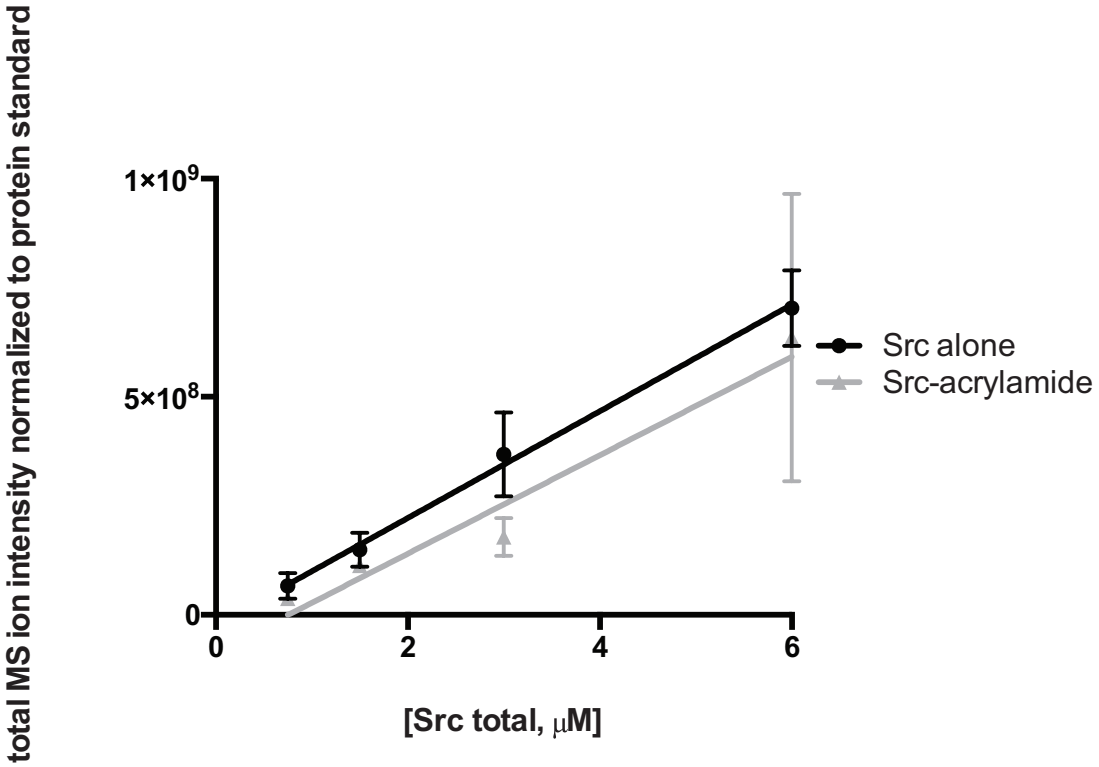


Figure S1 related to Figure 1 and 2. The line labeled "Src alone" represents the concentration of Src plotted against ion signal intensities normalized to 4 μM Histone H3 protein standard. "Src-acrylamide" corresponds to the same concentrations of Src reacted with crosslinker **2** to completion plotted against signal intensities normalized to 4 uM Histone H3 standard. Linear regression analysis by Prism 6 GraphPad software determined that the lines are not significantly different. Each point represents three replicates, and errors bars represent the standard error of the mean.

