Efficient Synthesis and Evaluation of Quorum Sensing Modulators Using Small Molecule Macroarrays

Thanit Praneenararat, Grant D. Geske, and Helen E. Blackwell*

Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, WI 53706-1322

e-mail: blackwell@chem.wisc.edu

Supporting Information.

* To whom correspondence should be addressed.

General experimental information.

General. All reagents were purchased from commercial sources (Aldrich, Acros, and NovaBiochem) and used without further purification. Solvents were purchased from commercial sources (Aldrich and J.T. Baker) and used as is, with the exception of dichloromethane (CH_2Cl_2), which was distilled over calcium hydride prior to use. Water was purified using a Millipore Analyzer Feed System. Planar cellulose membranes (Whatman 1Chr chromatography paper, 20 × 20 cm squares) utilized for macroarray construction were purchased from Fischer Scientific and stored in a desiccator at room temperature (rt) until ready for use.

Gas chromatography-mass spectrometry (GC-MS) data for PHLs **11a–g**, **11k**, and **11m** were obtained using a Shimadzu GCMS-QP2010S equipped with autoinjector and single quadrupole MS analyzer (by electron impact ionization, EI). The GC-MS was interfaced with a PC running the Shimadzu GCSolutions software package (version 2.50 SU3). A Shimadzu SHRXI-5MS capillary column (dimensions: 30 m × 0.25 mm × 0.25 μm) was used for all GC-MS work. Standard GC conditions were as follows: injection temperature = 280 °C; split mode with split ratio of 10; linear velocity = 36.1 cm/sec; initial column temperature = 120 °C; hold 1 min; ramp at 20 °C/min to 300 °C; ramp at 6 °C/min to 315 °C for a total run time of 12.5 min.

Reversed-phase high performance liquid chromatography (RP-HPLC) data for PHLs **11h–j** and **11l** and CHLs **12a–m** were obtained using a Shimadzu system equipped with an SCL-10Avp controller, LC-10AT pump, FCV-10ALvp solvent mixer, and SPD-10MAvp UV/vis diode array detector. A Shimadzu C18 column (5 μm, 4.6 mm × 250 mm) was used for analytical RP-HPLC work. Standard RP-HPLC conditions were as follows: flow rates were 1 mL/min; mobile phase A = 0.1% trifluoroacetic acid (TFA) in water; mobile phase B = 0.1% TFA in acetonitrile. Purities were determined by integration of peaks with UV detection at 268 nm for nitro PHLs **11h–j**, 254 nm for PHL **11l**, 275 nm for CHLs **12a–h** and **12j–m**, and 300 nm for CHL **12i**.

Electrospray ionization (ESI) MS data for PHLs **11h–j** and **11l** and CHLs **12a–m** were obtained using a Waters (Micromass) LCT™ system. This instrument uses a time-of-flight analyzer. Samples were dissolved in methanol and sprayed with a sample cone voltage of 20.

Luminescence and absorbance measurements in bioassays were obtained on a Synergy 2 multi-mode microplate reader (BioTek Inc.) and processed with Gen 5 software (version 1.05.11).

Initial derivatization of planar cellulose support.

Planar cellulose membrane amination protocol. The amination protocol was based in part on previous methods reported by our laboratory.[1] In brief, a grid was drawn on a 20 × 20 cm sheet of Whatman 1Chr paper **1** using a #2 lead pencil (distances between crosses = 1.5 × 1.5 cm). The sheet was then cut into four 10 × 10 cm pieces. The pieces were immersed in 100 mL of a 2.0 M solution of tosyl chloride in pyridine at rt for 90 min. The paper pieces were washed by subsequent immersion and shaking on a bench-top orbital shaker in DMF, methanol, and CH_2Cl_2 (150 mL, 5 min each), and dried under a stream of N_2. The tosylated paper was immersed in 60 mL of neat spacer unit, 4,7,10-trioxa-1,13-tridecanediamine (**2**), in a Pyrex dish, and placed in a

drying oven at 80 °C for 40 min. The amine solution was carefully decanted from the paper pieces. The pieces were then washed again using the same sequence as that performed after the tosylation procedure, and dried under a stream of N_2 to yield amino support **3**.

Linker installation protocol on support 3. 3,5-dichloro-4-hydroxybenzoic acid (**4**, 0.1157 g, 0.56 mmol) and 1-hydroxybenzotriazole (HOBt, 0.0767 g, 0.57 mmol) were dissolved in DMF (500 μL). A 100-μL aliquot of this solution was combined with *N*,*N*-diisopropylcarbodiimide (DIC, 17 μL, 0.11 mmol), and the mixture was vortexed at rt for 10 sec. Aliquots (2 μL each) of this activated solution were spotted onto each grid cross of amino support **3** using a laboratory Pipetteman (calibrated for 1–10 μL delivery).** This spotting process was performed twice, after which the support was allowed to stand at rt for 15 min. The planar support was then washed by subsequent immersion in DMF (2x) and MeOH (1x) (100 mL, 3 min each), and dried under a stream of N_2 to yield linker-functionalized support **5**.

***Note, all reagent spotting (and subsequent standing) steps in this study was performed under a N_2 atmosphere in an Aldrich© AtmosBag (size M, 39 in. x 48 in., 280 L gas volume capacity) to minimize the presence of adventitious moisture.*

AHL macroarray synthesis on linker-functionalized support 5.

