Electronic Supplementary Material (ESI) for Polymer Chemistry.

Mechanistic studies of the copolymerization between ethyl diazoacetate and cinnamaldehyde

*Yan Li, Cheng Chen, Feifei Li, Liqiong Liao, Lijian Liu**

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

Electronic Supplementary Information

Contents

1. Supplementary Materials and Methods

Cell imaging. Human cervix carcinoma (HeLa) cells were incubated in a complete medium (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS)) with 5% CO_2 at 37 ºC. The cells were seeded in a NEST confocal dish at a density of 1×10^5 cells and cultured in 2 mL of complete medium for 24 h. Then, a solution of copolymer (100 μg) dissolved in 20 μL of DMSO was added, followed by further incubation for another 4 h at 37 ºC. Imaging examination under UV-Vis excitation (458 nm) was conducted with a Zeiss Axiovert 200 fluorescence microscope. The two-photon-excitation fluorescence imaging experiment was performed using a Zeiss LSM 510 META NLO confocal microscope over the range of 720-950 nm.

2. Supplementary Results and Discussion

2.1 FT-IR spectroscopy

The FT-IR spectrum of the copolymer of EDA with CA (**Fig. S1**) shows that the bending vibration of *–CHO* is located at approximately 2,850 cm^{-1}. The intense bands at approximately 1,700 cm^{-1} belong to the carbonyl and aldehyde groups. The peak at approximately 1,550 cm^{-1} can be assigned to the azo group derived from EDA.[1]

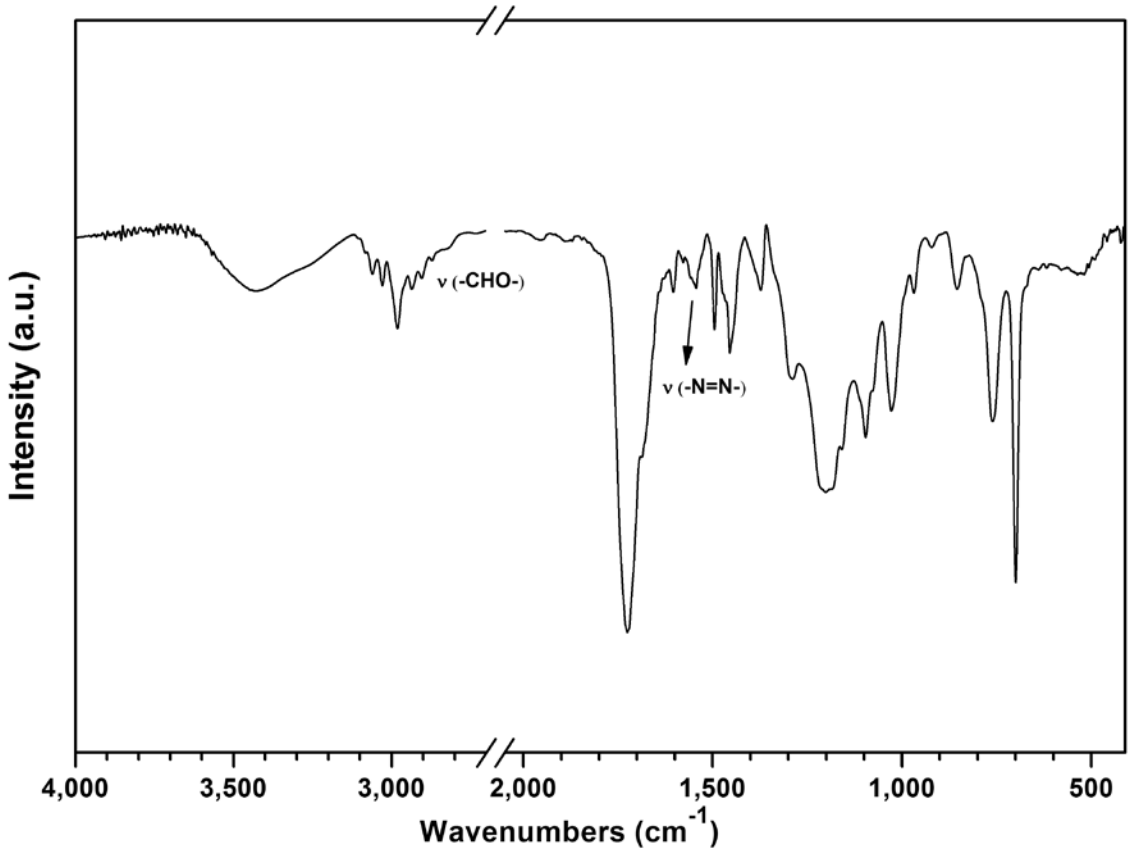


Fig. S1. FT-IR spectrum of the copolymer of EDA with CA.

2.2 XRD curve

XRD curve (**Fig. S2**) reveals that the copolymer is random.

