Supporting Information

Development of PET Tracers for Noninvasive Imaging of the Reactive Oxygen Species, Superoxide, *in Vivo*

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Experimental procedures, NMR spectra, elemental analysis data.

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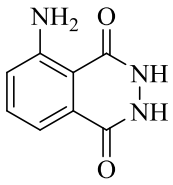
Figure S5. Biodistribution of [^{18}F]**12** in DOX-treated and untreated mice.

Figure S6. Stability of [^{18}F]**12** in DOX-treated and untreated mice *in vitro* and *in vivo*

Table S1. Reagent Concentrations

Compounds: **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, **12**, **13**, and **14** ^{1}H, ^{13}C NMR spectra.

Figure. S1



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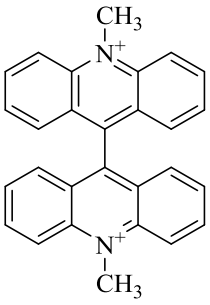

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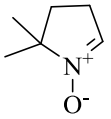

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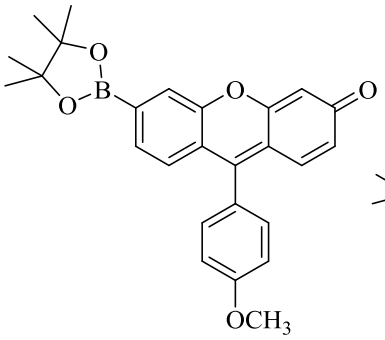
(DMPO)



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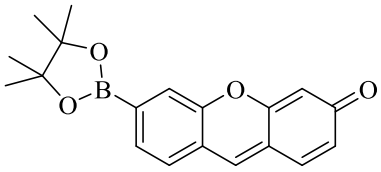

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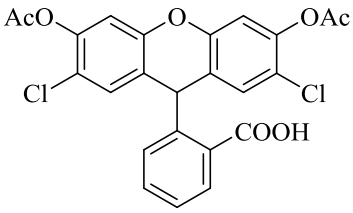
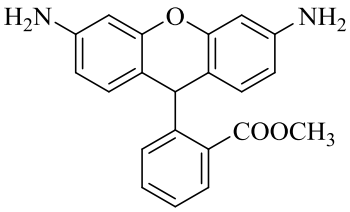
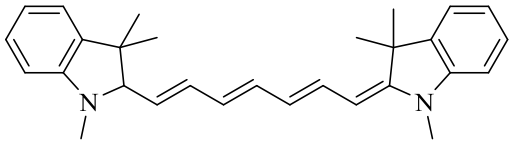


2,7-Dichlorodihydrofluorescein
Diacetate
(DCFH-DA)



Dihydrorhodamine 123 (DHR),



Dihydrocyanine
Hydro-Cy7

Experimental

Preparation of stock solutions

Reagent sources and final well concentrations are summarized in Table S1. Phosphate buffer saline

(PBS, pH 7.4) was prepared using Milli-Q plus system (Millicore Corp.). The following stock solutions were prepared in PBS: 0.1 mM. All solutions were prepared monthly and stored at 4 °C except for xanthine oxidase, which was diluted from commercial stock just prior to use. Hypoxanthine was prepared fresh on the day of experiment using slightly alkaline PBS to dissolve the powder. The cold (non-radiolabeled) compounds were synthesized and stored under anaerobic conditions in the dark until they were ready for use. All reagents were warmed to room temperature just prior to use. Two reaction solutions were prepared prior to each experiment, using the above stock solutions. The solutions were made to match the final well concentration summarized in Table S1. Both solutions contained PBS buffer and salmon sperm DNA (fluorescence enhancer, 250 μg/ml final concentration): solution A consisted of hypoxanthine and xanthine oxidase, solution B was made up of SIN-1 chloride and CPTIO.

Table S1. Reagent Concentrations

Reagent	Plate reader assay concentration[a]		Source
	μM	U/ml	
horseradish peroxidase (HRP)		0.2	Sigma-Aldrich
hypoxanthine (HX)	1×10^3		Sigma-Aldrich
xanthine oxidase (XO)		0.05 2	Sigma-Aldrich
hydrogen peroxide	1×10^3		Fisher-Scientific
12	158		
dihydroethidium (DHE)	158		Invitrogen
Linsidomine (SIN-1 chloride)	15.8		Invitrogen
superoxide dismutase (SOD)		575	Calbiochem
catalase		60	Calbiochem
2-4-carboxyphenyl-4,4,5,5-tetramethy-limidazoline-1-oxyl-3-oxide (CPTIO)	262		Calbiochem

[a] These amounts reflect the final concentration inside each well after 12 or DHE compound was added.

In Vitro Fluorescence of 12

Preliminary evaluation of the **12** was performed by fluorescence plate reader assay to compare specificity and kinetics of **12** and DHE oxidation by superoxide. The assay is performed in duplicates, using a Corning 96-well (12 columns × 8 rows) plate with a final reaction volume of 275 µl per well. All wells except for blank contained PBS-DNA buffer as described above. The final concentrations of all reagents are listed in Table S1. Well 2 contained only hypoxanthine and buffer, and served as the marker for autofluorescence. Wells 3-5 contained solution A, by itself (3), with addition of catalase (4), and with SOD (5). Hydrogen peroxide was added to wells 5 and 6, with and without horseradish peroxidase respectively. The last 2 wells in the row contained solution B only, (8) and with SOD (9). After adding the reagents, the **12** was added using a multichannel pipette.

The plate was inserted into the plate reader (Biotek Instruments Inc. FL400 Fluorescence Plate reader, supported by Biotek-KC4 Kineticalc Software). The automix function was activated for 2 seconds prior to each 30 second reading, and the plate was read at 590 nm and excited at 485 nm. The temperature was held constant at 37 °C for the entire 60 min assay. Data points were collected then analyzed in Matlab software. Each plate was dedicated to one fluorescent compound to avoid accidental mixing. Dihydroethidium and compound **12** were read in duplicates. Sampling of kinetic points from 5-14 min was used to compare reactivity of compound **12** with the oxidative radical and contrast it with the kinetics of DHE.

Platereader Kinetics Assay Results

After testing various concentrations of reagents, we found those listed in Table S1 showed

consistent linear kinetics within the 5-15 minute range. One method of testing specificity of **12** towards ROS is using fluorescence kinetic plate reader assays. Using fluorescence as an indicator of the oxidation of **12**, we contrasted the kinetics of oxidation using various biological oxidative species including superoxide, hydrogen peroxide, peroxynitrite and hydroxyl radical. Typical kinetic assay results are shown in Figure S2, which contrast the kinetics DHE and **12**.

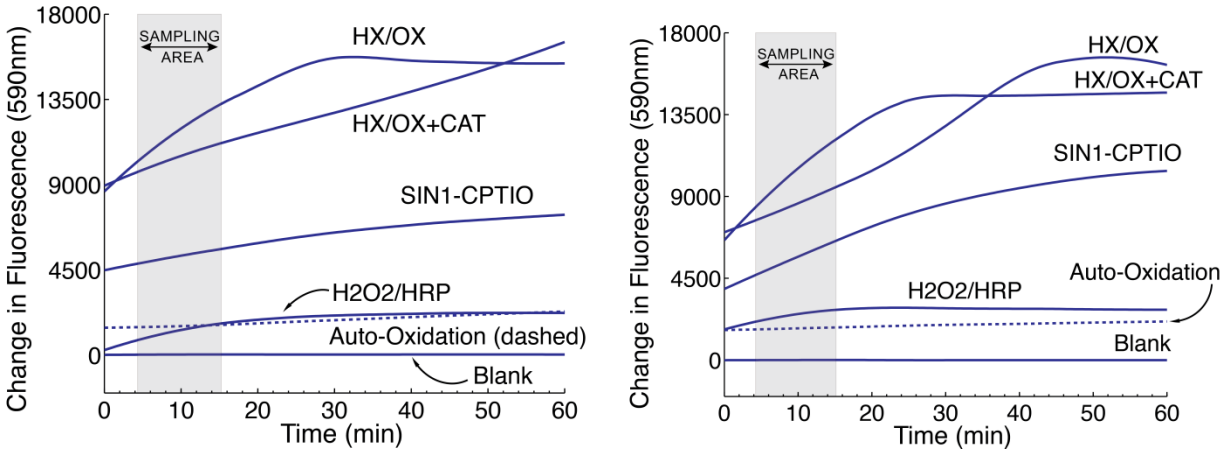


Figure S2. Kinetic plot of DHE oxidation (left) and 12 oxidation (right). Superoxide sources include catalysis of hypoxanthine by xanthine oxidase (HX/OX) and decomposition of SIN-1 chloride.

Superoxide was generated via catalysis of hypoxanthine by bovine-derived xanthine oxidase (HX/OX), which produces superoxide as well as hydrogen peroxide. The reagent concentrations were chosen for the reaction to shift to one-electron transfers, favoring O_2^- production. We also added catalase (CAT) to remove any hydrogen peroxide that might be generated, converting it to water and oxygen. However when hydrogen peroxide was added by itself, it acted like a mild reducing agent towards the compound **12** as well as DHE (data not shown). Superoxide dismutase (SOD) capture of superoxide was found to be an effective negative control; in its presence, auto-fluorescence kinetics was observed (data not shown). We implemented a secondary superoxide source that made use of the spontaneous decomposition of SIN-1 chloride in aqueous solutions to

produce superoxide and nitric oxide, which together form peroxynitrite. Under these conditions, no fluorescence was observed (data not shown), but with addition of 2-4-carboxyphenyl-4,4,5,5-tetramethy-limidazoline-1-oxyl-3-oxide (CPTIO), an effective NO scavenger, superoxide radical remained in solution and was able oxidize its substrate. Hydroxyl radical, which is often closely linked to superoxide generating systems, was generated using hydrogen peroxide and horseradish peroxidase, but it did not appear to any effects on the redox of **12** or DHE.

Microscopic fluorescence imaging of ROS.

EMT6 mouse breast cancer cells were grown in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The cell lines were maintained at 37 °C in a humidified 5% CO_2/ 95% air atmosphere. Aliquots of ~2,000 EMT6 cells were plated in chamber slides and cultured for 48 h; 400 nM doxorubicin was then added for the treatment group; after 1 h incubation, cells were replenished with fresh cell culture medium containing 2 µM DHE or **12** for another period of incubation of 30 min. The cell culture medium was then removed and the chamber slides were coverslipped and imaged with a Nikon E600 upright fluorescence microscope.

EMT-6 Cell uptake assays.

EMT-6 murine breast cancer cells were used as a model system to determine the uptake and retention of [^{18}F]**12** and [^{18}F]**14** by fluorescence microscopy. The EMT6 parental cell line used for experiments in Figure 3 was specifically selected because it has minimal expression of the p-glycoprotein transporter, and has been used as a "low MDR" control by many groups (1-3). Although DOX may induce MDR under some experimental conditions, the original paper by Twentyman et al. (4), describes escalating exposure to DOX over several weeks, and selection of

MDR clones, to produce the EMT6/AR1 drug-resistant cell line. That subline was not used in the current experiments. The 5 hour exposure to DOX used for **Figure 3** has not been shown to induce MDR. In **Figure 4**, DOX was not present and thus the issue of DOX-mediated inhibition of the MDR is not relevant. Our results showing increased uptake of the DHE analogue [^{18}F]**12** in response to DOX-induced ROS both in cell culture (EMT-6 cells) and in vivo (DOX-induced cardiotoxicity) is in agreement with previously published reports using the parent compound, DHE. (5, 6)

Method. Aliquots of 4,000 EMT6 cells suspended in culture medium were added to each well of Costar 24-well cell culture plates 48 h before the uptake assays to achieve log growth phase with approximately 70% confluence at the time of the uptake assay (approximately 15,000 cells per well). Cell culture medium was removed and freshly prepared cell culture medium containing ~5.6 × 10^6 Bq/ml [^{18}F]**12** or the oxidized compound [^{18}F]**14** was added to each well; after 30 min incubation at 37˚C, cells were rinsed twice with 1 ml ice cold 1 × PBS and then the cells were detached and harvested using 500 µl 0.05% EDTA/trypsin (Invitrogen, Grand Island, NY) treatment for ~2 min; protein concentration of the cell suspension was used to determine with a DC protein assay (Bio-Rad, Hercules, CA). A Wizard 1480 gamma counter (Perkin Elmer, Boston, MA) was used to count the radioactivity in each sample; uptake was expressed as ID%/mg protein.

