Supplement methods (for supplement Figure 6 and Figure 8)

Topo IIα relaxation activity assay

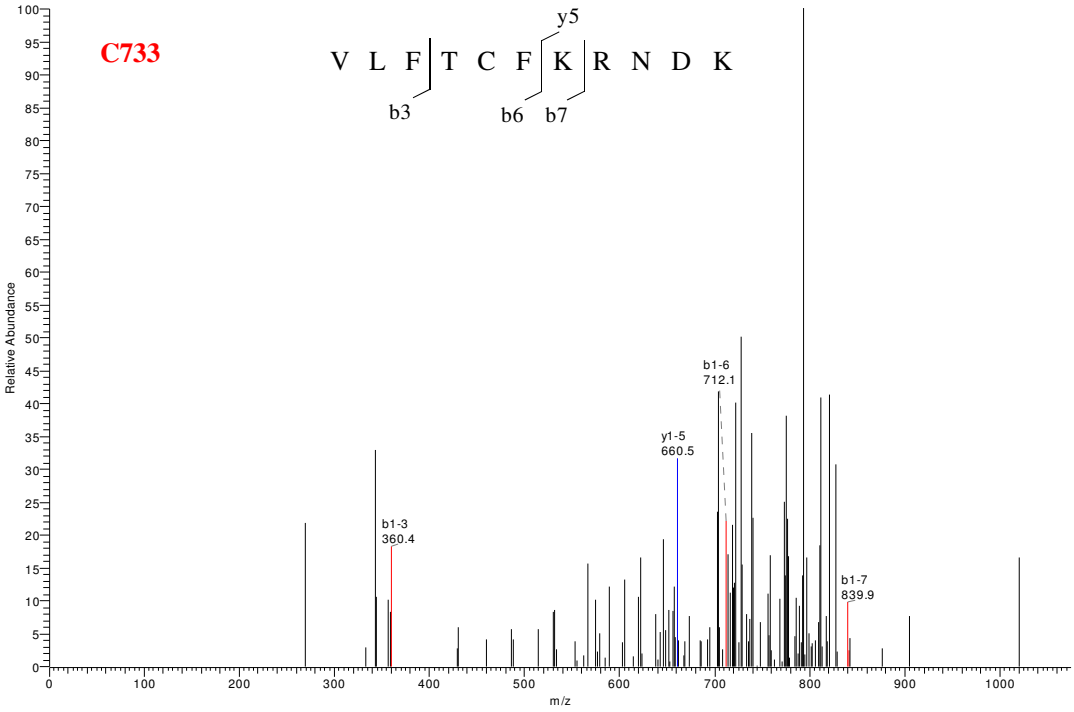
Topo IIα relaxation activity assay was performed in a 20-μL reaction mixture containing 400 ng supercoiled DNA and 8 ng recombinant Topo IIα (the amount that gives approximately 90% relaxation) in the Topo IIα reaction buffer. The reaction was performed at 37℃ for indicated time periods. All reactions were stopped by adding 4 μL of loading buffer containing 7 mM EDTA, 1% SDS, 50% glycerol and 0.2% bromophenol blue. The reactions were then resolved in 1% agarose gel by electrophoresis at 50 mV for 1.5 h to separate the supercoiled DNA, relaxed DNA and nicked DNA. The gels were stained with EtBr and photographed using a gel-imaging system. The percentages of supercoiled and relaxed forms of DNA in each lane were quantified by using ImageJ software.

Supplement Table 1. List of primer sequences

gene	sequence
DDIT3-forward	5’- ATGTTAAAGATGAGCGGGTGGCAGC-3’
DDIT3-reverse	5’-TTGAACACTCTCTCCTCAGGTTCCA-3’
GADD45A-forward	5’-CAAGCTGCTCAACGTCGACCC-3’
GADD45A-reverse	5’- GGTTGCTGACGCGCAGGATGT-3’
GADD45G-forward	5’- AGTCTTGAACGTGGACCCCGAC-3’
GADD45G-reverse	5’- TCGCCCACGCGCACTATGTC-3’
MDM2-forward	5’- TTTCGCAGCCAGGAGCACCG-3’
MDM2-reverse	5’- TGCACATTTGCCTGCTCCTCACC-3’
REX1-forward	5’- CGGGCCCACGCCAAGTTTCA-3’
REX1-reverse	5’- GCCCTGCGCCATCCCATTGT-3’
ATR-forward	5’- GCTGGTCACCACCAGACAGCC-3’
ATR-reverse	5’- ACATCACCCTTGGACCAGAGCCA -3’
GAPDH-forward	5’-GGCTCTCCAGAACATCATCC-3’
GAPDH-reverse	5’-GCTTCACCACCTTCTTGATG-3’

Supplement Figure 1. MS/MS spectra of cysteine-containing Topo IIα peptides with no HQ17(3) modification obtained from Huh7 cells

A.



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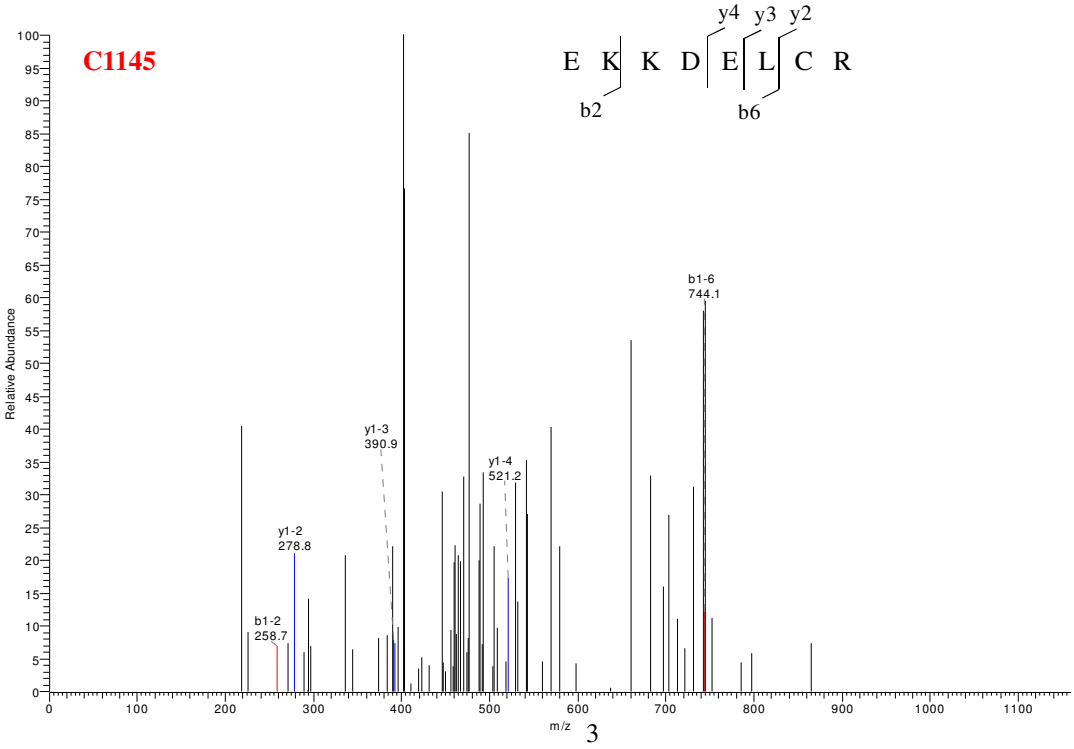
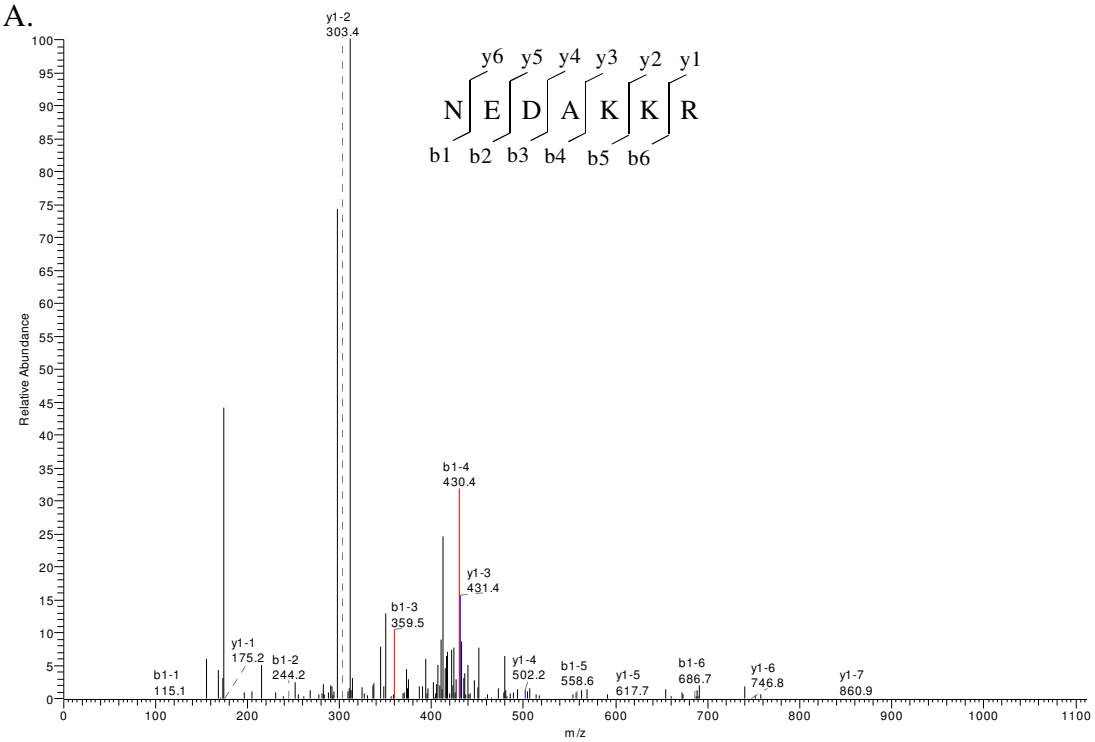


Supplement Figure 2. MS/MS spectra of tryptic Topo IIα peptides obtained from Huh7 cells

A.



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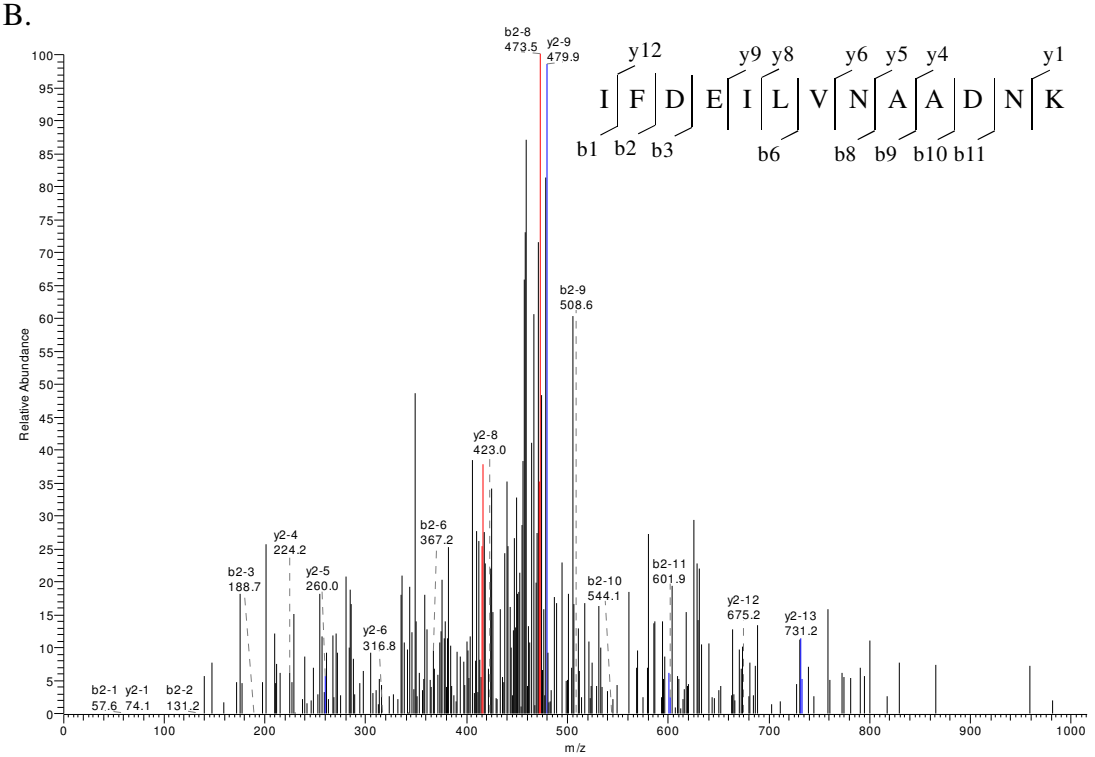

