Supporting Information

Of

Mechanistic Insight into the Chemiluminescent Decomposition of *Cypridina* Dioxetanone and the Chemiluminescent, Fluorescent Properties of the Light Emitter of *Cypridina* Bioluminescence

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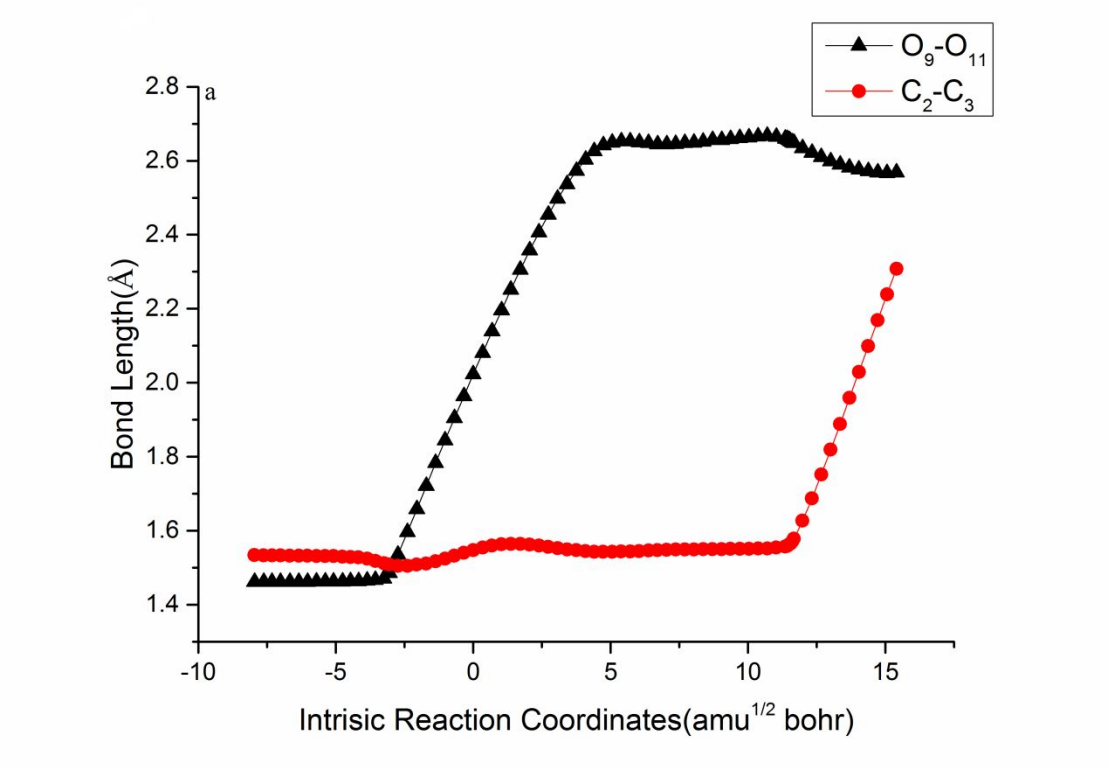
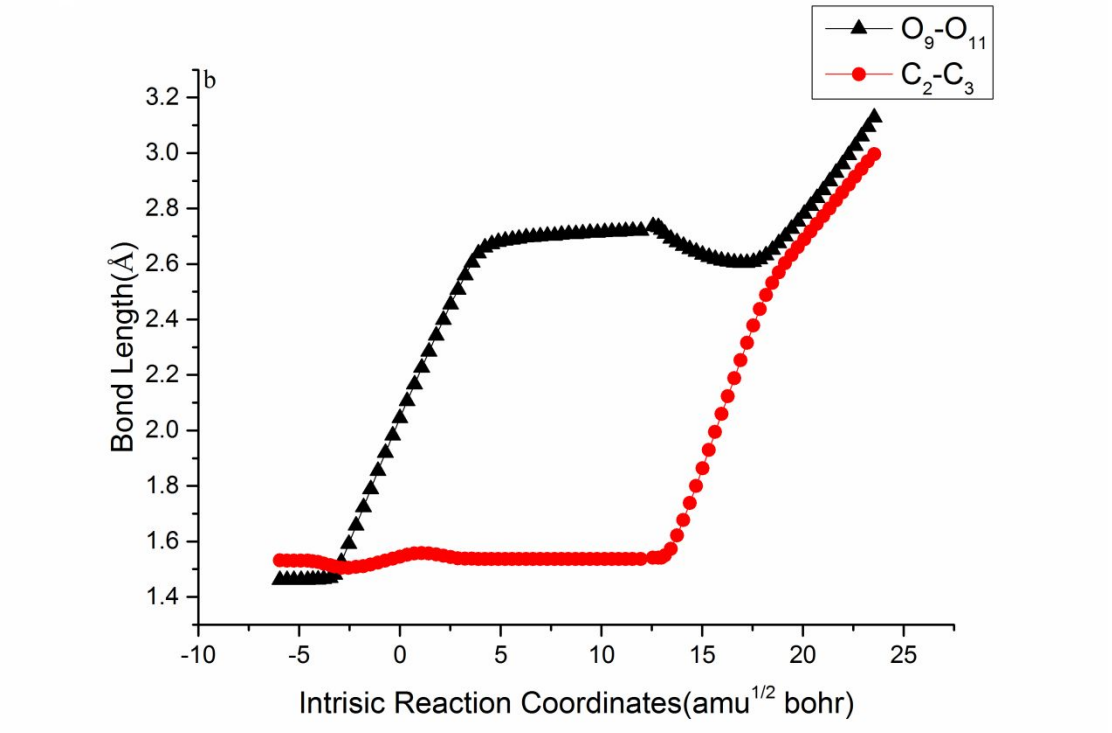

