Efficient Construction of Diketopiperazine Macroarrays Through a Cyclative-Cleavage Strategy and Their Evaluation as Luminescence Inhibitors in the Bacterial Symbiont *Vibrio fischeri*

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Supporting Information.

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Analytical instrumentation.

LC-MS analyses were performed using a Shimadzu LCMS-2010a (Columbia, MD) equipped with two pumps (LC-10ADvp), controller (SCL-10Avp), autoinjector (SIL-10Advp), UV diode array detector (SPD-M10Avp), and single quadrupole analyzer (by electrospray ionization, ESI). The LC-MS was interfaced with a PC running the Shimadzu LCSolutions software package (Version 2.04 Su2-H2). A Supelco (Bellefonte, PA) 15 cm × 2.1 mm C-18 wide-pore reverse-phase column was used for all LC-MS work. Standard reverse-phase HPLC conditions for LC-MS analyses were as follows: flow rate = 200 μL/min; mobile phase A = 0.1% formic acid in H_2O; mobile phase B = 0.1% formic acid in acetonitrile. HPLC purifications were performed using a Shimadzu HPLC equipped with a single pump (LC-10Atvp), solvent mixer (FCV-10Alvp), controller (SCL-10Avp), autoinjector (SIL-10AF), and UV diode array detector (SPD-M10Avp). A Grace Vydac® 25 cm × 22 mm C-18 reverse-phase column (CAT# 218TP1022) was used for all HPLC work. Standard reverse-phase HPLC conditions were as follows: flow rate = 9.0 mL/min; mobile phase A = 0.1% trifluoroacetic acid (TFA) in water; mobile phase B = 0.1% TFA in acetonitrile. UV detection at 218 nm was used for all LC analyses. Compound purities were determined by integration of the peaks in LC traces measured at this wavelength.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Unity 500 spectrometer in deuterated solvents at 500 MHz and 125 MHz, respectively. Chemical shifts are reported in parts per million. Couplings are reported in hertz.

Construction of DKP macroarrays (III).

Representative synthesis of amino acid-loaded cellulose support (I). The cellulose esterification procedure was adapted from a protocol reported by Frank.[1] Dots were marked on a 10 × 10 cm sheet of Whatman 1CHR paper at distances 0.9 cm apart using a #2 lead pencil. In this format, 80 compound spots (area/spot = 0.3 cm^2) could be accommodated on a single sheet without any detectable cross contamination (see Figure S-1 for macroarray layout). To ensure dryness, the sheets were washed in 50 mL of CH_2Cl_2 for 5 min (3x), dried under a stream of N_2, and placed under high vacuum at rt for 60 min.

Coupling solutions were prepared in glass vials, containing: 1 eq Fmoc-AA-OH, 1.2 eq *N,N'*-diisopropylcarbodiimide (DIC), and 2 eq *N*-methyl imidazole (NMI) in 3 mL of DMF. The concentrations of the solutions were adjusted to equalize the loadings of the amino acids.

Gly: 75 mg Fmoc-Gly-OH, 46 μL DIC, 35 μL NMI	480 nmol/cm^2
Ala: 105 mg Fmoc-Ala-OH, 65 μL DIC, 49 μL NMI	480 nmol/cm^2
Leu: 135 mg Fmoc-Leu-OH, 78 μL DIC, 59 μL NMI	510 nmol/cm^2
Phe: 195 mg Fmoc-Phe-OH, 104 μL DIC, 78 μL NMI	560 nmol/cm^2
Cph: 283 mg Fmoc-4-Cl-Phe-OH, 135 μL DIC, 101 μL NMI	510 nmol/cm^2

The dried paper sheets were placed in 15 cm Petri dishes and placed in an Atmosbag® (Aldrich) along with vials of the coupling solutions. The bag was purged with N_2 (3x) and filled until it resembled a soft pillow. The coupling solutions were poured onto and completely taken

up by the paper supports, the Petri dishes were covered, and the reactions were allowed to proceed at rt for 6 h. The supports were removed from the bag, washed by adding and then decanting 20 mL portions of DMF (3x) and EtOH (3x), and dried under a stream of N_2. Spots were punched from each support sheet to quantify the amino acid loadings (see procedure below), and the supports were blanket *N*-Fmoc deprotected by immersion in 20 mL of 4% 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF at rt for 30 min. The solutions were decanted, and the supports were washed by adding and then decanting 20 mL portions of DMF (3x) and EtOH (3x), and dried under a stream of N_2. Each support (**I**) was then cut into fourths prior to the Ugi 4CR (in order to avoid cross-contamination between the volatile isocyanide building blocks (**A**–**D**); see Figure S-1).

Representative Fmoc quantitation protocol. The amino acid loading of the esterified planar support was quantified according to standard UV Fmoc analysis procedures.[2] A spot (6 mm diameter) was punched from the array using a desktop hole-punch and immersed in a solution of 4% DBU in DMF. The spot was swirled in this mixture for 20 min. A 200 μL aliquot of this solution was removed and diluted with 800 μL of DMF, and the absorbance was read at 296 nm (ε_{296}= 9800 M^{-1} cm^{-1}) in a quartz cuvette. The value was multiplied by five to account for the dilution. Amino acid loadings of ~500 nmol/cm^2 were calculated using this method.

Representative Ugi 4CR procedure. Individual solutions (~250 μL) of 2 M Boc-AA-OH (**a**–**e**) were prepared in DMF. Aliquots (72 μL) of each solution were placed in four wells of a chemically resistant 96-well polystyrene plate. Aliquots of the corresponding aldehyde (**A**–**D**) were then added to these wells, and the solutions were mixed with repeated pipetting to generate 20 Boc-AA-OH/RCHO mixtures in the following configuration (see Figure S-1):

Column 1:	Boc-Gly-OH (**a**)	**Row a:**	Cyclohexanecarboxyaldehyde (**A**)
Column 2:	Boc-Ala-OH (**b**)	**Row c:**	Heptanal (**B**)
Column 3:	Boc-Leu-OH (**c**)	**Row e:**	3-phenylpropanal (**C**)
Column 4:	Boc-Phe-OH (**d**)	**Row g:**	Benzyloxyacetaldehyde (**D**)
Column 5:	Boc-Cph-OH (**e**)		

Supports **I** (now split into twenty 5 x 5 cm squares) were placed in glass Petri dishes, and a multi-channel pipetteman was used to deliver both the water and the Boc-AA-OH/RCHO mixtures onto the supports. First, 3 μL of Millipore water were spotted onto the support, followed by 2 μL of Boc-AA-OH/RCHO mixture. Second, 0.75 μL of isocyanide (**1:** *tert*-butyl isocyanide; **2:** 1-pentyl isocyanide; **3:** cyclohexyl isocyanide; **4:** benzyl isocyanide) were spotted on top of the other reagents. The library was built one isocyanide at a time to avoid cross-contamination with these volatile reagents. Five subarrays (pertaining to each ester-linked amino acid building block) were spotted with each of the 20 Boc-AA-OH/RCHO mixtures, followed by the appropriate isocyanide. The Petri dishes were covered, and the reactions were allowed to proceed at rt for 25 min. This procedure was then repeated with each of the three remaining isocyanides to generate a total of 400 library members. Thereafter, the supports were washed with 15 mL portions of MeOH (1x), DMF (3x), and MeOH (2x), and dried under a stream of N_2 to yield 20-member Ugi macroarrays **II**.

TFA vapor Boc deprotection procedure. Boc deprotection was performed on intact macroarrays **II** in Pyrex crystallization dishes. A 10 mL portion of TFA was added to the bottom

of a glass vacuum dessicator (interior diameter 21 cm, interior height 20 cm). Up to 10 arrays (**II**, 5 x 5 cm) were placed on a perforated ceramic platform in the dessicator that was situated 7 cm above the TFA. The dessicator was evacuated to 60 mm Hg over a 10 min period. The dessicator was sealed, disconnected from the vacuum, and allowed to stand for an additional 50 min at rt. The deprotected arrays were removed from the dessicator and allowed to vent in a fume hood at rt under N_2 for 2 h.

