# Electronic Supplementary Information For

## An Acid Catalyzed Reversible Ring-closure Reaction of Cyano-Rhodamine Spirolactam

Honglin Li,[a] Hong Guan,[a] Xinrui Duan,[a] Jun Hu,[a] Guiren Wang[b] and Qian Wang*[,a]

[a]*Department of Chemistry and Biochemistry,* [b]*Department of Mechanical Engineering & Biomedical Engineering Program, University of South Carolina, 631 Sumter Street, Columbia, SC 29208 (USA)*

*Corresponding authors E-mail: wang263@mailbox.sc.edu*

## Table of Contents:

## Materials and general methods:

Chemicals used in the syntheses were purchased from Aldrich without further purification. $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a Varian Mercury/VX 300 spectrometer and chemical shifts are reported as ppm (in $CDCl_3$ or with $CD_3OD$ and DCl mixture, TMS as internal standard). Mass spectra were recorded using Micromass Q-TOF I mass spectrometer. IR data were recorded with a Shimadzu FTIR Spectrophotometer. Fluorescence emission and excitation spectra were recorded using a Varian Cary Eclipse Fluorescence Spectrophotometer. All pH measurements were made on an Accumet Basic AB15 pH meter. X-ray intensity data were collected using a Bruker SMART APEX diffractometer. Cell images were obtained by Olympus X81 fluorescence microscopy.

### pH titration experiment

A 5 mM stock solution of **RBCN** in $CH_3CN$ was prepared and then diluted in $H_2O$ to give the 10 μM of **RBCN** sample. The **RBCN** sample was stirred at room temperature and pH was adjusted by 0.1 M, 2.0 M and 10.0 M of $HCl_{(aq)}$ and $NaOH_{(aq)}$, then absorption and fluorescence signals were recorded. For the reversible pH-dependent fluorescence changes experiment, the pH of **RBCN** sample was first adjusted to ~10 and then to ~2 and then adjusted between ~10 and ~2 for several cycles. Fluorescence signals were recorded in different cycle, respectively. 2.0 M and 10.0 M of $HCl_{(aq)}$ and $NaOH_{(aq)}$ were adopted in this assay to avoid changing the volume so much (totally ~2 % for 10 cycles).

### Cell imaging

SW620 cells were cultured in RPMI1640(VWR) supplemented with 10% FCS (Thermoscientific). One day before imaging, cells were seeded into 12-well flat bottomed plates. After 24 h, the cells were co-stained with 5 μM **RBCN** and trackers (15 nM for LysoTracker Green or 500 nM for Mitotracker Green) for 30 min at 37 °C under 5% $CO_2$ and washed with phosphate-buffered saline (PBS) three times. After replacement of medium, cells were imaged using Olympus X81 fluorescence microscopy.

### Synthesis of RBCN

In a 50 mL flask, rhodamine B (100.0 mg, 0.21 mmol) was dissolved in 25 mL ethanol. The red solution was heated to reflux under stirring, then cyanamide (50.0 mg, 1.2 mmol) was added. The resultant reaction mixture was refluxed for 72 h. TLC showed around 50% of the starting material was converted to product (**RBCN**) with much weaker polarity. After removal of ethanol under vacuum, the residue was purified by flash chromatography with $CH_2Cl_2$/EtOAc = 4/1 as eluent to give the light pink powder **RBCN** (53.5 mg, yield: 54.7%). $^{1}$H-NMR (400 MHz, $CDCl_3$), $\delta_H$ (ppm): 7.98 (d, 1H, $J$ = 7.4 Hz, $C_6H_4$), 7.65 (t, 1H, $J$ = 7.4 Hz, $C_6H_4$), 7.55 (t, 1H, $J$ = 7.4 Hz, $C_6H_4$), 7.16 (d, 1H, $J$ = 7.4 Hz, $C_6H_4$), 6.53 (d, 2H, $J$ = 8.8 Hz, Xanthene-H), 6.41 (s, 2H, Xanthene-H), 6.33 (d, 2H, $J$ = 8.8 Hz, Xanthene-H), 3.33 (q, 8H, $J$ = 6.8 Hz, $CH_2$), 1.17 (t, 12H, $CH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$), $\delta_C$ (ppm): 166.6489, 153.3394, 152.5031, 149.4970, 135.5330, 129.3200, 128.4062, 126.6485, 124.8635, 124.2857, 108.4320, 106.9820, 103.1700, 97.7815, 69.2468, 44.4259, 12.5743; IR (KBr): 2235 (s), -CN; HRMS *m/e* calculated for $(M+H)^+$ $C_{29}H_{30}N_4O_2$ 467.2447; found 467.2449.

