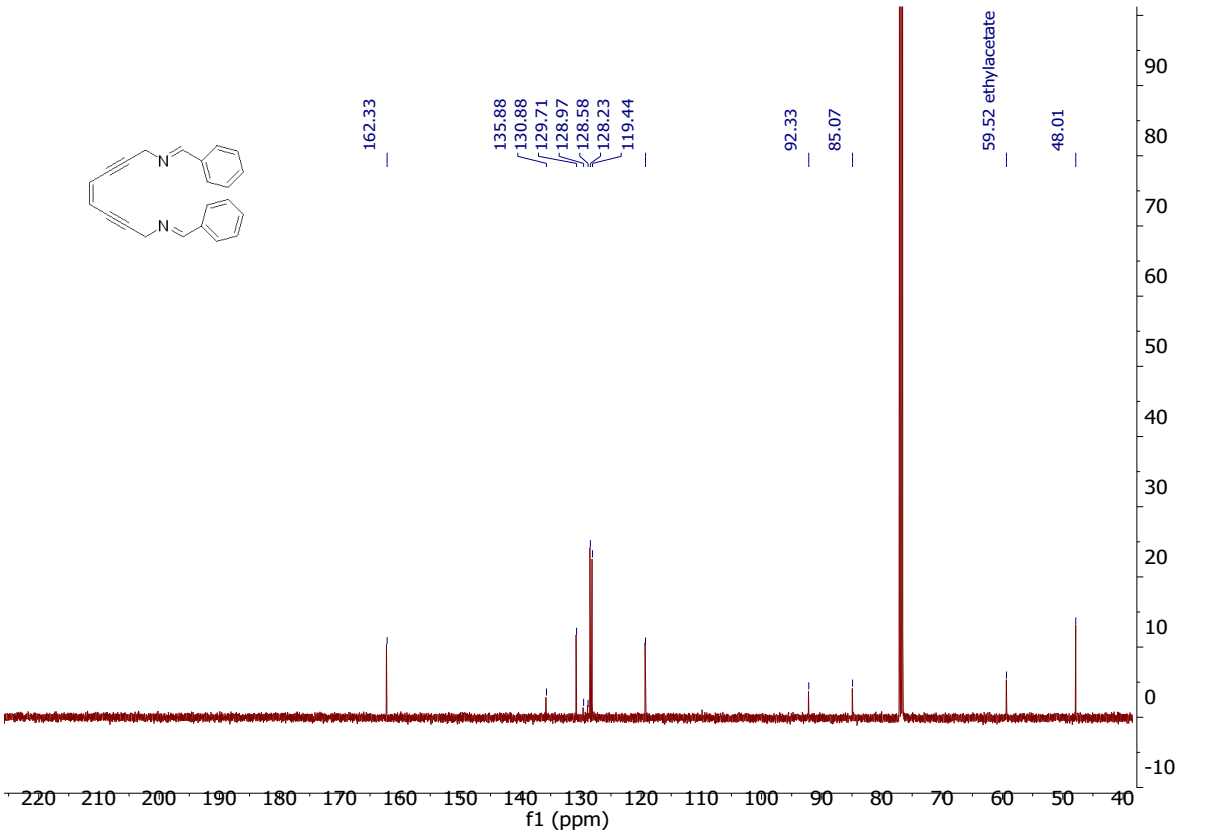


Figure S12 ^{13}C NMR spectrum of **EDY-III**

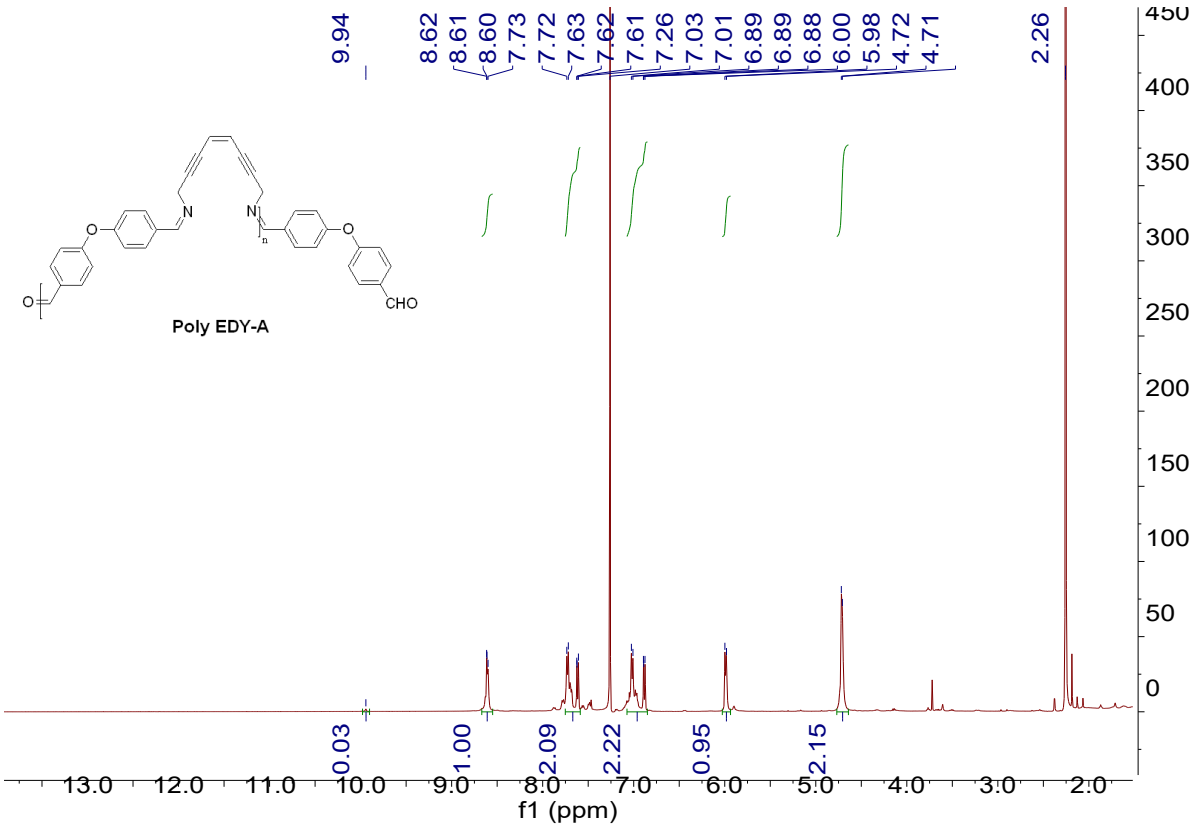


