Supplementary Information**:**

Benzothiazole Derivatives Augment Glucose Uptake in Skeletal Muscle Cells and Stimulate Insulin Secretion from Pancreatic β-Cells via AMPK Activation

L. Pasternak,[a] E. Meltzer-Mats,[b] G. Babai-Shani,[a] G. Cohen[a], O. Viskind,[b] J. Eckel,[c] E. Cerasi[d], S. Sasson,[a]* and A. Gruzman,[b]*

[a]Department of Pharmacology, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, 91120 Jerusalem, Israel

[b]Division of Medicinal Chemistry, Department of Chemistry, Faculty of Exact Sciences, Bar Ilan University, 52900 Ramat Gan, Israel

[c]German Diabetes Center, Integrative Physiology, Paul-Langerhans-Group, Auf'm Hennekamp 65, 40225 Düsseldorf, Germany

[d]The Endocrinology and Metabolism Service, Department of Medicine, Hadassah-Hebrew University Medical 91120 Center, Jerusalem, Israel

Corresponding Authors

Arie Gruzman: gruzmaa@biu.ac.il, Phone number: +972-(0)54-7489041
Shlomo Sasson: shlomo.sasson@mail.huji.ac.il, Phone number: +972-2-6758798

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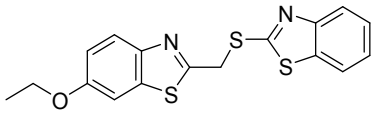
Supplemental Chart 1. Structures of the compounds that augment glucose transport in L6 myotubes in an AMPK-dependent manner



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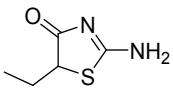

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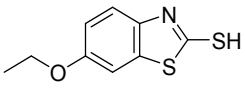

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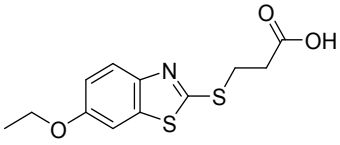

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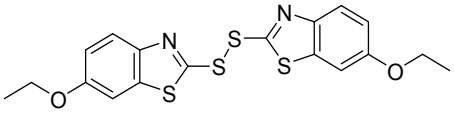

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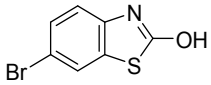

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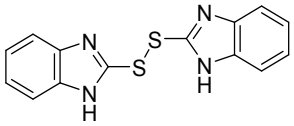

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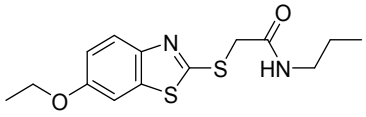

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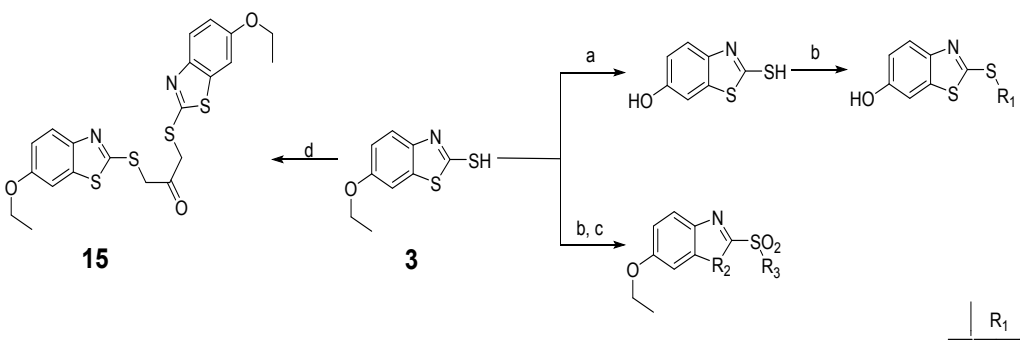
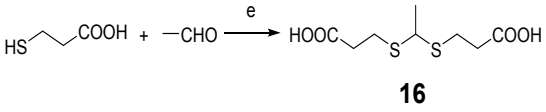
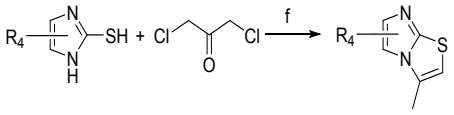
Supplemental Scheme 1. Synthesis of derivatives of the compound 1







(a) $AlCl_3$, $(CH_2Cl_2)_2$, rt, overnight.
(b) NaH, Alkylhalide, THF, 0° C, up to 2 h.
(c) *m*CPBA, THF, rt, overnight.
(d) NaH, 1,3-dichloroacetone, THF, 0° C, up to 2 h.
(e) $BF_3 \bullet OEt_2$, $CHCl_3$, rt, 2 h.
(f) K_2CO_3, KI, 18-cown-6, toluene, reflux, overnight.

	R_1
9	$-(CH_2)_2-Me$
10	$-CH(Me)_2$
11	$-(CH_2)_7-Me$
12	

	R_2	R_3
13	S=O	$-CH_2-COOH$
14	S	$-(CH_2)_2-COOH$

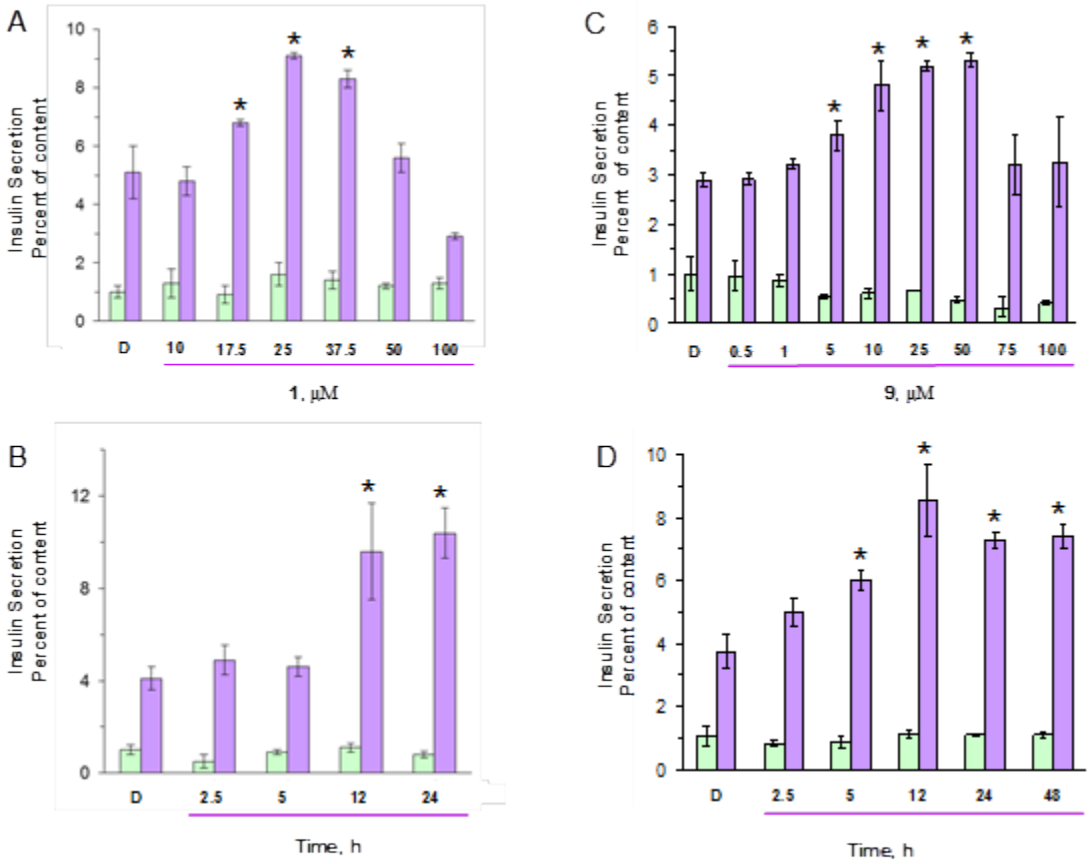
	R_4
17	H
18	
19	

Supplemental Table 1. Effect of test compounds on insulin secretion in INS-1E cells

Tested compound	Effect (EC_{50}, μM)
1	17.5±0.5
2	No effect
3	No effect
4	No effect
5	No effect
6	No effect
7	71±24.1
8	102±31.4
9	7.8±0.69
10	No effect
11	No effect
12	No effect
13	No effect
14	No effect
16	No effect
17	81±5.6
18	No effect
19	No effect

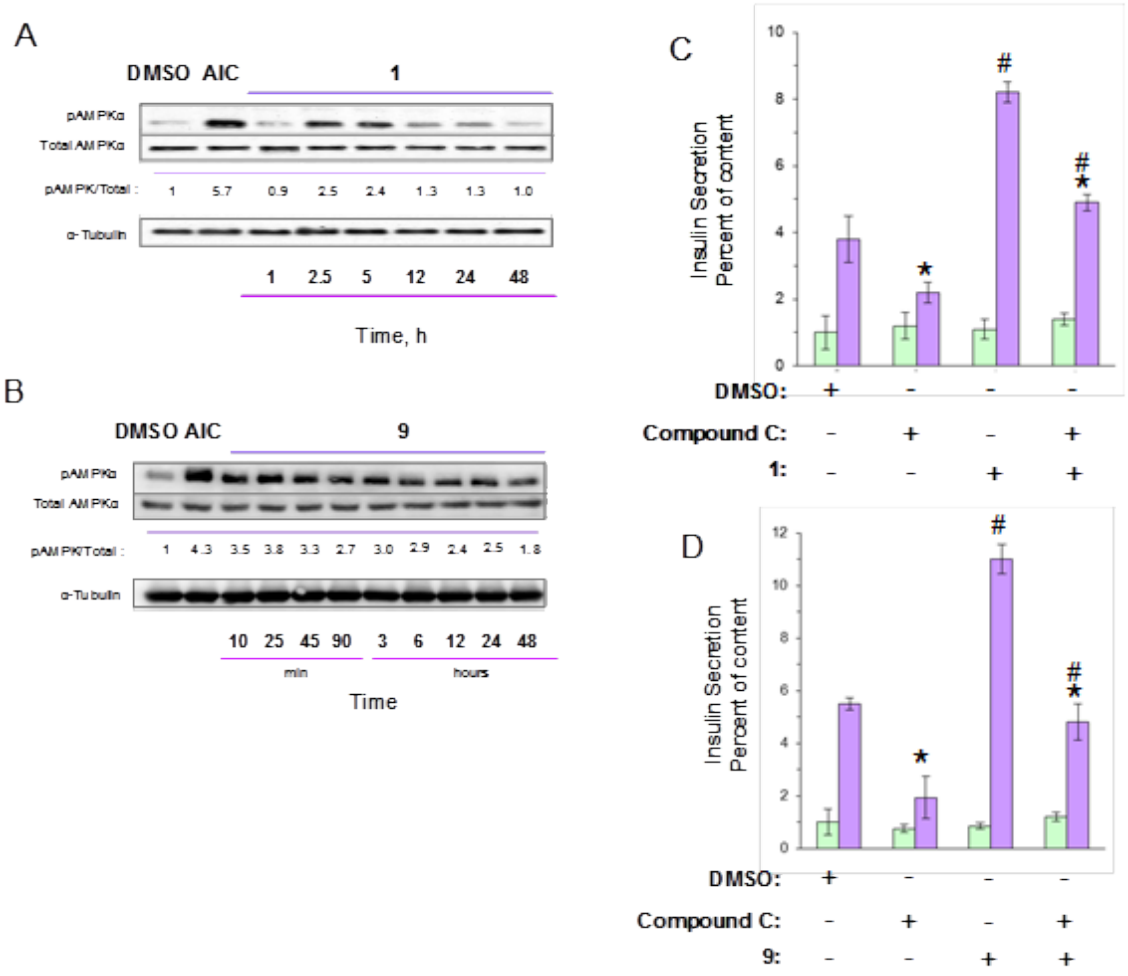
INS-1E cells were incubated with increasing concentrations of the indicated compounds for 12 h and then taken for the standard GSIS assay, as described below. EC_{50} values were calculated from the respective dose-response curves of the fold increase in insulin secretion at 16.7 mM glucose.

Supplemental Figure 1



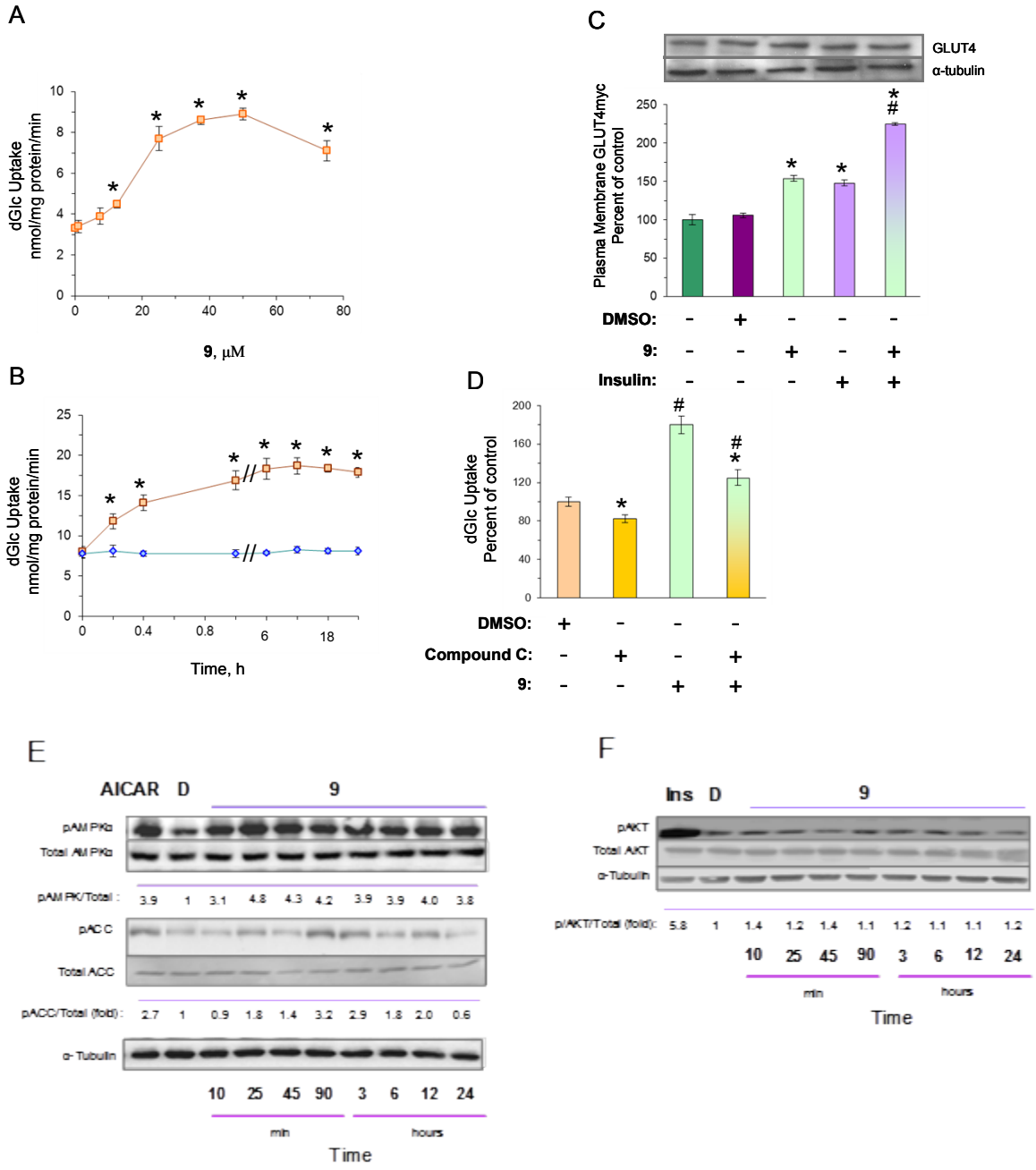
Role of AMPK in **1**- and **9**-induced insulin secretion. INS-1E cells were grown and treated with 25 μM of **1** (A) or 10 μM of **9** (B) for the indicated times. AICAR (AIC, 2 mM) was added for 2 h before the cells were lysed. Whole cell lysates were prepared at the indicated time periods and taken for Western blot analysis of AMPK and pThr^{172}-AMPK. WB of α-tubulin was used for protein loading control. The ratio of the band densities of AMPK and pThr^{172}-AMPK of the DMSO treated cells was taken as 1. Compounds **1** , 25 μM (C) and **9** , 10 μM (D**)** were added to INS-1E cells for 12 h, which were then treated for GSIS as described in the legend for Figure 1. Compound C (2.5 μM) was added to medium 1 h prior to the incubation with the test compounds.

Supplemental Figure 2



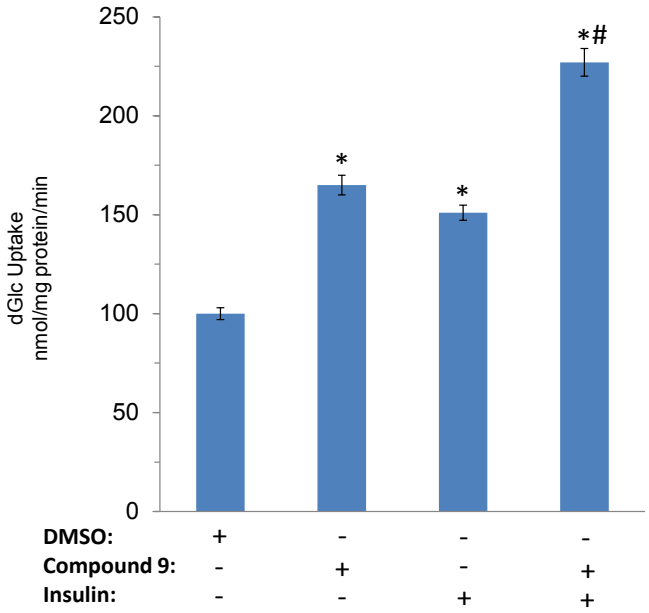
Dose- and time-dependent effect of **1** and **9** on insulin secretion from INS-1E cells. *Dose-response analysis*: the cells were grown and maintained as described. The culture medium was changed to fresh medium with increasing concentrations of 1 (A), 9 (C) or the vehicle [0.1% (v/v) DMSO; D] and incubated for 12 h. The cells were then taken for GSIS assay. Insulin content in the assay buffers and cell extracts was determined by RIA as described in under "Materials and Methods" in Supporting Information. *Time-course analysis*: INS-1E cells were incubated with 25 μM of 1 (B), 10 μM of 9 (D) or the vehicle (D) for the indicated periods of time. GSIS assay: green and purple bars represent insulin secretion following 1-h exposure to KRBH buffer containing 3.3 mM or 16.7 mM glucose, respectively. The secreted fraction of insulin is given as percent of total insulin content measured in whole cell extracts, which remained unaltered.

Supplemental Figure 3



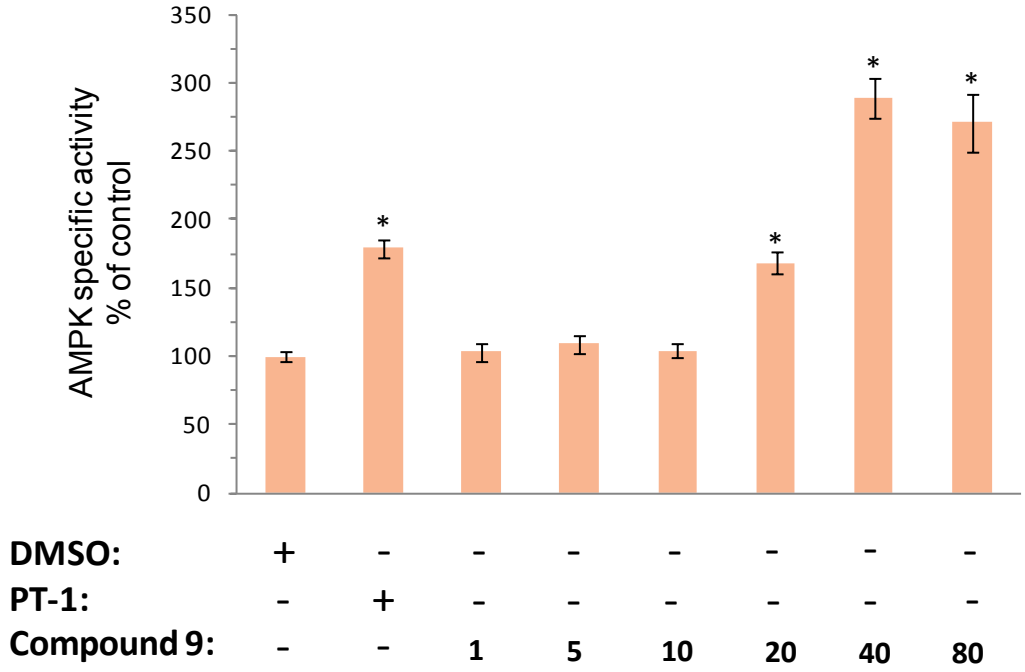
Compound **9** augments glucose uptake in L6 myotubes in an AMPK-dependent manner. (A) Dose response analysis: L6 myotubes were maintained in αMEM containing 23.0 mM glucose for 48 h, then washed and incubated with the same fresh medium supplemented with the indicated concentrations of **9** for 5 h. At the end of incubation the cells were taken for the standard [^{3}H]dGlc uptake assay. The basal rate of dGlc uptake of DMSO (0.1%, v/v)-treated L6 myotubes (3.2±0.11 nmol/mg protein/min) was taken as 100%. (B) Time course analysis: L6 myotubes were incubated with 37.5 μM of **9** (□) or the vehicle [DMSO, 0.1% (v/v)](◊), for the indicated periods of time. The cultures were then assayed for dGlc uptake at indicated times. The basal rate of [^{3}H]dGlc uptake at zero time (7.46 ±0.12 nmol/mg protein/min) was taken as 100%. (C) Compound **9** increased GLUT-4 abundance in the plasma membrane of L6 myotubes. L6 myotubes expressing GLUT-4myc were treated with 37.5 μM of **9** for 5 h and taken for cell surface transporter density determination as described in the under "Materials and Methods" in Supporting Information. DMSO-treated myotubes served as control. Insulin (100 nM) was introduced to cultures during the last 30 min of incubation. The representative Western blot depicts the total cell content of GLUT-4 in lysates of regular L6 myotube cultures. WB of α-tubulin was used as loading control. (D) Compound C (10 μM), **9** (37.5 μM) or DMSO (0.1%, v/v) were added to L6 myotubes as described in the legend to Figure 2. The cultures were then taken for the standard [^{3}H]dGlc assay. The rate of uptake at zero time (12.14 ±0.32 nmol/mg protein/min) was taken as 100%. (E) L6 myotubes treated with 37.5 μM of **9**. AICAR (2 mM) was added for 2 h. Whole cell lysates were prepared at the indicated time periods and taken for WB analysis of total or phosphorylated αAMPK and ACC. (F) L6 myotubes were treated with **9** or insulin as described above, and used for Western blot analysis of AKT and pAKT. Levels of α-tubulin were used as loading controls.