NH_3 vapor DKP cyclization procedure. Cyclization reactions were again performed on intact macroarrays. A 50 mL portion of NH_4OH was added to the bottom of a large Pyrex dish to generate an NH_3 chamber. Up to 10 deprotected arrays from above (5 x 5 cm) were placed in a small Pyrex dish, and the dish was placed inside the NH_3 chamber. The chamber was sealed with a plastic lid, and the cyclization reaction was allowed to proceed at rt for 60 min to yield DKPs (**III**). The arrays were removed from the chamber and allowed to vent in a fume hood at rt under N_2 for 2 h.

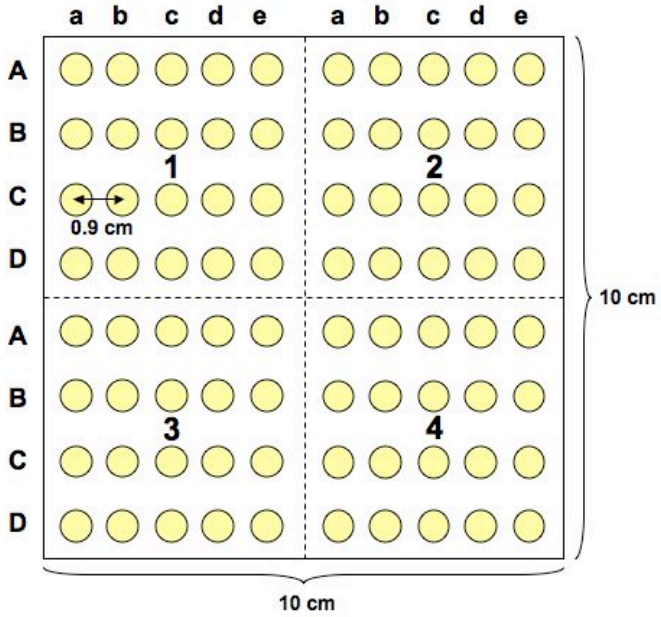


Figure S-1. Macroarray layout. Five of these arrays were blanket-esterified with amino acid building blocks (**Gly**, **Ala**, **Leu**, **Phe**, or **Cph**). The sheets were then cut into fourths to avoid isocyanide (**1**–**4**) cross-contamination during synthesis. Twenty Boc-AA-OH (**a**–**e**)/RCHO (**A**–**D**) solutions were generated and spotted onto the supports with a multichannel pipette.

For routine LC-MS characterization, individual compounds were eluted from the support by adding acetonitrile (1.0 mL) to a vial containing a punched out spot. The vials were shaken for 20 min, after which the solutions were transferred to clean labeled vials, and the acetonitrile was evaporated under reduced pressure. Compound residues were then dissolved in 200 μL of 1:1 AcCN/H_2O and filtered through a cotton plug for LC-MS analysis. For biological testing, the spots were punched directly into the wells of a 96-well plate (see Figure S-2 for a representative plate image). Purity data are compiled below in Table S-2.

Yield analysis of selected DKP macroarray members.

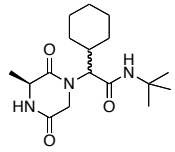
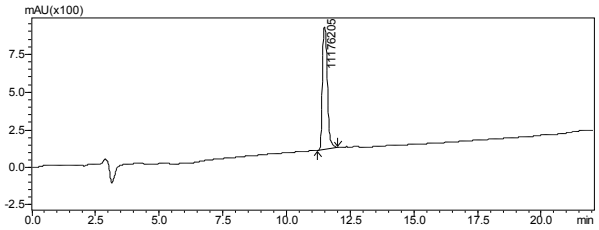
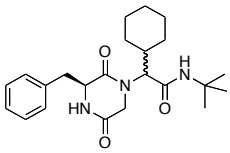
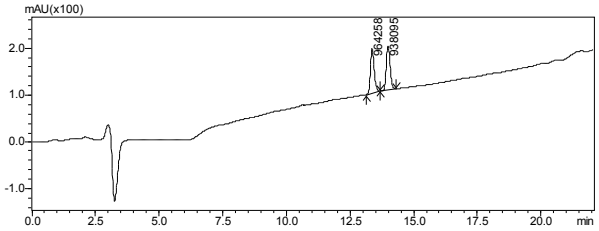
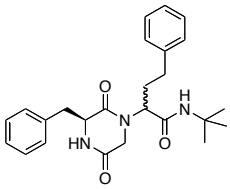
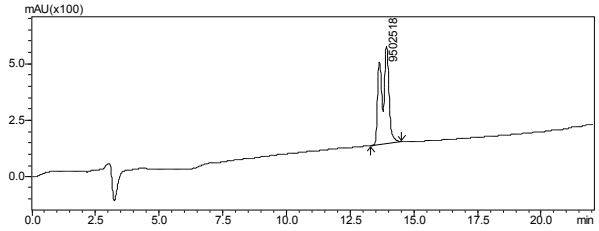
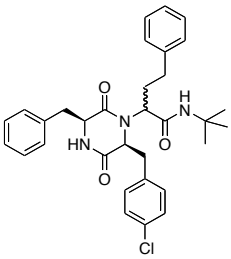
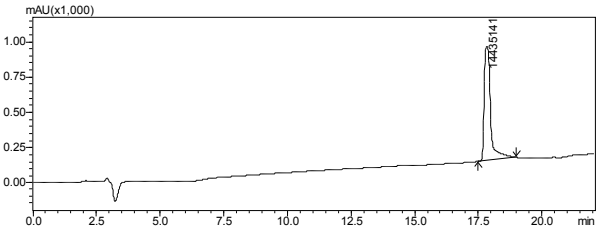
Synthesis of model DKPs (**GlyAb1**, **GlyAd1**, **GlyCd1**, and **CphCd1**)**.** Four model DKPs containing 0, 1, 2, or 3 aromatic groups were synthesized on planar supports in sufficient quantity to generate UV calibration curves on an LC-MS instrument. In brief, four amino acid-loaded supports (5 x 10 cm) were subjected to Ugi 4CRs by spotting (as described above) ~100 x in order to cover the entire support sheet. *N*-Boc deprotection and DKP cyclization steps were performed as described above on the entire sheets. The crude DKPs were eluted from the supports by swirling the supports in 20 mL AcCN (2x) for 20 min, and the solvent was removed under reduced pressure. The DKPs were purified by preparative HPLC to >95% purity, and 1–5 mg of each DKP were isolated. LC traces of purified compounds are shown in the table below (Table S-1). Calibration curves were generated for each DKP by plotting peak area at 218 nm vs. concentration (1.0, 0.5, 0.1, 0.05. and 0.01 mM). The calibration curves are shown below.

NMR data were obtained for the individual diastereomers of **GlyAd1** (see below), as these were the only diastereomers that gave baseline separation by HPLC.

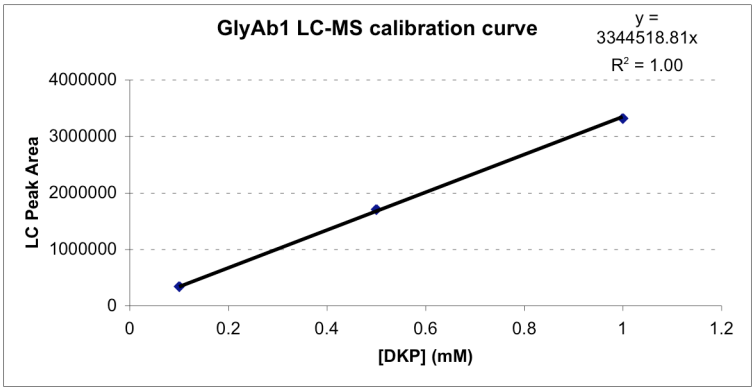
NMR data of GlyAd1 isomer A. ^{1}H: (500 MHz, CD_3OD) δ 7.79 (s, 1H), 7.16 (m, 3H), 7.29 (m, 2H), 4.60 (d, *J* = 11.7 Hz, 1H), 4.33 (t, *J* = 4.5 Hz, 1H), 3.59 (d, *J* = 17.7 Hz, 1H), 3.28 (dd, *J* = 13.2, 4.4 Hz, 1H), 3.00 (dd, *J* = 13.2, 4.4 Hz, 1H), 2.93 (d, *J* = 17.7 Hz, 1H), 1.74 (m, 5H), 1.61 (m, 1H), 1.37 (s, 9H), 1.24 (m, 4H), 1.08 (m, 1H), 0.90 (m, 1H); ^{13}C: (125 MHz, CD_3OD) δ 169.87, 169.51, 169.12, 136.62, 131.30, 130.02, 128.55, 62.63, 58.06, 52.72, 46.46, 40.96, 36.60, 30.64, 29.93, 29.08, 27.37, 26.76, 26.63.

