Electronic Supplementary Material (ESI) for CrystEngComm.

Molecular recognition and self-assembly mechanism of cocrystallization process

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Contents of Supporting Information:

1. Experimental section

1.1 Materials.

Analytical-grade urea was supplied by Tianjin Fengchuan Chemical Reagent Technologies Co., Ltd. of China. Its mass fraction purity is higher than 99.0%. The *m*-cresol (MC) with mass fraction purity higher than 99.0% was purchased from Tianjin Yuanli Chemical Co., Ltd. of China. Its purity was determined by gas chromatograph. The analytical-grade toluene was purchased from Tianjin Guangfu Fine Chemical Research Institute. The dimethyl sulfoxide-d_6 (DMSO-d6, 99.9 atom % D, contains 0.03% (v/v) TMS) was purchased from SIGMA-ALORICH Co., of USA. All chemicals were used without further purification. **Figure S1** indicates the molecular structures of urea and MC.

1.2 Analytical methods & equipments.

1.2.1 X-ray diffraction. Powder samples for X-ray powder diffraction (PXRD) analysis were piled on a glass slide. The patterns were collected by Rigaku D/MAX 2500 in 2θ range from 2° to 50° with a step size of 0.02°, voltage of 40 kV, and current of 100 mA. The single crystal X-ray diffraction data of MC_U cocrystal were collected on a Rigaku-Rapid II diffractometer with Mercury2 CCD area-detector by using graphite-monochromatized Mo Kα radiation (λ= 0.71073).

1.2.2 Thermal analysis. The thermodynamic properties of MC_U cocrystal were determined by DSC (DSC 1/500, Mettler-Toledo, Switzerland) under protection of nitrogen atmosphere (dry nitrogen; purging rate: 50 mL/min). The samples (5-10 mg) were placed into 50 μL aluminum pans, and the measurement temperature range was 25-100 °C with heating rate of 5 °C/min. The temperature deviations of the measurement were ± 0.3 K.

1.2.3 Fourier transformed infrared spectrometer (FTIR). Infrared spectra of urea, MC and MC_U cocrystal were collected by using FTS6000 infrared instrument (Bio-rad). 1-2 mg samples and 200 mg pure KBr were thoroughly ground to smaller than 2 μm and then homogeneously mixed by a mortar and pestle. The interaction between MC and urea was confirmed based on the wavenumbers.

1.2.4 ^{1}H-nuclear magnetic resonance (^{1}H NMR). ^{1}H NMR analyses were performed on a Varian Inova 500 MHz Spectrometer (Palo Alto, CA, USA). Approximately 6-8 mg samples were dissolved into 0.6 ml DMSO-d6. The existence of dMCUs were confirmed based on the changes of the chemical shifts of protons among MC, urea and MC_U cocrystal.

1.2.5 Raman spectroscopy (Raman). The ***RamanRXN2™ HYBRID*** analyzer (Kaiser Optical Systems, Inc. USA) which was equipped with both a *PhAT* probe head (for non-contact measurements) and a *MR* probe head (for direct measurements) was used to *in situ* monitor for the formation processes of MC_U cocrystal. The Raman spectra were collected from 100 to 1890 cm^{-1} at 1 min intervals during the isothermal process and 30 s intervals during the cooling process, respectively, and with 3 s exposure time. The *iC Raman*™ software (METTLER TOLEDO) was used to collect and analyze the Raman data. The *MR* probe head with immersion optics was used to *in situ* monitor the formation of MC_U cocrystal in solution while the *PhAT* probe head was used to collect the characteristic peaks for qualitative analysis.

1.2.6 Attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR). The ATR-FTIR ReactIR 45m (METTLER TOLEDO) which is designed to be flexible to use the full range of Comp probe and conduit technologies was used to *in situ* monitor the solute concentration. The *iC IR*™ software was used for data acquisition and analysis. The measurement duration was set at 1 min and spectra were collected from 1000 to 1890 cm^{-1}.

1.2.7 Focused Beam Reflectance Measurement (FBRM). A laboratory scale FBRM (model M400LF) system coupled with *iC FBRM*™ software from METTLER TOLEDO was employed to measure the particles with a 2 s duration. The total counts of particles were used to monitor the appearance and disappearance of crystals.

1.3 Experimental section.

1.3.1 Cocrystal preparation.

The single crystals of MC_U cocrystal were grown by using slow cooling crystallization. Certain amounts of urea were added into *m*-cresol at 25 °C. Then, the suspension was heated until urea was completely dissolved to form a clear solution. At last, the solution was cooled down to 25 °C slowly. And MC_U cocrystal seeds and toluene applied during the cooling process. The suitable single crystals were analyzed by single crystal X-ray diffractometer, DSC, FTIR, ^{1}H NMR and Raman spectroscopy.

1.3.2 Cooling cocrystallization. Cooling cocrystallization experiments were performed in a 250 mL double-jacketed glass crystallizer as shown in **Figure S2**. The temperature of jacketed crystallizer was controlled by a thermostat, and the accuracy of temperature control is ±0.01 °C. An overhead mechanical agitator was used to mix the solution. For all experiments, the agitation speed

was maintained at 300 rpm. Predetermined amounts of urea (24.00g) were completely dissolved in *m*-cresol (108.00g) without any other solvents in the crystallizer. At this ratio of materials, a clear solution can be obtained only when temperature is above 60 °C while it will remain as suspension at 60 °C. The suspension with urea particles was heated up to 76 °C rapidly and held at that temperature for 20 min to dissolve all urea solid particles. Then, the solution was cooled down to 60 °C rapidly. In the final step cooling process, the solution was cooled down to 50 °C at cooling rate of 0.1 °C/min. When the temperature dropped down to 59.7 °C, a burst of nucleation occurred. Then, the crystallizer was held at 50 °C for 30 min. The obtained suspension was filtered and the filtered cake was washed with appropriate amount of toluene, and then dried at ambient conditions for 24 hours. The final products were analyzed by PXRD, DSC, FTIR and ^{1}H NMR. During these experiments, Raman, ATR-FTIR spectroscopic analyzers and FBRM were used in combination to *in situ* monitor the cooling cocrystallization processes.

