Supporting Information for:

Using Enantioselective Indicator Displacement Assays to Determine the Enantiomeric Excess of α-Amino Acids

Diana Leung,* J. Frantz Folmer-Andersen,[‡] Vincent M. Lynch,* and Eric V. Anslyn*

Department of Chemistry and Biochemistry, The University of Texas at Austin, 1 University Station A5200, Austin, TX 78712

[‡]Institut de Science et d'Ingénierie Supramoléculaires. 8 Allée Gaspard Monge, BP 70028, 67083 Strasbourg (France)

anslyn@ccwf.cc.utexas.edu

Table of Contents:

II) ***Figures:***

III) ***Tables:***

General Experimental Details:

All commercially obtained reagents were used as received. Purity of commercially available chrome azurol S (**CAS**) is 37.5%. Deionized water was used throughout. All solvents used in spectrophotometric titrations were degassed by sparging with argon for one hour. A Varian Mercury 400 MHz and a Varian Unity 300 MHz NMR spectrometer were used to obtain ^{1}H and ^{13}C NMR spectra, which were referenced using the solvent residual peak. UV-vis spectra were recorded on a Beckman Coulter DU-800 spectrophotometer in a Starna 1 cm 7Q cuvette. All titrations and enantiomeric excess calibration curves were measured at 25°C in a 1:1 MeOH:H_2O solution, buffered to pH 7.5 with 50 mM HEPES. Ligands (*R*,*R*)-**1** and (*S*,*S*)-**1** were synthesized according to a published procedure.[1] Ligands (*R*,*R*)-**2** and (*S*,*S*)-**2** were synthesized according to a published procedure.[2]

Buffer. Degassed MeOH and H_2O were obtained by sparging with argon gas for 1 h. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; 11.915 g, 50 mmol) was dissolved in MeOH:H_2O (1:1, 800 mL). The pH of the solution was adjusted to 7.5 with NaOH (2 M). The solution was filtered and diluted to 1 L with 1:1 MeOH:H_2O.

I) ***Experimental Details on:***

a) ***UV-vis Titrations using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$**.**

Stock solutions for UV-vis Spectroscopy Analysis. (*R*,*R*)-**1** (2.6934 g, 6.50 mmol) was dissolved into 50 mL in MeOH to give a 130 mM stock solution. $Cu(OTf)_2$ (54.25 mg, 0.15 mmol) was diluted to 5 mL in H_2O to give a 30 mM stock solution. **CAS** (47 mg, 29.12 μmol, 37.5%) was dissolved into 10 mL of 1:1 MeOH:H_2O, buffered to pH 7.5 with 50 mM HEPES, to give a 2.912 mM stock solution. All amino acid stock solutions were prepared with 1:1 MeOH:H_2O, buffered to pH 7.5 with 50 mM HEPES.

Displacement Isotherms. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), and stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM) were mixed and diluted to

2 mL with buffered 1:1 MeOH:H_2O, to give solution **A**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM), and L-amino acid (different concentrations of amino acids were used, refer to **Table S-1**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the L-titrant **A**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM), and D-amino acid (different concentrations of amino acids were used, refer to **Table S-1**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the D-titrant **A**. L-titrant **A** was titrated into a cuvette containing solution **A** (600 μL) and absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the displacement isotherm measurements at 602 nm for the L-amino acid. D-titrant **A** was analogously titrated.

b) ***UV-vis Titrations using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$**.**

Stock solutions for UV-vis Spectroscopy Analysis. (*R*,*R*)-**2** (2.6215 g, 9.767 mmol) was dissolved in 25 mL in MeOH to give a 390 mM stock solution. The same stock solutions of **CAS**, and $Cu(OTf)_2$ were used as above.

Displacement Isotherms. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), and stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give solution **B**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM), and L-amino acid (different concentrations of amino acids were used, refer to **Table S-2**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the L-titrant **B**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM), and D-amino acid (different concentrations of amino acids were used, refer to **Table S-2**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the D-titrant **B**. L-titrant **B** was titrated into a cuvette containing solution **B** (600 μL) and absorbance measurements were

taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the displacement isotherm measurements for L-amino acid. D-titrant **B** was analogously titrated.

c) ***UV-vis Spectrophotometer Analysis: Enantiomeric Excess Calibration Curves using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$**.**

In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM), and L-amino acid (different concentrations of amino acids were used, refer to **Table S-3**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the L-titrant **C**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM), and D-amino acid (different concentrations of amino acids were used, refer to **Table S-3**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the D-titrant **C**. L-titrant **C** was titrated into a cuvette containing D-titrant **C** (600 μL) and absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the absorbance for *ee* calibration points at 602 nm for –100% to 0% *ee*. D-titrant **C** was titrated into a cuvette containing L-titrant **C** (600 μL) and absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the absorbance for *ee* calibration points at 602 nm for 0% to 100% *ee*.

d) ***UV-vis Spectrophotometer Analysis: Enantiomeric Excess Calibration Curves using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$**.**

In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM), and L-amino acid (different concentrations of amino acids were used, refer to **Table S-4**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to give the L-titrant **D**. In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM), and D-amino acid (different concentrations of amino acids were used, refer to **Table S-4**) were mixed and diluted to

