Supporting Information

Small molecule probes that target Abl kinase

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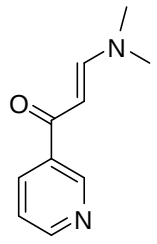
Contents:

1. General Information

All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. All reaction requiring anhydrous conditions were carried out under argon or nitrogen atmosphere using oven-dried glassware. HPLC-grade solvents were used for all reactions. Reaction progress was monitored by TCL on precoated silica plates (Merck 60 F_{254}, 0.25 µm) and spots were visualized by UV or iodine stain. Flash column chromatography was carried out using Merck 60 $F_{254,}$ 0.040-0.063 µm silica gel. All NMR spectra (^{1}H-NMR, ^{13}C-NMR) were recorded on a Bruker 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to appropriate internal standards or residual solvent peaks ($CDCl_3$ = 7.26 ppm, CD_3OD = 3.31 ppm, DMSO-d6 = 2.50 ppm). The following abbreviations were used in reporting spectra, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets. All enzymes were expressed in *E.coli* strain BL21-DE3 and purified as described previously.[1] They include Abl (human c-Abl, residues 229-512), Csk (human c-Src tyrosine kinase residues 1-450), Src (chicken c-Src residues 251-533) and ERK2 (human mitogen activated protein kinase 1) All analytical HPLC were carried out on Shimadzu LCMS (IT-TOF) system or Shimadzu LCMS-2010EV system equipped with an autosampler using reverse-phase Phenomenex Luna 5 µm $C_{18(2)}$ 100 Å 50 × 3 mm columns. Water with 0.1% TFA and acetonitrile with 0.1% TFA were used as eluents and the flow rate was 0.6 ml/min. For enzyme inhibition and IC_{50} measurements, Tecan microplate reader (Multimode Reader, Infinite®200) in luminescence mode with i-control™ software was used. Fluorescence scanning of the SDS-PAGE gels was carried out with Typhoon 9200 fluorescence gel scanner (Amersham Biosciences), and where applicable, the bands were quantified with ImageQuant 3.3 (Molecular Dynamics) software installed on the scanner

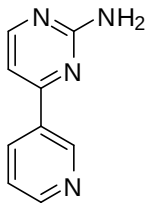
2. Synthetic procedures



(1)

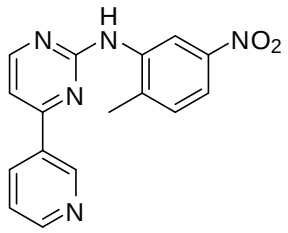
3-(Dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one (1): A 250 mL round-bottomed flask was charged with 6.4 mL (58.3 mmol) of 3-acetylpyridine, 9.4 mL (70 mmol, 1.2 eq.) of N,N-dimethylformamide dimethylacetal and 50 mL ethanol. The reaction mixture was refluxed overnight, cooled to room temperature and the solvent was removed under reduced pressure. To

the crude residue approximately 50 mL of diethyl ether was added and cooled to 0 ^{o}C. The product 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one was subsequently filtered off as yellow crystals (7.18 g, yield = 70%) and used in the subsequent steps without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.92 (3H, s), 3.14 (3H, s), 5.65 (1H, d, *J* = 12.33 Hz), 7.32 (1H, m), 7.81 (1H, d, *J* = 12.33 Hz), 8.15 (1H, d, *J* = 9.18 Hz), 8.62 (1H, d, *J* = 7.38 Hz), 9.04 (1H,s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 38.01, 45.85, 92.50, 123.93, 135.73, 136.30, 149.51, 152.05, 155.36, 187.02. ESI-MS (m/z) calculated = 177.102 [M+H^{+}], (m/z) observed = 177.099 [M+H^{+}]



(2)

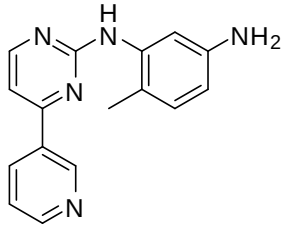
4-(Pyridin-3-yl) pyrimidin-2-amine (2): 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one (2.64 g, 15 mmol) and guanidinium hydrochloride (1.5 g, 15.75 mmol, 1.05 eq.) were mixed in 25 mL of 2-propanol. To the suspension was added 0.7 g of NaOH (17.5 mmol, 1.17 eq.) and the mixture was refluxed for 18 h. The reaction was then cooled to 0 ^{o}C and the precipitate was filtered off, suspended in water, filtered off once more and washed with 2-propanol and diethyl ether. The residue was dried in oven at 60 to 70 ^{o}C and the desired product was obtained as a white powder (1.68 g, yield = 65%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 6.77 (2H, s), 7.19 (1H, d, *J* = 5.1 Hz), 7.52 (1H, m), 8.34 (1H, d, *J* = 5.1 Hz), 8.39 (1H, d, *J* = 7.89 Hz), 8.68 (1H, d, *J* = 6.57 Hz), 9.22 (1H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 106.01, 123.71, 132.46, 134.11, 147.95, 151.10, 159.35, 161.56, 163.79. ESI-MS (m/z) calculated = 173.0827 (M+H)$^{+}$, (m/z) observed = (M+H)$^{+}$



(3)

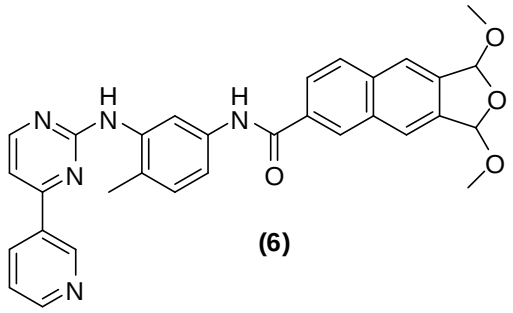
N-(2-methyl-5-nitrophenyl)-4-(pyridin-3-yl) pyrimidin-2-amine (3): 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one **3** (0.456 g, 2.65 mmol, 1.1 eq.), CuI (0.12 g, 0.6 mmol, 0.25 eq.) and anhydrous K_2CO_3 (0.67 g, 4.82 mmol, 2 eq.) were added to a sealed tube (50 mL) equipped with a rubber septum and a magnetic stirring bar. The tube was filled with N_2 gas and subsequently was added a mixture of 2-bromo-1-methyl-4-nitrobenzene (0.518 g, 2.41 mmol, 1 eq.) and DMEDA (0.064 mL, 0.6 mmol, 0.25 eq.) in anhydrous dioxane (20 mL) by syringe at

room temperature. The rubber septum was quickly replaced with a Teflon screw cap and the reaction mixture was stirred at 120 ^{o}C for 20 h. The reaction was cooled to room temperature and concentrated ammonia (10 mL) and a saturated solution of NaCl (40 mL) were added then extracted with ethyl acetate (5 × 40 mL). The organic layers were dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by column chromatography on silica gel to give 0.62 g (yield = 83.7%) of **4** as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (s, 1H), 9.27 (s, 1H), 8.75 (d, *J* = 4.8 Hz, 1H), 8.59 (d, *J* = 5.3 Hz, 1H), 8.54 (dt, 1H), 7.87 (dd, 1H), 7.52 – 7.48 (m, 1H), 7.36 (d, *J* = 8.2 Hz, 1H), 7.32 (d, *J* = 5.1 Hz, 1H), 7.18 (s, 1H), 2.48 (s, 3H). ESI-MS (m/z) calculated = 308.114 [M+H$^+$], (m/z) observed = 308.120 [M+H$^+$]



(4)

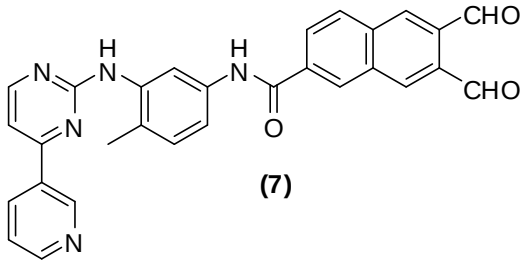
6-Methyl-N1-(4-(pyridin-3-yl) pyrimidin-2-yl) benzene-1,3-diamine (4): To a degassed solution of 0.4 g (1.302 mmol) of 3-(dimethylamino)-1-(pyridin-3-yl) prop-2-en-1-one in 50 mL of ethyl acetate was added 10% Pd/C (0.04 g). The reaction was stirred under a hydrogen atmosphere (balloon) for 3 h until complete disappearance of the starting material. The reaction was then filtered through celite, concentrated and the product was obtained as a yellow solid (0.36 g, quantitative yield) and used in the subsequent steps without further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.25 (3H, s), 6.40 (1H, m), 6.98 (1H, s), 7.01 (1H, m), 7.15 (1H, d, *J* = 5.1 Hz), 7.41 (1H, m), 7.60 (1H, s), 8.33 (1H, m), 8.49 (1H, d, *J* = 5.1 Hz), 8.71 (1H, m), 9.27 (1H,s). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.69 (d, *J* = 1.5 Hz, 1H), 8.68 (d, *J* = 1.6 Hz, 1H), 8.46 (d, *J* = 5.1 Hz, 1H), 8.41 (dt, 1H), 7.56 – 7.51 (m, 1H), 7.36 (d, *J* = 5.1 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 6.78 (s, 1H), 6.33 (dd, 1H), 4.85 (s, 2H), 2.05(s, 3H). ESI-MS (m/z) calculated = 278.140 [M+H$^+$], (m/z) observed = 278.101 [M+H$^+$]



(6)

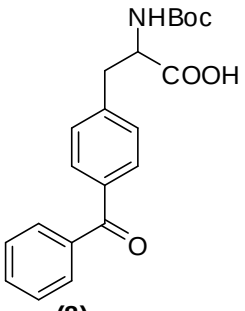
1,3-Dimethoxy-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-1,3-dihydronaphtho[2,3-c]furan-6-carboxamide (6): To a solution of 1,3-dimethoxy-1,3-dihydronaphtho[2,3-c]furan-6-carboxylic acid **(5)**[2] (0.05 g, 0.180 mmol, 1 eq.) in 10 mL of DMF

was added bromo-tris-pyrrolidino phosphonium hexafluorophosphate (PyBrOP) (0.126 g, 0.27 mmol, 1.5 eq.) and diisopropylethylamine (DIEA) (0.048 mL, 0.27 mmol, 1.5 eq.) and the mixture was stirred for 10 min. Compound **4** (0.05 g, 0.180 mmol, 1 equiv.) was subsequently added, and the mixture was stirred at room temperature overnight. The solvent was then removed *in vacuo* and the residue dissolved in ethyl acetate and washed with water and brine. The organic layer was dried with anhydrous Na_2SO_4 and concentrated. Purification by column chromatography afforded the pure product as a yellow powder (0.077 g, 80%). LC-MS: *m/z* found = 534.15 $[M + H]^+$ calculated = 534.21



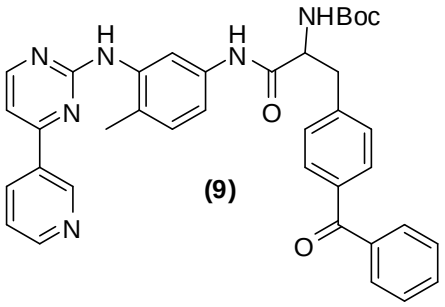
(7)

6,7-Diformyl-N-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenyl)-2-naphthamide (7): Compound **6** (0.077 g, 0.144 mmol) was dissolved in a mixture of trifluoroacetic acid (TFA):H_2O (1:1; total of 10 mL) and stirred at room temperature for 3 ho, after which the solvent was removed *in vacuo*. Traces of TFA was removed by the addition of methylene chloride and then removing the solvent under reduced pressure, which was repeated for several times to yield the product as an orange powder (0.066 g, 95%) which was further purified by reverse phase semi-prep HPLC using water and acetonitrile as eluents. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.55 (s, 2H), 9.40 (s, 1H), 9.08 (s, 1H), 8.87 (s, 1H), 8.87 – 8.82 (m, 2H), 8.75 – 8.72 (m, 2H), 8.68 (s, 1H), 8.57 (d, *J* = 5.1 Hz, 1H), 8.40 – 8.26 (m , 2H), 8.20 – 8.15 (m ,1H), 7.77 – 7.68 (m, 1H), 7.56 – 7.50 (m, 2H), 7.26 (d, *J* = 8.4 Hz, 1H), 2.26 (s, 3H). ESI-MS: *m/z* found = 488.14 $[M + H]^+$ calculated = 488.16.



