SUPPORTING INFORMATION

A theoretical and experimental study of cysteine-based perfluorinated derivatives

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*S-(perfluorophenyl)-L-cysteine (C_6F_5-S-Cys, **Ia**):*

Figure S1. HPLC chromatogram. RP-HPLC: [linear gradient H_2O/MeCN (100:0) to (40:60) over 8 min].

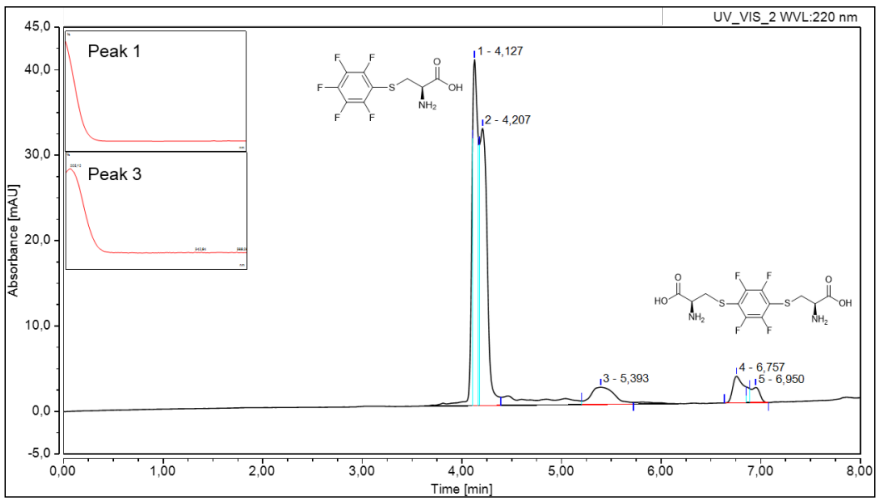


Figure S2. IR spectrum

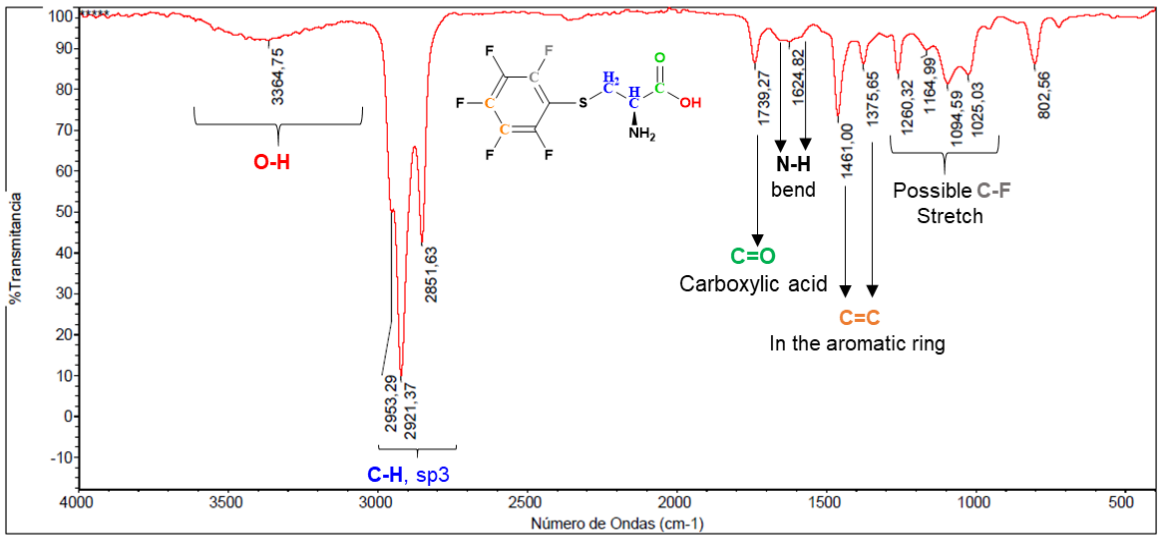
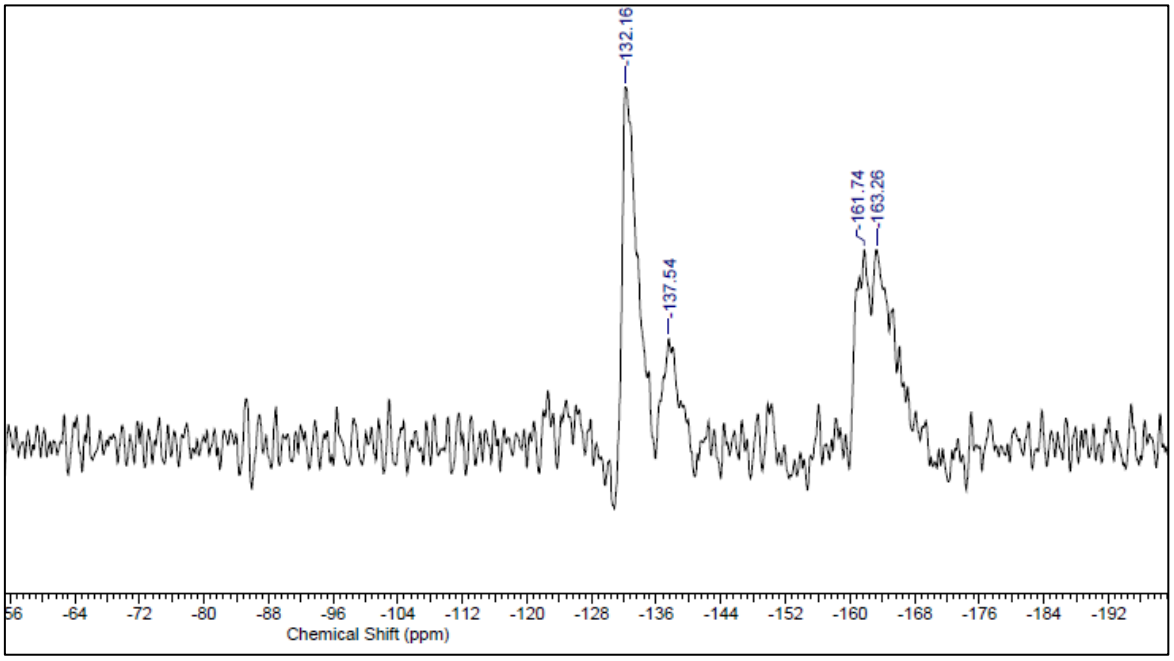


Figure S3. ^{19}F-NMR spectrum



*S-(perfluorophenyl)-L-Glutathione (C_6F_5-S-GSH, **Ib**):*

Figure S4. HPLC chromatogram of pure product. RP-HPLC: [linear gradient H_2O/MeCN (95:5) to (50:50) over 8 min]. ESI-MS spectrum.

