Neolymphostin A is a Covalent Phosphoinositide-3-kinase (PI3-K)/Mammalian Target of Rapamycin (mTOR) Dual Inhibitor that Employs an Unusual Electrophilic Vinylogous Ester

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Figure S1. HPLC chromatogram (450 nm) before and after labeling reaction

C18(2) Phenomenex Luna, 100 x 4.6 mm, 5 μ, 100 Å
10-100% CH_3CN in water (0.1% FA) over 20 min, 0.7 mL min^{-1}

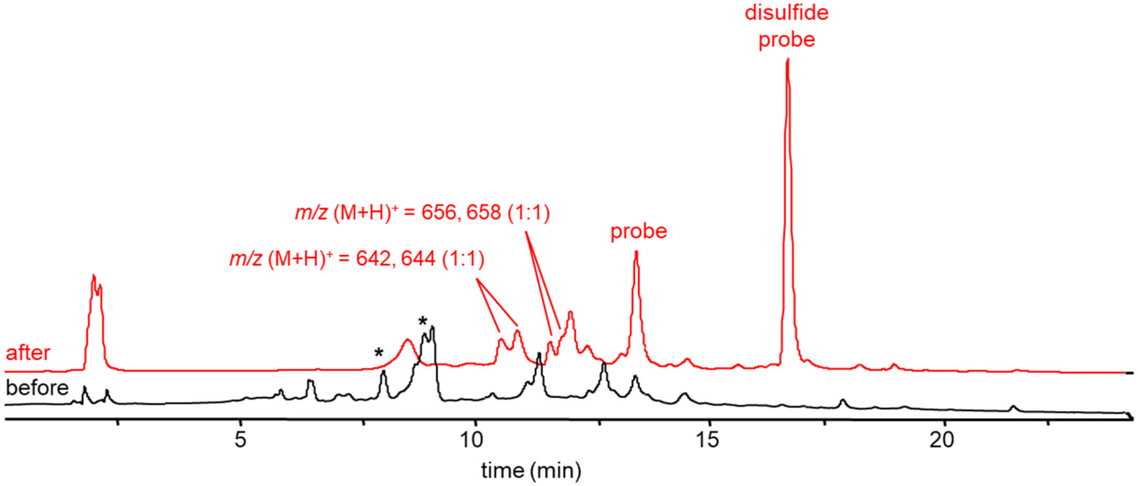
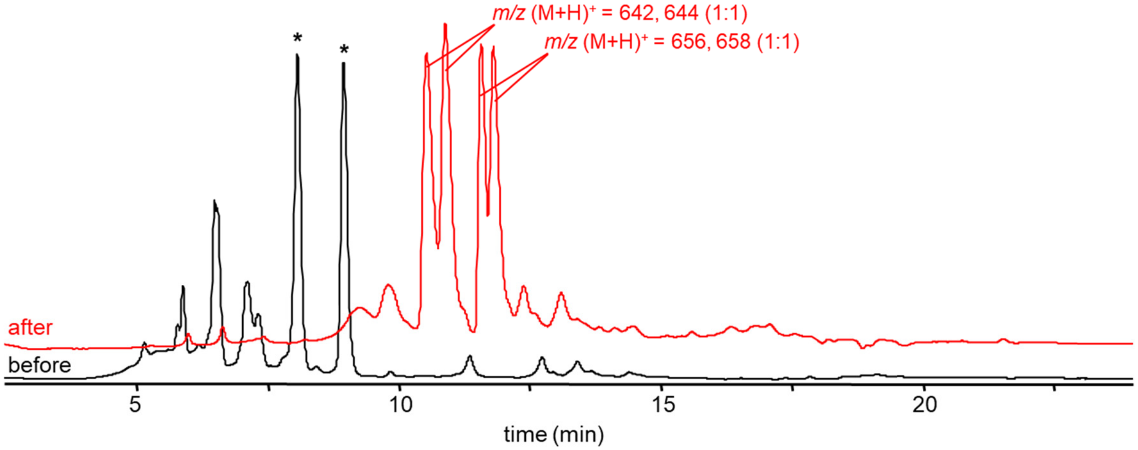


Figure S2. HPLC chromatogram (450 nm) before and after labeling reaction

C18(2) Phenomenex Luna, 100 x 4.6 mm, 5 μ, 100 Å
10-100% CH_3CN in water (0.1% FA) over 20 min, 0.7 mL min^{-1}



Asterisks (*) signal neolymphostin A (8.96 min) and neolymphostin B (8.08 min)

Figure S3. MS spectra of neolymphostin A/B–cysteine thiol probe adducts (**2a**/**2b**/**3a**/**3b**)

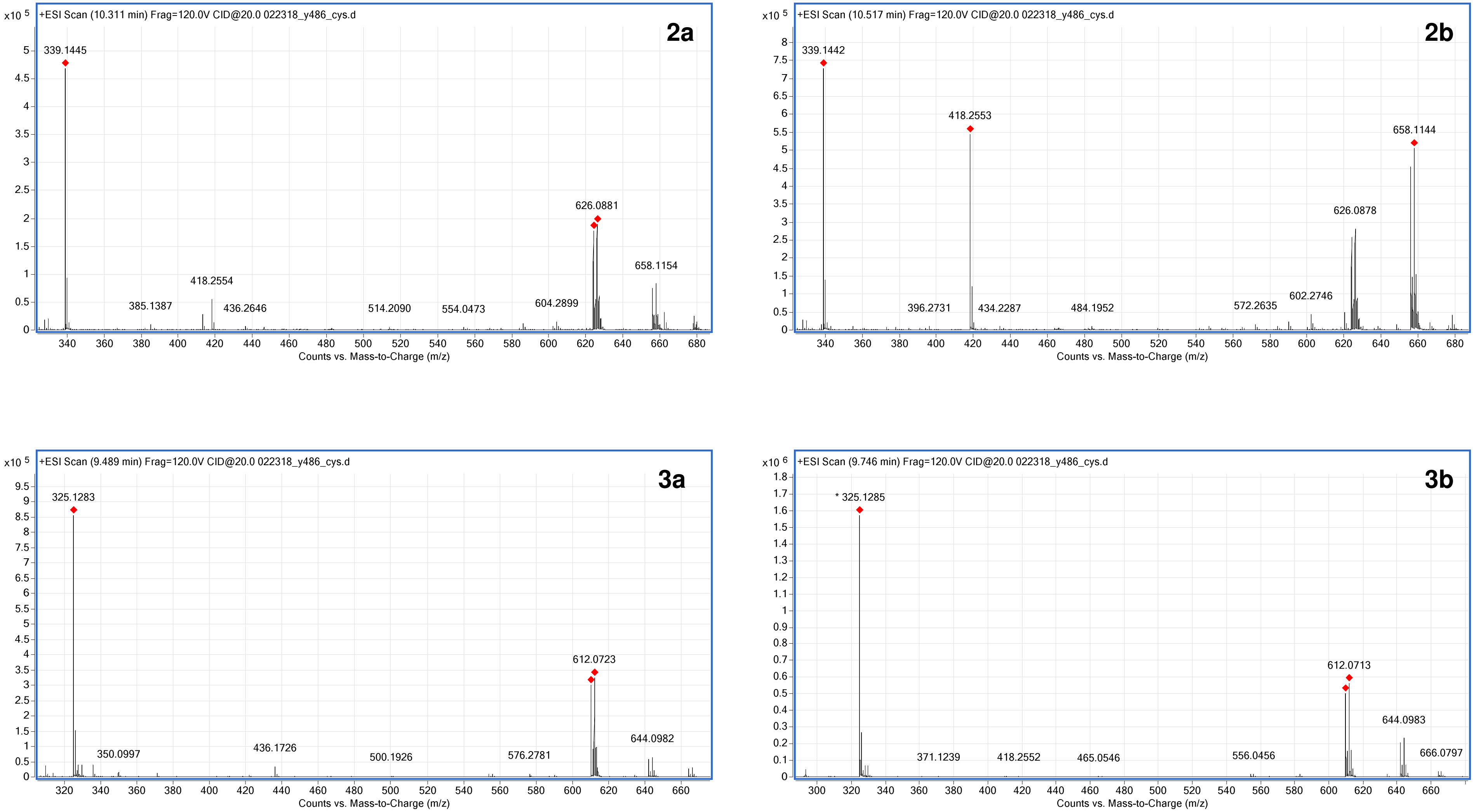
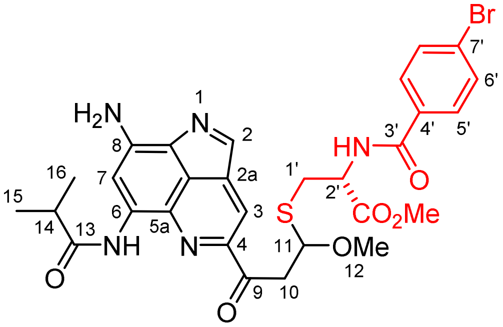


Table S1. NMR spectral data for neolymphostin A–cysteine thiol probe adduct (**2a**) in DMSO-d_6 at 500 MHz



diastereomer #1

Position #[a]	δ_H (mult., (J(Hz))
1	----
2	8.33 (s)
2a	----
3	8.47 (s)
4	----
5a	----
6	----
6-NH	9.82 (s)
7	8.04 (s)
8	----
8a	----
8b	----
9	----
10	a 3.78 (dd, 16.2, 5.6) b 4.12 (dd, 16.2, 8.0)
11	5.17 (dd, 7.8, 5.7)
12	3.27 (s)
13	----
14	2.92 (sept., 6.9)
15	1.14 (d, 6.8)
16	1.13 (d, 6.8)
1‘	a 3.02 (dd, 13.7, 9.4) b 3.20 (dd, 13.7, 5.1)
2‘	4.65 (m)
2‘-NH	8.93 (d, 7.8)
3‘	----
4‘	----
5‘	7.70 (d, 8.5)
6‘	7.59 (d, 8.5)
7‘	----
8‘	----
9‘	3.61 (s)

[a] Position numbering based on Aotani, et al. *J. Antibiot.* **1997**, *50*, 543–545.

^{1}H NMR (500 MHz, DMSO-d_6) of neolymphostin A–cysteine thiol probe adduct (**2a**)

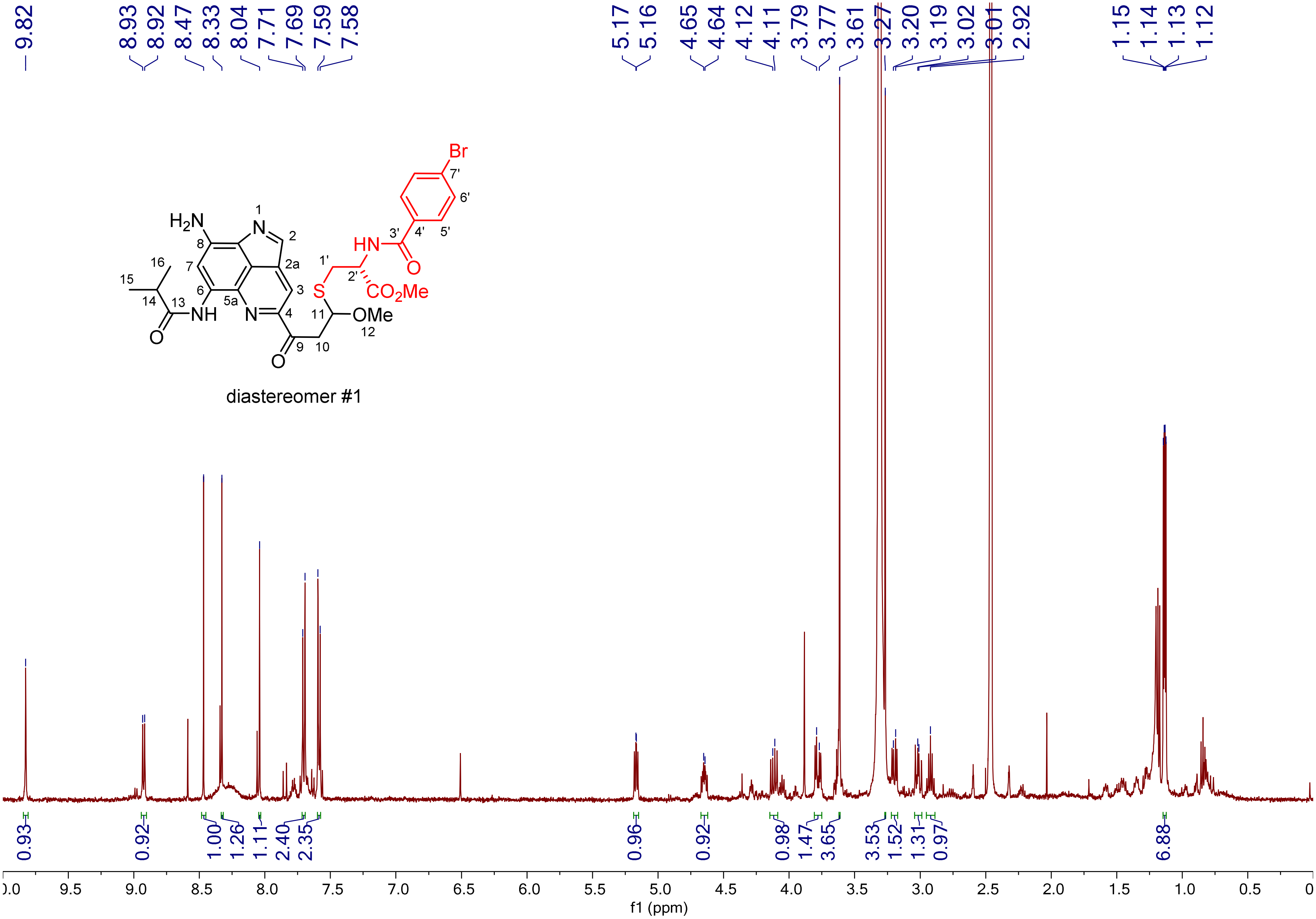
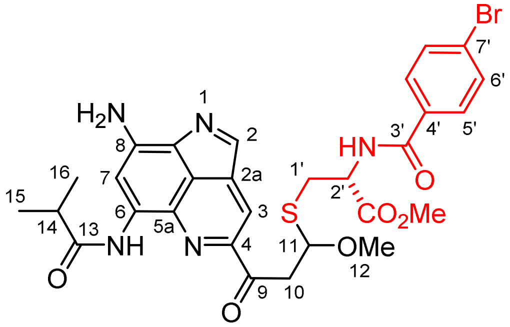


Table S2. NMR spectral data for neolymphostin A–cysteine thiol probe adduct (**2b**) in DMSO-d_6 at 500 MHz

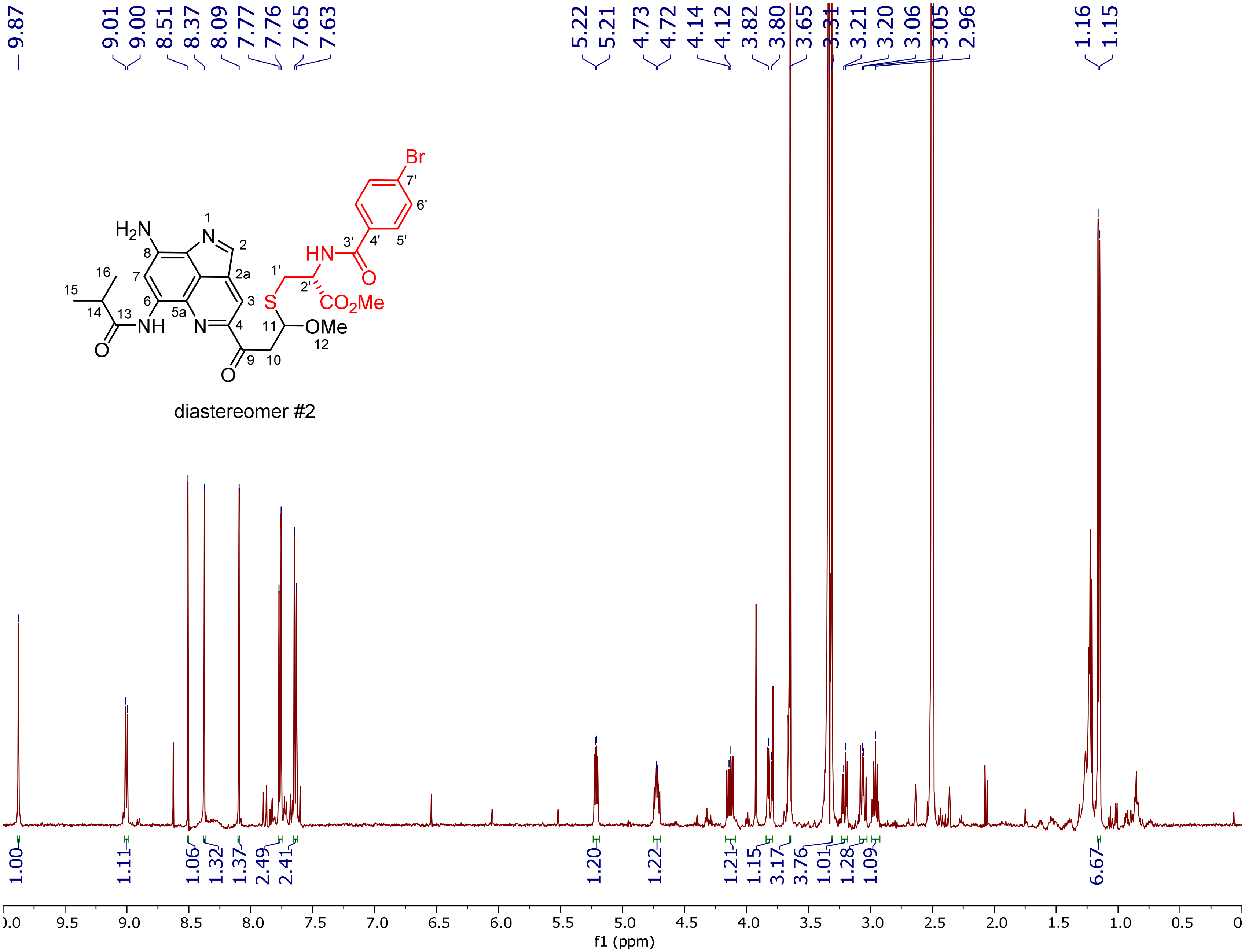


diastereomer #2

Position #[a]	δ_H (mult., J (Hz))	δ_C[b]	COSY	HMBC
1	----	----	----	----
2	8.37 (s)	145.8	----	2a, 8b
2a	----	136.6	----	----
3	8.51 (s)	117.6	----	4, 8b
4	----	146.2	----	----
5a	----	133.7	----	----
6	----	139.5	----	----
6-NH	9.87 (s)	----	----	5a, 7, 13
7	8.09 (s)	111.0	----	5a, 6, 8a
8	----	n.d.[c]	----	----
8a	----	124.6	----	----
8b	----	124.8	----	----
9	----	197.0	---	----
10	a 3.81 (dd, 16.5, 5.1) b 4.13 (dd, 16.5, 8.1)	44.4	10b, 11 10a, 11	9, 11 9, 11
11	5.22 (dd, 8.0, 5.1)	83.4	10a, 10b	12
12	3.31 (s)	54.8	----	11
13	----	176.1	----	----
14	2.96 (sept., 6.9)	35.1	15, 16	13, 15, 16
15	1.16 (d, 6.6)	19.3	14	13, 14, 16
16	1.16 (d, 6.6)	19.3	14	13, 14, 15
1‘	a 3.06 (dd, 13.7, 9.2) b 3.21 (dd, 13.7, 5.3)	29.5	1‘b, 2‘ 1‘a, 2‘	11, 8‘ 11, 8‘
2‘	4.73 (m)	53.2	1‘a, 1‘b, 2‘-NH	8‘
2‘-NH	9.01 (d, 7.8)	----	2‘	1‘, 3‘
3‘	----	165.7	----	----
4‘	----	132.6	----	----
5‘	7.77 (d, 8.6)	129.4	6‘	3‘, 7‘
6‘	7.64 (d, 8.6)	131.3	5‘	4‘, 7‘
7‘	----	125.2	----	----
8‘	----	171.1	----	----
9‘	3.65 (s)	52.0	---	8‘

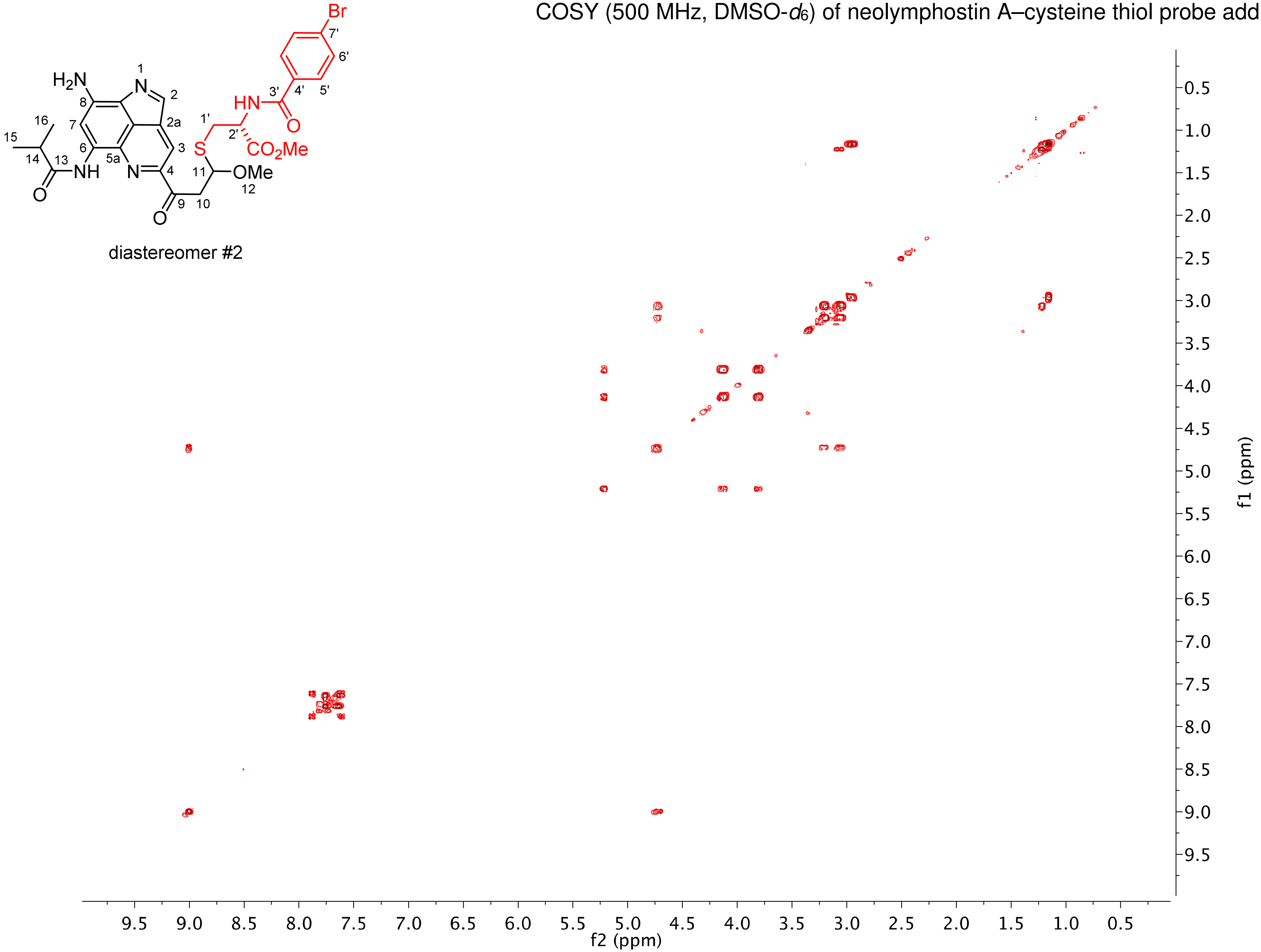
[a] Position numbering based on Aotani, et al. *J. Antibiot.* **1997**, *50*, 543–545.
[b] Carbon chemical shifts were based on HSQC and HMBC data
[c] n.d. = not determined

^{1}H NMR (500 MHz, DMSO-d_6) of neolymphostin A–cysteine thiol probe adduct (**2b**)



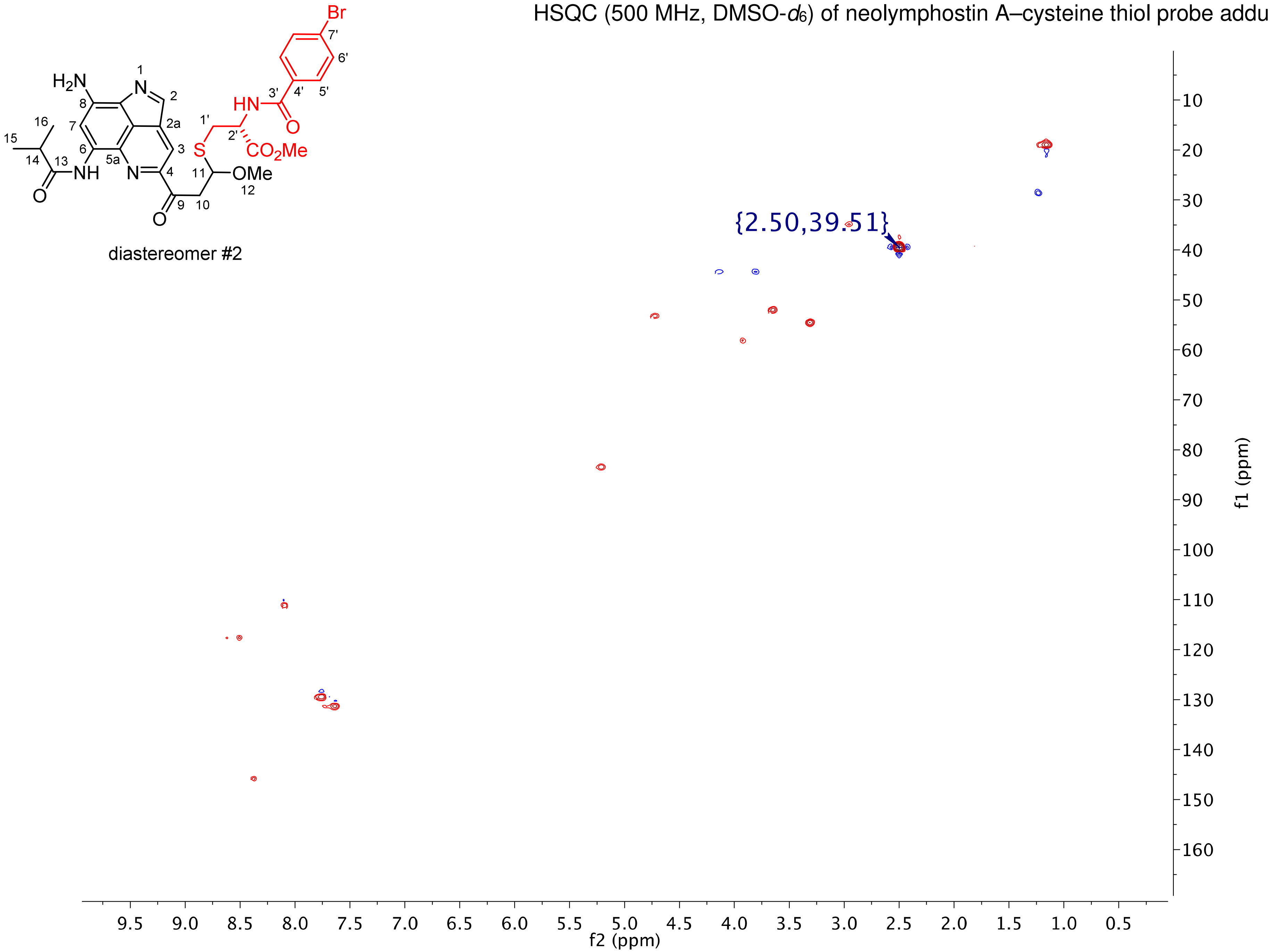
diastereomer #2

COSY (500 MHz, DMSO-d_6) of neolymphostin A–cysteine thiol probe adduct (**2b**)



diastereomer #2

HSQC (500 MHz, DMSO-d_6) of neolymphostin A–cysteine thiol probe adduct (**2b**)



