# Electronic supporting information

# for

# Isothiazolones; thiol-reactive inhibitors of cysteine protease cathepsin B and histone acetyl transferase PCAF

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## Experimental section

## Chemistry

**Table S1**. Interatomic distances (Å) in the crystal structures for compounds **1** and **5**.

| | | 2-phenyl-isothiazo-lone **1** | 5-chloro-2-phenyl-isothiazo-lone **5** | Literature values $sp^2$ [a] | Literature values $sp^3$ [a] |
|---|---|---|---|---|---|
| Cl | C1 | | 1.7084(18) | | 1.76 |
| S | N | 1.715(4) | 1.7038(15) | 1.56 | 1.78 |
| S | C1 | 1.702(6) | 1.7269(18) | 1.70 (thiophene)[b]<br>1.61 | 1.81 |
| O | C3 | 1.243(6) | 1.231(2) | 1.23-1.24 (amides) | 1.42-1.44 (ethers) |
| N | C3 | 1.401(7) | 1.396(2) | 1.35 (imines) | 1.46-1.48 (amines) |
| N | C4 | 1.433(7) | 1.433(2) | 1.35 (imines) | 1.46-1.48 (amines) |
| C1 | C2 | 1.345(7) | 1.346(3) | 1.31-1.34 (alkene) | |
| C2 | C3 | 1.452(7) | 1.454(3) | 1.45-1.46<br>(single conjugated) | |
| C4 | C5 | 1.399(7) | 1.396(3) | 1.38-1.40 (aromatic) | |
| C4 | C9 | 1.408(7) | 1.393(3) | 1.38-1.40 (aromatic) | |
| C5 | C6 | 1.383(7) | 1.384(3) | 1.38-1.40 (aromatic) | |
| C6 | C7 | 1.391(8) | 1.395(3) | 1.38-1.40 (aromatic) | |
| C7 | C8 | 1.402(8) | 1.392(3) | 1.38-1.40 (aromatic) | |
| C8 | C9 | 1.374(9) | 1.389(3) | 1.38-1.40 (aromatic) | |

Standard uncertainty in the last decimal place is given in parentheses
a [1], b CCDC 617103

