

Supplementary Information

Highly Specific, Bisubstrate-Competitive Src Inhibitors from DNA-Templated Macrocycles

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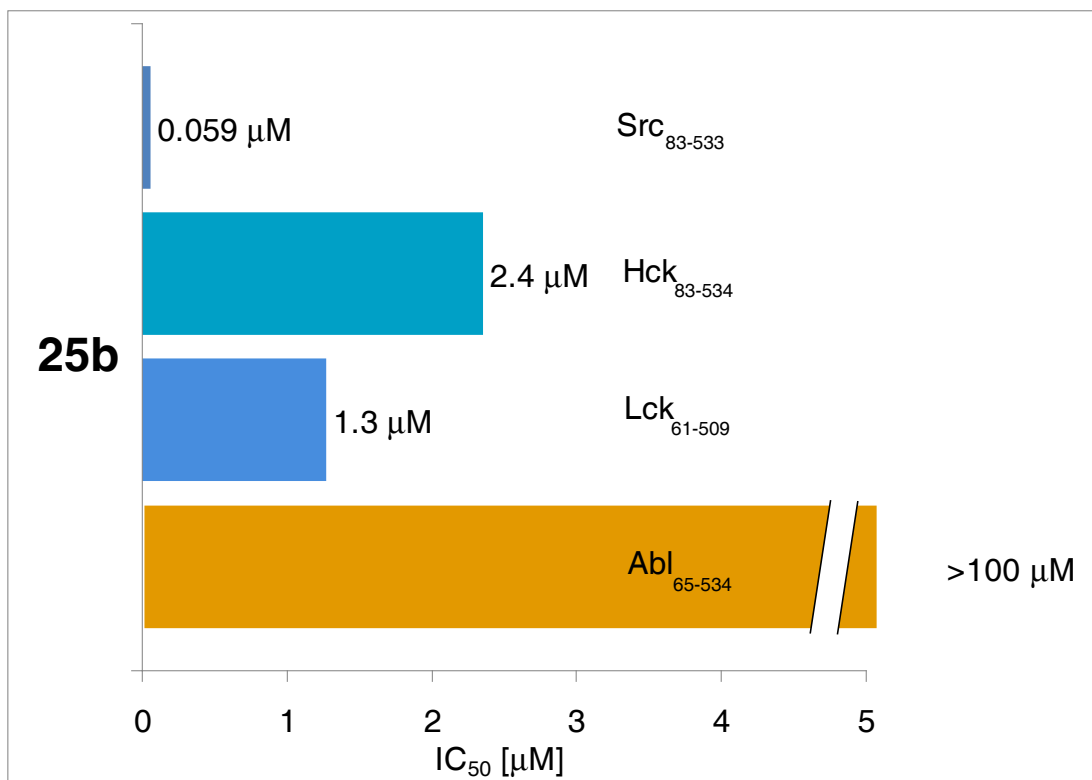
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Supplementary Results

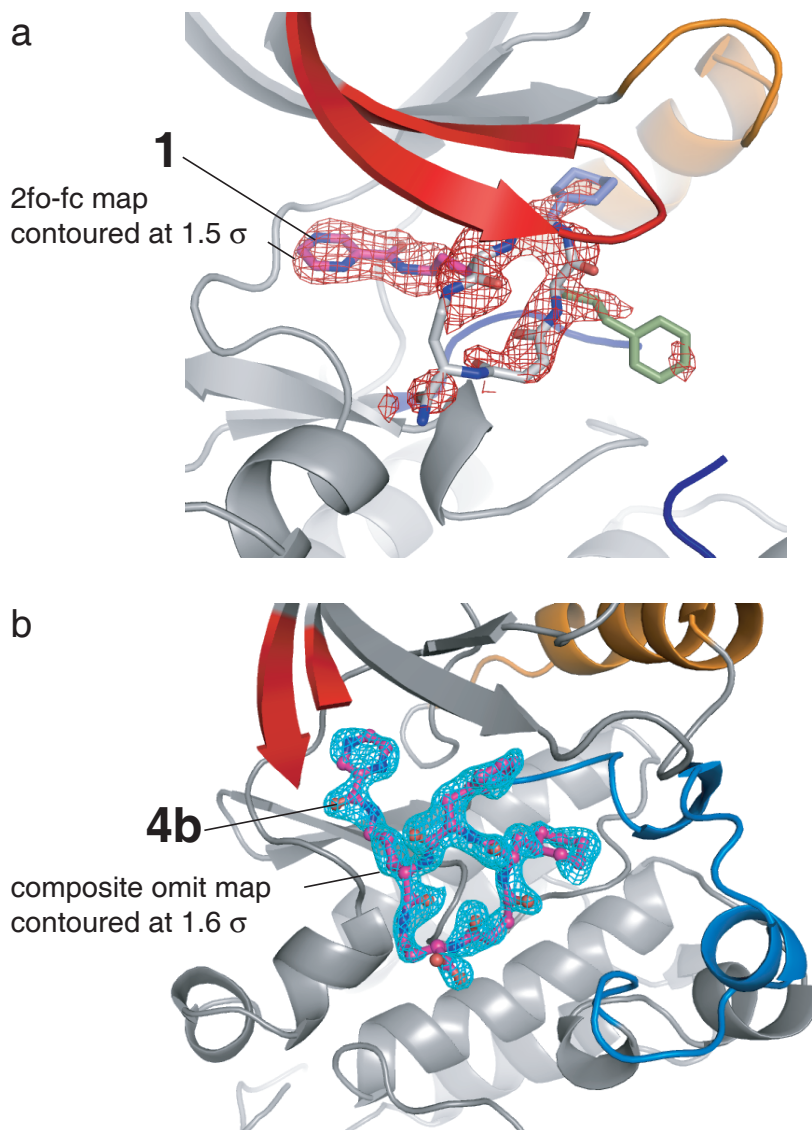
a	IC ₅₀ [μM]	Src	Hck	Lck	Abl
		1	60	> 100	> 100
2	15	> 100	> 100	> 100	
9	6.8	> 100	> 100	> 100	

b	IC ₅₀ [μM]	Src	Hck	Lck	Abl
		4b	0.13	0.86	2.4
25b	0.099	8.4	6.1	> 100	

Supplementary Figure 1. Specificity of macrocyclic kinase inhibitors. (a) IC₅₀ values of **1**, **2**, and **9** for kinase domains were determined in the presence of 5 μM ATP and 100 μM Src-optimal substrate peptide. (b) IC₅₀ values of **4b** and **25b** for kinase domains were determined in the presence of 250 μM ATP and 300 μM Src-optimal substrate peptide.



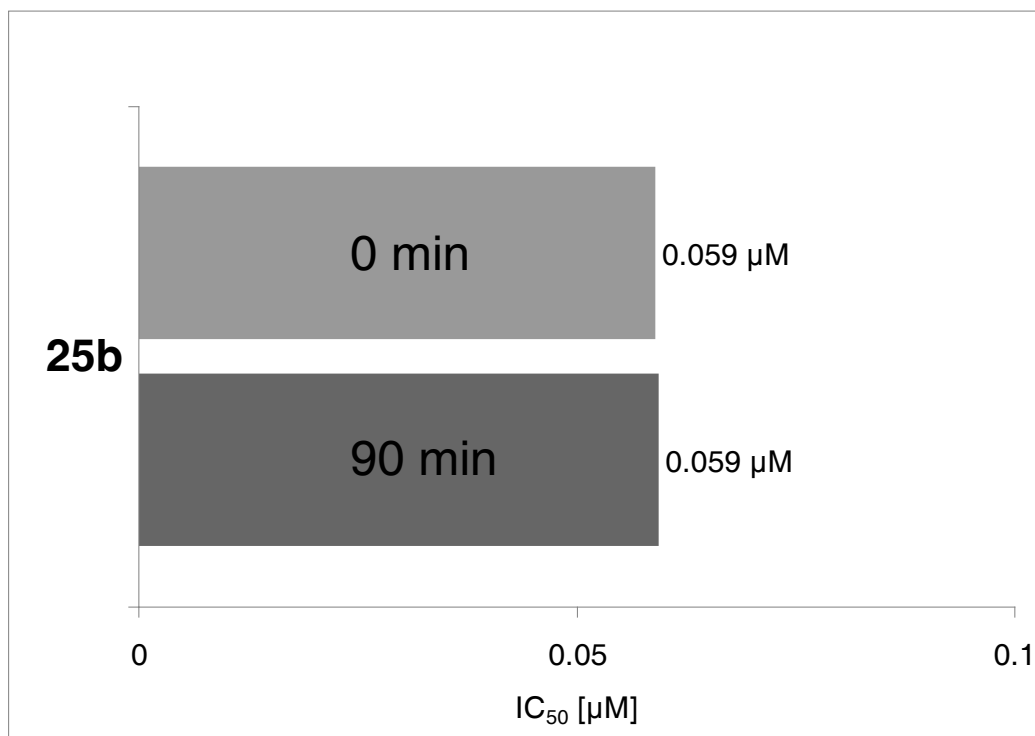
Supplementary Figure 2. In vitro potency of **25b** against three-domain constructs of Src, Hck, Lck, and Abl kinase. The inhibition activity of **25b** was measured using the continuous spectrophotometric kinase assay with 300 μM Src optimal substrate peptide, 250 μM ATP, 100 nM Src, 12.5 nM Hck, 42 nM Lck and 75 nM Abl.



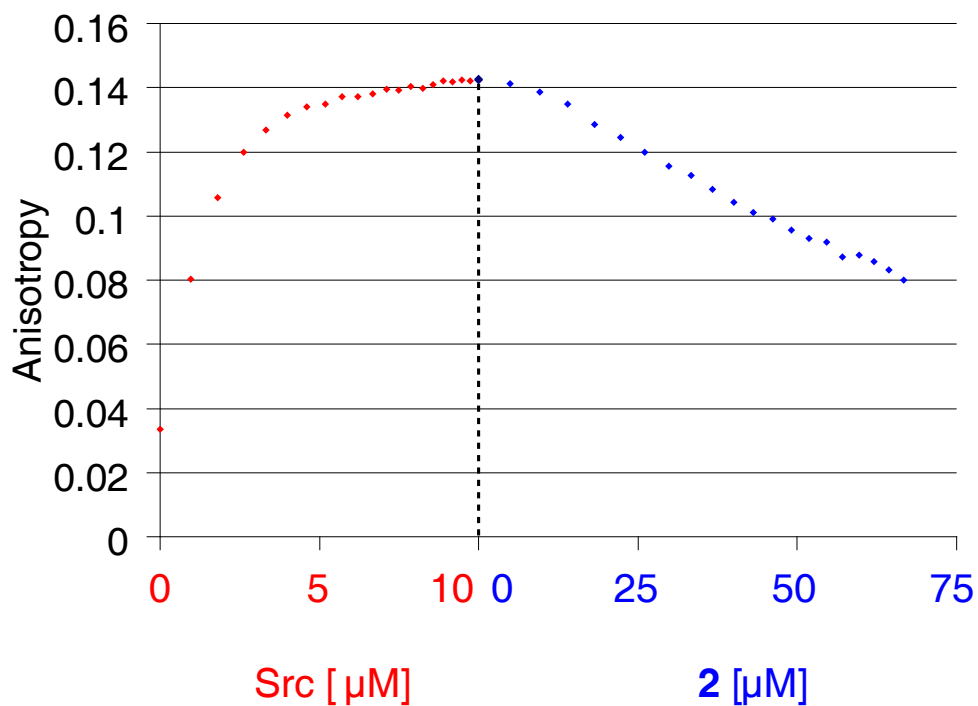
Supplementary Figure 3. Electron density maps and structural models of Src bound to macrocycles. (a) 2fo-fc electron density map around **1** contoured at 1.5 σ (red). (b) Composite omit map (cyan) around **4b**, contoured at 1.6 σ . The starting models for the macrocycle compounds were built in ChemDraw and a semiempirical quantumchemical AM1 geometry optimization was performed in REEL 0.9 (Restrains Editor Exclusively Ligands) (Nigel Moriarty) via the Phenix 1.6.1-336 interface (PHENIX: a comprehensive Python-based system for macromolecular structure solution. P. D. Adams, P. V. Afonine, G. Bunkóczi, V. B. Chen, I. W. Davis, N. Echols, J. J. Headd, L.-W. Hung, G. J. Kapral, R. W. Grosse-Kunstleve, A. J. McCoy, N. W. Moriarty, R. Oeffner), R. J. Read, D. C. Richardson, J. S. Richardson, T. C. Terwilliger and P. H. Zwart. *Acta Cryst. D*66, 213-221 (2010)). The optimized models of the macrocycles were built manually into the experimental electron density using COOT (Paul Emsley and Bernhard Lohkamp and William G. Scott and Kevin Cowtan; Features and Development of Coot; *Acta Crystallographica Section D - Biological Crystallography*).

	IC ₅₀ 4b	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	
Src	0.13 μM	E	V	K	L	G	Q	G	C	F	G	E	V	W	M	G	T	W	N	G	-	T	T	R	V	A	I	K	T	L	K	P	G
Hck	0.86 μM	E	K	K	L	G	A	G	Q	F	G	E	V	W	M	A	T	Y	N	K	-	H	T	K	V	A	V	K	T	M	K	P	G
Lck	2.4 μM	V	E	R	L	G	A	G	Q	F	G	E	V	W	M	G	Y	Y	N	G	-	H	T	K	V	A	V	K	S	L	K	Q	G
Abl	>100 μM	K	H	K	L	G	G	G	Q	Y	G	E	V	Y	E	G	V	W	K	K	Y	S	L	T	V	A	V	K	T	L	K	E	D

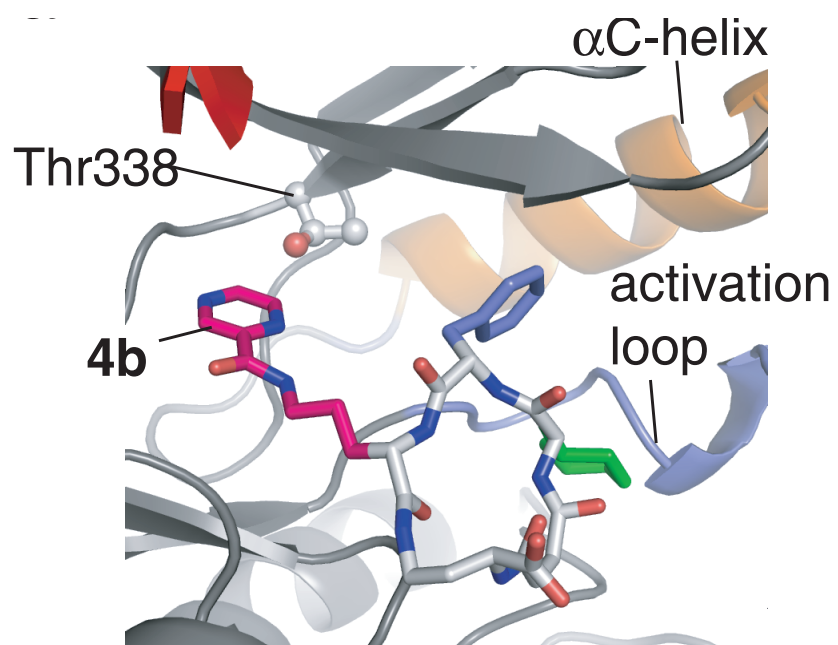
Supplementary Figure 4. Determinants of macrocycle specificity. Sequence alignment of the phosphate-binding loop and β 3- α C loop regions of the kinases tested here. Amino acids corresponding to Src residues that are within 5Å of **4b** in the co-crystal structure are highlighted in cyan. Amino acids differing in sequence from Src kinase domain are colored in red. The numbering of residues corresponds to chicken c-Src numbering. The half-maximal inhibitory concentration (IC₅₀) was determined in the presence of 250 μM ATP and 300 μM Src-optimal substrate peptide.



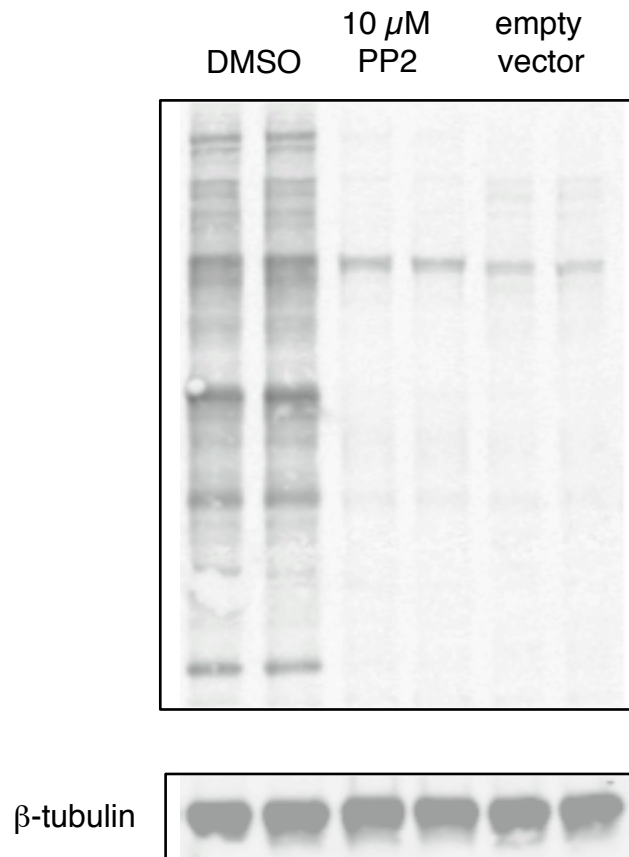
Supplementary Figure 5. Time-dependent inhibition study. Src₈₃₋₅₃₃ (100 nM) was incubated with 0-640 nM **25b** and kinase activity was measured either immediately (“0 min”) or after 90 min incubation at 30 °C (“90min”) using the spectrophotometric assay with Src optimal peptide at 300 μM and 250 μM ATP.



