Electronic Supplementary Information (ESI)

Corroboration of Zn(II)-Mg(II)-tertiary structure interplays essential to optimal catalysis of a phosphorothiolate thiolesterase ribozyme

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Running title: Divalent metal ions essential to phosphorothiolate thiolesterase RNA

catalysis

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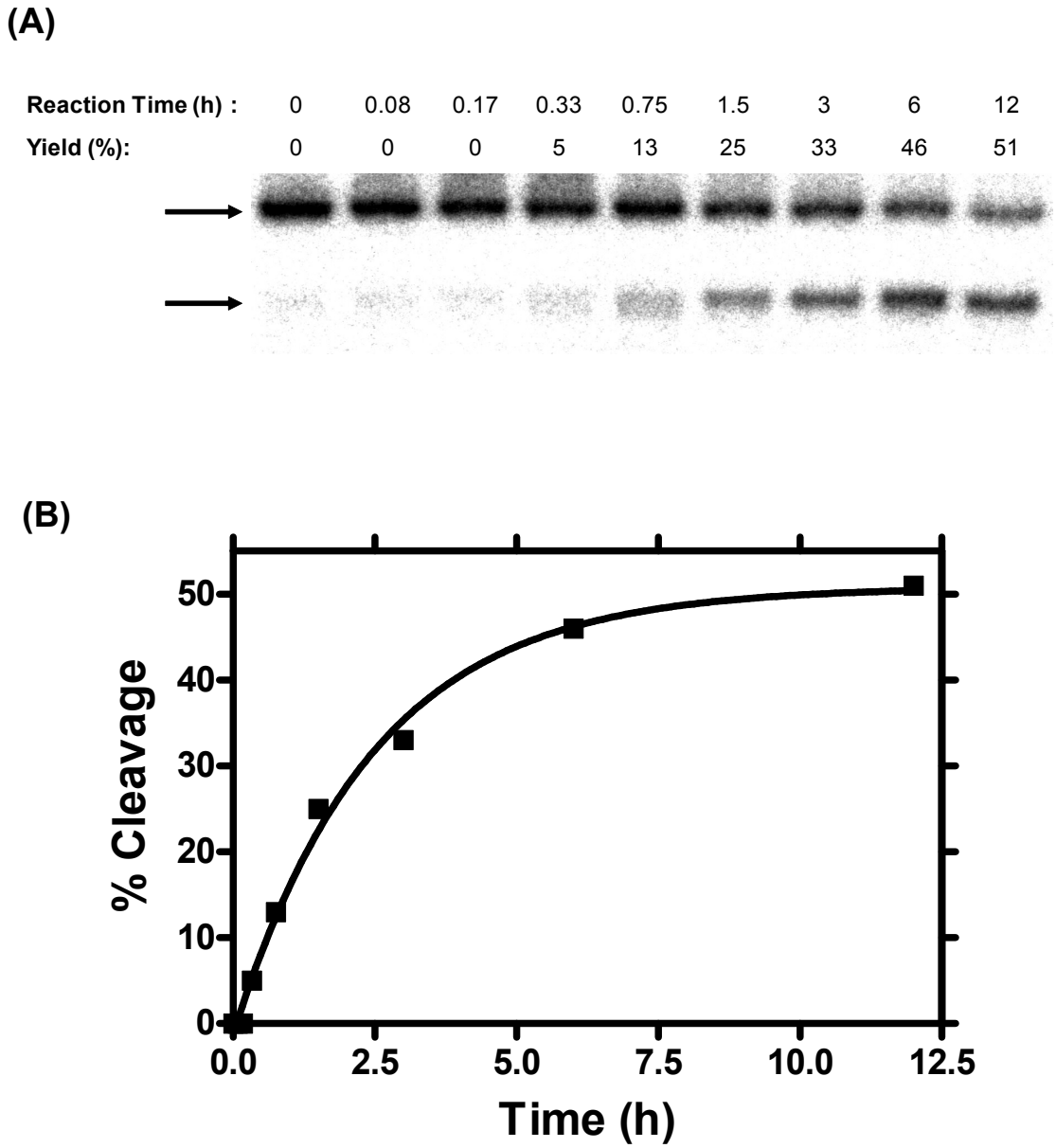


Fig. S1. Kinetics of TW17 ribozyme catalysis in the presence of 0.5 mM Zn^{2+} only. (**A**) Time-course study of catalysis for the ^{32}P-labeled GMPS-primed TW17 ribozyme previously conjugated with **18a**. The reaction products from each time point were separated by SAv gel shift assay in 8% urea-PAGE, and analyzed by an Amersham Typhoon PhosphorImager system. The top arrow indicates the location of the SAv-retarded ^{32}P-labeled TW17 ribozyme-**18a** conjugate; the bottom arrow represents the migration of the ^{32}P-labeled TW17 ribozyme-catalyzed reaction product. (**B**) Determination of the pseudo-first-order rate constant k_{obs} for the TW17 ribozyme catalysis in the presence of 0.5 mM Zn^{2+} only. Data obtained from the ImageQuant software analysis in Figure S1A were fitted into a single-exponential equation for the first-order kinetics, $F(t) = F_0 + F_{max}(1\text{-}e^{-k_{obs}t})$ [$F(t)$, percent cleavage of the reactant at a

specific time point *t*; GraphPad, La Jolla, CA, USA), to obtain the pseudo first-order rate constant k_{obs} = 0.005 min^{-1} for TW17 ribozyme catalysis.

Lane:	1	2	3	4	5	6	7	8
Mg^{2+} (100 mM):	+	+	-	-	-	-	-	-
Zn^{2+} (0.5 mM):	+	+	+	+	-	-	+	+
$[Co(NH_3)_6]^{3+}$ (50 μM)	-	-	-	-	+	+	+	+
Reaction Time (h):	0	3	0	3	0	3	0	3
Yield (%):	0	44	0	33	0	0	0	38

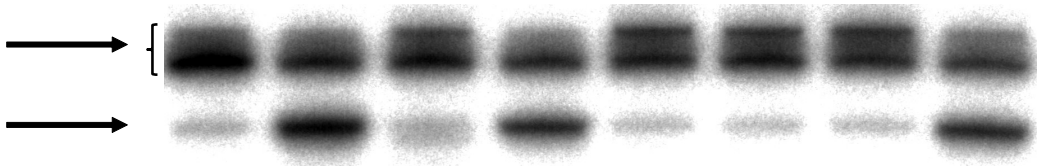


Fig. S2. Outer-sphere and inner-sphere Mg^{2+} all required for optimal TW17 ribozyme catalysis. The ^{32}P-body-labeled GMPS-primed TW17 RNA previously conjugated with **18a** catalyzed each reaction in the presence of specified metal ions. Reaction products were separated by streptavidin (SAv) gel shift assay in 8% urea-PAGE and analyzed by an Amersham Typhoon PhosphorImager system. The top arrow in the figure indicates the location of the SAv-retarded ^{32}P-labeled TW17 ribozyme-**18a** conjugate; the bottom arrow represents the migration of the ^{32}P-labeled TW17 ribozyme-catalyzed reaction product. The presence of doublet signals in the region indicated by the top arrow was the result of either one (lower band) or at least two (upper band) TW17 ribozyme-**18a** conjugates adsorbed onto an SAv molecule during the SAv gel shift assay due to significant excess of the TW17 ribozyme-**18a** conjugates in the samples.

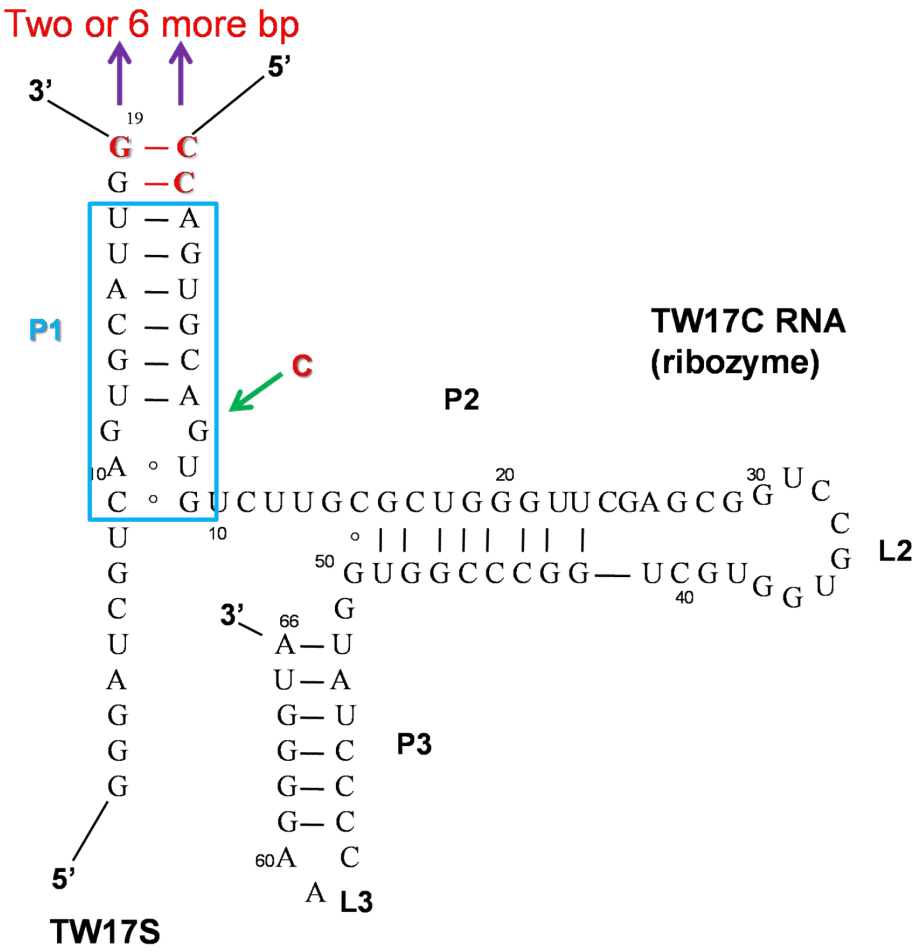


Fig. S3. Constructs of trans-acting TW17 ribozyme systems for studying of base-pairing (bp) effects on degrees of substrate RNA-catalyst RNA adsorption by classical Langmuir isotherm analysis. The black-colored RNA sequences and structures shown here are those for TW17S-1 RNA (named as $S_{1\text{-}18}$ RNA before){Wang, 2012 #527} and TW17C-1 RNA (named as $TW17_{22\text{-}87}$ RNA before){Wang, 2012 #527}. The RNA molecules complex to each other through the base pairs in the P1 helix (marked in the light blue box). Additional base-pairings in the P1 helix were acquired by either site-directed mutagenesis or sequence insertions in the RNA molecules to obtain more trans-acting TW17 ribozyme systems for Langmuir isotherm studies. The newly incorporated nucleotides in the RNA were either highlighted in red or shown as the purple arrows. All RNA molecules were acquired by *in vitro* transcription of the corresponding DNAs synthesized by procedures described on Table S1. Values of K_d (the dissociation constant for the TW17S RNA-TW17C RNA complex at equilibrium) and released binding free energy ($\Delta G_{binding}$) were obtained from Langmuir isotherm analysis as stated in Experimental.

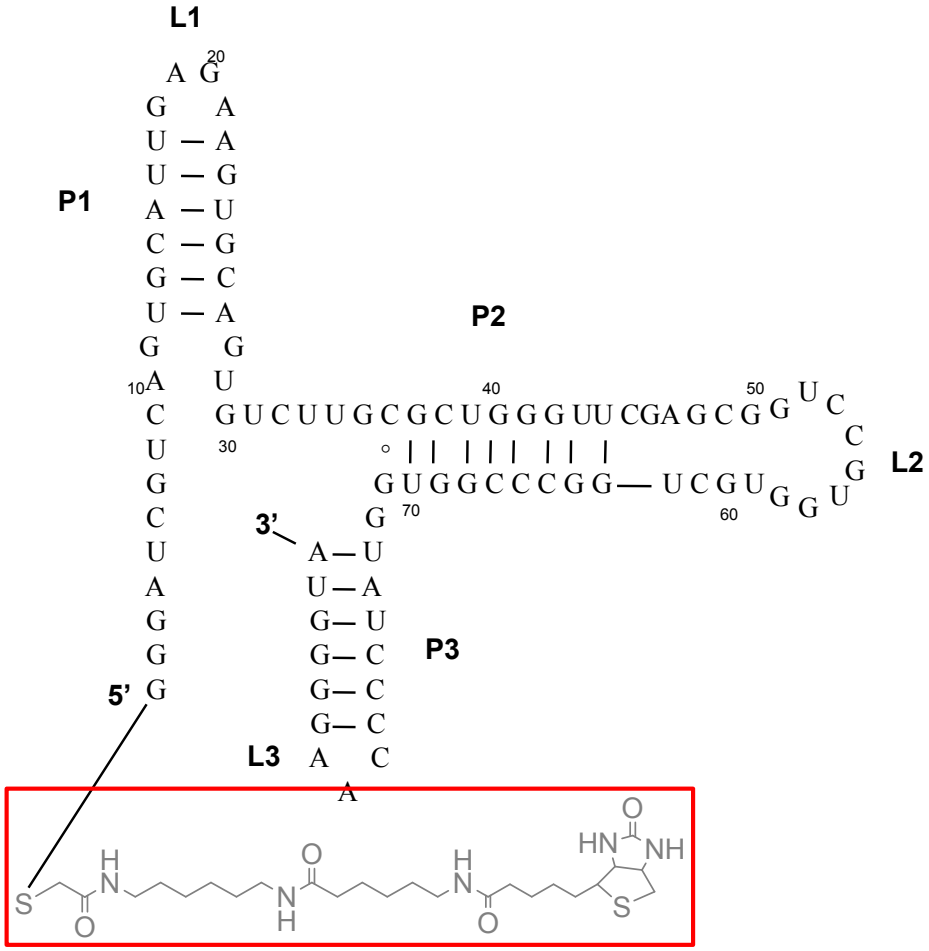
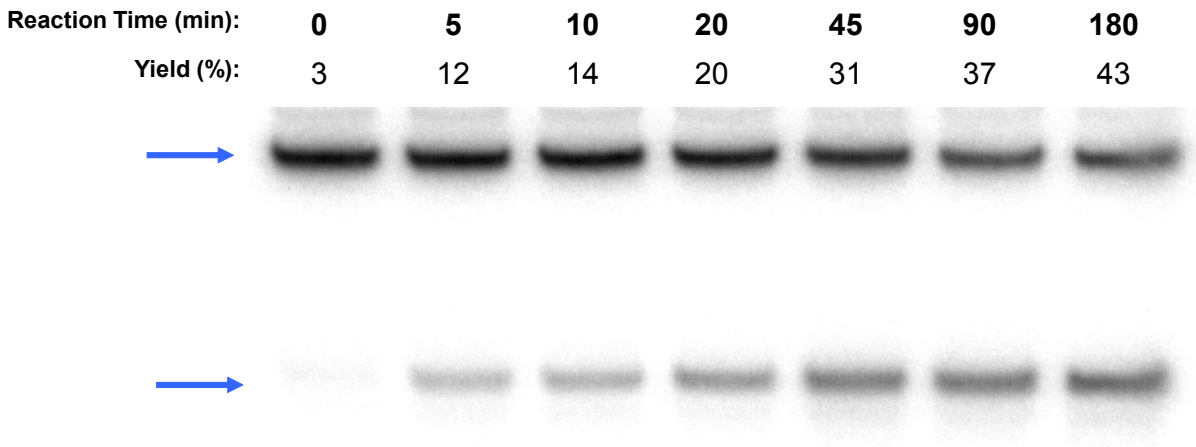


Figure S4. The secondary structure for the cis-acting GMPS-primed TW17 ribozyme when covalently linked to the substrate **18a** (gray color highlighted by a red-color box).

(A)



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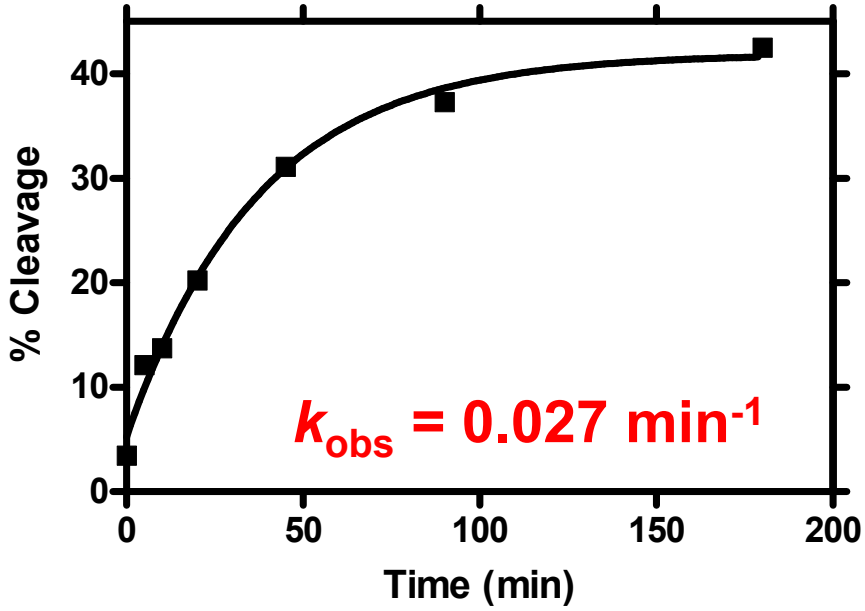
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20
30
40
0
50
100
150
200
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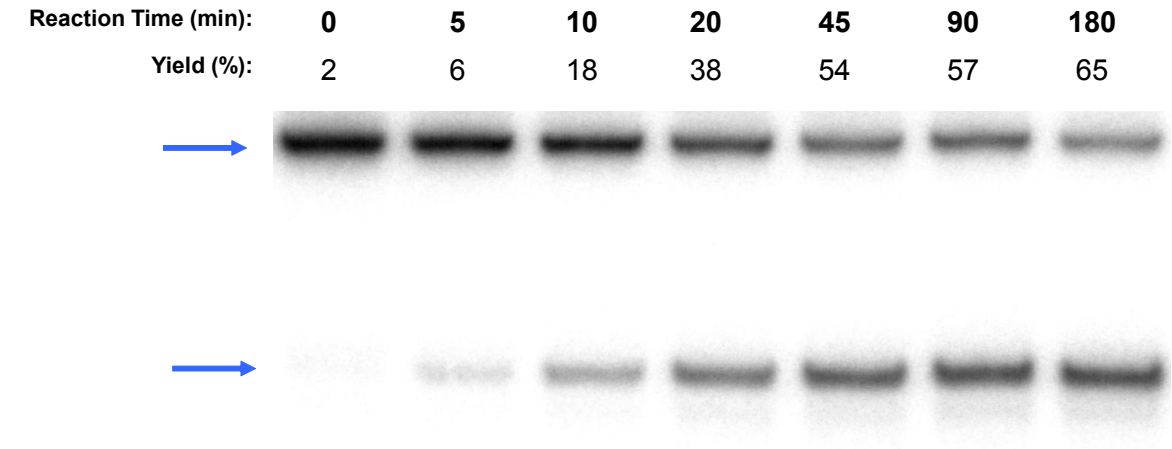
(C)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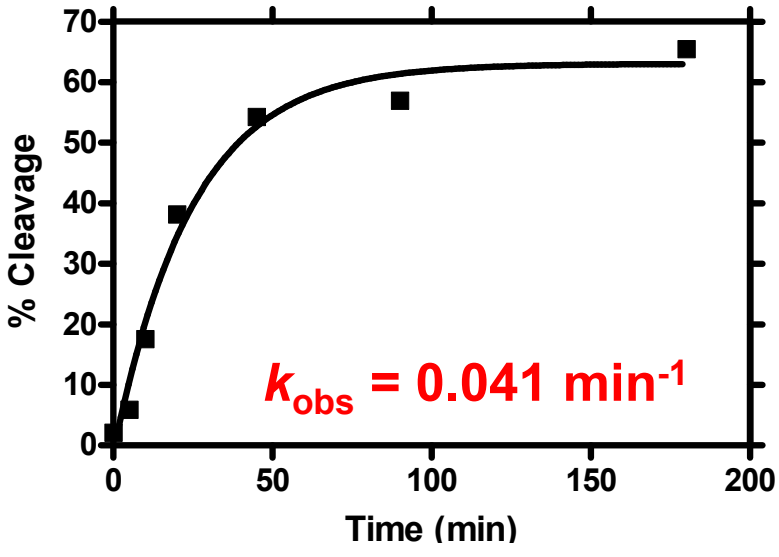
(D)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50
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50
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150
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Time (min)
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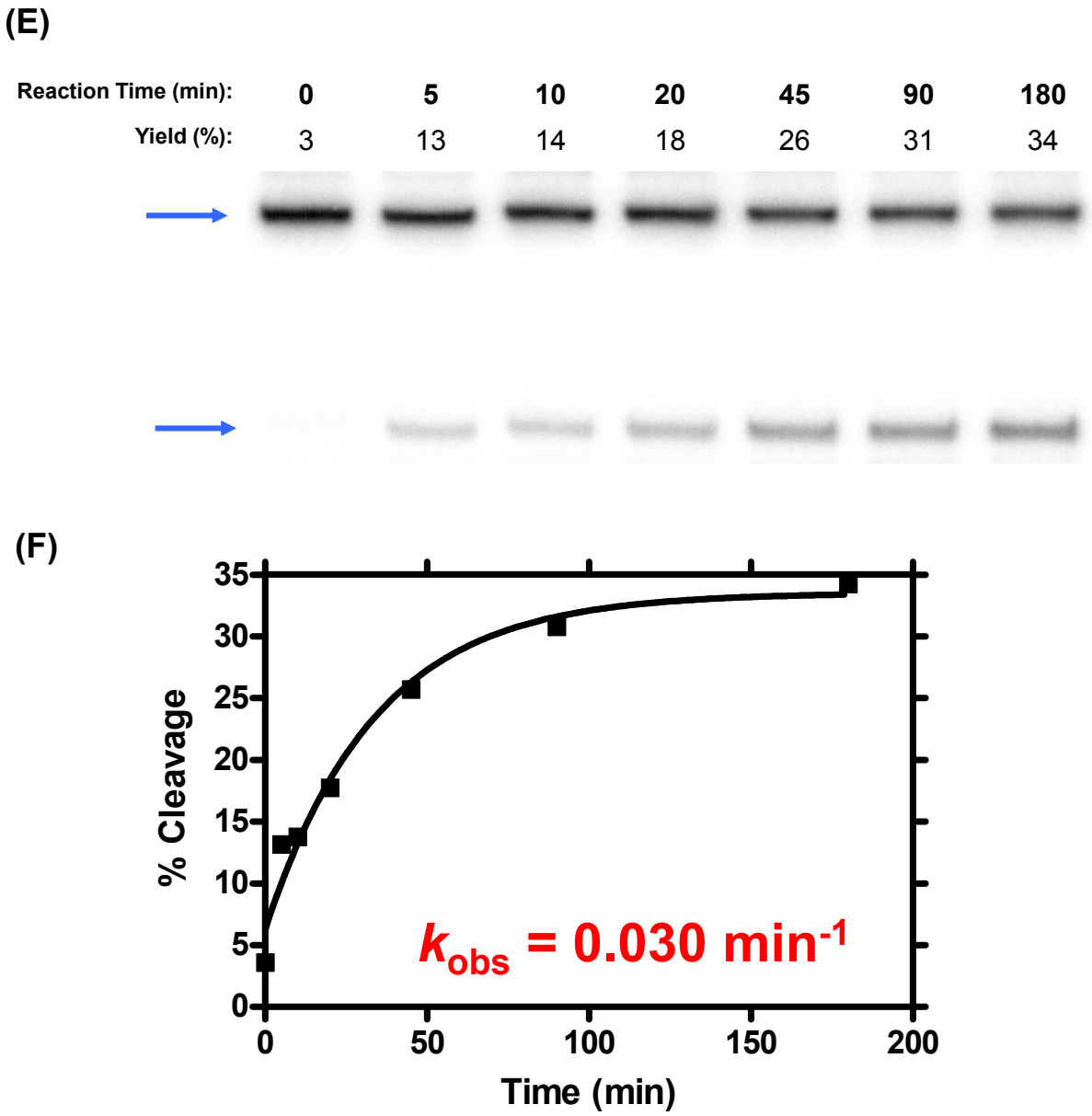
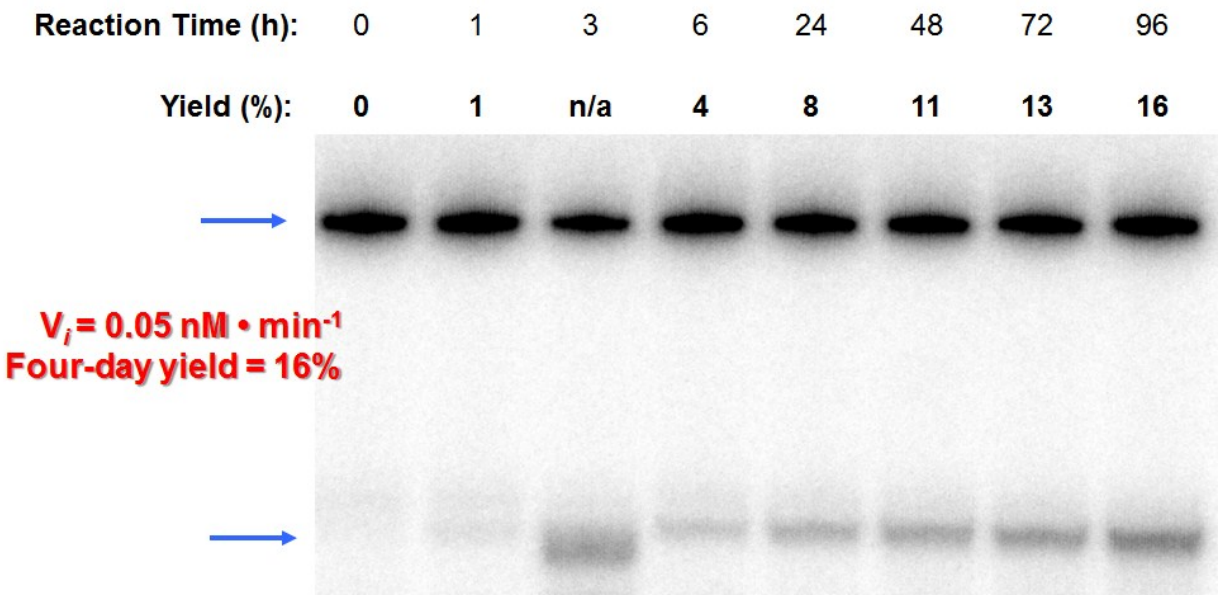


Fig. S5. *Cis*-actingTW17 ribozyme catalysis in the presence of metal concentrations different from those in the standard reaction condition ([Zn^{2+}] = 0.5 mM and [Mg^{2+}] = 100 mM). Streptavidin (SAv) gel-shift assay in 10% urea-PAGE was employed to separate products of TW17 ribozyme catalysis when (A) [Zn^{2+}] was adjusted to 1.25 mM, (C) [Mg^{2+}] was changed to 37.5 mM, or (E) both [Zn^{2+}] and [Mg^{2+}] were adjusted to 1.25 mM and 37.5 mM, respectively. The images of (A), (C) and (E) were acquired from the analyses of an Amersham Typhoon PhosphorImager system. The top blue arrow in each image represents the location of the SAv-retarded ^{32}P-labeled TW17 RNA-**18a** conjugate; the bottom blue arrow indicates the migration of the ^{32}P-labeled TW17 ribozyme-catalyzed reaction product. Each data set obtained from the ImageQuant software analysis in Figures S5A, S5C or S5E was plotted as (B), (D) or (F) respectively, and fitted into a single-exponential equation for the first-order kinetics, $F(t) = F_0 +$

$F_{max}(1-e^{-k_{obs}t})$ [$F(t)$, percent cleavage of the reactant at a specific time point t; GraphPad, La Jolla, CA, USA), to afford the corresponding pseudo first-order rate constant k_{obs} of 0.027 min^{-1}, 0.041 min^{-1} or 0.030 min^{-1} respectively for TW17 ribozyme catalysis.

