Supporting Information

A helically twisted imine macrocycle that allows for determining the absolute configurations of α-amino carboxylates

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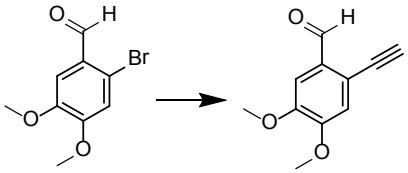
Contents

1. Syntheses and spectroscopic properties of new compounds

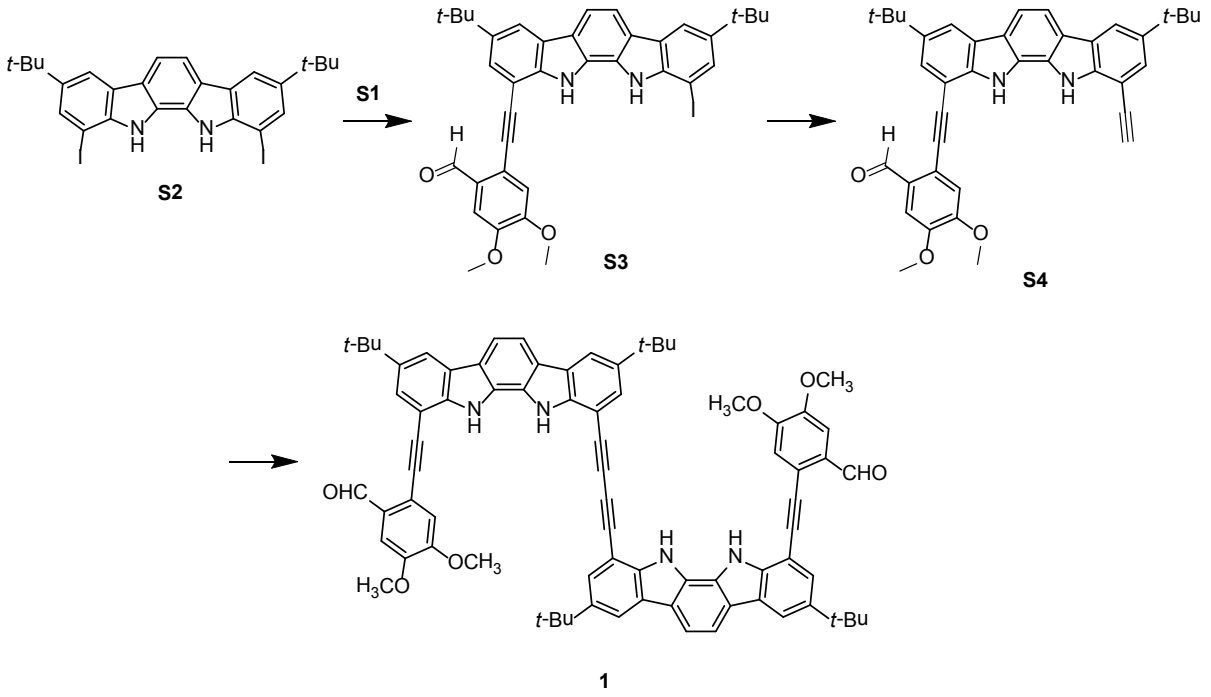
General: All chemicals were purchased from commercial suppliers and used without further purification unless otherwise specified. Triethylamine (Et_3N) and tetrahydrofuran (THF) were purchased as anhydrous grade. Dichloromethane (CH_2Cl_2) was purified by drying over calcium hydride (CaH_2), followed by distillation. The chemical shifts of ^1H NMR and ^{13}C NMR spectra were reported using the solvent signal as an internal reference, DMSO-d_6 (2.50 ppm for ^1H NMR, 39.5 ppm for ^{13}C NMR) and dichloromethane-d_2 (5.32 ppm for ^1H NMR). Column chromatography was performed using 230-400 mesh ultra pure silica. ^1H NMR and ^{13}C NMR spectra were obtained on Bruker (Avance II) 400 MHz spectrometer. FT-IR spectra were observed on Bruker (Vertex70). MALDI-TOF mass spectrometric measurements were performed on a Bruker (LRF20). Circular dichroism (CD) spectra were conducted on a JASCO (J-815). Melting points were determined with a Barnsted Electrochemical (IA9100). The elemental analysis data were obtained from *Center for research facilities* at Yonsei University.



S1

Compound **S1:** 2-Bromo-4,5-dimethoxy-benzaldehyde (2.3 g, 9.4 mmol), CuI (0.018 g, 0.094 mmol, 0.01 equiv), and $Pd(PPh_3)_2Cl_2$ (0.066 g, 0.094 mmol, 0.01 equiv) were added to a 100 mL Schlenk flask under N_2 gas. The flask was fitted with a rubber stopper, and then evacuated under vacuum and back-filled with N_2 (repeated three times). After addition of degassed Et_3N (40 mL), THF (40 mL), and trimethylsilylethyne (2.7 mL, 19 mmol), the solution was stirred at 85 °C during 12 h. The reaction mixture was allowed to cool down to room temperature, filtered through Celite and concentrated. The residue was dissolved in CH_2Cl_2, washed with distilled water. After concentration, the crude mixture was dissolved in CH_2Cl_2/MeOH = 2:1 (v/v) to which solution K_2CO_3 (0.13 g, 0.94 mmol, 0.1 equiv) was added. After stirred for 1 h, the mixture was washed with water and brine, and dried over anhydrous Na_2SO_4. The solution was concentrated under reduced pressure. The crude mixture was purified by flash column chromatography (silica gel, CH_2Cl_2/hexane = 2:1 (v/v)) to give **S1** (1.78 g, 98% for two steps) as a light yellow solid. Mp = 156-157 ºC; ^1H NMR

(400 MHz, DMSO-d_6, RT): 10.26 (s, 1H), 7.31 (s, 1H), 7.20 (s, 1H), 4.60 (s, 1H), 3.90 (s, 3H), 3.86 ppm (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, RT): 189.9, 154.0, 150.2, 130.5, 119.9, 115.8, 108.7, 86.8, 79.6, 56.7, 56.2 ppm; IR (KBr): 3238(C(sp)-H), 2101(C≡C), 1677(C=O) cm^{-1}; MS (MALDI-TOF): *m/z* $[M]^+$, 190; Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30, Found: C, 68.21; H, 5.21.



