Supplemental Information

Identification of the Molecular Targets of Disulfide Bond Disrupting Agents

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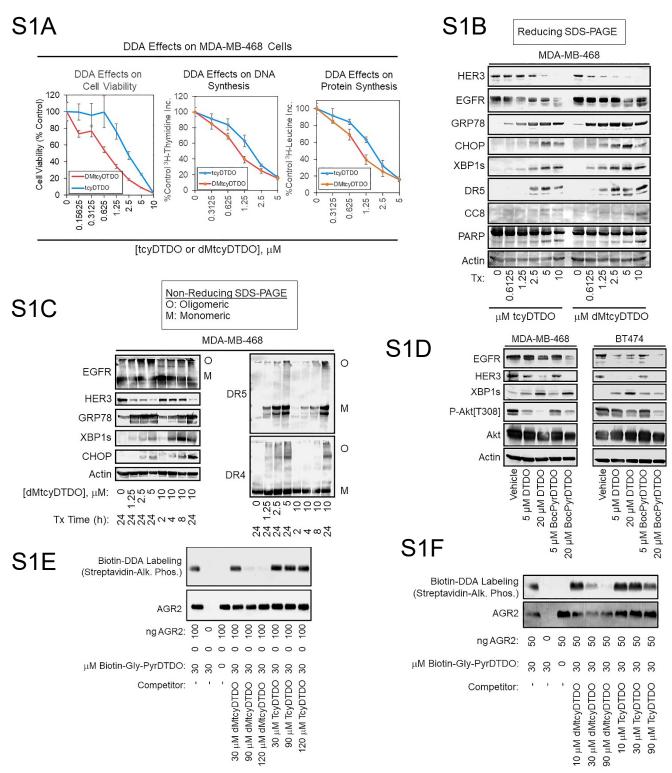
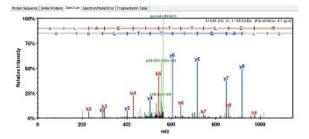


Fig. S1-Characterization of novel DDAs and an AGR2 DDA binding assay. S1A. MDA-MB-468 cells were treated for 24 h as indicated and assayed for viability, DNA synthesis, and protein synthesis in MTT assays, and tritiated thymidine and leucine incorporation assays, respectively. S1B. Reducing immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1C. Non-reducing immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1D. Immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1D. Immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1D. Immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1D. Immunoblot analysis of MDA-MB-468 cells treated for 24 h as indicated. S1E. and S1F. AGR2 binding assays employing recombinant AGR2 and Biotin-GlyPyrDTDO under the indicated conditions followed by immunoblot analysis for AGR2 or blotting with Streptavidin-conjugated Alkaline Phosphatase.

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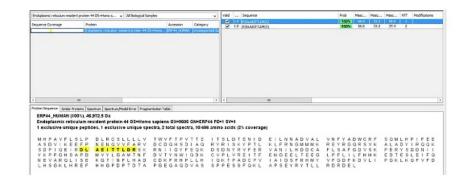
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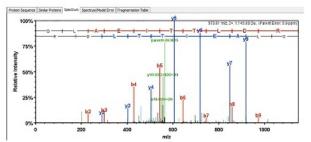
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Unique Peptide Search: ERp44 (one peptide sequence; two peptides)





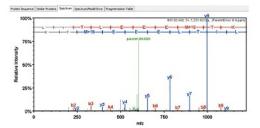
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Unique Peptide Search: PDIs (three peptides sequences; 13 peptides)

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2	227.2						1	1,109.5	555.3	1,092.5	1,091.5	9
3	328-2				310).2	т	996.5	498.7	979.4	978.4	- 8
4	441.3				423	3.3	L	895.4	448.2	878.4	877.4	7
5	570.3				552	2.3	E	782.3	391.7	765.3	764.3	6
6	699.4		350.2		681	.4	E	653.3		636.3	635.3	5
7	828.4		414.7		810).4	E	524.2		507.2	506.2	4
8	975.5		488.2		957	1.5	M+16	395.2		378.2	377.2	3
9	1,076.5		538.8		1,05	8.5	т	248.2		231.1	230.1	2
10	1,222.6	1	611.8	1.205	.6 1.20	4.6	К	147.1		130.1		1

Y-H20 Y

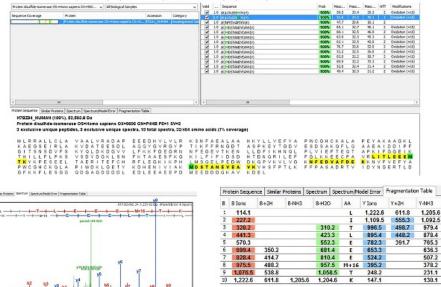
1,204.6 10 1,091.5 9

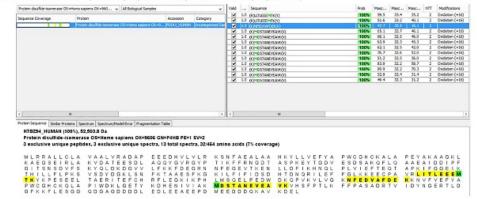
978.4 8

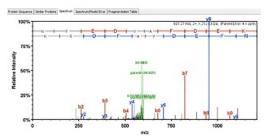
877.4 764.3 635.3 506.2 377.2

230.1

3



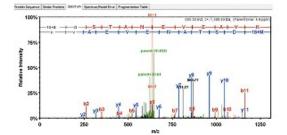




Pro	otein Sequer	ice	Similar B	Proteins	Spectrum	Spectr	um/M	lodel Error	Fragmentati	on Table		
в	B lons	8-	H2H	B-NH3	B-H20	A	A	Y Jons	Y+2H	Y-NH3	Y-H2O	Y
1	115.1			98.0)		N	1.213.5	607.3	1.196.5	1.195.5	10
2	262.1			245.	1		F	1,099.5	550.3	1,082.5	1,081.5	9
3	391.2			374.	1 37:	3.2	Ε	952.4	476.7	935.4	934.4	8
4	506.2			489.	2 48	3.2	D	823.4	412.2	806.4	805.4	7
5	605.3			588.	2 58	1.2	٧	708.4	354.7	691.3	690.3	6
6	676.3		338.7	659.	3 65	8.3	A	609.3		592.3	591.3	5
7	823.4		412.2	806.	3 80	5.4	F	538.3		521.2	520.2	4
8	938.4		469.7	921.	4 921	0.4	D	391.2		374.2	373.2	3
9	1.067.4		534.2	1.050	.4 1.04	9,4	Ε	276.2		259.1	258.1	2
10	1,213.5		607.3	1,196	.5 1,19	5.5	к	147.1		130.1		1

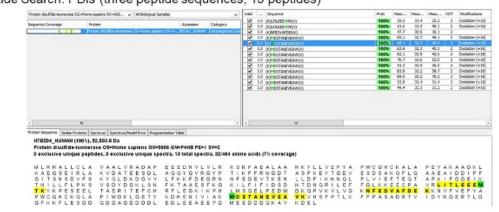
Unique Peptide Search: PDIs (three peptide sequences; 13 peptides)

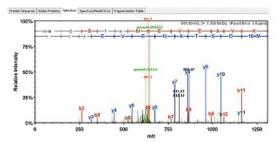
Protain clouifde-icomer	race OS «Hamo Is	perc 01 -960	 Al 20 	opca Sarpe				v									Prob		leec	Month -	Mex.	NT		led/cate	
equence Caverage	Prot	-				CONTRACTOR OF CO	Catego		1-8		o counter						190		39.5	22.4	25.2			xdation (
educe cases de		in chuilfide isome				AT HUMAN		oriend Se		2.0	o contrata						50		\$2.6	31.2	40.1	2	C.	edation ((+3
	21.2	10000000000	RIC LO HIL		-w. jr.	ALC: NO W	 Prost 	0.000			9 CONFIDER						190		42.7	36.6	26.1	2	_	viciation (_
											9 No DITA						100		68.1	32.5	46.0			vidation (
									1-8		ATEOHOD 0 ATEOHOD 0		_				100		63.9	323	45.3			xdation (
									l-8		0 (ICHOSTA)		_				100		62.2	32.5	42.0	-		edation (
									ll-S		0 (CHOOTA						50		26.2	32.6	12.0			withday (
									II-E		P (K)MDITA						50		55.2	32.5	34.0			viciation (
									II-E		ATERNO 0						50		83.5	32.2	55.7			viciation (
									비는중		0 (CHOSTA						50		09.9	32.2	- 30.3			xdation (
									IH-S		0 COMOSTA		-				100		12.0	32.4	11.4			xdation (
									IH-S		0 (checite	CALMAN (A)	_				100		40.4	31.3	11.7			ectation (
1				1		1		2	1																
stan Sequence Sam HT8254_HUWU Protein disuff Diecclusive un	Rer Protono Spo AH (1001.), 53 fide-is omena:	2.503.8 Da se OS=Homo	sapiens	0X-9606	CN-P4	-				cida	(7% cove														
HT8294_HUMU Protein disuff	Re Protono Spo AH (1001-), 63 Nde-isomera: nique peptid	2.503.8 Da se OS=Homo	sapiers tve unig	ox-esse ue spectr	CN-P4	HB FC+ tal spec			íns a		(TS cove	ragej	YLI		FYA	PW	co	H C	KA	LA	PEY	AK		AGK	
HT9294_HUMU Protein disuff Desclusive us	Re Protono Spo AH (1001-), 63 Nde-isomera: nique peptid	2,500.8 Da se OS=Homo les, 3 esclus	sapiens ive unig	OX-9606 us spects	CN=P4 a, 13 ta	Kal spec	ctra, 32A	464 am	íns a	AB		ragej			FYA GDV	PWES			K A Q F		PEY			AGK	
HT9294_HUW Protein disuff Decclusive un M L R R A L K A E G S E	Re Protono Soc AH (1001a), 63 Inde-Isomera: Inique peptid	Secore Da Se OS=Herric Ies, D esclus	R A D	AP E	CH=P4 a, 13 tz E E D	HIS FE-	VLR	464 am	ino a s N P	ABFR	EALA	га ра) А. Н.К. Т. А. S		ΥT			D S			L Q		A.L		DIP	5
HT9294_HUW Protein disuff Decclusive un M L R R A L K A E G S E	ReProtono Spo Al (1001), 62 Inde-Isomera: Inique peptid L L C L A E I R L A S D V F S	VAAL) KVDA	R A D R A D E E S X D G	AP E	CH=P4 a, 13 tz E E D Q Q Y	HIS FE-	VLR GYP	464 am	ino a S N P K F	ABFR	EALA	ragel A H K T A S N L L	PKE	Y T K H	GOV	E S	D S V I	AK	QF	L Q	AAE	A.L	DO	DIP	1
HT9294_HUW Protein disuff Decclusive un M L R R A L K A E G S E G I T S N S	Referencia AH (1001), 63 Note-isomeration inique peptid LLCLA EIRLA SDVFS FLPKS	A A A L Y K Y O A A L Y	R A D R A D E E S K D G S K L	AP E OL A VV L	CH=P4 a, 13 tz E E D Q Q Y	HS FC- tal spec	VLR GYP GRN	464 am	Ine a	AFEIF	EALA RNGO VTKE	TADOI A HK T AS N LL D HT	DPIDNO	KH RI	GOV	E S P L	D S VI LK	AK	Q F T E E C	Q T P A	AAE	AI	50	DIP	1
Protein disuff Decclusive un MLRRAL KAEGSE GITSNS THILLF	Na Protono Soc All (1001), 62 Inde-Isomera: Inique peptid L L C L A E I R L A S D V F S F L P K S E S E E L	X A A L Y K Y D A T K Y D A T K Y D A T K Y O L C V S D Y C	R A D R A D E E S K D G S K L T E F	AP E OL A VV L SN F	CH=P4 a, 13 tz E E D Q Q Y	HIS FC+ tal spectrum G V R F D E A E S I G K I	VLR GYP GRN FKG KPM	K 5 T 1 N 7 K 1 L V	Ine a N F K F E G L F	AFRICE	EALA RNGO VTKE FIDS LPED	TADAL A HK T AS N LL D HT W DK	DFIDNO	KH RI KV	G D V N O L L E F	E S P F G K	D S VI LK	A K E F K E	Q F E C A F		A A E A P K V R L K K N		50		8 10 1



