

Supporting Information

For

A Demonstration of the Feasibility of a Direct Solid Phase Split-Pool Biginelli Synthesis of 3,4-Dihydropyrimidinones

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Experimental Section

General Methods. Starting materials and reagents were purchased from commercial suppliers and used without further purification except for the following: dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), and diethyl ether (Et_2O) were passed through two activated alumina columns to remove water, peroxides, inhibitors, and other polar protic impurities as described by Grubbs, et al.¹⁶ 2,6-Lutidine was distilled from CaH_2 under an N_2 atmosphere. Silyl linker-functionalized polystyrene macrobeads (500-600 μm , 1% Divinylbenzene (DVB) crosslinked) were prepared as previously described⁶ from commercially available brominated polystyrene purchased from Polymer Laboratories, Inc., Amherst, MA (1462-9999).

NMR spectra were recorded on a Varian INOVA 500 MHz instrument and are referenced to residual solvent peaks (CDCl_3 : ^1H NMR at δ 7.27 and ^{13}C NMR at δ 77.0). LC/MS data were obtained on a Micromass Platform LCZ mass spectrometer in ESI mode attached to a Waters 2690 HPLC system. LC/MS chromatography was performed on a Waters Symmetry C18, 3.5 μm , 2.1 mm \times 50 mm column using a flow rate of 0.4 mL/min and a 5 min gradient of 15–100% MeCN in H_2O (constant 0.1% formic acid) with 200–450 nm detection on a Waters 996 photodiode array detector. Samples were usually dissolved in pure MeCN, or less frequently in pure MeOH.

Eppendorf-style pipettors were used for measurement of small liquid volumes. Very small scale solid-phase reactions (<25 beads) were done in either one dram glass vials sealed with Teflon-lined caps, or in 2 mL glass LC sample vials sealed with septum caps. Flushing with gas and injecting liquids through the septum was done via 27 gauge needles to minimize the size of punctures through which leakage could occur. Somewhat larger scale solid-phase reactions were performed in 2 mL (5-25 mg resin) or 10 mL (25-250 mg resin) PolyPrep[®] fritted polypropylene chromatography columns (BioRad) fitted with a nylon three-way stopcock (BioRad) at the lower end and a rubber septum-stopper or polypropylene cap at the upper end. Resin samples in polypropylene chromatography columns were rinsed on a Vac-Man[®] Laboratory Vacuum Manifold (Promega) fitted with nylon three-way stopcocks (BioRad).

Agitation of vials and plastic chromatography columns by 360° tumbling end-over-end was carried out on a Barnstead-Thermolyne Labquake™ tumbler. Vortex agitation was carried out at low speed (dial setting 3) on a Vortex Genie-2 mixer fitted with an Eppendorf microtube holder. Evaporation of solvents in vacuo from 1.5 mL polyethylene microcentrifuge tubes was done on a Savant Speed-Vac at medium heat.

Loading of 3-FMOC-aminopropanol onto Resin. Following the general procedure for bead loading,⁶ 250 mg (0.350 mmol Si) of silyl linker-functionalized polystyrene macrobeads (1.4 mmol Si per gram) were swollen in 1.0 mL of dry CH₂Cl₂ under N₂ and then treated with 4.65 mL (2.1 mmol, 6 equiv) of 4% (v/v) TfOH in dry CH₂Cl₂ for 2.5 h. The solution was then drained, the beads rinsed with 2 mL of dry CH₂Cl₂, and then resuspended in 1.0 mL of dry CH₂Cl₂, to which 330 µL (2.8 mmol, 8 equiv) of 2,6-lutidine was added. After 10 min a solution of 208 mg (0.700 mmol, 2 equiv) of 3-FMOC-amino-1-propanol (**6**) in 2.0 mL of CH₂Cl₂ (nearly saturated solution) was added and rinsed in with another 1 mL of CH₂Cl₂ (rinsing not recommended for subsequent loadings: solution more dilute, gives lower coupling levels). The resulting mixture was agitated by tumbling for 18 h, then the reaction solution drained off and the beads rinsed sequentially with 2 mL of THF (2 × 30 min), 2 mL of 3:1 THF/IPA [IPA = isopropyl alcohol] (2 × 75 min), 2 mL of 3:1 THF/H₂O (2 × 30 min), 2 mL of 3:1 THF/IPA (2 × 30 min), 2 mL of DMF (1 × 30 min), 2 mL of THF (2 × 30 min), and finally 2 mL of CH₂Cl₂ (2 × 30 min). The beads were then drained, dried under a flow of N₂, and finally in vacuo for 24 h to give **7**.

Cleavage of FMOC Group. Quantitative FMOC cleavage of 6-7 bead samples of **7** with 20% (v/v) piperidine in DMF¹¹ and UV analysis of the resulting solution of N-(9-fluorenylmethyl)piperidine adduct at 290 nm (ε 4950) indicated 0.88 mmol (79.5% loading yield) of FMOC per gram of resin (132.5 nmol per bead). The bulk of the resin was then deprotected by treating it with 3.0 mL of freshly prepared 20% (v/v) piperidine in DMF with tumbler agitation for 3.0 h. The liquid was then filtered away and the beads washed sequentially with 3

mL of DMF (2 × 30 min), 3 mL THF (2 × 30 min), and finally 3 mL of CH₂Cl₂. Removal of the last wash solution and drying in vacuo gave 0.2139 g of 3-aminopropanol-functionalized resin **8**.

Synthesis of Resin-Attached Urea 10. 3-Aminopropanol-functionalized beads (**8**, 0.2139 g, 0.250 mmol NH₂) were placed into a septum-stoppered 10 mL BioRad PolyPrep chromatographic column fitted with a stopcock, flushed with N₂ for 10 min, and then kept under this atmosphere with an N₂ balloon. To the beads were added 2 mL of dry CH₂Cl₂ and the beads allowed to swell for 10 min. Liquid trimethylsilylisocyanate¹² (**9**, TMSNCO) (144 mg, 1.250 mmol, 5 equiv) was then injected via syringe, followed by 20 µL (0.250 mmol, 1 equiv) of pyridine, and the mixture then agitated by end-over-end tumbling for 115 hr at room temperature. The reaction solution was filtered off by suction, the beads rinsed briefly with CH₂Cl₂, and then washed with 3 mL portions of CH₂Cl₂ (3 × 45 min) with tumbler agitation. After removal of the solvent by suction, the beads were air dried with suction for 15 min and finally vacuum dried overnight to give 0.3880 g of **10** as pale tan beads (>> 100%; odor of CH₂Cl₂ still present).

Lewis Acid Catalyzed Biginelli Reactions with Resin-Attached Urea 10. Testing of the effectiveness of Lewis acid/solvent combinations in catalyzing on-bead Biginelli condensations was accomplished in the following manner. Solutions of 0.1 M Lewis acid in either MeCN or THF were made up by weighing out 0.10 mmol of either LiClO₄¹⁴ (10.6 mg), LiOTf¹⁴ (15.6 mg), or Yb(OTf)₃¹⁵ (62.0 mg) into septum-capped 2 mL LC vials, flushing the vials briefly with N₂ introduced through a 27 gauge needle inserted through the slightly loosened septum cap, sealing the cap tightly, and then injecting 1.0 mL of solvent into the vial.

Urea-attached resin beads (**10**, 10.7 mg, 6.77 µmol urea) were weighed into Teflon-capped glass vials equipped with micro magnetic stirring bars, solid 4-bromobenzaldehyde (**11a**, 12.5 mg, 67.7 µmol, 10 equiv) was added, and then 136 µL of a 0.1 M Lewis acid stock solution (13.5 µmol, 20 mol% of the amount of ArCHO) was added via pipettor. The vials were flushed briefly with N₂ gas, sealed, and allowed to magnetically stir slowly for 15 min. Liquid ethyl acetoacetate (**13**, 8.8 mg, 67.7 µmol, 10 equiv) was added via pipettor and the vial again flushed

briefly with N₂ and sealed tightly. The resulting solution, which was now 0.1 M in Lewis acid and 0.5 M in both aldehyde and β -ketoester, was magnetically stirred slowly for another 15 min at room temperature, and then heated with magnetic stirring in a sand bath at 75 °C for 40 h.

After cooling to room temperature the supernatant solutions were removed from the beads with a pipettor and placed into labeled 1.5 mL Eppendorf polyethylene microcentrifuge tubes, the beads rinsed briefly with 100 μ L of THF, and the rinse solvents combined with their respective reaction solutions. These solutions were then evaporated in vacuo, the residues dissolved in 1.0 mL portions of MeCN, and then 100 μ L aliquots of these solutions were diluted with 1.0 mL of MeCN and subjected to LC/MS analysis. The LiClO₄ and LiOTf catalysts in MeCN both showed a major peak at 2.75 min for unreacted aldehyde and a small pair of peaks at 3.56 and 3.83 min for the two geometric isomers of the product of Knoevenagel condensation between aldehyde and β -ketoester. The only other peaks were a minor unidentified component at 2.53 min and a small peak at 3.00 min that has the same mass, but not the same fragmentation pattern or UV spectrum, as cleaved N-hydroxypropyldihydropyrimidinone **15a**. These same two catalysts in THF showed the same peaks, but had substantially larger 3.56/3.83 min peaks, and had a small additional unidentified peak at 3.25 min. The Yb(OTf)₃ catalyst in either MeCN or THF showed lower amounts of aldehyde and higher amounts of Knoevenagel condensation products than the other two catalysts, as well as a large peak at 3.90 min partially overlapping the 3.83 min peak. In none of the supernatants, however, did there appear to be substantial amounts of Biginelli product **15a** that had been separated from the beads.

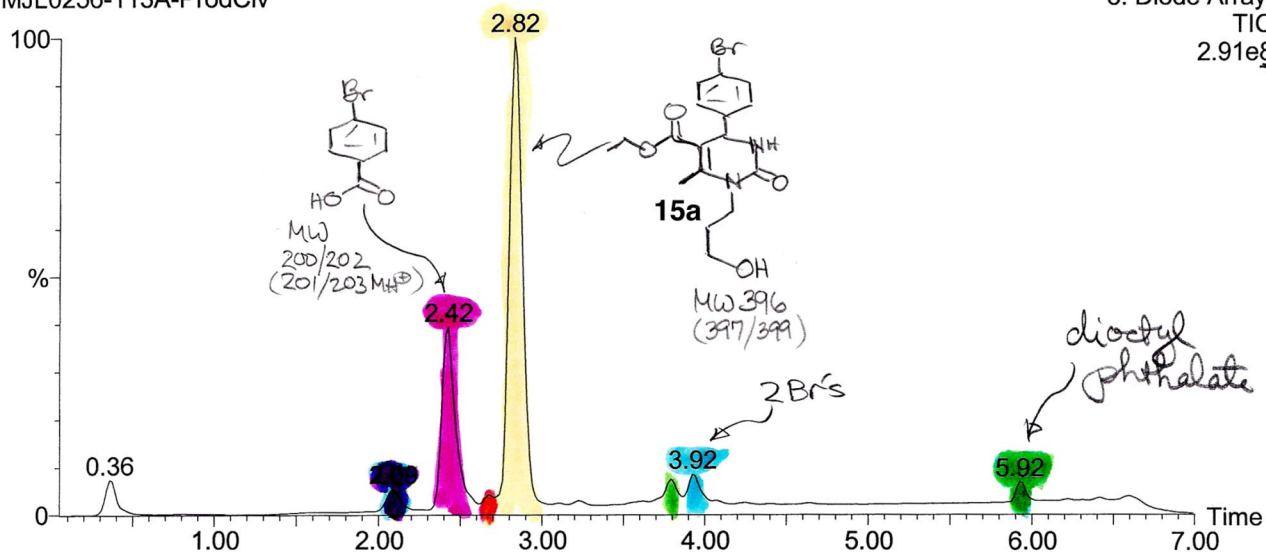
After removal of the supernatant solutions, the sets of beads were transferred to 1 mL fritted chromatography columns and subjected to a series of washings with 0.5 mL portions of the following solvents: THF (1 \times 45 min, 1 \times 30 min), 3:1 (v/v) THF/IPA (2 \times 30 min), 3:1 (v/v) THF/H₂O (2 \times 30 min), 3:1 (v/v) THF/IPA (2 \times 30 min), DMF (2 \times 30 min), THF (2 \times 30 min), and finally CH₂Cl₂ (2 \times 30 min). Following the washings the beads were dried in vacuo overnight.

The resin-attached products were cleaved from the beads and analyzed in the following manner. Eight (8) beads from each reaction were placed in separate 1.5 mL Eppendorf vials, 30 μ L of dry THF was added to each, and the vials were agitated gently on a vortex mixer for 30 min. Meanwhile, a cleavage cocktail was made up by placing 270 μ L of dry THF, 15 μ L of dry pyridine, and 15 μ L of 70:30 (w/w) HF:pyridine solution in a separate 1.5 mL polyethylene vial. The THF was removed from the beads with a pipettor, and 30 μ L of the cleavage cocktail was added to each set of beads, the vials were flushed briefly with N₂, and were then capped and sealed with parafilm. The vials were gently agitated on a vortex mixer for 3 h at room temperature, followed by the addition of 30 μ L of neat methoxytrimethylsilane (TMSOMe), and an additional 30 min of gentle vortex agitation. The liquid from each vial was then transferred via pipettor into separate 1.5 mL polyethylene vials, the beads in each vial rinsed with 30 μ L of fresh THF with gentle vortex agitation for 30 min, and the THF rinses combined with their respective cleavage solutions. The volatiles were removed from these vials in vacuo, and the residues were dissolved in 30 μ L of MeCN and subjected to LC/MS analysis.

The Yb(OTf)₃ catalyst in either MeCN or THF gave no Biginelli product from on-bead reaction. The LiClO₄ and LiOTf catalysts gave Biginelli product in either solvent, but gave the best results in MeCN, showing a major peak for Biginelli product at 2.88-2.92 min (λ_{max} =290.5 nm), with a smaller peak at 2.55 min with the correct mass for the imine adduct of aldehyde and 3-hydroxypropylamine, and another impurity peak at 3.00-3.06 min that might be the product of the hydroxypropyl group cyclizing by Michael addition to the dihydropyrimidinone double bond. This latter peak was largest for either catalyst in THF and smallest for the LiOTf/MeCN reaction, which was therefore concluded to be the best Lewis acid/solvent combination for on-bead Biginelli reactions. A subsequent repeat of the LiOTf/MeCN reaction, heated in an 80 °C sand bath for 72 h, and using a freshly opened bottle of LiOTf, gave, after cleavage from the resin, a strong peak at 2.82 min for the Biginelli product with little or no 3.00 min impurity, and a moderate peak at 2.42 min corresponding to 4-bromobenzoic acid, as the only other significant impurity (Figure S1).

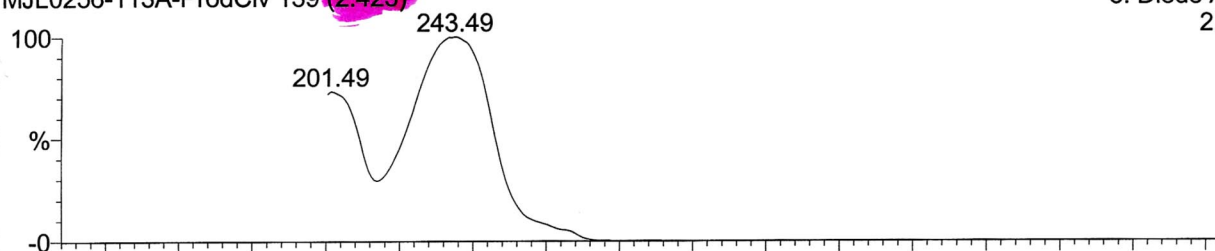
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MJL0256-113A-ProdClv

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TIC
2.91e8



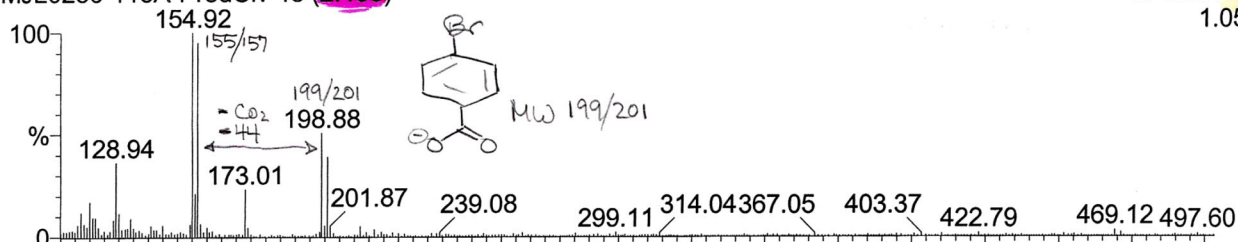
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3: Diode Array
2.63e6



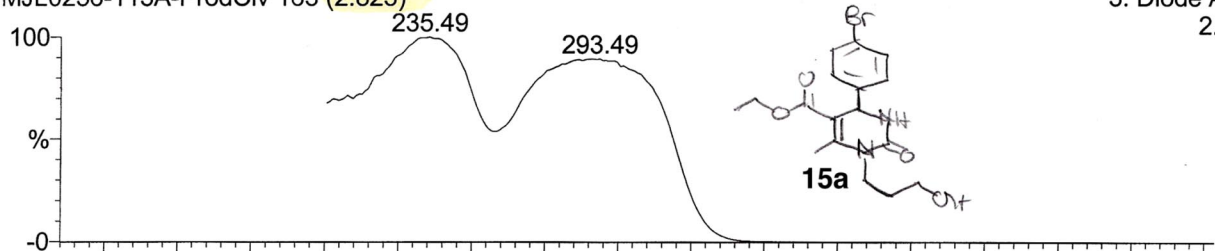
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1.05e4



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3: Diode Array
2.96e6



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1.77e6

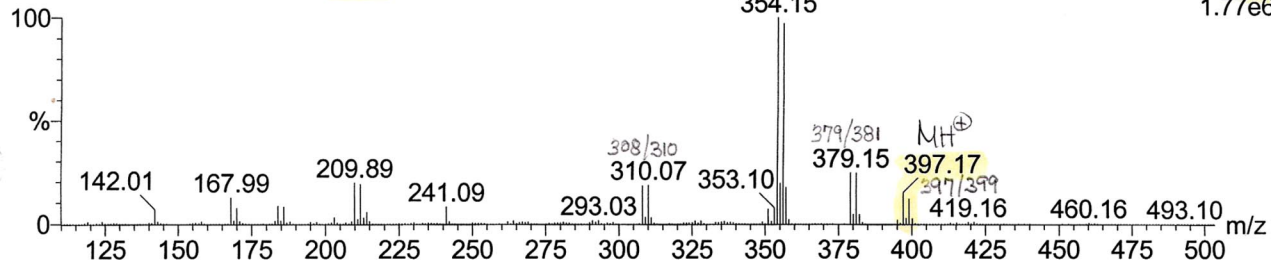


Figure S1. LC/MS of Compound 15a from LiOTf/MeCN/80 °C On-Bead Synthesis

Alternate Synthesis of Biginelli Product 15a. A. Preparation of 3-

(Triisopropylsiloxy)propylamine (17). Triisopropylsilyl trifluoromethanesulfonate (TIPSOTf; 2.38 g, 10.0 mmol, 1 equiv) was added dropwise via syringe over 7 min (slight exotherm) to a solution of 3-amino-1-propanol (751 mg, 10.0 mmol) and 2,6-lutidine (2.68 g, 25.0 mmol, 2.5 equiv) in 10.0 mL of CH₂Cl₂. After stirring for 3.5 h at room temperature, the reaction mixture was poured into 50 mL of ice and water, 10 mL of CH₂Cl₂ added, and the mixture thoroughly agitated. The organic layer was removed and the aqueous layer was extracted with CH₂Cl₂ (2 × 15 mL). The combined organic layers were washed successively with 50 mL of water and then 50 mL of 1 M aq. NaHCO₃, dried (MgSO₄), and evaporated in vacuo to give 1.83 g (79%) of **17** as a faint yellow liquid that was used as is. ¹H NMR (Figure S2; 500 MHz, CDCl₃): δ 1.07 (m, 21H); 1.73 (q, J=6.5 Hz, 2H); 2.80 (br s, 2H, D₂O exchangeable); 2.90 (br t, 2H); 3.80 (t, J≈5.75 Hz, 2H). ¹³C NMR (Figure S3; 125 MHz, CDCl₃): δ 12.10, 18.19, 35.55, 39.65, 61.95. LC/MS(UV/ES+): 2.65 min; m/z 232 (M+H); 188 (100%).