(A)



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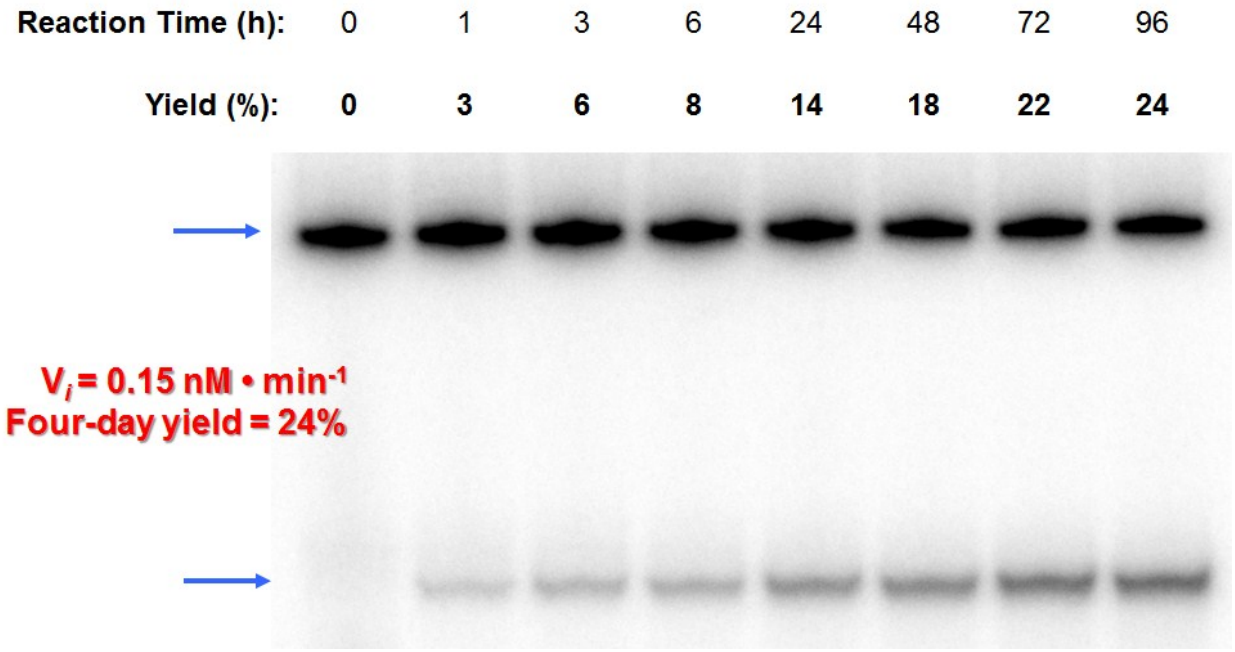


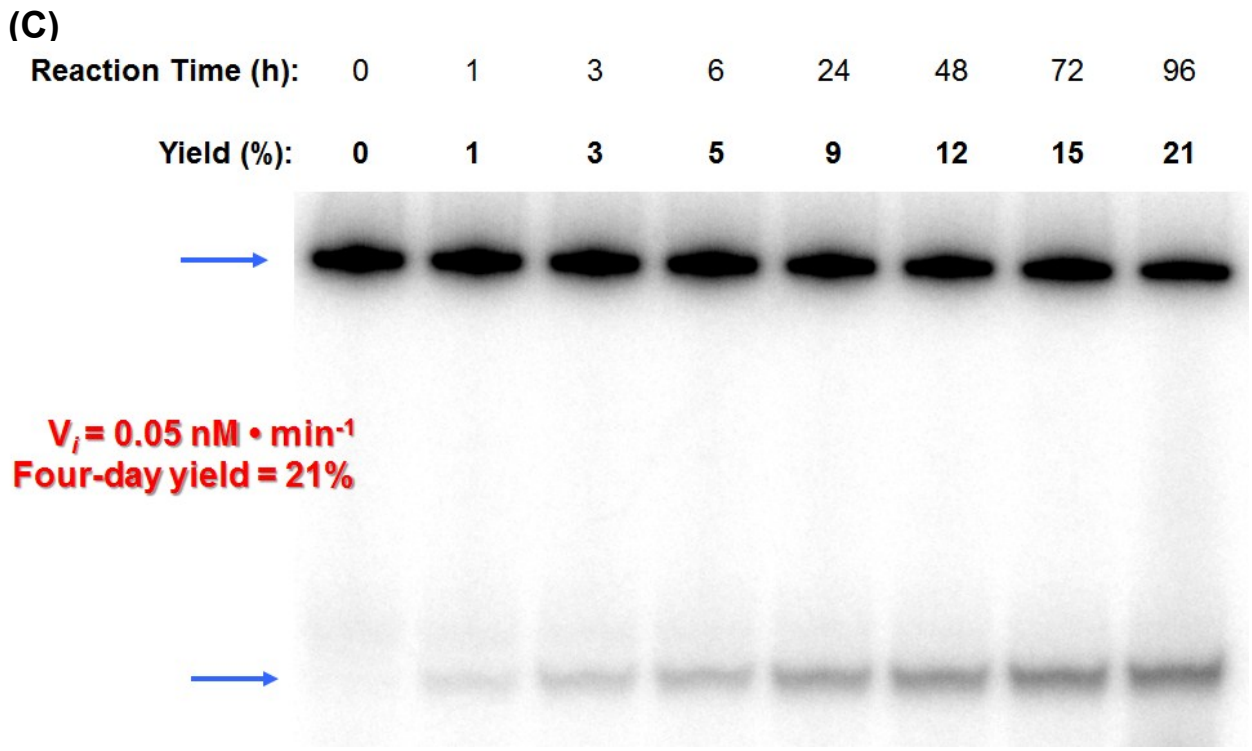


Fig. S6. Multiple substrate turnover of two *trans*-actingTW17 ribozyme systems in the presence of the optimal metal concentrations determined by the results of Figure S5 ($[Zn^{2+}]$ = 0.5 mM and $[Mg^{2+}]$ = 37.5 mM) and of those in the standard reaction condition ($[Zn^{2+}]$ = 0.5 mM and $[Mg^{2+}]$ = 100 mM). SAv gel-shift assay in 20% urea-PAGE was employed to separate catalytic products of two TW17 ribozyme systems: (A) and (C) of the $TW17S_{1\text{-}29}$ RNA-$TW17C_{30\text{-}87}$ RNA pair; (B) of the $TW17S_{1\text{-}29}$ RNA-TW17C-1 pair. Catalysis under multiple substrate turnover was attained by including 300 nM of the ^{32}P-labeled $TW17S_{1\text{-}29}$ RNA and 30 nM of $TW17C_{30\text{-}87}$ RNA/TW17C-1 RNA in the reactions. In addition, the TW17 ribozyme systems in (A) and (B) performed catalysis in the presence of 0.5 mM of Zn^{2+} and 100 mM of Mg^{2+}. On the other hand, TW17 ribozyme catalysis in (C) was carried out in the presence of 0.5 mM of Zn^{2+} and 37.5 mM of Mg^{2+}. All images were acquired from the analyses of an Amersham Typhoon PhosphorImager system and were quantified by the ImageQuant software. The top blue arrow in each image represents the location of the SAv-retarded ^{32}P-labeled $TW17S_{1\text{-}29}$ RNA-**18a** conjugate; the bottom blue arrow indicates the migration of the ^{32}P-labeled TW17 ribozyme-catalyzed reaction product. The initial velocities (v_i) were determined by measuring the slope of the time-course curve from 0 to 1 h in each reaction.

Supplemental Tables

Table S1. Procedures for construction of the *trans*-acting TW17 ribozyme systems.

Biomolecular *trans*-acting TW17 ribozyme systems	**Synthesis of the 5' fragment RNAs**	**Synthesis of the catalytic core RNAs**
TW17S-1 RNA + TW17C-1 RNA	**TW17S-1 RNA**: The DNA was acquired by a PCR reaction using Primer TC-20 as the template, and the shortened Normal 5'-primer and Primer TC-21 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-1 RNA**: The DNA was acquired by an extension reaction between Primer TC-2 and Primer TC-3, then a PCR reaction using the extension reaction product as the template, and Primer TC-2 and the normal 3' -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}
TW17S-2 RNA + TW17C-2 RNA	**TW17S-2 RNA**: The DNA was acquired by a PCR reaction using the modified 5'-primer as the template, and the shortened Normal 5'-primer and Primer TC-4 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-2 RNA**: The DNA was acquired by a 1st extension reaction between Primer TC-6 and Primer TC-11, a 2nd extension reaction using the 1st extension reaction product as the template and Primer TC-11 and the normal 3' -35 primer as the primer pair, then a PCR reaction using the extension reaction product as the template, and Primer TC-11 and the normal 3' -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}
TW17S-3 RNA + TW17C-3 RNA	**TW17S-3 RNA**: The DNA was acquired by an extension reaction between Primer TC-9 and Primer TC-10, then a PCR reaction using the extension reaction product as the template, and the shortened Normal 5'-primer and Primer TC-10 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-3 RNA**: The DNA was acquired by an extension reaction between Primer TC-6 and Primer TC-8, then a PCR reaction using the extension reaction product as the template, and the shortened normal 5'-primer and the normal 3' -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}
TW17S-4 RNA + TW17C-4 RNA	**TW17S-4 RNA**: The DNA was acquired by a PCR reaction using Primer TC-12 as the template, and the shortened Normal 5'-primer and Primer TC-13 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-4 RNA**: The DNA was acquired by an extension reaction between Primer TC-6 and Primer TC-14, then a PCR reaction using the extension reaction product as the template, and the shortened normal 5'-primer and the normal 3' -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}

TW17S-5 RNA + **TW17C-5 RNA**	**TW17S-5 RNA**: The DNA was acquired by a PCR reaction using Primer TC-15 as the template, and the shortened Normal 5'-primer and Primer TC-16 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-5 RNA**: The DNA was acquired by a 1st extension reaction between Primer TC-6 and Primer TC-17, a 2nd extension reaction using the 1st extension reaction product as the template and the shortened normal 5'-primer and the normal 3’ -35 primer as the primer pair, then a PCR reaction using the extension reaction product as the template, and the shortened normal 5'-primer and the normal 3’ -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}
TW17S-6 RNA + **TW17C-5 RNA**	**TW17S-6 RNA**: The DNA was acquired by a PCR reaction using Primer TC-18 as the template, and the shortened Normal 5'-primer and Primer TC-19 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C-5 RNA**: the same as the above.
TW17S-1 RNA + **TW17C-5 RNA**	**TW17S-1 RNA**: the same as the above	**TW17C-5 RNA**: the same as the above.
TW17S-2 RNA + **TW17C-3 RNA**	**TW17S-2 RNA**: the same as the above	**TW17C-3 RNA**: the same as the above.
TW17S$_{1\text{-}29}$ RNA + **TW17C$_{30\text{-}87}$ RNA**	**TW17S$_{1\text{-}29}$ RNA**: The DNA was acquired by an extension reaction between Primer TC-9 and Primer TC-22, then a PCR reaction using the extension reaction product as the template, and the shortened Normal 5'-primer and Primer TC-22 as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}	**TW17C$_{30\text{-}87}$ RNA**: The DNA was acquired by a 1st extension reaction between Primer TC-23 and Primer TC-24, a 2nd extension reaction using the 1st extension reaction product as the template and the shortened normal 5'-primer and the normal 3’ -35 primer as the primer pair, then a PCR reaction using the extension reaction product as the template, and the shortened normal 5'-primer and the normal 3’ -35 primer as the primer pair. The afforded DNA was *in vitro* transcribed by T7 RNA polymerase.{Wang, 2012 #527}
TW17S$_{1\text{-}29}$ RNA + **TW17C-1 RNA**	**TW17S$_{1\text{-}29}$ RNA**: the same as the above	**TW17C-1 RNA**: The synthesis was reported previously.{Wang, 2012 #527}

Table S2. Sequences of the primers for construction of the *trans*-acting TW17 ribozyme systems.

Primer name	**Primer sequence**

Shortened Normal 5’primer	5’-GGTAACACGCATATGTAATACG-3’
Normal 3’-35 primer	5’-ACCCCTTGGGGATACCACCGGGCCAGCACCACGGA-3’
Modified 5’-primer	5’-AACACGCATATGTAATACGACTCACTATAGGGATCGTCAGTGCATTGAG-3’
Primer TC-2	5’-AACACGCATATGTAATAGGACTCACTATAAGTGCAGTGTCTTGCGCTG-3’
Primer TC-3	5’-CACCGGGCCAGCACCACGGACCGCTCGAACCCAGCGCAAGAC-3’
Primer TC-4	5’-TCTCAATGCACTGACGATCC-3’
Primer TC-6	5’-CACCGGGCCAGCACCACGGACCGCTCGAACCCAGCGCAAGACACTGCAC-3’
Primer TC-8	5’-CACGCATATGTAATACGACTCACTATAGGGTGTCAGTGCAGTGTCTTGC-3’
Primer TC-9	5’-GGTAACACGCATATGTAATACGACTCACTATAGGGATCGTC-3’
Primer TC-10	5’-GGGTCTCAATGCACTGACGATCCCTATAGTGAGTCG-3’
Primer TC-11	5’-AACACGCATATGTAATACGACTCACTATAGTCTCAGTGCAGTGTCTTGC-3’
Primer TC-13	5’-GGAATGCACTGACGATCCC-3’
Primer TC-14	5’-AACACGCATATGTAATACGACTCACTATAGGAGTGCAGTGTCTTGCGC-3’
Primer TC-15	5’-AACACGCATATGTAATACGACTCACTATAGGGATCGTCAGTGCACC-3’
Primer TC-16	5’-GGTGCACTGACGATCCC-3’
Primer TC-17	5’-AACACGCATATGTAATACGACTCACTATAGGTGCAGTGTCTTGCGCTGG-3’
Primer TC-18	5’-AACACGCATATGTAATACGACTCACTATAGGGATCGTCAGTGCATC-3’

Primer TC-19	5’-GATGCACTGACGATCCC-3’
Primer TC-20	5’AACACGCATATGTAATACGACTCACTATAGGGATCGTCGTGCATTG-3’
Primer TC-21	5’-CAATGCACTGACGATCCC-3’
Primer TC-22	5’-ACT GCA CTT CTC AAT GCA CTG ACG ATC CCT ATA GTG AGT CG-3’
Primer TC-23	5’-GGT AAC ACG CAT ATG TAA TAC GAC TCA CTA TAGT CTT GCG CTG GG-3’
Primer TC-24	5’-CCG GGC CAG CAC CAC GGA CCG CTC GAA CCC AGC GCA AGA CTA TAG-3’

Table S3. Compositions of RNA solutions for the Langmuir isotherm analyses of *trans*-acting TW17 ribozyme systems. 4 X EK buffer: 400 mM EPPS, 4 M KCl , pH 7.5.

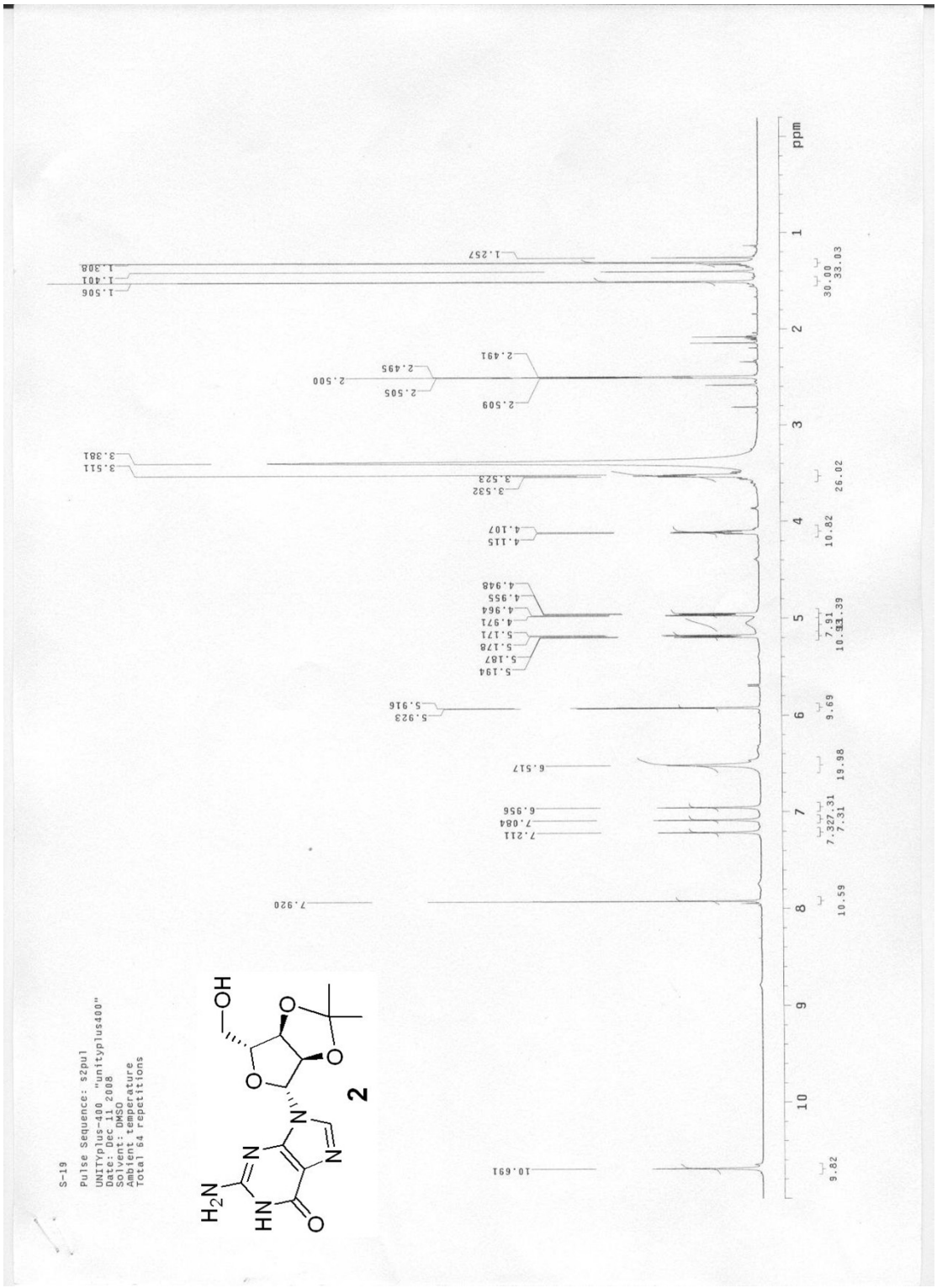
TW17S-X RNA: TW17C-Y RNA ratio	**TW17S-X RNA** solution volume (Stock solution conc.)	**Zn^{2+}** solution volume (Stock solution conc.)	**4X EK buffer** volume	**DEPC water** volume	**TW17C-Y RNA** solution volume (Stock solution conc.)	**Mg^{2+}** solution volume (Stock solution conc.)	**4X EK buffer** volume	**DEPC water** volume
1:0	0.5 µl (1.13 µM)	0.9 µl (0.01 M)	2 µl	0.6 µl	0 µl	0.188 µl (2 M)	2 µl	1.812 µl
1:0.5	0.5 µl (1.13 µM)	0.9 µl (0.01 M)	2 µl	0.6 µl	0.25 µl (1.13 µM)	0.188 µl (2 M)	2 µl	1.562 µl
1:1	0.5 µl (1.13 µM)	0.9 µl (0.01 M)	2 µl	0.6 µl	0.5 µl (1.13 µM)	0.188 µl (2 M)	2 µl	1.312 µl
1:5	0.5 µl (1.13 µM)	0.225 µl (0.2 M)	2 µl	1.47 µl	0.17 µl (16.32 µM)	0.924 µl (2 M)	2 µl	0.906 µl
1:10	0.5 µl (1.13 µM)	0.45 µl (0.2 M)	2 µl	1.45 µl	0.346 µl (16.32 µM)	1.232 µl (3 M)	2 µl	0.41 µl

1:20	0.74 µl (1.13 µM)	0.09 µl (2 M)	3 µl	2.17 µl	0.341 µl (49.16 µM)	1.56 µl (3 M)	3 µl	1.099 µl
1:25	0.74 µl (1.13 µM)	0.113 µl (2 M)	3 µl	2.14 µl	0.427 µl (49.16 µM)	1.56 µl (3 M)	3 µl	1.013 µl
1:40	0.74 µl (1.13 µM)	0.18 µl (2 M)	3 µl	2.08 µl	0.683 µl (49.16 µM)	1.56 µl (3 M)	3 µl	0.757 µl
1:50	0.74 µl (1.13 µM)	0.225 µl (2 M)	3 µl	2.03 µl	0.854 µl (49.16 µM)	1.56 µl (3 M)	3 µl	0.586 µl

Table S4. Determination of dissociation constant K_d (mM) and $\Delta G_{binding}$ (kcal/mol) of *trans*-acting TW17 ribozyme systems from Langmuir isotherm analyses.

Binary Systems	Dissociation Constant (K_d, µM)	$\Delta G_{binding}$ (kcal/mol)	Notes
TW17**S-1** RNA + TW17**C-1** RNA	0.18	-9.19	TW17S-1 RNA:18-mer RNA； TW17C-1 RNA:66-mer RNA
TW17**S-2** RNA + TW17**C-2** RNA	0.81	-8.30	TW17S-2 RNA:21-mer RNA； TW17C-2 RNA:71-mer RNA
TW17**S-3** RNA + TW17**C-3** RNA	1.61	-7.89	TW17S-3 RNA:24-mer RNA； TW17C-3 RNA:73-mer RNA
TW17**S-4** RNA + TW17**C-4** RNA	0.51	-8.57	TW17S-4 RNA: 19-mer RNA, the 3’ terminus ended with two C; TW17C-4 RNA :68-mer RNA, the 5’ terminus ended with two G.
TW17**S-5** RNA + TW17**C-5** RNA	0.19	-9.16	TW17S-5 RNA:17-mer RNA, the 3’ terminus ended with two C; TW17C-5 RNA:66-mer RNA, the 5’ terminus ended with two G.
TW17**S-6** RNA + TW17**C-5**	1.13	-8.10	TW17S-6 RNA: 17-mer RNA, the 3’ terminus ended with U and C.