References for Doxorubicin in EMT6 cells:

1 Mairinger S, Wanek T, Kuntner C, Doenmez Y, Strommer S, Stanek J, Capparelli E, Chiba P, Muller M, Colabufo NA, Langer O (2012) Synthesis and preclinical evaluation of the radiolabeled P-glycoprotein inhibitor [^{11}C]MC113. Nucl Med Biol 39:1219-1225.

2 Roy A, Murakami M, Ernsting MJ, Hoang B, Undzys E, Li SD (2014) Carboxymethylcellulose-based and docetaxel-loaded nanoparticles circumvent P-glycoprotein mediated multidrug resistance. Molecular Pharmaceutics.

3 Twentyman PR, Reeve JG, Koch G, Wright KA (1990) Chemosensitisation by verapamil and cyclosporin A in mouse tumour cells expressing different levels of P-glycoprotein and CP22 (sorcin). Br J Cancer 62:89-95.

4 Wanek T, Kuntner C, Bankstahl JP, Bankstahl M, Stanek J, Sauberer M, Mairinger S, Strommer S, Wacheck V, Loscher W, Erker T, Muller M, Langer O (2012) A comparative small-animal PET evaluation of [^{11}C]tariquidar, [^{11}C]elacridar and (*R*)-[^{11}C]verapamil for detection of P-glycoprotein-expressing murine breast cancer. Eur J Nucl Med Mol Imaging. 39:149-159.

5. Yoshida M, Shiojima I, Ikeda H, Komuro I. Chronic doxorubicin cardiotoxicity is mediated by oxidative DNA damage-ATM-p53-apoptosis pathway and attenuated by pitavastatin through the inhibition of Rac1 activity. **J Mol Cell Cardiol**. *2009*;47(5):698-705.

6 Luanpitpong S, Chanvorachote P, Nimmannit U, Leonard SS, Stehlik C, Wang L, Rojanasakul Y. Mitochondrial superoxide mediates doxorubicin-induced keratinocyte apoptosis through oxidative modification of ERK and Bcl-2 ubiquitination. **Biochem Pharmacol**. *2012*;83(12):1643-54; PubMed Central PMCID: PMC3337700.

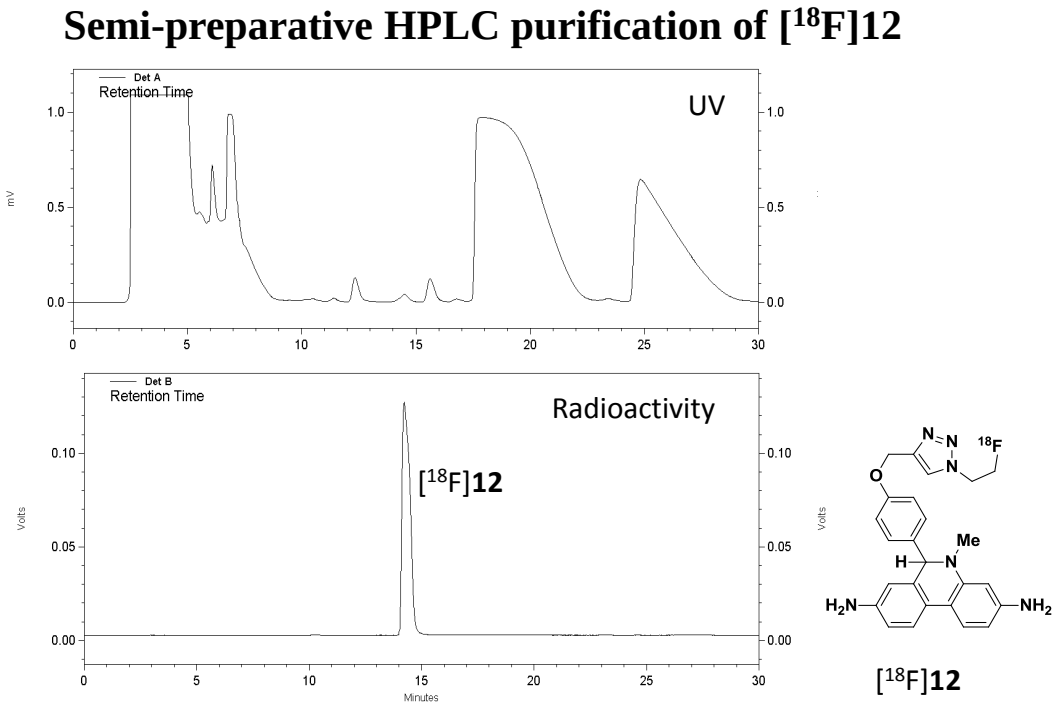
Image Analysis. Standardized uptake values (SUV) were determined in anterolateral region of the myocardium to assess [^{18}F]**12** uptake. A student's t-test was used to assess differences in uptake between groups.

***In vitro* blood stability studies**: An aliquot of [^{18}F]**12** in ethanol (20 µL) was added to 1 mL of heparinized whole rat blood (Sprague-Dawley rat, mature) and incubated at room temperature. At 5 min and 60 min, an aliquot (400 µL) was removed and ascorbic acid (1 mg in 50 µL water) was added; and the red blood cells and plasma separated by centrifugation. The plasma was deproteinated with ACN (1 mL), and centrifuged to separate protein pellet and supernatant. The supernatant was diluted with 0.1% TFA in water (4 mL), and analyzed by reversed phase HPLC (Agilent SB-C18 250 × 9.4 mm 5 µ; Gradient mobile phase A: 25% ACN/ 75% water/0.1% TFA, B: 30% ACN/ 70% water/0.1% TFA, from A to B over 15 min; 4 mL/min). The eluent was collected in 0.5 min/tube, and the fractions counted in a well counter. The counts were decay-corrected and plotted.

***In vitro* heart stability studi**es: The snap-frozen heart tissue was thawed in saline (1 mL) and homogenized; this homogenate was incubated with an aliquot of [^{18}F]**12** in ethanol (20 µL) at room temperature. At 5 min and 60 min, an aliquot (400 µL) was mixed with ascorbic acid (2 mg in 100 µL water) and was centrifuged to pellet the cell debris. The aqueous extract was deproteinated with ACN (1 mL), and centrifuged to separate protein pellet and supernatant. The supernatant was analyzed by reversed phase HPLC as described above.

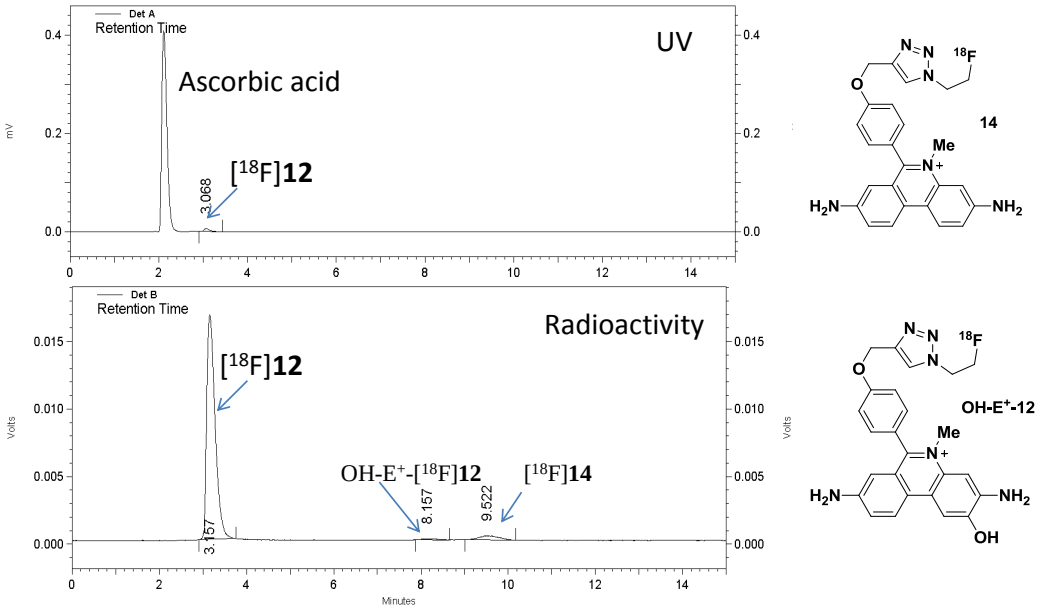
The *in vivo* stability of [^{18}F]**12** was evaluated in DOX-treated mice. Mice were euthanized 60 min post-injection, and whole blood (200 µl) and heart were collected and immediately snap-frozen in liquid nitrogen. Blood and tissue were later thawed in ACN (1 mL) with ascorbic acid (1 mg) and homogenized. Supernatant and pellet were separated by centrifugation and held on ice to prevent sample degradation. The supernatant was analyzed by reversed phase HPLC as described above.

Figure S3. Semi-preparative HPLC purification of [^{18}F]**12**



Column: Agilent SB-C18 250 9.4 mm 5µ
Mobile phase: 18% ACN, 82% water, 0.1% TFA
Flow rate: 3 mL/min; **UV**: 254 nm

Analytical HPLC of DHE analogue [^{18}F]12



Column: Agilent SB-C18 250X4.6 mm 5µ
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Figure S4. Analytical HPLC of DHE analogue **12**

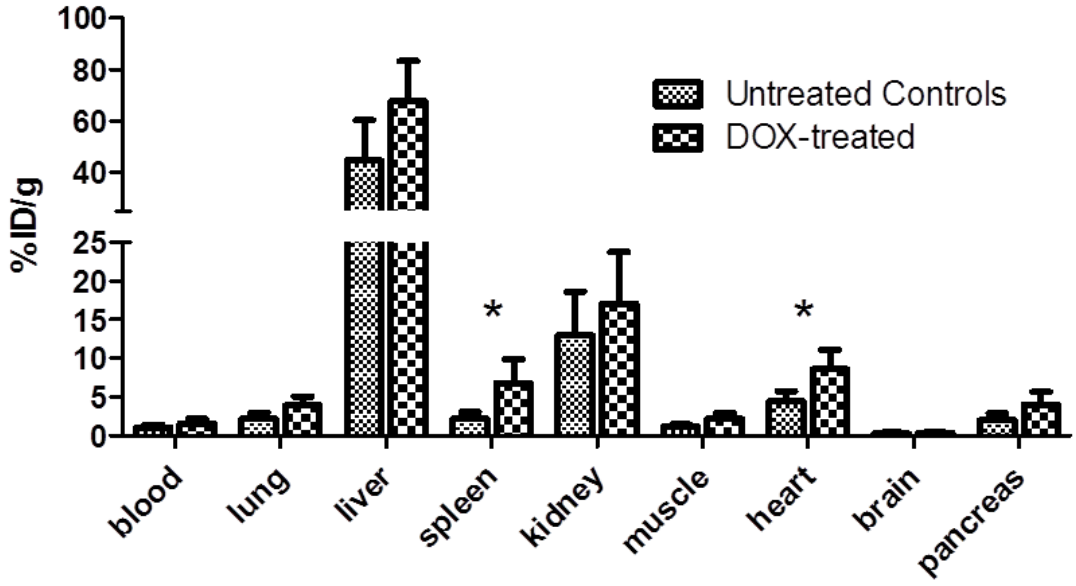
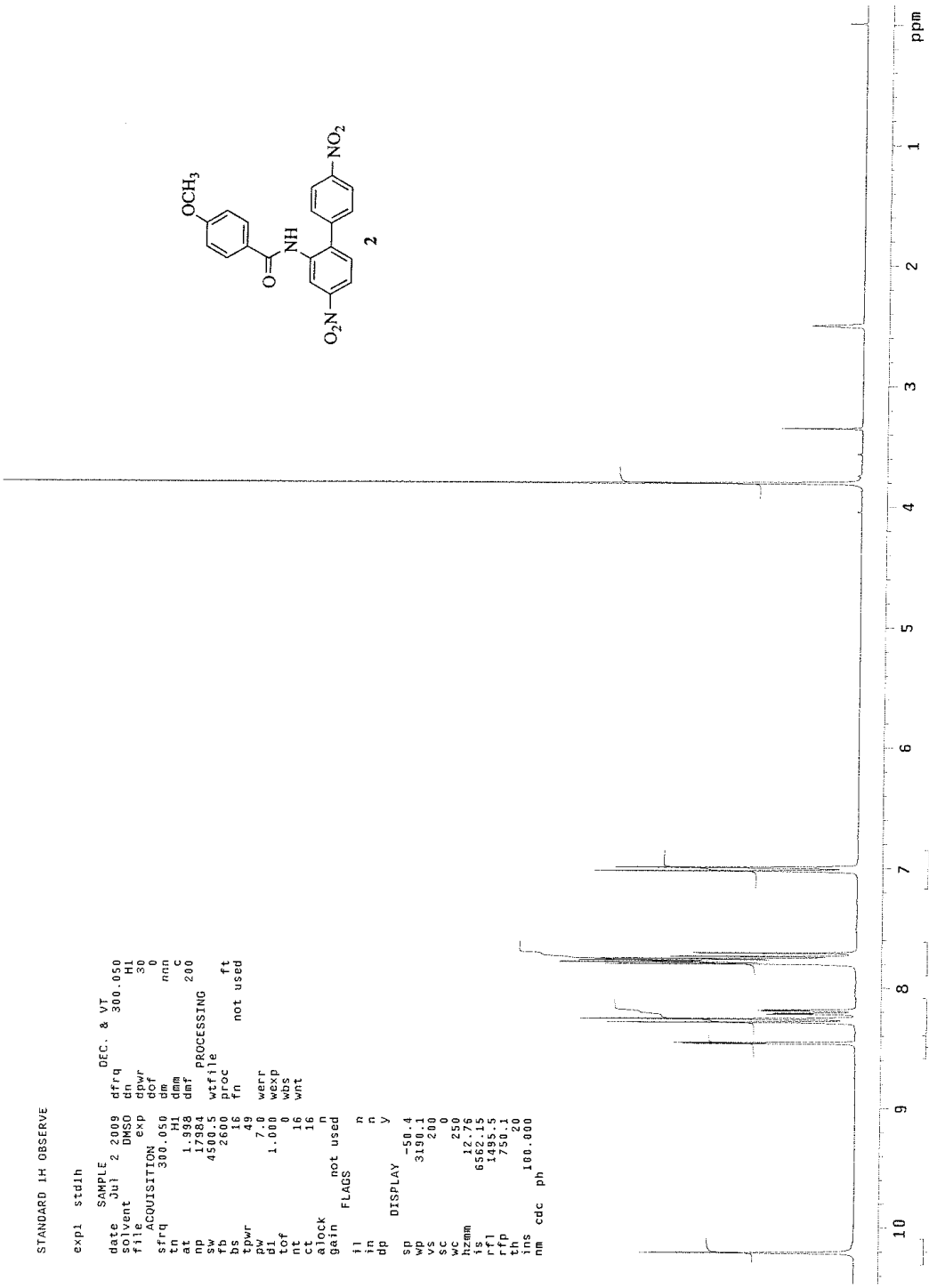