B. N-(3-Triisopropylsiloxypropyl)urea (18). 3-(Triisopropylsiloxy)propylamine **17** (1.328 g, 5.74 mmol) and 4-dimethylaminopyridine (DMAP; 77.1 mg, 0.631 mmol, 11 mol%) were dissolved in 30 mL of dry THF under N₂, and to this solution was added TMSNCO (**9**, 727 mg, 6.31 mmol, 1.1 equiv) dropwise via syringe over 2 min. After stirring at room temperature for 0.5 h the reaction mixture was refluxed for 103.5 h. The reaction mixture was cooled to room temperature and evaporated in vacuo, redissolved in CH₂Cl₂, filtered, and evaporated in vacuo again to give 1.54 g (97.8%) of **18** as a waxy solid that was used as is (contains DMAP). ¹H NMR (Figure S4; 500 MHz, CDCl₃): δ 1.07 (m, 21H); 1.74 (quintet, J=6.0 Hz, 2H); 3.32 (t, J=6.0 Hz, 2H); 3.82 (t, J≈5.5 Hz, 2H); 4.70 (br s, 2H), 5.36 (br s, 1H). LC/MS(UV/ES+): 4.30 min; m/z 275 (M+H); 258 (MH-NH₃;100%).

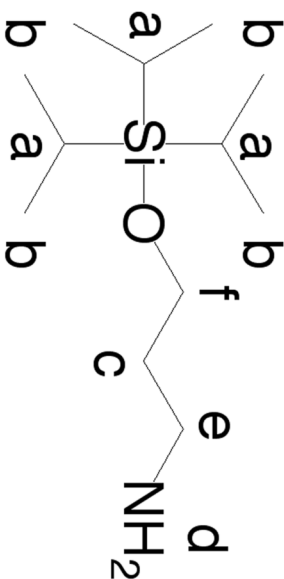
C. 1-(3-Triisopropylsiloxypropyl)-4-(4-bromophenyl)-5-carboethoxy-6-methyl-3,4-dihydropyrimidinone (19). Urea **18** (274 mg, 1.0mmol), 4-bromobenzaldehyde (185 mg, 1.0 mmol), and ethyl acetoacetate (130 mg, 1.0 mmol) were placed in 1.0 mL of MeCN, LiOTf was added (31.2 mg, 0.20 mmol, 20 mol%), and the mixture refluxed under N₂ for 116 h. The

MJL0256-48A, 25mg/0.6mL CDCl₃, Quant, 3-TIPSopropylamine

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luschi/MJL0256-48A,~                   dof           0
Quant
ACQUISITION
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tn           H1                      dmf            C
at           1.892                   dseq          200
np           30272                   dres          1.0
sw           8000.0                  homo           n
fb           4000                     temp          25.0
bs           4                       wffile         f
tpwr         53                      proc           ft
pw           6.0                     tn            not used
d1           20.000                  math           f
tof          0                       werr          0
nt           8                       wexp          8
ct           n                       wbs           wnt
atlock       not used                wnt           wft
gain         not used
il           n                       flags          wft
in           n                       in            n
dp           y                       dp            y
hs           nm                      hs            nm
DISPLAY
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nm          cdc ph

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3-Triisopropylsilyloxypropylamine

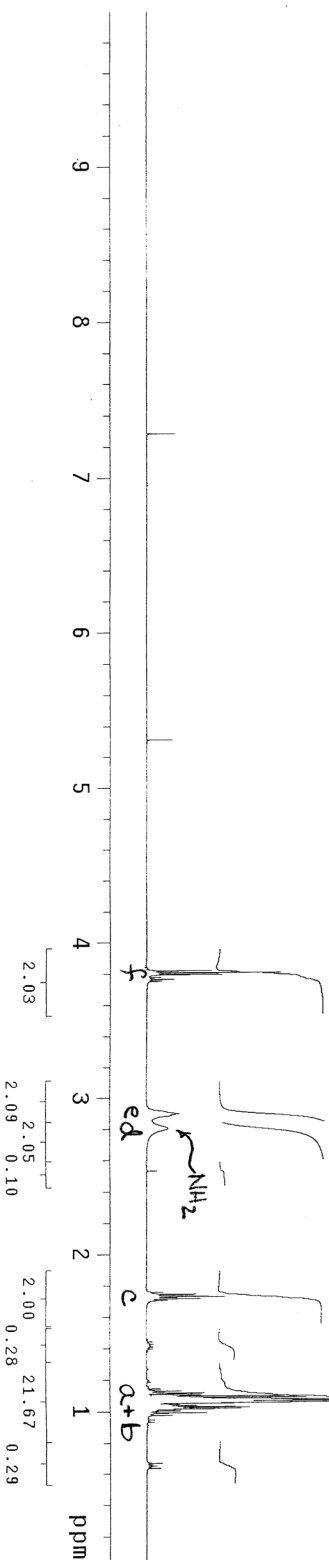


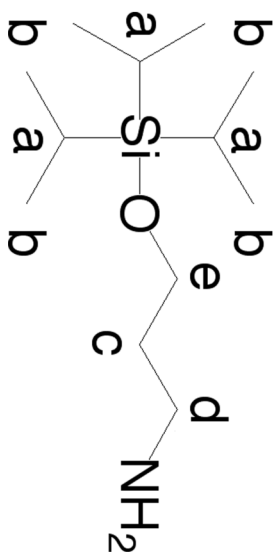
Figure S2. ¹H NMR Spectrum (500 MHz, CDCl₃) of Compound **17**: 3-(Triisopropylsilyloxy)propylamine

MJL0256-48A, 25mg/0.6mL CDCl₃, 3-TIPSOpr
opylamine

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at 1.300 dmf 7683
nd 65024 dseq 1.0
sv 25000.0 dres n
td 14000 homo n
bs 34 temp 25.0
tpwr 04 PROCESSING
pw 6.0 1b 0.50
di 0 wfile
to 0 proc ft
nt 2048 tn not used
ct 2048 math f
alock n
gain not used
il n werr
i1 n wexp
in n wbs
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nm cdc ph

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3-Triisopropylsilyloxypropylamine

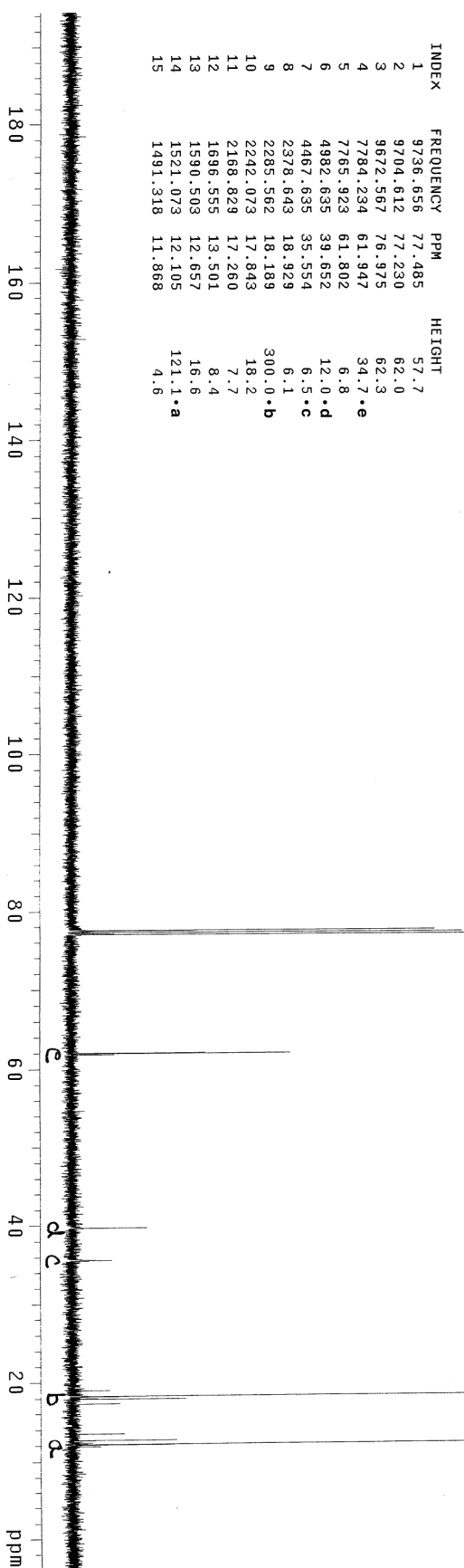


Figure S3. ¹³C NMR Spectrum (125 MHz, CDCl₃) of Compound **17**: 3-(Triisopropylsilyloxy)propylamine

MJL0256-57-104hr, Quant, 3-TIPSopropylam
ine+TMSNCO, THF/DMAP/103.5hr Reflux
expi s2pu1

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solvent	CDCl3	dn	H1
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tn	H1	dmm	nm
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nd	30272	dseq	
sw	8000.0	dres	1.0
fb	4000	homo	n
bs	4	temp	25.0
tpwr	53	PROCESSING	
pw	6.0	wtfile	ft
di	20.000	proc	fn
nt	0	math	not used
ct	16	werr	f
atlock	n	wexp	
gain	not used	wts	
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in	Y		
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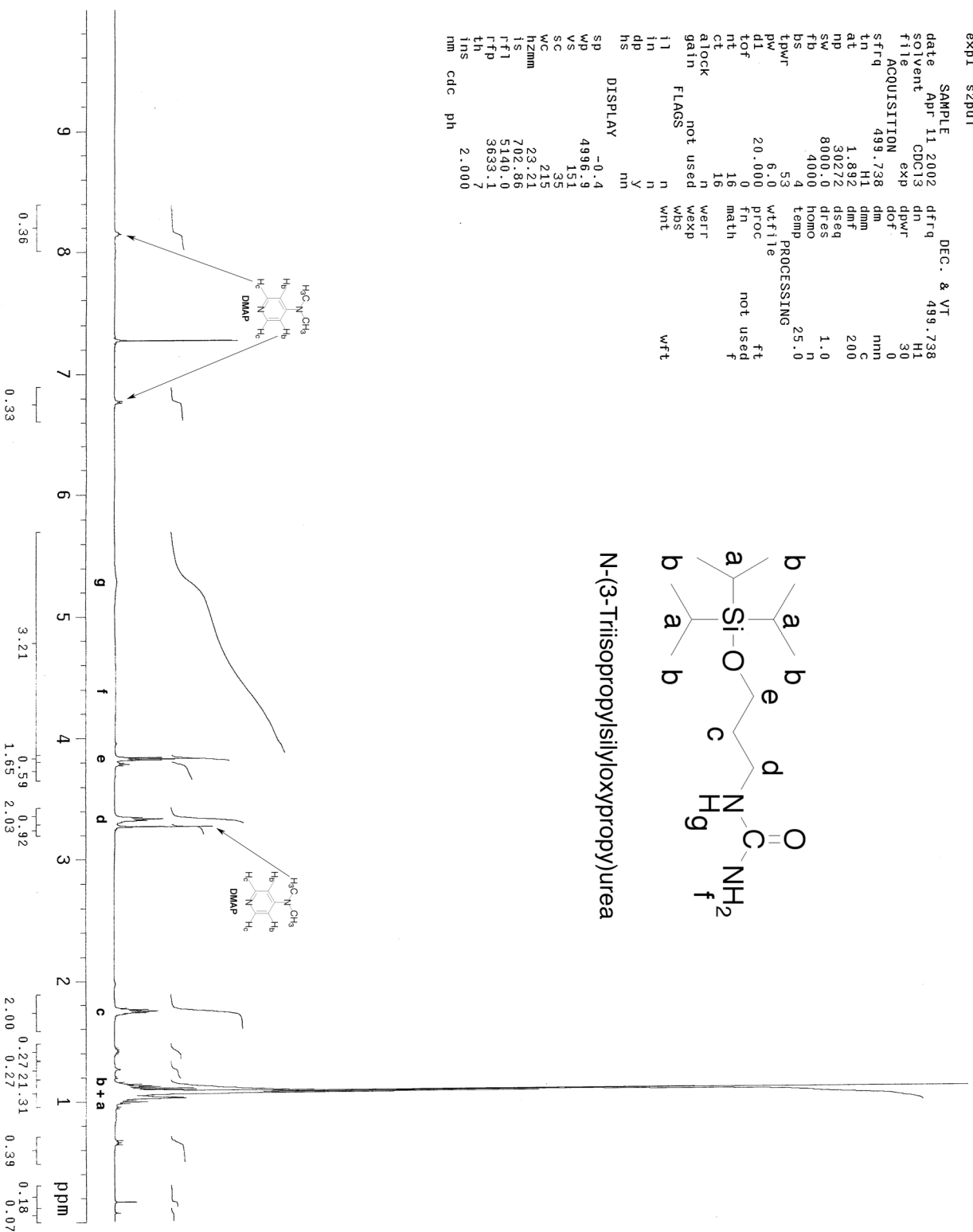
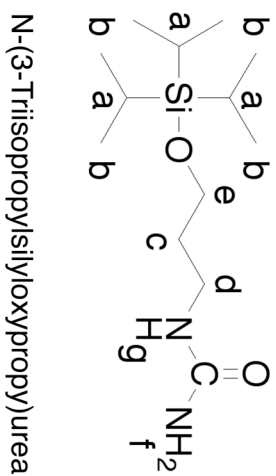


Figure S4. ^1H NMR Spectrum (500 MHz, CDCl_3) of Compound **18**: N-[3-(Triisopropylsilyloxy)propyl]urea

reaction was cooled, 1 mL of MeCN was added, the mixture was poured into 15 mL of 1 M aq. NaHCO₃, and extracted with EtOAc (1 × 15 mL, 1 × 10 mL). The combined organic extracts were dried (MgSO₄) and evaporated in vacuo to give 463 mg (83.6%) of **19** as an extremely viscous amber liquid. LC/MS (Figure S5; UV/ES+): major peak (**19**), 5.77-5.81 min (λ_{max} =290.5 nm); m/z 553/555 (M+H), 575/577 (M+Na), 379/381 (MH-TIPSOH, 100%). minor peak (**15a**), 2.90 min (λ_{max} =289.5 nm); m/z 397/399 (M+H), 379/381 (MH-H₂O), 354/356 (MH-HNCO, 100%).

D. 1-(3-Hydroxypropyl-4-(4-bromophenyl)-5-carboethoxy-6-methyl-3,4-dihydropyrimidinone (15a). Crude **19** from above (455 mg, 0.82 mmol) was placed in a 5 mL plastic vial with 4.5 mL of dry THF and 0.25 mL of dry pyridine, and 0.25 mL of 70:30 (w/w) HF/Pyridine were added. The mixture was then stirred at room temperature for 3.0 h, at which time 1.24 mL of TMSOMe was added and the solution stirred for another 30 min. Evaporation of the solution in vacuo gave 453.5 mg (>100%) of yellow-brown semisolid. LC/MS (Figure S6; UV/ES+): major peak (**15a**), 2.82 min (λ_{max} =292.5 nm); m/z 397/399 (M+H), 379/381 (MH-H₂O), 354/356 (MH-HNCO, 100%). minor peak (**19**), 5.72 min (λ_{max} =290.5 nm); m/z 553/555 (M+H), 379/381 (MH-TIPSOH, 100%). The major component of this product is identical by LC/MS to the product from the on-bead Biginelli reaction above.

