

Supporting Information

High Throughput Determination of Enantiopurity by Microplate Circular Dichroism

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1. General

All reagents were used as purchased. Chiral analytes were obtained from Sigma Aldrich or CombiBlocks, inc. Chiral analytes obtained as HCl salts were used as is and neutralized by using equimolar triethylamine during the derivatization reactions. Solvents were dried over 3Å molecular sieves (heated to 170 °C for 24 hours prior to use). All chiroptical analyses were performed using the EKKO CD microplate reader and a Hellma Suprasil quartz 96-well plate equipped with quartz lid and silicon buffer mat inlay. Spectral scanning was conducted every 5 nm from 375-410 nm with 0.8 sec (default) or 3.0 seconds (optimized) integration time and 40 mdeg sensitivity. All stock solutions of the sensor, additive/interferents and chiral analytes were prepared using a Mettler Toledo analytical balance (XS105 Series), Eppendorf electronic pipettes, and acetonitrile as solvent.

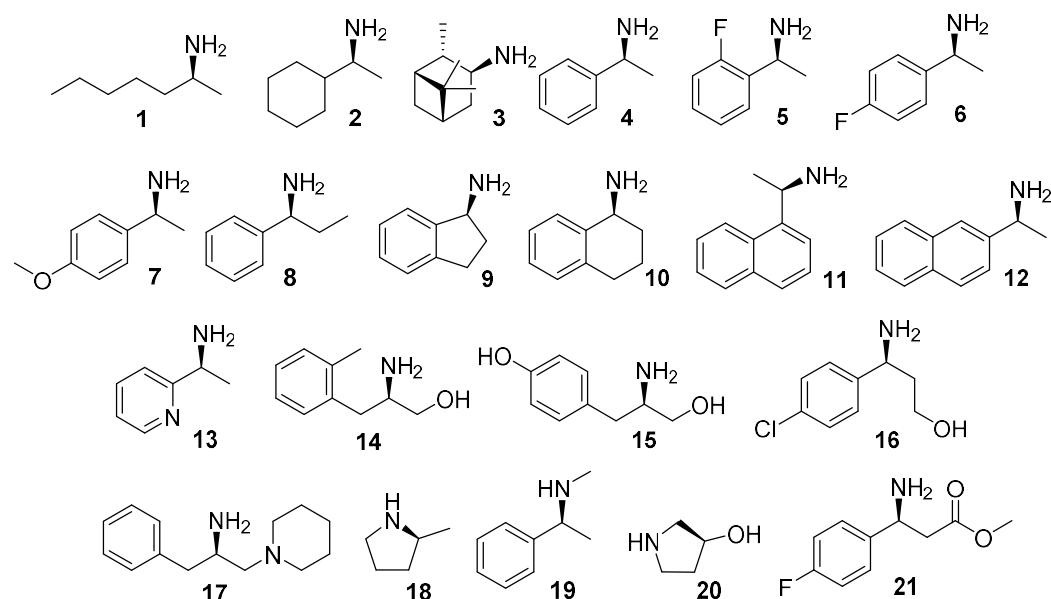


Figure S1. Structures of analytes tested.

2. Chiroptical sensing

Solutions of 4-chloro-3-nitrocoumarin, **A**, (5.00-6.00 mM) were prepared and distributed into vials. Stock solutions of each enantiomer of the chiral analyte of interest were prepared (0.025 M or 0.25 M) in acetonitrile for either 10.0 μ L or 100.0 μ L additions to the reagent (1:1 **A**/analyte ratio) allowing for the click reaction to occur at 5.00 mM. The reactions were conducted in the presence of 1.0-10.0 molar equivalents of triethylamine or DBU. After 20 minutes, aliquots of the reaction solutions were diluted to 600.0 μ M and/or 300.0 μ M. Calibrations were constructed with the quartz 96-well plate and varied *ee*'s (-100, -80, -40, 0, 40, 80, 100 %) of the analytes. EKKO parameters were set to scan every 5 nm at 375-410 nm with 40 mdeg sensitivity, 3 second integration time using the quartz plate and individual well blanking with acetonitrile.

2.1 Primary amines

CD sensing of heptan-2-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.

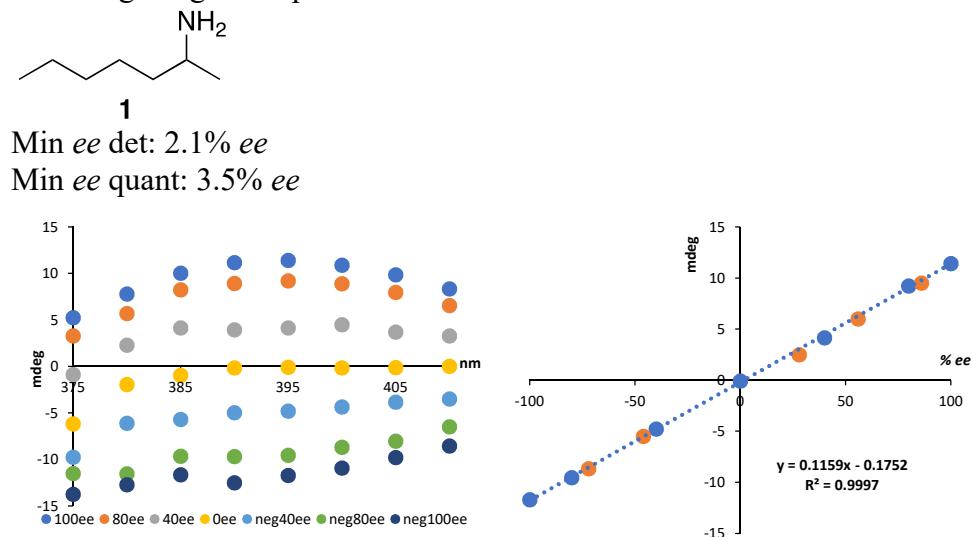


Table S1 (a). Calibration data for analyte **1** with **A**.

Calibration data at 395 nm (blue series):

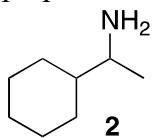
% ee	CD (mdeg)
100.0	11.4358
80.0	9.2282
40.0	4.1717
0.0	-0.0608
-40.0	-4.7708
-80.0	-9.5345
-100.0	-11.6960

Table S1 (b). Comparison of calculated and prepared *ee* data for analyte **1** with **A**.

Varied ee sample data run in parallel with calibration (orange series):

Actual ee	CD (mdeg)	Calculated ee	Difference in ee (% ee)
86.0	9.51	83.5	2.5
56.0	6.01	53.4	2.6
28.0	2.51	23.2	4.8
-46.0	-5.50	-45.9	0.1
-72.0	-8.64	-73.0	1.0

CD sensing of 1-cyclohexylethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min ee det: 2.6% ee

Min ee quant: 4.4% ee

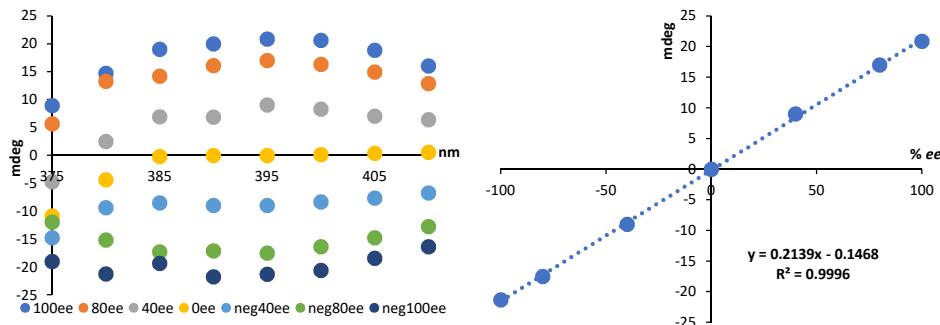


Figure S3. CD sensing of analyte **2** with **A**.

Table S2 (a). Calibration data for analyte **2** with **A**.

Calibration data at 395 nm (blue):

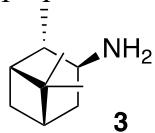
% ee	CD (mdeg)
100.0	20.869688
80.0	16.9756355
40.0	9.00441265
0.0	-0.0381829
-40.0	-8.9965038
-80.0	-17.511728
-100.0	-21.330919

Table S2 (b). Comparison of calculated and prepared ee data for analyte **2** with **A**.

Varied ee sample data run in parallel with calibration (orange):

Actual ee	CD (mdeg)	Calculated ee	Difference in ee (% ee)
28.0	2.24	24.4	3.6
-46.0	-3.75	-42.1	3.9
-72.0	-6.27	-70.3	1.7
-92.0	-8.29	-92.7	0.7

CD sensing of (+)-isopinocampheylamine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 5.4% *ee*

Min *ee* quant: 9.1% *ee*

(at 405 nm: MED=3.0%; MEQ=4.9%)

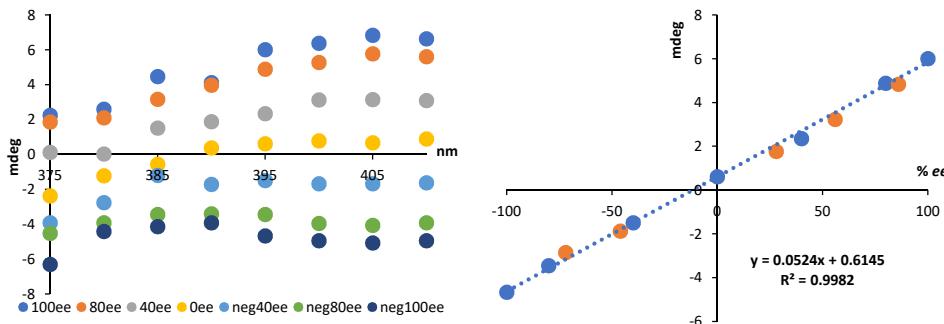


Figure S4. CD sensing of analyte **3** with **A**.

Table S3 (a). Calibration data for analyte **3** with **A**.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	6.02000284
80.0	4.89415121
40.0	2.35648561
0.0	0.61880469
-40.0	-1.4942828
-80.0	-3.4392319
-100.0	-4.6544204

Table S3 (b). Comparison of calculated and prepared *ee* data for analyte **3** with **A**.

Varied ee sample data run in parallel with calibration (orange):

Actual <i>ee</i>	CD (mdeg)	Calculated <i>ee</i>	Difference in <i>ee</i> (% <i>ee</i>)
86.0	4.84	80.6	5.3
56.0	3.23	49.9	6.1
28.0	1.77	22.1	5.9
-46.0	-1.87	-47.3	1.3
-72.0	-2.84	-66.0	6.0

Table S3 (c). Calibration data for analyte **3** with **A** (405 nm).

Calibration data at 405 nm:

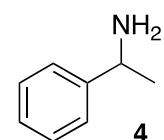
% ee	CD (mdeg)
100.0	6.85400343
80.0	5.78365088
40.0	3.16014671
0.0	0.68158883
-40.0	-1.6668742
-80.0	-4.0617595
-100.0	-5.0717402

Table S3 (d). Comparison of calculated and prepared *ee* data for analyte **3** with **A** (405 nm).

Varied ee sample data run in parallel with calibration at 405 nm:

actual ee	CD (mdeg)	calculated ee	Difference in ee (% ee)
86.0	6.32	91.3	5.26
56.0	4.08	54.2	1.85
28.0	2.41	26.5	1.50
-46.0	-2.19	-49.7	3.72
-72.0	-3.58	-72.7	0.74

CD sensing 1-phenylethan-1-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 0.9% *ee*

Min *ee* calc: 1.5% *ee*

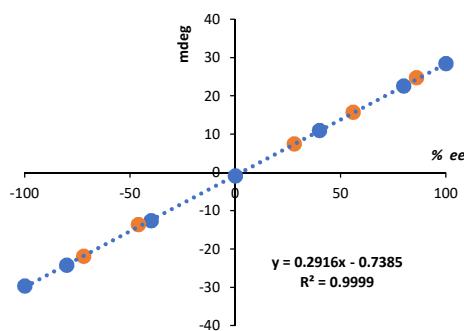
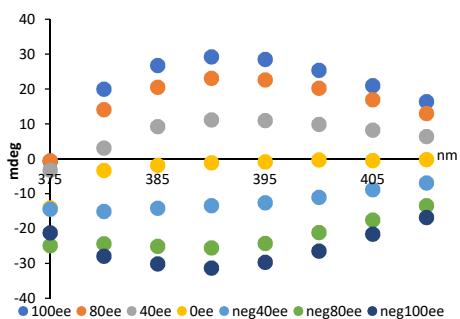


Figure S5. CD sensing of analyte **4** with **A**.

Table S4 (a). Calibration data for analyte **4** with **A**.

Calibration data at 395 nm (blue):

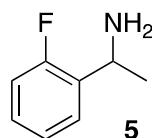
% ee	CD (mdeg)
100.0	28.4833374
80.0	22.6231594
40.0	10.9911451
0.0	-0.8774153
-40.0	-12.571907
-80.0	-24.199289
-100.0	-29.618732

Table S4 (b). Comparison of calculated and prepared *ee* data for analyte **4** with **A**.

