

Supplementary Information

Synthesis and Systematic Study on the Effect of Different PEG units on Stability of PEGylated, Integrin $\alpha v\beta 6$ -Specific A20FMDV2 Analogues in Rat Serum and Human Plasma

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Structure, RP-HPLC and MS profile of synthetic peptides 13-30

NAVPNLRGDLQVLAQKVART-OH (13) (A20FMDV2)

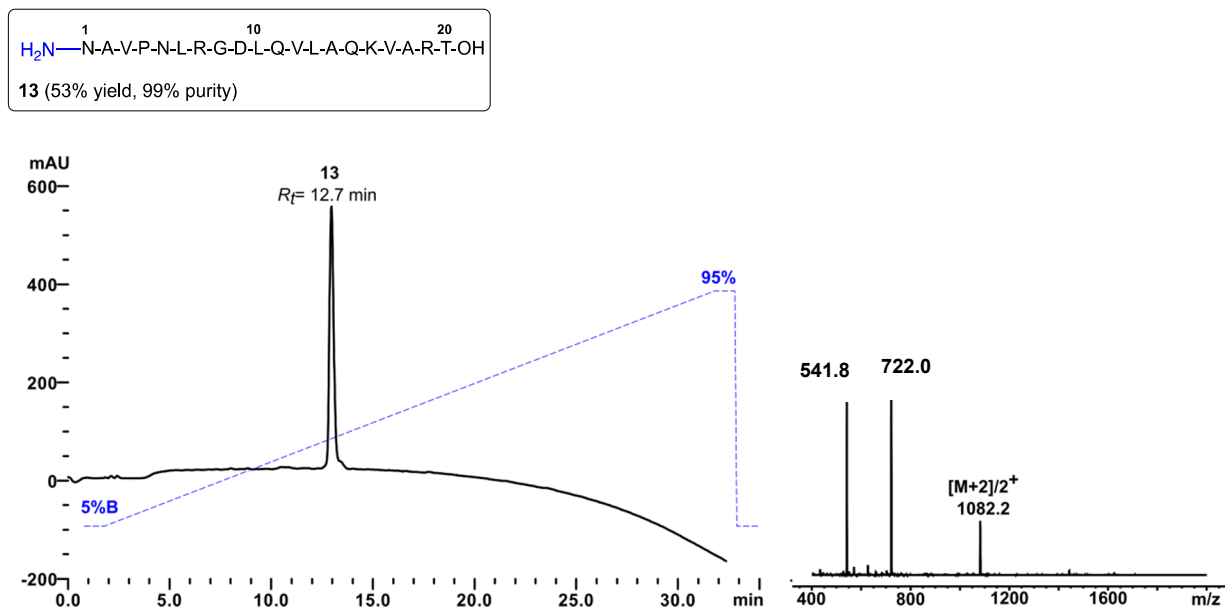


Figure S1. HPLC and MS traces of pure peptide 13 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 53% yield). R_t 12.7 min; m/z 541.8 $[M + 4H]^{4+}$ requires 541.9, m/z 722.0 $[M + 3H]^{3+}$ requires 722.2, m/z 1082.2 $[M + 2H]^{2+}$ requires 1082.8. Mass deconvolution calculated at 2162.87 Da with standard deviation of 0.42; theoretical mass calculated at 2163.50 Da.

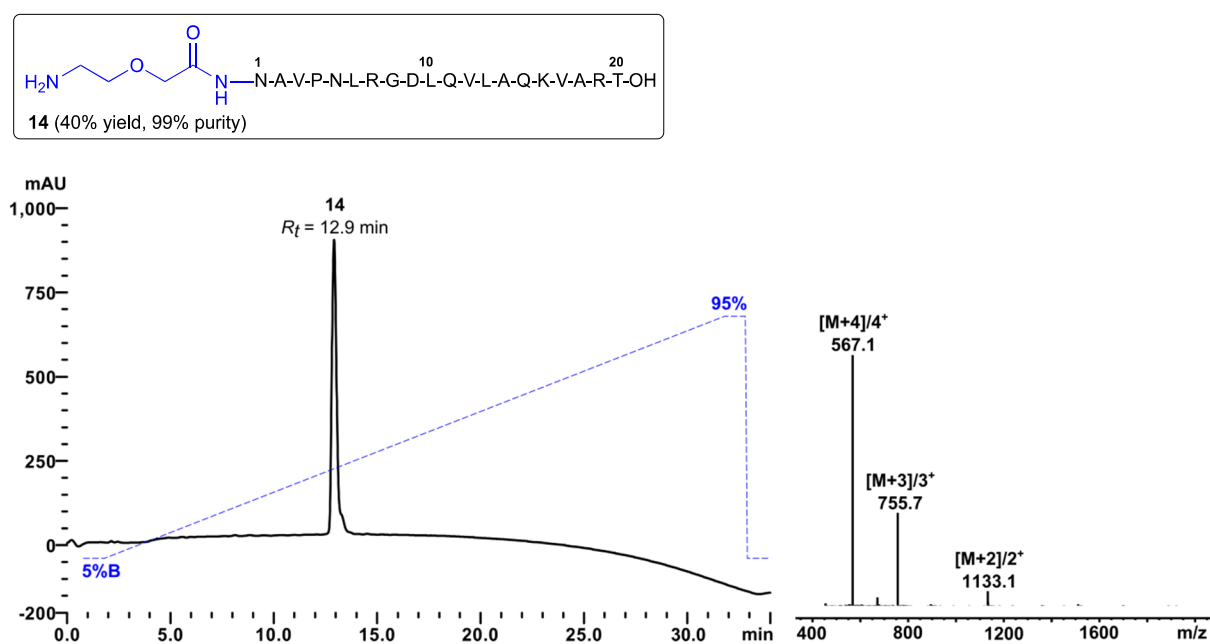
 $\text{H}_2\text{N-PEG}_1\text{-CH}_2\text{CO-NAVPNLRGDLQVLAQKVART-OH}$ (14)

Figure S2. HPLC and MS traces of pure peptide 14 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 40% yield). R_t 12.9 min; m/z 567.1 $[M + 4H]^{4+}$ requires 567.2, m/z 755.7 $[M + 3H]^{3+}$ requires 755.9, m/z 1133.1 $[M + 2H]^{2+}$ requires 1133.3. Mass deconvolution calculated at 2264.23 Da with standard deviation of 0.15; theoretical mass calculated at 2264.62 Da.