## Crystallography

X-ray intensity data from a colorless plate like crystal were collected at 100(2) K using a Bruker SMART APEX diffractometer (Mo Ka radiation, l = 0.71073 Å).[1] The raw area detector data frames were reduced with the SAINT+ program.[1] Final unit cell parameters were determined by least-squares refinement of 4087 reflections from the data set. Direct methods structure solution, difference Fourier calculations and full-matrix least-squares refinement against $F^2$ were performed with SHELXS/L[2] as implemented in OLEX2.[3]

The compound crystallizes in the triclinic crystal system. The space group P-1 (No. 2) was assumed and confirmed by the successful solution and refinement of the structure. The asymmetric unit consists of two crystallographically independent molecules and a methanol molecule of crystallization with a population of 50%. The methanol is disordered about an inversion center, and thus only half is present per asymmetric unit. It was further modeled as occupying two disordered sites with the asymmetric unit with occupancies O1S-C1S/O2S-C2S = 0.263(7)/0.237(7). These occupancies were constrained to sum to 0.5. The disordered methanol atoms were refined with C-O distances restrained to 1.45(2) Å, and were assigned a common isotropic displacement parameter. All other non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in geometrically idealized positions and included as riding atoms. Hydrogen atoms were not located or calculated for the disordered methanol atoms.

| | |
|---|---|
| Identification code | **RBCN** |
| Empirical formula | C29.25H31N4O2.25 |
| Formula weight | 474.58 |
| Temperature/K | 100(2) |
| Crystal system | triclinic |
| Space group | P-1 |
| a/Å | 11.2094(16) |
| b/Å | 14.479(2) |
| c/Å | 16.455(2) |
| α/° | 80.674(3) |
| β/° | 86.095(3) |
| γ/° | 75.836(3) |
| Volume/Å3 | 2554.1(6) |
| Z | 4 |
| ρcalcmg/mm3 | 1.234 |
| m/mm-1 | 0.079 |
| F(000) | 1010.0 |
| Crystal size/mm3 | 0.44 × 0.32 × 0.1 |
| 2Θ range for data collection | 2.5 to 50.06° |
| Index ranges | -13 ≤ h ≤ 13, -17 ≤ k ≤ 17, -19 ≤ l ≤ 19 |
| Reflections collected | 28263 |
| Independent reflections | 9012[R(int) = 0.0653] |
| Data/restraints/parameters | 9012/2/653 |
| Goodness-of-fit on F2 | 0.855 |
| Final R indexes [I>=2σ (I)] | R1 = 0.0459, wR2 = 0.0744 |
| Final R indexes [all data] | R1 = 0.0847, wR2 = 0.0843 |
| Largest diff. peak/hole / e Å-3 | 0.26/-0.19 |

