Electronic Supplementary Information

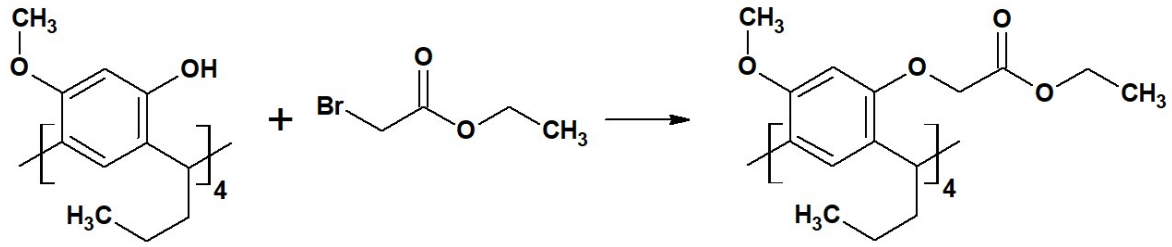
Calcium oxalate crystallization in synthetic urinary medium: the impact of resorcinares and calixarenes

Odin Bottrill[a], Matthew Boon[a], Franca Jones[a]*, Mauro Mocerino[a]

[a] *School of Molecular and Life Sciences, Curtin University, Kent Street, 6152, Perth Western Australia.*

Synthesis Methods

Synthesis of $1^6,3^6,5^6,7^6$-tetra(ethoxycarbonylmethyleneoxy)-$1^4,3^4,5^4,7^4$-tetramethoxy-2,4,6,8-tetrapropylresorcin[4]arene

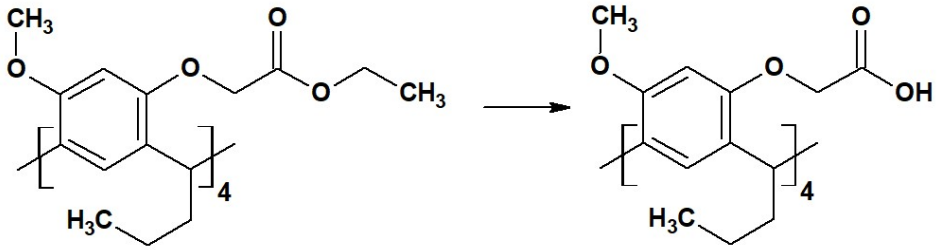


The tetrahydroxy tetramethoxyresorcin[4]arene (1.2 g, 1.68 mmol) was stirred with ethyl bromoacetate (1.5 mL, 13.5 mmol) in acetonitrile until the solid completely dissolved. Then potassium carbonate (1.8 g, 13.0 mmol) was added, forming a suspension as it stirred. This mixture was then heated to reflux for 40 hours. The solid was then filtered off and the solvent reduced under vacuum. The resulting oil was dissolved in diethyl ether (10 mL) and then washed in brine (2x20 mL). This was then dried with magnesium sulfate, filtered and final reduced under vacuum to give a white powder (1.256 g, 77%)

IR (ν/cm^{-1}): 2952 w (C-H), 1749 s (C=O), 1612 w (C-H aro), 1188 w (C-O).

^{1}H NMR (δ/ ppm, $CDCl_3$): 0.92 (t, 12H, $CH_2\boldsymbol{CH_3}$, J = 7.4 Hz), 1.28 - 1.31 (m, 12H, $OCH_2\boldsymbol{CH_3}$), 1.32 - 1.37 (m, 8H, $CH_2\boldsymbol{CH_2}CH$), 1.78 - 1.84 (m, 8H, $CH_3\boldsymbol{CH_2}CH_2$), 3.61 (s, 12H, $\boldsymbol{CH_3}O$), 4.10 – 4.16 (m, 8H, $CH_3\boldsymbol{CH_2}O$), 4.20 – 4.26 (m, 8H, $O\boldsymbol{CH_2}C{=}O$), 4.54 (t, 4H, $Ar\boldsymbol{CH}CH_2$, J = 7.6), 6.30 (s, 4H, H-Ar), 6.63 (s, 4H, H-Ar).

