Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

A structure-activity relationship study on multi-heterocyclic molecules: two linked thiazoles are required for cytotoxic activity

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Supporting Information Available

EXPERIMENTAL SECTION

GENERAL INFORMATION

All moisture- and air-sensitive reactions were carried out with dry solvents under argon. Silica gel 60 (230-400 mesh) was used for thin-layer chromatography (TLC), and flash chromatography. Reactions were monitored by LCMS or on TLC using UV light as visualizing method and $KMnO_4$, Bromocresol Green, and Ninhydrin as developing agents. ^{1}H and ^{13}C NMR spectra were recorded with instruments operating at 300MHz and 400MHz, respectively. Accurate mass spectra for the novel molecules were recorded high-resolution mass spectrometer, equipped with a conventional ESI source.

GENERAL PROCEDURES

Peptide Synthesis Procedure

To a stirred solution of Boc-protected free amino acid (1.0 eq) in CH_2Cl_2 (0.1 M) was added free amine amino ester (1.2 eq), DIPEA (4.0 eq), and coupling reagent (1.2 eq). The reaction mixture was stirred at room temperature for 45 min. Upon completion, the reaction was washed with aqueous HCl solution (pH = 1), and saturated aqueous $NaHCO_3$ solution. The collected organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The obtained crude residue was purified by flash column chromatography (silica gel, EtOAc/hexanes) to yield the desired dipeptide compound.

Amine Deprotection Procedure

The Boc-protected peptide was diluted to a 0.1 M concentration of 25% TFA and 75% CH_2Cl_2 (50% TFA and 50% CH_2Cl_2 for simultaneous Boc and *t*-Bu removal). Then 2 equivalents of anisole were added to the solution, followed by the addition of TFA. The reaction was run at room temperature and monitored via TLC every 15 minutes. Upon completion, the solution was concentrated *in vacuo* to yield the free amine peptide in quantitative yield.

Acid Deprotection Procedure

The methyl ester was diluted to a 0.1 M solution in MeOH/CH_2Cl_2 (1:1), followed by the addition of 8 equivalents of LiOH. The reaction was run at room temperature for 8 h. Upon completion, the reaction mixture was quenched with pH =1 aqueous solution and extracted with CH_2Cl_2. The collected organic layer was dried, and concentrated to afford the acid.

Thioamide Synthesis Procedure

The ester (methyl or ethyl) was diluted to a 0.1 M solution of MeOH/NH_4OH (1:1~1:3). The ester solution was stirred overnight for amide conversion. The solvent was removed *in vacuo* to afford the amide, which is diluted to a 0.05 M solution. The amide solution was added 0.75 equivalent of Lawesson's reagent for thioamideconversion. The solvent was then removed and the residue was purified by flash column chromatography to afford the thioamide.

Thiazole Synthesis Procedure

The thioamide was diluted to a 0.05 M solution of 1,2-dimethoxyethane (DME), followed by the addition of 8.0 equivalents of $KHCO_3$ and 3.0 equivalents of ethyl bromopyruvate. The reaction mixture was stirred for 16 h at room temperature. The solvent was removed and the residue was partitioned between water and chloroform. The collected organic layer was further washed with brine, driedand evaporated. The crude thiazoline residue was re-dissolved in DME (0.05M) and cooled to 0°C. Pyridine (9.0 equivalents) was slowly added, followed by drop-wise addition of TFAA (4.0 equivalents). After stirring at 0°C for 3 h, the reaction mixture was allowed to warm to room temperature, followed by slowly addition of TEA (2.0 equivalents) and the reaction was stirred for 1 h. The solvent was then evaporated and the residue was dissolved in chloroform, washed with pH =1 solution, saturated aqueous $NaHCO_3$ solution, dried and concentrated. The residue was purified by flash column chromatography to

yield the thiazole. Note: Thiazoletrifluoroacetate may be observed after the reaction is completed. Exposure of the trifluoroacetate to 1.2 equivalents of NaOEt in 0.05 M ethanol solution at 0°C for 1 h gave the desired thiazole ethyl ester.

Hydrogenolysis Procedure

The benzylated serine was dissolved in ethanol (0.1M), followed by addition of catalytic amount of 10% Pd/C. The solution was purged with H_2 and stirred overnight. Upon completion the reaction was filter through celite, rinsed with CH_2Cl_2 and concentrated to yield desired molecule.

Oxazole Synthesis Procedure

A) Oxazoline formation

1.1 equivalents of DASTwere added drop-wise to the serineinCH_2Cl_2(0.1M) at -78ºC under Ar. The reaction mixture was stirred for 1 h, followed by the addition of 2 equivalents of K_2CO_3 (use pyridine for the synthesis of phenyloxazole) and stirred for an additional 30min. The solution was then allowed to warm to room temperature. Upon completion, the organic solution was partitioned between saturated aqueous $NaHCO_3$ and CH_2Cl_2. The collected organic layer was dried and concentrated to give the oxazoline, which was subjected to oxidation without further purification.

B) Oxidation

2 equivalents of DBU were added drop-wise to a solution of oxazoline in CH_2Cl_2 at -46ºC and the solution was stirred for 15 min. 2 equivalents of $BrCCl_3$ were then added to the reaction mixture, which was allowed to proceed over 12 h. The reaction was worked up using the same method in peptide synthesis procedure and then purified by flash column chromatography to yield the oxazole product.

Boc-Thr(O^{t}Bu)-Oxazole-OMe (1)[1]

The dipeptide intermediate, Boc-Thr(O^{t}Bu)-Ser(Bn)-OMe,was synthesized using 1.2g of Boc-Thr(O^{t}Bu)-OH,1 g of free amine NH_2-Ser(Bn)-OMe, 3.02 mL of DIPEA, and 1.53 g of TBTUvia**"Peptide Synthesis Procedure"**.

The oxazole**1** was synthesized using 1.82 g of the dipeptide intermediate and 182 mg of 10% Pd/C in ethanol following **"Hydrogenolysis Procedure"** to remove benzyl ether then subjected to **"OxazoleSynthesis Procedure"** using 0.6 mL of DAST and 884 mg of K_2CO_3 for the first step, 0.97 mL of DBU and 0.63 mL of $BrCCl_3$ for the second step (colorless oil, 1.6 g, 85%).

R_f =0.74 (EtOAc:hexanes, 1:1)

^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.21 (s, 1H), 5.58 (d, *J* = 6.3 Hz, 1H), 4.85 (d, *J* = 6.9 Hz, 1H), 3.98 (s, 3H), 1.61 (s, 9H), 1.48 (d, *J* = 4.8 Hz, 3H), 0.98 ppm (s, 9H). 13C NMR: ($CDCl_3$, 400 MHz) δ = 164.22, 161.97, 155.89, 143.90, 133.87, 79.92, 74.35, 68.22, 55.34, 52.13, 28.81, 28.78, 20.13ppm ; HRMS(ESI): calcd for $C_{17}H_{28}N_2O_6Na^+$ [M + Na^+] 379.1845,found379.1832.

[1]D.Hernandez, M. Altuna, C. Cuevas, R.Aligue, F.Albericio, M. Alvarez,*J. Med. Chem*., 2008, **51**, 5722-5730

Boc-Thr(O^{t}Bu)-Dioxazole-OMe (2)[1]

The Boc-Thr(O^{t}Bu)-Oxazole-OH intermediate was synthesized using 1.6 g of **1**and 1.54 g of LiOH following **"Acid Deprotection Procedure"**. The obtained free acid was then coupled with 940 mg of NH_2-Ser(Bn)-OMe using 0.86 mL of DIPEA, and 1.6 g of TBTU following **"Peptide Synthesis Procedure"**to yield Boc-Thr(O^{t}Bu)-Oxazole-Ser(Bn)-OMe.

The dioxazole**2** was synthesized using 2.27 g of Boc-Thr(O^{t}Bu)-Oxazole-Ser(Bn)-OMeand 227 mg of 10% Pd/C following **"Hydrogenolysis Procedure"** to remove benzyl protecting group then subjected to **"OxazoleSynthesis Procedure"** using 1.5 mL of DAST and 714 mg of K_2CO_3 for the first step, 0.8 mL ofDBU and 0.5 mL of $BrCCl_3$ for the second step (colorless oil, 1.8 g, 85%).

R_f =0.43 (EtOAc:hexanes, 1:1).