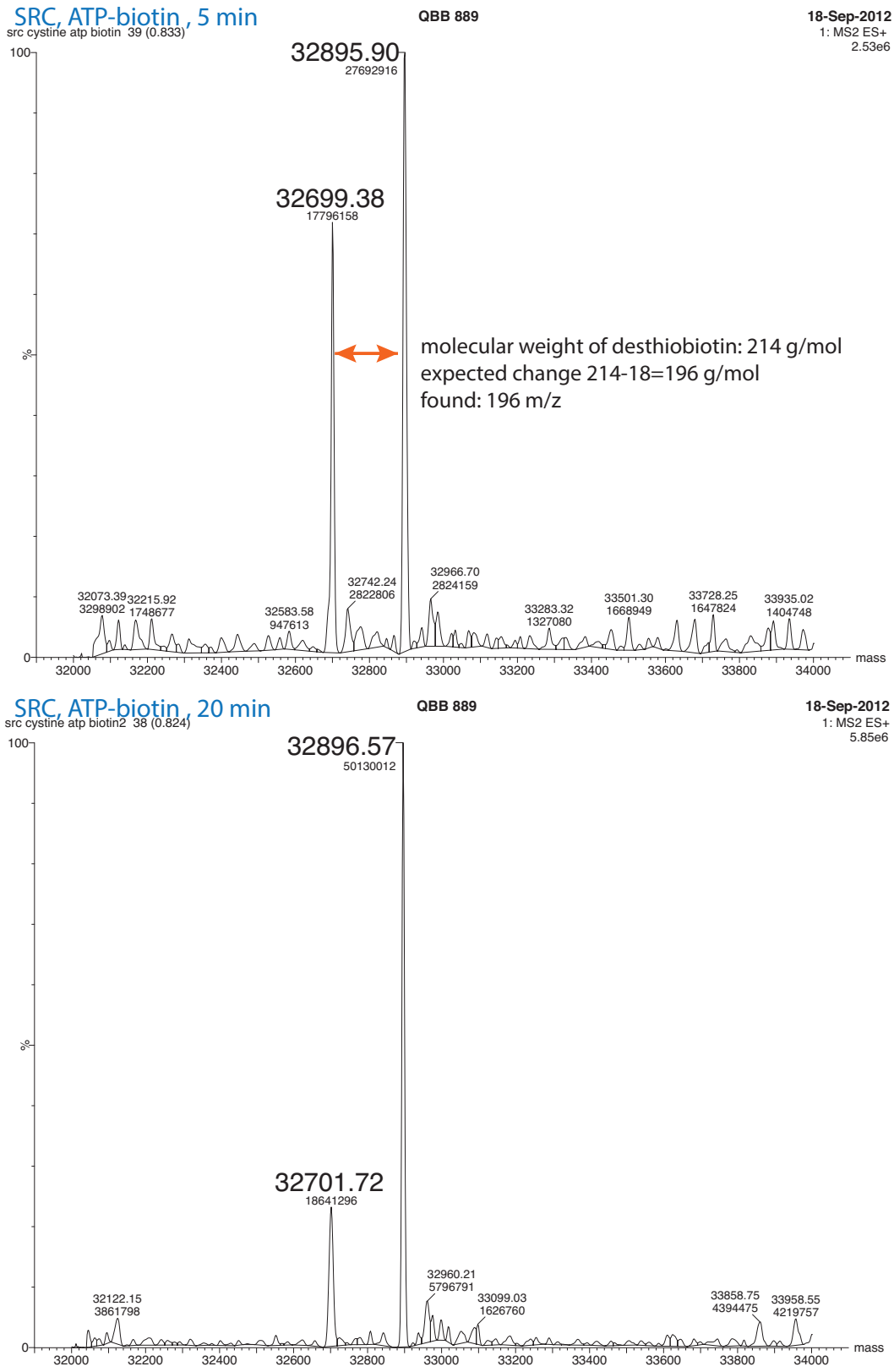


Figure S2 related to Figure 2C. LC-MS spectra showing addition of desthiobiotin to c-Src in 20 mins. Expected molecular weight of c-Src is 32690 Da, molecular weight calculated by MaxEnt deconvolution is 32690-32700 Da.

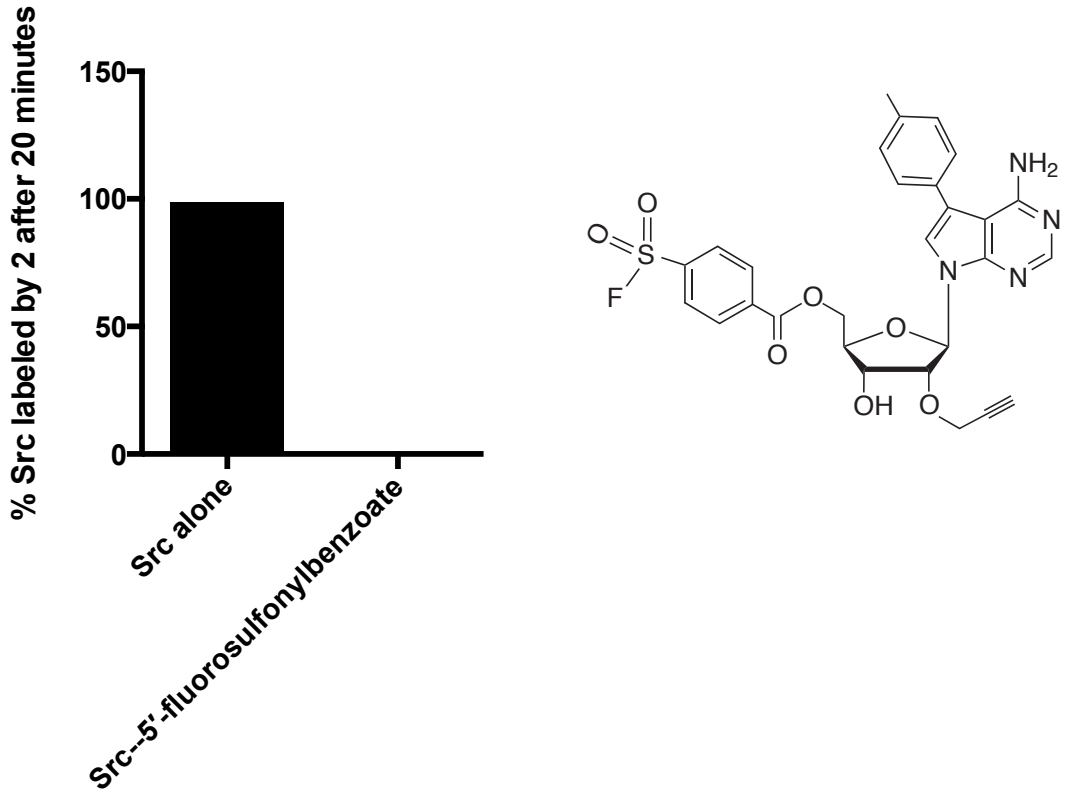


Figure S3 related to Figure 2C. Competition between 5'-fluorosulfonylbenzoate and crosslinker **2**. Src was treated with a 5'-fluorosulfonylbenzoate based Src inhibitor which targets the catalytic lysine (structure above)(Gushwa et al., 2012). The after the reaction between the inhibitor and Src had gone to competition, crosslinker **2** was added and allowed to react for 20 minutes. The mixture was analyzed by LC/MS, masses were determined by MaxEnt tool within Waters MassLynx software package. The crosslinker was unable to modify the kinase. This was preformed in triplicate.