$1^6,3^6,5^6,7^6$-tetra(carbonylmethyleneoxy)-$1^4,3^4,5^4,7^4$-tetramethoxy-2,4,6,8-tetrapropylresorcin[4]arene

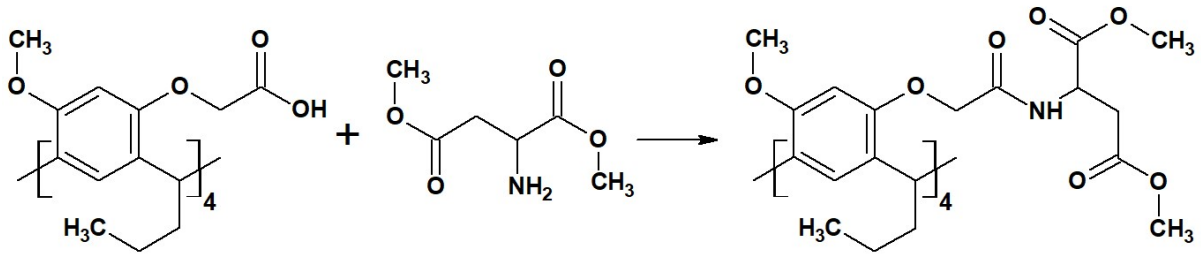


Sodium hydroxide pellets (0.15 g, 3.7 mmol) were dissolved in deionised water (4 mL), then added to tetra **(**ethoxycarbonylmethyleneoxy) tetramethoxyresorcin[4]arene (0.5 g, 0.47 mmol) in methanol (15 mL) and this was heated to reflux for 3 hours. The solvent was removed under vacuum and the resulting liquid was acidified with HCl and the solid which formed was then vacuum filtered to give a white powder (0.443 g, 88%).

ATR-IR: (ν/cm^{-1}): = 3446 w (O-H), 1731 (C=O), 1609 w (C-H aro), 1176 w (C-O).

^{1}H NMR (δ/ ppm, Acetone-d^6): 0.93 (t, 12H, $CH_2\boldsymbol{CH_3}$, J = 7.4 Hz), 1.32 - 1.34 (m, 8H, $CH_2\boldsymbol{CH_2}CH$), 1.86 - 1.88 (m, 8H, $CH_3\boldsymbol{CH_2}CH_2$), 2.87 (s, 4H, O***H***) , 3.68 (s, 12H, $\boldsymbol{CH_3}O$), 4.26 – 4.33 (m, 8H, $O\boldsymbol{CH_2}C{=}O$), 4.70 – 4.65 (m, Ar***CH***CH_2), 6.53 (s, 4H, H-Ar), 6.85 (s, 4H, H-Ar).

1^6,3^6,5^6,7^6-tetra[(dimethyl-*L*-aspartyl)-*N*-carbonylmethyleneoxy]-1^4,3^4,5^4,7^4-tetramethoxy-2,4,6,8-tetrapropylresorcin[4]arene

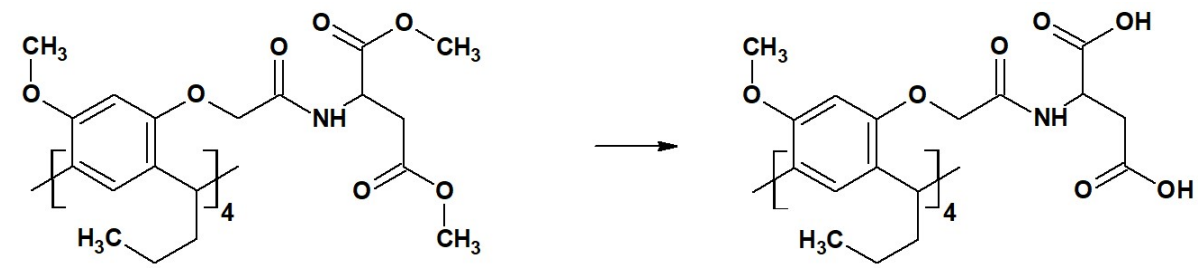


The tetra(carbonylmethyleneoxy) tetramethoxy tetrapropylresorcin[4]arene (0.5 g, 0.5 mmol), aspartic acid dimethyl ester (1.65 g, 11.4 mmol) and triethylamine (1.15 mL, 8.25 mmol) was dissolved in dichloromethane (30 mL) at room temperature and then TBTU (2.81 g. 5 mmol) was added. This was stirred at room temperature for 4 hours before being quenched by HCl. The organic layer was then extracted and washed with sodium bicarbonate (2 x 20 mL). The organic layer was then dried with magnesium sulfate, filter and finally the solvent was then reduced under vacuum to give a white powder (0.355 g, 45 %).

