

Electronic Supplementary Information (ESI)

Assessing guest-accessible volume in MOF by two-photon fluorescence microscopy

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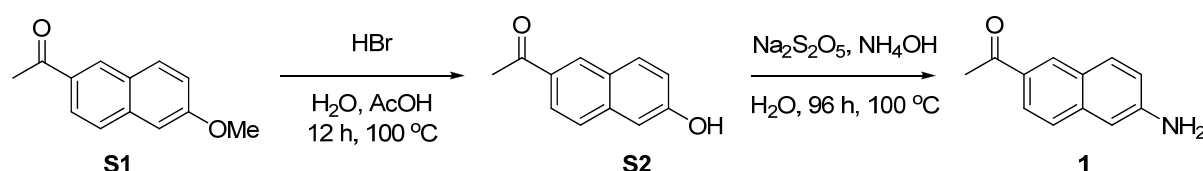
1. Synthesis of Dyes

1.1. General consideration for the synthesis

Unless otherwise noted, all materials were obtained from commercial suppliers Aldrich and TCI, and used without further purification. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone ketyl prior to use. Toluene was distilled from calcium hydride and stored over 4Å molecular sieve. Evaporation of organic solvents was conducted using a Büchi rotary evaporator with a desktop vacuum pump. Thin layer chromatography plates (Silica gel 60 F254, Merck) were visualized by ultraviolet light and/or treatment with acidic *p*-anisaldehyde stain followed by gentle heating. Chromatographic purification of products were accomplished by flash chromatography by using Merck silica gel 60(230~400mesh) with a mixture of hexane and ethyl acetate as eluent. ¹³C- NMR spectra were recorded on Varian Gemini 100 MHz spectrometer with complete proton decoupling. Chemical shifts are reported in ppm relative to the methyl peak of TMS. High performance liquid chromatography (HPLC) was conducted using a Waters model 600 pumping system with a Waters model 2487 ultraviolet detector at 254nm. Infrared (IR) spectra were recorded on a Bomem 102 FT-IR spectrometer. Optical rotations were measured at the 589nm sodium D-Line with RUDOLPH AUTOPOL automatic polarimeter. Low- and High-resolution FAB mass spectra were obtained by JEOL JMS-AX505WA mass spectrometer. Melting points were measured with capillary melting point apparatus of Thomas Hoover and are uncorrected.

1.2. Preparation of dyes

The synthesis was carried out according to the Scheme S1.



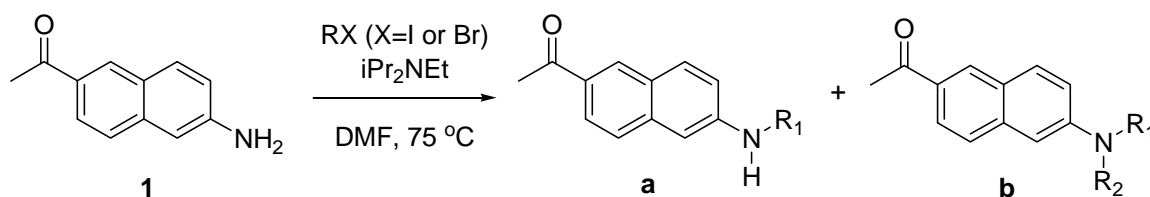
Scheme S1

1-(6-Hydroxynaphthalen-2-yl)ethanone (S2)

This compound was synthesized according to the previously reported method.¹

1-(6-Aminonaphthalen-2-yl)ethanone (1)

This compound was synthesized according to the previously reported method.²



General Procedure I.

To a solution of **1** (100 mg, 0.54 mmol) in DMF (2 mL) was added DIEA (0.2 mL, 1.08 mmol) and R₁X (1.08 mmol), and the mixture was gently heated with stirring at 75 °C for 9 - 13 hours. After cooling the reaction mixture

¹ H. M. Kim, C. Jung, B. R. Kim, S.-Y. Jung, J. H. Hong, Y.-G. Ko, K. J. Lee B. R. Cho, *Angew. Chem. Int. Ed.* 2007, **46**(19), 3460.

² H. M. Kim, B. R. Kim, M. J. An, J. H. Hong, K. J. Lee and B. R. Cho, *Chemistry An European Journal*, 2008, **14**(7), 2075.

to room temperature, the reaction mixture was diluted with ethyl acetate (10 mL). The organic layer was washed with brine (3 × 100 mL) and dried over anhydrous MgSO₄. After filtration, the filtrate was concentrated under vacuum. The crude product was purified by flash chromatography (*n*-Hexane/EA, 10:1 v/v) to give compounds **2-5**.

1-(6-(methylamino)naphthalen-2-yl)ethanone (2a)

This compound was synthesized according to the previously reported method.²

1-(6-(dimethylamino)naphthalen-2-yl)ethanone (2b)

Yellow solid, 45% yield. ¹H NMR (300 MHz, CDCl₃) δ 2.67 (s, 3H), 3.11 (s, 6H), 6.87 (s, 1H), 7.17 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.80 (d, *J* = 9 Hz, 1H), 7.91 (d, *J* = 9.4 Hz, 1H), 8.32 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 26.7, 40.7, 105.5, 116.5, 124.8, 125.2, 126.4, 130.6, 130.91, 130.93, 137.9, 150.4, 198.01; IR (KBr): 3233, 2925, 2886, 2823, 1667, 1623 cm⁻¹; HRMS (m/z):

[M+H]⁺ calcd for C₁₃H₁₃NO (M⁺): 214.1187 Found: 214.1230.