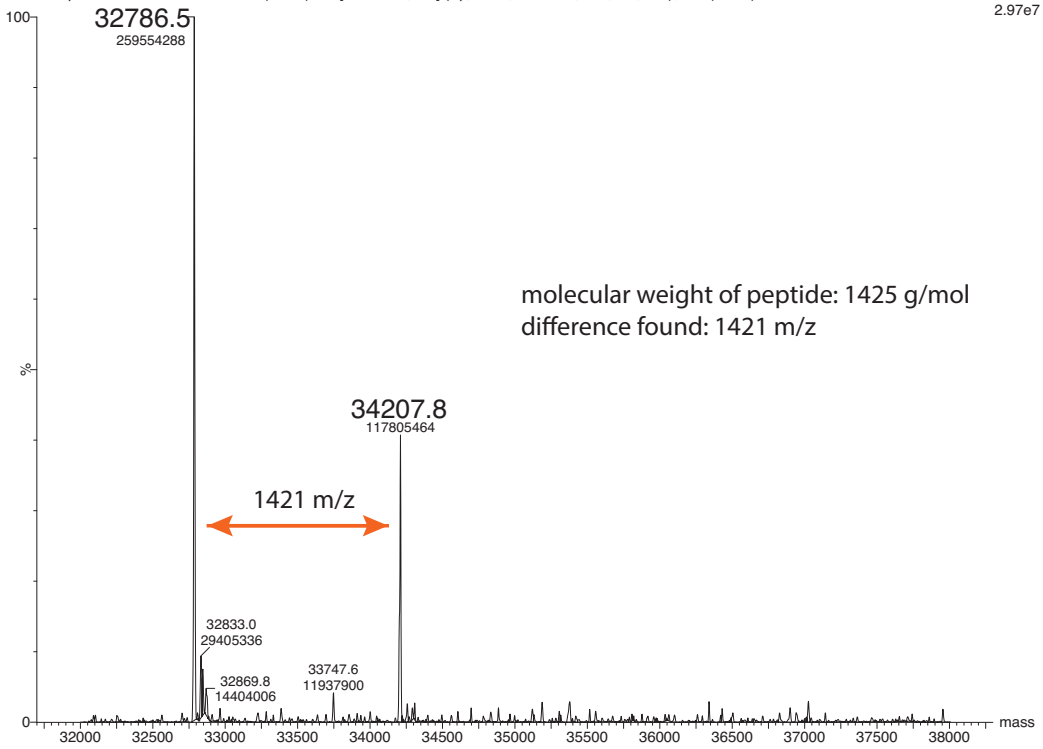
SRC, crosslinker **2**, Cys-Srctide, 80 mins

QBB 889

11-Aug-2013

081113 atpma 2 20 srctide 80 mins 35 (0.799) M1 [Ev-62503,It14] (Sp,0.750,726:1630,2.00,L33,R33); Cm (16:53)

1: MS2 ES+
2.97e7



molecular weight of peptide: 1425 g/mol
difference found: 1421 m/z

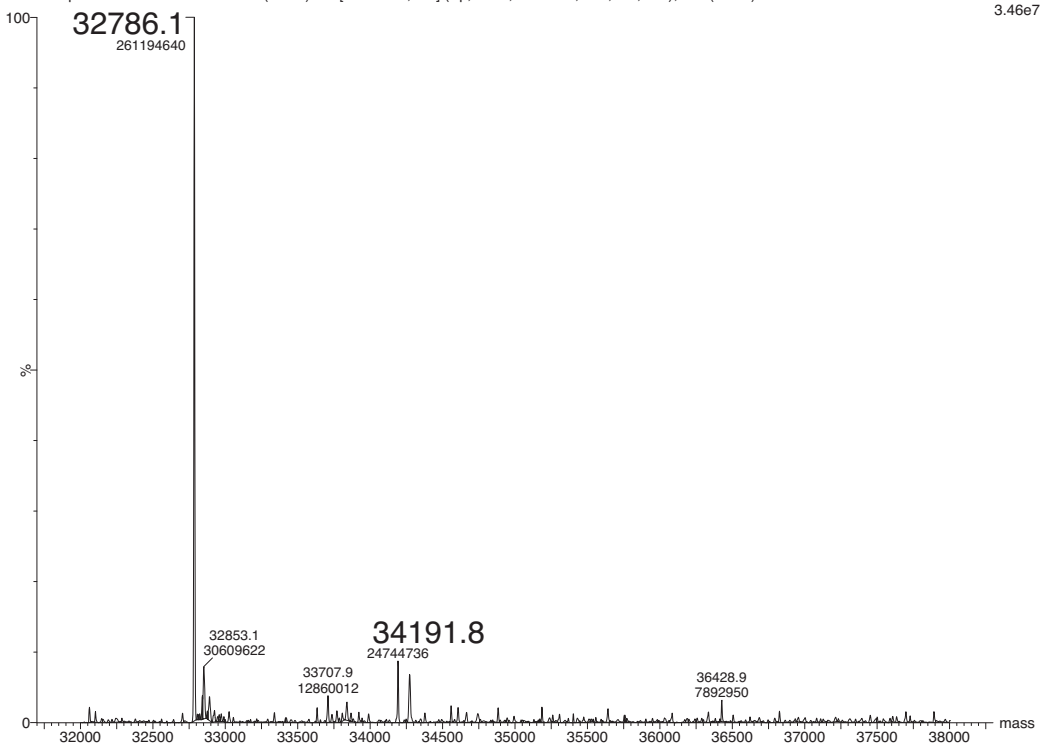
SRC, crosslinker **2**, Cys-Akttide, 80 mins

QBB 889

12-Aug-2013

081213 atpma 2 20 akttide 80 mins 35 (0.799) M1 [Ev-61664,It15] (Sp,0.750,725:1614,2.00,L33,R33); Cm (11:52)