ATR-IR: (ν/cm^{-1}) = 3405 (N-H), 1731 (C=O), 1676 (N**C=O**), 1498 (C-O), 1193 (C-O)

^{1}H NMR (δ/ ppm, $CDCl_3$): (Note: there is a 1:2 mixture of diastereoisomers) 0.91 – 0.95 (m, 12H, $CH_2\boldsymbol{CH_3}$), 1.32 - 1.34 (m, 8H, $CH_2\boldsymbol{CH_2}CH$), 1.82 - 1.85 (m, 8H, $CH_3\boldsymbol{CH_2}CH_2$), 2.23 (s, 4H, ***NH***) 2.83 (s, 12H, $\boldsymbol{CH_3}O$), 3.63 – 3.64 (m, 8H, $O\boldsymbol{CH_2}C{=}O$), 3.70 – 3.71 m, 8H, $O\boldsymbol{CH_2}(C{=}O)NH$), 4.50 - 4.57 (m, ***CH***NH), 4.88 – 4.91 (m, Ar***CH***CH_2), 6.30, 6.34, 6.72, 6.76 (4 s, 2 x 4H, ***H***-Ar),

1^6,3^6,5^6,7^6-tetra[(*L*-aspartyl)-*N*-carbonylmethyleneoxy]-1^4,3^4,5^4,7^4-tetramethoxy-2,4,6,8-tetrapropylresorcin[4]arene



Sodium hydroxide pellets (0.1 g, 2.5 mmol) were dissolved in deionised water (4 mL), then added to tetra **(**dimethyl -L-aspartyl) tetramethoxyresorcin[4]arene (0.35 g, 0.23 mmol) in methanol (15 mL), this was heated to reflux for 3 hours. The solvent was removed under vacuum and the resulting liquid was acidified with HCl and the solid which formed was then vacuum filtered and then dried over P_2O_5. Yield was a light orange powder (0.316 g, 90%)

ATR-IR: (ν/cm^{-1}) = 3389 (N-H), 2931 (C-H), 1732 (C=O), 1635 (N-**C=O**), 1192 (C-O)

^{1}H NMR (δ/ ppm, $CDCl_3$): (Note: there is a 1:2 mixture of diastereoisomers) 0.92 – 0.97 (m, 12H, $CH_2\boldsymbol{CH_3}$), 1.32 - 1.34 (m, 8H, $CH_2\boldsymbol{CH_2}CH$), 1.82 - 1.84 (m, 8H, $CH_3\boldsymbol{CH_2}CH_2$), 2.87 (s, 12H, $\boldsymbol{CH_3}O$), 3.63 – 3.64 (m, 8H, $O\boldsymbol{CH_2}C=O$), 3.70 – 3.71 m, 8H, $O\boldsymbol{CH_2}(C=O)NH$), 4.50 - 4.57 (m, ***CH***NH), 4.88 – 4.91 (m, Ar***CH***CH_2), 6.30, 6.34, 6.72, 6.76 (4 s, 2 x 4H, ***H***-Ar),