2 mL with buffered 1:1 MeOH:H_2O, to give the D-titrant **D**. L-titrant **D** was titrated into a cuvette containing D-titrant **D** (600 μL) and absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the absorbance for *ee* calibration points at 602 nm for –100% to 0% *ee*. D-titrant **D** was titrated into a cuvette containing L-titrant **D** (600 μL) and absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, affording the absorbance for *ee* calibration points at 602 nm for 0% to 100% *ee*.

e) ***UV-vis Spectrophotometer Analysis of Test Samples using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$**.**

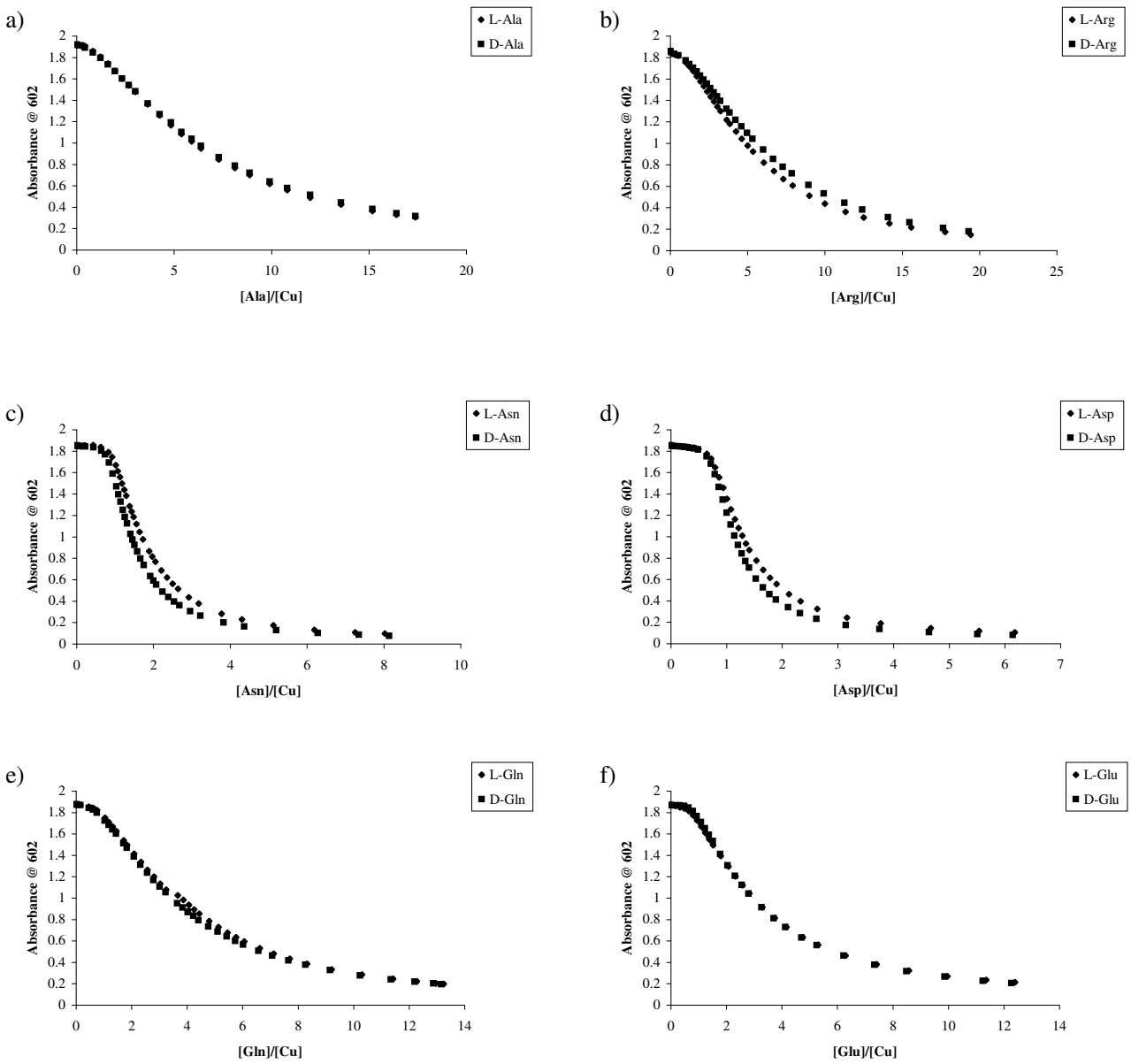
In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (13 μL, 200 μM), stock solution of (*R*,*R*)-**1** (37 μL, 2.5 mM), and a mixture of L- and D-amino acid (different concentrations of amino acids were used, refer to **Table S-3**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to afford test samples. Absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, providing the absorbance at 602 nm for determining *ee* of the test sample using the *ee* calibration curve developed for the specific amino acid (the *ee* of test samples are reported in **Table S-5**).

f) ***UV-vis Spectrophotometer Analysis of Test Samples using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$**.**