Supplemental Figure 4. Compound 9 and insulin augment rate of the glucose uptake in L6 myotubes in an additive manner



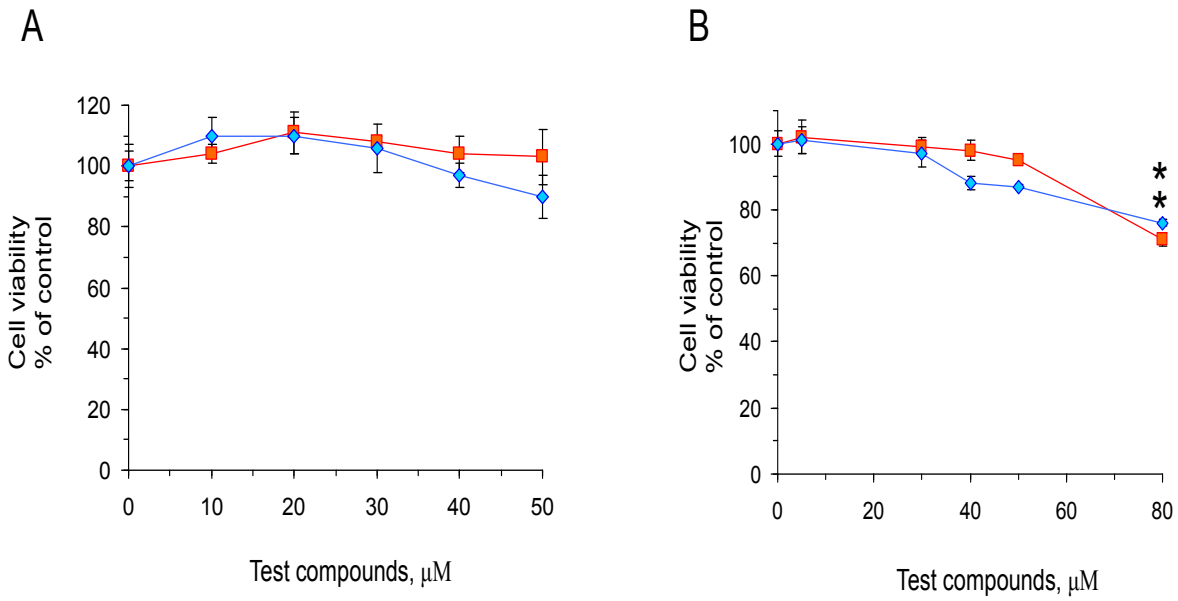
L6 myotubes expressing GLUT-4myc were treated with 25 μM of **9** for 5 h (with and without insulin). Insulin (100 nM) was introduced to cultures during the last 30 min of incubation. DMSO (0.1%, v/v) was used as a control treatment for the **9**. A this concentration DMSO does affect on rate of glucose transport. The cultures were then taken for the standard [^{3}H]dGlc assay as described in *Materials and Methods*. The rate of uptake of DMSO-treated cells (7.89 ±0.27 nmol/mg protein/min) was taken as 100%. Mean±SEM, n=3, *p<0.05 for differences between DMSO and treated by compound **9** and insulin-treated cells. #p <0.05 for differences between compound **9**- and insulin-treated cells.

Supplemental Figure 5. Compound 9 direct activates AMPK



The experiment was performed employing ADP-Glotm AMPK A1/B1/G1 kinase kit from SignalChem according to the manufacture protocol. The vehicle (DMSO, 0.1% v/v), PT-1 (20 μM) and **9** (at indicated concentrations) were added to the reaction mixture for 30 min at ambient temperature. The signals (the calibration curve and the compounds measurement) were detected by Synergy 4 Luminometer (BioTek Instruments, Winooski, VT). The basal AMPK specific activity with DMSO was taken as 100% (56.13±8.90 μmol/mg of AMPK/min), n=3, *$p<0.05$, in comparison with the respective controls.

Supplemental Figure 6. Compounds 1 and 9 do not compromise cell viability



INS-1E cells (**A**) and L6 myotubes (**B**) were grown and maintained as described in the legend to Figure 1. Increasing concentrations of **1** (blue circles) and **9** (red) were added to the medium and the cultures were incubated for additional 48 h. At the end of incubation period, the cultures were washed and incubated with the MTT reagent. The OD measurement for control INS-1E cells (2.96±0.6) or L6 myotubes (4.16±0.9) was were as a 100%. Mean±SEM, n=3, *p<0.05.

Materials

Human insulin (Actrapid) was purchased from Novo Nordisk (Bagsvaerd, Denmark). AICAR, BSA (bovine serum albumin, fraction V), 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT), compound C, dGlc, D-glucose, OPD, and the protease inhibitor cocktail were purchased from Sigma-Aldrich Chemicals (Rehovot, Israel). Glycerol and sodium fluoride were from Merck (Whitehouse Station, NJ). β-Mercaptoethanol, PMSF, sodium orthovanadate, sodium-β-glycerophosphate, sodium pyrophosphate and sodium dodecyl sulfate (SDS) were purchased from Alfa Aesar (Ward Hill, MA). PT-1 was supplied by Tocris (Bristol, UK). American Radiolabeled Chemicals (St. Louis, MO) supplied [^{3}H]dGlc [2.22 TBq/mmol (60 Ci/mmol)]. Linco Research Inc (St. Charles, MO) provided the ^{125}I labeled insulin. Antibodies against AMPKα, and pThr^{172}-AMPKα were from Cell Signaling Technology (Beverly, MD). Anti ACC, pSer^{79}-ACC and α-tubuline antibodies were from Millipore (Billerica, MA). The Anti-c-Myc (A-14) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The horseradish peroxidase-conjugated anti-rabbit IgG and the EZ-ECL chemoluminescence detection kit were from Jackson ImmunoResearch (West Grove, PA). Anti Akt/PKB (PH domain) and anti pSer^{473}-Akt/PKB antibodies were from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal anti-GLUT-4 antibodies were purchased from Abcame (Cambridge, MA); Goat serum, fetal calf serum (FCS), L-glutamine, α-MEM and antibiotics were purchased from Biological Industries (Beth-/Haemek, Israel). ADP-Glotm AMPK α 1 β1/γ1 kinase kit was purchased from SignalChem, Promega (Madison, Wisconsin, USA). Organic solvents (HPLC grade) were from Frutarom Ltd. (Haifa, Israel).

Synthetic procedures

General procedure for the synthesis of S-alkylbenzothiazoles (9-12). These compounds were synthesized according to the synthetic procedure for S-alkyl benzo-[*d*]thiazoles, using 2-mercaptobenzo[*d*]thiazol-6-ol[1] as the starting molecule[2].

General procedure for the ynthesis of (benzothiazole-sulfonyl)carboxylic acids (13-14). These compounds were synthesized according to the synthetic procedure described in the patent numbered WO2012129338 p. 254. Briefly, to a solution of 2-((6-ethoxybenzo[*d*]thiazol-2-yl)thio)acetic acid (0.03 g, 0.11 mmol) or 3-((6-ethoxybenzo[*d*]thiazol-2-yl)thio)propanoic acid (0.36 g, 1.27 mmol)[2] in DCM (10 mL) was added *m*-CPBA (0.06 g, 0.33 mmol and 0.66 g, 3.81 mmol, respectively) and the reaction mixture was stirred at room temperature for 16 h. The solution was then evaporated and purified by HPLC without quenching.

General procedure for the synthesis of 1,3-bis((6-ethoxybenzo[d]thiazol-2-yl)thio)propan-2-one (15). This compound was synthesized according to Di Nunno et al.[3] Briefly, to a stirred suspension of 6-ethoxy-2-mercaptobenzothiazole (1.00 g, 4.70 mmol) and $NaHCO_3$ (1.19 g, 14.2 mmol) in acetone (30 mL) was added dichloroacetone (1.19 g, 9.40 mmol in 20 mL acetone) dropwise. The reaction mixture was stirred under reflux overnight and then cooled to room temperature followed by further cooling to 0 °C and finally filtered. The filtrate was evaporated, dissolved in ethyl acetate and washed with 1N NaOH solution twice. The organic filtrates were combined, dried over Na_2SO_4 and filtered. Evaporation yielded in brown crude, which was purified using HPLC.

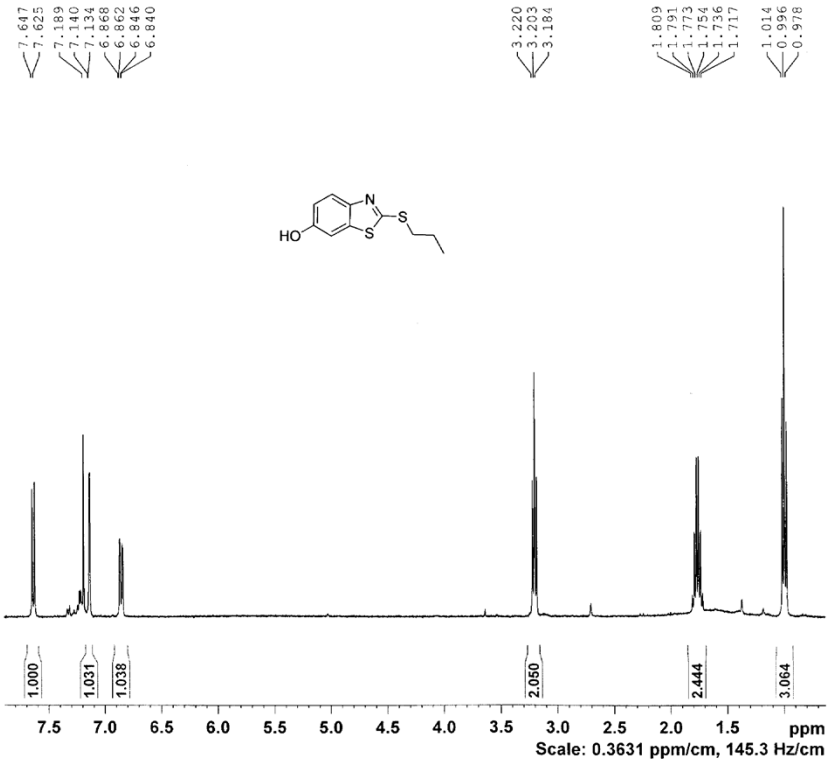
Synthesis of 3,3'-(ethane-1,1-diylbis(sulfanediyl))dipropionic acid (16). This compound was synthesized according to Fujita et al.[4] Briefly, to a stirred ice-cold solution of acetaldehyde (1.00 mL, 18.0 mmol) in $CHCl_3$ were added 3-mercaptopropionic acid (3.90 mL, 45.0 mmol) and boron trifluoro diethyl etherate (5.50 mL, 45.0 mmol). The reaction mixture was kept stirred under cooling for 2 h, and then diluted with $CHCl_3$ and poured into ice-cold water. The mixture was then acidified with concentrated HCl and extracted twice with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, filtered and evaporated to yield a clear yellow syrup. The crude was purified by precipitation of **19** as a sodium salt.

General procedure for the synthesis of 3-methylimidazo[2,1-*b*]thiazoles (17-19). These compounds were synthesized according to Abele et al.[5] Briefly, to a toluene (20 mL) solution of the appropriate mercaptoimidazole (1 equivalent), solid K_2CO_3 (4 equivalents) and solid KI (4 equivalents) were added 1,3-dichloropropane (1 equivalent) and freshly purified 18-crown-6 according to Gokel et al.[6] The reaction mixture was refluxed for 16 h, cooled to room temperature and filtered. The solid residue was washed three times with petroleum ether and ethyl acetate solution (1:1). The combined organic filtrates were evaporated to yield the desired product, which was further purified by HPLC.

Analytical data

The melting points of the various compounds were determined with Fisher-Johns melting point apparatus (Palmerton, PA). The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature in a Bruker Advanced NMR spectrometer (Vernon Hills, IL) operating at 200, 300, 400 and 600 MHz in accord with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) that was used as an internal standard. The samples were prepared by dissolving the synthesized compounds in DMSO-d_6 (δ_H = 2.50 ppm, δ_C = 39.52 ppm) or in $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.16 ppm). Chemical shifts were expressed in δ (ppm) and coupling constants (*J*) in hertz. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, unresolved multiplet due to the field strength of the instrument; dd, doublet of doublet. A QTof micro spectrometer (Micromass, Milford, MA) in the positive ion mode was used for mass spectrometry. Data were processed using massLynX v.4.1 calculation and deconvolution software (Waters Corporation, Milford, MA). Column chromatography was performed on Merck Silica gel 60 (230-400 mesh; Merck, Darmstadt, Germany). Analytical and preparative HPLC (Young Lin Instruments, Anyang, Korea) were performed on LUNA C18(2) preparative (10 μm, 100 x 30 mm) or analytical (5 μm, 250 x 4.6 mm) columns, both from Phenomenex Inc. (Torrance, CA). Acetonitrile and doubly distilled water were used as an eluent in different ratios. Analytical thin layer chromatography was carried out on pre-coated Merck Silica gel $60F_{254}$ (Merck) sheets using UV absorption and iodine physical adsorption for visualization. All the compounds gave satisfactory analytical results (within 0.4% of the theoretical values). The ≥95% purity of the final compounds was confirmed using HPLC analysis and elemental analysis.

2-(propylthio)benzo[*d*]thiazol-6-ol (9). Yield 40% white powder, mp 103-105 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.00 (t, 3H, *J* = 7.2 Hz, -$SCH_2CH_2C\underline{H}_3$), 1.76 (sextet, 2H, *J* = 7.4 Hz, -$SCH_2C\underline{H}_2CH_3$), 3.30 (t, 2H, *J* = 7.2 Hz, -$SC\underline{H}_2CH_2CH_3$), 6.85 (dd, 1H, *J* = 2.4, 6.4 Hz, HO-<u>Ph</u>-), 7.14 (d, 1H, *J* = 2.4 Hz, HO-<u>Ph</u>-), 7.64 (d, 1H, *J* = 8.8 Hz, HO-<u>Ph</u>-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 13.4 (-$SCH_2CH_2\underline{C}H_3$), 22.8 (-$SCH_2\underline{C}H_2CH_3$), 35.8 (-$S\underline{C}H_2CH_2CH_3$), 106.7 (HO-<u>Ph</u>-), 115.2 (HO-<u>Ph</u>-), 122.0 (HO-<u>Ph</u>-), 136.5 (HO-<u>Ph</u>-), 147.8 (HO-<u>Ph</u>-), 153.2 (HO-<u>Ph</u>-), 164.5 (HO-Ph-[*d*]<u>thiazole</u>-S-). MS (EI^+): 225.024 (M^+), 226.027 (MH^+). HRMS (EI^+) 225.0242 (M^+), 226.027 (MH^+). Anal. Calculated for $C_{10}H_{11}NOS_2$: C 53.30, H 4.92, N 6.22, S 28.46; Found: C 53.20, H 4.88, N 6.21, S 28.10. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 37.4 min.



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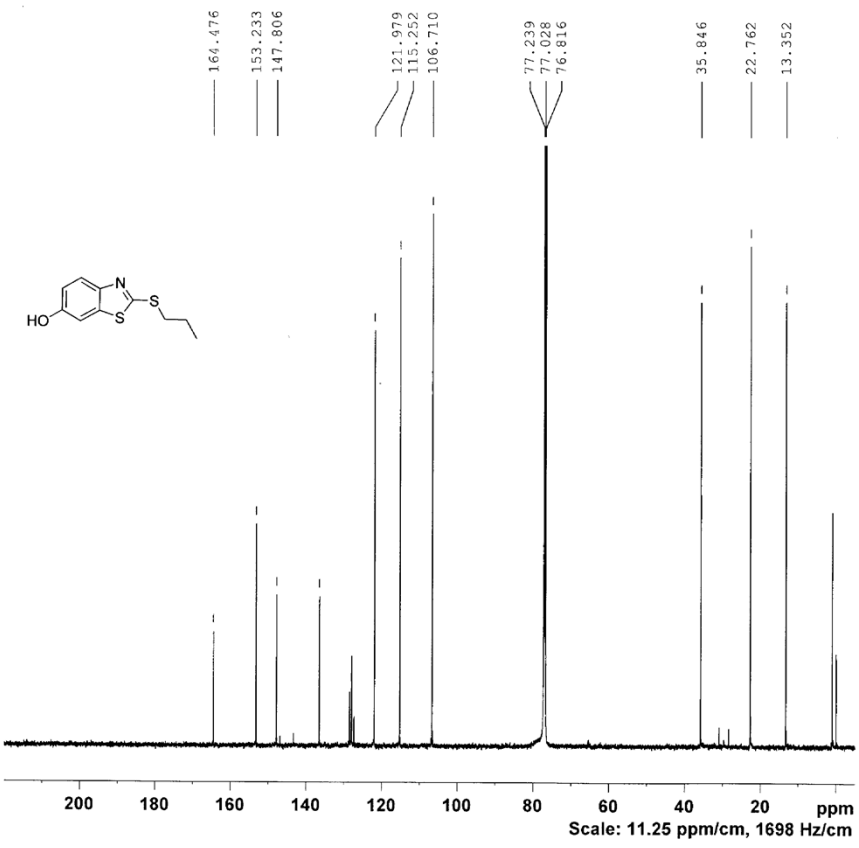

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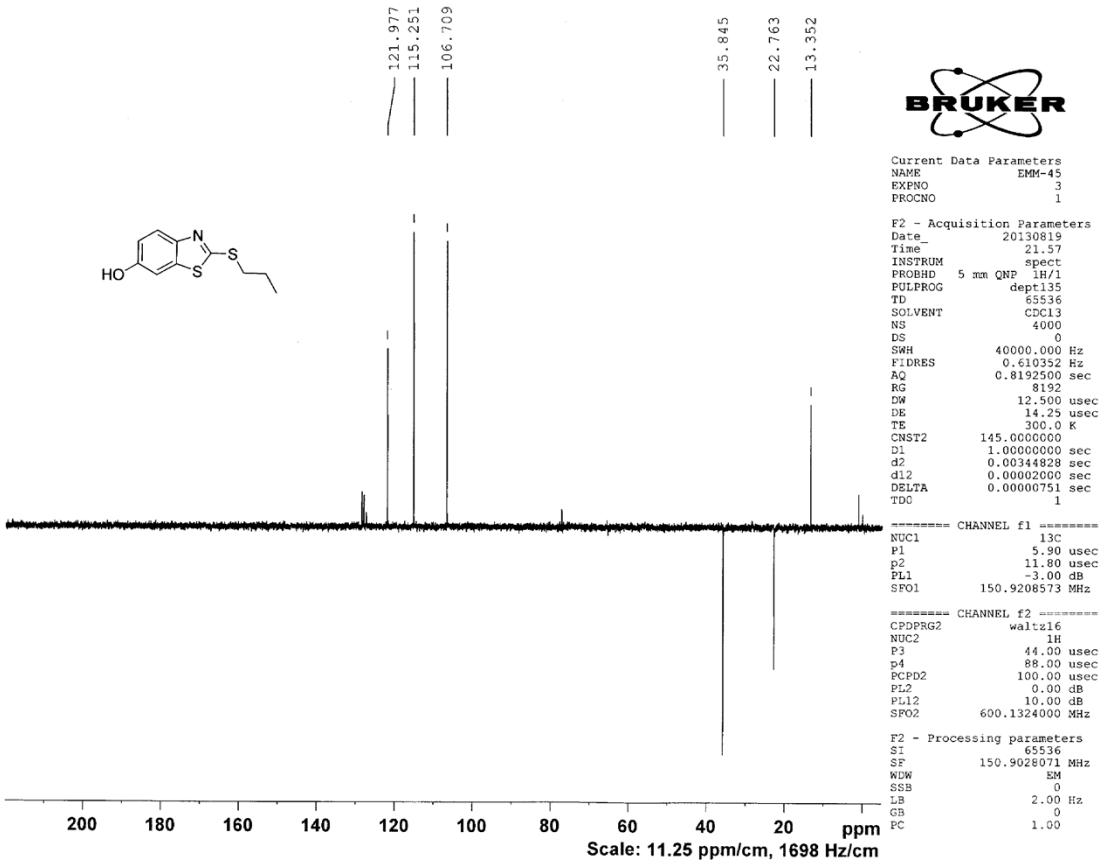