RNA			
TW17**S-1** RNA + TW17**C-5** RNA	0.15	-9.30	
TW17**S-2** RNA + TW17**C-3** RNA	0.25	-9.00	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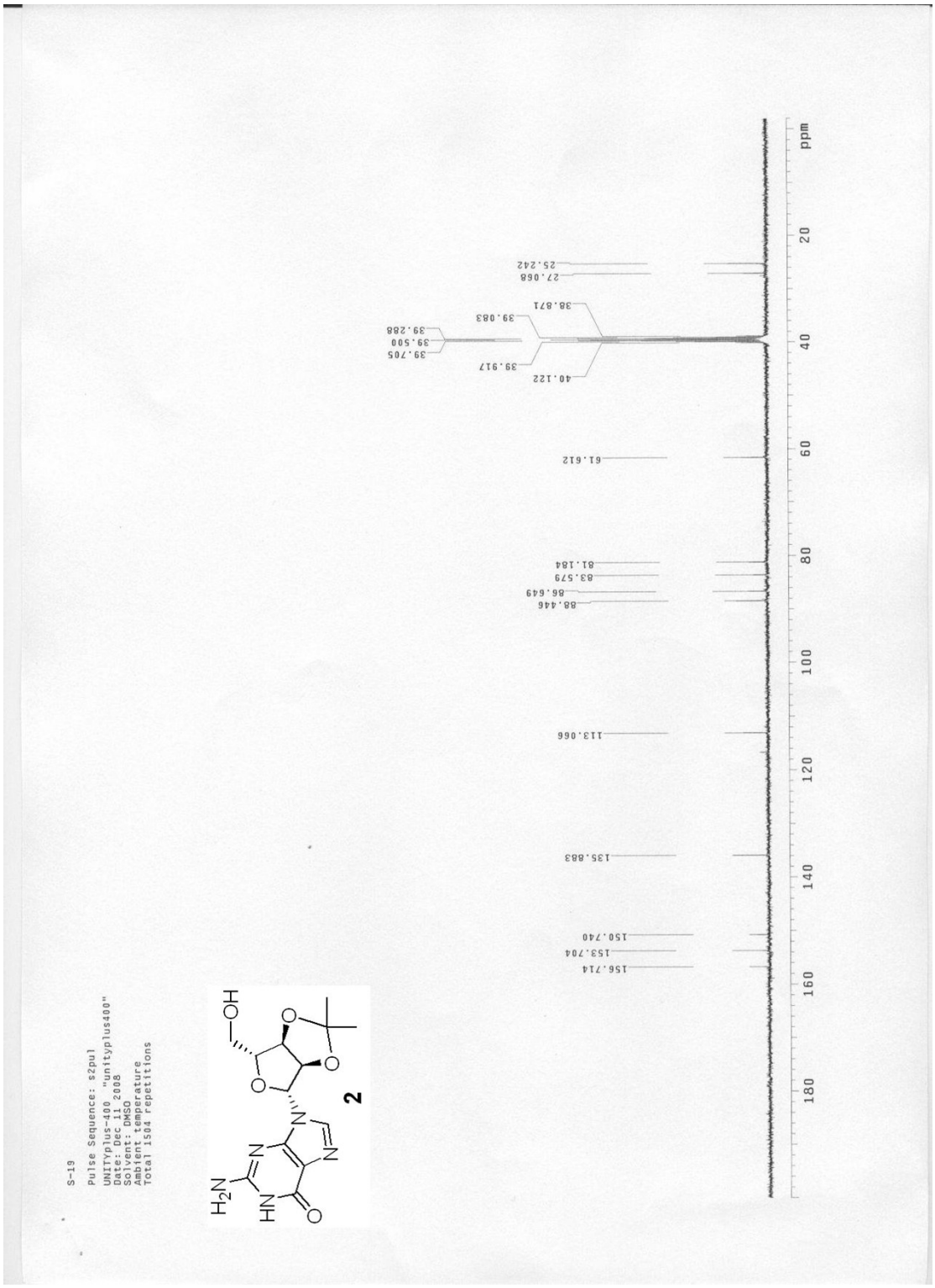
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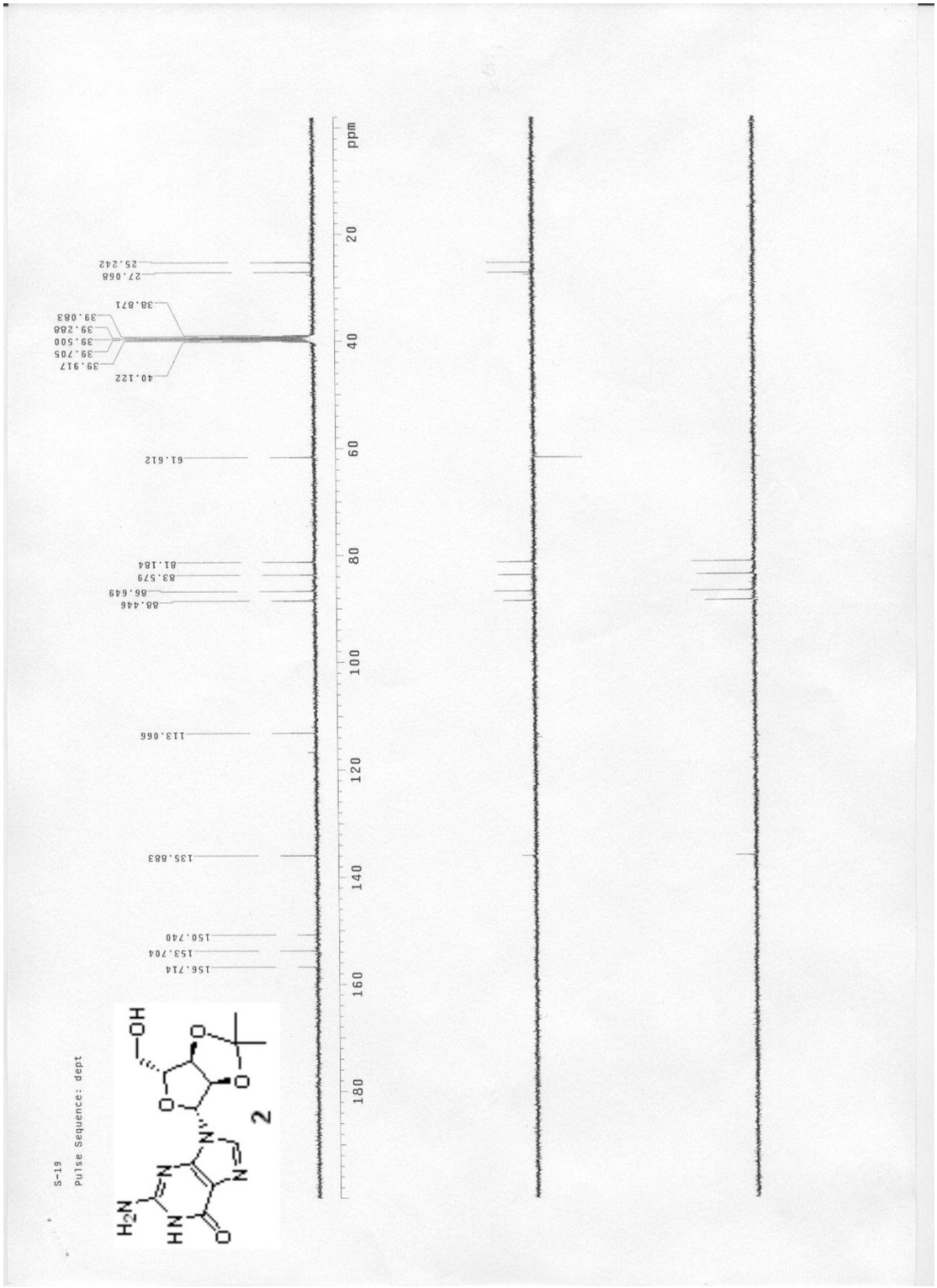
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39.500
39.288
39.083
38.871
27.068
25.242
2

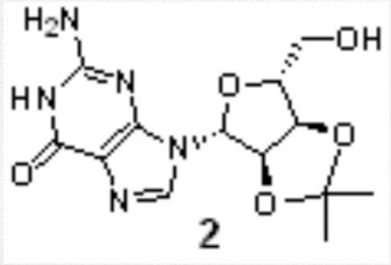
[Theoretical Ion Distribution] Page: 1

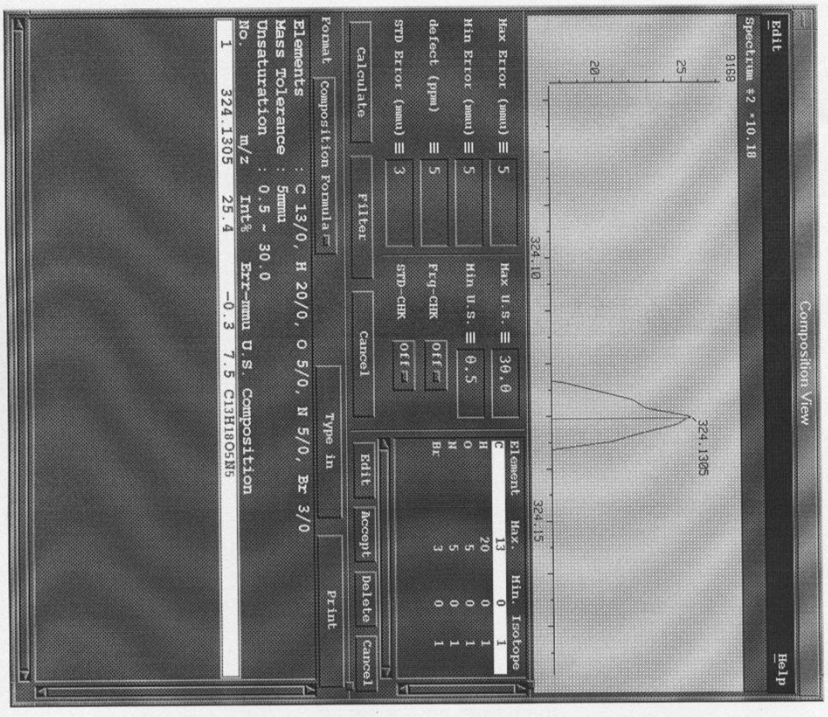
Molecular Formula : C13 H18 O5 N5

(m/z 324.1308, MW 324.3164, U.S. 7.5)

Base Peak : 324.1308, Averaged MW : 324.3172(a), 324.3178(w)

m/z	INT.	
324.1308	100.0000	**********
325.1335	16.7563	**********
326.1357	2.3224	*
327.1380	0.2325	
328.1403	0.0194	
329.1426	0.0014	





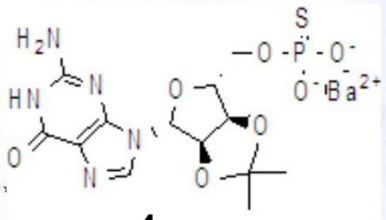
[Theoretical Ion Distribution] Page: 1

Molecular Formula : C13 H17 O7 N5 P S Ba

(m/z 555.9639, MW 555.6770, U.S. 10.5)

Base Peak : 555.9639, Averaged MW : 555.6729(a), 555.6757(w)

m/z	INT.	
547.9649	0.1426	
548.9675	0.0251	
549.9637	0.1463	
550.9662	0.0254	
551.9631	3.2621	**
552.9644	9.4432	*****
553.9636	12.3662	*******
554.9646	17.6518	**********
555.9639	100.0000	**********
556.9663	18.2164	**********
557.9632	7.2208	****
558.9650	1.0587	*
559.9654	0.1709	
560.9671	0.0191	
561.9684	0.0020	



4

[Elemental Composition] Page: 1

Data : EIHR333 Date : 09-Mar-2009 15:55

Sample: S-20

Note : NBA+glycerol

Inlet : Direct Ion Mode : FAB+

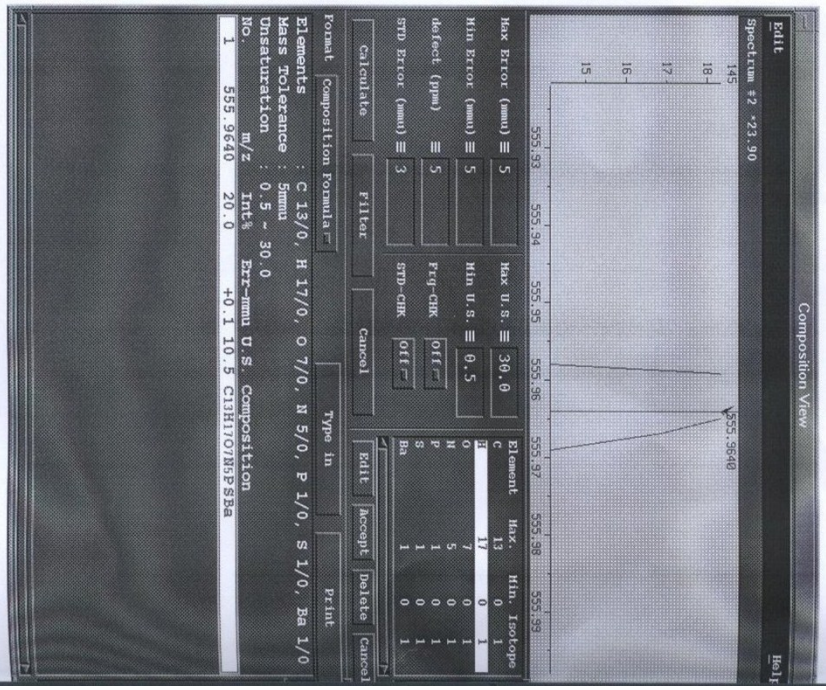
RT : 0.67 min Scan#: 5

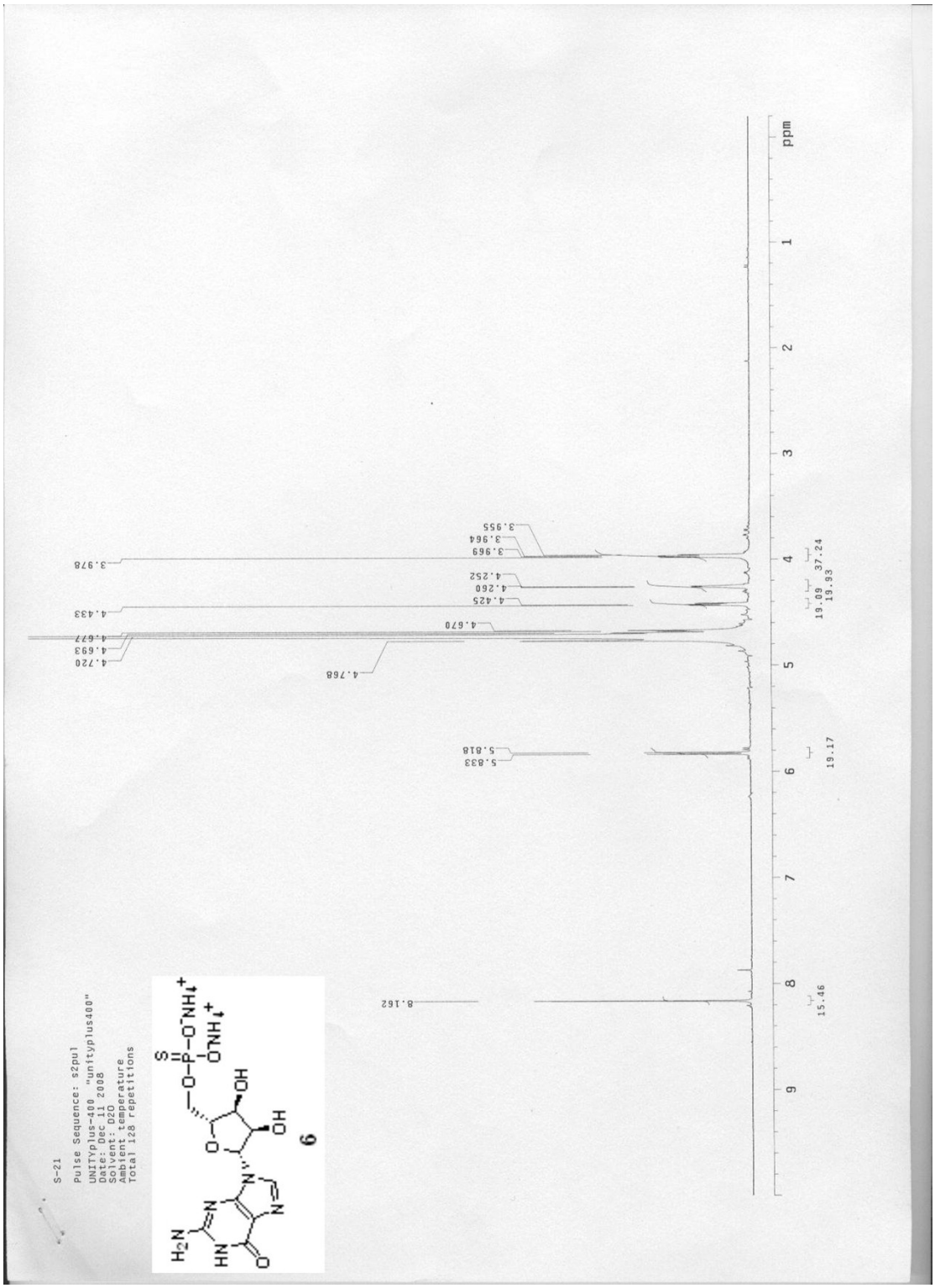
Elements : C 13/0, H 17/0, O 7/0, N 5/0, P 1/0, S 1/0, Ba 1/0

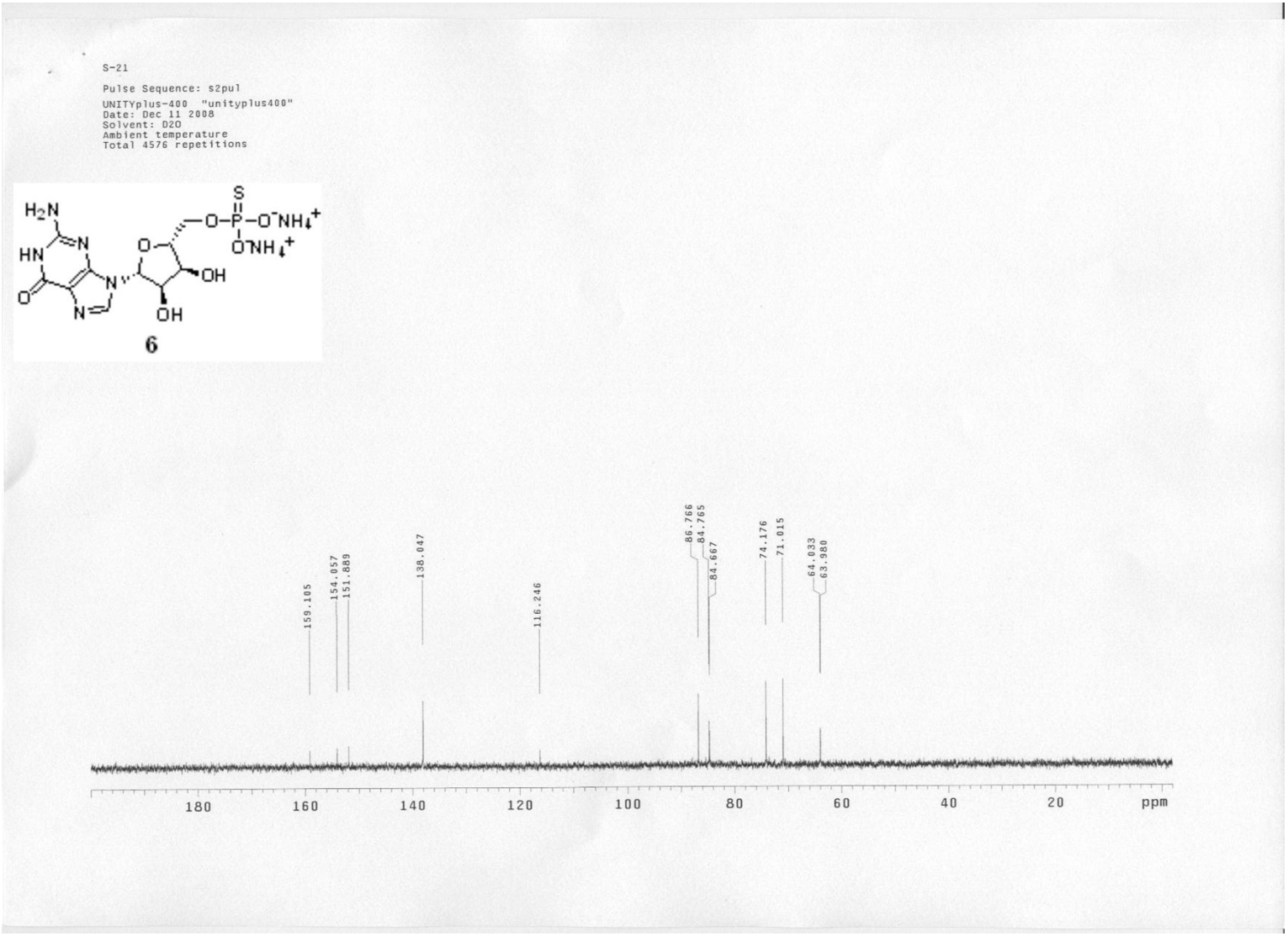
Mass Tolerance : 5mmu

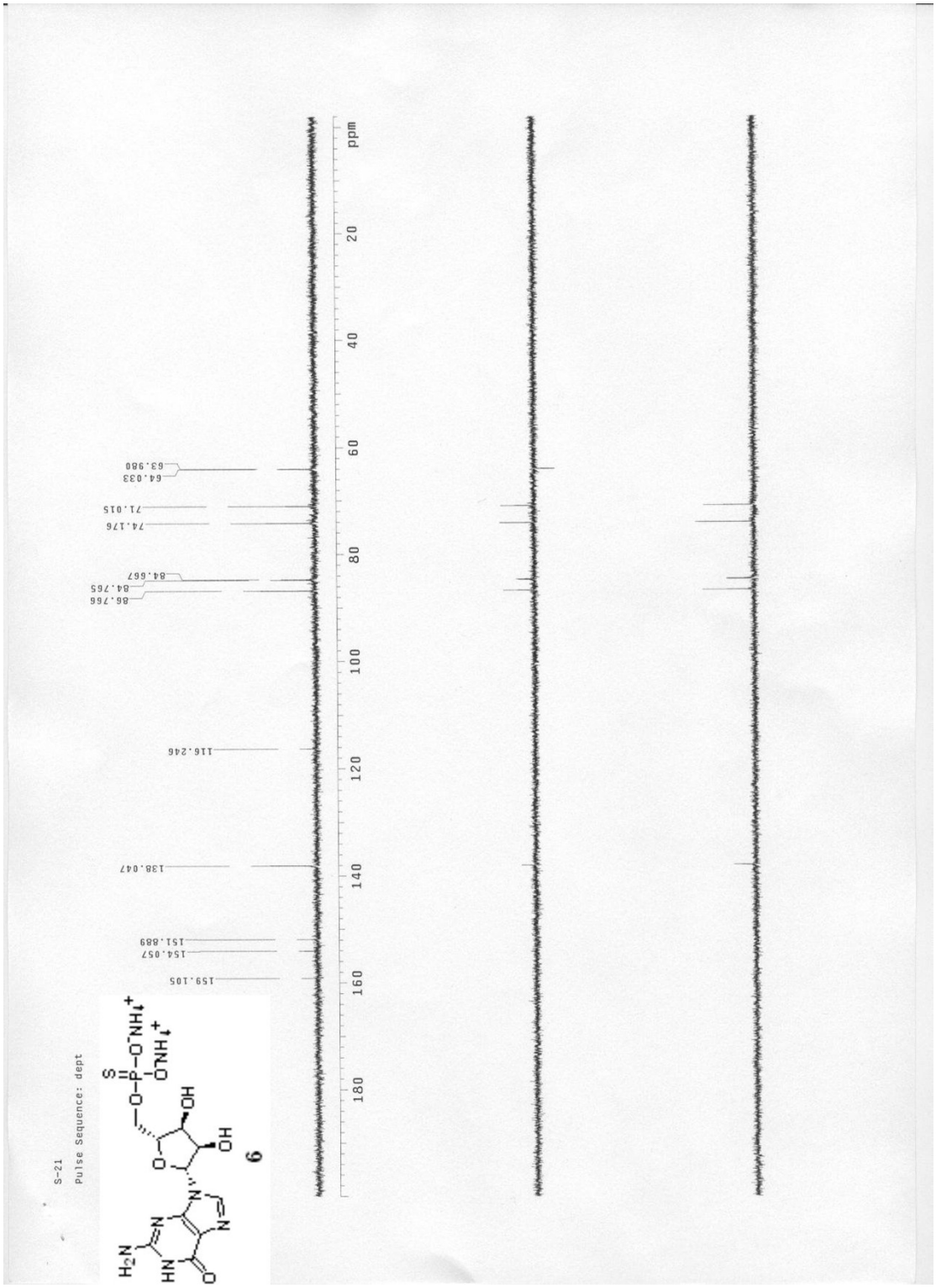
Unsaturation (U.S.) : 0.5 - 30.0

Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
555.9640	20.0	+0.2 / +0.1	10.5	C 13 H 17 O 7 N 5 P S Ba




S-21
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Dec 11 2008
Solvent: D2O
Ambient temperature
Total 128 repetitions
9
8.162
5.833
5.818
4.768
4.720
4.693
4.677
4.670
4.433
4.425
4.260
4.252
3.978
3.969
3.964
3.955
15.46
19.17
19.09
37.24
19.93
ppm

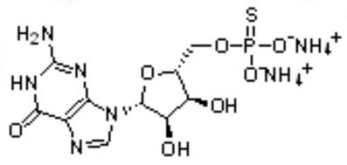

S-21
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Dec 11 2008
Solvent: D2O
Ambient temperature
Total 4576 repetitions
6
159.105
154.057
151.889
138.047
116.246
86.766
84.765
84.667
74.176
71.015
64.033
63.980
180
160
140
120
100
80
60
40
20
ppm


S-21
Pulse Sequence: dept
159.105
154.057
151.889
138.047
116.246
86.766
84.765
84.667
74.176
71.015
64.033
63.980
ppm
20
40
60
80
100
120
140
160
180
9

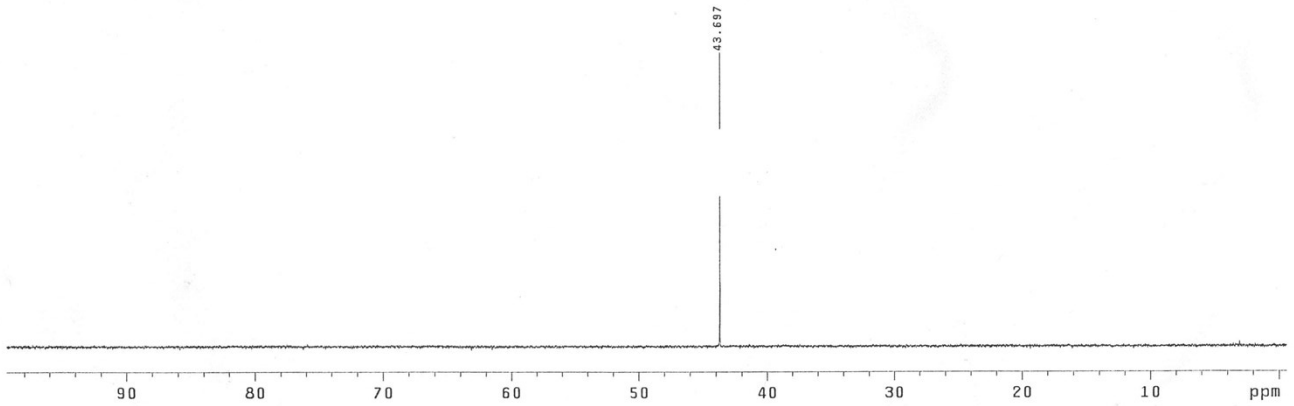
GMPS

Pulse Sequence: s2pul

INOVA-400 "unityplus400"
Date: Jan 11 2011
Solvent: D2O
Ambient temperature
Total 328 repetitions



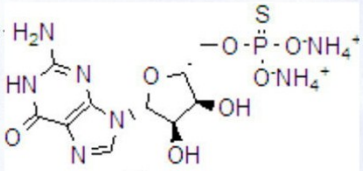
6



[Theoretical Ion Distribution]
Molecular Formula : C10 H21 O7 N7 P S
(m/z 414.0961, MW 414.3592, U.S. 6.5)
Base Peak : 414.0961, Averaged MW : 414.3582(a), 414.3591(w)