1-(6-(2-methylallylamino)naphthalen-2-yl)ethanone (3a)

Brown solid, 42% yield. ¹H NMR (300 MHz, CDCl₃) δ 1.83 (s, 3H), 2.67 (s, 3H), 3.82 (s, 2H), 4.94 (s, 1H), 5.01 (s, 1H), 6.77 (s, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.72 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 8.29 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 15.9, 21.9, 44.9, 99.4, 106.8, 113.9, 120.1, 121.4, 121.5, 125.9, 126.2, 126.3, 133.5, 137.3, 143.8, 193.4 IR (KBr): 3393, 3064, 2918, 1774, 1660, 1618 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₁₆H₁₇NO (M⁺): 239.1310 Found: 239.1308.

1-(6-(bis(2-methylallyl)amino)naphthalen-2-yl)ethanone (3b)

Yellow oil, 45% yield. ¹H NMR (300 MHz, CDCl₃) δ 1.79 (s, 6H), 2.66 (s, 3H), 3.94 (s, 4H), 4.79 (s, 2H), 4.89 (s, 2H), 6.81 (s, 1H), 7.02 (d, *J* = 6.6 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 1H), 8.29 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 15.6, 21.9, 51.8, 100.4, 105.9, 111.6, 119.9, 120.41, 121.6, 125.8, 126.0, 126.1, 133.1, 135.3, 144.4, 193.3; IR (KBr): 3077, 1970, 2915, 2852, 1670, 1620 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₂₀H₂₃NO (M⁺): 293.1780 Found: 293.1779.

1-(6-(Benzylamino)naphthalen-2-yl)ethanone (4a)

Brown solid, 36% yield. ¹H NMR (300 MHz, CDCl₃) δ 2.67 (s, 3H), 4.47 (s, 2H), 6.82 (s, 1H), 6.97 (d, *J* = 2.2 Hz, 1H), 7.31 - 7.40 (m, 5H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 5.8 Hz, 1H), 7.91 (d, *J* = 8.84 Hz, 1H), 8.31 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 26.7, 48.2, 104.2, 118.7, 124.9, 126.2, 126.3, 127.8, 129.02, 130.6, 131.1, 131.2, 138.2, 138.7, 148.2, 198.1; IR (KBr): 3370, 2919, 2848, 1657, 1616 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₇NO (M⁺): 275.1310 Found: 275.1310.

1-(6-(dibenzylamino)naphthalen-2-yl)ethanone (4b)

Yellow oil, 44% yield. ¹H NMR (300 MHz, CDCl₃) δ 2.66 (s, 3H), 4.80 (s, 4H), 6.97 (s, 1H), 7.17 (d, *J* = 9.1 Hz, 1H), 7.27 - 7.38 (m, 10H), 7.52 (d, *J* = 8.8 Hz, 1H), 7.76 (d, *J* = 9.0 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 1H), 8.31 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 21.9, 49.6, 101.1, 111.7, 120.12, 120.83, 121.77, 122.05, 122.72, 124.4, 125.8, 126.4, 126.6, 133.2, 144.7, 193.3; IR (KBr): 3063, 3025, 2922, 2849, 2244, 1943, 1735, 1669, 1612 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₂₆H₂₃NO (M⁺): 365.1780 Found: 365.1781

1-(6-(Naphthalen-2-ylmethylamino)naphthalen-2-yl)ethanone (5a)

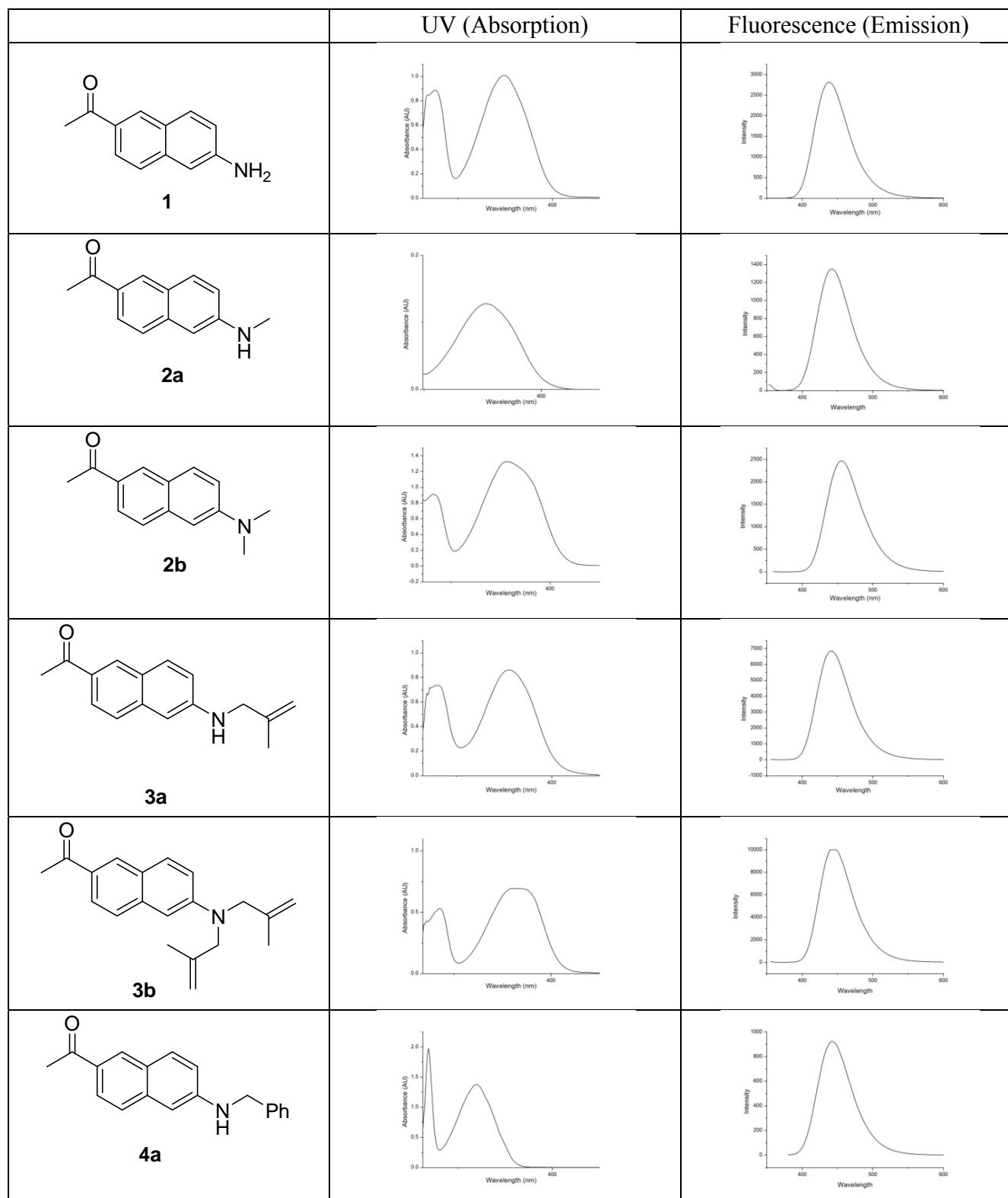
Yellow solid, 45% yield. ¹H NMR (300 MHz, CDCl₃) δ 2.66 (s, 3H), 4.62 (s, 2H), 6.84 (s, 1H), 6.98 (d, *J* = 8.6 Hz, 1H), 7.48 - 7.57 (m, 4H), 7.72 - 7.90 (m, 6H), 8.30 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 26.7, 48.4, 104.4, 118.7, 125.0, 125.8, 126.2, 126.3, 126.4, 126.6, 127.9, 128.0, 128.8, 130.6, 131.1, 131.2, 133.0, 133.7, 136.1, 138.2, 148.2, 198.0; IR (KBr): 3357, 3047, 2922, 2848, 1667, 1628 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₂₃H₁₉NO (M⁺): 325.1467 Found: 325.1465.