Figure S13 [1]H NMR spectrum of **Poly EDY-A**

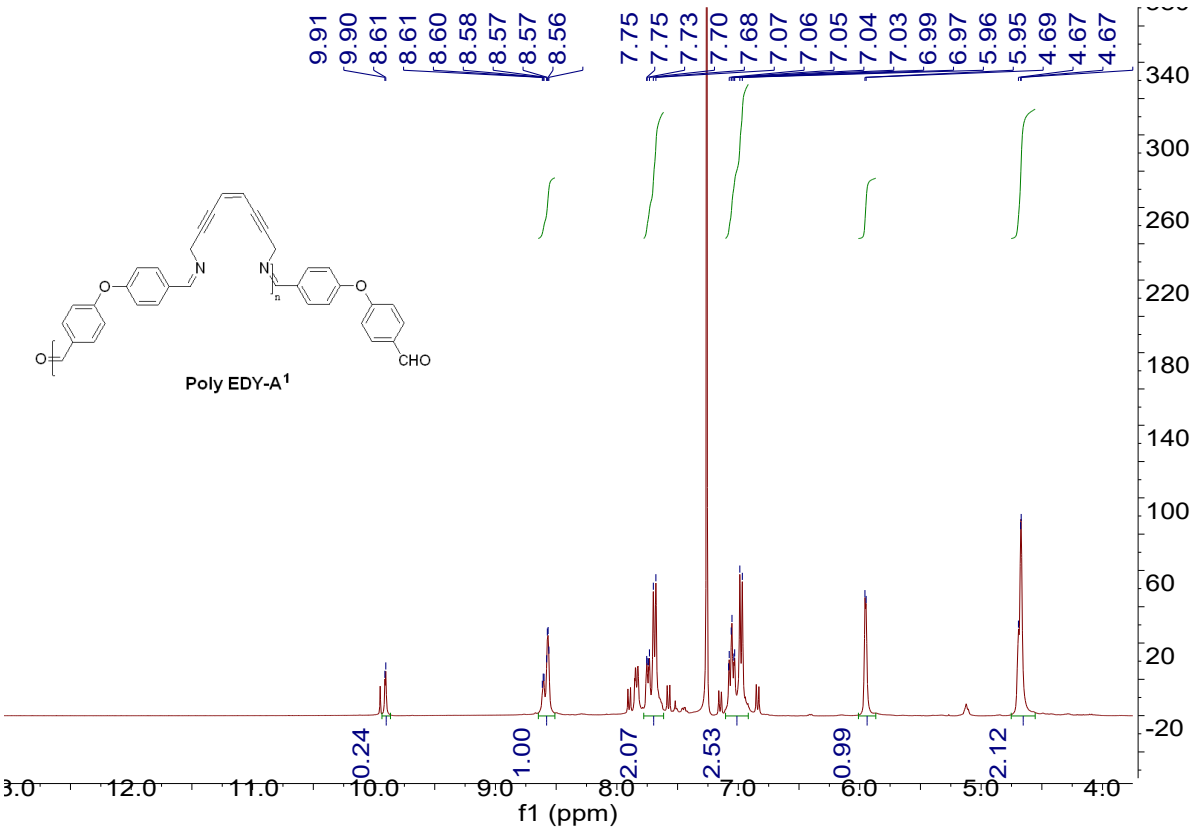


Figure S14 [1]H NMR spectrum of **Poly EDY-A[1]**