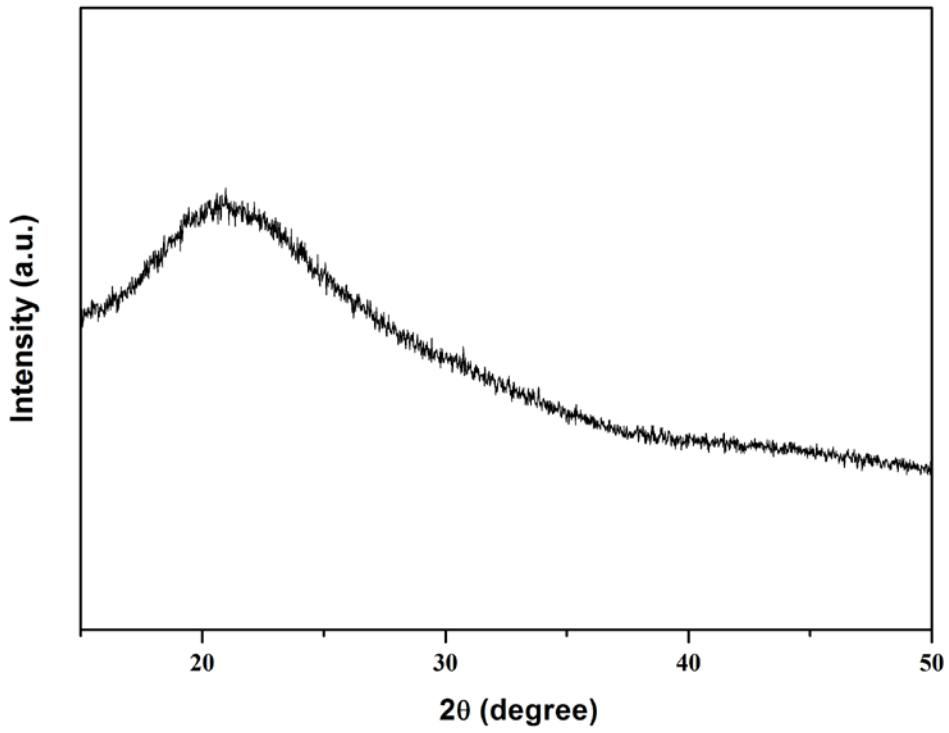


Fig. S2. XRD curve of the copolymer of EDA with CA.

2.3 Thermal properties

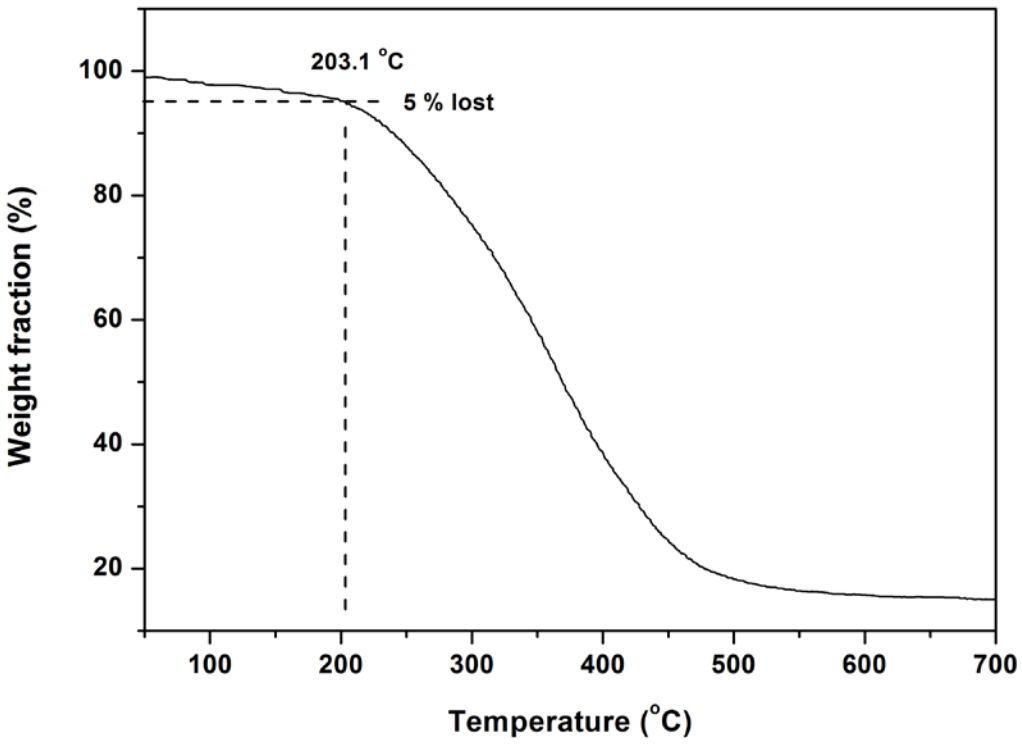


Fig. S3. TGA curve of the copolymer of EDA with CA.

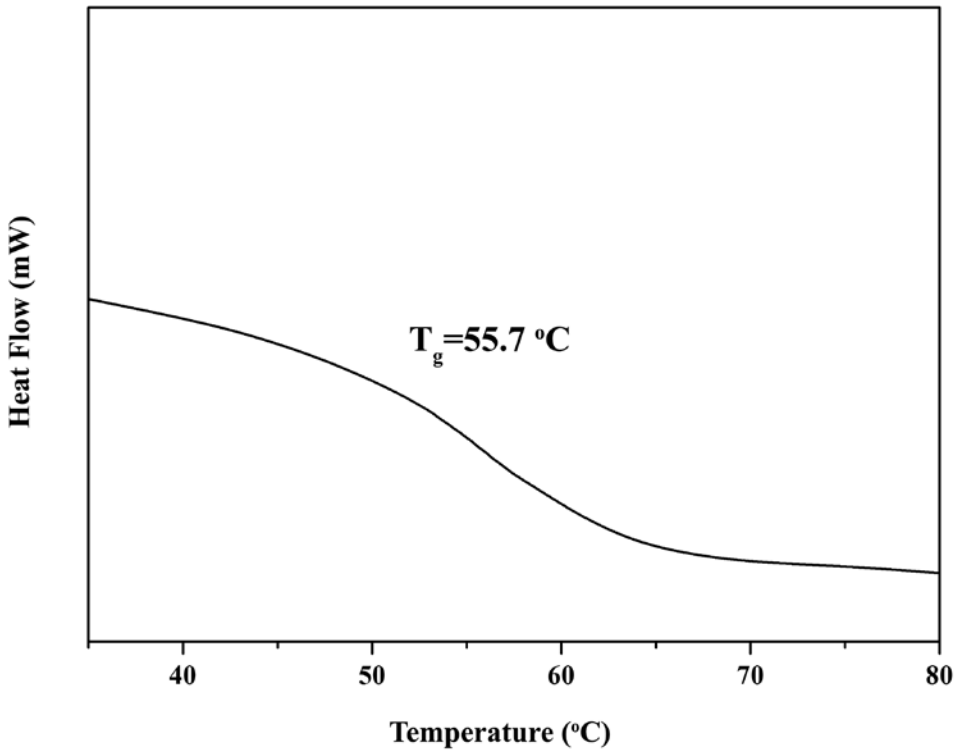


Fig. S4. DSC curve of the copolymer of EDA with CA. These data are recorded in the 2nd round. T_g means glass transition temperature.