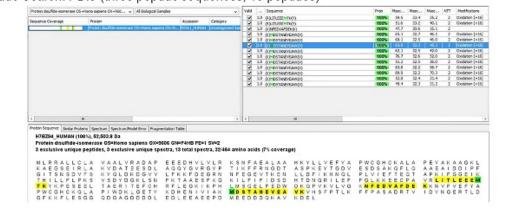
Protein Sequence Similar Proteins Spectrum Spectrum Model Error Pragmentation Table

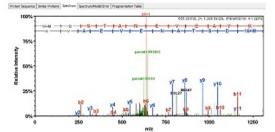
						-			_
8 Jons	B+2H	B-NH3	B4120	AA	Y Jons	Y+34	Y-NH3	Y-H20	Y
148.0				M+16	1.309.6	655.3	1.292.6	1.291.6	12
263.1			245.1	D	1,162.6	581.8	1,145.5	1,144.5	11
350.1			332.1	5	1.047.5	524.3	1.030.5	1.029.5	30
451.1			433.1	т	960.5	480.8	943.5	942.5	9
522.2			504.2	A	859.5	430.2	842.4	841.4	В
636.2	318.6	619.2	618.2	N	788.4	394.7	771.4	770.4	7
765.3	383.1	748.2	747.3	E	674.4	337.7	657.3	656.4	6
864.3	432.7	847.3	846.3	v	545.3		528.3	527.3	5
993.4	497.2	976.4	975.4	E	446.3		429.2	428.3	4
1,064.4	532.7	1,047.4	1,046.4	A	317.2		300.2		3
1.163.5	582.2	1.146.5	1.145.5	v	246.2	2	229.2		2
1,309.6	655.3	1,292.6	1,291.6	К	147.1		130.1		1
	148.0 263.1 350.1 451.1 522.2 636.2 765.3 864.3 993.4 1,064.4 1,163.5	148.0 263.1 350.1 451.1 522.2 636.2 318.6 765.3 383.1 864.3 432.7 993.4 497.2 1,064.4 532.7 1,064.5 582.2	148.0 263.1 350.1 451.1 522.2 765.3 363.1 748.2 765.3 984.3 984.3 983.4 497.2 976.4 1,064.4 532.7 1,047.4 1,064.4 532.7 1,047.4 1,044.5 1,045.5 1,044.5 1,045.5	140.0 203.1 245.1 350.1 350.1 350.1 352.2 504.2 504.2 504.2 505.3 383.1 748.2 745.3 903.4 497.2 976.4 975.4 1,044.4 1,046.4 1,	148.0 H+16 263.1 245.1 D 350.1 332.1 S 451.1 433.1 T 522.2 504.2 A 636.2 318.6 619.2 618.2 N 765.3 333.1 748.2 747.3 E 864.3 352.7 844.3 ¥ Y 903.4 497.2 976.4 975.4 E 1,064.4 532.7 1,047.4 1,046.4 A 1,165.5 582.2 1,146.5 1,445.5 Y	140.0 H+16 1,309.6 203.1 245.1 D 1,162.6 350.1 332.1 S 1,047.5 451.1 433.1 T 960.5 522.2 504.2 A 859.5 563.2 318.6 619.2 618.2 N 788.4 765.3 383.1 748.2 747.3 E 674.4 864.3 432.7 844.7 3 846.3 V 545.3 903.4 497.2 975.4 E 446.3 317.42 1,064.4 532.7 1,047.4 1.045.5 V 246.2	148.0 H+16 1.309.6 655.3 263.1 249.1 D 1.162.6 581.8 350.1 332.1 5 1.047.5 524.3 451.1 433.1 T 960.5 480.8 522.2 504.2 A 859.5 430.2 536.1 318.6 619.2 618.2 N 788.4 394.7 765.3 383.1 748.2 747.3 E 674.4 337.7 864.3 432.7 847.3 846.3 Y 545.3 37.7 963.4 497.2 975.4 E 446.3 1.465.5 1.445.5 Y 545.3 1.064.4 532.7 1.047.4 1.046.4 A 317.2 1.163.5 682.2 1.145.5 1.445.5 Y 545.3	148.0 H+16 1,309.6 655.3 1,292.6 263.1 245.1 D 1,162.6 581.8 1,145.5 350.1 332.1 S 1,047.5 524.3 1,030.5 451.1 433.1 S 960.5 480.8 943.5 522.2 504.2 A 859.5 430.2 842.4 636.2 318.5 619.2 618.2 N 788.4 394.7 771.4 765.3 333.1 749.2 747.3 E 674.4 337.7 657.3 864.3 342.7 847.3 846.3 429.2 1,064.4 347.2 300.2 1,064.4 532.7 1,047.4 1,046.4 317.2 300.2 249.2 1,064.4 532.7 1,047.4 1,046.4 317.2 300.2 249.2 1,064.4 532.7 1,047.4 1,046.4 347.2 300.2 249.2	140.0 PH+16 1,309.6 655.3 1,292.6 1,291.6 203.1 245.1 D 1,162.6 541.8 1,144.5 1,144.5 350.1 332.1 5 1,047.5 524.8 1,030.6 1,029.5 451.1 433.1 T 960.5 480.8 943.5 942.4 522.2 504.2 A 859.5 430.2 842.4 841.4 636.2 318.6 619.2 618.2 N 788.4 394.7 771.4 771.4 765.3 383.1 748.2 747.3 E 674.4 337.7 657.3 655.3 322.7 847.3 846.3 92.2 428.3 324.7 847.4 771.4 770.4 770.4 528.3 527.3 528.3 527.3 528.4 324.2 428.3 1,062.4 329.2 428.3 429.2 428.3 1,062.4 329.2 428.3 1,062.4 1,163.5 528.2 1,146.5 1,145.5 746.2





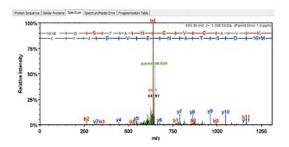
Pro	itein Sequen	ce :	Similar I	Proteins	Spectrum	Spe	ectrum/M	odel Error	Fragmentat	on Table		
в	B Jons	Bea	ж	B-NH3	840	0	AA	Y Jons	Y+2H	Y-NH3	Y-H20	Y
1	148.0						M+16	1,309.6	655.3	1.292.6	1,291.6	12
2	263.1				- 24	5.1	D	1,162.6	581.8	1,145.5	1,144.5	11
3	350.1				33	2.1	5	1,047.5	524.3	1,030.5	1,029.5	10
4	451.1				43	3.1	T	960.5	480.8	943.5	942.5	9
5	522.2				50	4.2	A	859.5	430.2	842.4	841.4	8
6	636.2	3	18.6	619.	2 61	8.2	N	788.4	394.7	771.4	770.4	7
7	765.3	3	83.1	748.	2 74	7.3	E	674.4	337.7	657.3	656.4	6
8	864.3	-4	32.7	847.	3 84	6.3	v	545.3		528.3	527.3	5
9	993.4	-4	97.2	976.	4 97	5.4	E	446.3		429.2	428.3	4
10	1.064.4	-5	32.7	1.047	.4 1.0	46.4	A	317.2		300.2		3
11	1,163.5	-5	82.2	1,146	i.5 1,1	45.5	v	246.2		229.2		2
12	1,309.6	6	55.3	1,292	.6 1,2	91.6	K	147.1		130.1		1





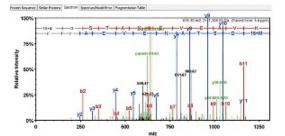
was sequen	NE.	Similar P	rotens	spectrum	spectrum	Model Error	Fragmentat	At table		
8 Jons	84	-21	B-NH3	8-1120	AA (Y lons	Y+2H	Y-NH3	Y-H20	۲
148.0					M+1	6 1,309.6	655.3	1,292.6	1,291.6	12
263.1				245	.1 D	1,162.6	581.8	1,145.5	1.144.5	11
350.1				332	.1 5	1,047.5	5 524.3	1,030.5	1,029.5	10
451.1				433	.1 Т	960.5	480.8	943.5	942.5	9
522.2				504	.2 A	859.5	430.2	842.4	841.4	8
636.2	:	318.6	619.	2 618	.2 N	788.4	394.7	771.4	770.4	7
765.3	:	383.1	748.	2 747	.3 E	674.4	337.7	657.3	656.4	6
864.3		432.7	847.	3 846	.3 V	545.3		528.3	527.3	5
993.4		497.2	976.	4 975	i,4 E	446.3		429.2	428.3	4
1,064.4		532.7	1,047	4 1.04	6.4 A	317.2		300.2		3
1,163.5		582.2	1,146	1.14	5.5 V	246.2		229.2		2
1.309.6	1	855.3	1,292	.6 1.29	1.6 K	147.1		130.1		1
	148.0 263.1 350.1 451.1 522.2 636.2 765.3 864.3 993.4 1,064.4 1,163.5	148.0 263.1 350.1 451.1 522.2 636.2 765.3 864.3 993.4 993.4 1,064.4 1,163.5	148.0 253.1 350.1 451.1 522.2 556.2 318.6 765.3 383.1 864.3 432.7 993.4 497.2 1,064.4 532.2	148.0 263.1 350.1 451.1 522.2 564.3 565.2 383.1 765.3 383.1 748. 664.3 497.2 976. 1,064.4 532.7 1,047 1,163.5 582.2 1,1047	148.0 263.1 245 263.1 264 332 350.1 333 345 451.1 433 522 504 504 619.2 616.2 318.6 619.2 618 705.3 383.1 748.2 744 964.3 322.7 947.3 846 993.4 497.2 976.4 975 1,064.4 532.7 1,047.4 1,04 1,163.5 582.2 1,145.5 1,44	148.0 PH+1 263.1 245.1 D 350.1 332.1 S 451.1 433.1 T 522.2 504.2 A 566.2 318.6 619.2 618.2 N 705.3 383.1 748.2 747.3 86.3 Y 993.4 492.7 976.4 975.4 E 1,046.4 A 1,065.4 632.7 1,047.4 1,046.4 A 1,455.5 Y	148.0 P+16 1,309.6 263.1 245.1 0 1,162.4 350.1 332.1 5 1,047.4 451.1 433.1 T 960.5 522.2 504.2 A 859.5 545.2 318.6 619.2 618.2 N 788.4 705.3 383.1 7.48.2 747.3 E 674.4 893.4 993.4 497.2 976.4 975.4 E 446.3 310.64.4 337.2 1,047.4 1,046.4 A 377.2 1,65.5 V 246.2 1,445.5 Y 245.2 1,245.5 Y 245.2 1,245.5 Y 246.2 1,445.5 Y 246.2 1,446.5 1,445.5 Y 246.2 1,446.5 1,445.5 Y 246.2 Y 246.2 1,445.5 Y 246.2 Y X <td< td=""><td>148.0 M+16 1.309.8 655.3 263.1 245.1 D 1.162.6 591.8 350.1 332.1 S 1.047.5 524.3 451.1 433.1 T 960.5 490.8 522.2 504.2 A 859.5 430.2 536.2 318.6 619.2 618.2 N 788.4 394.7 765.3 383.1 748.2 747.3 86.63 V 545.3 337.7 964.3 432.7 947.3 846.3 V 545.3 337.7 993.4 497.2 975.4 £ 446.3 317.2 1,064.4 532.7 1.047.4 1.046.4 A 317.2 1,064.4 532.7 1.047.4 5 1.455.5 V 246.2</td><td>148.0 P+16 1,309.6 655.3 1,292.6 263.1 245.1 0 1,162.6 501.8 1,145.5 350.1 332.1 S 1,047.5 524.3 1,030.6 451.1 433.1 T 960.5 490.8 943.5 522.2 504.2 A 859.5 430.2 842.4 505.3 318.6 619.2 618.2 N 788.4 394.7 771.4 705.3 383.1 7.48.2 747.3 E 674.4 337.7 657.3 984.3 432.7 947.3 846.3 V 545.3 528.3 993.4 497.2 976.4 975.4 E 446.3 429.2 1,064.4 532.7 1,044.5 1,445.5 Y 246.2 209.2</td><td>148.0 PH+16 1.309.6 655.3 1.292.6 1.291.6 263.1 245.1 D 1.162.6 581.8 1.145.5 1.144.5 350.1 332.1 5 1.047.5 524.3 1.030.6 1.023.5 451.1 433.1 T 860.5 480.8 943.5 942.4 552.2 504.2 A 859.5 430.2 842.4 841.4 562.3 318.6 619.2 618.2 N 788.4 394.7 771.4 770.4 765.3 383.1 7.48.2 744.3 E 674.4 337.7 657.3 655.3 993.4 497.2 975.4 £ 446.3 429.2 428.3 1,064.4 332.7 1.047.4 1.046.4 317.2 300.2 1.153.5 528.2 1.146.5 1.455.5 Y 246.2 229.2</td></td<>	148.0 M+16 1.309.8 655.3 263.1 245.1 D 1.162.6 591.8 350.1 332.1 S 1.047.5 524.3 451.1 433.1 T 960.5 490.8 522.2 504.2 A 859.5 430.2 536.2 318.6 619.2 618.2 N 788.4 394.7 765.3 383.1 748.2 747.3 86.63 V 545.3 337.7 964.3 432.7 947.3 846.3 V 545.3 337.7 993.4 497.2 975.4 £ 446.3 317.2 1,064.4 532.7 1.047.4 1.046.4 A 317.2 1,064.4 532.7 1.047.4 5 1.455.5 V 246.2	148.0 P+16 1,309.6 655.3 1,292.6 263.1 245.1 0 1,162.6 501.8 1,145.5 350.1 332.1 S 1,047.5 524.3 1,030.6 451.1 433.1 T 960.5 490.8 943.5 522.2 504.2 A 859.5 430.2 842.4 505.3 318.6 619.2 618.2 N 788.4 394.7 771.4 705.3 383.1 7.48.2 747.3 E 674.4 337.7 657.3 984.3 432.7 947.3 846.3 V 545.3 528.3 993.4 497.2 976.4 975.4 E 446.3 429.2 1,064.4 532.7 1,044.5 1,445.5 Y 246.2 209.2	148.0 PH+16 1.309.6 655.3 1.292.6 1.291.6 263.1 245.1 D 1.162.6 581.8 1.145.5 1.144.5 350.1 332.1 5 1.047.5 524.3 1.030.6 1.023.5 451.1 433.1 T 860.5 480.8 943.5 942.4 552.2 504.2 A 859.5 430.2 842.4 841.4 562.3 318.6 619.2 618.2 N 788.4 394.7 771.4 770.4 765.3 383.1 7.48.2 744.3 E 674.4 337.7 657.3 655.3 993.4 497.2 975.4 £ 446.3 429.2 428.3 1,064.4 332.7 1.047.4 1.046.4 317.2 300.2 1.153.5 528.2 1.146.5 1.455.5 Y 246.2 229.2