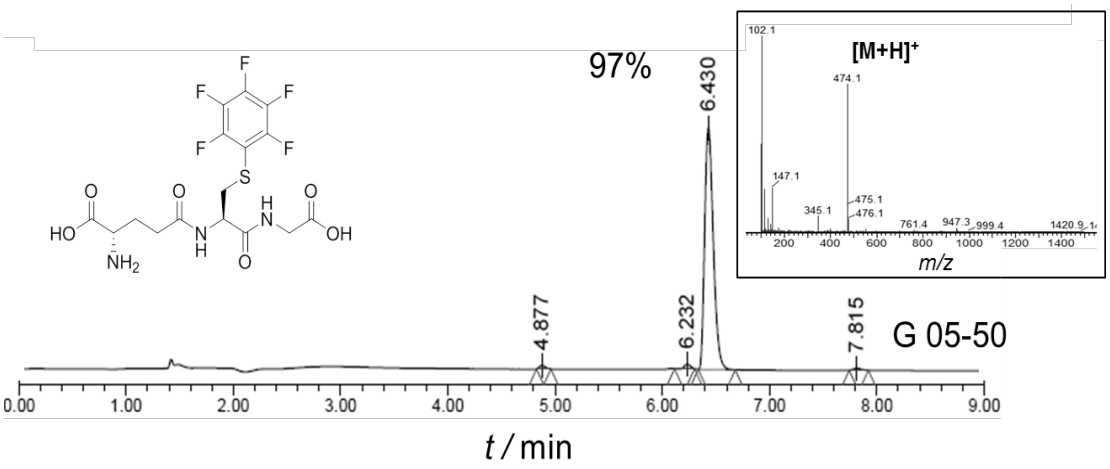
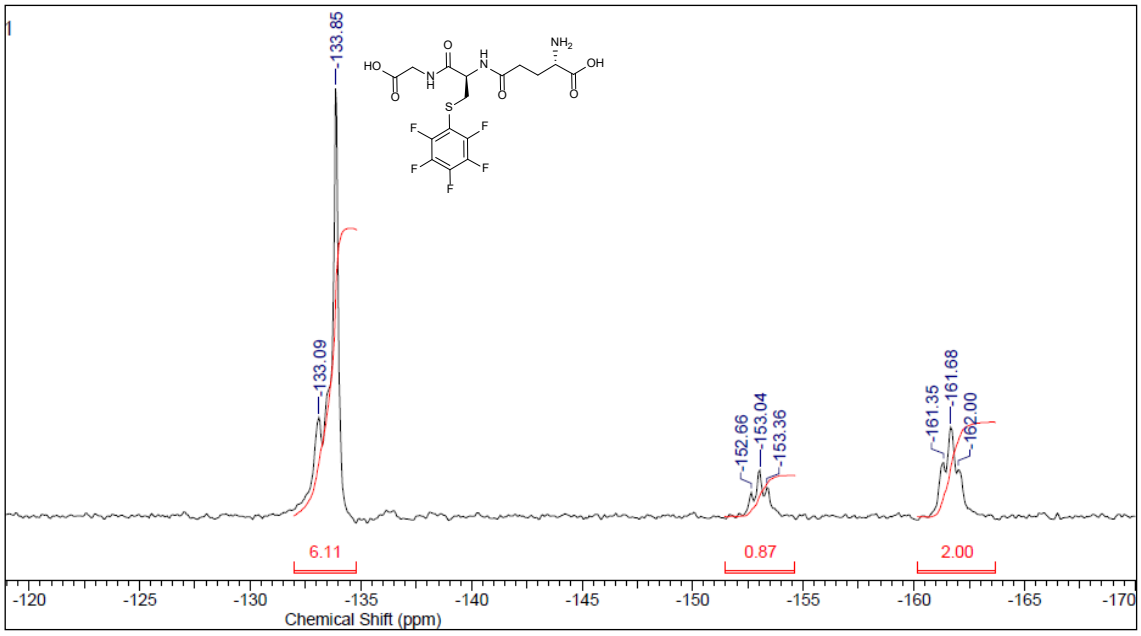
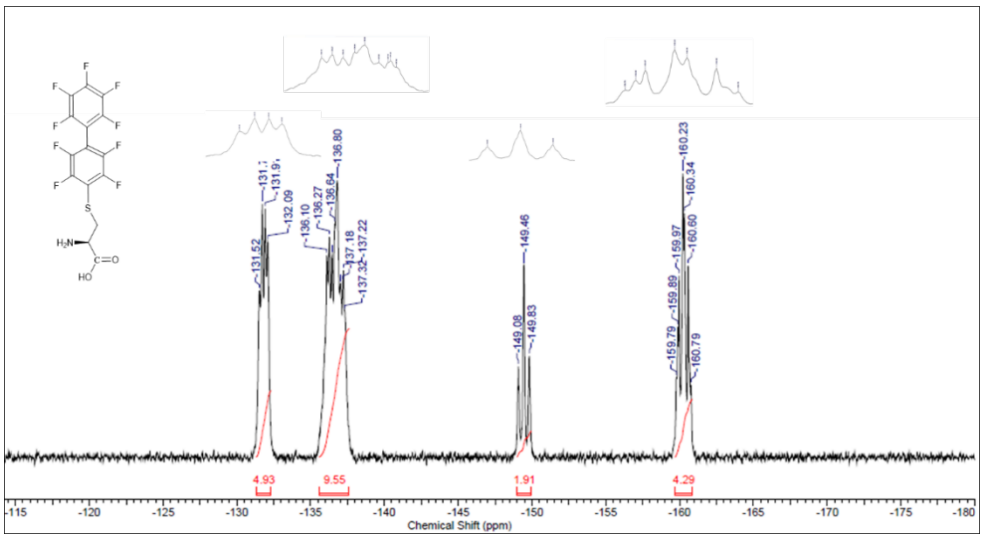


Figure S5. ^{19}F-NMR spectrum



*S-(perfluoro-[1,1′-biphenyl]-4-yl)-L-cysteine (**IIa**):*

Figure S6. ^{19}F-NMR spectrum



*S-(perfluoro-[1,1′-biphenyl]-4-yl)-L-glutathione (**IIb**):*

Figure S7. HPLC chromatogram. RP-HPLC: [linear gradient H_2O/MeCN (100:0) to (40:60) over 8 min].

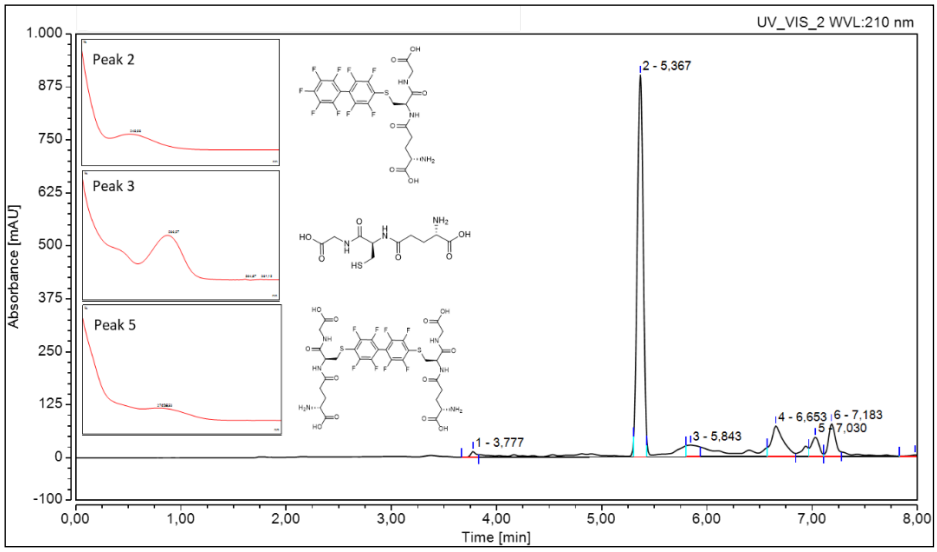


Figure S8. IR spectrum

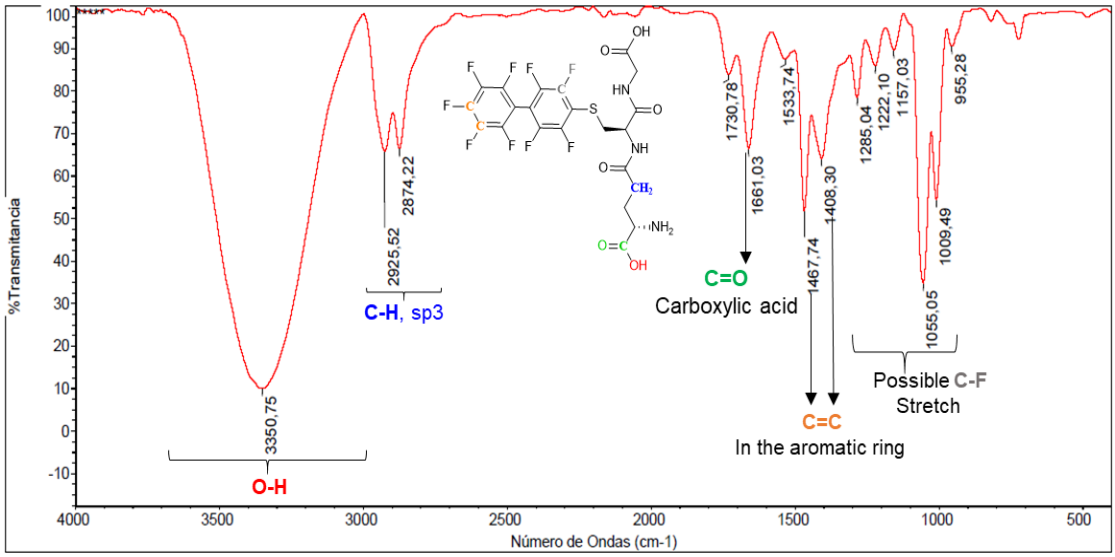


Figure S9. HPLC chromatogram of pure product. RP-HPLC: [linear gradient H_2O/MeCN (95:5) to (0:100) over 8 min]. ESI-MS spectrum.