Fig. S3 is the TGA curve of the copolymer of EDA with CA, which shows that the obtained copolymer is thermostable, losing merely 5% of their weights at 203.1 ^{o}C (T_L). **Fig. S4** is the DSC curve of the copolymer, and the T_g is 55.7 ^{o}C.

2.4 Mechanism studies of the block coupling by inhibition of TEMPO

EDA and CA can be restrained by the addition of radical inhibitor 2,2,6,6-tetramethylpiperidine-1-oxy (TEMPO), for which the peaks around 1.3 ppm and 4.2 ppm without TEMPO are broadened obviously compared to those with TEMPO, suggesting the polymerization of monomers by the change of chemical environment with the formation of main chain. In conclusion, the reaction is a possible radical mechanism. [2] (**Fig. S5**)

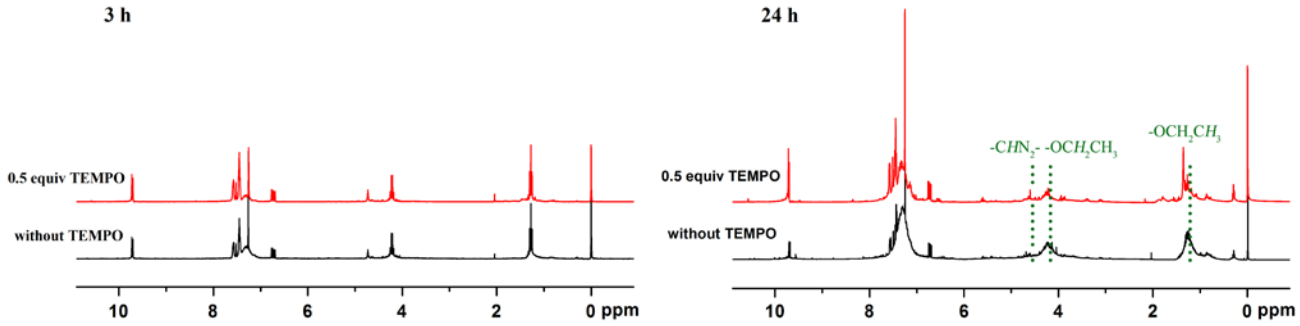


Fig. S5. TEMPO inhibition effects on the copolymerization. Conditions: CA (5.0 mmol, 1.0 equiv) and EDA (5.0 mmol, 1.0 equiv) are mixed at 60 ^{o}C under blowing nitrogen.

2.5 Mechanism studies of the block coupling by EPR

By RT-EPR spectroscopy, spin-trapping experiments using N-tert-butyl-α-phenylnitone (PBN) as spin trap was tried (**Fig. S6**), which shows triplet radical signals and confirms the existence of the radicals. Through addition of DMPO into EDA or CA separately, no signal was detected in EPR spectrum (**Fig. S7** or **Fig. S8**).

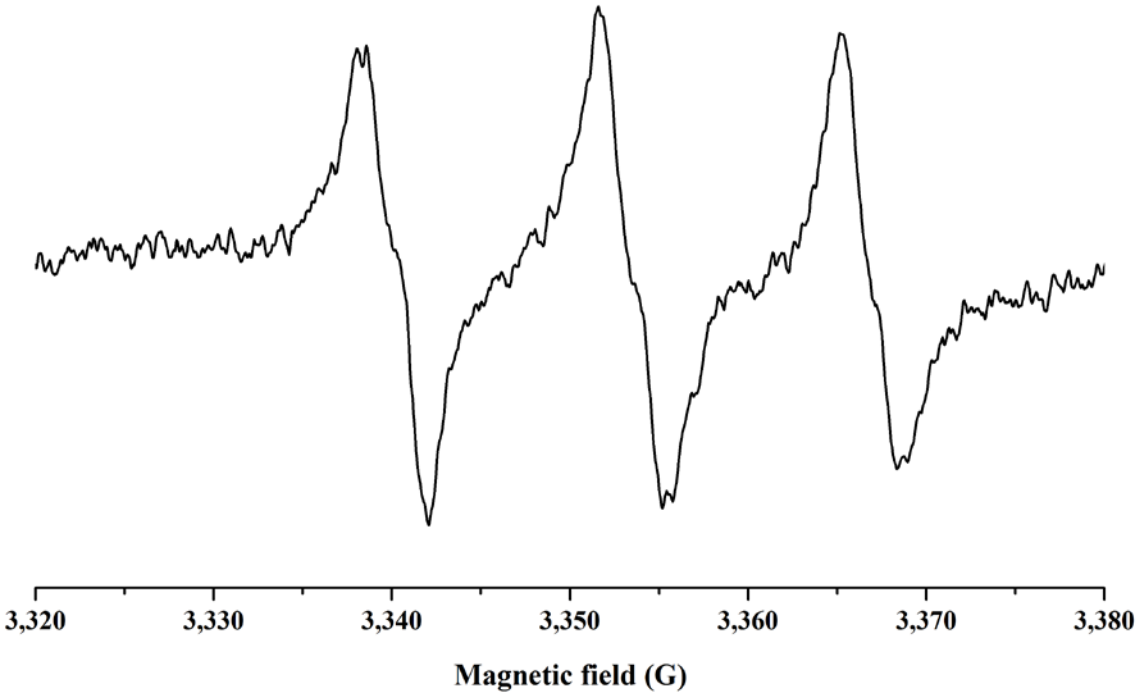


Fig. S6. **EPR measurement of the reaction mixture of EDA and CA trapped by PBN**. Conditions: cinnamaldehyde (5.0 mmol, 0.66 g) and EDA (5.0 mmol, 0.57 g) in 5 ml toluene at 60.0 ^{o}C with stream of dry nitrogen.