1.3.3 Dimer verification experiments. To verify the hypothesis that *m*-cresol-urea dimers (dMCUs) were firstly formed in solution before the formation of MC_U cocrystals, the following experiments which are similar to the cooling cocrystallization experiments were designed. And the devices for the dMCUs verification experiment are the same with the cooling cocrystallization experiments, as showed in **Figure 2**. In the experiments, in order to make the experimental phenomena and trends more prominent, 42.17g MC_U cocrystals were added into 90.00g *m*-cresol at 35 °C with agitation speed of 300 rpm. With this ratio of cocrystal to *m*-cresol, the saturation point of the solution should be 60 °C. The system was maintained at 35°C for 75 min to make the system to reach stability. Then, the system was rapidly heated up to 65 °C and was held at 65 °C for 20 min to dissolve all particles. Next, the clear solution was cooled down to 60 °C in 10 min and held for 30 min. At last, the clear solution was cooled down to 55 °C at cooling rate of 0.05 °C/min, then down to the final temperature 35 °C at cooling rate of 0.1 °C/min. When the temperature dropped down to 56.7 °C, flaky crystals appeared slowly. The first step cooling rate is slower than the second step, so as to avoid the occurrence of burst nucleation. The process was also *in situ* monitored by Raman, ATR-FTIR spectroscopy and FBRM.

In order to confirm the existence of dMCUs and to find the disappearing and appearing temperatures of dMCUs upon heating or cooling, we have also designed a cycle procedure of heating and cooling on the basis of the above experiments and the process was also *in situ* monitored by ATR-FTIR

spectroscopy and FBRM.

2. Figure Contents

Figure S1

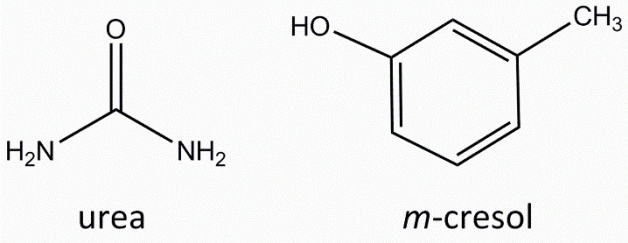


Figure S1. Molecular structures of urea and *m*-cresol

Figure S2

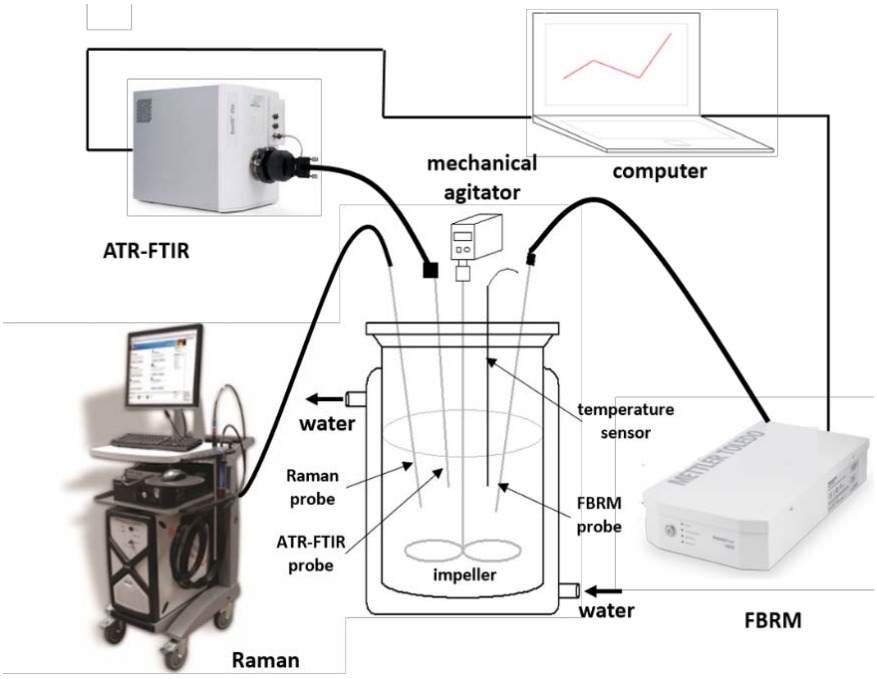


Figure S2. A schematic diagram of the experimental setup consisting of Raman, ATR-FTIR spectroscopic analysers and FBRM.