H₂N-PEG₂-CH₂CO-NAVPNLRGDLQVLAQKVART-OH (15)

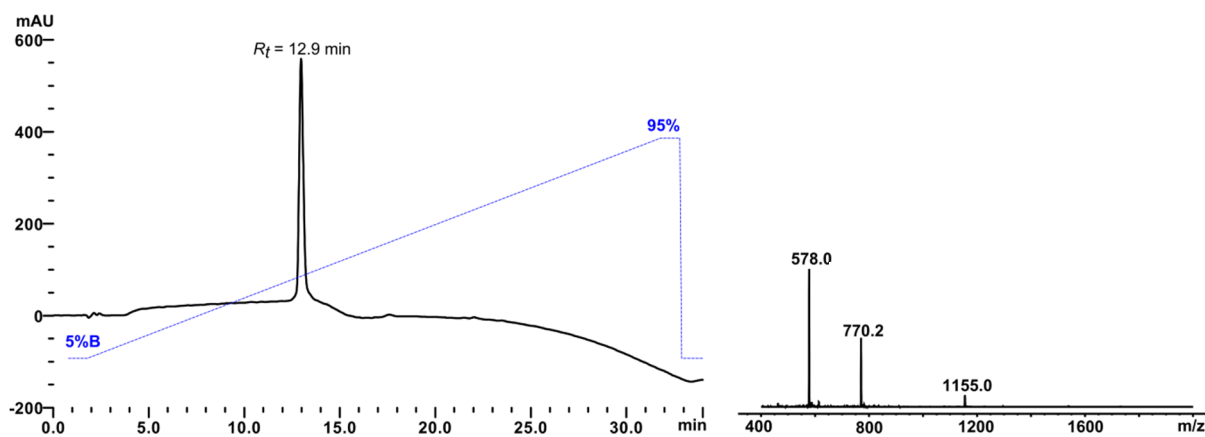
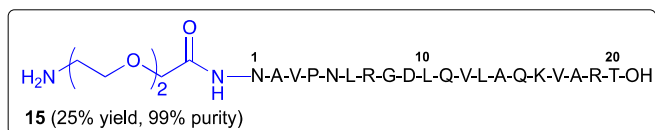


Figure S3. HPLC and MS traces of pure peptide 15 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 25% yield). R_t 12.9 min; m/z 578.0 $[M + 4H]^{4+}$ requires 578.2, m/z 770.2 $[M + 3H]^{3+}$ requires 770.6, m/z 1155.0 $[M + 2H]^{2+}$ requires 1155.3. Mass deconvolution calculated at 2307.87 Da with standard deviation of 0.23; theoretical mass calculated at 2308.67 Da.

H₂N-PEG₃-CH₂CO-NAVPNLRGDLQVLAQKVART-OH (16)

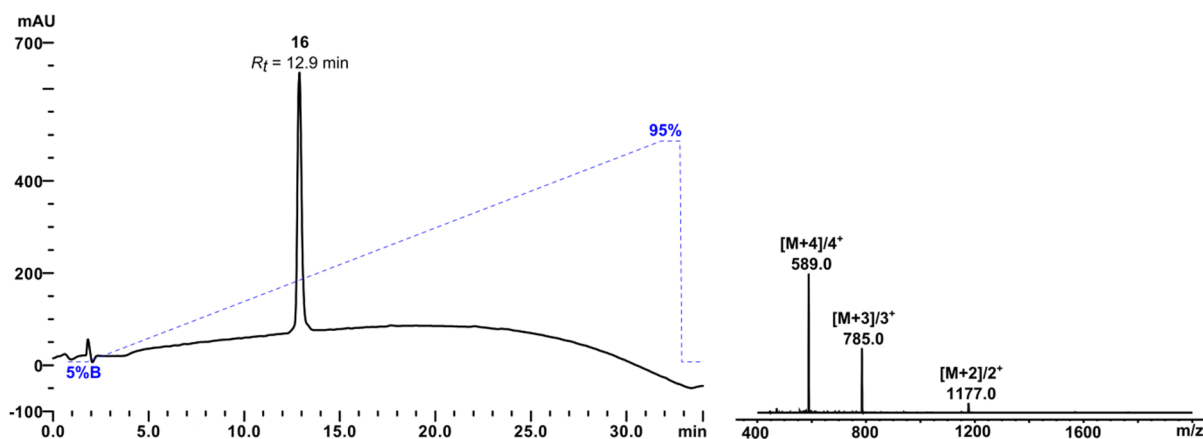
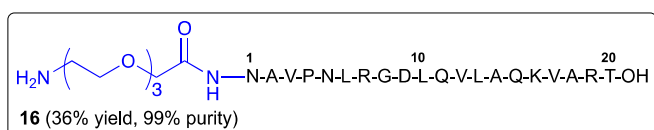


Figure S4. HPLC and MS traces of pure peptide 16 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 36% yield). R_t 12.9 min; m/z 589.0 $[M + 4H]^{4+}$ requires 589.2, m/z 785 $[M + 3H]^{3+}$ requires 785.3, m/z 1177.0 $[M + 2H]^{2+}$ requires 1177.4. Mass deconvolution calculated at 2352.00 Da with standard deviation of 0.00; theoretical mass calculated at 2352.73 Da.

H₂N-PEG₄-CH₂CO-NAVPNLRGDLQVLAQKVART-OH (17)

