Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Switch-on Fluorescent/FRET Probes to Study Human Histidine Triad Nucleotide Binding Protein 1 (hHint1), a Novel Target for Opioid Tolerance and Neuropathic Pain

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General Methods and Materials. Chloroformamidine hydrochloride was purchased from Oakwood Chemical, Inc (cat no: 075371), Methyl 4-aminothiophene-3-carboxylate was purchased from Matrix Scientific (cat no: 071956), Dimethyl sulfone (cat no: M81705-100G), Tin (IV) Chloride (cat no: 208930-250G), and Nitromethane (cat no: 360554-500 ml) were purchased from Sigma Aldrich. All solvents were purchased from Thermo Fisher Scientific and used as received unless otherwise noted. Anhydrous solvent such as DMF was used directly from solvent dispensing system (J. C. Meyer) packed with two columns of neutral alumina and dispensed under argon. Thin-layer chromatography was performed using EMD pre-coated silica gel 60 F-254 plates. All preparative separations were performed using Teledyne Isco combiflash system and using RediSepRf high performance gold silica pre-packed columns. High-resolution mass spectrometry was performed using LTQ Orbitrap Velos (Thermo ScientificTM). Samples and compounds during synthesis were freeze-dried with a lyophilizer available from Labonaco. All ^{1}H- and ^{13}C-NMR spectra were collected in d_6-DMSO (Cambridge Isotope Laboratories, Cambridge, MA) at 25 °C using AscendTM Bruker spectrometer 500 or 600 MHz at the Department of Medicinal Chemistry CCRB NMR facility at the University of Minnesota unless otherwise stated. All NMR chemical shifts were recorded in δ parts per million using d6-DMSO as internal reference. All the fluorescence intensity measurement experiments were performed using Varian Cary Eclipse Fluorescence Spectrophotometer. UV absorbance measurements for determining quantum yields were performed using Cary 5000 UV-Vis-NIR Spectrophotometer. Nickel nitrilotriacetic acid (Ni-NTA) was purchased from Qiagen and cobalt column agarose from Thermo Fisher Scientific. Biological buffers were purchased from Sigma-Aldrich. Protease inhibitor tablets were obtained from Roche.

Protein Expression and Purification

The full-length sequence of hHint1 and hHint2 was expressed from the pMCSG7 vector (N-terminal, tobacco etch virus (TEV) protease cleavable His6 tag) in Rosetta2 pLysS cells, while hLysRs was expressed from pM368 vector encoding full-length hLysRs appended with N-terminus His_6 tag (no TEV protease site) in BL21 (DE3) pLysS cells. The cells were grown in 2 x 1L LB (Fisher Scientific) media with ampicillin (100 mg/L, Sigma-Aldrich), chloramphenicol (34 mg/L, Sigma-Aldrich), and glucose (0.1% w/v, BD Difco) at 37 °C with shaking at 250 rpm. At OD_{600} = 0.7, cultures were induced to a final concentration of 1 mM IPTG (Denville Scientific Inc) and incubated at 30°C overnight. The cultures were harvested by centrifugation at 7,500 *g* at 4 °C for 10 min and the pellets were collected, then resuspended in buffer A (50 mM HEPES pH 7.0, 300 mM NaCl, 10% glycerol, 10 mM imidazole), which was then adjusted to 1 $mg \cdot mL^{-1}$ lysozyme and Benzonase nuclease (20 µl).

The resuspended cells were lysed by sonication (eight cycles of 30 s on, 30 s off) at 4 °C. The cell debris was removed from the lysate by centrifugation at 16,000 *g* at 4 °C for 45 min. The supernatant was loaded onto a nickel affinity column, washed with buffer A, and then eluted with an imidazole gradient using buffer B (50 mM HEPES pH 7.0, 300 mM NaCl, 10% glycerol, 500 mm imidazole). Fractions containing desired protein were combined and to it was added N-terminally His-tagged TEV protease 2% (w/w). The resulting solution was transferred to a dialysis tubing (molecular weight cut-off of 6000-7000 Da) and dialyzed against 2 L of TEV cleavage buffer (50 mM HEPES pH 7.0, 300 mM NaCl, 10% glycerol, 0.5 mM EDTA and 1 mM DTT) overnight at 4 °C. The dialyzed protein was then buffer exchanged into buffer A and passed through cobalt affinity chromatography to remove TEV protease. The flow through obtained was concentrated to 5 mL and further purified using size exclusion chromatography (SEC buffer, 20 mM Tris pH 7.5, 150 mM NaCl, 10% glycerol). Pure fractions were collected and concentrated. The protein concentration was then determined using A_{280} absorbance in nanodrop using the calculated extinction coefficient of 8480 and 44280 M^{-1} cm^{-1} and molecular weight of 14000 and 68000 Da for hHint1 and hLysRs. The final protein was stored at −80 °C until use.

The full-length sequence of hHint3-2 was expressed from the pLJCH3-2 pres plasmid vector in BL21(DE3) cells, while the 1DHFR[2] protein was expressed from the plasmid encoding cysteine free two DHFR proteins fused by a single amino acid linker reported previously. The procedure for the expression, purification and isolation was performed as described previously.

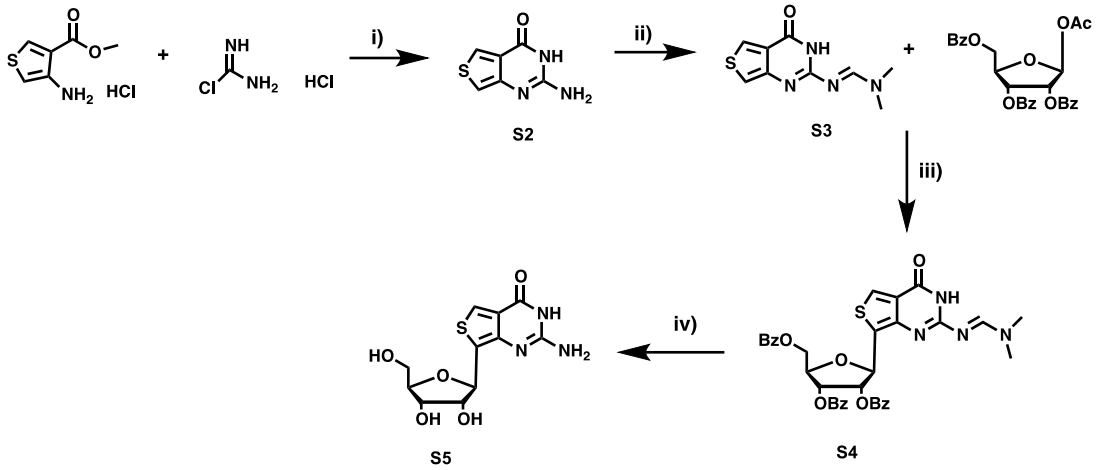
Analytical HPLC studies to determine the purity of the compounds

Analytical studies were performed on a Beckman coulter system gold operated by Karat software, with a Higgin analytical Targa C18 (250 x 4.6 mm) column. Stock solutions (10 mM) of the inhibitors were prepared in either water or DMSO. For stability, the stock solutions were diluted to a concentration of 200 μM using water (DI). A 200 μl aliquots of the sample volume were withdrawn and injected into the HPLC system. The samples were eluted using the gradient of solvent A (Water) and B (CH_3CN) (0-4 min: gradient 10% B, 4-24 min: gradient 100% B, 4min: gradient 100% B, flow rate 1.0 ml/min) with detection at 168-400 nm.

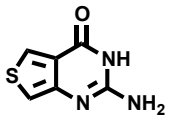
Synthesis of the dGth nucleoside:

dGth nucleoside was synthesized as previously reported by torr and co-worker with only a minor modification (see Scheme S1). See below for the details on the characterization and yields for each step.

Scheme S1[a]



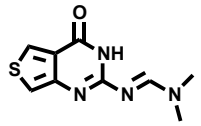
[a]**Reagents and conditions:** i) Dimethyl sulfone, 125 °C, 70%; ii) dimethylformamide dimethylacetal, DMF, overnight, 85 %; iii) $SnCl_4$, Nitromethane, 65 °C, 20 % iv) sat MeOH/NH_3, overnight and then 4N NaOH/MeOH, 2 hrs, 90 %



S2

2-Aminothieno[3,4-d]pyrimidin-4(3H)-one (S2)

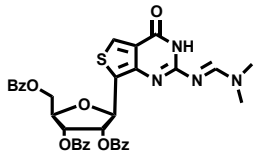
Same as described previously in reference.[20] Yield obtained 73.6%. The ^{1}H NMR spectrum was (DMSO-d_6): 6.07 (s, 2H), 6.96 (s, 1H) , 8.23 (s, 1H) and 10.54 (s, 1H). ^{13}C- DMSO-d_6: 159.01, 151.01, 150.74, 127.55, 124.18 and 109.22 ppm. Low resolution ESI-MS [M+H] 168.0



S3

N^2-DMF 2-aminothieno[3,4-d]pyrimidin-4(3H)-one (S3)

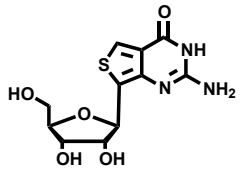
Same as described previously in reference.[20] Obtained in 95.9% yield. The ^{1}H NMR spectrum was (DMSO-d_6): 3.03 (s, 3H), 3.15 (s, 3H), 7.26 (d, 1H), 8.27 (d, 1H), 8.61 (s, 1H) and 11.04 (s, 1H). ^{13}C- DMSO-d_6: 159.70, 157.95, 155.46, 150.26, 127.15, 125.63, 112.15, 40.98 and 35.04 ppm. Low resolution ESI-MS [M+H] 223.0



S4

N^2-DMF 2-aminothieno[3,4-d]pyrimidine G mimic 2,3,5-tri-O-benzoylnucleoside (S4)

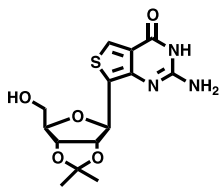
Same as described previously in reference.[20] Obtained in 20% yield. The crude product from this step was used without purification towards the next step. Low resolution ESI-MS [M+H] 667.2