Varied ee sample data run in parallel with calibration (orange):

Actual ee	CD (mdeg)	Calculated ee	Difference in ee (% ee)
86.0	24.76	87.5	1.5
56.0	15.76	56.6	0.6
28.0	7.53	28.4	0.4
-46.0	-13.56	-44.0	2.1
-72.0	-21.87	-72.5	0.5

CD sensing of 1-(2-fluorophenyl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 8.5% *ee*

Min *ee* calc: 14.1% *ee*

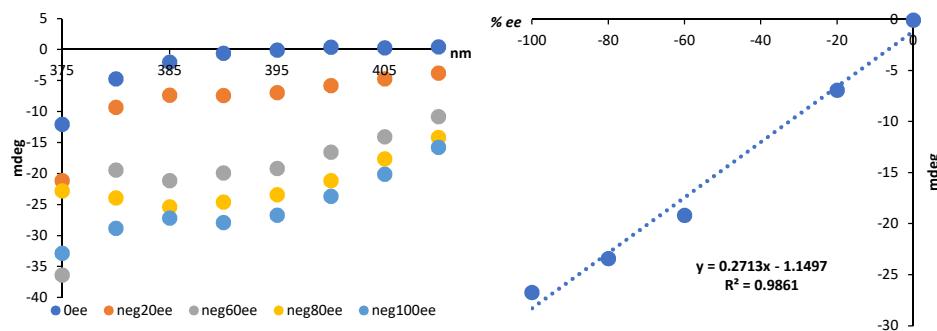


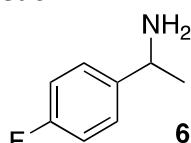
Figure S6. CD sensing of analyte **5** with **A**.

Table S5 (a). Calibration data for analyte **5** with A.

Calibration data at 395 nm:

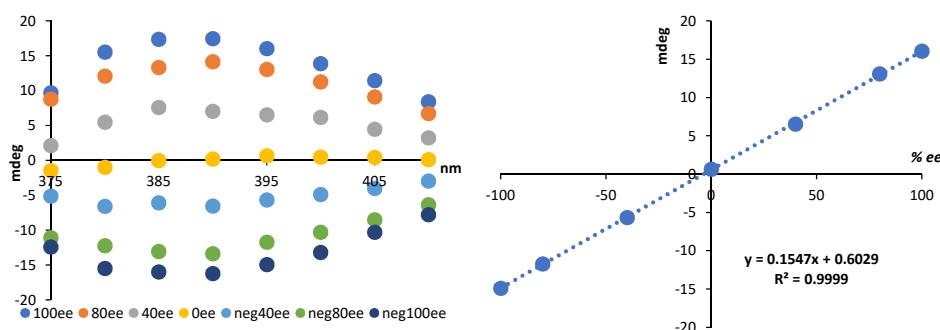
% ee	CD (mdeg)
0.0	-0.05807
-20.0	-6.92456
-60.0	-19.18521
-80.0	-23.41541
-100.0	-26.71606

CD sensing of 1-(4-fluorophenyl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 1.0 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min ee det: 1.2% ee

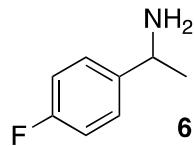
Min ee calc: 2.0% ee

**Figure S7.** CD sensing of analyte **6** with A.**Table S6.** Calibration data for analyte **6** with A.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	16.0775
80.0	13.0999
40.0	6.5736
0.0	0.6726
-40.0	-5.6247
-80.0	-11.6873
-100.0	-14.8916

CD sensing of with 4-chloro-3-nitrocoumarin and 1-(4-fluorophenyl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 1.8% *ee*

Min *ee* calc: 3.0% *ee*

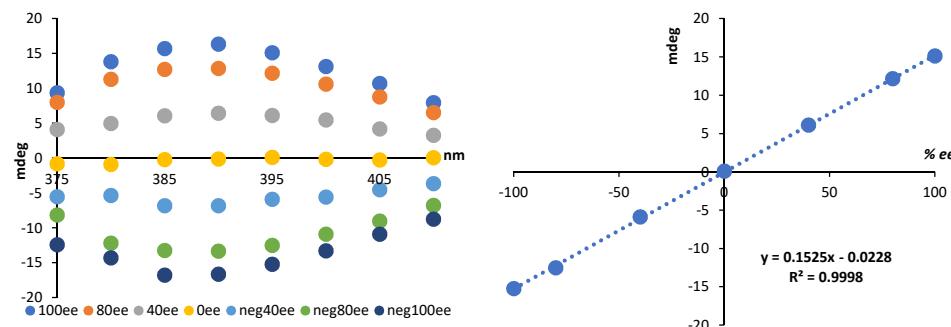


Figure S8. CD sensing of analyte **6** with **A** (low concentration data).

Table S7. Calibration data for analyte **6** with **A** (low concentration data).

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	15.1166
80.0	12.1680
40.0	6.1002
0.0	0.0932
-40.0	-5.8987
-80.0	-12.5183
-100.0	-15.2205

CD sensing of 1-(4-methoxyphenyl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.

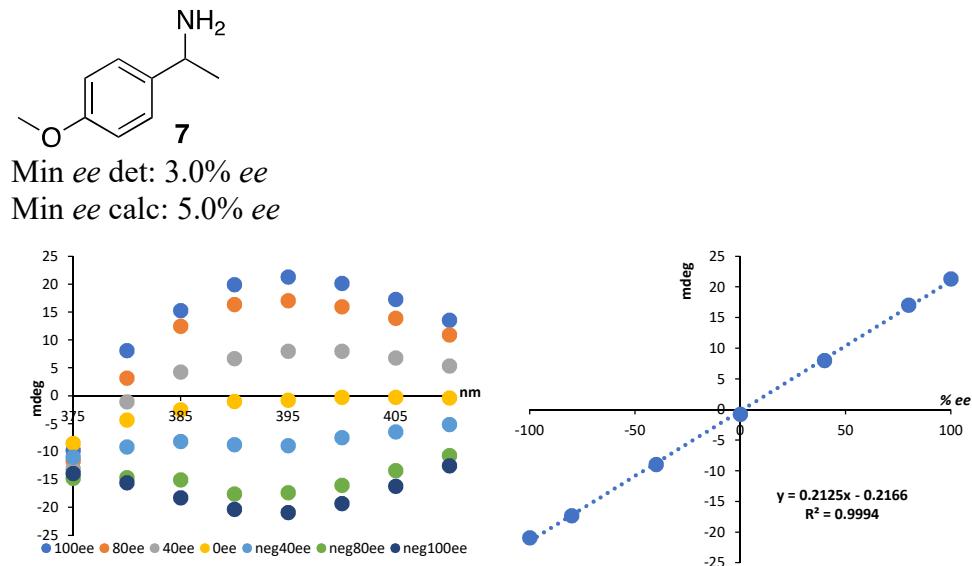


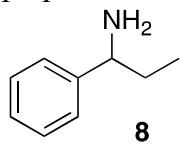
Figure S9. CD sensing of analyte 7 with A

Table S8. Calibration data for analyte 7 with A.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	21.3335
80.0	17.0425
40.0	8.0383
0.0	-0.7752
-40.0	-8.9360
-80.0	-17.3120
-100.0	-20.9073

CD sensing of 1-phenylpropan-1-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 2.9% *ee*

Min *ee* calc: 4.8% *ee*

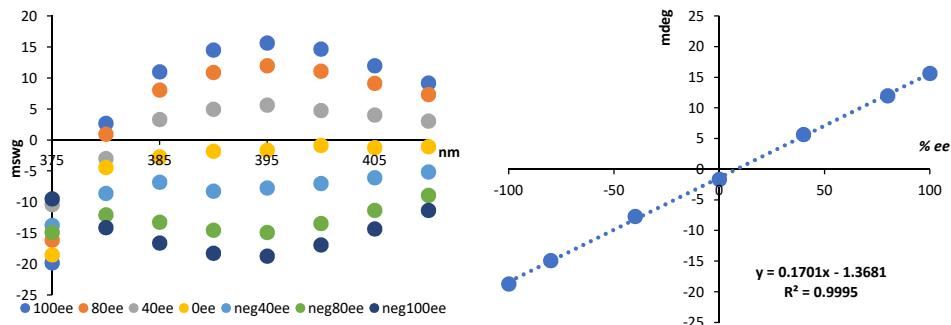


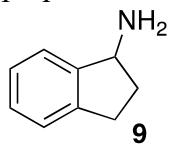
Figure S10. CD sensing of analyte **8** with A.

Table S9. Calibration data for analyte **8** with A.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	15.6794
80.0	11.9844
40.0	5.6772
0.0	-1.6336
-40.0	-7.7029
-80.0	-14.8823
-100.0	-18.6988

CD sensing of 2,3-dihydro-1*H*-inden-1-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of DBU mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 9.5% *ee*

Min *ee* calc: 15.8% *ee*

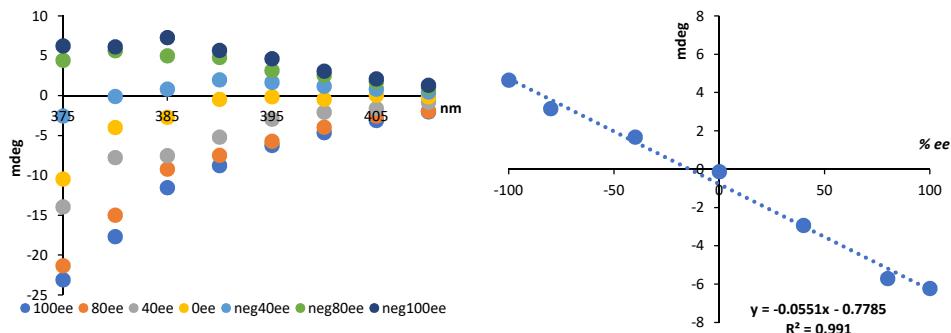


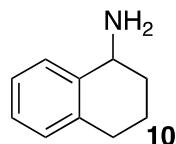
Figure S11. CD sensing of analyte **9** with **A**.

Table S10. Calibration data for analyte **9** with **A**.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	-6.221127
80.0	-5.698534
40.0	-2.9295716
0.0	-0.1207124
-40.0	1.68632865
-80.0	3.16899943
-100.0	4.66502714

CD sensing of 1,2,3,4-tetrahydronaphthalen-1-amine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min ee det: 6.7% ee

Min ee calc: 11.1% ee

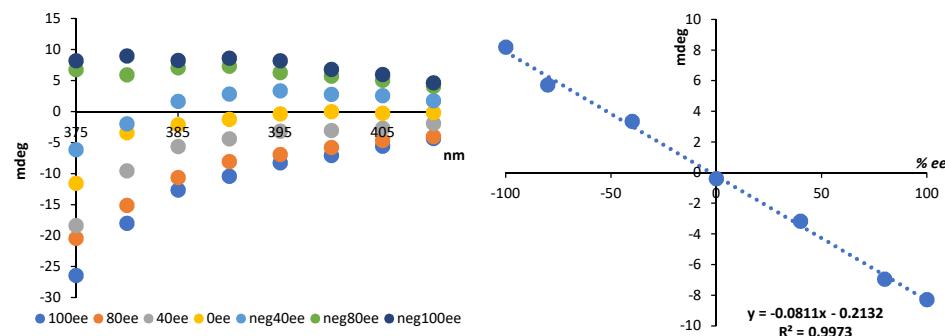


Figure S12. CD sensing of analyte **10** with **A**.

Table S11. Calibration data for analyte **10** with **A**.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	-8.2718
80.0	-6.9391
40.0	-3.1701
0.0	-0.3848
-40.0	3.3474
-80.0	5.7327
-100.0	8.1932

CD sensing of 1-(naphthalen-1-yl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.

Min *ee* det: 2.9% *ee*

Min *ee* calc: 4.9% *ee*

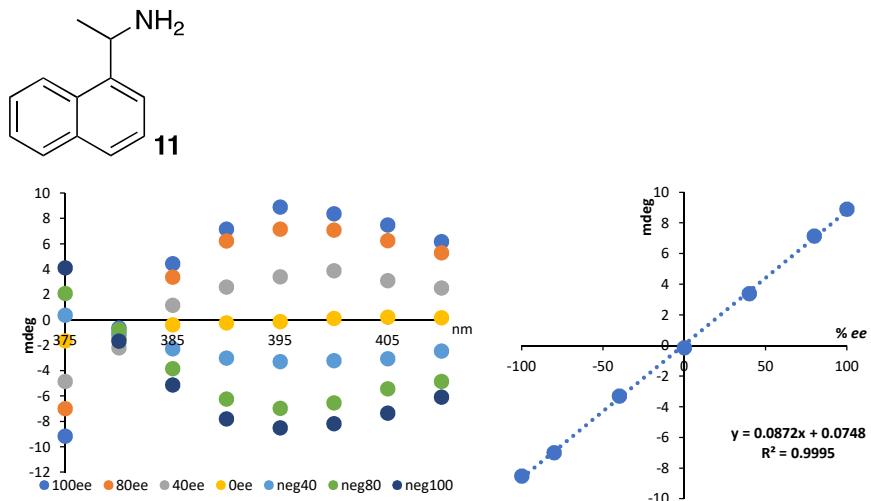


Figure S13. CD sensing of analyte **11** with **A**.