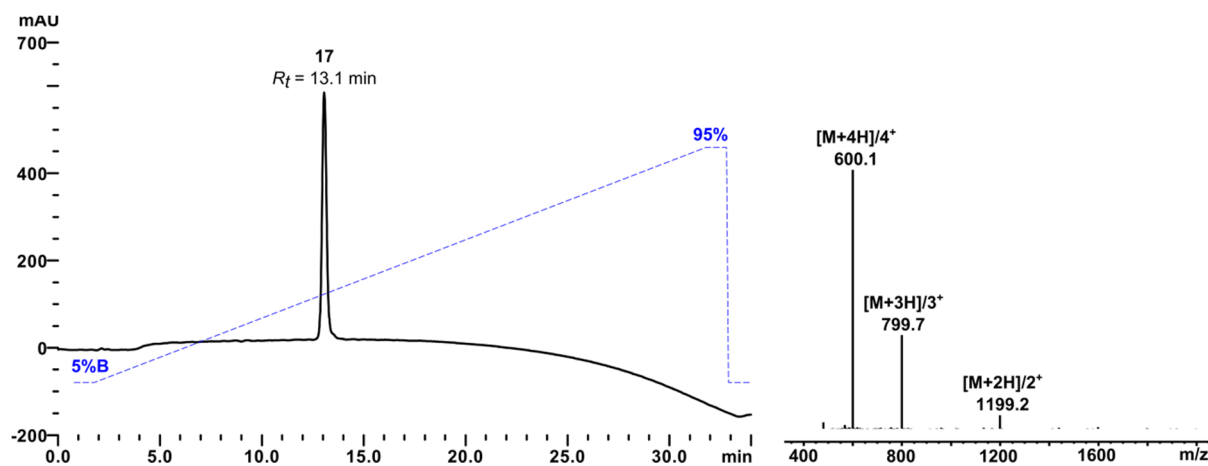
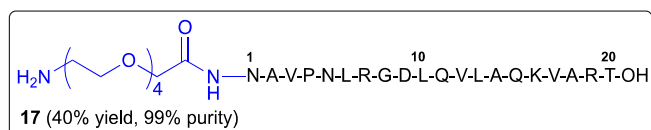


Figure S5. HPLC and MS traces of pure peptide 17 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 40% yield). R_t 13.1 min; m/z 600.1 $[M + 4H]^{4+}$ requires 600.2, m/z 799.7 $[M + 3H]^{3+}$ requires 799.9, m/z 1199.2 $[M + 2H]^{2+}$ requires 1199.4. Mass deconvolution calculated at 2396.30 Da with standard deviation of 0.17; theoretical mass calculated at 2396.78 Da.

H₂N-PEG₅-CH₂CO-NAVPNLRGDLQVLAQKVART-OH (18)

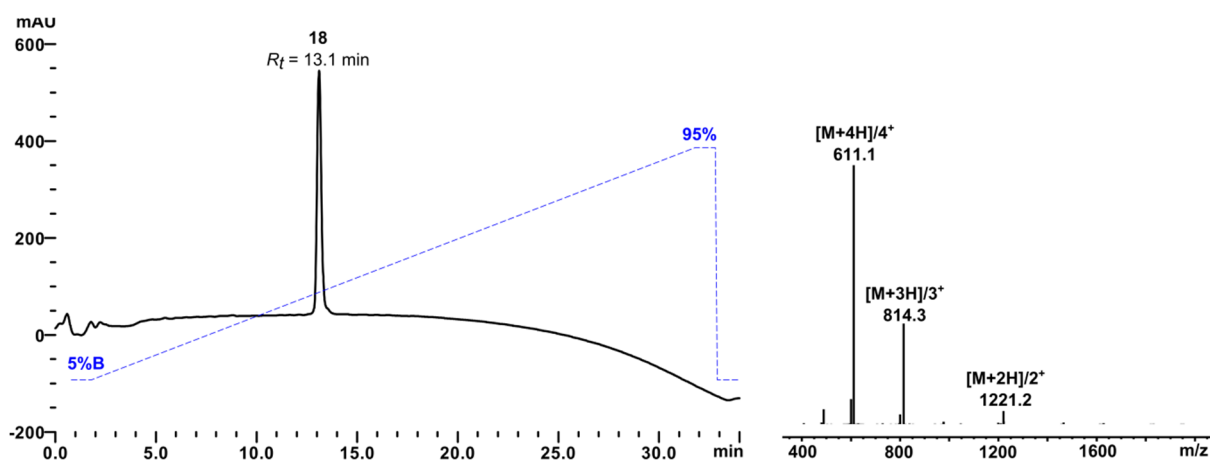
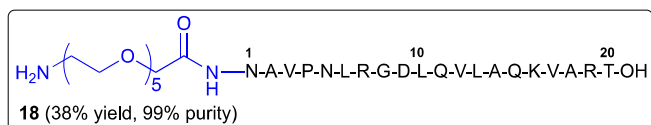


Figure S6. HPLC and MS traces of pure peptide 18 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 38% yield). R_t 13.1 min; m/z 611.1 $[M + 4H]^{4+}$ requires 611.2, m/z 814.3 $[M + 3H]^{3+}$ requires 814.6, m/z 1221.2 $[M + 2H]^{2+}$ requires 1221.4. Mass deconvolution calculated at 2440.23 Da with standard deviation of 0.29; theoretical mass calculated at 2440.83 Da.

H₂N-PEG₈-CH₂CO-NAVPNLRGDLQVLAQKVART-OH (19)

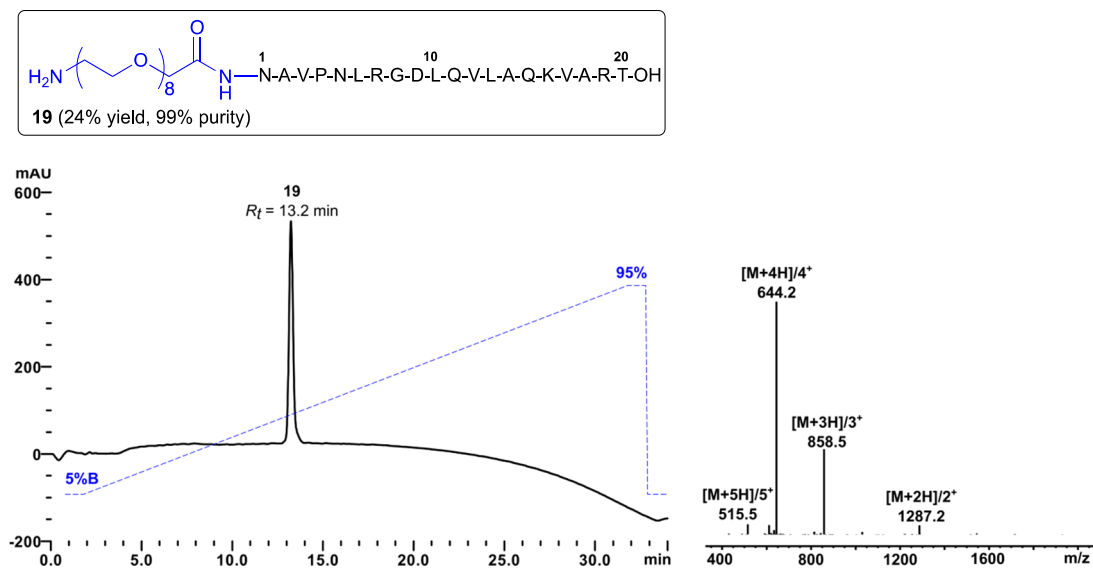


Figure S7. HPLC and MS traces of pure peptide 19 (*ca* 99% as judged by peak area of RP-HPLC at 210 nm, 24% yield). R_t 13.2 min; m/z 515.5 $[M+5H]^+/5^+$ requires 515.6, m/z 644.2 $[M+4H]^+/4^+$ requires 644.3, m/z 858.5 $[M+3H]^+/3^+$ requires 858.7, m/z 1287.2 $[M+2H]^+/2^+$ requires 1287.5. Mass deconvolution calculated at 2572.55 Da with standard deviation of 0.17; theoretical mass calculated at 2572.99 Da.