S5

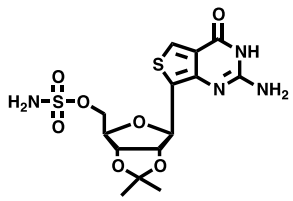
2-Aminothieno[3,4-d]pyrimidine G mimic nucleoside (S5)

Same as described previously in reference with minor modification in this step.[20] Treatment with saturated methanolic ammonia was found to result in the partial deprotection after overnight heating at 65 °C. Hence, the reaction was evaporated to dryness next day and stirred in 4N NaOH/MeOH (1:1, 5ml each) for 2 h. The reaction was then neutralized and organic was evaporated. The aqueous solvent was frozen and lyophilized to obtain the crude material. The material was loaded onto the reverse phase chromatography to purify and yield final product in 58.4% yield. The ^{1}H NMR spectrum was (DMSO-d_6): 3.52 (m, 2H), 3.78 (m, 1H), 3.94 (m, 1H), 4.02 (t, 1H), 4.85 (s, 1H), 5.12 (d, 1H), 6.21 (s 2H) and 8.13 (s, 1H). ^{13}C- DMSO-d_6: 161.06, 153.22, 147.71, 124.91,124.88, 124.81, 85.54, 77.42, 77.05, 72.29 and 62.85 ppm. Low resolution ESI-MS [M+H] 300.0



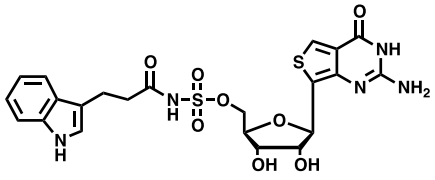
2′, 3′-O-O-isopropylidene-2-Aminothieno[3,4-d]pyrimidine G mimic nucleoside (5)

To a cold stirred suspension of **S5** (50.0 mg, 0.177 mmol) in acetone (3 ml) was added catalytic amount of perchloric acid (12.5 □l). The reaction was monitored using TLC (20:80:0.1 MeOH/$CHCl_3$/TEA solvent). After 2 h ammonium hydroxide (2 equivalent to perchloric acid, 27.5 □l) was added to neutralize the reaction mixture under an ice bath. The reaction mixture was then evaporated under rotary evaporator to complete dryness. The crude material was purified using reverse phase chromatography to isolate desired product (39.9 mg, 0.124 mmol) in 70 % yield. The purity of the compound was determine to be >98% upon HPLC analysis (Retention Time 21 min). The ^{1}H NMR spectrum was (DMSO-d_6): 1.31 (s, 3H), 1.50 (s, 3H), 3.53 (m, 2H), 3.90 (m, 1H), 4.71 (m, 1H), 4.81 (m, 1H), 4.97 (t, 1H), 5.30 (d, 1H), 6.20 (s, 2H) and 8.25 (s, 1H). ^{13}C- DMSO-d_6: 158.77, 151.25, 148.17, 126.79, 124.31, 123.90, 114.20, 86.10, 85.08, 82.21, 79.65, 62.16, 27.82, and 27.79 ppm. Low resolution ESI-MS [M+H] 340.1 HRMS (ESI+) calcd for $C_{14}H_{18}N_3O_5S$ [(M+H)+] 340.0967 found 340.09524



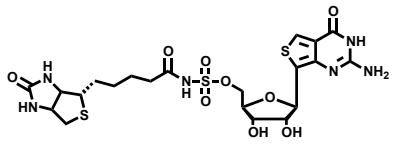
2′, 3′-O-O-isopropylidene-5’-O-(sulfamoyl)-2-Aminothieno[3,4-d]pyrimidine G mimic nucleoside (6)

A solution of **4** (30 mg, 0.088 mmol) in dimethyl formamide (5 mL) was stirred for 30 min at 0 °C. Next, sulfamoyl chloride (0.26 mmol, 30.6 mg) was added to the reaction mixture after which the reaction was brought to the room temperature and stirred for an additional one hour. An excess of TEA (12 µl, excess) was added and stirring was continued for an additional 10 min. The reaction mixture was finally quenched with MeOH (5 ml) under ice bath. The reaction mixture was evaporated to dryness and the crude reaction mixture was then purified by reverse phase chromatography to afford the title compound (33 mg, 0.079 mmol) in 89.6 % yield. The purity of the compound was determine to be >98% upon HPLC analysis (Retention Time 21 min). ^{1}H NMR spectrum was (DMSO-d_6): 1.32 (s, 3H), 1.53 (s, 3H), 4.15 (m, 3H), 4.83-4.90 (m, 2H), 5.37 (m, 1H), 6.24 (s, broad 2H), 7.61 (s, 2H), 8.28 (s, 1H) and 10.58 (s, 1H). ^{13}C- DMSO-d_6: 158.76, 151.37, 148.31, 127.13, 124.36, 123.1, 114.55, 85.94, 81.88, 81.75, 79.30, 68.76, 27.72 and 25.81 ppm. Low resolution ESI-MS [M+H] 419.1 HRMS (ESI+) calcd for $C_{14}H_{19}N_4O_7S_2$ [(M+H)+] 419.0695 found 419.0679



5′-O-[N-(3-Indole propionic acid)sulfamoyl]-2-Aminothieno[3,4-d]pyrimidine G mimic nucleoside (8)

To an ice cold stirred solution of **6** (20 mg, 0.072 mmol) and 22 (30.8 mg, 0.108 mmol) in DMF (0.2 mL) was added DBU (1.1 equiv, 11.8 µl, 0.079 mmol). After stirring for 10 min the reaction mixture was brought to room temperature and stirred overnight. Next, the volatiles were evaporated under reduced pressure and the mixture was used further for next step without any purification. In the next step, the crude mixture was dissolved in 80% aqueous TFA (0.2 ml) and stirred for 30 min. The reaction mixture was evaporated to dryness (co-evaporated 1% TEA/ethanol for removing TFA) and purified using reverse phase chromatography (A-ACN, B-Water + 0.1% TEA). The eluted peak was concentrated and lyophilized to obtain the desired final product in 60% yields (16.5 mg with 1.33 equivalent of TEA). The purity of the compound was determine to be >99% upon HPLC analysis (Retention Time 15.8 min). ^{1}H NMR spectrum was (DMSO-d_6): 1.10 (s, 12.0 H), 2.34 (m, 2.0 H), 2.85 (m, 9H), 3.88-3.96 (m, 4H), 5.12-5.18 (m, 2H), 6.18 (s, broad 2H), 6.94 (m, 1H), 7.07 (m, 2H), 7.29 (d, 1H), 7.48 (d, 1H), 8.16 (s, 1H), 10.48 (s, 1H) and 10.68 (s, 1H). ^{13}C-D_2O (600 MHz): 181.12, 168.61, 133.73, 126.06, 124.50, 120.28, 119.34, 116.84, 116.29, 111.68, 109.17, 79.92, 73.98, 68.98, 66.63, 56.62, 46.61, 44.26, 36.60, 19.08, 16.89, 7.98, 6.10 and 5.16 ppm. Low resolution ESI-MS 550.1 HRMS (ESI+) calcd for $C_{22}H_{24}N_5O_8S_2$ [(M+H)+] 550.1066 found 550.1045



5′-O-[N-(3-biotinyl butanoic acid)sulfamoyl]-2-Aminothieno[3,4-d]pyrimidine G mimic nucleoside (9)

To an ice cold stirred solution of 6 (30 mg, 0.072 mmol) and NHS-ester of biotin butanoic acid (61.3 mg, 0.18 mmol) in DMF (0.3 mL) was added DBU (1.1 equiv, 12 µl, 0.080 mmol). After stirring for 10 min the reaction mixture was brought to room temperature and stirred overnight. Next, the volatiles were evaporated under reduced pressure and the mixture was used further for next step without any purification. In the next step, the crude mixture was dissolved in 80% aqueous TFA (2 ml) and stirred for 30 min. The reaction mixture was evaporated to dryness (co-evaporated 1% TEA/ethanol for removing TFA) and purified using reverse phase

chromatography (A-ACN, B-Water + 0.1% TEA). The eluted peak was concentrated and lyophilized to obtain the desired final product in 68.8% yields (30 mg, with 1.85 equivalent of TEA). The purity of the compound was determine to be >99% upon HPLC analysis (Retention Time 14.1 min). ^{1}H NMR spectrum was (DMSO-d_6): 1.09 (t, 16.0H), 1.42 (m, 3H), 1.45 (m, 4H), 1.61 (m, 2H), 1.97 (t, 2H), 2.86 (m, 12H), 3.07 (m, 2H), 3.88-3.96 (m, 6H), 4.12 (m, 2H), 4.27 (m, 1H), 5.05 (s, 1H), 5.14 (s, 2H), 6.19 (s, broad 2H), 6.33 (s, 1H), 6.42 (s, 1H), 8.18 (s, 1H), and 10.49 (s, 1H). ^{13}C- D_2O (600 MHz): 183.73, 165.53, 165.14, 165.03, 155.45, 126.70, 123.13, 121.55, 82.11, 77.20, 76.68, 71.54, 68.68, 61.85, 60.17, 55.30,46.58, 39.77, 38.46, 28.07, 27.65, 25.64 and 8.25, HRMS (ESI+) calcd for $C_{21}H_{29}N_6O_9S_3$ [(M+H)+] 605.6848 found 605.6849

