Supplementary Material (ESI) for Chemical Communications

Supporting material for

Chiral Sensing using a Blue Fluorescent Antibody

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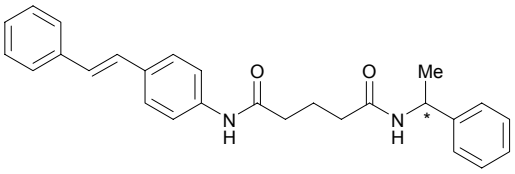
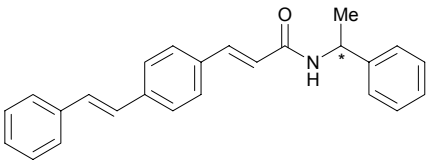
Synthesis of 4



To a solution of (*R*)-(+)-phenylethylamine (22.0 mg, 0.178 mmol) in DMF (3.5 mL) were added 4-(4-*trans*-styryl-phenylcarbamoyl)-butyric acid (50 mg, 0.162 mmol), triethylamine (56 μl, 0.405 mmol) and HBTU (68.0 mg, 0.178 mmol). After stirring for 3.5 hrs at rt, the mixture was diluted with water. The product was extracted with ethyl acetate twice. The combined organic layers were washed with water, followed by brine. After drying over $MgSO_4$ and concentration in vacuo, the residue was purified by silica gel column chromatography (50% ethyl acetate/hexane) to furnish the target product (43.9 mg, 66% yield) as white crystals. The above method was employed, starting from (*S*)-(+)-2-phenylpropionic acid (20 mg, 0.0956 mmol) to give the target product (48.5 mg, 73% yield).

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.51 (d, J = 7.2 Hz, 3H), 2.00-2.10 (m, 1H), 2.31-2.28 (m, 2H), 2.44 (t, J = 7.0 Hz, 2H), 5.15 (quint, J = 7.2 Hz, 1H), 5.93 (brd, J = 7.2 Hz, 1H), 7.03 (d, J = 16.4 Hz, 1H), 7.07 (d, J = 16.4 Hz, 1H), 7.20-7.40 (m, 5H), 7.44-7.56 (m, 4H), 8.11 (s, 1H) ppm. MALDI-FTMS calcd. for $C_{27}H_{29}NO_2$ (M^+ + H) 413.2223, found 413.2219. MALDI-FTMS calcd. for $C_{27}H_{28}NO_2Na$ (M^+ + Na) 435.2043, found 435.2040. (*R*)-**4**, $[\alpha]_D^{25}$ = +10.7 (*c* = 0.06 in DMF). (*S*)-**4**, $[\alpha]_D^{25}$ = -9.28 (*c* = 0.08 in DMF).

Synthesis of 5

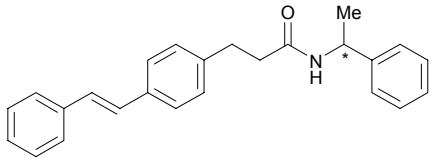


To a solution of (*R*)-(+)-phenylethylamine (10.0 mg, 0.0835 mmol) in DMF (5.0 mL) were added 4-*trans*-styryl-*trans*-4-cinnamic acid (*J. Am. Chem. Soc.* **1957**, *79*, 3514) (90 mg, 0.0759 mmol), triethylamine (29 μl, 0.209 mmol) and HBTU (32.0 mg, 0.0835 mmol). After stirring for 2 hrs at rt, the mixture was diluted with water. The product was extracted with ethyl acetate twice. The combined organic layers were washed with water, followed by brine. After drying over $MgSO_4$ and concentration in vacuo,

the residue was purified by silica gel column chromatography (100% dichloromethane) to furnish the target product (22.0 mg, 78 % yield) as white crystals. The same method was employed, starting from (*S*)-(+)-phenylethylamine (10.0 mg, 0.0835 mmol) to give the target product (14.8 mg, 61 % yield).