1-(6-(bis(naphthalen-2-ylmethyl)amino)naphthalen-2-yl)ethanone (5b)

Yellow solid, 40% yield. ¹H NMR (300 MHz, CDCl₃) δ 2.66 (s, 3H), 5.02 (s, 4H), 7.08 (s, 1H), 7.28 - 7.55 (m, 8H), 7.72 (m, 10H), 8.32 (s, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 26.7, 54.6, 105.9, 116.6, 124.9, 125.1, 125.2, 125.7, 126.1, 126.5, 126.6, 127.9, 128.0, 129.0, 130.5, 131.2, 131.4, 133.0, 133.7, 135.3, 137.9, 149.5, 198.0; IR (KBr): 3051, 2922, 2849, 2247, 1669, 1619 cm⁻¹; HRMS (m/z): [M+H]⁺ calcd for C₃₄H₂₇NO (M⁺): 465.2126 Found: 466.2169.

2. Absorption and Emission Spectra

Absorption spectra of DMF solutions were obtained using a UV-vis spectrometer (HP 8453, PDA type) in the wavelength range of 190–1100 nm. PL spectra were recorded with a Hitachi F-7000 FL spectrophotometer.



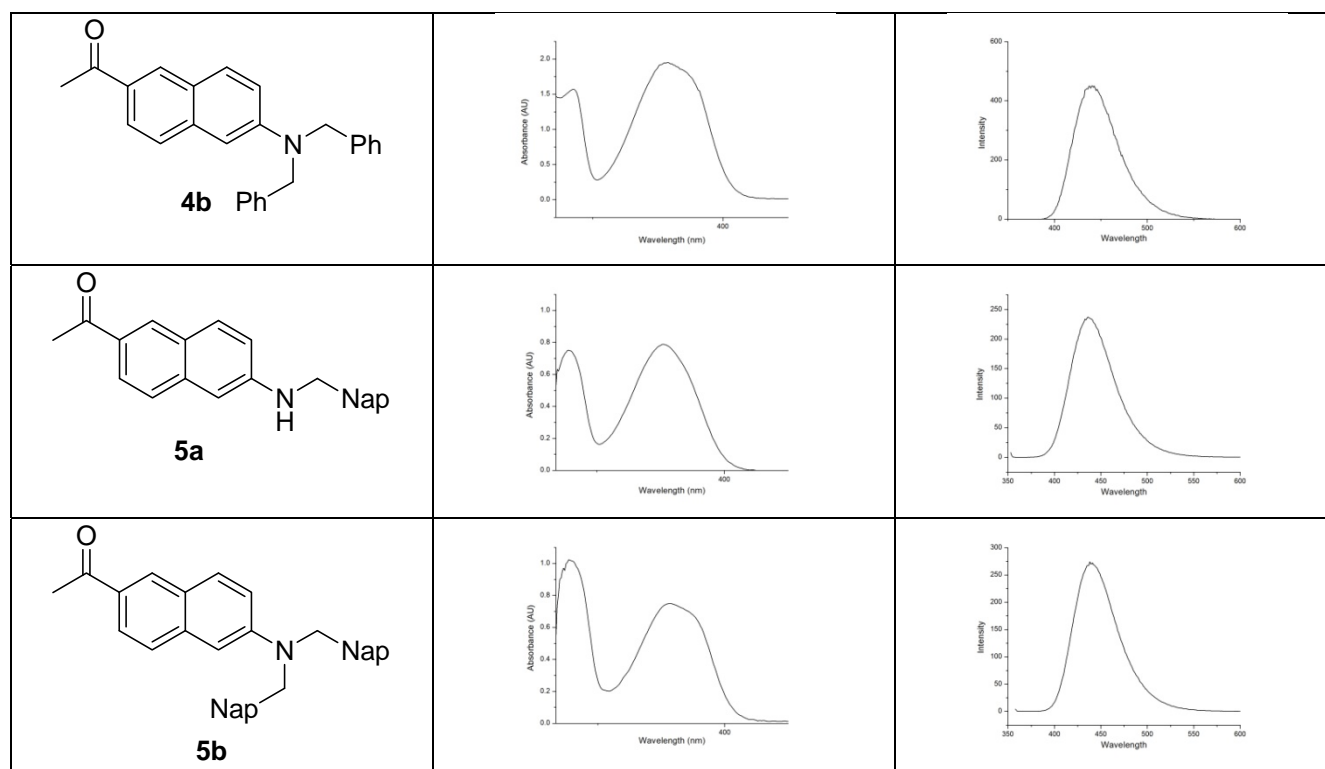


Table S1.