Demonstration of a Split-and-Pool Biginelli Reaction. A MeCN solution 0.1 M in LiOTf and 0.5 M in 4-bromobenzaldehyde (**11a**) was prepared by placing 15.6 mg (0.1 mmol) of LiOTf and 92.5 mg (0.5 mmol) of **11a** in a 2 mL septum-capped glass LC vial, flushing the vial briefly with N₂ via a 27 gauge needle inserted through the septum while loosening the cap, then sealing the cap tightly and injecting 1.0 mL of MeCN through the septum via syringe. In a similar fashion, a MeCN solution 0.1 M in LiOTf and 0.5 M in 4-chlorobenzaldehyde (**11b**) was prepared by dissolving 15.6 mg (0.1 mmol) of LiOTf and 70.3 mg (0.5 mmol) of **11b** in 1.0 mL of MeCN. Four glass vials equipped with Teflon-lined caps and micro magnetic stir bars were then loaded with 6 beads each (132.5 nmol urea/bead) of 3-hydroxypropylurea-loaded resin **10** (0.7-0.8 mg, 795 nmol). To two of these vials was added 100 μ L each of the LiOTf/**11a**/MeCN

EtAcetoAcetate+pBrPhCHO+3-TIPSOpropylUrea Biginelli, LiOTf/MeCN. Reflux 116hr
 MJL0256-88-R116hr

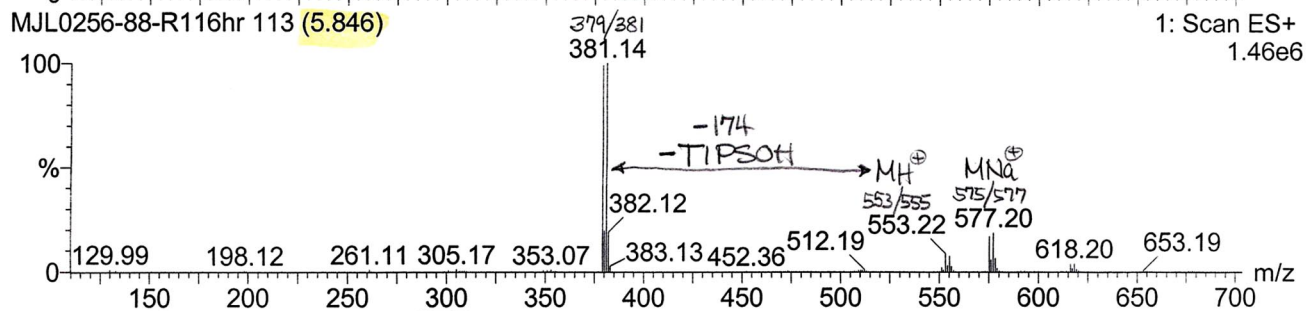
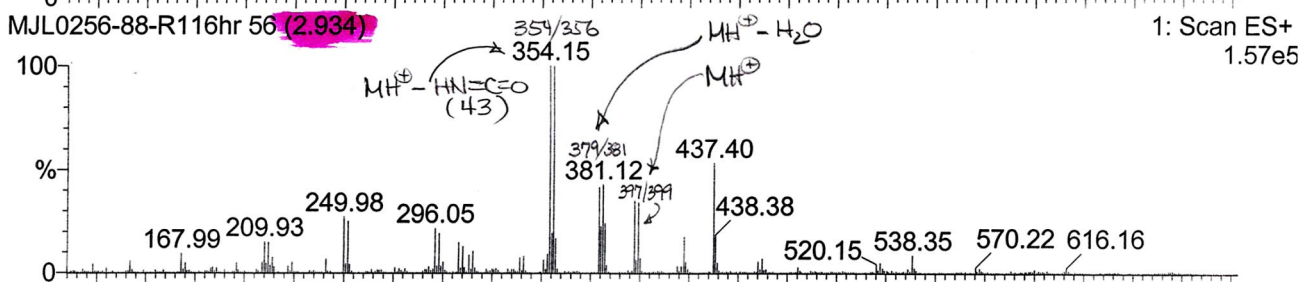
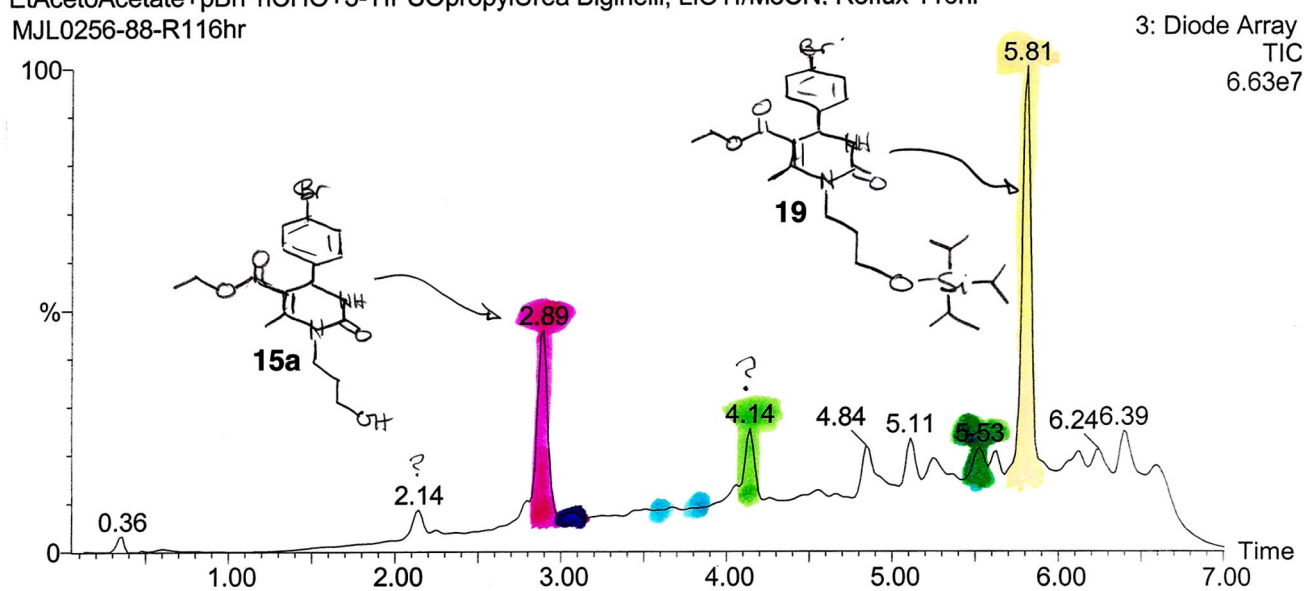


Figure S5. LC/MS of Compound **19** Alternate Synthesis

EtAcetoAcetate+pBrPhCHO+3-TIPSOpropylUrea Biginelli, HF/Pyr Cleavage Product

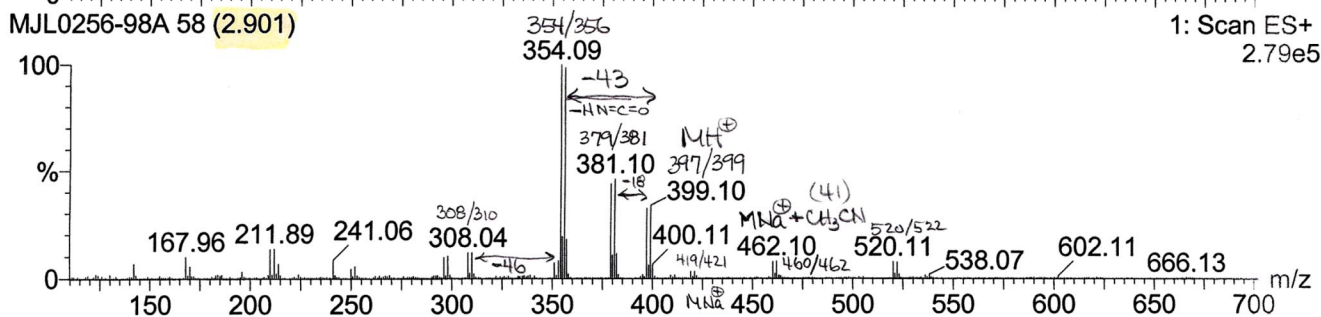
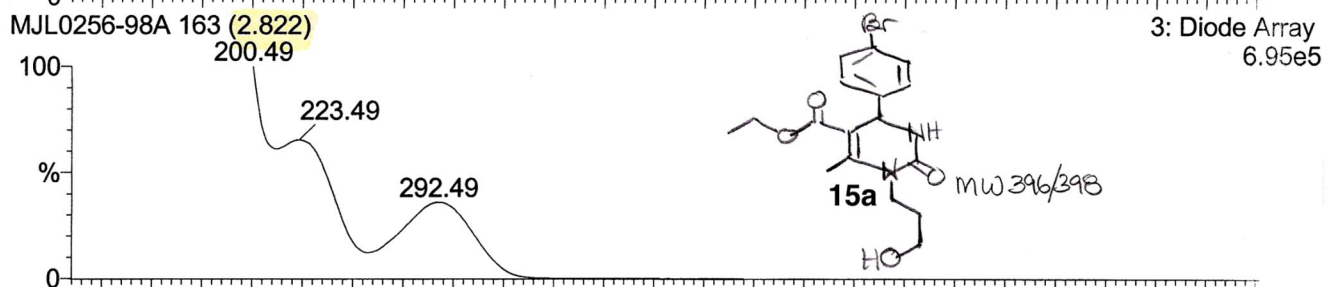
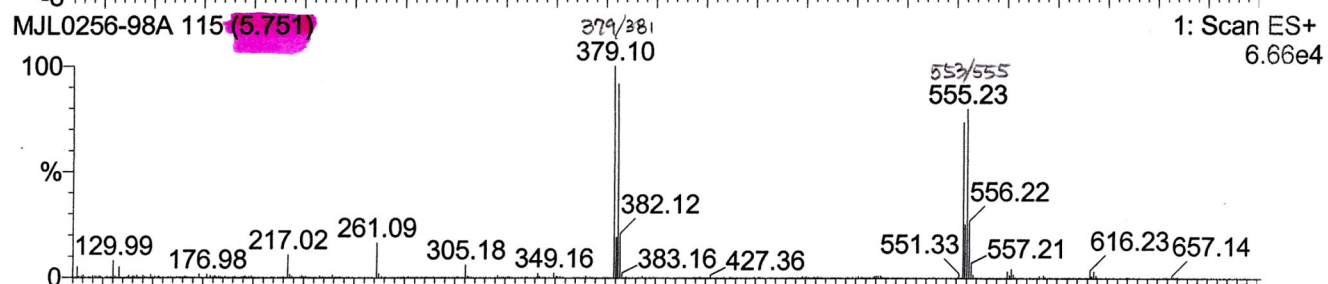
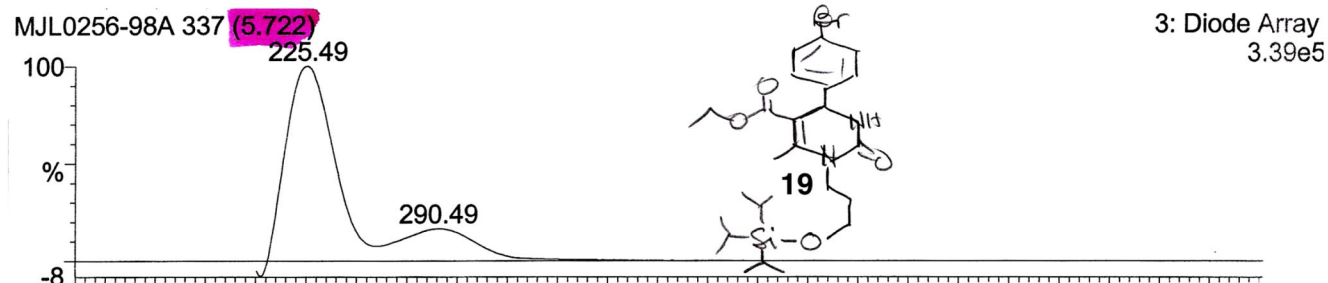
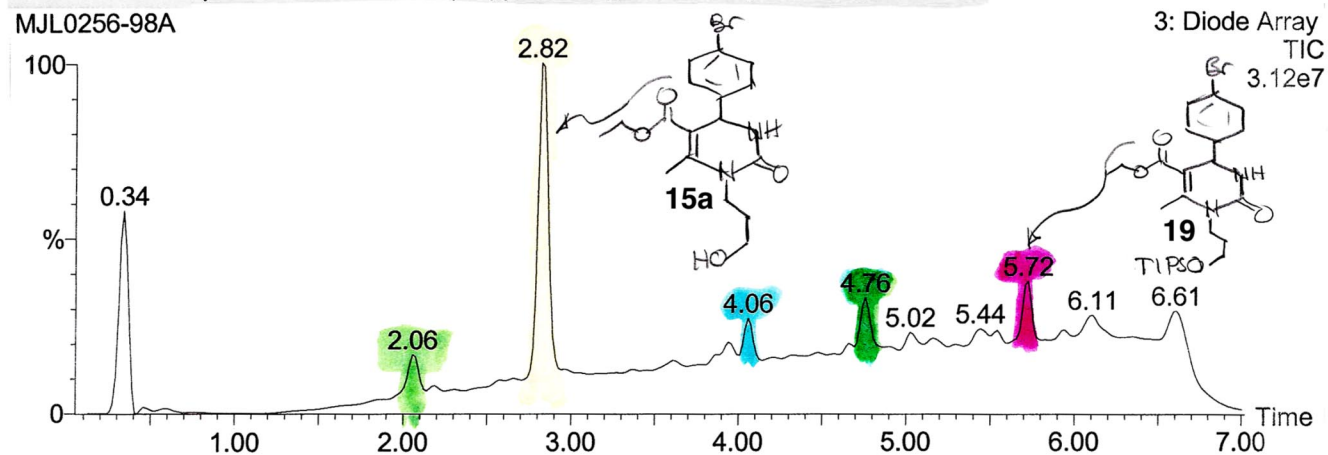


Figure S6. LC/MS of Compound **15a** Alternate Synthesis

solution, while to the other two vials was added 100 μ L each of the LiOTf/**11b**/MeCN solution. The vials were flushed briefly with N₂, stirred slowly at room temperature for 0.5 h, and then heated with magnetic stirring in an 80 °C sand bath for 43 h.

After cooling to room temperature, the supernatant solutions in the reaction vials were removed via pipettor, the resin beads rinsed briefly with fresh MeCN (2 \times 100 μ L), and the rinses discarded. A MeCN solution 0.1 M in LiOTf and 0.5 M in ethyl acetoacetate (**13**) was prepared in a fashion similar to that above by placing 15.6 mg (0.1 mmol) of LiOTf and 65.0 mg (0.5 mmol) of **13** in 1.0 mL of MeCN in a 2 mL septum-capped glass LC vial. One vial of 4-bromobenzaldehyde beads was then mixed with one vial of 4-chlorobenzaldehyde beads (12 beads total), 200 μ L of the LiOTf/**13**/MeCN solution was added via pipettor, and the vial flushed briefly with N₂ and sealed. The other two vials, one reacted with 4-bromobenzaldehyde (6 beads) and the other reacted with 4-chlorobenzaldehyde (6 beads), were similarly treated with 100 μ L each of LiOTf/**13**/MeCN solution, flushed briefly with N₂, and sealed. The three vials were then stirred slowly at room temperature for 0.5 h, and then heated in an 80 °C sand bath with gentle stirring for 69 h.

After cooling to room temperature, the supernatant solutions from each reaction vial were removed via pipettor, the resin beads rinsed briefly with fresh MeCN (100 μ L), and then washed with 200 μ L portions of MeCN (2 \times 30 min, vortex agitation), followed by 200 μ L portions of THF (2 \times 30 min, vortex agitation). The contents of each vial was then transferred to a separate 2 mL fritted chromatography column and washed with tumbler agitation with 200 μ L portions of the following solvents: THF (1 \times 30 min), 3:1 (v/v) THF/IPA (IPA = isopropyl alcohol) (2 \times 30 min), 3:1 (v/v) THF/H₂O (2 \times 30 min), 3:1 (v/v) THF/IPA (2 \times 30 min), THF (2 \times 30 min), DMF (2 \times 30 min), and finally THF (2 \times 30 min). Each of the twelve beads from the mixed Br/Cl vial was then transferred into a separate 0.5 mL Eppendorf microcentrifuge tube, while the 6 beads from each of the other two vials were transferred into their own individual 1.5 mL Eppendorf vials. The single bead samples were then cleaved with 15 μ L portions of freshly prepared 95:5:5 THF/pyridine/70:30 HF/pyridine cleavage cocktail for 3 h, followed by

quenching with 15 μ L portions of TMSOEt for 0.5 h, both at room temperature with gentle vortex agitation. The 6-bead samples were similarly cleaved with 90 μ L portions of cleavage cocktail and quenched with 90 μ L portions of TMSOEt.

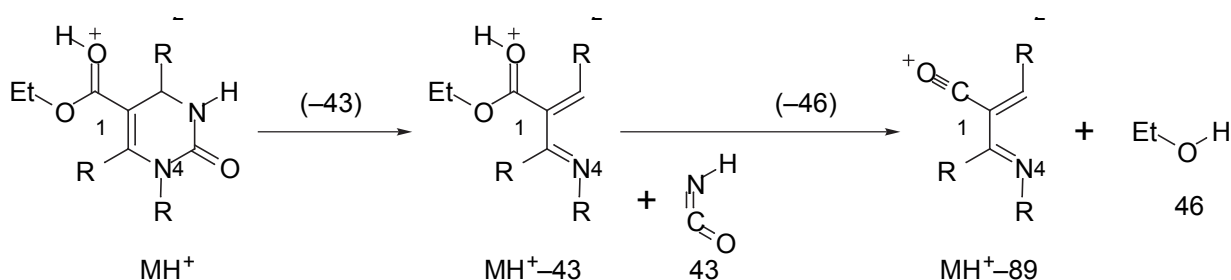
The solutions of cleavage products from each sample were then transferred to separate microcentrifuge tubes, and the single beads rinsed with 15 μ L portions of THF, while the 6-bead samples were rinsed with 90 μ L of THF, and the rinse solutions combined with their respective cleavage solutions. The beads were then washed with similar portions of THF with vortex agitation for 0.5 h, and these solutions also added to their respective cleavage solutions. These solutions were then evaporated in vacuo and the residues dissolved in 15 μ L (single bead samples) or 30 μ L (6-bead samples) of MeCN and subjected to LC/MS analysis.

The homogeneous 6 bead samples from 4-bromobenzaldehyde or 4-chlorobenzaldehyde reaction showed Biginelli product at 2.81 (**15a**) and 2.76 min (**15b**), respectively (Figures S7 and S8, pages S18 and S19). The twelve single bead samples showed Biginelli products **15** at 2.80 (Br), 2.80 (Br), 2.78 (Cl), 2.73 (Cl), 2.79 (Br), 2.73 (Cl), 2.84 (Br), 2.79 (Br), 2.72 (Cl), 2.72 (Cl), 2.80 (Br), and 2.72 (Cl) (Figures S9-S20, pages S20-S31), with no detectable evidence of mixtures of chloro- and bromo-products in any of the samples from the single beads. For each of these twelve pooled beads, examination of the MS scans prior to and following that of the peak for the Biginelli product in each case revealed no evidence of any of the crossover Biginelli compound.

Under the electrospray ionization conditions used for the LC/MS, the ES+ mode was best for obtaining the mass spectra of the Biginelli compounds, usually giving at least a small M+1 peak (MH^+), and sometimes also giving $MH^+ + MeCN$ ($MH^+ + 41$) or MNa^+ ($M + 23$) peaks as well. The main fragmentation seen with Biginelli compounds under these conditions resulted from the formal reverse Diels-Alder loss of H-N=C=O from the pyrimidinone ring to give what was usually the base peak at $MH^+ - 43$. The additional loss of the alkoxy group and a proton (e.g., -EtOH, -46) from the base peak gave what is presumed to be an acylium ion derived from the carboalkoxy group at C-5 of the pyrimidinone ring. Additionally, when the N-1 position was

substituted with a 3-hydroxypropyl group ($R^4 = -CH_2CH_2CH_2OH$) in on-bead derived products, loss of water from the molecular ion was also usually observed ($MH^+ - 18$). Of course, with bromo-substituted compounds, characteristic 2 mass unit-separated doublets of approximately equal intensity indicated bromine-containing ions, while in chloro-substituted compounds the 3:1 intensity of the 2 mass unit-separated doublets indicated chlorine-containing ions.

