Synthesis and Evaluation of 5-(Arylthio)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamines as Receptor Tyrosine Kinase and Thymidylate Synthase Inhibitors and as Antitumor Agents

Nilesh Zaware[a,d], Roy Kisliuk[b], Anja Bastian[c], Michael A. Ihnat[c], Aleem Gangjee[a,*],

[a]*Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, 600 Forbes Avenue, Pittsburgh, PA 15282, United States*

[b]*Department of Biochemistry, Tufts University School of Medicine, Boston, MA 02111, United States*

[c]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73117, United States*

[d]Present address for NZ: *Department of Pharmacological Sciences, Icahn School of Medicine at Mt. Sinai, 1425 Madison Avenue, New York, NY 10029, United States*

SUPPLEMENTAL DATA

Synthesis of compounds

Analytical samples were dried in vacuo (0.2 mm Hg) in a CHEM-DRY drying apparatus over P_2O_5 at 80 ^oC. Melting points were determined on a MEL-TEMP II melting point apparatus with FLUKE 51 K/J electronic thermometer and are uncorrected. Nuclear magnetic resonance spectra for proton (^1H NMR) were recorded on a Bruker WH-300 (300 MHz) or Bruker WH-400 (400 MHz) spectrometer. The chemical shift values are expressed in ppm (parts per million): s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet. Thin-layer chromatography (TLC) was performed on Whatman Sil G/UV254 silica gel plates with a fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for TLC are by volume. Column chromatography was performed on a 230-400 mesh silica gel (Fisher, Somerville, NJ) column. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within ±0.4% of the calculated values. Fractional moles of water or organic solvents frequently found in some analytical samples of antifolates could not be prevented in spite of 24-48 h of drying in vacuo and were confirmed where possible by their presence in the ^1H NMR spectra. All solvents and chemicals were purchased from Aldrich Chemical Co. or Fisher Scientific and were used as received.

2-Amino-4-chloro-1*H*-indole-3-carbonitrile (13). To an ice-cold solution of malononitrile (2.06 g, 31.2 mmol) in anhydrous DMF (100 mL) under N_2 was added sodium hydride (0.75 g, 31.2 mmol). The formed white suspension was stirred for 15 min then treated with 1,2-dichloro-3-nitrobenzene **11** (2 g , 15.4 mmol). The suspension was heated at 70-80 °C for 3 h. To the resulting solution was added 50 mL H_2O, and the aqueous mixture was acidified to pH 2 with concd HCl.

The mixture was extracted with ether (3x) and then the combined organic phases were dried using sodium sulfate and concentrated to give (2-Chloro-6-nitrophenyl) malononitrile **12** as a dark oil. TLC R_f 0.08 (hexane:EtOAc, 1:1). ^{1}H NMR (DMSO-d_6) δ 6.21 (s, 1 H, CH), 7.70-7.75 (m, 1 H, Ar), 7.90-7.92 (m, 1 H, Ar), 8.16-8.19 (m, 1 H, Ar). The material was used directly for the next step.

A solution of **12** (2 g, 9 mmol) in glacial AcOH (185 mL) was treated with a single charge of Zn dust (6 g, 3 parts by weight). The mixture was heated at 55 °C for 45 min, then treated with more Zn dust (2 g, 1 part by weight). After heating for another 105 min, the mixture was filtered through a pad of celite. The pad was washed well with AcOH and the filtrate was concentrated to a residue that was distributed between ethylacetate and H_2O. The organic phase was washed with 5% aq. $NaHCO_3$ and brine and concentrated to a residue that was purified by column chromatography, eluting with 1:1 hexane:EtOAc. The fractions containing the pure product (TLC) were pooled and evaporated to give a pink solid. The overall yield from **11** to **13** was 39%. TLC R_f 0.15 ($CHCl_3$/MeOH, 10:1 with two drops of concentrated NH_4OH); mp 247-247.2 ^{o}C; ^{1}H NMR (DMSO-d_6) δ 6.84-6.88 (m, 1 H, Ar), 6.90 (bs, 2 H, NH_2, exch), 6.92-6.94 (m, 1 H, Ar), 7.06-7.08 (m, 1 H, Ar), 10.97 (s, 1 H, NH, exch). Anal. calcd. for $C_9H_6ClN_3$: C, 56.41; H, 3.15; N, 21.92; Cl, 18.50; Found: C, 56.36; H, 3.11; N, 21.63; Cl, 18.20.

5-Chloro-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (14). A mixture of 2-Amino-4-chloro-1*H*-indole-3-carbonitrile **(13)** (215 mg , 1.12 mmol), carbamimidic chloride hydrochloride (142 mg, 1.23 mmol) and methyl sulfone (3 g) was stirred and heated at 110-120 °C for 8 h, after which it was recharged with carbamimidic chloride hydrochloride (142 mg, 1.23 mmol) and methyl sulfone (3 g) and was stirred and heated at 110-120 °C for 16 h. About 10 mL ammonia in methanol was

added to neutralize the reaction mixture. Purification by column chromatography, eluting sequentially with 1% and 5% MeOH in $CHCl_3$ afforded 40% of **14** as a white solid. TLC R_f 0.43 ($CHCl_3$/MeOH, 5:1); mp 245.2-246.3 °C; ^{1}H NMR (DMSO-d_6) δ 6.15 (bs, 2 H, 2-NH_2, exch), 6.85 (bs, 2 H, 4-NH_2, exch), 7.03-7.24 (m, 3 H, Ar), 11.54 (bs, 1 H, 9-NH, exch). Anal. calcd. for $C_{10}H_8ClN_5$: C, 51.40; H, 3.45; N, 29.97; Cl, 15.17; Found: C, 51.58; H, 3.46; N, 29.86; Cl, 15.05.

General procedure for the synthesis of 5-(substitutedphenylthio)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamines 3, **4.** Compound **14** (50 mg, 0.2 mmol), the appropriate thiol (0.9 mmol), potassium carbonate (120 mg, 0.9 mmol) were added to a 2-5 mL biotage® microwave vial. Around 3 mL NMP was added as solvent and the tube was sealed. The reaction was run in a microwave for 30 minutes at 250 °C. After cooling to room temperature, the reaction mixture was transferred on top of a silica gel column and eluted with 0% and 4% methanol in chloroform. Fractions containing the product (TLC) were pooled and evaporated to afford the product.

5-(2-Naphthylsulfanyl)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (3). Using the general procedure described above, the reaction of **14** with naphthalene-2-thiol **15** afforded **3** as an off-white solid: yield 88%. TLC R_f 0.35 ($CHCl_3$/MeOH 10:1 with two drops of concentrated NH_4OH); mp 251.9-252.6 °C; ^{1}H NMR (DMSO-d_6) δ 6.00 (bs, 2 H, 2-NH_2, exch), 7.14-7.16 (m, 1 H, Ar), 7.21-7.25 (m, 1 H, Ar), 7.34-7.36 (m, 1 H, Ar), 7.40-7.45 (m, 3 H, Ar), 7.42 (bs, 2 H, 4-NH_2, exch), 7.53 (m, 1 H, Ar), 7.67-7.69 (m, 1 H, Ar), 7.78-7.82 (m, 2 H, Ar), 11.49 (s, 1 H, 9-NH, exch). Anal. calcd. for $C_{20}H_{15}N_5S$: C, 67.20; H, 4.22; N, 19.59; S, 8.97; Found: C, 66.92; H, 4.20; N, 19.41; S, 8.80.