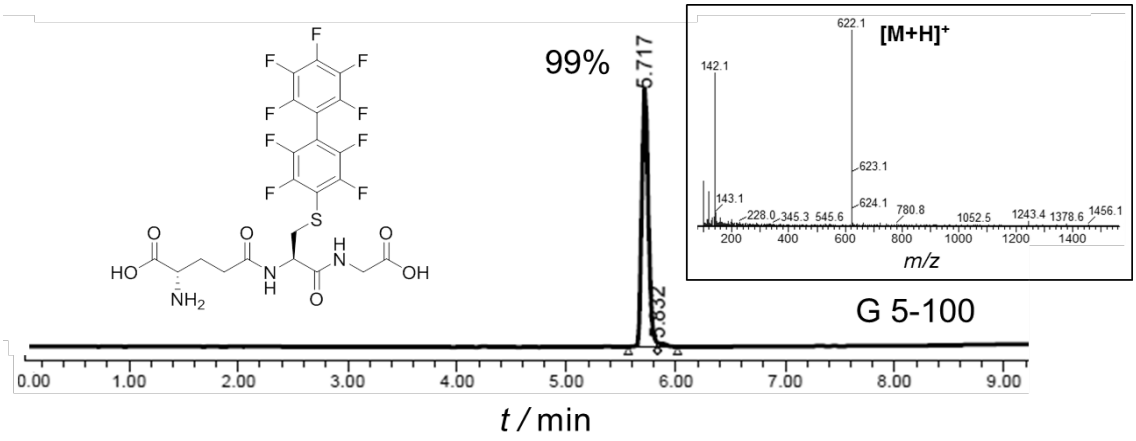
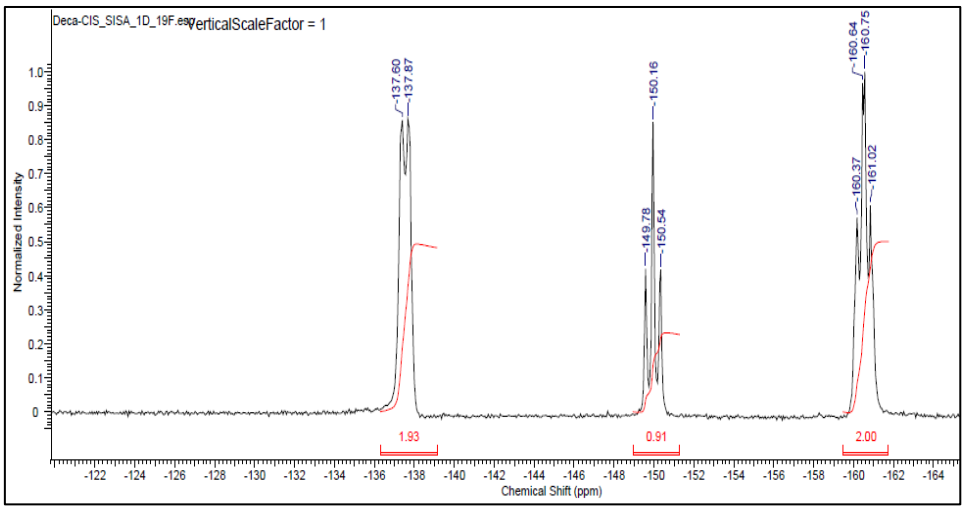
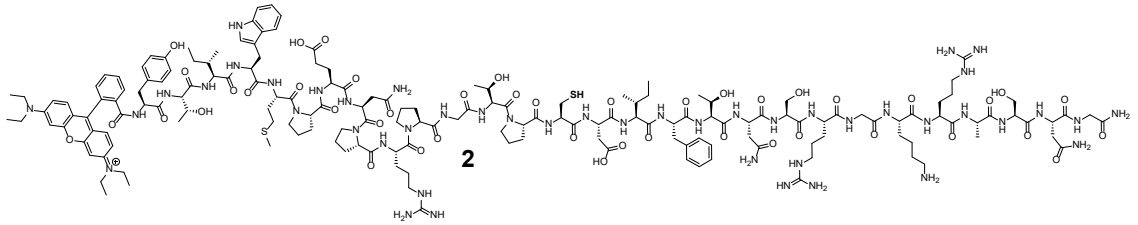


Figure S10. ^{19}F-NMR spectrum



Synthesis of $C_{12}F_9$-S-[RHB-peptide] (IIc)

A) Synthesis of RHB-Tyr-Thr-Ile-Trp-Met-Pro-Glu-Asn-Pro-Arg-Pro-Gly-Thr-Pro-Cys-Asp-Ile-Phe-Thr-Asn-Ser-Arg-Gly-Lys-Arg-Ala-Ser-Asn-Gly-NH_2



The automatically SPPS of YTIWMPENPRPGTP**C**DIFTNSRGKRASNG-NH_2 (RGV peptide) was carried out in CEM Discover Microwave Peptide Synthesizer following the Fmoc/tBu strategy and using Fmoc-Rink amide MBHA (200 mg, 0.73 mmol/g) as solid support. The peptide synthesis was monitored by RP-HPLC after the peptidyl resin cleavage of the intermediates (aliquot of 2 mg) using TFA/TIS/H_2O (95:2.5:2.5) during 1 h. When the peptide synthesis was achieved, rhodamine B (RHB) was manually conjugated to the RGV peptide. This reaction was carried out with rhodamine B (210 mg, 3 equiv), HBTU (166 mg, 3 equiv) and DIEA (152 µL, 6 equiv) in DMF for 45 min at room temperature. After completion of the synthesis, the resin was washed with dichloromethane (DCM) and the peptide was cleaved from the solid support with the concomitant amino acid side chains protecting groups elimination using the following acidolytic mixture: TFA/TIS/H_2O (95:2.5:2.5) for 1.5 h. Then the peptide crude was purified by semi-preparative HPLC-MS using linear gradient from 90:10 to 30:70 of H_2O/MeCN from over 15 min.

The purified peptide RHB-YTIWMPENPRPGTP**C**DIFTNSRGKRASNG-NH_2 was characterized by HPLC-MS: (H_2O/MeCN from 90:10 to 30:70 over 8 min): t_R: 4.9 and 5.2 min. *m/z* calculated for $C_{169}H_{247}N_{46}O_{44}S_2^+$ = 3691.18 Da, found: $[M+3H]^{3+}/3$ = 1231.5 and $[M+4H]^{4+}/4$ = 923.3. MALDI-TOF: *m/z* calculated for $C_{169}H_{247}N_{46}O_{44}S_2^+$ = 3691.18 Da, found $[M+H]^+$ = 3693.59 Da.

Figure S11. HPLC chromatogram of pure product. RP-HPLC: [linear gradient H_2O/MeCN (90:10) to (30:70) over 8 min]. ESI-MS spectrum.

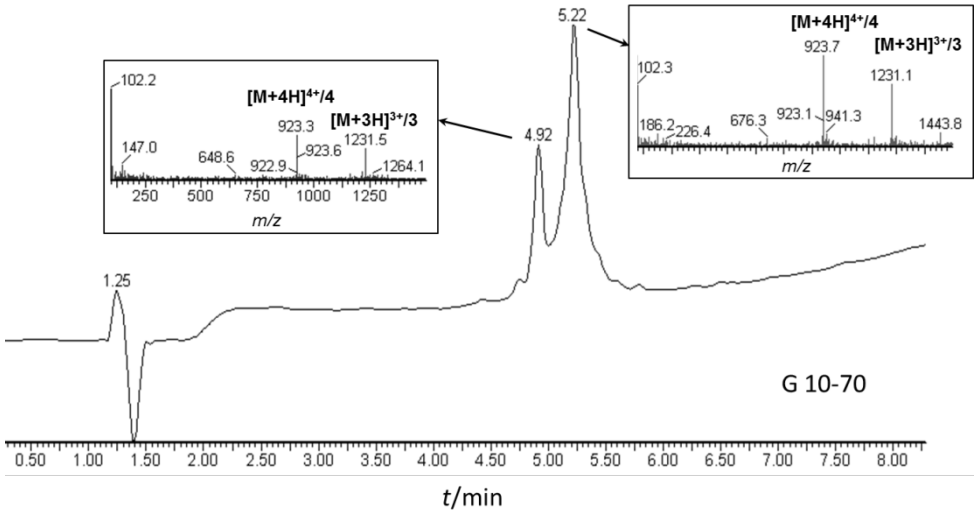


Figure S12. HPLC chromatogram of pure product **IIc**. ESI-MS spectrum.

