

Supporting information

Molecular Construction of HIV-gp120 Discontinuous Epitope Mimics by Assembly of Cyclic Peptides on an Orthogonal Alkyne functionalized TAC-scaffold

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General information

Chemicals were obtained from commercial sources and used without further purification, unless stated otherwise. Peptide grade DiPEA, CH₂Cl₂, NMP, TFA and HPLC grade solvents were purchased from Biosolve B.V. (Valkenswaard, The Netherlands). Fmoc-protected amino acids and BOP were purchased from GL Biochem Ltd. (Shanghai, China). Used amino acids with side chain protecting groups were as follows: Fmoc-Arg(Pbf), Fmoc-Asp(OtBu), Fmoc-Cys(Trt), Fmoc-Gln(Trt), Fmoc-Glu(OtBu), Fmoc-His(Trt), Fmoc-Thr(tBu) and Fmoc-Trp(Boc). TentaGel S RAM resin (particle size 90µm, capacity 0.25 mmol/g) was purchased from Rapp Polymere GmbH (Tübingen, Germany). Solid phase peptide synthesis was performed on a C.S. Bio Co. peptide synthesizer (model CS336X).

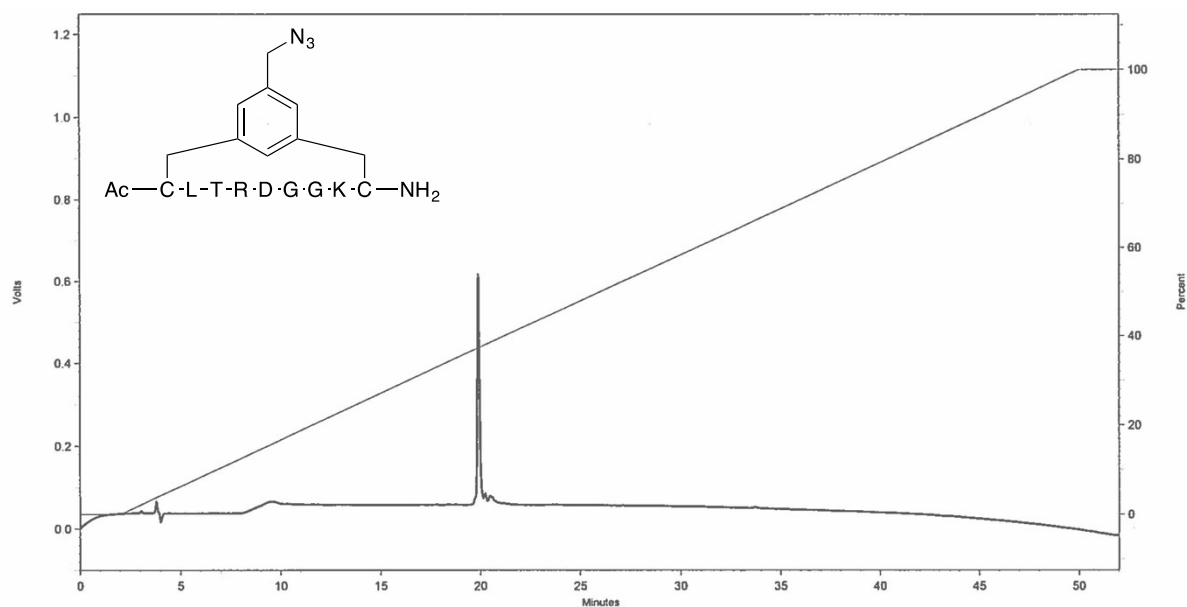
Analytical HPLC was performed on a Shimadzu-10Avp (Class VP) system using a Phenomenex Gemini C18 column (110 Å, 5 µm, 250×4.60 mm) at a flow rate of 1 mL min⁻¹. The used buffers were 0.1% trifluoroacetic acid in MeCN/H₂O 5:95 (buffer A) and 0.1% trifluoroacetic acid in MeCN/H₂O 95:5 (buffer B). Runs were performed by a standard protocol: 100% buffer A for 2 min, then a linear gradient of buffer B (0-100% in 48 min) and UV-absorption was measured at 214 and 254 nm.