5-(1-Naphthylsulfanyl)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (4). Using the general procedure described above, the reaction of **14** with naphthalene-1-thiol **16** afforded **4** as an off-white solid:

yield 70%. TLC R_f 0.35 ($CHCl_3$/MeOH 10:1 with two drops of concentrated NH_4OH); mp 308 °C; ^{1}H NMR (DMSO-d_6) δ 6.01 (bs, 2 H, 2-NH_2, exch), 7.18-7.30 (m, 4 H, Ar), 7.24 (bs, 2 H, 4-NH_2, exch), 7.39-7.42 (m, 1 H, Ar), 7.58-7.67 (m, 2 H, Ar), 7.73-7.75 (m, 1 H, Ar), 7.95-7.97 (m, 1 H, Ar), 8.27-8.29 (m, 1 H, Ar), 11.51 (s, 1 H, 9-NH, exch). Anal. calcd. for $C_{20}H_{15}N_5S$. 0.2 CH_3OH: C, 66.68; H, 4.37; N, 19.24; S, 8.81; Found: C, 66.83; H, 4.43; N, 18.99; S, 8.63.

2-Amino-4-bromo-1*H*-indole-3-carbonitrile (19). To an ice-cold solution of malononitrile (238 mg, 3,6 mmol) in anhydrous DMF (80 mL) under N_2 was added sodium hydride (130 mg, 5.2 mmol). The formed white suspension was stirred for 15 min then treated with 1,2-dibromo-3-nitrobenzene **17** (500 mg , 1.8 mmol). The suspension was heated at 70-80 °C for 3 h. To the resulting solution was added 50 mL H_2O, and the aqueous mixture was acidified to pH 2 with concd HCl. The mixture was extracted with ether (3x) and then the combined organic phases were dried using sodium sulfate and concentrated to give (2-bromo-6-nitrophenyl)malononitrile **(18)** as a dark oil. TLC R_f 0.42 ($CHCl_3$:MeOH, 10:1). ^{1}H NMR (DMSO-d_6) δ 6.18 (s, 1 H, CH), 7.62-7.66 (m, 1 H, Ar), 8.07-8.09 (m, 1 H, Ar), 8.19-8.21 (m, 1 H, Ar). The material was used directly for the next step.

A solution of **18** (196 mg, 0.73 mmol) in glacial AcOH (100 mL) was treated with a single charge of Zn dust (600 mg, 3 parts by weight). The mixture was heated at 55 °C for 45 min, then treated with more Zn dust (200 mg, 1 part by weight). After heating for another 105 min, the mixture was filtered through a pad of celite. The pad was washed well with AcOH and the filtrate was concentrated to a residue that was distributed between ethylacetate and H_2O. The organic phase was washed with 5% aq. $NaHCO_3$ and brine and concentrated to a residue that was purified by column chromatography, eluting with 1:1 hexane:EtOAc. The fractions containing the pure

product (TLC) were pooled and evaporated to give a pink solid. The overall yield from **17** to **19** was 78%. TLC R_f 0.25 ($CHCl_3$/MeOH, 10:1 with two drops of concentrated NH_4OH); mp 231.1-231.9 ^oC; ^1H NMR (DMSO-d_6) δ 6.78-6.82 (m, 1 H, Ar), 6.89 (bs, 2 H, NH_2, exch), 7.07-7.08 (m, 1 H, Ar), 7.10-7.12 (m, 1 H, Ar), 10.98 (s, 1 H, NH, exch). Anal. calcd. for $C_9H_6BrN_3$: C, 45.79; H, 2.56; N, 17.80; Br, 33.84; Found: C, 45.89; H, 2.59; N, 17.57; Br, 33.56.

5-Bromo-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (20). A mixture of 2-Amino-4-bromo-1*H*-indole-3-carbonitrile **(19)** (300 mg , 1.27 mmol), carbamimidic chloride hydrochloride (160 mg, 1.39 mmol) and methyl sulfone (3 g) was stirred and heated at 110-120 °C for 8 h, after which it was recharged with carbamimidic chloride hydrochloride (160 mg, 1.39 mmol) and methyl sulfone (3 g) and was stirred and heated at 110-120 °C for 12 h. About 10 mL ammonia in methanol was added to neutralize the reaction mixture. Purification by column chromatography, eluting sequentially with 1% and 5% methanol in chloroform afforded 45% of **20** as a white solid. TLC R_f 0.27 ($CHCl_3$/MeOH, 10:1 with two drops of concentrated NH_4OH); mp 240 ^oC; ^1H NMR (DMSO-d_6) δ 6.05 (bs, 2 H, 2-NH_2, exch), 6.95 (bs, 2 H, 4-NH_2, exch), 7.01-7.05 (m, 1 H, Ar), 7.24-7.26 (m, 2 H, Ar), 11.49 (bs, 1 H, 9-NH, exch). HRMS (ESI) calcd. for $C_{10}H_8BrN_5$ (^{79}Br, M+H)$^+$: 278.0036, found: 278.0029. Calcd. for $C_{10}H_8BrN_5$ (^{81}Br, M+H)$^+$: 280.0016, found: 280.0005.

General procedure for the synthesis of 5-(substitutedphenylthio)-9*H*-pyrimido[4,5-*b*]indole-2,4-diamines 5-7. Compound **20** (80 mg, 0.28 mmol), the appropriate thiol (1.15 mmol), copper iodide (219 mg, 1.15 mmol), potassium carbonate (318 mg, 2.3 mmol) were added to a 2-5 mL biotage® microwave vial. Around 3 mL DMF was added as solvent and the tube was sealed. The reaction was run in a microwave for 4 h at 180 °C. After cooling to room temperature, the DMF

was removed under reduced pressure and the crude product was purified by column chromatography, sequentially eluting with 0% and 4% methanol in chloroform. Fractions containing the product (TLC) were pooled and evaporated to afford the product.

5-[(2,5-Dimethoxyphenyl)sulfanyl]-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (5). Using the general procedure described above, the reaction of **20** with 2,5-dimethoxybenzenethiol **21** afforded **5** as an off white solid: yield 71%. TLC R_f 0.39 ($CHCl_3$/MeOH 10:1 with two drops concentrated NH_4OH); mp 281-281.5 °C; ^{1}H NMR (DMSO-d_6) δ 3.41 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 5.75-5.76 (m, 1 H, Ar), 5.98 (bs, 2 H, 2-NH_2, exch), 6.63-6.65 (m, 1 H, Ar), 6.93-6.95 (m, 1 H, Ar), 7.20-7.28 (m, 2 H, Ar), 7.26 (bs, 2 H, 4-NH_2, exch), 7.40-7.42 (m, 1 H, Ar), 11.46 (s, 1 H, 9-NH, exch). HRMS (ESI) calcd. for $C_{18}H_{17}N_5O_2S$: 367.1102, found: 367.1086.

5-[(4-Methoxyphenyl)sulfanyl]-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (6). Using the general procedure described above, the reaction of **20** with 4-methoxybenzenethiol **22** afforded **6** as an off white solid: yield 77%. TLC R_f 0.32 ($CHCl_3$/MeOH 10:1 with two drops concentrated NH_4OH); mp 263.1-263.8 °C; ^{1}H NMR (DMSO-d_6) δ 3.66 (s, 3 H, OCH_3), 6.02 (bs, 2 H, 2-NH_2, exch), 6.84-6.86 (m, 2 H, Ar), 7.09-7.17 (m, 3 H, Ar), 7.13 (bs, 2 H, 4-NH_2, exch), 7.25-7.33 (m, 2 H, Ar), 11.43 (s, 1 H, 9-NH, exch). Anal. calcd. for $C_{17}H_{15}N_5OS$: C, 60.51; H, 4.48; N, 20.75; S, 9.50; Found: C, 60.56; H, 4.37; N, 20.72; S, 9.63.