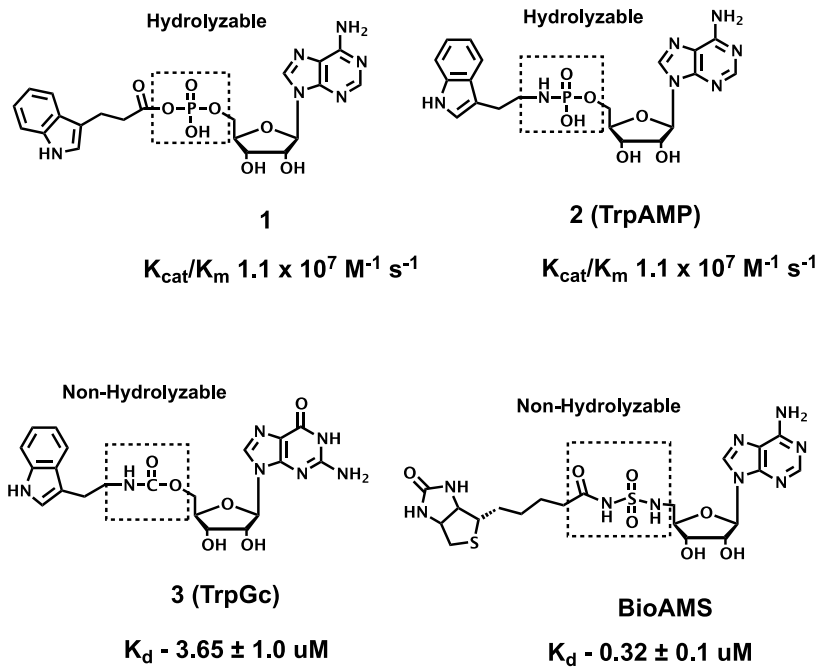


Figure S1. Chemical structures of the hHint1 substrates (compound **1** and **2**) and a previously reported hHint1 inhibitors compound **3** (TrpGc) and Bio-AMS

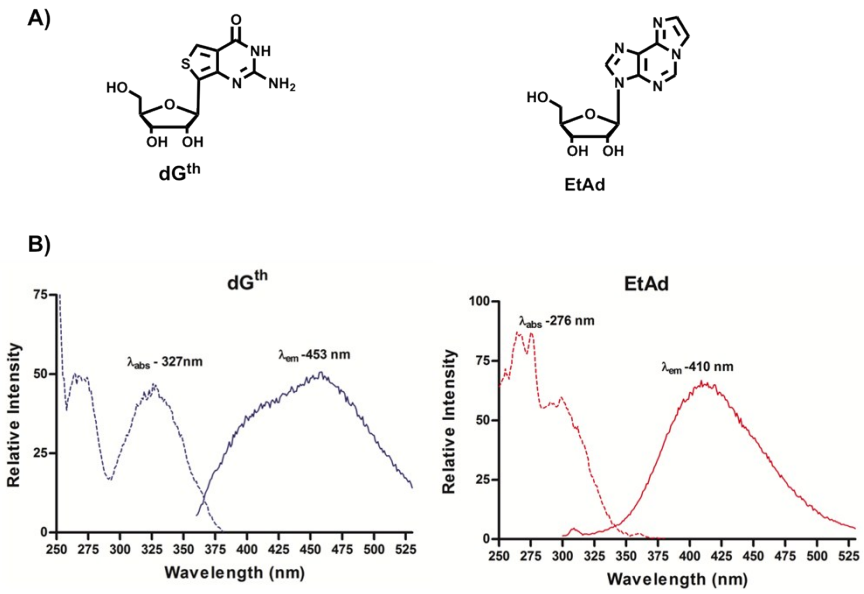


Figure S2. A) Chemical structures of the fluorescent nucleosides dGth (left) and EtAd (right) and B) Absorption (dashed lines) and Emission (solid lines) spectra of the respective nucleosides recorded in an aqueous buffer (20mM Tris, 150 mM NaCl, pH 7.4) at room temperature.

Table S1: Photophysical and fluorescence properties of EtAd and dGth nucleoside

Nucleoside	Solvent	λ_{abs} (nm)	λ_{emi} (nm)	Ø	Stokes shift (cm^{-1})	τ (ns)
EtAd	**Aqueous Buffer[a]**	**276**	**410**	**0.56[b]**	**11841**	**25**
dGth	**Aqueous Buffer[a]**	**327**	**453**	**0.34**	**8505**	**20**

[a] buffer- 20 mM Tris, 150 mM NaCl, pH 7.4 at room temperature and Ø means quantum yield
[b] Value taken as standard reference reported in the literature[2]

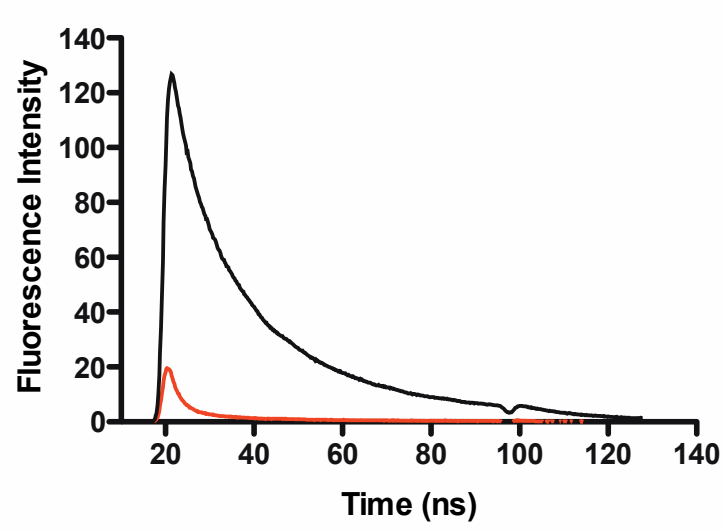


Figure S3. Time-resolved fluorescence studies on ethenoadenosine (black, fluorophore) and compound **7** (red, fluorophore + quencher). The decrease in the lifetime of fluorescence of **7** in comparison to parent nucleoside indicates dynamic quenching of the fluorophore.

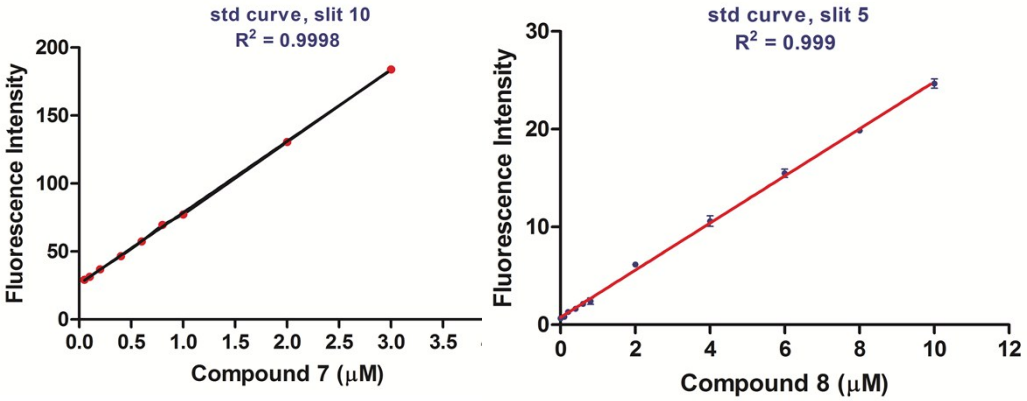


Figure S4. Standard curves: Measure fluorescence intensity of compound 7 and 8 in the absence of hHint1. The total increase in the fluorescence (λ_{ex}= 278 nm for **7**, λ_{em}= 410 slit 10 and 330 nm for **8**, λ_{em}= 453 slit 5) in the absence of hHint1 was plotted against the concentration of the respective compounds. Data points represent three measurements including the standard deviations.

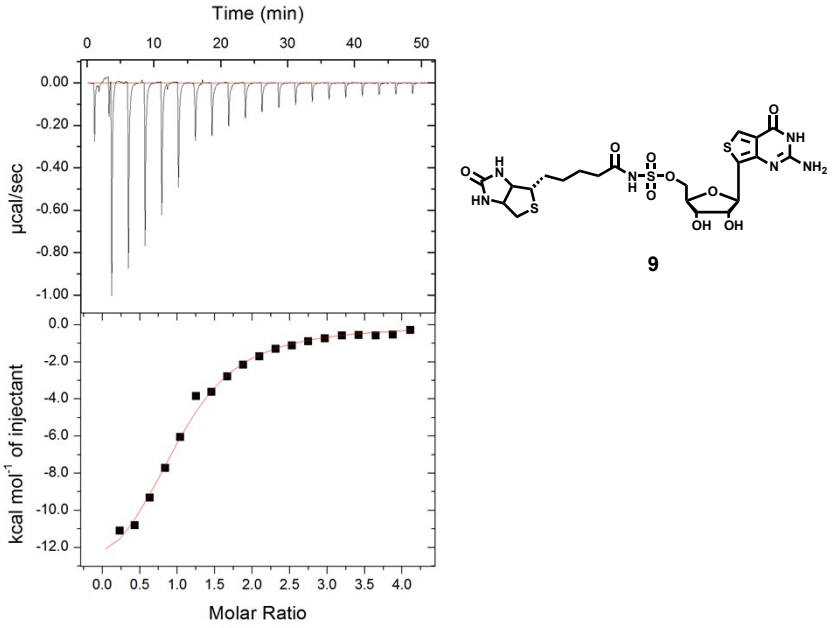


Figure S5. A typical binding isotherm created after plotting integrated heat peaks against the molar ratio of Compound 9 (500 μM, 10 mM Tris, 150 mM NaCl, pH 7.5) titrated into the solution of hHint1 (20 μM). Right: Chemical structure of 9.