Compound **S3**: The synthesis of **S2**[1] was described previously. **S1** (1.6 g, 8.1 mmol), **S2** (5.0 g, 8.1 mmol, 1.0 equiv), CuI (0.015 g, 0.081 mmol, 0.01 equiv), and $Pd(PPh_3)_2Cl_2$ (0.057 g, 0.081 mmol, 0.01 equiv) were added to a 250 mL Schlenk flask under N_2 gas. The Schlenk flask was fitted with a rubber stopper, and evacuated under vacuum and back-filled with N_2 (repeated three times). After addition of degassed Et_3N (30 mL) and THF (40 mL), the rubber stopper was replaced with a screw stopper and the solution was stirred at 55 °C for 16 h. The reaction mixture was allowed to cool down room temperature, filtered through Celite and concentrated. The crude mixture was dissolved in CH_2Cl_2, washed with brine, and dried over anhydrous Na_2SO_4. After concentration under reduced pressure, the residue was purified by flash column chromatography (silica gel, CH_2Cl_2/hexane = 2:1 (v/v)) to give **S3** (2.6 g, 48%) as a yellow solid. Mp > 273 ºC (dec); ^{1}H NMR (400 MHz, DMSO-d_6, RT): 11.27 (s, 1H), 10.78 (s, 1H), 10.59 (s, 1H), 8.32(s, 1H), 8.23(s, 1H), 8.05 (d, J = 8.0 Hz, 1H),

[1] K.-J. Chang, D. Moon, M. S. Lah and K.-S. Jeong, *Angew. Chem. Int. Ed.*, 2005, **44**, 7926.

8.00 (d, J = 8.0 Hz, 1H), 7.80 (s, 1H), 7.75 (s, 1 H), 7.48 (s, 1H), 7.46 (s, 1H), 4.00 (s, 3H), 3.93 (s, 3H), 1.46 (s, 9H), 1.42 ppm (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): 190.5, 154.2, 150.3, 144.5, 142.6, 139.5, 138.0, 131.2, 130.0, 126.3, 126.2, 126.0, 124.3, 121.6, 121.0, 120.9, 118.5, 116.7, 115.7, 113.0, 112.9, 108.9, 104.5, 92.1, 89.1, 77.1, 56.8, 56.2, 35.0, 34.9, 32.2, 32.1 ppm; IR (KBr): 3410(NH), 2188(C≡C), 1679(C=O) cm^{-1}; MS (MALDI-TOF): $[M–H]^+$, 683; Anal. Calcd for $C_{37}H_{35}IN_2O_3$: C, 65.10; H, 5.17; N, 4.10, Found: C, 65.64; H, 5.34; N, 4.08.

Compound **S4: S3** (2.5 g, 3.7 mmol), CuI (0.021 g, 0.11 mmol, 0.03 equiv), and $Pd(PPh_3)_2Cl_2$ (0.077 g, 0.11 mmol, 0.03 equiv) were added to a 100 mL Schlenk flask under N_2 gas. The flask was fitted with a rubber stopper, and then evacuated under vacuum and back-filled with N_2 (repeated three times). After addition of degassed Et_3N (20 mL), THF (30 mL) and trimethylsilylethyne (1.0 mL, 7.3 mmol, 2 equiv), the rubber stopper was replaced with a screw stopper and the solution was stirred at 55 °C during 16 h. The suspension was allowed to cool down to room temperature, filtered through Celite and concentrated. The residue was dissolved in CH_2Cl_2, washed with distilled water. After concentration, the crude mixture was dissolved in CH_2Cl_2/MeOH = 1:1 (v/v) to which solution K_2CO_3 (0.14 g, 1.0 mmol, 0.3 equiv) was added. After stirred for 2 h, the mixture was washed with brine, and dried over anhydrous Na_2SO_4. The solution was concentrated under reduced pressure. After concentration, the residue was purified by flash column chromatography (silica gel, CH_2Cl_2/hexane = 2:1 (v/v)) to give **S4** (1.7 g, 81% for two steps) as a yellow solid. Mp > 280 ºC (dec); ^{1}H NMR (400 MHz, DMSO-d_6, RT): 11.22 (s, 1H), 11.03 (s, 1H), 10.59 (s, 1H), 8.32 (s, 1H), 8.28 (s, 1H), 8.03 (s, 2H), 7.74 (s, 1H), 7.57 (s, 1H), 7.48 (s, 1H), 7.46 (s, 1H), 4.73 (s, 1H), 4.00 (s, 3H), 3.93 (s, 3H), 1.46 (s, 9H), 1.42 ppm (s, 9H); ^{13}C NMR (100 MHz, CD_2Cl_2-d_2, RT): 191.5, 153.7, 149.1, 142.8, 142.7, 139.7, 138.9, 129.3, 126.1, 126.0, 124.5, 124.0, 123.7, 121.5, 121.4, 119.1, 118.0, 117.8, 114.8, 112.9, 112.4, 112.1, 104.6, 103.9, 91.8, 91.2, 80.8, 80.5, 56.2, 55.9, 34.8, 34.7, 31.8, 31.7 ppm; IR (KBr): 3410(NH), 2186(C≡C), 1681(C=O) cm^{-1}; MS (MALDI-TOF): $[M–H]^+$, 581; Anal. Calcd for $C_{39}H_{36}N_2O_3$: C, 80.66; H, 6.25; N, 4.82, Found: C, 79.89; H, 6.37; N, 4.82.