Scheme S1. Electrospray Mass Spectral Fragmentation of 3,4-Dihydropyrimidin-2(1H)-ones.



The Biginelli 3,4-dihydropyrimidin-2(1H)-ones also typically show a λ_{max} of approximately 289-294 nm in the UV/Vis spectrum.

For the bromo-Biginelli compound **15a**: $MH^+ = m/z$ 397/399; $MH^+ - H_2O = m/z$ 379/381; $MH^+ - HNCO = m/z$ 354/356 (base peak); $MH^+ - (HNCO + EtOH) = m/z$ 308/310; $\lambda_{max} = 293.5$ nm.

For the chloro-Biginelli compound **15b**: $MH^+ = m/z$ 353/355; $MH^+ - H_2O = m/z$ 335/337; $MH^+ - HNCO = m/z$ 310/312 (base peak); $MH^+ - (HNCO + EtOH) = m/z$ 264/266; $\lambda_{max} = 293.5$ nm.

The impurities at about 2.05-2.13 min in the bromine-containing products and at about 1.90-2.00 min in the chlorine-containing products have not yet been identified, but the MS spectra showed that the 2.05-2.13 min material does contain a bromine, and the 1.90-2.00 min material does contain a chlorine.

Pure Br MJL0256-119AP
MJL0256-119AP

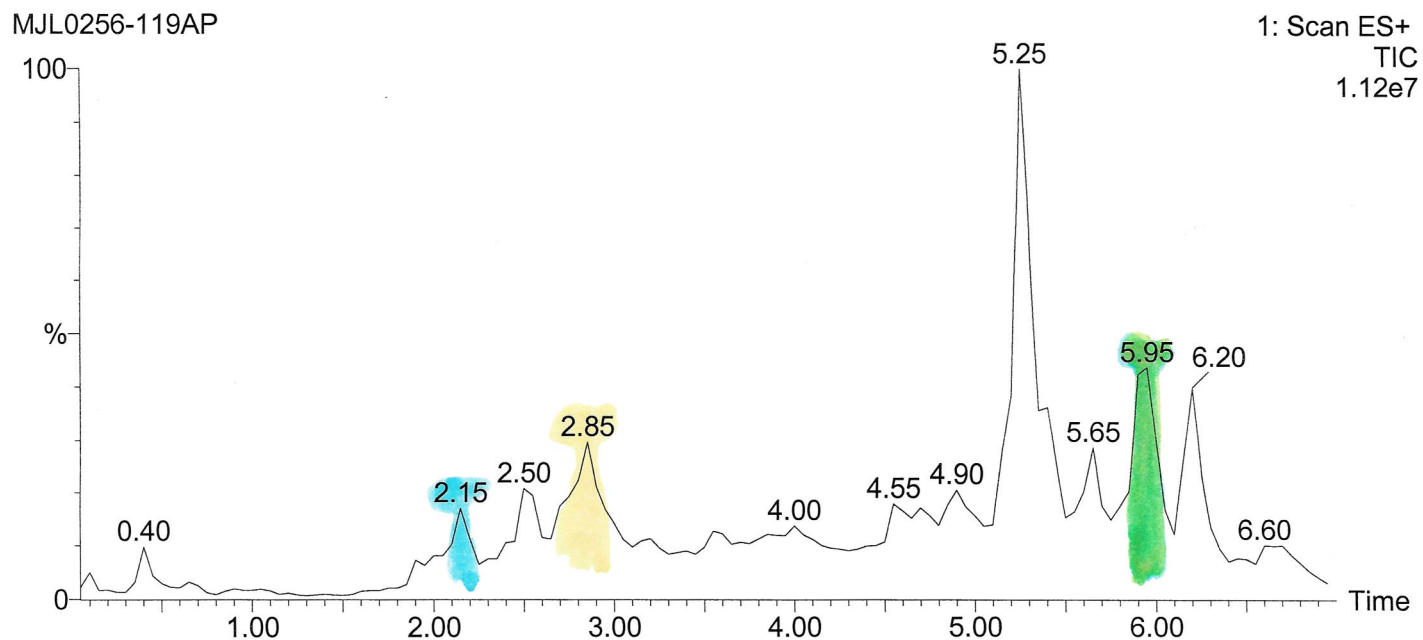
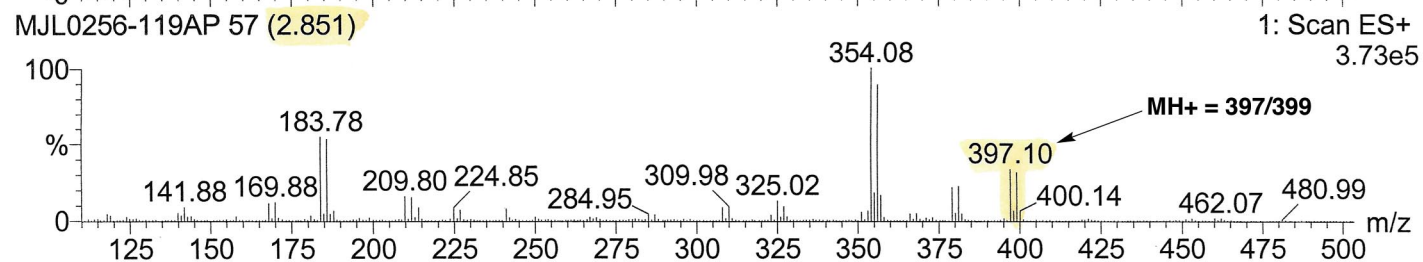
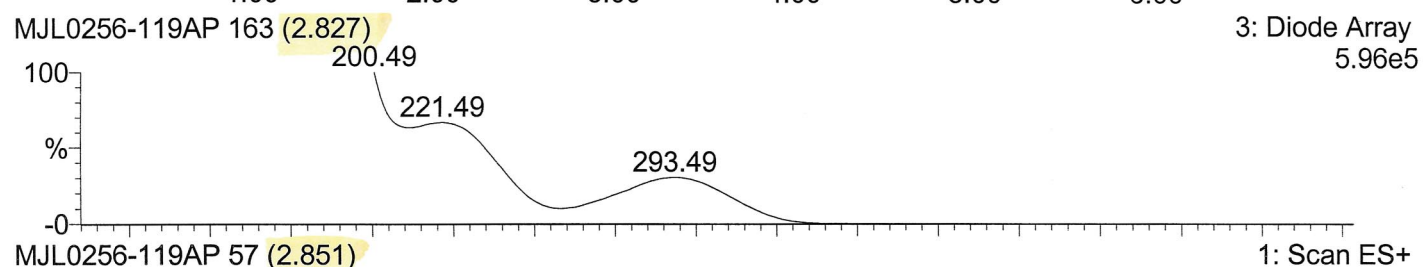
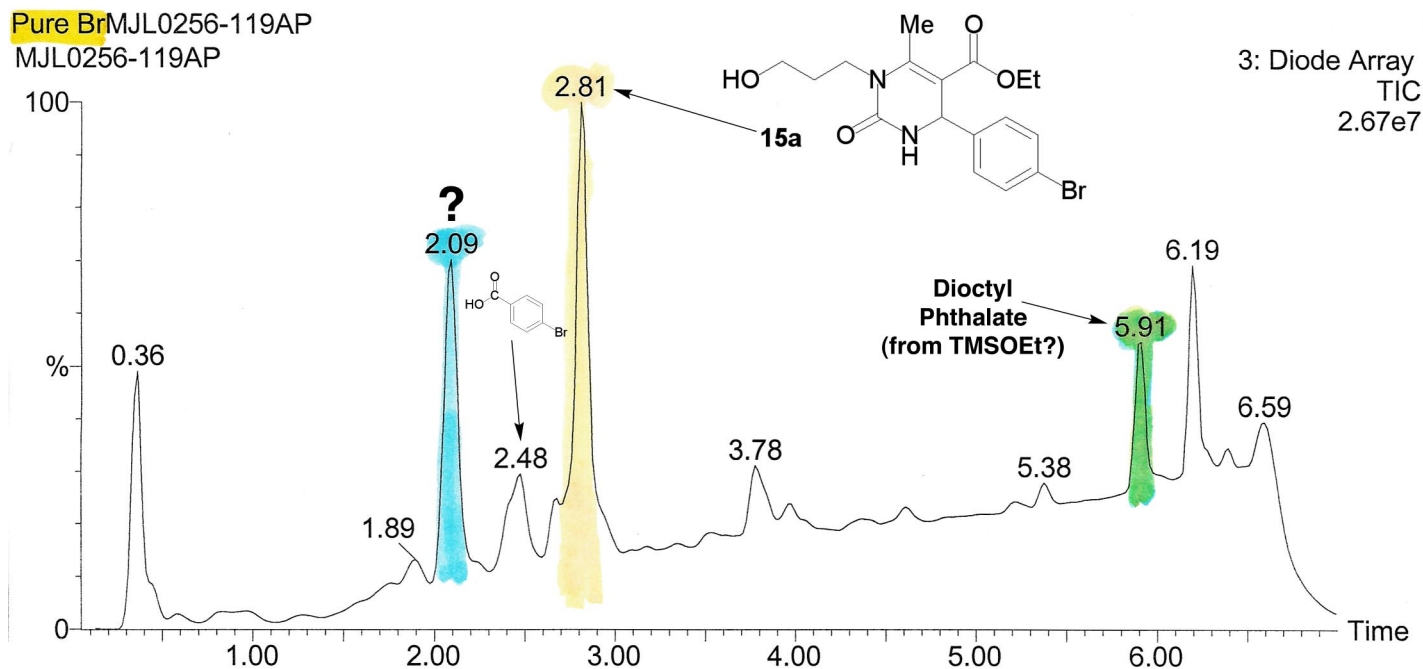


Figure S7. LC/MS of Pure Br Beads (Compound 15a, X = Br)

Pure Cl MJL0256-119CP-b
MJL0256-119CP-b

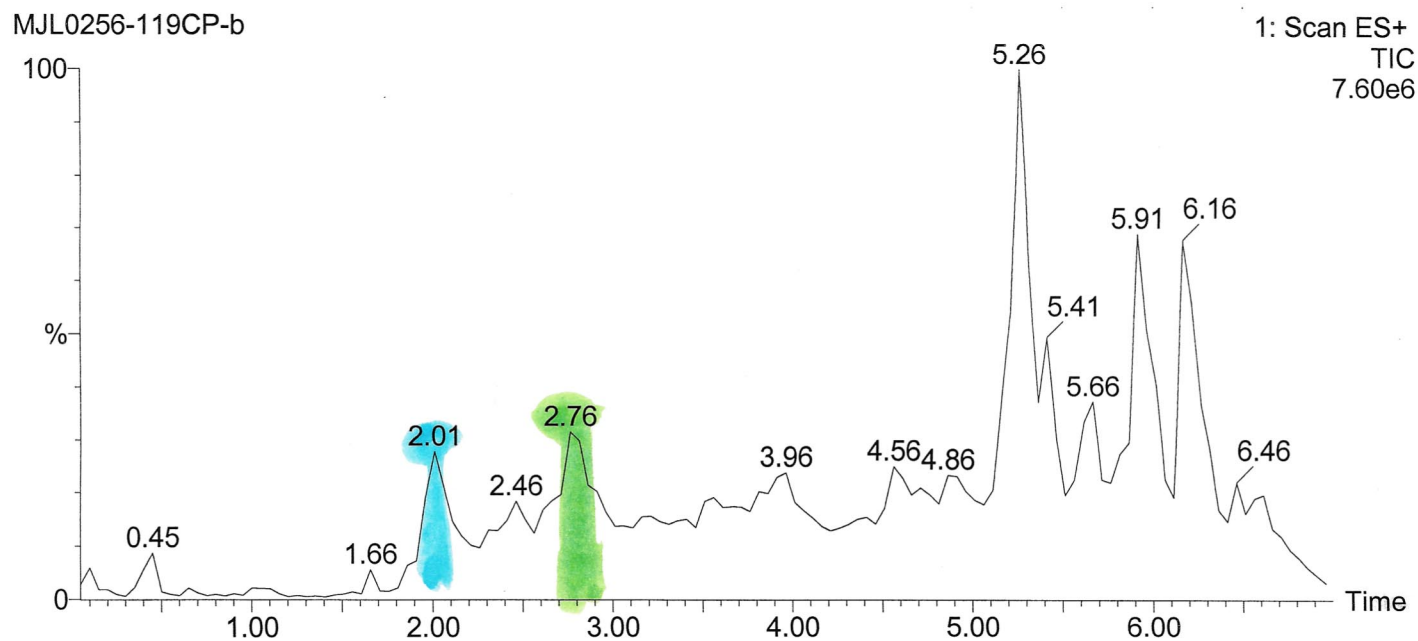
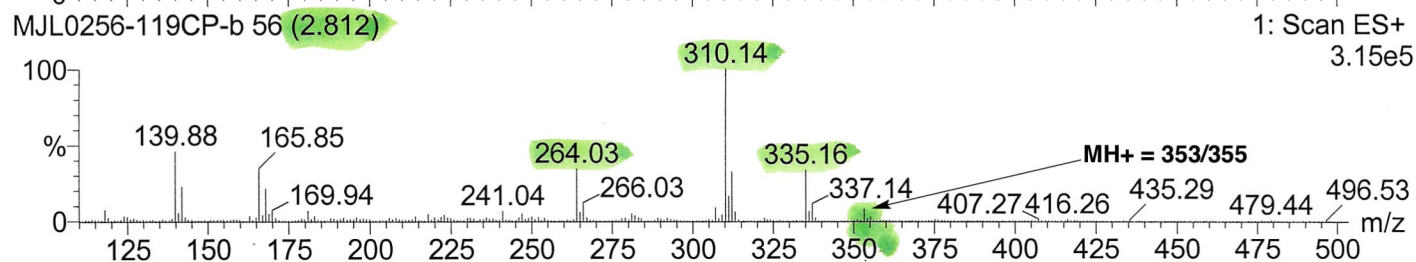
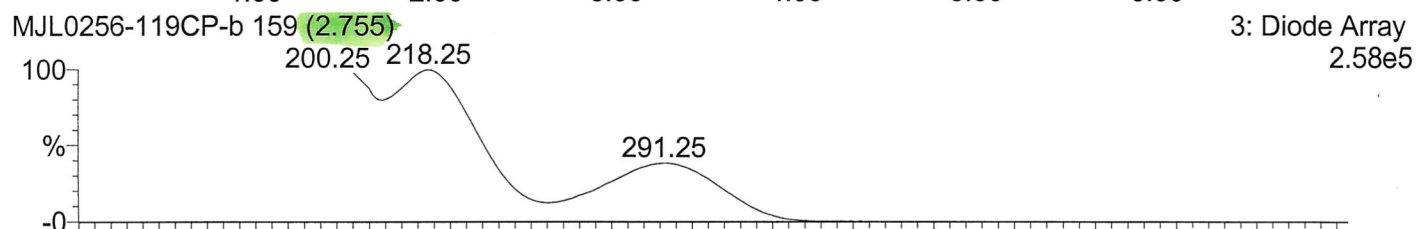
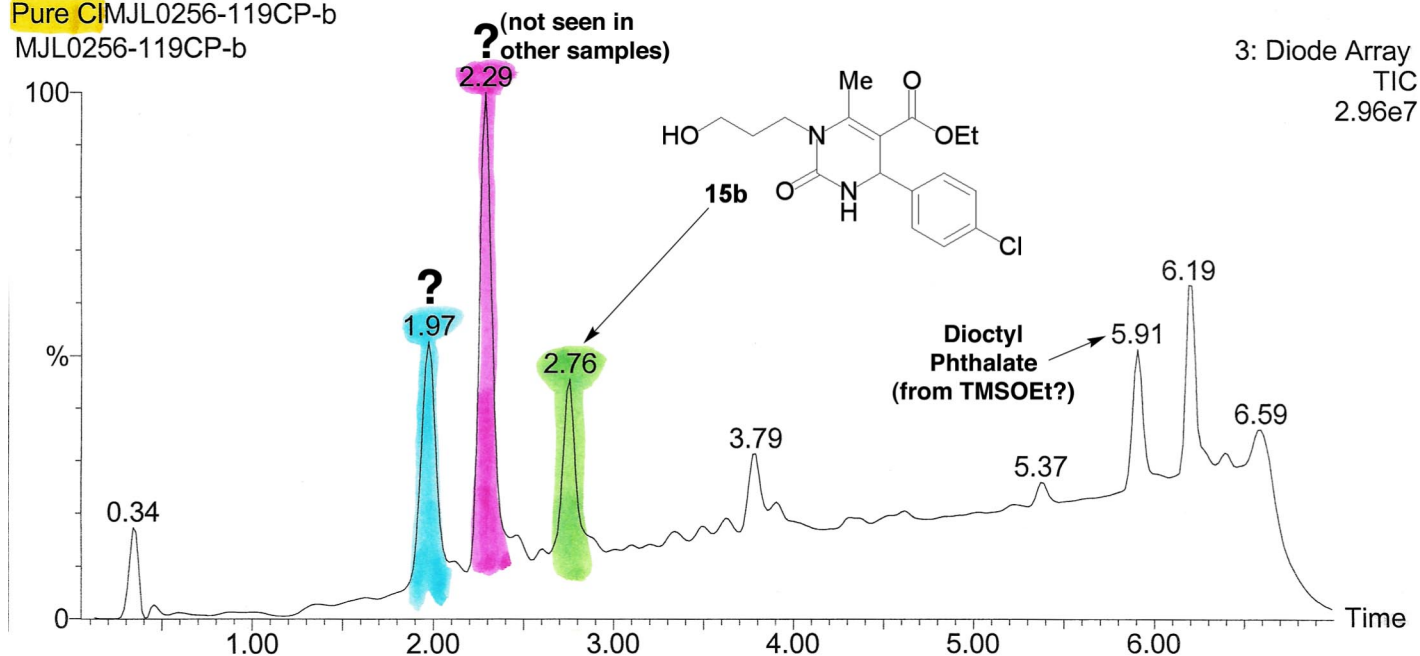
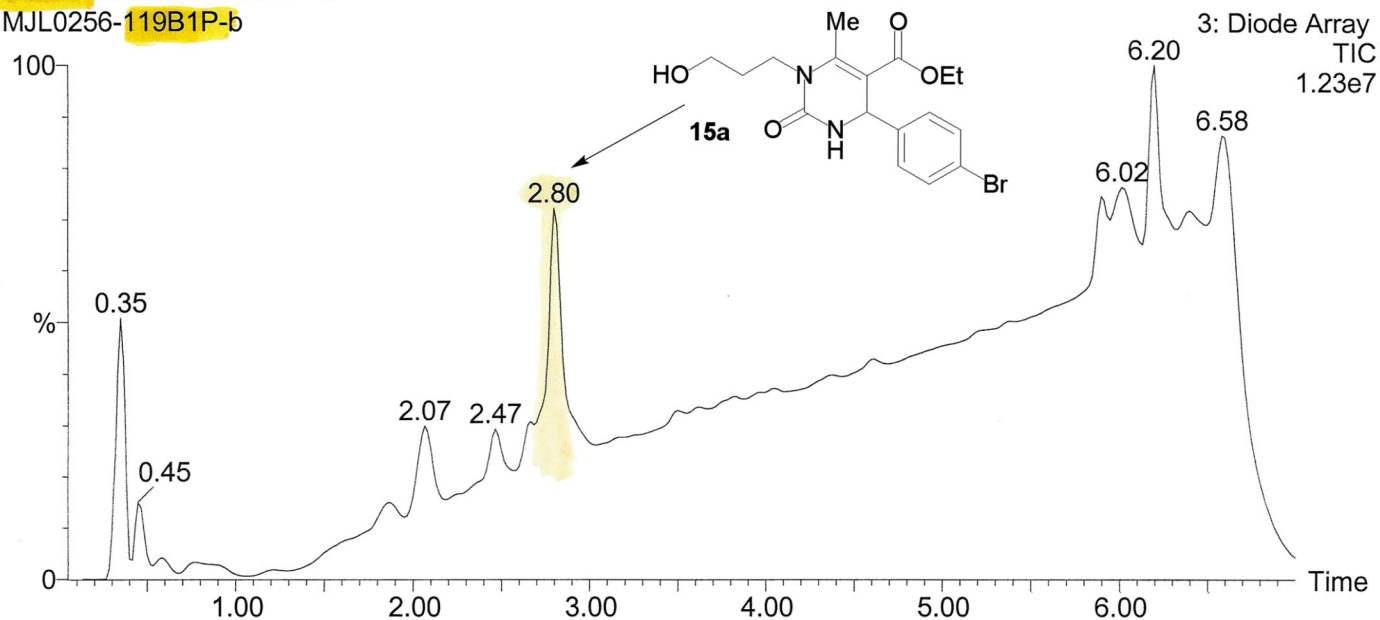