***Table S1***. X-ray crystallographic data for **RBCN**.

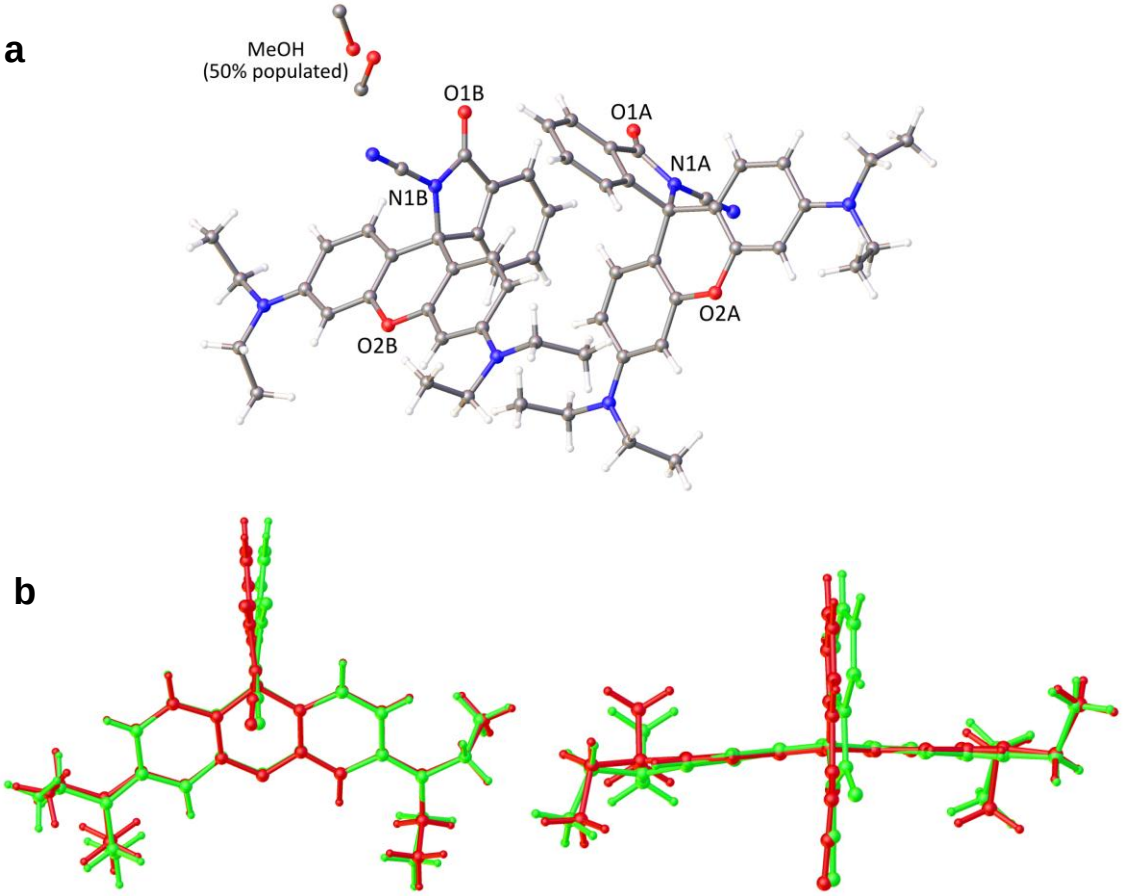


***Figure S1.*** (a) Components of the crystal structure. The asymmetric unit contains two crystallographically independent, chemically identical molecules and a disordered methanol molecule of crystallization. (b) The two independent molecules overlaid. Green = molecule with label suffix "A"; red = "B".

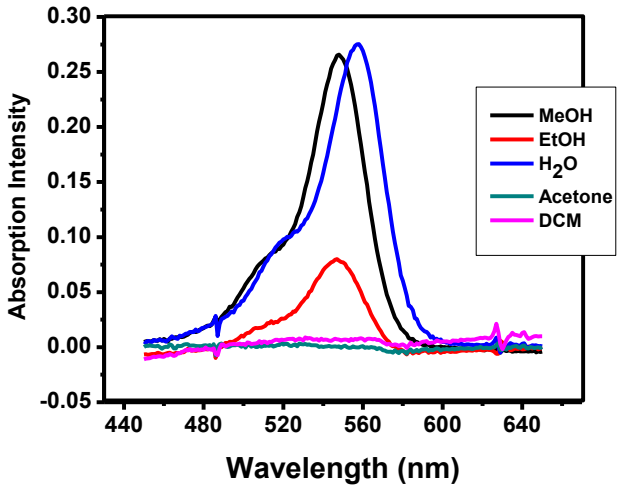

***Figure S2.*** Abso[illegible] different solvents.

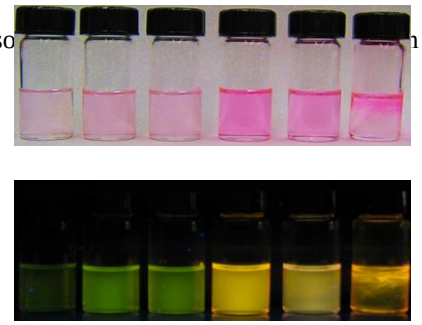


***Figure S3.*** Photos of **RBCN** in other protic solvents under sunlight (top) and UV light (bottom). From left to right: 1 = isopropanol alcohol, 2 = isobutyl alcohol, 3 = 1-butabol, 4 = ethylene glycol, 5 = diethylene glycol, 6 = glycerin.

