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Supporting Information

A Bifunctional Amino Acid Enables Both Covalent Chemical Capture and Isolation of in Vivo Protein–Protein Interactions

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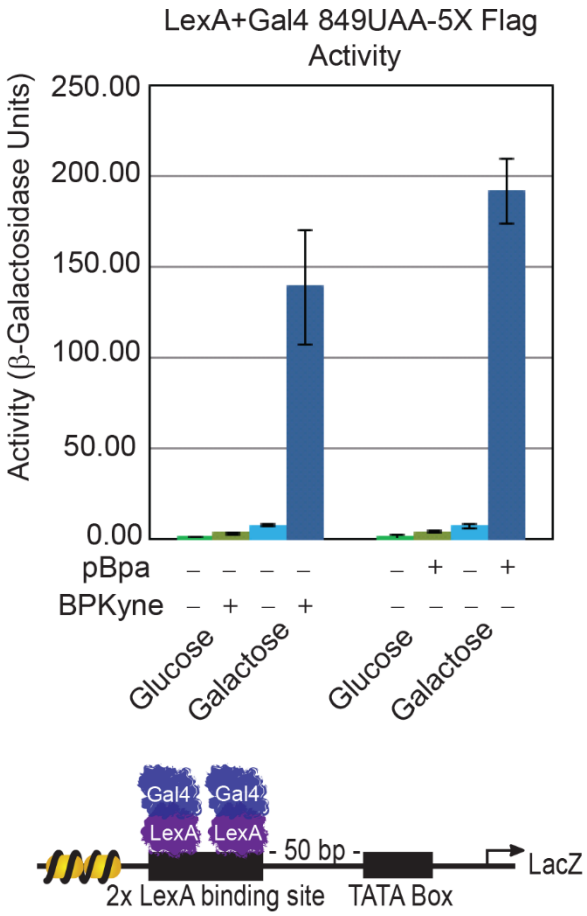
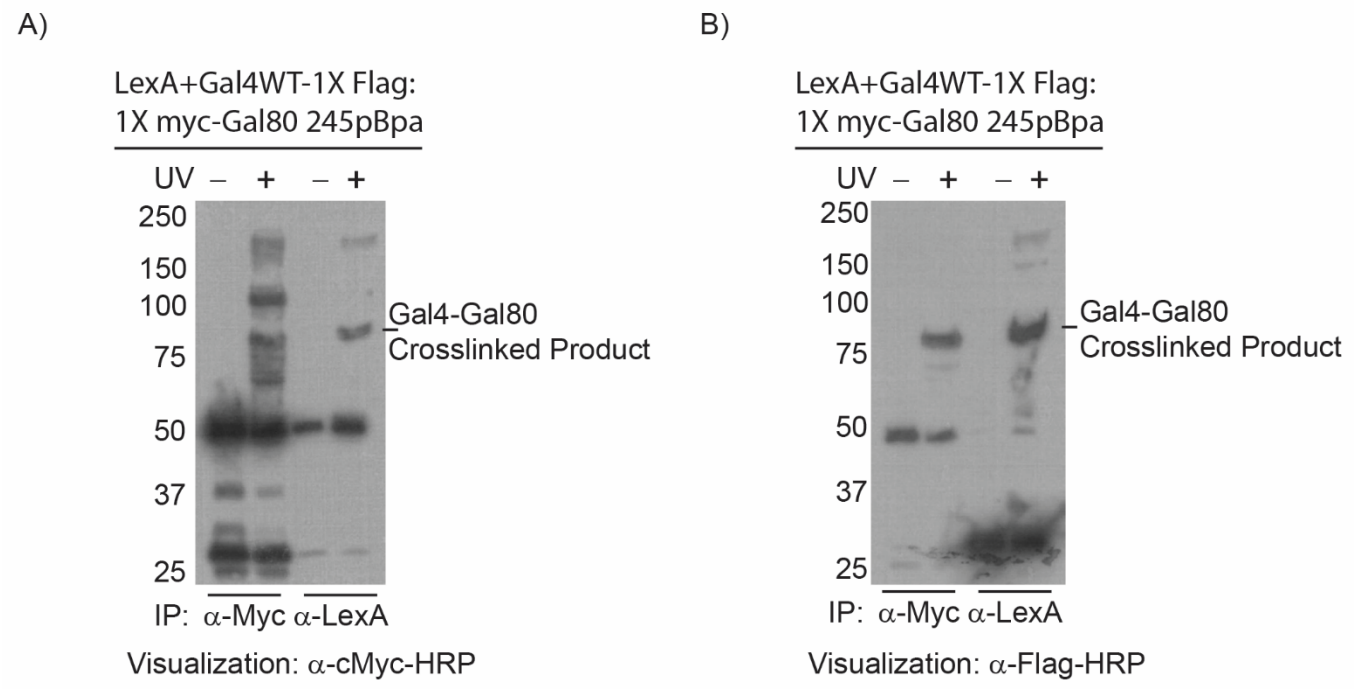
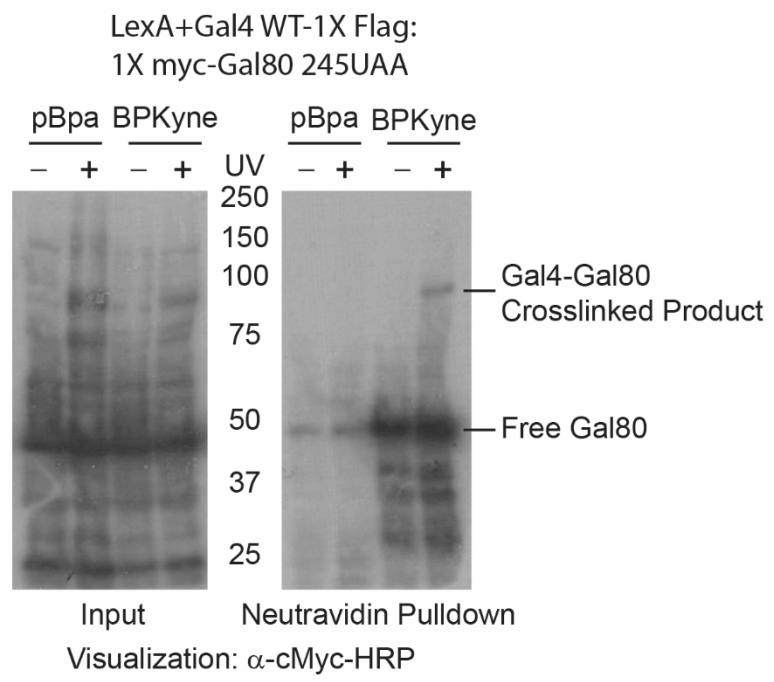


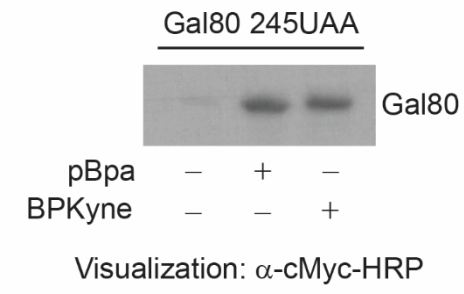
Supplemental Figure 1: Transcriptional activity of LexA+Gal4 849BPKyne under different growth conditions. β-Galactosidase assays were performed using yeast cells expressing the LexA+Gal4 849TAG-5X Flag and pSNRtRNA-pBpaRS plasmids to assess the ability of LexA+Gal4849Bpa and LexA-Gal4849BPKyne to upregulate transcription of an integrated pGal1-LacZ reporter gene in *Saccharomyces cerevisiae*. The cells were grown in media containing either 2% glucose or 2% galactose and 2% raffinose in the presence or absence of 1 mM UAA. Each experiment was run in triplicate with the indicated error (SDOM). There is no significant different in activity between the pBpa or BPKyne incorporated LexA+Gal4 protein under these experimental conditions when analyzed by students's t-test with $p \leq 0.05$.



