Thio-conjugation of benzofurazan substituents to Peptides: Molecular Sieves catalyze Nucleophilc Attack on Unsaturated fused Rings promoted by Molecular Sieves

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Supporting Information

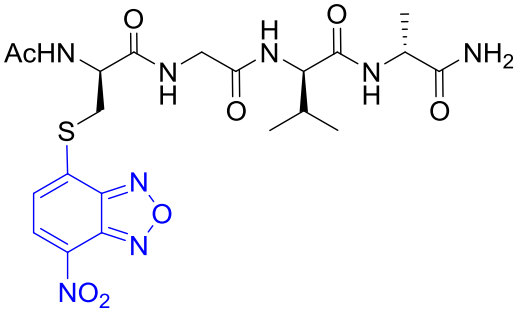
Table of contents

HPLC, MS and NMR characterization

Compound 1a strategy B

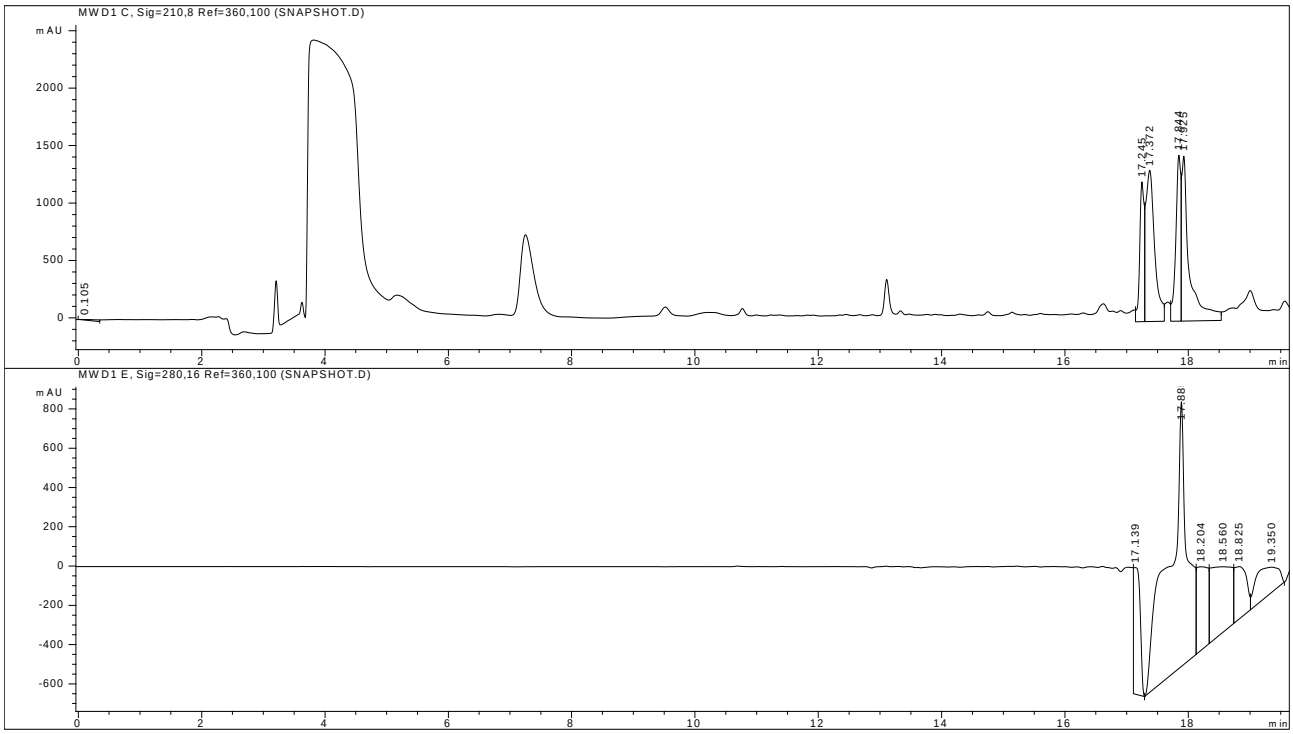
AcCys(a)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

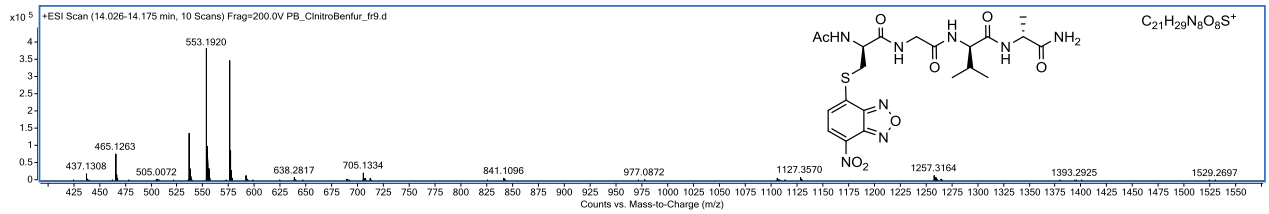


B) preparative HPLC *tR*= 17.844 min; ES-MS: calculated $[M + H]^+$, 553.1824, found m/z 553.1920 ($[M+H]^+$). Yellow solid

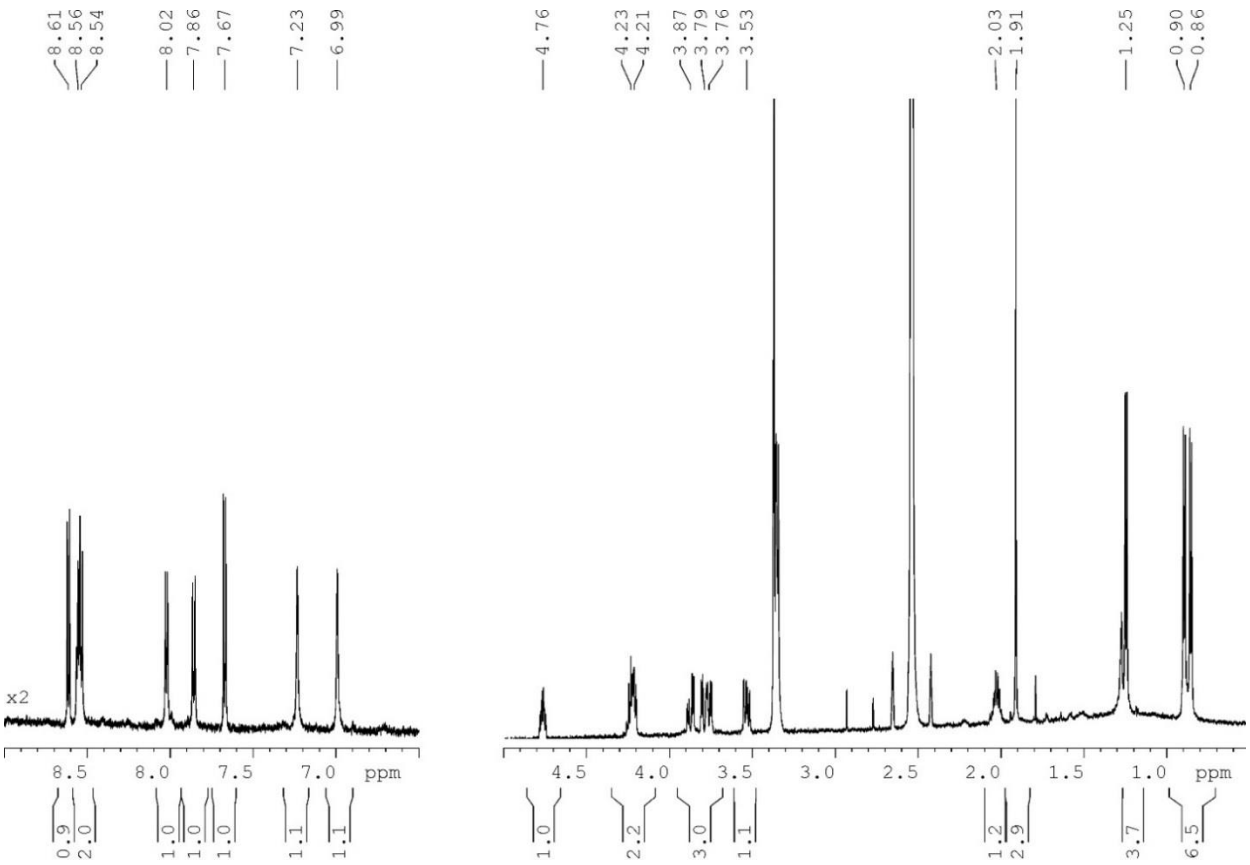
HPLC profile of the reaction crude product:



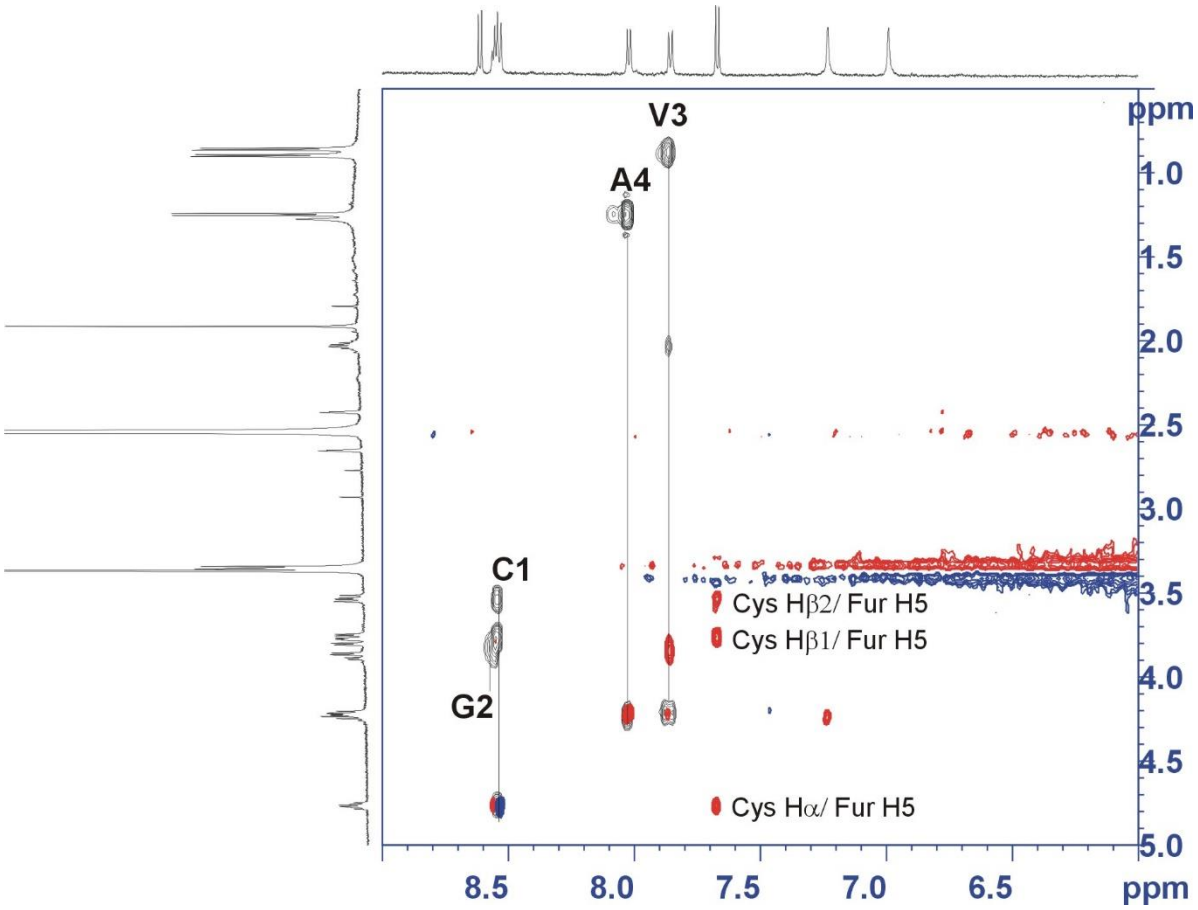
MS spectrum of **1a** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

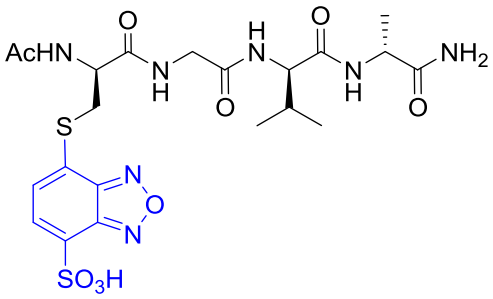
Compound 1a

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.91
CYS	H	1	8.54
CYS	HA	1	4.76
CYS	HB1	1	3.76
CYS	HB2	1	3.53
FU2	H5	5	7.67
FU2	H6	5	8.62
GLY	H	2	8.56
GLY	HA2	2	3.87
GLY	HA3	2	3.79
VAL	H	3	7.86
VAL	HA	3	4.21
VAL	HB	3	2.03
VAL	HG1	3	0.90
VAL	HG2	3	0.86
ALA	H	4	8.02
ALA	HA	4	4.23
ALA	HB	4	1.25
CO-NH2	NH1	4	7.23
CO-NH2	NH2	4	6.99

Compound 1b (X: Cl) strategy B

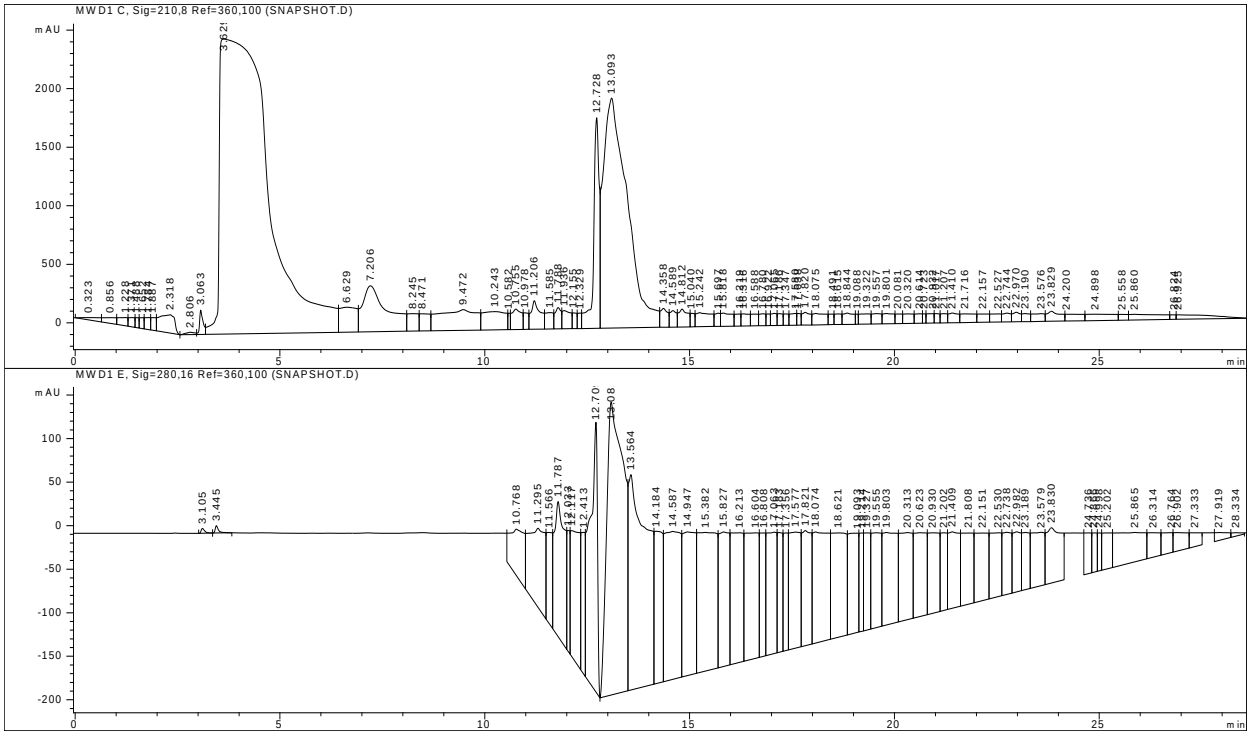
AcCys(b)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

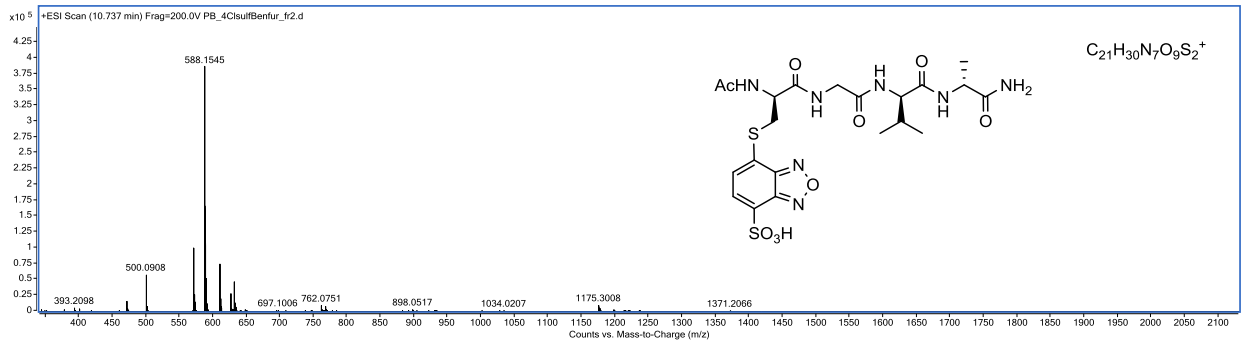


B) preparative HPLC *tR*= 12.728 min (close to the rt of the substrate employed b-Cl); ES-MS: calculated $[M + H]^+$, 588.1541, found m/z 588.1545 ($[M+H]^+$). Yellow solid

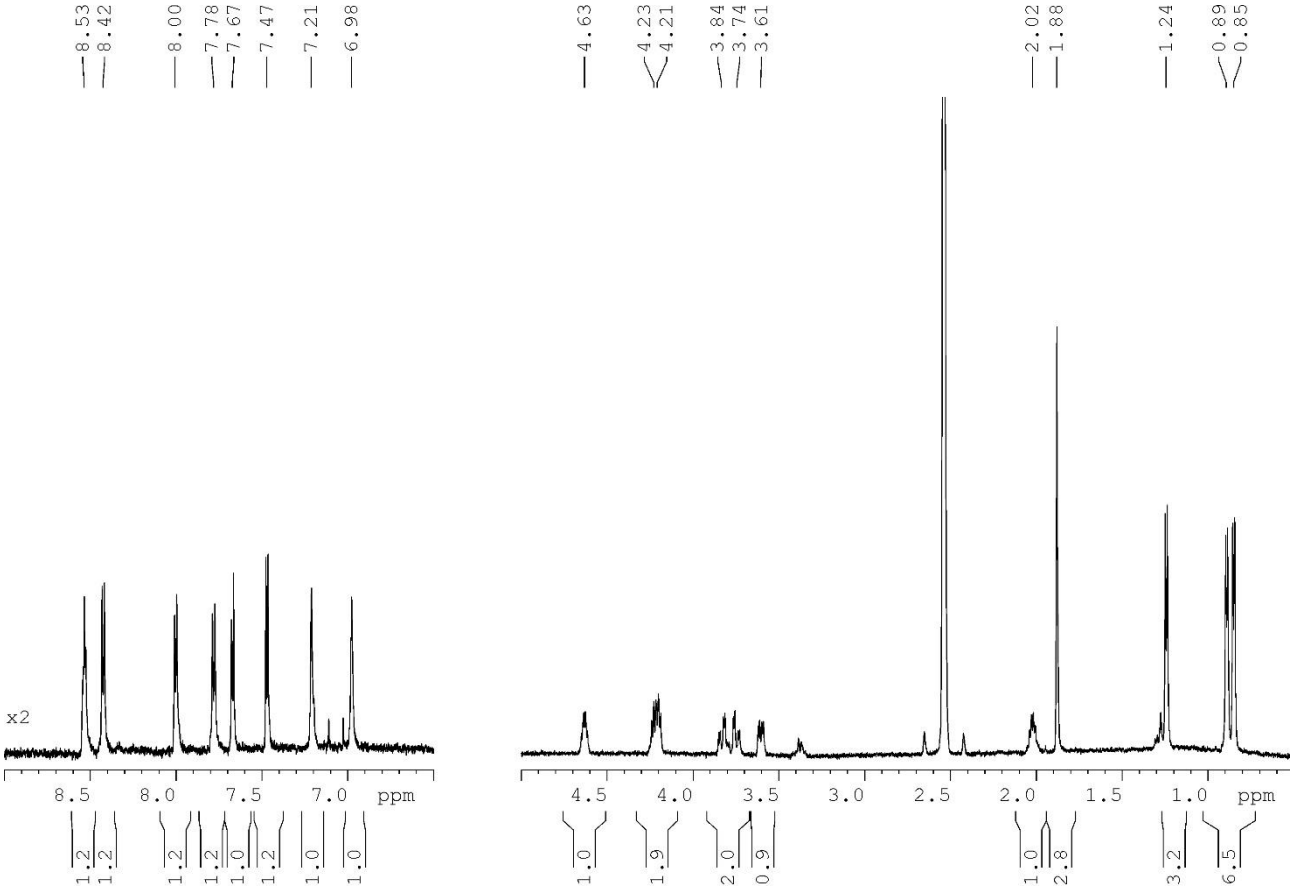
HPLC profile of the reaction crude product:



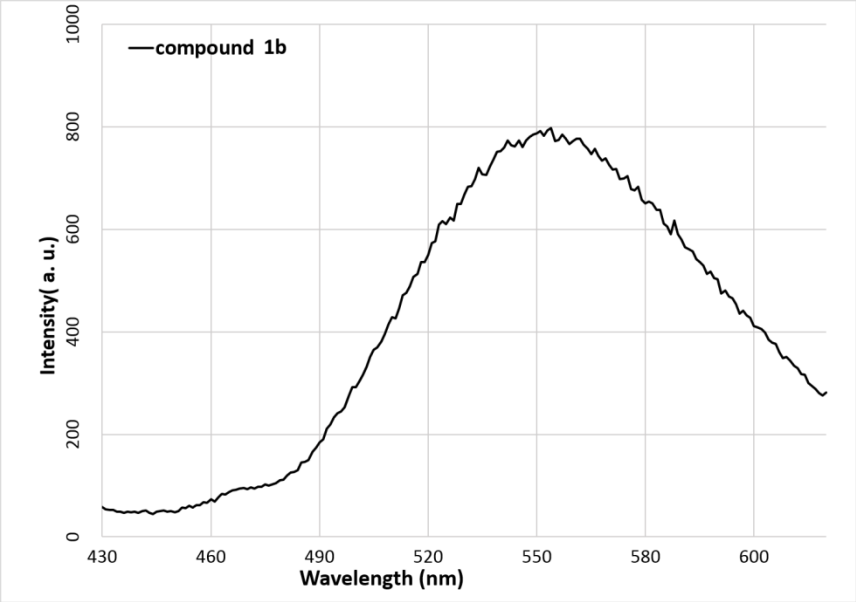
MS spectrum of **1b** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



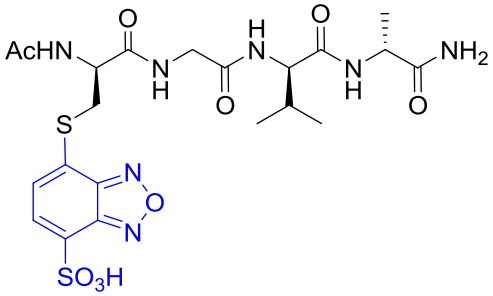
F) Fluorescence spectrum (λ_{ex} = 380 nm)



Compound 1b (X: F) strategy B

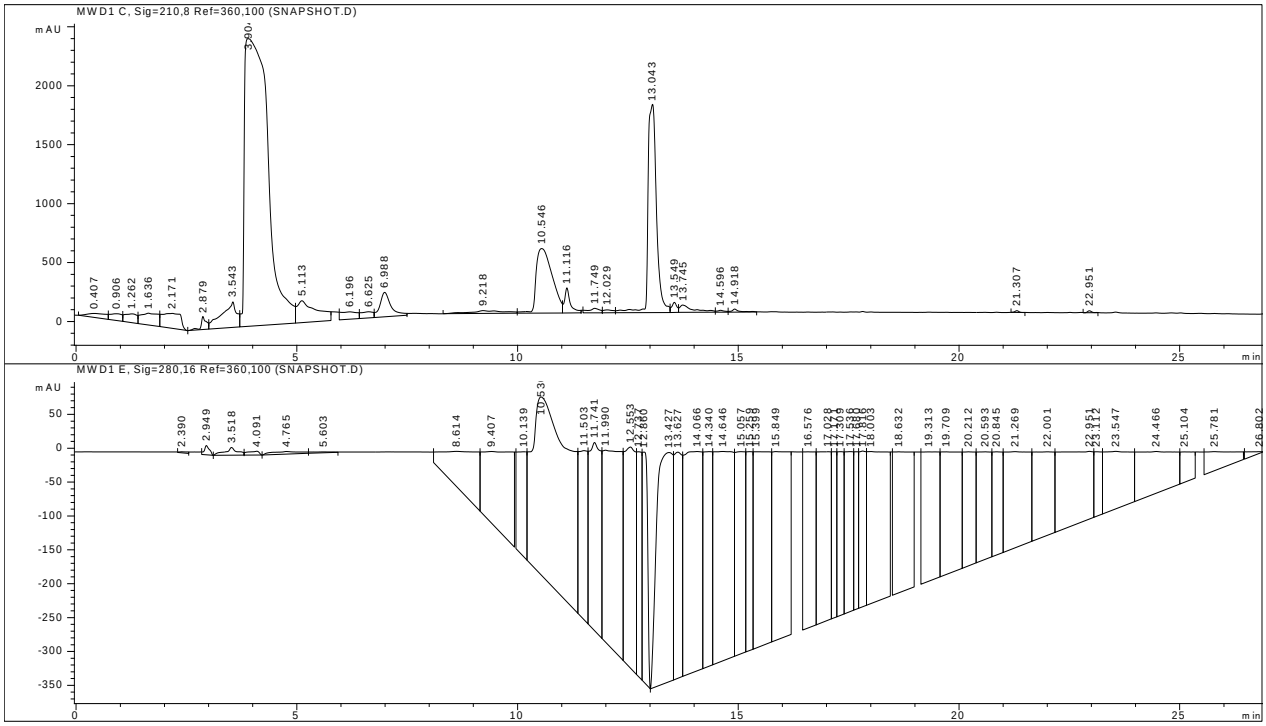
AcCys(b)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

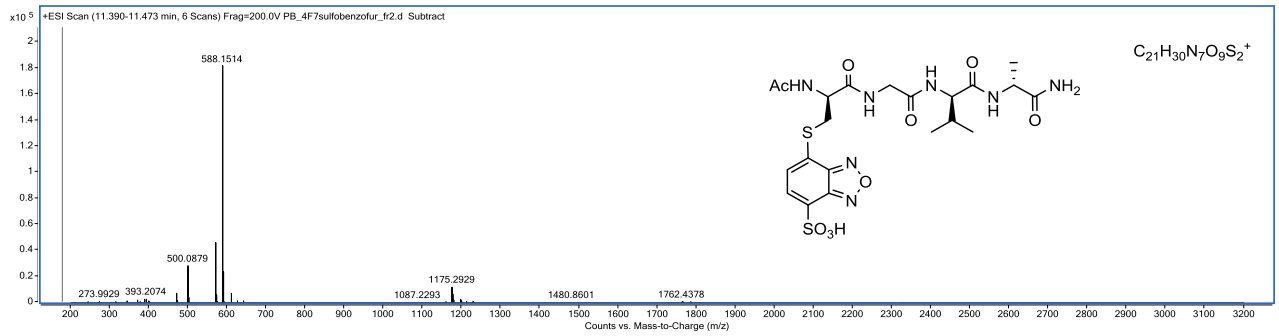


B) preparative HPLC *tR*= 13.043 min (co-eluted with the substrate employed b-F); ES-MS: calculated $[M + H]^+$, 588.1541, found m/z 588.1514 ($[M+H]^+$). Yellow solid

HPLC profile of the reaction crude product:



MS spectrum of **1b** after purification:



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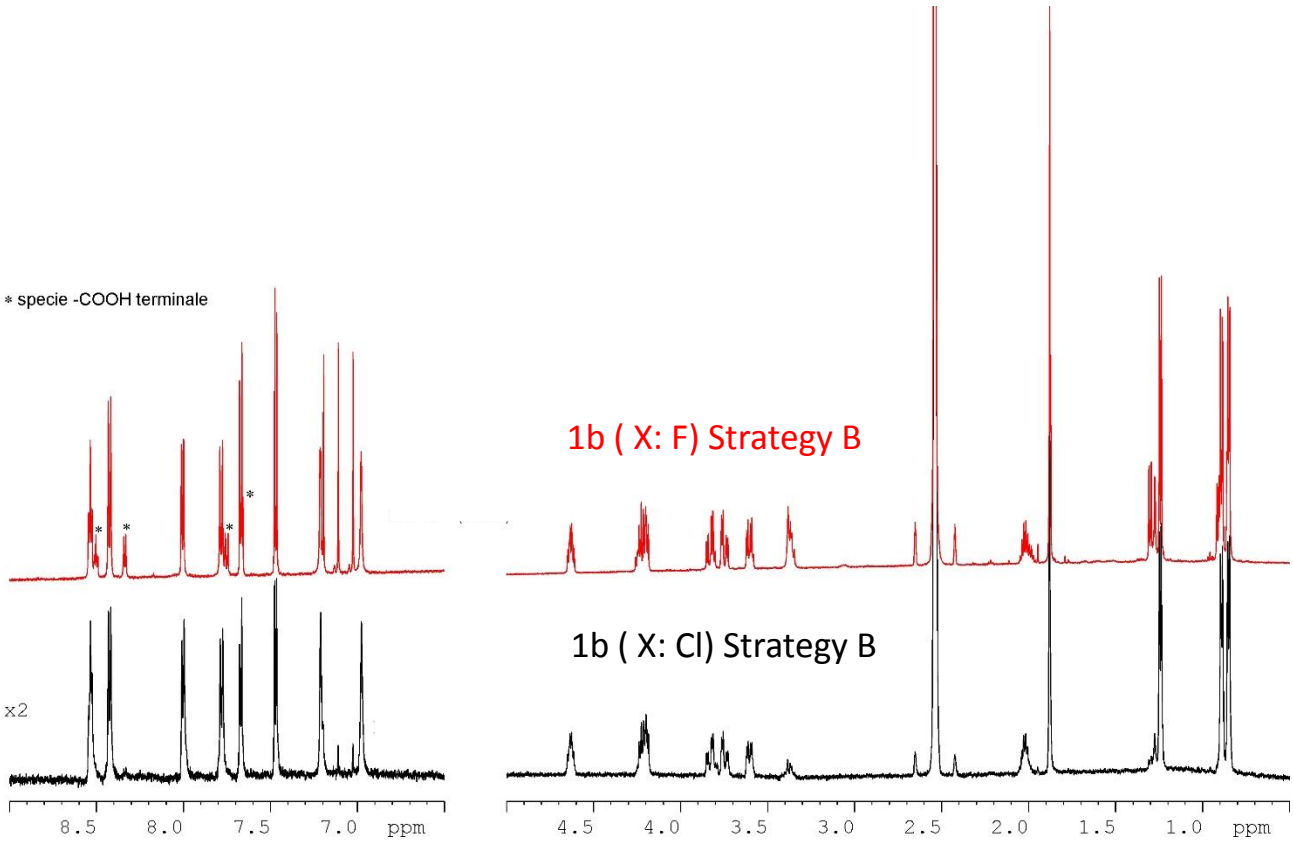
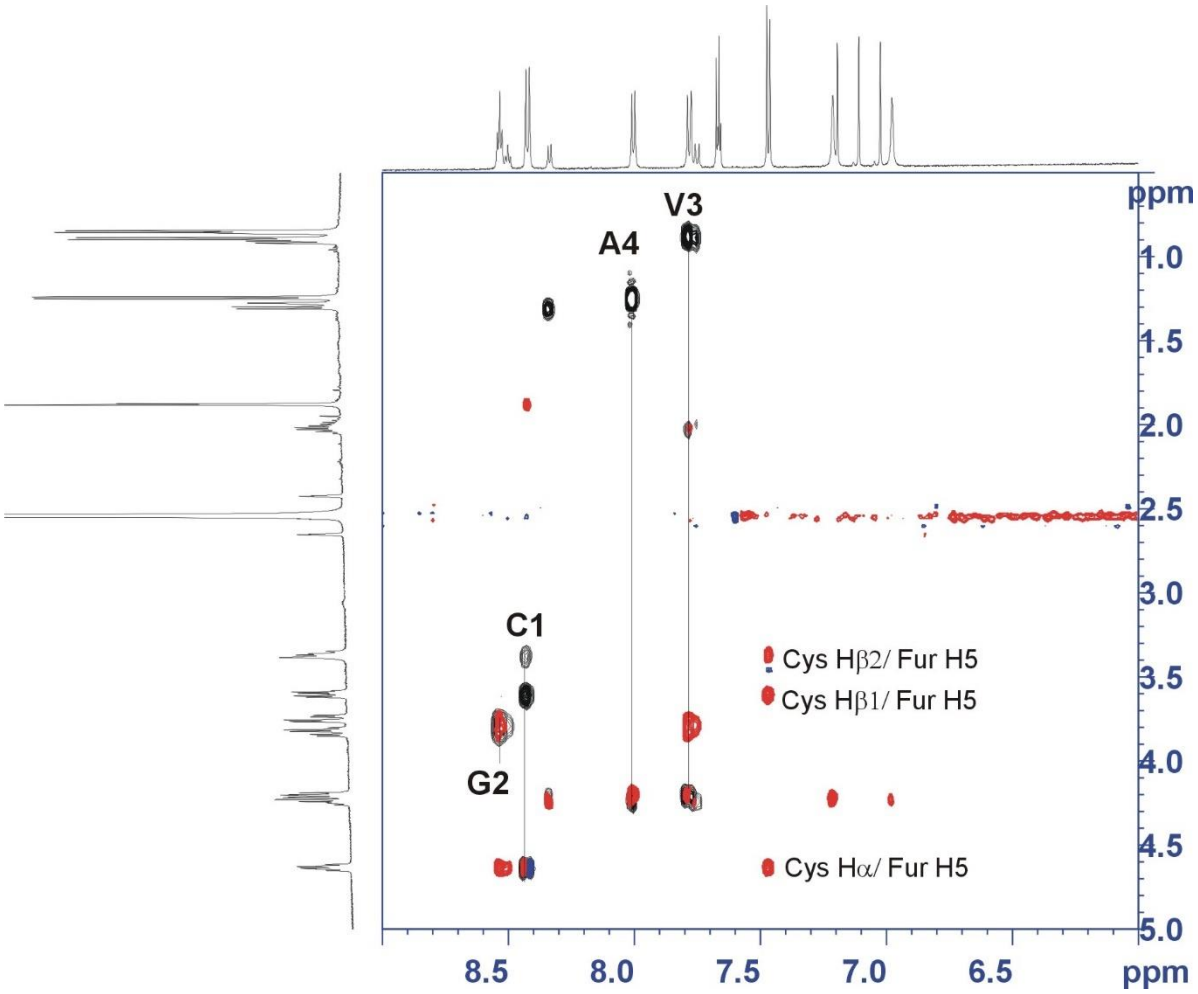


^{1}H-NMR spectra of **1b** obtained from the fluorine precursor (top) and the chlorine precursor (bottom). Asterisks denote an impurity due to transformation of the C-terminus –$CONH_2$ group into –COOH.