Additional SEM images

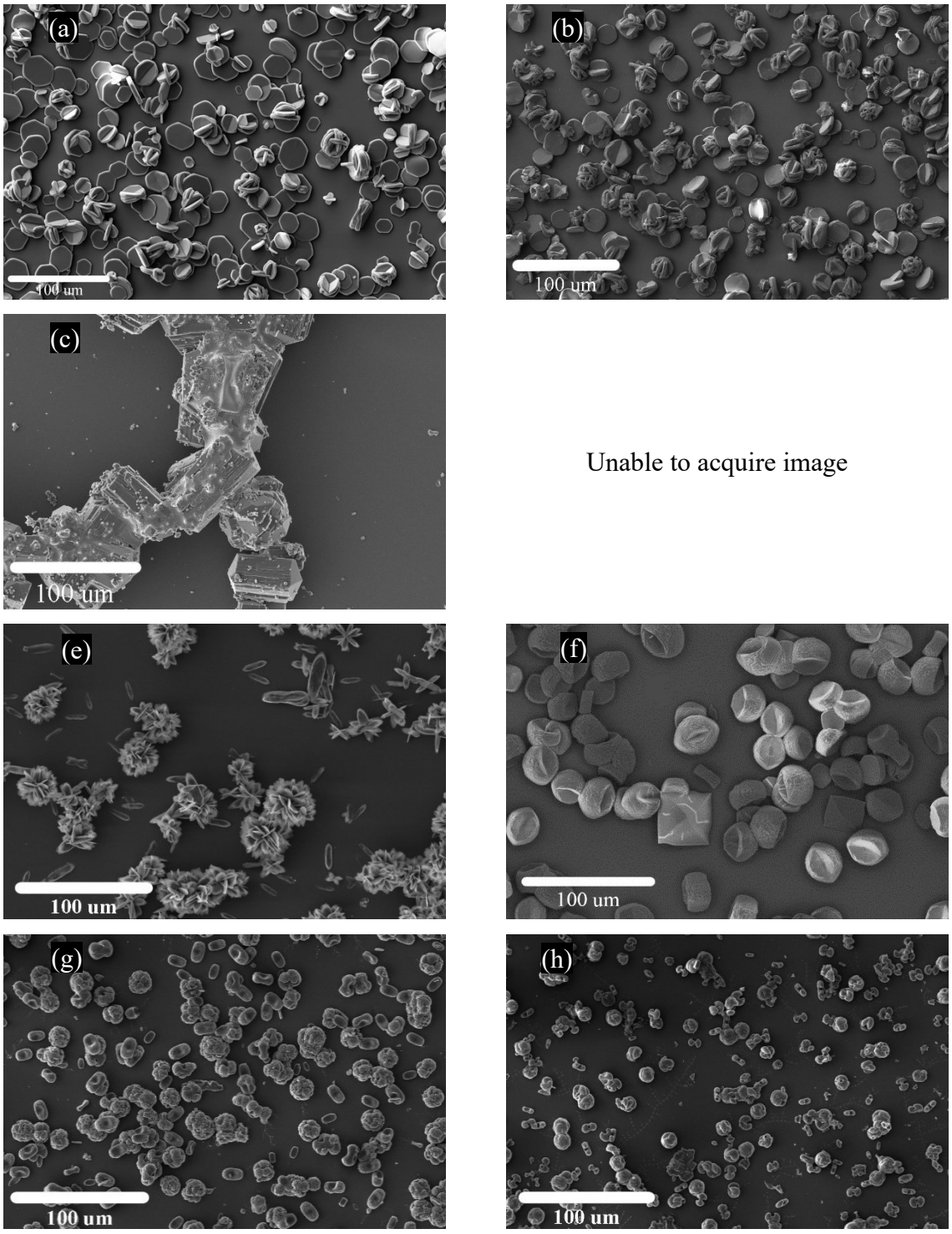


Figure S1. SEM micrographs of CaOx in (a) pure SUM; (b) SUM+Zn^{2+} (c) PRAsp, 1 mM; (d) PRAsp+Zn^{2+}; (e) CPro, 1 mM; (f) CPro+Zn^{2+}; (g) PCLys, 1 mM; (h) PCLys+Zn^{2+} 1mM. Scale bar 100 µm

