Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Liquid crystalline organization of self-assembling cyclic peptides

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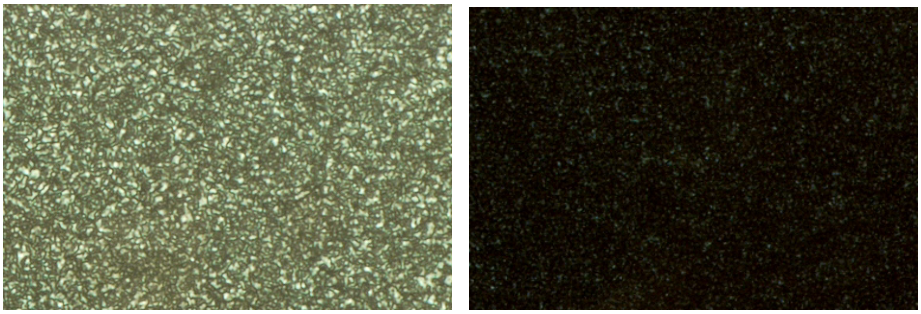


Figure S1. Polarized Optical Microscopy. Texture observed through crossed polarizers by compound **CP1** at 30 ºC, left, and compound **CP2** at 90 ºC, right, cooling from the isotropic liquid at a rate of 1 ºC/min.

Table S1: Summary of FT-IR (cm^{-1}) Data for Selected Peptides.

Cyclic Peptide		Amide A	Amide I_a	Amide I_b	Amide II	intersubunit distance (Å)[b]
CP-Bn	Solution[a]	3303	1667	1627	1532	4.80
	Solid	3302	1672	1626	1529	4.80
CP1	Solution[a]	3309	1673	1628	1534[c]	4.83
	Solid	3299	1678	1626	1539[c]	4.79
CP2	Solution[a]	3308	1673	1629	1535[c]	4.83
	Solid	3286	1674[c]	1643[c]	1540[c]	4.75
CP3	Solution[a]	3310	1671	1626	1530	4.83
	Solid	3300	[c]	1626	1541	4.80

[a] Solution in $CHCl_3$ (**CP-Bn**: 1 mM, **CP2**: 2.5 mM, **CP1** and **CP3**: 5 mM). [b] Estimated interpeptide distances derived from the Krimm's analysis.[1] [c]These bands are overlapped with nearby signals.

Table S2. X-ray data for the hexagonal columnar mesophase of compounds **CP1**, **CP2** and **CP3** at room temperature after cooling from the isotropic liquid. d_{obs} and d_{calc} are the observed and calculated spacings, respectively (vs: very strong; m: medium intensity; diff.: diffuse); *h k* are Miller indices; *a* is the hexagonal lattice constant; S_{col} is the cross-sectional area of the hexagonal unit cell; *Z* is the estimated number of molecules per column stratum.

Compound	d_{obs} (Å)	d_{calc} (Å)	*h k*	Lattice parameters (Å)	Z
CP1	43.8 (vs)	43.6	1 0	a = 50.3	2
	25.0 (m)	25.15	1 1	S_{col} = 2193 Å^2	
	4.4 (diff.)				
CP2	45.4 (vs)	45.1	1 0	a = 52.1	2
	25.7 (m)	26.05	1 1	S_{col} = 2350 Å^2	
	4.4 (diff.)				
CP3	40.5 (vs)	40.2	1 0	a = 46.4	1
	22.9 (m)	23.2	1 1	S_{col} =1865 Å^2	
	4.4 (diff.)				

[1] S. Krimm and J. Bandekar, *Adv. Protein Chem.*, 1986, **38**, 181–364.

General techniques:

N-[(dimethylamino)-1H-1,2,3-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (*N*-HATU), N-[(1H-benzotriazol-1-yl)-(dimethylamino) methylene]-N-methylmethanaminium hexafluorophosphate N-oxide (*N*-HBTU), N-[(1H-benzotriazol-1-yl)-(dimethylamino)methylene]-N-methylmethanaminium tetrafluoroborate N-oxide (*N*-TBTU),[2] *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), alpha-aminoacids were purchased from Novabiochem, Applied Biosystems, Aldrich or from Global Sales Manager, GL Biochem (Shanghai) Ltd, China. All reagents and solvents were used as received unless otherwise noted. Solvent mixtures for chromatography are re-ported as v/v ratios. Column chromatography was performed on EM Science silica gel 60 (230-400 mesh). HPLC purification was carried out on phenomenex Luna 5u Silica 100 Angstroms column with CH_2Cl_2/MeOH gradients between 100 and 90:10 or CH_2Cl_2/Isopropanol gradients between 100 and 90:10. CH_2Cl_2 and DIEA to be used as reaction solvents were distilled from CaH_2 over argon immediately prior to use. Tetrahydrofurane (THF) was dried and distilled over sodium/benzophenone.[3] **^{1}H NMR** spectra were recorded on Bruker AMX 500 MHz spectrometers, Espectrómetro Varian Inova 400, Varian Mercury 300 MHz, or Bruker WM 250 MHz spectrometers. Chemical shifts (δ) were reported in parts per million (ppm, δ) relative to tetramethylsilane (δ = 0.00 ppm) or by the deuterium solvent. ^{1}H NMR splitting patterns are designated as singlet (s), doublet (d), triplet (t), or quartet (q). All first-order splitting patterns were assigned on the basis of the appearance of the multiplet. Splitting patterns that could not be easily interpreted are designated as multiplet (m) or broad (br). **^{13}C NMR** spectra were recorded on Varian Mercury 300 MHz or Bruker WM 250 MHz spectrometers. Carbon resonances were assigned by using DEPT spectra obtained with phase angles of 135º. Electrospray (ESI) mass spectra were recorded on a Bruker BIOTOF II mass spectrometer. Mass Spectrometry of Laser Desorption/Ionization-Time of Flight (MALDI-TOF) was obtained on a Bruker Autoflex mass spectrometer. FTIR measurements were made on a JASCO FT/IR-400 spectrophotometer placing the sample on a CaF_2 pellet and in solution in a CaF_2 IR cell ($CHCl_3$). Differential scanning calorimetry (DSC) was performed with a DSC-MDSC TA instruments Q-20 and Q-2000. Liquid crystal textures were studied using an Olympus BX-50 polarizing microscope equipped with a Linkam TMS91 hot stage and a CS196 hot-stage central processor. Microphotographs were taken with an Olympus DP12-2 digital camera. X-ray Diffraction measurements were carried out at room temperature using a Pinhole camera (Anton-Paar)

[2] L. A. Carpino, H. Imazumi, A. El-Faham, F. J. Ferrer, C. Zhang, Y. Lee, B. M. Foxman, P. Henklein, C. Hanay, C. Mügge, H. Wenschuh, J. Klose, M. Beyermann and M. Bienert, *Angew. Chem. Int. Ed.,* 2002, **41**, 441–445.

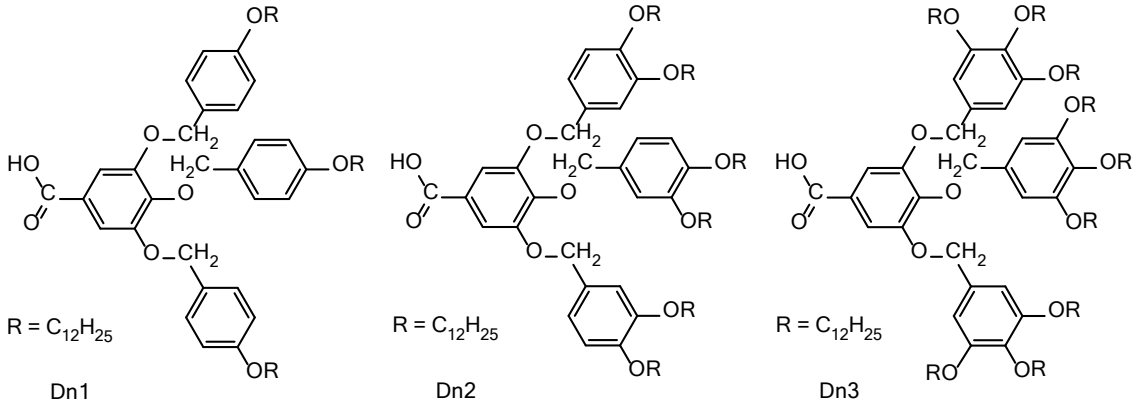
[3] a) H. C. Brown, "*Organic Synthesis via Boranes"*, Ed. John Wiley & Sons, 1975. b) D. D. Perrin, W. I. F. Armarego, *"Purification of Laboratory Chemicals"*, Ed. Pergamon Press, 1988.

operating with a point focused Ni-filtered Cu Kα beam. The sample was held in Lindemann glass capillaries (1 mm diameter) perfectly sealed and heated, when necessary, with a variable-temperature attachment. The diffraction patterns were collected on a flat photographic film perpendicular to the X-ray beam.