HMBC (500 MHz, DMSO-d_6) of neolymphostin A–cysteine thiol probe adduct (**2b**)

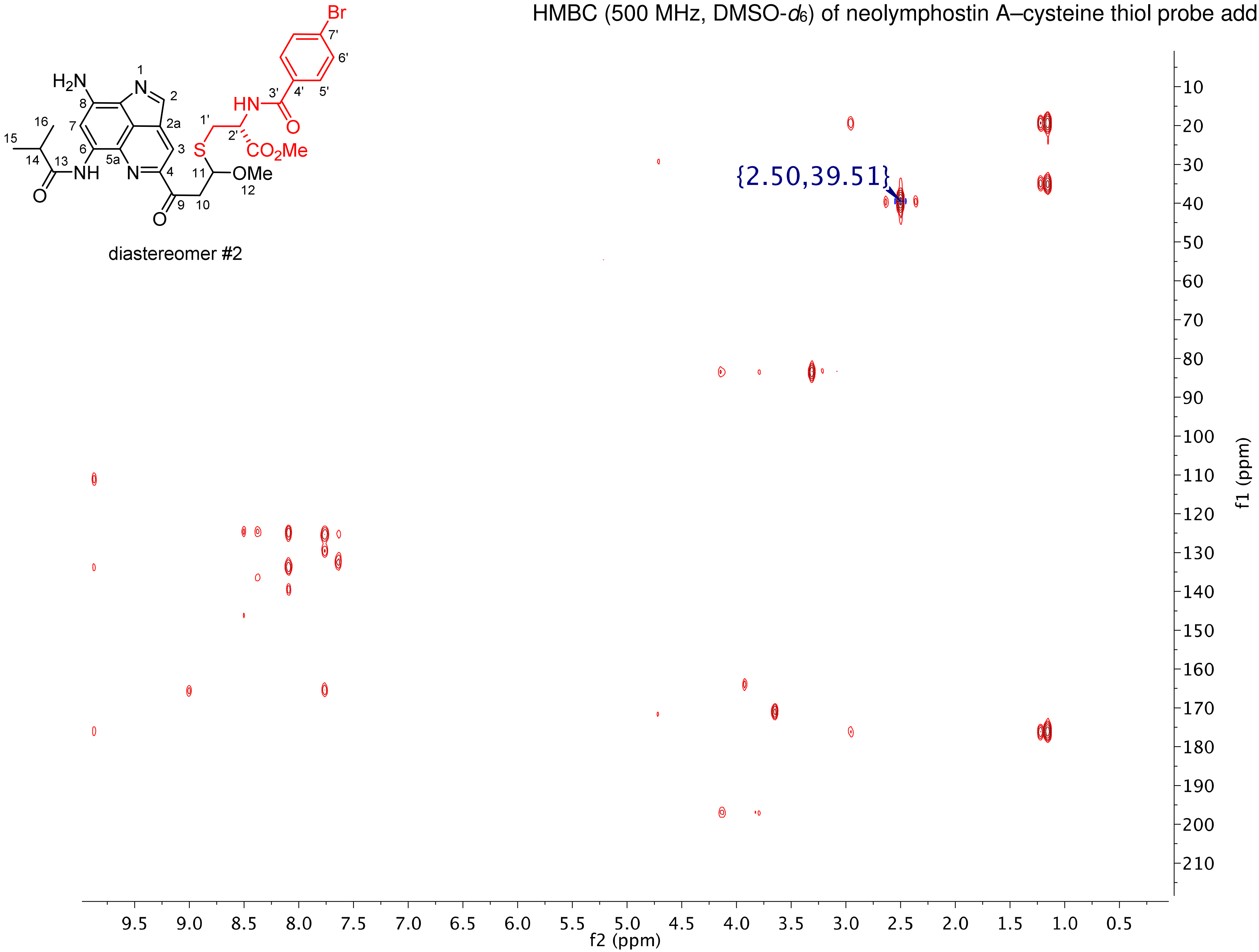
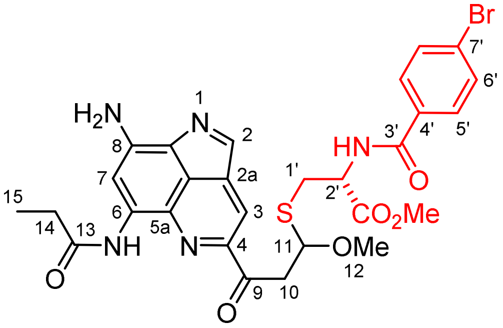


Table S3. NMR spectral data for neolymphostin B–cysteine thiol probe adduct (**3a**) in DMSO-d_6 at 500 MHz



diastereomer #1

Position #[a]	δ_H (mult., (J(Hz))
1	----
2	8.36 (s)
2a	----
3	8.51 (s)
4	----
5a	----
6	----
6-NH	9.90 (s)
7	8.12 (s)
8	----
8a	----
8b	----
9	----
10	a 3.83 (dd, 16.2, 5.6) b 4.19 (dd, 16.2, 7.8)
11	5.18 (dd, 7.6, 5.7)
12	3.31 (s)
13	----
14	2.60 (q, 7.5)
15	1.12 (t, 7.4)
1‘	a 3.07 (dd, 13.7, 9.3) b 3.27 (dd, 13.7, 5.3)
2‘	4.71 (m)
2‘-NH	8.96 (d, 7.8)
3‘	----
4‘	----
5‘	7.74 (d, 8.5)
6‘	7.61 (d, 8.5)
7‘	----
8‘	----
9‘	3.65 (s)

[a] Position numbering based on Aotani, et al. *J. Antibiot.* **1997**, *50*, 543–545.

^{1}H NMR (500 MHz, DMSO-d_6) of neolymphostin B–cysteine thiol probe adduct (**3a**)

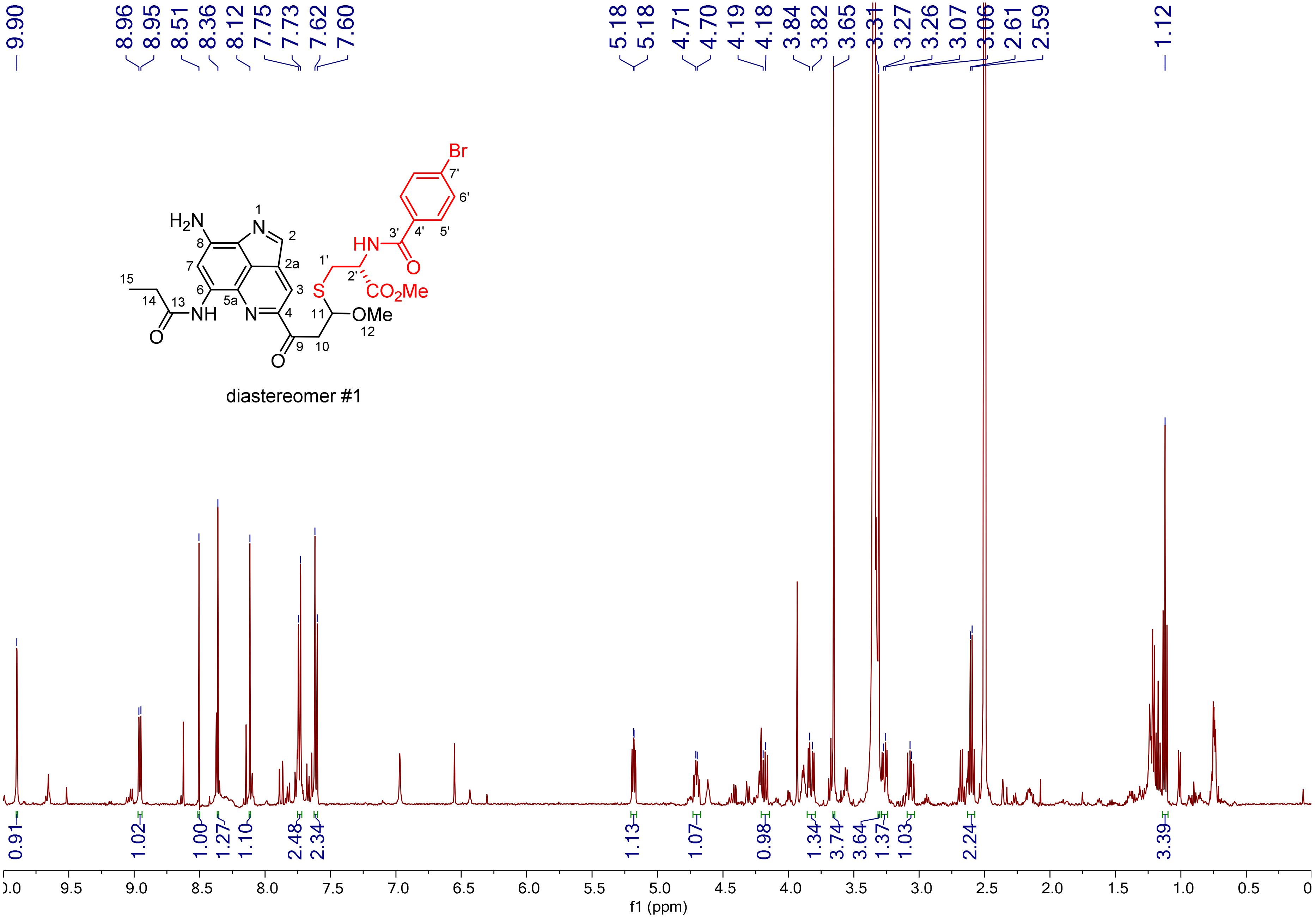
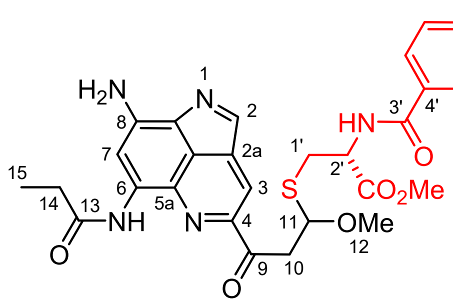


Table S4. NMR spectral data for neolymphostin B–cysteine thiol probe adduct (**3b**) in DMSO-d_6 at 500 MHz



diastereomer #2

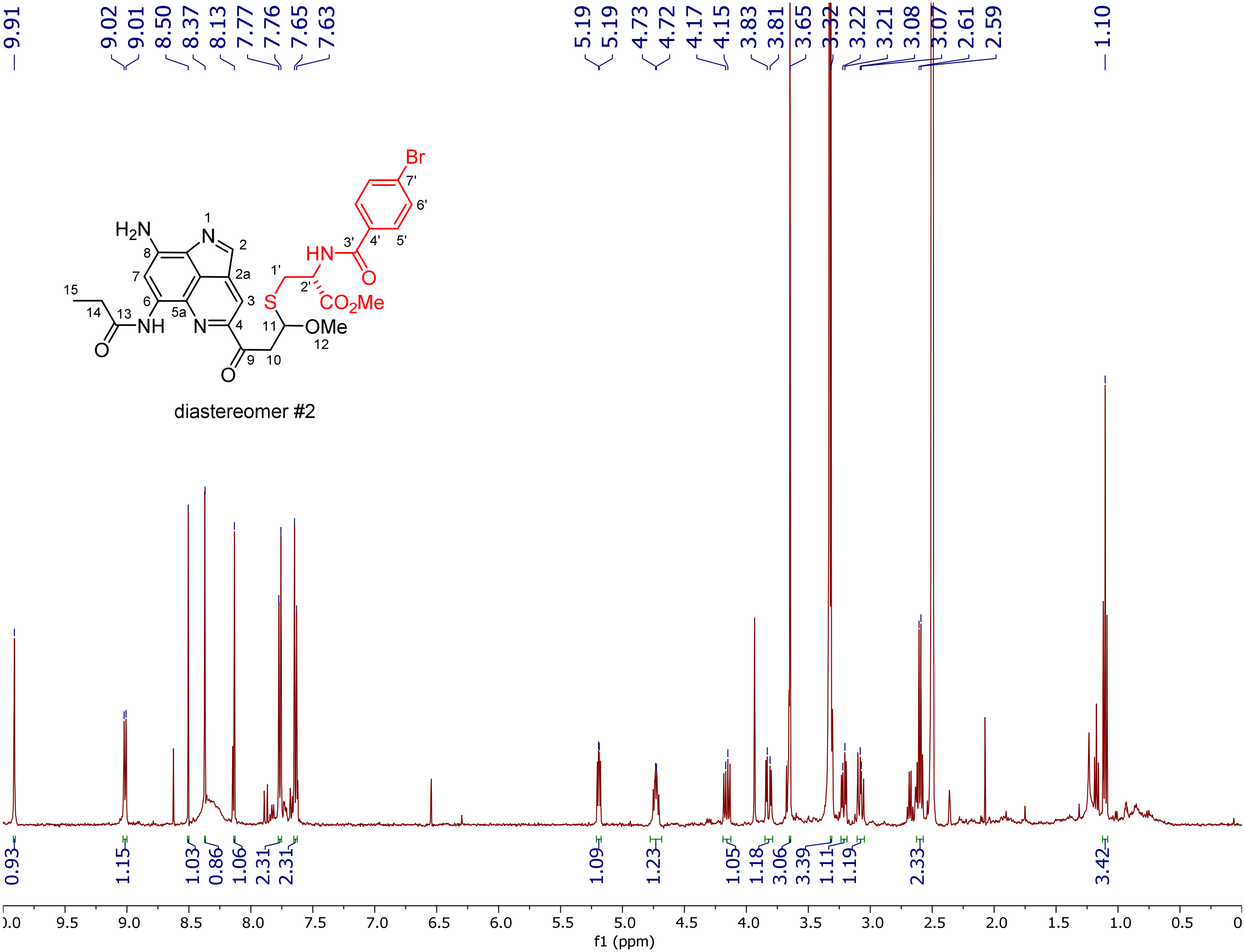
Position #[a]	δ_H (mult., (J(Hz))	δ_C[b]	COSY	HMBC
1	----	----	----	----
2	8.37 (s)	145.5	----	2a, 8b
2a	----	136.3	----	----
3	8.50 (s)	117.4	----	4, 8b
4	----	145.4	----	----
5a	----	133.6	----	----
6	----	139.3	----	----
6-NH	9.91 (s)	----	----	5a, 7, 13
7	8.13 (s)	110.4	----	5a, 6, 8a
8	----	n.d.[c]	----	----
8a	----	124.8	----	----
8b	----	124.4	----	----
9	----	198.0	---	----
10	a 3.82 (dd, 16.8, 5.1) b 4.16 (dd, 16.8, 8.2)	44.4	10b, 11 10a, 11	9, 11 9, 11
11	5.19 (dd, 8.0, 5.1)	83.4	10a, 10b	12, 1‘
12	3.32 (s)	54.6	----	11
13	----	173.2	----	----
14	2.60 (q, 7.5)	29.5	15	13, 15
15	1.10 (t, 7.5)	9.0	14	13, 14
1‘	a 3.08 (dd, 13.9, 9.5) b 3.22 (dd, 13.9, 5.6)	28.9	1‘b, 2‘ 1‘a, 2‘	11, 2‘ 11
2‘	4.73 (m)	53.2	1‘a, 1‘b, 2‘-NH	8‘
2‘-NH	9.02 (d, 7.8)	----	2‘	3‘
3‘	----	165.4	----	----
4‘	----	132.6	----	----
5‘	7.77 (d, 8.4)	129.2	6‘	3‘, 7‘
6‘	7.64 (d, 8.4)	131.1	5‘	4‘, 7‘
7‘	----	125.4	----	----
8‘	----	171.0	----	----
9‘	3.65 (s)	52.0	---	8‘

[a] Position numbering based on Aotani, et al. *J. Antibiot.* **1997**, *50*, 543–545.

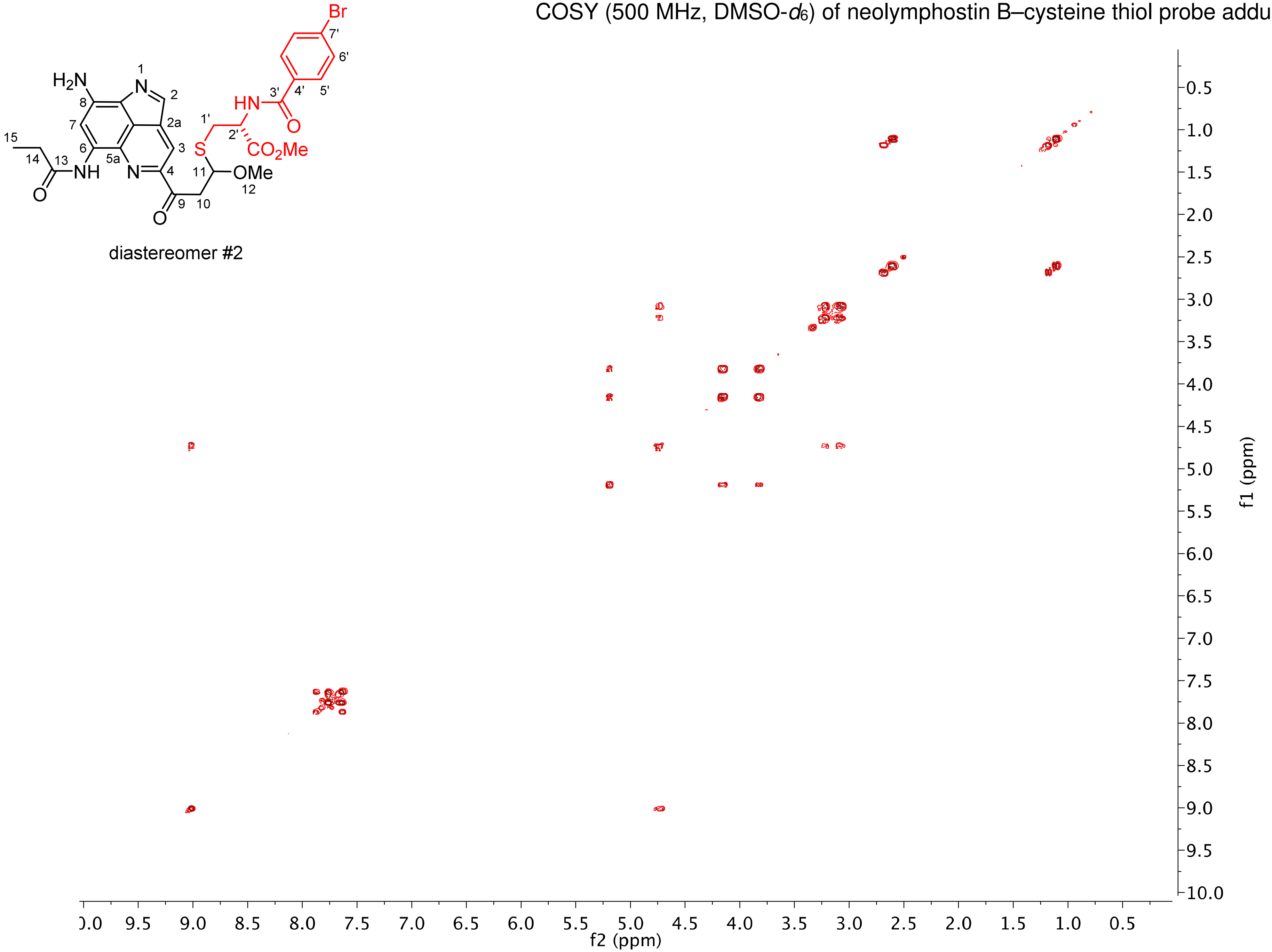
[b] Carbon chemical shifts were based on HSQC and HMBC data

[c] n.d. = not determined

^{1}H NMR (500 MHz, DMSO-d_6) of neolymphostin B–cysteine thiol probe adduct (**3b**)

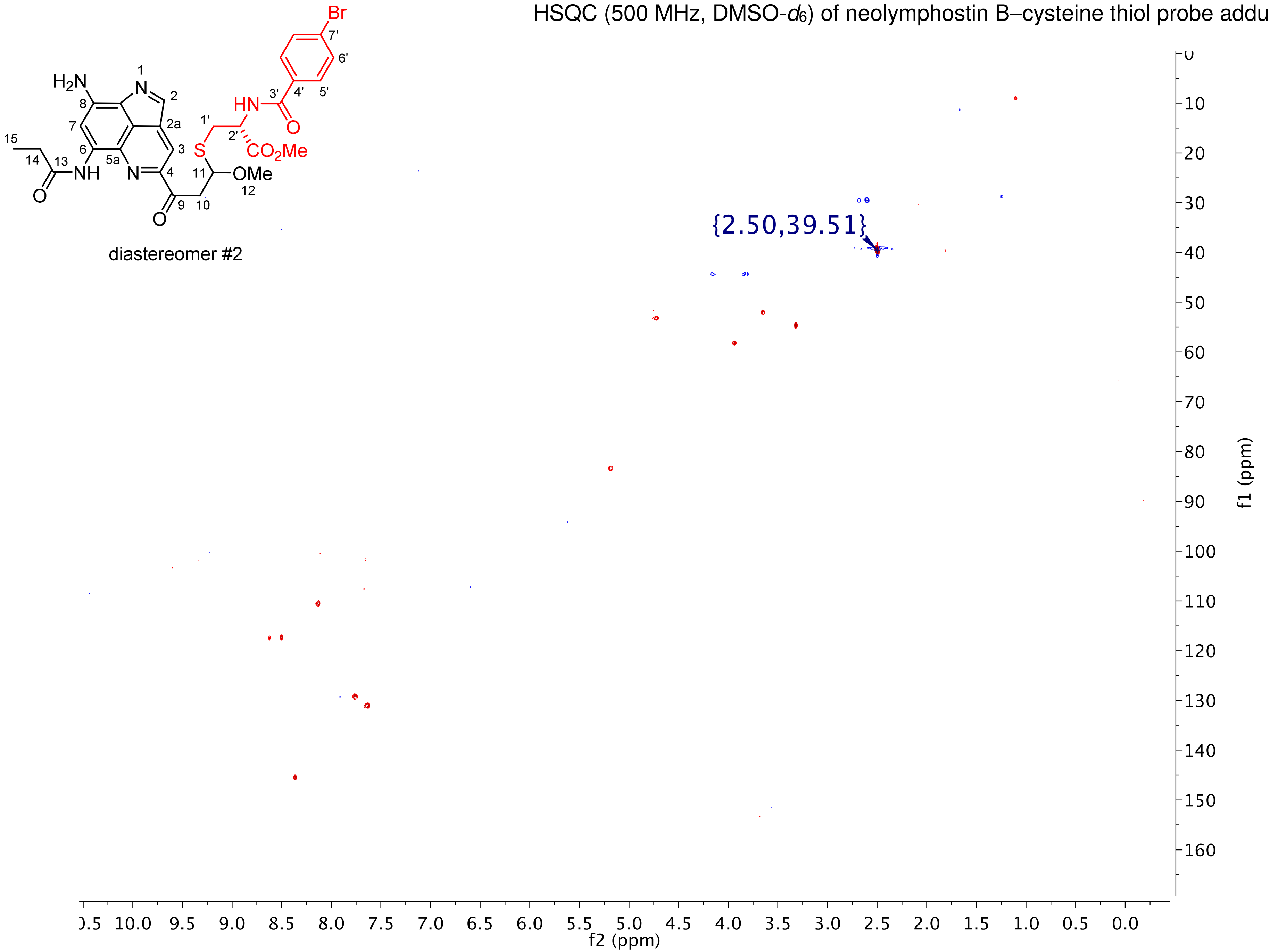


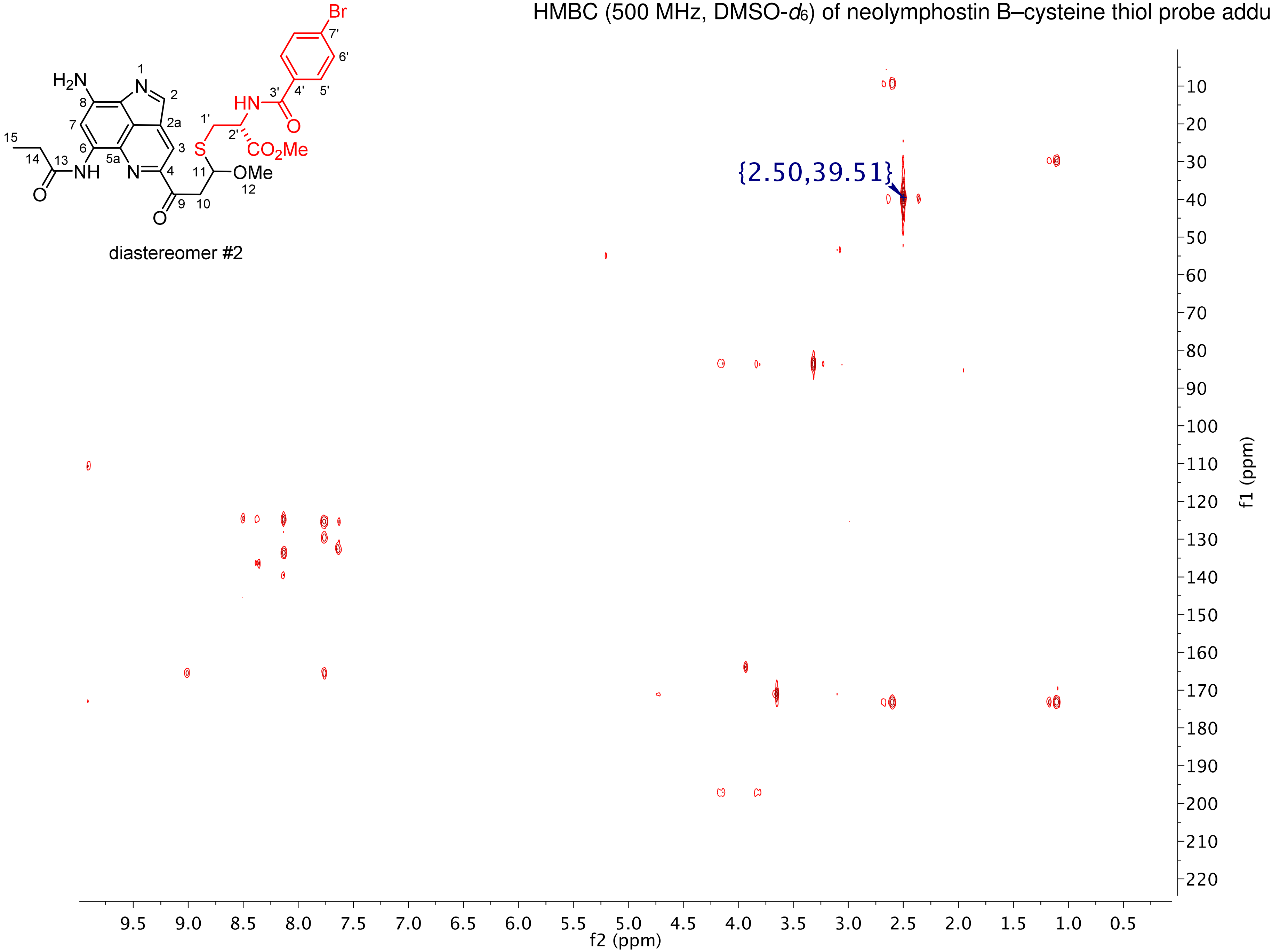
COSY (500 MHz, DMSO-d_6) of neolymphostin B–cysteine thiol probe adduct (**3b**)



diastereomer #2

HSQC (500 MHz, DMSO-d_6) of neolymphostin B–cysteine thiol probe adduct (**3b**)