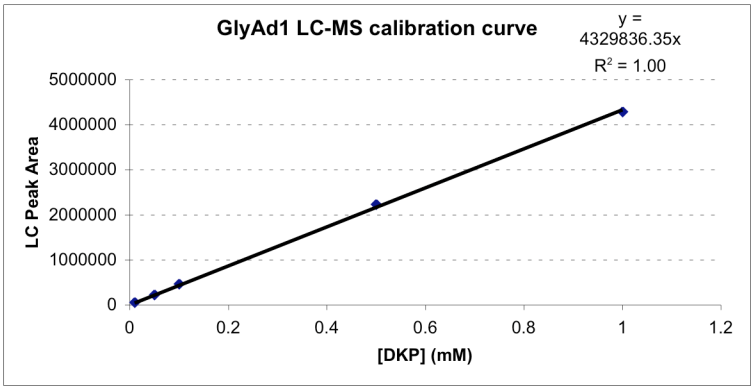
NMR data of GlyAd1 isomer B. ^{1}H: (500 MHz, CD_3OD) δ 7.64 (s, 1H), 7.28 (m, 3H), 7.21 (m, *J* = 7.6, 2.1 Hz, 2H), 4.50 (d, *J* = 11.1 Hz, 1H), 4.38 (d, *J* = 4.6 Hz, 1H), 4.35 (d, *J* = 17.5 Hz, 1H), 3.22 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.20 (d, *J* = 17.5 Hz, 1H), 3.05 (dd, *J* = 13.9, 5.5 Hz, 1H), 1.74 (m, 1H), 1.65 (m, 3H), 1.58 (m, 1H), 1.30 (s, 9H), 1.27–0.95 (m, 5H), 0.91 (d, *J* = 12.8 Hz, 1H), 0.66 (qd, *J* = 11.8, 3.1 Hz, 1H); ^{13}C: (125 MHz, CD_3OD) δ170.96, 170.88, 168.35, 136.82, 131.49, 129.85, 128.43, 62.50, 59.23, 52.59, 52.48, 46.51, 40.63, 36.60, 30.79, 30.62, 28.89, 28.88, 27.36, 26.76, 26.44.

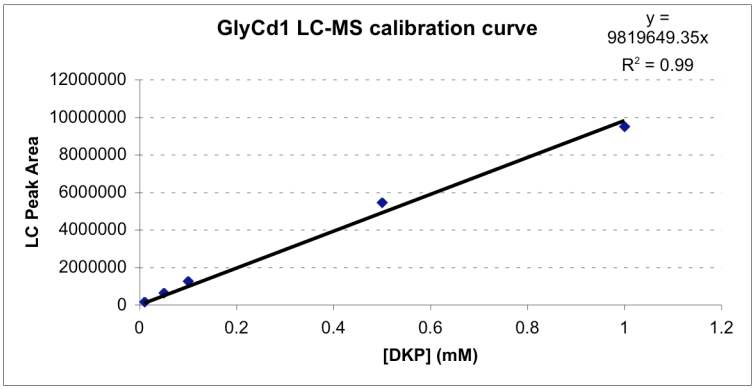
Table S-1. LC-MS analysis of model DKPs used for yield analysis

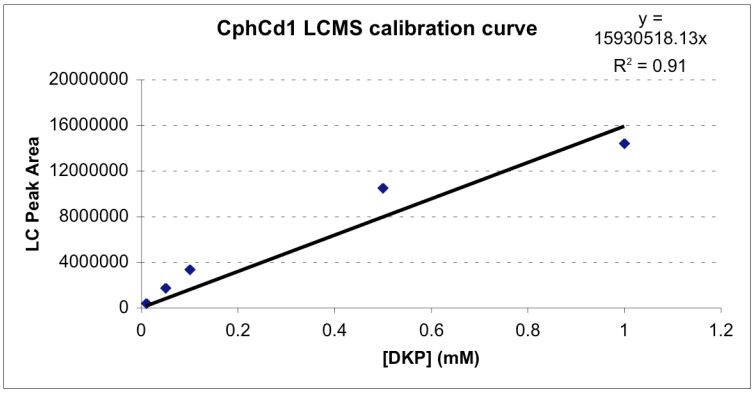
# Ar	% library	structure Mol. wt. MS data	LC trace (UV detection at 218 nm)
0	13.5	 **GlyAb1** calc: 323.4 obs: 324.1	
1	36	 **GlyAd1** calc: 399.5 obs: 400.1	
2	34.5	 **GlyCd1** calc: 421.5 obs: 444.1 ($M+Na^+$)	
3	14	 **CphCd1** calc: 546.1 obs: 546.2	

Calibration curves of model DKPs.









LC-MS purity analysis data.

Table S-2. LC-MS analysis of representative library members cleaved from macroarrays

entry	# Ar	retention time (min)[a]	calc. mass	obs. mass	purity (%)[b]
GlyAb1	0	11.54	323.4	346.1	82
GlyAd1	1	13.49/14.13	339.5	422.2	92
GlyCd1	2	13.76/14.05	421.5	444.1	97
CphCd1	3	17.91	572.1	568.1	94
GlyAc1	0	13.80/14.18	365.5	388.2	97
GlyBe2	1	16.67/16.86	450.0	472.1	84
GlyCb3	1	12.66	371.5	394.1	99
AlaAc2	0	15.72	393.6	416.2	89
AlaAd3	1	15.72/15.97	439.6	440.2	94
AlaBa3	0	13.96	351.5	374.1	91
AlaBd1	1	16.27	415.6	416.2	82
AlaCd2	2	15.46	449.6	450.1	98
AlaCe4	3	15.54	504.0	526.1	93
AlaDd1	2	14.41/14.63	451.6	474.2	99
LeuAb1	0	15.02	379.5	380.1	96
LeuAd2	1	17.68	469.7	470.3	94
LeuAd3	1	17.77	481.7	504.3	96
LeuBa2	0	16.10/16.26	381.6	382.2	94
LeuBb4	1	16.07	415.6	438.2	90
LeuCa1	1	14.37	387.5	410.1	82
LeuCd1	2	16.90/17.16	477.6	478.2	81
LeuDe2	2	17.84	542.1	564.2	95
PheAb4	2	14.62/15.07	447.6	470.2	91
PheBb3	1	17.03	441.6	442.2	96

entry	# Ar	retention time (min)[a]	calc. mass	obs. mass	purity (%)[b]
PheBe1	2	18.66/18.94	526.1	548.2	97
PheCa1	2	14.21	421.5	422.1	93
PheCc2	2	17.67	491.7	514.3	97
PheDa3	2	14.50/14.79	463.6	486.1	98
PheDd3	3	17.36	553.7	554.2	98
CphAa1	1	15.30/15.59	434.0	456.1	97
CphBb2	1	17.74	464.0	486.1	90
CphCb3	2	16.69/16.88	496.0	518.1	97
CphCd3	3	18.38	572.1	594.1	98
CphDc4	3	17.13/17.43	562.1	584.1	95

[a] Two retention times are given if the diastereomer peaks were resolved in the LC trace. [b] Determined by integration of LC traces (UV detection at 218 nm). DKP purities are reported as a mixture of diastereomers.