Synthesis.

Synthesis of Dendrons:

Dendron molecules **Dn1**, **Dn2** and **Dn3** were prepared following the synthetic strategy previously described.[4]



Scheme S1. Structure of **Dn1, Dn2 and Dn3** dendrons.

Peptide Synthesis:

γ-amino acids (Acp) and peptides were prepared following the synthetic strategy previously described.[5]

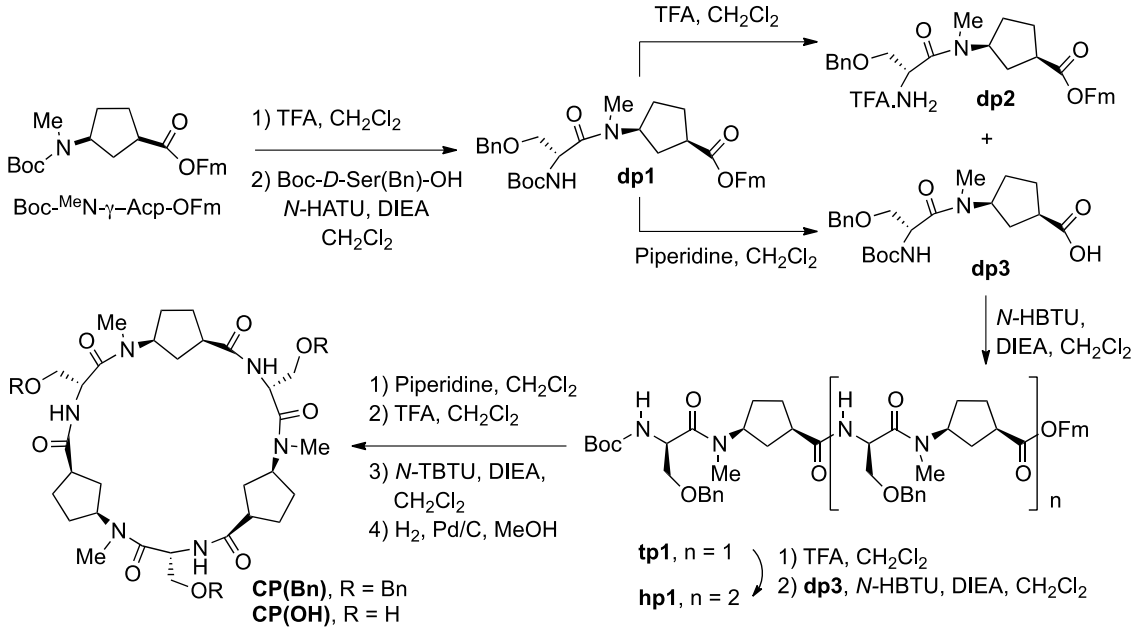
Cyclic Peptides containing dendron side chains *c*-{[*D*-Ser(Dn1-3)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$} (**CP1-3**) were prepared from cyclic hexapeptide **CP(OH)** following the synthetic strategy shown in the scheme S2.[5] Cyclic peptide **CP(OH)** was prepared by a convergent solution phase strategy starting from the fluorenylmethyl ester of N-methylated 3-aminocyclopentanecarboxylic acid ((*1R,3S*)-Boc-^{Me}N-γ-Acp-OFm) and the benzyl protected serine residues.[6] The TFA treatment of (*1R,3S*)-Boc-^{Me}N-γ-Acp-OFm provided a TFA salt that was then coupled with serine residue

[4] a) V. S. K. Balagurusamy, G. Ungar, V. Percec and G. Johansson, *J. Am. Chem. Soc.*, 1997, **119**, 1539–1555; b) V. Percec, C. H. Ahn, W. D. Cho, A. M. Jamieson, J. Kim, T. Leman, M. Schmidt, M. Gerle, M. Moller, S. A. Prokhorova, S. S. Sheiko, S. Z. D. Cheng, A. Zhang, G. Ungar and D. J. P. Yeardley, *J. Am. Chem. Soc.*, 1998, **120**, 8619–8631; c) V. Percec, W. D. Cho, P. E. Mosier, G. Ungar and D. J. P. Yeardley, *J. Am. Chem. Soc.*, 1998, **120**, 11061–11070.

[5] a) M. Amorín, L. Castedo and J. R. Granja, *J. Am. Chem. Soc.*, 2003, **125**, 2844–2845; b) M. Amorín, V. Villaverde, L. Castedo and J. R. Granja, *J. Drug Del. Sci. Tech.*, 2005, **15**, 87–92; c) R. J. Brea, M. Amorín, L. Castedo and J. R. Granja, *Angew. Chem. Int. Ed.*, 2005, **44**, 5710–5713; d) M. Amorín, L. Castedo and J. R. Granja, *Chem. Eur. J.*, 2005, **11**, 6543–6551; e) R. J. Brea, L. Castedo and J. R. Granja, *Chem. Commun.*, 2007, 3267–3269; f) R. J. Brea, M. E. Vázquez, M. Mosquera, L. Castedo and J. R. Granja, *J. Am. Chem. Soc.*, 2007, **129**, 1653–1657.

[6] a) N. Rodríguez-Vázquez, S. Salzinger, L. F. Silva, M. Amorín and J. R. Granja, *Eur. J. Org. Chem.*, 2013, 3477-3493; b) J. Bandekar, *Biochim. Biophys. Acta*, 1992, **1120**, 123–143.

(Boc-*D*-Ser(Bn)-OH) in the presence of *N*-HATU and DIEA to afford the basic building block **dp1**. This approach reduces the number of coupling with steric hindered N-methylated amino acids. After selective N-terminal deprotection (TFA treatment) of one third of **dp1** and C-terminal deprotection (20% piperidine/dichloromethane) of second third, the resulting dipeptides (**dp2** and **dp3**, respectively) were coupled in the presence of *N*-HBTU and DIEA to give tetrapeptide **tp1** in good yield. Finally, TFA treatment of resulting tetrapeptide (**tp1**) followed by coupling with C-unprotected dipeptide **dp3** provided hexapeptide **hp1**. The treatment of the unprotected linear hexapeptides, obtained by reaction with piperidine treatment (20% in dichloromethane) followed by TFA in dichloromethane, with *N*-TBTU and DIEA (1 mM in dichloromethane) gave rise the protected cyclic products **CP(Bn)** in good yield. The NMR spectrum showed the characteristic featured of dimeric structure denoted by the downfield shift of amide protons signals (8.10 ppm) in which the coupling constant of 9.3 Hz suggests the all trans conformation. The β-sheet type interaction characteristic of dimeric structure, with bands at 3302, 1672, 1626 and 1529 cm^{-1}, was also confirmed by FTIR (Table S1).[7] Benzyl groups were removed by treatment of **CP(Bn)** with 10% $Pd(OH)_2/C$ in MeOH for 24 h at rt under hydrogen atmosphere (balloon pressure). The resulting serine deprotected cyclic hexapeptide *c*-{[*D*-Ser-(*1R,3S*)-^{Me}N-γ-Acp-]$_3$} (**CP(OH)**) was not very soluble in non polar solvents, thus dimeric structure was not possible to study in this media.



Scheme S2. Synthesis of cyclic peptides by solution phase strategy.

[7] a) P. I. Haris and D. Chapman, *Biopolymers (Peptide Sci.)*, 1995, **37**, 251–263; b) S. Krimm and J. Bandekar, *Adv. Protein Chem.*, 1986, **38**, 181–364; c) J. Bandekar, *Biochim. Biophys. Acta*, 1992, **1120**, 123–143; d) J. Kubelka and T. A. Keiderling, *J. Am. Chem. Soc.*, 2001, **123**, 12048–12058.

