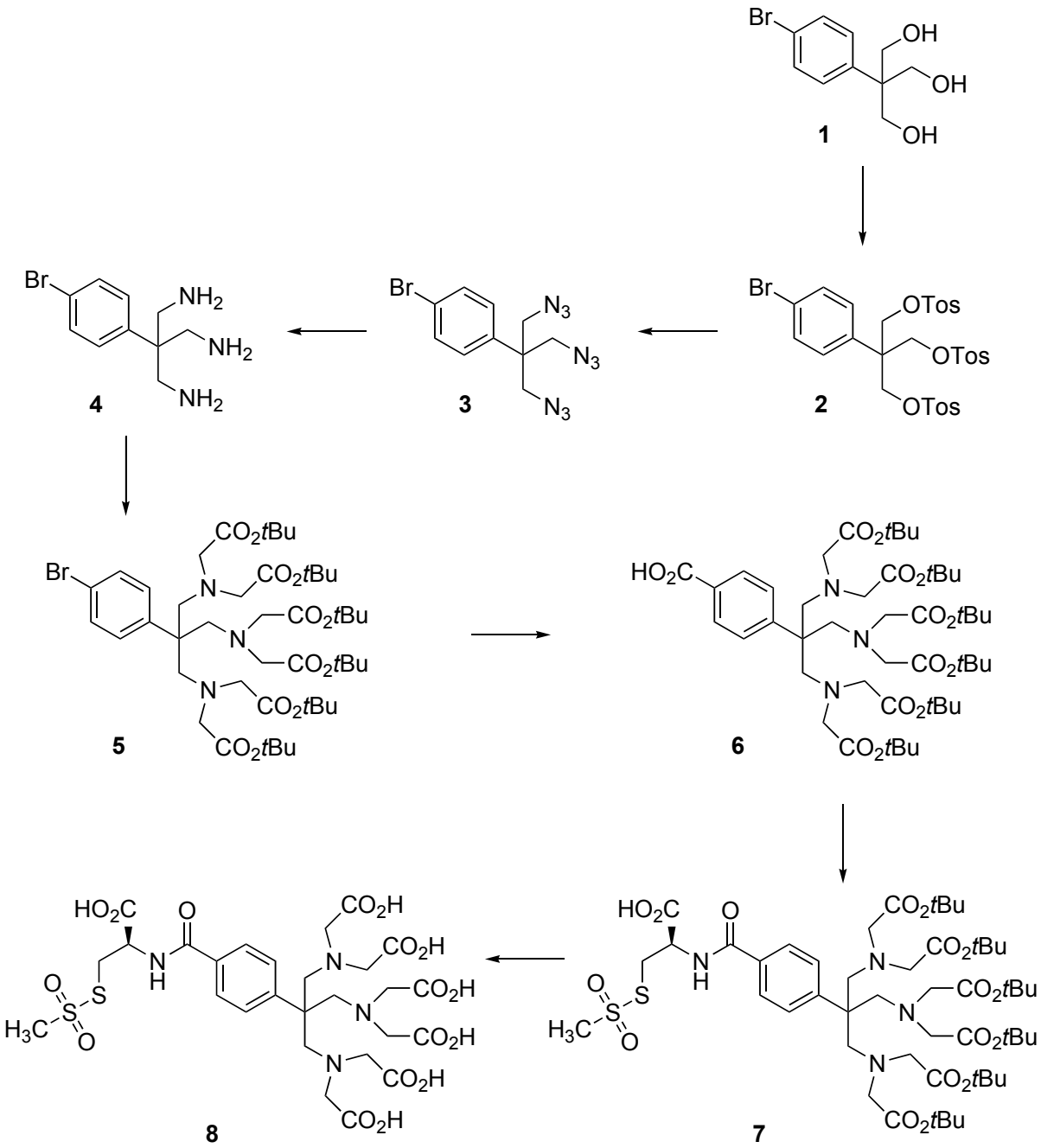
Supporting Information

Synthesis of Cys-Ph-TAHA

Materials and Methods

All used chemicals and solvents are commercially available. Reactions were controlled by thin layer chromatography (TLC) using Macherey-Nagel precoated sheets, 0.25 mm Polygram SIL G/UV_{254} plates. Spots were visualized by 10 wt% ethanolic phosphomolybdic acid reagent and were heated at 200 °C. Flash column chromatography was performed by using Merck silica gel 60 (0.015–0.040 mm). Analytical and preparative HPLC separations were carried out using a Jasco HPLC system with a DAD detector. HPLC was performed using a reversed phase (RP) column (Eurospher RP 18) from Knauer: 150 x 4.6 mm (analytical) and 250 x 8 mm (preparative) at flow rates of 1 mL·min^{-1} (analytical) and 3 mL·min^{-1} (preparative). The mobile phase contained of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitril (solvent B). ESI-MS spectra were measured using a Waters Micromass ZQ 4000 mass spectrometer. NMR spectra were recorded at 298 K using a 400 MHz Bruker Avance spectrometer equipped with a TXI HCN z-gradient probe head. Spectra were processed and analyzed with Topspin 2.0 (Bruker Biospin).



Scheme S1. Synthesis of Cys-Ph-TAHA.

4-Bromo-α-α-α-tris(tosyloxymethyl)toluene (2)

Compound **1** (4.70 g, 18.00 mmol) was dissolved in abs. pyridine (120 mL). Tosyl chloride (20.60 g, 108.05 mmol, 10.0 eq.) was added and the solution stirred at RT for 60 h. Pyridinium chloride was removed by filtration and the filtrate evaporated in vacuo. To the residue H_2O (250 mL) and CH_2Cl_2 (250 mL) were added and the phases separated. The aqueous layer was extracted with CH_2Cl_2 (3 x 200 mL) and the combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2) yielded **2** (11.85 g, 16.37 mmol, 91%) as a white foam. **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.59 (d, *J* = 8.2 Hz, 6H, 6 x Tos-Ar), 7.30 (d, *J* = 8.2 Hz, 6H, 6 x Tos-Ar), 7.22 (d, *J* = 8.6 Hz, 2H, H-3, H-5), 6.76 (d, *J* = 8.6 Hz, 2H, H-2, H-6), 4.14 (s, 6H, 3 x CH_2), 2.46 (s, 9H, 3 x CH_3) ppm. **^{13}C-NMR** ($CDCl_3$, 100.6 MHz): δ = 145.4 (3C, 3 x Tos-Ar), 133.8 (1C, C-1), 131.8 (2C, C-3, C-5), 131.5 (3C, 3 x Tos-Ar), 130.0 (6C, 6 x Tos-Ar), 127.8 (8C, C-2, C-6, 6 x Tos-Ar), 122.2 (1C, C-4), 68.4 (3C, 3 x CH_2), 45.9 (1C, C-α), 21.6 (3C, 3 x CH_3) ppm. **ESI-MS** *m/z* (methanol, positive mode): calcd. for $C_{31}H_{31}{}^{79}BrNaO_9S_3$ $[M+Na]^+$: 745.02, found: 744.94.