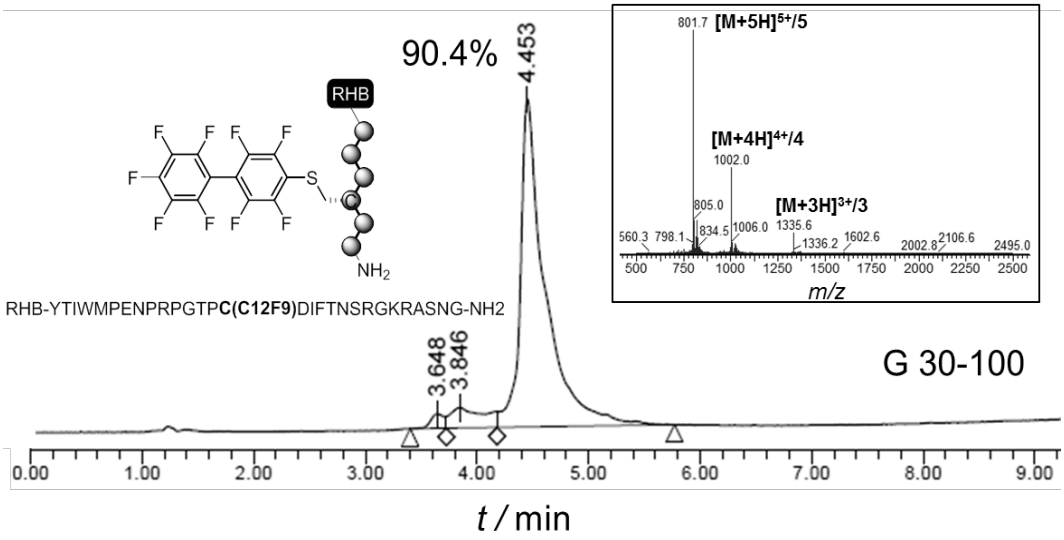
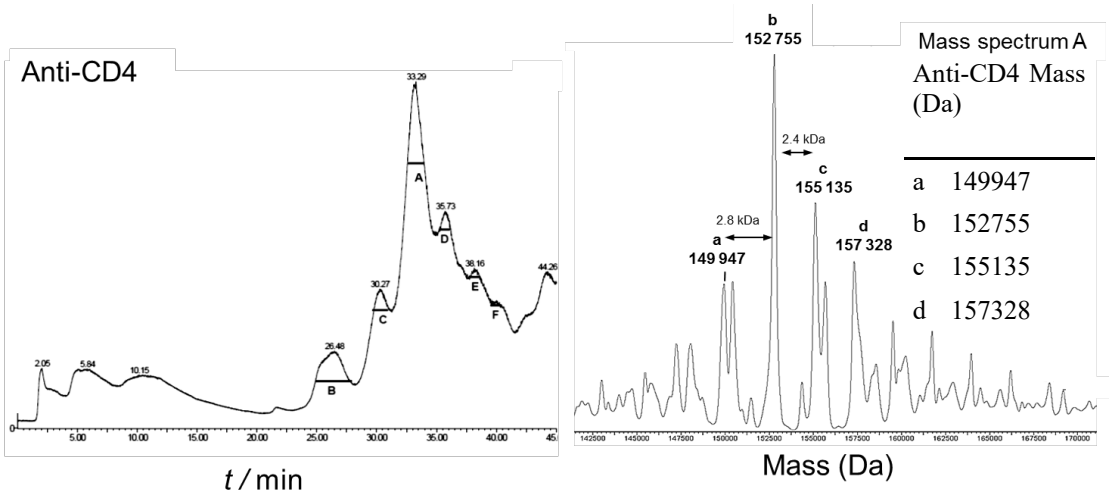
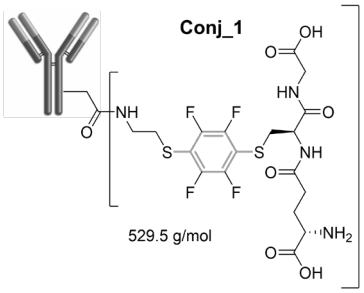


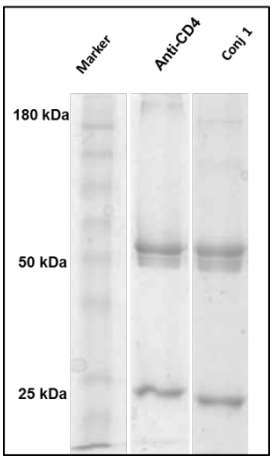
Figure S13. HPLC chromatogram and Mass spectrum of Anti-CD4



Conjugate 1. Anti-CD4-Cysteamine-C_6F_4-S-glutathione**.**

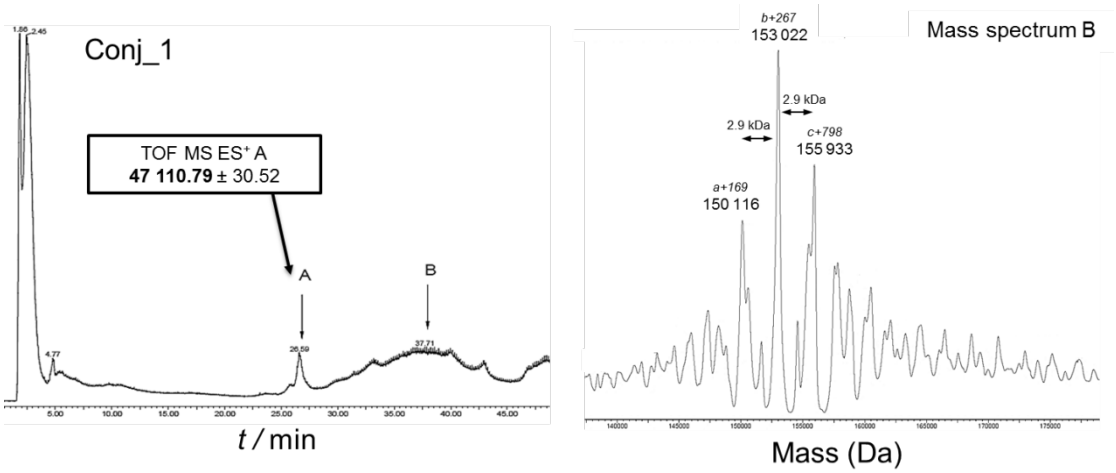


1 mL of Anti-CD4 solution (0.90 mg/mL, 6.0 µM), cystamine dihydrochloride (30 µg, 0.13 mM) and EDC·HCl (127 µg, 0.66 µmol, 0.66 mM). After PD-10 purification, 1 mL (0.85 mg/mL) of modified antibody was obtained. And then, it was treated with DTT (1 mg, 6.5 µmol). The solvent mixture was exchanged using a PD-10 column and approximately 1 mL (0.70 mg/mL) was obtained.



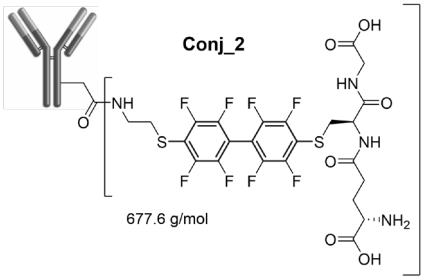
The previously synthetized C_6F_5-S-Glutathione (**Ia**) (2.0 mg, 4.2 µmol) was dissolved into DMF (50 µL) and it was added to the previously modified antibody solution (0.70 mg/mL). The reaction was left to react during 3 h at room temperature. After that, the crude was dialyzed (MWCO: 12-14,000) over PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH = 7.4) obtaining 1 mL of the conjugate (0.65 mg/mL). A sample of 0.64 mg/mL in water was diluted ½ with 1% formic acid aqueous solution to 2.1 µM. Then 12 µL was injected for MS analysis.

Figure S14. HPLC chromatogram and Mass spectrum of **Conjugate 1**

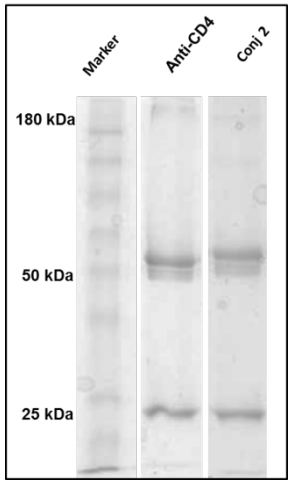


Conj_1	Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	150116	149947	169	530	0,3
2	153022	152755	267		0,5
3	155933	155135	798		1,5

Conjugate 2. Anti-CD4-Cysteamine-$C_{12}F_8$-S-glutathione.