D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

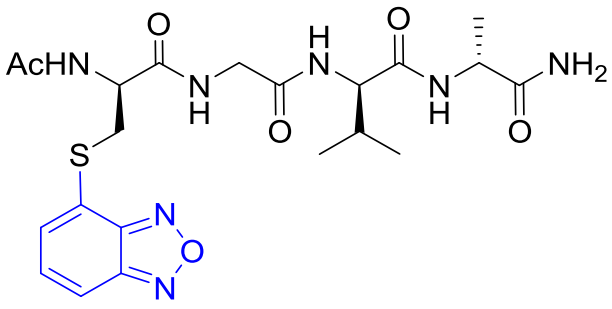
Compound 1b

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.88
CYS	H	1	8.42
CYS	HA	1	4.63
CYS	HB1	1	3.61
CYS	HB2	1	3.38
FU2	H5	5	7.47
FU2	H6	5	7.67
GLY	H	2	8.53
GLY	HA2	2	3.83
GLY	HA3	2	3.74
VAL	H	3	7.78
VAL	HA	3	4.21
VAL	HB	3	2.02
VAL	HG1	3	0.89
VAL	HG2	3	0.85
ALA	H	4	8.00
ALA	HA	4	4.23
ALA	HB	4	1.24
CO-NH2	NH1	4	7.21
CO-NH2	NH2	4	6.98

Compound 1c (X: F) strategy B

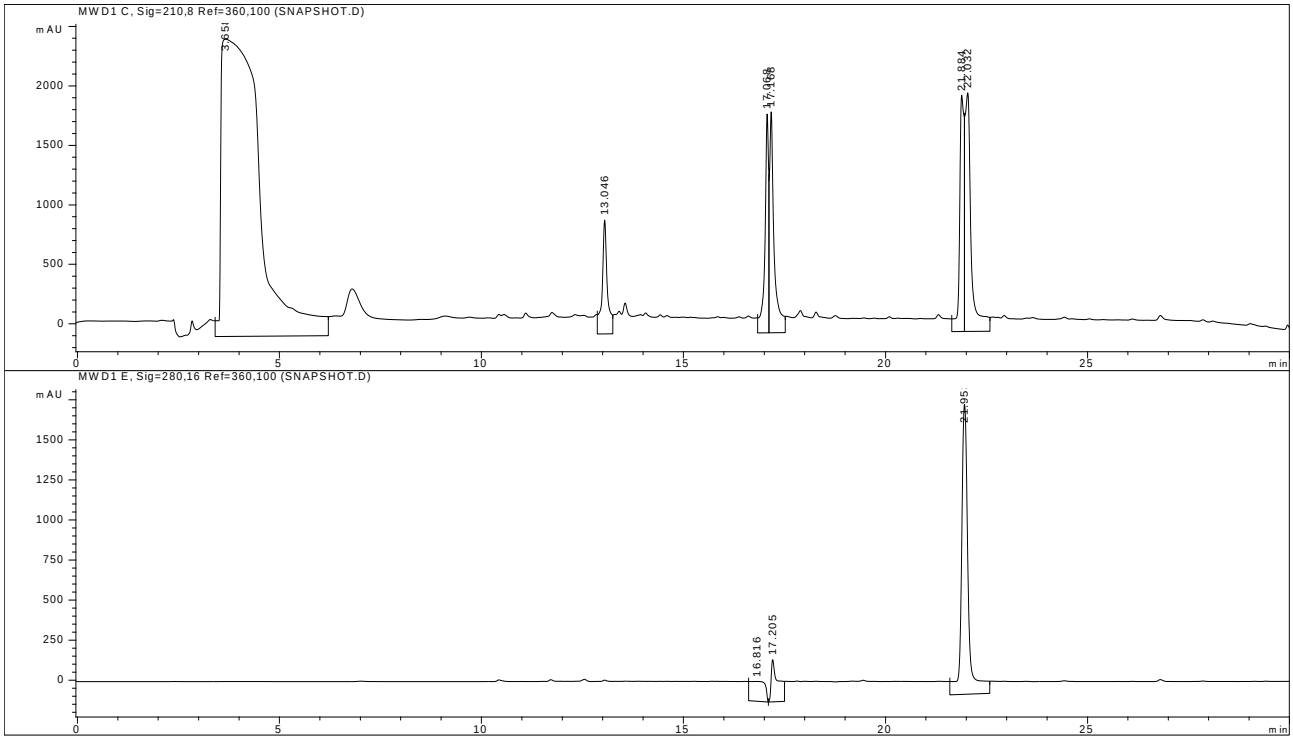
AcCys(c)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

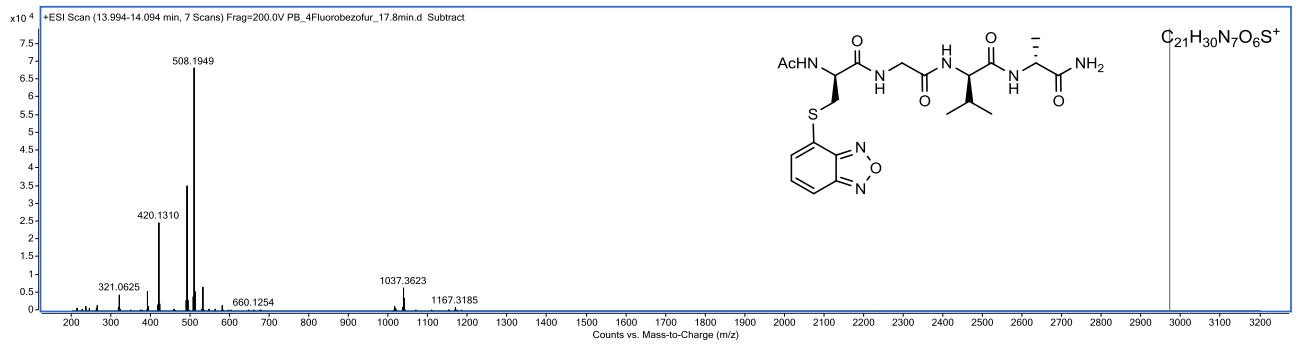


B) preparative HPLC *tR*= 17.068 min; ES-MS: calculated $[M + H]^+$, 508.1973, found m/z 508.1949 ($[M+H]^+$). White solid

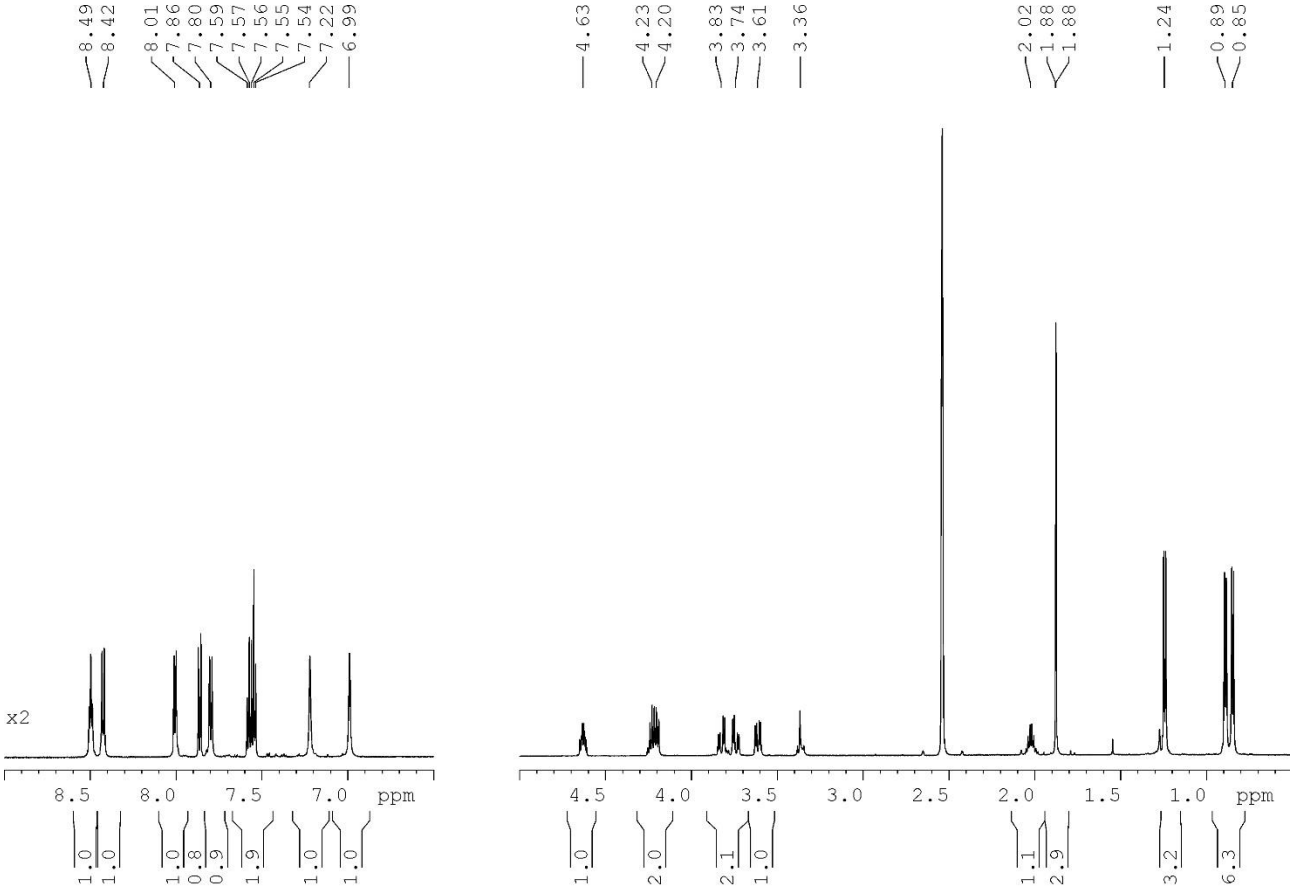
HPLC profile of the reaction crude product:



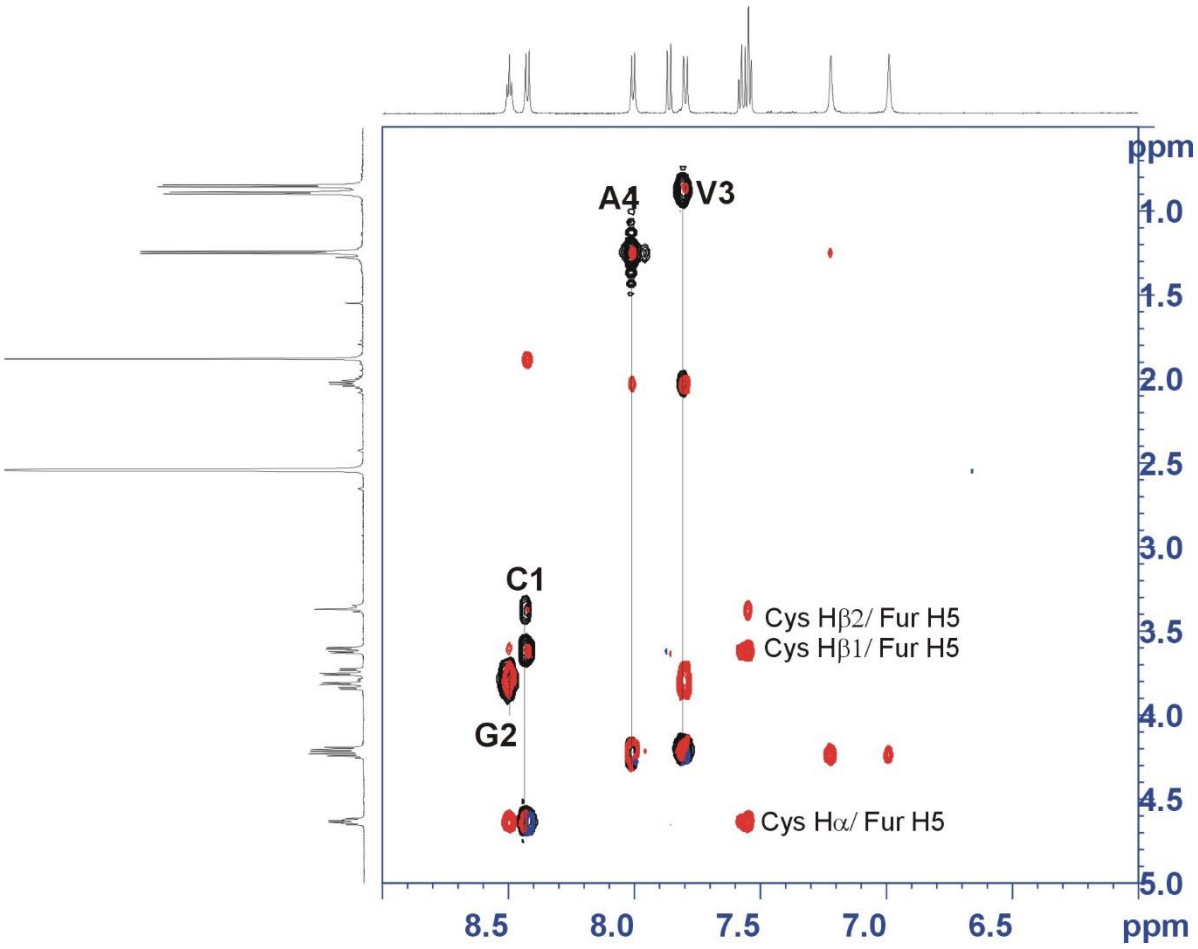
MS spectrum of **1c** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)

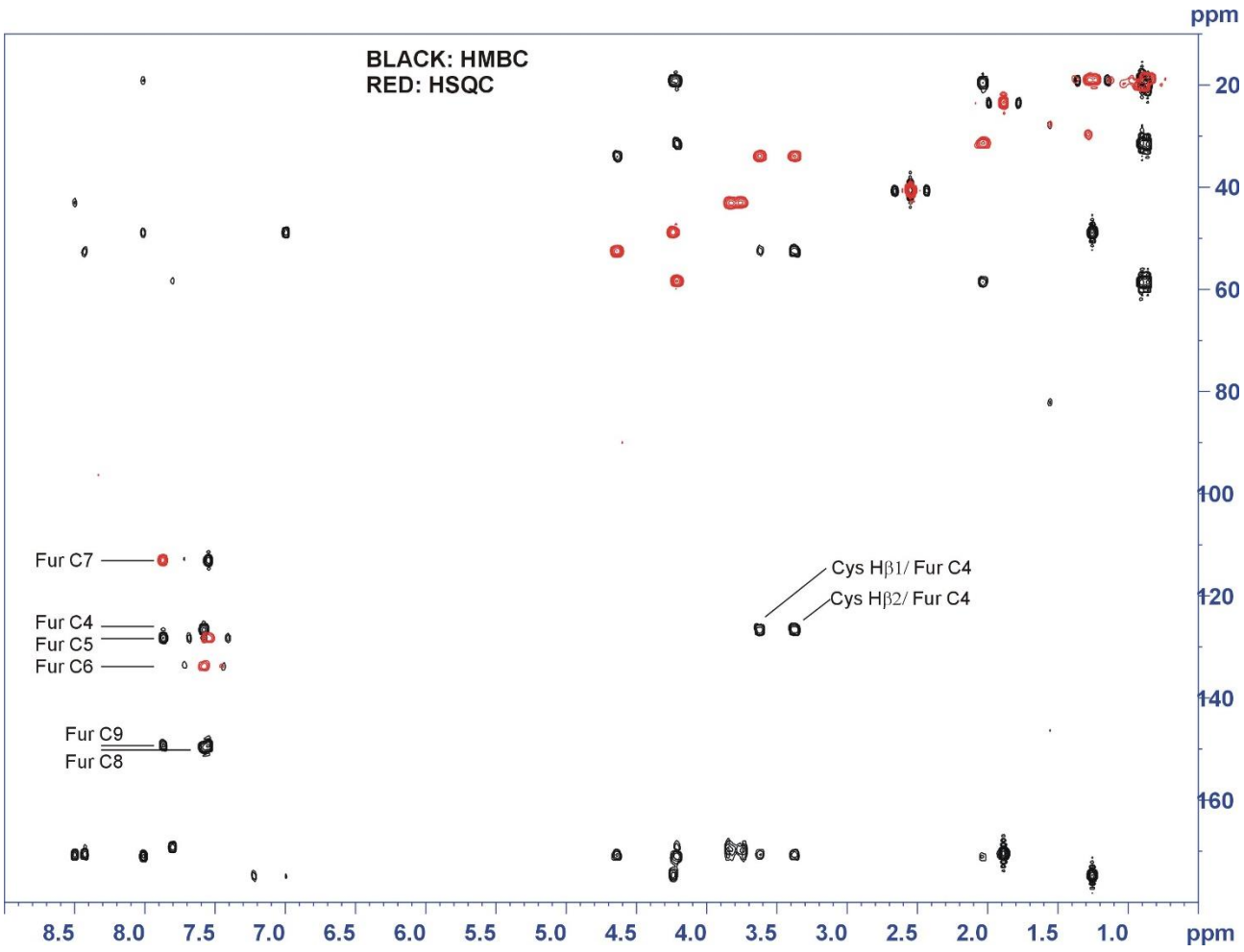


E) Assignment table

Compound 1c

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.88
CYS	H	1	8.43
CYS	HA	1	4.63
CYS	HB1	1	3.61
CYS	HB2	1	3.36
FU2	H5	5	7.55
FU2	H6	5	7.58
FU2	H7	5	7.87
GLY	H	2	8.49
GLY	HA2	2	3.83
GLY	HA3	2	3.74
VAL	H	3	7.80
VAL	HA	3	4.20
VAL	HB	3	2.03
VAL	HG1	3	0.89
VAL	HG2	3	0.85
ALA	H	4	8.01
ALA	HA	4	4.23
ALA	HB	4	1.24
CO-NH2	NH1	4	7.22
CO-NH2	NH2	4	6.99

G) Overlay of the $^{1}H,^{13}C$ HSQC (red) and $^{1}H,^{13}C$ HMBC (black) NMR spectra (600 MHz, DMSO-d_6, 298 K)



H) ^{13}C NMR assignment table (from ^{1}H,^{13}C HSQC and ^{1}H,^{13}C HMBC)

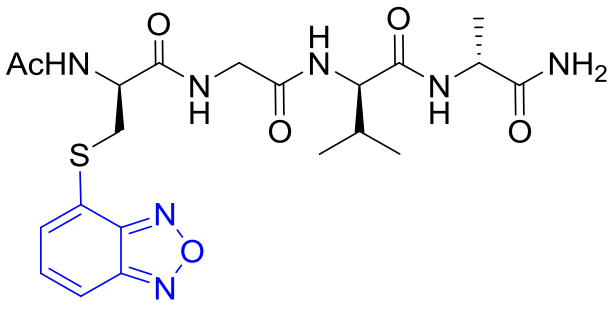
Compound 1c

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	23.3
N-term Ac	C	1	170.5
CYS	CA	1	52.3
CYS	CB	1	33.8
CYS	C	1	170.7
FUR	C5	5	128.3
FUR	C6	5	133.7
FUR	C7	5	112.8
FUR	C8	5	149.7
FUR	C9	5	149.3
FUR	C4	5	126.7
GLY	CA	2	43.0
GLY	C	2	169.3
VAL	CA	3	58.4
VAL	CB	3	31.2
VAL	CG1	3	19.8
VAL	CG2	3	18.6
VAL	C	3	171.0
ALA	CA	4	48.8
ALA	CB	4	18.8
C-term	CO-NH2	4	174.8

Compound 1c (X: Cl) strategy B

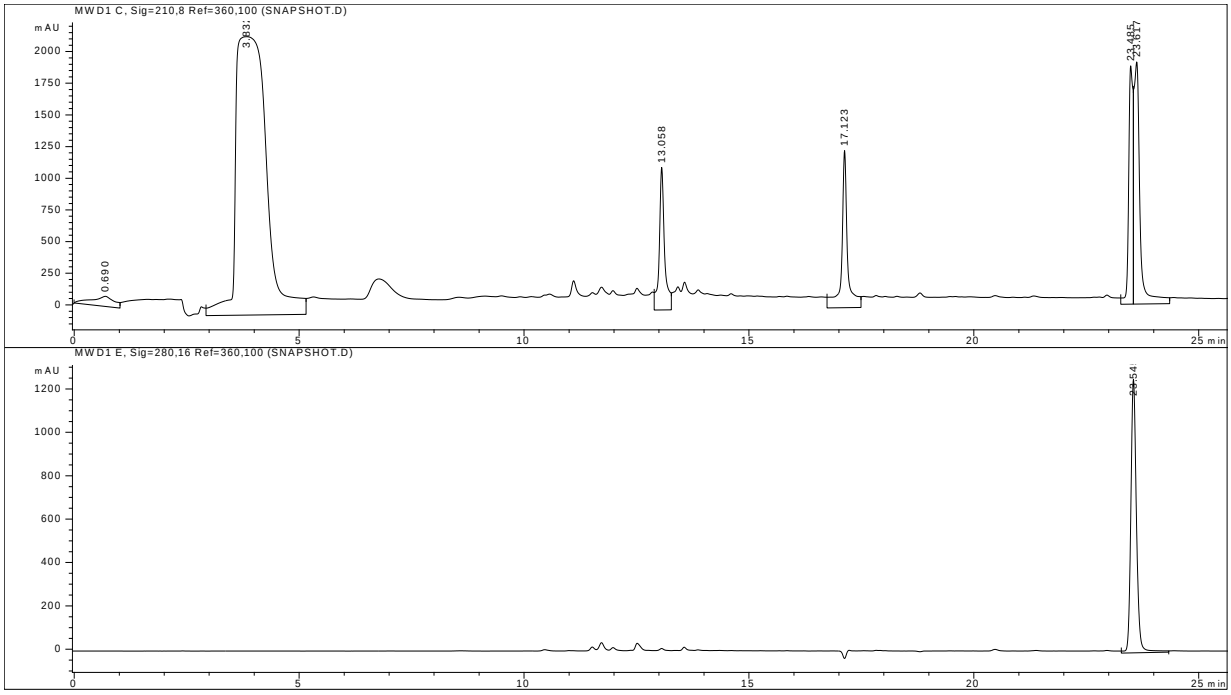
AcCys(c)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

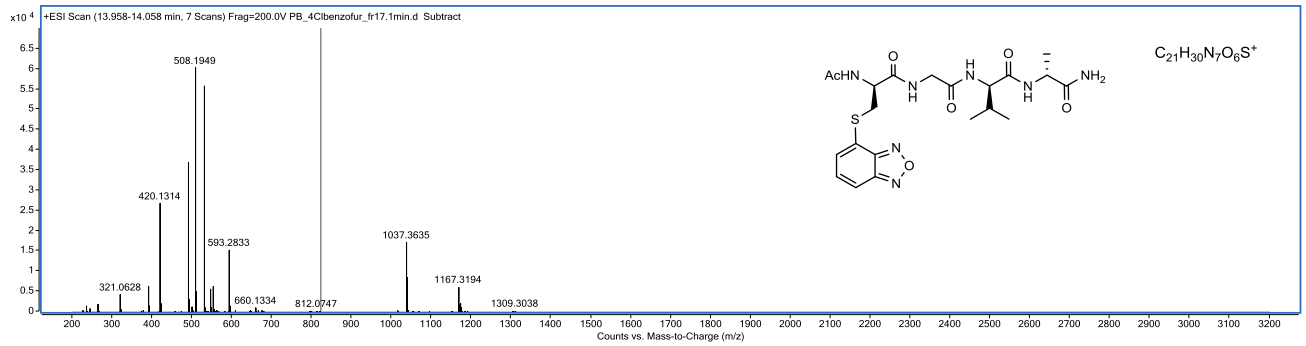


B) preparative HPLC *tR*= 17.123 min; ES-MS: calculated $[M + H]^+$, 508.1973, found m/z 508.1949 ($[M+H]^+$). White solid

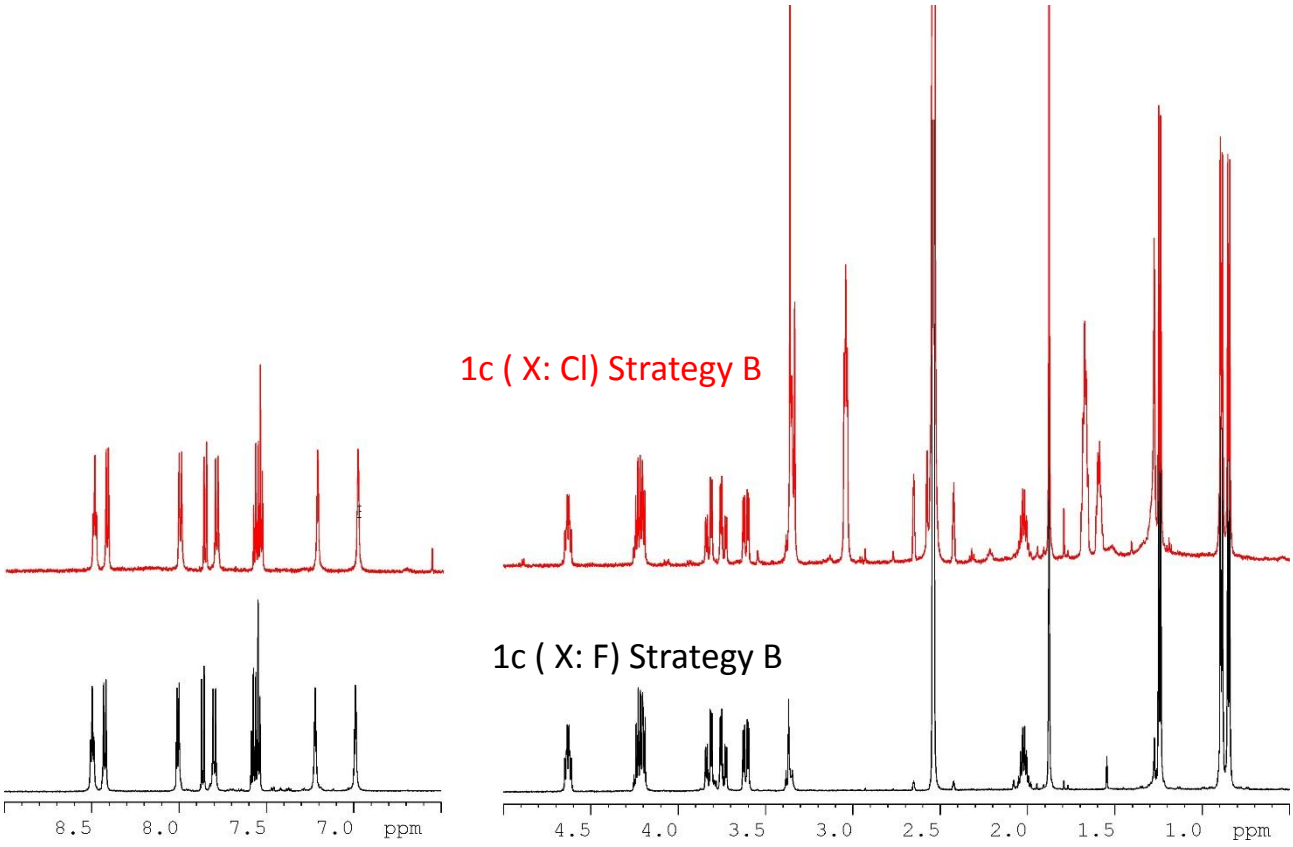
HPLC profile of the reaction crude product:



MS spectrum of **1c** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)

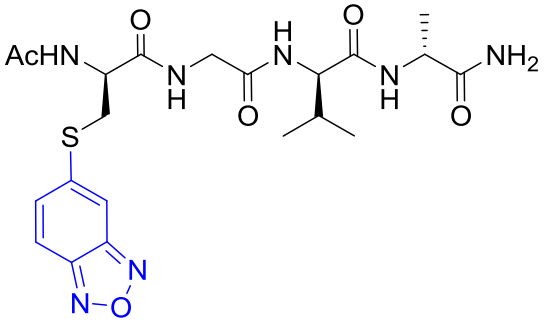


^{1}H-NMR spectra of **1c** obtained from the fluorine precursor (bottom) and the chlorine precursor (top).

