# **Supplementary information**

# Additive- and Column-free Synthesis of Rigid Biscoumarins as Fluorescent Dyes Sensing for Gquadruplex via Disaggregation-Induced Emission

Hui-Zi He, Kun Li, Kang-Kang Yu, Pei-Long Lu, Mei-Lin Feng , Shan-Yong Chen\* and Xiao-Qi Yu\*s

Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, No. 29 Wangjiang Road, Chengdu, P. R. of China 610064. E-mail: kli@scu.edu.cn, xqyu@scu.edu.cn; Fax: +86 28 85415886

# Contents

1. Experimental section	2
1.1 General methods	2
1.2 the synthesis of compounds 1-4	2
1.3 Optimization of reaction conditions and the synthesis of RBC 1-4	2
1.4 General procedure for products RCB 1-4	3
2. Proposed mechanism	3
3. X-ray Crystallographic Studies of Compound RBC2	5
4. Theoretical Calculation of RBC2 Based on the Single Crystal	7
5. Photophysical properties of RBC1-4	7
5.1 Fluorescent properties	7
5.2 Molar extinction coefficient of RBC1-4	8
5.3 Fluorescnece emissions of RBC2 in mixed solvent of 1,4-dioxane and water	9
5.4 Research on interaction between different G4 and RBC2	9
6. Docking method	11
7. Job's plot analysis	12
8. <sup>1</sup> H-NMR, <sup>13</sup> C-NMR and ESI-MS spectra of RBC1-4	12

**1. Experimental section** 

## 1.1 General methods

Unless otherwise noted, all chemical reagents, solvents and materials were commercially available and were used without further purification. n-butanol was distilled for purification. All the solvents used in the optical spectroscopic studies were either HPLC or spectroscopic grade. <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra were measured on a Bruker AM400 NMR spectrometer. Proton Chemical shifts of NMR spectra were given in ppm relative to internals reference TMS (1H, 0.00 ppm). ESI-HRMS spectral data were recorded on a Finnigan LCQDECA mass spectrometer. Fluorescence emission spectra were obtained using Hitachi F-7000 spectrometer at 298 K. Absorption spectra were recorded on a Hitachi Pharma Spec UV-1900UV-Visible Spectrophotometer. For measurement of basic photophysical properties, the stock solution of **RBC1-4** (5 uM) were prepared in DCM. Besides, while the dye **RBC2** was used for sensing G4, the stock solution (1 uM) was prepared in THF. After the addition of fluorophores, the solution was stirred.

The molecular docking studies were performed using GOLD (Genetic Optimization of Ligand Docking) 5.0.<sup>1</sup> The X-ray crystal structure (PDB entry 143D) of the hybridparallel basket NMR G-quadruplex structure was used in the docking studies and necessary modifications were carried out according to a literature report.<sup>2, 3</sup> The Discovery Studio 3.1 (Accelrys, Inc. USA) software package was used to prepare the G4 structure including adding hydrogen atoms, removing water molecules, and assigning force field (here the CHARMm force field was adopted).

## 1.2 the synthesis of compounds 1-4

Compounds 1-4 were synthesized according to the literature.<sup>4</sup>

# 1.3 Optimization of reaction conditions and the synthesis of RBC 1-4

## **Table S1 optimization of reaction conditions**

	N	2			
entry	solvents	Equiv of H <sub>2</sub> O	Temperature	Yields (%)	
			(°C)		
1	EtOH	0	80	5.5	
2	1,4-dioxane	0	110	trace	
3	Acetic acid	0	130	trace	
4	1-Hexanol	0	160	trace	
5	n-BuOH	0	130	10.5	
6	n-BuOH	4	130	8	
7	n-BuOH	8	130	69.4	
8	n-BuOH	16	130	36.5	
Compound 2: 0.5 mmol; Solvent: 1.5 mL; Stirred for 12 h at the given temperatures					

This novel transformation was first observed by heating compound 2 in ethanol (Table 1, entry 1). After screening solvents, we found n-Butanol was more effective, affording the product in 10.5% yield. We think water might take part in the reaction. Fortunately, when 8 equipment of water (*vs* compound 2), the isolated yield was high up to 69.4% (entry 7 vs entries 4 and 6). Therefore, all the rigid bis-coumarins were prepared according to reaction conditions listed in entry 7.