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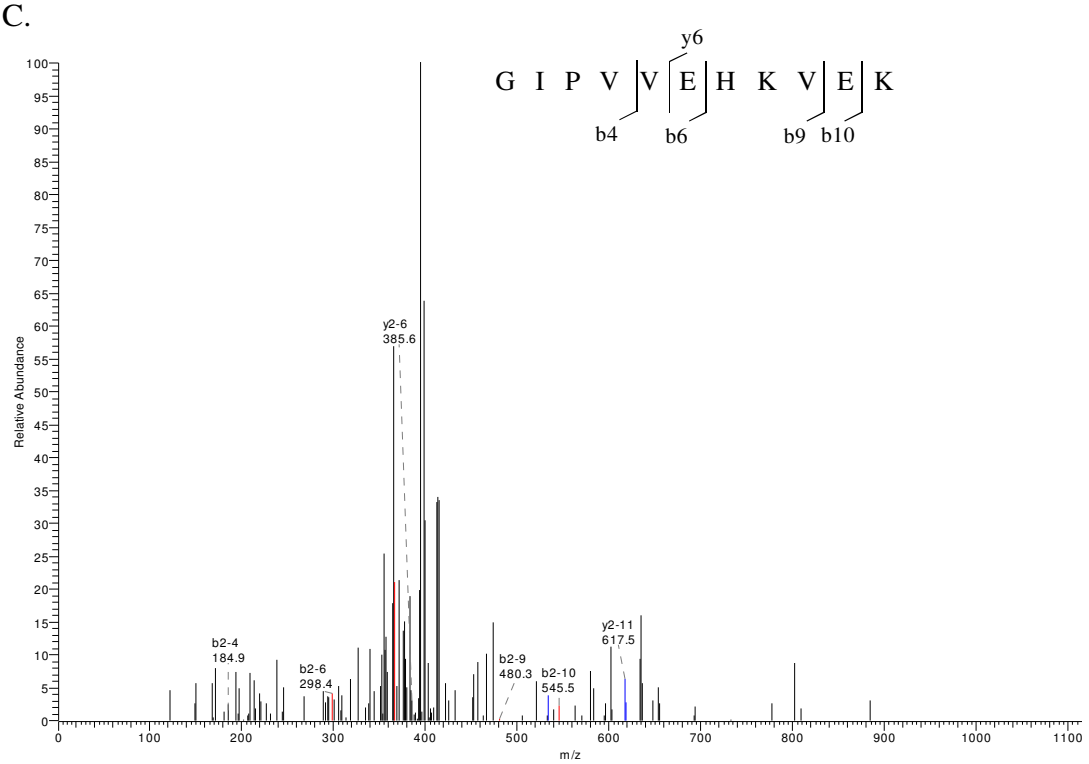
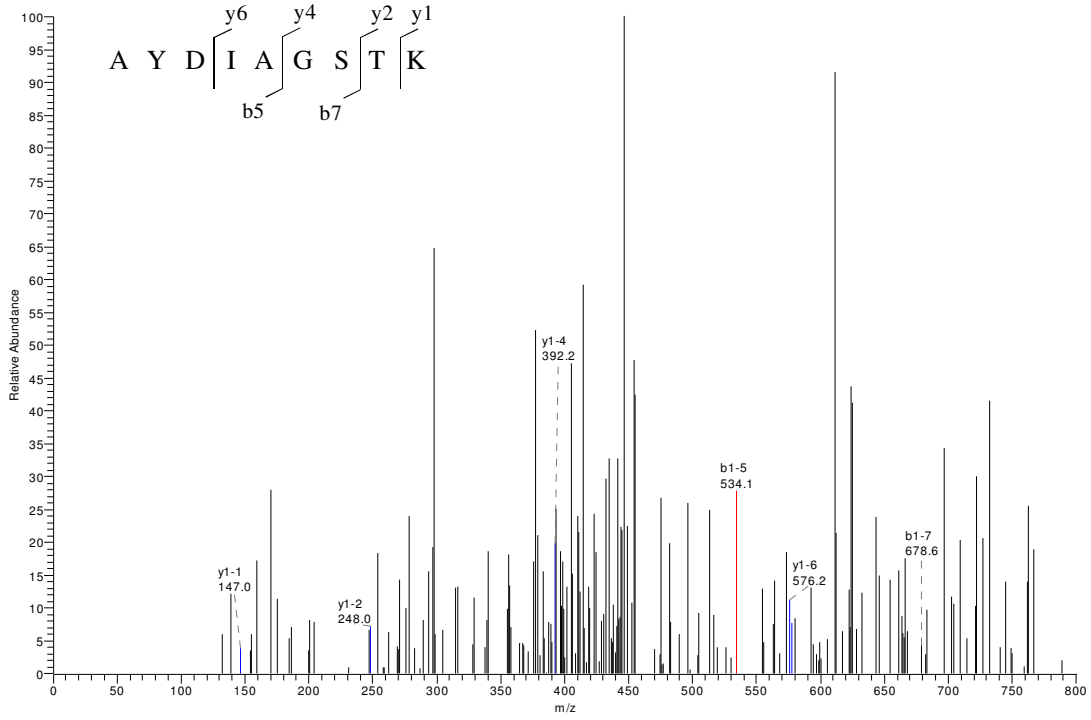
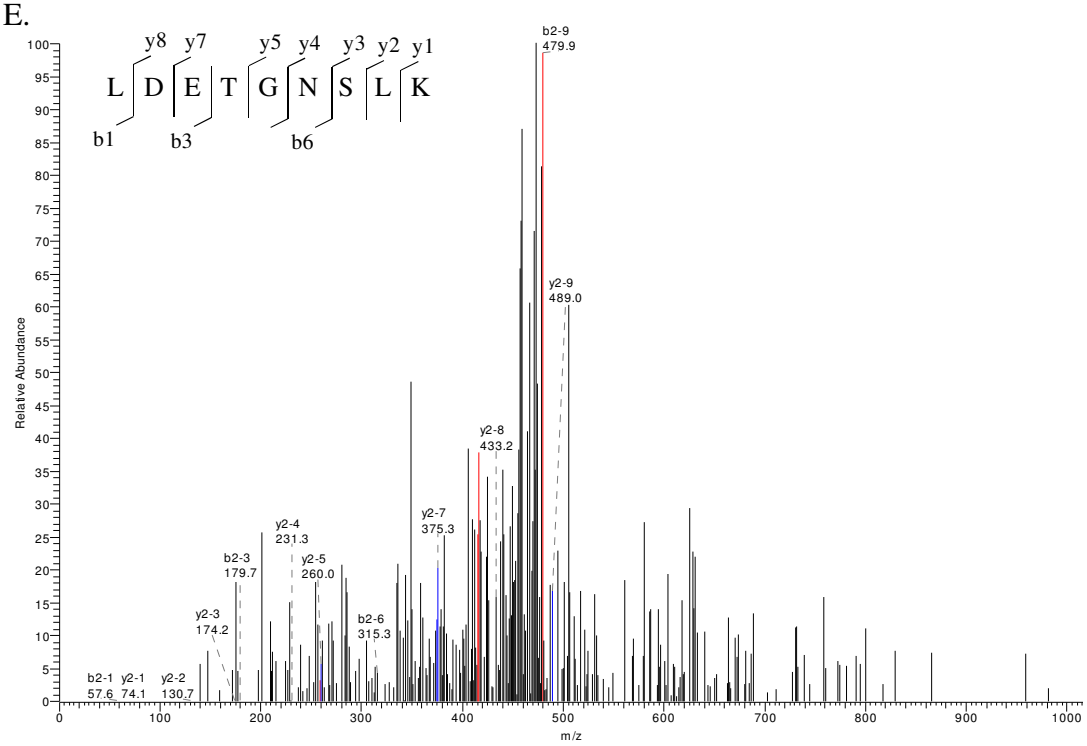

y6
G I P V V E H K V E K
b4
b6
b9
b10
Relative Abundance
y2-6
385.6
b2-4
184.9
b2-6
298.4
b2-9
480.3
b2-10
545.5
y2-11
617.5
m/z

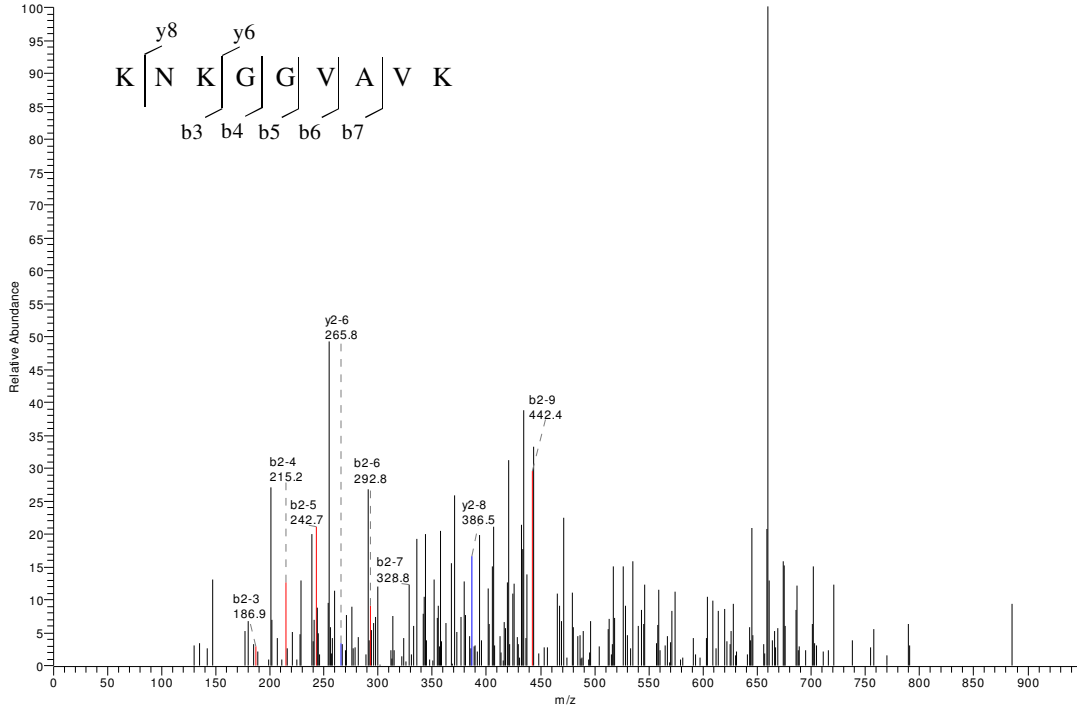
D.


y6
y4
y2
y1
A Y D I A G S T K
b5
b7
Relative Abundance
y1-4
392.2
b1-5
534.1
b1-7
678.6
y1-6
576.2
y1-1
147.0
y1-2
248.0
m/z

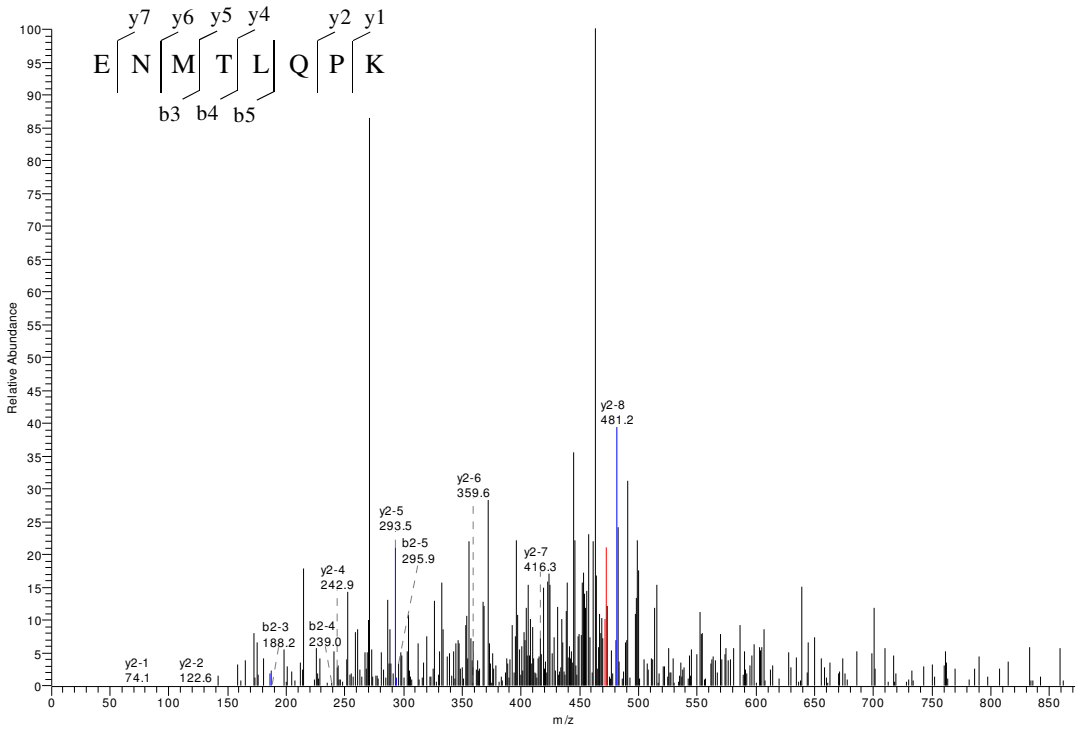
E.