a
O_9-O_{11}
C_2-C_3
Bond Length(Å)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
-10
-5
0
5
10
15
Intrisic Reaction Coordinates(amu$^{1/2}$ bohr)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O_9-O_{11}
C_2-C_3
Bond Length(Å)
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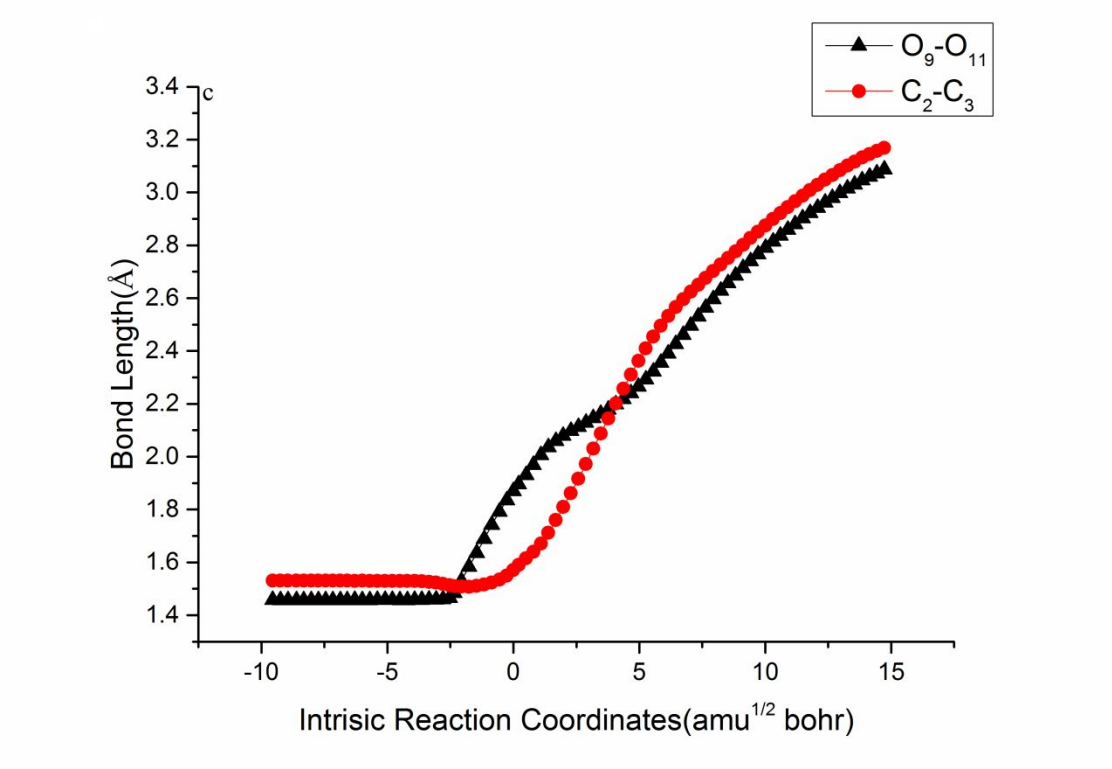
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
-10
-5
0
5
10
15
20
25
Intrisic Reaction Coordinates(amu$^{1/2}$ bohr)