ESI-TOF MS spectra were recorded on a microTOF mass spectrometer (Bruker). Samples were diluted with tuning mix (1:1, v/v) and infused at a speed of 300 µl/h using a nebulizer pressure of 5.8 psi, a dry gas flow of 3.0 L/min, a drying temperature of 180°C and a capillary voltage of 5.5 kV. Analytical LC-MS (electrospray ionization) was performed on Thermo- Finnigan LCQ Deca XP Max using same buffers and protocol as described for analytical HPLC. All reported mass values are monoisotopic.

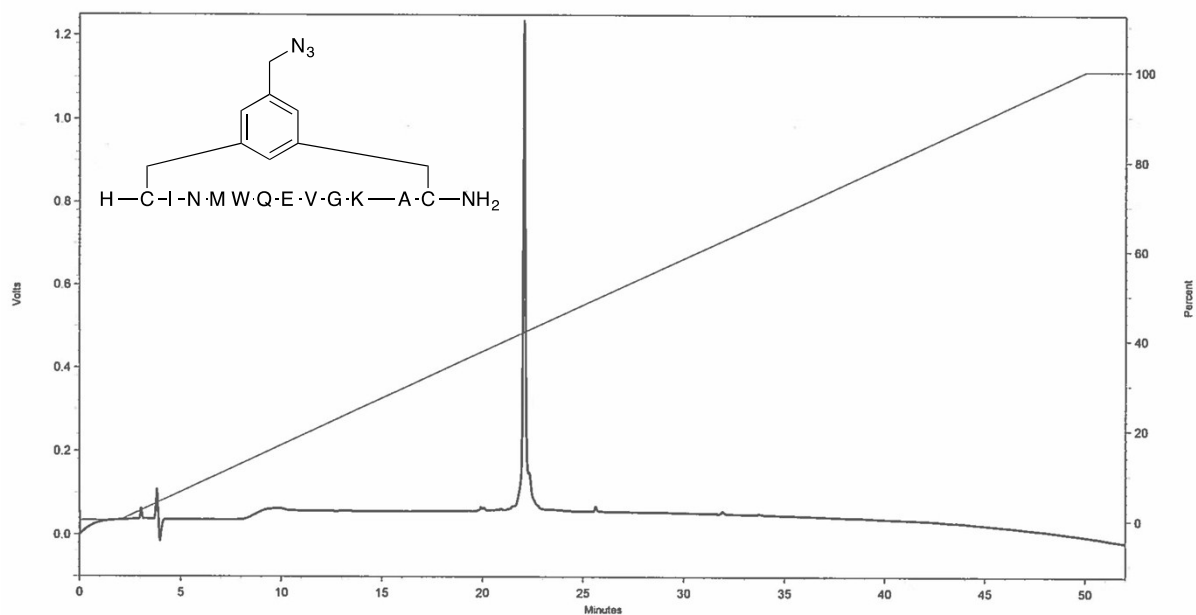
Analytical data for cyclic and linear peptides

Synthesized peptides:

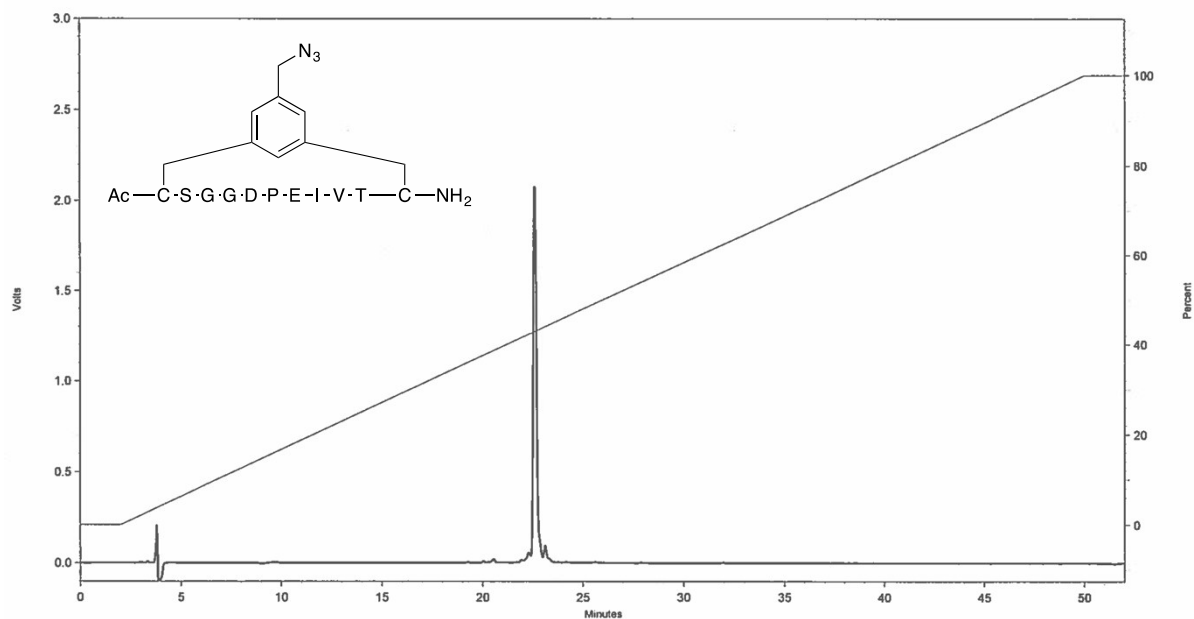
Cyclic peptide **16** (Ac-CLTRDGGKC-NH₂) 69 mg (60 μmol) 34%, (21 steps, average 95% per step) Exact mass calculated [M + H]⁺: 1150.5250; ESI-TOF MS found: 1150.5263. Rt = 19.90 min



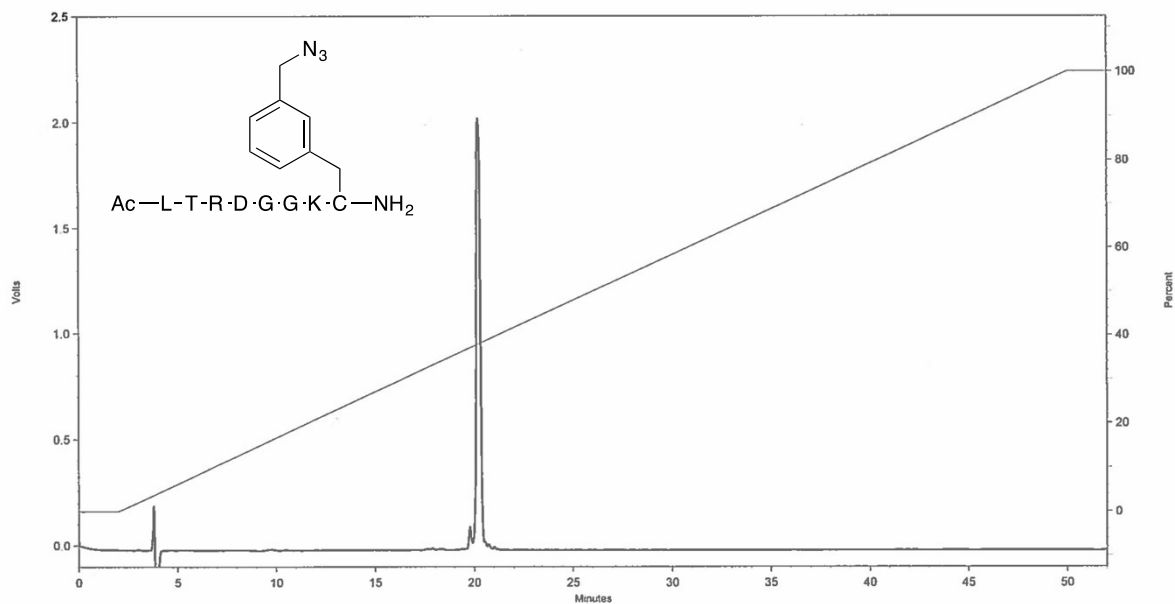
Cyclic peptide **17** (H-CINMWQEVGKAC-NH₂): 65 mg (42 μmol) 17%, (26 steps, average 93% per step) Exact mass calculated [M + 2H]²⁺: 769.3472; ESI-TOF MS found: 769,3464. Rt = 22.05 min