y8 y7 y5 y4 y3 y2 y1
L D E T G N S L K
b1 b3 b6
b2-9 479.9
y2-9 489.0
y2-8 433.2
y2-7 375.3
y2-4 231.3
b2-3 179.7
y2-5 260.0
y2-3 174.2
b2-6 315.3
b2-1 57.6
y2-1 74.1
y2-2 130.7
Relative Abundance
m/z

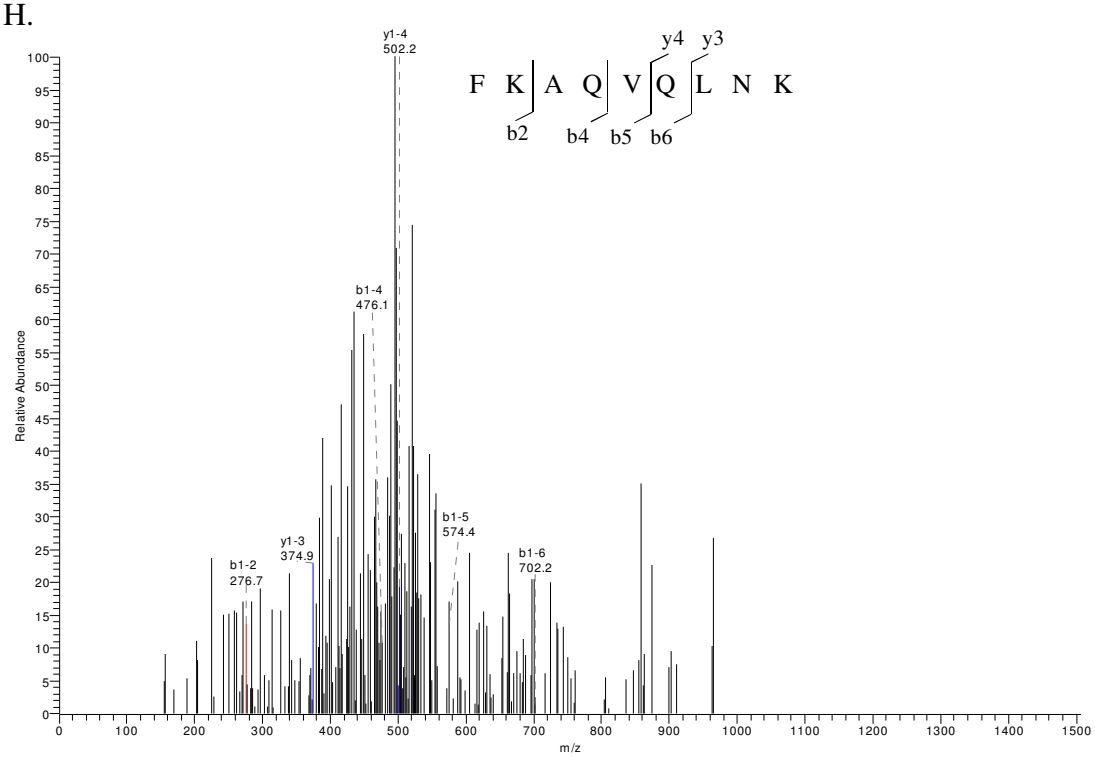
F.


y8 y6
K N K G G V A V K
b3 b4 b5 b6 b7
y2-6 265.8
b2-9 442.4
b2-4 215.2
b2-6 292.8
b2-5 242.7
y2-8 386.5
b2-7 328.8
b2-3 186.9
Relative Abundance
m/z

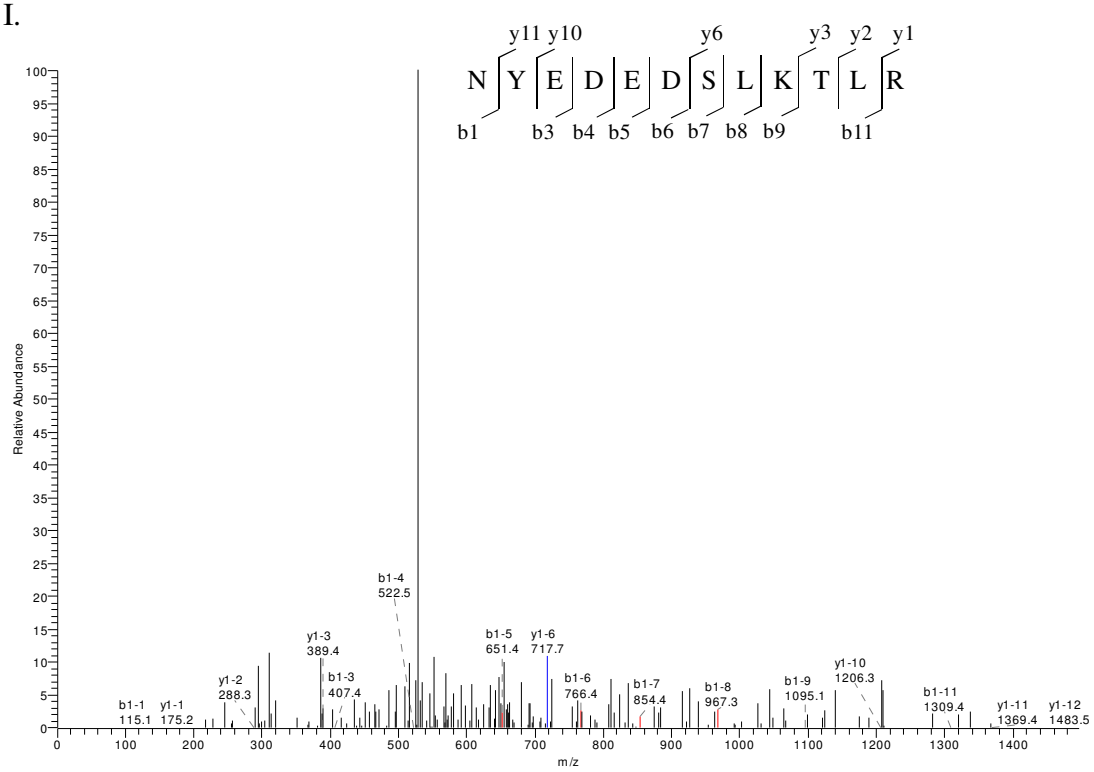
G.


y7 y6 y5 y4 y2 y1
E N M T L Q P K
b3 b4 b5
Relative Abundance
m/z
y2-8 481.2
y2-6 359.6
y2-5 293.5
b2-5 295.9
y2-7 416.3
y2-4 242.9
b2-3 188.2
b2-4 239.0
y2-1 74.1
y2-2 122.6

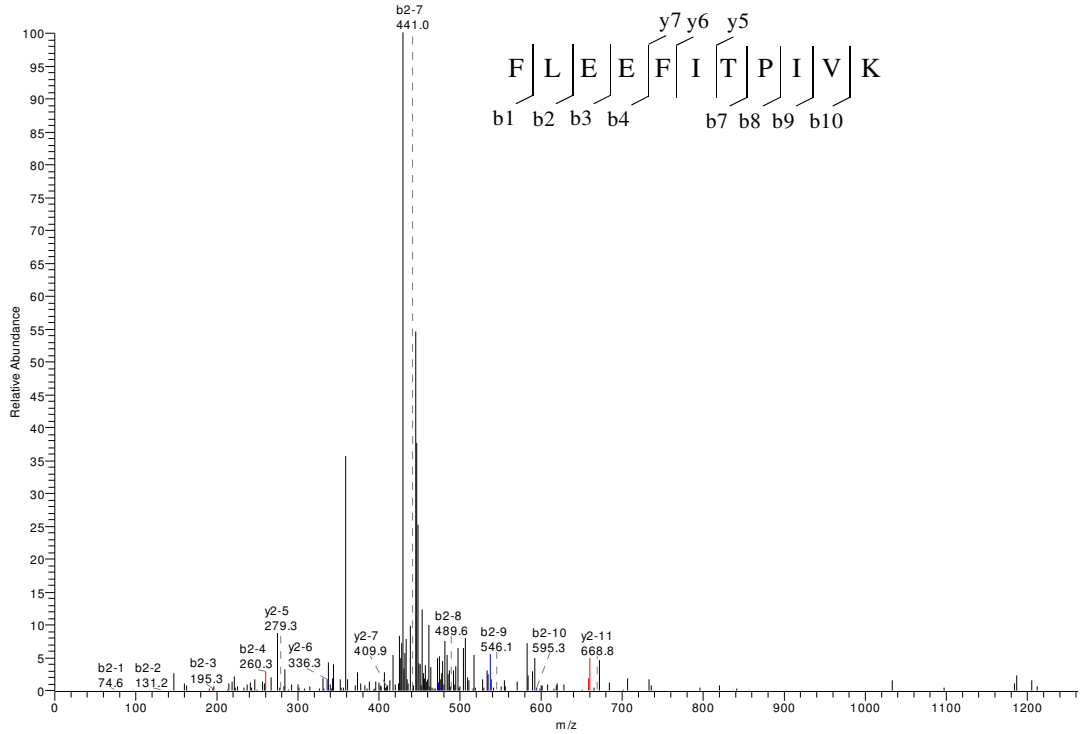
H.


y1-4 502.2
y4 y3
F K A Q V Q L N K
b2 b4 b5 b6
Relative Abundance
m/z
b1-4 476.1
b1-5 574.4
y1-3 374.9
b1-2 276.7
b1-6 702.2

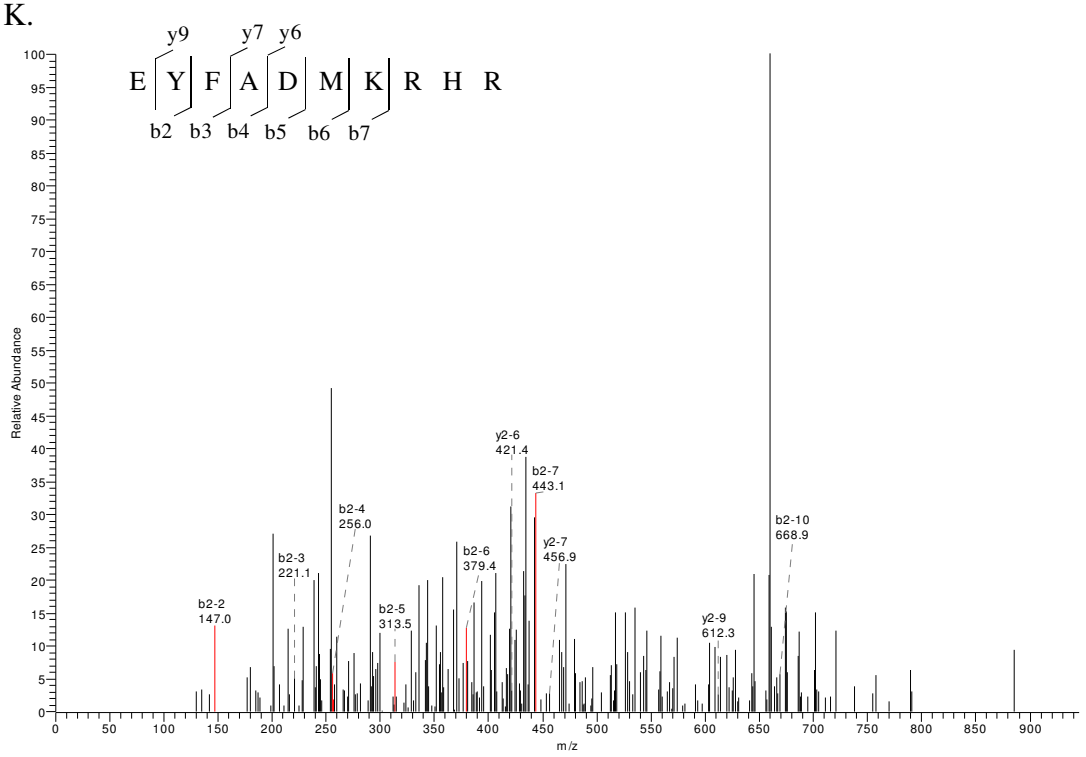
I.