Table S-3. Average purities of DKPs grouped by building block

R_1NH_2	average purity (%)
Gly	92
Ala	92
Leu	91
Phe	96
Cph	95

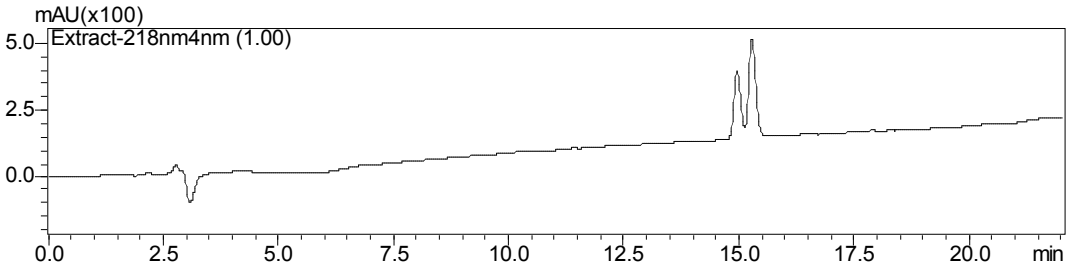
R_2CHO	average purity (%)
A	93
B	91
C	93
D	97

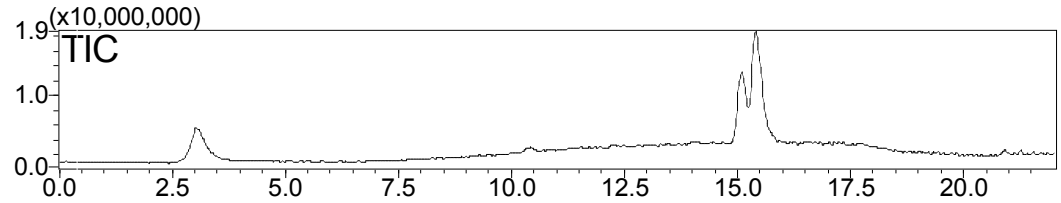
R_3CO_2H	average purity (%)
a	92
b	93
c	95
d	94
e	92

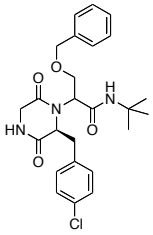
R_4NC	average purity (%)
1	91
2	93
3	96
4	92

LC traces of active DKPs cleaved from macroarrays.