c*-{[*D*-Ser(Bn)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$} [CP(Bn)]**. A solution of Boc-[*D*-Ser(Bn)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$OFm (365.0 mg, 0.303 mmol) in 20% piperidine in CH_2Cl_2 (5 mL) was stirred at rt for 30 min. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (10 mL) and washed with HCl (5%), dried over Na_2SO_4, filtered and concentrated. The resulting material was dissolved in TFA/CH_2Cl_2 (1:1, 2.5 mL) and stirred at rt for 10 min. The solvent was removal and the residue was dried under high vacuum for 2 h and used without further purification. The linear peptide was dissolved in CH_2Cl_2 (303 mL, 1.0 mM) and treated with *N*-TBTU (126.4 mg, 0.334 mmol), followed by dropwise addition of DIEA (0.212 mL, 1.213 mmol). After 12 h, the solvent was removed under reduced pressure, and the crude was purified by HPLC (Phenomenex Luna 5u column, CH_2Cl_2/MeOH) to afford 200 mg of **CP(Bn)** as white solid (73%). **^{1}H NMR** ($CDCl_3$, 300 MHz, δ): 8.10 (d, *J* = 9.3 Hz, 1 H), 7.35-6.99 (m, 5 H), 5.27 (q, *J* ~ 9 Hz, 1 H), 4.74 (m, 1 H), 4.40 (d, *J* = 12.5 Hz, 1 H), 4.30 (d, *J* = 12.5 Hz, 1 H), 3.47 (t, *J* = 9.0 Hz, 1 H), 3.27 (dd, *J* = 8.5, 5.3 Hz, 1 H), 2.95 (s, 3 H), 2.86 ppm (br, 1H). **^{13}C NMR** ($CDCl_3$, 75.4 MHz, δ): 175.0 (C=O), 171.7 (C=O), 137.9 (C), 128.3 (CH), 128.0 (CH), 127.2 (CH), 72.9 (CH_2), 70.4 (CH_2), 54.8 (CH), 47.9 (CH), 42.6 (CH), 36.2 (CH_2), 30.0 (CH_3), 27.2 (CH_2), 26.9 ppm (CH_2). **MS-ESI$^+$** [m/z*** (%)]: 907.5 ([MH]$^+$, 99); 929.5 ([MNa]$^+$, 100). **HRMS [MH$^+$] calculated:** 907.4964, **found**: 907.4954. **FTIR (CaF_2 pellet)**: ν = 3302 (amide A), 1672 (amide I_{II}), 1626 (amide I), 1529 cm^{-1} (amide II). **FTIR (1.0 mM, CaF_2 cell)**: ν = 3303 (amide A), 1667 (amide I_{II}), 1627 (amide I), 1532 cm^{-1} (amide II).

c*-{[*D*-Ser(Dn1)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$} (CP1)**. A solution of **CP(Bn)** (30 mg, 0.033 mmol) in $Pd(OH)_2$/C (15.0 mg, 20% in wt) in MeOH (1.0 mL) was stirred at rt for 24 h under hydrogen atmosphere (balloon pressure). The resulting peptide *c*-{[*D*-Ser-(*1R,3S*)-^{Me}N-γ-Acp-]$_3$} [**CP(OH)**] was filtered through celite pad, the residue was washed with methanol and the combined filtrates and washings were concentrated under reduced pressure; and dried at high vacuum for 6 h. The resulting material was dissolved in $CDCl_3$ (1.0 mL) and treated with **Dn1** (113.1 mg, 0.116 mmol), EDC (50.7 mg, 0.265 mmol), and DMAP (32.3 mg, 0.264 mmol). Each 24 h, additional EDC (25.0 mg, 0.232 mmol) was added during 3-5 days. It was diluted with $CHCl_3$ (5 mL) and washed with 5% HCl (2 x 5 mL), saturated solution of Na_2CO_3 (2 x 5 mL), dried over Na_2SO_4, filtered and concentrated. The crude was purified by HPLC (Phenomenex Luna 5u column, CH_2Cl_2/MeOH) to afford 38.4 mg of **CP1** as pale yellow solid (33%). **^{1}H NMR** ($CDCl_3$, 250 MHz, δ): 8.29 (br, 1 H), 7.35-7.26 (s and overlapping doublets, 6 H), 7.20 (d, *J* = 8.6 Hz, 2 H), 6.85 (d, *J* = 8.6 Hz, 4 H), 6.72 (d, *J* = 8.6 Hz, 2 H), 5.59 (br, 1 H), 5.30 (CH_2Cl_2), 4.97 and 4.93 (s, 6 H), 4.83 (br, 1 H), 4.63 (br, 1 H), 4.36 (br, 1 H), 3.95-3.85 (m, 6 H), 3.11 (s, 3 H), 2.98 ppm (br, 1 H). **MS-MALDI-TOF** (Dithranol matrix) [m/z*** (%)]: 3587.4 ([MNa]$^+$, 100). **FTIR (CaF_2 pellet)**: ν =

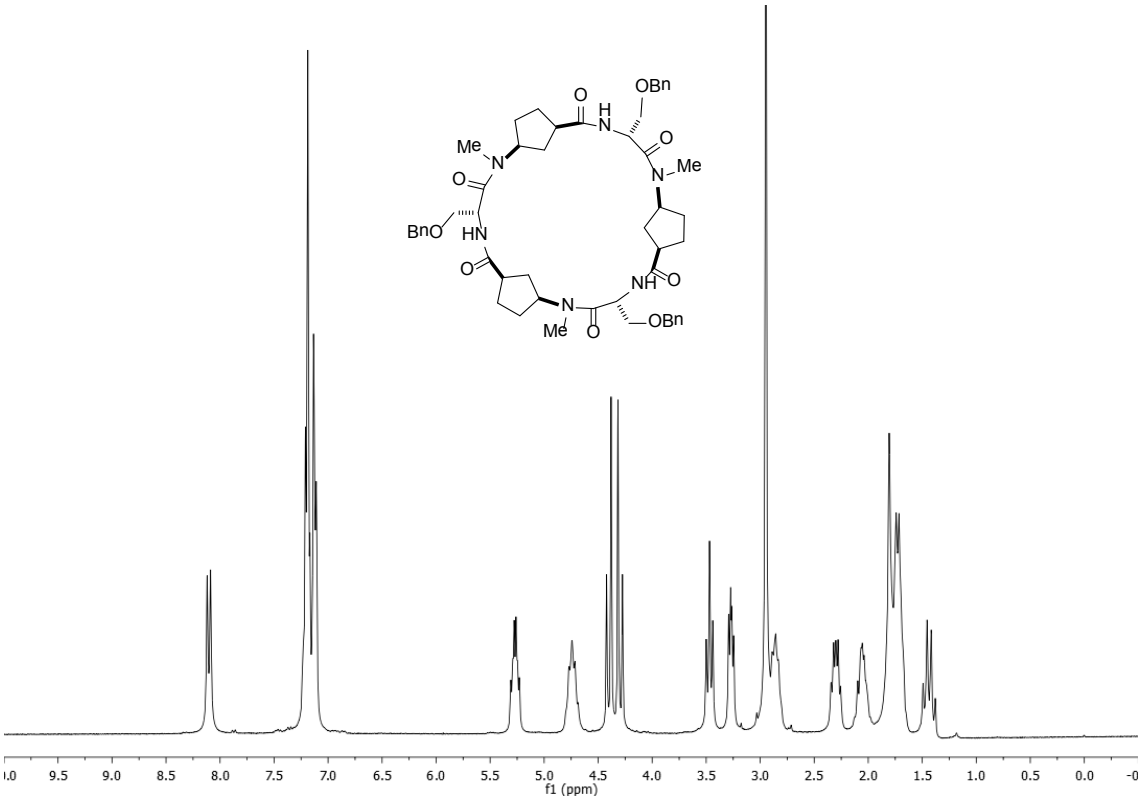
3299 (amide A), 1719 (ν C=O, ester), 1678 (amide I_{II}), 1626 (amide I), 1539 cm^{-1} (amide II). **FTIR (5.0 mM, CaF_2 cell)**: ν = 3309 (amide A), 1718 (ν C=O, ester), 1673 (amide I_{II}), 1628 (amide I), 1534 cm^{-1} (amide II).

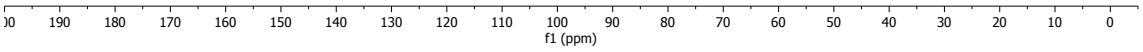
c*-{[*D*-Ser(Dn2)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$} (CP2)**. Prepared in the same way as **CP1** from **CP(Bn)** (25 mg, 0.028 mmol) in $Pd(OH)_2$/C (12.5 mg, 20% in wt) in MeOH (1.0 mL) and **Dn2** (149.2 mg, 0.096 mmol), EDC (42.3 mg, 0.221 mmol), and DMAP (26.9 mg, 0.221 mmol). The crude was purified by HPLC (Phenomenex Luna 5u column, CH_2Cl_2/MeOH) to afford 57.6 mg of **CP2** as pale yellow solid (39%). **^{1}H NMR** ($CDCl_3$, 500 MHz, δ): 8.29 (br, 1 H), 7.38 (s, 2 H), 6.98 and 6.90 (s, 3 H), 6.89 (d, *J* = 8.2 Hz, 2 H), 6.81 (d, *J* = 8.2 Hz, 2 H), 6.79 (d, *J* = 8.2 Hz, 1 H), 6.68 (d, *J* = 8.2 Hz, 1 H), 5.59 (br, 1 H), 5.30 (CH_2Cl_2), 4.98 and 4.93 (s, 6 H), 4.84 (br, 1 H), 4.64 (br, 1 H), 4.37 (br, 1 H), 3.95-3.65 (m, 12H), 3.14 (s, 3 H), 2.98 ppm (br, 1 H). **MS-MALDI-TOF** (Dithranol matrix) [m/z*** (%)]: 5246.9 ($[MNa]^+$, 100). **FTIR (CaF_2 pellet)**: ν = 3286 (amide A), 1731 (ν C=O, ester), 1674 (amide I_{II}), 1643 (amide I), 1540 cm^{-1} (amide II). **FTIR (2.5 mM, CaF_2 cell)**: ν = 3308 (amide A), 1717 (ν C=O, ester), 1673 (amide I_{II}), 1629 (amide I), 1535 cm^{-1} (amide II).