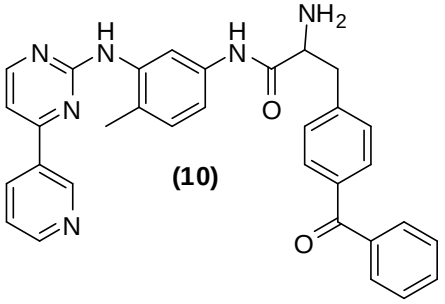
(8)

3-(4-Benzoylphenyl)-2-(tert-butoxycarbonylamino) propanoic acid (8): To 0.269 g (1 mmol) of commercially available 2-amino-3-(4-benzoylphenyl) propanoic acid (1 mmol) in 1:1 dioxane/water (5 mL each) was added 0.24 g of di-*tert*-butyl-dicarbonate (1.1 mmol) and 0.197 mL of triethylamine. The reaction was stirred for 4 h. The reaction the mixture was then concentrated on a rotary evaporator and the residue was diluted with water and ethyl acetate. The aqueous layer was acidified to *p*H 1 with 1 N HCl and back extracted with ethyl acetate. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to give 0.332 g (yield = 90%) of the protected amino acid as an off-white solid. The compound was used in the subsequent steps without further purification.



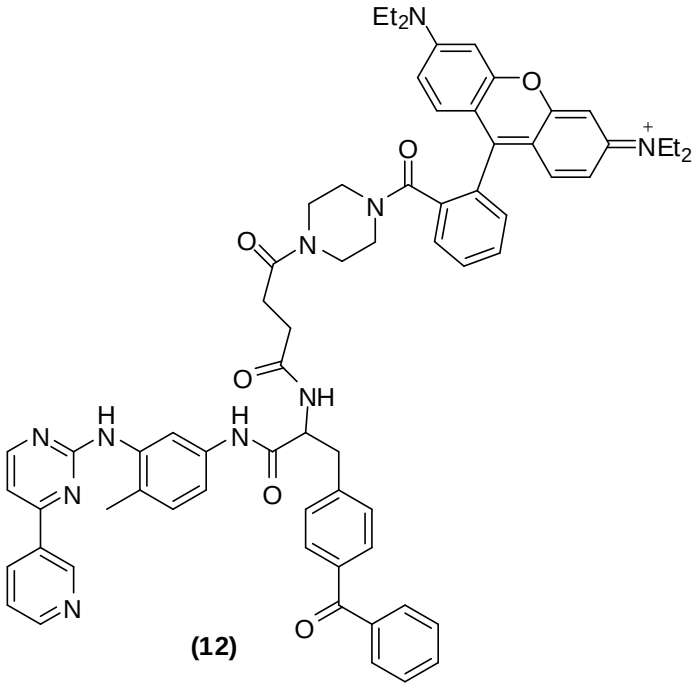
(9)

***Tert*-butyl 3-(4-benzoylphenyl)-1-(4-methyl-3-(4-(pyridin-3-yl) pyrimidin-2-ylamino) phenylamino)-1-oxopropan-2-ylcarbamate (9):** A 50 mL round-bottomed flask was charged with 0.29 g of **8** (0.785 mmol), 0.328 g of HATU (0.863 mmol), 0.162 mL of DIEA and 15 mL of DMF. After stirring for 5 min, 0.217 g of **4** (0.785 mmol) was added and the reaction was allowed to run overnight. The solvent was removed under reduced pressure and the crude reside was purified by column chromatography on silica gel using hexane and ethyl acetate as eluents to give 0.4 g of the product **9** (yield = 81%) as a yellow solid, and use directly in next steps.



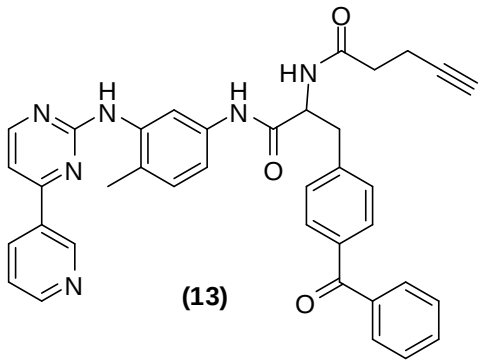
(10)

2-Amino-3-(4-benzoylphenyl)-N-(4-methyl-3-(4-(pyridin-3-yl) pyrimidin-2-ylamino) phenyl) propanamide (10): Compound **9** (0.33 g, 0.525 mmol) was dissolved in a 1:1 mixture of TFA and DCM with 1% water (5 mL total volume) and stirred at room temperature in an open round-bottomed flask. The reaction was completed in 30 min and the solvents were removed under reduced pressure to give 0.33 g of the product **10** (yield = 92%) which was used in the subsequent steps without further purification. ESI-MS: *m/z* found = 529.19 $[M + H]^+$ calculated = 529.22



(12)

N-(9-(2-(4-(4-(3-(4-benzoylphenyl)-1-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino)phenylamino)-1-oxopropan-2-ylamino)-4-oxobutanoyl)piperazine-1-carbonyl)phenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)-N-ethylethanaminium (12), Photo-Affinity Probe 1: A 25 mL round-bottomed flask was charged with 0.115 g of rhodamine B 4-(3-carboxypropionyl) piperazine amide **(11)** (0.189 mmol), 0.079 g of HATU (0.206 mmol), 0.045 mL of DIEA (0.258 mmol) and 5 mL of DMF. After stirring for 5 min, 0.11 g of **10** (0.172 mmol) was added and the stirring was continued for 3 h upon which all of the starting material **10** was used up. The solvent was removed on a rotary evaporator and the crude residue was purified by Semi-Prep HPLC on a C_{18} column, giving 0.08 g (yield = 41%) of the product **12** as a dark fine powder. ESI-MS: *m/z* found = 561.257 = $(M+2H^+)/2$, $[M]^+$ calculated = 1121.53



(13)

N-(3-(4-benzoylphenyl)-1-(4-methyl-3-(4-(pyridin-3-yl)pyrimidin-2-ylamino) phenylamino)-1-oxopropan-2-yl) pent-4-ynamide (13), Clickable Photo-Affinity Probe 2: A 25 mL round-bottomed flask was charged with 0.018 g of pent-4-ynoic acid (0.189 mmol), 0.079 g of HATU (0.206 mmol), 0.045 mL of DIEA (0.258 mmol) and 5 mL of DMF. After stirring for 5 min, 0.11 g of **10** (0.172 mmol) was added and the stirring was continued for 1 h upon which all of the starting material **10** was used up. The solvent was removed on a rotary evaporator and the crude residue was purified by column chromatography on silica gel using hexane and ethyl acetate as eluents to give 0.1 g of the product **9** (yield = 95%) as off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 2.19 (s, 3H), 2.29 (m, 4H), 2.70 (s, 1H), 2.94-3.18 (m, 2H), 4.77 (m, 1H), 7.15 (d, 1H, *J* = 8.4 Hz), 7.26 (d, 1H, *J* = 8.22 Hz), 7.42 (d, 1H, *J* = 8.4), 7.46 (d, 2H, *J* = 8.22 Hz), 7.53 (m, 3H), 7.66 (m, 5H), 7.92 (s, 1H), 8.40 (d, 1H, *J* = 8.22 Hz), 8.45 (d, 1H, *J* = 8.04 Hz), 8.50 (d, 1H, *J* = 5.10 Hz), 8.67 (d, 1H, *J* = 5.10 Hz), 8.97 (s, 1H), 9.3 (s, 1H), 10.1 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ = 14.10, 17.59, 33.98, 37.91, 54.46, 71.22, 83.68, 107.73, 115.87, 116.16, 124.72, 127.27, 128.53, 129.43, 129.49, 129.57, 130.22, 132.57, 133.04, 135.21, 136.67, 137.23, 137.73, 142.91, 149.42, 158.21, 159.58, 160.85, 160.98, 169.63, 170.45, 195.57. ESI-MS: *m/z* found = 609.24, $[M+H]^+$ calculated = 609.25

3. Labeling experiments

Schematic representation of the two different labeling strategies

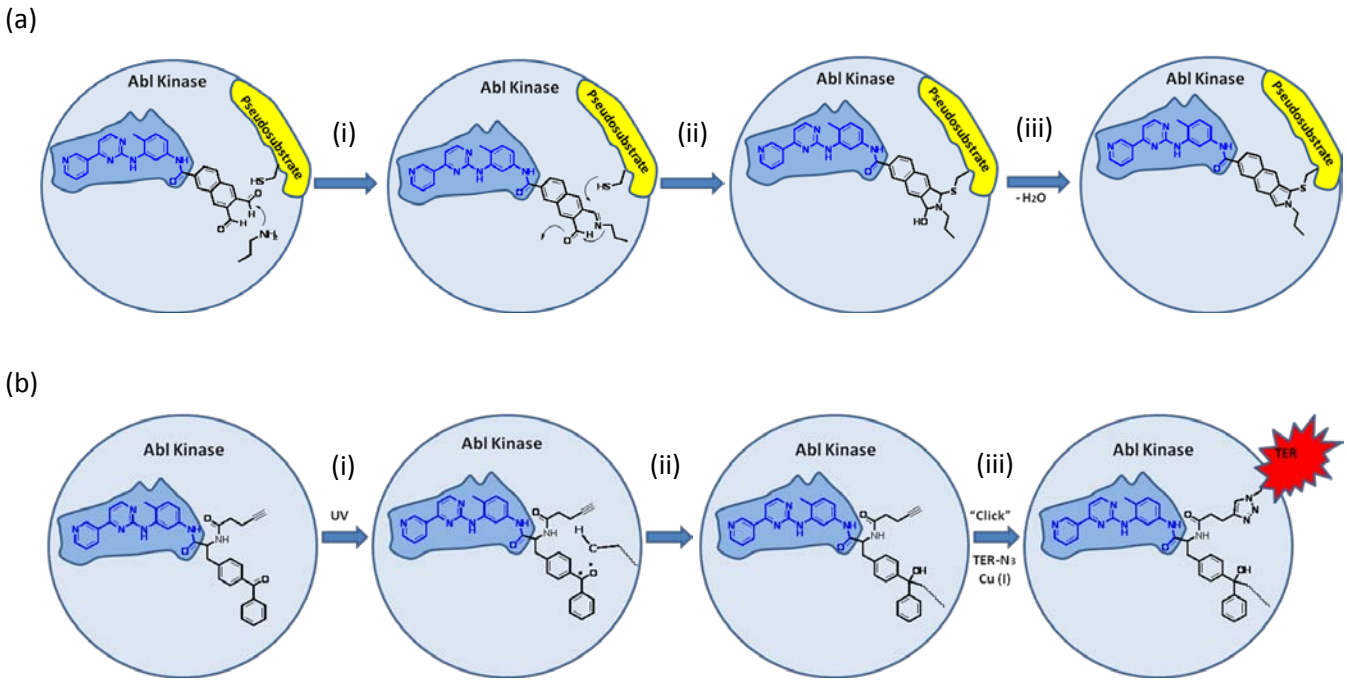


Figure S1. Two different methods to label Abl kinase. (a) A three-component reaction (kinase + pseudosubstrate + dialdehyde) mediated labeling of Abl using compound **7**. The compound **7** first occupies the ATP-binding pocket (shaded) of the kinase by exploiting the high binding affinity and selectivity of the imatinib core structure (blue). The catalytic lysine residue from the kinase active site forms a reversible imine adduct by reacting with the formyl group in **7**. The cysteine residue from the pseudosubstrate attacks the imino carbon with the subsequent removal of a water molecule, leading to the formation of a stable isoindole product. The cross-linked Abl-pseudosubstrate pair is subsequently visualized by in-gel fluorescent scanning. (b) Clickable photo-affinity probe (compound **13**) mediated labeling of Abl. Upon incubation with the kinase, compound **13** occupies the active site by virtue of the imatinib core structure. Subsequent UV irradiation generates a highly reactive diradical intermediate from the benzophenone unit of **13**, which quickly reacts with C-H bonds in the vicinity, leading to covalent binding of **13** with the kinase. The probe/kinase complex upon treatment with a rhodamine azide reporter (i.e. TER-N_3) under click chemistry conditions enables the fluorescence visualization of the labeled kinase with in-gel fluorescence scanning.