Table S2: Thermodynamic parameters of 9 binding to hHint1

Ligand	K_d (uM)	ΔH (kcal/mol)	-TΔS (kcal/mol)	ΔG (kcal/mol)	n
9	6.19 ± 0.34	-15.50 ± 0.71	8.39 ± 0.75	-7.14 ± 0.04	0.89 ± 0.1

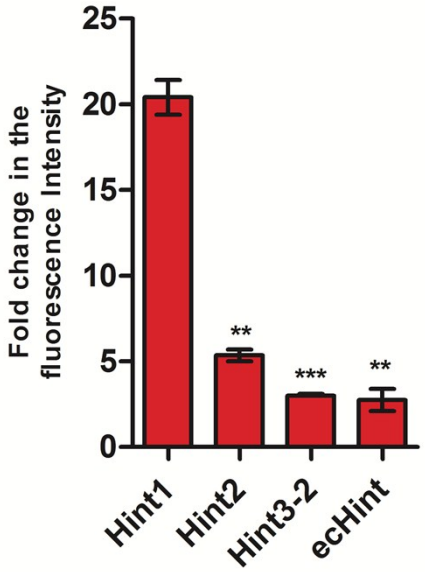
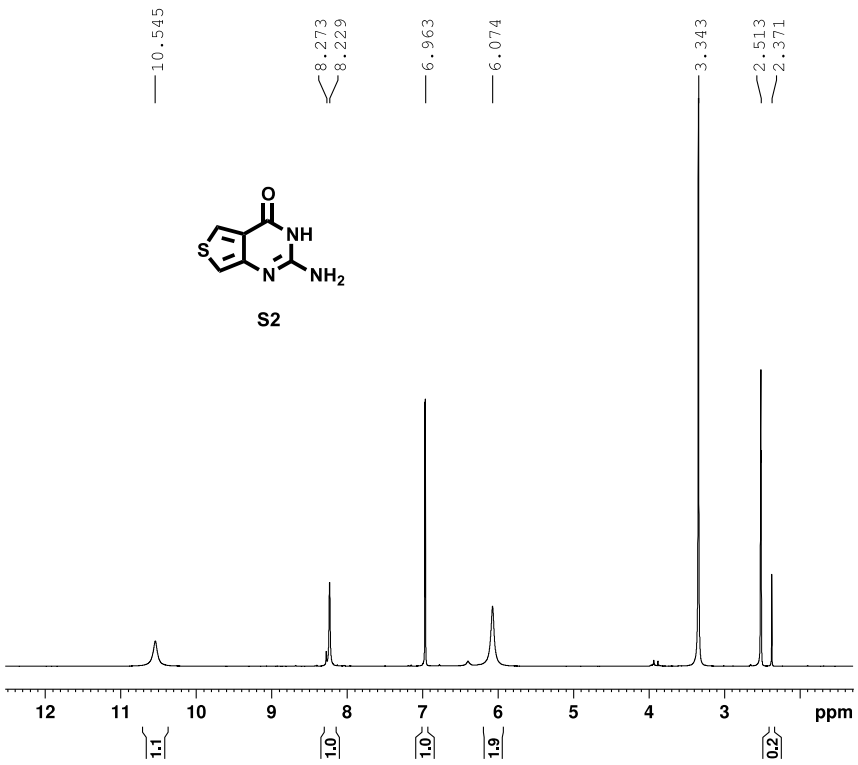


Figure S6. Selectivity test of compound **7** with other Hint proteins: Compound **7** (2 μM) was tested with 0.5 μM of Hint isoform proteins. Student t-test perform on the values indicate, ** p-values < 0.005, *** p-values ≥ 0.0001

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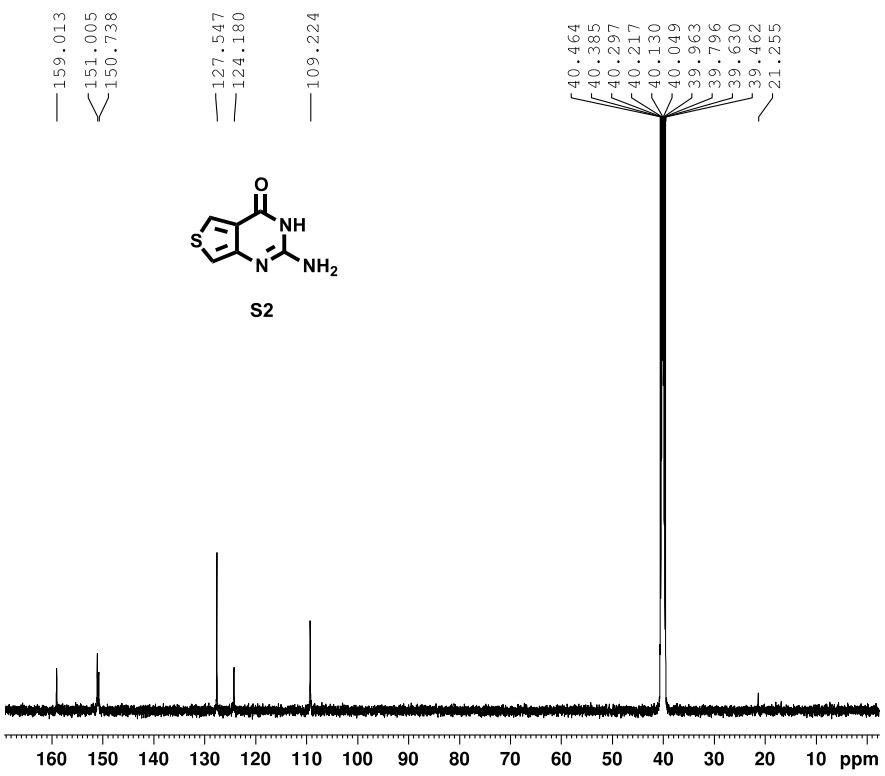


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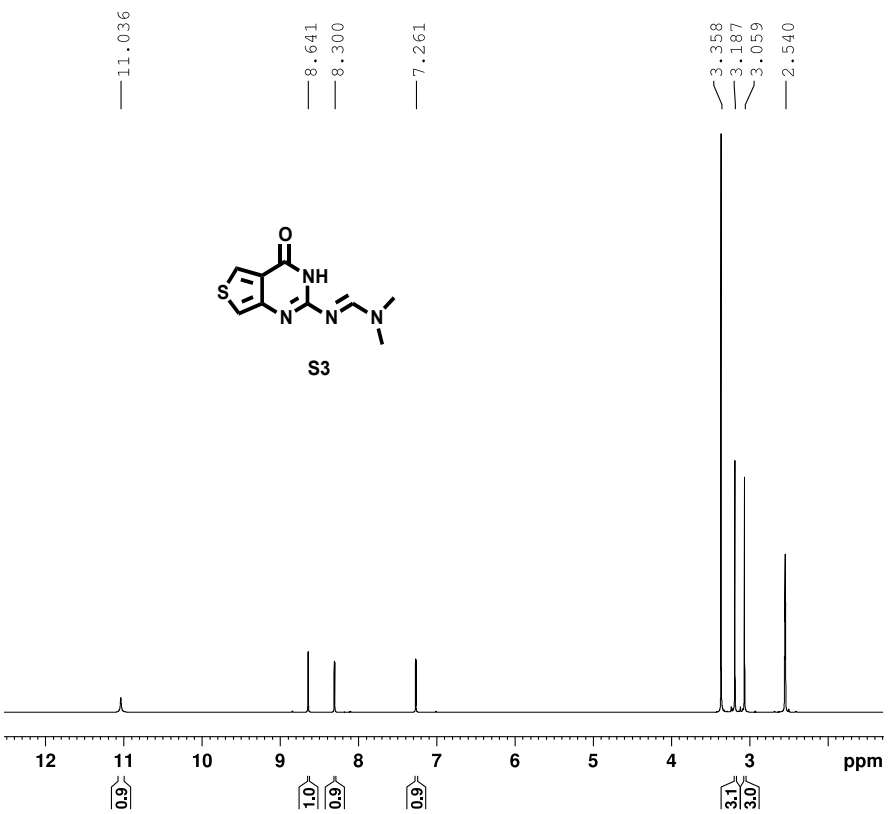
D11                0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1               125.7703637 MHz
NUC1                        13C
P1                       10.00 usec
PLW1               84.00000000 W

======== CHANNEL f2 ========
SFO2               500.1320005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                    80.00 usec
PLW2              15.00000000 W
PLW12              0.28308001 W
PLW13              0.18117000 W

F2 - Processing parameters
SI                       32768
SF                 125.7577890 MHz
WDW                         EM
SSB          0
LB                        1.00 Hz
GB           0
PC                        1.40
```

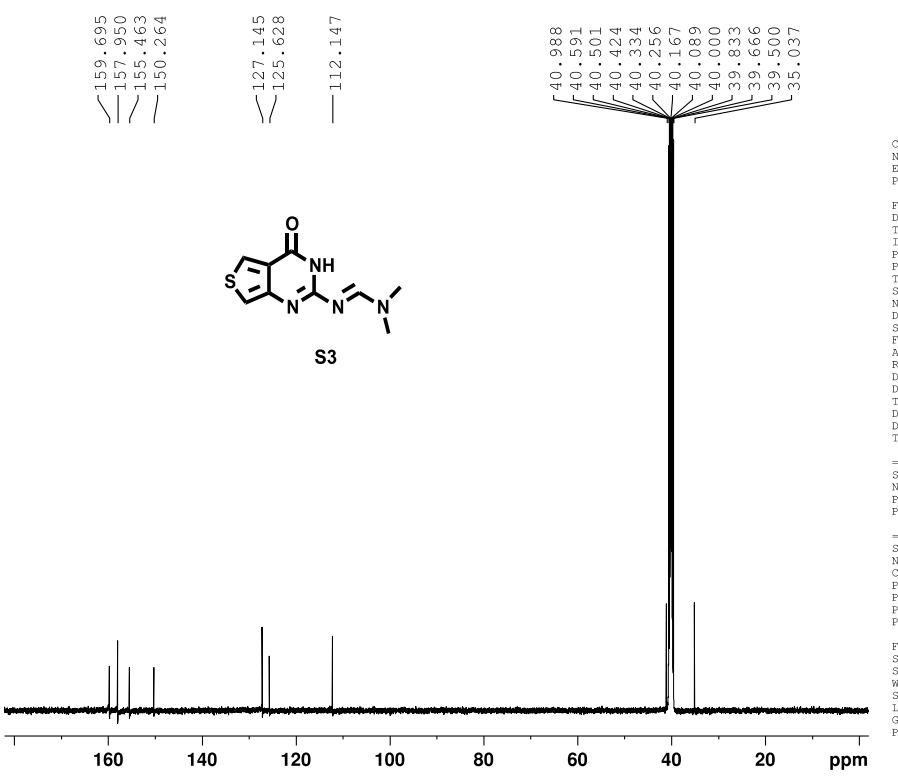