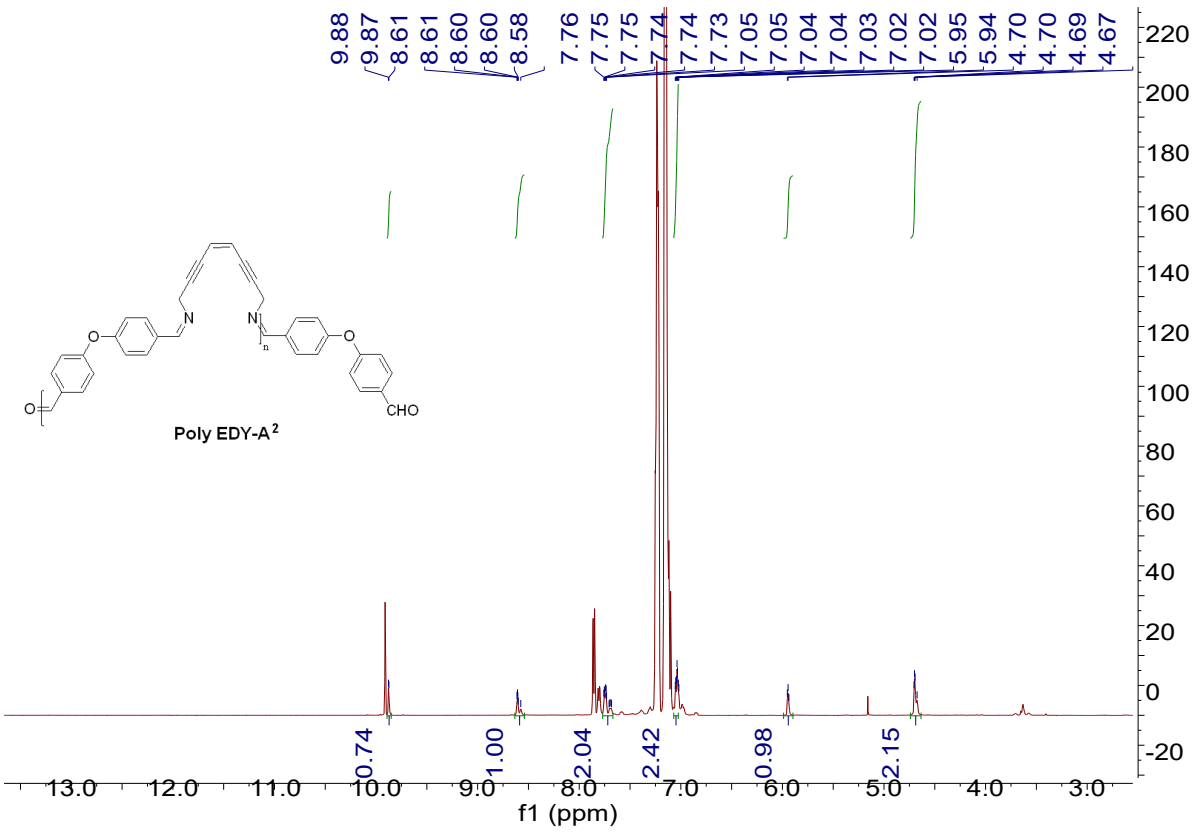


Figure S15 ^{1}H NMR spectrum of **Poly EDY-A^{2}**

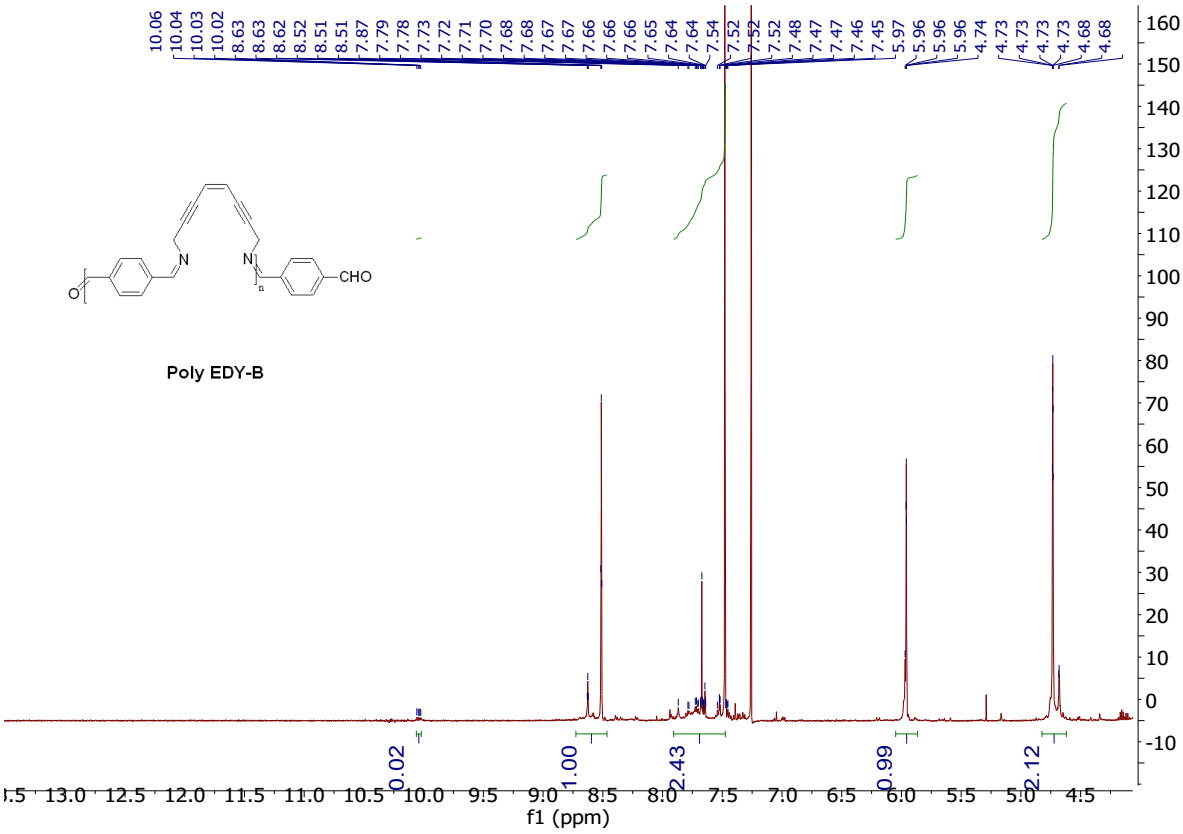