Page: 1

m/z	INT.	
414.0961	100.0000	**********
415.0982	15.0647	*********
416.0949	6.8990	****
417.0966	0.8903	*
418.0969	0.1502	
419.0985	0.0156	
420.0997	0.0016	
421.1013	0.0001	

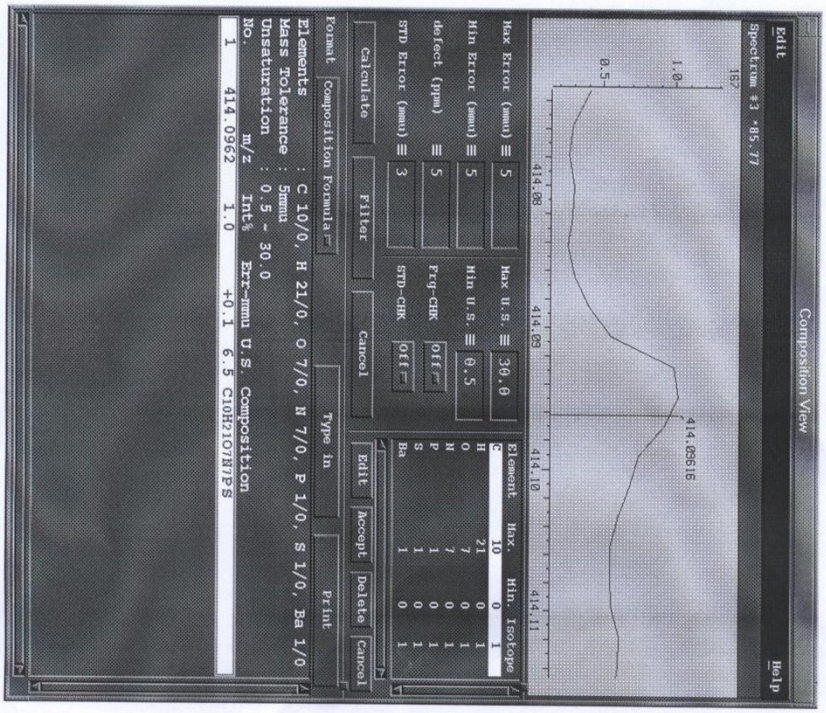


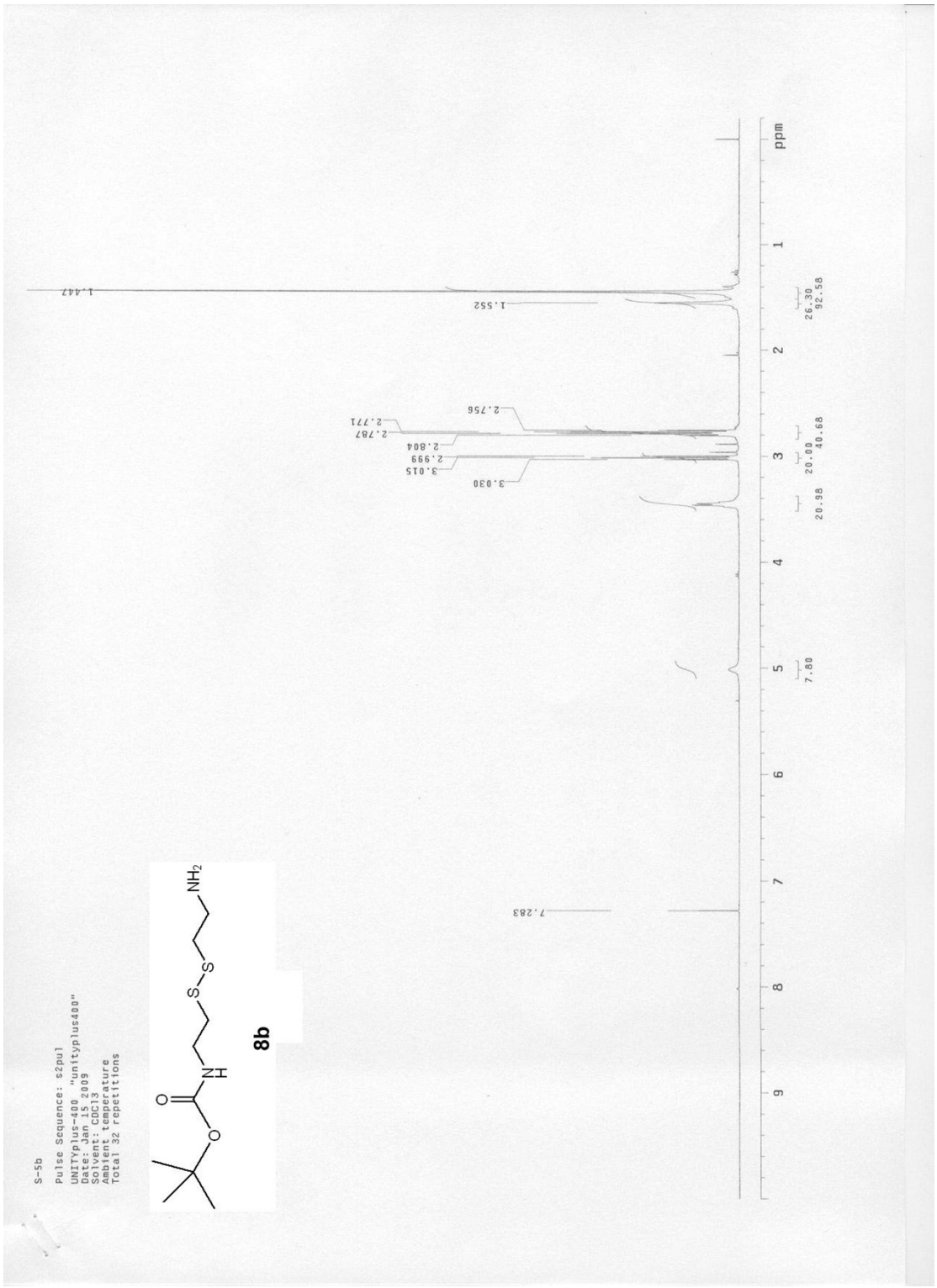
6

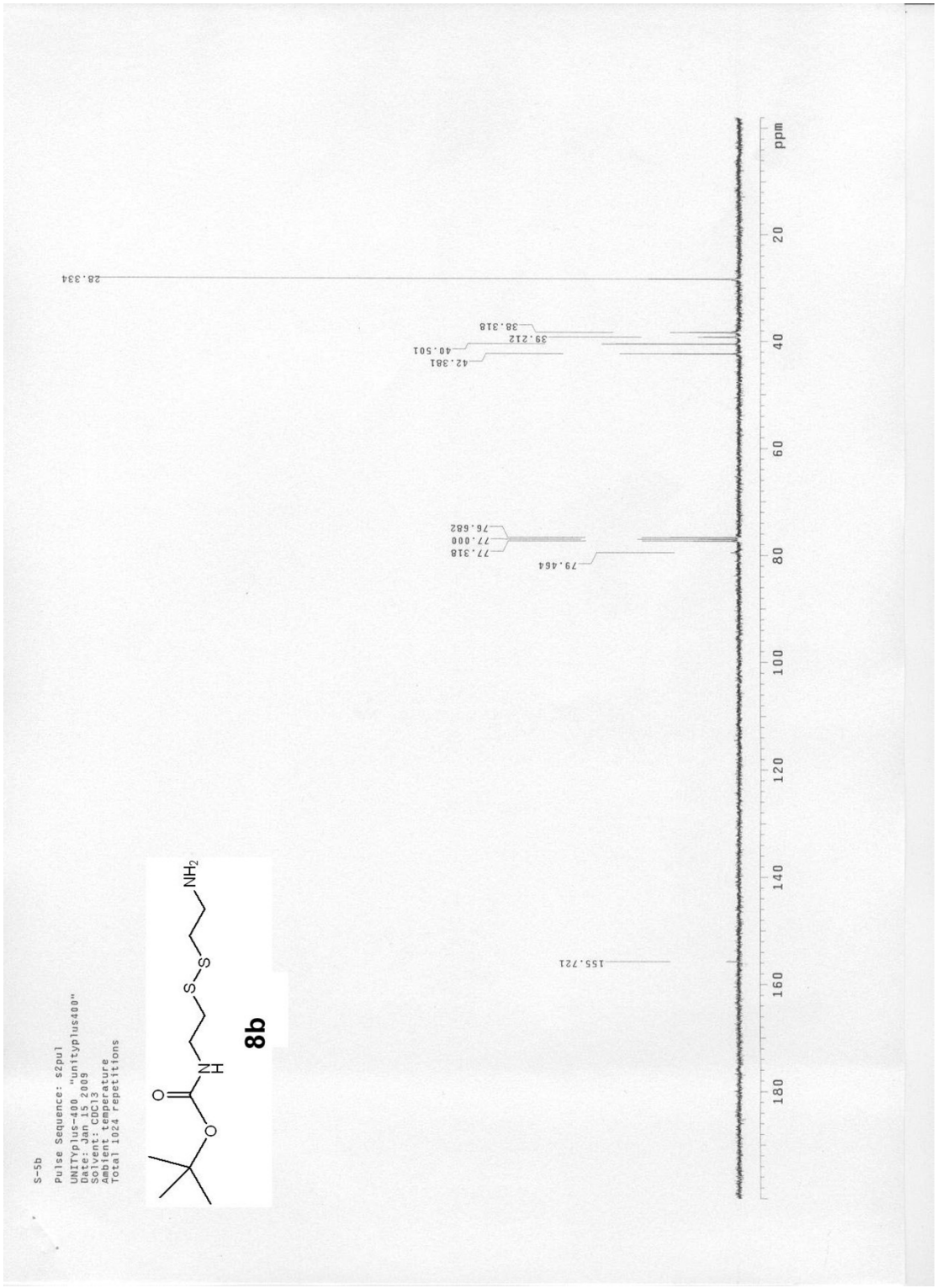
[Elemental Composition]
Data : EIHR334 Date : 09-Mar-2009 16:08
Sample: S-21
Note : NBA+glycerol
Inlet : Direct Ion Mode : FAB+
RT : 4.17 min Scan#: 26
Elements : C 10/0, H 21/0, O 7/0, N 7/0, P 1/0, S 1/0, Ba 1/0
Mass Tolerance : 5mmu
Unsaturation (U.S.) : 0.5 - 30.0

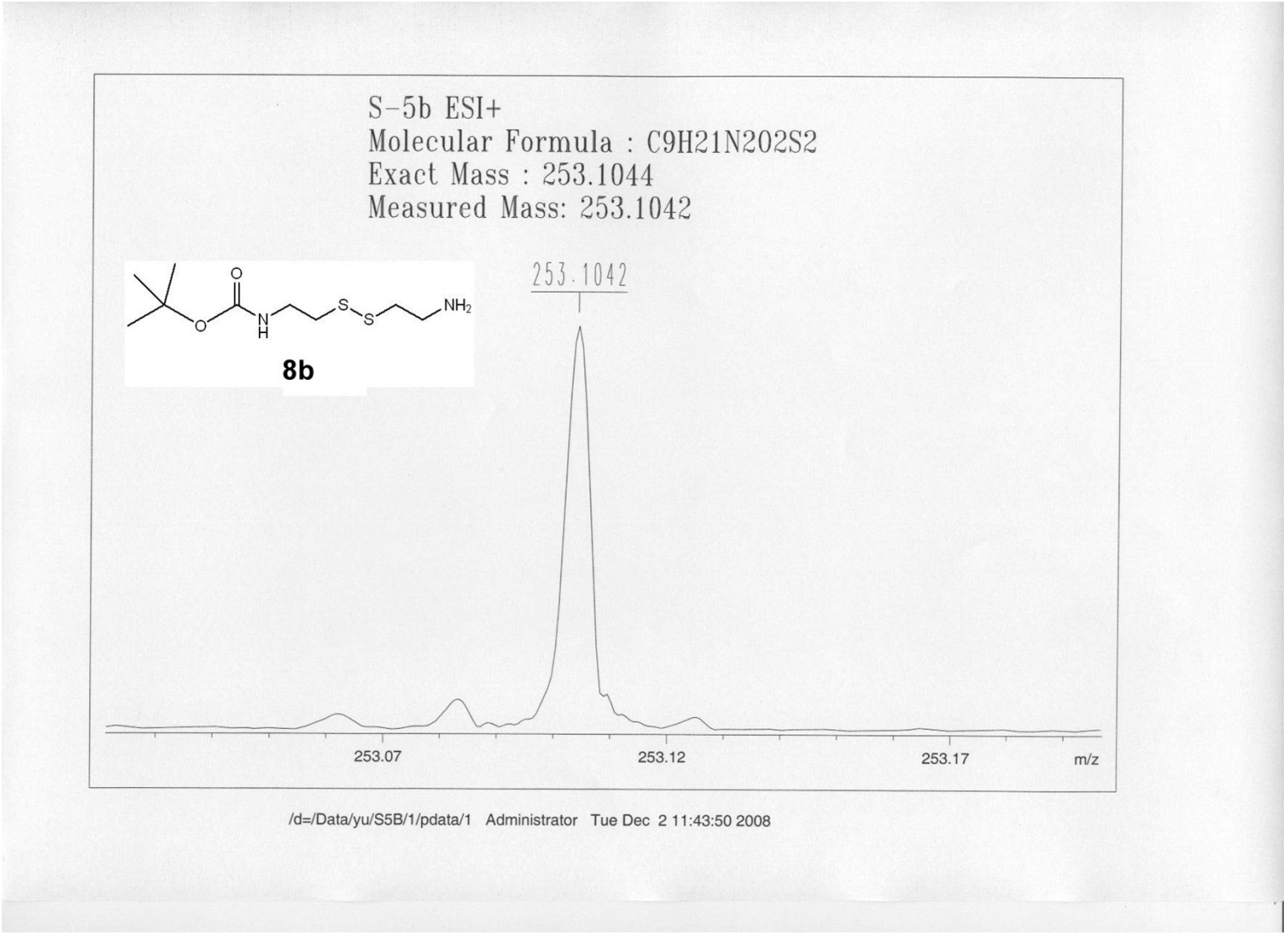
Page: 1

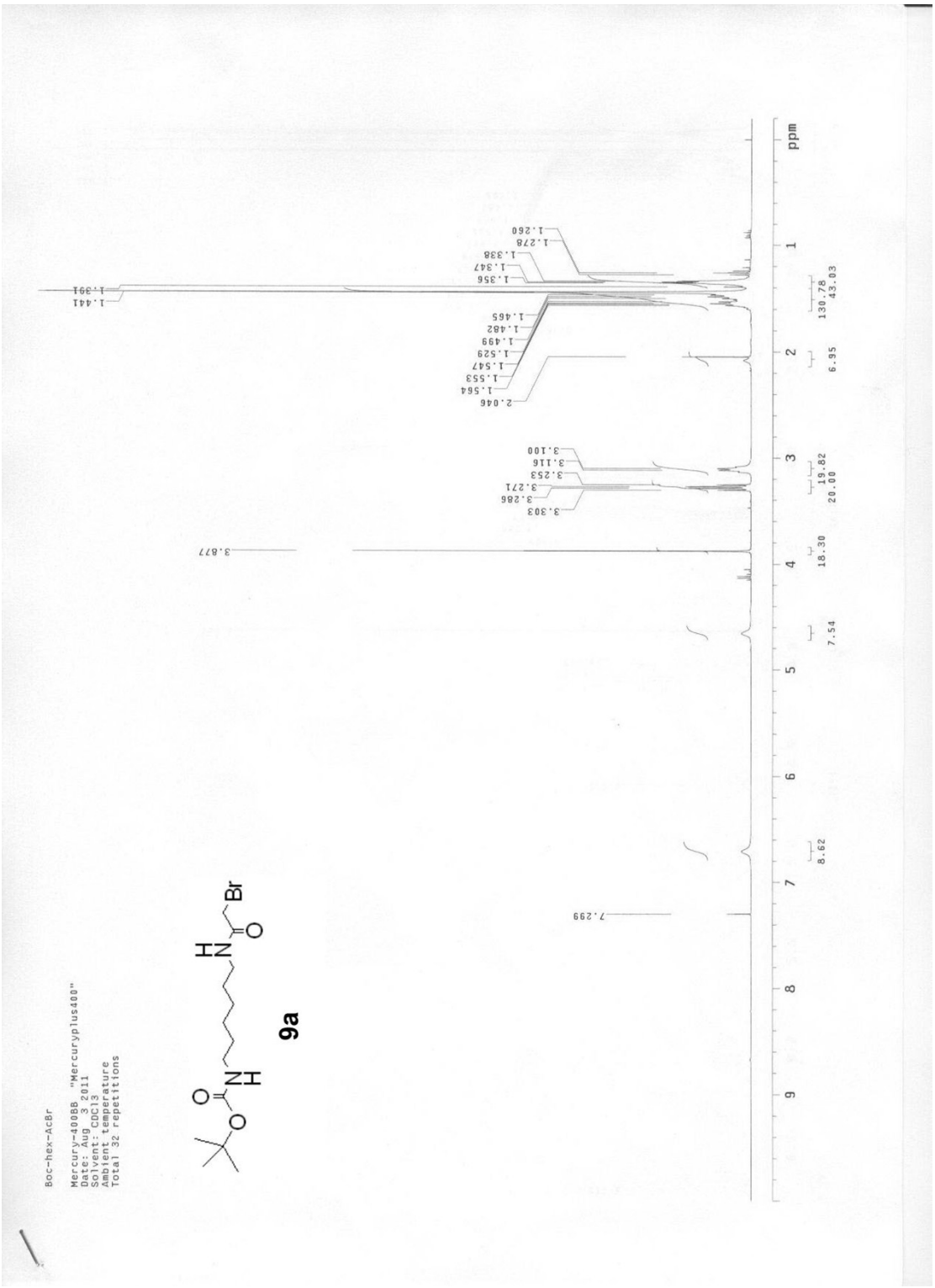
Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
414.0962	1.0	+0.2 / +0.1	6.5	C 10 H 21 O 7 N 7 P S

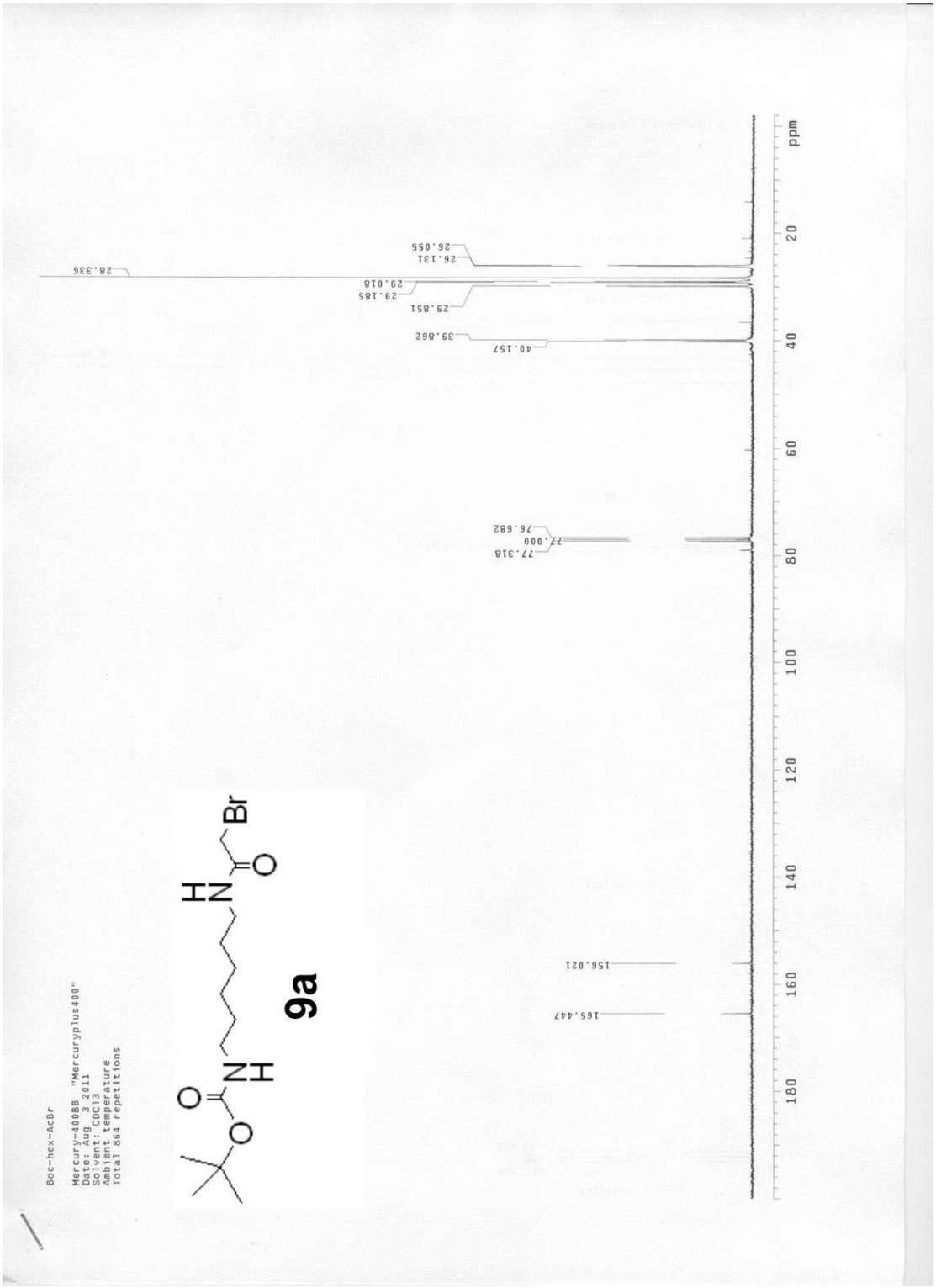


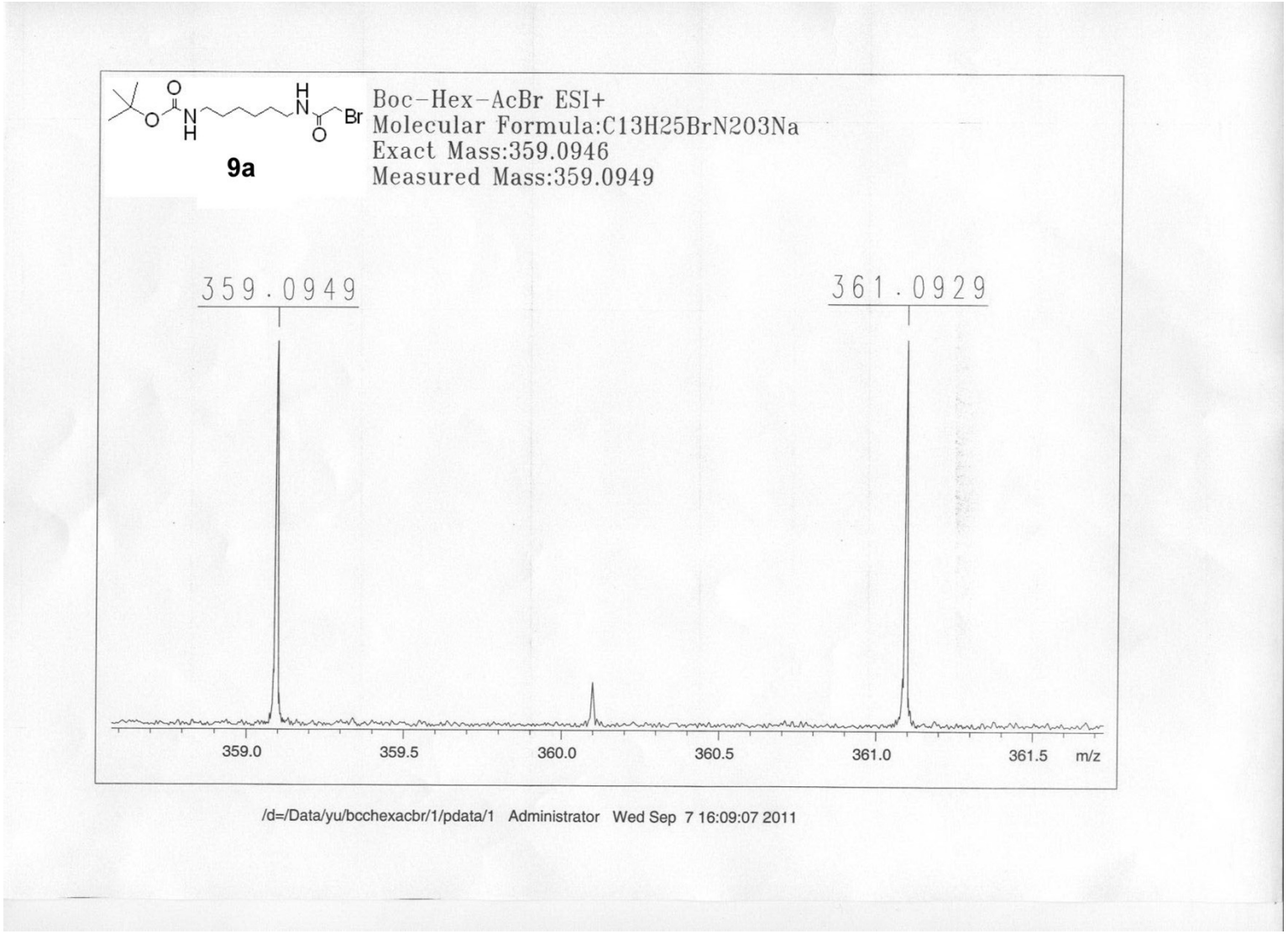

S-5b
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date_ Jan 15 2009
Solvent: CDCl3
Ambient temperature
Total 32 repetitions
8b
1.447
1.552
2.756
2.771
2.787
2.804
2.999
3.015
3.030
7.283
ppm
26.30
92.58
20.00
40.68
20.98
7.80