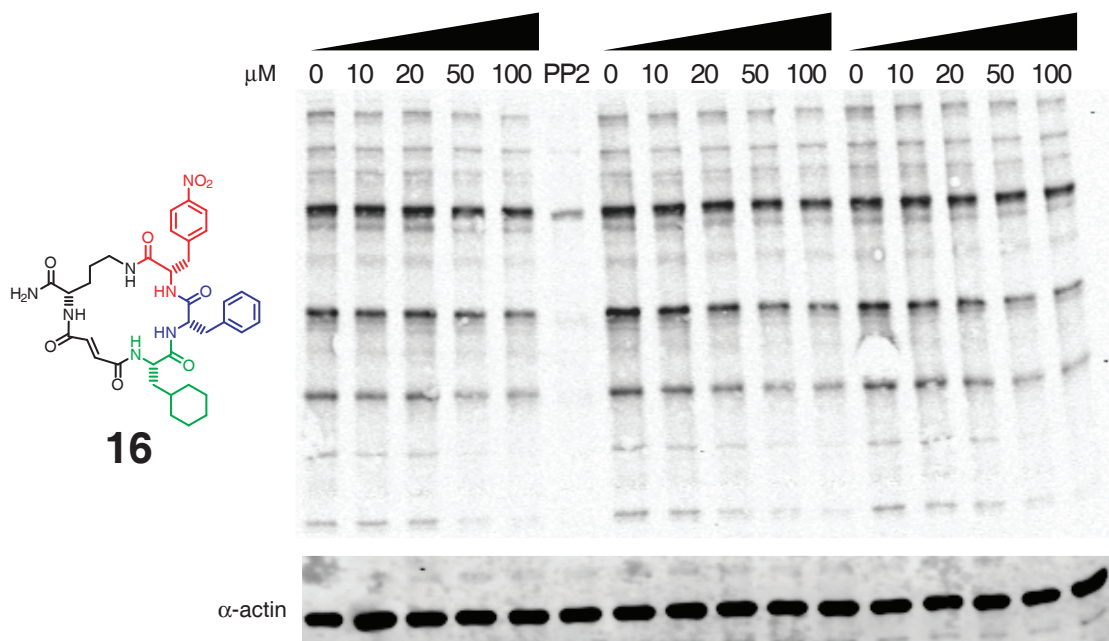
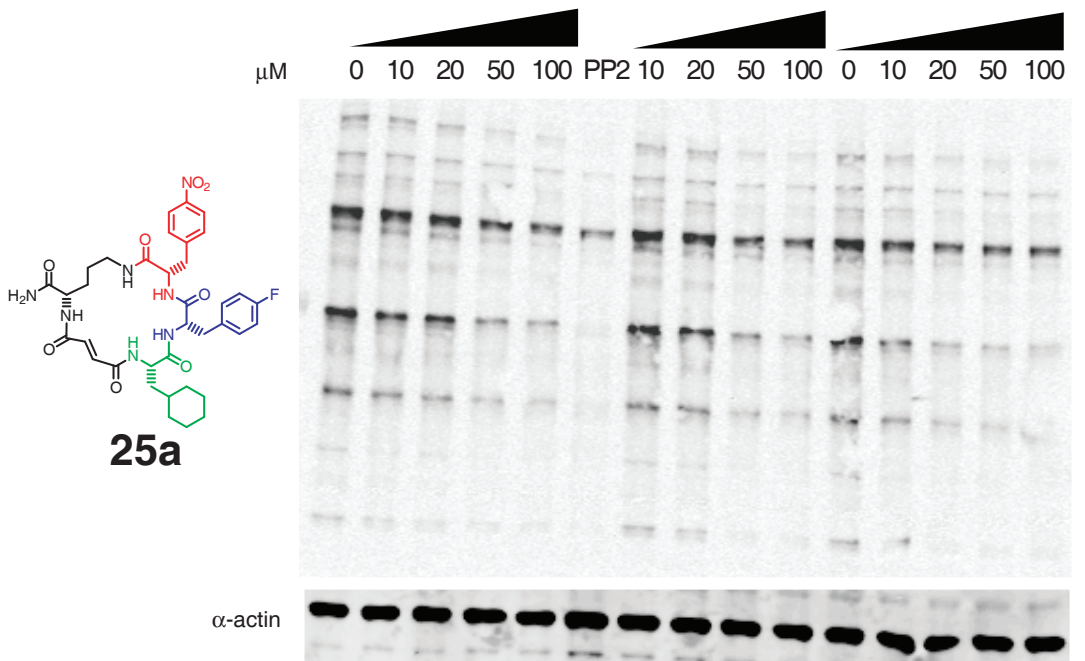
Supplementary Figure 6. Reversibility of inhibitor binding to Src kinase. Src kinase domain was added to 0.5 μM fluorescein-**2**. The anisotropy of fluorescein (left half of the plot, concentration referring to Src kinase domain added to the fluorescently labeled compound) was followed as Src concentrations increased. The resulting increase in anisotropy indicated binding of Src to fluorescein-**2**. Following saturation of fluorescein-**2** anisotropy, we added increasing amounts of unlabeled **2** to the mixture of Src and fluorescein-**2**. The decrease in anisotropy indicates the replacement of fluorescein-**2** with unlabeled **2** and confirms the reversibility of **2**-Src binding.



Supplementary Figure 7. Activity of the macrocycles against the Thr338Ile gatekeeper mutant of Src kinase. (a) Structure of the Src•**4b** complex with residues 272-283 of the P-loop (red) removed for clarity. The gatekeeper residue Thr338 is rendered in spheres and sticks.



Supplementary Figure 8. Phosphotyrosine levels in 3T3 (*src*^{-/-}) cells expressing Src Y529F. Treatment with the Src-family selective kinase inhibitor PP2 (lanes 3 and 4) reduces global phosphotyrosine levels detected by 4G10 antibody to comparable levels as in 3T3 (*src*^{-/-}) transfected with empty vector (lanes 5 and 6).



Supplementary Figure 9. Macrocycles **25a** and **16** inhibit Src kinase activity in cultured murine cells. 3T3 (src^{-/-}) cells transfected with a plasmid encoding Src Y529F were seeded in a 48-well plate and grown to confluence in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Individual wells were then treated with the indicated concentration of macrocycle in serum-free DMEM for six hours. The final concentration of DMSO in each treatment was 2%. After removing the small molecule and washing with phosphate-buffered saline, cells were lysed in radioimmunoprecipitation assay (RIPA) buffer, and global phosphotyrosine levels were quantified by Western blot using the 4G10 antibody.

Supplementary Table 1 Data collection and refinement statistics (molecular replacement)

	Src•1	Src•4b
Data collection		
Space group	P2 ₁	P321
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	42.2, 117.3, 62.7	143.6, 143.6, 41.5
α , β , γ (°)	90.0, 90.1, 90.0	90.0, 90.0, 120.0
Resolution (Å)	50-2.24	41.5-1.9
R_{sym} or R_{merge}	0.069 (0.30)	0.098 (0.42)
$I / \sigma I$	14.2 (3)	13.7 (4.1)
Completeness (%)	97.9 (85.7)	100 (99.9)
Redundancy	3.1	6.0
Refinement		
Resolution (Å)	2.24	1.9
No. reflections	29,870	38,763
$R_{\text{work}} / R_{\text{free}}$	0.1929 / 0.2496	0.1622 / 0.1849
No. atoms		
Protein	4140	2223
Ligand/ion	106	51
Water	107	324
<i>B</i> -factors		
Protein	Chain A: 48.4 Chain B: 48.9	17.03
Ligand/ion	Chain A: 53.2 Chain B: 53.4	24.08
Water	39.2	27.44
R.m.s. deviations		
Bond lengths (Å)	0.002	0.007
Bond angles (°)	0.687	1.117
PDB accession code	3U51	3U4W

* One crystal was used to solve each structure

*Highest-resolution shell is shown in parentheses.

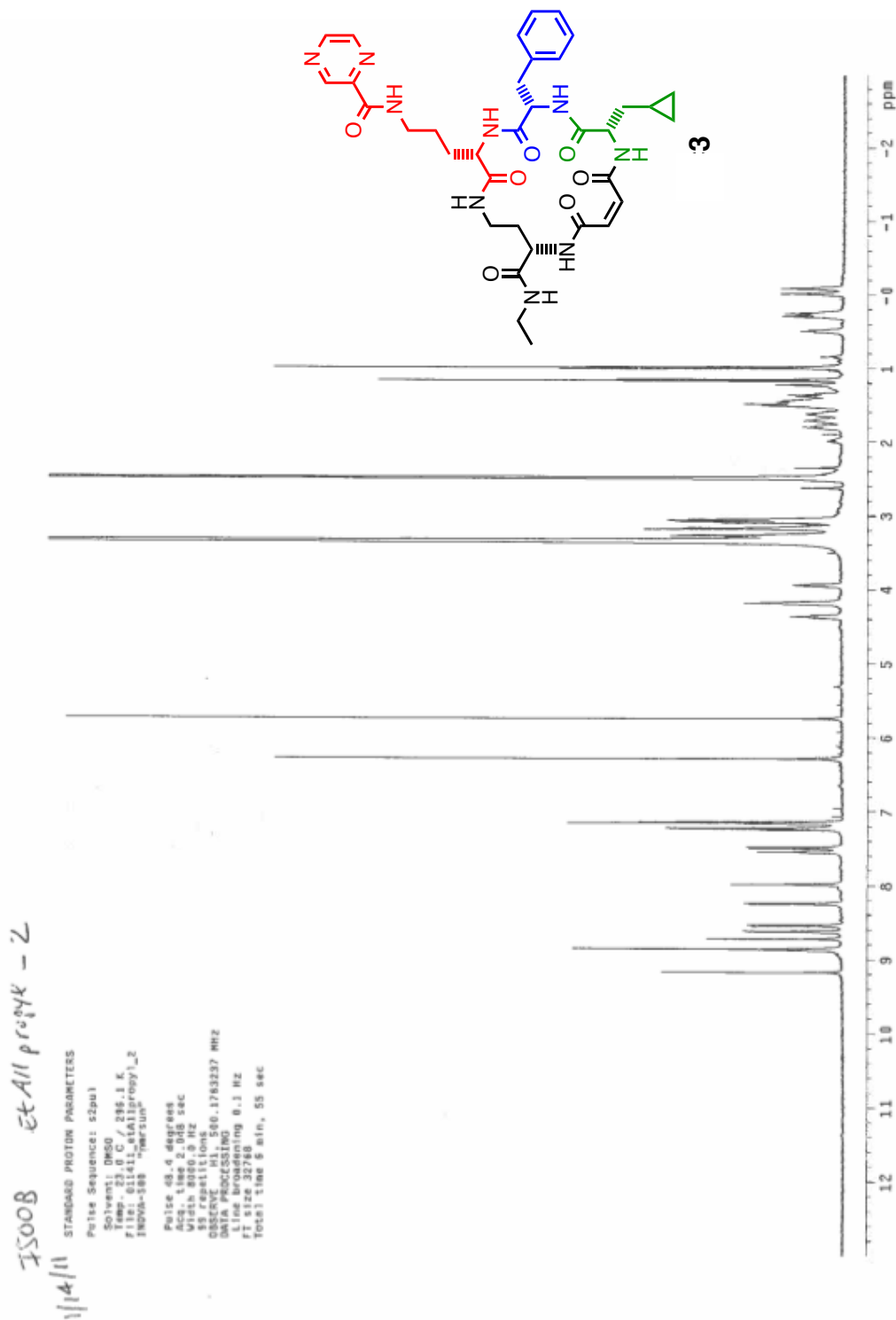
Supplementary Table 2. Mass spectrometry data for macrocycle compounds described in this work.

compound	expected [M+H] ⁺	observed [M+H] ⁺
1	744.4	characterized in ref. 1
2	666.3	characterized in ref. 1
3	704.4	704.4
4a	746.4	746.4
4b	719.4	719.4
5	751.4	751.4
6	751.4	751.4
7	751.4	751.4
8	751.4	751.4
9	652.3	characterized in ref. 1
10	662.3	662.3
11	668.3	668.3
12	752.2	752.2
13	698.3	698.3
14	774.3	774.3
15	748.3	748.3
16	704.3	704.3
17	673.4	673.4
18	693.3	693.3
19	737.3	737.3
20	727.3	727.3
21	684.4	684.4
22	702.4	702.4
23	715.4	715.4
24	720.3	720.3
25a	722.3	722.3
25b	723.3	723.3
26	718.4	718.4
27	737.3	737.3
28	737.3	737.3
29	737.3	737.3
30	737.3	737.3
31	737.3	737.3
fluorescein-1	1201.5	1201.5
fluorescein-2	1123.5	1123.5
fluorescein-9	1109.4	1109.4

Supplementary Reference

- 1) Kleiner, R.E., Dumelin, C.E., Tiu, G.C., Sakurai, K. & Liu, D.R. In vitro selection of a DNA-templated small-molecule library reveals a class of macrocyclic kinase inhibitors. *J Am Chem Soc* **132**, 11779-91 (2010).

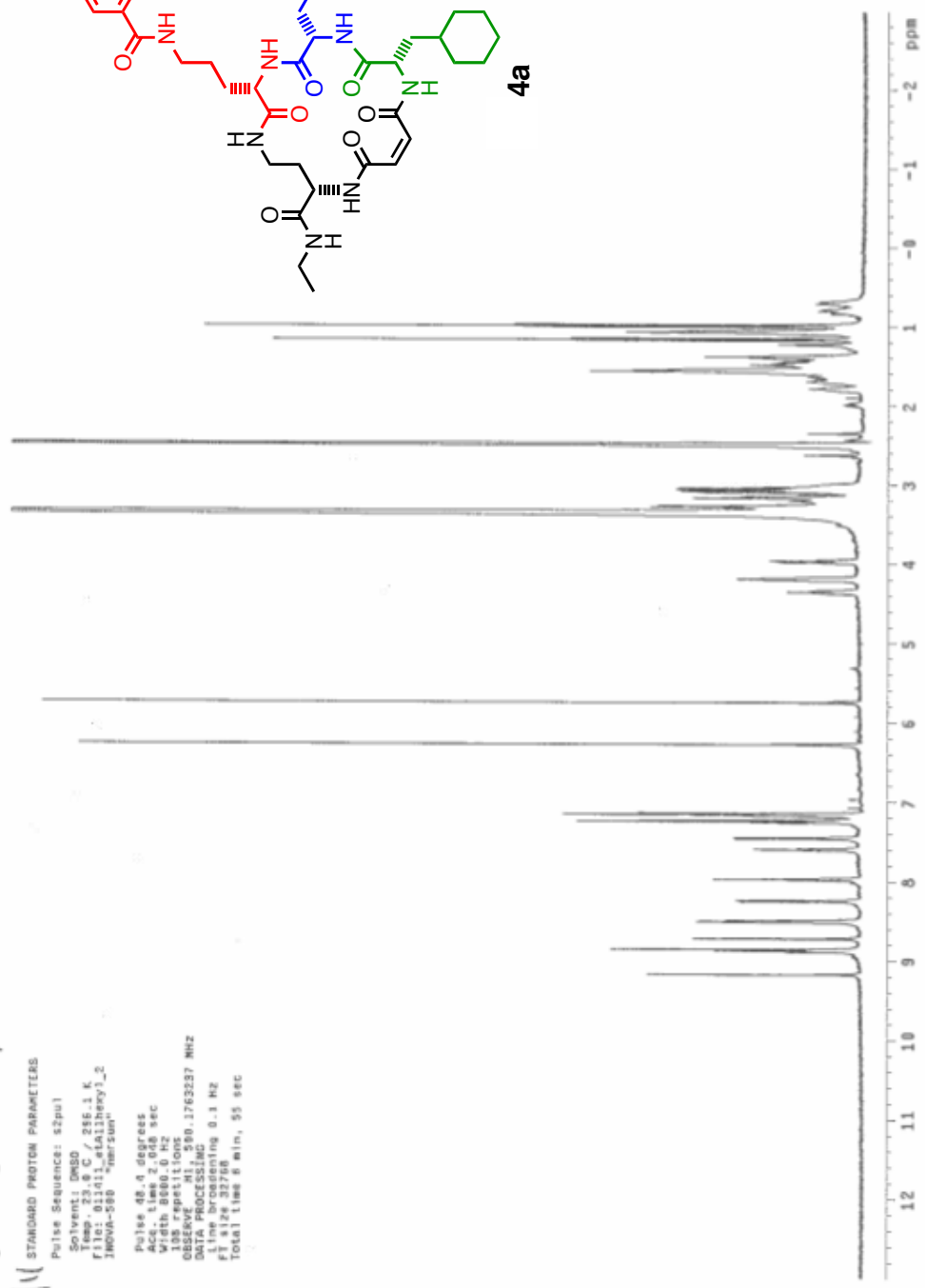
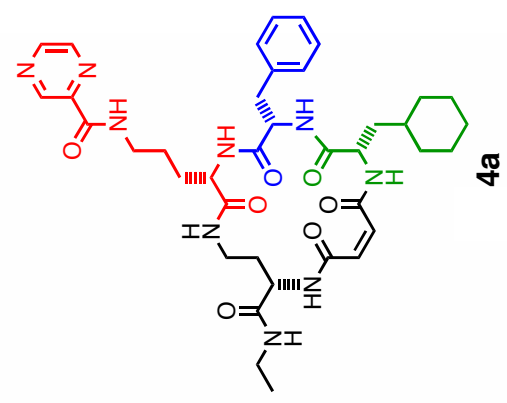
¹H NMR spectra of compounds described in this work



IS008 Ex A11 h2 rpk -2

1/14/11

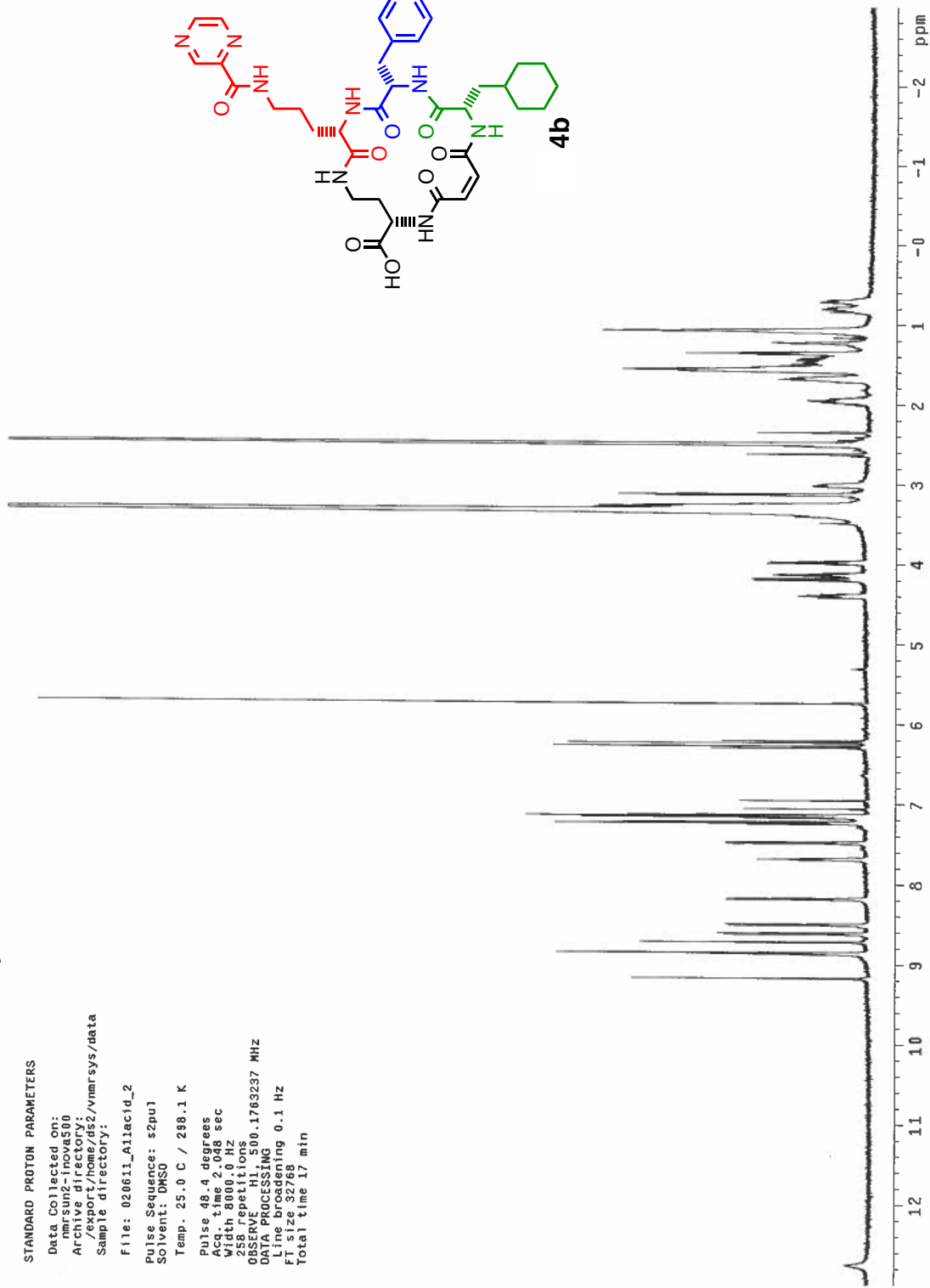
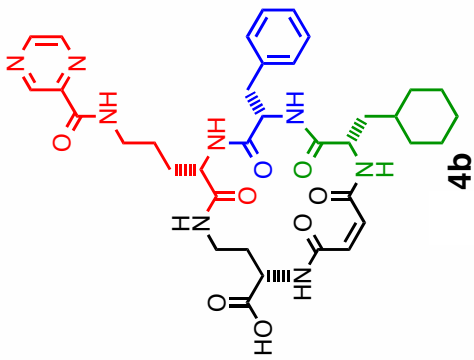
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DMSO-d6
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Total time 9 min, 55 sec