3. Preparation and characterization of (*S*)-KUMOF-2

3.1. Preparation of (*S*)-KUMOF-2

3.1.1 Synthesis of (*S*)-KUMOF-2

A reaction mixture in a small vial (10 mL) was prepared by dissolving of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (28.5 mg, 0.09 mmol) and (*S*)-2,2'-dihydroxy-6,6'-dimethylbiphenyl-4,4'-dicarboxylic acid ((*S*)-**KUBP**, 10 mg, 0.033 mmol) in DMF 5 mL. The tightly-capped vial was placed in an oven at 85 °C for 1 day. Colorless cubic crystals were obtained in 35% yield based on the used ligand. The crystals were separated and rinsed with DMF 5mL three times.

3.1.2 Formula determination of (*S*)-**KUMOF-2**: Based on the X-ray crystal structure, the framework of (*S*)-**KUMOF-2** has been formulated as $\text{Zn}_4\text{O}[(\text{S})\text{-KUBP}]_3$ with $Z = 1$ in the unit cell. As the free guest species could not be determined by the current X-ray data, the identification of the included molecules were identified by elemental analyses of the evacuated crystals and thermogravimetric analysis (TGA) as shown in the following information. Based on the accumulated results, the formula used was calculated to $\text{Zn}_4\text{O}[(\text{S})\text{-KUBP}]_3$.

3.1.3 Elemental analysis of the framework of (*S*)-**KUMOF-2**: As the free guest molecules were evaporated before the conduction of the elemental analysis. Element Analysis: (%) calcd. for (*S*)-**KUMOF-2**, $\text{Zn}_4\text{O}[(\text{S})\text{-KUBP}]_3(\text{DMF})_4(\text{H}_2\text{O})_5 = \text{C}_{60}\text{H}_{76}\text{O}_{29}\text{Zn}_4$: C, 47.32; H, 5.03; found: C, 47.57; H, 5.24.

3.1.4 IR (KBr) of (*S*)-**KUMOF-2**: 3410 (br), 2925 (w), 1655 (s), 1559 (s), 1414 (s), 1296 (w), 1248 (w), 1165 (w), 1103 (m), 1041 (m), 1000(m), 882 (w), 793 (m), 772 (m), 662(w).

3.1.5 Thermogravimetric Analysis (TGA) of (*S*)-**KUMOF-2**: Thermogravimetric analyses were carried out at a ramp rate of 10 °C/min in a N₂ flow using a Scinco TGA N-1000 instrument. Sample (~14.14 mg) was loaded and the temperature was increased by 10°C/min from 25°C to 700°C. The first weight loss (– 63.4 %) is due to the evaporation of DMFs. The second step (–36.6 %) is for the decomposition of the (*S*)-**KUMOF-2** and the residue is attributed to ZnO.

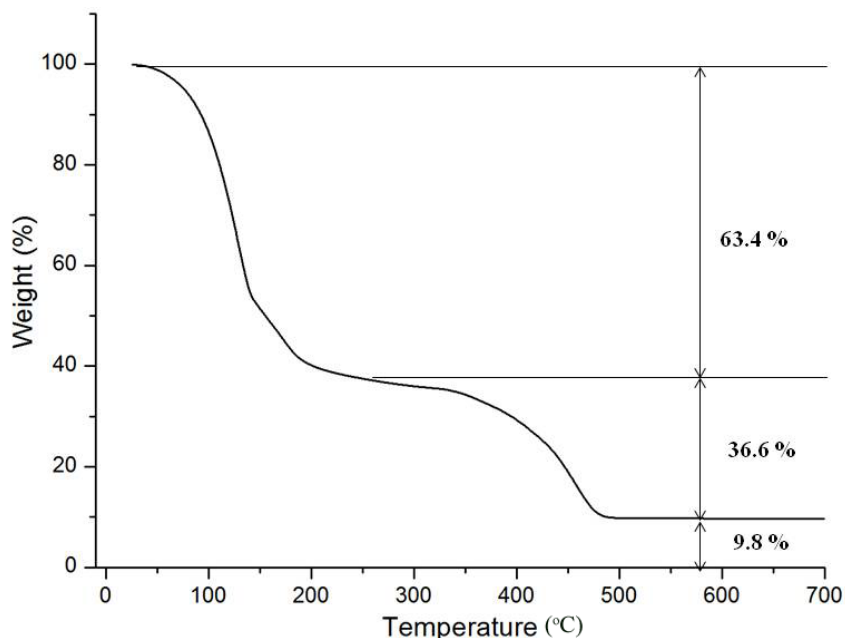


Figure S1. TGA thermogram of the (*S*)-**KUMOF-2** as-prepared.