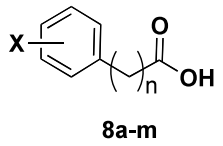
Homoserine coupling on support 5. *N*-Fmoc-L-HSe(OTrt)-OH (**6**, 0.1180 g, 0.20 mmol), 1,1'-carbonyldiimidazole (CDI, 0.1016 g, 0.63 mmol) and *N*-methylimidazole (NMI, 16 μL, 0.20 mmol) were dissolved in DMF (500 μL). The mixture was allowed to stand at rt for 5 min, after which 2-μL aliquots of the mixture were spotted onto each grid cross of linker-functionalized support **5**. The treated support was allowed to stand at rt for 20 min. This spotting and standing process was repeated three times (for a total reaction time of 1 h). The support was washed by subsequent immersion in DMF (2x) and MeOH (1x) (100 mL, 3 min each), and dried under a stream of N_2. Thereafter, any unreacted hydroxyl groups (and/or amines from the spacer) on the esterified support were "capped" by blanket acetylation. The support was immersed into 50 mL of 20% Ac_2O in DMF (v/v) in a Pyrex dish. The dish was covered and shaken at rt for 15 min on a bench-top orbital shaker, after which the solution was decanted. The support was subjected to the same washing sequence utilized in the previous esterification step and dried under a stream of N_2 to give *N*-Fmoc-L-HSe(OTrt)-functionalized support **7**.

Fmoc quantification protocol. The loading of *N*-Fmoc-L-HSe(OTrt)-OH onto support **7** was determined by UV quantification of the cleaved Fmoc protecting group. In brief, a spot (6 mm diameter) was punched from support **7** using a desktop hole-punch and placed into a 4-mL glass vial. A 1 mL-aliquot of 4% (v/v) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF was added to the vial, and the mixture was vortexed at rt for 30 sec and then shaken at rt for 5 min using an orbital shaker. A 200 μL-portion of this solution was removed and diluted with 800 μL of DMF. This solution was swirled at rt for 30 sec, after which the absorbance was measured at 296 nm (ε_{296} = 9880 $M^{-1}cm^{-1}$) in a quartz cuvette. The absorbance value was multiplied by 5 to account for the dilution. *N*-Fmoc-L-Hse(OTrt)-OH loadings in the range of 0.3–0.4 μmol/cm^2 were obtained using this method.

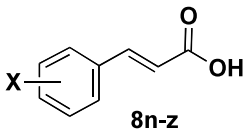
Blanket Fmoc cleavage protocol on planar support. Support **7** was immersed into 100 mL of 4% DBU (v/v) in DMF in a Pyrex dish and shaken at rt for 5 min on an orbital shaker. The solution was decanted from the support. The Fmoc-deprotected Hse-support was washed by subsequent immersion in DMF (2x) and MeOH (1x) (100 mL, 3 min each), and dried under a stream of N_2.

Carboxylic acid coupling protocol.
Method A (phenylacetic acid derivatives). Carboxylic acids **8a**–**m** and **8aa** (Figure S-1) and HOBt were dissolved in DMF (500 μL) to give ~1 M acid/~1.1 M HOBt solutions (note, acid **8aa** was included as necessary to generate control compound *N*-decanoyl L-homoserine lactone (DHL) on the array). Aliquots (100 μL) of these solutions were combined with DIC (to give ~1.1 M DIC), and the mixtures were vortexed at rt for 5 sec. Aliquots (2 μL) of these activated solutions were spotted in a spatially–addressed manner onto the grid crosses on the Fmoc-deprotected Hse support, and the treated support was allowed to stand at rt for 15 min. This spotting and standing process was repeated three times (for a total reaction time of 45 min). Thereafter, the support was washed with DMF (2x) and MeOH (1x) (100 mL, 3 min each) and dried under the stream of N_2 to give macroarray **9**.

Method B (cinnamic acid derivatives). Carboxylic acids **8n**–**z** (Figure S-1) and HOBt were dissolved in DMF (200 μL) to give ~1 M acid/~1.1 M HOBt solutions. These solutions were combined with DIC (to give ~1.1 M DIC), and the mixtures were vortexed at rt for 5 sec. Due to rapid compound precipitation, aliquots (2 μL) of these activated solutions were quickly spotted three times consecutively in a spatially–addressed manner onto the grid crosses on the Fmoc-deprotected Hse support, and the treated support was allowed to stand at rt for 15 min. Thereafter, the support was washed and dried according to the procedure for the phenyl acetic acids outlined above.

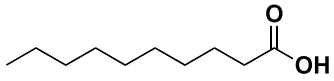


8a-m



8n-z

8a: X = H
8b: X = 4-Br
8c: X = 3-Br
8d: X = 2-Br
8e: X = 4-I
8f: X = 3-I
8g: X = 2-I
8h: X = 4-NO_2
8i: X = 3-NO_2
8j: X = 2-NO_2
8k: X = 4-CF_3
8l: X = 4-Ph
8m: X = 4-Br **
8n: X = H
8o: X = 4-F
8p: X = 3-F
8q: X = 2-F
8r: X = 2,6-F_2
8s: X = F_5
8t: X = 4-Cl
8u: X = 2-Br
8v: X = 4-NO_2
8w: X = 3-NO_2
8x: X = 2-NO_2
8y: X = 4-Br-2-F
8z: X = 4-Cl-3-NO_2



8aa

Figure S-1. Structures of carboxylic acid building blocks (**8a**–**z** and **8aa**) used to prepare the AHL library (**9**) and the control compound DHL. **For **8a**–**m**, n = 1 except for **8m** (n = 2).

TFA vapor-phase trityl deprotection and cyclization/cleavage of macroarray 9.
Trityl deprotection. A 10 mL-portion of TFA was added to the bottom of a glass vacuum dessicator (15 × 20 cm). To effect trityl cleavage, the intact array (**9**) was placed in a Petri dish (9 × 1 cm), and the dish was placed on a ceramic shelf positioned 5 cm above the bottom of the dessicator. The dessicator was evacuated to 60 mbar for 3 min to produce a TFA vapor atmosphere, sealed, and allowed to stand for 7 min. Thereafter, the dessicator was opened, and the array was removed. The array was washed with CH_2Cl_2 (100 mL, 2x) and dried under a stream of N_2 to yield **10**.