## 1.4 General procedure for products RCB 1-4

To a schlenk reaction tube was added a formyl coumarin (0.5 mmol), deionized water (72 uL, 4 mmol) and n-butanol (1.5 mL). The mixture was stirred at 130°C for 12 h, and then cooled to room temperature and filtered. The resulting solid was washed with n-butanol and dried under vacuum to generate the raw product. After recrystallization from  $CH_2Cl_2/MeOH$ , the rigid bis-coumarins (RBC) were obtained with high purity.

**RBC1**: Yield: 65.1%, green solid; <sup>1</sup>H NMR (400 MHz, TFA)  $\delta$  8.32 (d, J = 8.1 Hz, 2H), 7.86 (s, 2H), 7.81 (d, J = 8.4 Hz, 2H), 3.67 (s, 2H), 3.50 (s, 12H); <sup>13</sup>C NMR (101 MHz, TFA)  $\delta$  165.63, 156.72, 154.77, 147.18, 127.60, 119.90, 117.38, 112.07, 106.05, 49.35, 19.60. ESI-MS: calcd. for C<sub>23</sub>H<sub>21</sub>N<sub>2</sub>O<sub>5</sub>Na [M+Na]<sup>+</sup> 427.1264, Found 427.1266.

**RBC2**: Yield: 69.8%, green solid; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.65 (d, J = 9.0 Hz, 2H), 6.65 (dd, J = 9.0, 2.4 Hz, 2H), 6.47 (d, J = 2.4 Hz, 2H), 3.42 (q, J = 7.1 Hz, 8H), 3.38 (s, 2H), 1.22 (t, J = 7.1 Hz, 12H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  162.6, 155.1, 155.0, 151.1, 123.0, 108.8, 101.7, 97.3, 95.6, 44.9, 18.0, 12.6; ESI-MS: calcd. for C<sub>27</sub>H<sub>29</sub>N<sub>2</sub>O<sub>5</sub> Na [M+Na]<sup>+</sup> 483.1890, Found 483.1881.

**RBC3**: Yield: 20%, yellow solid: <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.66 (d, J = 9.0 Hz, 2H), 6.61 (d, J = 8.9 Hz, 2H), 6.44 (d, J = 2.2 Hz, 2H), 3.38 (s, 2H), 3.36 – 3.28 (m, 8H), 1.66 – 1.54 (m, 12H), 1.44 – 1.31 (m, 8H), 0.98 (t, J = 7.3 Hz, 12H); <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$  162.7, 155.1, 151.5, 123.0, 108.9, 101.7, 97.5, 95.7, 51.2, 29.3, 20.4, 18.0, 14.1; ESI-MS: calcd. for C<sub>35</sub>H<sub>45</sub>N<sub>2</sub>O<sub>5</sub> Na[M+Na]<sup>+</sup> 595.3142, Found 595.3140.

**RBC4**: Yield: 45.1%, yellow solid : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.22 (s, 2H), 3.38 (s, 2H), 3.34 – 3.22 (m, 8H), 2.96 – 2.73 (m, 8H), 2.09 – 1.91 (m, 8H). <sup>13</sup>C NMR (151 MHz, CDCl<sub>3</sub>)  $\delta$  162.8, 155.2, 150.0, 146.1, 118.8, 118.4, 106.6, 101.5, 95.1, 62.8, 50.0, 49.6, 34.9, 27.9, 21.5, 20.5. ESI-MS: calcd. for C<sub>31</sub>H<sub>29</sub>N<sub>2</sub>O<sub>5</sub> [M+H]<sup>+</sup> 509.2071, Found 509.2076.