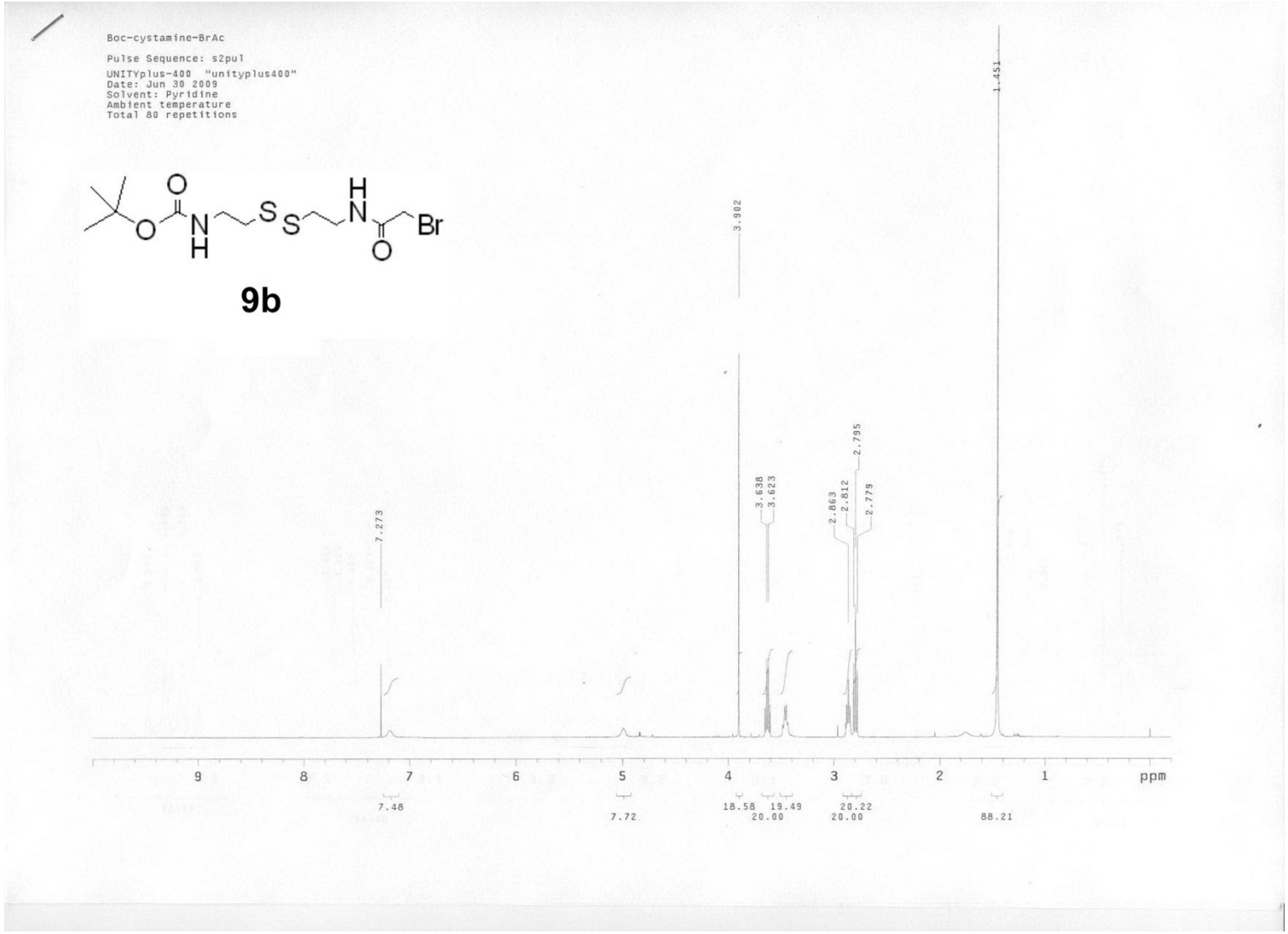

S-5b
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date_ Jan 15 2009
Solvent: CDCl3
Ambient temperature
Total 1024 repetitions
8b
155.721
79.464
77.318
77.000
76.682
42.381
40.501
39.212
38.318
28.334
ppm

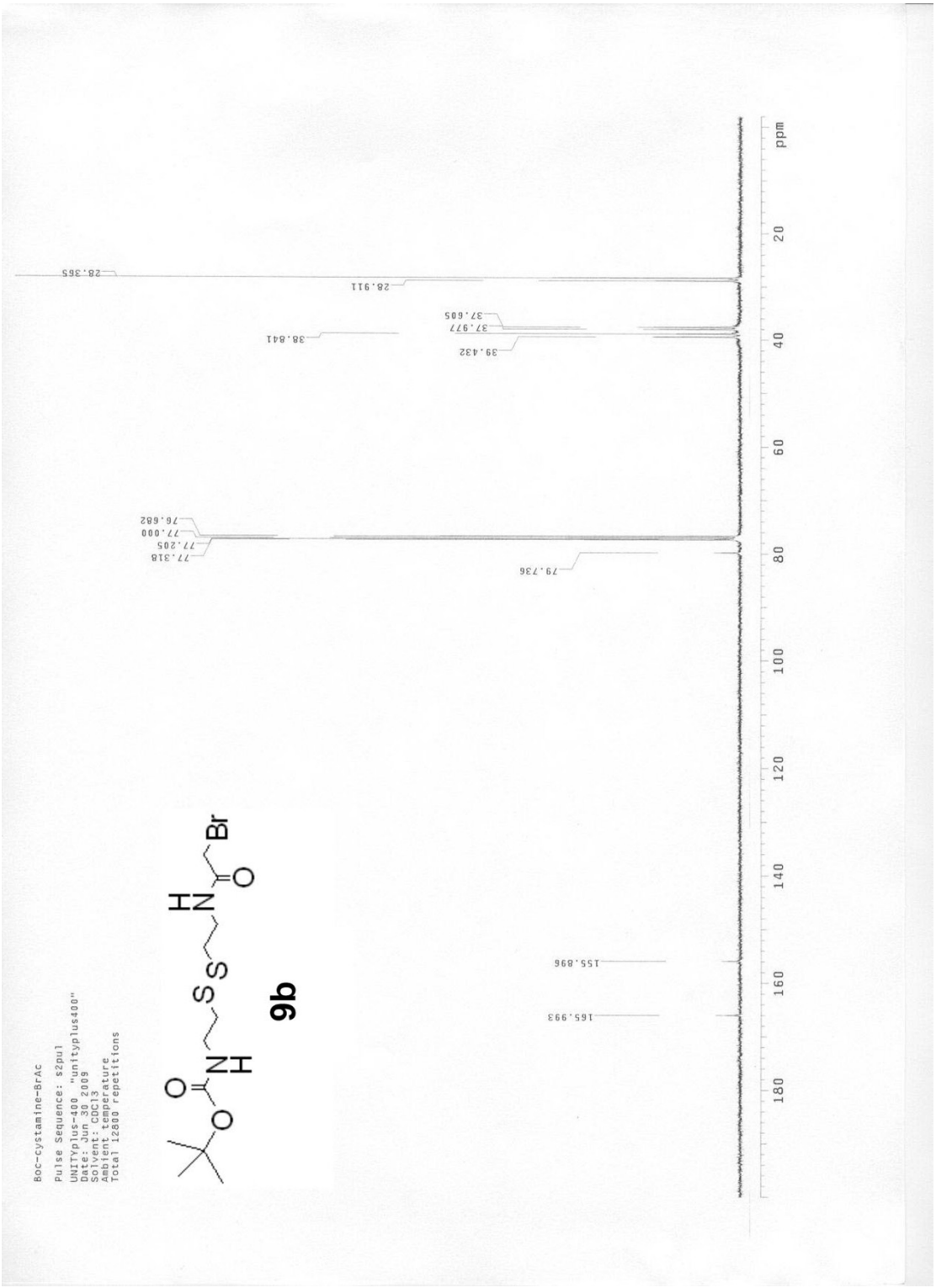

S-5b ESI+
Molecular Formula : C9H21N2O2S2
Exact Mass : 253.1044
Measured Mass: 253.1042
8b
253.1042
253.07
253.12
253.17
m/z
/d=/Data/yu/S5B/1/pdata/1 Administrator Tue Dec 2 11:43:50 2008

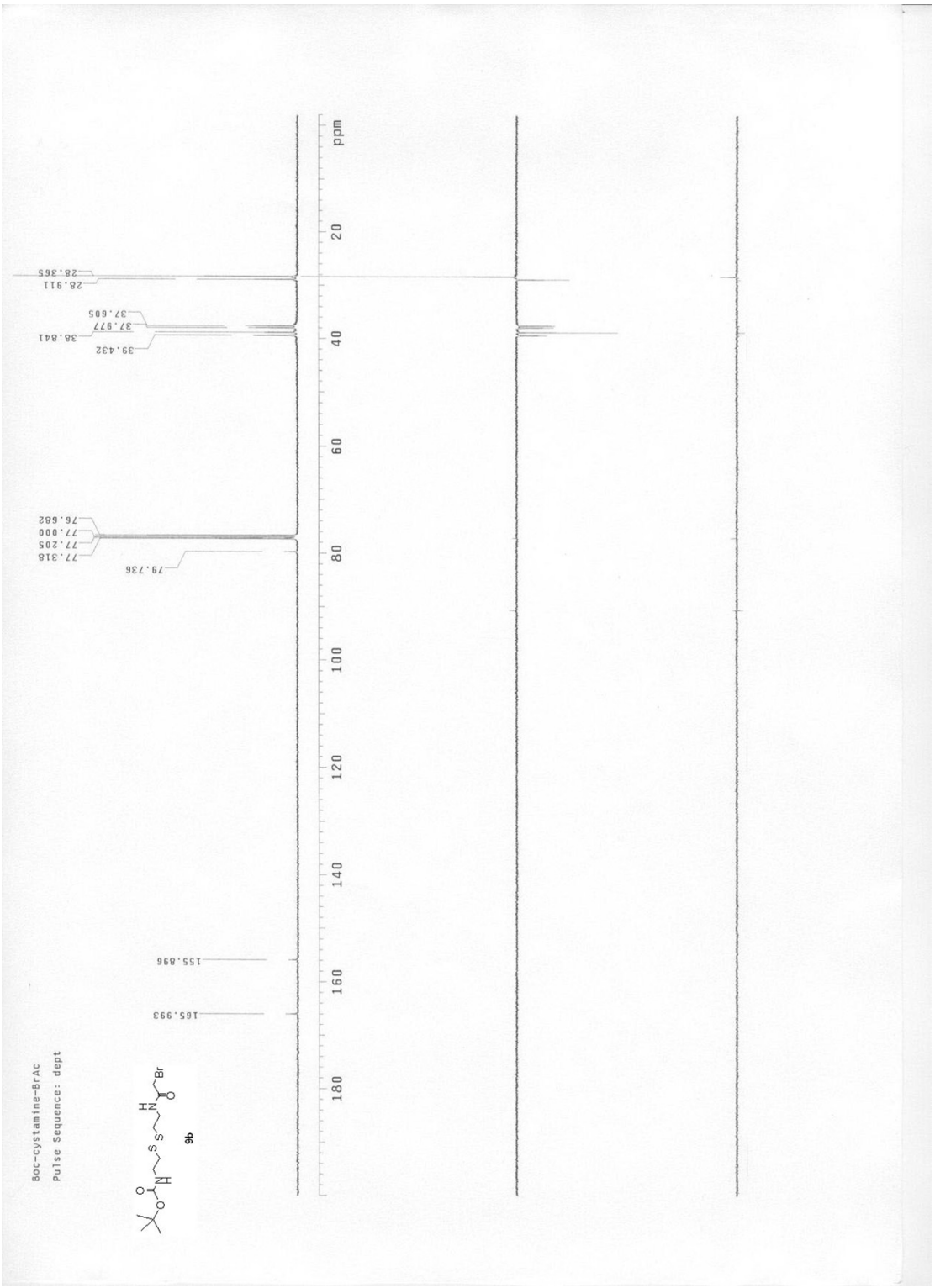

Boc-hex-AcBr
Mercury-400BB "Mercuryplus400"
Date: Aug 3 2011
Solvent: CDCl3
Ambient temperature
Total 32 repetitions
9a
ppm

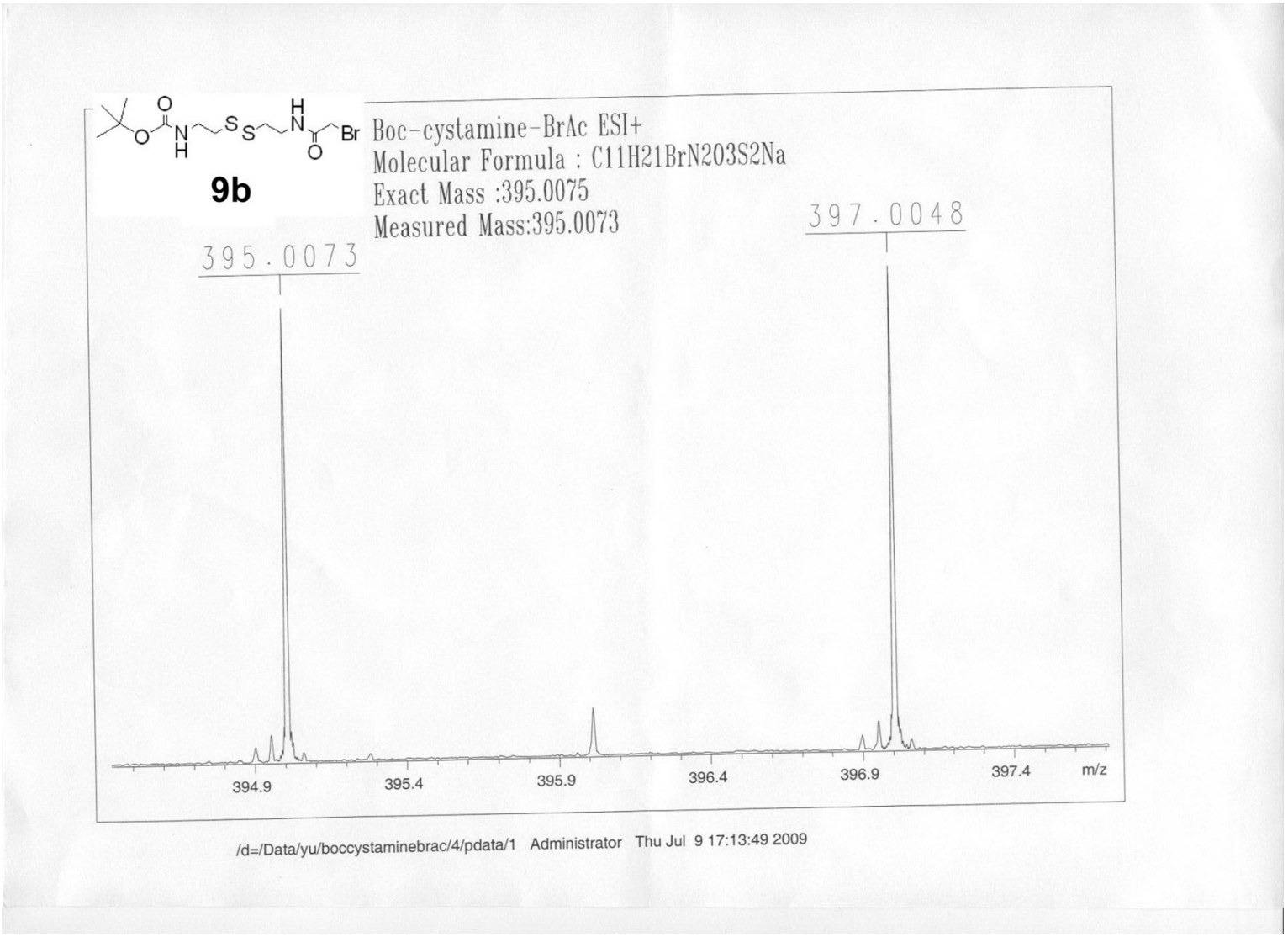

Boc-hex-AcBr
Mercury-400BB "Mercuryplus400"
Date: Aug 3 2011
Solvent: CDCl3
Ambient temperature
1 Ambient temperature
Total 864 repetitions
9a
Br
O
N
H
O
O
N
H
O
156.021
165.447
77.318
77.000
76.682
39.862
40.157
29.185
29.018
28.336
26.131
26.055
ppm
20
40
60
80
100
120
140
160
180

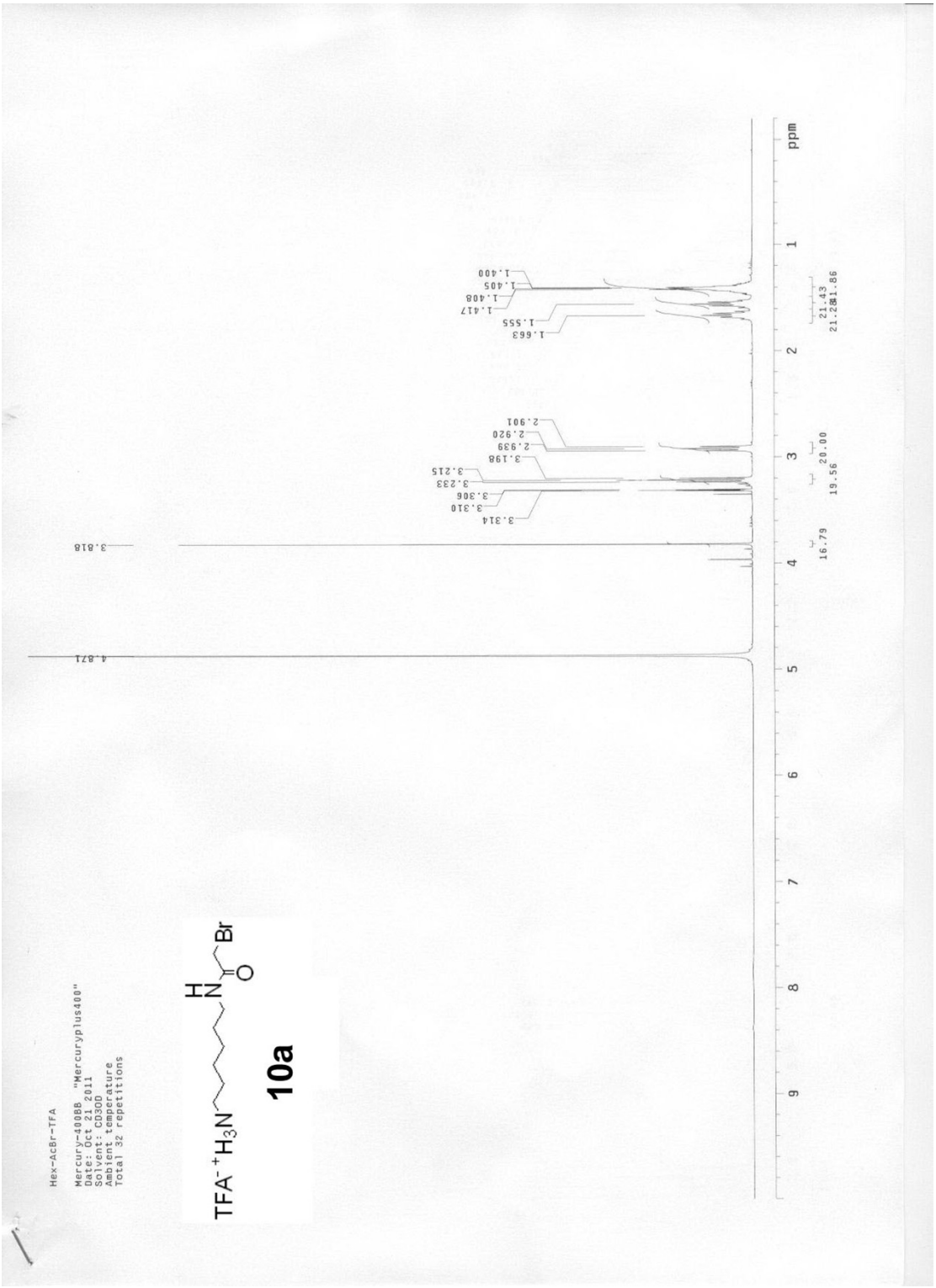

9a
Boc-Hex-AcBr ESI+
Molecular Formula:C13H25BrN2O3Na
Exact Mass:359.0946
Measured Mass:359.0949
359.0949
361.0929
359.0
359.5
360.0
360.5
361.0
361.5
m/z
/d=/Data/yu/bcchexacbr/1/pdata/1 Administrator Wed Sep 7 16:09:07 2011

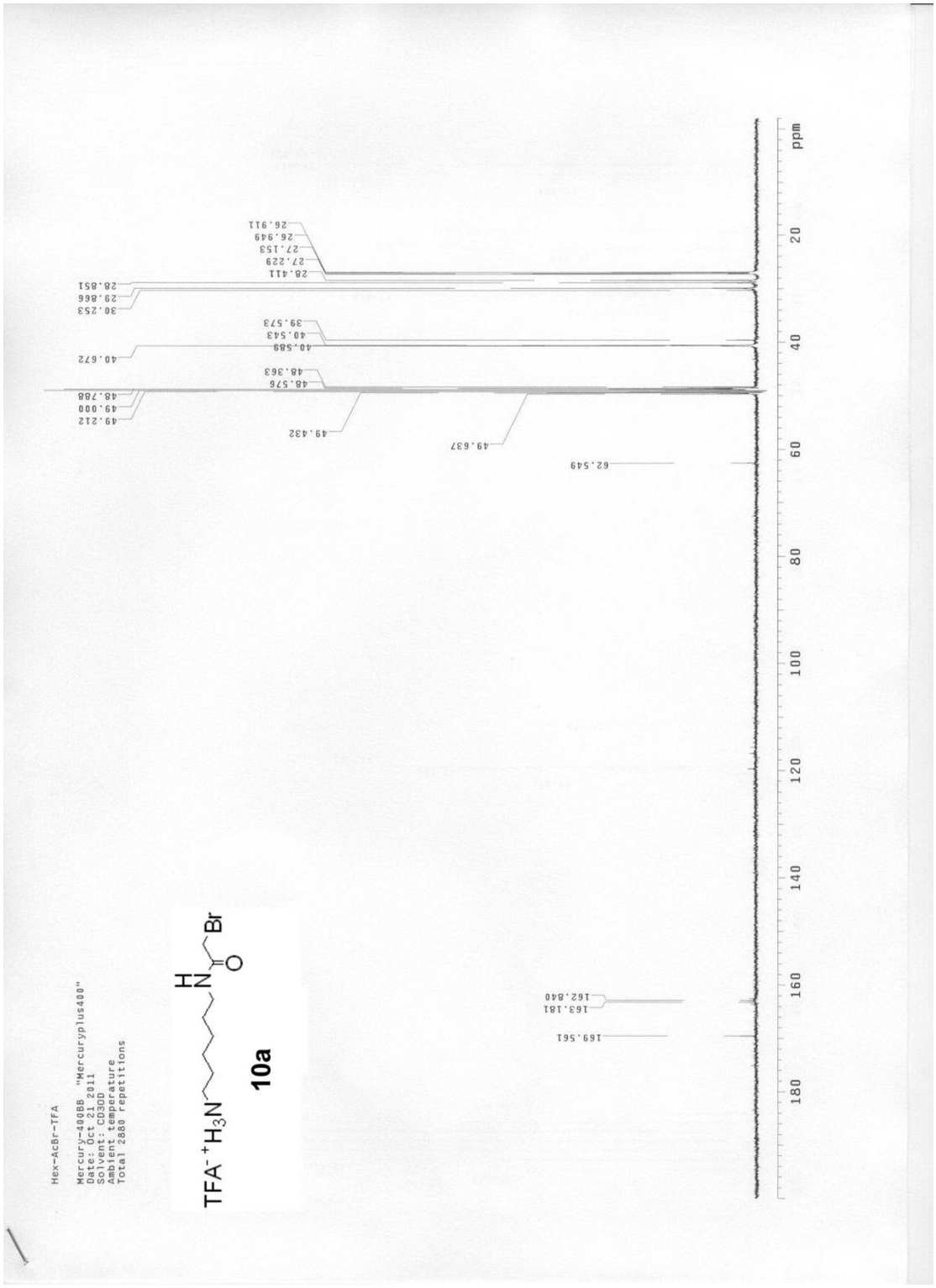

Boc-cystamine-BrAc
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Jun 30 2009
Solvent: Pyridine
Ambient temperature
Total 80 repetitions
9b
7.273
3.902
3.638
3.623
2.863
2.812
2.795
2.779
1.451
9 8 7 6 5 4 3 2 1 ppm
7.48
7.72
18.58
19.49
20.00
20.22
20.00
88.21

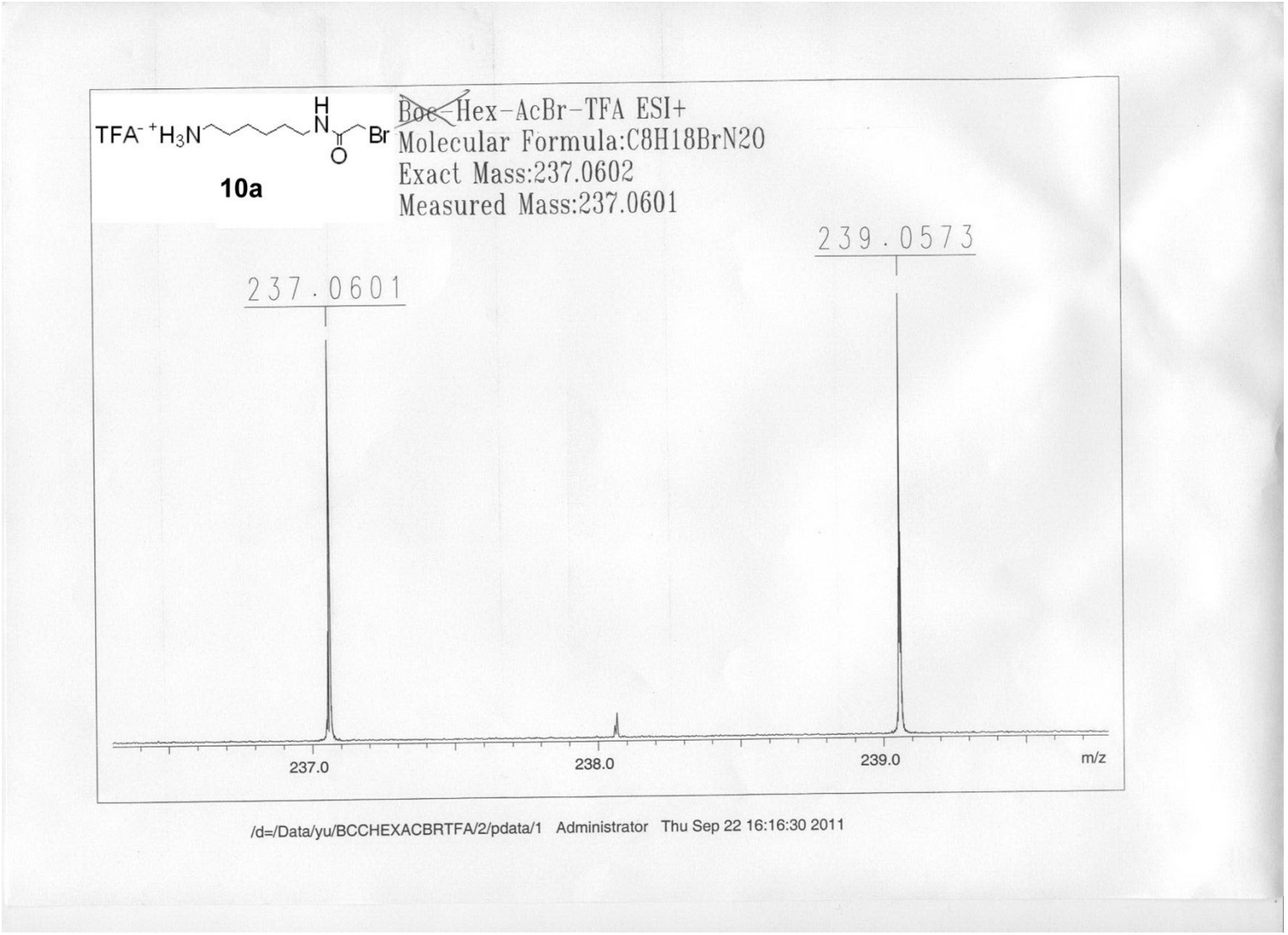

Boc-cystamine-BrAc
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Jun 30 2009
Solvent: CDCl3
Ambient temperature
Total 12800 repetitions
9b
ppm
20
40
60
80
100
120
140
160
180
28.365
28.911
37.605
37.977
38.841
39.432
76.682
77.000
77.205
77.318
79.736
155.896
165.993