Figure S16 ^{1}H NMR spectrum of **Poly EDY-B**

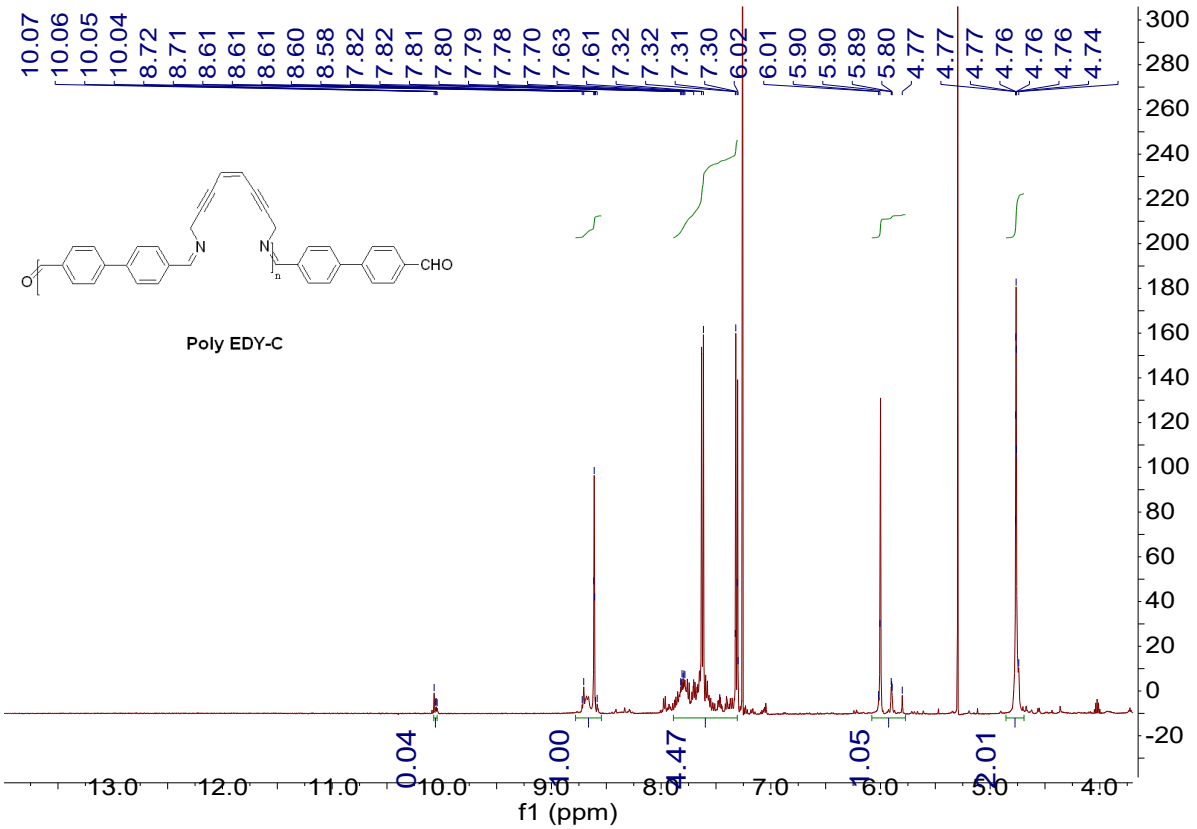


Figure S17 ^{1}H NMR spectrum of **Poly EDY-C**

