Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

Uracil grafted imine-based covalent organic framework for nucleobase recognition

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General methods. All reactions with air-sensitive materials were carried out under Ar using standard Schlenk techniques. TLC was performed using pre-coated silica gel 60 F254 and developed in the indicated solvent system. Compounds were visualized under UV light (λ = 254 nm). Merck 60 (230–400 Mesh) silica gel was used for column chromatography.

^{1}H NMR and ^{13}C NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported in ppm and referenced to the residual non-deuterated solvent frequencies ($CDCl_3$: δ 7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C). CP/MAS-^{13}C NMR were recorded on a 400 MHz spectrometer Wide Bore (probe: 4 mm MAS WB DVT). The sample rotation frequency was 12 kHz and a 2.5 mm ZrO_2 rotor was used. Mass spectra were recorded by means of MALDI-TOF or FAB/IE techniques. Solids were analysed on a diamond plate (ATR) or as films on sodium chloride.

Powder X-ray diffraction

Powder X-ray diffraction measurements were carried out with X'PERT MPD with conventional Bragg-Brentano geometry using Kα (λ = 1.5406 Å) for values of 2θ from 2° to 40°.

Thermogravimetric analysis

Thermogravimetric analysis was performed on a TGA-Q-50 instrument on a platinum plate, heating the samples under nitrogen atmosphere at a heating rate of 10 °C/min. Prior to the measurements the samples were activated at 120 ºC under vacuum.

UV-visible spectroscopy

UV-visible spectra data were recorded in a Synergy H4 Hybrid reader using 96 well plates.

Materials

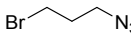
The following reagents were commercially available and were used as received: 2,5-dimethoxyterephtaldehyde (**DMTA**), CuI, *o*-DCB, *n*-butanol, NaN_3 and DIPEA.
2,5-dihydroxyterephtaldehyde (**DHTA**),[1] 2,5-bis(prop-2-in-1-yloxy)terephtaldehyde (**BPTA**),[1] 1,3,5-tris-(4-aminophenyl)benzene (**TAPB**),[2] **[HC≡C]$_{0.5}$-TPB-DMTP-COF**,[1] were prepared according to reported procedures.

Adenine, cytosine, uracil and thymine were obtained from Sigma-Aldrich. All the compounds were used without further purification.

Nucleobase interaction studies

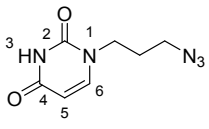
Stock solutions of each nucleobase (3.7 mM) in water were prepared. Each COF (0.5 mg) was vortexed for 1 min with a solution of each nucleobase at three different concentrations (50, 100 and 150 μM). The final volume of each sample was 600 μL. Control experiments using the nucleobases were done at the same concentrations. The samples were incubated at room temperature for the time indicated in the figures and centrifuged at 4 °C for 45 min at 13000 rpm (around 15700 g). The supernatants were collected, the absorbance recorded and the amount of free nucleobases quantified applying Beer-Lambert law. All the experiments were done in triplicates. Statistical analysis was performed using one-way ANOVA Tukey's test (each group vs Control). *P<0.01, **P<0.001, and ***P<0.0001.

Synthesis of the azide-substituted Uracil



1-azido-3-bromopropane was synthesized following a described procedure[3b] using 1,3-dibromopropane (10.7 mL, 105.4 mmol), NaN_3 (7.5 g, 115.4 mmol) and DMF (150 mL). The crude was purified by column chromatography using ciclohexane/AcOEt (10:1) as eluent. 5.0 g of **1-azido-3-bromopropane** was obtained as a colourless oil (29% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 3.49 (t, J = 6.4 Hz, 2H, BrCH_2CH$_2$CH$_2$N$_3$), 3.48 (t, J = 6.3 Hz, 2H, BrCH$_2$CH$_2$CH_2N$_3$), 2.08 (p, 6.4 Hz, 2H, N^{1}CH$_2$CH_2CH$_2$N$_3$).



1-(3-azidoprop-1-yl)-Uracil. Cs_2CO_3 (9.67 g, 70.0 mmol) was added over a suspension of **Uracil** (10 g, 89.2 mmol) in dry DMF (225 mL). The mixture was heated to 40°C. After 30 min, **1-azido-3-bromopropane** (4.2 g, 25.6 mmol) was added and the mixture was heated to 40°C. After 24 h, the solvent was removed over low pressure and H_2O (200 mL) was added. After sonication, the mixture was extracted with a $CHCl_3$/MeOH mixture (10:1, 3x200 mL). The combined organic phases were dried with $MgSO_4$ and filtered, and the solvent

was eliminated under low pressure. The crude was purified by column chromatography using $CHCl_3$/THF (3:1) as eluent. 1.45 g of **UR3N3** was obtained as a white solid (29% yield).

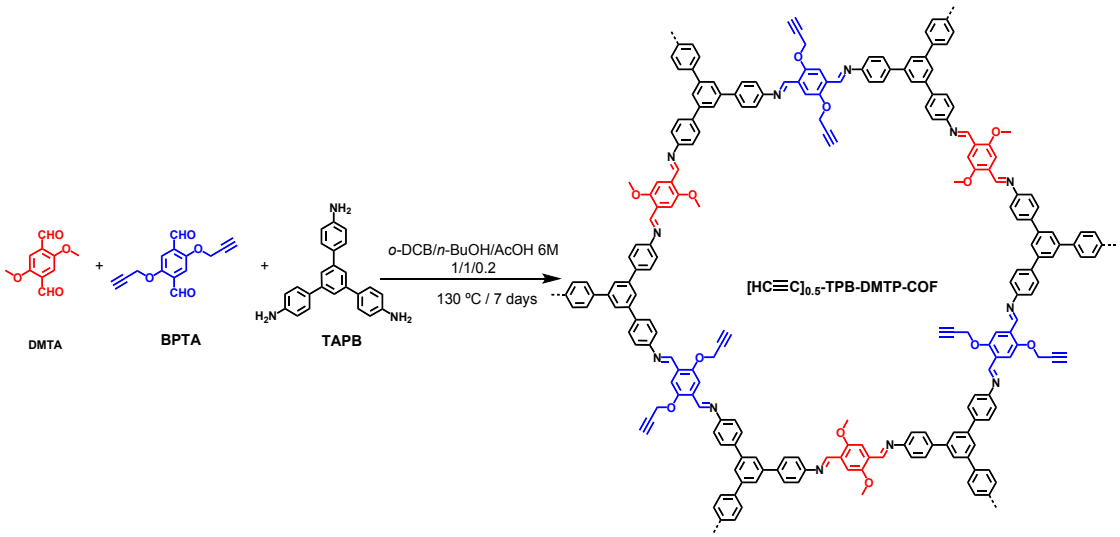
^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) = 9.12 (s, 1H, N^3H), 7.17 (d, J = 7.9 Hz, 1H, H^6), 5.72 (d, J = 7.9 Hz, 1H, H^5), 3.83 (t, J = 6.8 Hz, 2H, $N^1CH_2CH_2CH_2N_3$), 3.41 (t, J = 6.3 Hz, 2H, $N^1CH_2CH_2CH_2N_3$), 1.97 (p, 6.7 Hz, 2H, $N^1CH_2CH_2CH_2N_3$).

^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm) = 163.5, 150.8, 144.5, 102.4, 48.2, 46.5, 28.0.

HRMS (FAB): calculated for $C_7H_{10}N_5O_2$ $[M+H]^+$: 196.0834. Found: 196.0833 $[M+H]^+$.

Synthesis of COFs and characterization

- **Synthesis of $[HC\equiv C]_{0.5}$-TPB-DMTP-COF:**[1]



Scheme S1. Synthesis of **$[HC\equiv C]_{0.5}$-TPB-DMTP-COF**.

Following the procedure previously described, from **DMTA** (215.3 mg, 1.109 mmol), **BPTA** (268.8 mg, 1.110 mmol), **TAPB** (520.4 mg, 1.481 mmol) and *o*-DCB/*n*-Butanol (10 mL/10 mL) and acetic acid (6 M, 2.1 mL) in a Pyrex vessel (ϕ = 29 mm, h = 10 cm) were obtained, after Soxhlet extraction in THF, 866.0 mg (94 %) of a yellow solid. FTIR (ATR) (cm^{-1}): 3288, 2957, 2127, 1689, 1592, 1504, 1415, 1291, 1208, 1146, 1036, 878, 829, 694.