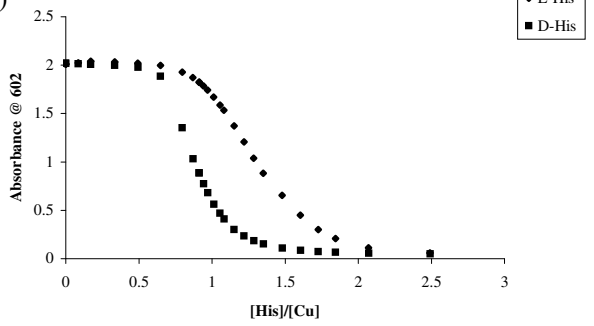
In a separate solution, stock solution of **CAS** (7 μL, 10 μM), stock solution of $Cu(OTf)_2$ (7 μL, 105 μM), stock solution of (*R*,*R*)-**2** (45 μL, 8.8 mM), and a mixture of L- and D-amino acid (different concentrations of amino acids were used, refer to **Table S-4**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O, to afford the test samples. Absorbance measurements were taken from 300 – 900 nm on a UV-vis spectrophotometer, providing the absorbance at 602 nm for determining *ee* of the test sample using the *ee* calibration curve developed for the specific amino acid (the *ee* of test samples are reported in **Table S-6**).

II) ***Figures:***

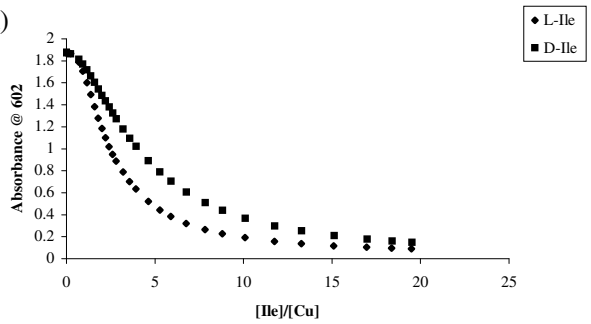
a) ***Displacement Isotherms using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$.



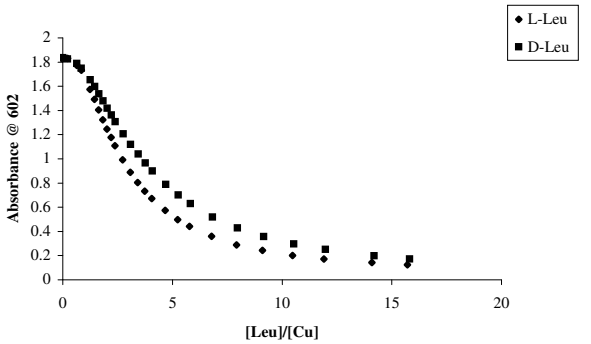
g)


L-His
D-His
Absorbance @ 602
[His]/[Cu]

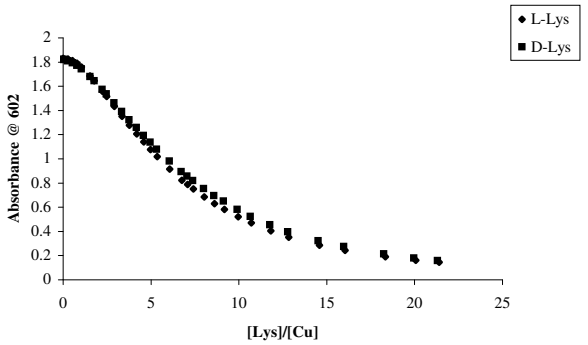
h)


L-Ile
D-Ile
Absorbance @ 602
[Ile]/[Cu]

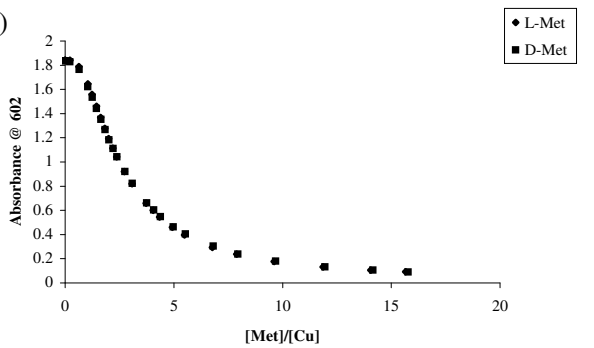
i)


L-Leu
D-Leu
Absorbance @ 602
[Leu]/[Cu]

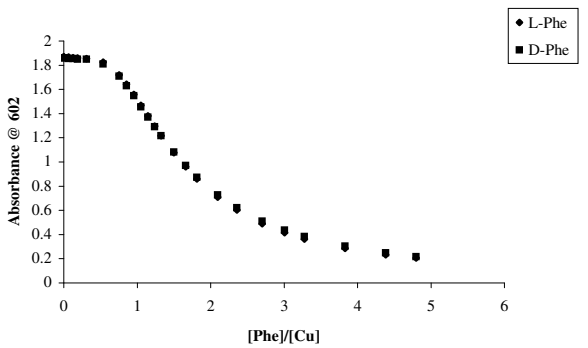
j)


L-Lys
D-Lys
Absorbance @ 602
[Lys]/[Cu]