Figure S3

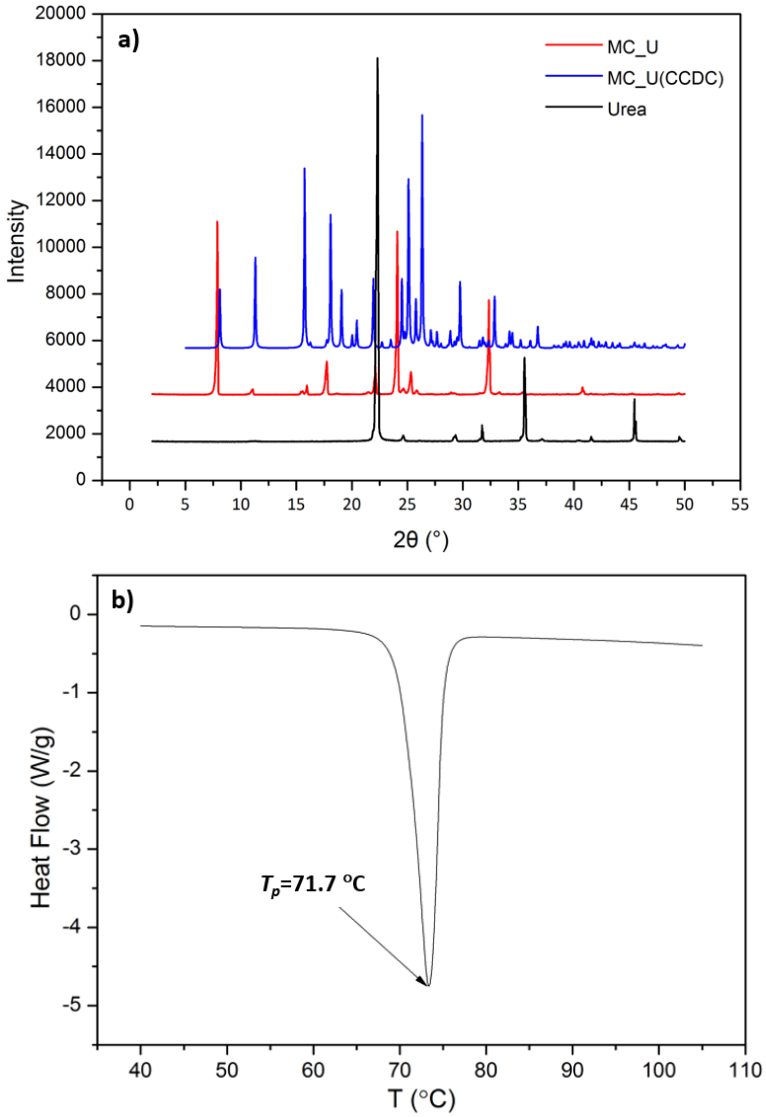
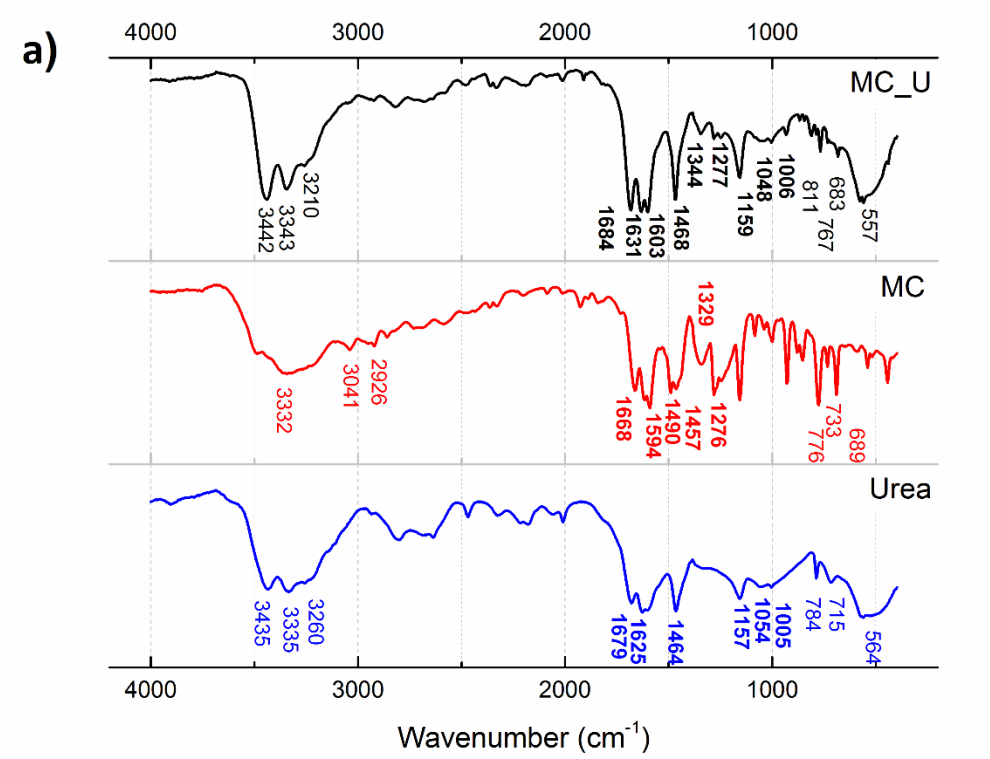
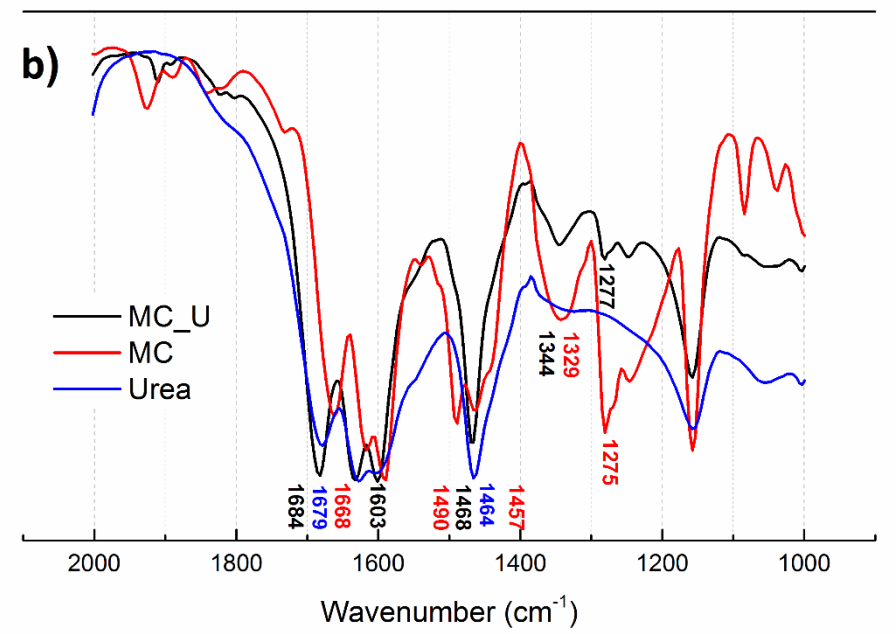


Figure S3. PXRD patterns and DSC thermogram of MC_U cocrystal. a) shows the PXRD pattern of urea, MC_U cocrystal (CCDC) calculated by single crystal crystallography and MC_U cocrystal obtained by experiments, respectively. b) shows the DSC data of MC_U cocrystal with peak melting temperature (T_p) of 71.7 °C.

Figure S4


a)
4000
3000
2000
1000
MC_U
3210
3343
3442
1684
1631
1603
1468
1344
1277
1159
1048
1006
811
767
683
557
MC
3332
3041
2926
1668
1594
1490
1457
1329
1276
776
733
689
Urea
3435
3335
3260
1679
1625
1464
1157
1054
1005
784
715
564
Wavenumber (cm-1)


b)
MC_U
MC
Urea
1684
1679
1668
1603
1490
1468
1464
1457
1344
1329
1277
1275
2000
1800
1600
1400
1200
1000
Wavenumber (cm-1)