3.2 Structure determination of (*S*)-**KUMOF-2**

3.2.1 Single Crystal Structure Determination

The diffraction data set from a colorless block crystal sealed in a capillary was collected at 296 K on a Bruker APEX CCD diffractometer with CuK α radiation ($\lambda = 1.54178 \text{ \AA}$). Bruker SMART program^{S1} was used for data collection, and SAINT^{S2} was used for cell refinement, and reduction. Absorption correction was applied using SADABS^{S3}. Crystal structure was solved using SHELX-TL software package^{S4} with a chiral cubic space group, *P*-43*m* (No. 211). An initial structure was obtained by direct methods using XS, and improved by subsequent refinements using XL. Both methyl and hydroxyl groups were disordered over two sites and almost overlapped with each other. Due to this disorder, it was not possible to define a right configuration of the organic ligand, and the resulting structure looked centrosymmetric. Therefore, the Flack's *x* parameter was meaningless in this structure. In fact, the crystal structure could be also solved with a centrosymmetric space group *P*-43*m* because its "centrosymmetric" structural feature was essentially identical with the disordered model obtained from a chiral space group *P*23. Non-hydrogen atoms were refined anisotropically. An hydrogen atom bonded to C3 was placed in calculated positions and refined by applying a riding model. Hydrogen atoms for methyl and hydroxy groups were not included due to their disorder. Without including solvent molecules, the refinement converged to R1 = 0.0261 for 16062 reflections of $I > 2\sigma(I)$ and 0.0291 for all 836 data. The large volume fraction of disordered solvents was calculated by PALTON SOLV CALC^{S5} to 23971 \AA^3 which corresponds to 87.1% of the unit cell volume. PALTON SQUEEZE^{S5} routine calculated that 7298 electrons per unit cell were attributed to the disordered solvents. With a modified reflection data excluding the solvent contribution, the final refinement process converged to R1 = 0.0613, wR2 = 0.1891 ($I > 2\sigma(I)$).

Table S2. Crystal data and structure refinement for (*S*)-**KUMOF-2**

Empirical formula	$C_{48}H_{36}O_{19}Zn_4$	
Formula weight	1178.25	
Temperature	296(2) K	
Wavelength	1.54178 Å	
Crystal system	Cubic	
Space group	<i>P</i> -43 <i>m</i>	
Unit cell dimensions	$a = 17.1607(1)$ Å	$\alpha = 90^\circ$.
	$b = 17.1607(1)$ Å	$\beta = 90^\circ$.
	$c = 17.1607(1)$ Å	$\gamma = 90^\circ$.
Volume	5053.65(5) Å ³	
Z	1	
Density (calculated)	0.387 Mg/m ³	
Absorption coefficient	0.698 mm ⁻¹	
F(000)	596	
Crystal size	0.10 x 0.10 x 0.10 mm ³	
Theta range for data collection	2.57 to 45.14°.	
Index ranges	-15 ≤ h ≤ 15, -15 ≤ k ≤ 15, -15 ≤ l ≤ 14	
Reflections collected	16062	
Independent reflections	836 [R(int) = 0.0979]	
Completeness to theta = 45.14°	99.8 %	
Refinement method	Full-matrix least-squares on F ²	
Data / restraints / parameters	836 / 0 / 47	
Goodness-of-fit on F ²	0.927	
Final R indices [I > 2σ(I)]	R1 = 0.0261, wR2 = 0.0595	
R indices (all data)	R1 = 0.0291, wR2 = 0.0603	
Extinction coefficient	0.00006(3)	
Largest diff. peak and hole	0.121 and -0.177 e.Å ⁻³	

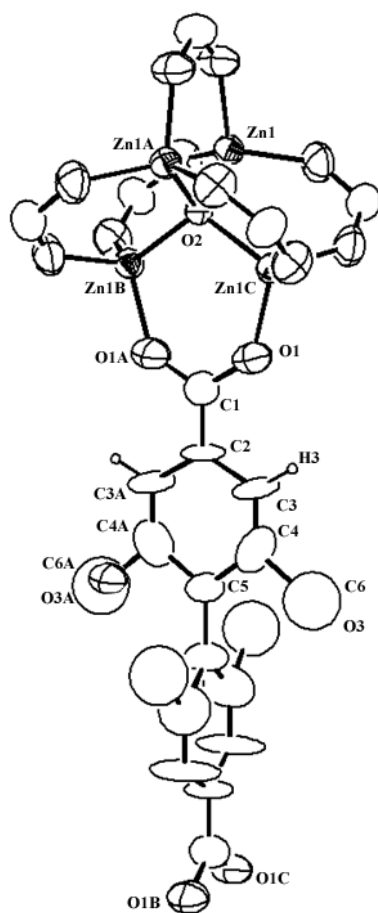


Figure S2. ORTEP drawing (20% probability) of a fragment in (*S*)-**KUMOF-2** is displayed with selected atomic labels. C6 (-CH₃) and O3(-OH) atoms are disordered over two sites with a half-occupancy, respectively. Therefore, two atoms look superimposed onto same positions.

3.3. XRPD of (*S*)-**KUMOF-2**

The X-ray powder diffraction (XRPD) data of the evacuated (*S*)-**KUMOF-2** were obtained as it is and after solvent exchange. The occluded solvents in the MOF crystals were replaced by continuous washing with relatively inert solvents, such as toluene, and/or dichloromethane. For example, the colorless crystals were collected and rinsed with dry dichloromethane or toluene (3 × 5 mL) to remove DEF and MeOH and soaked in dry dichloromethane or toluene for 3 days. The XRPD study confirmed that the MOF structure was intact.