Supplemental Figure 2: *In vivo* photo-crosslinking of Gal80 245Bpa using traditional immunological techniques. The isolation of Gal4-Gal80 crosslinked product was performed using a protein- or epitope tag-specific antibody, anti-LexA and anti-cMyc, respectively. The blot was analyzed by Western blot using either A) cMyc antibody B) Flag antibody. For both techniques the Gal4-Gal80 crosslinked product was observed only after UV irradiation



Supplemental Figure 3: Full Western blot visualizing the biotin-labeling of Gal4-Gal80 245UAA crosslinked product after CuAAC cycloaddition



Supplemental Figure 4: Myc-Gal80 245UAA expression. BPKyne incorporation was compared to that of pBpa in the 245 position of Gal80 using the *E. coli* tyrosyl tRNA/tRNA synthetase pair ($tRNA^{Tyr}_{CUA}$-TyrRS) in the presence or absence of 1 mM pBpa or BPKyne.

General information (molecular biology)

LS41 [JPY9::pZZ41, Matα his3Δ200 leu2Δ1 trp1Δ63 ura3-52 lys2Δ385 gal4 URA::pZZ41] yeast was used for all experiments. pBpa was purchased from Chem-Impex International (Wood Dale, IL). All plasmids described below were constructed using standard molecular biology techniques and the sequences of all isolated plasmids were validated by sequencing at the University of Michigan Core Facility (Ann Arbor, MI).

Table of plasmids used in this study

Plasmid Name	Function
pLexA+Gal4WT-5X Flag	Expresses LexA(1-202)+Gal4(840-881)+5X Flag tag
pLexA+Gal4849TAG-5X Flag	Expresses LexA(1-202)+Gal4(840-881)+5X Flag tag with a TAG codon replacing the codon of the existing amino acid
pSNRtRNA-pBpaRS	Expressed tRNA under the control of the SNR52 promoter and contains synthetase specific for pBpa
p6XMyc Gal80	Expresses full-length Gal80+6X c-Myc tag
pLexA-Gal4WT-1X Flag	Expresses LexA(1-202)+Gal4(840-881)+1X Flag tag
pMyc Gal80 245TAG	Expresses full-length Gal80+1X myc tag with a TAG codon replacing the codon of the existing amino acid

UAA incorporation and expression

LS41 yeast was transformed with pLexA+Gal4 849 TAG-5X Flag, pLexA+Gal4WT-5X Flag, or Myc-Gal80 245TAG and pSNRtRNA-pBpaRS plasmids. Individual colonies were grown in 5 mL SC media containing 2% raffinose, but lacking histidine, tryptophan, and uracil for selection. The cultures were incubated at 30 °C with 250 rpm agitation. Following incubation, these cultures were used to inoculate 5 mL cultures of SC media containing 2% raffinose and 2% galactose, with or without 1 mM pBpa / 1 mM BPKyne (dissolved in 50 µL 1 M NaOH), and 50 µL 1 M HCl. The cultures were incubated at 30 °C with agitation to an OD_{660} of 1.0. Three ODs were isolated, washed with sterile water, and stored at -20 °C. The samples were lysed in 10 µL 4X NuPAGE LDS Sample loading buffer (Invitrogen), 10 µL 1X lysis buffer (50 mM Tris-Acetate pH 7.9, 100 mM potassium acetate, 20% glycerol, 0.2% Tween20, 2 mM β-mercaptoethanol, and 2 mM magnesium acetate), and 10 µL 1 M DTT by boiling at 95 °C for 10 min. The samples were run on a 3-8% Tris-acetate SDS-PAGE gel and analyzed by Western blot with the anti-Flag (M2) antibody (Sigma Aldrich) for the pLexA-Gal4 expression samples and anti-cMyc-HRP (9E10) antibody (Santa Cruz) for the Myc-Gal80 expression samples. Expression levels were quantified using ImageJ and relative levels of LexA-Gal4 protein for each experiment were expressed as follows ((experimental/WT LexA-Gal4)*100).

β-Galactosidase assays

LS41 yeast was transformed with pLexA+Gal4 849 TAG-5X Flag or pLexA+Gal4WT-5X Flag and pSNRtRNA-pBpaRS plasmids. Individual colonies were grown in 5mL SC media containing 2% raffinose,

but lacking histidine, tryptophan, and uracil for selection. The cultures were incubated at 30 °C with 250 rpm agitation. Following incubation, these cultures were used to inoculate 5 mL cultures of SC media containing either 2% glucose or 2% Raffinose and 2% galactose with or without 1 mM pBpa / 1 mM BPKyne (dissolved in 50 µL 1 M NaOH), and 50 µL 1M HCl which were subsequently incubated at 30 °C with agitation to an OD_{660} of 1.0 and harvested. The activity of each construct was assessed using B-Galactosidase assay as previously described.[1]

LexA+Gal4 849TAG-5X Flag *in vivo* photo-crosslinking

For *in vivo* photo-crosslinking, a colony of LexA+Gal4 849 TAG-5X Flag was grown in 5 mL SC media containing 2% raffinose, but lacking histidine, tryptophan, and uracil for selection. The cultures were incubated at 30 °C with 250 rpm agitation. Following incubation, these cultures were used to inoculate 10 mL cultures of SC media containing 2% glucose, with 1 mM pBpa or 1 mM BPKyne (dissolved in 100 µL 1 M NaOH), and 100 µL 1 M HCl. The cultures were incubated at 30 °C with agitation to an OD_{660} of 1.0. For each culture, the cells were isolated by centrifugation and washed with the SC media lacking histidine, tryptophan, and uracil. The cell pellets were resuspended in 2 mL SC media containing 2% glucose and then transferred to small culture dishes and subjected to UV irradiated at 365nm light (Eurosolar 15W UV lamp) with cooling for 30 minutes. The cells were isolated by centrifugation and stored at -20 °C until lysis. The control samples were washed with 1 mL SC media containing 2% glucose, isolated by centrifugation, and stored at -20 °C until lysis.

For crosslinking studies with Myc-Gal80, the procedure was identical except that cells were grown in SC media lacking histidine, leucine, tryptophan, and uracil. For lysis, cells were resuspended in 350 µL lysis buffer (50 mM HEPES – KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini, EDTA Free Protease Inhibitor (Roche)) and lysed using glass beads by vortexing at 4 °C. Subsequently, the lysate was pelleted and the supernatant incubated with anti-LexA antibody (sc-1725, Santa Cruz Biotechnologies) for 2 hours at 4 °C for immunoprecipitation. The proteins bound to the antibody were isolated by incubation for 1 hour with 8 µL pre-washed protein G magnetic beads (Millapore) at 4 °C. After immunoprecipitation, the beads were washed six times with 1 mL Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.1% Na-Deoxycholate, and 1 mM EDTA) and stored at -20 °C until elution.

The samples were eluted from the beads by heating at 95 °C for 10 minutes in 10 µL NuPAGE 4X LDS Sample Loading Buffer (Invitrogen), 10 µL water, and 10 µL 1M DTT. The samples were run on a 3-8% Tris-acetate SDS-PAGE gel and analyzed by Western blot using either anti-Flag (M2) antibody (Sigma Aldrich) for endogenous crosslinking profile or anti-cMyc antibody (SC-40, Santa Cruz Biotechnology) for Myc-Gal80 crosslinking. Crosslinking yields were quantified using ImageJ and the relative amount of Gal4-Gal80 crosslinked product for each experiment was expressed as follows ((Gal4 BPKyne-Gal80 crosslinked product/Gal4 Bpa-Gal80 crosslinked product)*100).

LexA+Gal4 WT-1X Flag:1X myc-Gal80 245TAG whole cell lysate preparation

A colony of LexA+Gal4 WT-1X Flag:1X myc-Gal80 245TAG was grown in 5 mL SC media containing 2% raffinose, but lacking histidine, tryptophan, leucine and uracil for selection. The cultures were incubated at 30 °C with 250rpm agitation. Following incubation, these cultures were used to inoculate 100 mL

cultures of SC media containing 2% glucose, with 1 mM pBpa or 1 mM BPKyne (dissolved in 100 µL 1 M NaOH), and 1 mM 1 M HCl which were subsequently incubated at 30 °C with agitation to an OD_{660} of 2.0. For each culture, the cells were isolated by centrifugation and washed with the SC media lacking histidine, tryptophan, leucine, and uracil. The cell pellets were resuspended in 2 mL SC media containing 2% glucose and then transferred to small culture dishes and subjected to UV irradiated at 365 nm light (Eurosolar 15W UV lamp) with cooling for 45 min. The cells were isolated by centrifugation and stored at -80 °C until lysis. The control samples were washed with 1 mL SC media containing 2% glucose, isolated by centrifugation, and stored at -80 °C until lysis. For lysis, cells were resuspended in 600 µL lysis buffer (50 mM HEPES – KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini, EDTA Free Protease Inhibitor (Roche)) and lysed using glass beads by vortexing at 4 °C. The supernatant was split into 2 mg protein aliquots and stored at -80 °C until CuAAC labeling.