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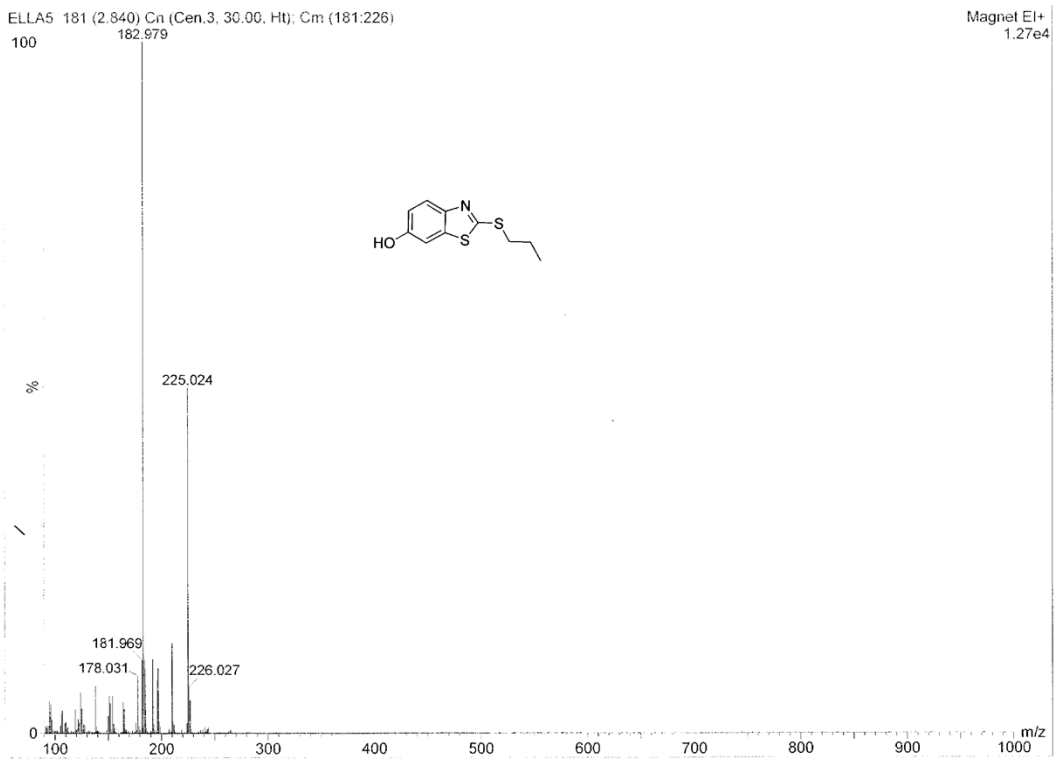
NAME                  EMM-45
EXPNO                      3
PROCNO                     1

F2 - Acquisition Parameters
Date_              20130819
Time                  21.57
INSTRUM                spect
PROBHD    5 mm QNP  1H/1
PULPROG             dept135
TD                    65536
SOLVENT                CDC13
NS                      4000
DS                         0
SWH               40000.000 Hz
FIDRES             0.610352 Hz
AQ                 0.8192500 sec
RG                      8192
DW                    12.500 usec
DE                    14.25 usec
TE                    300.0 K
CNST2             145.0000000
D1                 1.00000000 sec
d2                 0.00344828 sec
d12                0.00002000 sec
DELTA              0.00000751 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                     13C
P1                     5.90 usec
p2                    11.80 usec
PL1                   -3.00 dB
SFO1              150.9208573 MHz

======== CHANNEL f2 ========
CPDPRG2               waltz16
NUC2                     1H
P3                    44.00 usec
p4                    88.00 usec
PCPD2                100.00 usec
PL2                   0.00 dB
PL12                  10.00 dB
SFO2              600.1324000 MHz

F2 - Processing parameters
SI                       65536
SF                150.9028071 MHz
WDW                         EM
SSB                          0
LB                    2.00 Hz
GB                           0
PC                    1.00
```

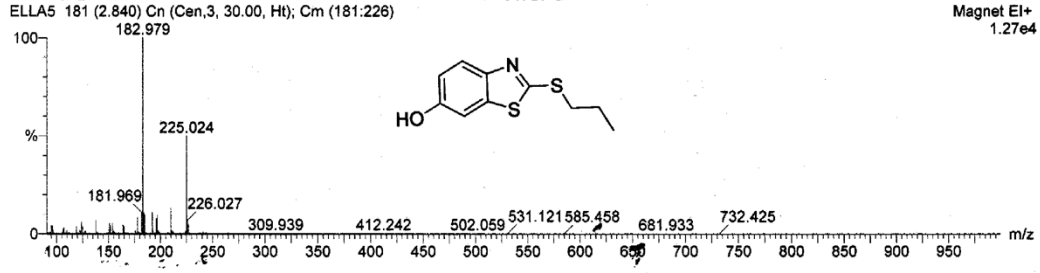


Elemental Composition Report **Page 1**

Multiple Mass Analysis: 2 mass(es) processed
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

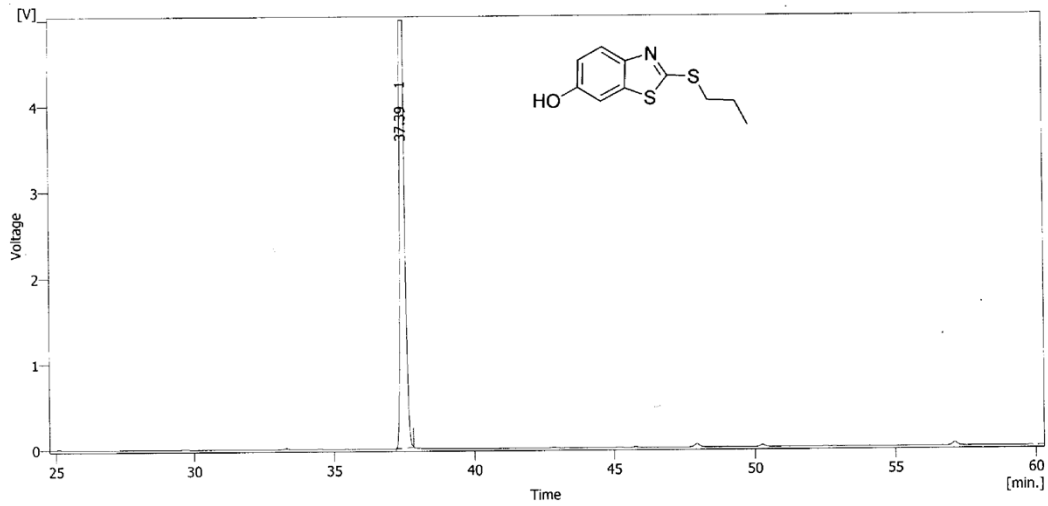
Monoisotopic Mass, Odd and Even Electron Ions
71587 formula(e) evaluated with 9 results within limits (up to 50 closest results for each mass)

EMM-45 EI 17-Oct-2012
ELLA5 181 (2.840) Cn (Cen,3, 30.00, Ht); Cm (181:226) Magnet EI+ 1.27e4



Minimum:	15.00				-1.5		
Maximum:	100.00		5.0	5.0	50.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
182.9792	100.00	182.9786	0.6	3.4	1.5	n/a	12C4 1H7 16O4 32S2
		182.9779	1.3	7.2	11.0	n/a	12C10 1H 16O 14N 32S
		182.9813	-2.1	-11.2	6.0	n/a	12C7 1H5 16O 14N 32S2
		182.9752	4.0	21.8	6.5	n/a	12C7 1H3 16O4 32S
225.0242	49.88	225.0248	-0.6	-2.8	11.0	n/a	12C13 1H7 16O 14N 32S
		225.0255	-1.3	-5.9	1.5	n/a	12C7 1H13 16O4 32S2
		225.0222	2.0	9.1	6.5	n/a	12C10 1H9 16O4 32S
		225.0215	2.7	12.2	16.0	n/a	12C16 1H3 16O 14N
		225.0282	-4.0	-17.8	6.0	n/a	12C10 1H11 16O 14N 32S2

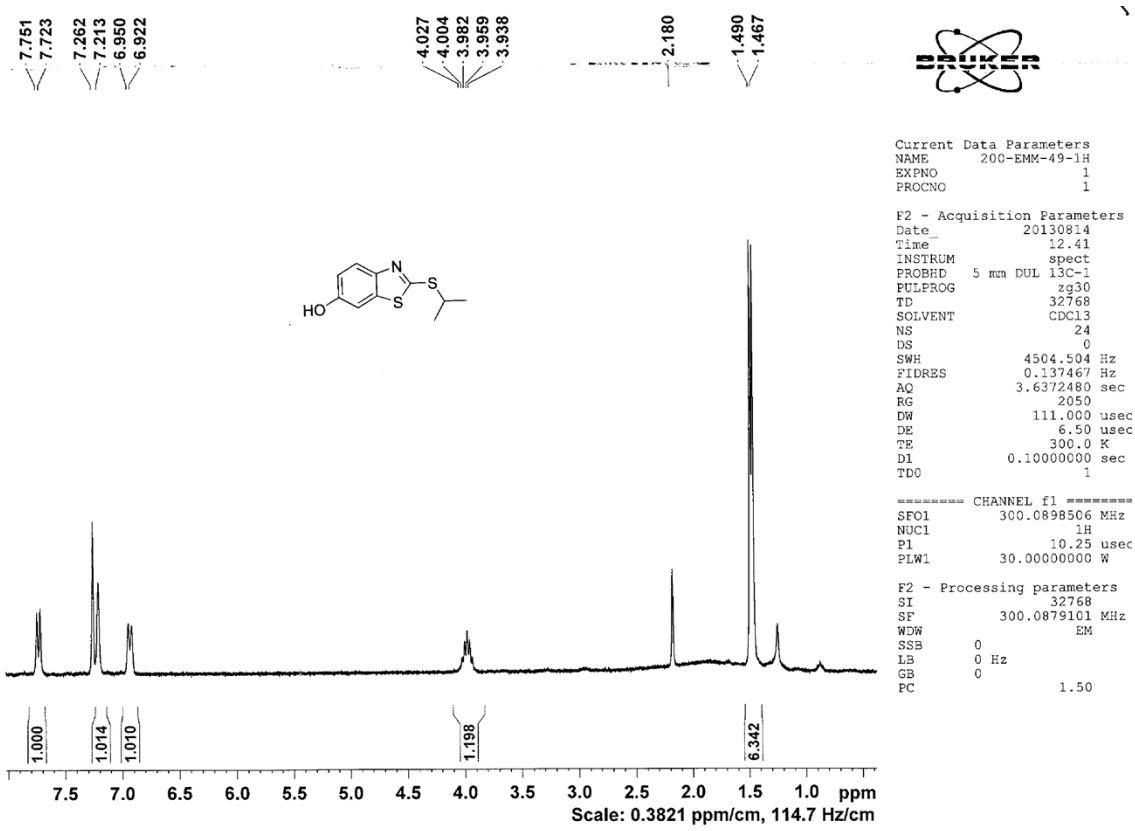
09/2013 12:17 Chromatogram E:\EMM-45.PRM Page 2 of 2



Result Table (Uncal - E:\EMM-45 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	37.393	65856.839	4992.630	100.0	100.0	0.19	808	
	Total	65856.839	4992.630	100.0	100.0			

2-(isopropylthio)benzo[*d*]thiazol-6-ol (10). Yield 50% white powder, mp too broad to be determined. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.48 (d, 6H, *J* = 6.9 Hz, -SCH($C\underline{H}_3$)$_2$), 3.98 (quint, 1H, *J* = 6.7 Hz, -SC$\underline{H}$(CH_3)$_2$), 6.94 (d, 1H, *J* = 8.4 Hz, HO-$\underline{Ph}$-), 7.21 (s, 1H, HO-$\underline{Ph}$-), 7.74 (d, 1H, *J* = 8.4 Hz, HO-$\underline{Ph}$-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 23.4 (-SCH($\underline{C}H_3$)$_2$), 39.8 (-S$\underline{C}$H(CH_3)$_2$), 106.6 (HO-$\underline{Ph}$-), 115.2 (HO-$\underline{Ph}$-), 122.3 (HO-$\underline{Ph}$-), 136.9 (HO-$\underline{Ph}$-), 148.0 (HO-$\underline{Ph}$-), 153.2 (HO-$\underline{Ph}$-), 163.3 (HO-Ph-[*d*]$\underline{thiazole}$-S-). MS (EI$^+$): 225.032 (M^+), 226.040 (MH^+). HRMS (CI$^+$) 225.032 (M^+), 226.0404 (MH^+). HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 37.2 min.

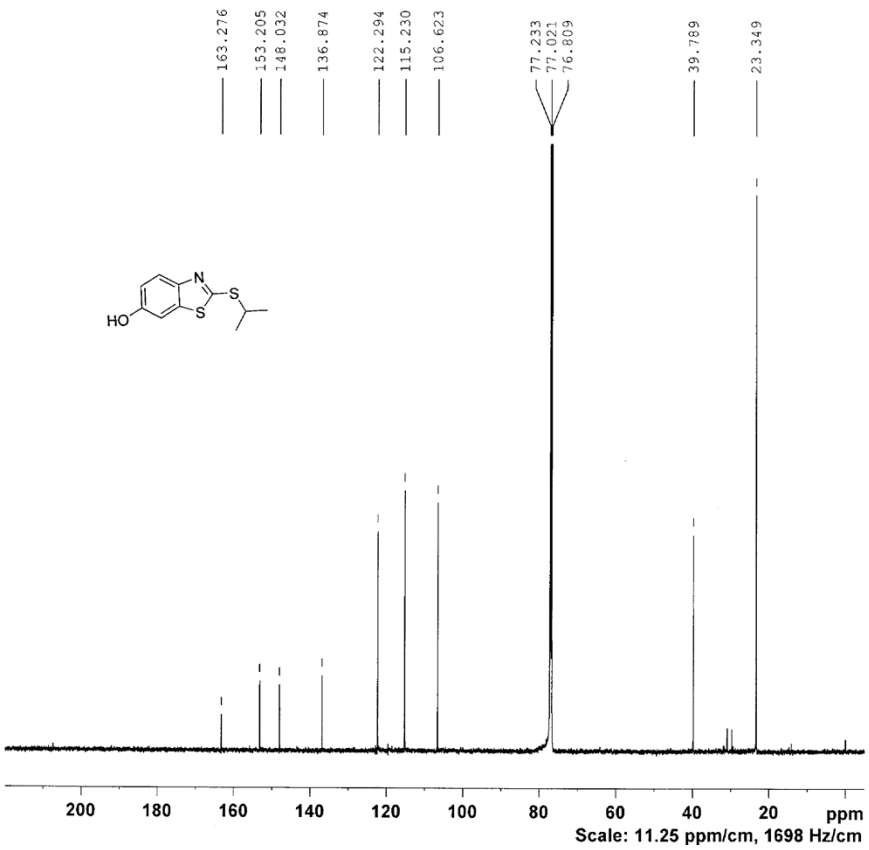


Current Data Parameters
NAME 200-EMM-49-1H
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20130814
Time 12.41
INSTRUM spect
PROBHD 5 mm DUL 13C-1
PULPROG zg30
TD 32768
SOLVENT CDCl3
NS 24
DS 0
SWH 4504.504 Hz
FIDRES 0.137467 Hz
AQ 3.6372480 sec
RG 2050
DW 111.000 usec
DE 6.50 usec
TE 300.0 K
D1 0.10000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 300.0898506 MHz
NUC1 1H
P1 10.25 usec
PLW1 30.00000000 W

F2 - Processing parameters
SI 32768
SF 300.0879101 MHz
WDW EM
SSB 0
LB 0 Hz
GB 0
PC 1.50



BRUKER

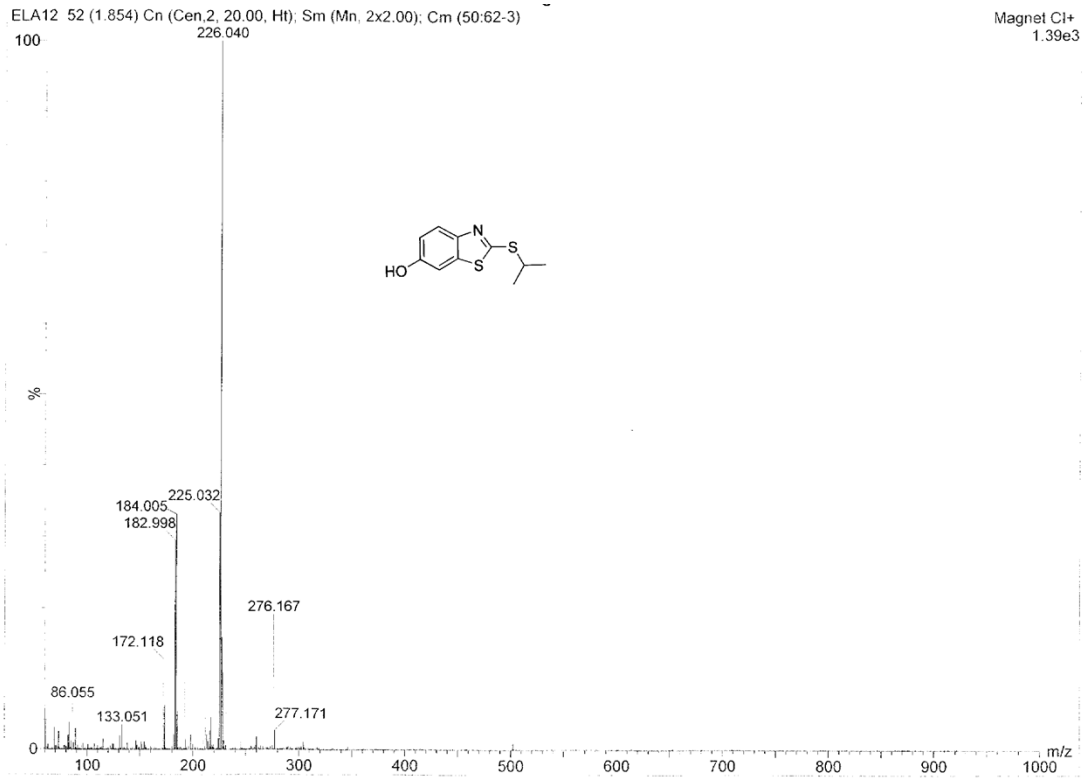
Current Data Parameters
NAME EMM-49
EXPNO 2
PROCNO 1

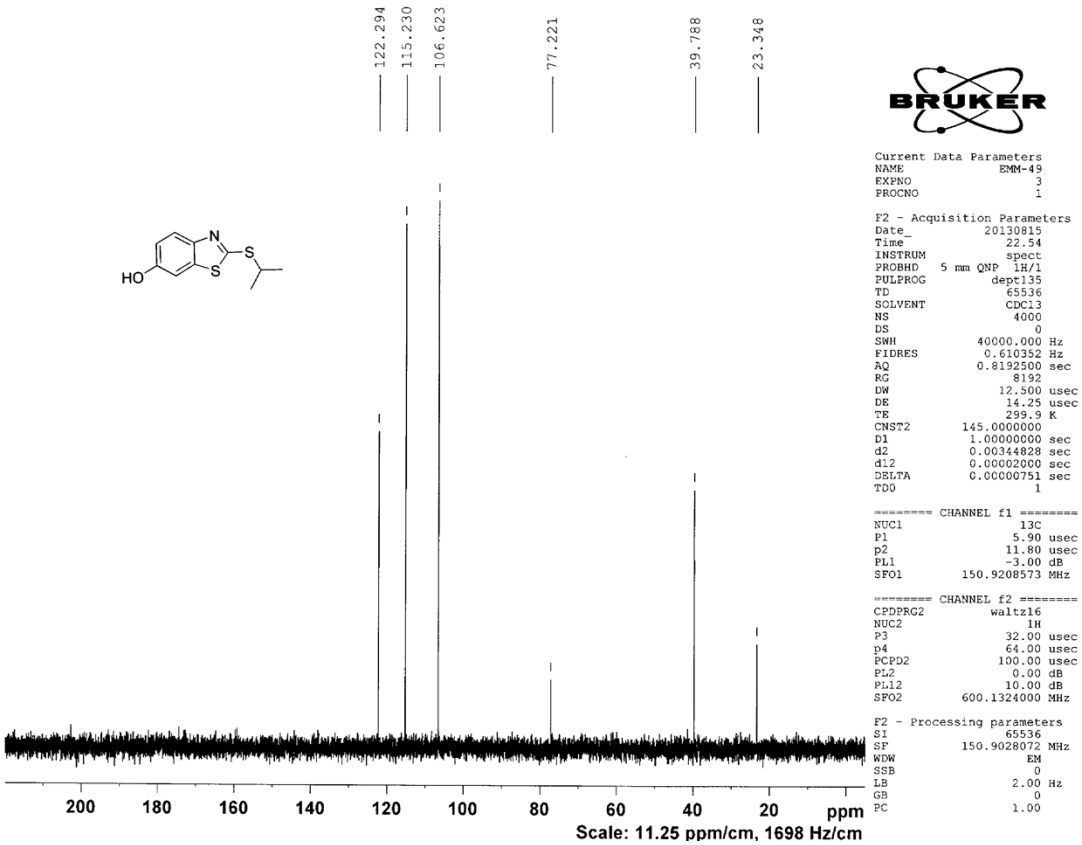
F2 - Acquisition Parameters
Date_ 20130815
Time 15.06
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG zgdc30
TD 65536
SOLVENT CDCl3
NS 40000
DS 0
SWH 40000.000 Hz
FIDRES 0.610352 Hz
AQ 0.8192500 sec
RG 8192
DW 12.500 usec
DE 14.25 usec
TE 300.1 K
D1 0.50000000 sec
d11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 5.90 usec
PL1 -3.00 dB
SFO1 150.9208573 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 100.00 usec
PL2 0.00 dB
PL12 10.00 dB
SFO2 600.1324000 MHz

F2 - Processing parameters
SI 65536
SF 150.9028072 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00





BRUKER

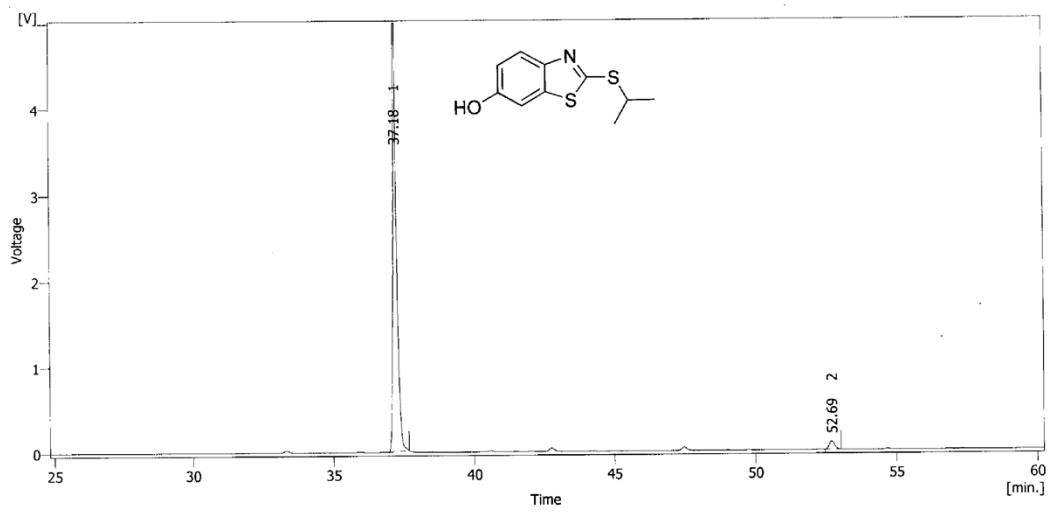
```
Current Data Parameters
NAME                  EMM-49
EXPNO                      3
PROCNO                     1