Compound **1: S4** (1.6 g, 2.8 mmol) was dissolved in pyridine (80 mL) and $Cu(OAc)_2 \cdot H_2O$ (0.66 g, 3.4 mmol, 1.2 equiv) was added. The mixture was stirred for 5 h at ambient

temperature. The mixture was filtered through Celite and concentrated. The residue was taken up in CH_2Cl_2, washed with saturated $NaHCO_3$ solution and brine, and dried over anhydrous Na_2SO_4. After concentration, the residue was purified by flash column chromatography (silica gel, DCM/Hexane = 2:1 (v/v)) to give **1**(0.96 g, 60%) as a yellow solid. Mp > 297 ºC (dec); ^{1}H NMR (400 MHz, DMSO-d_6, RT): 11.40 (s, 2H; NH), 11.20 (s, 2H; NH), 10.53 (s, 2H), 8.42 (s, 2H), 8.33 (s, 2H), 8.09 (s, 4H), 7.73 (s, 4H), 7.40 (s, 2H), 7.25 (s, 2H), 3.85 (s, 6H), 3.70 (s, 6H), 1.47 ppm (s, 36H); ^{13}C NMR (100 MHz, CD_2Cl_2-d_2, RT): 191.7, 152.7, 147.8, 143.1, 142.2, 140.1, 140.0, 128.2, 126.0, 125.7, 125.6, 124.4, 122.7, 122.6, 121.4, 121.0, 118.5, 117.6, 116.6, 116.1, 114.8, 113.1, 111.5, 105.1, 103.7, 95.1, 91.2, 79,5, 77.9, 55.7, 54.4, 34.8, 34.7, 31.9, 31.7 ppm; IR (KBr): 3404(NH), 2193(C≡C), 1672(C=O) cm^{-1}; MS (ESI) [*M*-H]$^+$, 1159.4; Anal. Calcd for $C_{78}H_{70}N_4O_6 \cdot 2H_2O$: C, 78.36; H, 6.24; N, 4.69, Found: C, 78.15; H, 6.34; N, 4.75.

2. 1D- and 2D ^{1}H NMR Spectra

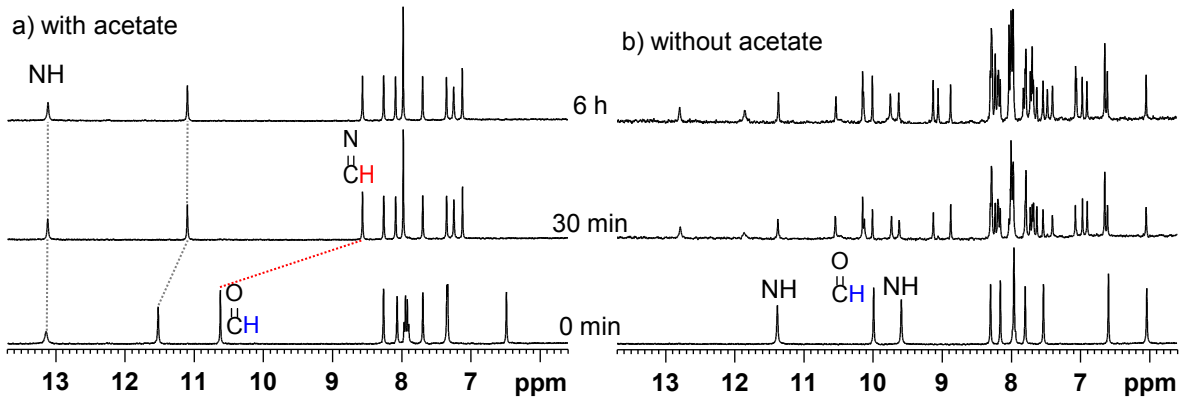


Fig. S1 Partial ^{1}H NMR (400 MHz, CD_2Cl_2, RT) spectra of time-dependent imine formation between **1** (1.0 mM) and **2a** (2 equiv) in the presence (left column) and in the absence (right column) of $Bu_4N^+AcO^-$(2 equiv).