Cleavage of individual AHL array members. A 10 mL-portion of TFA was added to the bottom of a glass vacuum dessicator (15 × 20 cm). Spots of array **10** were punched out and placed in individual 4-mL glass vials. The vials were placed in a Petri dish (9 × 1 cm), and the dish was placed on the ceramic shelf in the dessicator. The dessicator was evacuated to 60 mbar for 10 min to produce a TFA vapor atmosphere, sealed, and allowed to stand for 18 h. The dessicator was then opened, and the vials were removed. AHLs (**11** and **12**) were eluted from the spots with MeOH (1 mL). The eluent was removed *in vacuo*, and the concentrated samples were submitted for either GC-MS or HPLC analysis. Fully dried samples were stored at rt for later analysis.

Cleavage of entire AHL arrays. Intact arrays (**10**) were subjected to the same TFA cyclization/cleavage protocol described for individual array members. Thereafter, the cleaved array was removed from the dessicator, placed in a 12.5 × 6.5 cm glass dish, and allowed to stand at rt for 15 min (to facilitate TFA evaporation). This dish was then carefully placed in a larger Pyrex dish containing 500 mL of 25% aq. NH_4OH (to neutralize any residual TFA on the array surface; note, TFA can impede bacterial growth). The Pyrex dish was covered and allowed to stand at rt for 1 h. The glass dish was removed from the NH_4OH bath, and allowed to stand in a fume hood at rt for an additional 15 min. The arrays of cleaved AHLs (**11** and **12**) were then subjected to bacterial agar overlay assays, as described below.

Solution-phase synthesis of PHL 11b.

In a 50-mL round-bottom flask equipped with a magnetic stirbar, 4-bromophenylacetic acid (0.7687 g, 3.57 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 0.6840 g, 3.57 mmol) were dissolved in 10 mL of DMF. (*S*)-aminobutyrolactone·HBr (0.3269, 1.80 mmol) was dissolved in 20 mL of distilled water in a 50 mL test tube. Triethylamine (248 μL, 1.78 mmol) was added to the aminobutyrolactone solution and mixed for 10 min. Thereafter, the aminobutyrolactone solution was carefully added dropwise to the activated acid solution over 5 min at rt, and the reaction mixture was allowed to stir at rt overnight (~18 h). The mixture was concentrated *in vacuo* and resuspended in EtOAc (50 mL). This solution was washed with 10% aq. citric acid solution (50 mL, 2x), saturated $NaHCO_3$ solution (40 mL, 2x), and brine (40 mL, 2x). The organic layer was isolated, dried over $MgSO_4$, and concentrated *in vacuo* to yield crude **11b**. This material was purified by flash silica gel column chromatography (eluent: 60% ethyl acetate in *n*-hexane) to give 121 mg of **11b** as a white solid. 23% yield. ^{1}H NMR, ^{13}C NMR, IR, and MS data matched those previously reported for **11b**.[2]

GC-MS calibration curve for PHL 11b.

Macroarray member **11b** served as a representative compound to gauge the efficiency of PHL macroarray synthesis. Solid stock of PHL **11b** (1.50 mg; synthesized according to the method above) was dissolved in 5 mL of MeOH to yield a 1.01 mM solution. This solution was diluted with MeOH to make ~0.5, 0.4, 0.3, 0.2, 0.1 and 0.05 mM solutions. A 40-μL portion of 0.4 mM internal standard (4-bromophenylpropionyl HL, **11m**) was added to each solution, and the total volume was adjusted to 200 μL (to make each solution 0.2 mM in internal standard). The solutions were analyzed by GC-MS, and the peak areas of **11b** and the standard were determined. A calibration curve was constructed by plotting the ratio of peak area of **11b**/peak area of the internal standard versus the ratio of concentration of **11b**/concentration of the internal standard (shown in Figure S-2). This curve was utilized to quantitate the amount of PHL **11b** generated on the PHL macroarray.

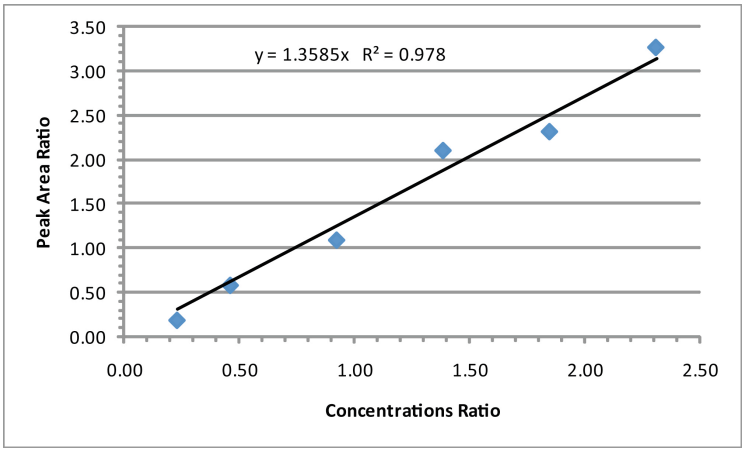


Figure S-2. Calibration curve generated for PHL **11b** by GC-MS.

HPLC calibration curve for CHL 12a.

Macroarray member **12a** served as a representative compound to gauge the efficiency of CHL macroarray synthesis. Solid stock of CHL **12a** (2.49 mg; synthesized according to our previous report[2]) was dissolved in 2 mL of ACN:H_2O (1:1) to yield a 5.38 mM solution. This solution was diluted with ACN:H_2O (1:1) to make ~1.1, 0.5, 0.4, 0.3, 0.2, 0.1 and 0.05 mM solutions. The solutions were analyzed by RP-HPLC, and the peak area of **12a** at 275 nm was determined. A calibration curve was constructed by plotting the peak area of **12a** versus the concentration of **12a** (shown in Figure S-3). This curve was utilized to quantitate the amount of CHL **12a** generated on the CHL macroarray.