c
O_9-O_{11}
C_2-C_3
Bond Length(Å)
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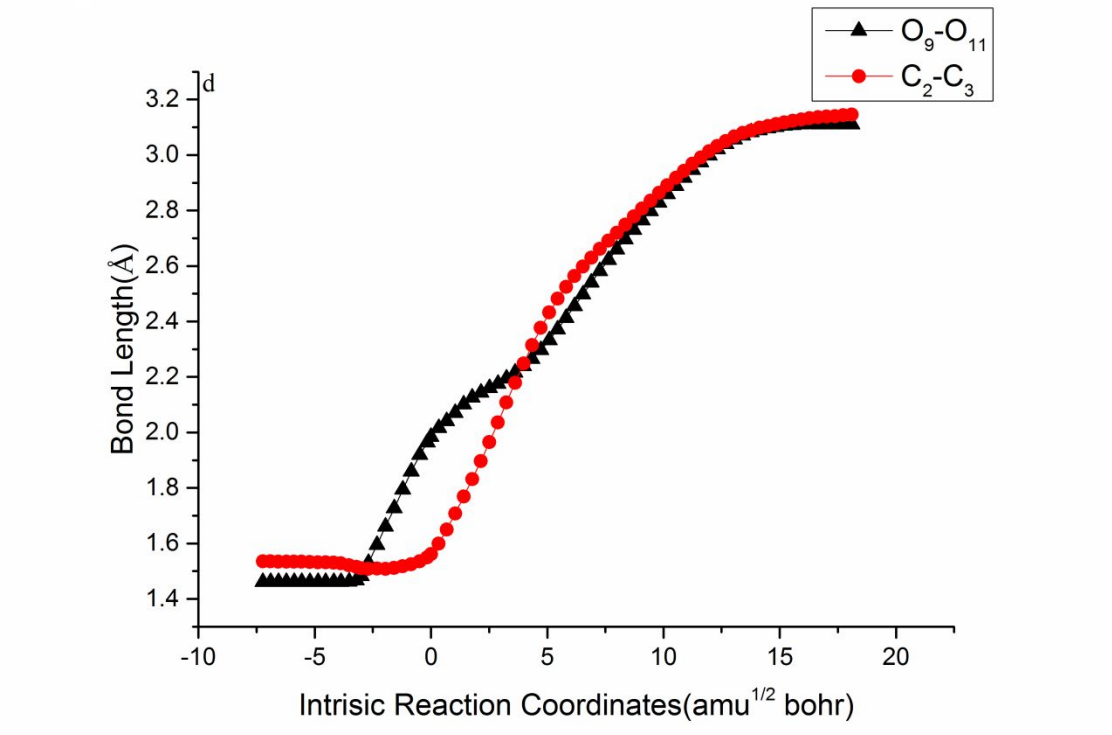
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
-10
-5
0
5
10
15
Intrisic Reaction Coordinates(amu$^{1/2}$ bohr)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O_9-O_{11}
C_2-C_3
Bond Length(Å)
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3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
-10
-5
0
5
10
15
20
Intrisic Reaction Coordinates(amu$^{1/2}$ bohr)