Figure S5. Biodistribution of [^{18}F]**12** in DOX-treated and untreated mice.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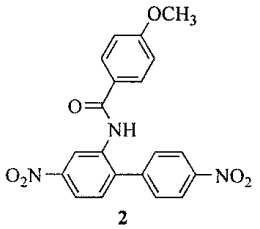

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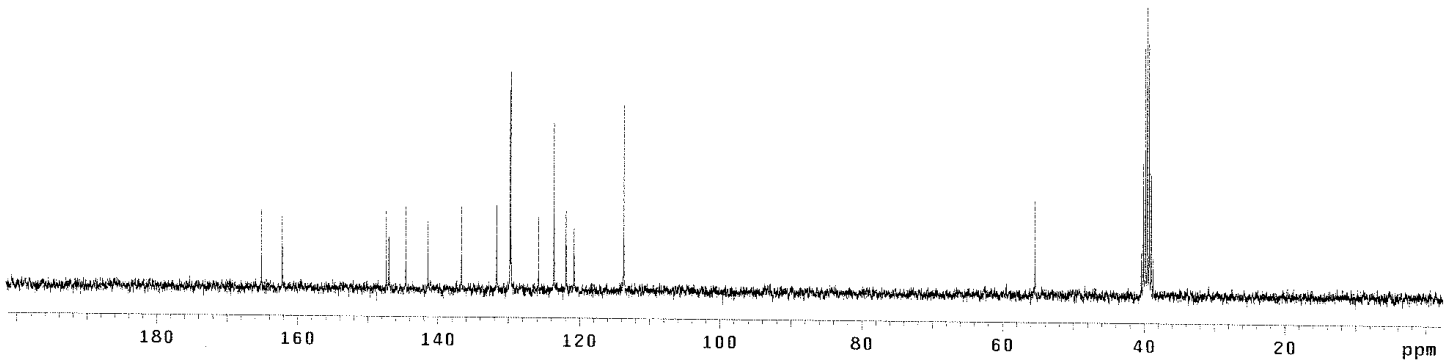

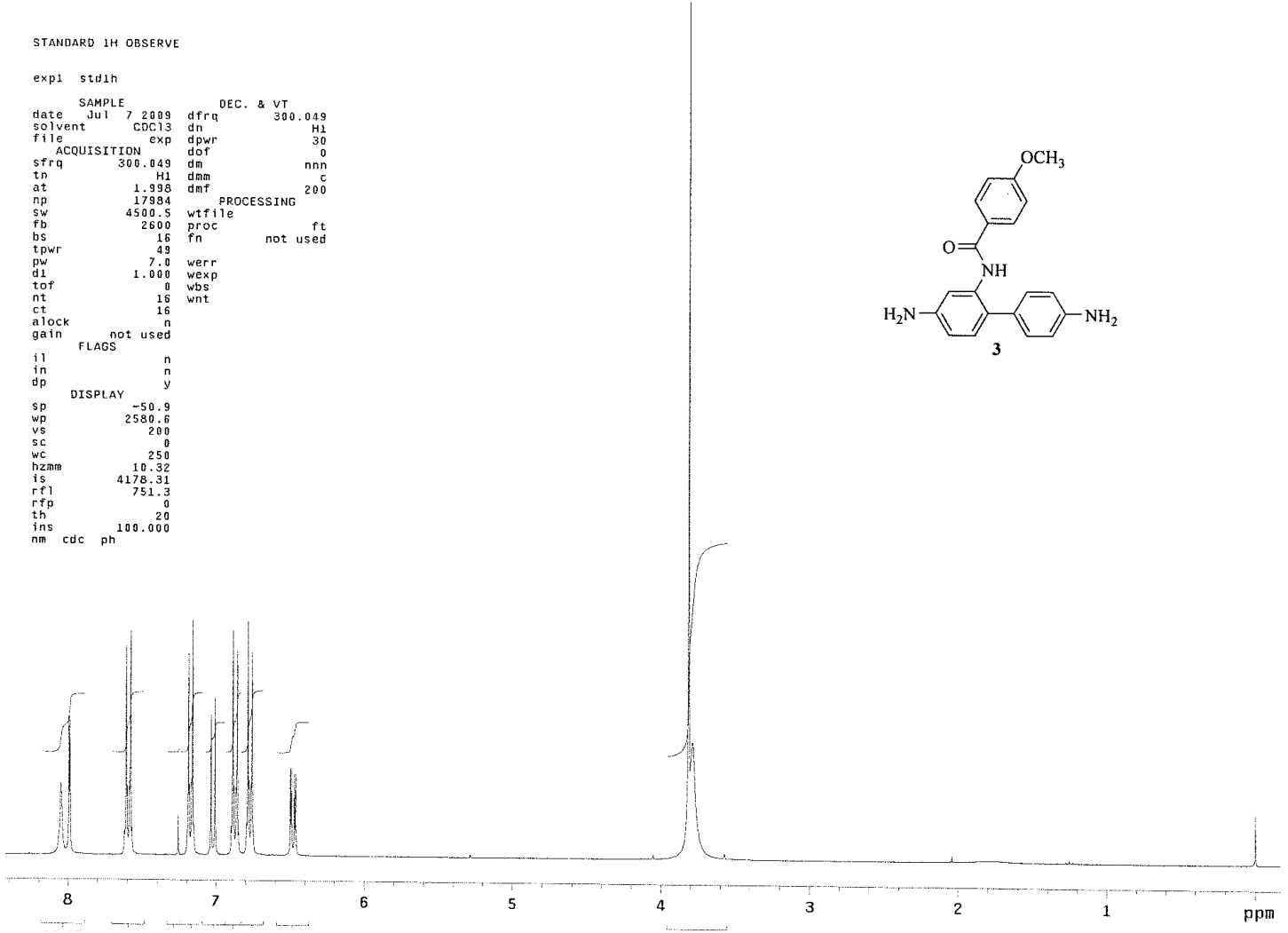
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13C OBSERVE

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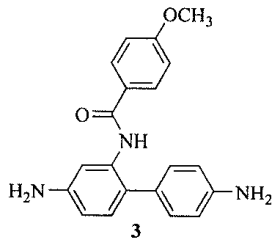

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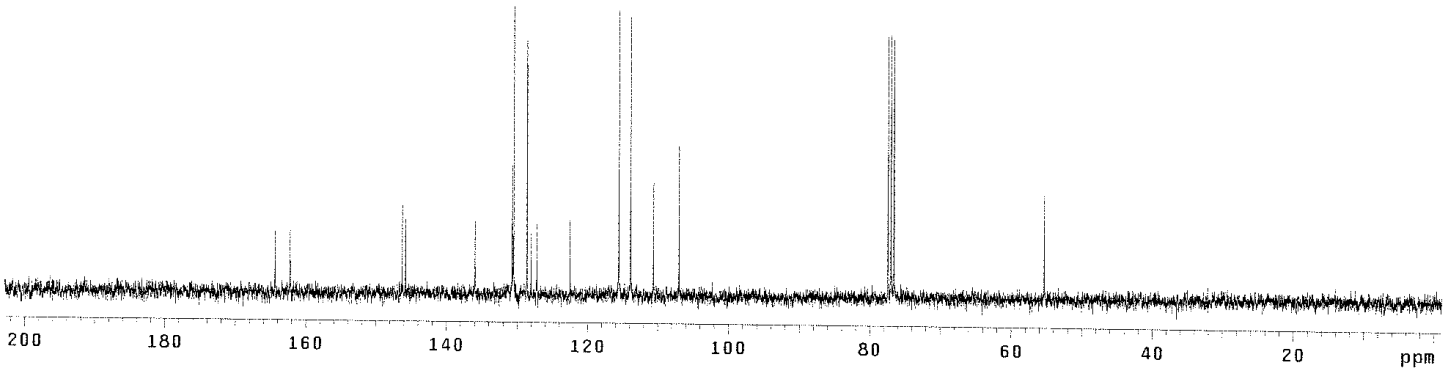

