Supporting Information

Substrate-Induced Self-Assembly of Cooperative Catalysts

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## Table of Contents

1. Abbreviations ................................................................. S3

2. General Information ..................................................... S4

3. Procedures and Characterisation Data for Novel Compounds .... S6

4. Further detail for the experiments shown in the Figures of the manuscript ........................... S12

5. $^1$H-NMR and $^{13}$C-NMR Spectra for Novel Compounds .......... S22

6. References ................................................................. S31
1. Abbreviations

- cac: critical aggregation concentration
- DLS: Dynamic Light Scattering
- h: hour(s)
- HPNPP: hydroxypropyl p-nitrophenyl phosphate
- HRMS: high resolution mass spectrometry
- IR: infrared
- NMR: nuclear magnetic resonance
- ppm: parts per million
- PMA: phosphomolybdic acid
- rt: room temperature
- s: second(s)
- T: temperature
- TACN: 1,4,7-triazacyclononane
- TEM: Transmission electron microscopy
- UV: ultraviolet
2. General Information

All fine chemicals were sourced from commercial suppliers and were used directly without purification unless mentioned. Compounds lacking experimental details were prepared according to the literature as cited. Flash column chromatography was performed using silica gel 60 (Aldrich) and a suitable eluent. Analytical TLC was performed on aluminium backed plates pre-coated (0.25 mm) with Merck Silica Gel 60 F254 with a suitable solvent system and was visualised using UV fluorescence (254 nm) and/or developed with ninhydrin, phosphomolybdic acid (PMA) or potassium permanganate.

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma-Aldrich and used without further purification. The pH of buffer solutions was determined at room temperature using a Metrohm-632 pH-meter equipped with a Ag/AgCl/KCl reference electrode. In all cases, stock solutions were prepared using deionised water filtered with a MilliQ-water-purifier (Millipore) and stored at 4 °C unless stated otherwise. Zn(NO$_3$)$_2$ were analytical grade products and the concentration of stock solutions were determined by atomic absorption spectroscopy. Stock solutions of the HPNPP[S1] substrate were prepared by weight and their concentrations were confirmed by UV spectroscopy and adjusted if necessary.

$^1$H, $^{13}$C and $^{31}$P NMR spectra were recorded using a Bruker AV300 spectrometer operating at 300 MHz for $^1$H at ambient temperature or using a Bruker AV500 operating at 500 MHz for $^1$H. Chemical shifts ($\delta$) are quoted in parts per million (ppm) and coupling constants ($J$) are in Hertz (Hz). Residual solvent peaks were used as the internal reference for $^1$H and $^{13}$C NMR. A solution of K$_2$HPO$_4$ at pH 7 (HEPES buffer 10 mM) was used as a coaxial reference ($\delta = 0$ ppm) for $^{31}$P-NMR spectra. Abbreviations for multiplicity are as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, etc.).

Infrared (IR) spectra were recorded using a Perkin Elmer Spectrum 100 FT-IR spectrometer on a film ATR sampling accessory. Absorption maxima are expressed in wavenumbers (cm$^{-1}$) and recorded using a range of 450-4000 cm$^{-1}$. High resolution mass spectrometry (HRMS) measurements were obtained using a Bruker microTOF-Q II mass spectrometer operating at a nominal accelerating voltage of 70 eV.

UV-Vis measurements were recorded on Varian Cary 50 or Cary 100 equipped with thermostatted multiple cell holders spectrophotometers with thermostatted cell holders. Calibration curves were carried out by measuring the absorbance at 405 nm and 40 °C of increasing amounts of the leaving group (4-nitrophenol) to an aqueous solution buffered at pH 7.0 (10 mM). The obtained ε·l values obtained from applying the Lambert-Beer law were used to convert the kinetic signals (dA/dt) into reaction rate (dC/dt). Molar absorption coefficients used for monitoring p-nitrophenolate formation were: $\varepsilon_{405} = 10800$ M$^{-1}$ cm$^{-1}$, $\varepsilon_{400} = 11000$ M$^{-1}$ cm$^{-1}$.

Fluorescence measurements were recorded on a PerkinElmer LS 55 Luminescence Spectrometer, or on a Varian Cary Eclipse Fluorescence spectrophotometer equipped with a thermostatted cell holder. Nile red was added from a concentrated solution in EtOH (typically 5 µL of a 1 mM solution were present in a 1 mL solution). To confirm that the substrate was not substantially consumed in the course of the cac determination experiments, the formation of PNP was monitored at 405 nm, a wavelength at which the contribution to absorbance of Nile Red is negligible compared to that of PNP. The amount of formed PNP at the end of the experiment was always less than 20% of [HPNPP], and typically around 10% (also in this case at the end of the experiment).

