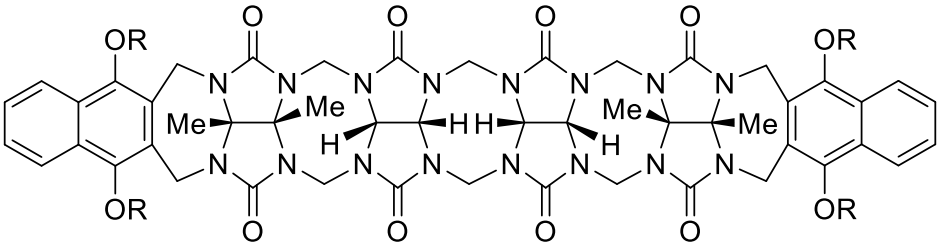
**Contents**

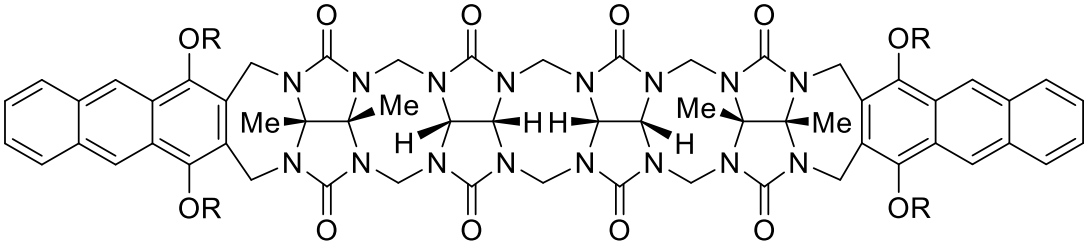
## 1. General information

All commercially available reagents and solvents were used without further purification. Circular dichroism spectra were recorded on a JASCO J-710 spectropolarimeter. CD spectra were collected with a standard sensitivity of 100 mdeg, a data pitch of 2.0 nm, a band width of 1 nm, a scanning speed of 500 nm $min^{-1}$, and a response of 0.5 s using a quartz cuvette. The data were baseline corrected and smoothed using a binomial equation. NMR spectra were measured on 600 MHz spectrometer at room temperature in $D_2O$. Hosts **M2** and **M3** were prepared by the literature procedures (**M2**: Ma, D.; Hettiarachchi, G.; Nguyen, D.; Zhang, B.; Wittenberg, J. B.; Zavalij, P. Y.; Briken, V.; Isaacs, L. *Nat. Chem.* **2012**, *4*, 503-510; **M3**: Murkli, S.; Klemm, J.; King, D.; Zavalij, P. Y.; Isaacs, L. *Chem. Eur. J.* **2020**, *26*, 15249-15258).

**Sensor structures**




**M2**, R = $(CH_2)_3SO_3Na$

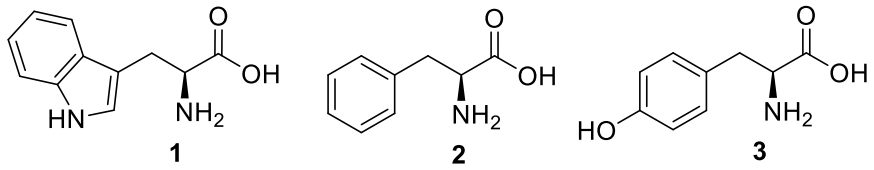



**M3**, R = $(CH_2)_3SO_3Na$

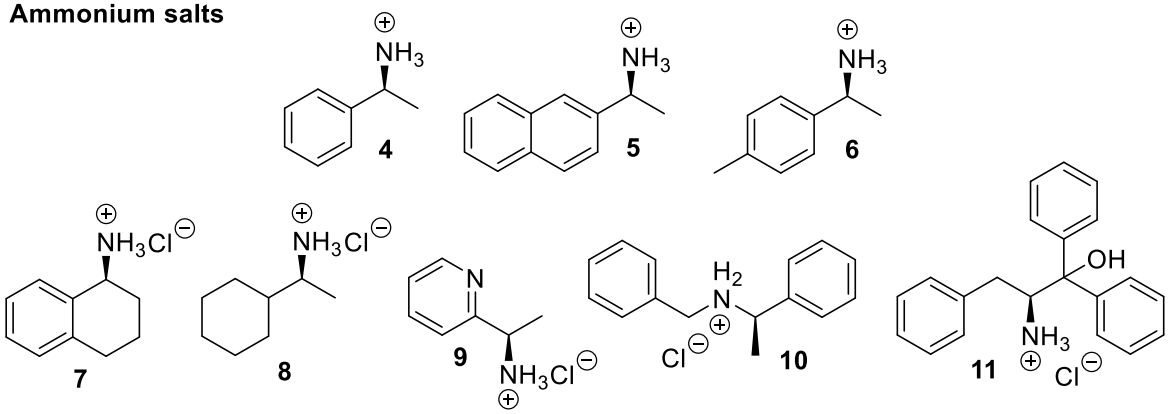
## 2. Substrate scope

The utility of the sensing assay was tested with amino acids **1-3**, ammonium salts **4-14**, alcohols **15-19**, and terpenes **20** and **21** (only one enantiomer is shown for simplicity). Control experiments showed that the substrates did not have any CD signals in the region of interest in the absence of the sensor.

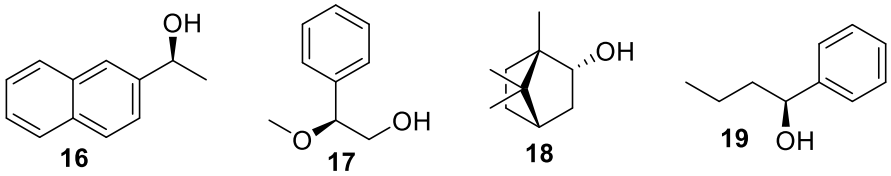
**Amino acids**



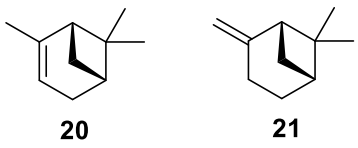

**Ammonium salts**




**Alcohols**



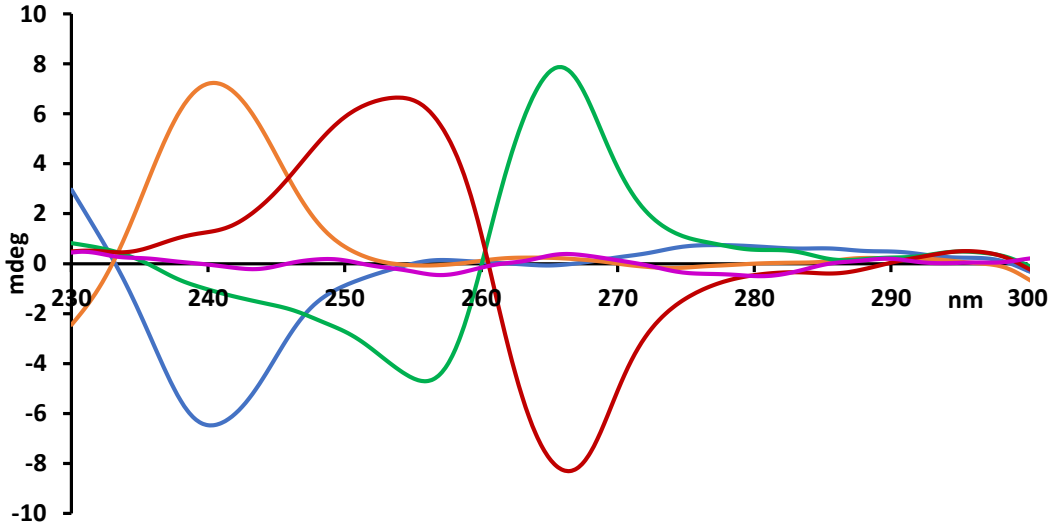

**Terpenes**



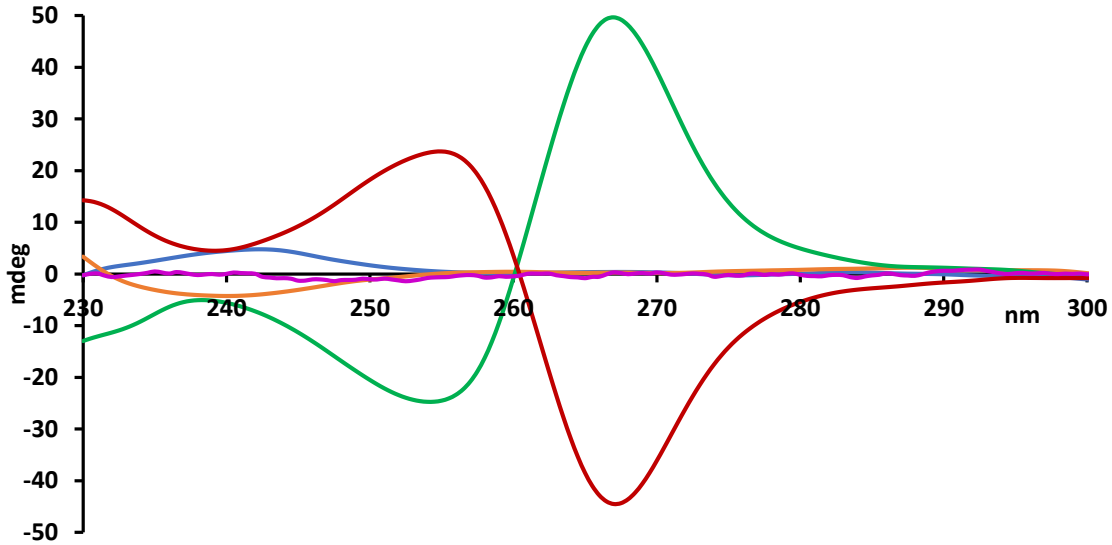
## 3. Comparison between M2 and M3

Stock solutions of **M2** and **M3** (1.0 mM) were prepared in deionized $H_2O$ and 0.2 mL portions were placed into 4 mL vials followed by addition of 0.8 mL of $H_2O$. In separate vials, stock solutions of the analytes (0.2 mM in $H_2O$) were prepared and an equimolar amount of HCl (1.0 M) was added to generate the ammonium salts. To each vial containing 0.5 mL of the sensor was added 1 equivalent (0.5 mL) of the analyte. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted by diluting the solution to a final concentration of 5.0 μM by adding 1.9 mL of deionized $H_2O$ into 0.1 mL of the mixture using a quartz cuvette that has a 10 mm path length.

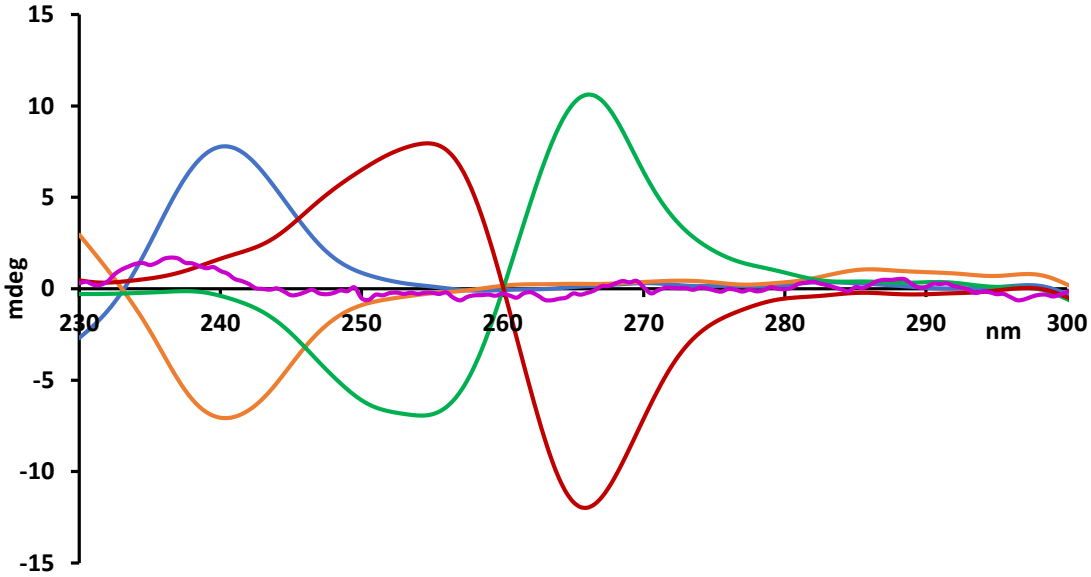
CD spectra of the assembly obtained with **M2** and (*R*)-**4** (blue) or (*S*)-**4** (orange) and **M3** and (*R*)-**4** (green) or (*S*)-**4** (red). A control experiment with (*R*)-**4** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



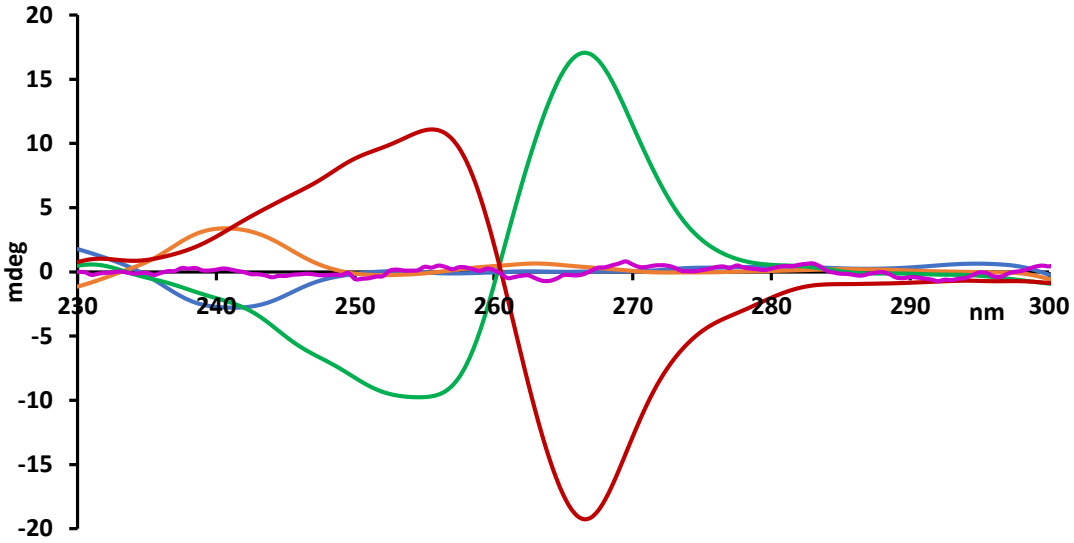
CD spectra of the assembly obtained with **M2** and (*R*)-**5** (blue) or (*S*)-**5** (orange) and **M3** and (*R*)-**5** (green) or (*S*)-**5** (red). A control experiment with (*R*)-**5** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



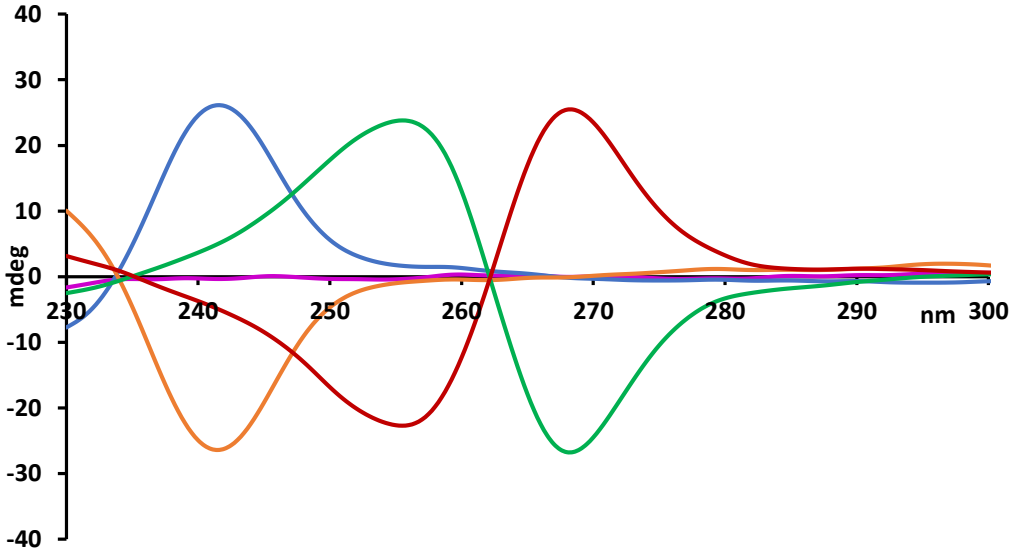

CD spectra of the assembly obtained with **M2** and (*R*)-**8** (blue) or (*S*)-**8** (orange) and **M3** and (*R*)-**8** (green) or (*S*)-**8** (red). A control experiment with (*R*)-**8** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



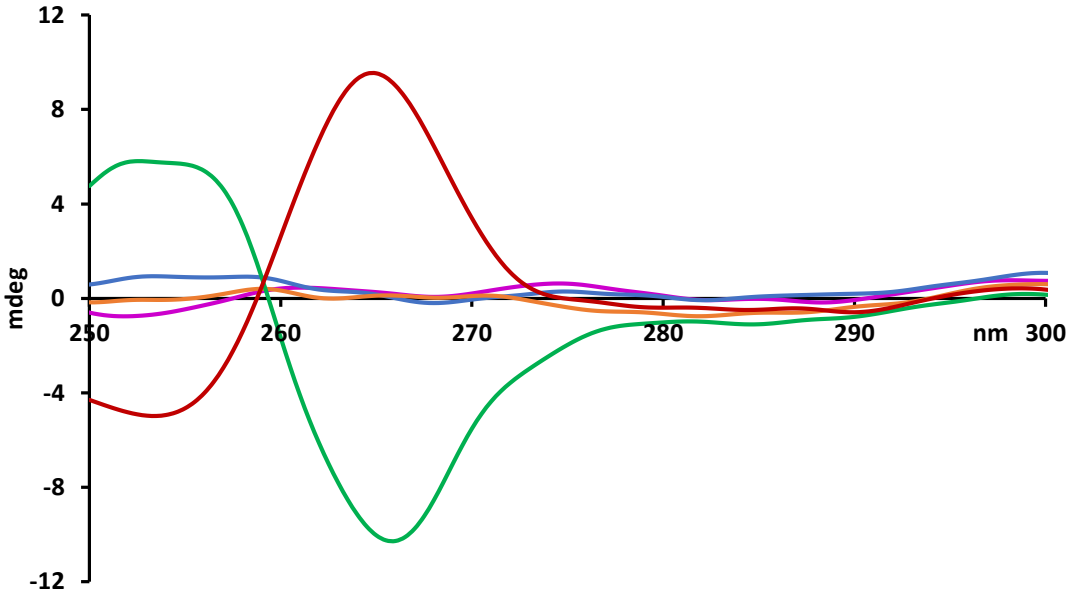
CD spectra of the assembly obtained with **M2** and (*R*)-**10** (blue) or (*S*)-**10** (orange) and **M3** and (*R*)-**10** (green) or (*S*)-**10** (red). A control experiment with (*R*)-**10** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