3. 1. Labeling experiments with the dialdehyde (7)

Unless otherwise indicated, typical labeling reactions with the dialdehyde **7** were carried out in the following optimized conditions: kinase (400 to 600 nM), pseudosubstrate (2 μM) and dialdehyde **7** (10 μM) in the reaction buffer (25 mM HEPES at *p*H = 7.5, 150 mM NaCl, 2 mM $MgCl_2$) were incubated for 20 min at RT before adding 6X loading dye (reducing). The sample was heated at 95 ^oC for 10 min before SDS-PAGE and in-gel fluorescence scanning (excitation at 480 nm, emission collected at 520 nm filter)

pH-dependence of the cross-linking reaction

HEPES buffer in the pH range of 6 to 9 were used to determine the *p*H dependence of the labeling reaction of Abl kinase with the dialdehyde **7**. As shown below, efficient labeling was observed at pH > 7 while a low pH was found to be unfavorable for the reaction.

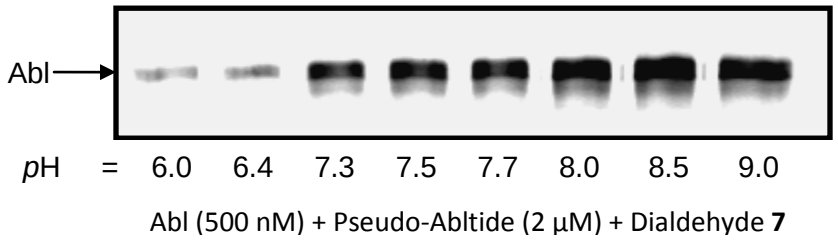


Figure S2. *p*H-dependence of the labeling profiles of the dialdehyde **7** mediated cross-linking of Abl kinase with the pseudosubstrate

Effect of exogenous thiols on the efficiency of the labeling

In order to determine the exogenous thiol tolerance of the dialdehyde-guided cross-linking reaction of the kinase with the thiol peptide pseudosubstrate, reactions were done at varying concentrations of an exogenous competing thiol, β-mercaptoethanol. Briefly, 500 nM of Abl kinase was incubated in the buffer (25 mM HEPES at *p*H = 7.5, 150 mM NaCl, 2 mM $MgCl_2$) with varying amounts of β-mercaptoethanol (BME). To this mixture was added the dialdehyde **7** (10 μM) and the Abl-pseudosubstrate (2 μM). The reactions were incubated for 20 min at RT and subsequently denatured and subjected to SDS-PAGE and in-gel fluorescence scanning. As shown in Figure S2, 5 to 10 fold excess of the exogenous thiol was found to have no significant effect in the cross-linking efficiency while higher concentrations (> 50 fold excess) of the thiol was found to significantly shield the kinase from cross-linking with the substrate peptide.

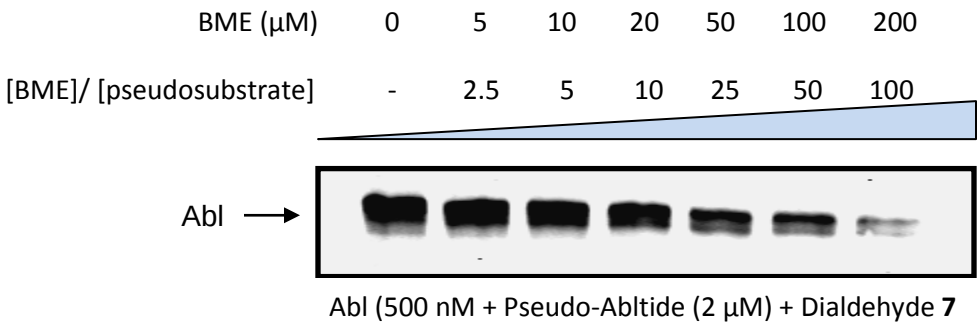


Figure S3. Effect of β-mercaptoethanol (BME) on the three-component reaction of Abl kinase with the dialdehyde **7** and Pseudo-Abltide.

Effect of exogenous amines on the efficiency of the labeling

Since the labeling reaction was based on the selectivity of the dialdehyde **7** towards Abl kinase, we tested if the reaction is tolerated in the presence of exogenous amines (e.g. lysine). Thus cross-linking reactions were set up in the presence of varying amounts of lysine. As shown in Figure S3, even 800 fold excess of lysine was found to have no significant effect in the labeling efficiency. This indicates that the labeling reaction is selective towards the kinase and is highly shielded from exogenous amines.

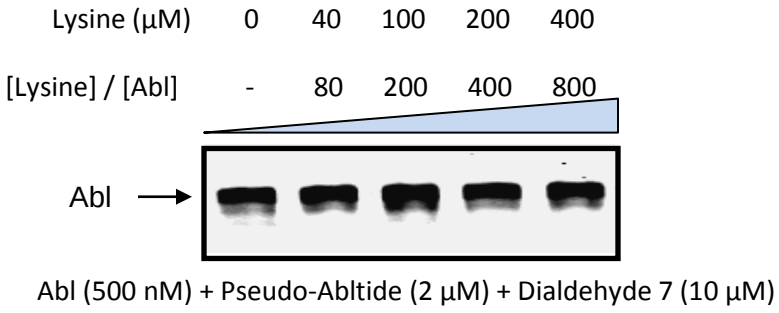


Figure S4. Effect of exogenous lysine on the labeling reaction.

IC_{50} evaluation of the probe

Concentration-dependent experiments were performed to determine the inhibition potency and hence the binding affinity of the probes towards the Abl kinase. The inhibition assay was performed with Kinase-Glo® Plus Luminescent Kinase assay kit from Promega following the manufactures instructions. Briefly, recombinant Abl kinase, a consensus Abl peptide substrate

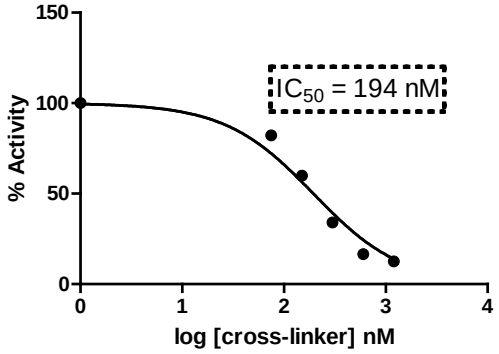
with sequence KKGEAIYAAPFA-NH_2, ATP and the probe were mixed in the kinase reaction buffer (100 mM Tris, *p*H = 7.5, 10 mM $MgCl_2$) at a volume of 55 µL in a flat-bottom solid white 96-well plate. The incubation was allowed to continue for 20 min at 37 ^oC and the kinase reaction was subsequently quenched by the addition of an equal volume of the Kinase-Glo reagent. After 5 min of incubation, the luminescence readouts from the wells were measured using Tecan microplate reader with i-control software. The ATP and substrate peptide concentrations used in the assay were 10 µM and 50 µM, respectively. The following control reactions were also performed simultaneously.

1. Enzyme control (No kinase, Buffer+Substrate+ATP),
2. Inhibitor control (No inhibitor, Buffer+Kinase+Substrate+ATP),
3. Substrate control (No substrate, Buffer+Kinase+ATP)
4. Buffer alone.

The luminescence intensity from each well was measured and the inhibition potency was calculated using the following relation,

Potency = 1 - Remaining activity

Dose-dependent inhibition assays were performed by varying the concentration of the probes under fixed enzyme concentration of 50 nM as mentioned before. The IC_{50} values of the probes were calculated from the percentage activity vs. log [concentration of probe] curves generated using GraphPad Prism software.



The dialdehyde **7** was found to be a potent inhibitor of the Abl kinase as evident from the IC_{50} value of 194 nM at kinase concentration of 50 nM. The % remaining activity vs log (concentration of the probe) plot is shown on left.

3.2. Labeling experiments with compound (12).

The general procedure for the labeling studies with the photo-affinity probe-1 (compound **12**) is as follows; 2 µL of the enzyme stock solutions (0.4 µg/µL) were diluted with 17.6 µL of Tris.HCl buffer (50 mM, *p*H = 7.5). Different concentrations of **12** (stock solutions in DMSO)

were added (0.4 µL) and the reactions were incubated at room temperature in the dark for 30 min. Subsequently the reaction mixtures were irradiated with a handheld UV lamp under the long UV channel for 20 min. The reactions were quenched by the addition of 4 µL of 6X SDS loading buffer followed by boiling at 95 ^{o}C for 10 min. Samples were resolved on a 10% denaturing SDS-PAGE gel and fluorescence was detected with a fluorescence gel scanner (Typhoon 9200, Amersham).

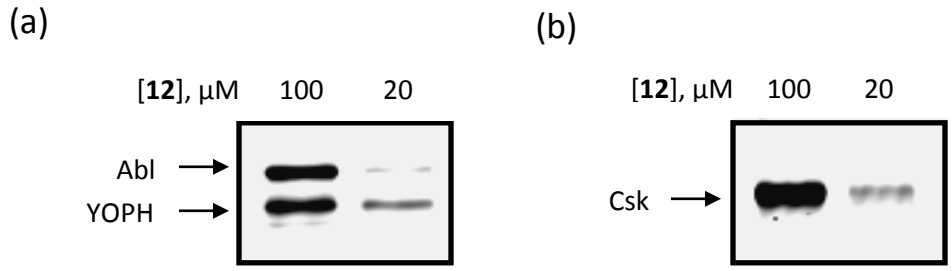


Figure S5. Labeling of (a) Abl and (b) Csk kinases with compound (**12)**.

As the probe was designed to target Abl kinase, the photo-affinity labeling experiments were performed with Abl kinase and another kinase Csk as a control to evaluate the selectivity of the labeling reaction. As shown in Figure S4, the affinity labeling was found to be non-selective with intense labeling from Csk and YOPH under identical conditions (The phosphatase YOPH was present as an impurity in the Abl preparation used in the experiments). Moreover intense protein labeling required more than 50 µM of the cross-linker and at concentrations below 5 µM the cross-linker was found to be very inefficient with very faint labeling and the labeling was completely abolished at concentrations below 1 µM. The failure of the CL-1 in detecting the Abl kinase selectively and efficiently could be due to its bulky structure mainly contributed from the fluorescent dye unit which may hinder binding of this cross-linker to the active site of the kinase. In other words the mere presence of the ATP site targeting fragments from Imatinib (2-phenylaminopyridine motif (blue), 3'-pyridyl group (purple), flag methyl group(black) and the amide functionality(red)) are not just enough to confer selective and efficient binding of the molecule to the active site of the Abl kinase. In order to minimize these drawbacks in the first photo-affinity probe design, we eliminated the bulky fluorescent dye unit and incorporated an alkyne handle suitable for a sequential click-chemistry based visualization of the cross-linking, leading to the clickable photo-affinity probe (**13**).

3.3. Labeling experiments with the clickable probe, compound (13).

The general procedure for the photo cross-linking with subsequent click-chemistry is as follows; 2 µL of the enzyme stock solutions (0.1 to 0.5 µg/µL) were diluted with 17.6 µL of Tris.HCl

buffer (50 mM, *p*H = 7.5). The clickable probe, **13**, (stock solution in DMSO) was added (0.4 µL) and the reactions were incubated at room temperature in the dark for 30 min. Subsequently the reaction mixtures were irradiated with a handheld UV lamp under the long UV channel (~ 350 nm) for 20 min in the absence of other light sources. The reaction mixtures were then subjected to click-chemistry with a TER-azide dye in the following conditions. To each reaction was added 1 µL each of TER-N_3 fluorescent dye (concentration in reaction = 50 µM, stock solution in DMSO), $CuSO_4$ (concentration in reaction = 1 mM, stock solution in water), TBTA (concentration in reaction = 100 µM, stock solution in DMSO) and sodium ascorbate (concentration in reaction = 1 mM, stock solution in water) and incubated for 2 h at room temperature. The reactions were quenched by the addition of 5 µL of 6X SDS loading buffer followed by boiling at 95 ^{o}C for 10 min. Samples were analyzed on a 10% denaturing SDS-PAGE gel and fluorescence was detected with the fluorescence gel scanner.

The clickable photo cross-linker (CL-2) was found to be selective for c-Abl kinase. It was found that cross linker concentration of 0.5 µM is sufficient to get good labeling and this concentration was used for all subsequent labeling experiments (with CL-1, the labeling was very faint even at 20 µM cross-linker)

Detection limit of the pure Abl with (13).

In order to determine the fluorescent detection limit of the Abl kinase by the clickable photo-affinity probe-2, photo-cross-linking reactions were set up as mentioned before with decreasing amounts of Abl. As shown in Figure S5, 0.3 to 0.2 µg of pure Abl kinase was readily detected with the probe at a concentration of 0.5 µM.