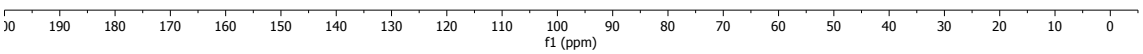
c*-{[*D*-Ser(Dn3)-(*1R,3S*)-^{Me}N-γ-Acp-]$_2$} (CP3)**. Prepared in the same way as **CP1** from **CP(Bn)** (20 mg, 0.022 mmol) in $Pd(OH)_2$/C (10.0 mg, 20% in wt) in MeOH (1.0 mL) and **Dn3** (162.0 mg, 0.077 mmol), EDC (33.8 mg, 0.176 mmol), and DMAP (21.6 mg, 0.176 mmol). The crude was purified by HPLC (Phenomenex Luna 5u column, CH_2Cl_2/MeOH) to afford 92.5 mg of **CP3** as white solid (61%). **^{1}H NMR** ($CDCl_3$, 400 MHz, δ): 8.28 (br, 1 H), 7.42 and 7.38 (s, 2H), 6.63, 6.57 and 6.55 (s, 6 H), 5.60 (br, 1 H), 5.30 (CH_2Cl_2), 5.00 and 4.95 (s, 6 H), 4.84 (br, 1 H), 4.63 (br, 1 H), 4.40 (br, 1 H), 3.95-3.65 (m, 18 H), 3.15 (s, 3 H), 3.02 ppm (br, 1 H). **MS-MALDI-TOF** (Dithranol matrix) [m/z*** (%)]: 6886.8 ($[MH]^+$, 100). **FTIR (CaF_2 pellet)**: ν = 3300 (amide A), 1699 (ν C=O, ester), 1626 (amide I), 1541 cm^{-1} (amide II).). **FTIR (5.0 mM, CaF_2 cell)**: ν = 3310 (amide A), 1708 (ν C=O, ester), 1671 (amide I_{II}), 1626 (amide I), 1530 cm^{-1} (amide II).

Nuclear Magnetic Resonance

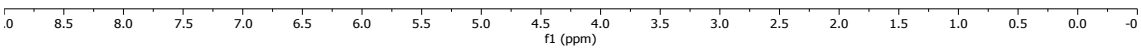
CP(Bn):



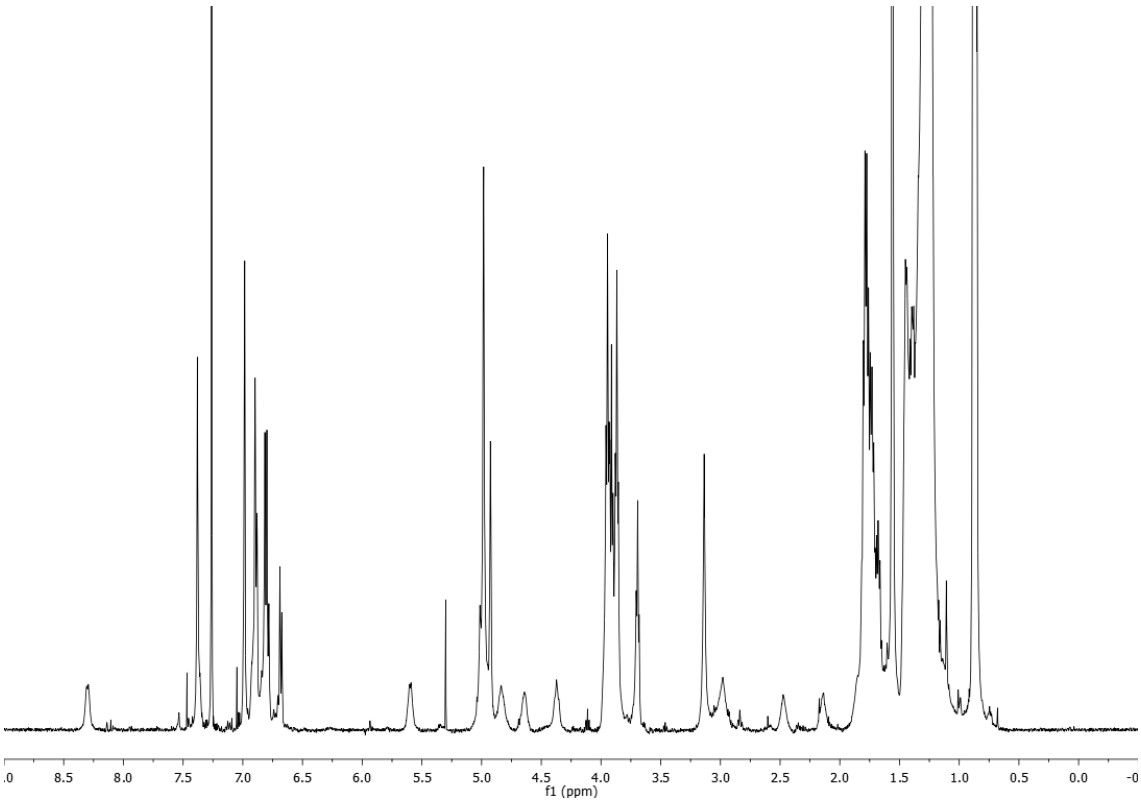




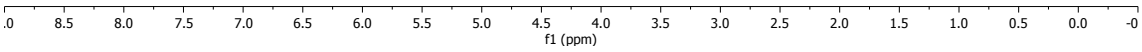
CP1:



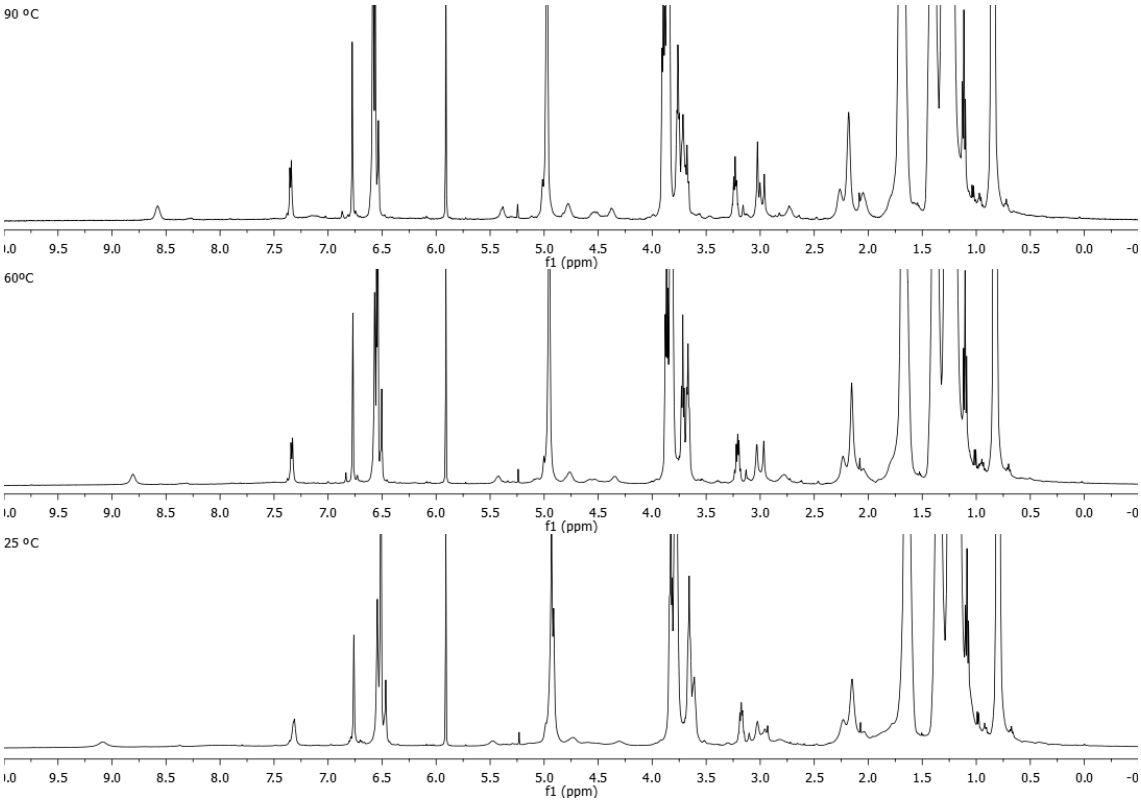
CP2:



CP3:

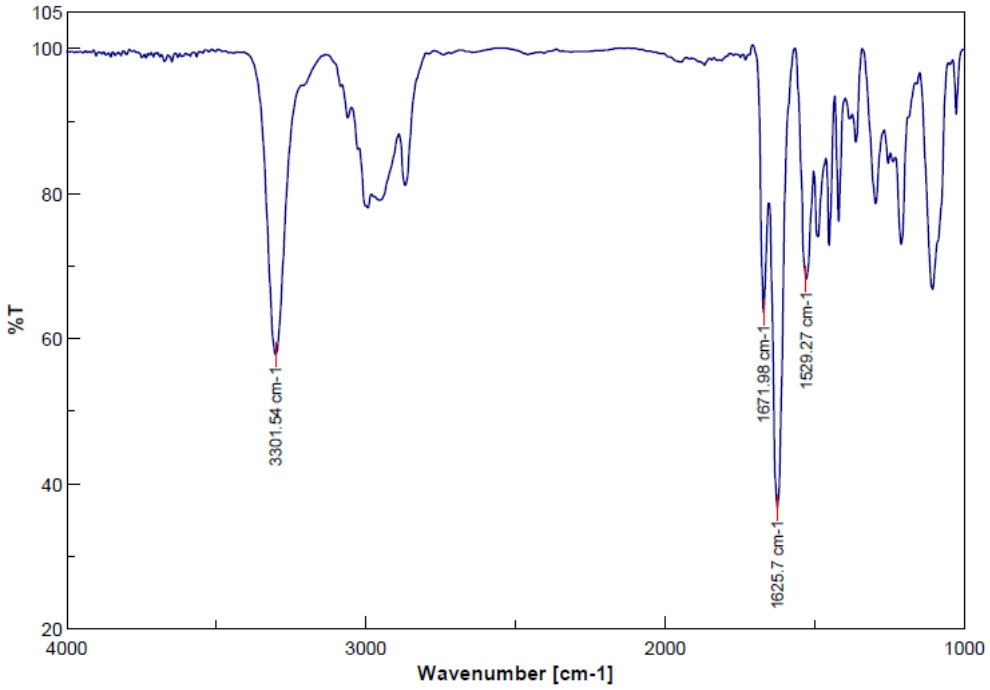


CP3 at 25 ºC, 60 ºC and 90 ºC in $C_2D_2Cl_4$ (NH at 9.09 ppm, 8.81 ppm, and 8.58 ppm respectively. 500 MHz):

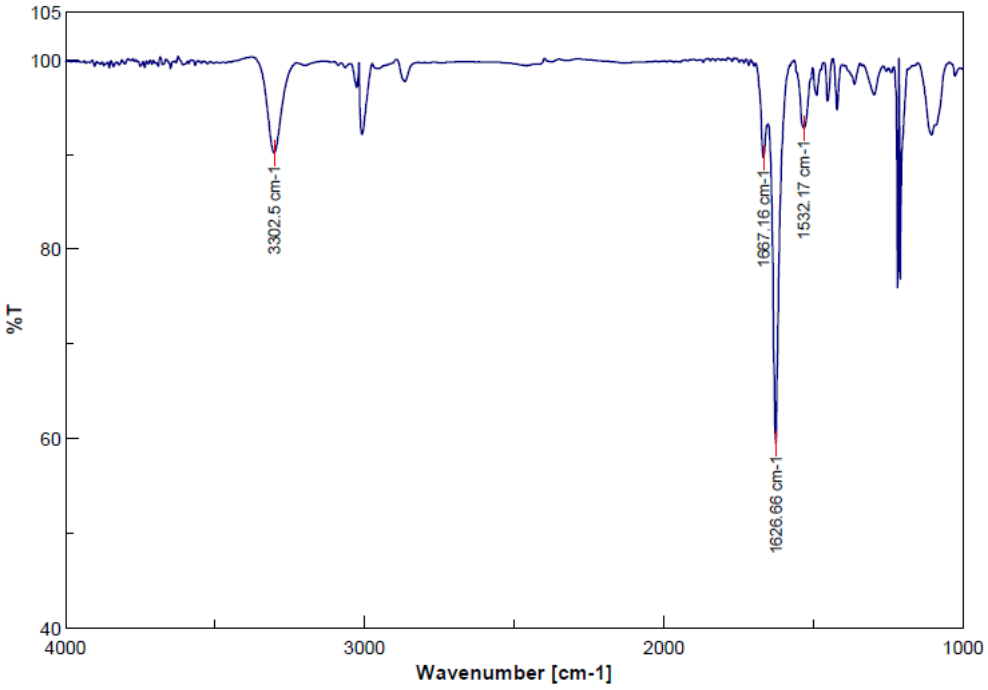


IR spectroscopy

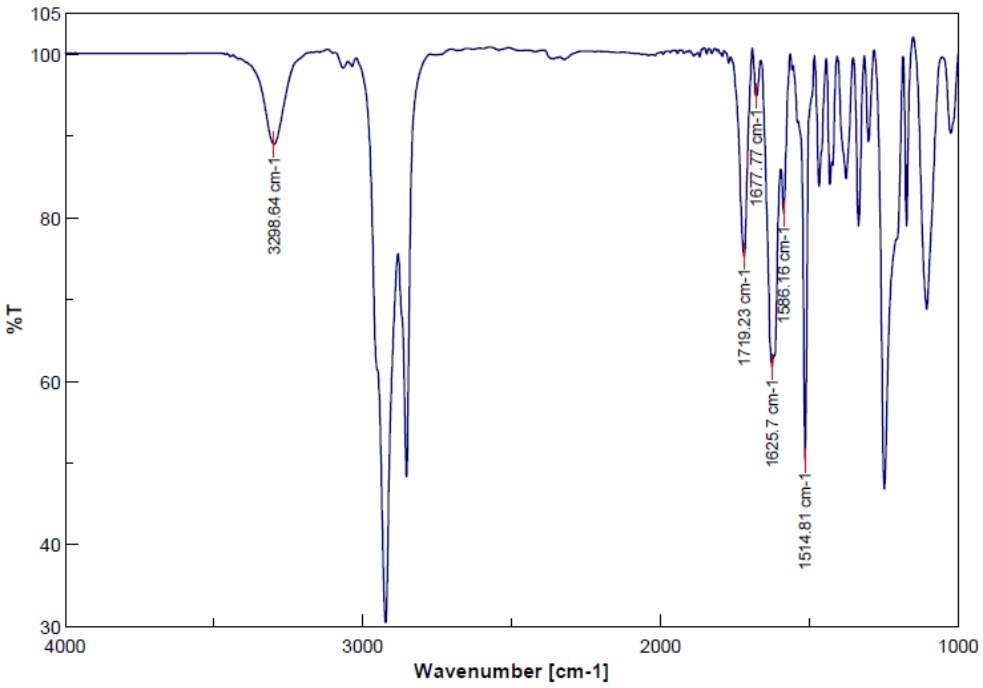
For FTIR spectra on thin films, the sample was prepared by casting from CH_2Cl_2 solutions and subsequent thermal treatment. According to DSC thermograms (see below), heating above the clearing point and cooling at a rate of 10º/min ensures that the mesophase is present at room temperature for IR measurements.



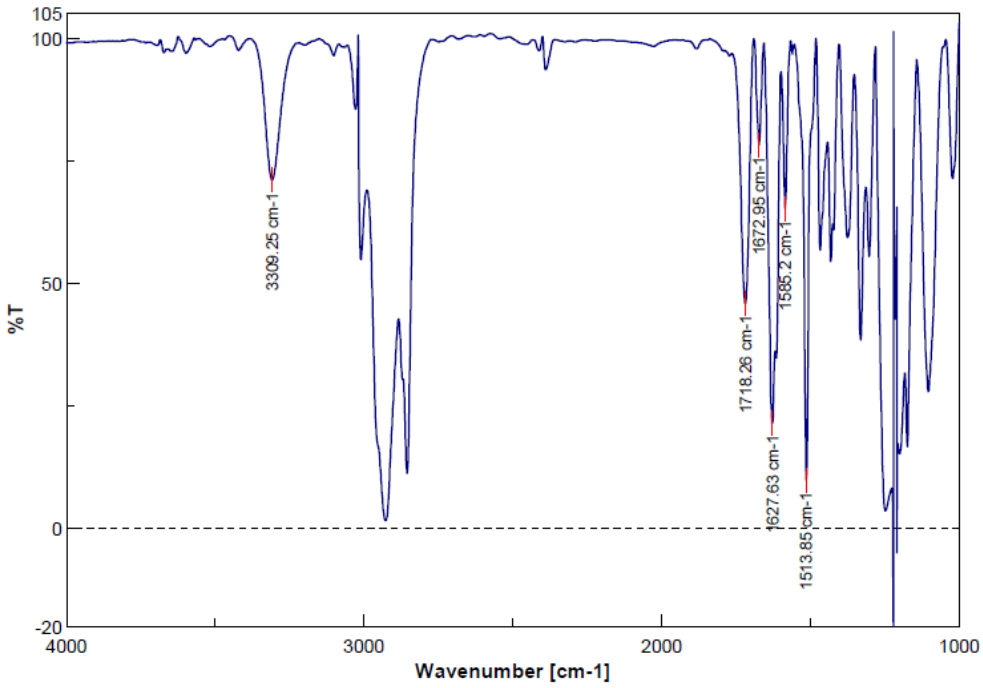
CP(Bn): FTIR (CaF_2 pellet).