Allacid - 2 015

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Total time: 17 min



STANDARD PROTON PARAMETERS

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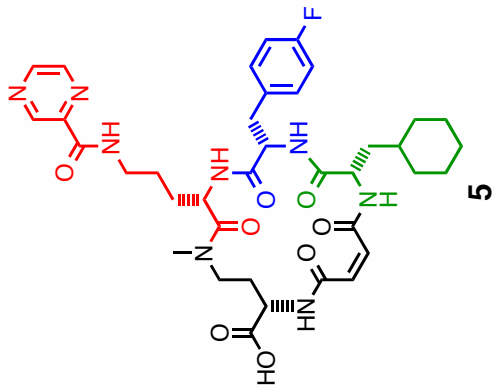
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DATA PROCESSING

Line broadening 0.1 Hz

FT size 131072

Total time 37 min, 0 sec



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Acq. time 3.414 sec

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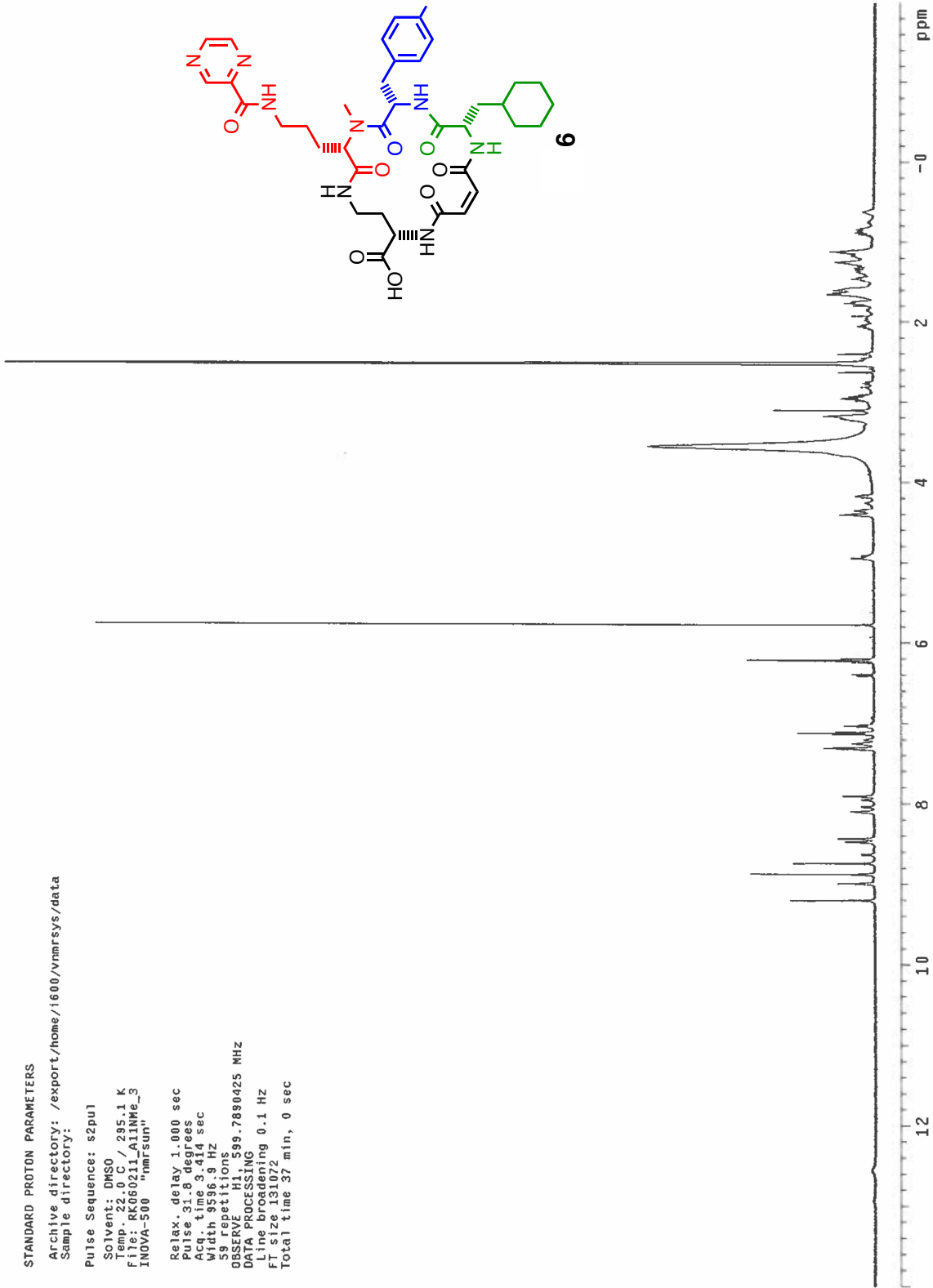
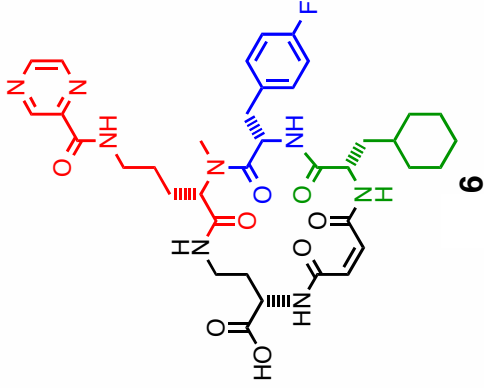
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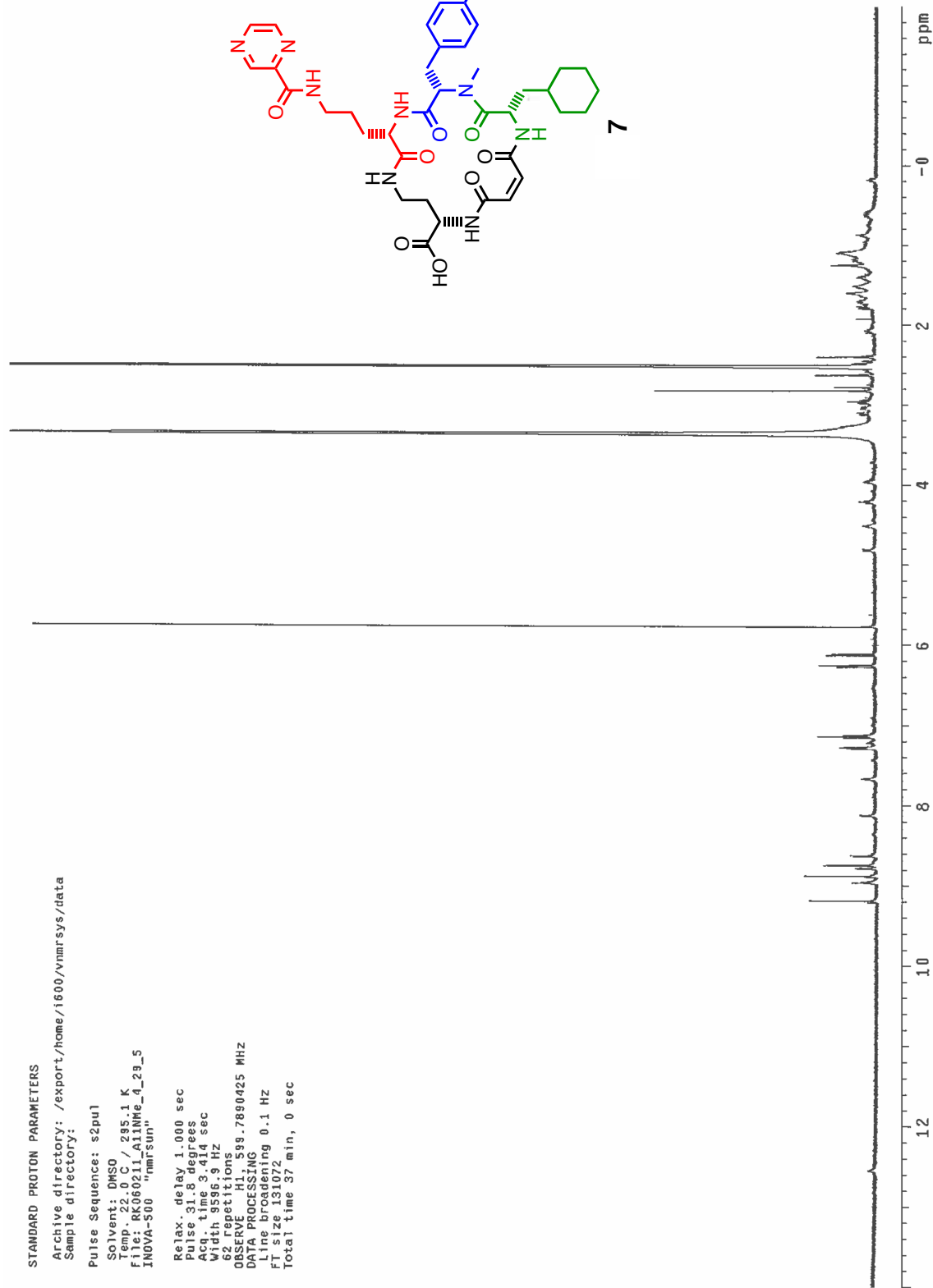
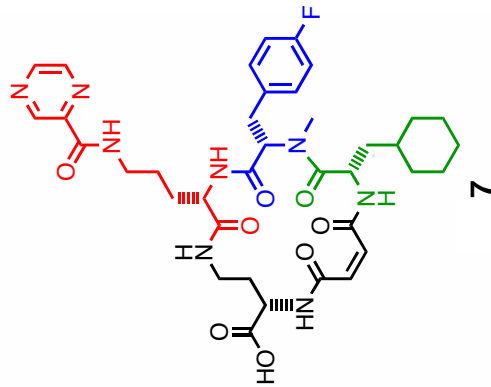
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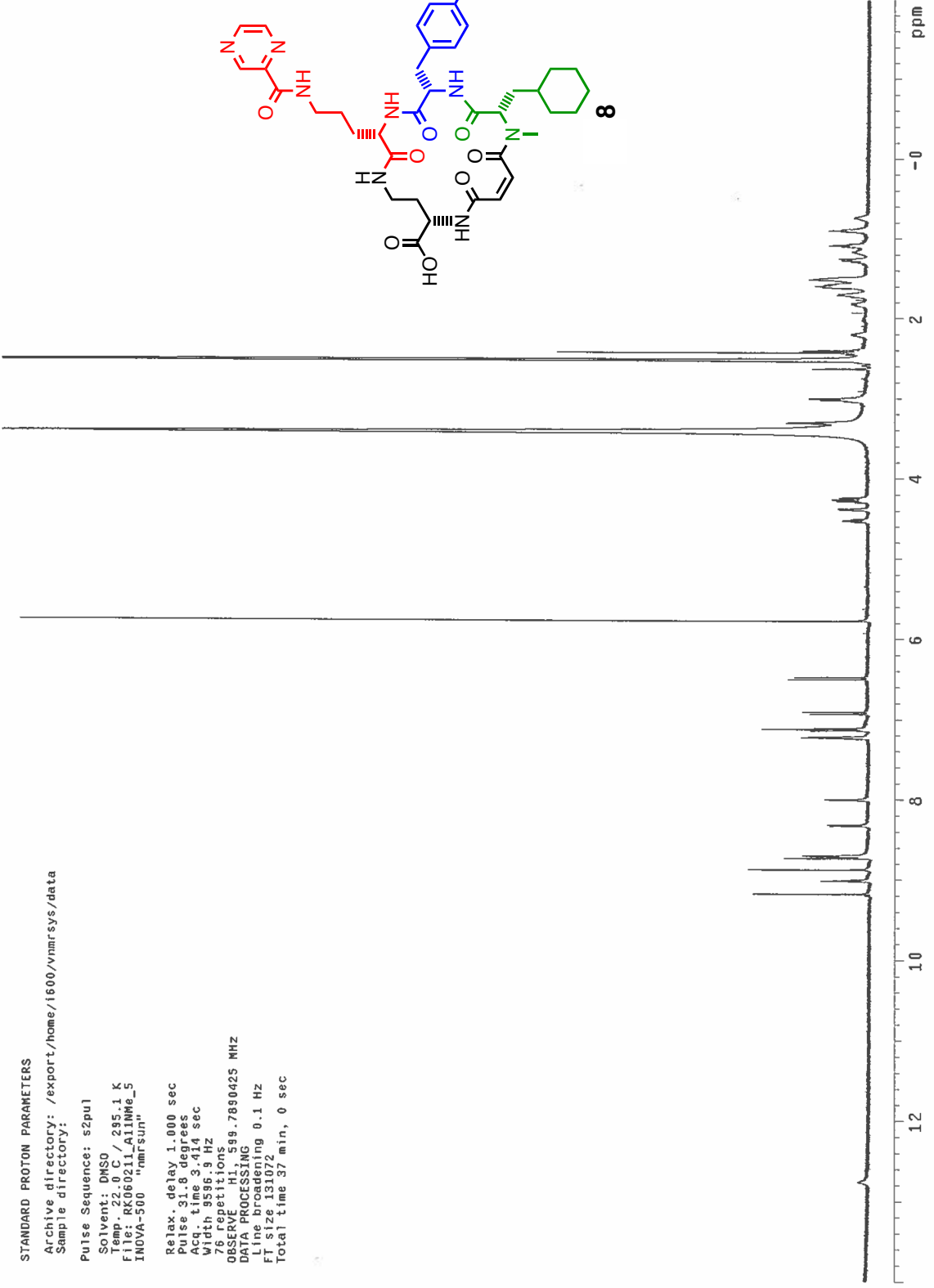
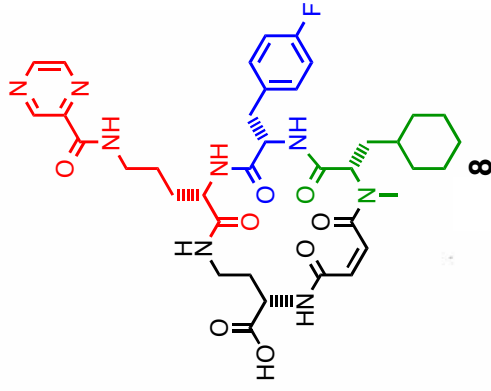
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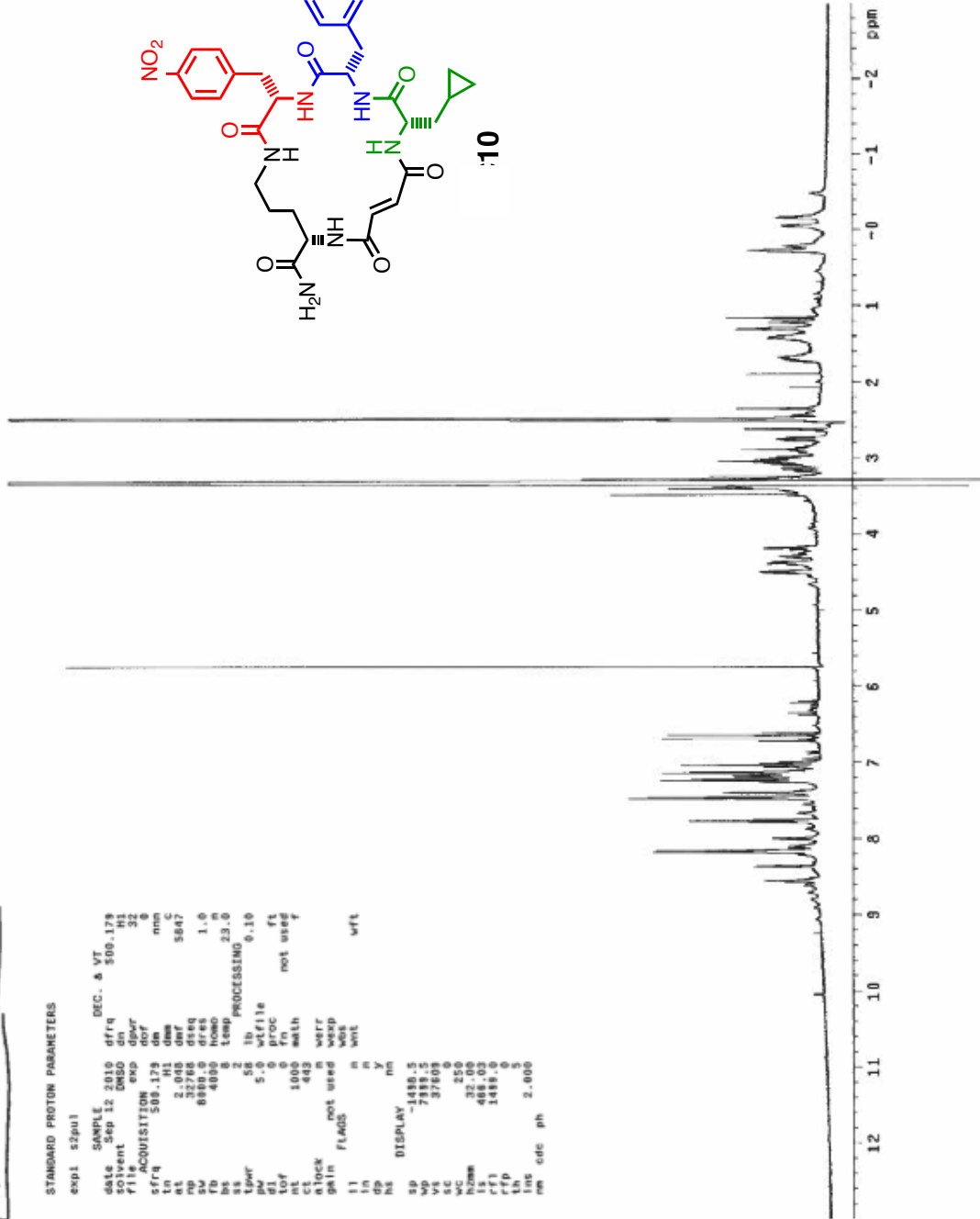
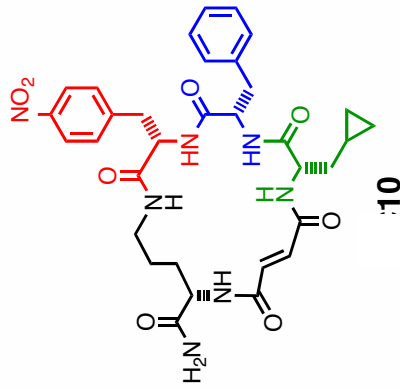