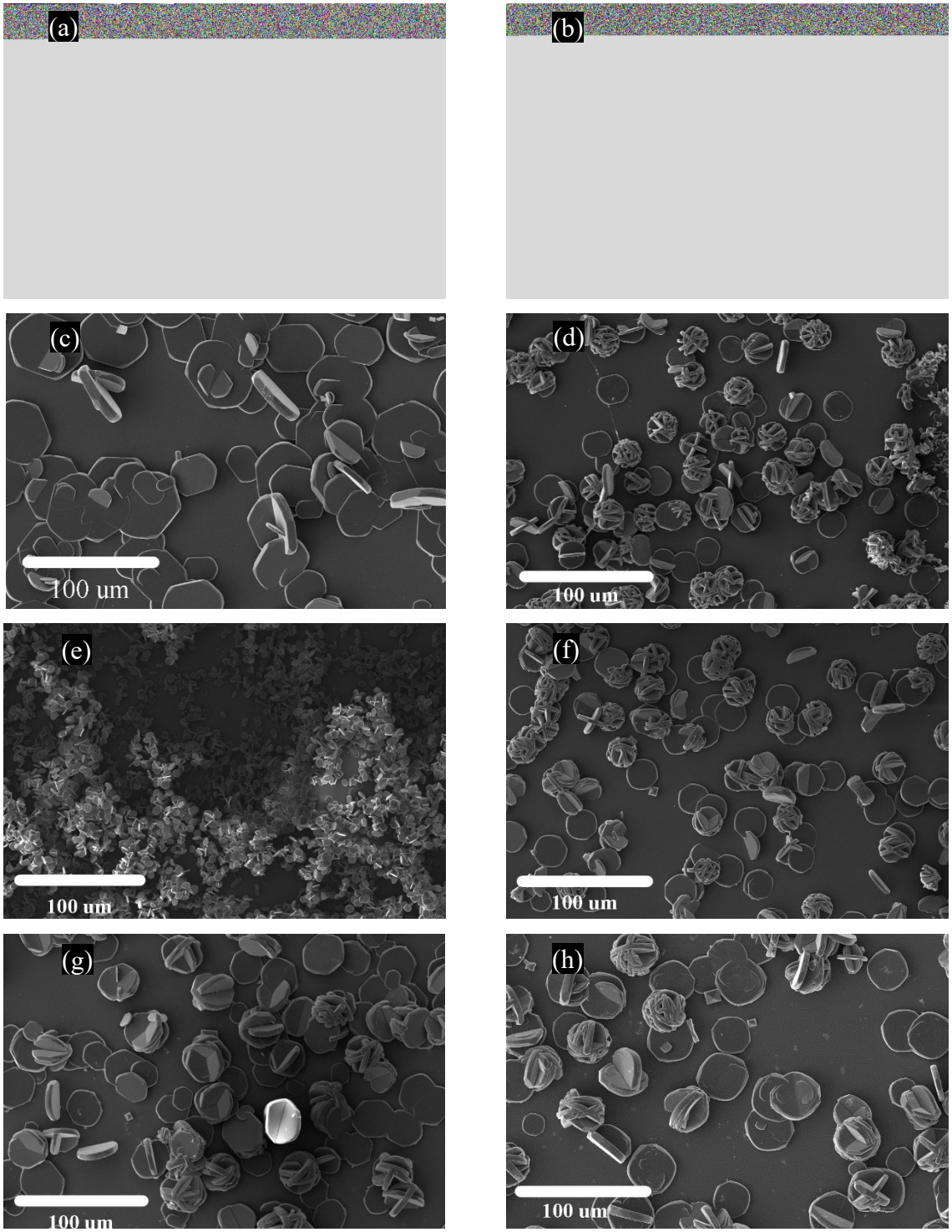


Figure S2. SEM micrographs of CaOx in (a) pure SUM; (b) SUM+Zn^{2+} (c) Asp, 4 mM; (d) Asp+Zn^{2+}; (e) Pro, 4 mM; (f) Pro+Zn^{2+}; (g) Lys, 4 mM; (h) Lys+Zn^{2+} 1mM. Scale bar 100 µm

