ELECTRONIC SUPPORTING INFORMATION

Chirality Sensing of Terpenes, Steroids, Amino Acids, Peptides and Drugs with Acyclic Cucurbit[*n*]urils and Molecular Tweezers

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1. Abbreviation

CD	Circular Dichroism
ICD	Induced Circular Dichroism
PMT	Photomultiplier tube
HT	High tension voltage
BW	Bandwidth
LP-Filter	Long pass filter
λ_{obs}	Monitoring wavelength
D.I.T	Digital integration time
$t_{measure}$	Measuring time
T	Temperature
CB8	Cucurbit[8]uril
MDPP	N,N'-dimethyl-2,9-diazaperopyrenium dication
MDAP	2,7-dimethyldiazapyrenium dication
L-Phe-L-Ala	L-Phenylalanyl-L-alanine
L-Phe-Gly	L-Phenylalanylglycine
L-Phe-L-Val	L-Phenylalanyl-L-valine
L-Ala-L-Phe	L-Alanyl-L-phenylalanine
D-Phe	D-Phenylalanine
L-Phe	L-Phenylalanine
L-Trp-NH_2	L-Tryptophanamide hydrochloride
L-Trp-OMe	L-Tryptophan methyl ester hydrochloride
D-Trp-OMe	D-Tryptophan methyl ester hydrochloride
D-Trp	D-Tryptophan
L-Trp	L-Tryptophan
L-Arg	L-Arginine hydrochloride
D-Arg	D-Arginine hydrochloride
L-Lys	L-Lysine hydrochloride
Ac-L-Arg-OMe	N-acetyl-L-arginine methyl ester hydrochloride
Ac-L-Lys-OMe	N-acetyl-L-lysine methyl ester hydrochloride
MPCP	*N*-methyl-4-pyridinylium[2.2]paracyclophane
DI-water	Distilled water
EtOH	Ethanol
ACN	Acetonitrile

2. Materials

All solvents were used as received from Aldrich or Fluka without further purification. All chemicals were purchased and used as received unless stated otherwise. The acyclic CB*n* molecular container C1[1] and C2[1] and the molecular tweezer CLR01[2] were synthesized according to the literature procedures. The chiral tetracyclic hydrocarbon analytes, (*R*)-trinorbornane and (*S*)-trinorbornane were synthesized according to the literature procedures.[3, 4] CB8 was synthesized according to literature procedures[5] but can also be purchased from Strem or Sigma. MDPP[6] was synthesized from 1,3,8,10-tetrahydro-2,9-dimethyl-2,9-diazadibenzoperylene, according to literature procedures.[6] Likewise, MDAP[7] was synthesized from 1,3,6,8-tetrahydro-2,7-dimethyl-2,7-diazapyrene, following the DDQ-oxidation procedure for MDPP.[6]

3. Instrumentation

Absorption spectra were measured on a Jasco V-730 double-beam UV–VIS spectrophotometer and baseline corrected. CD spectra were recorded on a Jasco J-1500 CD spectrometer attached with a Fluorescence Detected Circular Dichroism (FDCD)-550 accessory. The fluorescence intensity of the samples was collected using the FDCD-550 attachment of the CD spectrometer using appropriate long pass filters to avoid the scattered light from the excitation wavelength. The HT voltage applied to the PMT of the CD detector was kept in auto mode and the HT voltage applied to the PMT of the FDCD detector was kept in manual mode and adjusted accordingly so that a fluorescence signal saturation is not reached in each set of measurements. The HT voltage on the PMT of FDCD detector and all other measurement parameters were kept constant when the fluorescence intensity of two or more samples were compared. Blank measurements of H_2O or buffer provided no induced CD signals in the regions examined. All CD spectra reported are baseline corrected for the appropriate solvent system used. For spectroscopy analysis in quartz cuvettes, suprasil (type 111-QS) emission cuvettes with a light path of 10 mm and dimensions of 10x10 mm from Hellma-Analytics were utilized.

4. Sample Preparation

The stock solutions of water-soluble molecules were prepared in DI-water and kept in the fridge at +8°C for storage, except for CLR01, Ac-L-Lys-OMe and H-Lys-Leu-Val-Phe-Phe-OH, which were stored in the freezer at -20°C. For water-insoluble molecules such as (*R*)-limonene, (1*R*)-endo-(+)-fenchol, clopidogrel, testosterone and camptothecin, the stock solutions were prepared in ethanol and then diluted in the host-containing DI-water for the CD measurements. For the water-insoluble chiral bridged-alkane trinorbornanes studied the stock solutions were prepared in acetonitrile and then diluted in the host-containing DI-water for the CD measurements. All the stock solutions prepared in ethanol and acetonitrile were stored in the freezer at -20°C. Nandrolone and prednisolone has a solubility of 810 µM[8] and 483 µM[8] respectively in water. Hence, their stock solutions were prepared in water.

The concentration of the stock solutions of the dyes and the analytes were determined by UV- Vis absorption titration measurements unless stated otherwise. The molar extinction coefficient of the samples used for the determination of the concentration of their stock solutions by UV-Vis absorption titration are given in Table S 1.

Table S 1: Absorption maxima (λ_{max}) and molar extinction coefficients ($\varepsilon_{\lambda max}$) of the dyes and analytes used for the determination of the concentration of their stock solutions by UV-Vis absorption titration measurements.

Sample	λ_{max} *(nm)*	$\varepsilon_{\lambda max}$ *($M^{-1}cm^{-1}$)*
C1	290	12161
C2	290	8610
CLR01	285	8425
MDPP	413	26000
MDAP	393	7800
CB8•MDPP	443	55000
CB8•MDAP	419	7600
D-Phe	257.6	195[9]
L-Phe	257.6	195[9]
L-Trp	278	5579[9]
D-Trp	278	5579[9]
L-Trp-NH_2	278	5579[9]
L-Trp-OMe	278	5579[9]
D-Trp-OMe	278	5579[9]
L-Phe-L-Ala	257.6	195[9]
L-Phe-Gly	257.6	195[9]
L-Phe-L-Val	257.6	195[9]
L-Ala-L-Phe	257.6	195[9]

For samples whose molar extinction coefficient could not be found in literature, the stock solutions were prepared by appropriately weighing in the required amount of the pure sample to attain the desired concentration (see Fig. S 24 - Fig. S 26) for the determination of the molar extinction coefficient.

The concentration of the stock solution of the host CB8 was determined by fluorescence titration against the high-affinity MPCP[10] dye by exciting the sample at 368 nm and collecting the emission intensity at 531 nm.

5. CD Measurements

5.1. Analyte spectra with acyclic CB*n* molecular container

5.1.1. Detection of phenylalanine and tryptophan-based amino acids in aqueous media

The acyclic CB*n* type molecular containers and their derivatives have been reported to bind several N-terminal amino acids with aromatic and cationic side chains with binding constants ranging from $<10^2$ (for anionic and small side chains) to $\approx 10^6$ M^{-1} for amino acid amides with aromatic (Phe, Trp, Tyr) and even cationic (Lys and Arg) side chains.[11-14] The acyclic CB*n* molecular containers show similar molecular recognition features as cucurbit[*n*]uril hosts, but since they are acyclic they can flex their methylene bridged glycoluril oligomer backbone to accommodate more voluminous guests.[13] Moreover they also allow the guest substituents to protrude through the side of the host•guest complex rather than through the portals as is the case for macrocyclic cucurbit[*n*]urils.[13] The acyclic CB*n* molecular hosts C1 and C2 are chromophoric and show absorbance signals in the near UV and visible wavelength region. Hence, we monitored the CD signals arising from C1 and C2 in the presence of chiral guests such as amino acids, amino acid derivatives and drugs. Addition of L-Phe, D-Phe and Phe derivatives to C1 shows induced CD signals (Fig. S 1 (a) and Fig. S 3 (a)). This is however not accompanied by any change in the fluorescence of the C1 receptor (Fig. S 1(b) and Fig. S 3 (b)). The addition of L-Trp, D-Trp and Trp derivatives to C1 also shows induced CD signals, however the signals are much stronger when compared to the addition of phenylalanine derivatives (Fig. S 2 (a) and Fig. S 4 (a)). Binding of Trp-species is accompanied by an enhancement in the fluorescence of the C1 receptor (Fig. S 2 (b) and Fig. S 4 (b)).

The induced CD signals arising on addition of chiral analytes to C1 was also compared to the induced CD signals arising on addition of the chiral analytes to the CB8•MDPP[15] (Fig. S 1 (c), Fig. S 2 (c), Fig. S 3 (c) and Fig. S 4 (c)) and CB8•MDAP[15] (Fig. S 5) receptor. This shows that the acyclic C1 receptor is a general binder for the chiral analytes studied which shows signals, which mostly differ in the signal magnitude but not the type or positioning of the ICD bands. In contrast, CB8•MDPP and CB8•MDAP receptor shows analyte-indicative induced CD signals for the different phenylalanine and tryptophan derivatives (Fig. S 3 (c), Fig. S 4 (c) and Fig. S 5).

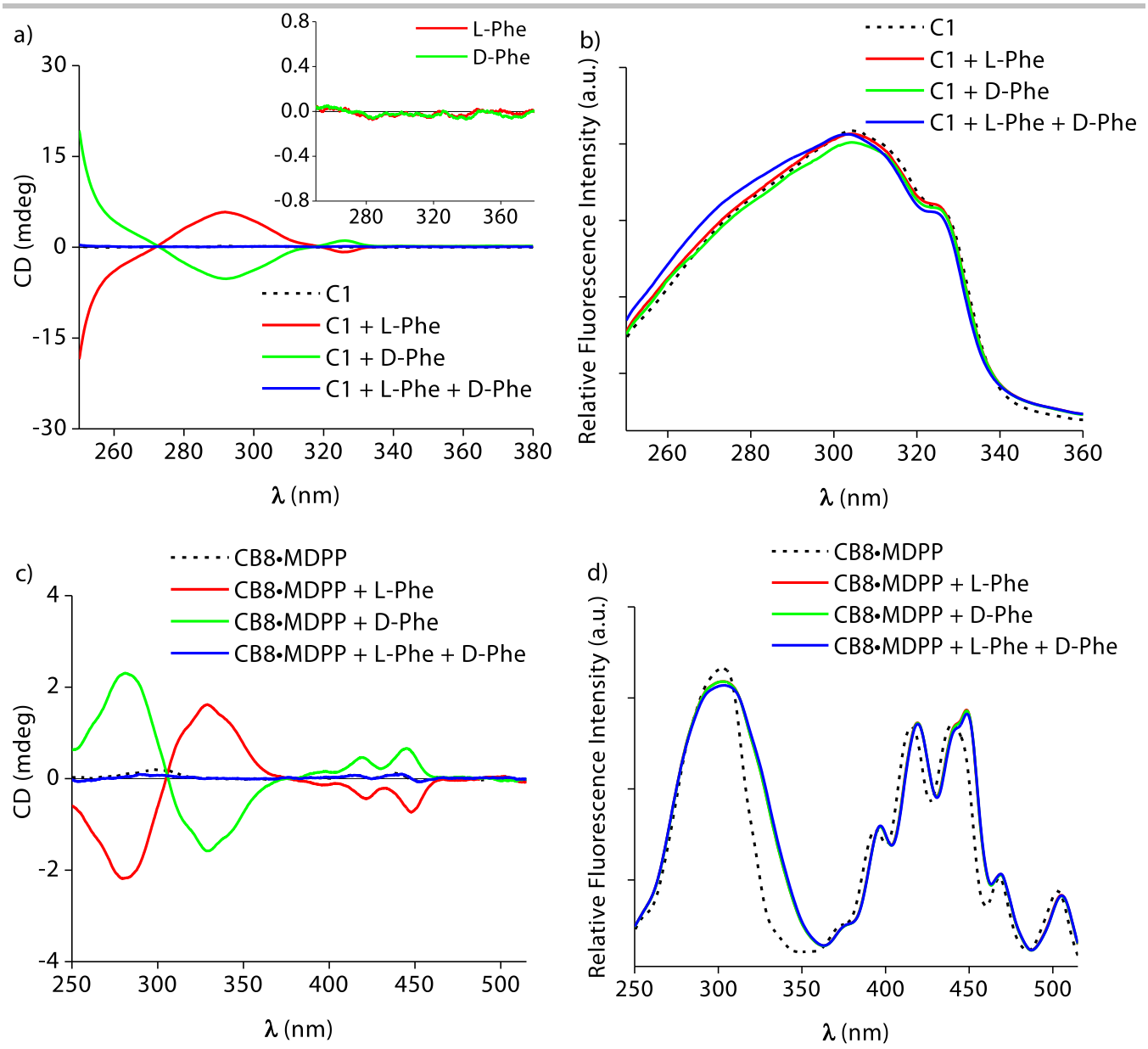


Fig. S 1: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of L-Phe (100 µM), D-Phe (100 µM) and a racemic mixture of L-Phe (100 µM) and D-Phe (100 µM) in DI-water. The inset shows the CD signals arising from L-Phe (100 µM) and D-Phe (100 µM) alone in the absence of C1; the phenylalanine derivatives do not show any CD signals in the region examined. (b) The excitation spectra of C1 (100 µM) and C1 (100 µM) in the presence of L-Phe (100 µM), D-Phe (100 µM) and a racemic mixture of L-Phe (100 µM) and D-Phe (100 µM) in DI-water which shows the equilibration of the system. The excitation spectra was collected using a long pass filter of 380 nm and at an HT voltage of 800 V. (c) CD spectrum of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of L-Phe (50 µM), D-Phe (50 µM) and a racemic mixture of L-Phe (50 µM) and D-Phe (50 µM) in DI-water. (d) The excitation spectra of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of L-Phe (50 µM), D-Phe (50 µM) and a racemic mixture of L-Phe (50 µM) and D-Phe (50 µM) in DI-water collected using a long pass filter of 515 nm and at an HT voltage of 510 V. For molar ellipticity and molar circular dichroism values for C1 and CB8•MDPP complexes with the chiral analytes, please refer to Table S 2 and Table S 3.