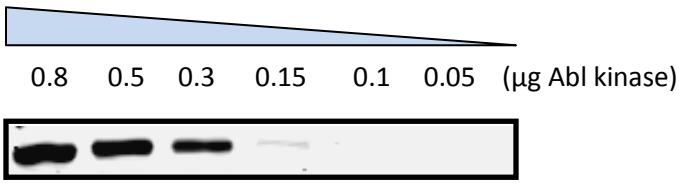


Figure S6. Labeling of decreasing amounts of pure Abl kinase with 0.5 µM clickable probe (**13**)

Labeling experiments with the clickable probe (13) in the presence of K562 cell lysate

Human leukemic cell line K562 is a p-210 Bcr-Abl expressing (chronic myelogenous leukemia) cell line. Total cell lysates were prepared as mentioned elsewhere and used for labeling reactions with the clickable probe, **13**. We could not detect the endogenous Bcr-Abl (MW = 210 kDa) or endogenous c-Abl (MW = 130 kDa) from the lysate with our probe. This is presumably due to

the insufficient detection limit of the probe as well as due to the insufficient expression levels of these proteins in the cell lines. So we carried out labeling experiments from this mammalian proteome with varying amounts of spiked Abl kinase as follows. Decreasing amounts of recombinant Abl kinase was spiked to 30 µg of K562 total cell lysate (50 mM Tris buffer, *p*H = 7.5, 150 mM NaCl). The cell lysates were prepared from approximately 1.5×10^7 cells cultured in T75 cell culture flasks. To 30 µg of the lysate was added 0.5 µM clickable probe (**13**) and the reaction was allowed to continue for 20 min. Subsequently the reactions were irradiated with UV light (~ 350 nm) for 20 min followed by click-chemistry with rhodamine azide (TER-N_3) as described above. After 2 h of click reaction, 6X SDS loading dye were added, heated to 95 ^oC for 10 min and the proteins were resolved by SDS-PAGE. In-gel fluorescent scanning was used to visualize the labeled protein bands. As shown below in Figure S6, up to ~ 1% of the Abl kinase in the total proteome could be readily detected using our probe.

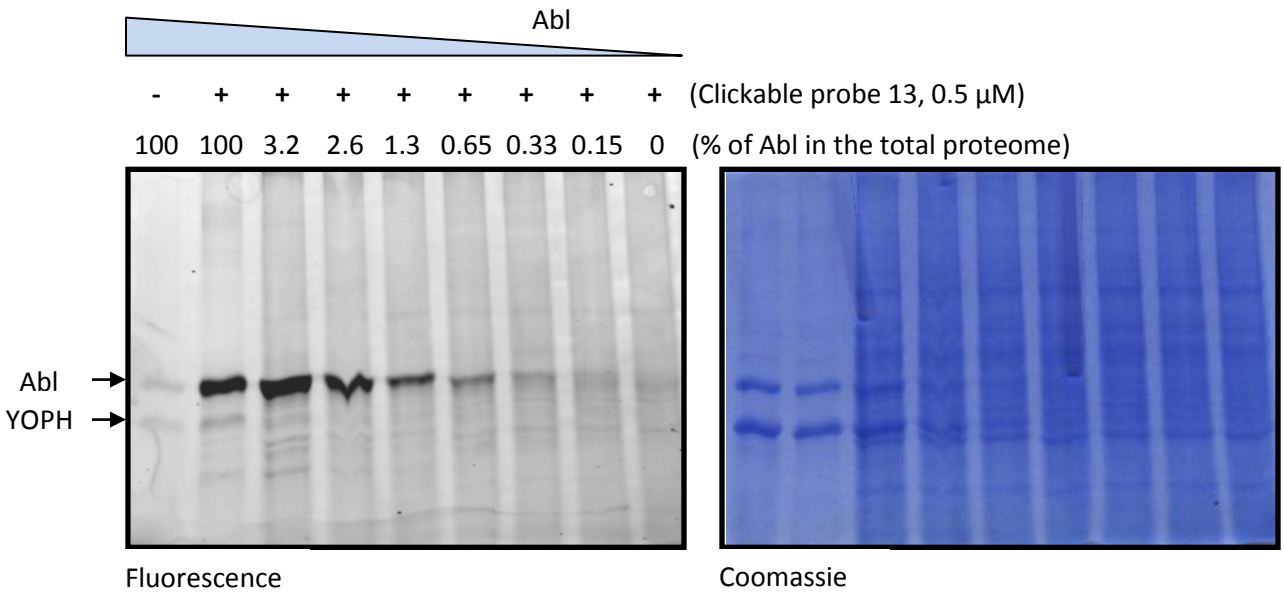
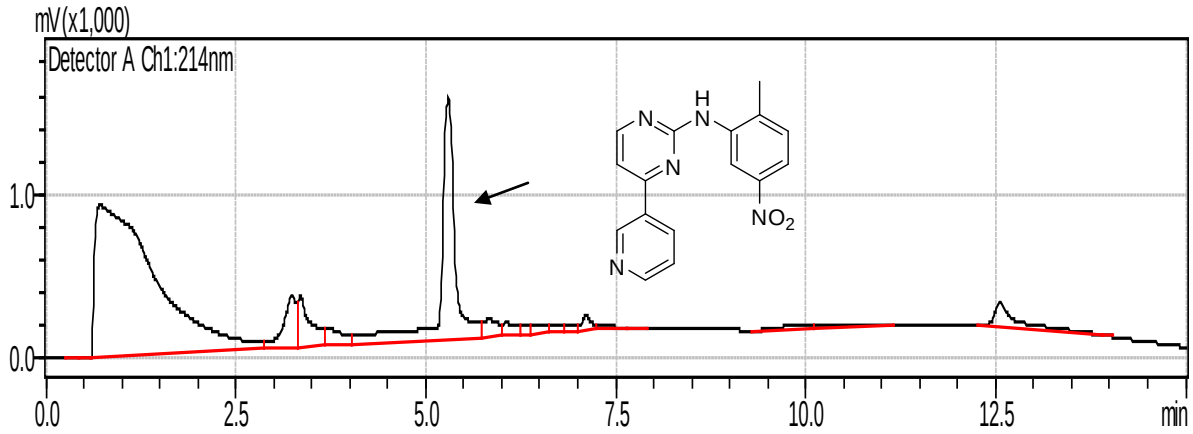
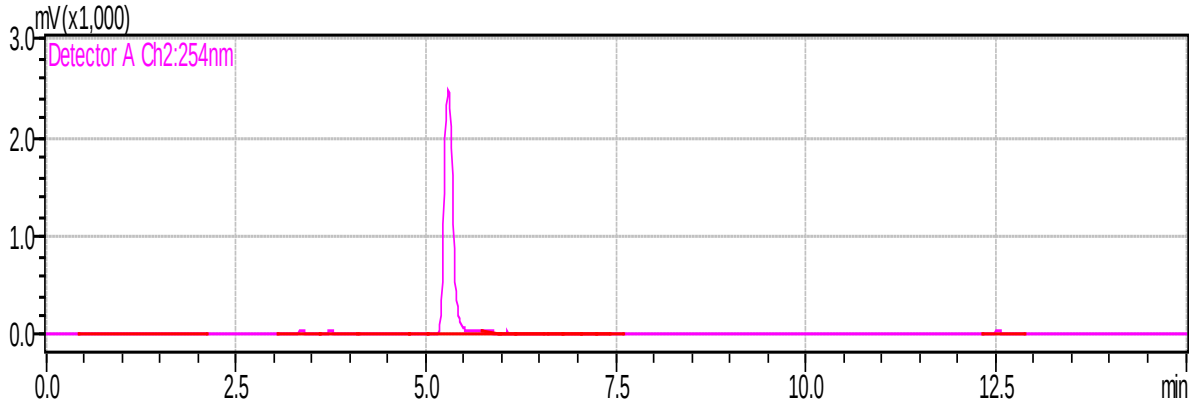


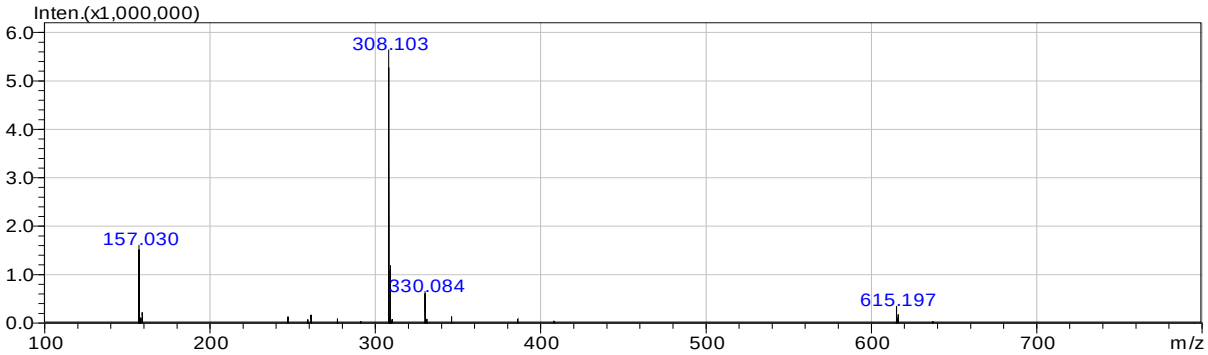
Figure S7. Labeling of decreasing amounts of spiked Abl kinase in the presence of K562 cell lysate (30 µg total proteins per lane).