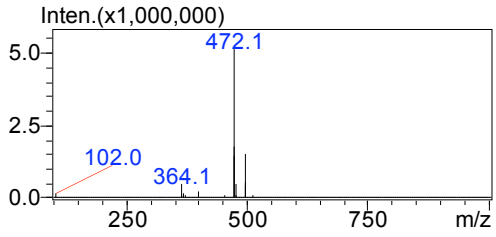
CphDa1



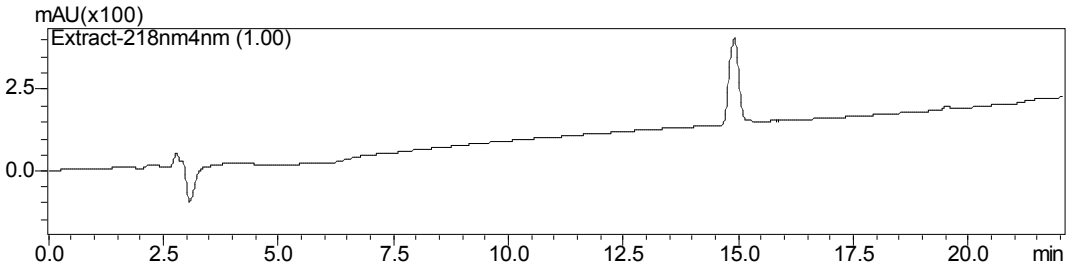


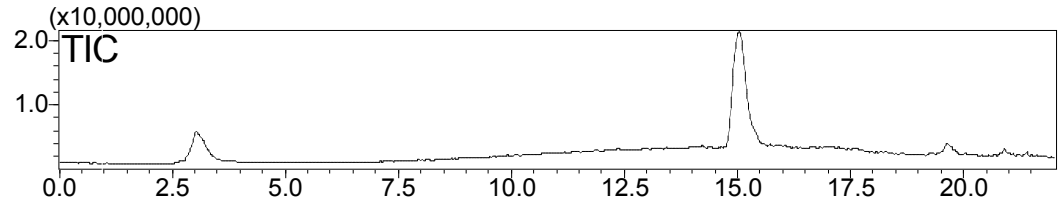


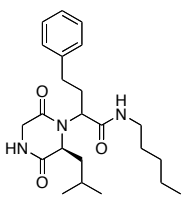
Cph Da1
Mol. Wt.: 471.98



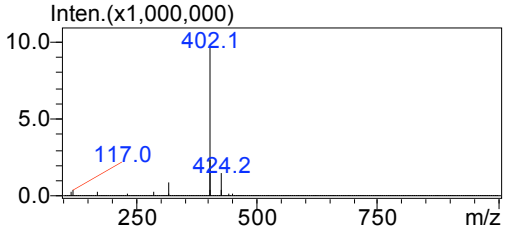
LeuCa2



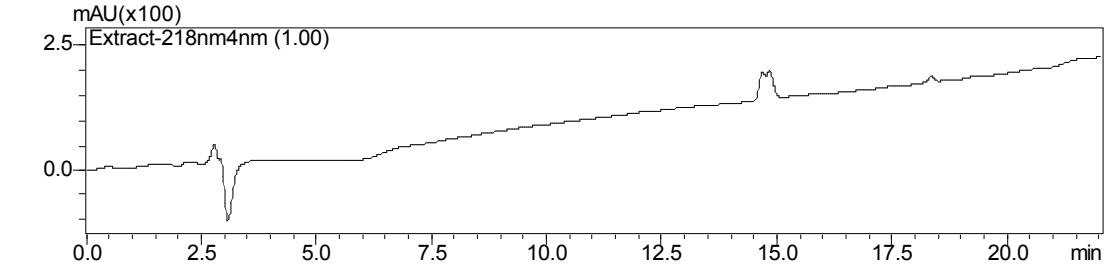


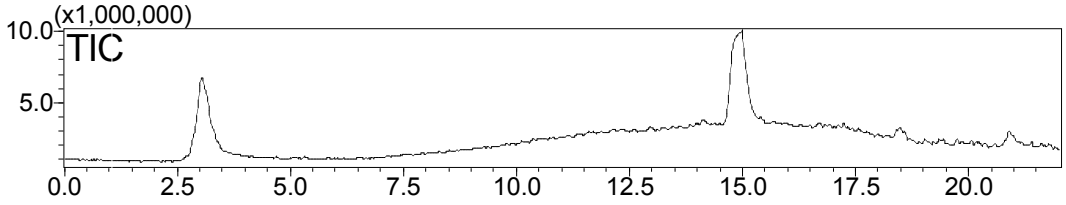


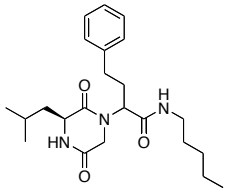
Leu Ca2
Mol. Wt.: 401.54



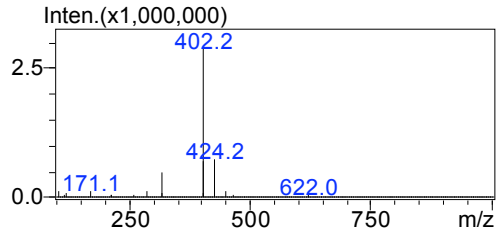
GlyCc2



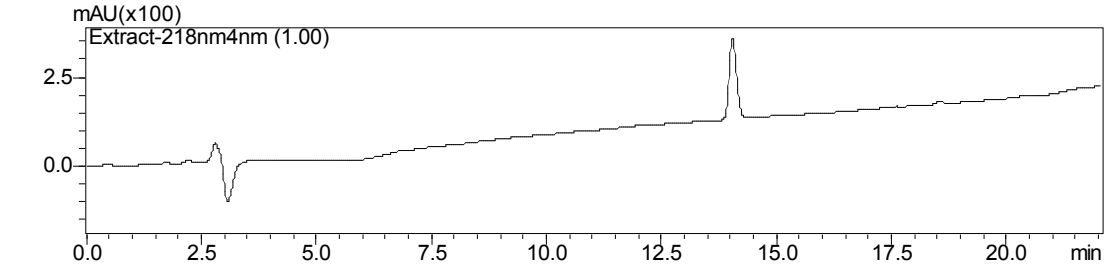


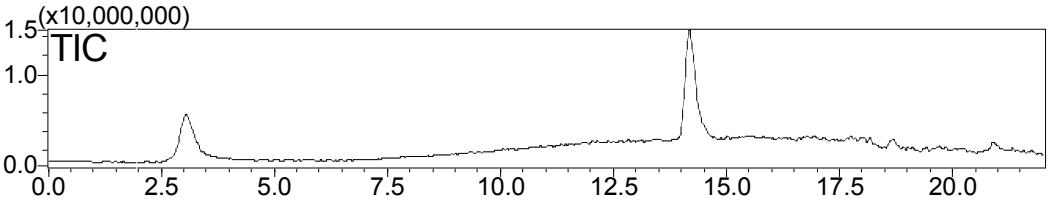


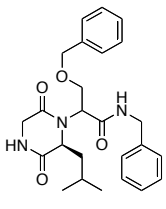
Gly Cc2
Mol. Wt.: 401.54



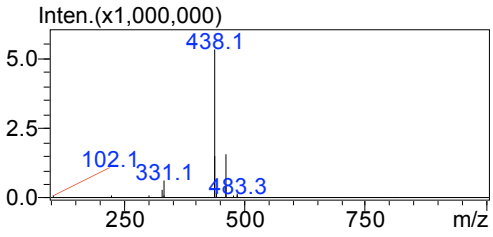
LeuDa4



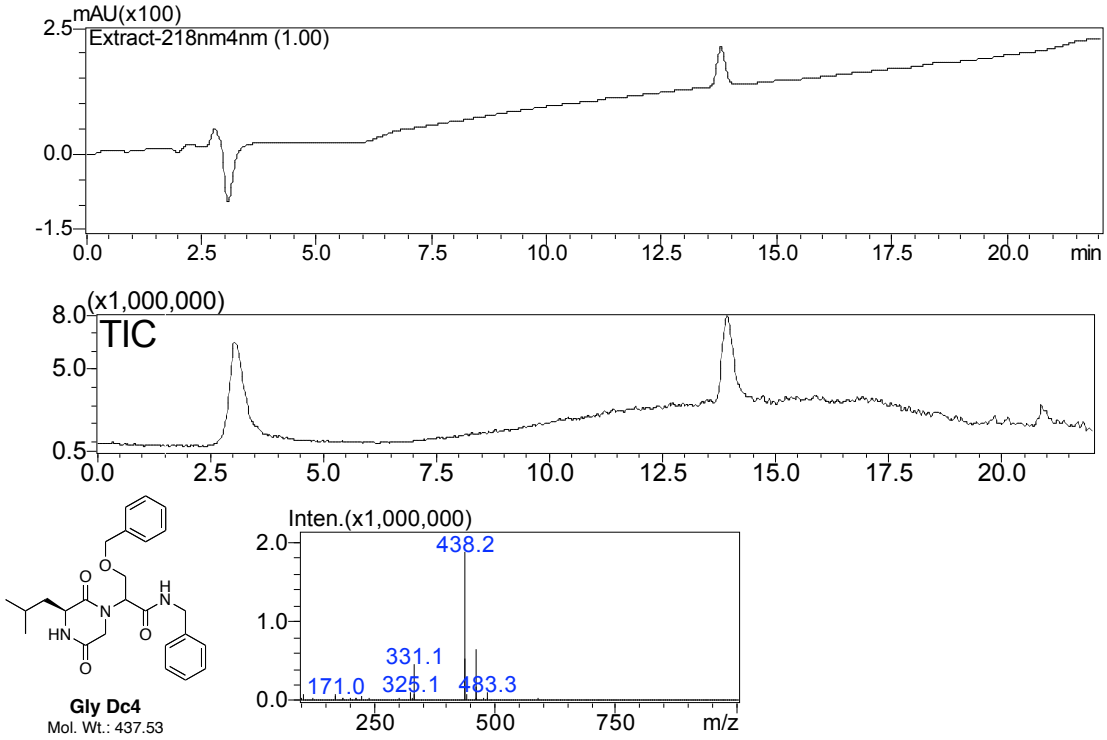




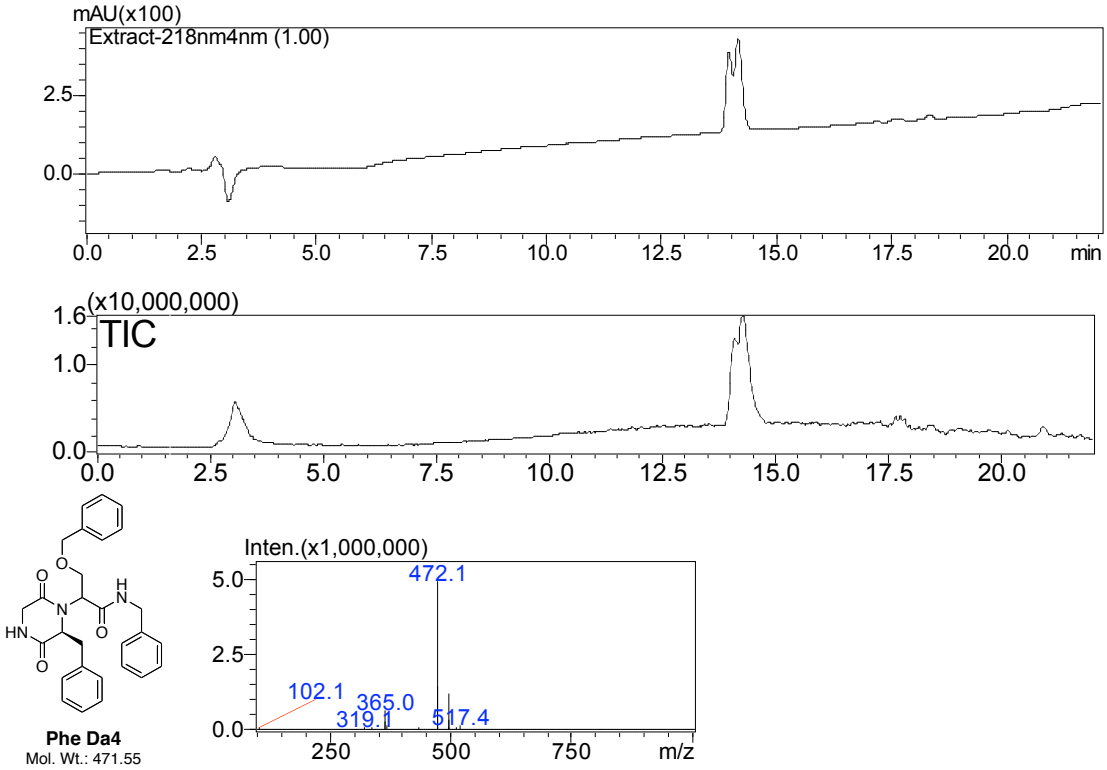
Leu Da4
Mol. Wt.: 437.53



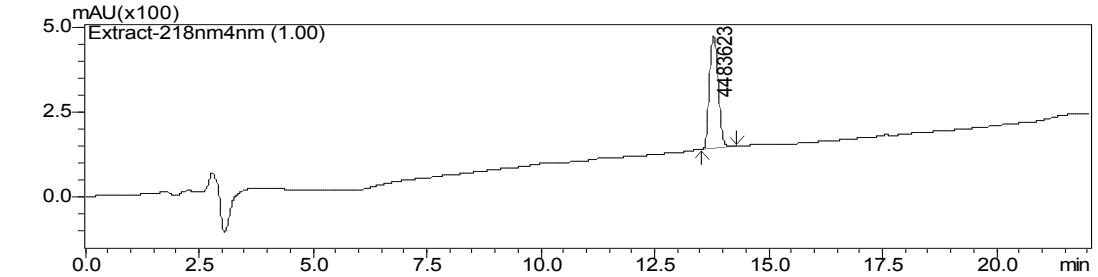
GlyDc4

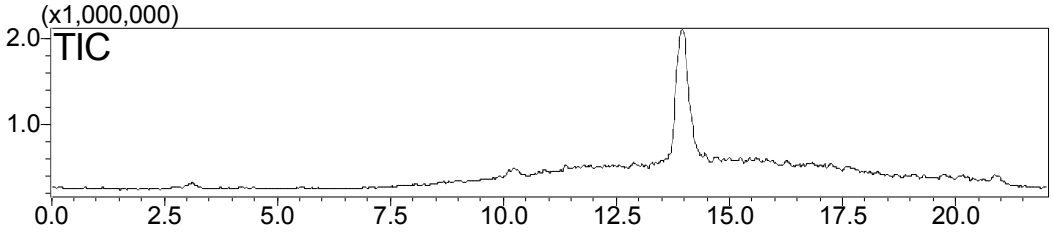


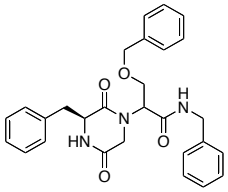
PheDa4



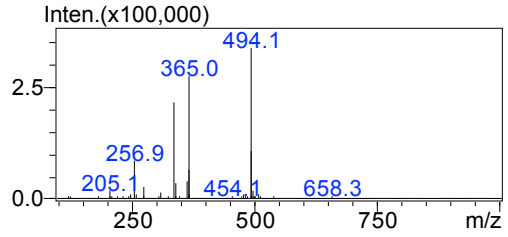
GlyDd4







Gly Dd4
Mol. Wt.: 471.55



Scaled-up synthesis of active DKPs.