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.58 (d, J = 7.2 Hz, 3H), 5.25-5.34 (m, 1H), 5.81 (brd, J = 8.0 Hz, 1H), 6.39 (d, J = 15.2 Hz, 1H), 7.09 (d, J = 16.4 Hz, 1H), 7.16 (d, J = 16.4 Hz, 1H), 1.26-7.40 (m, 8H), 7.46-7.54 (m, 6H), 7.63 (d, J = 15.2 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ 21.6, 49.0, 120.2, 126.3, 126.6, 126.9, 127.5, 127.87, 127.94, 128.2, 128.7, 129.8, 134.0, 137.0, 138.8, 140.8, 143.1, 164.9 ppm. MALDI-FTMS calcd. for $C_{25}H_{24}NO$ (M^+ + H) 354.1852, found. 354.1846. (*R*)-**5**, $[\alpha]_D^{25}$ = -157 (*c* = 0.0204 in DMF). (*S*)-**5**, $[\alpha]_D^{25}$ = +158 (*c* = 0.0114 in DMF).

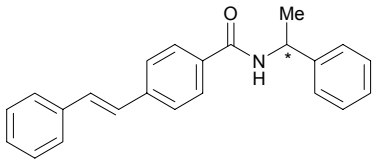
Synthesis of 6



To a solution of (*R*)-(+)-phenylethylamine (12.0 mg, 0.0951 mmol) in DMF (5.0 mL) were added 3-(4-*trans*-styryl-phenyl)-propionic acid (20 mg, 0.0793 mmol), triethylamine (28 µl, 0.198 mmol) and HBTU (36.0 mg, 0.0951 mmol). After stirring for 24 hrs at 4 C, the mixture was diluted with water. The product was extracted with ethyl acetate twice. The combined organic layers were washed with water, followed by brine. After drying over $MgSO_4$ and concentration in vacuo, the residue was purified by silica gel column chromatography (100% dichloromethane) to furnish the target product (20.7 mg, 70 % yield) as white crystals. The same method was employed, starting from (*S*)-(+)-phenylethylamine (12.0 mg, 0.0951 mmol) to yield the target product (23.0 mg, 78 % yield).

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.41 (d, J = 6.8 Hz, 3H), 2.40-2.55 (m, 2H), 2.97 (t, J = 7.6 Hz, 2H), 5.10 (quint, J=7.2Hz, 1H), 5.57 (brd, J = 8.4 Hz, 1H), 7.08 (brs, 2H), 7.14-7.21 (m, 4H), 7.21-7.32 (m, 4H), 7.33-7.39 (m, 2H), 7.39-7.44 (m, 2H), 7.49-7.54 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ 21.6, 31.4, 38.5, 126.1, 126.4, 126.6, 127.3, 127.5, 128.1, 128.6, 128.7, 128.8, 135.4, 137.3, 140.3, 142.9, 171.0 ppm. MALDI-FTMS calcd. for $C_{25}H_{26}NO$ (M^+ + H) 356.2009, found. 356.2005. (*R*)-**6**,, $[\alpha]_D^{25}$ = -25.6 (*c* = 0.0312 in DMF). (*S*)-**6**, $[\alpha]_D^{25}$ = +28.4 (*c* = 0.0134 in DMF).

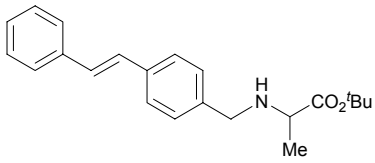
Synthesis of 7



To a solution of (*R*)-(+)- α-phenylethylamine (32.5 mg, 0.268 mmol) in DMF (4.5 mL) were added *trans*-stilbene-4-carboxylic acid (*J. Am. Chem. Soc.* 1959, ***81***, 2564) (50.0 mg, 0.223 mmol), triethylamine (78 μl, 0.558 mmol) and HBTU (102 mg, 0.268 mmol). After stirring for 2 hrs at rt, the mixture was diluted with water and diethylether to generate a crystalline product, which was purified by filtration to yield the target product (65.9 mg, 90% yield) as white crystals. The same method was employed, starting from (*S*)-(+)- α-phenylethylamine (25.0 mg, 0.211 mmol) to give the target product (30.3 mg, 83%).