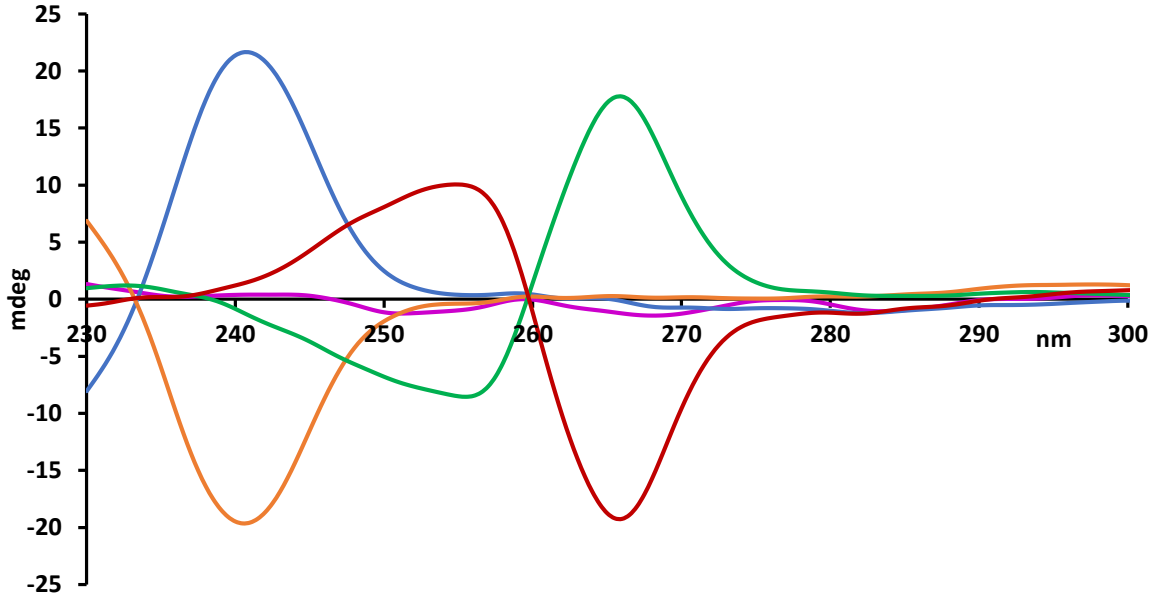

CD spectra of the assembly obtained with **M2** and (*R*)-**11** (blue) or (*S*)-**11** (orange) and **M3** and (*R*)-**11** (green) or (*S*)-**11** (red). A control experiment with (*R*)-**11** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



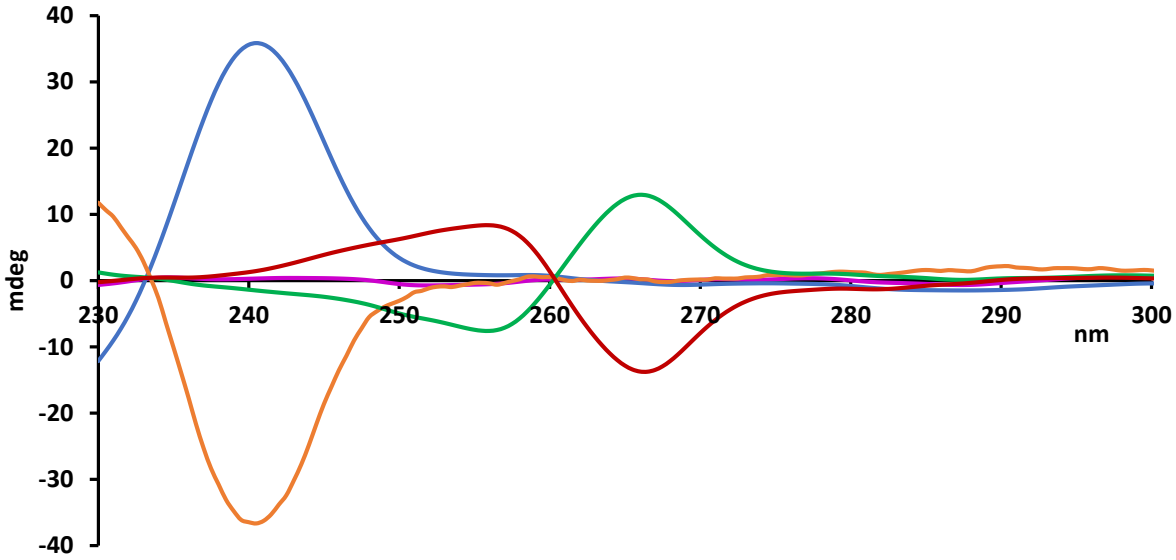
CD spectra of the assembly obtained with **M2** and (*R*)-**12** (blue) or (*S*)-**12** (orange) and **M3** and (*R*)-**12** (green) or (*S*)-**12** (red). A control experiment with (*R*)-**12** (purple) in the absence of sensor shows negligible CD effects in the region of interest.




CD spectra of the assembly obtained with **M2** and (*R,R*)-**13** (blue) or (*S,S*)-**13** (orange) and **M3** and (*R,R*)-**13** (green) or (*S,S*)-**13** (red). A control experiment with (*R,R*)-**13** (purple) in the absence of sensor shows negligible CD effects in the region of interest



CD spectra of the assembly obtained with **M2** and (*R,S*)-**14** (blue) or (*S,R*)-**14 (**orange) and **M3** and (*R,S*)-**14** (green) or (*S,R*)-**14** (red). A control experiment with (*R,S*)-**14** (purple) in the absence of sensor shows negligible CD effects in the region of interest

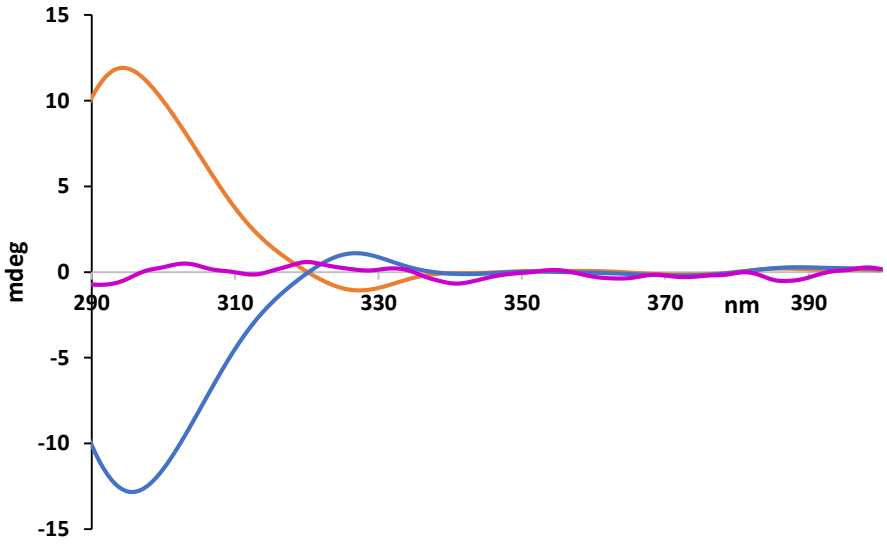


## 4. Enantioselective sensing with M2

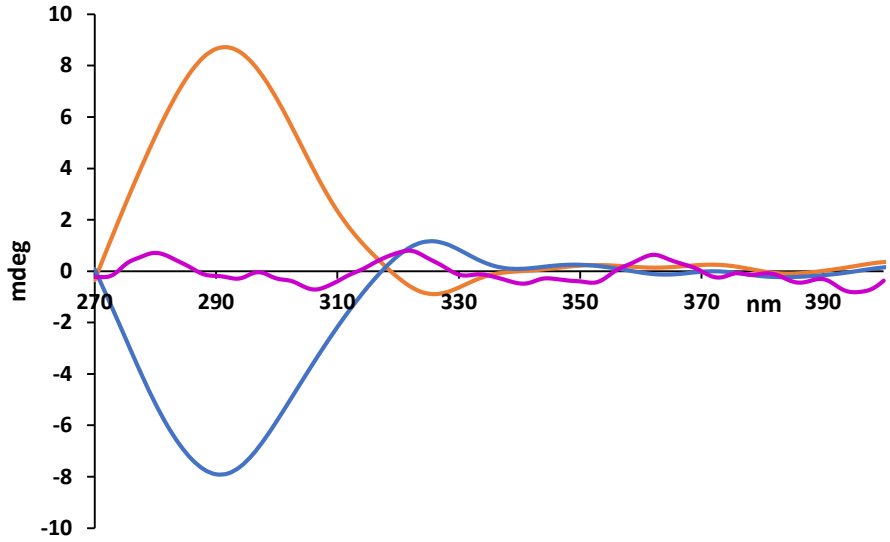
### 4.1 Sensing of amino acids

A stock solution of **M2** (1.0 mM) was prepared in deionized $H_2O$ and 0.1 mL portions were placed into 4 mL vials followed by addition of 0.9 mL of deionized $H_2O$. In separate vials, stock solutions of the analytes (1.0 mM in $H_2O$) were prepared. To each vial containing 1.0 mL of **M2** were added 10 equivalents (1.0 mL) of the analytes. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted as described above after dilution to 50.0 μM with a quartz cuvette that has a 10 mm path length.

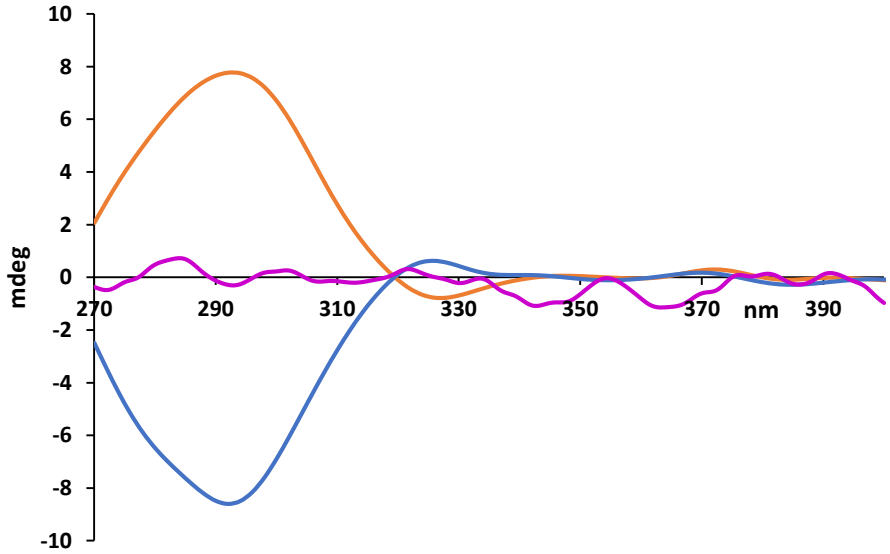
CD spectra of the assembly obtained from **M2** and (*R*)-**1** (blue) or (*S*)-**1** (orange). A control experiment with (*R*)-**1** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



CD spectra of the assembly obtained from **M2** and (*R*)-**2** (blue) or (*S*)-**2** (orange). A control experiment with (*R*)-**2** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



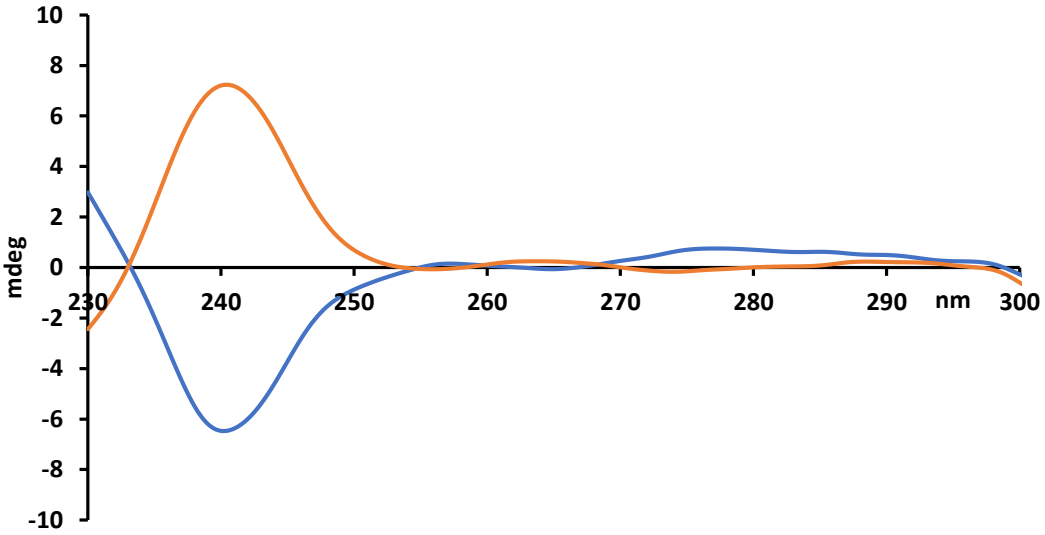

CD spectra of the assembly obtained from **M2** and (*R*)-**3** (blue) or (*S*)-**3** (orange). A control experiment with (*R*)-**3** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



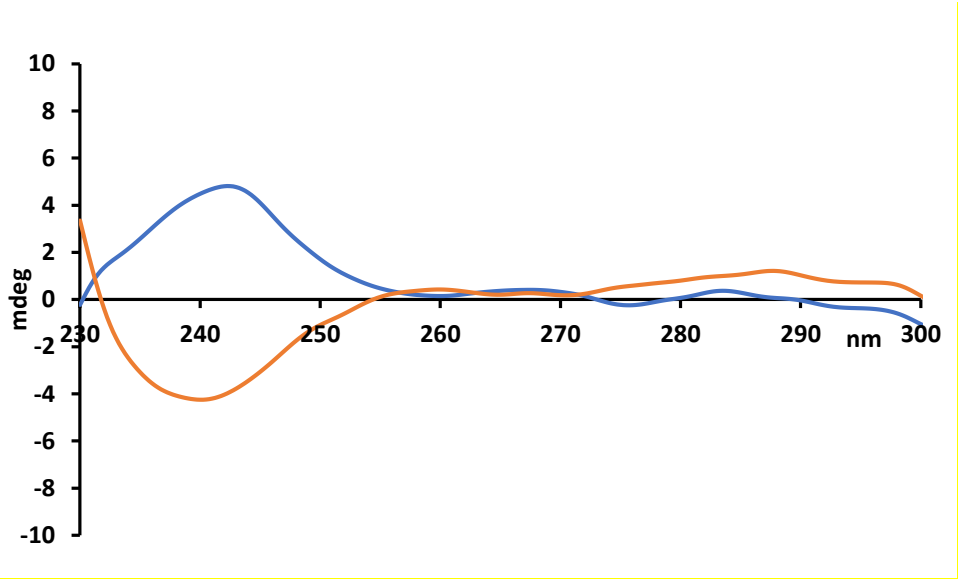
### 4.2 Sensing of amines and amino alcohols

A stock solution of **M2** (1.0 mM) was prepared in deionized $H_2O$ and 0.2 mL portions were placed into 4 mL vials followed by addition of 0.8 mL of deionized $H_2O$. In separate vials, stock solutions of the analytes (0.2 mM in $H_2O$) were prepared. To each vial containing 0.5 mL of **M2** was added 1 equivalent (0.5 mL) of the analyte. For amines **4**-**14**, an equimolar amount of HCl (1.0 M) was added to generate the ammonium salts. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted as described above at 5.0 μM with a quartz cuvette that has a 10 mm path length unless noted otherwise.

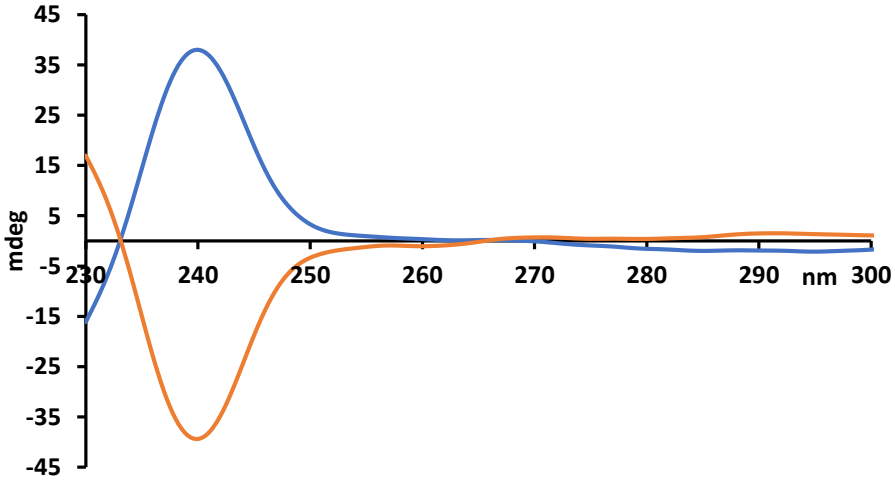
CD spectra of the assembly obtained from **M2** (*R*)-**4** (blue) or (*S*)-**4** (orange).



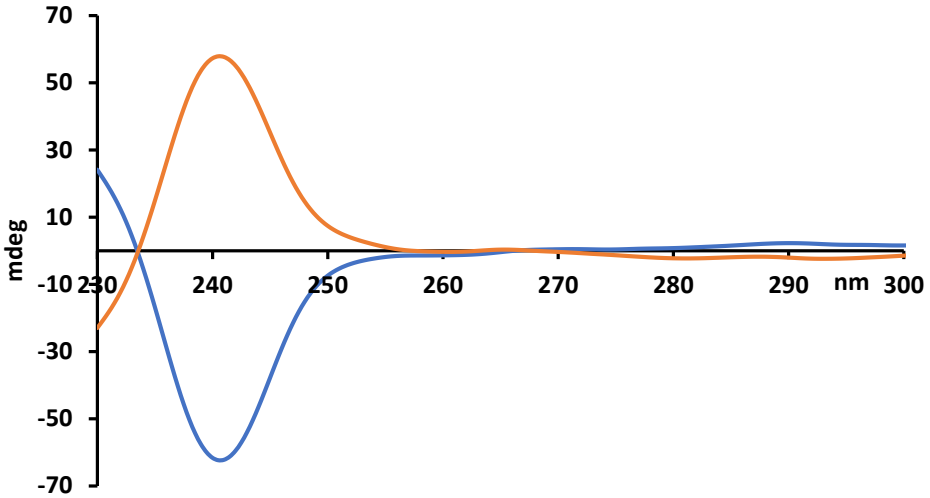
CD spectra of the assembly obtained from **M2** and (*R*)-**5** (blue) or (*S*)-**5** (orange).



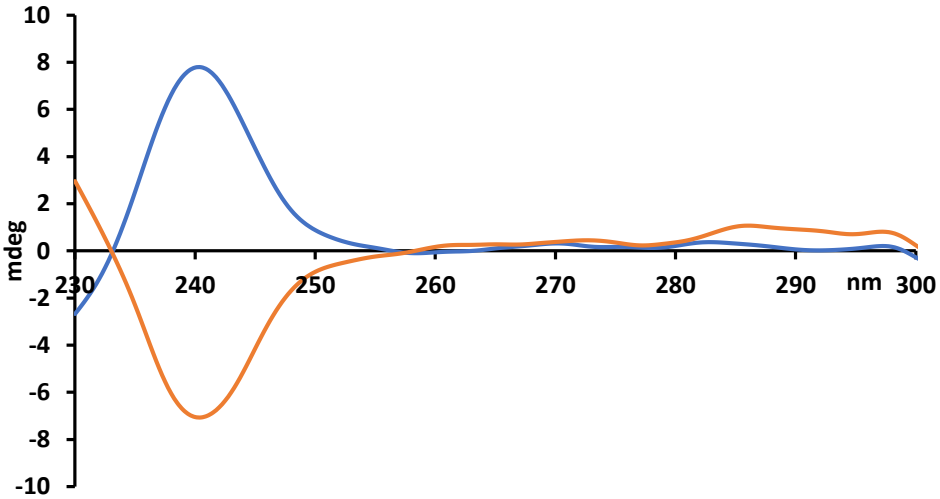

CD spectra of the assembly obtained from **M2** and (*R*)-**6** (blue) or (*S*)-**6** (orange).



