Supplementary Information for:

**Dynamic Combinatorial Libraries for the Recognition of Heavy Metal Ions**

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**General remarks**

Solvents were purchased from Romil (LC-MS grade CHCl₃), Fisher (HPLC grade MeOH) and Aldrich (TFA). Metal salts were purchased from Sigma-Aldrich (RbCl), Lancaster (LiCl), Breckland (NaCl, KCl), Alpha Aesar (CsCl). 4-Methoxypyridine-2,6-dicarbaldehyde¹ 1 and 2,6-bis(2-carboxyphenoxyethyl)pyridine² 2a were synthesized according to literature procedures.

¹H and ¹³C NMR spectra were recorded on Bruker DPX-400, DRX 500 or AV 600 or Advance 500 TCI Cryo Spectrometers. All signals were internally referenced to the solvent residue (3.31 ppm for MeOD). All high-resolution (HR) electrospray ionisation (ESI) mass spectra were recorded on a Waters LCT Premier XE instrument.

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UV-Vis spectra were recorded in a 1 cm quartz cuvette using a Lambda 14 spectrometer from Perkin Elmer at 22 °C, unless otherwise stated. The DCLs were analysed by LCMS using an Agilent 1100 series HPLC coupled to an Agilent XCT Ion-Trap.

**Synthesis**

![Diagram](image)

Fig. 1 Synthesis of 2b. a) K$_2$CO$_3$, acetone, reflux, 7 d, 55%; b) N$_2$H$_4$, reflux, 12 h, 99%.

1,7-Bis[2-(methoxycarbonyl)phenyl]-1,4,7-trioxaheptane was prepared as described in the literature.$^3$

1,7-Bis[2-(hydrazinocarbonyl)phenyl]-1,4,7-trioxaheptane.

1,7-Bis[2-(methoxycarbonyl)phenyl]-1,4,7-trioxaheptane (374 mg, 1.00 mmol) was suspended in a solution composed of 5.00 mL of EtOH and 254 μL (4.00 mmol) of hydrazine monohydrate. The reaction was refluxed for 12 h. The final product was obtained by recrystallization from EtOH in quantitative yield.

$^1$H-NMR (600 MHz, CDCl$_3$): $\delta$ = 9.22 (s, 2H, NH), 8.15 (dd, $^3$J = 7.8 Hz, $^4$J = 1.8 Hz, 2H, 6-C$_{Ar}$-H), 7.42 (ddd, $^3$J = 8.2 Hz, $^2$J = 7.3 Hz, $^4$J = 1.8 Hz, 2H, 4-C$_{Ar}$-H), 7.09 (m, 2H, 5-C$_{Ar}$-H), 6.98 (dd, $^3$J = 8.3 Hz, $^4$J = 0.8 Hz, 2H, 3-C$_{Ar}$-H), 4.40 (m, 4H, Ar-O-CH$_2$), 4.02 (s, 4H, NH$_2$), 3.98 (m, 4H, CH$_2$O-CH$_2$) ppm.

$^{13}$C-NMR (150 MHz, CDCl$_3$): $\delta$ = 166.3 (COON$_2$H$_3$), 156.5 (2-C$_{Ar}$), 132.7 (4-C$_{Ar}$), 132.0 (6-C$_{Ar}$), 121.9 (5-C$_{Ar}$), 120.9 (1-C$_{Ar}$), 113.3 (3-C$_{Ar}$), 69.3 68.3 (2 CH$_2$) ppm.

ESI-MS (pos. mode): C$_{18}$H$_{22}$N$_4$O$_5$Na$^+$ calcd. 397.1482, found 397.1416.

C$_{18}$H$_{22}$N$_4$O$_5$: calcd. C 57.57, H 5.92, N 14.96; found. C 57.15, H 6.04, N 14.02

**Library Preparation**

Templated DCLs were prepared by mixing stock solutions of building blocks (20 mM in CHCl$_3$) and metal salts (50 mM in MeOH) and adding the appropriate amount of CHCl$_3$ and MeOH. Exchange was initiated by addition of 5% TFA to the DCLs. DCLs were typically prepared on a 1 mL scale (1 mM, analytical for metal screening) or on a 10 mL scale (10 mM, preparative to isolate the 2+2 macrocycles).