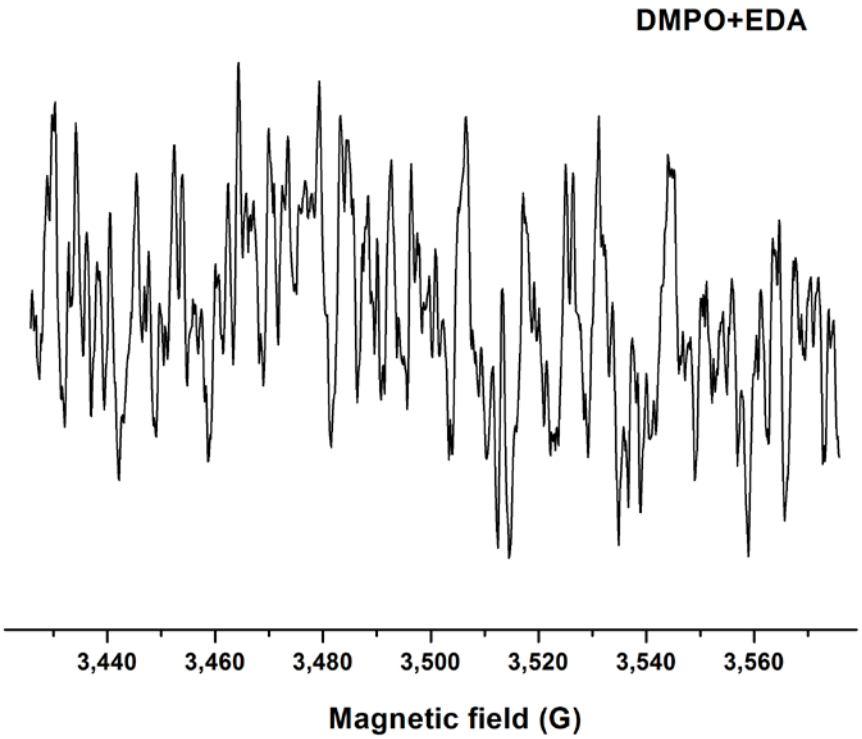


Fig. S7. **EPR measurement of EDA trapped by DMPO**.

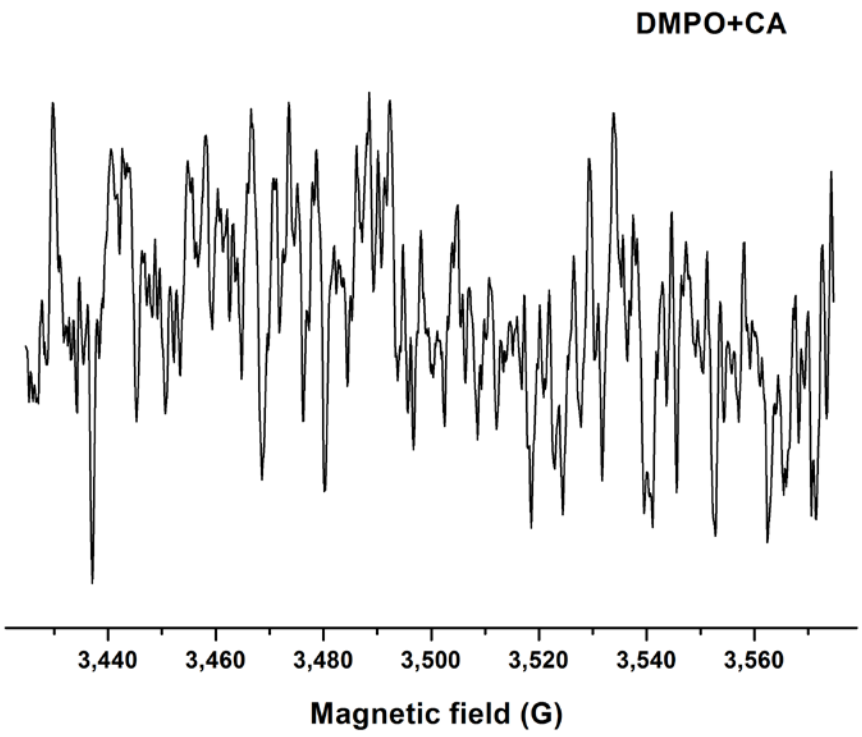


Fig. S8. EPR measurement of CA trapped by DMPO.

2.6 IR kinetics

Fig. S9 plots the relationship between peak area of the product and the reaction time in 480 minutes. From the curve, it can be observed that the peak area of -N=N- group keeps increasing but follows with a sudden drop periodically, which may be the complicated behaviours of azo-participated reactions, or a result of instrument/probe error and something not inherent to the reaction. Thus, we are discussing the kinetics behaviour in initial 119.5 minutes in the main text.

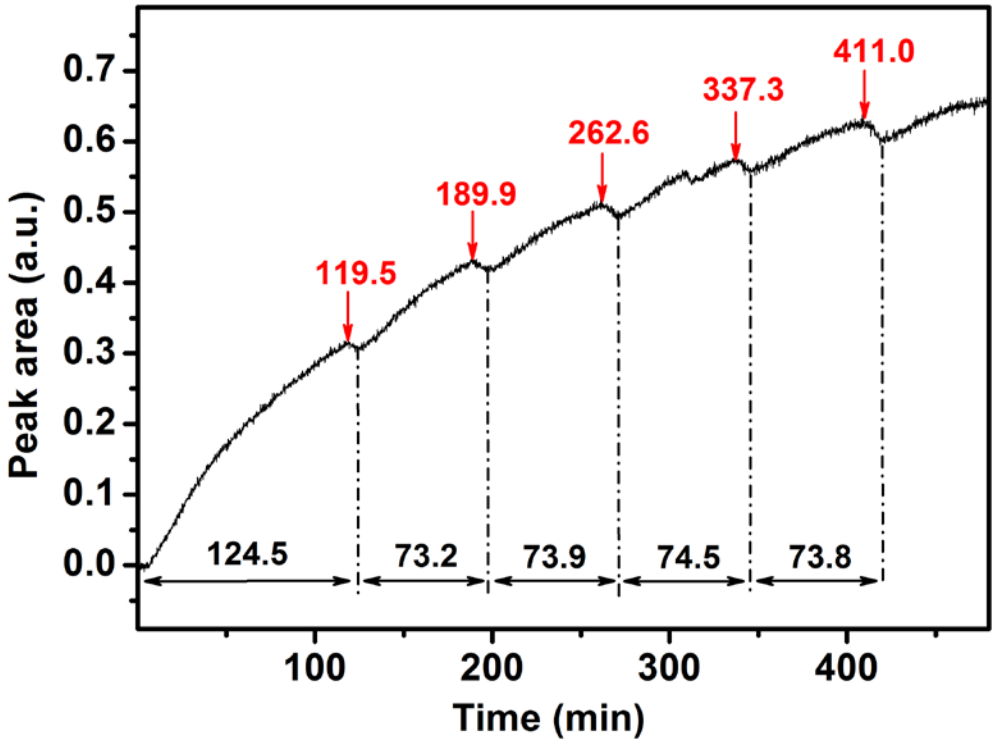


Fig. S9. The plot between the peak area from 1528 to 1565 cm^{-1} and the reaction time in 480 minutes.