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.62 (d, J = 7.0 Hz, 3H), 5.36 (quint, J = 7.0 Hz, 1H), 6.31 (brd, J = 7.0 Hz, 1H), 7.11 (d, J = 16.4 Hz, 1H), 7.19 (d, J = 16.4 Hz, 1H), 7.26-7.32 (m, 2H), 7.35-7.43 (m, 6H), 7.51-7.58 (m, 2H), 7.55 (brd, J = 8.4 Hz, 2H), 7.76 (brd, J = 8.4 Hz, 2H) ppm. ^{13}C NMR (d_6-DMSO, 100MHz) δ 22.18, 48.29, 126.04, 126.15, 126.54, 126.66, 127.54, 127.82, 127.98, 128.19, 128.73, 130.09, 133.18, 136.73, 139.74, 144.91, 164.98. MALDI-FTMS calcd. for $C_{23}H_{22}NO$ (M^+ + H) 328.1696, found. 328.1702. (*R*)-**7**, $[\alpha]_D^{25}$ = +158.5 (*c* = 0.041 in DMF). (*S*)-**7**, $[\alpha]_D^{25}$ = -159.9 (*c* = 0.284 in DMF).

Synthesis of 9

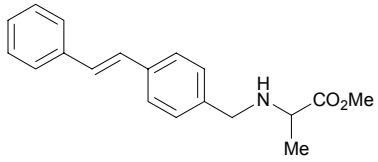


To a solution of L-alanine *tert*-butylester hydrochloride (196 mg, 1.08 mmol) in dichloromethane (20 mL) were added 4-*trans*-4-styrylbene carboxylic aldehyde (150 mg, 0.720 mmol), acetic acid (410 μl, 7.20 mmol) and sodium triacetoxyborohydride (458 mg, 2.16 mmol). After stirring overnight at rt, the mixture was diluted with sat. aq. $NaHCO_3$. The product was extracted with dichloromethane twice. The combined organic layers were washed with water, followed by brine. After drying over $MgSO_4$ and concentration in vacuo, the residue was purified by silica gel column chromatography (10% methanol/dichloromethane) to give the target product (214 mg, 88 % yield) as white crystals. The same method was employed, starting from D-alanine *tert*-butylester hydrochloride (150 mg, 0.827 mmol) to give the target product (48.1 mg, 17 % yield).

^{1}H NMR (CD_3CN, 400MHz) δ 1.19 (d, J = 7.2Hz, 3H), 1.45 (s, 9H), 3.13 (q, J = 7.2 Hz, 1H), 3.61 (d, J = 13.4 Hz, 1H), 3.77 (d, J = 13.4 Hz, 1H), 7.18 (brd, J = 0.8 Hz, 2H), 7.24-7.29 (m, 1H), 7.29-7.39 (m, 4H), 7.49-7.58 (m, 4H) ppm. ^{13}C NMR (CD_3CN, 100MHz) δ 19.1, 28.0, 51.6, 56.5, 80.8, 126.4, 126.5, 127.4,

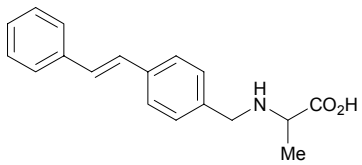
128.3, 128.4, 128.5, 128.6, 136.1, 137.3, 139.4, 175.0. MALDI-FTMS calcd. for $C_{22}H_{28}NO_2$ (M^+ + H) 338.2114, found. 338.2110. (*R*)-**9**, $[\alpha]_D^{25}$ = +49.2 (*c* = 0.13 in DMF). (*S*)-**9**, $[\alpha]_D^{25}$ = -52.8 (*c* = 0.197 in DMF).