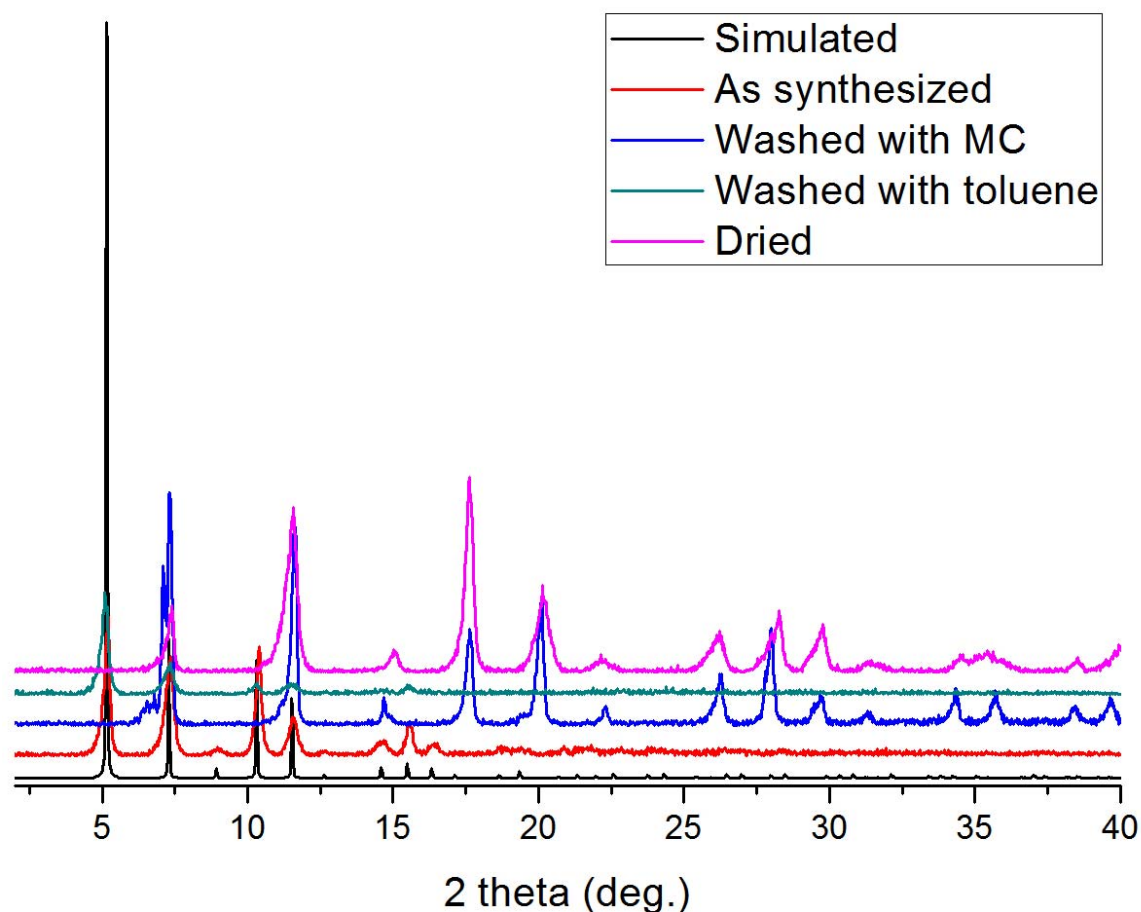


Figure S3. (a) XRPD patterns of the simulation based on the single-crystal structure of (*S*)-**KUMOF-1**, (b) the as-synthesized (*S*)-**KUMOF-2**, (c) solvent exchanged (*S*)-**KUMOF-2**,

3.4. Preparation of **COF-1**

This compound was synthesized according to the previously reported method. The guest molecules, mesitylene, trapped inside pores were removed by heating at 200 °C before the incubation with dyes as described in the literature.³

³ A. P. Côte', A. I. Benin, N. W. Ockwig, M. O'Keeffe, A. J. Matzger, O. M. Yaghi, *Science*, 2005, **310**, 1166.

4. Two-photon fluorescence microscopy

4.1. Incubation of MOF with various dyes

MOF crystals (roughly 4 mg) were treated with dyes in DMF stock solution (1.0 mL of 0.02 M solution) at r.t. for 1 hr.

4.2. TPM images

4.2.1. general procedure for measurement of two-photon fluorescence microscopy

The incubated crystals were placed on the paratone-N oil, which was applied to a glass slide in advance of the placement of crystals, and then wet crystals were blotted dry with tissue paper. Prior to the two-photon microscopy measurement, proper crystals (with a size of 75 – 100 μm) are screened using wide-field fluorescence and white-light transmission microscopes. The spatial distribution of dyes inside the selected MOFs were imaged with a two-photon confocal microscope (Leica TCS SP2) equipped with a femtosecond laser (Chameleon, Coherent Inc., $\lambda = 750$ nm, 200 fs, 10 mW) and an objective lens (NA 0.5, HC PL Fluotar 20x). By carefully adjusting the position of focal plane, we were able to obtain the cross sectional fluorescence images across the center of the crystals, which reflect the local concentration of dyes. Internal PMT2 were used to collect the signals in an 8 bit unsigned 512 x 512 pixels at 400 Hz scan speed. Compared with a more conventional one-photon fluorescence microscopy technique, the two-photon microscopy with near-infrared light source (750 nm) does not significantly suffer from the attenuation of excitation light, which provides more reliable information on the 2-dimensional distribution of dyes.