5. DSC Curves of Model Compound and Polymers

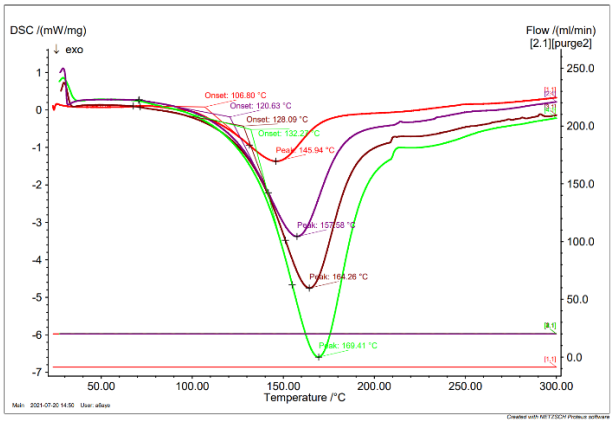


Figure S18 DSC curves of **EDY-III** under heating rate as 5/10/15/20 K/min

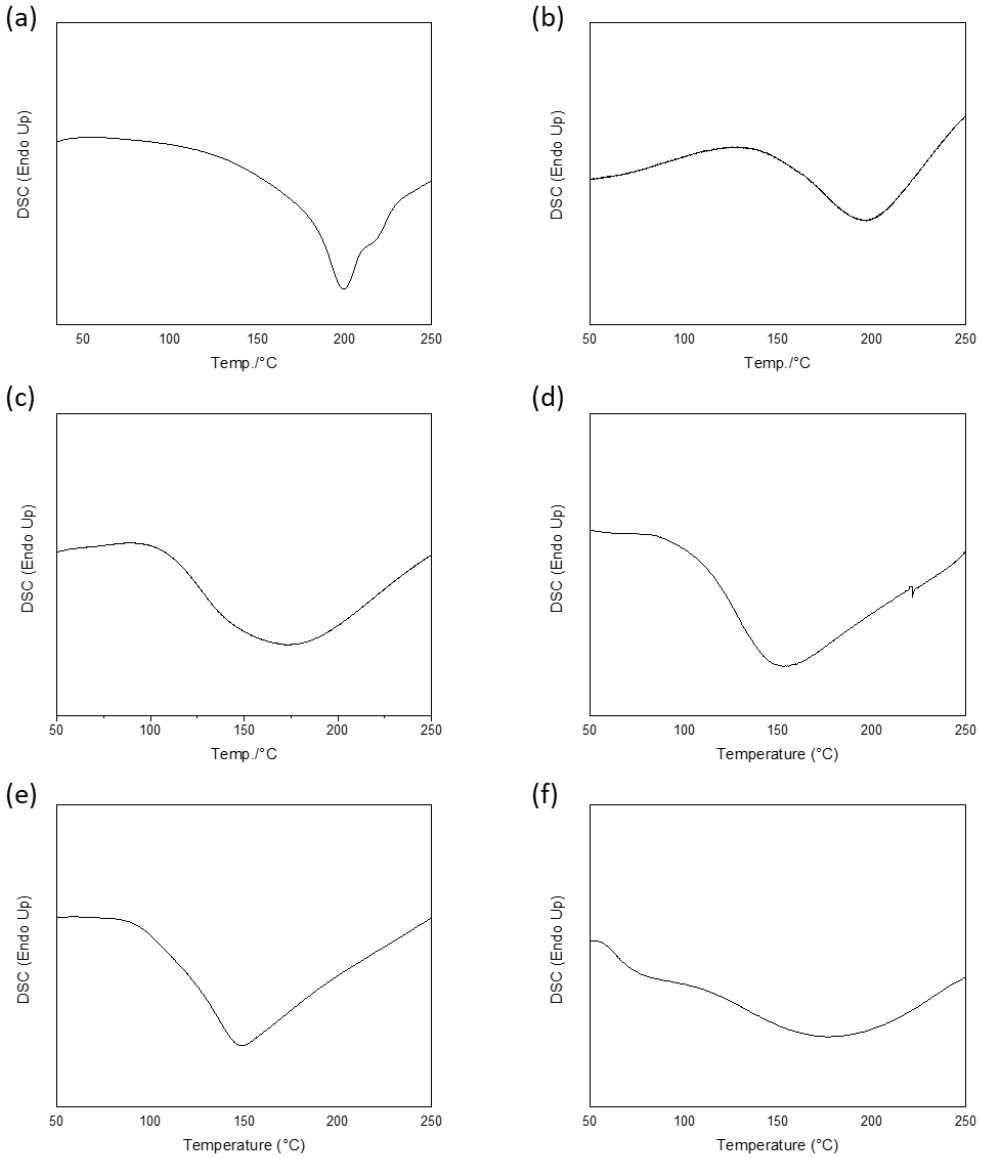
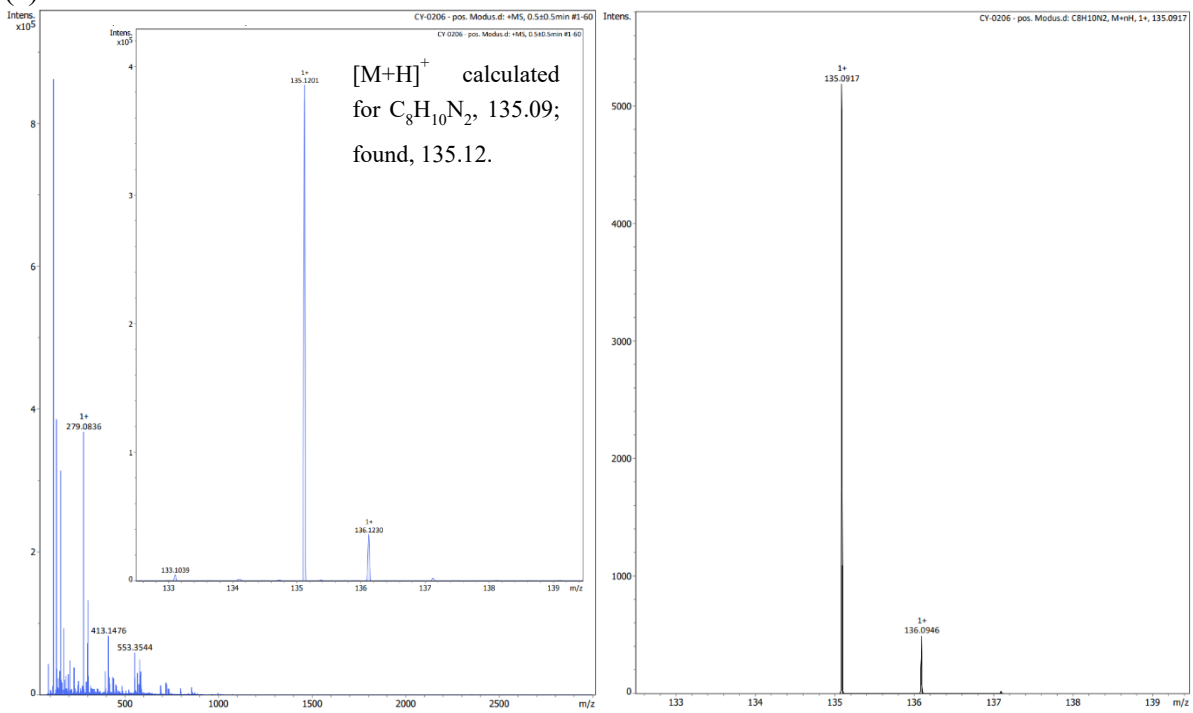