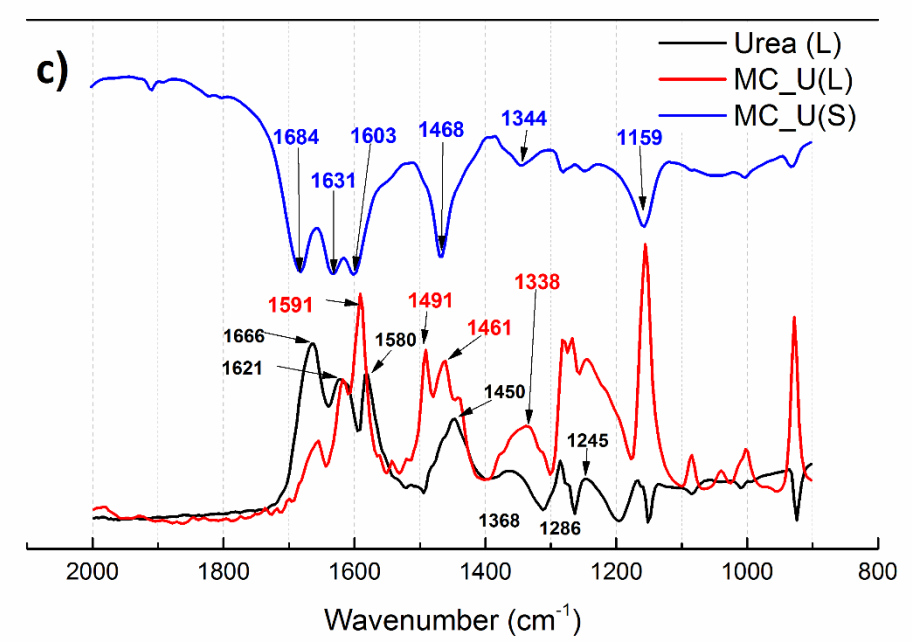


Figure S4. FTIR spectra of MC_U cocrystal, MC and urea respectively. a) displays the solid FTIR spectra of MC, urea and MC_U cocrystal from 4000 to 400 cm^{-1}; b) displays the magnified FTIR spectra from 2000 to 1000 cm^{-1}; and c) shows the characteristic absorption peaks corresponding to solid FTIR (marked with S) and liquid ATR-FTIR data (marked with L).