Protein doulf de nomerase (05-Hana sapiens OX-960.	 v Al Bological Sample 	ies .		y Vald	Seque			Prab	Marc			NT.	Modificati	
iequence Coverage	Presen		Accession	Category	3	10 (0000			1001		33.4	35.2	2	Oxidation	
adminis coverage					3	1.0 (R)LITL			1001		38.2	40.1	- 2	Cedation	(+26
	Protein deutfide-eon	ensee CS+Home septeme C	OX+8 POSA1_HUMHI	Uncategorized	2	1.0 (K)MPR			1001		30.6	36.1	2		
					1		TANEVEAWN(V)		1001		32.7	46.1	- 2	Codetion	
					1		TANK/WAR(V)		1001		32.5	46.0	5	Oxdelien	
					1		TANEVEAW(V)		1001		32.3	45.3	2	Crictation	
					<u> </u>		WENTANG()		1905		32.5	42.0	1.3	Ondeten	
					1		TANEVEAVA()/)		1001		32.6	\$2.0 36.0	2	Oxdetion	
					1		TANEYEARA(V)		1001		32.5		- 2	Oxidation	
					-6-		TANEVEAVA(V)		1905		32.2	50.7	2	Oxidation	
					- * -		TANEYEARN(V)		1005		32.2	30.3	- 2	Credation	
					-6-		TANEYEAW(0)		1905		32.4	31.4	2	Oxidation	
					×	1.0 (0/125	TANEVEAVO()		1301	48.4	32.3	31.2	- 2	Cristetian	1(+3
<	e Secture Sector	m/Hodel Error Presenente	ation Table		2 4		8								
Protein Securical Senter P HT8234_HUMAN (Protein disulfide- 3 exclusive unique M L R R A L L	otere Spectrum Spectru 100%), 52,503,8 Da isomerase OS#Hom as peptides, 3 exclu C L A V A A L	o sapiens 011-960 sive unique spect	E CN=P4HB PE= tra, 53 total spec E E E D H V L	1 \$V=2 tra, 32:464 a V L R K			verage) A.A. HKYL		PWCGH			PEY			
Protein Securical Senter P HTB234_HUMAN (Protein disumde- 3 esclusive unique M L R R A L L K A E G S E I	oterna Spectrum Spectru 100 N., 52,503,8 Da Isomerase OS#Hon as peptides, 3 exclu C L A V A A L R L A K V D A	o sapiens OX=960 sive unique spect VRADAP E TEESDL /	E CINHPAHB PEH Ira, 13 total spec E E E D H V L A Q Q Y G V R	1 \$V=2 tra, 32:464 a V L R K G Y P T	mino aci SNF IKFI	AEAL	verage) A.A. HKYL DT. ASPK	EYTGOV	ESDSA	KQF	LQ	AAE	AL	DDIP	e p
Protein Securical HT5234_HUMAN (Protein disuttide 3 esclusive uniqu M L R R A L L K A E G S E I G I T S N S D	otatna Spectrum Spectru 100N), 52,503,8 Da Isomerase OSHiban se peptides, 3 exclu C L A V A A L R L A K V D A V F S K V O L	o sapiens 00.*960 sive unique spect VRADAP E TEESDL A DKDGVV L	E GN=P4HB PE= tra,13 total spec E E E D H V L A G G Y G V R L F K K F D E	1 \$V=2 tra, 32:464 a VLR K GYP T GRN N	mino aci S N F /	A E A L F R N GI E V T K	verage) A.A. HKYL D.T. A.S.P.K E.N. LLD.F.	EYTGOV	ESDSA	FTE	L Q Q T	AAEAPK	A L L	GGEI	P
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Protein Securica: Senter P HT5234, HUMANI(Protein disuttide Desclustive unique M L R R A L L K A E G S E I G I T S N S O T H I L L P L T K Y K P E S	otem Spectrum Spectru 100%, 52,503.8 Da isomerase Officient as peptidet, 3 exclu CLA VAAL RLA KVDA VFS KYOL PKS VSOL PKS TAER	VRADAP E TEESDL / DKDGVV I DGKLSN /	E GN=P4HB PE= tra,13 total spec E E E D H V L A G G Y G V R L F K K F D E	180-2 tra, 02-464 з VLR К GYP N GRN N FKG К КРН L	mino aci SNF IKFI	AEAL FRNGI EVTK IFID ELPEI	AA HKYL DT ASPK EN LLDP SD HTDN W DK OP	EYTGOV	ESDSA PLVIE FGLKK	FTE	D E	A A E A P K V R L K N	A L L L F L L T V F	GGEI	H

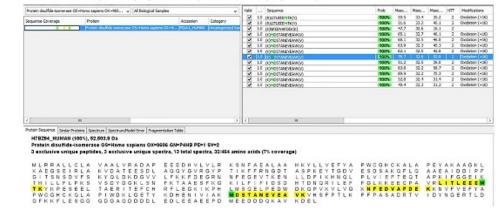


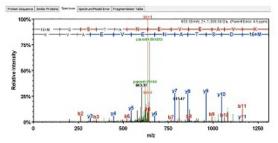
Pro	otein Sequenc	æ	Similar F	roteins	Spectrum	Spe	ectrum/Ma	odel Error	Fragmentati	on Table		
з	B Ions	84	24	B-NH3	8-H20		AA	Y Jons	Y+2H	Y-NH3	Y+H2O	Y
1	148.0						M+16	1,309.6	655.3	1,292.6	1,291.6	12
2	263.1				245	i.1	D	1,162.6	581.8	1,145.5	1,144.5	11
3	350.1				332	2.1	s	1,047.5	524.3	1,030.5	1,029.5	10
4	451.1				433	3.1	т	960.5	480.8	943.5	942.5	9
5	522.2				504	1.2	A	859.5	430.2	842.4	841.4	8
6	636.2	;	318.6	619.	2 618	3.2	N	788.4	394.7	771.4	770.4	7
7	765.3		383.1	748.	2 747	.3	E	674.4	337.7	657.3	656.4	6
8	864.3	- 4	432.7	847.	3 840	i.3	v	545.3	1	528.3	527.3	5
9	993.4		497.2	976.	4 975	i.4	E	446.3		429.2	428.3	4
10	1,064.4		532.7	1,047	.4 1,04	6.4	A	317.2	1.00	300.2		3
11	1,163.5	1	582.2	1,146	.5 1.14	5.5	v	246.2		229.2		2
12	1.309.6		355.3	1.292	.6 1.29	1.6	K	147.1		130.1		1





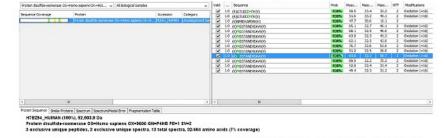
Pre	stein Sequen	ce Smlar	Proteins Sp	sectrum S	Spectrum/M	odel Error	Fragmentab	on Table		
в	B Jons	B+2H	B-NH3	8(H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	γ
1	148.0				M+16	1,309.6	655.3	1,292.6	1,291.6	12
2	263.1			245.1	D	1,162.6	581.8	1.145.5	1,144.5	11
3	350.1			332.1	S 5	1,047.5	524.3	1,030.5	1,029.5	10
4	451.1			433.1	T	960.5	480.8	943.5	942.5	9
5	522.2			504.2	2 A	859.5	430.2	842.4	841.4	8
6	636.2	318.6	619.2	618.2	N	788.4	394.7	771.4	770.4	7
7	765.3	383.1	748.2	747.3	E	674.4	337.7	657.3	656.4	6
8	864.3	432.7	847.3	846.3	V V	545.3		528.3	527.3	5
9	993.4	497.2	976.4	975.4	E	446.3		429.2	428.3	4
10	1.064.4	532.7	1.047.4	1,046.	4 A	317.2		300.2		3
11	1,163.5	582.2	1,146.5	1,145.	5 V	246.2		229.2		2
12	1,309.6	655.3	1,292.6	1,291.	6 K	147.1		130.1		1



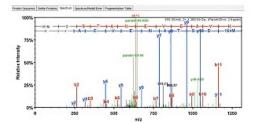


	n Table	"ragmentatio	del Error	ctrum/Mo	rum Sp	Spec	Proteins	Similar P	Sequence	roð
Y-H2O	Y-NH3	Y+2H	Y Jons	AA	H20		B-NH3	+2H	na Bi	1
1,291.6	1.292.6	655.3	1.309.6	M+16					8.0	
1,144.5	1,145.5	581.8	1,162.6	D	245.1				53.1	
1,029.5	1,030.5	524.3	1,047.5	s	332.1				50.1	1
942.5	943.5	480.8	960.5	т	433.1				51.1	
841.4	842.4	430.2	859.5	A	504.2				2.2	
770.4	771.4	394.7	788.4	N	618.2	2	619.	318.6	6.2	
656.4	657.3	337.7	674.4	E	747.3	2	748.	383.1	5.3	
527.3	528.3		545.3	v	846.3	3	847.	432.7	\$4.3	
428.3	429.2		446.3	E	975.4	4	976.	497.2	3.4	
	300.2		317.2	A	1.046.4	.4	1.047	532.7	64.4	
	229.2		246.2	٧	1.145.5	1.5	1.146	582.2	63.5	1
	130.1		147.1	ĸ	1,291.6	2.6	1,292	655.3	09.6	2