#### 2. Proposed mechanism

Although the mechanism for this novel transformation is unclear, a Cannizzaro reaction should be involved. Cannizzaro reaction is known to take place in strong alkaline medium, but is has also been claimed to occur in the presence of a Lewis acid<sup>5</sup>. While H<sup>18</sup><sub>2</sub>O was used to replace H<sub>2</sub>O, it's discovered that oxygen atom of about 30% of the product RBC2 was replaced by <sup>18</sup>O from mass spectrum (figure S1). Based on these results, we think that the weak acidity of glass in combination with high temperature is helpful for the disproportionation of a coumarin aldehyde to an

equimolar mixture of alcohol and carboxylic acid in our reaction conditions. A proposed mechanism was outlined in scheme S1:





Figure S1. ESI-HRMS spectrum of the product produced after adding  $H_2^{18}O$ 

# 3. X-ray Crystallographic Studies of Compound RBC2



Figure S2. The angle between the coumarin plane and linking ring



Figure S3. X-ray crystal structure of RBC2. Deposition number : 1968182

Identification code	RBC2	
Empirical formula	$C_{27}H_{28}N_2O_5$	
Formula weight	460.51	
Temperature/K	293.15	
Crystal system	monoclinic	
Space group	P2/c	
a/Å	5.1905(5)	
b/Å	9.3677(7)	
c/Å	23.713(3)	

Table S3. Crystal data and structure refinement for RBC2

α/°	90
β/°	94.729(12)
$\gamma/^{\circ}$	90
Volume/Å <sup>3</sup>	1149.0(2)
Z	2
$\rho_{calc}g/cm^3$	1.331
µ/mm⁻¹	0.092
F(000)	488.0
Crystal size/mm <sup>3</sup>	$0.35 \times 0.3 \times 0.25$
Radiation	MoKa ( $\lambda = 0.71073$ )
2@ range for data collection/°	6.758 to 52.736
Index ranges	$\textbf{-6} \leq h \leq 6,  \textbf{-11} \leq k \leq 11,  \textbf{-29} \leq \textbf{l} \leq \textbf{14}$
Reflections collected	2976
Independent reflections	2976 [ $R_{int} = ?, R_{sigma} = 0.0459$ ]
Data/restraints/parameters	2976/0/158
Goodness-of-fit on F <sup>2</sup>	1.049
Final R indexes [I>= $2\sigma$ (I)]	$R_1 = 0.0630, wR_2 = 0.1520$
Final R indexes [all data]	$R_1 = 0.1006, wR_2 = 0.1644$
Largest diff. peak/hole / e Å-3	0.46/-0.25

Table 51. Dona lenguis of 1002						
Atom Atom		Length/Å	Atom Atom		Length/Å	
01	C1	1.378(4)	C3	C4	1.424(4)	
01	C9	1.378(3)	C4	C5	1.398(4)	
O2	C1	1.206(4)	C4	C9	1.395(4)	
03	C3	1.381(3)	C5	C6	1.356(4)	
O3	C31	1.381(3)	C6	C7	1.420(4)	
N1	C7	1.362(4)	C7	C8	1.395(4)	
N1	C11	1.523(5)	C8	C9	1.368(4)	
N1	C13	1.452(4)	C10	C21	1.506(4)	
C1	C2	1.439(5)	C11	C12	1.396(6)	
C2	C3	1.344(4)	C13	C14	1.503(5)	
C2	C10	1.506(4)				

 Table S4. Bond lengths of RBC2

# Table S5. Bond angles of RBC2

Table 55. Dond angles of RDC2							
Atom	Atom	Atom	Angle/°	Atom	Atom	Atom	Angle/°
C9	01	C1	121.7(3)	C9	C4	C3	116.9(3)
C3 <sup>1</sup>	03	C3	117.4(3)	C9	C4	C5	116.6(3)
C7	N1	C11	121.2(3)	C6	C5	C4	121.9(3)
C7	N1	C13	121.9(3)	C5	C6	C7	121.1(3)
C13	N1	C11	116.5(3)	N1	C7	C6	120.6(3)
				-			