4-Bromo-α-α-α-tris(azidomethyl)toluene (3)

Compound **2** (11.50 g, 15.89 mmol) was dissolved in 500 mL abs. DMF. NaN_3 (6.19 g, 95.23 mmol, 6.0 eq.) was added and the suspension stirred at 100 °C for 18 h. After cooling, 1.4 L of H_2O were added and the aqueous layer was extracted with CH_2Cl_2 (3 x 500 mL). The combined organic layers were washed with H_2O (500 mL), dried over Na_2SO_4 and concentrated in vacuo to a solvent residue of 30 mL. THF (50 mL) was added and again the volume was reduced in vacuo to 30 mL. This procedure was repeated twice. The resulting THF solution was filled up to 400 mL under argon atmosphere and directly used for the following reaction.

4-Bromo-α-α-α-tris(aminomethyl)toluene (4)

To the THF solution of the azide **3** triphenylphosphine (37.53 g, 143.10 mmol, 9.0 eq.) was added and stirred for 2 h at 65 °C. After gas formation has stopped, **4** was set free by adding 2M NaOH solution (200 mL) and stirring for another 2 h at 65 °C. Afterwards the reaction mixture was acidified to pH 1 and the aqueous phase was washed with $CHCl_3$ (3 x 300 mL). Subsequently, solid NaOH was added to set pH 12 and extracted with $CHCl_3$ (5 x 300 mL). The combined organic layers dried

over Na_2SO_4 and concentrated in vacuo to yield the product **4** (3.80 g, 14.72 mmol, 93% over 2 steps) as yellow oil. **^{1}H-NMR** (MeOH-d_4, 400 MHz): δ = 7.55 (d, J = 8.6 Hz, 2H, H-3, H-5), 7.30 (d, J = 8.6 Hz, 2H, H-2, H-6), 2.90 (s, 6H, 3 x CH_2) ppm. **^{13}C-NMR** (MeOH-d_4, 100.6 MHz): δ = 141.4 (1C, C-1), 131.4 (2C, C-3, C-5), 128.6 (2C, C-2, C-6), 120.6 (1C, C-4), 48.3 (1C, C-α), 45.0 (3C, CH_2) ppm. **ESI-MS** *m/z* (methanol, positive mode): calcd. for $C_{10}H_{17}{}^{79}BrN_3$ $[M+H]^+$: 258.06, found: 257.98.

4-Bromo-α-α-α-tris[[*N,N*-di(*tert*-butoxycarbonylmethyl)amino]methyl]toluene (5)

Compound **4** (3.30 g, 12.78 mmol) was dissolved under argon atmosphere in abs. MeCN (330 mL). DIPEA (26.75 mL, 156.27 mmol, 12.2 eq.) and *tert*-butyl bromoactetate (17.0 mL, 115.05 mmol, 9.0 eq.) were added and the resulting solution refluxed for 42 h. The solvent was removed in vacuo and the residue dissolved in CH_2Cl_2. The solution was washed with 5% citric acid solution (2 x 200 mL) and H_2O (200 mL), dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/MeOH 100:1) yielded **5** (11.69 g, 12.40 mmol, 97%) as a yellow foam. **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.31 (d, J = 8.7 Hz, 2H, H-3, H-5), 7.25 (d, J = 8.7 Hz, 2H, H-2, H-6), 3.17 (s, 18H, 9 x CH_2), 1.39 (s, 54H, 6 x $C(CH_3)_3$) ppm. **^{13}C-NMR** ($CDCl_3$, 100.6 MHz): δ = 170.9 (6C, 6 x CO_2t-Bu), 143.7 (1C, C-1), 131.3 (2C, C-3, C-5), 128.6 (2C, C-2, C-6), 119.8 (1C, C-4), 80.4 (6C, 6 x $C(CH_3)_3$), 59.3 (3C, 3 x C-αCH_2), 56.5 (6C, 6 x NCH_2CO_2t-Bu), 48.5 (1C, C-α), 28.0 (18C, 18 x $C(CH_3)_3$) ppm. **ESI-MS** *m/z* (methanol, positive mode): calcd. for $C_{46}H_{77}{}^{79}BrN_3O_{12}$ $[M+H]^+$: 944.47, found: 944.44.

α-α-α-Tris[[*N,N*-di(*tert*-butoxycarbonylmethyl)amino]methyl]-*p*-toluic acid (6)

Compound **5** (360 mg, 0.382 mmol) was dissolved in abs. DMF (6.5 mL). Dried lithium formate (179 mg, 3.44 mmol, 9.0 eq.) und $PdCl_2$(dppf) (28 mg, 36.2 μmol, 10 mol%) were added. Argon gas was directed through the suspension for 20 min. Afterwards, acetic anhydride (216 μL, 2.29 mmol, 6.0 eq.) and DIPEA (392 μL, 2.29 mmol, 6.0 eq.) were added and the suspension stirred for 44 h at 120 °C. After cooling, ethyl acetate (80 mL) was added and the organic phase washed with H_2O (2 x 50 mL), dried over Na_2SO_4 and concentrated in vacuo. Purification by column chromatography (CH_2Cl_2/MeOH 20:1) yielded **6** (282 mg, 0.311 mmol, 81%) as a brown oil. **^{1}H-NMR** ($CDCl_3$, 400 MHz): δ = 7.99 (d, J = 8.2 Hz, 2H, H-3, H-5), 7.55 (d,