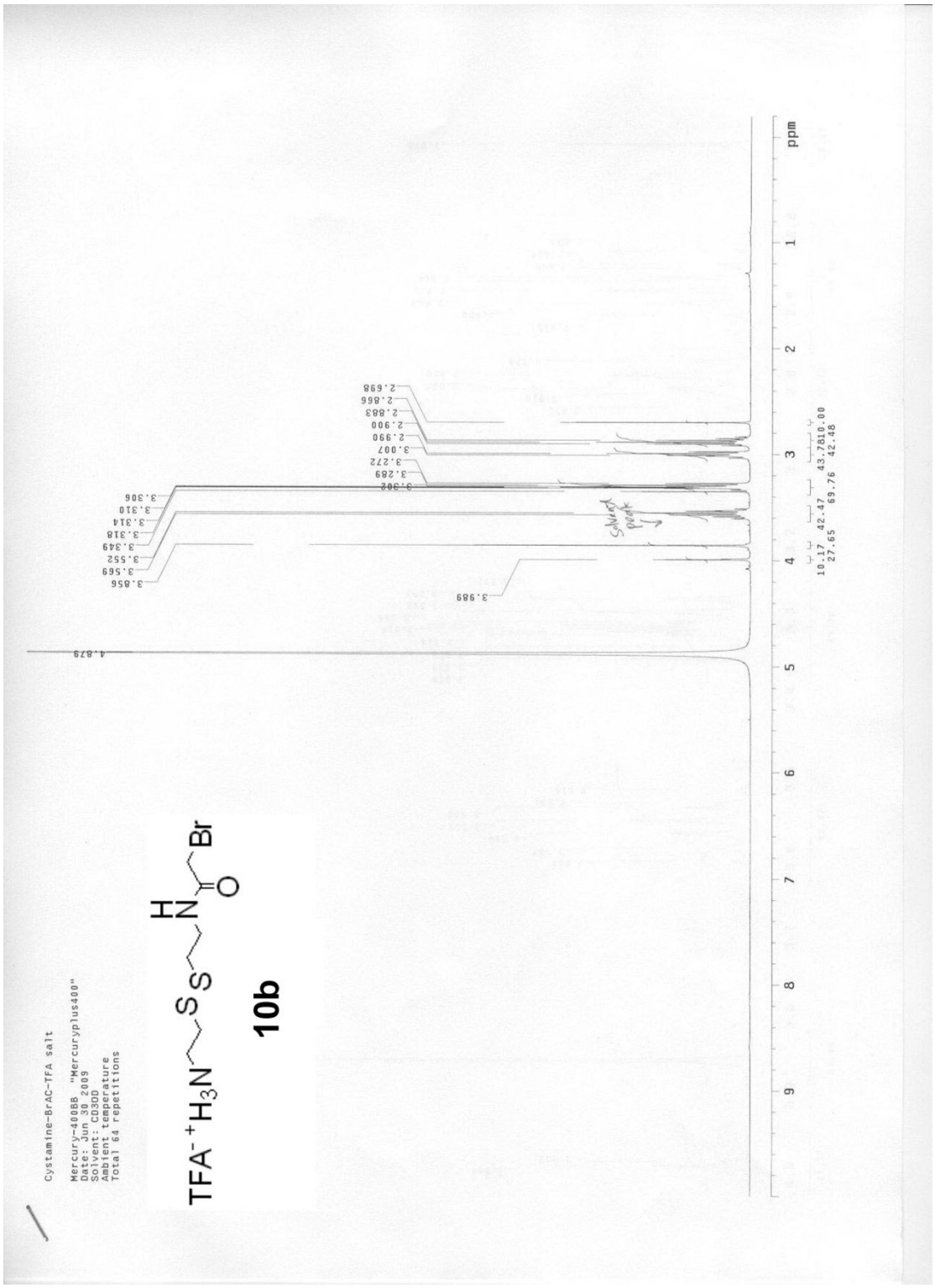

Boc-cystamine-BrAc
Pulse Sequence: dept
9b
165.993
155.896
79.736
77.318
77.205
77.000
76.682
39.432
38.841
37.977
37.605
28.911
28.365
ppm
20
40
60
80
100
120
140
160
180

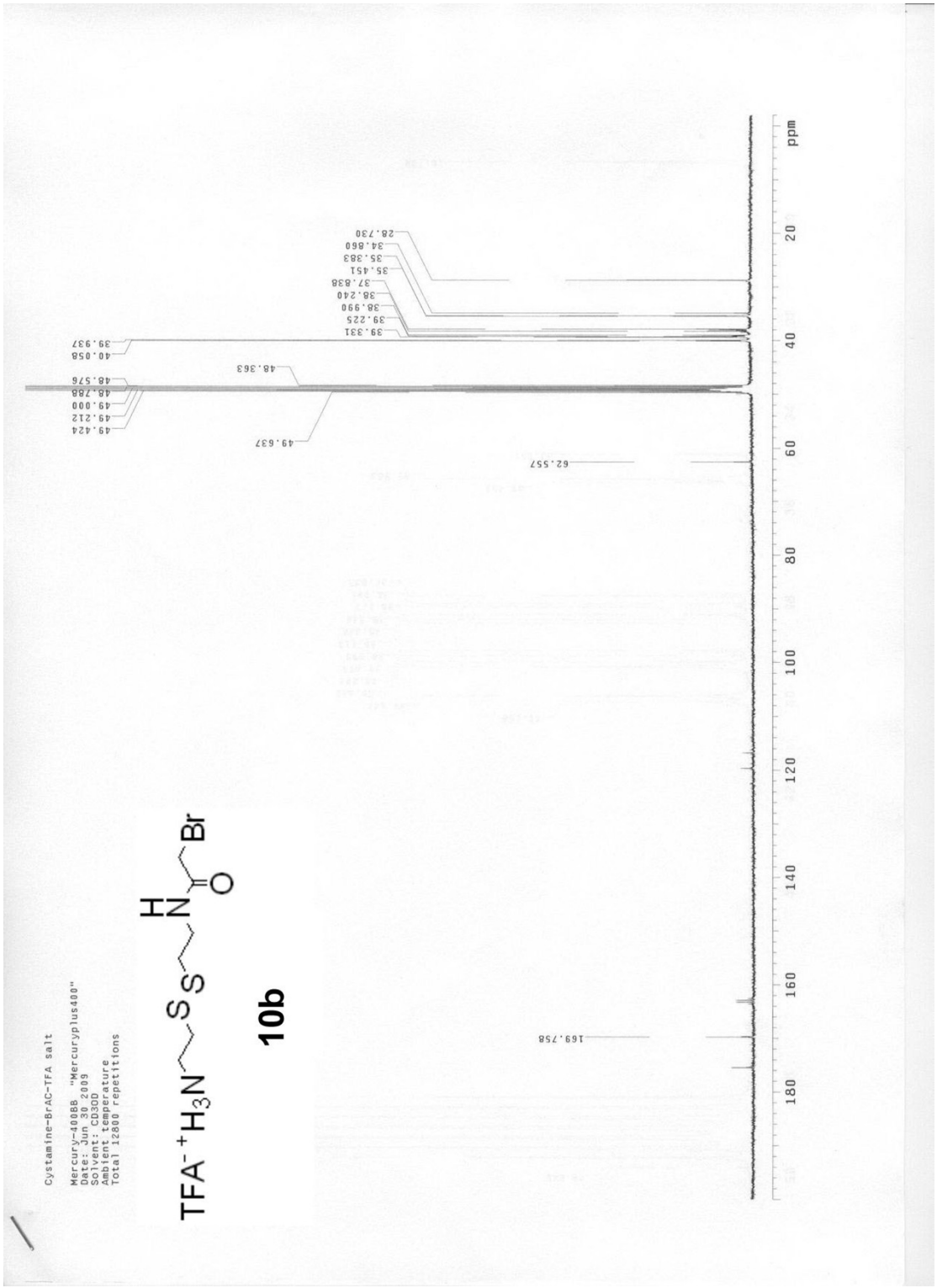

9b
Boc-cystamine-BrAc ESI+
Molecular Formula : C11H21BrN2O3S2Na
Exact Mass :395.0075
Measured Mass:395.0073
395.0073
397.0048
394.9
395.4
395.9
396.4
396.9
397.4
m/z
/d=/Data/yu/boccystaminebrac/4/pdata/1 Administrator Thu Jul 9 17:13:49 2009

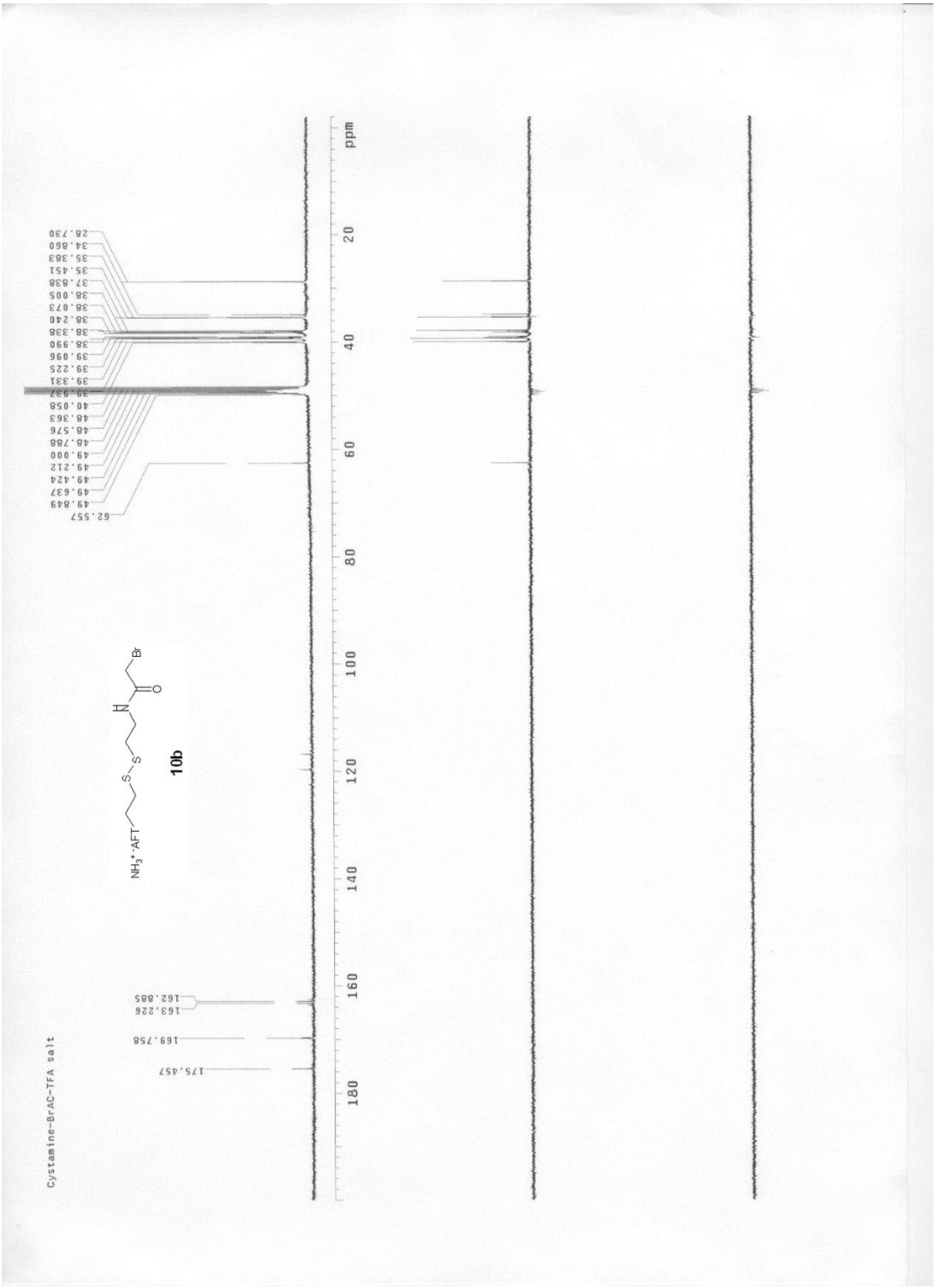

Hex-AcBr-TFA
Mercury-400BB "Mercuryplus400"
Date: Oct 21 2011
Solvent: CD3OD
Ambient temperature
Total 32 repetitions
TFA⁻ ⁺H3N
Br
O
10a
4.821
3.818
3.314
3.310
3.306
3.233
3.215
3.198
2.939
2.920
2.901
1.663
1.555
1.417
1.408
1.405
1.400
ppm
16.79
20.00
19.56
21.43
21.28
41.86

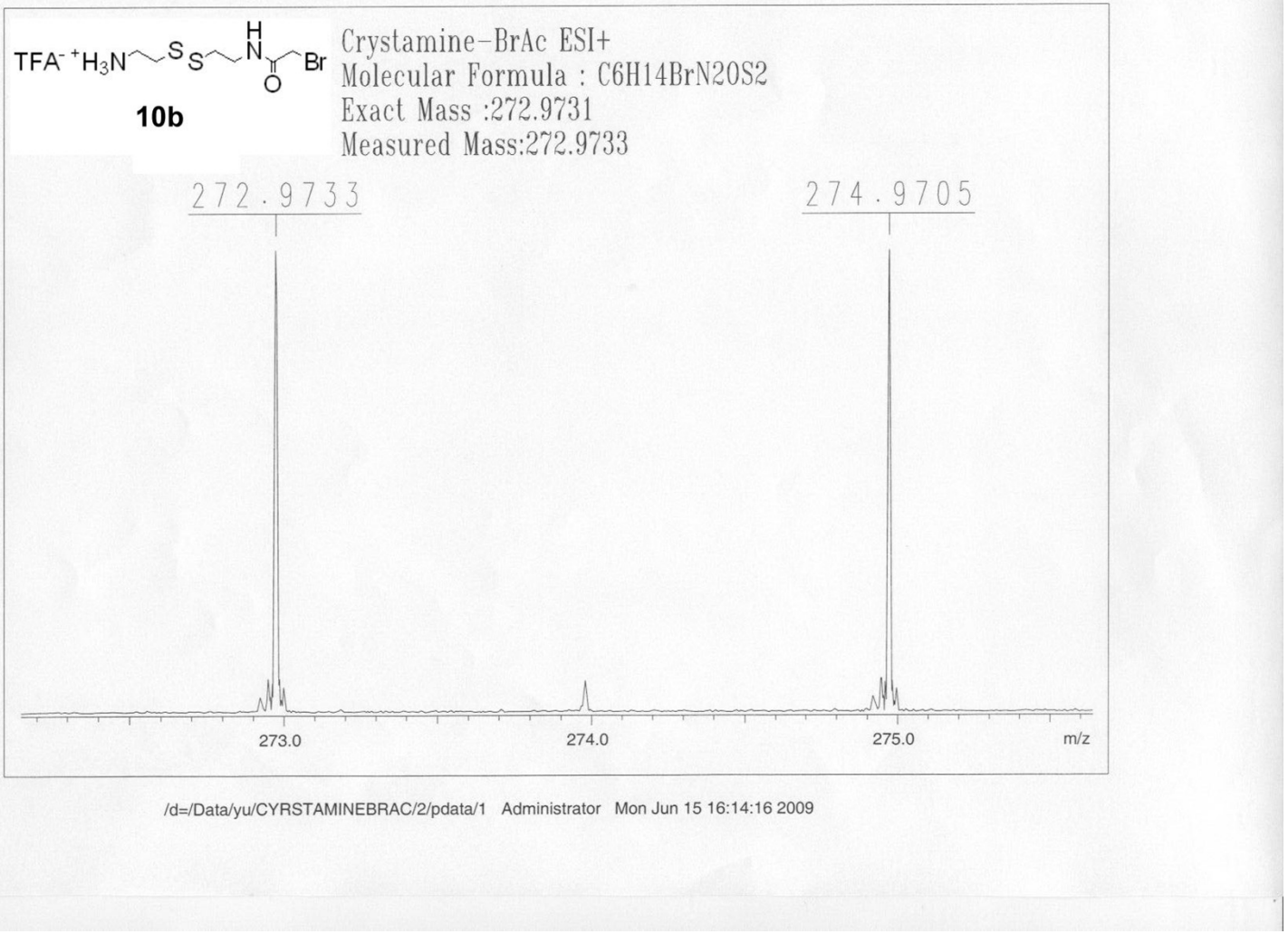

Hex-AcBr-TFA
Mercury-400BB "Mercuryplus400"
Date: Oct 21 2011
Solvent: CD3OD
Ambient temperature
Total 2880 repetitions
TFA⁻ ⁺H3N
Br
O
10a
ppm
20
40
60
80
100
120
140
160
180
26.911
26.949
27.153
27.229
28.411
28.851
29.866
30.253
39.573
40.543
40.589
40.672
48.363
48.576
48.788
49.000
49.212
49.432
49.637
62.549
162.840
163.161
169.561

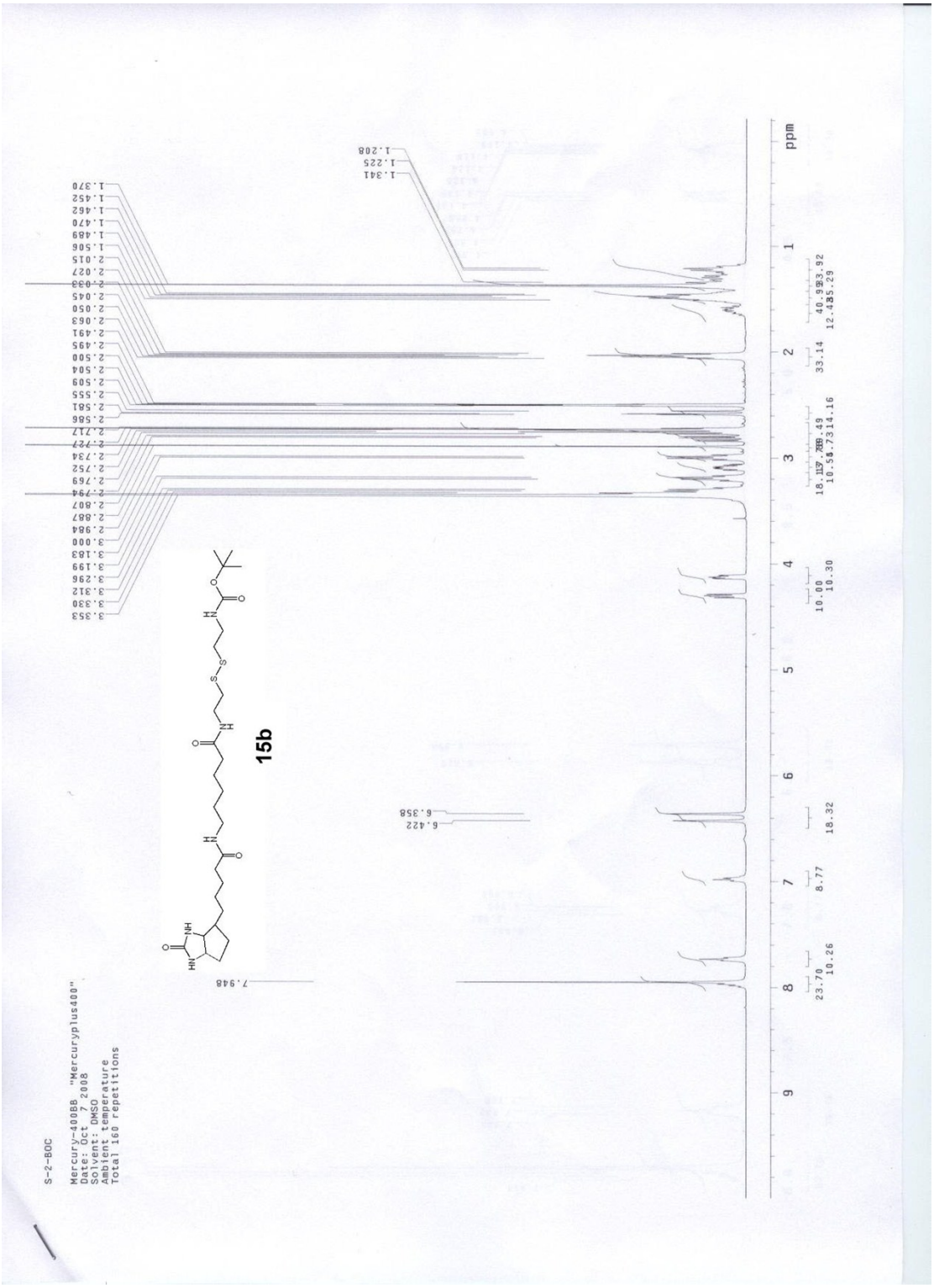

TFA⁻ ⁺H₃N
H
N
O
Br
10a
Boc-Hex-AcBr-TFA ESI+
Molecular Formula:C8H18BrN20
Exact Mass:237.0602
Measured Mass:237.0601
239.0573
237.0601
237.0
238.0
239.0
m/z
/d=/Data/yu/BCCHEXACBRTFA/2/pdata/1 Administrator Thu Sep 22 16:16:30 2011

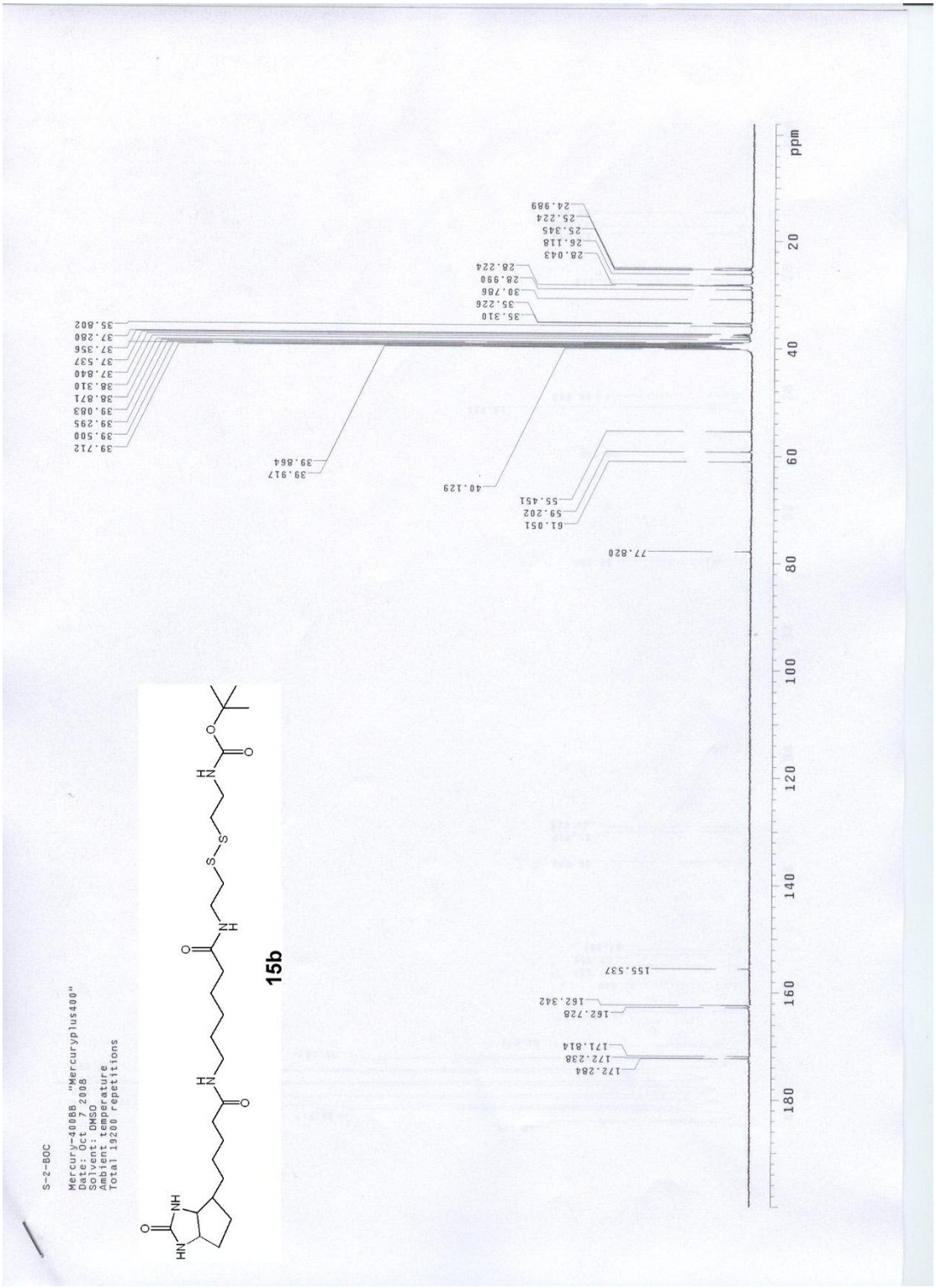

Cystamine-BrAC-TFA salt
Mercury-400BB "Mercuryplus400"
Date Jun 7 2009
Solvent CD3OD
Ambient temperature
Total 64 repetitions
TFA⁻ ⁺H3N S S H N O Br
10b
4.879
3.989
3.856
3.569
3.552
3.349
3.318
3.314
3.310
3.306
3.302
3.289
3.272
3.007
2.990
2.900
2.883
2.866
2.698
Solvent peak
10.17 42.47 43.7810.00
27.65 69.76 42.48
ppm