^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.23 (d, *J* = 2.4 Hz, 2H), 5.54 (d, *J* =6.3 Hz, 1H), 4.87 (d, *J* = 6.9 Hz, 1H), 4.10 (d, *J* = 2.4 Hz, 1H), 3.87 (s, 3H), 1.39 (s, 9H), 1.21 (s, 3H), 0.97 ppm (s, 9H).^{13}C NMR: ($CDCl_3$, 400 MHz) δ = 165.03, 161.29, 155.76, 155.67, 143.65, 139.25, 134.29, 129.81, 80.03, 74.38, 68.60, 54.99, 52.22, 28.27, 28.00, 20.23ppm ; HRMS(ESI): calcd for $C_{20}H_{29}N_3O_7Na^+$ [M + Na^+] 446.1904,found446.1891.

[1]D.Hernandez, M. Altuna, C. Cuevas, R.Aligue, F.Albericio, M. Alvarez,*J. Med. Chem*., 2008, **51**, 5722-5730

Boc-Thr(O^{t}Bu)-Dioxazole-Thiazole-OEt (5)

The thioamideintermediateProtected Dioxazole-Thioamidewassynthesizedfollowing**"ThioamideSynthesis Procedure"**using 973.8 mg of**2**and 960 mg ofLawesson'sreagent.

5 was synthesized following **"Thiazole Synthesis Procedure"** using 960 mg of Protected Dioxazole-Thioamide, 1.4 g of $KHCO_3$, and 0.7 mL of ethyl bromopyruvate for the first step; 1.0 mL of TFAA, 0.9 mL of pyridine, and 0.5 mL of TEA for the second step. (yellow oil,810 mg, 69%).

R_f = 0.53 (EtOAc: hexanes, 1:2).

^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.36 (s, 1H), 8.20 (s, 1H), 8.17 (s, 1H), 5.56 (d, *J* = 6.9 Hz, 1H), 4.87 (d, *J* = 6.9 Hz, 1H), 4.38 (q, *J* = 19.2 Hz, 2H), 4.15 (d, *J* = 2.4 Hz, 1H), 1.41 (s, 9H), 1.38 (s,3H), 1.18 (s, 3 H), 0.97 ppm (s, 9H).^{13}C NMR: ($CDCl_3$, 300 MHz) δ = 139.05, 136.42, 127.11, 68.71, 61.62, 54.87, 28.43, 28.05, 20.33, 14.02ppm ; HRMS(ESI): calcd for $C_{24}H_{32}N_4O_7SNa^+$ [M + Na^+] 543.1889,found543.1876.

Boc-Thr(O^{t}Bu)-Dioxazole-Dithiazole-Phenyloxazole-OMe (6)

The protected dioxazole thioamideintermediatewas synthesized following **"ThioamideSynthesis Procedure"** using 810 mg of **5** and 520 mg of Lawesson's reagent.

6was synthesized following the "**Thiazole Synthesis Procedure**" utilizing 560 mg of protecteddioxazolethioamide, 880 mg of $KHCO_3$, and 1.07 g of bromoketophenyloxazole-OMe[1] for the first step; 0.6 mL of pyridine, 0.6 mL of TFAA and 0.3 mLof TEA for thesecondstep. (yellowoil, 560 mg, 69%).

R_f: 0.26 (EtOAc:hexanes, 1:3)

^{1}H NMR: ($CDCl_3$, 300 MHz) δ = 8.31 (s, 1H), 8.22 (s, 1H), 8.19(s, 1H), 8.15-8.11 (m, 3H), 7.51-7.39 (m, 3H), 5.59 (d, J = 6.9 Hz, 1H), 4.86 (d, J = 6.9 Hz, 1H), 4.15 (d, J = 2.4 Hz, 1H), 3.91(s, 3H), 1.40 (s, 9H), 1.20 (s, 3 H), 0.98 ppm (s, 9H). ^{13}C NMR: ($CDCl_3$, 300 MHz) δ = 68.61, 61.60, 28.44, 28.02, 20.56 ppm ; HRMS(ESI): calcd for $C_{35}H_{36}N_6O_8S_2Na^+$ [M + Na^+] 755.1934, found 755.1920.

[1]C-M.Pan, C-C. Lin, S. J. Kim, R. P. Sellers and S. R. McAlpine, Tetrahedron Lett., 2012, 53, 4065-4069

Boc-Thr(O^{t}Bu)-Trioxazole-OMe (3)

The intermediateprotected dioxazole-Ser(Bn)-OMewas synthesized using 889 mg of **2**and 700 mg of LiOH following **"Acid Deprotection Procedure"**. The obtained free acid was then coupled with 430 mg of NH_2-Ser(Bn)-OMe using 1.3 mL of DIPEA, and 662 mg of TBTU following **"Peptide Synthesis Procedure"** The trioxazole**3** was synthesized using 1.03 g of protected dioxazole-Ser(Bn)-OMe and 103 mg of 10% Pd/C following **"Hydrogenolysis Procedure"** to remove benzyl ether then subjected to **"OxazoleSynthesis Procedure"** using 0.6 mL of DAST and 677 mg of K_2CO_3 for the first step, 0.5 mL of DBU and 0.3 mL of $BrCCl_3$ for the second step (colorless oil, 578mg, 70%).

R_f =0.4 (EtOAc:hexanes, 1:1).

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.42 (s, 1H), 8.31 (s, 1H), 8.30 (s, 1H), 5.60 (d, J = 9.3 Hz, 1H), 4.90 (d, J = 9.3 Hz, 1H), 4.19 (q, J =6.9 Hz, 1H), 3.95 (s, 3H), 1.47 (s, 9H) 1.27 (dd, J = 6.0, 1.2 Hz, 3H), 0.98 ppm (s, 9H).HRMS(ESI): calcd for $C_{23}H_{30}N_4O_8Na^+$ [M + Na^+] 513.1962,found513.1951.

Boc-Thr(O^{t}Bu)-Trioxazole-Thiazole-Phenyloxazole-OMe (4)

The intermediate protected trioxazole-thioamidewassynthesizedfollowing**"ThioamideSynthesis Procedure"**using 538 mg of**3**and 408 mg ofLawesson'sreagent. **4**was synthesized following the "**Thiazole Synthesis Procedure**" utilizing 272 mg of protected trioxazole thioamide, 596 mg of $KHCO_3$, and 0.6 g of bromoketophenyloxazole-OMe[1] for the first step; 0.4 mL of pyridine, 0.3 mL of TFAA and 0.2 mL of TEA for the second step (yellow oil, 278 mg, 72%).

R_f: 0.3 (EtOAc:hexanes, 1:1)

^{1}H NMR: ($CDCl_3$, 400 MHz) δ= 8.50 (s, 1H), 8.40 (s, 1H), 8.38 (s, 1H), 8.23 (s, 1H), 8.21-8.17 (m, 2H), 7.55-7.47 (m, 3H), 5.62 (d, *J* =9.8 Hz, 1H), 4.92 (d, *J* = 8.9 Hz, 1H), 4.20 (q, *J* = 7.0 Hz, 1H), 3.99 (s, 3H), 1.42 (s, 9H), 1.21 (d, *J* = 6.4 Hz, 3H), 0.98 ppm (s, 9H) ; HRMS(ESI): calcd for $C_{35}H_{37}N_6O_9SH^+$ [M + 1] 717.2343,found717.1958.

[1]C-M.Pan, C-C. Lin, S. J. Kim, R. P. Sellers and S. R. McAlpine, Tetrahedron Lett., 2012, 53, 4065-4069

Boc-Thr(O^{t}Bu)-Thiazole-OEt (7)

To a stirredsolutionofacid Boc-Thr(O^{t}Bu)-OH (1.5 g, 5.45 mmol) in benzene (40.8 mL) and MeOH (13.6 mL) was added TMSD (2M solution in diethyl ether) drop-wise until slightly yellow. The mixture was stirred at room temperature for 1 h. The solvent was removed and the residue was co-evaporated with CH_2Cl_2 (100 mL × 5). The obtained crude ester wassubjecttothe"**Thioamide Synthesis Procedure**" to yield the thioamide intermediate Boc-Thr(O^{t}Bu)-thioamide.The thiazole**7**was synthesized following the "**Thiazole Synthesis Procedure**" using 832 mg of thioamideBoc-Thr(O^{t}Bu)-thioamide, 2.3g of $KHCO_3$, and 1.2 mL of ethyl bromopyruvate for the first step; 2.1 mL of pyridine, 1.6 mL of TFAA and 0.8 mL of TEA for the second step. (yellow oil, 860 mg, 78%).