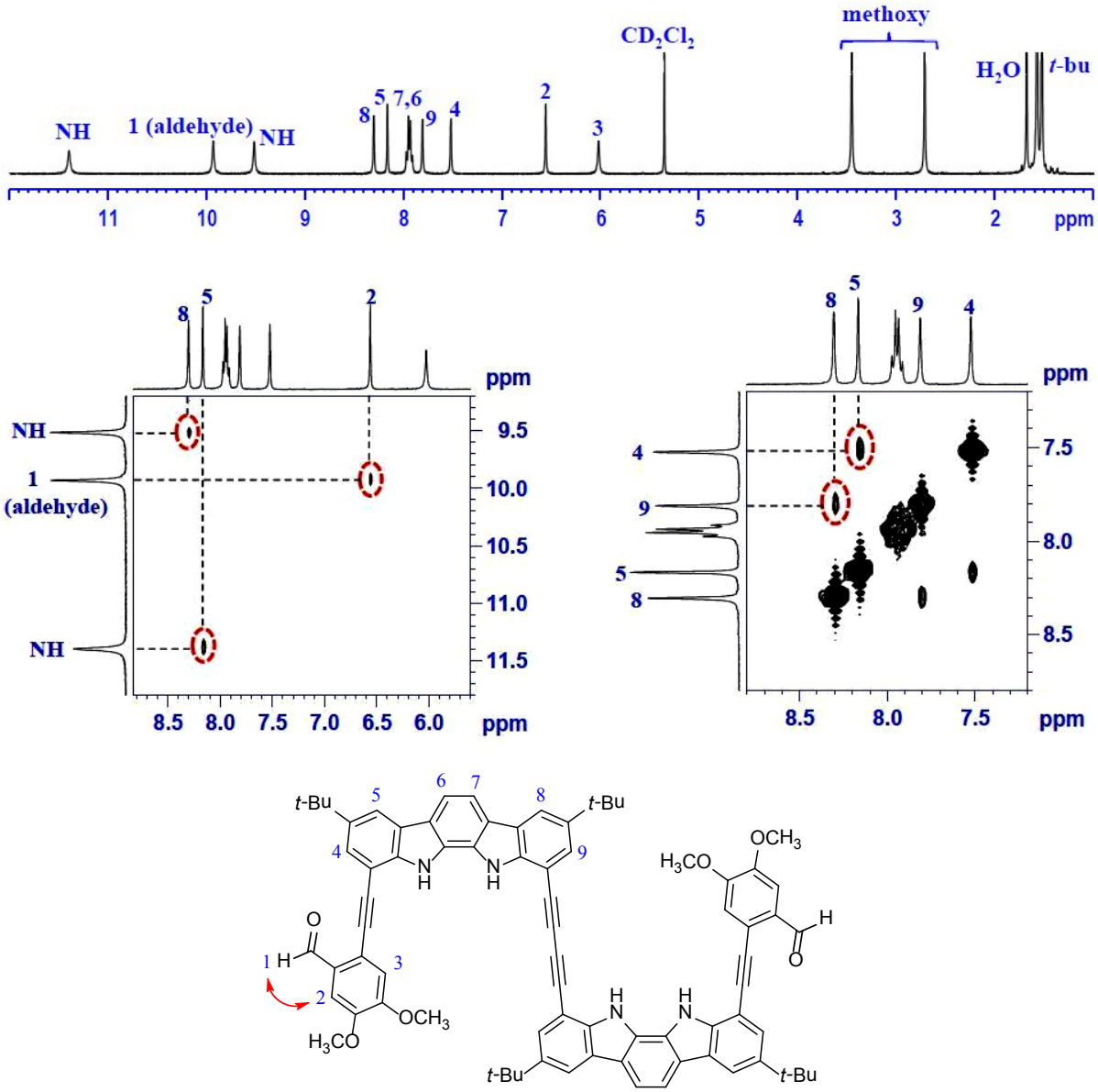


Fig. S2 Partial COSY spectrum (400 MHz, CD_2Cl_2, RT, pulse program: cosygpqf) of **1** (5.0 mM).