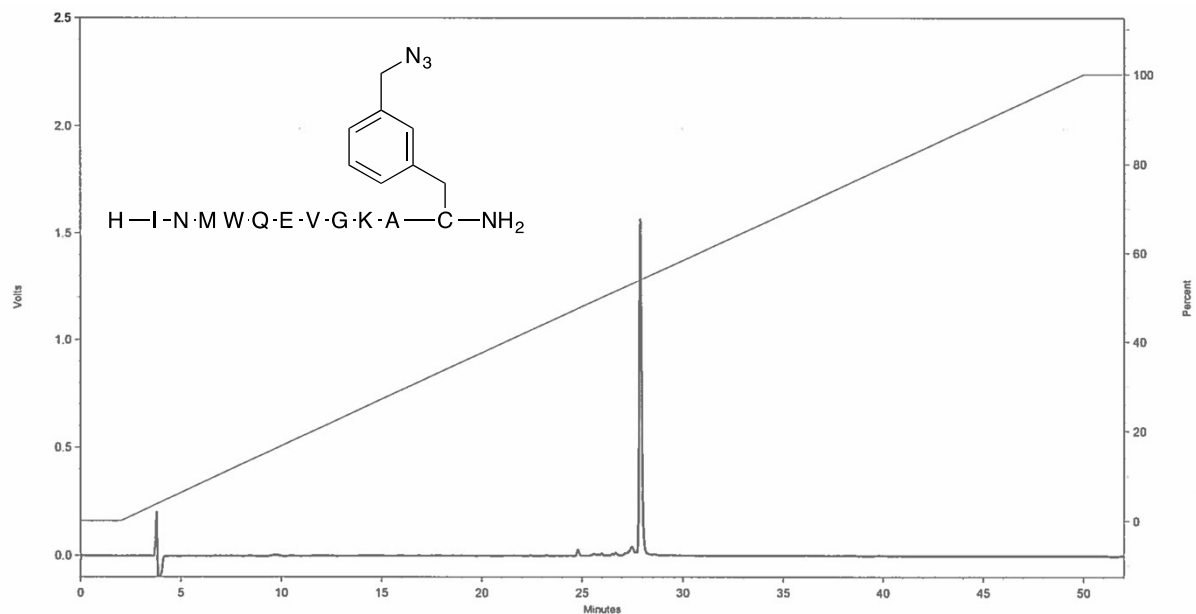
Cyclic peptide **18** (Ac-CSGGDPEIVTC-NH₂) 92 mg (72 μmol) 29%, (25 steps, average 95% per step) Exact mass calculated [M + H]⁺: 1278.5247; ESI-TOF MS found: 1278.5269. Rt = 22.58 min



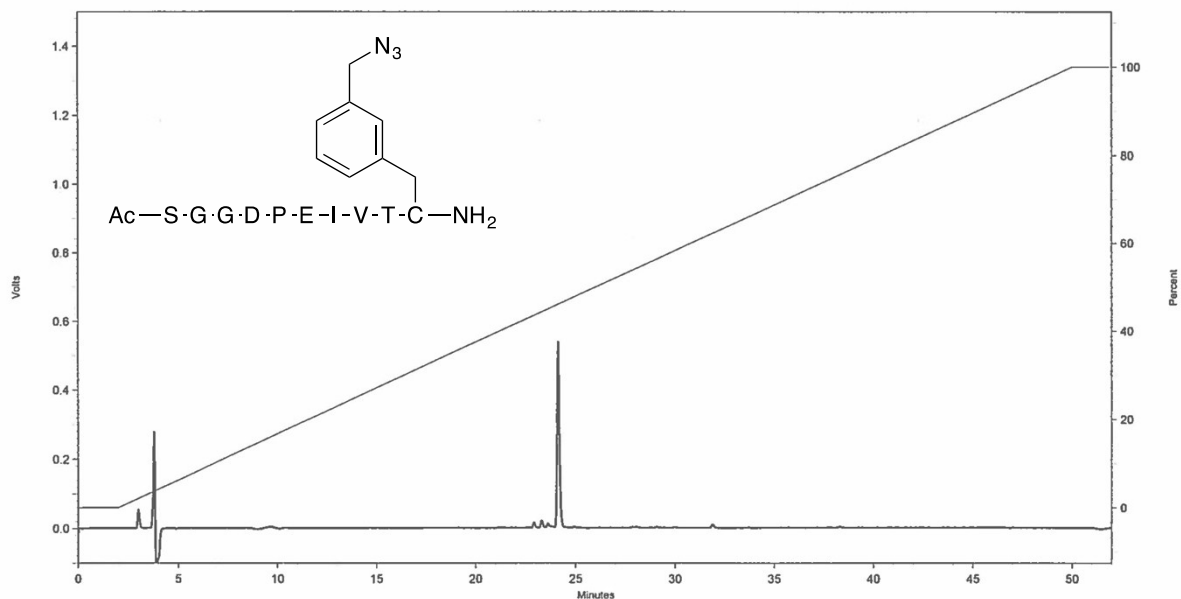
Linear peptide **19** (Ac-LTRDGGKC-NH₂) 36 mg (34 μmol) 19%, (19 steps, average 92% per step) Exact mass calculated [M + H]⁺: 1035.5158; ESI-TOF MS found: 1035.5170. Rt = 20.19 min