J = 8.2 Hz, 2H, H-2, H-6), 3.31 (s, 6H, 3 x C-αCH$_2$), 3.22 (s, 12H, 6 x CH$_2$CO$_2$*t*-Bu), 1.42 (s, 54H, 6 x C(CH$_3$)$_3$) ppm. **^{13}C-NMR** (CDCl$_3$, 100.6 MHz): δ = 170.0 (7C, 6 x CO$_2$*t*-Bu, C-4*C*O$_2$H), 151.3 (1C, C-1), 130.2 (2C, C-3, C-5), 126.9 (2C, C-2, C-6), 127.3 (1C, C-4), 80.6 (6C, 6 x *C*(CH$_3$)$_3$), 59.5 (3C, 3 x C-α*C*H$_2$), 56.6 (6C, 6 x N*C*H$_2$CO$_2$*t*-Bu), 49.4 (1C, C-α), 28.1 (18C, 18 x C(*C*H$_3$)$_3$) ppm. **ESI-MS** *m/z* (methanol, positive mode): calcd. for C$_{47}$H$_{78}$N$_3$O$_{14}$ [M+H]$^+$: 908.55, found: 908.57.

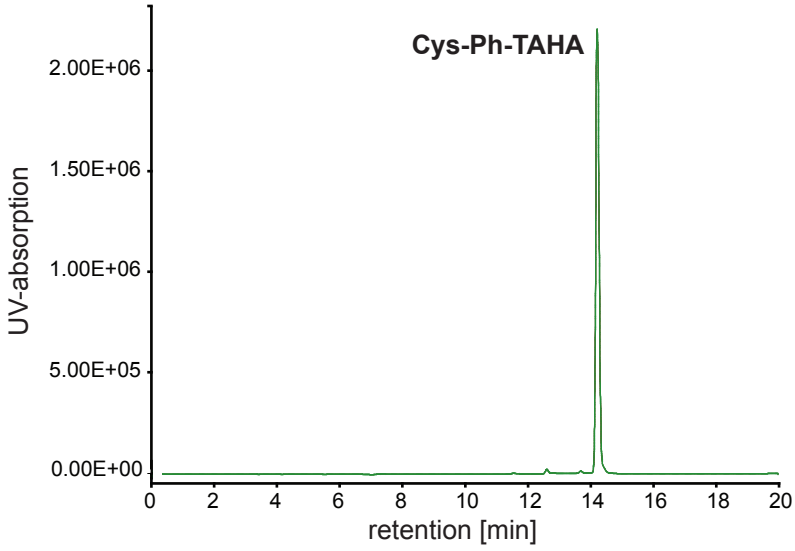
***N*-[4-[α-α-α-Tris[[*N,N*-di(*tert*-butoxycarbonylmethyl)amino]methyl]methyl]-benzoyl]-*S*-mesyl-(*R*)-cysteine (7)**

Compound **6** (178 mg, 196 μmol) was dissolved in 10 mL abs. DMF. HATU (75 mg, 196 μmol, 1.0 eq.) and DIPEA (33.5 μL, 196 mmol, 1.0 eq.) were added and the solution stirred for 2.5 h at RT. Subsequently, *S*-mesyl-(*R*)-cysteine (39 mg, 196 μmol, 1.0 eq.) and again DIPEA (33.5 μL, 196 μmol, 1.0 eq.) were added and the reaction mixture was stirred for another 20 h. Afterwards the solvent was removed in vacuo, to the residue H$_2$O and CHCl$_3$ (30 mL each) were added and the phases separated. The aqueous layer was extracted with CHCl$_3$ (2 x 20 mL) and the combined organic phase was dried over Na$_2$SO$_4$ and concentrated in vacuo. Purification by column chromatography (CH$_2$Cl$_2$/MeOH from 25:1 to 8:1) yielded **7** (147 mg, 135 μmol, 69%) in form of a brown oil. Additionally, educt **6** (31 mg, 34 μmol, 17%) was re-isolated. **^{1}H-NMR** (CDCl$_3$, 400 MHz): δ = 8.02 (s, 1H, NH), 7.82 (s, 2H, H-3, H-5), 7.52 (s, 2H, H-2, H-6), 4.71–4.89 (m, 1H, SCH$_2$C*H*), 3.60–4.00 (m, 2H, SCH$_2$), 3.36 (s, 3H, SCH$_3$), 3.28 (s, 6H, 3 x C-αCH$_2$), 3.22 (s, 12H, 6 x NCH$_2$CO$_2$*t*-Bu), 1.43 (s, 54H, 6 x C(CH$_3$)$_3$) ppm. **^{13}C-NMR** (CDCl$_3$, 100.6 MHz): δ = 171.0 (6C, 6 x CO$_2$*t*-Bu), 167.6 (1C, CO$_2$H), 162.9 (1C, NHCO), 149.3 (1C, C-1), 130.6 (1C, C-4), 127.6 (2C, C-3, C-5), 126.9 (2C, C-2, C-6), 80.7 (6C, 6 x *C*(CH$_3$)$_3$), 59.6 (3C, 3 x C-α*C*H$_2$), 56.6 (6C, 6 x N*C*H$_2$CO$_2$*t*-Bu), 54.6 (1C, SCH$_2$*C*H), 50.3 (1C, C-α), 34.7 (1C, S*C*H$_2$CH), 28.2 (18C, 18 x C(*C*H$_3$)$_3$) ppm. **ESI-MS** *m/z* (methanol, positive mode): calcd. for C$_{51}$H$_{85}$N$_4$O$_{17}$S$_2$ [M+H]$^+$: 1089.54, found: 1089.49.