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**LC-MS Analysis**

The DCLs were analysed by LCMS using an Agilent 1100 series HPLC coupled to an Agilent XCT Ion-Trap. Water was obtained from a MilliQ purification system. MeOH (Fisher Scientific, LC-MS grade) and formic acid (Fluka, p.a. puriss. for mass spectrometry) were purchased. Samples were analysed using either method A or B:

**Method A:**
- **Column:** Phenomenex Prodigy 5 ODS-2 column (150 x 4.60 mm, particle size: 5 µm)
- **Injection volume:** 5 µl
- **Flow rate:** 1.0 ml/min
- **Gradient:** MeOH in water (60% to 70% in 10 min, then to 100% in 1 min, both containing 0.1% formic acid) at 323 K. UV absorption was detected at 290 nm and referenced against 550 nm.

**Method B:**
- **Column:** Water Symmetry C₈ (150 x 3.90 mm, particle size: 5 µm)
- **Injection volume:** 1 µl
- **Flow rate:** 1.0 ml/min
- **Gradient:** MeCN in water (35% 0-15 min, then to 100% in 5 min, both containing 0.1% formic acid) at 323 K. UV absorption was detected at 290 nm and referenced against 550 nm.

Positive ion mass spectra were acquired in standard enhanced mode using electrospray ionisation (drying temperature: 350 °C; nebuliser pressure: 18 psi; drying gas flow: 8 l/min; ICC target: 200 000; range: 400-1700 m/z)
**Reversibility**

![Diagram](image)

**Fig. 2** Conversion of 4a into 3a over time.

The isolated sample of 4a was dissolved in CHCl₃/MeOH/TFA (50/50/5, 1 mM) and stirred. A sample of the mixture was taken daily, basified (to halt the exchange) and stored at -20 °C. After 19 days, the samples were analysed by LCMS and the relative peak of the two products (3a and 4a) were plotted over time. Original DCL distribution was reached after approx. 10 days.
Fig. 3 Conversion of 4b into 3b over time.

The isolated sample of 4b was dissolved in CHCl$_3$/MeOH/TFA (70/30/5, 1 mM) and stirred. A sample of the mixture was taken daily, basified (to halt the exchange) and stored at -20 °C. After 19 days, the samples were analysed by LCMS and the relative peak of the two products (3b and 4b) were plotted over time. Original DCL distribution was reached after approx. 24 days.
Isolation of Macrocycle and Characterisation

The free macrocycles were isolated as described below. Metal complexes were synthesised by adding 1.0 eq. of a metal chloride (as a 50 mM solution in MeOD) to a 1.0 mM solution of the free macrocycle.

Dialdehyde 1 (33.0 mg, 200 mmol) and dihydrazide 2a (81.4 mg, 200 mmol) were dissolved in 200 ml of CHCl₃/MeOH/TFA (90:10:5). After 17 days, exchange was stopped by adding NEt₃ (11 ml, 1.1 eq. with respect to TFA). The solution was concentrated in vacuo to ca. 10 ml and the product was precipitated by addition of Na₂CO₃ (aq, sat, 50 ml) and H₂O (150 ml). The precipitate was collected by filtration and washed with H₂O (100 ml) to give a beige powder (97.0 mg, 90%).

1H-NMR (500 MHz, 300 K, CDCl₃/MeOD, 1:3, v/v), δ = 8.99 (s, 1H, e), 8.24 (dd, 1H, 3J = 7.8 Hz, 4J = 1.7 Hz, h), 8.19 (dd, 1H, 3J = 7.2 Hz, 4J = 2.3 Hz, h), 8.14 (d, 1H, 3J = 2.3 Hz, c), 7.74 (t, 1H, 3J = 7.7 Hz, j), 7.67 (dt, 1H, 3J = 7.8 Hz, 4J = 1.8 Hz, p), 7.59 (s, 1H, e), 7.46 (d, 1H, 3J = 7.8 Hz, o), 7.41 (d, 1H, 3J = 8.2 Hz, k), 7.20 - 7.28 (m, 4H, 2i, j, c), 7.05 (d, 1H, 3J = 7.6 Hz, o), 6.61 (dd, 1H, 3J = 7.7 Hz, 4J = 1.5 Hz, k), 5.39 (s, 2H, m), 5.25 (s, 2H, m), 4.01 (s, 3H, a), ppm.