1: MS2 ES+
3.46e7



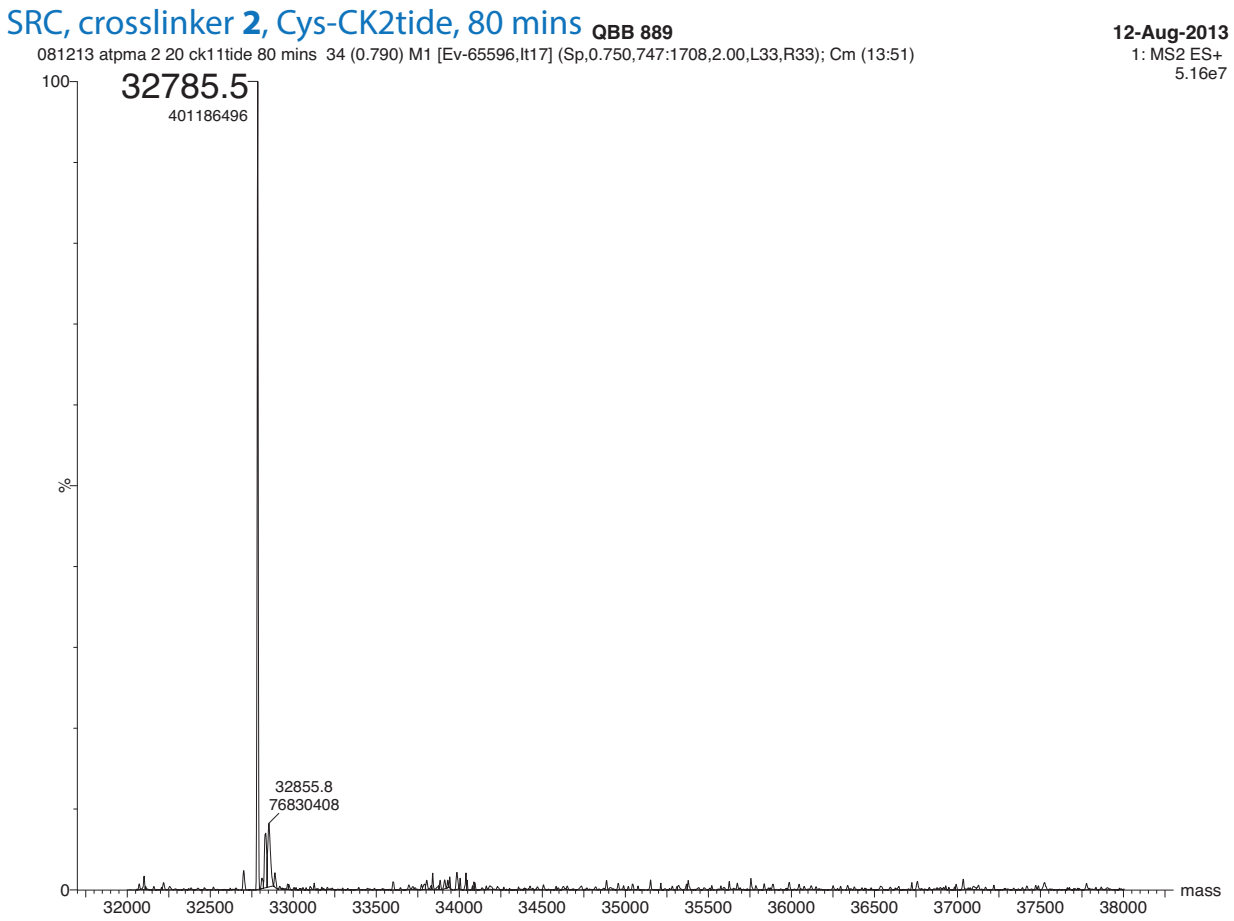
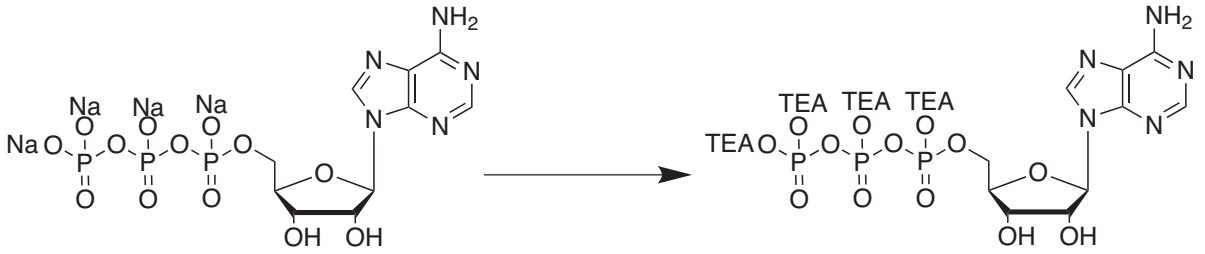


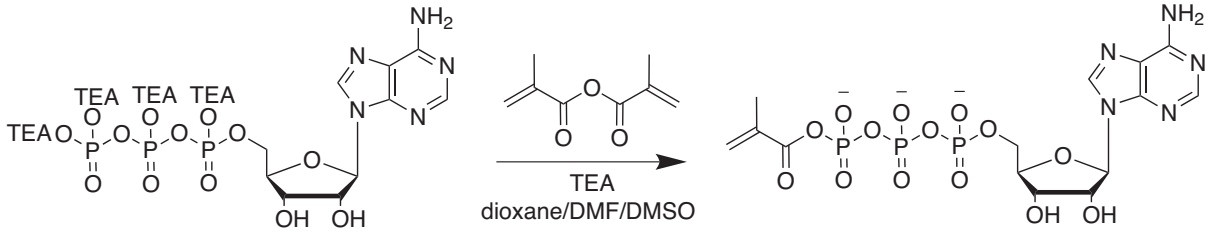
Figure S4 related to Figure 3. Representative LC/MS data. Quantification of these spectra was used to generate Figure 3. Masses were determined by MaxEnt tool within Waters MassLynx software package.

Supplementary Experimental Procedures

Unless noted, chemical reagents and solvents were used without further purification from commercial sources. Reaction mixtures were magnetically stirred. Thin layer chromatography was preformed on Merck pre-coated silica gel F-254 plates (0.25 mm). Concentration in vacuo was generally preformed using a Buchi rotary evaporator. Nuclear magnetic resonance spectra was recorded on a Varian 400 MHz instrument. Proton NMR spectra were recorded in ppm using the residual solvent signal as an internal standard: D_20 (4.416 ppm). Carbon NMR were recorded in ppm. All compounds are greater than 95% pure based on LCMS. Mass spectrometry (ESI-MS) was carried out using a Waters Acquity UPLC/ESI-TQD with a 2.1 x 50 mm Acquity UPLC BEH C18 column.