Standard TEM images were recorded on either a Jeol 300 PX electron microscope or a FEI Tecnai FEG20 after staining with a 1% uranyl acetate solution for 30 s. Cryo-TEM samples were prepared using a FEI Vitrobot for plunge freezing and the images were recorded using a FEI Tecnai 12 with Gatan cold stages and a Gatan Ultrascan 100 4 Mpixel digital camera.
Confocal images were taken using a laser scanning confocal microscope (BX51WI-FV300-Olympus) coupled to a frequency doubled Ti:Sapphire femtosecond laser at 400 nm, 76 MHz (VerdiV5-Mira900-F Coherent). The laser beam was scanned on a 40x40 μm sample area with a 512x512 px resolution, using a 60x water immersion objective (UPLSAPO60xW-Olympus). The emission was collected with a photomultiplier tube using a 435 nm longpass filter.

DLS analysis were performed on a Malvern Zetasizer Nano-S instrument using filtered milli-Q water and a filtered buffer solution.

Curve fitting was performed using Origin fitting tools.
3. Procedures and Characterisation Data for Novel Compounds

General procedure 1 (GP1): N-Alkylation of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate

To a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (1 equiv.) in MeCN (0.1 M) were added K$_2$CO$_3$ (3 equiv.), NaHCO$_3$ (3 equiv.) and the corresponding 1-bromoalkane (2.5 equiv.). The reaction mixture was further stirred overnight at 70 ºC. The next day, after evaporation under reduced pressure, the crude product was purified by flash chromatography to obtain the alkylation product.

General procedure 2 (GP2): Boc deprotection of di-tert-butyl 7-alkyl-1,4,7-triazacyclononane-1,4-dicarboxylate

To a solution of the corresponding di-tert-butyl 7-alkyl-1,4,7-triazacyclononane-1,4-dicarboxylate in MeOH (0.5 M) was added 6M HCl (45 mL / mmol). The reaction mixture was further stirred for 3 hours at 60 ºC. The crude was evaporated under reduced pressure and the deprotected product was obtained without further purification.
di-tert-Butyl 7-ethyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S1)

According to GP1, to a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (76 mg, 0.23 mmol) in MeCN (5 mL) were added K$_2$CO$_3$ (99 mg, 0.69 mmol), NaHCO$_3$ (63 mg, 0.69 mmol) and 1-bromoethane (0.06 mg, 0.55 mmol). The crude product was purified by flash chromatography (50% EtOAc:hexanes) to obtain the title compound as a yellow oil (54 mg, 66%).

$^1$H NMR (400 MHz, CDCl$_3$) δ 3.55–3.41 (m, 4H, NC$_2$H$_2$C$_2$H$_2$N), 3.33–3.18 (m, 4H, NCH$_2$C$_2$H$_2$N), 2.67–2.51 (m, 6H, CH$_3$C$_2$H$_2$N and NC$_2$H$_2$CH$_2$N), 1.47 (s, 18H, C(C$_3$H$_7$)$_3$), 1.00 (t, J = 7.3 Hz, 3H, C$_3$H$_7$CH$_2$N).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.9 (CO), 79.5 (C(CH$_3$)$_3$), 79.4*, 79.4*, 53.6 (NCH$_2$CH$_2$N), 53.5*, 53.4 (NCH$_2$CH$_2$N), 53.1*, 51.0 (CH$_2$CH$_2$N), 50.9*, 50.8 (NCH$_2$CH$_2$N), 50.6*, 50.5*, 50.3*, 50.2 (NCH$_2$CH$_2$N), 50.1*, 50.0*, 49.9*, 28.7 (C(CH$_3$)$_3$), 28.7*, 12.9 (CH$_3$CH$_2$N). Spectral data is concordant with what is reported in the literature.$^{[62]}$

*Extra signals due to the presence of rotamers.

1-Ethyl-1,4,7-triazacyclononane (C$_2$TACN)

According to GP2, to a solution of S1 (30 mg, 0.08 mmol) in MeOH (1 mL) was added 6M HCl (3 mL). The crude was evaporated under reduced pressure and the product C$_2$TACN was obtained without further purification as a white solid (22 mg, 97%).

$^1$H NMR (400 MHz, D$_2$O) δ 3.43 (s, 4H, NC$_2$H$_2$CH$_2$N ), 3.36 (dd, J = 6.9, 4.5 Hz, 4H, NCH$_2$CH$_2$N), 3.26 (dd, J = 6.7, 4.7 Hz, 4H, NCH$_2$CH$_2$N), 3.06 (q, J = 7.2 Hz, 2H, NCH$_2$CH$_2$), 1.19 (t, J = 7.2 Hz, 3H, NCH$_2$CH$_2$).

