SUPPORTING INFORMATION

A BODIPY-tagged Phosphono Peptide as Activity-Based Probe for Human Leukocyte Elastase

Anna-Christina Schulz-Fincke,[†] Michael Blaut,[§] Annett Braune,[§,*] and Michael Gütschow[†,*]

[†] Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, 53121 Bonn, Germany

[§] Department of Gastrointestinal Microbiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany

Table of Contents

General methods and materials ……..….. S2

Synthetic procedures and characterization of all compounds ……..…….…… S3

General enzymatic methods …….……. S11

Enzyme inhibition assays ……..…… S11

Figure S1. Inhibition of HLE with compound **1A** …… S14

Figure S2. Inhibition of HLE with compound **1B** …… S14

Figure S3. Inhibition of HLE with probe **6** ……... S15

Figure S4. Inhibition of HLE with sivelestat …… S15

Figure S5. Reactivation experiments …….. S16

Figure S6. Normalized absorption and emission spectra of probe **6** …… S16

Detection of HLE with probe **6** ……...……...……. S16

References ……. S19

NMR spectra and LC/MS data of synthesized compounds ……...……... S21

General Methods and Materials

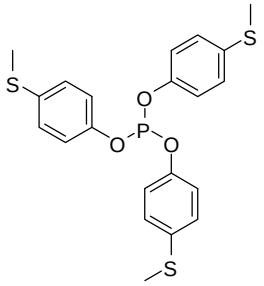
Melting points were determined on a Büchi 50 oil bath apparatus. Thin layer chromatography was performed using Merck aluminum sheets coated with silica gel 60 F_{254}. NMR spectra were recorded using Bruker Avance III-600 MHz and Bruker Avance DRX-500 MHz instruments. LC-DAD chromatograms and ESI-MS spectra were recorded on an Agilent 1100 HPLC system with an Applied Biosystems API-2000 mass spectrometer. HRMS was performed on a microTOF-Q mass spectrometer (Bruker, Köln, Germany) with ESI-source coupled with a HPLC Dionex Ultimate 3000 (Thermo Scientific, Braunschweig, Germany) using a EC50/2 Nucleodur C18 Gracity 3 µm column (Macherey-Nagel, Düren, Germany). A volume of 1 µL of a sample solution (1.0 mg/mL) was injected. Mobile phase was a mixture of 2 mM aqueous ammonium acetate solution (A) and acetonitrile (B). Elution was performed from 90:10 (A:B) up to 0:100 (A:B) in 9 min, 0:100 (A:B) for 5 min.

Bromoacetic acid and boron trifluoride diethyl etherate were purchased from Sigma Aldrich (Steinheim, Germany), 4-(methylthio)phenol, isobutyraldehyde and benzyl carbamate from Alfa Aesar (Karlsruhe, Germany), Boc-Pro-OH from Carl Roth (Karlsruhe, Germany), Boc-Val-OH from Nova Biochem (Merck, Germany), 4-ethynylbenzaldehyde from Fluorochem (Hadfield, United Kingdom) and 2,4-dimethylpyrrole from TCI (Eschborn, Germany).

Absorption spectra were recorded on a Varian Cary 50 Bio, emission spectra on a Hitachi F-7000 fluorescence spectrophotometer (Tokyo, Japan) adjusted to a PMT value of 400 V. The 10 mM stock solution of **6** was prepared in DMSO. UV and fluorescence spectra were recorded in CH_2Cl_2, MeOH or H_2O, all containing 1% DMSO. The final concentration of compound **6** was 5 µM and 1 µM for recording of UV and fluorescence spectra, respectively.

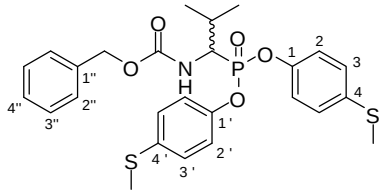
Synthetic procedures and characterization of all compounds

Tris(4-(methylthio)phenyl)phosphite (7)[1]



Phosphorus trichloride (0.69 g, 5.0 mmol) was added to a solution of 4-(methylthio)phenol (2.1 g, 15.0 mmol) in acetonitrile (25 mL) at room temperature, and the mixture was refluxed for 3 h. The volatile components were removed under reduced pressure, and the resulting crude phosphite, an unstable compound, was immediately used for the next step without further purification.

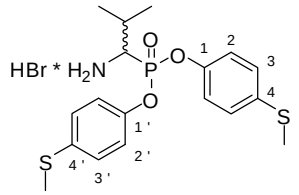
Benzyl 1-(bis(4-(methylthio)phenoxy)phosphoryl)-2-methylpropylcarbamate (8)



Tris(4-(methylthio)phenyl) phosphite (**7**, 2,24 g, 5.0 mmol) was dissolved in glacial acetic acid (25 mL), and isobutyraldehyde (0.43 g, 6.0 mmol) and benzyl carbamate (0.76 g, 5.0 mmol) were subsequently added. The mixture was heated to 90 °C for 3 h, before the solvent was evaporated. The resulting oil was dissolved in MeOH (25 mL) and stored at -20 °C for crystallization. The colorless product was filtered off, washed with cold MeOH and dried (1.2 g, 46%); mp 112–115 °C; lit.[1] mp 108 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.02 (d, ^{3}J = 3.1 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.03 (d, ^{3}J = 3.0 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 2.18–2.28 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.43 (s, 3H, SCH$_3$), 2.45 (s, 3H, SCH$_3$), 4.14–4.21 (m, 1H, CHPO), 5.03–5.12 (m, 2H, CH$_{arom}$), 7.06–7.10 (m, 4H, CH$_{arom}$), 7.21–7.28 (m, 4H, CH$_{arom}$), 7.33 (s, 3H, CH$_{arom}$), 7.34 (s, 2H, PhC$\underline{H}_2$), 8.00 (d, ^{3}J = 10.2 Hz, 1H, OCONH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.42, 15.48 (SCH$_3$), 18.69 (d, ^{3}J = 7.6 Hz, CH(C$\underline{H}_3$)$_2$), 20.18 (d, ^{3}J = 10.4 Hz, CH(C$\underline{H}_3$)$_2$), 28.81 (d, ^{2}J = 5.5 Hz, C$\underline{H}$(CH$_3$)$_2$), 54.52 (d, ^{2}J = 153.8 Hz, CHPO), 66.04 (CH$_2$), 121.02 (d, ^{3}J = 4.2 Hz, C-2 or C-2`), 121.28 (d, $^{3}J$ = 3.9 Hz, C-2 or C-2`), 127.72, 127.79, 127.86,

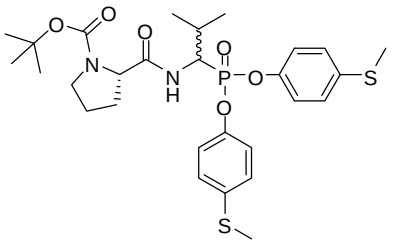
127.99, 128.46, 134.55, 134.85, 137.01 (C-1'', C-2'', C-3'', C-4'', C-3', C-4', C-3, C-4), 147.47 (d, J = 9.5 Hz, C-1 or C-1`), 147.85 (d, $J$ = 9.7 Hz, C-1 or C-1`), 156.70 (d, ^3J = 5.9 Hz, OCONH); LC/MS (ESI) (H_2O/MeOH, 90:10 to 0:100) *m/z*: 531.9 $[M + H]^+$; 93% purity. HRMS (ESI^+) *m/z*: calcd for $C_{26}H_{30}NO_5PS_2$ $[M + NH_4^+]$ 549.1641, found 549.1641.

Bis(4-(methylthio)phenyl) 1-amino-2-methylpropylphosphonate (9)[2]



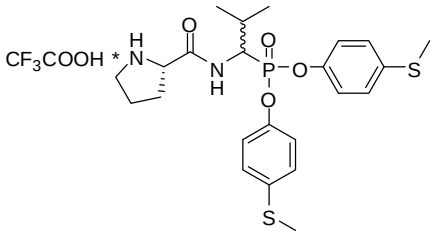
Benzyl 1-(bis(4-(methylthio) phenoxy)phosphoryl)-2-methylpropylcarbamate (**8**, 1.1 g, 2.0 mmol) was dissolved in 33% HBr/AcOH solution (25 mL) and stirred at room temperature for 30 min. The volatile components were removed under reduced pressure. The resulting oil was dissolved in ethyl acetate and left for crystallization at 4 °C. The hydrobromide salt was filtered off, washed extensively with cold diethyl ether and used without further purification (940 mg, 98% yield); mp 150–154 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 1.14 (d, J = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 1.17 (d, J = 6.9 Hz, 3H, CHC<u>H</u>$_3$), 2.36-2.42 (m, 1H, C<u>H</u>$(CH_3)_2$), 2.451 (s, 3H, SCH_3), 2.454 (s, 3H, SCH_3), 4.10 (dd, J = 4.8, 15.4 Hz, 1H, POCH), 7.15 (d, J = 8.3 Hz, 2H, 2-H or 2'-H), 7.17 (d, J = 8.4 Hz, 2H, 2-H or 2'-H), 7.27-7.32 (m, 4H, 3-H and 3'-H), 8.70 (s, 3H, NH_3), ^{13}C NMR (151 MHz, DMSO) δ 15.30, 15.31 (SCH_3), 18.46 (d, J = 4.7 Hz, CH<u>C</u>H$_3$), 19.34 (d, J = 8.9 Hz, CH_3), 28.05 (<u>C</u>H$(CH_3)_2$), 51.78 (d, J = 152.6 Hz, POCH), 121.28, (d, J = 4.1 Hz, C-2 or C-2`), 121.34 (d, $J$ = 3.7 Hz, C-2 or C-2`), 127.73, 135.62 (C-3, C-3', C-4, C-4'), 146.75 (d, J = 10.1 Hz, C-1 or C-1`), 146.91 (d, $J$ = 9.4 Hz, C-1 or C-1`); LC/MS (ESI) (m/z): 398.1 $[M + H]^+$; 74% purity.

(*S*)-*tert*-Butyl 2-(1-(bis(4-(methylthio)phenoxy)phosphoryl)-2-methylpropylcarbamoyl) pyrrolidine-1-carboxylate (10)[2]



The bis(4-(methylthio)phenyl) 1-amino-2-methylpropylphosphonate hydrobromide (**9**, 0.97 g, 2.0 mmol) was suspended in acetonitrile (40 mL), and DIPEA (0.65 g, 5.0 mmol) was added. After complete dissolving, Boc-Pro-OH (0.43 g, 2.0 mmol) was added followed by the addition of HBTU (0.91 g, 2.4 mmol). The reaction was performed at room temperature over night. After completion of the reaction, the solvent was evaporated under reduced pressure and the resulting oil was dissolved in ethyl acetate (50 mL), washed with $NaHCO_3$ (50 mL), $KHSO_4$ (50 mL), and brine (50 mL). After drying (Na_2SO_4), the solution was concentrated *in vacuo* and the crude oil was used without further purification. LC/MS (ESI) (m/z): 595.4 $[M + H]^+$; 89% purity.

(*S*)-Bis(4-(methylthio)phenyl) 2-methyl-1-(pyrrolidine-2-carboxamido) propyl-phosphonate (11)[2]



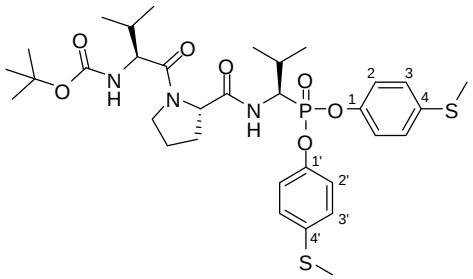
(S)-*tert*-Butyl 2-(1-(bis(4-(methylthio)phenoxy)phosphoryl)-2-methylpropylcarbamoyl) pyrrolidine-1-carboxylate (**10**, 1.19 g, 2.0 mmol) was dissolved in DCM/TFA (1:1) and stirred for 2 h at room temperature. The volatile components were removed under reduced pressure. The resulting oil was dissolved in toluene (4 × 10 mL) and evaporated. The obtained phosphonic dipeptide was used without further purification.

(*S,S*)-*tert*-Butyl 1-(2-(1-(bis(4-(methylthio)phenoxy)phosphoryl)-2-methylpropyl-carbamoyl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-yl-carbamate (1)[3]

The trifluoroacetate **11** (1.22 g, 2.0 mmol) was dissolved in acetonitrile (20 mL) followed by the addition of DIPEA (0.65 g, 5.0 mmol). Next, the Boc-Val-OH (0.43 g, 2.0 mmol) and HBTU (0.91 g, 2.4 mmol) were added. The reaction was performed over night at room temperature. After completion of the reaction, the solvent was evaporated and the resulting oil was dissolved in ethyl acetate (50 mL). The solution was washed with 5% $NaHCO_3$ (50 mL), 5% $KHSO_4$ (50 mL), and brine (50 mL), dried over Na_2SO_4, filtered and concentrated under

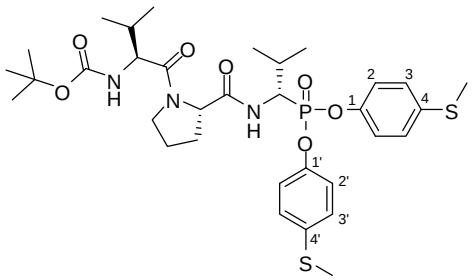
reduced pressure resulting in a crude phosphonic tripeptide. Purification and diastereomeric separation was performed by column chromatography on silica gel using CH_2Cl_2/ethyl acetate (4:1). Epimer **1B** was eluted first. Compounds **1A** (375 mg, 27% yield) and **1B** (278 mg, 20% yield) were obtained as oils, forming colorless foams under reduced pressure.