4. LC-MS Characterization

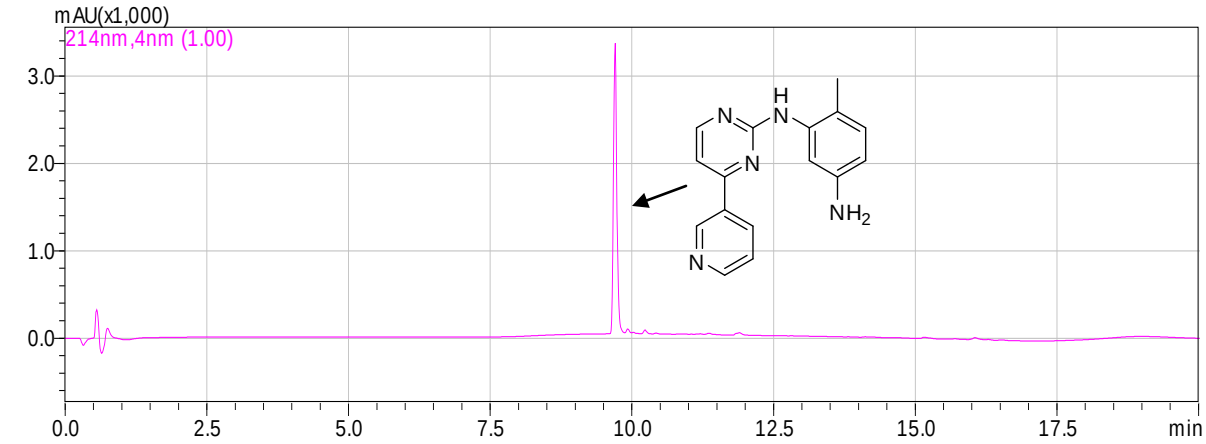
Compound 3







Compound 4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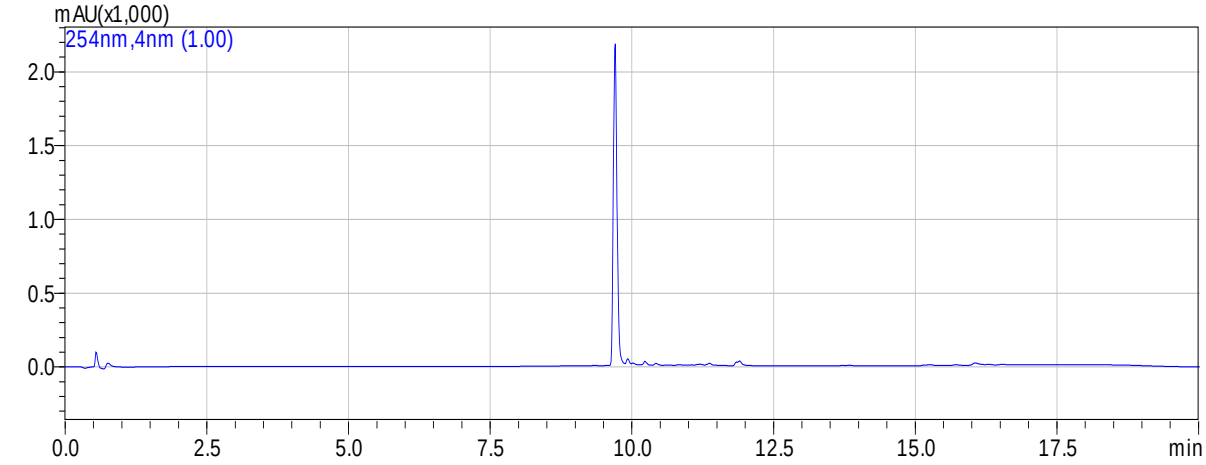
214nm,4nm (1.00)
3.0
2.0
1.0
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
min
H
N
N
N
N
NH2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254nm,4nm (1.00)
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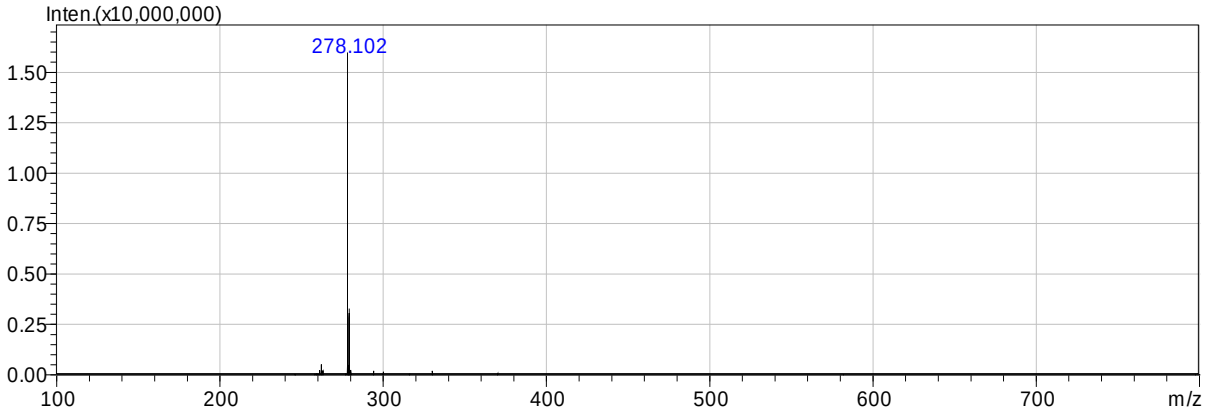
1.5
1.0
0.5
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
min


Inten.(x10,000,000)
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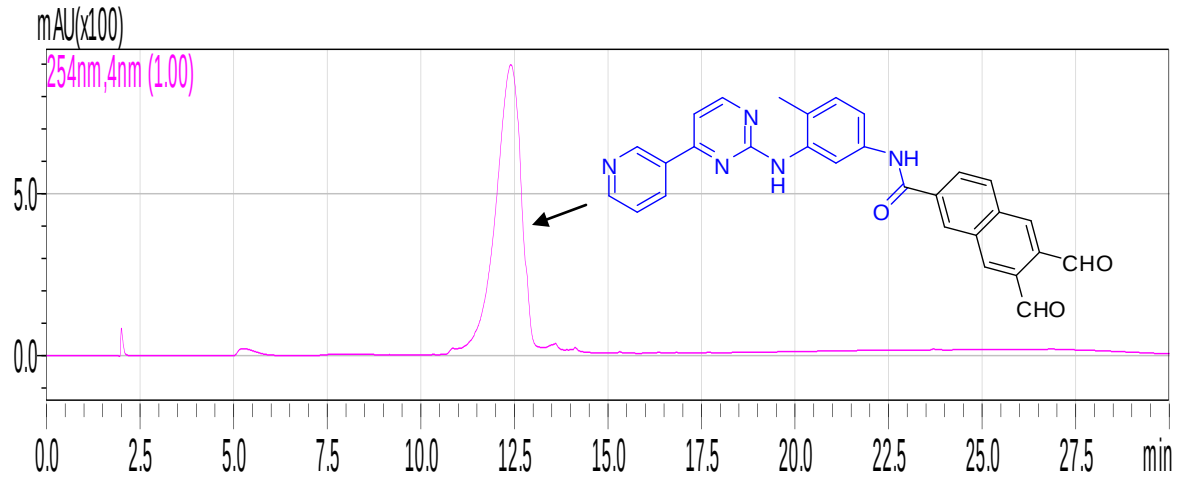
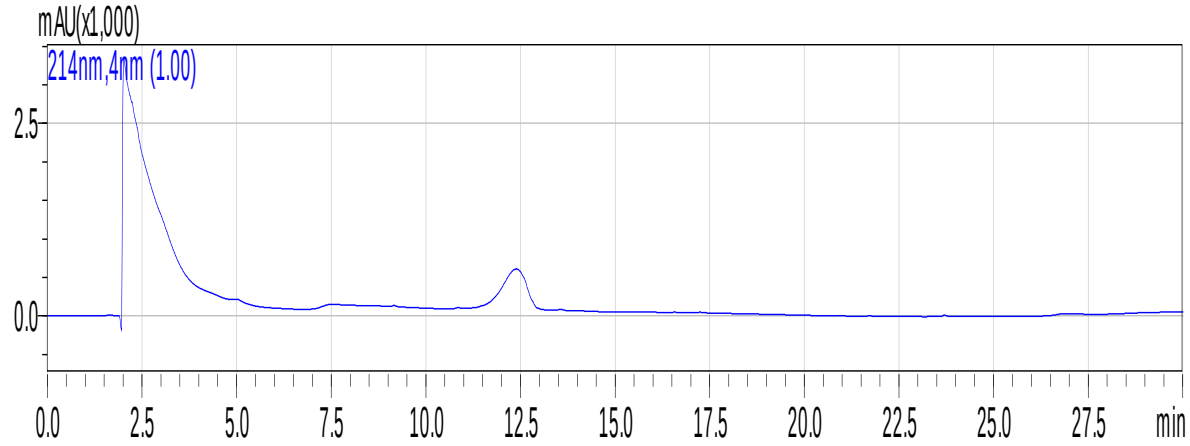
1.50
1.25
1.00
0.75
0.50
0.25
0.00
100
200
300
400
500
600
700
m/z

Compound 7


mAU(x100)
254nm,4nm (1.00)
5.0
0.0
N
N
N
H
NH
O
CHO
CHO
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 min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214nm,4nm (1.00)
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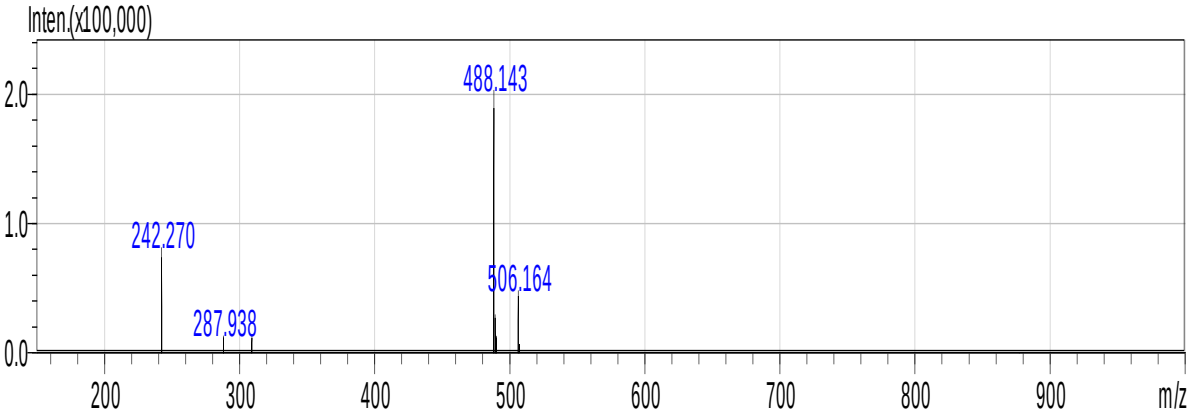
0.0
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 22.5 25.0 27.5 min


Inten.(x100,000)
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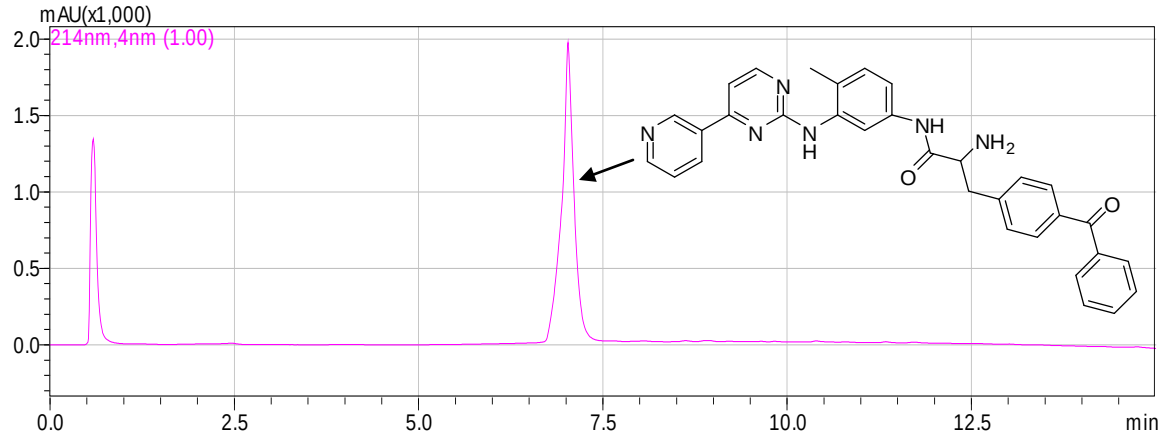
1.0
242.270
506.164
287.938
0.0
200 300 400 500 600 700 800 900 m/z

Compound 10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214nm,4nm (1.00)
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1.0
0.5
0.0
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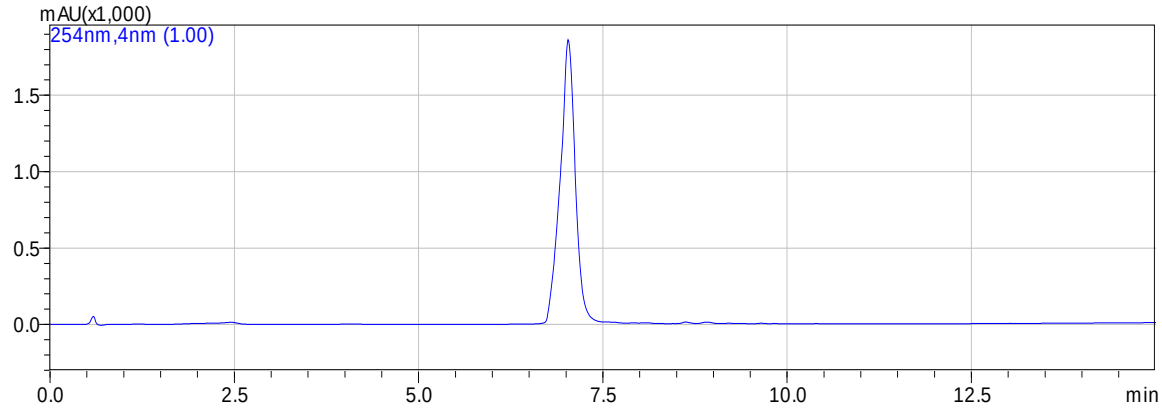
2.5
5.0
7.5
10.0
12.5
min
N
N
N
H
NH
NH2
O
O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254nm,4nm (1.00)
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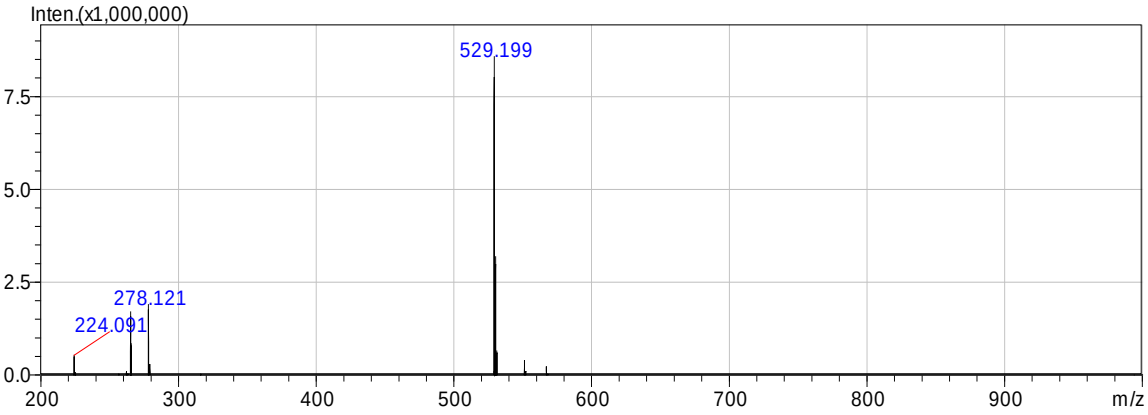
7.5
10.0
12.5
min