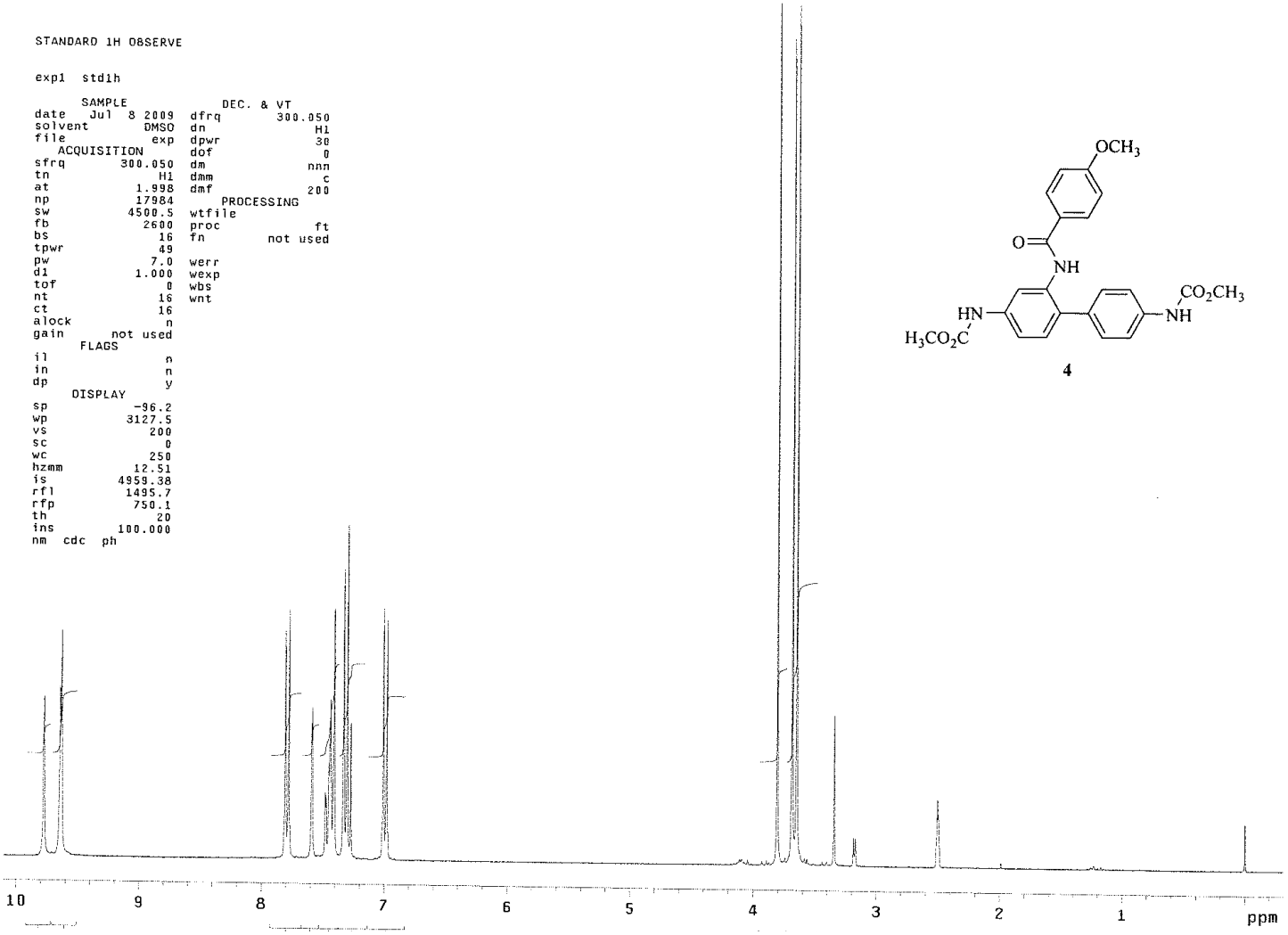
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13C OBSERVE

expl std13c

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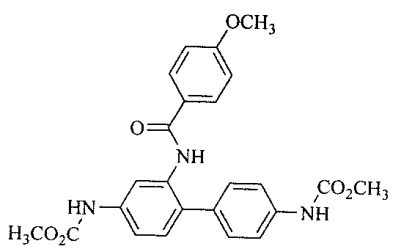
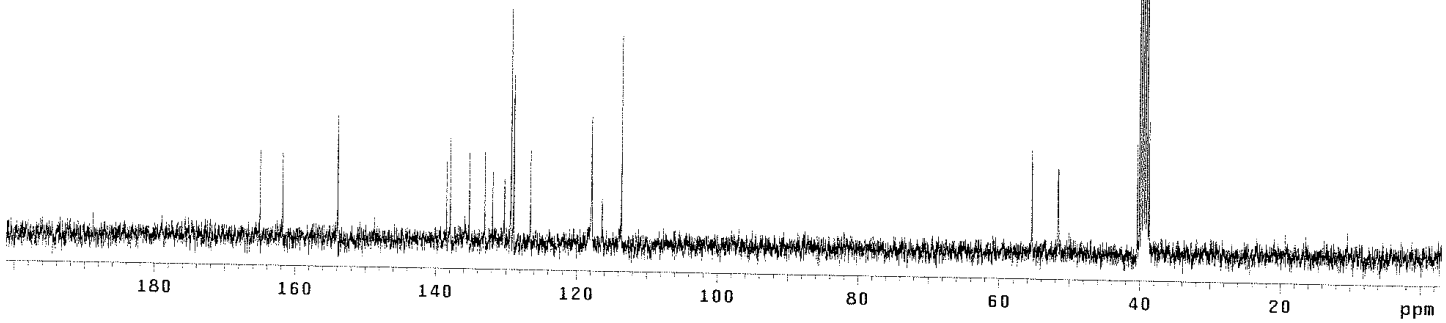
fb	10400	wtfile	
bs	16	proc	ft
tpwr	53	fn	not used
pw	4.5		
di	1.327	werr	
tof	0	wexp	
nt	1024	wbs	
ct	480	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-233.2		
wp	15412.0		
vs	150		
sc	0		
wc	250		
hzmm	61.65		
is	500.00		
rfl	4851.0		
rfp	2980.2		
th	7		
ins	100.000		

nm no ph



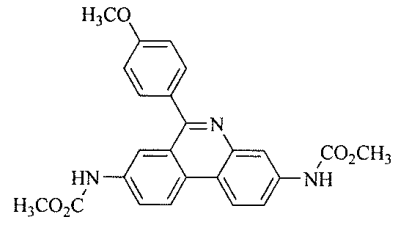
4



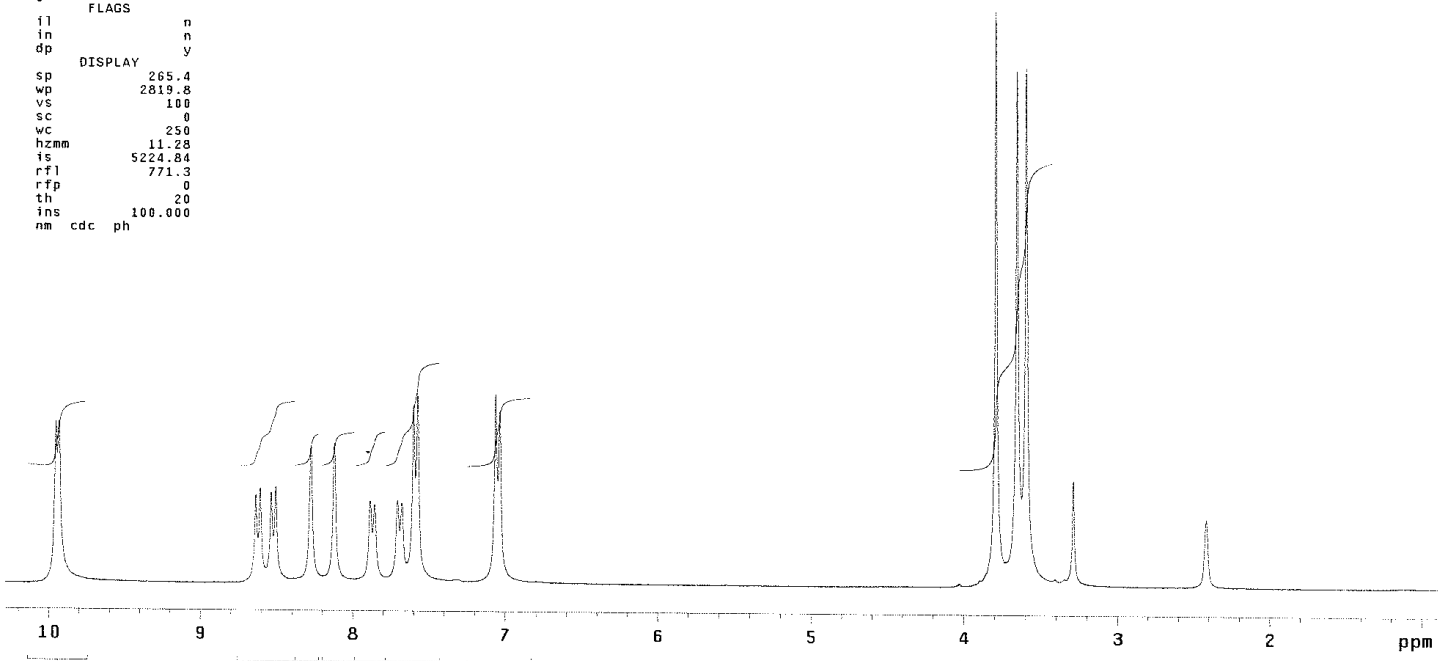
STANDARD 1H OBSERVE

exp1 std1h

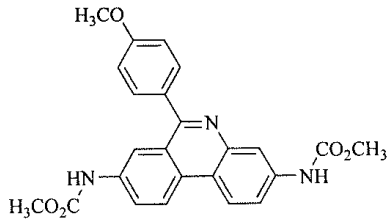
SAMPLE		DEC. & VT	
date	Apr 11 2011	dfrq	300.050
solvent	DMSO	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.050	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	17984	PROCESSING	
sw	4500.5	wtfile	
fb	2600	proc	ft
bs	16	fn	not used
tpwr	49		
pw	7.0	werr	
d1	1.000	wexp	
tof	0	wbs	
nt	64	wnt	
ct	32		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	265.4		
wp	2819.8		
vs	100		
sc	0		
wc	250		
hzmm	11.28		
is	5224.84		
rfl	771.3		
rfp	0		
th	20		
ins	100.000		
nm cdc ph			



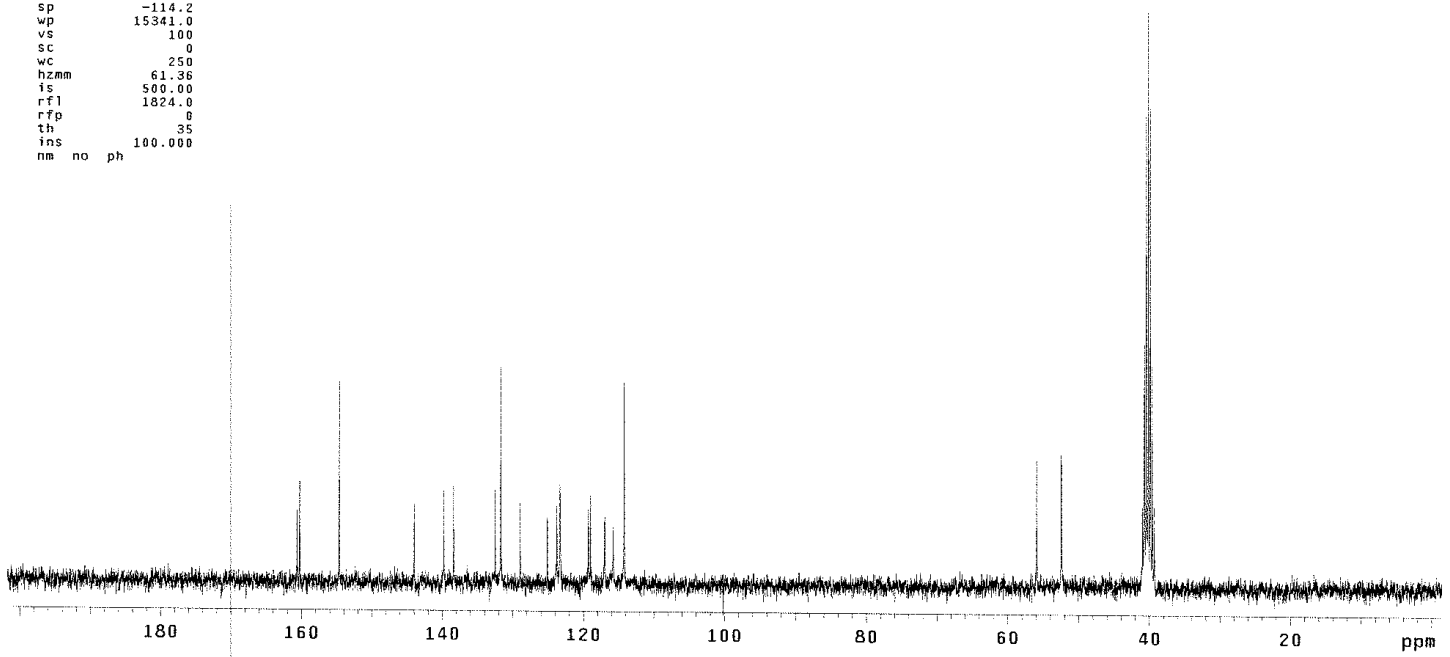
5



```
13C OBSERVE

exp1  std13c

      SAMPLE            DEC. & VT
date    Apr 11 2011  dfrq       300.050
solvent       DMSO   dn              H1
file           exp   dpwr            39
   ACQUISITION       dof              0
sfrq         75.455   dm             yyy
tn              C13   dmm              w
at            0.873   dmf          10316
np            32758      PROCESSING
sw         18761.7    lb            1.15
fb           10400    wtfile
bs              16    proc             ft
tpwr            53    fn         not used
pw             4.5
d1           1.327    werr
tof              0    wexp
nt            1024    wbs
ct             336    wnt
alock            n
gain      not used
      FLAGS
il               n
in               n
dp               y
     DISPLAY
sp          -114.2
wp         15341.0
vs             100
sc               0
wc             250
hzmm         61.36
is          500.00
rfl         1824.0
rfp              0
th              35
ins         100.000
nm  no  ph
```