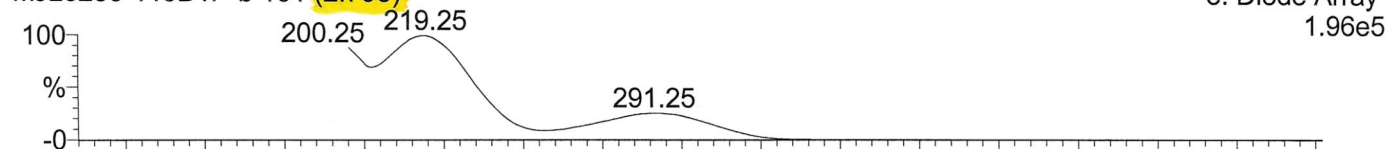
Figure S8. LC/MS of Pure Cl Beads (Compound **15b**, X = Cl)

Mixed 1 MJL0256-119B1P-b

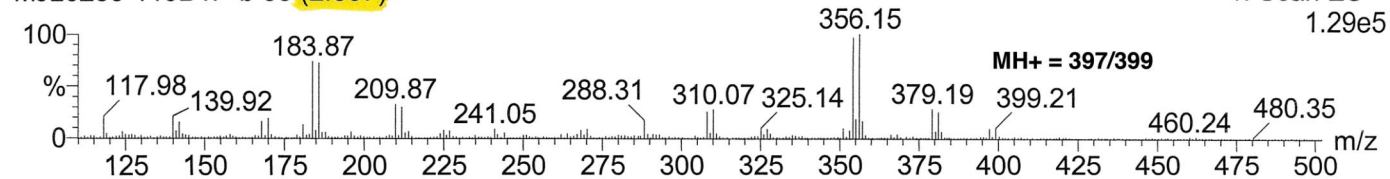
MJL0256-119B1P-b



MJL0256-119B1P-b 161 (2.798)



MJL0256-119B1P-b 53 (2.837)



MJL0256-119B1P-b

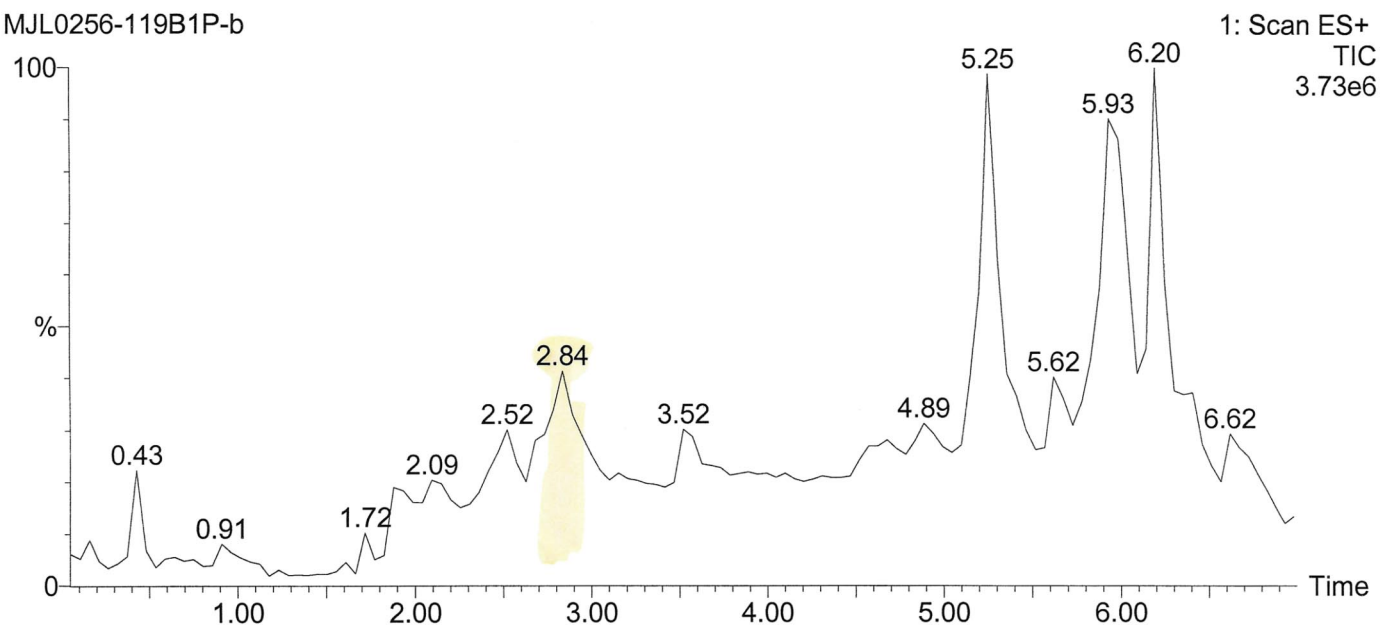
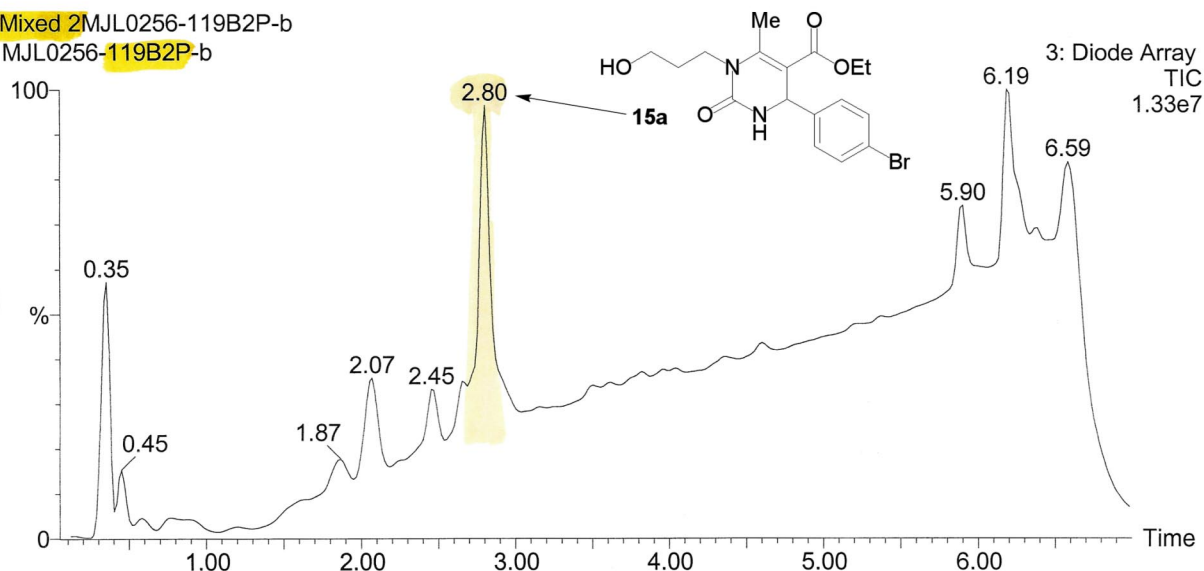
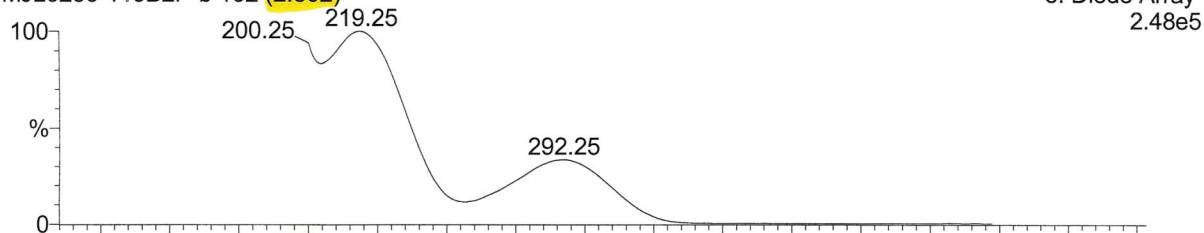


Figure S9. LC/MS of Pooled Bead No. 1 (Compound 15a, X = Br)

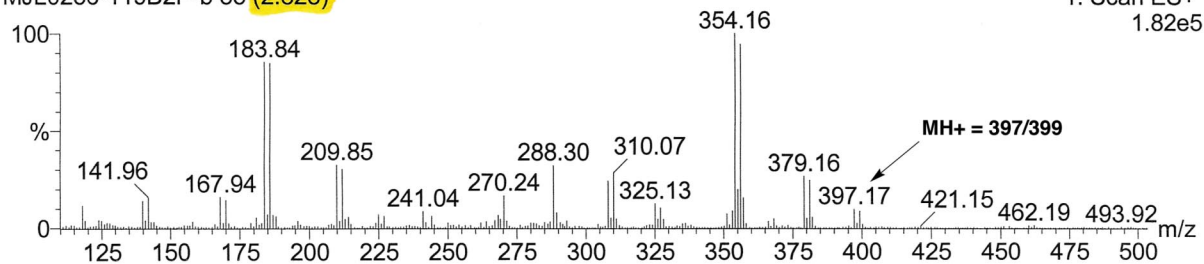
Mixed 2 MJL0256-119B2P-b
MJL0256-119B2P-b



MJL0256-119B2P-b 162 (2.802)



MJL0256-119B2P-b 55 (2.828)



MJL0256-119B2P-b

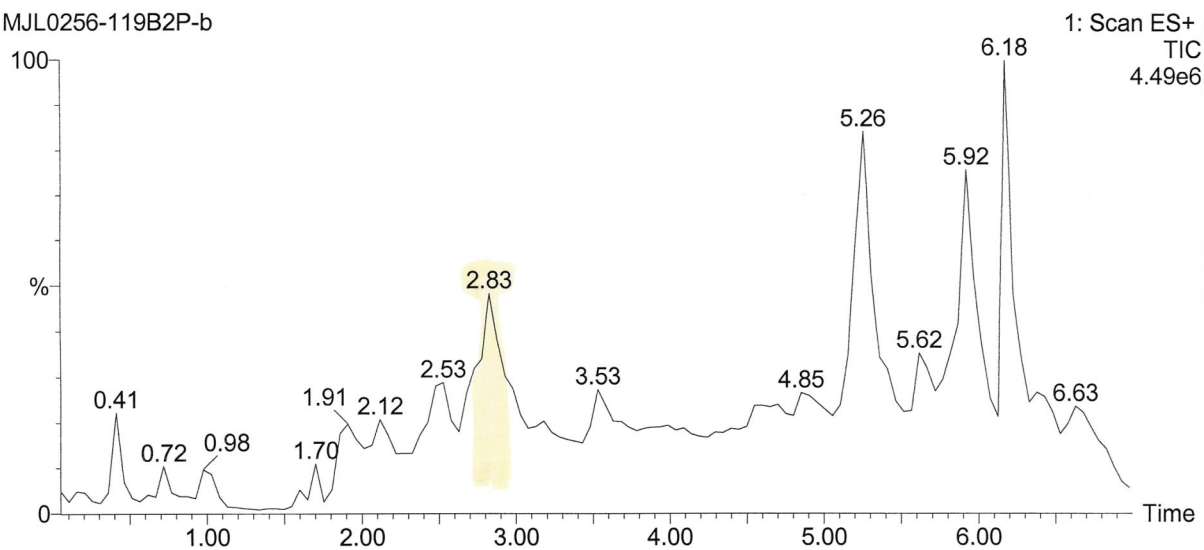


Figure S10. LC/MS of Pooled Bead No. 2 (Compound **15a**, X = **Br**)