13C-NMR (125 MHz, 300 K, CDCl₃/MeOD, 1:3, v/v). δ = 169.0, 165.1, 165.1, 158.6, 158.3, 157.5, 157.1, 156.7, 153.7, 149.2, 141.3, 139.1, 135.8, 134.6, 133.9, 133.7, 126.5, 125.0, 125.0, 123.4, 123.3, 116.2, 114.6, 107.3, 78.1, 72.5, 57.0 ppm.

HRMS (ESI+) calcd. for C₂₉H₂₄N₆O₇: [MH]+ (m/z) = 537.1881, found: 537.1913.

Dialdehyde 1 (8.30 mg, 50.0 mmol) and dihydrazide 2b (18.7 mg, 50.0 mmol) were dissolved in 50 ml of CHCl₃/MeOH/TFA (90:10:5). After three days the solution was washed with Na₂CO₃ (aq, sat, 3 × 50.0 ml) and the combined organic layers were evaporated to dryness. After recrystallization from Et₂O/hexanes, a beige powder was obtained (12.0 mg, 48%).

1H-NMR (400 MHz, 298 K, CDCl₃/MeOD, 3:1, v/v). δ = 8.51 (s, 1H, e), 8.16 (dd, 1H, 3J = 7.8 Hz, 4J = 1.7 Hz, h), 8.02 (m, 2H, h, c), 7.56 (dt, 1H, 3J = 7.8 Hz, 4J = 1.8 Hz, j), 7.51 (dt, 1H, 3J = 7.8 Hz, 4J = 1.8 Hz, j), 7.49 (s, 1H, e), 7.28 (d, 1H, 3J = 8.2 Hz, k), 7.24 (t, 1H, 3J = 7.6 Hz, i), 7.18 (t, 1H, 3J = 7.6 Hz, i), 7.14 (d, 1H, 3J = 8.3
Hz, k), 7.05 (d, 1H, \(^3J = 2.3\) Hz, c), 4.42 (m, 2H, m), 4.28 (m, 2H, m), 4.02 (m, 2H, n), 3.98 (s, 3H, a), 3.82 (m, 2H, n) ppm. 

\(^{13}\)C-NMR (100 MHz, 300 K, CDCl\(_3\)/MeOD, 3:1, v/v). \(\delta = 167.9, 164.6, 163.8, 157.4, 157.2, 155.2, 154.3, 152.7, 151.7, 150.0, 123.1, 122.9, 121.2, 115.0, 114.8, 106.2, 77.7\) (under solvent peak, located by HMQC), 74.5, 69.7, 69.5, 56.3 ppm.

HRMS (ESI+) calced. for C\(_{26}\)H\(_{25}\)N\(_5\)O\(_6\): [MH\(^+\)] (m/z) = 504.1878, found: 504.2041.

4a and its complex with Ba\(^{2+}\) were described earlier.\(^4\)

Dialdehyde 1 (33.0 mg, 200 mmol) and dihydrazide 2b (75.0 mg, 200 mmol) were dissolved in 10 ml of CHCl\(_3\)/MeOH/TFA (90:10:5). After 14 days, a white precipitate was collected by filtration, suspended in Na\(_2\)CO\(_3\) (sat. aq., 10 ml) and sonicated (5 min). The white solid was collected by filtration, washed with water (5 ml) and dried in vacuo (95.0 mg, 95%).

\(^1\)H-NMR (400 MHz, 298 K, CDC\(_3\)/MeOD, 7:3, v/v). \(\delta = 8.05\) (dd, 4H, \(^3J = 8.0\) Hz, \(^4J = 1.7\) Hz, h), 8.02 (s, 4H, e), 7.53 (s, 4H, c), 7.37 (t, 4H, \(^3J = 7.8\) Hz, j), 7.08 (m, 8H, k, i), 4.43 (t, 8H, \(^3J = 4.2\) Hz, m), 4.13 (t, 8H, \(^3J = 4.2\) Hz, n), 3.92 (s, 6H, a) ppm.