Traditional immunoprecipitation of LexA+Gal4 WT-1X Flag: 1X myc-Gal80 245pBpa crosslinked products

Whole cell lysate was incubated at 4 °C for 2 hours with either 10 µL anti-LexA (sc-1725, Santa Cruz Biotechnologies) or anti-cMyc (9E10) (SC 40, Santa Cruz Biotechnologies) antibody. The proteins bound to the antibody were isolated by incubation for 1 hour with 40 µL pre-washed Dynabeads® protein G magnetic beads (ThermoFisher) at 4 °C. After immunoprecipitation, the beads were washed six time with 1 mL Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.1% Na-Deoxycholate, and 1 mM EDTA) and stored at -20 °C until elution. The crosslinked samples were eluted from the beads by heating at 95 °C for 10 minutes in 10 µL NuPAGE 4X LDS Sample Loading Buffer (Invitrogen), 10 µL water, and 10 µL 1M DTT. The samples were run on a 3-8% Tris-acetate SDS-PAGE gel and analyzed by Western blot using either anti-Flag (M2) antibody (Sigma Aldrich) for the Gal4-containing crosslinked products or anti-cMyc-HRP antibody (SC-40, Santa Cruz Biotechnology) for Gal80-containing crosslinked products.

Copper-catalyzed alkyne-azide cycloaddition (CuAAC) labeling of LexA+Gal4 WT-1X Flag: 1X myc-Gal80 245BPKyne crosslinked products

2 mg/mL proteome was combined with 500 µM biotin-PEG3-azide (Sigma-Aldrich), 1% sodium dodecyl sulfate, 5 mM Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, Sigma-Aldrich), 1 mM $CuSO_4$ in water, and 5 mM sodium ascorbate in water at 37 °C with 250rpm agitation. After 2 hours, the samples were purified through acetone precipitation overnight at -20 °C followed by resuspension in 1X PBS pH 7.4 (ThermoFisher) and 0.5% SDS. Samples were combined with 50 µL GE Sera-Mag™ SpeedBeeds™ Neutravidin Particles and incubated at room temperature with rocking for 1.5 hours. After pulldown, the beads were washed with 1 mL Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.1% Na-Deoxycholate, and 1 mM EDTA) and stored at -80 °C until elution. The click samples were eluted from the beads by heating at 95 °C for 10 minutes in 10 µL NuPAGE 4X LDS Sample Loading Buffer (Invitrogen), 10 µL water, and 10 µL 1 M DTT. The samples were run on a 3-8% Tris-acetate SDS PAGE gel and analyzed by Western blot using anti-cMyc-HRP antibody (SC-40, Santa Cruz Biotechnology) for Myc-Gal80 crosslinking.

General information (organic synthesis)

Reactions were performed in oven-dried glassware containing PFTE-coated magnetic stir bars and fitted with rubber septa. Reactions performed under inert atmosphere were run under a positive pressure of dry N_2.

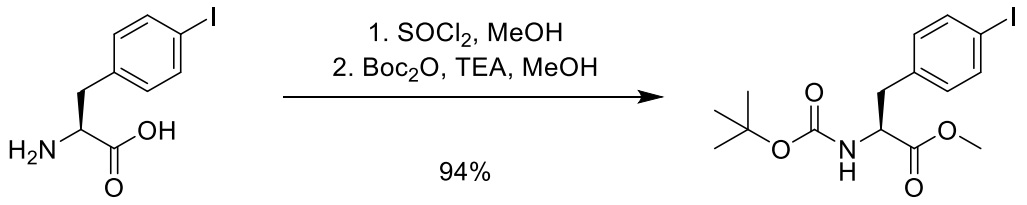
Thin layer chromatography (TLC) was performed using commercially available pre-coated glass plates (0.25 mm) (EMD Chemicals Inc. TLC Silica Gel 60 F_{254}) and visualized using a combination of UV light at 254 nm, ninhydrin, and bromophenol blue. Flash chromatography was carried out using E. Merck Silica Gel 60 (230-400 mesh).

Commercial purification system MBraun-MB-SPS #08-113 provided dry tetrahydrofuran (THF). Anhydrous methanol and 1,4-dioxane were used as supplied by Sigma Aldrich. All other solvents and commercially obtained reagents were used as received or purified using standard procedures. 4-Iodo-L-phenylalanine and 4-bromophenylboronic acid were purchased from Chem-Impex International. $PdCl_2$, $Pd_2(dba)_3$, and CuI were purchased from Strem Chemicals Inc. All other reagents were purchased from Sigma Aldrich.

NMR spectra were recorded on Varian 400 and 600 MHz spectrometers. The chemical shifts (δ) are reported in ppm. Coupling constants (*J*) are reported in hertz (Hz) rounded to 0.1 Hz and were calculated using MestReNova. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination of these. Solvents were used as internal standards when assigning NMR spectra (δH: $CDCl_3$ 7.24 ppm, d_6-DMSO 2.49 ppm; δC: $CDCl_3$ 77.0 ppm, d_6-DMSO 39.5 ppm).

High resolution mass spectra (HRMS) were recorded on an Agilent Technologies quadrapole time-of-flight 6520 Accurate-Mass Q-TOF HPLC-MS mass spectrometer by electrospray ionization (ESI).

Synthesis of BPKyne

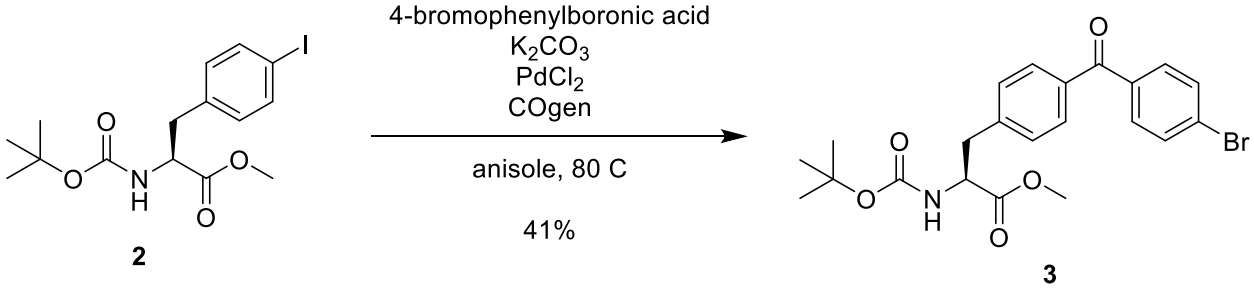


2

Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-iodophenyl)propanoate (**2**). In a 100 mL round bottom flask under N_2, 4-iodo-L-phenylalanine (3 g, 10.3 mmol) was suspended in 30 mL of anhydrous methanol. The flask was cooled to 0 °C in an ice bath, then thionyl chloride (1.3 mL, 18.2 mmol) was added slowly dropwise. The reaction was allowed to slowly warm to room temperature, and then was heated at reflux overnight (~12 h). The reaction was cooled to room temperature and the solvent was removed under reduced pressure. The resulting solid was dissolved in 15 mL of methanol. The solution was cooled to 0 °C in an ice bath, then triethylamine (4.3 mL, 30.9 mmol) was added. A solution of di-*tert*-butyl dicarbonate (2.48 g, 11.4 mmol) dissolved in minimal methanol was then added. The reaction was allowed to stir at room temperature overnight. The organic solvent was removed under reduced

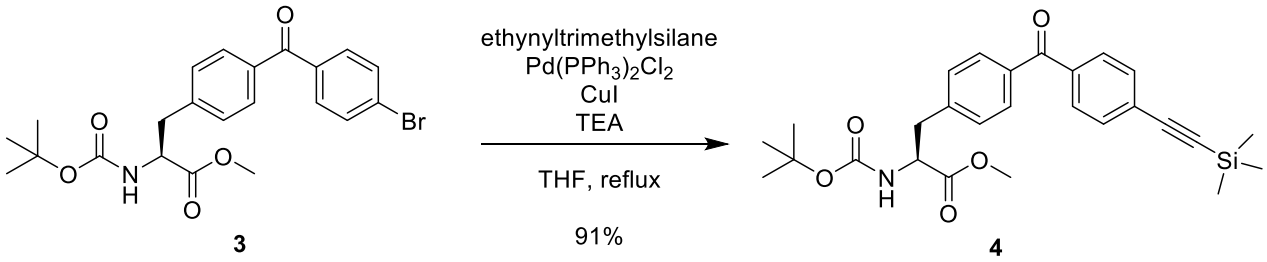
pressure, and the remaining solution was extracted with ethyl acetate (3 x 15 mL). The combined organic extracts were washed with 1 M HCl (2 x 15 mL), 1 M NaOH (1 x 15 mL), and brine (1 x 20 mL), then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **2** as a white solid (3.92 g, 94% yield) that was used without further purification.