Compound 1d (X: Br) strategy B

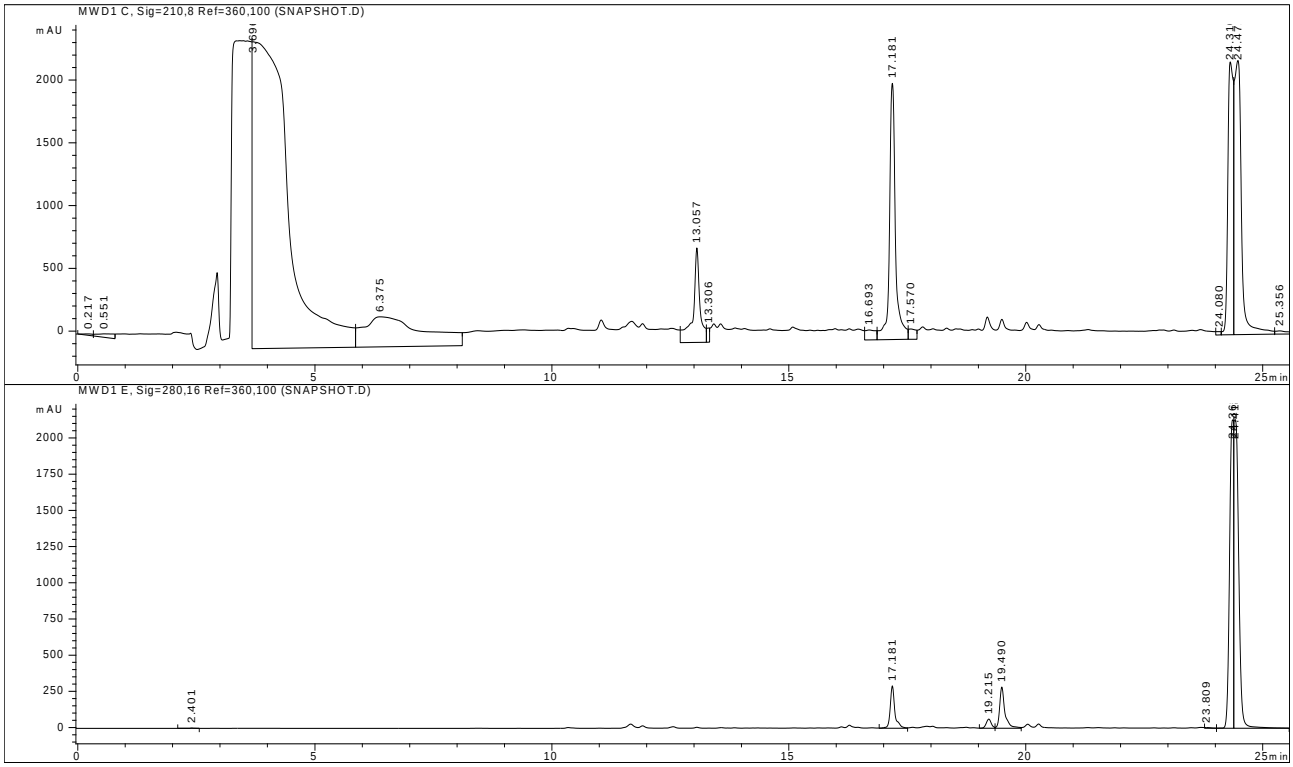
AcCys(d)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

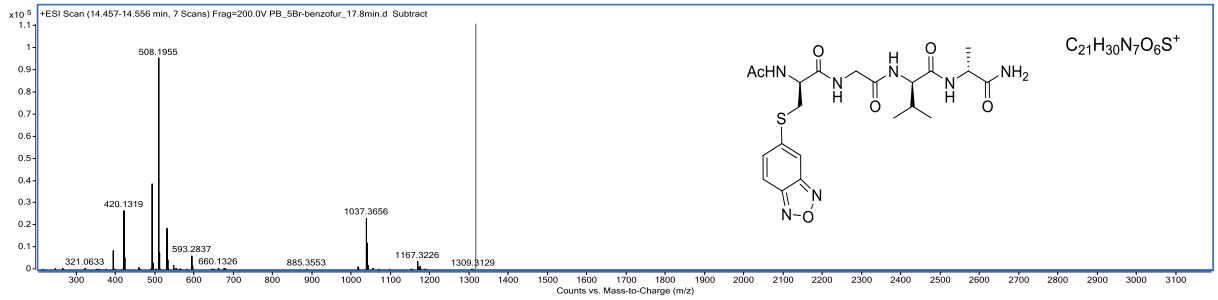


B) preparative HPLC *tR*= 17.181 min; ES-MS: calculated $[M + H]^+$, 508.1973, found m/z 508.1955 ($[M+H]^+$). White solid

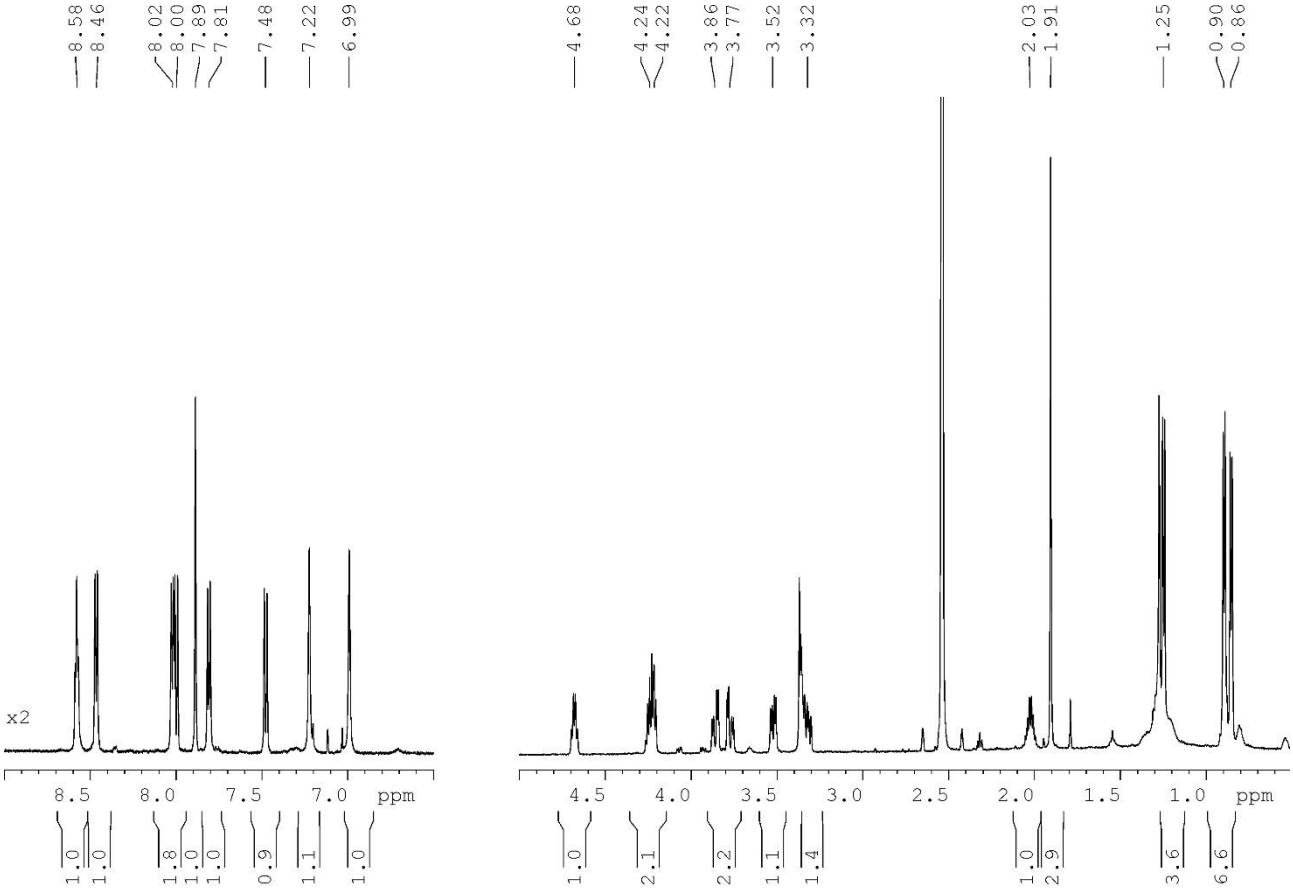
HPLC profile of the reaction crude product:



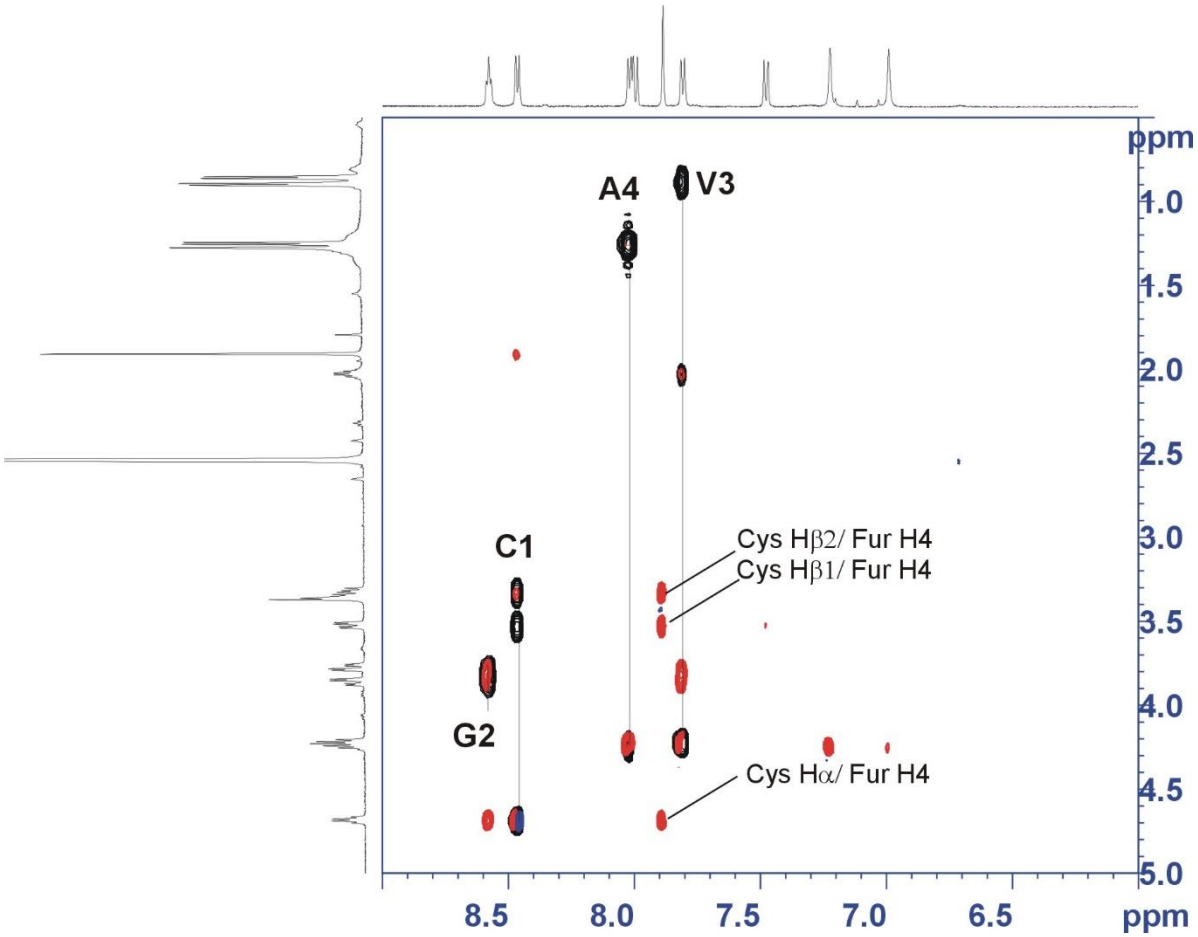
MS spectrum of **1d** after purification:



C) ^{1}H-NMR (600 MHz, DMSO-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, DMSO-d_6, 298 K)

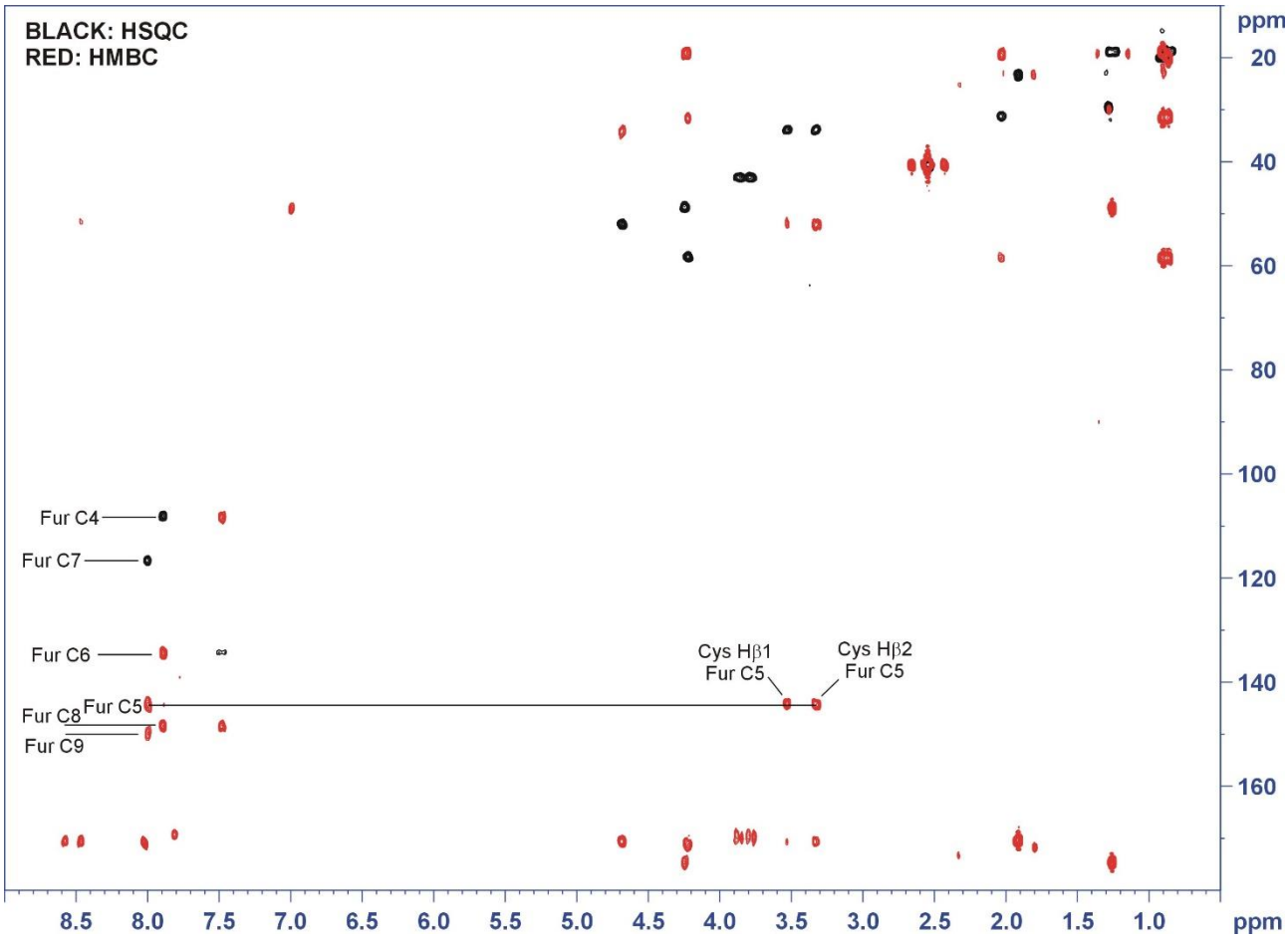


E) ^{1}H NMR assignment table

Compound 1d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.91
CYS	H	1	8.46
CYS	HA	1	4.68
CYS	HB1	1	3.52
CYS	HB2	1	3.33
FUR	H6	5	7.48
FUR	H7	5	8.00
FUR	H4	5	7.89
GLY	H	2	8.57
GLY	HA2	2	3.86
GLY	HA3	2	3.77
VAL	H	3	7.81
VAL	HA	3	4.22
VAL	HB	3	2.03
VAL	HG1	3	0.90
VAL	HG2	3	0.86
ALA	H	4	8.02
ALA	HA	4	4.24
ALA	HB	4	1.25
CO-NH2	NH1	4	7.22
CO-NH2	NH2	4	6.99

G) Overlay of the $^{1}H,^{13}C$ HSQC (black) and $^{1}H,^{13}C$ HMBC (red) NMR spectra (600 MHz, DMSO-d_6, 298 K)



H) ^{13}C NMR assignment table (from ^{1}H,^{13}C HSQC and ^{1}H,^{13}C HMBC)

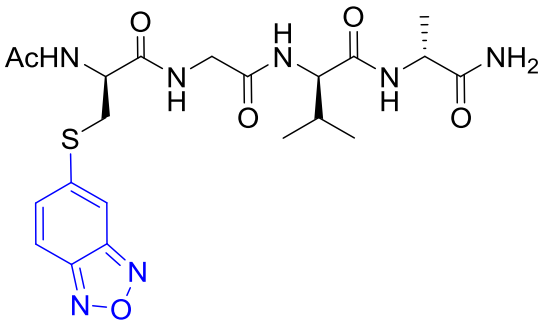
Compound 1d

RES	SPIN ID	SEQ		ppm
N-term Ac	CH3		1	23.3
N-term Ac	C		1	170.4
CYS	CA		1	52.0
CYS	CB		1	33.8
CYS	C		1	170.6
FUR	C5		5	144.3
FUR	C6		5	134.1
FUR	C7		5	116.6
FUR	C8		5	148.4
FUR	C9		5	149.7
FUR	C4		5	107.9
GLY	CA		2	42.9
GLY	C		2	169.3
VAL	CA		3	58.3
VAL	CB		3	31.1
VAL	CG1		3	19.8
VAL	CG2		3	18.7
VAL	C		3	171.0
ALA	CA		4	48.6
ALA	CB		4	18.8
C-term	CO-NH2		4	174.5

Compound 1d (X: Cl) strategy B

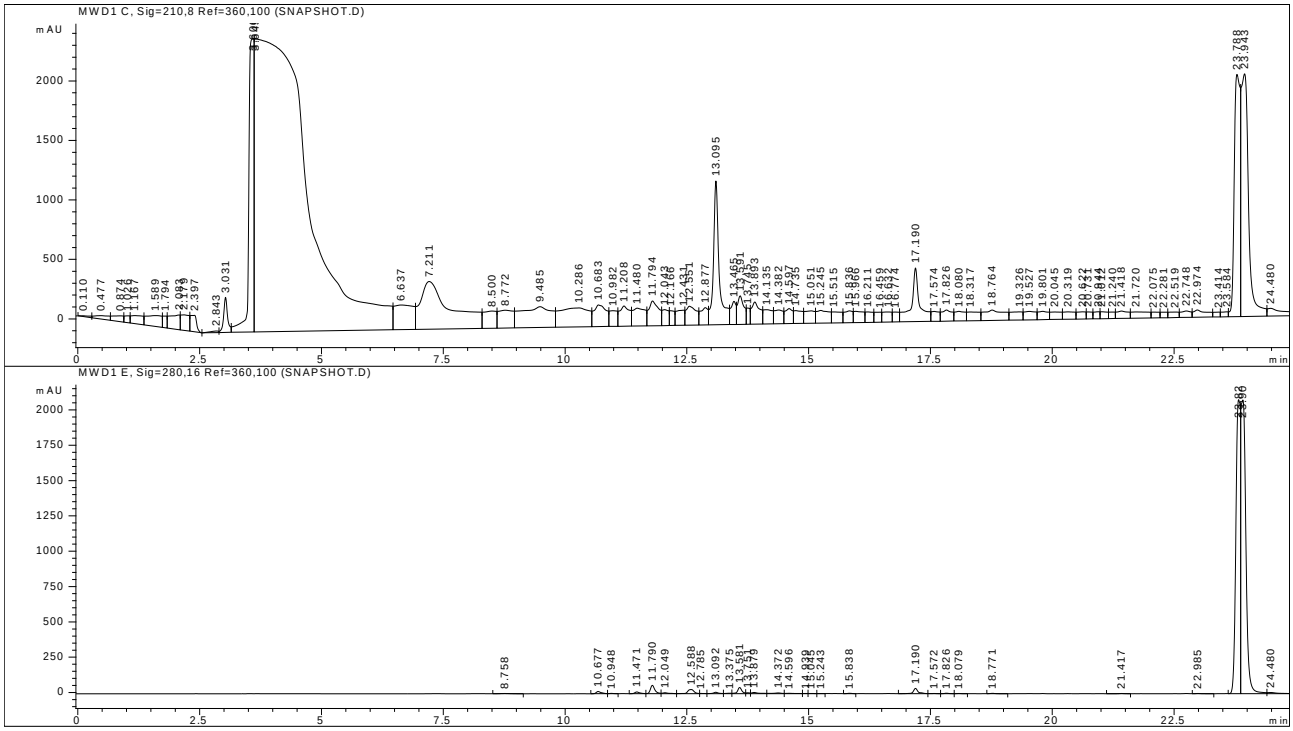
AcCys(d)GlyValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

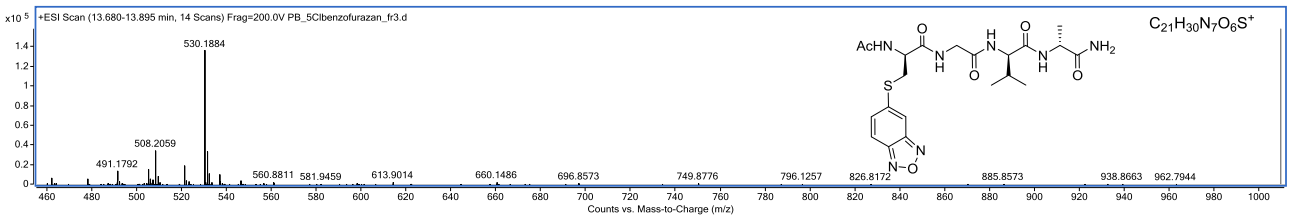


B) preparative HPLC *tR*= 17.190 min; ES-MS: calculated $[M + H]^+$, 508.1973, found m/z 508.2059 ([M+H]). White solid

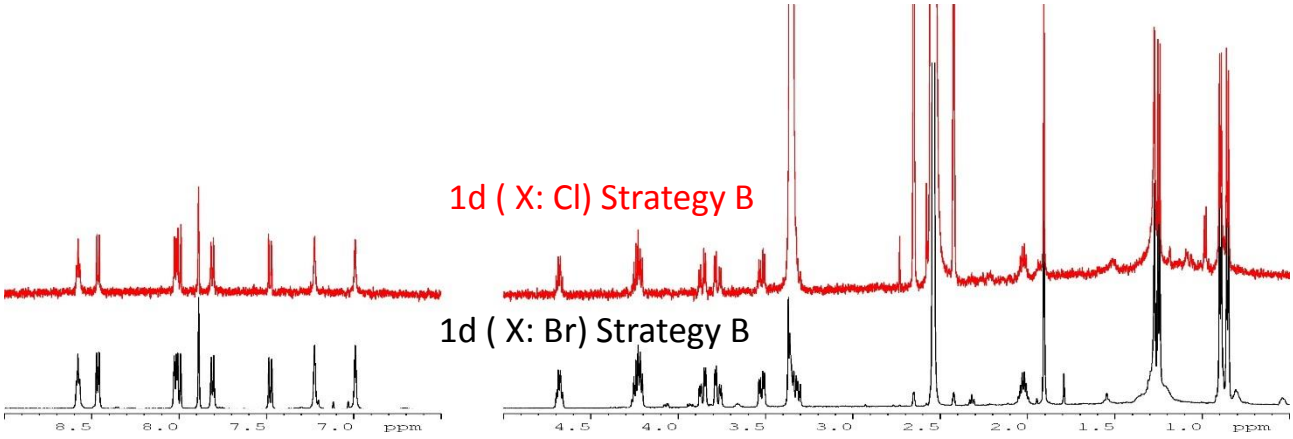
HPLC profile of the reaction crude product:



MS spectrum of **1d** after purification:



C) ^{1}H-NMR (600 MHz, DMSO-d_6 , 298 K)

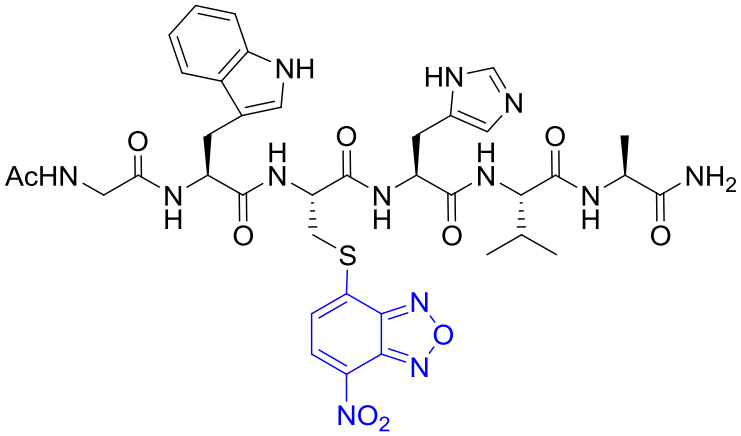


^{1}H-NMR spectra of 1**d** obtained from the bromine precursor (bottom) and the chlorine precursor (top)

Compound 2a strategy A

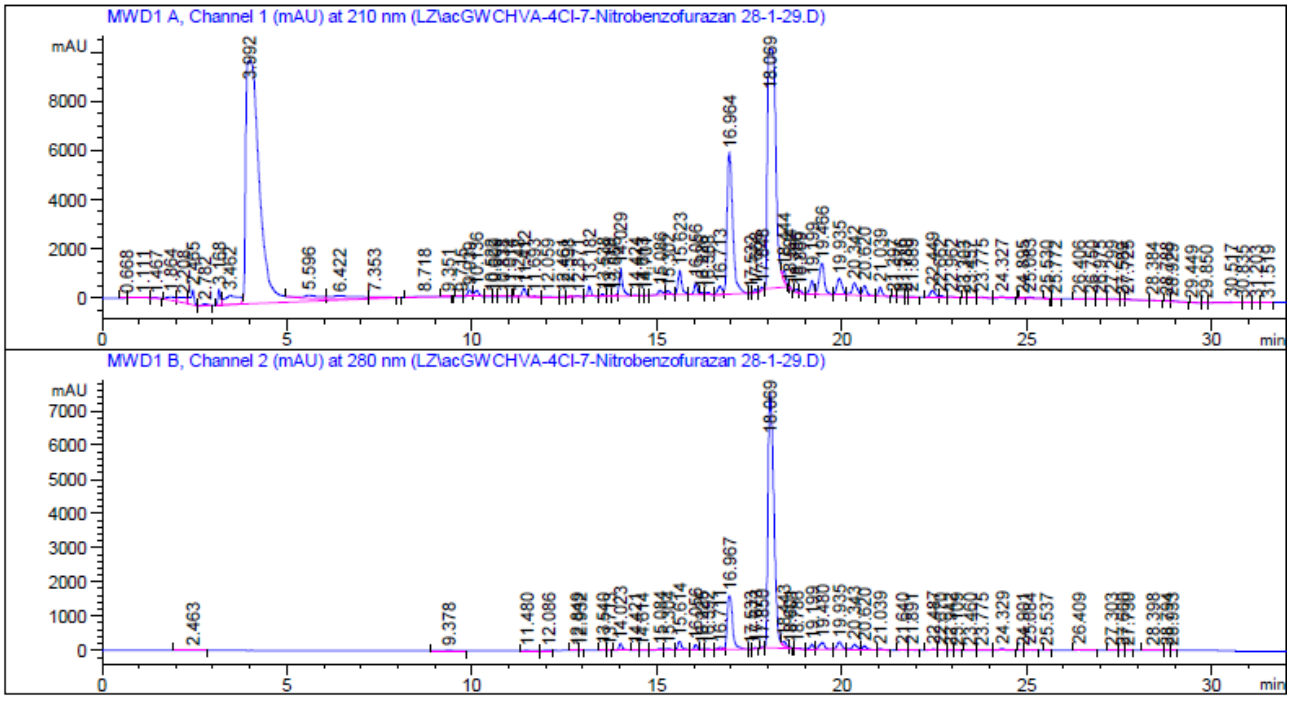
AcGlyTrpCys(a)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

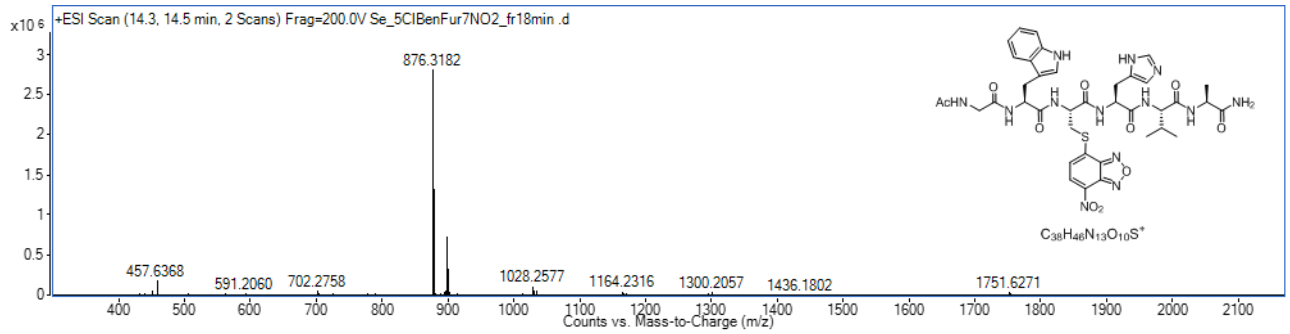


B) preparative HPLC *tR*= 18.069 min; ES-MS: calculated $[M + H]^+$, 876.3206, found m/z 876.3182 ($[M+H]^+$). yellow solid

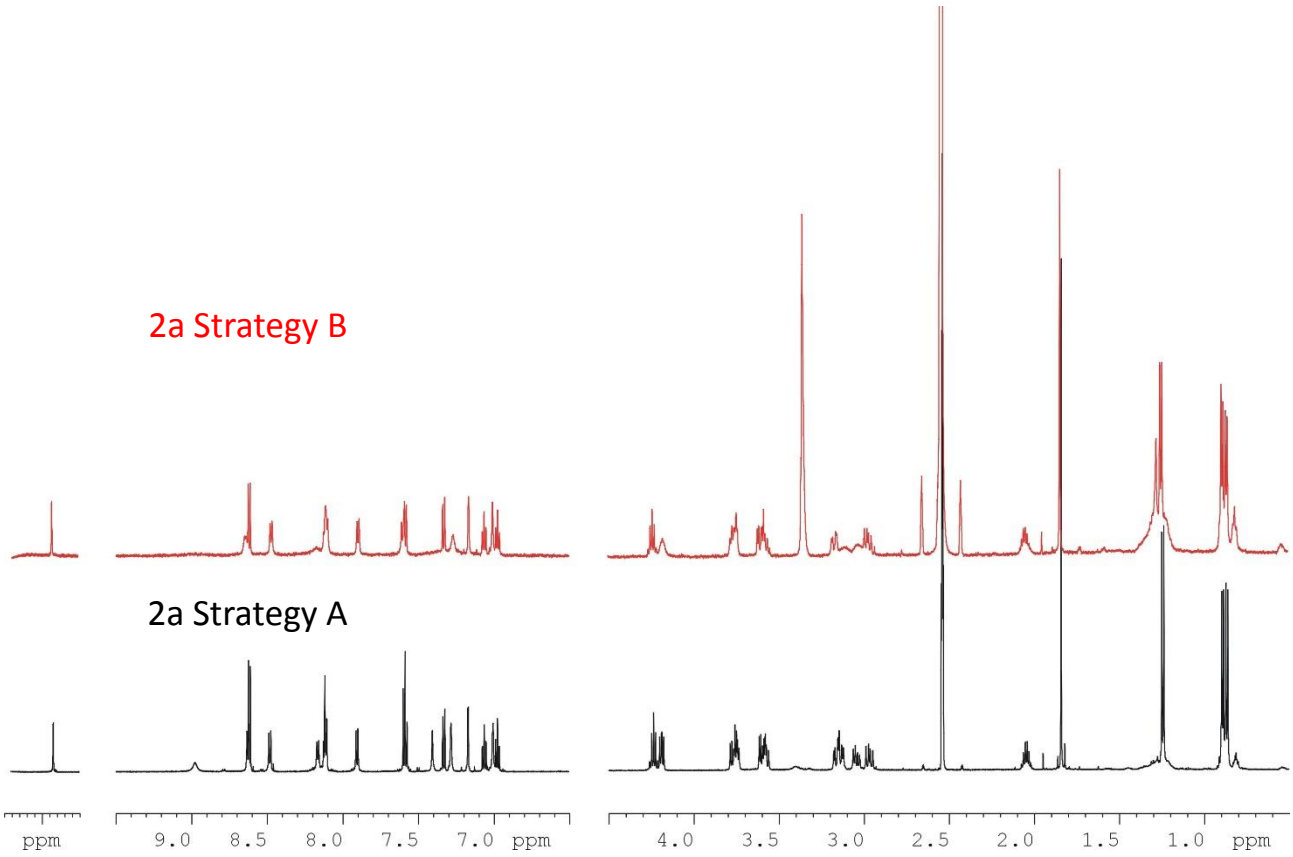
HPLC profile of the reaction crude product:



MS spectrum of **2a** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)

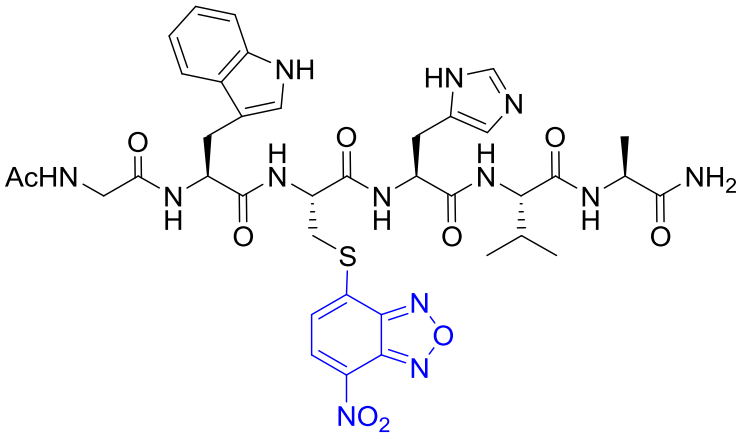


^{1}H NMR spectra of product **2a** obtained by means of the activated molecular sieve synthetic approach (top) and by the standard approach with DIPEA as the base (bottom).

