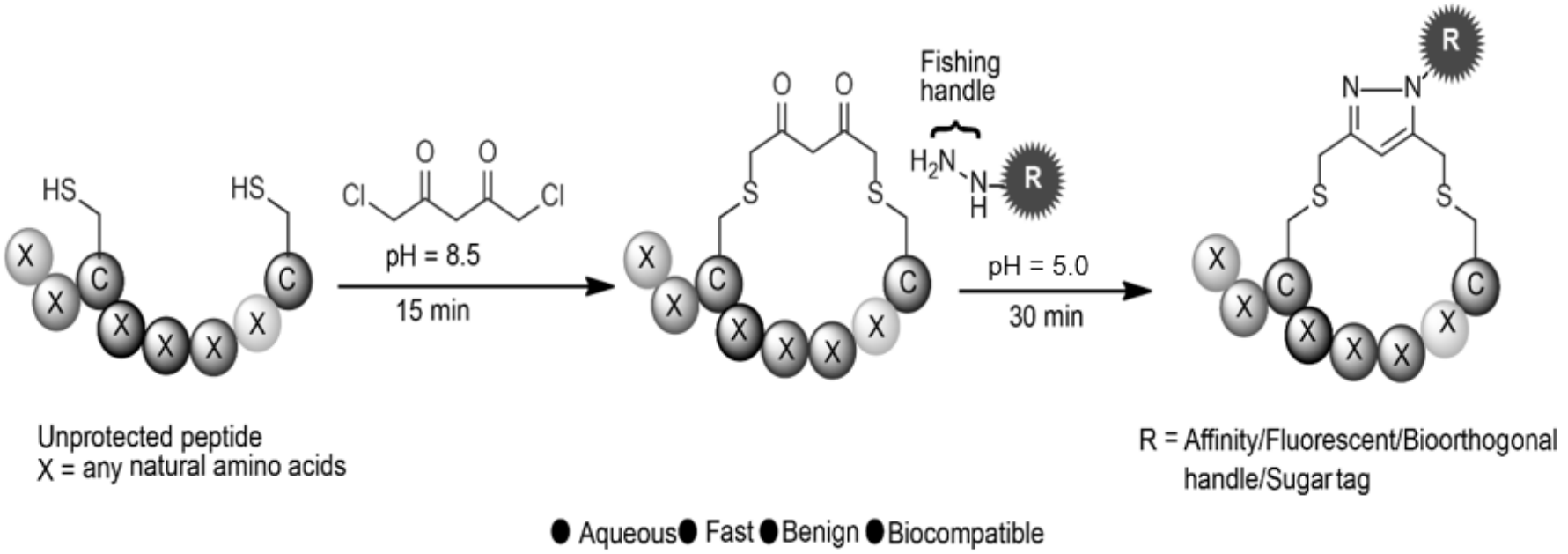
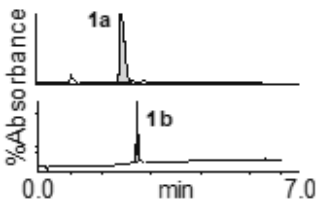

HS
HS
X
X
C
X
X
X
C
X
Cl
Cl
O
O
pH = 8.5
15 min
S
S
Fishing handle
H_2N
N
H
R
pH = 5.0
30 min
N
N
R
Unprotected peptide
X = any natural amino acids
R = Affinity/Fluorescent/Bioorthogonal handle/Sugar tag
Aqueous
Fast
Benign
Biocompatible

Figure 1

A

	Sequences	DKMP
1a	H-SWCDYRC-NH_2	**1b**
2a	H-SLCFDNGC-NH_2	**2b**
3a	H-FCPWCR-NH_2	**3b**
4a	H-SWCWDYRGDLEWC-NH_2	**4b**
5a	H-SQCVRSC-NH_2	**5b**