```
Current Data Parameters
NAME                  AZ-I-60
EXPNO                       1
PROCNO                      1

F2 - Acquisition Parameters
Date_              20150422
Time                  15.57
INSTRUM               spect
PROBHD    5 mm PABBO BB/
PULPROG               zg30
TD                   65536
SOLVENT               DMSO
NS                      64
DS                       2
SWH               10000.000 Hz
FIDRES                0.152588 Hz
AQ                 3.2767999 sec
RG                    186.69
DW                  50.000 usec
DE                   13.29 usec
TE                   298.1 K
D1              1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1           500.1330885 MHz
NUC1                     1H
P1                    10.99 usec
PLW1          15.00000000 W

F2 - Processing parameters
SI                       65536
SF             500.1299871 MHz
WDW                         EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```



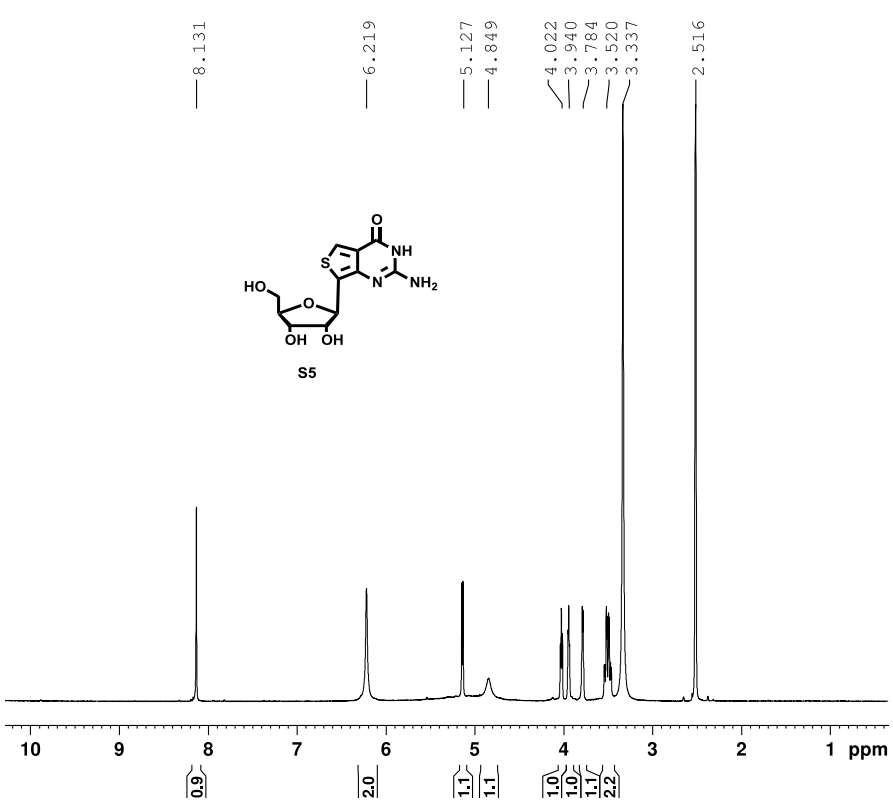

```
Current Data Parameters
NAME                  AZ-I-60
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_              20150423
Time                   5.10
INSTRUM               spect
PROBHD    5 mm PABBO BB/
PULPROG             zgpg30
TD                   65536
SOLVENT               DMSO
NS                    5000
DS                       4
SWH               29761.904 Hz
FIDRES                0.454131 Hz
AQ                 1.1010048 sec
RG                    186.69
DW                  16.800 usec
DE                   6.50 usec
TE                   298.1 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1           125.7703637 MHz
NUC1                    13C
P1                   10.00 usec
PLW1          84.00000000 W

======== CHANNEL f2 ========
SFO2           500.1320005 MHz
NUC2                     1H
CPDPRG[2          waltz16
PCPD2                80.00 usec
PLW2          15.00000000 W
PLW12          0.28308001 W
PLW13          0.18117000 W

F2 - Processing parameters
SI                       32768
SF             125.7577890 MHz
WDW                         EM
SSB          0
LB                    1.00 Hz
GB           0
PC                    1.40
```



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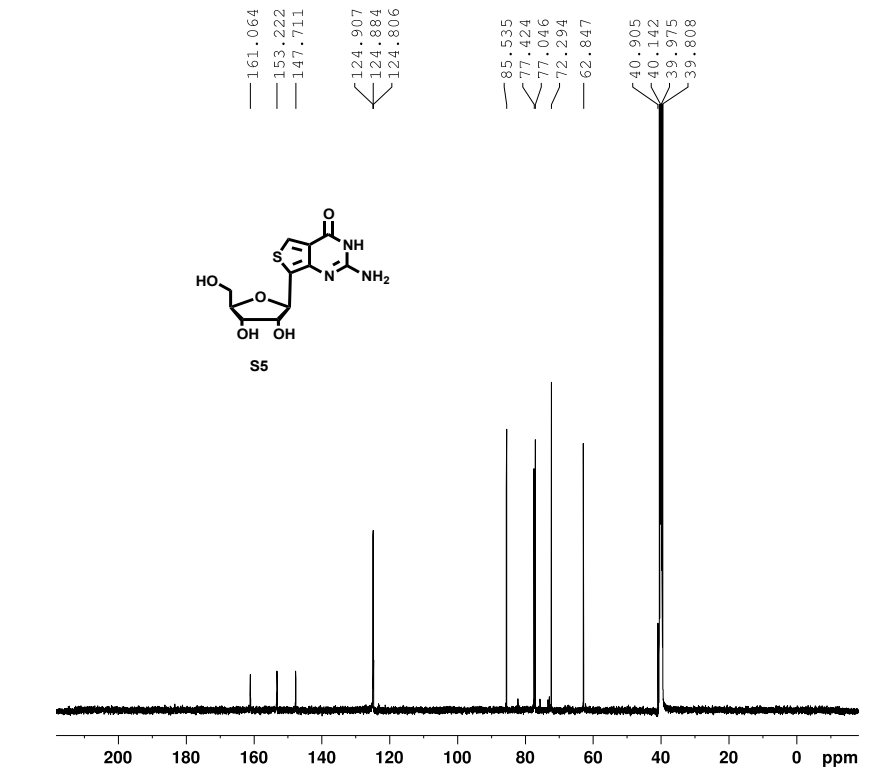
```
Current Data Parameters
NAME        RS_dGth_dmso
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150726
Time               16.21
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    64
DS                     2
SWH            10000.000 Hz
FIDRES             0.152588 Hz
AQ              3.2767999 sec
RG                 148.37
DW                 50.000 usec
DE                  6.50 usec
TE                 298.1 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1330885 MHz
NUC1                   1H
P1                  10.99 usec
PLW1          15.00000000 W

F2 - Processing parameters
SI                 65536
SF            500.1299995 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



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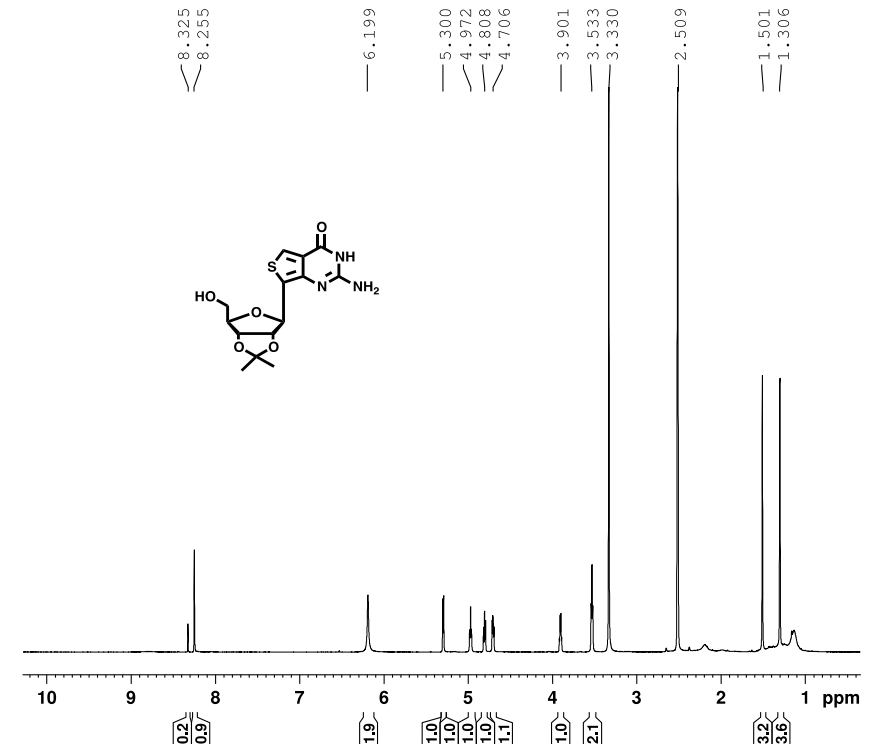
```
Current Data Parameters
NAME          dgth_rerun
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20170710
Time               1.19 h
INSTRUM            spect
PROBHD   Z119470_0114 (
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                  6000
DS                     4
SWH            29761.904 Hz
FIDRES             0.908261 Hz
AQ              1.1010048 sec
RG                 186.69
DW                 16.800 usec
DE                  6.50 usec
TE                 298.1 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1
SFO1          125.7703643 MHz
NUC1                  13C
P1                  9.40 usec
PLW1          84.00000000 W
SFO2          500.1320005 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2          15.00000000 W
PLW12          0.30996001 W
PLW13          0.15591000 W

F2 - Processing parameters
SI                 32768
SF            125.7577890 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