Unique Peptide Search: PDIs (three peptide sequences; 13 peptides)

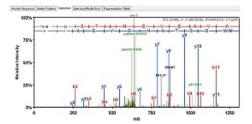


MIRRALUCIA VAALVRADAP EEEDHVLVLR KSNFAEALAA HKYLLVEFYA PWCOHCKALA PEYAKAAOKL KAAGSEIRIA KVOATEESDI AGGYGVEGYP TIKFFRNGGT ASPKEYYGDV ESDSAKGFIG AAFAIDDIPF DITSNSDYFS KYGLDKDGVV FFKKFDEGRN NFEGEVTKEN LLDFIKHNGL PLVIEFTEGT APKIFGETK THILFLPKS VSDVDGKLSN FKTAAESFKG KILFIFIDSD HTDNGRIEF FOLKKEECPA VRITLEEF TKYKPESEL TAERITEFCK RFLEGRIKPH LMSGELPEDW DOPVKVLVG KMFEDVAFGC KKNVYFYFYA PWCGHCKGLA PIWDKLGETY KDHENIVIAK MOSTANEVYAK VKVHSPTLK FPPASADRTV IDVNGERTLD GFKKFLESOG QDARAODODDL

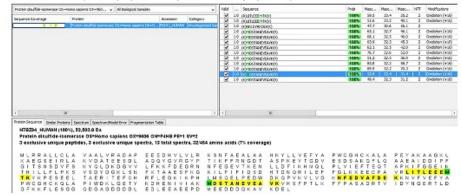


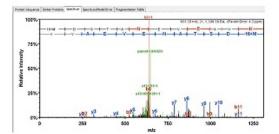
Pro	otein Sequenc	e S	mlar P	roteins	Spectrum	Spe	ctrum/Mi	del Error	Fragmentab	on Table		
8	8 Ions	0+2	1	8-1413	8-1120	,	AA	Y Jons	Y+21	Y-1913	Y-1120	Y
1	148.0						M+16	1.309.6	655.3	1.292.6	1.291.6	12
2	263.1				24	5.1	D	1,162.6	581.8	1,145.5	1,144.5	11
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в	864.3	43	2.7	847.	3 846	5.3	v	545.3	1	528.3	527.3	5
9	993.4	49	72	976.	4 97	5.4	E	446.3	1	429.2	428.3	4
10	1.064.4	- 53	2.7	1.047	.4 1.04	6.4	A	317.2	10.00	300.2		3
11	1,163.5	-58	2.2	1,146	.5 1,14	5.5	v	246.2	1.0	229.2		2
12	1,309.6	65	5.3	1,292	.6 1,29	1.6	к	147.1		130.1		1

Prater: double-kanesaar 5	All v	Bulugical Samples		v Veld				Photo	Mer	Max		PET	Nodif	
leguence Concerner	Prettin	Accession	Grapsy	NXX N	1.6 dourner			100%	59.5	33.4	35.2	2		67 (4)
editive Care also				×.	L0 (OLTER			100%	51.5	35.2	40.1	5	Guides	ion Cali
	Proton-daulto: bonenase Q2+	Home suggery GANON PERAL HUR	GRE (Unestopped		1.0 (09940)			100%	45.7	30.8	16.1			
				2	1.0 004057			100%	63.5	21.7	40.1	T		ion (+5
				8	1.0 00HDST			100%	68.5	32.5	46.8	- 2		07 (+)
				N S	L8 compar			100%	63.9	32.3	45.3	5	Chester	
				1	LE (c)epse			10005	83.5	32.5	42.8	- 2	Oxidet	
				M	1.0 (c)rectri			100%	26.7	32.h	32.8	- 2		kini (+1
				X	1.0 00-057			100%	51.2	31.5	36.8	1	Chocad	
				8	1.0 00H057			100%	93.9	32.2	96.7	2	Oxeces	
					LA DO DER			1025		32.2	203	1 2	Owner	84(4)
				N N	L0 (c)-pin	WMM.MID(V)		100%	52.5	32.4	31.4	- 2	Guide	
				N N	1.0 004007	AND CAREGO		100%	48.4	22.3	31.2	1	Coded	ion (+)
t International Terration	-			2 6		-								
HORDA HUMAN (Protein disuttide-		ns alt=seae alt=P4HB PI			ids (7% cov									
HORDAL HUMAN (Protein disuttide- bisclusive uniqui MILRRALL	olem (gentus (gentus/hold in foch), 62,600,805 Isomerase C&Homa capie is peptidet, 9 eachtive or O L A V A A L V R A	ns otraste dar Pena P ique spectra, 13 total sp DAP EEEDHV	ectra, 3246 LVLR	kamino aci KSNP	AEALA	eragej La Hryel		wcgh			PEY			
HORDAL HUMAN (Protein disuttate Disclusive unique MILRRALL KAEGSCI	iden Spectra Spectra Modifi Iden (2,560,80) Idenerate Contents capie a peptider, 3 acclusive un O LA VAALVRA R LA K VOATEE	na Otroleo a Morrena Pr lique spectra, 13 total sp DAP EEEDH V SDL ACOVGV	ectra, 2246 LVLR RGVP	K S N P T I K F	AEALA	eragej A HRYLL IT ASPKE	YTGDV D	SDSA	KQF	LQ	AAE	A.L	D D I	PF
HORDAL HUMAN (Protein disutide Sectodive unique M L R R A L L K A E G S C I G I T S N S D	iden Spectra Spectra Modifi Iden (2,560,80) Idenerate Contents capie a peptider, 3 acclusive un O LA VAALVRA R LA K VOATEE	ns 00 = 9606 0 M Pohl P lique spectra, 13 total sp DAP EEEDH V SDL AOOYGV GVV LFKKFD	EVLR RGVP EGRN	K SNF TIKF NFEG	AEALA FRNGD EVTKE	A HRYLL T ASPKE N LLOFI	NTGDV E	SDSA	KOF	L Q Q T		A.L	D D I	PF
HORDAL HUMAN (Protein dikutide- Desclutive unique M L R R A L L K A E G S C L G I T S N S D	iden Spectra Spectra Modifi Iden (2,560,80) Idenerate Contents capie a peptider, 3 acclusive un O LA VAALVRA R LA K VOATEE	nt 00 * 9606 0 M Pohle Pr igue spectra, 13 total sp DAP EEEDH V SDL AOOYGV GVV LFKKFO	EVLR RGVP EGRN	K S N P T I K F	AEALA FRNGD EVTKE	erage) A HKYLL T ASPXE N LLOFI	NTGDV E	SDSA	KOF	L Q Q T	AAE	A I I F	GGE	I K
HORDAL HUMAN (Protein disuttate Sectorive unique M L R R A L L K A E G S C I G I T S N S D T H I L L F L	International Spectrum (Spectrum (Sp	ns 00 = 9606 0 M Pohl P lique spectra, 13 total sp DAP EEEDH V SDL AOOYGV GVV LFKKFD	EVLR RGVP EGRN	K SNF TIKF NFEG	AEALA FRNGD EVTXE IFIOS	eragei A HKYLL T ASPKE N LLOFI D HTONG	NTGDV E KHNQL P RILEF F	SDSA	K Q F F T E E E C		AAEAPK	AI IF		PP
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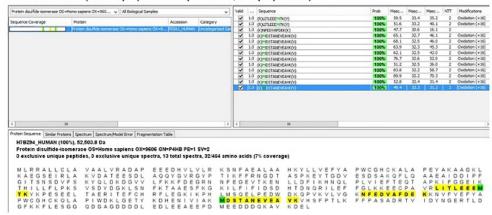


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2	263.1			245	.1 D	1.162.6	681.8	1.145.5	1.144.5	11
3	350.1			332	.1 S	1.047.5	524.3	1.030.5	1.028.5	10
4	451.1			433	.1 т	960.5	480.8	943.5	942.5	9
5	522.2			504	.2 A	859.5	430.2	842.4	841.4	8
6	636.2	318.6	619.3	2 618	.2 N	788.4	394.7	771.4	770.4	7
7	765.3	383.1	748.3	2 747	.3 E	674.4	337.7	657.3	656,4	6
8	864.3	432.7	847.	3 846	13 V	545.3		528.3	527.3	5
9	993.4	497.2	976/	4 975	iA E	446.3		429.2	428.3	4
10	1,064.4	532.7	1,047	4 1.84	6.4 A	317.2		300.2		3
11	1.163.5	582.2	1,145	5 1.14	5.5 V	246.2		229.2		2
12	1.309.6	655.3	1.292	.6 1.29	1.6 K	147.1		130.1		1