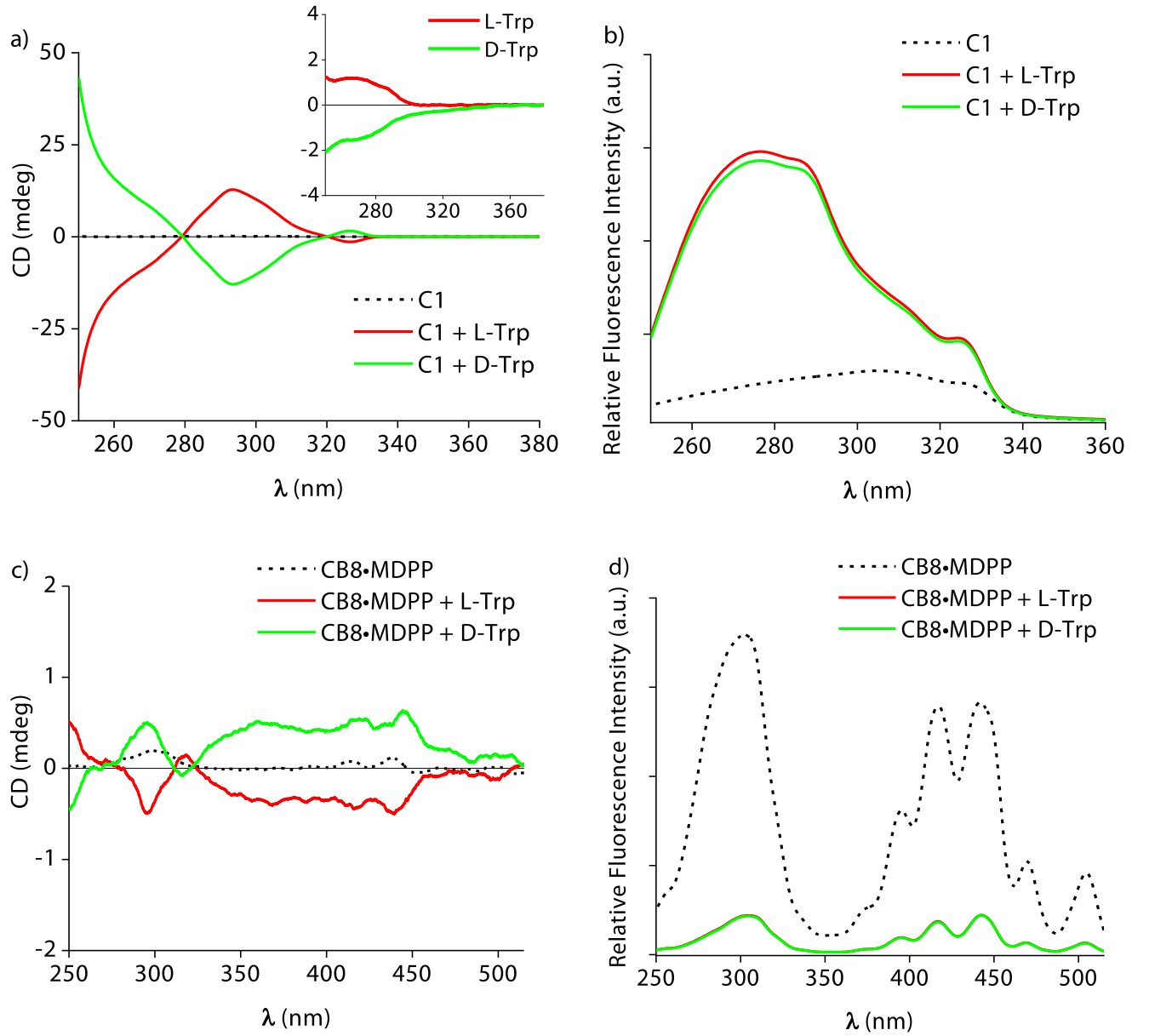


Fig. S 2: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of L-Trp (100 µM) and D-Trp (100 µM) in DI-water. The inset shows the CD signals arising from L-Trp (100 µM) and D-Trp (100 µM) alone in the absence of C1. The tryptophan derivatives show weak CD signals in the region examined compared to the signals observed in presence of C1. (b) The excitation spectra of C1 (100 µM) and C1 (100 µM) in the presence of the tryptophan derivatives (100 µM) in DI-water collected using a long pass filter of 380 nm and at an HT voltage of 620 V (c) CD spectrum of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of L-Trp (50 µM) and D-Trp (50 µM) in DI-water. (d) The excitation spectra of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of the tryptophan derivatives (50 µM) in DI-water collected using a long pass filter of 515 nm and at an HT voltage of 510 V. For molar ellipticity and molar circular dichroism values for C1 and CB8•MDPP complexes with the chiral analytes, please refer to Table S 2 and Table S 3.

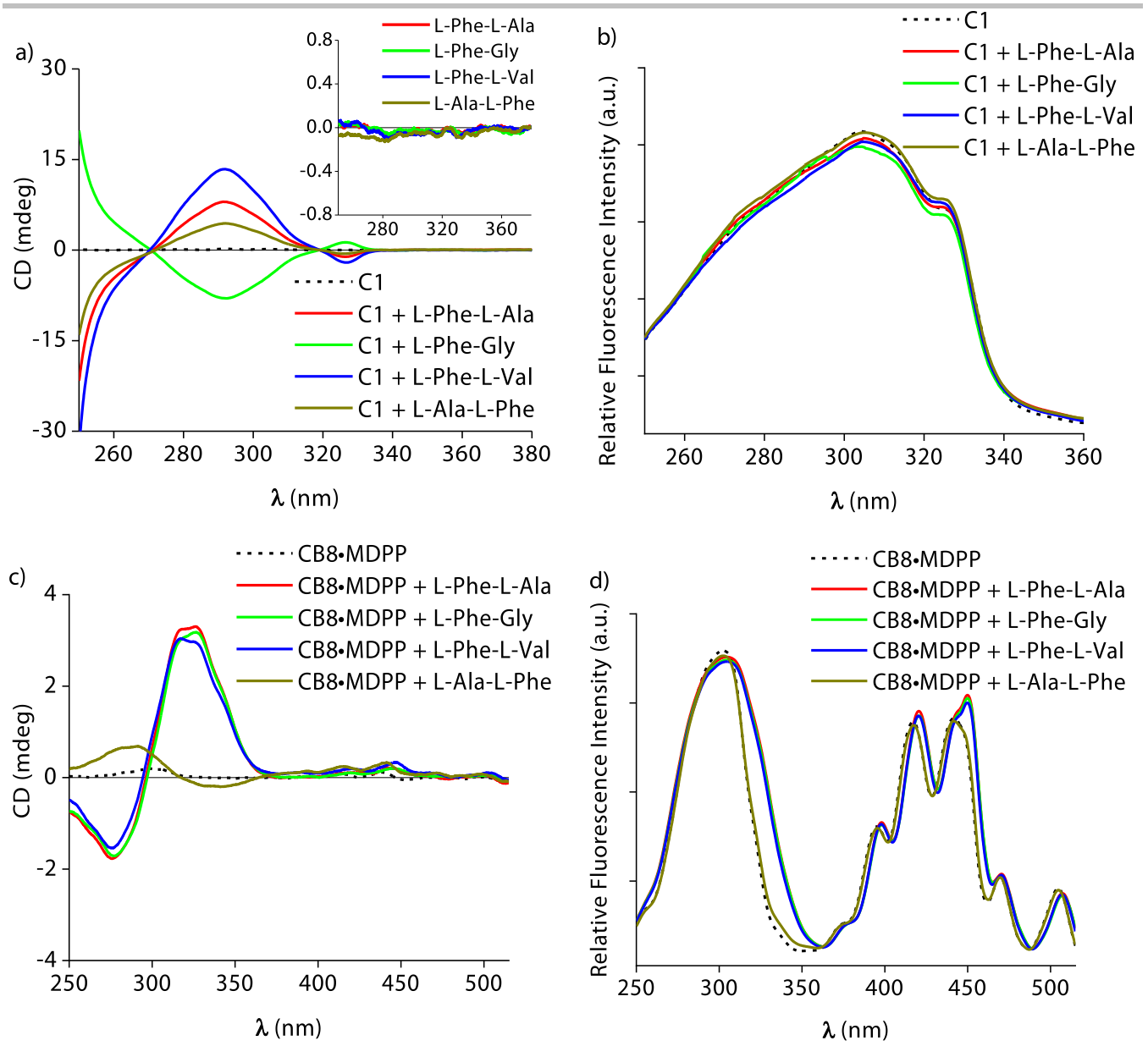


Fig. S 3: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of L-Phe-L-Ala (100 µM), L-Phe-Gly (100 µM), L-Phe-L-Val (100 µM) and L-Ala-L-Phe (100 µM) in DI-water. The inset shows the CD signals arising from L-Phe-L-Ala (100 µM), L-Phe-Gly (100 µM), L-Phe-L-Val (100 µM) and L-Ala-L-Phe (100 µM) alone in the absence of C1; the phenylalanine derivatives do not show any CD signals in the region examined. (b) The excitation spectra of C1 (100 µM) and C1 (100 µM) in the presence of the phenylalanine derivatives (100 µM) in DI-water which shows the equilibration of the system. The excitation spectra was collected using a long pass filter of 380 nm and at an HT voltage of 800 V. (c) CD spectrum of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of L-Phe-L-Ala (50 µM), L-Phe-Gly (50 µM), L-Phe-L-Val (50 µM) and L-Ala-L-Phe (50 µM) in DI-water. (d) The excitation spectra of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of the phenylalanine derivatives (50 µM) in DI-water collected using a long pass filter of 515 nm and at an HT voltage of 510 V. For molar ellipticity and molar circular dichroism values for C1 and CB8•MDPP complexes with the chiral analytes, please refer to Table S 2 and Table S 3.

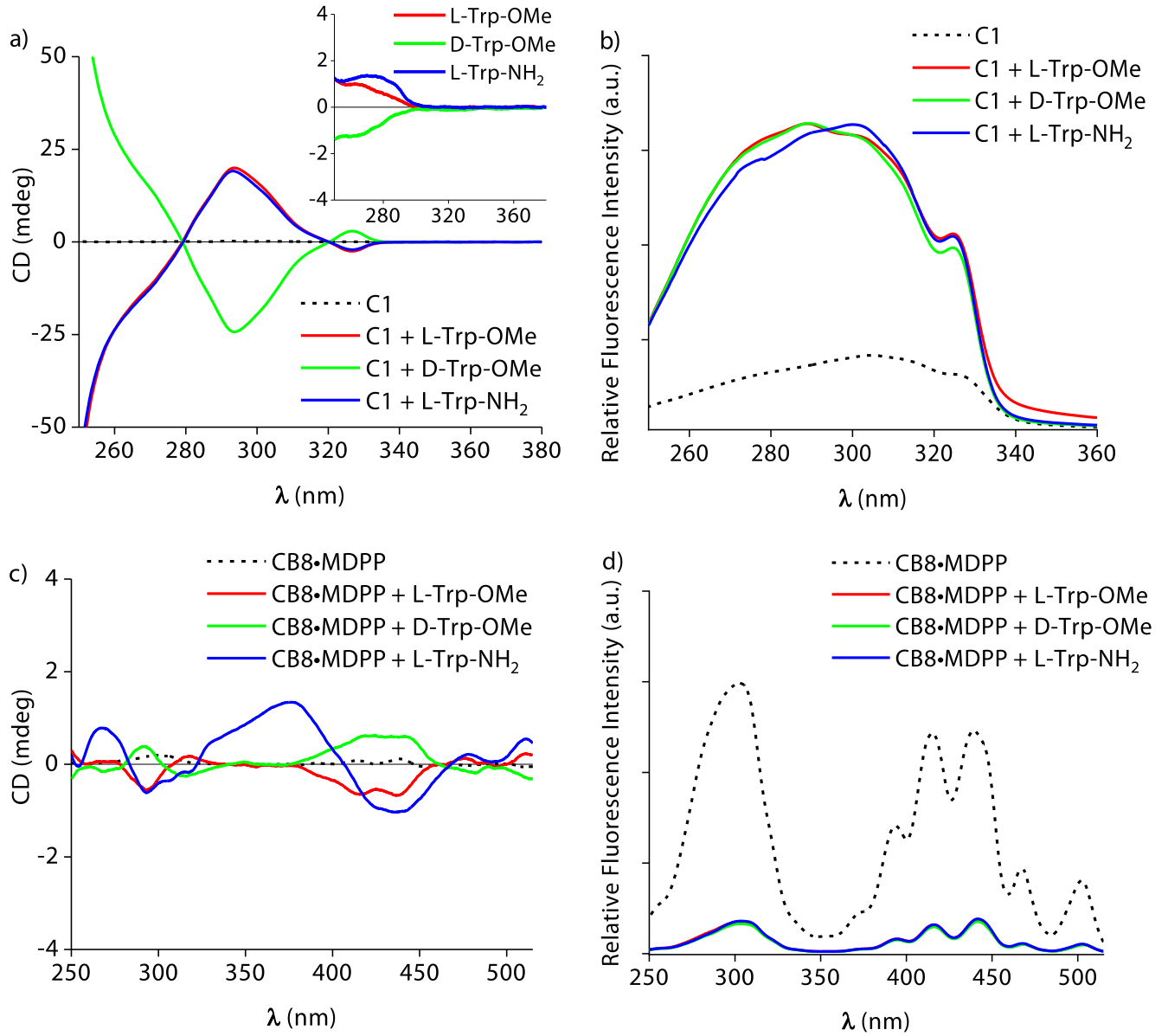


Fig. S 4: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of L-Trp-OMe (100 µM), D-Trp-OMe (100 µM) and L-Trp-NH_2 (100 µM) in DI-water. The inset shows the CD signals arising from L-Trp-OMe (100 µM), D-Trp-OMe (100 µM) and L-Trp-NH_2 (100 µM) alone in the absence of C1. The tryptophan derivatives show only weak CD signals in the region examined when compared to the signals observed in presence of C1. (b) The excitation spectra of C1 (100 µM) and C1 (100 µM) in the presence of the tryptophan derivatives (100 µM) in DI-water which shows the equilibration of the system The excitation spectra was collected using a long pass filter of 380 nm and at an HT voltage of 800 V.(c) CD spectrum of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of L-Trp-OMe (50 µM), D-Trp-OMe (50 µM) and L-Trp-NH_2 (50 µM) in DI-water. (d) The excitation spectra of CB8•MDPP (20 µM) and CB8•MDPP (20 µM) in the presence of the tryptophan derivatives (50 µM) in DI-water collected using a long pass filter of 515 nm and at an HT voltage of 510 V. For molar ellipticity and molar circular dichroism values for C1 and CB8•MDPP complexes with the chiral analytes, please refer to Table S 2 and Table S 3.