Table S12. Calibration data for analyte **11** with **A**.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	8.8984
80.0	7.1578
40.0	3.3868
0.0	-0.1450
-40.0	-3.2873
-80.0	-6.9810
-100.0	-8.5060

CD sensing of 1-(naphthalen-2-yl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.

Min ee det: 2.3% ee

Min ee calc: 3.8% ee

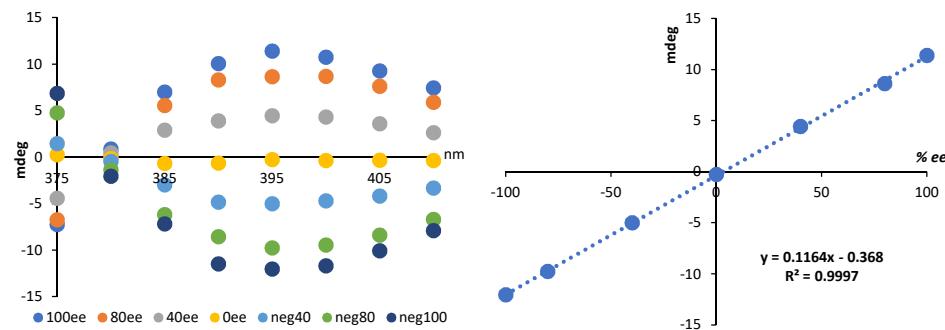
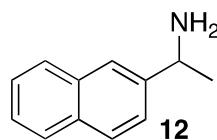


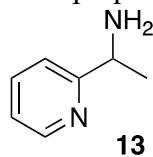
Figure S14. CD sensing of analyte **12** with **A**.

Table S13. Calibration data for analyte **12** with **A**.

Calibration data at 395 nm:

% ee	CD (mdeg)
100.0	11.3915
80.0	8.6311
40.0	4.4450
0.0	-0.2547
-40.0	-5.0051
-80.0	-9.7505
-100.0	-12.0333

CD sensing of 1-(pyridin-2-yl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 9.5% *ee*

Min *ee* calc: 15.8% *ee*

(at 405 nm: MED=2.6%; MEQ=4.3%)

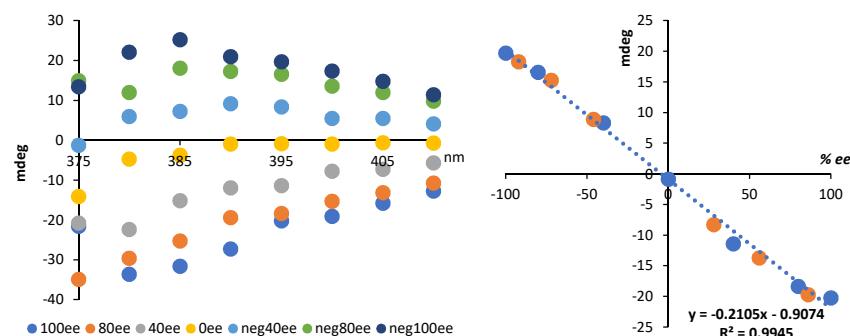


Figure S15 (a). CD sensing of analyte **13** with **A**.

Table S14 (a). Calibration data for analyte **13** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	-20.2468
80.0	-18.3840
40.0	-11.4047
0.0	-0.8733
-40.0	8.3173
-80.0	16.5375
-100.0	19.7018

Table S14 (b). Comparison of calculated and prepared *ee* data for analyte **13** with **A**.

Varied ee sample data run in parallel with calibration (orange):

Actual <i>ee</i>	CD (mdeg)	Calculated <i>ee</i>	Difference in <i>ee</i> (% <i>ee</i>)
86.0	-19.70	89.3	3.3
56.0	-13.73	60.9	4.9
28.0	-8.27	35.0	7.0
-46.0	8.88	-46.5	0.5
-72.0	15.24	-76.7	4.7
-92.0	18.31	-91.3	0.7

Calibration data at 405 nm (blue):

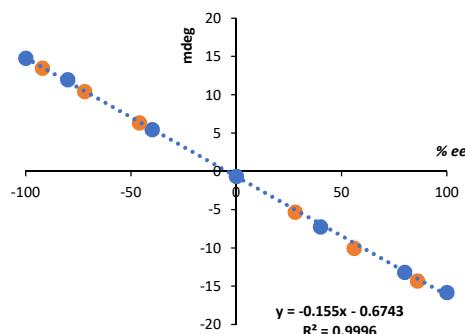


Figure S15 (b). CD sensing of analyte **13** with **A** (405 nm calibration).

Table S14 (c). Calibration data for analyte **13** with **A** (405 nm).

Calibration data at 405 nm (blue):

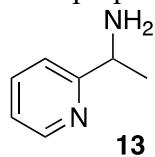
% ee	CD (mdeg)
100.0	-15.8350
80.0	-13.1643
40.0	-7.2669
0.0	-0.6469
-40.0	5.4427
-80.0	11.9774
-100.0	14.7731

Table S14 (d). Comparison of calculated and prepared ee data for analyte **13** with **A**.

Varied ee sample data run in parallel with calibration (orange):

Actual ee	CD (mdeg)	calculated ee	Difference in ee (% ee)
86.0	-14.30	87.9	1.9
56.0	-10.07	60.6	4.6
28.0	-5.31	29.9	1.9
-46.0	6.32	-45.2	0.9
-72.0	10.42	-71.6	0.4
-92.0	13.45	-91.1	0.9

CD sensing of 1-(pyridin-2-yl)ethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 3.9% *ee*

Min *ee* calc: 6.5% *ee*

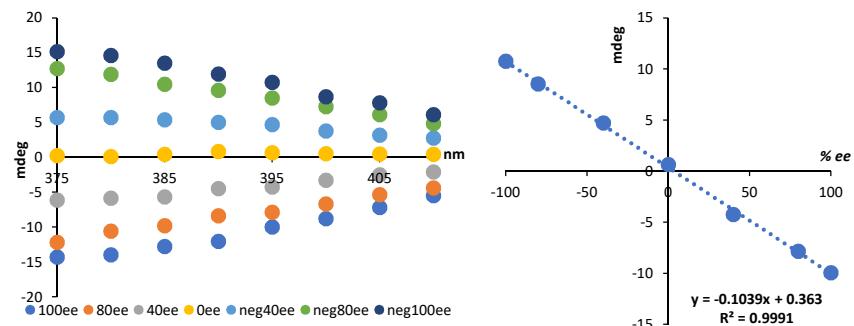


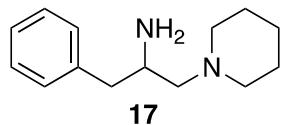
Figure S16. CD sensing of analyte **13** with A (low concentration data).

Table S15. Calibration data for analyte **13** with A (low concentration data).

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	-9.9669
80.0	-7.8554
40.0	-4.2522
0.0	0.6519
-40.0	4.7000
-80.0	8.5198
-100.0	10.7440

CD sensing of 1-phenyl-3-(piperidin-1-yl)propan-2-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 13.2% *ee*
Min *ee* calc: 22.0% *ee*

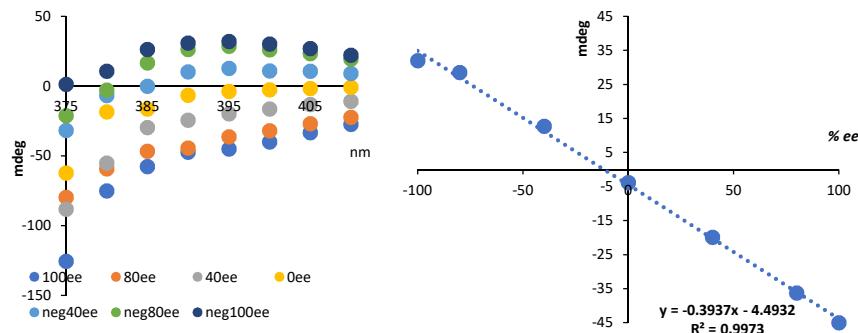


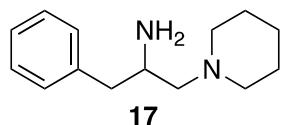
Figure S17. CD sensing of analyte **17** with **A**.

Table S16. Calibration data for analyte **17** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100	-44.9296
80	-36.2014
40	-19.8397
0	-3.7801
-40	12.7776
-80	28.5462
-100	31.9750

CD sensing of with 4-chloro-3-nitrocoumarin with 1-phenyl-3-(piperidin-1-yl)propan-2-amine and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min ee det: 3.8% ee

Min ee calc: 6.4% ee

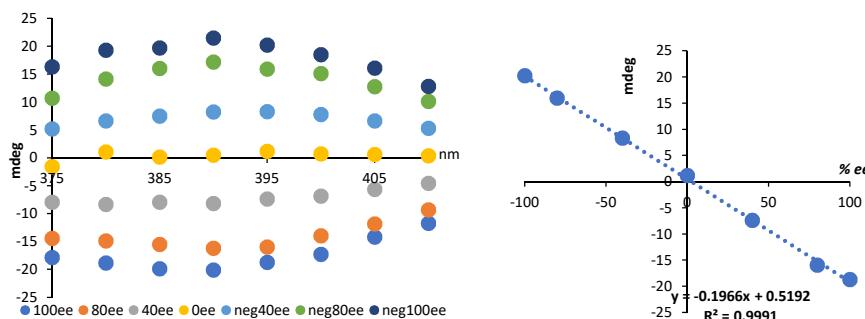


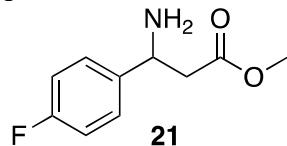
Figure S18. CD sensing of analyte **17** with **A** (low concentration data).

Table S17. Calibration data for analyte **17** with **A** (low concentration data).

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100	-18.7236
80	-15.9499
40	-7.3802
0	1.1705
-40	8.3206
-80	15.9470
-100	20.2504

CD sensing of 3-amino-3-(4-fluorophenyl)propanoate (mixtures of *R* enantiomer and raceme) with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 2.7% *ee*
Min *ee* calc: 4.4% *ee*

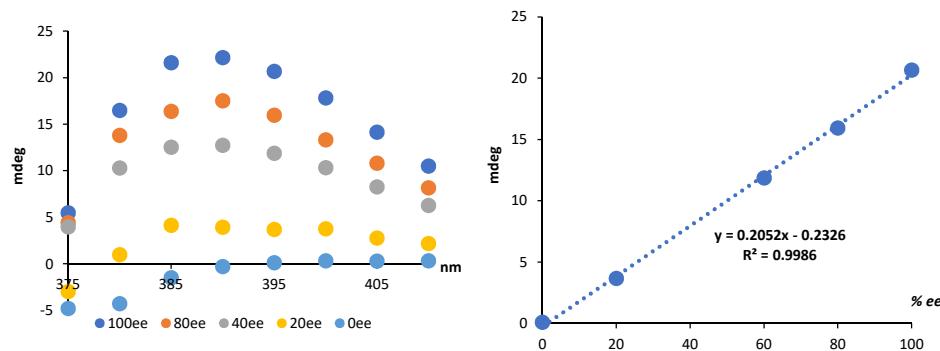


Figure S19. CD sensing of analyte **21** with A.

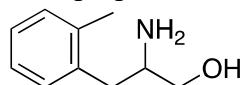
Table S18. Calibration data for analyte **21** with A.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	20.6664
80.0	15.9409
60.0	11.8506
20.0	3.6595
0.0	0.0784

2.2 Amino alcohols

CD sensing of 2-amino-3-(o-tolyl)propan-1-ol with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



14

Min *ee* det: 3.4% *ee*

Min *ee* calc: 5.6% *ee*

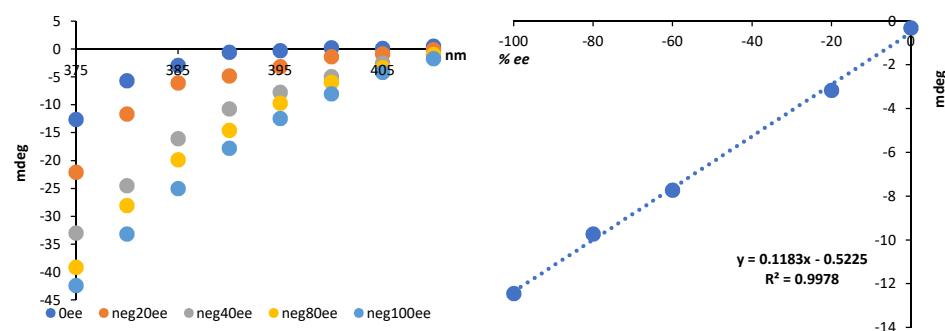


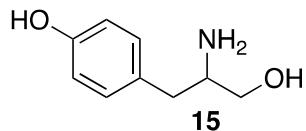
Figure S20. CD sensing of analyte **14** with **A**.