CphDa1. A 5 x 10 cm section of Whatman 1CHR filter paper loaded with 360 nmol/cm^2 4-Cl-Phe was spotted ~100 times with the appropriate reagents as described above. The array was washed, dried, and subjected to TFA vapor deprotection and NH_3 vapor cyclization. The product was eluted from the array with 25 mL AcCN (2x, 20 min), which was subsequently removed *in vacuo*. The crude product was purified by preparatory HPLC to afford **CphDa1** as a white powder; MS: expected, 471.98; observed, m/z 472.1 $[M+H]^+$.

LeuCa2. A 5×10 section of Whatman 1CHR filter paper loaded with 375 nmol/cm^2 Leu was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution, and purification analogous to **CphDa1** afforded **LeuCa2** as a white powder; MS: expected, 401.54; observed, m/z 402.1 $[M+H]^+$.

GlyCc2. A 5×10 section of Whatman 1CHR filter paper loaded with 360 nmol/cm^2 Gly was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution, and purification analogous to **CphDa1** afforded **GlyCc2** as a white powder; MS: expected, 401.54; observed, m/z 402.1 $[M+H]^+$.

LeuDa4. A 5×10 section of Whatman 1CHR filter paper loaded with 375 nmol/cm^2 4-Cl-Phe was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution and purification analogous to **CphDa1** afforded **LeuDa4** as a white powder; MS: expected, 437.53; observed, m/z 438.1 $[M+H]^+$.

GlyDc4. A 5×10 section of Whatman 1CHR filter paper loaded with 360 nmol/cm^2 4-Cl-Phe was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution, and purification analogous to **CphDa1** afforded **GlyDc4** as a white powder; MS: expected, 437.53; observed, m/z 438.2 $[M+H]^+$.

PheDa4. A 5×10 section of Whatman 1CHR filter paper loaded with 380 nmol/cm^2 4-Cl-Phe was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution, and purification analogous to **CphDa1** afforded **PheDa4** as a white powder; MS: expected, 471.55; observed, m/z 472.1 $[M+H]^+$.

GlyDd4. A 5×10 section of Whatman 1CHR filter paper loaded with 380 nmol/cm^2 Gly was spotted ~100 times with the appropriate reagents as described above. Washing, TFA vapor deprotection, NH_3 vapor cyclization, elution, and purification analogous to **CphDa1** afforded **GlyDd4** as a white powder; MS: expected, 471.6; observed, m/z 494.1 $[M+Na]^+$.

Full bacteriological assay protocols and data.

Compound handling and reagents. Stock solutions of synthetic compounds (25 mM) were prepared in DMSO and stored in sealed vials. All biological reagents were purchased from Fisher and used according to enclosed instructions. LBS (Luria-Bertani Salt) medium was prepared from 20 g dehydrated LB broth, 15 g NaCl, 3 mL glycerol, and 7.8 g Tris-HCl with a final pH = 7.5. OHHL was synthesized according to our previously reported procedure.[3]

Instrumentation. Absorbance and luminescence measurements were obtained using a PerkinElmer Wallac 2100 EnVision™ multilabel plate reader using Wallac Manager v1.03 software. A 595 nm filter was used for measuring bacterial cell density (OD_{600}).

***Vibrio fischeri* assay procedures.** For luminescence antagonism assays, macroarray spots were punched directly into the wells of a 96-well plate. Unfunctionalized Whatman 1CHR spots were punched into positive and negative control wells to normalize cell growth across the wells. Aliquots of DMSO (5 μL) were added to assist in compound solubilization during the bioassay. An overnight culture of *V. fischeri* ES114 (Δ–*luxI*)[4] was diluted 1:10 with LBS medium, and 250 μL portions were added to the negative control wells. An appropriate volume of concentrated OHHL (10 mM in DMSO) was then added to the remaining cell culture to give a final concentration of 3–5 μM (*ca.* EC_{50} of autoinducer in this strain). A 250-μL portion of the diluted culture was added to each remaining well of the plate. Plates were grown at rt with shaking (200 rpm) until the OD_{600} = 0.35–0.4 (4–6 h). Aliquots of each well (200 μL) were then transferred to fresh, white-walled 96-well plates for analysis. Luminescence was measured and normalized to cell density per well. The primary data are shown below. IC_{50} values were calculated using GraphPad Prism software using a sigmoidal curve fit.