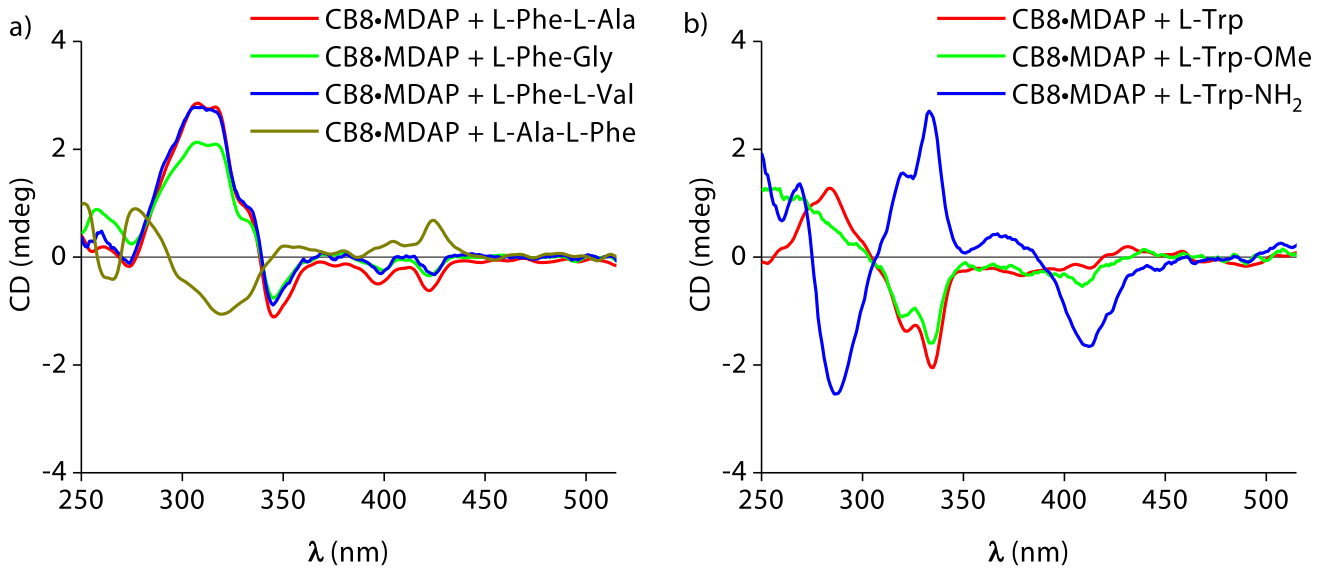


Fig. S 5: (a) CD spectrum of CB8•MDAP (100 µM) in the presence of L-Phe-L-Ala (400 µM), L-Phe-Gly (350 µM), L-Phe-L-Val (350 µM) and L-Ala-L-Phe (1800 µM) in DI-water. (b) CD spectrum of CB8•MDAP (100 µM) in the presence of L-Trp (176 µM), L-Trp-OMe (200 µM) and L-Trp-NH_2 (176 µM) in DI-water.

5.1.2. Analysis of mixture of amino acids using acyclic CB*n* molecular container C1 and CB8•MDPP receptor in aqueous media

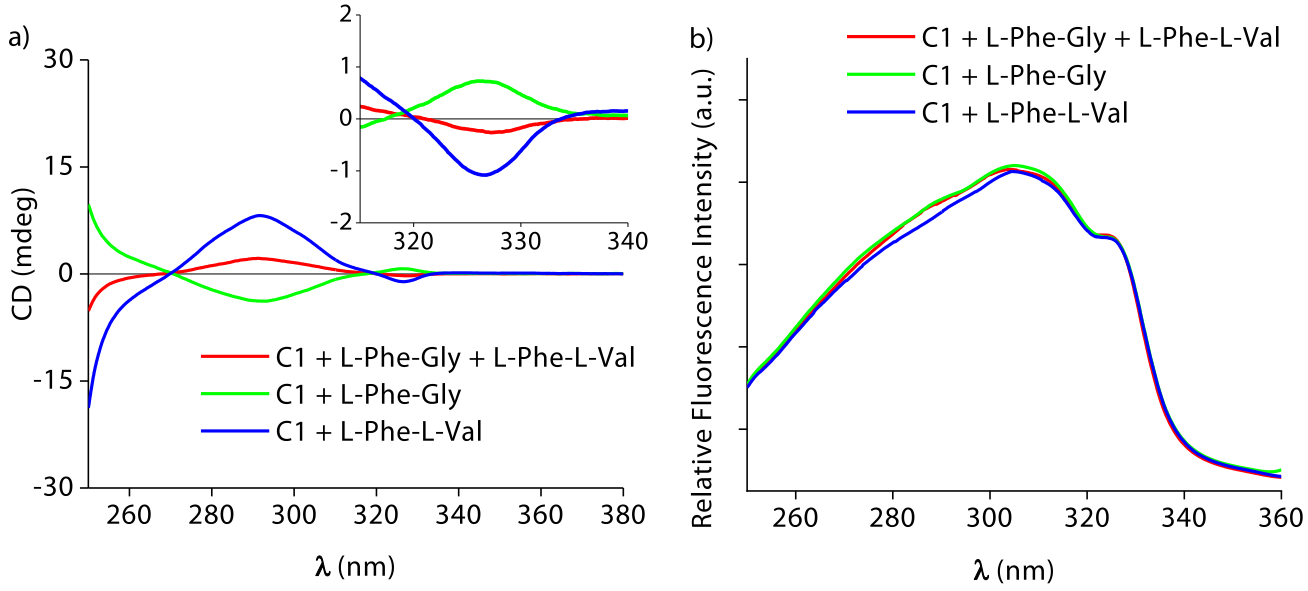


Fig. S 6: (a) CD spectrum of C1 (100 µM) in the presence of a mixture of L-Phe-Gly (50 µM) and L-Phe-L-Val (50 µM) in DI-water (red). The green and blue line represents the CD spectrum of C1 (100 µM) in the presence of L-Phe-Gly (50 µM) and L-Phe-L-Val (50 µM) respectively. (b) The excitation spectrum of the sample collected using a long pass filter of 380 nm and at an HT voltage of 800 V.

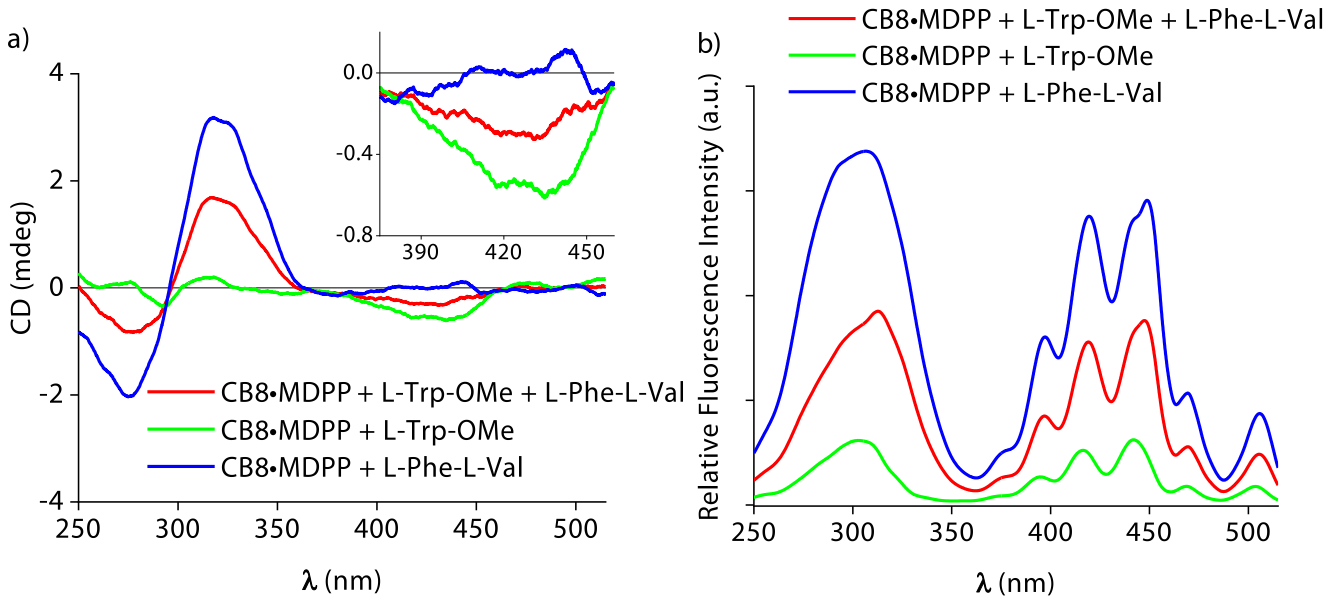


Fig. S 7: (a) CD spectrum of CB8•MDPP (20 µM) in the presence of a mixture of L-Trp-OMe (50 µM) and L-Phe-L-Val (50 µM) in DI-water (red). The green and blue line represents the CD spectrum of CB8•MDPP (20 µM) in the presence of L-Trp-OMe (50 µM) and L-Phe-L-Val (50 µM) respectively. (b) The excitation spectrum of the sample collected using a long pass filter of 515 nm and at an HT voltage of 510 V.

5.1.3. Monitoring the racemization of amino acids and dipeptides

CD spectroscopy can be a useful spectroscopic technique for the sensitive, label-free monitoring of reactions of chiral analytes in real time. We made use of the induced CD signals arising for the supramolecular chemosensors in presence of chiral analytes for the monitoring of chemical reactions.

Several neutral free amino acids such as L-Phe are reported to undergo serious racemization and decomposition in polar organic solvents such as DMF and ethylene glycol under alkaline conditions, while this phenomena do not occur, or are largely decreased, in water under the same alkaline conditions.[16] However, in order to monitor the racemization event, a lengthy derivatization procedure was adopted in the literature before the samples were analyzed by HPLC. We used a supramolecular reaction monitoring method: Both C1 and the CB8•MDPP receptor shows induced CD signals in the presence of the chiral analytes, L-Phe and L-Phe-Gly in DI-water (Fig. S 1 (a, c) and Fig. S 3 (a, c)). Hence, we monitored the racemization of L-Phe and L-Phe-Gly with the help of C1 and CB8•MDPP receptor in real time by recording the induced CD signals arising in these systems. L-Phe and L-Phe-Gly were heated in the presence of 1.2 eq. of K_2CO_3 at 130^{o}C for 2 hours in DMF, ethylene glycol and water and the resulting racemization was evaluated by measuring the induced CD signals arising on adding aliquots of L-Phe and L-Phe-Gly from the reaction mixture to C1 and CB8•MDPP receptor in DI-water before and at fixed time points of the reaction (Fig. S 8 and Fig. S 9).

The racemization of L-Phe and L-Phe-Gly was also monitored at room temperature (25^{o}C) in the presence of 1.2 eq. of K_2CO_3 for 24 hours in DMF with the help of C1 and CB8•MDPP receptor (Fig. S 8 and Fig. S 9 – DMF (control)). The amino acids/ dipeptide was found to be quite stable under these conditions; the racemization of the amino acid/ dipeptide occurs only strongly upon heating in DMF.