R_f = 0.37 (EtOAc: hexanes, 0.2:0.8)

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.05 (s, 1H), 5.80 (d, *J* =7.9 Hz, 1H), 4.95 (d, *J* =7.9 Hz, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.34 (d, *J* = 5.6 Hz, 1H), 1.48 (s, 9H), 1.39 (t, *J* = 6.9 Hz, 3H), 1.19 (d, *J* = 6.4 Hz, 3H), 0.96 ppm (s, 9H).HRMS(ESI): calcd for $C_{18}H_{30}N_2O_5SNa^+$ [M + Na^+] 409.1773,found409.1760.

Boc-Thr(O^{t}Bu)-Dithiazole-OEt (8)

The thioaimdeintermediate Boc-Thr(OtBu)-thiazole-thioamidewas synthesized following the "**Thioamide Synthesis Procedure**" using867 mg of thiazole**7**and 544 mg of Lawesson's reagent in 50 mL of dry DME at 60°C for 5 h. The thiazole**8**was synthesized following the "**Thiazole Synthesis Procedure**" utilizing 832 mg of Boc-Thr(OtBu)-thiazole-thioamide, 940 mg of $KHCO_3$, and 0.5 mL of ethyl bromopyruvate for the first step; 0.86 mL of pyridine, 0.66 mL of TFAA and 0.33 mL of TEA for the second step. Finally, 86 mg of NaOEt was used to yield the thiazole**8** as yellow oil (545 mg, 97%).

R_f = 0.6 (EtOAc: hexanes, 0.35:0.65)

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.17 (s, 1H), 8.05 (s, 1H), 5.76 (d, *J* =8.0 Hz, 1H), 4.94 (d, *J* =9.2 Hz, 1H), 4.46 (q, *J* = 6.9 Hz, 2H), 4.35 (d, *J* = 6.3 Hz, 1H), 1.54 (s, 9H), 1.44 (t, *J* = 7.5 Hz, 3H), 1.25 (d, *J* = 6.7 Hz, 3H), 0.99 ppm (s, 9H). HRMS(ESI): calcd for $C_{21}H_{31}N_3O_5S_2Na^+$ [M + Na^+] 492.1603, found 492.1588.

Boc-Thr(O^{t}Bu)-Trithiazole-OEt (9)

The thioaimdeBoc-Thr(OtBu)-dithiazole-thioamidewas synthesized following the"**Thioamide Synthesis Procedure**" utilizing 538 mg of thiazole**8**and 320 mg of Lawesson's reagent in 46 mL of dry benzeneat 60°C for 5 h. The thiazole**9**was synthesized following the "**Thiazole Synthesis Procedure**" utilizing 382 mg of thioamide**CCL-05**, 670 mg of $KHCO_3$, and 0.35 mL of ethyl bromopyruvate for the first step; 0.61 mL of pyridine, 0.47 mL of TFAA and 0.24 mL of TEA for the second step. Finally, 68 mg of NaOEt was used to yield the desired thiazole**9** as yellow oil (400 mg, 87%).

R_f = 0.5 (EtOAc: hexanes, 0.35:0.65)

^{1}H NMR: ($CDCl_3$, 400 MHz) δ = 8.20 (s, 1H), 8.16 (s, 1H), 7.97 (s, 1H), 5.78 (d, *J* =7.9 Hz, 1H), 4.96 (d, *J* =7.9 Hz, 1H), 4.47 (q, *J* = 7.4 Hz, 2H), 4.38 (d, *J* = 4.6 Hz, 1H), 1.52 (s, 9H), 1.45 (t, *J* = 7.3 Hz, 3H), 1.26 (d, *J* = 7.0 Hz, 3H), 1.01 ppm (s, 9H).HRMS(ESI): calcd for $C_{24}H_{32}N_4O_5S_3Na^+$ [M + Na^+] 575.1433,found575.1416.

Boc-Thr(O^{t}Bu)-Tetrathiazole-Phenyloxazole-OMe (10)

The Boc-Thr(OtBu)-trithiazole-thioamidewas synthesized following the"**Thioamide Synthesis Procedure**" utilizing 400 mg of thiazole**9**and 250 mg of Lawesson's reagent in 30 mL of dry benzenerefluxing for 5 h. **10**was synthesized following the "**Thiazole Synthesis Procedure**" utilizing 286 mg of Boc-Thr(OtBu)-trithiazole-thioamide, 424 mg of $KHCO_3$, and 528 mg of bromoketophenyloxazole-OMe[1] for the first step; 0.4 mL of pyridine, 0.3 mL of TFAA and 0.2 mL of TEA for the second step (yellow oil, 360 mg, 88%).

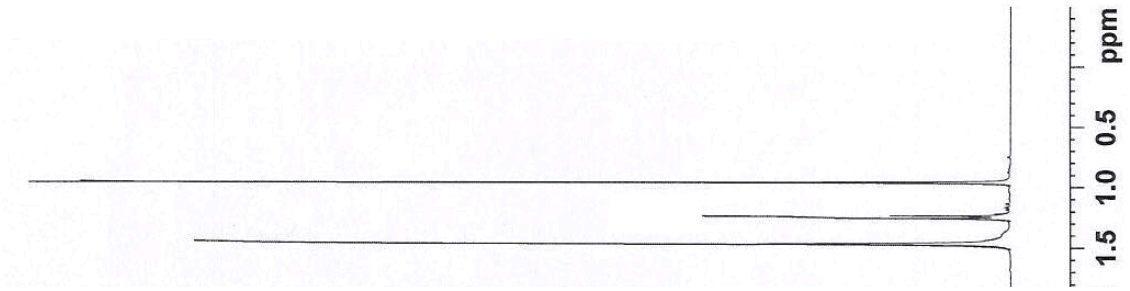
R_f: 0.25 (EtOAc:hexanes, 2:3)

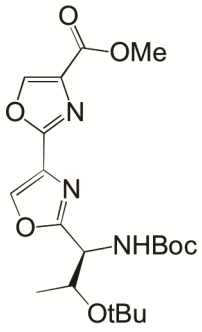
[1]C-M. Pan, C-C. Lin, S. J. Kim, R. P. Sellers and S. R. McAlpine, Tetrahedron Lett., 2012, 53, 4065-4069