R*f*: 0.36 (1:3 ethyl acetate:hexanes); HRMS (ESI): expected 405.0437 ($C_{15}H_{20}INO_4$), found 406.0529 ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ7.63 – 7.55 (m, 2H), 6.85 (d, *J* = 8.0 Hz, 2H), 4.95 (d, *J* = 8.3 Hz, 1H), 4.54 (q, *J* = 6.6 Hz, 1H), 3.69 (s, 3H), 3.05 (dd, *J* = 13.9, 5.8 Hz, 1H), 2.96 (dd, *J* = 13.8, 6.1 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (125, $CDCl_3$) δ171.96, 154.91, 137.50, 135.68, 131.24, 92.44, 79.98, 54.13, 52.25, 37.83, 28.22.



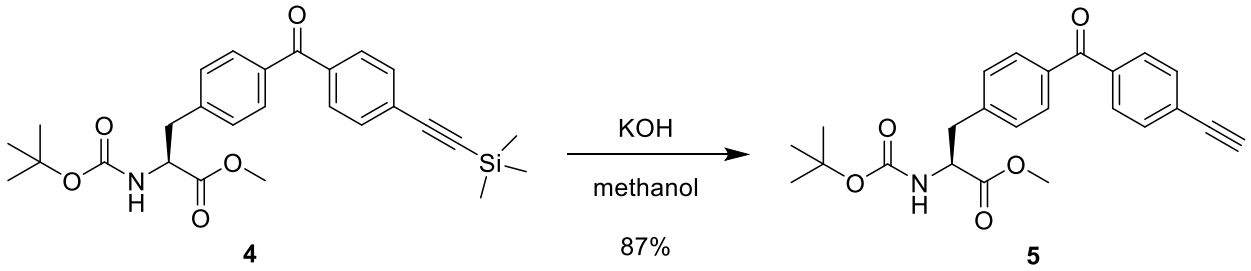
Methyl (S)-3-(4-(4-bromobenzoyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (**3**). Compound **3** was prepared following a procedure similar to that reported by Ahlberg and coworkers.[2] To chamber A of a 100 mL COware reaction vessel (Sigma Aldrich product STW5) was added **2** (1 g, 2.5 mmol), 4-bromophenyl boronic acid (493 mg, 2.45 mmol), K_2CO_3 (1023 mg, 7.4 mmol), and $PdCl_2$ (4.4 mg, 0.025 mmol). To chamber B was added 9-methyl-9*H*-fluorene-9-carbonyl chloride (895 mg, 3.7 mmol), tri-*tert*-butylphosphonium tetrafluoroborate (50 mg, 0.17 mmol), and $Pd_2(dba)_3$ (100 mg, 0.11 mmol). Anisole (8 mL) was added to each chamber, then the vessel was sealed and diisopropylethylamine (1.3 mL, 7.5 mmol) was added to chamber B. The reaction was heated at 80 °C overnight. After cooling to room temperature, the contents of chamber A were transferred to a separatory funnel, diluted with 40 mL water, and extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with brine (1 x 20 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography using a stepwise 0→25% ethyl acetate in hexanes gradient (2.5% step increases) to give **3** as a white solid (462, 41% yield).

R*f*: 0.26 (1:3 ethyl acetate:hexanes); HRMS (ESI): expected 461.0838 and 463.0817 ($C_{22}H_{24}BrNO_5$), found 462.0903 and 464.0906 ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8 Hz, 2H), 7.68-7.60 (m, 4H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.68 – 4.61 (m, 1H), 3.74 (s, 3H), 3.24 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.11 (dd, *J* = 13.7, 6.3 Hz, 1H), 1.42 (s, 9H); ^{13}C NMR (125, $CDCl_3$) δ 195.11, 171.89, 154.93, 141.45, 136.26, 135.80, 131.54, 131.40, 130.15, 129.37, 127.38, 80.06, 54.16, 52.34, 38.36, 28.22.



Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(4-((trimethylsilyl)ethynyl)benzoyl)phenyl)propanoate (**4**). To a 100 mL round bottom flask under N_2 was added compound **3** (1144 mg, 2.5 mmol), $Pd(PPh_3)_2Cl_2$ (87 mg, 0.13 mmol), and CuI (24 mg, 0.13 mmol). Anhydrous THF (10 mL) was added followed by ethynyltrimethylsilane (0.53 mL, 3.8 mmol) and triethylamine (3.3 mL, 23.7 mmol). The reaction was heated at reflux overnight. After cooling to room temperature, the reaction was concentrated to minimal volume under reduced pressure. The reaction was diluted with ethyl acetate (30 mL) and washed with 1 M HCl (3 x 15 mL) and brine (1 x 15 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash chromatography using a stepwise 0→40% ethyl acetate in hexanes gradient (10% step increases) to give **4** as a yellow crystalline solid (1088 mg, 91% yield).

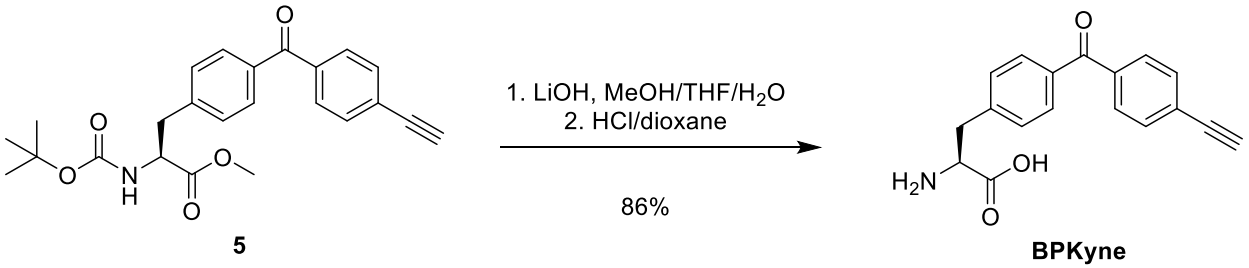
R*f*: 0.39 (1:3 ethyl acetate:hexanes); HRMS (ESI): expected 479.2128 ($C_{27}H_{33}NO_5Si$), found 480.2214 ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 8.3, 3.1 Hz, 4H), 7.59 – 7.50 (m, 2H), 7.26-7.21 (m, 2H), 5.01 (d, *J* = 3.1 Hz,1H), 4.62 (q, *J* = 6.7 Hz, 1H), 3.72 (s, 3H), 3.21 (dd, *J* = 13.5, 5.7 Hz, 1H), 3.09 (dd, *J* = 13.7, 6.4 Hz, 1H), 1.40 (s, 9H), 0.25 (s, 9H); ^{13}C NMR (125, $CDCl_3$) δ 195.48, 171.94, 154.96, 141.30, 136.96, 136.06, 131.75, 130.22, 129.78, 129.35, 127.28, 104.02, 97.82, 80.11, 54.19, 52.37, 38.41, 28.26, -0.17.



Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(4-ethynylbenzoyl)phenyl)propanoate (**5**). In a 25 mL round bottom flask, compound **4** (1 g, 2.1 mmol) was dissolved in 10 mL methanol. Potassium hydroxide (20 mg, 0.36 mmol) was added and the reaction was stirred at room temperature for 45 minutes. The solvent was removed under reduced pressure, and the resulting residue was diluted with 20 mL H_2O then extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were washed with brine (1 x 15 mL), dried over $MgSO_4$, filtered, and concentrated. The resulting residue was purified by flash chromatography using a stepwise 0→40% ethyl acetate in hexanes gradient (10% step increases) to give **5** as a yellow crystalline solid (740 mg, 87% yield).