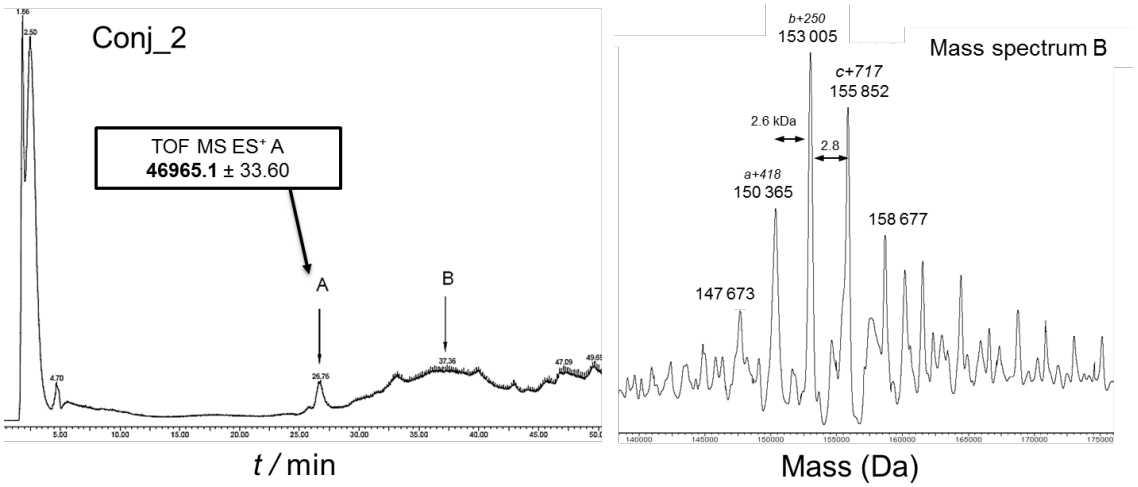


1 mL of Anti-CD4 solution (0.91 mg/mL, 6.0 µM), cystamine dihydrochloride (30 µg, 0.13 mM) and EDC·HCl (127 µg, 0.66 µmol, 0.66 mM). After PD-10 purification, approximately 1 mL (0.90 mg/mL) of modified antibody was obtained. And then, it was treated with DTT (1 mg, 6.5 µmol). The solvent mixture was exchanged using a PD-10 column and approximately 1 mL (0.85 mg/mL) was obtained.



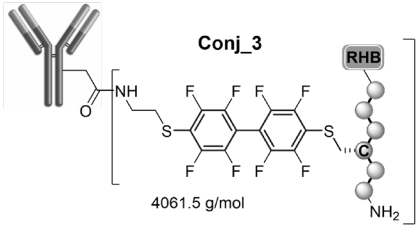
The previously synthetized $C_{12}F_9$-S-Glutathione (**IIa**) (2.0 mg, 3.2 µmol) was dissolved into DMF (50 µL) and it was added to the previously modified antibody solution (0.85 mg/mL). The reaction was left to react during 3 h at room temperature. After that, the crude was dialyzed (MWCO: 12-14,000) over PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH = 7.4) and over water obtaining 0.5 mL (0.64 mg/mL) and 0.5 mL (0.65 mg/mL) respectively. A sample of 0.64 mg/mL in water was diluted ½ with 1% formic acid aqueous solution to 2.1 µM. Then 12 µL was injected for MS analysis.

Figure S15. HPLC chromatogram and Mass spectrum of **Conjugate 2**

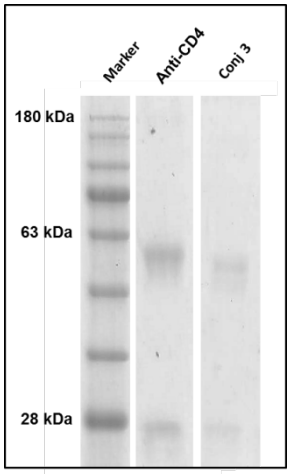


Conj_2	Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	150365	149947	418	677,6	0,6
2	153005	152755	250		0,4
3	155852	155135	717		1,1

Conjugate 3. Anti-CD4-Cysteamine-$C_{12}F_8$-S-RHB-Peptide.

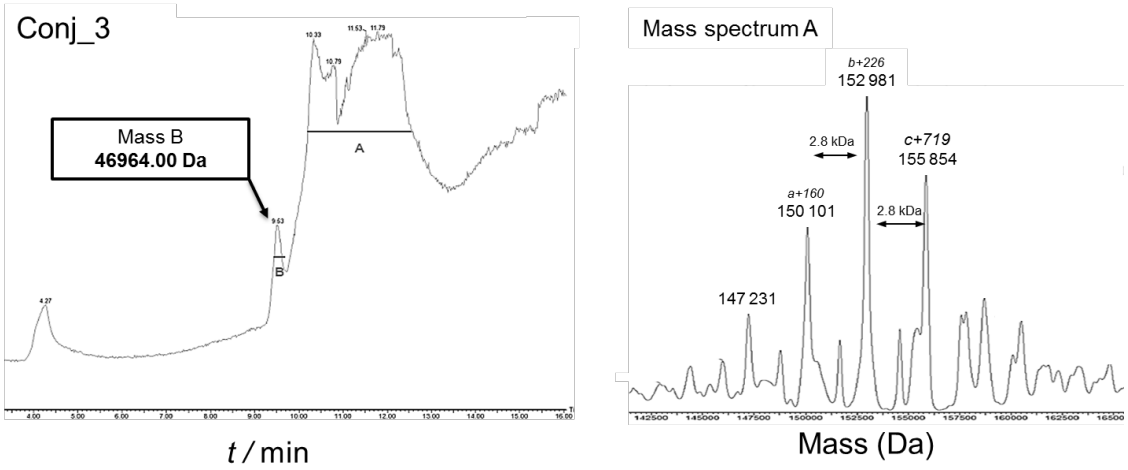


1 mL of the non-reduced cystamine modified antibody (0.60 mg/mL, 4.0 µM) was treated with DTT (1 mg, 6.5 µmol). The solvent mixture was exchanged using a PD-10 column and approximately 1.5 mL (0.44 mg/mL) was obtained.



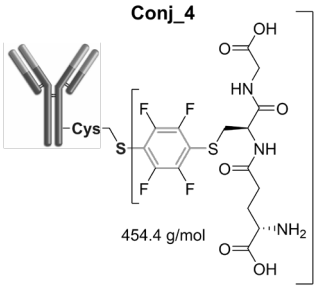
The previously synthetized $C_{12}F_9$-S-RHB-peptide (**IIb**) (1.0 mg, 0.25 µmol) was dissolved DMF (80 µL) and TEA (2 µL, 0.02 µmol) and it was added to 1 mL of the previously modified antibody solution (0.44 mg/mL). The reaction was left to react during 3 h at room temperature. After that, the crude was dialyzed (MWCO: 12-14,000) over PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH = 7.4) obtaining approximately 1.5 mL of the conjugate (0.49 mg/mL). Moreover, 0.5 mL of sample was dialyzed (MWCO: 12-14,000) in front of water in order to perform their mass analysis (0.33 mg/mL). A sample of 0.33 mg/mL in water was diluted ½ with 1% formic acid aqueous solution to 1.1 µM. Then 12 µL was injected for MS analysis.

Figure S16. HPLC chromatogram and Mass spectrum of **Conjugate 3**

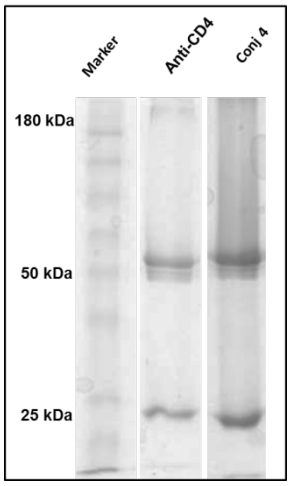


Conj_3	Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	150101	149947	154	4081,5	0,0
2	152981	152755	226		0,1
3	155854	155135	719		0,2

Conjugate 4. Anti-CD4-S-C_6F_4-S-Glutathione.