y11 y10 y6 y3 y2 y1
N Y E D E D S L K T L R
b1 b3 b4 b5 b6 b7 b8 b9 b11
Relative Abundance
m/z
b1-4 522.5
y1-3 389.4
b1-5 651.4
y1-6 717.7
y1-2 288.3
b1-3 407.4
b1-6 766.4
b1-7 854.4
b1-8 967.3
b1-9 1095.1
y1-10 1206.3
b1-11 1309.4
y1-11 1369.4
y1-12 1483.5
b1-1 115.1
y1-1 175.2

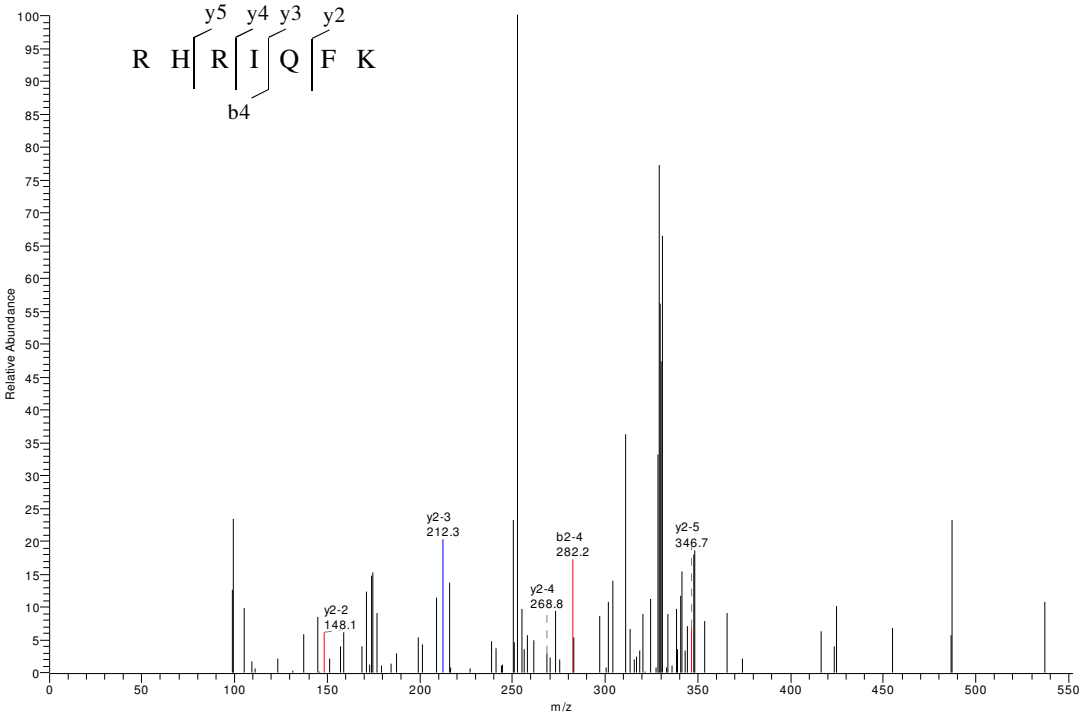
J.


y7 y6 y5
F L E E F I T P I V K
b1 b2 b3 b4 b7 b8 b9 b10
Relative Abundance
m/z
b2-7 441.0
y2-5 279.3
b2-8 489.6
b2-9 546.1
b2-10 595.3
y2-11 668.8
y2-7 409.9
b2-4 260.3
y2-6 336.3
b2-3 195.3
b2-1 74.6
b2-2 131.2

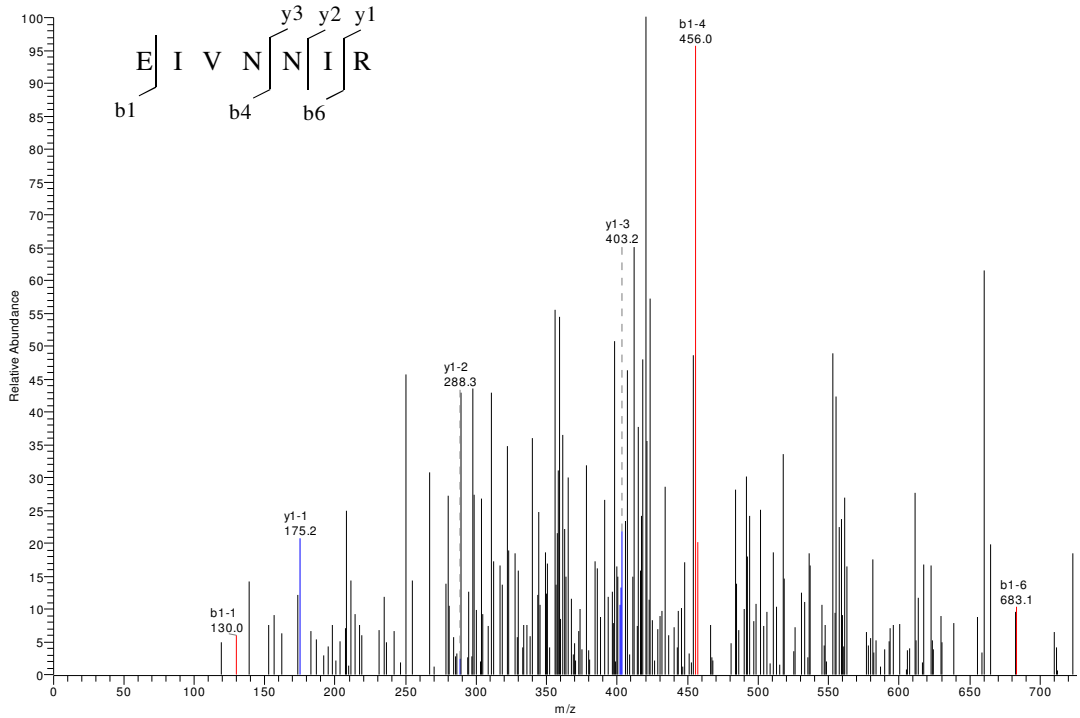
K.


y9 y7 y6
E Y F A D M K R H R
b2 b3 b4 b5 b6 b7
Relative Abundance
m/z
b2-2 147.0
b2-3 221.1
b2-4 256.0
b2-5 313.5
b2-6 379.4
y2-6 421.4
b2-7 443.1
y2-7 456.9
y2-9 612.3
b2-10 668.9

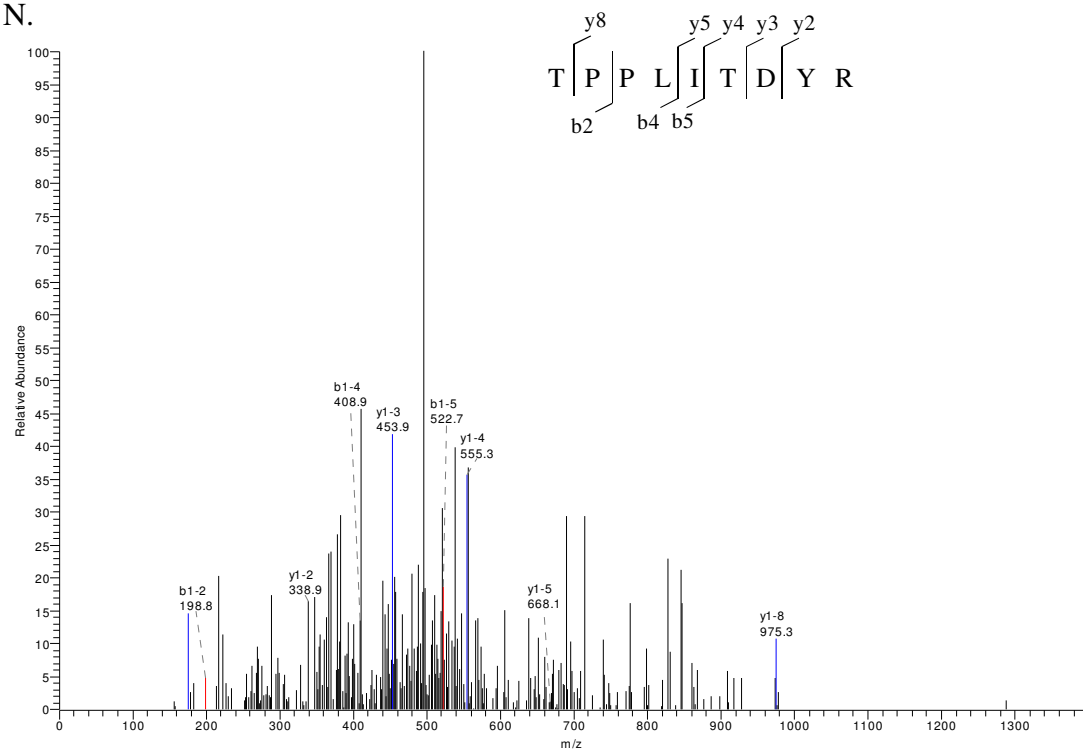
L.


y5 y4 y3 y2
R H R I Q F K
b4
Relative Abundance
m/z
y2-2 148.1
y2-3 212.3
y2-4 268.8
b2-4 282.2
y2-5 346.7

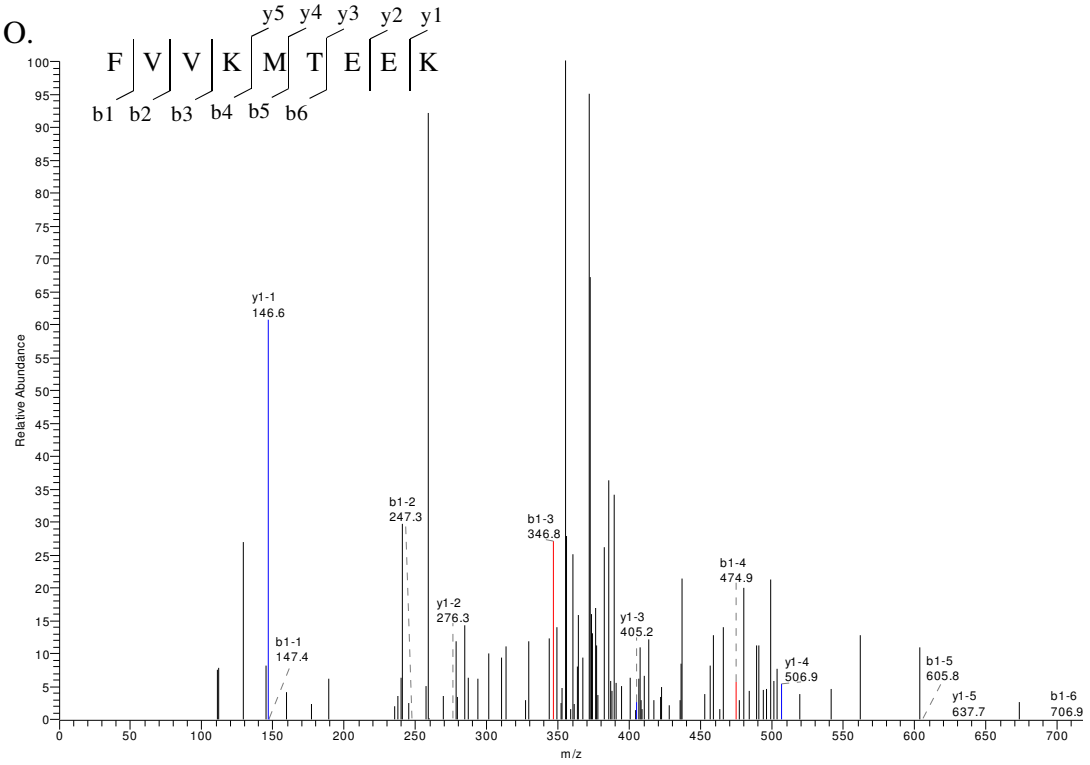
M.