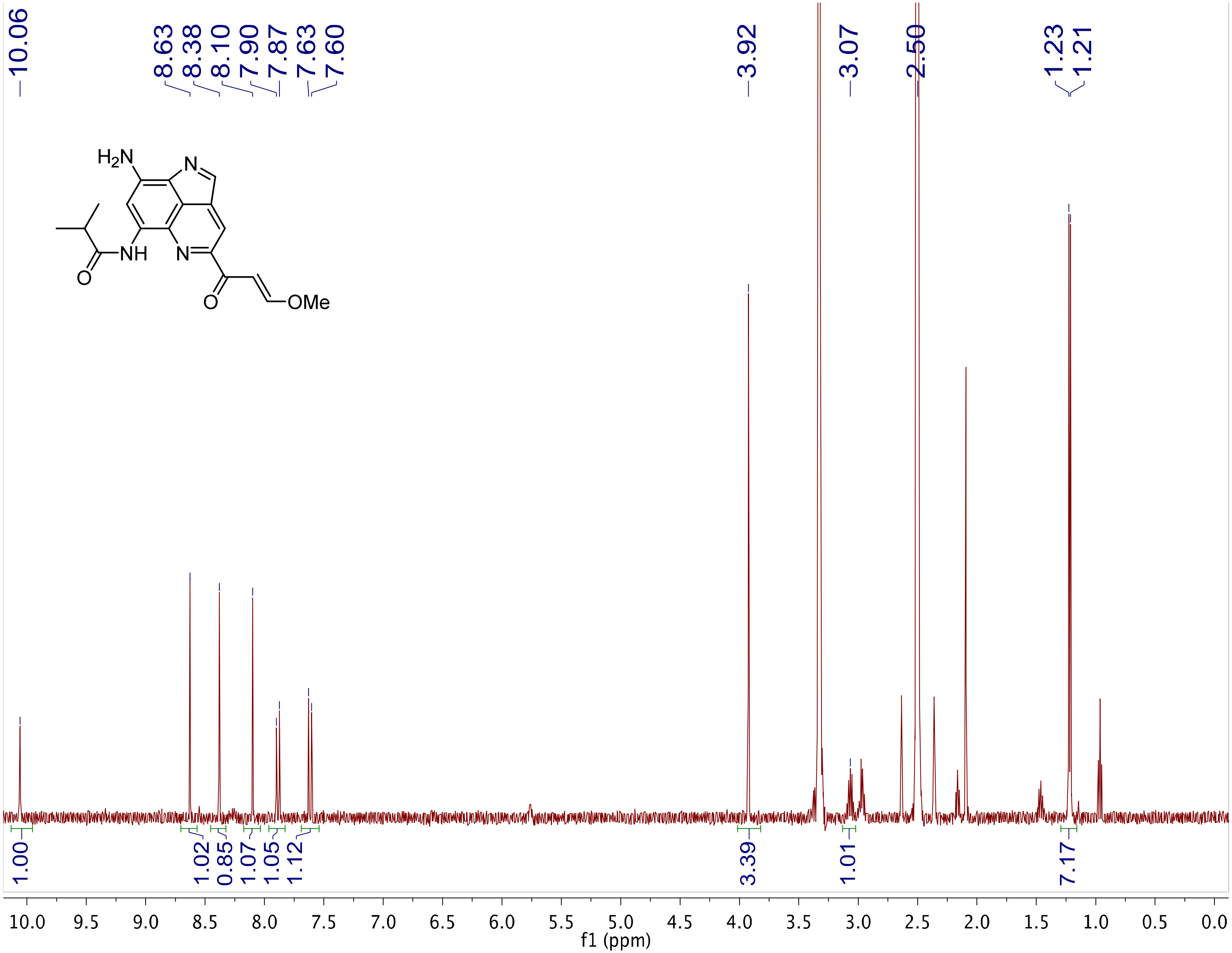




diastereomer #2

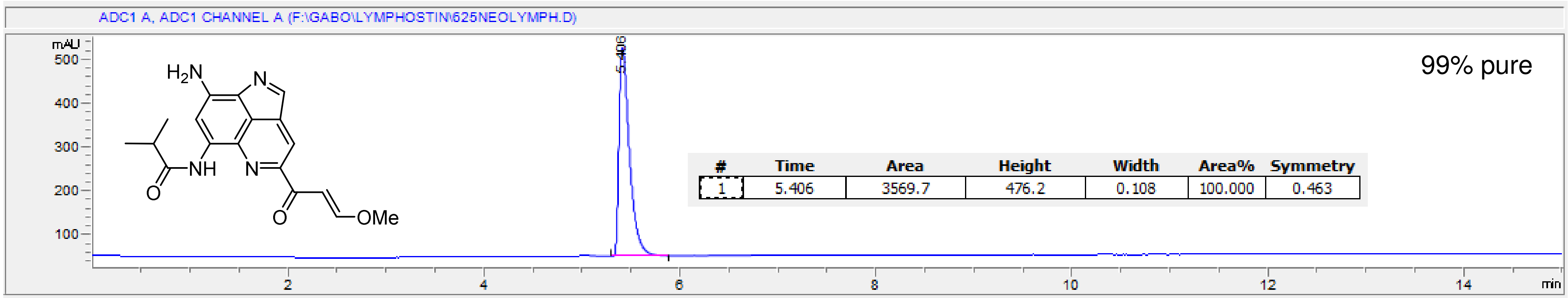
HMBC (500 MHz, DMSO-*d*6) of neolymphostin B–cysteine thiol probe adduct (**3b**)

^{1}H NMR (500 MHz, DMSO-d_6) of neolymphostin A (**4**)



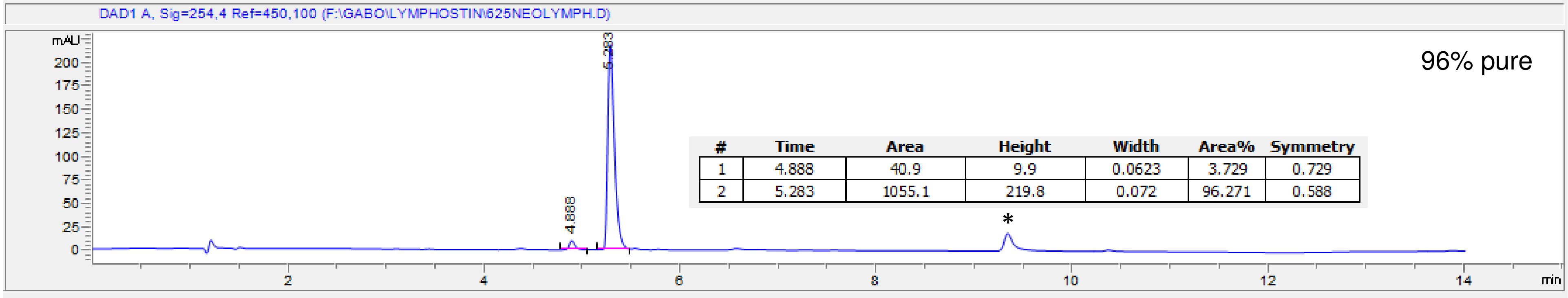
HPLC chromatogram of neolymphostin A (**4**)

C18(2) Luna Phenomenex, 100 x 4.6 mm, 5 µ, 100 Å
10-100% CH_3CN in water (0.1% FA) over 10 min, 1.0 mL min^{-1}
Top: ELSD; Bottom: 254 nm



99% pure

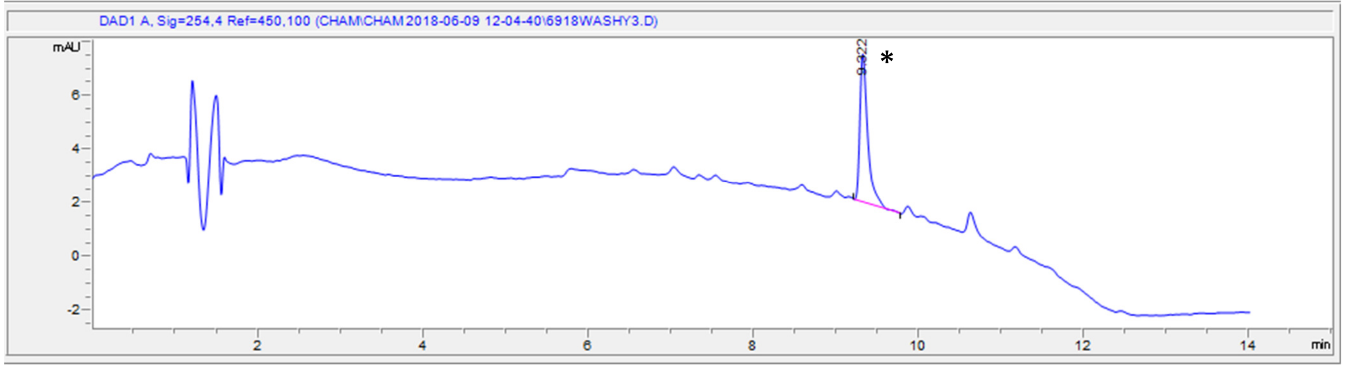
#	Time	Area	Height	Width	Area%	Symmetry
1	5.406	3569.7	476.2	0.108	100.000	0.463



96% pure

#	Time	Area	Height	Width	Area%	Symmetry
1	4.888	40.9	9.9	0.0623	3.729	0.729
2	5.283	1055.1	219.8	0.072	96.271	0.588

Blank run showing “ghost peak” at 9.3 min (254 nm)



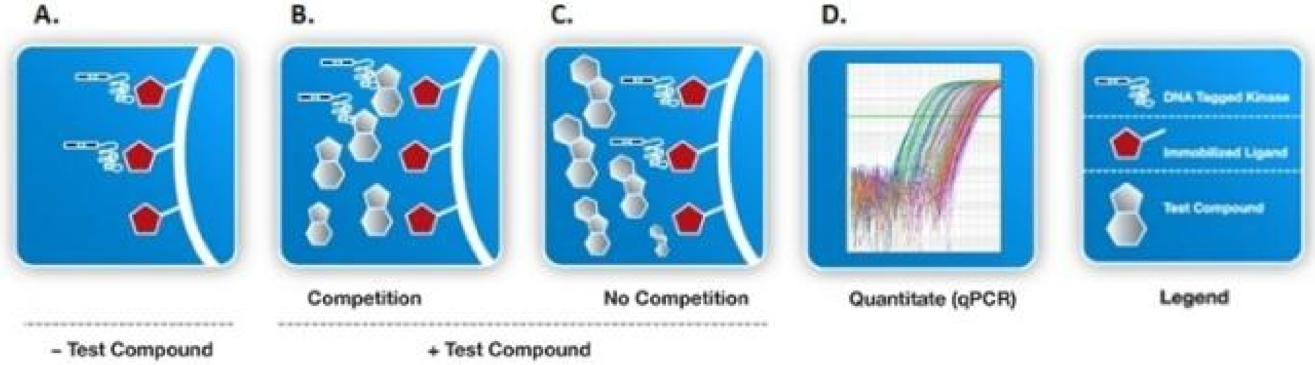
Description of KINOMEscan

Technology Overview

The KINOMEscan™ screening platform employs a novel and proprietary active site-directed competition binding assay to quantitatively measure interactions between test compounds and more than 450 human kinases and disease relevant mutant variants. This robust and reliable assay technology affords investigators the ability to extensively annotate compounds with accurate, precise and reproducible data. KINOMEscan™ assays do not require ATP and thereby report true thermodynamic interaction affinities, as opposed to IC_{50} values, which can depend on the ATP concentration.

How KINOMEscan™ Works

Compounds that bind the kinase active site and directly (sterically) or indirectly (allosterically) prevent kinase binding to the immobilized ligand, will reduce the amount of kinase captured on the solid support (A & B). Conversely, test molecules that do not bind the kinase have no effect on the amount of kinase captured on the solid support (C). Screening "hits" are identified by measuring the amount of kinase captured in test versus control samples by using a quantitative, precise and ultra-sensitive qPCR method that detects the associated DNA label (D). In a similar manner, dissociation constants (Kds) for test compound-kinase interactions are calculated by measuring the amount of kinase captured on the solid support as a function of the test compound concentration.



Kinase assays

For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2µm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 % SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05 % Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

Binding Constants (K_ds)

Binding constants (K_ds) were calculated with a standard dose-response curve using the Hill equation:

$$\text{Response} = \text{Background} + \frac{\text{Signal} - \text{Background}}{1 + \left(\text{K}_\text{d}^{\text{Hill Slope}} / \text{Dose}^{\text{Hill Slope}}\right)}$$

The Hill Slope was set to -1.

Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

Compound Handling

An 11-point 3-fold serial dilution of each test compound was prepared in 100% DMSO at 100x final test concentration and subsequently diluted to 1x in the assay (final DMSO concentration = 1%). Most K_ds were determined using a compound top concentration = 30,000 nM. If the initial K_d determined was < 0.5 nM (the lowest concentration tested), the measurement was repeated with a serial dilution starting at a lower top concentration. A K_d value reported as 40,000 nM indicates that the K_d was determined to be >30,000 nM.

scanKINETIC platform

This protocol offers dissociation kinetics to classify inhibitors as irreversible, reversible-slow dissociation, or reversible-rapid dissociation. Kinetic dissociation constants were measured like described above but equilibration time and dilutions were varied in a set of four different experiments, study arms A-D. In study arm A and C, the compound and kinase were combined and equilibrated for 6 h and 1 h, respectively, before measuring the K_d. In study arm B the compound and kinase were combined and equilibrated for 1 h, diluted 30-fold, and re-equilibrated for 5 h. In study arm D the compound and kinase were pre-diluted, combined, and equilibrated for 5 h

Percent Control (%Ctrl)

Neolymphostin A was screened at 1000nm against a panel of kinases and results for primary screen binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits.

$$\%\text{Ctrl} = \left(\frac{\text{test compound signal} - \text{positive control signal}}{\text{negative control signal} - \text{prositive control signal}}\right) \text{X } 100$$

test compound = neolymphostin A, negative control = DMSO (100%Ctrl), positive control = control compound (0%Ctrl)

Selectivity Score (S-scores)

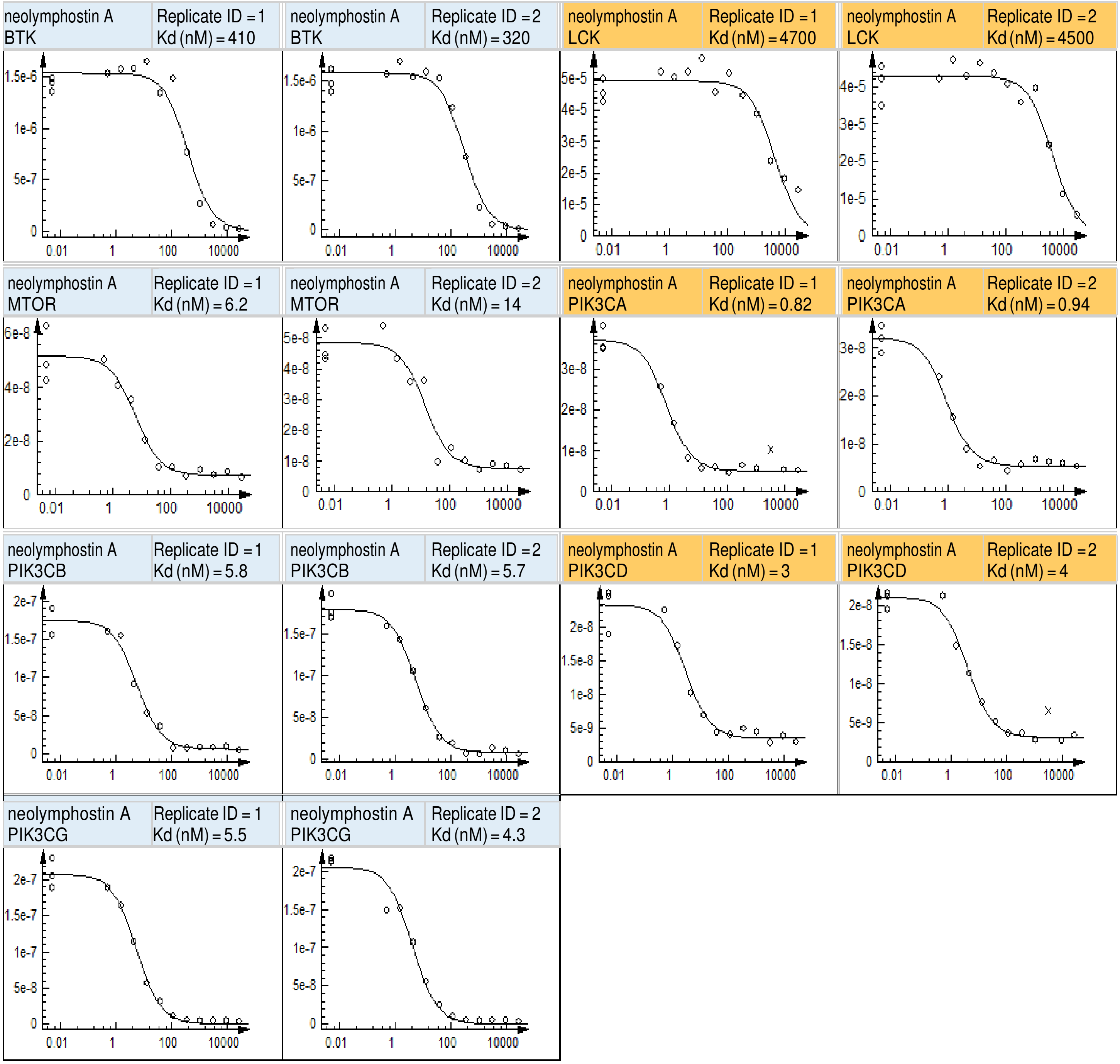
Selectivity Score or S-score is a quantitative measure of compound selectivity. It is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested, excluding mutant variants. This value can be calculated using %Ctrl as a potency threshold (below) and provides a quantitative method of describing compound selectivity to facilitate comparison of different compounds.

S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested)
S(10) = (number of non-mutant kinases with %Ctrl <10)/(number of non-mutant kinases tested)
S(1) = (number of non-mutant kinases with %Ctrl <1)/(number of non-mutant kinases tested)

Table S5. Dissociation constants (K_d) for neolymphostin A and selected kinases

Target	neolymphostin A
Gene Symbol	K_d (nM)
BTK	3370
LCK	4600
MTOR	10
PIK3CA	0.88
PIK3CB	5.7
PIK3CD	3.5
PIK3CG	4.9

Curve images for dissociation constants (K_d) for neolymphostin A and selected kinases

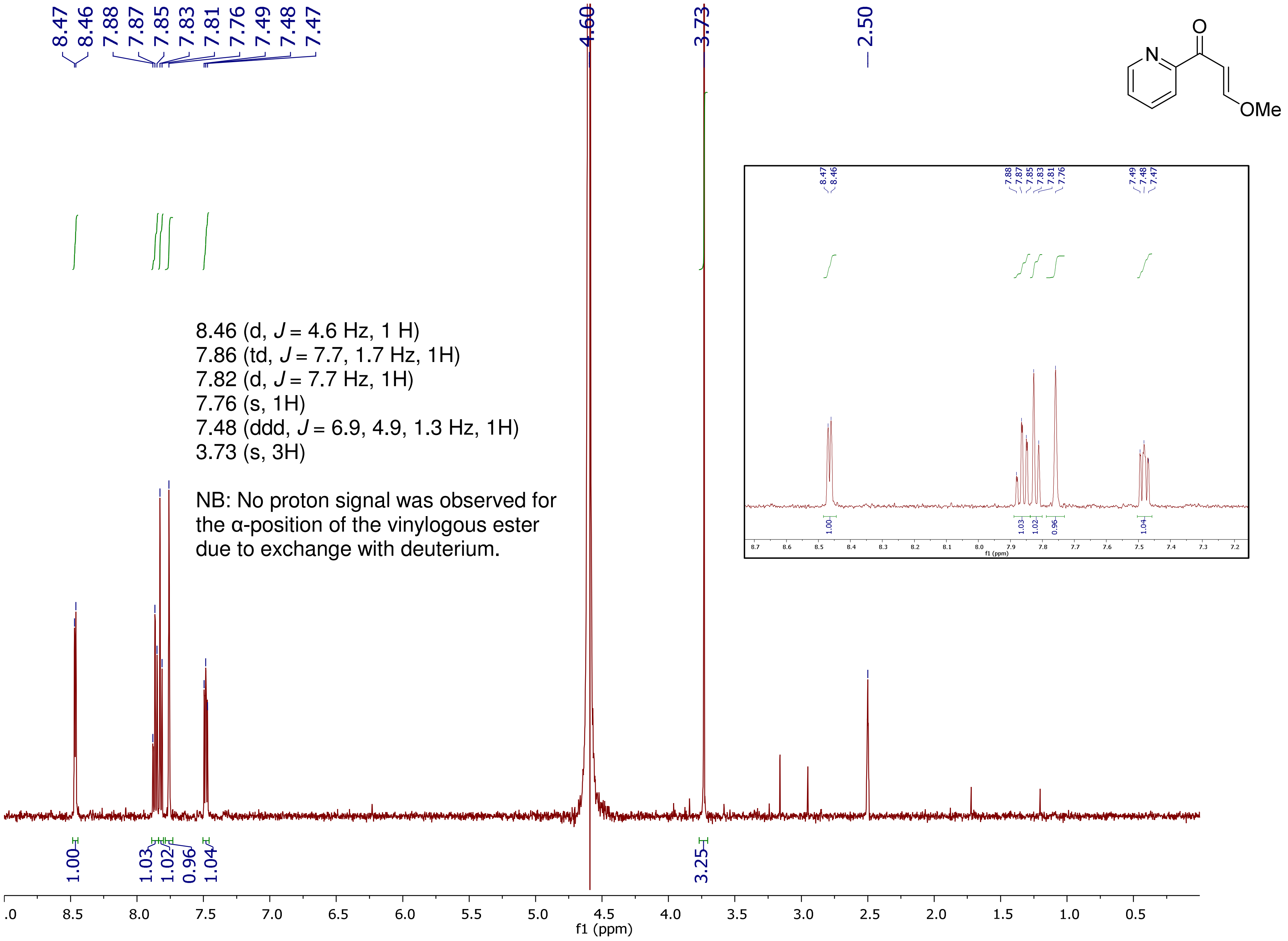


The amount of kinase measured by qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Data points marked with an "x" were not used for K_d determination.

Table S6. Dissociation constants (K_d) for wortmannin and selected kinases

Target	neolymphostin A
Gene Symbol	K_d (nM)
MTOR	9200
PIK3CA	5.4
PIK3CB	7.6
PIK3CD	15
PIK3CG	5.5

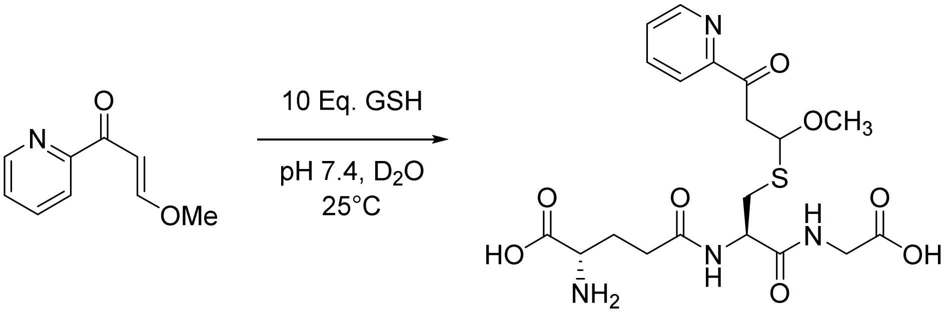
H NMR (500 MHz, phosphate-buffered D_2O) of **6**



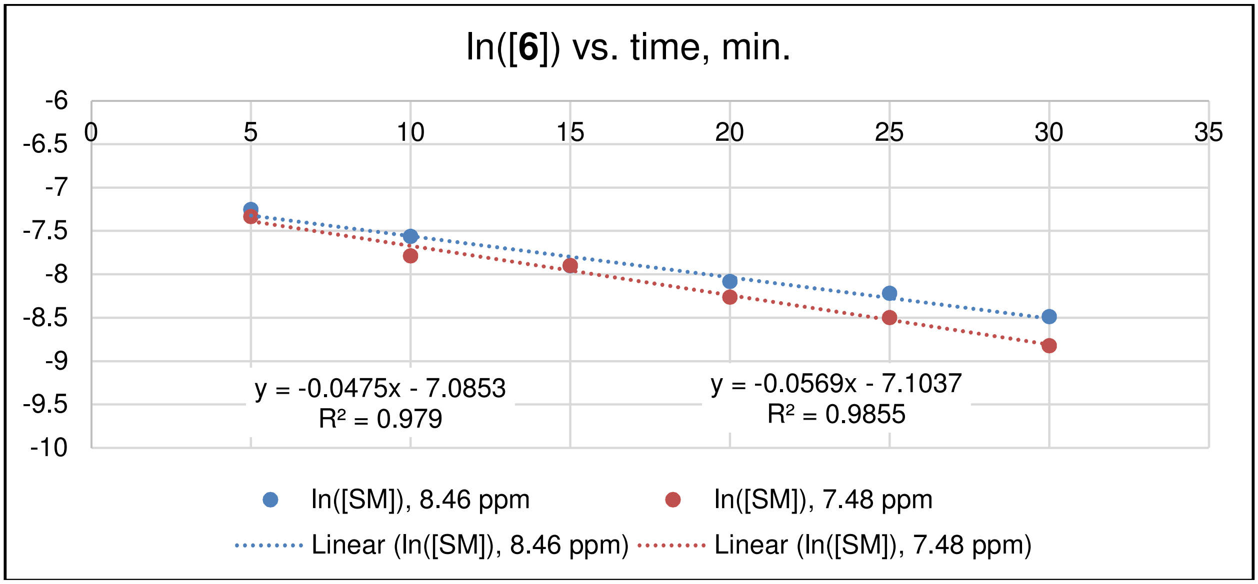
8.46 (d, *J* = 4.6 Hz, 1 H)
7.86 (td, *J* = 7.7, 1.7 Hz, 1H)
7.82 (d, *J* = 7.7 Hz, 1H)
7.76 (s, 1H)
7.48 (ddd, *J* = 6.9, 4.9, 1.3 Hz, 1H)
3.73 (s, 3H)

NB: No proton signal was observed for the α-position of the vinylogous ester due to exchange with deuterium.