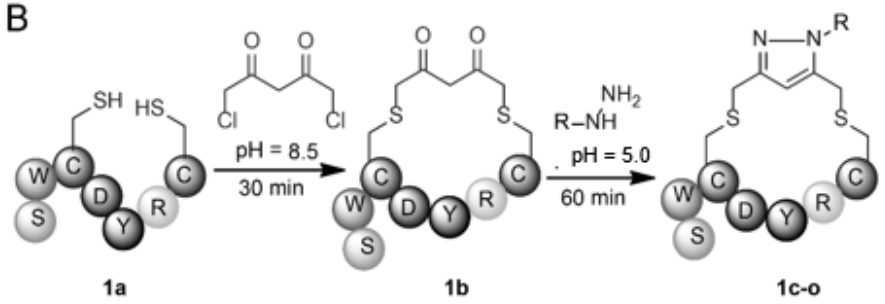


C

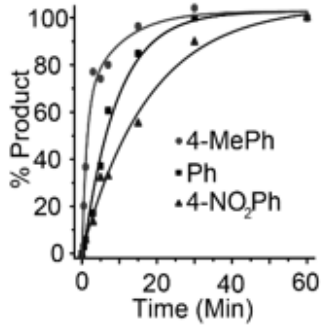
DKMP	DAMP														
1b	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**	**1k**	**1l**	**1m**	-	**1o**	**1p**	**1q**
2b	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**	**2m**	-	-	**2p**	**2q**
3b	**3c**	-	-	**3f**	-	-	-	-	**3k**	**3l**	**3m**	-	**3o**	-	**3q**
4b	**4c**	-	-	-	-	-	-	**4j**	**4k**	**4l**	**4m**	-	-	-	-
5b	**5c**	**5d**	**5e**	-	-	**5h**	**5i**	-	-	-	-	-	-	-	-

For c; R = Ph
For d; R = Toluyl
For e; R = 4-nitrophenyl
For f; R = Methyl
For g; R = H
For h; R = 4-fluorophenyl
For i; R = Pentafluorophenyl
For j; R = 2-hydroxyethyl
For k; R = Mannosyl
For l; R = Acetamido fluorescein
For m; R = Acetamido PEG2 biotin
For n; R = 3,6,9-Trioxa-decan
For o; R = Decyl
For p; R = Propargyl
For q; R = 4-Sulfonamidophenyl