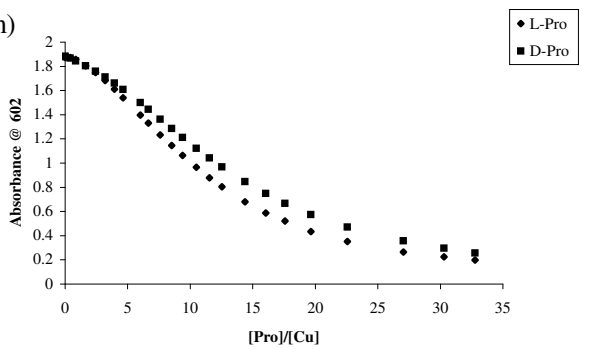
k)


L-Met
D-Met
Absorbance @ 602
[Met]/[Cu]

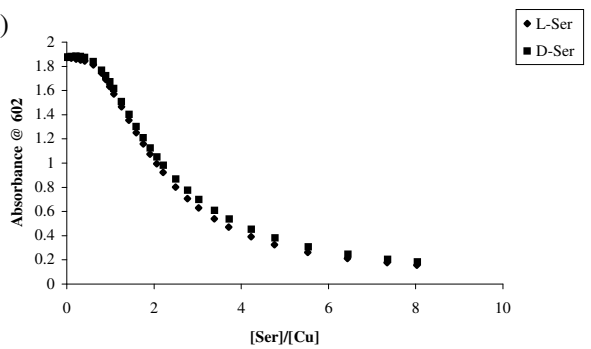
l)


L-Phe
D-Phe
Absorbance @ 602
[Phe]/[Cu]

m)


L-Pro
D-Pro
Absorbance @ 602
[Pro]/[Cu]

n)


L-Ser
D-Ser
Absorbance @ 602
[Ser]/[Cu]

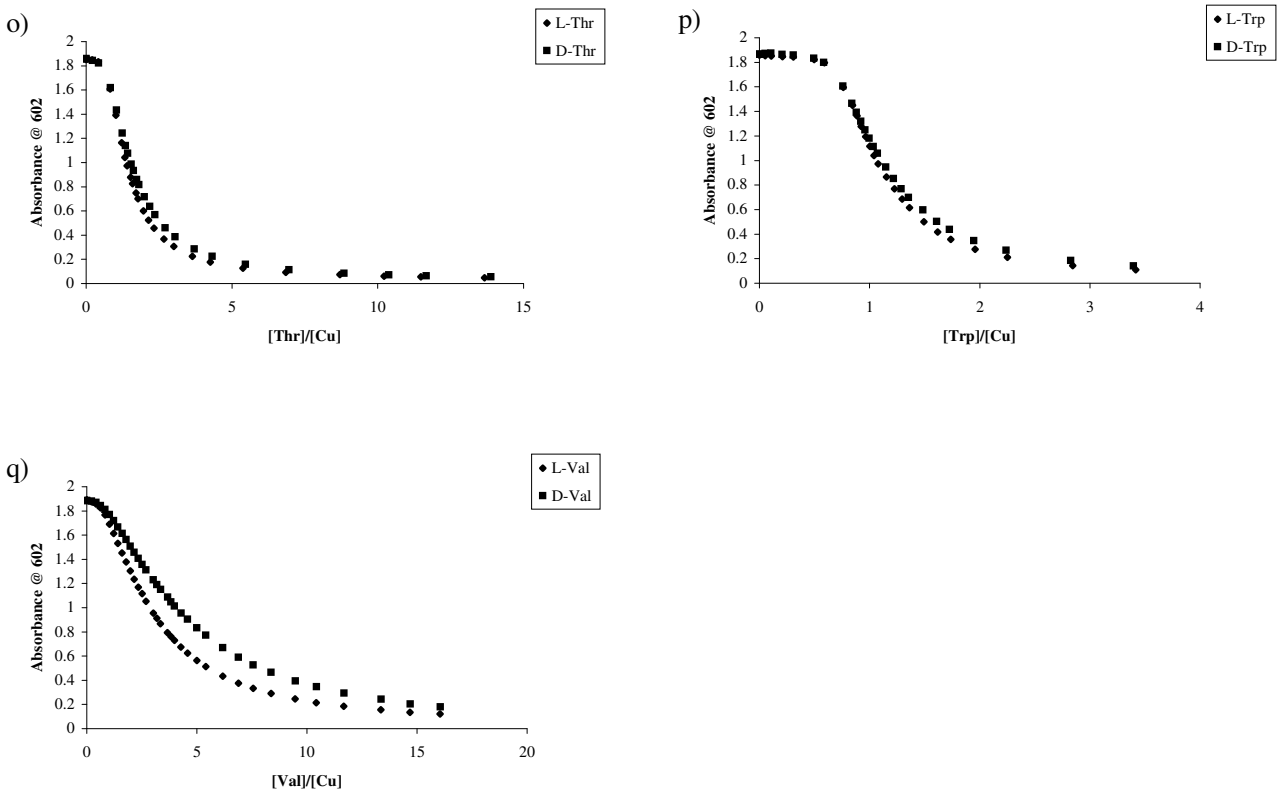
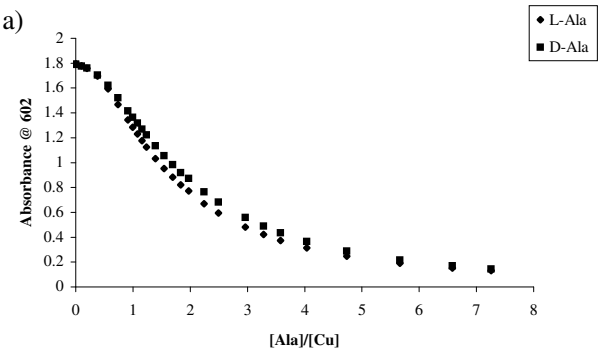