NMR Spectra of Compounds

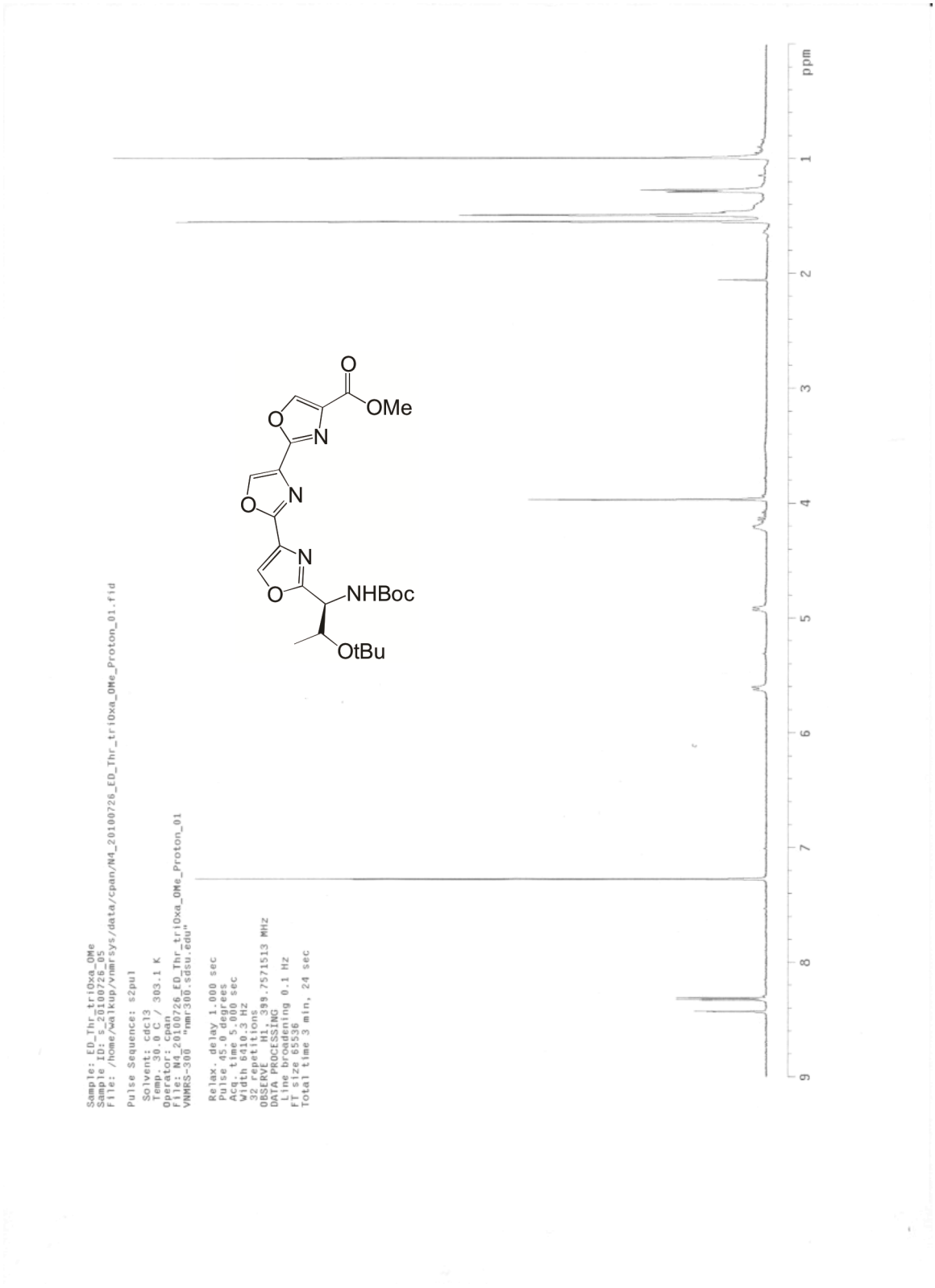
^{1}H NMR Spectra

Boc-Thr(O^{t}Bu)-Oxazole-OMe (**1**)

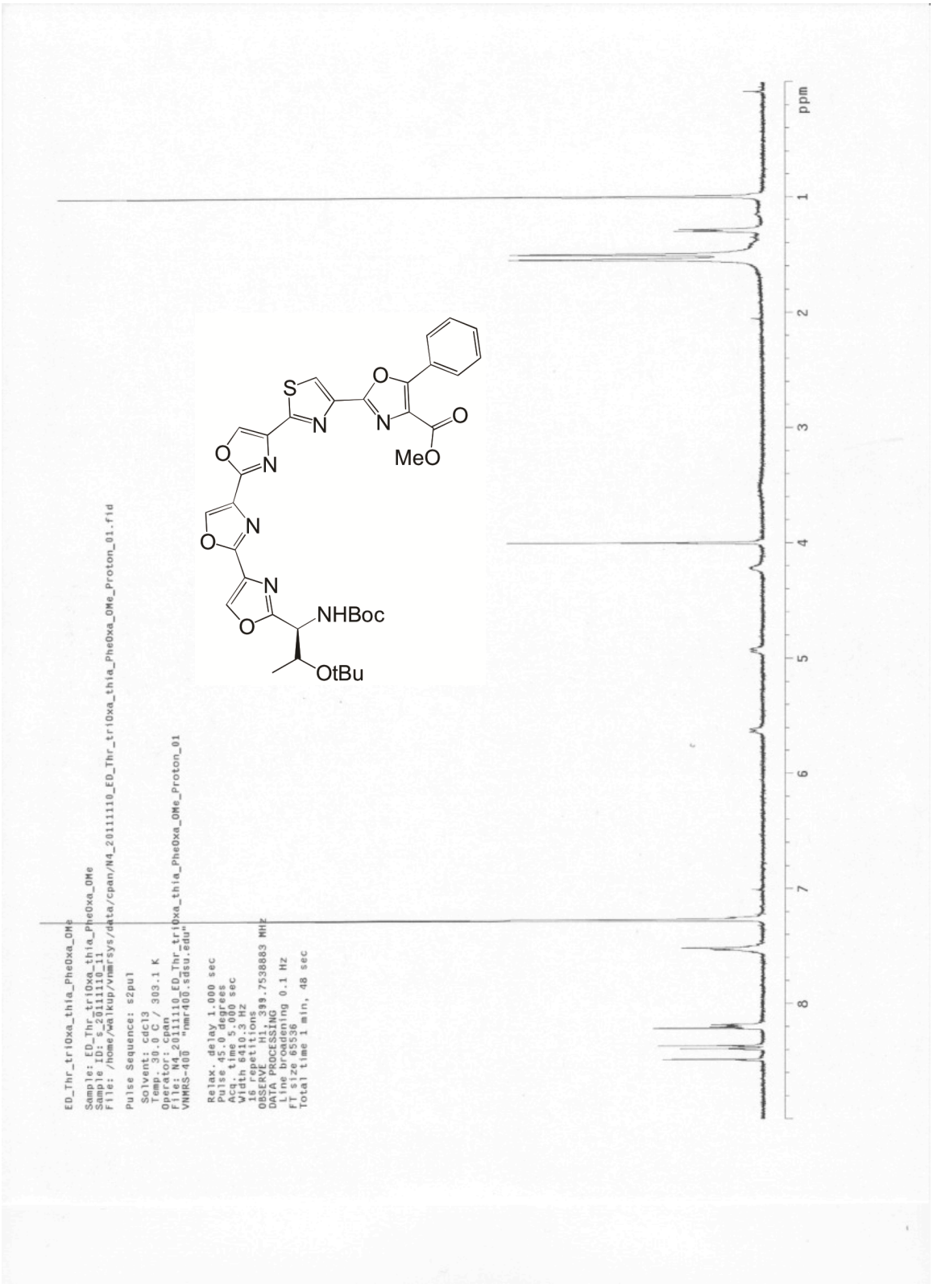




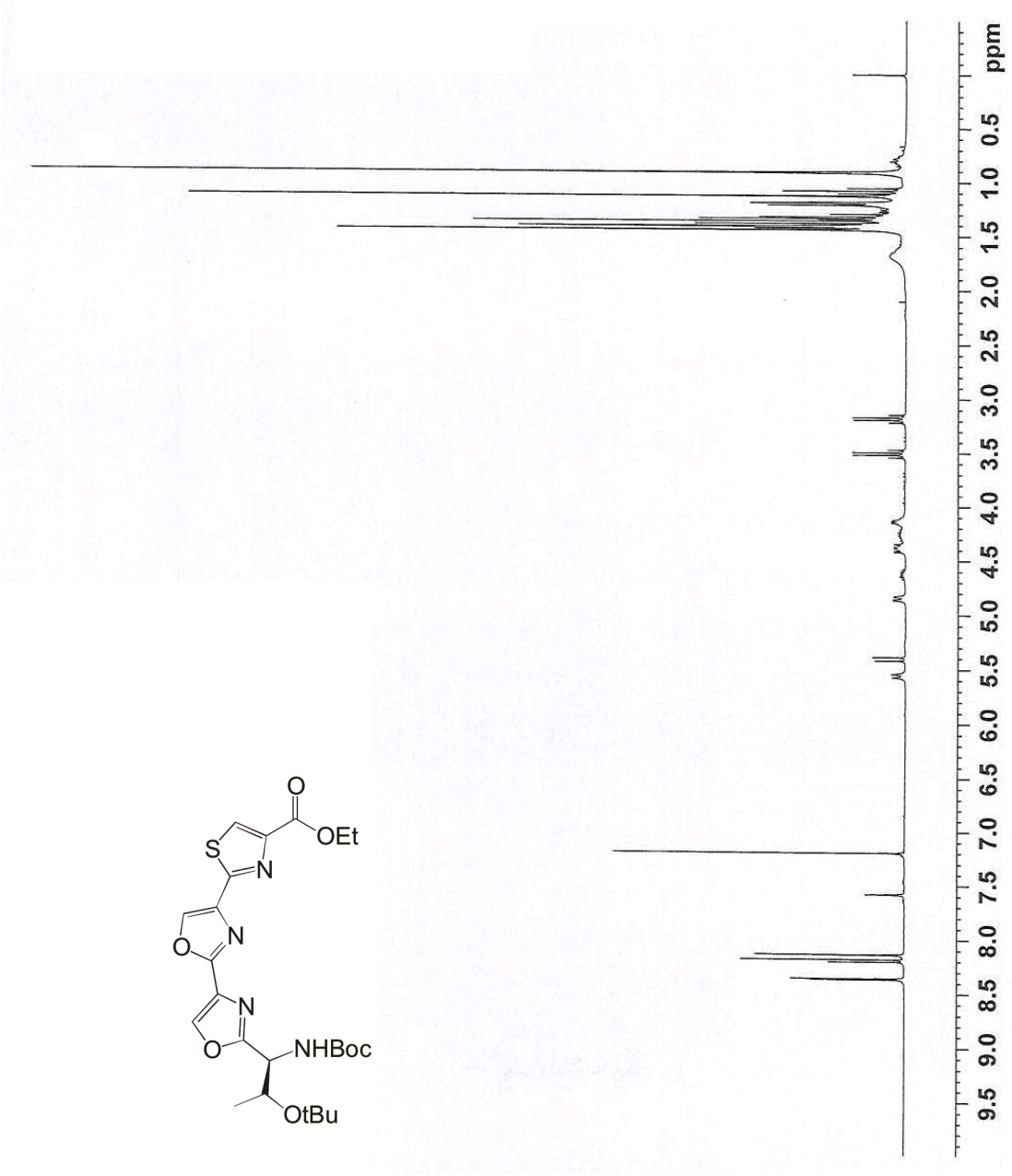
Boc-Thr(O^{t}Bu)-Dioxazole-OMe (**2**)