_								
	01	C1	C2	118.9(3)	N1	C7	C8	122.2(3)
	O2	C1	01	116.1(3)	C8	C7	C6	117.2(3)
	O2	C1	C2	125.0(3)	C9	C8	C7	120.5(3)
	C1	C2	C10	118.1(3)	01	C9	C4	120.7(3)
	C3	C2	C1	118.4(3)	C8	C9	01	116.7(3)
	C3	C2	C10	123.4(3)	C8	C9	C4	122.6(3)
	O3	C3	C4	113.3(3)	$C2^1$	C10	C2	109.1(4)
	C2	C3	03	123.3(3)	C12	C11	N1	110.8(5)
	C2	C3	C4	123.4(3)	N1	C13	C14	114.0(3)
	C5	C4	C3	126.5(3)				

#### 4. Theoretical Calculation of RBC2 Based on the Single Crystal



Figure S4. Molecularorbital amplitude plots of the HOMO and LUMO levels of RBC2.

To understand the photophysical properties of **RBC2**, density functional theory (DFT) calculations were performed. The groundstate geometries of BIN-COE and BIN-COP were optimized using DFT with B3LYP hybrid functions at the basis-set level of 6-31G\* by using the single-crystal structure determined by X-ray analysis. The calculation results showed that the majority of the electron distribution of the HOMO located on the amino moiety. As expected, **RBC2**, having symmetric molecular structures, showed similar electron distributions in the LUMO. The electron distributions were primarily located on the two lactone rings. As predicted by DFT, the DE values was were 3.75 eV, indicating a green emission. These data suggest an electron transfer within **RBC2**.

## 5. Photophysical properties of RBC1-4

#### 5.1 Fluorescent properties



Figure S5. Fluorescent spectra of RBC1 in different organic solvents



Figure S6. Fluorescent spectra of RBC2 in different organic solvents



Figure S7. Fluorescent spectra of RBC3 in different organic solvents



Figure S8. Fluorescent spectra of RBC4 in different organic solvents

# 5.2 Molar extinction coefficient of RBC1-4



Figure S9. UV spectra and absorption-concentration curve of RBC1 in DCM  $(\lambda abs = 366 \text{ nm})$ 



Figure S10. UV spectra and absorption-concentration curve of RBC2 in DCM  $(\lambda abs = 371 \text{ nm})$ 



Figure S11. UV spectra and absorption-concentration curve of RBC3 in DCM  $(\lambda abs = 372 \text{ nm})$ 



Figure S12. UV spectra and absorption-concentration curve of RBC4 in DCM  $(\lambda abs = 385 \text{ nm})$ 

# 5.3 Fluorescent emissions of RBC2 in mixed solvent of 1,4-dioxane and water



Figure S13. emissions of RBC2 in mixed solvent of 1,4-dioxane and water

## 5.4 Research on interaction between different G4 and RBC2

## Table S6 DNA samples used in this study

Name	Sequence	Structure in K <sup>+</sup> solution
22AG	5'-d(AGGGTTAGGGTTAGGGTTAGGG)	Hybrid-Type G4
G4TTA	5'-d(TTAGGGTTAGGGTTAGGGTTAGGG)-3'	Hybrid-Type G4
G3T3	5'-d(GGGTTTGGGTTTGGGTTTGGG)-3'	Hybrid-Type G4
Ckit*	5'-d(GGCGAGGAGGGGGGGGGGGCGTGGCCGGC)-3'	Hybrid-Type G4
Ckit	5'-d(AGGGAGGGCGCTGGGAGGAGGG)-3'	Parallel G4
CM22	5'-d(TGAGGGTGGGTAGGGTGGGTAA)-3'	Parallel G4
HRAS	5'-d(TCGGGTTGCGGGCGCAGGGCACGGGCG)-3'	Antiparallel G4
S15a	CGC GCG TTT CGC GCG	Single-Strand
S15b	CGC GCG AAA CGC GCG	Single-Strand
ds15	s15a/s15b	Double-Strand
ds26	5'-d(CAATCGGATCGAATTCGATCCGATTG)-3'	Double-Strand



**Figure S14**. Fluorescence titration of 1µM **RBC2** with stepwise addition of the Gquadruplex-forming oligonucleotides (22AG, G4TTA, G3T3, Ckit\*, CM22, Ckit and HRAS), single-stranded (s15a, s26a) and double-stranded DNA(ds15 and ds26) in 10mM Tris-HCl buffer, 50mM KCl, pH 7.4.  $\lambda_{ex}$ =394 nm.