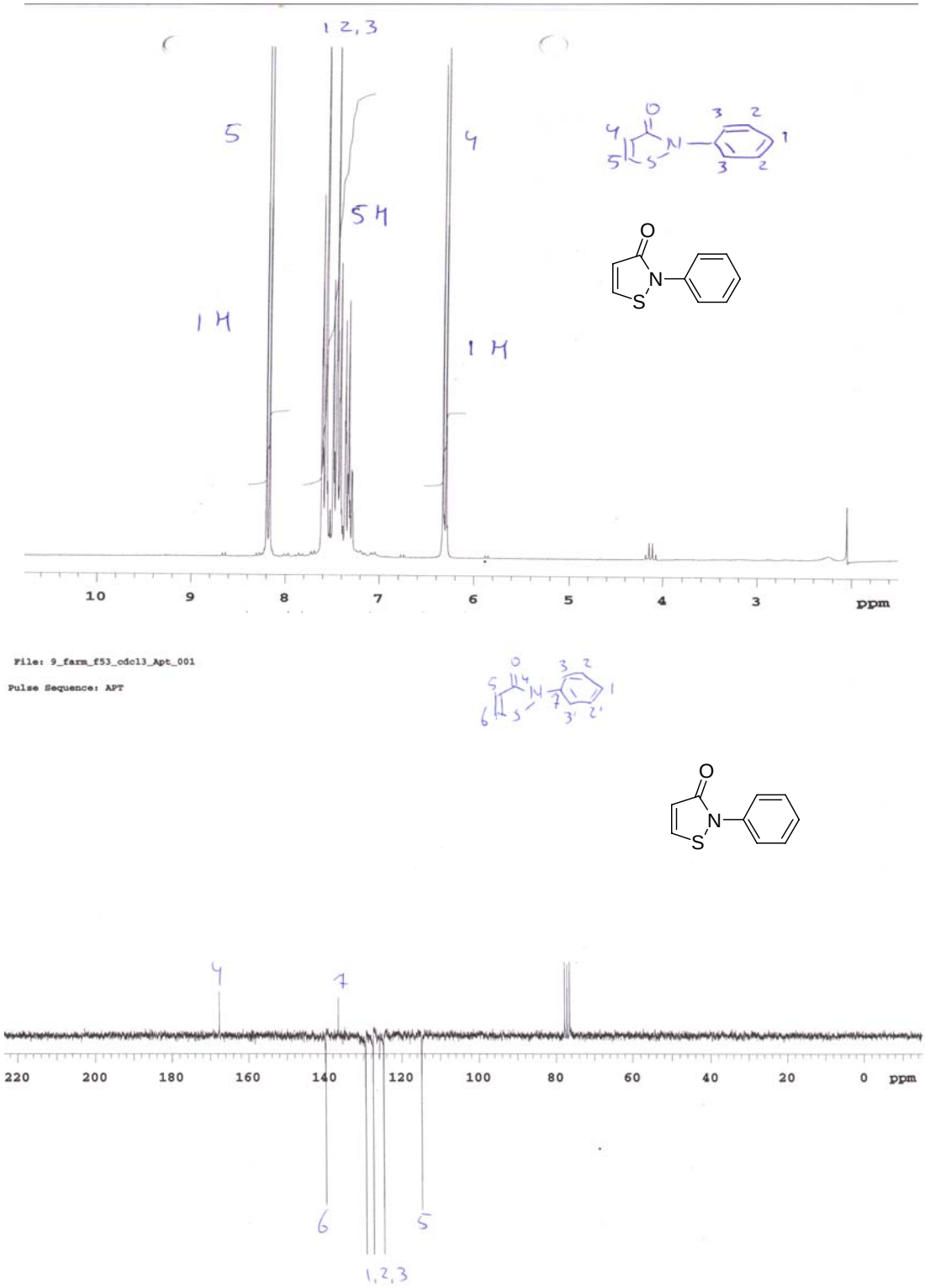



**Figure S1**. $^{1}H$ and $^{13}C$ NMR spectra of **1** .

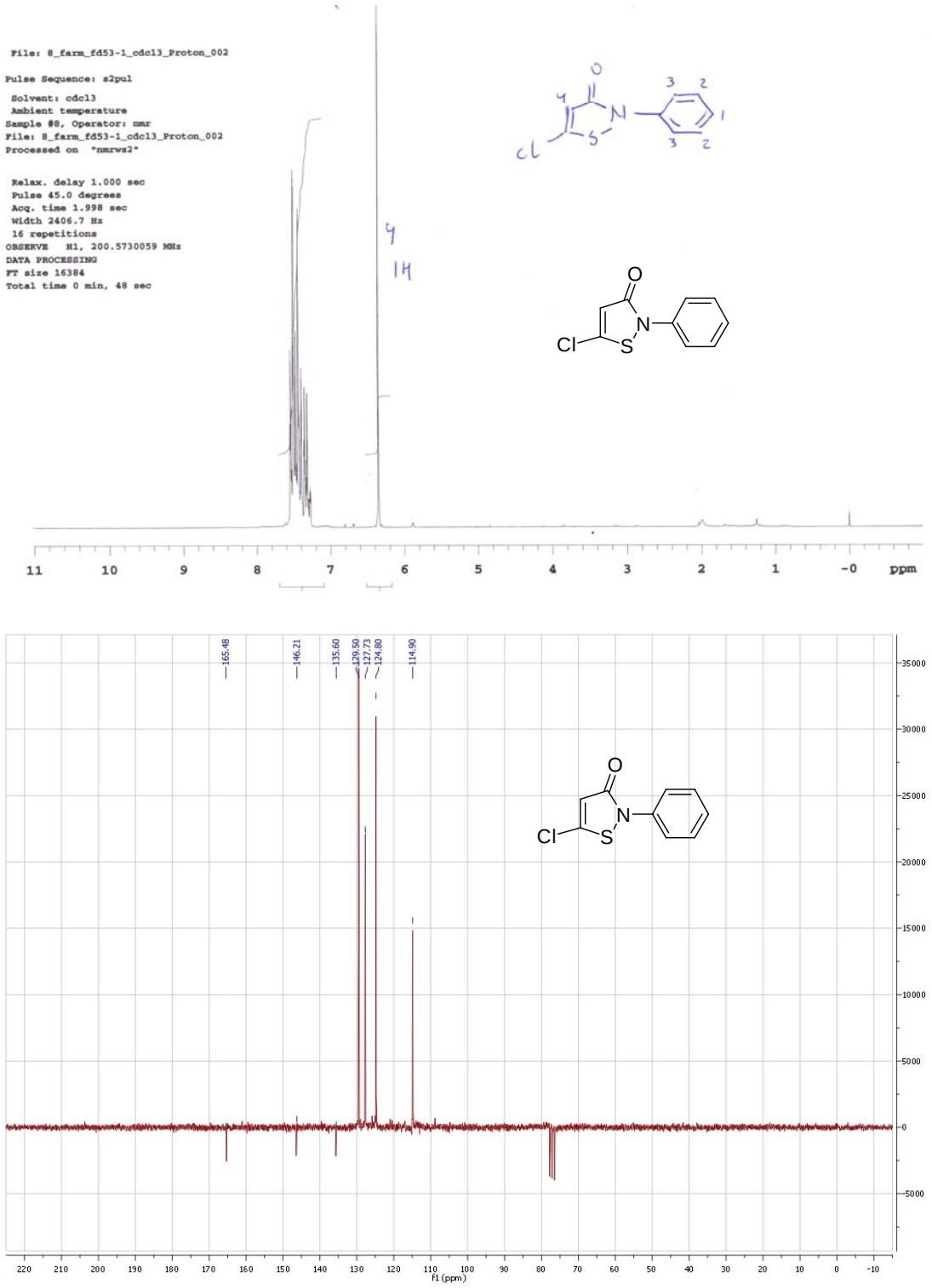



**Figure S2**. $^{1}H$ and $^{13}C$ NMR spectra of **5** .