$^{13}$C NMR (101 MHz, D$_2$O) δ 50.7 (NCH$_2$CH$_2$N), 47.8 (NCH$_2$CH$_2$N), 42.0 (NCH$_2$CH$_2$N), 41.8 (NCH$_2$CH$_2$), 9.0 (NCH$_2$CH$_2$). IR ν$_{max}$(film)/cm$^{-1}$: 3351, 1646, 1457, 1319, 1156, 1043, 877, 669. HRMS Found (ESI): MH$,^+$ 158.1651 C$_8$H$_{20}$N$_3$ requires 158.1652.
di-tert-Butyl 7-dodecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S2)

According to GP1, to a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (75 mg, 0.23 mmol) in MeCN (5 mL) were added K$_2$CO$_3$ (94 mg, 0.68 mmol), NaHCO$_3$ (57 mg, 0.68 mmol) and 1-bromodecane (142 mg, 0.57 mmol). The crude product was purified by flash chromatography (20% EtOAc:hexanes) to obtain the title compound as a yellow oil (72 mg, 71%).

$^1$H NMR (400 MHz, CDCl$_3$) δ 3.53–3.40 (m, 4H, NC$_2$H$_2$C$_8$H$_{15}$), 3.32–3.16 (m, 4H, NC$_2$H$_2$CH$_2$N), 2.66–2.57 (m, 4H, NCH$_2$C$_8$H$_{15}$), 2.51–2.41 (m, 2H, CH$_3$(CH$_2$)$_9$C$_8$H$_2$N), 1.47 (s, 18H, C(CH$_3$)$_3$), 1.26 (s, 20H, CH$_3$(C$_8$H$_2$)$_9$CH$_2$N), 0.87 (t, $J$ = 7.0 Hz, 3H, C(CH$_3$)$_3$CH$_2$N).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.7(CO), 79.3 (C(CH$_3$)$_3$), 79.3*, 79.3*, 79.2*, 57.0 (NCH$_2$C$_8$H$_2$N), 54.1(NCH$_2$CH$_2$N), 54.1*, 54.0*, 50.7 (CH$_3$(CH$_2$)$_9$C$_8$H$_2$N), 50.6*, 50.4*, 49.8 (NCH$_2$C$_8$H$_2$N), 31.9, 29.7, 29.7, 29.6, 29.3, 28.6*, 28.6*, 27.9, 27.5, 22.7 (CH$_3$(CH$_2$)$_9$C$_8$H$_2$N), 14.1(CH$_3$(CH$_2$)$_9$C$_8$H$_2$N).

IR $\nu_{max}$ (film)/cm$^{-1}$ 2922, 2846, 1696, 1458, 1411, 1365, 1260, 1152, 1094, 1027, 801.

HRMS Found (ESI): $\text{MH}^+$, 498.4251 C$_{28}$H$_{56}$N$_3$O$_4$ requires 498.4265.

*Extra peaks due to the presence of rotamers.

1-Dodecyl-1,4,7-triazacyclononane (C$_{12}$TACN)

According to GP2, to a solution of S2 (34 mg, 0.07 mmol) in MeOH (1 mL) was added 6M HCl (3 mL). The crude was evaporated under reduced pressure and the product C$_{12}$TACN was obtained without further purification as a white solid (26 mg, 96%).

$^1$H NMR (400 MHz, D$_2$O) δ 3.51 (s, 4H, NCH$_2$C$_8$H$_2$N), 3.28 (t, $J$ = 5.7 Hz, 4H, NCH$_2$C$_8$H$_2$N), 3.02 (d, $J$ = 6.2 Hz, 4H, NCH$_2$C$_8$H$_2$N), 2.84-2.70 (m, 2H, NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 1.52–1.45 (m, 2H, NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 1.18 (s, 18H, NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 0.82–0.73 (m, 3H, NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N).

$^{13}$C NMR (101 MHz, D$_2$O) δ 55.3 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 47.6 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 43.0 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 41.9 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 31.9, 29.8, 29.8, 29.7, 29.6, 29.4, 27.3, 24.1, 22.6 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N), 13.9 (NCH$_2$CH$_2$(CH$_2$)$_9$C$_8$H$_2$N). IR $\nu_{max}$ (film)/cm$^{-1}$ 3379, 2922, 2852, 1456, 689. HRMS Found (ESI): $\text{MH}^+$ 298.3206 C$_{18}$H$_{36}$N$_3$O$_4$ requires 298.3217.
di-tert-Butyl 7-tetradecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S3)

According to GP1, to a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (76 mg, 0.23 mmol) in MeCN (5 mL) were added K$_2$CO$_3$ (96 mg, 0.69 mmol), NaHCO$_3$ (58 mg, 0.69 mmol) and 1-bromo-12-tetradecane (160 mg, 0.58 mmol). The crude product was purified by flash chromatography (20% EtOAc:hexanes) to obtain the title compound as a yellow oil (68 mg, 82%).