Preparation of ATP-triethylammonium salt. Dowex 50W resin (4.0g) was incubated in saturated triethylamine (50 mL) for 1 hour. The liquid was removed and the beads were washed with H_20 (pH=9.0) (2 x 5 mL). ATP (4.0 g) dissolved in H_20 (5 mL) was poured over beads and incubated for 30 mins. The flow through was removed and saved. ATP-TEA was eluted with H_20 (2 x 5 mL). The flow through and elution fractions were frozen and lyophilized.

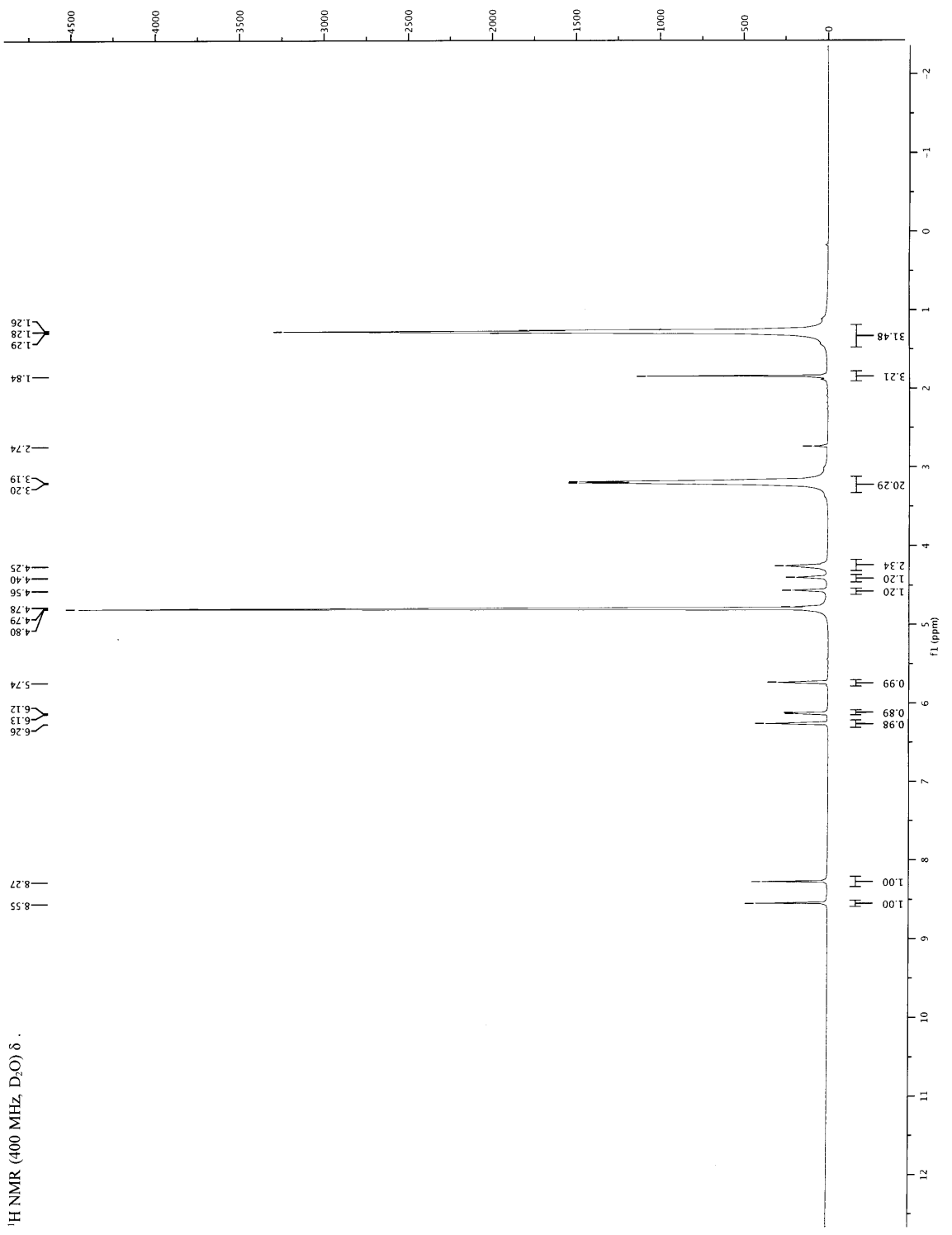


Synthesis of 2. This synthesis was adapted from the synthesis of ATP-Biotin (Patricelli et al., 2007). To a solution of methacrylic anhydride (74 µL, 0.50 mmol) in 3 mL of a dioxane/DMF/DMSO mixture (1:1:1) was added triethylamine (50 µL) and a solution of ATP triethylammonium salt (0.40 g, 0.49 mmol) in anhydrous DMSO (3 mL) and stirred overnight at room temperature. The reaction was quenched with water (4 mL) and the solution was quickly extracted with ethyl acetate (3 x 4 mL). The aqueous layer was immediately frozen and lyophilized. The yellow solid was suspended in water and transferred to a pre-equilibrated 30 x 250mm preparative C18, 5 μM column (Waters), and purified by HPLC with 100% water over 25 minutes. Fractions containing product were frozen and lyophilized to yield 0.224 g of **2** (56%) as the tris(triethylammonium) salt. ^{1}H NMR (400 MHz, D_2O) δ 8.55 (s, 1H), 8.27 (s, 1H), 6.26 (s, 1H), 6.13 (d, J = 5.9,