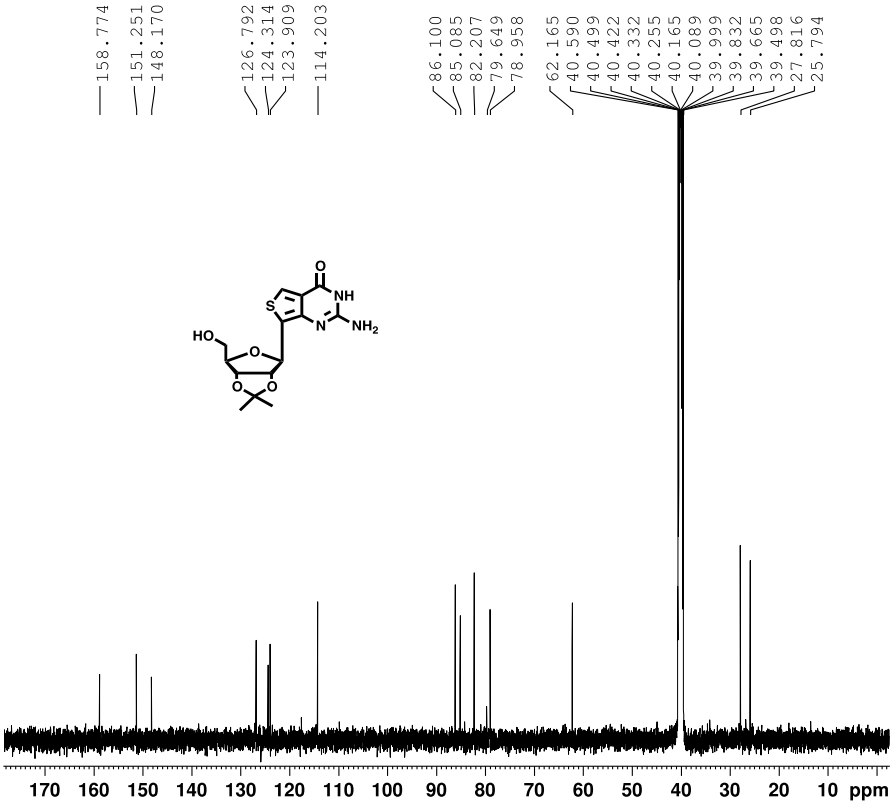


Current Data Parameters
NAME dgthKp
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150522
Time 19.29
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 64
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 186.69
DW 50.000 usec
DE 13.29 usec
TE 298.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 500.1330885 MHz
NUC1 1H
P1 10.99 usec
PLW1 15.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300006 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



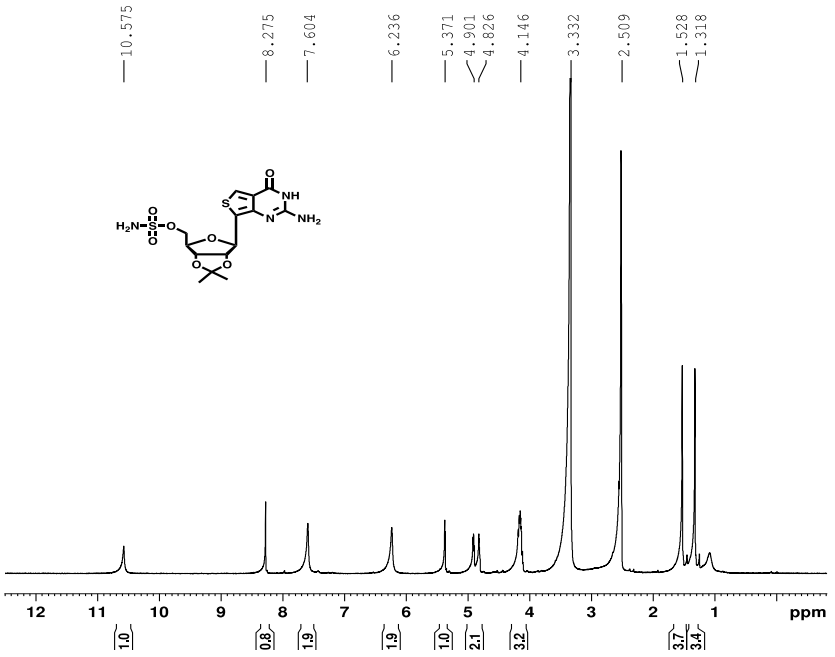
Current Data Parameters
NAME dgthKp
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150524
Time 5.00
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5000
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 186.69
DW 16.800 usec
DE 6.50 usec
TE 298.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 125.7703637 MHz
NUC1 13C
P1 10.00 usec
PLW1 84.00000000 W

======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.00000000 W
PLW12 0.28308001 W
PLW13 0.18117000 W

F2 - Processing parameters
SI 32768
SF 125.7577890 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

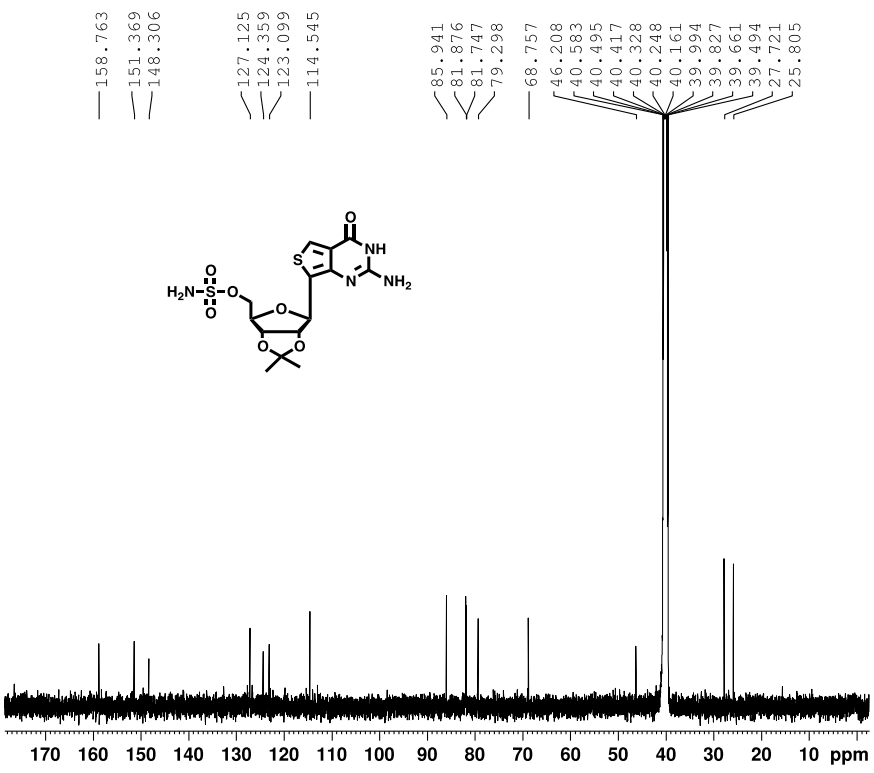



```
Current Data Parameters
NAME      RS_dGthKpSONH2
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150726
Time               16.29
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 65536
SOLVENT             DMSO
NS                    64
DS                     2
SWH            10000.000 Hz
FIDRES             0.152588 Hz
AQ                 3.2767999 sec
RG                   166.64
DW                  50.000 usec
DE                  13.29 usec
TE                  298.1 K
D1              1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1330885 MHz
NUC1                    1H
P1                  10.99 usec
PLW1          15.00000000 W

F2 - Processing parameters
SI                  65536
SF            500.1299991 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



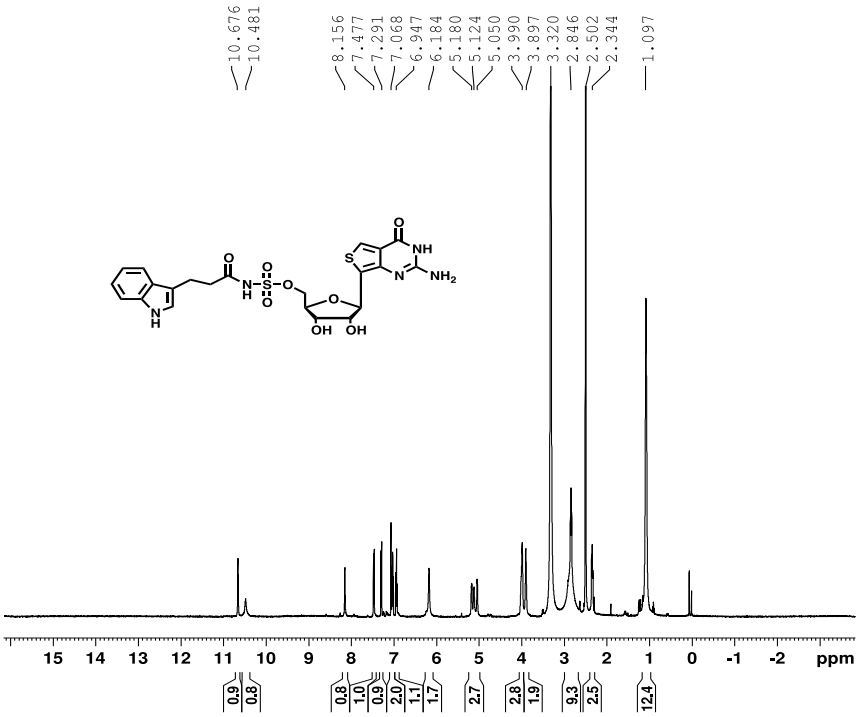
```
Current Data Parameters
NAME      RS_dGthKpSONH2
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150727
Time               6.59
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                 65536
SOLVENT             DMSO
NS                  5000
DS                     4
SWH            29761.904 Hz
FIDRES             0.454131 Hz
AQ                 1.1010048 sec
RG                   186.69
DW                  16.800 usec
DE                   6.50 usec
TE                  298.1 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          125.7703637 MHz
NUC1                   13C
P1                  10.00 usec
PLW1           84.00000000 W