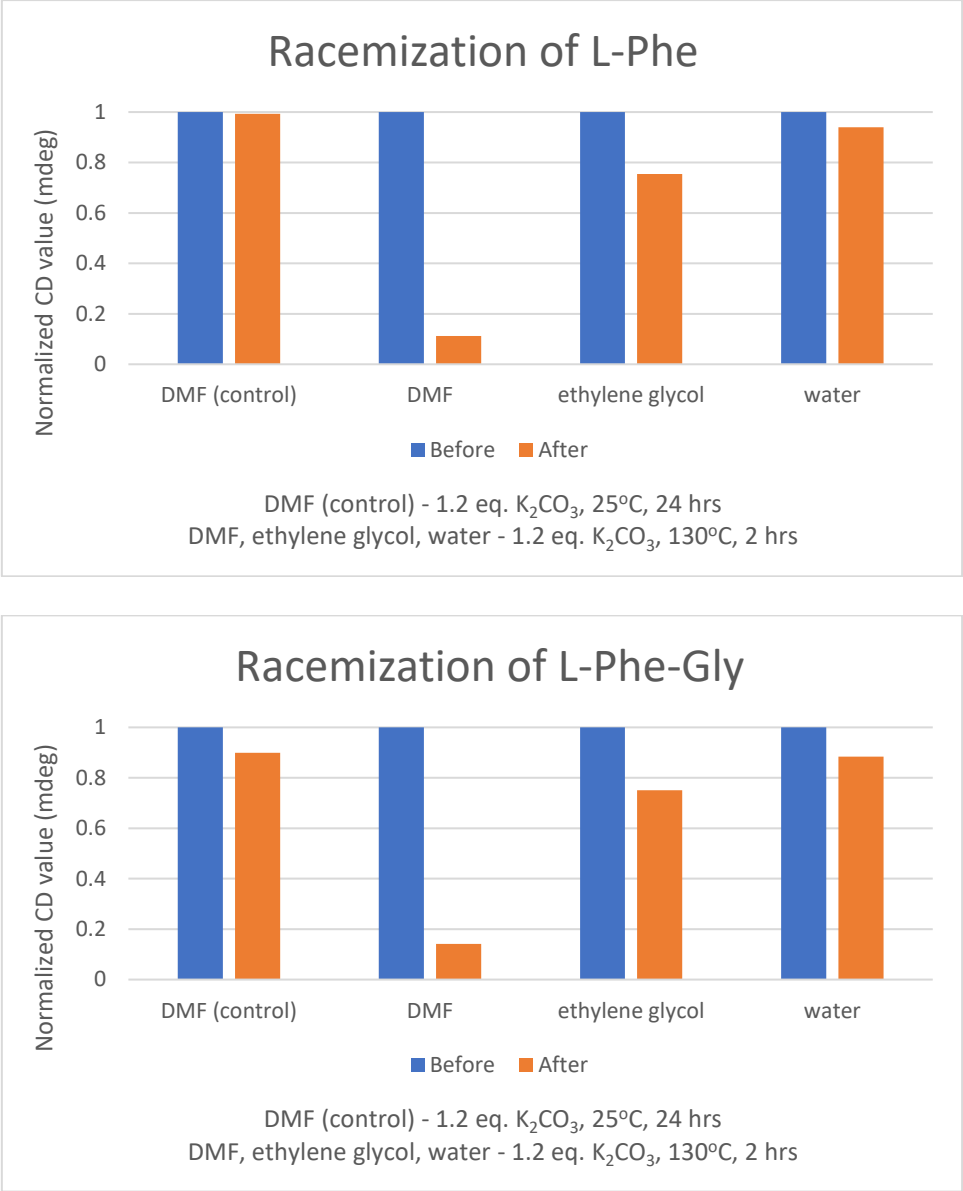
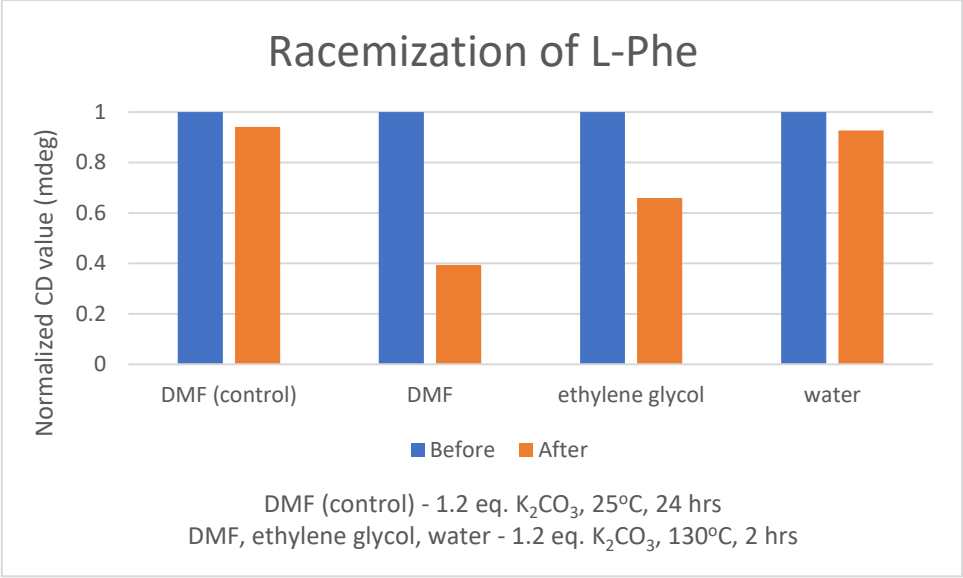


Fig. S 8: Monitoring the racemization of (a) L-Phe and (b) L-Phe-Gly in the presence of C1 before and after the completion of reaction in DMF, ethylene glycol and water using single point time course CD measurements. Measured at λ_{obs} = 292 nm, BW = 4 nm, Data Pitch = 10 s, D.I.T = 16 s, $t_{measure}$ = 6 min. The signals are monitored in DI-water at a concentration of C1 of 100 µM in the presence of excess of L-Phe and L-Phe-Gly (≈200 µM) from the reaction mixture. The DMF (control) shows the control reaction in DMF when the reaction mixture was kept at room temperature instead of heating to 130°C.



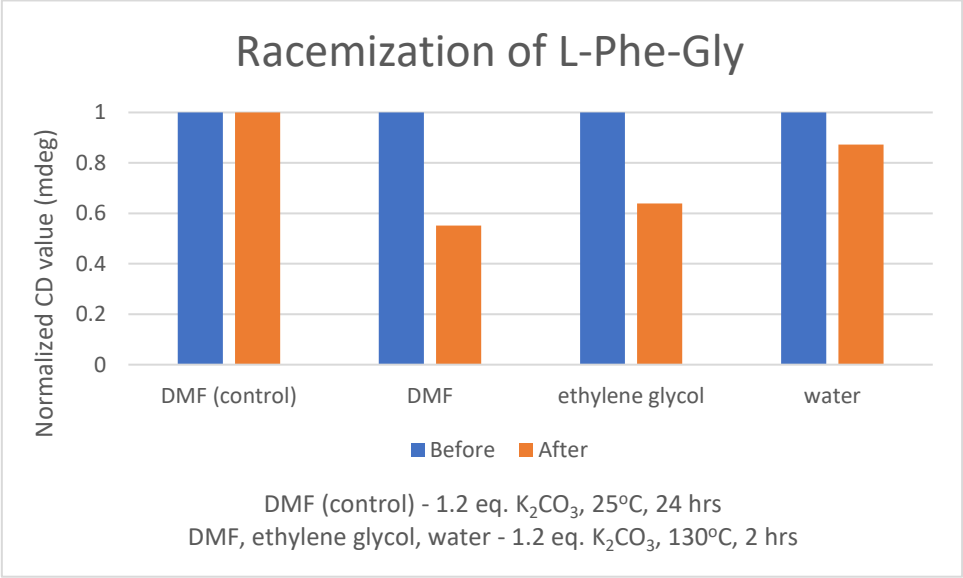


Fig. S 9: Monitoring the racemization of (a) L-Phe and (b) L-Phe-Gly in the presence of CB8•MDPP before and after the completion of reaction in DMF, ethylene glycol and water using single point time course CD measurements. Measured at λ_{obs} = 338 nm (for L-Phe) and 333 nm (for L-Phe-Gly), BW = 4 nm, Data Pitch = 30 s, D.I.T = 30 s, $t_{measure}$ = 10 min. The signals are monitored in DI-water at a concentration of CB8•MDPP of 20 µM in the presence of excess of L-Phe and L-Phe-Gly (≈100 µM) from the reaction mixture. The DMF (control) shows the control reaction in DMF when the reaction mixture was kept at room temperature instead of heating to 130°C.

5.1.4. Detection of water-insoluble and partially water-soluble drugs in aqueous media

The acyclic CB*n* type molecular container (C1 and C2) has been reported to bind and enhance the solubility and bioactivity of a wide range of poorly soluble pharmaceuticals. [17] Hence, we monitored the CD signals arising in the achiral C1 receptor in presence of chiral analytes such as (*R*)-limonene, (1*R*)-endo-(+)-fenchol, L-menthone, chiral bridged-alkane trinorbornanes, clopidogrel, testosterone, camptothecin, vecuronium, nandrolone and prednisolone in DI-water.

The addition of (*R*)-limonene and (1*R*)-endo-(+)-fenchol to C1 leads to appearance of CD bands in the 250 nm to 350 nm region, while the chiral analytes alone do not show any CD signals in this wavelength region (Fig. S 10 (a)). The addition of the chiral trinorbornane enantiomers, (*R*)-trinorbornane and (*S*)-trinorbornane to acyclic CB*n* C2 also causes induced CD signals in the 250 nm to 350 nm region (Fig. S 11 (a)), while the analyte alone does not show any CD signal. The addition of vecuronium to C1 also causes induced CD signals in the 250 nm to 350 nm region, while vecuronium alone does not show any CD signals in this region (Fig. S 12 (a)).The addition of excess of vecuronium to C1 shows that a complete complexation has been achieved even at a 1:1 ratio of C1 and vecuronium (Fig. S 13). Nandrolone and prednisolone possess CD signals on their own, but on binding to C1 shows characteristic shifts and increase in the signal magnitudes in the CD spectra (Fig. S 12 (a)). The addition of both nandrolone and prednisolone in excess to C1 shows a gradual shift in the spectra which tells that after a 1:1 complexation has been achieved, the CD signals arising from the excess nandrolone or prednisolone alone results in a shift in the spectra to appear more like the analyte alone (Fig. S 14 (a, b)). Moreover the addition of water-insoluble drugs such as testosterone, clopidogrel and camptothecin (from their ethanolic stock solution) to C1 shows induced CD signals (Fig. S 15 (a)) which was compared to the signals arising from the analytes alone by taking the CD spectra of testosterone, clopidogrel and camptothecin in ethanol (Fig. S 15 (c)). This shows characteristic shifts and changes in the CD spectra of testosterone, clopidogrel and camptothecin on binding to C1. Besides the addition of testosterone, clopidogrel and camptothecin to C2 gives rise to differences in the induced CD spectra when compared to C1 which can be useful for pattern recognition based steroid identifications (Fig. S 15 (b)).

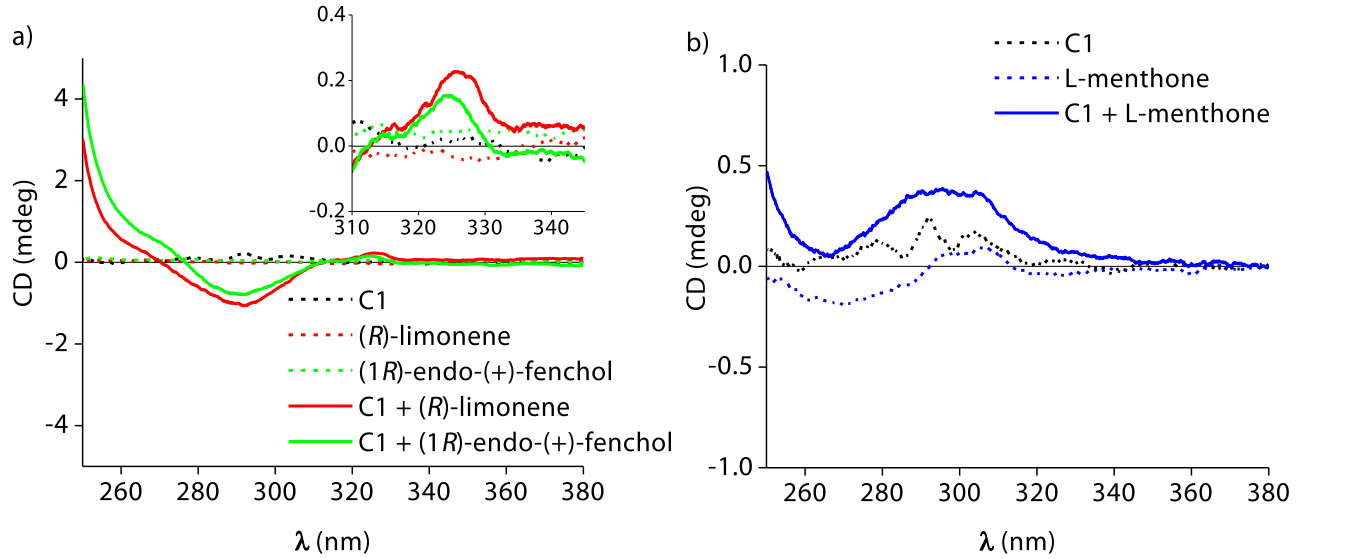


Fig. S 10: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of (*R*)-limonene (100 µM) (red) and (1*R*)-endo-(+)-fenchol (100 µM) (green) in DI-water (with ≤ 5 vol% ethanol). The dashed line represents the CD signals arising from (*R*)-limonene (100 µM) and (1*R*)-endo-(+)-fenchol (100 µM) alone. (b) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of L-menthone (100 µM) in DI-water. The dashed line represents the CD signals arising from L-menthone (100 µM) alone.