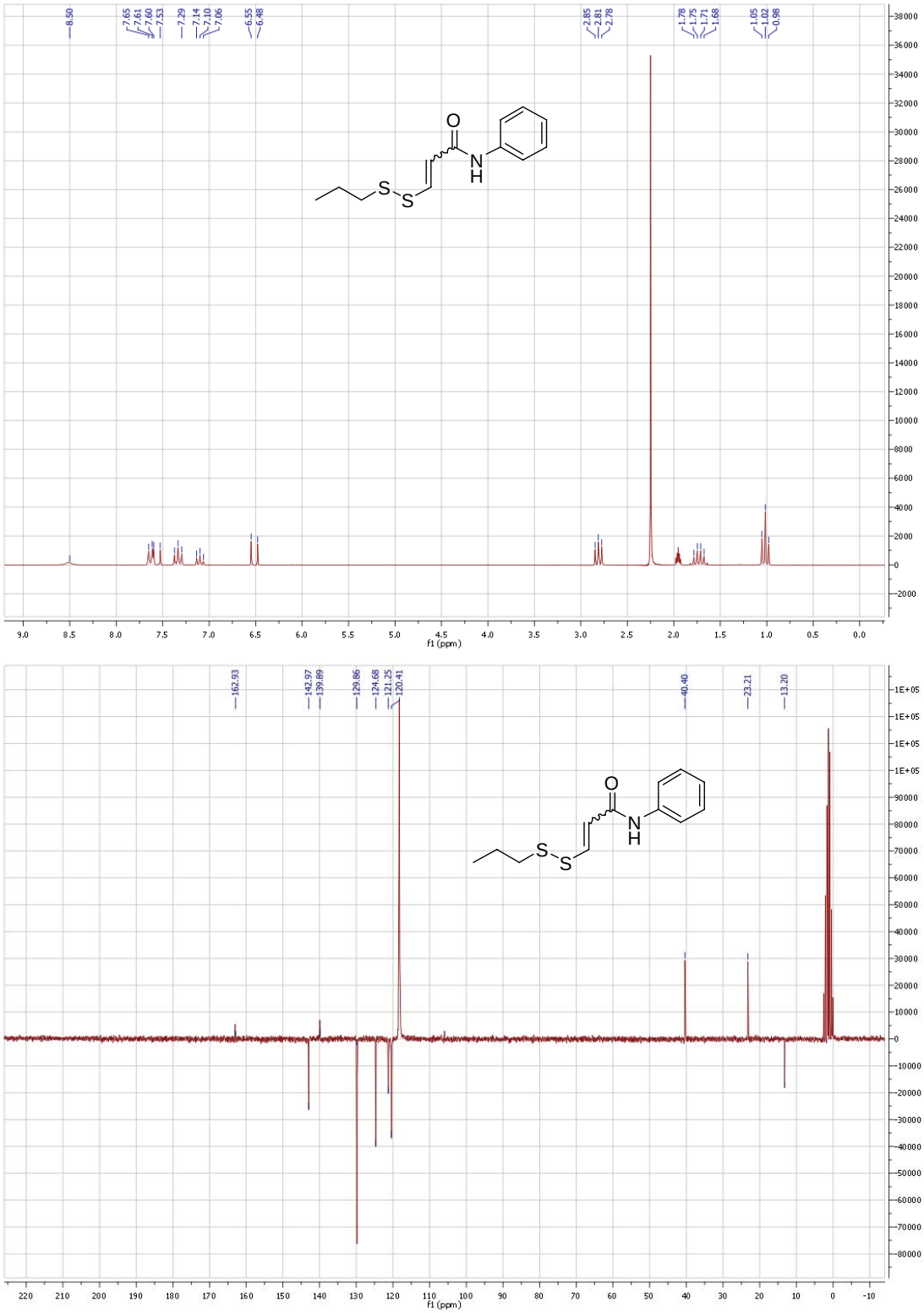



**Figure S3**. $^{1}H$ and $^{13}C$ NMR spectra of **3** .

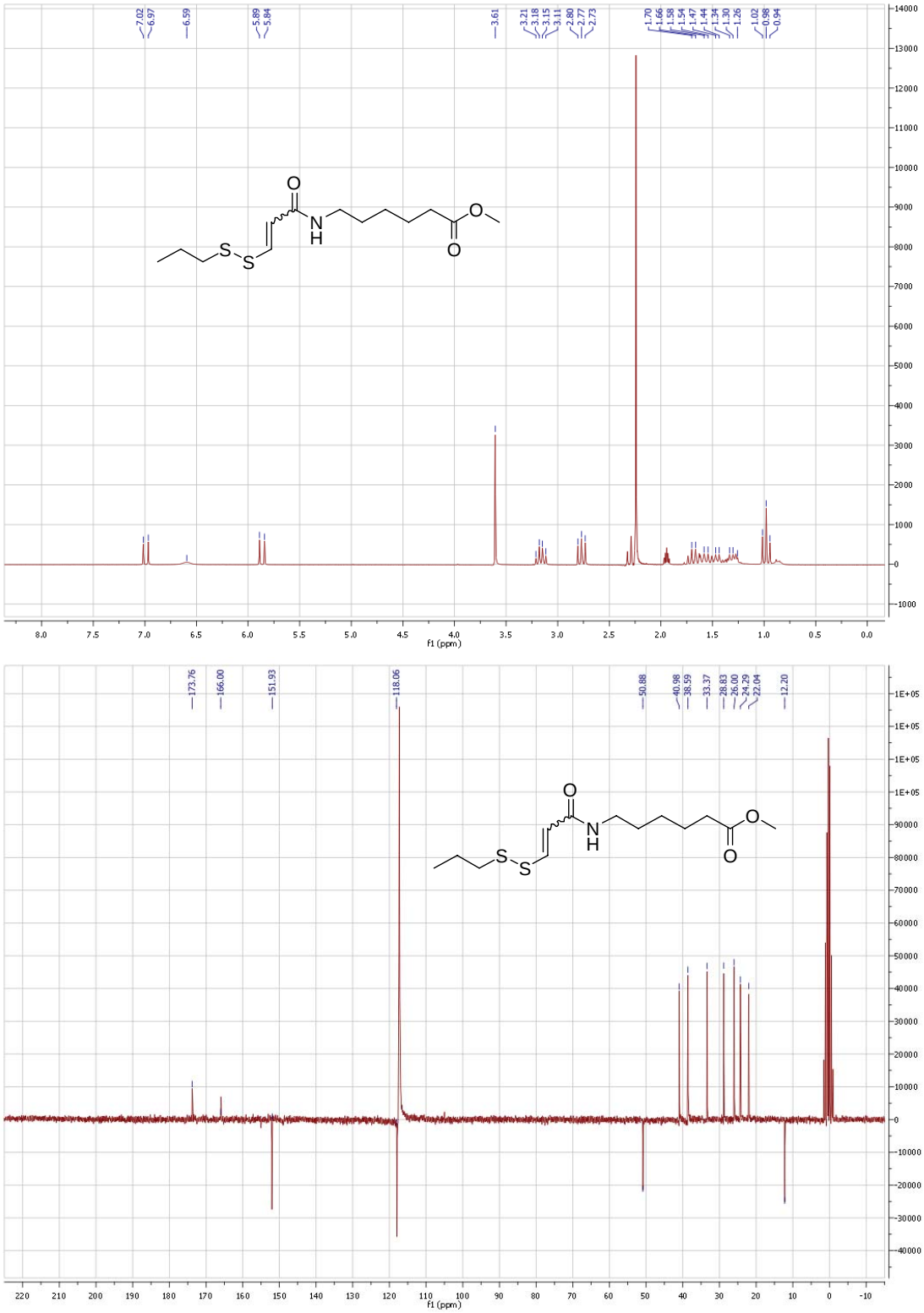



**Figure S4**. $^{1}H$ and $^{13}C$ NMR spectra of **4** .