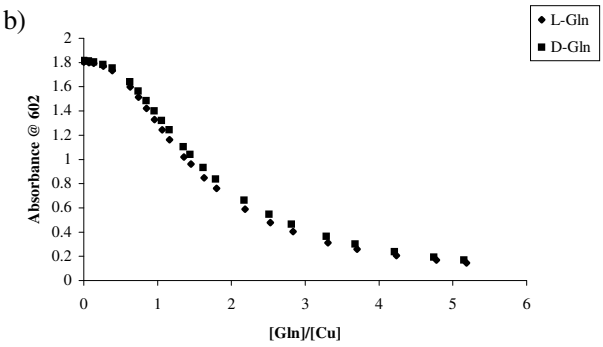
Figure S-1. Displacement isotherms at 602 nm measured with a UV-vis spectrophotometer. Displacement isotherms obtained for a solution containing **CAS** (10 μM), $Cu(OTf)_2$ (200 μM) and (*R*,*R*)-**1** (2.5 mM) in 1:1 MeOH:H_2O, 50 mM HEPES buffered to pH 7.5 with the addition of: a) L- or D-alanine. b) L- or D-arginine. c) L- or D-asparagine. d) L- or D-aspartate. e) L- or D-glutamine. f) L- or D-glutamate. g) L- or D-histidine. h) L- or D-isoleucine. i) L- or D-leucine. j) L- or D-lysine. k) L- or D-methionine. l) L- or D-phenylalanine. m) L- or D-proline. n) L- or D-serine. o) L- or D-threonine. p) L- or D-tryptophan. q) L- or D-valine. The concentrations of amino acids used are shown in **Table S-1**.

b) ***Displacement Isotherms using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$.

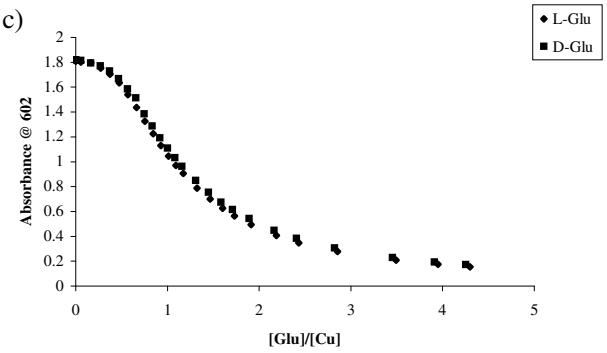
a)



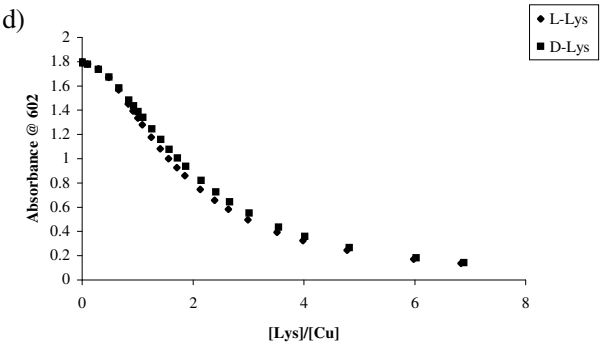
b)



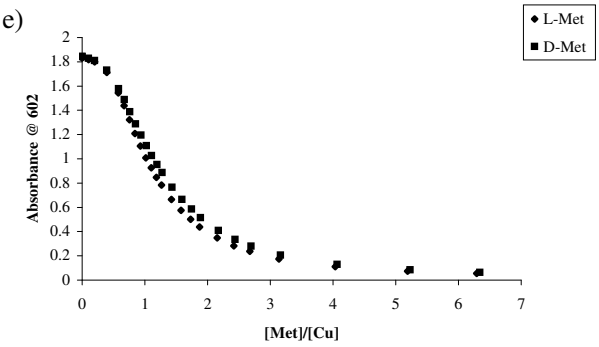
c)



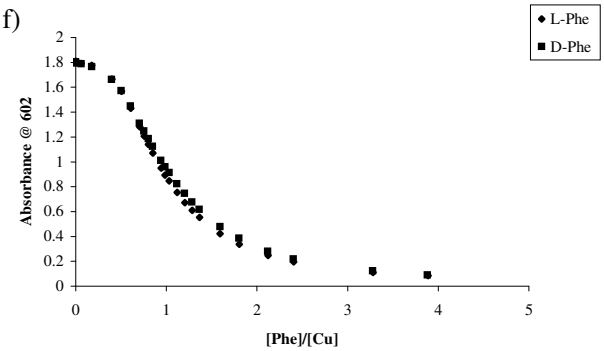
d)