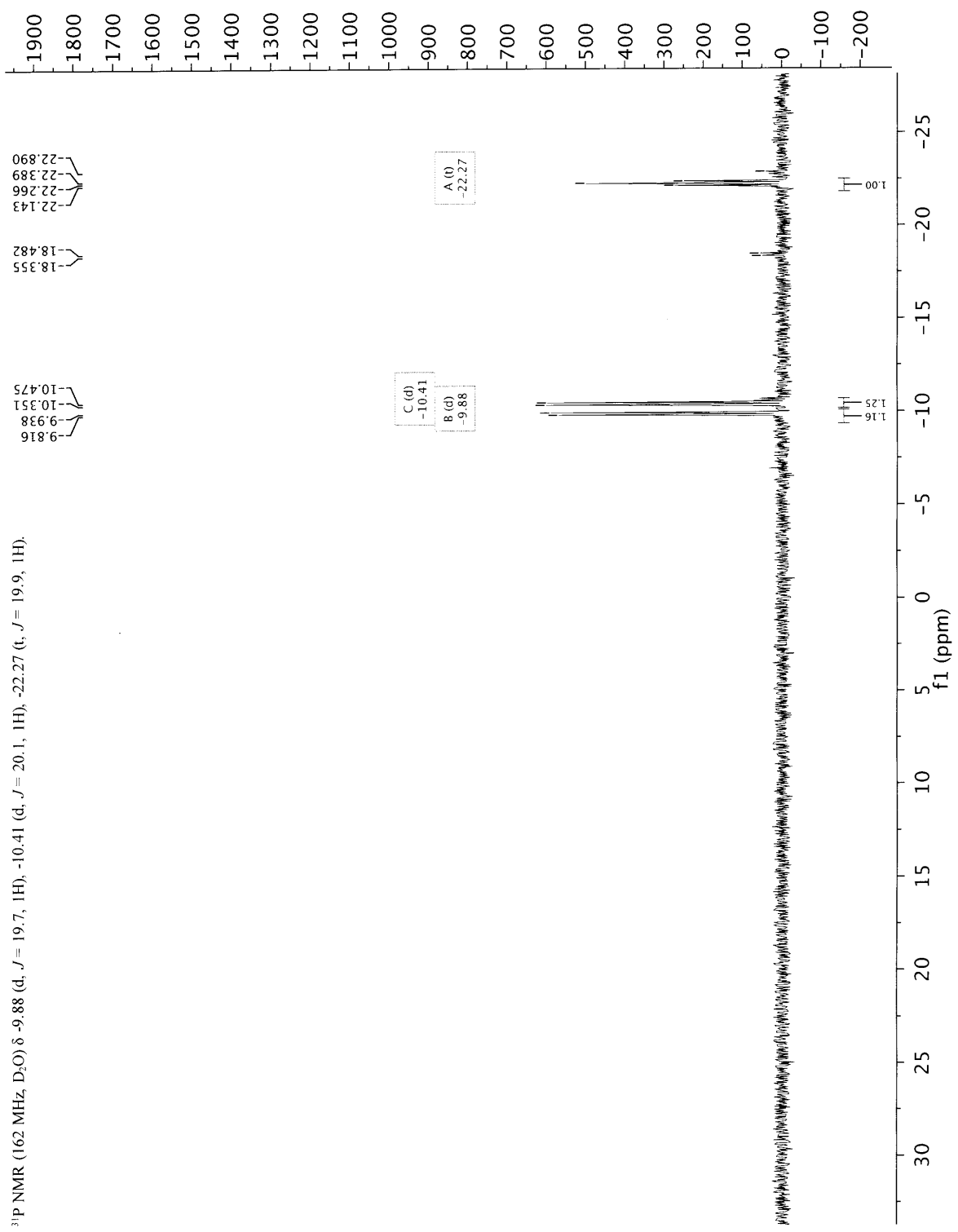
1H), 5.74 (s, 1H), 4.56 (d, *J* = 3.3, 1H), 4.40 (s, 1H), 4.24 (d, *J* = 11.8, 3H), 3.20 (d, *J* = 6.9, 20H), 1.87 (d, *J* = 17.6, 3H), 1.50 – 1.05 (m, 32H). ^{13}C NMR (100 MHz, D_2O) δ 148.66, 141.35, 138.07, 135.42, 129.63, 125.14, 87.39, 84.24, 74.76, 70.43, 65.34, 46.78, 38.87, 17.97, 8.36. MS calculated for $C_{14}H_{20}N_5O_{14}P_3$ 575.02, found 575.10.

Expression and Purification of c-Src. Hexahistidine-tagged recombinant chicken c-Src (residues 251-533) was prepared as previously described (Seeliger et al., 2005) with the modifications used by Blair et al(Blair et al., 2007). The hexahistidine tag was removed with AcTev protease (Invitrogen) and concentrations were determined spectrophotometrically at 280 nm using an extinction coefficient of 52,370 M-1cm-1. Protein aliquots were stored at -80 °C in 50 mM Tris (pH 8), 100 mM NaCl, and 1 mM DTT.