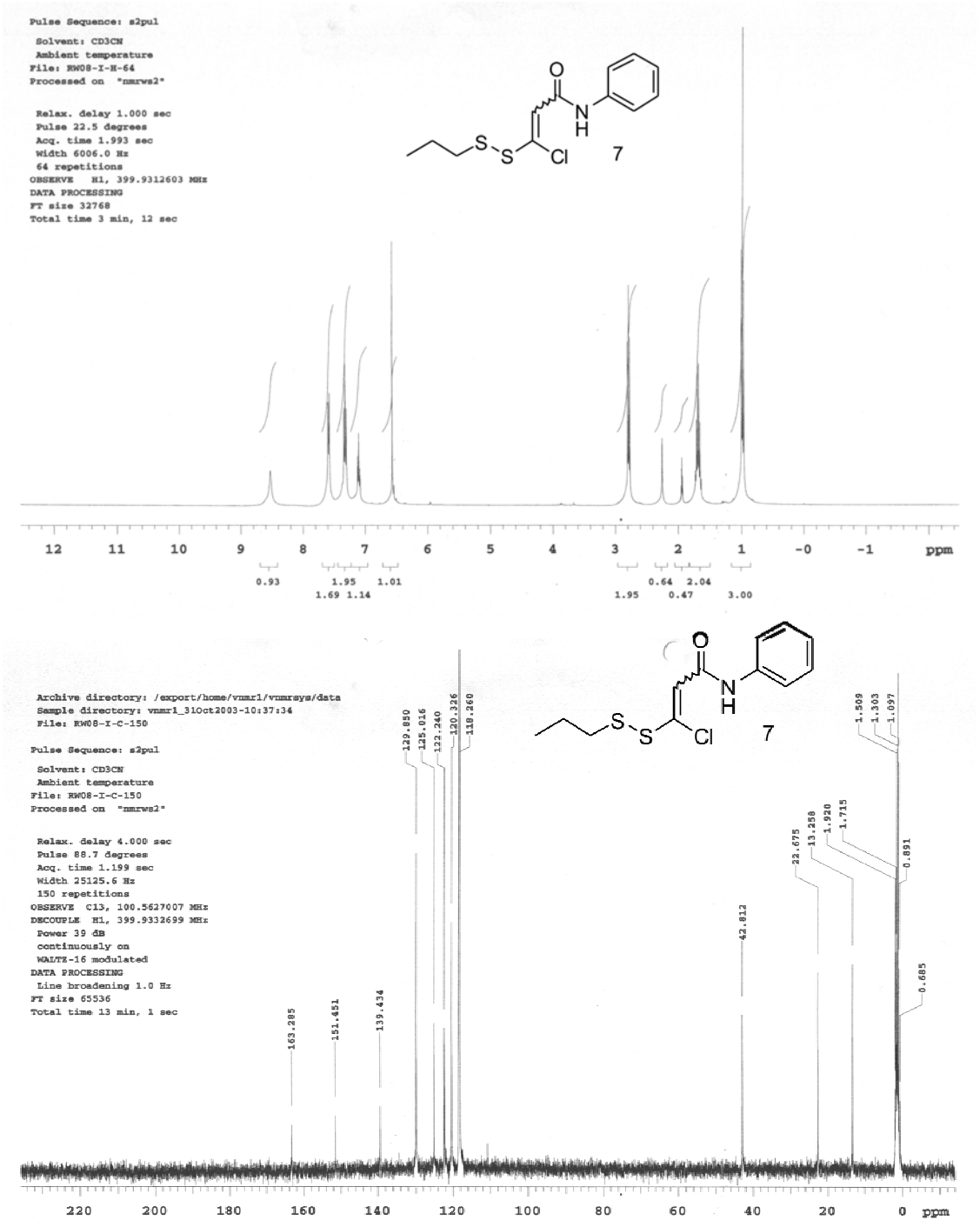



**Figure S5**. $^{1}H$ and $^{13}C$ NMR spectra of **7**.

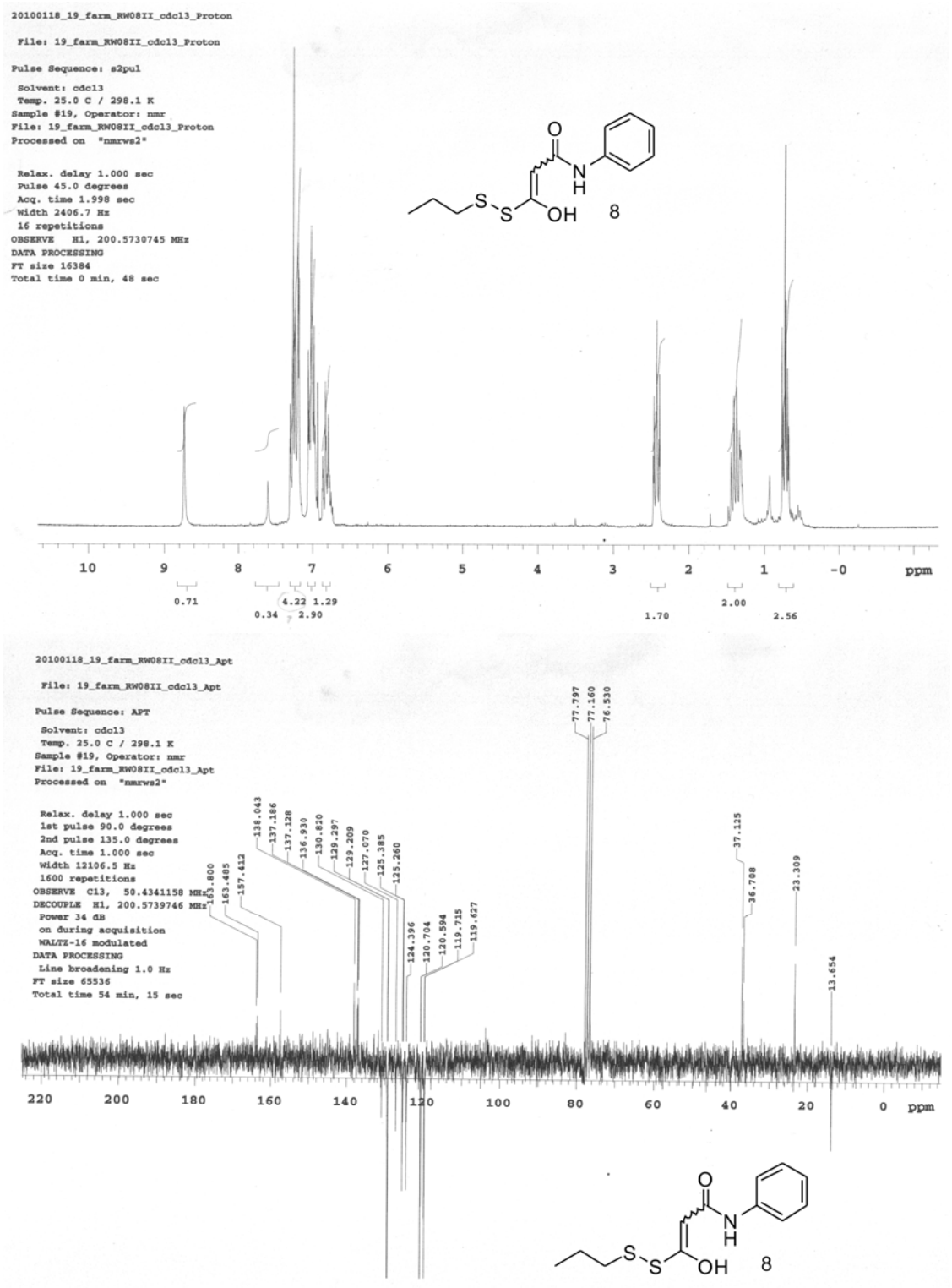
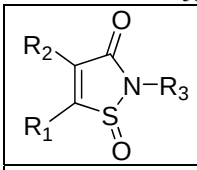