Synthesis of 10



To a solution of L-alanine methylester hydrochloride (100 mg, 0.720 mmol) in dichloromethane (9.6 mL) were added 4-*trans*-4-styrylbene carboxylic aldehyde (100 mg, 0.480 mmol), acetic acid (275 µl, 4.80 mmol) and sodium triacetoxyborohydride (305 mg, 1.44 mmol). After stirring for 2 hrs at rt, the mixture was diluted with sat. aq. $NaHCO_3$. The product was extracted with dichloromethane twice. The combined organic layers were washed with water followed by brine. After drying over $MgSO_4$ and concentration in vacuo, the residue was purified by silica gel column chromatography (10% methanol/dichloromethane) to yield the target product (75.7 mg, 53 % yield) as white crystals. Employing this method, starting from D-alanine methylester hydrochloride (100 mg, 0.480 mmol), gave the target product (78.4 mg, 55 % yield). ^{1}H NMR ($CDCl_3$, 400MHz) δ 1.33 (d, J = 6.8 Hz, 3H), 3.40 (q, J = 6.8 Hz, 1H), 3.68 (d, J = 13.0 Hz, 1H), 3.75 (s, 3H), 3..81 (d, J = 13.0 Hz, 1H), 7.10 (brs, 2H), 7.23-7.28 (m, 2H), 7.30-7.39 (m, 4H), 7.45-7.53 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ 21.6, 31.4, 38.5, 126.1, 126.4, 126.6, 127.3, 127.5, 128.1, 128.6, 128.7, 128.8, 135.4, 137.3, 140.3, 142.9, 171.0 ppm. MALDI-FTMS calcd. for $C_{19}H_{22}NO_2$ (M^+ + H) 296.1645, found. 296.1645. (*R*)-**10**, $[\alpha]_D^{25}$ = +56.2 (*c* = 0.105 in DMF). (*S*)-**10**, $[\alpha]_D^{25}$ = -5.75 (*c* = 0.115 in DMF).

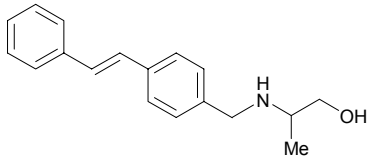
Synthesis of 11



To L-2-(4-*trans*-styryl-benzylamino)-propionic acid *tert*-butyl ester (50 mg, 0.461 mmol) was added pre-cooled trifluoroacetic acid (1.0 mL). After stirring for 1 hr at rt, the mixture was concentrated in vacuo to yield the target product (58.0 mg, 99 % yield) as white crystals. The same method was employed, starting from D-2-(4-*trans*-styryl-benzylamino)-propionic acid *tert*-butyl ester (41.8 mg, 0.461 mmol) to give the target product (55.3 mg, 98 % yield).

^{1}H NMR (CD_3OD, 400MHz) δ 1.56 (d, J = 6.8 Hz, 3H), 3.75 (q, J = 6.8 Hz, 1H), 4.15 (d, J = 12.8 Hz, 1H), 4.24 (d, J = 12.8 Hz, 1H), 7.20-7.30 (m, 3H), 7.32-7.38 (m, 2H), 7.45-7.51 (m, 2H), 7.54-7.59 (m, 2H), 7.63-7.70 (m, 2H) ppm. MALDI-FTMS calcd. for $C_{18}H_{20}NO_2$ (M^+ + H) 282.1488, found. 282.1495.