2.7 DFT calculations for diradicals

EDA **N^{diazo}, C^{3}-diradical**, EDA-CA **N^{diazo}, C^{5}-diradical** and EDA-EDA-CA **N^{diazo}, C^{8}-diradical** are calculated by DFT using M06-2X/def2-SVP, and the details are shown in **Table S1**-4.

Two possible diradical structures of **N^{diazo}, C^{5}-diradical** (**N^{diazo}, C^{5}-diradical 1** and **N^{diazo}, C^{5}-diradical 2**) were proposed and the results revealed that electrons are distributed on N atom and C atom, respectively.

The total energy of **N^{diazo}, C^{5}-diradical 1** is -837.90391759 a.u., while that of **N^{diazo}, C^{5}-diradical 2** is -837.90059171 a.u.. The energy difference between the two structures is calculated as below.

$$-837.90391759-(-837.90059171)*627.51 \text{ kcal/mol} \approx -2.09 \text{ kcal/mol},$$

which means that the energy of **N^{diazo}, C^{5}-diradical 1** is 2.09 kcal/mol lower than that of **N^{diazo}, C^{5}-diradical 2**. So **N^{diazo}, C^{5}-diradical 1** is more stable and acts as the practical **N^{diazo}, C^{5}-diradical** in the main text.

Fig. S10 shows the calculated result of EDA-EDA **N^{diazo}, C^{6}-diradical**, which is unstable and easy to release the N_2 and generate a closed-shell structure. **Fig. S11** is CA calculated by DFT and the total energy is -422.4738922 a.u.

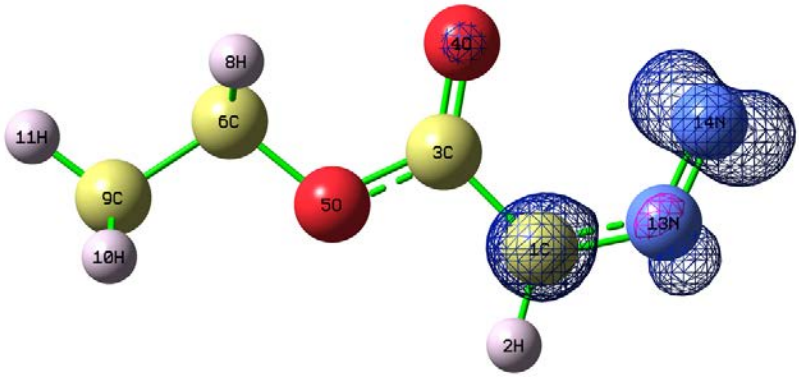
The binding energy of **N^{diazo}, C^{5}-diradical** relative to that of the sum of **N^{diazo}, C^{3}-diradical** and CA is:

$$-837.90391759-(-422.4738922-415.38942340)*627.51 \text{ kcal/mol} = -25.48 \text{ kcal/mol}.$$

The binding energy of **N^{diazo}, C^{8}-diradical** relative to that of the sum of **N^{diazo}, C^{5}-diradical** and **N^{diazo}, C^{3}-diradical** is:

$$-1253.36671276-(-837.90391759-415.38942340)*627.51 \text{ kcal/mol} = -46.04 \text{ kcal/mol}.$$

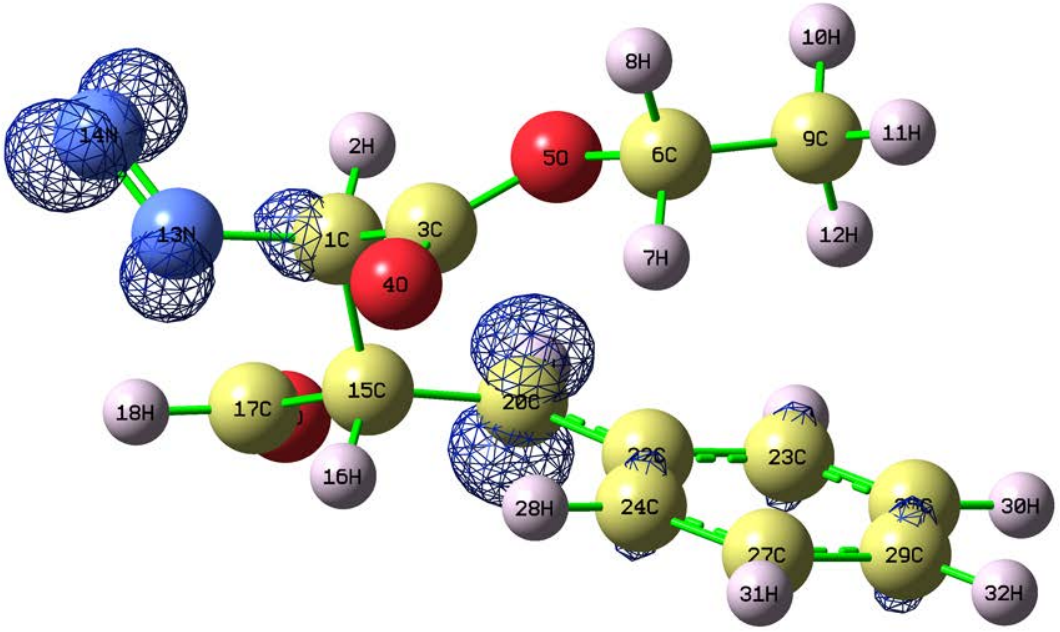
Table S1. Population of atoms of **EDA N^{diazo}, C^3-diradical**.