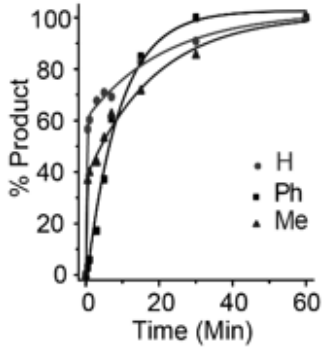
B



D



E



F

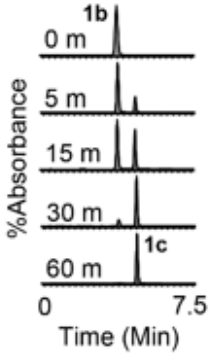


Figure 2

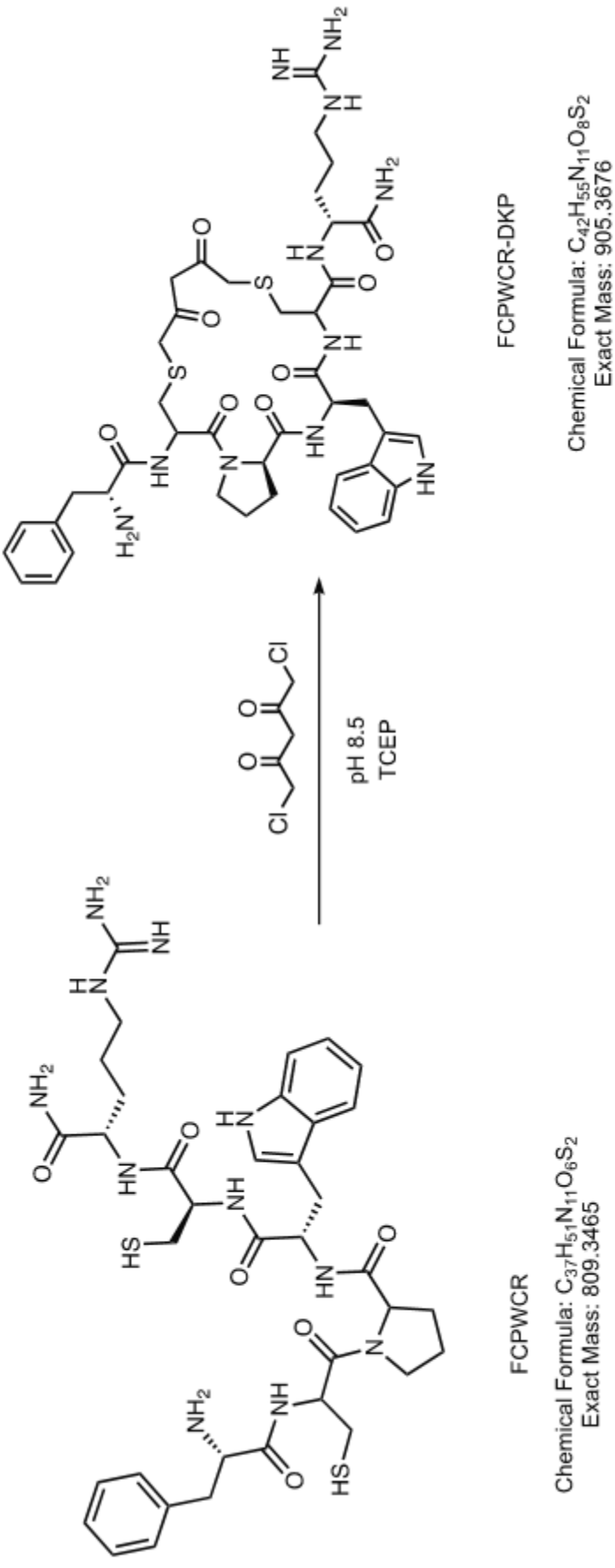

pH 8.5
TCEP
FCPWCR
Chemical Formula: $C_{37}H_{51}N_{11}O_6S_2$
Exact Mass: 809.3465
FCPWCR-DKP
Chemical Formula: $C_{42}H_{55}N_{11}O_8S_2$
Exact Mass: 905.3676

Figure 3A

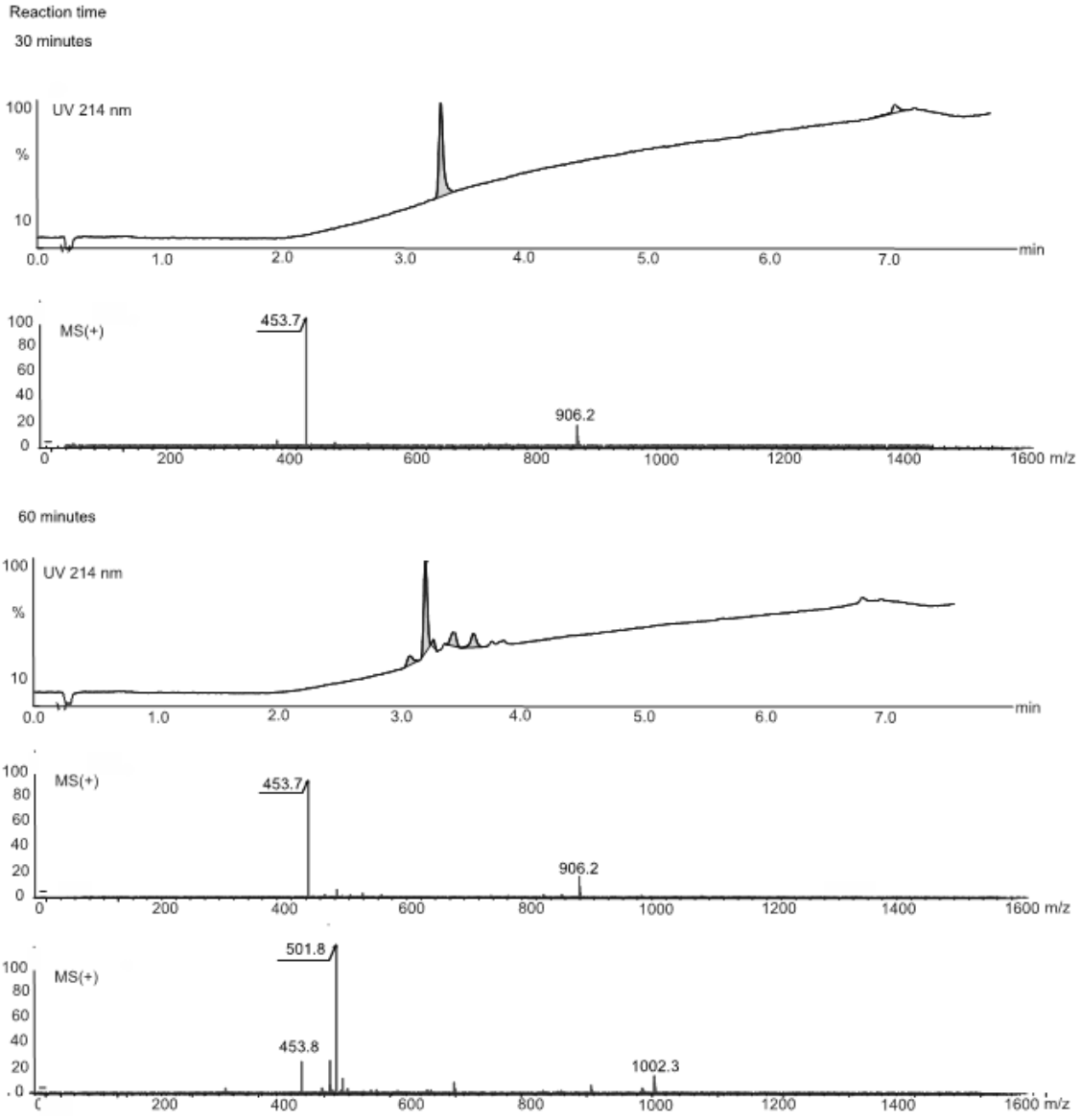

Reaction time
30 minutes
UV 214 nm
%
min
MS(+)
453.7
906.2
m/z
60 minutes
UV 214 nm
MS(+)
453.7
906.2
MS(+)
501.8
453.8
1002.3