***N*-[4-[α-α-α-Tris[[*N,N*-di(carboxymethyl)amino]methyl]methyl]benzoyl]-*S*-mesyl-(*R*)-cysteine (8)**

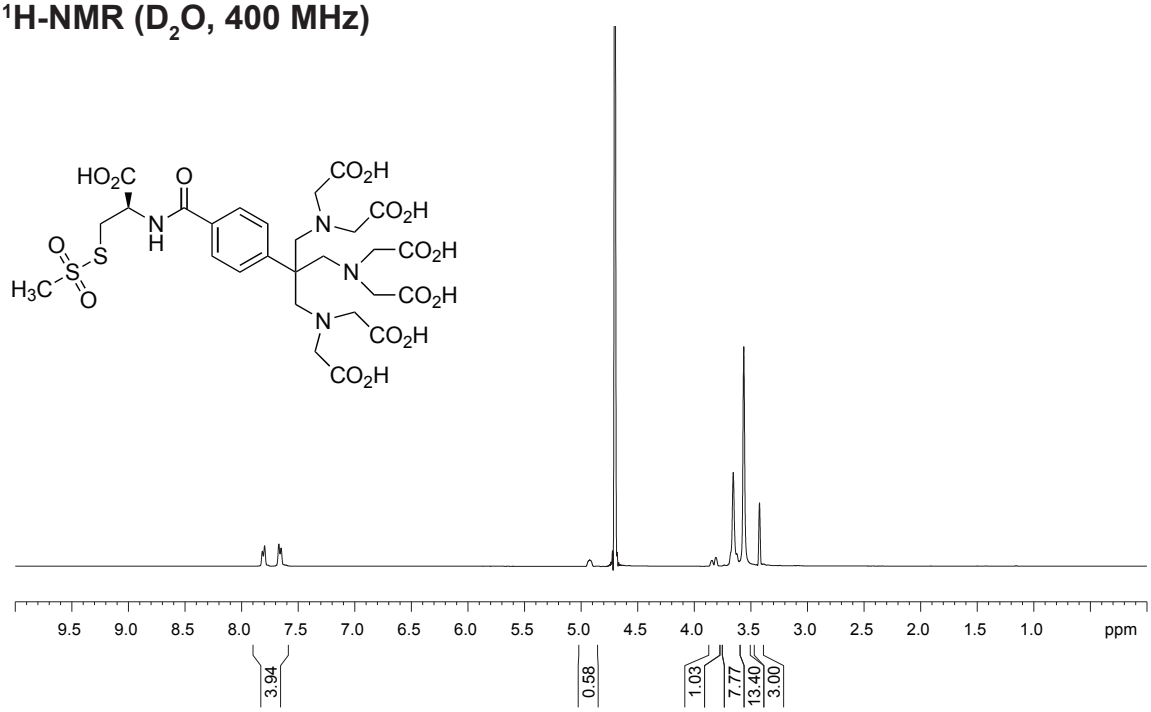
Compound **7** (209 mg, 192 μmol) was dissolved in 20 mL concentrated TFA and stirred at RT overnight. Afterwards the reaction mixture was concentrated in vacuo.

The residue was dissolved in H_2O (16 mL) and centrifuged to remove insoluble particles. The solution was purified by preparative HPLC (0–30% A in 30 min). Combined product fractions yielded after lyophylisation **Cys-Ph-TAHA**, **8** (89 mg, 118 μmol, 61%) as a white powder. **^{1}H-NMR** (D_2O, 400 MHz): δ = 7.81 (d, J = 7.9 Hz, 2H, H-3, H-5), 7.66 (d, J = 7.9 Hz, 2H, H-2, H-6), 4.86–4.95 (m, 1H, SCH_2CH), 3.83 (d, J = 13.8 Hz, 1H, SCH_aH_b), 3.69–3.61(m, 7H, SCH_aH_b}, 3 x C-αCH_2), 3.56 (s, 12H, 6 x NCH_2CO_2H), 3.42 (s, 3H, CH_3) ppm. **^{13}C-NMR** (D_2O, 100.6 MHz): δ = 172.5 (7C, 7 x CO_2H), 169.3 (1C, C-4*C*O), 143.3 (1C, C-1), 131.9 (1C, C-4), 127.9 (2C, C-3, C-5), 126.8 (2C, C-2, C-6), 60.7 (3C, 3 x C-αCH_2), 55.7 (6C, 6 x NCH_2CO_2H), 52.6 (1C, SCH_2CH), 49.4 (1C, CH_3), 46.1 (1C, C-α), 36.2 (1C, SCH_2CH) ppm. **ESI-MS** *m/z* (water, positive mode): calcd. for $C_{27}H_{37}N_4O_{17}S_2$ $[M+H]^+$: 753.16, found: 753.08. **ESI-HRMS** *m/z* (water, positive mode): calcd. for $C_{27}H_{37}N_4O_{17}S_2$ $[M+H]^+$: 753.1590, found: 753.1588.