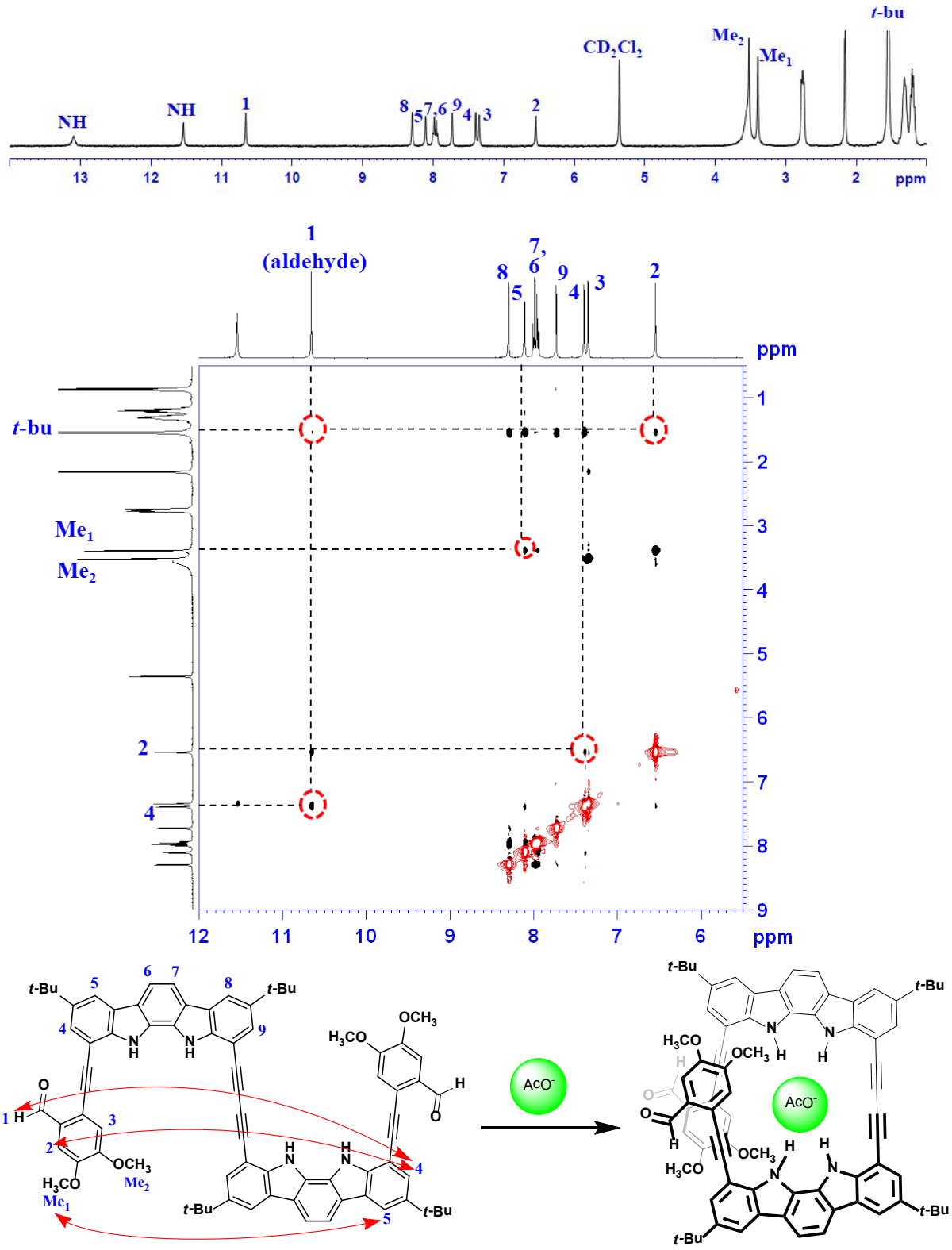


Fig. S3 Partial ROESY spectra (400 MHz, CD_2Cl_2, RT, mixing time: 400 ms, pulse program: roesyph) of **1** (5.0 mM) in the presence of $Bu_4N^+AcO^-$ (2 equiv).

3. Circular dichroism (CD) experiments

3.1 Time-dependent CD spectra for the formation of imine macrocycle **3a**

To a solution of compound **1** (1.0 mM in CH_2Cl_2) and tetrabutylammonium acetate (2 equiv) was added cyclohexane-(1*R*,2*R*)-diamine **2a** (2 equiv), and the reaction progress was monitered at 24 °C by CD spectroscopy. After given time period, an aliquot was taken from the reaction mixture and was diluted with pure CH_2Cl_2 until the concentration is 5.0×10^{-5} M based on **1**. The CD spectrum was recorded under the conditions (scanning rate: 500 nm min^{-1}, band width: 1.0 nm, response time: 1.0 sec, accumulations: 2 scans).

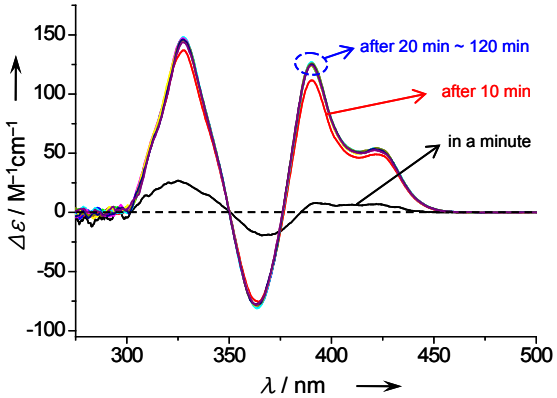


Fig. S4 Time-dependent CD spectra of **3a** in the presence of (*R*,*R*)-**2a** (2 equiv) and $Bu_4N^+AcO^-$ (2 equiv) at 24 °C.

3.2 Time-dependent CD spectra for the formation of imine macrocycle **3b**

To a solution of compound **1** (1.0 mM in CH_2Cl_2), *N*-Boc-D-Ala (5 equiv), tetrabutylammonium hydroxide (5 equiv) was added ethane-1,2-diamine (**2b**) (4 equiv), and the reaction progress was monitered at 24 °C by CD spectroscopy. After given time period, an aliquot was taken from the reaction mixture and was diluted with pure CH_2Cl_2 until the concentration is 5.0×10^{-5} M based on **1**. The CD spectrum was recorded under the conditions described in 3.1.

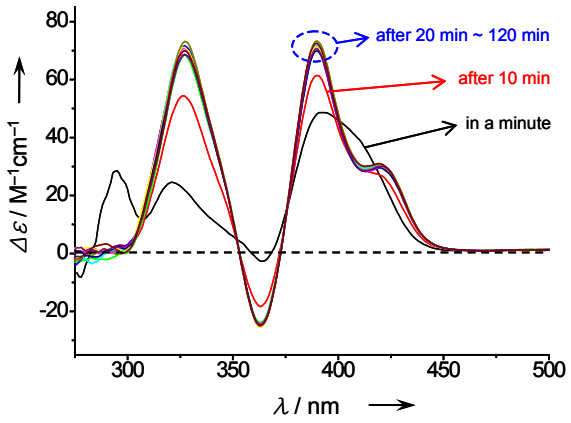


Fig. S5 Time-dependent CD spectra of **3b** in the presence of *N*-Boc-D-Ala (5 equiv), tetrabutylammonium hydroxide (5 equiv), and ethane-1,2-diamine **2b** (4 equiv) at 24 °C.

3.3 CD spectra and CD values of **3b** in the presence of anionic amino acids with different *N*-protective groups (Boc, Cbz, and Ac)

To a solution of compound **1** (1.0 mM in CH_2Cl_2), *N*-protected amino acids (5 equiv), tetrabutylammonium hydroxide (5 equiv) was added ethane-1,2-diamine (**2b**) (4 equiv). After an hour, an aliquot was taken from the reaction mixture and was diluted with pure CH_2Cl_2 until the concentration is 5.0×10^{-5} M based on **1**. The CD spectrum was recorded under the conditions described in 3.1.