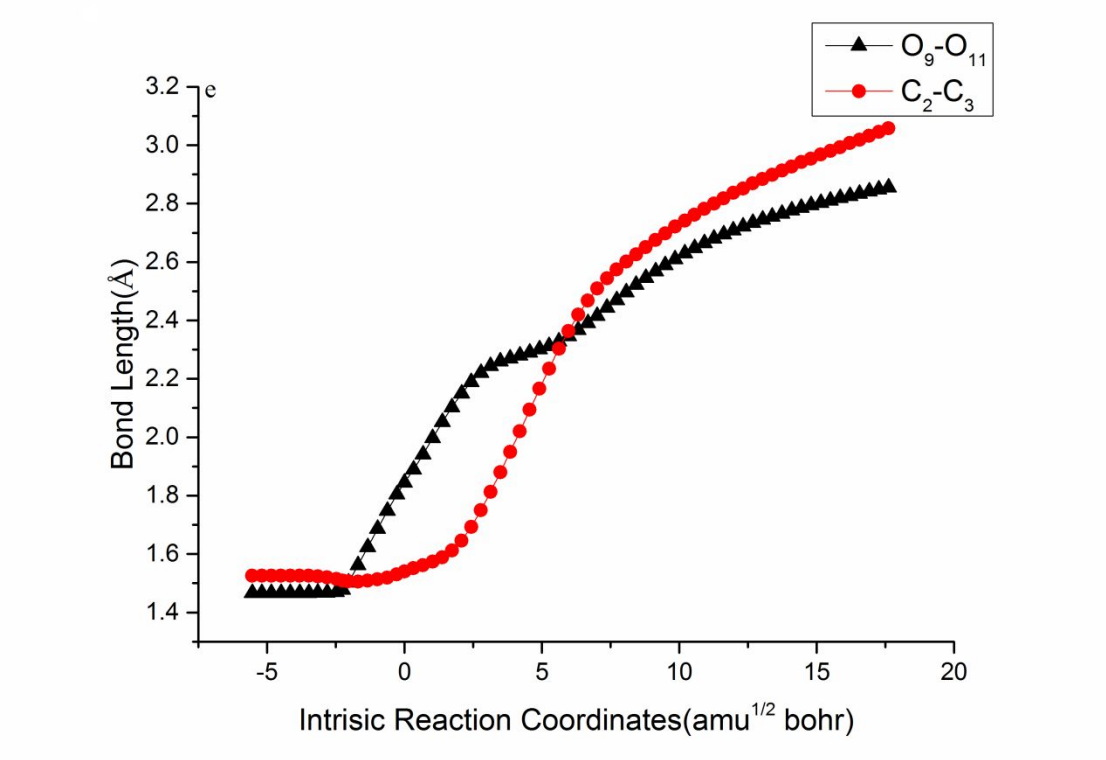


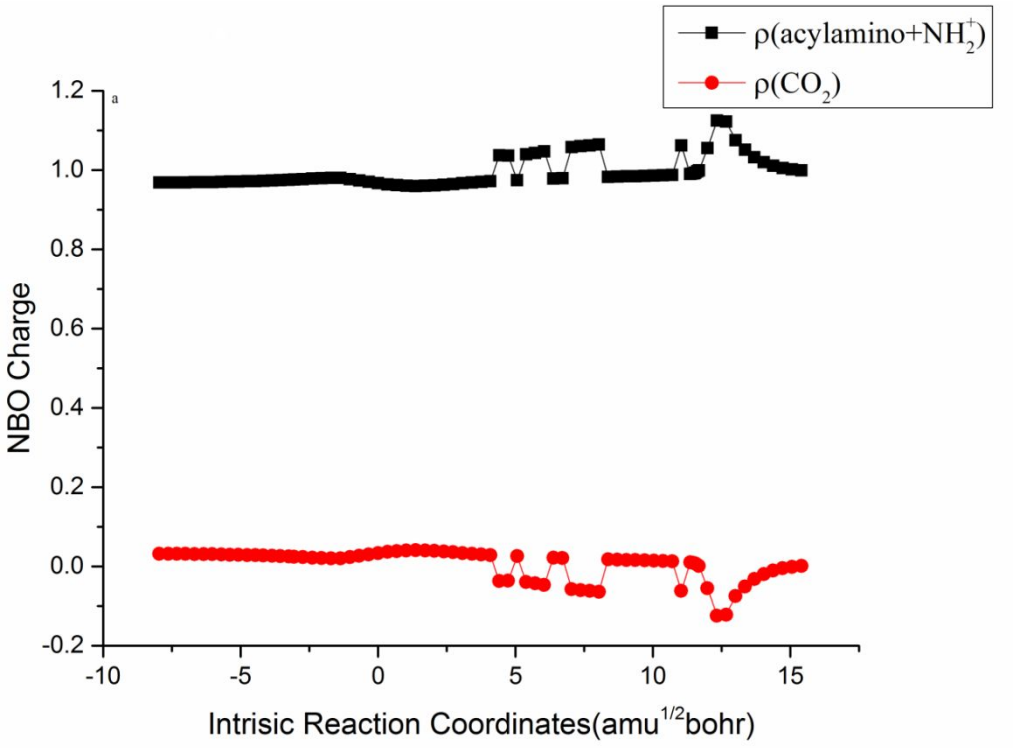
Figure S1. Variation of O_9-O_{11} and C_2-C_3 bond length (in Å) during the thermolysis reaction of C-DO acylamino+NH_2^+ (a), C-DO acylamino (b), C-DO amide+NH_2^+ (c), C-DO acylimino (d) and C-DO amide ion (e), as a function of intrinsic reaction coordinates. The calculations were made at the ωB97XD/6-31+G(d) level of theory.