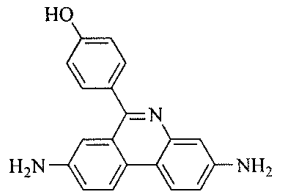
5



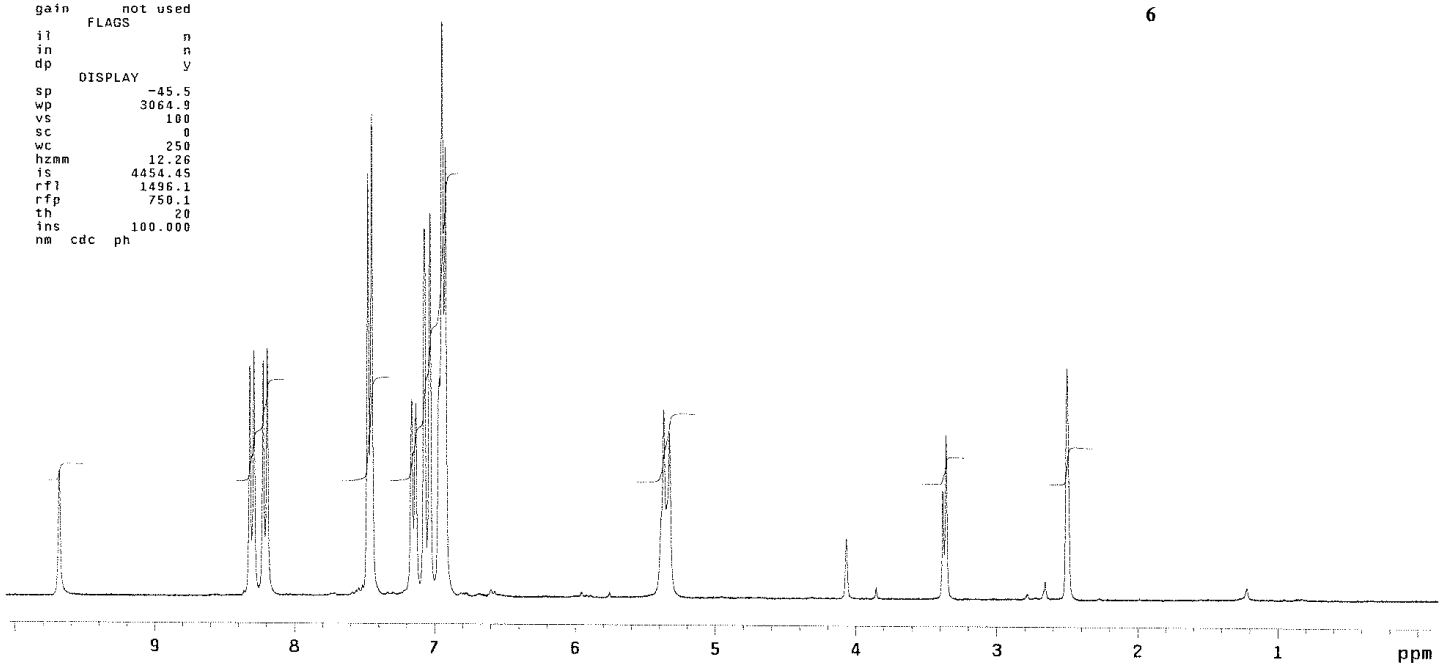
STANDARD 1H OBSERVE

expl stdih

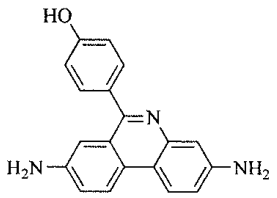
SAMPLE		DEC. & VT	
date	Jun 24 2011	dfrq	300.050
solvent	DMSO	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.050	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	17984	PROCESSING	
sw	4500.5	wtfile	
fb	2600	proc	ft
bs	16	fn	not used
tpwr	48		
pw	7.0	werr	
dl	1.000	wexp	
tof	0	wbs	
nt	64	wnt	
ct	32		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-45.5		
wp	3064.9		
vs	100		
sc	0		
wc	250		
hzmm	12.26		
is	4454.45		
rfl	1496.1		
rfp	750.1		
th	20		
ins	100.000		
nm cdc ph			



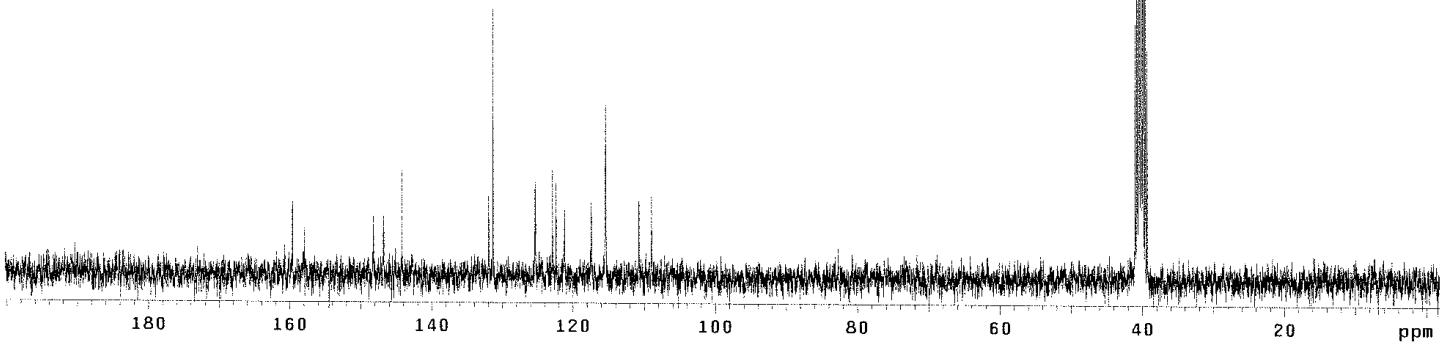
6



```
13C OBSERVE

expl  std13c

      SAMPLE            DEC. & VT
date   Jun 24 2011  dfrq       300.050
solvent       DMSO  dn              H1
file           exp  dpwr            39
   ACQUISITION      dof              0
sfrq        75.455  dm             yyy
tn             C13  dmm              w
at           0.873  dmf          10316
np           32758      PROCESSING
sw        18761.7  lb            1.15
fb          10400  wtfile
bs             16  proc            ft
tpwr           53  fn        not used
pw            4.5
d1          1.327  werr
tof            0  wexp
nt          1024  wbs
ct           960  wnt
alock          n
gain     not used
      FLAGS
il             n
in             n
dp             y
     DISPLAY
sp         -138.3
wp        15246.0
vs            300
sc              0
wc            250
hzmm        60.98
is         500.00
rfl        1824.0
rfp             0
th             20
ins       100.000
nm  no  ph
```



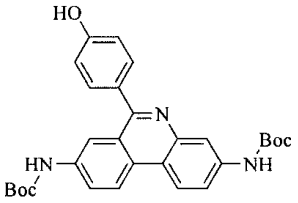
6



STANDARD 1H OBSERVE

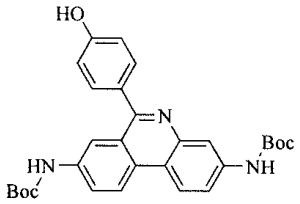
expl stdlh

SAMPLE		DEC. & VT	
date	Apr 12 2011	dfrq	300.050
solvent	DMSO	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.050	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	17984	PROCESSING	
sw	4500.5	wtfile	
fb	2600	proc	ft
bs	16	fn	not used
tpwr	49		
pw	7.0	werr	
dl	1.000	wexp	
tof	0	wbs	
nt	64	wnt	
ct	32		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-42.2		
wp	3070.3		
vs	300		
sc	0		
wc	250		
hzmm	12.28		
is	7662.06		
rfl	771.3		
rfp	0		
th	20		
ins	100.000		
nm cdc ph			

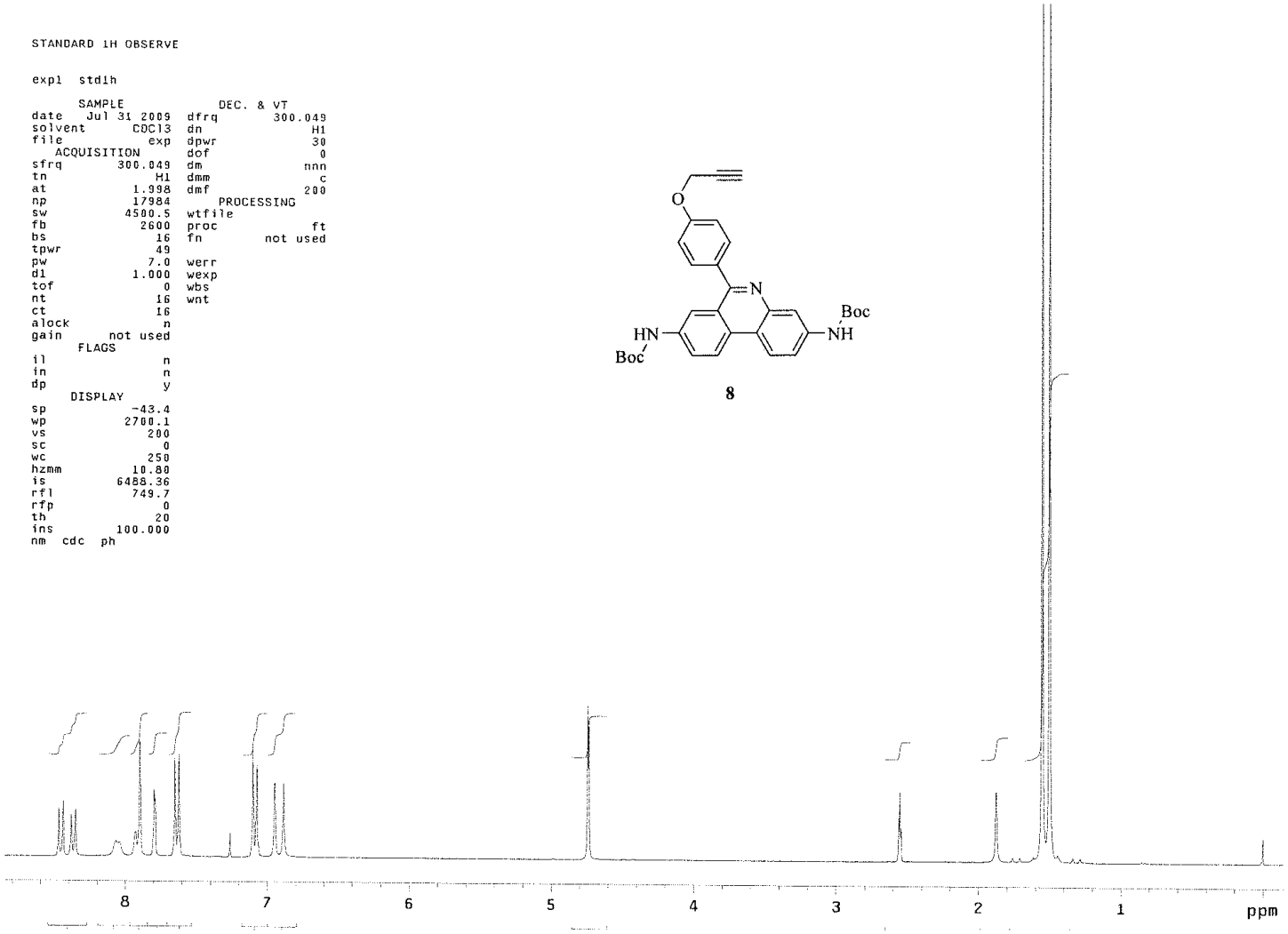


7

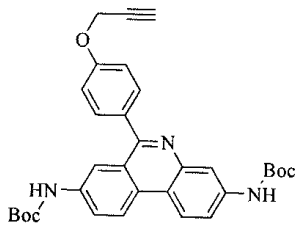
```
13C OBSERVE

exp1  std13c

      SAMPLE           DEC. & VT
date   Apr 12 2011  dfrq       300.050
solvent       DMSO  dn              H1
file           exp  dpwr            39
   ACQUISITION      dof             0
sfrq         75.455  dm            yyy
tn              C13  dmm             w
at            0.873  dmf         10316
np            32758      PROCESSING
sw          18761.7  lb           1.15
fb            10400  wtfile
bs               16  proc           ft
tpwr             53  fn       not used
pw              4.5
d1            1.327  werr
tof               0  wexp
nt             1024  wbs
ct              320  wnt
alock             n
gain      not used
      FLAGS
il                n
in                n
dp                y
     DISPLAY
sp           -138.3
wp          15270.0
vs              150
sc                0
wc              250
hzmm          61.08
is           500.00
rfl         1824.0
rfp               0
th                6
ins         100.000
nm  no  ph
```