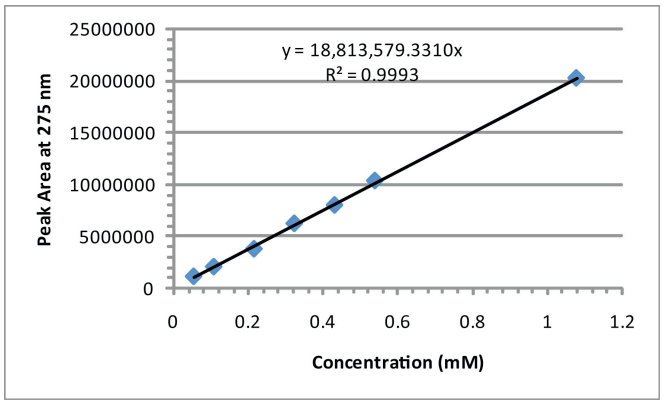
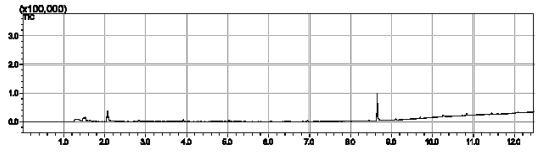
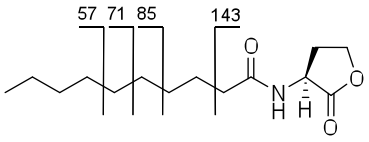
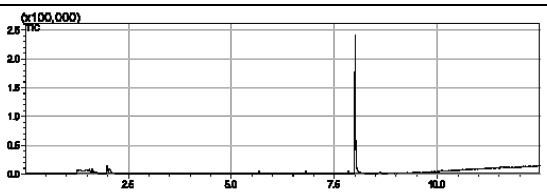
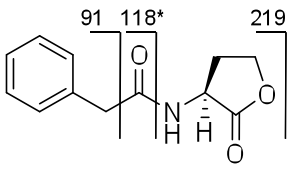
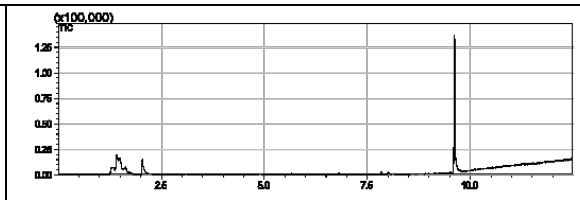
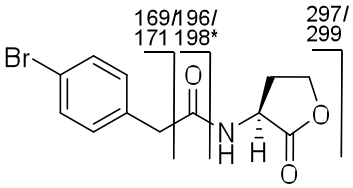
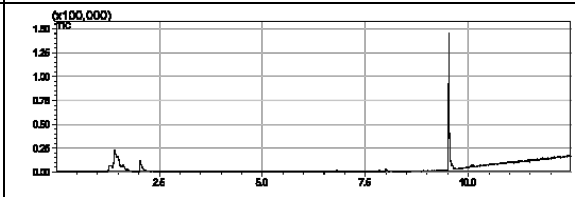
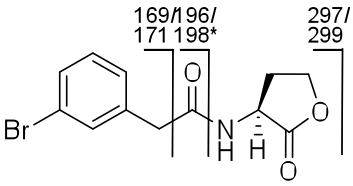
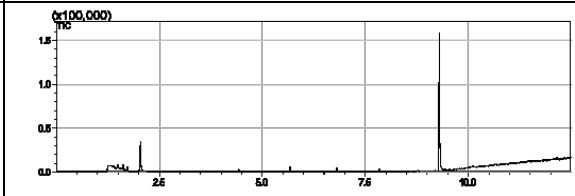
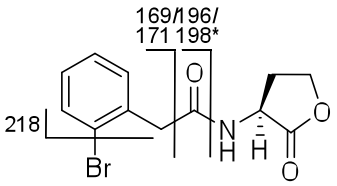
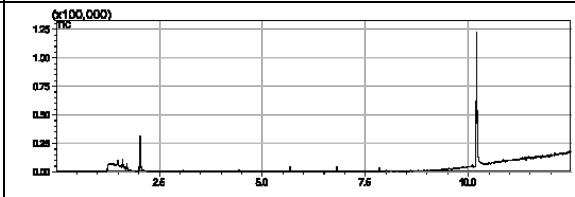
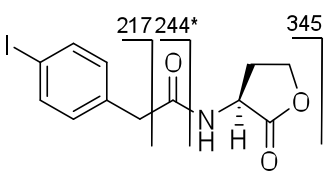
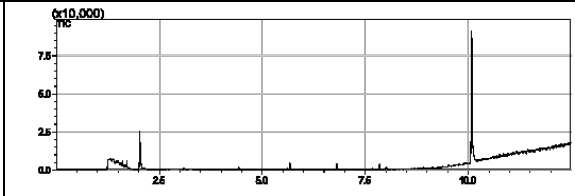
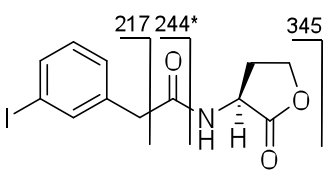
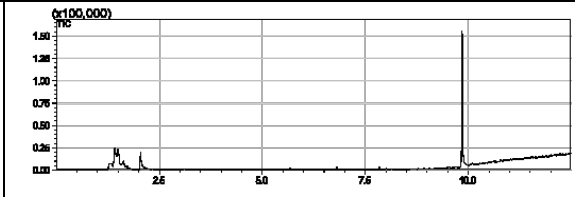
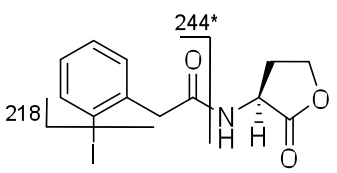
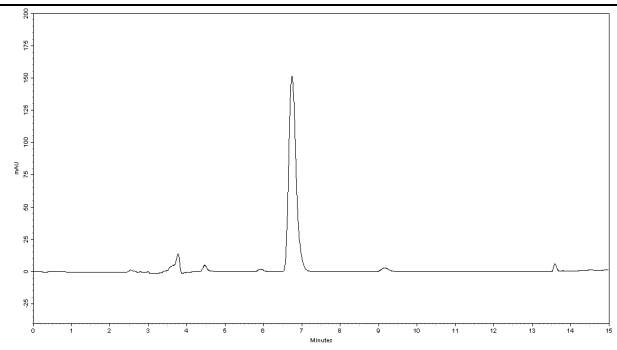