5-[(4-Chlorophenyl)sulfanyl]-9*H*-pyrimido[4,5-*b*]indole-2,4-diamine (7). Using the general procedure described above, the reaction of **20** with 4-chlorobenzenethiol **23** afforded **7** as a brown solid: yield 42%. TLC R_f 0.3 ($CHCl_3$/MeOH 5:1 with two drops concentrated NH_4OH); mp 260 °C;

H NMR (DMSO-d_6) δ 6.03 (bs, 2 H, 2-NH_2, exch), 6.98-7.00 (m, 2 H, Ar), 7.19-7.23 (m, 1 H, Ar), 7.28-7.32 (m, 3 H, Ar), 7.30 (bs, 2 H, 4-NH_2, exch), 7.39-7.41 (m, 1 H, Ar), 11.50 (s, 1 H, 9-NH, exch). HRMS (ESI) calcd. for $C_{16}H_{12}ClN_5S$ $(M+H)^+$: 342.0580, found: 342.0568.

Cells.

All cells were maintained at 37 °C in a humidified environment containing 5% CO_2 using media from Mediatech (Hemden, NJ). A-431 cells were from the American Type Tissue Collection (Manassas, VA).

Chemicals.

All growth factors (bFGF, VEGF, EGF, and PDGF-β) were purchased from Peprotech (Rocky Hill, NJ). PD153035, SU5416, AG1295, and CB676475 (4-[(4′-chloro-2′-fluoro)phenylamino]-6,7-dimethoxyquinazoline) were purchased from Calbiochem (San Diego, CA). The CYQUANT cell proliferation assay was from Molecular Probes (Eugene, OR). All other chemicals were from Sigma Chemical unless otherwise noted.

Antibodies.

The PY-HRP antibody was from BD Transduction Laboratories (Franklin Lakes, NJ). Antibodies against EGFR, PDGFR-β, FGFR-1, Flk-1, and Flt-1 were purchased from Upstate Biotech (Framingham, MA).

Phosphotyrosine ELISA.

Cells used were tumor cell lines naturally expressing high levels of EGFR (A431), Flk-1 (U251),

Flt-1 (A498), PDGFR-β (SF-539), and FGFR-1 (NIH OVCAR-8). Expression levels at the RNA level were derived from the NCI Developmental Therapeutics Program (NCI-DTP) web site public molecular target information (http://www.dtp.nci.nih.gov/mtargets/mt_index.html). Briefly, cells at 60–75% confluence were placed in serum-free medium for 18 h to reduce the background of phosphorylation. Cells were always >98% viable by Trypan blue exclusion. Cells were then pretreated for 60 min with 10, 3.33, 1.11, 0.37, and 0.12 μM compounds followed by 100 ng/ml EGF, VEGF, PDGF-BB, or bFGF for 10 min. The reaction was stopped and cells permeabilized by quickly removing the media from the cells and adding ice-cold Tris-buffered saline (TBS) containing 0.05% Triton X-100, protease inhibitor cocktail, and tyrosine phosphatase inhibitor cocktail. The TBS solution was then removed and cells fixed to the plate for 30 min at 60 °C and further incubation in 70% ethanol for an additional 30 min. Cells were further exposed to block (TBS with 1% BSA) for 1 h, washed, and then a horseradish peroxidase (HRP)-conjugated phosphotyrosine (PY) antibody added overnight. The antibody was removed, cells were washed again in TBS, exposed to an enhanced luminal ELISA substrate (Pierce Chemical, Rockford, IL), and light emission measured using a UV product (Upland, CA) BioChemi digital darkroom. The known RTK-specific kinase inhibitor, PD153035, was used as a positive control compound for EGFR kinase inhibition; SU5416 for Flk1 kinase inhibition; AG1295 for PDGFR-β kinase inhibition; and CB676475 (4-[(4′-chloro-2′-fluoro)phenylamino]-6,7-dimethoxyquinazoline) was used as a positive control for both Flt1 and Flk1 kinase inhibition. Data were graphed as a percent of cells receiving growth factor alone and IC_{50} values were estimated from two to three separate experiments (n = 8–24) using non-linear dose-response regression analysis using Prism 6.0 software (GraphPad, San Diego, CA). In each case, the activity of a positive control inhibitor did not deviate more than 10% from the IC_{50} values listed in the text.

CYQUANT cell proliferation assay.

As a measure of cell proliferation, the CYQUANT cell counting/proliferation assay was used as previously described.[1] Briefly, cells are first treated with compounds for 12 h and then allowed to grow for an additional 36 h. The cells are then lysed and the CYQUANT dye, which intercalates into the DNA of cells, is added and after 5 min the fluorescence of each well measured using a Biotek FLx800 fluorescence plate reader . A positive control used for cytotoxicity in each experiment was cisplatin, **23** with an apparent average IC_{50} value of 8.2 ± 0.65 μM. Data are graphed as a percent of cells receiving growth factor alone and IC_{50} values estimated from two to three separate experiments (n = 6–15) using non-linear dose-response regression analysis using Prism 6.0 software (GraphPad, San Diego, CA).

Statistics.

All analysis was done using Prism 4.0. (GraphPad Software, San Diego, CA).

Acknowledgements

This work was supported, in part, by the National Institutes of Health, Grants AI41743 (A.G.); AI44661 (A.G.), CA89300 (A.G.), and AI47759 (A.G.) from the National Institute of Allergy and Infectious Diseases

Appendix: Elemental Analysis

Compd	Formula	Calcd, %					Found, %				
		C	H	N	S	Cl/Br	C	H	N	S	Cl/Br
13	$C_9H_6ClN_3$	56.41	3.15	21.92		18.50	56.36	3.11	21.63		18.20
14	$C_{10}H_8ClN_5$	51.40	3.45	29.97		15.17	51.58	3.46	29.86		15.05
3	$C_{20}H_{15}N_5S$	67.20	4.22	19.59	8.97		66.92	4.20	19.41	8.80	
4	$C_{20}H_{15}N_5S$. 0.2 CH_3OH	66.68	4.37	19.24	8.81		66.83	4.43	18.99	8.63	
19	$C_9H_6BrN_3$	45.79	2.56	17.80		33.84	45.89	2.59	17.57		33.56
6	$C_{17}H_{15}N_5OS$	60.51	4.48	20.75	9.50		60.56	4.37	20.72	9.63	