H₂N-PEG₅-CH₂CH₂CO-NAVPNLRGDLQVLAQKVART-OH (20)

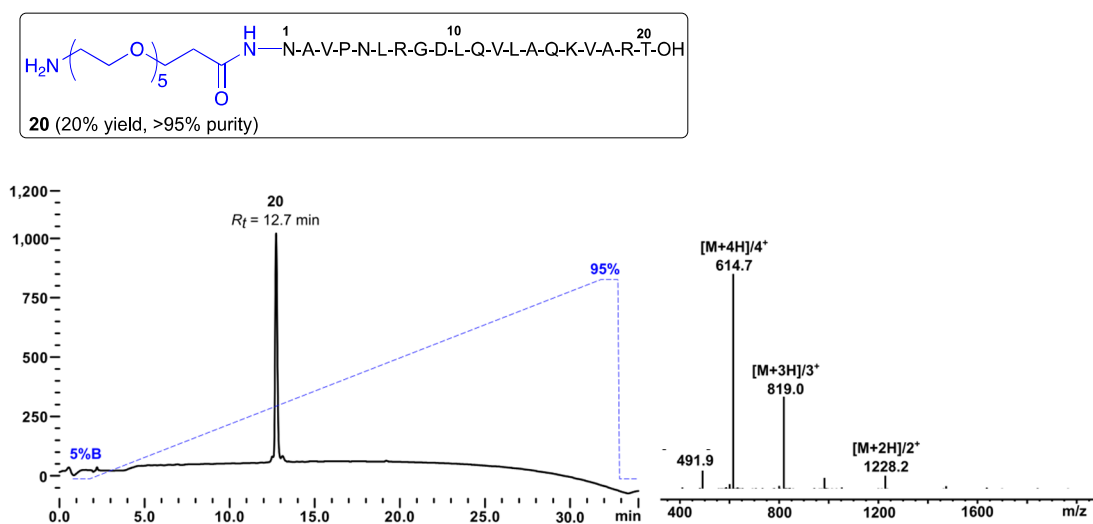


Figure S8. HPLC and MS traces of pure peptide 20 (>95% as judged by peak area of RP-HPLC at 210 nm, 20% yield). R_t 12.7 min; m/z 491.9 $[M+5H]^+/5^+$ requires 492.0, m/z 614.7 $[M+4H]^+/4^+$ requires 614.7, m/z 819.0 $[M+3H]^+/3^+$ requires 819.3, m/z 1228.2 $[M+2H]^+/2^+$ requires 1228.4. Mass deconvolution calculated at 2454.43 Da with standard deviation of 0.17; theoretical mass calculated at 2454.86 Da.

H₂N-PEG₁₀-CH₂CH₂CO-NAVPNLRGDLQVLAQKVART-OH (21)

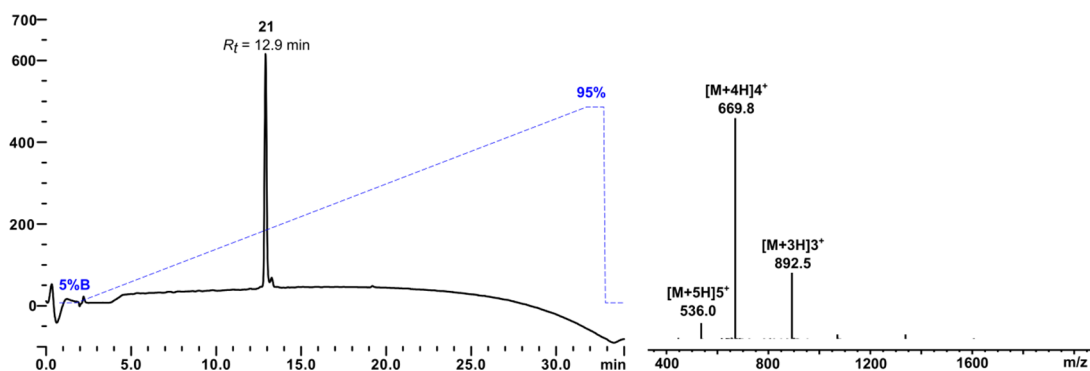
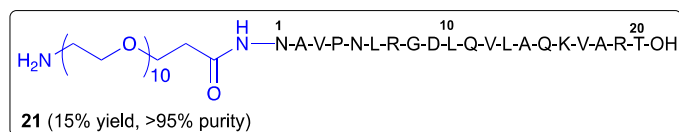


Figure S9. HPLC and MS traces of pure peptide 21 (>95% as judged by peak area of RP-HPLC at 210 nm, 15% yield). R_t 12.9 min; m/z 536.0 $[M + 5H]^{5+}$ requires 536.0, m/z 669.8 $[M + 4H]^{4+}$ requires 669.8, m/z 892.5 $[M + 3H]^{3+}$ requires 892.7. Mass deconvolution calculated at 2674.90 Da with standard deviation of 0.36; theoretical mass calculated at 2675.12 Da.