Diastereomer 1A[3] with (*S*,*S*,*R*)-configuration



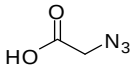
^{1}H NMR (600 MHz, $CDCl_3$) δ 0.91 (d, *J* = 6.7 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 0.97 (d, *J* = 6.7 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.00 (d, *J* = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.07 (d, *J* = 6.9 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.41 (s, 9H, C(CH_3)$_3$), 1.80–1.87 (m, 1H, CH), 1.92–2.00 (m, 2H, CH_2), 2.16–2.20 (m, 1H, C$\underline{H}$(CH_3)$_2$), 2.31–2.37 (m, 1H, C$\underline{H}$(CH_3)$_2$), 2.42 (s, 3H, SCH_3), 2.43 (s, 3H, SCH_3), 3.59 (m, 1H, CH), 3.70–3.75 (m, 1H, CH), 4.25–4.28 (m, 1H, CH), 4.46–4.48 (m, 1H, CH), 4.65–4.71 (m, 1H, CH), 5.25 (d, ^3J = 9.3 Hz, 1H, CONH), 7.05 (d, ^3J = 8.4 Hz, 2H, 2-H or 2`-H), 7.12 (d, $^3J$ = 8.4 Hz, 2H, 2-H or 2`-H), 7.18 (dd, ^3J = 8.5, ^4J = 4.8 Hz, 4H, 3-H and 3`-H), 7.26 (d, ^3J = 10.6 Hz, 1H, CONH), one CH signal is missing; ^{13}C NMR (150 MHz, $CDCl_3$) δ 16.51 (SCH_3), 16.54 (SCH_3), 17.42 (CH(C$\underline{H}_3$)$_2$), 17.94 (d, *J* = 4.4 Hz, CH(C$\underline{H}_3$)$_2$), 19.59 (CH(C$\underline{H}_3$)$_2$), 20.20 (d, *J* = 13.7 Hz, CH(C$\underline{H}_3$)$_2$), 25.23, 27.35 (C_{Pro}), 28.31 (C(C$\underline{H}_3$)$_3$), 29.22 (d, ^2J = 3.8 Hz, C$\underline{H}$(CH_3)$_2$), 31.35 (C$\underline{H}$(CH_3)$_2$), 47.77 (C_{Pro}), 51.13 (d, *J* = 152.1 Hz, POCH), 56.78 (NHC$\underline{H}$CO), 60.04 (C_{Pro}), 79.70 (C$\underline{(}$$CH_3$)$_3$), 120.89 (d, ^3J = 4.1 Hz, C-2 or C-2`), 121.17 (d, $^3J$ = 4.1 Hz, C-2 or C-2`), 128.31 (C-3 or C-3`), 128.38 (C-3 or C-3`), 135.04 (C-4 or C-4`), 135.22 (C-4 or C-4`), 147.75 (d, ^2J = 9.1 Hz, C-1 or C-1`), 148.06 (d, $^2J$ = 9.9 Hz, C-1 or C-1`), 155.79 (OCONH), 171.14 (d, ^3J = 6.7 Hz, CH_{Pro}C$\underline{O}$), 172.81 (CON_{Pro}); ^{31}P NMR (243 MHz, $CDCl_3$) δ 17.87; LC/MS (ESI) (H_2O/MeOH, 90:10 to 0:100) *m/z*: 694.2 $[M + H]^+$; 98% purity; HRMS (ESI^+) *m/z*: calcd for $C_{33}H_{48}N_3O_7PS_2$ $[M + H^+]$ 694.2744, found 694.2759.

Diastereomer 1B[3] with (*S*,*S*,*S*)-configuration



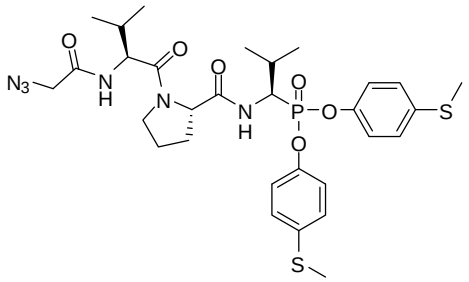
^{1}H NMR (600 MHz, $CDCl_3$) δ 0.82 (d, ^3J = 6.7 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 0.86 (d, ^3J = 6.7 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.03 (d, ^3J = 6.7 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.10 (d, ^3J = 6.8 Hz, 3H, CH(C$\underline{H}_3$)$_2$), 1.40 (s, 9H, C(CH_3)$_3$), 1.76–1.83 (m, 2H, CH_2), 1.91–1.98 (m, 2H, CH_2), 2.35–2.41 (m, 2H, C$\underline{H}$(CH_3)$_2$), 2.42 (s, 3H, SCH_3), 2.43 (s, 3H, SCH_3), 3.45–3.48 (m, 1H, CH), 3.67–3.72 (m, 1H, CH), 4.21–4.24 (m, 1H, CH), 4.66–4.71 (m, 2H, CH), 5.13 (d, ^3J = 9.3 Hz, 1H, CONH), 7.05 (d, ^3J = 8.3 Hz, 2H, 2-H or 2`-H), 7.09 (d, $^3J$ = 8.4 Hz, 2H, 2-H or 2`-H), 7.17 (d, ^3J = 8.5 Hz, 4H, 3-H and 3`-H), 7.31 (d, ^3J = 10.3 Hz, 1H, CONH); ^{13}C NMR (150 MHz, $CDCl_3$) δ 16.54 (SCH_3), 17.35 (CH(C$\underline{H}_3$)$_2$), 17.89 (d, ^3J = 4.3 Hz, CH(C$\underline{H}_3$)$_2$), 19.58 (CH(C$\underline{H}_3$)$_2$), 20.49 (d, ^3J = 14.0 Hz, CH(C$\underline{H}_3$)$_2$), 25.02, 27.25 (C_{Pro}), 28.31 (C(C$\underline{H}_3$)$_3$), 29.23 (d, ^2J = 3.6 Hz, C$\underline{H}$(CH_3)$_2$), 31.28 (C$\underline{H}$(CH_3)$_2$), 47.73 (C_{Pro}), 51.01 (d, ^2J = 153.0 Hz, POCH), 56.76 (NH$\underline{C}$HCO), 60.27 (C_{Pro}), 79.72 ($\underline{C}$(CH_3)$_3$, 120.97 (d, ^3J = 4.2 Hz, C-2 or C-2`), 121.26 (d, $^3J$ = 4.0 Hz, C-2 or C-2`), 128.18 (C-3 or C-3`), 128.39 (C-3 or C-3`), 134.99 (C-4 or C-4`), 135.17 (C-4 or C-4`), 147.73 (d, J = 8.9 Hz, C-1 or C-1`), 148.01 (d, $J$ = 10.0 Hz, C-1 or C-1`), 155.77 (OCONH), 171.16 (d, J = 5.5 Hz, $CH_{Pro}\underline{C}O$), 173.48 (CON_{Pro}); ^{31}P NMR (243 MHz, $CDCl_3$) δ 18.25; LC/MS (ESI) (H_2O/MeOH, 90:10 to 0:100) *m/z*: 694.2 $[M + H]^+$; 95% purity; HRMS (ESI^+) *m/z*: calcd for $C_{33}H_{48}N_3O_7PS_2$ $[M + H^+]$ 694.2744, found 694.2751.

2-Azidoacetic acid (3)[4]



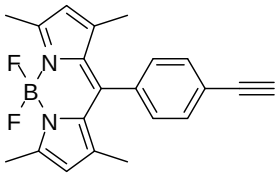
Bromoacetic acid (0.69 g, 5.0 mmol) was dissolved in H_2O (10 mL). Sodium azide (0.65 g, 10 mmol) was added and the mixture stirred overnight at room temperature. It was acidified with concd HCl, extracted with diethyl ether (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried (Na_2SO_4) and evaporated at room temperature. The azido compound was used without further purification.

Bis(4-(methylthio)phenyl) (*R*)-1-((*S*)-1-((*S*)-2-(2-azidoacetamido)-3-methylbutanoyl)-pyrrolidine-2-carboxamido)-2-methylpropylphosphonate (4)



Compound **1A** (0.17 g, 0.25 mmol) was dissolved in CH_2Cl_2/TFA (1:1) and stirred for 2 h at room temperature. The volatile components were removed under reduced pressure. The resulting oil was dissolved in toluene (4 × 10 mL) and evaporated. The obtained TFA salt **2** was dissolved in acetonitrile (14 mL) followed by the consecutive addition of DIPEA (0.065 g, 0.5 mmol), 2-azidoacetic acid (**3**, 0.025 g, 0.25 mmol) and HBTU (0.091 g, 0.24 mmol). The reaction mixture was stirred over night at room temperature. The solution was evaporated and the resulting crude oil was partially purified by column chromatography using CH_2Cl_2/ethyl acetate (4:1) to obtain a yellowish oil (0.13 g, 76%). This material was used without a further purification in the next step. LC/MS (ESI) (H_2O/MeOH, 60:40 to 0:100) *m/z*: 677.2 $[M + H]^+$; 74% purity.

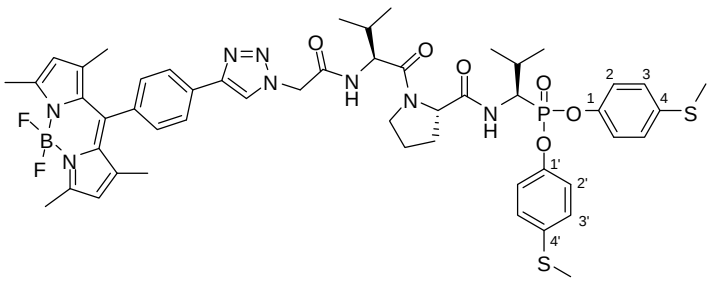
4,4-Difluoro-8-(4-ethynylphenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (ethynyl-BODIPY, 5)[5]



To a solution of 4-ethynylbenzaldehyde (0.13 g, 1.0 mmol) and 2,4-dimethylpyrrole (0.22 g, 2.3 mmol) in CH_2Cl_2 (75 mL), TFA (0.023 g, 0.20 mmol) was added under nitrogen atmosphere. The mixture was stirred at room temperature overnight. After adding *p*-chloranil (0.25 g, 1.0 mmol), the mixture was stirred at room temperature for additional 30 min. Et_3N (1.82 g. 18 mmol) and $BF_3 \times OEt_2$ (2.98 g, 21 mmol) were slowly added and stirring was continued at room temperature for 6 h. The reaction mixture was washed with water (3 × 50 mL), brine (1 × 50 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography (CH_2Cl_2/cyclohexane 3:1) to give a magenta solid (89 mg, 26%); mp 238–242°C; lit.[5] mp 252–253 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ

1.36 (s, 6H, CH_3), 2.44 (s, 6H, CH_3), 4.32 (s, 1H, C≡CH), 6.18 (s, 2H, CH_{pyr}), 7.43–7.38 (m, 2H, CH_{arom}), 7.68–7.63 (m, 2H, CH_{arom}); ^{13}C NMR (126 MHz, DMSO-d_6) δ 14.22 (CH_3), 14.35 (CH_3), 82.25 (C≡CH), 83.00 (C≡CH), 121.67, 122.80, 128.56, 130.57, 132.70, 134.69, 141.03, 142.76, 155.31 (C_{arom}); LC/MS (ESI) (H_2O/MeOH, 90:10 to 0:100) *m/z*: 349.1 [M + H]$^+$; 97% purity. HRMS (ESI$^+$) *m/z*: calcd for $C_{21}H_{19}BF_2N_2$ [M + H$^+$] 349.1682, found 349.1686.

10-(4-(1-(2-((*S*)-1-((*S*)-2-((*R*)-1-(Bis(4-(methylthio)phenoxy)phosphoryl)-2-methylpropylcarbamoyl)pyrrolidin-1-yl)-3-methyl-1-oxobutan-2-ylamino)-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)phenyl)-5,5-difluoro-1,3,7,9-tetramethyl-5*H*-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (6)



The azido component (**4**, 128 mg, 0.19 mmol) and ethynyl-BODIPY (**5**, 55 mg, 0.16 mmol) were dissolved in DMSO (10 mL). A brown cloudy solution of $CuSO_4 \times 5\ H_2O$ (1.97 mg, 0.0079 mmol) and sodium ascorbate (3.17 mg, 0.016 mmol) in H_2O (1 mL) was added. During the following 72 h, $CuSO_4 \times 5\ H_2O$ (1.97 mg) and sodium ascorbate (3.17 mg) were added seven times and the mixture was stirred at room temperature. The solution was quenched with H_2O (20 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with H_2O (30 mL), brine (30 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography using cyclohexane/ethyl acetate (1:2 to 0:1) to give an orange solid (80 mg, 49%); >134°C, decomposition; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.92 (d, ^3J = 6.6 Hz, 3H, $CH(C\underline{H}_3)_2$), 0.96 (d, ^3J = 6.6 Hz, 3H, $CH(C\underline{H}_3)_2$), 1.01 (d, ^3J = 6.7, 3H, $CH(C\underline{H}_3)_2$), 1.03 (d, ^3J = 6.8, 3H, $CH(C\underline{H}_3)_2$), 1.40 (s, 6H, $C_{pyr}CH_3$), 1.89–1.99 (m, 4H, CH_{Pro}), 2.21–2.26 (m, 1H, $C\underline{H}(CH_3)_2$), 2.27–2.32 (m, 1H, $C\underline{H}(CH_3)_2$), 2.39 (s, 3H, SCH_3), 2.43 (s, 3H, SCH_3), 2.53 (s, 6H, $C_{pyr}CH_3$), 3.74–3.78 (m, 1H, CH_{Pro}), 3.84–3.88 (m, 1H, CH_{Pro}), 4.66 (t, ^3J = 8.3 Hz, 1H, CHPO), 4.74–4.79 (m, 1H, CH_{Pro}), 4.83 (s, 2H, CH_{pyr}, inapplicable integration), 4.95–5.01 (m, 1H, NHC$\underline{H}$CO), 5.62 (s, 1H, CONH), 5.95 (s, 2H, CH_2), 7.01 (d, ^3J = 8.3 Hz, 2H, 2-H or 2`-H), 7.04 (d, $^3J$ = 8.4 Hz, 2-H or 2`-H), 7.09 (d, ^3J = 8.3 Hz, 2H, 3-H or 3`-H), 7.14 (d, ^3J = 8.3 Hz,

2H, 3-H or 3`-H), 7.32 (d, ^{3}J = 7.6 Hz, 2H, CH_{arom}), 7.96 (d, ^{3}J = 8.0 Hz, 2H, CH_{arom}), 8.09 (s, 1H, triazole-H), 8.25 (s, 1H, CONH), ^{13}C NMR (150 MHz, $CDCl_3$) δ 14.63 ($\underline{C}H_3C_{pyr}$, $\underline{C}H_3C_{pyr}$), 16.33 (SCH_3), 16.43 (SCH_3), 18.25 (d, ^{3}J = 7.6 Hz, $CH(\underline{C}H_3)_2$), 19.01 (C_{aliph}), 20.24 (d, ^{3}J = 12.7 Hz, $CH(\underline{C}H_3)_2$), 25.08, 28.98, (C_{aliph}), 29.33 (br s, $\underline{C}H(CH_3)_2$), 29.71, 31.87, 48.07 (C_{aliph}), 51.16 (d, J = 155.1 Hz, CHPO), 52.55 (CH_2), 56.14 (NH$\underline{C}$HCO), 59.79 ($\underline{C}_{Pro}CO$), 120.96 (t, ^{3}J = 4.2 Hz, C-2 and C-2`), 121.30, 121.89, 126.43 ($C_{arom}$), 128.10 (C-3 or C-3`), 128.29 (C-3 or C-3`), 128.65, 131.07, 131.37, 134.97 ($C_{arom}$), 135.38 (C-4 or C-4`), 135.41 (C-4 or C-4`), 141.12, 143.08 ($C_{arom}$), 147.80 (d, $^{2}J$ = 9.8 Hz, C-1 or C-1`), 147.90 (d, ^{2}J = 9.9 Hz, C-1 or C-1`), 155.63 (C_{arom}), 165.25 ($CH_2\underline{C}ONH$), 170.99 ($CH\underline{C}ON_{Pro}$), 172.59 (d, ^{3}J = 5.8 Hz, $CH_{Pro}\underline{C}ONH$); a signal for one aromatic carbon is missing. ^{31}P NMR (243 MHz, $CDCl_3$) δ 17.85; LC/MS (ESI) (H_2O/MeOH, 60:40 to 0:100) *m/z*: 1005.7 $[M - F]^+$; 94% purity; HRMS (ESI) *m/z*: calcd for $C_{51}H_{60}BF_2N_8O_6PS_2$ $[M - F]^+$ 1005.3886, found 1005.3895.