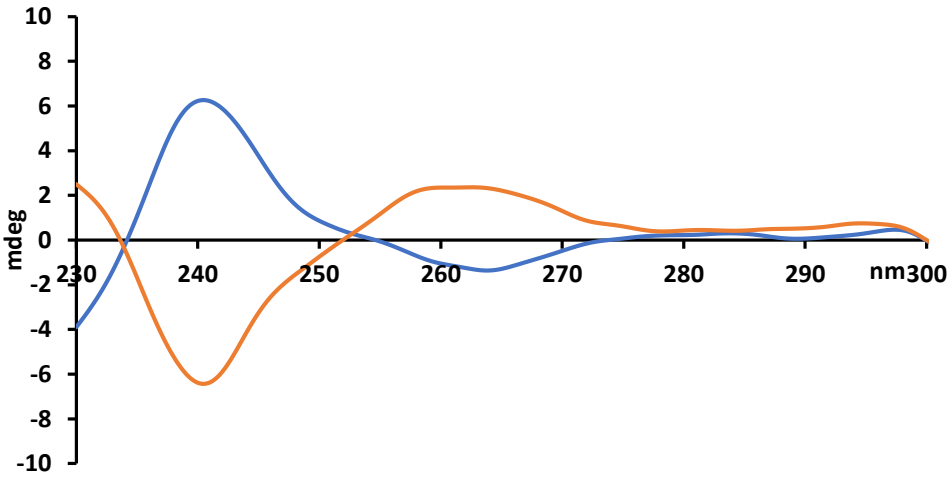
CD spectra of the assembly obtained from **M2** and (*R*)-**7** (blue) or (*S*)-**7** (orange).



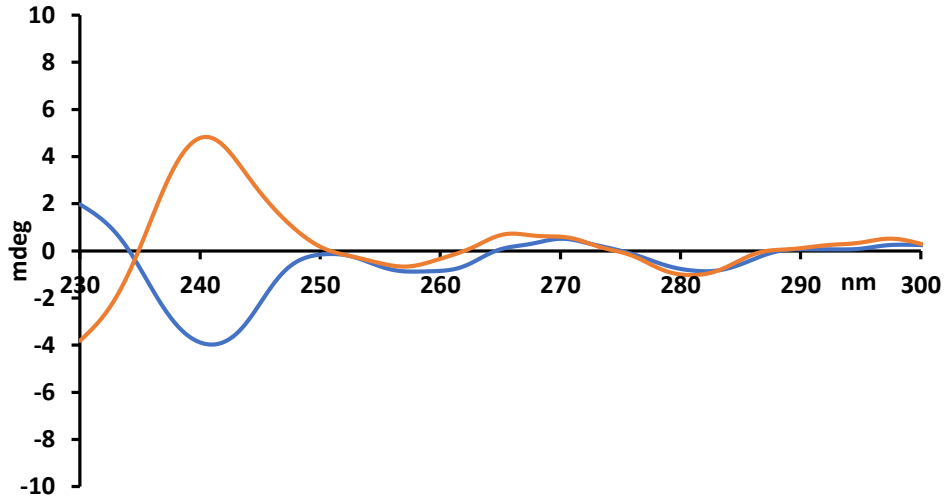

CD spectra of the assembly obtained from **M2** and (*R*)-**8** (blue) or (*S*)-**8** (orange).



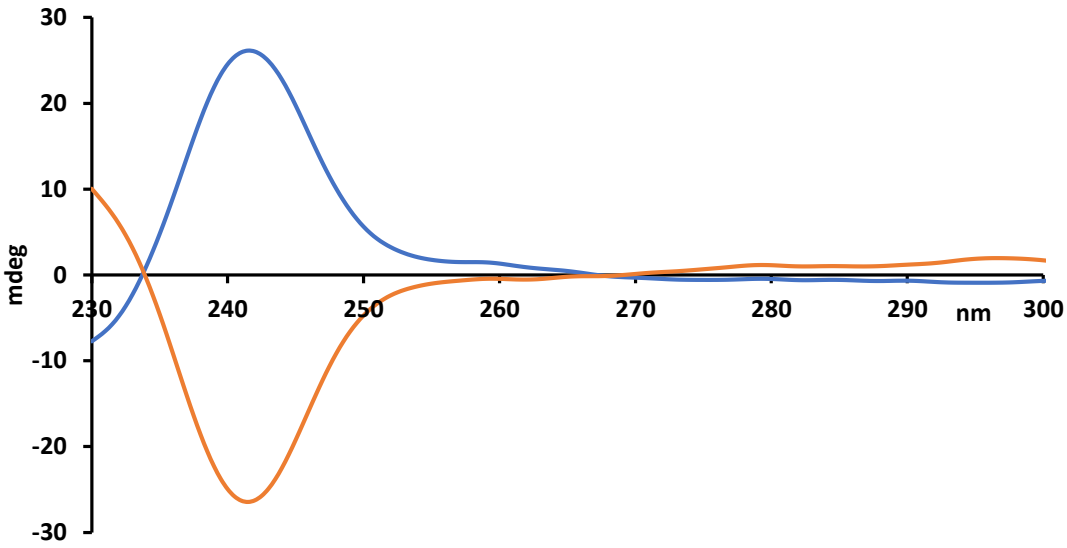
CD spectra of the assembly obtained from **M2** and (*R*)-**9** (blue) or (*S*)-**9** (orange).



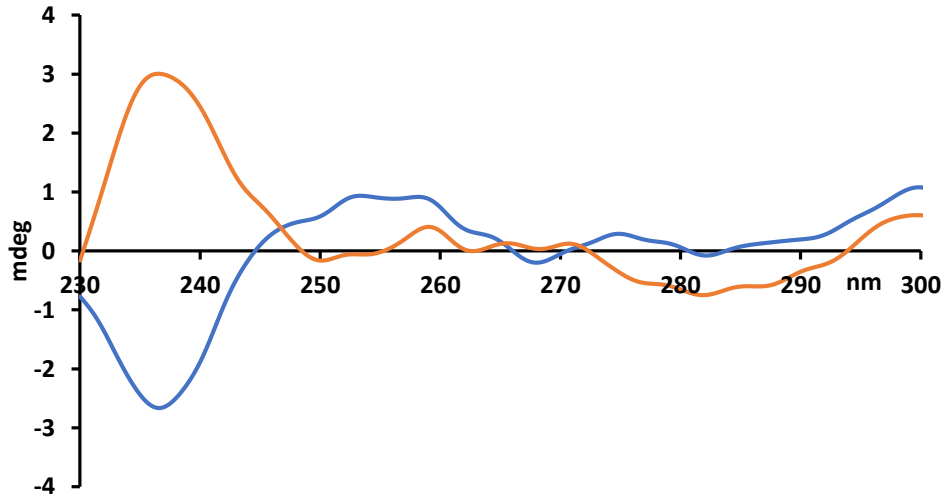

CD spectra of the assembly obtained from **M2** and (*R*)-**10** (blue) or (*S*)-**10** (orange).



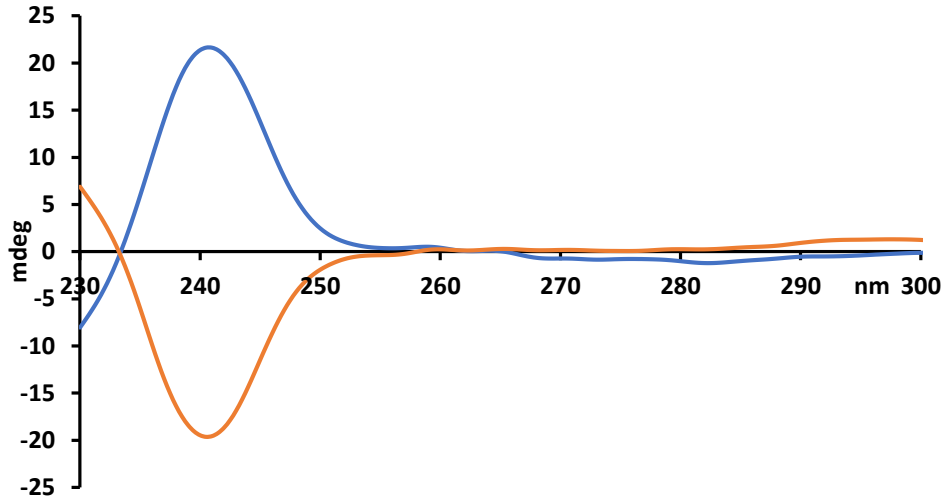
CD spectra of the assembly obtained from **M2** and (*R*)-**11** (blue) or (*S*)-**11** (orange).



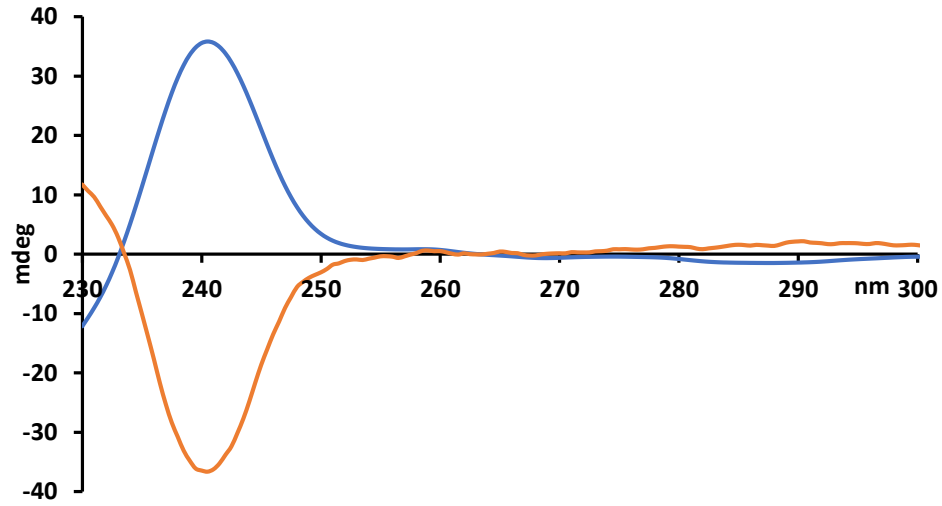

CD spectra of the assembly obtained from **M2** and (*R*)-**12** (blue) or (*S*)-**12** (orange).



CD spectra of the assembly obtained from **M2** and (*R,R*)-**13** (blue) or (*S,S*)-**13** (orange).

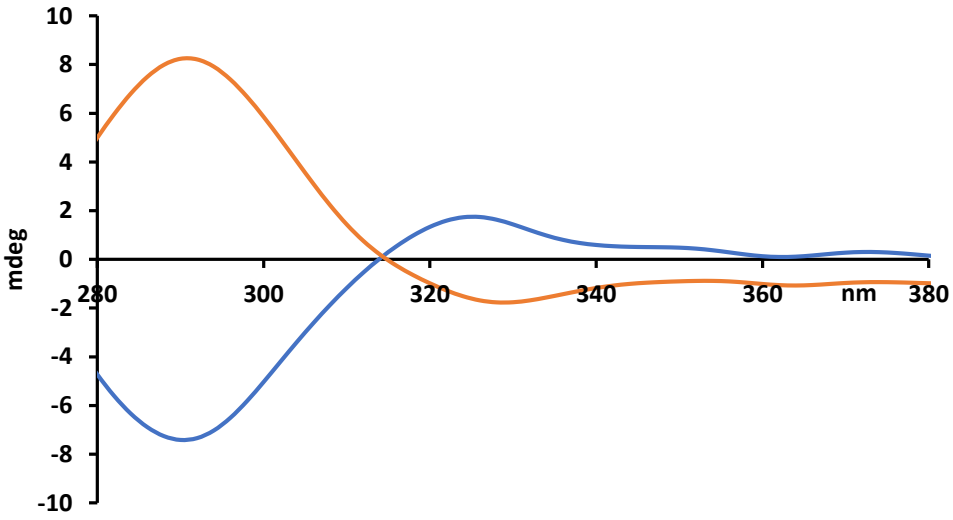



CD spectra of the assembly obtained from **M2** and (*R,S*)-**14** (blue) or (*S,R*)-**14** (orange).

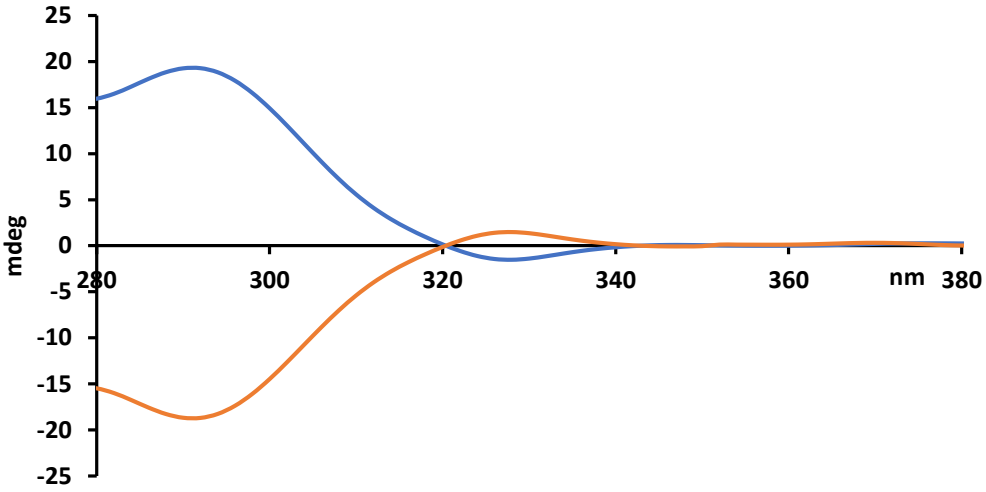


When selected mixtures were prepared as described above but subjected to CD analysis at concentrations between 30.0 and 50.0 µM we observed new CD signals at higher wavelengths and a maximum at approximately 290 nm. These results are in agreement with the chiroptical amino acid sensing conducted with **M2** and **1**-**3** at 50.0 µM, see Section 4.1. At these concentrations strong UV absorption at lower wavelengths occurs which precludes recording of the CD signals at 240 nm.

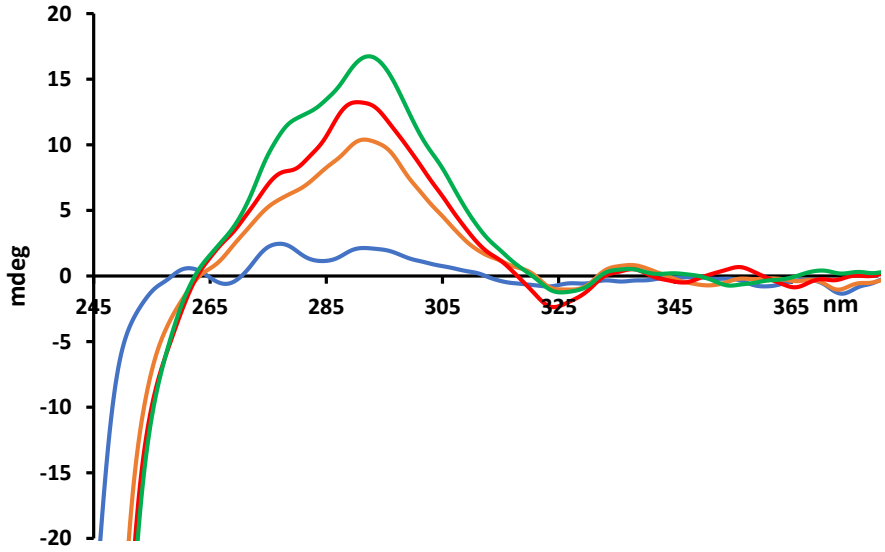
CD spectra of the assembly obtained from **M2** and (*R*)-**6** (blue) or (*S*)-**6** (orange) measured at 50 µM.



CD spectra of the assembly obtained from **M2** and (*R*)-**7** (blue) or (*S*)-**7** (orange) measured at 50 μM.




CD spectra of the assembly obtained from **M2** and (*R*)-**7** at 5.0 (blue), 30 (orange), 40 (red) and 50 (green) μM.

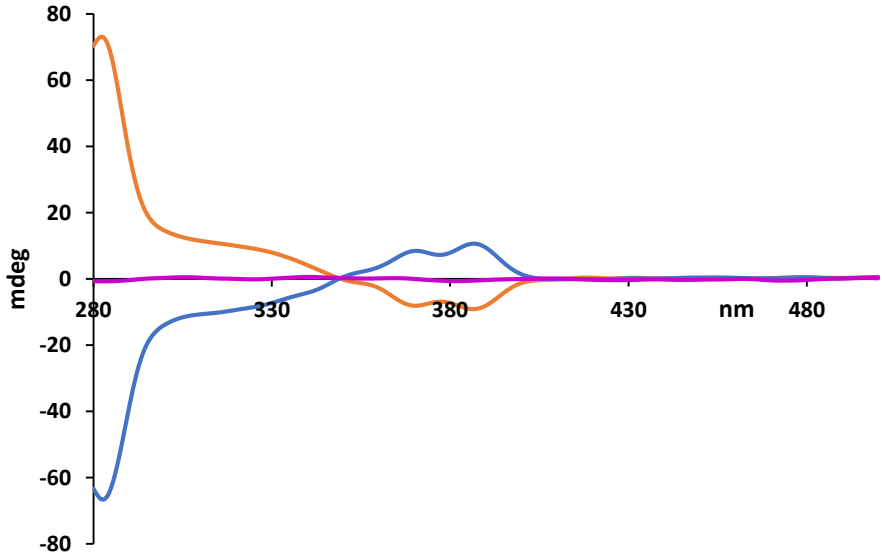


## 5. Enantioselective sensing with M3

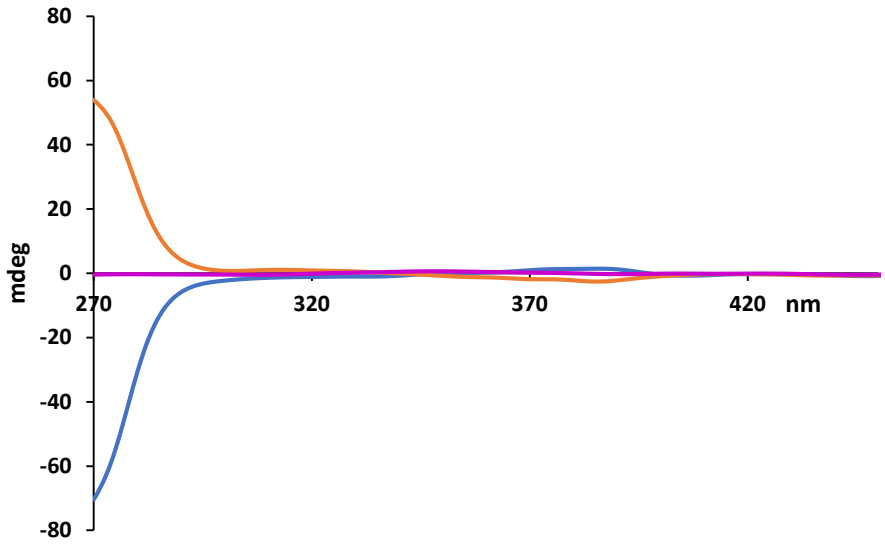
### 5.1 Sensing of amino acids

A stock solution of **M3** (1.0 mM) was prepared in deionized $H_2O$ and 0.25 mL portions were placed into 4 mL vials. In separate vials, stock solutions of the analytes (10.0 mM in $H_2O$) were prepared. To each vial containing 0.25 mL of **M3** was added 10 equivalents (0.25 mL) of the analyte. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted as described above at 0.5 mM with a quartz cuvette that has a 1 mm path length.