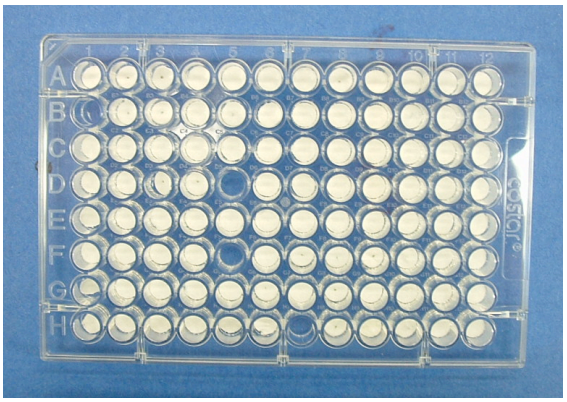
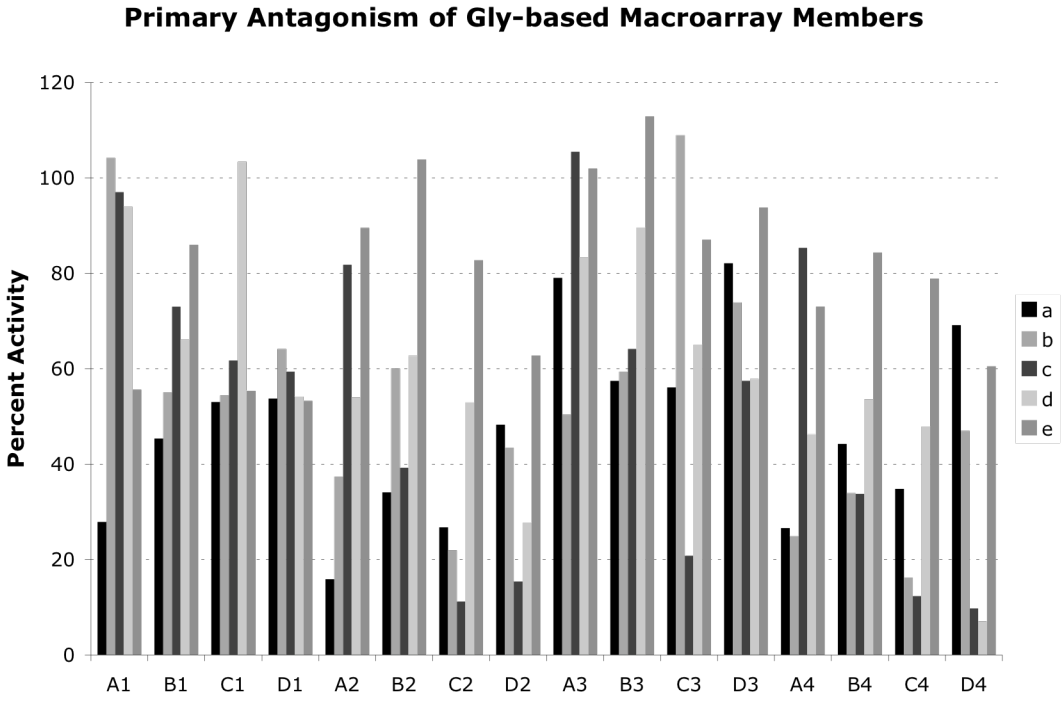
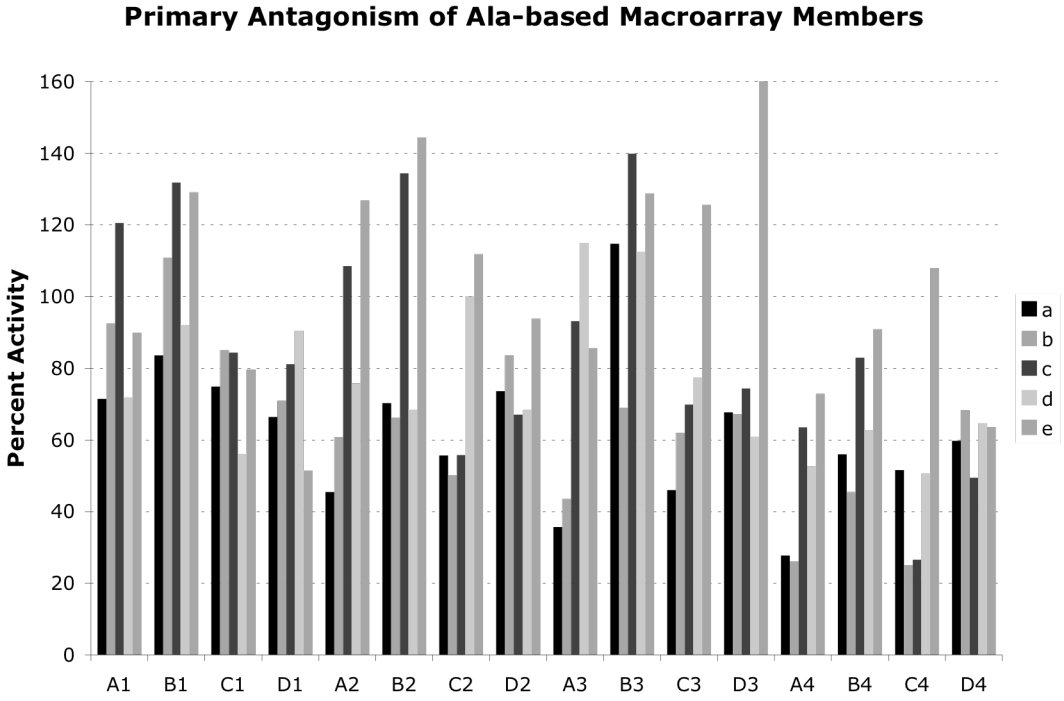
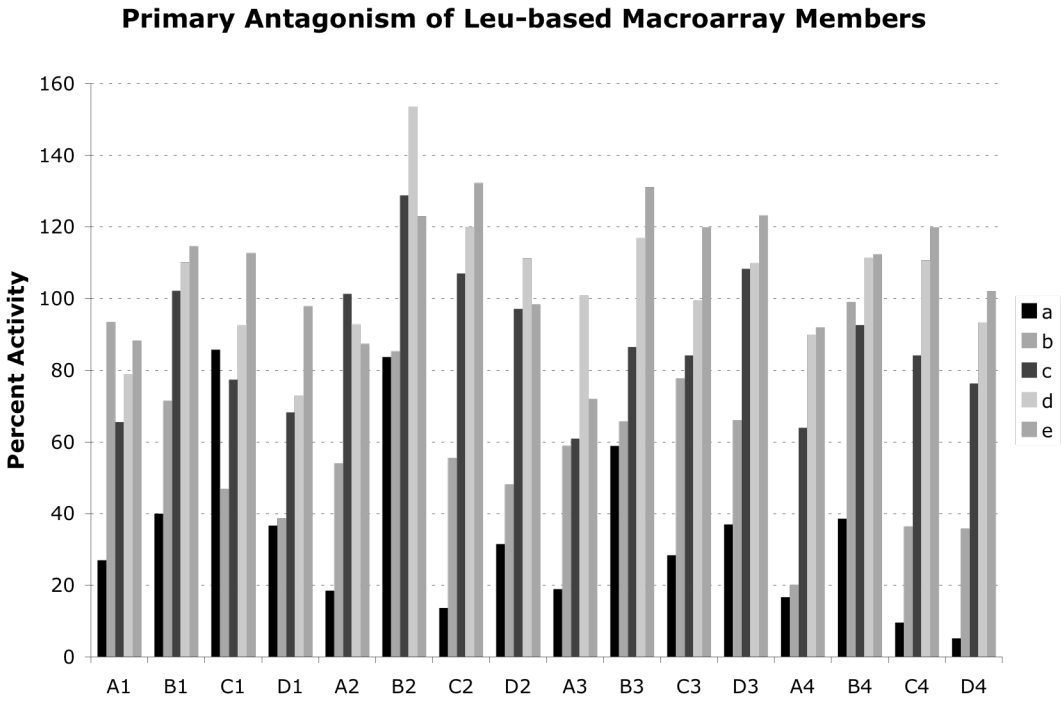


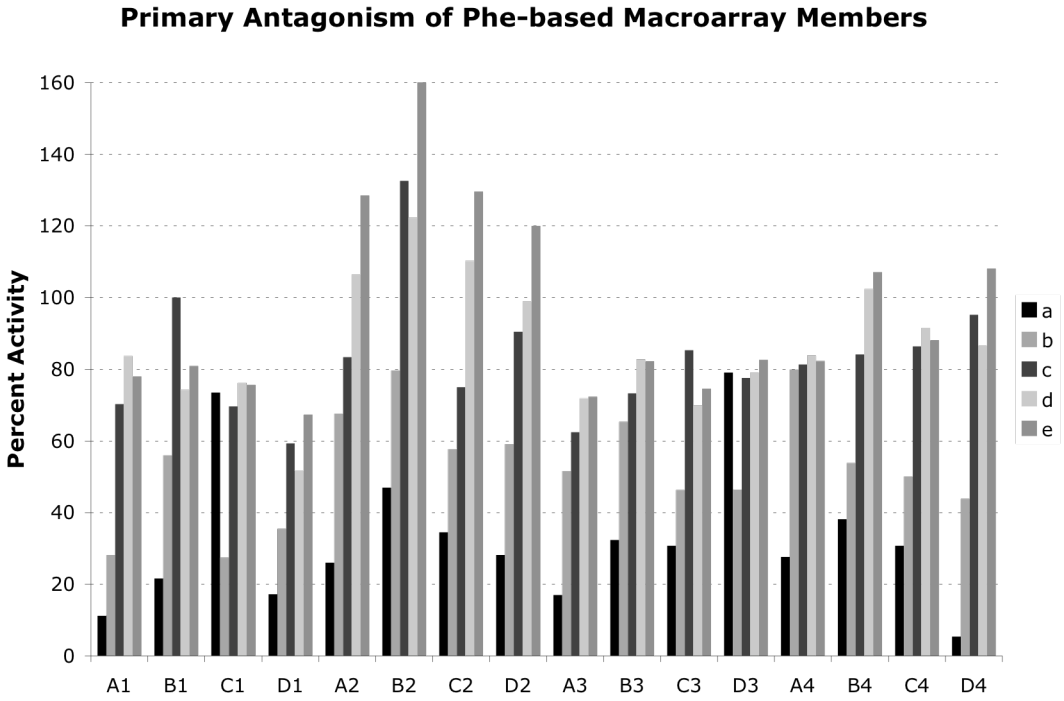
Figure S-2. DKPs were screened in 96-well plates against *V. fischeri* in the presence of the cellulose support spots.

Primary antagonism assay data for macroarray members. DKPs were tested at ~550 μM against 5 μM OHHL in *V. fischeri* (Δ–*luxI*). Each graph shows the biological data obtained from one of the five subarrays generated on ester-linked amino supports **I**. The title of the graph gives the side chain of the amine component (**Gly**, **Ala**, **Leu**, **Phe**, or **Cph**). Array members are then grouped according to aldehyde (**A**–**D**) and isocyanide (**1**–**4**) components. The individual bars in each group show the effect of the second amino acid side chain (**a**–**e**) on the activity of the DKP.