R*f*: 0.42 (3:7 ethyl acetate:hexanes); HRMS (ESI): expected 407.1733 ($C_{27}H_{33}NO_5Si$), found 408.1812 ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (dd, *J* = 8.2, 6.3 Hz, 4H), 7.63 – 7.53 (m, 2H), 7.24 (d, *J* = 8.0 Hz,

2H), 5.01 (d, *J* = 8.2 Hz, 1H), 4.62 (q, *J* = 7.9 Hz, 1H), 3.72 (s, 3H), 3.27 – 3.17 (m, 1H), 3.23 (s, 1H), 3.09 (dd, *J* = 13.7, 6.4 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (125, $CDCl_3$) δ 195.43, 171.94, 154.96, 141.41, 137.44, 135.95, 131.97, 130.25, 129.82, 129.39, 126.21, 82.78, 80.08 (2 overlapping signals), 54.20, 52.39, 38.44, 28.27.



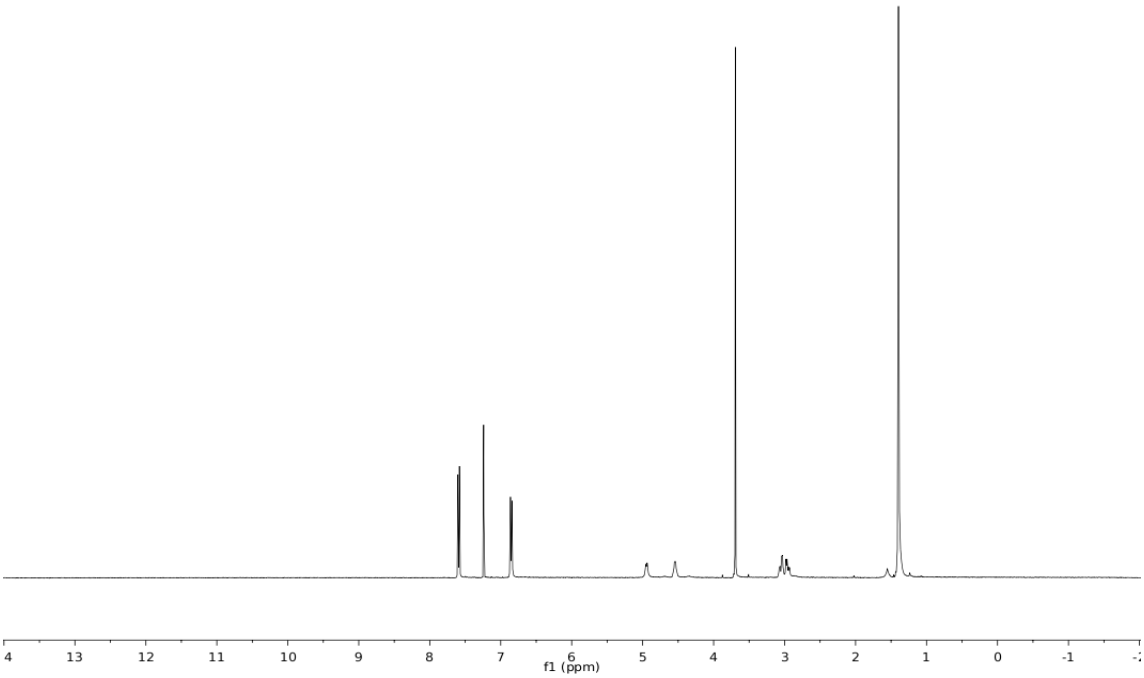
5 **BPKyne**

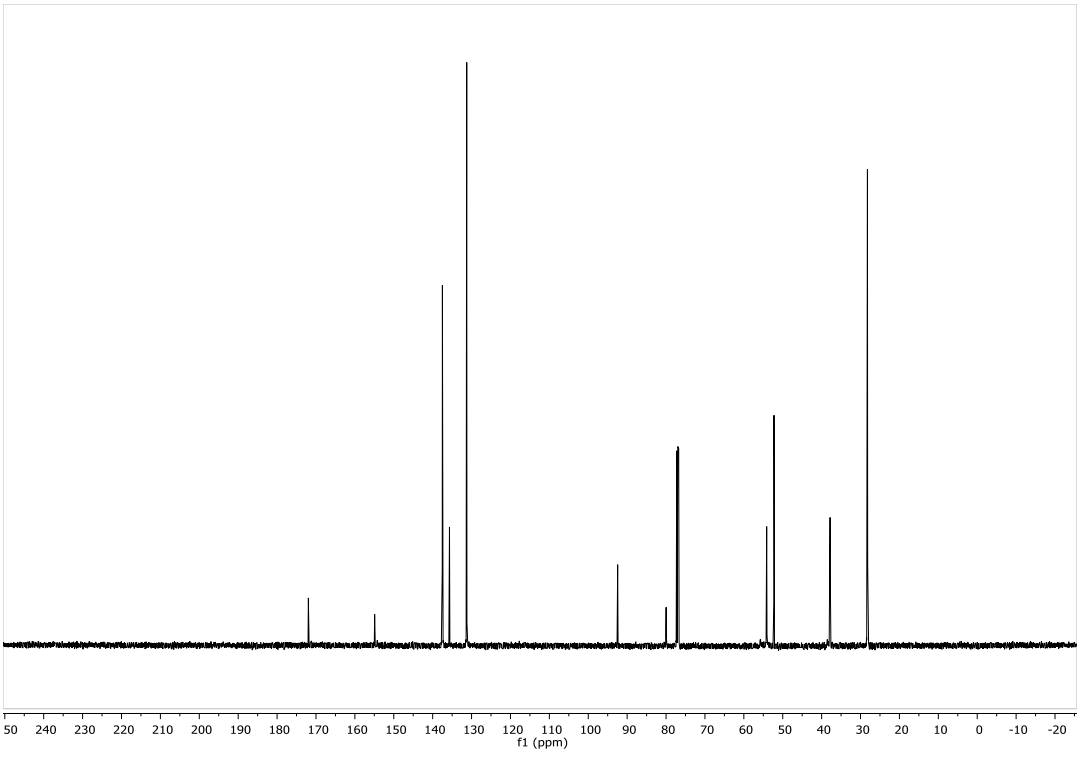
(S)-2-amino-3-(4-(4-ethynylbenzoyl)phenyl)propanoic acid (**BPKyne**). In a 25 mL round bottom flask, compound **5** (600 mg, 1.5 mmol) was dissolved in 4:1 methanol:THF. A solution of lithium hydroxide (71 mg, 3.0 mmol) dissolved in 1 mL H_2O was added and the reaction was stirred at room temperature for 2 h. The organic solvent was removed under reduced pressure, and the remaining volume was diluted with 15 mL H_2O. The solution was adjusted to pH = 1 with 1 M HCl, then extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were washed with brine (1 x 10 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was dried under vacuum to ensure removal of any trace methanol, then immediately moved forward without further purification. In a 25 mL round bottom flask under N_2, the intermediate was dissolved in 2 mL of anhydrous dioxane. The solution was cooled to 0 °C in an ice bath, and 4 M HCl in dioxane was added (0.5 mL, 2 mmol). The reaction was stirred at room temperature for 6 h. The solution was removed under reduced pressure, and the resulting residue was resuspendsed in 2 mL of anhydrous dioxane and treated a second time with 0.5 mL of 4 M HCl in dioxane. After stirring at room temperature for 6 hours, the reaction was concentrated to minimal volume under reduced pressure. Cold Et_2O was added, and the solid was isolated by vacuum filtration. The solid was washed with cold Et_2O (5 x 5 mL) and dried under vacuum to give the HCl salt of **BPKyne** as a light brown solid (424 mg, 86% yield).

HRMS (ESI): expected 293.1052 ($C_{18}H_{15}NO_3$), found 294.1130 ($M+H^+$); ^{1}H NMR (400 MHz, d_6-DMSO) δ 8.42 (s, 3H), 7.77 – 7.63 (m, 6H), 7.47 (d, *J* = 8.2 Hz, 2H), 4.50 (s, 1H), 4.29-4.22 (m, 1H), 3.25-3.15 (m, 2H); ^{13}C NMR (125 MHz, d_6-DMSO) δ194.72, 170.17, 140.63, 136.89, 135.56, 131.86, 129.82, 129.80, 125.85, 83.93, 82.69, 53.12, 35.68.