Figure S5

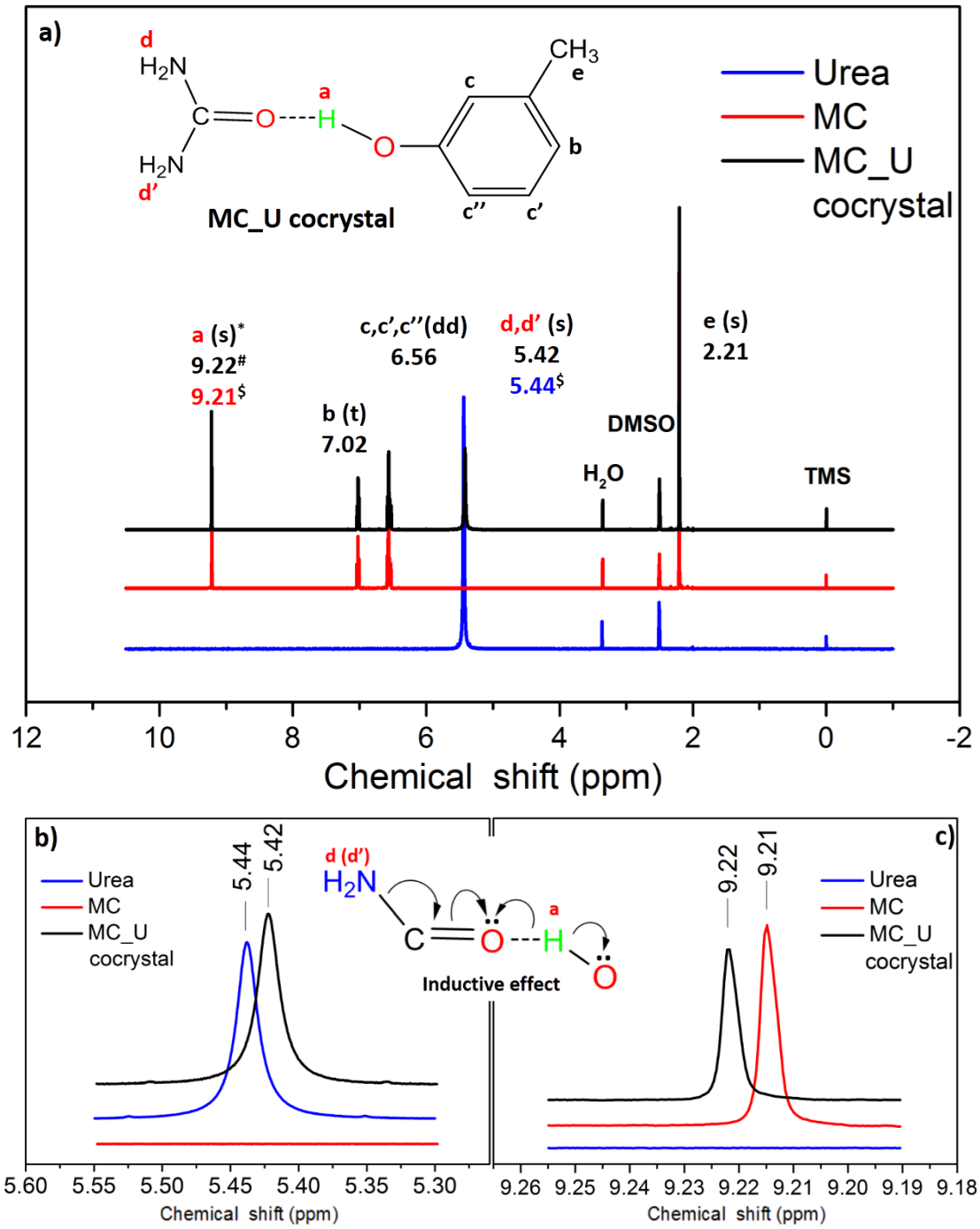
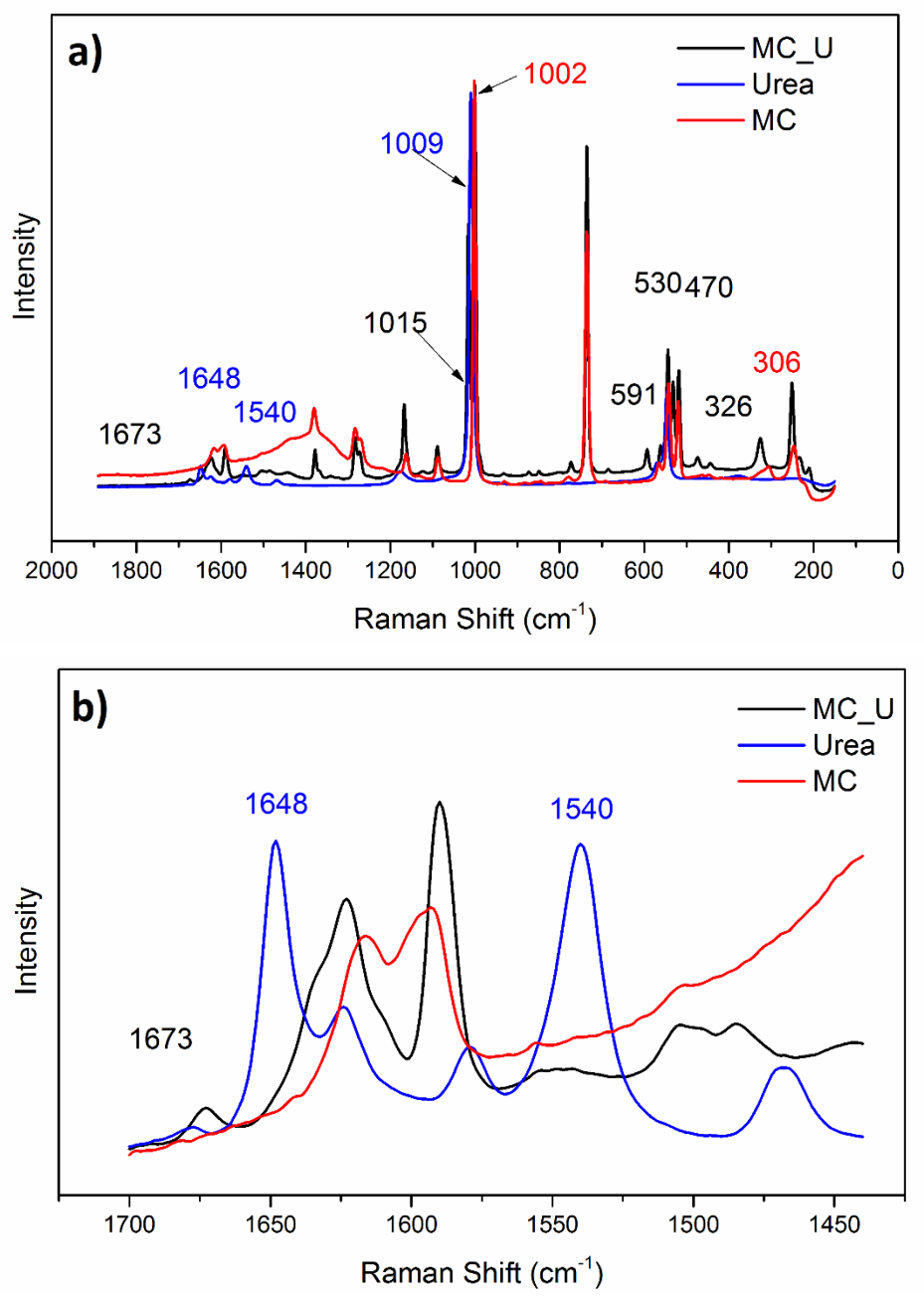
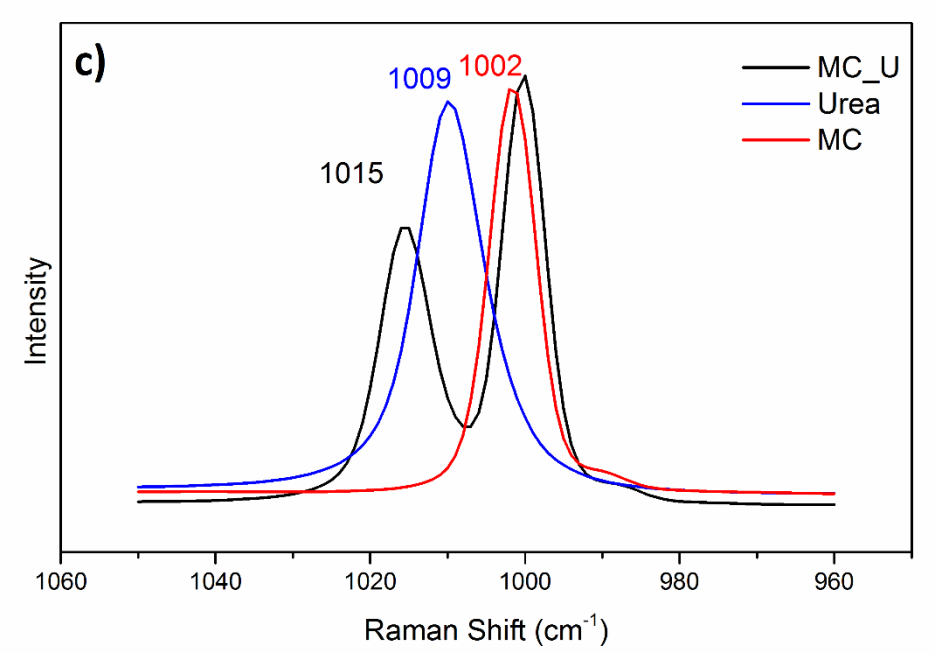
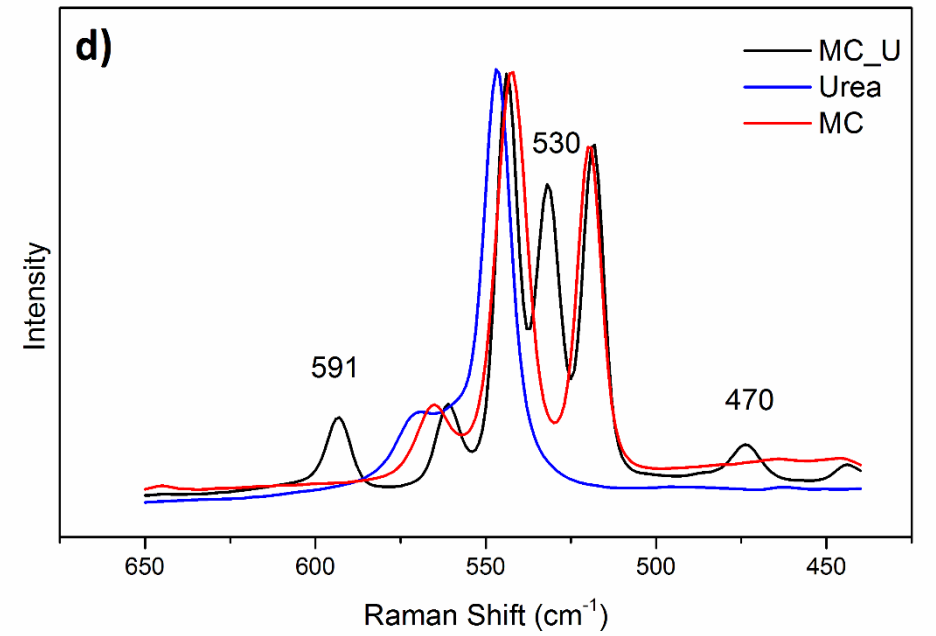