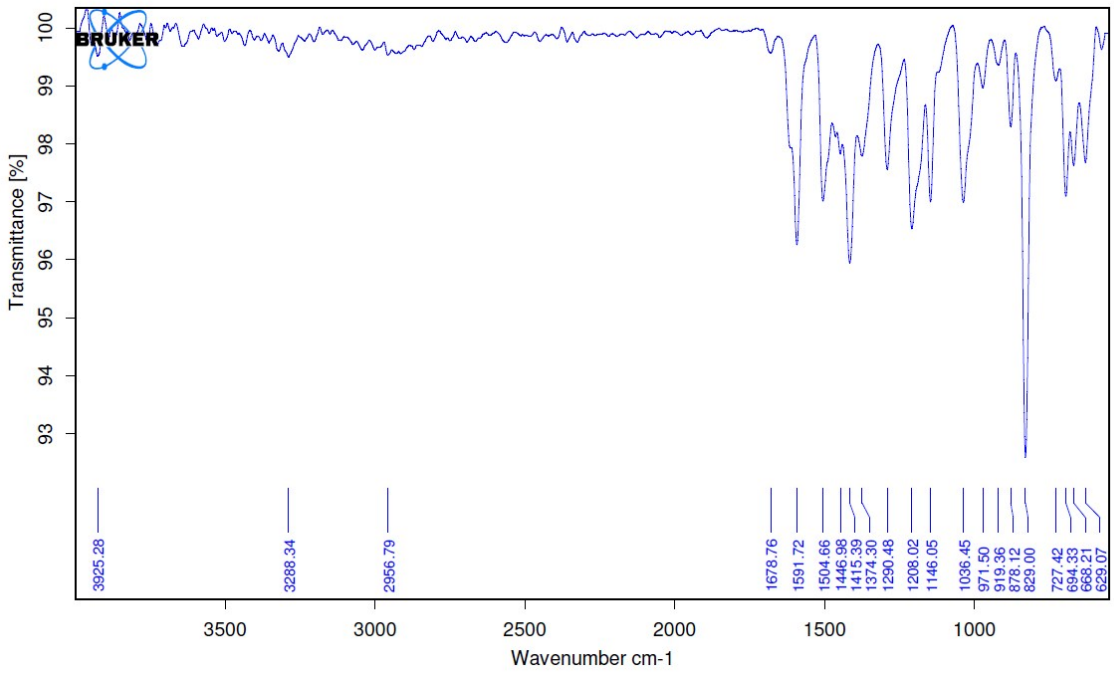


Figure S1. FTIR (ATR) spectrum of **[HC≡C]$_{0.5}$-TPB-DMTP-COF**.

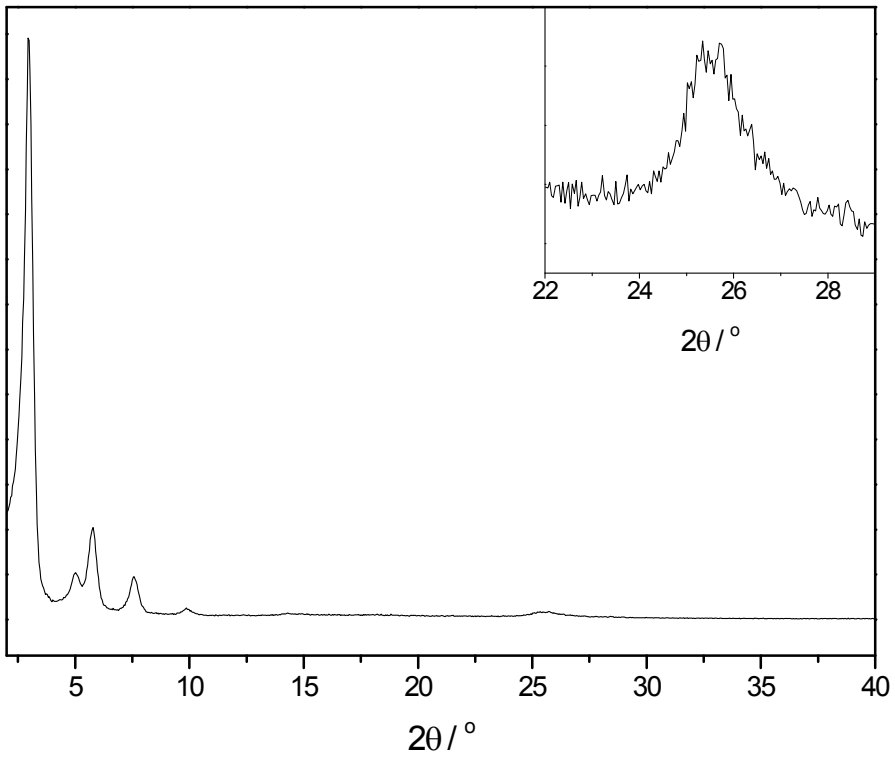
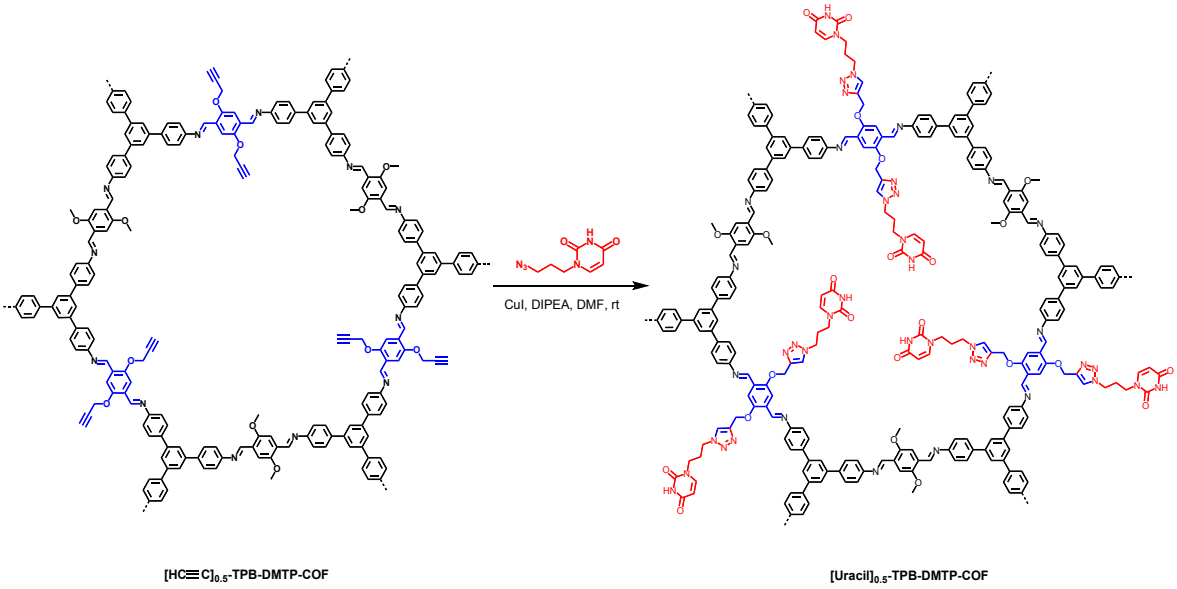


Figure S2. PXRD pattern of **[HC≡C]$_{0.5}$-TPB-DMTP-COF**.

Table S1. PXRD of **[HC≡C]$_{0.5}$-TPB-DMTP-COF**.

Pos. (°2θ)	d-spacing (Å)	Rel. Int. (%)	FWHM (°2θ)
2.9750	29.698	100	0.3149
4.9836	17.732	7.81	0.3149
5.7930	15.256	15.63	0.3542
7.5515	11.707	7.27	0.3542
9.8914	8.942	1.64	0.1968
25.457	3.499	1.21	0.1447

- **Synthesis of [Uracil]$_{0.5}$-TPB-DMTP-COF:**



Scheme S2. Synthesis of **[Uracil]$_{0.5}$-TPB-DMTP-COF**.

To 100.9 mg of **[HC≡C]$_{0.5}$-TPB-DMTP-COF** in 2 mL of anhydrous DMF were added 24.3 mg (0.128 mmol) of CuI and 75 µL of *N,N*-diisopropylethylamine (DIPEA). The suspension was purged with argon for 5 min and then 51 mg (0.261 mmol) of 1-(3-azidopropyl)uracil were added. The mixture was stirred overnight at room temperature under argon and centrifuged at 6000 rpm for 5 min. After washing with DMF, H_2O, acetonitrile and THF the solid was dried under vacuum at 120 ºC yielding an orange powder (151.8 mg, quantitative yield). FTIR (ATR) (cm^{-1}): 3026, 2946, 1682, 1618, 1592, 1507, 1455, 1416, 1373, 1289, 1209, 1145, 1040, 829, 697.