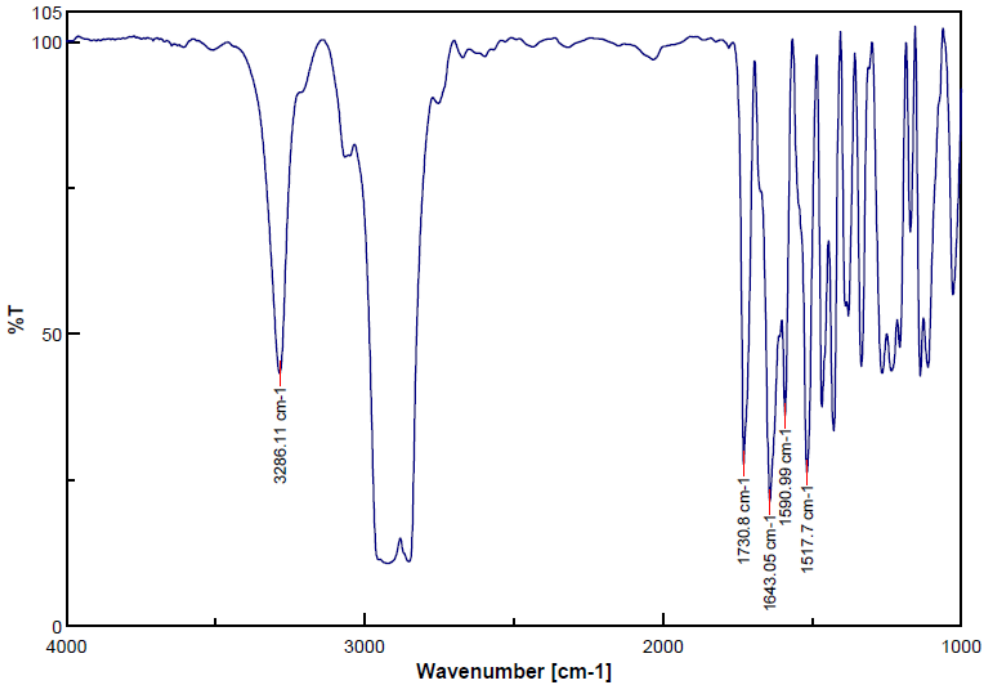
CP(Bn): FTIR (1.0 mM, CaF_2 cell).



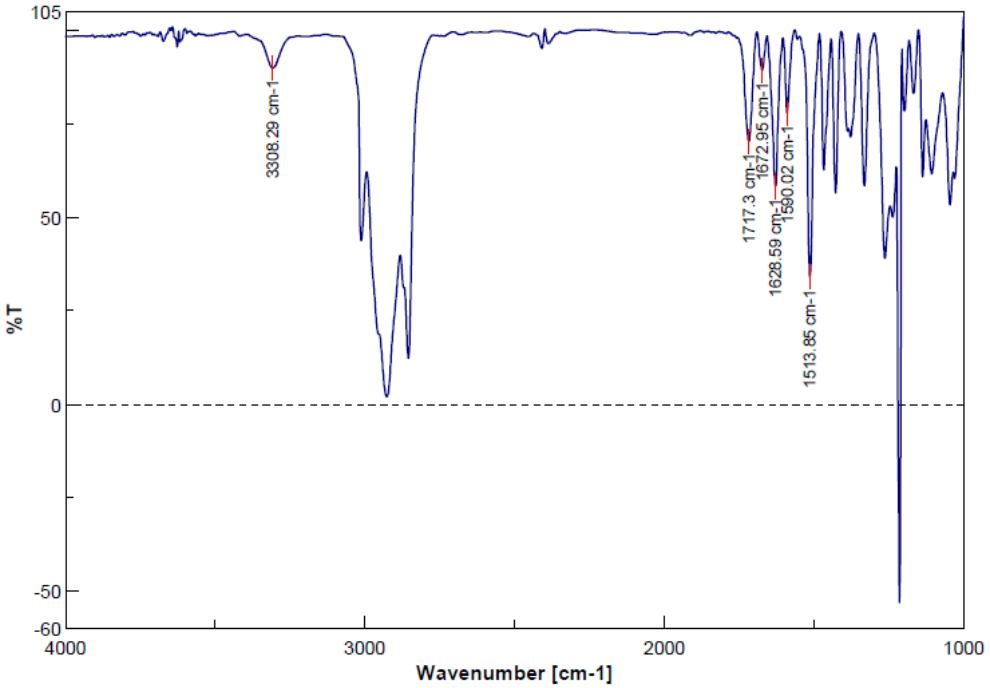
CP1: FTIR (CaF_2 pellet):



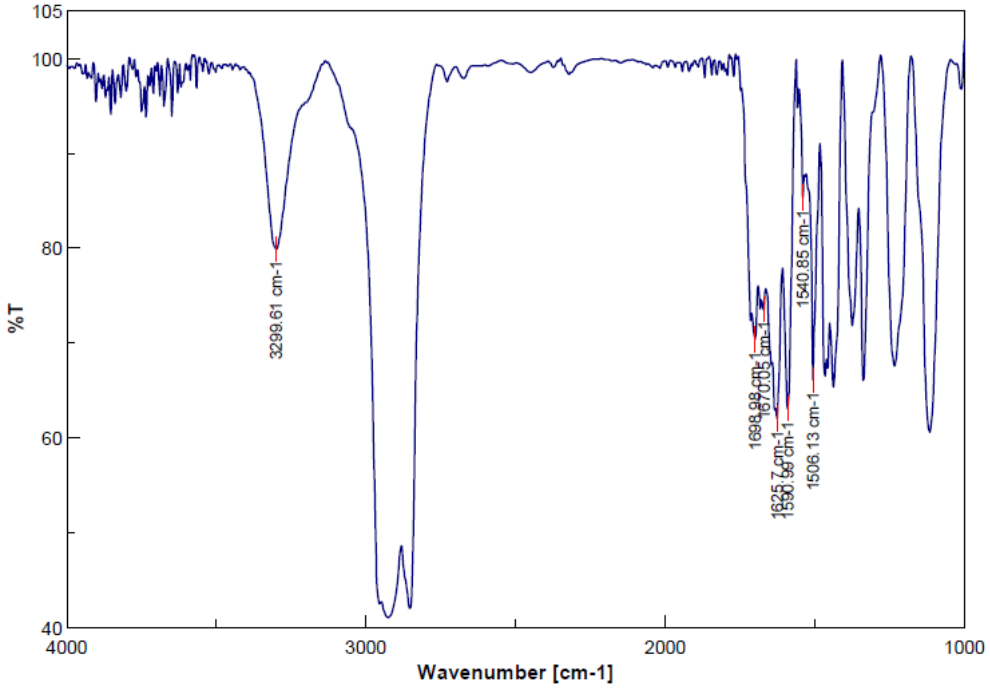
CP1: FTIR (5.0 mM, CaF_2 cell):



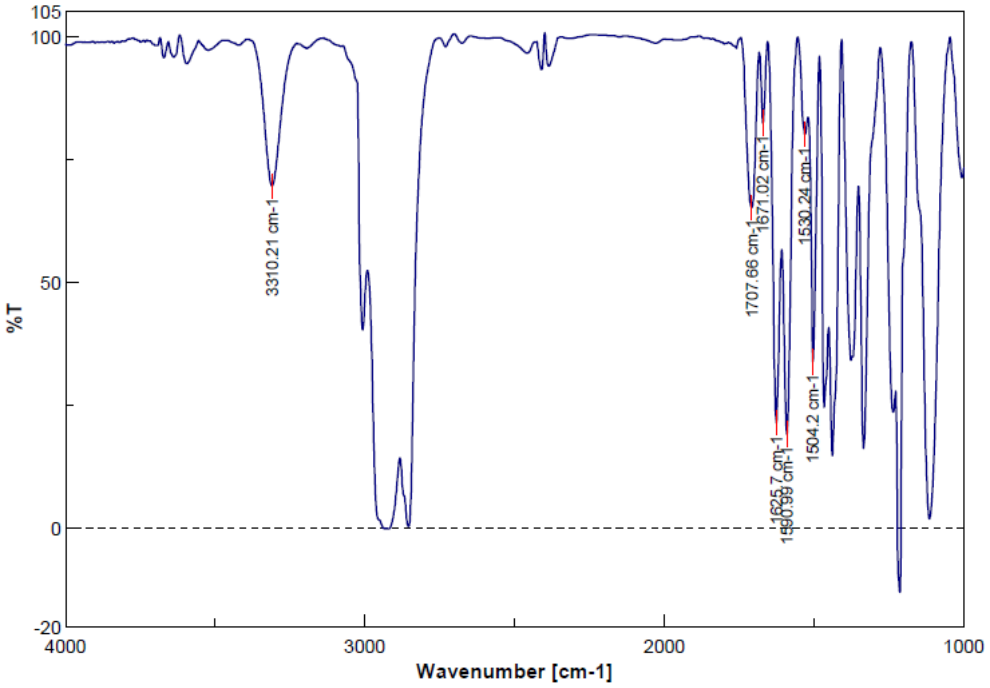
CP2: FTIR (CaF_2 pellet).