Figure S4a. ^{1}H NMR kinetics data for **6** reacting with GSH in phosphate-buffered D_2O, pH 7.4, 25°C



time, min.	ln([**6**]), 8.46 ppm	ln([**6**]), 7.48 ppm
5	-7.253256922	-7.331238893
10	-7.56062856	-7.785473736
15	-7.901007052	-7.898153983
20	-8.08047554	-8.259364082
25	-8.218337818	-8.496990484
30	-8.485104877	-8.822574841



	signal at 8.46 ppm	signal at 7.48 ppm	average
slope of ln([**6**]) vs. time	-0.0475	-0.0569	-0.0522[a]
calculated half-life, min.	14.6	12.2	13.3[b]

[a] Average of slopes of each linear regression line

[b] Calculated by $t_{1/2} = \ln(2)/(-1*\text{average slope})$

Stacked ^{1}H NMR spectra of **6** reacting with GSH in phosphate-buffered D_2O, pH 7.4, 25°C

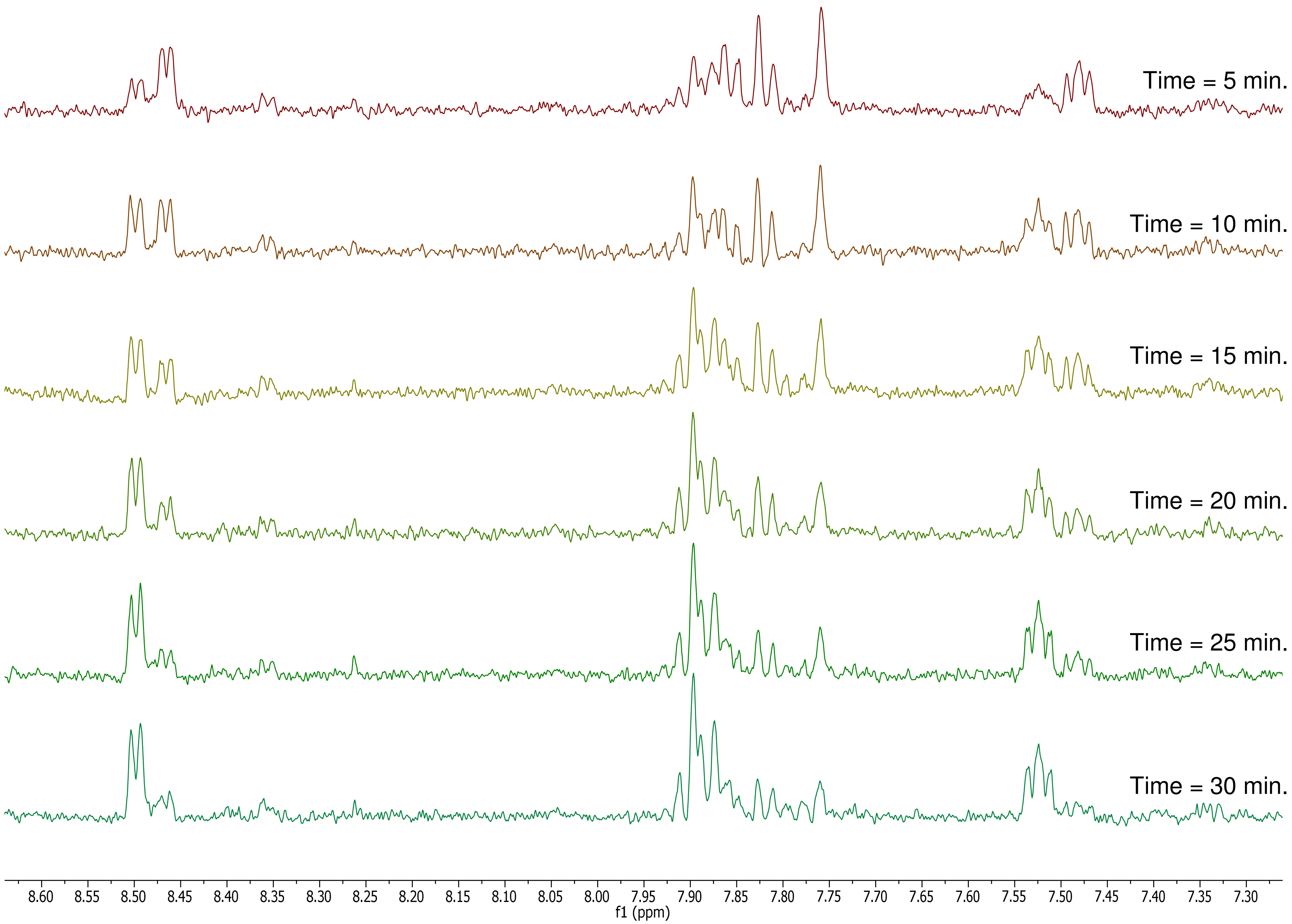
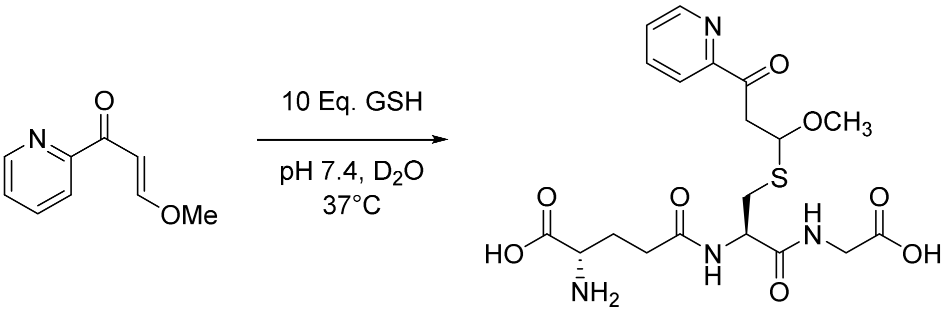
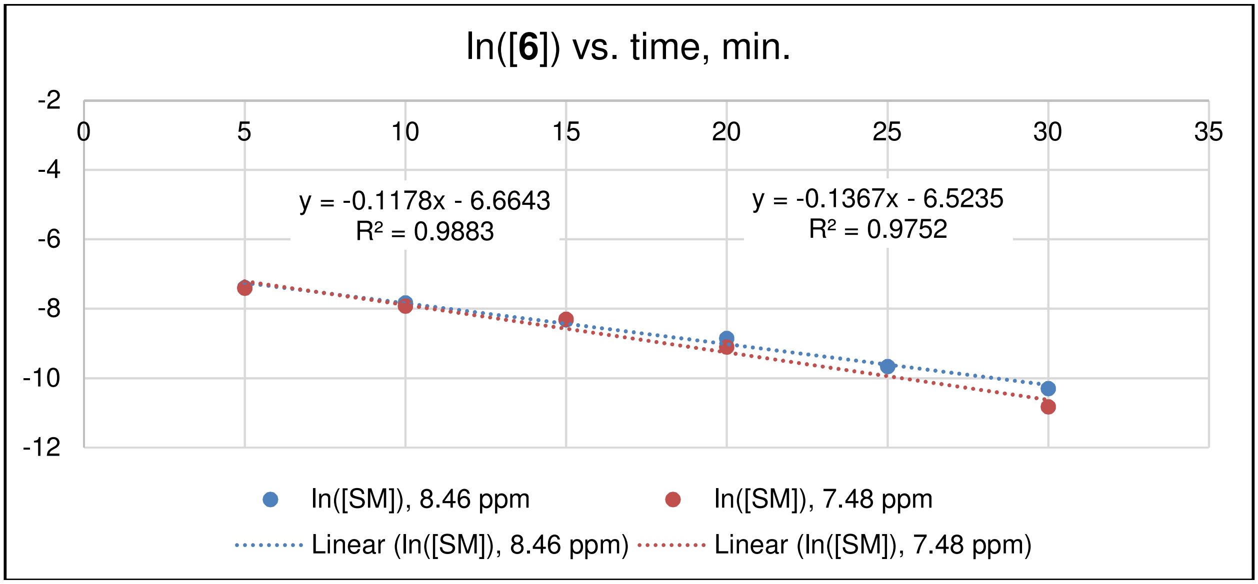


Figure S4b. ^{1}H NMR kinetics data for **6** reacting with GSH in phosphate-buffered D_2O, pH 7.4, 37°C



time, min.	ln([**6**]), 8.46 ppm	ln([**6**]), 7.48 ppm
5	-7.385382833	-7.407998793
10	-7.830088325	-7.919356191
15	-8.317580138	-8.303619091
20	-8.853665428	-9.104979856


ln([**6**]) vs. time, min.

	signal at 8.46 ppm	signal at 7.48 ppm	average
slope of ln([**6**]) vs. time	-0.1178	-0.1367	-0.12725[a]
calculated half-life, min.	5.9	5.1	5.4[b]

[a] Average of slopes of each linear regression line

[b] Calculated by $t_{1/2} = \ln(2)/(-1*\text{average slope})$

Stacked ^{1}H NMR spectra of **6** reacting with GSH in phosphate-buffered D_2O, pH 7.4, 37°C

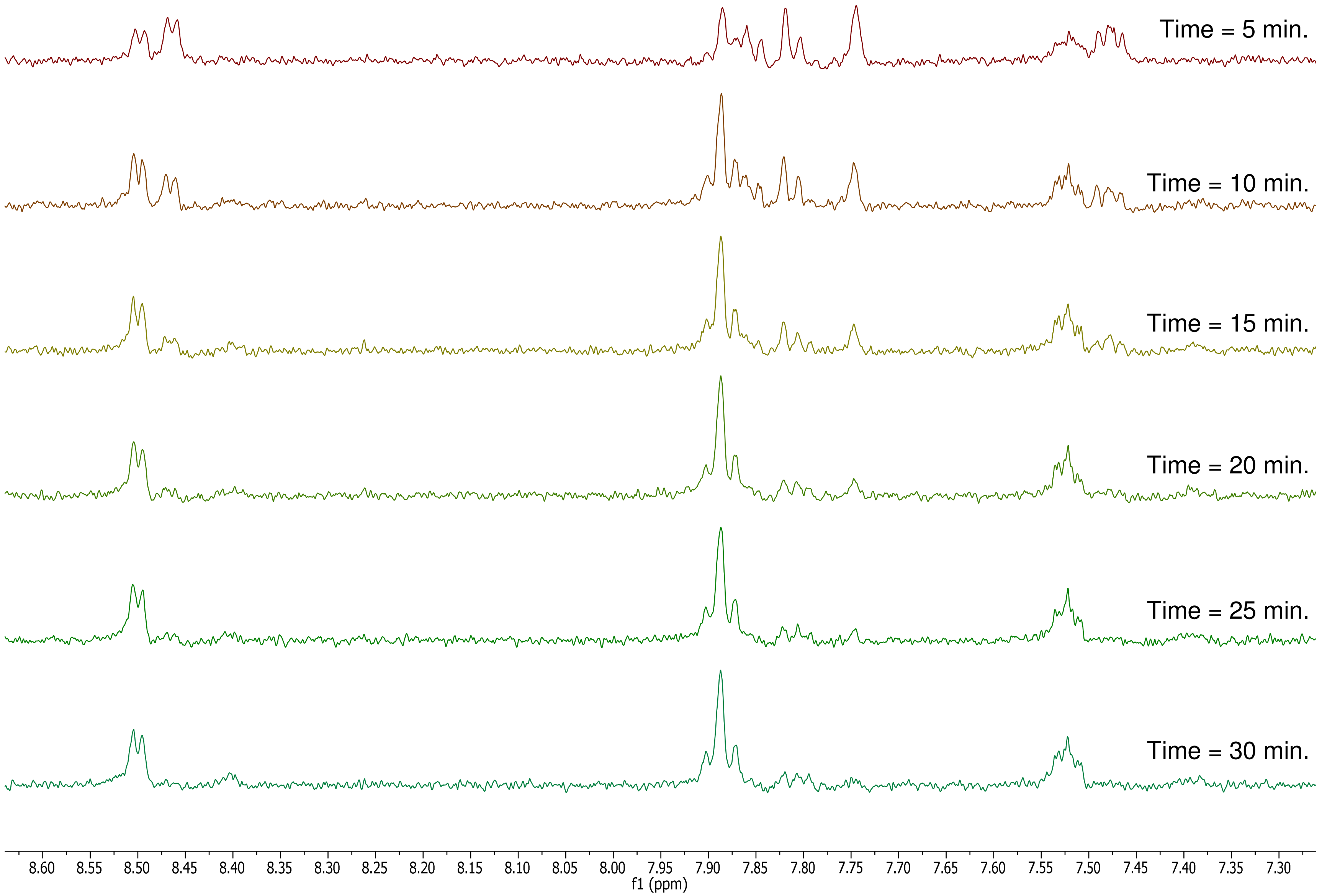
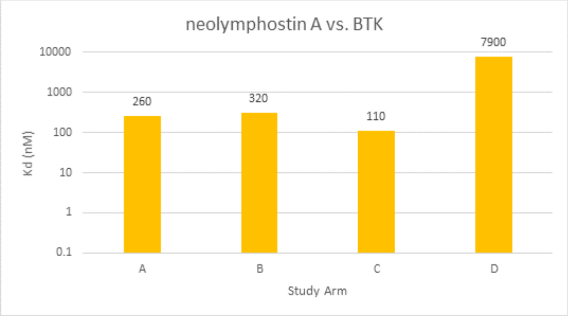
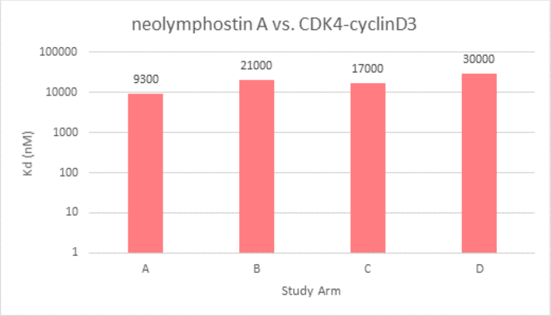
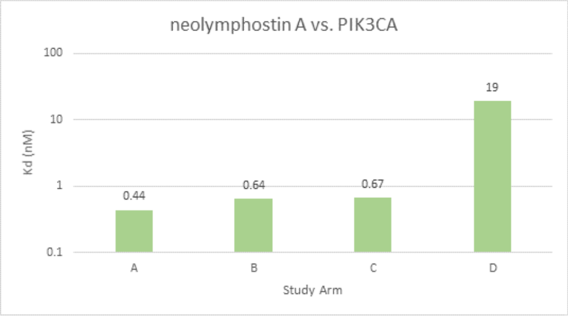


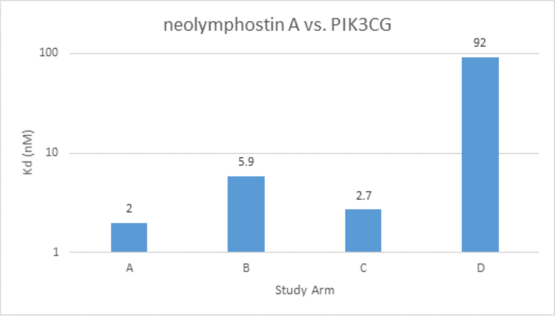
Table S7. Dissociation constants (K_d) for neolymphostin A and selected kinases under four conditioning experiments (Arms A–D)

Compound	BTK	CDK4-CYCLIND3	PIK3CA	PIK3CG
Compound name (conditioning)	K_d (nm)	K_d (nm)	K_d (nm)	K_d (nm)
Neolymphostin A (Arm A)	260	9300	0.44	2
Neolymphostin A (Arm B)	320	21000	0.64	5.9
Neolymphostin A (Arm C)	110	17000	0.67	2.7
Neolymphostin A (Arm D)	7900	>30000	19	92





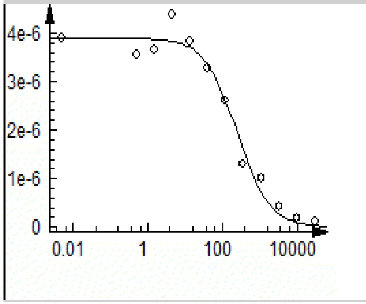




Curve images for dissociation constants (K_d) for neolymphostin A and selected kinases under four conditioning experiments (Arms A–D)

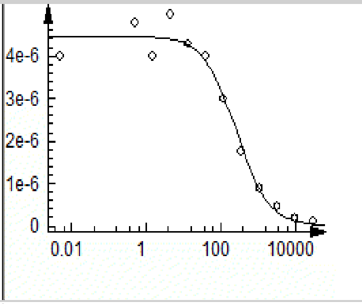
neolymphostin A_Arm A BTK

Replicate ID = 1 Kd (nM) = 250



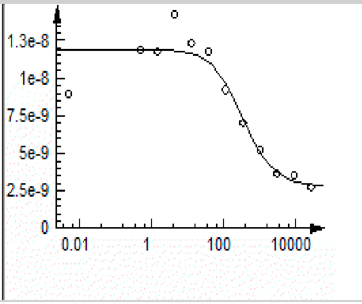
neolymphostin A_Arm A BTK

Replicate ID = 2 Kd (nM) = 260



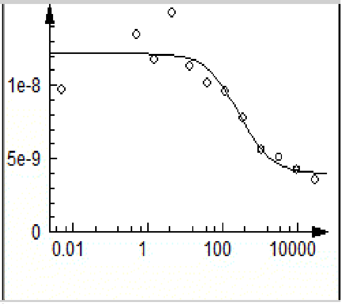
neolymphostin A_Arm B BTK

Replicate ID = 1 Kd (nM) = 350



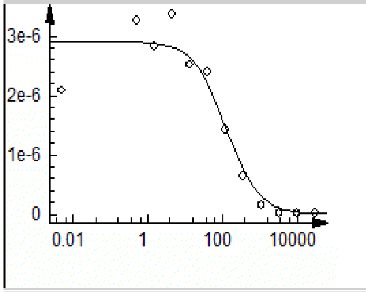
neolymphostin A_Arm B BTK

Replicate ID = 2 Kd (nM) = 280



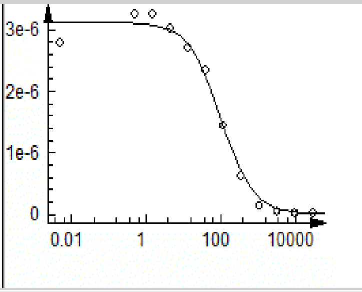
neolymphostin A_Arm C BTK

Replicate ID = 1 Kd (nM) = 130



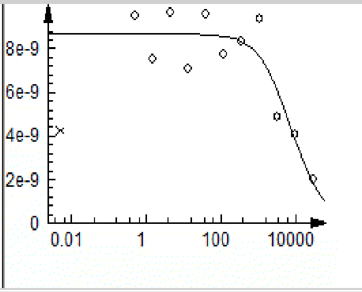
neolymphostin A_Arm C BTK

Replicate ID = 2 Kd (nM) = 100



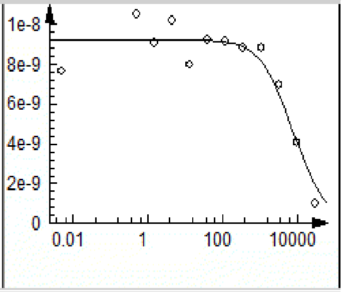
neolymphostin A_Arm D BTK

Replicate ID = 1 Kd (nM) = 7900



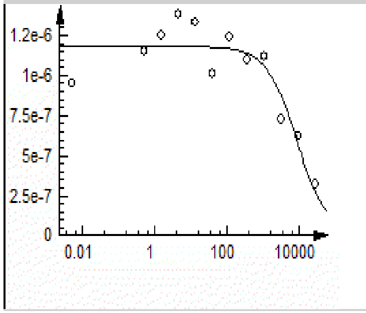
neolymphostin A_Arm D BTK

Replicate ID = 2 Kd (nM) = 7900



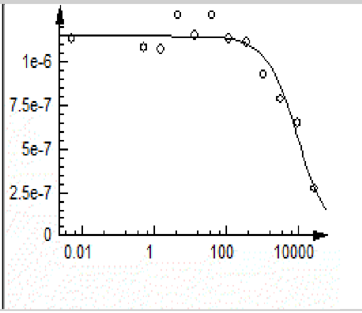
neolymphostin A_Arm A CDK4-cyclinD3

Replicate ID = 1 Kd (nM) = 9100



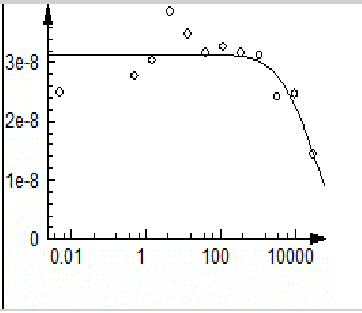
neolymphostin A_Arm A CDK4-cyclinD3

Replicate ID = 2 Kd (nM) = 9600



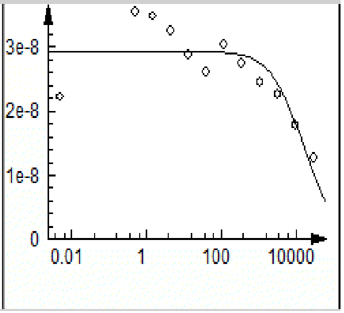
neolymphostin A_Arm B CDK4-cyclinD3

Replicate ID = 1 Kd (nM) = 26000



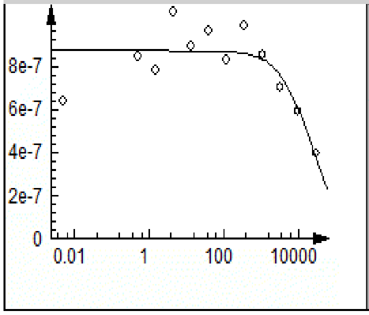
neolymphostin A_Arm B CDK4-cyclinD3

Replicate ID = 2 Kd (nM) = 16000



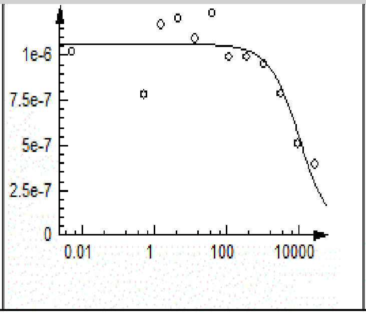
neolymphostin A_Arm C CDK4-cyclinD3

Replicate ID = 1 Kd (nM) = 22000



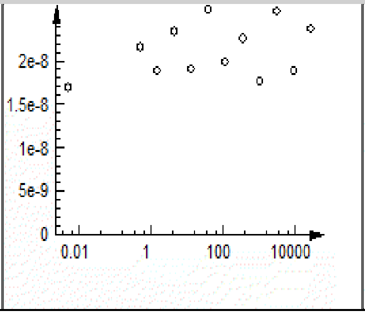
neolymphostin A_Arm C CDK4-cyclinD3

Replicate ID = 2 Kd (nM) = 11000



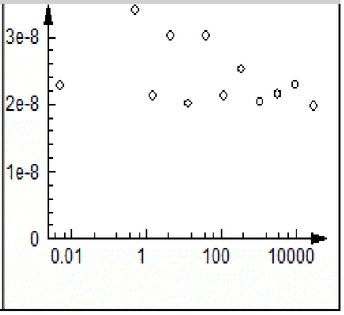
neolymphostin A_Arm D CDK4-cyclinD3

Replicate ID = 1 Kd (nM) = >40000



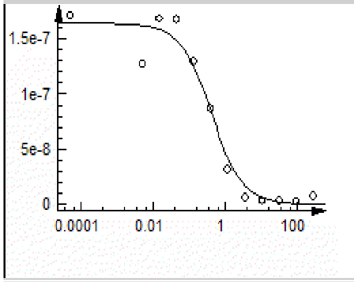
neolymphostin A_Arm D CDK4-cyclinD3

Replicate ID = 2 Kd (nM) = >40000

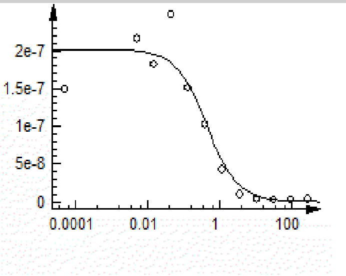


Curve images for dissociation constants (K_d) for neolymphostin A and selected kinases under four conditioning experiments (Arms A–D)...(continued)

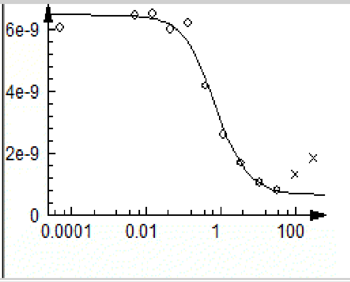
neolymphostin A_Arm A PIK3CA Replicate ID = 1 Kd (nM) = 0.43



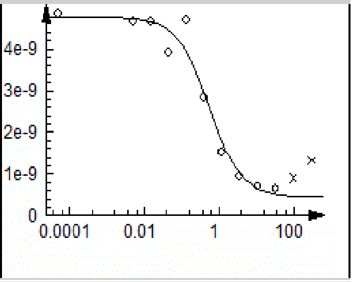
neolymphostin A_Arm A PIK3CA Replicate ID = 2 Kd (nM) = 0.45



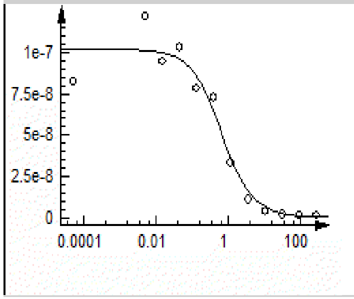
neolymphostin A_Arm B PIK3CA Replicate ID = 1 Kd (nM) = 0.72



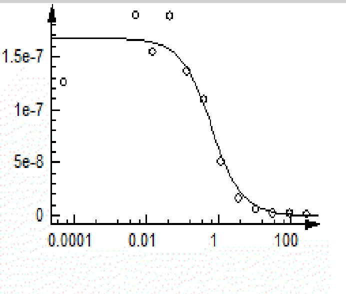
neolymphostin A_Arm B PIK3CA Replicate ID = 2 Kd (nM) = 0.56



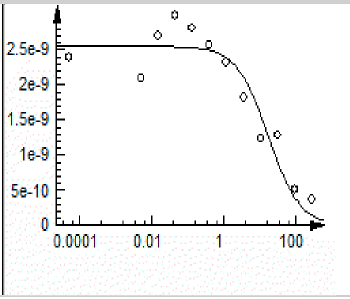
neolymphostin A_Arm C PIK3CA Replicate ID = 1 Kd (nM) = 0.68



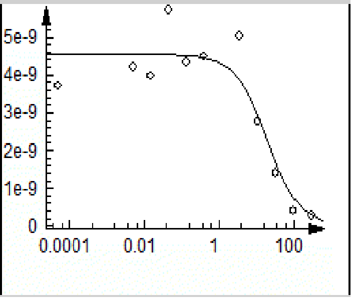
neolymphostin A_Arm C PIK3CA Replicate ID = 2 Kd (nM) = 0.65



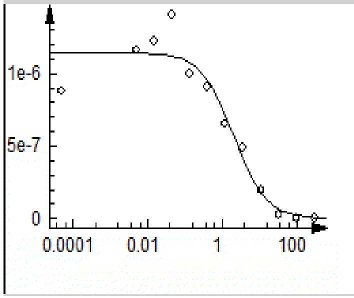
neolymphostin A_Arm D PIK3CA Replicate ID = 1 Kd (nM) = 18



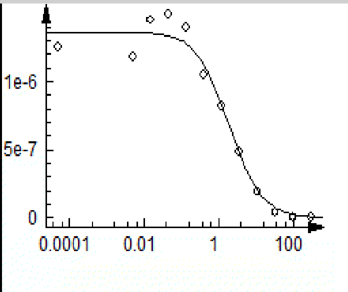
neolymphostin A_Arm D PIK3CA Replicate ID = 2 Kd (nM) = 20



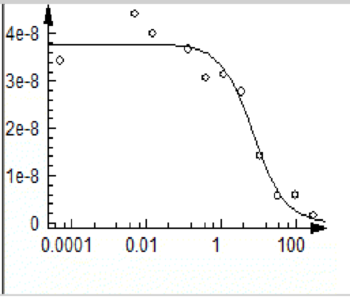
neolymphostin A_Arm A PIK3CG Replicate ID = 1 Kd (nM) = 2



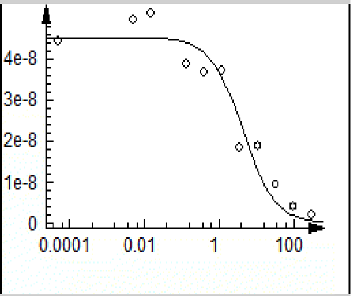
neolymphostin A_Arm A PIK3CG Replicate ID = 2 Kd (nM) = 1.9



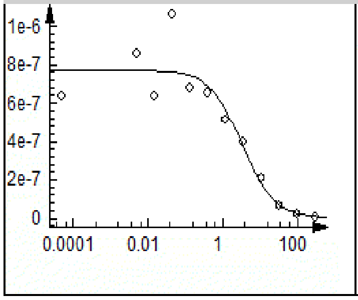
neolymphostin A_Arm B PIK3CG Replicate ID = 1 Kd (nM) = 7.3



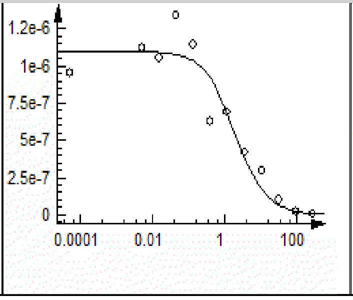
neolymphostin A_Arm B PIK3CG Replicate ID = 2 Kd (nM) = 4.4



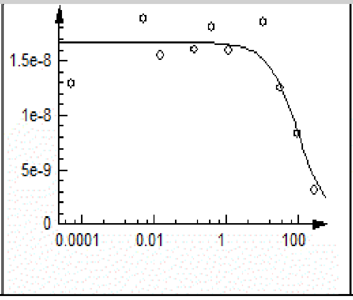
neolymphostin A_Arm C PIK3CG Replicate ID = 1 Kd (nM) = 3.3



neolymphostin A_Arm C PIK3CG Replicate ID = 2 Kd (nM) = 2



neolymphostin A_Arm D PIK3CG Replicate ID = 1 Kd (nM) = 100



neolymphostin A_Arm D PIK3CG Replicate ID = 2 Kd (nM) = 80

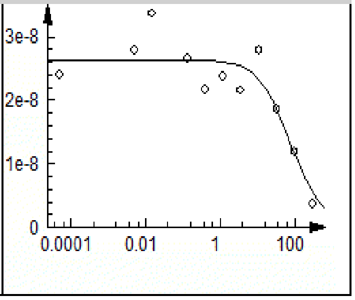
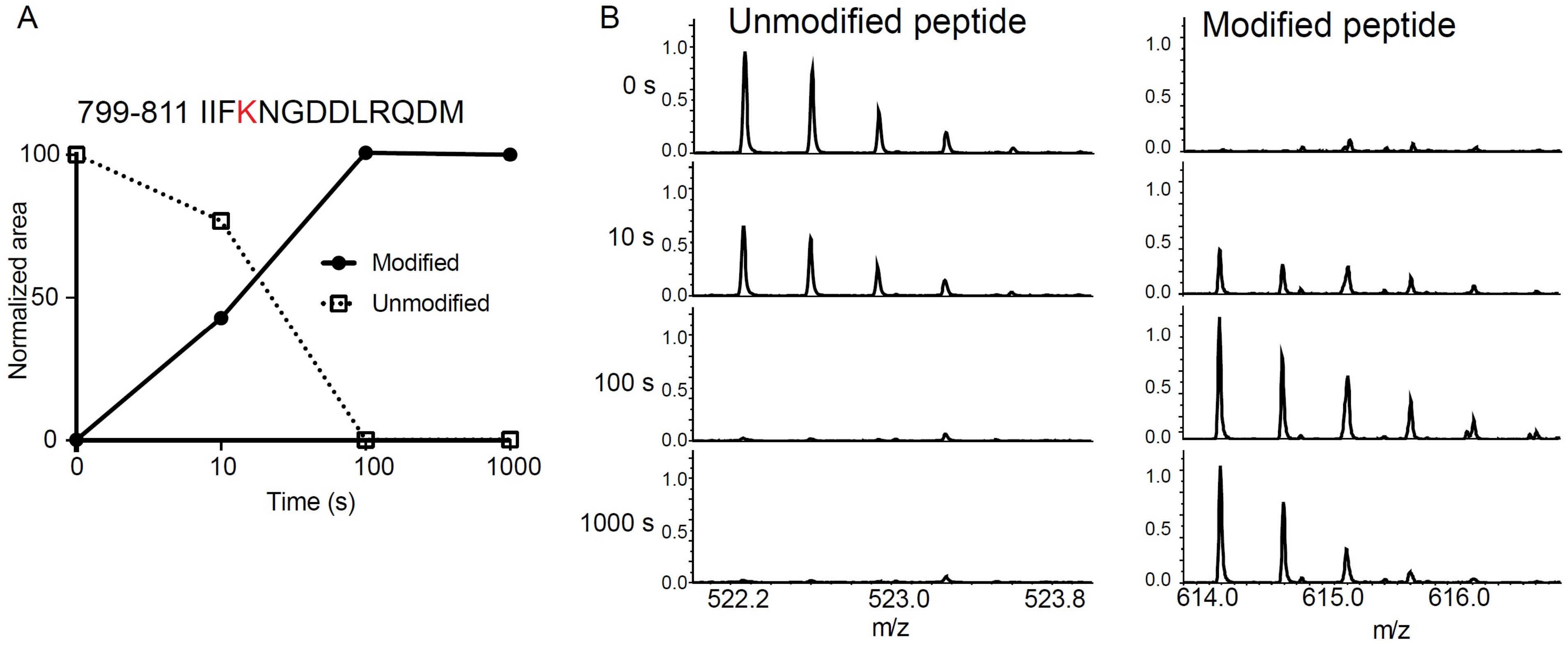


Figure S5. Timecourse for covalent modification of PI3K



A. The intensity for the modified and unmodified variant of the peptide indicated is shown over a time course of incubation with neolymphostin. B. Raw peptide traces of the unmodified and modified peptide over time.