$^1$H NMR (400 MHz, CDCl$_3$) δ 3.55–3.38 (m, 4H, NC$_2$H$_4$C$_2$H$_2$N), 3.32–3.17 (m, 4H, NC$_2$H$_2$CH$_2$N), 2.67–2.56 (m, 4H, NCH$_2$C$_2$H$_2$N), 2.51–2.43 (m, 2H, CH$_3$(CH$_2$)$_{11}$CH$_3$), 1.47 (s, 18H, C(CH$_3$)$_3$), 1.26 (s, 24H, CH$_3$(C$_2$H$_2$)$_{12}$CH$_2$N), 0.9–0.86 (m, 3H, C$_3$H$_3$(CH$_2$)$_{11}$CH$_3$).

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 155.7 (CO), 155.6*, 155.4*, 79.4 (C(CH$_3$)$_3$), 56.9 (NC$_2$H$_2$C$_2$H$_2$N), 54.0 (NC$_2$H$_2$CH$_2$N), 53.6*, 50.8 (CH$_3$(C$_2$H$_2$)$_{13}$N), 49.7 (NC$_2$H$_2$C$_2$H$_2$N), 31.9, 29.7, 29.7, 29.4 (C(CH$_3$)$_3$), 28.6 (C(CH$_3$)$_3$), 28.6*, 27.6, 27.5, 22.7 (CH$_3$(C$_2$H$_2$)$_{13}$N), 14.1 (C(CH$_3$)$_3$).

IR ν$_{max}$ (film)/cm$^{-1}$: 2923, 2853, 1687, 1460, 1412, 1365, 1247, 1153, 731.

HRMS Found (ESI): MH$^+$ 526.4565 C$_{30}$H$_{60}$N$_3$O$_4$ requires 526.4578.

*Extra peaks due to the presence of rotamers.

1-Tetradecyl-1,4,7-triazacyclononane (C$_{14}$TACN)

According to GP2, to a solution of S3 (41 mg, 0.08 mmol) in MeOH (1 mL) was added 6M HCl (3 mL). The crude was evaporated under reduced pressure and the product C$_{14}$TACN was obtained without further purification as a white solid (12 mg, 40%).

$^1$H NMR (400 MHz, D$_2$O) δ 3.49 (s, 4H, NC$_2$H$_2$C$_2$H$_2$N), 3.24 (t, J = 5.7 Hz, 4H, NCH$_2$C$_2$H$_2$N), 2.95 (d, J = 5.7 Hz, 4H, NCH$_2$C$_2$H$_2$N), 2.69 (m, 2H, NCH$_2$C$_2$H$_2$(CH$_2$)$_{11}$CH$_3$), 2.44 (s, 2H, NCH$_2$C$_2$H$_2$(CH$_2$)$_{11}$CH$_3$), 1.44 (s, 2H, NCH$_2$C$_2$H$_2$(CH$_2$)$_{11}$CH$_3$), 1.14 (d, J = 10.3 Hz, 22H, NCH$_2$C$_2$H$_2$(CH$_2$)$_{11}$CH$_3$), 0.82–0.66 (m, 3H, NCH$_2$C$_2$H$_2$(CH$_2$)$_{11}$CH$_3$).

$^{13}$C NMR (101 MHz, D$_2$O) δ 55.2 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 47.6 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 46.7 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 43.1 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 41.9, 32.0, 30.0, 30.0, 29.9, 29.9, 29.8, 29.5, 27.5 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 24.2, 22.7 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 13.9 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$), 8.2 (NC$_2$H$_2$(CH$_2$)$_{12}$CH$_3$).

IR ν$_{max}$ (film)/cm$^{-1}$: 3360, 2923, 2853, 1687, 1460, 1412, 1365, 1247, 1153, 731. HRMS Found (ESI): MH$^+$ 326.4565 C$_{20}$H$_{44}$N$_3$ requires 326.4578.

S9
di-tert-Butyl 7-hexadecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S4)

According to GP1, to a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (10 mg, 0.03 mmol) in MeCN (0.6 mL) were added K$_2$CO$_3$ (27 mg, 0.09 mmol), NaHCO$_3$ (7.6 mg, 0.09 mmol) and 1-bromohexadecane (24.4 mg, 0.08 mmol). The crude product was purified by flash chromatography (20% EtOAc:hexanes) to obtain the title compound as a yellow oil (8.8 mg, 52%).