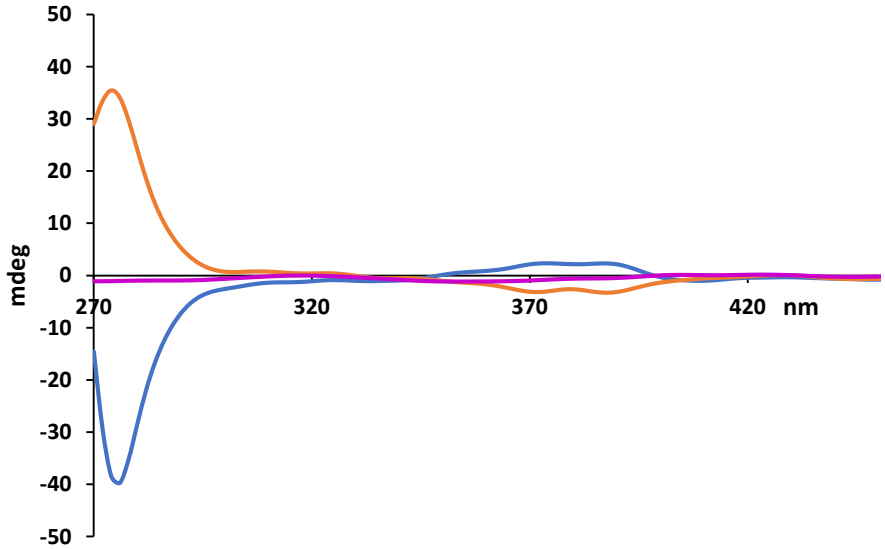
CD spectra of the assembly obtained from **M3** and (*R*)-**1** (blue) or (*S*)-**1** (orange). A control experiment with (*R*)-**1** (purple) in the absence of sensor shows negligible CD effects in the region of interest.



CD spectra of the assembly obtained from **M3** and (*R*)-**2** (blue) or (*S*)-**2** (orange). A control experiment with (*R*)-**2** (purple) in the absence of sensor shows negligible CD effects in the region of interest.




CD spectra of the assembly obtained from **M3** and (*R*)-**3** (blue) or (*S*)-**3** (orange). A control experiment with (*R*)-**3** (purple) in the absence of sensor shows negligible CD effects in the region of interest.

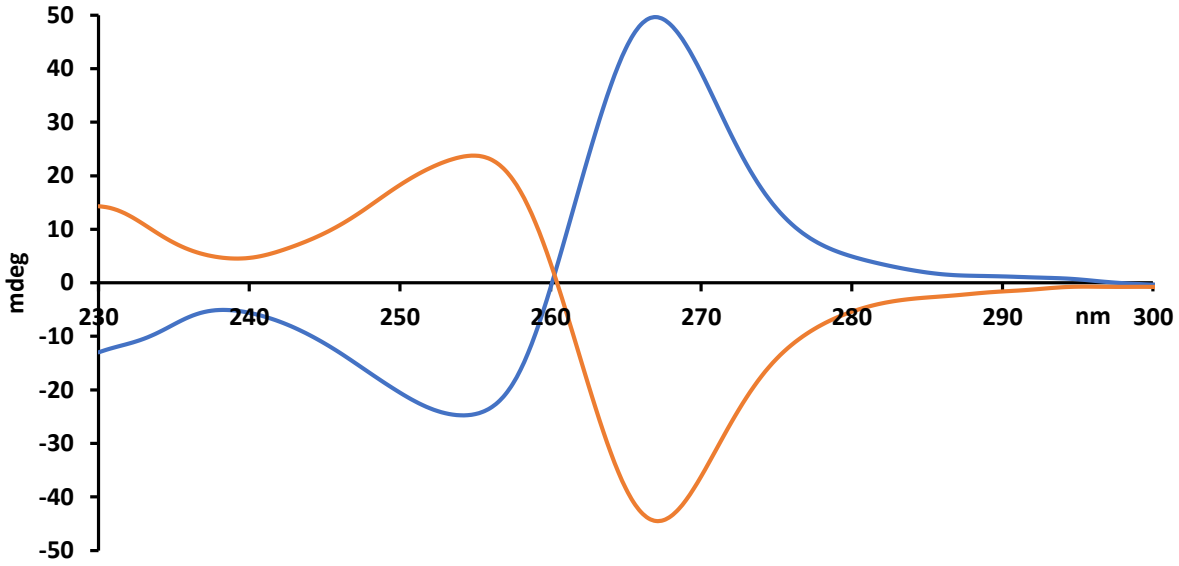


### 5.2 Sensing of amines, amino alcohols, alcohol and terpenes with M3

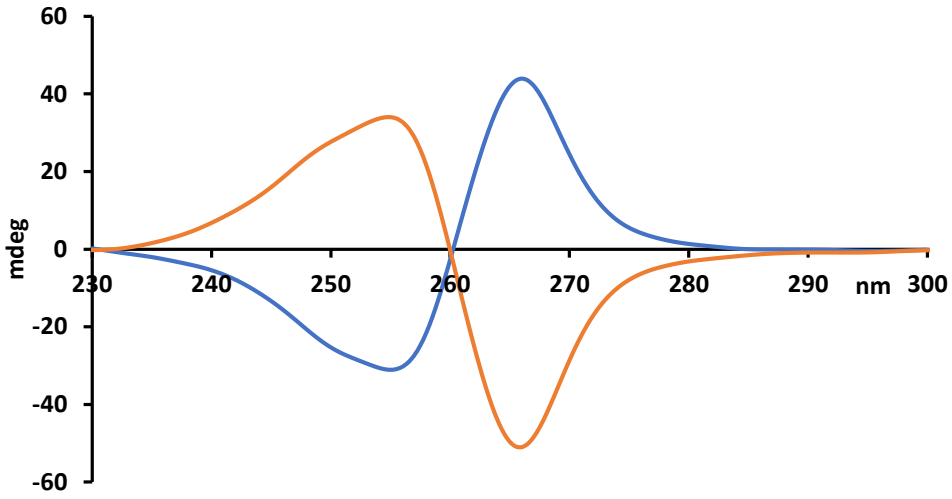
A stock solution of **M3** (1.0 mM) was prepared in deionized $H_2O$ and 0.2 mL portions were placed into 4 mL vials followed by addition of 0.8 mL of $H_2O$. In separate vials, stock solutions of the analytes (0.2 mM in $H_2O$) were prepared. To each vial containing 0.5 mL of **M2** was added 1 equivalent (0.5 mL) of the analyte. For amines **4**-**14**, an equimolar amount of HCl (1.0 M) was added to generate the ammonium salts. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted as described above at 5.0 μM with a quartz cuvette that has a 10 mm path length.

CD spectra of the assembly obtained from **M3** and (*R*)-**4** (blue) or (*S*)-**4** (orange)

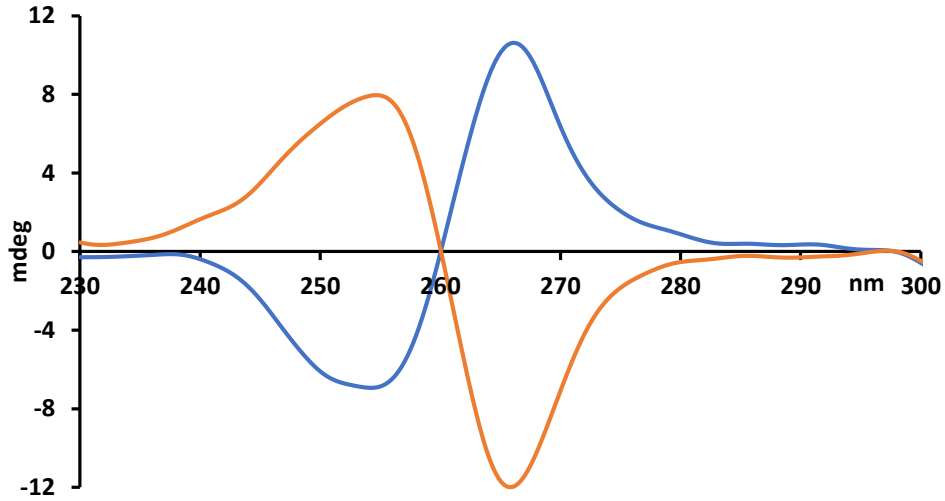
CD spectra of the assembly obtained from **M3** and (*R*)-**5** (blue) or (*S*)-**5** (orange)



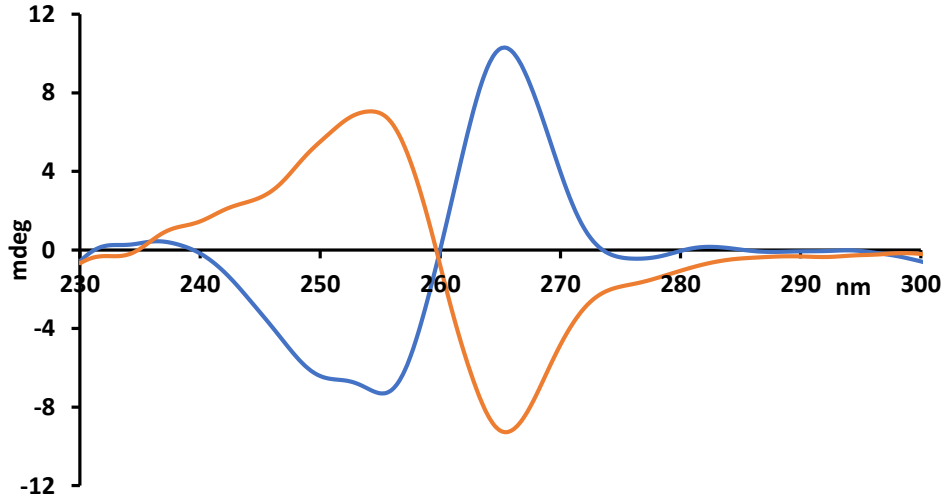

CD spectra of the assembly obtained from **M3** and (*R*)-**6** (blue) or (*S*)-**6** (orange)



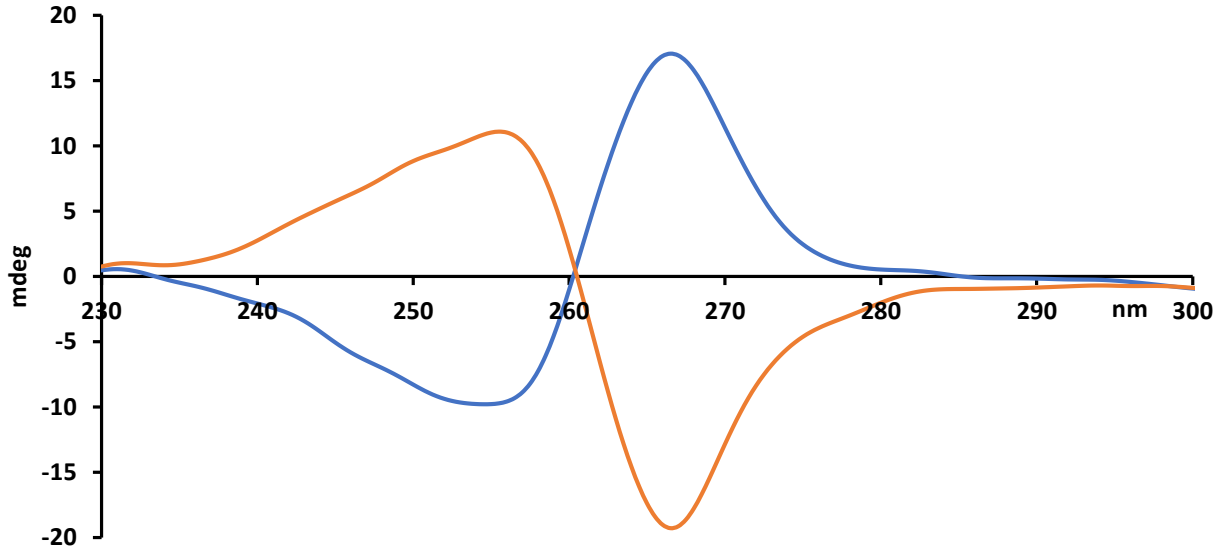
CD spectra of the assembly obtained from **M3** and (*R*)-**8** (blue) or (*S*)-**8** (orange)



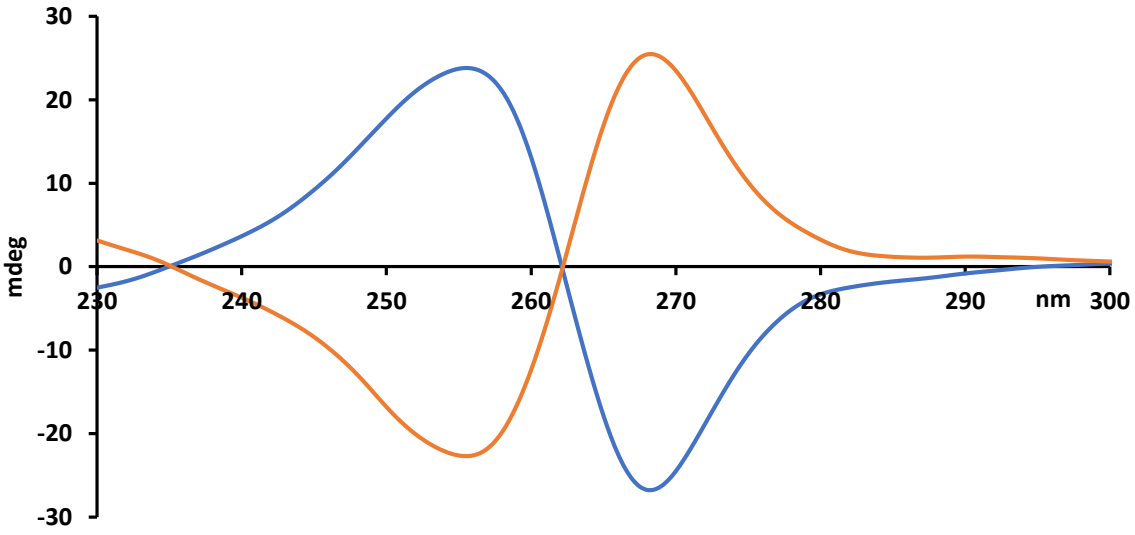

CD spectra of the assembly obtained from **M3** and (*R*)-**9** (blue) or (*S*)-**9** (orange)



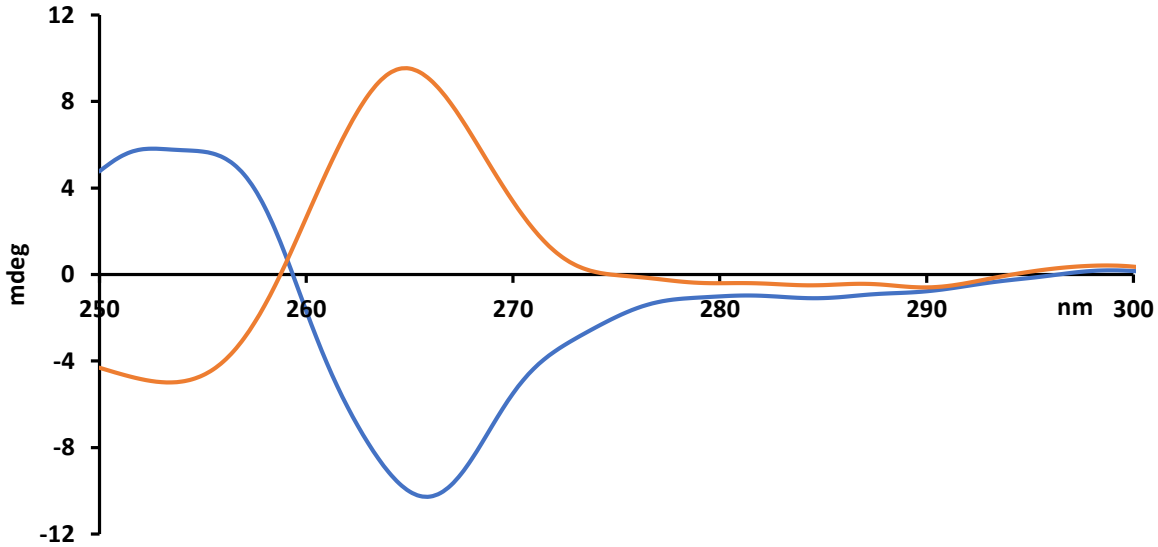
CD spectra of the assembly obtained from **M3** and (*R*)-**10** (blue) or (*S*)-**10** (orange)



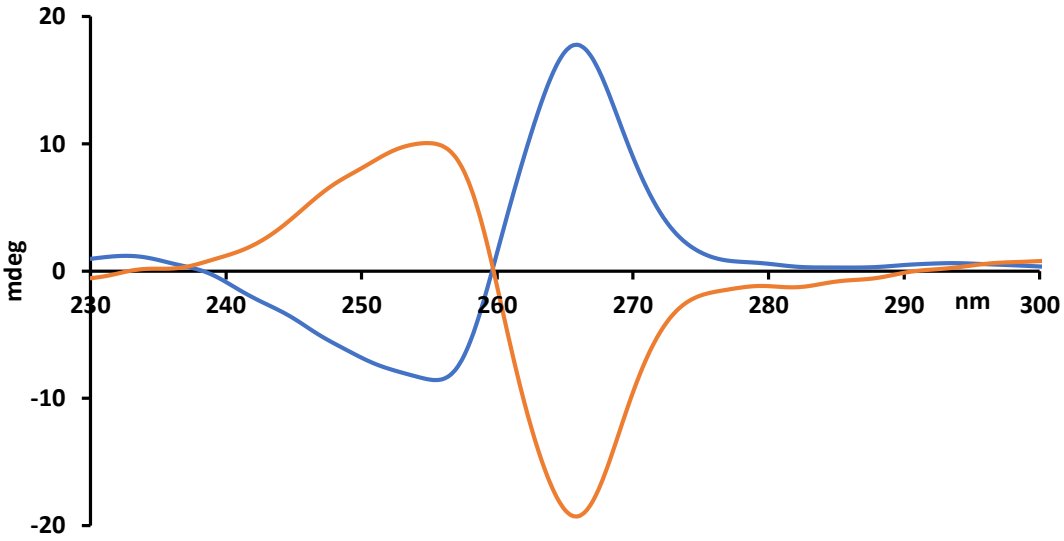

CD spectra of the assembly obtained from **M3** and (*R*)-**11** (blue) or (*S*)-**11** (orange)



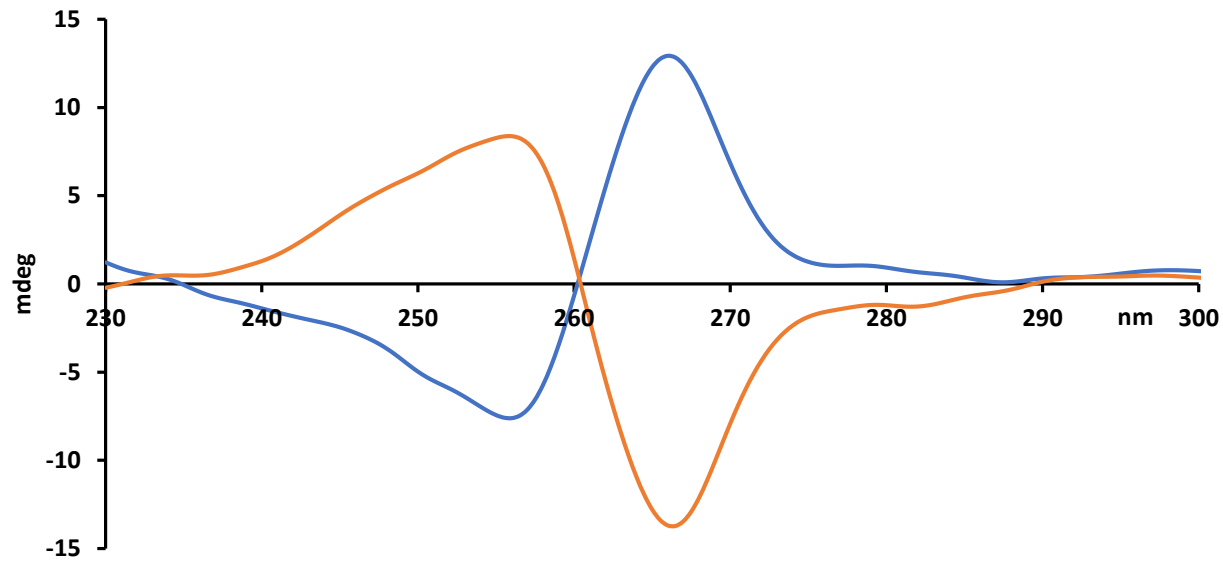
CD spectra of the assembly obtained from **M3** and (*R*)-**12** (blue) or (*S*)-**12** (orange)