H₂N-PEG₁₅-CH₂CH₂CO-NAVPNLRGDLQVLAQKVART-OH (22)

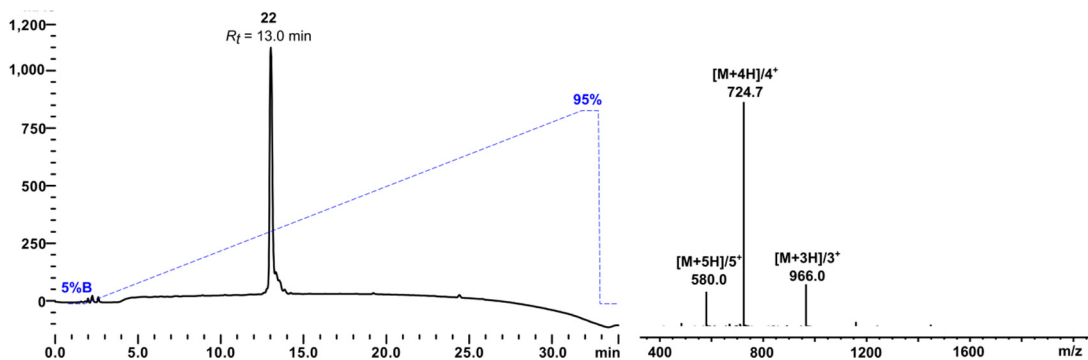
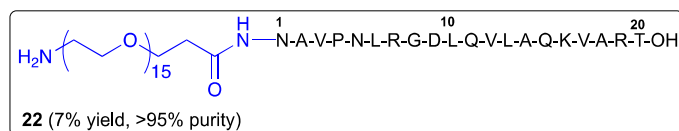


Figure S10. HPLC and MS traces of pure peptide 22 (>95% as judged by peak area of RP-HPLC at 210 nm, 7% yield). R_t 13.0 min; m/z 580.0 $[M + 5H]^{5+}$ requires 580.1, m/z 724.7 $[M + 4H]^{4+}$ requires 724.8, m/z 966.0 $[M + 3H]^{3+}$ requires 966.1. Mass deconvolution calculated at 2894.93 Da with standard deviation of 0.12; theoretical mass calculated at 2895.36 Da.

H₂N-PEG₂₀-CH₂CH₂CO-NAVPNLRGDLQVLAQKVART-OH (23)

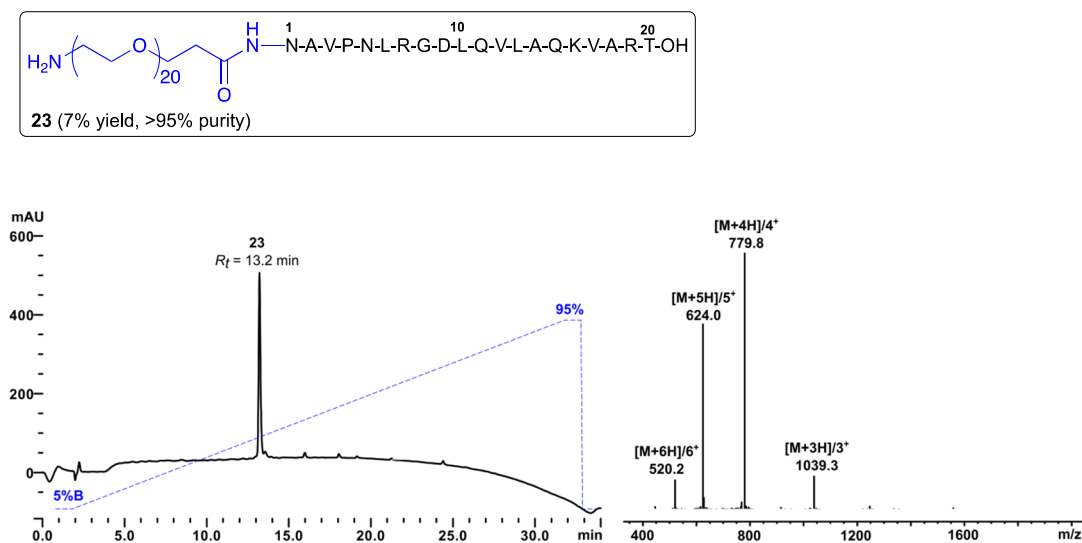


Figure S11. HPLC and MS traces of pure peptide 23 (>95% as judged by peak area of RP-HPLC at 210 nm, 7% yield). R_t 13.2 min; m/z 520.2 $[M + 6H]^{6+}$ requires 520.3, m/z 624.0 $[M + 5H]^{5+}$ requires 624.1, m/z 779.8 $[M + 4H]^{4+}$ requires 779.9, m/z 1039.3 $[M + 3H]^{3+}$ requires 1039.6. Mass deconvolution calculated at 3115.08 Da with standard deviation of 0.15; theoretical mass calculated at 3115.65 Da.

DTPA-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (24)