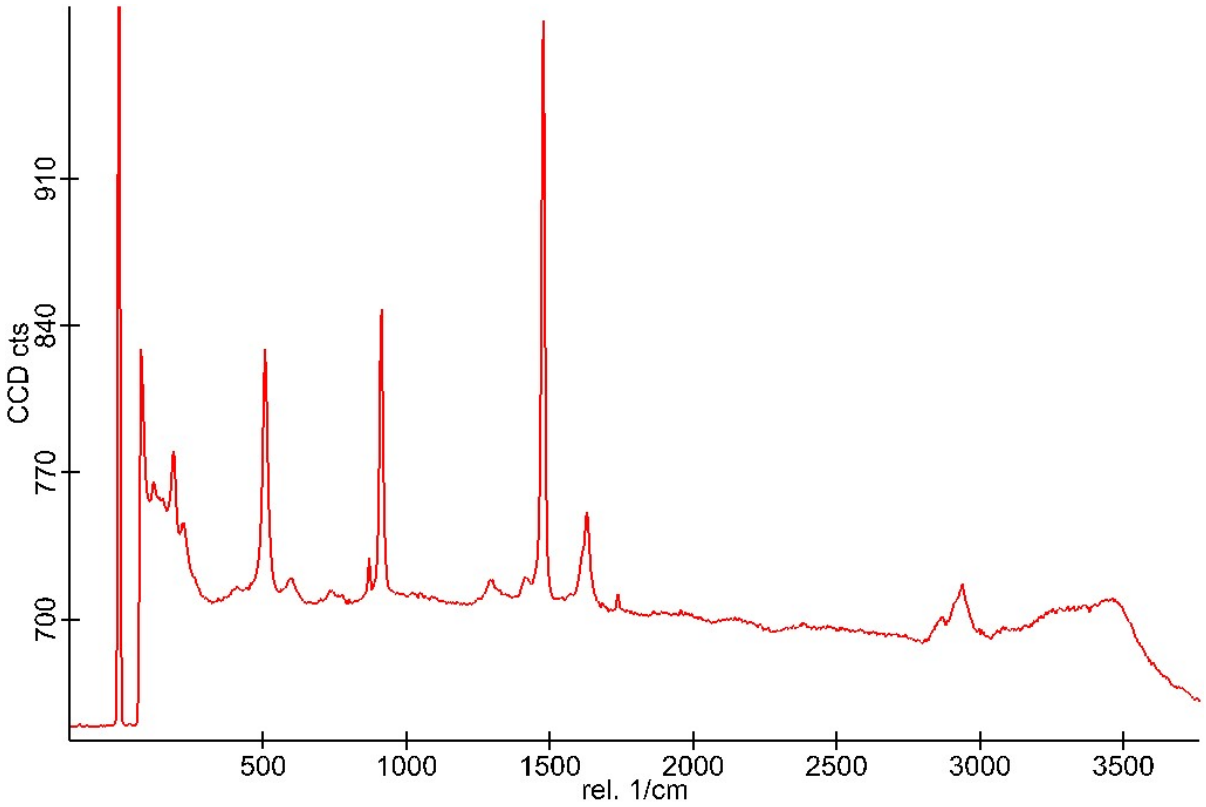


Figure S3. Recorded signal for the PRAsp crystals formed showing singlet at 1500 1/cm representing COD

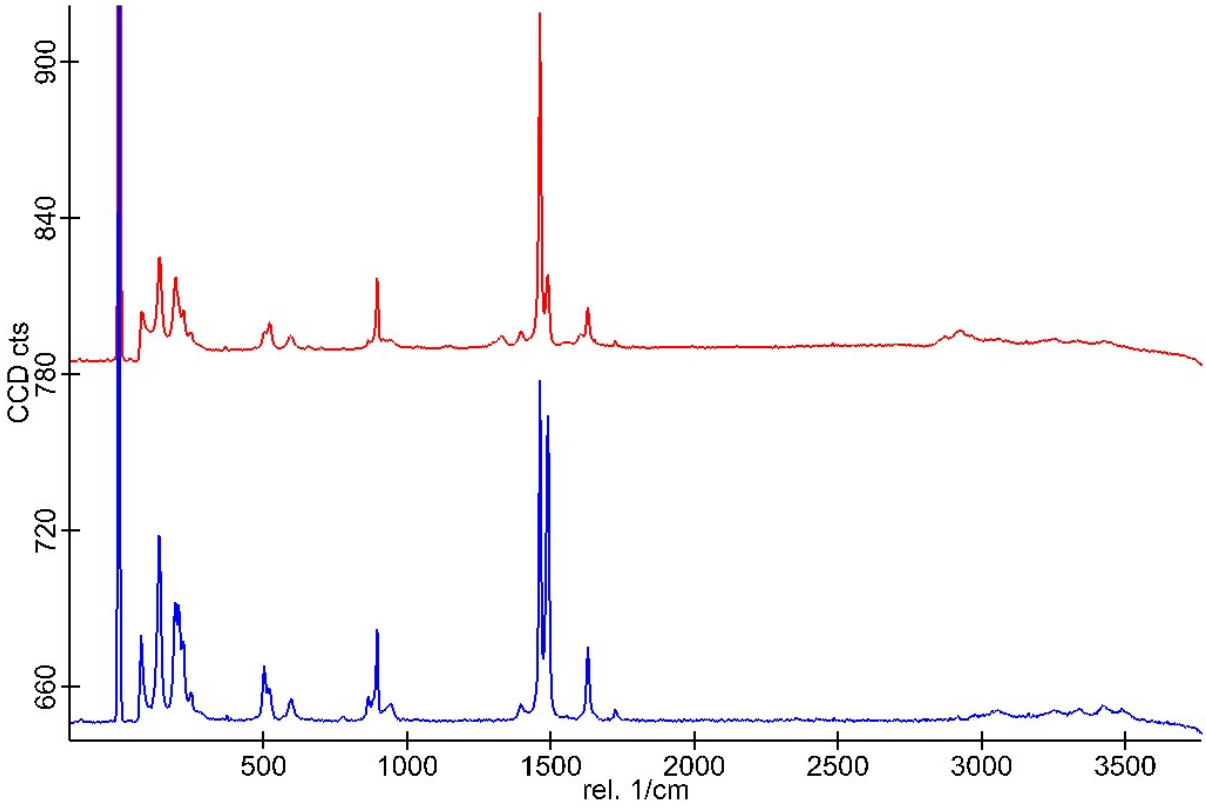


Figure S4. Raman spectrum collected with 532 nm laser. PCLys (red) vs COM pure (blue) showing the signals from COM on the crystals formed in the presence of PCLys