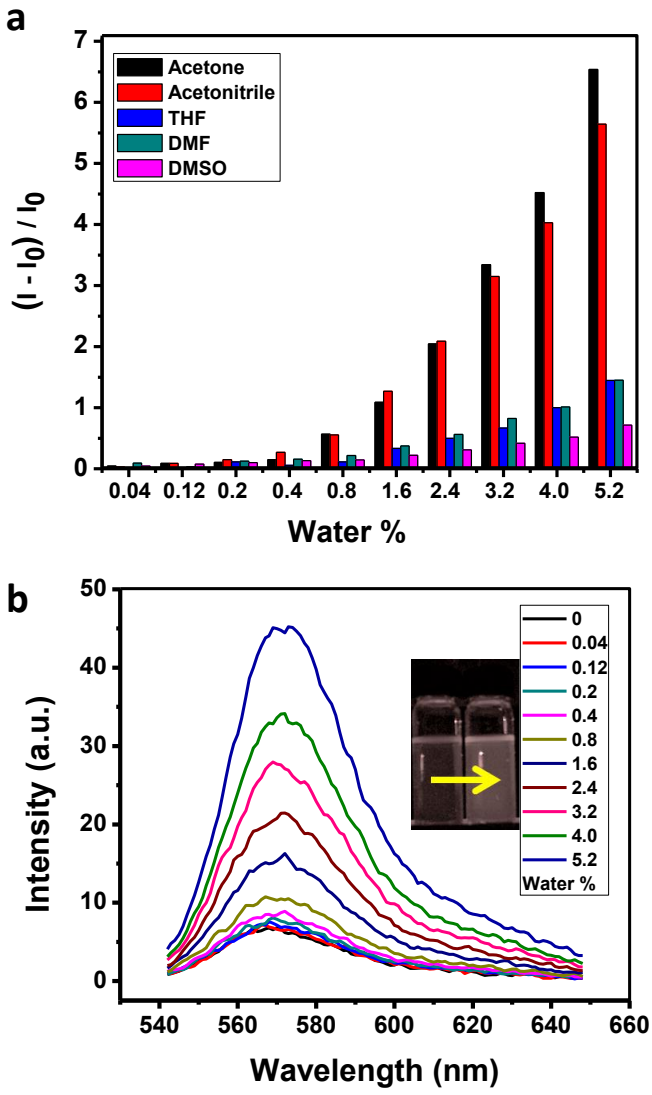


***Figure S4.*** (a) Fluorescence intensity changes of **RBCN** (at 570 nm) in acetone, acetonitrile, THF, DMF and DMSO with different portions (v/v) of water. (b) Fluorescence spectrum changes of **RBCN** in acetonitrile with different portions of water. Inset shows the photo of **RBCN** before and after addition of

5.2 % water under UV-light. 5 μM **RBCN** for each sample and the excitation was performed at 530 nm.

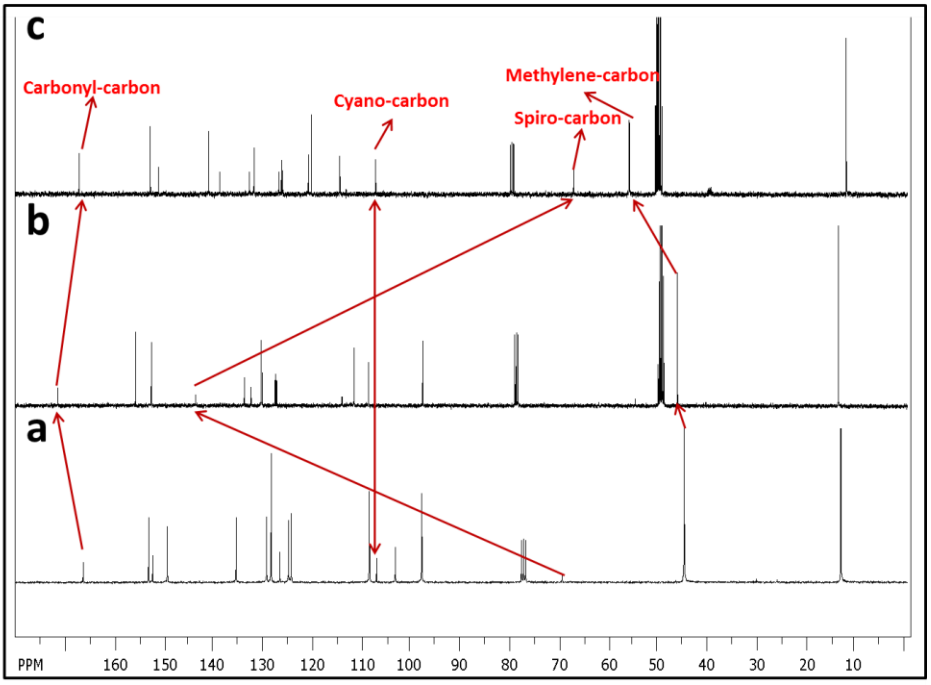


***Figure S5*** $^{13}$C-NMR spectrums of **RBCN** in (a) $CDCl_3$, (b) $CDCl_3$ (40%) + $CD_3OD$ (60%) and (c) $CDCl_3$ (33.3%) + $CD_3OD$ (50%) + DCl (16.7%).

