Enantiomeric NMR discrimination of carboxylic acids using actinomycin D as a chiral solvating agent

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1 General information
CSA 1 was purchased from Push-herb chem Biotech Inc. The compounds 2-15 were purchased from Aladdin biochemical technology co. Ltd (Shanghai, China), which were all used without further purification. $^1$H NMR data were collected on a Bruker Avance 600 MHz spectrometer at 20 °C. Chemical shifts (ppm) internally referenced to CDCl$_3$ signal ($\delta$H = 7.26 ppm) or C$_6$D$_6$ ($\delta$H = 7.16 ppm) were obtained.

2 Carboxylic Acids recognized by CSA 1
(a) The CSA 1 and (±)-mandelic acid were dissolved in the solvent CDCl$_3$, both concentrations are 20 mM. The solutions were distributed among five NMR tubes with the mole ratio of the CSA 1 and (±)-mandelic acid increased from 0 to 1, the total concentration of host and guest in the NMR tubes (total volume of 500 µL) was 20 mM which remained unchanged.

(b) The CSA 1 and racemic carboxylic acids were dissolved, with their concentrations being 20 mM in CDCl$_3$, 50 µL of CSA 1 and 500 µL of each racemic carboxylic acid were mixed together, with the mole ratio being 1:10. The total concentration in the NMR tubes (total volume was 550 µL) was 20 mM.

3 Determination of enantiomeric purity of mandelic acid
To determine the enantiomeric purity of the carboxylic acids, samples with ee values of -100%, -90%, -80%, -60%, -40%, -20%, 0% were prepared by racemic mandelic acid and (R)-mandelic acid at the concentration of 20 mM in CDCl₃, expressed as % R in the data. The CAS 1 was also dissolved in CDCl₃ at a concentration of 20 mM. Then 50 µL of CAS 1 and 500 µL of racemic mandelic acid and (R)-mandelic acid mixtures with different ee’s were added to the NMR tube (total concentration of 20 mM) with a molar ratio of 1:10. Then the enantiomeric purity of the carboxylic acids was determined by ¹H NMR method. The plotting of gravimetry ee value (y axis) versus NMR observed ee value (x axis) presented excellent linearity with $R^2 = 0.9996$.

4. ¹H NMR spectroscopy of CSA 1 and racemic carboxylic acids

Figure S1: ¹H NMR (600 MHz, CDCl₃) of CSA 1.
Figure S2: $^1$H NMR (600 MHz, CDCl$_3$) of racemic mandelic acid.

Figure S3: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid with the corresponding molar ratio 1:1.
Figure S4: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid with the corresponding molar ratio 1:5.

Figure S5: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid with the
corresponding molar ratio 1:10.

Figure S6: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid with the corresponding molar ratio 1:20.

Figure S7: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-bromomandelic acid (3).
Figure S8: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-fluoromandelic acid (4).

Figure S9: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 4-chloromandelic acid (5).
Figure S10: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 4-bromomandelic acid (6).
Figure S11: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 4-fluoromandelic acid (7).

Figure S12: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 4-methoxymandelic acid (8).
Figure S13: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 3,5-difluoromandelic acid (9).

Figure S14: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic α-methoxyphenylacetic acid (10).
Figure S15: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-naphthaleneacetic acid (11).

Figure S16: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-phthalimidopropionic acid (12).
Figure S17: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic phenylsuccinic acid (13).

Figure S18: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-hydroxy-3-methylbutyric acid (14).
Figure S19: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-hydroxycaprylic acid (15).

Figure S20: $^1$H NMR (600 MHz, C$_6$D$_6$) of CSA 1 and racemic mandelic acid (2) (The CSA 1 and racemic carboxylic acids were dissolved in C$_6$D$_6$, with their concentrations being 20 mM. 50 µL of CSA 1 and 500 µL of racemic mandelic acid were mixed, with the mole ratio being 1:10. The total concentration in the NMR tubes was 20 mM).
Studies of the stoichiometry of CSA 1/(R)- and (S)-4-methoxymandelic acid by $^1$H NMR titration (Job Plots)

Figure S21: Job plot of CSA 1 with (R)-8 and (S)-8. (The CSA 1, (R)-8 and (S)-8 were separately dissolved in CDCl$_3$, with their concentrations being 20 mM. The solutions were distributed among 22 NMR tubes with the host-guest mole ratio increasing from 0 to 1, the total concentration of host and guest was 20mM).

According to Figure S21, the $\alpha$-H signal in the upfield belong to
(R)-4-methoxymandelic acid and the α-H signal in the downfield belong to (S)-4-methoxymandelic acid.

7, $^{19}$F NMR spectroscopy of CSA 1 and racemic 2-fluoromandelic acid

Figure S22: $^{19}$F NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-fluoromandelic acid (4). (The CSA 1 and racemic 2-fluoromandelic acid were dissolved in CDCl$_3$, with their concentrations being 20 mM. 50 µL of CSA 1 and 500 µL of racemic 2-fluoromandelic acid were mixed, with the mole ratio being 1:10. The total concentration in the NMR tubes was 20 mM).

8, $^1$H NMR spectroscopy of CSA 1 and racemic mandelic acid under 10mM

Figure S23: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid (2) under the concentration of 10mM, with the molar ration being 1:10. (The sample of Act-D and mandelic acid in CDCl$_3$ (molar ratio being 1:10, the concentration was 20 mM), was diluted and the final concentration was 10 mM. The result shows that the ΔΔδ value of α-H resonance is 0.046 ppm).
9, 1H NMR spectroscopy of discrimination of a single enantiomer of racemic carboxylic acids measured in the presence of CSA 1

Equal volume of 20 mM enantiomeric pure isomer ((R)-(−)-mandelic acid, (S)-(−)-2-chloromandelic acid) and the respective racemic compounds solutions were added into NMR tubes containing 20 mM Act-D, the total molar ratio was 1:10, total concentration in the NMR tubes was 20 mM.

Figure S24: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic mandelic acid with the addition of (R)-(−)-mandelic acid.
Figure S25: $^1$H NMR (600 MHz, CDCl$_3$) of CSA 1 and racemic 2-bromomandelic acid with the addition of (S)-(+)2-chloromandelic acid.