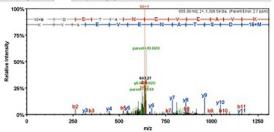




dein Sequer	ice.	Similar	Proteins	Spectrum	Spectru	n/M	del Error	Fragmentad	on table		
6 Jons	04	-211	0-1413	04120	AA		Y Jons	Y+2H	Y-1013	Y-H20	۲
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263.1				245	1 1	D	1,162.6	581.8	1.145.5	1.144.5	11
350.1				332	4 1	5	1,047.5	524.3	1,030.5	1,029.5	10
451.1				433	4	T	960.5	480.8	943.5	942.5	9
522.2				504	2 1	A	859.5	430.2	842.4	841.4	8
636.2		318.6	619.	2 618	2 1	M	788.4	394.7	771.4	770.4	7
765.3		383.1	748.	2 747	3	E	674.4	337.7	657.3	656.4	6
864.3		432.7	847.	3 840	3 1	v	545.3		528.3	527.3	5
993.4		497.2	976.	4 975	4	t	446.3		429.2	428.3	4
1.064.4		532.7	1.047	4 1.04	6.4	A.	317.2		300.2		3
1,163.5		582.2	1,146	.5 1,14	5.5	v	246.2		229.2		Z
1,309.6	1	655.3	1.292	.6 1.29	1.6	ĸ	147.1		130.1		1
	8 Jons 148.0 263.1 350.1 451.1 522.2 636.2 765.3 864.3 993.4 1.064.4 1,163.5	B Jons B- 148.0 263.1 350.1 451.1 522.2 636.2 765.3 864.3 993.4 1.084.4 1.084.4	B lons B+21 148.0 263.1 330.1 451.1 522.2 666.2 666.2 318.6 765.3 303.1 864.3 432.7 903.4 437.2 1.064.4 532.7 1.463.5 582.2	B Jons B+2H B+2H B+2H3 148.0 263.1 350.1 451.1 522.2 765.3 303.1 748. 564.2 318.6 649. 765.3 303.1 748. 564.3 1.064.4 532.7 1.064.4 532.7 1.047 1.064.4 532.7 1.047 1.064.4 532.7 1.047 1.064.5 532.7 1.047 1.065.2 1.047	B Jons B +2h B +2h B +8h3 C +120 148.0 263.1 245 350.1 352 350.1 352 350.1 352 451.1 453 562.2 504 504 504 504 504 562.2 318.6 619.2 618 619.2 614 503.3 303.1 748.2 747 846 903.4 497.2 976.4 975 1.064.4 532.7 1.047.4 1.04 51.4 51.4 51.4	B Jons B+2H D+N13 D+123 AA 148.0 M- M- M- M- 263.1 245.1 332.1 S0.1 332.1 S0.1 332.1 S0.1 S0.2 S0.4 AA S0.3 S0.3 TAR.2 F47.3 B46.3 S0.4 A7.3 B46.3 S0.3 S0.3 S0.4 A97.2 976.4 375.4 S0.4 S0.4 S0.2 T.146.5 T.144.5 T.144.5 TA44.5 S0.4 S0.2 S0.4 S0.2 S0.4 A45.5 T.144.5 S0.4 S0.4 S0.2 S0.4 S0.2 S0.4 S0.2 S0.4 S0.2 S0.4 S0.2 S0.4<	B Jons B+21 D+N13 D+120 AA 148.0 M+16 M+16 M+16 263.1 245.1 D M+16 350.1 332.2 S S 451.1 433.1 T S 566.2 318.6 619.2 648.2 N 765.3 303.1 748.2 747.3 E 864.3 497.2 976.4 975.4 F 1.064.4 532.7 1.044.5 1.044.5 X	B Jons B+21 D+N13 B+120 AA Y Jons 148.0 M+16 1,309.6 263.1 245.1 D 1,162.6 350.1 332.1 S 1,047.5 511.1 433.1 T 969.5 522.2 504.2 A 859.5 536.5 318.6 619.2 648.2 N 788.4 765.3 303.1 748.2 747.3 E 674.4 854.3 497.2 976.4 975.4 E 446.3 1,064.4 532.7 1,047.4 1,048.4 A 317.2 1,163.5 582.2 1,445.5 1,445.5 V 246.3	B Jons B+2ri D-1413 D-123 AA Y Jons Y+2ri 148.0 M+16 1,309.6 655.3 561.8 561.9 263.1 245.1 D 1,162.6 561.9 561.9 350.1 332.1 5 1,047.5 524.2 541.9 565.3 502.2 504.2 A 859.5 430.2 563.6 302.1 337.7 564.2 318.6 619.2 618.2 M 788.4 394.7 765.3 303.1 748.2 747.3 L 674.4 337.7 903.4 497.2 975.4 975.4 446.3 1.044.4 1.046.4 A 317.2 1.163.15 582.21 1.045.4 1.045.4 A 317.2 1.163.15 582.21 1.445.5 1.442.3 Y 446.3 1.163.15 1.445.21 Y 246.2 246.2 1.445.3 1.445.3 Y 246.2 1.445.3 Y 246.2 1.445.3	148.0 M+16 1,309.6 655.3 1,292.6 203.1 245.1 D 1,162.6 691.8 1,145.5 350.1 352.1 S 1,047.5 524.3 1,030.5 4561.4 1,030.5 451.1 433.1 7 960.5 480.8 943.5 522.2 504.2 A 859.5 430.2 842.4 636.2 318.6 619.2 618.2 N 788.4 394.7 771.4 765.3 303.1 748.2 747.3 E 674.4 337.7 657.3 804.3 497.2 976.4 975.4 £ 446.3 429.2 1,064.4 532.7 1.047.4 1.046.4 A 317.2 300.2 1,064.4 532.7 1.047.4 7.462.2 229.2 300.2 229.2	B Jons D+2H D+NH3 D+H2D AA Y Jons Y+2H Y4H1 Y1H2 148.0 M+36 1,309.6 655.3 1,292.6 1,291.6 283.1 245.1 D 1,162.6 561.3 1,292.5 1,291.6 350.1 332.1 S 1,447.5 524.3 1,145.5 541.4 1,030.5 1,029.5 451.1 433.1 T 860.5 400.8 943.5 942.5 562.2 504.2 A 859.5 430.2 842.4 841.4 616.2 318.6 619.2 648.2 A 788.4 394.7 71.4 770.4 765.3 303.1 748.2 747.3 C 674.4 337.7 657.3 657.4 8943.3 427.7 846.3 V 546.3 428.2 428.3 1,064.4 632.7 1,047.4 1,048.4 A 317.2 300.2 1,145.5 1,165.5 1,414.5.5 <



Protein Sequence Similar Proteine Solicitrum Spectrum/Hodel Error Pragmentation Table



Pro	tein Sequer	ce	Similar P	roteins	Spectrum	Spe	ctrum/Mo	del Error	Fragmentati	on Table		
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1	148.0						M+16	1.309.6	655.3	1.292.6	1.291.6	12
2	263.1				245	.1	D	1,162.6	581.8	1,145.5	1,144.5	11
3	350.1				332	.1	5	1,047.5	524.3	1,030.5	1,029.5	10
4	451.1				433	.1	т	960.5	480.8	943.5	942.5	9
5	522.2				504	.2	A	859.5	430.2	842.4	841.4	8
6	636.2		318.6	619.	2 618	.2	N	788.4	394.7	771.4	770.4	7
7	765.3		383.1	748.	2 747	.3	E	674.4	337.7	657.3	656.4	6
8	864.3		432.7	847.	3 846	.3	V	545.3		528.3	527.3	5
9	993.4		497.2	976.	4 975	.4	E	446.3		429.2	428.3	4
10	1,064.4		532.7	1,047	.4 1,04	6.4	Α	317.2		300.2		3
11	1,163.5		582.2	1,146	.5 1.14	5.5	٧	246.2		229.2		2
12	1,309.6		655.3	1,292	.6 1.29	1.6	ĸ	147.1		130.1		1

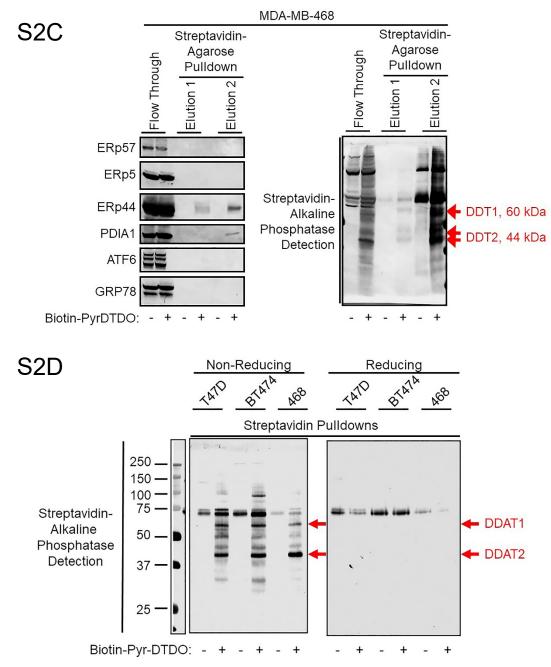
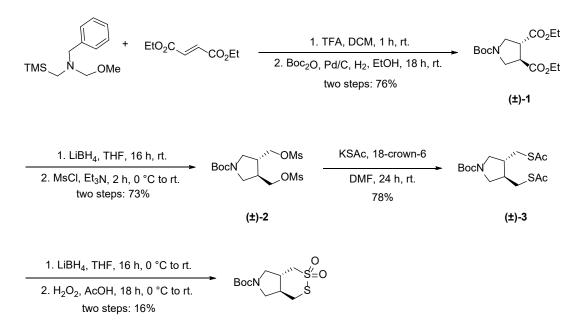


Fig. S2-Confirmation of ERp44 and PDIA1 as DDA targets. S2A. Peptide coverage of ERp44, and S2B. Peptide coverage of PDIA1 identified in Biotin-PyrDTDO/Streptavidin-Agarose pulldowns from MDA-MB-468 cells by tandem mass spectrometry. S2C. Streptavidin-Agarose pulldowns of Biotin-PyrDTDO treated cells in which the flow through, elution one (250 μ M dMtcyDTDO + 100 μ M 2-Mercaptoethnol), and elution 2 (2X SDS-PAGE Laemmli sample buffer + 2 mM Biotin) were analyzed by immunoblot and Streptavidin-Alkaline Phosphatase detection. S2D. Streptavidin-Agarose pulldowns of Biotin-PyrDTDO treated cells performed in the presence of 100 mM NEM. Half of each sample was brought to 1 M 2-mercaptoethanol and boiled for 20 minutes to generate reduction samples. These non-reducing (left panel) or reducing samples (right panel) were analyzed by blotting with Streptavidin-Alkaline Phosphatase. Molecular weight markers are indicated in kiloDaltons.

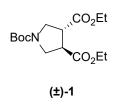
General Methods

Reagents and solvents were purchased from commercial sources and used without further purification unless otherwise specified. Anhydrous solvents were obtained using a commercial solvent drying system (using activated alumina for THF, diethyl ether, dichloromethane; molecular sieves for DMF) and transferred via syringe to flame-dried glassware that had been cooled under an argon atmosphere. ¹H and ¹³C NMR spectra were recorded using commercially-obtained deuterated solvents on Bruker-600 (¹H at 600 MHz; ¹³C at 151 MHz) and Varian Inova-500 (¹H at 500 MHz; ¹³C at 125 MHz) spectrometers. Chemical shifts (δ) are given in parts per million (ppm) relative to TMS and referenced to residual protonated solvent (CDCl₃: δ_H 7.26 ppm, δ_C 77.16 ppm; CD₃OD: δ_H 4.87 ppm, δ_C 49.00 ppm; DMSO-*d*₆: δ_H 2.50 ppm, δ_C 39.52 ppm; D₂O: δ_H 4.79 ppm). Coupling constants (*J*) are reported in Hz. Spin multiplicities are presented by the following symbols: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quartet), p (pentet), and m (multiplet). Electrospray ionization (ESI) high-resolution mass spectra (HRMS) were recorded on an ESI-TOF instrument, operating in positive mode as stated, with methanol as the carrier solvent otherwise as mentioned.

Synthesis of (±)-BocPyrDTDO:



1-(tert-Butyl)-3,4-diethyl-(3S,4S)- and (3R,4R)-pyrrolidine-1,3,4-tricarboxylate, (±)-1



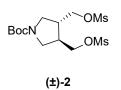
Intermediate (±)-1 was synthesized following a procedure described in a patent.^{1a} To a stirred solution of diethyl fumarate (3.8 mL, 23 mmol) in DCM (50 mL) at 0 °C was added *N*-benzyl-1-methoxy-*N*-((trimethylsilyl)methyl)methanamine (5.4 mL, 21 mmol) followed by dropwise addition of a solution of TFA in DCM (0.1 mL of TFA in 1 mL of DCM) over 10 min. After completion of addition, the cold bath was removed, and the reaction mixture was stirred at rt for 1 h. The reaction mixture was diluted with DCM (20 mL), washed with a saturated solution of sodium bicarbonate (20 mL), and the organic layer was dried over

Na₂SO₄ and concentrated under reduced pressure. The residue was dissolved in ethyl acetate (25 mL) and to this was added Boc₂O (8.00 g, 36.6 mmol) followed by 10% palladium on carbon (1 g, wet). The resulting reaction mixture was subjected to catalytic hydrogenation using hydrogen gas for 18 h with stirring. The catalyst was filtered through a pad of celite, and the filtrate was concentrated. The residue was purified by flash silica gel column chromatography (0–25% EtOAc/hexanes) to provide the Boc-protected diester (±)-1 (5.1 g, 16 mmol, 76% yield) as a pale-yellow oil. The ¹H NMR data matches the literature.¹

¹**H NMR** (CDCl₃, 500 MHz): δ 4.16 (q, *J* = 7.1 Hz, 4H), 3.78–3.69 (m, 2H), 3.57–3.44 (m, 2H), 3.42–3.29 (m, 2H), 1.45 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 6H).