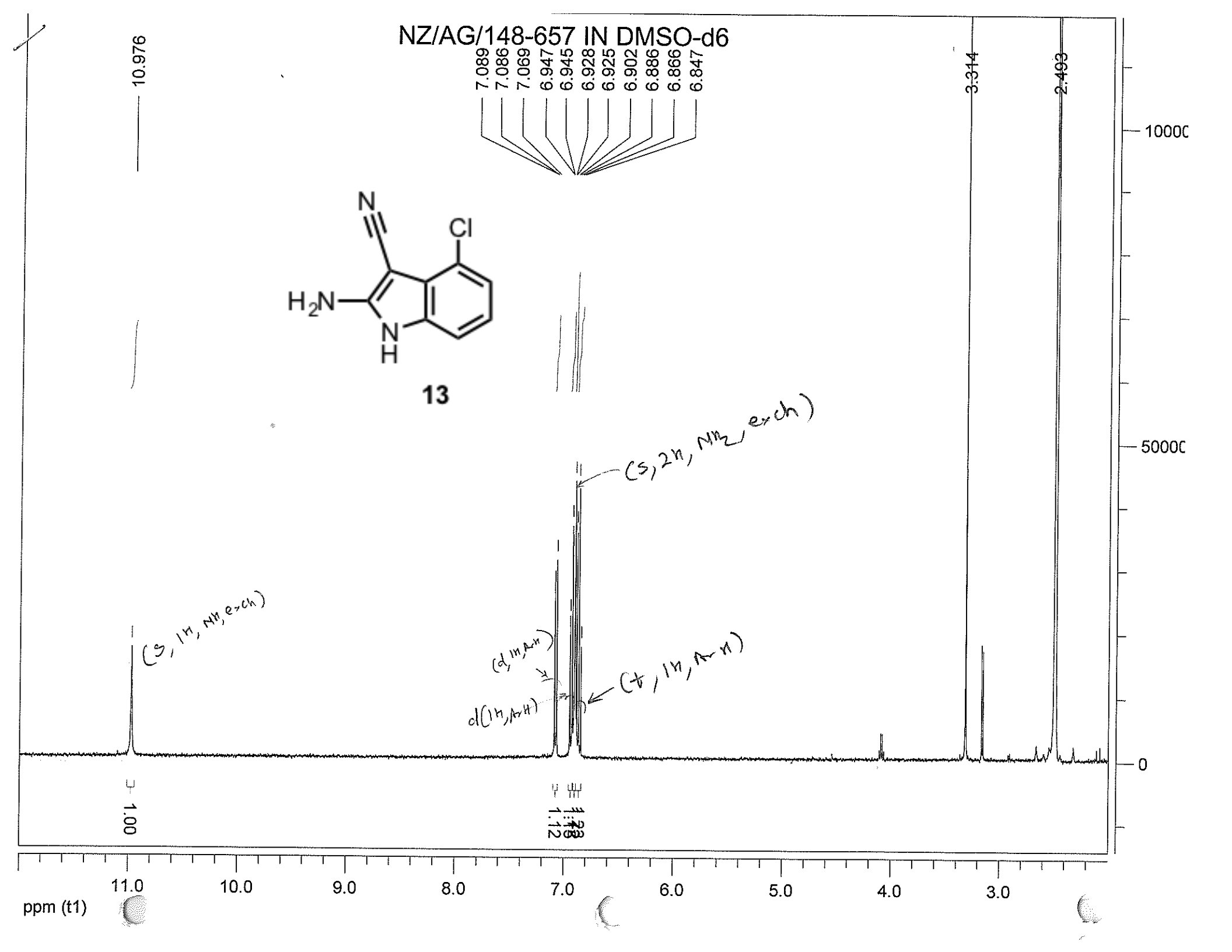
High-resolution mass spectra (HRMS) (ESI)

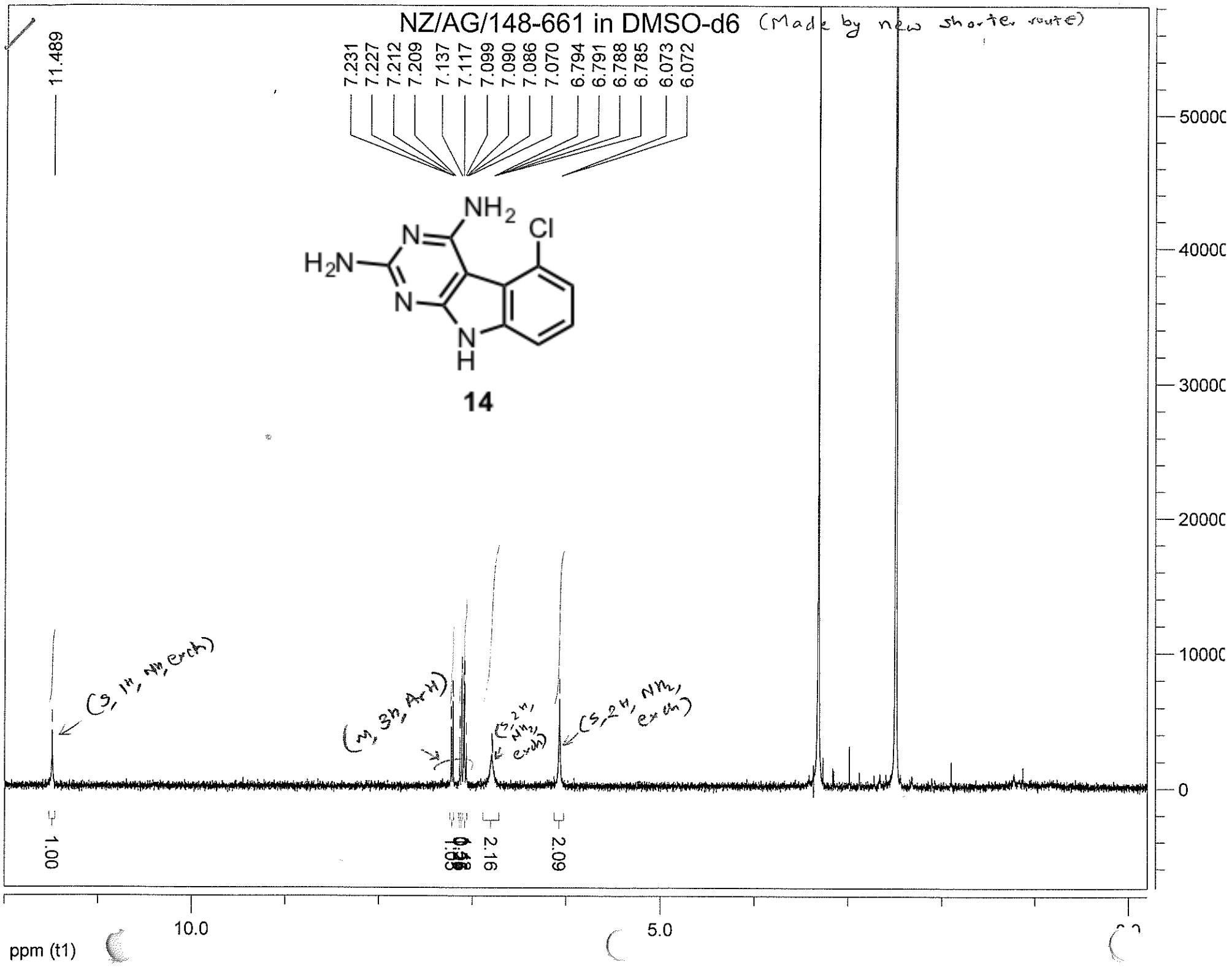
Compd	Formula	Calcd. mass	Found mass
20	$C_{10}H_8BrN_5$	280.0016	280.0005
5	$C_{18}H_{17}N_5O_2S$	367.1102	367.1086
7	$C_{16}H_{12}ClN_5S$	342.0580	342.0568