General enzymatic methods

Enzymatic reactions with bovine chymotrypsin, bovine trypsin, human cathepsin B, human cathepsin L, human leukocyte elastase and porcine pancreatic elastase were recorded on a Cary 50 or 100 Bio, Varian spectrophotometer (Agilent Technologies, USA) with an emission wavelength of 405 nm. Human thrombin and bovine factor Xa were assayed on a FLUOstar Optima fluorimeter (BMG labtech, Germany) in 96 well plates with flat bottom (Sarstedt, USA). The wavelength for excitation was 340 nm and for emission 460 nm. The substrates Suc-Ala-Ala-Pro-Phe-pNA (bovine chymotrypsin), Boc-Ile-Glu-Gly-Arg-AMC (bovine factor Xa), Suc-Ala-Ala-Pro-Arg-pNA (bovine trypsin), Z-Arg-Arg-pNA (human cathepsin B), Z-Phe-Arg-pNA (human cathepsin L), and Z-Gly-Gly-Arg-AMC (human thrombin) were obtained from Bachem (Switzerland), while MeO-Suc-Ala-Ala-Pro-Val-pNA (human leukocyte elastase, porcine pancreatic elastase) was purchased from Calbiochem (Germany). The stock solutions of inhibitors (10 mM) were prepared in DMSO. All measurements were performed over 60 min in duplicate with five different inhibitor concentrations.

Enzyme inhibition assays

Bovine chymotrypsin inhibition assay.[6] Assay buffer was 20 mM Tris-HCl buffer containing 150 mM NaCl, pH 8.4. An enzyme stock solution of 1 mg/mL was prepared in 1 mM aqueous HCl, diluted with assay buffer and kept at 0°C. A 40 mM stock solution of chromogenic substrate Suc-Ala-Ala-Pro-Phe-pNA was prepared in DMSO and diluted with assay buffer containing 10% DMSO. The final concentrations were as follows: substrate, 200 μM (= 2.68 × K_m);[7] DMSO, 6%; bovine chymotrypsin, 50 ng/mL. Into a cuvette containing 845 μL assay buffer, 55 μL inhibitor solution in DMSO and 50 μL substrate solution (4 mM) were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 50 μL of the enzyme solution.

Bovine factor Xa inhibition assay.[8] Assay buffer was 50 mM Tris-HCl buffer containing 100 mM NaCl and 10 mM $CaCl_2$, pH 8.0. The enzyme stock solution (1 U/μL) was prepared in water, diluted with assay buffer and kept at 0°C. A 20 mM stock solution of fluorogenic substrate Boc-Ile-Glu-Gly-Arg-AMC in DMSO was diluted with assay buffer. The final concentrations were as follows: substrate, 100 μM (= 1.69 × K_m); DMSO, 6%; bovine factor

Xa, 0.5 U/mL. Into each well containing 174 µL assay buffer, 11 µL inhibitor solution in DMSO and 10 µL substrate solution (2 mM) were added and thoroughly mixed. The reaction was performed at 37 °C, initiated by adding 5 µL of the enzyme solution.

Bovine trypsin inhibition assay.[9] Assay buffer was 20 mM Tris-HCl buffer containing 150 mM NaCl, pH 8.4. The trypsin stock solution (10 µg/mL) was prepared in 1 mM HCl, diluted with assay buffer and kept at 0°C. A 40 mM stock solution of the chromogenic substrate Suc-Ala-Ala-Pro-Arg-pNA in DMSO was diluted with assay buffer. The final concentrations were as follows: substrate, 200 µM (= 2.70 × K_m); DMSO, 6%; bovine trypsin, 40 ng/mL. Into a cuvette containing 845 µL assay buffer, 50 µL substrate solution (4 mM) and 55 µL inhibitor solution in DMSO were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 50 µL of the enzyme solution.

Human leukocyte elastase.[6] Assay buffer was 50 mM sodium phosphate buffer containing 500 mM NaCl, pH 7.8. An enzyme stock solution of 100 µg/mL was prepared in 100 mM sodium acetate buffer, pH 5.5. An aliquot was kept at 0°C and diluted with assay buffer immediately before the measurement. A 50 mM stock solution of the chromogenic substrate MeO-Suc-Ala-Ala-Pro-Val-pNA was prepared in DMSO and diluted with assay buffer containing 10% DMSO. The final concentrations were as follows: substrate, 100 µM (= 1.85 × K_m); DMSO, 1.5%; human leukocyte elastase, 35 ng/mL. Into a cuvette containing 890 µL assay buffer, 10 µL inhibitior solution in DMSO and 50 µL substrate solution (2 mM) were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 50 µL of enzyme solution.

Human thrombin inhibition assay.[10] Assay buffer was 50 mM Tris-HCl buffer containing 150 mM NaCl, pH 8.0. The enzyme stock solution (10000 U/mL) was prepared in water, diluted with assay buffer and kept at 0°C. A 10 mM stock solution of fluorogenic substrate Z-Gly-Gly-Arg-AMC in DMSO was diluted with assay buffer. The final concentrations were as follows: substrate, 40 µM (= 1.0 × K_m); DMSO, 6%; human thrombin, 1.5 U/mL. Into each well containing 173.8 µL buffer, 11.2 µL of an inhibitor solution in DMSO and 10 µL of a substrate solution (2 mM) were added and thoroughly mixed. The reaction was performed at 25°C, initiated by adding 5 µL of enzyme solution.

Porcine pancreatic elastase inhibition assay. Assay buffer was 50 mM sodium phosphate buffer containing 500 mM NaCl, pH 7.8. An enzyme stock solution of 100 U/mL was prepared in 100 mM sodium acetate buffer, pH 5.5. An aliquot was kept at 0°C and diluted with assay buffer immediately before the measurement. A 50 mM stock solution of the chromogenic substrate MeO-Suc-Ala-Ala-Pro-Val-pNA was prepared in DMSO and diluted with assay buffer containing 10% DMSO. In accordance to literature,[11] a K_m value greater than 1000 µM was determined using 18 different substrate concentration in triplicate measurements. For the inhibition assay, the final concentrations were as follows: substrate, 100 µM (<< K_m); DMSO, 1.5%; porcine pancreatic elastase, 0.01 U/mL. Into a cuvette containing 890 µL assay buffer, 10 µL of the inhibitor solution in DMSO and 50 µL substrate solution (2 mM) were added and thoroughly mixed. The reaction was performed at 25 °C, initiated by adding 50 µL of the enzyme solution.

Human cathepsin B inhibition assay.[12] Assay buffer was 100 mM sodium phosphate buffer containing 100 mM NaCl, 5 mM EDTA and 0.01% Brij 35, pH 6.0. An enzyme stock solution of 0.47 mg/mL in 20 mM sodium acetate buffer containing 1 mM EDTA, pH 5.0, was diluted 1:500 with assay buffer containing 5 mM DTT, incubated for 30 min at 37 °C and kept at 0°C. A 100 mM stock solution of the chromogenic substrate Z-Arg-Arg-pNA was prepared with DMSO. The final concentrations were as follows: substrate, 500 µM (= 0.45 × K_m); DMSO, 2%; human cathepsin B, 18.8 ng/mL. Into a cuvette containing 960 µL assay buffer, 15 µL of the inhibitor solution in DMSO, and 5 µL of the substrate solution were added and thoroughly mixed. The reaction was performed at 37 °C and initiated by adding 20 µL of enzyme solution.

Human cathepsin L inhibition assay.[12] Assay buffer was 100 mM sodium phosphate buffer containing 100 mM NaCl, 5 mM EDTA and 0.01% Brij 35, pH 6.0. An enzyme stock solution of 135 µg/mL in 20 mM malonate buffer containing 400 mM NaCl and 1 mM EDTA, pH 5.5, was diluted 1:100 with assay buffer containing 5 mM DTT, incubated for 30 min at 37 °C and kept at 0°C. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-pNA was prepared with DMSO. The final concentrations were as follows: substrate, 100 µM (= 5.88 × K_m); DMSO, 2%; human cathepsin L, 54 ng/mL. Into a cuvette containing 940 µL assay buffer, 10 µL of the inhibitor solution in DMSO and 10 µL of the substrate solution were added and thoroughly mixed. The reaction was performed at 37 °C, initiated by adding 20 µL of the enzyme solution.

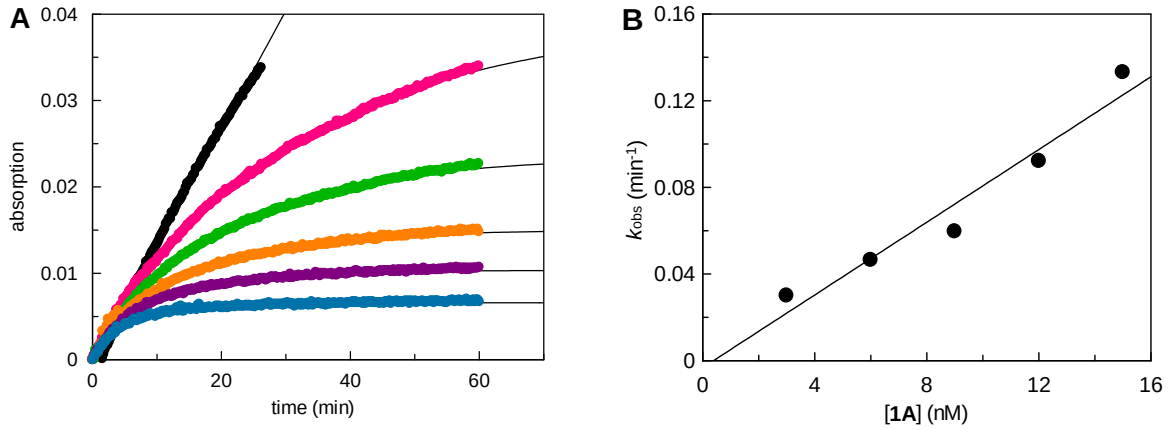


Figure S1. Inhibition of HLE with compound 1A. (A) Product formation in the presence of varying inhibitor concentrations. From top to bottom: 0 nM, 3 nM, 6 nM, 9 nM, 12 nM, 15 nM. Progress curves were analyzed using the equation $[P] = v_i\ (1 - \exp(-k_{obs}\ t))/k_{obs} + d$. (B) The first-order rate constants k_{obs} (mean values from duplicate measurements) were plotted against the inhibitor concentrations.

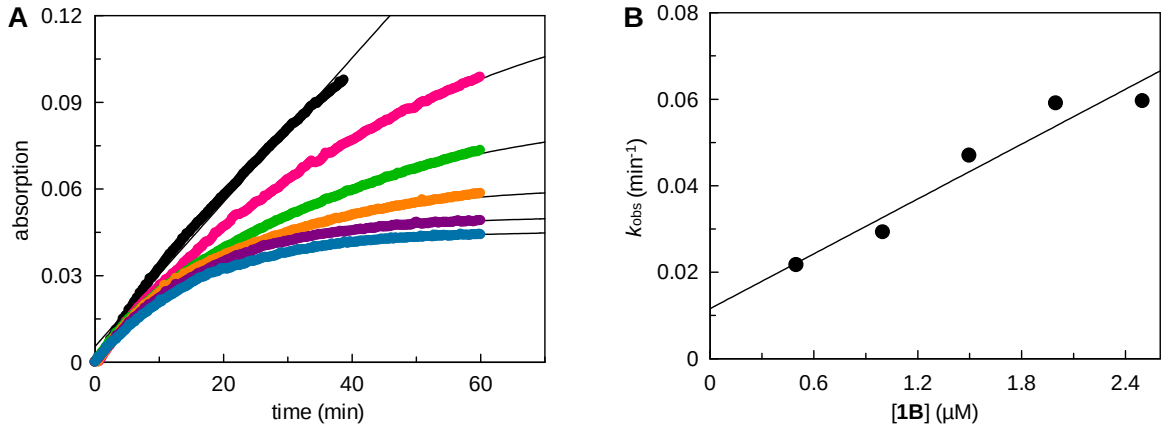


Figure S2. Inhibition of HLE with compound 1B. (A) Product formation in the presence of varying inhibitor concentrations. From top to bottom: 0 μM, 0.5μM, 1 μM, 1.5 μM, 2 μM, 2.5 μM. Progress curves were analyzed using the equation $[P] = v_i\ (1 - \exp(-k_{obs}\ t))/k_{obs} + d$. (B) The first-order rate constants k_{obs} (mean values from duplicate measurements) were plotted against the inhibitor concentrations.

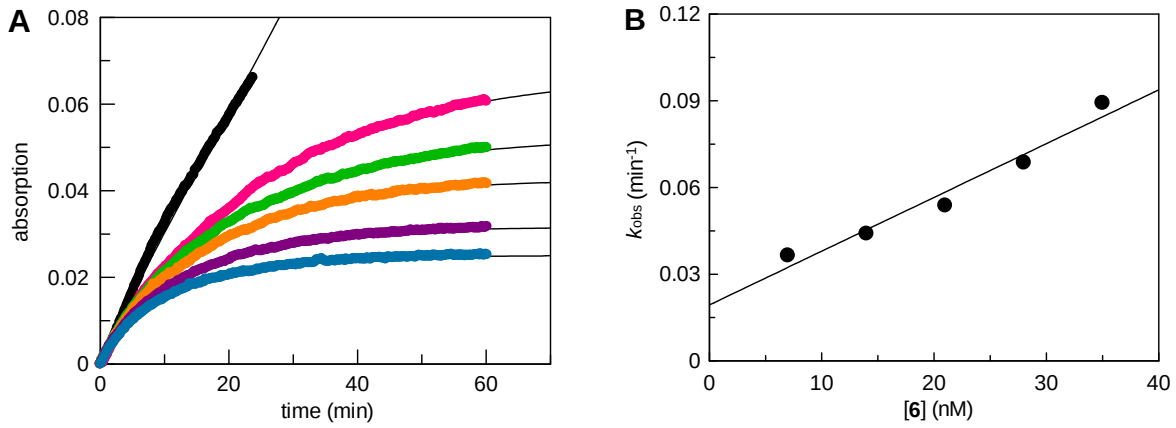


Figure S3. Inhibition of HLE with probe 6. (A) Product formation in the presence of varying inhibitor concentrations. From top to bottom: 0 nM, 7 nM, 14 nM, 21 nM, 29 nM, 35 nM. Progress curves were analyzed using the equation [P] = v_i (1 – exp(-k_{obs} t))/k_{obs}+ d. (B) The first-order rate constants k_{obs} (mean values from duplicate measurements) were plotted against the inhibitor concentrations.