Inten.(x1,000,000)
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0.0
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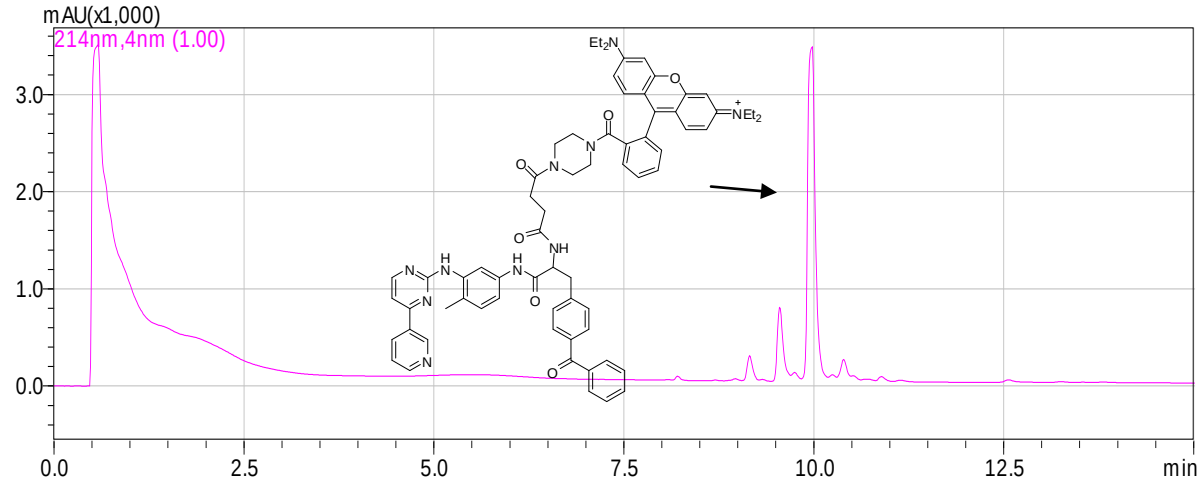
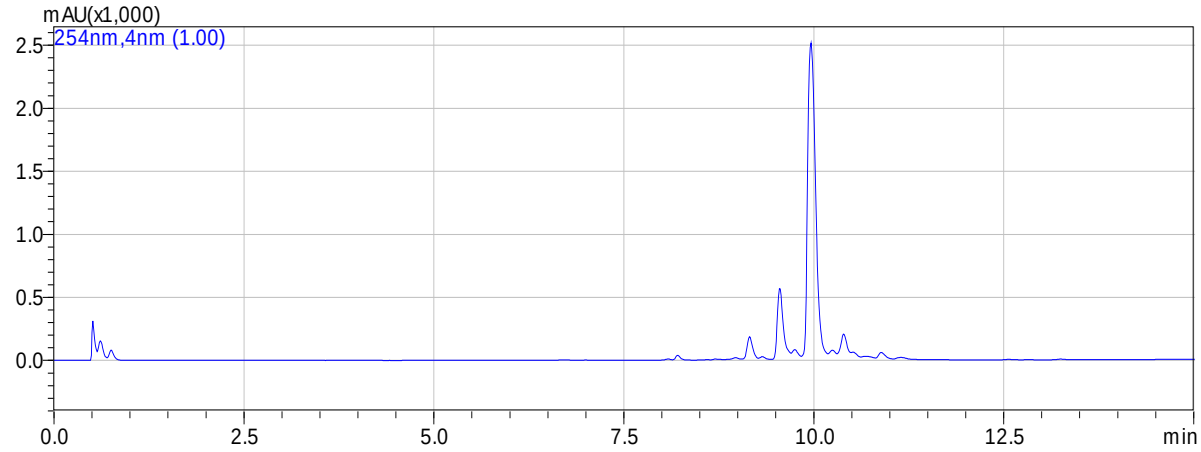
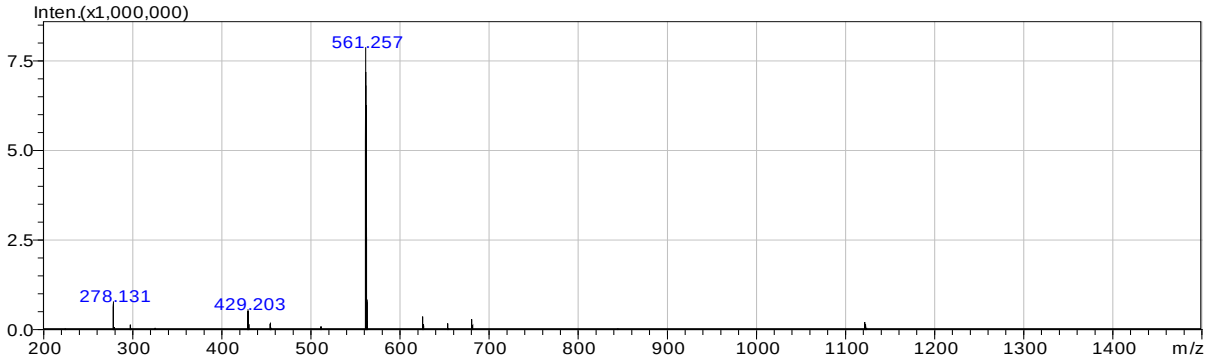
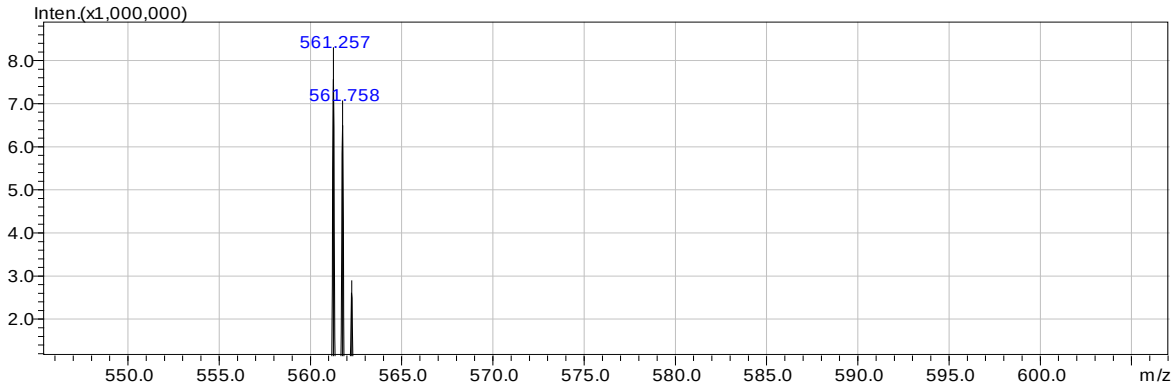
200
300
400
500
600
700
800
900
m/z

Compound 12 (Photo-affinity probe-1)

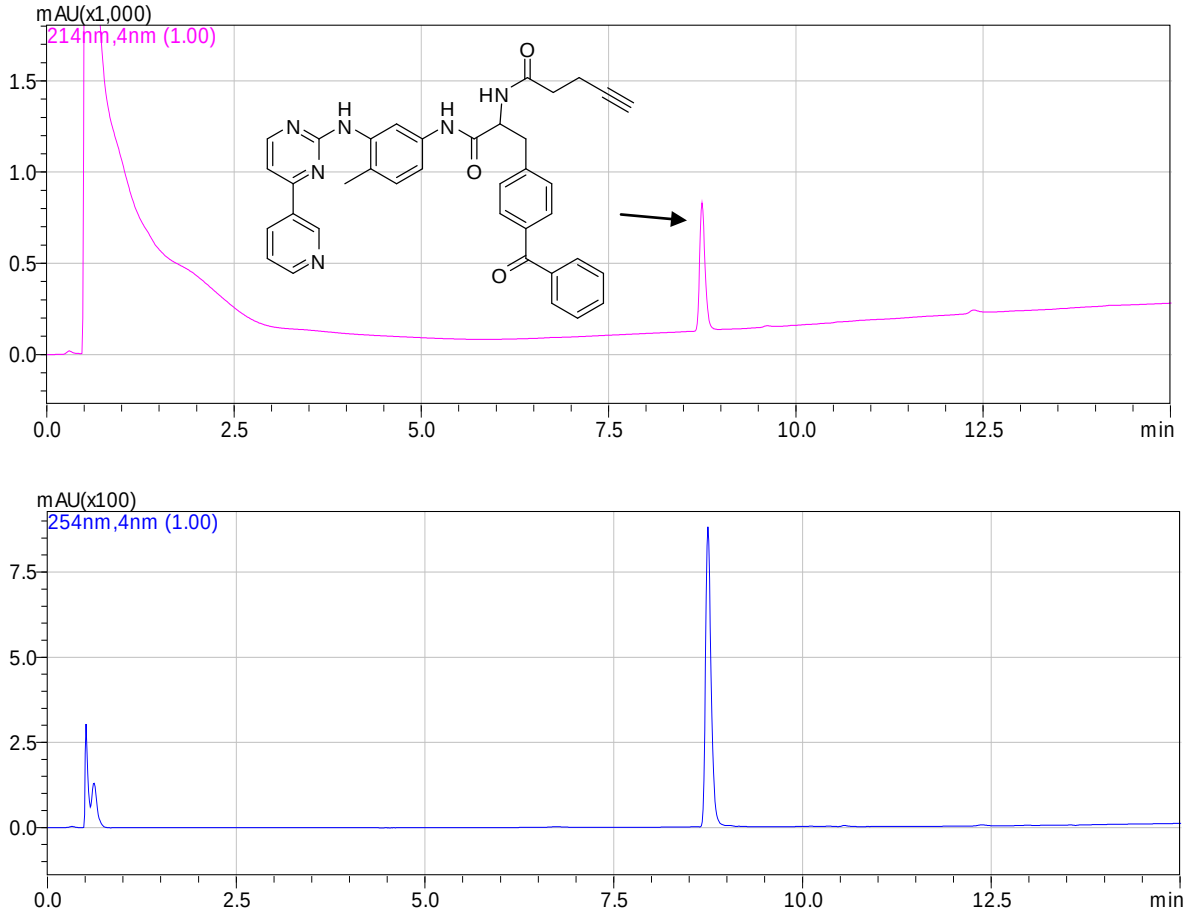


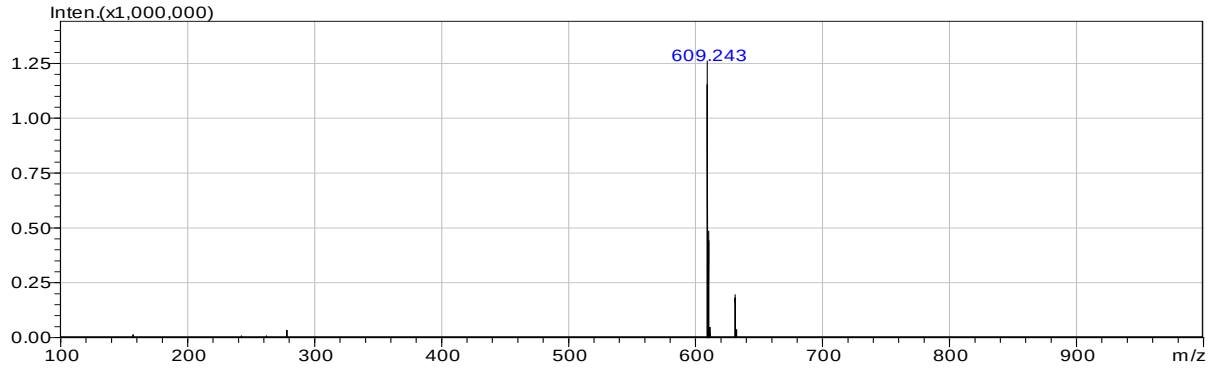






Compound 13 (Clickable photo-affinity probe-2)