Linear peptide **20** (H-INMWQEVGKAC-NH₂) 95 mg (34 μmol) 27%, (24 steps, average 95% per step) Exact mass calculated [M + H]⁺: 1422.6775; ESI-TOF MS found: 1422.6839. Rt = 27.89 min

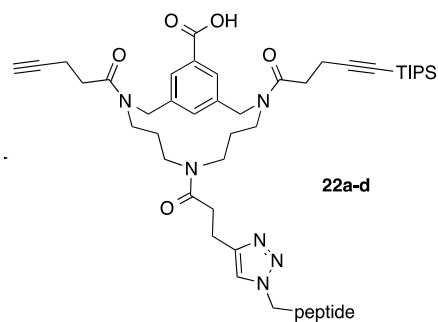


Linear peptide **21** (Ac-SGGDPEIVTC-NH₂) 52 mg (44 μmol) 23%, (23 steps, average 94% per step) Exact mass calculated [M + H]⁺: 1163.5155; ESI-TOF MS found: 1163.5139. Rt = 24.12 min

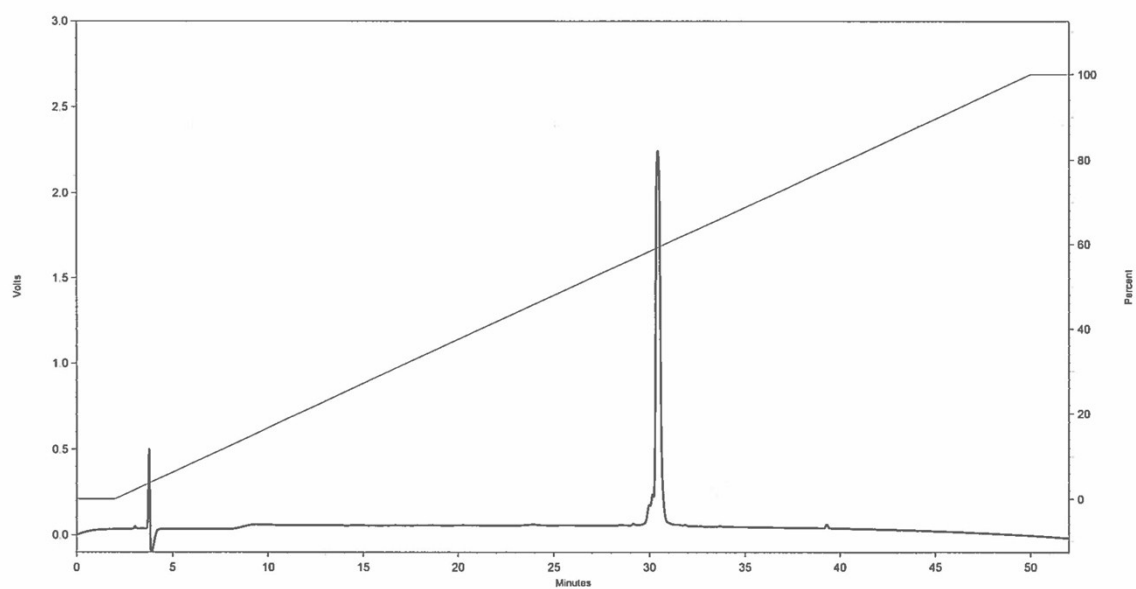


Analytical data for constructs

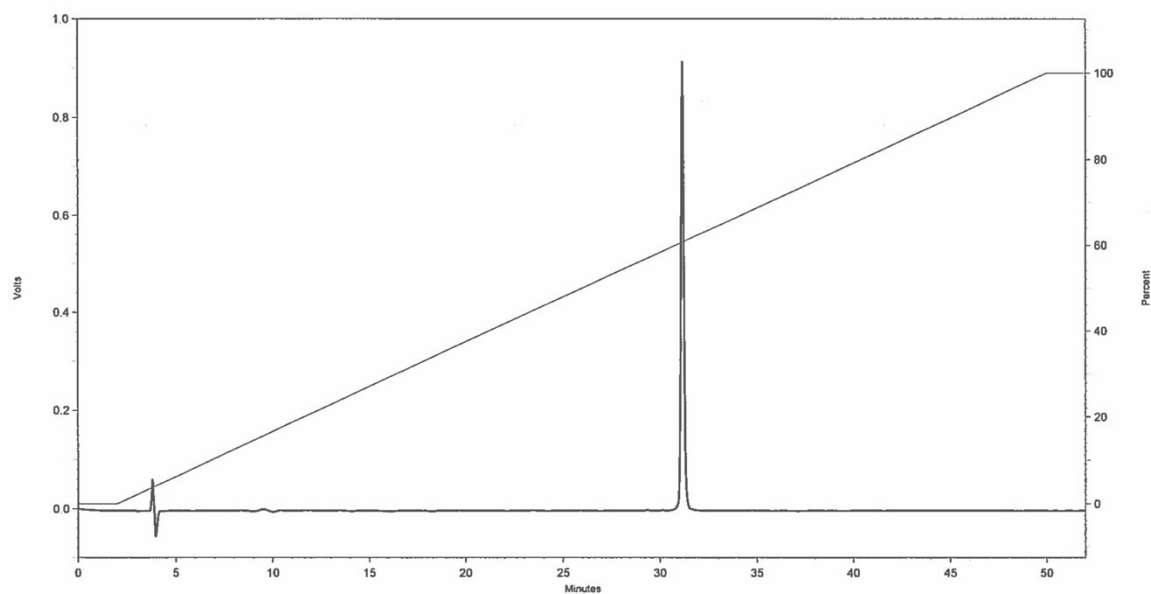
Constructs bearing one peptide and a free alkyne 22a-d



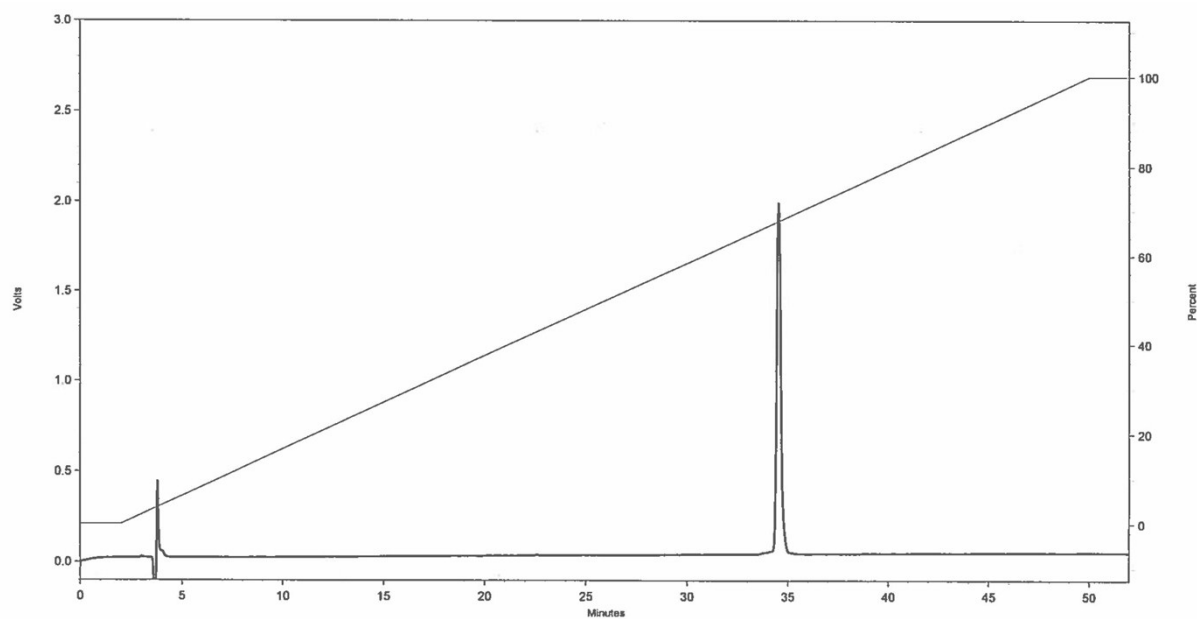
22a Peptide **16** (12.7 μmol scale) yield: 11.5 mg (6.3 μmol) 50%, Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 912.4620; ESI-TOF MS found: 912.4684. Rt = 30.42 min