Compound 2a strategy B

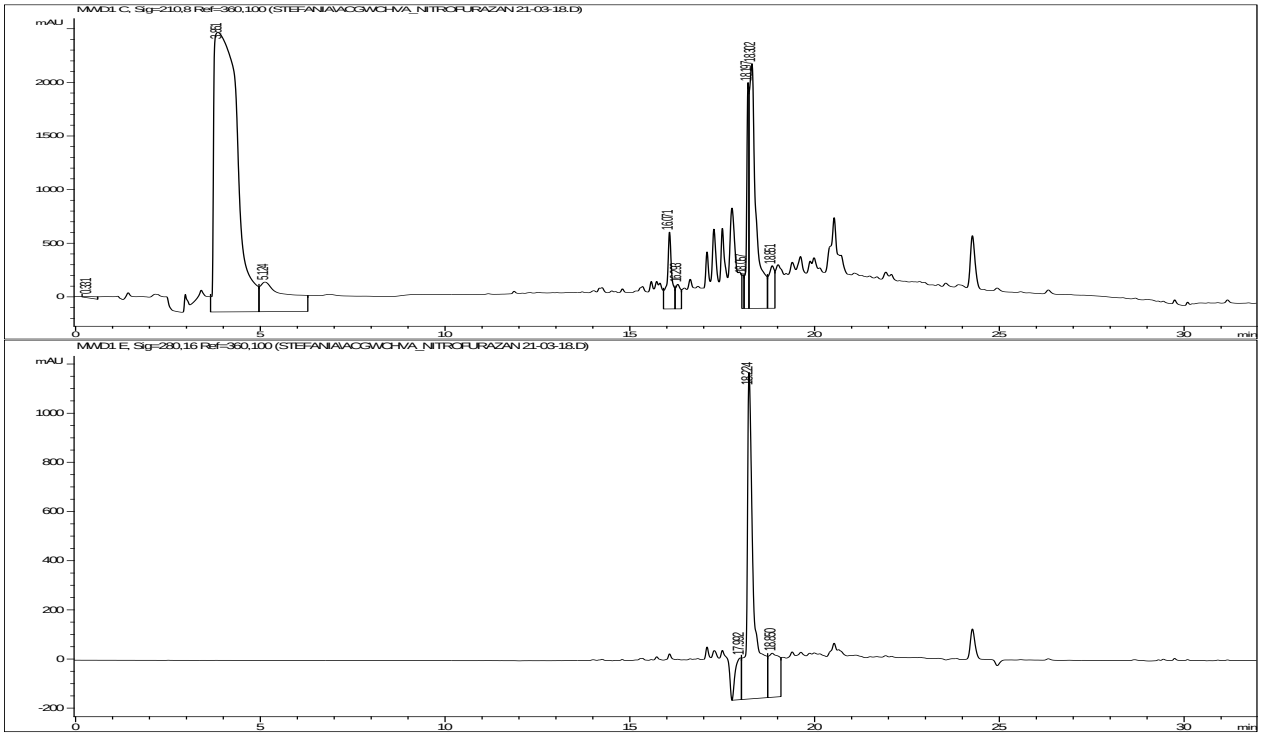
AcGlyTrpCys(a)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

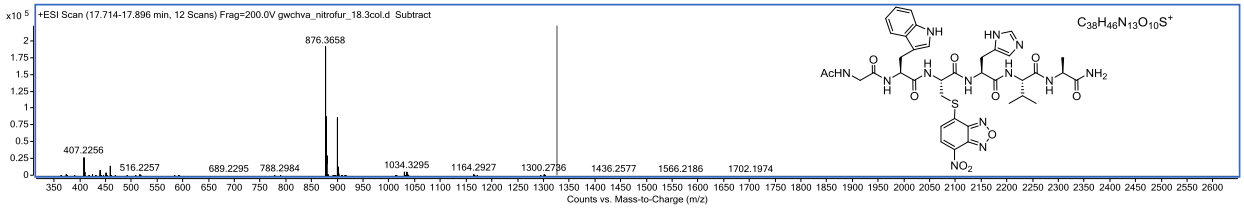


B) preparative HPLC *tR*= 18.302 min; ES-MS: calculated $[M + H]^+$, 876.3206, found m/z 876.3658 ($[M+H]^+$). yellow solid

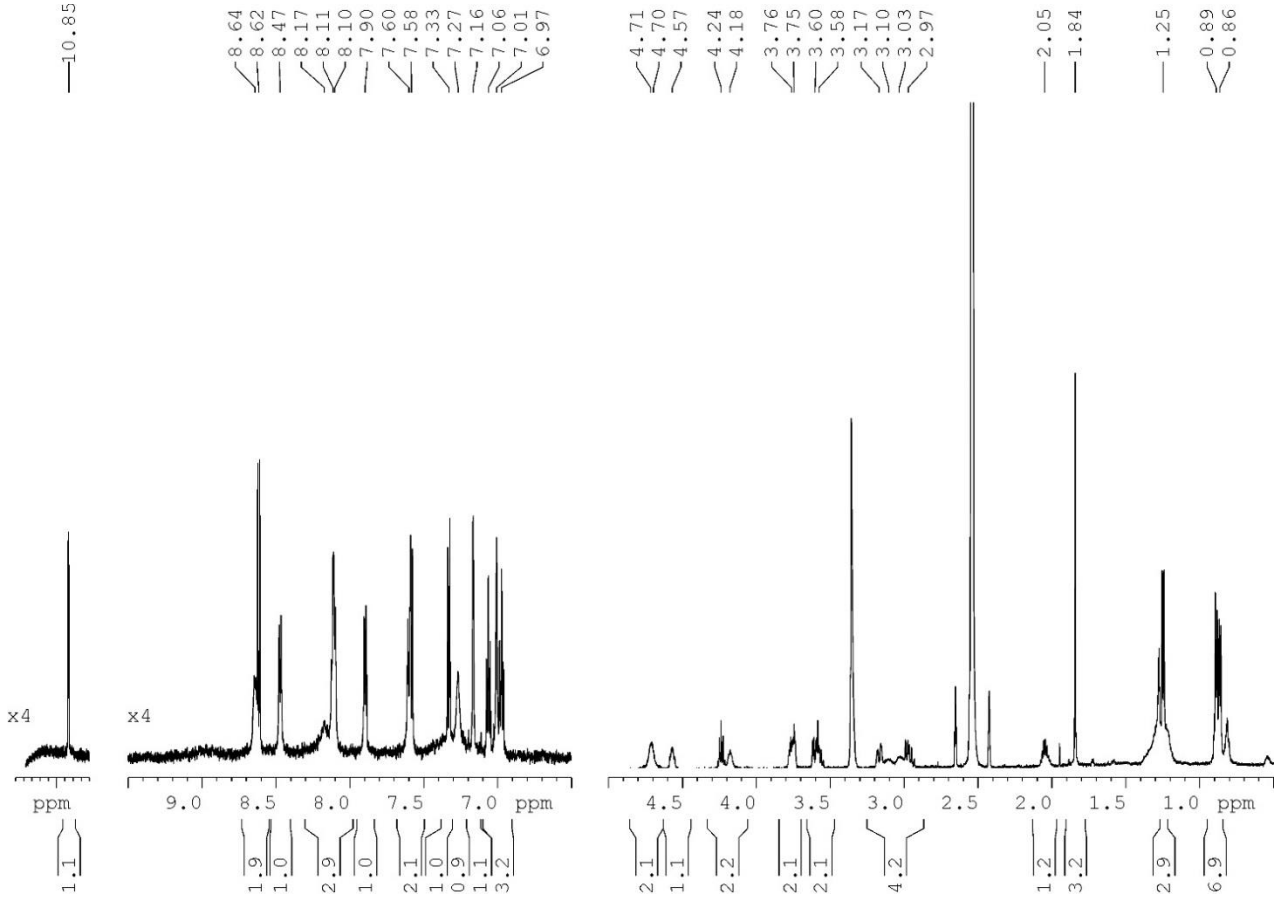
HPLC profile of the reaction crude product:



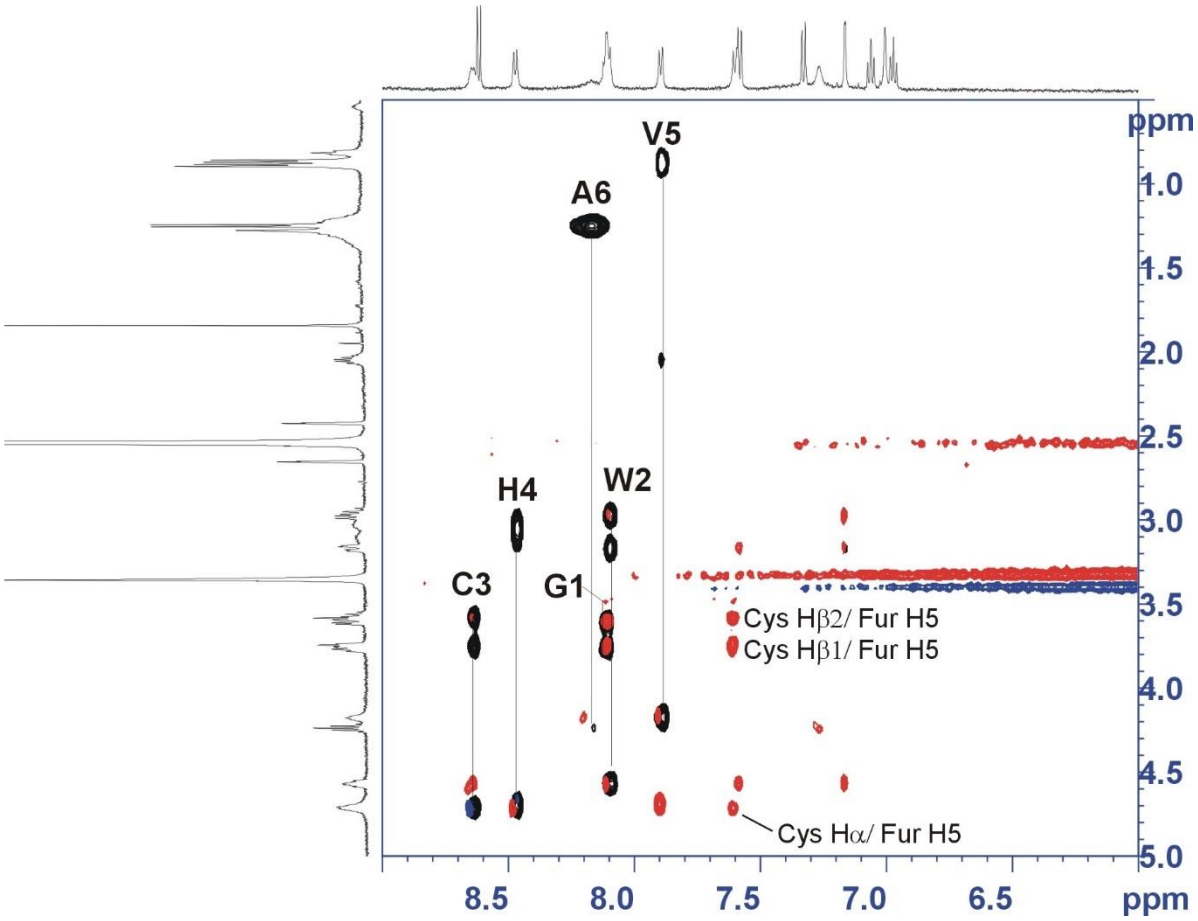
MS spectrum of **2a** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, *298K)*



E) Assignment table

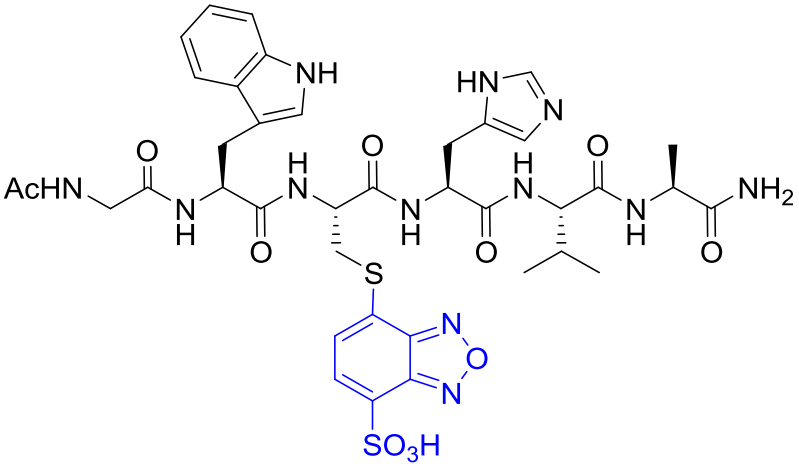
Compound 2a

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.84
GLY	H	1	8.11
GLY	HA1	1	3.76
GLY	HA2	1	3.60
TRP	H	2	8.10
TRP	HA	2	4.57
TRP	HB1	2	3.17
TRP	HB2	2	2.97
TRP	HD1	2	7.16
TRP	HE1	2	10.85
TRP	HZ2	2	7.33
TRP	HH2	2	7.06
TRP	HZ3	2	6.97
TRP	HE3	2	7.58
CYS	H	3	8.64
CYS	HA	3	4.71
CYS	HB1	3	3.70
CYS	HB2	3	3.58
FUR	H5	7	7.60
FUR	H6	7	8.62
HIS	H	4	8.47
HIS	HA	4	4.70
HIS	HB1	4	3.10
HIS	HB2	4	3.03
HIS	HD2	4	7.37
HIS	HE1	4	8.97
VAL	H	5	7.90
VAL	HA	5	4.18
VAL	HB	5	2.05
VAL	HG1	5	0.89
VAL	HG2	5	0.86
ALA	H	6	8.17
ALA	HA	6	4.24
ALA	HB	6	1.25
CO-NH2	NH1	6	7.27
CO-NH2	NH2	6	7.01

Compound 2b (X: Cl) strategy A

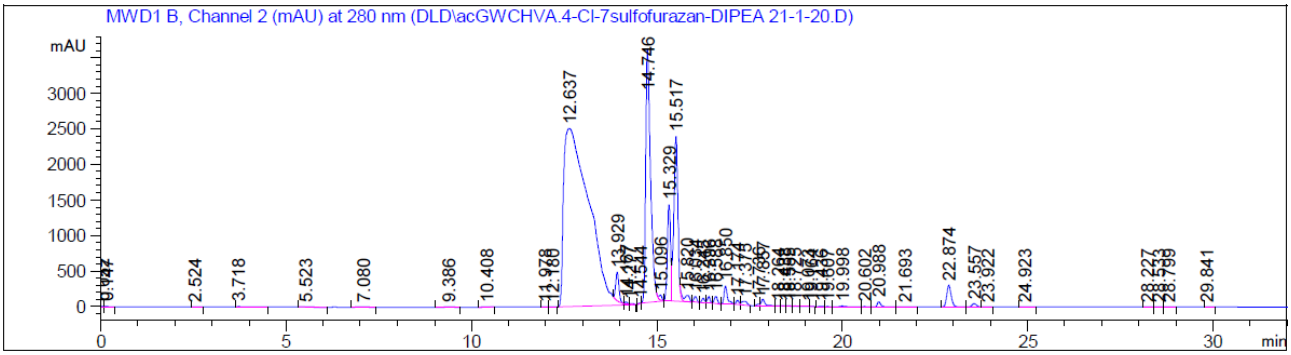
AcGlyTrpCys(b)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

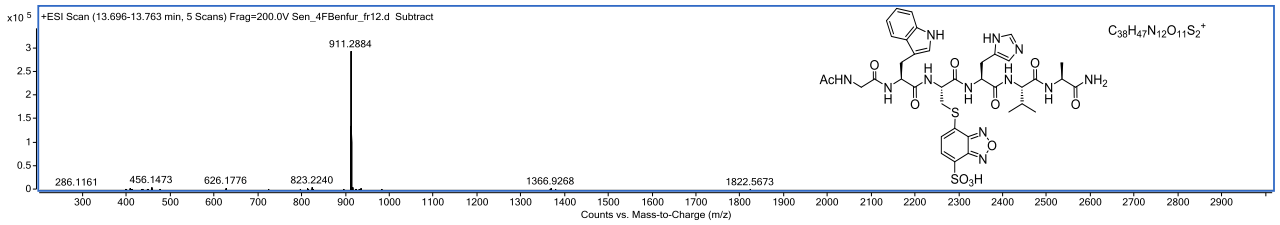


B) preparative HPLC *tR*= 15.329 min; ES-MS: calculated $[M + H]^+$, 911.2923, found m/z 911.2884($[M+H]^+$). Yellow solid

HPLC profile of the reaction crude product:



MS spectrum of **2b** after purification:



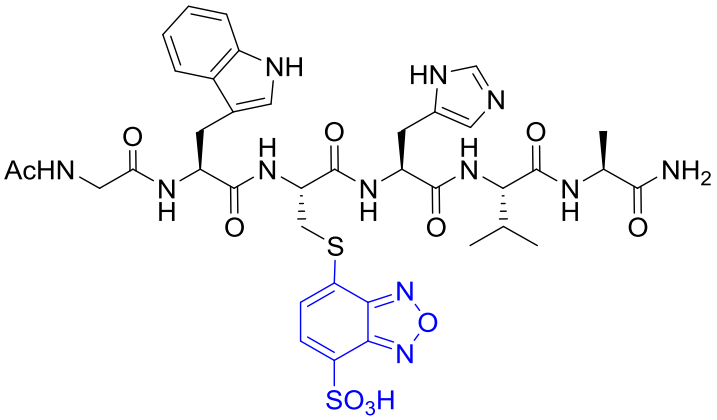
C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)

^{1}H NMR spectra of product **2b** obtained by means of the activated molecular sieve synthetic approach (top) and by the standard approach with DIPEA as the base (bottom). The chlorinated furazan precursor was used.

Compound 2b (X: Cl) strategy B

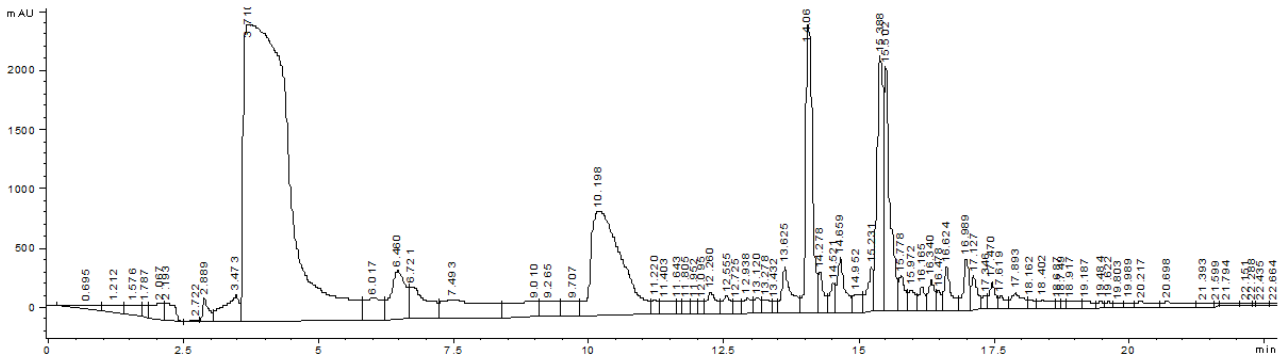
AcGlyTrpCys(b)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

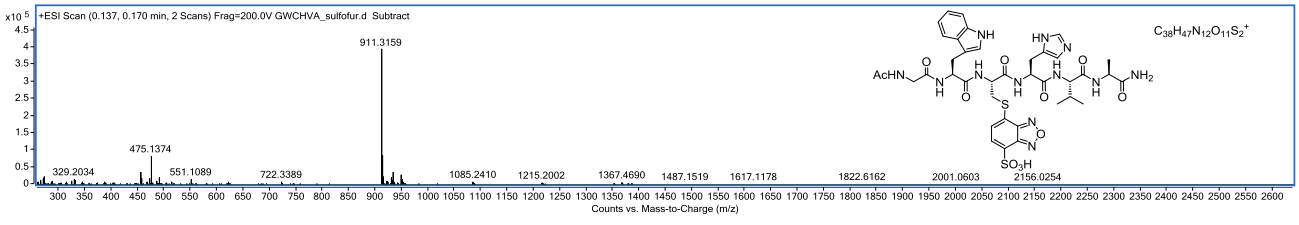


B) preparative HPLC *tR*= 15.388 min; ES-MS: calculated $[M + H]^+$, 911.2923, found m/z 911.3159 ($[M+H]^+$). Yellow solid

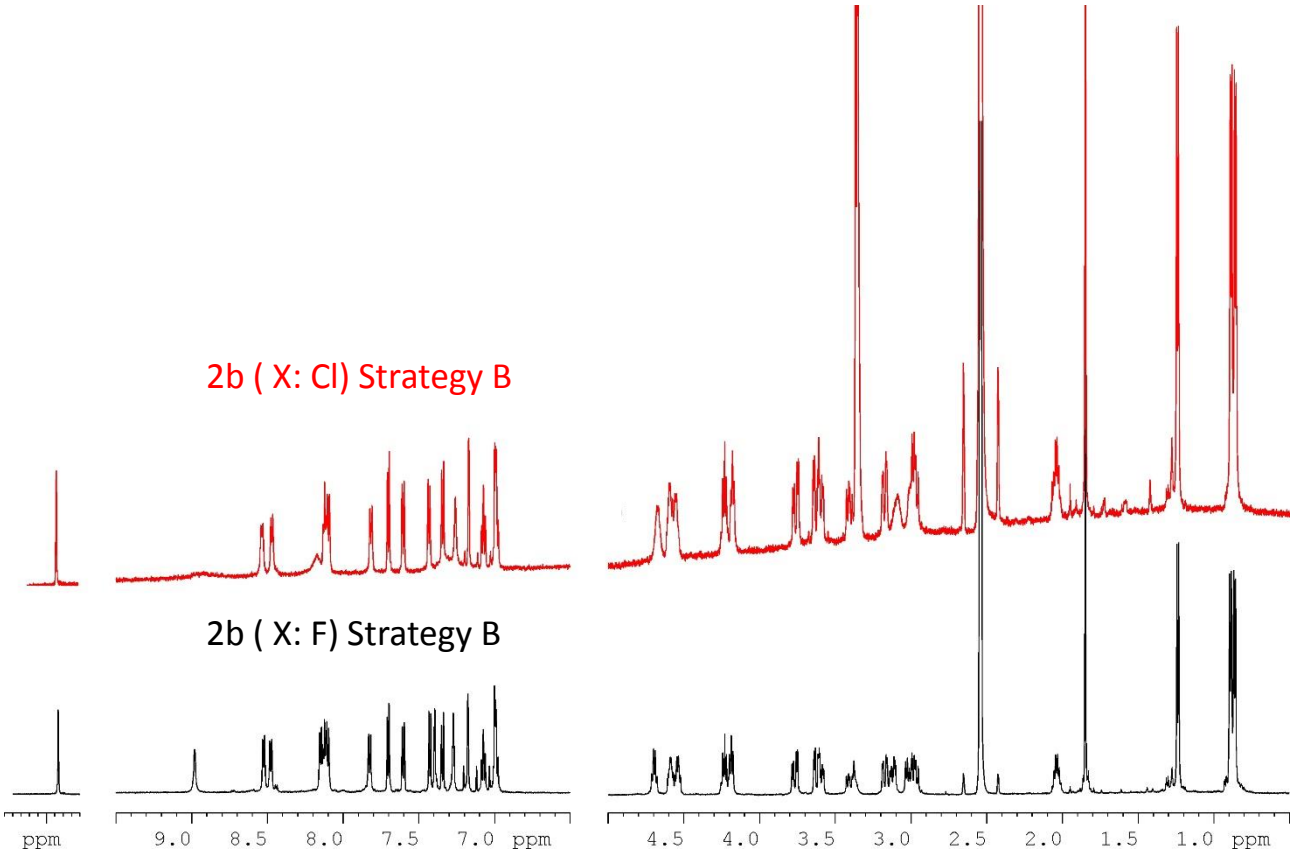
HPLC profile of the reaction crude product:



MS spectrum of **2b** after purification:

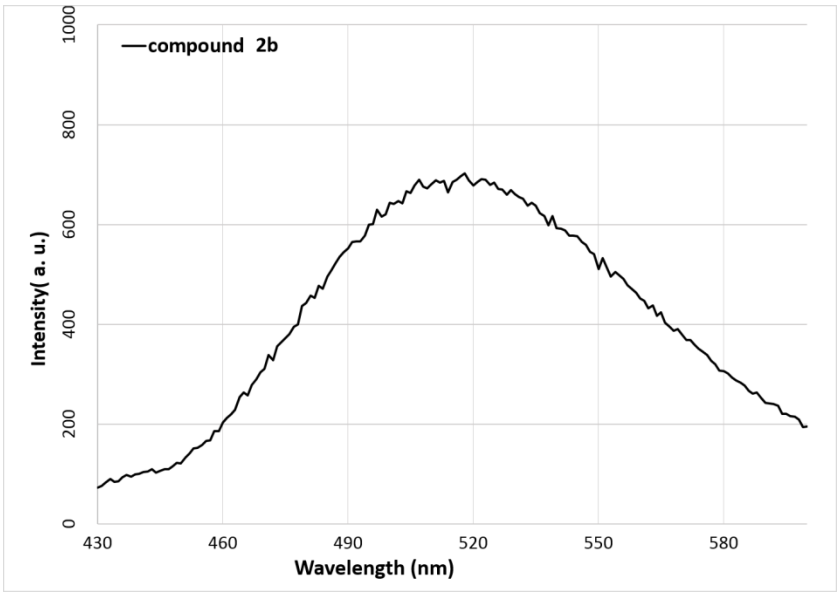


C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



^{1}H-NMR spectra of **2b** obtained from the fluorine precursor (bottom) and the chlorine precursor (top).

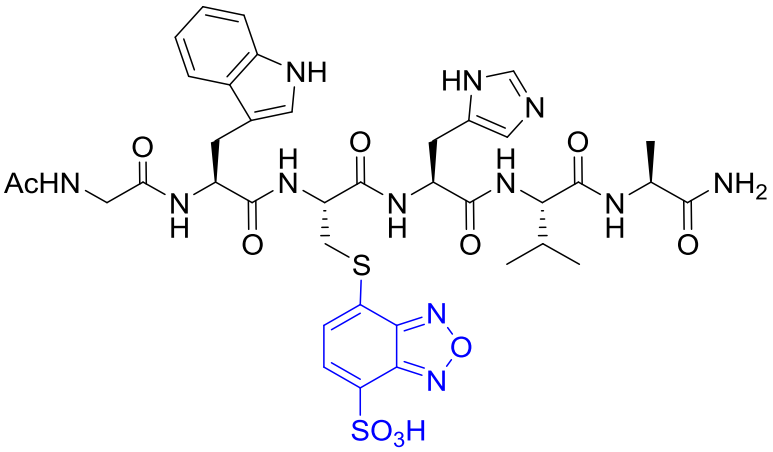
F) Fluorescence spectrum (λ_{ex} = 380 nm);



Compound 2b (X: F) strategy A

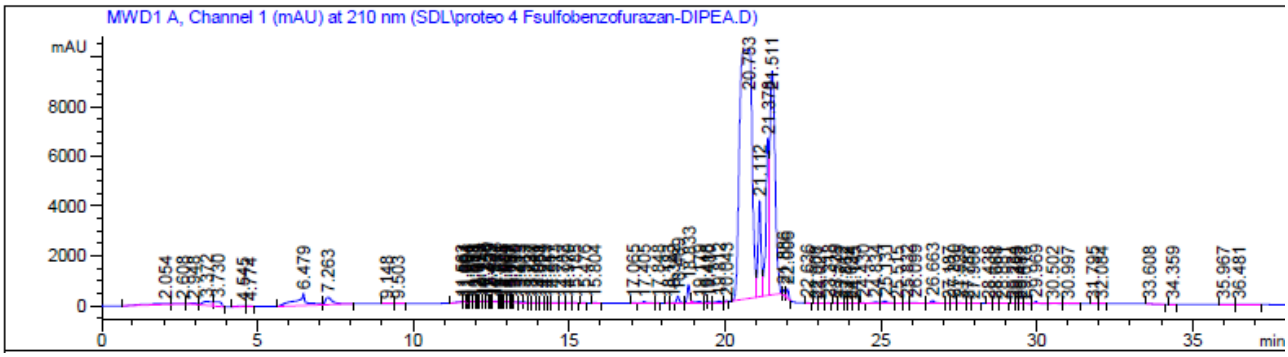
AcGlyTrpCys(b)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

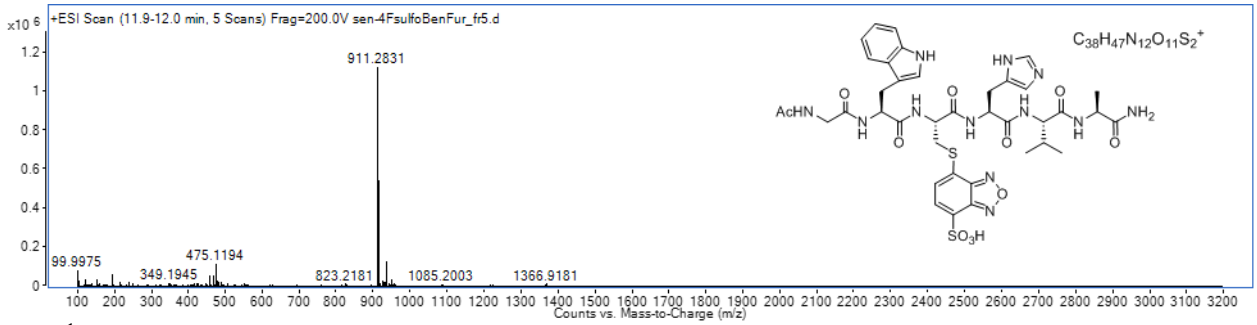


B) preparative HPLC *tR*= 21.112 min; ES-MS: calculated $[M + H]^+$, 911.2923, found m/z 911.2831 ($[M+H]^+$). Yellow solid

HPLC profile of the reaction crude product:



MS spectrum of **2b** after purification:

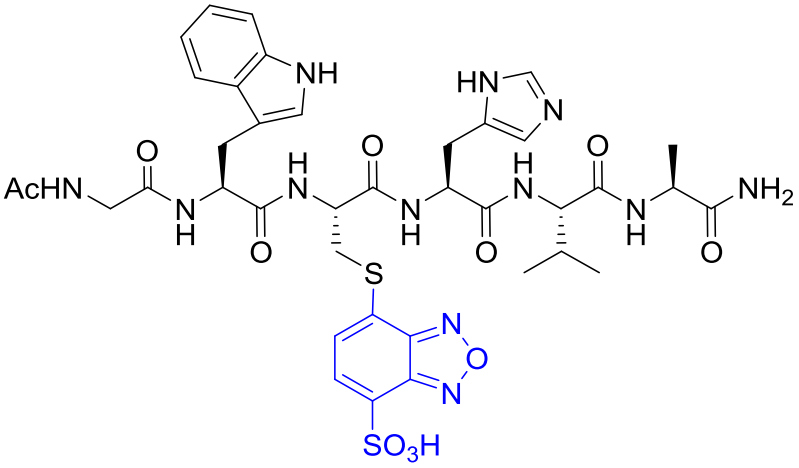


C) ^{1}H-NMR (600 MHz, dmso-d$_6$, 298 K): The NMR characterization was not performed, since the product was isolated after several HPLC, so the amount collected resulted insufficient

Compound 2b (X: F) strategy B

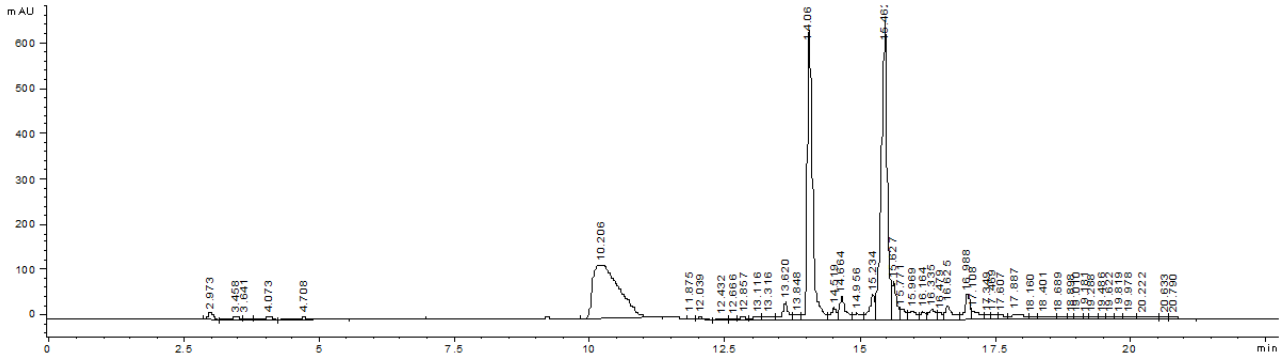
AcGlyTrpCys(b)HisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

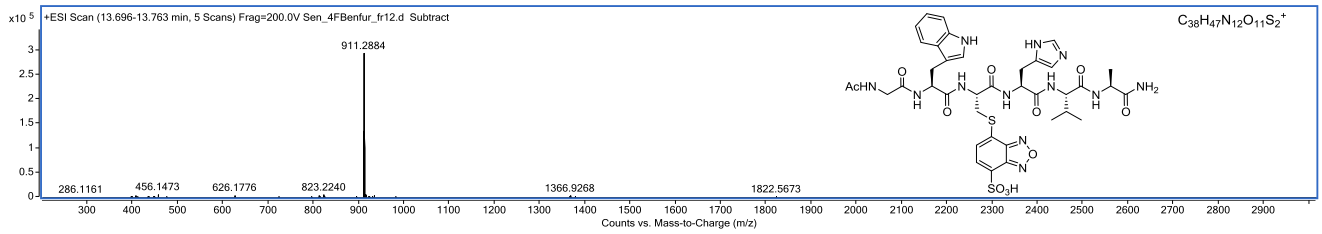


B) preparative HPLC *tR*= 15.462 min; ES-MS: calculated $[M + H]^+$, 911.2923, found m/z 911.2884 ($[M+H]^+$). Yellow solid