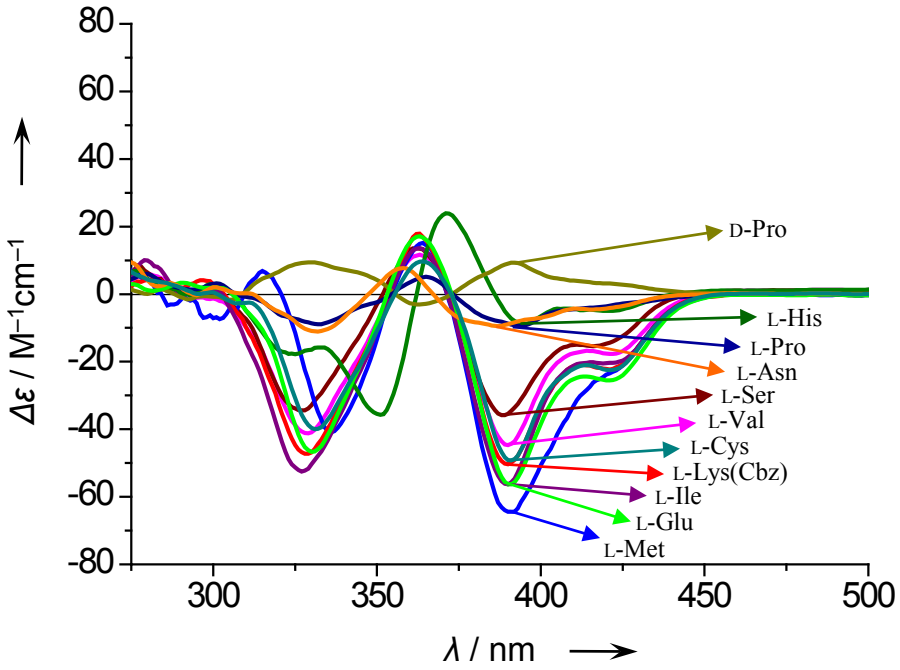


Fig. S6 CD spectra of **3b** (5.0×10^{-5} M) in the presence of *N*-Boc amino acids which are not shown in Fig 3a for clarity.

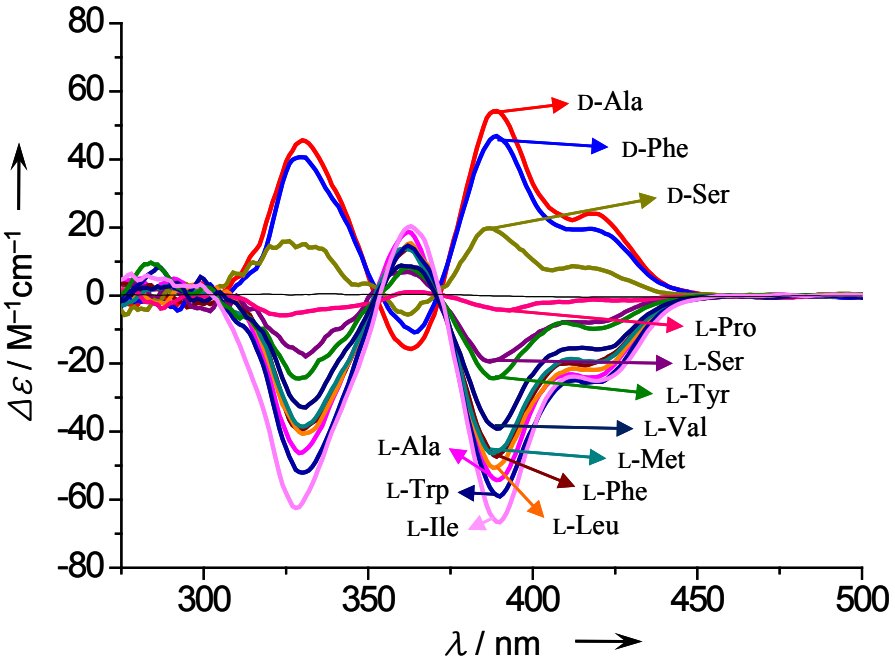


Fig. S7 CD spectra of **3b** (5.0×10^{-5} M) in the presence of *N*-Cbz-protected amino acids.

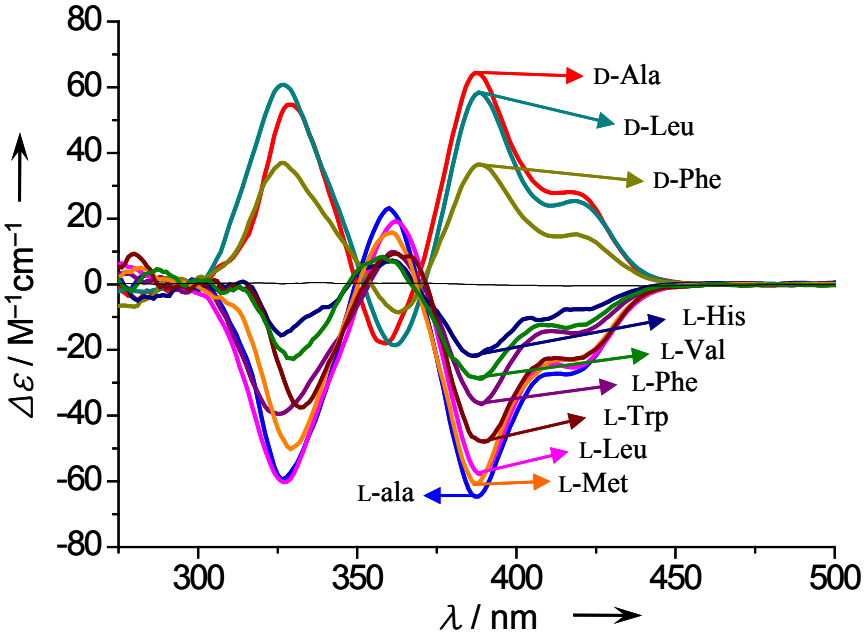


Fig. S8 CD spectra of **3b** (5.0×10^{-5} M) in the presence of *N*-acetyl (Ac)-protected amino acids.