Figure 3B

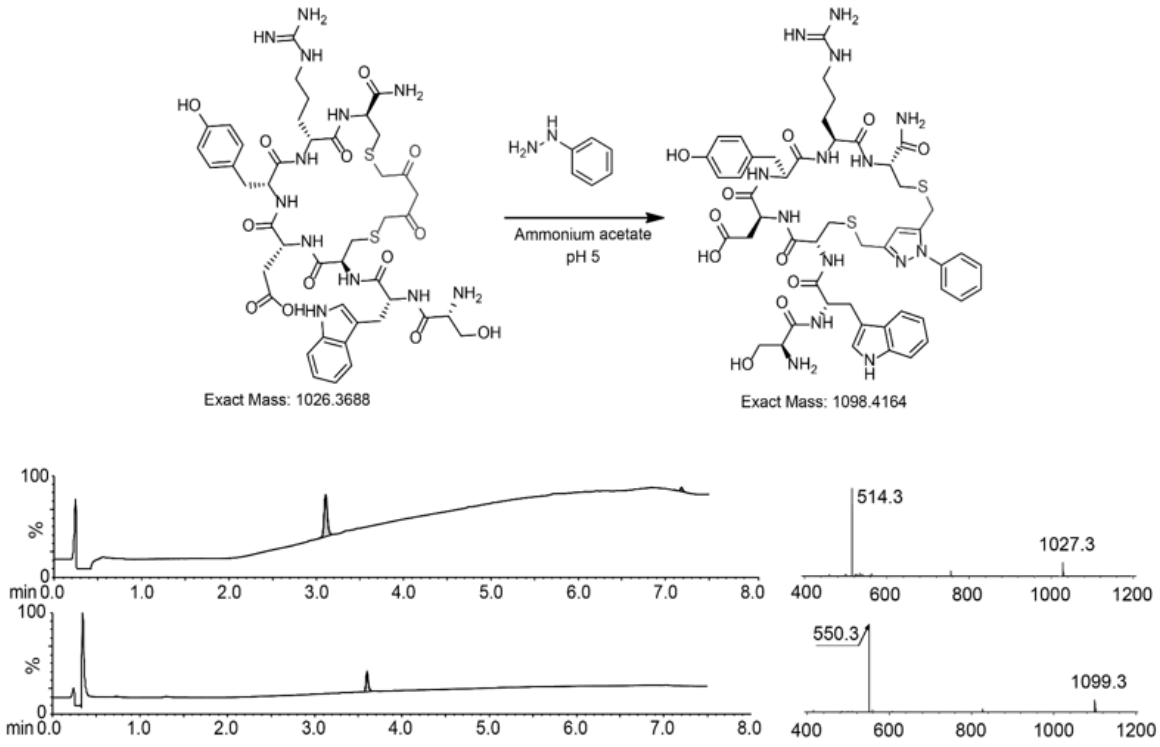

Ammonium acetate
pH 5
Exact Mass: 1026.3688
Exact Mass: 1098.4164
100
%
0
min 0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0
514.3
1027.3
400 600 800 1000 1200
550.3
1099.3