**Figure S6**. $^{1}H$ and $^{13}C$ NMR spectra of **8**.

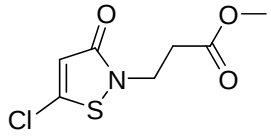
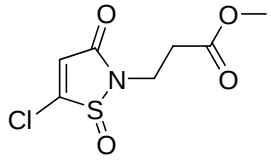
## Biochemistry

**Table S2.** $IC_{50}$ values for inhibition of Cathepsin B and PCAF

|  | $R_1$ | $R_2$ | $R_3$ | Cat B [a] $IC_{50}$ (μM) | PCAF [b] $IC_{50}$ (μM) |
|---|---|---|---|---|---|
| **19** | Cl | H | -Ethyl | 27.4 ± 1.1 | >10 |
| **20** | Cl | Cl | -Ethyl | 39.0 ± 0.5 | >10 |
| **21** | Cl | Metyl | -Ethyl | 17.4 ± 1.3 | >10 |
| **22** | Cl | H | $-(CH_2)_2CO_2Me$ | 21.3 ± 1.5 | 5.6 ± 0.2 |
| **23** | Cl | Cl | $-(CH_2)_2CO_2Me$ | 34.5 ± 1.2 | >10 |
| **24** | Cl | Metyl | $-(CH_2)_2CO_2Me$ | 23.1 ± 3.1 | >10 |
| **25** | $-S(CH_2)_{11}CH_3$ | H | $-(CH_2)_2CO_2Me$ | >100 | >10 |
| **26** | $-S(CH_2)_{11}CH_3$ | Cl | $-(CH_2)_2CO_2Me$ | >100 | >10 |

[a] maximal concentration in the assay 100 μM.
[b] data reported previously.[2, 3] maximal concentration in the assays 10 μM

**Table S3.** Enzyme kinetics parameters for Cathepsin B inhibition (n = 3)

| | Conc inhibitor (μM) | $V_{max}$ (nmol/s) | $K_m$ (mM) | $K_S$ (mM) | $R^2$ |
|---|---|---|---|---|---|
|  **14** | 0 | 0.65 ± 0.66 | 1.2 ± 1.3 | 0.10 ± 0.11 | 0.989 |
| | 6 | 0.51 ± 0.24 | 1.4 ± 0.7 | 0.10 ± 0.051 | 0.998 |
| | 12 | 0.23 ± 0.13 | 1.2 ± 0.7 | 0.20 ± 0.13 | 0.995 |
|  **22** | 0 | 0.56 ± 0.67 | 1.1 ± 1.4 | 0.098 ± 0.13 | 0.982 |
| | 11 | 0.52 ± 0.59 | 1.1 ± 1.2 | 0.11 ± 0.15 | 0.972 |
| | 22 | 0.50 ± 0.70 | 1.1 ± 1.9 | 0.090 ± 0.15 | 0.972 |

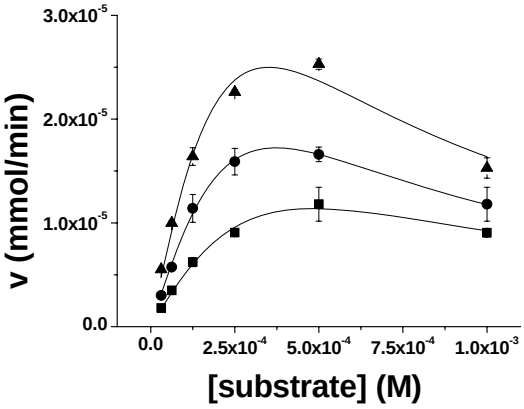



**Figure S7**. Conversion of the substrate Cbz-Arg-Arg-AMC by the enzyme Cathepsin B with no inhibitor present (▲) shows substrate inhibition at high substrate concentrations. The same is observed for the enzyme activity after preincubation with 6 μM inhibitor **14** (●) and the enzyme activity after preincubation with 12 μM inhibitor **14** (■). The data were fitted to equation 1 and the parameters are shown in table S3.

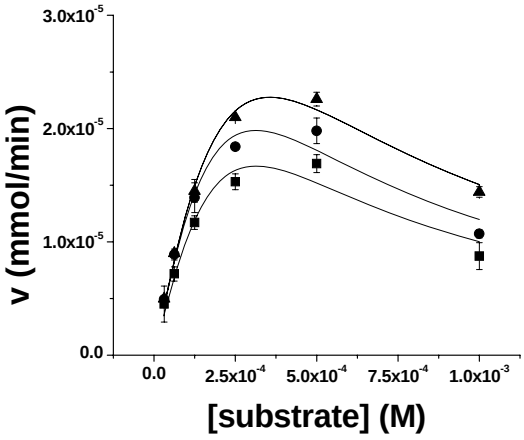



**Figure S8**. Conversion of the substrate Cbz-Arg-Arg-AMC by the enzyme Cathepsin B with no inhibitor present (▲) shows substrate inhibition at high substrate concentrations. The same is observed for the enzyme activity after preincubation with 11 μM inhibitor **22** (●) and the enzyme activity after preincubation with 22 μM inhibitor **22** (■). The data were fitted to equation 1 and the parameters are shown in table S3. The data represented by the blocks (■) and circles (●) did not fit well to equation 1.

**Table S4.** Time dependent inhibition of Cathepsin B by **5**. The $IC_{50}$ of the residual enzyme activity was determined after 1, 15, 40 or 120 minutes pre-incubation of the enzyme with the inhibitor (n = 3).