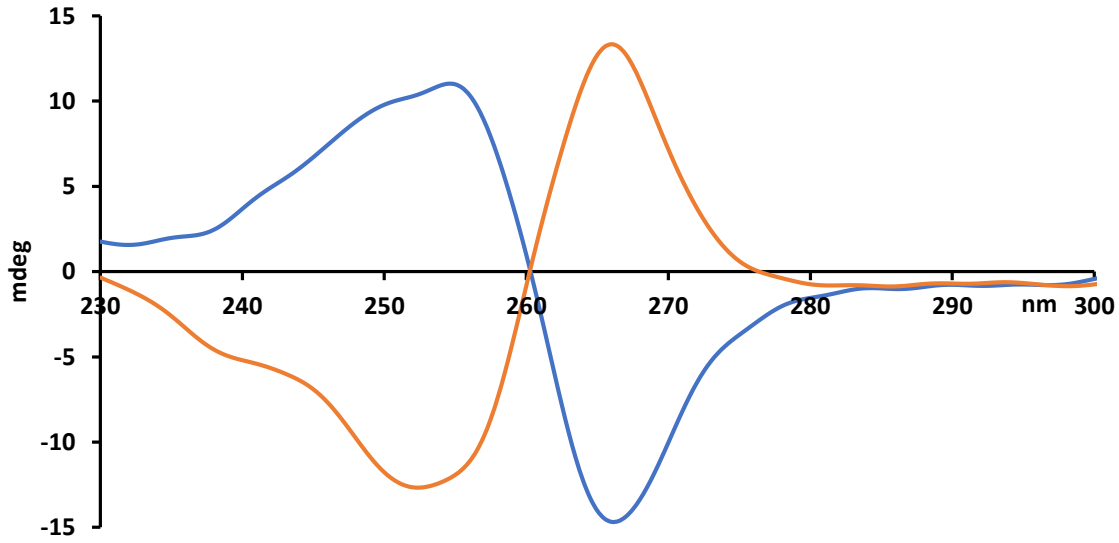

CD spectra of the assembly obtained from **M3** and (*R,R*)-**13** (blue) or (*S,S*)-**13** (orange)



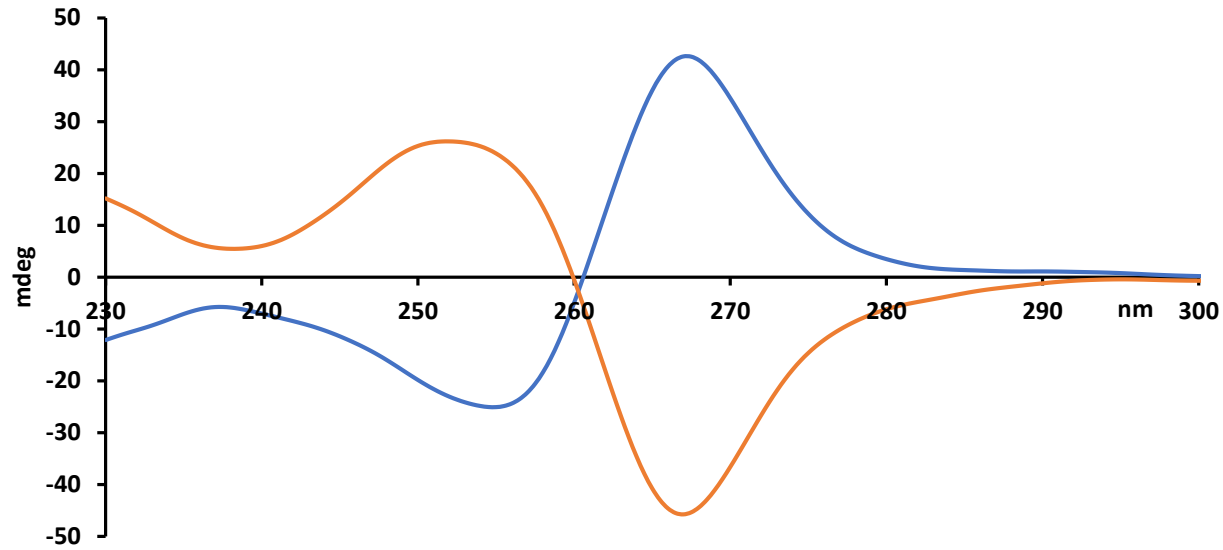
CD spectra of the assembly obtained from **M3** and (*R*,*S*)-**14** (blue) or (*S*,*R*)-**14** (orange)



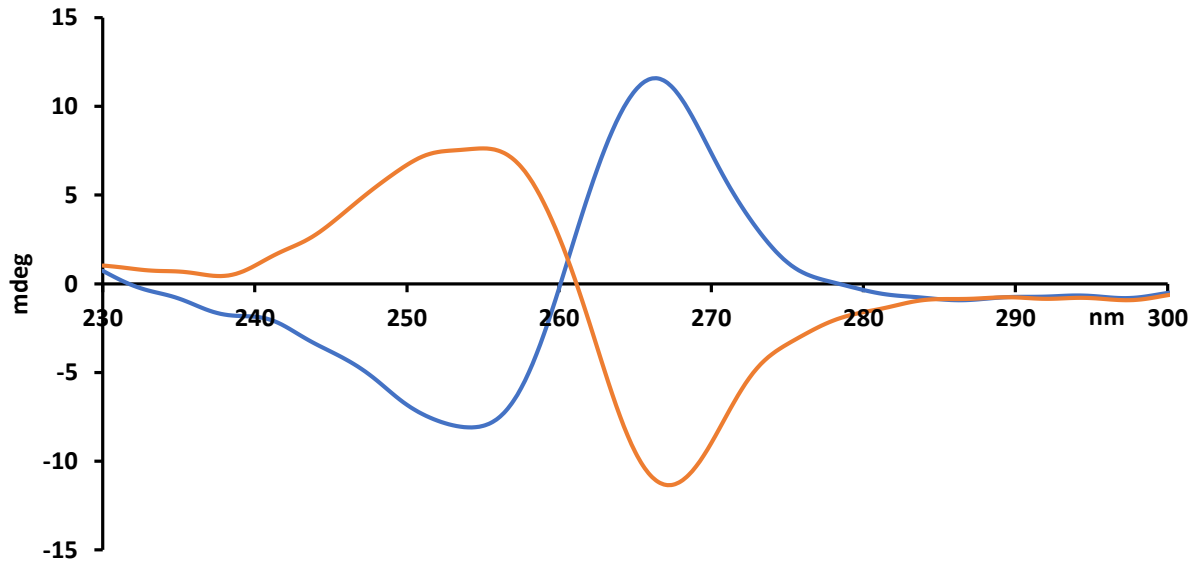

CD spectra of the assembly obtained from **M3** and (*R*,*S*)-**15** (blue) or (*S*,*R*)-**15** (orange)



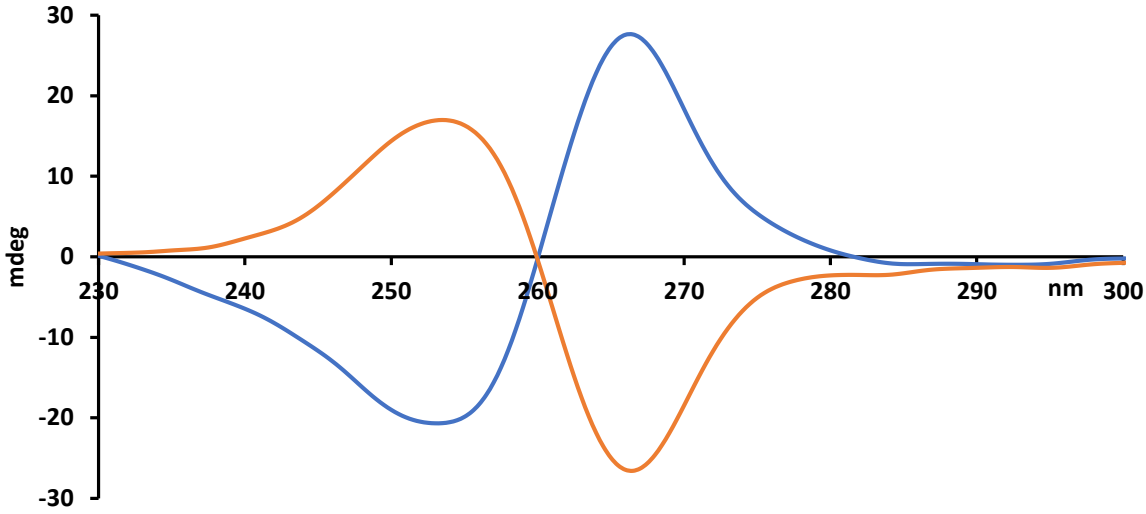
CD spectra of the assembly obtained from **M3** and (*R*)-**16** (blue) or (*S*)-**16** (orange)



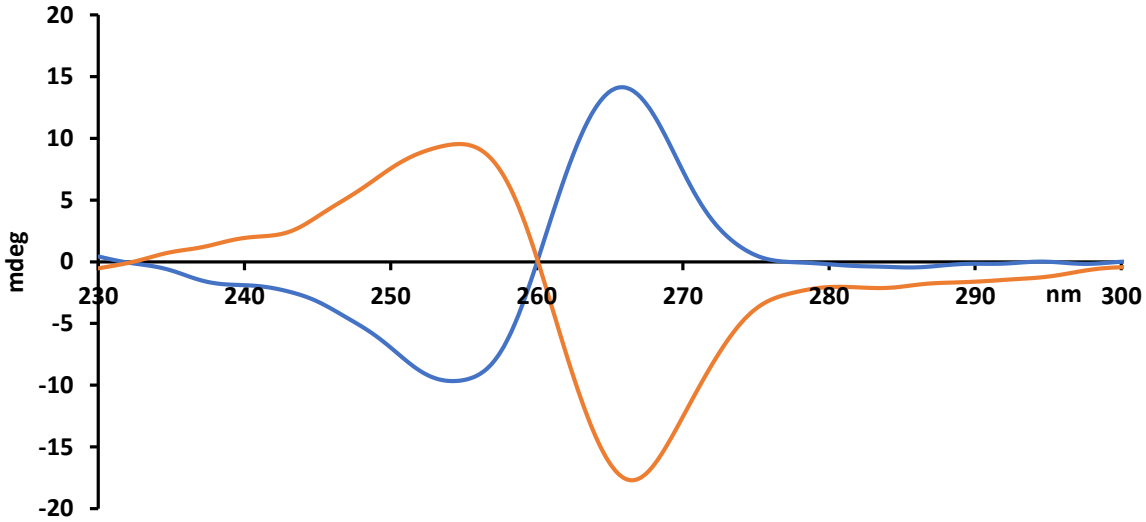

CD spectra of the assembly obtained from **M3** and (*R*)-**17** (blue) or (*S*)-**17** (orange)



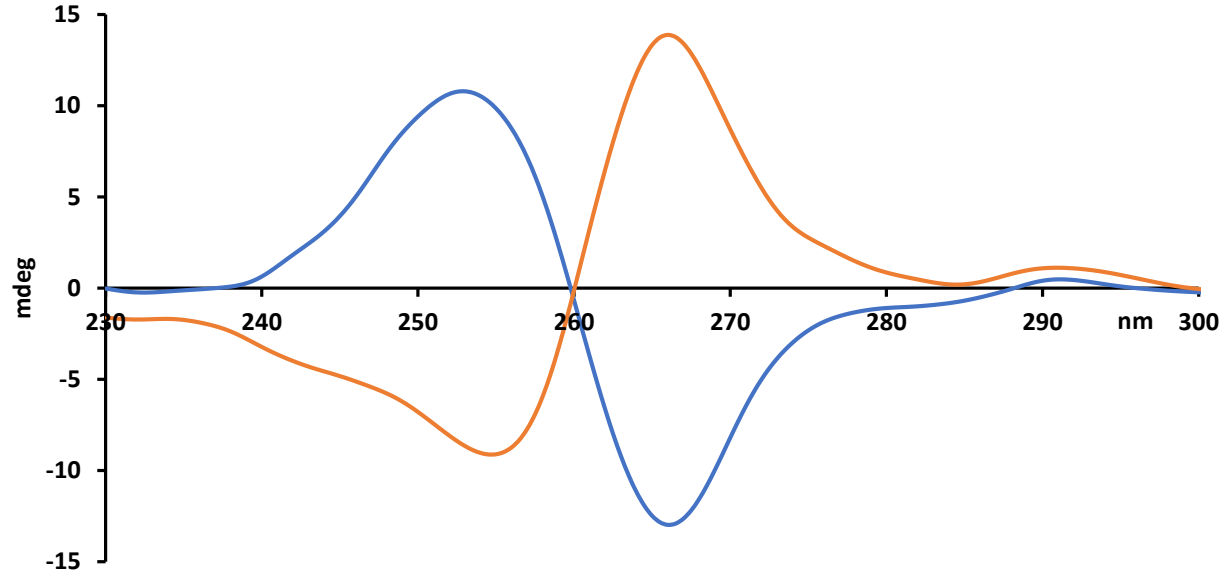
CD spectra of the assembly obtained from **M3** and (+)-**18** (blue) or (-)-**18** (orange)



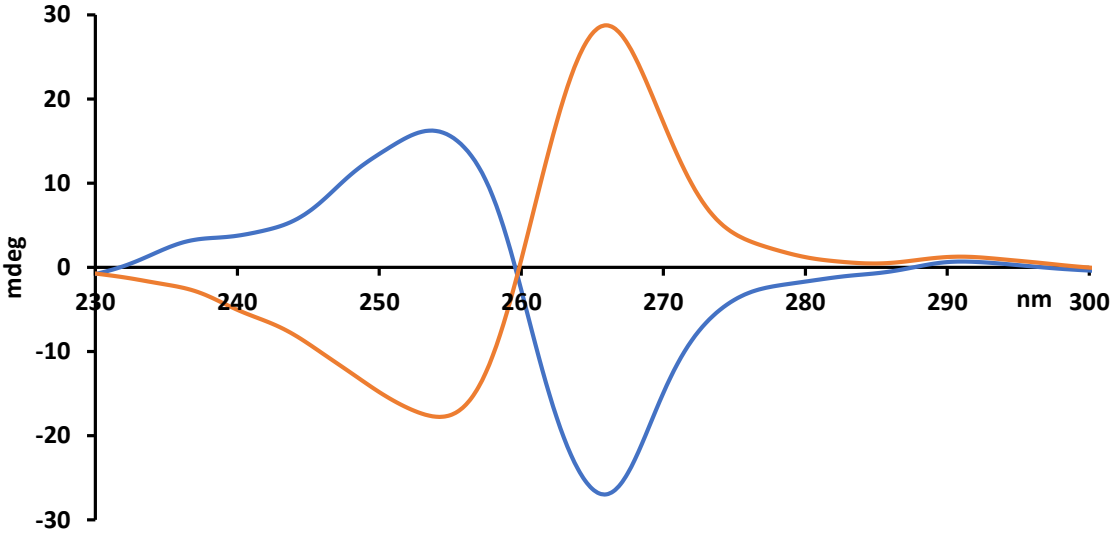

CD spectra of the assembly obtained from **M3** and (*R*)-**19** (blue) or (*S*)-**19** (orange)



CD spectra of the assembly obtained from **M3** and (+)-**20** (blue) or (-)-**20** (orange)

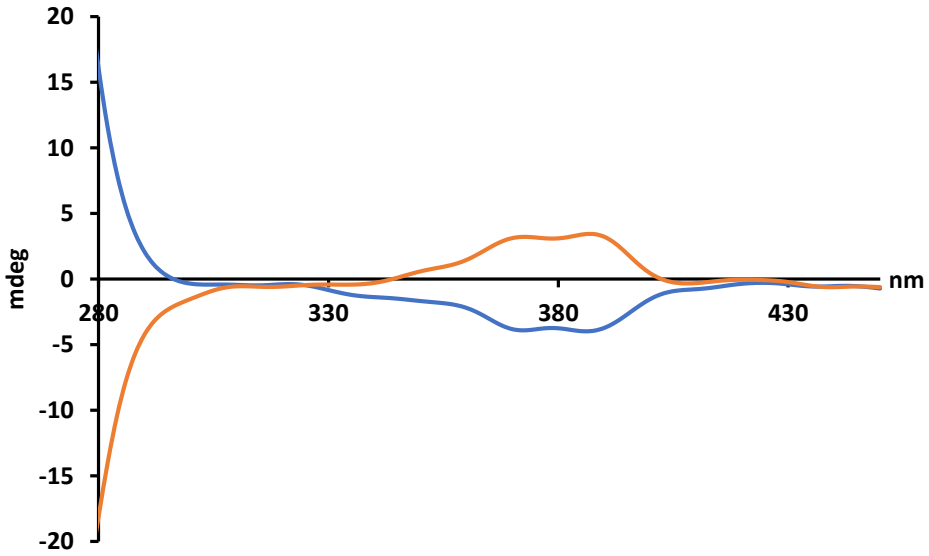



CD spectra of the assembly obtained from **M3** and (+)-**21** (blue) or (-)-**21** (orange)



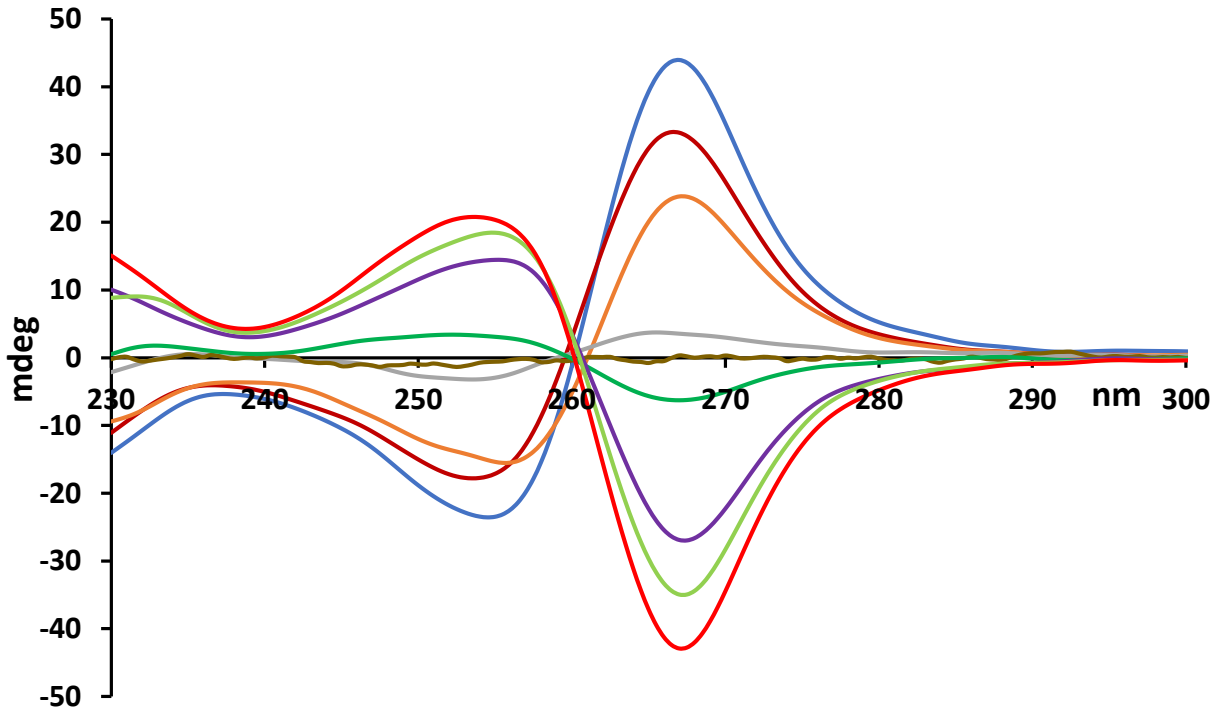
When mixtures were prepared as described above but subjected to CD analysis at 50.0 μM we observed CD signals at approximately 380 nm. The strong UV absorption at lower wavelengths under these conditions precludes simultaneous CD analysis of the signals below 280 nm.