Figure S5. ^{1}H NMR spectra of MC_U cocrystal, MC and urea in DMSO-d6, respectively. a) displays the ^{1}H NMR spectra of MC, urea and MC_U cocrystal with the schematic diagram of MC_U cocrystal: the characteristic chemical shift of MC_U cocrystal, -OH of MC and -NH_2 of urea were marked by different colors; # indicates the chemical shift values of characteristic protons of MC_U cocrystal; $ indicates the chemical shift of active hydrogen and the value in red is the -OH of MC while the value in blue is the -NH_2 of urea. b) displays the different chemical shift of -NH_2 in urea and MC_U cocrystal, respectively. and c) shows the different chemical shift of -OH in MC and MC_U cocrystal, respectively. The schematic diagram of "inductive effect" which is shown between b) and c) diagrams can explain the cause of the phenomenon and the deviation of chemical shift.
^{1}H NMR (MC, 500 MHz, [D_6]DMSO, 25 °C, TMS): δ = 9.21 (s, 1H; OH), 7.03 (t, 1H; =CH-), δ = 6.56 (dd, 3H; =CH-), 2.21 ppm (s, 3H; CH_3).
^{1}H NMR (Urea, 500 MHz, [D_6]DMSO, 25 °C, TMS): δ = 5.44 ppm (s, 4H; NH_2).

Figure S6


a)
MC_U
Urea
MC
1002
1009
1015
530
470
306
591
326
1648
1540
1673
Intensity
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Raman Shift (cm-1)
b)
MC_U
Urea
MC
1648
1540
1673
Intensity
1700
1650
1600
1550
1500
1450
Raman Shift (cm-1)


c)
1009
1002
1015
MC_U
Urea
MC
Intensity
1060
1040
1020
1000
980
960
Raman Shift (cm-1)


d)
MC_U
Urea
MC
530
591
470
Intensity
650
600
550
500
450
Raman Shift (cm-1)

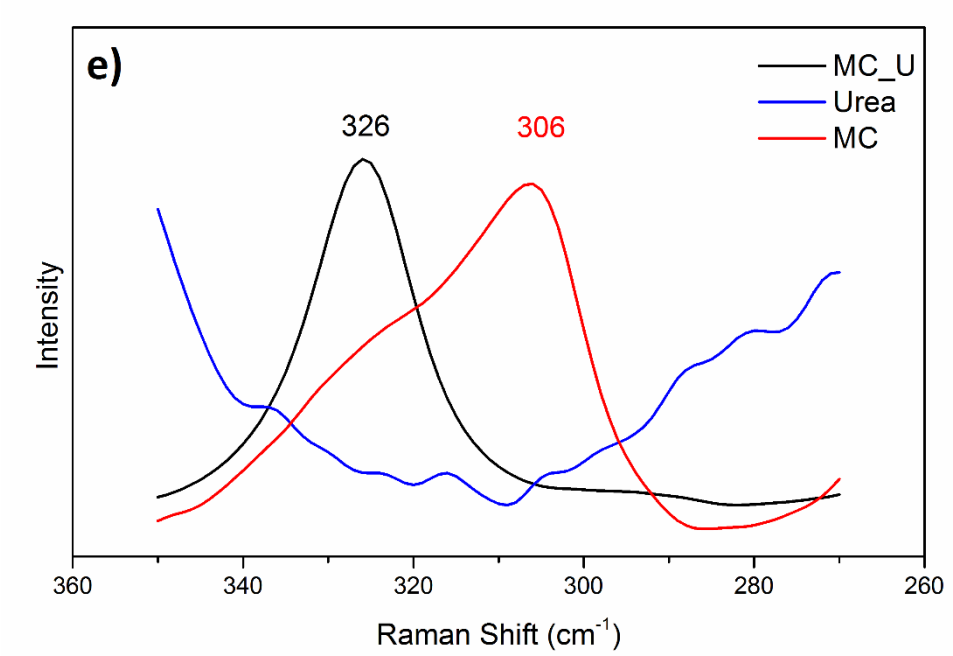


Figure S6. Raman spectra of urea, MC and MC_U cocrystal in the range of a) 1890-150 cm^{-1}; b) 1700-1450 cm^{-1}; c) 1050-960 cm^{-1}; d) 650-425 cm^{-1} and e) 350-270 cm^{-1}.