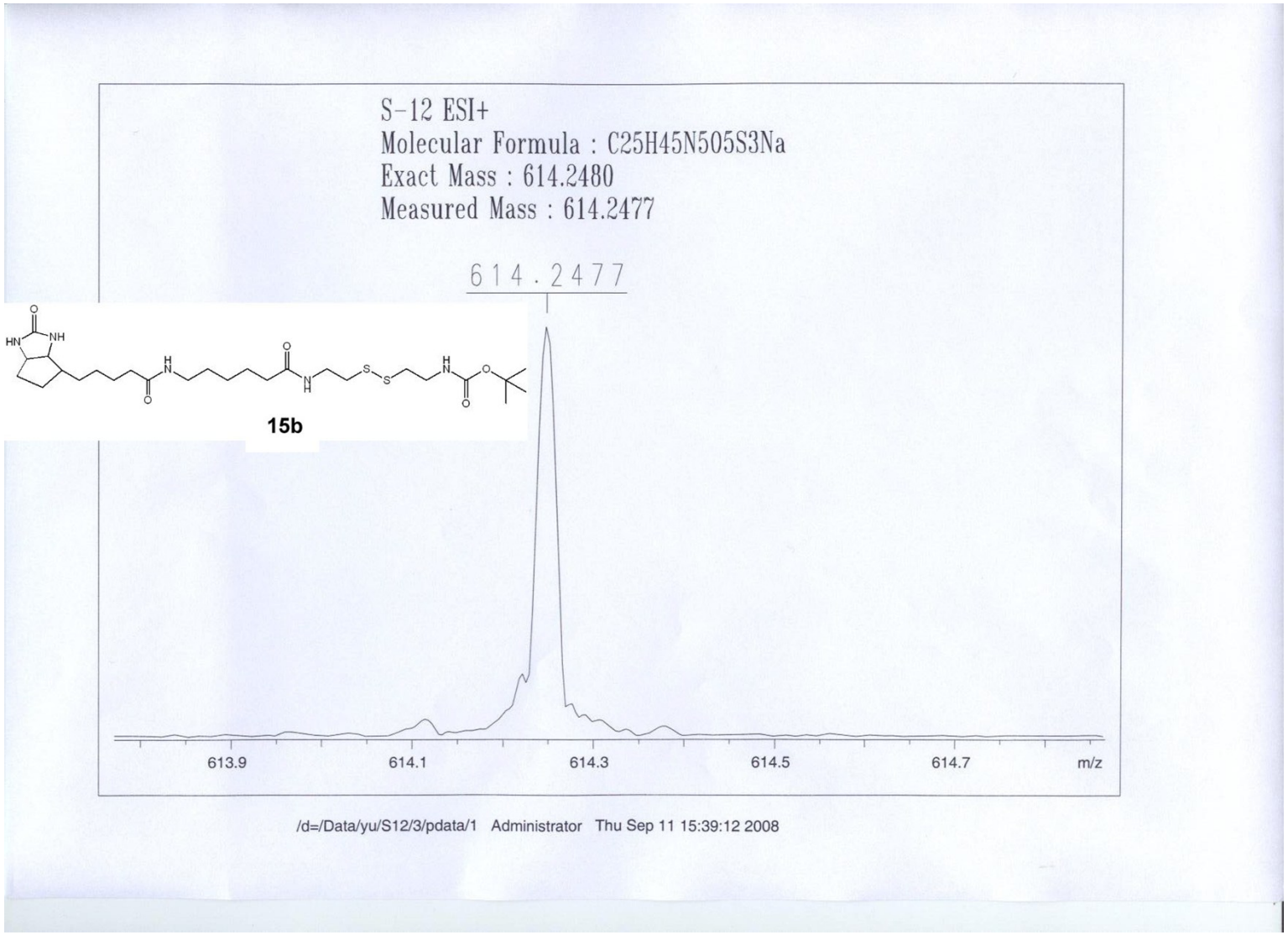

Cystamine-BrAC-TFA salt
Mercury-400BB "Mercuryplus400"
Date Jun 8 2009
Solvent: D2O
Ambient temperature
Total 12800 repetitions
TFA⁻ ⁺H3N
S
S
H
N
O
Br
10b
169.758
62.557
49.637
48.363
49.424
49.212
49.000
48.788
48.576
40.058
39.937
39.331
39.225
38.990
38.240
37.838
35.451
35.383
34.860
28.730
ppm
20
40
60
80
100
120
140
160
180

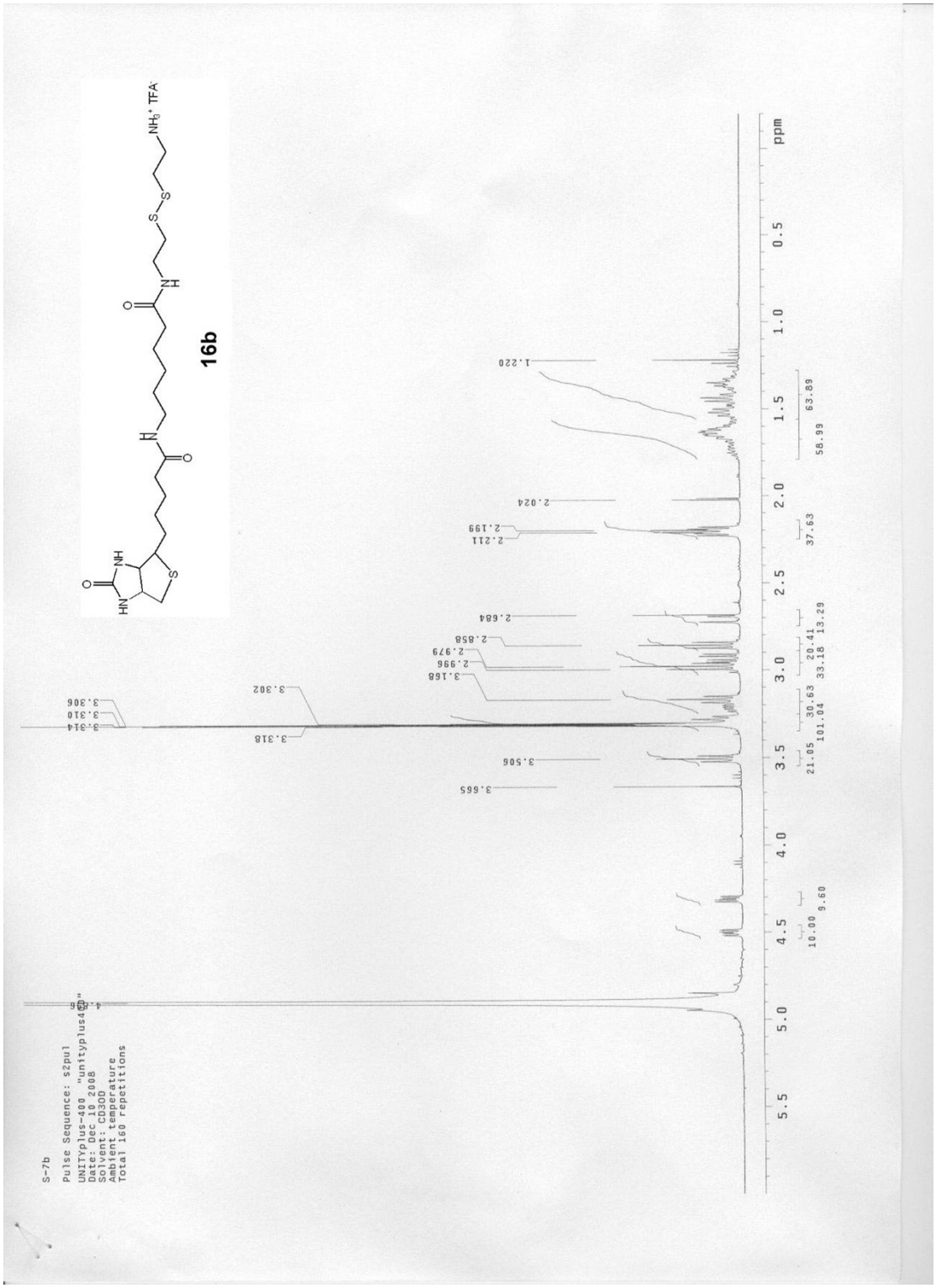

Cystamine-BrAC-TFA salt
ppm
20
40
60
80
100
120
140
160
180
175.457
169.758
163.226
162.885
62.557
49.849
49.637
49.424
49.212
49.000
48.788
48.576
48.363
40.058
39.937
39.331
39.225
39.096
38.990
38.338
38.240
38.073
38.005
37.838
35.451
35.383
34.860
28.730
10b
Br
O
H
N
S
S
NH3+·AFT

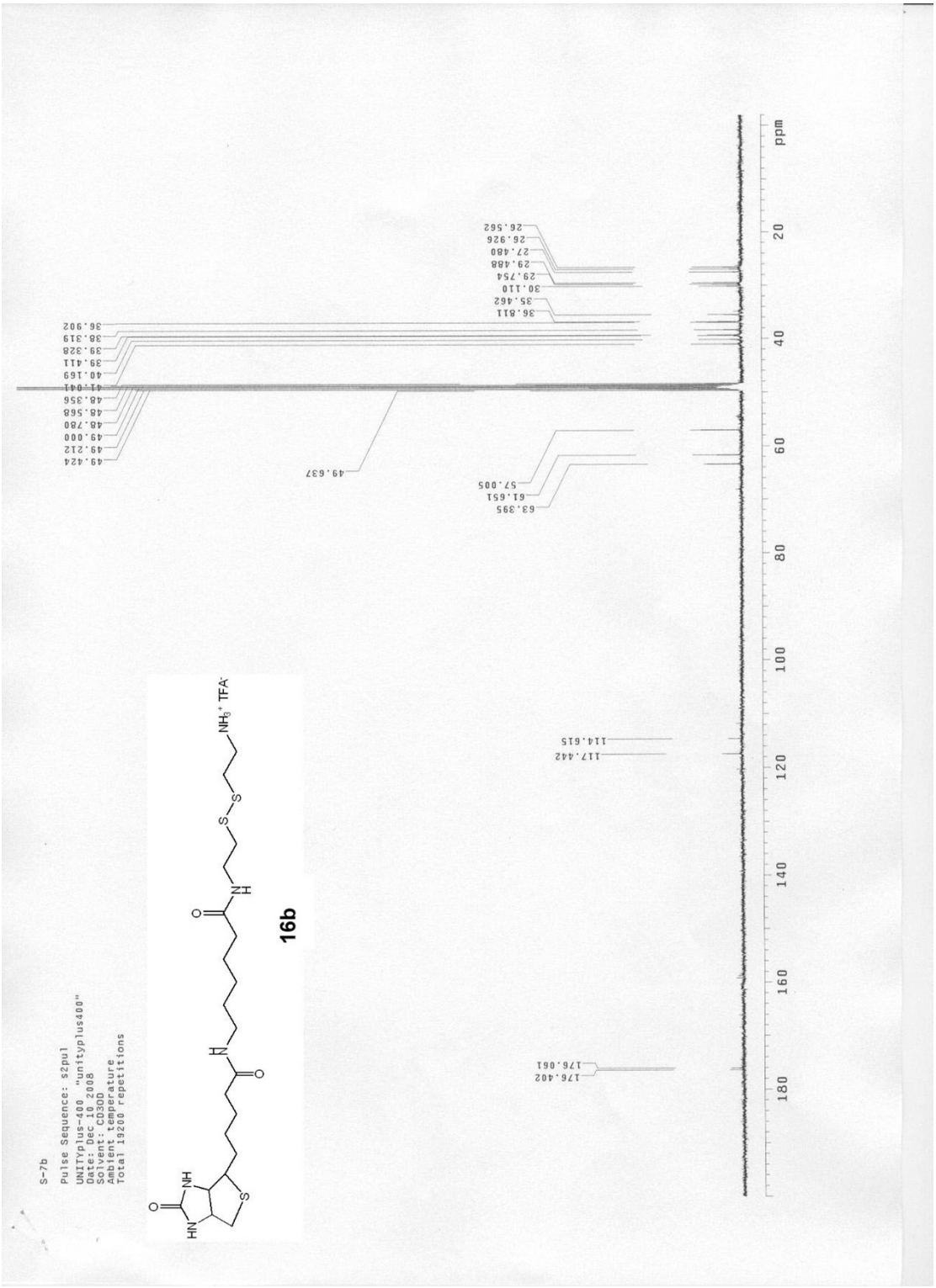

TFA⁻ ⁺H₃N S S H N Br O
10b
Crystamine-BrAc ESI+
Molecular Formula : C6H14BrN2OS2
Exact Mass :272.9731
Measured Mass:272.9733
272.9733
274.9705
273.0
274.0
275.0
m/z
/d=/Data/yu/CYRSTAMINEBRAC/2/pdata/1 Administrator Mon Jun 15 16:14:16 2009

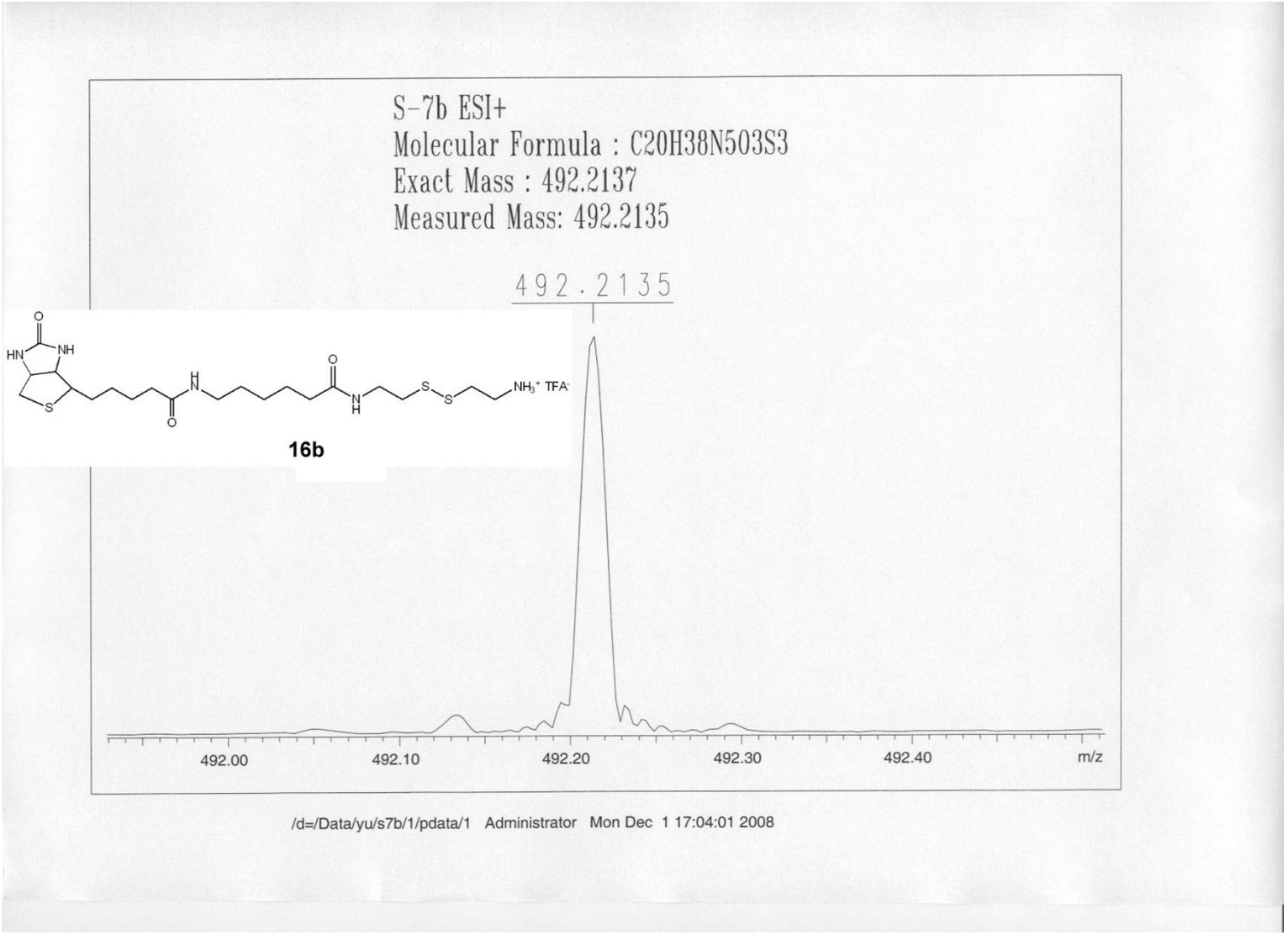

S-2-BOC
Mercury-400BB "Mercuryplus400"
Date: Oct 7 2008
Solvent: DMSO
Ambient temperature
Total 160 repetitions
15b
ppm

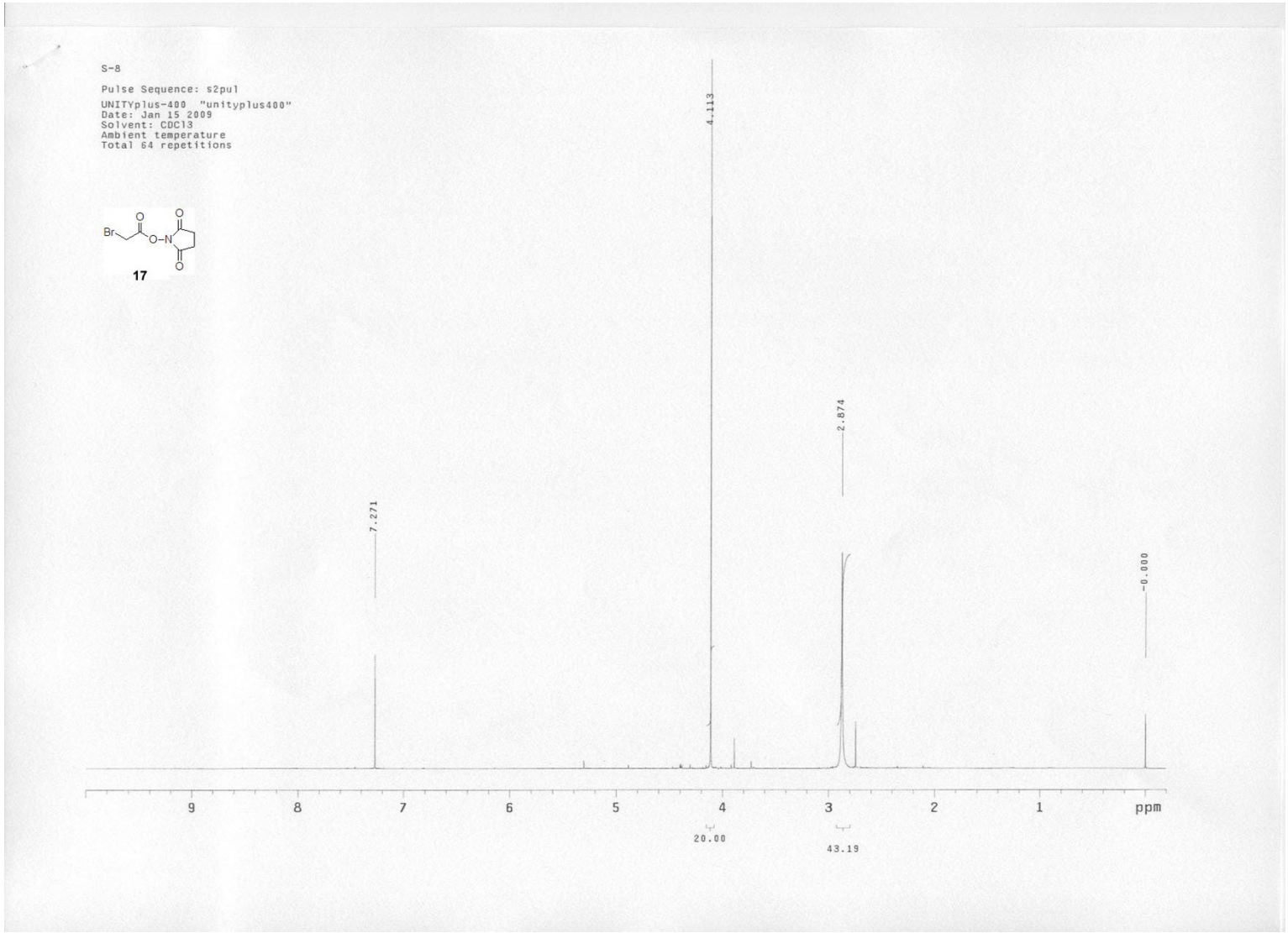

S-2-BOC
Mercury-400BB "Mercuryplus400"
Date 07 Jul 2008
Solvent: DMSO
Ambient temperature
Total 19200 repetitions
15b
ppm
20
40
60
80
100
120
140
160
180
24.989
25.224
25.345
26.118
28.043
28.224
28.990
30.786
35.226
35.310
35.802
37.280
37.356
37.537
37.840
38.310
38.871
39.083
39.295
39.500
39.712
39.864
39.917
40.129
55.451
59.202
61.051
77.820
155.537
162.342
162.728
171.814
172.238
172.284

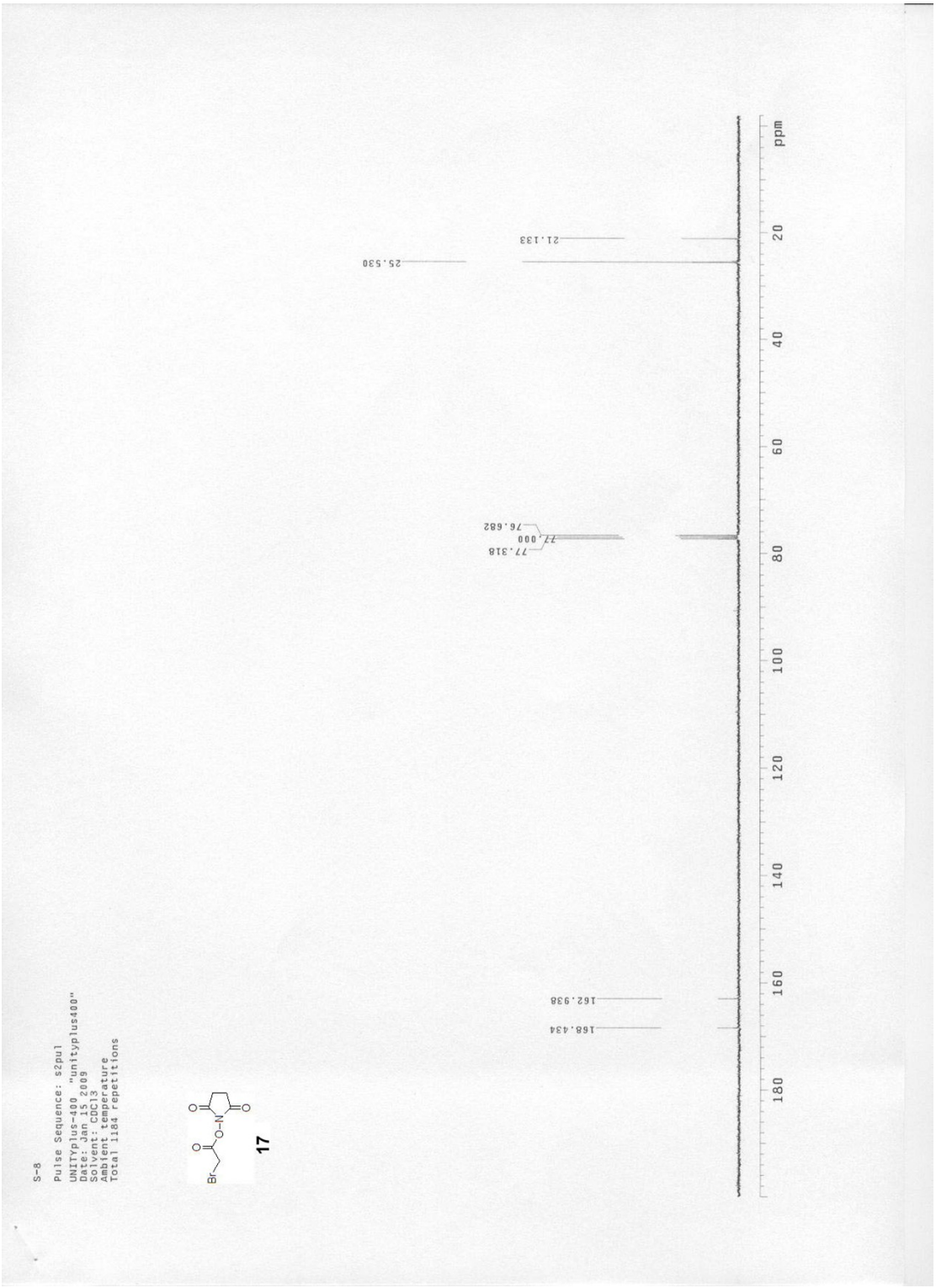

S-12 ESI+
Molecular Formula : C25H45N5O5S3Na
Exact Mass : 614.2480
Measured Mass : 614.2477
614.2477
15b
613.9
614.1
614.3
614.5
614.7
m/z
/d=/Data/yu/S12/3/pdata/1 Administrator Thu Sep 11 15:39:12 2008


S-7b
Pulse Sequence: s2pul
UNITyplus-400 "unityplus400"
Date: Dec 10 2008
Solvent: CD3OD
Ambient temperature
Total 160 repetitions
16b
3.314
3.310
3.306
3.302
3.318
3.665
3.506
3.168
2.996
2.979
2.858
2.684
2.211
2.199
2.024
1.220
ppm
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
10.00
9.60
21.05
101.04
30.63
33.18
20.41
13.29
37.63
58.99
63.89