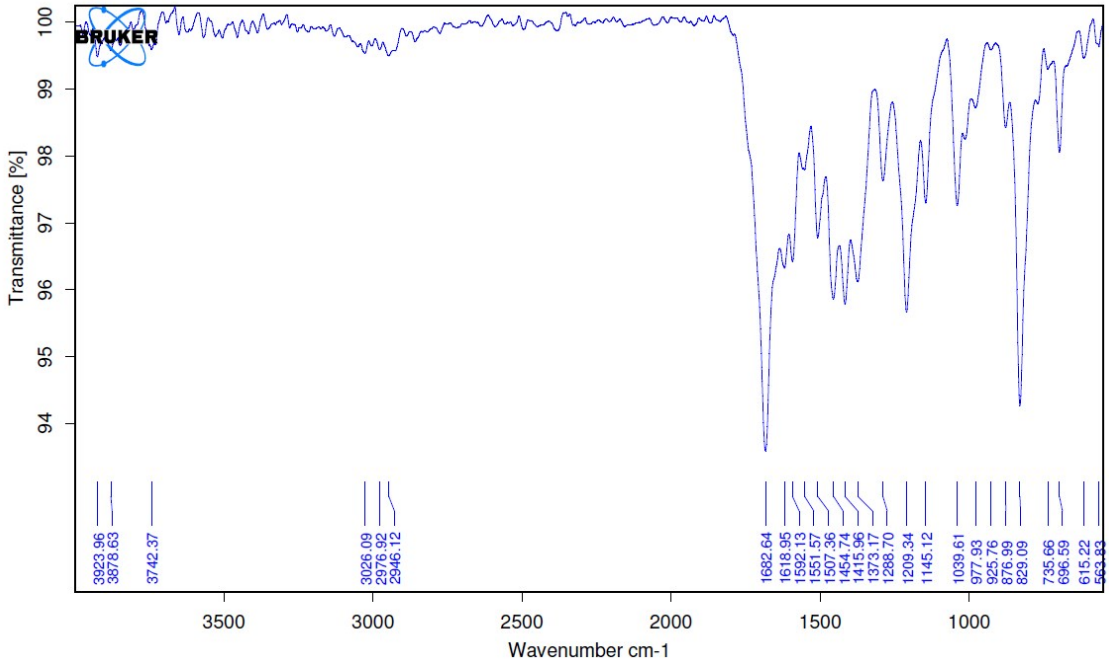


Figure S3. FTIR (ATR) spectrum of **[Uracil]$_{0.5}$-TPB-DMTP-COF**.

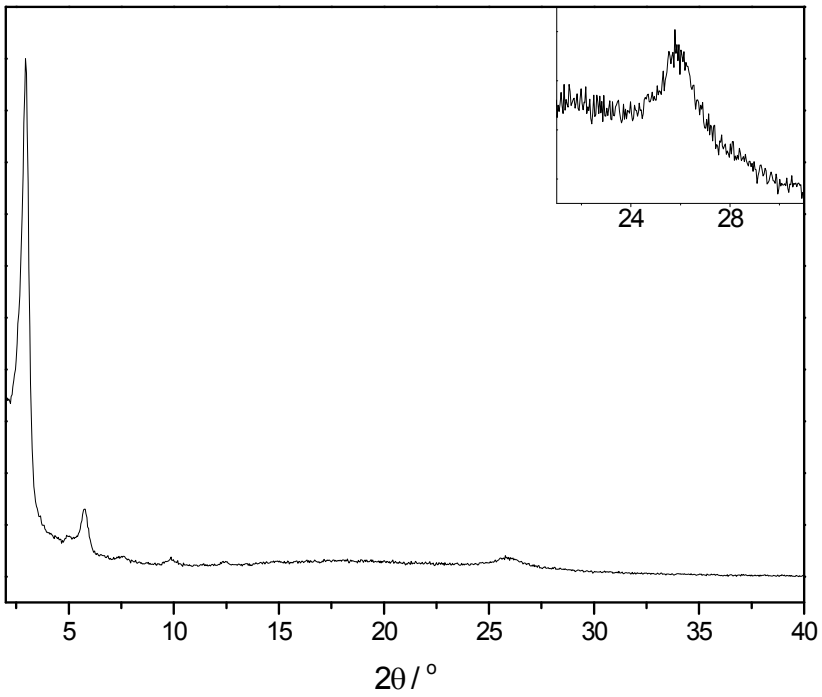


Figure S4. PXRD pattern of **[Uracil]$_{0.5}$-TPB-DMTP-COF**.

Table S2. PXRD of **[Uracil]$_{0.5}$-TPB-DMTP-COF**.

Pos. (°2θ)	d-spacing (Å)	Rel. Int. (%)	FWHM (°2θ)
2.9724	29.724	100	0.2755
4.9368	17.900	7.85	0.1968
5.7669	15.325	13.24	0.2755
7.6366	11.577	3.84	0.3149
9.8292	8.999	3.37	0.1574
25.5176	3.491	3.49	0.1574

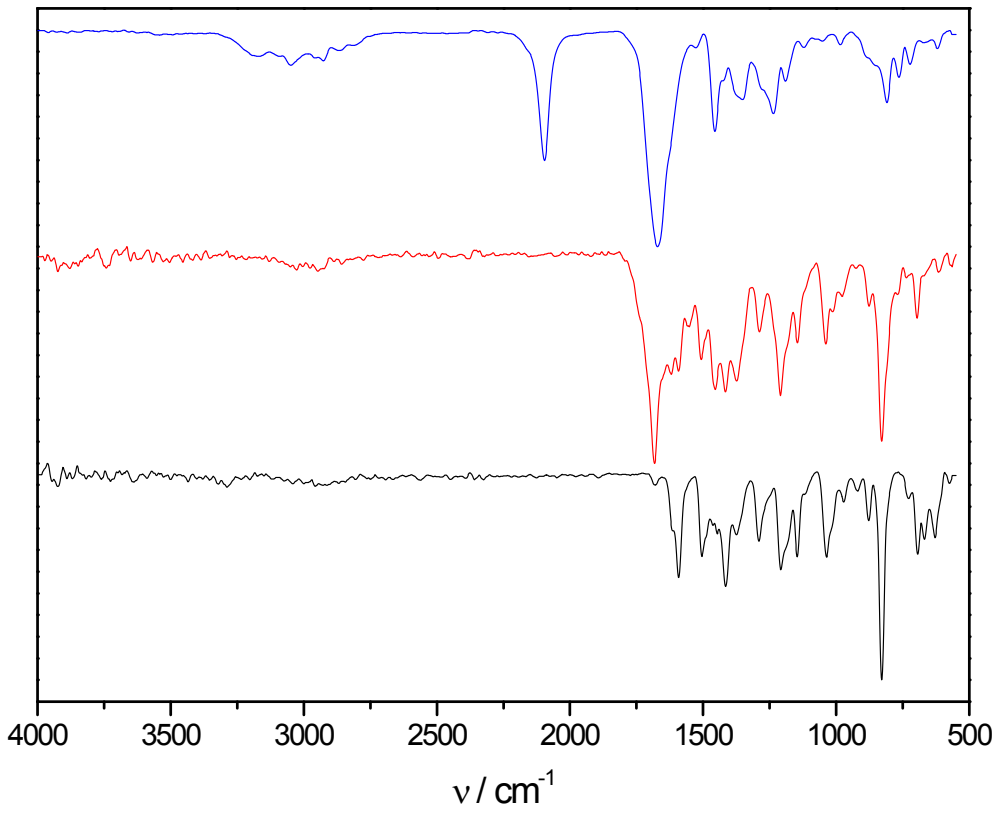
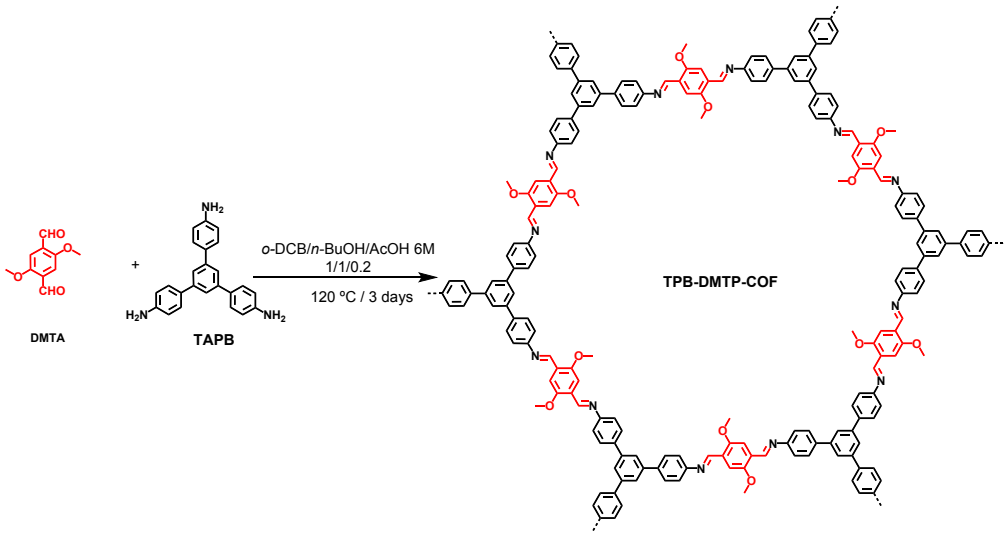


Figure S5. Comparative FTIR (ATR) spectra of **[HC≡C]$_{0.5}$-TPB-DMTP-COF** (black), **[Uracil]$_{0.5}$-TPB-DMTP-COF** (red), and 1-(3-azidopropyl)uracil (blue).

- **Synthesis of TPB-DMTP-COF:**[1]



Scheme S3. Synthesis of **TPB-DMTP-COF**.