Table S19. Calibration data for analyte **14** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
0.0	-0.3104
-20.0	-3.1702
-40.0	-7.7239
-80.0	-9.7244
-100.0	-12.4519

CD sensing of 4-(2-amino-3-hydroxypropyl)phenol with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 3.1% *ee*

Min *ee* calc: 5.2% *ee*

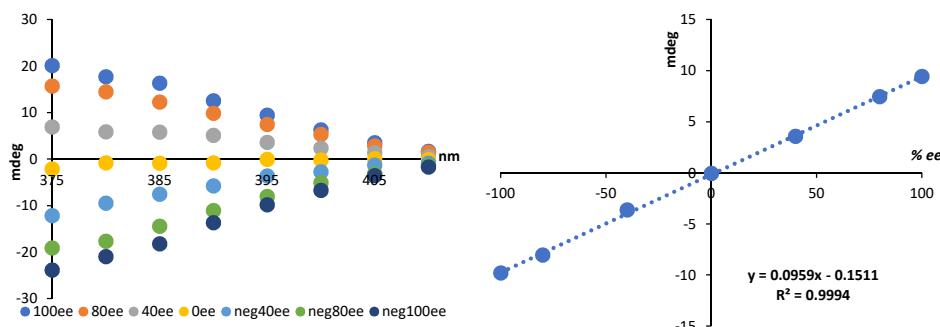


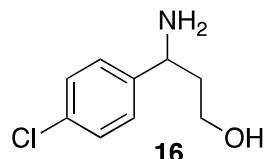
Figure S21. CD sensing of analyte **15** with A.

Table S20. Calibration data for analyte **15** with A.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	9.4333
80.0	7.4694
40.0	3.5805
0.0	-0.0682
-40.0	-3.6350
-80.0	-8.0452
-100.0	-9.7922

CD sensing of 3-amino-3-(4-chlorophenyl)propan-1-ol (using racemate and *S* enantiomer) with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 3.9% *ee*

Min *ee* calc: 6.5% *ee*

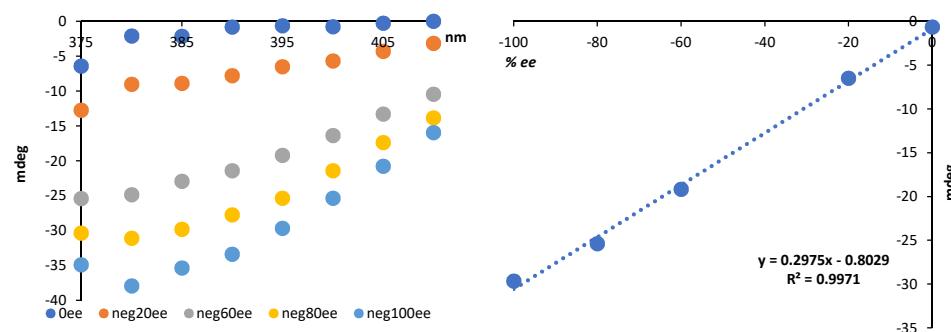


Figure S22. CD sensing of analyte **16** with **A**.

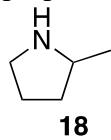
Table S21. Calibration data for analyte **16** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
0.0	-0.6434
-20.0	-6.4890
-60.0	-19.1915
-80.0	-25.3734
-100.0	-29.6622

2.3 Secondary amines

CD sensing of 2-methylpyrrolidine with 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 1.1% *ee*

Min *ee* calc: 1.8% *ee*

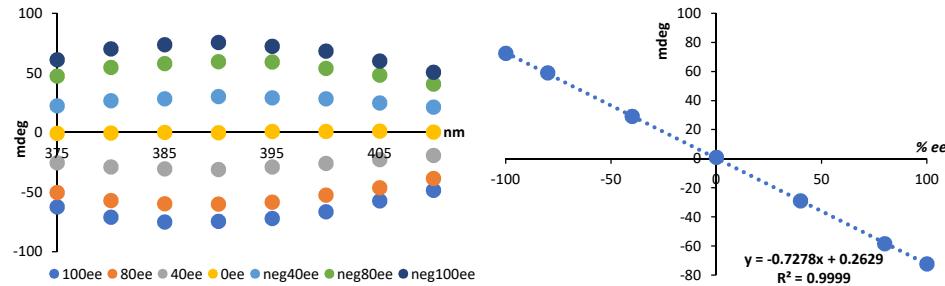


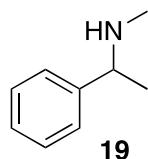
Figure S23. CD sensing of analyte **18** with **A**.

Table S22. Calibration data for analyte **18** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	-72.2418
80.0	-58.4289
40.0	-29.0504
0.0	0.8538
-40.0	29.1702
-80.0	59.0911
-100.0	72.4464

CD sensing of *N*-methyl-1-phenylethan-1-amine with 4-chloro-3-nitrocoumarin and 10 equivalents of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 7.4% *ee*

Min *ee* calc: 12.3% *ee*

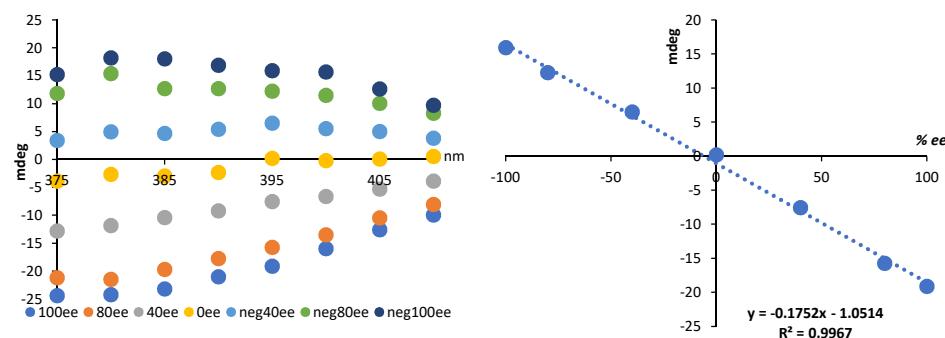


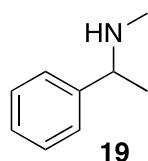
Figure S24. CD sensing of analyte **19** with **A**.

Table S23. Calibration data for analyte **19** with **A**.

Calibration data at 395 nm (blue):

% ee	CD (mdeg)
100.0	-19.1155
80.0	-15.7032
40.0	-7.5257
0.0	0.2388
-40.0	6.5119
-80.0	12.2732
-100.0	15.9607

CD sensing of with 4-chloro-3-nitrocoumarin with *N*-methyl-1-phenylethan-1-amine and 2 equivalents of triethylamine reacted at 5.00 mM and diluted to 0.300 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm.



Min *ee* det: 2.2% *ee*

Min *ee* calc: 3.6% *ee*

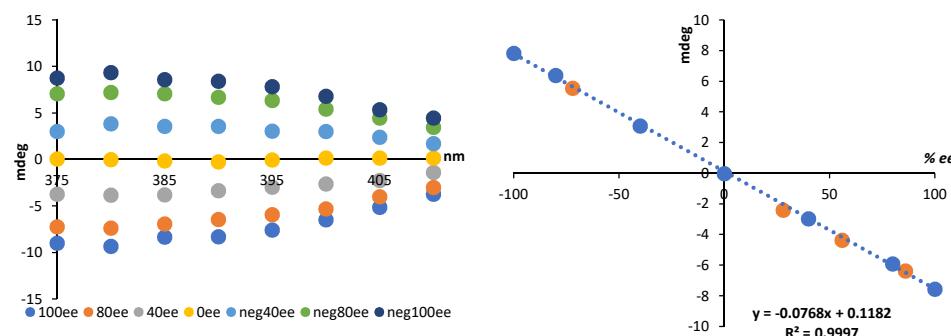


Figure S22. CD sensing of analyte **19** with **A** (low concentration data).

Table S24 (a). Calibration data for analyte **19** with **A** (low concentration data).

Calibration data at 395 nm (blue):

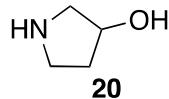
% ee	CD (mdeg)
100.0	-7.5616
80.0	-5.9090
40.0	-2.9740
0.0	-0.0185
-40.0	3.0795
-80.0	6.3824
-100.0	7.8288

Table S24 (b). Comparison of calculated and prepared *ee* data for analyte **19** with **A** (low concentration data).

Varied ee sample data run in parallel with calibration (orange):

Actual <i>ee</i>	CD (mdeg)	Calculated <i>ee</i>	Difference in <i>ee</i> (% <i>ee</i>)
86.0	-6.36	84.41	1.6
56.0	-4.38	58.54	2.6
28.0	-2.41	32.87	4.9
-72.0	5.56	-70.88	1.1

CD sensing of with 4-chloro-3-nitrocoumarin with pyrrolidin-3-ol and 1 equivalent of triethylamine reacted at 5.00 mM and diluted to 0.600 mM for chiroptical sensing. Reactions were prepared following the general protocol above. Calibration data were taken at 410 nm.



Min *ee* det: 3.4% *ee**

Min *ee* calc: 5.6% *ee**

*For readings at 410 nm

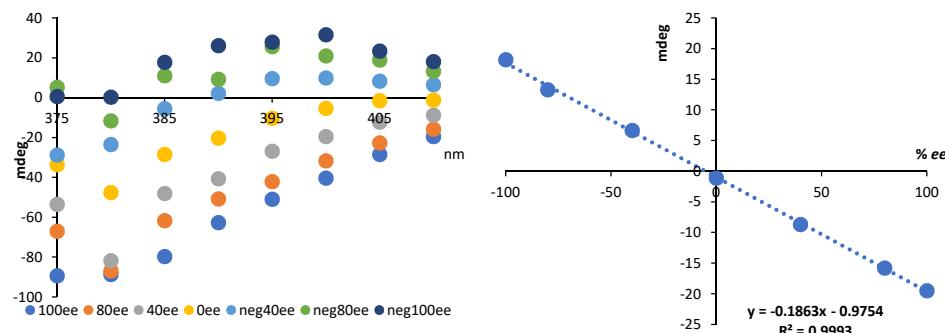


Figure S23. CD sensing of analyte **20** with A.

Table S25. Calibration data for analyte **20** with A.

Calibration data at 410 nm (blue):

<i>ee</i>	mdeg
100.0	-19.4685
80.0	-15.7860
40.0	-8.6630
0.0	-1.0948
-40.0	6.6828
-80.0	13.2848
-100.0	18.2167

3. Accuracy and precision analysis

3.1 Formulae for minimum ee detectable and quantifiable (MED/MEQ)

Minimum ee detectable (MED) and minimum ee quantifiable (MEQ) determined by using the standard error of linear fit for each analyte calibration described in SI section 2.

$$\text{MED} = \frac{(ee \text{ cal std. error}) \times 3}{2}$$

$$\text{MEQ} = \frac{(ee \text{ cal std. error}) \times 5}{2}$$

3.2 Summary of accuracy metrics by %ee

Table S26. Tabulated accuracy metrics by analyte.

Entry	Analyte	Sample (% ee)	EKKO (% ee)	Absolute error (% ee)	Averaged abs. error	MED, MEQ
1	1	86.0 (R)	83.5 ^a	2.5		
2	1	56.0 (R)	53.4 ^a	2.6		
3	1	28.0 (R)	23.2 ^a	4.8		
4	1	-46.0 (S)	-45.9 ^a	0.1		
5	1	-72.0 (S)	-73.0 ^a	1.0	2.2	2.1, 3.5
6	2	28.0 (R)	24.4 ^b	3.6		
7	2	-46.0 (S)	-42.1 ^b	3.9		
8	2	-72.0 (S)	-70.3 ^b	1.7		
9	2	-92.0 (S)	-92.7 ^b	0.7	2.5	2.6, 4.4
10	3 ^e	86.0 (R)	80.7 ^a	5.3		
11	3 ^e	56.0 (R)	49.9 ^a	6.1		
12	3 ^e	28.0 (R)	22.1 ^a	5.9		
13	3 ^e	-46.0 (S)	-47.3 ^a	1.3		
14	3 ^e	-72.0 (S)	-66.0 ^a	6.0	4.9	3.0, 5.0
15	4	86.0 (R)	87.4 ^a	1.5		
16	4	56.0 (R)	56.6 ^a	0.6		
17	4	28.0 (R)	28.4 ^a	0.4		
18	4	-46.0 (S)	-44.0 ^a	2.1		
19	4	-72.0 (S)	-72.5 ^a	0.5	1.0	0.9, 1.5
20	13 ^e	86.0 (R)	89.3 ^c	3.3		
21	13 ^e	56.0 (R)	60.9 ^c	4.9		
22	13 ^e	28.0 (R)	35.0 ^c	7.0		
23	13 ^e	-46.0 (S)	-46.5 ^c	0.5		
24	13 ^e	-72.0 (S)	-76.7 ^c	4.7		
25	13 ^e	-92.0 (S)	-91.3 ^c	0.7	3.5	2.6, 4.3
26	19	86.0 (R)	84.4 ^d	1.6		
27	19	56.0 (R)	58.5 ^d	2.5		
28	19	28.0 (R)	32.9 ^d	4.9		
29	19	-72.0 (S)	-70.9 ^d	1.1	2.5	2.2, 3.6

Solutions were prepared as described above. ^aMeasurements at 0.6 mM (1 equiv. of Et₃N) in ACN. ^b0.3 mM (10 equiv. of Et₃N). ^c0.6 mM (10 equiv. of Et₃N). ^d0.3 mM (2 equiv. of Et₃N).