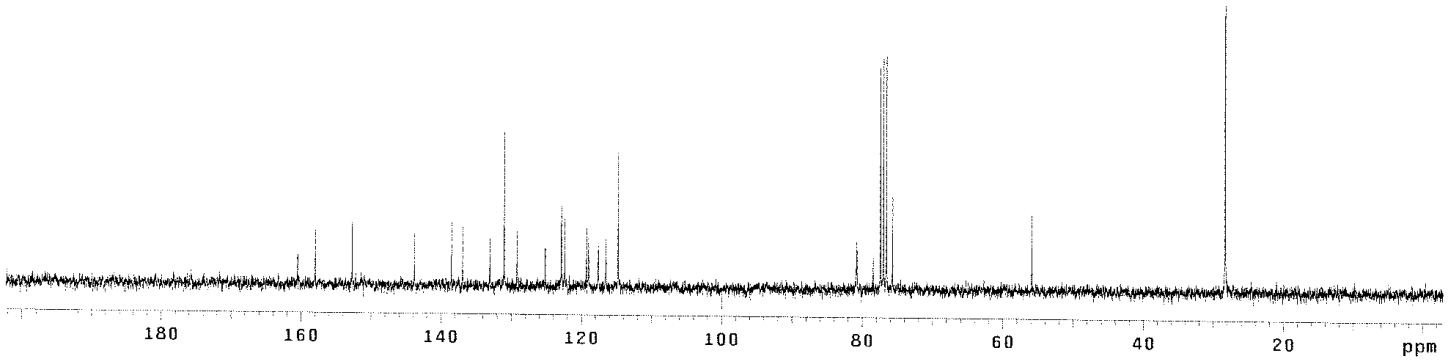
7

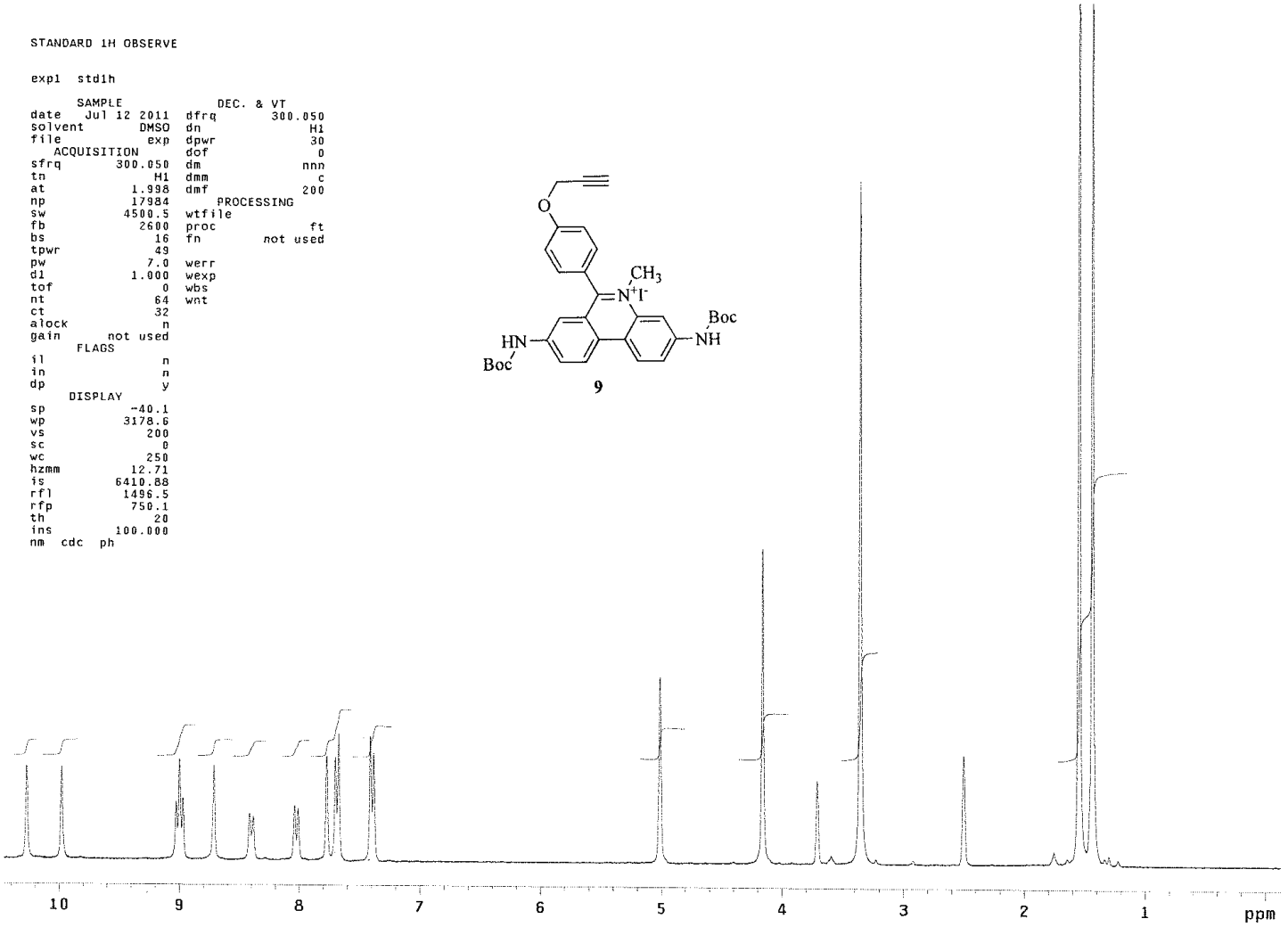

STANDARD 1H OBSERVE
expl std1h
SAMPLE
date Jul 31 2009
solvent CDCl3
file exp
ACQUISITION
sfrq 300.049
tn H1
at 1.998
np 17984
sw 4500.5
fb 2600
bs 16
tpwr 49
pw 7.0
d1 1.000
tof 0
nt 16
ct 16
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -43.4
wp 2700.1
vs 200
sc 0
wc 250
hzmm 10.80
is 6488.36
rfl 749.7
rfp 0
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 300.049
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
O
N
HN
Boc
Boc
NH
8
8
7
6
5
4
3
2
1
ppm

```
13C OBSERVE

expl  std13c

      SAMPLE              DEC. & VT
date    Jul 31 2009  dfrq        300.049
solvent      CDCl3   dn              H1
file           exp   dpwr            39
   ACQUISITION       dof              0
sfrq         75.455  dm             yyy
tn              C13  dmm              w
at            0.873  dmf          10316
np            32758     PROCESSING
sw          18761.7  lb            1.15
fb            10400  wtfile
bs               16  proc            ft
tpwr             53  fn         not used
pw              4.5
d1            1.327  werr
tof               0  wexp
nt             1024  wbs
ct              608  wnt
alock             n
gain      not used
      FLAGS
il                n
in                n
dp                y
     DISPLAY
sp           -224.0
wp         15484.2
vs              50
sc               0
wc             250
hzmm         61.94
is          500.00
rfl         7648.2
rfp         5809.4
th               4
ins         100.000
nm  no  ph
```



8



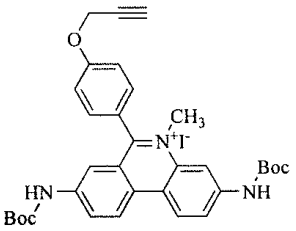

STANDARD 1H OBSERVE
expl std1h
SAMPLE
date Jul 12 2011
solvent DMSO
file exp
ACQUISITION
sfrq 300.050
tn H1
at 1.998
np 17984
sw 4500.5
fb 2600
bs 16
tpwr 49
pw 7.0
d1 1.000
tof 0
nt 64
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
DISPLAY
sp -40.1
wp 3178.6
vs 200
sc 0
wc 250
hzmm 12.71
is 6410.88
rfl 1496.5
rfp 750.1
th 20
ins 100.000
nm cdc ph
DEC. & VT
dfrq 300.050
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
PROCESSING
wtfile
proc ft
fn not used
werr
wexp
wbs
wnt
O
CH3
N+I-
HN
Boc
Boc
NH
9
10
9
8
7
6
5
4
3
2
1
ppm

13C OBSERVE

exp1 std13c

SAMPLE		DEC. & VT	
date	Jul 19 2011	dfrq	300.050
solvent	DMSO	dn	Hi
file	exp	dpwr	39
ACQUISITION		dof	0
sfrq	75.455	dm	yyy
tn	C13	dmm	w
at	0.873	dmf	10316
np	32758	PROCESSING	
sw	18761.7	lb	1.15
fb	10400	wtfile	
bs	16	proc	ft
tpwr	53	fn	not used
pw	4.5		
d1	1.327	werr	
tof	0	wexp	
nt	5000	wbs	
ct	2624	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-138.3		
wp	15318.1		
vs	500		
sc	0		
wc	250		
hzmm	61.27		
is	500.00		
rfl	1824.0		
rfp	0		
th	20		
ins	100.000		

nm no ph



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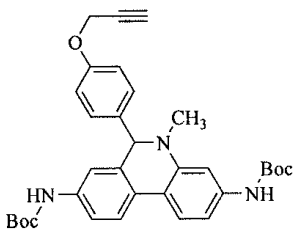
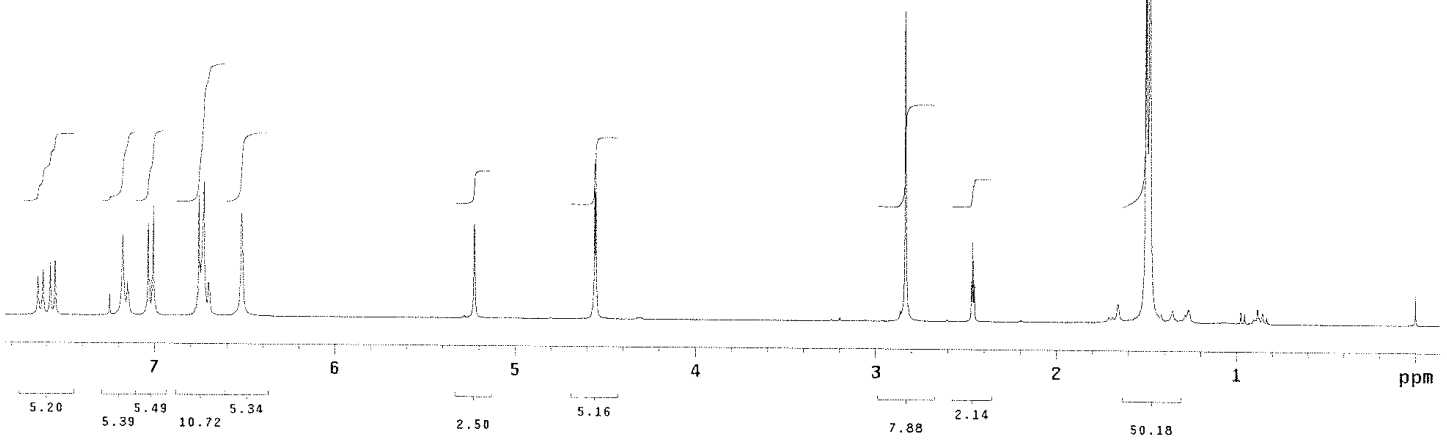
STANDARD 1H OBSERVE

expl stdlh

SAMPLE		DEC. & VT	
date	Jun 4 2009	dfrq	300.049
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.049	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	17984	PROCESSING	
sw	4500.5	wtfile	
fb	2600	proc	ft
bs	16	fn	not used
tpwr	49		
pw	7.0	werr	
d1	1.000	wexp	
tof	0	wbs	
nt	16	wnt	
ct	16		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-41.0		
wp	2392.4		
vs	200		
sc	0		
wc	250		
hzmm	9.57		
is	7416.33		
rfl	753.0		
rfp	0		
th	9		
ins	100.000		
nm cdc ph			