F2 - Acquisition Parameters
Date_              20130815
Time                  22.54
INSTRUM               spect
PROBHD   5 mm QNP  1H/1
PULPROG             dept135
TD                    65536
SOLVENT               CDCl3
NS                     4000
DS                        0
SWH               40000.000 Hz
FIDRES            0.610352 Hz
AQ                0.8192500 sec
RG                     8192
DW                  12.500 usec
DE                  14.25 usec
TE                   299.9 K
CNST2             145.0000000
D1                1.00000000 sec
d2                0.00344828 sec
d12               0.00002000 sec
DELTA             0.00000751 sec
TD0                        1

========== CHANNEL f1 ========
NUC1                     13C
P1                    5.90 usec
p2                   11.80 usec
PL1                  -3.00 dB
SFO1           150.9208573 MHz

========== CHANNEL f2 ========
CPDPRG2              waltz16
NUC2                     1H
P3                   32.00 usec
P4                   64.00 usec
PCPD2               100.00 usec
PL2                   0.00 dB
PL12                 10.00 dB
SFO2           600.1324000 MHz

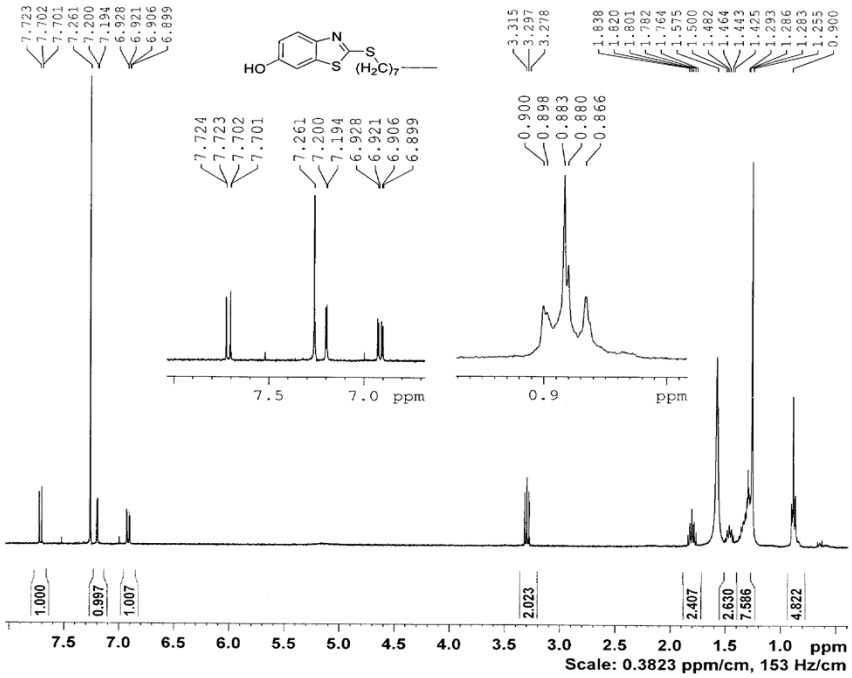
F2 - Processing parameters
SI                    65536
SF             150.9028072 MHz
WDW                      EM
SSB                       0
LB                   2.00 Hz
GB                        0
PC                     1.00
```



Result Table (Uncal - E:\EMM-49 - Channel 1)

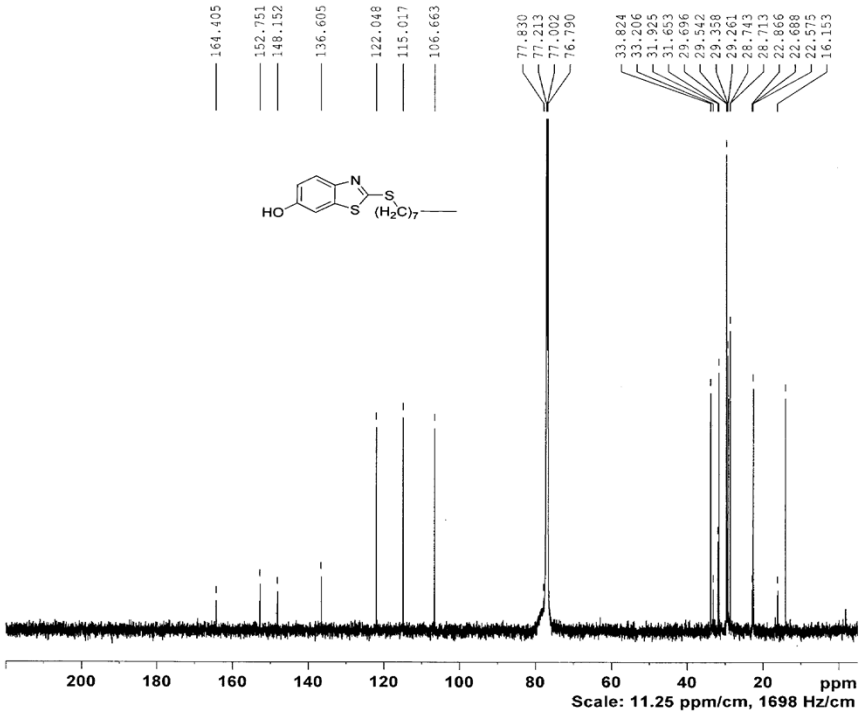
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	37.183	45612.484	4990.840	97.4	98.1	0.12	767	
2	52.687	1240.294	95.726	2.6	1.9	0.20	883	
	Total	46852.778	5086.566	100.0	100.0			

2-(octylthio)benzo[*d*]thiazol-6-ol (11). Yield 65% amorphic clear solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 – 0.09 (m, 3H, -S(CH_2)$_7$C$\underline{H}_3$), 1.26−1.29 (m, 6H, -S(CH_2)$_3$($C\underline{H}_2$)$_4$$CH_3$), 1.46 (quint, 2H, *J* = 7.5 Hz, -S$CH_2$$CH_2$$C\underline{H}_2$($CH_2$)$_4$$CH_3$), 1.80 (quint, 2H, *J* = 7.4 Hz, -S$CH_2$$C\underline{H}_2$$CH_2$($CH_2$)$_4$$CH_3$) 3.30 (t, 2H, *J* = 7.4 Hz, -S$\underline{CH}_2$(CH_2)$_6$$CH_3$), 6.91 (dd, 1H, *J* = 2.8, 6.0 Hz, HO-$\underline{Ph}$-), 7.20 (d, 1H, *J* = 2.4 Hz, HO-$\underline{Ph}$-), 7.71 (d, 1H, *J* = 8.4 Hz, HO-$\underline{Ph}$-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 14.0 (-S(CH_2)$_7$$\underline{C}H_3$), 22.6 (-S($\underline{C}H_2$)$_7$$CH_3$), 28.7 (-S($\underline{C}H_2$)$_7$$CH_3$), 29.3 (-S($\underline{C}H_2$)$_7$$CH_3$), 29.7 (-S($\underline{C}H_2$)$_7$$CH_3$), 31.6 (-S($\underline{C}H_2$)$_7$$CH_3$), 33.8 (-S$\underline{C}H_2$($CH_2$)$_6$$CH_3$), 106.7 (HO-$\underline{Ph}$-), 115.0 (HO-$\underline{Ph}$-), 122.0 (HO-$\underline{Ph}$-), 136.6 (HO-$\underline{Ph}$-), 148.2 (HO-$\underline{Ph}$-), 152.8 (HO-$\underline{Ph}$-), 164.4 (HO-Ph-[*d*]$\underline{thiazole}$-S-). MS ($ES^+$): 282 ($MH^+$). HPLC (gradient water/$CH_3CN$ from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 51.8 min.




```
NAME                  EMM-47
EXPNO                      2
PROCNO                     1
Date_               20130825
Time_                  12.14
INSTRUM                spect
PROBHD   5 mm PABBO BB-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        13
DS                         0
SWH                7183.908 Hz
FIDRES             0.109618 Hz
AQ                 4.5613556 sec
RG                       362
DW                    69.600 usec
DE                     6.50 usec
TE                    299.1 K
D1                 0.10000000 sec
TD0                        1

======== CHANNEL f1 ========
NUC1                      1H
P1                     10.10 usec
PL1                     0.00 dB
SFO1          400.1032008 MHz
SI                     32768
SF            400.1000104 MHz
WDW                       EM
SSB                        0
LB                     0.00 Hz
GB                         0
PC                      1.00
```



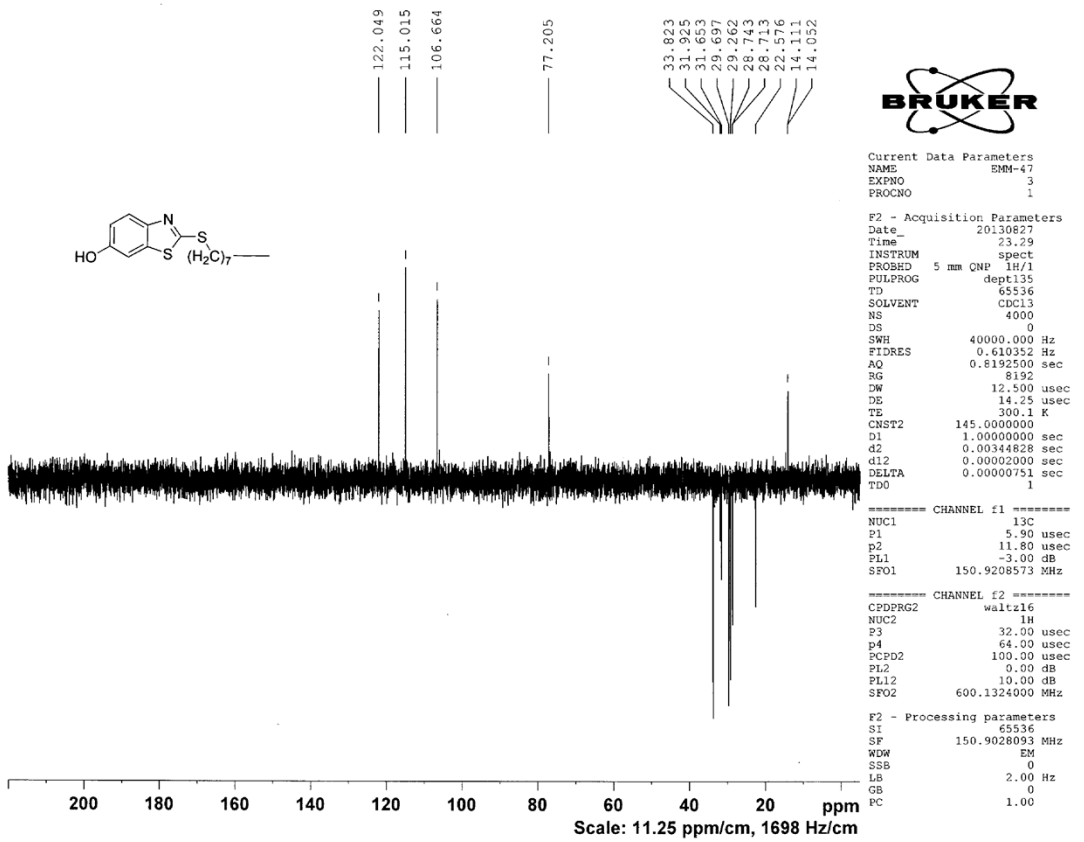

```
Current Data Parameters
NAME                  EMM-47
EXPNO                      2
PROCNO                     1

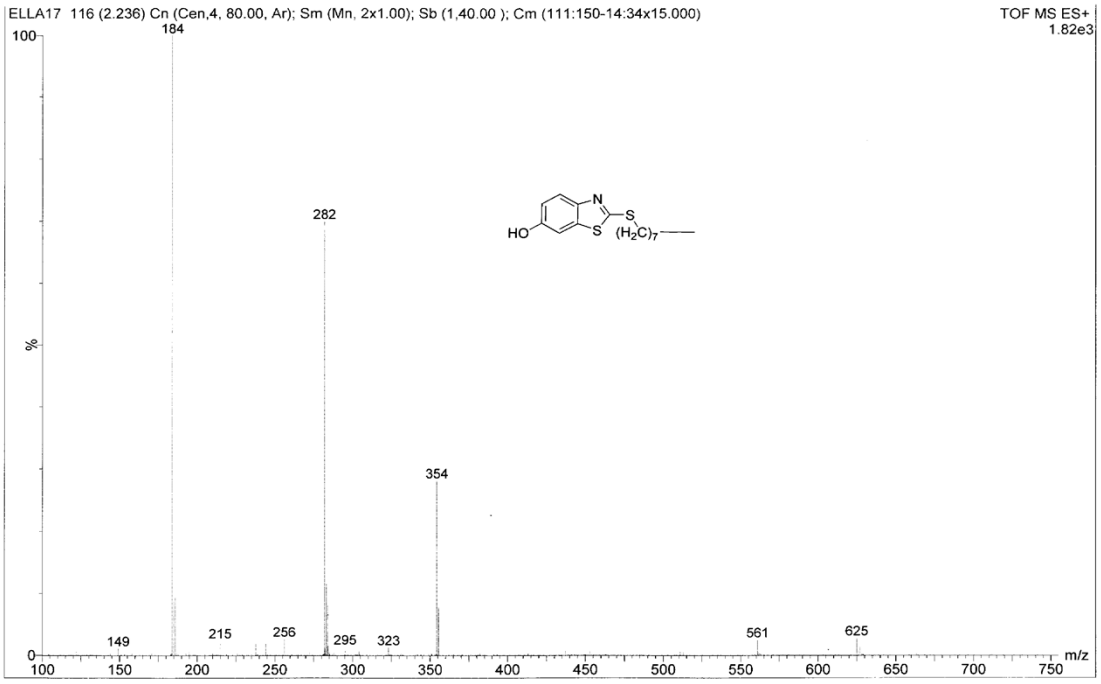
F2 - Acquisition Parameters
Date_               20130827
Time_                  15.10
INSTRUM                spect
PROBHD   5 mm QNP  1H/1
PULPROG             zgdc30
TD                    65536
SOLVENT               CDCl3
NS                        39068
DS                         0
SWH               40000.000 Hz
FIDRES             0.610352 Hz
AQ                 0.8192500 sec
RG                      8192
DW                    12.500 usec
DE                     14.25 usec
TE                    300.1 K
D1                 0.50000000 sec
d11                0.03000000 sec
TD0                        1

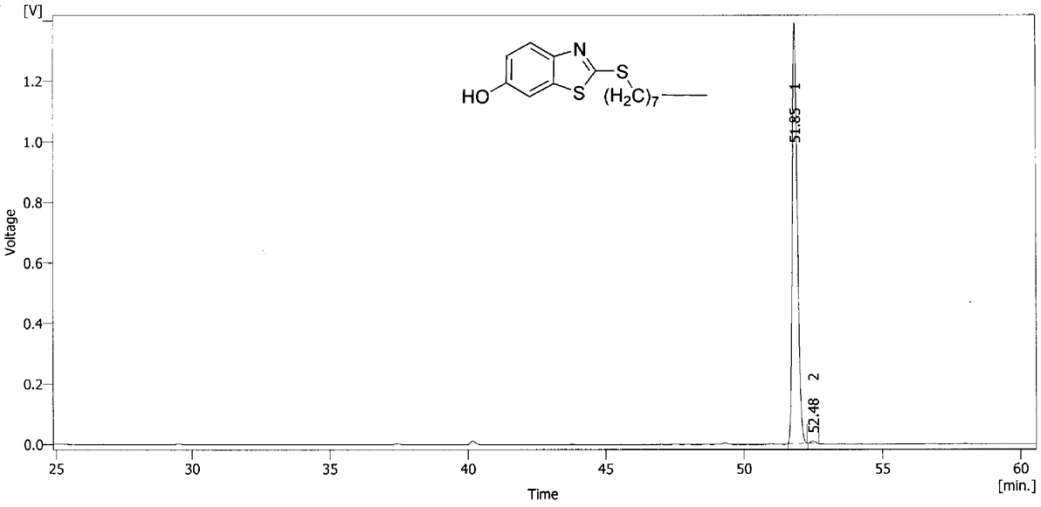
======== CHANNEL f1 ========
NUC1                      13C
P1                      5.90 usec
PL1                    -3.00 dB
SFO1          150.9208573 MHz

======== CHANNEL f2 ========
CPDPRG2               waltz16
NUC2                      1H
PCPD2                 100.00 usec
PL2                     0.00 dB
PL12                   10.00 dB
SFO2          600.1324000 MHz

F2 - Processing parameters
SI                        65536
SF            150.9028093 MHz
WDW                       EM
SSB                        0
LB                     2.00 Hz
GB                         0
PC                      1.00
```


122.049
115.015
106.664
77.205
33.823
31.925
31.653
29.697
29.262
28.743
28.713
22.576
14.111
14.052
BRUKER
HO
N
S
S
(H2C)7
200
180
160
140
120
100
80
60
40
20
ppm
Scale: 11.25 ppm/cm, 1698 Hz/cm
Current Data Parameters
NAME EMM-47
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130827
Time 23.29
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 4000
DS 0
SWH 40000.000 Hz
FIDRES 0.610352 Hz
AQ 0.8192500 sec
RG 8192
DW 12.500 usec
DE 14.25 usec
TE 300.1 K
CNST2 145.0000000
D1 1.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000751 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 5.90 usec
p2 11.80 usec
PL1 -3.00 dB
SFO1 150.9208573 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 32.00 usec
p4 64.00 usec
PCPD2 100.00 usec
PL2 0.00 dB
PL12 10.00 dB
SFO2 600.1324000 MHz
F2 - Processing parameters
SI 65536
SF 150.9028093 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00

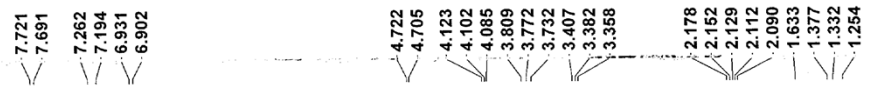

ELLA17 116 (2.236) Cn (Cen,4, 80.00, Ar); Sm (Mn, 2x1.00); Sb (1,40.00); Cm (111:150-14:34x15.000)
TOF MS ES+
1.82e3
100
%
0
184
282
354
149
215
256
295
323
561
625
HO
N
S
S
(H2C)7
100
150
200
250
300
350
400
450
500
550
600
650
700
750
m/z

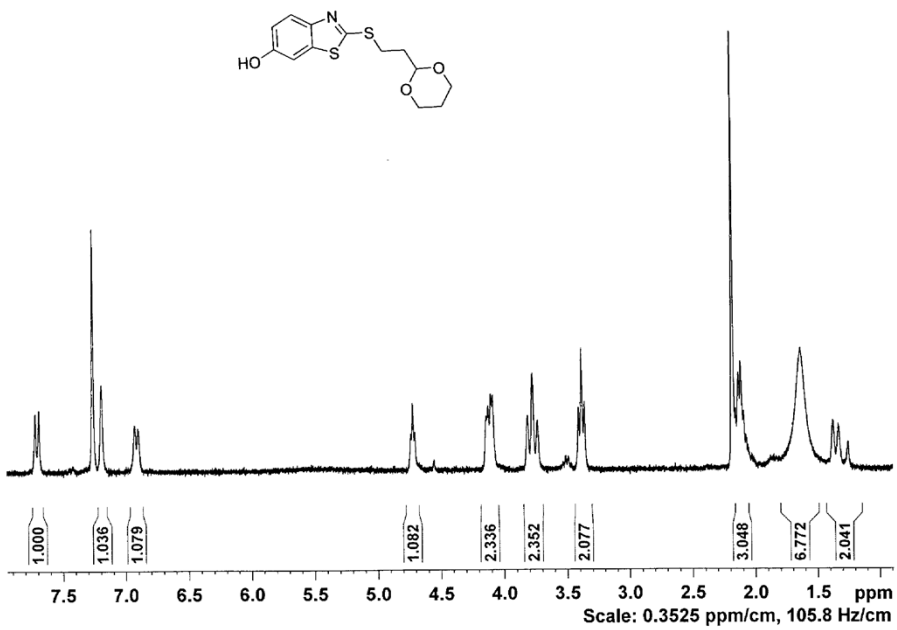


Result Table (Uncal - E:\EMM-47 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	51.850	17625.296	1392.347	99.5	99.4	0.20	791	
2	52.483	93.409	8.563	0.5	0.6	0.18	993	
	Total	17718.705	1400.910	100.0	100.0			

2-((2-(1,3-dioxan-2-yl)ethyl)thio)benzo[*d*]thiazol-6-ol (12). Yield 30% white powder, mp too broad to be determined. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.24 – 1.38 (m, 2H, -$SCH_2CH_2(C_4\underline{H}_7O_2)$), 2.09−2.15 (q, 2H, *J* = 6.2 Hz, -$SCH_2C\underline{H}_2(C_4H_7O_2)$), 3.38 (t, 2H, *J* = 7.4 Hz, -$SC\underline{H}_2CH_2(C_4H_7O_2)$), 3.77 (t, 2H, *J* = 11.6 Hz, -$SCH_2CH_2(C_4\underline{H}_7O_2)$), 4.08 – 4.12 (m, 2H, -$SCH_2CH_2(C_4\underline{H}_7O_2)$), 4.72 (t, 1H, *J* = 5.1 Hz, -$SCH_2CH_2(C_4\underline{H}_7O_2)$), 6.92 (d, 1H, *J* = 8.7 Hz, HO-Ph-), 7.19 (s, 1H, HO-Ph-), 7.71 (d, 1H, *J* = 9.0 Hz, HO-Ph-). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 25.7 (-$SCH_2CH_2(\underline{C}_4H_7O_2)$), 28.4 (-$S\underline{C}H_2CH_2(C_4H_7O_2)$), 34.7 (-$SCH_2\underline{C}H_2(C_4H_7O_2)$), 66.9 (-$SCH_2CH_2(\underline{C}_4H_7O_2)$), 100.4 (HO-Ph-), 106.7 (HO-Ph-), 115.1 (HO-Ph-), 122.1 (HO-Ph-), 136.6 (HO-Ph-), 147.9 (HO-Ph-), 153.1 (HO-Ph-), 163.7 (HO-Ph-[*d*]thiazole-S-). MS (CI^+): 297.066 (M^+), 298.062 (MH^+). HRMS (CI^+) 297.0664 (M^+), 298.0621 (MH^+). HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 32.8 min.