CD spectra of the assembly obtained from **M3** and (*R*)-**6** (blue) or (*S*)-**6** (orange) measured at 50 μM.

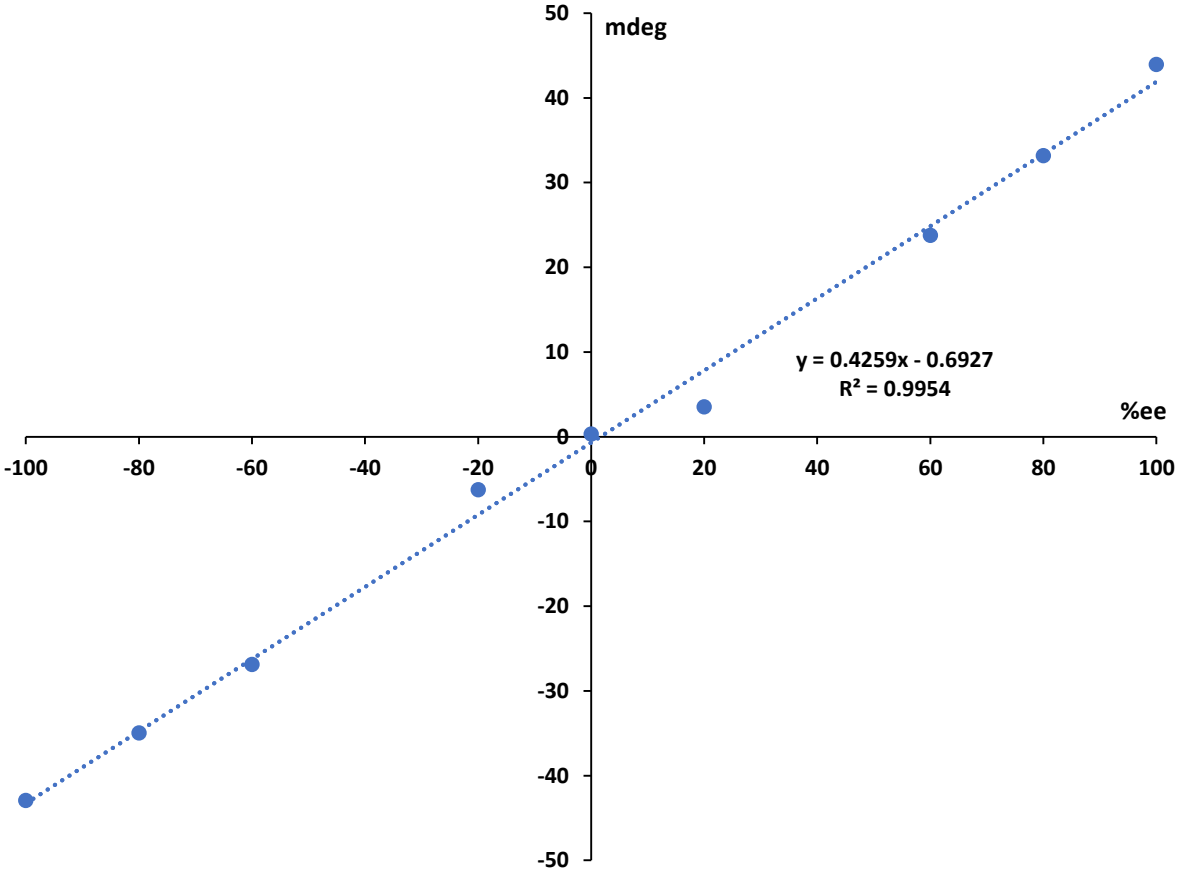


## 6. Determination of the enantiomeric purity of 5

A stock solution of **M3** (0.2 mM) was prepared in deionized $H_2O$. Stock solutions of **5** (0.2 mM in $H_2O$) at varying *ee* compositions (+100.0, +80.0, +60.0, +20.0, 0.0, -20.0, -60.0, -80.0, -100.0) were prepared by mixing enantiopure (*R*)- or (*S*)-enantiomers at various ratios. An equimolar amount of HCl (1.0 M) was added to **5** to generate the ammonium salt. To each vial containing 0.5 mL of **M3** was added 1 equivalent (0.5 mL) of the analyte. The mixtures were stirred for 15 minutes at 25 °C and CD analysis was conducted as described above at 5.0 μM with a quartz cuvette that has a 10 mm path length. The CD analysis was carried out as described above. The CD amplitudes measured at 268 nm were plotted against %*ee* of the analyte.



Linear relationship between the CD amplitudes at 268 nm and the *ee* of **5**



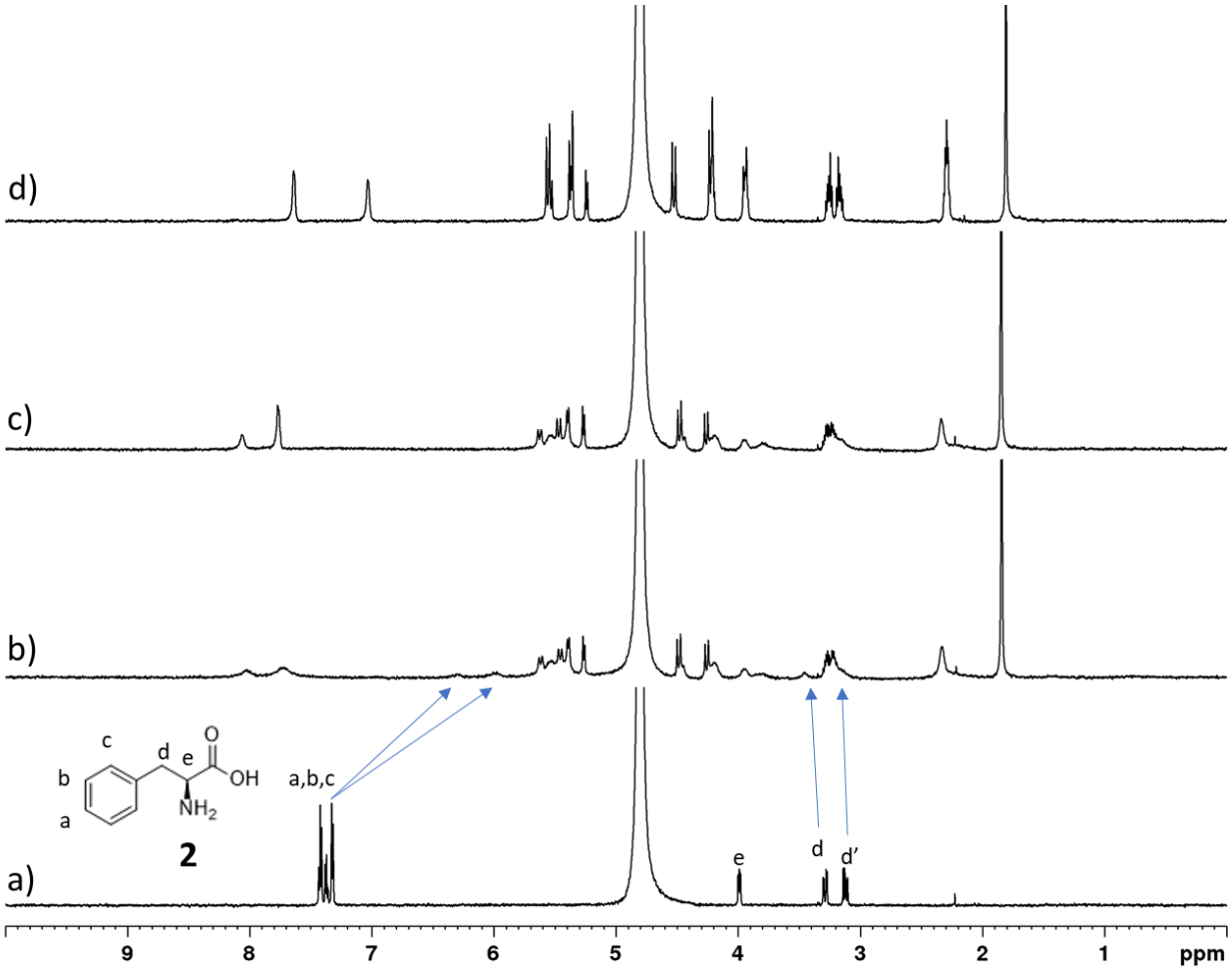

Six scalemic samples of **5** were prepared and then treated with **M3** as described above. Using the linear regression equation from the calibration curve and the measured CD amplitudes at 268 nm, the enantiomeric ratio and the absolute configuration of the major enantiomer were determined.

| Sample composition | | | Chiroptical sensing results | |
|---|---|---|---|---|
| **Entry** | **Absolute config.** | ***er* (268 nm) (*R*) : (*S*)** | **Absolute config.[a]** | ***er* [b] (268 nm) (*R*) : (*S*)** |
| 1 | (*S*) | 30.0 : 70.0 | (*S*) | 26.2 : 73.7 |
| 2 | (*R*) | 95.0 : 5.0 | (*R*) | 95.7 : 4.3 |
| 3 | (*R*) | 85.0 : 15.0 | (*R*) | 82.1 : 17.9 |
| 4 | (*S*) | 45.0 : 55.0 | (*S*) | 48.4 : 51.6 |
| 5 | (*S*) | 25.0 : 75.0 | (*S*) | 24.1 : 75.9 |
| 6 | (*S*) | 35.0 : 65.0 | (*S*) | 39.8 : 60.2 |

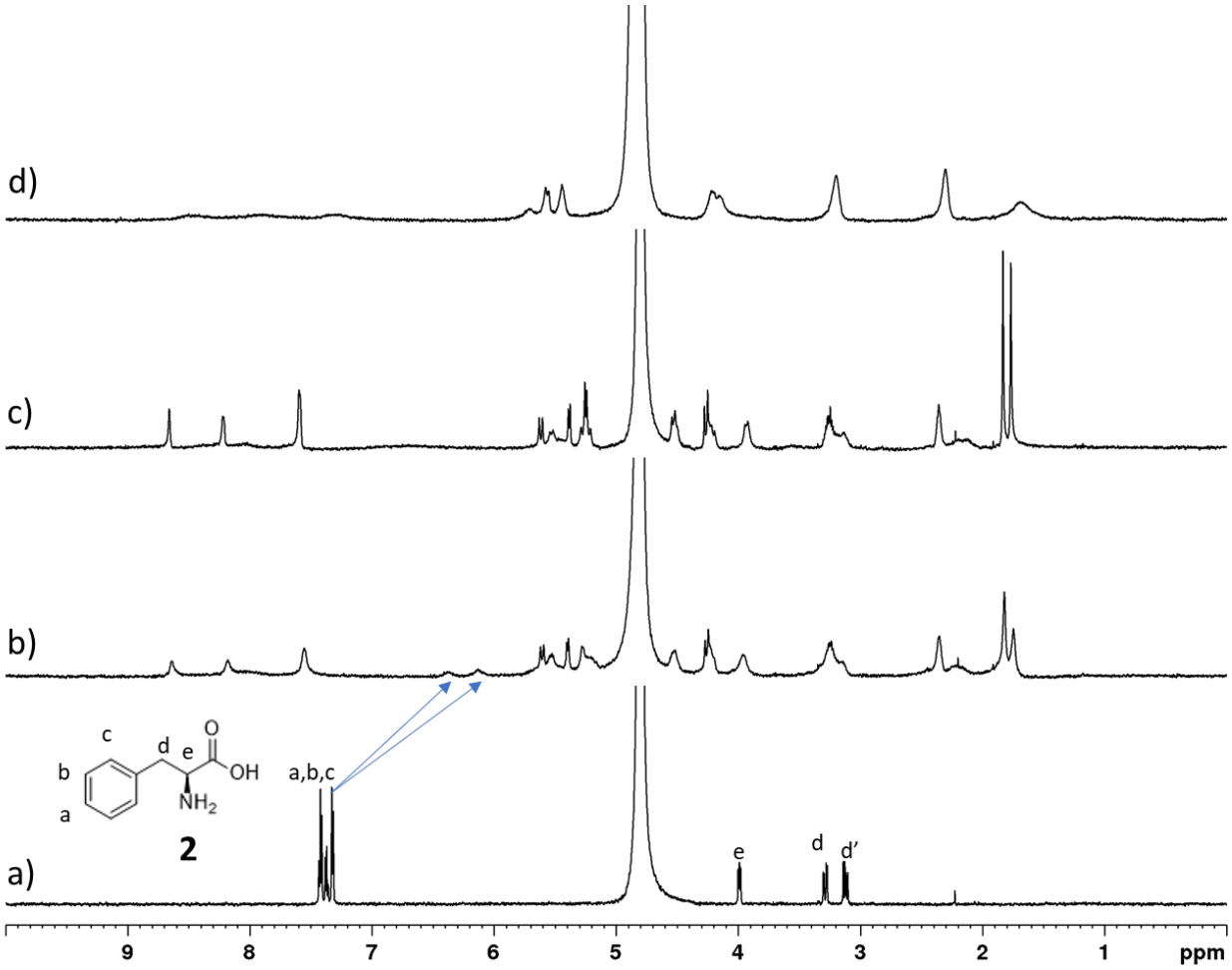
[a]Based on the sign of CD response. [b]Based on the amplitude of the CD response.

## 7. $^1$H NMR study of the interactions between selected guests and M2 and M3, respectively

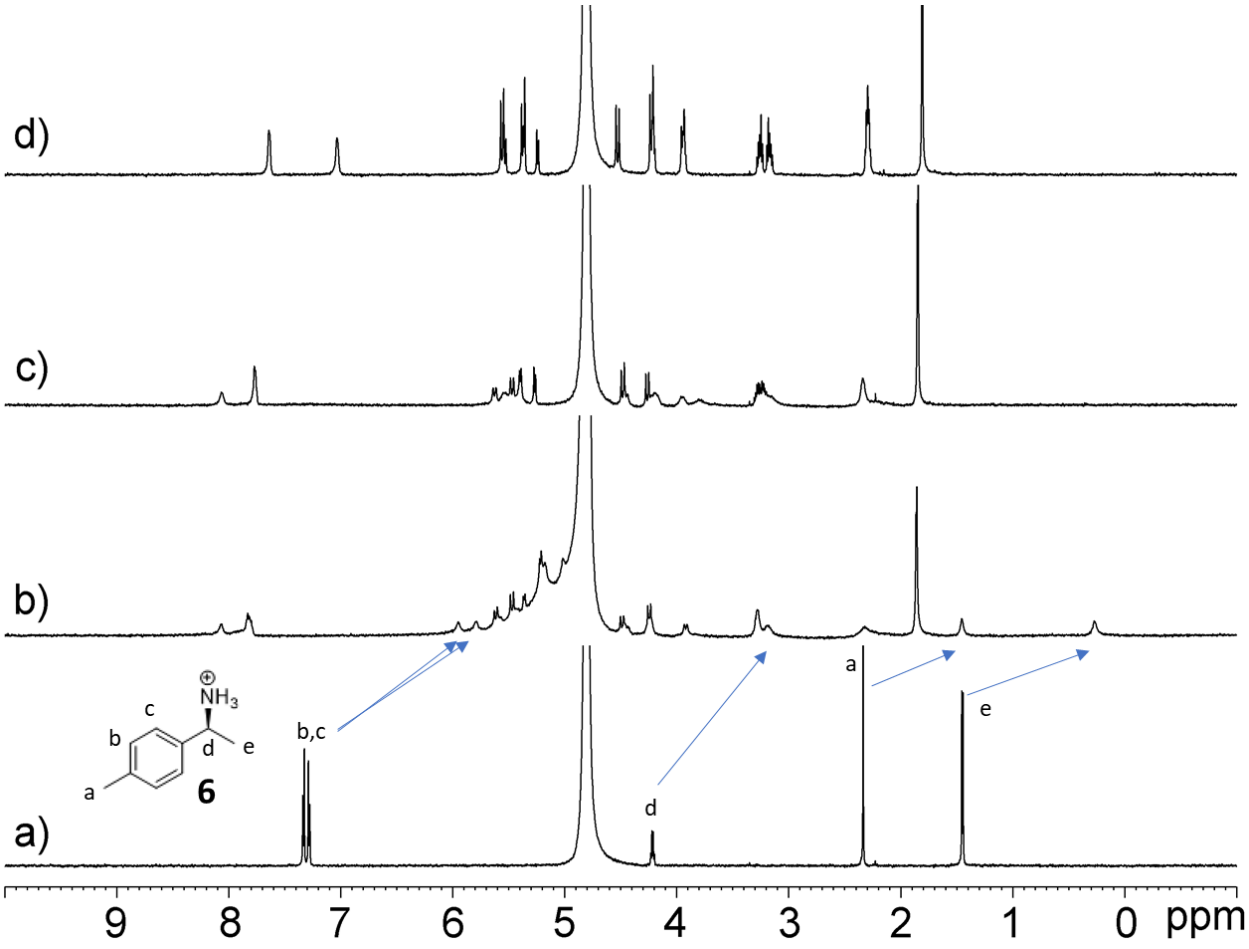
$^1$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**2** (0.50 mM), b) a 1:1 mixture of **M2** (0.25 mM) and (*S*)-**2** (0.25 mM), c) a 1:2 mixture of **M2** (0.25 mM) and (*S*)-**2** (0.50 mM), and d) **M2** (0.25 mM).