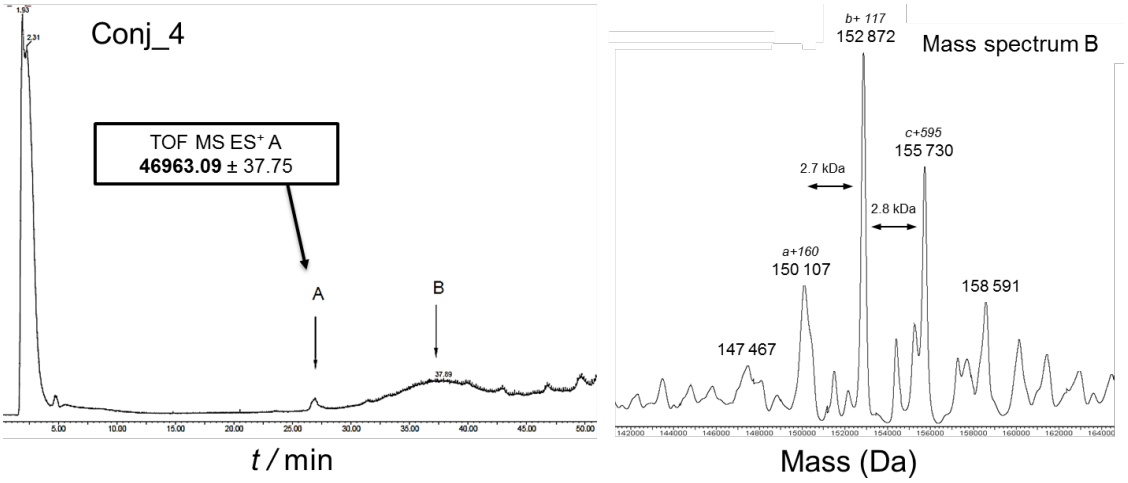
1 mL of Anti-CD4 solution (1.21 mg/mL, 8.1 µM) was treated with DTT (4 µg, 0.026 µmol). After PD-10 purification, 1 mL of the reduced antibody (1.08 mg/mL) was treated with a solution of C_6F_5-S-Glutathione (**3**) (2.0 mg, 4.2 µmol) in DMF (50 µL). The reaction was left to react during 3 h at room temperature. After PD-10 purification, approximately 1 mL of the modified antibody was obtained (0.91 mg/mL).



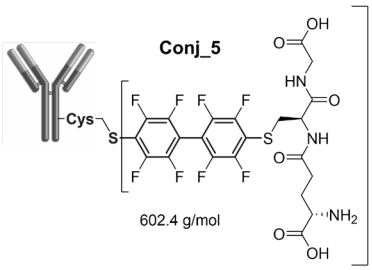
A sample of 0.78 mg/mL in water was diluted 1/5 with 1% formic acid aqueous solution to 1.0 µM. Then 12 µL was injected for MS analysis.

Conj_4	Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	150107	149947	160	454,4	0,4
2	152872	152755	117		0,3
3	155730	155135	595		1,3

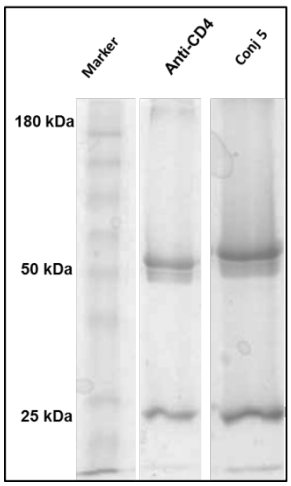
Figure S17. HPLC chromatogram and Mass spectrum of **Conjugate 4**



Conjugate 5. Anti-CD4-S-$C_{12}F_8$-S-Glutathione.



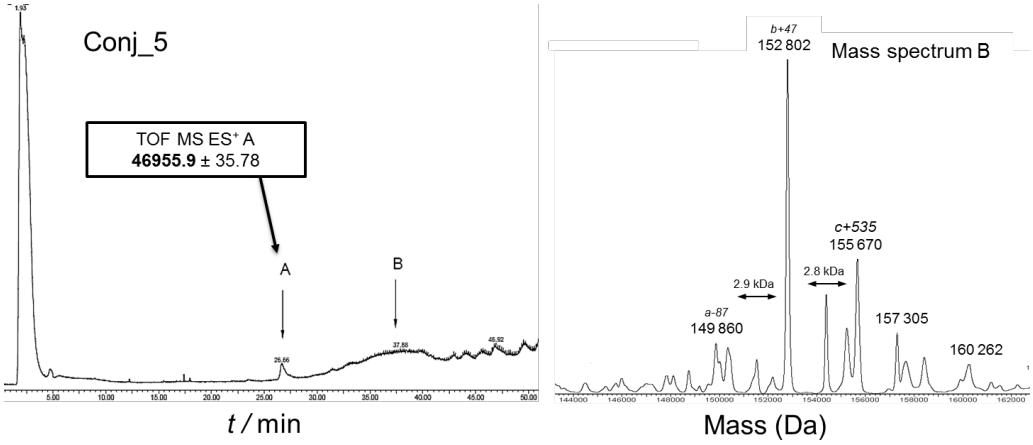
1 mL of Anti-CD4 solution (1.27 mg/mL, 8.5 µM) was treated with DTT (4 µg, 0.026 µmol). After PD-10 purification, 1 mL of the reduced antibody (0.95 mg/mL) was treated with a solution of $C_{12}F_9$-S-Glutathione (**IIa**) (2.0 mg, 3.2 µmol) in DMF (50 µL). The reaction was left to react during 3 h at room temperature. After PD-10 purification, approximately 1 mL of the modified antibody was obtained (0.80 mg/mL).



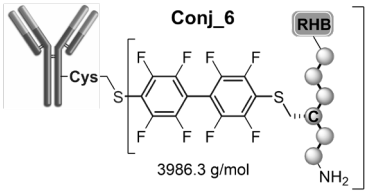
A sample of 0.80 mg/mL in water was diluted 1/5 with 1% formic acid aqueous solution to 1.1 µM. Then 12 µL was injected for MS analysis.

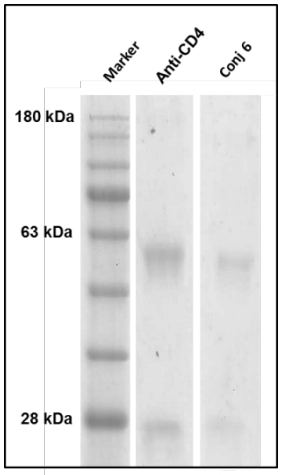
	Conj_5 Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	149860	149947	-87	602,4	-0,1
2	152802	152755	47		0,1
3	155670	155135	535		0,9

Figure S18. HPLC chromatogram and Mass spectrum of **Conjugate 5**



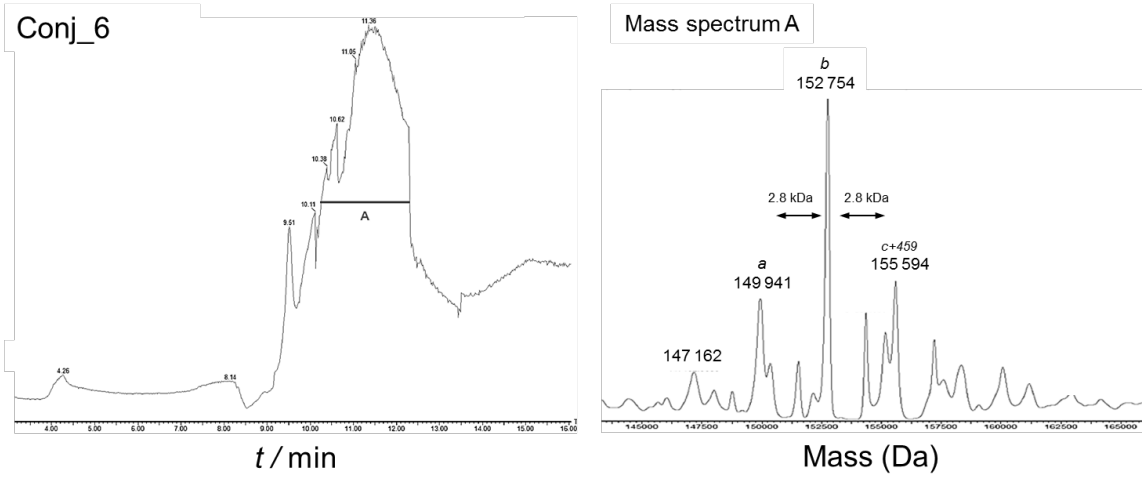
Conjugate 6. Anti-CD4-S-$C_{12}F_8$-S-RHB-Peptide.