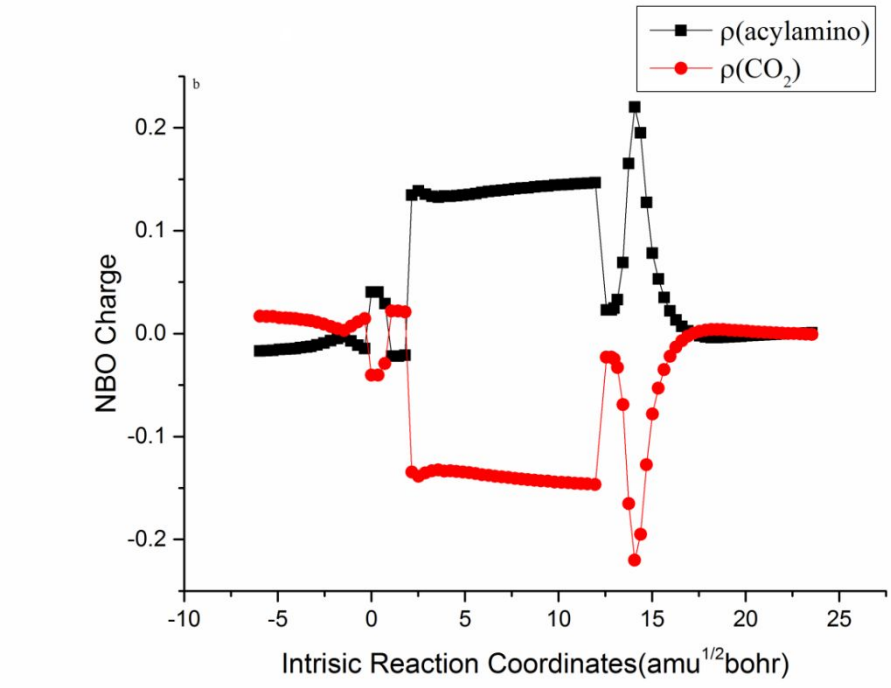
For C-DO acylamino+NH_2^+, the O_9-O_{11} bond is elongated by 0.561 Å from 1.462 to 2.023 Å in TS, while C_2-C_3 remains nearly unchanged (slightly increasing from 1.534 to 1.547 Å). The C_2-C_3 bond begins to break after TS, which indicates that the broken of the O_9-O_{11} and C_2-C_3 bonds is asynchronous.

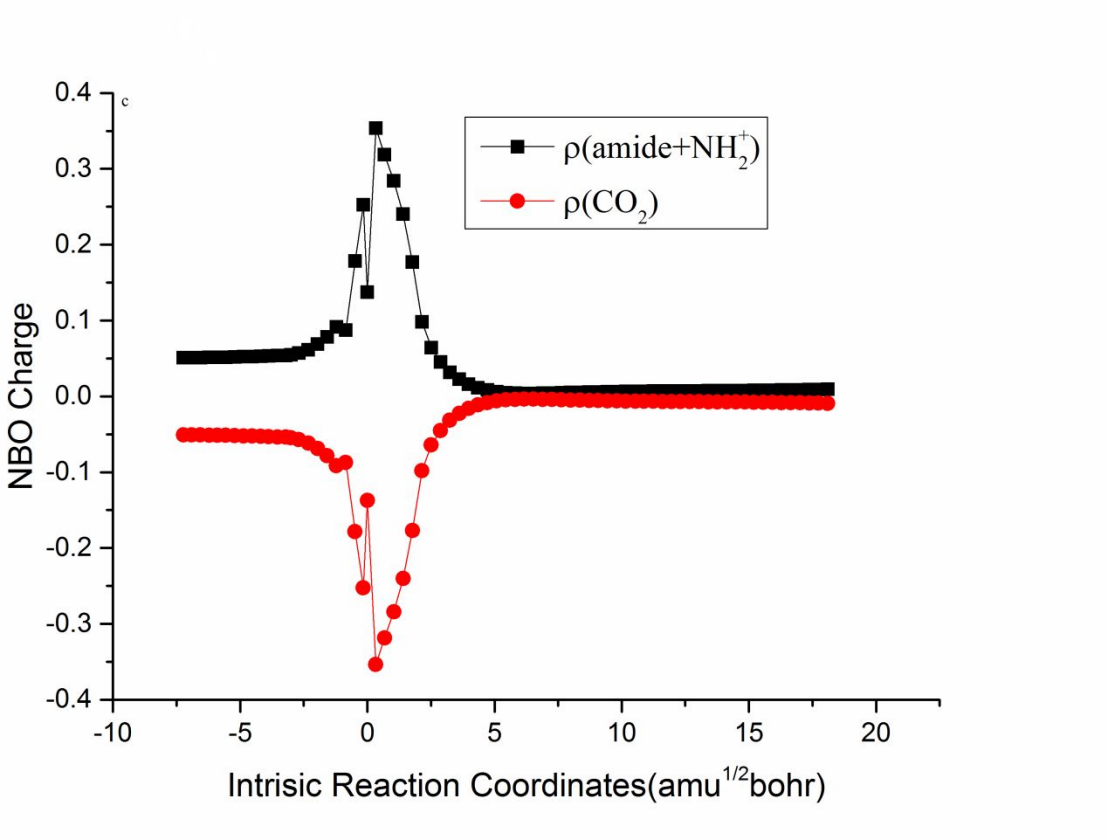
The decomposition of C-DO acylamino starts with dissociation of the O_9-O_{11} bond, and the following step is the dissociation of the C_2−C_3 bond. The bond length of O_9-O_{11} in TS_{O-O} (2.045 Å) is 0.585 Å longer than that in the R, but the C_2-C_3 bond lengths in the R and TS_{O-O} are 1.534 and 1.545 Å, respectively, it changes not much. The bond lengths of O_9−O_{11} are 2.735 and 2.709 Å in Int and TS_{C-C}, respectively. The bond lengths of C_2−C_3 are 1.540 and 1.549 Å in Int and TS_{C-C}, respectively. It indicates that the O_9−O_{11} bond is broken completely in Int and the C_2−C_3 bond gradually breaks after Int. So the dissociation of the O_9−O_{11} and C_2−C_3 bonds occur