DMTA (84.4 mg, 0.435 mmol), **TAPB** (102.5 mg, 0.292 mmol) and *o*-DCB/*n*-Butanol (2 mL/2 mL) and acetic acid (6 M, 0.4 mL) in a Pyrex vessel (ϕ = 18 mm, h = 10 cm) were obtained, after Soxhlet extraction in THF, 162.1 mg (95 %) of a yellow solid. FTIR (ATR) (cm^{-1}): 2957, 1592, 1504, 1464, 1410, 1290, 1211, 1144, 1042, 879, 823, 639.

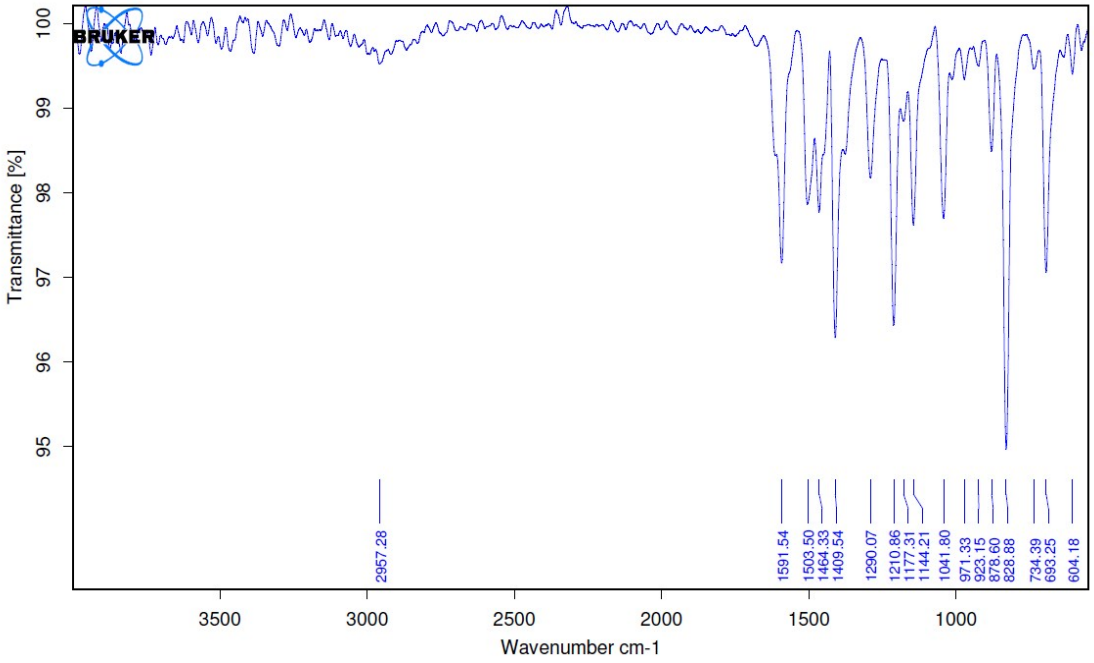


Figure S6. FTIR (ATR) spectrum of **TPB-DMTP-COF**.

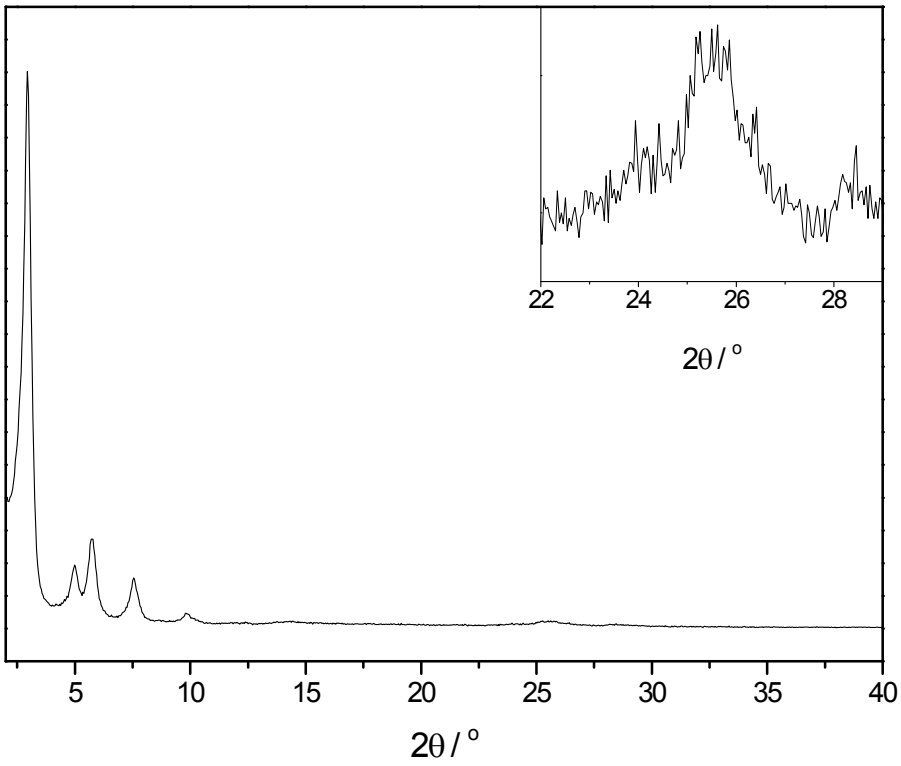
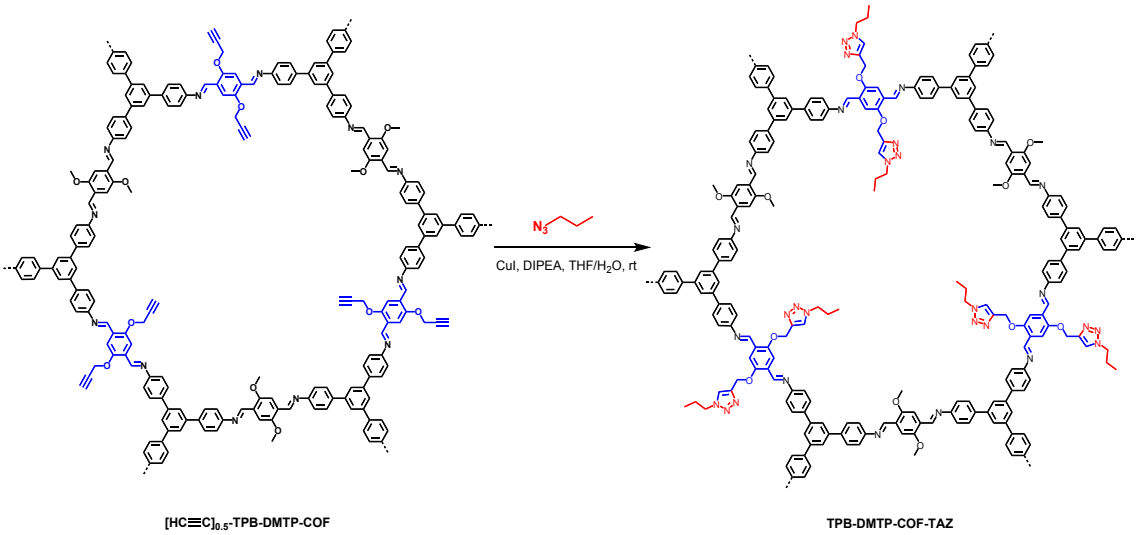


Figure S7. PXRD pattern of **TPB-DMTP-COF**.

Table S3. PXRD of **TPB-DMTP-COF**.

Pos. (°2θ)	**d-spacing** (Å)	**Rel. Int.** (%)	**FWHM** (°2θ)
2.9531	29.919	100	0.2362
4.9972	17.684	11.25	0.2755
5.7309	15.422	15.95	0.3149
7.5433	11.720	8.92	0.2755
9.8263	9.001	2.51	0.1968
25.5271	3.499	1.03	0.1378

- **Synthesis of TPB-DMTP-COF-TAZ:**[4]



Scheme S4. Synthesis of **TPB-DMTP-COF-TAZ**.

34.4 mg (0.18 mmol) of CuI and 129.2 mg of **[HC≡C]$_{0.5}$-TPB-DMTP-COF** were suspended in a mixture of THF/H_2O (3.9/1.7 mL). The suspension was purged with Argon for 5 min and then *N,N*-diisopropylethylamine (DIPEA) was added (92.7 μL). The mixture was purged with Argon for 5 min and toluene (0.2 mL) and 60 mg of 1-azidopropane[3] were added. The suspension was stirred overnight at room temperature under argon. The solid was centrifuged with 5 mL of THF. Then, it was washed thoroughly with water, THF and dried, yielding a yellow solid (166 mg). FTIR (ATR) (cm^{-1}): 2965, 2971, 1769, 1591, 1503, 1463, 1414, 1380, 1290, 1146, 1042, 877, 829, 733, 696, 607.