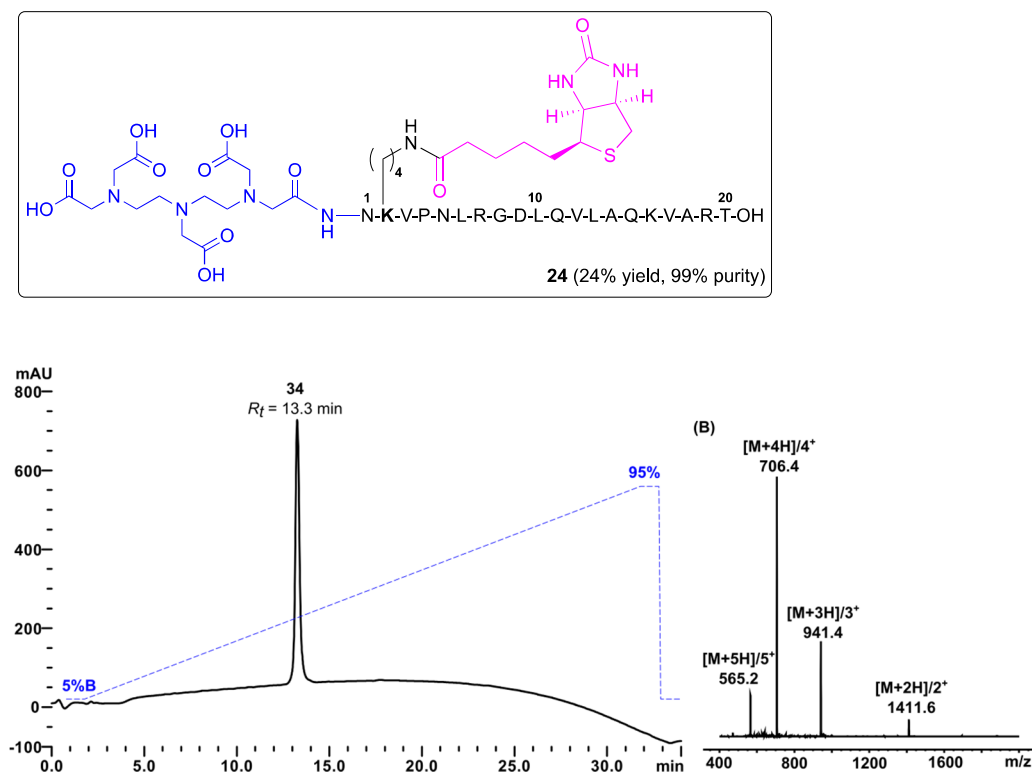


Figure S12. HPLC and MS traces of pure peptide 24 (ca 99% as judged by peak area of RP-HPLC at 210 nm, 24% yield). R_t 13.3 min; m/z 565.2 $[M + 5H]^{5+}$ requires 565.2, m/z 706.4 $[M + 4H]^{4+}$ requires 706.2, m/z 941.4 $[M + 3H]^{3+}$ requires 941.3, m/z 1411.6 $[M + 2H]^{2+}$ requires 1411.4. Mass deconvolution calculated at 2821.25 Da with standard deviation of 0.25; theoretical mass calculated at 2820.77 Da.

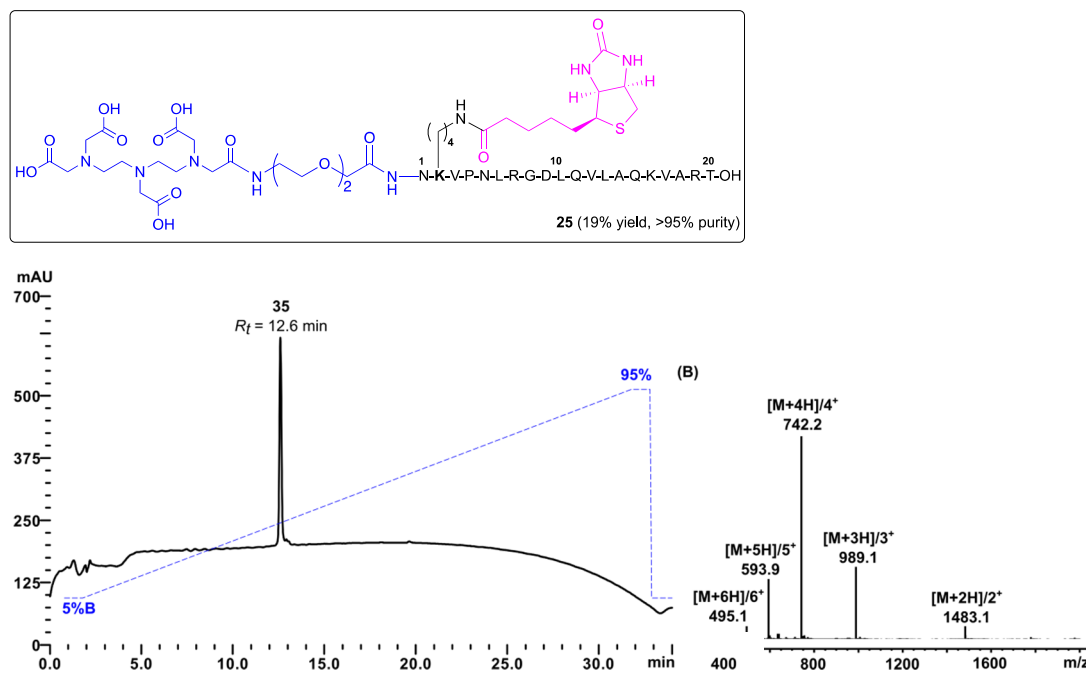
DTPA-PEG₂-CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (25)

Figure S13. HPLC and MS traces of pure peptide 25 (>95% as judged by peak area of RP-HPLC at 210 nm, 19% yield). R_t 12.6 min; m/z 495.1 $[M + 6H]^{6+}$ requires 495.6, m/z 593.9 $[M + 5H]^{5+}$ requires 594.5, m/z 742.2 $[M + 4H]^{4+}$ requires 742.9, m/z 989.1 $[M + 3H]^{3+}$ requires 990.1, m/z 1483.1 $[M + 2H]^{2+}$ requires 1484.7. Mass deconvolution calculated at 2964.48 Da with standard deviation of 0.24; theoretical mass calculated at 2967.42 Da.

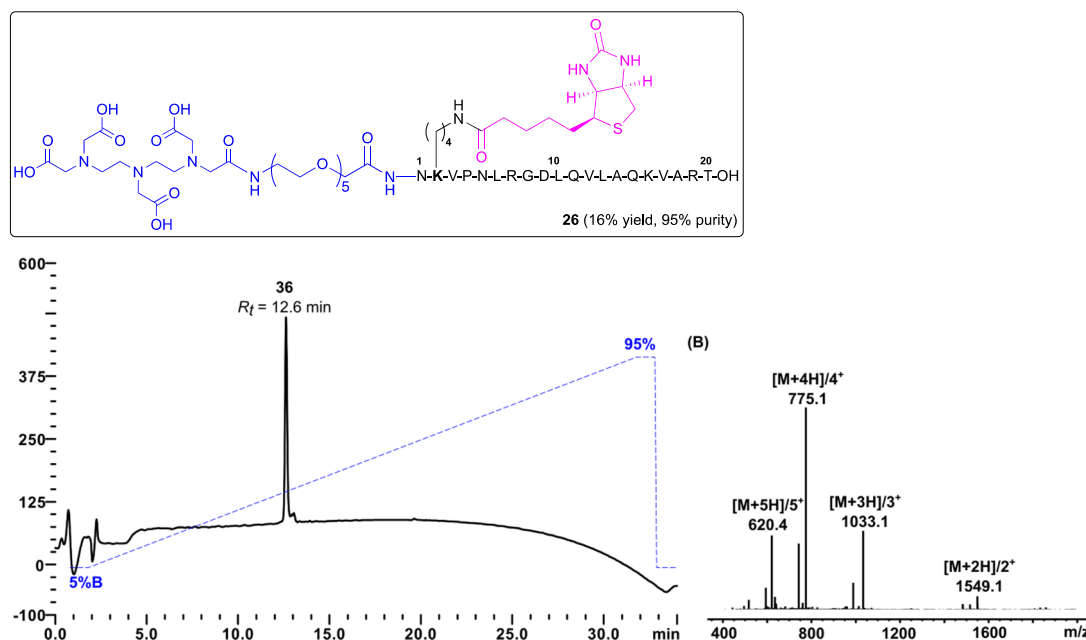
DTPA-PEG₃-CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (26)

Figure S14. HPLC and MS traces of pure peptide 26 (ca 95% as judged by peak area of RP-HPLC at 210 nm, 16% yield). R_t 12.6 min; m/z 620.4 $[M + 5H]^{5+}$ requires 620.7, m/z 775.1 $[M + 4H]^{4+}$ requires 775.7, m/z 1033.1 $[M + 3H]^{3+}$ requires 1033.9, m/z 1549.1 $[M + 2H]^{2+}$ requires 1550.3. Mass deconvolution calculated at 3096.48 Da with standard deviation of 0.36; theoretical mass calculated at 3098.58 Da.