^e405 nm.

3.3 Assay precision estimation between wells

EKKO parameters were set to scan every 5 nm 375-410 nm with 40 mdeg sensitivity, 3 second integration time and single data accumulation. Individual well blanking with acetonitrile was applied before sample scans were collected. CD sensing was carried out with enantiopure (*R*)-1-cyclohexylethan-1-amine using 4-chloro-3-nitrocoumarin and 1 equivalent of triethylamine mixed at 5.00 mM and diluted to 0.600 mM for chiroptical sensing after 20 minutes. CD signals for the different wells in the quartz plate were compared at 395 nm with average and standard deviation values reported by CD intensities (mdeg).

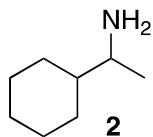


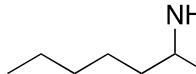
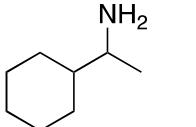
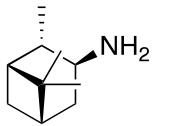
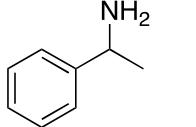
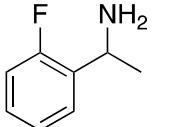
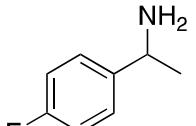
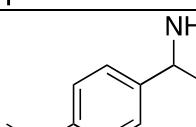
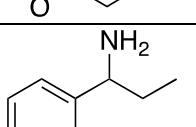
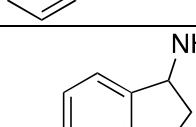
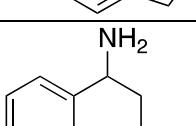
Table S27. Replicate CD measurements for enantiopure **2** with A.

Entry	CD (mdeg)
1	18.3808
2	19.7558
3	17.3792
4	18.0662
5	18.0745
6	19.1789
7	19.1064
8	19.6535
9	19.4360
10	19.8321
11	18.8046
12	19.9456
Average	18.9678
1 std. dev.	0.8303

3.4 Tabulated MED/MEQ by analyte

Table S28. Tabulated MED/MEQ data.

All data from measurements at 395nm. Accuracy is reported as averaged %*ee* difference from actual values.

Analyte	Conditions (base & sensing conc.)	Signal at +100% <i>ee</i> (<i>R</i>)	Signal at - 100% <i>ee</i> (<i>S</i>)	MED	MEQ	UV at 395 nm	λ_{\max}	Accuracy (<i>ee</i>)
	1 eq Et ₃ N 0.600 mM	11.436	-11.696	2.1	3.5	.522	395	2.2%
	10 eq Et ₃ N 0.600 mM	20.870	-21.331	2.6	4.4	.652	395	2.5%
	1 eq Et ₃ N 0.600 mM	6.020	-4.654	5.4	9.1	.503	405	4.9%
	1 eq Et ₃ N 0.600 mM	28.483	-29.619	0.9	1.5	.547	390	1.8%
	10 eq Et ₃ N 0.600 mM	n/a	-27.911	7.9	13.1	.666	390	
	10 eq Et ₃ N 0.300 mM	15.117	-15.221	1.8	3.0	.272	390	
	1 eq Et ₃ N 0.600 mM	21.334	-20.907	3.0	5.0	.558	395	
	1 eq Et ₃ N 0.600 mM	15.679	-18.699	2.9	4.8	.508	395	
	1 eq DBU 0.600 mM	-6.221	4.665	9.5	15.8	.570	385	
	1 eq Et ₃ N 0.600 mM	-8.272	8.193	6.7	11.1	.543	390	

	10 eq Et ₃ N 0.300 mM	8.898	-8.506	2.9	4.9	.370	395	
	10 eq Et ₃ N 0.300 mM	11.3915	-12.033	2.3	3.8	.285	395	
	10 eq Et ₃ N 0.600 mM	-20.247	19.702	9.5	15.8	1.141	385	3.5%
	10 eq Et ₃ N 0.600 mM	n/a	-12.452	3.4	5.6	.668	375	
	10 eq Et ₃ N 0.300 mM	9.433	-9.792	3.1	5.2	.363	<375	
	1 eq Et ₃ N 0.600 mM	n/a	-29.662	3.9	6.5	.746	380	
	10 eq Et ₃ N 0.300 mM	-18.723	20.250	3.8	6.4	0.54	390	
	1 eq Et ₃ N 0.300 mM	-72.242	72.446	1.1	1.9	.976	390	
	10 eq Et ₃ N 0.600 mM	-19.116	15.961	7.4	12.3	1.152	380	
	2 eq Et ₃ N 0.600 mM	-15.401	15.292	3.3	5.5	.7848	380	
	2 eq Et ₃ N 0.300 mM	-7.562	7.829	2.2	3.6	.377	380	2.5%
	1 eq Et ₃ N 0.600 mM	-40.489	38.259	8.5	14.2	1.584	395	

<chem>NCC(C(=O)OC)c1ccc(F)cc1</chem>	10 eq Et ₃ N 0.600 mM	20.666	n/a	2.7	4.4	.618	390	
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4. Effects of base

CD sensing with 4-chloro-3-nitrocoumarin and 1-phenylpropan-1-amine and 1 equivalent of triethylamine or DBU reacted at 5.00 mM and diluted to 0.600 mM were compared. Reactions were prepared following the general protocol above. Calibration data were taken at 395 nm (tabulated in lower right quadrant).

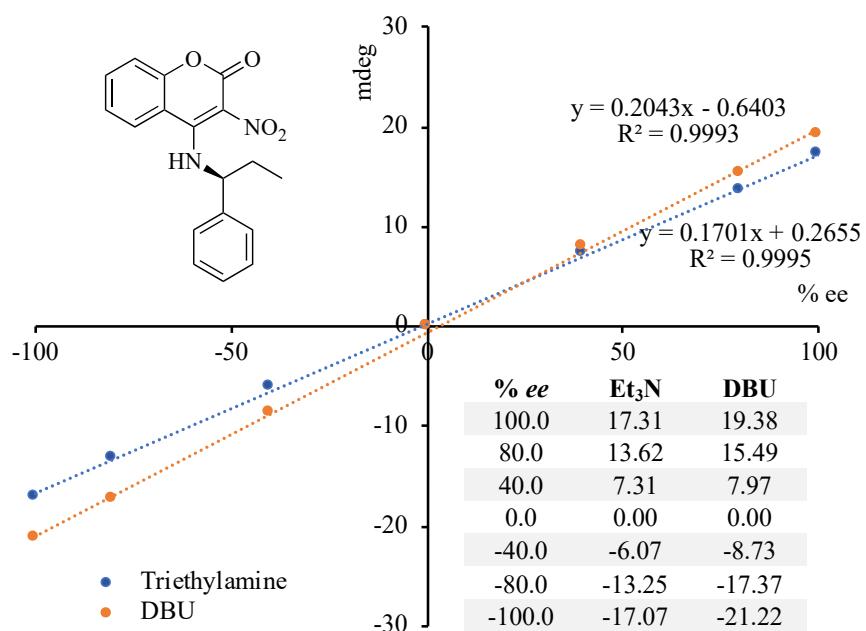


Figure S24. CD calibration data using Et₃N and DBU with analyte **8** with **A**.

5. Interferent analysis

Interferent studies were conducted using 1-cyclohexylethyl-1-amine, 1 equivalent of triethylamine and a stoichiometric amount of the coumarin probe. Reactions were carried out at 5.00 mM consistent with the analyte screening experiments described above. Sensing using the EKKO then allowed for the assessment of interferent significance at the standard 0.600 mM analytical concentration. Various interferent amounts were added to each reaction vial containing **A** and Et₃N in acetonitrile. After 10 minutes, a stoichiometric equivalent of (*R*)-1-cyclohexylethyl-1-amine was added and the mixture was allowed to equilibrate for 30 minutes. The samples were then diluted to 0.600 mM for plating into the quartz 96-well plate where chiroptical analysis was carried out. EKKO parameters were set to scan every 5 nm 375-410 nm with 40 mdeg sensitivity, 3 second integration time.

i. DMF

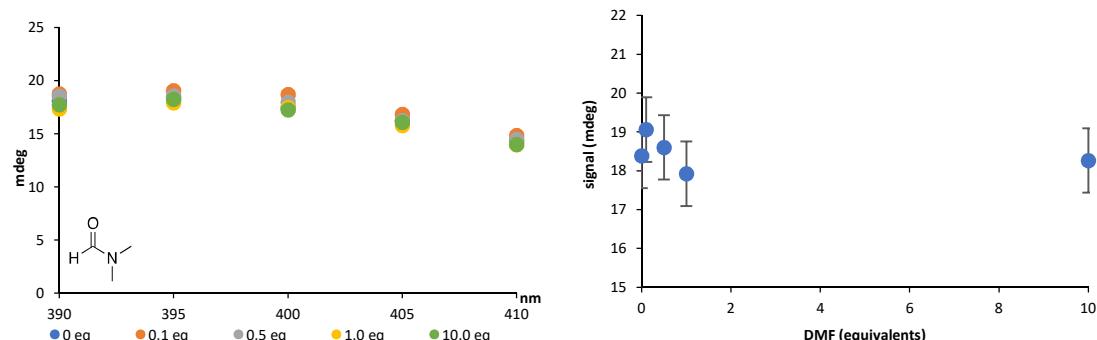


Figure S25. Interferent data for **2** and **A** with varied amounts of DMF.

Table S29 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of DMF.

DMF (equiv)	CD (mdeg)
0.0	18.38081
0.1	19.05924
0.5	18.60195
1.0	17.92278
10.0	18.26321

Table S29 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of DMF.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	3.701092	8.292312	4.965341	6.120441	7.795395	2.074932
380	12.96051	13.65538	12.39335	14.9835	11.06632	12.52703
385	15.58515	17.54167	15.25019	15.8854	15.66294	16.65219
390	18.1039	18.77794	18.5661	17.35267	15.34996	17.75563
395	18.38081	19.05924	18.60195	17.92278	16.37927	18.26321
400	17.47841	18.70522	17.98386	17.48511	16.43598	17.27659
405	16.26408	16.84489	16.27087	15.78196	14.60638	16.092
410	14.30861	14.84353	14.48851	13.98264	12.74981	14.02224
UV						
375	1.718048	1.733231	1.710347	1.670493	1.615953	1.691837
380	1.383134	1.392421	1.371531	1.341805	1.298872	1.366439
385	1.085446	1.090286	1.07103	1.049117	1.016154	1.074102
390	0.840675	0.842016	0.824899	0.8088	0.784242	0.832675
395	0.640674	0.63885	0.623686	0.611743	0.593833	0.633286
400	0.478229	0.475117	0.461502	0.452853	0.440297	0.471749
405	0.346385	0.341429	0.329618	0.323946	0.315705	0.34012
410	0.244222	0.239373	0.230379	0.226793	0.222288	0.239626

ii. Toluene

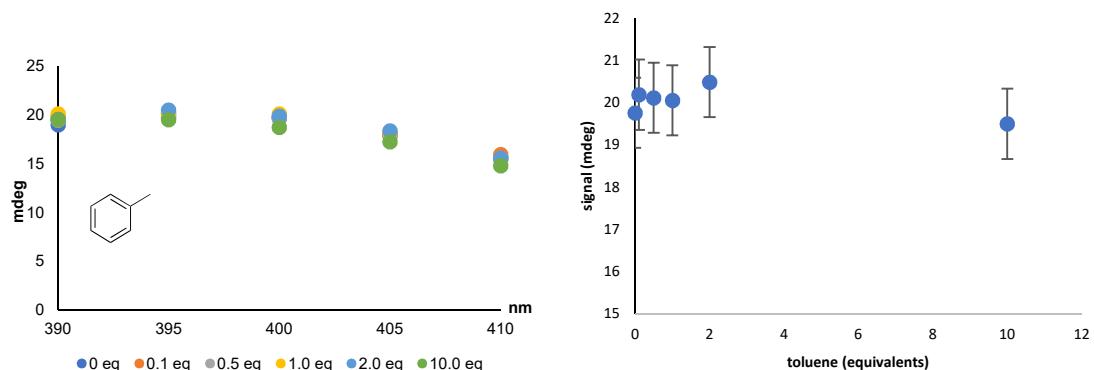


Figure S26. Interferent data for **2** and **A** with varied amounts of toluene.