======== CHANNEL f2 ========
SFO2          500.1320005 MHz
NUC2                    1H
CPDPRG[2          waltz16
PCPD2               80.00 usec
PLW2          15.00000000 W
PLW12          0.28308001 W
PLW13          0.18117000 W

F2 - Processing parameters
SI                  32768
SF            125.7577890 MHz
WDW                    EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```



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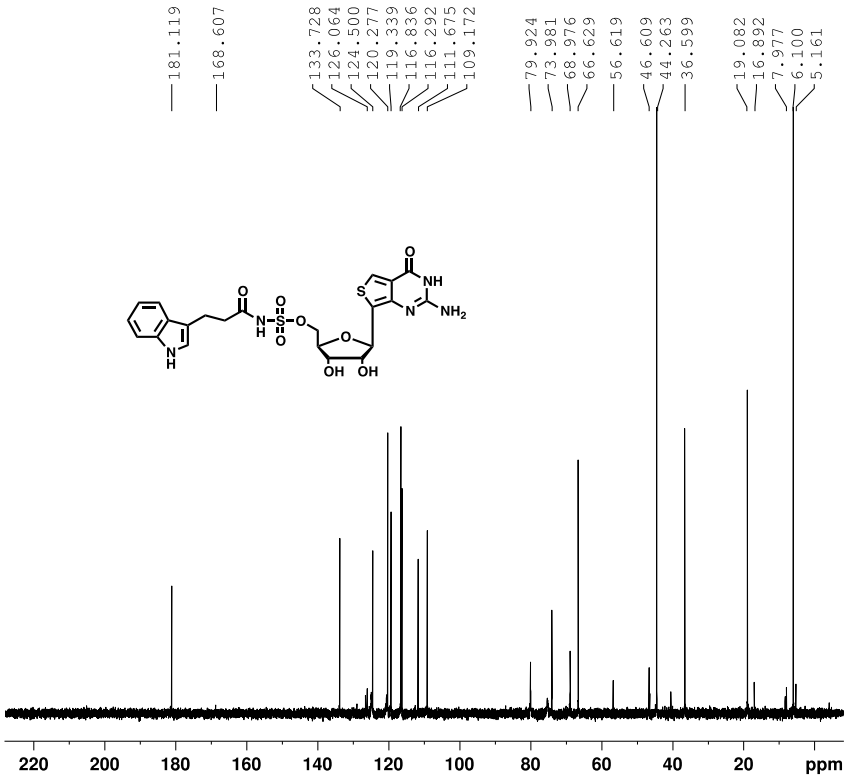
```
Current Data Parameters
NAME      RS_dGthIndSoNh2
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20150719
Time               13.32
INSTRUM            spect
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT            DMSO
NS                    64
DS                     2
SWH           10000.000 Hz
FIDRES         0.152588 Hz
AQ             3.2767999 sec
RG                186.69
DW                50.000 usec
DE                13.29 usec
TE                298.1 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          500.1330885 MHz
NUC1                    1H
P1                  10.99 usec
PLW1         15.00000000 W

F2 - Processing parameters
SI                 65536
SF            500.1300057 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```



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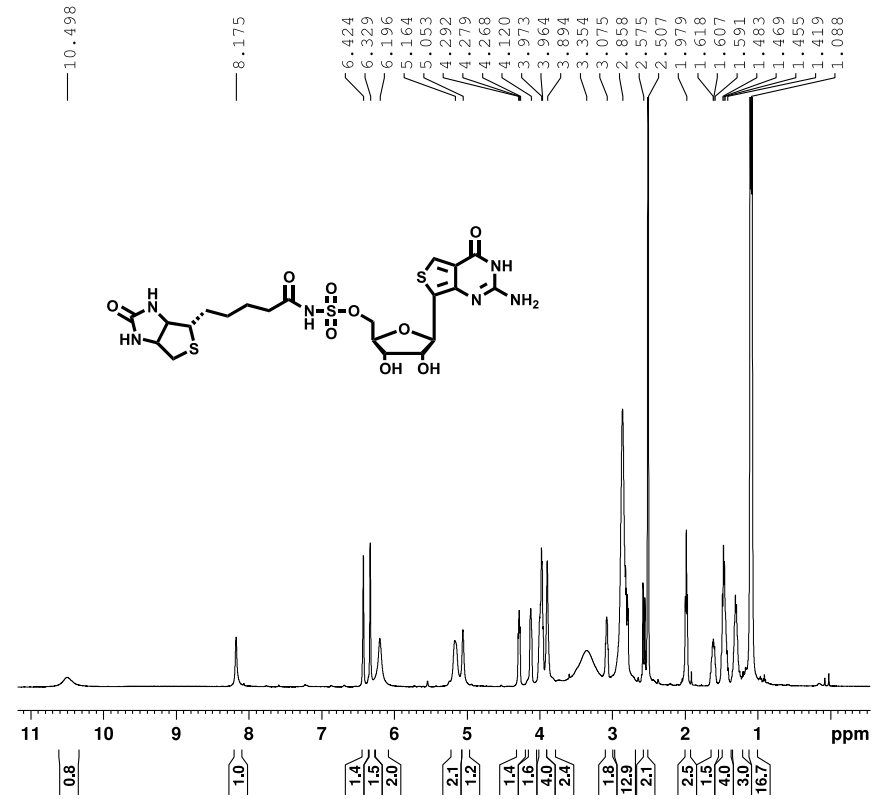
```
Current Data Parameters
NAME      20171023_rachit_13C
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_           20171025
Time               5.35 h
INSTRUM            spect
PROBHD   Z148997_0006 (
PULPROG          zgpg30
TD               131072
SOLVENT             DMSO
NS                 10240
DS                     0
SWH           35714.285 Hz
FIDRES         0.544957 Hz
AQ             1.8350080 sec
RG                   101
DW                14.000 usec
DE                40.00 usec
TE                298.0 K
D1            5.00000000 sec
D11           0.03000000 sec
TD0                    1
SFO1          151.0363450 MHz
NUC1                   13C
P1                 12.00 usec
PLW1          88.27500153 W
SFO2          600.5974024 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2              80.00 usec
PLW2           6.20429993 W
PLW12          0.06075503 W
PLW13          0.03045042 W

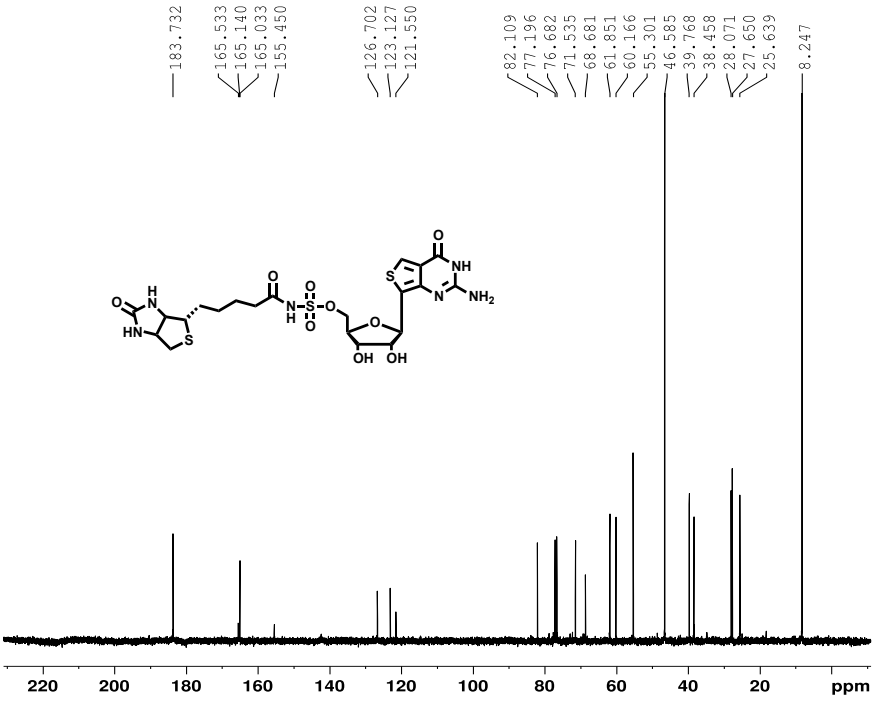
F2 - Processing parameters
SI                 32768
SF            151.0197329 MHz
WDW                   EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```



Current Data Parameters
NAME AZ-1-160_8 MG
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20170905
Time 14.25 h
INSTRUM spect
PROBHD Z119470_0114 (
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 64
DS 2
SWH 10000.000 Hz
FIDRES 0.305176 Hz
AQ 3.2767999 sec
RG 86.1
DW 50.000 usec
DE 13.27 usec
TE 298.1 K
D1 1.00000000 sec
TD0 1
SFO1 500.1330885 MHz
NUC1 1H
P1 11.10 usec
PLW1 15.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300018 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