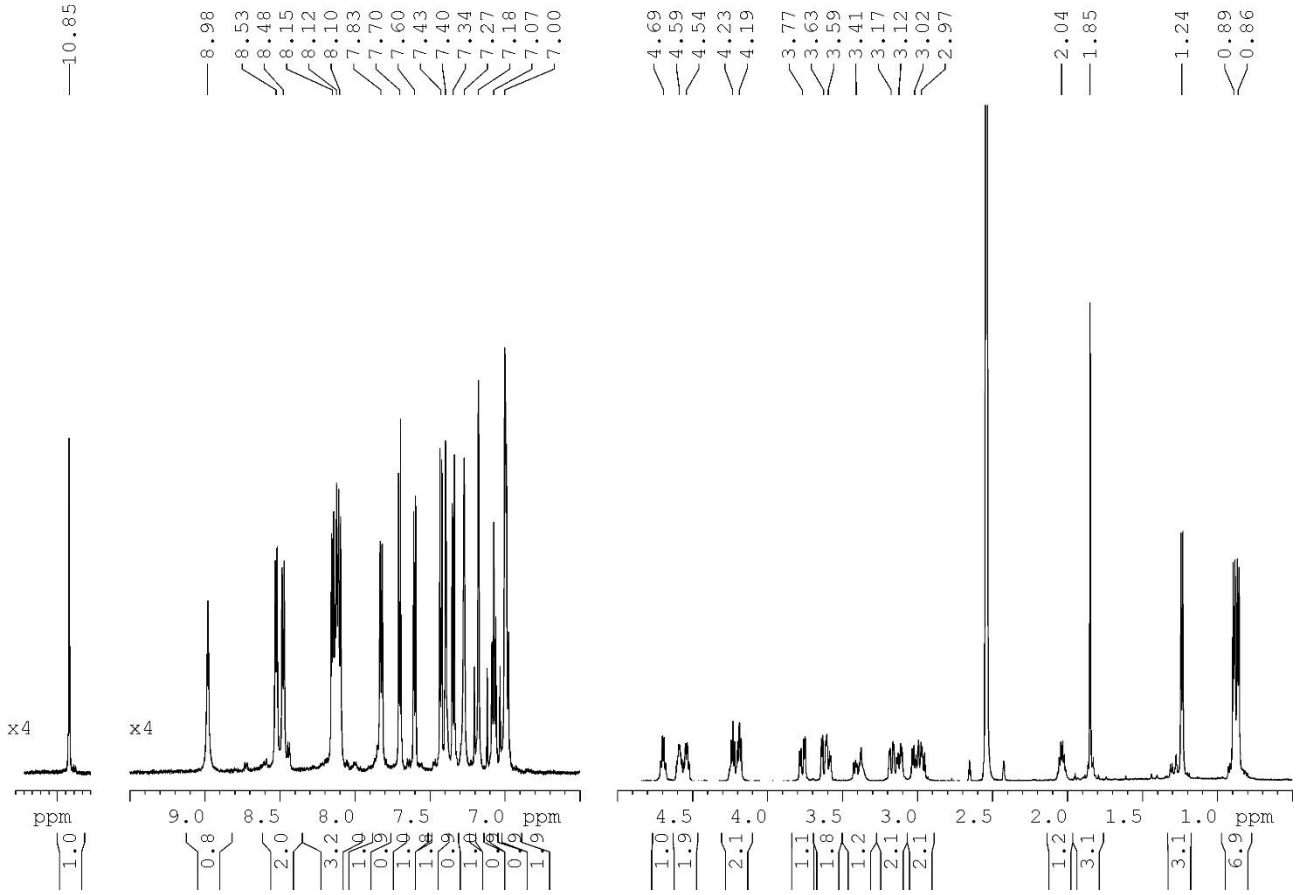
HPLC profile of the reaction crude product:



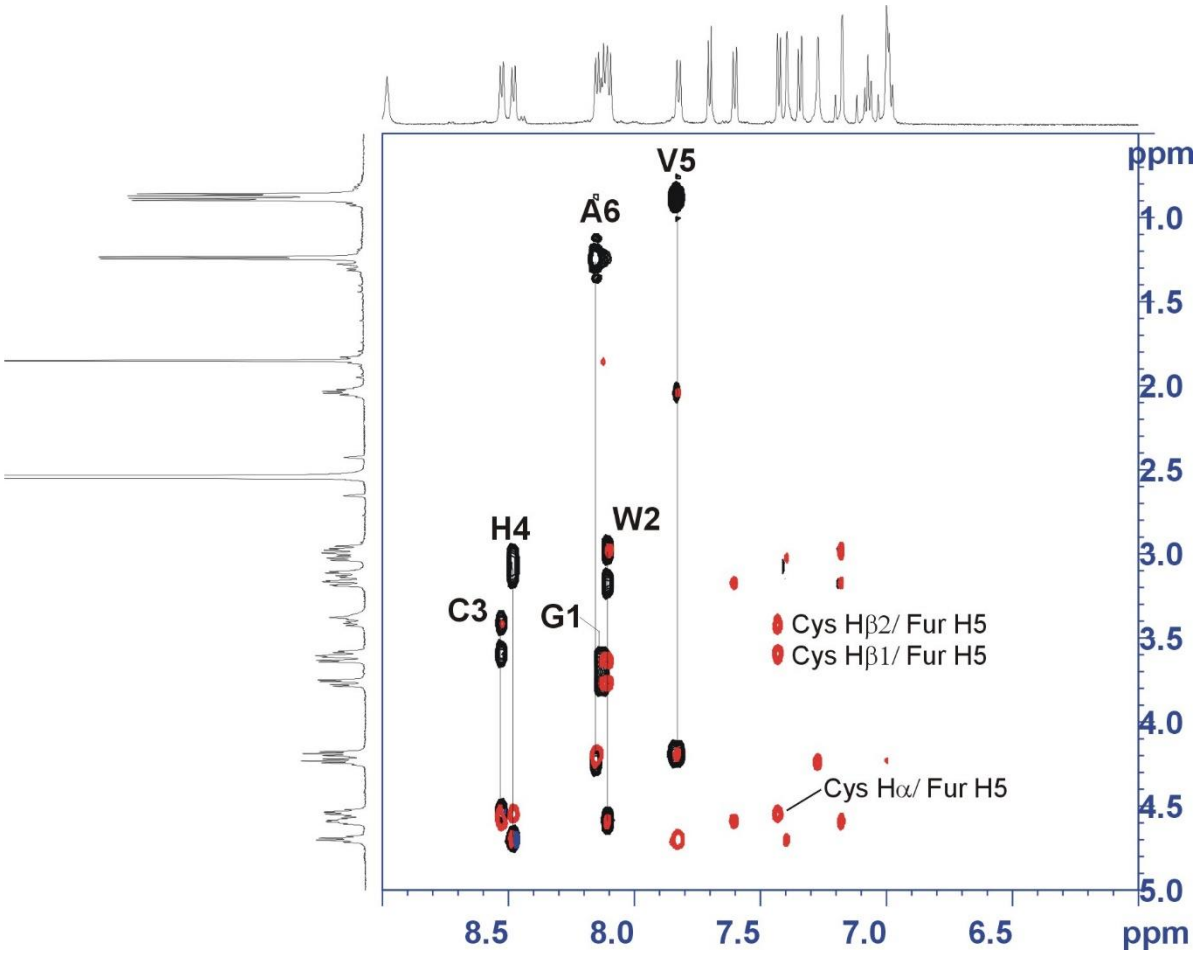
MS spectrum of **2b-F** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

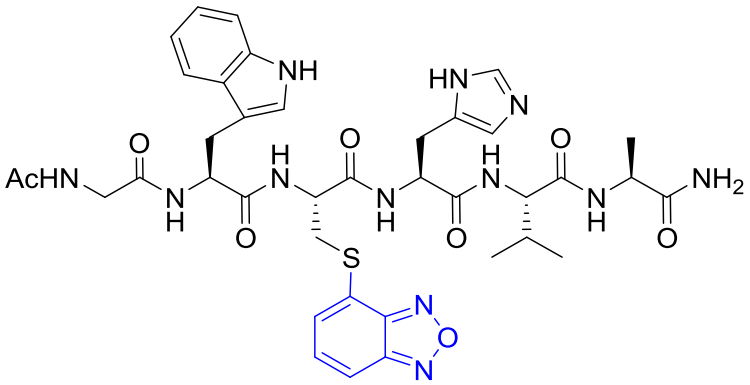
Compound 2b

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.85
GLY	H	1	8.12
GLY	HA1	1	3.77
GLY	HA2	1	3.63
TRP	H	2	8.10
TRP	HA	2	4.59
TRP	HB1	2	3.17
TRP	HB2	2	2.97
TRP	HD1	2	7.18
TRP	HE1	2	10.85
TRP	HZ2	2	7.34
TRP	HH2	2	7.07
TRP	HZ3	2	6.99
TRP	HE3	2	7.60
CYS	H	3	8.53
CYS	HA	3	4.54
CYS	HB1	3	3.59
CYS	HB2	3	3.41
FUR	H5	7	7.43
FUR	H6	7	7.70
HIS	H	4	8.48
HIS	HA	4	4.69
HIS	HB1	4	3.12
HIS	HB2	4	3.02
HIS	HD2	4	7.40
HIS	HE1	4	8.98
VAL	H	5	7.83
VAL	HA	5	4.19
VAL	HB	5	2.04
VAL	HG1	5	0.89
VAL	HG2	5	0.86
ALA	H	6	8.15
ALA	HA	6	4.23
ALA	HB	6	1.24
CO-NH2	NH1	6	7.27
CO-NH2	NH2	6	7.00

Compound 2c (X: F) strategy A

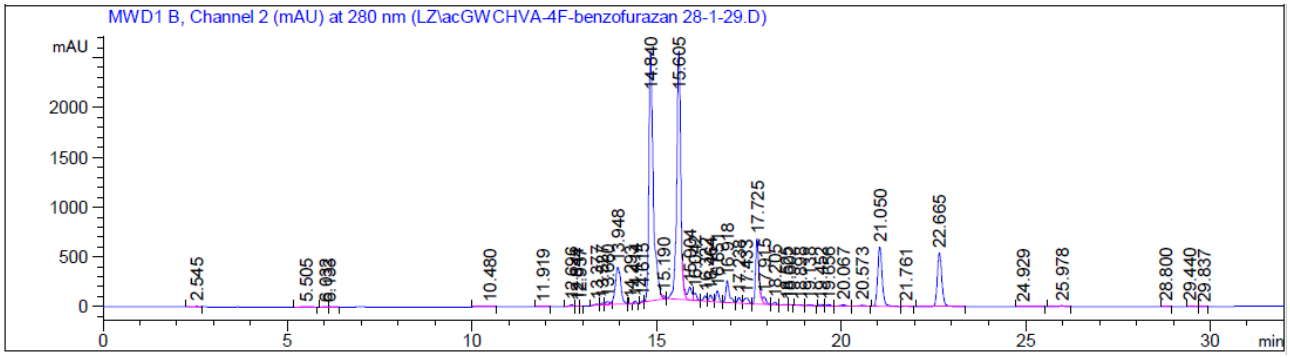
AcGly(c)TrpCysHisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

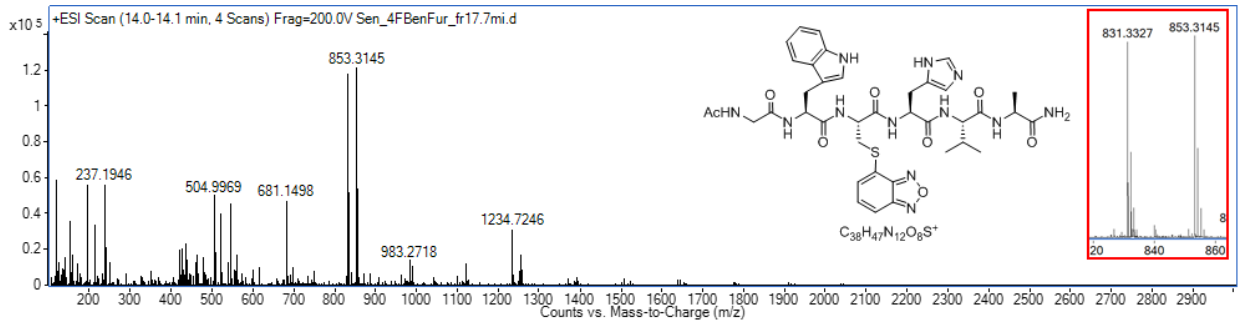


B) Preparative HPLC *tR*= 17.725 min; ES-MS: calculated $[M + H]^+$, 831.3355, found m/z 831.3327 ($[M+H]^+$). White solid

HPLC profile of the reaction crude product:



MS spectrum of **2c** after purification:

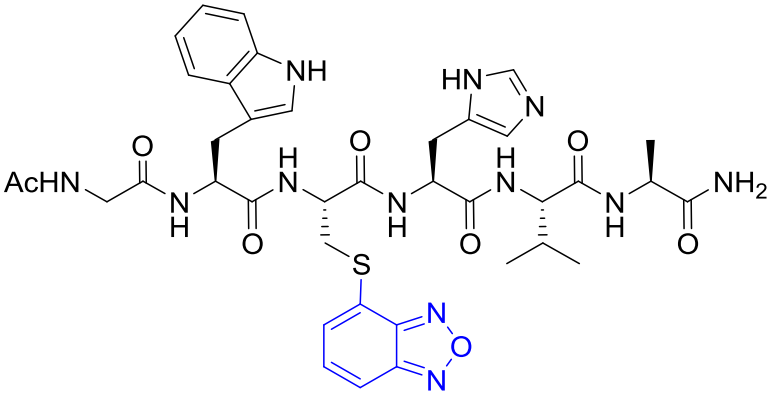


C) 1H-NMR (600 MHz, dmso-d6, 298 K): the collected amount of the final product resulted insufficient for the NMR analysis

Compound 2c (X: F) strategy B

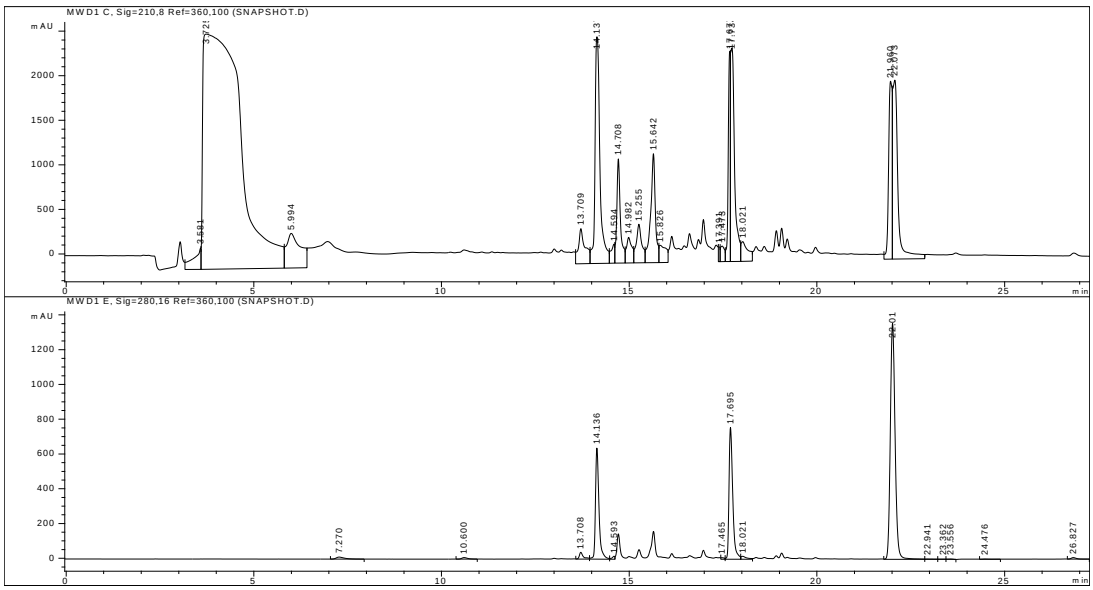
AcGly(c)TrpCysHisValAlaNH_2

A) Structure of benzofurazan/peptide conjugates

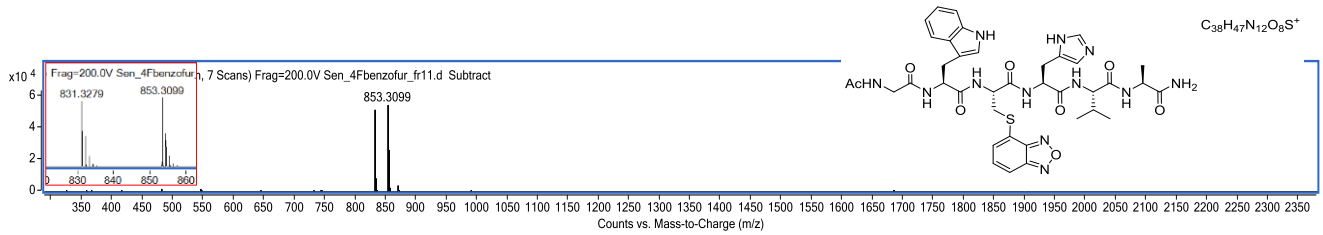


B) preparative HPLC *tR*= 17.670 min; ES-MS: calculated $[M + H]^+$, 831.3355, found m/z 831.3279 ($[M+H]^+$). White solid

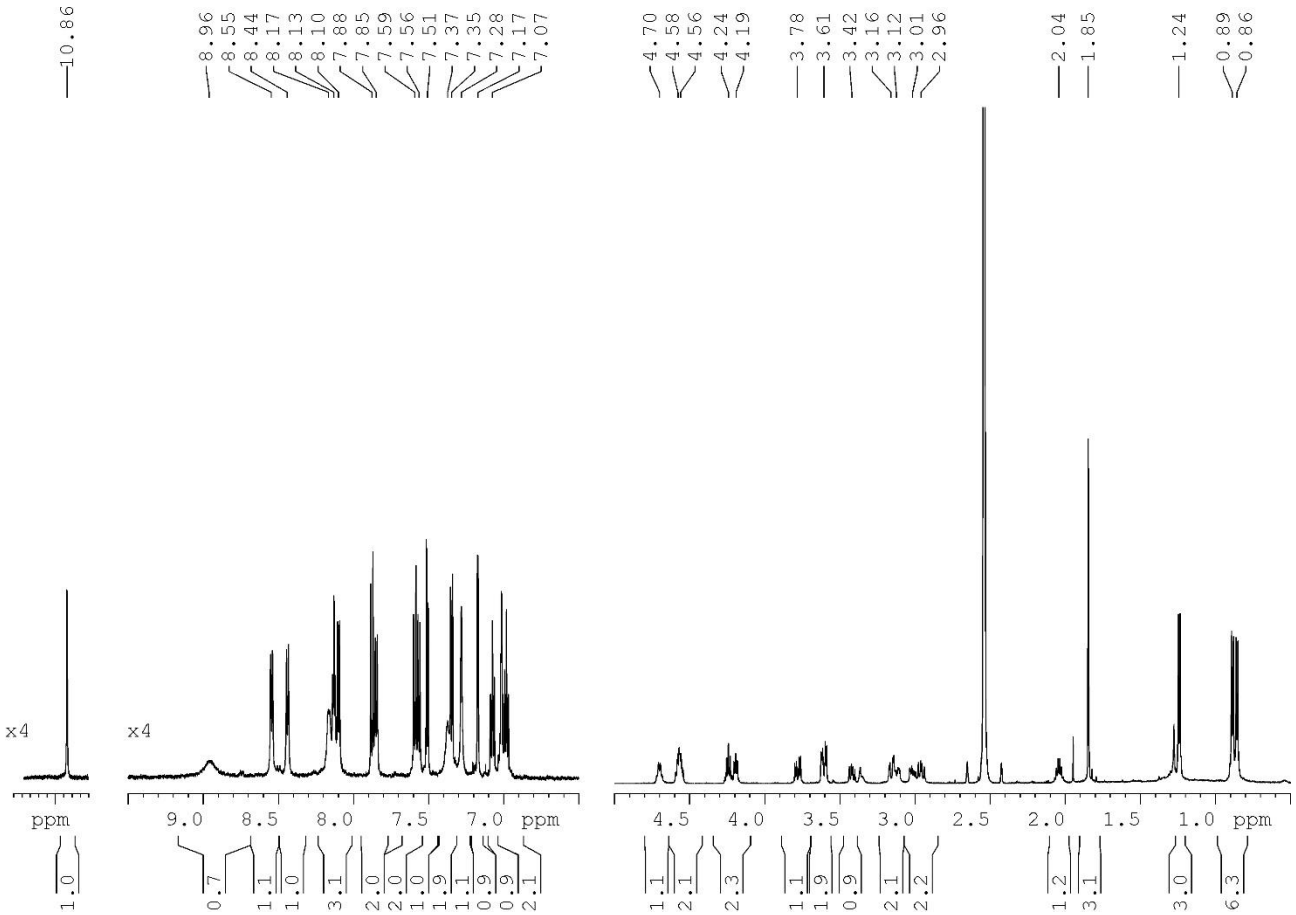
HPLC profile of the reaction crude product:



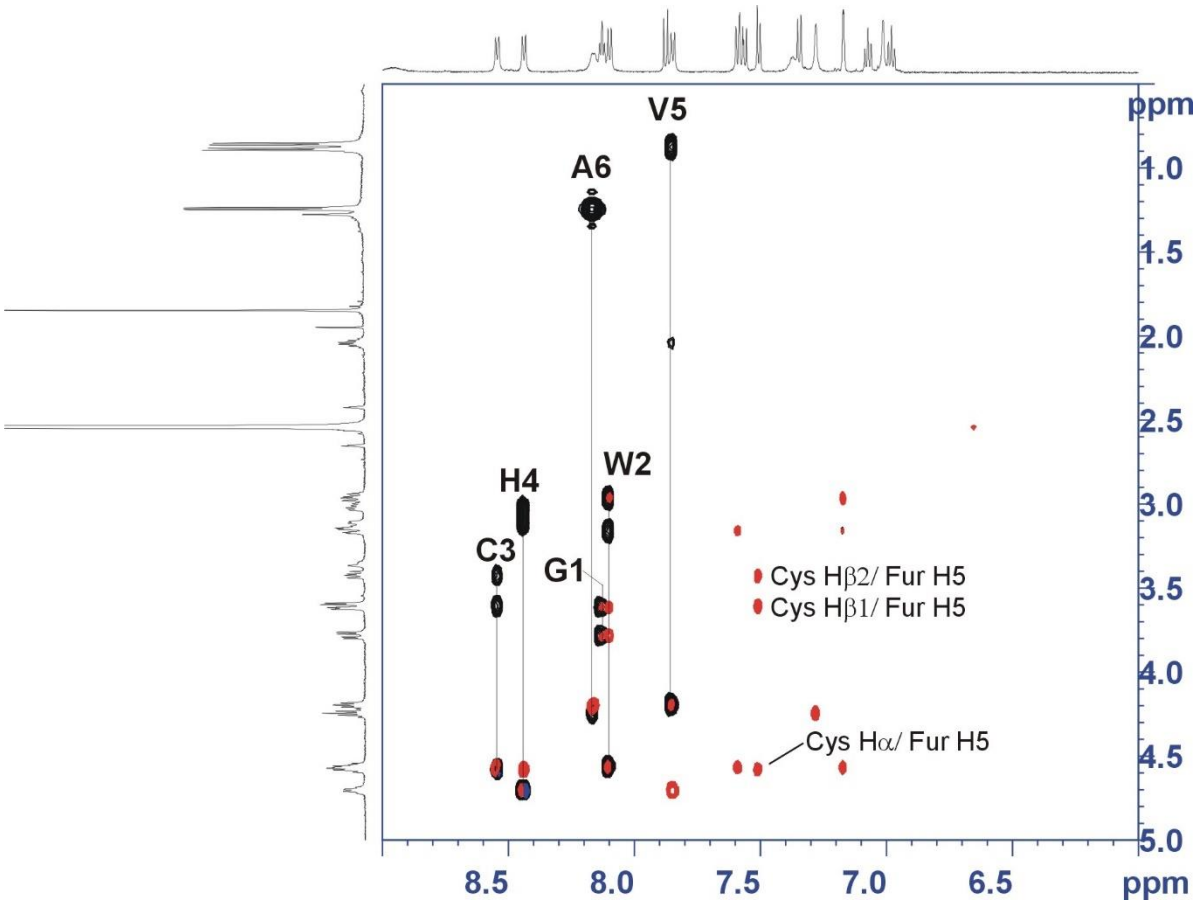
MS spectrum of **2c** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

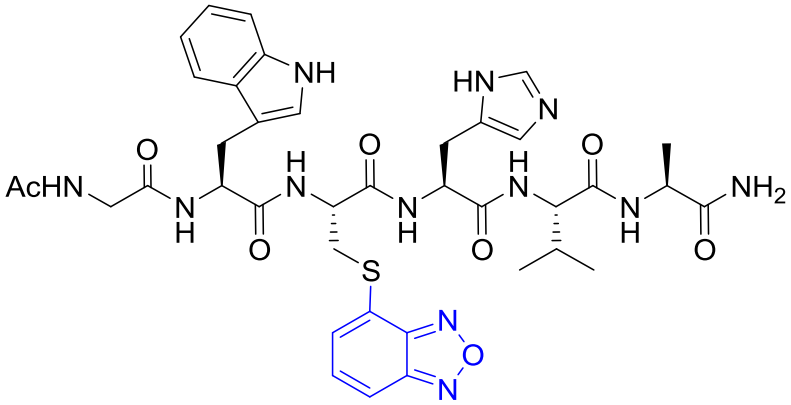
Compound 2c

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.85
GLY	H	1	8.13
GLY	HA1	1	3.78
GLY	HA2	1	3.61
TRP	H	2	8.10
TRP	HA	2	4.56
TRP	HB1	2	3.16
TRP	HB2	2	2.96
TRP	HD1	2	7.17
TRP	HE1	2	10.87
TRP	HZ2	2	7.35
TRP	HH2	2	7.07
TRP	HZ3	2	6.98
TRP	HE3	2	7.59
CYS	H	3	8.55
CYS	HA	3	4.58
CYS	HB1	3	3.61
CYS	HB2	3	3.42
FUR	H5	7	7.51
FUR	H6	7	7.56
FUR	H7	7	7.88
HIS	H	4	8.44
HIS	HA	4	4.70
HIS	HB1	4	3.12
HIS	HB2	4	3.01
HIS	HD2	4	7.37
HIS	HE1	4	8.96
VAL	H	5	7.85
VAL	HA	5	4.19
VAL	HB	5	2.04
VAL	HG1	5	0.89
VAL	HG2	5	0.86
ALA	H	6	8.17
ALA	HA	6	4.24
ALA	HB	6	1.24
CO-NH2	NH1	6	7.28
CO-NH2	NH2	6	7.01

Compound 2c (X: Cl) strategy A

AcGly(c)TrpCysHisValAlaNH_2

A) Structure of benzofurazan/peptide conjugates

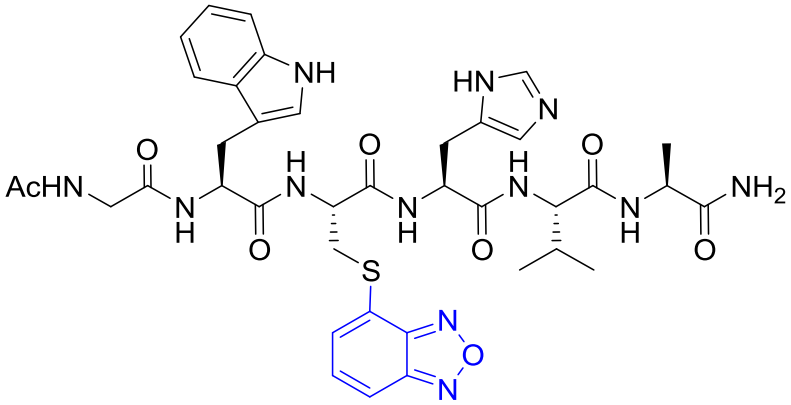


B) Yield (2**c**) after RP-HPLC purification: 0% .

Compound 2c (X: Cl) strategy B

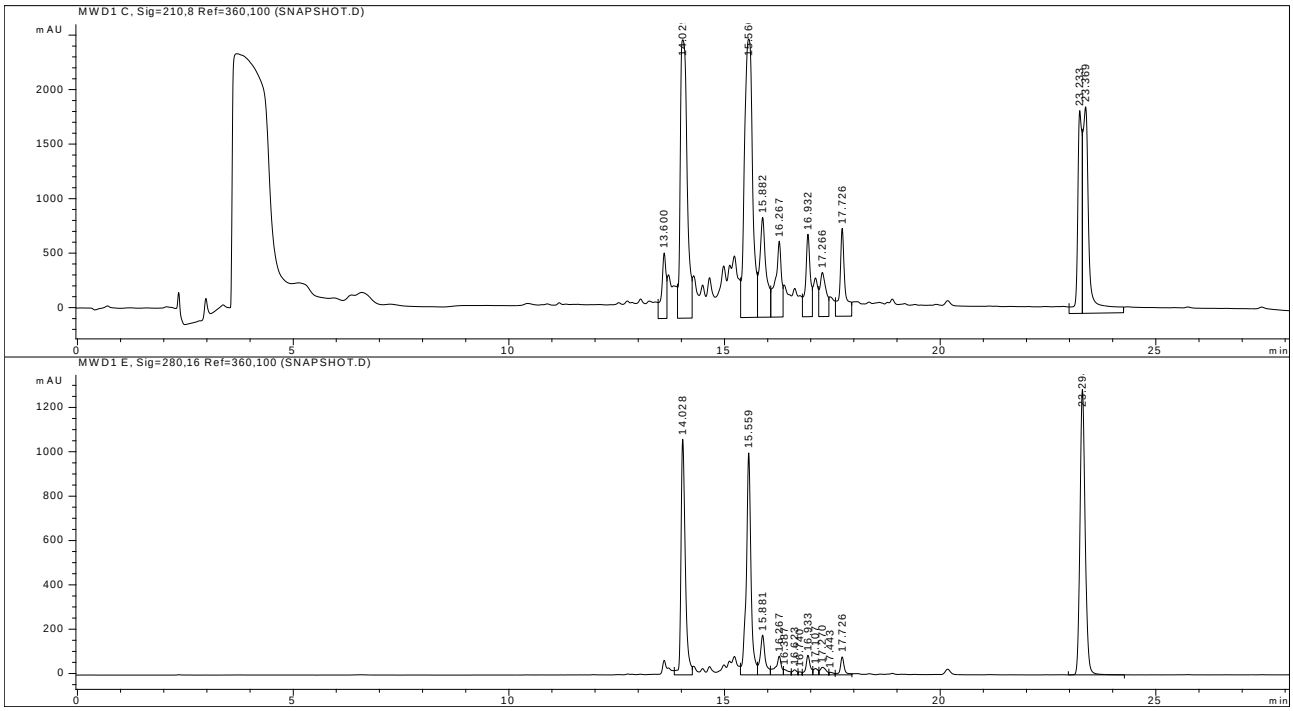
AcGly(c)TrpCysHisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

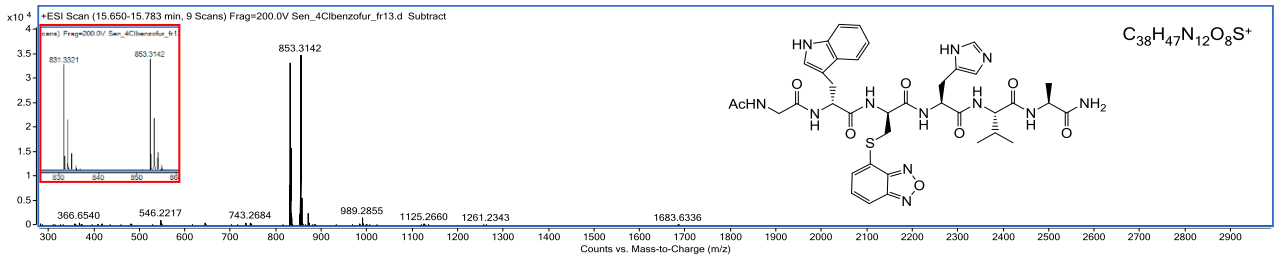


B) preparative HPLC *tR*= 17.726 min; ES-MS: calculated $[M + H]^+$, 831.3355, found m/z 831.3321 ($[M+H]^+$). White solid

HPLC profile of the reaction crude product:



MS spectrum of 2**c** after purification:

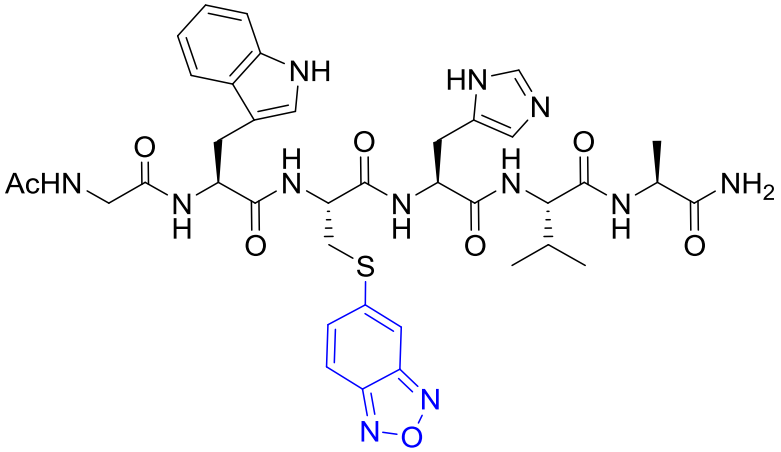


C) 1H-NMR (600 MHz, dmso-d6, 298 K): the collected amount of the final product resulted insufficient for the NMR analysis

Compound 2d (X: Br) strategy A

AcGly(d)TrpCysHisValAlaNH_2

A) Structure of benzofurazan/peptide conjugates

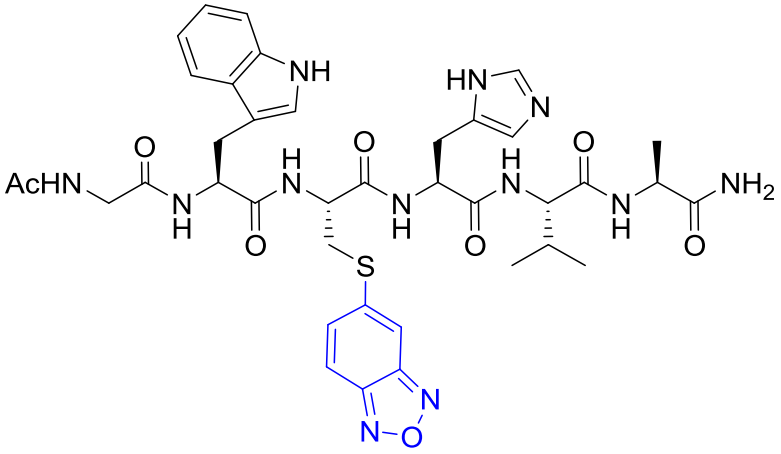


B) Yield (**2d-Br**) after RP-HPLC purification: 0%.