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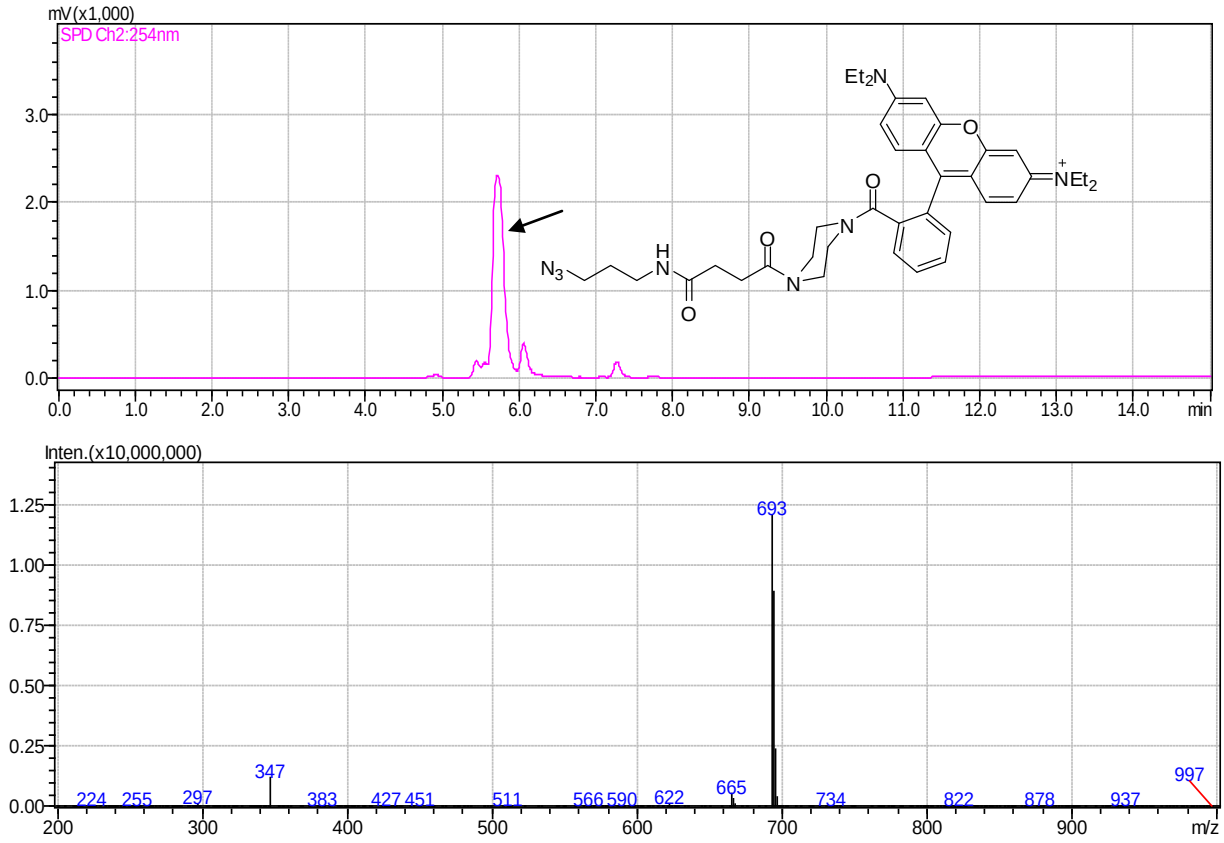
0.00
100
200
300
400
500
600
700
800
900
m/z

TER-N_3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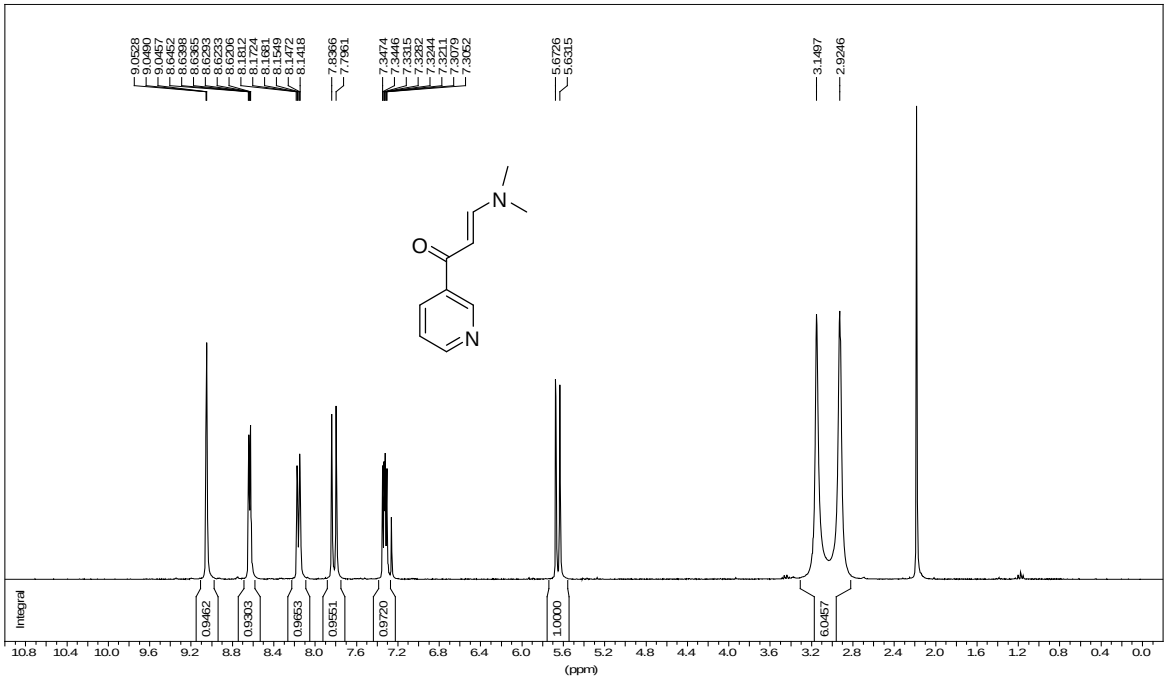
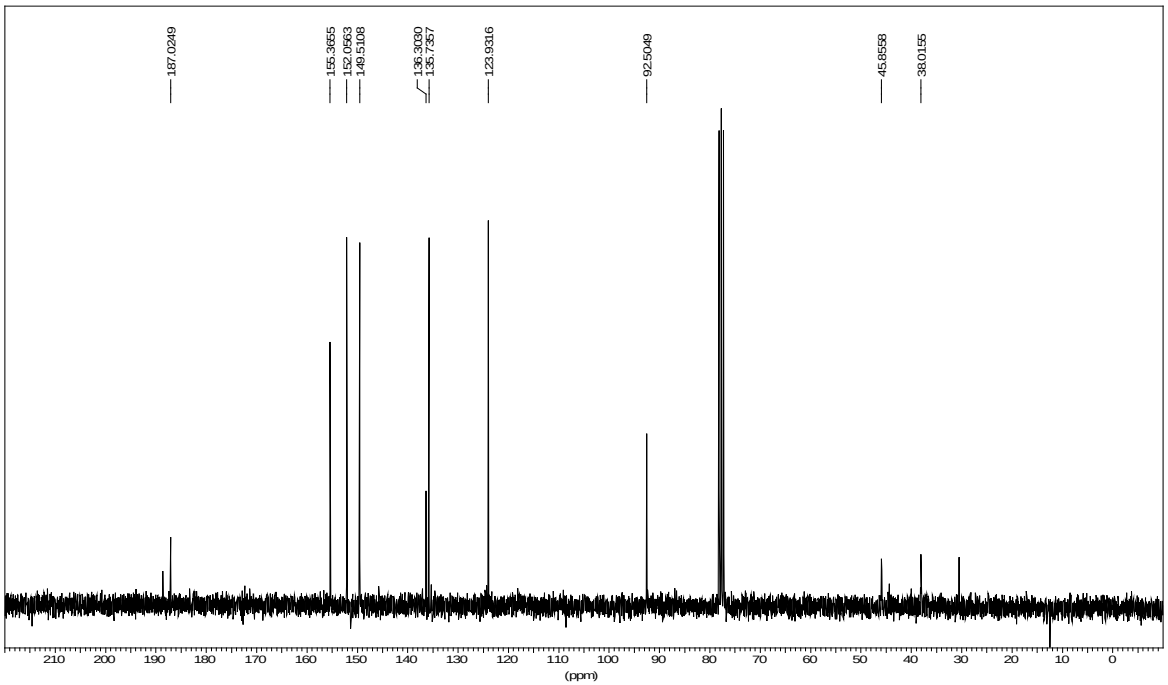
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
min
Et2N
O
NEt2
O
N
O
H
N
N3
O
N
O
Inten.(x10,000,000)
693
1.25
1.00
0.75
0.50
0.25
0.00
224
255
297
347
383
427
451
511
566
590
622
665
734
822
878
937
997
200
300
400
500
600
700
800
900
m/z