¹³**C NMR** (CDCl₃, 125 MHz): δ 171.81, 154.02, 80.02, 61.44, 48.12, 48.02, 46.12, 45.39, 28.54, 14.23.

tert-Butyl (3S,4S)- and (3R,4R)-3,4-bis(((methylsulfonyl)oxy)methyl)pyrrolidine-1-carboxylate, (±)-2



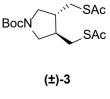
To an ice-cooled solution of the diester (\pm) -1 (5.0 g, 16 mmol) in anhydrous THF (135 mL) was added dropwise a solution of LiBH₄ in THF (16 mL of a 2.0 M LiBH₄ solution in THF diluted with an additional 20 mL of anhydrous THF). The reaction mixture was stirred overnight at rt and quenched with an aqueous 2 M NaOH solution. Ether was added, the layers were separated, and the aqueous phase was back extracted twice with ether. The combined organic extracts were dried over Na₂SO₄, filtered, and concentrated to give the diol which was used without further purification. To a stirred solution of the diol in DCM

(110 mL) cooled to 0 °C was added triethylamine (11 mL, 80 mmol) followed by methanesulfonyl chloride (3.1 mL, 40 mmol). The reaction mixture was stirred at 0 °C for 15 min, then at rt for 1.5 h. The reaction mixture was poured into water (76 mL) and the phases were separated. The aqueous phase was extracted with DCM (2 × 76 mL). The combined organics were dried (MgSO₄), filtered, and concentrated. The residue was purified by flash chromatography (0–100% EtOAc/DCM) to afford the desired dimesylate (±)-2 (4.5 g, 12 mmol, 73% yield) as a white solid.²

¹H NMR (CDCl₃, 500 MHz): δ 4.25 (m, 4H), 3.65 (m, 2H), 3.24 (m, 2H), 3.05 (s, 6H), 2.53 (m, 2H), 1.46 (s, 9H).

¹³C NMR (CDCl₃, 125 MHz): δ 154.24, 80.23, 68.86, 47.95, 47.46, 40.52, 39.42, 37.70, 28.58.

tert-Butyl-(3S,4S)- and (3R,4R)-3,4-bis((acetylthio)methyl)pyrrolidine-1-carboxylate, (±)-3



A mixture of the dimesylate (±)-2 (7.8 g, 20 mmol), KSAc (4.6 g, 40 mmol), and 18-crown-6 (1.3 g, 5.0 mmol) in anhydrous DMF (392 mL) was stirred for 24 h at rt. After completion of the reaction, water (392 mL) was added and the mixture was extracted with EtOAc (3 × 392 mL). The combined organic phases were washed with water (392 mL) and brine (392 mL), dried (Na₂SO₄), concentrated, and purified by column chromatography (0–10% DCM/EtOAc) to afford the dithioacetate (±)-3 (5.4 g, 16 mmol, 78% yield) as a colorless oil.

¹**H NMR** (CDCl₃, 500 MHz): δ 3.69–3.50 (m, 2H), 3.20–3.09 (m, 2H), 3.09–2.93 (m, 2H), 2.85 (dd, *J* = 13.7, 7.3 Hz, 2H), 2.34 (s, 6H), 2.22–2.05 (m, 2H), 1.44 (s, 9H).

¹³**C NMR** (CDCl₃, 125 MHz): δ 195.26, 154.37, 79.63, 50.64, 50.29, 43.13, 42.40, 30.74, 30.65, 28.60.

HRMS (ESI-TOF): Calculated for [C₁₅H₂₅NO₄S₂ + Na]⁺: 370.1117; found: 370.1104.

tert-Butyl-(4a*S*,7a*S*)- and (4a*R*,7a*R*)-tetrahydro-1*H*-[1,2]dithiino[4,5-*c*]pyrrole-6(4*H*)-carboxylate 2,2-dioxide, (±)-BocPyrDTDO

(±)-BocPyrDTDO

To an ice-cooled solution of the dithioacetate (±)-3 (5.42 g, 15.6 mmol) in anhydrous THF (132 mL) was added dropwise a solution of LiBH₄ in THF (7.8 mL of 4.0 M LiBH₄ in THF diluted with additional 27 mL of anhydrous THF). The reaction mixture was stirred overnight at rt and quenched with an aqueous 2 M NaOH solution. Ether (25 mL) was added, the layers were separated, and the aqueous phase was back extracted with ether (2 × 25 mL). The combined organic extracts were dried over Na₂SO₄, filtered, and

concentrated to give the crude dithiol (3.81 g, 14.5 mmol, 93% yield), which was used without further purification for the next step. A solution of the crude dithiol (1.91 g, 7.23 mmol) in AcOH (9.0 mL) was cooled in an ice bath and a solution of H_2O_2 in water/AcOH (2.59 mL of 30% H_2O_2 in water diluted with 3.0 mL of AcOH) was added slowly such that the reaction temperature did not rise above 35 °C. After stirring for 18 h, the solvent was removed under vacuum, and the residue was diluted with water (15 mL), neutralized with NaHCO₃, and extracted with EtOAc (3 × 50 mL). The organic extract was dried with Na₂SO₄, filtered, and concentrated under vacuum. The crude material was purified by column chromatography (0–100% EtOAc/DCM) to afford the desired (±)-BocPyrDTDO (0.66 g, 2.25 mmol, 16% yield).

¹**H NMR** (CDCl₃, 500 MHz): δ 3.75 (m, 1H), 3.67 (m, 1H), 3.52 (m, 1H), 3.41 (t, *J* = 12.6 Hz, 1H), 3.33 (t, *J* = 12.6, 1H), 3.14 (m,1H), 3.07 (t, *J* = 10.8 Hz, 1H), 3.01 (t, *J* = 10.8 Hz, 1H), 2.8–2.67 (m, 1H), 2.40–2.28 (m, 1H), 1.45 (s, 9H).

¹³**C NMR** (CDCl₃, 125 MHz): δ 154.09, 80.27, 62.62, 62.50, 50.16, 49.76, 49.63, 49.19, 43.14, 43.01, 42.44, 42.36, 35.43, 35.30, 28.55.

HRMS (ESI-TOF): Calculated for [C₁₁H₁₉NO₄S₂ + Na]⁺: 316.0648; found: 316.0646.

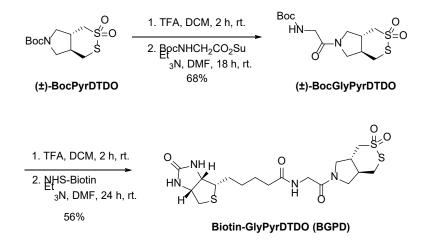
Slow *s*-cis/*s*-trans interconversion with respect to the *N*-acylpyrrolidine ring³ produces two conformational diastereomers and additional NMR signals at room temperature.

The interconversion is fast on the NMR timescale at 75 °C in DMSO-d₆:

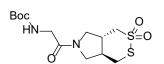
¹**H NMR** (DMSO-*d*₆, 500 MHz) at 75 °C: δ 3.80 (dd, *J* = 13.1, 2.8 Hz, 1H), 3.63–3.53 (m, 3H), 3.43 (dd, *J* = 13.5, 2.9 Hz, 1H), 3.32 (dd, *J* = 13.5, 11.4 Hz, 1H), 3.03 (t, *J* = 10.6 Hz, 1H), 2.97 (t, *J* = 10.6 Hz, 1H), 2.65–2.53 (m, 1H), 2.41–2.31 (m, 1H), 1.43 (s, 9H).

¹³**C NMR** (DMSO-*d*₆, 125 MHz) at 75 °C: *δ* 153.87, 79.12, 62.83, 50.04, 49.58, 42.78, 42.01, 35.55, 28.70. High temperature 1D and 2D NMR spectra for this compound are included.

Synthesis of BioGlyPyrDTDO (BGPD):



tert-Butyl-(2-((4aS,7aS)- and (4aR,7aR)-2,2-dioxidotetrahydro-1*H*-[1,2]dithiino[4,5-*c*]pyrrol-6(4*H*)-yl)-2-oxoethyl)carbamate, (±)-BocGlyPyrDTDO



A solution of (±)-BocPyrDTDO (0.10 g, 0.34 mmol) was stirred in TFA:DCM (3.4 mL, 1:1) for 2 h, at rt. The reaction mixture was then concentrated under vacuum to provide the deprotected ammonium trifluoroacetate salt **HPyrDTDO.TFA** as a brown solid, which was used in the next step without further purification.

BOCGLYPYIDTOO 1H NMR (DMSO- d_6 , 500 MHz): δ 9.25 (bs, 2H), 3.91 (dd, J = 13.0, 2.9 Hz, 1H), 3.68 (t, J = 12.6 Hz, 1H), 3.53–3.42 (m, 3H), 3.33 (dd, J = 13.6, 11.5 Hz, 1H), 2.95 (tq, J = 13.4, 6.6 Hz, 2H), 2.62–2.53 (m, 1H), 2.40–2.30 (m, 1H).

¹³**C NMR** (DMSO-*d*₆, 125 MHz): δ 60.97, 47.55, 47.30, 41.98, 40.64, 33.97.

To the solution of the resulting solid in DMF (4.8 mL) was added Et_3N (0.10 mL, 0.68 mmol) and 2,5dioxopyrrolidin-1-yl (*tert*-butoxycarbonyl)glycinate (0.09 g, 0.34 mmol), respectively, and the reaction mixture was stirred at rt for 24 h. After this time the solvent was evaporated, and the crude product was purified by flash chromatography (0–1% MeOH/DCM) to afford (±)-BocGlyPyrDTDO (0.08 g, 0.23 mmol 68%) as a viscous white solid.

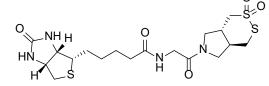
¹**H NMR** (CDCl₃, 500 MHz): δ 5.38 (s, 1H), 4.01–3.95 (m, 1H), 3.93–3.79 (m, 2H), 3.74 (dt, *J* = 16.0, 8.5 Hz, 1H), 3.58 (t, *J* = 13.9 Hz, 1H), 3.48–3.34 (m, 2H), 3.26–3.04 (m, 3H), 2.9–2.7 (m, 1H), 2.53–2.29 (m, 1H), 1.44 (s, 9H).

¹³**C NMR** (CDCl₃, 125 MHz): *δ* 167.38, 155.92, 80.06, 62.42, 62.14, 49.89, 49.38, 48.94, 43.39, 43.14, 42.96, 42.88, 41.72, 41.60, 35.18, 34.95, 28.46.

HRMS (ESI-TOF): Calculated for [C₁₃H₂₂N₂O₅S₂ + Na]⁺: 373.0862; found: 373.0863.

Slow *s*-cis/*s*-trans interconversion with respect to the *N*-acylpyrrolidine ring³ produces two diastereomers and additional NMR signals (e.g., PyrDTDO ring peak doubling) at room and even elevated (in DMSO- d_6) temperature.

N-(2-((4a*S*,7a*S*)- and (4a*R*,7a*R*)-2,2-Dioxidotetrahydro-1*H*-[1,2]dithiino[4,5-*c*]pyrrol-6(4*H*)-yl)-2-oxoethyl)-5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide, Biotin-GlyPyrDTDO (BGPD)



Biotin-GlyPyrDTDO (BGPD)

A solution of (±)-BocGlyPyrDTDO (0.08 g, 0.23 mmol) was stirred in TFA:DCM (6 mL, 1:2) for 2 h at rt. After this time the reaction was concentrated under vacuum to provide the deprotected ammonium trifluoroacetate salt as a brown oily solid, which was used in the next step without further purification. To a solution of the resulting solid and (+)-biotin *N*-succinimidyl ester (0.08 g, 0.23 mmol) in DMF was added Et₃N (0.06 mL, 0.46 mmol) and the reaction mixture was stirred at rt for 24 h. Then the solvent was evaporated and the crude product was purified by flash chromatography (0–10% MeOH/DCM) to afford **Biotin-GlyPyrDTDO** (0.06 g, 0.13 mmol, 56%) as a white solid.

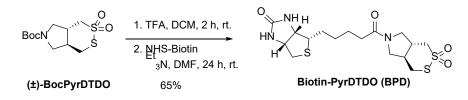
¹**H NMR** (DMSO-*d*₆, 500 MHz) δ 7.94 (s, 1H), 6.41 (s, 1H), 6.35 (s, 1H), 4.33–4.27 (m, 1H), 4.14–4.11 (m, 1H), 3.90–3.68 (m, 5H), 3.64 (td, *J* = 12.9, 2.0 Hz, 1H), 3.46 (ddd, *J* = 13.5, 6.4, 2.8 Hz, 1H), 3.35–3.30 (m, 1H), 3.24–3.17 (m, 1H), 3.11–3.05 (m, 1H), 3.02–2.91 (m, 1H), 2.82 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.69–2.47 (m, 2H), 2.46–2.25 (m, 1H), 2.14 (t, *J* = 7.0 Hz, 2H), 1.65–1.58 (m, 1H), 1.55–1.42 (m, 3H), 1.39–1.25 (m, 2H).