Primary Antagonism of Leu-based Macroarray Members
Percent Activity
160
140
120
100
80
60
40
20
0
A1
B1
C1
D1
A2
B2
C2
D2
A3
B3
C3
D3
A4
B4
C4
D4
a
b
c
d
e


Primary Antagonism of Phe-based Macroarray Members
Percent Activity
160
140
120
100
80
60
40
20
0
A1
B1
C1
D1
A2
B2
C2
D2
A3
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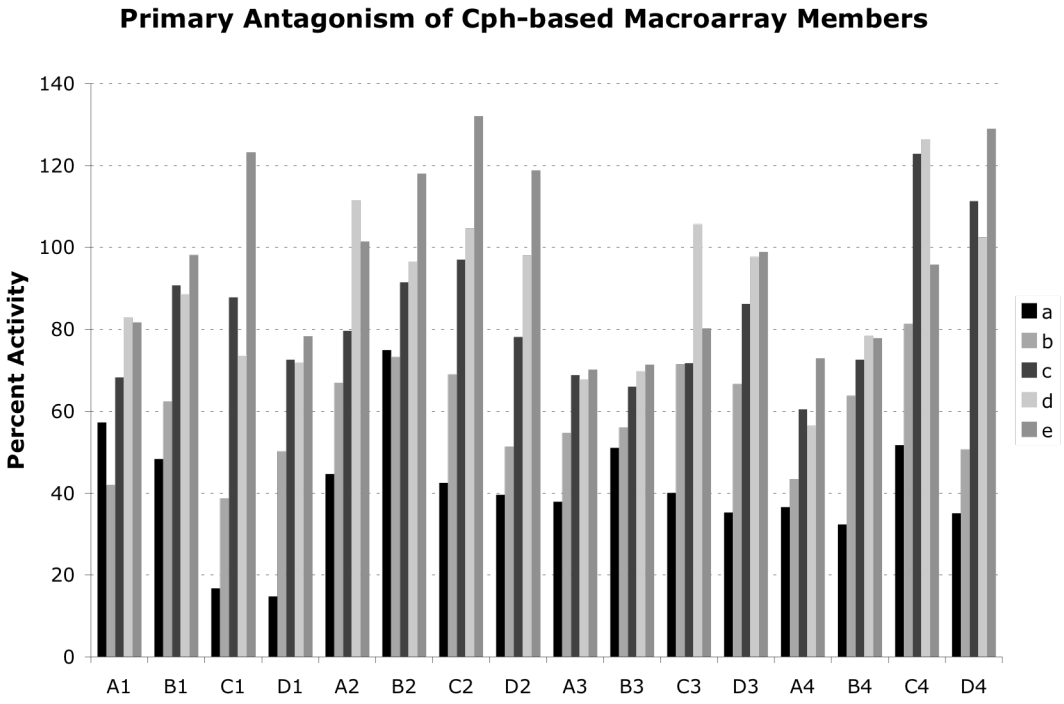
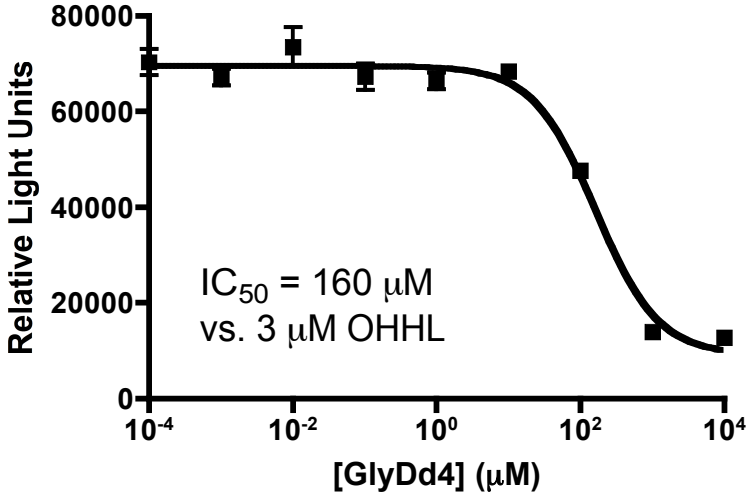
B4
C4
D4
a
b
c
d
e



Inhibition dose response curve for GlyDd4.

A luminescence inhibition dose response curve was generated from an authentic sample of **GlyDd4**. The compound concentrations tested ranged from 10^{-4} to 10^{4} μM against 3 μM OHHL in *V. fischeri* (Δ–*luxI*). Bacterial samples were prepared as described above. A best curve fit was calculated using PrismGraph.



Antagonism assay data obtained from authentic DKP samples.

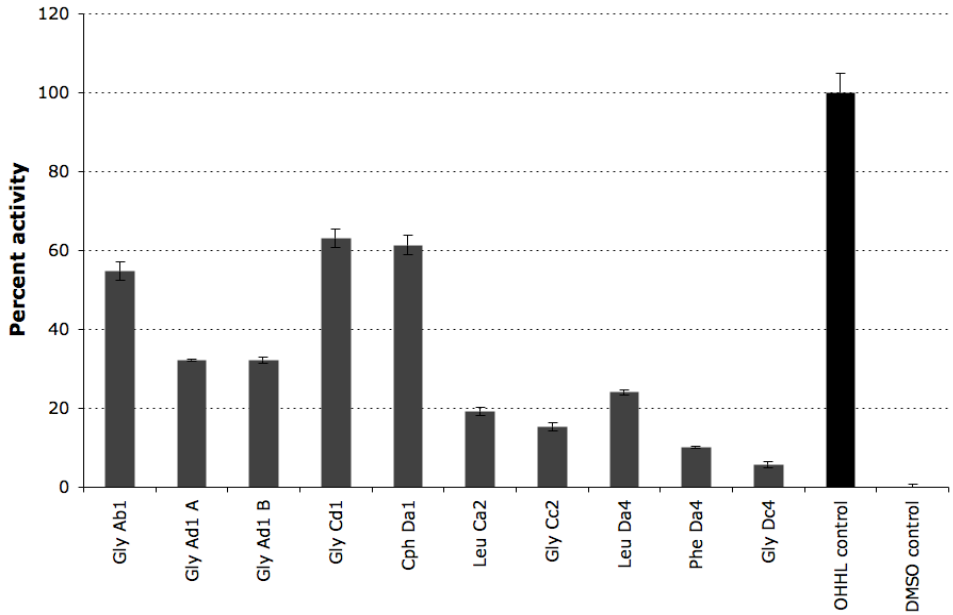


Figure S-3. Authentic samples of active and control DKPs were tested at 500 μM against 5 μM OHHL in *V. fischeri* (Δ–*luxI*). Luminescence was normalized to cell density for each well, and these values were then normalized to the OHHL (5 μM OHHL only) and DMSO controls to give percent activity. The DMSO control contained no autoinducer and gave the background luminescence of the *V. fischeri* strain.

Optical density measurements obtained from authentic DKP samples at 500 μM.

DKP	OD600	% survival
Gly Cc2	0.524	98
Leu Ca2	0.530	99
Gly Dc4	0.511	95
Leu Da4	0.514	96
Gly Dd4*	0.541	101
Phe Da4	0.525	98
Cph Da1	0.526	98
Gly Ab1	0.532	99
Gly Ad1 A	0.534	100
Gly Ad1 B	0.528	99
Gly Cd1	0.548	102
positive	0.536	100

* Average of optical densities at all dose response concentrations ranging from 0.0001–1000 μM. Positive control = 5 μM OHHL only.

References.

(1) Frank, R. *Tetrahedron* **1992**, *48*, 9217–9232.

(2) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404-3409.

(3) Geske, G. D.; O'Neill, J. C.; Miller, D. M.; Mattmann, M. E.; Blackwell, H. E. *J. Am. Chem. Soc.* **2007**, *129*, 13613-13625.

(4) Lupp, C.; Urbanowski, M.; Greenberg, E. P.; Ruby, E. G. *Mol. Microbiol.* **2003**, *50*, 319-331.