Figure S6. Neolymphostin docked into PI3Kα

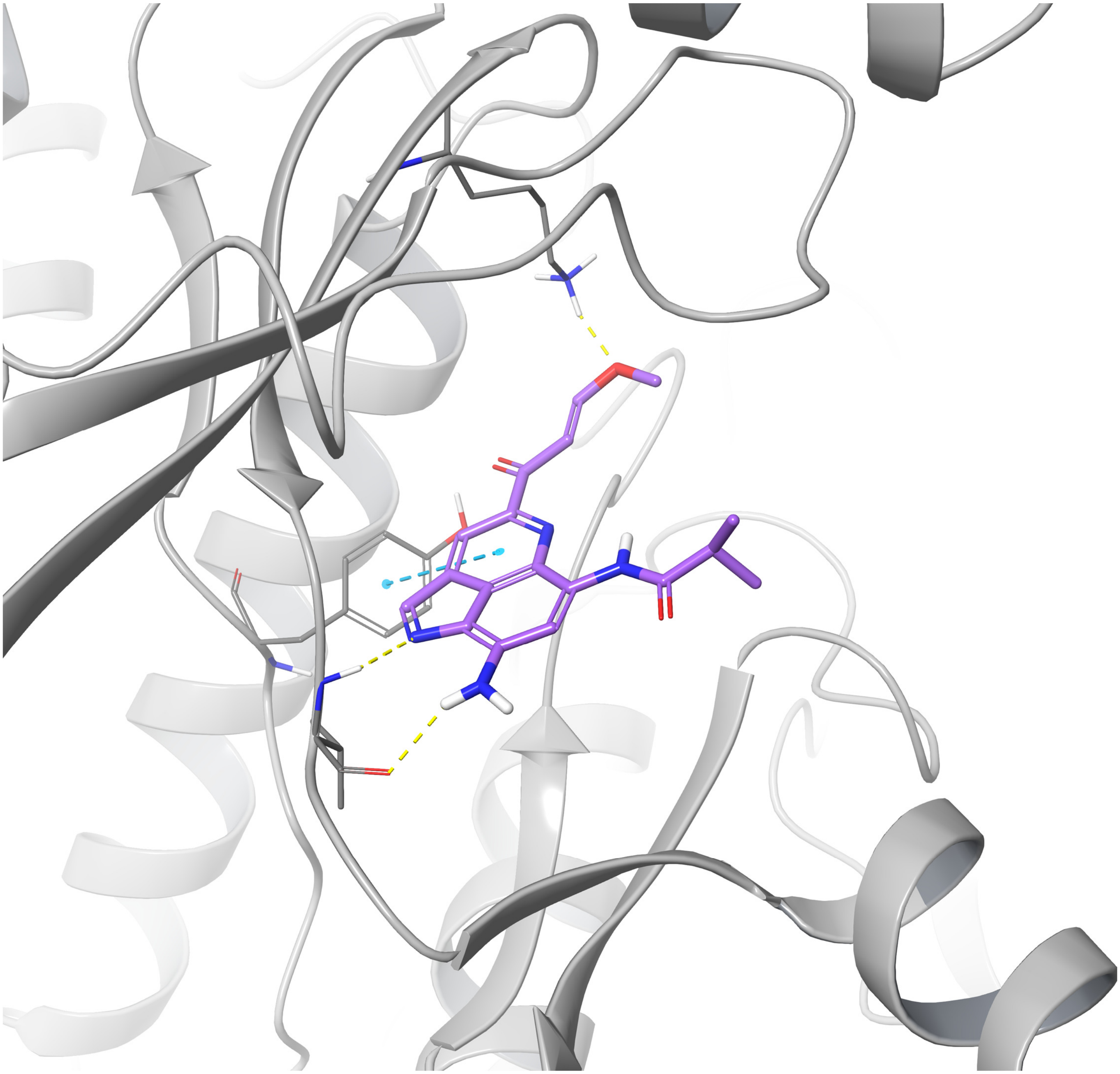


Figure S7. Neolymphostin docked into mTOR

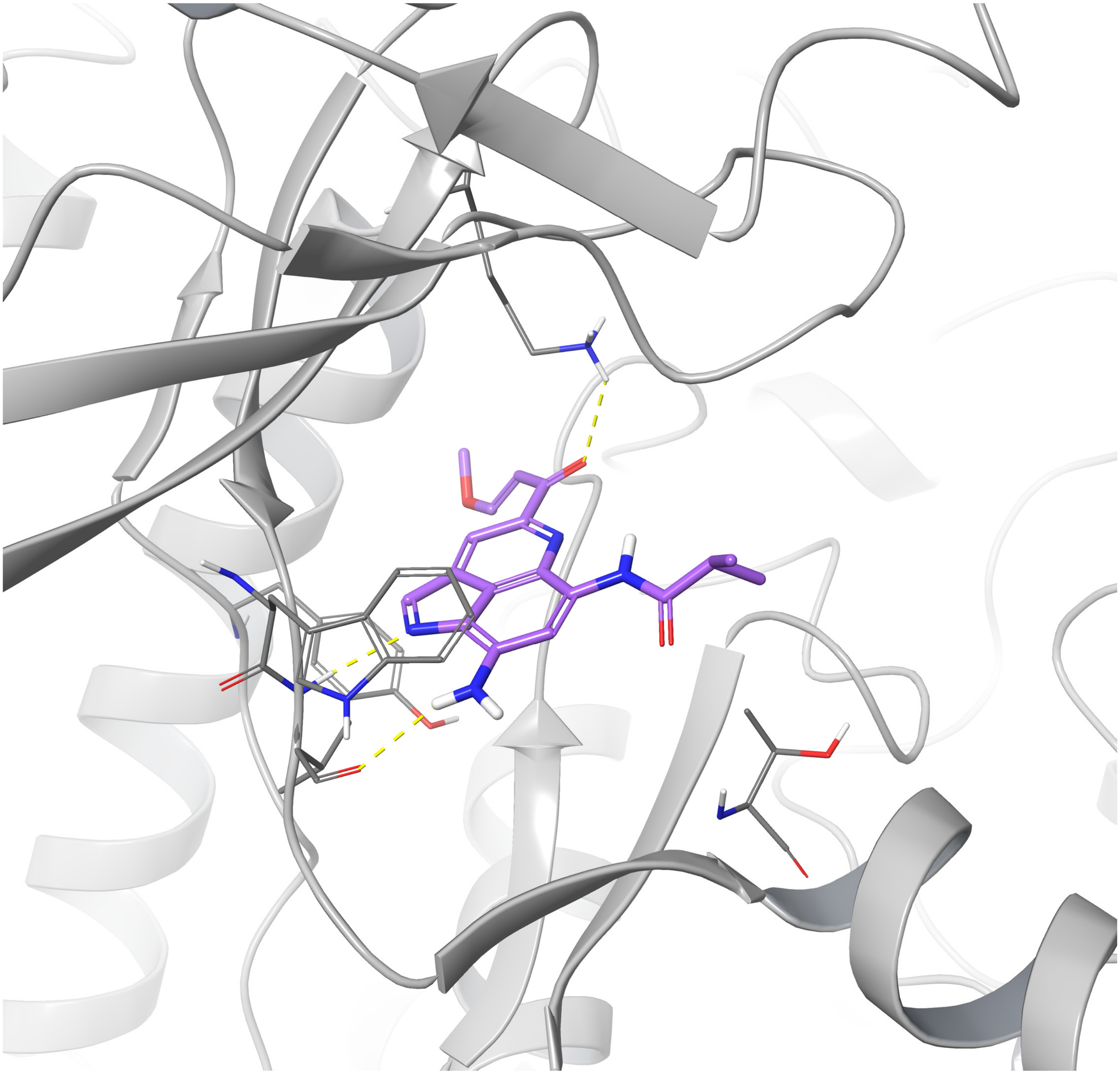


Figure S8. Neolymphostin docked into PI3Kα (induced fit)

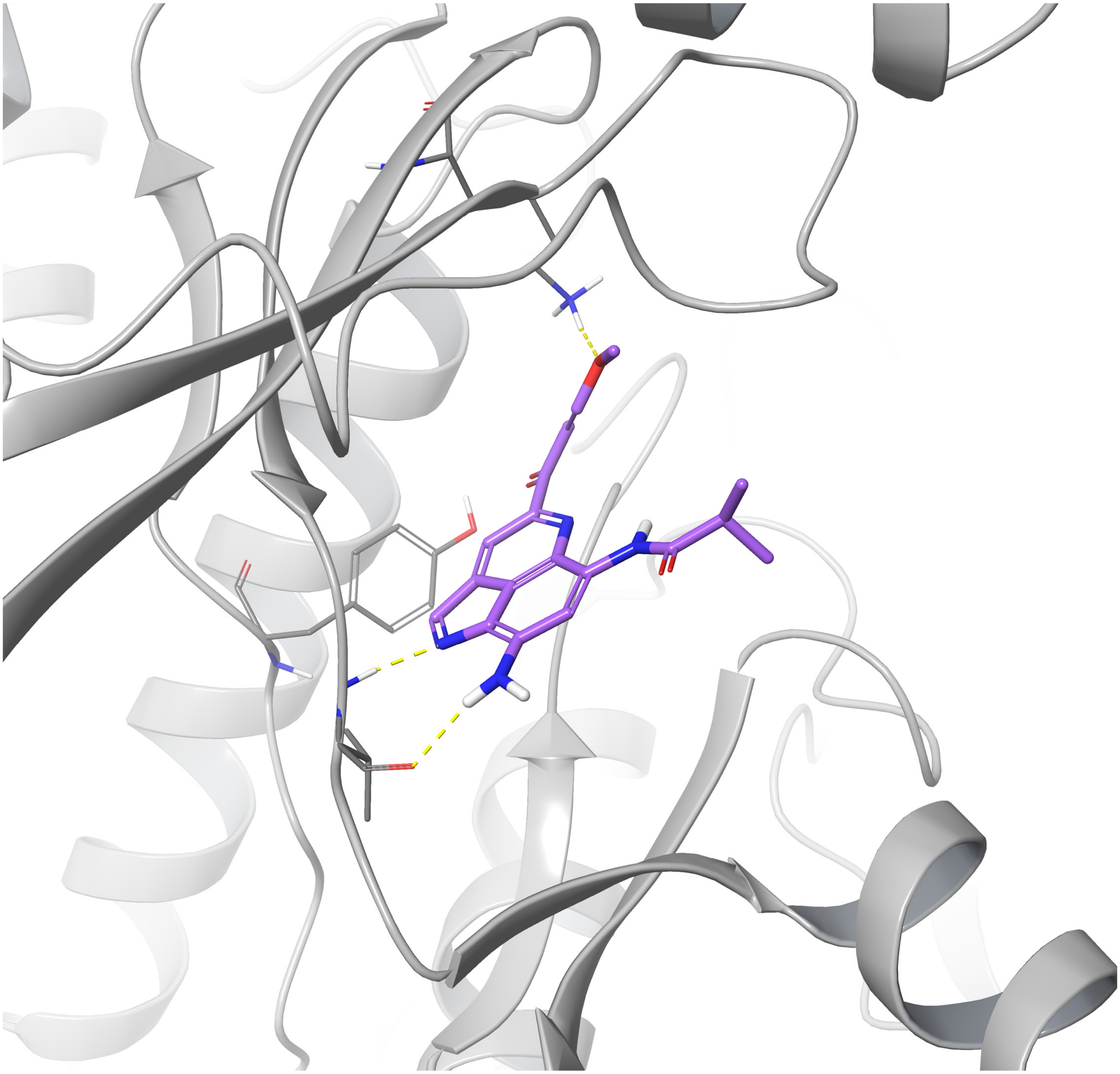


Figure S9. Neolymphostin docked into mTOR (induced fit)

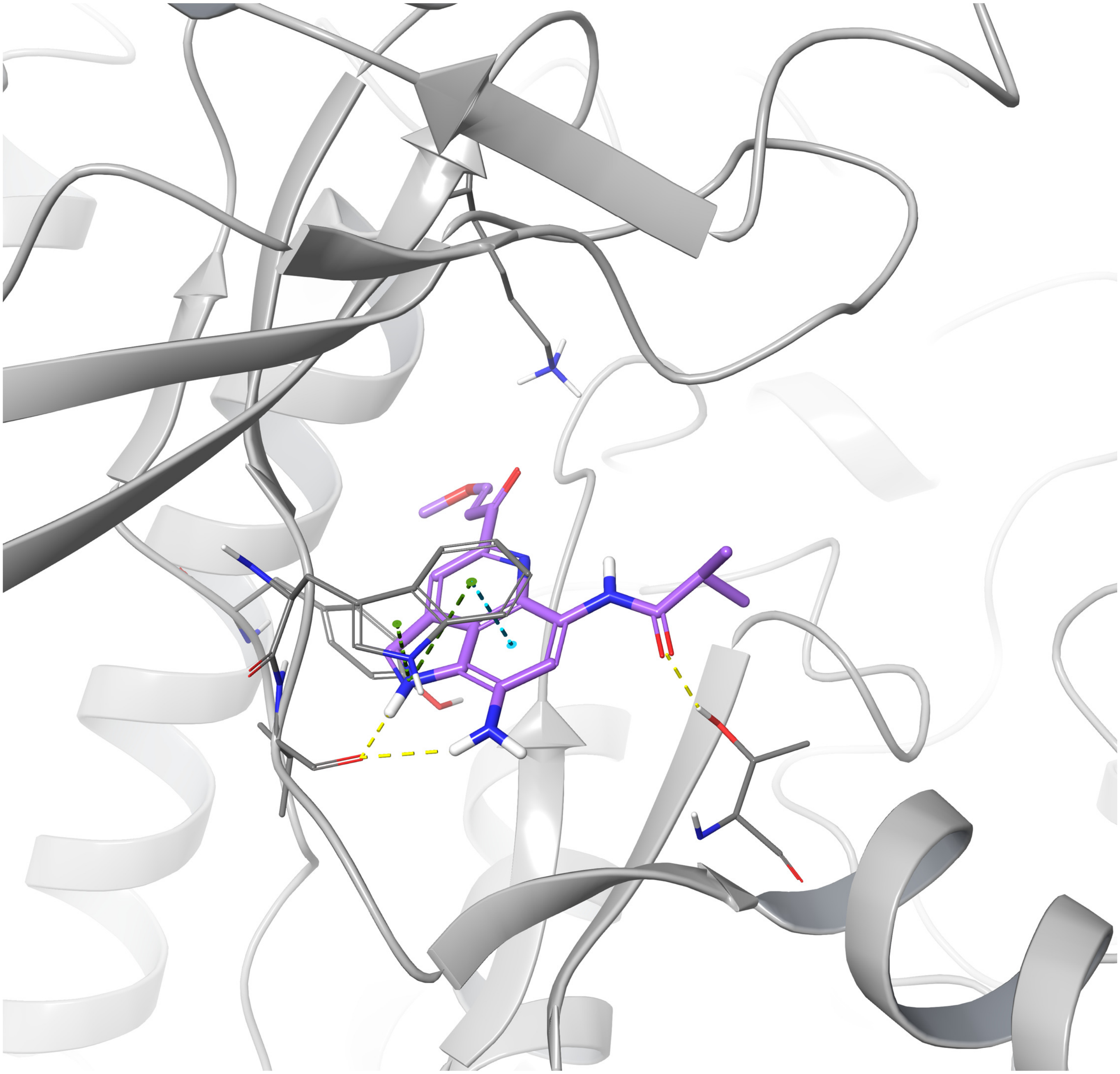


Figure S10. Neolymphostin docked into PI3Kα (covalent model)

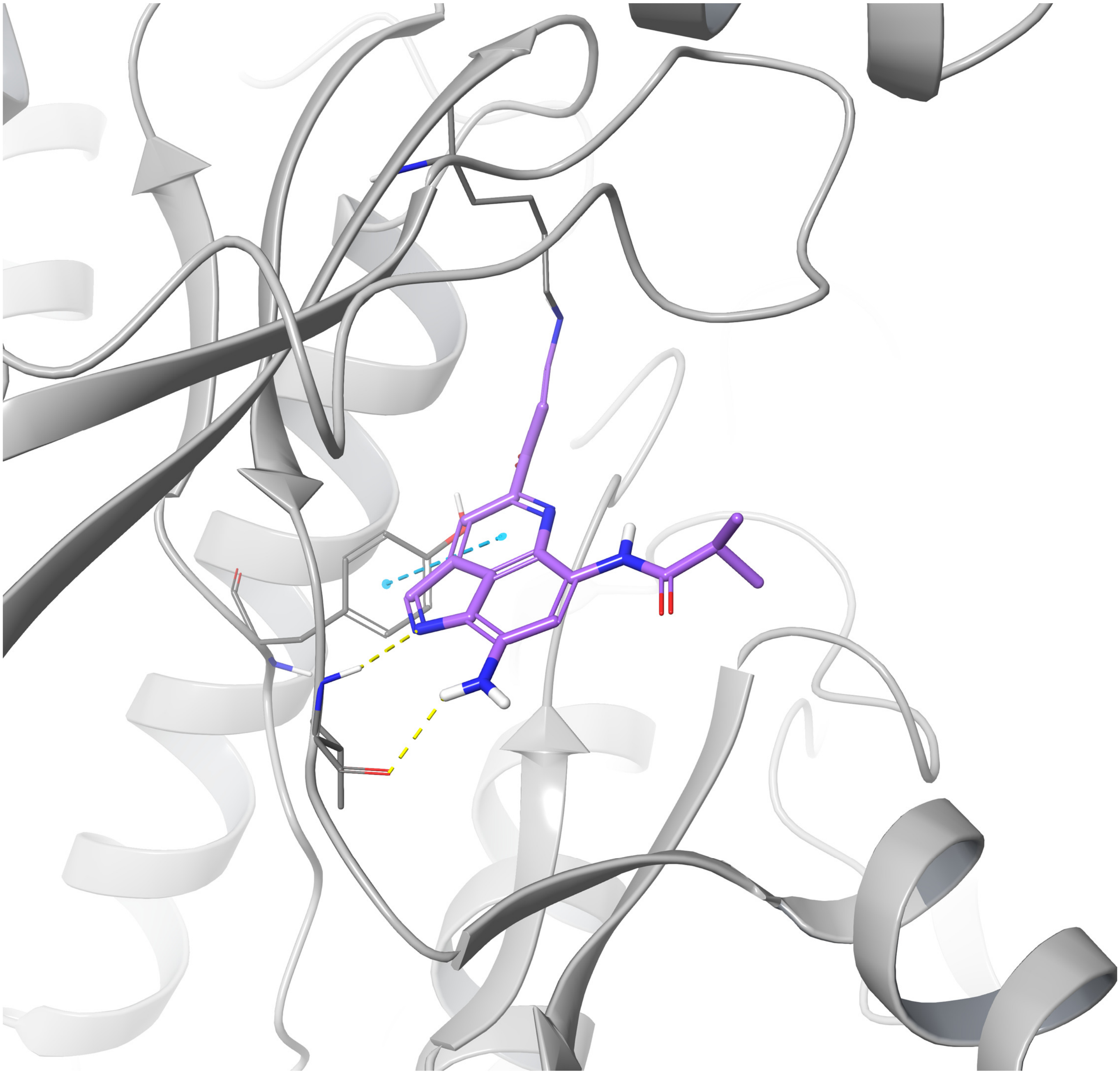
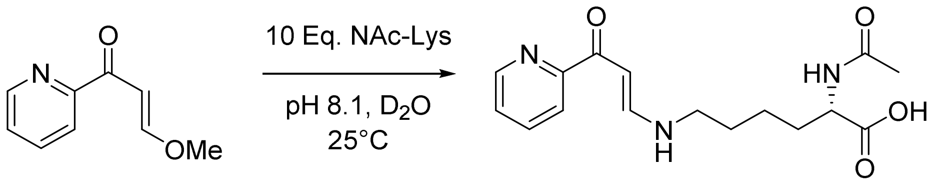