¹³**C NMR** (DMSO-*d*₆, 125 MHz): *δ* 172.31, 166.93, 166.86, 162.68, 61.79, 61.65, 61.00, 59.18, 55.41, 49.25, 48.76, 48.31, 42.72, 41.67, 41.05, 40.87, 40.78, 40.11, 34.94, 34.85, 34.80, 28.14, 28.02, 25.23.

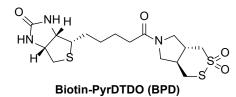
HRMS (ESI-TOF): Calculated for [C₁₈H₂₈N₄O₅S₃ + H]⁺: 477.1295; found: 477.1287.

Slow *s*-cis/*s*-trans interconversion with respect to the *N*-acylpyrrolidine ring³ produces two diastereomers and additional NMR signals (e.g., PyrDTDO ring peak doubling).

Synthesis of Biotin-PyrDTDO (BPD):



(3aS,4S,6aR)-4-(5-((4aR,7aR) and (4aS,7aS)-2,2-dioxidotetrahydro-1H-[1,2]dithiino[4,5-c]pyrrol-6(4H)-yl)-5-oxopentyl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one, Biotin-PyrDTDO (BPD)



A solution of (±)-BocPyrDTDO (0.20 g, 0.68 mmol) was stirred in TFA:DCM (6 mL, 1:1) for 2 h at rt, monitoring by TLC. After reaction completion, the mixture was concentrated under vacuum to provide the deprotected ammonium trifluoroacetate salt as a brown solid, which was used in the next step without further purification. To a solution of the resulting solid and (+)-biotin *N*-succinimidyl ester in DMF was added Et₃N (0.20 mL, 1.40 mmol) and the reaction mixture was stirred at rt for 24 h.

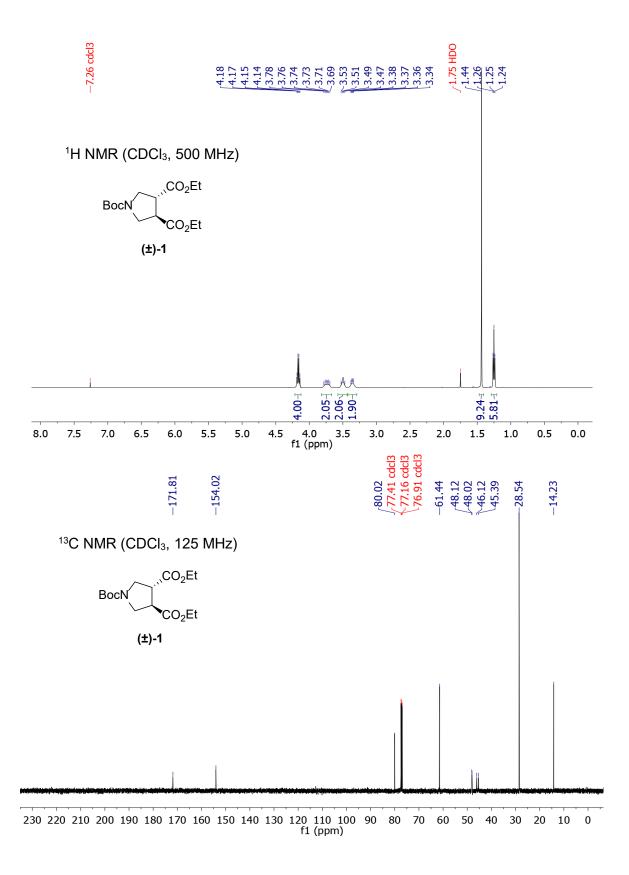
Then the solvent was evaporated and the crude product was purified by flash chromatography (0–10% MeOH/DCM) to afford **Biotin-PyrDTDO** (0.19 g, 0.45 mmol, 65%) as a white solid.

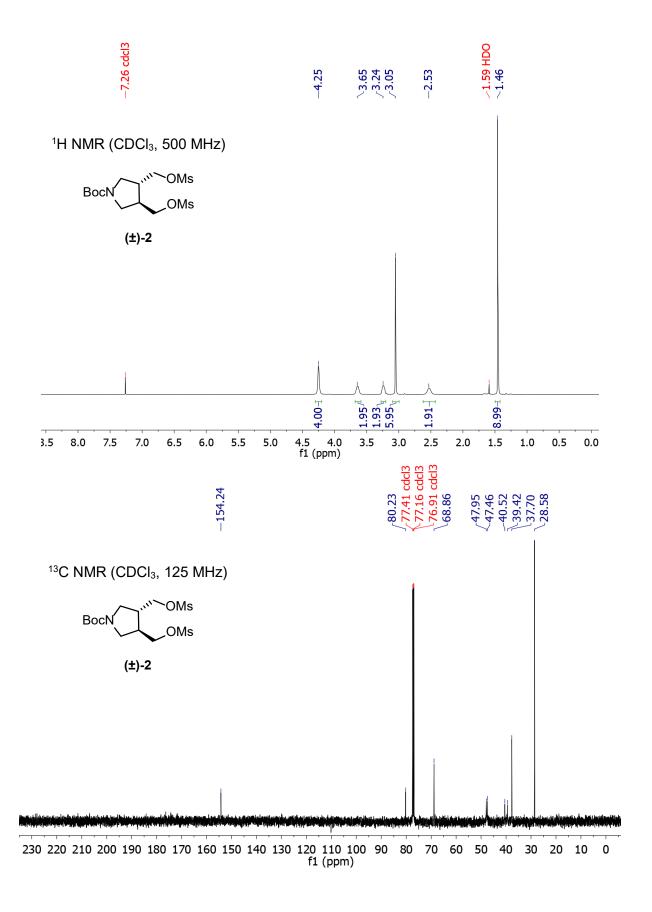
¹**H NMR** (DMSO-*d*₆, 600 MHz) δ 6.44 (s, 1H), 6.36 (s, 1H), 4.31 (dd, *J* = 7.8, 5.0 Hz, 1H), 4.14 (ddd, *J* = 7.1, 4.5, 1.8 Hz, 1H), 3.88–3.82 (m, 1H), 3.76–3.66 (m, 3H), 3.65–3.60 (m, 1H), 3.49–3.40 (m, 1H), 3.28–3.35 (m, 1H), 3.23–3.13 (m, 1H), 3.13–3.07 (m, 1H), 2.99–2.87 (m, 1H), 2.83 (dd, *J* = 12.4, 5.1 Hz, 1H), 2.58 (d, *J* = 12.4 Hz, 1H), 2.67–2.47 (m, 1H), 2.45–2.28 (m, 1H), 2.26–2.13 (m, 2H), 1.68–1.57 (m, 1H), 1.56–1.42 (m, 3H), 1.40–1.27 (m, 2H).

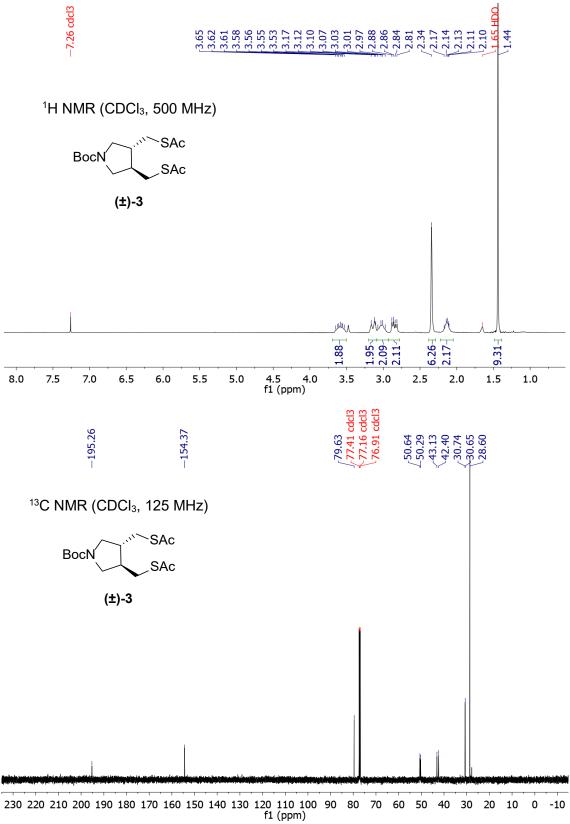
¹³**C NMR** (DMSO-*d*₆, 151 MHz): *δ* 170.42, 170.35, 162.71, 61.89, 61.69, 61.05, 59.18, 55.45, 49.67, 49.22, 49.02, 48.52, 42.70, 41.71, 41.23, 40.34, 39.87, 35.06, 34.86, 33.18, 33.11, 28.29, 28.11, 24.27.

HRMS (ESI-TOF): Calculated for [C₁₆H₂₅N₃O₄S₃ + Na]⁺: 442.0899; found: 442.0899.

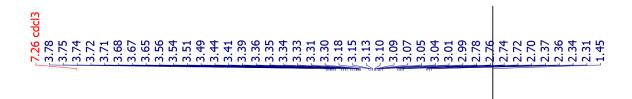
Slow *s*-cis/*s*-trans interconversion with respect to the *N*-acylpyrrolidine ring³ produces two diastereomers and additional NMR signals (e.g., PyrDTDO ring peak doubling).