\(^{13}\)C-NMR (100 MHz, 300 K, CDCl\(_3\)/MeOD, 7:3, v/v). \(\delta = 167.5, 163.0, 157.2, 154.3, 147.5, 134.3, 132.5, 122.6, 120.4, 114.1, 108.6, 70.0, 69.4, 56.1\) ppm.

HRMS (ESI+) calced. for C\(_{52}\)H\(_{50}\)N\(_{10}\)O\(_{12}\): [MH\(^+\)] (m/z) = 1007.3682, found: 1007.3770; [MH\(^+\)\(^2+\)] (m/z): 504.1884. found: 504.1920.

4a[Sr\(^{2+}\)]

\(^1\)H-NMR (500 MHZ, 300 K, CDCl\(_3\)/MeOD, 1:1, v/v), \(\delta = 8.33\) (s, 4H, e), 7.93 (t, 2H, \(^3J = 7.8\) Hz, p), 7.55 (d, 4H, \(^3J = 7.8\) Hz, o), 7.48 (dt, 4H, \(^3J = 7.8\) Hz, \(^4J = 1.7\) Hz, j), 7.41 (dd, 4H, \(^3J = 7.8\) Hz, \(^4J = 1.7\) Hz, h), 7.26 (s, 4H, c), 7.18 (d, 4H, \(^3J = 8.4\) Hz, k), 6.94 (dt, 4H, \(^3J = 7.5\) Hz, \(^4J = 0.6\) Hz, i), 5.44 (s, 8H, m), 4.04 (s, 6H, a) ppm.

$^{13}$C-NMR (125 MHz, 300 K, CDCl₃/MeOD, 1:1, v/v). $\delta$ = 169.1, 167.0, 157.4, 156.2, 153.7, 147.8, 139.1, 134.9, 131.7, 123.4, 122.2, 120.4, 114.2, 113.3, 70.7, 56.7 ppm.

HRMS (ESI+) calcd. for C₅₈H₄₆N₁₂O₁₀Sr: [MH]$^+$ (m/z) = 1159.2589, found: 1159.2614; [MH$^2$$^+$] (m/z): 580.1331. found: 580.1315.

$^1$H-NMR (400 MHz, 298 K, CDCl₃/MeOD, 7:3, v/v). $\delta$ = 8.30 (s, 4H, e), 7.46 (m, j overlaps with residual solvent peak for CDCl₃), 7.30 (d, 4H, $^3$J = 7.8 Hz, h), 7.07 (s, 4H, c), 7.02 (d, 4H, $^3$J = 8.4 Hz, k), 6.90 (t, 4H, $^3$J = 7.5 Hz, i), 4.40 (t, 8H, $^3$J = 4.2 Hz, m), 4.04 (br s, 8H, n), 3.91 (s, 6H, a), ppm.

In HRMS only the free macrocycle is observed.

$^1$H-NMR (500 MHz, 300 K, CDCl₃/MeOD, 7:3, v/v). $\delta$ = 8.38 (s, 4H, e), 7.48 (dt, 4H, $^3$J = 7.8 Hz, $^4$J = 1.7 Hz, j), 7.38 (dd, 4H, $^3$J = 7.8 Hz, $^4$J = 1.7 Hz, h), 7.14 (s, 4H, c), 7.02 (t, 4H, $^3$J = 8.4 Hz, k), 6.94 (t, 4H, $^3$J = 7.5 Hz, i), 4.40 (br s, 8H, m), 3.96-4.20 (br s, 8H, n), 3.96 (s, 6H, a) ppm.