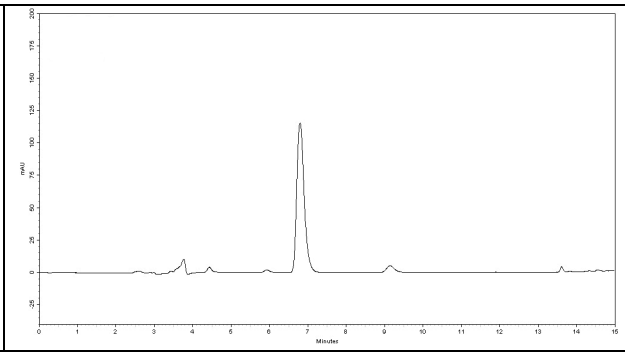
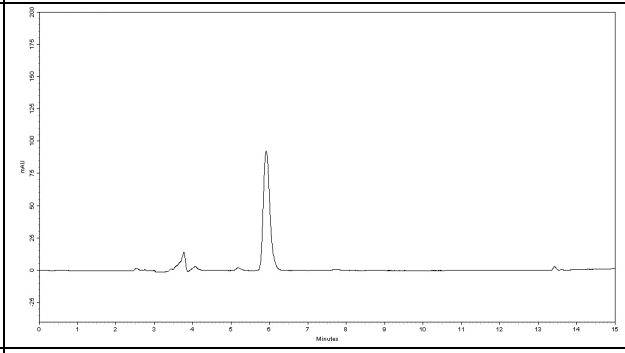
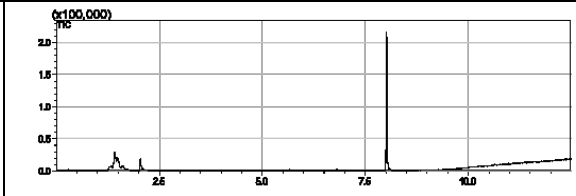
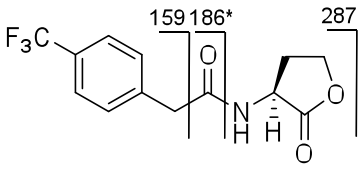
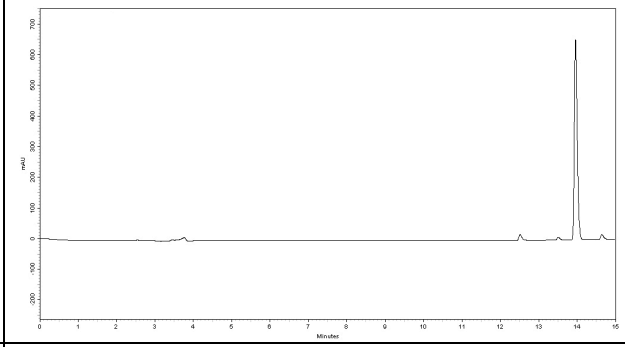
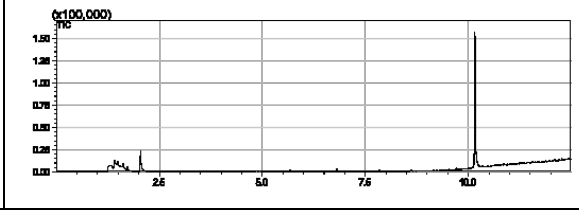
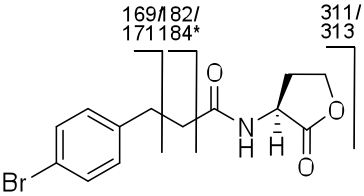
Figure S-3. Calibration curve generated for CHL **12a** by HPLC.

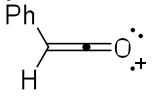
GC-MS data for 13-member PHL macroarray.

Table S-1. Purity data and ESI-MS fragmentation patterns for the PHL macroarray members **11**.

Compound % Purity	GC chromatogram	Structures with observed mass fragmentation patterns[a]
DHL 92%		
11a 95%		

11b 96%		
11c 99%		
11d 97%		
11e 98%		
11f 94%		
11g 94%		
11h[b] 95%		Cal. $[M]^+$ = 264.2; ESI-MS obs. $[M+Na]^+$ = 287.2