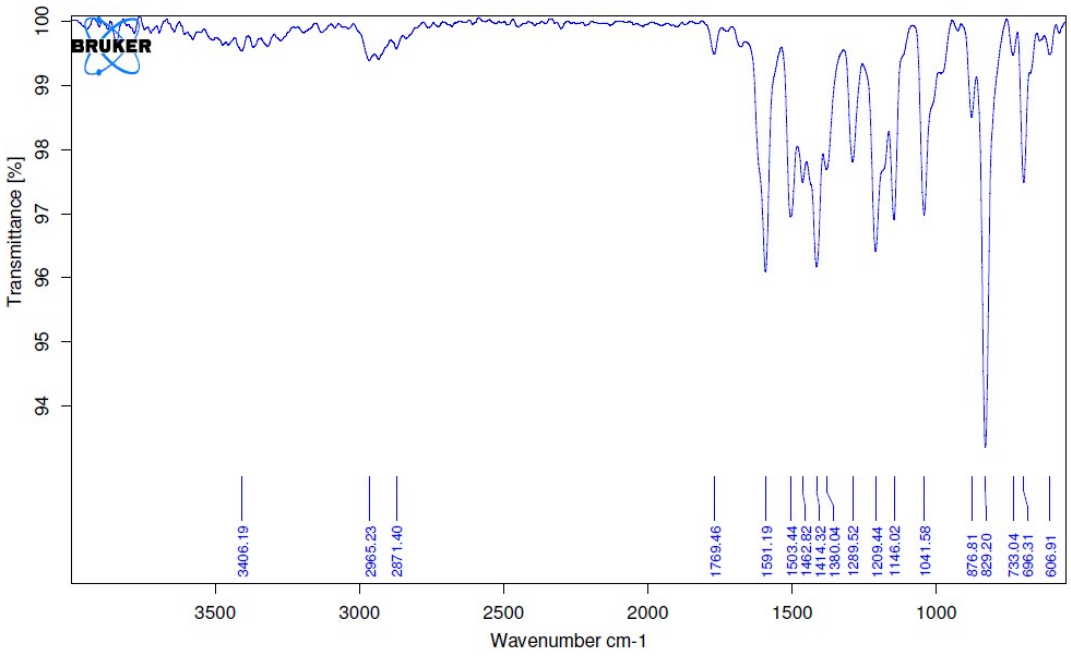


Figure S8. FTIR (ATR) spectrum of **TPB-DMTP-COF-TAZ**.

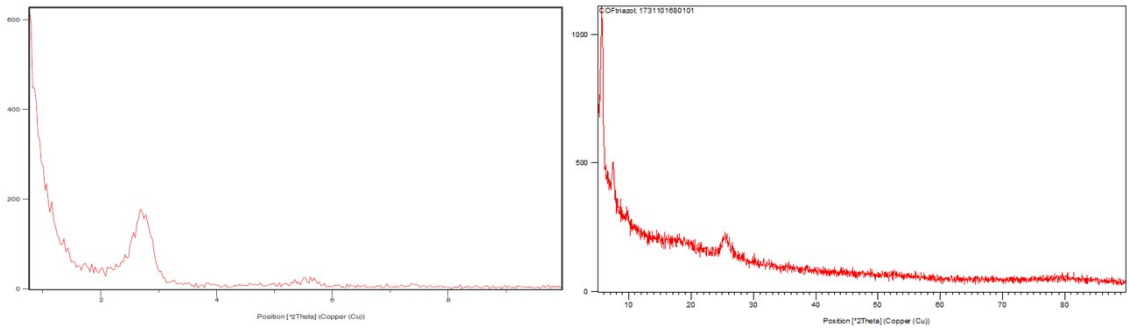


Figure S9. PXRD patterns of **TPB-DMTP-COF-TAZ**.

Table S4. PXRD of **TPB-DMTP-COF-TAZ**.

Small-angle range			Wide-angle range		
Pos. (°2θ)	**d-spacing** (Å)	**Rel. Int.** (%)	**Pos.** (°2θ)	**d-spacing** (Å)	**Rel. Int.** (%)
2.7299	32.338	100	5.6783	15.564	100
5.6243	15.714	9.23	7.4671	11.829	45.80
			25.4213	3.504	18.83

Table S5. Lattice parameters of the synthesized COFs.

	a=b / Å	c / Å	α=β / º	γ / º
[HC≡C]$_{0.5}$-TPB-DMTP-COF	35.3	3.50	90	120
[Uracil]$_{0.5}$-TPB-DMTP-COF	35.3	3.49	90	120
TPB-DMTP-COF	35.4	3.49	90	120
TPB-DMTP-COF-TAZ	36.6	3.50	90	120

- **Nitrogen adsorption isotherms data**

Table S6. Surface area, pore volume and pore size of COFs.

	BET surface area (m^2 g^{-1})	Pore volume (cm^3 g^{-1})	Pore size (nm)
[HC≡C]$_{0.5}$-TPB-DMTP-COF (ref 1)	1642	1.02	3.03
[HC≡C]$_{0.5}$-TPB-DMTP-COF (this work)	1510	0.94	2.53
[Uracil]$_{0.5}$-TPB-DMTP-COF	105	0.088	1.30

TGA of COFs

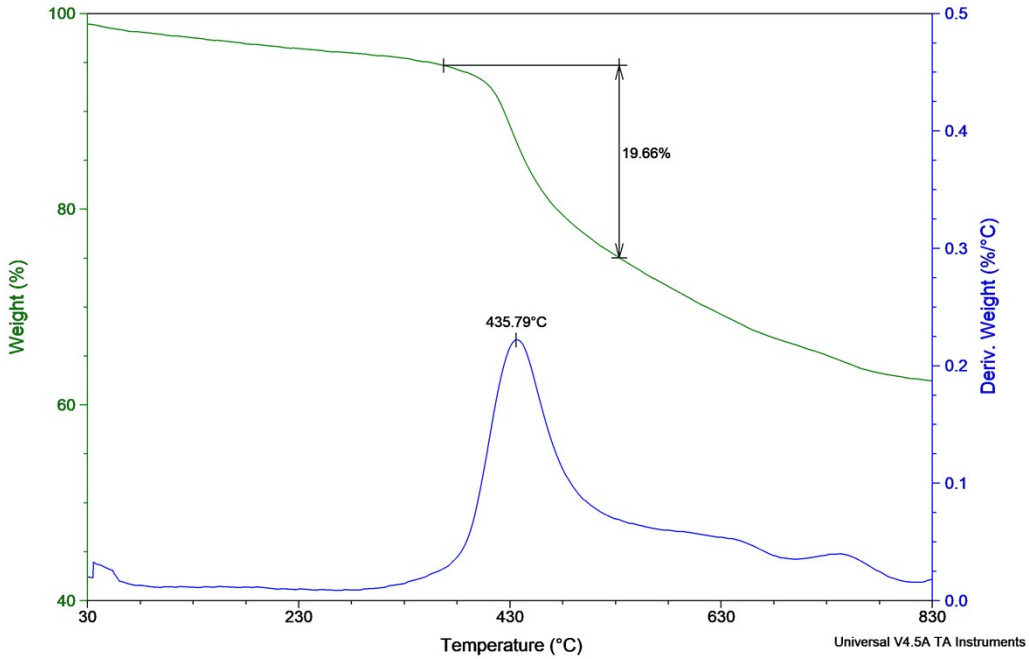


Figure S10. TGA profile of **[HC≡C]$_{0.5}$-TPB-DMTP-COF**. Heating ramp: 10 °C/min.

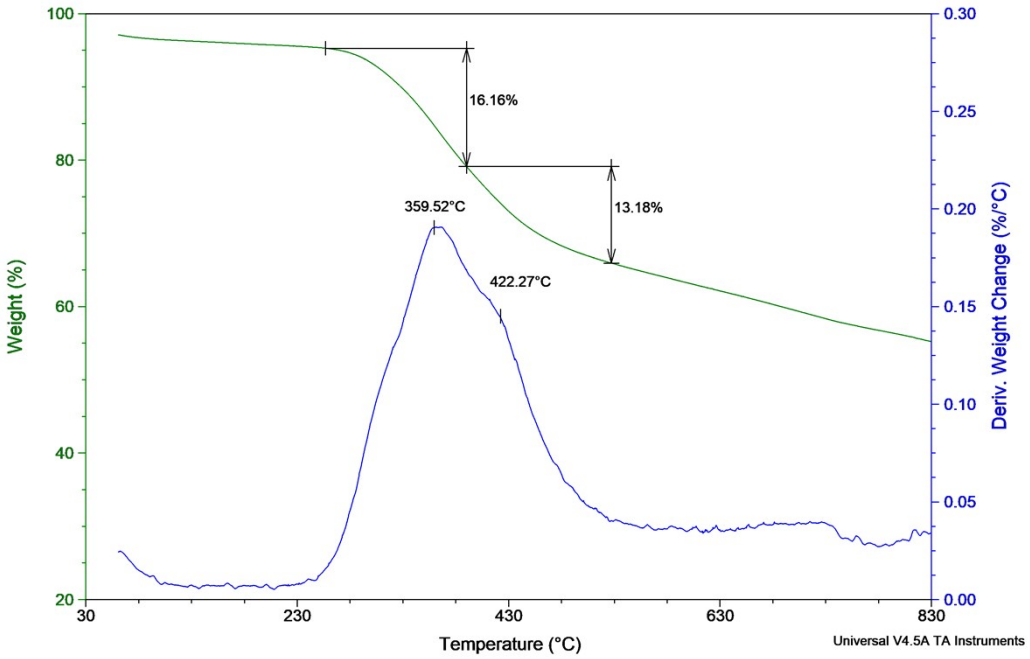


Figure S11. TGA profile of **[Uracil]$_{0.5}$-TPB-DMTP-COF**. Heating ramp: 10 °C/min.