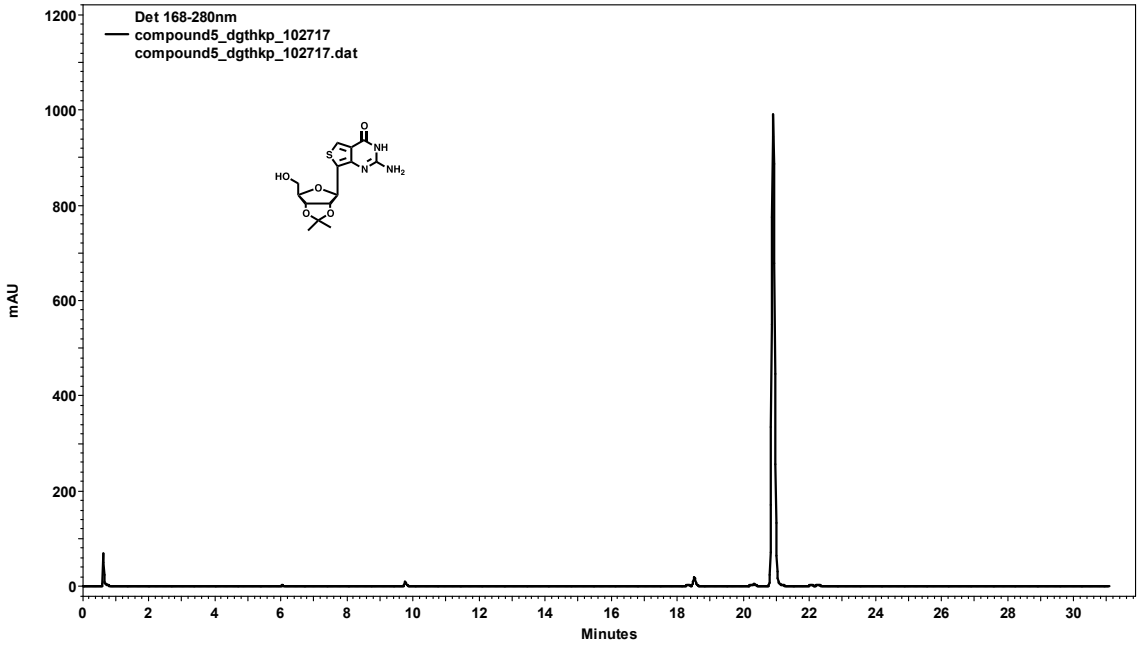
Current Data Parameters
NAME 20171025_Rachit_13C_2
EXPNO 1
PROCNO 1

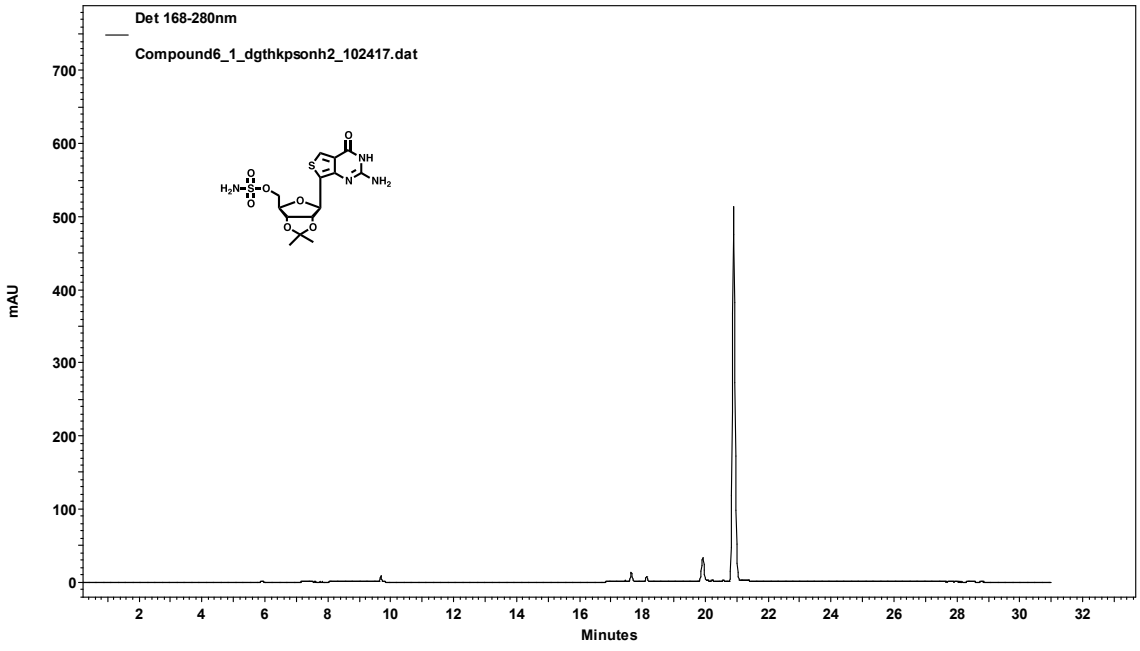
F2 - Acquisition Parameters
Date_ 20171026
Time 11.02
INSTRUM spect
PROBHD 1.7 mm CPTCI 1
PULPROG zgpg30
TD 131072
SOLVENT D2O
NS 12280
DS 0
SWH 42613.637 Hz
FIDRES 0.325116 Hz
AQ 1.5379114 sec
RG 175.87
DW 11.733 usec
DE 40.00 usec
TE 298.2 K
D1 5.00000000 sec
D11 0.03000000 sec
TD0 1

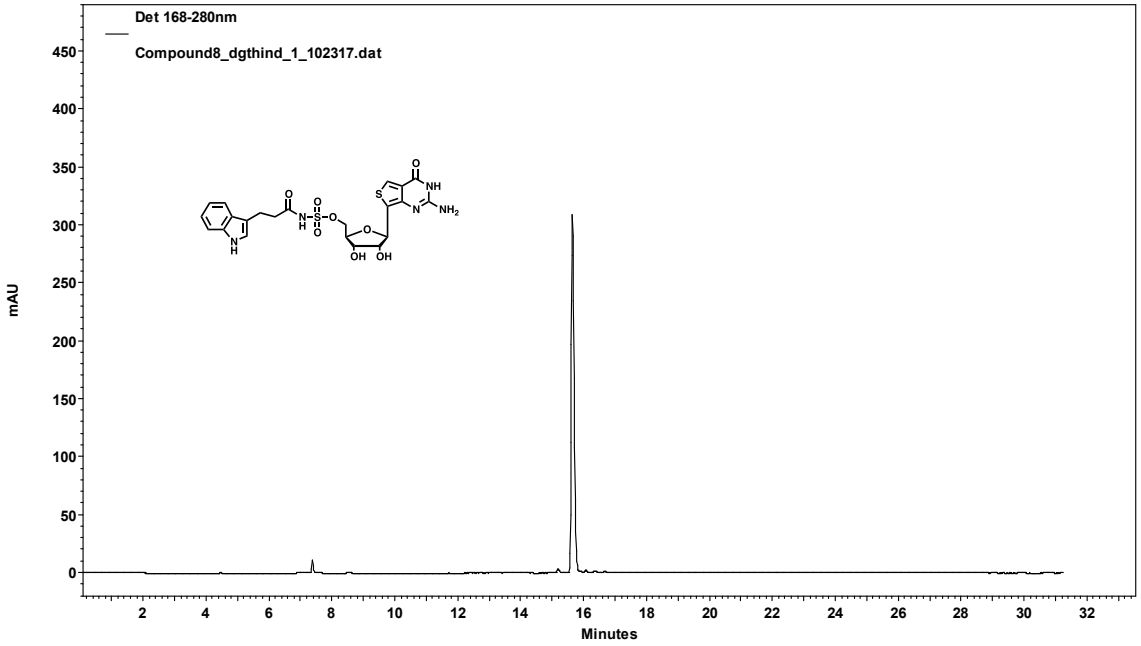
======== CHANNEL f1 ========
SFO1 176.1174898 MHz
NUC1 13C
P1 12.00 usec
PLW1 86.00000000 W

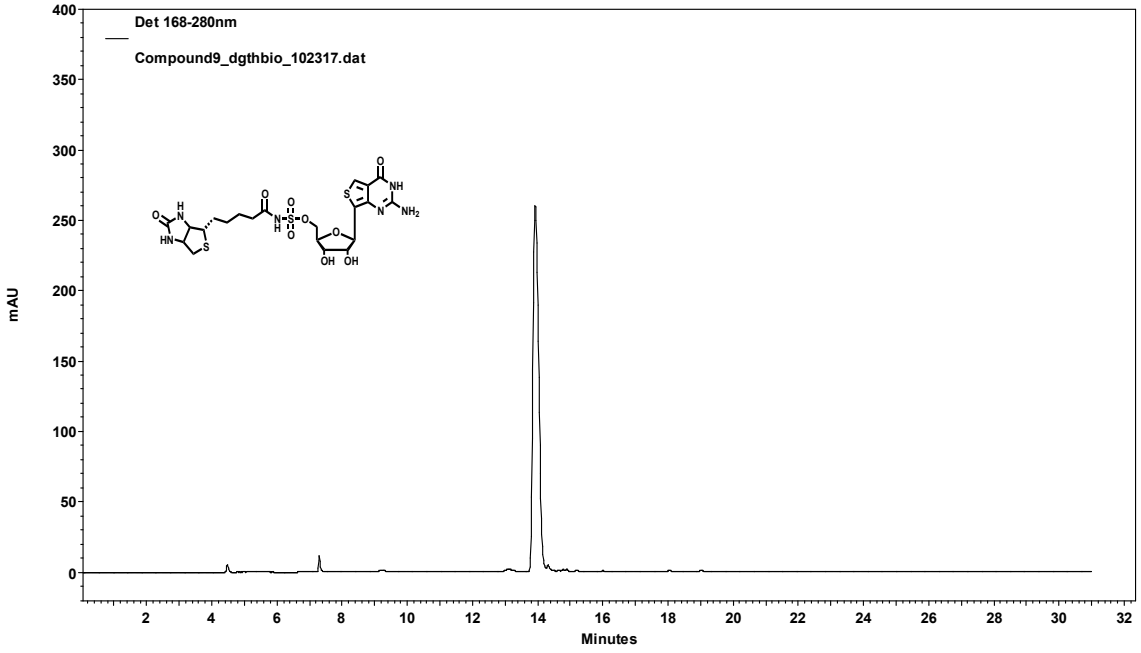
======== CHANNEL f2 ========
SFO2 700.3328013 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 0.62500000 W
PLW12 0.00976560 W
PLW13 0.00625000 W

F2 - Processing parameters
SI 32768
SF 176.0981190 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00


Det 168-280nm
compound5_dgthkp_102717
compound5_dgthkp_102717.dat
mAU
Minutes


Det 168-280nm
Compound6_1_dgthkpsonh2_102417.dat
mAU
Minutes


Det 168-280nm
Compound8_dgthind_1_102317.dat
mAU
Minutes


Det 168-280nm
Compound9_dgthbio_102317.dat
mAU
Minutes