Table S30 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of toluene.

Toluene (equiv)	CD (mdeg)
0.0	19.7558
0.1	20.1864
0.5	20.1140
1.0	20.0526
2.0	20.4845
10.0	19.4970

Table S30 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of toluene.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	0.0 eq
375	7.083969	2.818225	6.045797	3.772065	7.903865	5.064323
380	12.56743	13.00495	12.68393	13.94949	14.49623	9.4914
385	16.96258	17.62398	18.26528	19.02011	17.75866	18.30842
390	18.97308	19.83142	19.45918	20.0986	19.54636	19.50221
395	19.75584	20.1864	20.11397	20.05257	20.48446	19.49697
400	19.65828	19.81603	19.64947	20.10437	19.87865	18.72258
405	17.88028	18.01678	18.09863	18.18659	18.37712	17.25384
410	15.56483	15.93171	15.48895	15.53776	15.56283	14.78889
UV						
375	1.742633	1.748625	1.7416	1.755353	1.742738	1.681584
380	1.381489	1.390053	1.383918	1.396007	1.388289	1.338851
385	1.06113	1.071255	1.065477	1.076622	1.071019	1.032254
390	0.801897	0.811202	0.806053	0.815485	0.810949	0.780258
395	0.592663	0.600061	0.595846	0.604269	0.600417	0.576462
400	0.426577	0.43251	0.428912	0.435385	0.432428	0.414058
405	0.293942	0.298835	0.295581	0.300892	0.29821	0.284843
410	0.197894	0.201049	0.199072	0.202848	0.200641	0.191482

iii. Triphenylphosphine

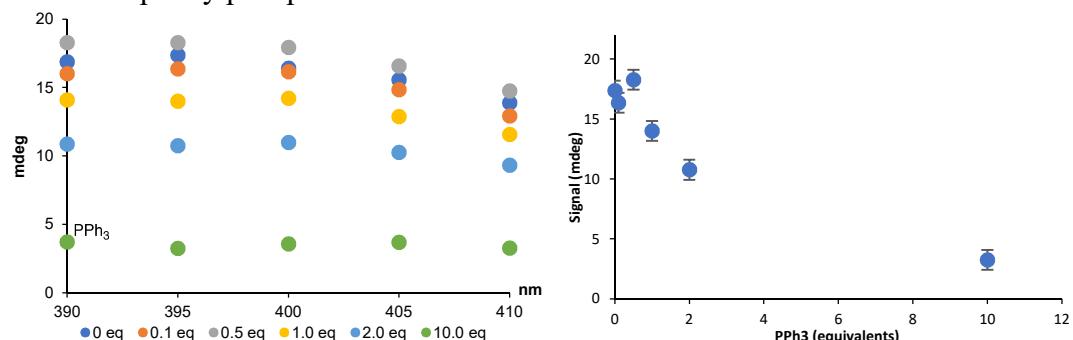


Figure S27. Interferent data for **2** and **A** with varied amounts of triphenylphosphine.

Table S31 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of triphenylphosphine.

PPh ₃ (equiv)	CD (mdeg)
0.0	17.37922
0.1	16.36394
0.5	18.28477
1.0	14.01682
2.0	10.76414
10.0	3.249209

Table S31 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of triphenylphosphine.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	8.54564	4.866404	12.66656	9.284744	1.837116	-2.44786
380	14.88412	12.36525	13.34874	9.884215	5.206082	-2.33699
385	15.65549	15.94776	16.76504	13.07539	10.17251	4.24934
390	16.88801	16.01717	18.28445	14.10031	10.86694	3.720726
395	17.37922	16.36394	18.28477	14.01682	10.76414	3.249209
400	16.42408	16.15882	17.93624	14.21902	10.98211	3.58857
405	15.56214	14.85555	16.56844	12.87517	10.25904	3.703028
410	13.89939	12.92723	14.7573	11.57629	9.337602	3.284698
UV						
	1.716079	1.692594	1.800917	1.765264	1.732444	1.762746
	1.400286	1.384659	1.47871	1.459009	1.446314	1.505728
	1.118751	1.107806	1.187532	1.179176	1.179194	1.25546
	0.885432	0.875495	0.942942	0.939606	0.947361	1.02743
	0.689973	0.678635	0.734941	0.726623	0.732503	0.790903
	0.528799	0.515116	0.560858	0.540528	0.537177	0.550939
	0.395308	0.37926	0.415608	0.383671	0.370418	0.342134
	0.287836	0.271959	0.299294	0.264494	0.247455	0.202611

iv. Dimethylsulfoxide

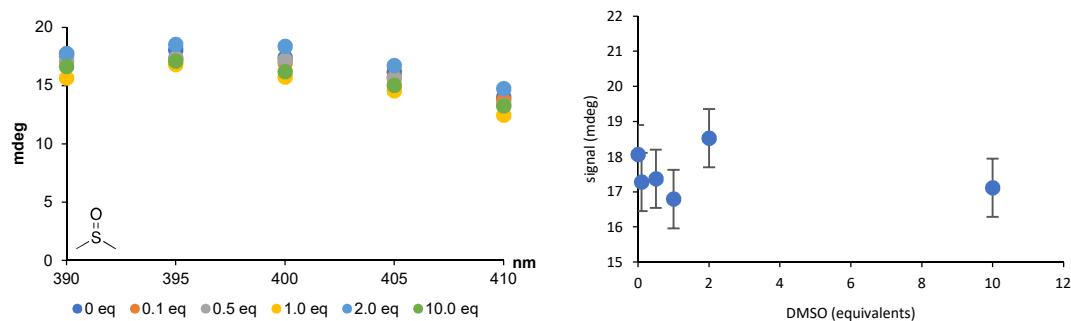


Figure S28. Interferent data for **2** and **A** with varied amounts of dimethylsulfoxide.

Table S32 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of dimethylsulfoxide.

DMSO (equiv)	CD (mdeg)
0.0	18.06618
0.1	17.2803
0.5	17.36935
1.0	16.7928
2.0	18.52839
10.0	17.11227

Table S32 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of dimethylsulfoxide.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	7.023398	9.169786	4.45887	8.969941	9.459262	13.56309
380	13.88426	12.05777	11.82059	12.51915	13.45562	10.86051
385	17.27999	14.78185	16.0864	14.38782	17.68922	16.49466
390	17.73993	17.51911	17.09217	15.65627	17.72781	16.63178
395	18.06618	17.2803	17.36935	16.7928	18.52839	17.11227
400	17.40174	16.96609	17.22019	15.7242	18.37616	16.2069
405	16.17075	15.7191	15.58869	14.56176	16.72548	15.02619
410	14.00302	13.74776	13.32117	12.46518	14.73882	13.24656
UV						
375	1.717594	1.712273	1.675421	1.610834	1.735062	1.654324
380	1.391157	1.386544	1.348464	1.289535	1.406037	1.334974
385	1.098275	1.095013	1.056985	1.003432	1.107402	1.045336
390	0.856234	0.854208	0.816948	0.768637	0.858931	0.806266
395	0.6553	0.653663	0.619586	0.577094	0.652976	0.609509
400	0.491796	0.490363	0.459462	0.422547	0.486109	0.450309
405	0.357418	0.356967	0.329162	0.299297	0.350181	0.321518
410	0.253266	0.25322	0.231226	0.207668	0.245565	0.224366

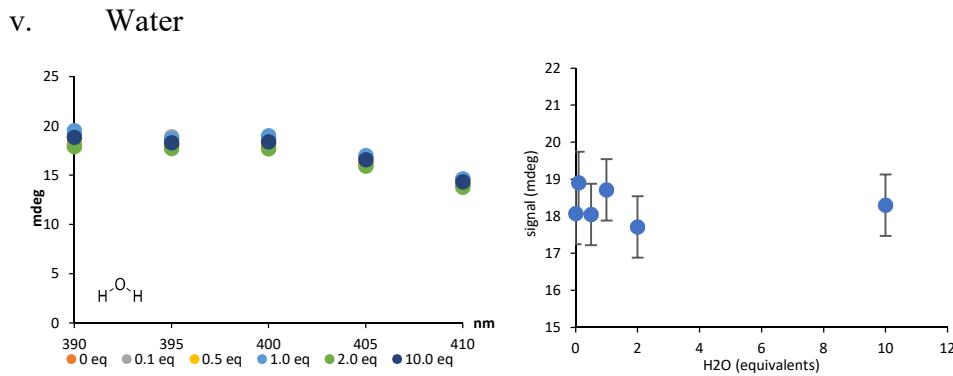


Figure S29. Interferent data for **2** and **A** with varied amounts of water.

Table S33 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of water.

H ₂ O (equiv)	CD (mdeg)
0.0	18.0745
0.1	18.9078
0.5	18.0516
1.0	18.7149
2.0	17.7119
10.0	18.3002

Table S33 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of water.

	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	4.529279	3.870102	9.927652	5.545256	8.423003	11.04102
380	13.73541	11.69039	12.50507	12.36893	10.8599	12.51319
385	16.98852	16.41388	15.96192	17.56314	15.91163	16.38819
390	18.14568	19.18778	17.97178	19.51287	17.8993	18.82341
395	18.07451	18.90784	18.05158	18.71489	17.71194	18.30025
400	17.90979	18.73347	18.18899	18.99775	17.65592	18.39157
405	16.10045	16.777	16.44233	17.00239	15.90509	16.56408
410	14.02888	14.61895	14.19094	14.60323	13.77857	14.33543
375	1.668566	1.695892	1.674304	1.704789	1.637653	1.660507
380	1.326978	1.350175	1.330835	1.359494	1.303475	1.324884
385	1.021542	1.041704	1.025121	1.050567	1.004606	1.022823
390	0.771008	0.788174	0.774318	0.794889	0.757769	0.773502
395	0.569884	0.583242	0.572191	0.589653	0.559772	0.57284
400	0.410121	0.420758	0.411838	0.425966	0.402594	0.413158
405	0.28264	0.290113	0.283679	0.294533	0.277444	0.286433
410	0.191226	0.196688	0.19195	0.200168	0.188119	0.194821

vi. Ethyl acetate

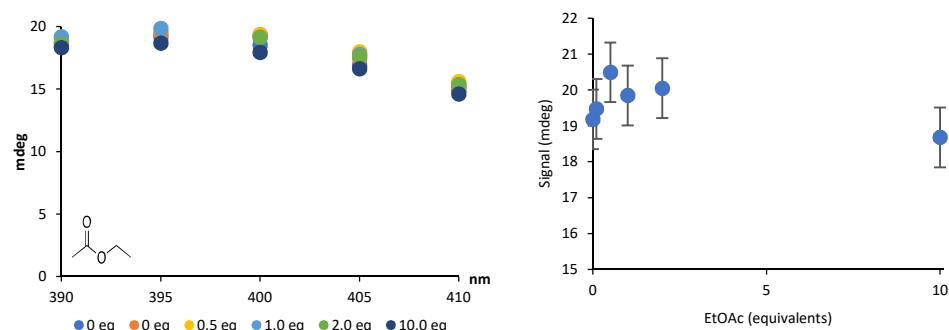


Figure S30. Interferent data for **2** and **A** with varied amounts of ethyl acetate.

Table S34 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of ethyl acetate.

EtOAc (equiv)	CD (mdeg)
0.0	19.17887
0.1	19.4692
0.5	20.49106
1.0	19.8442
2.0	20.04825
10.0	18.67634

Table S34 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of ethyl acetate.

CD	0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	-0.48919	8.146048	6.885185	6.838701	6.198051	8.118895
380	15.12203	15.05639	14.73077	11.45069	13.86383	15.29953
385	16.02069	17.11142	18.30594	16.32957	17.7578	15.83015
390	18.73287	18.44296	19.04935	19.1876	18.64363	18.30497
395	19.17887	19.4692	20.49106	19.8442	20.04825	18.67634
400	18.52238	19.36867	19.33053	19.14042	19.16359	17.92607
405	16.90826	17.44444	17.99143	17.77835	17.62524	16.62409
410	14.94242	15.13527	15.592	15.33779	15.35586	14.61088
UV						
375	1.725108	1.745141	1.762389	1.752099	1.738615	1.651683
380	1.373423	1.39185	1.407673	1.404252	1.393468	1.322029
385	1.061168	1.076546	1.08961	1.089195	1.081109	1.024327
390	0.804896	0.817425	0.828588	0.828821	0.822727	0.777755
395	0.597753	0.608059	0.61698	0.617639	0.612953	0.577785
400	0.431107	0.439734	0.446718	0.448024	0.444224	0.417644
405	0.298452	0.30525	0.310052	0.311363	0.309017	0.289417
410	0.202428	0.207523	0.210765	0.211953	0.210037	0.196722

vii. Tetrahydrofuran

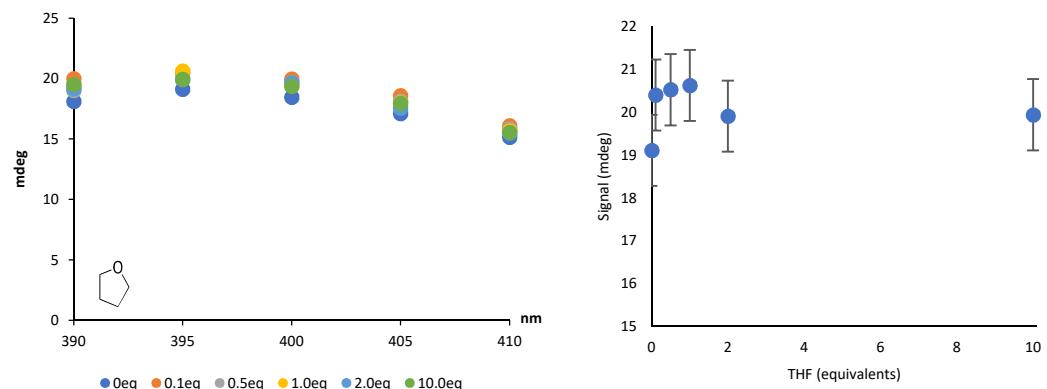


Figure S31. Interferent data for **2** and **A** with varied amounts of tetrahydrofuran.