CP2: FTIR (2.5 mM, CaF_2 cell).



CP3: FTIR (CaF_2 pellet):



CP3: FTIR (5.0 mM, CaF_2 cell):

Differential Scanning Calorimetry

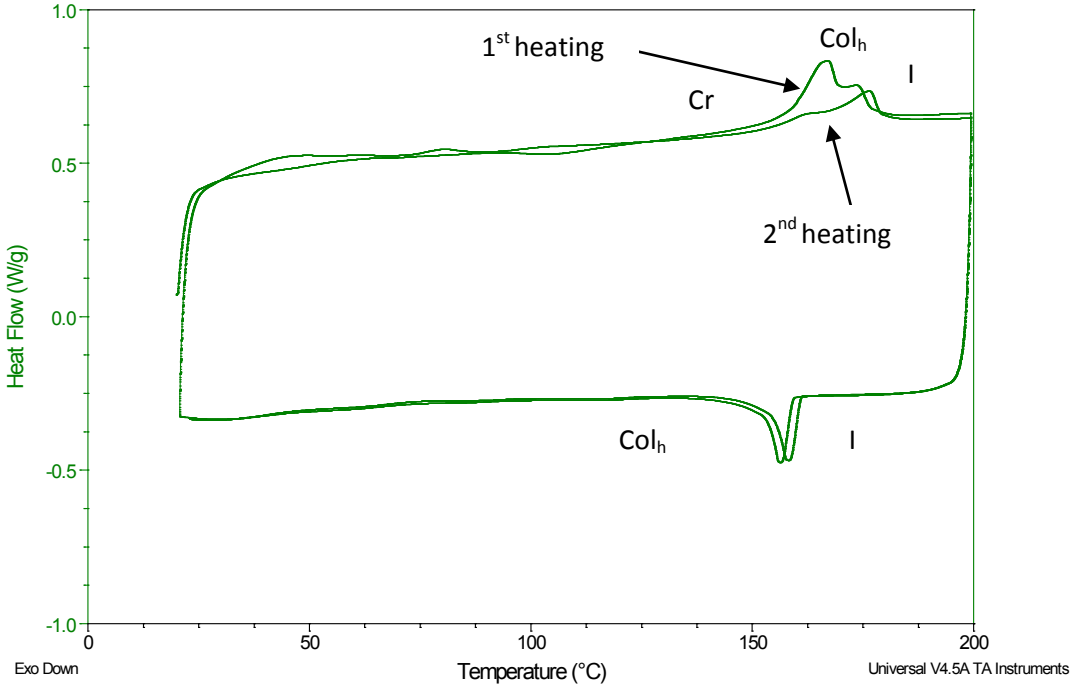
The DSC thermogram of compound **CP1** shows two overlapped peaks in both the first and second heating process, being the lower temperature peak smaller in the second heating scan than in the first heating scan. This lower temperature peak was confirmed to correspond to a crystal-mesophase transition, and the peak at higher temperature was assigned to the mesophase-isotropic liquid transition after examining the cooling thermogram in which only one peak is observed that corresponds to the formation of the mesophase from the isotropic liquid as observed by POM. In the second heating scan, the peak corresponding to the crystal melting process is much smaller than in the first heating, what indicates that crystallization was not complete.

For compound **CP2**, two peaks that can be assigned to crystal-crystal and crystal-mesophase transitions, respectively, are observed in the first heating cycle. A small third peak with lower associated enthalpy is ascribed to the clearing process. In the second heating process, the lowest temperature small peak is assigned to the crystal to mesophase transition and comes from partial crystallization as observed for compound **CP1**.

No crystallization peak is observed on cooling for **CP1** and **CP2**.

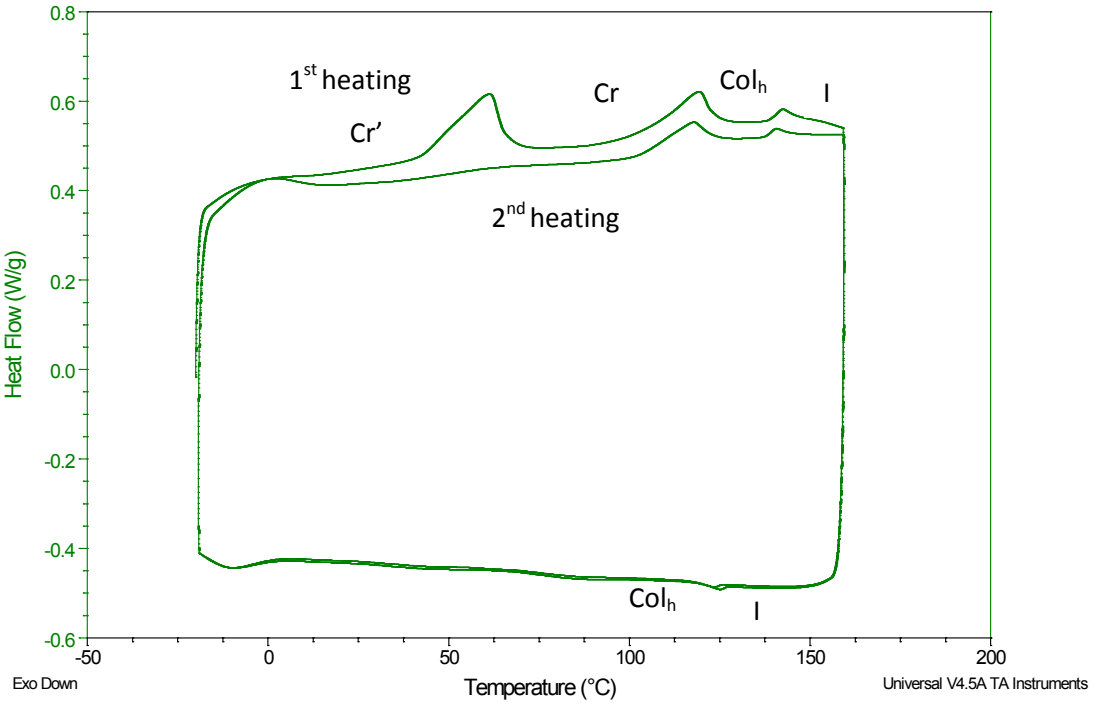
Compound **CP3** shows a broad peak corresponding to the crystal to mesophase transition in the first heating process. In addition, a small peak, with an enthalpy value similar to that measured for compound **CP2**, corresponding to the transition between the mesophase and the isotropic liquid, is observed in the first heating process. The crystal to mesophase transition is not observed in subsequent cycles either in the heating or the cooling process until 0ºC.

CP1



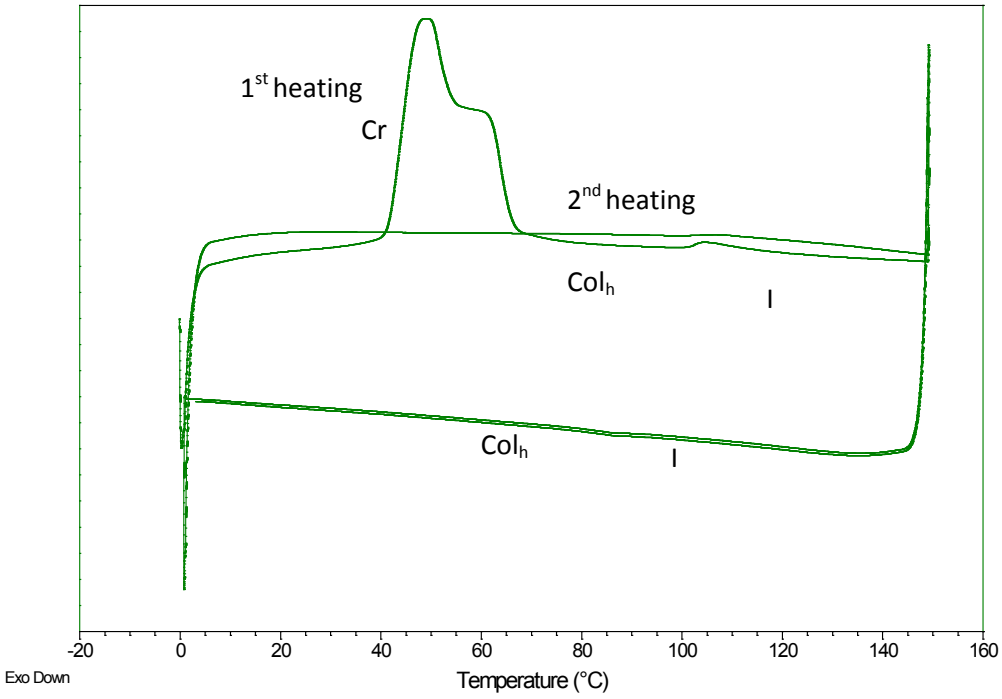
DSC thermograms recorded at a rate of 10 ºC/min for **CP1**. First and second heating-cooling cycles.