Table S8. HDX-MS experiment

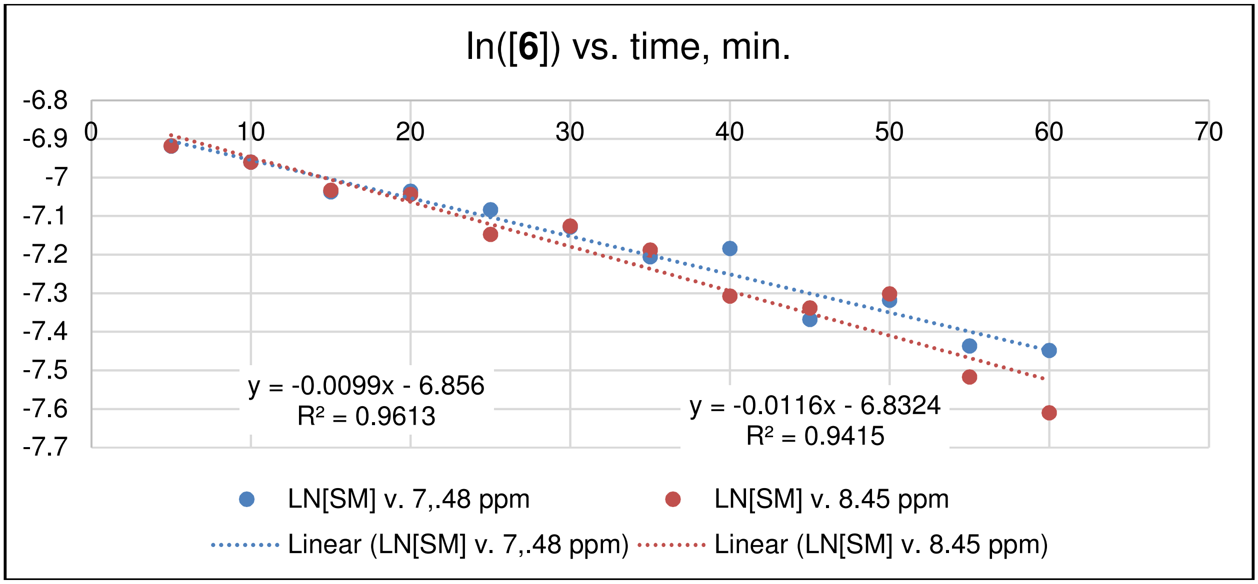
					PI3Kα alone				PI3Kα + neolymphostin			
Start	End	Z	RT	Sequence	3s on ice	SD	300s	SD	3s on ice	SD	300s	SD
11	23	2	12.3	WGIHLMPPRILVE	8.8	0.1	40.8	0.5	8.5	0.5	41.3	1.2
31	35	1	8.17	IVTLE	40.5	1.4	55.4	0.4	41.2	2.6	56.4	1.1
37	42	1	7.05	LREATL	1.3	0.2	4.9	0.3	1.5	0.5	5.4	0.4
43	62	4	9.34	ITIKHELFKEARKYPLHQLL	2.9	0.1	21.2	0.2	2.9	0.2	21.4	0.8
50	67	2	8.67	FKEARKYPLHQLLQDESS	6.9	0.0	34.0	0.5	6.5	0.0	35.5	2.0
69	76	1	9.08	IFVSVTQE	2.0	0.4	14.2	0.1	2.1	0.6	14.6	0.3
71	82	2	9.24	VSVTQEAEREEF	1.6	0.2	21.8	0.2	1.7	0.3	22.1	1.3
77	82	1	6.99	AEREEF	3.1	0.4	29.7	0.2	3.5	1.0	30.6	1.4
83	91	3	6.99	FDETRRLCD	2.2	0.4	6.8	0.3	2.5	0.7	7.9	0.2
83	92	3	9.87	FDETRRLCDL	1.0	0.1	3.8	0.1	1.0	0.0	4.4	0.3
92	99	1	13.96	LRLFQPFL	0.9	0.3	1.6	0.0	1.0	0.2	2.0	0.2
93	99	2	12.8	RLFQPFL	1.5	0.2	1.7	0.2	1.7	0.3	1.9	0.2
99	103	1	6.67	LKVIE	0.9	0.4	2.7	0.1	1.3	0.3	2.9	0.3
100	119	4	9.76	KVIEPVGNREEKILNREIGF	6.7	0.0	33.8	0.4	6.7	0.3	34.6	1.3
120	127	1	9.56	AIGMPVCE	5.8	0.3	35.1	0.5	6.0	0.9	36.6	0.8
128	139	2	10.93	FDMVKDPEVQDF	11.4	0.5	31.2	0.3	11.4	0.8	32.2	0.6
145	152	1	5.21	NVCKEAVD	7.2	1.3	15.0	2.4	7.6	2.7	15.8	1.0
147	152	1	3.96	CKEAVD	5.2	0.8	13.8	1.5	5.8	0.5	14.6	0.9
153	163	3	4.58	LRDLNSPHSRA	19.6	0.3	36.8	0.7	19.5	0.4	38.0	2.1
154	164	3	4.56	RDLNSPHSRAM	13.3	0.5	34.6	0.6	13.5	0.5	35.7	1.7
165	193	5	10.35	YVYPPNVESSPELPKHIYNKLDKGQIIVV	12.6	0.1	34.2	0.2	12.6	0.2	34.9	1.5
193	209	3	11.67	VIWVIVSPNNDKQKYTL	16.9	0.4	39.9	0.3	16.4	0.1	40.4	1.6
196	223	5	9.13	VIVSPNNDKQKYTLKINHDCVPEQVIAE	9.7	0.1	22.7	0.0	9.7	0.4	23.4	1.0
223	233	3	5.12	EAIRKKTRSML	30.1	0.1	43.2	0.5	29.6	0.4	44.2	1.7
224	233	3	4.56	AIRKKTRSML	29.4	1.1	41.5	0.5	29.6	0.1	42.7	1.6
244	252	1	10.63	LEYQGKYIL	35.1	0.3	52.6	0.3	34.8	0.5	54.3	2.5
253	259	1	4.94	KVCGCDE	3.0	0.5	12.9	0.3	3.4	1.0	14.2	0.6
260	269	2	10.82	YFLEKYPLSQ	11.8	0.3	18.7	0.1	11.8	0.7	19.2	1.0
261	275	4	9.46	FLEKYPLSQYKYIRS	10.1	0.3	16.1	0.1	9.7	0.3	16.6	0.3
279	287	2	11.93	LGRMPNLML	13.0	0.1	25.0	0.5	12.9	0.3	25.4	1.0
294	301	1	10.13	YSQLPMDC	36.0	0.4	65.3	0.6	36.0	0.6	66.8	1.5
302	327	4	9.64	FTMPSYSRRISTATPYMNGETSTKSL	38.1	0.6	45.3	0.6	37.7	0.9	46.6	2.1
328	334	1	12.03	WVINSAL	29.0	0.2	41.7	0.2	29.0	0.5	42.7	0.8
343	369	4	10.94	YVNVNIRDIDKIYVRTGIYHGGEPLCD	12.6	0.6	23.7	0.7	12.4	0.4	24.2	1.1
370	389	3	11.81	NVNTQRVPCSNPRWNEWLNY	5.0	0.3	32.5	0.2	4.3	0.5	32.8	2.4
390	402	3	11.4	DIYIPDLPRAARL	1.9	0.4	10.6	0.2	2.1	0.8	11.1	0.1
405	429	3	10.62	SICSVKGRKGAKEEHCPLAWGNINL	3.7	0.2	6.4	0.1	3.6	0.1	6.9	0.1
430	436	1	10.77	FDYTDTL	1.7	0.2	9.2	0.1	2.0	0.2	10.5	0.6
437	443	1	7.53	VSGKMAL	14.7	0.3	41.5	0.4	15.0	1.3	42.4	0.8
444	474	3	13.81	NLWPVPHGLEDLLNPIGVTGSNPNKETPCLE	5.5	0.2	30.1	0.9	5.3	0.2	31.2	2.1
456	474	2	9.29	LNPIGVTGSNPNKETPCLE	8.6	0.3	38.7	0.5	8.7	0.6	39.5	1.6
474	478	1	10.11	ELEFD	3.4	0.5	3.7	0.6	4.8	2.6	5.4	0.5
483	491	2	10.71	VVKFPDMSV	21.9	0.0	41.4	0.8	21.8	0.4	42.0	1.8
484	491	2	10.4	VKFPDMSV	27.0	0.1	51.4	0.6	27.4	0.4	52.8	1.6
492	498	1	8.23	IEEHANW	9.5	0.8	39.9	0.3	9.6	1.4	39.0	2.1
495	506	3	9.19	HANWSVSREAGF	35.2	0.4	45.3	0.3	35.2	0.8	45.9	1.0
507	522	3	6.27	SYSHAGLSNRLARDNE	41.5	0.3	43.9	0.4	41.1	1.3	44.3	1.5
523	531	3	5.7	LRENDKEQL	14.9	0.2	44.3	0.3	15.3	0.3	45.4	1.5
532	542	2	6.99	KAISTRDPLSE	7.1	0.6	29.9	0.4	7.2	0.5	31.4	1.7
546	551	1	9.19	QEKDFL	2.3	0.2	2.8	0.6	2.4	0.7	3.2	0.2
550	565	4	12.43	FLWSHRHYCVTIPEIL	1.4	0.2	16.2	0.1	1.1	0.4	16.8	0.8
552	570	4	13.05	WSHRHYCVTIPEILPKLLL	0.3	0.1	11.6	0.2	0.3	0.2	12.0	0.6
571	583	2	8.53	SVKWNSRDEVAQM	1.4	0.2	7.6	0.1	1.5	0.3	8.1	0.3
584	600	3	11	YCLVKDWPPIKPEQAME	1.2	0.1	17.5	0.8	1.3	0.3	18.7	0.8
602	632	5	13.87	LDCNYPDPMVRGFAVRCLEKYLTDDKLSQYL	3.0	0.1	18.2	0.7	3.0	0.1	18.6	1.5
631	635	1	12.55	YLIQL	0.4	0.5	0.7	0.3	1.0	1.8	0.9	0.4
636	649	2	13.67	VQVLKYEQYLDNLL	0.9	0.2	15.2	0.2	0.8	0.2	15.6	0.3
649	666	4	9.71	LVRFLLKKALTNQRIGHF	0.3	0.0	0.6	0.1	0.5	0.2	0.7	0.1
667	671	1	13.61	FFWHL	1.7	0.5	2.1	0.9	0.8	0.3	2.0	0.6
672	687	2	9.21	KSEMHNKTVSQRFGLL	2.5	0.1	16.9	0.3	2.5	0.3	17.1	0.5
688	692	1	7.65	LESYC	1.8	0.6	2.1	0.6	2.6	0.9	2.7	0.6
691	697	2	6.93	YCRACGM	13.9	0.2	30.3	0.5	14.1	1.1	30.7	0.7
698	709	2	6.83	YLKHLNRQVEAM	0.7	0.2	2.8	0.1	0.8	0.3	3.1	0.2
716	734	3	7.95	TDILKQEKKDETQKVQMKF	27.3	0.1	54.0	0.4	27.1	0.7	55.5	1.0
735	744	2	8.59	LVEQMRRPDF	5.2	0.4	61.6	0.8	4.8	0.6	63.3	3.3
745	766	3	14.06	MDALQGFLSPLNPAHQLGNLRL	2.5	0.3	8.7	0.1	2.7	0.5	8.9	0.3
769	781	4	10.35	CRIMSSAKRPLWL	11.3	0.1	26.2	0.1	10.8	0.0	24.0	1.0
782	791	1	10.79	NWENPDIMSE	23.0	0.2	51.4	0.2	22.3	0.5	52.3	1.5
793	797	1	6.57	LFQNN	48.0	0.8	64.9	0.2	45.7	2.5	65.0	1.8
794	798	1	4.57	FQNNE	22.8	0.9	60.0	2.1	20.6	0.4	57.5	2.4
798	806	2	8.11	EIIFKNGDD	5.0	0.5	18.3	0.4				
799	807	1	9.65	IIFKNGDDL	6.3	0.4	22.7	0.8				
799	811	3	9.5	IIFKNGDDLRQDM	3.2	0.2	24.6	0.3				
799	813	3	10.3	IIFKNGDDLRQDMLT	2.2	0.1	16.5	0.4				
802	811	2	6.94	KNGDDLRQDM	4.3	0.5	30.4	0.4				
815	821	2	9.55	QIIRIME	0.3	0.1	0.4	0.1	0.4	0.1	0.6	0.2
822	830	1	10.57	NIWQNQGLD	9.2	0.2	26.8	0.3	9.5	1.1	27.9	0.6
830	839	2	13.11	DLRMLPYGCL	1.0	0.2	10.8	1.6	1.0	1.0	9.4	0.3
840	847	1	10.93	SIGDCVGL	2.9	0.4	8.1	0.3	3.0	0.8	8.6	0.3
847	858	2	8.77	LIEVVRNSHTIM	9.6	0.4	21.7	0.2	4.5	0.5	20.5	1.2
847	859	3	8.43	LIEVVRNSHTIMQ	8.6	0.2	23.6	0.6	4.1	0.5	20.7	1.5
848	858	3	7.53	IEVVRNSHTIM	10.4	0.4	26.0	0.4	5.4	0.1	24.0	0.7
848	859	2	7.12	IEVVRNSHTIMQ	9.7	0.2	27.4	0.4	5.2	0.7	24.1	0.6
860	872	3	10.03	IQCKGGLKGALQF	63.6	0.3	66.5	0.2	63.3	0.9	66.4	1.3
873	893	2	8.12	NSHTLHQWLKDKNKGEIYDAA	6.5	0.1	23.5	0.2	6.9	0.4	24.4	0.6
880	893	2	7.8	WLKDKNKGEIYDAA	8.6	0.2	32.4	0.2	9.0	0.5	33.2	0.9
892	896	1	9.46	AAIDL	2.3	1.1	2.8	0.8	2.4	1.6	3.1	0.4
909	922	2	10.83	FILGIGDRHNSNIM	1.8	0.2	7.7	0.1	1.8	0.3	7.8	0.4
921	930	2	10.62	IMVKDDGQLF	3.2	0.5	4.5	0.8	3.4	1.0	5.4	0.5
930	956	5	10.24	FHIDFGHFLDHKKKKFGYKRERVPFVL	4.1	0.1	17.0	0.2	3.9	0.3	16.9	0.6
957	961	1	10.45	TQDFL	1.5	0.1	10.4	0.1	1.8	0.5	10.7	0.5
961	980	4	9.11	LIVISKGAQECTKTREFERF	11.7	1.3	23.4	0.5	10.9	1.3	24.3	2.0
977	983	2	9.51	FERFQEM	2.0	1.6	3.4	0.9	0.4	0.6	4.3	0.6
984	989	1	8.97	CYKAYL	2.3	0.7	1.6	0.1	2.8	1.9	2.6	0.8
990	997	2	5.07	AIRQHANL	2.8	0.2	30.7	0.5	2.8	0.4	32.0	1.2
1002	1006	1	13.15	FSMML	0.9	0.4	10.5	0.2	1.1	0.8	14.2	0.4
1006	1013	1	10.77	LGSGMPEL	9.2	0.8	40.0	0.7	8.8	2.1	40.2	2.5
1014	1020	1	9.36	QSFDDIA	16.2	0.6	18.9	0.3	16.4	0.4	19.5	1.2
1017	1021	1	7.84	DDIAY	3.5	0.5	24.0	0.2	3.3	1.1	24.5	0.8
1029	1037	2	6.21	DKTEQEALE	10.3	0.1	32.8	0.8	10.3	0.4	33.3	1.0
1039	1055	2	7.82	FMKQMNDAHHGGWTTKM	15.7	0.1	23.4	0.2	15.8	0.1	24.0	0.7
1039	1057	4	9.9	FMKQMNDAHHGGWTTKMDW	15.2	0.2	22.1	0.1	15.2	0.0	22.7	0.8
1060	1068	2	3.95	HTIKQHALN	46.2	0.2	52.2	0.8	45.8	0.1	53.4	1.9

% Relative Deuterium Incorporation
10
20
30
40
50
60
70
80
90

Figure S11. ^{1}H NMR kinetics data for **6** reacting with N^{α}-acetyl lysine in phosphate-buffered D_2O, pH 8.1, 25°C



time, min.	ln([**6**]), 8.46 ppm	ln([**6**]), 7.48 ppm
5	-6.918118066	-6.918566195
10	-6.96067768	-6.960123264
15	-7.032918422	-7.037289331
20	-7.044091723	-7.03558865
25	-7.148035113	-7.083645945
30	-7.125757431	-7.128426641
35	-7.188657664	-7.205387682
40	-7.308078988	-7.184008656
45	-7.338538195	-7.3678788
50	-7.301659565	-7.318497444
55	-7.517132576	-7.437014604
60	-7.6102922	-7.448322872



	signal at 8.46 ppm	signal at 7.48 ppm	average
slope of ln([**6**]) vs. time	-0.0116	-0.0099	-0.01075[a]
calculated half-life, min.	59.8	70.0	64.5[b]

[a]Average of slopes of each linear regression line

[b]Calculated by $t_{1/2} = \ln(2)/(-1 \times \text{average slope})$

Stacked ^1H NMR spectra of **6** reacting with N$^{\alpha}$-acetyl lysine in phosphate-buffered D_2O, pH 8.1, 25°C

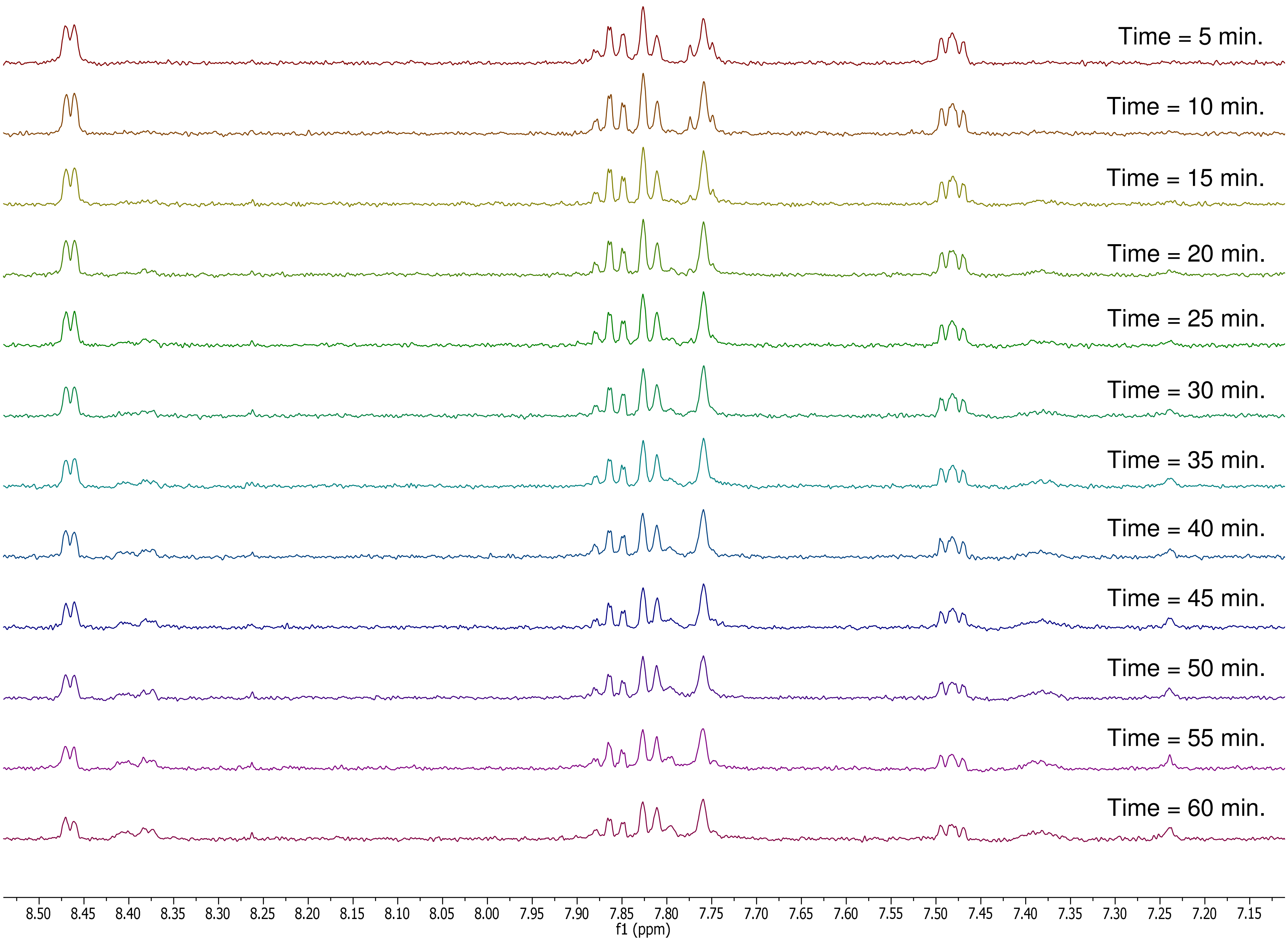
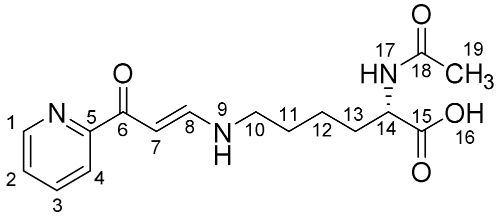


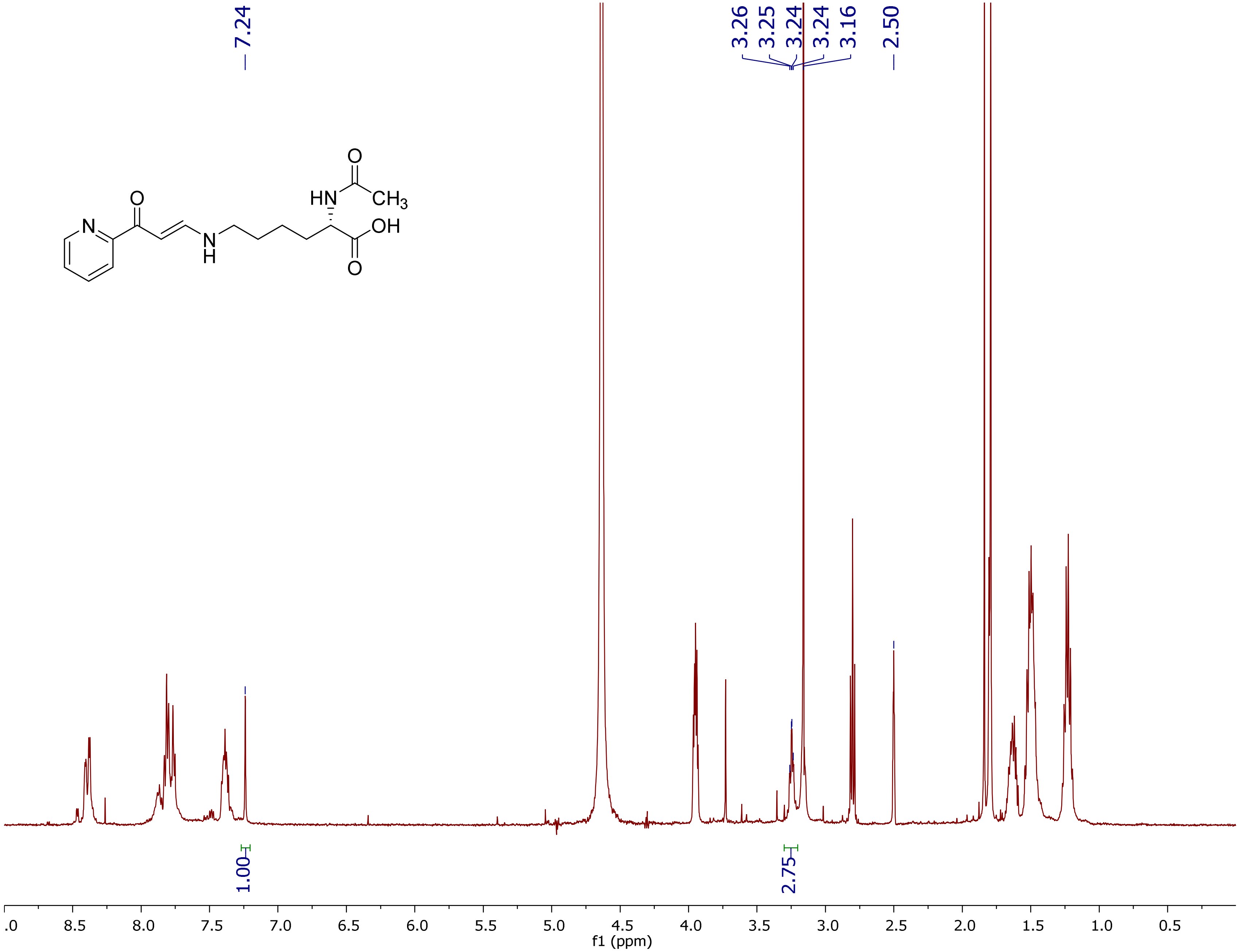
Table S9. NMR spectral data for **8** in phosphate-buffered D_2O, pH 8.1, at 500 MHz

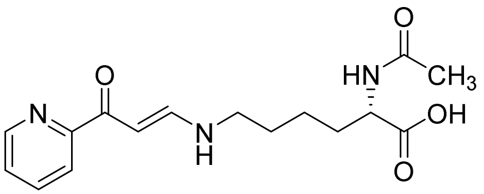


Position #	δH (mult., J(Hz))	δC[b]	COSY	HMBC
1	8.39 (dd, 13.5, 4.4)	149.8	2	3
2	7.39 (m)	127.8	1, 3	4
3	7.80 (m, 7.5)	139.9	2, 4	1, 5
4	7.76 (m, 7.5)	123.5	3	2
5	---	156.0	---	---
6	---	188.0	---	---
7	---[a]	90.6	---	---
8	7.24 (s)	160.0	---	6, 7, 10
9-NH	---[a]	---	---	---
10	3.25 (td, 6.5, 2.3)	50.4	11	8, 11, 12
11	1.52 (m)	27.8	10, 12	10, 12
12	1.23 (m)	23.6	11, 13	10, 11, 13, 14
13	a 1.52 (m) b 1.63 (m)	32.5	14 12, 13a, 14	11, 12, 14, 15 12, 14, 15
14	3.95 (m)	56.4	13a, 13b	12, 13, 15
15	---	180.4	---	---
16-OH	---[a]	---	---	---
17-NH	---[a]	---	---	---
18	---	174.8	---	---
19	1.81 (s)	23.2	---	18

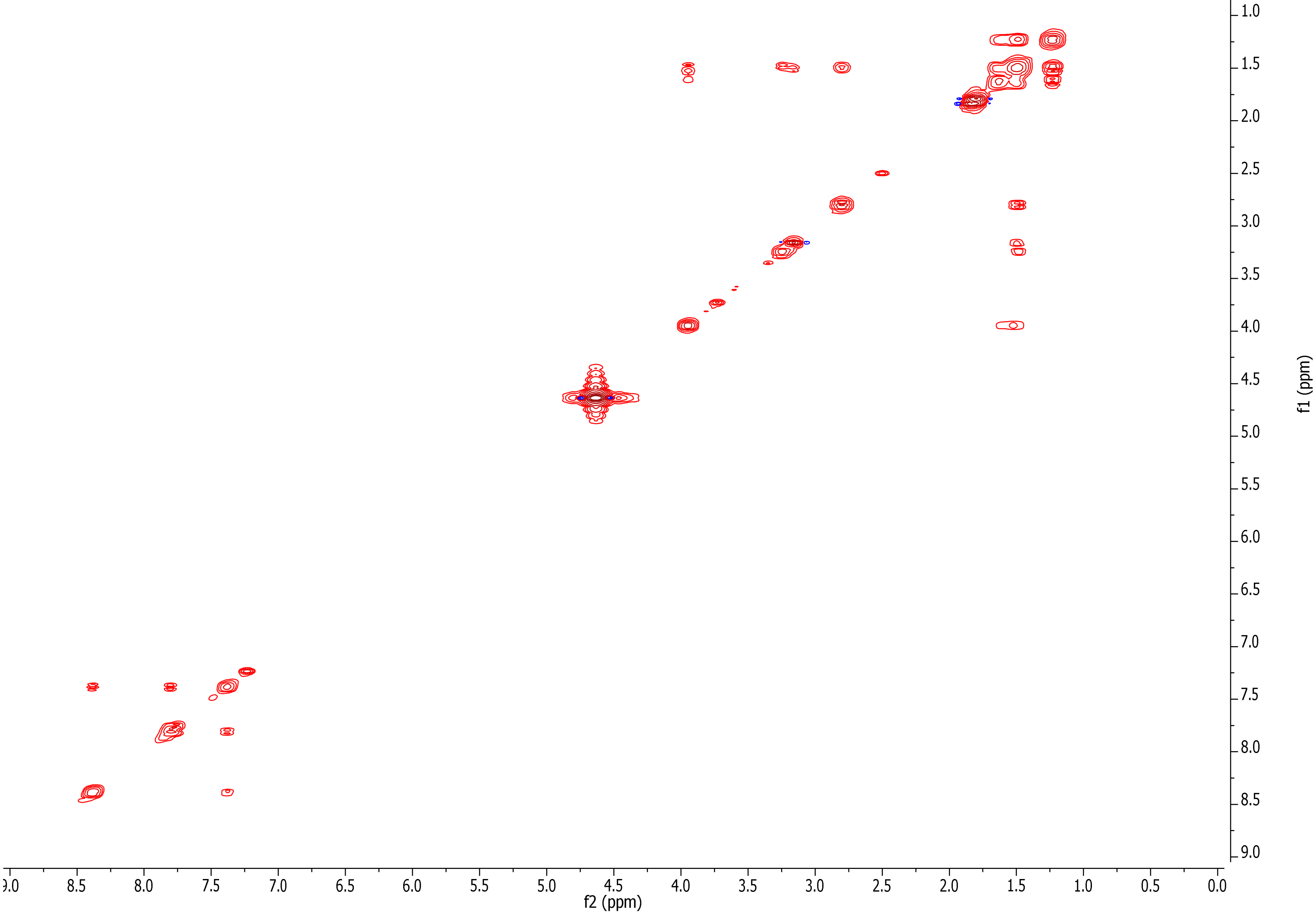
[a] Proton signals were not observed due to exchange with deuterium.
[b] Carbon chemical shifts were based on HSQC and HMBC data.