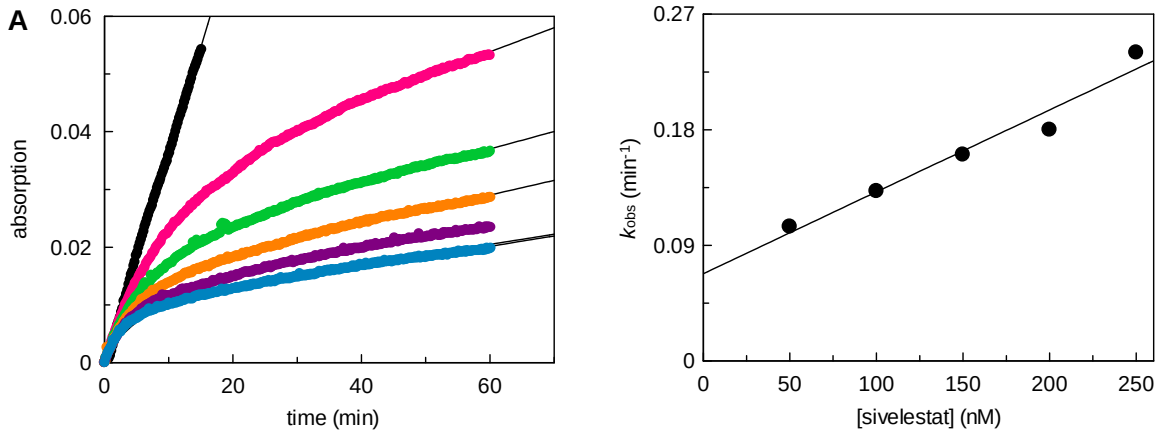


Figure S4. Inhibition of HLE with sivelestat. (A) Product formation in the presence of varying inhibitor concentrations. From top to bottom: 0 nM, 50 nM, 100 nM, 150 nM, 200 nM, 250 nM. Progress curves were analyzed by non-linear regression using the equation [P] = v_st + (v_i - v_s)(1-exp(-k_{obs} t))/k_{obs} + d, where [P] is the product concentration, v_s is the steady state rate, v_i is the initial rate, k_{obs} is the observed first-order rate constant and d is the offset. (B) Values k_{obs} were plotted against inhibitor concentrations [I] and k_{on} and k_{off} values were obtained by linear regression using the equation k_{obs} = [I] k_{on} /(1+ [S]/K_m) + k_{off}, from the slope and the intercept, respectively. The standard errors refer to this linear regression The K_i value was calculated from the equation K_i = k_{off} / k_{on}.

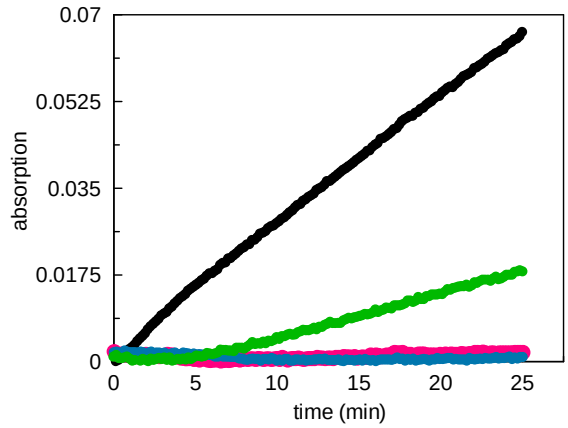


Figure S5. Reactivation experiments. Green: The enzyme (HLE) was incubated in assay buffer with sivelestat for 30 min at 25 °C in a volume of 985.5 µL. A volume of 14.5 µL of a substrate (MeO-Suc-Ala-Ala-Pro-Val-pNA) solution was added and the reaction was immediately monitored. The final concentrations were as follows, sivelestat 200 nM, substrate 1 mM, DMSO 1.5%. Blue: The same experiment was performed with compound **1A** in a final concentration of 20 nM. Magenta: The same experiment was performed with activity-based probe **6** in a final concentration of 50 nM. Black: The same experiment was performed in the absence of inhibitors.

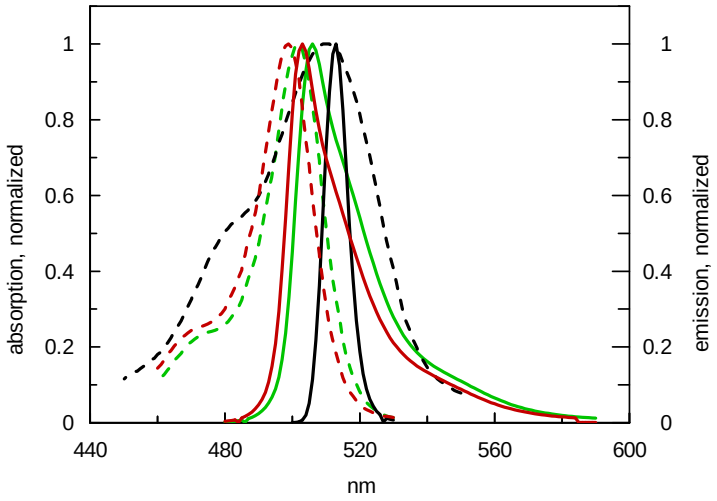


Figure S6. Normalized absorption and emission spectra of probe 6. Absorption (dashed lines) and emission (solid lines) spectra were recorded at concentrations of 5 µM and 1 µM, respectively, in the corresponding solvent containing 1% DMSO. Red: MeOH, λ_{ex}= 499 nm, λ_{em} = 503 nm; green: CH_2Cl_2, λ_{ex} = 502 nm, λ_{em} = 506 nm; black: H_2O, λ_{ex}= 509 nm, λ_{em} = 513 nm.

Detection of human leukocyte elastase with probe 6

Estimation of the detection limit. A 200 µM solution of probe **6** was prepared in DMSO. A HLE solution of 267 µg/mL was prepared in 100 mM sodium acetate buffer, pH 5.5. Elastase assay buffer, *i.e.* 50 mM sodium phosphate buffer containing 500 mM NaCl, pH 7.8, was used to prepare mixtures of a total volume of 40 µL containing 2.5 µM of the probe, 2.5% DMSO and varying concentrations of HLE (10.7 ng/µL, 18.0 ng/µL, 25.3 ng/µL, 32.7 ng/µL, 40.0 ng/µL). These mixtures were incubated at 25 °C for 20 min. To 18 µL of each mixture, 6 µL of reducing 4 × Roti-Load 1 buffer (Roth, Germany) was added followed by incubation for 5 min at 95 °C. After centrifugation (14,000 g, 5 min), aliquotes of 20 µL were applied to SDS-PAGE analysis. Gels (13%) were run in Tris/glycine/SDS buffer (25 mM Tris, 192 mM glycine, 0.1% SDS). The enzyme was visualized by in-gel fluorescence detection using a Typhoon Trio scanner (GE Healthcare, UK) with a 488-nm blue laser and an emission 520-nm band-pass filter (520 BP 40). A PMT value of 350 V and a pixel size scanning resolution of 100 µm were adjusted. PageRuler Plus Prestained Protein Ladder (ThermoFisher Scientific, USA) served as standards.

Competition experiment. A 400 µM solution of sivelestat (Sigma Aldrich, Germany) was prepared in DMSO. Two mixtures of a total volume of 39 µL containing HLE (41.08 ng/µL) were prepared in elastase assay buffer. A volume of 0.5 µL sivelestat solution or 0.5 µL DMSO were added and both mixtures were incubated at 25 °C for 5 min. A volume of 0.5 µL of a 200 µM solution of probe **6** in DMSO was added to both samples to reach the following concentrations: probe **6**, 2.5 µM; DMSO, 2.5%; HLE, 40 ng/µL; sivelestat, 5.0 µM. These mixtures were incubated at 25 °C for 20 min. SDS-PAGE and in gel fluorescence detection were performed as described above.

Preparation of cell lysate.[13] HEK 293 cells were cultured in DMEM (Dulbecco`s Modified Eagle`s Medium) supplemented with penicillin (100 U/mL; Invitrogen, Germany), streptomycin (100 µg/mL; Invitrogen, Germany) and fetal bovine serum (FBS) (10%; PPA Laboratories, Germany) under a humified atmosphere of 5% CO_2 at 37 °C. For the preparation of the cell lysate, confluent HEK cells seeded on a 5 cm^2 growth area were washed two times with phosphate buffer saline (PBS) and scraped off with 1 mL of PBS buffer followed by homogenization *via* ten aspirations through a 23-gauge needle. Intact cells and nuclei were removed by centrifugation (2000 *g*, 5 min, 4 °C). The resulting supernatants were subjected to protein quantification with Roti-Nanoquant (Roth, Germany).

Survey of the probe's selectivity. HEK lysate was received as described above. Three mixtures were prepared in elastase assay buffer, all containing probe **6**. A volume of 6.0 µL of HLE was added to the first and second mixture. A volume of or 4.9 µL HEK cell lysate spiked with 6.0 µL of HLE was added to the third mixture. The mixtures were incubated at 25 °C for 20 min. After incubation, 4.9 µL of HEK cell lysate was added to the second mixture. In the final volume of 40 µL, the mixtures contained the following concentrations: probe **6**, 2.5 µM; DMSO, 2.5%; HLE, 40 ng/µL; HEK cell lysate, 0.60 µg/µL. SDS-PAGE and in-gel fluorescence detection were performed as described above.

Colloidal Coomassie G-250 staining. Proteins were stained over night with PageBlue Protein Staining Solution (ThermoFisher Scientific, USA). Gels were captured with a G:BOX F3 Gel Documentation System (Syngene, Cambridge, UK) using a visible light converter screen with the UV transilluminator.

References

(1) Sieńczyk, M.; Winiarski, Ł.; Kasperkiewicz, P.; Psurski, M.; Wietrzyk, J.; Oleksyszyn, J. Simple phosphonic inhibitors of human neutrophil elastase. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1310−1314.

(2) Sienczyk, M.; Grzywa, R.; Pietrusewicz, E.; Oleksyszyn, J.; Winiarski, L.; Burster, T.; Boehm, O. Eur. Pat. Appl. (2013), EP 2676962 A1 20131225.

(3) Winiarski, Ł.; Oleksyszyn, J.; Sieńczyk, M. Human neutrophil elastase phosphonic inhibitors with improved potency of action. *J. Med. Chem.* **2012**, *55*, 6541−6553.

(4) Schmitz, J.; Li, T.; Bartz, U.; Gütschow, M.; Cathepsin B inhibitors: Combining dipeptide nitriles with an occluding loop recognition element by click chemistry. *ACS Med. Chem. Lett.* 2015, *7*, 211−216.

(5) Benstead, M.; Rosser, G. A.; Beeby, A.; Mehl, G. H.; Boyle, R. W. Mesogenic BODIPYs: an investigation of the correlation between liquid crystalline behaviour and fluorescence intensity. *Photochem. Photobiol. Sci.* **2011**, *10*, 992−999.

(6) Sisay, M. T.; Hautmann, S.; Mehner, C.; König, G. M.; Bajorath, J.; Gütschow, M. Inhibition of human leukocyte elastase by brunsvicamides a-C: cyanobacterial cyclic peptides. ChemMedChem **2009**, *4*, 1425−1429.

(7) Steinebach, C.; Schulz-Fincke, A. C.; Schnakenburg, G.; Gütschow, M. In situ generation and trapping of thioimidates: An intermolecular tandem reaction to 4-acylimino-4*H*-3,1-benzothiazines *RSC Adv.* **2016**, *6*, 15430−15440.

(8) Häußler, D.; Scheidt, T.; Stirnberg, M.; Steinmetzer, T.; Gütschow, M. A bisbenzamidine phosphonate as a Janus-faced inhibitor for trypsin-like serine proteases. *ChemMedChem* **2015**, *10*, 1641−1646.

(9) Sisay, M. T.; Steinmetzer, T.; Stirnberg, M.; Maurer, E.; Hammami, M.; Bajorath, J.; Gütschow, M. Identification of the first low-molecular-weight inhibitors of matriptase-2. *J. Med. Chem.* **2010**, *53*, 5523−5535.

(10) Dosa, S.; Stirnberg, M.; Lülsdorff, V.; Häußler, D.; Maurer, E.; Gütschow M. Active site mapping of trypsin, thrombin and matriptase-2 by sulfamoyl benzamidines. *Bioorg. Med. Chem.* **2012**, *20*, 6489−6505.

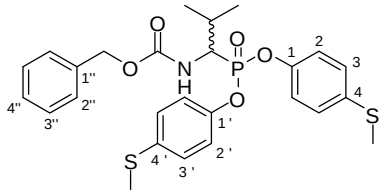
(11) Castillo, M. J.; Nakajima, K.; Zimmerman, M.; Powers, J. C. Sensitive substrates for human leukocyte and porcine pancreatic elastase: a study of the merits of various chromophoric and fluorogenic leaving groups in assays for serine proteases. *Anal. Biochem.* **1979**, *99*, 53−64.

(12) Frizler, M.; Lohr, F.; Lülsdorff, M.; Gütschow, M. Facing the gem-dialkyl effect in enzyme inhibitor design: preparation of homocycloleucine-based azadipeptide nitriles. *Chem. Eur. J.* **2011**, *17*, 11419−11423.