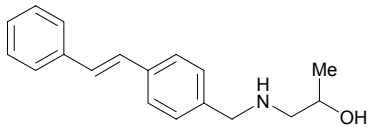
Synthesis of 12



To a solution of lithium aluminum hydride (4.6 mg, 0.122 mmol) in tetrahydrofuran (2.0 mL) was added L-2-(4-*trans*-styryl-benzylamino)-propionic acid methyl ester (18 mg, 0.0609 mmol) at 0 °C. After stirring for 10 min at rt, 10 μL of water, 10 μL of 10% aq. NaOH,and then 30 μL of water were added. The mixture was filtrated over Celite and the filtrate was evaporated. The residue was purified by silica gel column chromatography (10% methanol/dichloromethane) to give the target product (12.7 mg, 78 % yield) as white crystals.

^{1}H NMR ($CDCl_3$, 400MHz) δ 1.11 (d, J = 6.4 Hz, 3H), 2.82-2.91 (m, 1H), 3.29 (dd, J = 6.8 Hz, 10.4Hz, 1H), 3.62 (dd, J = 4.0 Hz, 10.4 Hz, 1H), 3.76 (d, J =13.2 Hz, 1H), 3.89 (d, J = 13.2 Hz, 1H), 7.10 (brs, 2H), 7.23-7.29 (m, 1H), 7.30-7.39 (m, 2H), 7.46-7.54 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ21.6, 31.4, 38.5, 126.1, 126.4, 126.6, 127.3, 127.5, 128.1, 128.6, 128.7, 128.8, 135.4, 137.3, 140.3, 142.9, 171.0 ppm. MALDI-FTMS calcd. for $C_{18}H_{22}NO$ (M^+ + H) 268.1696, found. 268.1699. (*R*)-**12**, $[\alpha]_D^{25}$ = -27.2 (*c* = 0.025 in DMF). (*S*)-**12**, $[\alpha]_D^{25}$ = +26.4 (*c* = 0.0125 in DMF).

Synthesis of 13



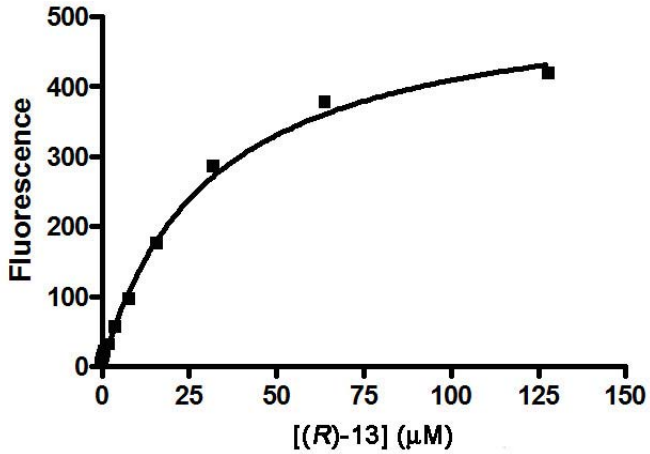
To a solution of (S)-(+)-amino-2-propanol (35 mg, 0.461 mmol) in dichloromethane (4.0 mL) were added 4-*trans*-4-styrylbene carboxylic aldehyde (80 mg, 0.384 mmol), acetic acid (220 μl, 3.84 mmol) and sodium triacetoxyborohydride (98 mg, 0.461 mmol). After stirring overnight at rt, the mixture was diluted with sat. aq. $NaHCO_3$. The product was extracted with dichloromethane twice. The combined organic layers were washed with water, followed by brine. After drying over $MgSO_4$ and concentration in vacuo, the residue was purified by silica gel column chromatography (20% methanol/dichloromethane) to give the target product (47.4 mg, 46 % yield) as white crystals. The same method was employed, starting from (R)-(-)-amino-2-propanol (35 mg, 0.461 mmol) to give the target product (63.3 mg, 62 % yield).