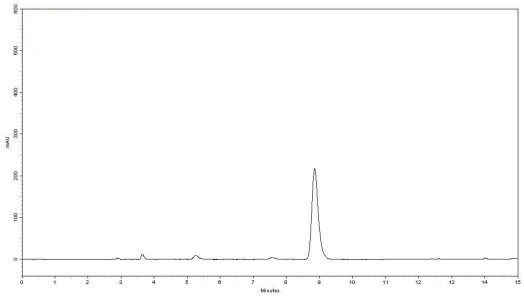
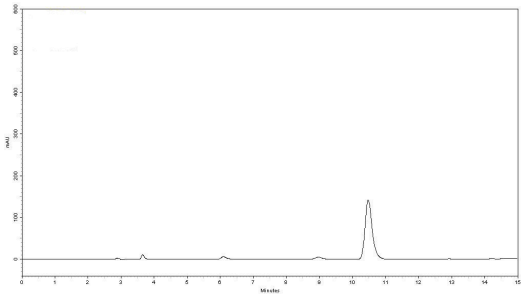
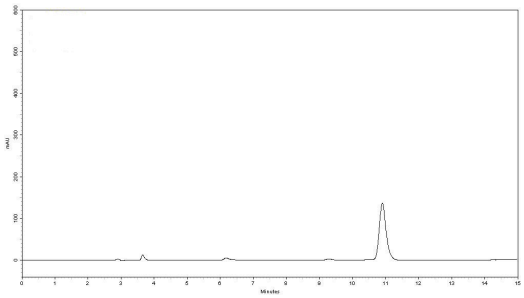
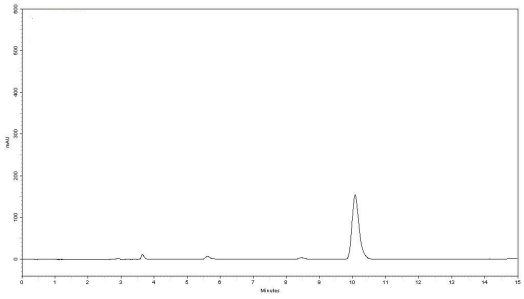
11i[b] 92%		Cal. $[M]^+$ = 264.23; ESI-MS obs. $[M+Na]^+$ = 287.2
11j[b] 95%		Cal. $[M]^+$ = 264.23; ESI-MS obs. $[M+Na]^+$ = 287.2
11k 99%		
11l[b] 94%		Cal. $[M]^+$ = 295.33; ESI-MS obs. $[M+Na]^+$ = 318.3
11m 97%		

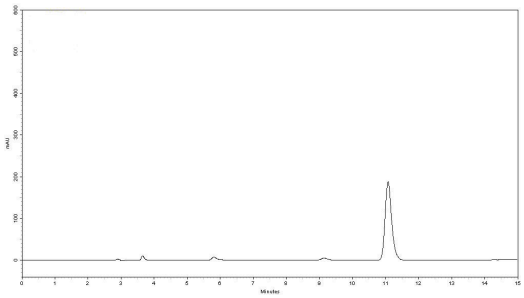
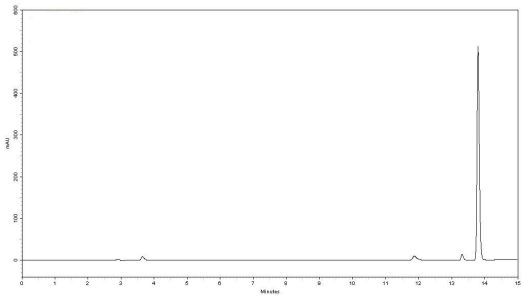
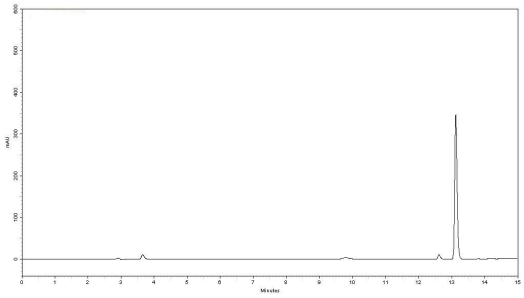
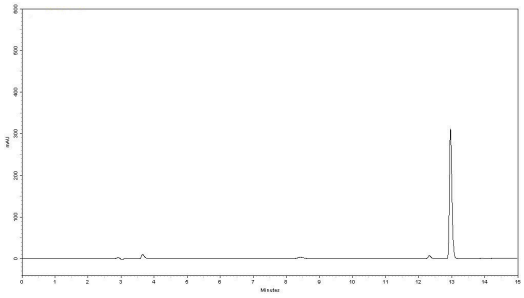
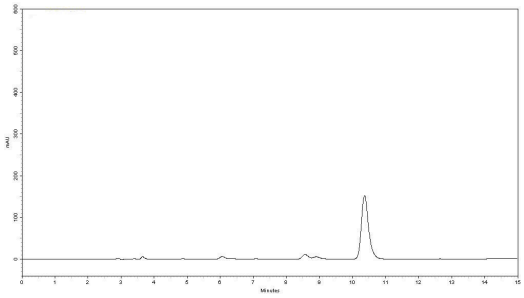
[a] Fragmentations from the loss of homoserine lactone moiety (with asterisks) have one extra mass unit loss. This can be attributed to the formation of ketene radical cation, *e.g.*, 