$^{13}$C-NMR (125 MHz, 300 K, CDCl₃/MeOD, 7:3, v/v). $\delta$ = 168.6, 166.6, 157.3, 152.4, 146.6, 134.9, 131.7, 121.9, 119.6, 113.3, 112.4, 69.4, 68.0, 56.5 ppm.
$^{1}H$-NMR (400 MHz, 298 K, CDCl$_3$/MeOD, 7:3, v/v). $\delta$ = 8.35 (s, 4H, e), 7.42-7.46 (m, 8H, h, j), 7.19 (s, 4H, c), 6.98 (d, 4H, $^3J$ = 8.3 Hz, k), 6.94 (t, 4H, $^3J$ = 7.6 Hz, i), 4.34 (s, 8H, m), 4.00 (broad s, 8H, n), 3.98 (s, 6H, a) ppm.

$^{13}C$-NMR (100 MHz, 300 K, CDCl$_3$/MeOD, 1:1, v/v). $\delta$ = 168.0, 165.4, 162.3, 156.6, 153.6, 146.7, 133.7, 130.7, 121.1, 119.7, 113.1, 112.7, 68.5, 67.6, 55.7 ppm.

HRMS (ESI+) calcd. for C$_{52}$H$_{48}$N$_{10}$O$_{12}$Ba: [MH]$^+$ (m/z) = 1143.2578, found: 1143.2592; [MH$_2$$^{2+}$] (m/z): 572.1325. found: 572.1287.
Fig. 4: LCMS and HRMS analysis of the 1+1 macrocycle with pyridyl linker 3a (Method A). a) HPLC trace recorded at 290 nm showing the isolated material to consist of 1+1 macrocycle (3a, 97%, blue peak) and the 2+2 macrocycle (4a, 3%, peak at 8 mins); b) low resolution mass spectrum of the blue peak in a); c) ESI(+) - HRMS spectrum of the isolated material. The isotope pattern in b) and c) show that the isolated material is the 1+1 and not the 2+2 macrocycle.
Fig. 5: LCMS and HRMS analysis of the 1+1 macrocycle with ethyleneglycol linker 3b (Method A). a) HPLC trace recorded at 290 nm showing the isolated material to consist of 1+1 macrocycle (3b, >99%, blue peak); b) low resolution mass spectrum of the blue peak in a); c) ESI(+)-HRMS spectrum of the isolated material. The isotope pattern in b) and c) show that the isolated material is the 1+1 and not the 2+2 macrocycle.
Fig. 6: LCMS and HRMS analysis of the 2+2 macrocycle with pyridyl linker 4a (Method A). a) HPLC trace recorded at 290 nm showing the isolated material to consist of 1+1 macrocycle (3a, 6%, 6.8 mins) and the 2+2 macrocycle (4a, 91%, red peak, the MS of the peak at 8 mins is identical to that of the red peak, it is possibly a conformational isomer of 4b, 3%); b) mass spectrum of the red peak in a); c) ESI(+) - HRMS spectrum of the isolated material. The isotope pattern in b) and c) show that the isolated material is the 2+2 and not the 1+1 macrocycle.
Fig. 7: LCMS and HRMS analysis of the 2+2 macrocycle with ethyleneglycol linker 4b (Method A). a) HPLC trace recorded at 290 nm showing the isolated material to consist of two isomers of the 2+2 macrocycle (4b, 95%, red peak and peak at 6.1 mins, 5%); b) mass spectrum of the red peak in a); c) ESI(+) HRMS spectrum of the isolated material. The isotope pattern in b) and c) show that the isolated material is the 2+2 (4b) and not the 1+1 macrocycle (3b).
NMR Titrations

Fig. 8: NMR spectra of 4a in CDCl₃/MeOD (1:1) after addition of different amounts of MgCl₂. Residual solvent signals are marked with asterisk.

Fig. 9: NMR spectra of 4a in CDCl₃/MeOD (1:1) after addition of different amounts of CaCl₂. Residual solvent signals are marked with asterisk.
Fig. 10: NMR spectra of 4a in CDCl₃/MeOD (1:1) after addition of different amounts of SrCl₂. Residual solvent signals are marked with asterisk.

Fig. 11: NMR spectra of 4a in CDCl₃/MeOD (1:1) after addition of different amounts of BaCl₂. Residual solvent signals are marked with asterisk.
Fig. 12: NMR spectra of 4b in CDCl$_3$/MeOD (7:3) after addition of different amounts of MgCl$_2$. Residual solvent signals are marked with asterisk.