^{1}H NMR (500 MHz, phosphate-buffered D_2O) of **8**

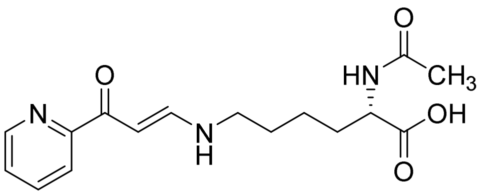


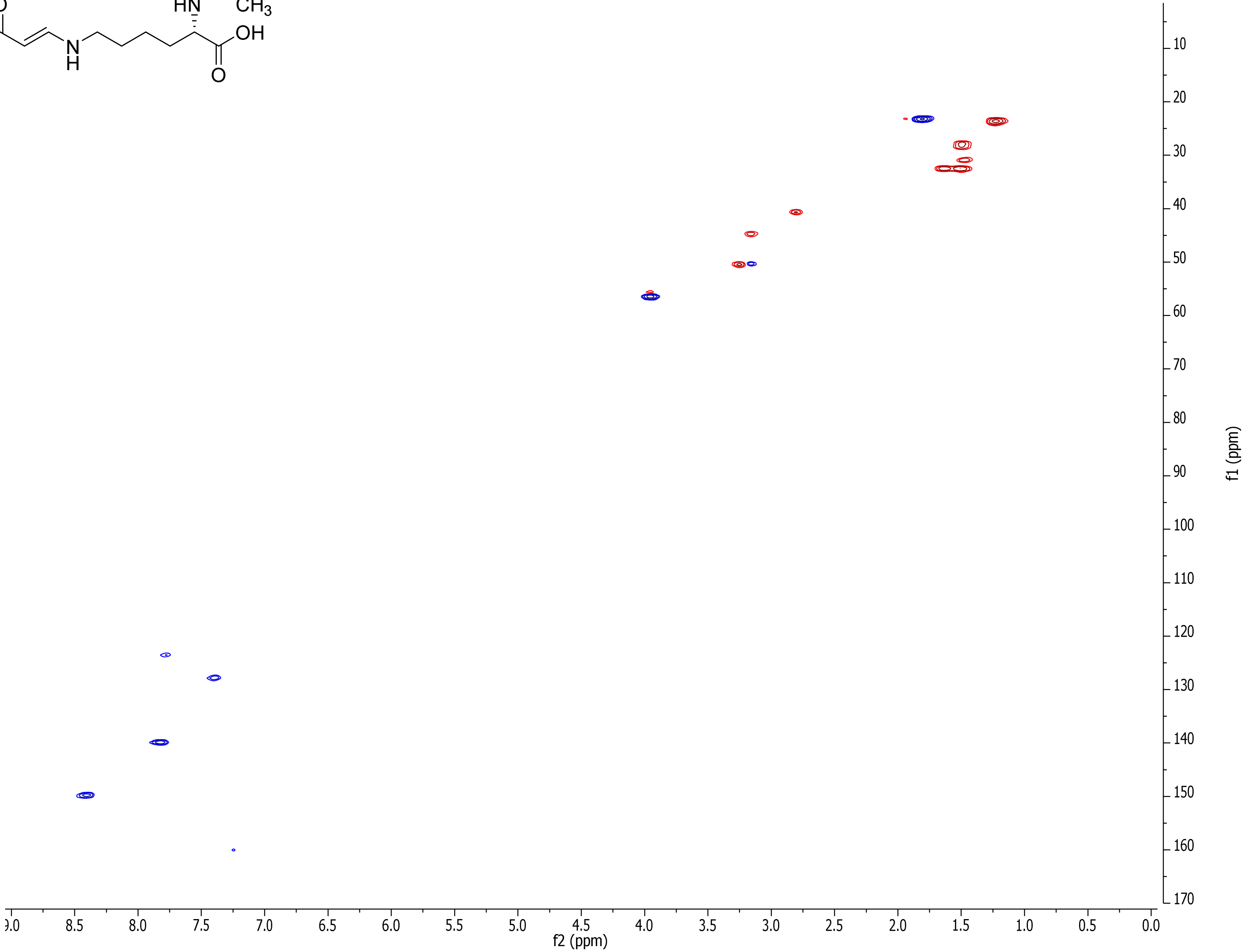


COSY NMR (500 MHz, phosphate-buffered D_2O) of **8**

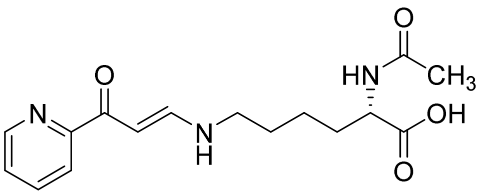


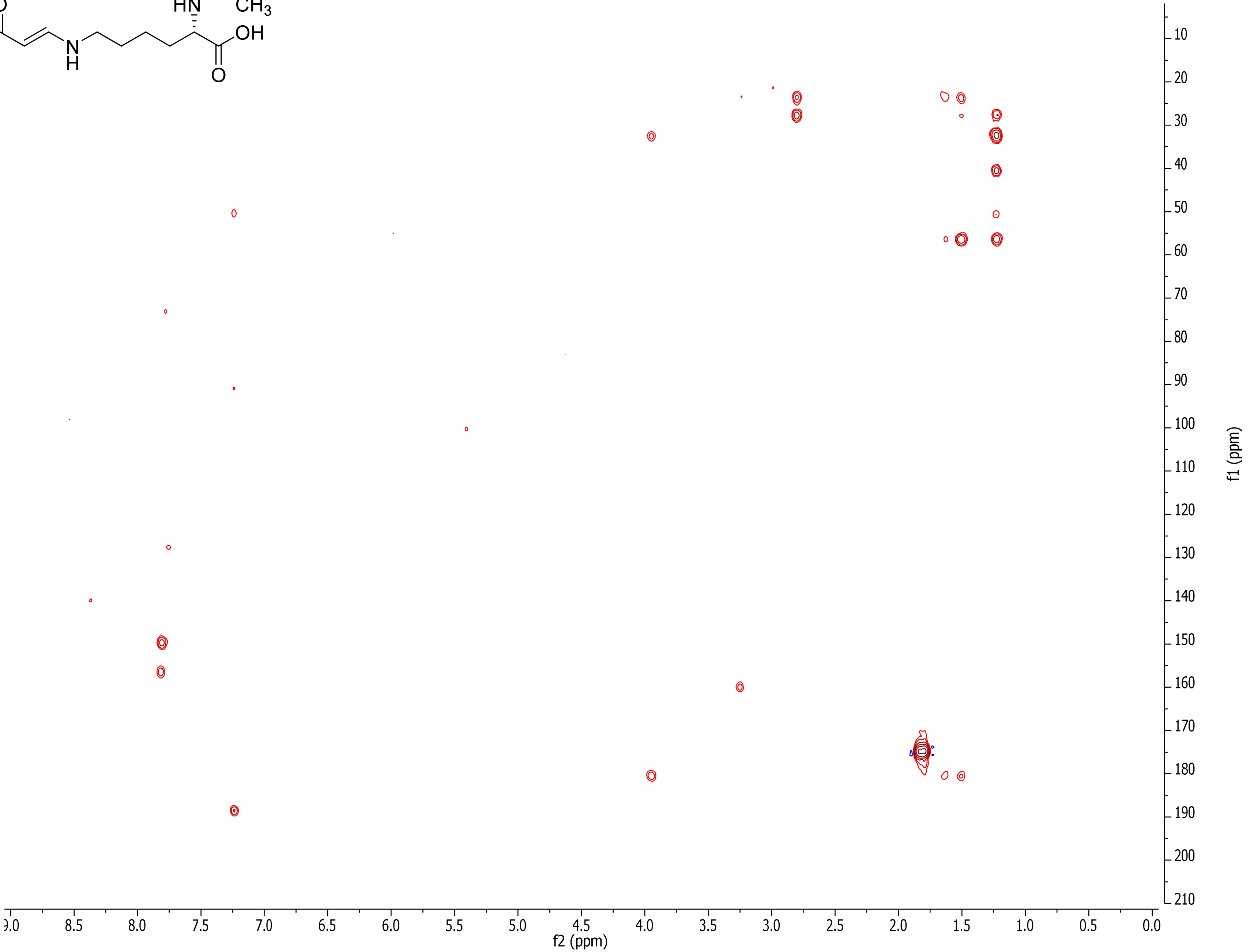
HSQC NMR (500 MHz, phosphate-buffered D_2O) of **8**



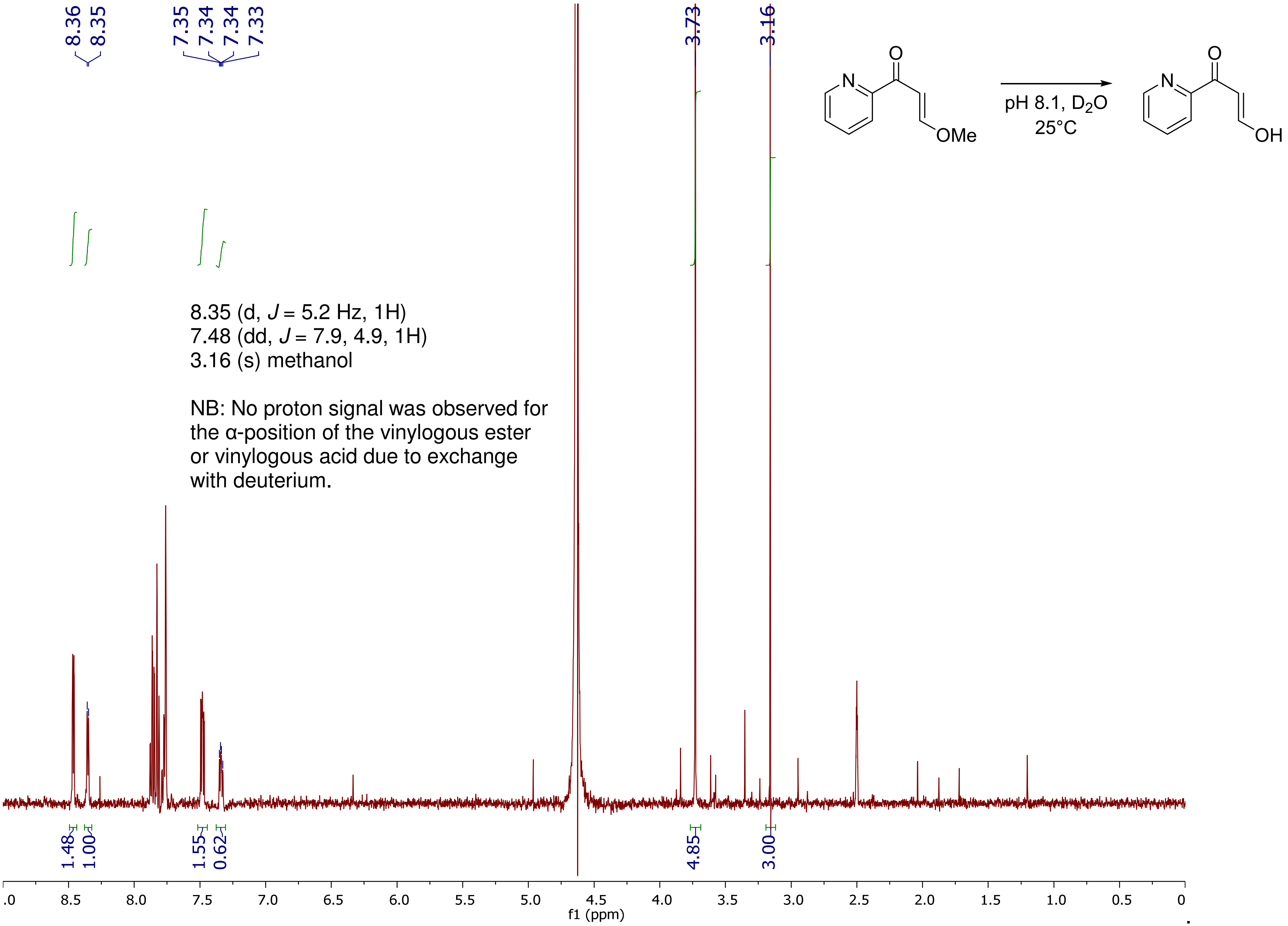


HMBC NMR (500 MHz, phosphate-buffered D_2O) of **8**





^{1}H NMR (500 MHz, phosphate-buffered D_2O) spectrum of **6** and hydrolysis product **9**



8.35 (d, *J* = 5.2 Hz, 1H)
7.48 (dd, *J* = 7.9, 4.9, 1H)
3.16 (s) methanol

NB: No proton signal was observed for the α-position of the vinylogous ester or vinylogous acid due to exchange with deuterium.

Table S10. Results of competition binding assay for neolymphotin against a panel of 97 kinases

Target	Neolymphostin A
Gene Symbol	%Ctrl @ 1000nM
ABL1(E255K)-phosphorylated	70
ABL1(T315I)-phosphorylated	75
ABL1-nonphosphorylated	39
ABL1-phosphorylated	76
ACVR1B	96
ADCK3	87
AKT1	100
AKT2	97
ALK	100
AURKA	1.5
AURKB	1.9
AXL	39
BMPR2	57
BRAF	77
BRAF(V600E)	77
BTK	15
CDK11	59
CDK2	87
CDK3	96
CDK7	50
CDK9	89
CHEK1	88
CSF1R	82
CSNK1D	75
CSNK1G2	33
DCAMKL1	83
DYRK1B	80
EGFR	58
EGFR(L858R)	57
EPHA2	96
ERBB2	28
ERBB4	90
ERK1	90
FAK	98
FGFR2	94
FGFR3	91
FLT3	74
GSK3B	89
IGF1R	96
IKK-alpha	78
IKK-beta	83
INSR	93
JAK2(JH1domain-catalytic)	62
JAK3(JH1domain-catalytic)	18
JNK1	0.25
JNK2	0.15
JNK3	0.2
KIT	84
KIT(D816V)	91

%Ctrl Legend:

0≤x<.1	.1≤x<1	1≤x<10	10≤x<35	x≥35

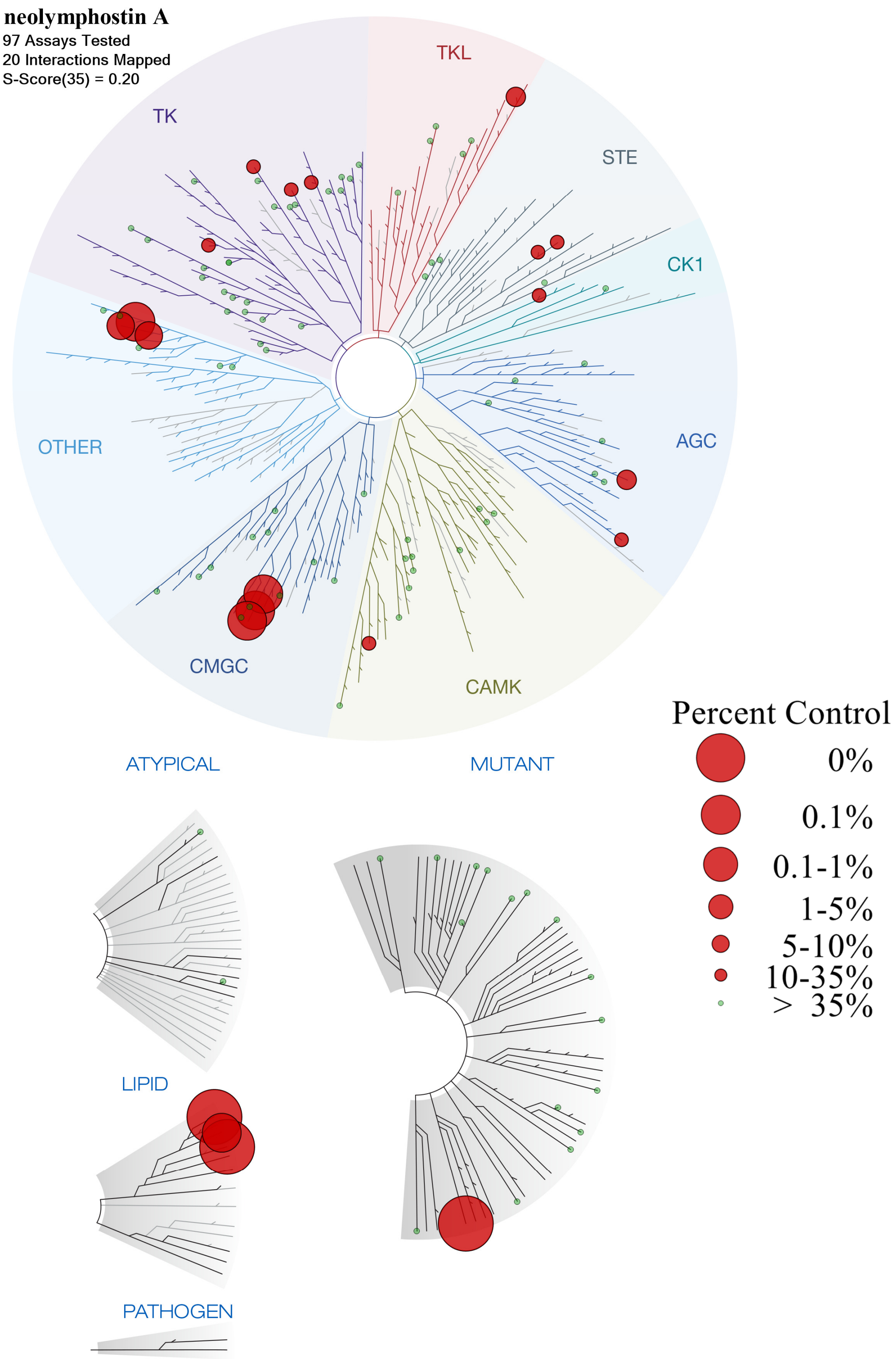
Table S10. (continued)

Target Gene Symbol	Neolymphostin A %Ctrl @ 1000nM
KIT(V559D,T670I)	73
LKB1	93
MAP3K4	88
MAPKAPK2	98
MARK3	100
MEK1	10
MEK2	12
MET	60
MKNK1	87
MKNK2	97
MLK1	8
p38-alpha	100
p38-beta	100
PAK1	100
PAK2	98
PAK4	100
PCTK1	70
PDGFRA	61
PDGFRB	95
PDPK1	100
PIK3C2B	0
PIK3CA	0
PIK3CG	0.55
PIM1	100
PIM2	100
PIM3	91
PKAC-alpha	89
PLK1	100
PLK3	76
PLK4	0.95
PRKCE	18
RAF1	90
RET	86
RIOK2	41
ROCK2	93
RSK2(Kin.Dom.1-N-terminal)	6.1
SNARK	27
SRC	89
SRPK3	69
TGFBR1	97
TIE2	61
TRKA	12
TSSK1B	93
TYK2(JH1domain-catalytic)	60
ULK2	75
VEGFR2	68
YANK3	99
ZAP70	72

%Ctrl Legend:

0≤x<.1	.1≤x<1	1≤x<10	10≤x<35	x≥35

Figure S12. TREEspot™ interaction map for neolymphostin A @ 1000nM

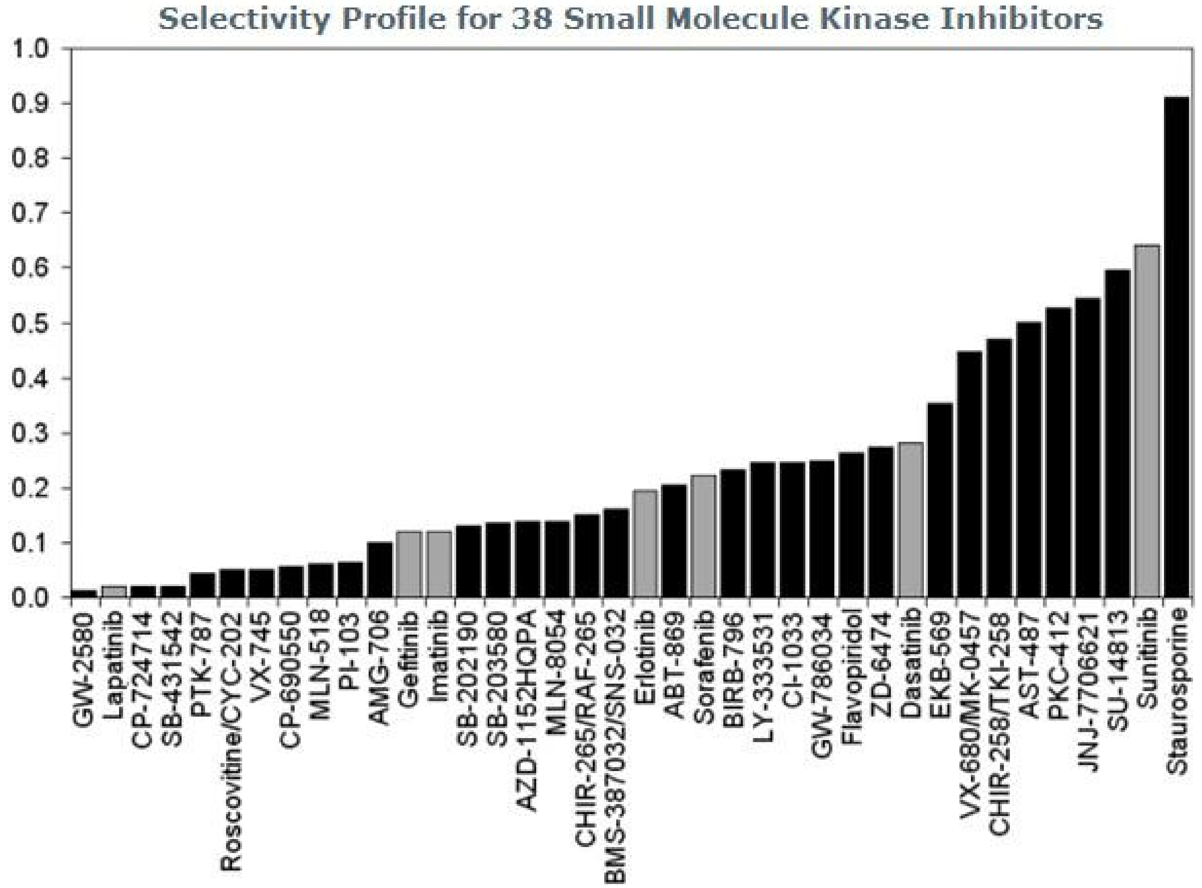


TREEspot™ is a proprietary data visualization software tool developed by KINOMEscan. Kinases found to bind are marked with red circles, where larger circles indicate higher-affinity binding

Table S11. Selectivity S-Score for neolymphostin A against a 97 kinase panel

Compound Name	Selectivity Score Type	Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
Neolymphostin A	S(35)	20	90	1000	0.222
Neolymphostin A	S(10)	11	90	1000	0.122
Neolymphostin A	S(1)	7	90	1000	0.078

Selectivity S35-Score for 38 kinase inhibitors against a panel of 287 kinases



KINOMEscan's in vitro competition binding assay was used to evaluate 38 kinase inhibitors against a panel of 287 distinct human protein kinases (~55% of the predicted human protein kinome), and three lipid kinases. The compounds tested included 21 tyrosine kinase inhibitors, 15 serine-threonine kinase inhibitors, 1 lipid kinase inhibitor and staurosporine. S(35) = (number of non-mutant kinases with %Ctrl <35)/(290 kinases tested; 27 mutant variants were excluded from this analysis). Compounds approved for use in humans (as of August, 2007) are highlighted (gray bars).

Figure S13. NCI 60-cell line dose response curves for neolymphostin A

National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: D - 790970 / 1

SSPL: 0ZNH

EXP. ID: 1610RS07

Report Date: February 11, 2017

Test Date: October 31, 2016

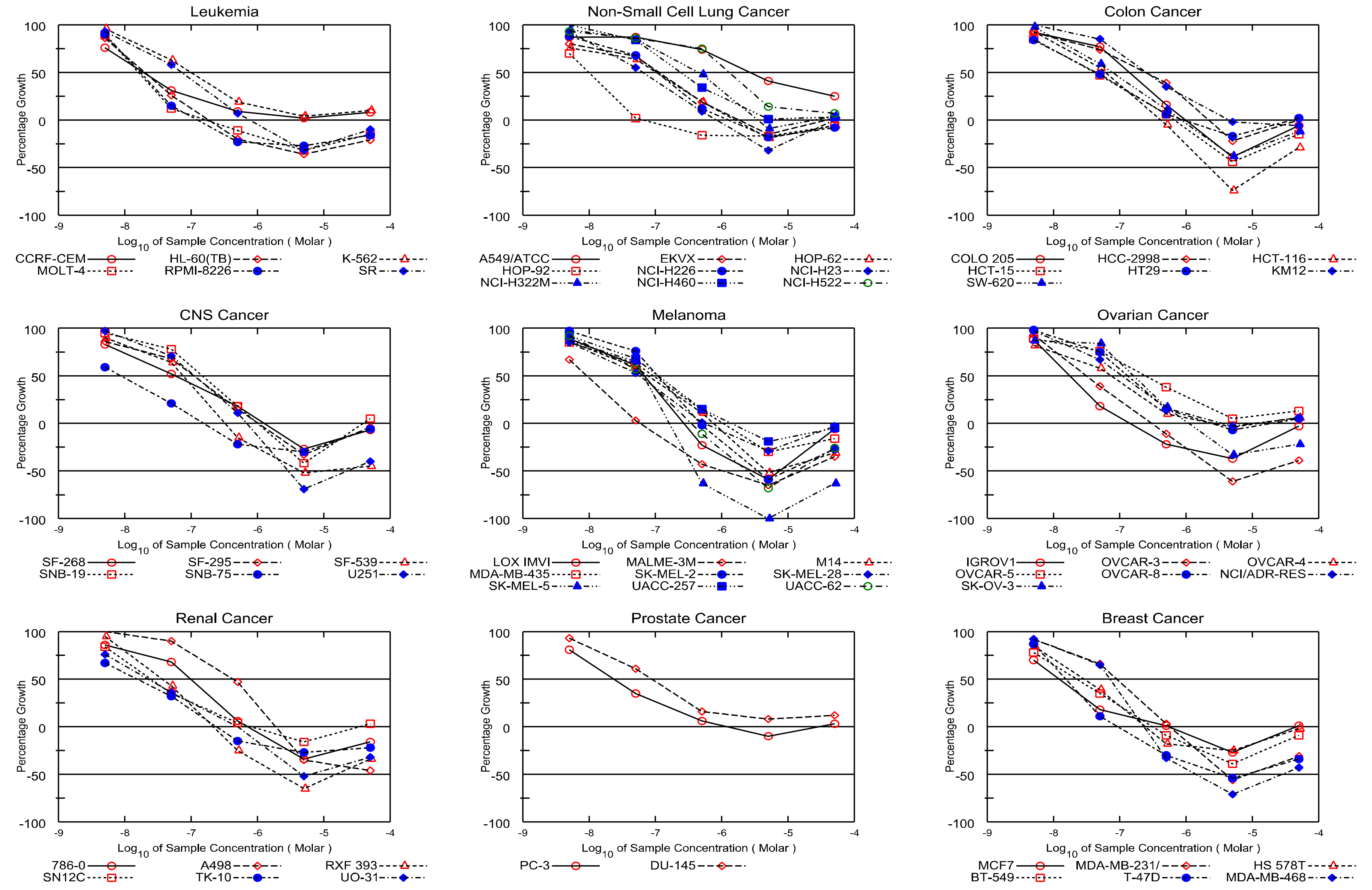


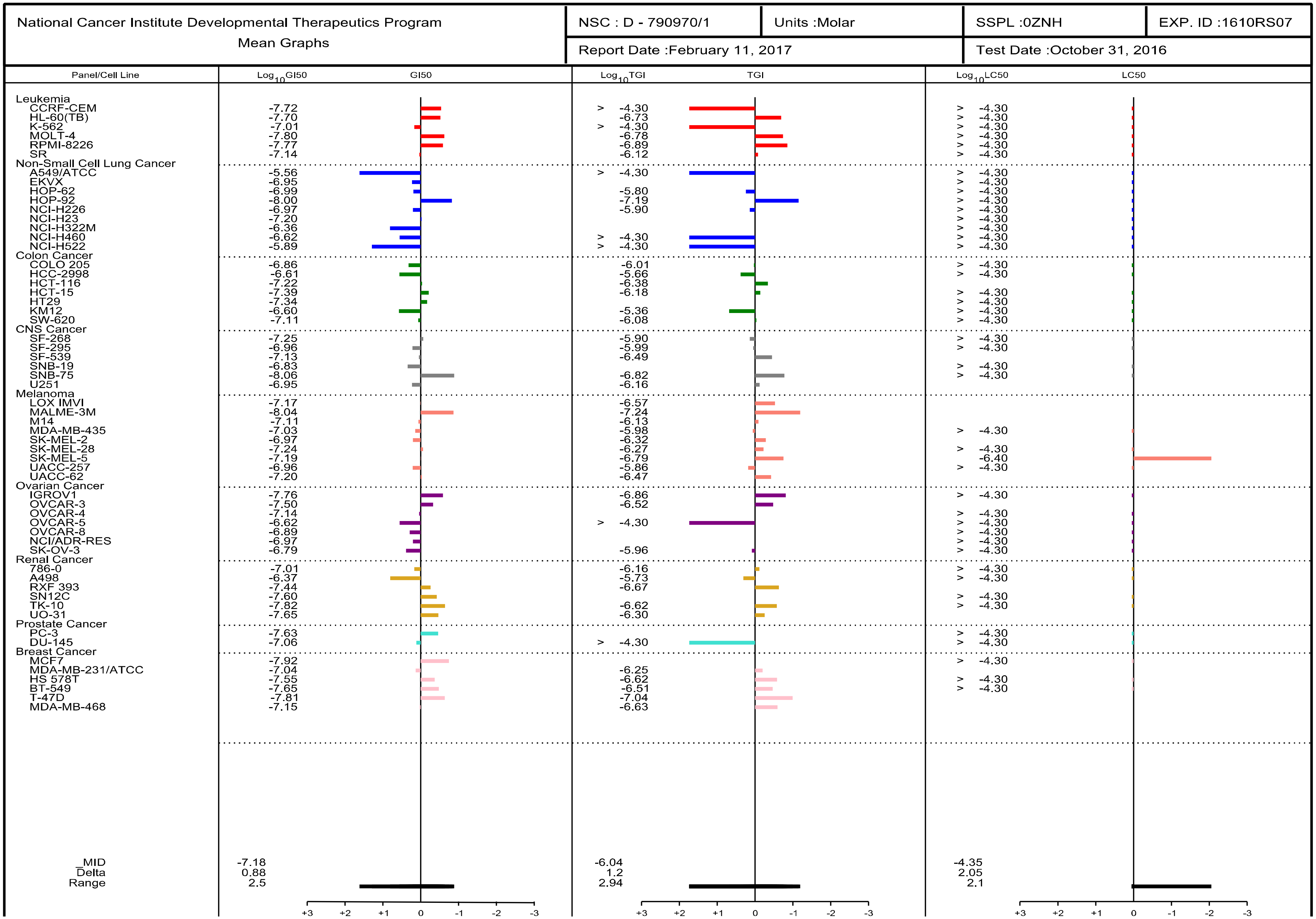
Figure S14. NCI 60-cell line testing results for neolymphostin A