```
Current Data Parameters
NAME      200-EMM-57 fr.34-39-1
EXPNO                         2
PROCNO                        1

F2 - Acquisition Parameters
Date_               20130814
Time                   12.48
INSTRUM                spect
PROBHD    5 mm DUL 13C-1
PULPROG                zg30
TD                    32768
SOLVENT               CDCl3
NS                       24
DS                        0
SWH                4504.504 Hz
FIDRES            0.137467 Hz
AQ                3.6372480 sec
RG                     2050
DW                 111.000 usec
DE                    6.50 usec
TE                   300.0 K
D1              0.10000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            300.0898506 MHz
NUC1                      1H
P1                     10.25 usec
PLW1            30.00000000 W

F2 - Processing parameters
SI                    32768
SF              300.0879096 MHz
WDW                      EM
SSB          0
LB           0 Hz
GB           0
PC                     1.50
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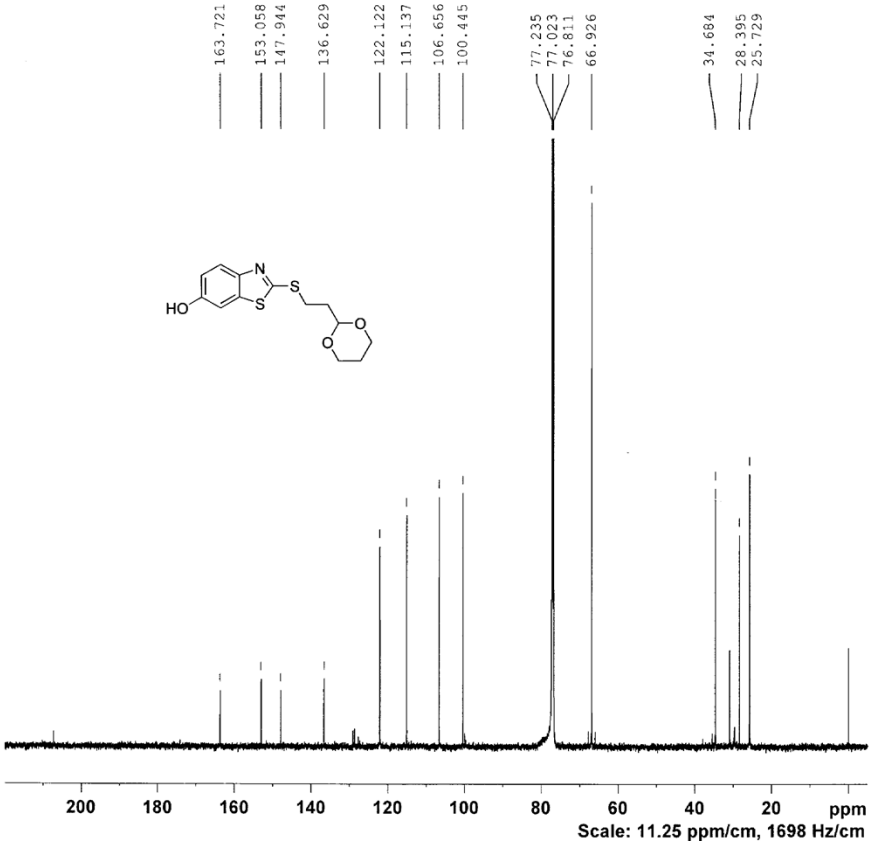


```
Current Data Parameters
NAME                  EMM-57
EXPNO                      2
PROCNO                     1

F2 - Acquisition Parameters
Date_            20130818
Time                13.38
INSTRUM             spect
PROBHD   5 mm QNP  1H/1
PULPROG          zgdc30
TD                65536
SOLVENT           CDCl3
NS                40000
DS                    0
SWH           40000.000 Hz
FIDRES         0.610352 Hz
AQ             0.8192500 sec
RG                 8192
DW               12.500 usec
DE               14.25 usec
TE                296.6 K
D1          0.50000000 sec
d11         0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 13C
P1                5.90 usec
PL1              -3.00 dB
SFO1       150.9208573 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2           100.00 usec
PL2               0.00 dB
PL12             10.00 dB
SFO2       600.1324000 MHz

F2 - Processing parameters
SI                65536
SF          150.9028083 MHz
WDW                  EM
SSB                   0
LB                 2.00 Hz
GB                    0
PC                 1.00
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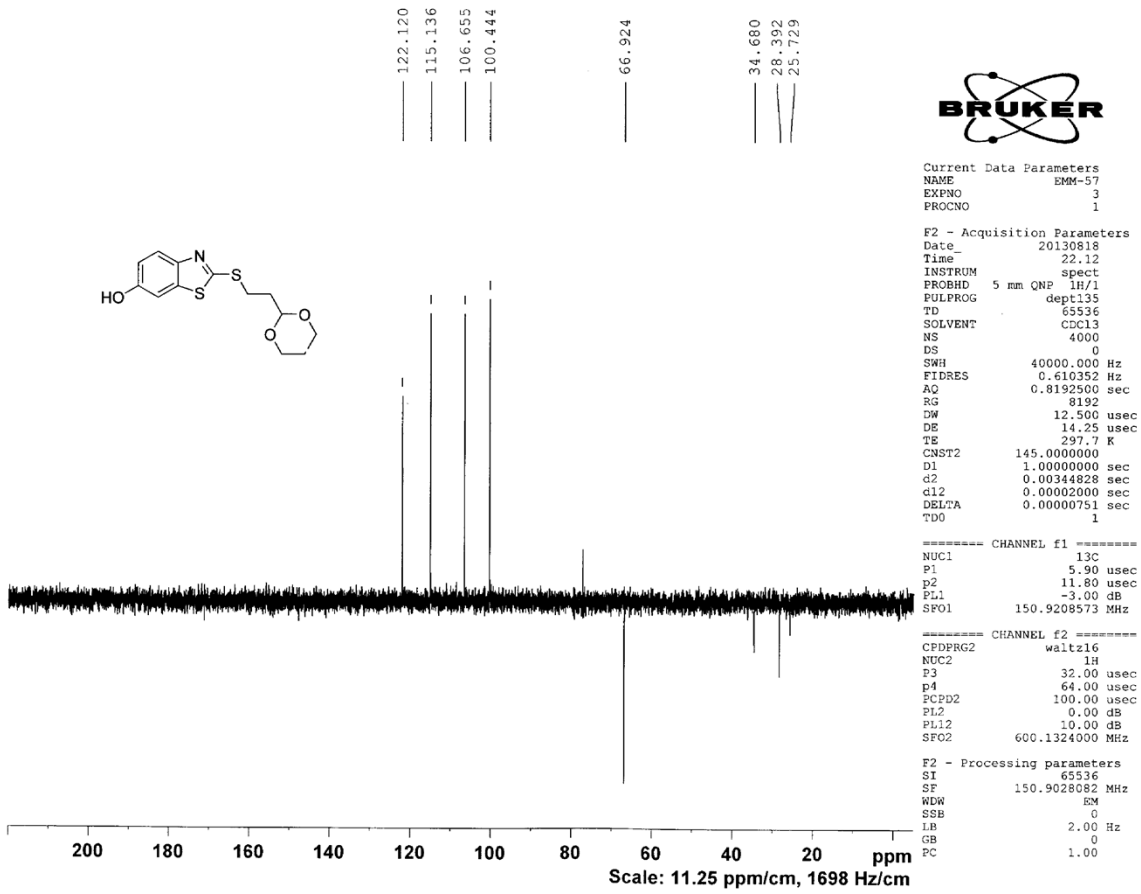
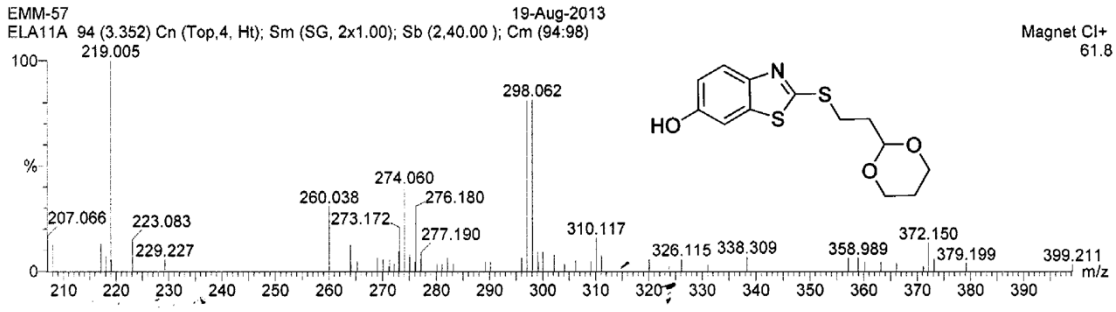
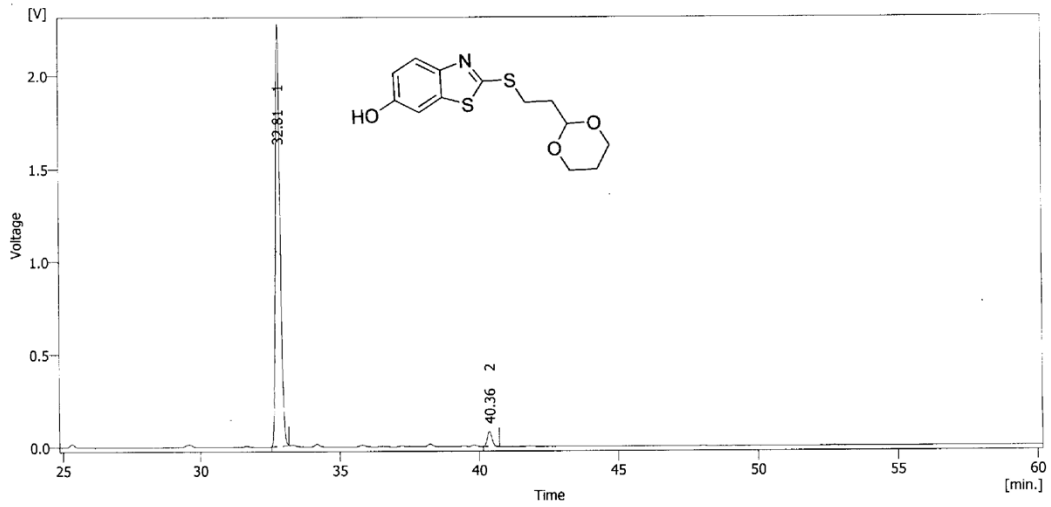

122.120
115.136
106.655
100.444
66.924
34.680
28.392
25.729
HO
N
S
S
O
O
200
180
160
140
120
100
80
60
40
20
ppm
Scale: 11.25 ppm/cm, 1698 Hz/cm
BRUKER
Current Data Parameters
NAME EMM-57
EXPNO 3
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130818
Time 22.12
INSTRUM spect
PROBHD 5 mm QNP 1H/1
PULPROG dept135
TD 65536
SOLVENT CDCl3
NS 4000
DS 0
SWH 40000.000 Hz
FIDRES 0.610352 Hz
AQ 0.8192500 sec
RG 8192
DW 12.500 usec
DE 14.25 usec
TE 297.7 K
CNST2 145.0000000
D1 1.00000000 sec
d2 0.00344828 sec
d12 0.00002000 sec
DELTA 0.00000751 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 5.90 usec
p2 11.80 usec
PL1 -3.00 dB
SFO1 150.9208573 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
P3 32.00 usec
p4 64.00 usec
PCPD2 100.00 usec
PL2 0.00 dB
PL12 10.00 dB
SFO2 600.1324000 MHz
F2 - Processing parameters
SI 65536
SF 150.9028082 MHz
WDW EM
SSB 0
LB 2.00 Hz
GB 0
PC 1.00

Multiple Mass Analysis: 3 mass(es) processed
Tolerance = 8.0 mDa / DBE: min = 0.0, max = 100.0
Isotope cluster parameters: Separation = 1.0 Abundance = 1.0%

Monoisotopic Mass, Odd and Even Electron Ions
395896 formula(e) evaluated with 52 results within limits (up to 50 closest results for each mass)



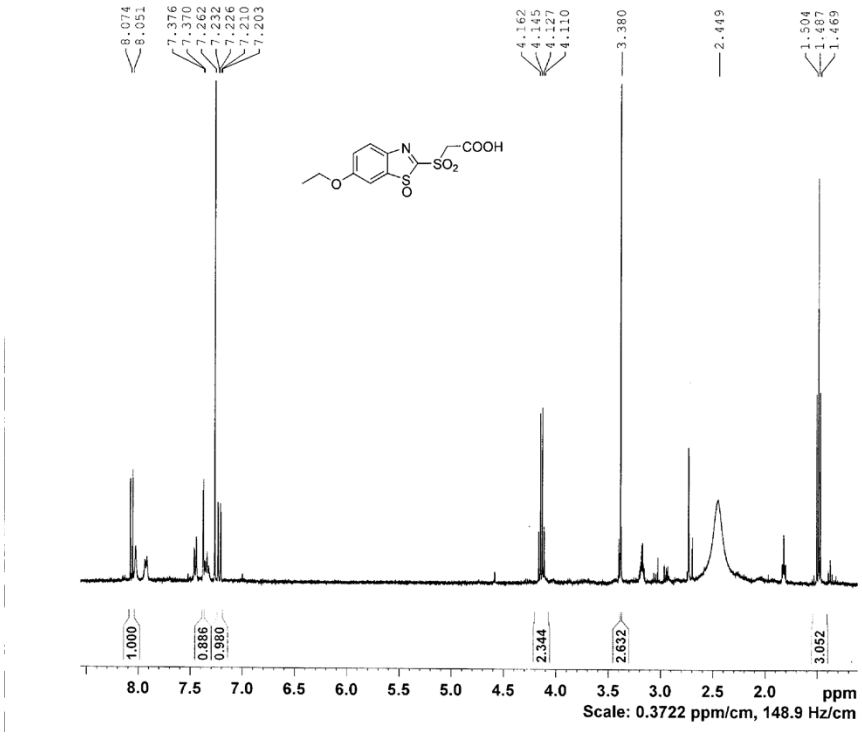
Minimum:	40.00				0.0		
Maximum:	100.00		8.0	5.0	100.0		
Mass	RA	Calc. Mass	mDa	PPM	DBE	Score	Formula
298.0621	81.30	298.0617	0.4	1.5	16.5	n/a	12C18 1H8 14N3 16O2
		298.0630	-0.9	-3.0	16.0	n/a	12C20 1H10 16O3
		298.0632	-1.1	-3.7	6.0	n/a	12C13 1H18 14N2 32S3
		298.0605	1.6	5.3	1.5	n/a	12C10 1H20 14N 16O3 32S3
		298.0637	-1.6	-5.3	12.0	n/a	12C13 1H10 14N6 16O 32S



Result Table (Uncal - E:\EMM-57 - Channel 1)

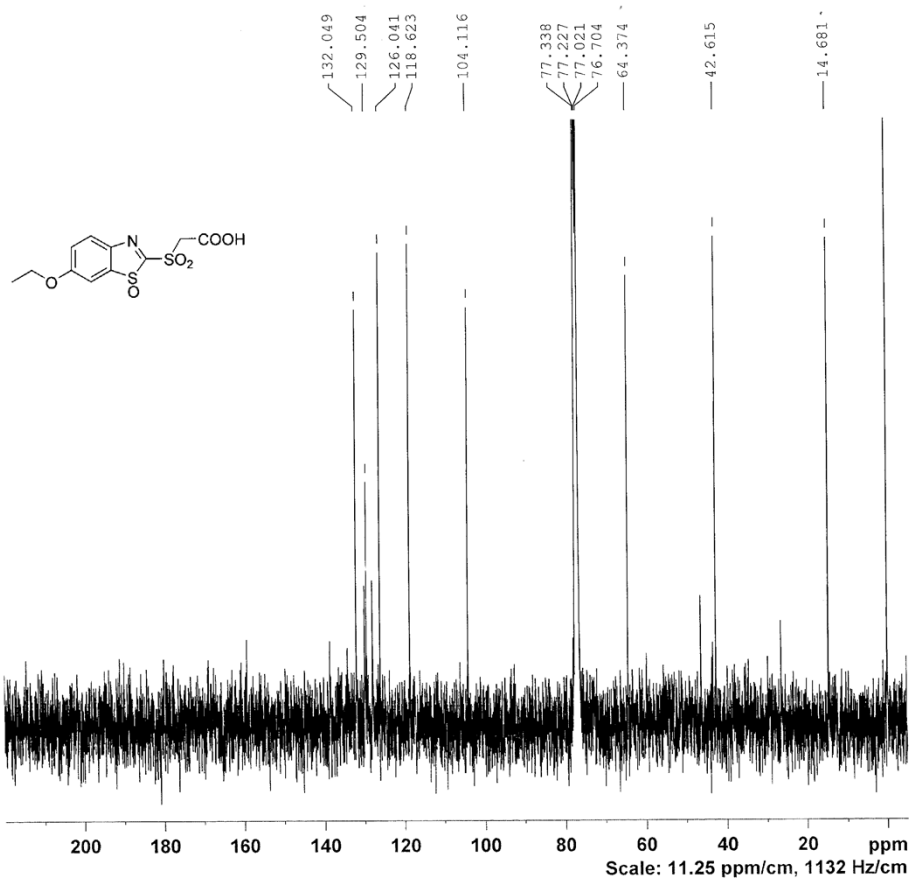
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	32.813	25828.184	2274.640	96.6	96.6	0.18	789	
2	40.360	912.055	79.721	3.4	3.4	0.18	873	
	Total	26740.239	2354.361	100.0	100.0			

2-((6-ethoxybenzo[*d*]thiazol-2-yl)sulfonyl)acetic acid (13). Yield 25% yellow powder, mp decomposes too rapidly. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (t, 3H, *J* = 7.0 Hz, -Ph-$OCH_2C\underline{H}_3$), 3.38 (s, 2H, -S-$C\underline{H}_2$-COOH), 1.65−1.70 (m, 1H, -Ph-$OC\underline{H}_2CH_3$), 4.14 (q, 2H, *J* = 6.9 Hz, -Ph-$OC\underline{H}_2CH_3$), 7.22 (dd, 1H, *J* = 2.6, 6.4 Hz, -$\underline{Ph}$-OCH_2CH_3), 7.37 (d, 1H, *J* = 2.4 Hz, -$\underline{Ph}$-OCH_2CH_3), 8.06 (d, 1H, *J* = 9.2 Hz, -$\underline{Ph}$-OCH_2CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.7 (-Ph-$OCH_2\underline{C}H_3$), 42.6 (-S-$\underline{C}H_2$-COOH), 64.4 (-Ph-$O\underline{C}H_2CH_3$), 104.1 (-$\underline{Ph}$-OCH_2CH_3), 118.6 (-$\underline{Ph}$-OCH_2CH_3), 126.0 (-$\underline{Ph}$-OCH_2CH_3), 129.5 (-$\underline{Ph}$-OCH_2CH_3), 132.0 (-$\underline{Ph}$-OCH_2CH_3), 159.2 (-Ph-[*d*]$\underline{thiazole}$-S-). Anal. Calculated for $C_{11}H_{11}NO_6S_2$: C 41.63, H 3.49, N 4.41; Found: C 42.15, H 3.43, N 4.49. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 35.6 min.



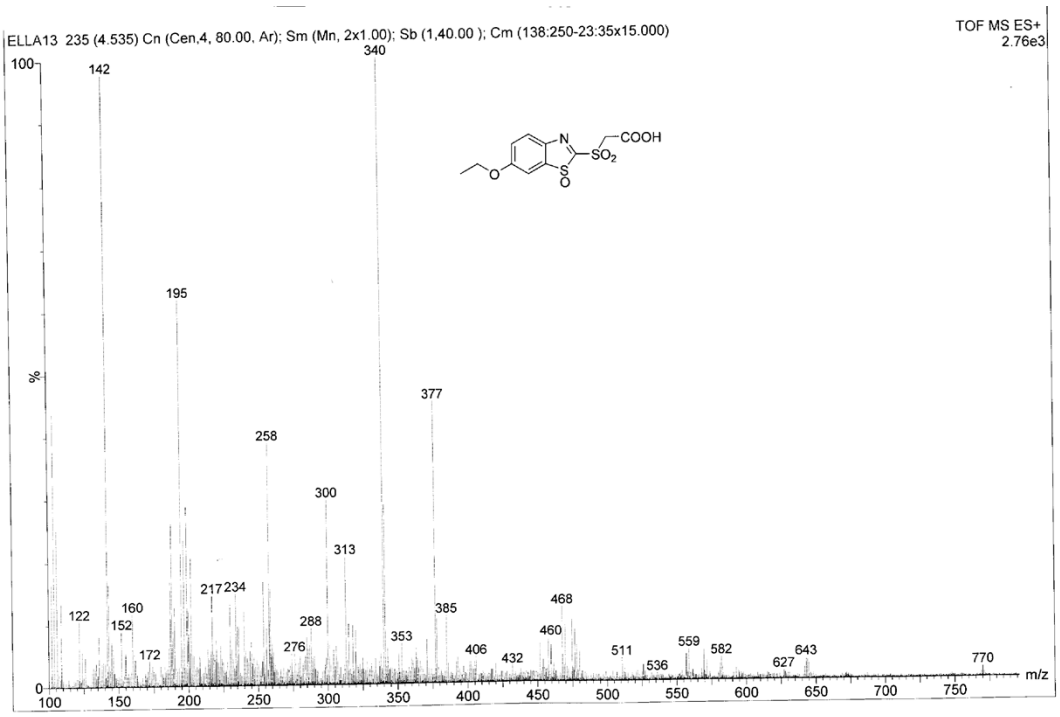
BRUKER

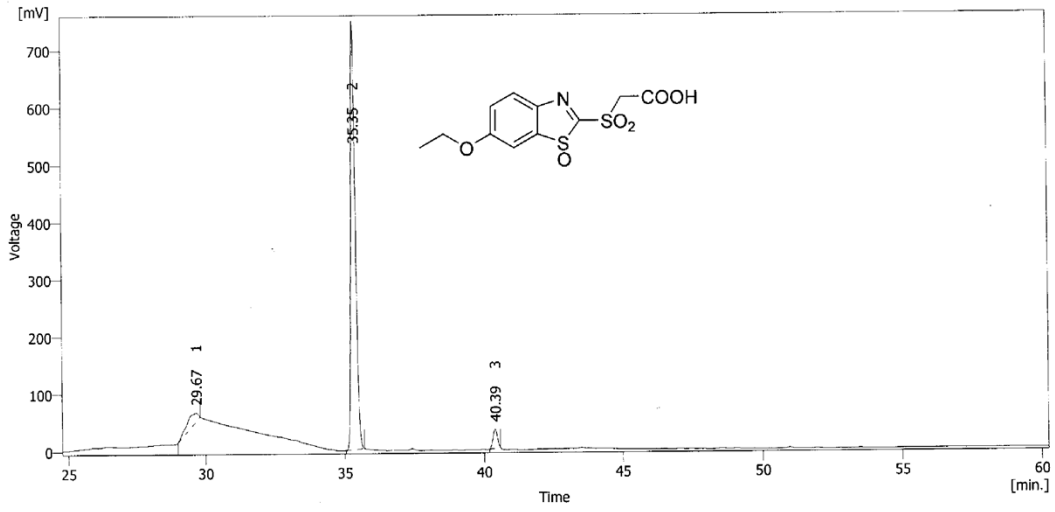
NAME	EMM-69(O) fr. 14-21
EXPNO	1
PROCNO	1
Date_	20130825
Time	12.24
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	8
DS	0
SWH	7183.908 Hz
FIDRES	0.109618 Hz
AQ	4.5613556 sec
RG	362
DW	69.600 usec
DE	6.50 usec
TE	299.1 K
D1	0.10000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.10 usec
PL1	0.00 dB
SFO1	400.1032008 MHz
SI	32768
SF	400.1000097 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00



BRUKER

NAME	EMM-69(O)
EXPNO	1
PROCNO	1
Date_	20131016
Time	7.51
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgdc30
TD	32768
SOLVENT	CDCl3
NS	4673
DS	0
SWH	24154.590
FIDRES	0.737140
AQ	0.6783476
RG	71.8
DW	20.700
DE	12.00
TE	299.5
D1	0.10000000
D11	0.03000000
TD0	1
======== CHANNEL f1 ==========	
NUC1	13C
P1	9.20
PL1	3.00
SFO1	100.616292
======== CHANNEL f2 ==========	
CPDPRG2	waltz1
NUC2	1H
PCPD2	80.0
PL2	0.0
PL12	18.0
SFO2	400.100920
SI	32768
SF	100.605224
WDW	EM
SSB	0
LB	2.00
GB	0
PC	1.00

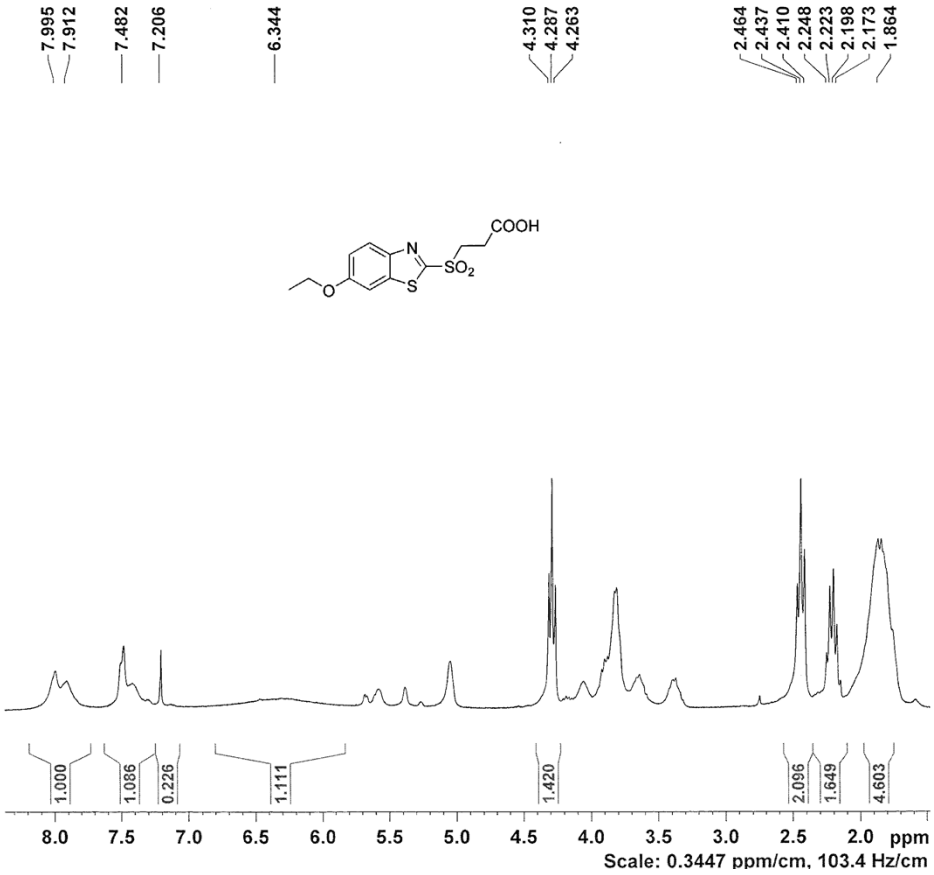




Result Table (Uncal - E:\EMM-69(O) - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	29.667	566.696	14.330	5.9	1.8	0.38	991	
2	35.350	8643.433	748.275	90.3	94.0	0.18	795	
3	40.393	359.700	33.079	3.8	4.2	0.18	966	
	Total	9569.829	795.684	100.0	100.0			

3-((6-ethoxybenzo[*d*]thiazol-2-yl)sulfonyl)propanoic acid (14). Yield 30% yellow powder, mp decomposes too rapidly. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86 (m, 3H, -Ph-$OCH_2C\underline{H}_3$), 2.21 (q, 2H, *J* = 7.5 Hz, -Ph-$OC\underline{H}_2CH_3$), 2.44 (t, 2H, *J* = 8.1 Hz, -SO_2-$(C\underline{H}_2)_2$-COOH), 4.29 (t, 2H, *J* = 8.2 Hz, -SO_2-$(C\underline{H}_2)_2$-COOH), 6.34 (broad pick, 1H, -$(CH_2)_2$-COO$\underline{H}$), 7.21 (s, 1H, EtO-$\underline{Ph}$-), 7.48 (m, 1H, EtO-$\underline{Ph}$-), 7.95 (m, 1H, EtO-$\underline{Ph}$-). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 22.1 (-Ph-$OCH_2\underline{C}H_3$), 27.8 (-Ph-$O\underline{C}H_2CH_3$), 33.1 (-SO_2-$(\underline{C}H_2)_2$-COOH), 68.5 (-SO_2-$(\underline{C}H_2)_2$-COOH), 98.4 (EtO-$\underline{Ph}$-), 100.0 (EtO-$\underline{Ph}$-), 103.7 (EtO-$\underline{Ph}$-), 103.8 (EtO-$\underline{Ph}$-), 107.5 (EtO-$\underline{Ph}$-), 133.4 (EtO-$\underline{Ph}$-), 177.9 (-Ph-[*d*]$\underline{thiazole}$-S-), 202.5 (-$(CH_2)_2$-$\underline{C}$OOH). MS (EI^+): 225.024 (M^+), 226.027 (MH^+). Anal. Calculated for $C_{12}H_{13}NO_5S_2$: C 45.70, H 4.15, N 4.44, S 20.34; Found: C 46.11, H 3.99, N 4.74, S 20.30. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 29.0 min.



BRUKER