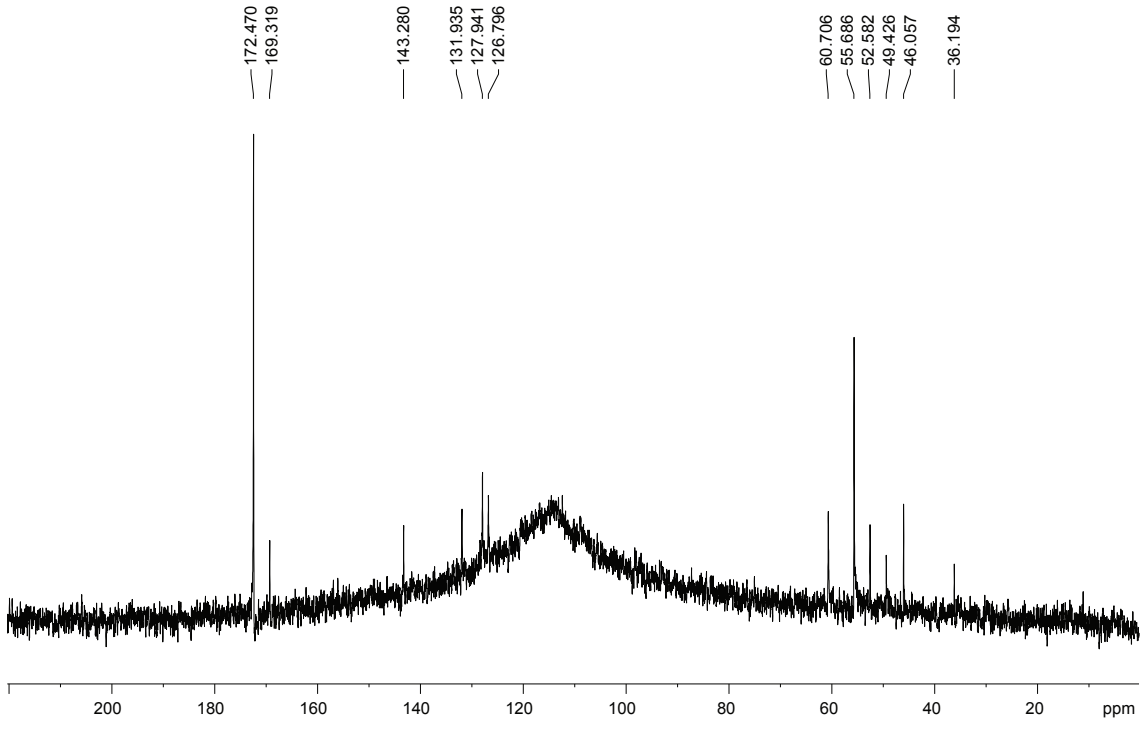


HPLC-chromatogram of purified Cys-Ph-TAHA. The mobile phase contained of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). Gradient: 0–30% B in 15 min.

^{1}H-NMR (D_2O, 400 MHz)



^{13}C-NMR (D_2O, 100.6 MHz)



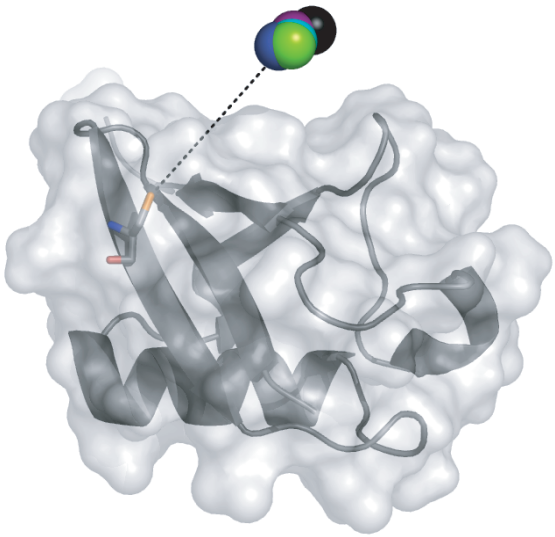


***Figure* S1.** 3D-structure (1D3Z) of ubiquitin-T12C with the back-calculated metal positions (12.8 Å at 298 K) for thulium at different temperatures: 278 K = black; 288 K = dark blue; 298 K = purple; 308 K = cyan; 315 K = green. The structure shows that there is no direct interaction of the metal with protein side chains.

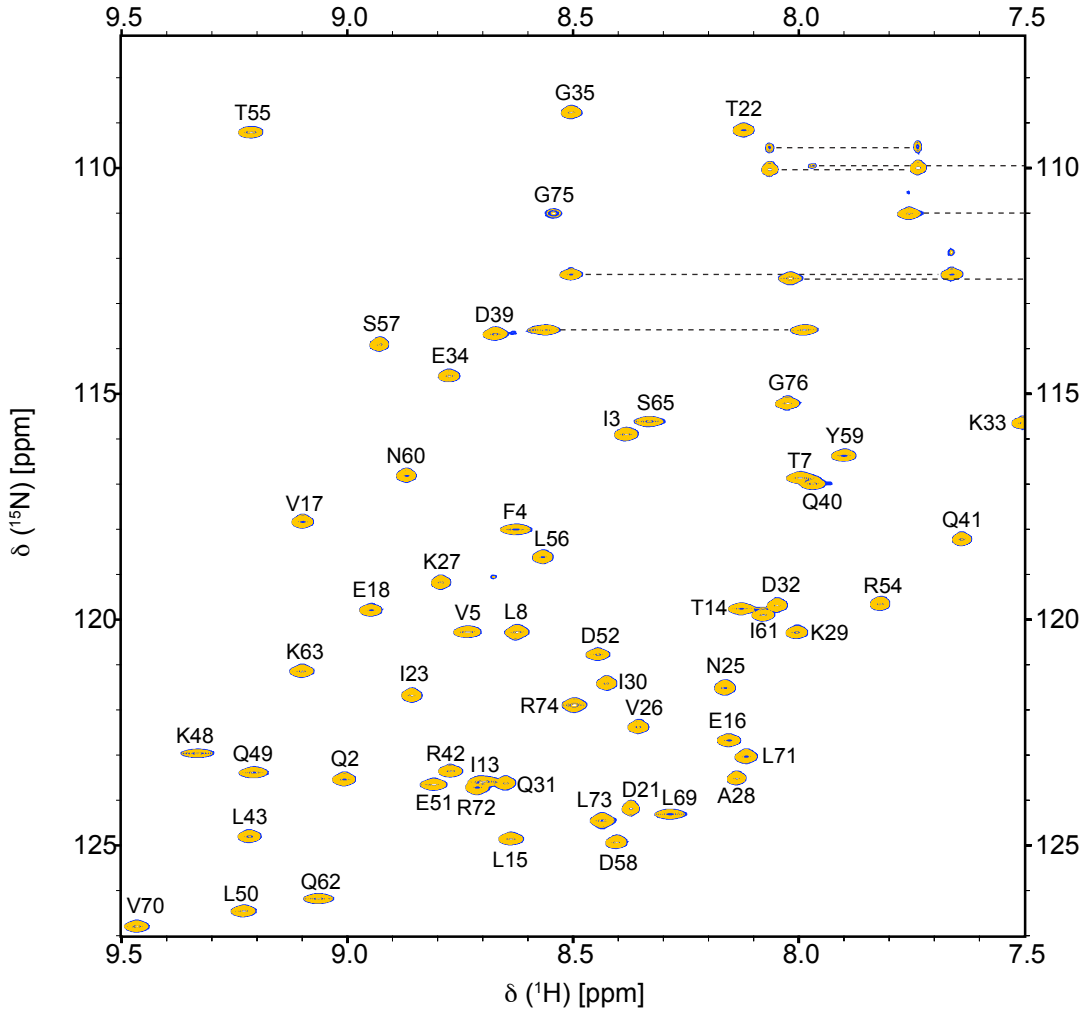


Figure S2. T12C Tm: sections of superimposed ^{1}H-^{15}N HSQC spectra, measured at 308 K. The spectra were recorded before (blue) and after (orange) an overnight incubation at 308 K. The dashed lines connect side chain signals.