5. ^{1}H and ^{13}C NMR spectra

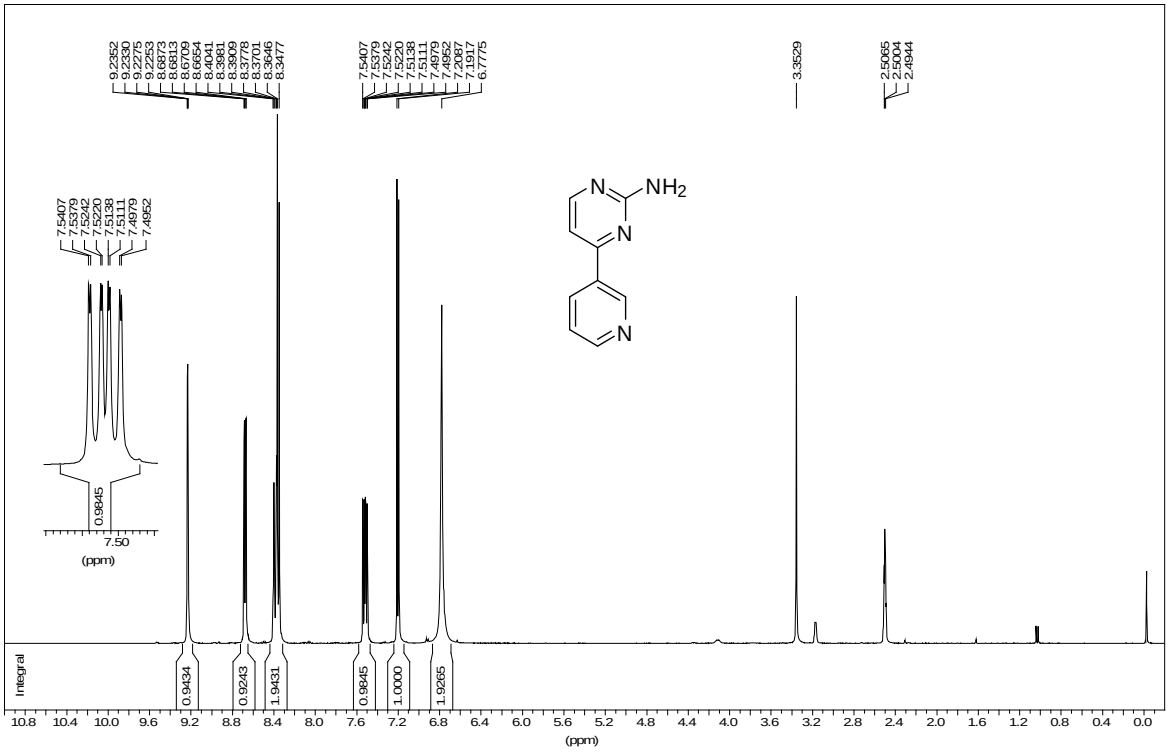
1H normal range AC300, 256



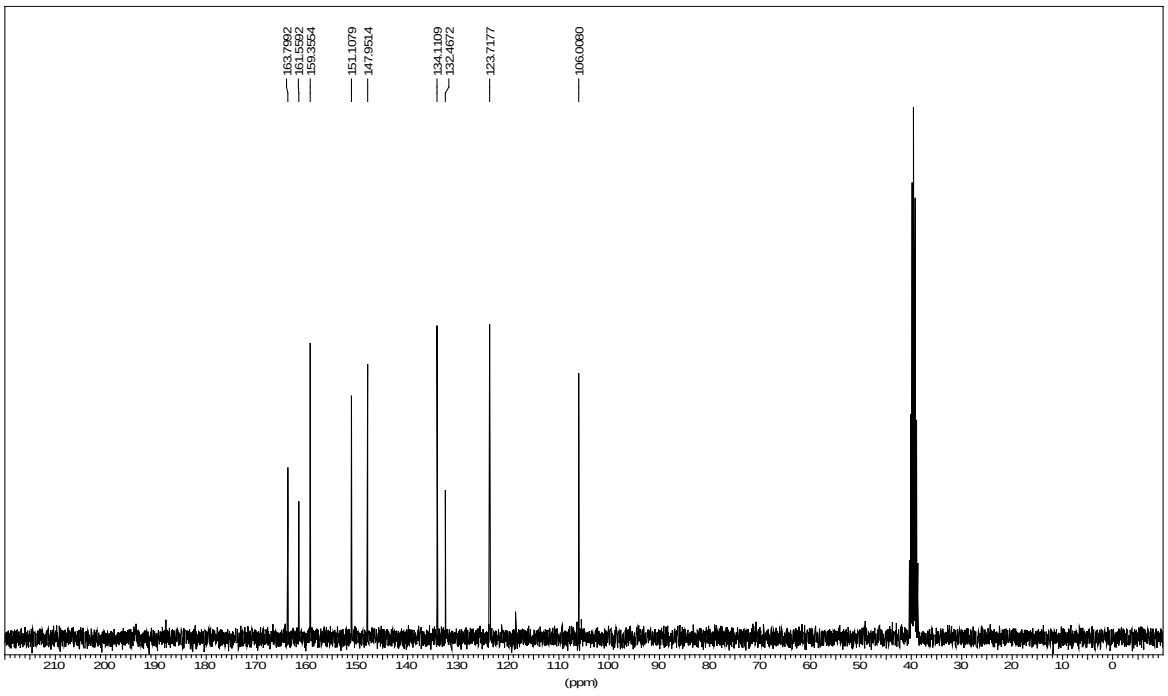
13C Standard AC300, 256



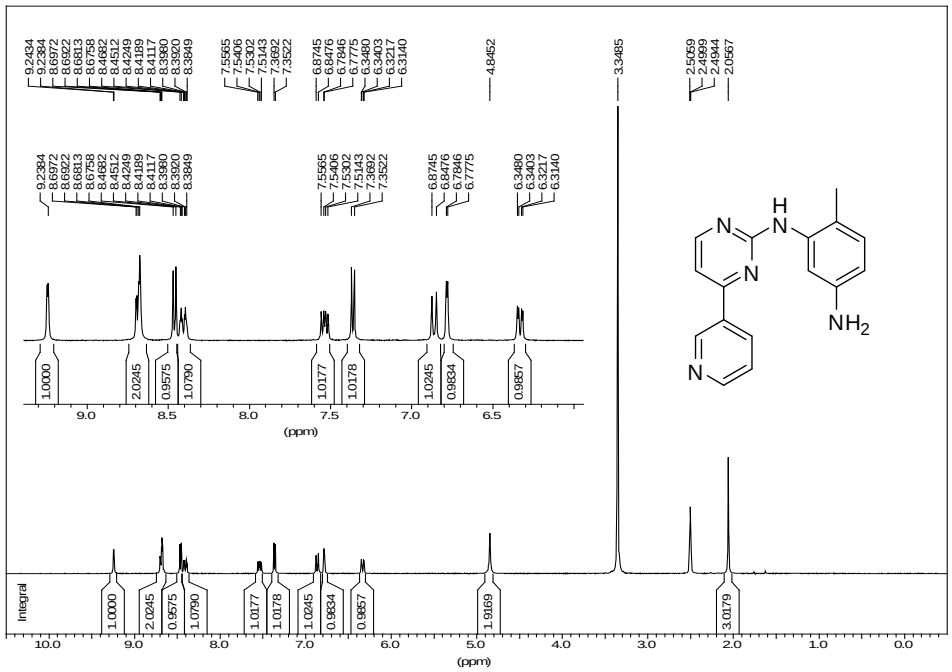
1H normal range AC300



13C Standard AC300



1H Imatinib nitro reduction with SnCl2 AC300



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NS	:	8	
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O2	:	1853.43	Hz
O3	:	1853.43	Hz
SFO1		300.1318534	MHz
SFO2		300.1318534	MHz
SFO3		300.1318534	MHz
SOLVENT	:	DMSO	
*** Processing Parameters ***			
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*** 1D NMR Plot Parameters ***			
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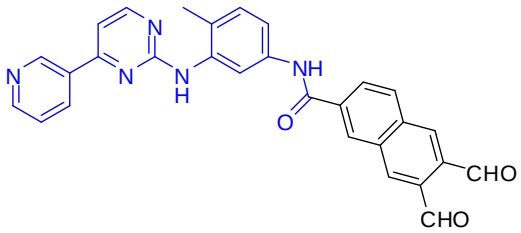
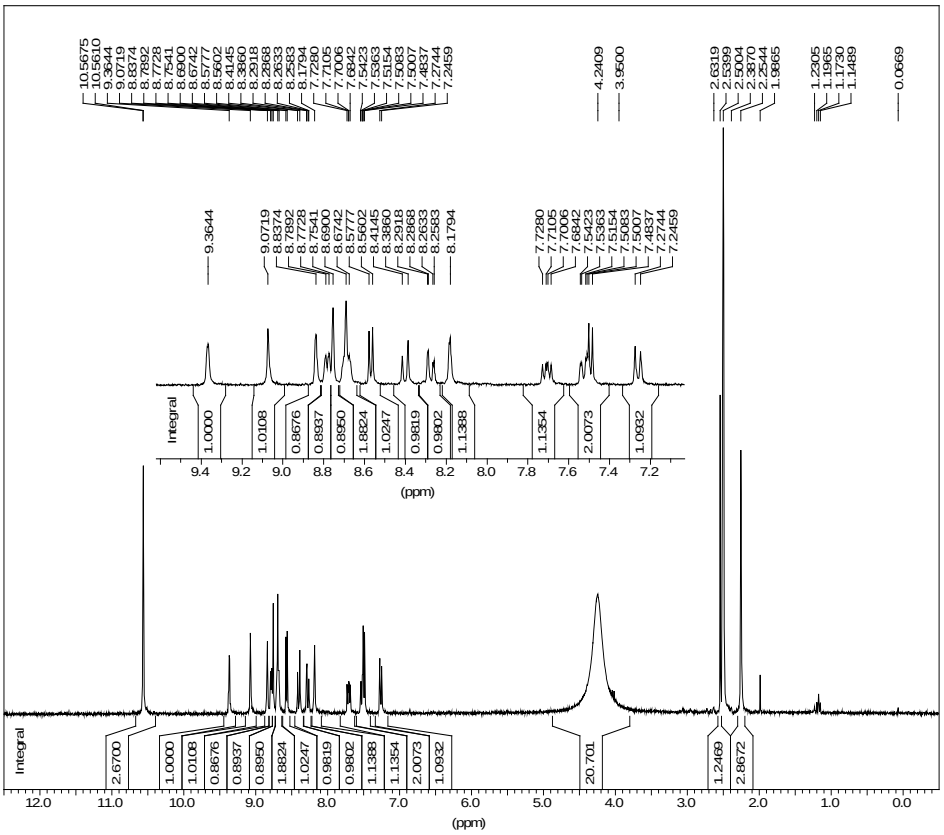


1H Imatinib-NDA dialdehyde final (prep) in DMSO-d6 AC300



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PROCNO : 1
*** Acquisition Parameters ***
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DATE_d : Jul 17 2009
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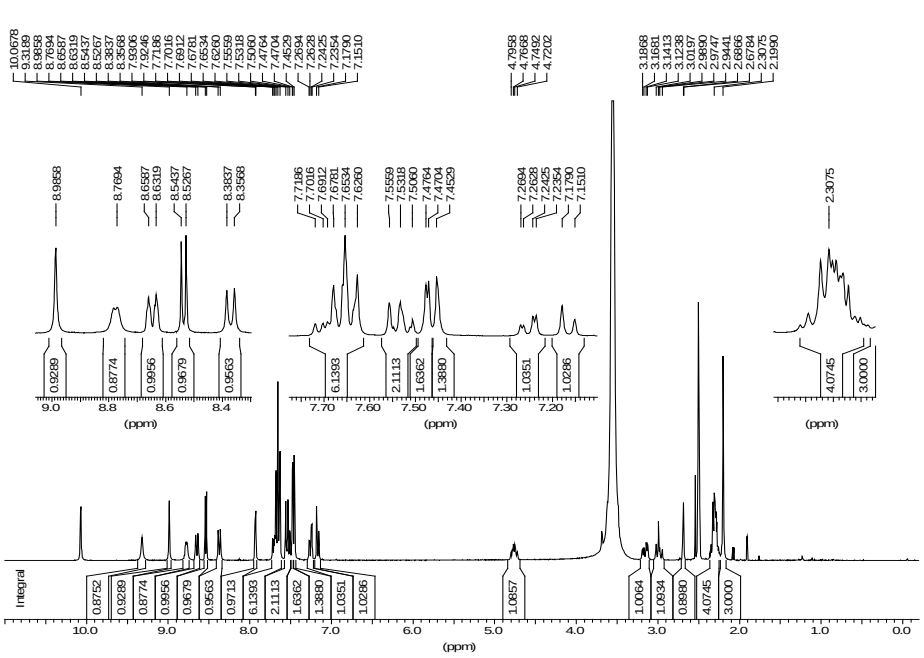
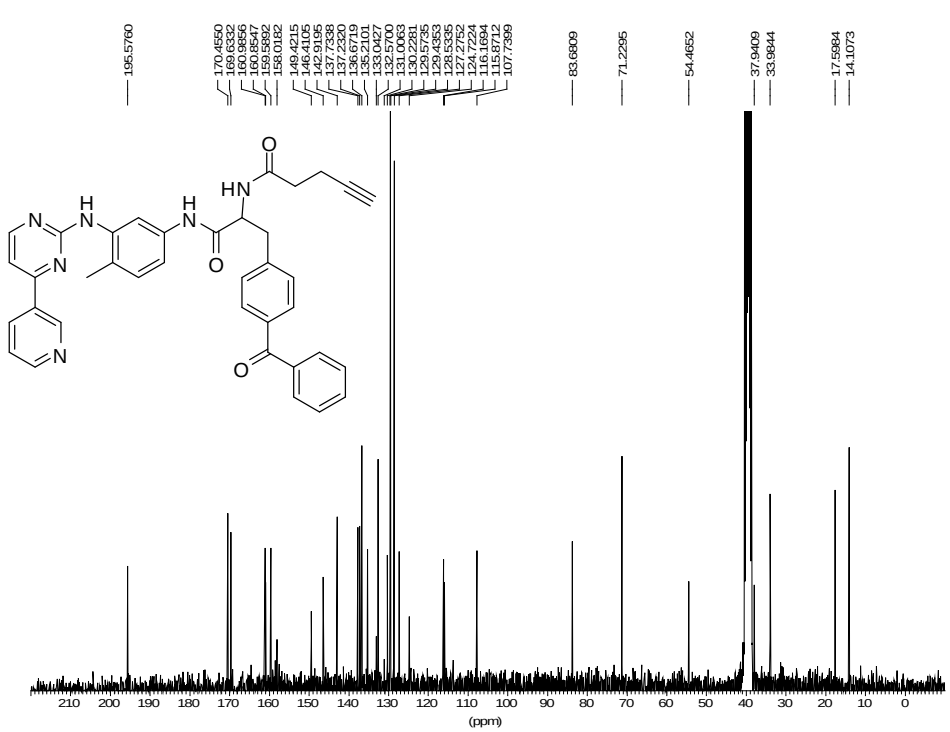
NS : 8
NUCLEUS : off
O1 : 1853.43 Hz
SFO1 300.1318534 MHz
SOLVENT : DMSO
*** 1D NMR Plot Parameters ***
NUCLEUS : off

1H normal range AC300, Photo CL-2



13C Standard AC300, CL-2



6. References

1. Seeliger, M. A.; Young, M.; Henderson, M. N.; Pellicena, P.; King, D. S.; Falick, A. M.; Kuriyan, J. *Protein Sci*. **2005**, 14, 3135-3139. (b) Khokhlatchev, A.; Xu, S.; English, J.; Wu, P.; Schaefer, E.; Cobb, M. H. *J. Biol. Chem.* **1997**, 272, 11057-11062. (c) Bougeret, C.; Rothhut, B.; Jullien, P.; Fischer, S.; Benarous, R. *Oncogene* **1993**, 8, 1241-1247. (d) Seeliger, M. A.; Nagar, B.; Frank, F.; Cao, X.; Henderson, M. N.; Kuriyan, J. *Structure* **2007**, 15, 299-311.
2. Liu, K.; Kalesh, K. A.; Ong, L. B.; Yao, S. Q. *ChemBioChem* **2008**, 9, 1883-1888.