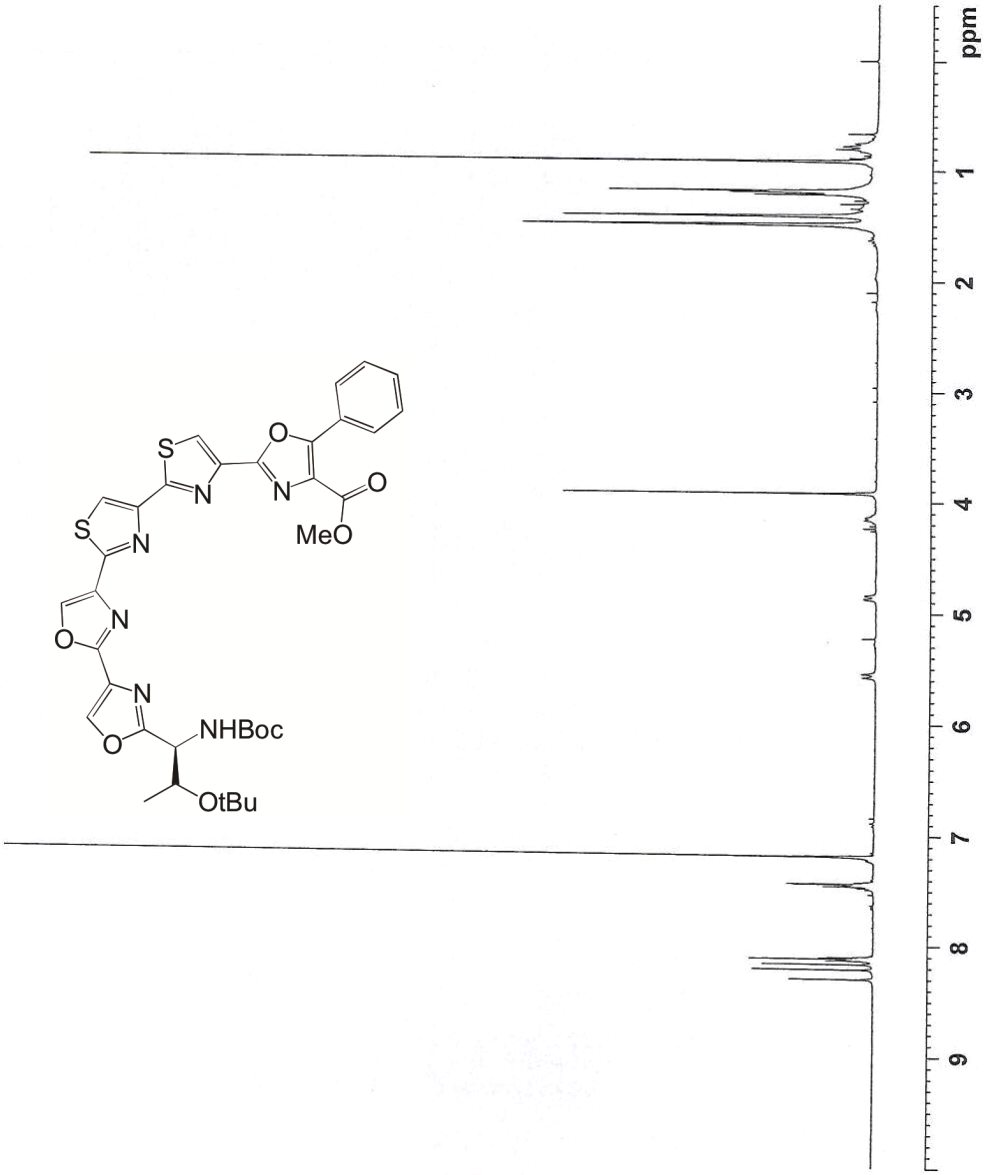
Boc-Thr(O^{t}Bu)-Trioxazole-OMe (**3**)



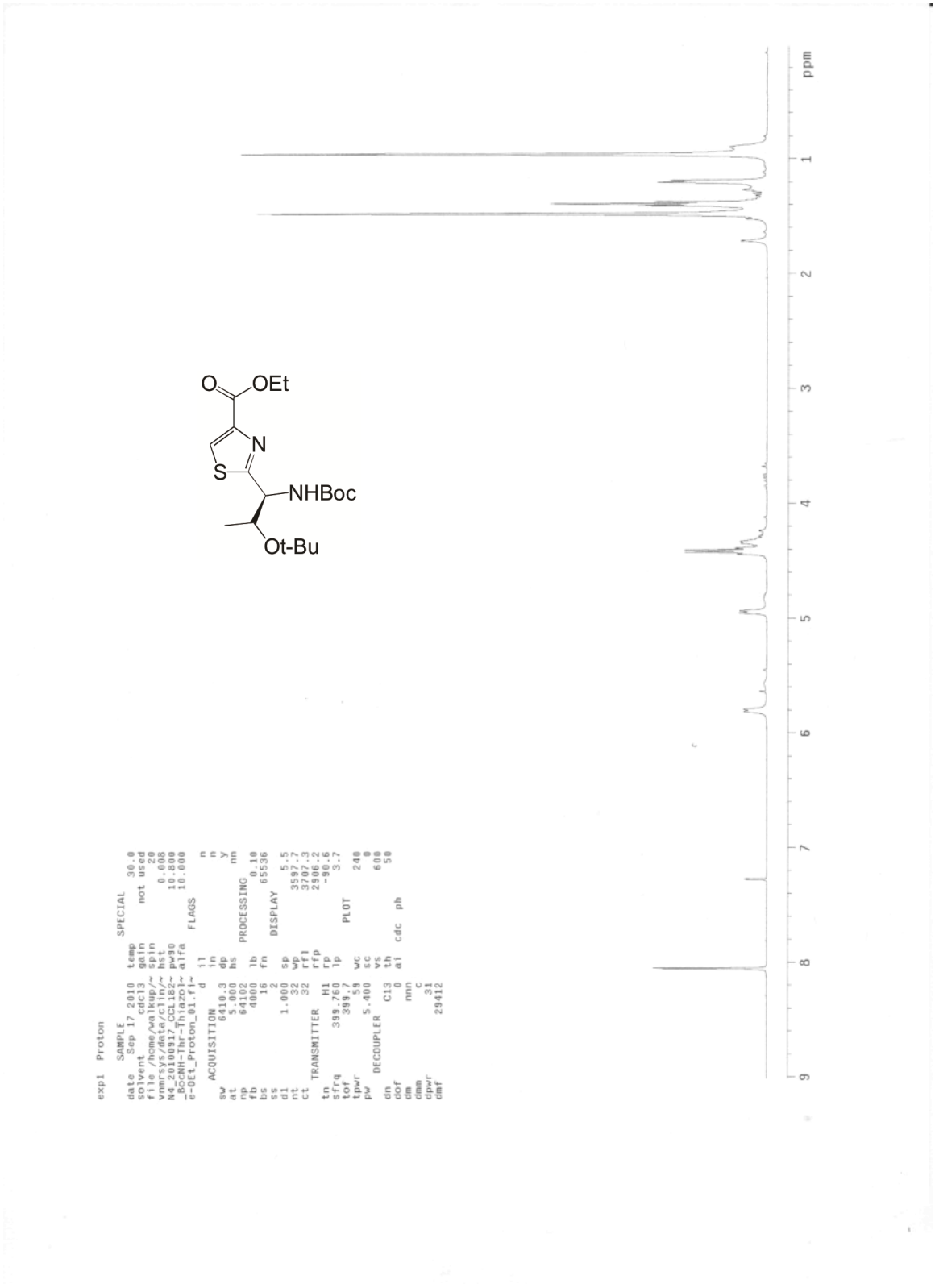
Boc-Thr(O^{t}Bu)-Trioxazole-Thiazole-Phenyloxazole-OMe (**4**)