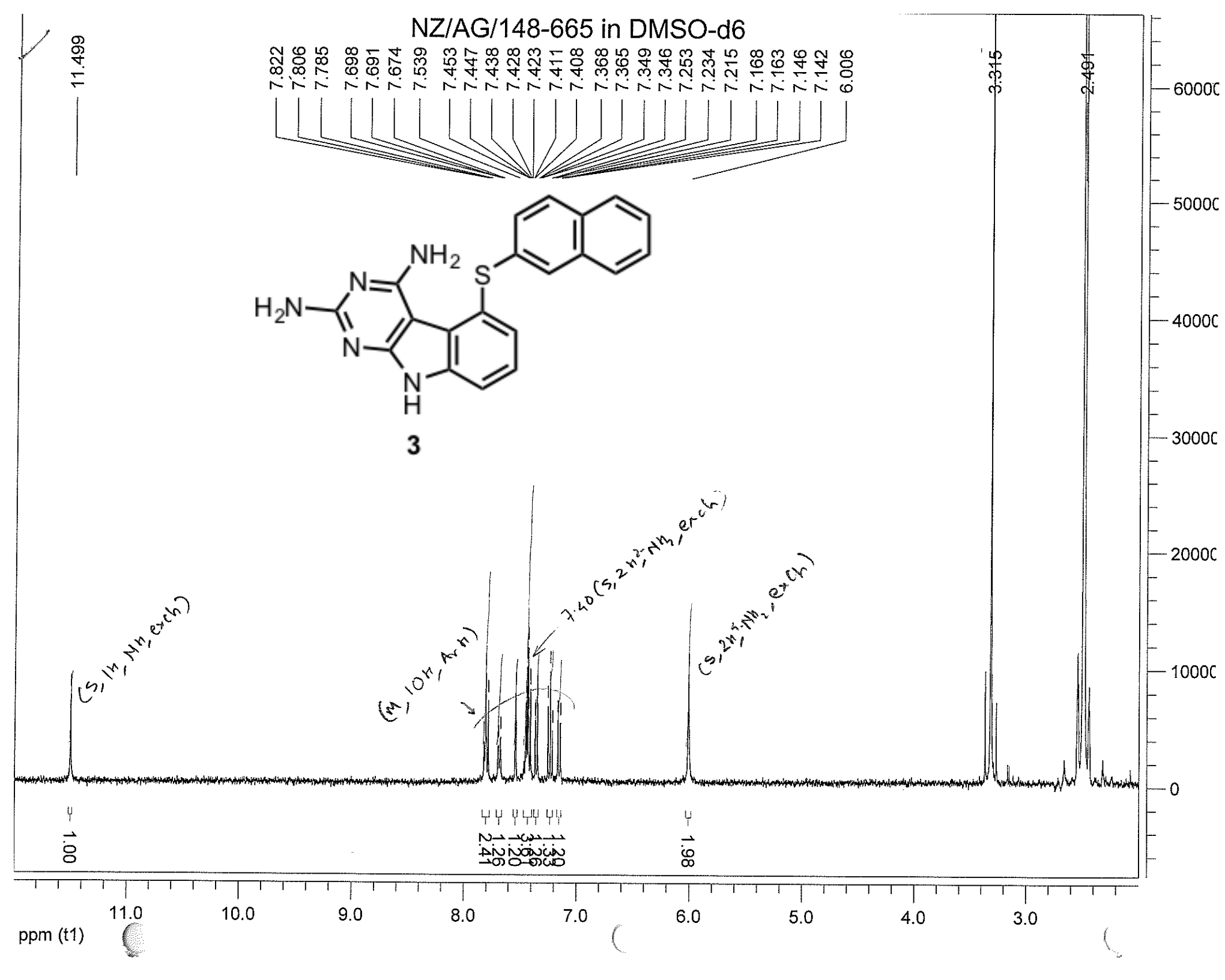
References and notes

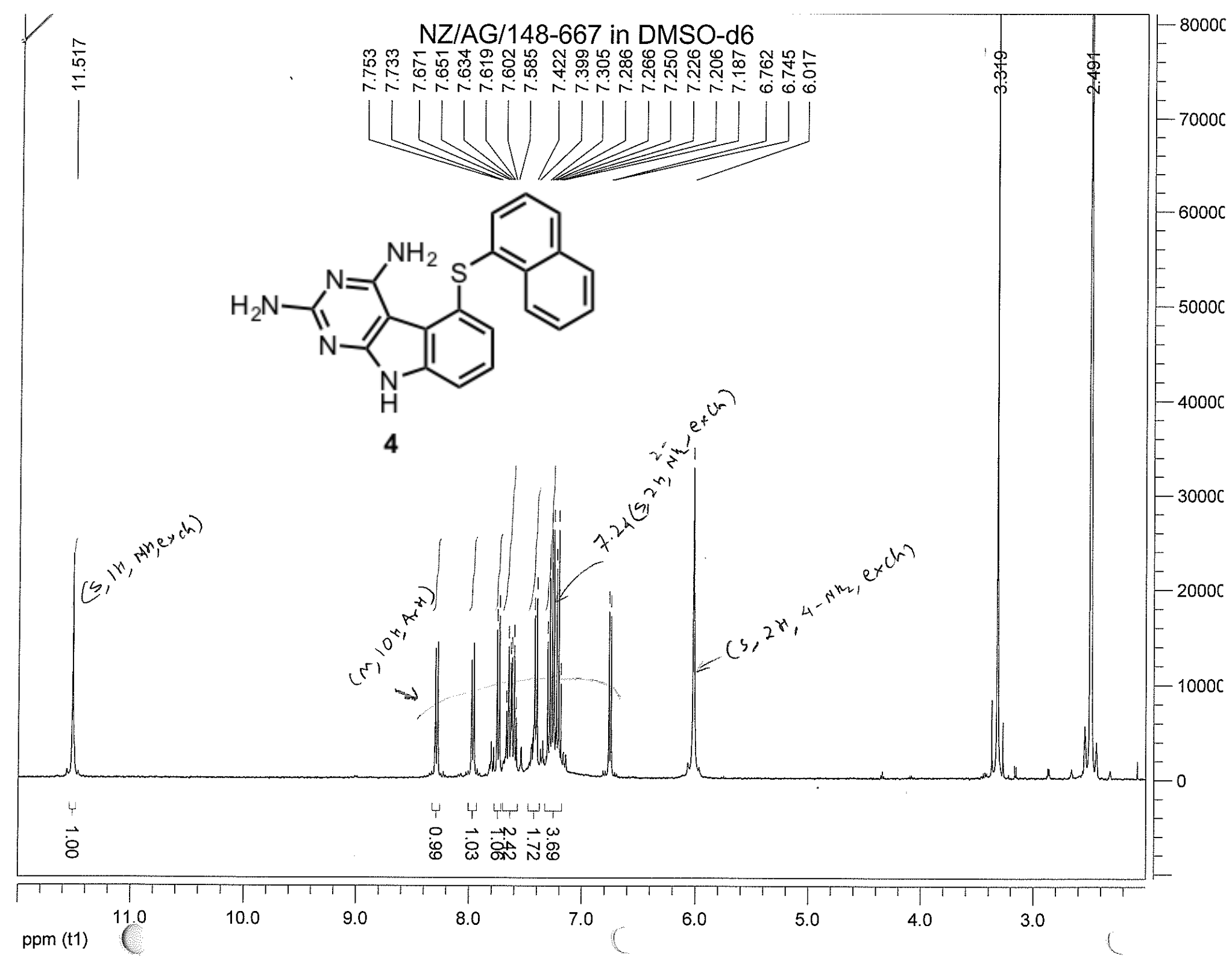
(1) Wilson, S. M.; Barsoum, M. J.; Wilson, B. W.; Pappone, P. A.: Purine nucleotides modulate proliferation of brown fat preadipocytes. *Cell Proliferation* **1999**, *32*, 131-140