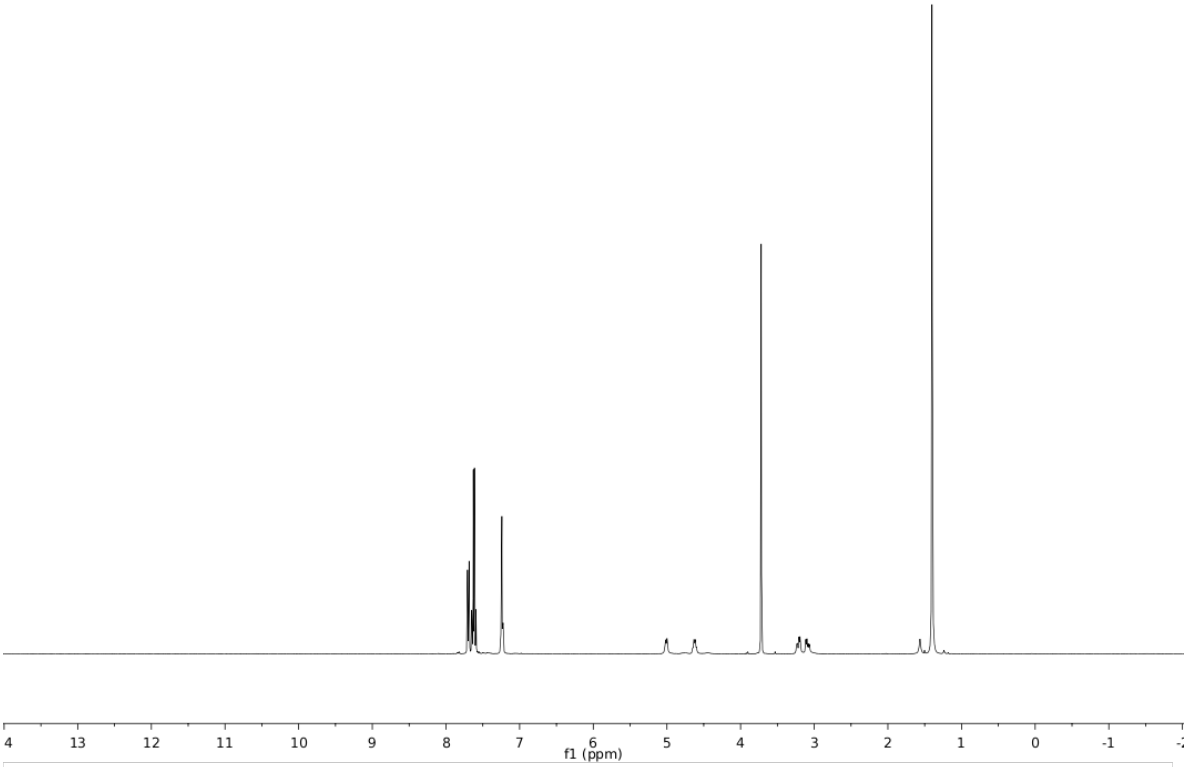
^{1}H and ^{13}C NMR Spectra

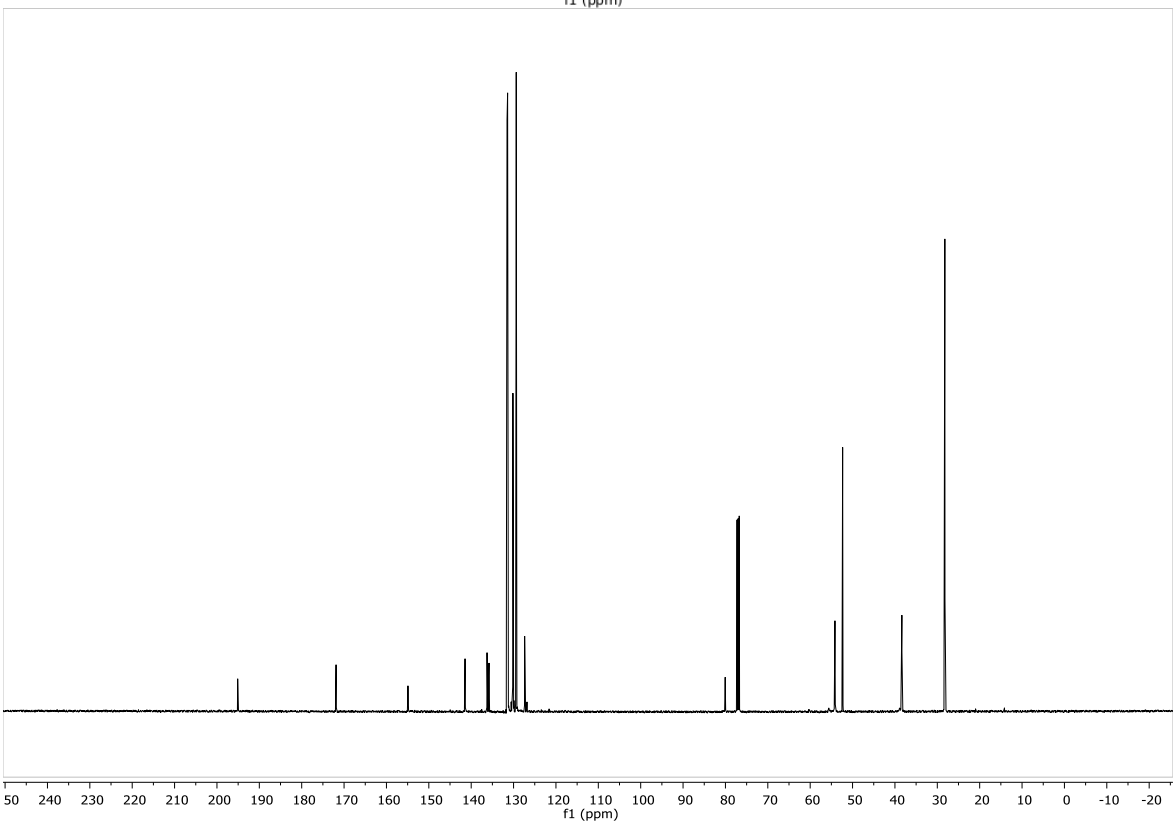
Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-iodophenyl)propanoate (**2**)