CP2



DSC thermograms recorded at a rate of 10 ºC/min for **CP2**. First and second heating-cooling cycles.

CP3



DSC thermograms recorded at a rate of 10 ºC/min for **CP3**. First and second heating-cooling cycles.

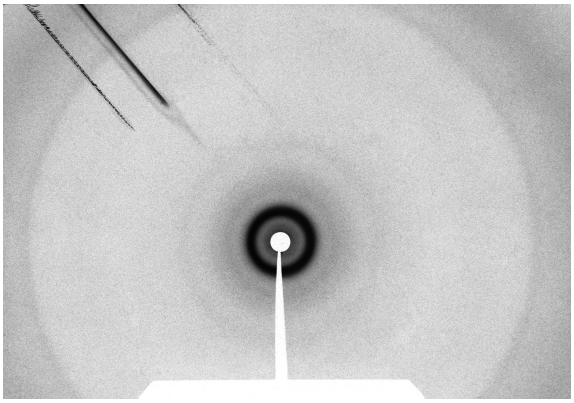
X-ray diffraction

For X-ray studies a sample of each compound was heated to the isotropic liquid and then cooled to room temperature.

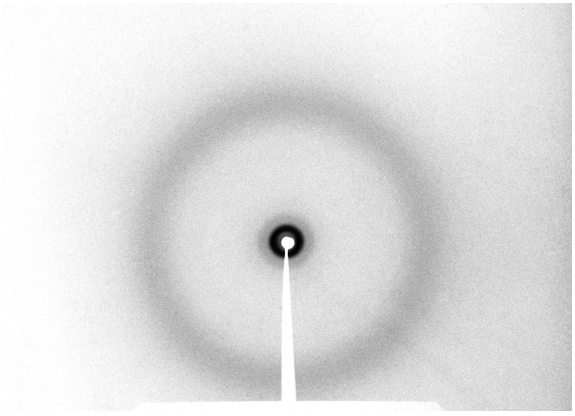
The small-angle region contains a very strong and a medium-intensity sharp maxima, the spacing of which are in the reciprocal ratio $1:3^{1/2}$, and they can be indexed, respectively, as the (*10*) and (*11*) reflections of a two-dimensional hexagonal lattice (see SI). Apart from this, the patterns show a broad, diffuse halo in the large-angle region at ca. 4.4 Å, characteristic of the liquid-like arrangement of the hydrocarbon chains and of the absence of a regular stacking parameter.

The hexagonal unit-cell cross-section can be calculated as $S_{col} = a\ d_{10}$ (Table S2). The column cross-section S_{col} and the stacking periodicity h along the columnar axis are linked through the $hS_{col} = ZV_m$ relationship, where Z is the number of molecules within a column stratum h-thick and V_m the molecular volume. However, in the XRD patterns of these compounds a scattering maximum related to the stacking periodicity h (inter-dimer spacing) has not been observed. Then, from the previous relationship, the thickness of the column stratum can be estimated considering the number (Z) of molecules as a variable: $h = (Z\ V_m)/(a\ d_{10})$. The molecular volume V_m is related to the density ρ and the molar mass M by the following equation: $\rho = (M \times 10^{24}/N)/V_m$, where N is Avogadro's number. Assuming that the density must be close to 1 $g{\cdot}cm^{-3}$, the following relationship is deduced: $h = (Z\ M \times 10^{24})/(N\ a\ d_{10})$. From this equation it is deduced that there should be two molecules (one dimer) per stratum for compound **CP1** (Z = 2, h = 5.4 Å) and compound **CP2** (Z = 2, h = 7.4 Å). This solution is equivalent to consider that must be four molecules (two dimers) occupying a stratum of double thickness (Z = 4, h = 10.8 Å for compound **CP1** and Z = 4, h = 14.8 Å). For Z = 2 the deduced h values are too short considering that the intra-dimer distance estimated from the IR vibration frequency is around 4.8 Å. Therefore it is reasonable to consider that four molecules (Z = 4) fill a column stratum. This means that two dimers are needed to cover the column cross-section, which implies that each dimer fills half of the column cross-section and has to associate with another dimer to generate a twin pair which is able to fill the whole cross section of the column. These pairs of dimers generate the columns through stacking, thus generating two parallel nanotubes.

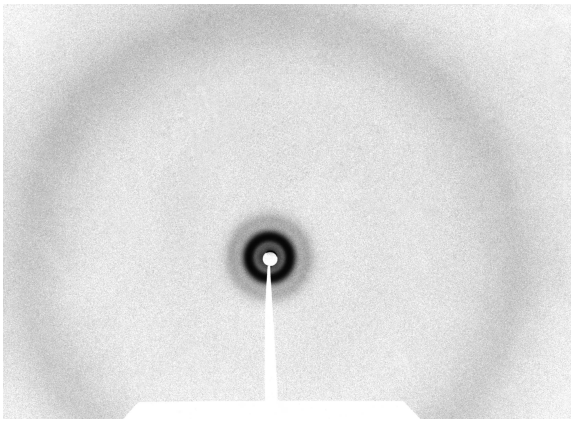
In the case of compound **CP3**, analogous calculations lead to the conclusion that the columns of the Col_h mesophase are generated by stacking of one-dimer entities at a mean distance h = 11.8 Å, thus generating a nanotube along the column axis.



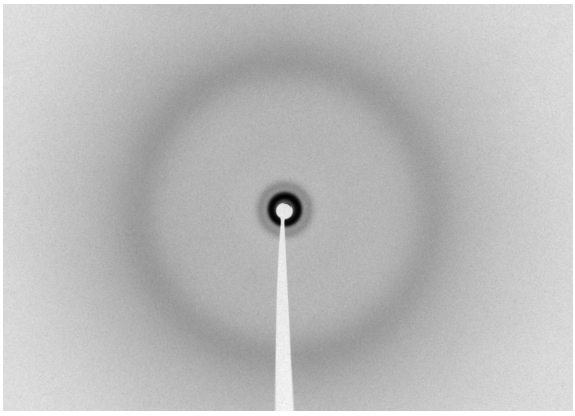
Small-angle X-ray scattering (SAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP1** recorded at room temperature. Partial crystallization took place during the experiment due to the long exposure time. However, the reflections from the hexagonal columnar mesophase are unambiguously observed at small angles (the two first maxima).



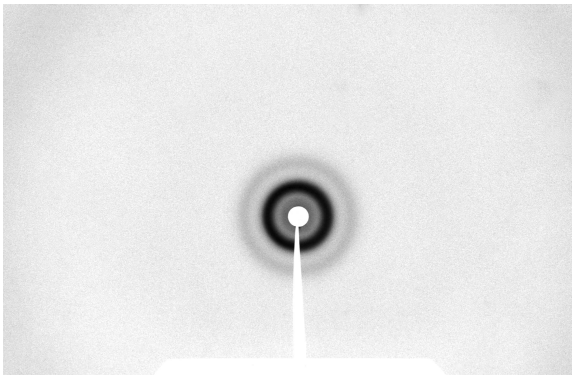
Wide-angle X-ray scattering (WAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP1** recorded at room temperature.



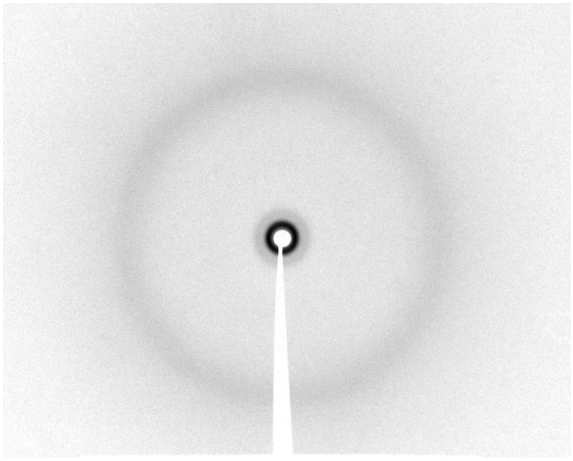
Small-angle X-ray scattering (SAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP2** recorded at room temperature.



Wide-angle X-ray scattering (WAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP2** recorded at room temperature.



Small-angle X-ray scattering (SAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP3** recorded at room temperature.



Wide-angle X-ray scattering (WAXS) pattern of the hexagonal columnar (Col_h) mesophase of compound **CP3** recorded at room temperature.