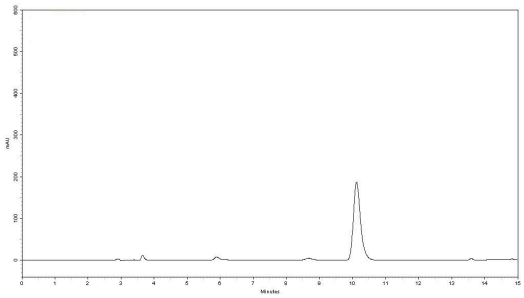
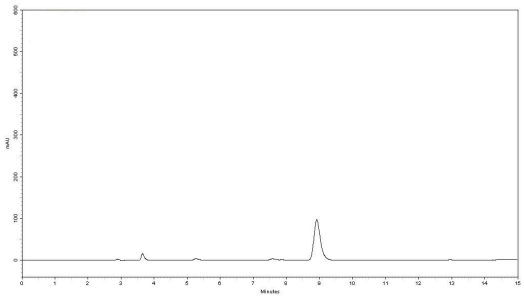
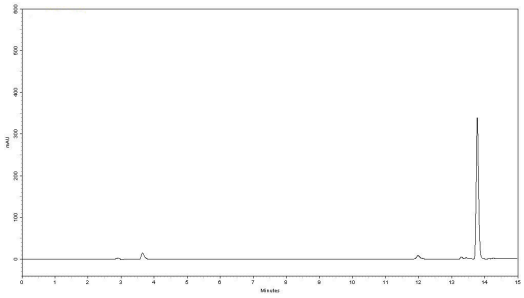
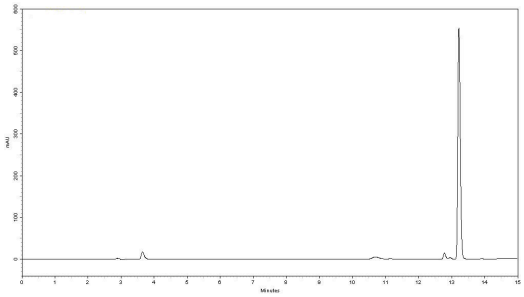
[b] The GC signal intensities for nitro PHLs **11h–j** and **11l** were weak and did not allow a quantitative measure of purity. HPLC was used instead to measure purities (with UV detection at 268 nm for **11h–j** and 254 nm for **11l**). ESI-MS was used to confirm the identities of the main product peaks.

HPLC and ESI-MS data for 13-member CHL macroarray.

Table S-2. Purity and ESI-MS data for the CHL macroarray members **12**.

Compound % Purity Cal. Mass; ESI-MS Obs. Mass	HPLC chromatogram (UV detection at 275 nm)
12a 93% Cal. $[M]^+$ = 231.25; Obs. $[M+Na]^+$ = 254.2	
12b 92% Cal. $[M]^+$ = 249.24; Obs. $[M+Na]^+$ = 272.3	
12c 92% Cal. $[M]^+$ = 249.24; Obs. $[M+Na]^+$ = 272.3	
12d 94% Cal. $[M]^+$ = 249.24; Obs. $[M+Na]^+$ = 272.1	

12e 94% Cal. $[M]^+$ = 267.23; Obs. $[M+Na]^+$ = 290.3	
12f 94% Cal. $[M]^+$ = 321.2; Obs. $[M+Na]^+$ = 344.3	
12g 94% Cal. $[M]^+$ = 265.69; Obs. $[M+Na]^+$ = 288.3	
12h 94% Cal. $[M]^+$ = 310.40; Obs $[M+Na]^+$ = 332.2+334.2 (isotopes)	
12i[a] 89% Cal. $[M]^+$ = 276.25; Obs. $[M+Na]^+$ = 299.3	

12j 94% Cal. $[M]^+$ = 276.25; Obs. $[M+Na]^+$ = 299.3	
12k 93% Cal. $[M]^+$ = 276.25; Obs. $[M+Na]^+$ = 299.3	
12l 93% Cal. $[M]^+$ = 328.14; Obs. $[M+Na]^+$ = 350.2+352.2 (isotopes)	
12m 94% Cal. $[M]^+$ = 310.69; Obs. $[M+Na]^+$ = 333.3	

[a] Based on UV absorbance at 300 nm.

V. fischeri **LuxR antagonism assay protocol and data.**

Strain and media. *V. fischeri* ES114 (Δ-*luxI*) was cultured according to published procedures.[3] This mutant strain lacks a functional LuxI synthase, and LuxR activation or inhibition is reported by luciferase production and readily measured by luminescence. The native AHL ligand for *V. fischeri*, *N*-(3-oxo-hexanoyl)-L-homoserine lactone (OHHL), was synthesized according to our previously reported procedures.[4]

Procedures. Small molecule LuxR antagonism assays in *V. fischeri* ES114 were performed according to previously reported procedures.[4] Only key details specific to macroarray experiments are provided here. The antagonism assays were performed at ~5 μM AHL against 5 μM OHHL (its EC_{50} value). For the PHL library, MeOH solutions of PHLs **11e**, **11i**, **11l**, and **11m** obtained after macroarray cleavage and GC-MS characterization were allowed to evaporate, and redissolved in ~60 μL of DMSO to give final concentrations of ~500 μM. Aliquots (2 μL) of these solutions were added to a 96-well microtiter plate, and bacterial culture was added to make a final volume of 200 μL per well. Authentic enantiopure samples of PHLs **11e**, **11i**, **11l**, and **11m** from earlier studies[2,4,5] were utilized as controls. All experiments were performed in triplicate. For the CHL library, the antagonism assay was performed in the same manner, except there was only one authentic enantiopure CHL (**12a**)[2] as a control (see text).

Figures S-4 shows LuxR antagonism assay data for PHLs **11e**, **11i**, **11l**, and **11m**. The activities of the macroarray-derived AHLs and authentic samples were comparable. These data suggest that our estimations of compound concentration in macroarray stock solution generation are reasonable. In addition, they indicate that there was minimal AHL racemization in the macroarray synthesis and cleavage methods (D-AHLs are known to have significantly reduced or no activity in these assays).[4,5]