Table S35 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et tetrahydrofuran.

THF (equiv)	CD (mdeg)
0.0	19.1064
0.1	20.3974
0.5	20.5231
1.0	20.6233
2.0	19.9035
10.0	19.9389

Table S35 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of tetrahydrofuran.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	7.895279	5.954861	8.549965	8.21884	3.968195	8.412551
380	13.57993	14.58251	12.83173	12.94309	13.07442	12.75243
385	16.9769	17.60412	18.02289	16.919	17.32295	17.45985
390	18.09744	19.9966	18.99892	19.4864	19.1757	19.50885
395	19.10635	20.39739	20.52312	20.62328	19.90353	19.93892
400	18.44282	19.96537	19.65747	19.49072	19.64772	19.34087
405	17.10894	18.58819	18.12525	17.9905	17.55352	17.94613
410	15.14092	16.09671	15.81724	15.66071	15.47214	15.54338
UV						
375	1.742156	1.852016	1.837712	1.828598	1.785967	1.813445
380	1.400724	1.487459	1.479824	1.467923	1.434403	1.456966
385	1.098722	1.165446	1.162817	1.149771	1.121694	1.139859
390	0.848808	0.899294	0.900171	0.886015	0.86272	0.876849
395	0.642426	0.680277	0.683396	0.669822	0.64991	0.661422
400	0.475498	0.503199	0.507965	0.494649	0.478152	0.487099
405	0.339888	0.359348	0.365111	0.353249	0.339676	0.346081
410	0.236111	0.248629	0.254554	0.244477	0.234052	0.238545

viii. (*S*)-BINOL

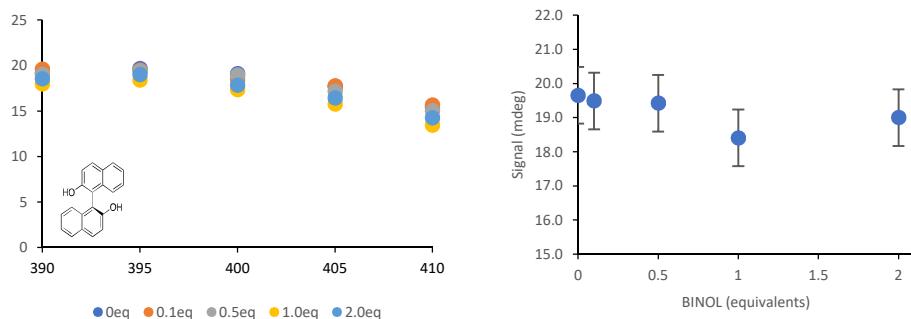


Figure S32. Interferent data for **2** and **A** with varied amounts of (*S*)-BINOL.

Table S36 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et (*S*)-BINOL.

(<i>S</i>)- BINOL (equiv)	CD (mdeg)
0.0	19.6535
0.1	19.4858
0.5	19.4201
1.0	18.4063
2.0	19.0014

Table S36 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of (*S*)-BINOL.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq
375	5.582212	5.234453	9.923106	11.05803	8.375974
380	11.77665	12.72403	14.15745	15.32597	12.35943
385	16.87861	17.49616	17.91014	17.03139	16.85476
390	19.17328	19.60485	19.01182	17.97823	18.54933
395	19.65345	19.48577	19.42013	18.40625	19.00135
400	19.0915	18.46898	18.94696	17.33868	17.83717
405	17.69638	17.75105	17.18179	15.74854	16.4312
410	15.39126	15.65046	15.01221	13.44825	14.26149
UV					
375	1.820479	1.824241	1.801844	1.678293	1.742228
380	1.470496	1.469771	1.453746	1.354318	1.407023
385	1.160803	1.155759	1.145959	1.067146	1.108719
390	0.904358	0.896655	0.890672	0.828879	0.861745
395	0.692931	0.68331	0.680454	0.631785	0.657149
400	0.519461	0.508724	0.507693	0.469479	0.489308
405	0.377955	0.366733	0.36677	0.337124	0.351994
410	0.26657	0.256244	0.256864	0.235538	0.246317

ix. [Rh(COD)Cl]₂

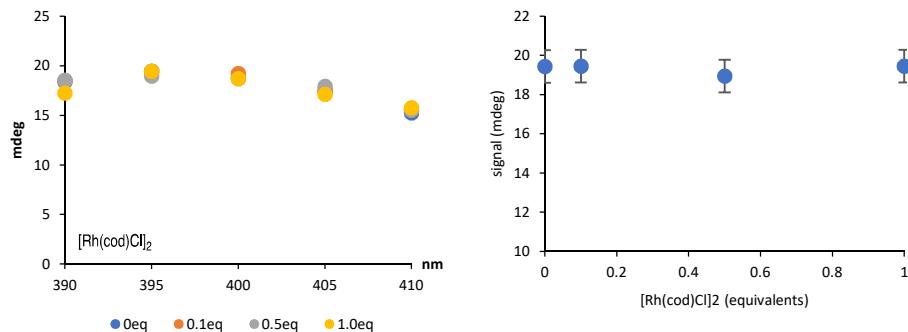


Figure S33. Interferent data for **2** and **A** with varied amounts of [Rh(COD)Cl]₂.

Table S37 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et [Rh(COD)Cl]₂.

[Rh(cod)Cl] ₂ (equiv)	CD (mdeg)
0.0	19.4360
0.1	19.4468
0.5	18.9465
1.0	19.4445

Table S37 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of [Rh(COD)Cl]₂.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq
375	6.694352	12.05132	8.87196	11.56723
380	15.12419	12.78723	11.23852	13.45279
385	16.63083	17.48959	15.70633	14.5928
390	18.39106	18.50842	18.52775	17.24779
395	19.436	19.44683	18.94648	19.44451
400	18.86852	19.21828	18.70327	18.68979
405	17.37842	17.57623	17.91316	17.10875
410	15.2384	15.51771	15.54418	15.74621

UV	0.0 eq	0.1 eq	0.5 eq	1.0 eq
1.809521	1.852397	1.97485	2.085597	
1.468701	1.503888	1.635029	1.758958	
1.164571	1.195696	1.333662	1.467441	
0.911105	0.941066	1.084627	1.227369	
0.701541	0.730154	0.873614	1.019206	
0.528456	0.552408	0.685187	0.822153	
0.386777	0.404527	0.518608	0.640574	
0.274321	0.287163	0.381743	0.489058	

x. Methanol

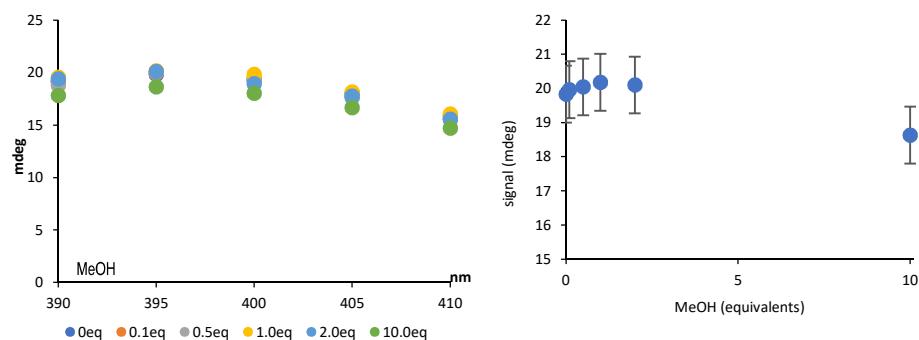


Figure S34. Interferent data for **2** and **A** with varied amounts of methanol.

Table S38 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et methanol.

MeOH (equiv)	CD (mdeg)
0.0	19.83215
0.1	19.96178
0.5	20.04448
1.0	20.17724
2.0	20.09847
10.0	18.63258

Table S38 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of methanol.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	3.601986	4.698122	9.378057	5.986872	10.45049	4.881062
380	11.96795	14.72664	14.28138	12.44053	13.62449	12.14835
385	17.934	17.02359	17.11359	17.56534	17.13455	16.1223
390	19.16995	19.14585	18.78837	19.56112	19.40186	17.83314
395	19.83215	19.96178	20.04448	20.17724	20.09847	18.63258
400	19.28558	19.46462	19.36391	19.81905	18.93614	18.02641
405	18.0432	17.7535	17.61235	18.14332	17.75216	16.64985
410	15.90397	15.65961	15.60666	16.06345	15.56104	14.71105
UV						
375	1.836311	1.820588	1.802461	1.843751	1.808769	1.826828
380	1.478489	1.46259	1.450484	1.480987	1.454445	1.474535
385	1.16066	1.145585	1.136396	1.157567	1.137825	1.159191
390	0.896867	0.883182	0.875911	0.889406	0.875567	0.896787
395	0.679372	0.667086	0.661418	0.669386	0.660123	0.680417
400	0.503172	0.492731	0.487795	0.491671	0.48612	0.504533
405	0.36017	0.351269	0.347031	0.347981	0.344832	0.361696
410	0.249533	0.242896	0.239749	0.238805	0.2376	0.251599

xi. Chloroform

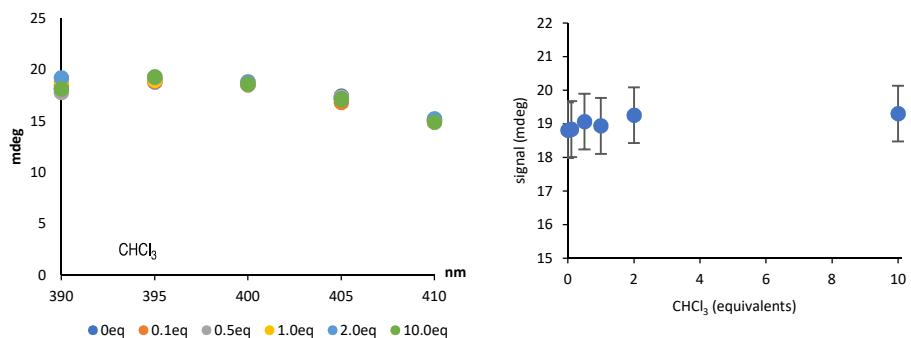


Figure S35. Interferent data for **2** and **A** with varied amounts of chloroform.

Table S39 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et chloroform.

CHCl ₃ (equiv)	CD (mdeg)
0.0	18.8046
0.1	18.8396
0.5	19.0639
1.0	18.9368
2.0	19.2524
10.0	19.3025

Table S39 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of chloroform.