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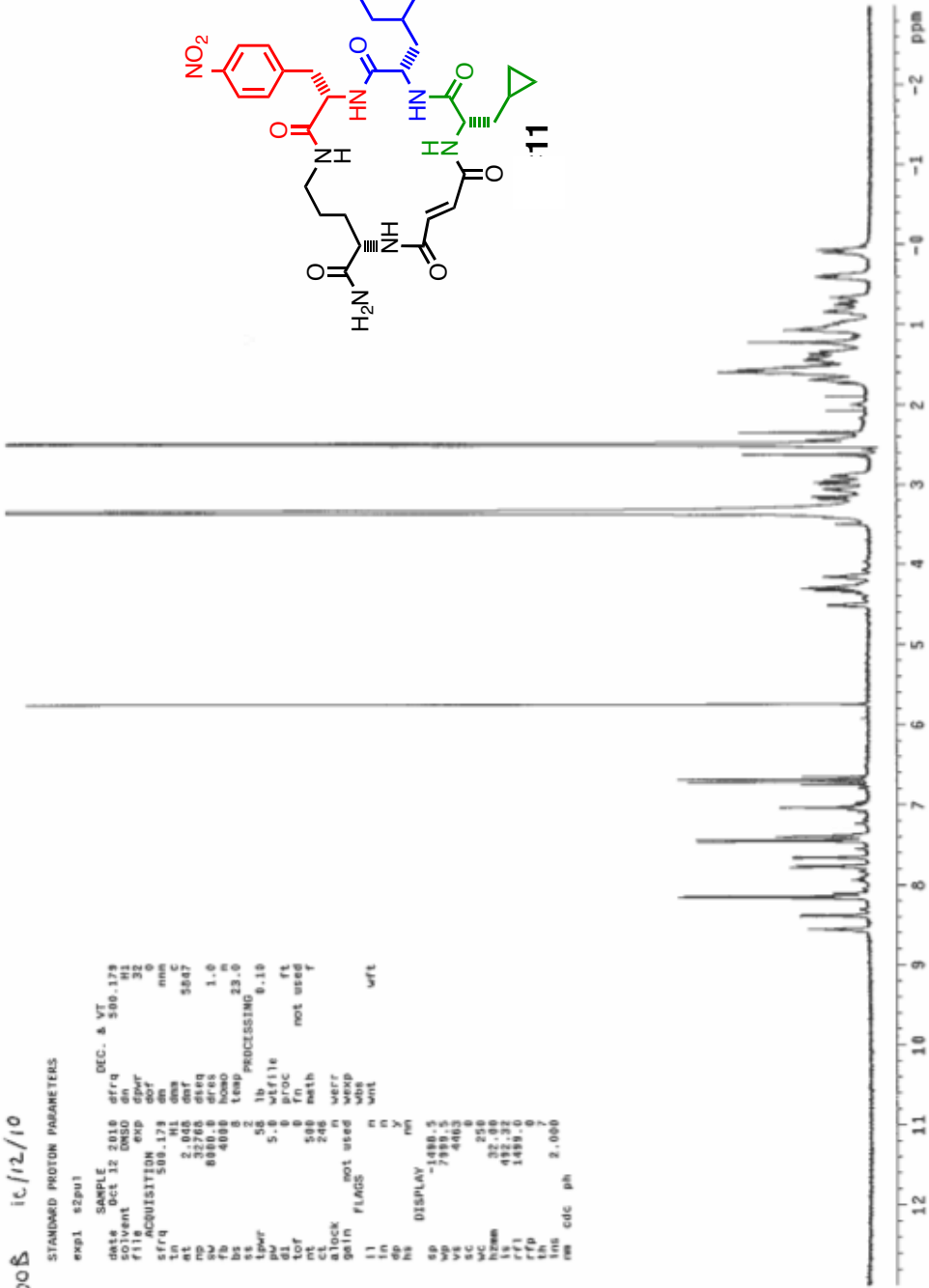
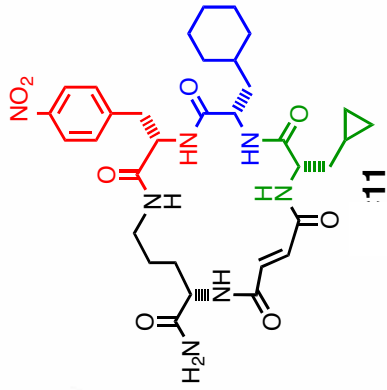


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15008 10/12/10

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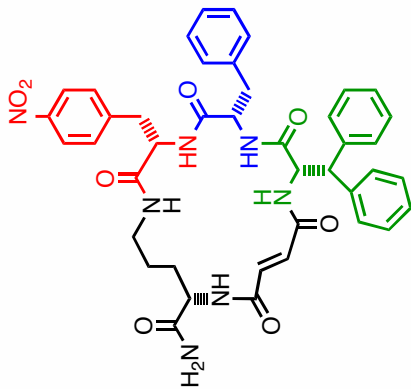
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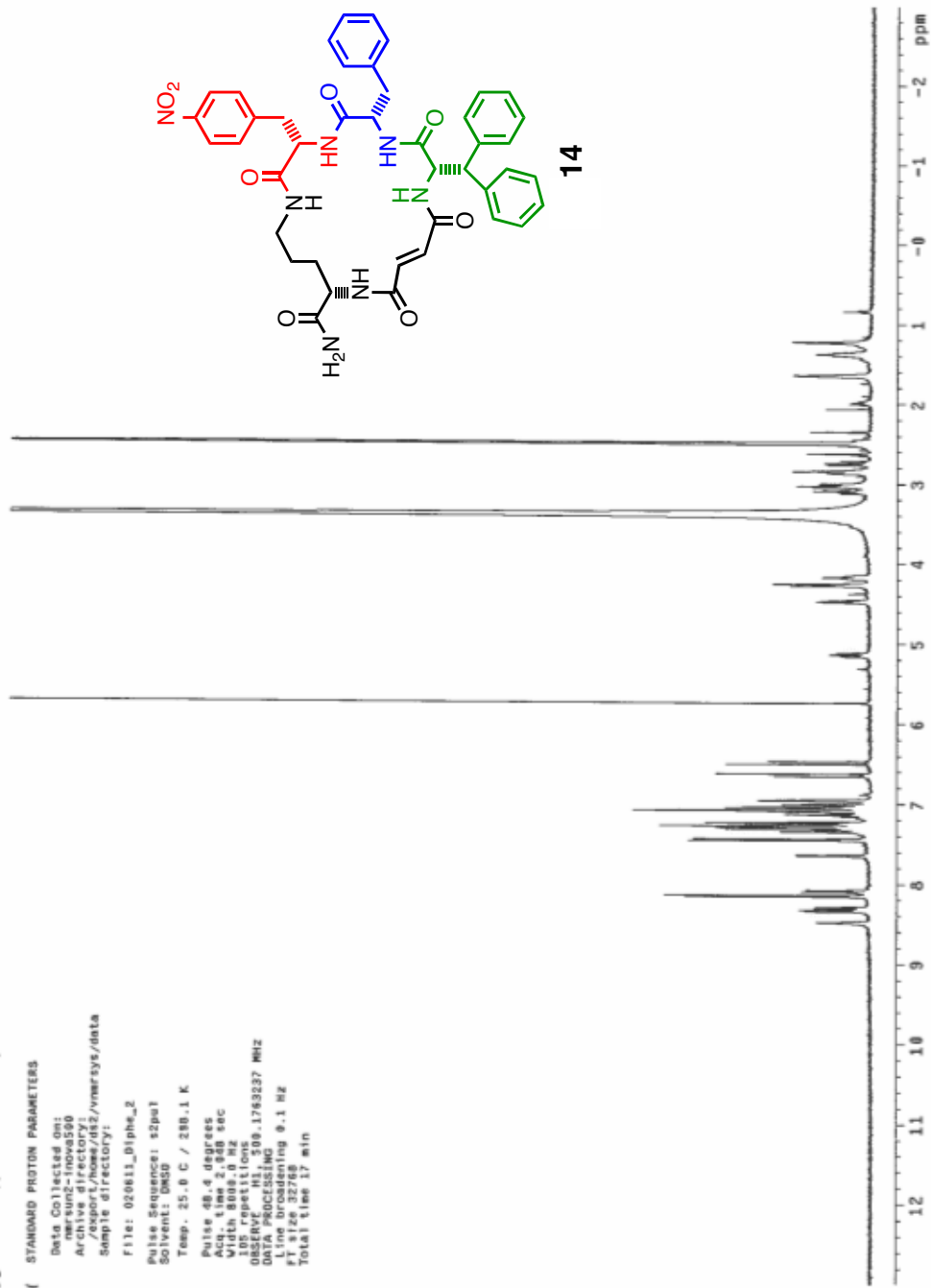
IS008 Diphe - 2 trans
2/6/11

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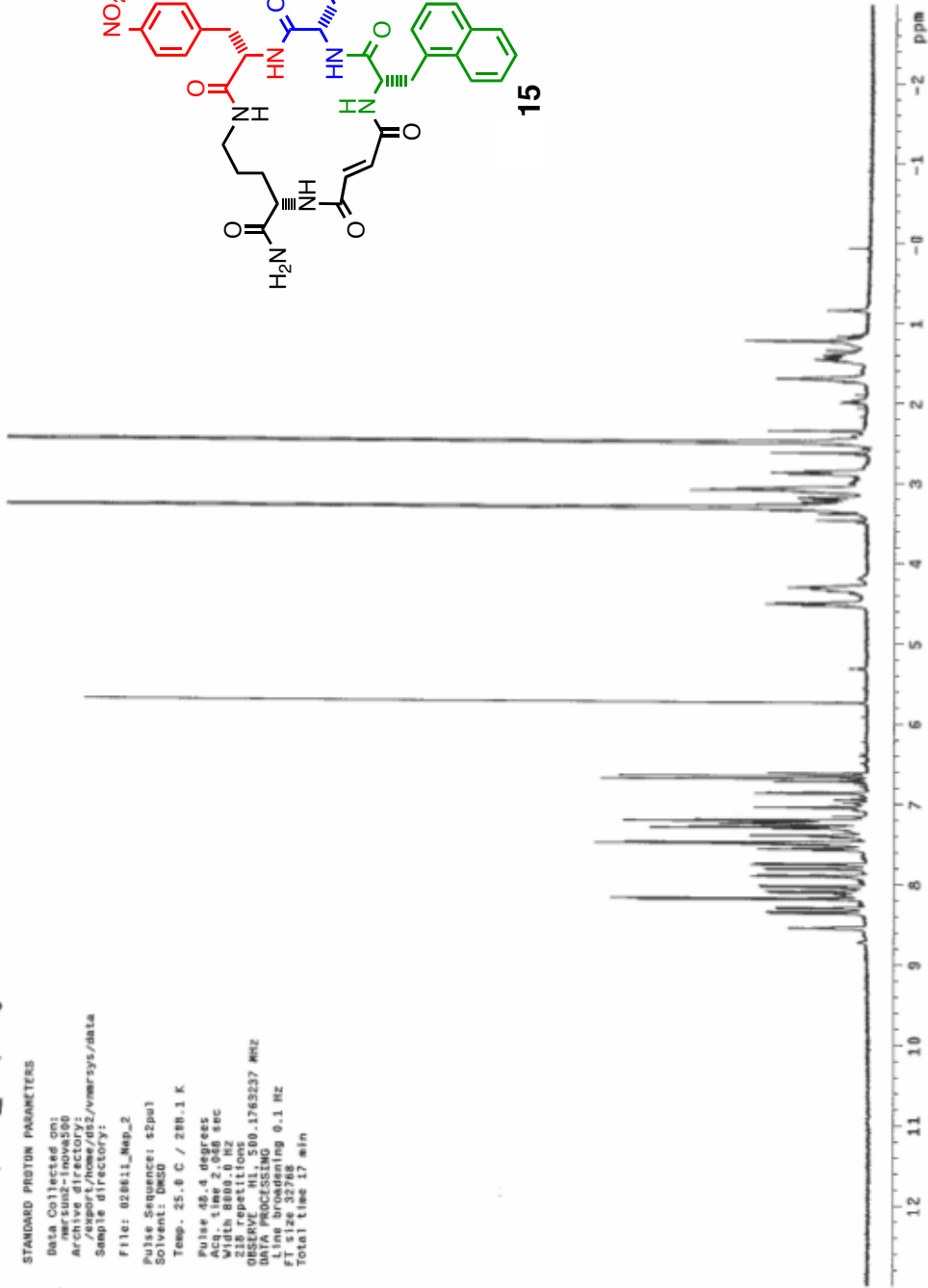
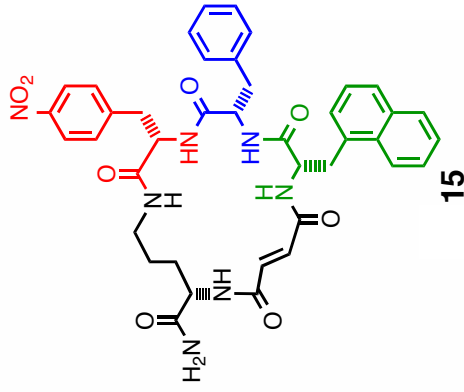
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2/6/11
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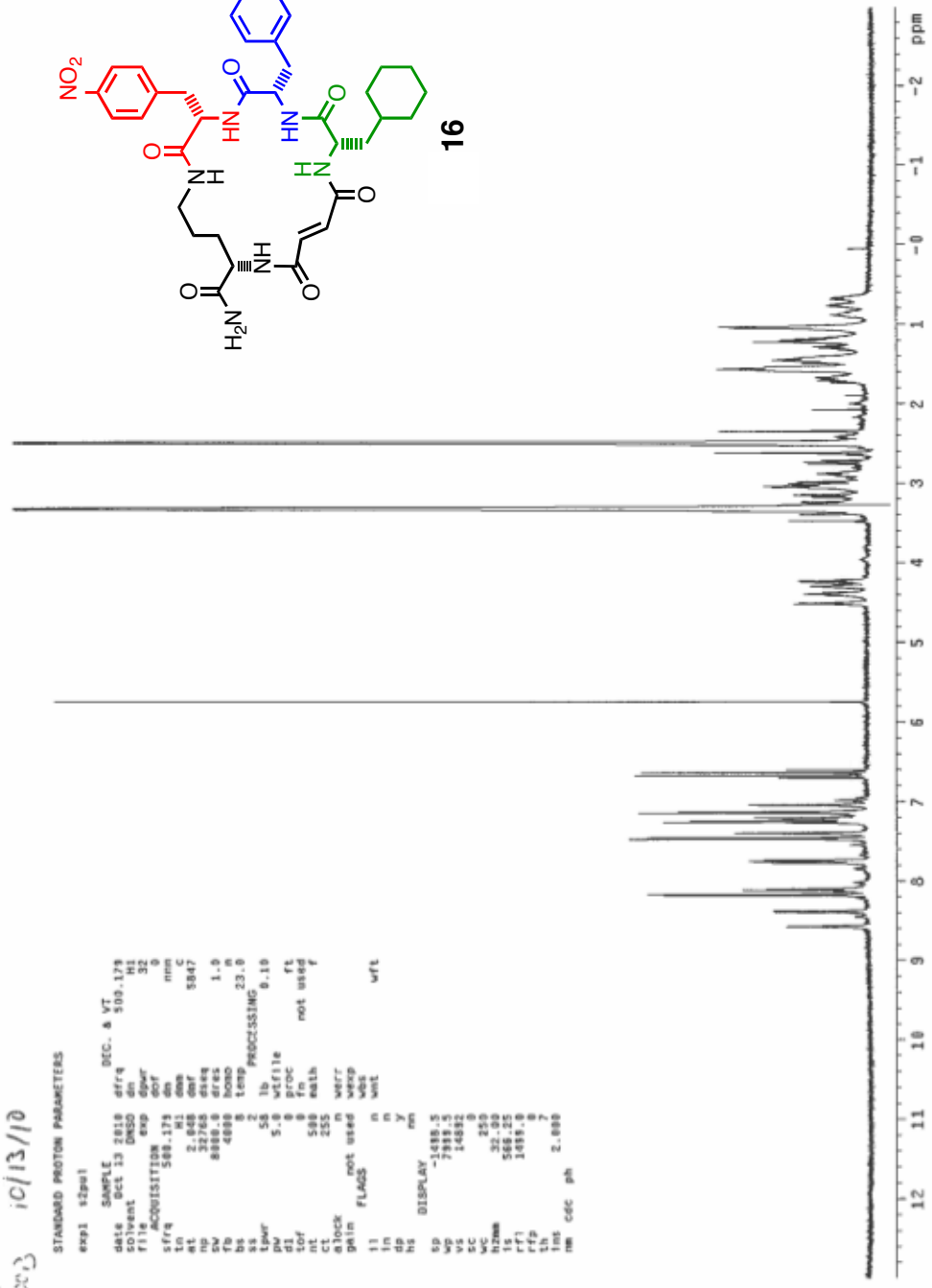
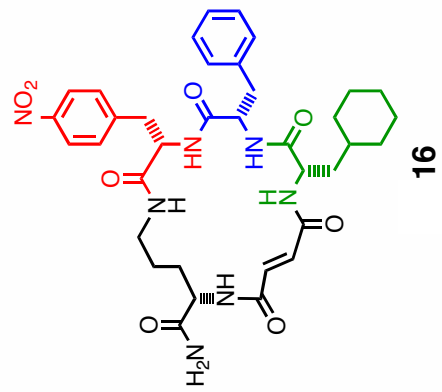


1005 AION 26 46 -31.5-
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STANDARD PROTON PARAMETERS

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fl100 exp 32
  
```



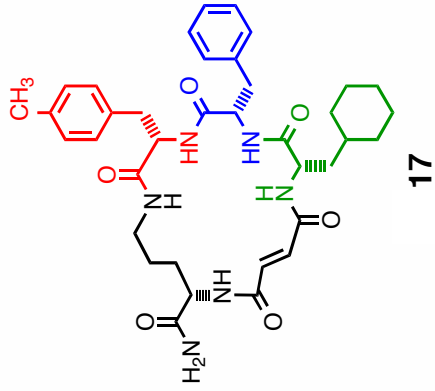
Amephe - 2
 11/12/10
 550B

STANDARD PROTON PARAMETERS

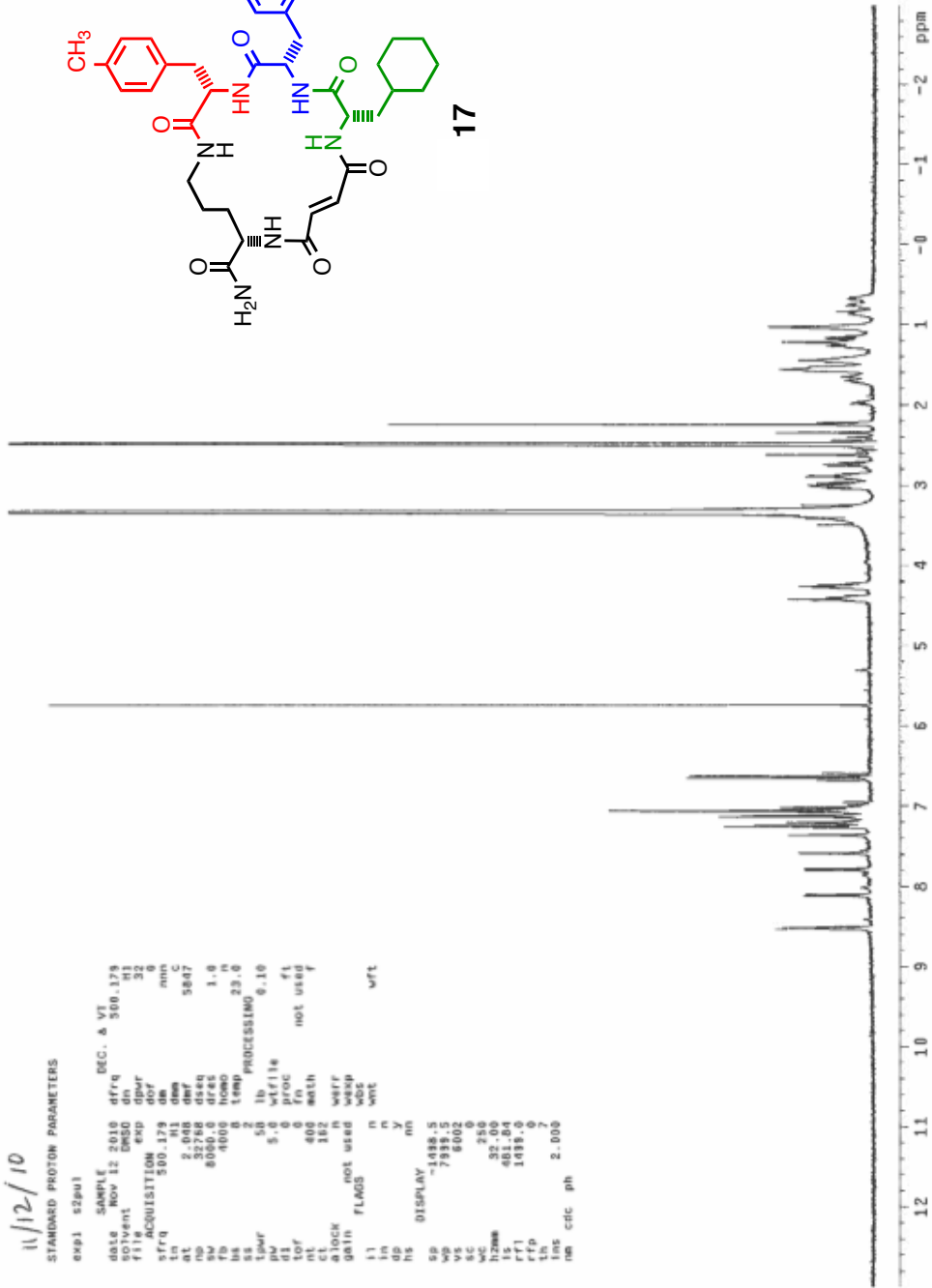
```

exp1 s2p03          DEC. & V1
=====
SAMPLE            dfrq 500.179
NOV 12 2010      dfrq 500.179
SOLVENT           exp 32
FILE             exp dot 32
ACQUISITION      dm 5807
IN              2.808 dmf
NU              32768 dsdq
SV              8000.0 dras 1.8
F2              4000 homo 23.6
SS              2 2 1000 PROCESSING
TPMR            50 lb 6.10
PU              5.0 wflf1e
DI              0 proc fl
AT              400 math not used
CL              182 math
ALOCK           not used
GAIN           not used
=====
FLAGS           n wff
              n wwp
              n wpc
              n wte
              wft