Table S1. Chemical shifts, PCS and RDCs of T12C Tb and T12C Tm. The asterisks indicate values that were excluded from the calculations either due to overlapped/ weak peaks or mobility. The bars reflect values that were not detectable due to PRE.

	T12C Lu		T12C					T12C Tm				
Residue	**δ (^{1}H)**	**δ (^{15}N)**	**δ (^{1}H)**	**δ (^{15}N)**	**^{1}H-PCS**	**^{15}N-PCS**	**RDC**	**δ (^{1}H)**	**δ (^{15}N)**	**^{1}H-PCS**	**^{15}N-PCS**	**RDC**
Q2	8.951	123.475	9.187	123.653	0.236	0.178	-6.60	8.987	123.488	0.036	0.013	-1.39
I3	8.391	115.876	8.616	116.103	0.225	0.227	5.22	8.301	115.792	-0.090	-0.084	-6.27
F4	8.793	118.262	8.799	118.436	0.006	0.174	14.44	8.578	117.883	-0.215	-0.379	-9.65
V5	9.254	120.774	9.655	121.027	0.401	0.253	17.81	8.629	120.212	-0.625	-0.562	-9.93
K6	9.113	129.453	-	-	-	-	-	8.620	128.677	-0.493	-0.776	-6.93
T7	8.887	117.897	10.127	119.039	1.240	1.142	*	7.843	116.999	-1.044	-0.898	-4.82
L8	9.065	120.995	9.837	122.067	0.772	1.072	-6.26	8.569	120.369	-0.496	-0.626	5.50
T9	7.657	106.085	8.657	107.144	1.000	1.059	-5.67	7.045	105.467	-0.612	-0.618	6.32
G10	7.989	110.048	9.732	111.644	1.743	1.596	-	6.886	109.036	-1.103	-1.012	3.47
K11	7.210	121.375	-	-	-	-	-	6.388	120.404	-0.822	-0.971	-1.98
C12	8.726	123.497	-	-	-	-	-	-	-	-	-	-
I13	9.575	124.879	10.568	126.161	0.993	1.282	-	8.581	123.731	-0.994	-1.148	-9.27
T14	8.696	120.484	9.553	121.323	0.857	0.839	13.19	8.085	119.876	-0.611	-0.608	-8.43
L15	8.845	125.132	9.211	125.494	0.366	0.362	6.29	8.569	124.860	-0.276	-0.272	-5.66
E16	8.148	122.747	8.307	122.902	0.159	0.155	-0.71	8.093	122.676	-0.055	-0.071	-5.36
V17	8.979	117.766	8.998	117.728	0.019	-0.038	-6.81	9.058	117.848	0.079	0.082	1.22
E18	8.701	119.472	8.585	119.315	-0.116	-0.157	-6.04	8.866	119.628	0.165	0.156	-1.21
S20	7.072	103.568	6.890	103.393	-0.182	-0.175	10.30	7.301	103.788	0.229	0.220	-7.39
D21	8.111	124.124	7.920	123.907	-0.191	-0.217	-6.05	8.344	124.345	0.233	0.221	4.62
T22	7.917	109.099	7.754	108.949	-0.163	-0.150	5.77	8.098	109.284	0.181	0.185	-3.07
I23	8.559	121.431	8.307	121.212	-0.252	-0.219	13.37	8.826	121.686	0.267	0.255	-7.06
N25	7.965	121.551	7.815	121.453	-0.150	-0.098	4.51	8.121	121.651	0.156	0.100	-3.79
V26	8.148	122.367	7.988	122.258	-0.160	-0.109	14.35	8.319	122.496	0.171	0.129	-7.37
K27	8.602	119.163	8.458	119.047	-0.144	-0.116	6.96	8.750	119.288	0.148	0.125	-1.07
A28	7.980	123.583	7.899	123.546	-0.081	-0.037	0.42	8.063	123.629	0.083	0.046	1.15
K29	7.883	120.383	7.848	120.412	-0.035	0.029	10.15	7.933	120.388	0.050	0.005	-6.82
I30	8.363	121.579	8.371	121.629	0.008	0.050	10.59	8.371	121.564	0.008	-0.015	-4.01
Q31	8.580	123.688	8.594	123.730	0.014	0.042	1.71	8.573	123.592	-0.007	-0.096	2.62
D32	8.009	119.792	8.039	119.863	0.030	0.071	1.55	7.989	119.734	-0.020	-0.058	-0.55
K33	7.468	115.775	7.561	115.883	0.093	0.108	12.59	7.397	115.688	-0.071	-0.087	-7.76
E34	8.801	114.662	8.939	114.746	0.138	0.084	4.40	8.691	114.559	-0.110	-0.103	0.85
G35	8.501	108.935	8.580	108.964	0.079	0.029	-9.30	8.424	108.861	-0.077	-0.074	5.48
I36	6.198	120.455	6.268	120.486	0.070	0.031	-7.94	6.127	120.384	-0.071	-0.071	-1.27
D39	8.572	113.785	8.503	113.757	-0.069	-0.028	6.78	8.615	113.800	0.043	0.015	-5.26
Q40	7.866	117.011	7.800	116.955	-0.066	-0.056	12.94	7.901	117.041	0.035	0.030	-7.67
Q41	7.524	118.286	7.428	118.146	-0.096	-0.140	-8.39	7.593	118.372	0.069	0.086	11.03
R42	8.539	123.329	8.321	123.065	-0.218	-0.264	-3.27	8.713	123.502	0.174	0.173	8.08
L43	8.816	124.468	8.374	123.977	-0.442	-0.491	-2.13	9.234	124.920	0.418	0.452	7.27
I44	9.169	122.725	8.095	121.711	-1.074	-1.014	-	10.166	123.682	0.997	0.957	-2.70
F45	8.859	124.713	7.507	123.263	-1.352	-1.450	-	10.349	126.476	1.490	1.763	-4.93
A46	9.142	133.633	-	-	-	-	-	-	-	-	-	-
G47	8.295	106.775	-	-	-	-	-	-	-	-	-	-
K48	8.034	122.131	-	-	-	-	-	9.440	123.246	1.406	1.115	-6.03
Q49	8.664	122.923	8.141	122.341	-0.523	-0.582	14.78	9.257	123.565	0.593	0.642	-8.21
L50	8.631	125.972	8.001	125.369	-0.630	-0.603	3.65	9.266	126.595	0.635	0.623	1.82
E51	8.432	123.360	8.065	122.973	-0.367	-0.387	*	8.826	123.731	0.394	0.371	-3.26
D52	8.211	120.650	7.974	120.400	-0.237	-0.250	-4.78	8.44	120.895	0.229	0.245	-0.04