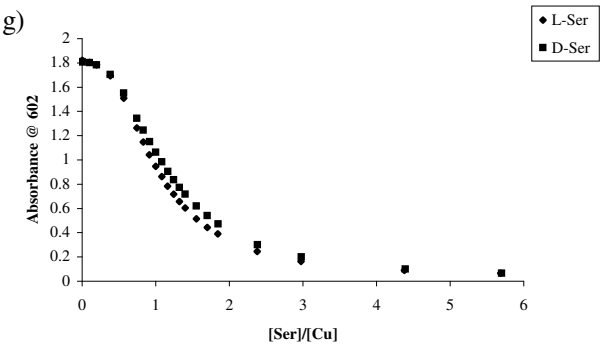
e)



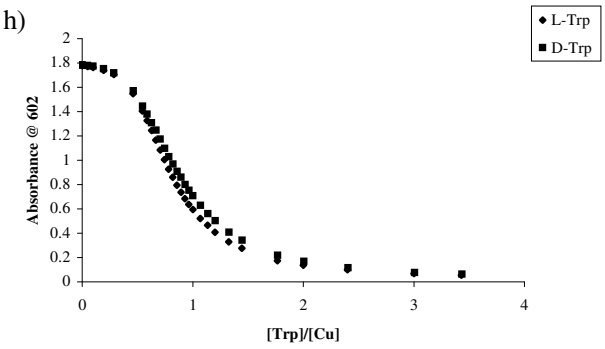
f)



g)



h)



i)

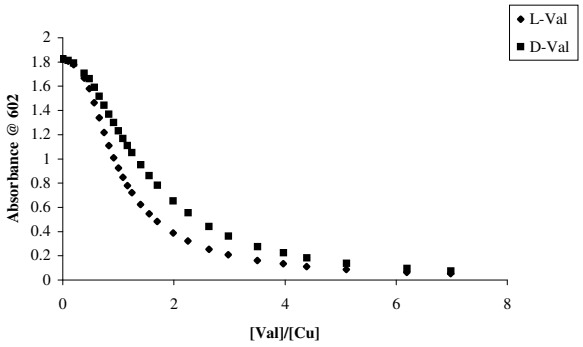


Figure S-2. Displacement isotherms at 602 nm measured with a UV-vis spectrophotometer. Displacement isotherms obtained for a solution containing **CAS** (10 μM), $Cu(OTf)_2$ (105 μM) and (*R*,*R*)-**2** (8.8 mM) in 1:1 MeOH:H_2O, 50 mM HEPES buffered to pH 7.5 with the addition of: a) L- or D-alanine. b) L- or D-glutamine. c) L- or D-glutamate. d) L- or D-lysine. e) L- or D-methionine. f) L- or D-phenylalanine. g) L- or D-serine. h) L- or D-tryptophan. i) L- or D-valine. The concentrations of amino acids used are shown in **Table S-2**.

c) ***Enantiomeric Excess Calibration Curves using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$ ***on a UV-vis Spectrophotometer.***