CD	0.0 eq	0.1 eq	0.5 eq	1.0 eq	2.0 eq	10.0 eq
375	7.335403	5.960458	9.38448	10.27618	3.096751	5.291764
380	13.15671	14.54055	13.46599	13.0278	12.61094	11.47759
385	17.85132	16.14495	17.06184	17.73589	15.08928	17.00699
390	18.17275	18.50821	17.76669	18.87009	19.19333	18.09718
395	18.80465	18.83965	19.0639	18.93675	19.2524	19.30248
400	18.62235	18.51533	18.52288	18.83091	18.79042	18.56628
405	17.41822	16.79387	17.17839	17.30508	17.20239	17.08762
410	15.03594	15.01989	15.09315	15.14193	15.19831	14.88156
UV						
375	1.817432	1.796224	1.791505	1.793464	1.790103	1.805425
380	1.476105	1.454319	1.449965	1.450343	1.44519	1.457407
385	1.170595	1.149186	1.143591	1.142617	1.136102	1.145454
390	0.915295	0.89554	0.889228	0.887255	0.879556	0.88691
395	0.704409	0.685951	0.679078	0.676499	0.668252	0.674417
400	0.530666	0.514337	0.50733	0.504099	0.496012	0.501018
405	0.38796	0.374059	0.367077	0.363784	0.356035	0.359981
410	0.275216	0.263634	0.257715	0.254615	0.248154	0.251119

xii. Acetone

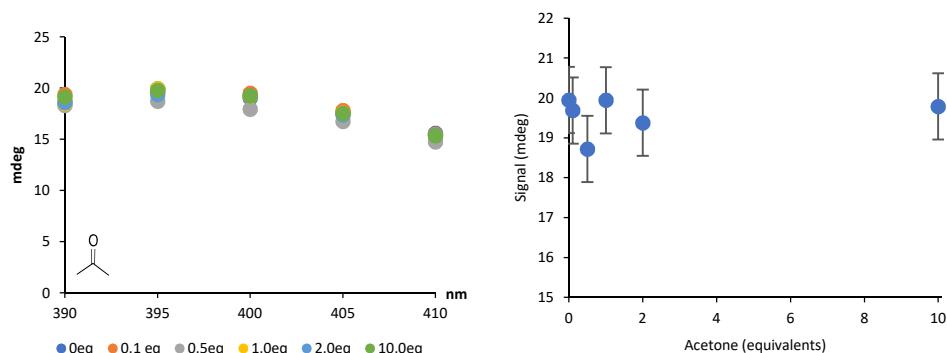


Figure S36. Interferent data for **2** and **A** with varied amounts of acetone.

Table S40 (a). CD intensities at 395 nm for **2** with **A** in the presence of varied equivalents of et acetone.

Acetone (equiv)	CD (mdeg)
0.0	19.9456
0.1	19.6864
0.5	18.7184
1.0	19.9424
2.0	19.3773
10.0	19.7858

Table S40 (b). CD and UV data for **2** with **A** in the presence of varied equivalents of acetone.

CD	0eq	0.1 eq	0.5eq	1.0eq	2.0eq	10.0eq
375	6.567243	11.55389	-0.13636	3.376545	3.851742	10.90334
380	13.17048	12.04944	12.81627	12.74063	12.47968	12.80129
385	16.81995	16.93356	15.72308	15.93156	17.1831	18.42176
390	18.59289	19.37165	18.30937	18.50717	18.58438	19.12793
395	19.94559	19.68639	18.71841	19.94242	19.37732	19.78577
400	19.04192	19.48736	17.92139	19.18628	19.23324	19.25965
405	17.62345	17.8037	16.73364	17.55724	17.41498	17.53588
410	15.60427	15.50334	14.76451	15.39844	15.39437	15.35124
UV						
375	1.812631	1.830012	1.830676	1.822182	1.808843	1.817193
380	1.459008	1.47415	1.479225	1.469665	1.462111	1.468762
385	1.14464	1.157158	1.166943	1.156197	1.150393	1.156381
390	0.884732	0.895286	0.907935	0.89625	0.891452	0.896009
395	0.671729	0.680456	0.695377	0.682951	0.67848	0.682008
400	0.497855	0.505047	0.521479	0.508237	0.504418	0.507199
405	0.356963	0.362597	0.37993	0.366198	0.362722	0.36458
410	0.248702	0.252649	0.268869	0.256219	0.253142	0.254383

6. Reconstitution Assays

6.1 Plate preparation

A concentrated solution of 4-chloro-3-nitrocoumarin was prepared using dry acetonitrile in a 25.00 mL volumetric flask. With an electronic pipettor, 20.0 μ L aliquots were dispensed into 200 nm glass-coated polypropylene (Thermofisher Plate+) 96-well plates where the wells have a maximum capacity of 270.0 μ L and introduced into Genevac instrumentation for 5 hours at 27 °C. The crystalline residues in the wells were then preserved using teflon sealing covers placed on the wells directly after removal from the Genevac instrument and then stored in the fridge, freezer, or benchtop. The plates were subsequently used for 6.800 mM sensing reactions in acetonitrile.



Figure S37. Crystalline residues of the concentrated 4-chloro-3-nitrocoumarin *in vacuo*.

6.2 Sensing of 1-cyclohexylethyl-1-amine

4-chloro-3-nitrocoumarin was plated and subjected to the Genevac instrument where 0.0017 mmol sensor was present in each well according to the protocol above. A stock solution of 1-cyclohexylethyl-1-amine with 1 equivalent of Et₃N was added (250 μ L of the stock solution contained 0.0017 mmol of analyte and of Et₃N, all stoichiometrically 1:1:1). Different ratios of the *S* and *R* enantiomers of 1-cyclohexylethyl-1-amine were added to establish a calibration curve. The reactions were left to proceed in the wells (recovered with the Teflon cover) for 1 hour prior to dilution and sensing was conducted at 0.600 mM.

Calibration data were taken at 395 nm (*plate stored on the bench top*).

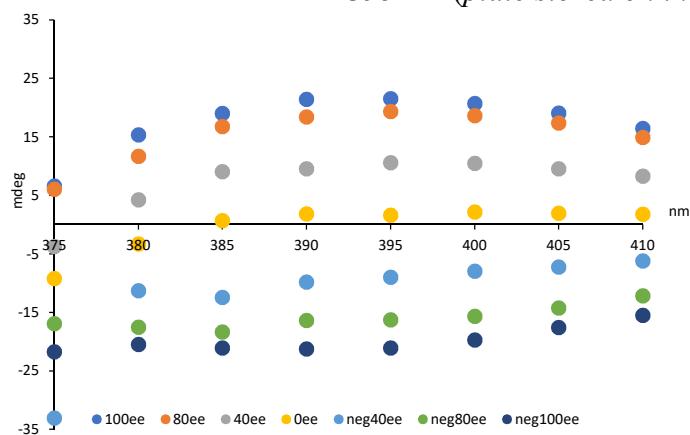


Figure S38. CD sensing of **2** with **A** (reconstitution protocol).

Table S41. CD and UV data for **2** with **A** (reconstitution protocol).

CD	100 ee	80 ee	40 ee	0 ee	-40 ee	-80 ee	-100 ee
375	6.57766	6.023612	-3.74385	-9.19674	-33.038	-16.92	-21.7364
380	15.32561	11.66193	4.256193	-3.33633	-11.3038	-17.5479	-20.4826
385	18.98047	16.78611	9.070425	0.677777	-12.4569	-18.3366	-21.1068
390	21.3917	18.41192	9.524222	1.835502	-9.82112	-16.401	-21.2234
395	21.52259	19.349	10.59341	1.632693	-9.00198	-16.2678	-21.0841
400	20.70177	18.64543	10.45983	2.18412	-7.97571	-15.6442	-19.6959
405	19.08269	17.37144	9.545595	1.962564	-7.25499	-14.2452	-17.5829
410	16.43538	14.9159	8.260969	1.760908	-6.21147	-12.1441	-15.4867

UV	375	380	385	390	395	400	405	410
	1.779539	1.814467	1.807316	1.726074	2.285851	1.691752	1.671077	
	1.412378	1.444103	1.436731	1.378577	1.814046	1.349631	1.326638	
	1.085967	1.115037	1.109013	1.065815	1.378475	1.042131	1.020561	
	0.820558	0.845362	0.840132	0.80801	1.022376	0.787682	0.769811	
	0.608413	0.630214	0.624165	0.600428	0.745019	0.583541	0.568527	
	0.439202	0.456683	0.451435	0.434353	0.527352	0.420816	0.408114	
	0.304024	0.317081	0.312617	0.301236	0.355474	0.290575	0.28062	
	0.205411	0.214676	0.211234	0.20443	0.233094	0.196482	0.18911	

Calibration data was taken at 395 nm (*plate stored in the refrigerator*).

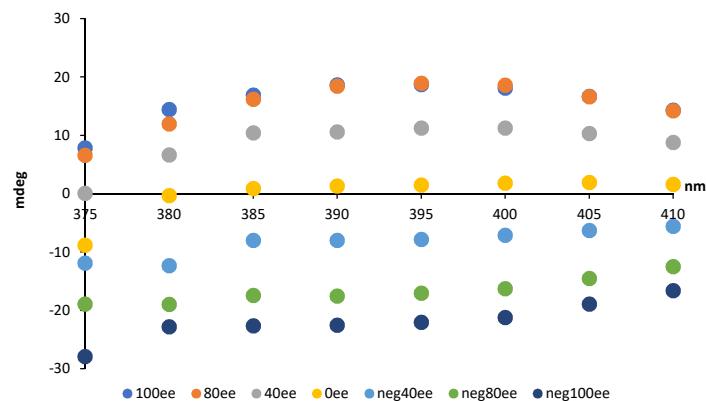


Figure S39. Kit-style CD sensing of **2** with **A** (reconstitution protocol; refrigerated plate).

Table S42. CD and UV data for **2** with **A** (reconstitution protocol; refrigerated plate).

CD	100 ee	80 ee	40 ee	0 ee	-40 ee	-80 ee	-100 ee
375	7.837451	6.58992	0.070779	-8.84166	-11.9026	-18.9204	-27.9002
380	14.42016	11.95655	6.638533	-0.33125	-12.3287	-18.9759	-22.7991
385	16.90556	16.18059	10.43069	0.895075	-8.00212	-17.4183	-22.6526
390	18.62923	18.41934	10.6016	1.290508	-8.0083	-17.5768	-22.5515
395	18.68109	18.94347	11.2319	1.485833	-7.83627	-17.0414	-22.042
400	18.08165	18.58766	11.25743	1.821021	-7.12407	-16.2706	-21.2234
405	16.68827	16.59246	10.29611	1.906835	-6.30684	-14.5302	-18.9256
410	14.32594	14.20559	8.778873	1.595331	-5.58393	-12.5238	-16.5965
UV							
	1.616056	1.639727	1.767039	1.747296	1.787824	1.772572	1.787811
	1.285842	1.30038	1.406386	1.392028	1.42489	1.412166	1.423345
	0.990466	1.001661	1.085144	1.0736	1.100112	1.089061	1.097229
	0.746423	0.75698	0.820633	0.810993	0.831813	0.823594	0.829783
	0.550224	0.559999	0.6084	0.600302	0.617186	0.609389	0.614754
	0.393877	0.403217	0.437854	0.431574	0.444092	0.438732	0.442545
	0.269674	0.277716	0.301433	0.297017	0.306384	0.301732	0.30535
	0.179785	0.187709	0.201947	0.198953	0.205242	0.201776	0.204835

7. Asymmetric reaction screening

The catalyst and ligand were premixed under argon for 1 hour prior to exposure to the imine and subsequent introduction of the reaction vials into the hydrogenation apparatus. Reactions were conducted in CH₂Cl₂:MeOH (5:1) for 16 hours. The crude reaction mixture was passed through a cotton plug and then diluted with ACN to 5.00 mL for sensing using A and 2 equivalents of Et₃N as described above. Sensing data were collected at 0.600 mM and 0.300 mM.

CD intensities for the diluted crude reaction mixture at 0.600 mM (left) and 0.300 mM (right).

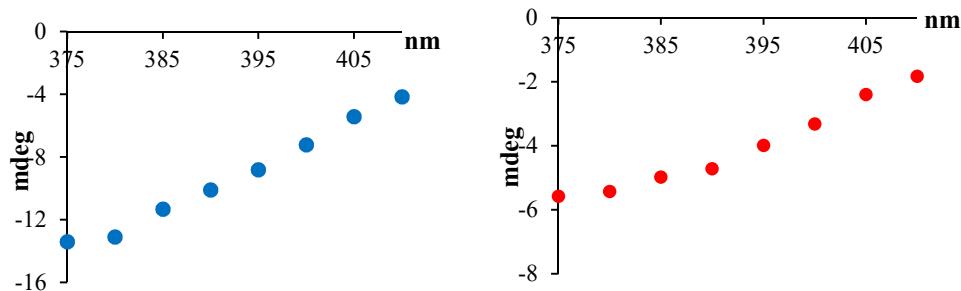


Figure S40. CD sensing of the crude asymmetric reaction mixture with A.

HPLC comparison was conducted by subjecting the crude reaction mixture to Boc protection according to a published procedure.¹ After purification, chiral HPLC analysis with a Whelk-O1 column using hexanes:IPA (94:6) as mobile phase was performed and the enantiomeric excess was determined as 61 % ee. The enantiomers eluted at 7.9 and 9.0 minutes which was verified with racemic Boc-protected *N*-methyl-1-phenylethan-1-amine. The ee values were compared to those calculated from CD analysis at 0.600 mM and 0.300 mM using calibration reference data determined above.

Table S43. ee analysis using sensing data collected on the EKKO instrument with 0.600 mM (top) and 0.300 mM (bottom) concentrations.

Calculated yield (UV)	Conversion (¹ H NMR)	ee by CD (390 nm)	ee by CD (395 nm)	ee by CD (400 nm)
90.7%	97%	64.2%	62.0%	57.4%
90.7%	97%	63.0%	58.7%	56.6%

8. References

- Thanzeel, F. Y.; Balaraman, K.; Wolf, C., Click chemistry enables quantitative chiroptical sensing of chiral compounds in protic media and complex mixtures. *Nature Comm.* **2018**, 9.