Figure 4A

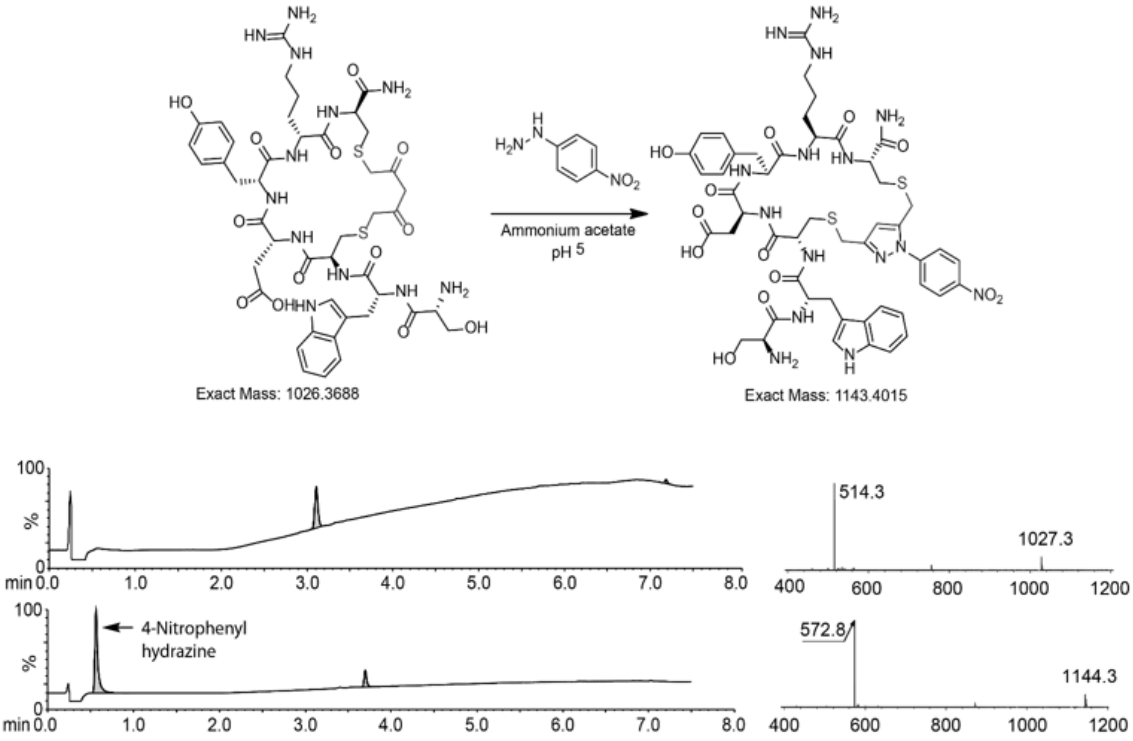
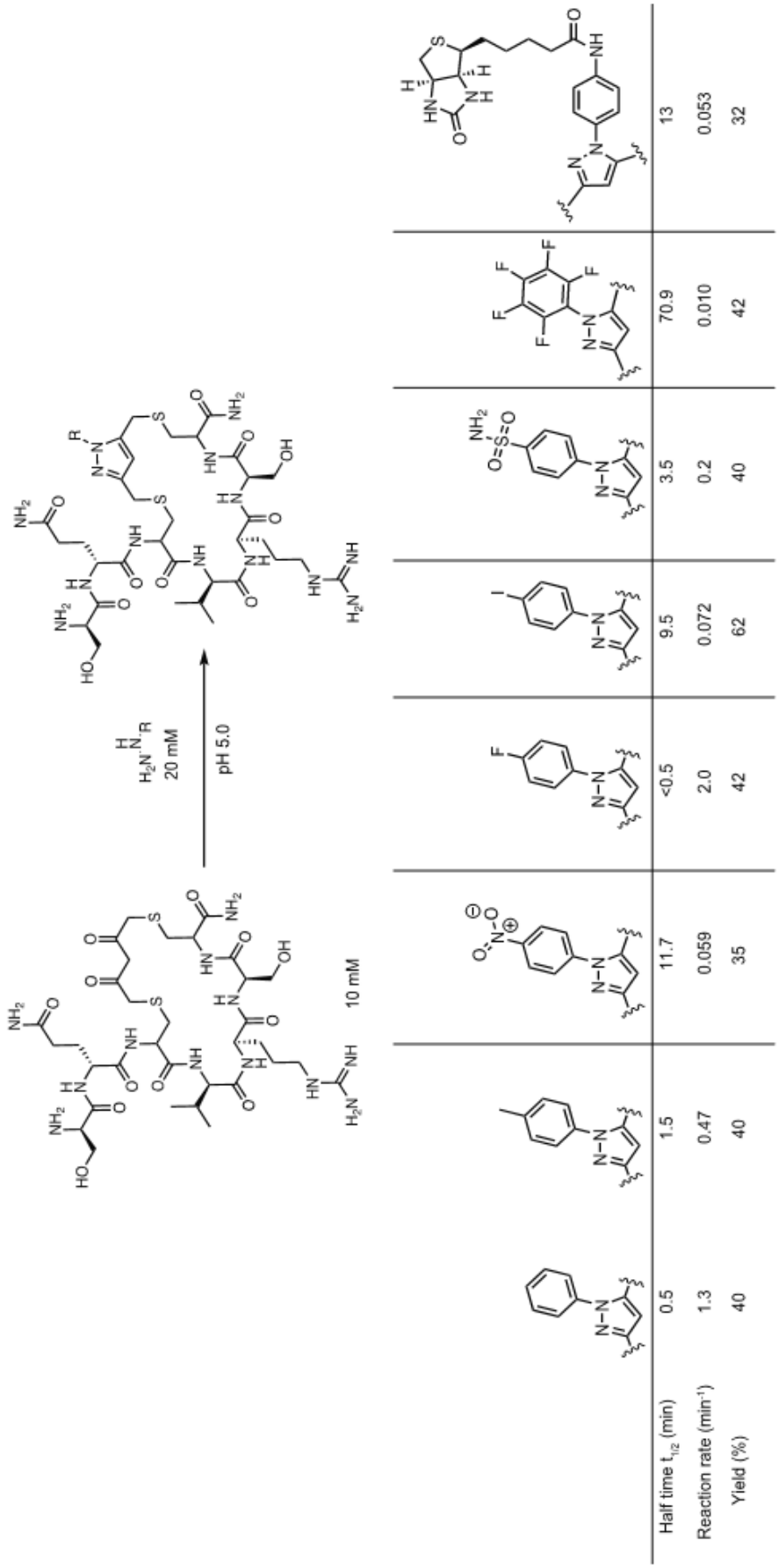