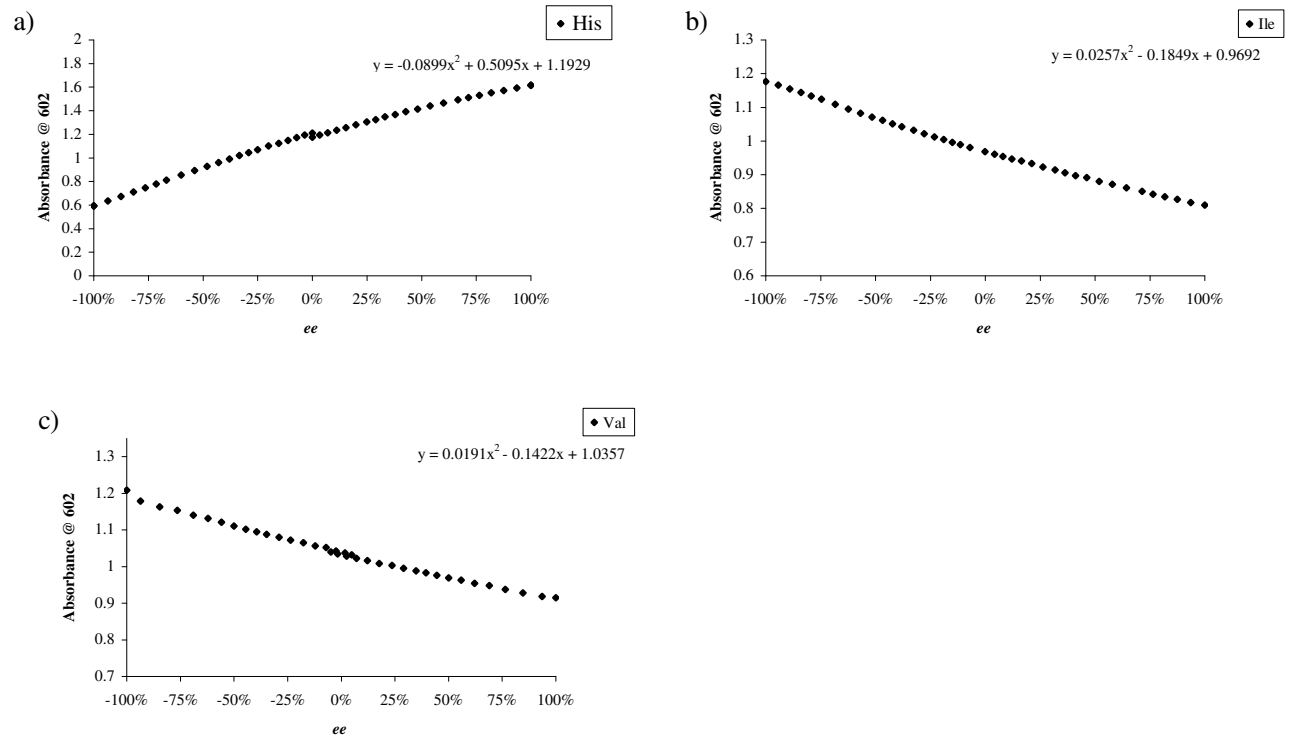


Figure S-3. Absorbance at 602 nm as a function of *ee* for displacement experiments measured with a UV-vis spectrophotometer performed in a solution containing **CAS** (10 μM), $Cu(OTf)_2$ (200 μM) and (*R*,*R*)-**1** (2.5 mM) in 1:1 MeOH:H_2O, 50 mM HEPES buffered to pH 7.5, with the addition of: a) histidine (202 μM). b) isoleucine (697 μM). c) valine (714 μM).

d) ***Enantiomeric Excess Calibration Curves using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$ ***on a UV-vis Spectrophotometer.***

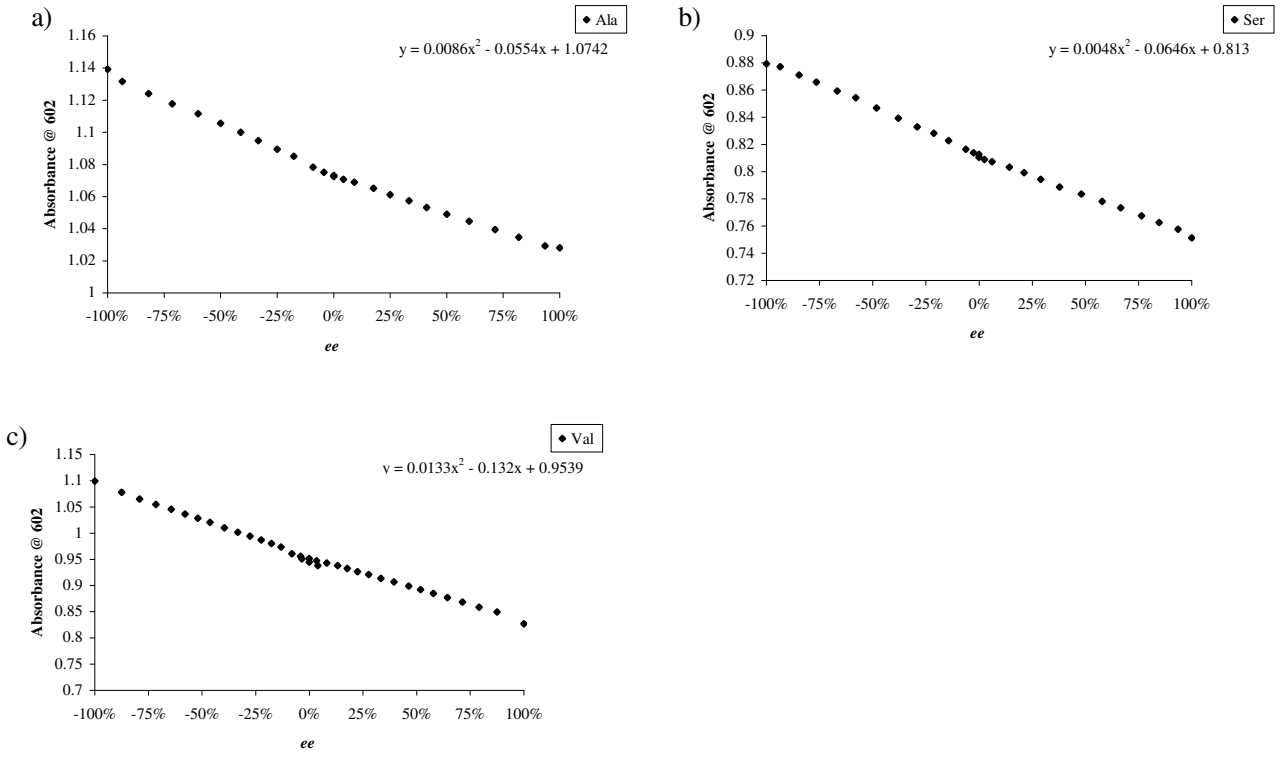


Figure S-4. Absorbance at 602 nm as a function of *ee* for displacement experiments measured with a UV-vis spectrophotometer performed in a solution containing **CAS** (10 μM), $Cu(OTf)_2$ (105 μM) and (*R*,*R*)-**2** (8.8 mM) in 1:1 MeOH:H_2O, 50 mM HEPES buffered to pH 7.5, with the addition of: a) alanine (149 μM). b) serine (125 μM). c) valine (125 μM).

III) ***Tables:***

a) ***Concentrations of Amino Acids used in UV-vis Displacement Isotherms using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$.

Table S-1. Concentrations of α-amino acids used to produce UV-vis displacement isotherms with $[Cu^{II}((R,R)\text{-}\mathbf{1})]^{2+}$.