y3 y2 y1
E I V N N I R
b1
b4
b6
b1-4
456.0
y1-3
403.2
y1-2
288.3
y1-1
175.2
b1-1
130.0
b1-6
683.1
Relative Abundance
m/z

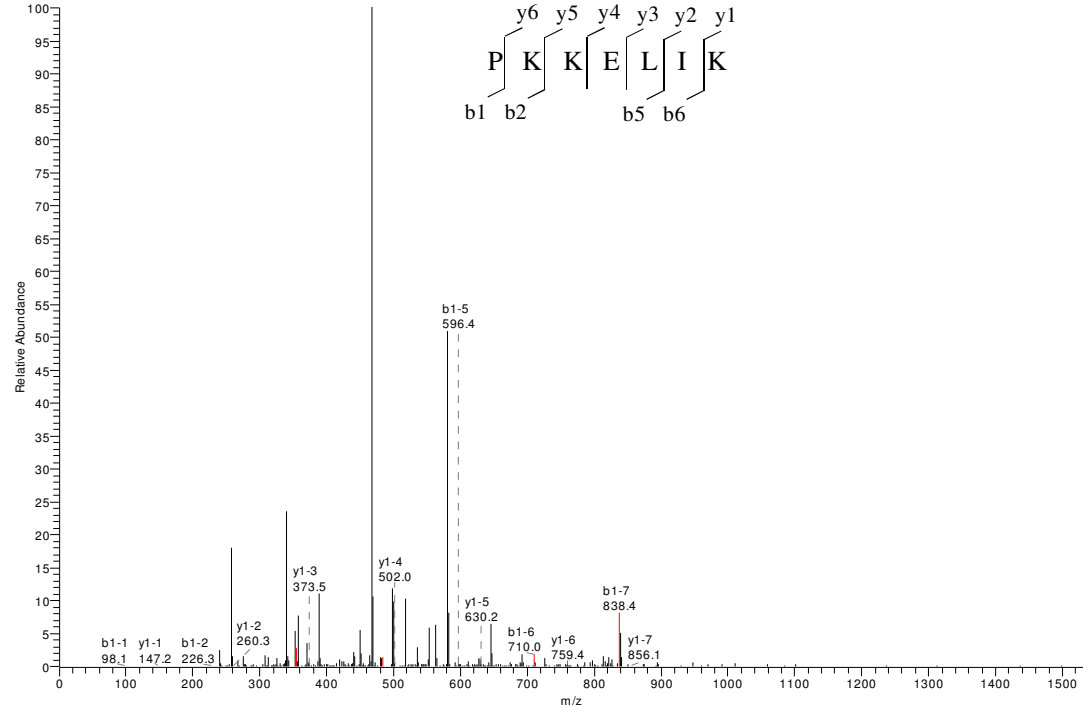
N.


y8
y5 y4 y3 y2
T P P L I T D Y R
b2
b4 b5
b1-4
408.9
y1-3
453.9
b1-5
522.7
y1-4
555.3
b1-2
198.8
y1-2
338.9
y1-5
668.1
y1-8
975.3
Relative Abundance
m/z

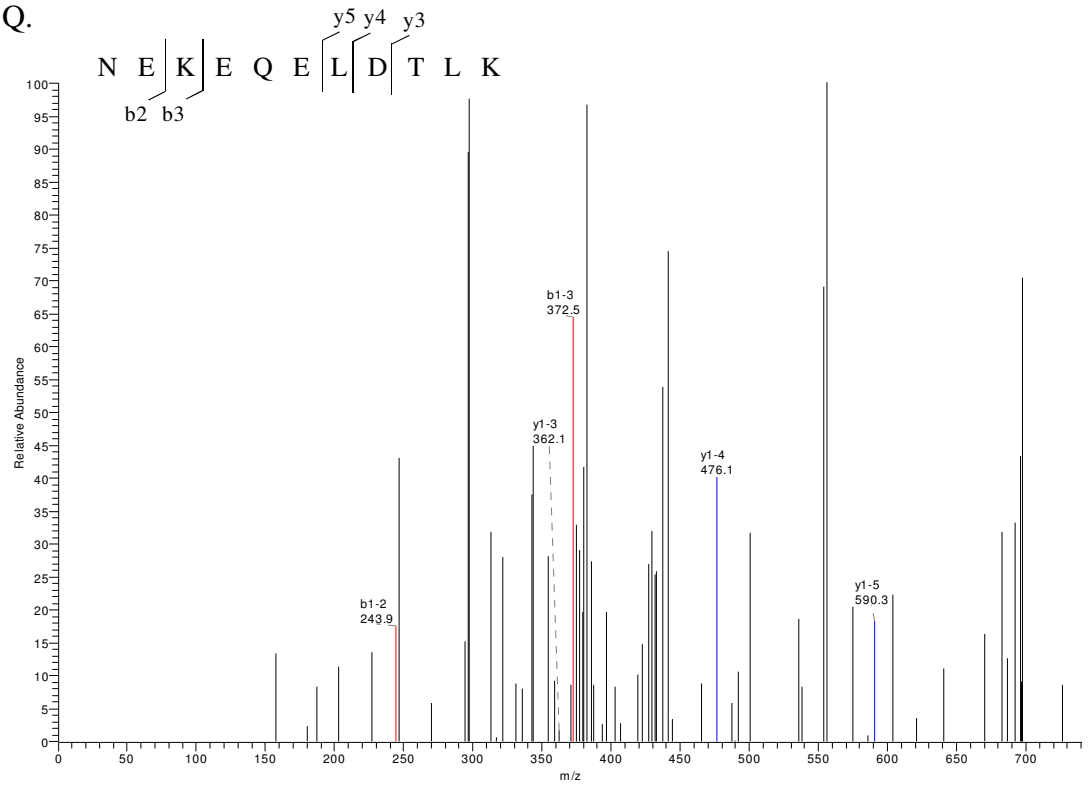
O.


y5 y4 y3 y2 y1
F V V K M T E E K
b1 b2 b3 b4 b5 b6
Relative Abundance
m/z
y1-1
146.6
b1-2
247.3
b1-3
346.8
b1-4
474.9
y1-2
276.3
y1-3
405.2
b1-1
147.4
y1-4
506.9
b1-5
605.8
y1-5
637.7
b1-6
706.9

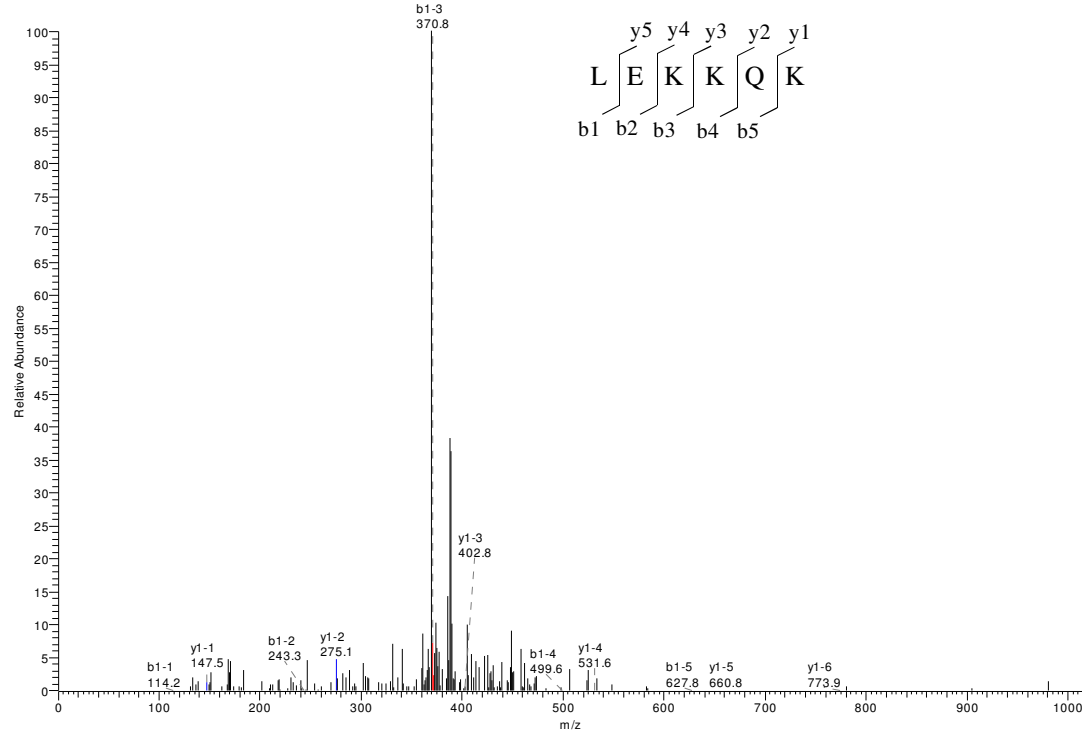
P.


y6 y5 y4 y3 y2 y1
P K K E L I K
b1 b2 b5 b6
Relative Abundance
m/z
b1-5
596.4
y1-4
502.0
y1-3
373.5
y1-5
630.2
b1-7
838.4
y1-2
260.3
b1-6
710.0
y1-6
759.4
y1-7
856.1
b1-1
98.1
y1-1
147.2
b1-2
226.3

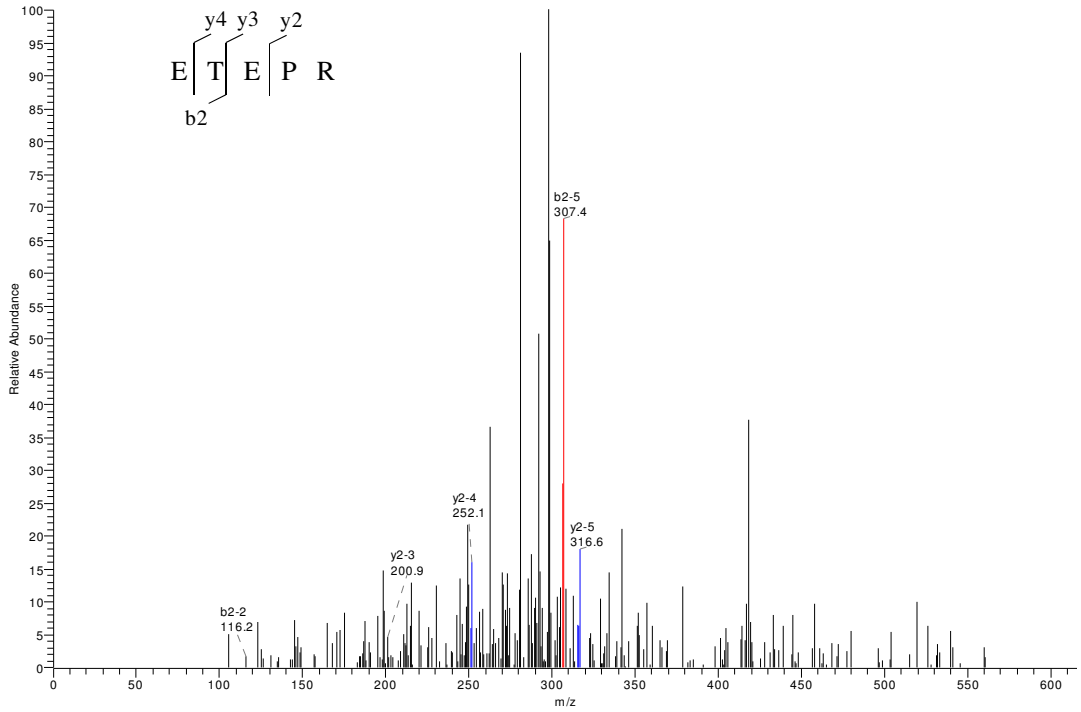
Q.


y5 y4 y3
N E K E Q E L D T L K
b2 b3
b1-3
372.5
y1-3
362.1
y1-4
476.1
b1-2
243.9
y1-5
590.3
Relative Abundance
m/z