Ammonium acetate
pH 5
Exact Mass: 1026.3688
Exact Mass: 1143.4015
514.3
1027.3
572.8
1144.3
4-Nitrophenyl
hydrazine
min

Figure 4B



Half time $t_{1/2}$ (min)	0.5	1.5	11.7	<0.5	9.5	3.5	70.9	13
Reaction rate (min^{-1})	1.3	0.47	0.059	2.0	0.072	0.2	0.010	0.053
Yield (%)	40	40	35	42	62	40	42	32

Figure 5A

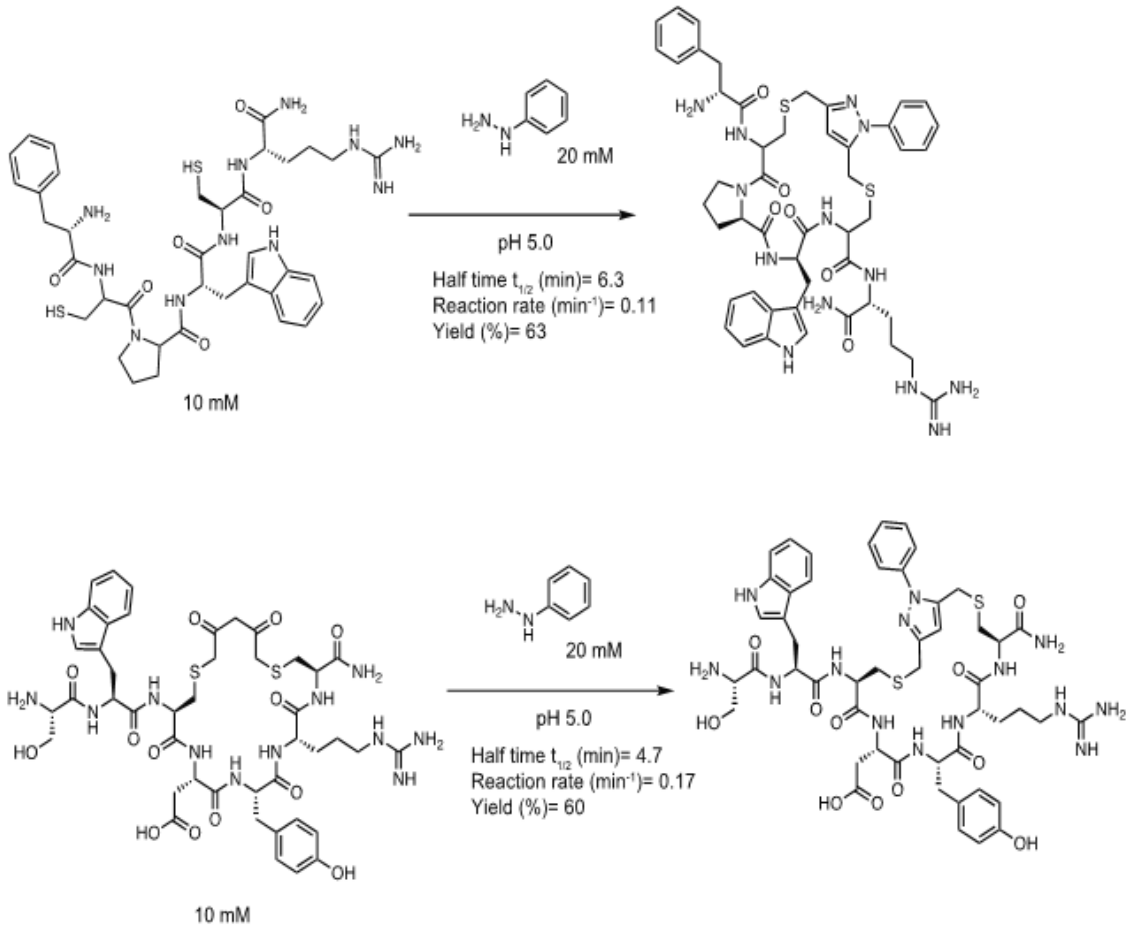

20 mM
pH 5.0
Half time $t_{1/2}$ (min)= 6.3
Reaction rate (min^{-1})= 0.11
Yield (%)= 63
10 mM
20 mM
pH 5.0
Half time $t_{1/2}$ (min)= 4.7
Reaction rate (min^{-1})= 0.17