```
Current Data Parame
NAME      200-EMM-71
EXPNO
PROCNO

F2 - Acquisition Pa
Date_           2013
Time              1
INSTRUM           sp
PROBHD   5 mm DUL 1
PULPROG           :
TD                3
SOLVENT           CD
NS
DS
SWH            4504
FIDRES         0.13
AQ             3.637
RG
DW               111
DE                 6
TE                 3
D1           0.1000
TD0

======== CHANNEL f1
SFO1         300.089
NUC1
P1                 1
PLW1        30.0000

F2 - Processing par
SI                 3
SF           300.087
WDW
SSB        0
LB         0 Hz
GB         0
PC
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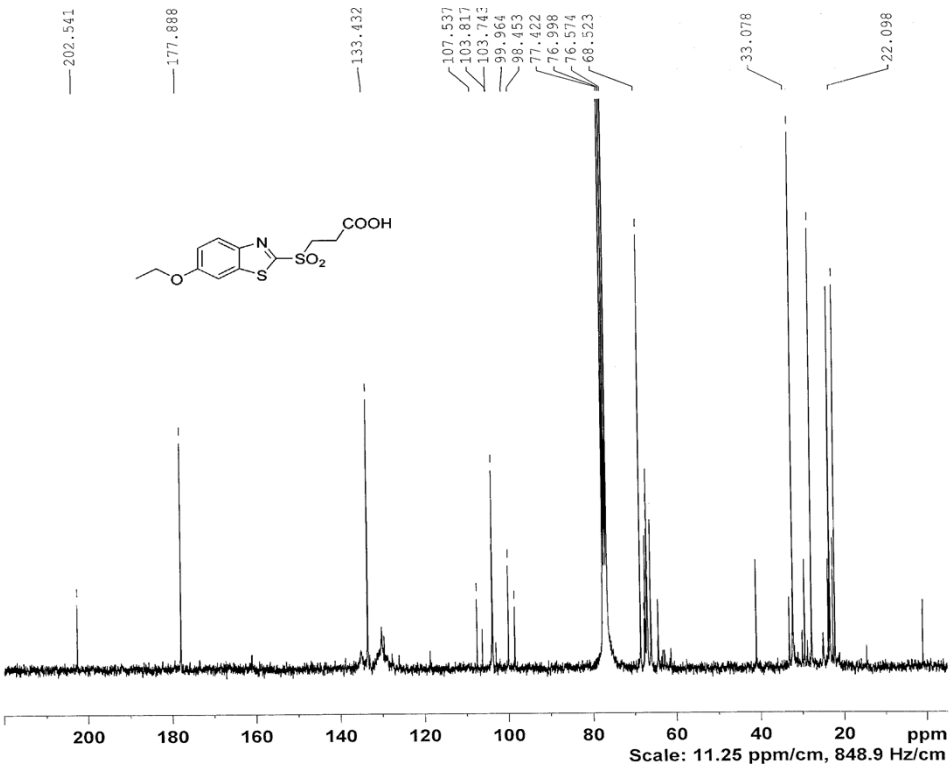


BRUKER

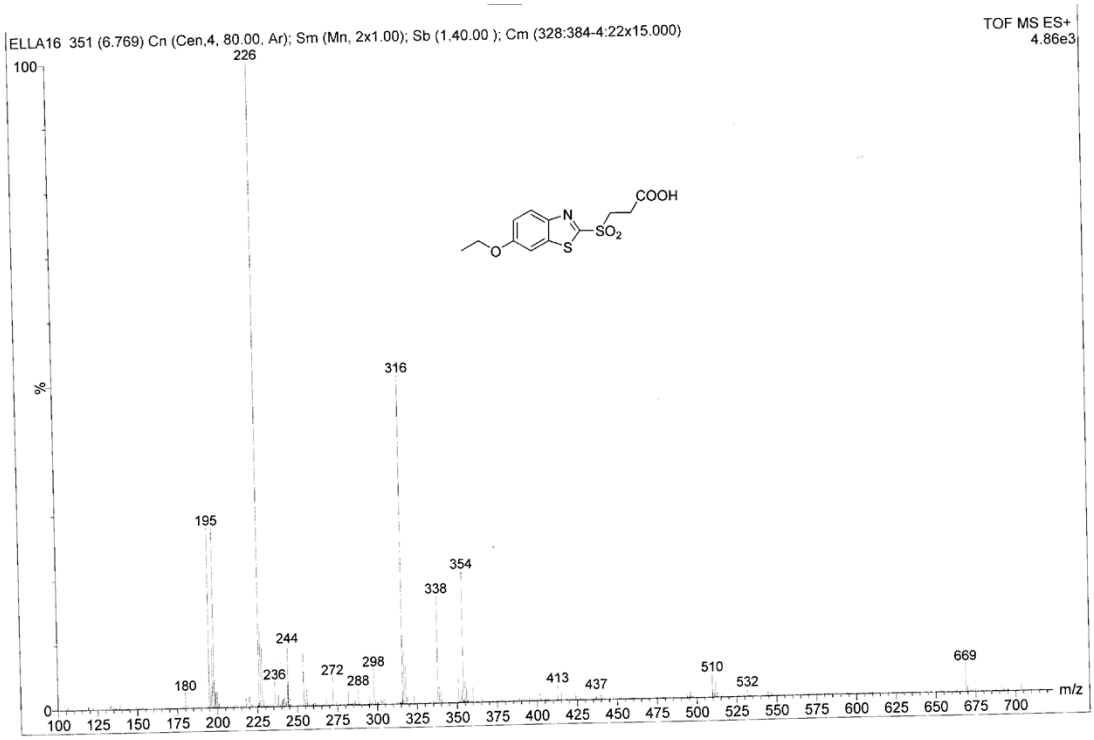
```
Current Data Parameter
NAME      200-EMM-71(O
EXPNO
PROCNO

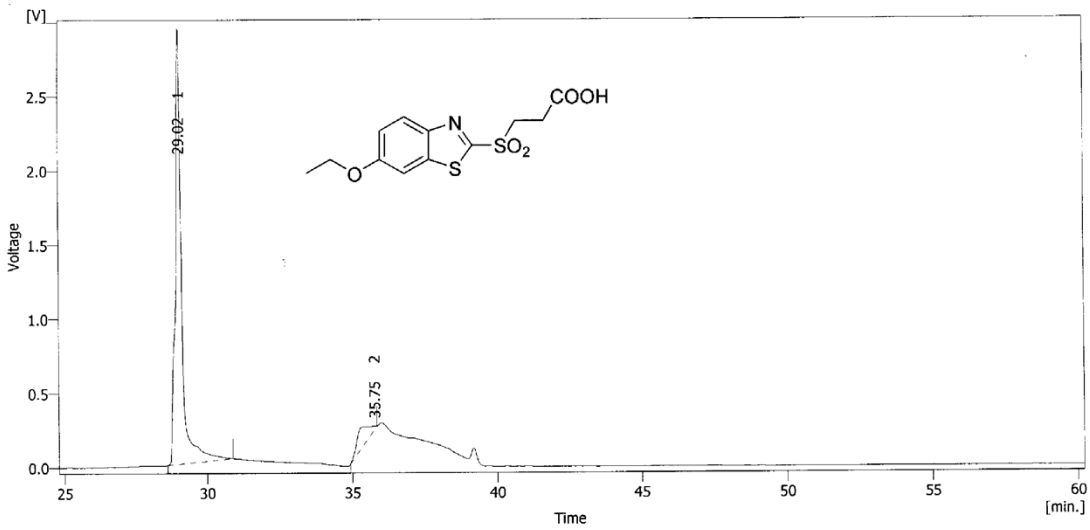
F2 - Acquisition Para
Date_          201310
Time              8.
INSTRUM           spe
PROBHD   5 mm DUL 13C
PULPROG         zgdc
TD               327
SOLVENT          CDC
NS               356
DS
SWH          19531.2
FIDRES        0.5960
AQ            0.8388
RG               20
DW             25.6
DE                6.
TE              300
D1          0.100000
D11         0.030000
TD0

======== CHANNEL f1 =
SFO1         75.4662
NUC1              1
P1               11.
PLW1        70.66739

======== CHANNEL f2 =
SFO2         300.0882
NUC2
CPDPRG[2        waltz
PCPD2           100.
PLW2         29.4698
PLW12         0.3796

F2 - Processing param
SI               327
SF           75.4571
WDW
SSB        0
LB                2.
GB         0
PC                1.
```

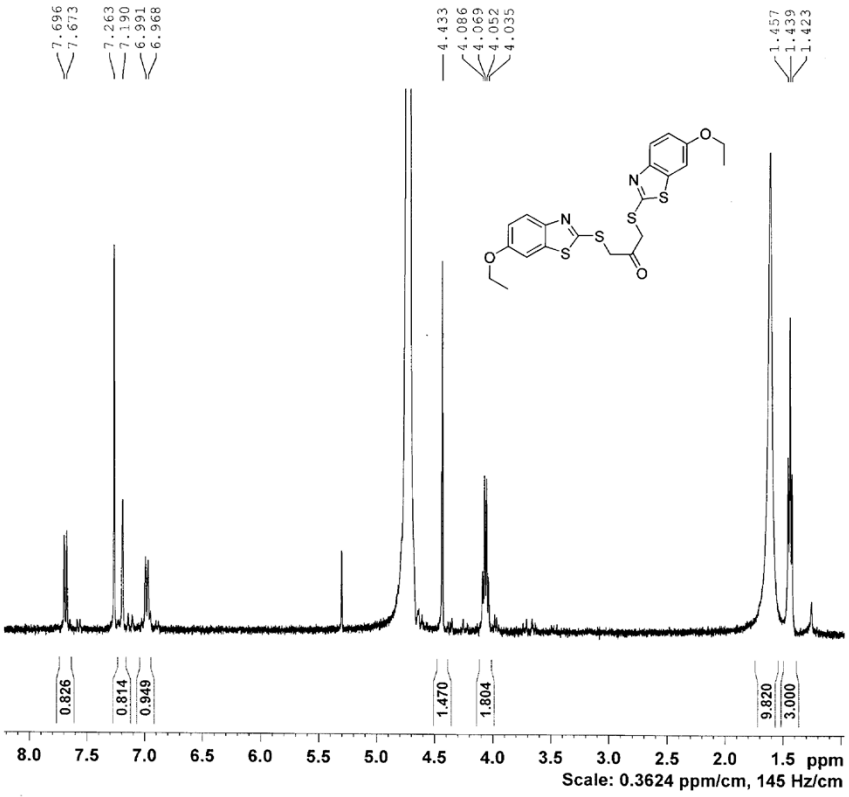




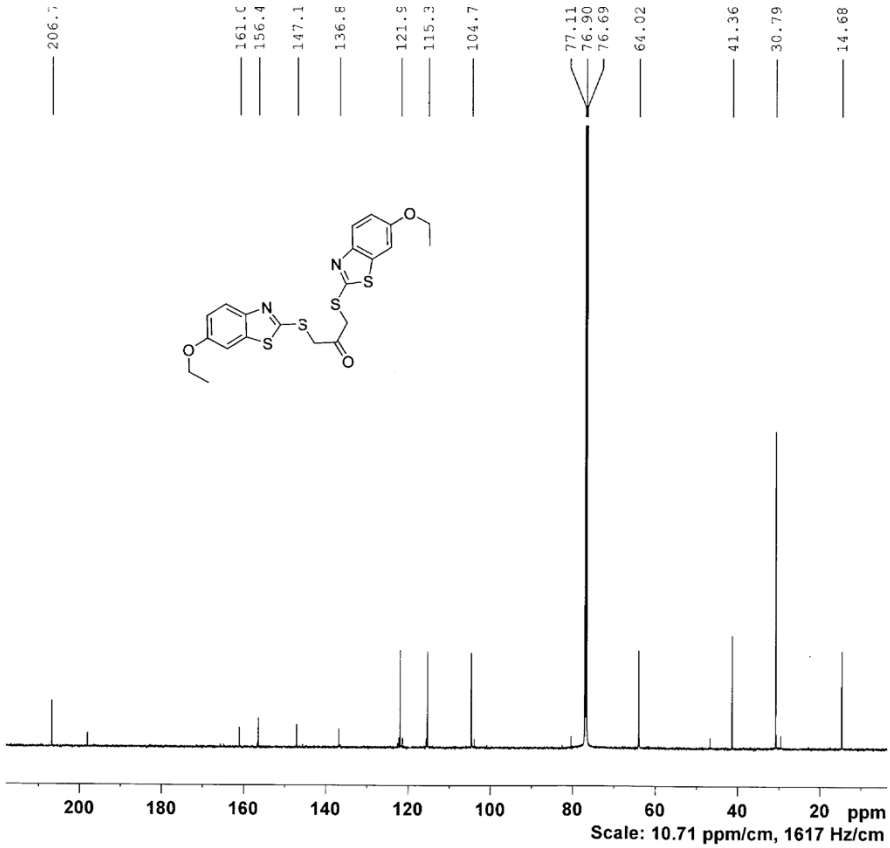
Result Table (Uncal - E:\EMM-71(O) - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	29.020	44114.367	2933.857	91.9	99.1	0.17	704	
2	35.753	3911.882	25.270	8.1	0.9	0.56	990	
	Total	48026.250	2959.127	100.0	100.0			

1,3-bis((6-ethoxybenzo[d]thiazol-2-yl)thio)propan-2-one (15). Yield 25% white powder, mp 48-52 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44 (t, 6H, *J* = 6.8 Hz, (-Ph-$OCH_2C\underline{H}_3)_2$), 4.06 (q, 4H, *J* = 6.8 Hz, (-Ph-$OC\underline{H}_2CH_3)_2$), 4.43 (s, 4H, -$SC\underline{H}_2COC\underline{H}_2$S-), 6.99 (d, 2H, *J* = 9.2 Hz, (CH_3CH_2O-$\underline{Ph}$-)$_2$), 7.19 (s, 2H, (CH_3CH_2O-$\underline{Ph}$-)$_2$), 7.68 (d, 2H, *J* = 9.2 Hz, (CH_3CH_2O-$\underline{Ph}$-)$_2$). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 14.7 ((-Ph-$OCH_2\underline{C}H_3)_2$), 41.4 (-$S\underline{C}H_2CO\underline{C}H_2$S-), 64.0 (-Ph-$O\underline{C}H_2CH_3)_2$), 104.7 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 115.3 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 122.0 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 136.8 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 147.1 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 156.5 (($CH_3CH_2O$-$\underline{Ph}$-)$_2$), 161.1 (HO-Ph-[*d*]$\underline{\text{thiazole}}$-S-), 206.7 (-$SCH_2\underline{C}OCH_2$S-). MS ($ES^+$): 477 ($MH^+$), 499 ($MNa^+$). Anal. Calculated for $C_{21}H_{20}N_2O_3S_4$: C 52.92, H 4.23, N 5.88; Found: C 52.59, H 4.53, N 5.83. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 52.2 min.