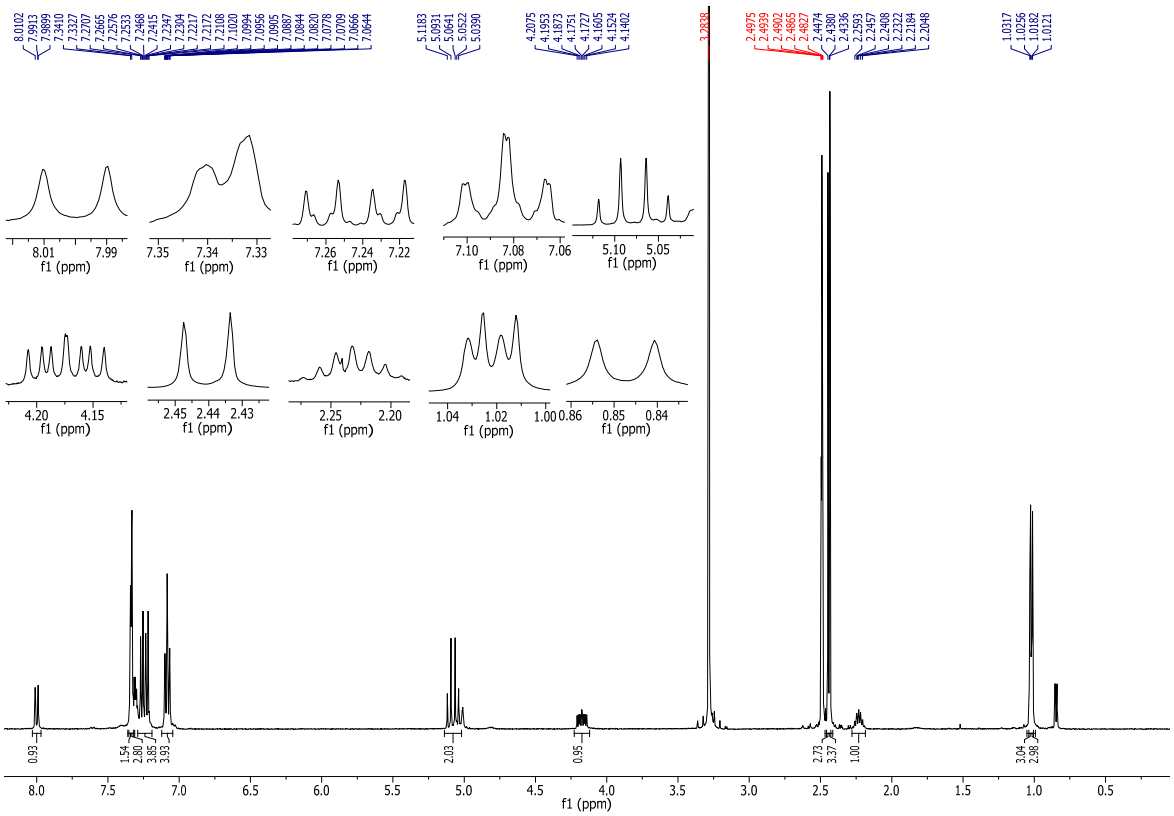
(13) Häußler, D.; Schulz-Fincke, A. C.; Beckmann, A. M.; Keils, A.; Gilberg, E.; Mangold, M.; Bajorath, J.; Stirnberg, M.; Steinmetzer, T.; Gütschow, M. A fluorescent-labeled phosphono bisbenzguanidine as an activity-based probe for matriptase. *Chem. Eur. J.* **2017**, *23*, 5205−5209.

NMR spectra and LC/MS data of synthesized compounds

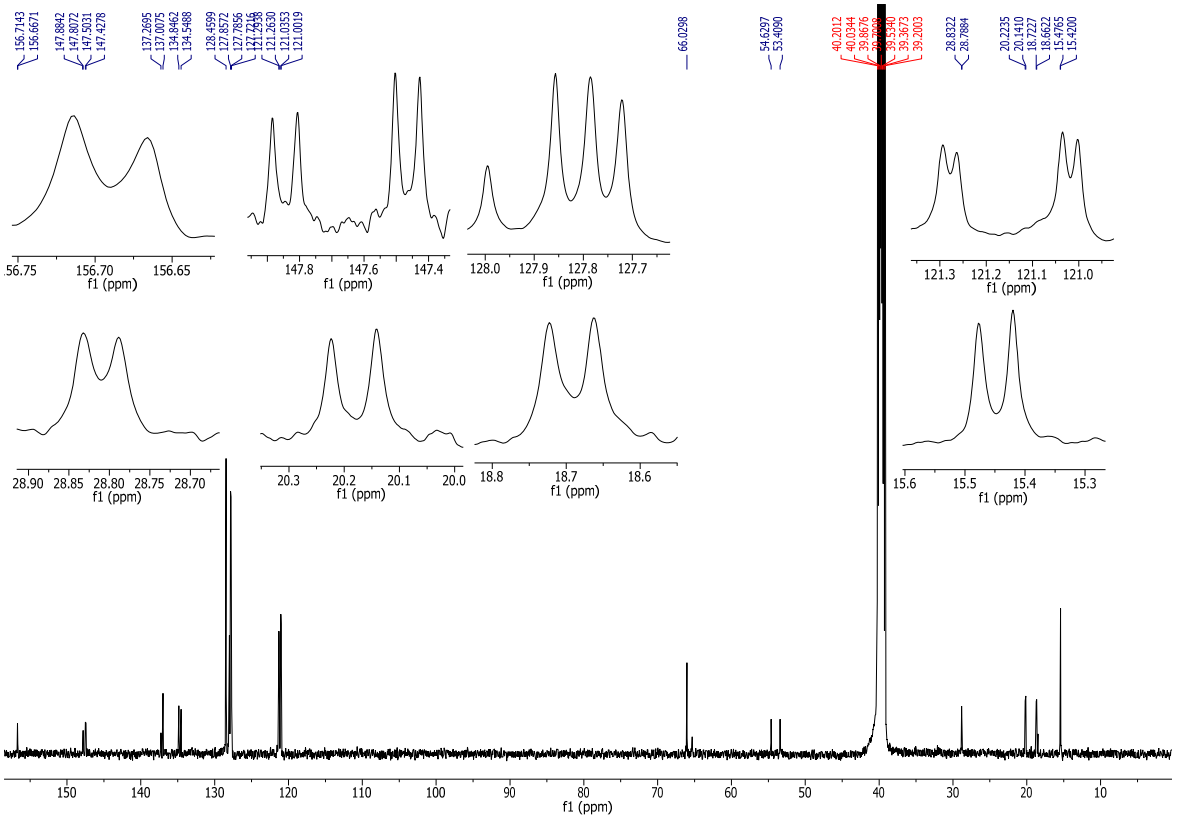
NMR spectra of compound **8**



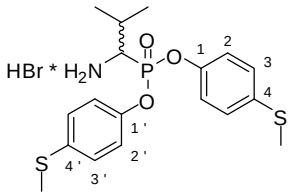
^{1}H NMR (DMSO-d_6)



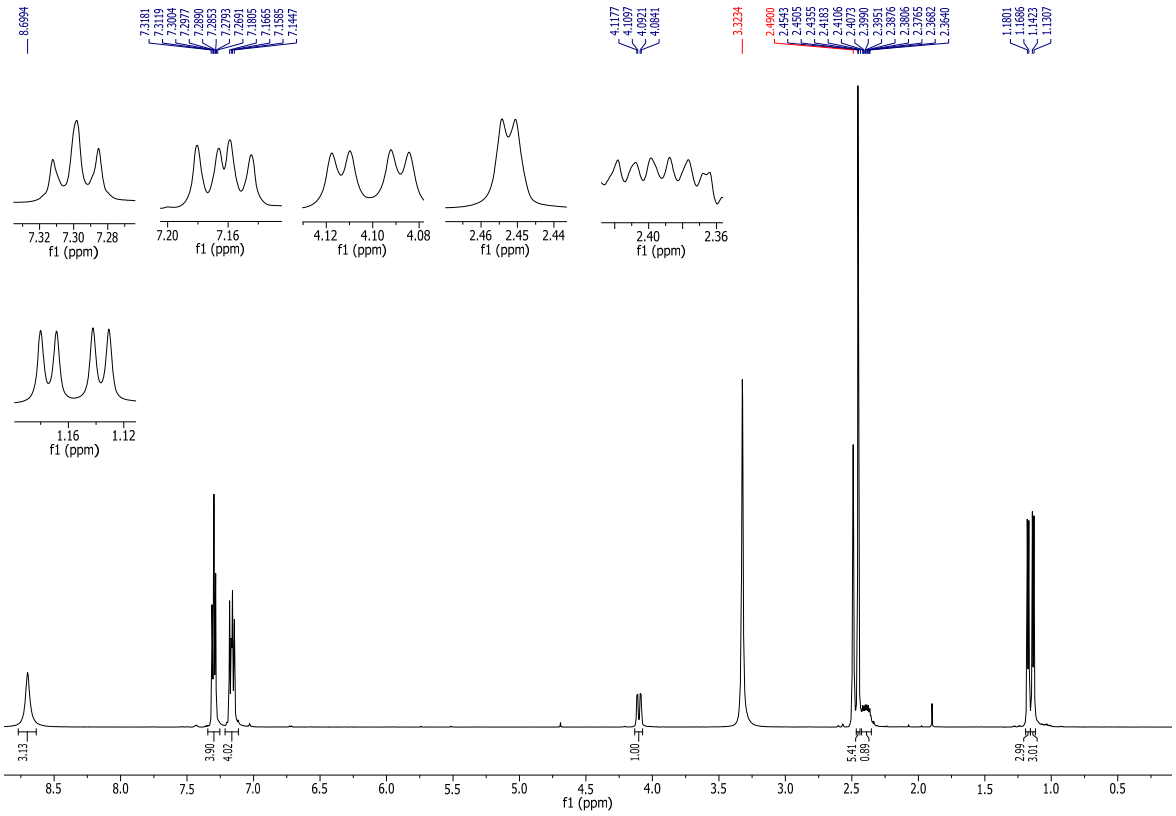
^{13}C NMR


156.7143
156.6671
147.8842
147.8072
147.5031
147.4278
137.2695
137.0075
134.8462
134.5488
128.4599
127.8572
127.7056
121.2630
121.0353
121.0019
66.0298
54.6297
53.4090
40.2012
40.0344
39.8676
39.7008
39.5340
39.3673
39.2003
28.8322
28.7884
20.2235
20.1410
18.7227
18.6622
15.4765
15.4200
f1 (ppm)

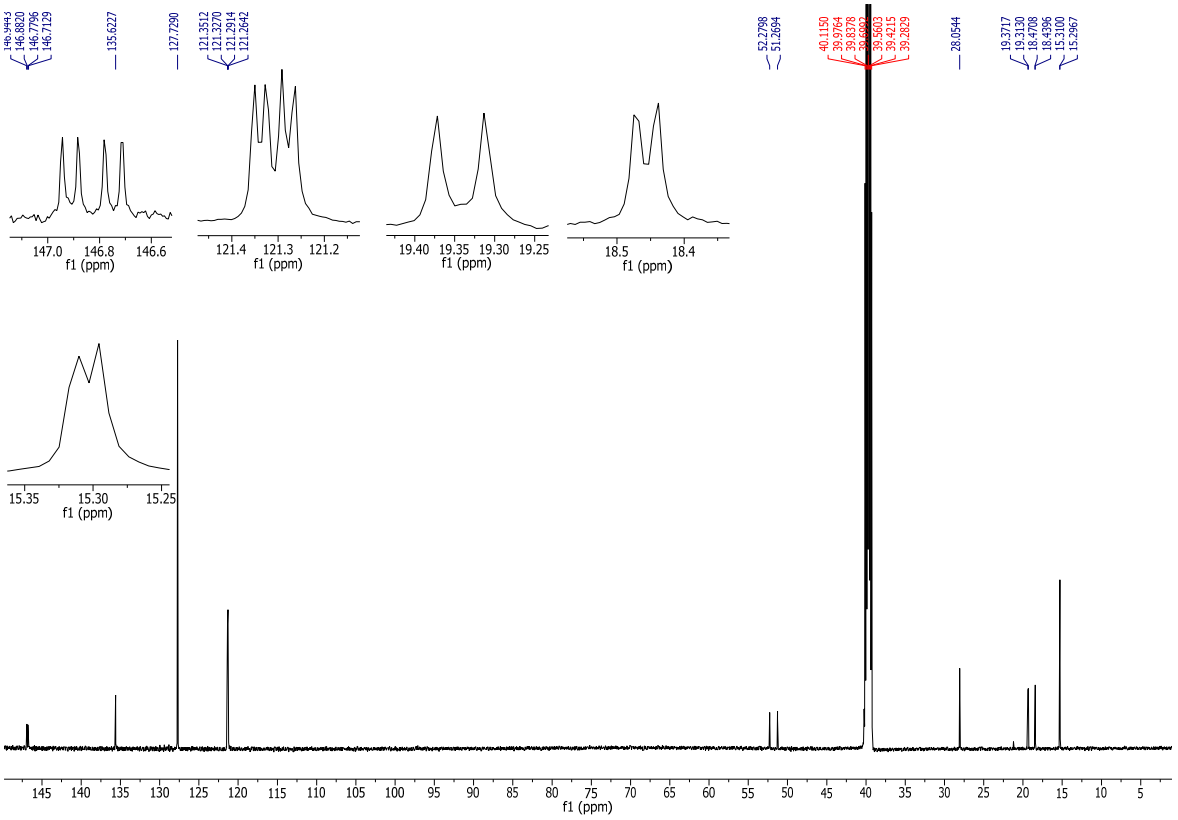
NMR spectra of compound **9**



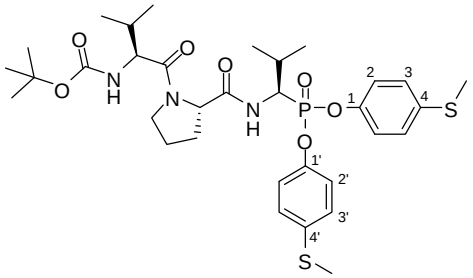
^{1}H NMR (DMSO-d_6)