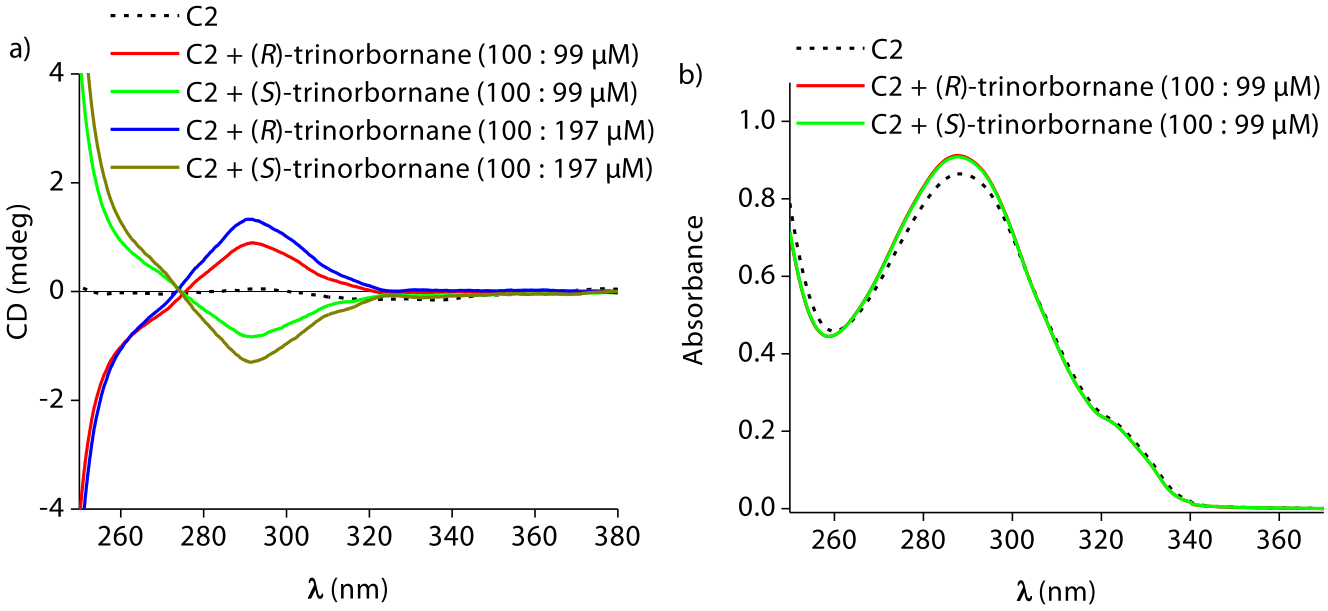


Fig. S 11: (a) CD spectrum of C2 (100 µM) and C2 (100 µM) in the presence of the enantiomers, (*R*)-trinorbornane (99 µM) and (*S*)-trinorbornane (99 µM) as well as excess of (*R*)-trinorbornane (197 µM) and (*S*)-trinorbornane (197 µM) in DI-water (with ≤ 1.2 vol% ACN).(b) Absorbance spectrum of C2 (100 µM) and C2 (100 µM) in the presence of (*R*)-trinorbornane (99 µM) and (*S*)-trinorbornane (99 µM) in DI-water (with ≤ 1.2 vol% ACN).

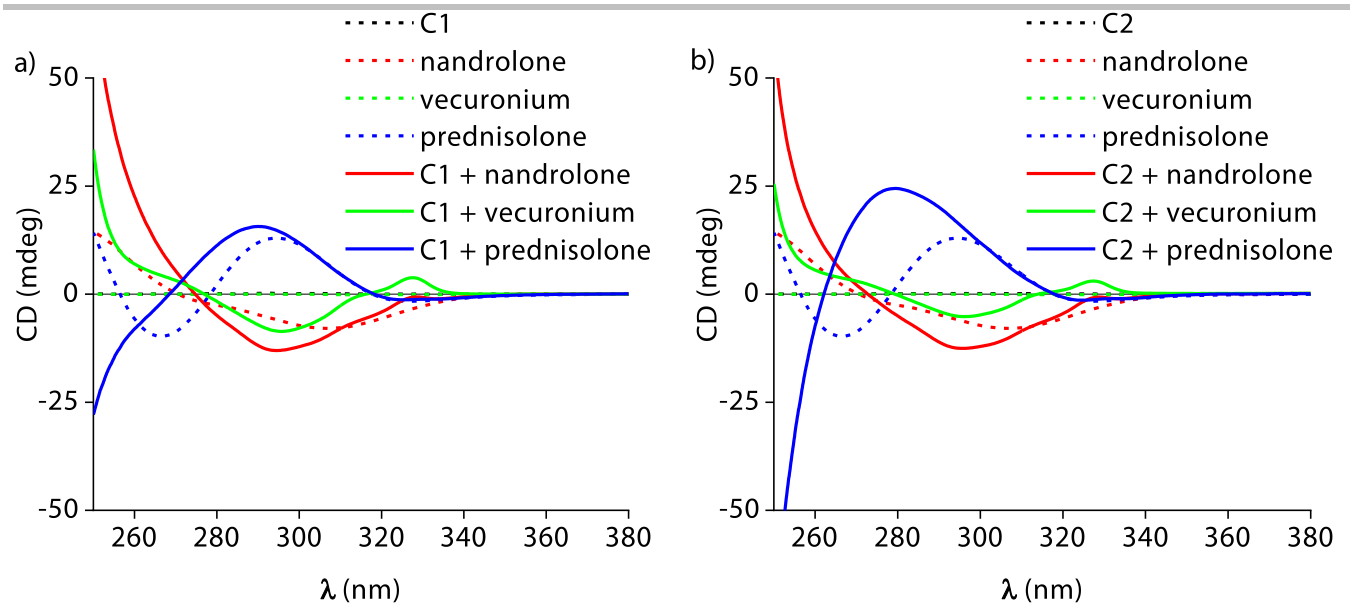


Fig. S 12: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of nandrolone (100 µM), vecuronium (100 µM) and prednisolone (100 µM) in DI-water. (b) CD spectrum of C2 (100 µM) and C2 (100 µM) in the presence of nandrolone (100 µM), vecuronium (100 µM) and prednisolone (100 µM) in DI-water. The dashed line shows the signals arising from nandrolone (100 µM), vecuronium (100 µM) and prednisolone (100 µM) alone in DI-water.

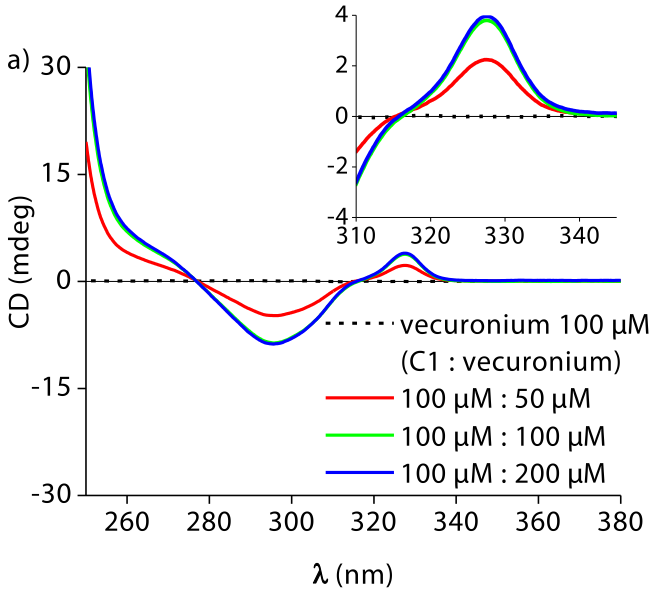


Fig. S 13: CD spectrum of C1 (100 µM) in the presence of vecuronium (50 µM) (red), vecuronium (100 µM) (green) and vecuronium (200 µM) (blue) in DI-water. The dashed black line represents the CD spectra corresponding to vecuronium (100 µM) alone in DI-water.

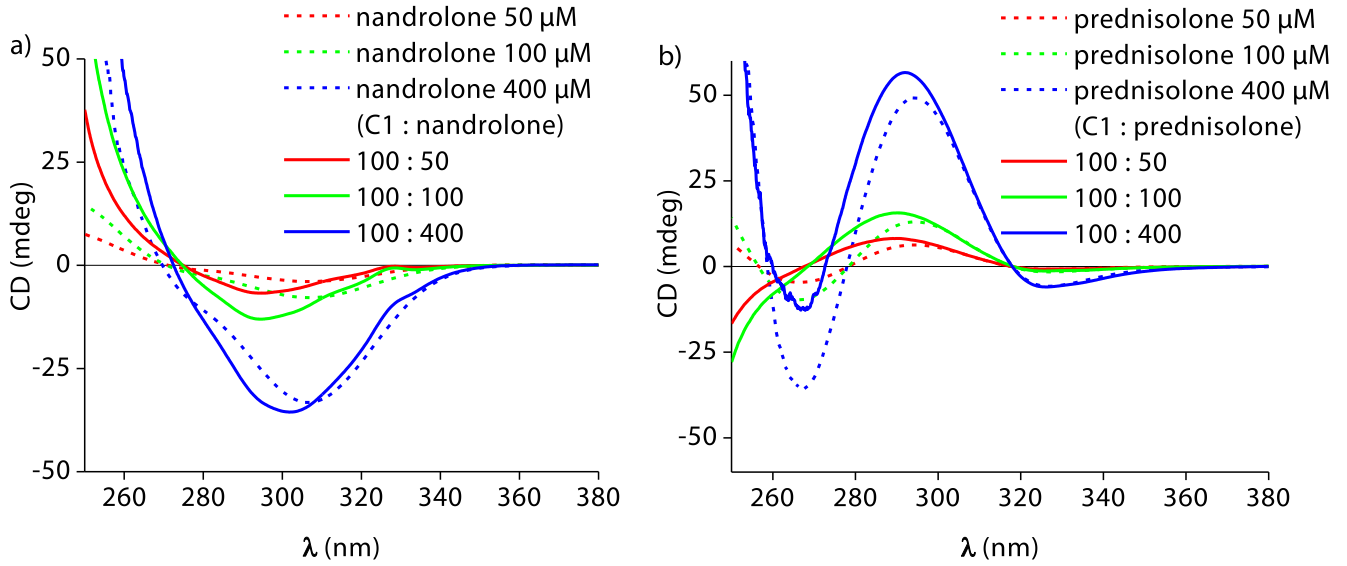


Fig. S 14: (a) CD spectrum of C1 (100 µM) in the presence of nandrolone (50 µM) (red), nandrolone (100 µM) (green), and nandrolone (400 µM) (blue) in DI-water. The dashed line represents the CD signals arising from nandrolone alone. (b) CD spectrum of C1 (100 µM) in the presence of prednisolone (50 µM) (red), prednisolone (100 µM) (green) and prednisolone (400 µM) (blue) in DI-water. The dashed line represents the CD signals arising from prednisolone alone.

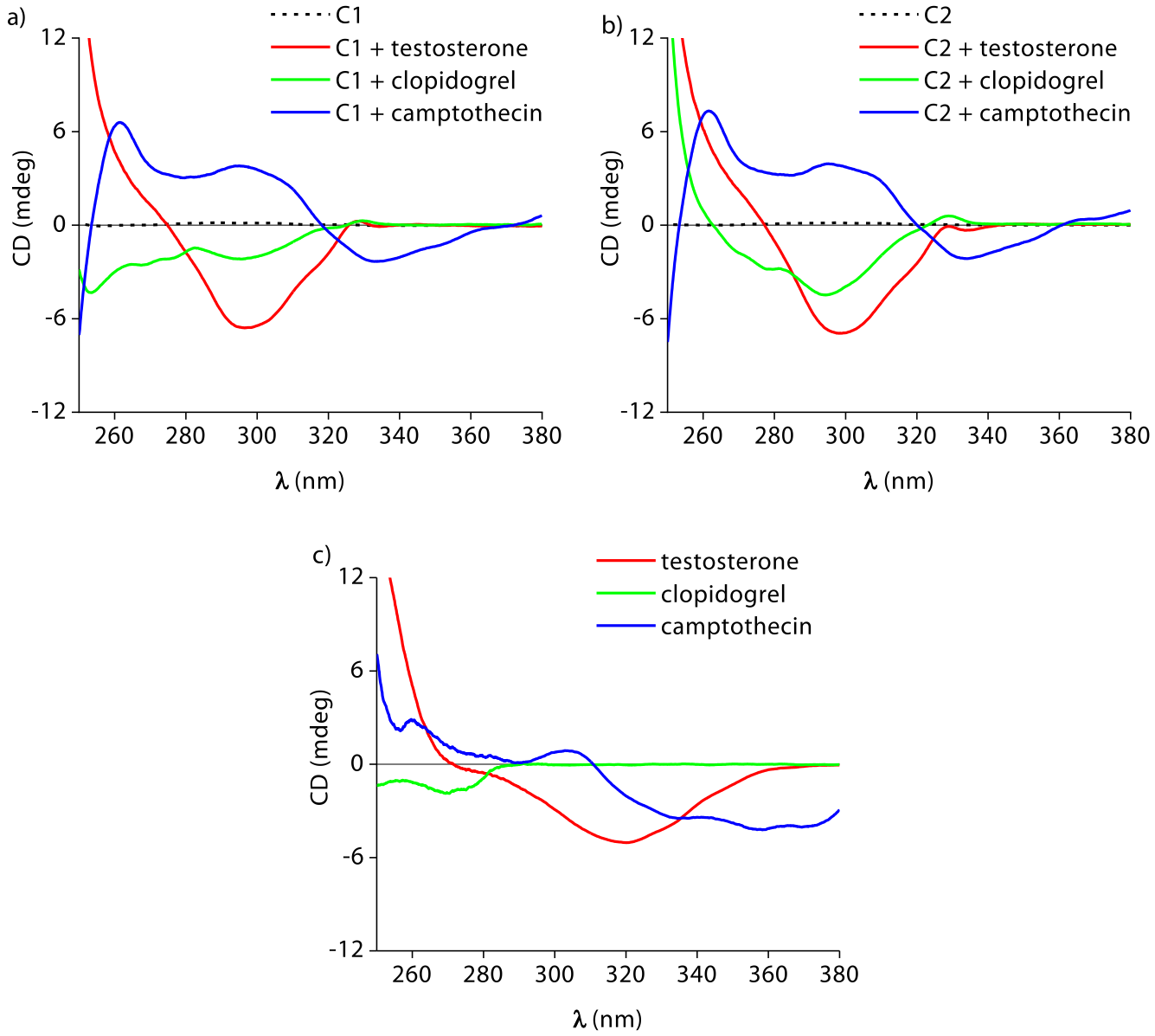


Fig. S 15: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of testosterone (100 µM), clopidogrel (100 µM), and camptothecin (100 µM) in DI-water (with ≤ 5 vol% ethanol) (b) CD spectrum of C2 (100 µM) and C2 (100 µM) in the presence of testosterone (100 µM), clopidogrel (100 µM), and camptothecin (100 µM) in DI-water (with ≤ 5 vol% ethanol) (c) CD signals arising from testosterone (100 µM), clopidogrel (100 µM), and camptothecin (100 µM) alone in ethanol.