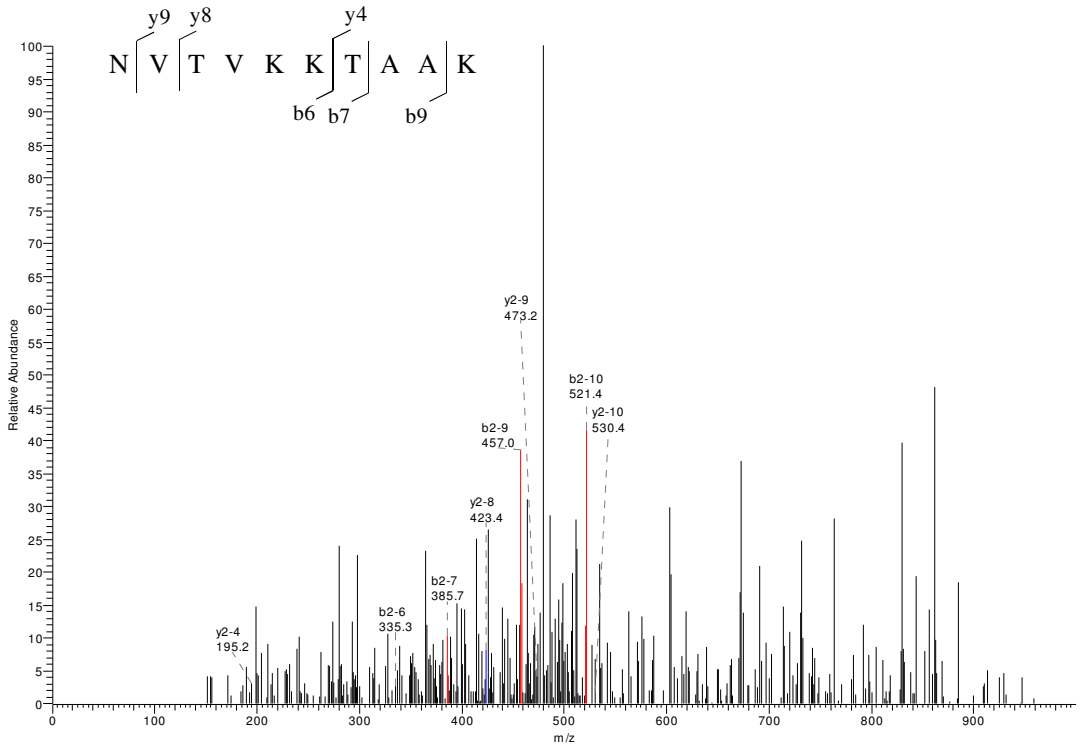
R.


b1-3
370.8
y5 y4 y3 y2 y1
L E K K Q K
b1 b2 b3 b4 b5
y1-3
402.8
y1-1
147.5
b1-2
243.3
y1-2
275.1
b1-1
114.2
b1-4
499.6
y1-4
531.6
b1-5
627.8
y1-5
660.8
y1-6
773.9
Relative Abundance
m/z

S.

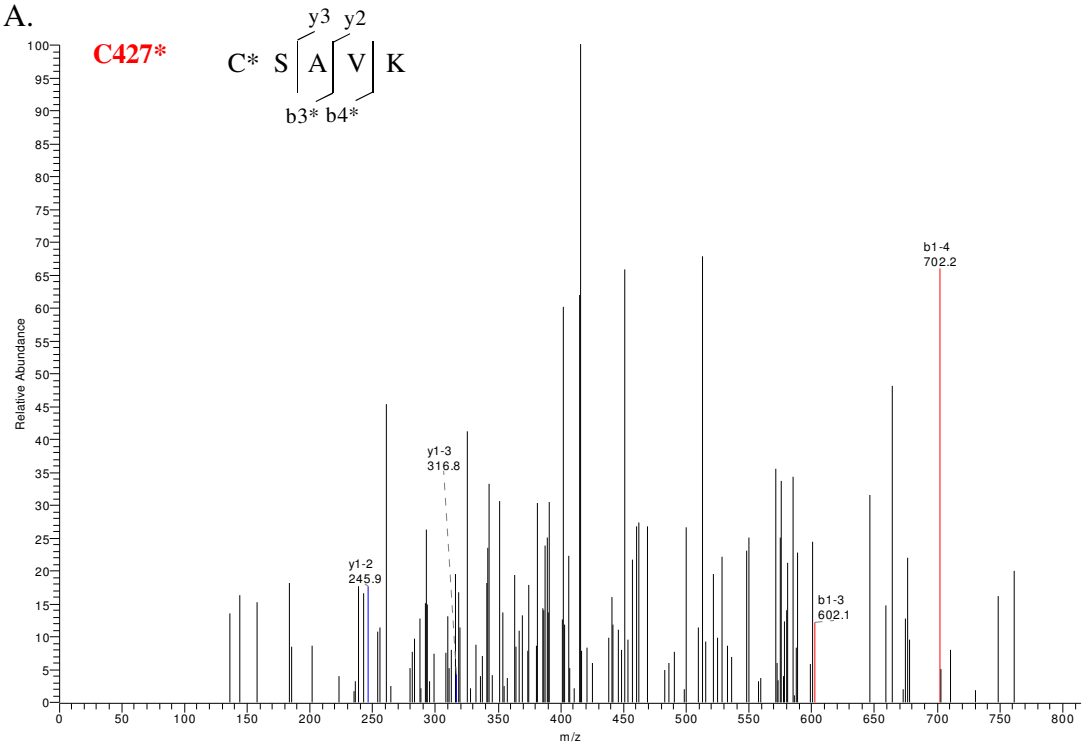

y4 y3 y2
E T E P R
b2
b2-5
307.4
y2-4
252.1
y2-5
316.6
y2-3
200.9
b2-2
116.2
Relative Abundance
m/z

T.

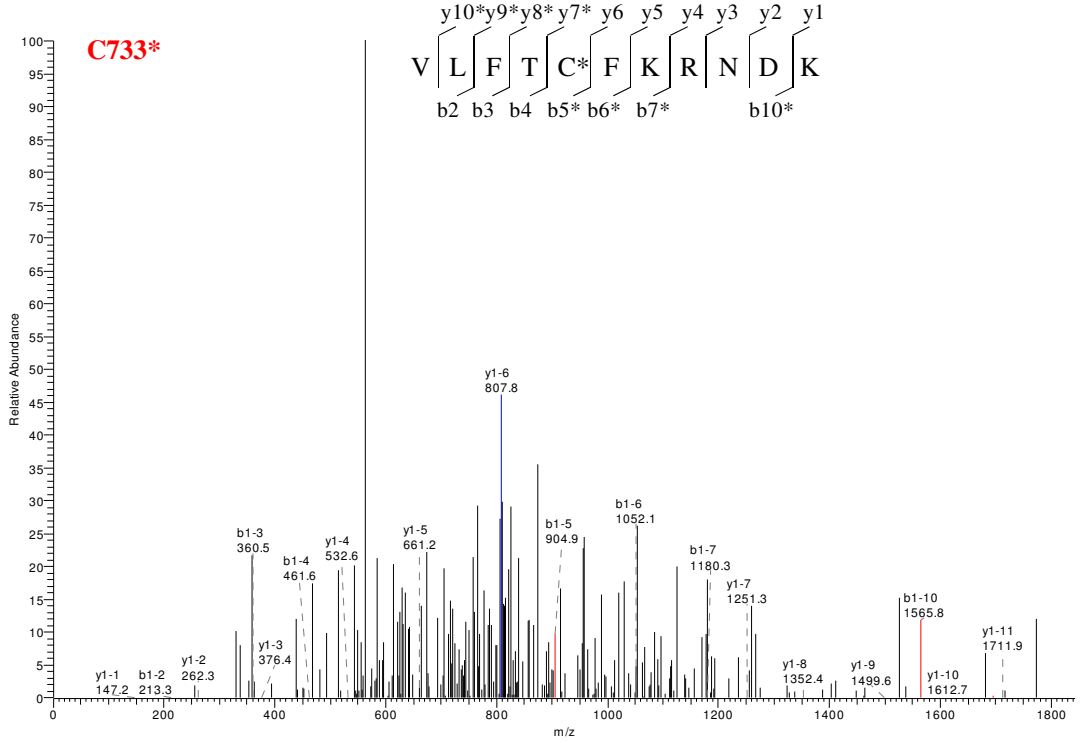

y9 y8 y4
N V T V K K T A A K
b6 b7 b9
y2-9
473.2
b2-10
521.4
y2-10
530.4
b2-9
457.0
y2-8
423.4
b2-7
385.7
b2-6
335.3
y2-4
195.2
Relative Abundance
m/z

Supplement Figure 3. MS/MS Spectra of HQ17(3)-Topo IIα peptide adducts formed *in vitro*

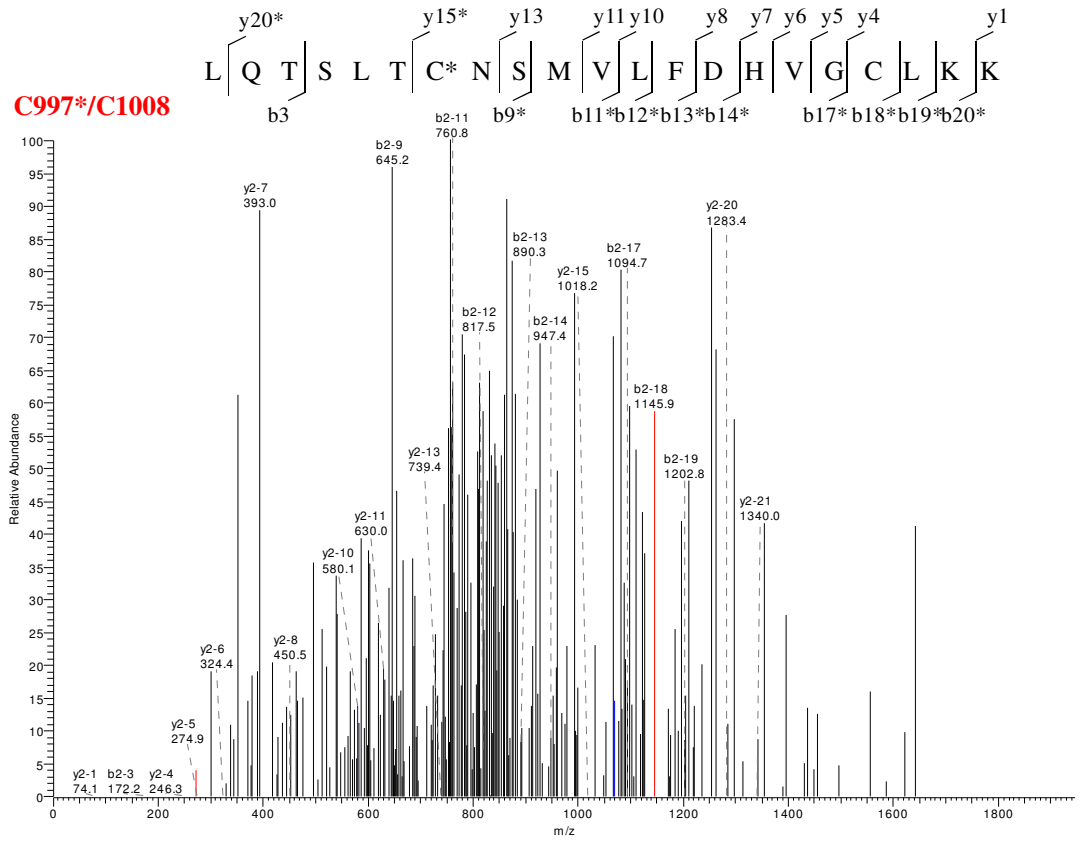
A.



B.

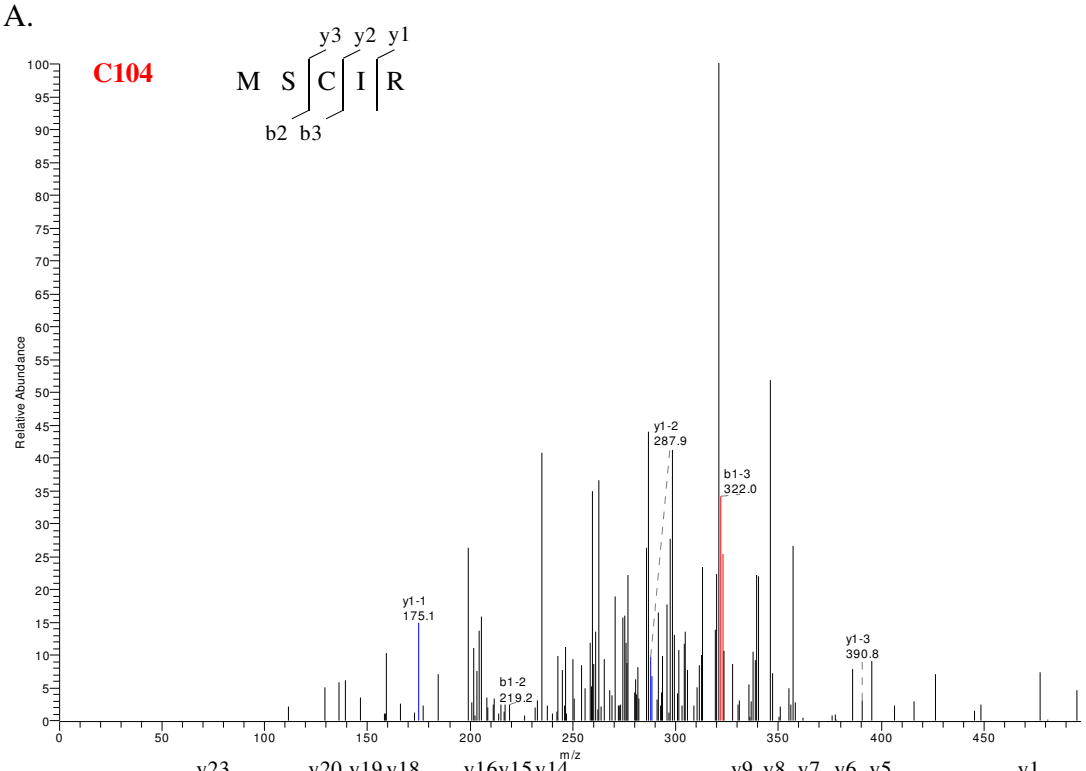


C.