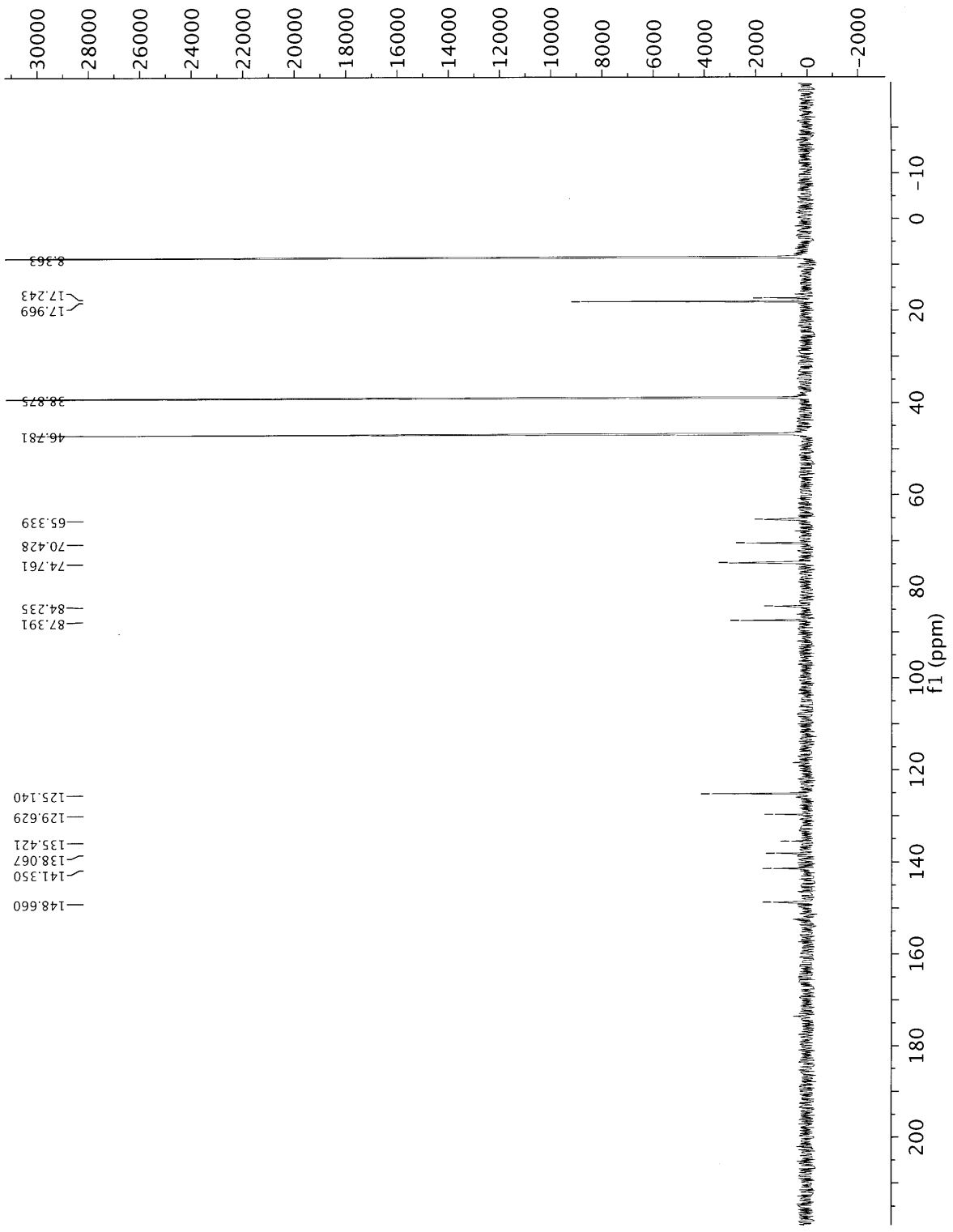
Statistical analysis. Data are presented as mean ± SEM with n=3 unless otherwise indicated. Statistical analysis was performed using Prism 6 software for either linear regression analysis, one-way ANOVA for comparisons or Student t-test for comparison depending on the number of groups.