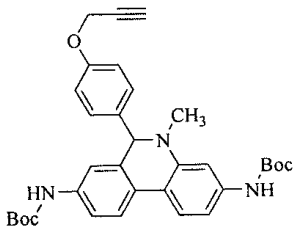
10



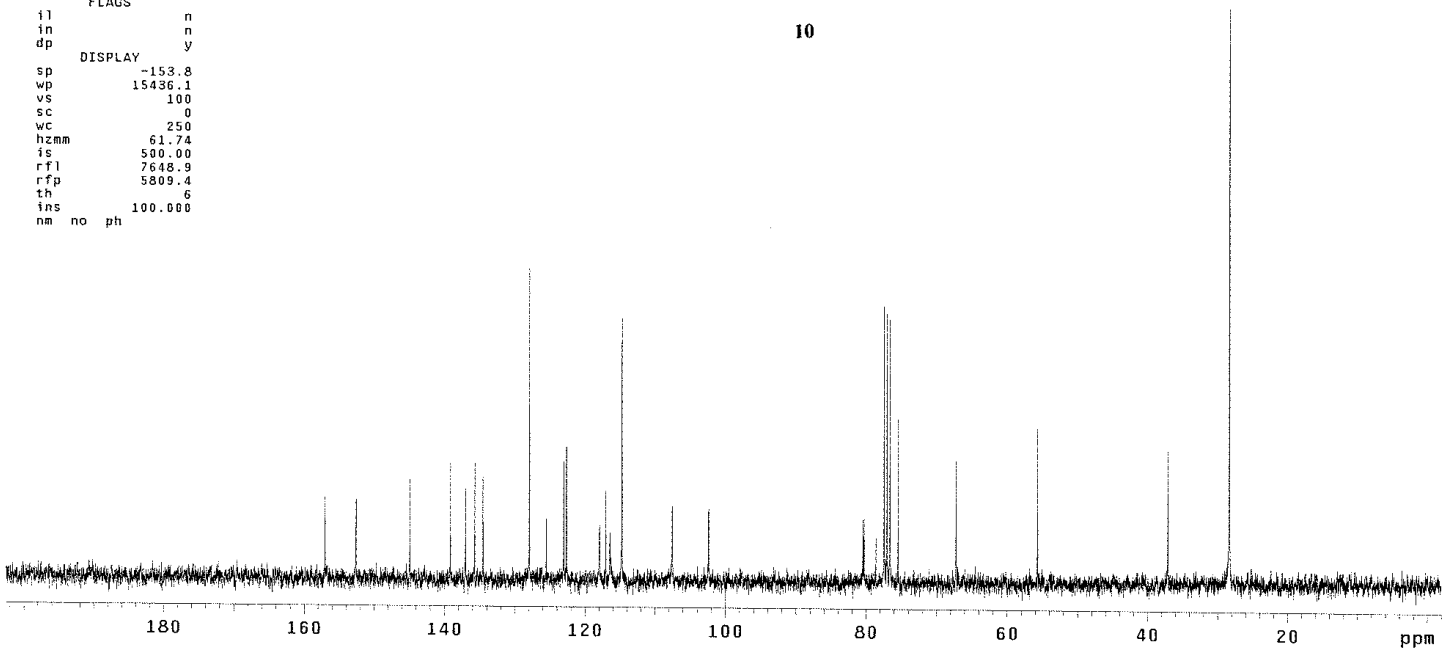
```
13C OBSERVE

expl  std13c

      SAMPLE                DEC. & VT
date   Jun  4 2009   dfrq        300.049
solvent      CDCl3   dn               Hi
file             exp dpwr             39
   ACQUISITION       dof               0
sfrq         75.455  dm              yyy
tn               C13 dmm               w
at             0.873 dmf           10316
np             32758     PROCESSING
sw           18761.7   lb             1.15
fb             10400   wtfile
bs                16   proc              ft
tpwr              53   fn         not used
pw               4.5
d1             1.327   werr
tof                0   wexp
nt              1024   wbs
ct               320   wnt
alock              n
gain       not used
      FLAGS
il                 n
in                 n
dp                 y
     DISPLAY
sp            -153.8
wp          15436.1
vs              100
sc                0
wc              250
hzmm          61.74
is           500.00
rfl          7648.9
rfp          5809.4
th                 6
ins         100.000
nm  no  ph
```



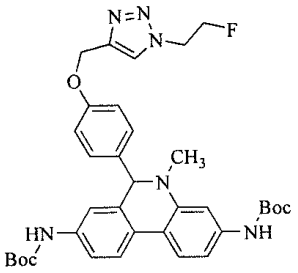
10



STANDARD 1H OBSERVE

expl stdlh

SAMPLE		DEC. & VT	
date	Jun 5 2009	dfrq	300.049
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	300.049	dm	nnn
tn	H1	dmm	c
at	1.998	dmf	200
np	17984	PROCESSING	
sw	4500.5	wtfile	
fb	2600	proc	ft
bs	16	fn	not used
tpwr	49		
pw	7.0	werr	
dl	1.000	wexp	
tof	0	wbs	
nt	16	wnt	
ct	16		
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-58.4		
wp	2500.6		
vs	200		
sc	0		
wc	250		
hzmm	10.00		
is	8090.22		
rfl	747.6		
rfp	0		
th	20		
ins	100.000		
nm cdc ph			



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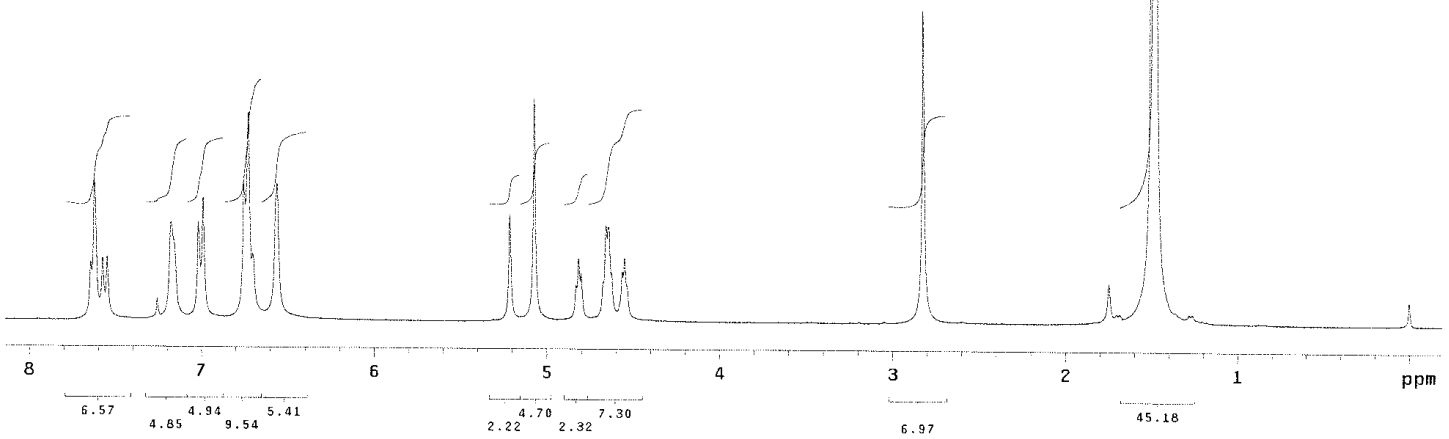
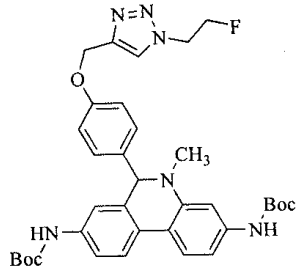


13C OBSERVE

expl std13c

SAMPLE		DEC. & VT	
date	Jun 5 2009	dfrq	300.049
solvent	CDCl3	dn	H1
file	exp	dpwr	39
ACQUISITION		dof	0
sfrq	75.455	dm	yyy
tn	C13	dmm	w
at	0.873	dmf	10316
np	32758	PROCESSING	
sw	18761.7	lb	1.15
fb	10400	wtfile	
bs	16	proc	ft
tpwr	53	fn	not used
pw	4.5		
dl	1.327	werr	
tof	0	wexp	
nt	1024	wbs	
ct	448	wnt	
alock	n		
gain	not used		
FLAGS			
il	n		
in	n		
dp	y		
DISPLAY			
sp	-153.8		
wp	15318.1		
vs	100		
sc	0		
wc	250		
hzmm	61.27		
is	500.00		
rfl	7648.9		
rfp	5809.4		
th	20		
ins	100.000		
nm no ph			