Boc-Thr(O^{t}Bu)-Dioxazole-Thiazole-OEt (**5**)



Boc-Thr(O^{t}Bu)-Dioxazole-Dithiazole-Phenyloxazole-OMe (**6**)

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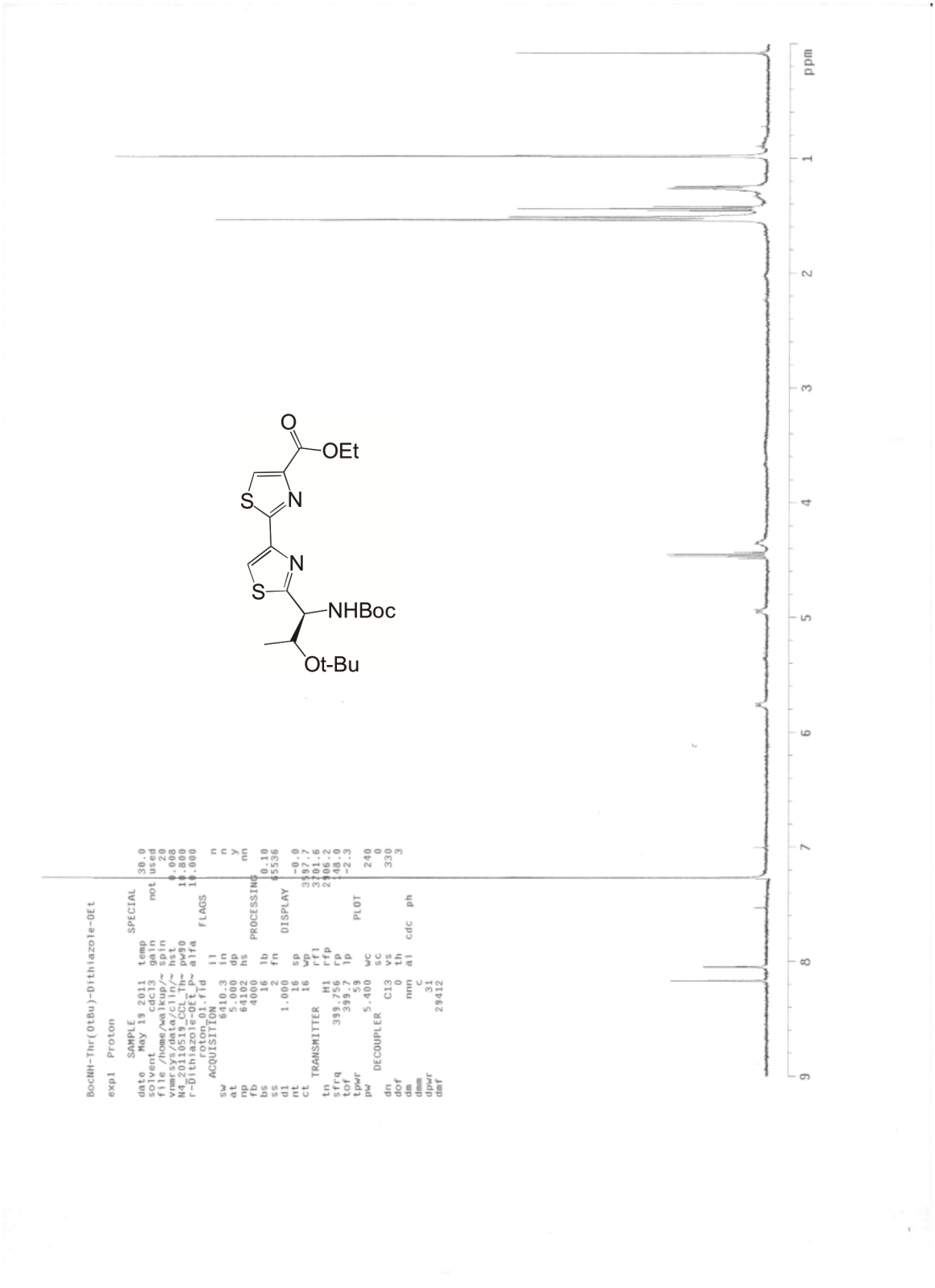
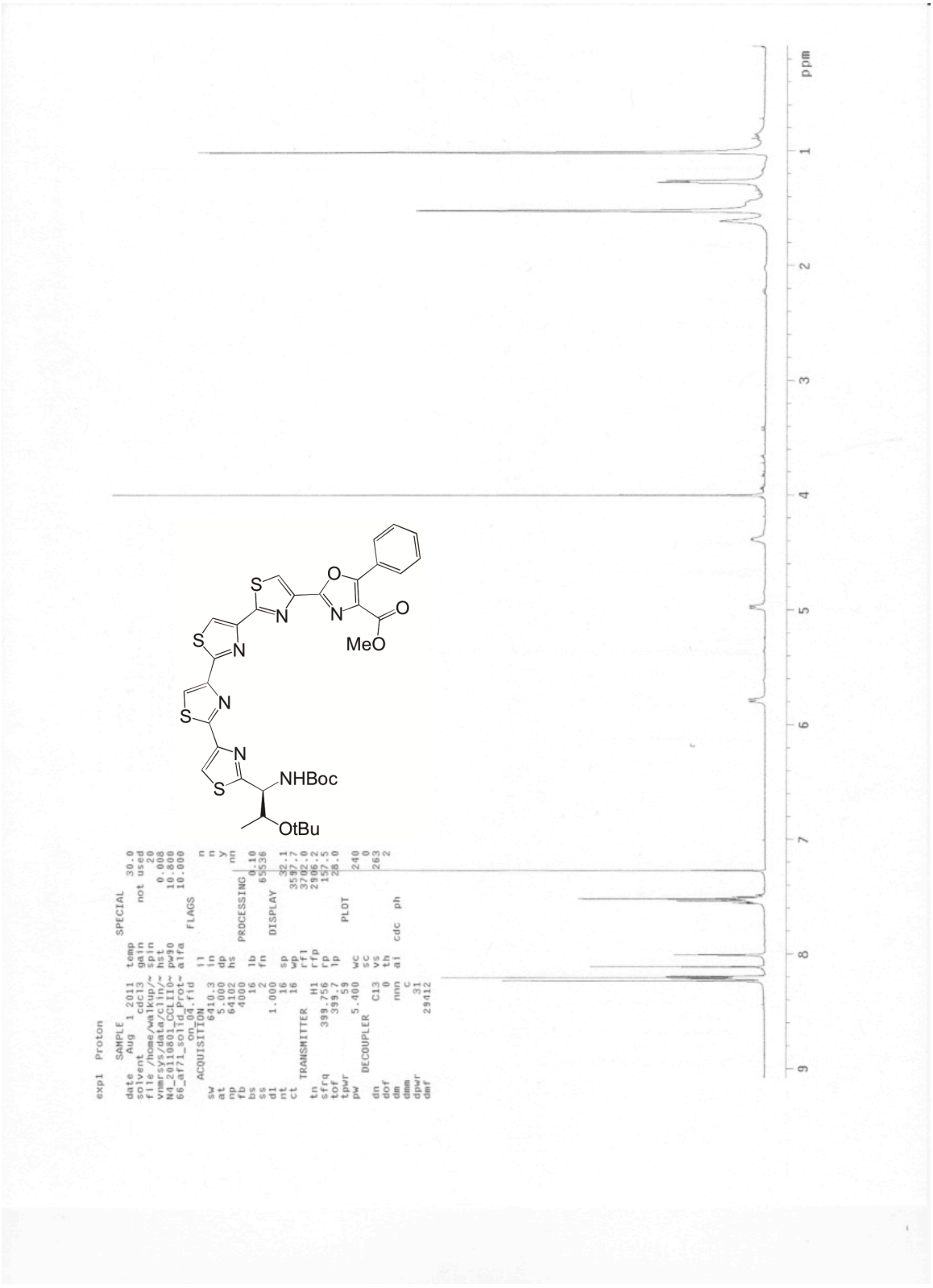
Boc-Thr(O^{t}Bu)-Thiazole-OEt (**7**)