	Atom	Atomic charge	Spin_pop.
1	C	0.081858	0.890425
2	H	0.082476	-0.040214
3	C	0.246185	-0.078617
4	O	-0.223099	0.108744
5	O	-0.364215	0.021489
6	C	0.109590	0.000343
7	H	0.058392	0.001300
8	H	0.058411	0.001295
9	C	-0.084782	0.000004
10	H	0.058970	0.000066
11	H	0.055581	0.000088
12	H	0.058963	0.000066
13	N	-0.090739	-0.057549
14	N	-0.047591	1.152561

Total energy: -415.38942340 a.u.

Table S2. Population of atoms of EDA-CA **N^{diazo}, C^{5}-diradical 1**.

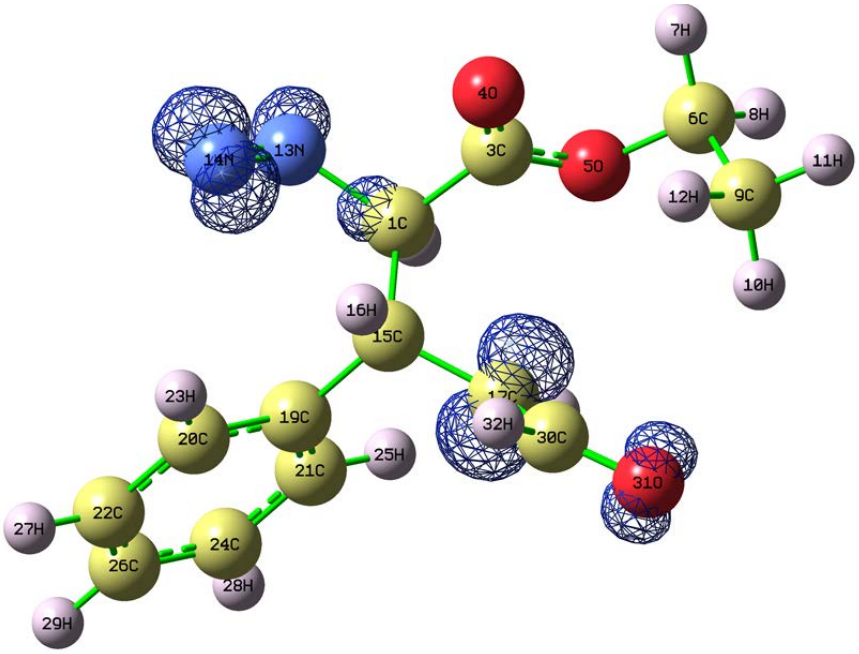


	Atom	Atomic charge	Spin_pop.
1	C	0.049285	0.183519
2	H	0.072562	0.004693
3	C	0.251802	-0.008825
4	O	-0.231363	0.005274
5	O	-0.336847	0.001128
6	C	0.114620	0.000233
7	H	0.058088	0.000446
8	H	0.058543	0.000509
9	C	-0.070908	0.001418
10	H	0.055959	-0.000046
11	H	0.050833	-0.000071
12	H	0.055343	-0.000707
13	N	-0.083927	0.115345

14	N	-0.081044	0.740526
15	C	-0.155210	-0.087778
16	H	0.086702	0.011413
17	C	0.192059	0.015480
18	H	0.052709	0.000367
19	O	-0.193237	-0.003074
20	C	-0.024745	0.859985
21	H	0.046210	-0.039289
22	C	0.052587	-0.220464
23	C	-0.036243	0.203693
24	C	-0.06394	0.225360
25	C	-0.017284	-0.105403
26	H	0.019665	-0.008893
27	C	-0.016270	-0.110173
28	H	0.030354	-0.010308
29	C	-0.016872	0.229707
30	H	0.026513	0.003335
31	H	0.027336	0.003411
32	H	0.026723	-0.010813

Total energy: -837.90391759 a.u.

Table S3. Population of atoms of EDA-CA **N^{diazo}, C^{5}-diradical 2**.

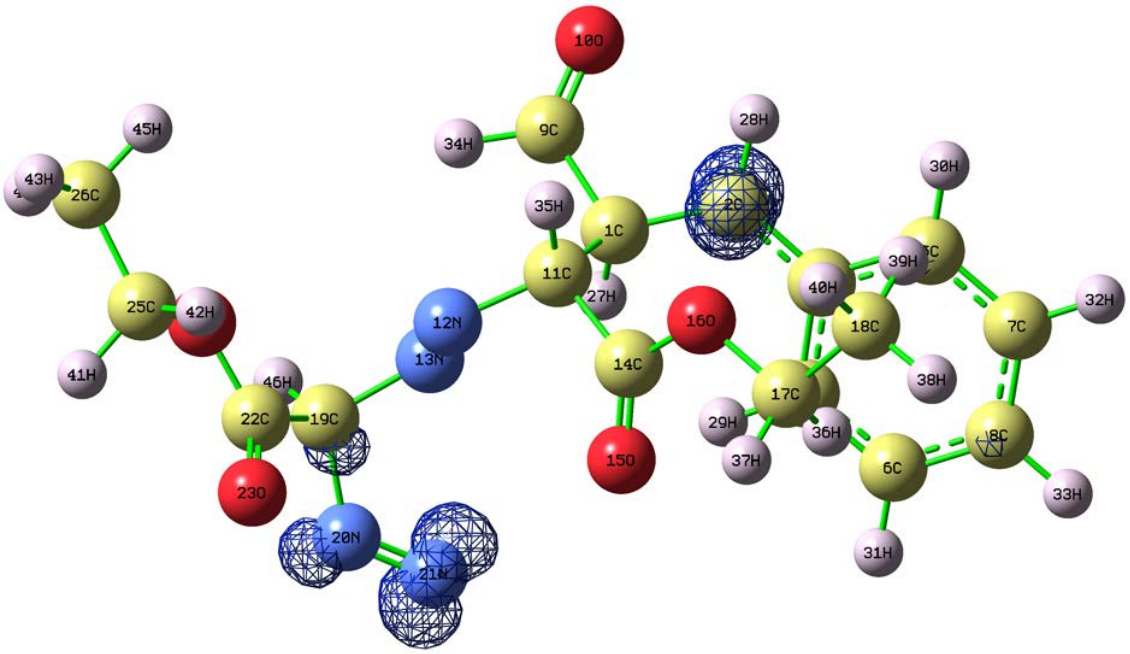


	Atom	Atomic charge	Spin_pop.
1	C	0.083259	0.159859
2	H	0.070036	-0.008451
3	C	0.244528	0.002556
4	O	-0.223491	-0.003768
5	O	-0.34728	0.004563
6	C	0.124532	-0.000212
7	H	0.064885	0.000017
8	H	0.057473	0.000085
9	C	-0.129893	0.001441
10	H	0.072272	-0.001207
11	H	0.061027	-0.000226
12	H	0.070631	0.000861
13	N	-0.080281	0.125326
14	N	-0.080038	0.723458
15	C	-0.103863	-0.065189