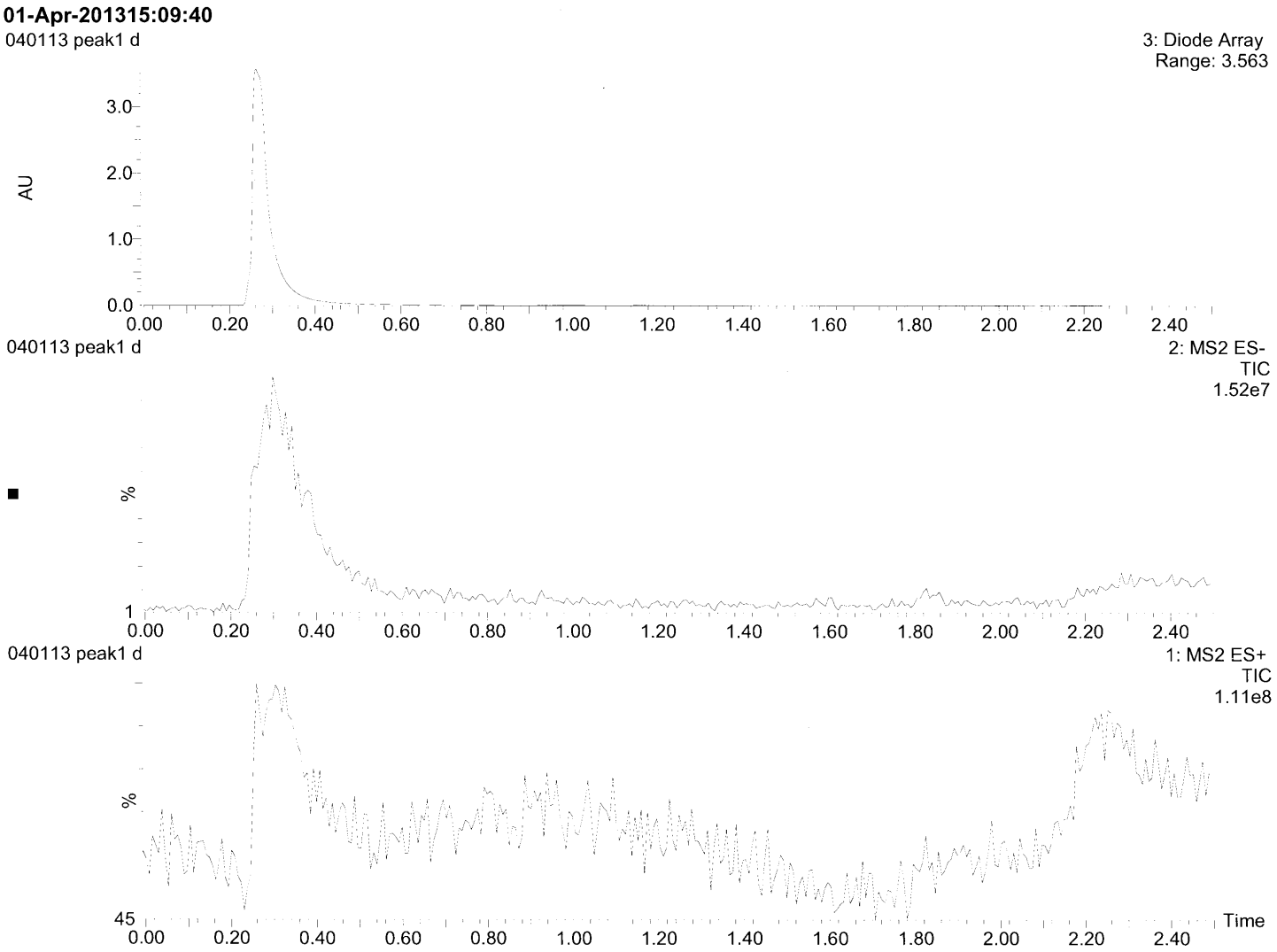
^{1}H NMR of compound **2**



^{31}P NMR spectrum of compound **2**



^{13}C NMR spectrum of compound **2**


01-Apr-201315:09:40
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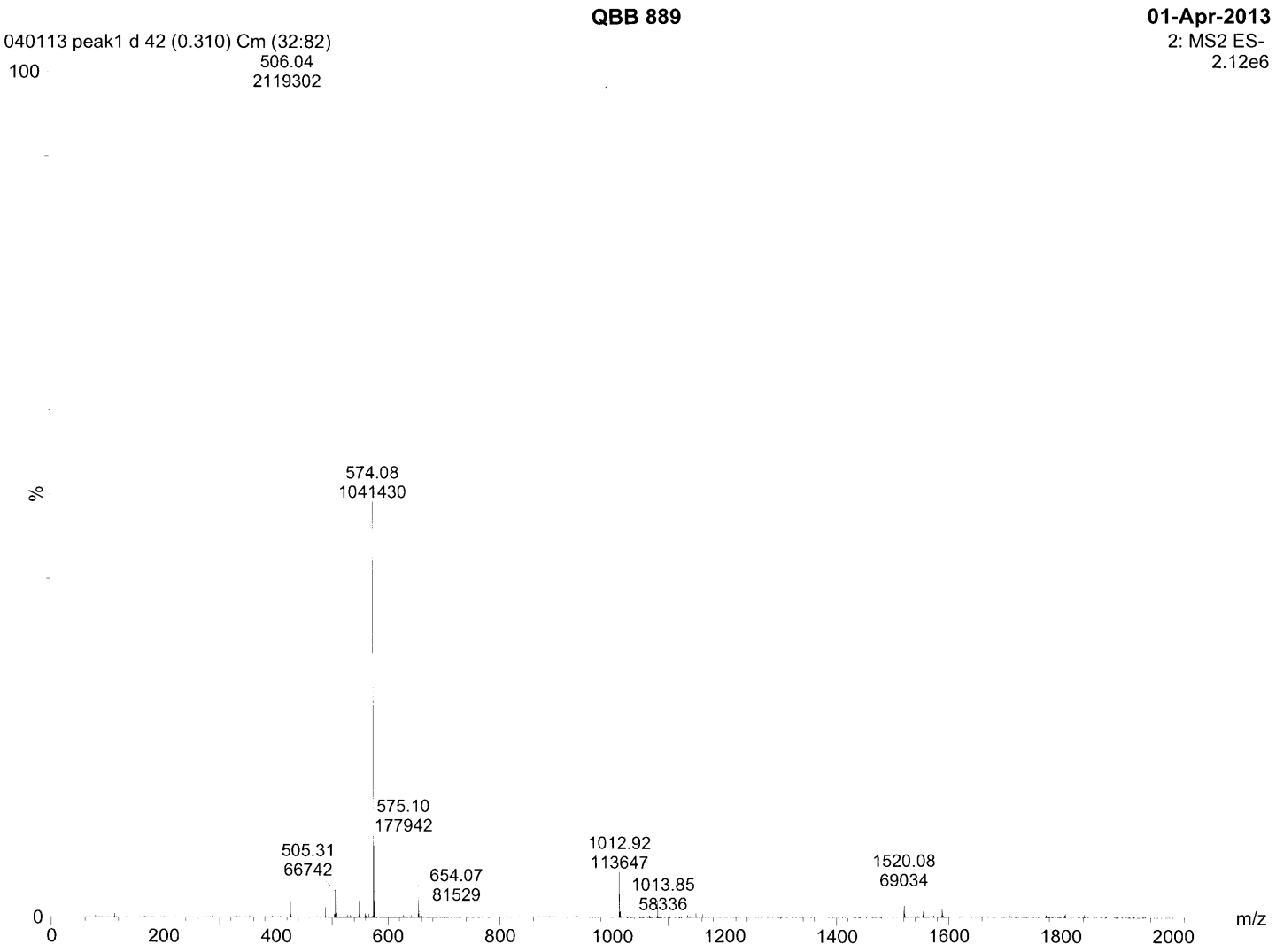
Range: 3.563
AU
3.0
2.0
1.0
0.0
0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40
040113 peak1 d
2: MS2 ES-
TIC
1.52e7
%
1
0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40
040113 peak1 d
1: MS2 ES+
TIC
1.11e8
%
45
Time
0.00 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40

LCMS chromograph and spectrum of compound **2**



Supplemental References

Blair, J.A., Rauh, D., Kung, C., Yun, C.-H., Fan, Q.-W., Rode, H., Zhang, C., Eck, M.J., Weiss, W.A., and Shokat, K.M. (2007). Structure-guided development of affinity probes for tyrosine kinases using chemical genetics. Nat Chem Biol *3*, 229–238.

Seeliger, M.A., Young, M., Henderson, M.N., Pellicena, P., King, D.S., Falick, A.M., and Kuriyan, J. (2005). High yield bacterial expression of active c-Abl and c-Src tyrosine kinases. Protein Sci. *14*, 3135–3139.

Gushwa, N.N., Kang, S., Chen, J., and Taunton, J. (2012). Selective Targeting of Distinct Active Site Nucleophiles by Irreversible Src-Family Kinase Inhibitors. J. Am. Chem. Soc. *134*, 20214–20217.