IN              n
DP              y
HS              n
=====
SP              -1498.5
WD              7895.5
VS              6002
WC              350
H2ZMR          32.00
F1              481.84
F2              1498.0
F3              1498.0
F4              7
F5              2.000
=====
nm cfc ph
  
```



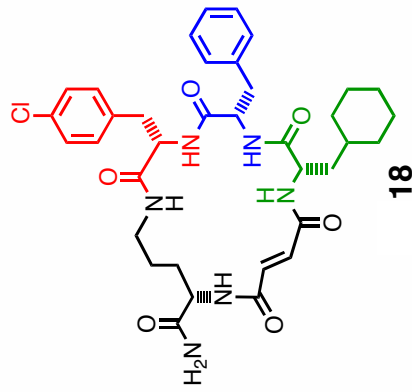
17



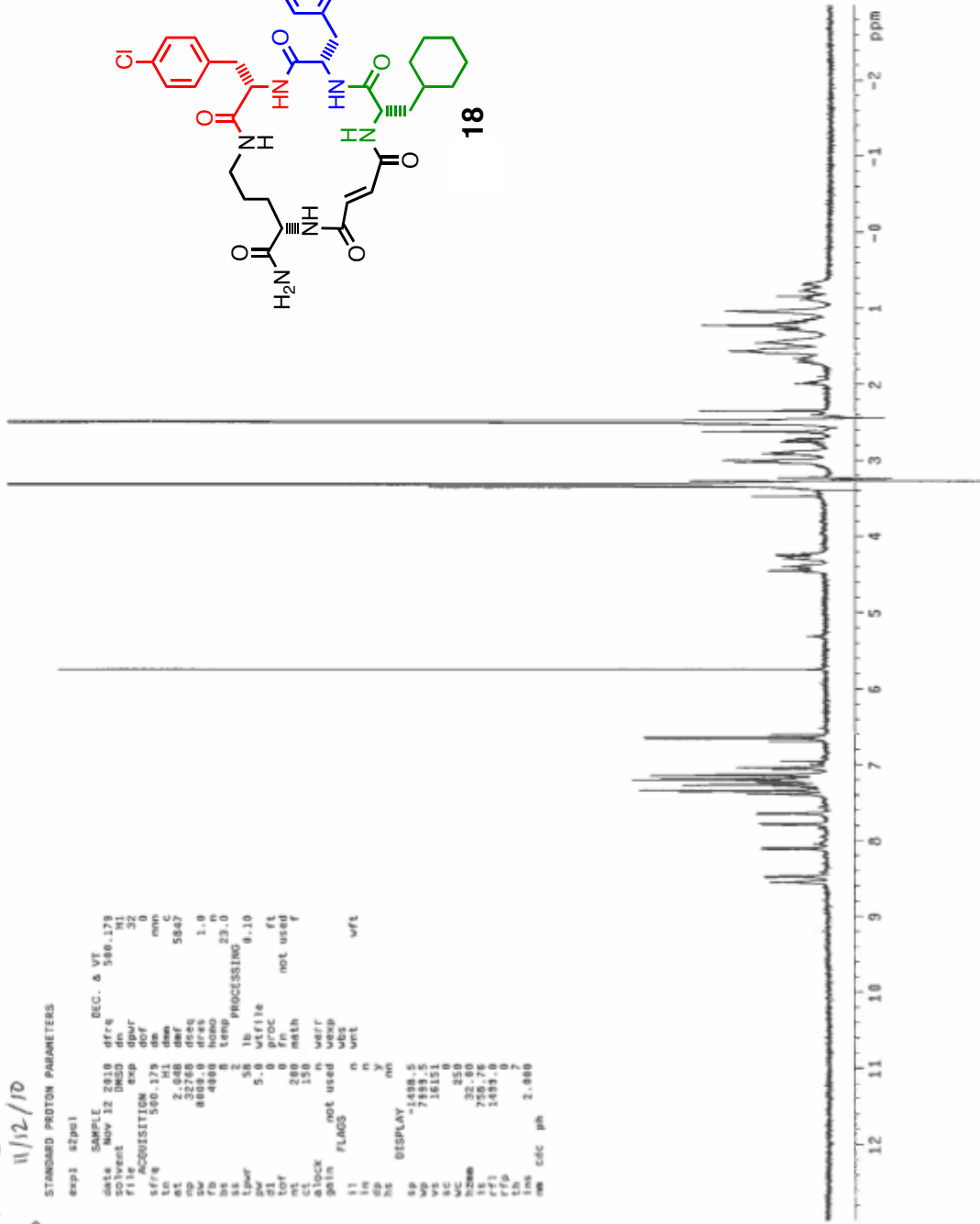
4c1Ahe-2
IC00B 11/12/10

STANDARD PROTON PARAMETERS

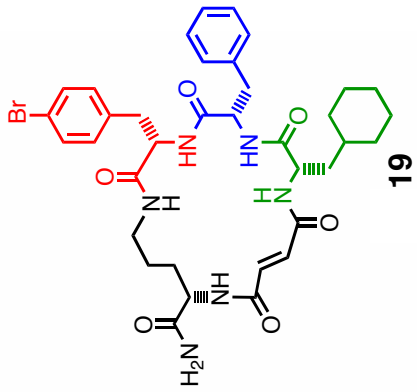
```
exp1 42pel          DEC. & VT
date Nov 12 2010   dfrq 500.179
solvent DMSO-d6    dmpr 32
file              exp 0
ACQUISITION
sfrq 500.179      ds   mm
at 2.600          dmr 5847
rg 32768          dpaq
sw 8000.0         dres 1.0
fb 4000           h2o0
ss 2              tely PROCESSING
ls 56             lb 0.10
pwr 5.0          wfile
dl 0             pproc
acq 200          meth not used
ct 150           werr
allok not used   wexp
gain not used    wps
fls 11           wft
in n
op y
hs n
DISPLAY
sp -1.000-5
pp 7.918-5
vs 16151
sc 0
b2nm 32.000
ls 755.76
rf1 1.403-0
rfp 0
lra 2
lra cdc ph 2.000
```



18



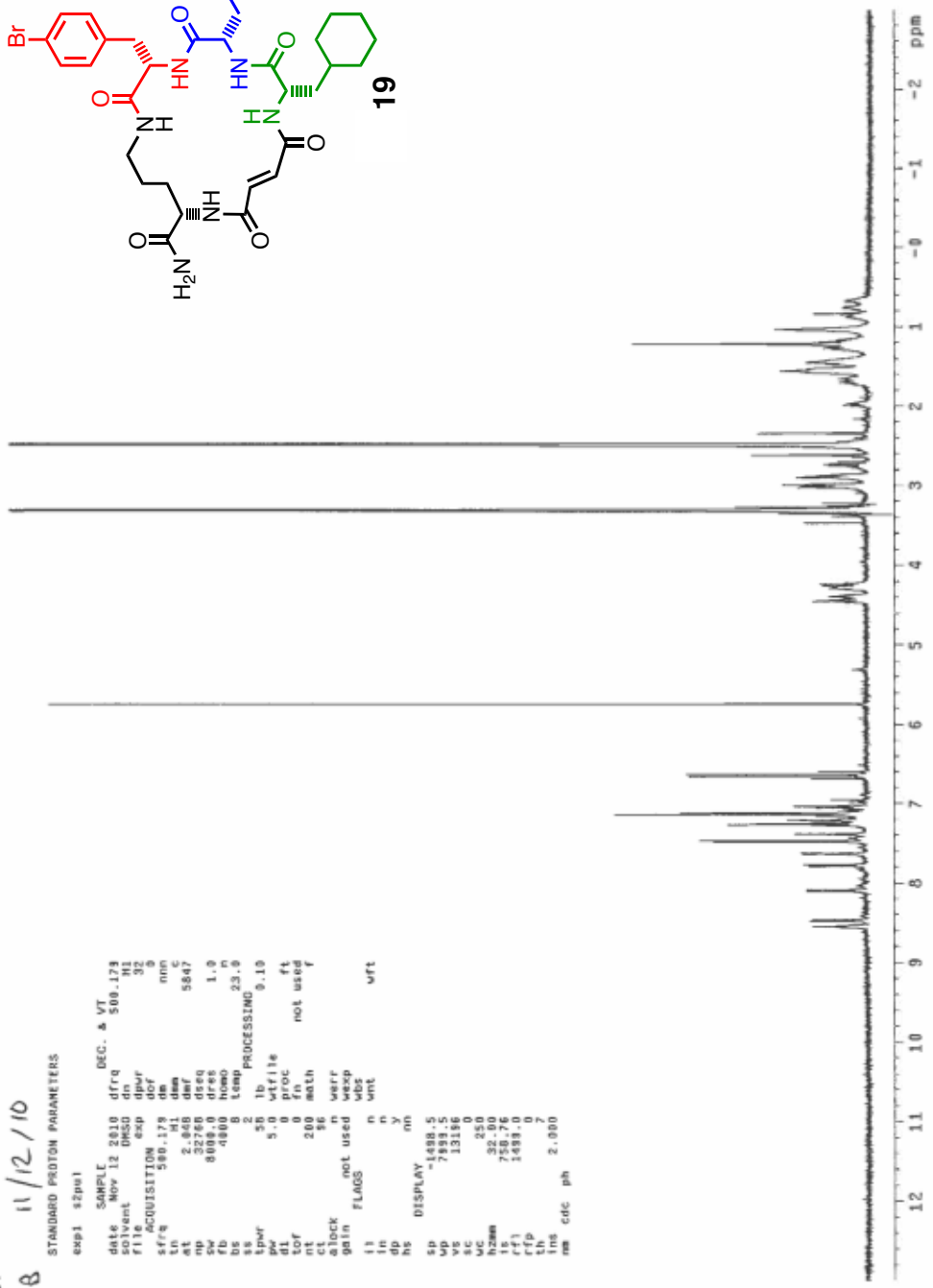
4-BrPhc-2
 55008 11/12/10



STANDARD PROTON PARAMETERS

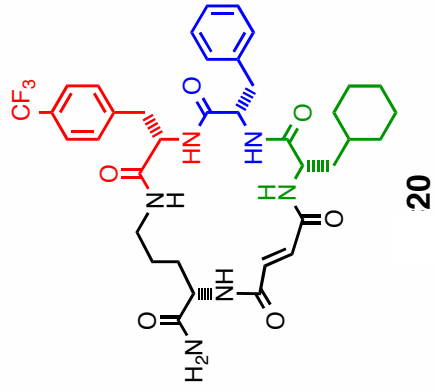
```

exp1 42pe1
SAMPLE
date Nov 12 2010 dfrq DEC. & VT
file 55008.d EXPNO 32
PROCNO 1
F1 ACQUISITION exp1 dprf 9
SFRQ 500.179 dm nmh
AQ 2.861 dmw 5847
RG 32768 dreq
SV 8008.0 dfe8 1.0
EB -1800 homo
US 0 Temp PROCESSING 23.0
TPW 56 lb
PW 5.0 wfile
DL 0 proc fl
OCR 0 fn not used
CT 200 meth
SE 56
elock n verr
gain not used wexp
FLAGS not used vds
l1 n vnt
l2 n vnt
l3 n vnt
l4 n vnt
l5 n vnt
l6 n vnt
l7 n vnt
l8 n vnt
l9 n vnt
l10 n vnt
l11 n vnt
l12 n vnt
hs DISPLAY nm
SP -1498.5
VP 7893.5
VS 13196
SC 0
NS 0
NS2MM 32.00
IS 758.76
RF1 1433.0
RF2 0
RF3 0
RF4 0
RF5 0
RF6 0
RF7 0
RF8 0
RF9 0
RF10 0
RF11 0
RF12 0
ins cdc ph
  
```



4-CF₃-Ph - 2

1500
1000
500



20

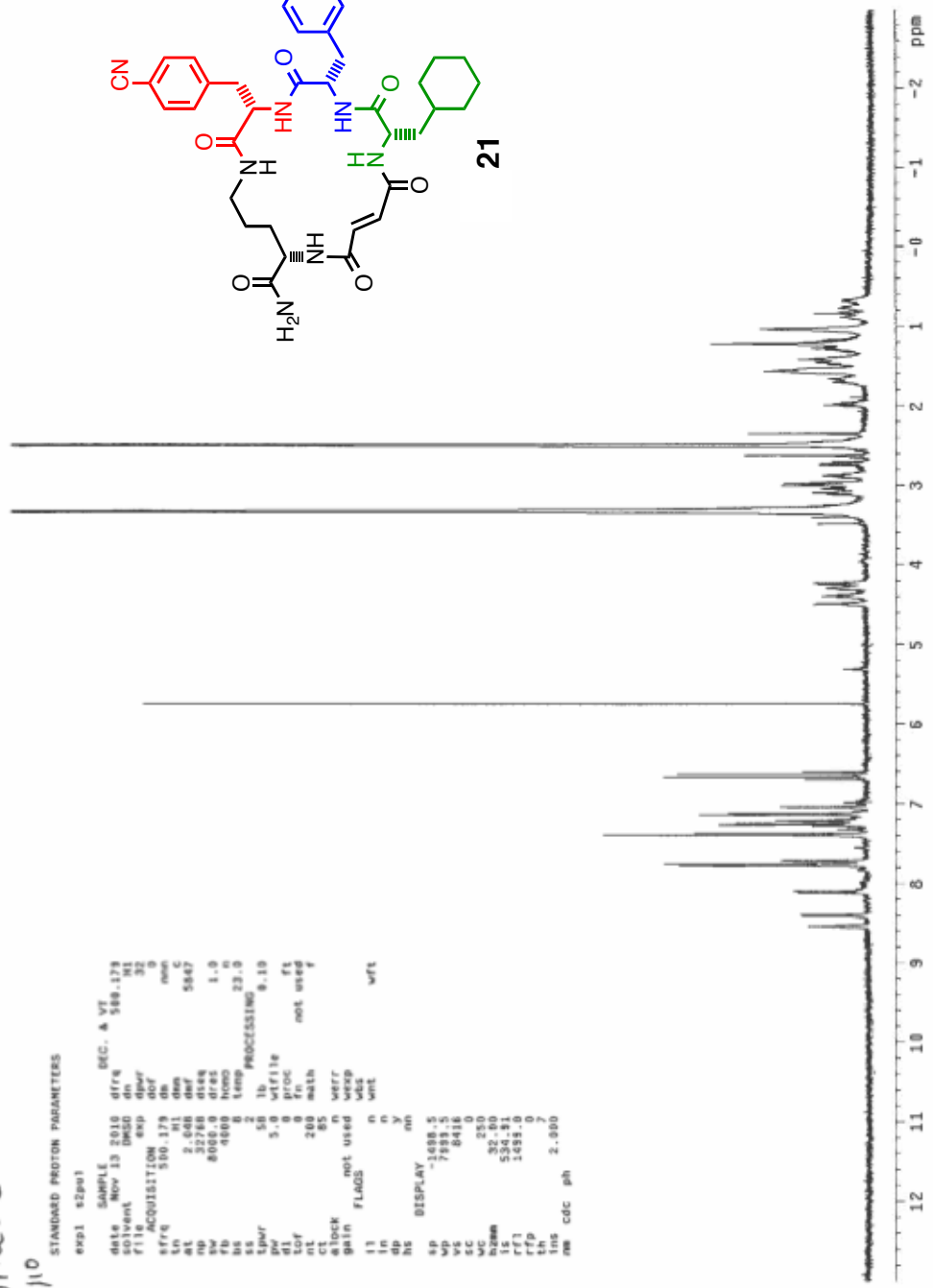
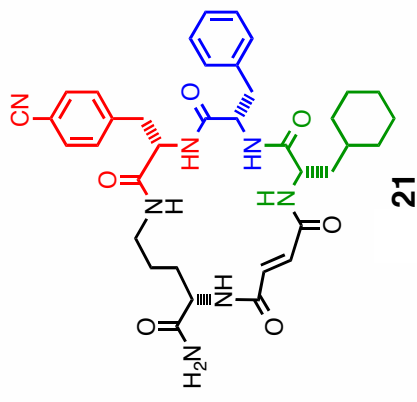


4CNphe-2
11/13/10
15008

STANDARD PROTON PARAMETERS

```

#991 #2901
date Nov 13 2010 09:58:17 DEC. & VT
file wnt 32
f1 ACQUISITION exp dof
sfrq 510.179 db
ln 11 dm 5847
rl 22748 d148
sv 8000.0 dr45 1.0
fb 1800 homo 23.0
bs 0 temp
tput 58 lb PROCESSING 8.10
pv 5.0 v1file 0
dl 0 proc ft
tdf 200 fn not used
ct 85 data
atock n wrrr
gain not used wexp
ll 11 FLAGS n wnt wft
ln 3 y
dp 3
hs DISPLAY mh
sp 1688.5
wp 7893.5
vs 8416
sc 0
hcnm 32.80
ls 534.81
rf1 1495.0
rfp 0
lms cdc ph
mw 2.900
  
```

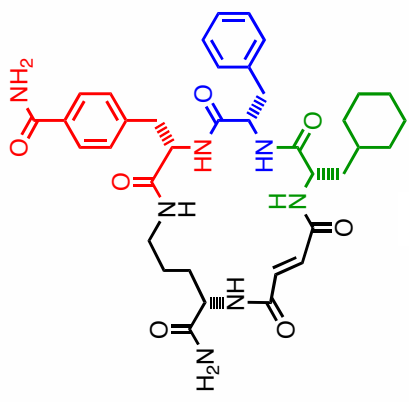


11/13/10
 Isaac
 4CONH₂ phe - 2

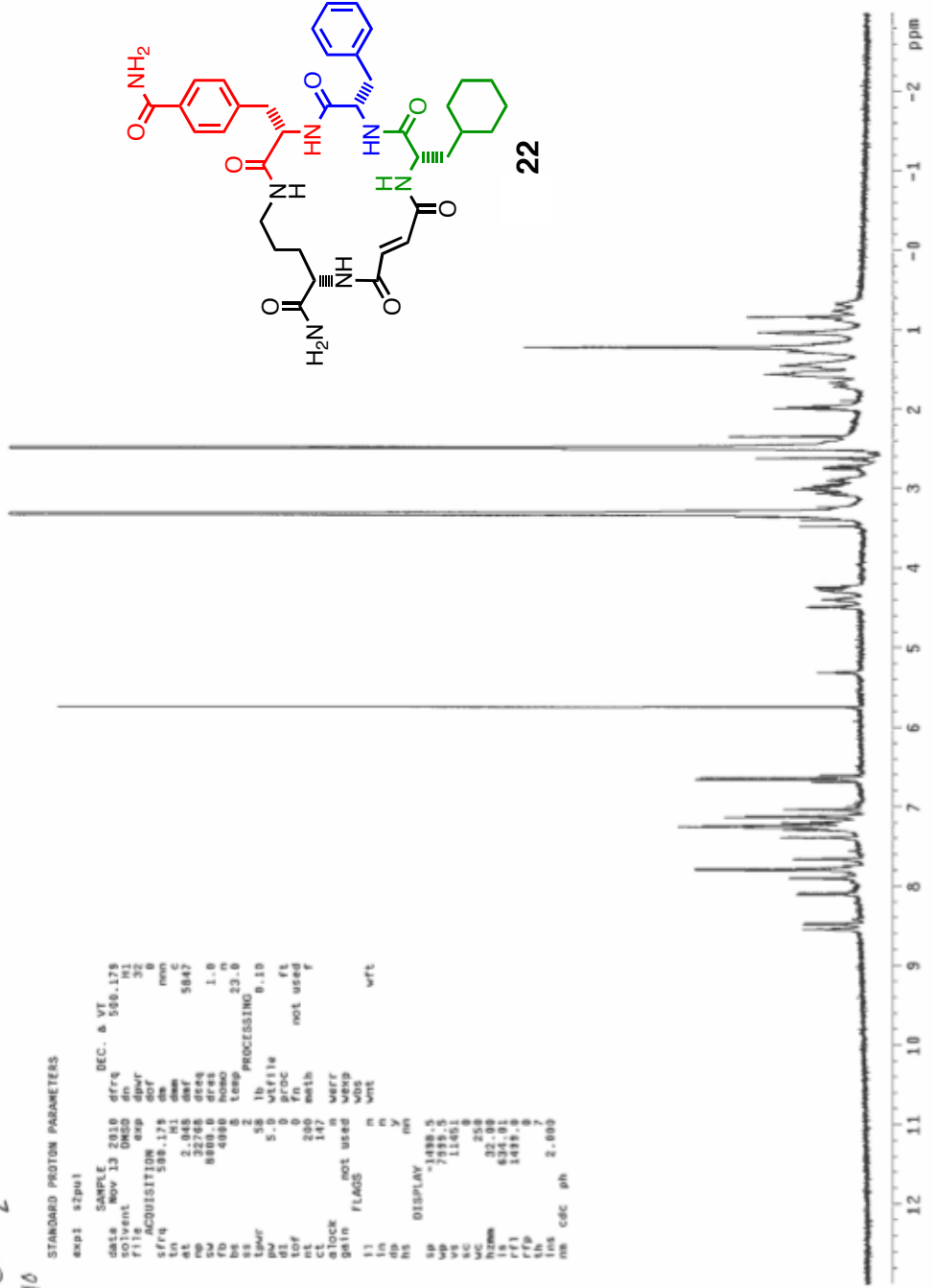
STANDARD PROTON PARAMETERS