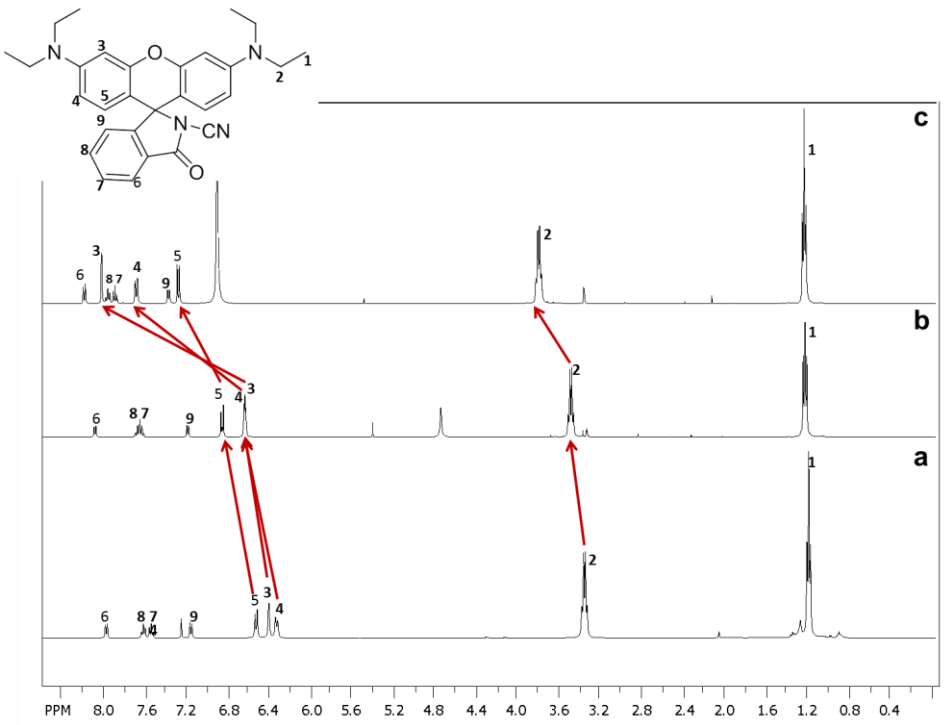


***Figure S6***. $^{1}$H-NMR spectrums of **RBCN** in (a) $CDCl_3$, (b) $CDCl_3$ (40%) + $CD_3OD$ (60%) and (c) $CDCl_3$

(33.3%) + $CD_3OD$ (50%) + DCl (16.7%).