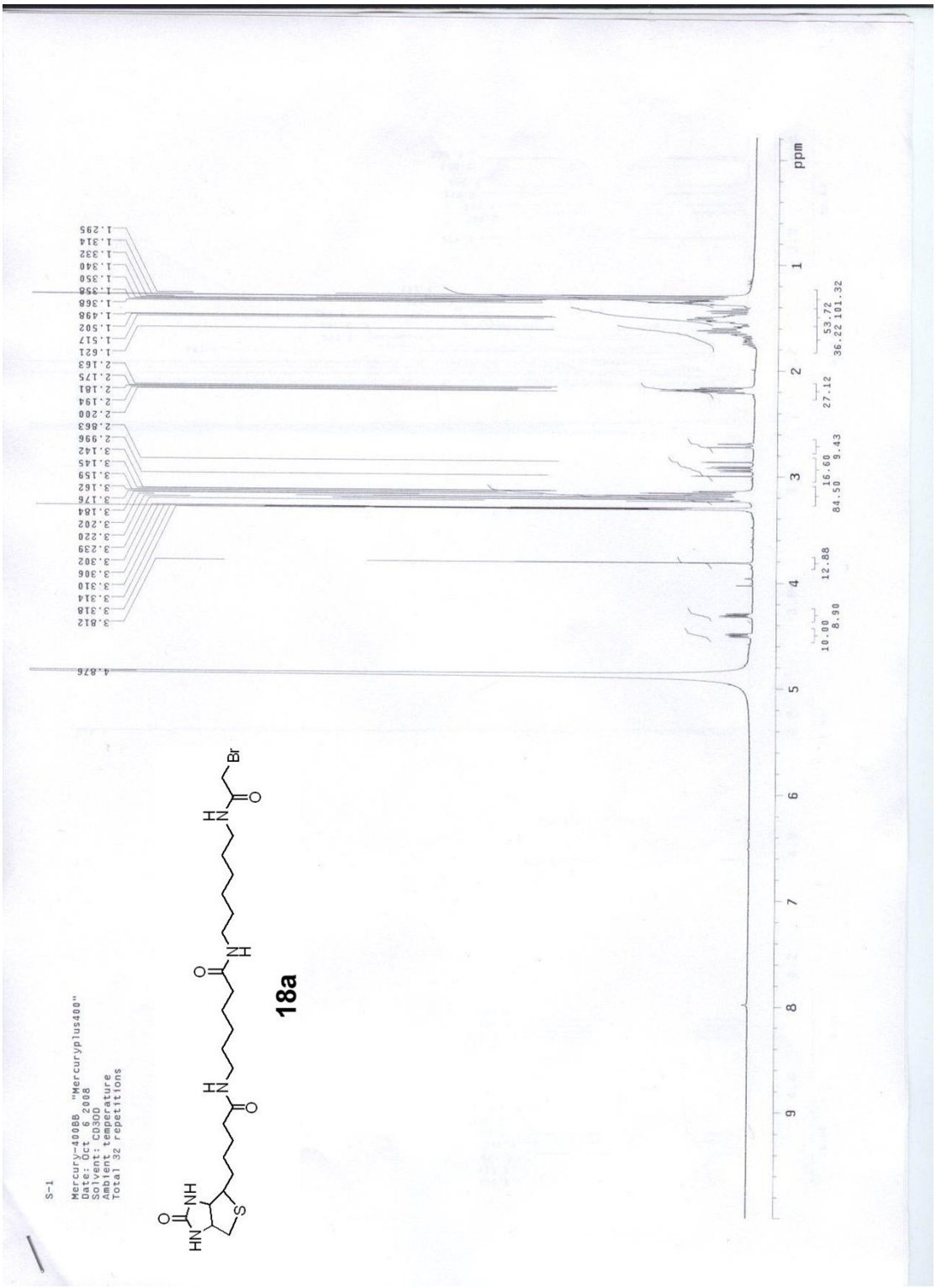

S-7b
Pulse Sequence: s2pul
UNITyplus-400 "unityplus400"
Date: Dec 10 2008
Solvent: CD3OD
Ambient temperature
Total 19200 repetitions
16b
ppm
20
40
60
80
100
120
140
160
180
26.562
26.926
27.480
29.488
29.754
30.110
35.462
36.811
36.902
36.319
39.328
39.411
40.169
41.041
48.356
48.568
48.780
49.000
49.212
49.424
49.637
57.005
61.651
63.395
114.615
117.442
176.061
176.402

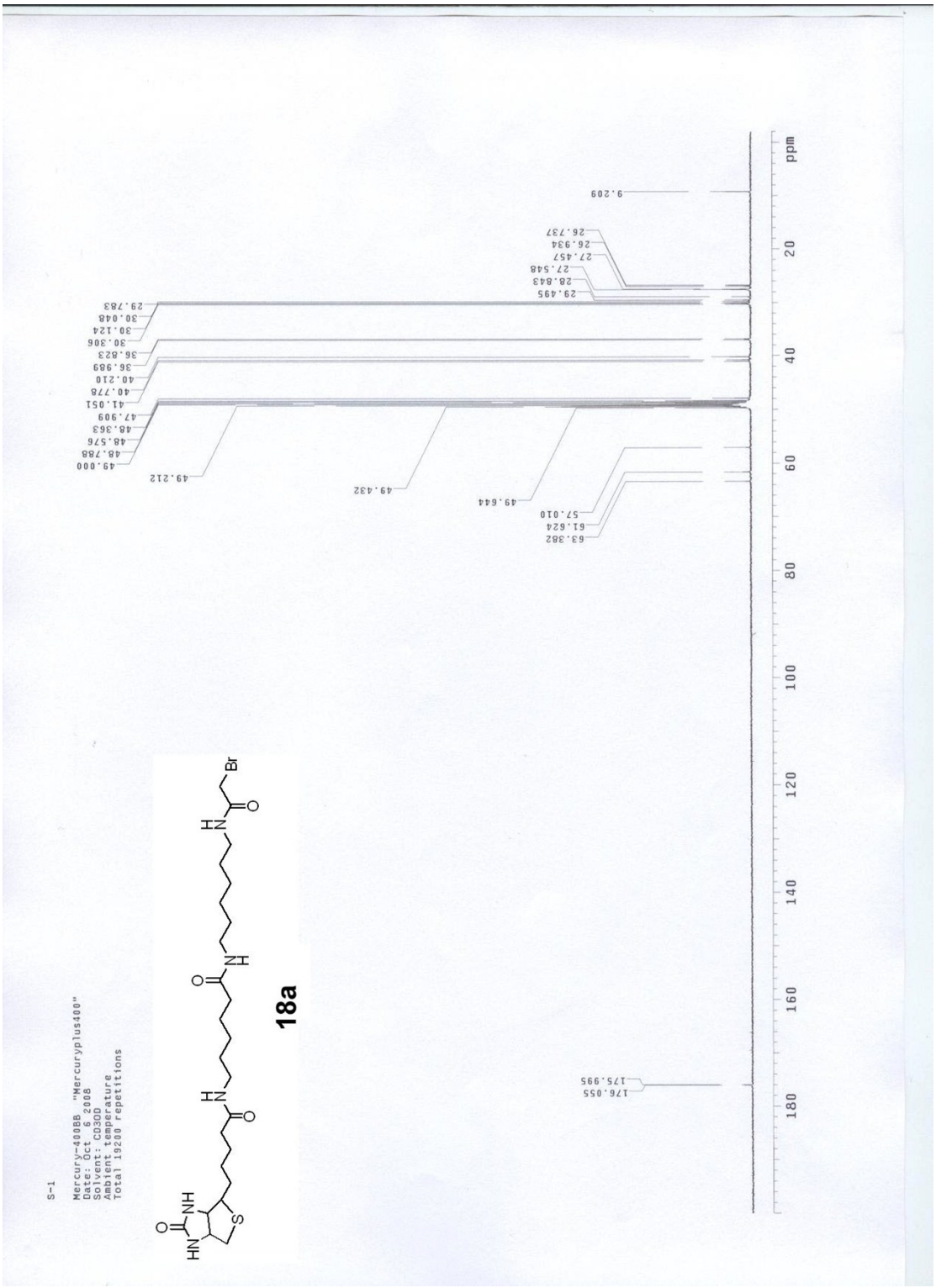

S-7b ESI+
Molecular Formula : C20H38N5O3S3
Exact Mass : 492.2137
Measured Mass: 492.2135
492.2135
16b
492.00
492.10
492.20
492.30
492.40
m/z
/d=/Data/yu/s7b/1/pdata/1 Administrator Mon Dec 1 17:04:01 2008

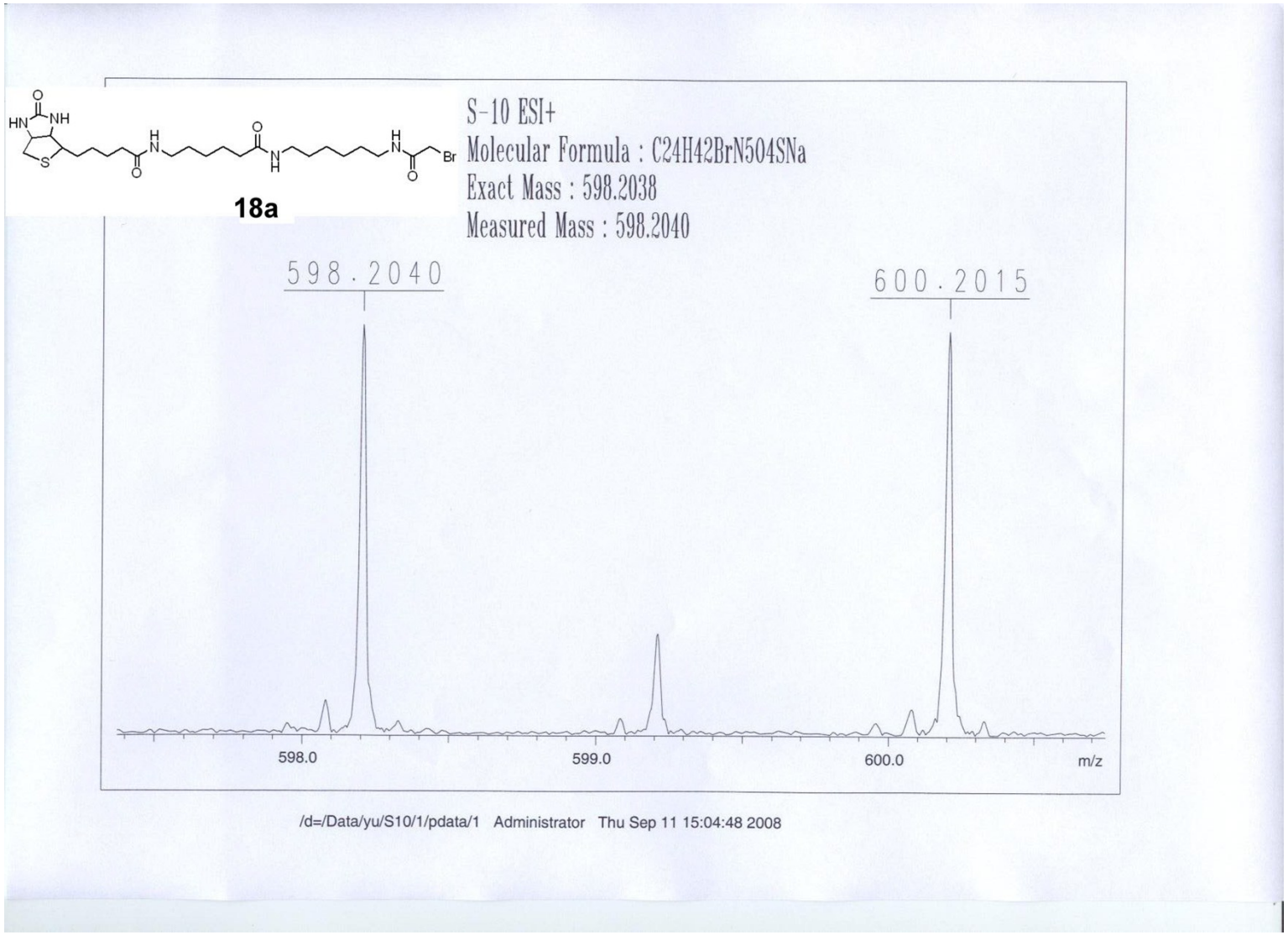

S-8
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Jan 15 2009
Solvent: CDCl3
Ambient temperature
Total 64 repetitions
Br
O
O-N
O
O
17
4.113
2.874
7.271
-0.000
9
8
7
6
5
4
3
2
1
ppm
20.00
43.19

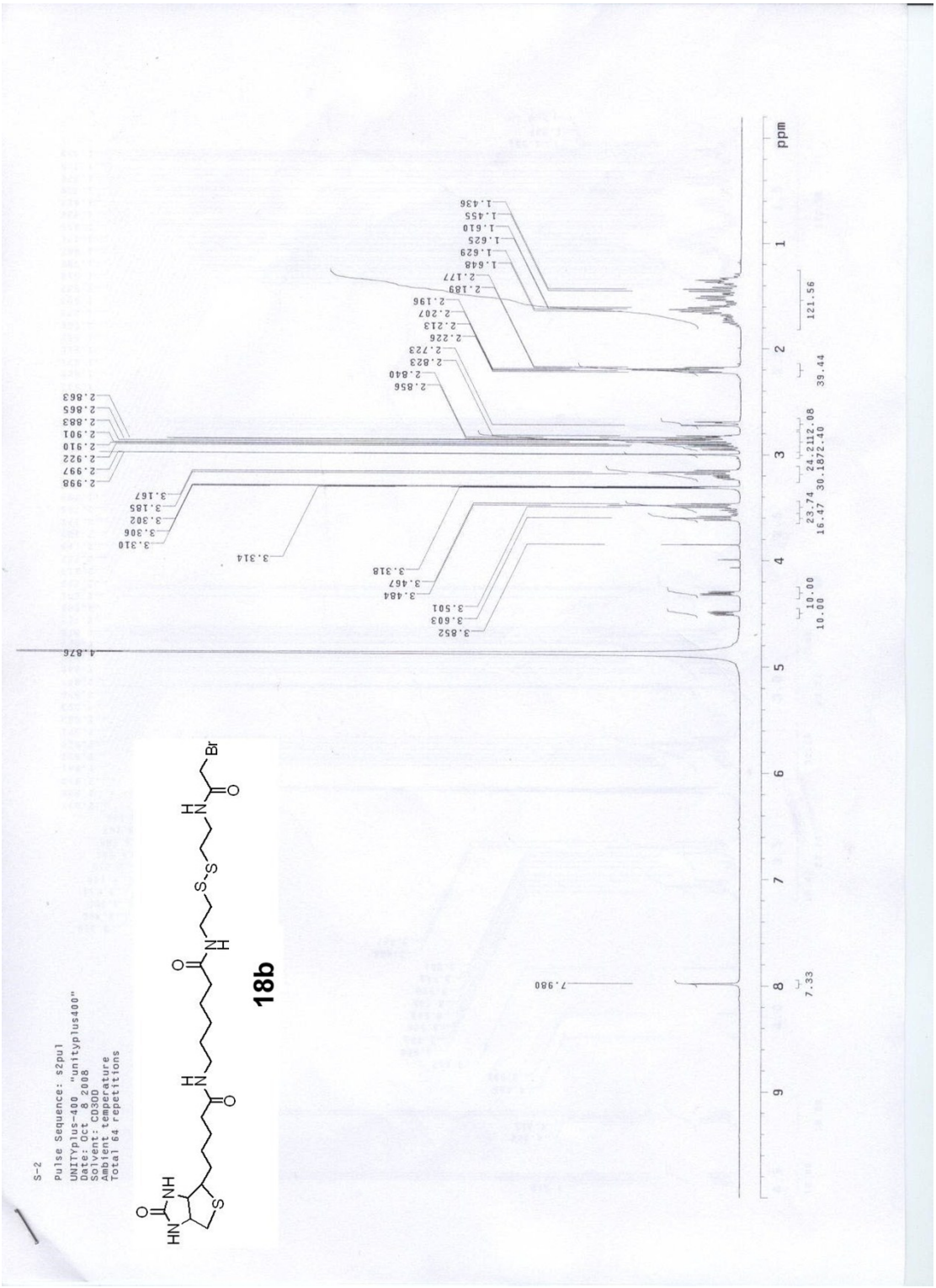

S-8
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Jan 15 2009
Solvent: CDCl3
Ambient temperature
Total 1184 repetitions
17
Br
168.434
162.938
77.318
77.000
76.682
25.530
21.133
ppm
180
160
140
120
100
80
60
40
20

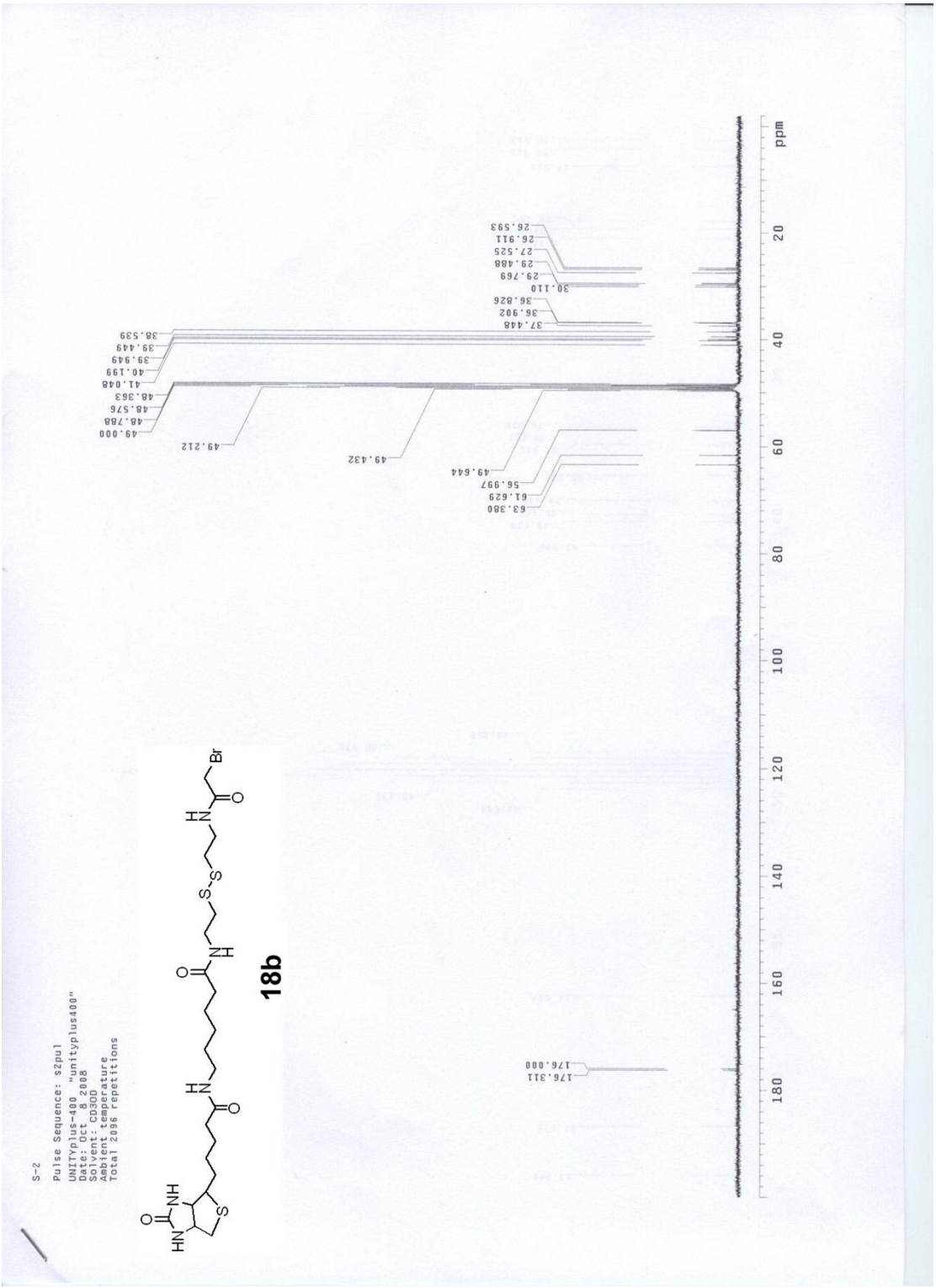

Br
O
O
N
O
O
17
S-8 ESI+
Molecular Formula : C6H604NBrNa
Exact Mass : 257.9378
Measured Mass: 257.9380
257.9380
259.9358
258.0
259.0
260.0
m/z
/d=/Data/yu/s8/1/pdata/1 Administrator Wed Jan 21 14:49:06 2009

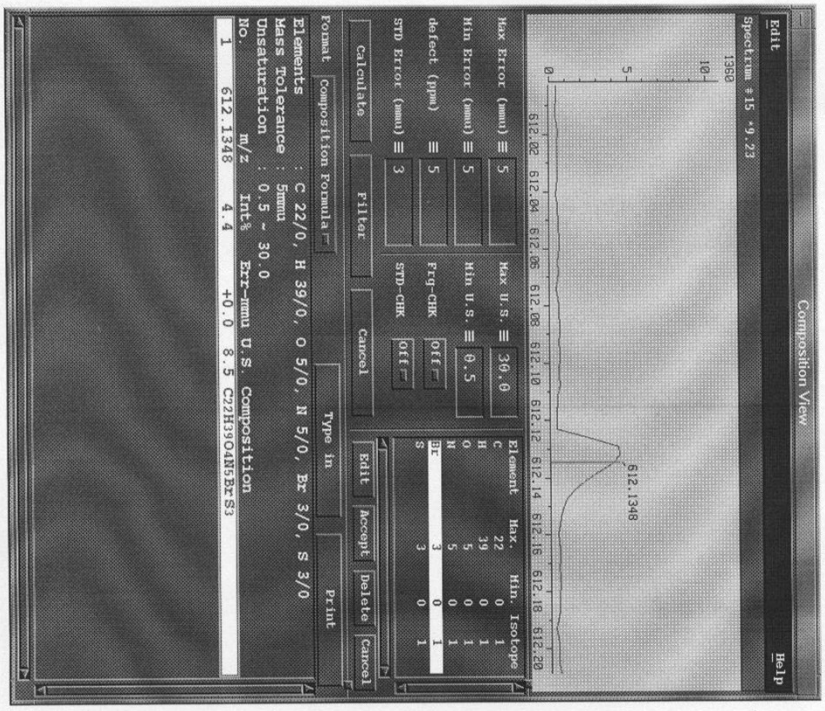

S-1
Mercury-400BB "Mercuryplus400"
Date: Oct 6 2008
Solvent: CD3OD
Ambient temperature
Total 32 repetitions
4.876
3.812
3.318
3.314
3.310
3.306
3.302
3.239
3.220
3.202
3.184
3.176
3.162
3.159
3.145
3.142
2.996
2.863
2.200
2.194
2.181
2.175
2.163
1.621
1.517
1.502
1.498
1.368
1.358
1.350
1.340
1.332
1.314
1.295
10.00
8.90
12.88
16.60
84.50
9.43
27.12
53.72
36.22
101.32
ppm
18a

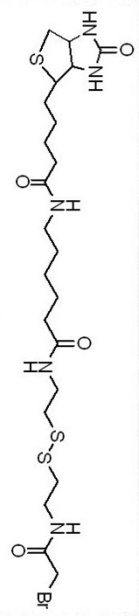

S-1
Mercury-400BB "Mercuryplus400"
Date: Oct 6 2008
Solvent: CD3OD
Ambient temperature
Total 19200 repetitions
18a
Br
O
NH
NH
NH
O
O
O
NH
NH
S
ppm
20
40
60
80
100
120
140
160
180
9.209
26.737
26.934
27.457
27.548
28.843
29.495
29.783
30.048
30.124
30.306
36.823
36.989
40.210
40.778
41.051
47.909
48.363
48.576
48.788
49.000
49.212
49.432
49.644
57.010
61.624
63.382
175.995
176.055


18a
S-10 ESI+
Molecular Formula : C24H42BrN5O4SNa
Exact Mass : 598.2038
Measured Mass : 598.2040
598.2040
600.2015
598.0
599.0
600.0
m/z
/d=/Data/yu/S10/1/pdata/1 Administrator Thu Sep 11 15:04:48 2008


S-2
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date: Oct 8 2008
Solvent: CD3OD
Ambient temperature
Total 64 repetitions
18b
Br
O
N
H
S
HN
NH
ppm
4.876
7.980
3.852
3.603
3.501
3.484
3.467
3.318
3.314
3.310
3.306
3.302
3.185
3.167
2.998
2.997
2.922
2.910
2.901
2.883
2.865
2.863
2.856
2.840
2.823
2.723
2.226
2.213
2.207
2.196
2.189
2.177
1.648
1.629
1.625
1.610
1.455
1.436
10.00
10.00
23.74
16.47
24.2112.08
30.1872.40
39.44
121.56
7.33


S-2
Pulse Sequence: s2pul
UNITYplus-400 "unityplus400"
Date_ Oct 3 2008
Solvent CD3OD
Ambient temperature
Total 2096 repetitions
18b
ppm
20
40
60
80
100
120
140
160
180
176.311
176.000
63.380
61.629
56.997
49.644
49.432
49.212
49.000
48.788
48.576
48.363
41.048
40.199
39.949
39.449
38.539
37.448
36.902
36.826
30.110
29.769
29.488
27.525
26.911
26.593





18b

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[ Theoretical Ion Distribution ]                                    Page: 1
Molecular Formula : C22 H39 O4 N5 Br S3
                          (m/z   612.1348, MW   613.6848, U.S.  8.5)
Base Peak :   614.1328, Averaged MW :   613.6814(a),   613.6843(w)

    m/z       INT.
  612.1348  86.5444 ***************************************************
  613.1375  25.4538 ***************
  614.1328 100.0000 ************************************************************
  615.1353  28.5872 ******************
  616.1311  16.5450 **********
  617.1326   3.9507 **
  618.1298   1.1769 *
  619.1305   0.2293
  620.1291   0.0446
  621.1294   0.0069
  622.1292   0.0010
  623.1297   0.0001
```