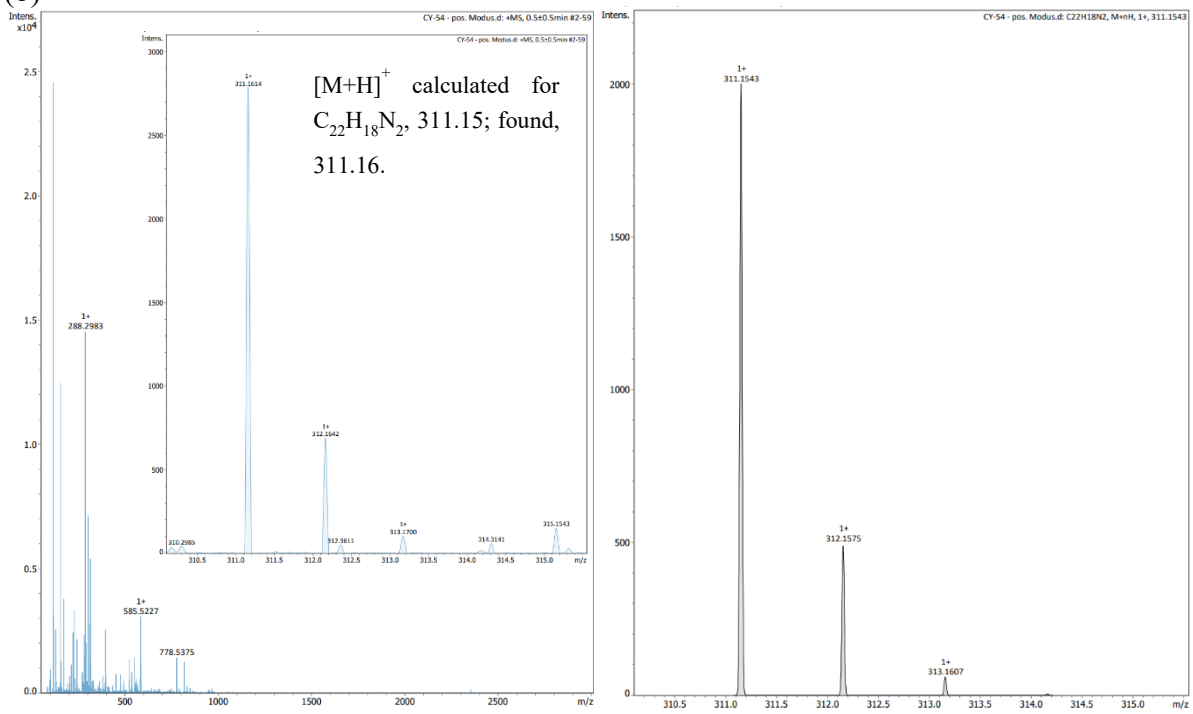
Figure S19 DSC curves of (a) **Poly EDY-A;** (b) **Poly EDY-A[1];** (c) **Poly EDY-A[2];** (d) **Poly EDY-B;** (e) **Poly EDY-C;** (f) **Poly EDY-D** under heating rate as 5 K/min