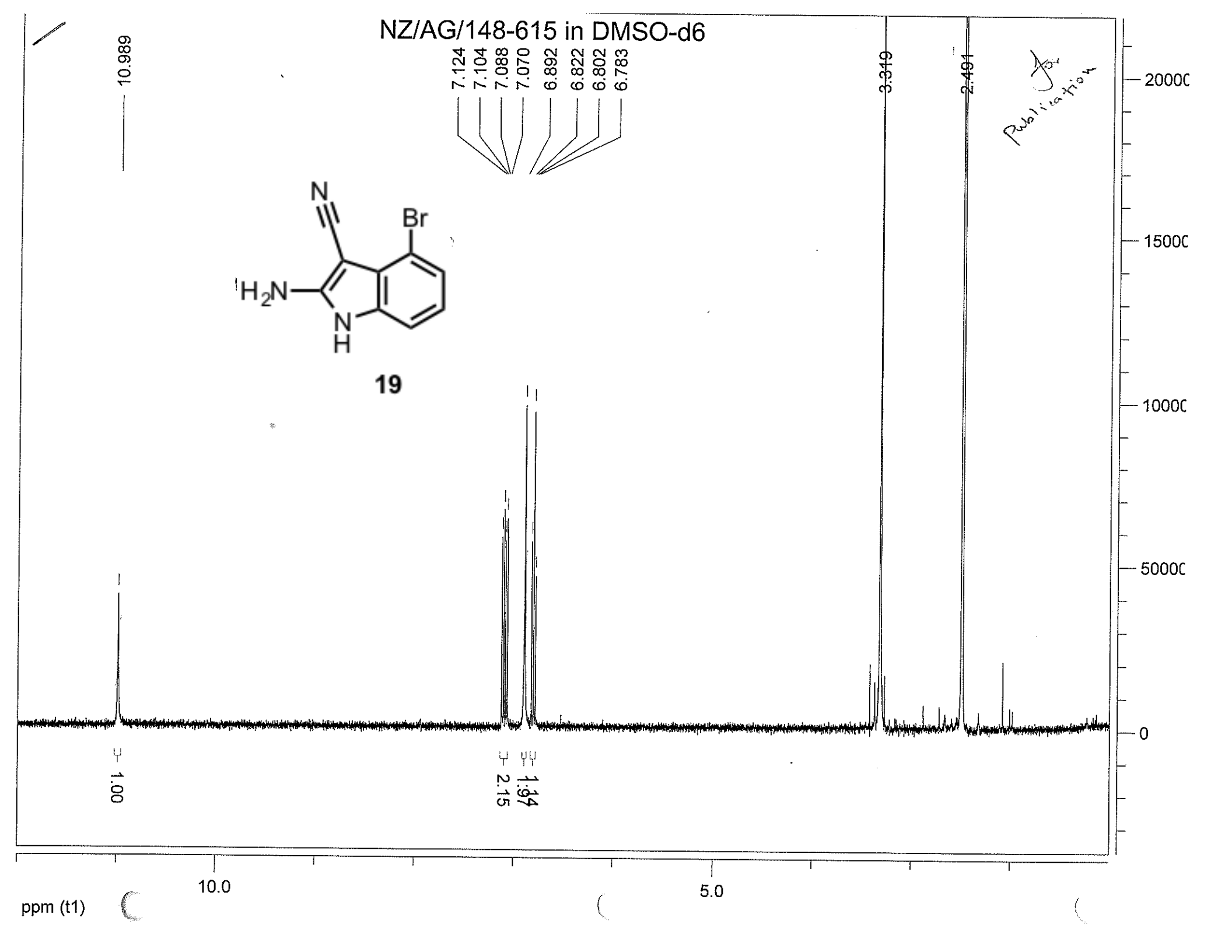
NZ/AG/148-657 IN DMSO-d6

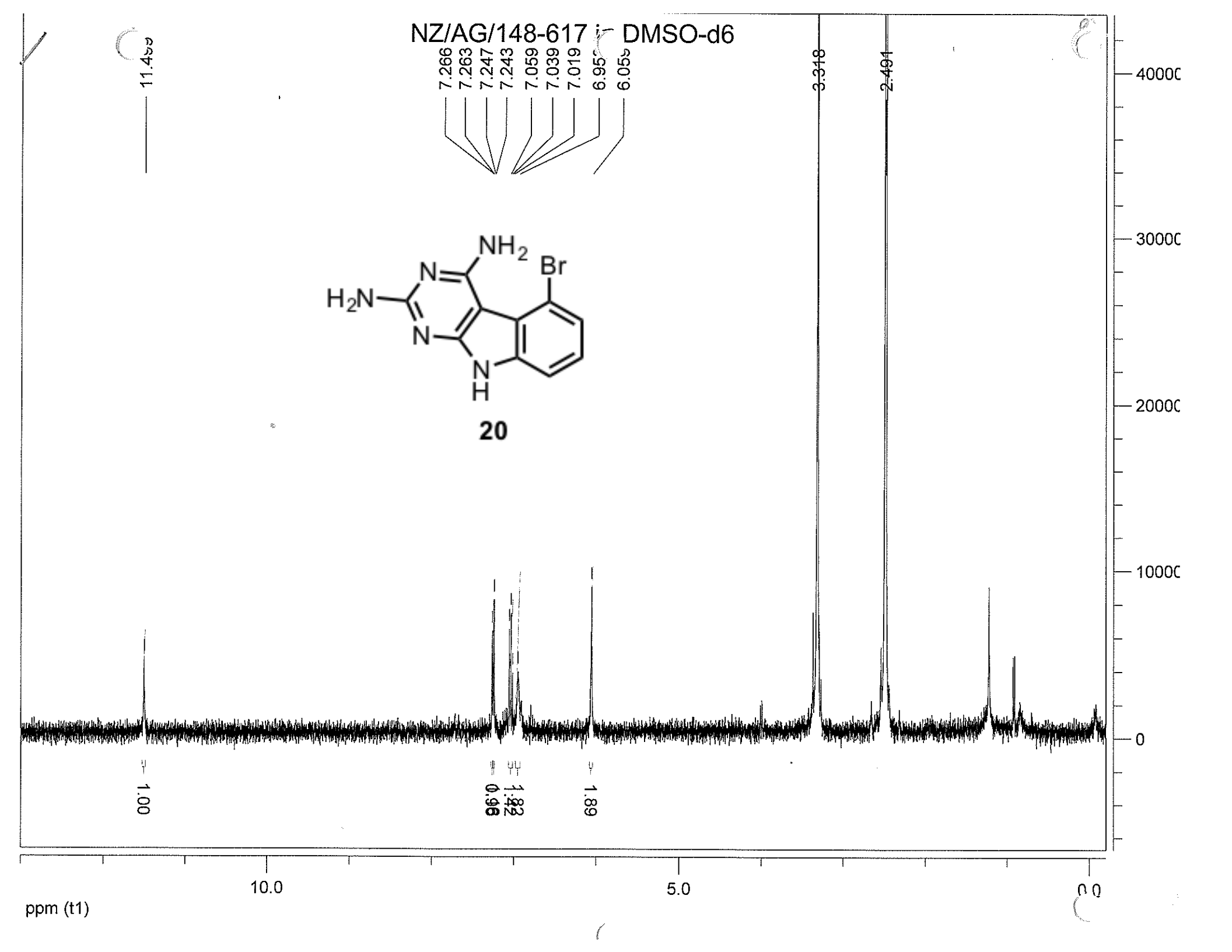


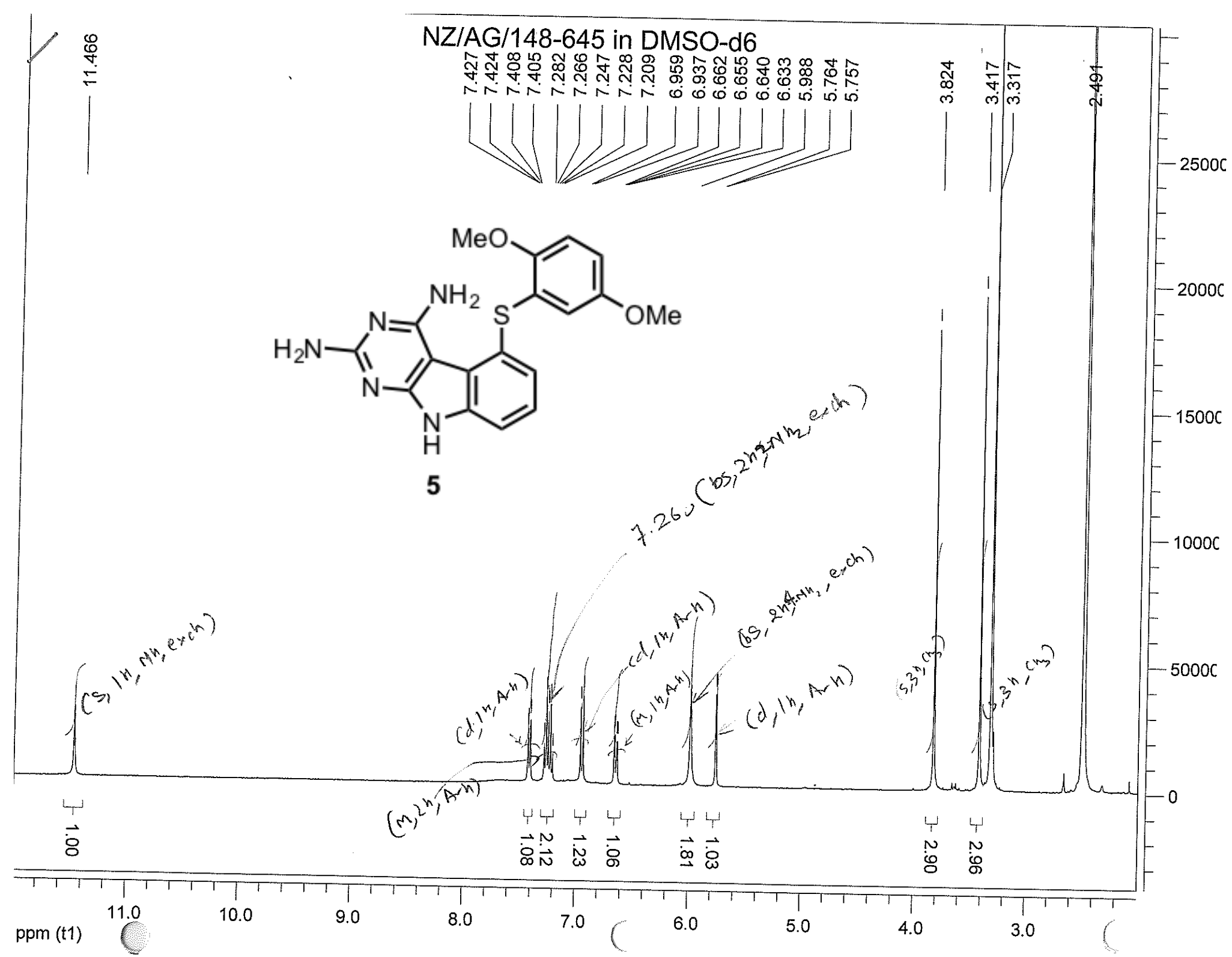

NZ/AG/148-661 in DMSO-d6 (Made by new shorter route)
11.489
7.231
7.227
7.212
7.209
7.137
7.117
7.099
7.090
7.086
7.070
6.794
6.791
6.788
6.785
6.073
6.072
NH2
N
H2N
N
Cl
N
H
14
(s, 1H, NH, exch)
(m, 3H, Ar-H)
(s, 2H, NH2, exch)
(s, 2H, NH2, exch)
1.00
2.16
2.09
50000
40000
30000
20000
10000
0
10.0
5.0
ppm (t1)