Table S1 Comparison of CD value of macrocycles with *N*-protected amino acids

N-protected amino acids	$\Delta\varepsilon$ (421 nm) / $M^{-1}cm^{-1}$			$\Delta\varepsilon$ (390 nm) / $M^{-1}cm^{-1}$		
	N-Boc	*N*-Cbz	*N*-Ac	*N*-Boc	*N*-Cbz	*N*-Ac
L-Ala	–27	–23	–26	–66	–54	–63
L-Glu(OtBu)	–26	_[a]	_	–55	_	_
L-Cys	–23	_	–8	–49	_	–20
L-Met	–23	–19	–22	–64	–45	–58
L-Lys(Cbz)	–22	_	_	–50	_	_
L-Leu	–21	–22	–25	–54	–50	–57
L-Ile	–20	–25	_	–56	–67	_
L-Gln	–19	_	_	–44	_	_
L-Phe	–19	–19	–14	–48	–47	–36
L-Val	–18	–16	–12	–44	–39	–28
L-Trp	–18	–25	–22	–45	–59	–48
L-Arg(Boc)$_2$	–17	_	_	–40	_	_
L-Asp(OtBu)	–16	_	_	–38	_	_
L-Ser	–14	–8	_	–35	–19	_
L-Tyr	–10	–10	_	–22	–24	_
L-His	–5	_	_	–15	_	_
L-Asn	–4	_	_	–9	_	_
L-Pro	–3	–1	_	–9	–4	_
D-Ala	+27	+23	+27	+66	+54	+63
D-Leu	+24	_	+25	+58	_	+57
D-Phe	+20	+19	+15	+49	+46	+36
D-Ser	_	+8	_	_	+19	_
D-Pro	+3	_	_	+9	_	_

[a] Not measured.

4. Computer modeling structures

Energy-minimized structures of complexes **3b** complexed with anionic *N*-Boc-D-Ala were generated using MacroModeling 9.1[2] program. The structures were found with MMFFs force field[3] in the gas phase via 3000 separated search steps in Monte Carlo conformational search.[4]

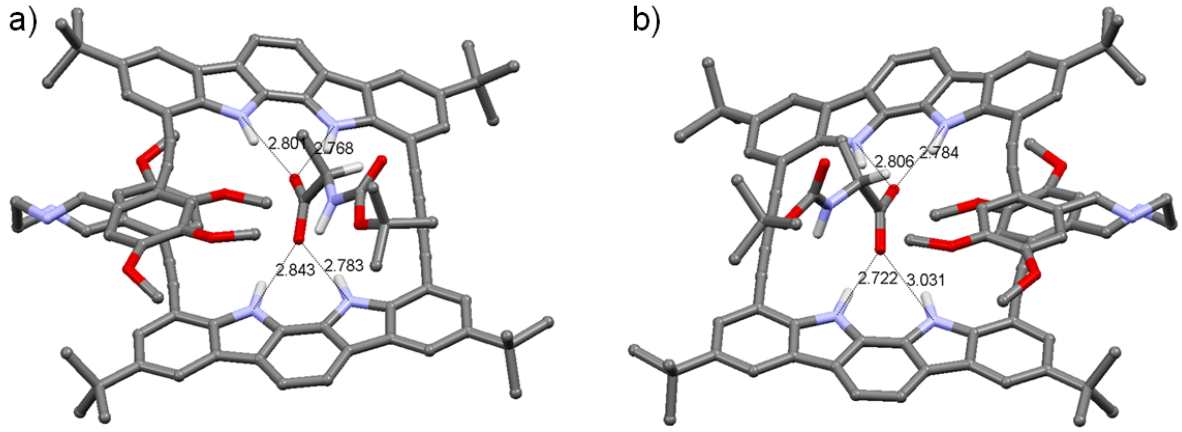


Fig. S9 Energy minimized structure of **3b** complexed with anionic *N*-Boc-D-Ala. a) *P*-helix (energy = 805 kJ/mol) and b) *M*-helix (energy = 810 kJ/mol).

[2] F. Mohamedi, N. G. T. Richards, W. C. H. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W. C. Still, *J. Comp. Chem*., 1990, **11**, 440.
[3] T. A. Halgren, *J. Comp. Chem*., 1996, **17**, 490.
[4] M. Saunders, K. N. Houk, Y. D. Wu, W. C. Still, M. Lipton, G. Chang and W. C. Guida, *J. Am. Chem. Soc*., 1990, **112**, 1419; E. Polak and G. Ribiere, Revenue Francaise Informat. Recherche Operationelle, Serie Rouge, 1969**,** **16**, 35.