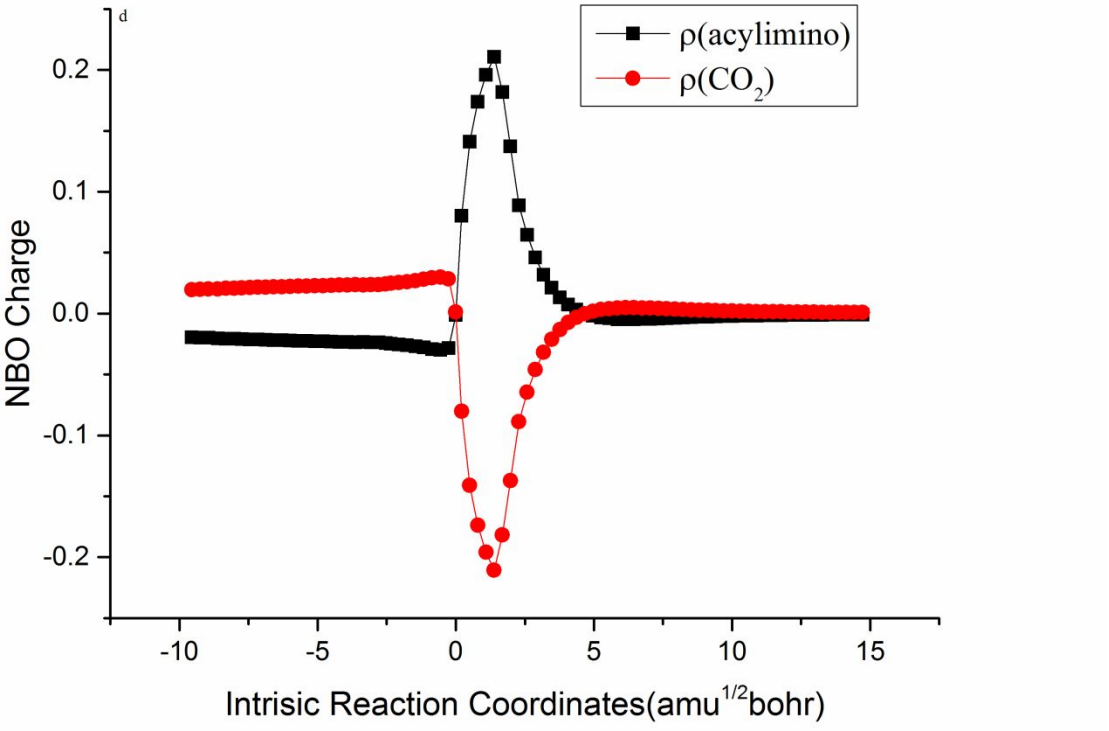
sequentially.