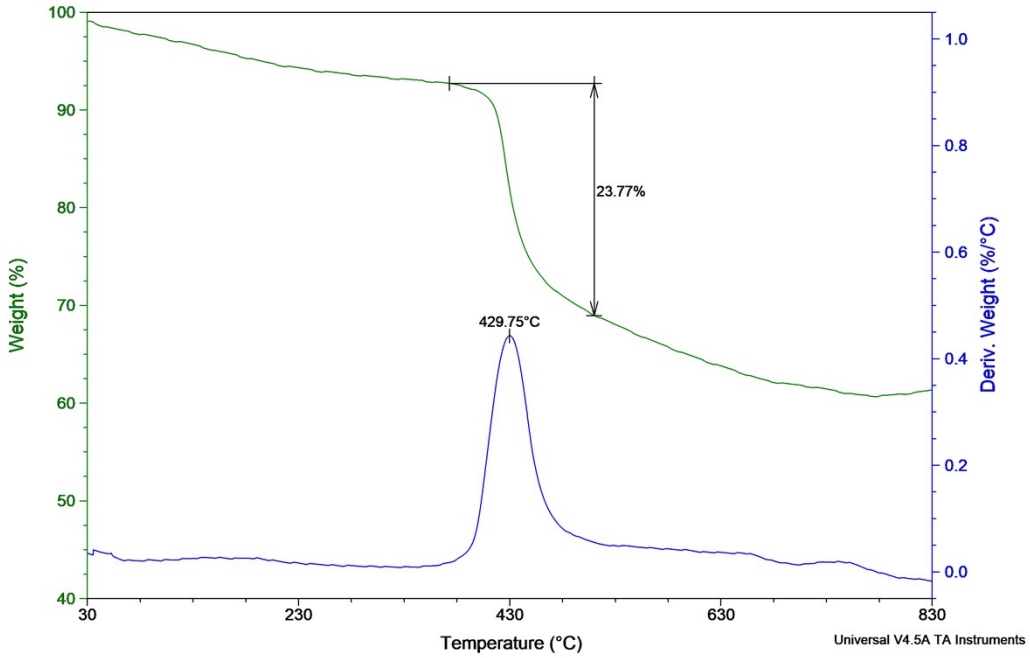


Figure S12. TGA profile of **TPB-DMTP-COF**. Heating ramp: 10 °C/min.

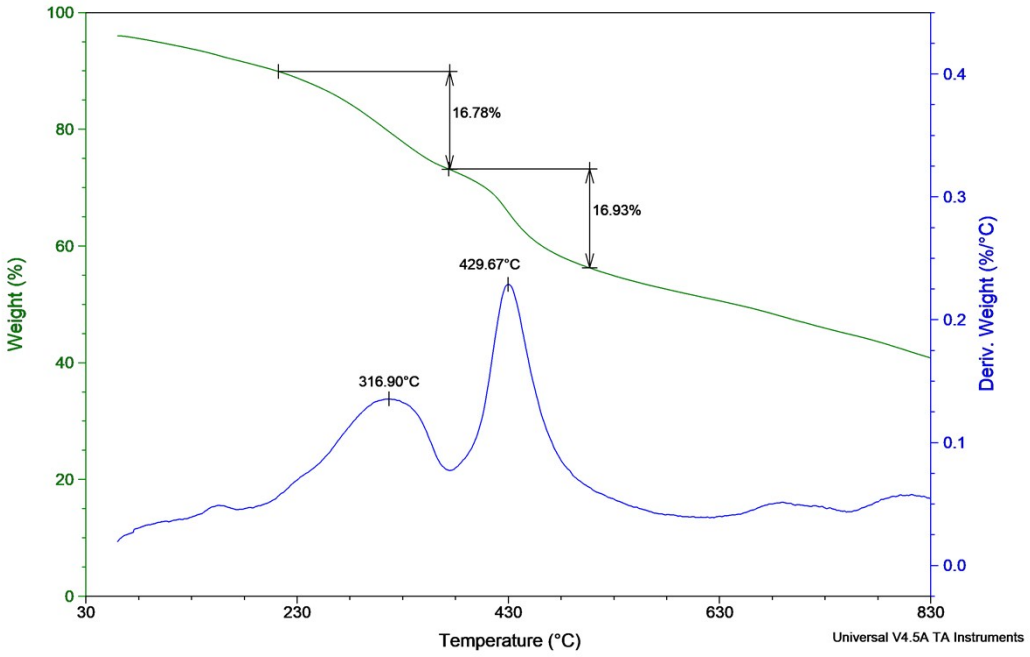
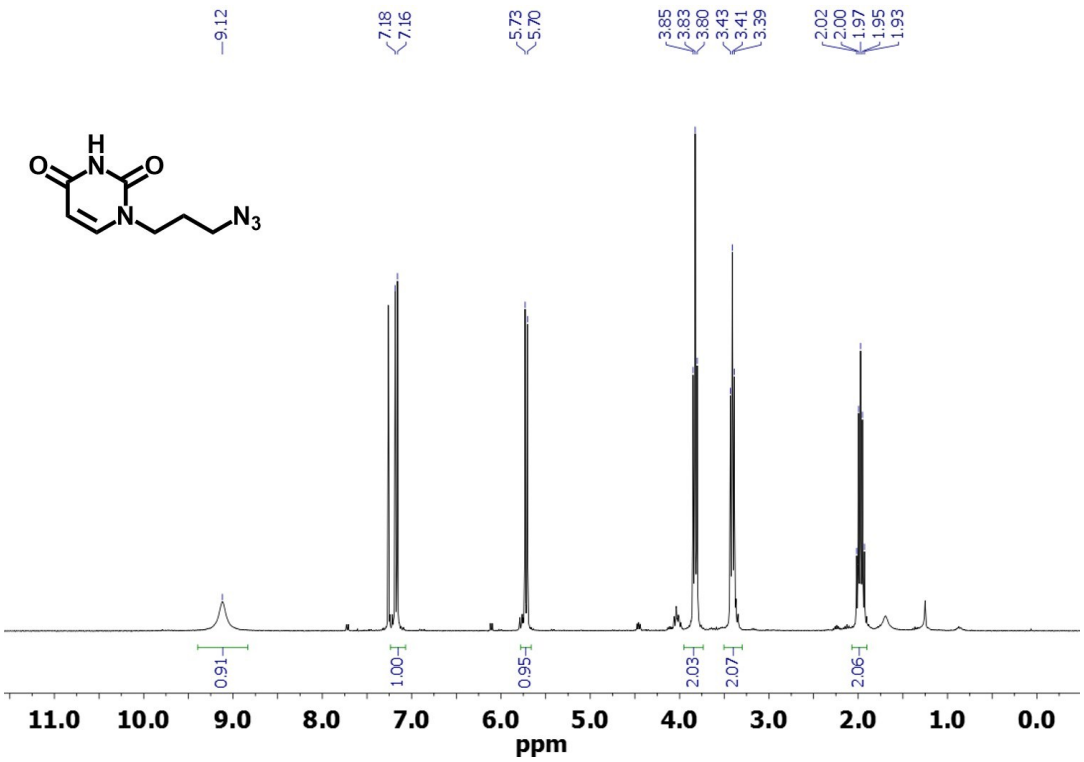