1 mL of Anti-CD4 solution (2.0 mg/mL, 8.5 µM) was treated with DTT (4 µg, 0.026 µmol). After PD-10 purification, 1 mL of the reduced antibody (1.15 mg/mL) was treated with a solution of $C_{12}F_9$-S-RHB-peptide (**IIb**) (1.0 mg, 0.25 µmol) dissolved in DMF (80 µL) and TEA (2 µL, 0.02 µmol). The reaction was left to react during 1 h at 37 ºC. After that, the crude was dialyzed (MWCO: 12-14,000) over PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl and 2.7 mM KCl, pH = 7.4) obtaining two fractions of 0.5 mL of conjugate (0.88 and 0.33 mg/mL). Moreover, 0.5 mL of the crude was dialyzed (MWCO: 12-14,000) in front of water in order to perform their mass analysis (0.34 mg/mL). A sample of 0.33 mg/mL in water was diluted ½ with 1% formic acid aqueous solution to 1.1 µM. Then 12 µL was injected for MS analysis.

Figure S19. HPLC chromatogram and Mass spectrum of **Conjugate 5**



Conj_6	Mass detected (Da)	Anti-CD4 Mass (Da)	Δ_{mass} (Da)	MW	DAR
1	149941	149947	-6	3986,3	0,0
2	152754	152755	-1		0,0
3	155594	155135	459		0,1

Figura S20. Optimized geometric structures DFT/B3LYP level: (Ia) S-(perfluorophenyl)-L-cysteine, (Ib) S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-cysteine, (IIa) S-(perfluorophenyl)-L-glutathione, and (IIb) S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-glutathione.

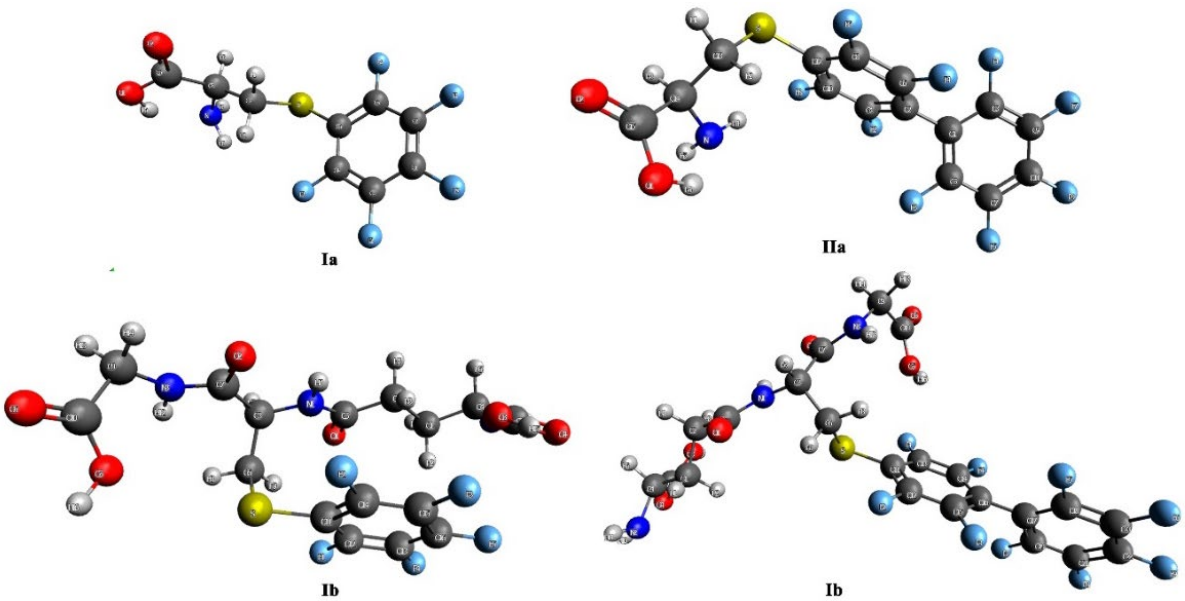


Figura S21 Optimized geometric structures DFT/B3LYP level of S-(perfluorophenyl)-L-cysteine (Ia).

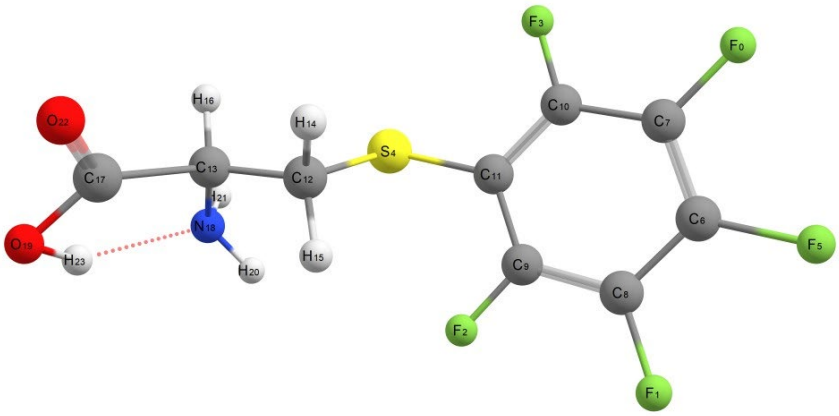


Table S11. Selected bond lengths (Å) of compound **Ia** using DFT-method from ORCA calculations.

Bond	Length(Å)	Bond	Length(Å)	Bond	Length(Å)
C(1)-C(2)	1.387	C(1)-F(5)	1.330	S-C(7)	1.833
C(1)-C(3)	1.389	C(2)-F(1)	1.333	C(7)-H(1)	1.087
C(2)-C(5)	1.388	C(3)-F(2)	1.333	C(7)-H(2)	1.091
C(3)-C(4)	1.385	C(4)-F(3)	1.338	C(7)-C(8)	1.525
C(4)-C(6)	1.395	C(5)-F(4)	1.333	C(8)-H(3)	1.093
C(5)-C(6)	1.395	C(6)-S	1.767	C(8)-N	1.467
C(8)-C(9)	1.547	N-H(4)	1.013	N-H(5)	1.011
C(9)-O(2)	1.201	C(9)-O(1)	1.333	O(1)-H(6)	0.987

Table S2. Selected bond angles (°) of compound Ia using DFT-method from ORCA calculations.

Angle	Degree (°)	Angle	Degree (°)
C(6)-S-C(7)	100.24	C(2)-C(1)-C(3)	119.91
F(5)-C(1)-C(3)	119.99	F(5)-C(1)-C(2)	120.10
F(1)-C(2)-C(1)	119.78	F(1)-C(2)-C(5)	120.48
C(1)-C(2)-C(5)	119.74	C(1)-C(3)-C(4)	119.49
F(2)-C(3)-C(4)	120.65	C(3)-C(4)-C(6)	121.97
F(2)-C(3)-C(1)	119.86	F(3)-C(4)-C(6)	120.08
F(3)-C(4)-C(3)	117.95	F(4)-C(5)-C(6)	120.52
F(4)-C(5)-C(2)	117.85	C(2)-C(5)-C-(6)	121.63
S-C(6)-C(4)	121.77	C(4)-C(6)-C(5)	117.26
S-C(6)-C(5)	120.94	H(1)-C(7)-H(2)	109.17
C(8)-C(7)-H(2)	110.06	S-C(7)-H(2)	109.30
C(8)-C(7)-H(1)	109.56	S-C(7)-H(1)	109.68
S-C(7)-C(8)	109.06	H(3)-C(8)-N	109.06
C(7)-C(8)-N	114.37	H(3)-C(8)-C(9)	105.19
C(7)-C(8)-C(9)	108.55	C(9)-C(8)-N	110.32
C(7)-C(8)-H(3)	108.95	O(1)-C(9)-O(2)	123.78
C(8)-C(9)-O(2)	122.56	C(8)-C(9)-O(1)	113.65
H(4)-N-H(5)	107.58	C(8)-N-H(4)	111.72
C(8)-N-H(5)	112.37	C(9)-O(1)-H(6)	105.06

Figura S22. Optimized geometric structures DFT/B3LYP level of S-(perfluorophenyl)-L-glutathione (Ib).