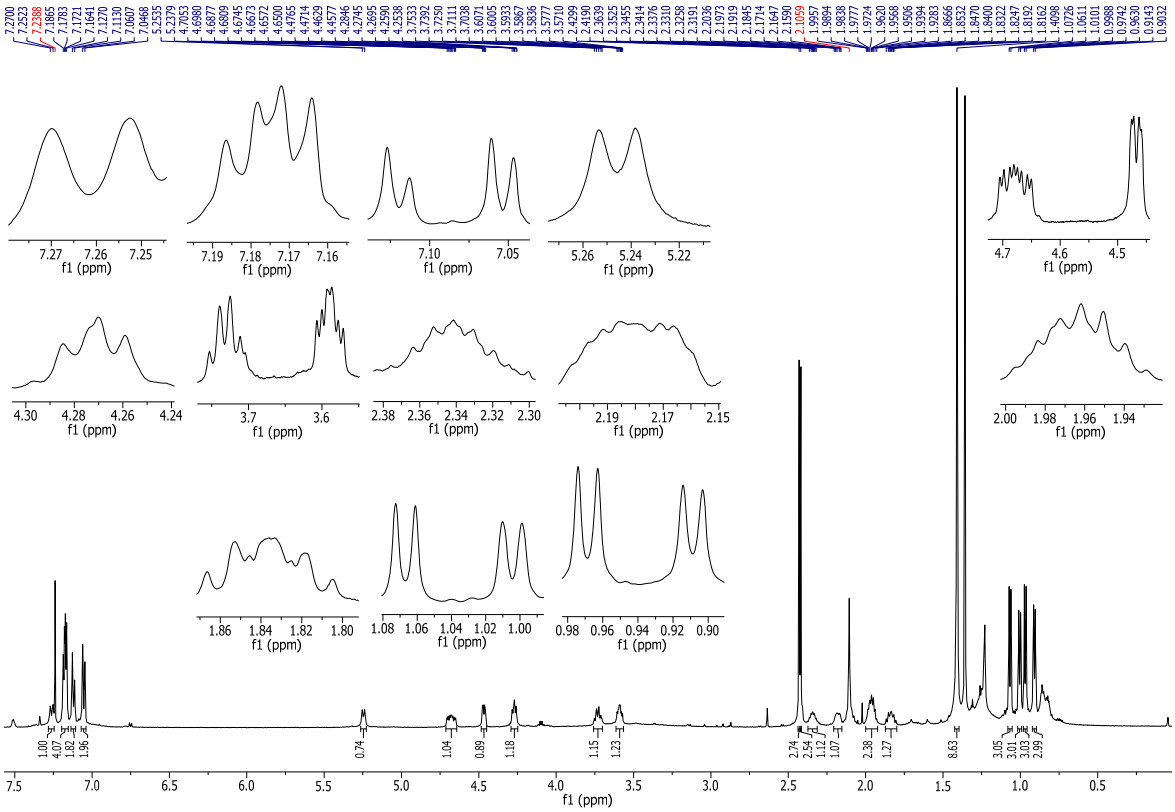
^{13}C NMR


146.9443
146.8820
146.7796
146.7129
135.6227
127.7290
121.3512
121.3270
121.2914
121.2642
52.2798
51.2694
40.1150
39.9704
39.8258
39.6812
39.5603
39.4215
39.2829
28.0544
19.3717
19.3130
18.4708
18.4096
15.3100
15.2967
f1 (ppm)

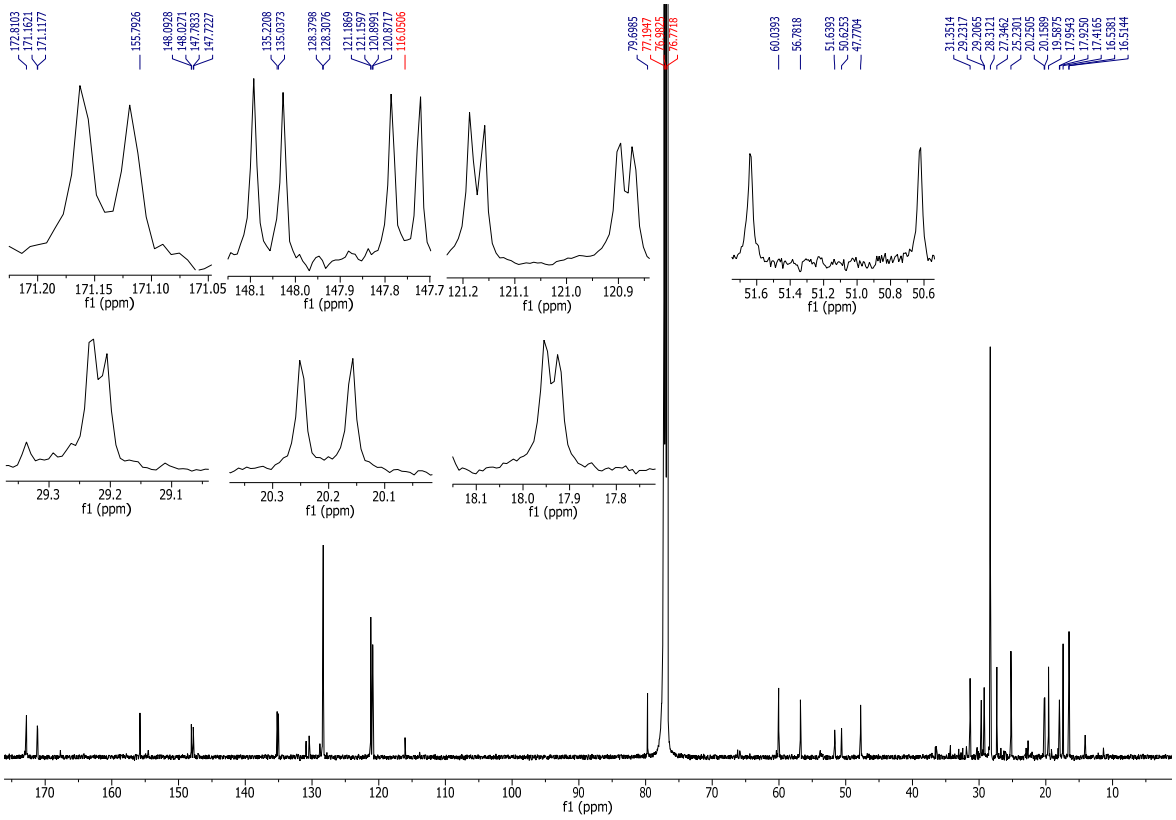
NMR spectra of compound **1A**



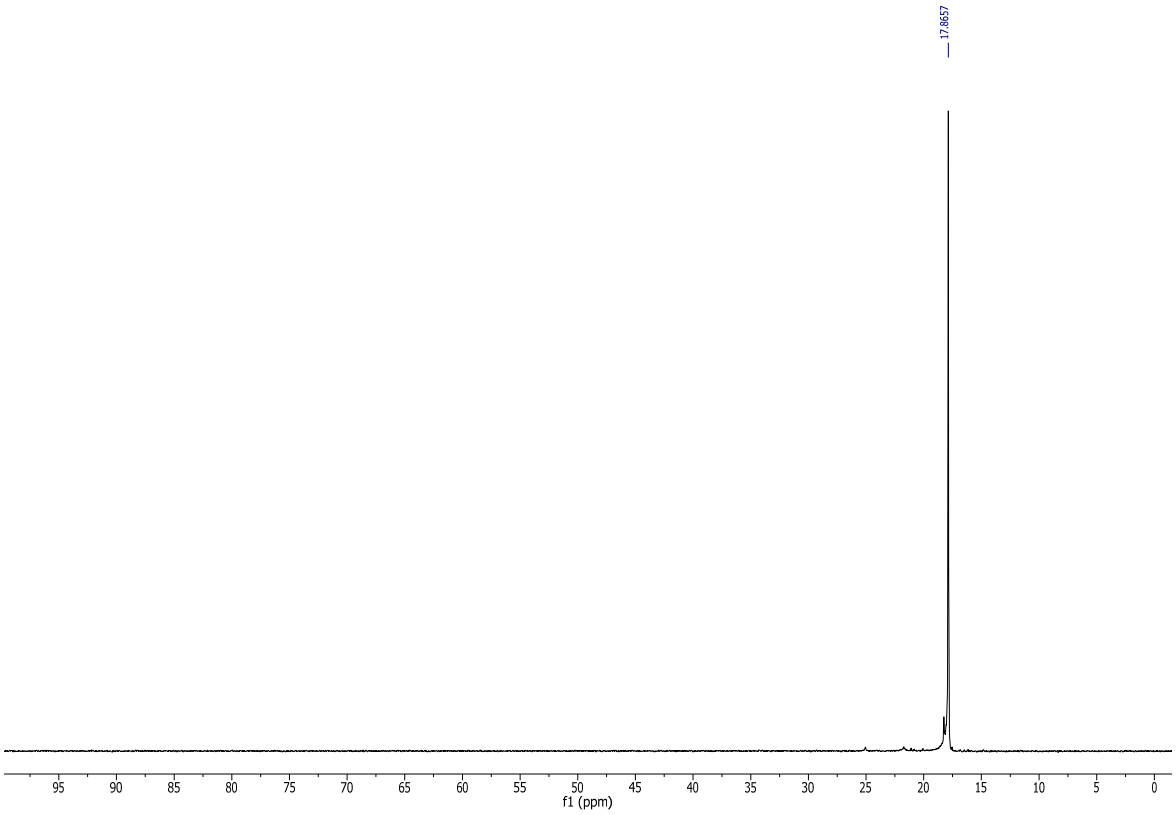
^{1}H NMR ($CDCl_3$)



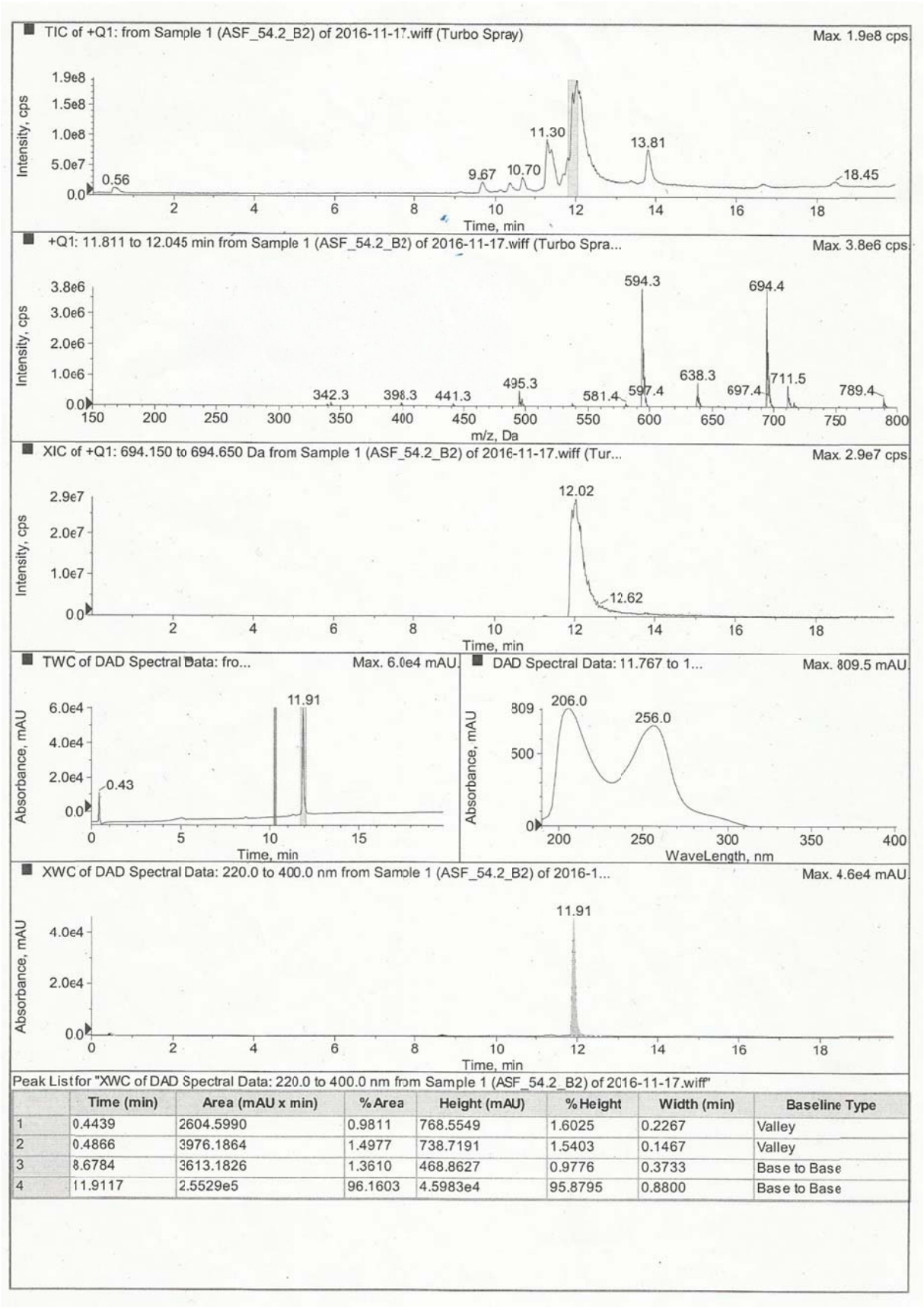
^{13}C NMR



^{31}P NMR



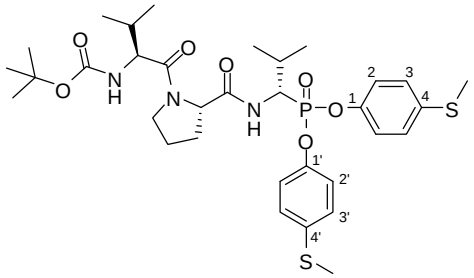
LC/MS analysis of compound **1A**



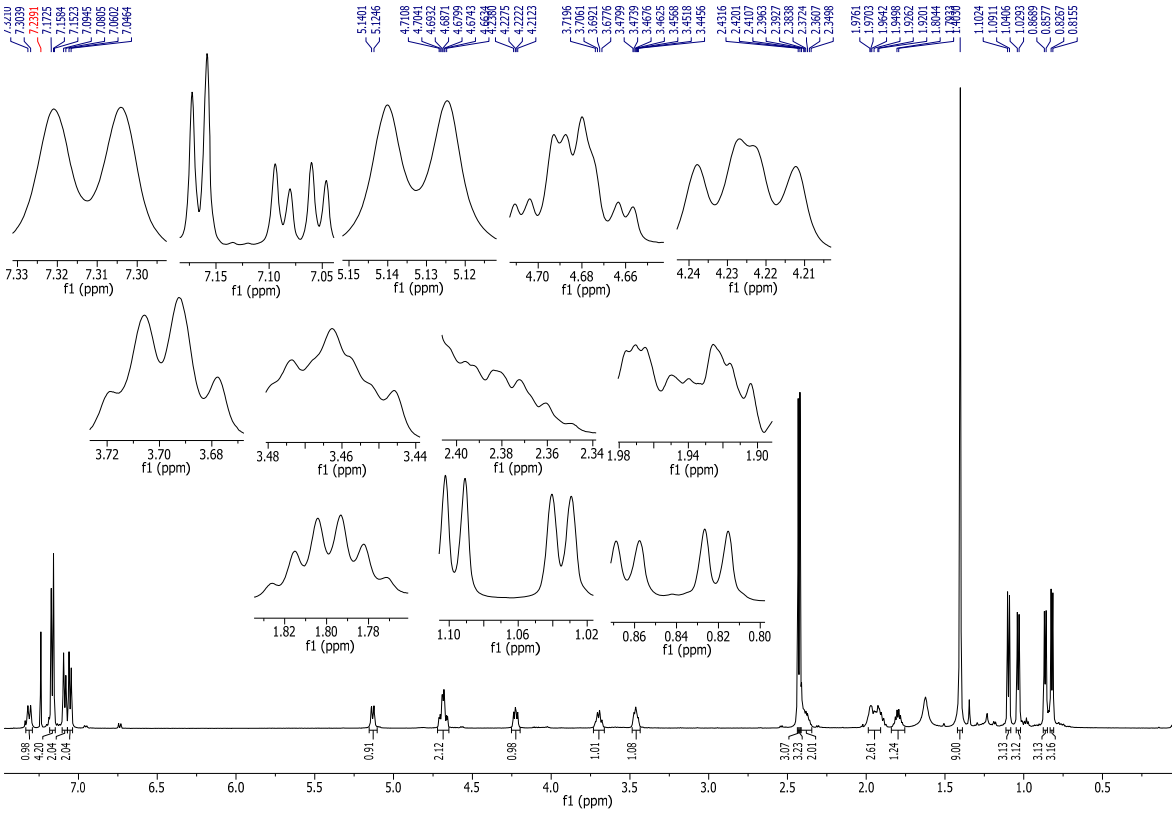
Peak List for "XWC of DAD Spectral Data: 220.0 to 400.0 nm from Sample 1 (ASF_54.2_B2) of 2016-11-17.wiff"