```

exp1 szpu1
data Nov 13 2010 DEC. & VT
file 00200000000000000000
file 00200000000000000000
ACQUISITION exp 32
SFRq 588.175 dm
AQ 2.045 dm
NU 32768 dft8
SI 8880.0 dft8 1.0
FB 4098 homo
SI 2 Temp 23.0
LAMP 58 1b PROCESSING 0.10
PA 5.0 vrf1e
DI 0 proc
HD 0 not used
CT 200 math
ALOCK 147 n voff
GAIN not used wexp
SI 11 n voff
IN n wft
DS n
HS y
SI 015PLAY nm
SP -1488.5
WP 7955.5
VS 11451
VC 258
WZ 32.00
IR 634.01
RFI 1435.8
SFP 7
INS 2.000
mb csc ph
  
```



22

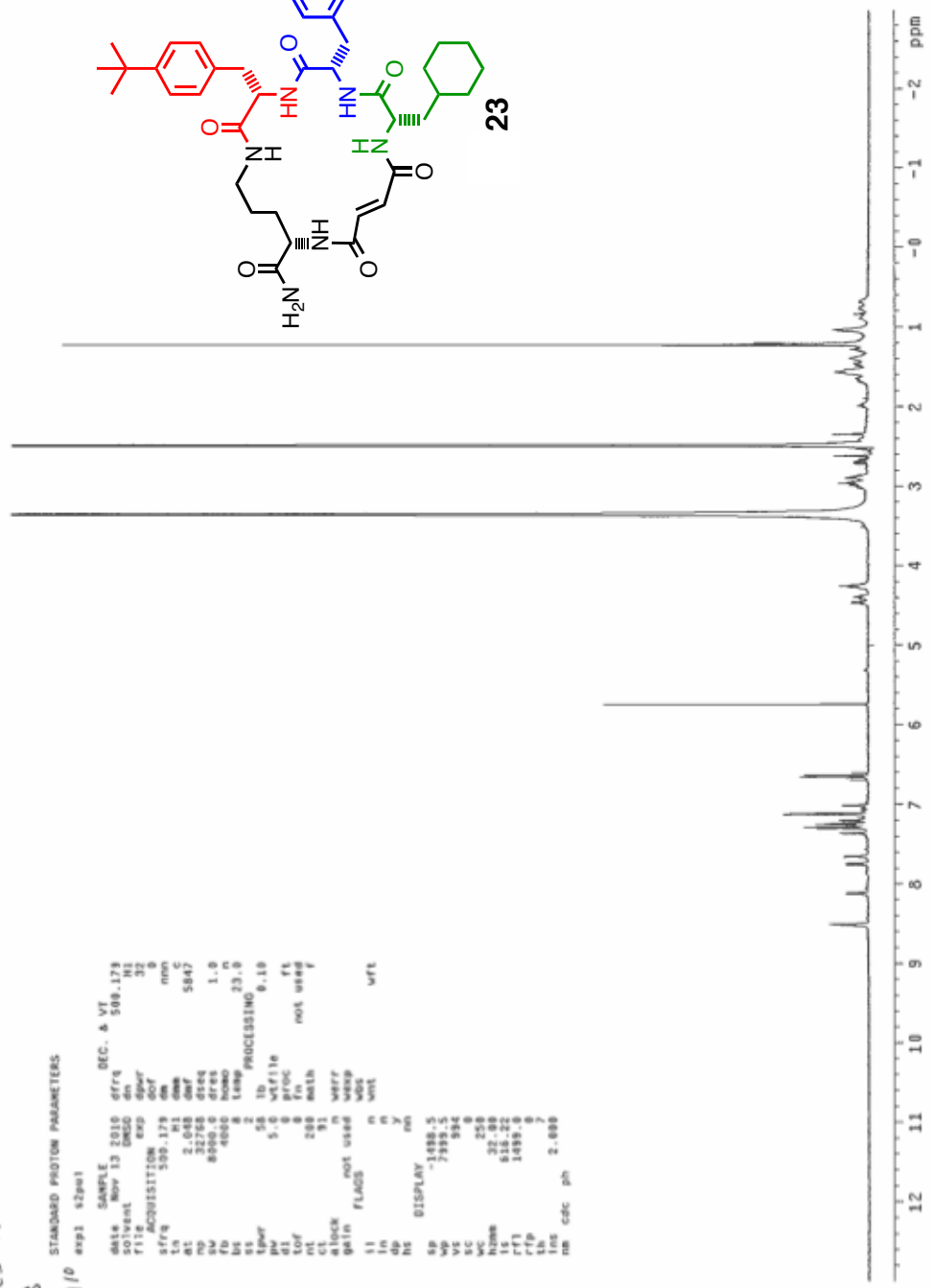
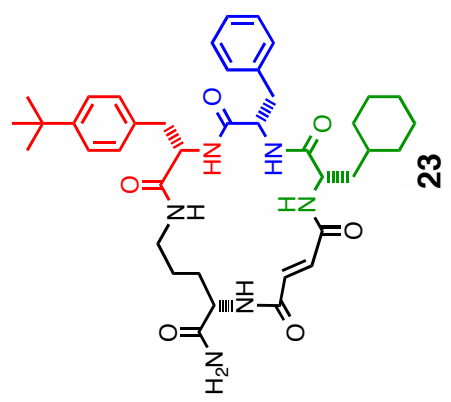


4tBuAhe-2
 11/12/10
 ITC008

STANDARD PHOTON PARAMETERS

```

  exp1 szpet
  date Nov 13 2010 08:58:17 DEC. & VT
  file ITC008
  title ACQUISITION exp szpet
  sfrq 500.179 dm min
  ln 2.01 dm
  md 32768 dft 5847
  sv 8000.0 dft 1.0
  zb 4000 homo 23.0
  us 8 temp PROCESSING 0.10
  spwr 55 lb vstfile
  pr 5.0 vstfile
  di 8 proc fl
  cor 248 fin not used
  cl 91 msh
  atlock n wfff
  gain not used wss
  ll flags n wff
  ln n
  dp n
  hs n
  sp DISPLAY 1488.5
  wp 7385.5
  vs 594
  sc 8
  vc 258
  hnm 31.22
  rff 515.22
  rfp 1488.8
  line 9
  nb cdc ph 2.000
  
```

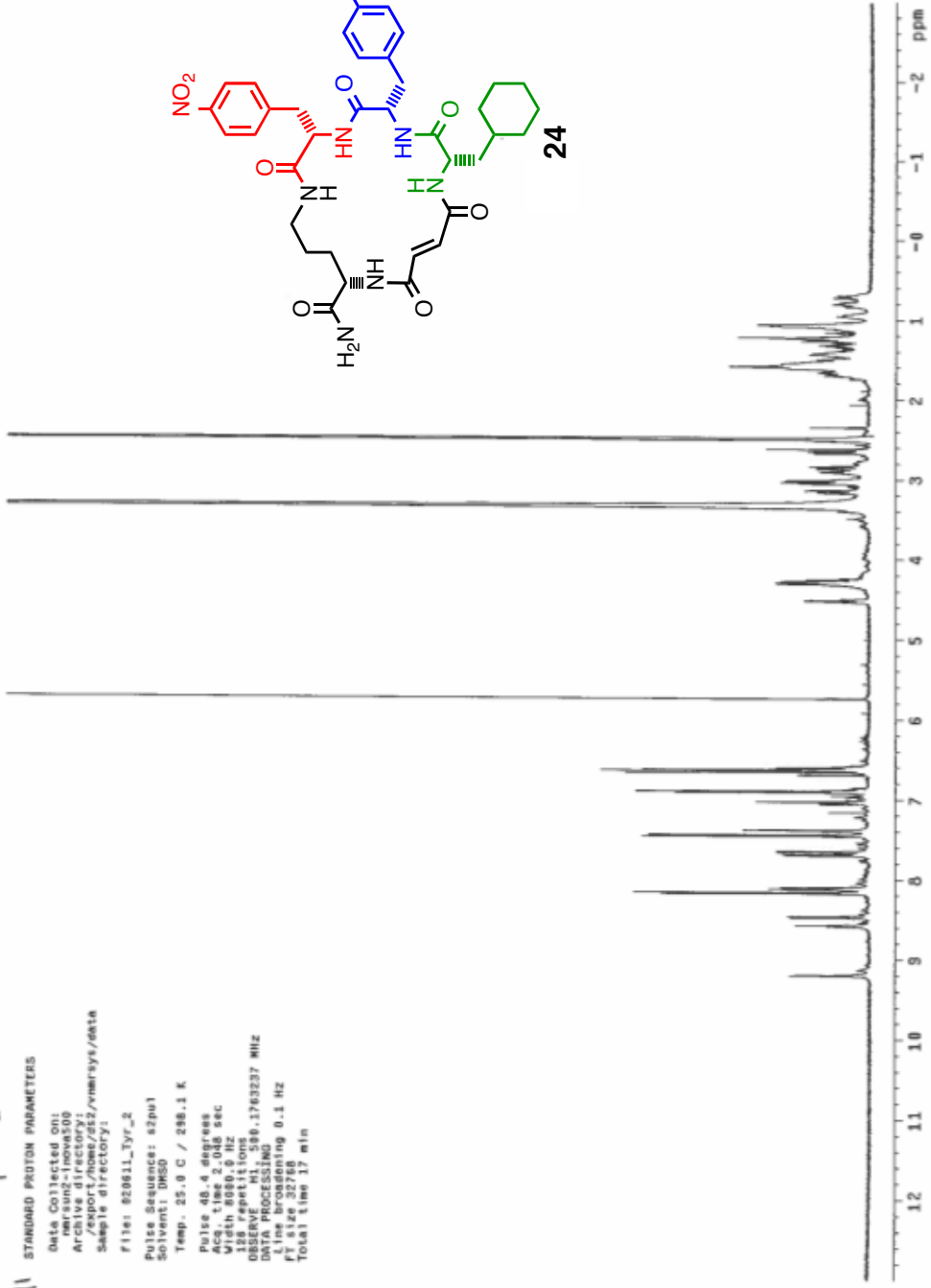
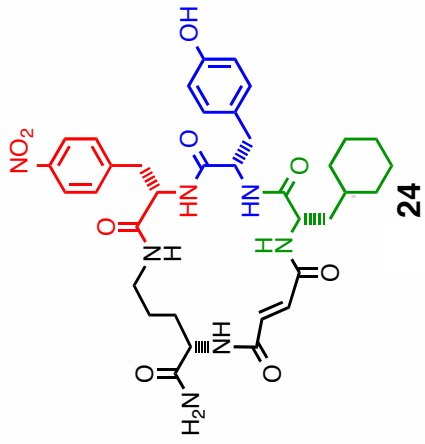


3500B
2/6/11

Tyr-2 trans

STANDARD PROTON PARAMETERS

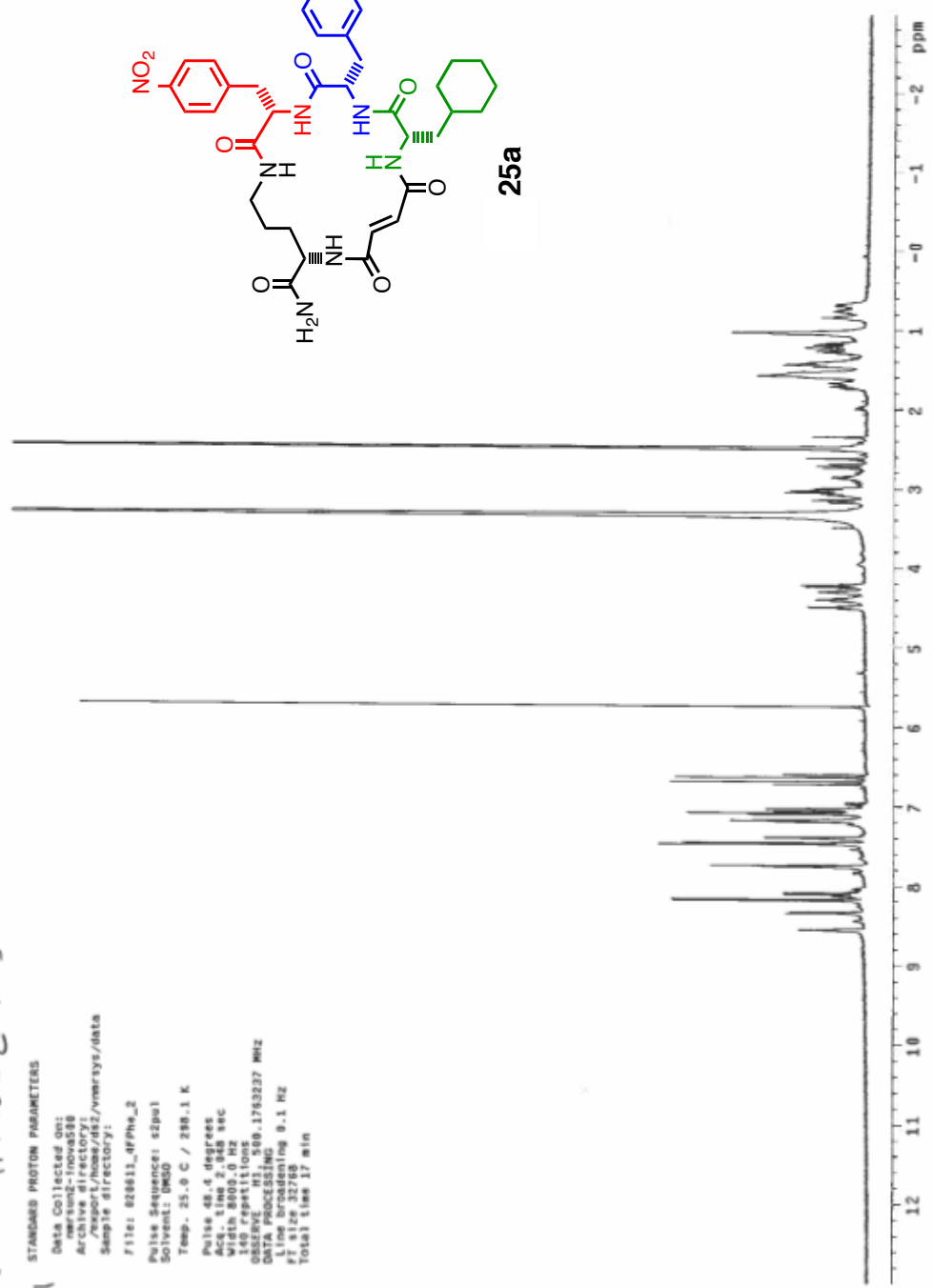
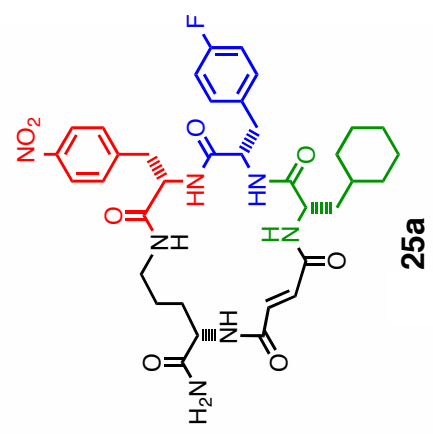
Date Collected on: 11/15/08
Archive directory: /export/home/652/vmmr/sys/desta
Sample directory: /
File: 829811_Tyr_2
Pulse Sequence: s2pu1
Solvent: DMSO
Temp: 25.0 C / 298.1 K
Pulse 48.4 degrees
Acq. time 2.048 sec
Width 8080.0 Hz
Height 1.000000
Observer: ML
Date_Time: 11/15/08 11:05:00
DATA PROCESSING
Line broadening 0.1 Hz
SI size 32768
Total time 17 min



55008 4FPhu-2 trans

2/6/11
STANDARD PROTON PARAMETERS
Data Collected on:
merino2-100a588
Archive directory:
/export/home/ds2/vvartsys/data
Sample directory:

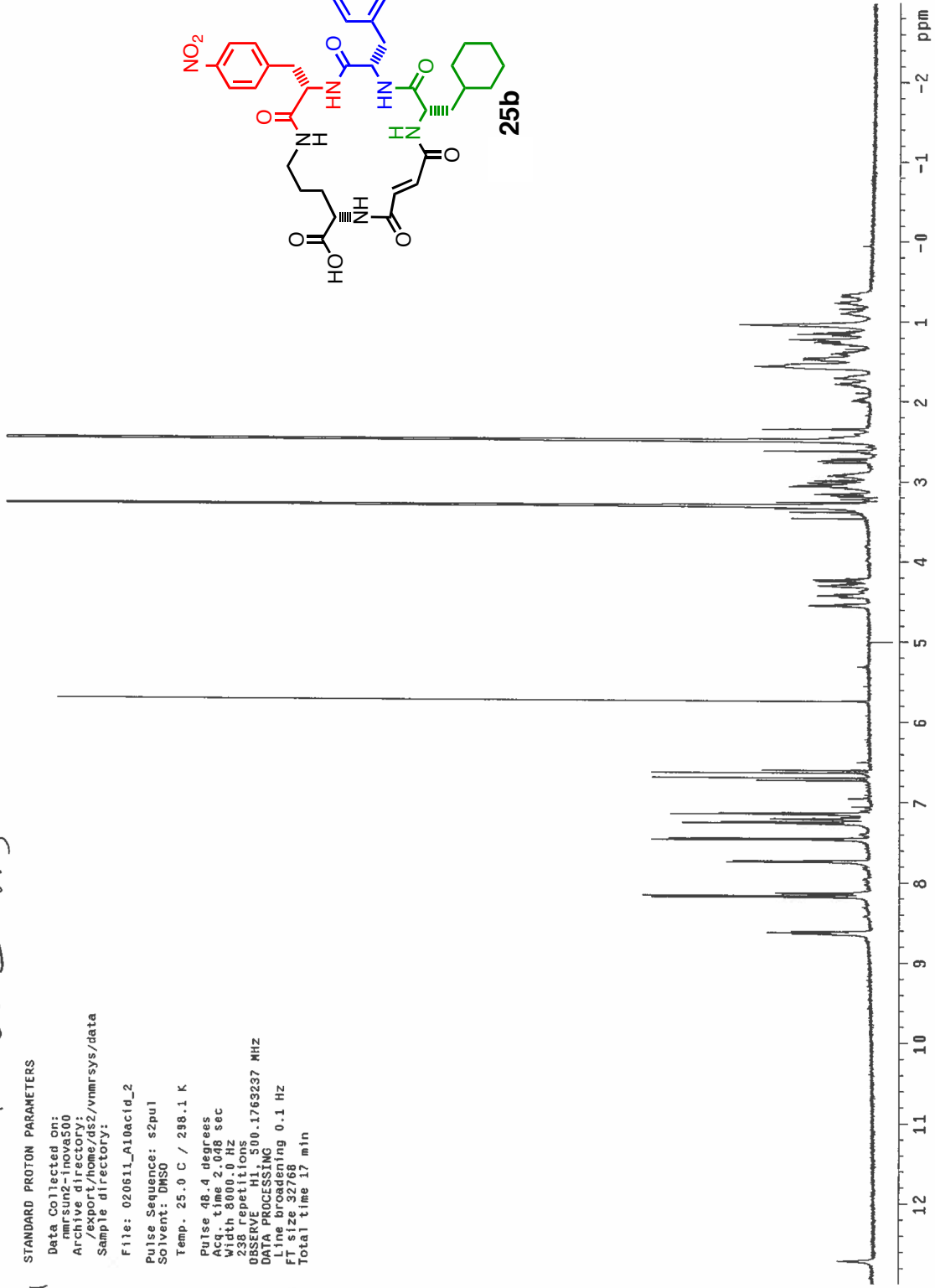
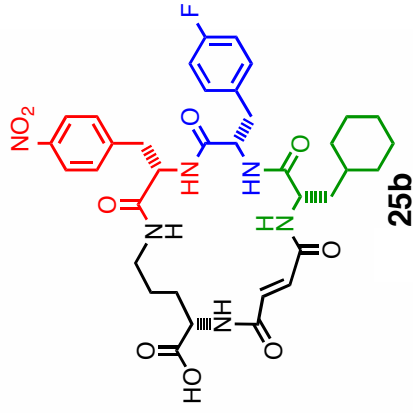
File: 828813_4FPhu_2
Pulse Sequence: s2pu1
Solvent: DMSO
Temp. 25.0 C / 298.1 K
Pulse 48.4 degrees
Acq. time 2.948 sec
Width 8609.0 Hz
149 repetitions
SOLVENT: DMSO
DATA PROCESSING:
Line broadening 0.1 Hz
FT size 32768
Total time 17 min



13C NMR 110acid-2 trans

STANDARD PROTON PARAMETERS

Data Collected on: mmrsum2-inova500
Archive directory: ex4por/home/ds2/vnmrsys/data
Sample directory:
File: 020611_A10acid_2
Pulse Sequence: s2pul
Solvent: DMSO
Temp: 25.0 C / 298.1 K
Pulse 48.4 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
238 repetitions
OBSERVE H1, 500.1763237 MHz
DATA PROCESSING 0.1 Hz
Line broadening 0.1 Hz
SI size 32717
Total time 17 min

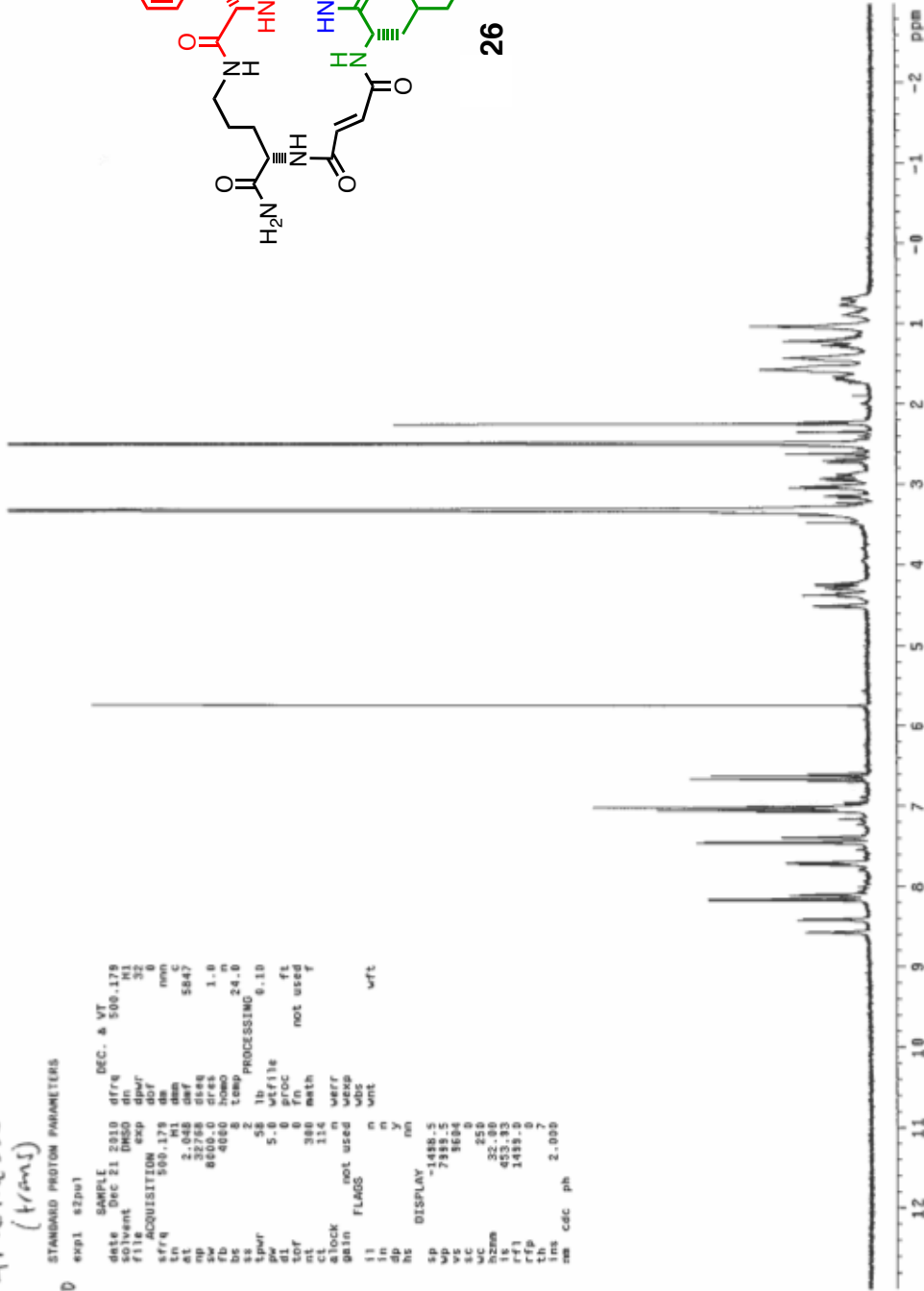
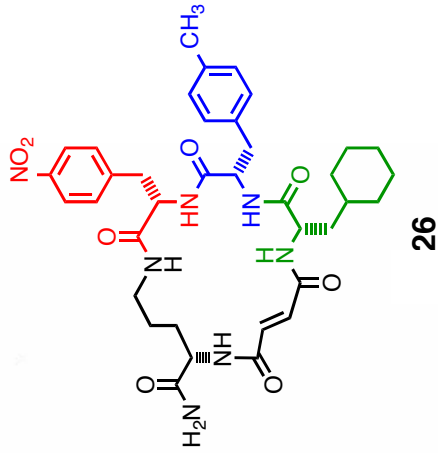


4MePhe-2
(trans)

T00B
12/21/10

STANDARD PROTON PARAMETERS