Amino Acids	[L-amino acid] (mM)	[D-amino acid] (mM)
Ala	5.00	5.00
Arg	6.05	6.01
Asn	2.58	2.61
Asp	2.01	2.00
Gln	3.67	3.65
Glu	3.85	3.81
His	2.02	2.02
Ile	5.60	5.61
Leu	5.11	5.14
Lys	6.26	6.23
Met	5.09	5.13
Phe	1.50	1.50
Pro	9.96	9.96
Ser	2.50	2.50
Thr	4.98	5.06
Trp	1.26	1.26
Val	5.01	5.01

b) ***Concentrations of Amino Acids used in UV-vis Displacement Isotherms using Receptor*** ***$[Cu^{II}((R,R)\text{-}2)]^{2+}$.***

Table S-2. Concentrations of α-amino acids used to produce UV-vis displacement isotherms with $[Cu^{II}((R,R)\text{-}\mathbf{2})]^{2+}$.

Amino Acids	[L-amino acid] (mM)	[D-amino acid] (mM)
Ala	1.24	1.24
Gln	0.856	0.851
Glu	0.714	0.706
Lys	1.26	1.27
Met	1.27	1.28
Phe	0.759	0.757
Ser	1.25	1.25
Trp	0.631	0.632
Val	1.25	1.25

c) ***Concentrations used for Enantiomeric Excess Calibration Curves using Receptor*** ***$[Cu^{II}((R,R)\text{-}1)]^{2+}$.***

Table S-3. Concentrations of α-amino acids used to produce *ee* calibration curves using $[Cu^{II}((R,R)\text{-}\mathbf{1})]^{2+}$.

Amino Acids	[Amino Acid] (mM)
His	0.202
Ile	0.697
Val	0.714

d) ***Concentrations used for Enantiomeric Excess Calibration Curves using Receptor*** $[Cu^{II}((R,R)\text{-}2)]^{2+}$**.**

Table S-4. Concentrations of α-amino acids used to produce *ee* calibration curves using $[Cu^{II}((R,R)\text{-}\mathbf{2})]^{2+}$.

Amino Acids	[Amino Acid] (mM)
Ala	0.149
Ser	0.125
Val	0.125

e) ***Test Samples Analyzed on a UV-vis Spectrophotometer using Receptor*** $[Cu^{II}((R,R)\text{-}1)]^{2+}$**.**

Table S-5. Enantiomeric excess determination of test samples by analysis using $[Cu^{II}((R,R)\text{-}\mathbf{1})]^{2+}$ through UV-vis measurements.

Amino Acids	*ee* (Actual)	*ee* (Experimental)[a]
His		
a	-25.1%	-20.0%
b	-50.0%	-67.1%
c	75.0%	54.7%
d	-0.1%	-9.7%
Ile		
a	-57.2%	-56.7%
b	-21.5%	-14.8%
c	42.8%	39.4%
d	78.5%	90.6%
Val		
a	-57.9%	-68.1%
b	78.9%	60.9%
c	-22.8%	-39.2%
d	93.0%	89.6%

[a] Enantiomeric excess determination of test samples of α-amino acid through UV-vis measurements, and *ee* calibration curves made for each amino acid using receptor $[Cu^{II}((R,R)\text{-}\mathbf{1})]^{2+}$. **CAS** (10 μM), $Cu(OTf)_2$ (200 μM), (*R*,*R*)-**1** (2.5 mM), and a mixture of L- and D-amino acid (different concentrations of amino acids were used, refer to **Table S-3**) were mixed and diluted to 2 mL with buffered 1:1

MeOH:H_2O.

f) ***Test Samples Analyzed on a UV-vis Spectrophotometer using Receptor [Cu^{II}((R,R)-2)]$^{2+}$.***

Table S-6. Enantiomeric excess determination of test samples by analysis using [Cu^{II}((*R*,*R*)-**2**)]$^{2+}$ through UV-vis measurements.

Amino Acids	***ee* (Actual)**	***ee* (Experimental)[a]**
Ala		
a	0.0%	-17.6%
b	33.3%	68.2%
c	100.0%	66.4%
d	-33.3%	-37.5%
Ser		
a	-100.0%	-90.8%
b	59.9%	82.6%
c	19.9%	13.9%
d	-60.1%	-73.3%
Val		
a	20.0%	19.0%
b	-20.0%	-6.8%
c	60.0%	67.5%
d	-60.0%	-60.3%

[a] Enantiomeric excess determination of test samples of α-amino acid through UV-vis measurements, and *ee* calibration curves made for each amino acid using receptor [Cu^{II}((*R*,*R*)-**2**)]$^{2+}$. **CAS** (10 μM), $Cu(OTf)_2$ (105 μM), (*R*,*R*)-**2** (8.8 mM), and a mixture of L- and D-amino acid (different concentrations of amino acids were used, refer to **Table S-4**) were mixed and diluted to 2 mL with buffered 1:1 MeOH:H_2O.

(1) Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986-7987.

(2) Mimoun, H.; de Laumer, J. Y.; Giannini, L.; Scopelliti, R.; Floriani, C. *J. Am. Chem. Soc.* **1999**, *121*, 6158-6166.