Figure S7

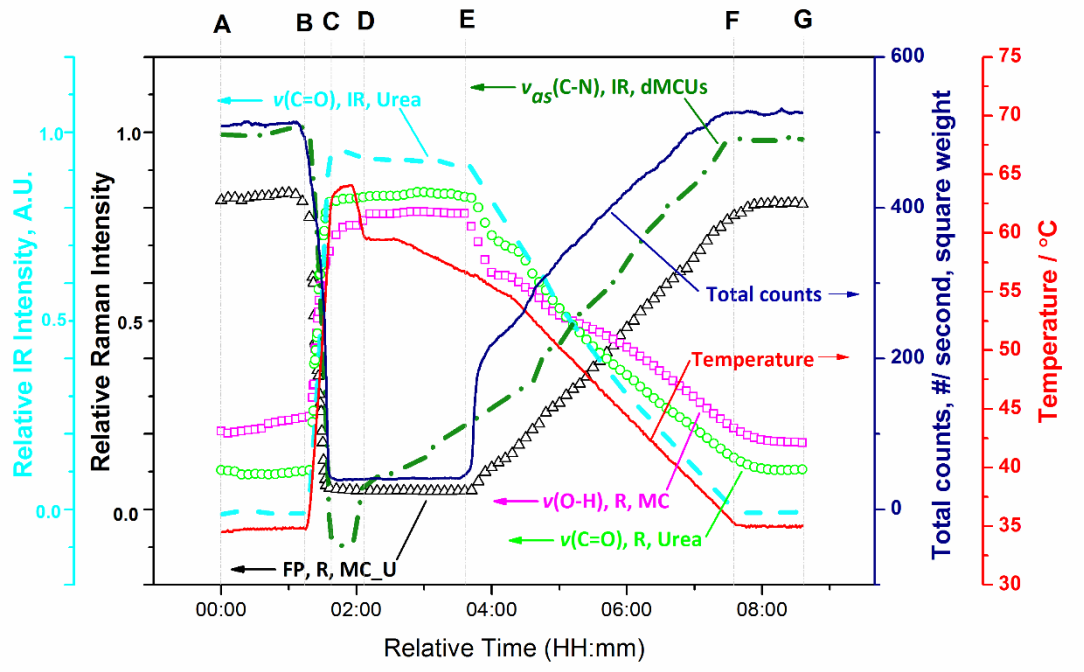


Figure S7. Changing trends of Raman, ATR-FTIR and FBRM data during cooling crystallization process of dimer verification experiments.

R: Raman data, IR: ATR-FTIR data, FP: fingerprint region of MC_U cocrystal

Figure S8

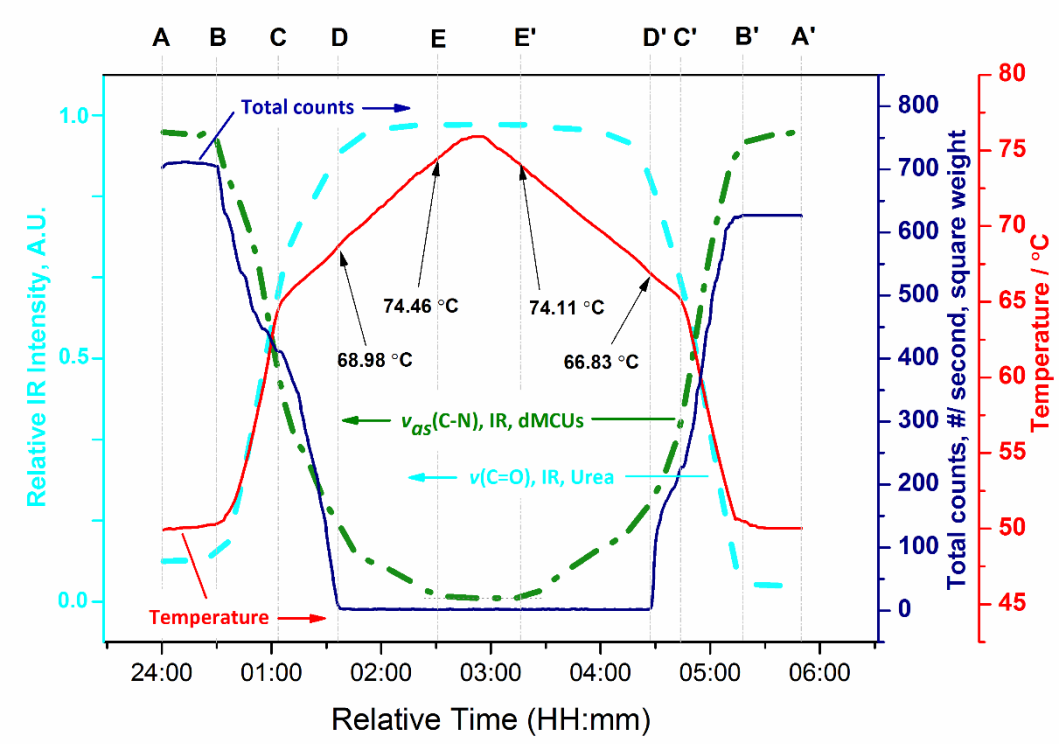


Figure S8. Changing trends of ATR-FTIR and FBRM data during heating and cooling processes for investigating the effect of temperature on formation of dMCUs: dMCUs disappear when temperature is higher than 74.46 °C while dMCUs are formed when temperature is lower than 74.11 °C.

3. Table contents

Table S1

Table S1. Crystal Data and Structure Refinement

empirical formula	$C_8H_{12}O_2N_2$
formula weight	168.20 g/mol
temperature	123 K
wavelength	0.71073 Å
crystal system, space group	orthorhombic, *Pbca* (61)
unit cell dimensions	a = 11.2502(5) Å, alpha =90 deg b = 7.1880(4) Å, beta = 90 deg c = 21.7868(15) Å, gamma = 90 deg
Volume	1761.82(17) $Å^3$
Z, calculated density	8, 1.26817 g/cm^3
F(000)	720
μ	0.092 mm^{-1}
limiting indices	$-12 \leq h \leq 13$, $-4 \leq k \leq 8$, $-18 \leq l \leq 25$
completeness to theta = 25.010	99.80%
T_{min}/T_{max}	0.989/0.995
data/restrains/parameters	2036/0/111
goodness-of-fit on $\hat{F}_2$	1.098
R-Factor (%)	5.52

Table S2

Table S2. Hydrogen Bonds For MC_U Cocrystal

D−H···A	*d*(D−H)	*d*(H···A)	*d*(D···A)	∠(DHA)	symop_for_A
O(11)-H(11)···O(1)	0.82	1.91	2.710(2)	164	.
N(3)-H(3A)···O(1)	0.86	2.07	2.929(2)	173	3/2-x, -1/2+y, z
N(4)-H(4A)···O(1)	0.86	2.09	2.946(2)	171	3/2-x, 1/2+y, z
N(3)-H(3B)···O(11)	0.86	2.20	2.969(2)	148	-1/2+x, y, 1/2-z
N(4)-H(4B)···O(11)	0.86	2.26	3.009(2)	146	-1/2+x, y, 1/2-z