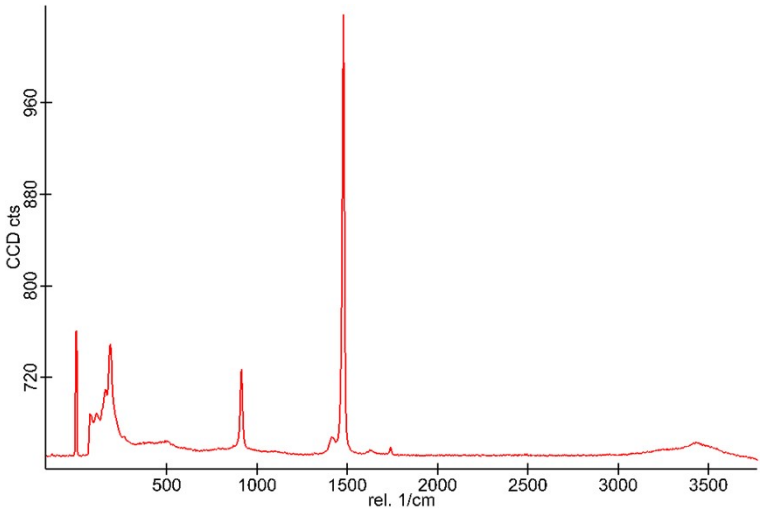


Figure S5. Raman spectrum of pure COD in SUM obtained from crystallisation at approximately 4ºC for 24 hours

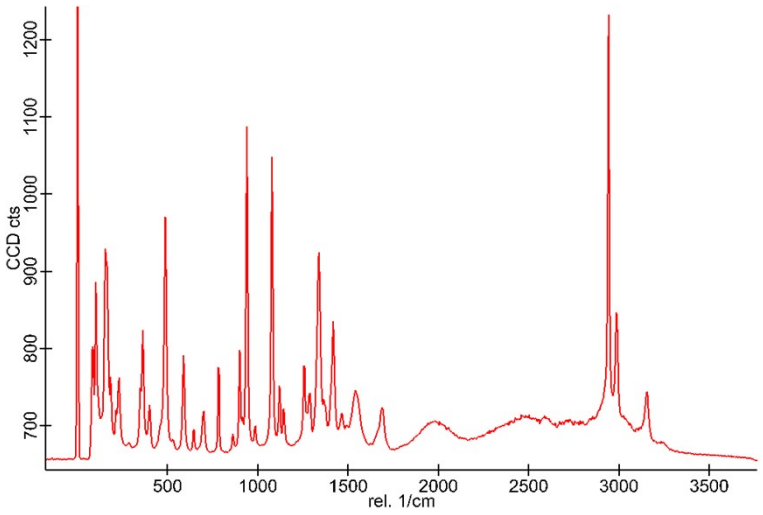


Figure S6. Raman spectrum of DL-Asp amino acid showing strong signals in the 2800-3000 1/cm region

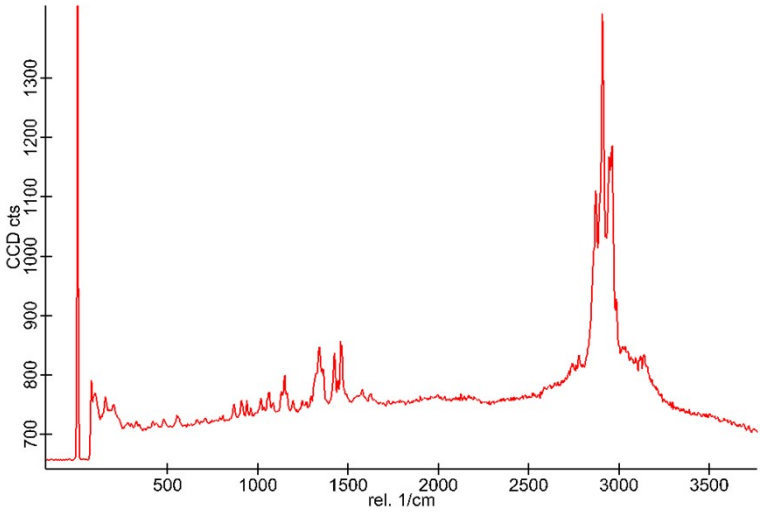


Figure S7. Raman spectrum of DL-Lys-HCl amino acid showing strong signals in the 2800-3000 1/cm region