NAME	EMM-151
EXPNO	1
PROCNO	1
Date_	20121127
Time_	15.04
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zg30
TD	65536
SOLVENT	CDCl3
NS	16
DS	0
SWH	7183.908 Hz
FIDRES	0.109618 Hz
AQ	4.5613556 sec
RG	9
DW	69.600 usec
DE	6.50 usec
TE	299.3 K
D1	0.10000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.10 usec
PL1	0.00 dB
SFO1	400.1032008 MHz
SI	32768
SF	400.1000095 MHz
WDW	EM
SSB	0
LB	0.00 Hz
GB	0
PC	1.00



BRUKER

```
Current Data Parameters
NAME                  EMM-151
EXPNO                       4
PROCNO                      1

F2 - Acquisition Parameters
Date_              20130107
Time                    8.01
INSTRUM                spect
PROBHD    5 mm QNP  1H/1
PULPROG                 zgdc
TD                     65536
SOLVENT                CDCl3
NS                     20000
DS                         0
SWH                40000.000 Hz
FIDRES             0.610352 Hz
AQ                 0.8192500 sec
RG                      8192
DW                    12.500 usec
DE                    14.25 usec
TE                    300.1 K
D1                 0.50000000 sec
d11                0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                       6.00 usec
PL1                      3.00 dB
SFO1              150.9208573 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  100.00 usec
PL2                     16.50 dB
PL12                    14.80 dB
SFO2              600.1324000 MHz

F2 - Processing parameters
SI                         65536
SF                150.9028258 MHz
WDW                           EM
SSB                            0
LB                          2.00 Hz
GB                             0
PC                          1.00
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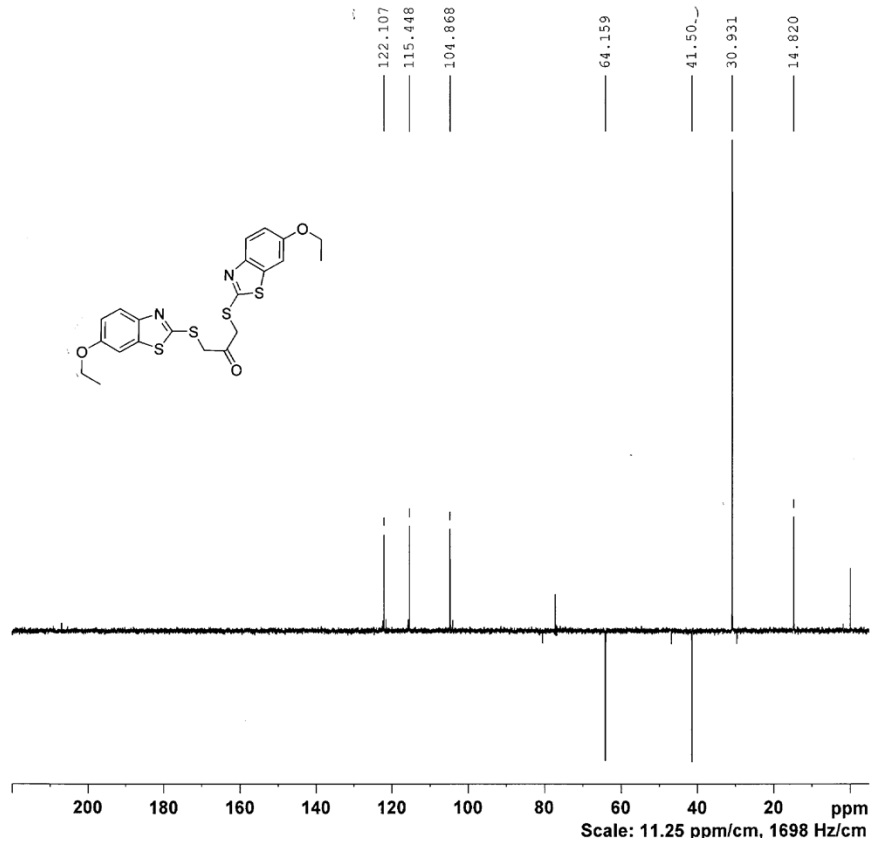

BRUKER

```
Current Data Parameters
NAME                  EMM-151
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_              20130106
Time                   13.33
INSTRUM                spect
PROBHD    5 mm QNP  1H/1
PULPROG              dept135
TD                     65536
SOLVENT                CDCl3
NS                      4000
DS                         4
SWH                40000.000 Hz
FIDRES             0.610352 Hz
AQ                 0.8192500 sec
RG                      8192
DW                    12.500 usec
DE                    14.25 usec
TE                    299.9 K
CNST2             145.0000000
D1                 2.00000000 sec
d2                 0.00344828 sec
d12                0.00002000 sec
DELTA              0.00000751 sec
TD0                         1

======== CHANNEL f1 ========
NUC1                      13C
P1                       5.90 usec
p2                      11.80 usec
PL1                     -3.00 dB
SFO1              150.9208026 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
P3                      14.50 usec
p4                      29.00 usec
PCPD2                  100.00 usec
PL2                      0.00 dB
PL12                    14.80 dB
SFO2              600.1324000 MHz

F2 - Processing parameters
SI                         65536
SF                150.9028052 MHz
WDW                           EM
SSB                            0
LB                          2.00 Hz
GB                             0
PC                          1.00
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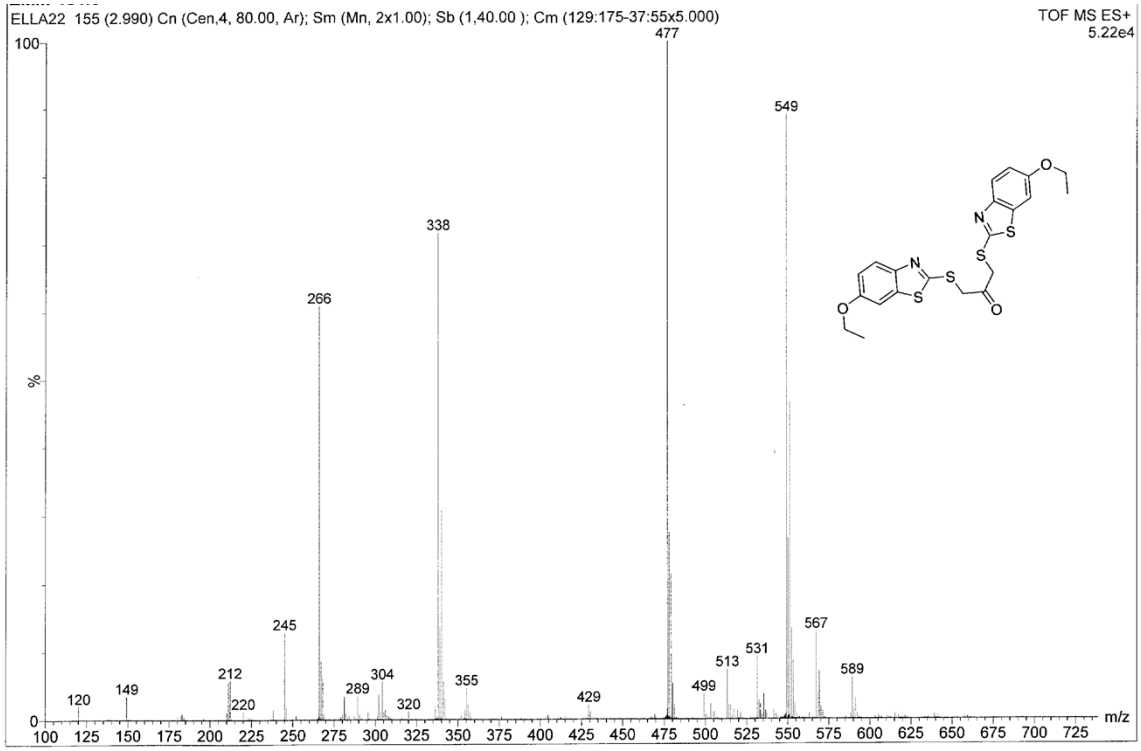
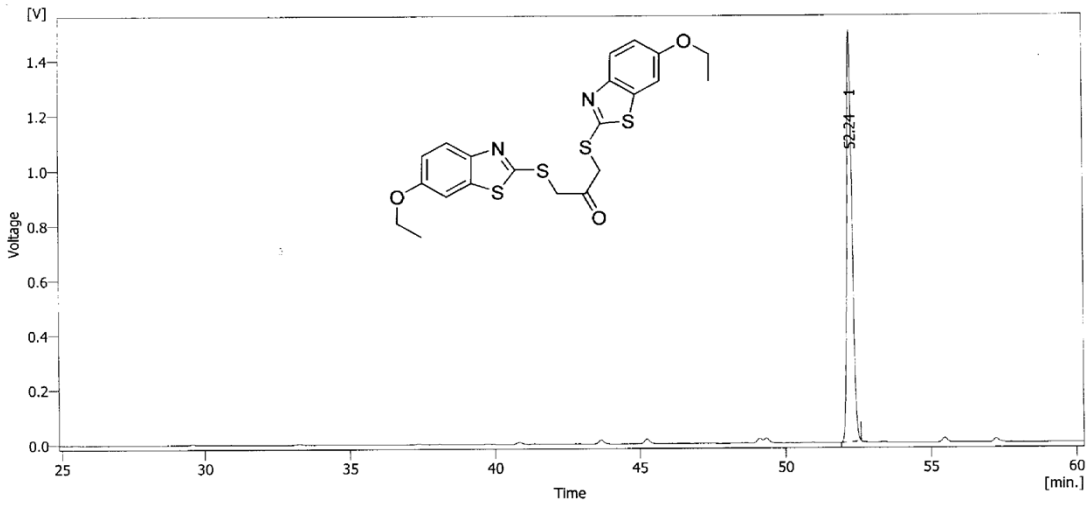


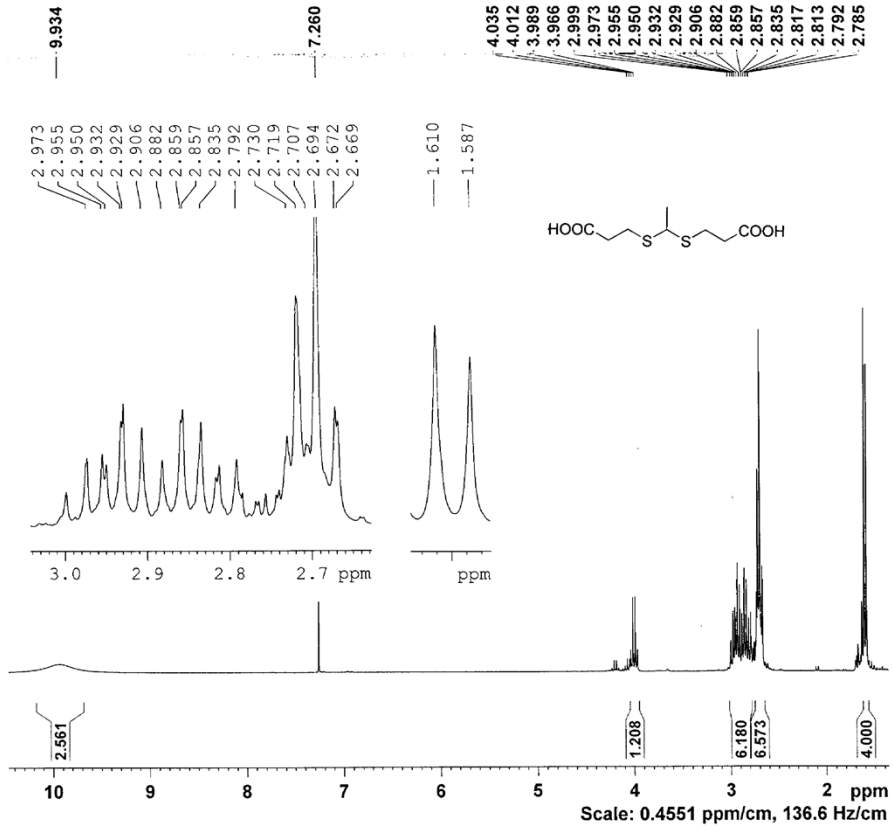


Result Table (Uncal - E:\EMM-151 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	52.240	18637.054	1501.020	100.0	100.0	0.19	779	
	Total	18637.054	1501.020	100.0	100.0			

3,3'-(ethane-1,1-diylbis(sulfanediyl))dipropionic acid (16). Yield 60% clear yellow syrup. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.60 (d, 3H, *J* = 6.9 Hz,

$C\underline{H}_3CH(SCH_2CH_2COOH)_2$), 2.78 – 3.00 (m, 8H, $CH_3CH(SC\underline{H}_2C\underline{H}_2COOH)_2$), 4.00 (q, 1H, *J* = 6.9 Hz, $CH_3C\underline{H}(SCH_2CH_2COOH)_2$), 9.93 (broad pick, 2H, $CH_3CH(SC\underline{H}_2C\underline{H}_2COO\underline{H})_2$). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.9 ($\underline{C}H_3CH(SCH_2CH_2COOH)_2$), 25.0 ($CH_3CH(S\underline{C}H_2CH_2COOH)_2$), 34.4 ($CH_3CH(SCH_2\underline{C}H_2COOH)_2$), 46.6 ($CH_3\underline{C}H(SCH_2CH_2COOH)_2$), 178.1 ($CH_3CH(SCH_2CH_2\underline{C}OOH)_2$). MS ($ES^+$): 239 ($M^+$), 261 ($MNa^+$). Anal. Calculated for $C_8H_{14}O_4S_2$: C 40.32, H 5.92, S 26.91; Found: C 40.47, H 6.01, S 25.82. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 21.7 min.

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EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameter
Date_           20130815
Time               14.38
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                   24
DS                    0
SWH           4504.504 Hz
FIDRES        0.137467 Hz
AQ            3.6372480 se
RG                  575
DW              111.000 us
DE                6.50 us
TE               300.0 K
D1            0.10000000 se
TD0                   1

======== CHANNEL f1 ======
SFO1         300.0898506 MH
NUC1                1H
P1                10.25 us
PLW1         30.00000000 W

F2 - Processing parameters
SI                32768
SF           300.0879101 MH
WDW                  EM
SSB       0
LB        0 Hz
GB        0
PC                 1.50
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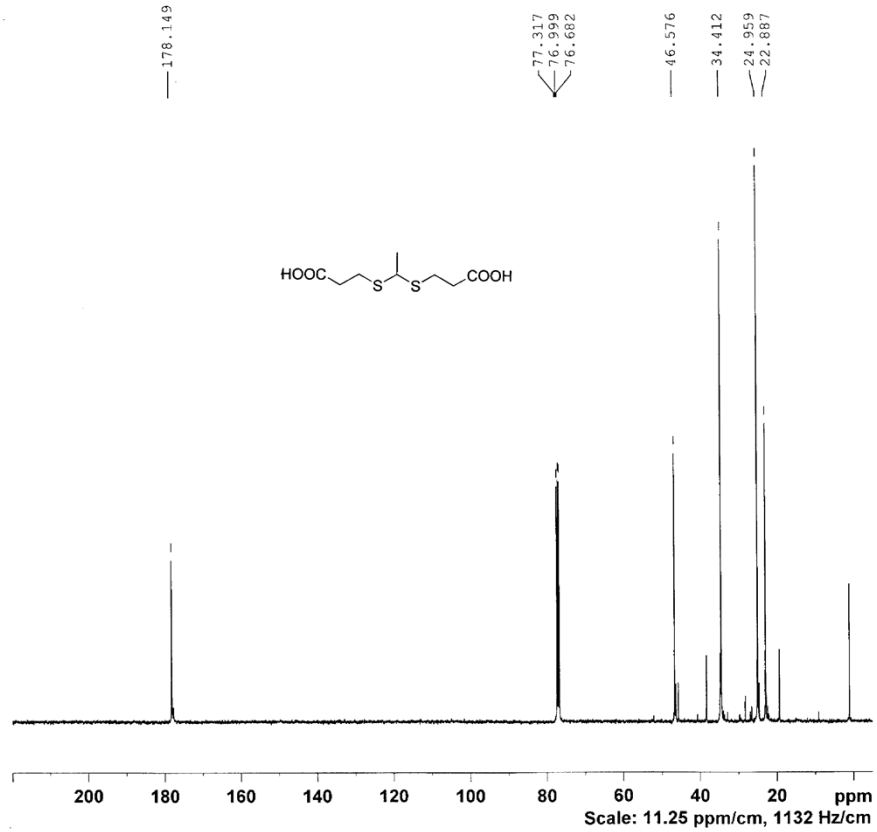
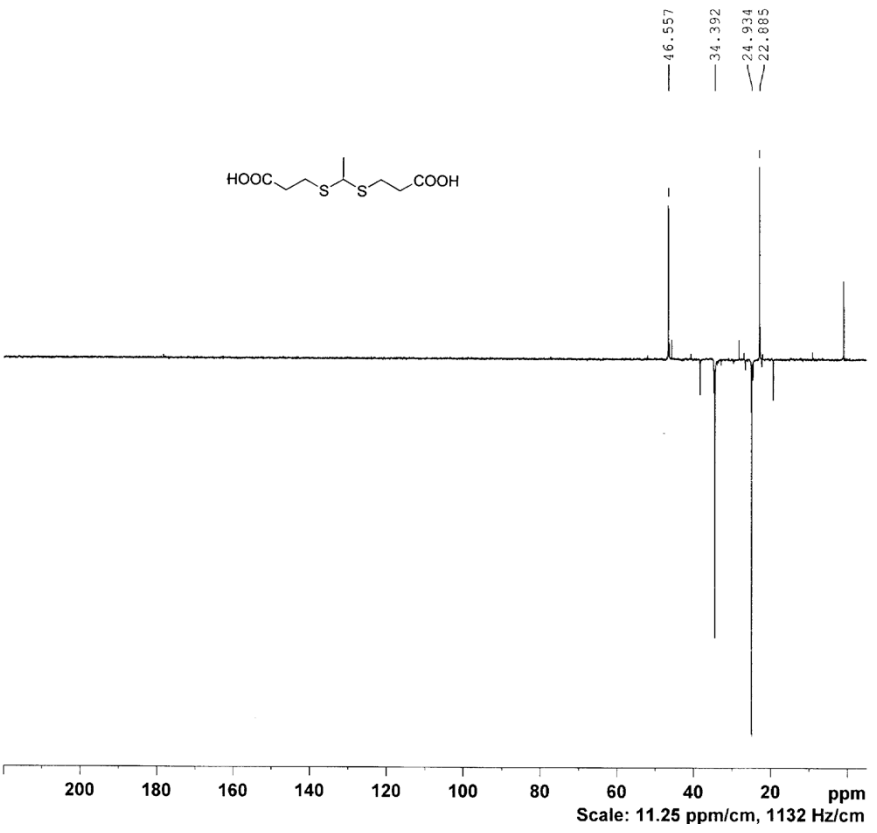


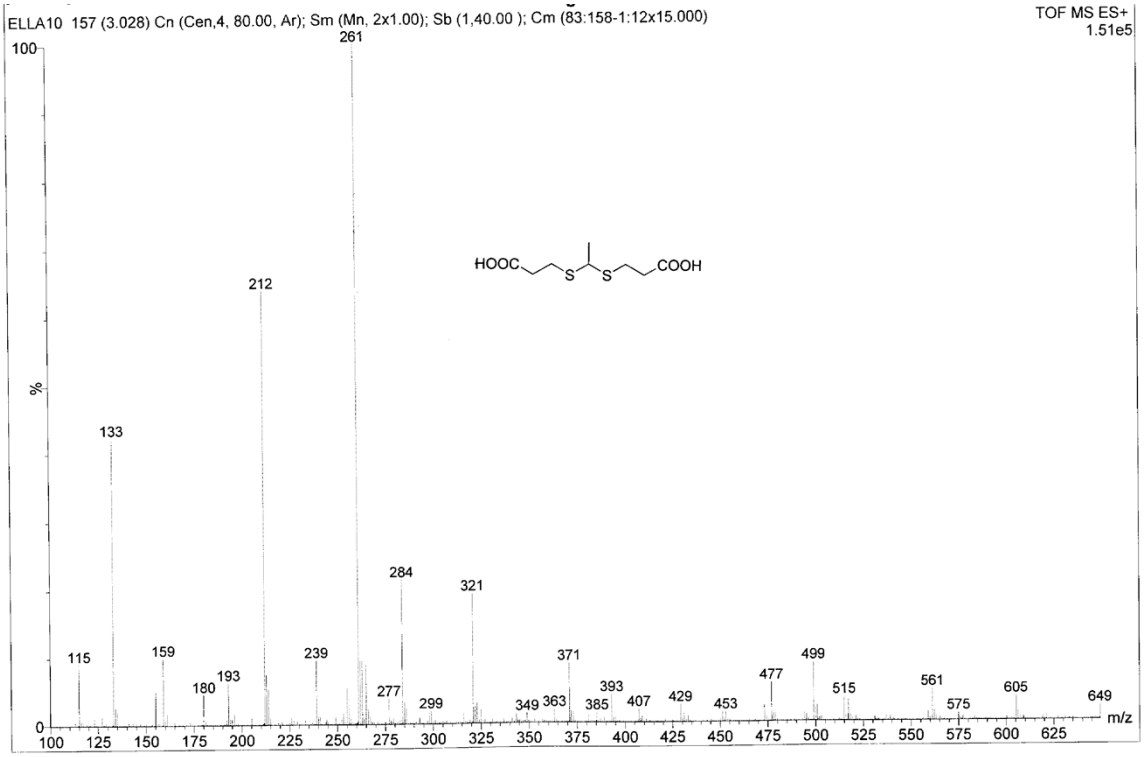
BRUKER

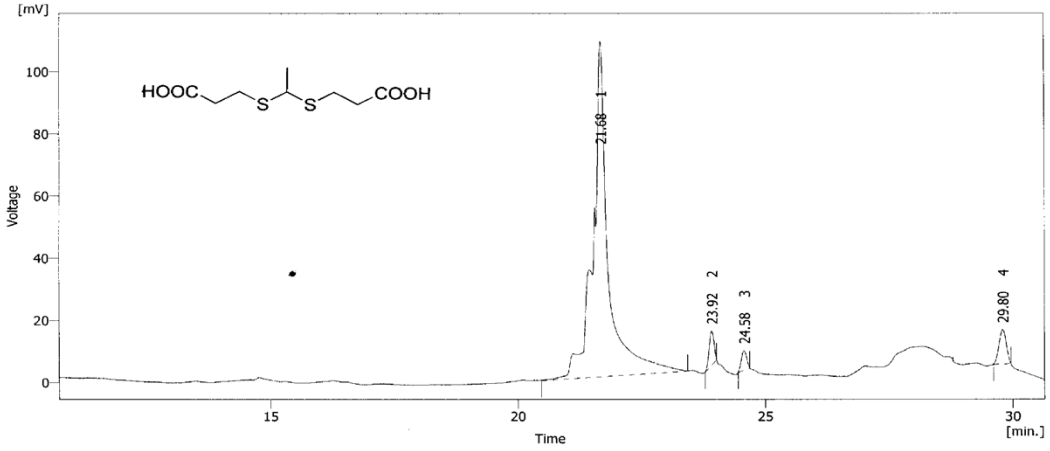
NAME	EMM-11
EXPNO	2
PROCNO	1
Date_	20130821
Time	7.33
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	zgdc30
TD	32768
SOLVENT	CDCl3
NS	22267
DS	0
SWH	24154.590 Hz
FIDRES	0.737140 Hz
AQ	0.6783476 sec
RG	71.8
DW	20.700 usec
DE	12.00 usec
TE	301.7 K
D1	0.10000000 sec
D11	0.03000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	9.20 usec
PL1	3.00 dB
SFO1	100.6162926 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
PCPD2	80.00 usec
PL2	0.00 dB
PL12	18.00 dB
SFO2	400.1009202 MHz
SI	32768
SF	100.6052280 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00