16	H	0.051845	0.004411
17	C	0.004043	0.904715
18	H	0.067488	-0.044349
19	C	0.01751	0.021073
20	C	-0.005769	0.006552
21	C	-0.039709	0.006856
22	C	-0.015977	-0.000241
23	H	0.024537	0.000754
24	C	-0.013127	-0.002159
25	H	0.015519	0.000501
26	C	-0.009639	0.008715
27	H	0.028928	0.000266
28	H	0.026387	0.000173
29	H	0.028958	-0.000421
30	C	0.152835	-0.159185
31	O	-0.242369	0.312752
32	H	0.024745	0.000474

Total energy: -837.90059171 a.u.

Table S4. Population of atoms of **EDA-EDA-CA N^{diazo}, C^{8}-diradical**.



	Atom	Atomic charge	Spin_pop.
1	C	-0.146085	-0.085275
2	C	-0.005553	0.869410
3	C	0.023609	-0.216341
4	C	-0.037863	0.214528
5	C	-0.039378	0.199946
6	C	-0.012420	-0.104896
7	C	-0.018244	-0.103581
8	C	-0.019954	0.218683
9	C	0.197336	0.012757
10	O	-0.198508	-0.003040
11	C	0.056107	0.048185
12	N	-0.12564	0.023259
13	N	-0.145807	-0.003326
14	C	0.237363	-0.005588
15	O	-0.218017	0.005137
16	O	-0.339173	0.001059

17	C	0.116648	0.001411
18	C	-0.071799	0.002067
19	C	0.195602	0.160142
20	N	-0.091142	0.101537
21	N	-0.065563	0.737811
22	C	0.242041	-0.005974
23	O	-0.215738	-0.001015
24	O	-0.358724	0.000676
25	C	0.114437	0.000546
26	C	-0.085588	0.000000
27	H	0.101484	0.009659
28	H	0.037958	-0.040496
29	H	0.025749	-0.010981
30	H	0.016690	-0.008960
31	H	0.028476	0.003270
32	H	0.022476	0.003247
33	H	0.024291	-0.010277
34	H	0.024181	0.000015
35	H	0.068931	-0.001836
36	H	0.060306	-0.001325
37	H	0.058467	0.000577
38	H	0.052351	-0.000015
39	H	0.051510	-0.001705
40	H	0.055333	0.000035
41	H	0.059190	0.000039
42	H	0.057955	-0.000023
43	H	0.055298	0.000028
44	H	0.058581	0.000001
45	H	0.057461	0.000002
46	H	0.095365	-0.009371

Total energy: -1253.36671276 a.u.

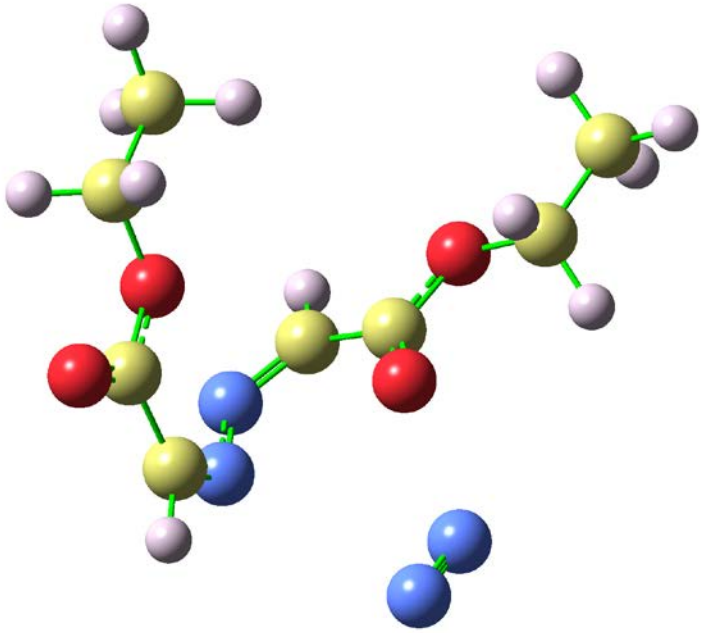


Fig. S10. EDA-EDA N^{diazo}, C^{6}-diradical calculated by DFT.

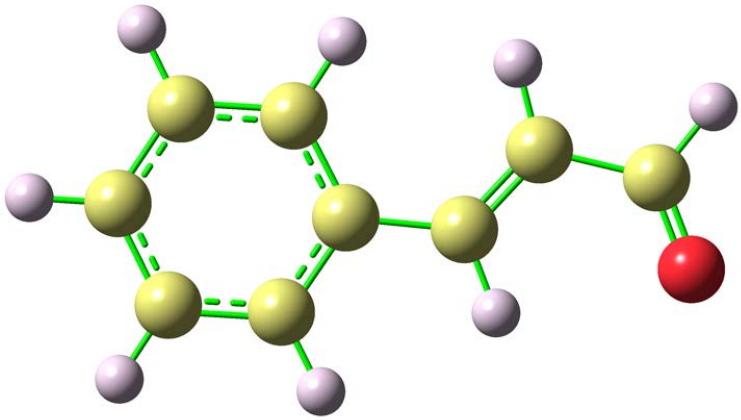


Fig. S11. CA calculated by DFT. Total energy: -422.4738922 a.u.