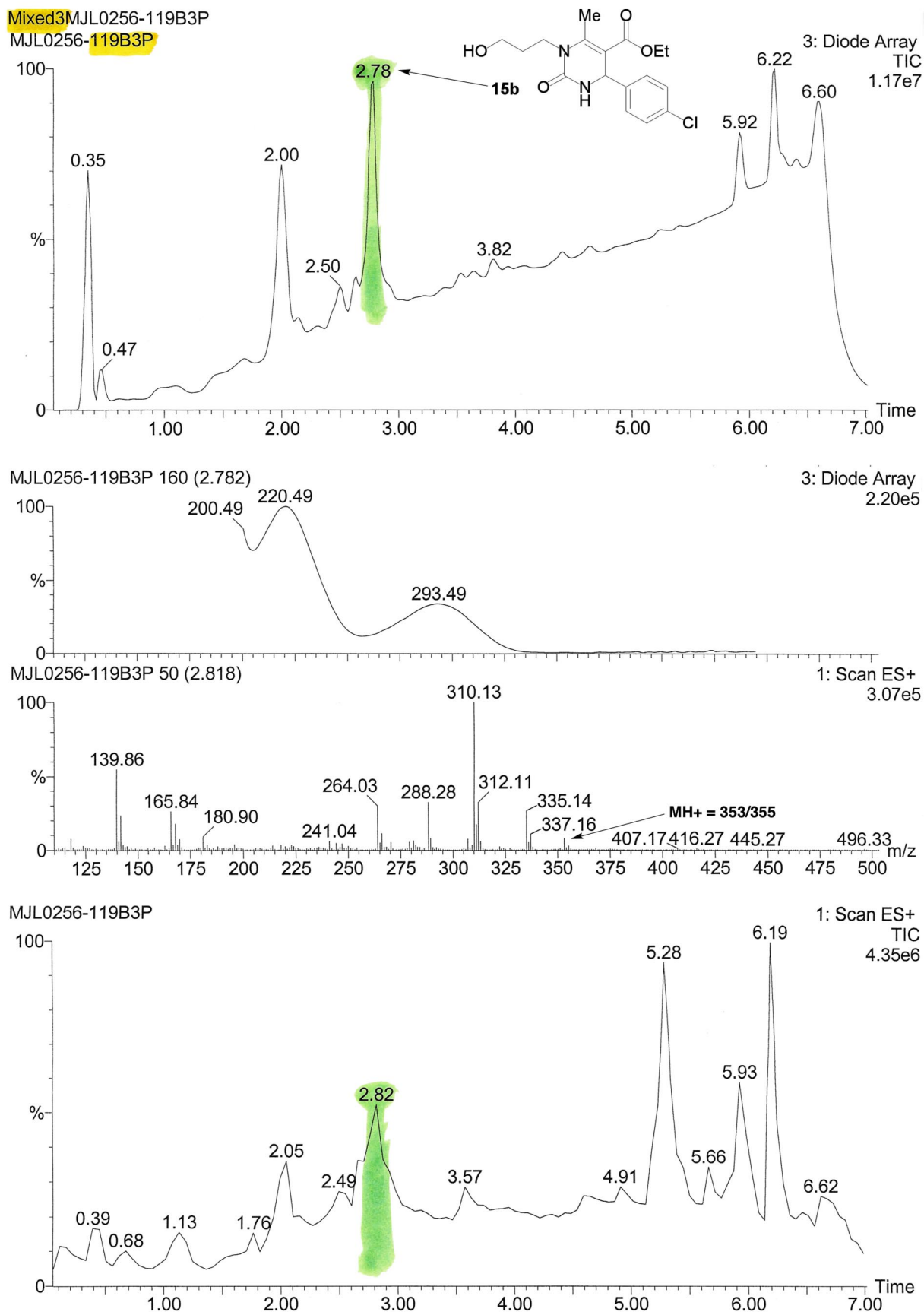


Figure S11. LC/MS of Pooled Bead No. 3 (Compound **15b, X = Cl)**

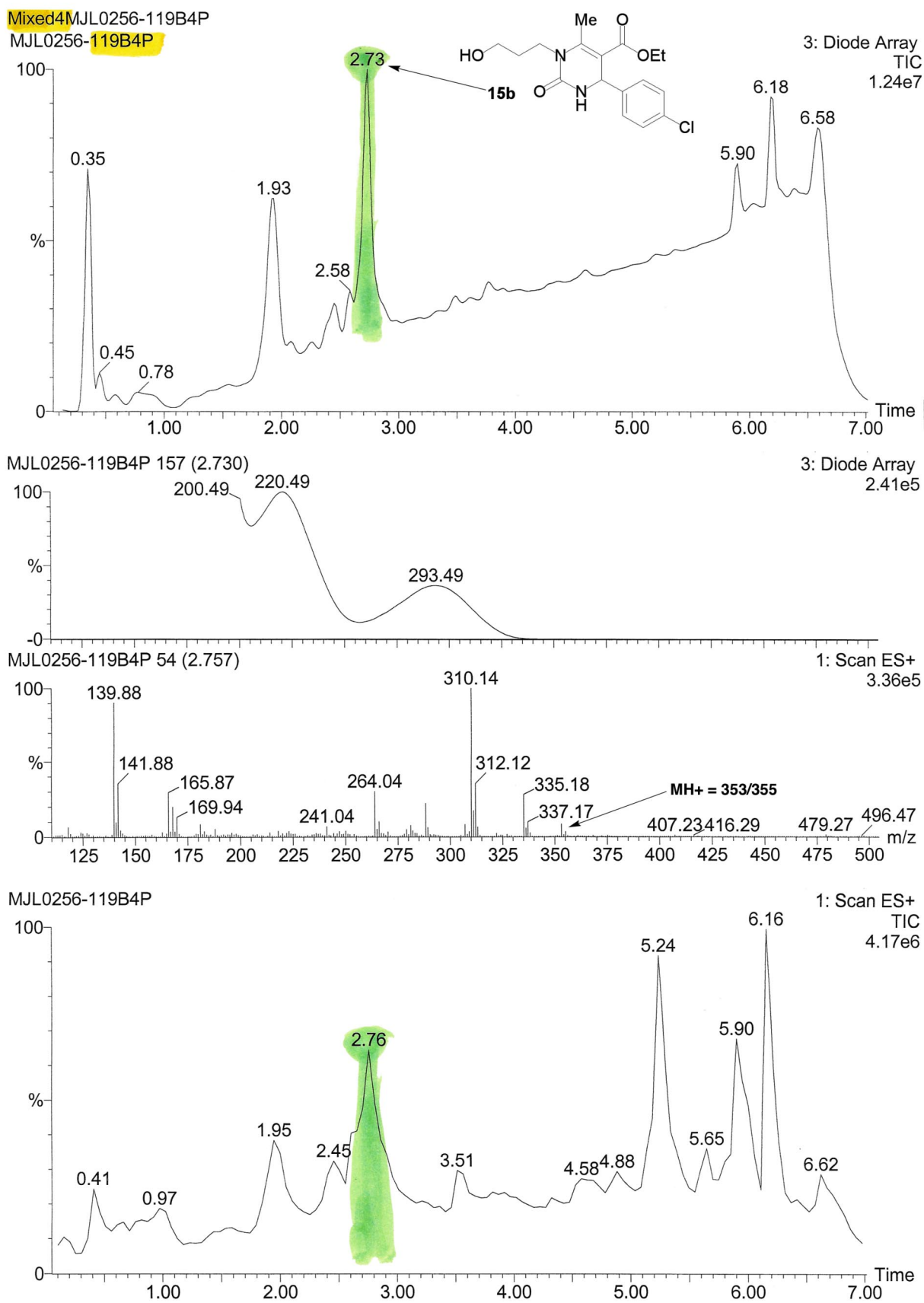


Figure S12. LC/MS of Pooled Bead No. 4 (Compound **15b**, X = **Cl**)

Mixed5 MJL0256-119B5P
 MJL0256-119B5P

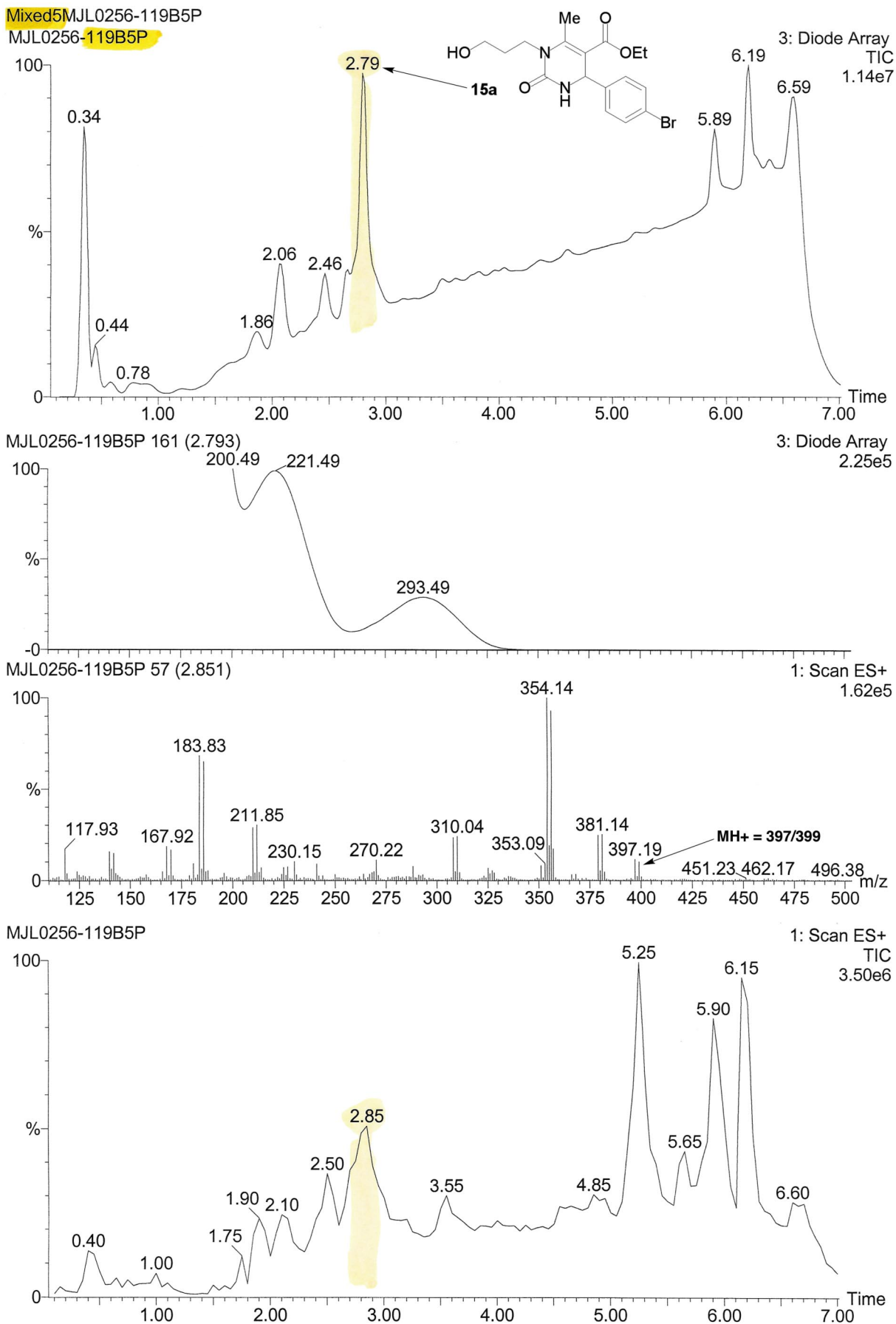
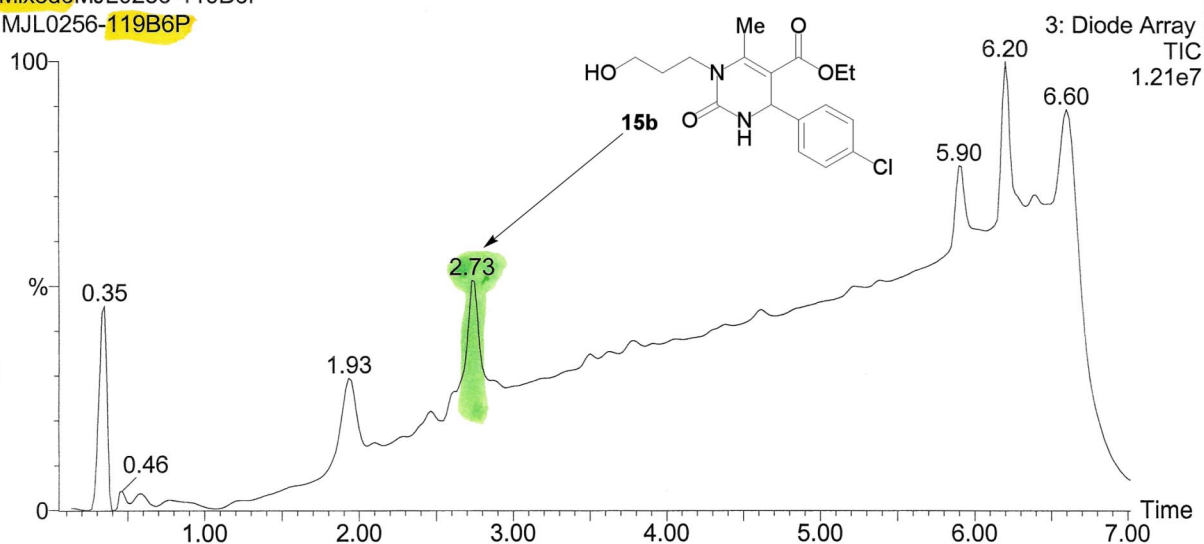


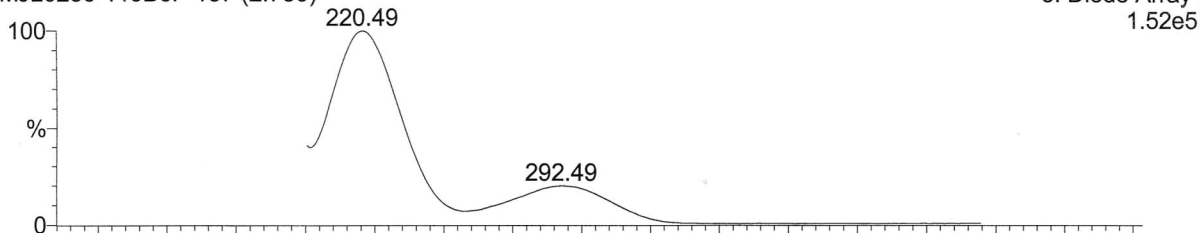
Figure S13. LC/MS of Pooled Bead No. 5 (Compound 15a, X = Br)

Mixed6 MJL0256-119B6P

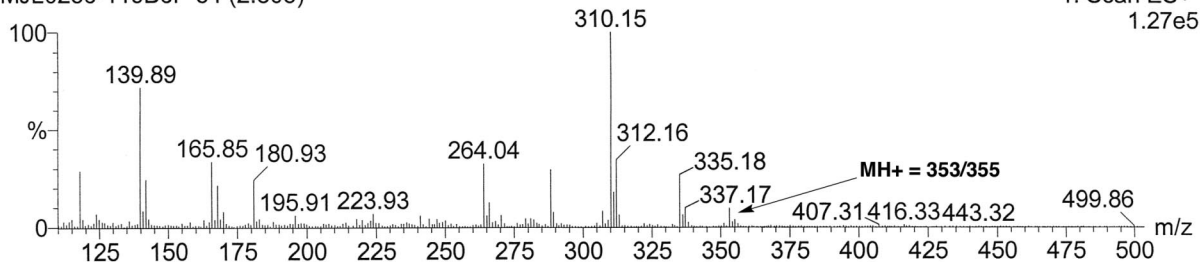
MJL0256-119B6P



MJL0256-119B6P 157 (2.730)



MJL0256-119B6P 54 (2.805)



MJL0256-119B6P

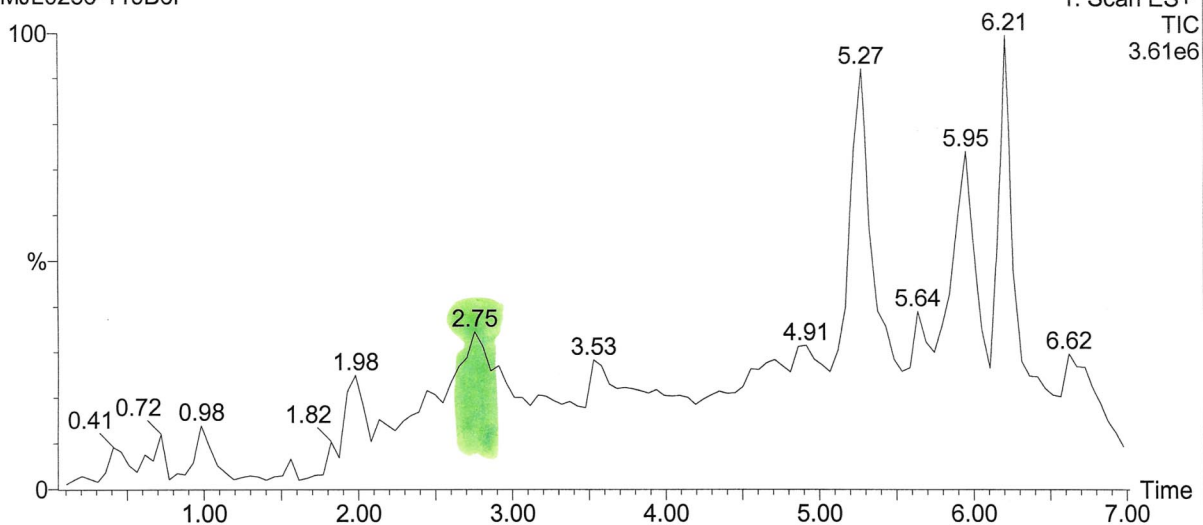
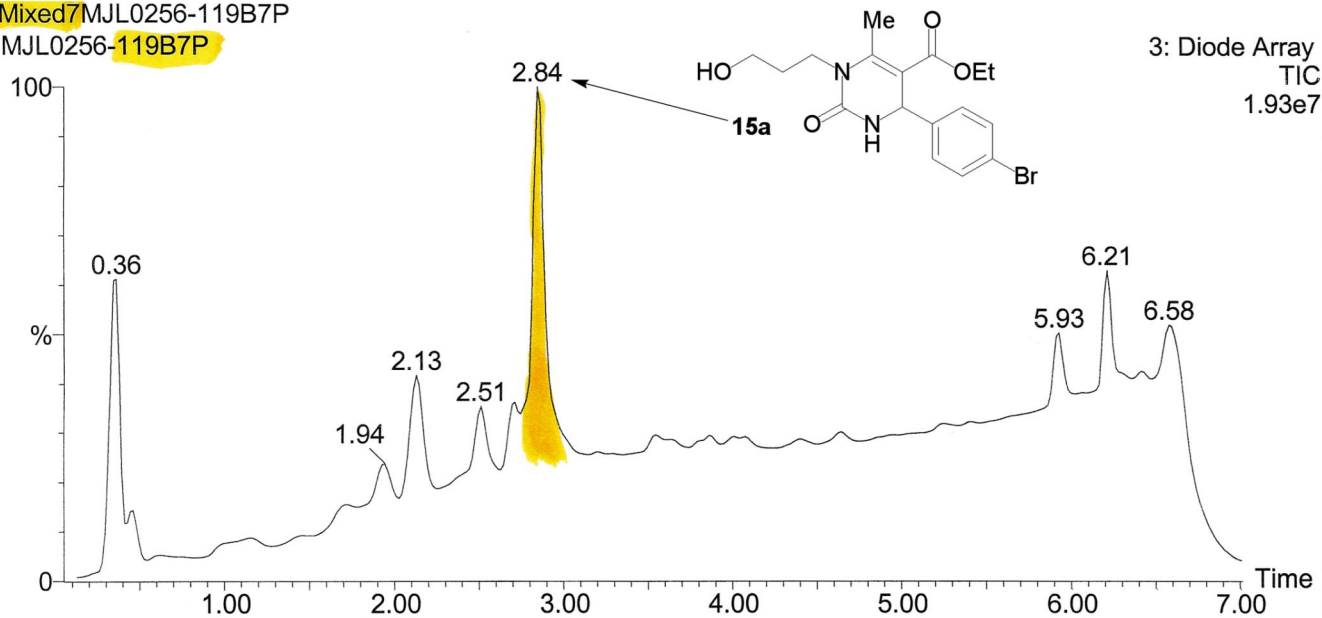


Figure S14. LC/MS of Pooled Bead No. 6 (Compound **15b**, X = Cl)

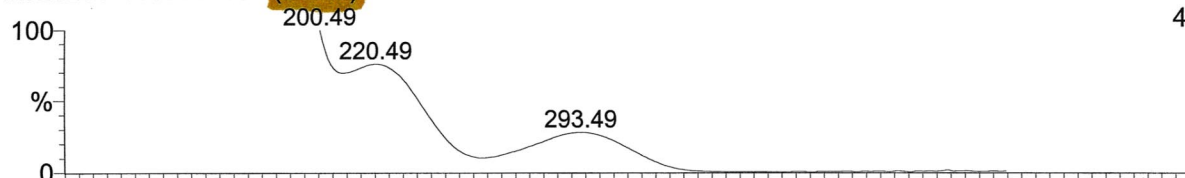
Mixed7 MJL0256-119B7P

MJL0256-119B7P



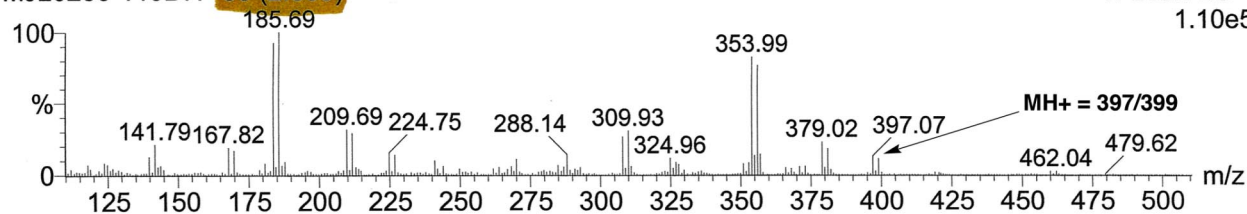
MJL0256-119B7P 164 (2.843)

3: Diode Array
4.35e5



MJL0256-119B7P 56 (2.869)