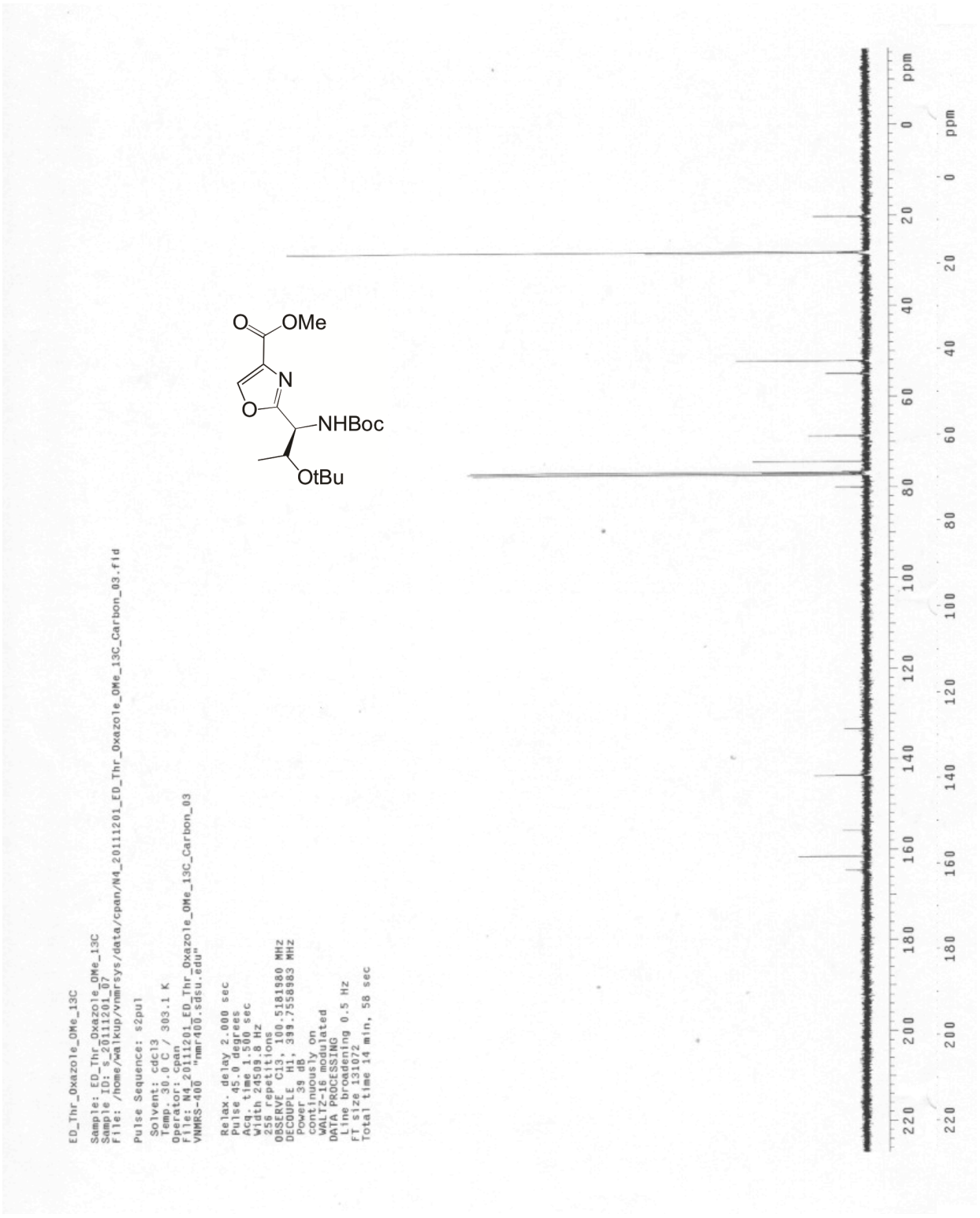
Boc-Thr(O^{t}Bu)-Dithiazole-OEt (**8**)

Boc-Thr(O^{t}Bu)-Trithiazole-OEt (**9**)

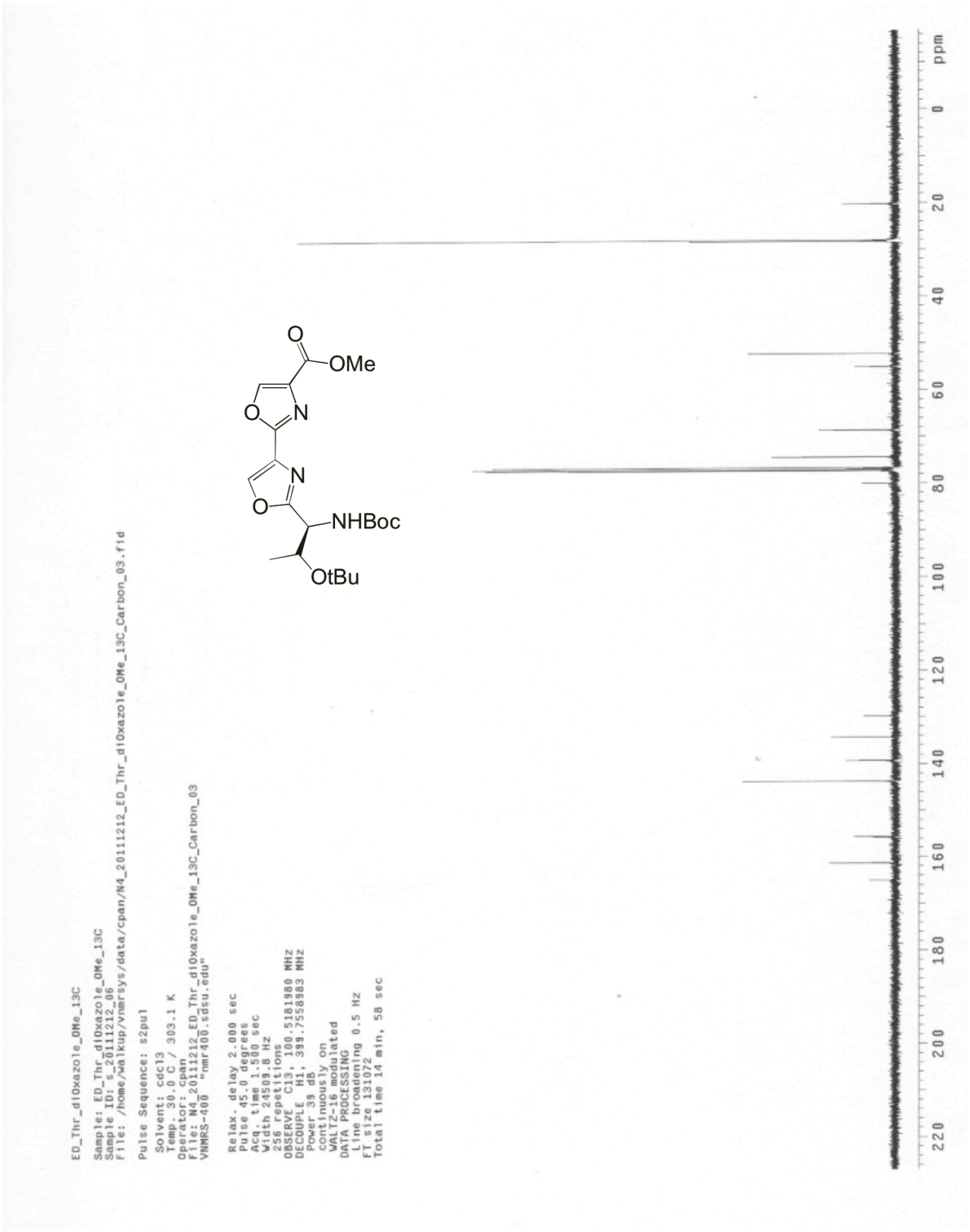


Boc-Thr(O^{t}Bu)-Tetrathiazole-Phenyloxazole-OMe (**10**)

^{13}C NMR Spectra



Boc-Thr(O^{t}Bu)-Oxazole-OMe (**1**)



Boc-Thr(O^{t}Bu)-Dioxazole-OMe (**2**)

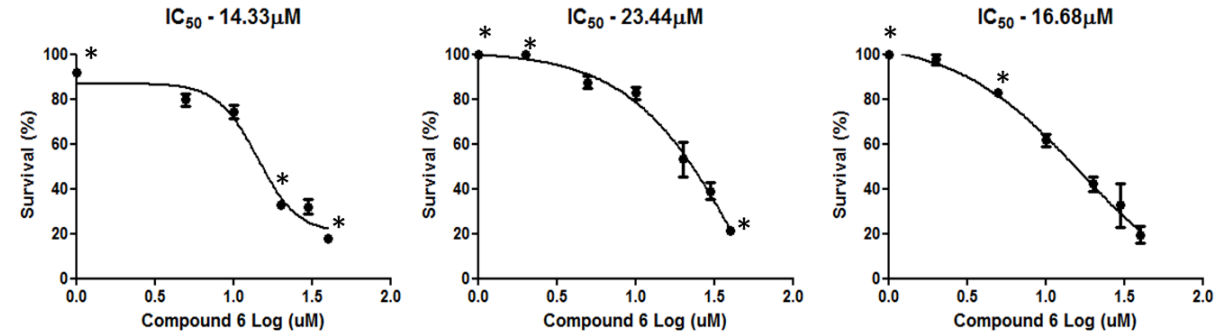
Methods: Cytotoxicity

Cytotoxicity of the compounds against human colon cancer cell line HCT-116 cells was determined using Cell Counting Kit-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] reduction assay.