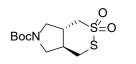




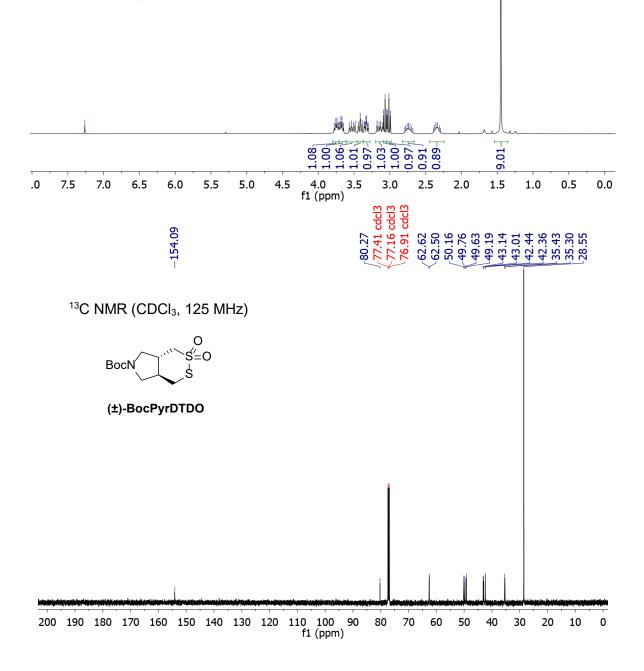


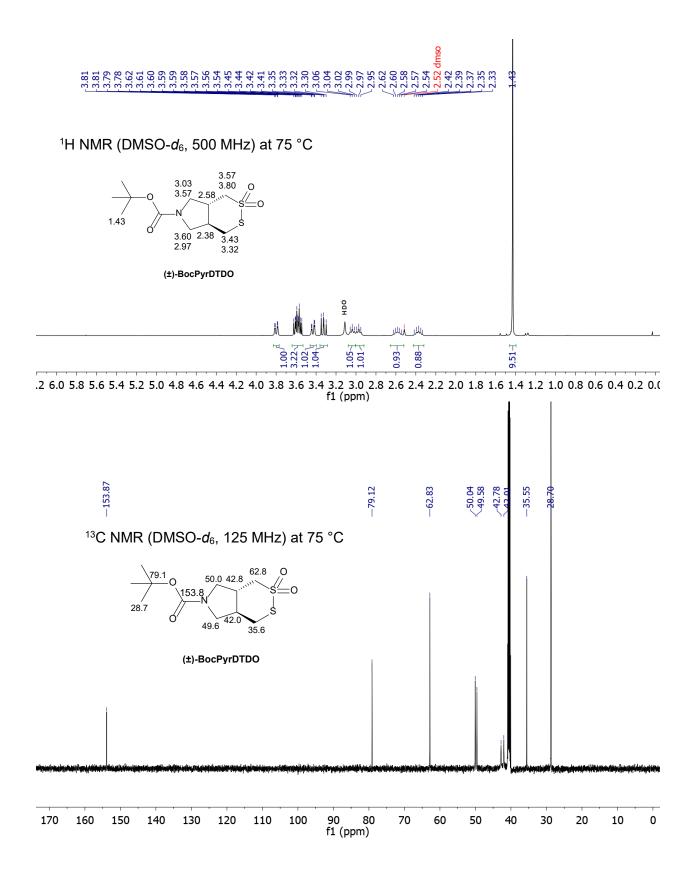


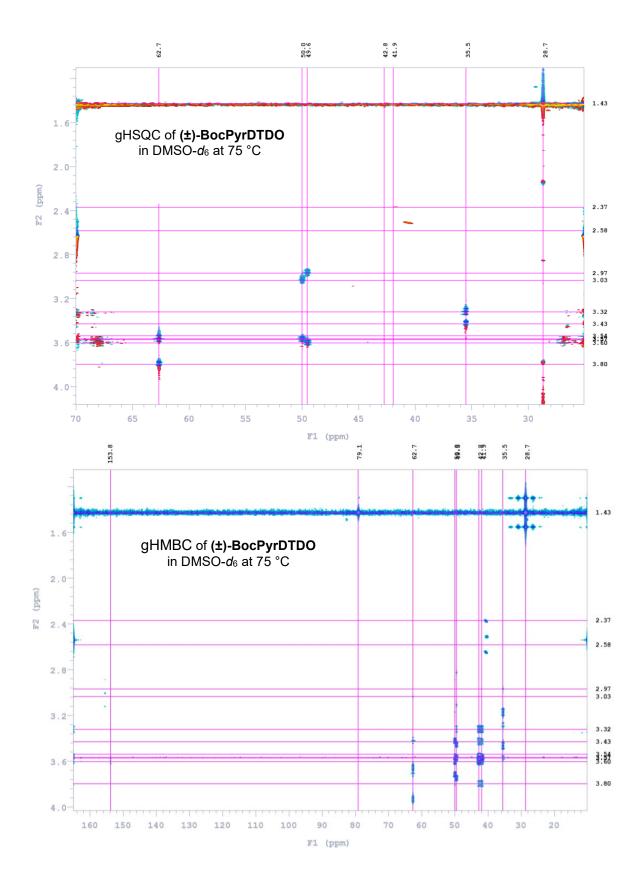
¹H NMR (CDCl₃, 500 MHz)

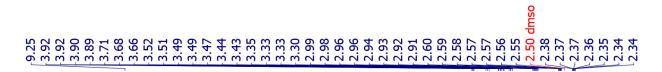


(±)-BocPyrDTDO

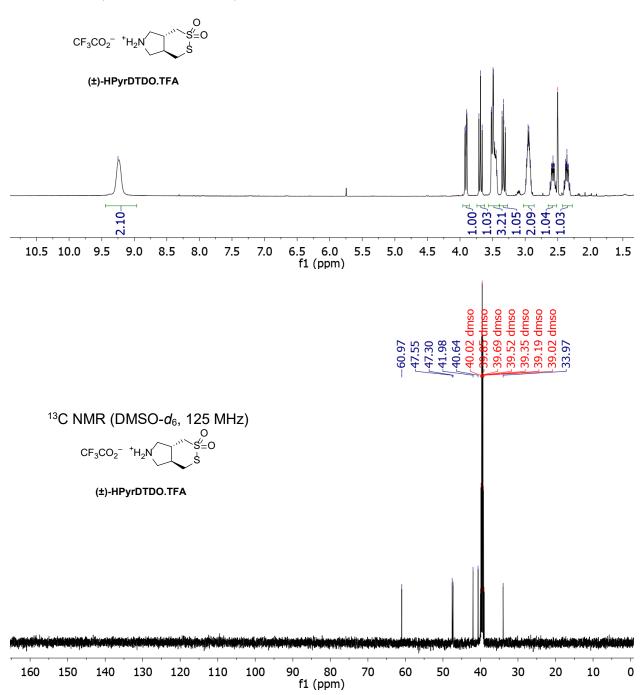


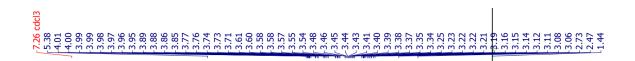


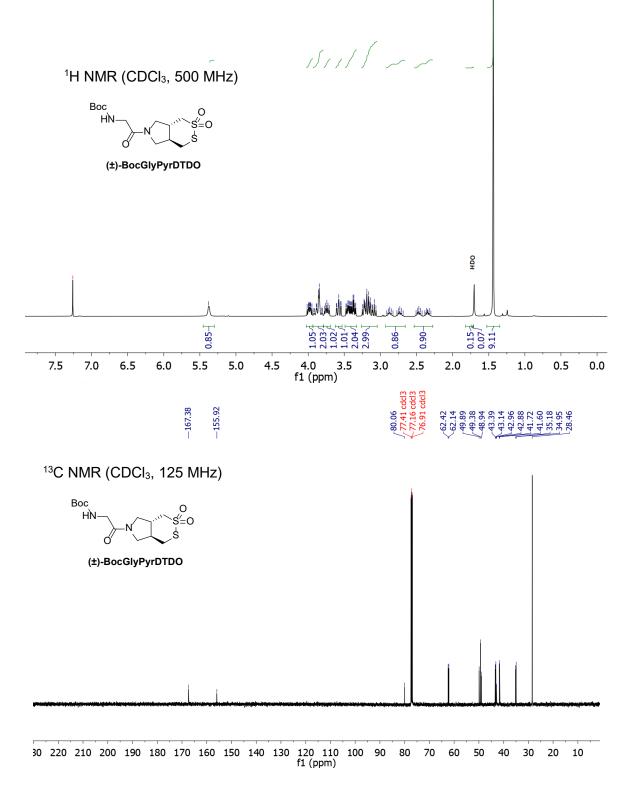


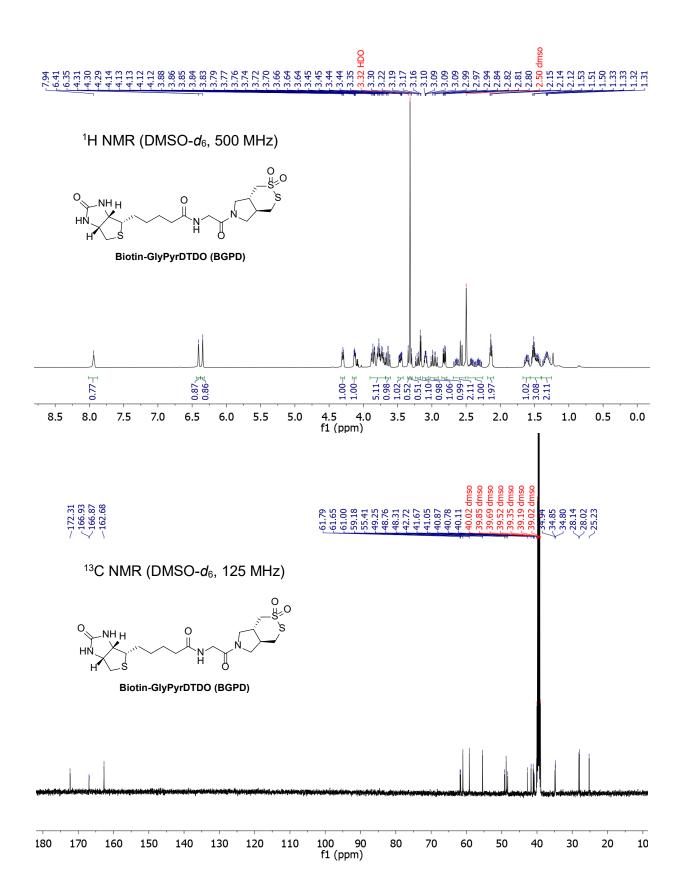


¹H NMR (DMSO-*d*₆, 500 MHz)

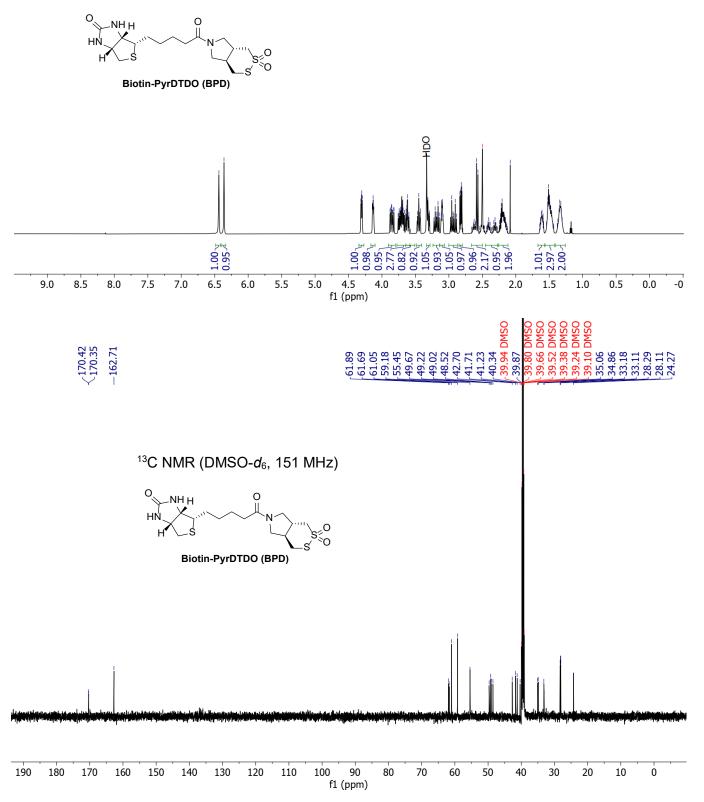








¹H NMR (DMSO-*d*₆, 600 MHz)



References

1. a) Gohimukkula, D. R.; Jones, D.; Qabaja, G.; Zhu, J. J.; Cooper, J. T.; Banner, W. K.; Sundermann, K.; Bondlela, M.; Rao, M.; Wang, P.; Gowda, R. B.; Andrews, R. C.; Gupta, S.; Hari, A. Preparation of phenylheteroaryl derivatives as inhibitors od advanced glycosylation end product receptors (RAGE). Assignee: TransTech Pharma, Inc.; WO 2011103091 (A1) Augest 25, **2011**. b) Rodríguez Sarmiento, R. M. a.; Wirz, B.; Iding, H., Chemoenzymatic preparation of non-racemic N-Boc-pyrrolidine-3,4-dicarboxylic acid 3-ethyl esters and their 4-hydroxymethyl derivatives. *Tetrahedron: Asymmetry* **2003**, *14* (11), 1547.

2. J.; Hunziker, D.; Mattei, P.; Mauser, H.; Tang, G.; Wang, L. Preparation of bicyclic derivatives as autotaxin inhibitors for therapy. Assignee: F. Hoffmann-La Roche AG, Switz.; Hoffmann-La Roche Inc. WO 20144048865 (A1), Apryl 3, **2014**.

3. For some case studies of N-acyl-prolyl isomerization please see: a) Kubyshkin, V.; Durkin, P.; Budisa, N., Energetic contribution to both acidity and conformational stability in peptide models. *New J. Chem.*, **2016**, *40* (6), 5209. b) Kubyshkin, V.; Budisa, N., Amide rotation trajectories probed by symmetry. *Org. Biomol. Chem.*, **2017**, *15* (32), 6764.