5.1.5. Solubilization of water-insoluble drugs and detection in aqueous media

Since the acyclic CB*n* type molecular containers are known to exhibit high water solubility and to enhance the solubility and bioactivity of a wide range of poorly soluble pharmaceuticals, [17] we solubilized several water-insoluble steroids such as testosterone, clopidogrel and camptothecin using both C1 and C2 and the CD signals arising in the achiral receptor upon complexation with these chiral analytes were measured in DI-water. The host•guest complex for the measurements was obtained by stirring solutions containing known concentration of C1 and C2 with an excess of solid drug in DI water at room temperature for 12 hrs. The excess insoluble drug was removed by centrifugation and the supernatant containing the complex was then collected. All the three steroids tested were solubilized in water upon complexation by both C1 and C2 and shows distinct induced CD signals. The C2●steroid complexes show a higher CD magnitude when compared to the C1●steroid complexes tested implying a higher solubilization power of C2.

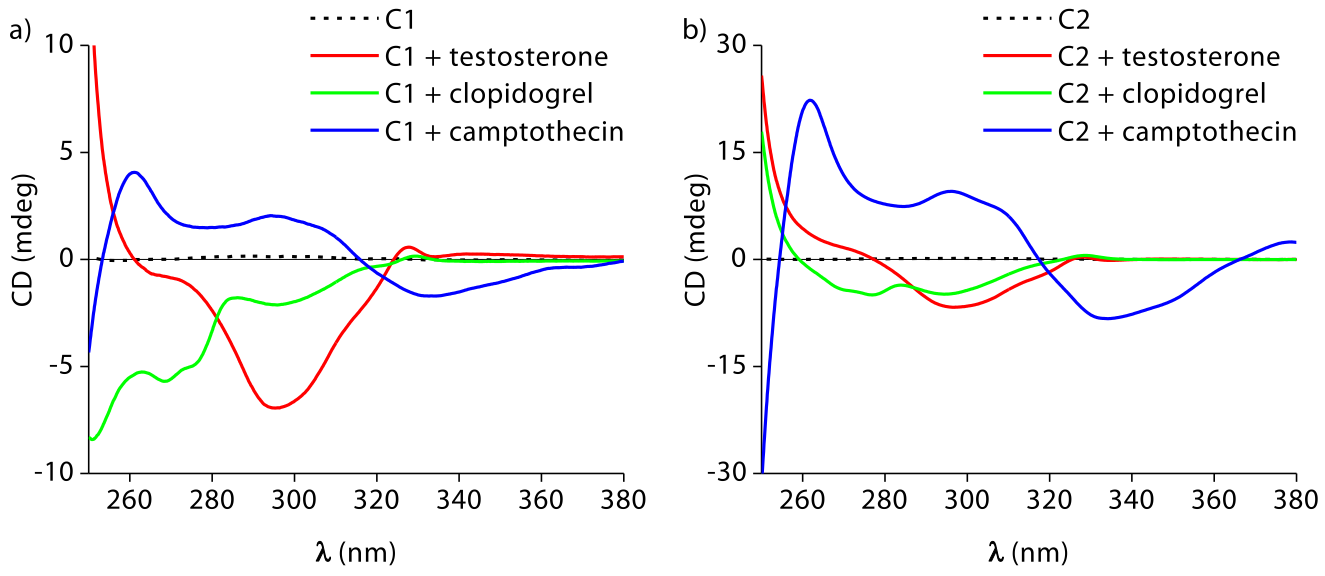


Fig. S 16: (a) CD spectrum of C1 (100 µM) and C1 (100 µM) in the presence of excess of testosterone, clopidogrel and camptothecin in DI-water. (b) CD spectrum of C2 (100 µM) and C2 (100 µM) in the presence of excess of testosterone, clopidogrel and camptothecin in DI-water.

5.1.6. Analysis of mixture of drugs using acyclic CB*n* molecular container C1 in aqueous media

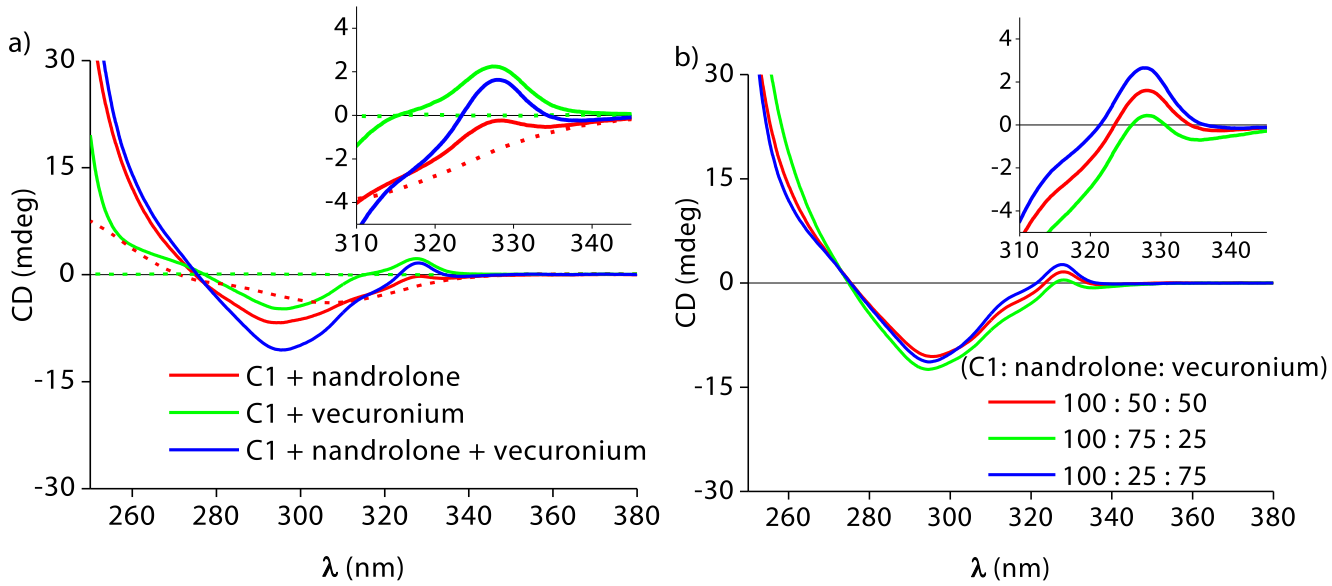


Fig. S 17: (a) CD spectrum of C1 (100 µM) in the presence of a mixture of nandrolone (50 µM) and vecuronium (50 µM) in DI-water (blue). The red and green line represents the CD spectrum of C1 (100 µM) in the presence of nandrolone (50 µM) and vecuronium (50 µM) respectively. (b) CD spectrum of C1 (100 µM) in the presence of varying ratios of nandrolone and vecuronium (C1: nandrolone: vecuronium). The dashed red and green line shows the CD signals arising from nandrolone (50 µM) and vecuronium (50 µM) alone in DI-water.

5.2. Analyte spectra with tweezers receptor

The molecular tweezer, CLR01 has been reported to selectively complex lysine and arginine derivatives and reject all other amino acids.[18] Hence, we monitored the CD signals arising in the achiral CLR01 receptor in presence of chiral lysine and arginine containing amino acids such as L-Arg, D-Arg, L-Lys Ac-L-Arg-OMe, Ac-L-Lys-OMe and peptide derivatives such as H-Lys-Leu-Val-Phe-Phe-OH. The addition of both L-Arg and the arginine derivative Ac-L-Arg-OMe shows induced CD signal with the CLR01 receptor (Fig. S 18(a) and Fig. S 19 (a)) Moreover the enantiomers L-Arg and D-Arg shows induced CD signals which roughly behave as mirror images exhibiting opposite signs (Fig. S 18 (a)). Both L-Lys and the lysine derivative Ac-L-Lys-OMe also shows an induced CD signals with the CLR01 receptor (Fig. S 20(a) andFig. S 21 (a)), but the signals are not that intense when compared with the arginine derivatives. The addition of the peptide derivative H-Lys-Leu-Val-Phe-Phe-OH to CLR01 is also accompanied by a small induced CD signal (Fig. S 22 (a)).

5.2.1. Detection of arginine and lysine derivatives in aqueous media

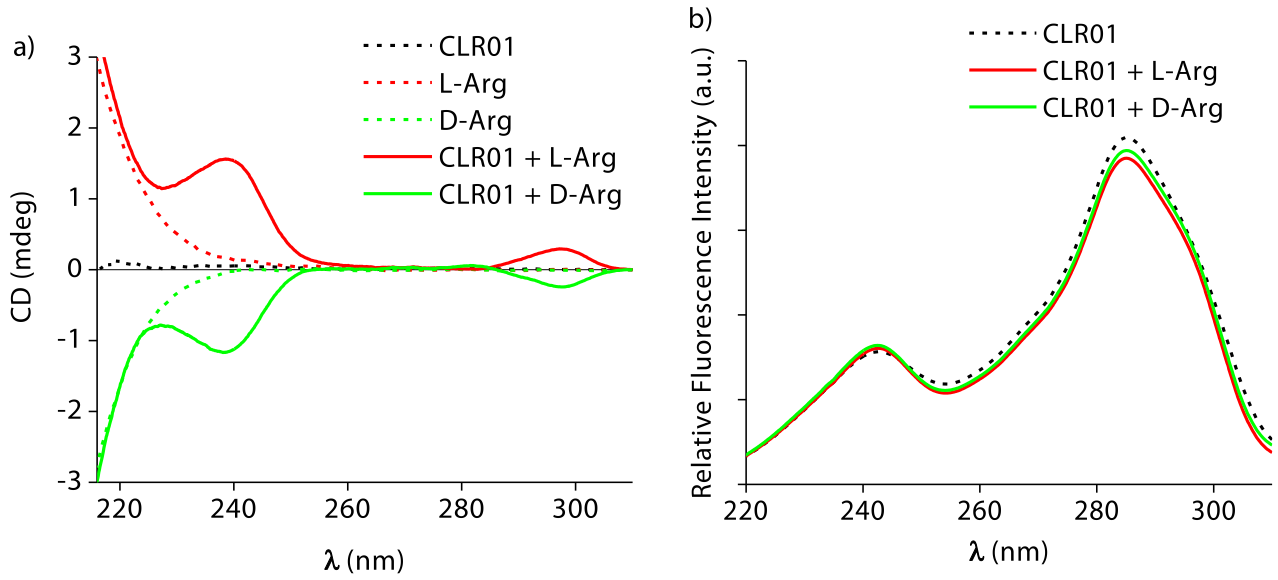


Fig. S 18: (a) CD spectrum of CLR01 (20 µM) in the absence and presence of L-Arg (200 µM) and D-Arg (200 µM) in DI-water. The dashed red and green line represents the CD spectrum of the analytes, L-Arg (200 µM) and D-Arg (200 µM) alone. (b) The excitation spectra of CLR01 (20 µM) in the absence and presence of L-Arg (200 µM) and D-Arg (200 µM) in DI-water collected using a long pass filter of 320 nm and at an HT voltage of 690 V.

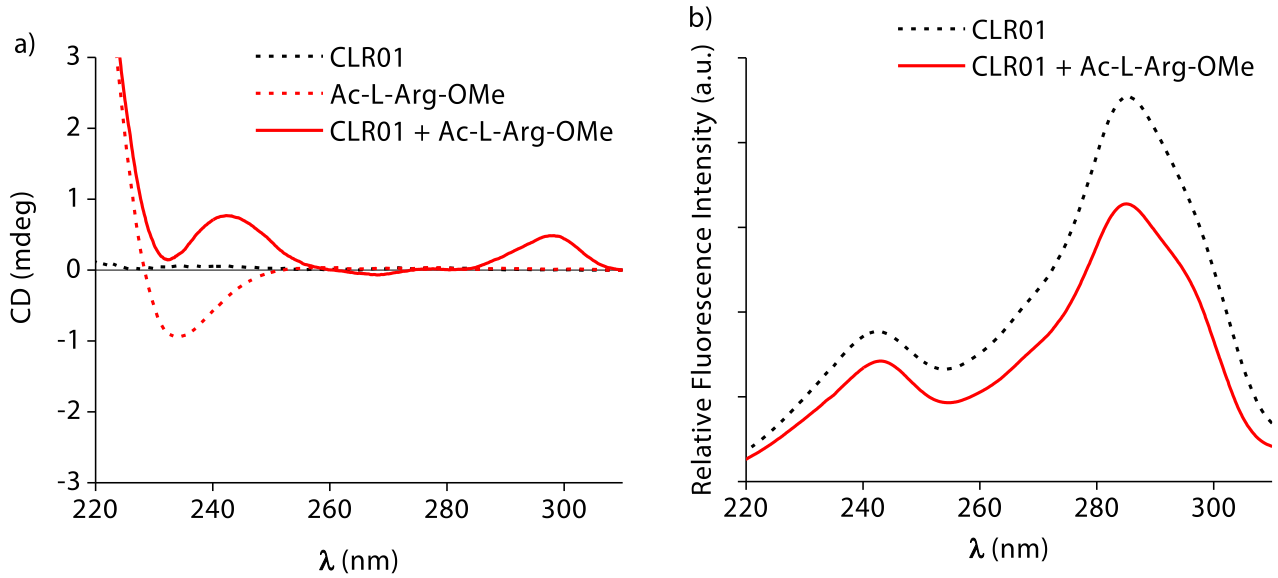


Fig. S 19: (a) CD spectrum of CLR01 (20 µM) in the absence and presence of Ac-L-Arg-OMe (200 µM) in DI-water. The dashed red line represents the CD signals arising from the analyte, Ac-L-Arg-OMe (200 µM) alone.(b) The excitation spectra of CLR01 (20 µM) in the absence and presence of Ac-L-Arg-OMe (200 µM) in DI-water collected using a long pass filter of 320 nm and at an HT voltage of 690 V.