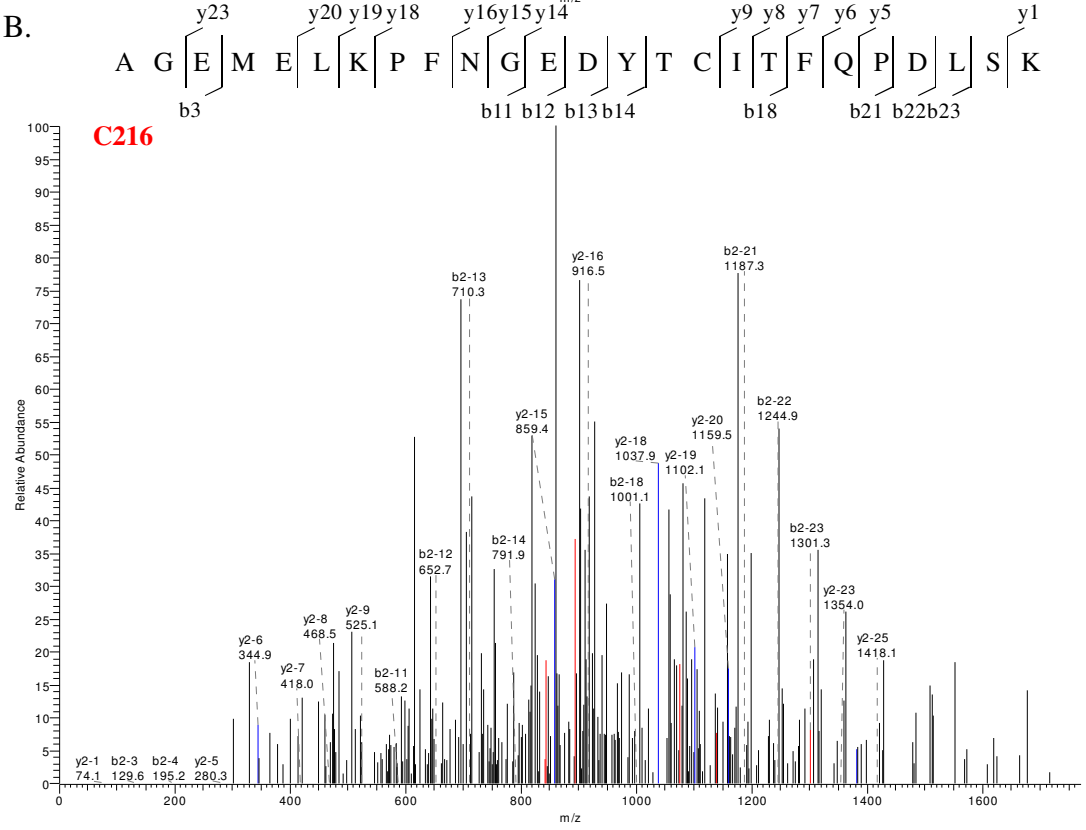

y20* y15* y13 y11 y10 y8 y7 y6 y5 y4 y1
L Q T S L T C* N S M V L F D H V G C L K K
b3 b9* b11*b12*b13*b14* b17* b18*b19*b20*
C997*/C1008
b2-11 760.8
b2-9 645.2
y2-7 393.0
y2-20 1283.4
b2-13 890.3
b2-17 1094.7
y2-15 1018.2
b2-12 817.5
b2-14 947.4
b2-18 1145.9
y2-13 739.4
b2-19 1202.8
y2-21 1340.0
y2-11 630.0
y2-10 580.1
y2-8 450.5
y2-6 324.4
y2-5 274.9
y2-1 74.1
b2-3 172.2
y2-4 246.3
Relative Abundance
m/z

Supplement Figure 4. MS/MS spectra of cysteine-containing Topo IIα peptides with no HQ17(3) modification obtained *in vitro*

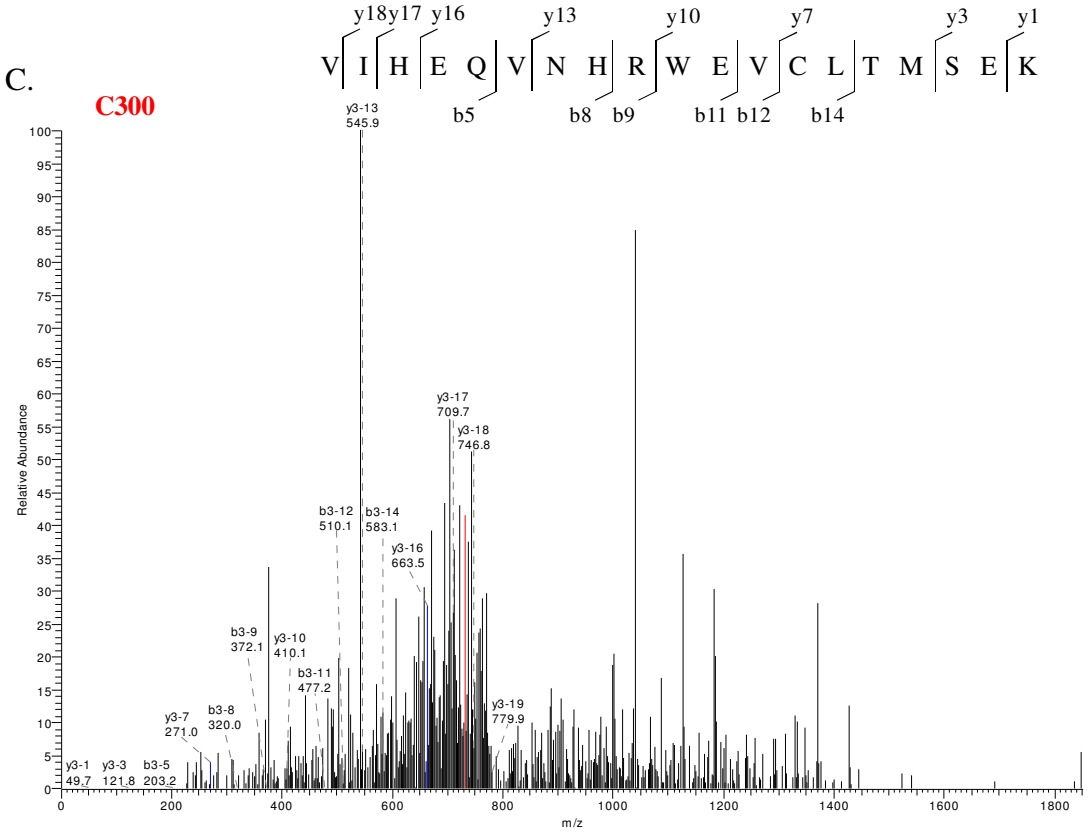
A.



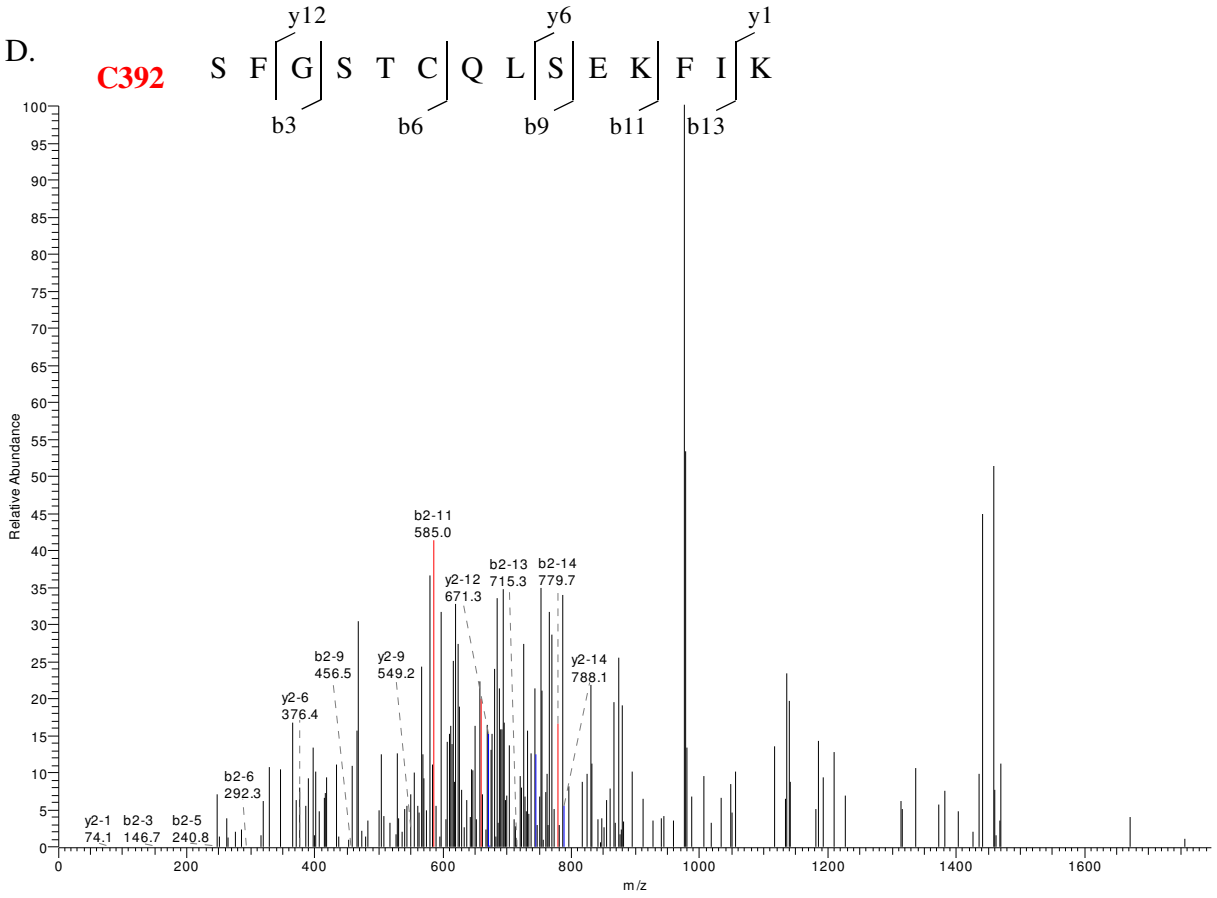
B.