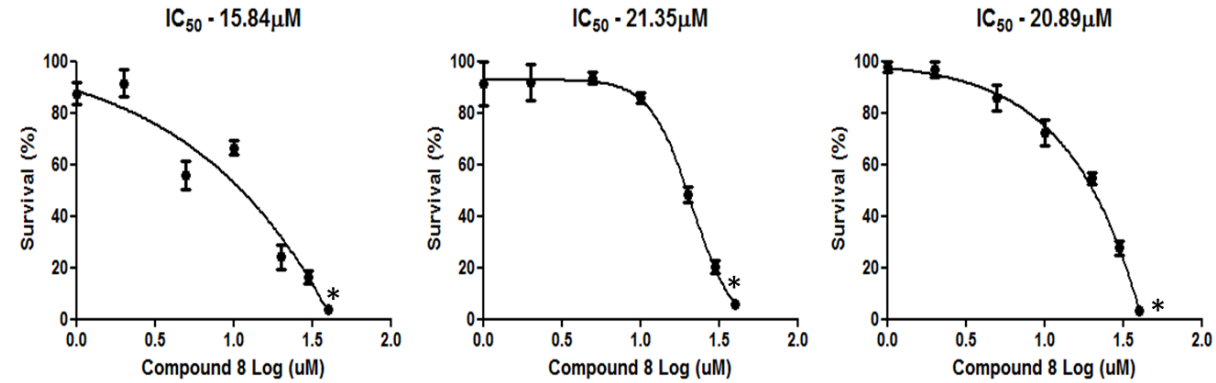
HCT-116 cells (3000 cells/well) in Dulbecco's modified eagle's medium (DMEM; Life Technologies) with 10% fetal bovine serum were seeded in 96 well tissue culture dish. The cells were allowed to adhere for 24 hoursin CO_2 incubator at 37^0C before being treated for 72 hours with compounds 1-10 at 40uM concentration. "Media only" lanes and media with 1% DMSO were used as controls. Post treatment the media was aspirated off and 90µl of fresh serum free media was added to each well. 10µl of room temperature CCK8 reagent (Dojindo Molecular Technologies) was added per well, and the plate was covered in aluminium foil and incubated for 1 hour in CO_2 incubator at 37^0C. The cell viability was determined colorimetrically using Chromate Microplate Reader (Awareness Technology) at 450nm, and development of an orange-color from reduction of the formazan dye was directly proportional to the number of living cells. Graphs were plotted for log of the concentrations of the compound versus percentage cell survival at 40uM concentration using the GraphPad Prism software.

IC_{50} values were determined for compounds that showed 70% or more growth inhibition. The same methodology as mentioned above was applied for treating the cells for 72 hours with compound **6**, **8**, **9** and **10** at 1µM, 2µM, 5µM, 10µM, 20 µM, 30µM and 40µM concentrations. For each compound, the data was analyzed and IC_{50} values were determined by plotting curves for curves for log of the concentrations of the compound versus percentage cell survival using GraphPad Prism software. The experiments were repeated three times with each data point performed in quadruplicate.

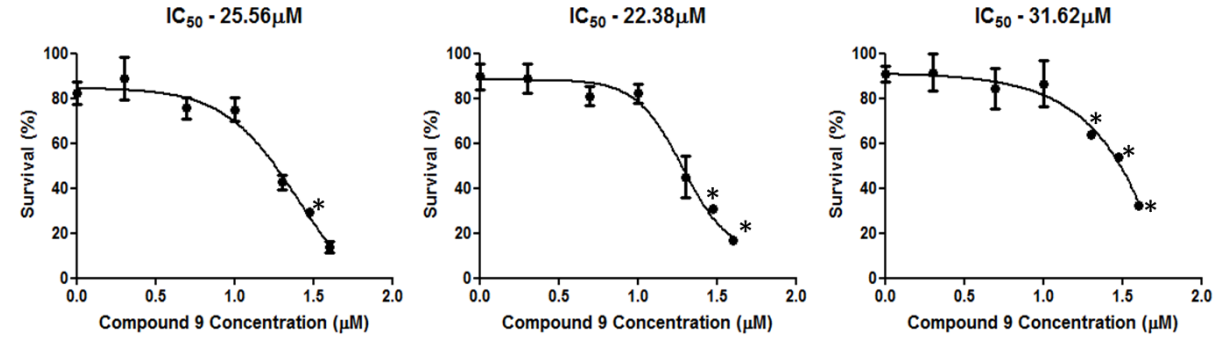
IC_{50} Curves for Compound 6



IC_{50} Curves for Compound 8



IC_{50} Curves for Compound 9



IC_{50} Curves for Compound 10

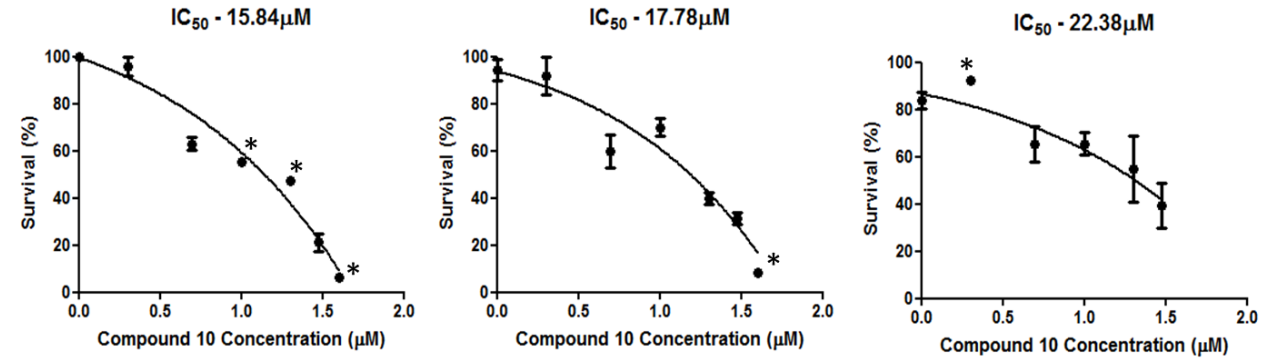


Figure S1. IC_{50} Values of **6, 8, 9** and **10**.

HCT-116 cells were treated with **6**, **8**, **9** and **10** at concentration range 1μM, 2μM, 5μM, 10μM, 20 μM, 30 μM and 40 μM for 72 hours and cell viability and IC_{50} values were determined by CCK-8 assay as described in the methods.

A. IC_{50} values from the growth inhibition curves of three separate experiments for **6**.

B. IC_{50} values from the growth inhibition curves of three separate experiments for **8**.

C. IC_{50} values from the growth inhibition curves of three separate experiments for **9**.

D. IC_{50} values from the growth inhibition curves of three separate experiments for **10**.

*** The set of readings for the point were consistent within a very narrow range and did not generate an error bar. All the error bars represent the Standard Errors of Mean (SEM)**

Methods: Confocal Microscopy

Confocal Microscopy. HCT 116 cells were seeded 24 hours prior to treatment in 35-mm Fluorodish cell culture dishes (World Precision Instruments, Sarasota, Florida, USA), at a density of 20,000 cells/dish. Cells were treated with 1% (v/v) dimethyl sulfoxide (DMSO; Sigma-Aldrich) as a control, or compound **5** or **9** at a concentration of 10 μM. After 24 hours post-treatment, cells were fixed with 1.5% glutaraldehyde in PBS for 1 hour. Cells were washed with PBS and stained with 5 μg/ml bisbenzimide (Hoechst 33342; Invitrogen/Life Technologies) for 20 minutes at room temperature. Confocal images were obtained with a FluoView 1000 confocal microscope (Olympus; 405 nm laser),housedat the Biomedical Imaging Facility of UNSW. Images were acquired with a 100X oil immersion objective.