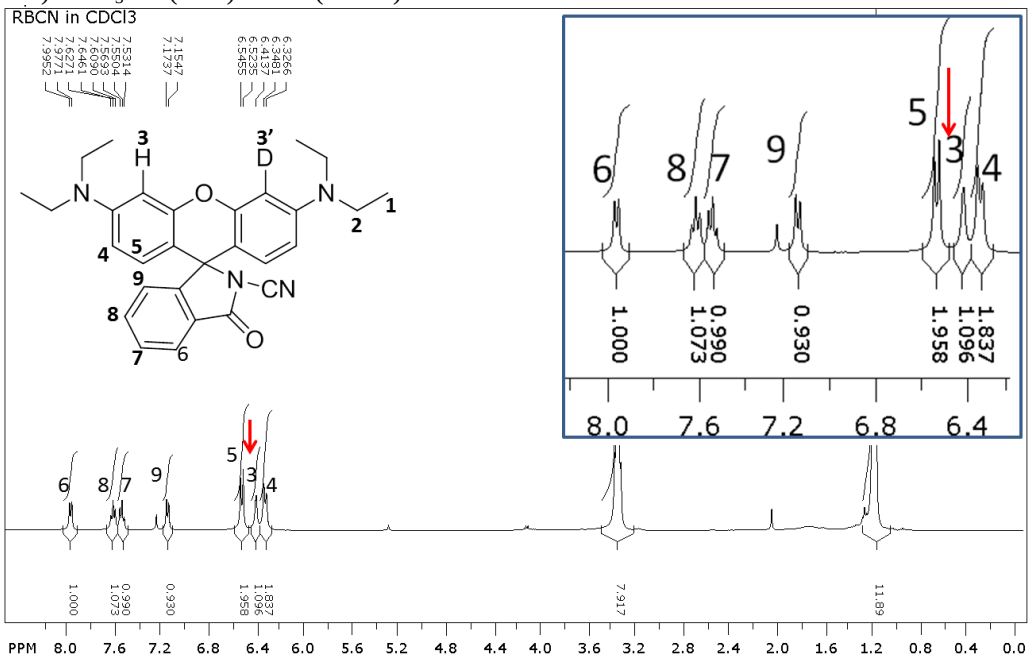


***Figure S7***. $^1$H-NMR spectrum of **RBCN** after treatment of DCl (3days later).

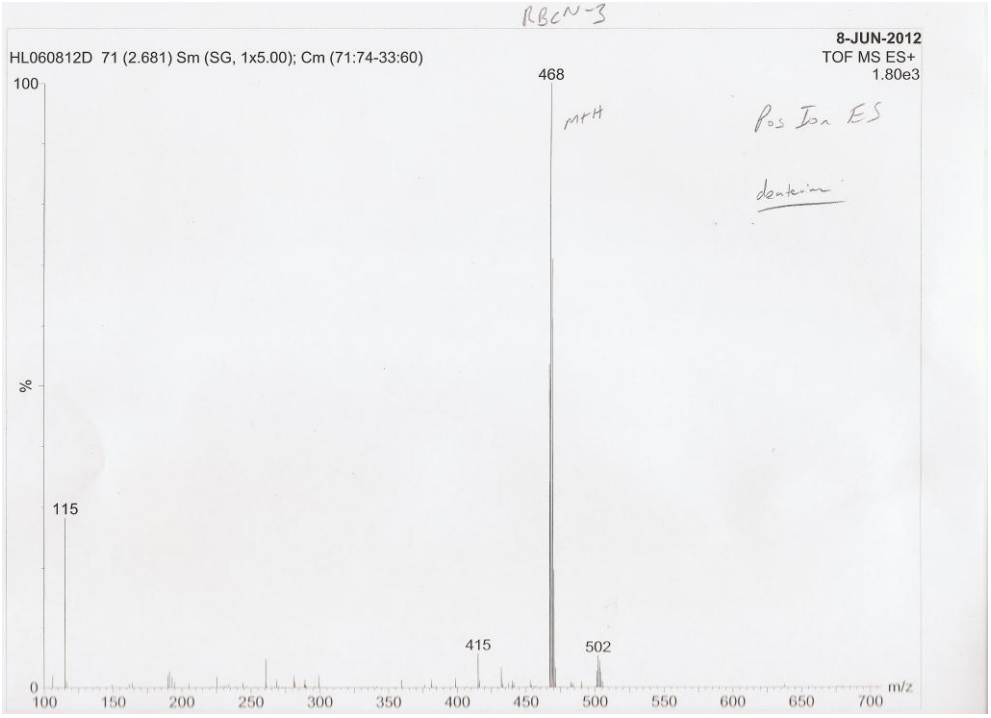


***Figure S8***. MS spectrum of **RBCN** after treatment of DCl (3days later).

After treatment by DCl for 3 days, 3'-H was deuterated (Figure S7) that was also supported by MS result (Figure S8).

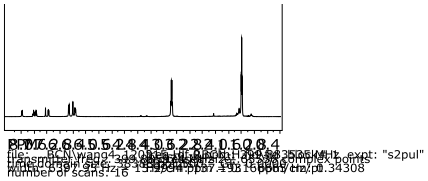

***Figure S9.*** $^{1}$H-NMR of **RBCN** (in $CDCl_3$).

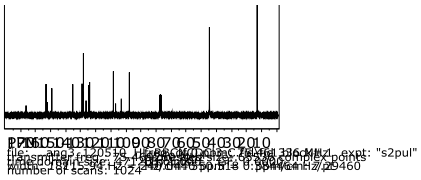

***Figure S10.*** $^{13}$C-NMR of **RBCN** (in $CDCl_3$).

**References:**

(1) SMART Version 5.630, SAINT+ Version 6.45. Bruker Analytical X-ray Systems, Inc., Madison, Wisconsin, USA, **2003**.
(2) Sheldrick, G.M. *Acta Cryst*. **2008**, A64, 112-122.
(3) Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard J. A. K.; Puschmann, H. *J. Appl. Cryst.* **2009**, *42*, 339-341.