Yield (%)= 60
10 mM

Figure 5B

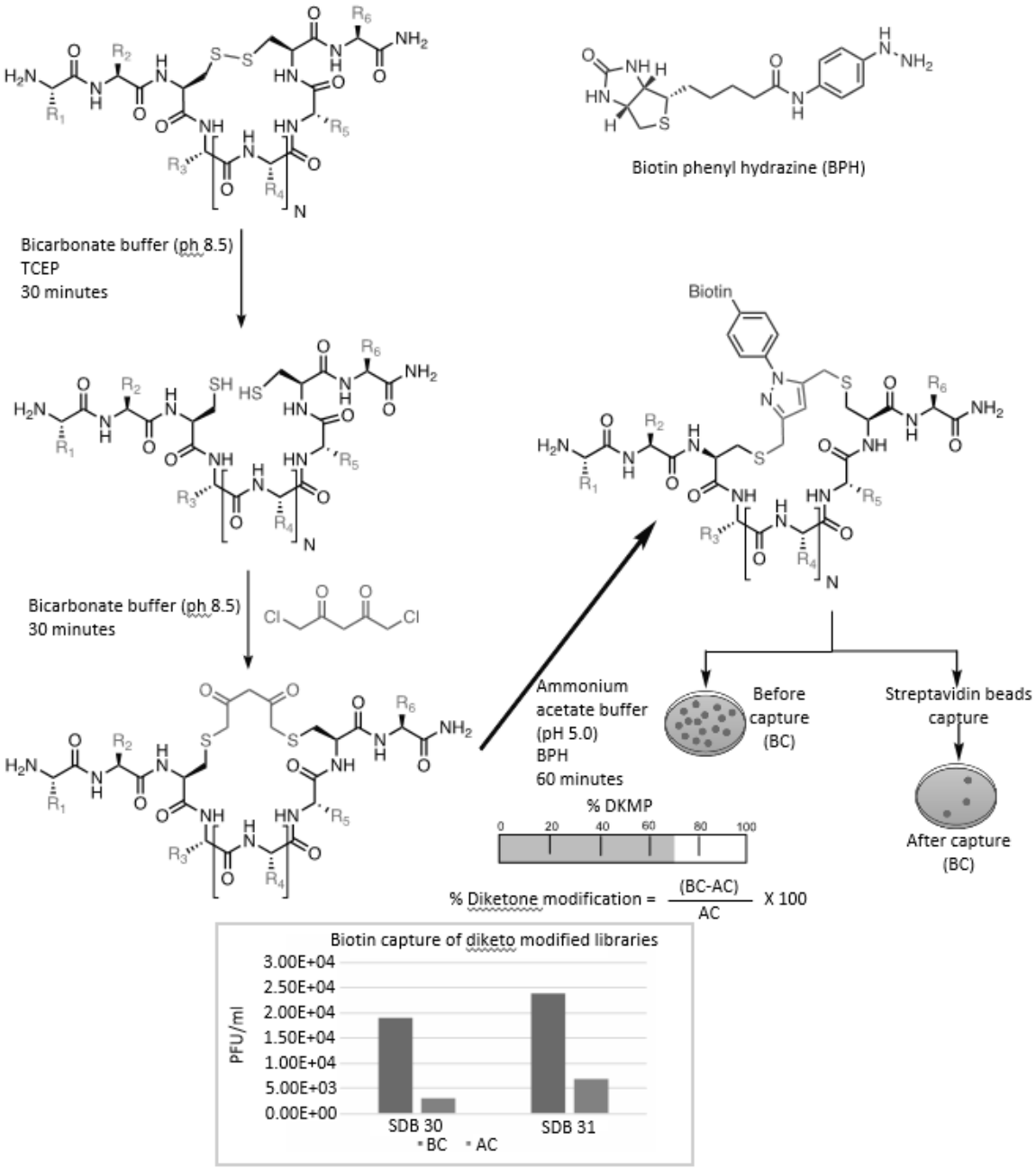

Biotin phenyl hydrazine (BPH)
Bicarbonate buffer (ph 8.5)
TCEP
30 minutes
Bicarbonate buffer (ph 8.5)
30 minutes
Ammonium
acetate buffer
(pH 5.0)
BPH
60 minutes
Biotin
Before
capture
(BC)
Streptavidin beads
capture
After capture
(BC)
% DKMP
0
20
40
60
80
100
% Diketone modification = (BC-AC) / AC X 100
Biotin capture of diketo modified libraries
PFU/ml
3.00E+04
2.50E+04
2.00E+04
1.50E+04
1.00E+04
5.00E+03
0.00E+00
SDB 30
SDB 31
BC
AC