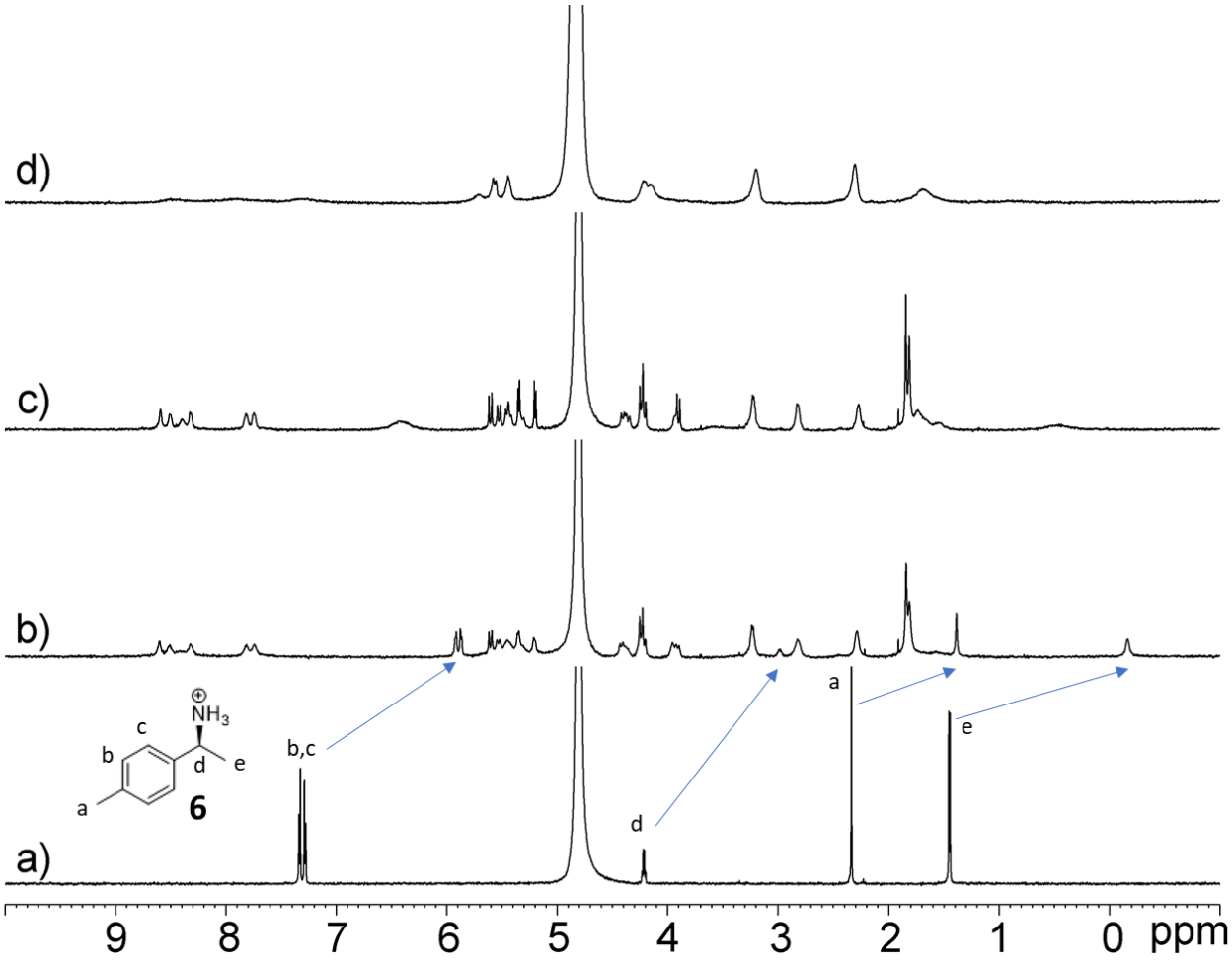
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**2** (0.50 mM), b) a 1:1 mixture of **M3** (0.25 mM) and (*S*)-**2** (0.25 mM), c) a 1:2 mixture of **M3** (0.25 mM) and (*S*)-**2** (0.50 mM), and d) **M3** (0.25 mM).



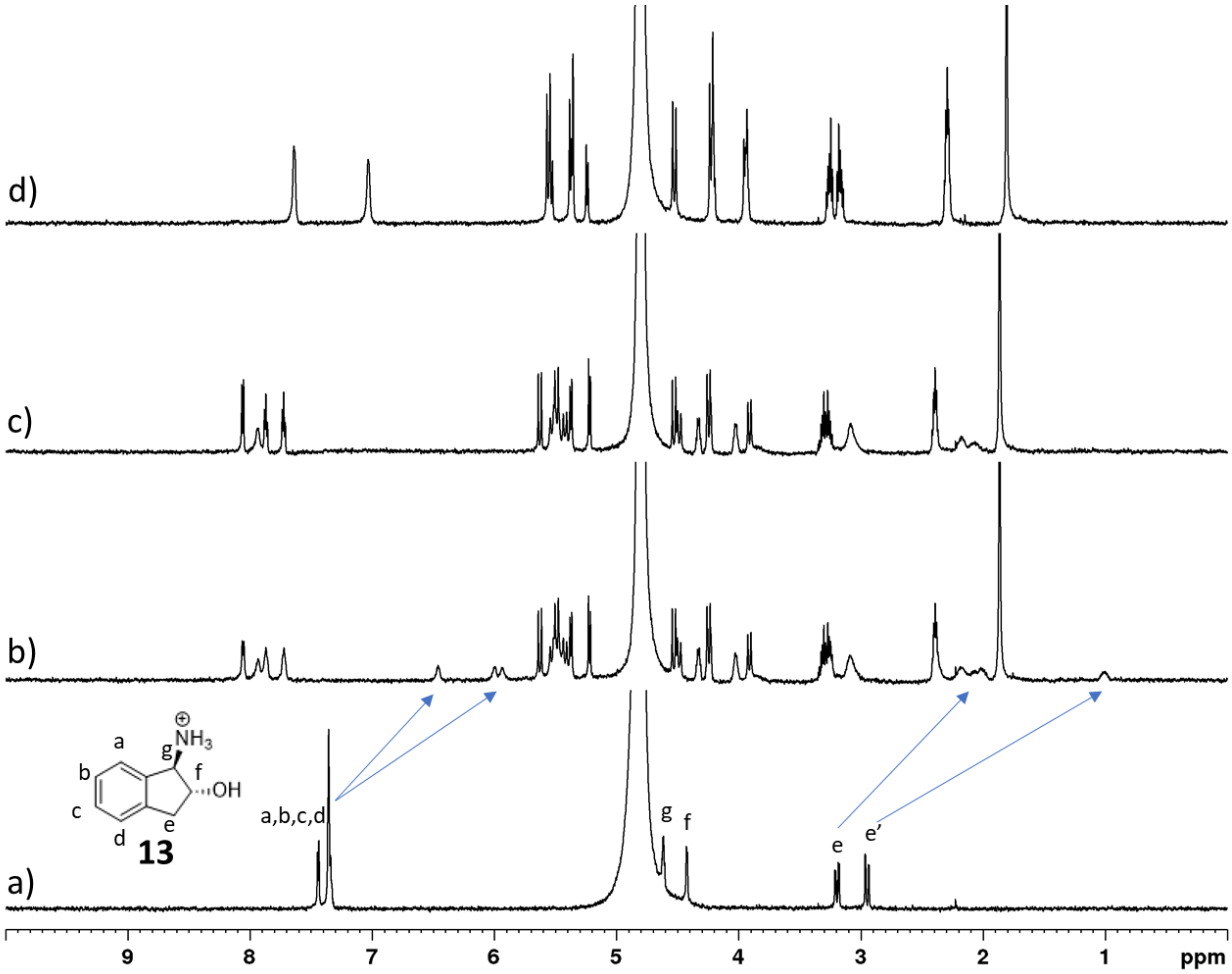
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**6** (0.50 mM), b) a 1:1 mixture of **M2** (0.25 mM) and (*S*)-**6** (0.25 mM), c) a 1:2 mixture of **M2** (0.25 mM) and (*S*)-**6** (0.50 mM), and d) **M2** (0.25 mM).



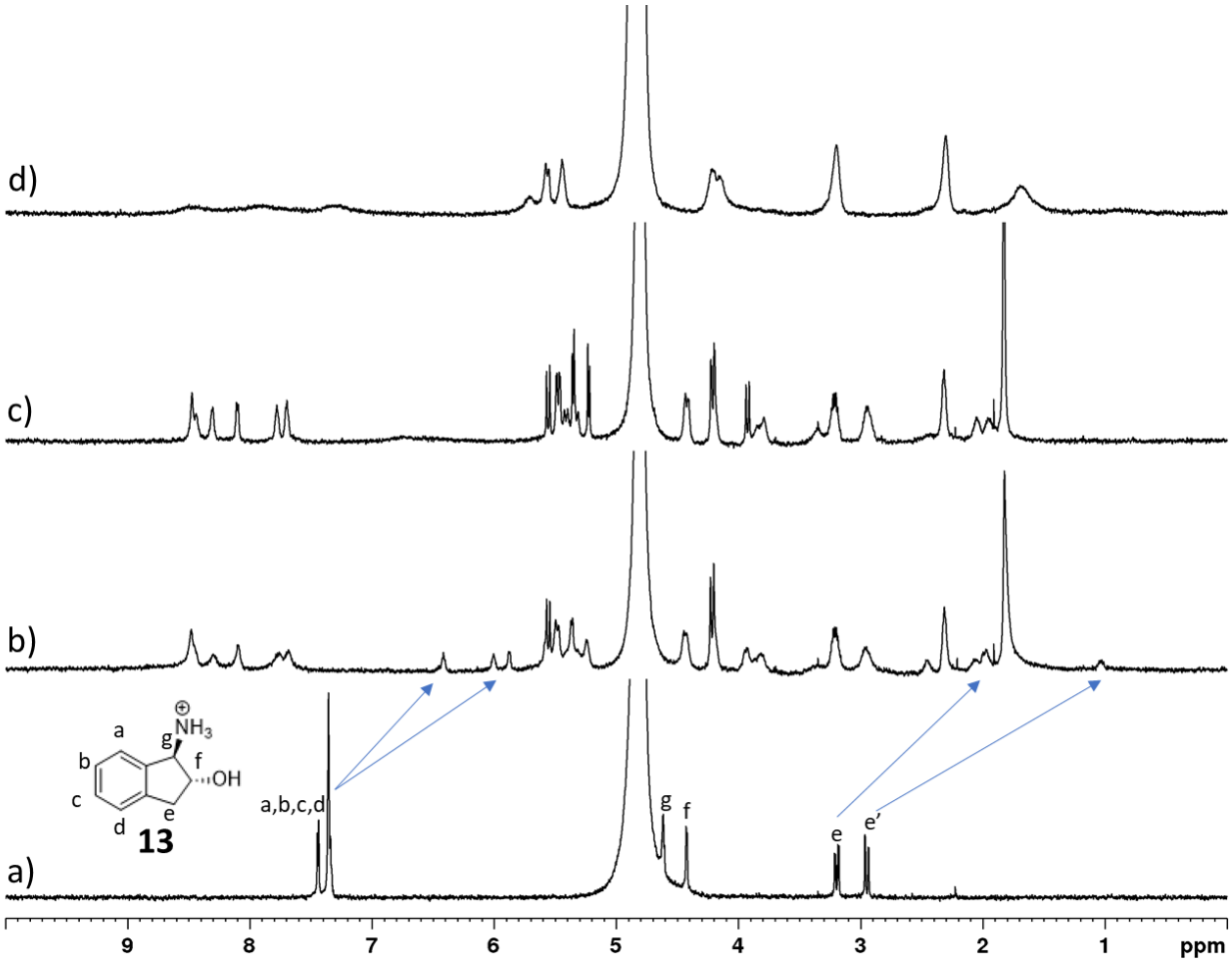
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**6** (0.50 mM), b) a 1:1 mixture of **M3** (0.25 mM) and (*S*)-**6** (0.25 mM), c) a 1:2 mixture of **M3** (0.25 mM) and (*S*)-**6** (0.50 mM), and d) **M3** (0.25 mM).



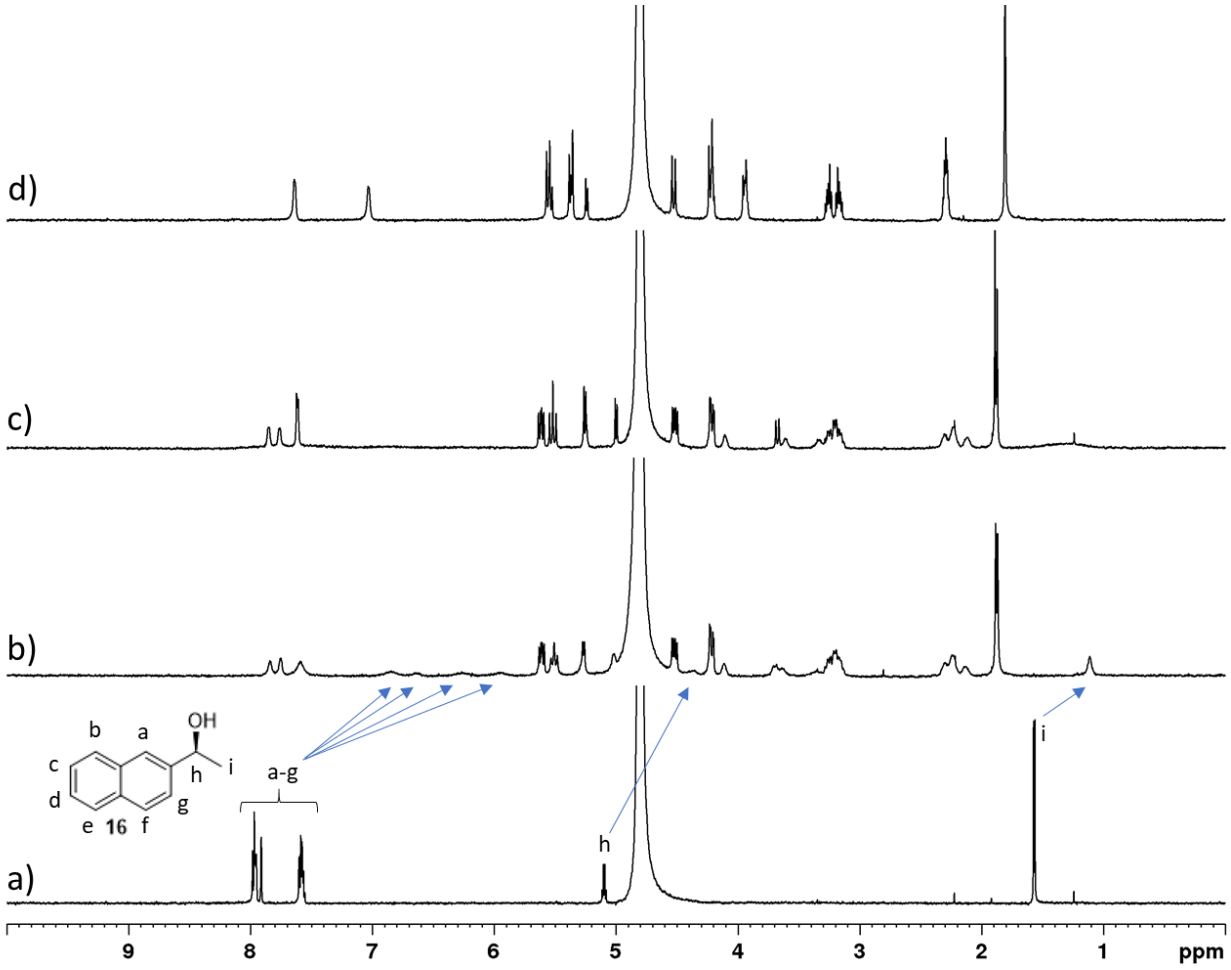
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 °C) for: a) (*R,R*)-**13** (0.50 mM), b) a 1:1 mixture of **M2** (0.25 mM) and (*R,R*)-**13** (0.25 mM), c) a 1:2 mixture of **M2** (0.25 mM) and (*R,R*)-**13** (0.50 mM), and d) **M2** (0.25 mM).



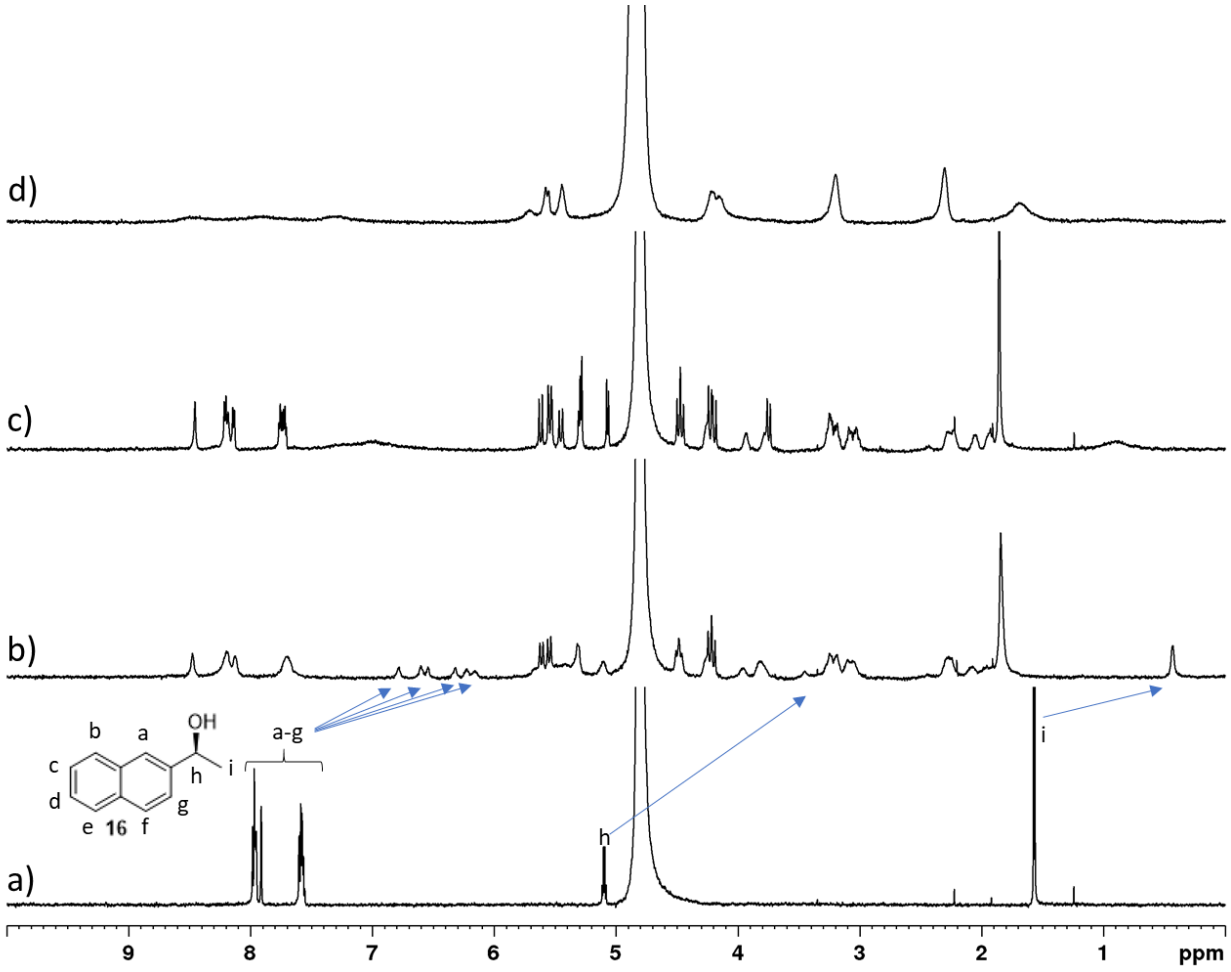
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 °C) for: a) (*R,R*)-**13** (0.50 mM), b) a 1:1 mixture of **M3** (0.25 mM) and (*R,R*)-**13** (0.25 mM), c) a 1:2 mixture of **M3** (0.25 mM) and (*R,R*)-**13** (0.50 mM), and d) **M3** (0.25 mM).