6. ESI-ToF MS Spectra of Model Compounds

(a)



(b)



(c)

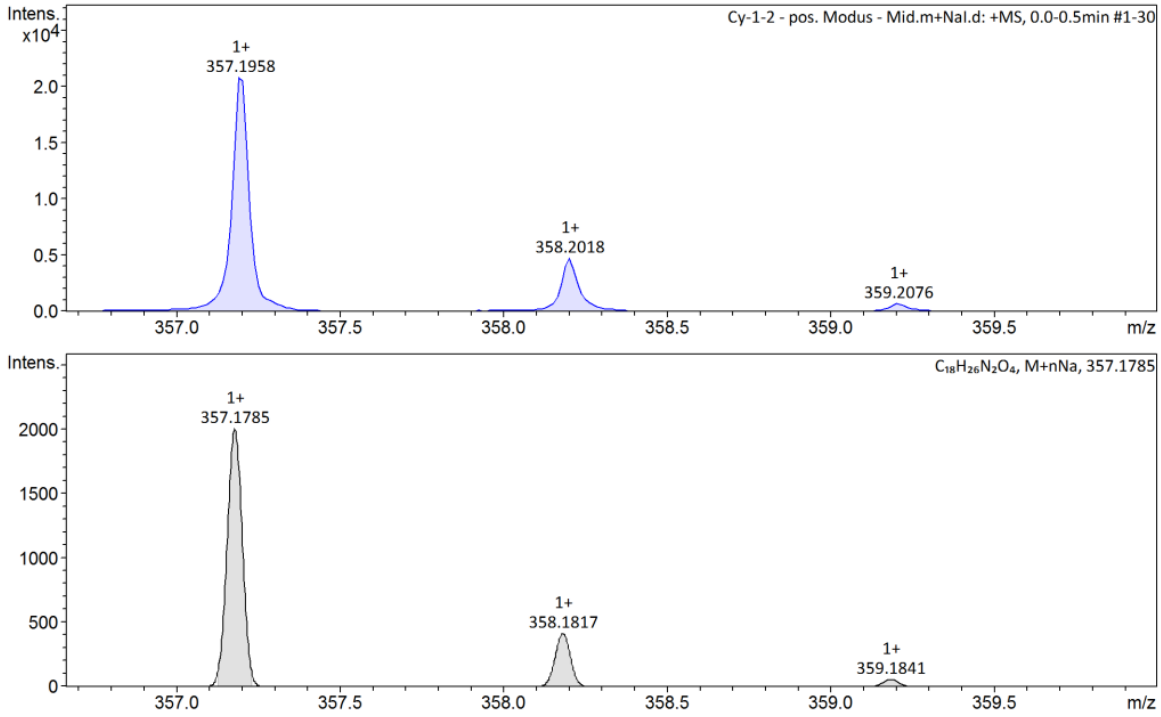


Figure S20 Left: the full experimental ESI-ToF MS spectra and insert: the compound spectra; and right: the simulated isotope pattern of compound (a) **EDY II**, (b) **EDY III**. (c) **EDY-I**.

7. Reference

1. G. A. Molander and F. Cadoret, *Tetrahedron Letters*, 2011, **52**, 2199-2202.
2. D. S. Rawat and J. M. Zaleski, *Chemical Communications*, 2000, DOI: 10.1039/b007360l, 2493-2494.
3. D. S. Rawat and J. M. Zaleski, *Journal of the American Chemical Society*, 2001, **123**, 9675-9676.