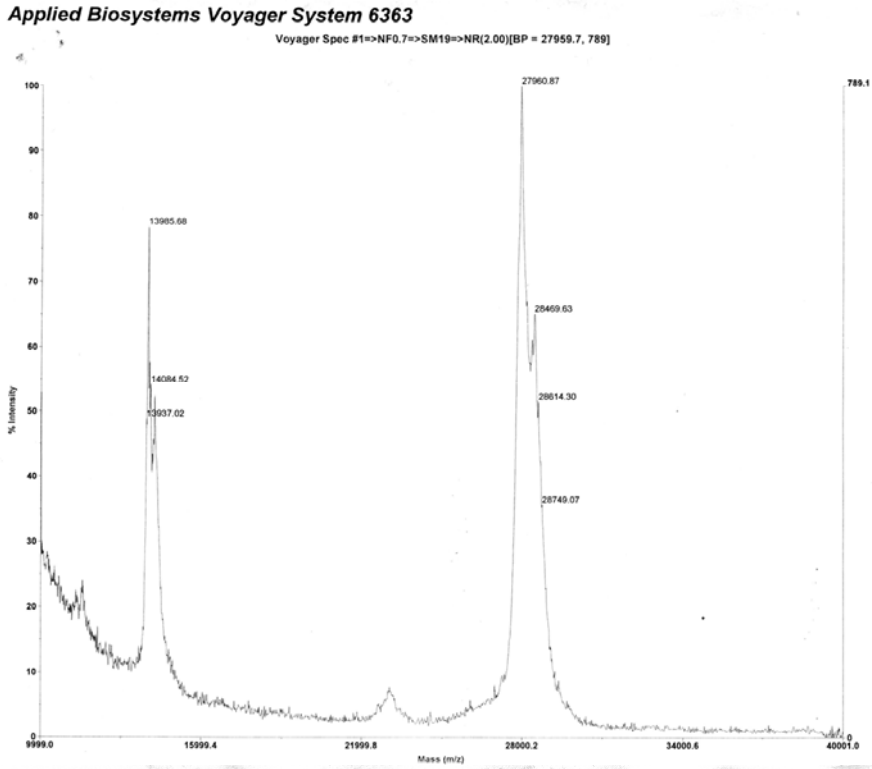
| Time (min) | $IC_{50}$ (μM) |
|---|---|
| 1 | 13.6 ± 1.5 |
| 15 | 12.7 ± 1.3 |
| 40 | 9.3 ± 1.1 |
| 120 | 8.1 ± 1.0 |

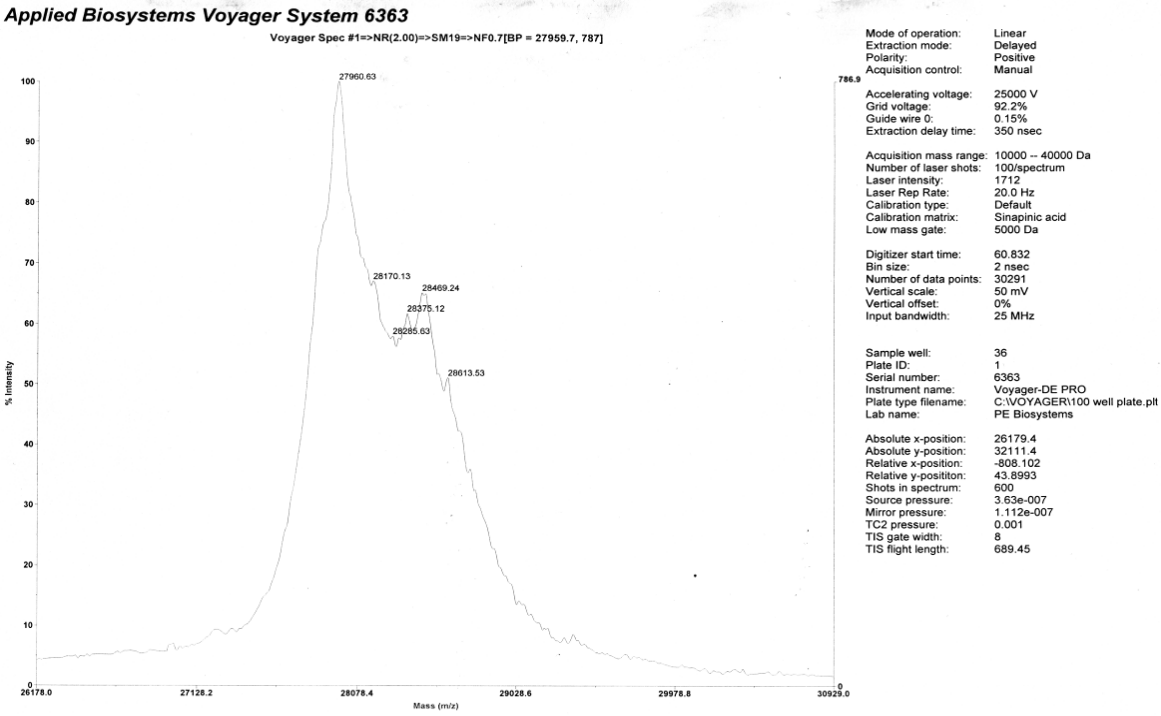
**Table S5.** Dilution experiments after pre-incubation of Cathepsin B (20 nM) and inhibitor **5** (17 μM) with a substrate solution (100 μM) demonstrates a reduction of activity that is proportional to the dilution. This demonstrates covalent binding of the enzyme to the inhibitor (n = 3) [6].

| Dilution | v (fluorescence units/min) | Reduction in activity |
|---|---|---|
| 1 | 13.3 ± 0.5 | 1.00 |
| 2 | 6.9 ± 0.1 | 1.93 |
| 5 | 2.7 ± 0.1 | 4.93 |

**Table S6.** Reduction rate of cathepsin B by **5**. The initial rate of cathepsin B (1.5 nM) activity with or without inhibitor **5** (14 μM) were determine after 20 second reaction of the enzyme with the substrate (33.3 μM). (n = 3) The result shows a very quick reduction of the enzyme activity in presence of the inhibitor.

| Inhibitor | V (fluorescence units/s) |
|---|---|
| No Inhibitor | 25.3 ± 1.2 |
| **5** (14 μM) | 11.9 ± 5.0 |