R54	7.532	119.598	7.294	119.331	-0.238	-0.267	-8.05	7.783	119.849	0.251	0.251	6.63
T55	8.870	108.963	8.584	108.727	-0.286	-0.236	8.87	9.179	109.229	0.309	0.266	-6.37
L56	8.183	118.229	7.866	117.873	-0.317	-0.356	10.88	8.537	118.629	0.354	0.400	-5.90
S57	8.535	113.605	8.172	113.197	-0.363	-0.408	3.01	8.943	114.059	0.408	0.454	-0.27
D58	7.977	124.679	7.595	124.312	-0.382	-0.367	3.79	8.397	125.090	0.420	0.411	-4.87
Y59	7.312	115.960	6.765	115.420	-0.547	-0.540	15.29	7.904	116.552	0.592	0.592	-9.24
N60	8.189	116.145	7.514	115.306	-0.675	-0.839	-9.61	8.896	117.020	0.707	0.875	10.91
I61	7.254	119.080	6.444	118.083	-0.810	-0.997	-8.72	8.146	120.157	0.892	1.077	8.61
Q62	7.770	125.201	6.545	124.197	-1.225	-1.004	-4.41	9.243	126.416	1.473	1.215	5.90
K63	8.500	120.591	8.080	120.178	-0.420	-0.413	-10.47	9.168	121.296	0.668	0.705	4.77
E64	9.465	114.976	9.686	115.181	0.221	0.205	1.80	9.656	115.252	0.191	0.276	-1.03
S65	7.729	115.217	-	-	-	-	-	8.397	115.897	0.668	0.680	-
T66	8.872	117.747	-	-	-	-	-	-	-	-	-	-
L67	9.340	127.737	-	-	-	-	-	9.476	128.116	0.136	0.379	-13.29
H68	9.329	120.108	-	-	-	-	-	-	-	-	-	-
L69	8.213	123.981	-	-	-	-	-	8.242	124.313	0.029	0.332	3.50
V70	9.184	126.626	8.881	126.443	-0.303	-0.183	-8.14	9.466	126.920	0.282	0.294	9.18
L71	8.117	122.964	8.041	122.853	-0.076	-0.111	*	8.093	123.030	-0.024	0.066	8.58
R72	8.610	123.734	8.487	123.616	-0.123	-0.118	3.20	8.646	123.772	0.036	0.038	-0.64
L73	8.401	124.667	8.248	124.525	-0.153	-0.142	4.14	8.422	124.689	0.021	0.022	-1.61
R74	8.480	122.133	8.369	122.011	*	*	*	8.469	122.127	*	*	*
G75	8.518	111.191	8.426	111.072	*	*	*	8.512	111.191	*	*	*
G76	7.989	115.258	7.883	115.149	*	*	*	7.985	115.248	*	*	*

Table S2. Chemical shifts, PCS and RDCs of S57C Tb and S57C Tm. The asterisks indicate values that were excluded from the calculations either due to overlapped/ weak peaks or mobility. The bars reflect values that were not detectable due to PRE.