^{1}H NMR ($CDCl_3$, 400MHz) δ1.15 (d, J = 6.4 Hz, 3H), 2.46 (dd, J = 9.4 Hz, 12.0 Hz, 1H), 2.75 (dd, J = 2.8 Hz, 12.0 Hz, 1H), 3.78-3.89 (m, 1H), 3.80 (d, J = 13.4 Hz, 1H), 3.85 (d, J = 13.4 Hz, 1H), 7.10 (brs, 2H), 7.23-7.29 (m, 1H), 7.30-7.39 (m, 4H), 7.46-7.54 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$, 100MHz) δ20.4, 53.2, 56.1, 65.5, 77.2, 126.5, 126.6, 127.6, 128.3, 128.5, 128.6, 128.7, 136.4, 137.3, 139.0. MALDI-FTMS calcd. for $C_{18}H_{22}NO$ (M^+ + H) 268.1696, found. 268.1693. (*R*)-**13**, $[\alpha]_D^{25}$ = -2.66 (*c* = 1.09 in DMF). (*S*)-**13**, $[\alpha]_D^{25}$ = +2.52 (*c* = 0.33 in DMF).

Determination of Kds of (*R*)- and (*S*)-13 for mAb 19G2

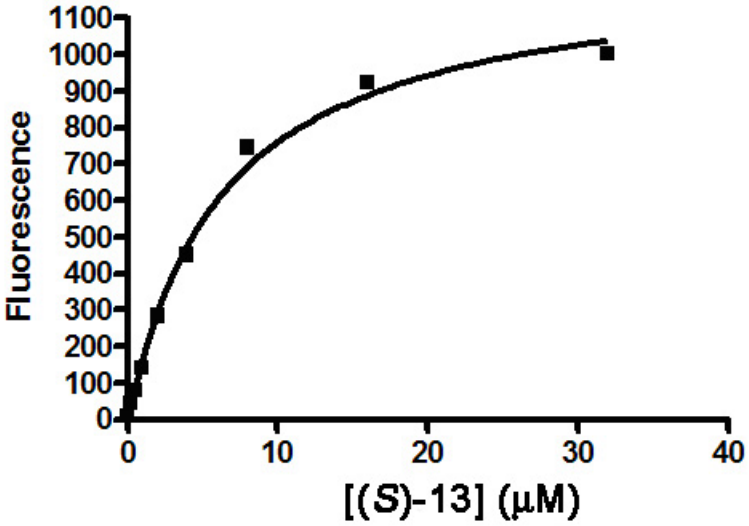
Final Concentration: 19G2 2.5 μM, stilbene substrate 0-256 μM in PBS (5 % DMF)

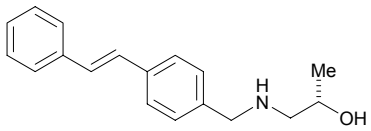
total volme 150 μL. λ_{exc} = 327 nm, λ_{em} = 410 nm. Curve fitting was performed by using GraphPad Prism (GraphPad Software, Inc.).



One site binding (hyperbola)
Best-fit values
BMAX 536.0
KD 31.11
Std. Error
BMAX 18.93
KD 2.854
95% Confidence Intervals
BMAX 493.2 to 578.8
KD 24.65 to 37.56
Goodness of Fit
Degrees of Freedom 9
R^2 0.9959
Absolute Sum of Squares 996.1
Sy.x 10.52
Constraints
BMAX BMAX > 0.0

KD	KD > 0.0
Data	
Number of X values	11
Number of Y replicates	1
Total number of values	11
Number of missing values	0





One site binding (hyperbola)

Best-fit values	
BMAX	1245
KD	6.404
Std. Error	
BMAX	50.49
KD	0.7145
95% Confidence Intervals	
BMAX	1125 to 1364
KD	4.714 to 8.094
Goodness of Fit	
Degrees of Freedom	7
R^2	0.9941
Absolute Sum of Squares	7232
Sy.x	32.14
Constraints	
BMAX	BMAX > 0.0
KD	KD > 0.0
Data	
Number of X values	9
Number of Y replicates	1
Total number of values	9
Number of missing values	0