4.2.2. TPM images of MOFs

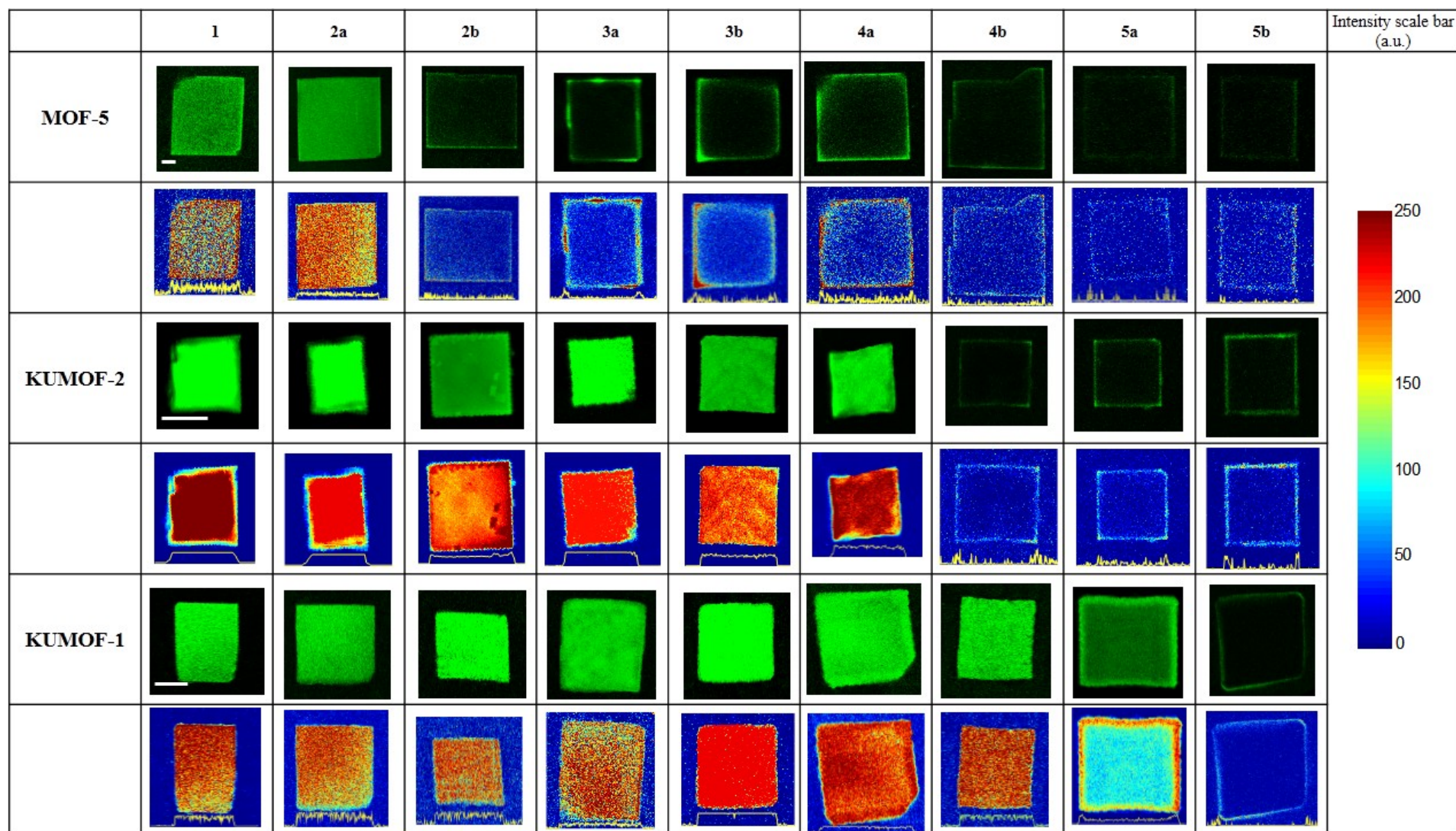


Figure S4. Scale bar = 50 μm

4.2.3. TPM images of COF-1

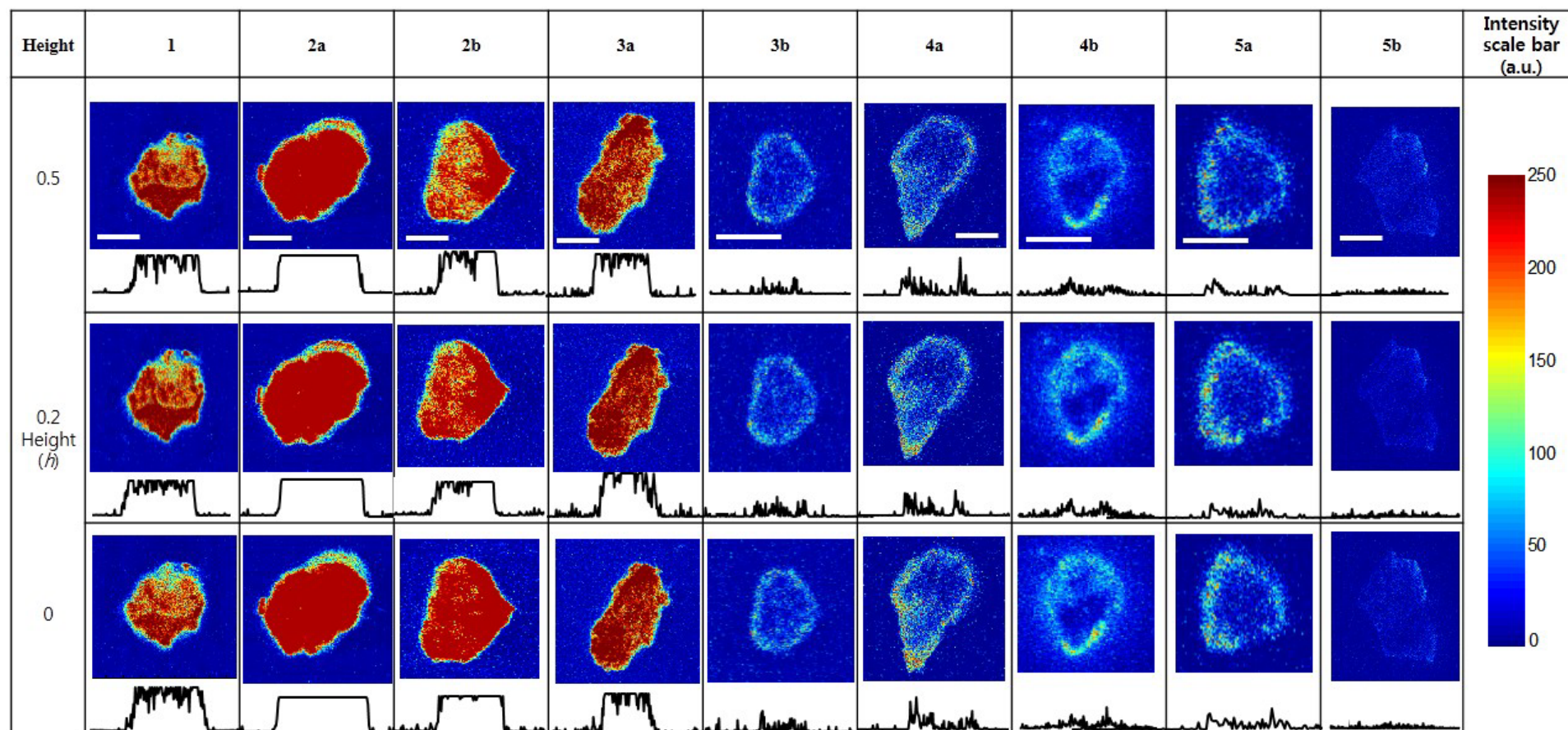
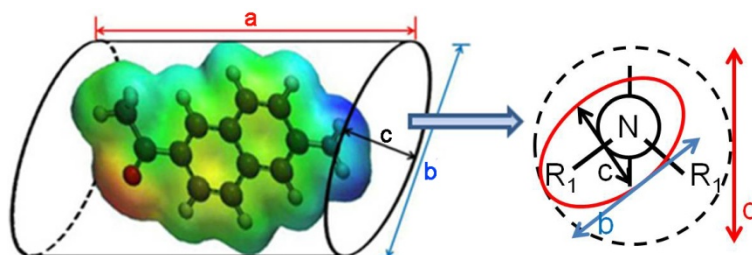