**Figure S15.** CD spectra of 4µM G-quadruplex-forming oligonucleotides 22AG, G4TTA, G3T3, Ckit\*, CM22, Ckit and HRAS in 10mM Tris-HCl buffer, 50mM KCl, pH 7.4, with 1 µM **RBC2.** 

## 6. Docking method

The molecular docking studies were performed using GOLD (Genetic Optimization of Ligand Do cking) 5.0.[1] The X-ray crystal structure (PDB entry 143D) of the anti-parallel basket NMR Gquadruplex structure was used in the docking studies. Prepare ligands module in Discovery Studio 3.1 (Accelrys, Inc. USA) software package was adopted. When prepare for Docking DNA and lig and, necessary modifications were carried out including: (1) "Change ionization" was set as "false "; (2) "generate tautomers" was set as "false"; (3) "generate isomers" was set as "true"; (4) "fix bad valencies" was set as "true"; (5) "generate coordinates" was set as "3D"; (6) The Discovery Studio 3.1 (Accelrys, Inc. USA) software package was used to prepare the protein structure including ad ding hydrogen atoms to the protein, removing water molecules, and assigning force field (here the CHARMm force field was adopted); (7) The necessary modifications of crystal structure (PDB ent ry 143D) were carried out according to a literature report.[2,3] (8) Define and edit binding site mo dule was used and active site was set with site size of 10.10 Å; (9) For GOLD, the "predefined generic algorithm (GA)" setting was set as "automatic", scoring function was set as GoldS core, and others were set to default.

#### REFERENCES

- 1. Jones G, Willett P, Glen R C, et al. Development and validation of a genetic algorithm for flexible docking. Journal of molecular biology, 1997, 267(3): 727-748.
- Agrawal, Saurabh, Rajendra Prasad Ojha, and Souvik Maiti. "Energetics of the human Tel
   -22 quadruplex- telomestatin interaction: a molecular dynamics study." The Journal of
   Physical Chemistry B 112.22 (2008): 6828-6836.

3 Shi, Shuo, et al. "Molecular "light switch" for G-

quadruplex DNA: cycling the switch on and off." Dalton Transactions41.19 (2012): 5789-5793."

7. Job's plot analysis



**Figure S16**. Job's plot of **RBC2** and various G4s including 22AG, G4TTA, G3T3, Ckit\*, CM22. The total concentration of **RBC2** and G4 were kept at 1  $\mu$ M in Tris-HCl buffer (10mM, pH = 7.4).

8. <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and ESI-MS spectra of RBC1-4



Figure S17. <sup>1</sup>H NMR (400 MHz, CF<sub>3</sub>COOD) and <sup>13</sup>C NMR (100 MHz, CFCOOD) and ESI-MS spectraum of RBC1.





Figure S18. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) and ESI-MS spectraum of RBC2.





Figure S19. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) and ESI-MS spectraum of RBC3.





Figure S20. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) and ESI-MS spectraum of RBC4.

Reference

- 1. G. Jones, P. Willett and R. C. Glen, J. Mol. Biol., 1997, 267(3), 727-748.
- 2. A. Saurabh, R. P. Ojha and S. Maiti, J. Phy. Chem. B, 2008, 112, 6828-6836.
- 3. S. Shi, J. Zhao, X. Gao, C. Lv, L. Yang, J. Hao, H. Huang, J. Yao, W. Sun, T. Yao and L. Ji, *Dalton Trans.* 2012, **41** (19), 5789–5793.
- 4. P. Zhang, W. Liu, G. Niu, M. Wang, J. Ge, H. Zhang, Y. Li and P. J. Wang, Org.Chem., 2017,82, 3456-3462.
- (a) K. M. A. Santos, E. M. Albuquerque, L. E. P. Borges and M. A. Fraga, *Mol. Catal.*, 2018, 45,198-205. (b) Y. K. K. Nakajima and M. K. M. Hara, *Chem. Lett.* 2013, 42, 873-875.