Applied Biosystems Voyager System 6363
Voyager Spec #1=>NF0.7=>SM19=>NR(2.00)[BP = 27959.7, 789]
27960.87
789.1
13985.68
28469.63
14084.52
13937.02
28614.30
28749.07
% Intensity
Mass (m/z)
9999.0
15999.4
21999.8
28000.2
34000.6
40001.0
Mode of operation: Linear
Extraction mode: Delayed
Polarity: Positive
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 92.2%
Guide wire 0: 0.15%
Extraction delay time: 350 nsec
Acquisition mass range: 10000 -- 40000 Da
Number of laser shots: 100/spectrum
Laser intensity: 1712
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: Sinapinic acid
Low mass gate: 5000 Da
Digitizer start time: 60.832
Bin size: 2 nsec
Number of data points: 30291
Vertical scale: 50 mV
Vertical offset: 0%
Input bandwidth: 25 MHz
Sample well: 36
Plate ID: 1
Serial number: 6363
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems
Absolute x-position: 26179.4
Absolute y-position: 32111.4
Relative x-position: -808.102
Relative y-posititon: 43.8993
Shots in spectrum: 600
Source pressure: 3.63e-007
Mirror pressure: 1.112e-007
TC2 pressure: 0.001
TIS gate width: 8
TIS flight length: 689.45


Applied Biosystems Voyager System 6363
Voyager Spec #1=>NR(2.00)=>SM19=>NF0.7[BP = 27959.7, 787]
27960.63
786.9
28170.13
28469.24
28375.12
28285.63
28613.53
% Intensity
Mass (m/z)
26178.0
27128.2
28078.4
29028.6
29978.8
30929.0
Mode of operation: Linear
Extraction mode: Delayed
Polarity: Positive
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 92.2%
Guide wire 0: 0.15%
Extraction delay time: 350 nsec
Acquisition mass range: 10000 -- 40000 Da
Number of laser shots: 100/spectrum
Laser intensity: 1712
Laser Rep Rate: 20.0 Hz
Calibration type: Default
Calibration matrix: Sinapinic acid
Low mass gate: 5000 Da
Digitizer start time: 60.832
Bin size: 2 nsec
Number of data points: 30291
Vertical scale: 50 mV
Vertical offset: 0%
Input bandwidth: 25 MHz
Sample well: 36
Plate ID: 1
Serial number: 6363
Instrument name: Voyager-DE PRO
Plate type filename: C:\VOYAGER\100 well plate.plt
Lab name: PE Biosystems
Absolute x-position: 26179.4
Absolute y-position: 32111.4
Relative x-position: -808.102
Relative y-posititon: 43.8993
Shots in spectrum: 600
Source pressure: 3.63e-007
Mirror pressure: 1.112e-007
TC2 pressure: 0.001
TIS gate width: 8
TIS flight length: 689.45

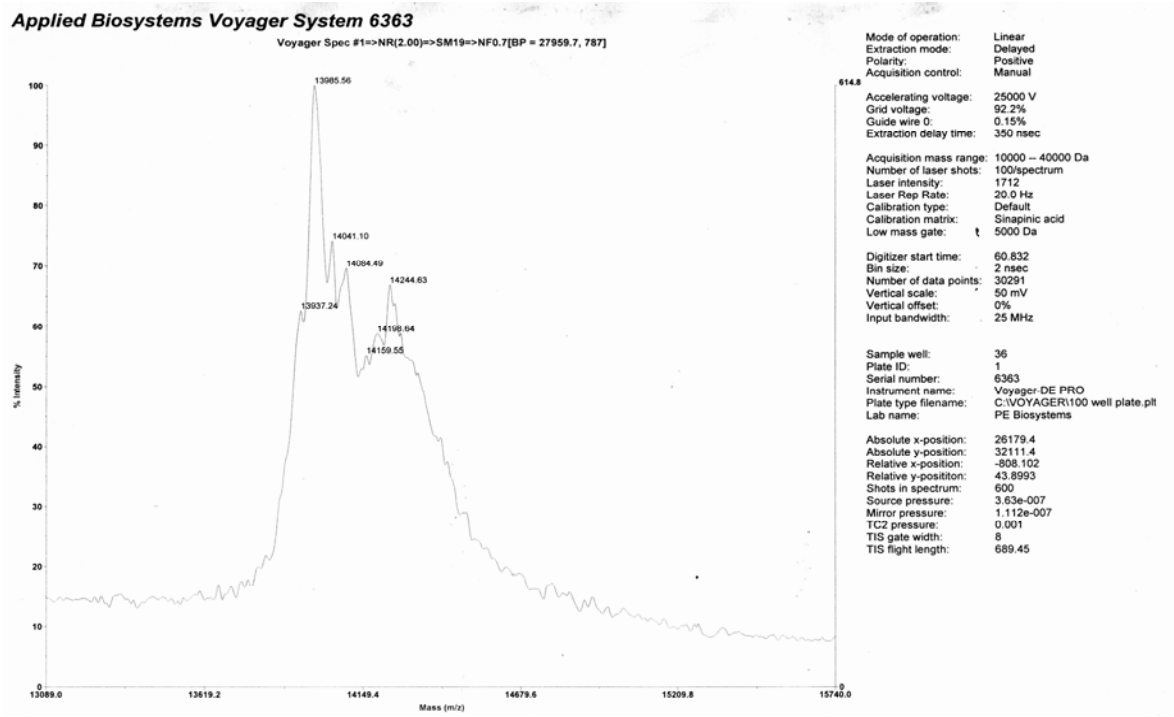
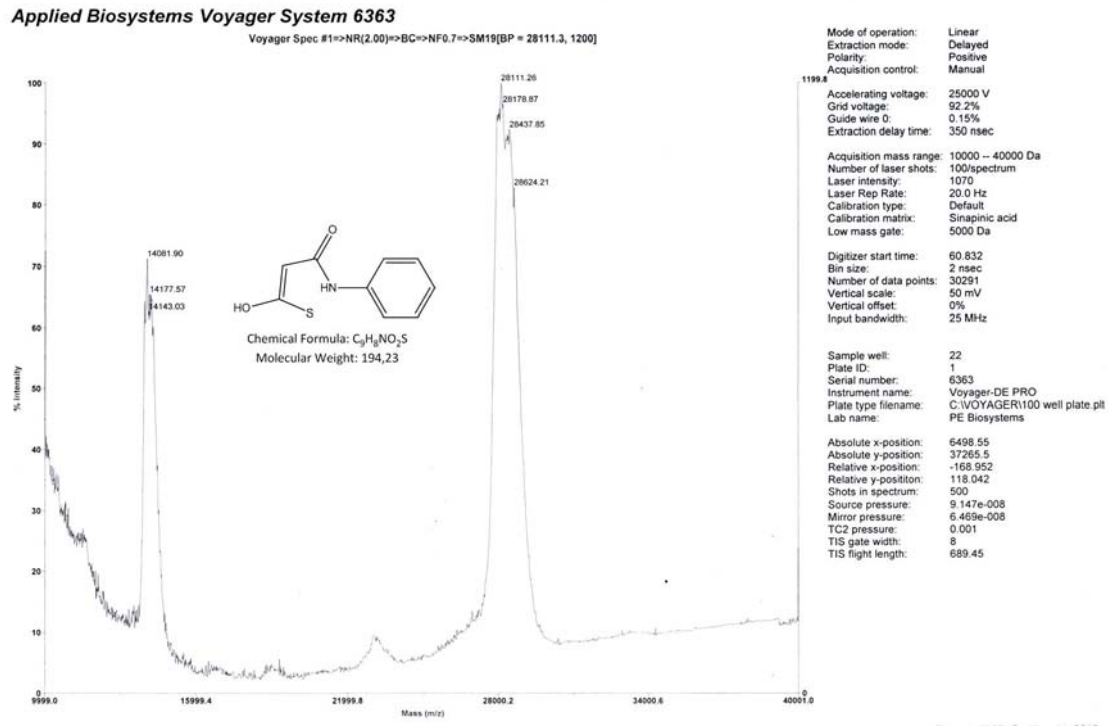