	Time (min)	Area (mAU x min)	% Area	Height (mAU)	% Height	Width (min)	Baseline Type
1	0.4439	2604.5990	0.9811	768.5549	1.6025	0.2267	Valley
2	0.4866	3976.1864	1.4977	738.7191	1.5403	0.1467	Valley
3	8.6784	3613.1826	1.3610	468.8627	0.9776	0.3733	Base to Base
4	11.9117	2.5529e5	96.1603	4.5983e4	95.8795	0.8800	Base to Base

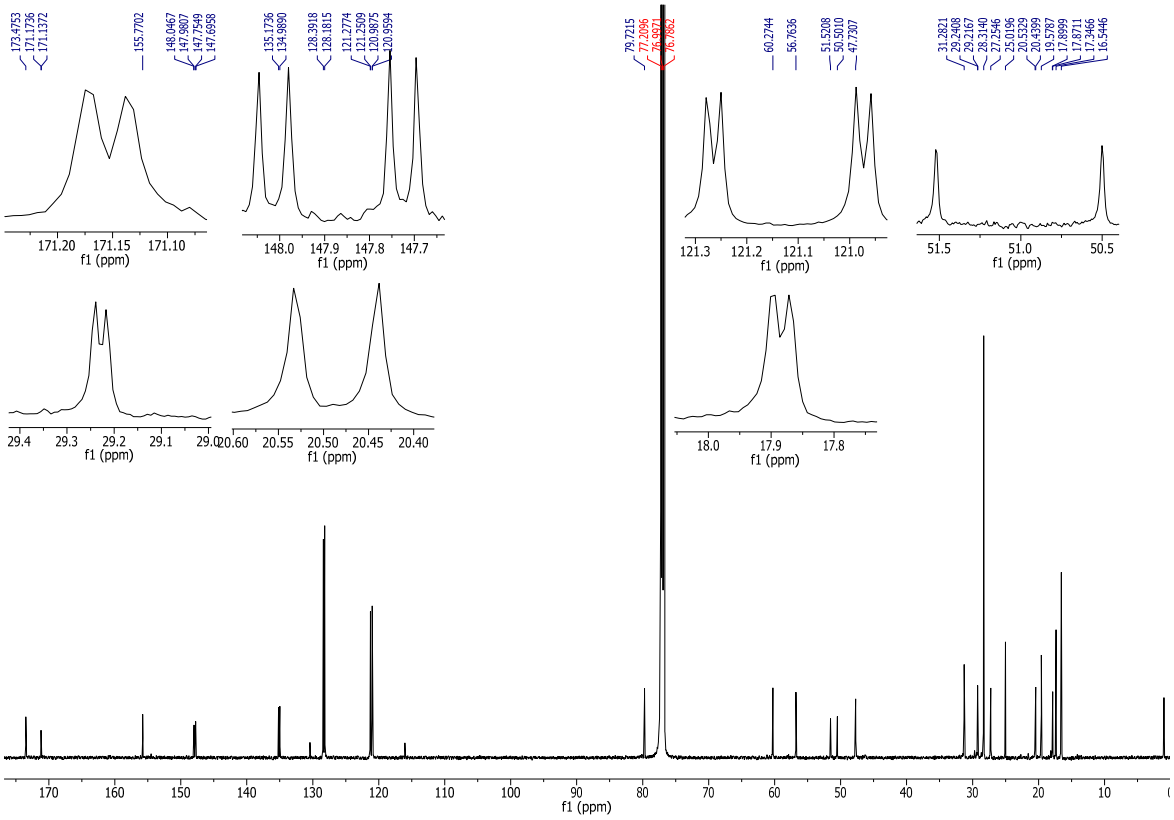
NMR spectra of compound **1B**



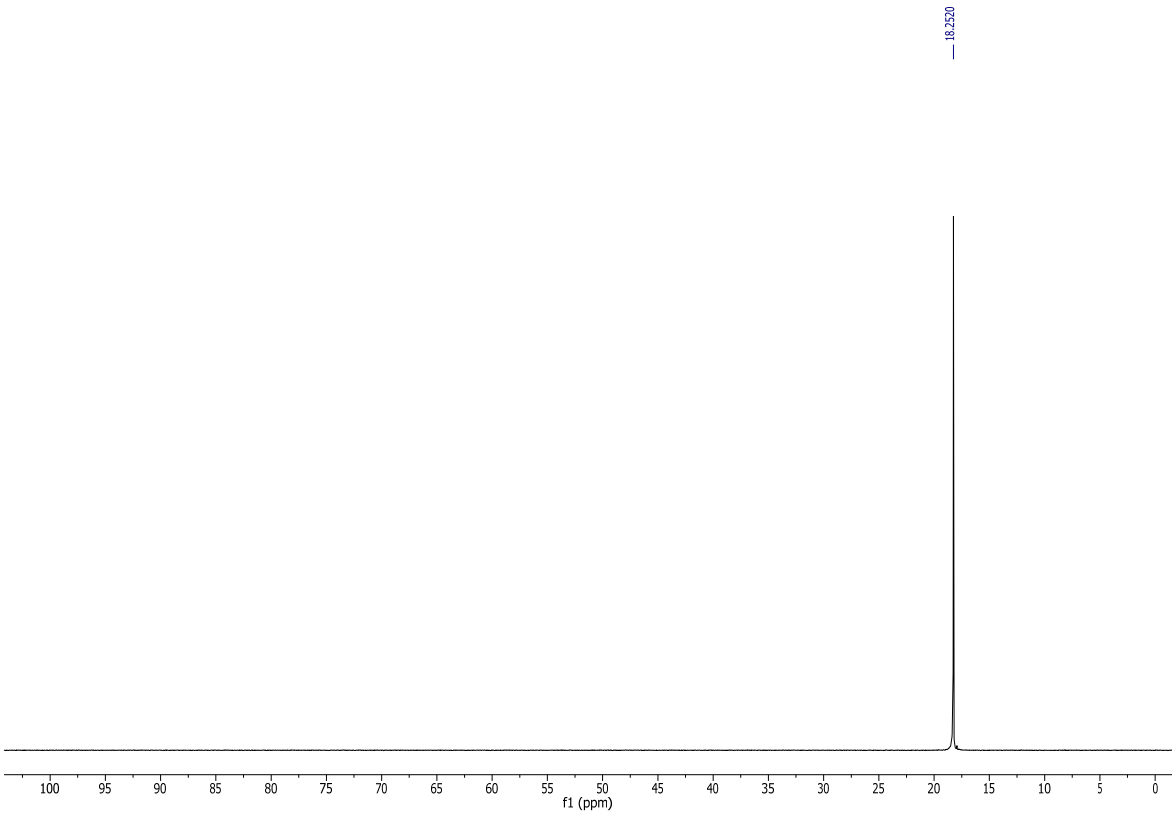
^{1}H NMR ($CDCl_3$)



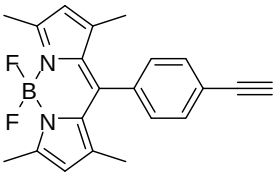
^{13}C NMR



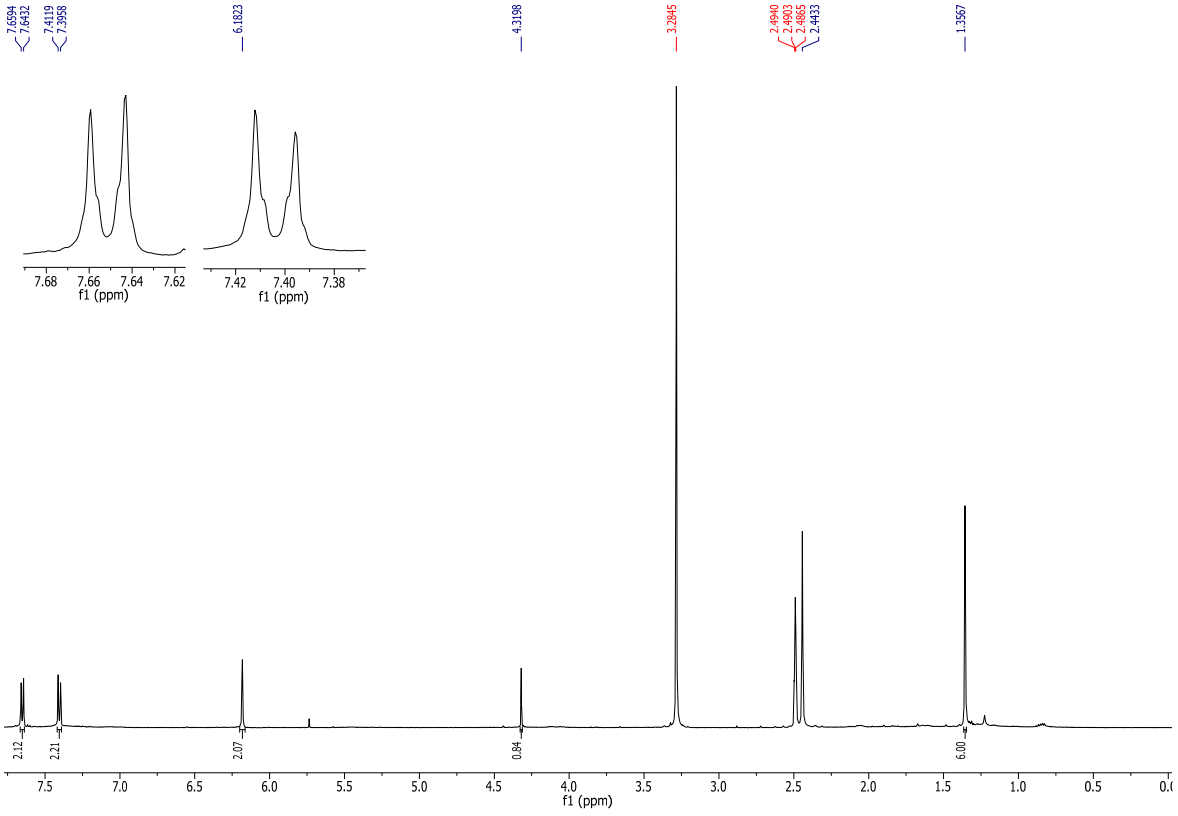
^{31}P NMR



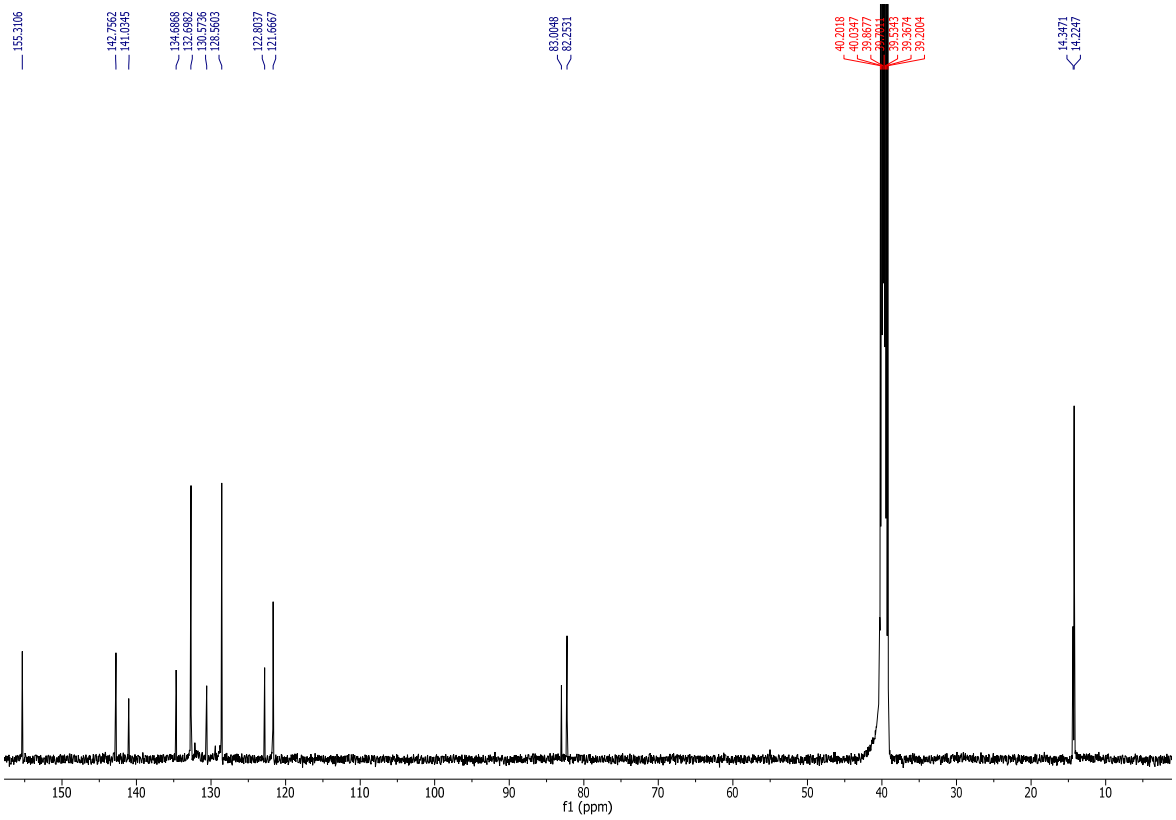
NMR spectra of compound **5**



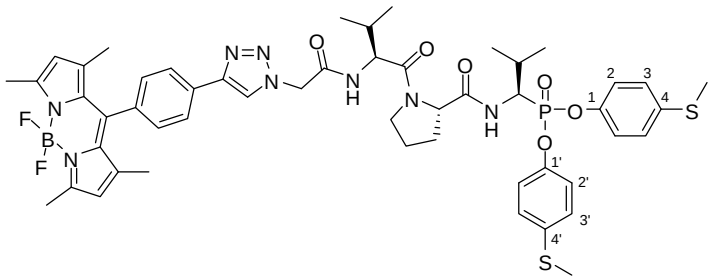
^{1}H NMR (DMSO-*d6*)