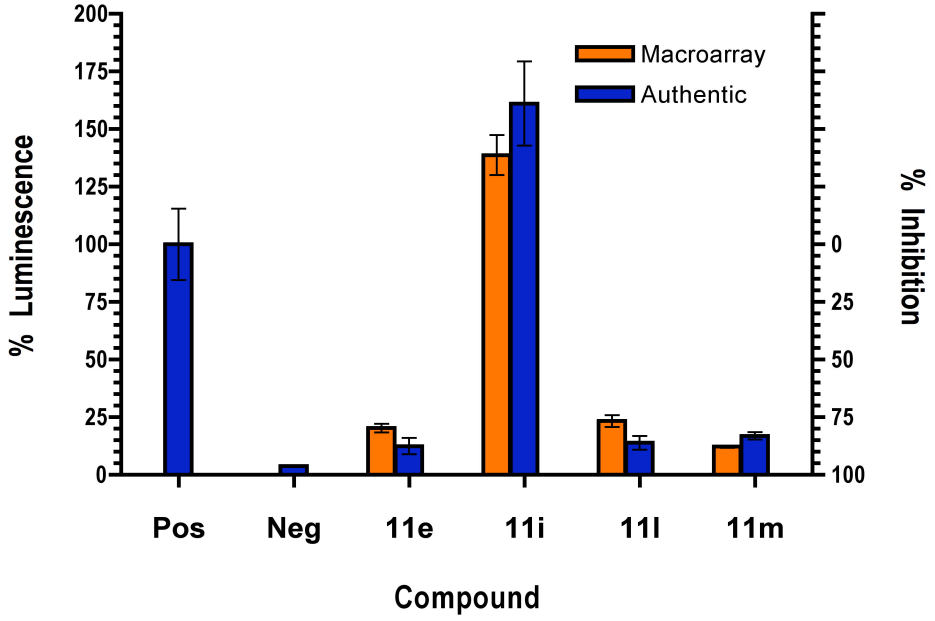


Figure S-4. *V. fischeri* ES114 (Δ-*luxI*) antagonism assay data for PHLs **11e**, **11i**, **11l**, and **11m**. Assay performed at 1:1 against OHHL; concentration of all compounds ~5 μM. Authentic = authentic sample of AHL. Macroarray = compounds obtained in this work. Pos = positive control, OHHL at 5 μM. Neg = negative control, DMSO (1%).

C. violaceum **overlay assay protocol.**

Strain and media. *C. violaceum* CV026 was cultured according to published procedures.[6] This strain lacks a functioning CviI synthase (transposon insertion into *cviI*), and CviR activity is reported by the formation of a deep purple pigment (the antibiotic violacein). The native AHL ligand for *C. violaceum*, *N*-hexanoyl L-homoserine lactone (HHL), was synthesized according to our previously reported procedures.[4]

Procedures. A 2-mL aliquot of *C. violaceum* overnight culture (OD_{660} ~ 0.8–1.0) was added to 40 mL of a molten mixture of Luria-Bertani medium and agar (1% w/v) at 45 °C. HHL was added to the molten-agar to give a final concentration of 1 μM. The mixture was swirled vigorously, and 20-mL portions of this mixture were transferred into sterile polystyrene Petri dishes (100 × 15 mm). Macroarrays of cleaved library members (along with the control DHL) were gently submerged into the molten-agar, and the plates were allowed to stand at rt until they solidified. The plates then were incubated at 30 °C for 16–20 h, or until violacein pigmentation levels were sufficiently strong for visual analysis.

C. violaceum **pigment extraction assay protocol.**

Strain and media. The same strain used in the agar overlay assays, *C. violaceum* CV026, was utilized in these solution-phase assays. Culture conditions were identical.

Procedures. The violacein extraction assay procedure was adapted from Blosser *et al*.[7] This assay was performed in an antagonism dose response format against HHL, the native AHL ligand for *C. violaceum*. In brief, aliquots of DHL, PHL **11k**, and PHL **11l** stock solutions (in DMSO) were added to 4-mL sterile glass vials to give final concentrations of 1 pM – 10 μM solutions after dilution with culture. HHL was also added to each vial to give a final concentration of 400 nM (~EC_{50}). An overnight culture of *C. violaceum* CV026 was diluted to an OD_{660} of ~0.07–0.08 and then added to each vial to make 1 mL solutions.

The vials were capped loosely with septa and incubated overnight (22 h) at 30 °C with shaking at 150 rpm. Thereafter, the vials were vortexed for 10 sec, and 400-μL aliquots were transferred to 2-mL Eppendorf tubes. (In addition, aliquots (150 μL) from each vial were transferred to a 96-well microtiter plate for OD_{660} cell density measurements.) A 10% SDS solution (400 μL) was added to each Eppendorf tube. The tubes were vortexed for 5 sec and allowed to stand for 5 min. A 1-mL aliquot of water-saturated butanol was added to each Eppendorf tube, and tubes were vortexed for 5 sec and centrifuged at 13,000 rpm for 15 min. The upper butanol phase (200 μL) was removed and transferred to a 96-well microtiter plate. Violacein production was measured at 585 nm using a plate reader, normalized to cell density per well, and plotted against compound concentration (see Figure S-5). IC_{50} values for DHL, **11k**, and **11l** were determined with GraphPad Prism software (v. 4.0) using a sigmoidal curve fit. Note, no compound was observed to inhibit bacterial growth over the time course of this assay (as measured by OD_{660}).

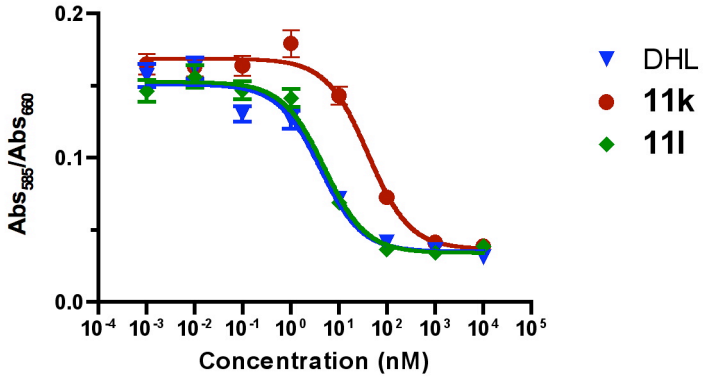


Figure S-5. *C. violaceum* CV026 dose-response curves for DHL, **11k**, and **11l** against HHL at 400 nM μM. IC_{50} values = 4.12 nM, 40.5 nM, and 4.98 nM, respectively.

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