```
exp1 822u1
SAMPLE DEC. A VT
date Dec 21 2010 dfrq 500.179
solvent DMSO d1
f1file exp dpr 32
acq 0
aquisition exp dpr 0
sfreq 500.179 dmz nonc
in4 2.048 dmz
at 32708 dseq 5847
np 6500 dms 1.0
fb 4000 dms 1.0
bc 8 temp 24.0
ss 2 PROCESSING
spmr 58 lb f1file 6.10
dl 5.0 fproc
tor 0 frn not used
nt 380 math
cl 114 usrf
clock not used n usrf
gain not used wds
flags n vnt wft
l1 n
l2 n
l3 n
l4 n
l5 n
l6 n
l7 n
l8 n
l9 n
l10 n
l11 n
l12 n
DISPLAY -1488-5
sp 7988.5
wp 800.8
sc 250
wc 250
hzom 32.00
fz 453.93
f1 1435.0
rfp 7
th 7
ims cdc ph 2.000
```

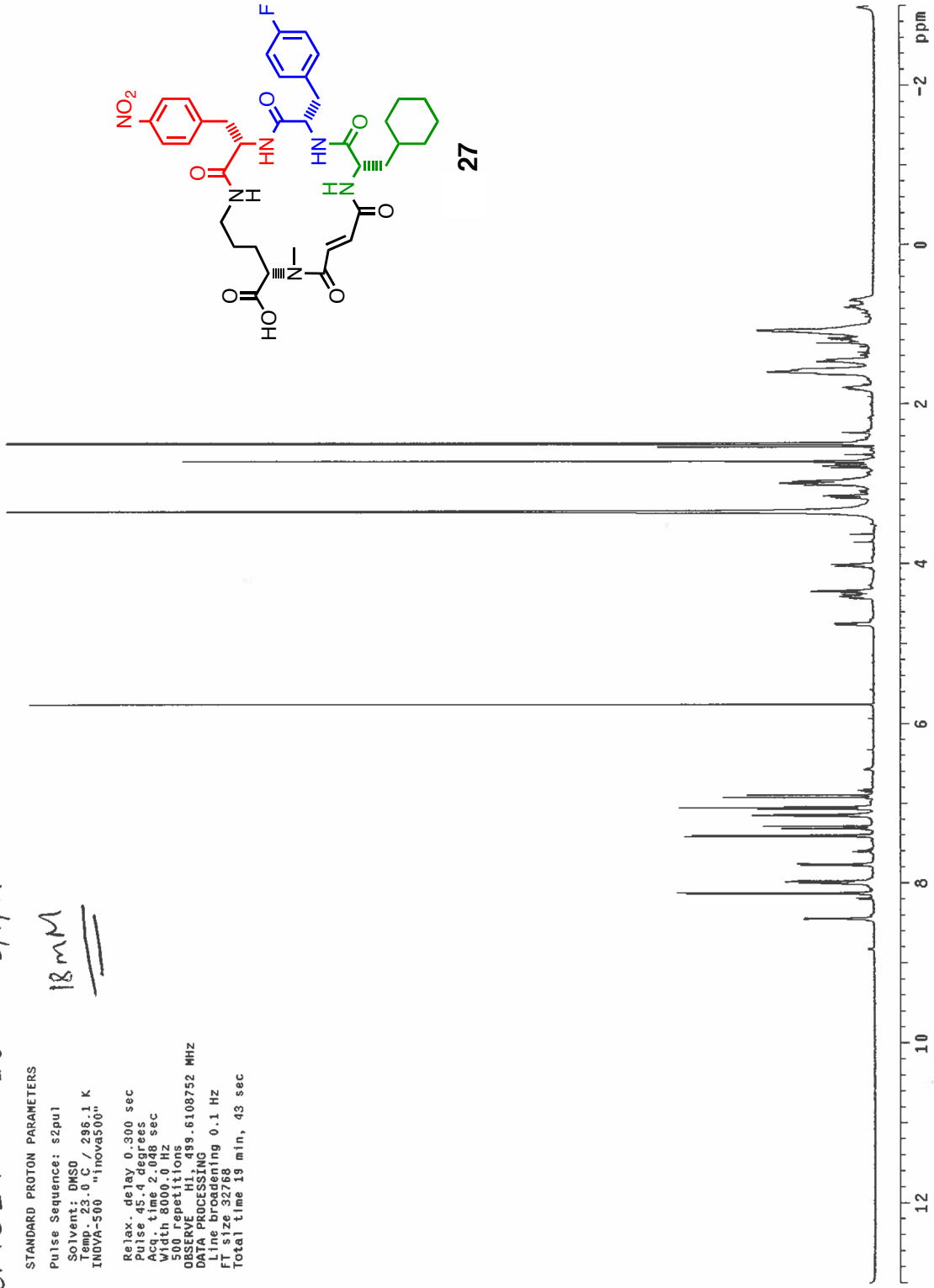
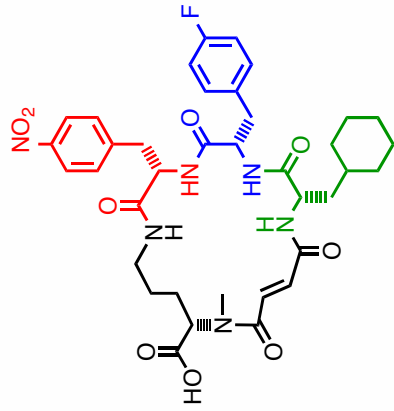


110ME - 1 1500 6/1/11

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pu1
Solvent: DMSO
Temp.: 23.0 C / 296.1 K
INVA-500 "inova500"
Relax. delay 0.300 sec
Pulse 45.4 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
500 repetitions
OBSERVED F1 499.6108752 MHz
LINE PROCESSING
FT size 32768
Total time 19 min, 43 sec

18 mM



10 mM

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul

Solvent: DMSO

Temp: 23.0 C / 296.1 K

File: 060111_A10NHE_2

INOVA-500 ¹Hmfsun¹

Relax. delay 0.300 sec

Pulse 45.4 degrees

Acq. time 2.048 sec

Math 8000.0 Hz

50

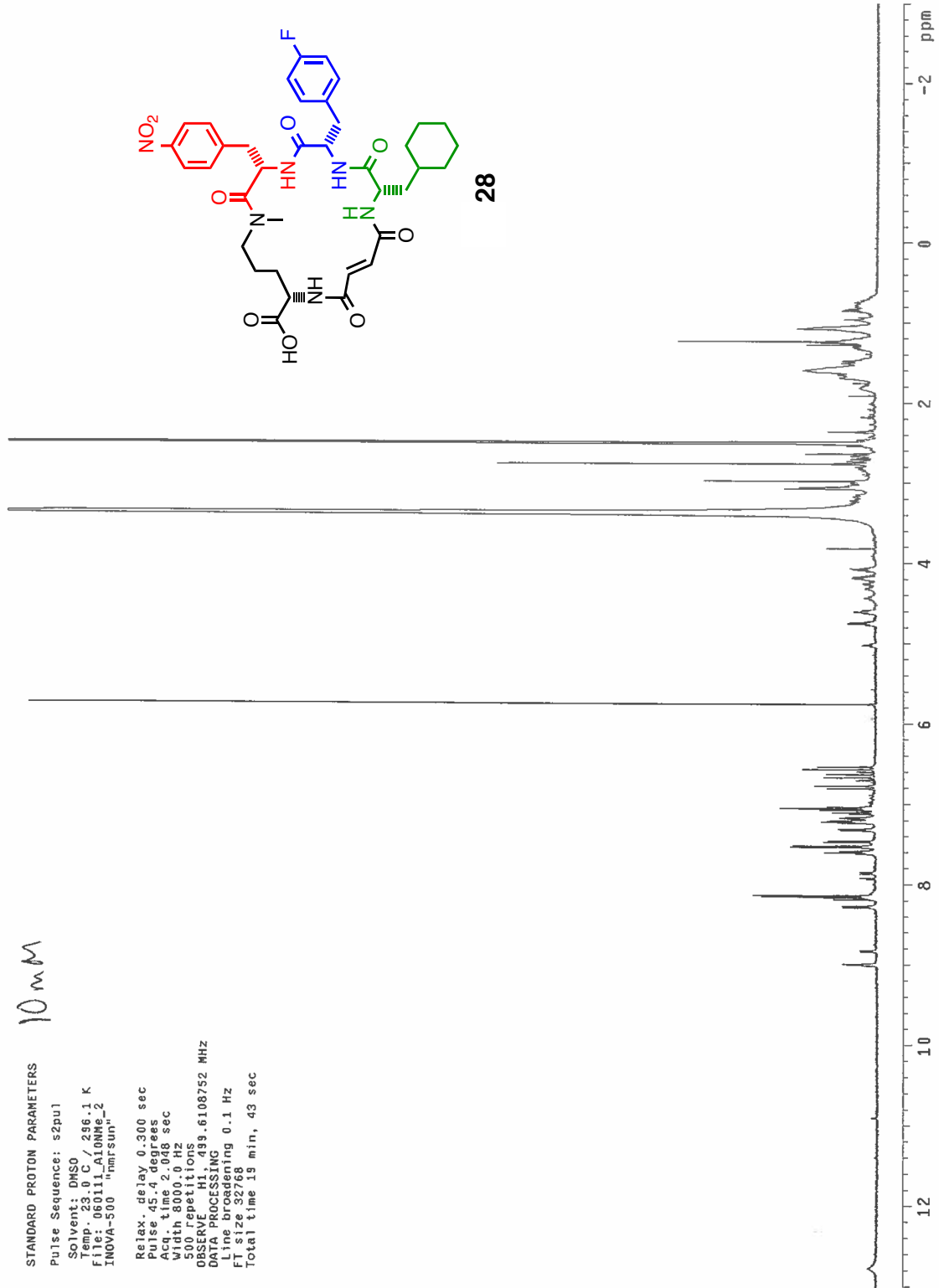
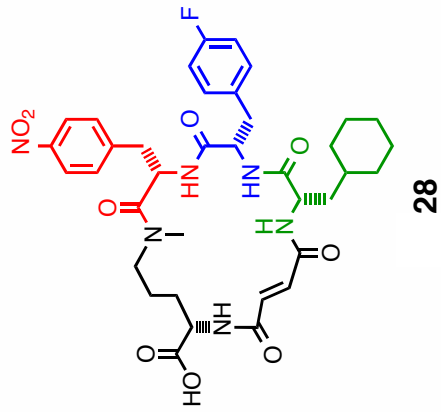
OBSERVE F1 0099.6108752 MHz

DATA PROCESSING

Line broadening 0.1 Hz

FT size 32768

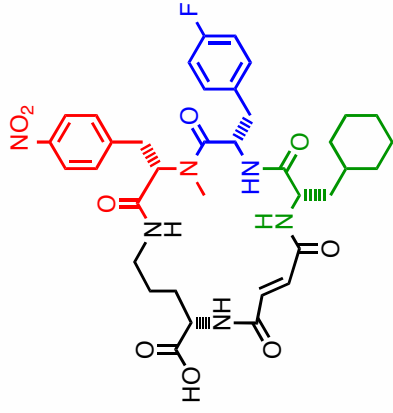
Total time 19 min, 43 sec



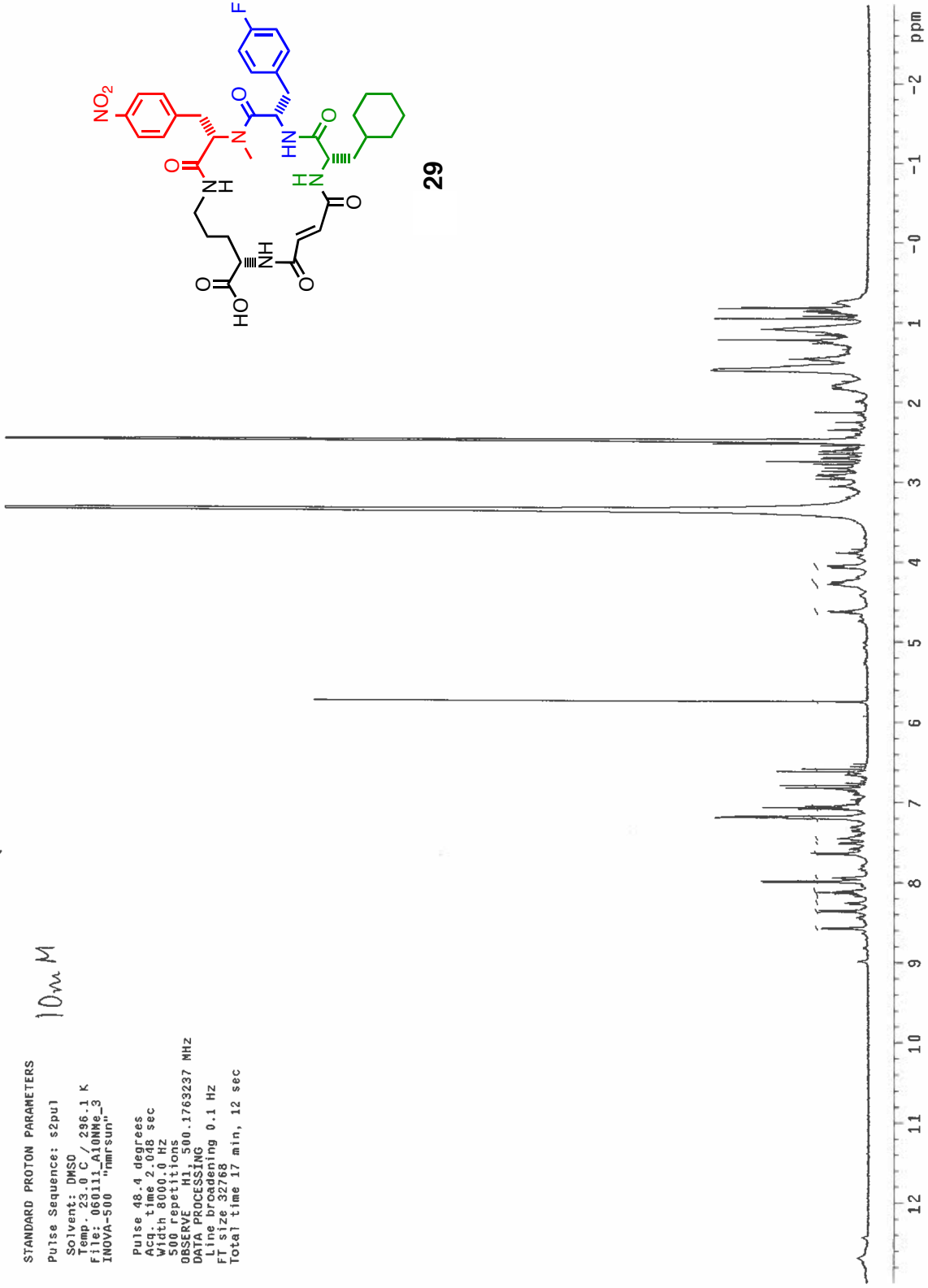
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pu1
 Solvent: DMSO
 Temp: 23.0 C / 296.1 K
 File: 060111_A10NMe_3
 INOVA-500 "hmrSun"
 Pulse 48.4 degrees
 Acq. time 2.048 sec
 Width 8000.0 Hz
 500 repetitions
 OBSERVE H1, 500.1763237 MHz
 DATA PROCESSING
 Line broadening 0.1 Hz
 FT size 32768
 Total time 17 min, 12 sec

10mM



29

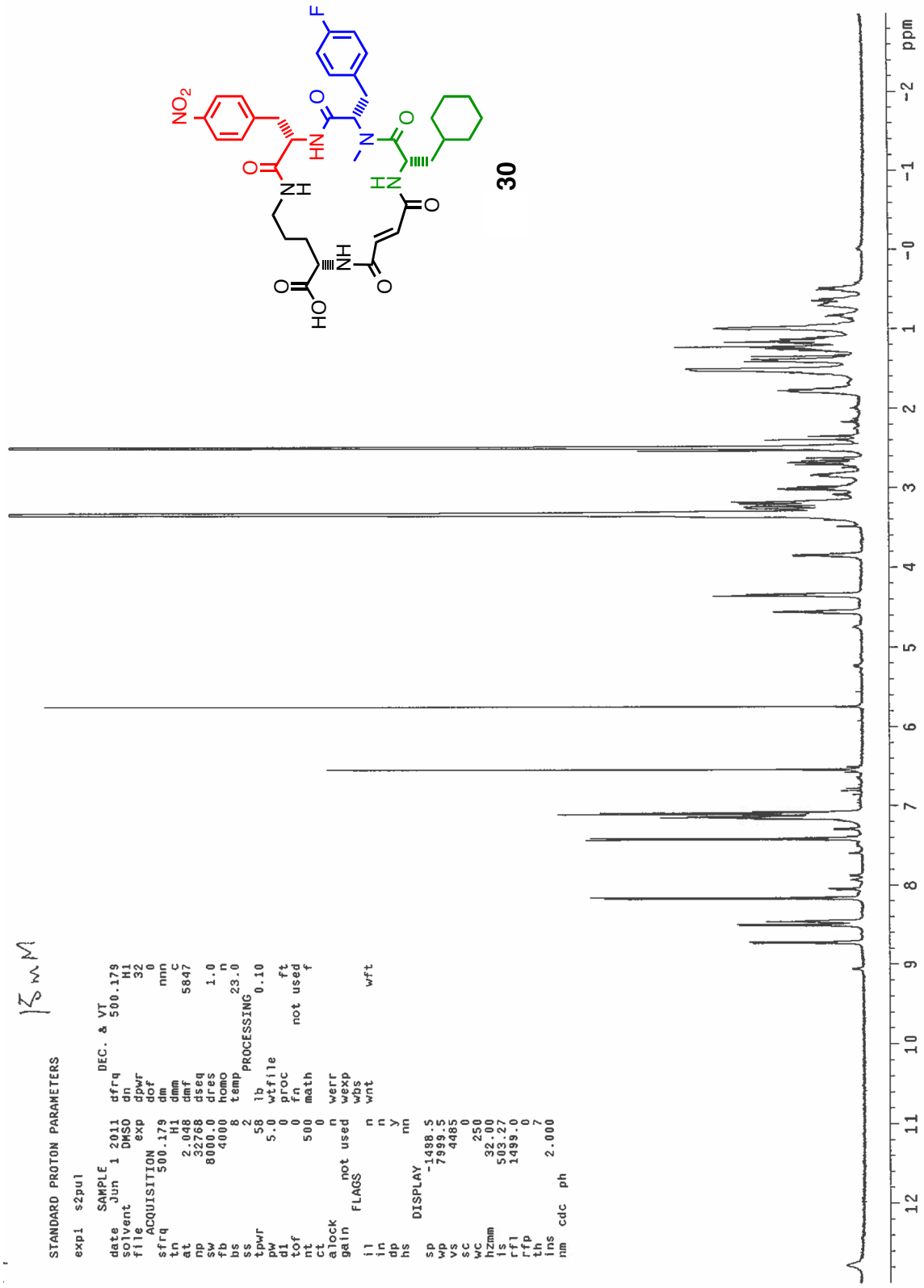
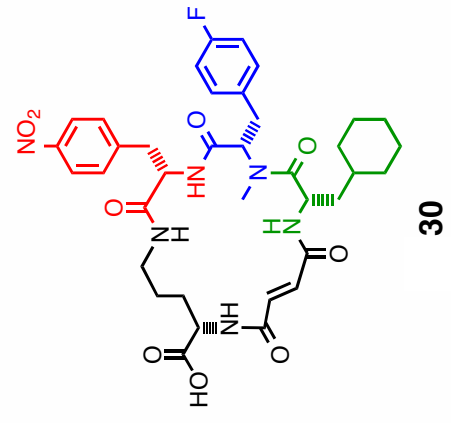


15 mm

STANDARD PROTON PARAMETERS