2.8 DFT calculations for Step 1

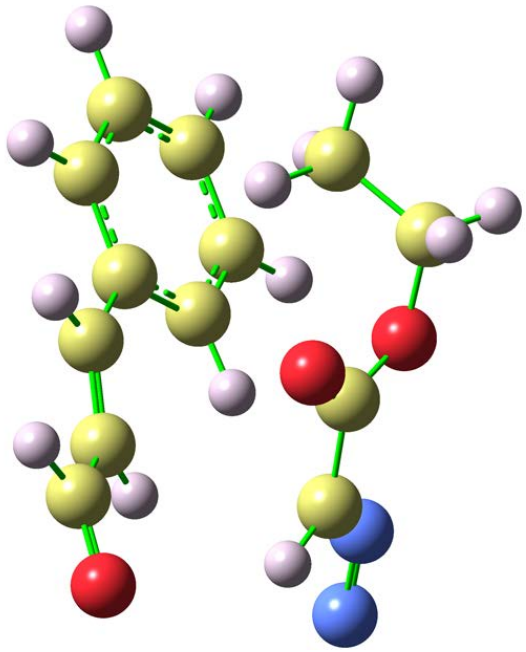


Fig. S12. The computed reactants (EDA and CA) in Step 1.

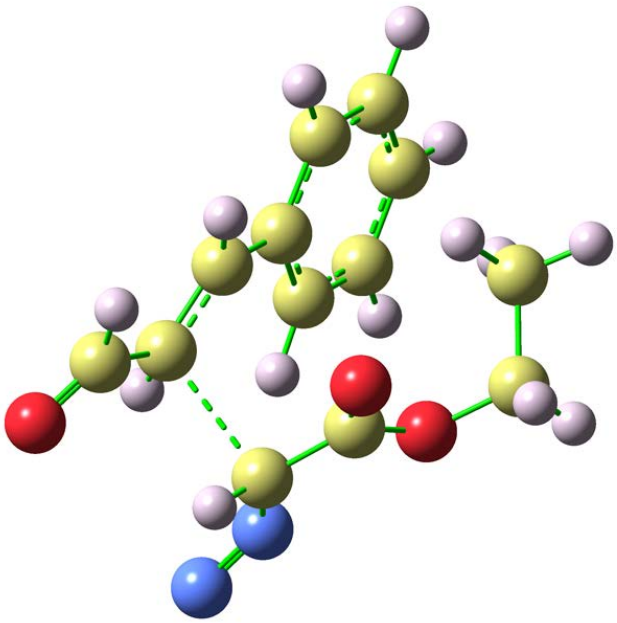


Fig. S13. The computed transition state TS1in Step 1.

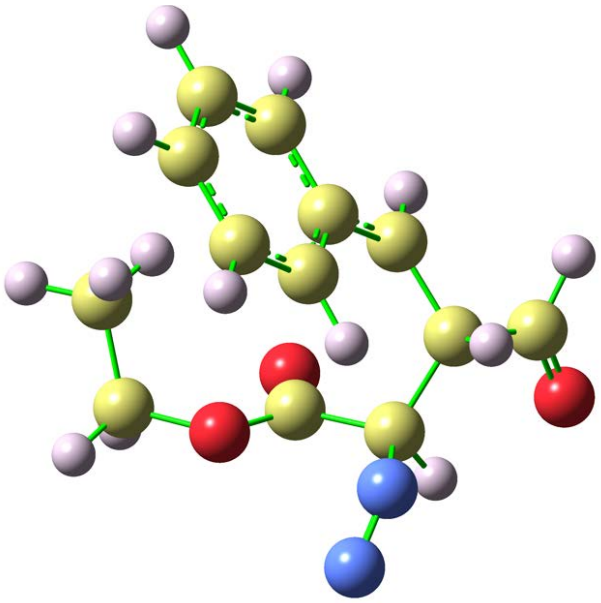


Fig. S14. The computed product in Step 1.

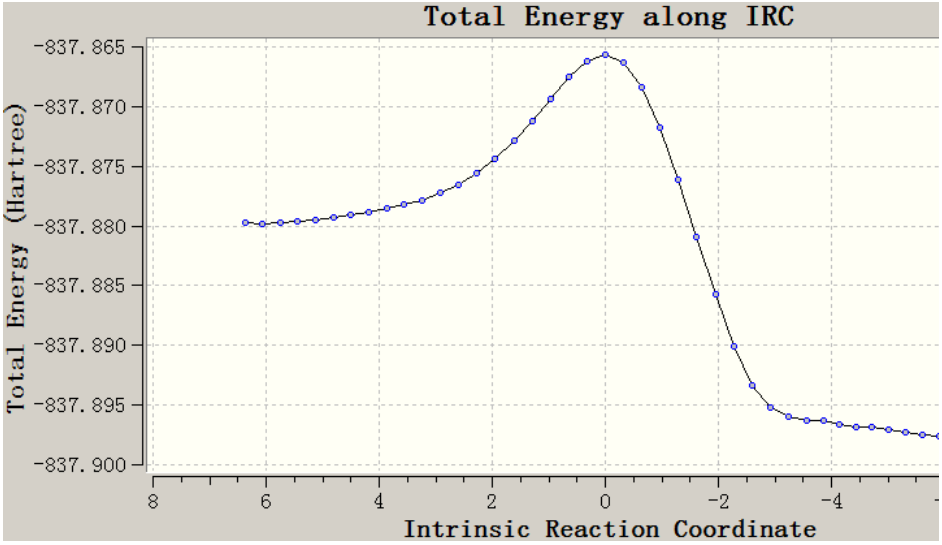


Fig. S15. The total energy along intrinsic reaction coordinate (IRC).

The structures and IRC are optimized by M06-2X/def2-SVP. To get more accurate transformation of the energy, M06-2X/def2-TZVP is used. The data are shown below.

Reactants：-838.8335443 a.u.

TS1：-838.8179971 a.u.

Product：-838.8518032 a.u.

The energy transformation of Step 1=627.51*(-838.8518032+838.8335443)= -11.46 kcal/mol.

The barrier=627.51*(-838.8179971+838.8335443)= +9.76 kcal/mol.

3. Supplementary Reference:

1. K. Maruoka, M. Oishi and H. Yamamoto, *Macromolecules*, 1996, **29**, 3328-3329.
2. H. Lu, W. I. Dzik, X. Xu, L. Wojtas, B. de Bruin and X. P. Zhang, *J. Am. Chem. Soc*., 2011, **133**, 8518-8521.