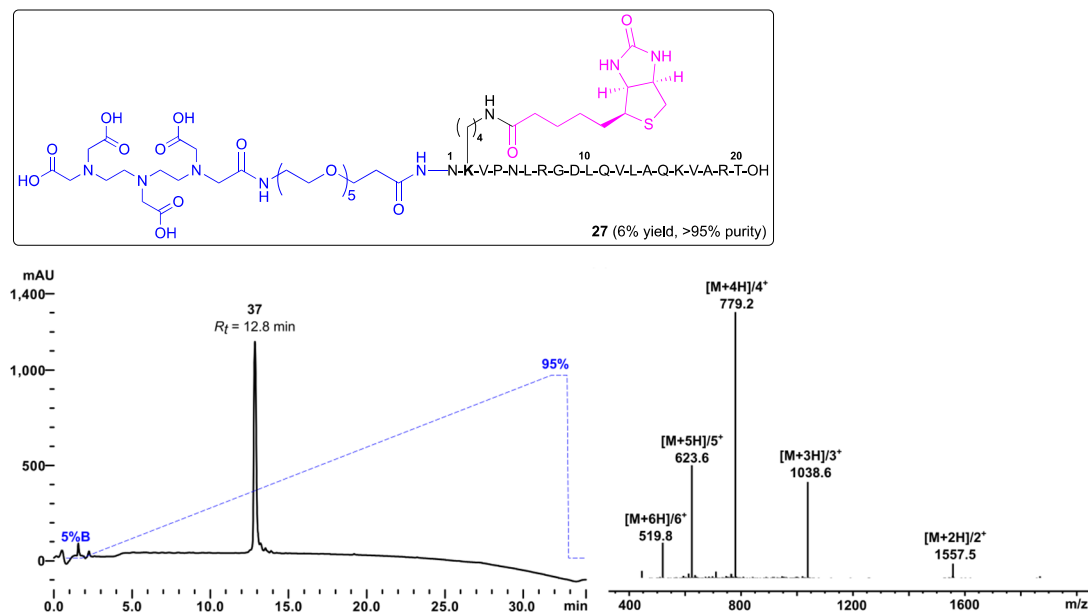
DTPA-PEG₅-CH₂CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (27)

Figure S15. HPLC and MS traces of pure peptide 27 (>95% as judged by peak area of RP-HPLC at 210 nm, 6% yield). R_t 12.8 min; m/z 519.8 $[M + 6H]^{6+}$ requires 519.9, m/z 623.6 $[M + 5H]^{5+}$ requires 623.7, m/z 779.2 $[M + 4H]^{4+}$ requires 779.4, m/z 1038.6 $[M + 3H]^{3+}$ requires 1038.9, m/z 1557.5 $[M + 2H]^{2+}$ requires 1557.8. Mass deconvolution calculated at 3112.88 Da with standard deviation of 0.11; theoretical mass calculated at 3113.61 Da.

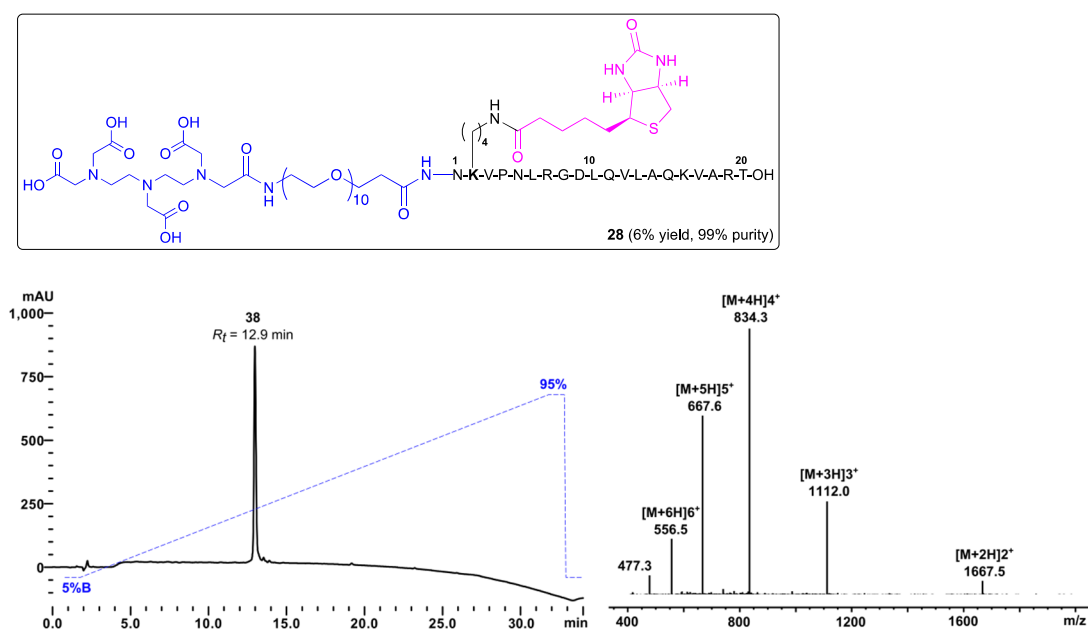
DTPA-PEG₁₀-CH₂CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (28)

Figure S16. HPLC and MS traces of pure peptide 28 (ca 99% as judged by peak area of RP-HPLC at 210 nm, 6% yield). R_t 12.9 min; m/z 477.3 $[M + 7H]^{7+}$ requires 477.3, m/z 556.5 $[M + 6H]^{6+}$ requires 556.6, m/z 667.6 $[M + 5H]^{5+}$ requires 667.8, m/z 834.3 $[M + 4H]^{4+}$ requires 834.5, m/z 1112.0 $[M + 3H]^{3+}$ requires 1112.3, m/z 1667.5 $[M + 2H]^{2+}$ requires 1667.9. Mass deconvolution calculated at 3333.04 Da with standard deviation of 0.11; theoretical mass calculated at 3333.87 Da.