**Figure S9**. MALDI – TOF mass spectrum of Cathepsin B.



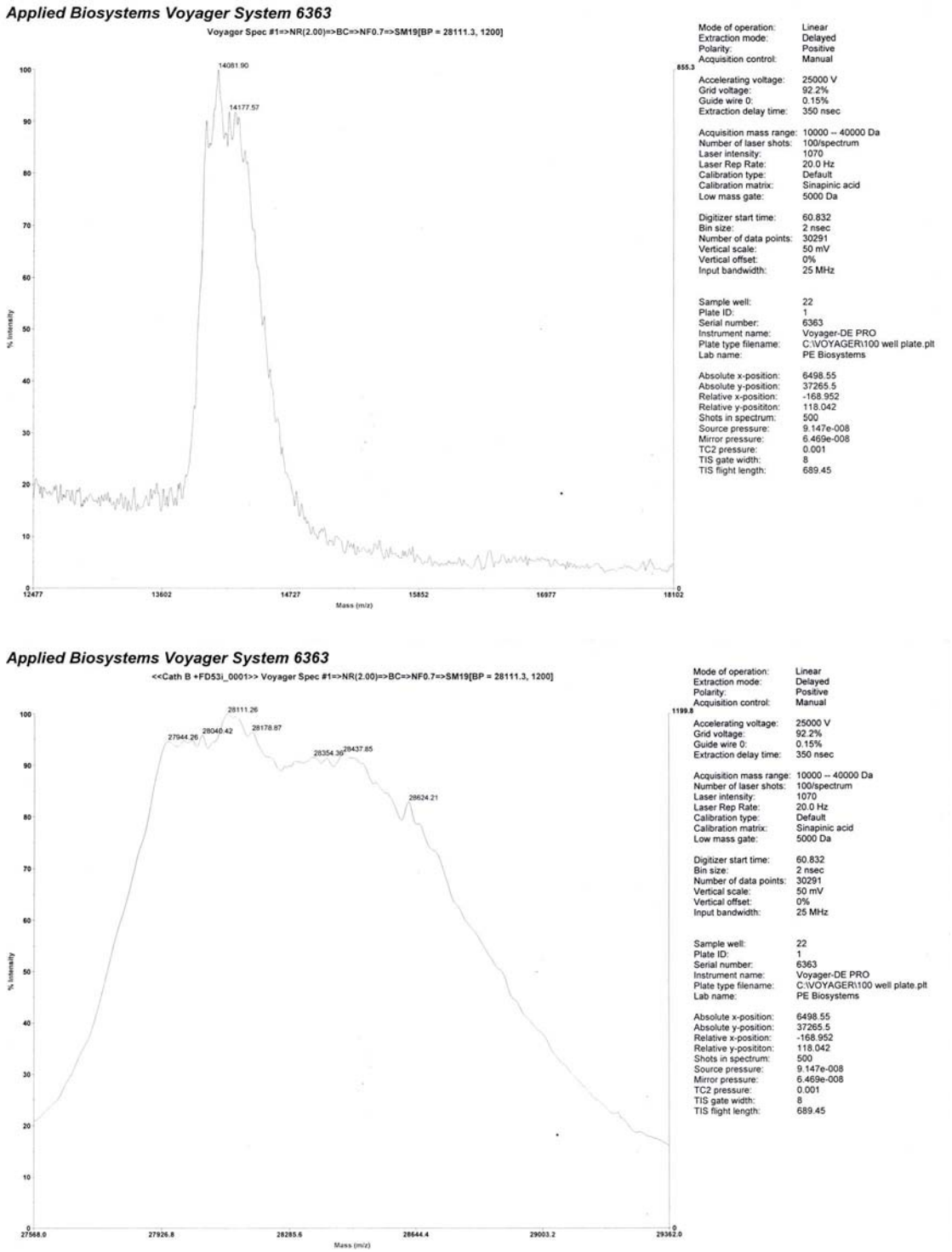



**Figure S10**. MALDI – TOF mass spectrum of Cathepsin B that was pre-incubated with inhibitor **5**.

**References**

1. E. V. Anslyn and D. A. Dougherty, *University Science Books*, 2006, 22.
2. F. J. Dekker, M. Ghizzoni, N. Van der Meer, R. Wisastra and H. J. Haisma, *Bioorg. Med. Chem.*, 2009, **17**, 460-466.
3. M. Ghizzoni, H. J. Haisma and F. J. Dekker, *Eur J Med Chem*, 2009, **44**, 4855-4861.