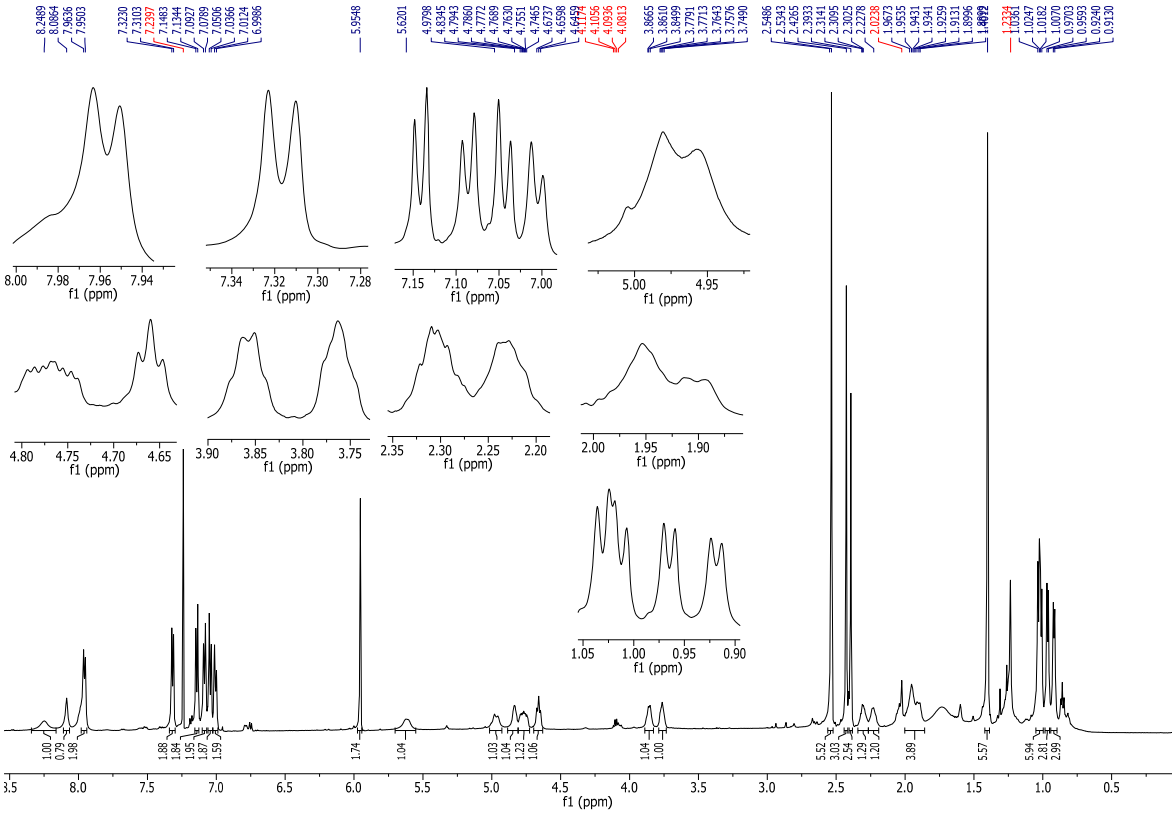
^{13}C NMR


155.3106
142.7562
141.0945
134.6868
132.6982
130.5736
128.5603
122.8037
121.6667
83.0048
82.2531
40.2018
40.0347
39.8677
39.7007
39.5343
39.3674
39.2004
14.3471
14.2247
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

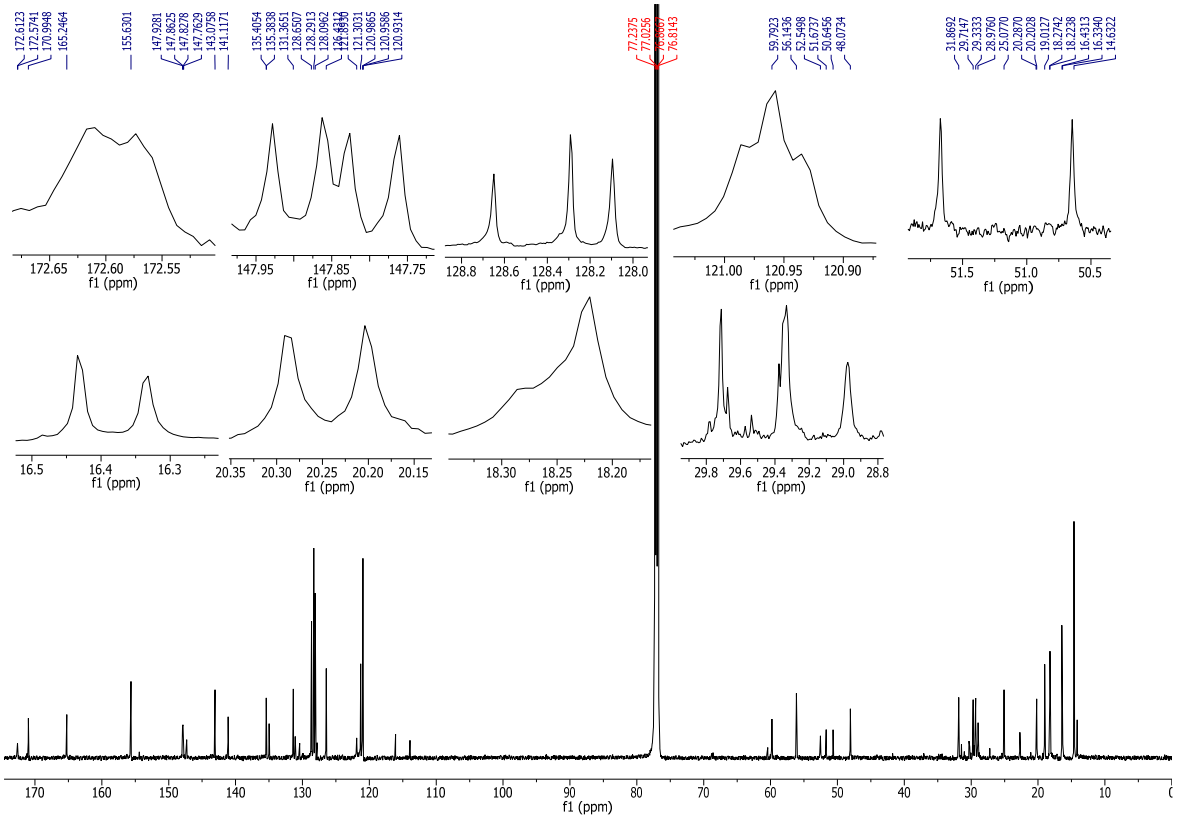
NMR spectra of compound **6**



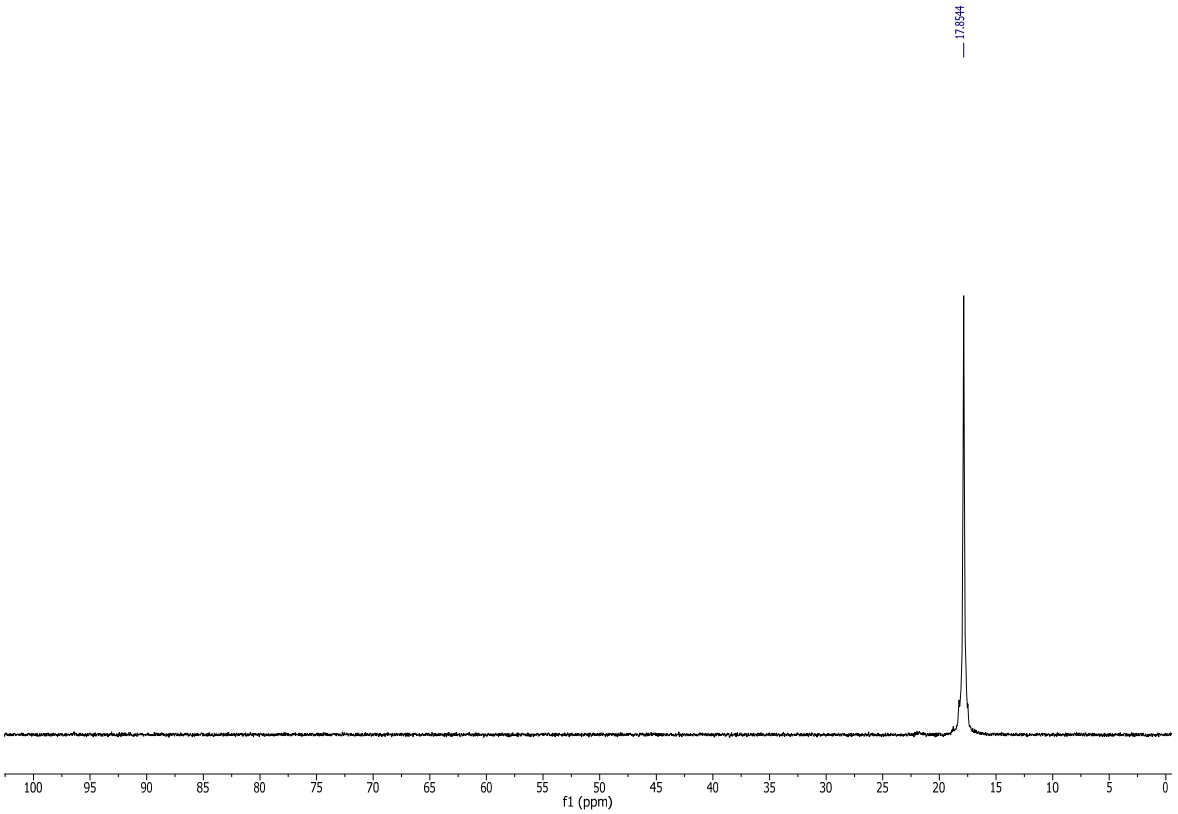
^{1}H NMR ($CDCl_3$)



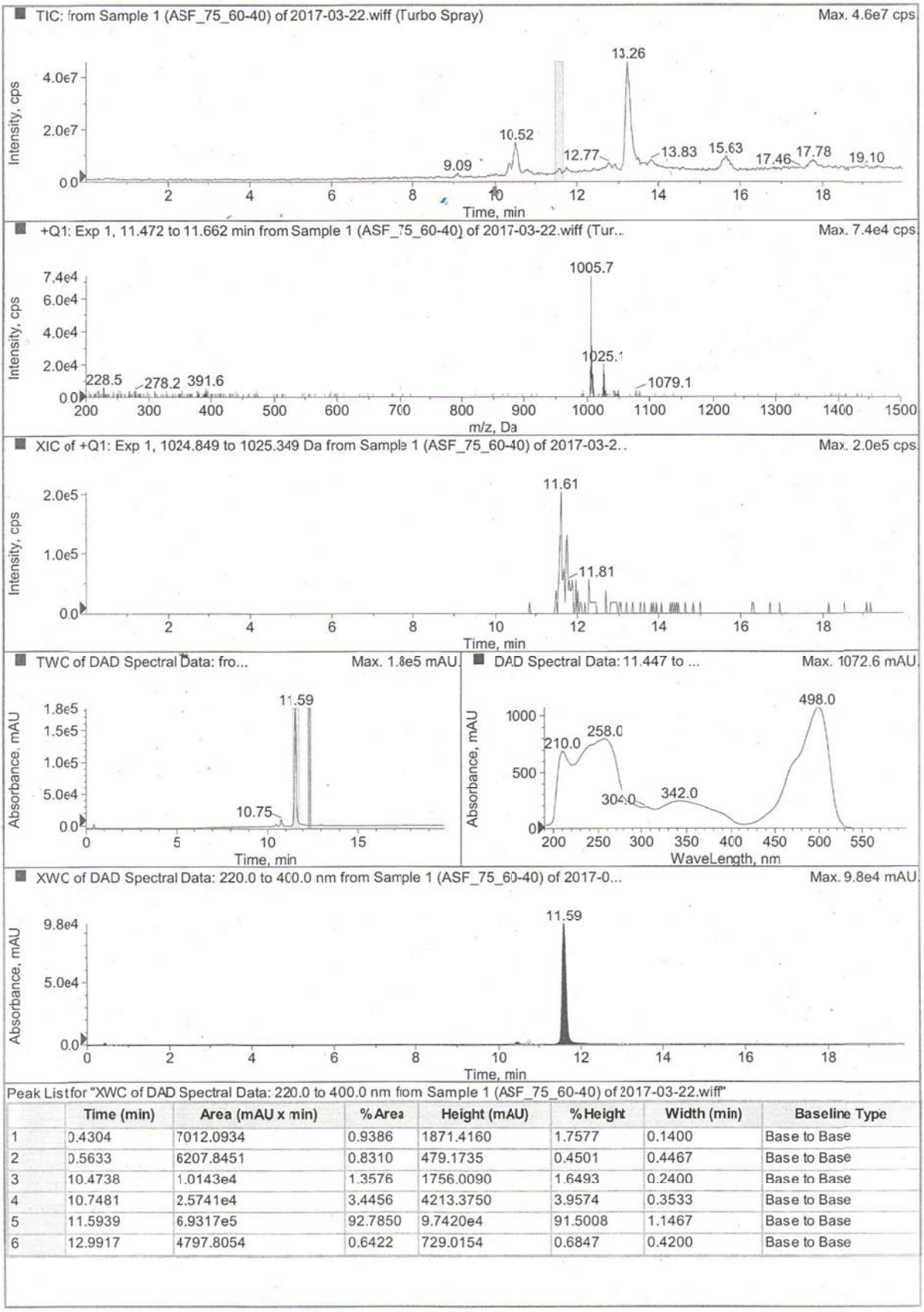
^{13}C NMR



^{31}P NMR



LC/MS analysis of compound **6**



Peak List for "XWC of DAD Spectral Data: 220.0 to 400.0 nm from Sample 1 (ASF_75_60-40) of 2017-03-22.wiff"

	Time (min)	Area (mAU x min)	%Area	Height (mAU)	%Height	Width (min)	Baseline Type
1	0.4304	7012.0934	0.9386	1871.4160	1.7577	0.1400	Base to Base
2	0.5633	6207.8451	0.8310	479.1735	0.4501	0.4467	Base to Base
3	10.4738	1.0143e4	1.3576	1756.0090	1.6493	0.2400	Base to Base
4	10.7481	2.5741e4	3.4456	4213.3750	3.9574	0.3533	Base to Base
5	11.5939	6.9317e5	92.7850	9.7420e4	91.5008	1.1467	Base to Base
6	12.9917	4797.8054	0.6422	729.0154	0.6847	0.4200	Base to Base