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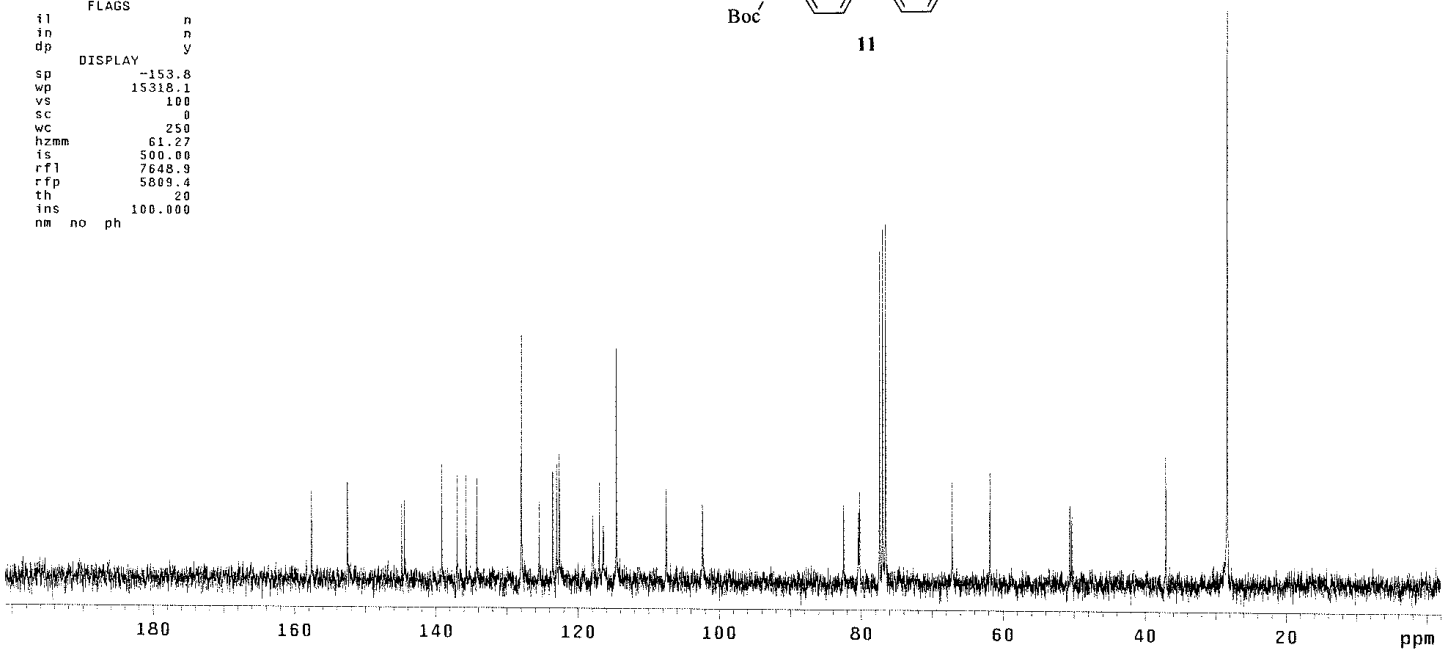
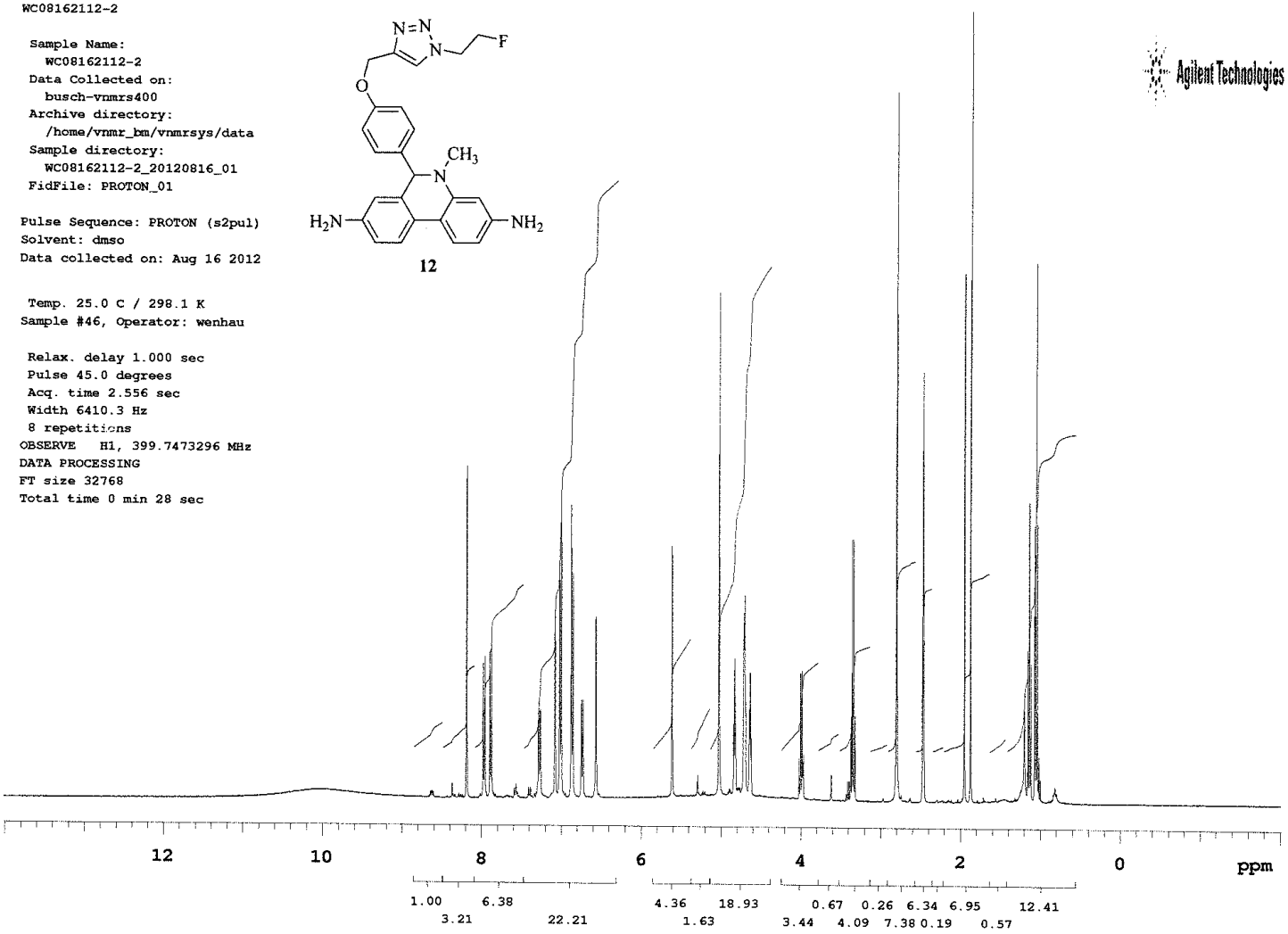



WC08162112-2
Sample Name:
WC08162112-2
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_bm/vnmrsys/data
Sample directory:
WC08162112-2_20120816_01
FidFile: PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Aug 16 2012
Temp. 25.0 C / 298.1 K
Sample #46, Operator: wenhau
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
8 repetitions
OBSERVE H1, 399.7473296 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 28 sec
12
Agilent Technologies
12
10
8
6
4
2
0
ppm
1.00
6.38
3.21
22.21
4.36
18.93
1.63
0.67
0.26
6.34
6.95
12.41
3.44
4.09
7.38
0.19
0.57

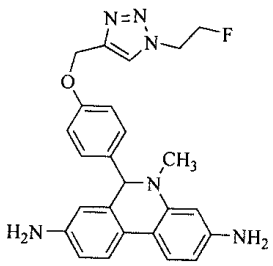
WC08162112-2

Sample Name:
WC08162112-2
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_bm/vnmrsys/data
Sample directory:
WC08162112-2_20120816_01
FidFile: CARBON_01

Pulse Sequence: CARBON (s2pul)
Solvent: dmso
Data collected on: Aug 16 2012

Temp. 25.0 C / 298.1 K
Sample #46, Operator: wenhau

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.285 sec
Width 25510.2 Hz
256 repetitions
OBSERVE C13, 100.5165460 MHz
DECOUPLE H1, 399.7493284 MHz
Power 34 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 9 min 45 sec



12

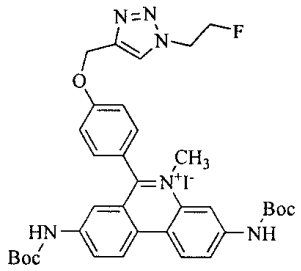
wc08172112

Sample Name:
wc08172112
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_bm/vnmrsys/data
Sample directory:
wc08172112_20120817_01
FidFile: PROTON_01

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Aug 17 2012

Temp. 25.0 C / 298.1 K
Sample #46, Operator: wenhau

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
8 repetitions
OBSERVE H1, 399.7473296 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 28 sec



13

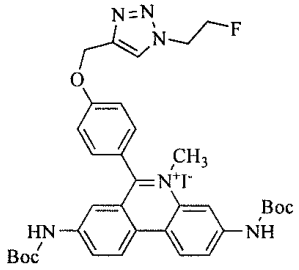
wc08172112

Sample Name:
wc08172112
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_bm/vnmrsys/data
Sample directory:
wc08172112_20120817_01
FidFile: CARBON_01

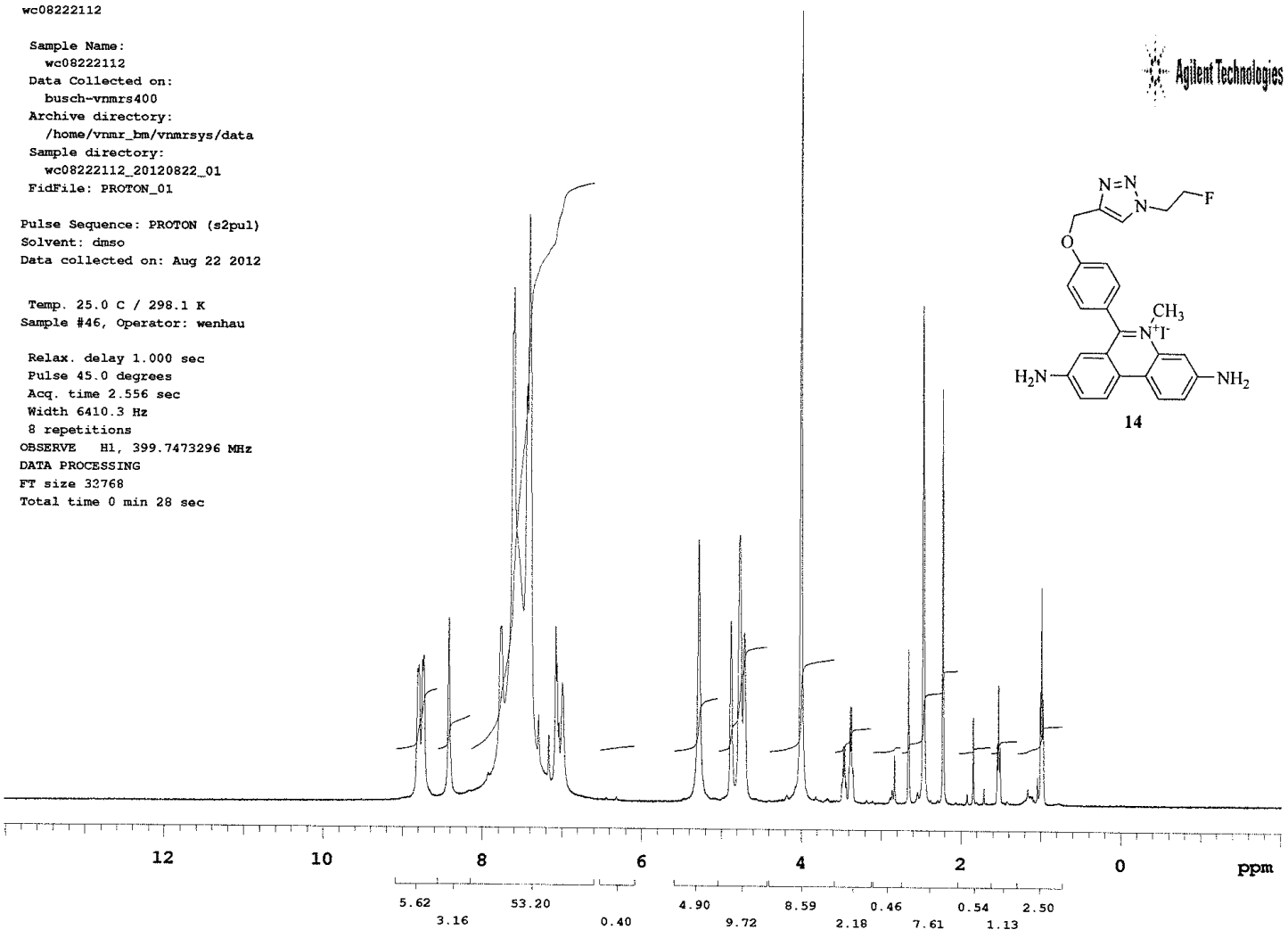
Pulse Sequence: CARBON (s2pul)
Solvent: dmso
Data collected on: Aug 17 2012

Temp. 25.0 C / 298.1 K
Sample #46, Operator: wenhau

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.285 sec
Width 25510.2 Hz
256 repetitions
OBSERVE C13, 100.5165460 MHz
DECOUPLE H1, 399.7493284 MHz
Power 34 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 9 min 45 sec



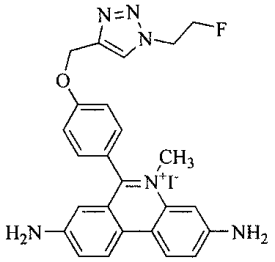
13


wc08222112
Sample Name:
wc08222112
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_bm/vnmrsys/data
Sample directory:
wc08222112_20120822_01
FidFile: PROTON_01
Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Aug 22 2012
Temp. 25.0 C / 298.1 K
Sample #46, Operator: wenhau
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
8 repetitions
OBSERVE H1, 399.7473296 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 28 sec
Agilent Technologies
N=N
N
F
O
CH3
N+I-
H2N
NH2
14
12
10
8
6
4
2
0
ppm
5.62
3.16
53.20
0.40
4.90
9.72
8.59
2.18
0.46
7.61
0.54
1.13
2.50

wc08222112

exp114 CARBON

SAMPLE		PRESATURATION	
date	Aug 22 2012	satmode	n
solvent	dmso	wet	n
file	/home/vnmr_bm~	SPECIAL	
/vnmrsys/Automatio~		temp	25.0
n/auto_20120822_01~		gain	30
/enterQ.macdir/loc~		spin	not used
46_001/current		hst	0.008
ACQUISITION		pw90	6.500
sw	25510.2	alfa	10.000
at	1.285	FLAGS	
np	65536	il	n
fb	17000	in	n
bs	64	dp	y
dl	1.000	hs	nn
nt	256	PROCESSING	
ct	64	lb	0.50
TRANSMITTER		fn	not used
tn	C13	DISPLAY	
sfrq	100.528	sp	-91.4
tof	1530.6	wp	20402.4
tpwr	59	rfl	1698.3
pw	3.250	rfp	0
DECOUPLER		rp	-90.7
dn	H1	lp	0
dof	0	PLOT	
dm	yyy	wc	250
decwave	w	sc	0
dpwr	34	vs	119821
dmf	10101	th	11
		ai cdc ph	



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