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.53–3.42 (m, 4H, NC$_2$H$_2$C$_6$H$_2$N), 3.29–3.20 (m, 4H, NC$_2$H$_2$CH$_2$N), 2.62 (dt, $J$=10.9, 4.8 Hz, 4H, NCH$_2$C$_6$H$_2$N), 2.47 (t, $J$=7.6 Hz, 2H, CH$_3$(CH$_2$)$_{14}$C$_6$H$_2$N ), 1.47 (s, 18H, C(CH$_3$)$_3$), 1.25 (s, 28H, CH$_3$(CH$_2$)$_{14}$C$_6$H$_2$N), 0.9 –0.85 (m, 3H, CH$_3$(CH$_2$)$_{14}$C$_6$H$_2$N), $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 155.7 (CO), 155.6*, 79.3 (C(CH$_3$)$_3$), 57.0 (NCH$_2$C$_6$H$_2$N), 54.0 (NCH$_2$C$_6$H$_2$N), 50.6 (CH$_3$(CH$_2$)$_{14}$C$_6$H$_2$N), 49.7 (NCH$_2$C$_6$H$_2$N), 31.9, 31.6, 29.7, 29.7, 29.4 (CH$_3$(CH$_2$)$_{14}$N), 28.6 (C(CH$_3$)$_3$), 28.6*, 27.5, 22.7 (CH$_3$(CH$_2$)$_{14}$N), 14.1(CH$_3$(CH$_2$)$_{14}$N). IR $\nu_{max}$(film)/cm$^{-1}$ 2922, 2853, 1694, 1411, 1364, 1247, 1151, 1096, 989, 772. HRMS Found (ESI): MH$^+$ 554.4878 C$_{32}$H$_{64}$N$_3$O$_4$ requires 554.4891.

*Extra peaks due to the presence of rotamers.

1-Hexadecyl-1,4,7-triazacyclononane (C$_{16}$TACN)

This compound was synthesized according to published procedures.$^{[S3]}$
di-tert-Butyl 7-octadecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S5)

According to GP1, to a solution of di-tert-butyl 1,4,7-triazacyclononane-1,4-dicarboxylate (76 mg, 0.23 mmol) in MeCN (5 mL) were added K$_2$CO$_3$ (96 mg, 0.69 mmol), NaHCO$_3$ (58 mg, 0.69 mmol) and 1-bromooctadecane (192 mg, 0.58 mmol). The crude product was purified by flash chromatography (20% EtOAc:hexanes) to obtain the title compound as a yellow oil (43 mg, 32%).

**$^1$H NMR** (400 MHz, CDCl$_3$) $\delta$ 3.55–3.43 (m, 4H, NC$_2$H$_2$C$_2$H$_2$N), 3.30–3.19 (m, 4H, NC$_2$H$_2$CH$_2$N), 2.67–2.57 (m, 4H, NCH$_2$C$_2$H$_2$N), 2.52–2.42 (m, 2H, CH$_3$(CH$_2$)$_{16}$C$_2$H$_2$N), 1.47 (s, 18H, C(CH$_3$)$_3$), 1.26 (s, 24H, CH$_3$(C$_2$H$_2$)$_{16}$CH$_2$N), 0.92–0.84 (m, 3H, C$_2$H$_3$(CH$_2$)$_{16}$CH$_2$N).

**$^{13}$C NMR** (101 MHz, CDCl$_3$) $\delta$ 155.7 (CO), 79.3 (C(CH$_3$)$_3$), 54.1 (NCH$_2$C$_2$H$_2$N), 53.7 (NCH$_2$C$_2$H$_2$N), 50.7(CH$_3$(CH$_2$)$_{16}$C$_2$H$_2$N), 50.4* 50.1(NCH$_2$C$_2$H$_2$N), 49.8*, 31.9, 29.7, 29.7, 29.7(C$_2$H$_3$(CH$_2$)$_{16}$CH$_2$N), 28.6 (C(CH$_3$)$_3$), 28.6*, 28.0, 27.6, 27.5, 22.7 (CH$_3$(CH$_2$)$_{16}$CH$_2$N), 14.1(C$_2$H$_5$CH$_3$N), 10.5 (CH$_3$(CH$_2$)$_{16}$CH$_3$).

**IR** $\nu$ max (film)/cm$^{-1}$ 2922, 2852, 1693, 1459, 1247, 1150, 1094, 989, 860, 732.  

**HRMS** Found (ESI): MH$^+$ 582.5184 C$_{34}$H$_{68}$N$_3$O$_4$ requires 582.5204.  

*Extra peaks due to the presence of rotamers.

1-Octadecyl-1,4,7-triazacyclononane (C$_{18}$TACN)

According to GP2, to a solution of S5 (22 mg, 0.04 mmol) in MeOH (1 mL) was added 6M HCl (3 mL). The crude was evaporated under reduced pressure and the product C$_{18}$TACN was obtained without further purification as a white solid (17 mg, 89%).