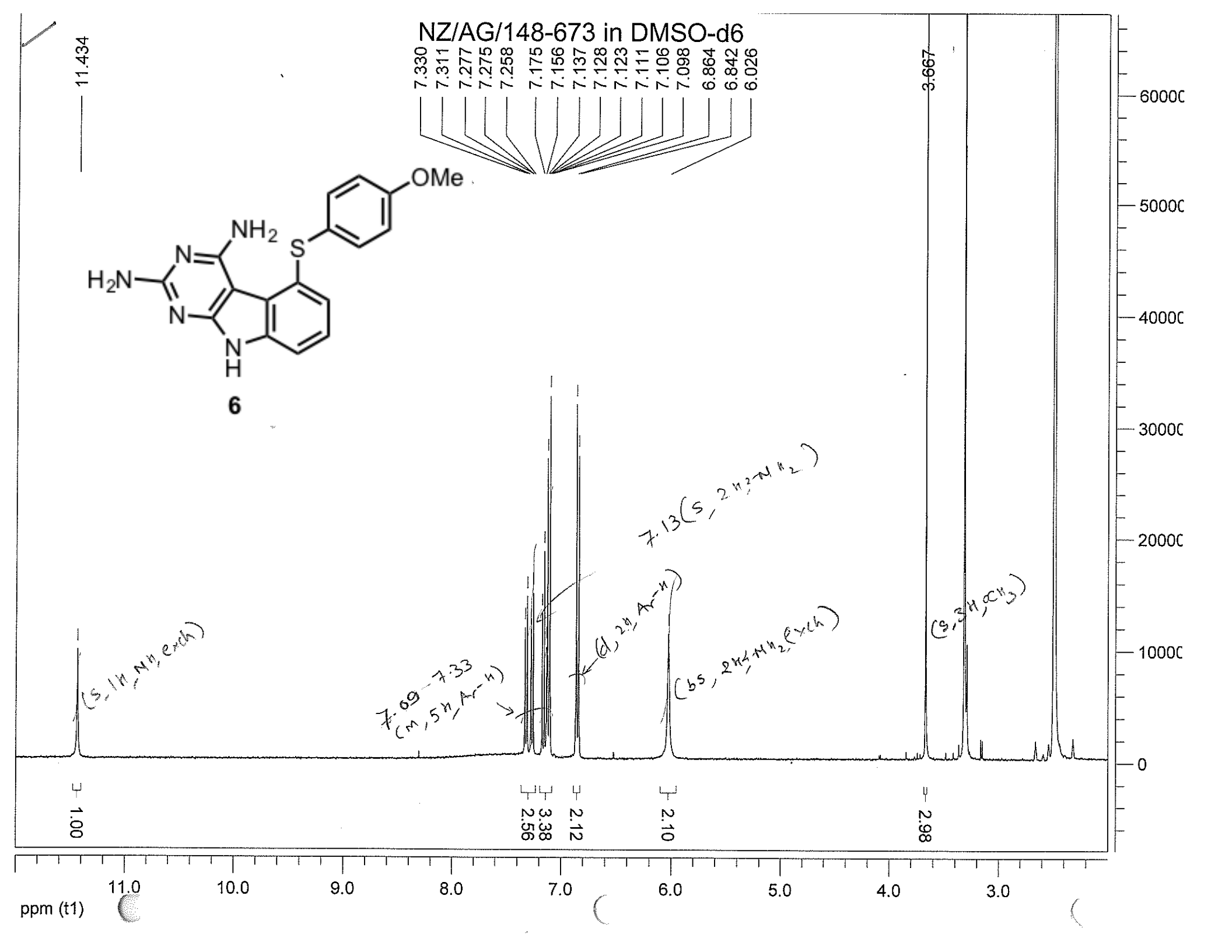

NZ/AG/148-665 in DMSO-d6
11.499
7.822
7.806
7.785
7.698
7.691
7.674
7.539
7.453
7.447
7.438
7.428
7.423
7.411
7.408
7.368
7.365
7.349
7.346
7.253
7.234
7.215
7.168
7.163
7.146
7.142
6.006
3.315
2.491
NH2
N
S
H2N
N
N
H
3
(s, 1H, NH, exch)
(m, 10H, Arh)
7.40 (s, 2H, NH2, exch)
(s, 2H, NH2, exch)
1.00
2.41
1.26
1.20
3.66
1.33
1.29
1.98
6000
5000
4000
3000
2000
1000
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)