1: Scan ES+
1.10e5



MJL0256-119B7P

1: Scan ES+
TIC
4.75e6

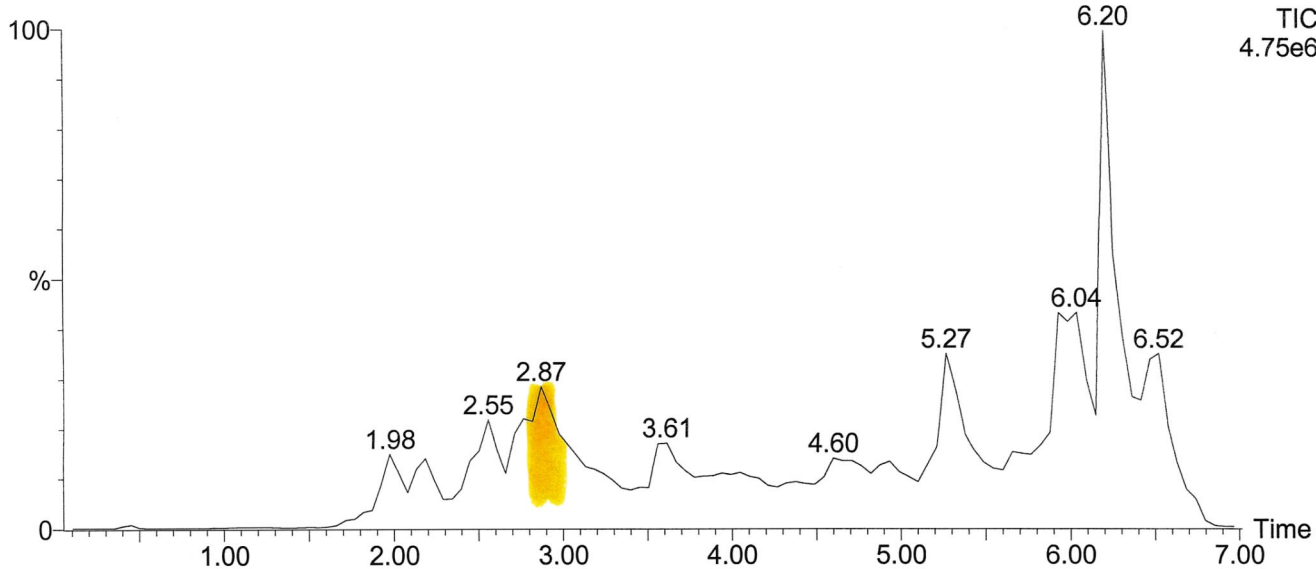


Figure S15. LC/MS of Pooled Bead No. 7 (Compound **15a**, X = **Br**)

Mixed8MJL0256-119B8P
MJL0256-119B8P

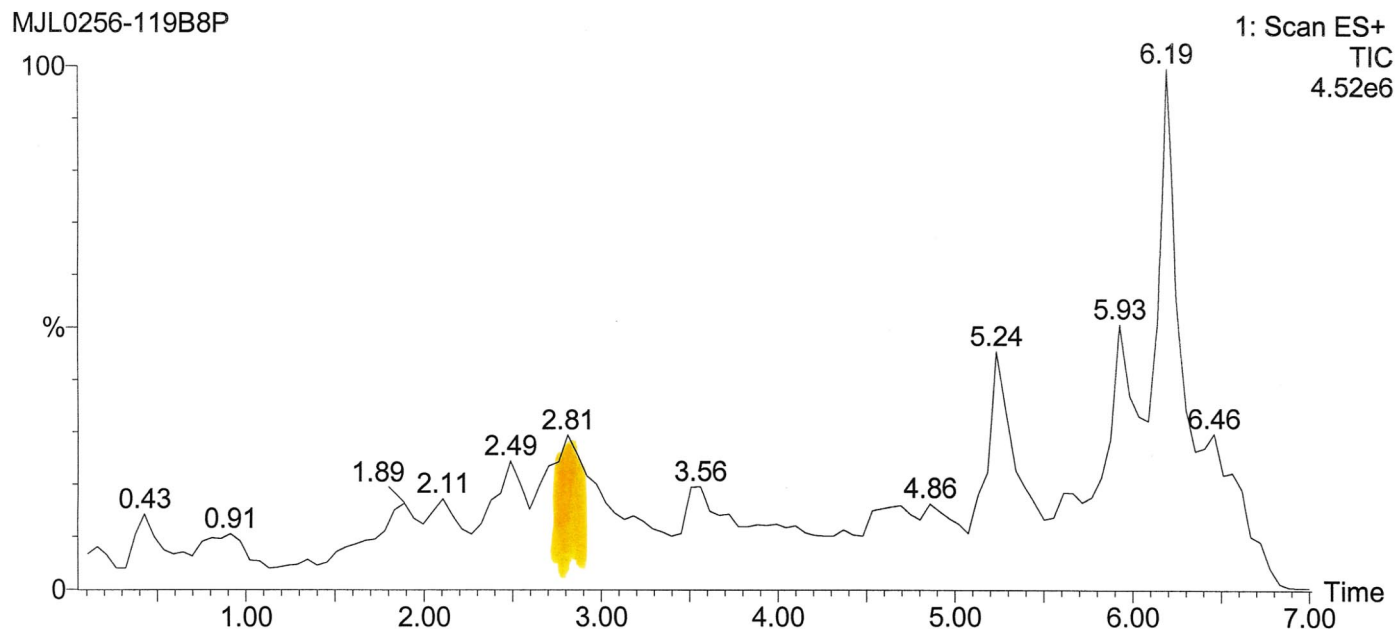
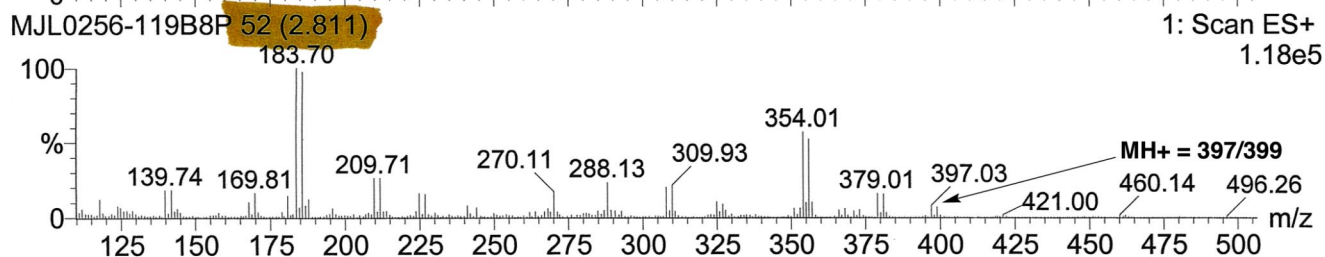
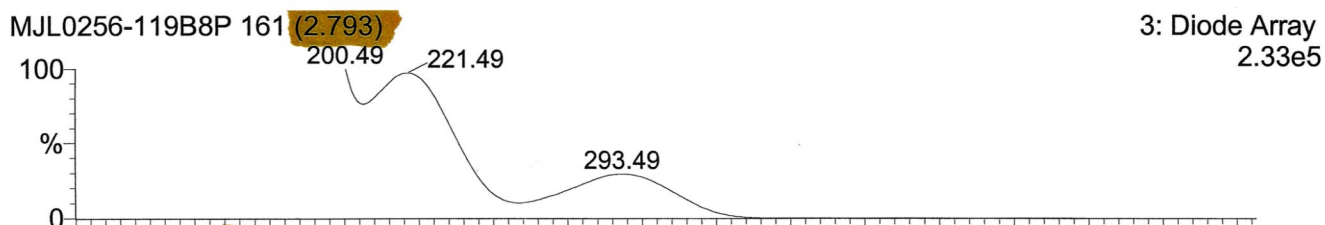
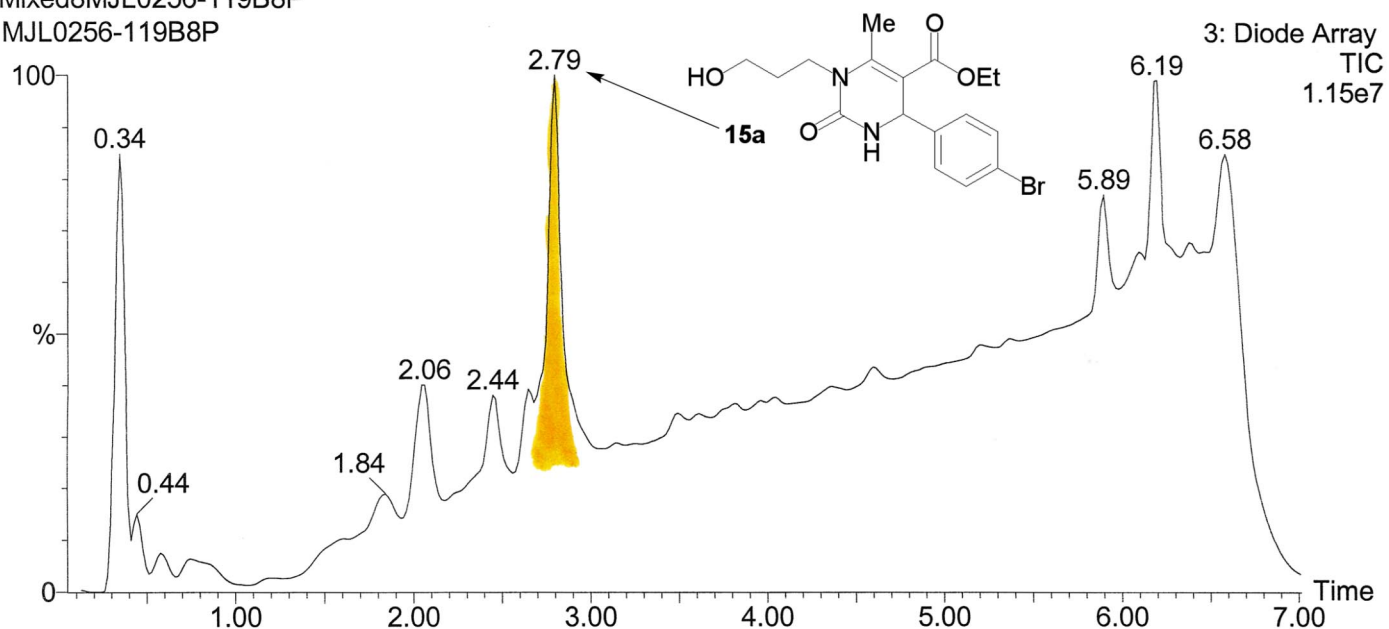
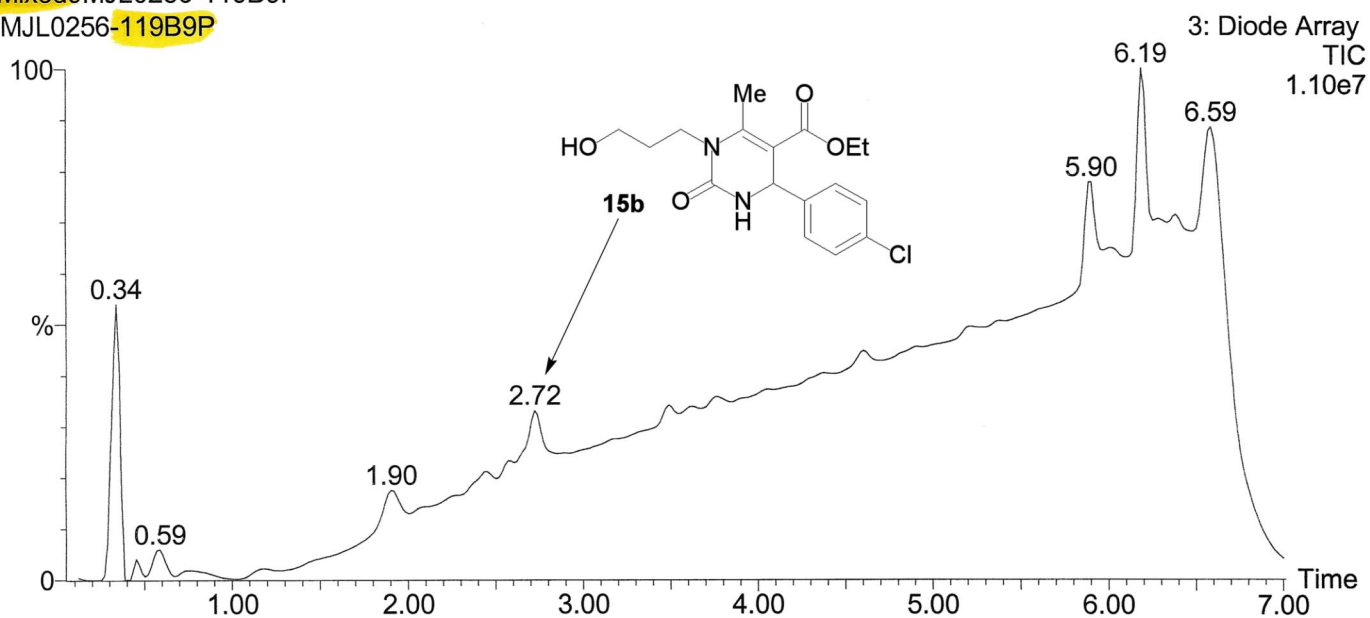


Figure S16. LC/MS of Pooled Bead No. 8 (Compound 15a, X = Br)

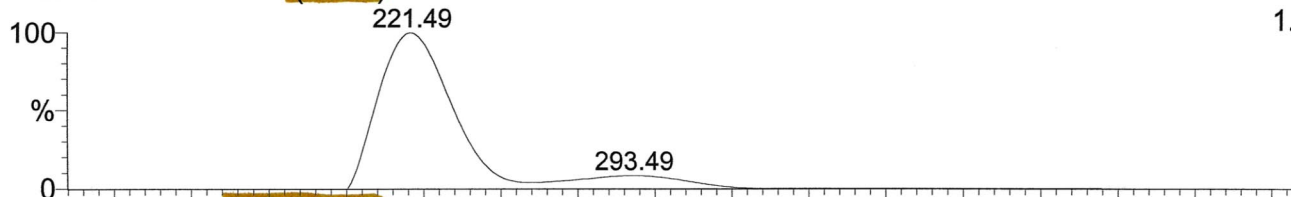
Mixed9MJL0256-119B9P

MJL0256-119B9P



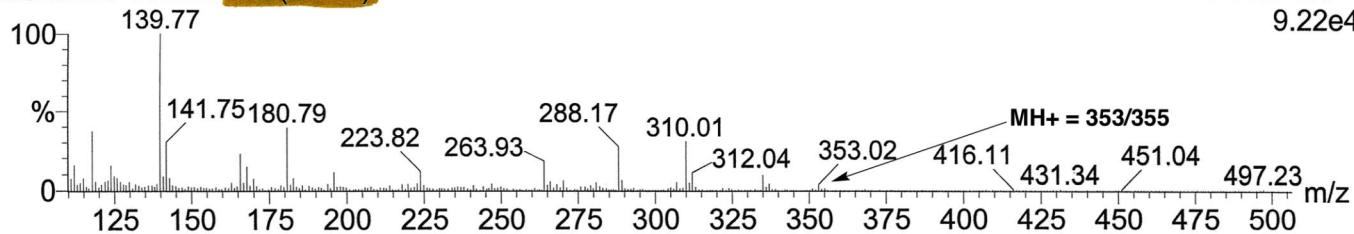
MJL0256-119B9P 157 (2.720)

3: Diode Array
1.19e5



MJL0256-119B9P 53 (2.761)

1: Scan ES+
9.22e4



MJL0256-119B9P

1: Scan ES+
TIC
4.75e6

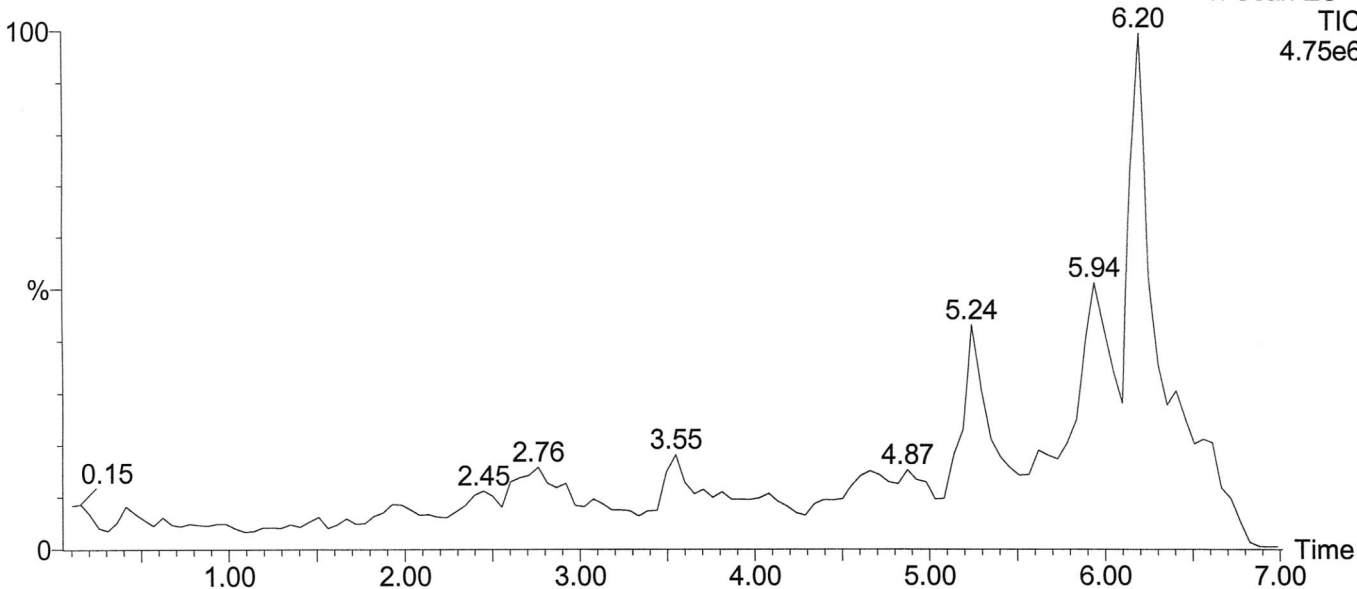
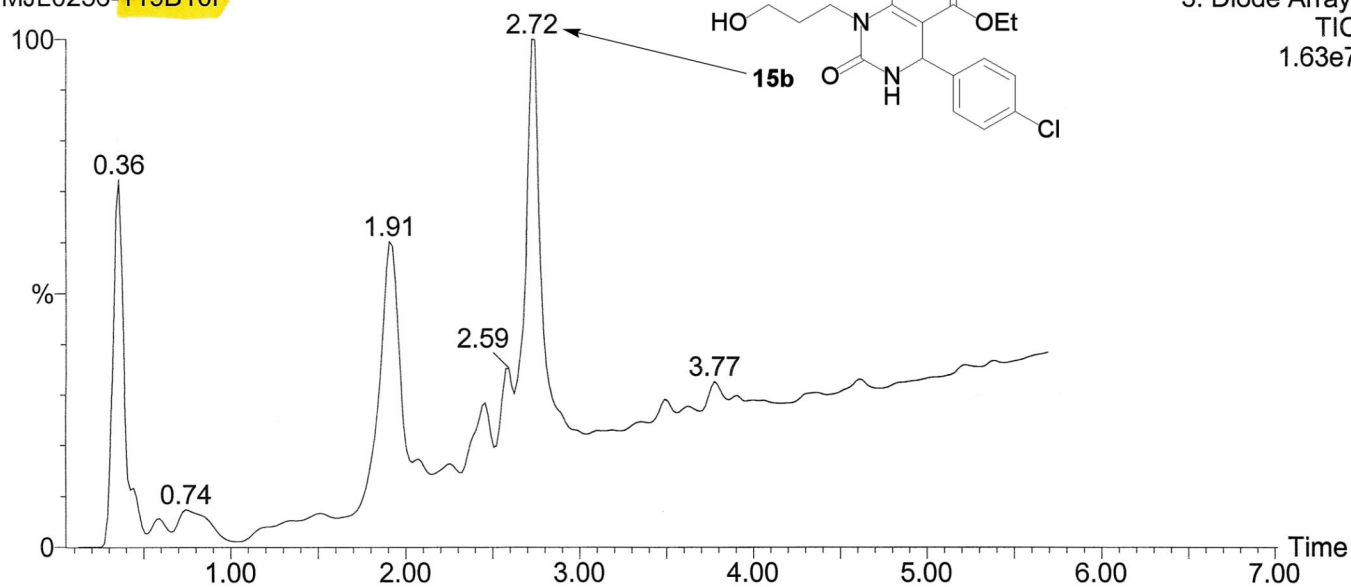