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 790970 / 1	Experiment ID : 1610RS07	Test Type : 08	Units : Molar
Report Date : February 11, 2017	Test Date : October 31, 2016	QNS :	MC :
COMI : Y486.338	Stain Reagent : SRB Dual-Pass Related	SSPL : 0ZNH	

					Log10 Concentration										
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.382	1.593	1.305	0.763	0.492	0.404	0.476	76	31	9	2	8	1.93E-8	> 5.00E-5	> 5.00E-5
HL-60(TB)	0.809	3.021	2.720	1.384	0.649	0.521	0.640	86	26	-20	-36	-21	2.00E-8	1.84E-7	> 5.00E-5
K-562	0.228	2.145	2.069	1.434	0.595	0.301	0.416	96	63	19	4	10	9.86E-8	> 5.00E-5	> 5.00E-5
MOLT-4	0.563	2.041	1.872	0.737	0.503	0.383	0.477	89	12	-11	-32	-15	1.59E-8	1.66E-7	> 5.00E-5
RPMI-8226	0.926	2.303	2.162	1.139	0.717	0.675	0.777	90	15	-23	-27	-16	1.71E-8	1.28E-7	> 5.00E-5
SR	0.568	2.337	2.220	1.601	0.692	0.387	0.511	93	58	7	-32	-10	7.28E-8	7.57E-7	> 5.00E-5
Non-Small Cell Lung Cancer															
A549/ATCC	0.470	1.819	1.645	1.639	1.470	1.029	0.807	87	87	74	41	25	2.73E-6	> 5.00E-5	> 5.00E-5
EKVX	0.853	2.298	2.016	1.824	1.121	0.725	0.882	80	67	19	-15	2	1.13E-7	.	> 5.00E-5
HOP-62	0.945	2.068	1.793	1.668	1.156	0.770	0.892	76	64	19	-19	-6	1.03E-7	1.59E-6	> 5.00E-5
HOP-92	1.002	1.574	1.405	1.014	0.837	0.834	0.939	70	2	-16	-17	-6	9.94E-9	6.42E-8	> 5.00E-5
NCI-H226	0.768	1.285	1.222	1.122	0.832	0.627	0.705	88	68	12	-18	-8	1.06E-7	1.26E-6	> 5.00E-5
NCI-H23	0.648	2.032	1.938	1.403	0.771	0.441	0.649	93	55	9	-32	.	6.29E-8	.	> 5.00E-5
NCI-H322M	0.967	2.254	2.185	2.061	1.582	0.884	1.017	95	85	48	-9	4	4.36E-7	.	> 5.00E-5
NCI-H460	0.183	1.894	1.889	1.617	0.771	0.195	0.241	100	84	34	1	3	2.41E-7	> 5.00E-5	> 5.00E-5
NCI-H522	0.922	2.081	2.006	1.924	1.791	1.088	1.006	93	86	75	14	7	1.29E-6	> 5.00E-5	> 5.00E-5
Colon Cancer															
COLO 205	0.580	1.888	1.785	1.587	0.787	0.357	0.546	92	77	16	-39	-6	1.38E-7	9.76E-7	> 5.00E-5
HCC-2998	0.864	2.666	2.528	2.206	1.568	0.677	0.858	92	74	39	-22	.	2.45E-7	2.20E-6	> 5.00E-5
HCT-116	0.252	2.145	2.016	1.296	0.240	0.065	0.179	93	55	-5	-74	-29	6.09E-8	4.16E-7	.
HCT-15	0.330	2.224	1.941	1.213	0.442	0.185	0.281	85	47	6	-44	-15	4.08E-8	6.56E-7	> 5.00E-5
HT29	0.228	1.362	1.181	0.778	0.293	0.189	0.256	84	48	6	-17	2	4.53E-8	.	> 5.00E-5
KM12	0.566	2.852	2.866	2.518	1.358	0.555	0.535	101	85	35	-2	-6	2.49E-7	4.40E-6	> 5.00E-5
SW-620	0.254	1.734	1.699	1.131	0.415	0.157	0.223	98	59	11	-38	-12	7.76E-8	8.33E-7	> 5.00E-5
CNS Cancer															
SF-268	0.752	2.376	2.096	1.594	1.048	0.546	0.699	83	52	18	-27	-7	5.67E-8	1.25E-6	> 5.00E-5
SF-295	0.611	2.825	2.510	2.123	0.937	0.408	0.585	86	68	15	-33	-4	1.10E-7	1.01E-6	> 5.00E-5
SF-539	1.194	3.050	2.861	2.375	1.019	0.578	0.662	90	64	-15	-52	-45	7.46E-8	3.25E-7	.
SNB-19	0.265	1.047	1.007	0.878	0.404	0.154	0.302	95	78	18	-42	5	1.47E-7	.	> 5.00E-5
SNB-75	0.941	1.875	1.496	1.134	0.734	0.663	0.889	59	21	-22	-30	-6	8.74E-9	1.52E-7	> 5.00E-5
U251	0.495	1.932	1.896	1.519	0.656	0.154	0.297	97	71	11	-69	-40	1.13E-7	6.90E-7	.
Melanoma															
LOX IMVI	0.462	2.614	2.386	1.769	0.357	0.188	0.435	89	61	-23	-59	-6	6.72E-8	2.67E-7	.
MALME-3M	0.588	1.044	0.892	0.602	0.333	0.204	0.385	67	3	-43	-65	-35	9.11E-9	5.79E-8	.
M14	0.406	1.751	1.558	1.200	0.549	0.195	0.281	86	59	11	-52	-31	7.69E-8	7.39E-7	.
MDA-MB-435	0.495	2.356	2.084	1.672	0.759	0.347	0.415	85	63	14	-30	-16	9.31E-8	1.05E-6	> 5.00E-5
SK-MEL-2	1.000	2.074	2.037	1.816	0.982	0.412	0.729	97	76	-2	-59	-27	1.08E-7	4.74E-7	.
SK-MEL-28	0.560	1.718	1.543	1.177	0.571	0.398	0.543	85	53	1	-29	-3	5.77E-8	5.38E-7	> 5.00E-5
SK-MEL-5	0.727	2.593	2.345	1.932	0.270	-0.002	0.266	87	65	-63	-100	-63	6.50E-8	1.61E-7	3.96E-7
UACC-257	1.025	1.941	1.866	1.648	1.161	0.834	0.969	92	68	15	-19	-5	1.09E-7	1.38E-6	> 5.00E-5
UACC-62	0.899	2.891	2.729	2.025	0.799	0.292	0.663	92	57	-11	-68	-26	6.25E-8	3.42E-7	.
Ovarian Cancer															
IGROV1	0.532	1.905	1.735	0.778	0.414	0.336	0.516	88	18	-22	-37	-3	1.73E-8	1.39E-7	> 5.00E-5
OVCAR-3	0.441	1.409	1.341	0.821	0.393	0.170	0.271	93	39	-11	-61	-39	3.15E-8	3.03E-7	.
OVCAR-4	0.705	1.594	1.438	1.219	0.798	0.680	0.759	82	58	10	-4	6	7.30E-8	.	> 5.00E-5
OVCAR-5	0.733	2.242	2.077	1.876	1.304	0.807	0.932	89	76	38	5	13	2.39E-7	> 5.00E-5	> 5.00E-5
OVCAR-8	0.522	2.037	2.009	1.658	0.747	0.487	0.592	98	75	15	-7	5	1.30E-7	.	> 5.00E-5
NCI/ADR-RES	0.647	2.289	2.235	1.746	0.907	0.627	0.745	97	67	16	-3	6	1.07E-7	.	> 5.00E-5
SK-OV-3	1.104	2.092	1.968	1.931	1.275	0.742	0.862	87	84	17	-33	-22	1.61E-7	1.11E-6	> 5.00E-5
Renal Cancer															
786-0	0.636	2.392	2.154	1.836	0.736	0.419	0.533	86	68	6	-34	-16	9.81E-8	6.95E-7	> 5.00E-5
A498	1.153	1.897	1.931	1.823	1.503	0.744	0.626	105	90	47	-35	-46	4.27E-7	1.86E-6	> 5.00E-5
RXF 393	0.641	1.212	1.186	0.885	0.480	0.224	0.426	95	43	-25	-65	-34	3.65E-8	2.13E-7	.
SN12C	0.522	2.076	1.835	1.068	0.588	0.438	0.564	84	35	4	-16	3	2.50E-8	.	> 5.00E-5
TK-10	1.429	2.506	2.149	1.772	1.218	1.040	1.113	67	32	-15	-27	-22	1.51E-8	2.41E-7	> 5.00E-5
UO-31	0.508	1.898	1.560	1.015	0.511	0.244	0.345	76	36	.	-52	-32	2.26E-8	5.04E-7	.
Prostate Cancer															
PC-3	0.588	1.649	1.443	0.957	0.655	0.530	0.623	81	35	6	-10	3	2.32E-8	.	> 5.00E-5
DU-145	0.346	1.584	1.493	1.099	0.547	0.447	0.497	93	61	16	8	12	8.75E-8	> 5.00E-5	> 5.00E-5
Breast Cancer															
MCF7	0.360	1.954	1.472	0.642	0.373	0.262	0.384	70	18	1	-27	1	1.20E-8	.	> 5.00E-5
MDA-MB-231/ATCC	0.573	1.251	1.199	1.023	0.595	0.255	0.394	92	66	3	-56	-31	9.08E-8	5.68E-7	.
HS 578T	1.105	2.145	1.989	1.506	0.904	0.832	1.087	85	39	-18	-25	-2	2.83E-8	2.39E-7	> 5.00E-5
BT-549	1.113	2.212	1.967	1.496	1.013	0.681	1.017	78	35	-9	-39	-9	2.21E-8	3.11E-7	> 5.00E-5
T-47D	0.851	1.610	1.513	0.933	0.592	0.392	0.563	87	11	-30	-54	-34	1.53E-8	9.13E-8	.
MDA-MB-468	0.913	1.855	1.778	1.525	0.616	0.268	0.524	92	65	-33	-71	-43	7.12E-8	2.32E-7	.

Figure S15. NCI 60-cell line mean graphs for neolymphostin A

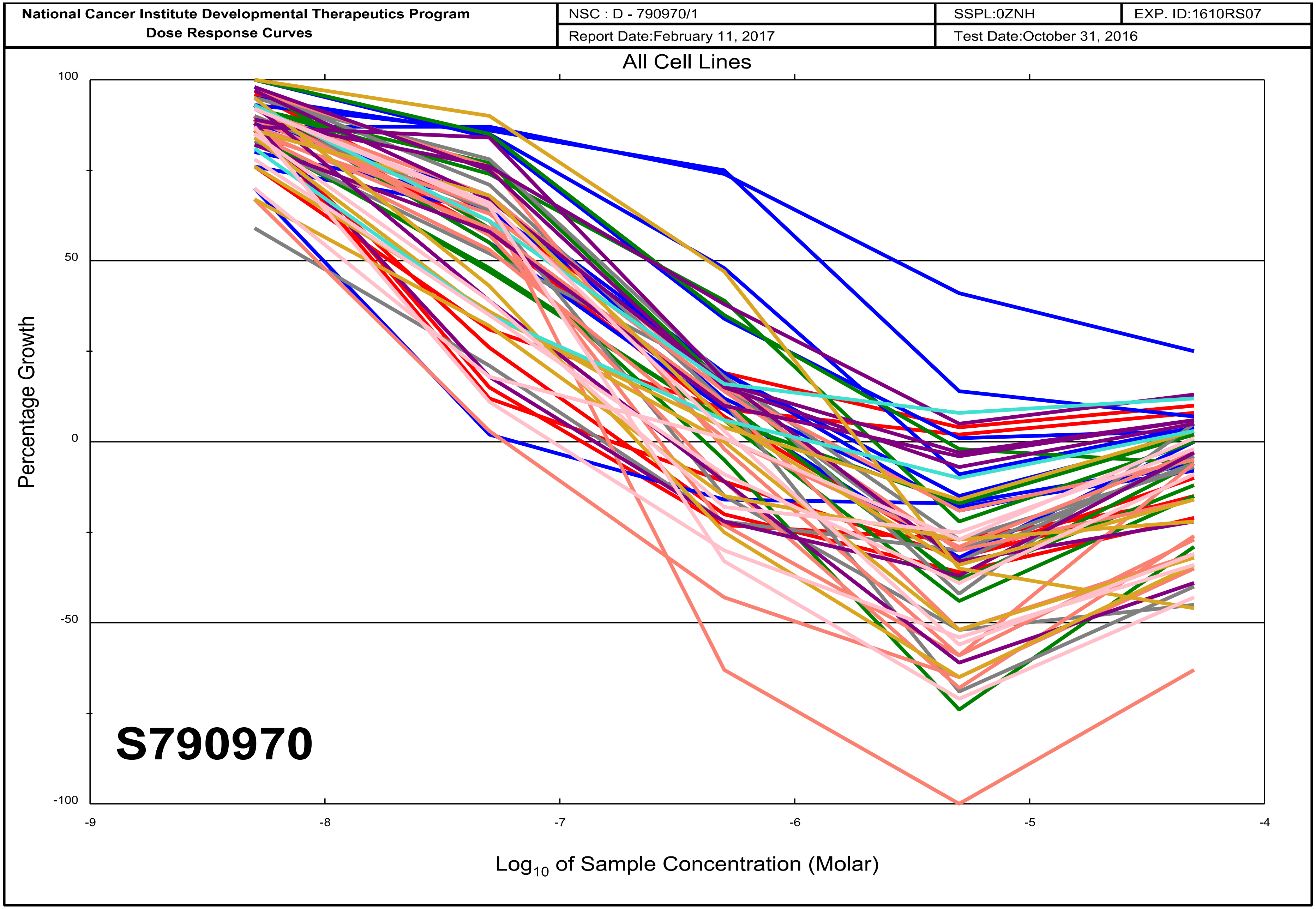


National Cancer Institute Developmental Therapeutics Program
Mean Graphs

NSC : D - 790970/1
Units :Molar
SSPL :0ZNH
EXP. ID :1610RS07
Report Date :February 11, 2017
Test Date :October 31, 2016

Panel/Cell Line	Log_{10}GI50	Log_{10}TGI	Log_{10}LC50
Leukemia			
CCRF-CEM	-7.72	> -4.30	> -4.30
HL-60(TB)	-7.70	-6.73	> -4.30
K-562	-7.01	> -4.30	> -4.30
MOLT-4	-7.80	-6.78	> -4.30
RPMI-8226	-7.77	-6.89	> -4.30
SR	-7.14	-6.12	> -4.30
Non-Small Cell Lung Cancer			
A549/ATCC	-5.56	> -4.30	> -4.30
EKVX	-6.95		> -4.30
HOP-62	-6.99	-5.80	> -4.30
HOP-92	-8.00	-7.19	> -4.30
NCI-H226	-6.97	-5.90	> -4.30
NCI-H23	-7.20		> -4.30
NCI-H322M	-6.36		> -4.30
NCI-H460	-6.62	> -4.30	> -4.30
NCI-H522	-5.89	> -4.30	> -4.30
Colon Cancer			
COLO 205	-6.86	-6.01	> -4.30
HCC-2998	-6.61	-5.66	> -4.30
HCT-116	-7.22	-6.38	
HCT-15	-7.39	-6.18	> -4.30
HT29	-7.34		> -4.30
KM12	-6.60	-5.36	> -4.30
SW-620	-7.11	-6.08	> -4.30
CNS Cancer			
SF-268	-7.25	-5.90	> -4.30
SF-295	-6.96	-5.99	> -4.30
SF-539	-7.13	-6.49	
SNB-19	-6.83		> -4.30
SNB-75	-8.06	-6.82	> -4.30
U251	-6.95	-6.16	
Melanoma			
LOX IMVI	-7.17	-6.57	
MALME-3M	-8.04	-7.24	
M14	-7.11	-6.13	
MDA-MB-435	-7.03	-5.98	> -4.30
SK-MEL-2	-6.97	-6.32	
SK-MEL-28	-7.24	-6.27	> -4.30
SK-MEL-5	-7.19	-6.79	-6.40
UACC-257	-6.96	-5.86	> -4.30
UACC-62	-7.20	-6.47	
Ovarian Cancer			
IGROV1	-7.76	-6.86	> -4.30
OVCAR-3	-7.50	-6.52	
OVCAR-4	-7.14		> -4.30
OVCAR-5	-6.62	> -4.30	> -4.30
OVCAR-8	-6.89		> -4.30
NCI/ADR-RES	-6.97		> -4.30
SK-OV-3	-6.79	-5.96	> -4.30
Renal Cancer			
786-0	-7.01	-6.16	> -4.30
A498	-6.37	-5.73	> -4.30
RXF 393	-7.44	-6.67	
SN12C	-7.60		> -4.30
TK-10	-7.82	-6.62	> -4.30
UO-31	-7.65	-6.30	
Prostate Cancer			
PC-3	-7.63		> -4.30
DU-145	-7.06	> -4.30	> -4.30
Breast Cancer			
MCF7	-7.92		> -4.30
MDA-MB-231/ATCC	-7.04	-6.25	
HS 578T	-7.55	-6.62	> -4.30
BT-549	-7.65	-6.51	> -4.30
T-47D	-7.81	-7.04	
MDA-MB-468	-7.15	-6.63	
_MID	-7.18	-6.04	-4.35
Delta	0.88	1.2	2.05
Range	2.5	2.94	2.1

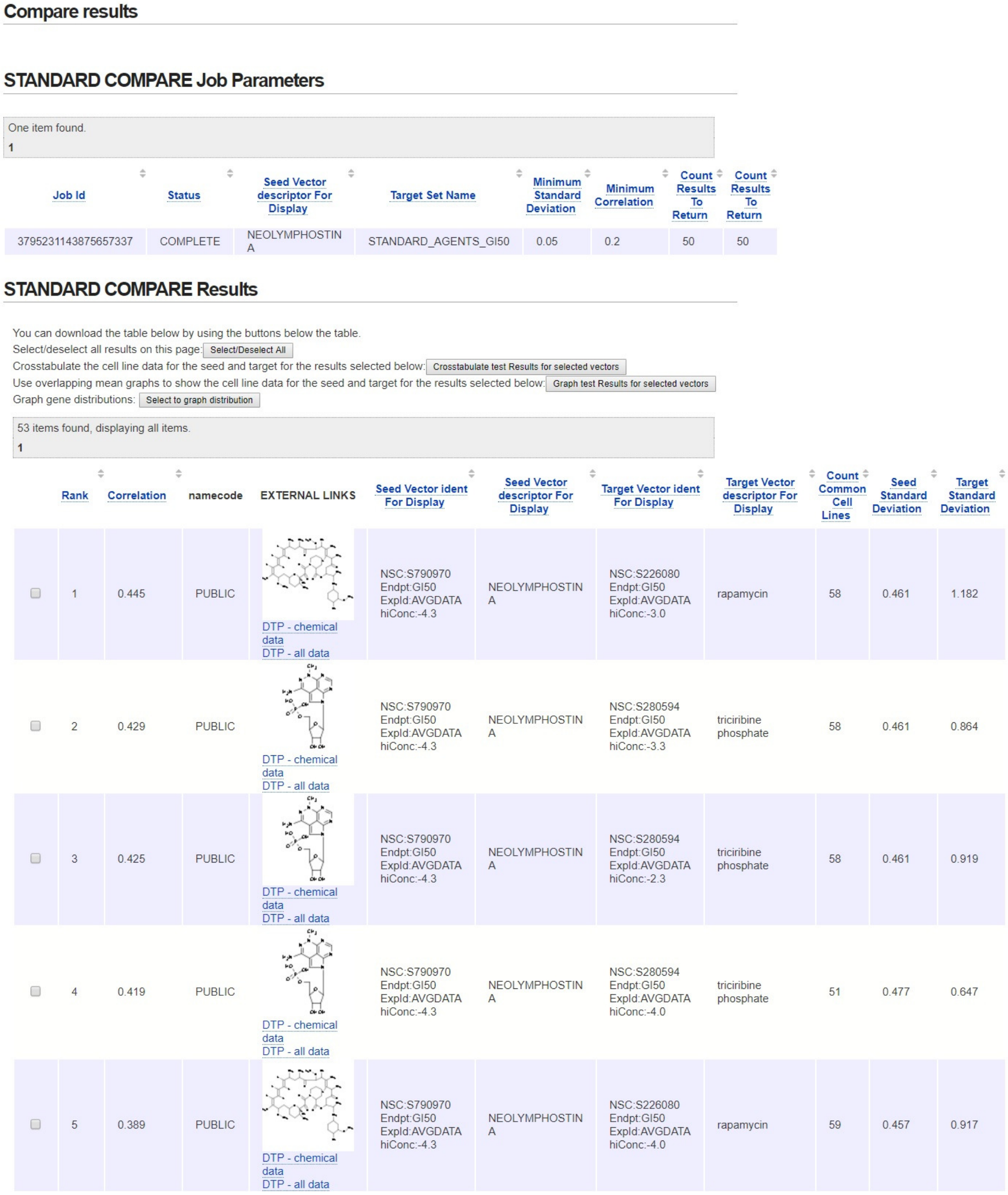
Figure S16. NCI 60-cell line dose response curves for neolymphostin A (all)



National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC : D - 790970/1	SSPL:0ZNH	EXP. ID:1610RS07	
Report Date:February 11, 2017	Test Date:October 31, 2016		

Figure S17. COMPARE results



Compare results

STANDARD COMPARE Job Parameters

One item found.
1

Job Id	Status	Seed Vector descriptor For Display	Target Set Name	Minimum Standard Deviation	Minimum Correlation	Count Results To Return	Count Results To Return
3795231143875657337	COMPLETE	NEOLYMPHOSTIN A	STANDARD_AGENTS_GI50	0.05	0.2	50	50

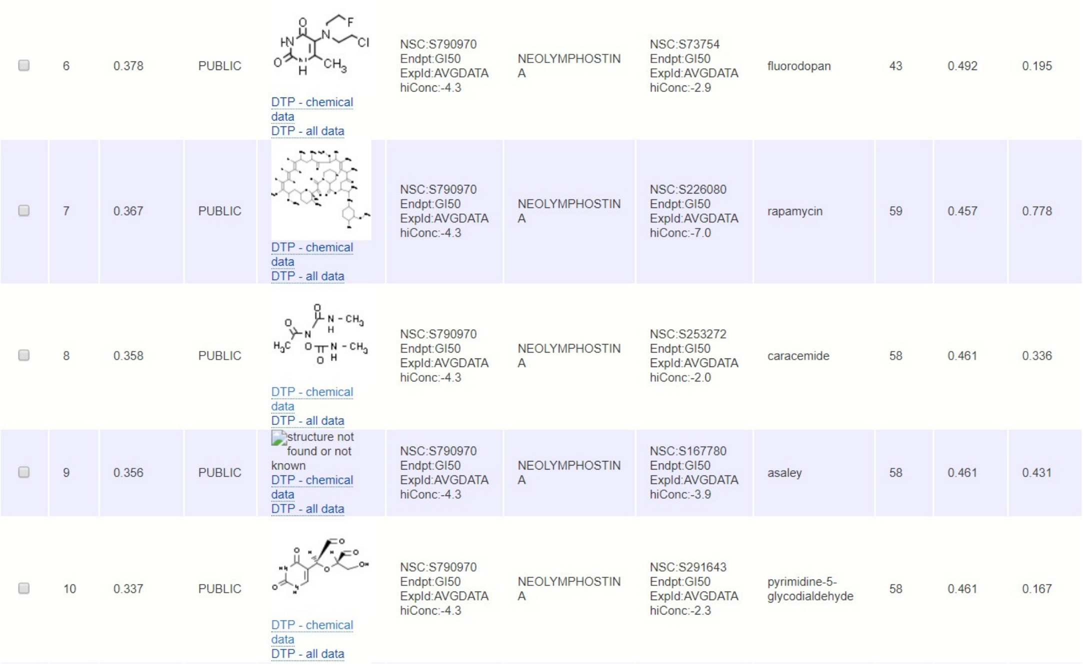
STANDARD COMPARE Results

You can download the table below by using the buttons below the table.
Select/deselect all results on this page: Select/Deselect All
Crosstabulate the cell line data for the seed and target for the results selected below: Crosstabulate test Results for selected vectors
Use overlapping mean graphs to show the cell line data for the seed and target for the results selected below: Graph test Results for selected vectors
Graph gene distributions: Select to graph distribution

53 items found, displaying all items.
1

	Rank	Correlation	namecode	EXTERNAL LINKS	Seed Vector ident For Display	Seed Vector descriptor For Display	Target Vector ident For Display	Target Vector descriptor For Display	Count Common Cell Lines	Seed Standard Deviation	Target Standard Deviation
☐	1	0.445	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 Expld:AVGDATA hiConc:-4.3	NEOLYMPHOSTIN A	NSC:S226080 Endpt:GI50 Expld:AVGDATA hiConc:-3.0	rapamycin	58	0.461	1.182
☐	2	0.429	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 Expld:AVGDATA hiConc:-4.3	NEOLYMPHOSTIN A	NSC:S280594 Endpt:GI50 Expld:AVGDATA hiConc:-3.3	triciribine phosphate	58	0.461	0.864
☐	3	0.425	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 Expld:AVGDATA hiConc:-4.3	NEOLYMPHOSTIN A	NSC:S280594 Endpt:GI50 Expld:AVGDATA hiConc:-2.3	triciribine phosphate	58	0.461	0.919
☐	4	0.419	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 Expld:AVGDATA hiConc:-4.3	NEOLYMPHOSTIN A	NSC:S280594 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	triciribine phosphate	51	0.477	0.647
☐	5	0.389	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 Expld:AVGDATA hiConc:-4.3	NEOLYMPHOSTIN A	NSC:S226080 Endpt:GI50 Expld:AVGDATA hiConc:-4.0	rapamycin	59	0.457	0.917

Figure S17. COMPARE results (continued)



☐	6	0.378	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 ExpId:AVGDATA hiConc:-4.3	NEOLYMPHOSTINA	NSC:S73754 Endpt:GI50 ExpId:AVGDATA hiConc:-2.9	fluorodopan	43	0.492	0.195
☐	7	0.367	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 ExpId:AVGDATA hiConc:-4.3	NEOLYMPHOSTINA	NSC:S226080 Endpt:GI50 ExpId:AVGDATA hiConc:-7.0	rapamycin	59	0.457	0.778
☐	8	0.358	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 ExpId:AVGDATA hiConc:-4.3	NEOLYMPHOSTINA	NSC:S253272 Endpt:GI50 ExpId:AVGDATA hiConc:-2.0	caracemide	58	0.461	0.336
☐	9	0.356	PUBLIC	structure not found or not known DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 ExpId:AVGDATA hiConc:-4.3	NEOLYMPHOSTINA	NSC:S167780 Endpt:GI50 ExpId:AVGDATA hiConc:-3.9	asaley	58	0.461	0.431
☐	10	0.337	PUBLIC	DTP - chemical data DTP - all data	NSC:S790970 Endpt:GI50 ExpId:AVGDATA hiConc:-4.3	NEOLYMPHOSTINA	NSC:S291643 Endpt:GI50 ExpId:AVGDATA hiConc:-2.3	pyrimidine-5-glycodialdehyde	58	0.461	0.167