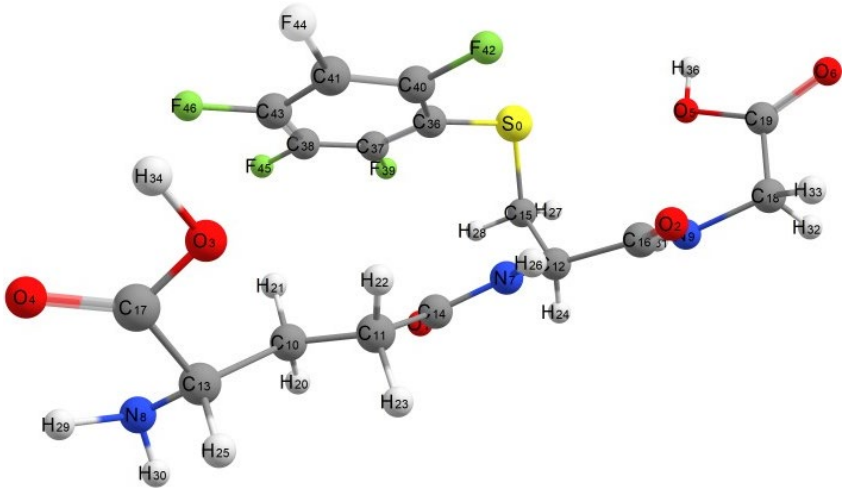


Table S3. Selected bond lengths (Å) of compound Ib using DFT-method from ORCA calculations.

Bond	Length(Å)	Bond	Length(Å)	Bond	Length(Å)
C(11)-C(10)	1.5234	C(12)-N(7)	1.4397	C(13)-N(8)	1.4548
C(13)-C(10)	1.5375	C(14)-N(7)	1.3563	C(14)-C(11)	1.5192
C(14)-O(1)	1.2225	C(15)-C(12)	1.5391	C(15)-S(0)	1.8247
C(16)-C(12)	1.5326	C(16)-N(9)	1.3592	C(16)-O(2)	1.2170
C(17)-C(13)	1.5206	C(17)-O(4)	1.2024	C(17)-O(3)	1.3517
C(18)-N(9)	1.4421	C(19)-C(18)	1.5202	C(19)-O(6)	1.1995
C(19)-O(5)	1.3512	H(20)-C(10)	1.0897	H(21)-C(10)	1.0909
H(22)-C(11)	1.0891	H(23)-C(11)	1.0964	H(24)-C(12)	1.0967
H(25)-C(13)	1.1052	H(26)-N(7)	1.0105	H(27)-C(15)	1.0906
H(28)-C(15)	1.0855	H(29)-N(8)	1.0127	H(30)-N(8)	1.0111
H(31)-N(9)	1.0060	H(32)-C(18)	1.0919	H(33)-C(18)	1.0885
H(34)-O(3)	0.9707	H(35)-O(5)	0.9707	C(36)-S(0)	1.7691
C(37)-C(36)	1.3948	C(38)-C(37)	1.3860	F(39)-C(37)	1.3345
C(40)-C(36)	1.3948	F(42)-C(40)	1.3342	C(43)-C(41)	1.3863
C(43)-C(38)	1.3876	F(44)-C(41)	1.3375	F(45)-C(38)	1.3323
zF(46)-C(43)	1.3309				

Table S4 Selected bond angles (°) of compound Ib using DFT-method from ORCA calculations.

Angle	Degree (°)	Angle	Degree (°)
C(15)-S(0)-C(36)	101.75	C(17)-O(3)-H(34)	107.39
C(19)-O(5)-H(35)	107.26	C(14)-N(7)-H(26)	121.99
C(12)-N(7)-H(26)	115.25	C(12)-N(7)-C(14)	122.63
H(29)-N(8)-H(30)	109.34	C(13)-N(8)-H(30)	111.17
C(13)-N(8)-H(29)	110.68	C(16)-N(9)-H(31)	118.12
C(18)-N(9)-H(31)	117.60	C(16)-N(9)-C(18)	121.35
C(13)-C(10)-H(20)	107.12	C(13)-C(10)-H(21)	109.26
C(11)-C(10)-H(20)	108.35	C(11)-C(10)-C(13)	115.28
H(20)-C(10)-H(21)	105.43	C(11)-C(10)-H(21)	110.86
C(10)-C(11)-H(22)	111.71	C(10)-C(11)-H(23)	109.77
C(14)-C(11)-H(22)	109.69	C(10)-C(11)-C(14)	112.23
C(14)-C(11)-H(23)	106.98	H(22)-C(11)-H(23)	106.17
C(16)-C(12)-H(24)	108.97	C(15)-C(12)-C(16)	112.15
N(7)-C(12)-C(16)	107.07	N(7)-C(12)-H(24)	109.42
C(15)-C(12)-H(24)	106.56	N(7)-C(12)-C(15)	112.62
C(10)-C(13)-H(25)	107.81	C(17)-C(13)-H(25)	104.00
N(8)-C(13)-H(25)	113.11	N(8)-C(13)-C(10)	109.72
C(10)-C(13)-C(17)	114.46	N(8)-C(13)-C(17)	107.74

N(7)-C(14)-C(11)	114.72	O(1)-C(14)-C(11)	123.36
O(1)-C(14)-N(7)	121.91	H(27)-C(15)-H(28)	107.64
C(12)-C(15)-H(28)	108.97	S(0)-C(15)-H(28)	110.10
C(12)-C(15)-H(27)	111.03	S(0)-C(15)-H(27)	104.29
S(0)-C(15)-C(12)	114.55	N(9)-C(16)-C(12)	114.93
O(2)-C(16)-C(12)	121.87	O(2)-C(16)-N(9)	123.21
O(3)-C(17)-O(4)	122.30	O(4)-C(17)-C(13)	124.83
O(3)-C(17)-C(13)	112.83	H(32)-C(18)-H(33)	107.54
C(19)-C(18)-H(33)	107.45	N(9)-C(18)-H(33)	108.25
C(19)-C(18)-H(32)	107.16	N(9)-C(18)-H(32)	110.90
N(9)-C(18)-C(19)	115.24	O(6)-C(19)-C(18)	123.45
O(5)-C(19)-C(18)	112.92	O(5)-C(19)-O(6)	123.61
S(0)-C(36)-C(37)	122.28	C(37)-C(36)-C(40)	117.25
S(0)-C(36)-C(40)	120.40	C(36)-C(37)-F(39)	120.18
C(36)-C(37)-C(38)	121.86	C(38)-C(37)-F(39)	117.96
C(43)-C(38)-F(45)	119.79	C(37)-C(38)-F(45)	120.64
C(37)-C(38)-C(43)	119.56	C(36)-C(40)-F(42)	120.47
C(43)-C(41)-F(44)	119.70	C(41)-C(43)-F(46)	120.03
C(38)-C(43)-F(46)	120.14	C(38)-C(43)-C(41)	119.83

Figura S23. Optimized geometric structures DFT/B3LYP level of S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-cysteine (IIa).

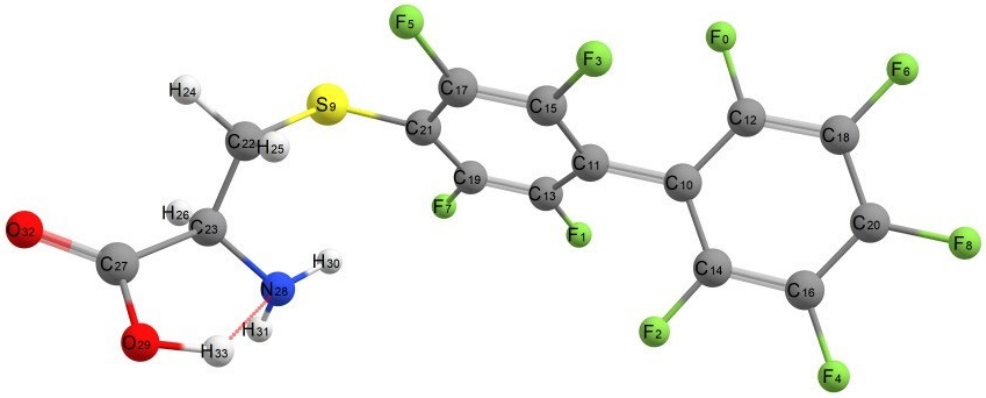


Figura S24. Optimized geometric structures DFT/B3LYP level of S-(perfluoro-[1,1'-biphenyl]-4-yl)-L-glutathione (IIb).