Fig. 13: NMR spectra of 4b in CDCl$_3$/MeOD (7:3) after addition of different amounts of CaCl$_2$. Residual solvent signals are marked with asterisk.
Fig. 14: NMR spectra of 4b in CDCl$_3$/MeOD (7:3) after addition of different amounts of SrCl$_2$. Residual solvent signals are marked with asterisk.

Fig. 15: NMR spectra of 4b in CDCl$_3$/MeOD (7:3) after addition of different amounts of BaCl$_2$. Residual solvent signals are marked with asterisk.
NOESY Spectra

Fig. 16: NOESY spectrum of 4a (1 mM in CDCl$_3$/MeOD, 1:1, 298 K, 500 MHz, mixing time = 800 ms). a) whole spectrum; b) aromatic region. Cross-peaks are indicated with corresponding colours in the spectrum and the structures next to the spectra. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k) and green cross-peaks were assigned to the protons of green pyridine ring (o, p).
Fig. 17: NOESY spectrum of 4a[Sr$^{2+}$] (1 mM in CDCl$_3$/MeOD, 1:1, 298 K, 500 MHz, mixing time = 800 ms). Cross-peaks are indicated with corresponding colours in the spectrum and the structure below the spectrum. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k) and green cross-peaks were assigned to the protons of green pyridine ring (o, p).
Fig. 18: NOESY spectrum of 4a[Ba^{2+}] (1 mM in CDCl\textsubscript{3}/MeOD, 1:1, 298 K, 500 MHz, mixing time = 800 ms). Cross-peaks are indicated with corresponding colours in the spectrum and the structure below the spectrum. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k) and green cross-peaks were assigned to the protons of green pyridine ring (o, p).
Fig. 19: NOESY spectrum of 4b (1 mM in CDCl$_3$/MeOD, 7:3, 298 K, 500 MHz, mixing time = 800 ms). a) whole spectrum; b) aromatic region. Cross-peaks are indicated with corresponding colours in the spectrum and the structures next to the spectra. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k).
Fig. 20: NOESY spectrum of 4b[Ca^{2+}] (1 mM in CDCl<sub>3</sub>/MeOD, 7:3, 298 K, 500 MHz, mixing time = 800 ms). Cross-peaks are indicated with corresponding colours in the spectrum and the structure below the spectrum. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k).
Fig. 21: NOESY spectrum of 4b[Sr^{2+}] (1 mM in CDCl₃/MeOD, 7:3, 298 K, 500 MHz, mixing time = 800 ms). Cross-peaks are indicated with corresponding colours in the spectrum and the structure below the spectrum. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k).
**Fig. 22:** NOESY spectrum of $4b[\text{Ba}^{2+}]$ (1 mM in CDCl$_3$/MeOD, 7:3, 298 K, 500 MHz, mixing time = 800 ms). Cross-peaks are indicated with corresponding colours in the spectrum and the structure below the spectrum. Light blue cross-peaks were assigned to the protons on the light blue ring (h, i, j, k).
**UV-Vis Titrations**

The results for 4a have been reported previously\(^5\) and the results for 4b are shown in Fig. 23.

Host 4b was dissolved in CHCl₃/MeOH (7:3, v/v) to give a solution of 0.010 mM. Metal salts were dissolved in the solution of 4b to give solutions containing 0.010 mM 4b and 1.0 mM.

Procedure of the binding studies involved making sequential additions (20 × 2.0 µl, then 5 × 5.0 µl, then 5 × 10 µl) of metal salts using Eppendorf pipettes to a 2.0 ml solution of 4a or 4b in a spectrometric cell (\(b = 1.0\) cm). UV–Vis spectra were then combined to produce plots that showed the changes in the spectral features as a function of changes in the concentration of metal salts.

Binding constants were calculated using a model derived by Maarten Smoulders.\(^6\)

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Fig. 23 UV spectra of 4b with different amounts of metal guests (left). Binding isotherms for the binding of different guests to 4b at 23 °C (right).
Crystallographic Data

The .cif files for 3b and 4b can be downloaded separately and the data for 4a has been published.7