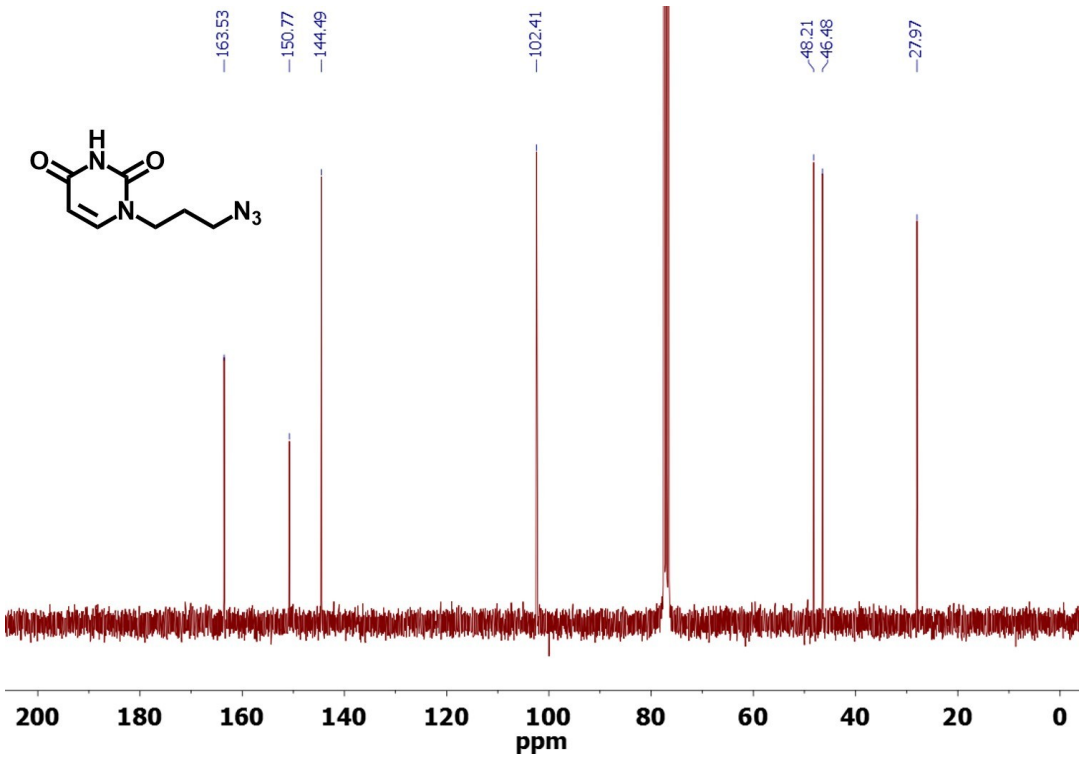
Figure S13. TGA profile of **TPB-DMTP-COF-TAZ**. Heating ramp: 10 °C/min.

- **^{1}H NMR, ^{13}C NMR and FTIR spectra of starting materials and COFs**

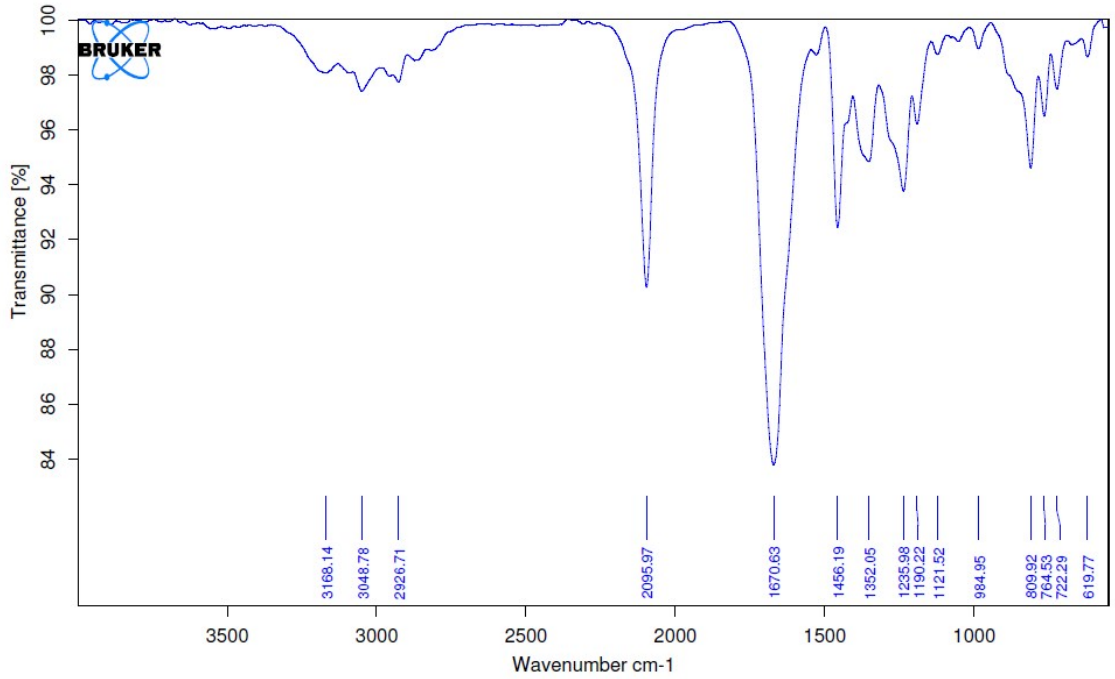
^{1}H NMR 1-(3-azidoprop-1-yl)-Uracil



^{13}C NMR 1-(3-azidoprop-1-yl)-Uracil



FTIR 1-(3-azidoprop-1-yl)-Uracil



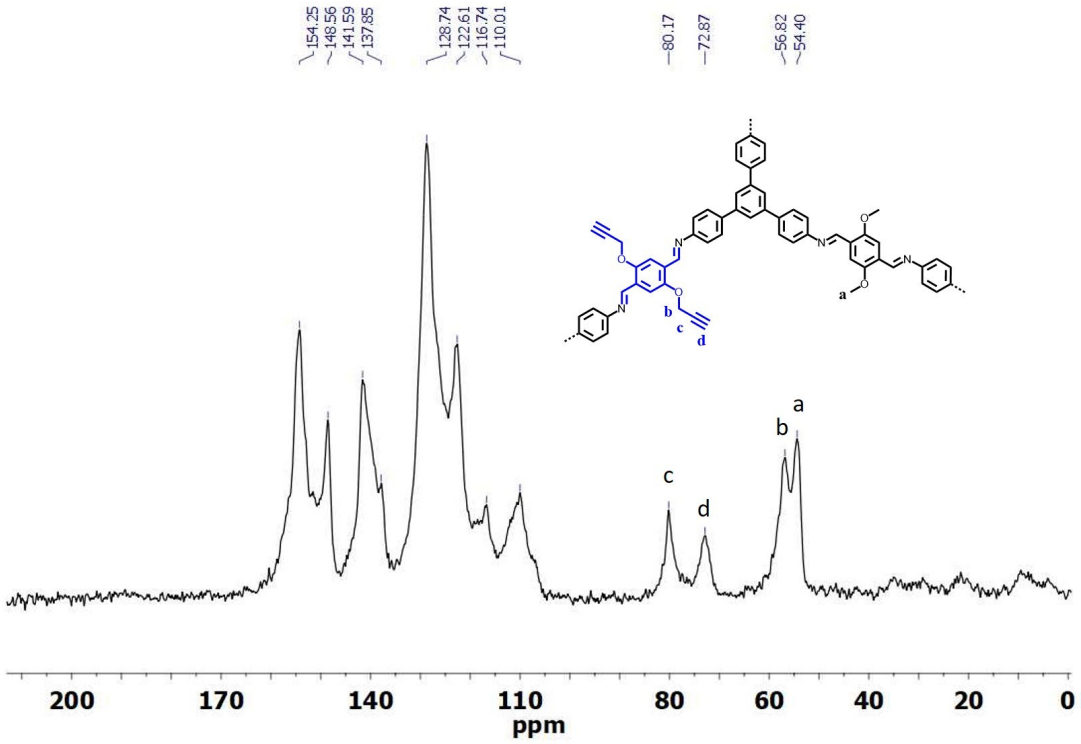


Figure S14. CP/MAS-^{13}C NMR of **[HC≡C]$_{0.5}$-TPB-DMTP-COF**.

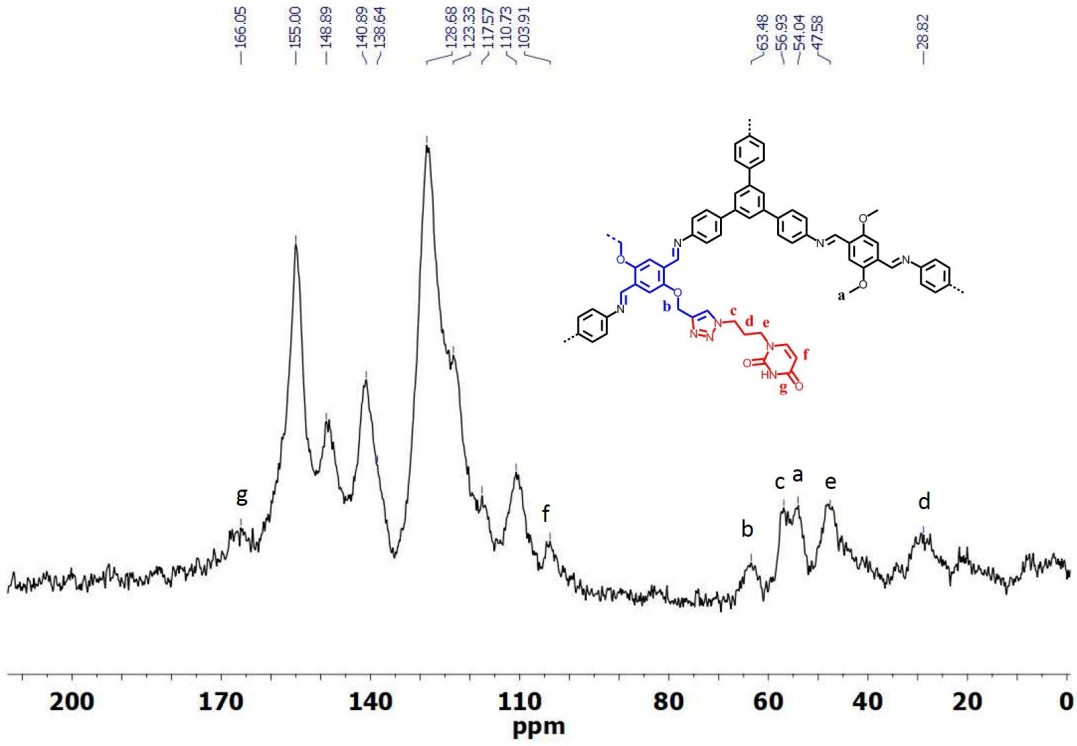


Figure S15. CP/MAS-^{13}C NMR of **[Uracil]$_{0.5}$-TPB-DMTP-COF**.