**$^1$H NMR** (400 MHz, D$_2$O) $\delta$ 3.60 (s, 4H, NC$_2$H$_2$C$_2$H$_2$N), 3.34 (d, $J$ = 5.9 Hz, 4H, NCH$_2$C$_2$H$_2$N), 3.04 (d, $J$ = 5.9 Hz, 4H, NCH$_2$C$_2$H$_2$N), 2.77 (dd, $J$ = 10.5, 2H, NCH$_2$CH$_2$(CH$_2$)$_{15}$CH$_3$), 1.53 (s, 2H, NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 1.14 (s, 30H, NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 0.83 (t, $J$ = 6.4 Hz 3H, NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$).

**$^{13}$C NMR** (101 MHz, D$_2$O) $\delta$ 55.2 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 47.5 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 43.2 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 41.9 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 32.1, 30.3, 30.0, 29.6, 27.6, 24.2, 22.7 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$), 13.9 (NCH$_2$CH$_2$(CH$_2$)$_{16}$CH$_3$).  

**IR** $\nu$ max (film)/cm$^{-1}$ 3413, 2917, 2850, 1635, 1468, 669.  

**HRMS** Found (ESI): MH$^+$ 382.4141 1 C$_{24}$H$_{52}$N$_3$ requires 382.4156.
4. Further detail for the experiments shown in the Figures of the manuscript

(a) Additional TEM images related to Figure 2d.

(i) TEM images of C\textsubscript{16}TACN\cdotZn\textsuperscript{2+} (50 μM) in the absence of substrate HPNPP. The majority of the grid is free of structures. In some areas, small structures can be seen, but this is rare.

![TEM image](image1)

(ii) TEM images of C\textsubscript{16}TACN\cdotZn\textsuperscript{2+} (50 μM) in the presence of substrate HPNPP (250 μM). Numerous structures are seen throughout the grid.

![TEM images](image2)
(iii) TEM images of $\text{C}_{16}\text{TACN} \cdot \text{Zn}^{2+}$ (50 μM) in the presence of waste products (250 μM). Smaller, less distinct structures are seen throughout the grid.

Conditions of the TEM experiments: (i) $[\text{C}_{16}\text{TACN} \cdot \text{Zn}^{2+}] = 50$ μM in the absence of substrate HPNPP; (ii) $[\text{C}_{16}\text{TACN} \cdot \text{Zn}^{2+}] = 50$ μM in the presence of HPNPP (250 μM) and (iii) $[\text{C}_{16}\text{TACN} \cdot \text{Zn}^{2+}] = 50$ μM in the presence of waste (with HPNPP and left for 48 h). All experiments were performed in aqueous buffer solution (HEPES, 10 mM, pH 7.0) at 25 °C and TEM images were stained with 1% uranyl acetate solution. Residence time of grid on sample: 60 sec, residence time of grid on uranyl acetate solution: 30 sec.
(b) Additional information related to Figure 3b.

(i) Cac data related to Figure 3b

This curve shows that the onset of catalysis (at around 55 \( \mu \text{M} \) C\(_{16}\)TACN·Zn\(^{2+}\), shown in Figure 3b in the manuscript) in the presence of 62.5 \( \mu \text{M} \) HPNPP matches the cac determined by fluorescence titrations (Figure below).

![Figure S1. Emission intensity profiles for Nile Red (2 \( \mu \text{M}, \lambda_{\text{ex}} = 570 \text{ nm}, \lambda_{\text{em}} = 643 \text{ nm} \)) at increasing C\(_{16}\)TACN·Zn\(^{2+}\) concentrations in the presence of 62.5 \( \mu \text{M} \) HPNPP.](image)

(ii) Comment on experimental conditions: kinetic experiments monitoring the rate of HPNP cleavage are all performed at 40°C instead of room temperature in order to have significant reaction rates. We have measured cac at both room temperature (25°C) and 40°C to show that there is no significant difference in the cac and that we are always working above the cac. Below is shown the fluorescence titration performed at 40°C, which shows the cac is about 10 \( \mu \text{M} \), which is the same with what is calculated at 25°C (see Figure S2, below).

![Figure S2. Emission intensity profiles for Nile Red (2 \( \mu \text{M}, \lambda_{\text{ex}} = 570 \text{ nm}, \lambda_{\text{em}} = 615 \text{ nm} \)) at increasing C\(_{16}\)TACN·Zn\(^{2+}\) concentrations in the presence of 500 \( \mu \text{M} \) HPNPP at 40°C.](image)
(c) $^{31}$P NMR data confirming the cleavage of HPNPP.