For C-DO amide-NH_2^+, C-DO acylimino and C-DO amide ion, The O_9-O_{11} bonds are elongated by 0.524, 0.411 and 0.378 Å, respectively, while C_2-C_3 remains nearly unchanged. The C_2-C_3 bond gets down breaking after TS, which illustrates that the broken of the O_9-O_{11} and C_2-C_3 bonds is asynchronous.






c
ρ(amide+NH2+)
ρ(CO2)
NBO Charge
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-10
-5
0
5
10
15
20
Intrisic Reaction Coordinates(amu1/2bohr)


d
ρ(acylimino)
ρ(CO2)
NBO Charge
0.2
0.1
0.0
-0.1
-0.2
-10
-5
0
5
10
15
Intrisic Reaction Coordinates(amu1/2bohr)

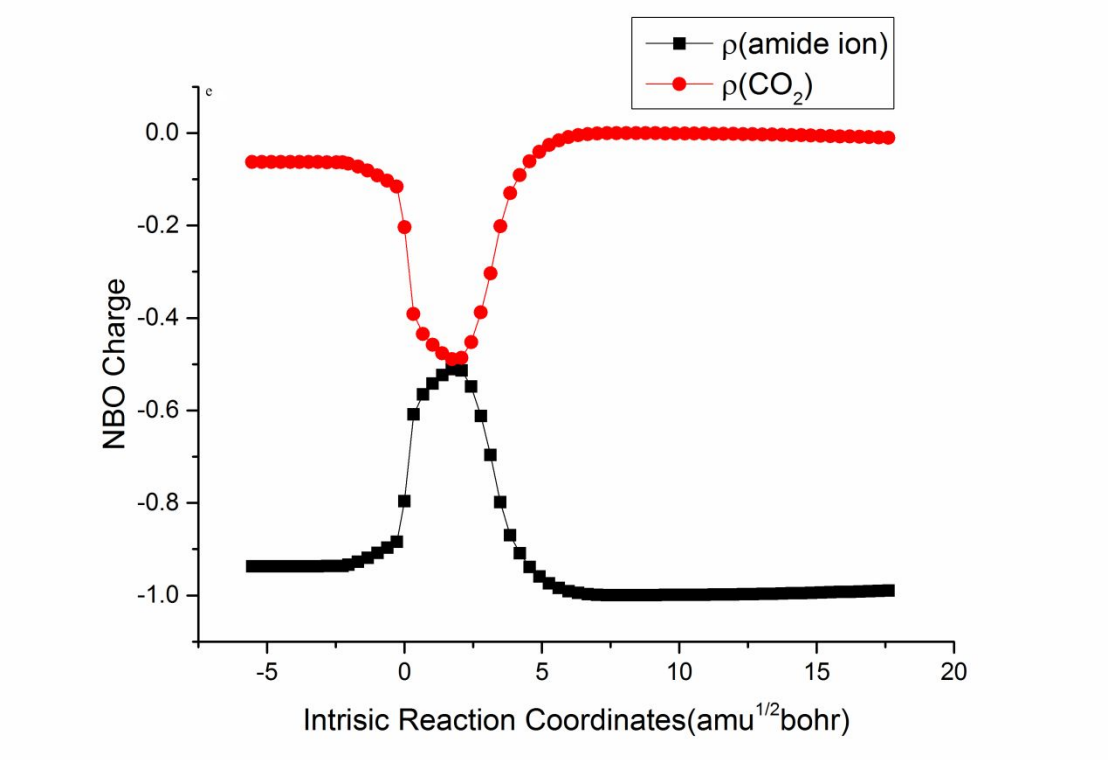


Figure S2. NBO charge of the CO_2 and oxyluciferin moieties of C-DO acylamino+NH_2^+ (a), C-DO acylamino (b), C-DO amide+NH_2^+ (c), C-DO acylimino (d) and C-DO amide ion (e).

Table S1 Computed ground states dissociation constants (pK) of all forms of C-DO in diethyl ether and water.

	diethyl ether	water		diethyl ether	water
pK_A	12.53	17.01	pK_B	20.33	25.87
pK_C	16.91	15.92	pK_D	2.98	-2.48
pK_E	6.40	7.47	pK_F	-4.81	-11.34
pK_G	29.22	21.57	pK_H	21.42	12.71
pK_I	24.84	22.65			