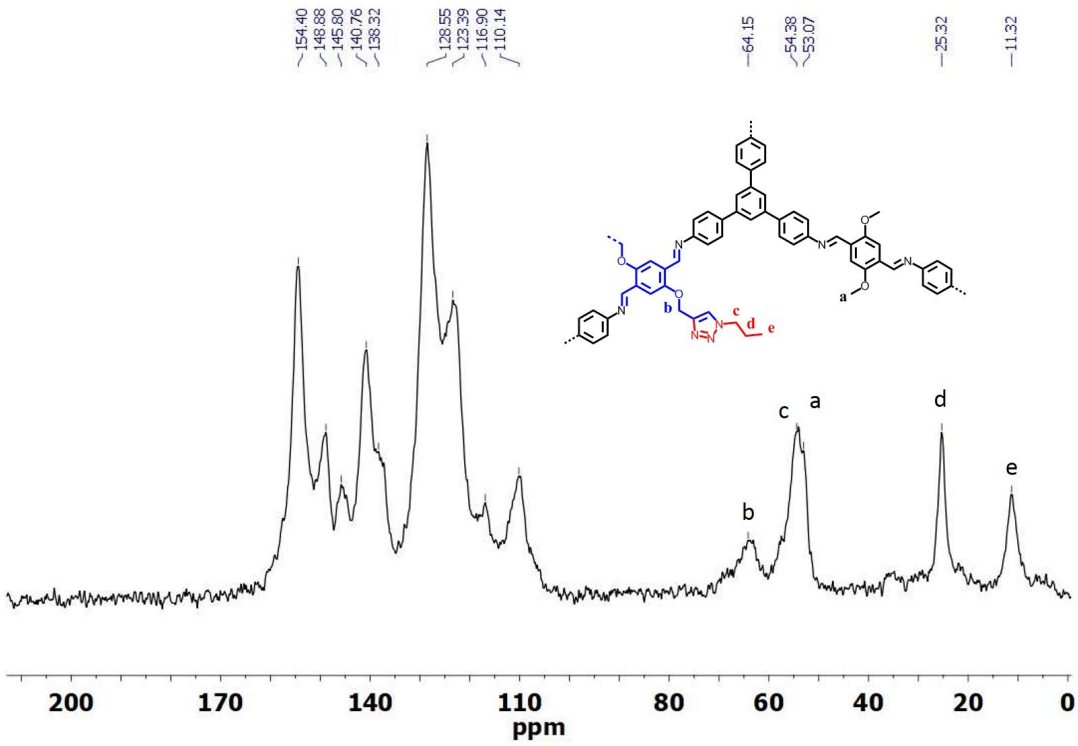


Figure S16. CP/MAS-^{13}C NMR of **TPB-DMTP-COF-TAZ**.

- **Elemental Analysis**

  - **$[HC\equiv C]_{0.5}$-TPB-DMTP-COF**

    Calculated - C: 80.75 %, H: 4.84 %, N: 6.73 %

    Experimental - C: 78.73 %, H: 5.06 %, N: 6.46 %

    Experimental - C: 79.20 %, H: 4.99 %, N: 6.48 %

    Experimental - C: 79.04 %, H: 5.05 %, N: 6.47 %

  - **$[Uracil]_{0.5}$-TPB-DMTP-COF**

    Calculated – C: 68.73 %, H: 4.78 %, N: 16.03 %

    Experimental – C: 60,91 %, H: 4,72 %, N: 13,71 %

    Experimental – C: 61,50 %, H: 4,80 %, N: 14,00 %

    Experimental – C: 61,39 %, H: 4,76 %, N: 13,78 %

  - **TPB-DMTP-COF**

    Calculated - C: 79.57 %, H: 5.14 %, N: 7.14 %

    Experimental - C: 78.38 %, H: 5.67 %, N: 6.06 %

    Experimental - C: 75.56 %, H: 5.43 %, N: 5.83 %

    Experimental - C: 78.36 %, H: 5.69 %, N: 6.12 %

  - **TPB-DMTP-COF-TAZ**

    Calculated - C: 73.60 %, H: 5.56 %, N: 14.31 %

    Experimental - C: 64.14 %, H: 4.99 %, N: 10.24 %

    Experimental - C: 64.58 %, H: 5.00 %, N: 10.27 %

    Experimental - C: 64.10 %, H: 4.91 %, N: 10.13 %

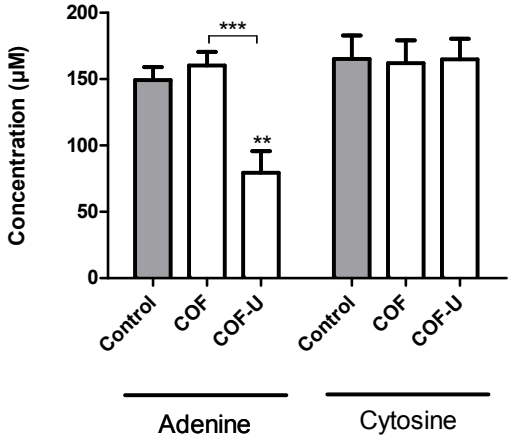


Fig S17. Concentration of nucleobases (adenine & cytosine) after incubation for 18 h with TPB-DMTP-COF-TAZ (COF-P) **and** $[Uracil]_{0.5}$-TPB-DMTP-COF (COF-U) in methanol. All the experiments were done in triplicates. Statistical analysis was performed using one-way ANOVA Tukey's test (each group vs Control). *P<0.01, **P<0.001, and ***P<0.0001.

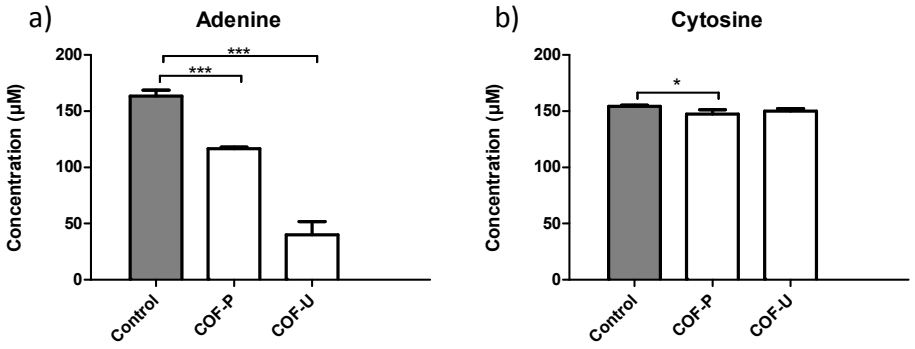


Figure S18. Concentration of nucleobases in the media after incubation at 150 µM using **TPB-DMTP-COF-TAZ (COF-P)** and **$[Uracil]_{0.5}$-TPB-DMTP-COF (COF-U)** for 22 hours. (a) Adenine, (b) Cytosine. The interaction of adenine with **$[Uracil]_{0.5}$-TPB-DMTP-COF** is superior compared with any other combination. All the experiments were done in triplicates. Statistical analysis was performed using one-way ANOVA Tukey's test (each group vs Control). *P<0.01, **P<0.001, and ***P<0.0001.

- References

1. H. Xu, J. Gao and D. Jiang, *Nat. Chem.*, 2015, **7**, 905.
2. A. de la Peña Ruigómez, D. Rodríguez-San-Miguel, K. C. Stylianou, M. Cavallini, D. Gentili, F. Liscio, S. Milita, O. M. Roscioni, M. L. Ruiz-González, C. Carbonell, D. Maspoch, R. Mas-Ballesté, J. L. Segura and F. Zamora, *Chem. Eur. J.*, 2015, **21**, 10666-10670.
3. S. Balabadra, M. Kotni, V. Manga, A. D. Allanki, R. Prasad and P. S. Sijwali, *Bioorg. Med. Chem.*, 2017, **25**, 221-232.

4. L. Meri-Bofi, S. Royuela, F. Zamora, M. L. Ruiz-Gonzalez, J. L. Segura, R. Munoz-Olivas and M. J. Mancheno, *J. Mater. Chem. A*, 2017, **5**, 17973-17981.