(i) Summary of data:

Figure S3. Partial $^{31}$P-NMR spectra ($H_2O:D_2O$ 9:1, 202 MHz, 303 K) with 1 mM HPNPP alone (I), just after addition of 75 µM CuTACN-Zn$^{2+}$ (II) and after 13 h (III).

(ii) Full data:

Figure S4. Full $^{31}$P-NMR (9:1 H$_2$O:D$_2$O, 202 MHz, 303 K) with 1 mM HPNPP alone (a), just after addition of 75 µM CuTACN-Zn$^{2+}$ (b) and at equal intervals of 47 min up to 13 h (c to r). The signal at 0 ppm originates from the coaxial reference (K$_2$HPO$_4$ in HEPES buffer at pH 7).
(d) Additional data related to Figure 3c.

To confirm the presence of aggregates at all conditions of this experiment, the cac of $C_{16}{\text{TACN}}$ was measured in the presence of 500 $\mu M$ HPNPP and in the absence of Zn$^{2+}$ (in HEPES buffer 5 mM).

Figure S5. Emission intensity profiles for Nile Red (5 $\mu M$, $\lambda_{\text{ex}} = 570$ nm, $\lambda_{\text{em}} = 643$ nm) at increasing $C_{16}{\text{TACN}}$ concentrations, in the presence of 500 $\mu M$ HPNPP; the dotted lines are the linear fit to the first two and last five data points, indicating a cac of 13 $\mu M$. 


(e) Additional data related to Figure 4a.

(i) Plot of the rate of HPNPP hydrolysis with increasing concentrations of C\textsubscript{14}TACN·Zn\textsuperscript{2+} (linear scale):

The curve for C\textsubscript{14}TACN·Zn\textsuperscript{2+} from Figure 4a is shown here in linear scale to allow the increase in reaction rate once the cac is reached.

![Graph showing rate of HPNPP hydrolysis with increasing concentrations of C\textsubscript{14}TACN·Zn\textsuperscript{2+}](image)

*Figure S6. Initial speed of HPNPP hydrolysis at increasing C\textsubscript{14}TACN·Zn\textsuperscript{2+} concentrations, [HPNPP] = 500 µM, [HEPES buffer] = 5 mM*

(ii) Determination of the cac data in Figure 4a

The cac of C\textsubscript{12}TACN·Zn\textsuperscript{2+}, C\textsubscript{14}TACN·Zn\textsuperscript{2+}, C\textsubscript{16}TACN·Zn\textsuperscript{2+} and C\textsubscript{18}TACN·Zn\textsuperscript{2+} in the presence of 500 µM HPNPP was determined by the cumulative addition of C\textsubscript{n}TACN·Zn\textsuperscript{2+} to a solution containing HEPES buffer (5 mM) and nile red (2 µM). The fluorescence intensity was measured at 615 nm.

![Graph showing emission intensity profiles for Nile red at increasing C\textsubscript{n}TACN·Zn\textsuperscript{2+} concentrations](image)

*Figure S7. Emission intensity profiles for Nile red (2 µM, λ\textsubscript{ex} = 570 nm, λ\textsubscript{em} = 615 nm) at increasing C\textsubscript{n}TACN·Zn\textsuperscript{2+} concentrations (see legend for details), in the presence of 500 µM HPNPP at 25°C.*
(f) Experimental procedure related to Figure 4b.

This experiment was performed to demonstrate the Michaelis-Menten characteristics of this system and required the addition of increasing concentrations of substrate HPNPP in order to approach $V_{\text{max}}$. Because the concentration of substrate HPNPP needed was high (up to 3 mM), we required higher concentrations of buffer to ensure that the buffer capacity was not breached, thus the reason for using $[\text{HEPES buffer}] = 50$ mM.

Conditions: $([\text{C}_16\text{TACN} \cdot \text{Zn}^{2+}] = 50 \, \mu\text{M}, [\text{HEPES buffer}] = 50$ mM, pH 7.0), $T = 40$ °C; absorbance measured at 405 nm. Initial rates ($v_0$) were calculated by converting the absorbance signals into concentration using the $\varepsilon \cdot l$ calculated from a calibration curve. Michaelis-Menten parameters were then determined by fitting the corresponding saturation profile with the Michaelis-Menten model (Origin 8 Pro, function: Hill; constraints: $n = 1$, i.e. Michaelis-Menten kinetics).

(g) Additional data related to Figure 3c, refuelling experiments.