Compound 2d (X: Br) strategy B

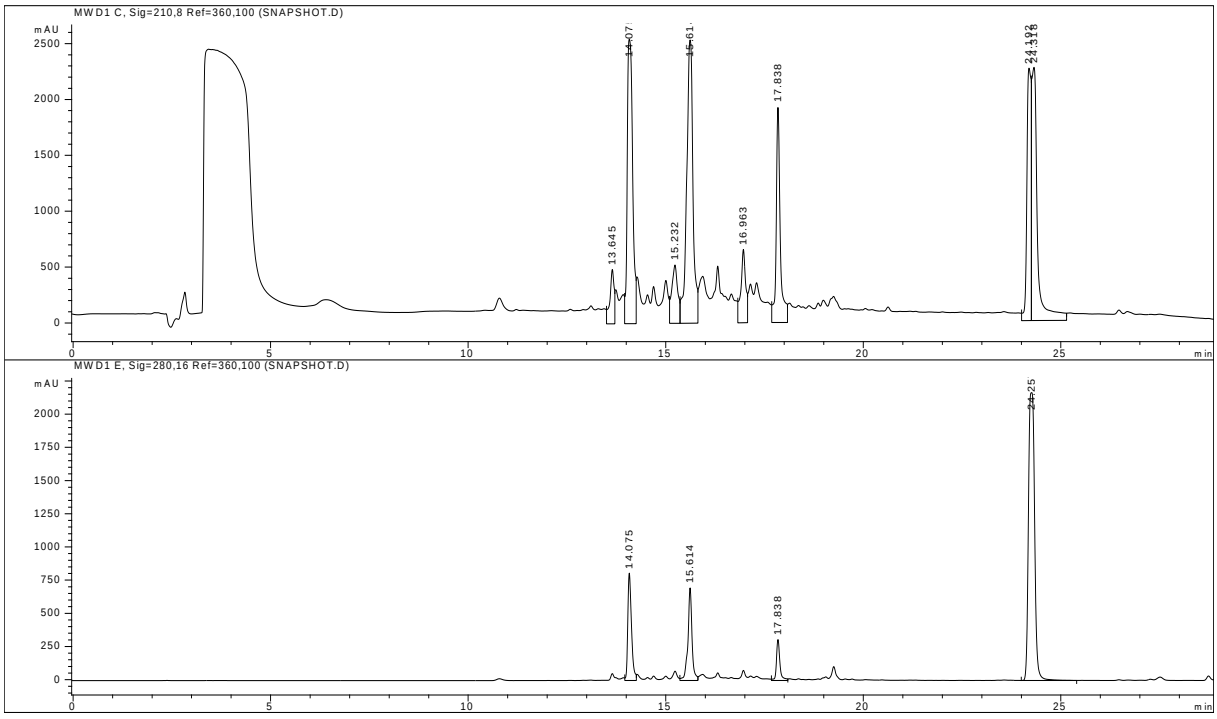
AcGly(d)TrpCysHisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

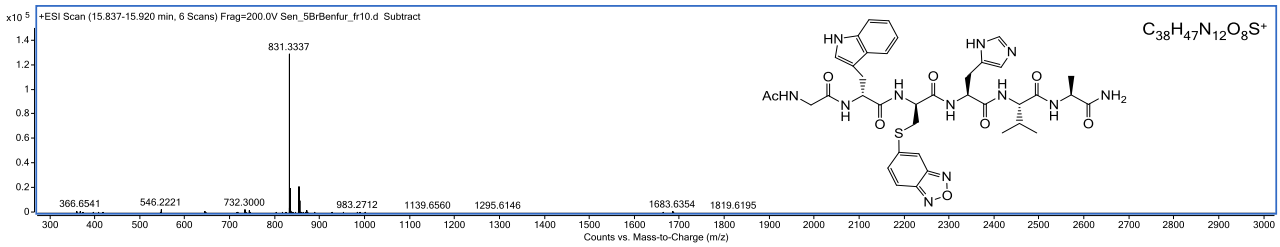


B) preparative HPLC *tR*= 17.838 min; ES-MS: calculated $[M + H]^+$, 831.3355, found m/z 831.3337 ($[M+H]^+$). White solid

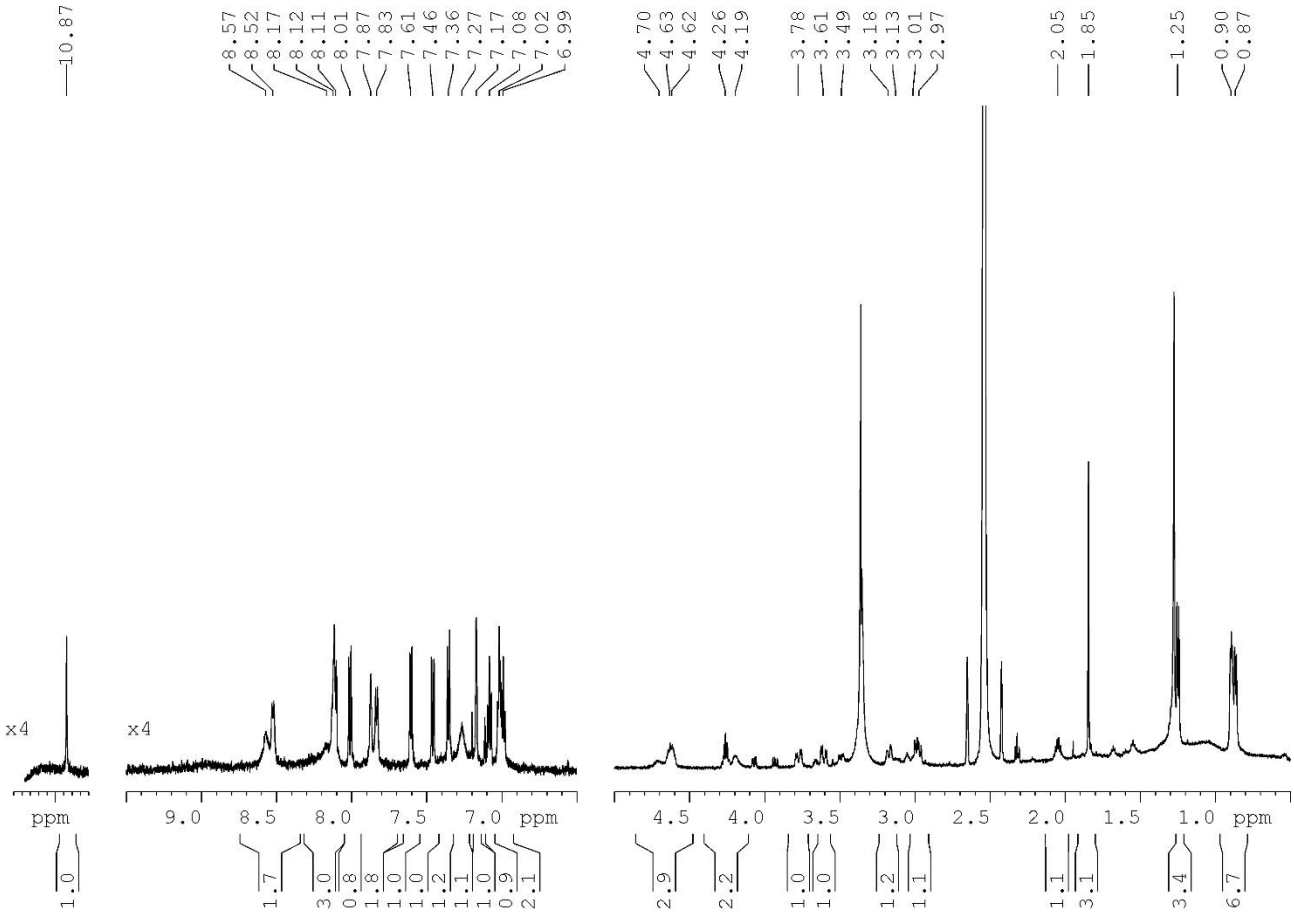
HPLC profile of the reaction crude product:



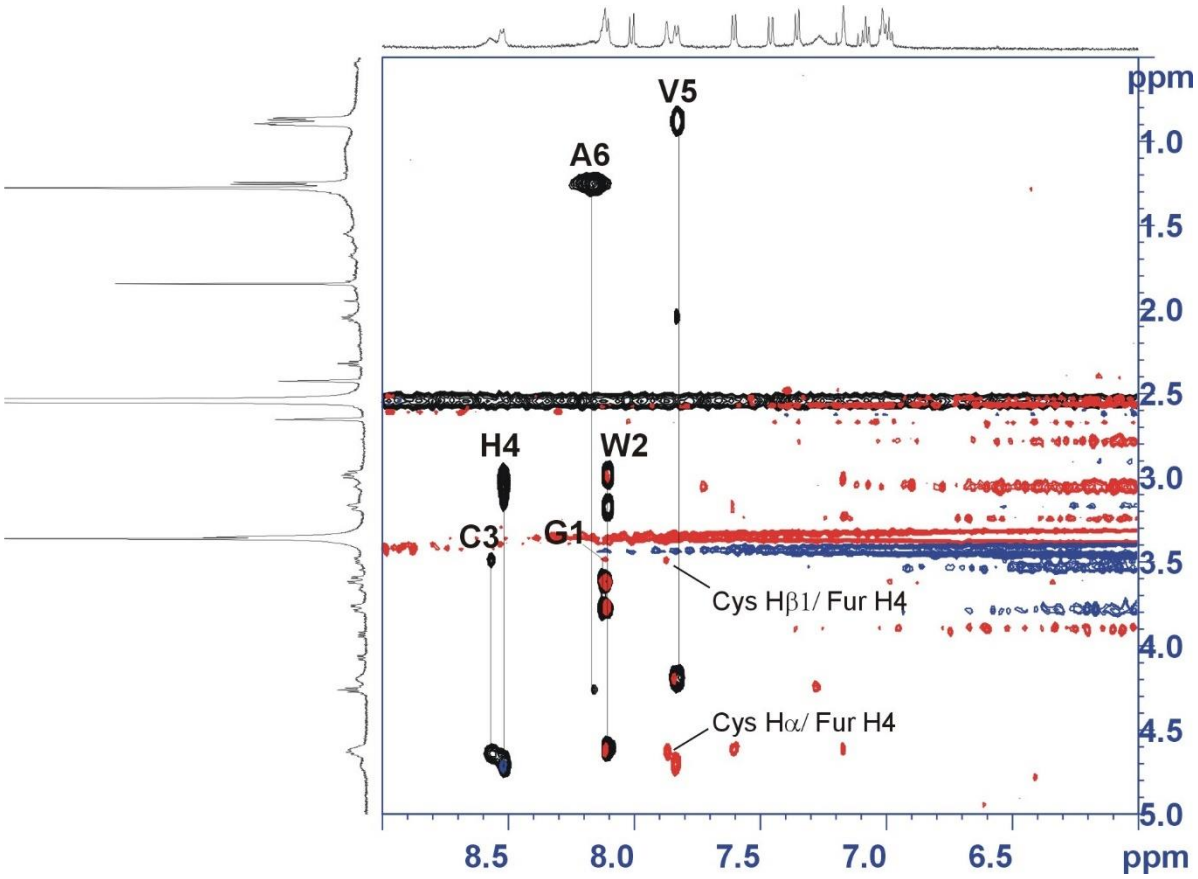
MS spectrum of **2d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

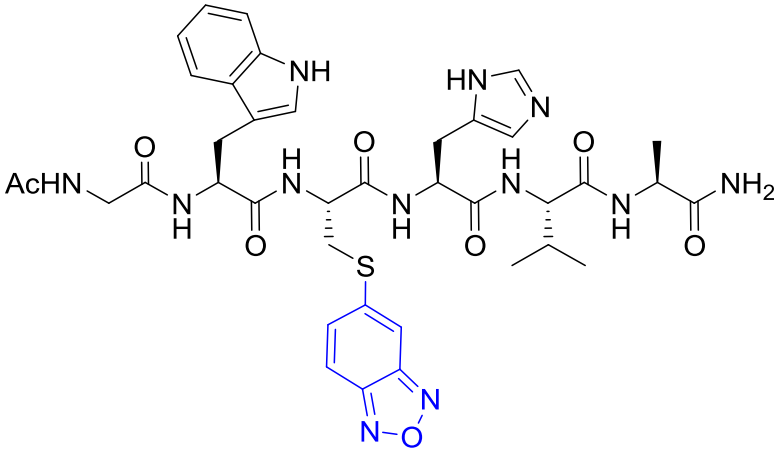
Compound 2d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.85
GLY	H	1	8.12
GLY	HA1	1	3.78
GLY	HA2	1	3.61
TRP	H	2	8.11
TRP	HA	2	4.62
TRP	HB1	2	3.18
TRP	HB2	2	2.97
TRP	HD1	2	7.17
TRP	HE1	2	10.87
TRP	HZ2	2	7.36
TRP	HH2	2	7.08
TRP	HZ3	2	6.99
TRP	HE3	2	7.61
CYS	H	3	8.57
CYS	HA	3	4.63
CYS	HB1	3	3.49
CYS	HB2	3	nd
FUR	H6	7	7.46
FUR	H7	7	8.01
FUR	H4	7	7.87
HIS	H	4	8.52
HIS	HA	4	4.70
HIS	HB1	4	3.13
HIS	HB2	4	3.01
HIS	HD2	4	nd
HIS	HE1	4	8.97
VAL	H	5	7.83
VAL	HA	5	4.19
VAL	HB	5	2.05
VAL	HG1	5	0.90
VAL	HG2	5	0.87
ALA	H	6	8.17
ALA	HA	6	4.26
ALA	HB	6	1.25
CO-NH2	NH1	6	7.27
CO-NH2	NH2	6	7.02

Compound 2d (X: Cl) strategy A

AcGly(d)TrpCysHisValAlaNH_2

A) Structure of benzofurazan/peptide conjugates

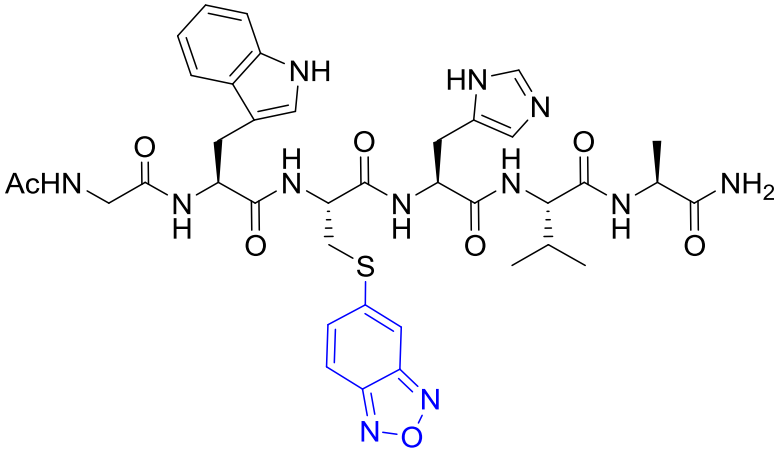


B) Yield (**2d-Cl**) after RP-HPLC purification: 0%.

Compound 2d (X: Cl) strategy B

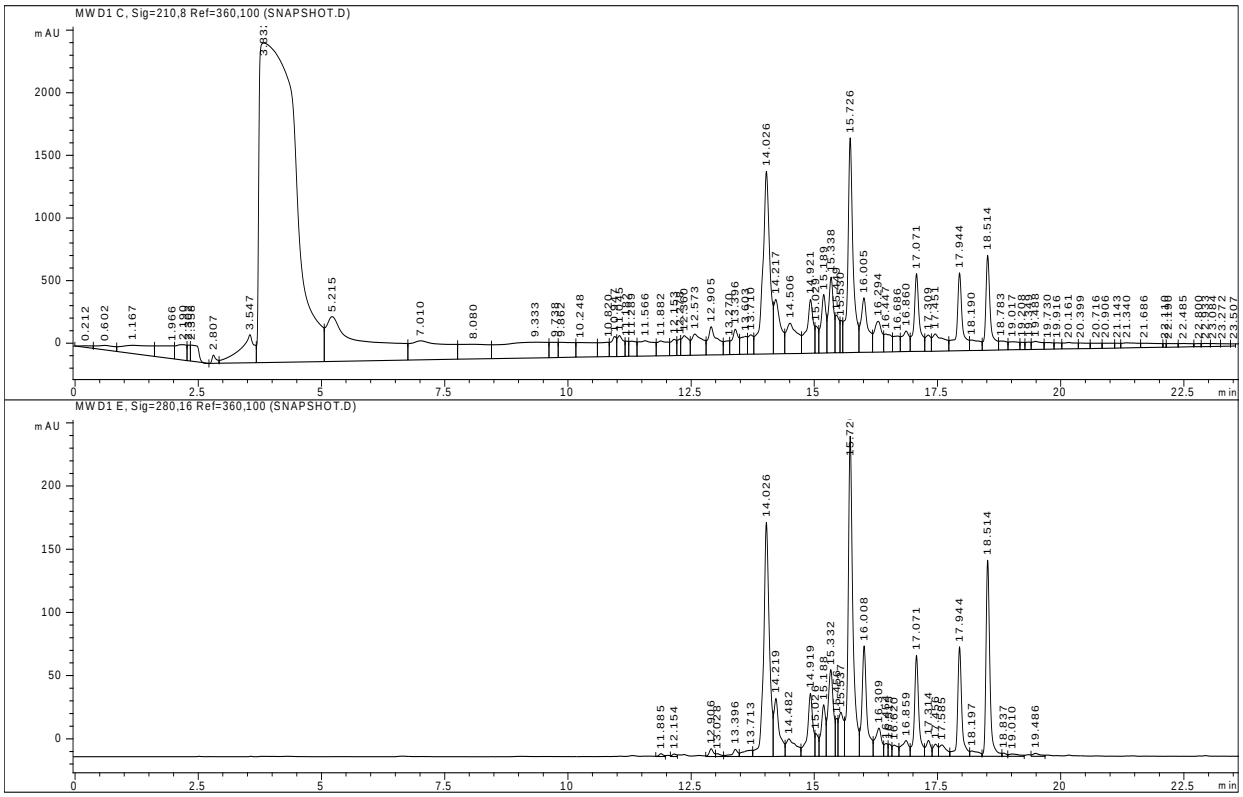
AcGly(d)TrpCysHisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

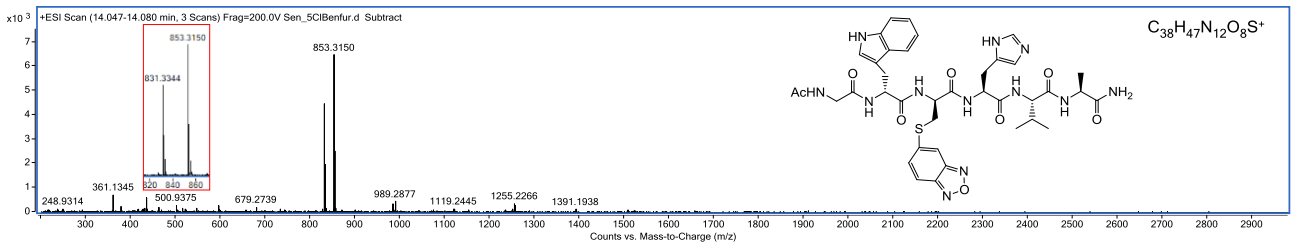


B) preparative HPLC *tR*= 17.944 min; ES-MS: calculated $[M + H]^+$, 831.3355, found m/z 831.3344 ($[M+H]^+$). White solid

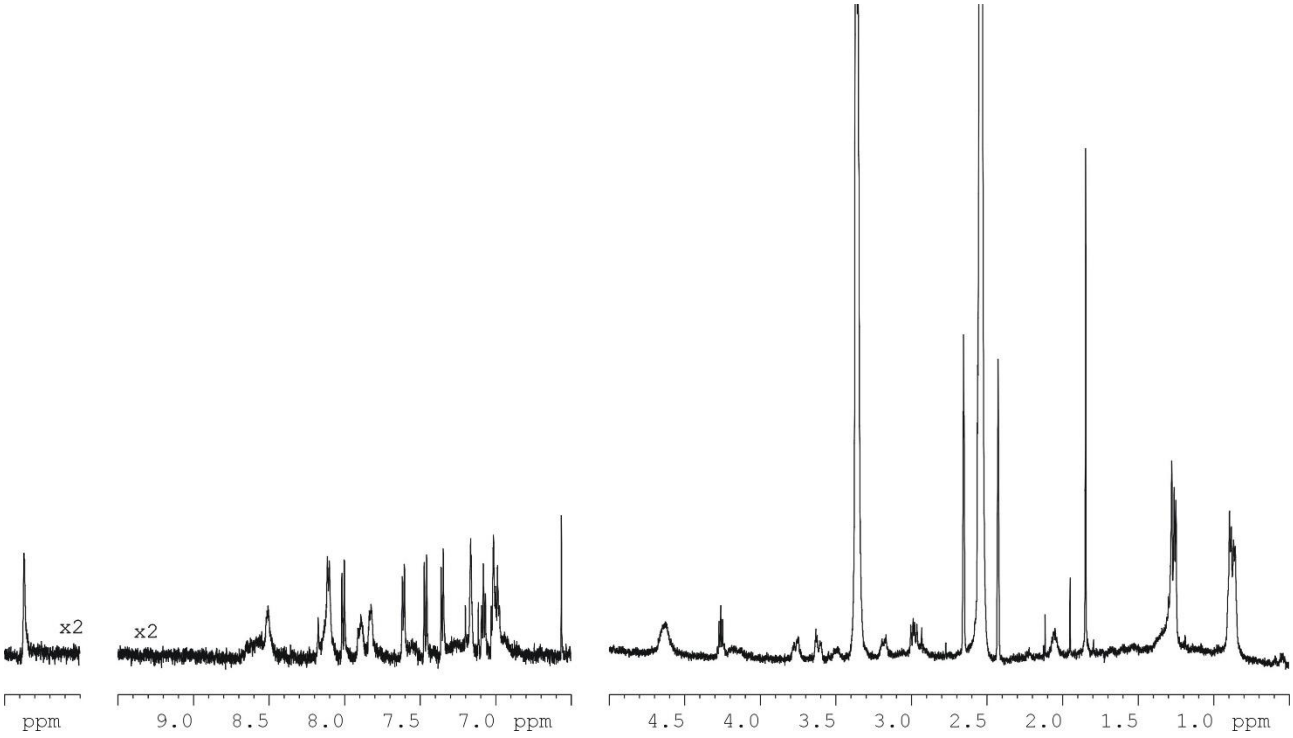
HPLC profile of the reaction crude product:



MS spectrum of **2d** after purification:



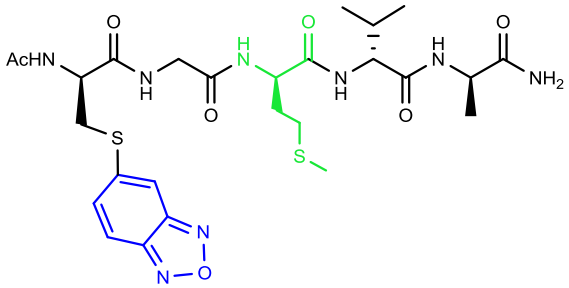
C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K): the collected amount of the final product resulted not sufficient for for a good quality ^{1}HNMR spectrum



Compound 3d (X: Br) strategy B

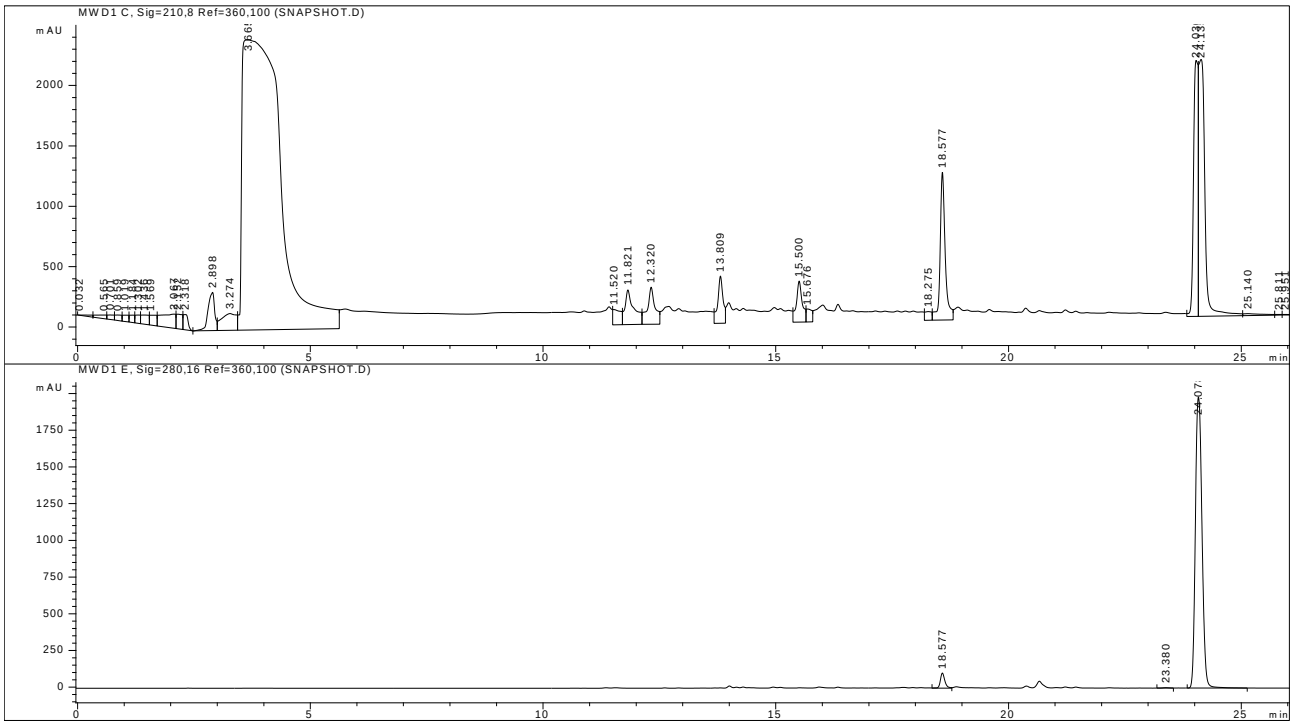
AcCys(d)GlyMetValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

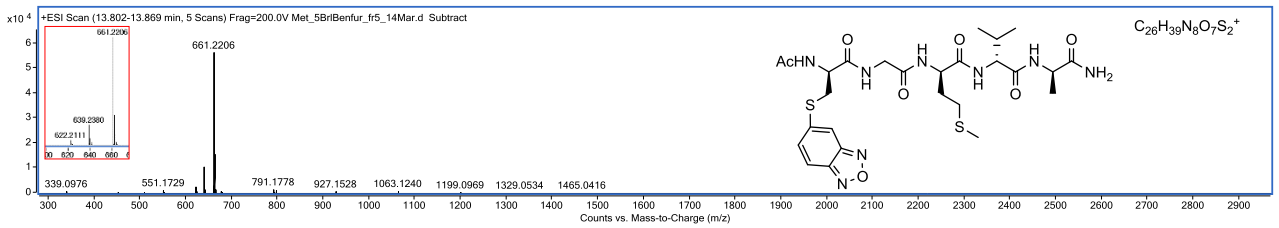


B) preparative HPLC *tR*= 18.577 min; ES-MS: calculated $[M + H]^+$, 639.2378, found m/z 639.2380 ($[M+H]^+$). White solid

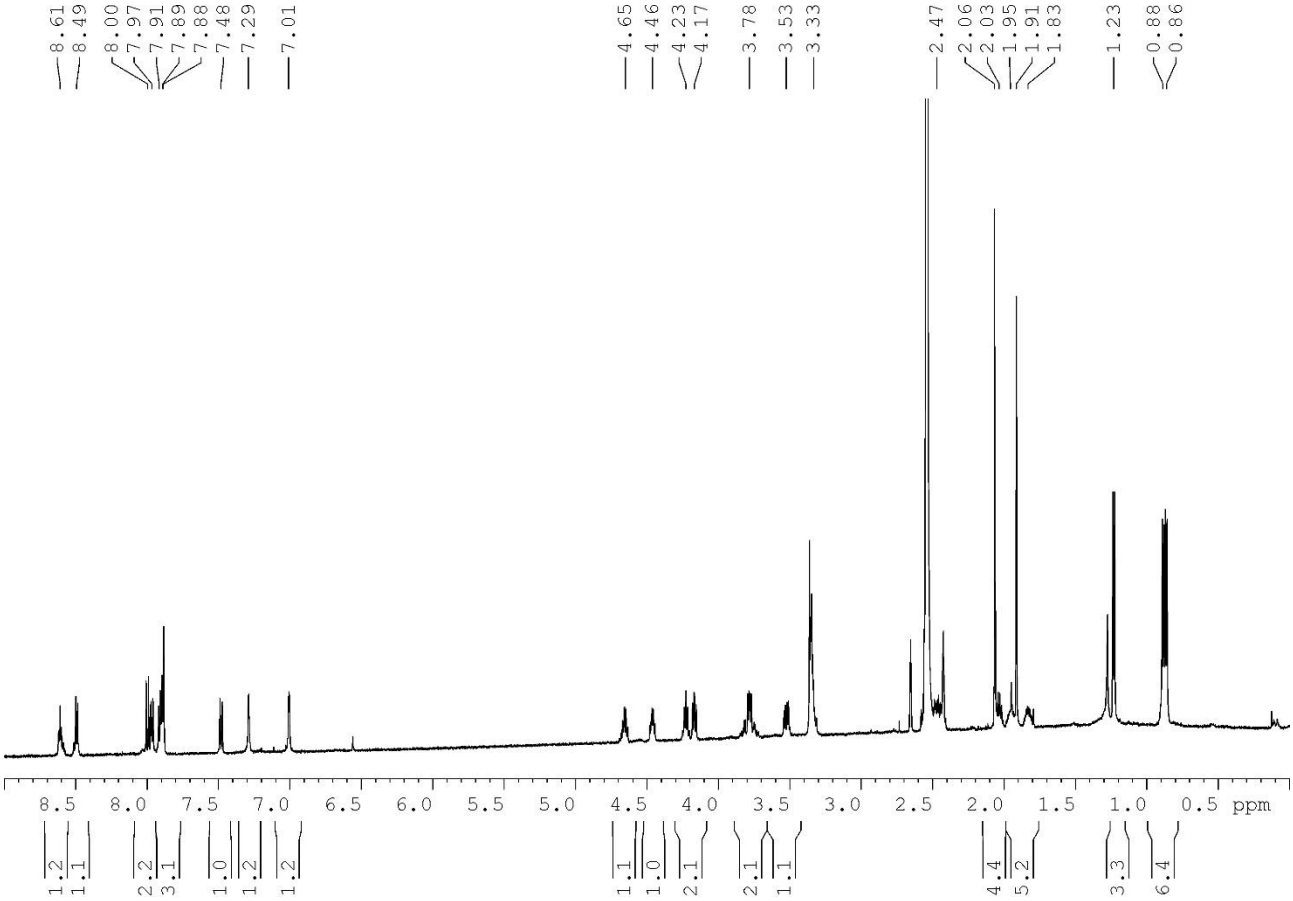
HPLC profile of the reaction crude product:



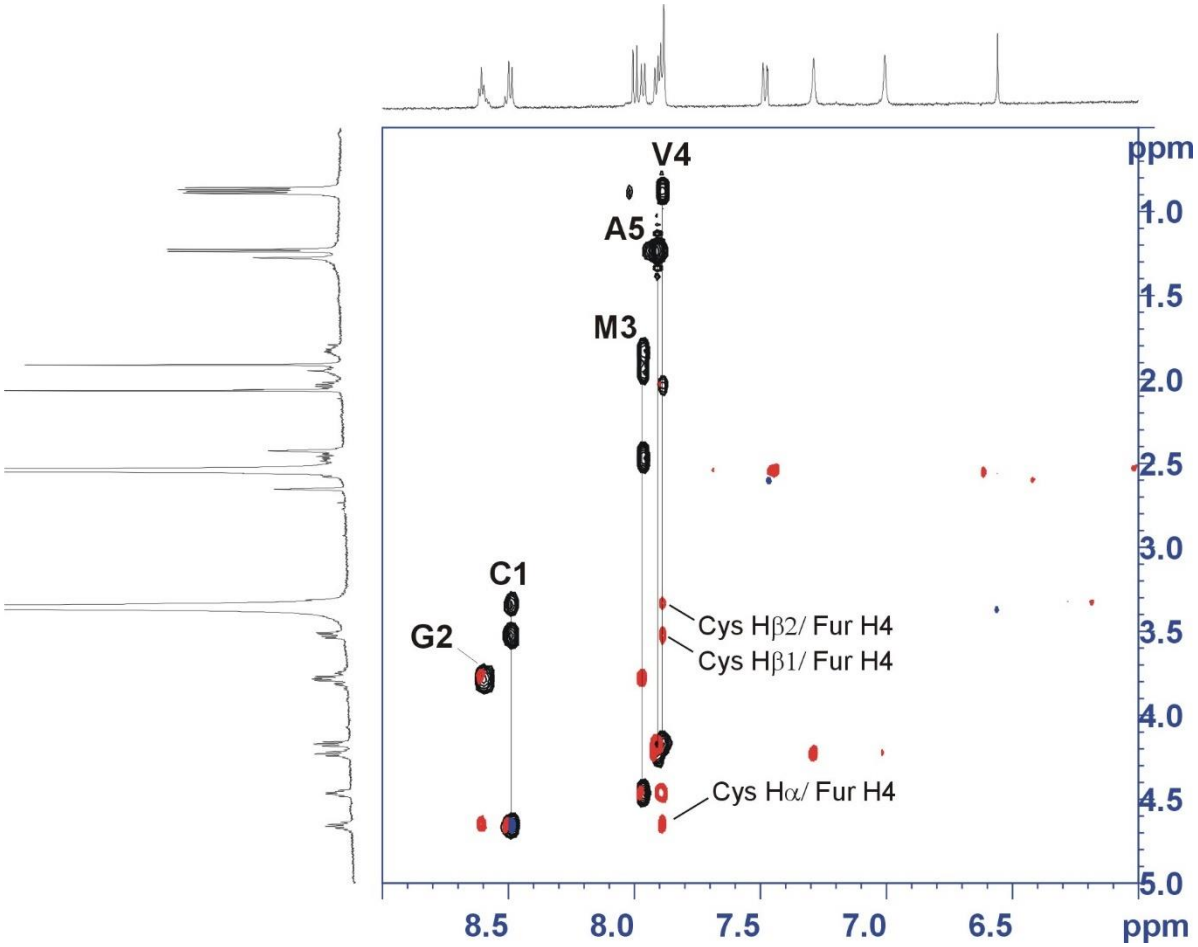
MS spectrum of **3d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

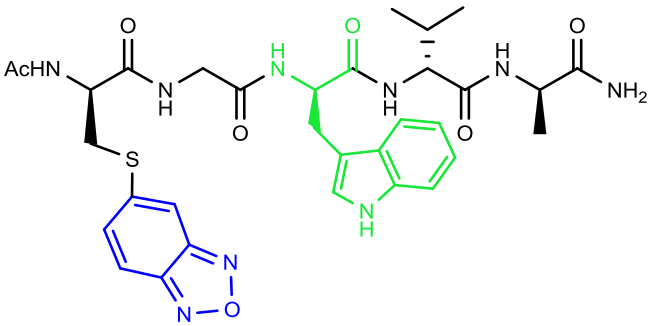
Compound 3d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.91
CYS	H	1	8.49
CYS	HA	1	4.65
CYS	HB1	1	3.53
CYS	HB2	1	3.33
FUR	H6	6	7.48
FUR	H7	6	8.00
FUR	H4	6	7.88
GLY	H	2	8.61
GLY	HA2	2	3.78
GLY	HA3	2	3.78
MET	H	3	7.97
MET	HA	3	4.46
MET	HB2	3	1.95
MET	HB3	3	1.83
MET	HG1	3	2.47
MET	HG2	3	2.47
MET	HD	3	2.07
VAL	H	4	7.89
VAL	HA	4	4.17
VAL	HB	4	2.03
VAL	HG1	4	0.88
VAL	HG2	4	0.86
ALA	H	5	7.91
ALA	HA	5	4.23
ALA	HB	5	1.23
CO-NH2	NH1	5	7.01
CO-NH2	NH2	5	7.29

Compound 4d (X: Br) strategy B

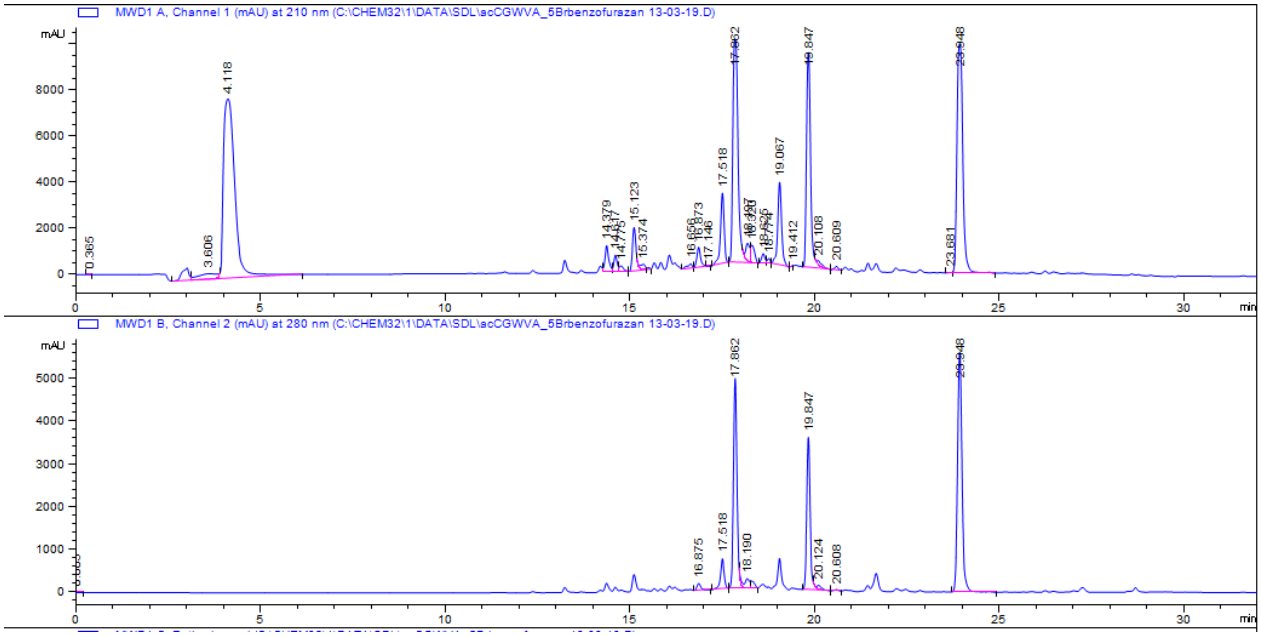
AcCys(d)GlyTrpValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

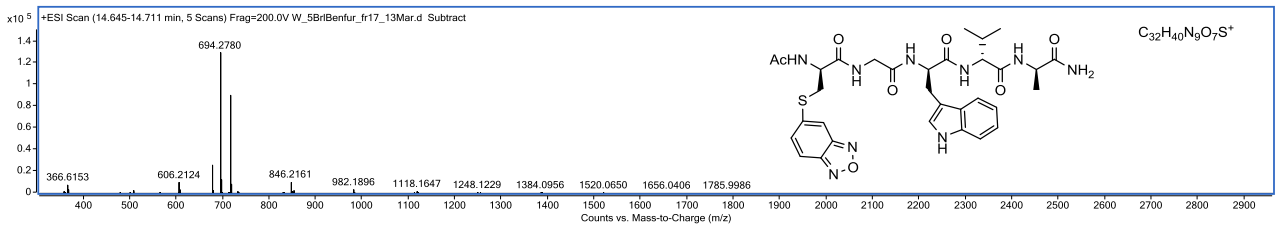


B) preparative HPLC *tR*= 19.847 min; ES-MS: calculated $[M + H]^+$, 694.2766, found m/z 694.2780 ($[M+H]^+$). White solid

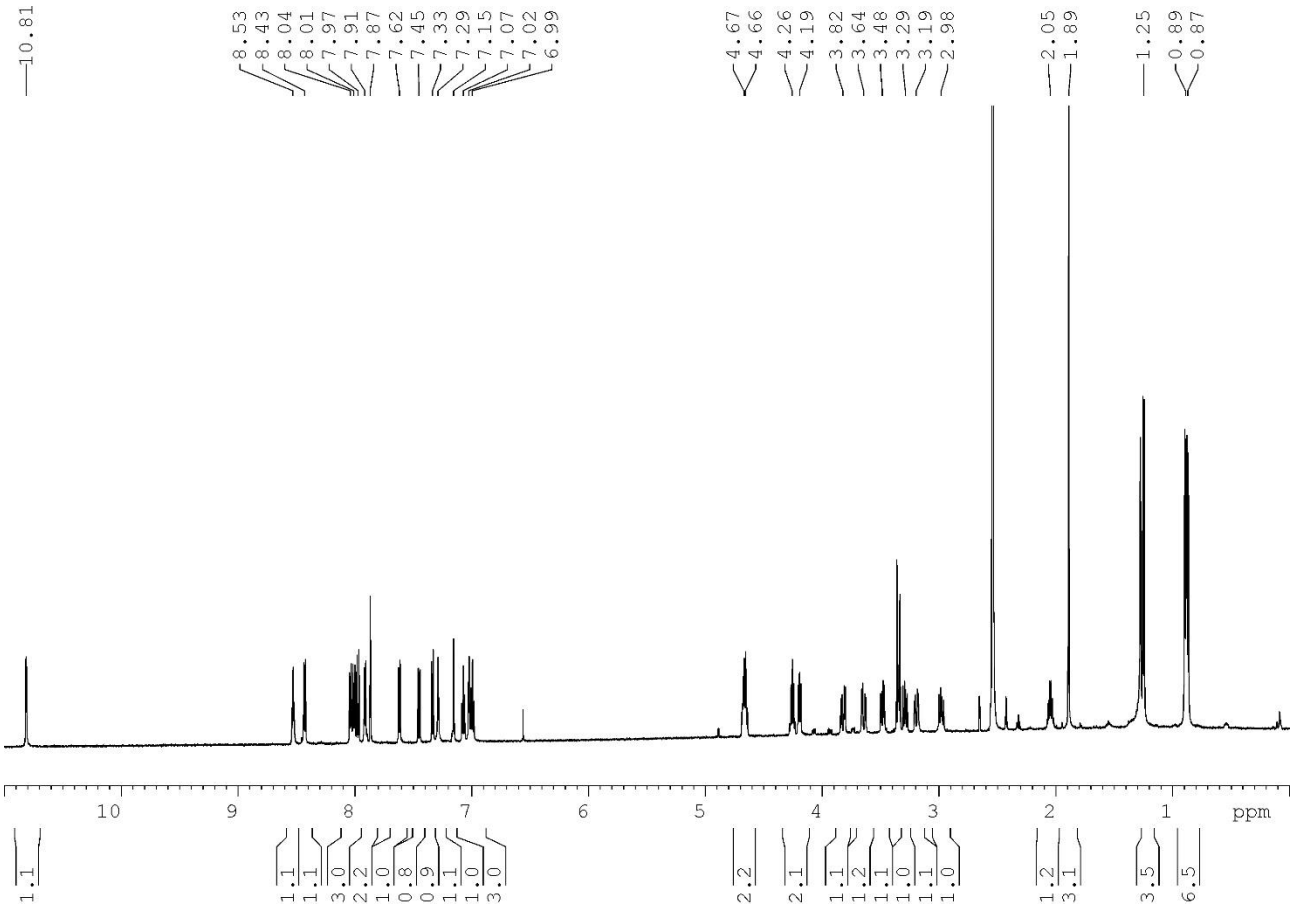
HPLC profile of the reaction crude product:



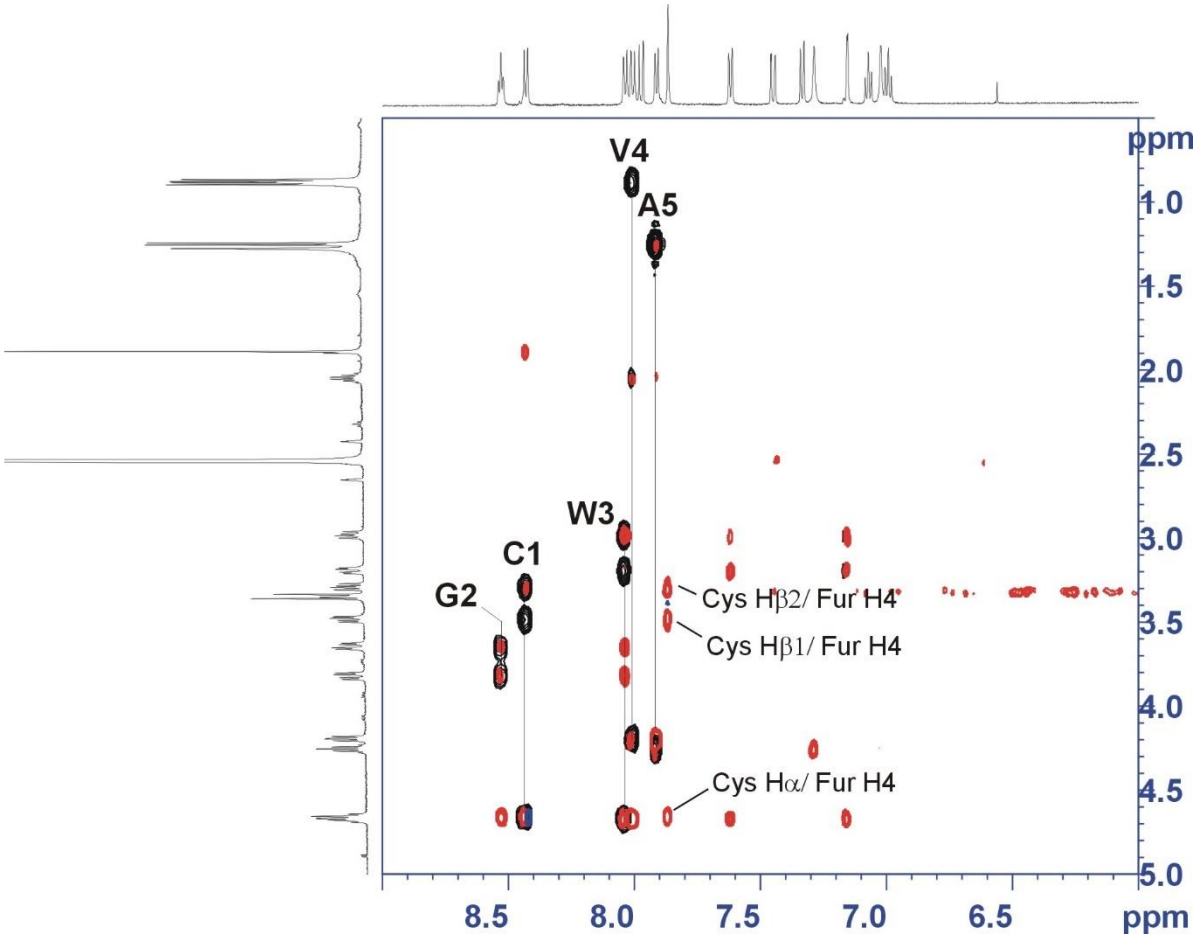
MS spectrum of **4d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)

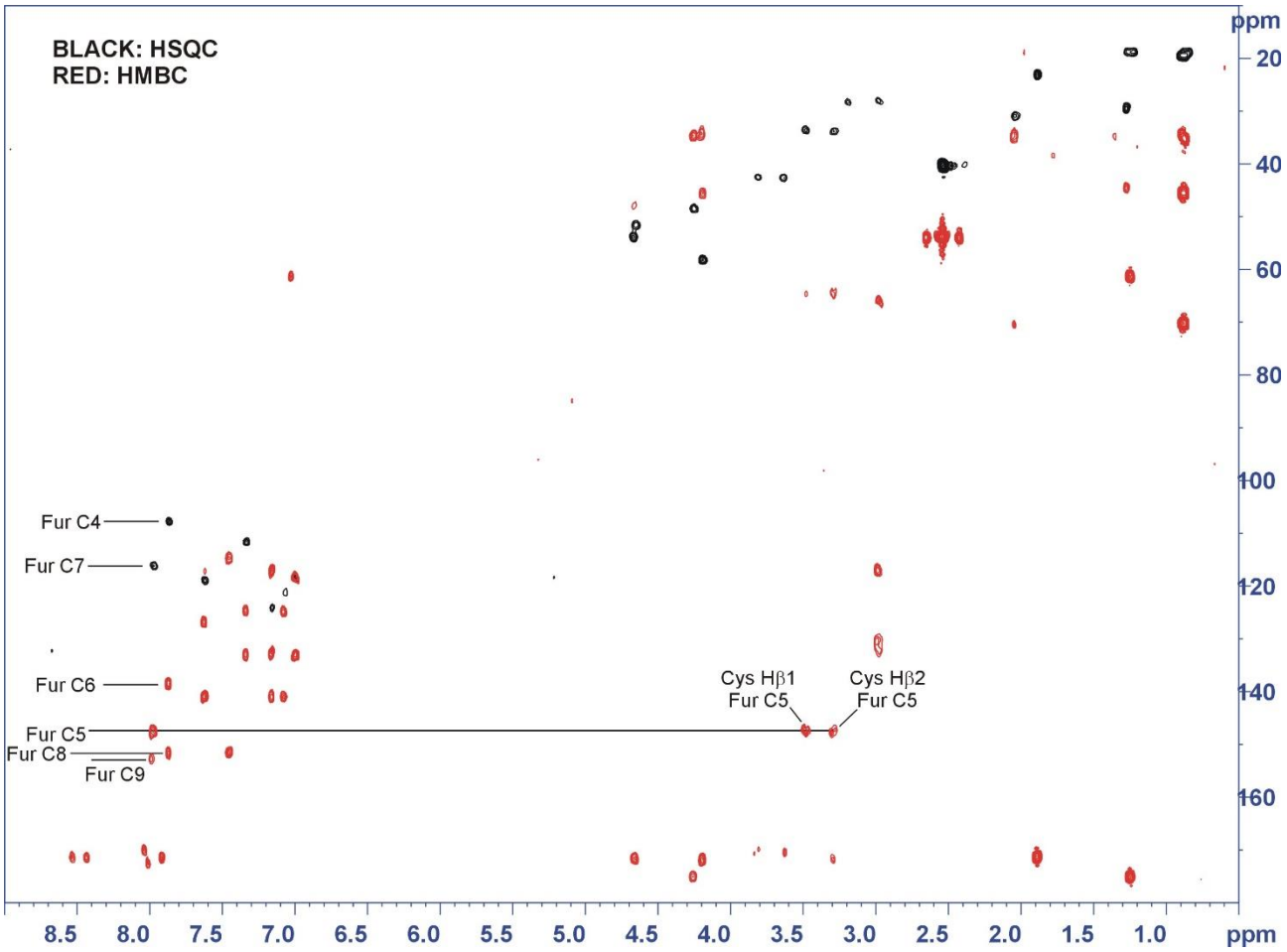


E) Assignment table

Compound 4d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.89
CYS	H	1	8.43
CYS	HA	1	4.66
CYS	HB1	1	3.48
CYS	HB2	1	3.29
FUR	H6	6	7.45
FUR	H7	6	7.97
FUR	H4	6	7.87
GLY	H	2	8.53
GLY	HA2	2	3.82
GLY	HA3	2	3.64
TRP	H	3	8.04
TRP	HA	3	4.67
TRP	HB1	3	3.19
TRP	HB2	3	2.98
TRP	HD1	3	7.15
TRP	HE1	3	10.81
TRP	HZ2	3	7.33
TRP	HH2	3	7.07
TRP	HZ3	3	6.99
TRP	HE3	3	7.62
VAL	H	4	8.01
VAL	HA	4	4.19
VAL	HB	4	2.05
VAL	HG1	4	0.89
VAL	HG2	4	0.87
ALA	H	5	7.91
ALA	HA	5	4.26
ALA	HB	5	1.25
CO-NH2	NH1	5	7.02
CO-NH2	NH2	5	7.29

G) Overlay of the $^{1}H,^{13}C$ HSQC (black) and $^{1}H,^{13}C$ HMBC (red) NMR spectra (600 MHz, DMSO-d_6, 298 K)



H) ^{13}C NMR assignment table (from ^{1}H,^{13}C HSQC and ^{1}H,^{13}C HMBC)

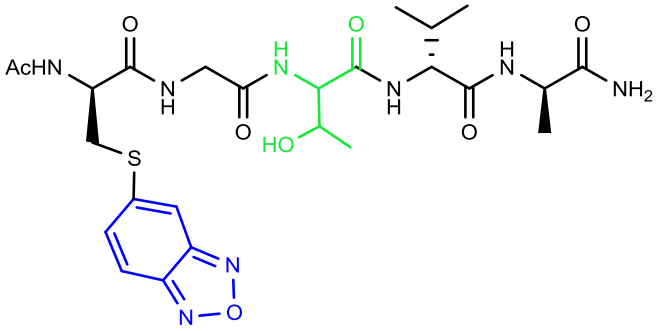
Compound 4d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	23.2
N-term Ac	C=O	1	170.5
CYS	CA	1	51.8
CYS	CB	1	33.7
CYS	C	1	171.0
FUR	C5q	6	144.1
FUR	C6	6	134.2
FUR	C7	6	116.3
FUR	C8q	6	148.6
FUR	C9q	6	149.9
FUR	C4	6	107.9
GLY	CA	2	42.7
GLY	C	2	169.6
TRP	CA	3	54.0
TRP	CB	3	28.3
TRP	CG quat	3	110.3
TRP	CD1	3	124.3
TRP	CD2 quat	3	127.7
TRP	CE2 quat	3	136.9
TRP	CZ2	3	111.8
TRP	CH2	3	121.4
TRP	CZ3	3	118.7
TRP	CE3	3	119.1
TRP	C	3	170.8
VAL	CA	4	58.4
VAL	CB	4	31.1
VAL	CG1	4	19.6
VAL	CG2	4	18.8
VAL	C	4	171.1
ALA	CA	5	48.7
ALA	CB	5	18.9
ALA	CO-NH2	5	174.8

Compound 5d (X: Br) strategy B

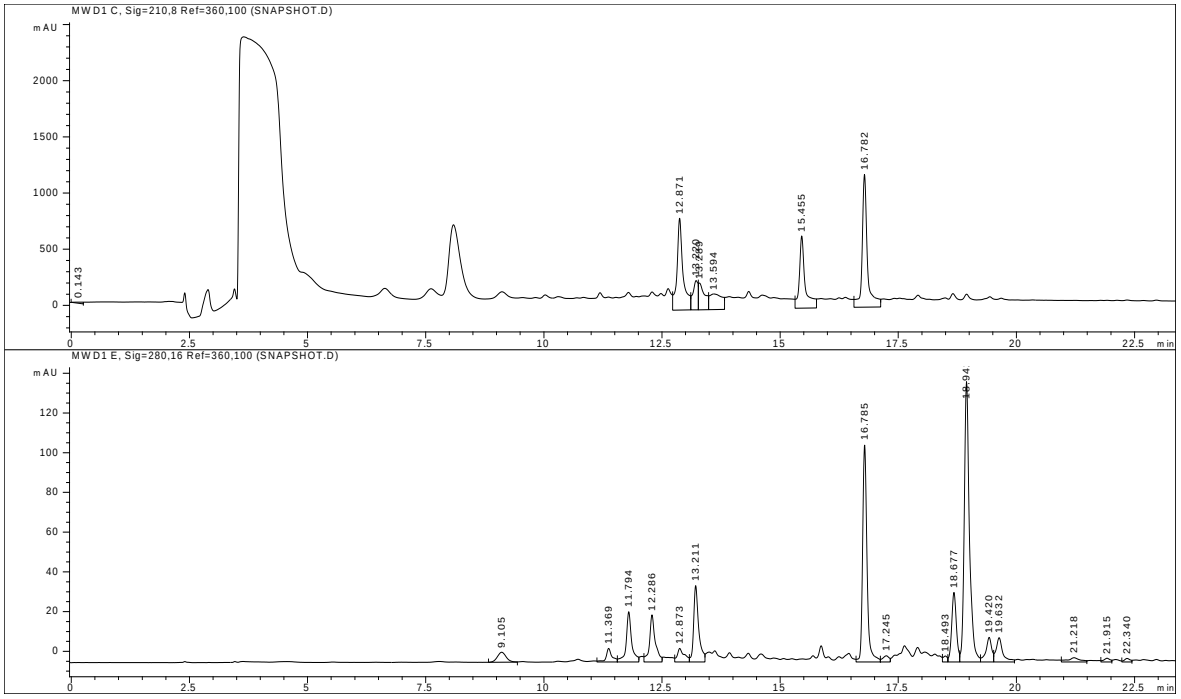
AcCys(d)GlyThrValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

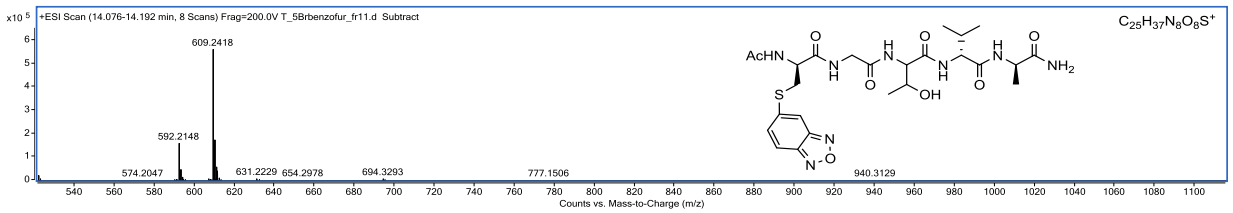


B) preparative HPLC *tR*= 16.782 min; ES-MS: calculated $[M + H]^+$, 609.2450, found m/z 609.2418 ($[M+H]^+$). White solid

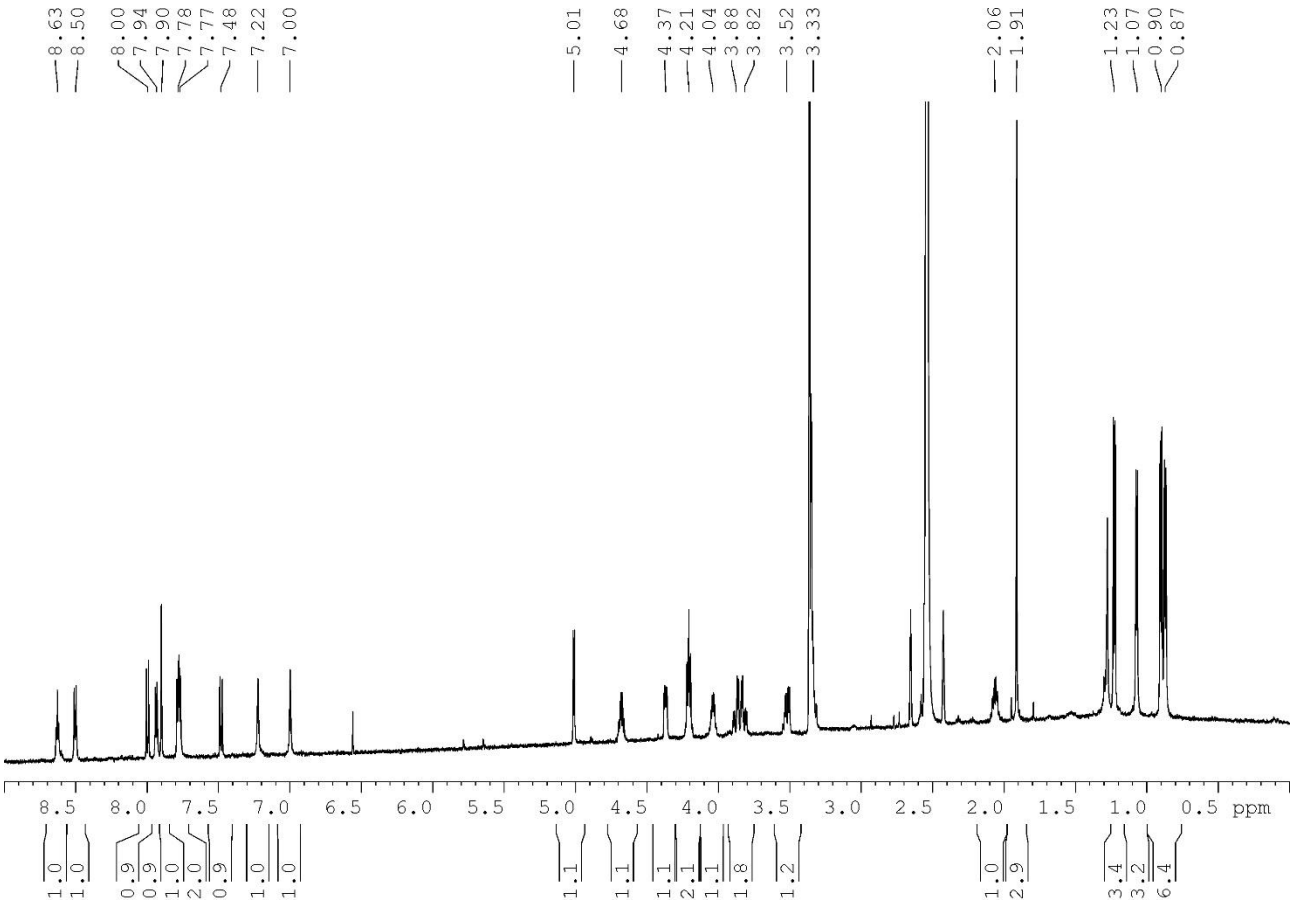
HPLC profile of the reaction crude product:



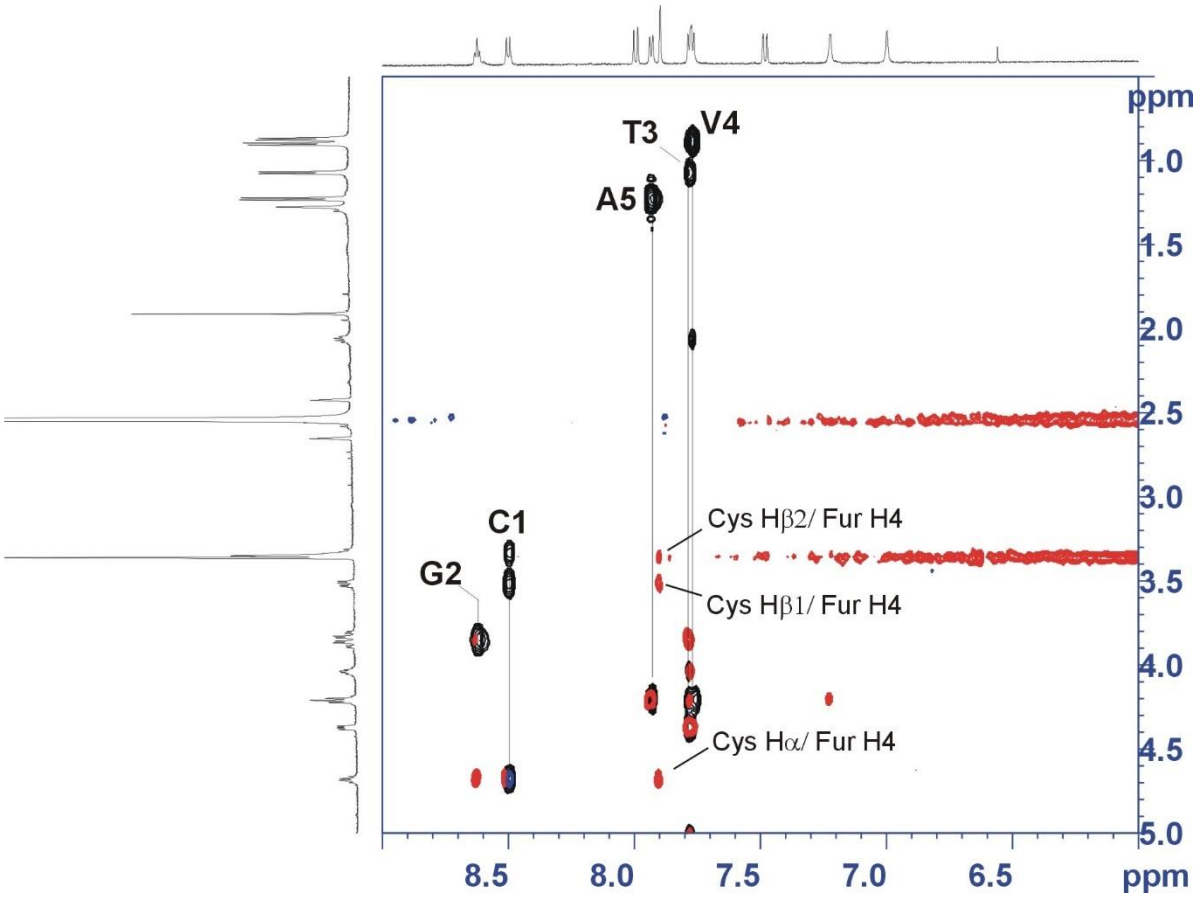
MS spectrum of **5d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