	S57C Lu		S57C Tb					S57C Tm				
Residue	**δ (^{1}H)**	**δ (^{15}N)**	**δ (^{1}H)**	**δ (^{15}N)**	**^{1}H-PCS**	**^{15}N-PCS**	**RDC**	**δ (^{1}H)**	**δ (^{15}N)**	**^{1}H-PCS**	**^{15}N-PCS**	**RDC**
Q2	8.872	123.052	-	-	-	-	-	-	-	-	-	-
I3	8.206	115.198	-	-	-	-	-	-	-	-	-	-
F4	8.542	118.579	9.268	119.195	0.726	0.616	-1.82	8.133	118.137	-0.409	-0.442	-
V5	9.189	121.155	9.540	121.565	0.351	0.410	-1.74	8.921	120.856	-0.268	-0.299	*
K6	8.902	128.063	9.232	128.356	0.330	0.293	-5.18	8.710	127.868	-0.192	-0.195	-1.39
T7	8.653	115.460	8.807	115.656	0.154	0.196	-3.23	8.531	115.345	-0.122	-0.115	4.31
L8	9.035	121.227	9.165	121.353	0.130	0.126	-1.02	8.944	121.135	-0.091	-0.092	4.78
T9	7.552	105.878	7.641	105.982	0.089	0.104	2.90	7.482	105.813	-0.070	-0.065	-1.23
G10	7.747	109.246	7.832	109.319	0.085	0.073	-7.35	7.674	109.194	-0.073	-0.052	1.56
K11	7.179	121.891	7.250	121.975	0.071	0.084	0.230	7.094	121.813	-0.085	-0.078	5.67
T12	8.553	120.649	8.613	120.743	0.060	0.094	-4.97	8.460	120.554	-0.093	-0.095	3.11
I13	9.483	127.65	9.657	127.747	0.174	0.097	-1.32	9.304	127.454	-0.179	-0.196	-0.45
T14	8.649	121.672	8.655	121.703	0.006	0.031	-3.12	8.443	121.409	-0.206	-0.263	-5.05
L15	8.696	125.51	9.133	125.956	0.437	0.446	-0.23	-	-	-	-	-
E16	8.017	122.542	-	-	-	-	-	-	-	-	-	-
V17	8.848	117.494	-	-	-	-	-	-	-	-	-	-
E18	8.616	120.298	-	-	-	-	-	-	-	-	-	-
S20	7.006	103.385	-	-	-	-	-	-	-	-	-	-
D21	7.903	123.324	-	-	-	-	-	-	-			-
T22	7.661	108.069	-	-	-	-	-	-	-	-	-	-
I23	8.443	121.284	9.198	122.096	0.755	0.812	-5.91	-	-	-	-	-
N25	7.843	121.392	-	-	-	-	-	-	-	-	-	-
V26	7.996	122.212	-	-	-	-	-	8.006	122.153	0.010	-0.059	*
K27	8.448	118.807	9.053	119.302	0.605	0.495	-6.02	8.378	118.643	-0.070	-0.164	0.15
A28	7.899	123.482	8.332	123.862	0.433	0.380	-5.77	7.884	123.409	-0.015	-0.073	-2.60
K29	7.777	120.248	8.266	120.618	0.489	0.370	-4.63	7.579	120.026	-0.198	-0.222	-5.12
I30	8.201	121.474	8.609	121.835	0.408	0.361	-6.17	7.956	121.234	-0.245	-0.240	-0.75
Q31	8.455	123.495	8.688	123.673	0.233	0.178	-7.24	8.301	123.307	-0.154	-0.188	-1.80
D32	7.922	119.736	8.052	119.817	0.130	0.506	-5.49	8.574	124.729	-0.174	-0.166	-3.61
K33	7.350	115.493	7.441	115.531	0.091	0.421	-4.29	8.693	132.653	-0.208	-0.217	-2.86
E34	8.641	114.355	8.731	114.422	0.090	0.401	-6.36	8.623	124.432	-0.092	-0.064	-0.09
G35	8.404	108.875	8.459	108.959	0.055	0.398	4.30	8.882	122.162	-0.165	-0.182	-2.11
I36	6.068	120.303	6.169	120.399	0.101	0.316	1.76	8.331	123.167	-0.078	-0.050	4.10
D39	8.442	113.616	8.565	113.773	0.123	0.268	1.03	7.866	102.306	-0.133	-0.130	-2.66
Q40	7.739	116.861	7.883	117.058	0.144	0.238	4.41	7.353	118.084	-0.043	-0.040	-2.09
Q41	7.396	118.124	7.580	118.362	0.184	0.197	-0.26	7.713	116.824	-0.026	-0.037	1.25
R42	8.409	123.217	8.646	123.533	0.237	0.157	-6.32	8.440	113.575	-0.002	-0.041	*
L43	8.715	124.496	9.072	124.897	0.357	0.096	*	5.964	120.222	-0.104	-0.081	*
I44	9.047	122.344	9.449	122.742	0.402	0.084	-6.36	8.272	108.751	-0.132	-0.124	1.36
F45	8.748	124.895	9.194	125.401	0.446	0.081	*	7.734	119.532	-0.188	-0.204	*
A46	8.901	132.870	9.317	133.291	0.416	0.067	-5.36	8.470	114.193	-0.171	-0.162	1.81
G47	7.999	102.436	8.274	102.704	0.275	0.038	-5.02	7.119	115.262	-0.231	-0.231	-4.40
K48	7.909	122.058	8.203	122.325	0.294	0.267	3.41	7.789	121.991	-0.120	-0.067	3.47
Q49	8.546	123.058	8.784	123.349	0.238	0.291	1.63	8.526	122.985	-0.020	-0.073	-0.78
L50	8.458	125.632	8.853	125.966	0.395	0.334	-7.29	8.366	125.53	-0.092	-0.102	-0.12
E51	8.342	123.136	8.706	123.519	0.364	0.383	*	-	-	-	-	-

D52	8.074	120.255	8.330	120.620	0.256	0.365	4.43	8.162	120.374	0.088	0.119	0.21
R54	7.346	119.270	7.694	119.556	0.348	0.286	0.04	-	-	-	-	-
T55	8.885	109.171	-	-	-	-	-	-	-	-	-	-
L56	8.093	117.596	10.126	119.349	2.033	1.753	-	-	-	-	-	-
C57	7.935	116.710	-	-	-	-	-	-	-	-	-	-
D58	7.838	122.117	-	-	-	-	-	-	-	-	-	-
Y59	7.089	115.262	8.248	116.265	1.159	1.003	3.43	6.269	114.455	-0.820	-0.807	*
N60	8.113	116.590	9.415	117.952	1.302	1.362	-1.69	6.854	115.398	-1.259	-1.192	3.29
I61	7.308	118.889	9.021	120.505	1.713	1.616	-2.05	-	-	-	-	-
Q62	7.406	124.638	8.922	126.552	1.516	1.914	3.90	-	-	-	-	-
K63	8.503	120.763	-	-	-	-	-	-	-	-	-	-
E64	9.203	114.330	-	-	-	-	-	-	-	-	-	-
S65	7.560	114.865	-	-	-	-	-	-	-	-	-	-
T66	8.621	117.415	9.179	118.011	0.558	0.596	0.19	8.341	117.071	-0.280	-0.344	-5.41
L67	9.309	127.736	9.897	128.337	0.588	0.601	0.69	8.990	127.401	-0.319	-0.335	-2.44
H68	9.122	119.647	9.555	120.036	0.433	0.389	-6.58	8.914	119.395	-0.208	-0.252	0.14
L69	8.190	123.755	8.468	124.061	0.278	0.306	-5.83	8.043	123.634	-0.147	-0.121	3.81
V70	9.085	126.492	9.331	126.674	0.246	0.182	-4.57	8.980	126.362	-0.105	-0.130	0.33
L71	8.026	123.064	8.180	123.234	0.154	0.170	-0.68	7.950	122.999	-0.076	-0.065	2.02
R72	8.503	123.679	8.643	123.873	0.140	0.194	4.88	8.447	123.607	-0.056	-0.072	-2.45
L73	8.265	124.509	8.379	124.649	0.114	0.140	*	8.229	124.454	-0.036	-0.055	-2.59
R74	8.349	121.956	8.443	122.073	*	*	*	8.307	121.915	*	*	*
G75	8.392	111.040	8.478	111.140	*	*	*	8.367	111.011	*	*	*
G76	7.858	115.077	7.926	115.160	*	*	*	7.844	115.056	*	*	*