Figure 6

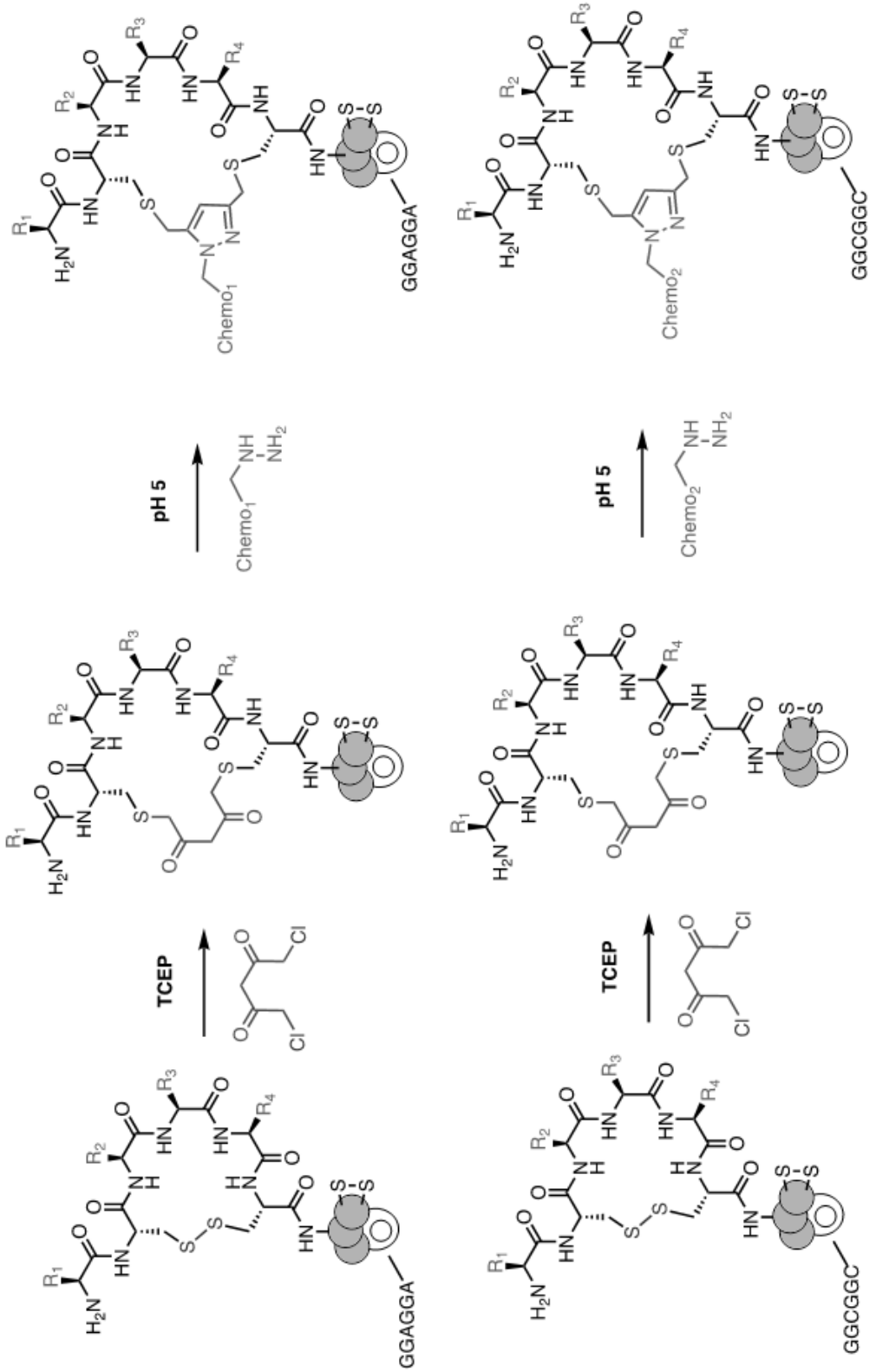


Figure 7