Table S3

Table S3. The Characteristic Frequencies (cm^{-1}) of Urea, *m*-Cresol and MC_U

	Frequencies (cm^{-1})		Assignments		Frequencies (cm^{-1})		Assignments		Frequencies (cm^{-1})		Assignments
	IR	**Raman**			**IR**	**Raman**			**IR**	**Raman**	
U [a]	3435 3335 3260		$\nu_{as}(NH_2)$ $\nu_s(NH_2)$	MC	3332 3041 2926 1668		ν(OH) ν(=C-H) $\nu(CH_3)$ γ(=C-H)	MC_U	3442 3343 3210 1684	1673	$\nu_{as}(NH_2)$ $\nu_s(NH_2)$ ν(OH) ν(C=O)
	1679 1666[L]	1678 1648	ν(C=O)		1594 1490	1617 1593	ν(C=C)		1655[L] 1631 1618[L]	1623	$\delta_s(NH_2)$
	1625 1621[L] 1614[L]	1624	$\delta_s(NH_2)$		1457	1380	$\delta_{as}(CH_3)$ $\delta_s(CH_3)$		1603 1591[L]	1590	ν(C=C)
	1580[L]	1579 1540	ν(C=O)		1329 1275	1271	ν(C-O)			1544	ν(C=O)
	1464 1450[L]	1468	ν_{as}(C-N)			1163 1002	ν(C=C) ν(C=C)		1491[L] 1468 1461[L] 1442[L]	1484	ν_{as}(C-N)
	1167[L] 1157	1176	$\rho_s(NH_2)$		776 733 689	780 736 690	meta		1344 1338[L] 1277	1341 1283	ν(C-O)
	1054 1005 784 715	1010 781 701	$\rho_{as}(NH_2)$ ν_s(C-N) ω(C=O) $\tau_{as}(NH_2)$			445 306	ν(C-O) ν(O-H)		1267[L] 1159 1155[L]	1167	$\rho_s(NH_2)$
	564	570 547	ρ(C=O)						1059[L] 1048 1039[L] 1006	1089 1015 1000	FP region [b]
									811 767 683	735 685	meta
										591	C=O···H-O
										444 325	ν(C-O) ν(O-H)

Symbols

ν: Stretching

δ: Deformation Vibration

τ: Torsion

γ: Out-of-Plane Bending

β: In-Plane Bending

ω: Out-of-Plane Waging

ρ: In-Plane Waging

Subscript

$_s$: Symmetric

$_{as}$: Antisymmetric

Superscript

^{L}: ATR-FTIR data

^{a}: Urea

^{b}: fingerprint region

Table S4

With the help of MestReNova software which is used for analysis of NMR data, we get the following Table S4 to explain the dMCUs. Samples of 7.1 mg (MC_U cocrystal), 7.0 mg (MC) and 7.3 mg (urea) were dissolved into 0.6 ml of DMSO-d6 (0.03% (v/v) TMS), respectively. The original and normalized NMR data are shown in Table S4. The normalization process for the "normalized 2 data" in the last column of the Table S4 is as follows:

Firstly, the concentrations of the three samples were normalized to the same concentration.

Secondly, normalized data of MC and urea were converted to the value of the same scale with MC_U cocrystal, that is, the "normalized data" of MC will be multiplied by 64.29% to get the "normalized 2 data" column, while the "normalized data" of urea will be multiplied by 35.71% to get the corresponding "normalized 2 data", in the column "Normalized 2". And 64.29% and 35.71% are the proportion of MC and urea in MC_U cocrystal, respectively.

Take the OH in MC for example, the "normalized 2 data" is 12.16.

And 12.16 = 18.65 * 7.1 / 7.0 * 64.19%.

In ^{1}H NMR, the peak area is proportional to the number of protons and the ratio of the peak area is the height of the integral curve (T. Pan, Y. Zhang and Suchman, Spectrum analytical method, East China University of Science and Technology Press, Shanghai, 2009.). From the Normalized column and the Component 1 (MC_U cocrystal) in Table S4, we can know that the ratio of protons in the group is 1:1:3:4:3 (11.30 : 11.51 : 33.74 : 52.27 : 36.96 ≈1:1:3:4:3 = (1H; OH) : (1H; =CH-) : (3H; =CH-) : (4H; NH_2) ; (3H; CH_3)). And the ratio means that MC_U cocrystal solution contains equimolar amounts of MC and urea. Therefore, the dimers (dMCUs) are present in the solution. What's more, by comparing Component 2 and 3 with Component 1 in the Normalized 2 column, we can know that the normalized values are very close , for example, 12.16/11.30 ≈ 1 and 55.30/52.27 ≈ 1. It means that dMCUs content only one part of MC and one part of urea. So, we can conclude that the *m*-cresol-urea dimers (dMCUs), rather than trimers and tetramers, exist in solution even before MC_U cocrystals appear as a solid.

Table S4. The original and normalized ^{1}H NMR data of Urea, *m*-Cresol and MC_U

Components		Assignment	Chemical Shift /ppm	Range [a] /ppm	Normalized [b]	Normalized 2 [c]
1	MC_U cocrystal	s, 1H; OH	9.22	9.24 ~ 9.20	11.30	11.30
		t, 1H; =CH-	7.02	7.06 ~ 6.99	11.51	11.51
		dd, 3H; =CH-	6.56	6.61 ~ 6.51	33.74	33.74
		s, 4H; NH_2	5.42	5.54 ~ 5.30	52.27	52.27
		s, 3H; CH_3	2.21	2.23 ~ 2.19	36.96	36.96
	TMS	-	0.00	0.02 ~ -0.02	1.00 [d]	1.00 [d]
2	MC	s, 1H; OH	9.21	9.23 ~ 9.19	18.65	12.16
		t, 1H; =CH-	7.03	7.06 ~ 6.99	19.12	12.47
		dd, 3H; =CH-	6.56	6.61 ~ 6.51	55.21	36.00
		s, 3H; CH_3	2.21	2.23 ~ 2.19	60.60	39.52
	TMS	-	0.00	0.02 ~ -0.02	1.00 [d]	-
3	Urea	s, 4H; NH_2	5.44	5.55 ~ 5.32	159.19 [e]	55.30
	TMS	-	0.00	0.02 ~ -0.02	1.00 [d]	-

[a]: Integral range of a certain characteristic peak

[b]: Normalized values compared with the standard peak is processed by MestReNova software. In this process, the peak of TMS was selected as the standard peak.

[c]: Normalized values at the same concentration compared with the standard peak

[d]: Standard peak, and the normalized value of the standard peak is 1.00.

[e]: The relative content of TMS is too low and the relative content of urea is too high, resulting in the normalized value of NH_2 in urea more than 100.

4. CCDC number

1541360