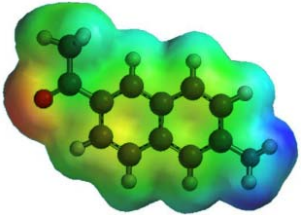
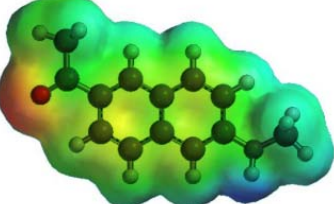
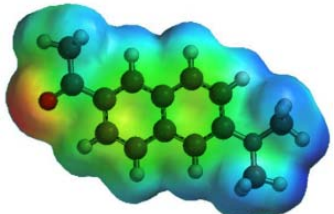
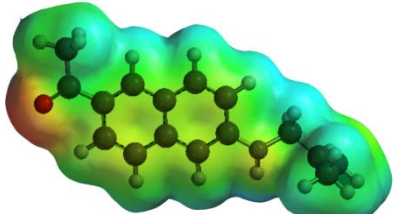
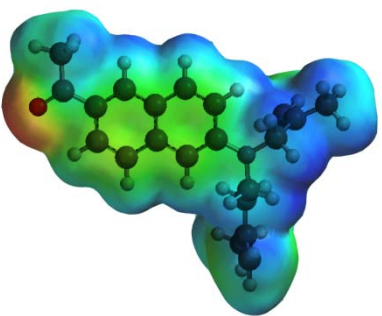
Figure S5. TPM images of COF-1 after incubation with dyes. Scale bar; 20 μm

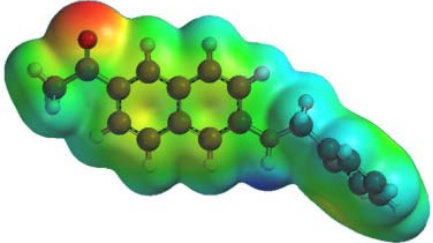
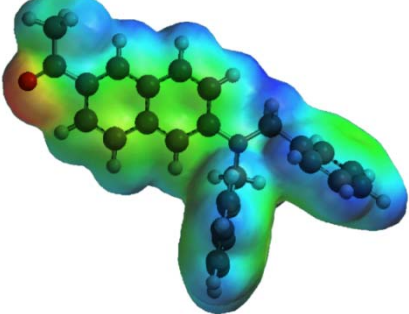
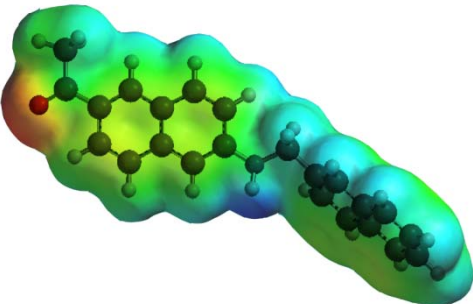
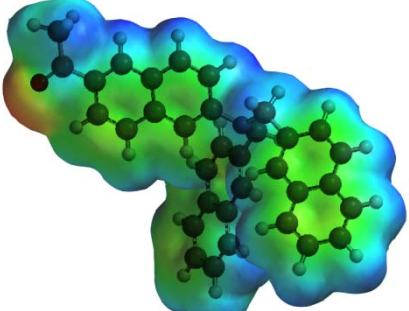
5. Comparison of size; Dyes vs products of Carbonyl-ene reaction

To estimate the geometry and electrodensity potential maps of dyes were performed using the Spartan'10 program.



5.1. Dyes

Dyes		Dimension (a x b x c) (Å)
1		11.5 x 6.5 x 3.2
2a		12.6 x 6.7 x 3.5
2b		12.6 x 6.7 x 3.5
3a		16.1 x 6.7 x 5.8
3b		16.1 x 10.5 x 10.5

4a		17.3 x 7.2 x 6.1
4b		17.3 x 13.7 x 13.7
5a		19.5 x 11.4 x 8.4
5b		19.5 x 16.3 x 16.3