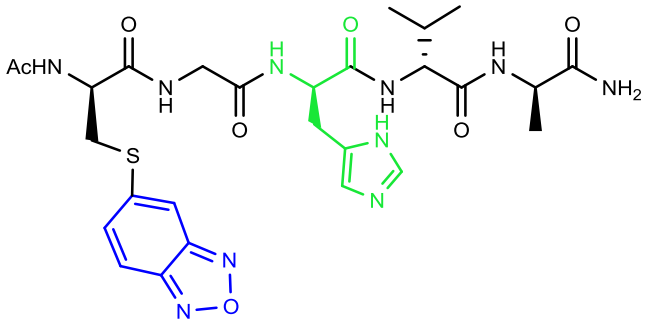
Compound 5d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.91
CYS	H	1	8.50
CYS	HA	1	4.68
CYS	HB1	1	3.52
CYS	HB2	1	3.33
FUR	H6	6	7.48
FUR	H7	6	8.00
FUR	H4	6	7.90
GLY	H	2	8.63
GLY	HA2	2	3.88
GLY	HA3	2	3.82
THR	H	3	7.78
TRP	HA	3	4.37
THR	HB	3	4.04
THR	HG1	3	5.01
THR	HG2	3	1.07
VAL	H	4	7.77
VAL	HA	4	4.21
VAL	HB	4	2.06
VAL	HG1	4	0.90
VAL	HG2	4	0.87
ALA	H	5	7.94
ALA	HA	5	4.21
ALA	HB	5	1.23
CO-NH2	NH1	5	7.00
CO-NH2	NH2	5	7.22

Compound 6d (X: Br) strategy B

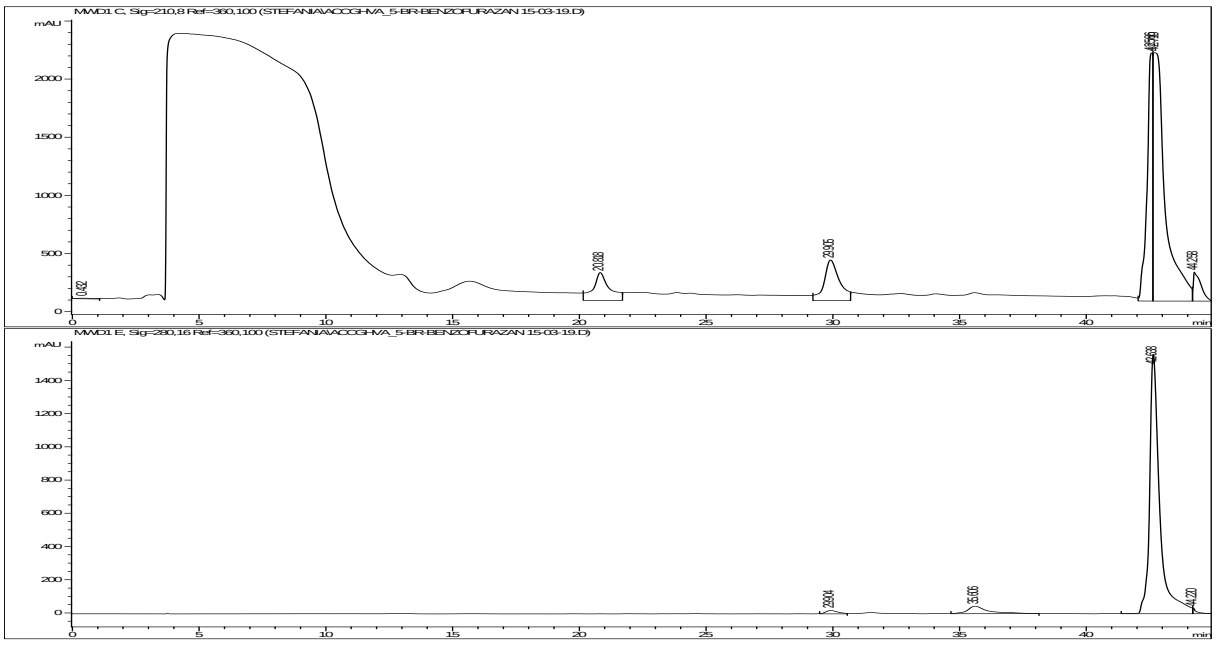
AcCys(d)GlyHisValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

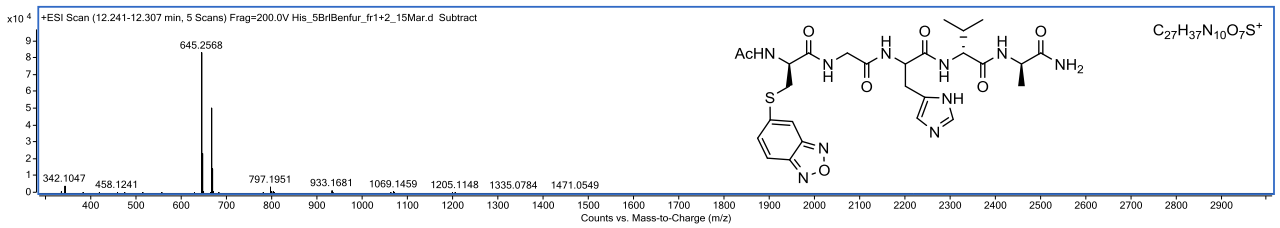


B) preparative HPLC *tR*= 29.905 min; ES-MS: calculated $[M + H]^+$, 645.2562, found m/z 645.2568 ($[M+H]^+$). White solid

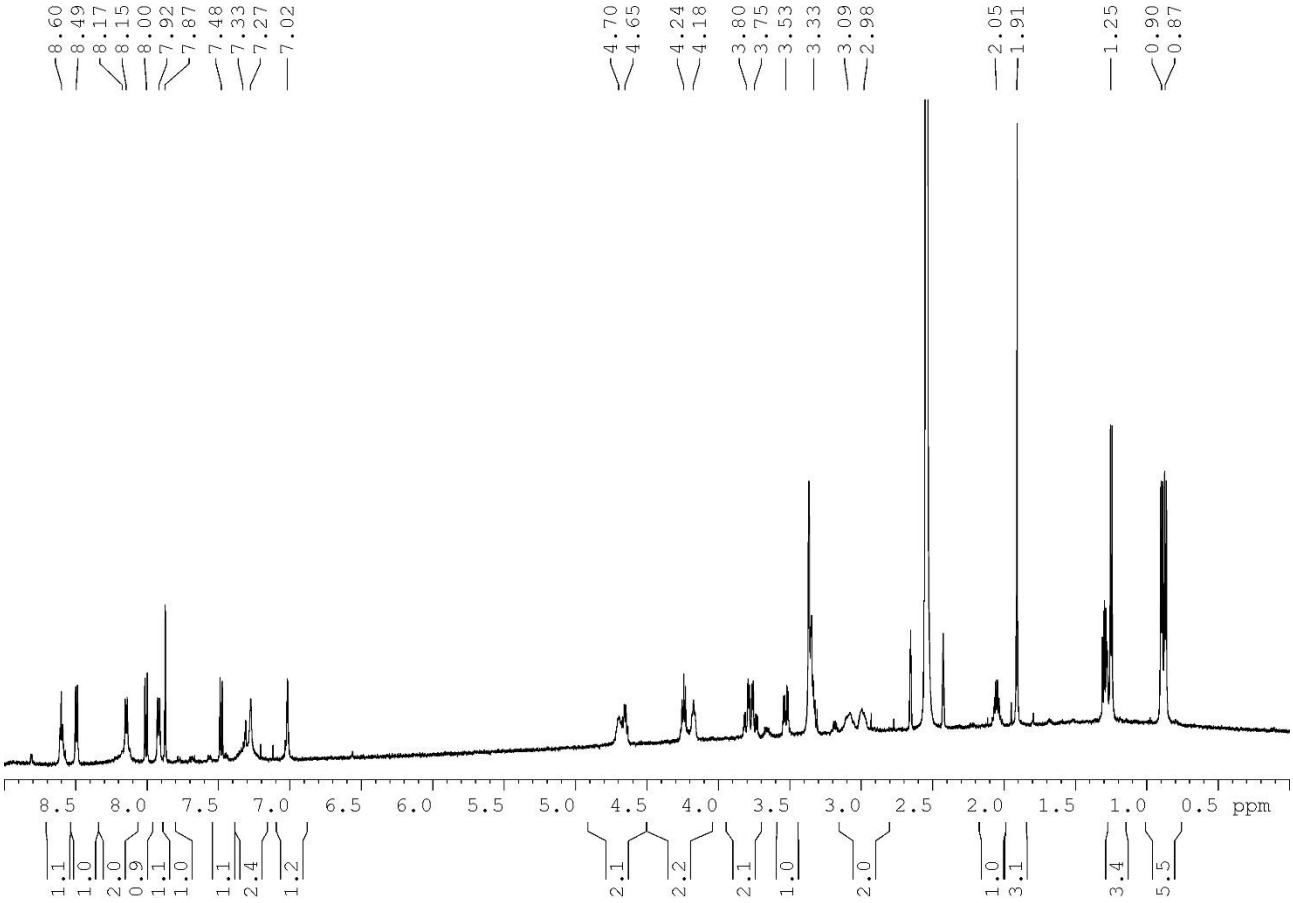
HPLC profile of the reaction crude product:



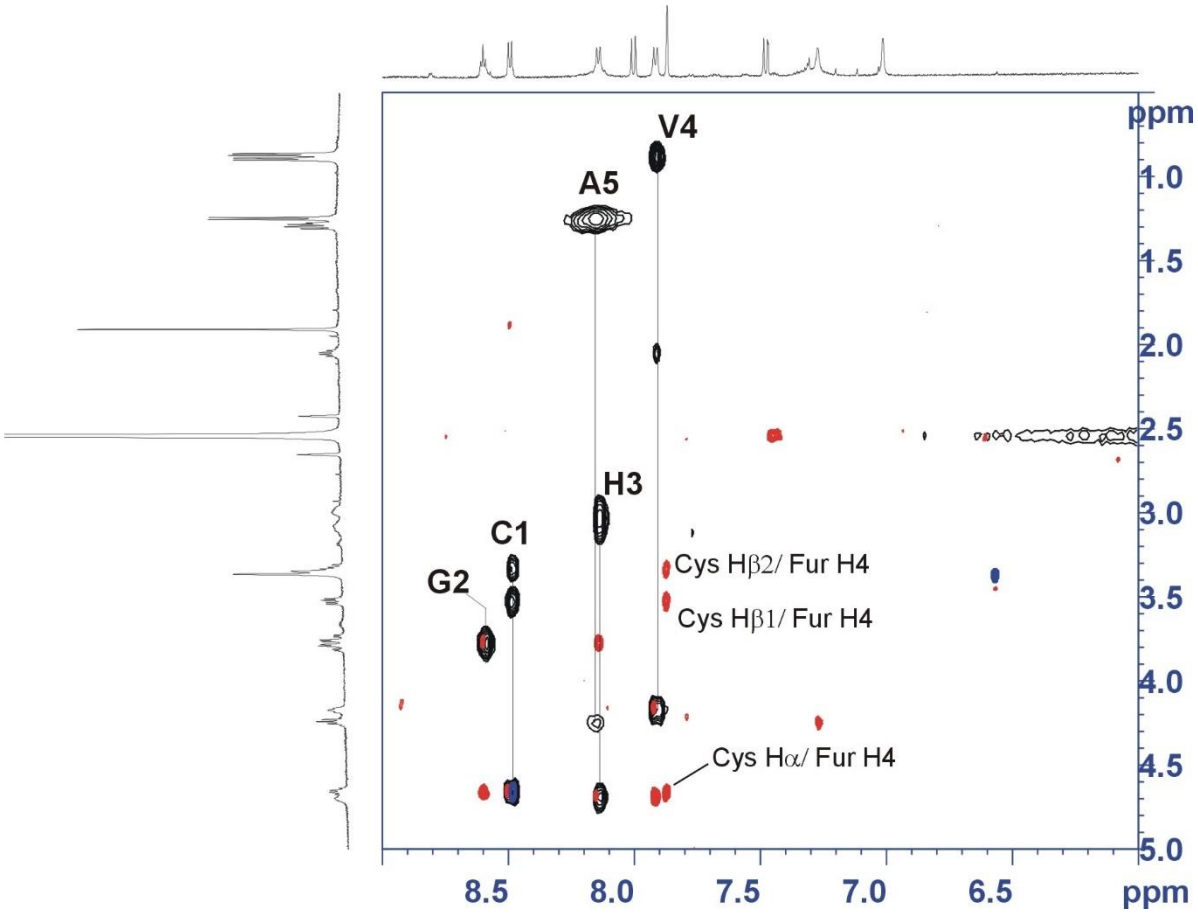
MS spectrum of **6d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

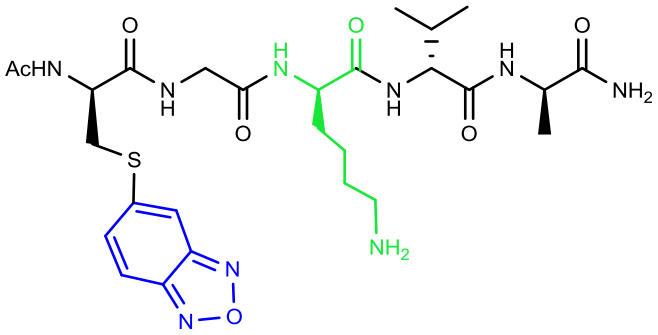
Compound 6d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1.91
CYS	H	1	8.49
CYS	HA	1	4.65
CYS	HB1	1	3.53
CYS	HB2	1	3.33
FUR	H6	6	7.48
FUR	H7	6	8.00
FUR	H4	6	7.87
GLY	H	2	8.60
GLY	HA2	2	3.80
GLY	HA3	2	3.75
HIS	H	3	8.15
HIS	HA	3	4.70
HIS	HB1	3	3.09
HIS	HB2	3	2.98
HIS	HD2	3	7.33
HIS	HE1	3	8.94
VAL	H	4	7.92
VAL	HA	4	4.17
VAL	HB	4	2.05
VAL	HG1	4	0.90
VAL	HG2	4	0.87
ALA	H	5	8.17
ALA	HA	5	4.24
ALA	HB	5	1.25
CO-NH2	NH1	5	7.02
CO-NH2	NH2	5	7.27

Compound 7d (X: Br) strategy B

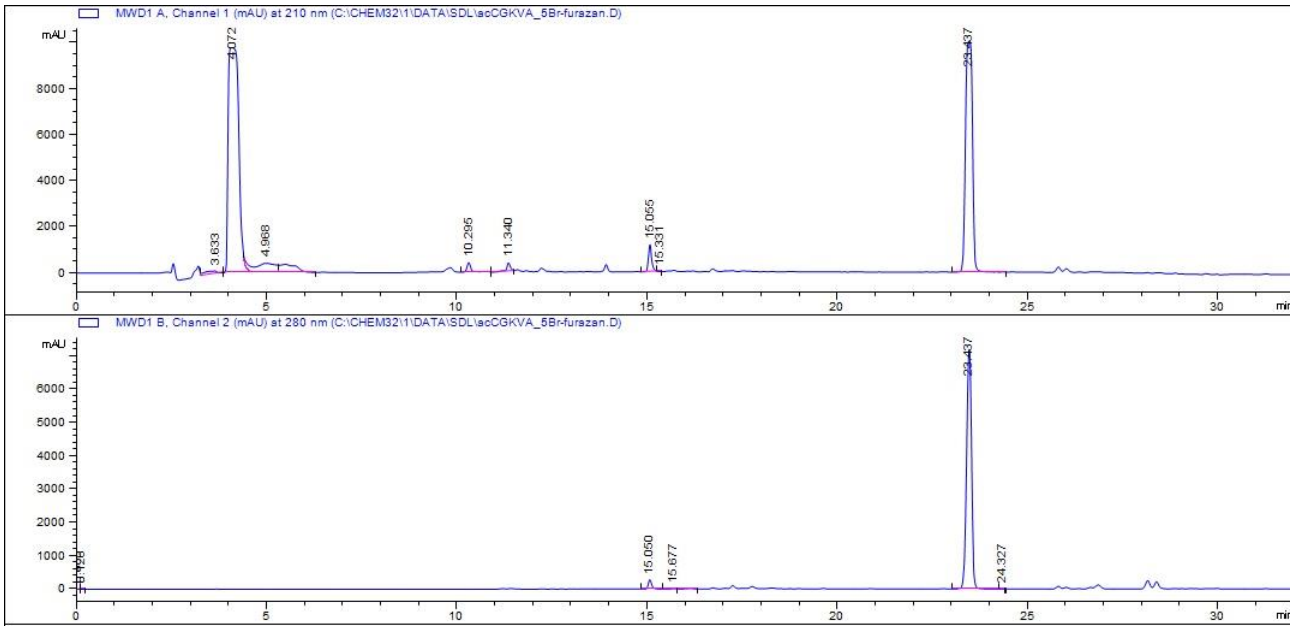
AcCys(d)GlyLysValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

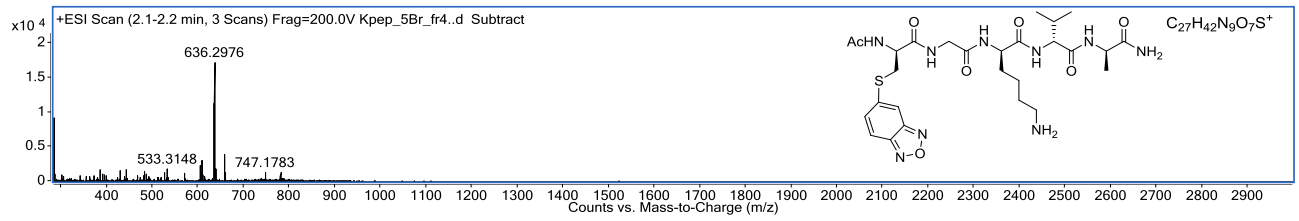


B) preparative HPLC *tR*= 15.055 min; ES-MS: calculated $[M + H]^+$, 636.2943, found m/z 636.2976 ($[M+H]^+$). White solid

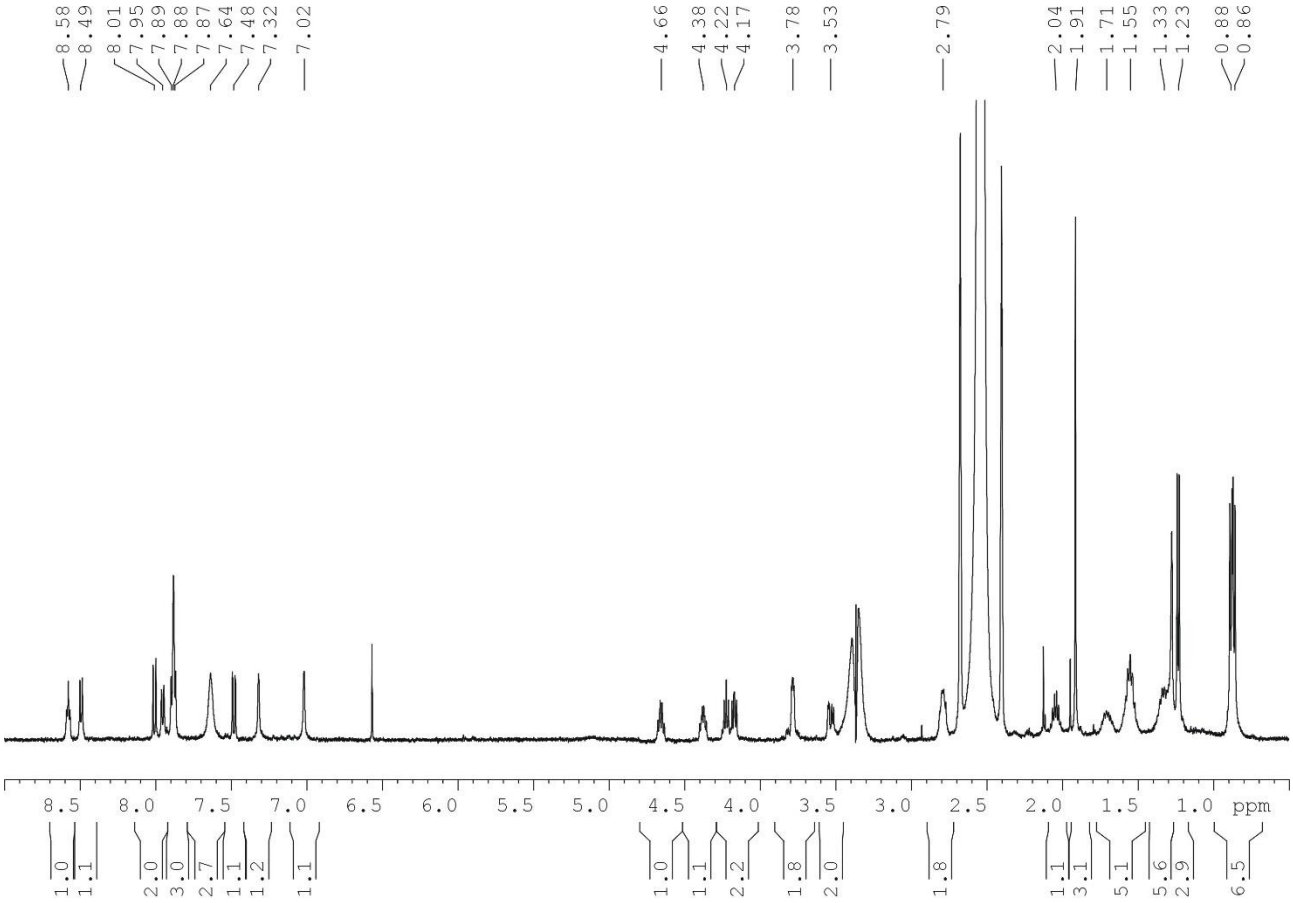
HPLC profile of the reaction crude product:



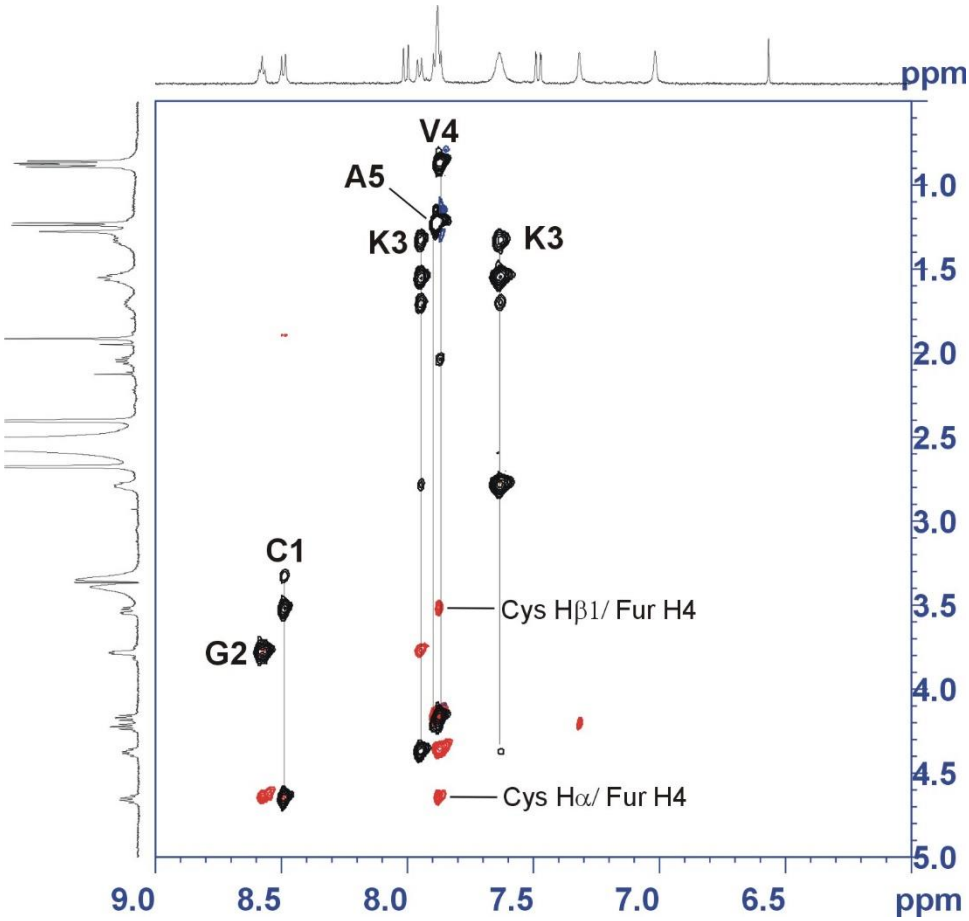
MS spectrum of **7d** after purification:



C) ^{1}H-NMR (600 MHz, dmso-d_6, 298 K)



D) Expansions of 2D TOCSY and 2D ROESY NMR (600 MHz, dmso-d_6, 298 K)



E) Assignment table

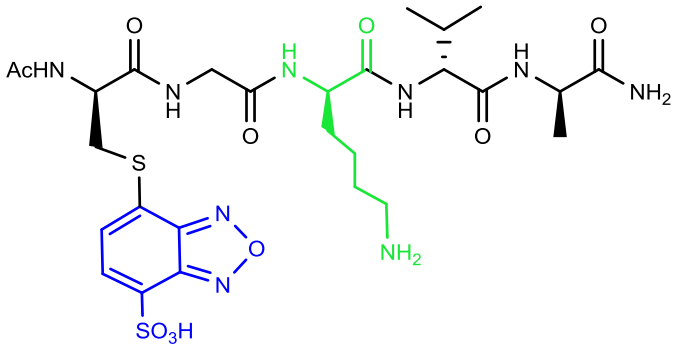
Compound 7d

RES	SPIN ID	SEQ	ppm
N-term Ac	CH3	1	1,91
CYS	H	1	8,49
CYS	HA	1	4,66
CYS	HB1	1	3,5
CYS	HB2	1	3,32
FUR	H6	6	7,48
FUR	H7	6	8,01
FUR	H4	6	7,88
GLY	H	2	8,58
GLY	HA2	2	3,78
GLY	HA3	2	3,78
LYS	H	3	7,95
LYS	HA	3	4,38
LYS	HB/HG/HD	3	1,71 – 1,55 – 1,33
LYS	HE	3	2,79
LYS	HZ	3	7,64
VAL	H	4	7,87
VAL	HA	4	4,17
VAL	HB	4	2,04
VAL	HG1	4	0,88
VAL	HG2	4	0,86
ALA	H	5	7,89
ALA	HA	5	4,22
ALA	HB	5	1,23
CO-NH2	NH1	5	7,02
CO-NH2	NH2	5	7,32

Compound 7b (X: Cl) strategy B

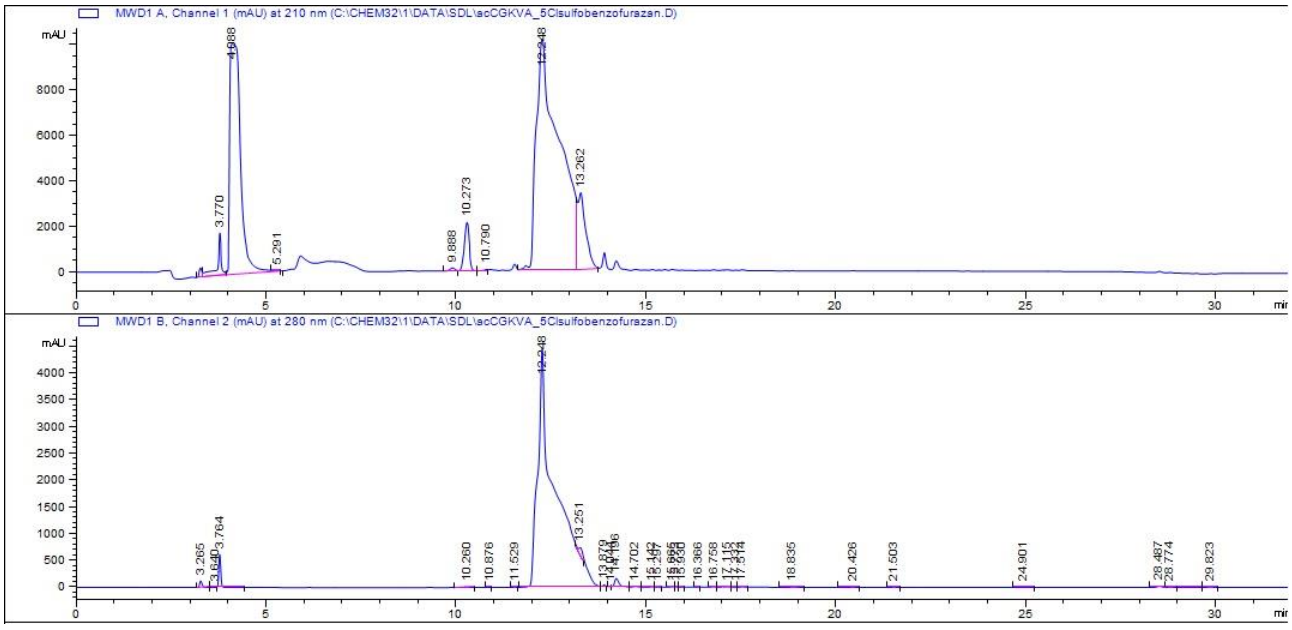
AcCys(b)GlyLysValAlaNH$_2$

A) Structure of benzofurazan/peptide conjugates

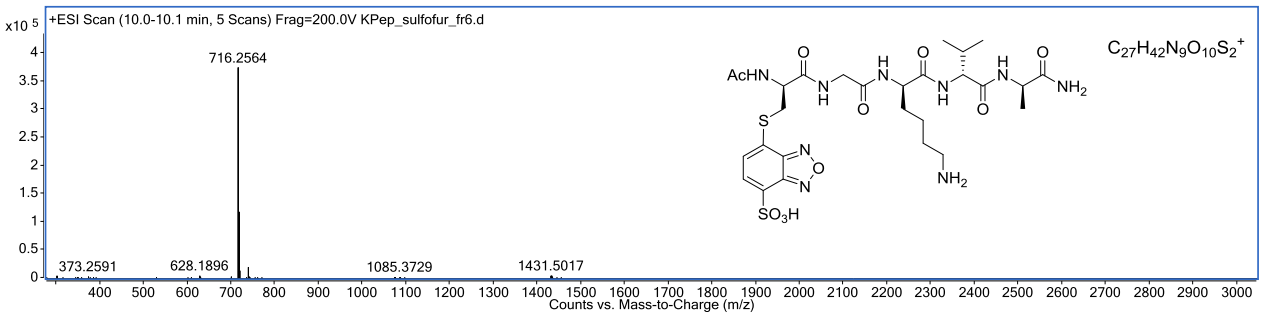


B) preparative HPLC *tR*= 12.248 min (co-eluted with the substrate employed b-Cl); ES-MS: calculated $[M + H]^+$, 716.2503, found m/z 716.2564 ($[M+H]^+$). yellow solid

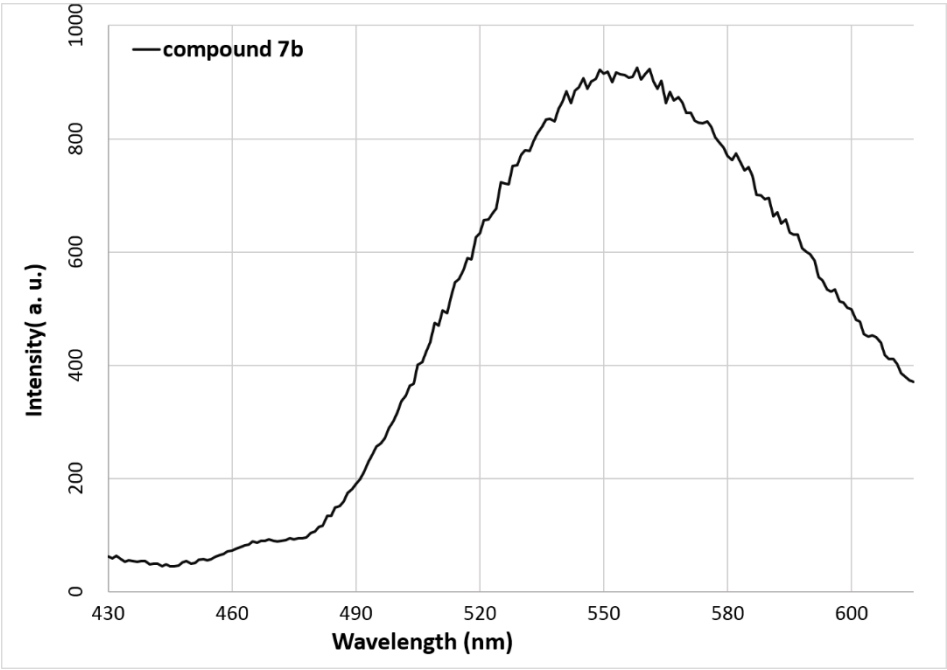
HPLC profile of the reaction crude product:



MS spectrum of **7b** after purification:



F) Fluorescence spectrum (λex = 380 nm)



Abbreviations

Boc: *tert*-butoxycarbonyl

DBU:1,8-diazabyciclo[5.4.0]undec-7-ene

DCM: Dichloromethane

DIPEA:diisopropylethylamine

DMF: *N*,*N*-Dimethylformamide

DMSO-d_6: Dimethylsulfoxide-d_6

Fmoc: 9-Fluorenylmethoxycarbonyl

Fur: Benzofurazan

HOBt: *N*-hydroxybenzotriazole

PyBOP: benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium

^{t}Bu: *tert*-butyl

TFA: Trifluoroacetic acid

TEA:Triethylamine

TIS:Triisopropylsilane