```

exp1 s2pu1
SAMPLE
date Jun 1 2011 DEC. & VT
solvent DMSO dfrq 500.179
file ACQUISITION exp dn 32
sfrq 500.179 dm dnm nnn
tn 2.048 dmf 5847
mp 32768 dseq
sw 8000.0 dres 1.0
fb 4000 homo n
bs 8 temp 23.0
ss 2 lb PROCESSING 0.10
tpwr 58 wf file
pw 5.0 wffile ft
t1 0 proc ft
t2 0 an not used
ct 500 math
atock n werr
gain not used wexp wft
il n n y wnt
in dp n y wnt
hs nn
DISPLAY
sp -1488.5
wp 7999.5
vs 4485
sc 0
vc 250
l2mm 35.29
rf1 505.29
rfp 1488.0
th 7
ins cdc ph
nm 2.000
  
```

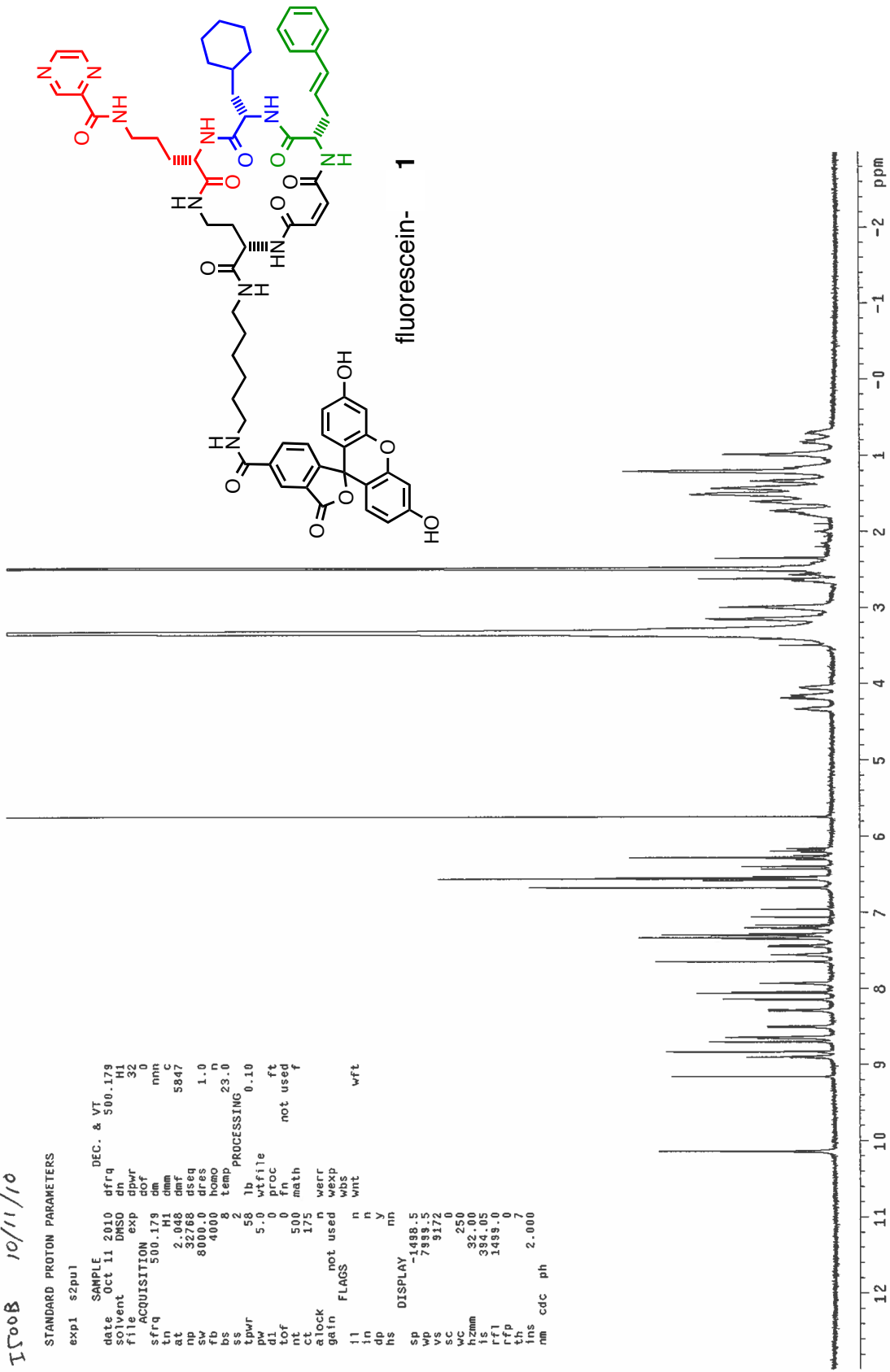


A1188C1001 - 1000
 1500B 10/11/10

STANDARD PROTON PARAMETERS

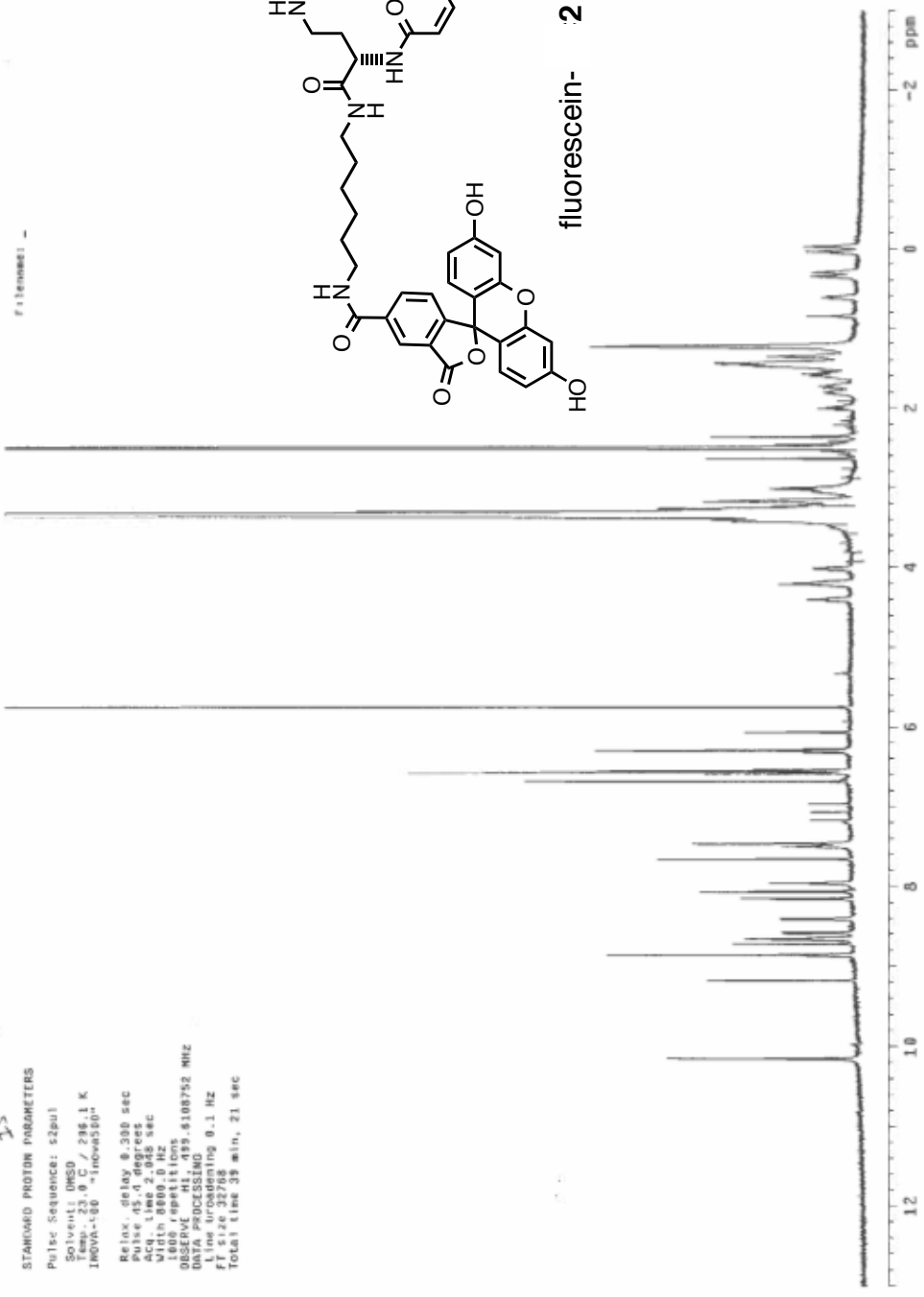
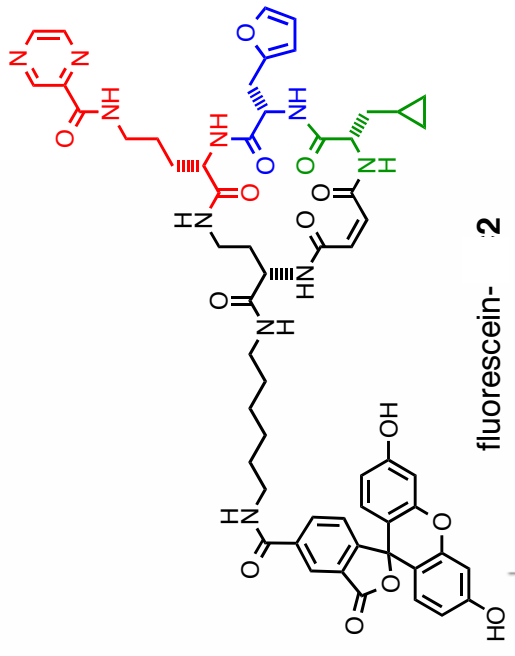
```

exp1 s2pu1
SAMPLE
date Oct 11 2010 dfrq DEC. & VT 500.179
solvent Oct 11 DMSO dn H1 32
file exp dpwr 32
ACQUISITION exp dof 0
sfrq 500.179 dm nmr 5847
tn H1 dm C
at 2.048 dmf 5847
np 32768 dseq
pw 80000 pres 1.0
ts 40000 temp 23.0
ss 2 PROCESSING
tpwr 58 lb 0.10
pw 5.0 wfile ft
d1 0 proc not used
tof 0 fn not used
nt 500 math f
ct 175 n verr
atlock not used nsep
gain not used wss
flgs not used wnt
f1 n n wft
in n y
dp n
hs nn
DISPLAY
sp -1489.5
wp 7999.5
vs 9172
vc 0
bc 250
hzmm 32.00
ls 394.05
rfl 1489.0
rfp 0
th 7
ins 2.000
nm cdc ph
  
```



9/30/10
1500 A1181C507-fluor

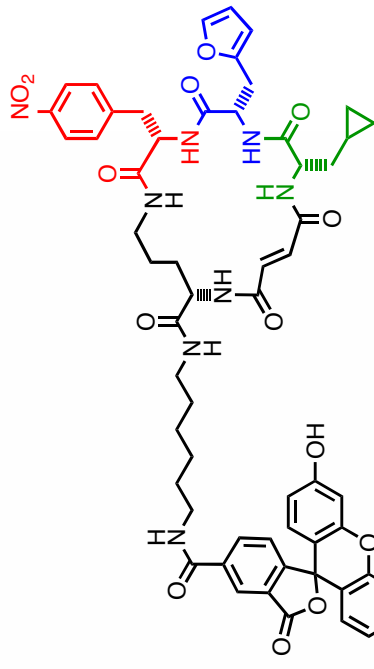
STANDARD PROTON PARAMETERS
Pulse Sequence: zgpg30
Solvent: DMSO
Temp: 30.0 C / 295.1 K
INSTRUM: spect
Relax. delay: 8.300 sec
Pulse: 15.0 degrees
Acq. time: 2.045 sec
Width: 8000.0 Hz
SFO: 500.136 MHz
OBSERVE: 1H 100.6108752 MHz
DATA PROCESSING
Line broadening: 0.1 Hz
SI size: 32768
Total time: 35 min, 21 sec



00B 10/11/10

STANDARD PROTON PARAMETERS

```
exp1 s2pu1
SAMPLE DEC. & VT
date Oct 11 2010 dfrq 500.179
solvent DMSO dn H1
file exp 32
ACQUISITION exp dof 0
sfrq 500.179 dm nnn
in H1 dm C
ac 2.068 dmr 5847
sv 32768 dpc 0
sh 8000.0 dpe 1.0
fb 4000 homo
bs 8 temp 23.0
ss 2 PROCESSING
tpwr 56 lb wfile 0.10
pw 5.0 wproc ft
d1 0 proc
tof 0 fn not used
nt 1000 math
ct 203 n verr
atlock not used n wexp
gain not used wps
il n wnt
in n
dp n
hs nm
DISPLAY
sp -1488.5
wp 7989.5
vs 5586
sc 0
wc 250
hzmhm 32.00
fs 406.01
f1 1499.0
rfp 0
th 7
ins 2.000
nm cdc ph
```



fluorescein- 9