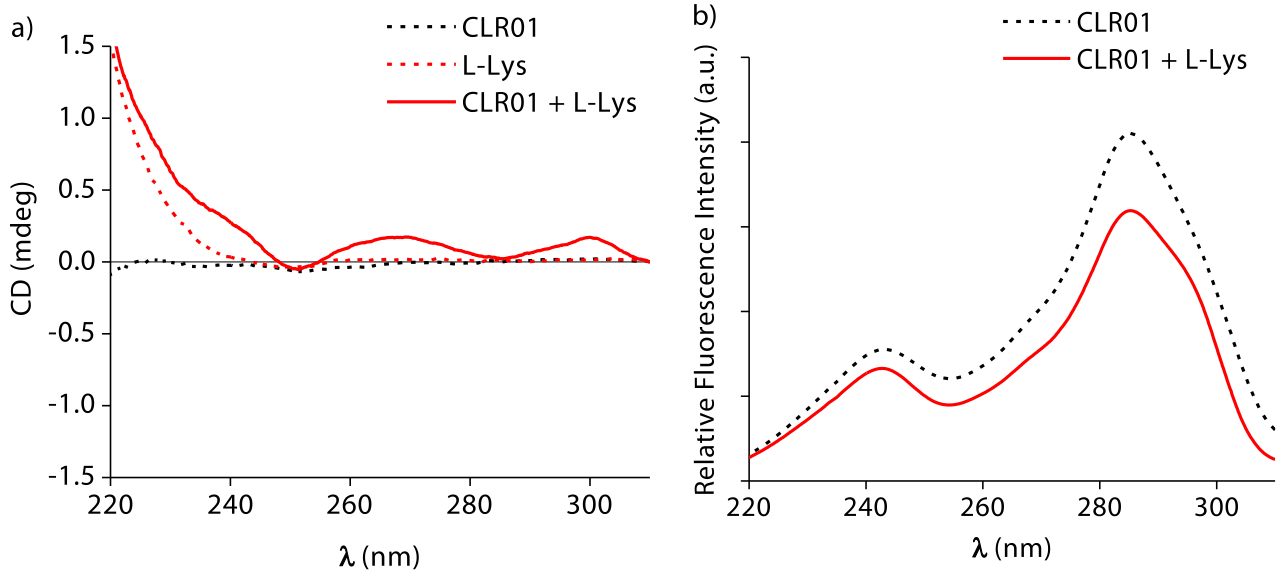


Fig. S 20: (a) CD spectrum of CLR01 (20 µM) in the absence and presence of L-Lys (200 µM) in DI-water. The dashed red line represents the CD signals arising from the analyte, L-Lys (200 µM) alone.(b) The excitation spectra of CLR01 (20 µM) in the absence and presence of L-Lys (200 µM) in DI-water collected using a long pass filter of 320 nm and at an HT voltage of 690 V.

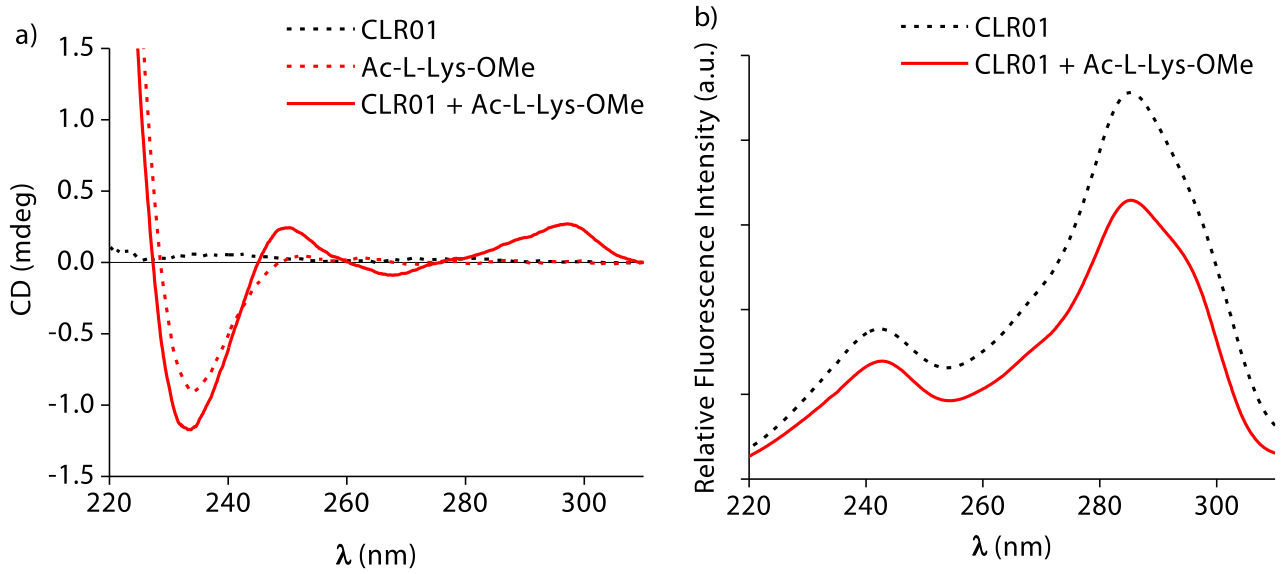


Fig. S 21: (a) CD spectrum of CLR01 (20 µM) in the absence and presence of Ac-L-Lys-OMe (200 µM) in DI-water. The dashed red line represents the CD signals arising from the analyte, Ac-L-Lys-OMe (200 µM) alone. (b) The excitation spectra of CLR01 (20 µM) in the absence and presence of Ac-L-Lys-OMe (200 µM) in DI-water collected using a long pass filter of 320 nm and at an HT voltage of 690 V.

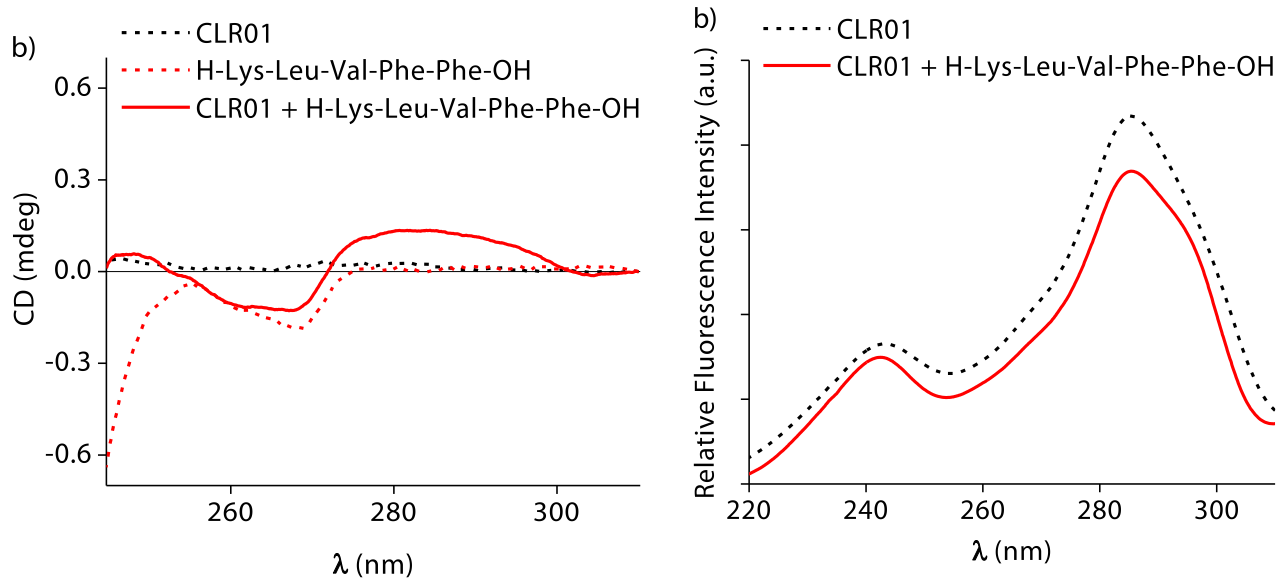


Fig. S 22: (a) CD spectrum of CLR01 (20 µM) in the absence and presence of H-Lys-Leu-Val-Phe-Phe-OH (200 µM) in DI-water. The dashed red line represents the CD signals arising from the analyte, H-Lys-Leu-Val-Phe-Phe-OH (200 µM) alone. b) The excitation spectra of CLR01 (20 µM) in the absence and presence of H-Lys-Leu-Val-Phe-Phe-OH (200 µM) in DI-water collected using a long pass filter of 320 nm and at an HT voltage of 690 V.

5.2.2. Analysis of mixture of amino acids using molecular tweezer CLR01 in aqueous media

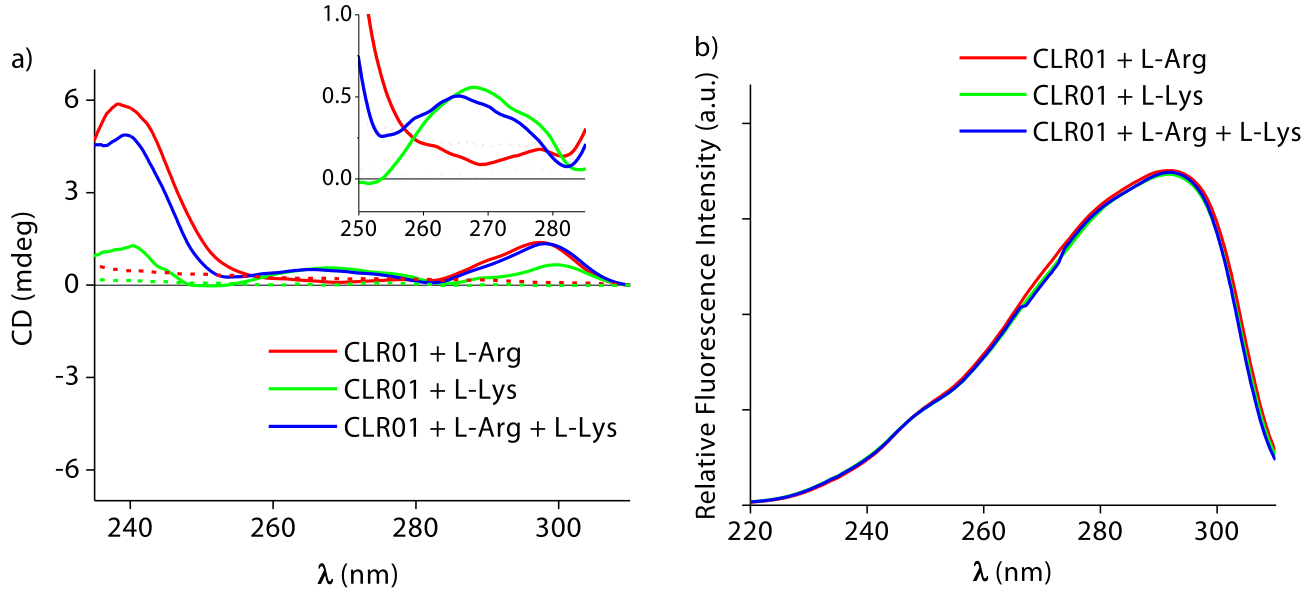


Fig. S 23: (a) CD spectrum of CLR01 (140 µM) in the presence of a mixture of L-Arg (70 µM) and L-Lys (70 µM) in DI-water (blue). The red and green line represents the CD spectrum of CLR01 (100 µM) in the presence of L-Arg (70 µM) and L-Lys (70 µM) respectively. The dashed line represents the CD signals arising from the amino acids alone in DI-water. (b) The excitation spectrum of the sample collected using a long pass filter of 320 nm and at an HT voltage of 550 V.

5.3. Molar ellipticity ([θ]) and molar circular dichroism (Δε) for the host•guest complexes studied

Table S 2: Molar ellipticity and molar circular dichroism data for complexes of C1 (100 µM) with chiral analytes in DI-water at 25°C.