Table S2. Selected bond lengths (Å) and dihedral angles (deg) of the possible emitters in their fluorescent and chemiluminescent states[a]

	fluorescent state						chemiluminescent state					
	gas phase		diethyl ether		water		gas phase		diethyl ether		water	
	θ	$R(C_2\text{-}O_9)$	θ	$R(C_2\text{-}O_9)$	θ	$R(C_2\text{-}O_9)$	θ	$R(C_2\text{-}O_9)$	θ	$R(C_2\text{-}O_9)$	θ	$R(C_2\text{-}O_9)$
acylamino-NH_2^+	179	1.238	179	1.219	179	1.236	-127	1.383	-129	1.380	-132	1.376
acylamino	179	1.212	180	1.218	180	1.234	-127	1.389	-130	1.382	-134	1.380
acylimino	180	1.246	-180	1.250	179	1.263	-134	1.380	-138	1.376	-131	1.385
amide+NH_2^+	-174	1.230	-174	1.228	-177	1.251	-125	1.405	-127	1.397	-131	1.386
amide ion	175	1.226	175	1.233	176	1.260	-134	1.388	-131	1.385	-130	1.380

[a] The angle θ is the torsional angle $O_9\text{-}C_2\text{-}N_1\text{-}C_{10}$. The atom numbering is shown in Figure 3.

Table S3. Computed fluorescent (pK^{F*}) and chemiluminescent (pK^{C*}) states dissociation constants of all possible emitters in diethyl ether and water.

	diethyl ether	water		diethyl ether	water
pK_J^{F*}	11.33	16.69	pK_J^{C*}	12.30	17.46
pK_K^{F*}	9.54	17.50	pK_K^{C*}	10.00	14.78
pK_L^{F*}	12.38	18.53	pK_L^{C*}	15.30	15.73
pK_M^{F*}	-0.34	0.45	pK_M^{C*}	1.61	-3.13
pK_N^{F*}	-3.18	-0.59	pK_N^{C*}	-3.69	-4.07
pK_O^{F*}	1.45	-0.36	pK_O^{C*}	3.91	-0.45
pK_P^{F*}	24.74	18.34	pK_P^{C*}	20.04	12.34
pK_K^{F*}	26.52	17.53	pK_K^{C*}	22.34	15.02
pK_R^{F*}	23.68	16.50	pK_R^{C*}	17.04	14.08

$$\chi_n^{Acylamino} = \chi_n^{Acylamino-NH2+} \times \frac{k_A}{[H]^+} \quad \text{(S1)}$$

$$\chi_n^{Amide-NH2+} = \chi_n^{Acylamino-NH2+} \times \frac{k_A \times k_C \times k_D}{[H]^{+2}} \quad \text{(S2)}$$

$$\chi_n^{Acylimino} = \chi_n^{Acylamino-NH2+} \times \frac{k_A \times k_B \times k_C \times k_D \times k_E \times k_F}{[H]^{+3}} \quad \text{(S3)}$$

$$\chi_n^{Amide} = \chi_n^{Acylamino-NH2+} \times \frac{k_A \times k_B \times k_C \times k_D \times k_E \times k_F \times k_G \times k_H \times k_I}{[H]^{+6}} \quad \text{(S4)}$$

$$\chi_n^{Acylamino-NH2} = \frac{1}{1 + \frac{k_A}{[H]^+} + \frac{k_A \times k_C \times k_D}{[H]^{+2}} + \frac{k_A \times k_B \times k_C \times k_D \times k_E \times k_F}{[H]^{+3}} + \frac{k_A \times k_B \times k_C \times k_D \times k_E \times k_F \times k_G \times k_H \times k_I}{[H]^{+6}}} \quad \text{(S5)}$$

$$\chi_n^{Acylamino} = \chi_n^{Acylamino-NH2+} \times \frac{k_J}{[H]^+} \quad \text{(S6)}$$

$$\chi_n^{Amide-NH2+} = \chi_n^{Acylamino-NH2+} \times \frac{k_J \times k_L \times k_M}{[H]^{+2}} \quad \text{(S7)}$$

$$\chi_n^{Acylimino} = \chi_n^{Acylamino-NH2+} \times \frac{k_J \times k_K \times k_L \times k_M \times k_N \times k_O}{[H]^{+3}} \quad \text{(S8)}$$

$$\chi_n^{Amide} = \chi_n^{Acylamino-NH2+} \times \frac{k_J \times k_K \times k_L \times k_M \times k_N \times k_O \times k_P \times k_Q \times k_R}{[H]^{+6}} \quad \text{(S9)}$$

$$\chi_n^{Acylamino-NH2} = \frac{1}{1 + \frac{k_J}{[H]^+} + \frac{k_J \times k_L \times k_M}{[H]^{+2}} + \frac{k_J \times k_K \times k_L \times k_M \times k_N \times k_O}{[H]^{+3}} + \frac{k_J \times k_K \times k_L \times k_M \times k_N \times k_O \times k_P \times k_Q \times k_R}{[H]^{+6}}} \quad \text{(S10)}$$