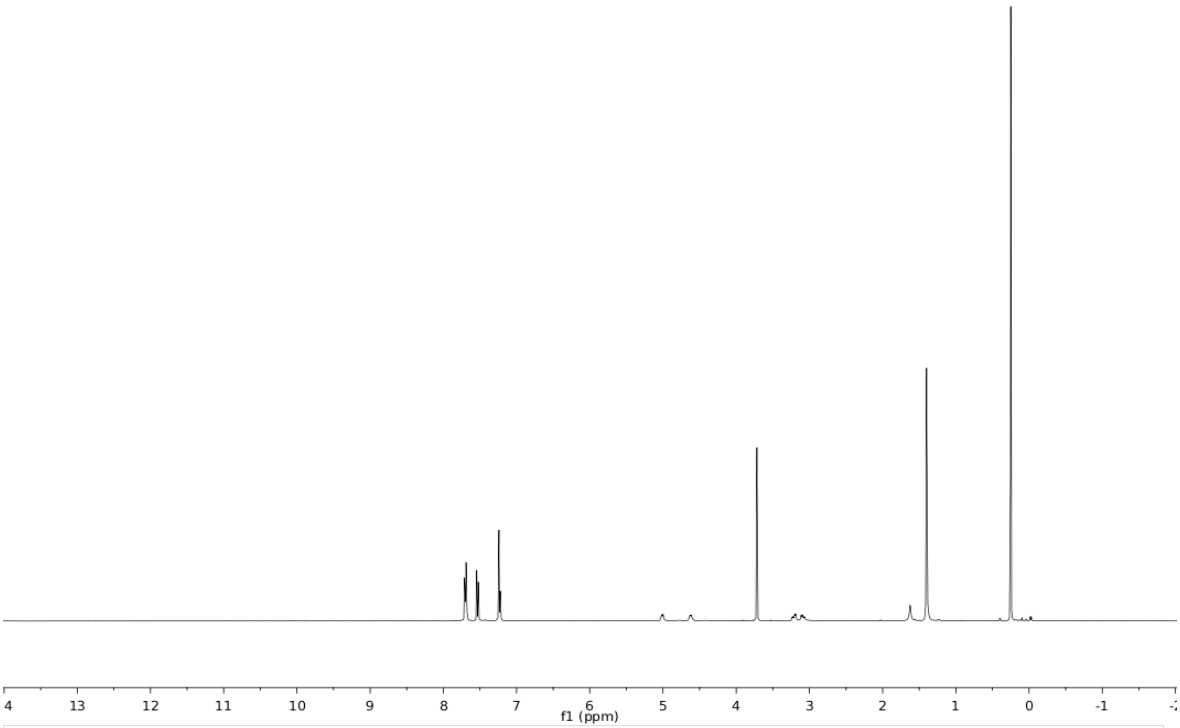


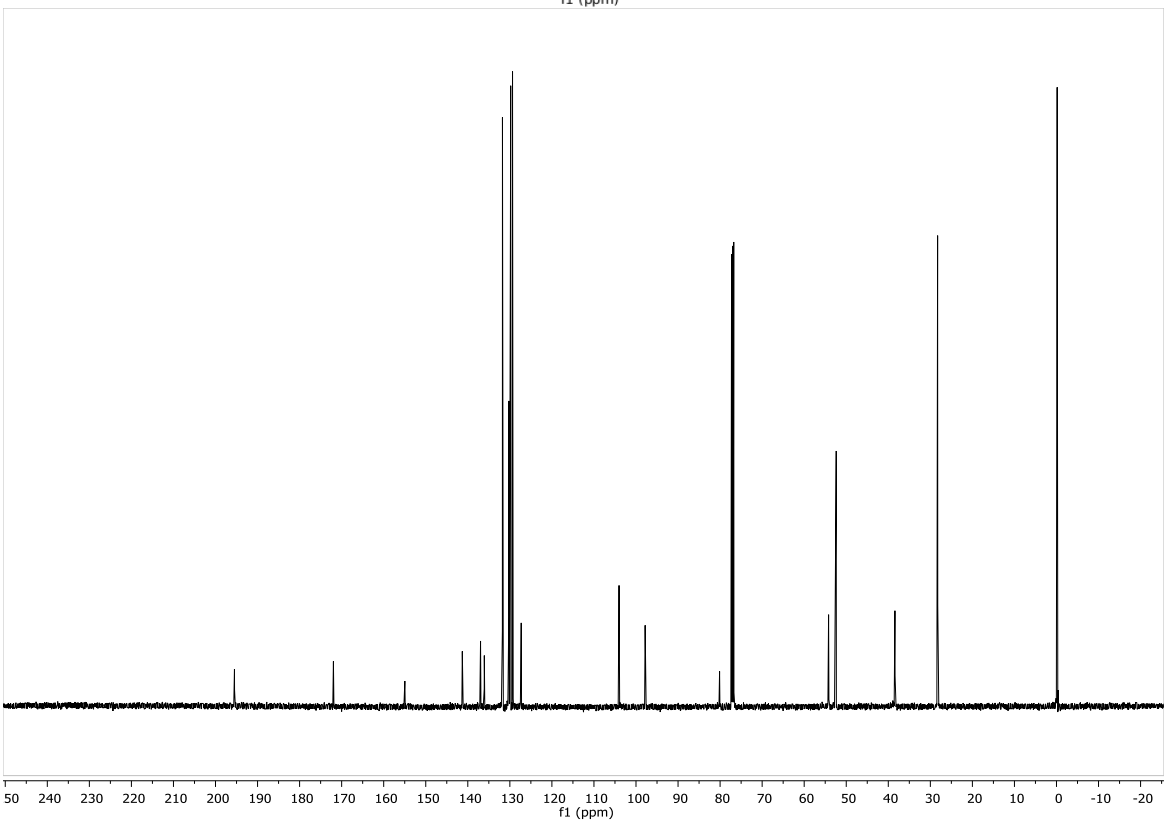
Methyl (S)-3-(4-(4-bromobenzoyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (**3**)



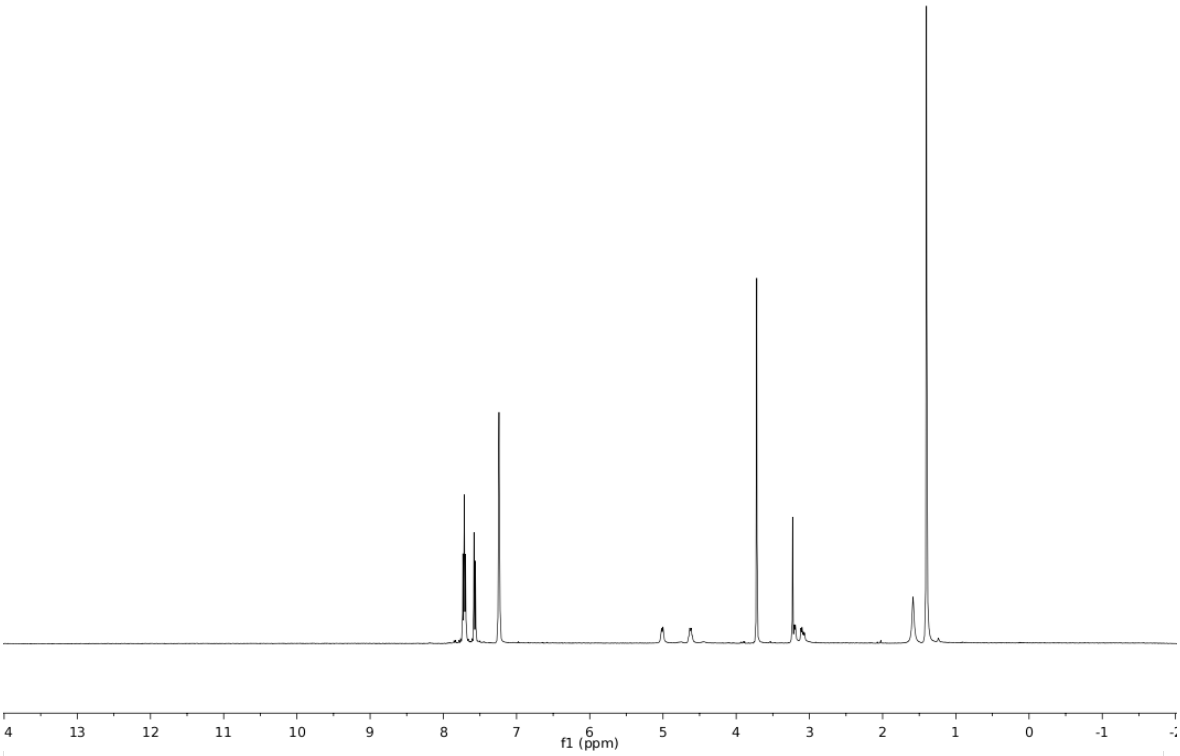


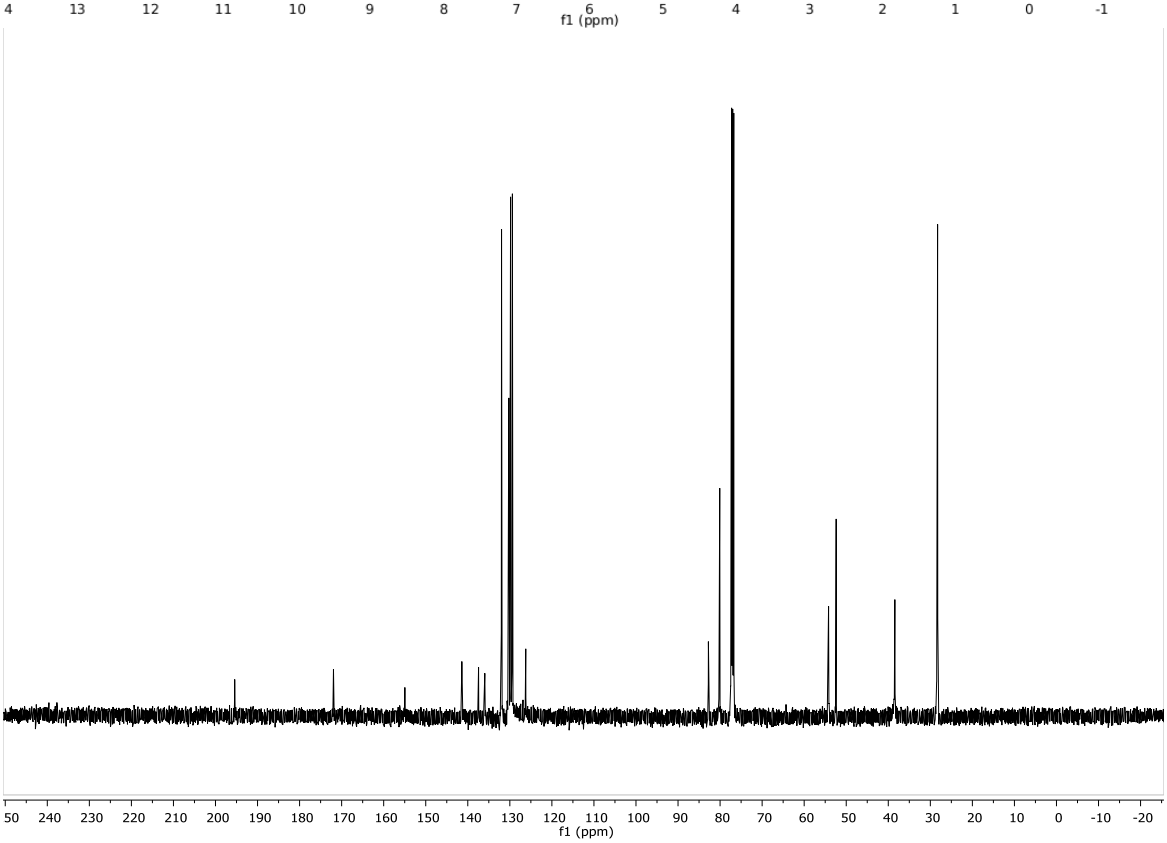
Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(4-((trimethylsilyl)ethynyl)benzoyl)phenyl)propanoate (**4**)