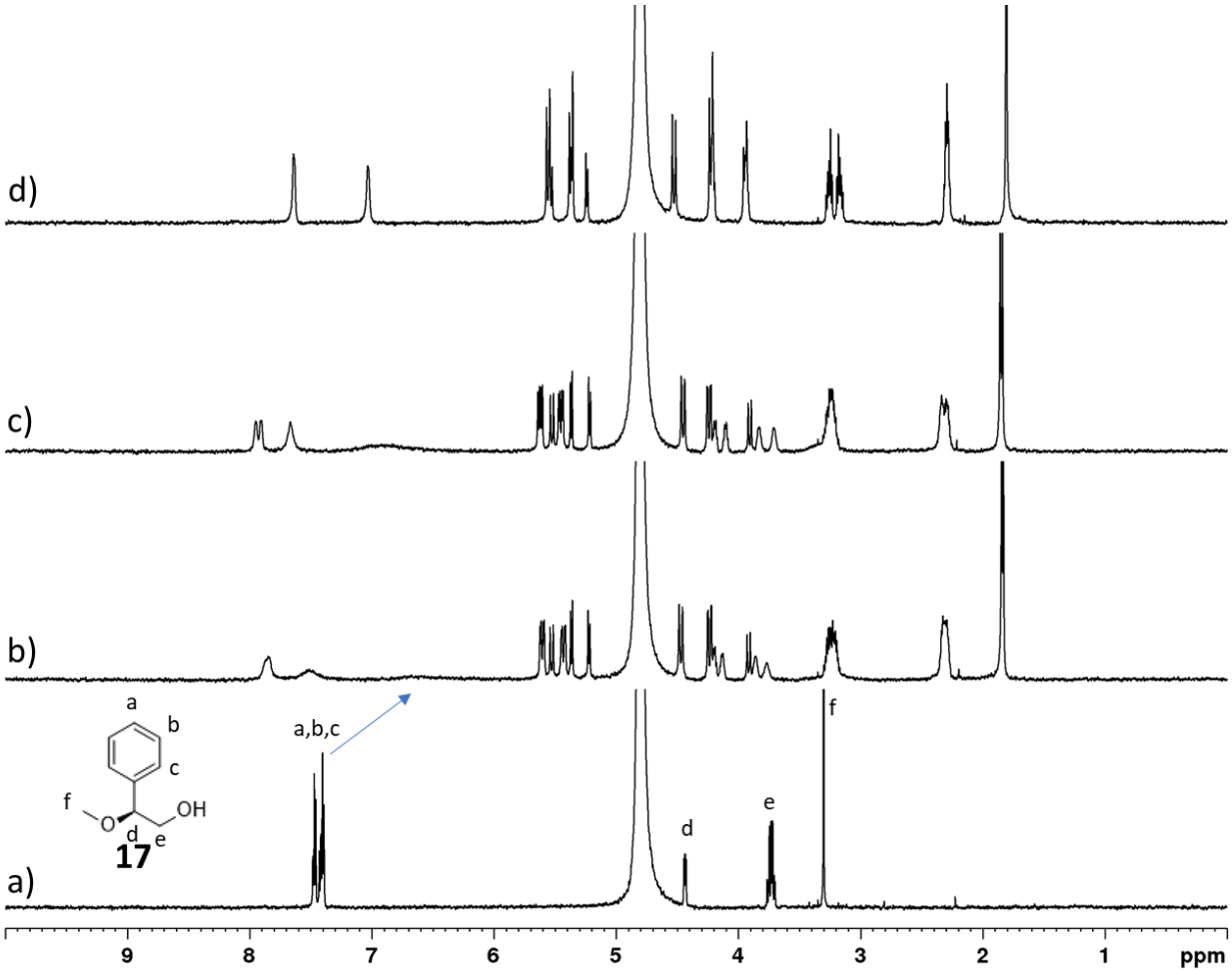
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**16** (0.50 mM), b) a 1:1 mixture of **M2** (0.25 mM) and (*S*)-**16** (0.25 mM), c) a 1:2 mixture of **M2** (0.25 mM) and (*S*)-**16** (0.50 mM), and d) **M2** (0.25 mM).



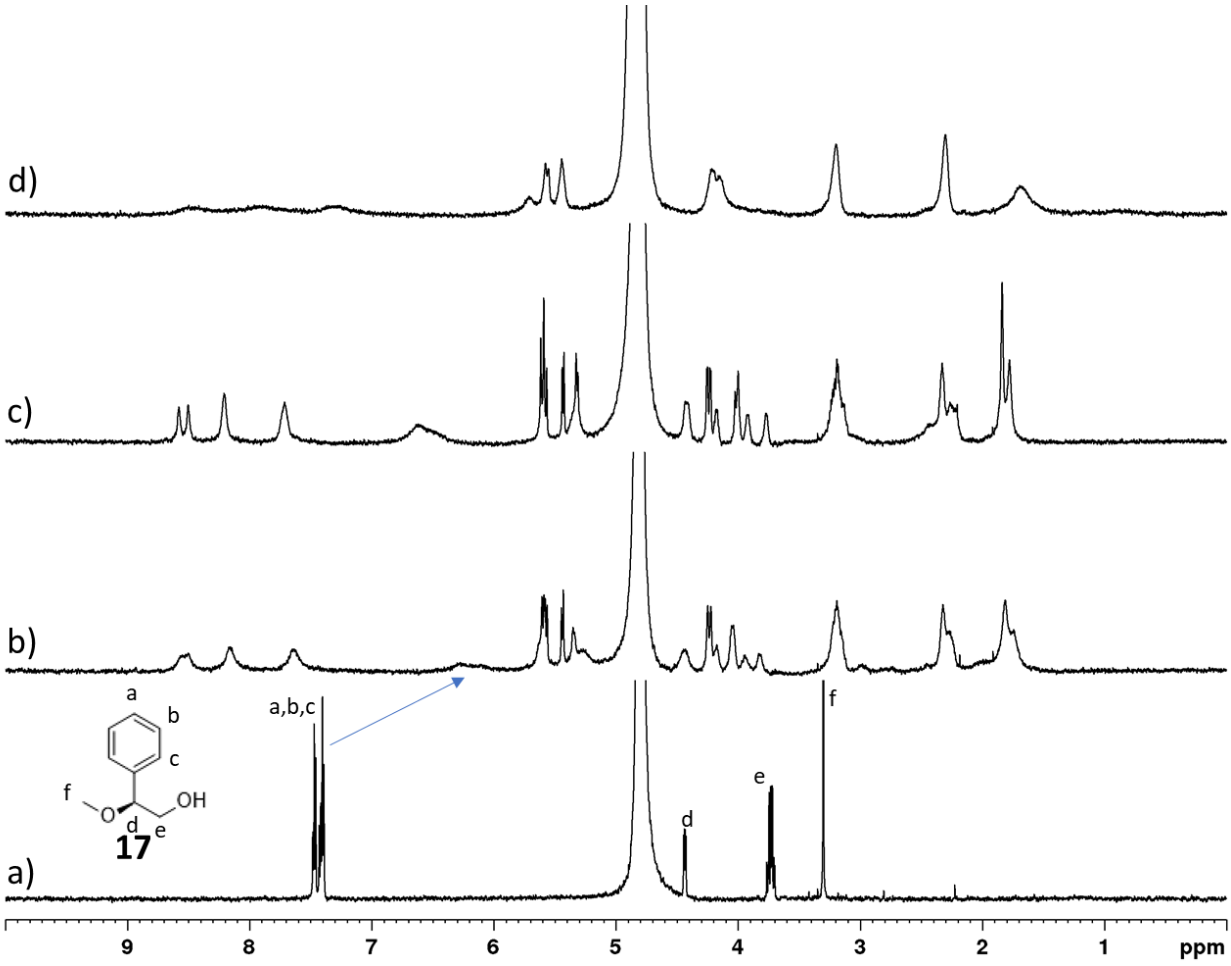
$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**16** (0.50 mM), b) a 1:1 mixture of **M3** (0.25 mM) and (*S*)-**16** (0.25 mM), c) a 1:2 mixture of **M3** (0.25 mM) and (*S*)-**16** (0.50 mM), and d) **M3** (0.25 mM).



$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**17** (0.50 mM), b) a 1:1 mixture of **M2** (0.25 mM) and (*S*)-**17** (0.25 mM), c) a 1:2 mixture of **M2** (0.25 mM) and (*S*)-**17** (0.50 mM), and d) **M2** (0.25 mM).

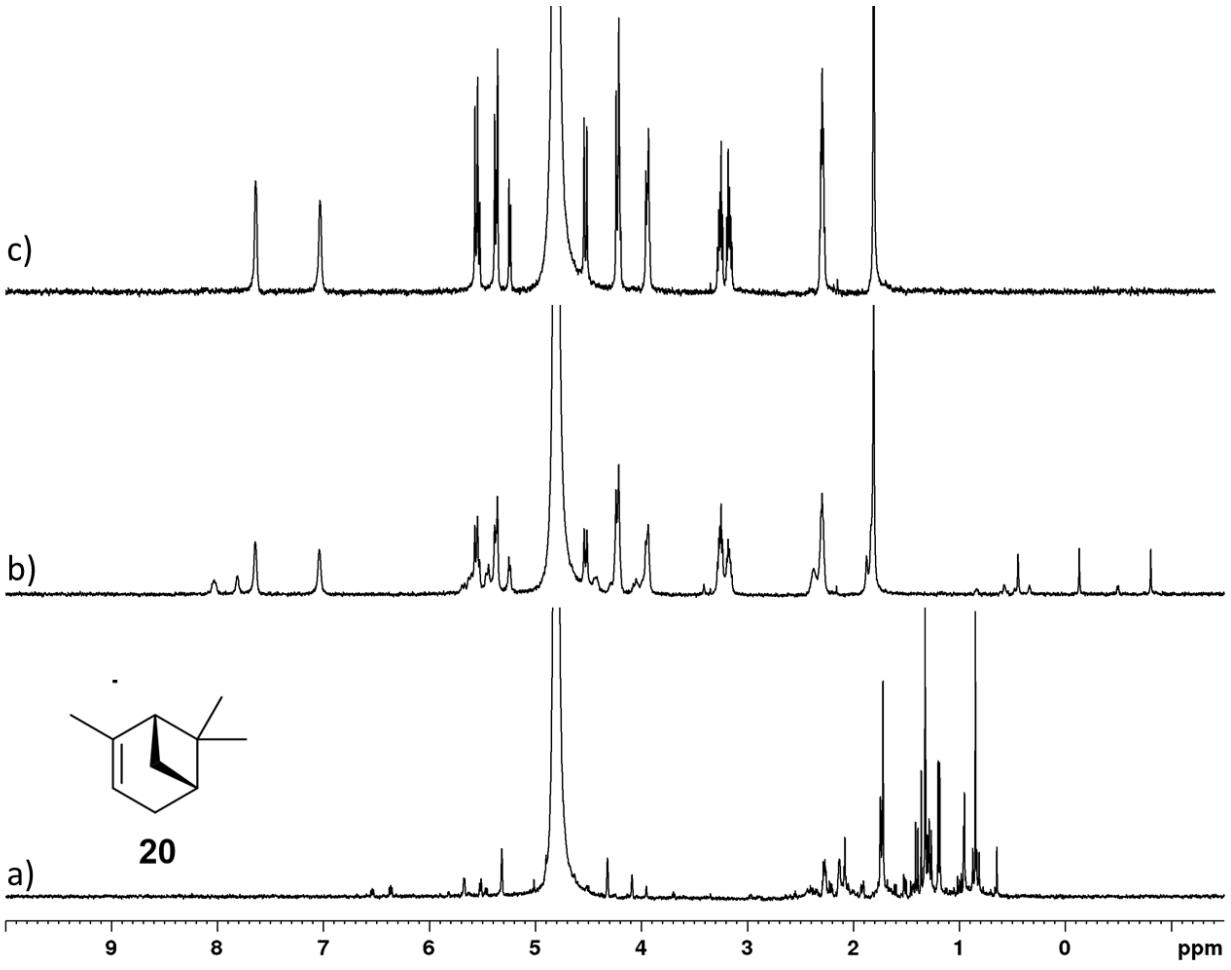


$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (*S*)-**17** (0.50 mM), b) a 1:1 mixture of **M3** (0.25 mM) and (*S*)-**17** (0.25 mM), c) a 1:2 mixture of **M3** (0.25 mM) and (*S*)-**17** (0.50 mM), and d) **M3** (0.25 mM).

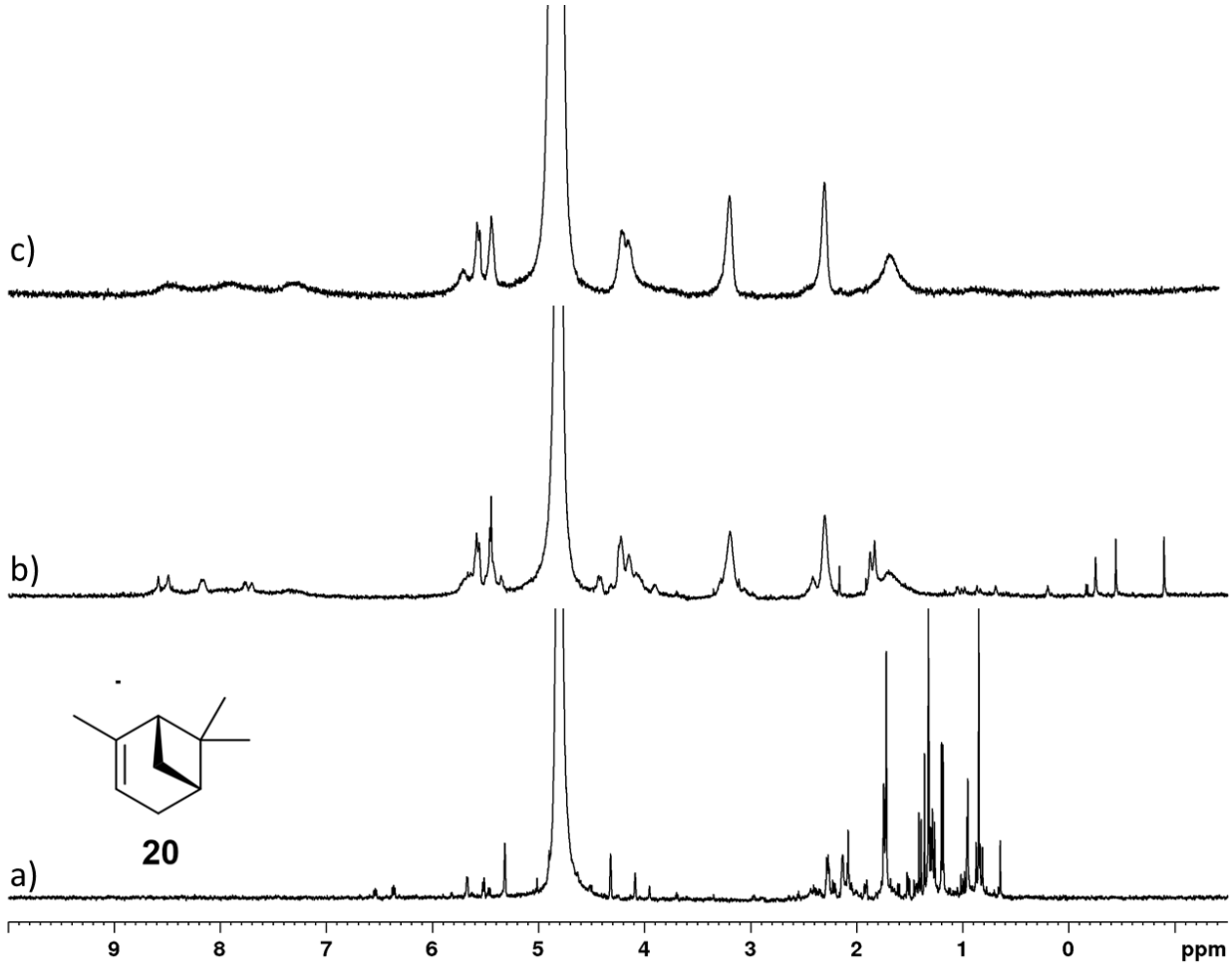


Host·terpene mixtures were prepared by adding a drop of either (+)-**20** or (+)-**21** into a solution of either **M2** or **M3** (0.25 mM) in $D_2O$.

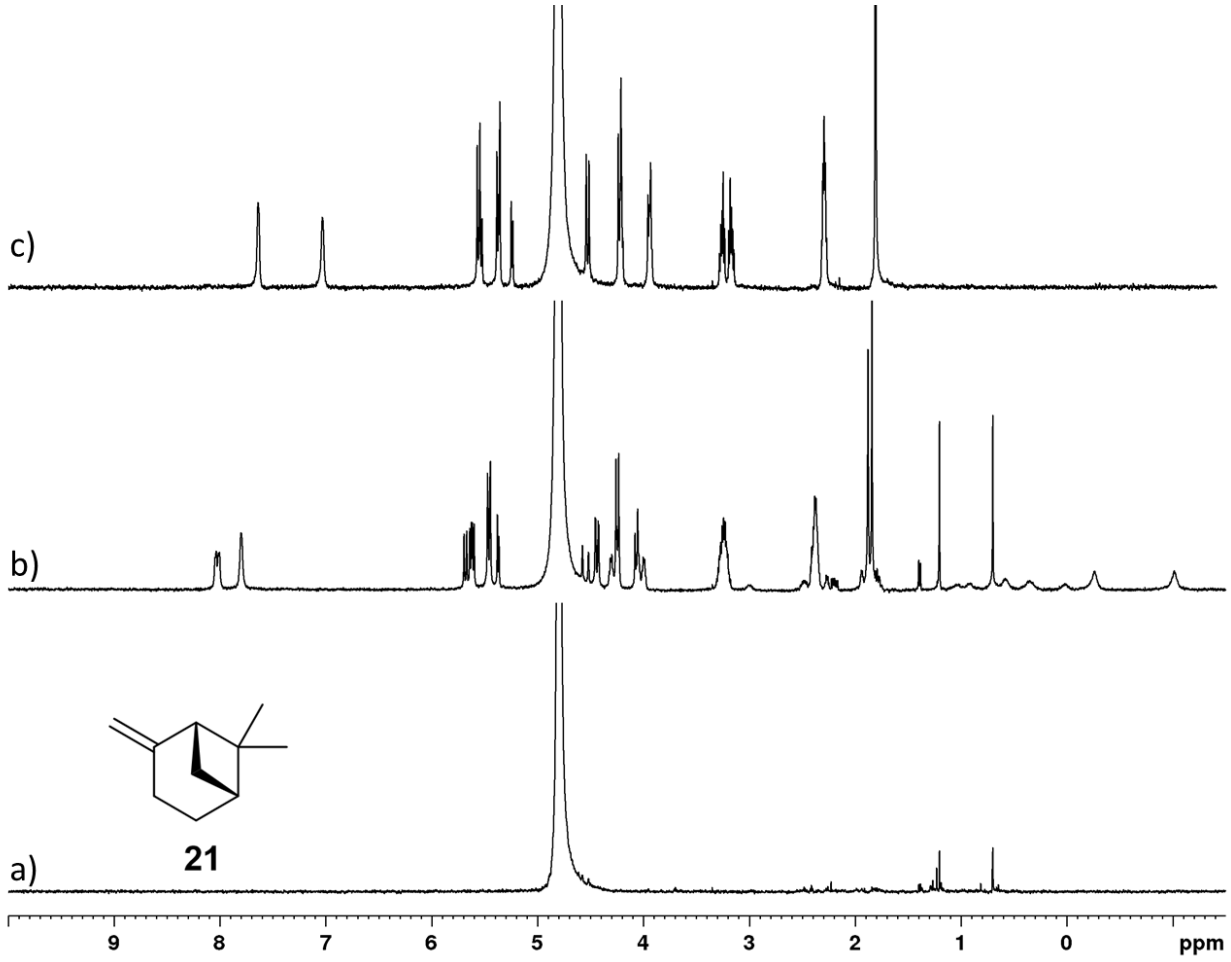
$^1$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (+)-**20** alone, b) a mixture of **M2** and (+)-**20**, c) **M2** alone.



$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (+)-**20** alone, b) a mixture of **M3** and (+)-**20**, c) **M3** alone.



$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (+)-**21** alone, b) a mixture of **M2** and (+)-**21**, c) **M2** alone.



$^{1}$H NMR spectra recorded (600 MHz, $D_2O$, 25 ºC) for: a) (+)-**21** alone, b) a mixture of **M2** and (+)-**21**, c) **M2** alone.