(i) Experiment overview

To confirm the possibility of the system to be ‘refuelled’, an experiment was performed whereby aliquots of HPNPP (125 µM) was sequentially added to a solution of 50 µM of $\text{C}_16\text{TACN} \cdot \text{Zn}^{2+}$ in 5 mM HEPES pH 7. This low concentration of HPNPP was chosen so that HPNPP cleavage could be completed within a reasonable timeframe. The initial rates of HPNPP cleavage was measured directly following the addition of each aliquot of HPNPP (black data points) and again after completion of the hydrolysis (after 48 h, grey data points). After each successive addition of HPNPP, it can be seen that the system is successfully reactivated, though not to the same reaction rate. The reduced reaction rate following successive additions is due to the ability of the waste product to also bind to the surface of the self-assembled catalysts, thereby acting as a competitive inhibitor.
(ii) Kinetics data for refuelling experiments

Experiments were performed in duplicate and the results averaged. Below represents one set of data:

Figure S8. Initial rates of HPNPP hydrolysis after successive additions of HPNPP (125 µM each addition) in the presence [HEPES buffer] = 5 mM and [CoTACN Zn²⁺] = 50 µM at 40 °C (i) change in absorbance directly after 1st addition of HPNPP; (ii) change in absorbance 48h after 1st addition; (iii) change in absorbance directly after 2nd addition; (iv) change in absorbance 48h after 2nd addition; (v) change in absorbance directly after 3rd addition; (vi) change in absorbance 48h after 3rd addition; (v) change in absorbance directly after 4th addition of HPNPP.
**SUPPORTING INFORMATION**

(h) **Comment on the concentration of Nile red used in the fluorescence titration experiments**

Throughout the manuscript, Nile red is used as the fluorescent probe to determine the cac values of different precursors in the presence of the substrate **HPNPP**. Either 2 or 5 µM of Nile red is used in the experiments, which may seem too high to be ‘probing.’ We have attempted to use concentrations of Nile red that are much lower but found that with concentrations of Nile red lower than 2 µM, the system becomes too insensitive and the data unreliable. We have also compared the cac's obtained with 2 or 5 µM of Nile red and have found them to be within experimental error (see below), though the profiles of the curves can vary, meaning that one of the concentrations can be easier to interpret. We believe the ‘actual’ concentration of Nile red in solution is much lower than 2 or 5 µM, as during the experiment, the Nile red can be seen accumulated on the cuvette cap, as its solubility in water is so low. However, using concentrations of Nile red below 2 µM gives poor data, possibly due to the slow kinetics of dissolution of the Nile red from the hydrophobic cap of the cuvette. Comparison of the cac's obtained with the fluorescent probe against the concentrations of catalyst where we begin to observe onset of catalysis shows that these data match closely (i.e. matching the data obtained in Figures 2b and 3b). We therefore believe that the Nile red is not having a major impact on the self-assembly of the amphiphiles.

![Emission intensity profiles for Nile red 2 µM and 5 µM at increasing C16TACN Zn²⁺ concentrations in the presence of 500 µM HPNPP at 25°C (λex = 570 nm, λem = 615 nm)](image)

*Figure S8. Emission intensity profiles for Nile red 2 µM and 5 µM at increasing C16TACN Zn²⁺ concentrations in the presence of 500 µM HPNPP at 25°C (λex = 570 nm, λem = 615 nm)*
(i) Comment on the concentration of HEPES used in the fluorescence titration experiments

We found no significant differences in cac from using 5 mM or 10 mM HEPES. Slight differences in the cac observed were within experimental error (see below).

**Figure S9.** Emission intensity profiles for Nile red 2 µM at increasing C<sub>16</sub>TACN Zn<sup>2+</sup> concentrations in the presence of 500 µM HPNPP in either 5 mM or 10 mM of HEPES at 25°C (λ<sub>ex</sub> = 570 nm, λ<sub>em</sub> = 615 nm)
5. $^1$H-NMR and $^{13}$C-NMR Spectra for Novel Compounds

Di-tert-butyl 7-ethyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S1)
1-Ethyl-1,4,7-triazacyclonane (C₃TACN)

[Chemical structure image]

[Graph showing NMR spectral data]

- δ values for various peaks
  - 8.975
  - 41.782
  - 42.027
  - 47.763
  - 50.717
di-tert-Butyl 7-dodecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S2)
1-Dodecyl-1,4,7-triazacyclononane (C\textsubscript{12}TACN)
di-tert-Butyl 7-tetradecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S3)

N
Boc

Boc

1H NMR spectrum of di-tert-Butyl 7-tetradecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S3)

"NMR Spectrum Image"
1-Tetradecyl-1,4,7-triazacyclononane (C₁₄TACN)
SUPPORTING INFORMATION

di-tert-Butyl 7-hexadecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S4)
di-tert-Butyl 7-octadecyl-1,4,7-triazacyclononane-1,4-dicarboxylate (S5)
SUPPORTING INFORMATION

1-Octadecyl-1,4,7-triazacyclononane (C\textsubscript{18}TACN)
6. References

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