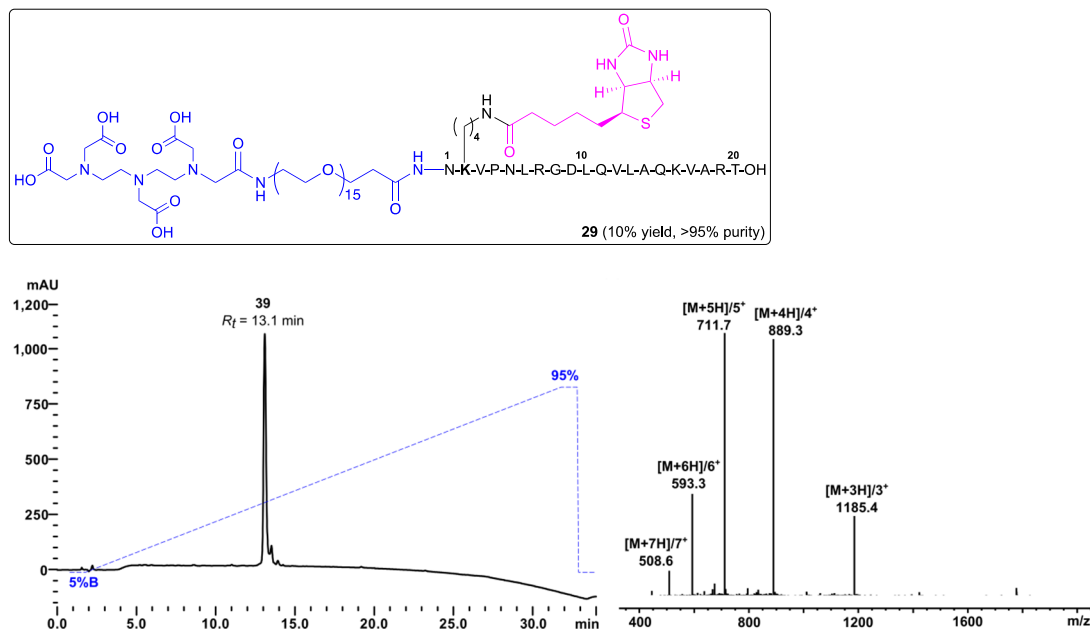
DTPA-PEG₁₅-CH₂CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (29)

Figure S17. HPLC and MS traces of pure peptide 29 (>95% as judged by peak area of RP-HPLC at 210 nm, 10% yield). R_t 13.1 min; m/z 508.6 $[M+7H]^+$ requires 508.7, m/z 593.3 $[M+6H]^+$ requires 593.4, m/z 711.7 $[M+5H]^+$ requires 711.8, m/z 889.3 $[M+4H]^+$ requires 889.5, m/z 1185.4 $[M+3H]^+$ requires 1185.7. Mass deconvolution calculated at 3553.43 Da with standard deviation of 0.29; theoretical mass calculated at 3554.11 Da.

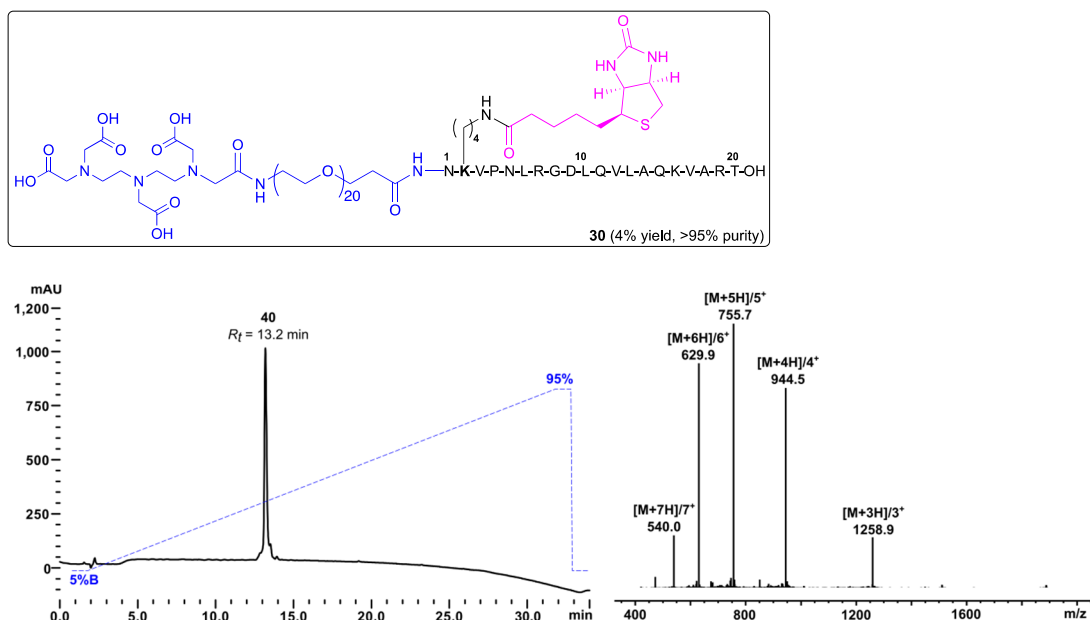
DTPA-PEG₂₀-CH₂CH₂CO-NK(D-biotin)VPNLRGDLQVLAQKVART-OH (30)

Figure S18. HPLC and MS traces of pure peptide 30 (>95% as judged by peak area of RP-HPLC at 210 nm, 4% yield). R_t 13.2 min; m/z 540.0 $[M+7H]^+$ requires 540.2, m/z 629.9 $[M+6H]^+$ requires 630.1, m/z 755.7 $[M+5H]^+$ requires 755.9, m/z 944.5 $[M+4H]^+$ requires 944.6, m/z 1258.9 $[M+3H]^+$ requires 1259.1. Mass deconvolution calculated at 3773.65 Da with standard deviation of 0.26; theoretical mass calculated at 3774.40 Da.