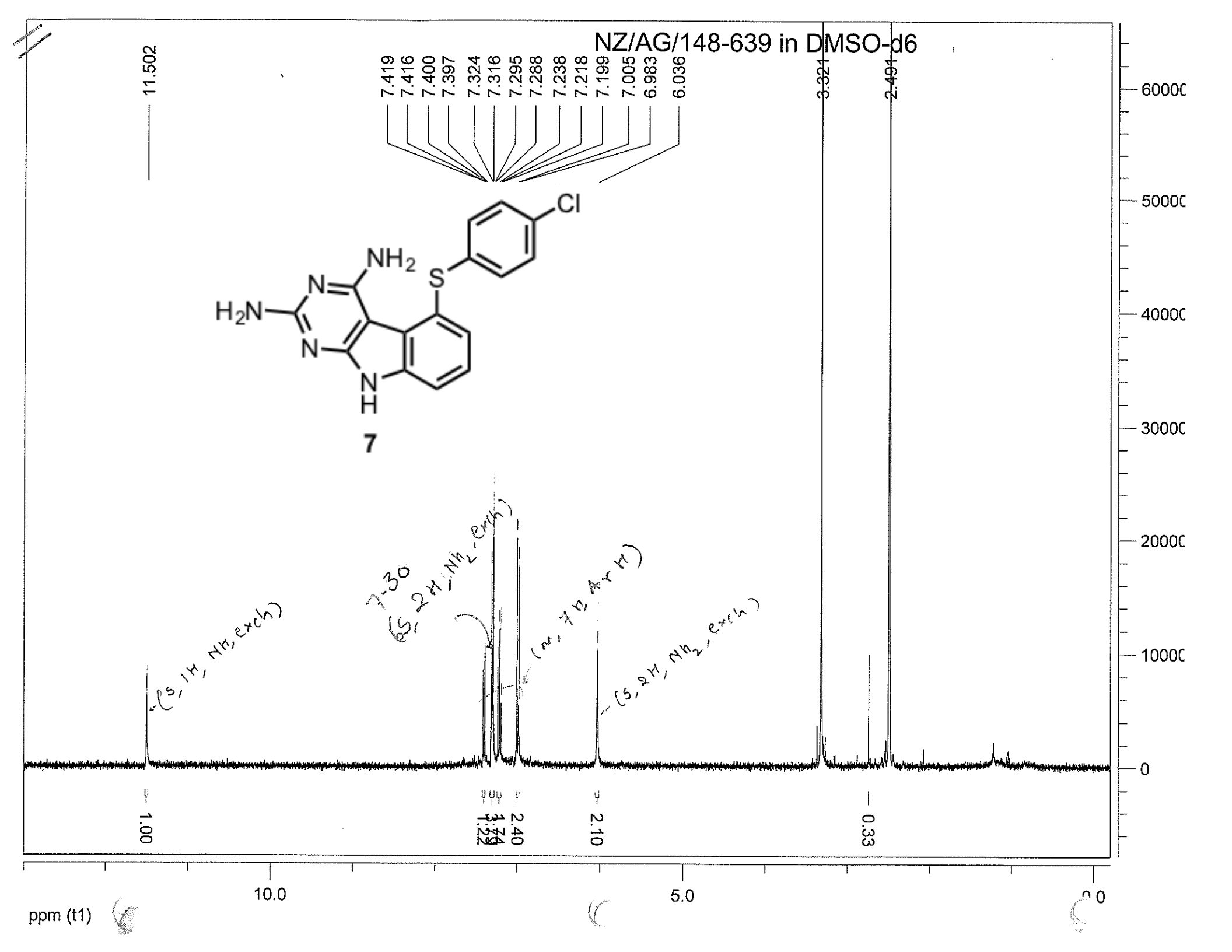

NZ/AG/148-667 in DMSO-d6
11.517
7.753
7.733
7.671
7.651
7.634
7.619
7.602
7.585
7.422
7.399
7.305
7.286
7.266
7.250
7.226
7.206
7.187
6.762
6.745
6.017
3.319
2.491
NH2
H2N
N
N
S
N
H
4
(s, 1H, NH, exch)
(m, 10H, Ar-H)
7.24 (s, 2H, NH2, exch)
(s, 2H, 4-NH2, exch)
1.00
0.99
1.03
1.06
2.42
1.72
3.69
ppm (t1)


NZ/AG/148-615 in DMSO-d6
10.989
7.124
7.104
7.088
7.070
6.892
6.822
6.802
6.783
3.349
2.491
Publication
N
Br
H2N
N
H
19
20000
15000
10000
5000
0
1.00
2.15
1.97
1.94
10.0
5.0
ppm (t1)


NZ/AG/148-617 in DMSO-d6
11.455
7.266
7.263
7.247
7.243
7.059
7.039
7.019
6.955
6.059
3.318
2.491
NH2
Br
H2N
N
N
N
H
20
1.00
0.96
1.42
1.82
1.89
40000
30000
20000
10000
0
10.0
5.0
0.0
ppm (t1)


NZ/AG/148-645 in DMSO-d6
11.466
7.427
7.424
7.408
7.405
7.282
7.266
7.247
7.228
7.209
6.959
6.937
6.662
6.655
6.640
6.633
5.988
5.764
5.757
3.824
3.417
3.317
2.491
MeO
NH2
S
OMe
H2N
N
N
NH
5
(s, 1H, NH, exch)
(d, 1H, A-h)
(m, 2H, A-h)
7.26 (bs, 2H, NH2, exch)
(d, 1H, A-h)
(m, 1H, A-h)
(bs, 2H, NH2, exch)
(d, 1H, A-h)
3H, (s)
3H, (s)
1.00
1.08
2.12
1.23
1.06
1.81
1.03
2.90
2.96
25000
20000
15000
10000
5000
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)


NZ/AG/148-673 in DMSO-d6
11.434
7.330
7.311
7.277
7.275
7.258
7.175
7.156
7.137
7.128
7.123
7.111
7.106
7.098
6.864
6.842
6.026
3.667
OMe
NH2
S
H2N
N
N
H
6
7.13 (s, 2H, NH2)
(d, 2H, Ar-H)
(bs, 2H, NH2 Exch)
(s, 3H, CH3)
7.69 - 7.33
(m, 5H, Ar-H)
(s, 1H, NH, exch)
1.00
2.56
3.38
2.12
2.10
2.98
6000
5000
4000
3000
2000
1000
0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
ppm (t1)


NZ/AG/148-639 in DMSO-d6
11.502
7.419
7.416
7.400
7.397
7.324
7.316
7.295
7.288
7.238
7.218
7.199
7.005
6.983
6.036
3.321
2.491
Cl
NH2
S
H2N
N
N
N
H
7
7.30
(s, 2H, NH2, exch)
(s, 1H, NH, exch)
(m, 7H, Ar-H)
(s, 2H, NH2, exch)
1.00
2.40
2.10
0.33
6000
5000
4000
3000
2000
1000
0
10.0
5.0
0
ppm (t1)