Chiral analyte	λ_{max} (nm)	[θ] x 10^3 (deg M^{-1} m^{-1})	Δε (M^{-1} cm^{-1})
L-Phe	292	5.80	1.76
	326	-1.09	-0.33
D-Phe	292	-5.22	-1.58
	326	1.08	0.33
L-Phe-L-Ala	292	7.98	2.42
	326	-1.09	-0.33
L-Phe-Gly	292	-7.99	-2.42
	326	1.26	0.38
L-Phe-L-Val	292	13.39	4.06
	326	-2.02	-0.61
L-Ala-Phe	292	4.43	1.34
	326	-0.60	-0.18
L-Trp	293	12.79	3.88
	326	-1.38	-0.41
D-Trp	293	-12.86	-3.40
	326	1.59	0.48
L-Trp-OMe	293	19.88	6.03
	326	-2.47	-0.75
D-Trp-OMe	293	-24.18	-7.33
	326	2.85	0.86
L-Trp-NH2	293	19.10	5.79
	326	-2.06	-0.62
vecuronium	296	-8.75	-2.65
	328	3.94	1.19
(*R*)-Limonene[a]	291	-1.03	-0.31
	326	0.23	0.07
((1*R*)-endo-(+)-Fenchol[a]	291	-0.79	-0.24
	324	0.15	0.05
(*R*)-trinorbornane[b]	291	1.34	0.40
(*S*)-trinorbornane[b]	291	-1.30	-0.39

[a] with ≤ 5 vol% ethanol in DI-water
[b] with ≤ 1.2 vol% ACN in DI-water

Table S 3: Molar ellipticity and molar circular dichroism data for complexes of CB8•MDPP (20 μM) with chiral analytes in DI-water at 25°C.

Chiral analyte	λ_{max} (nm)	[θ] x 10^3 (deg M^{-1} m^{-1})	Δε (M^{-1} cm^{-1})
L-Phe	280	-10.91	-3.31
	330	8.02	2.43
D-Phe	280	11.48	3.41
	330	-7.89	-2.39
L-Phe-L-Ala	276	-8.86	-2.69
	322	16.29	4.94
L-Phe-Gly	276	-8.53	-2.55
	322	15.55	4.72
L-Phe-L-Val	276	-7.69	-2.33
	322	14.90	4.52
L-Ala-Phe	286	3.27	0.99
	326	-0.95	-0.29
L-Trp	293	-2.19	-0.66
	439	-2.50	-0.76
D-Trp	293	2.30	0.70
	439	2.42	0.73
L-Trp-OMe	287	-1.88	-0.57
	418	-3.20	-0.97
D-Trp-OMe	287	1.52	0.46
	418	3.04	0.92
L-Trp-NH2	375	6.68	2.03
	435	-5.16	-1.56

Experimentally recorded ellipticity values (θ in mDeg) were converted into molar ellipticity values according to [θ] = θ/(10*L*C), with the pathlength L in cm and the concentration C of the receptor in mol/L. The molar circular dichroism (Δε) was obtained via Δε = [θ] /3298.

5.4. Molar extinction coefficient of acyclic CB*n* molecular container C1 and C2 and molecular tweezer CLR01

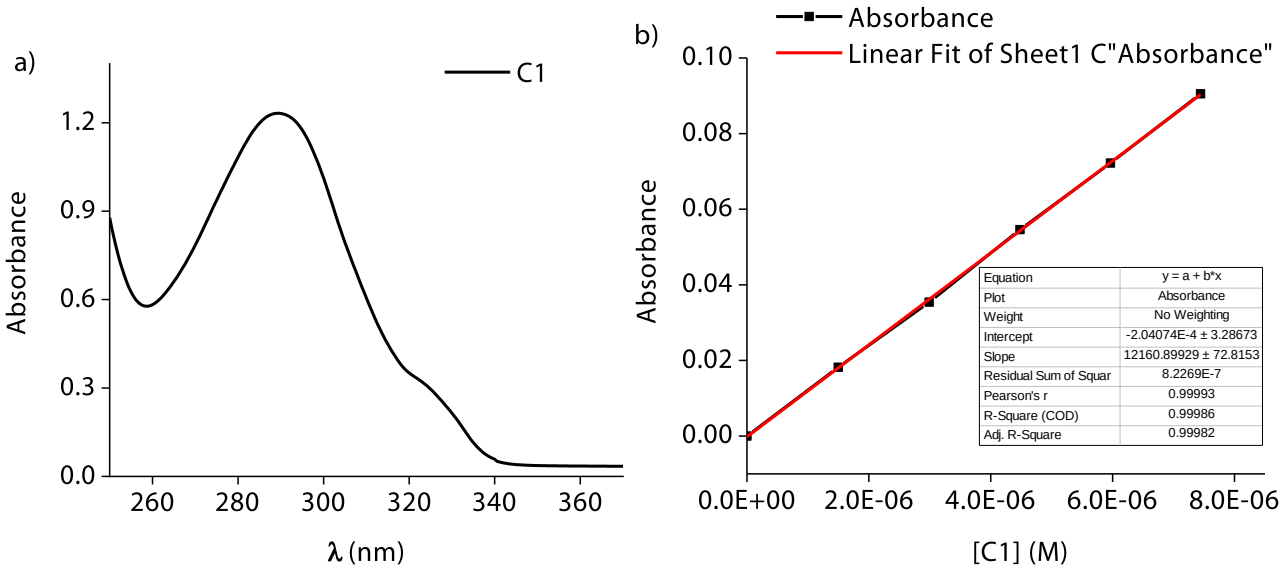


Fig. S 24: (a) Absorbance spectra of C1 (100 µM) in DI-water. (b) Determination of molar extinction coefficient of C1 using UV-Vis titration at 290 nm and Beer Lamberts Law. Molar extinction coefficient at 290 nm = (12161 ± 72.8) $M^{-1}cm^{-1}$.

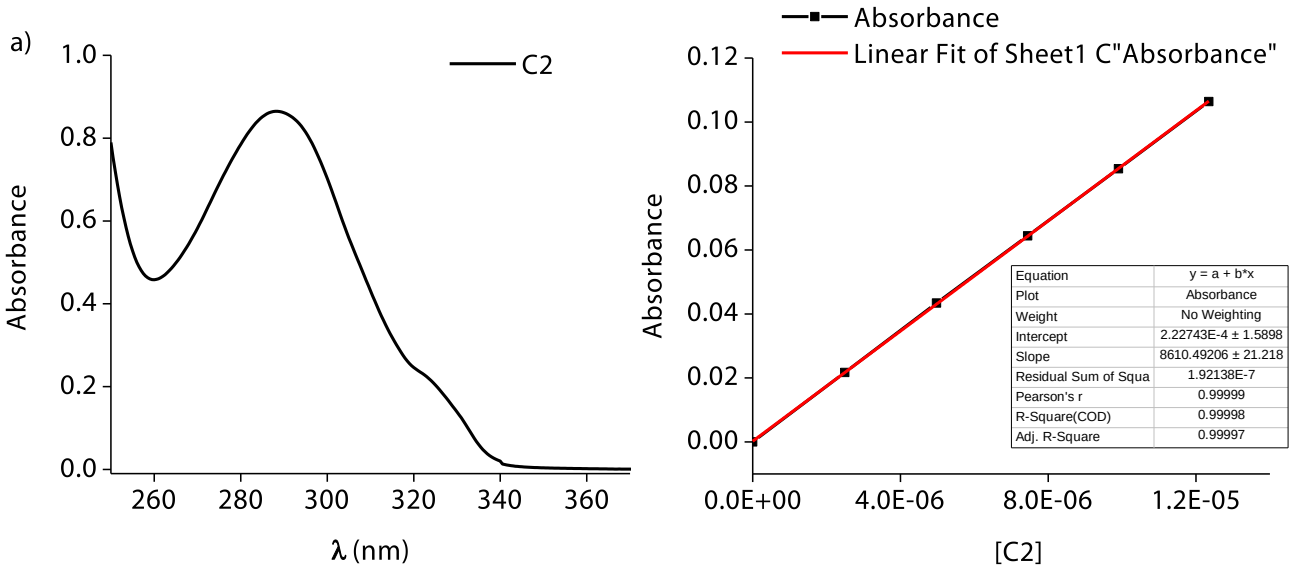


Fig. S 25: (a) Absorbance spectra of C2 (100 µM) in DI-water. (b) Determination of molar extinction coefficient of C2 using UV-Vis titration at 290 nm and Beer Lamberts Law. Molar extinction coefficient at 290 nm = (8610 ± 21.2) $M^{-1}cm^{-1}$.

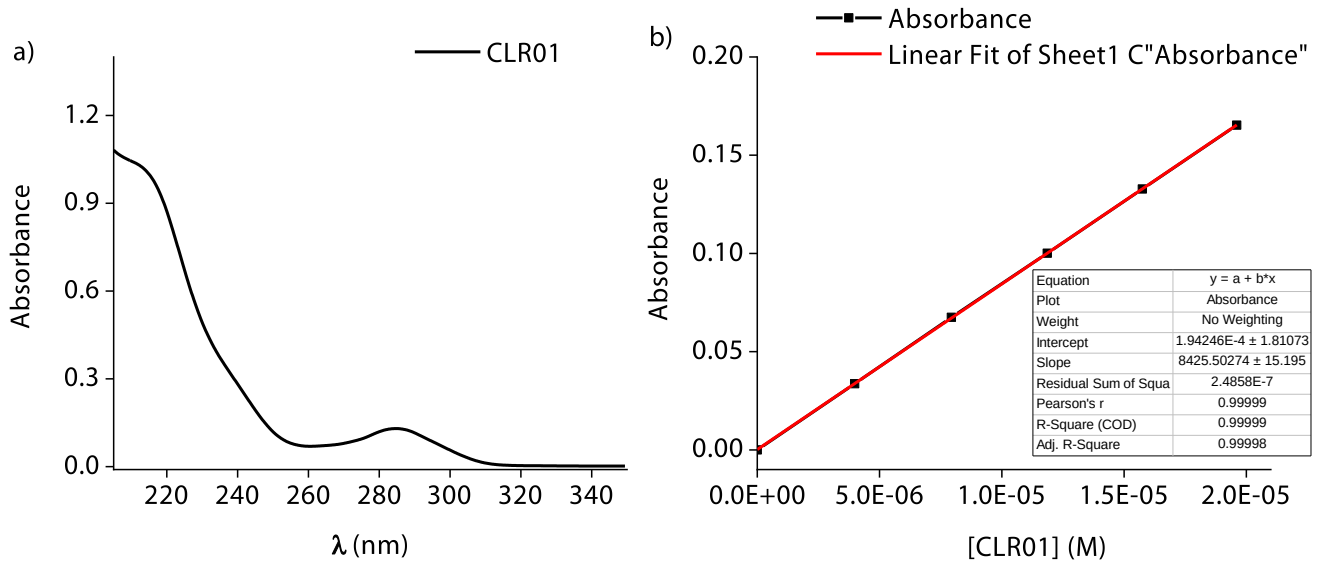


Fig. S 26: (a) Absorbance spectra of CLR01 (20 µM) in DI-water. (b) Determination of molar extinction coefficient of CLR01 using UV-Vis titration at 285 nm and Beer Lamberts Law. Molar extinction coefficient at 285 nm = (8425 ±15.2) $M^{-1}cm^{-1}$.

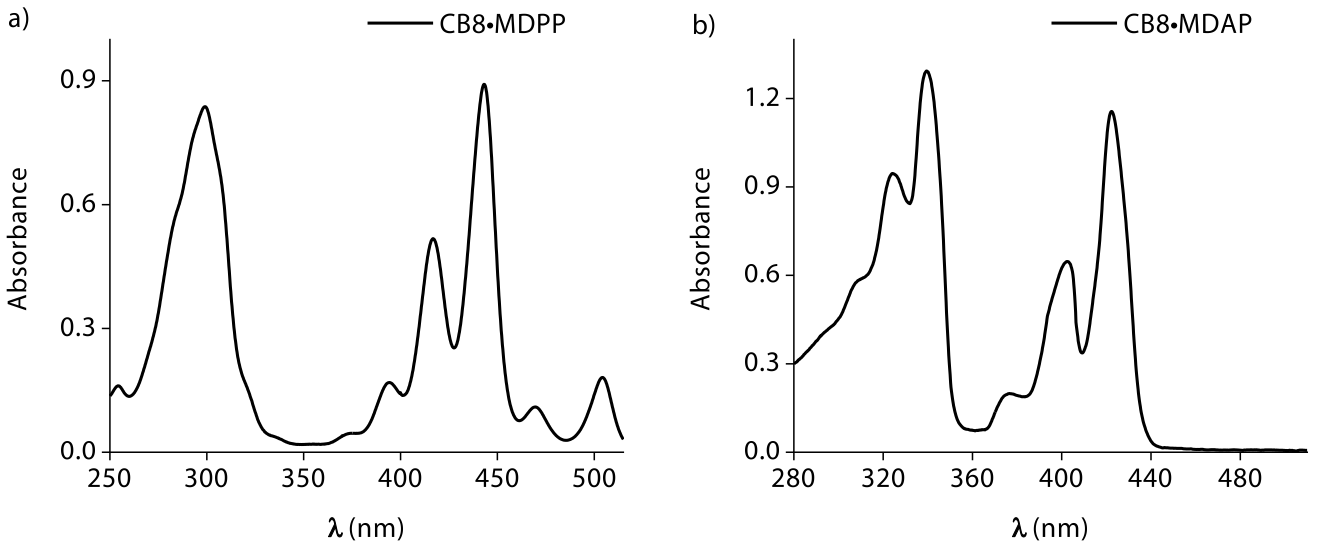


Fig. S 27: (a) Absorbance spectra of CB8•MDPP (20 µM) in DI Water. (b) Absorbance spectra of CB8•MDAP (20 µM) in DI-water. The reported extinction coefficient of CB8•MDPP in DI-water at 443 nm is 55000 $M^{-1}cm^{-1}$ and CB8•MDAP in DI-water at 419 nm is 7600 $M^{-1}cm^{-1}$.[19]

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