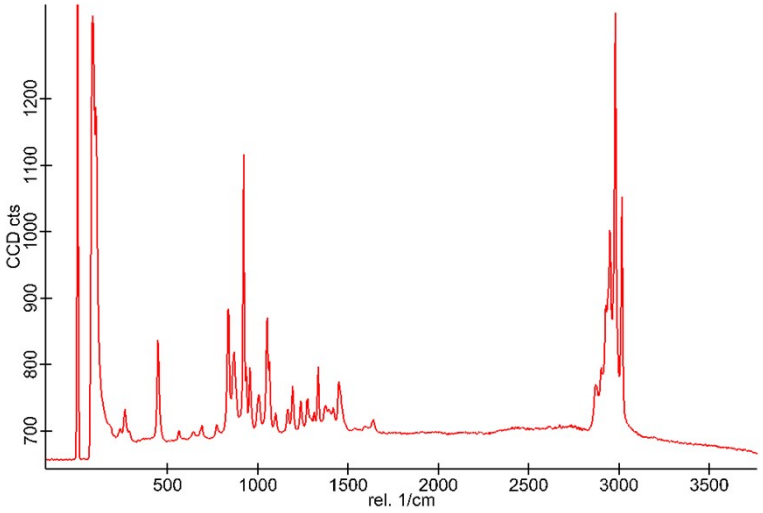


Figure S8. Raman spectrum of DL-Pro amino acid showing strong signals in the 2800-3000 1/cm region

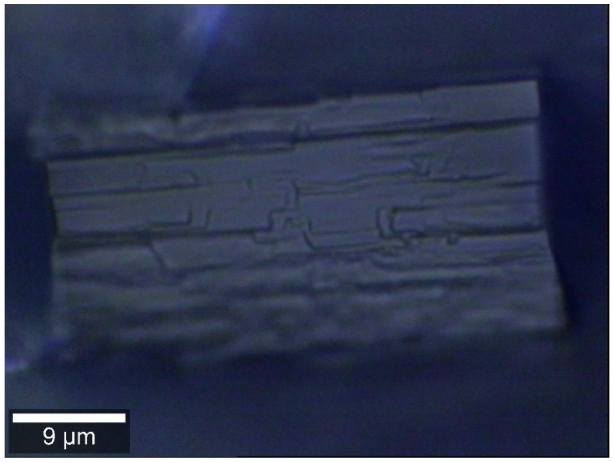


Figure S9. Optical image of the COD crystal formed in the presence of PRAsp scanned in depth analysis

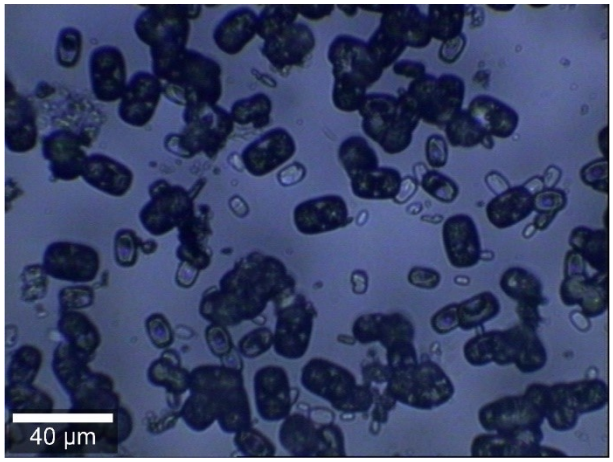


Figure S10. Optical image of the COD crystals formed in the presence of PCLys scanned in depth analysis

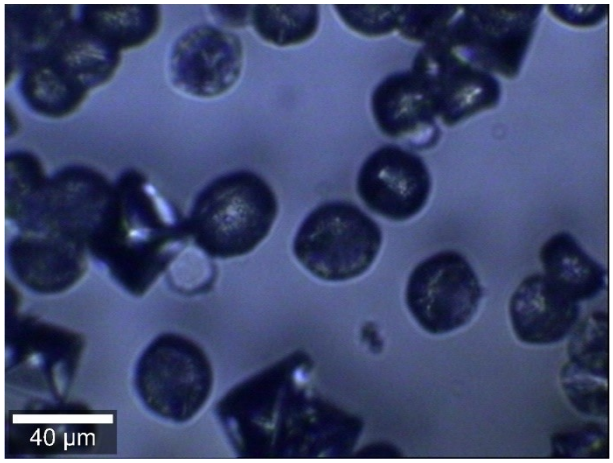


Figure S11. Optical image of the COM crystals formed in the presence of CPro scanned in depth analysis