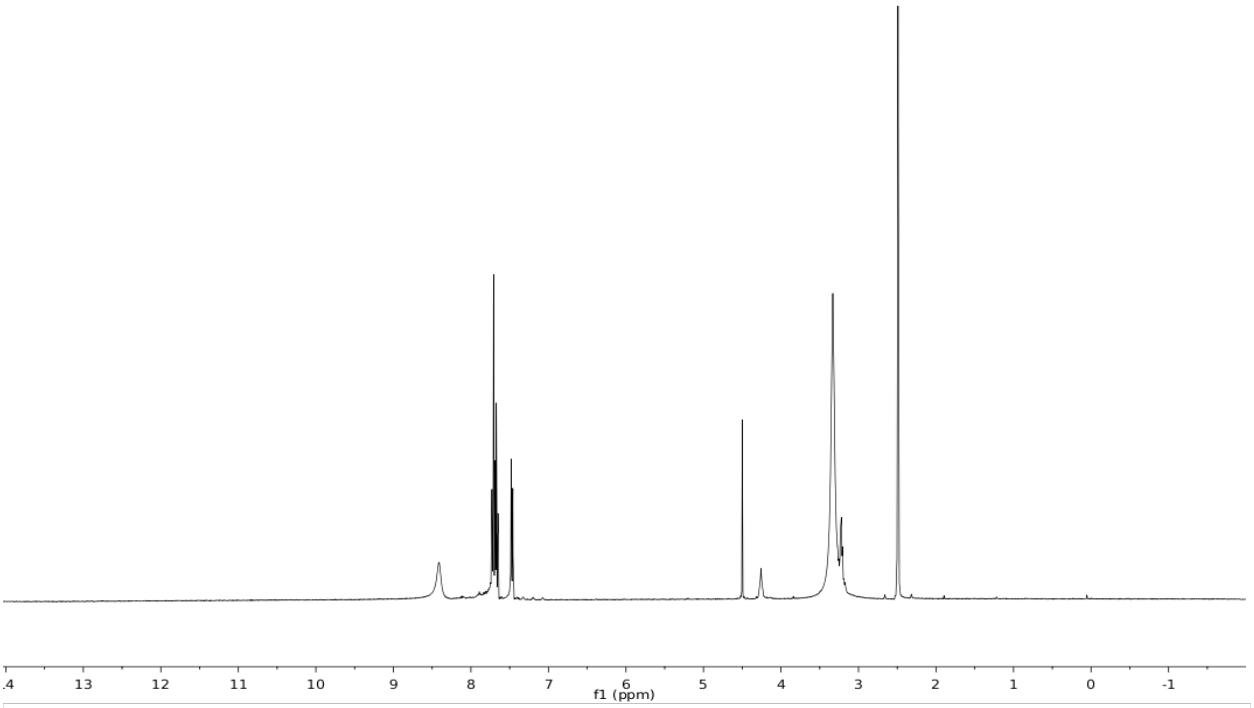


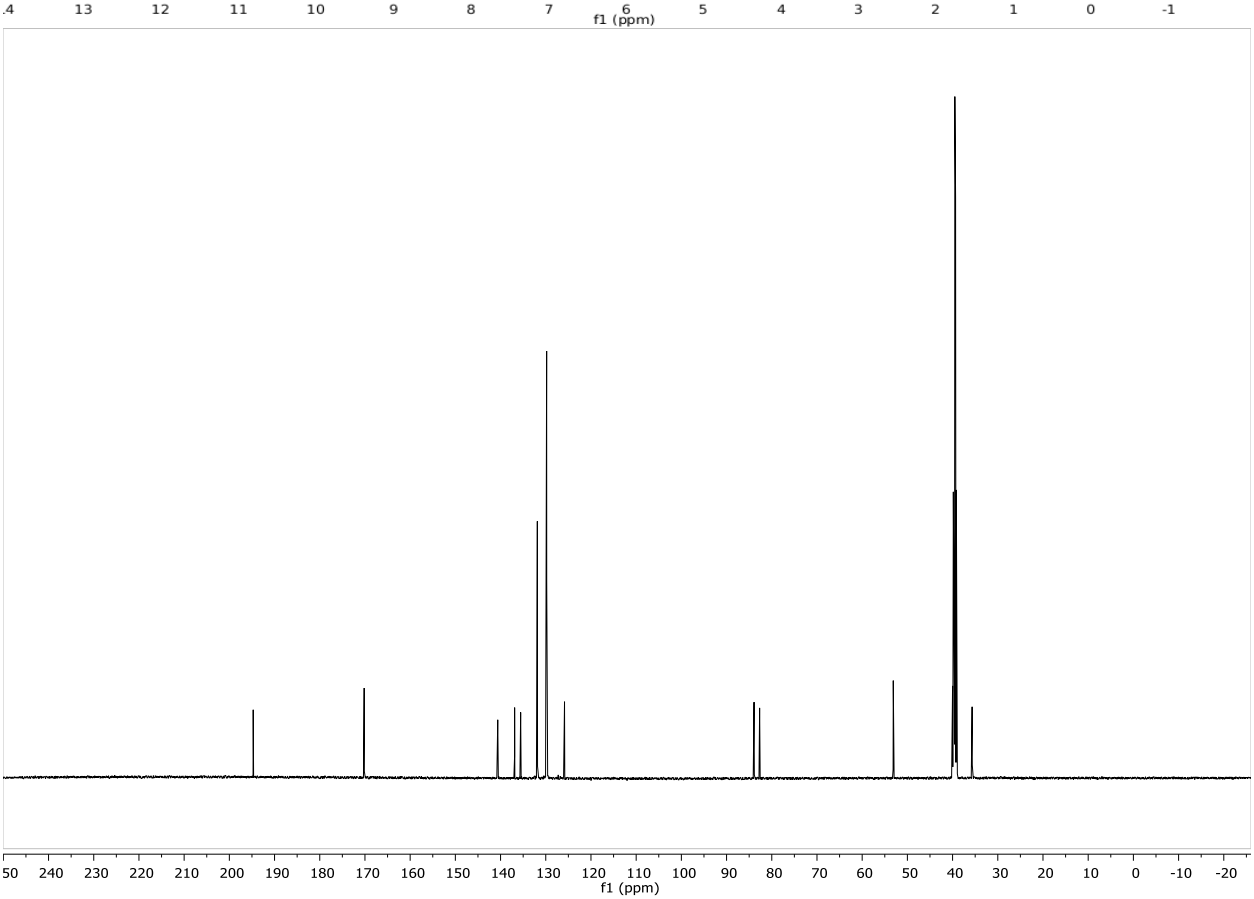
Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(4-ethynylbenzoyl)phenyl)propanoate (**5**)





(S)-2-amino-3-(4-(4-ethynylbenzoyl)phenyl)propanoic acid (**BPKyne**)





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[2] A. Ahlburg, A. T. Lindhardt, R. H. Taaning, A. E. Modvig, T. Skrydstrup, *Journal of Organic Chemistry* **2013**, *78*, 10310-10318.