Figure S17. LC/MS of Pooled Bead No. 9 (Compound **15b**, X = **Cl**)

Mixed10MJL0256-119B10P

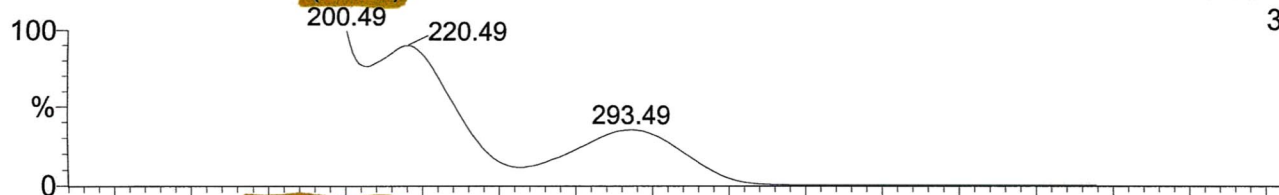
MJL0256-119B10P

3: Diode Array
TIC
1.63e7



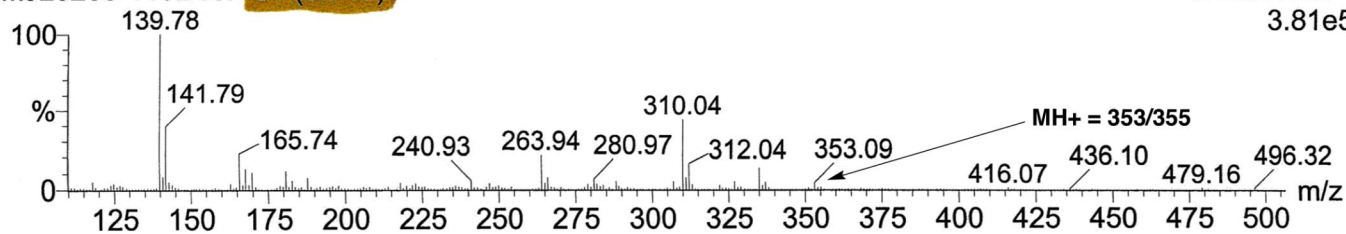
MJL0256-119B10P 157 (2.723)

3: Diode Array
3.24e5



MJL0256-119B10P 54 (2.743)

1: Scan ES+
3.81e5



MJL0256-119B10P

1: Scan ES+
TIC
2.52e6

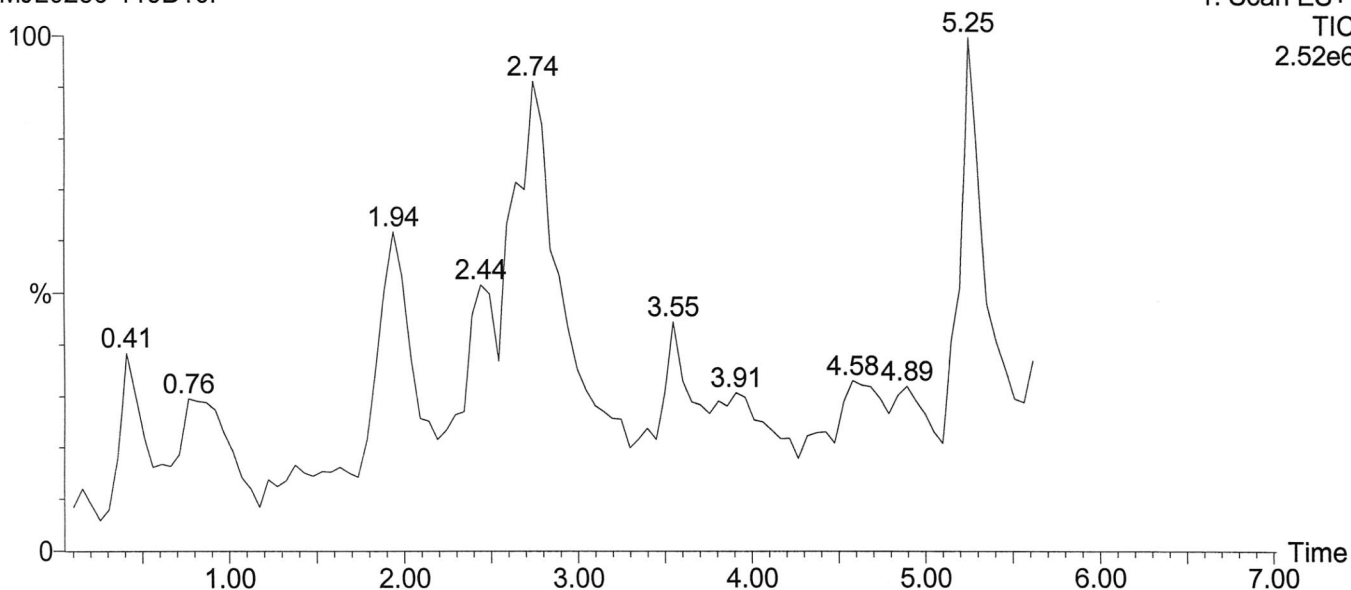


Figure S18. LC/MS of Pooled Bead No. 10 (Compound **15b**, X = Cl)

Mixed11 MJL0256-119B11P

MJL0256-119B11P

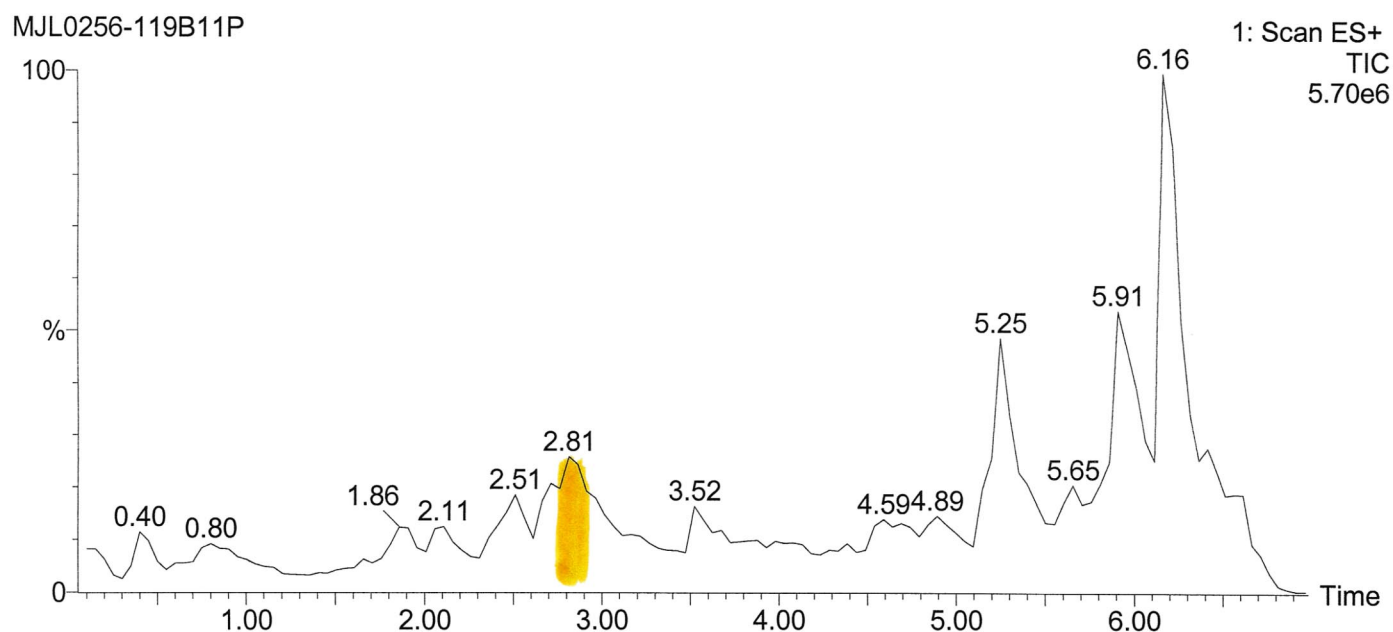
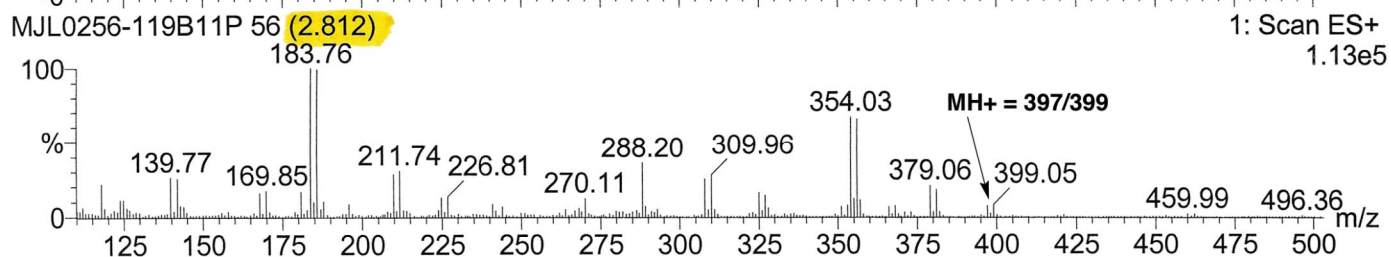
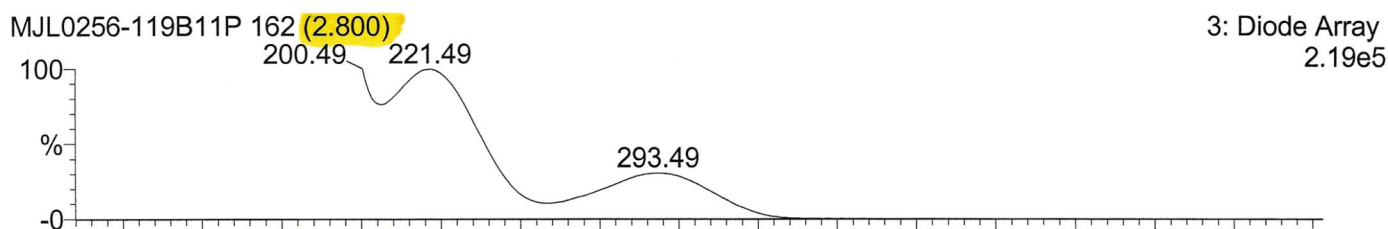
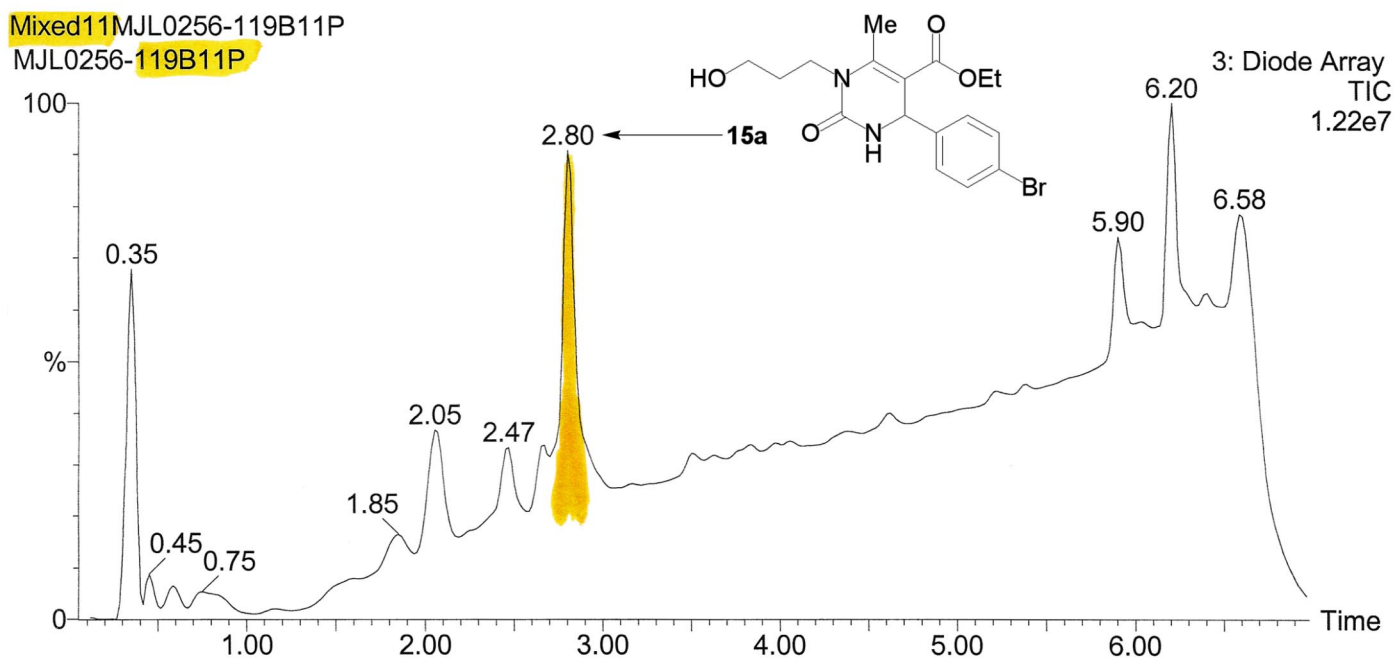


Figure S19. LC/MS of Pooled Bead No. 11 (Compound 15a, X = Br)

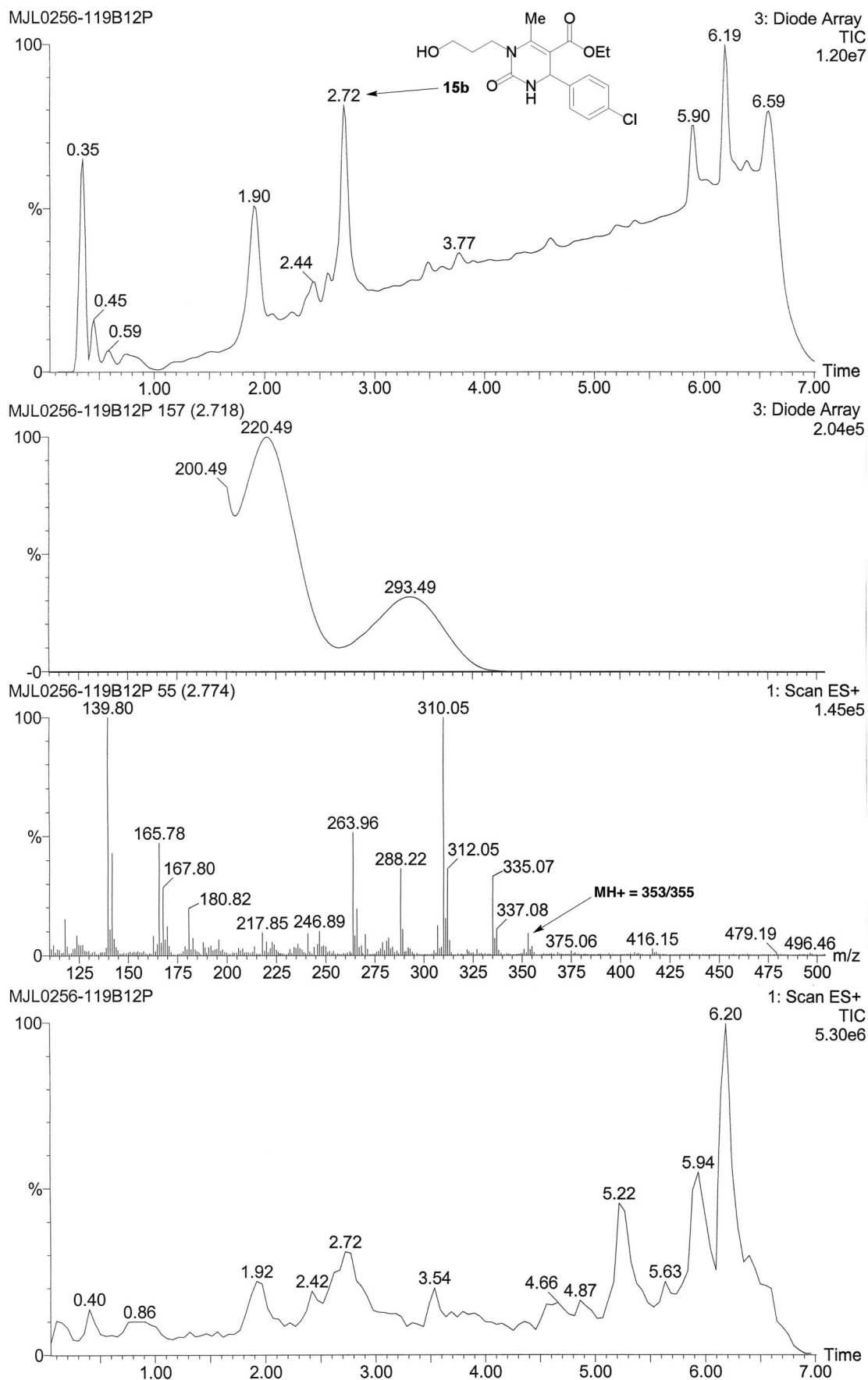


Figure S20. LC/MS of Pooled Bead No. 12 (Compound 15b, X = Cl)

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