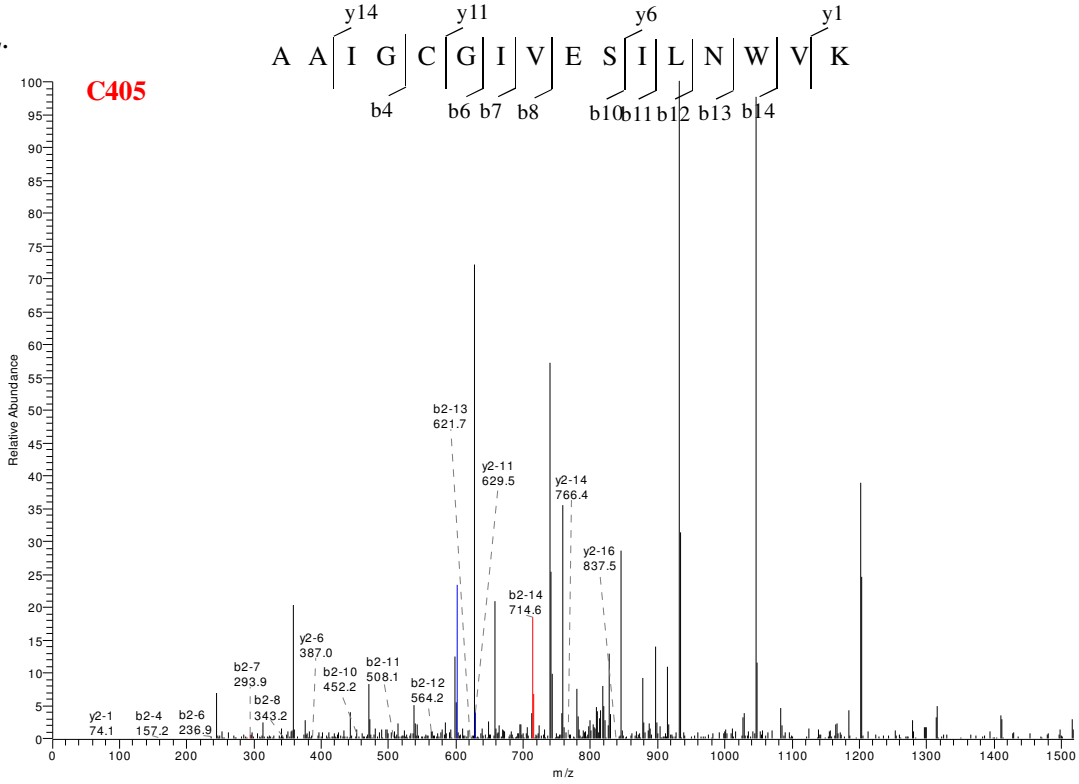
C.


C300
y18 y17 y16 y13 y10 y7 y3 y1
V I H E Q V N H R W E V C L T M S E K
b5 b8 b9 b11 b12 b14
y3-13 545.9
y3-17 709.7
y3-18 746.8
b3-12 510.1
b3-14 583.1
y3-16 663.5
b3-9 372.1
y3-10 410.1
b3-11 477.2
y3-7 271.0
b3-8 320.0
y3-19 779.9
y3-1 49.7
y3-3 121.8
b3-5 203.2
Relative Abundance
m/z

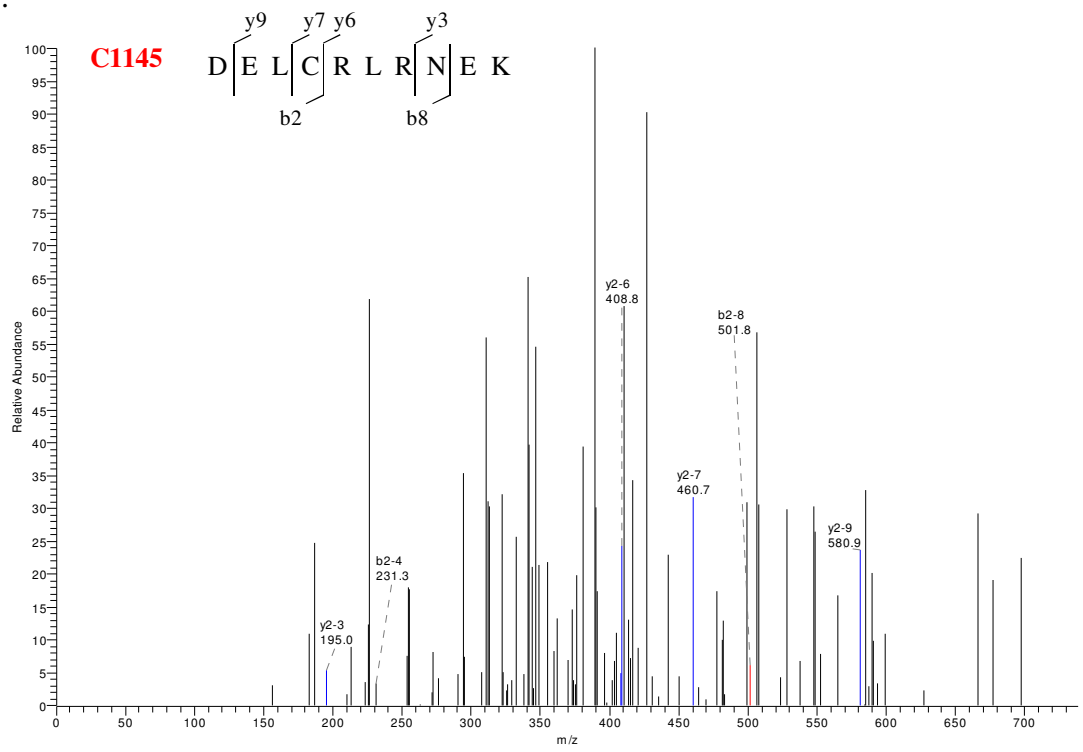
D.


C392
y12 y6 y1
S F G S T C Q L S E K F I K
b3 b6 b9 b11 b13
b2-11 585.0
b2-13 715.3
b2-14 779.7
y2-12 671.3
b2-9 456.5
y2-9 549.2
y2-14 788.1
y2-6 376.4
b2-6 292.3
y2-1 74.1
b2-3 146.7
b2-5 240.8
Relative Abundance
m/z

E.

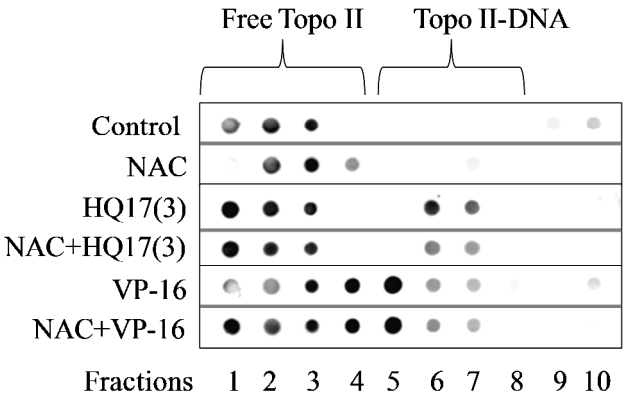

A A I G C G I V E S I L N W V K
y14
y11
y6
y1
b4
b6 b7 b8
b10 b11 b12 b13 b14
C405
Relative Abundance
m/z
b2-13 621.7
y2-11 629.5
y2-14 766.4
y2-16 837.5
b2-14 714.6
y2-6 387.0
b2-11 508.1
b2-10 452.2
b2-12 564.2
b2-7 293.9
b2-8 343.2
y2-1 74.1
b2-4 157.2
b2-6 236.9

F.

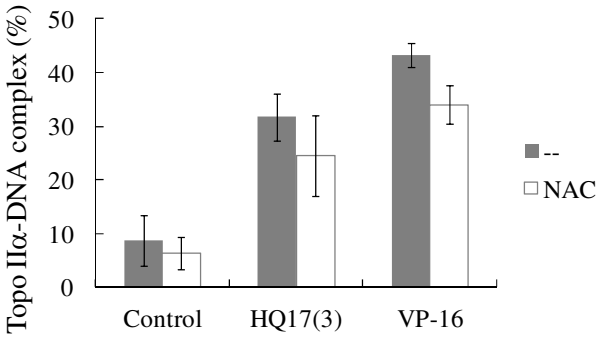

D E L C R L R N E K
y9
y7 y6
y3
b2
b8
C1145
Relative Abundance
m/z
y2-6 408.8
b2-8 501.8
y2-7 460.7
y2-9 580.9
b2-4 231.3
y2-3 195.0

Supplement Figure 5. NAC exerts no effect on HQ17(3)-mediated Topo IIα-DNA complex formation. Huh7 cells were pretreated with NAC (1 mM) for 3 h and followed by treating with HQ17(3) (30 μM), or VP-16 (50 μM), for 90 min and then lysed in 1% sarkosyl in TE buffer. (A) ICE bioassay was then performed to monitor Topo IIα-DNA complex formation. A representative result from three experiments is shown. (B) The percentage of Topo IIα-DNA (Fractions 5~8) in each lane was calculated. The error bars represent the standard deviation.

A.

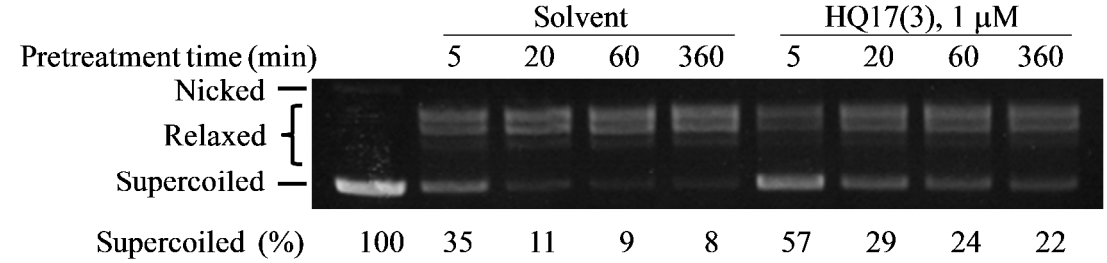


B.

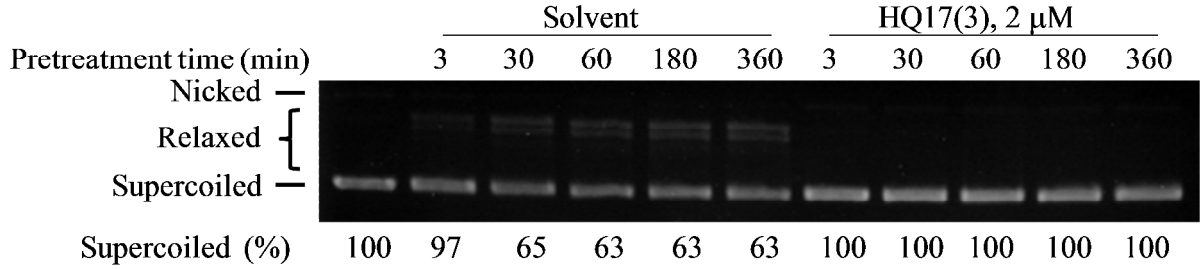


Supplement Figure 6. Pretreatment of Topo IIα with HQ17(3) (A) decreases enzyme activity or (B) inactivates enzyme. Topo IIα and HQ17(3) a molar ratio of 1:200 or 1:800 were incubated on ice for 20 min, and then the supercoiled DNA was added to the mixture to perform relaxation reaction at 37℃.

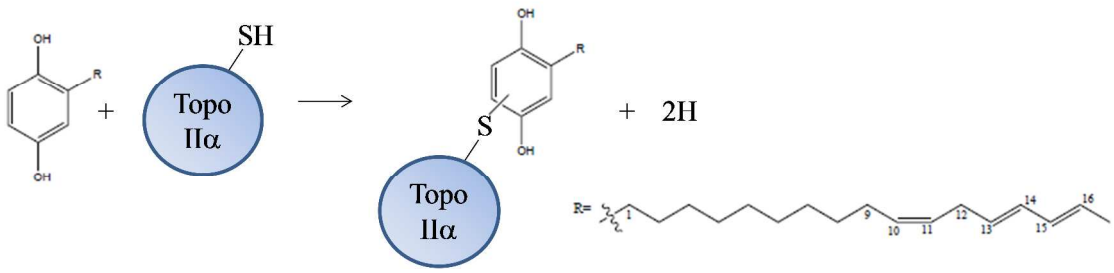
A.



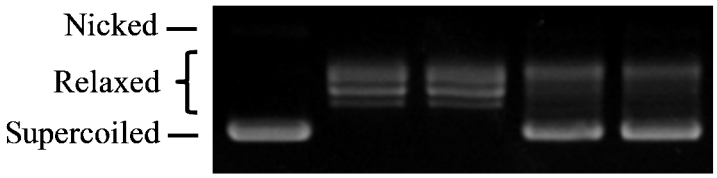
B.



Supplement Figure 7. Hypothetical reaction of HQ17(3) with cysteine residue of Topo IIα



Supplement Figure 8. DTT exerts no effect on HQ17(3)-mediated Topo IIα poisoning. Topo IIα relaxation reaction was performed at 37℃ for 20 min to dissect whether DTT affects HQ17(3)-caused Topo IIα inactivation.



Lane	1	2	3	4	5
Topo IIα	-	+	+	+	+
HQ17(3) (μM)	-	-	-	**40**	**40**
200 μM DTT	-	-	+	-	+
Solvent	-	+	+	+	+
Supercoiled DNA (%)	**100**	**1**	**1**	**50**	**58**