5. X-ray crystallographic analysis

Single crystals were grown as follow: **1** (20 mg), $Bu_4N^+OAc^-$ (2 equiv) and **2a** (2 equiv) were dissolved in CH_2Cl_2 (1 mM) and stirred for 1 h. After evaporate organic solvent, imine macrocycle **3a** was dissolved in ethyl acetate containing CH_2Cl_2 (1-2 drops) and *n*-pentane was added to the solution until no precipitate was formed. Slow diffusion of *n*-pentane into an ethyl acetate/CH_2Cl_2 solution over a few days yielded single crystals suitable for the X-ray diffraction.

A specimen of suitable size and quality was coated with Paratone oil and mounted onto a glass capillary. Reflection data were collected on a Bruker D8 Venture PHOTON 100 area detector diffractometer, with Cu IμS microfocus tube radiation (λ = 1.54178 Å). The full sphere of reflection data were collected as ω scan frames with 0.5°/frame and an exposure time of 20 s/frame. Cell parameters were determined and refined by APEX2 program[5].

Data reduction was performed using SAINT software.[6] The data were corrected for Lorentz and polarization effects. An empirical absorption correction was applied using the SADABS program.[7] The structure was solved by direct methods and all nonhydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares on F^2 by using the SHELXTL/PC package.[8] Hydrogen atoms were placed at their geometrically calculated positions and refined riding on the corresponding carbon atoms with isotropic thermal parameters. The disordered solvent molecules, pentane and ethylacetate, are treated by solvent mask with Olex 2.[9]

A summary of the crystal and some crystallography data are given in Table S7. CCDC-951760 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK.

[5] APEX2, version 2012.2-0, Data collection software, Bruker AXS, Inc., Madison, WI, 2011.
[6] SAINT, version 6.0, Data integration software, Bruker AXS Inc., Madison, WI, 2011.
[7] G. M. Sheldrick, version 2.05 SADABS, Program for absorption correction with the Bruker SMART system, Universitat Gottingen, Germany, 2011.
[8] G. M. Sheldrick, SHELXL-93: Program for the refinement of crystal structures; Universitat Gottingen: Germany, 2004.
[9] O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program. J. Appl. Cryst., 2009, **42**, 339.

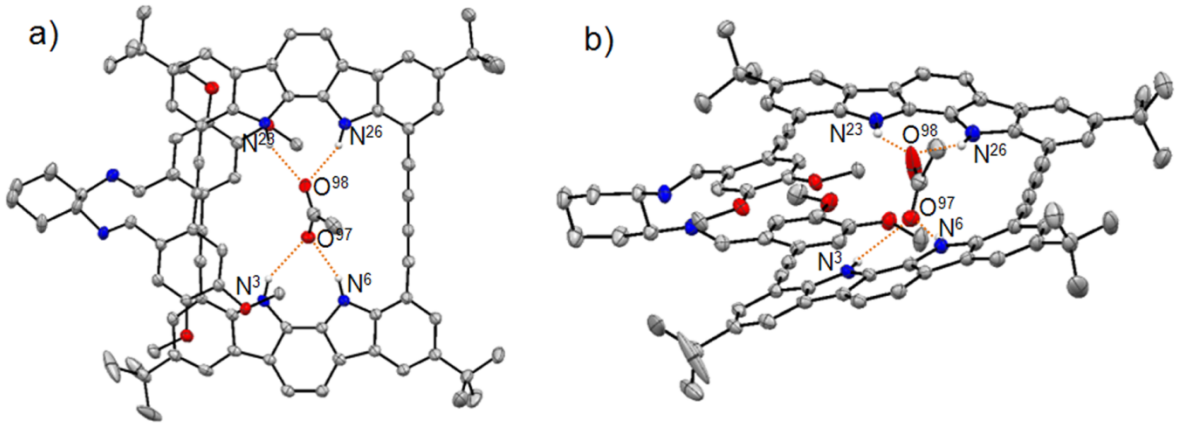


Fig. S10 ORTEP views of **3a**·TBA^{+}OAc^{-} with 20% probability ellipsoids. The countercation and hydrogen atoms except NH have been omitted for clarity.

Table S2 Crystal data and structure refined for **3a**·TBA^{+}OAc^{-}

Identification code	cu_Moon115_0m
Empirical formula	$C_{102}H_{119}N_7O_6$
Formula weight	1539.03
Temperature/K	140.0
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	16.0521(4)
b/Å	16.1874(4)
c/Å	38.9167(10)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	10112.2(4)
Z	4
ρ_{calc}mg/mm^3	1.011
m/mm^{-1}	0.486
F(000)	3312.0
Crystal size/mm^3	0.1 × 0.1 × 0.07
2Θ range for data collection	4.54 to 160°
Index ranges	$-20 \le h \le 20$, $-18 \le k \le 20$, $-47 \le l \le 49$
Reflections collected	321916
Independent reflections	21096[R(int) = 0.1129]
Data/restraints/parameters	21096/0/1057
Goodness-of-fit on F^2	1.075

Final R indexes [I>=2σ (I)]	R_1 = 0.0674, wR_2 = 0.1790
Final R indexes [all data]	R_1 = 0.0978, wR_2 = 0.1956
Largest diff. peak/hole / e Å^{-3}	1.43/-0.28
Flack parameter	0.12(8)

Table S3 Hydrogen bond distances and angles for **3a**• $Bu_4N^+OAc^-$ [Å and °]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N^3-H(N^3)···O^{97}	0.88(3)	2.42(3)	3.19(5)	145(2)
N^6-H(N^6)···O^{97}	0.88(3)	2.03(3)	2.85(5)	156(2)
N^{23}-H(N^{23})···O^{98}	0.88(3)	2.25(5)	3.03(6)	149(2)
N^{26}-H(N^{26})···O^{98}	0.88(3)	2.03(5)	2.85(6)	156(3)