BRUKER

NAME	EMM-11
EXPNO	1
PROCNO	1
Date_	20130821
Time	2.52
INSTRUM	spect
PROBHD	5 mm PABBO BB-
PULPROG	dept135
TD	32768
SOLVENT	CDCl3
NS	5000
DS	0
SWH	24154.590 Hz
FIDRES	0.737140 Hz
AQ	0.6783476 sec
RG	71.8
DW	20.700 usec
DE	12.00 usec
TE	300.3 K
CNST2	145.0000000
D1	2.00000000 sec
D2	0.00344828 sec
D12	0.00002000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	13C
P1	9.20 usec
P2	18.40 usec
PL1	3.00 dB
SFO1	100.6162926 MHz
======== CHANNEL f2 ========	
CPDPRG2	waltz16
NUC2	1H
P3	10.10 usec
P4	20.20 usec
PCPD2	80.00 usec
PL2	0.00 dB
PL12	18.00 dB
SFO2	400.1009202 MHz
SI	32768
SF	100.6052280 MHz
WDW	EM
SSB	0
LB	2.00 Hz
GB	0
PC	1.00

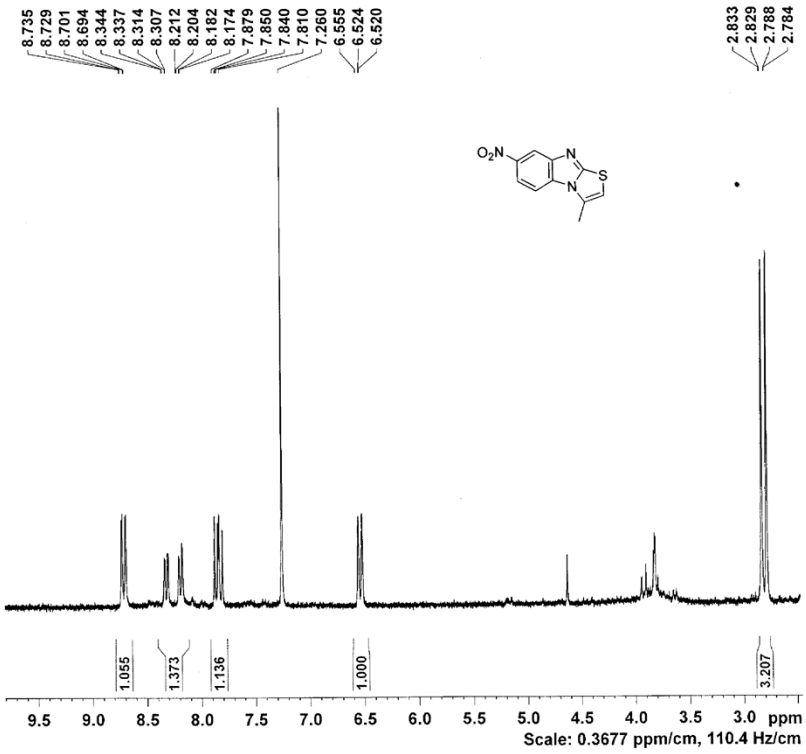




Result Table (Uncal - E:\EMM-11 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	21.680	2335.129	107.888	91.0	79.3	0.18	924	
2	23.923	73.498	10.934	2.9	8.0	0.11	970	
3	24.577	45.285	6.191	1.8	4.5	0.12	991	
4	29.797	111.770	11.091	4.4	8.1	0.17	997	
	Total	2565.683	136.105	100.0	100.0			

3-methyl-7-nitrobenzo[4,5]imidazo[2,1-*b*]thiazole (19). Yield 30% yellow powder, mp too broad to be determined. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.81 (dd, 3H, *J* = 1.2, 12.3 Hz, -thiazole-C$\underline{H_3}$), 6.54 (dd, 1H, , *J* = 1.2, 9.3 Hz, -thiazole-CH_3), 7.81 – 7.88 (m, 1H, NO_2-Ph-), 8.17 – 8.34 (m, 1H, NO_2-Ph-), 8.72 (dd, 1H, *J* = 2.0, 8.4 Hz, NO_2-Ph-). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.5 (-thiazole-$\underline{C}H_3$), 106.9 (-thiazole-CH_3), 107.1 (-thiazole-CH_3), 107.5 (NO_2-Ph-), 110.3 (NO_2-Ph-), 115.6 (NO_2-Ph-), 116.2 (NO_2-Ph-), 118.8 (NO_2-Ph-), 119.4 (NO_2-Ph-). MS (ES^+): 234 (MH^+). Anal. Calculated for $C_{10}H_7N3O_2S$: C 51.49, H 3.02, N 18.02; Found: C 51.40, H 3.05, N 17.90. HPLC (gradient water/CH_3CN from 100% of water in 60 min, flow = 1 mL/min, λ = 254 nm): t_R = 63.5 min.

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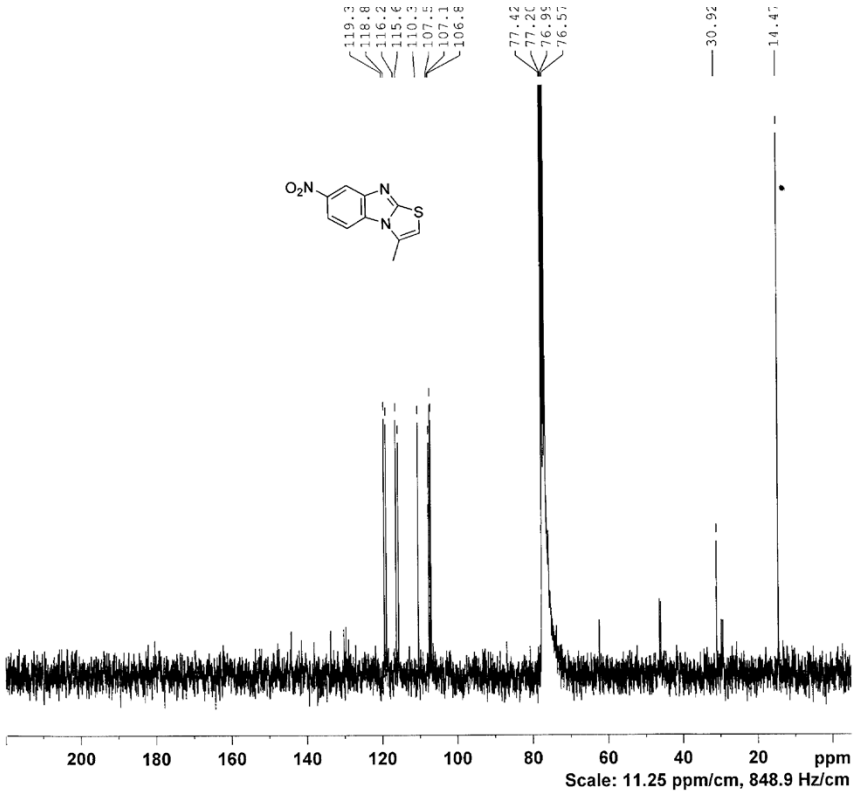
Current Data Parameters
NAME      200-EMM-130-1H
EXPNO                  4
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131106
Time              13.56
INSTRUM           spect
PROBHD   5 mm DUL 13C-1
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    24
DS                     0
SWH            4504.504 Hz
FIDRES        0.137467 Hz
AQ            3.6372480 sec
RG                  2050
DW             111.000 usec
DE               6.50 usec
TE               300.0 K
D1            0.10000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         300.0898506 MHz
NUC1                   1H
P1                 10.25 usec
PLW1         30.00000000 W

F2 - Processing parameters
SI                 32768
SF           300.0879102 MHz
WDW                   EM
SSB        0
LB         0 Hz
GB         0
PC                  1.50
```



BRUKER

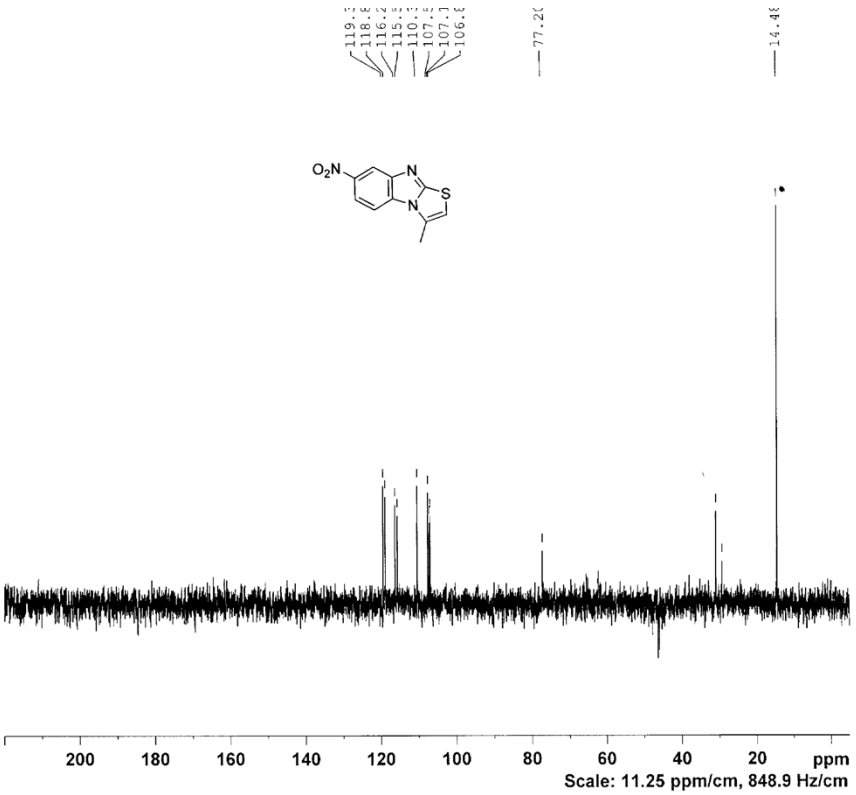
```
Current Data Parameters
NAME                  EMM-130
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_              20131108
Time                   9.53
INSTRUM               spect
PROBHD    5 mm DUL 13C-1
PULPROG            zgdc30
TD                  32768
SOLVENT             CDCl3
NS                  40000
DS                      0
SWH             19531.250 Hz
FIDRES          0.596046 Hz
AQ              0.8388608 sec
RG                   2050
DW                 25.600 usec
DE                  6.50 usec
TE                 300.0 K
D1              0.10000000 sec
D11             0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1           75.4662169 MHz
NUC1                   13C
P1                   11.00 usec
PLW1          70.66739655 W

======== CHANNEL f2 ========
SFO2          300.0882901 MHz
NUC2                    1H
CPDPRG[2            waltz16
PCPD2              100.00 usec
PLW2          29.46981049 W
PLW12          0.37964469 W

F2 - Processing parameters
SI                  32768
SF             75.4571651 MHz
WDW                    EM
SSB       0
LB                  2.00 Hz
GB        0
PC                  1.50
```



BRUKER

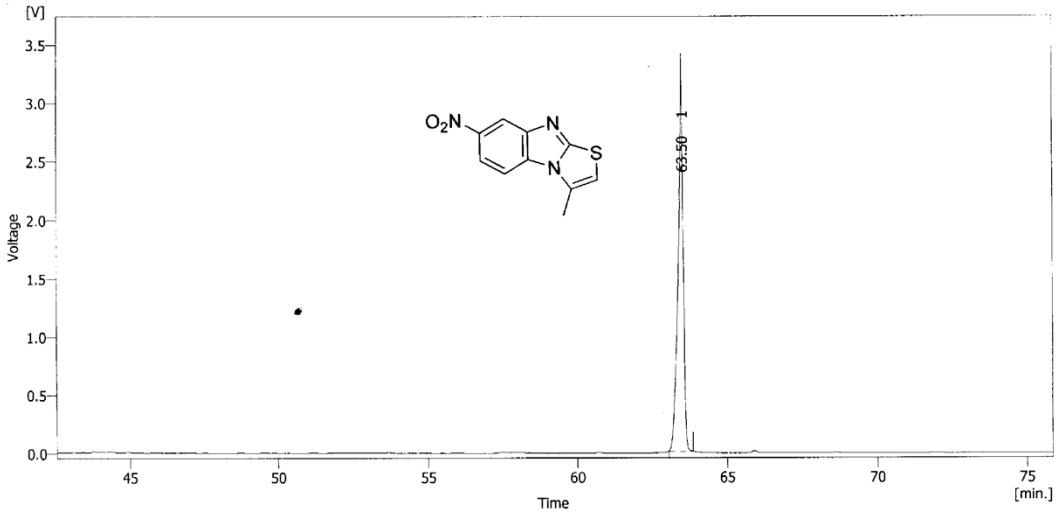
```
Current Data Parameters
NAME                  EMM-130
EXPNO                       1
PROCNO                      1

F2 - Acquisition Parameters
Date_              20131107
Time                  22.45
INSTRUM               spect
PROBHD    5 mm DUL 13C-1
PULPROG           dept135
TD                  32768
SOLVENT             CDCl3
NS                   6000
DS                      0
SWH             19531.250 Hz
FIDRES          0.596046 Hz
AQ              0.8388608 sec
RG                   2050
DW                 25.600 usec
DE                  6.50 usec
TE                 300.0 K
CNST2          145.00000000
D1               2.00000000 sec
D2               0.00344828 sec
D12              0.00002000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1           75.4662169 MHz
NUC1                   13C
P1                   11.00 usec
P2                   22.00 usec
PLW1          70.66739655 W

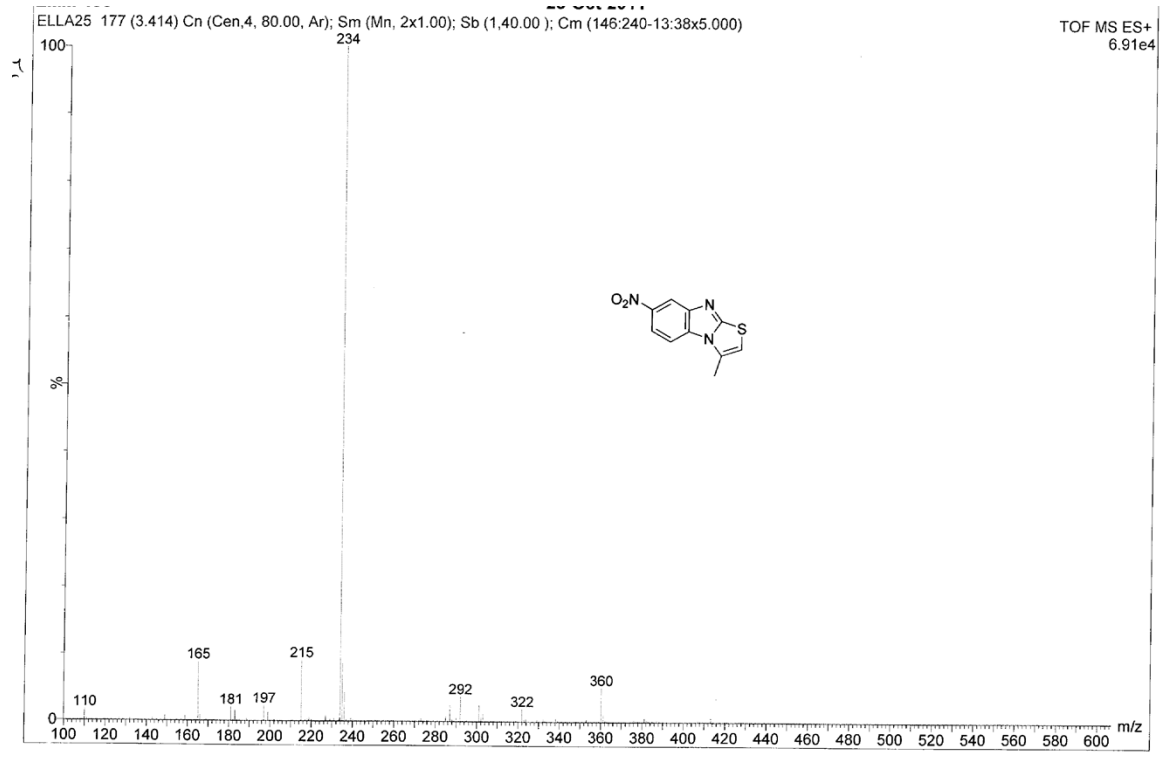
======== CHANNEL f2 ========
SFO2          300.0882901 MHz
NUC2                    1H
CPDPRG[2            waltz16
P3                   10.25 usec
P4                   20.50 usec
PCPD2              100.00 usec
PLW2          29.46981049 W
PLW12          0.37964469 W

F2 - Processing parameters
SI                  32768
SF             75.4571651 MHz
WDW                    EM
SSB       0
LB                  2.00 Hz
GB        0
PC                  1.50
```



Result Table (Uncal - E:\EMM'S 11, 1302 - Channel 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	63.497	37986.987	3408.005	100.0	100.0	0.16	762	
	Total	37986.987	3408.005	100.0	100.0			



Experimental Part

a. Cell cultures

L6 and L6-GLUT4myc myotubes.

L6 and L6-GLUT4myc myocytes and myotube cultures were prepared, grown and maintained as described.[7] The L6-GLUT4myc cell line was the courtesy of Dr. Amira Klip (Hospital for Sick Children, Toronto, Canada).

INS-1E insulinoma cell line.

INS-1E cell cultures were grown and maintained as described.[8] Cells in passage from 60-90, which maintain normal GSIS, were used in the study.

b. Isolation of rat islets of Langerhans

Male Wistar rats (150–250 g) were purchased from Harlan (Jerusalem, Israel). The joint ethics committee for animal welfare of the Hebrew University approved the study protocol. The Hebrew University is an Association for Assessment and Accreditation of Laboratory Animal Care International–accredited institute. Pancreatic islets were isolated from rats after collagenase digestion, as previously described.[9] Pooled islets from three to five animals were preincubated in RPMI-1640 medium (11 mM glucose) for a 16-h recovery period, then divided into the experimental groups, each consisting of 30-50 islets of a similar size.

c. Glucose uptake assay

The [^{3}H]dGlc uptake assay in L6 myotubes was performed as described.[10]

d. MTT cell viability test

We conducted this test as described.[11] The results were normalized to total protein content in culture wells, as by the Bradford method.[12]

e. Western blots

Whole cell lysates were prepared as previously described[13] with some minor modifications: the lysis buffer contained 50 mmol/L Tris-HCl, pH 7.5, 1 mmol/L EDTA, 1mmol/L EGTA, 1 mmol/L Na3VO4, 150 mmol/L NaCl, 50 mmol/L NaF, 10 mmol/L sodium-glycerophosphate, 5 mmol/L sodium pyrophosphate, and 1 mmol/L PMSF, supplemented with 0.1% (v/v) IGEPAL, 0.1% (v/v) 2-β-mercaptoethanol, and protease inhibitor cocktail (1:100 dilution). The cells were washed with ice-cold PBS, and 1 ml of lysis buffer was then added at 4°C for 40 min. The resulting cell lysates were centrifuged at 8,700 g x 30 min at 4°C, and the supernatant fractions were separated and kept at -70°C until used. Protein content in the supernatant was determined by Bradford analysis, using a BSA standard dissolved in the same buffer. Aliquots (15-50 μg of protein) were mixed with the sample buffer [62.5 mmol/L Tris-HCl, pH 6.8, 2% (w/v) SDS, 10% (v/v) glycerol, 50 mmol/L DTT, and 0.01% (w/v) bromophenol blue] and heated at 95°C for 5 min. The proteins were separated on 10% SDS-PAGE and Western blot analyses was performed using antibodies according to our previously established protocols.[2]

f. Colorimetric assay for detection of GLUT4
The colorimetric detection of surface GLUT4myc in L6 myotubes was performed as described.[14]

g. GSIS and Insulin RIA
The assays were performed as described.[8]

Statistical analysis
Statistical significance *, # ($p < 0.05$) was calculated among the various experimental groups using the Student t-test (two tailed) test. Results are given as mean ± SEM (n =3-6).

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