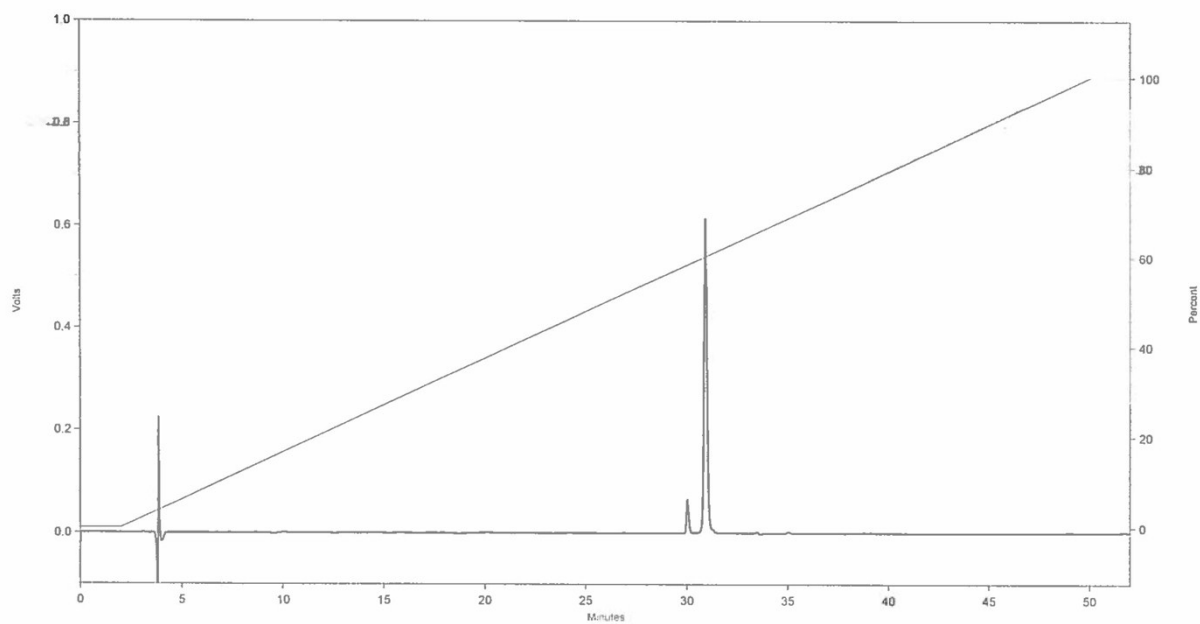
22b Peptide **17** (5 μmol scale) 5.0 mg (2.3 μmol) 45%, Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 1106.0428; ESI-TOF MS found: 1106.0408. RT = 31.14 min



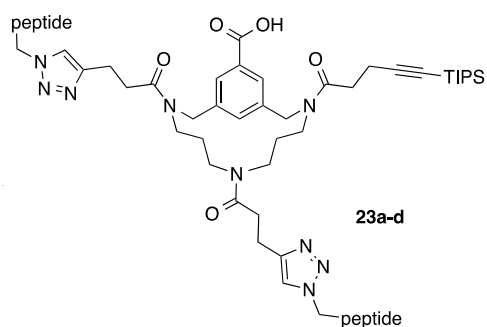
22c Peptide **18** (10 μmol scale) yield: 13 mg (6.7 μmol) 67%, Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 976.4618; ESI-TOF MS found: 976.4619. Rt = 34.54 min



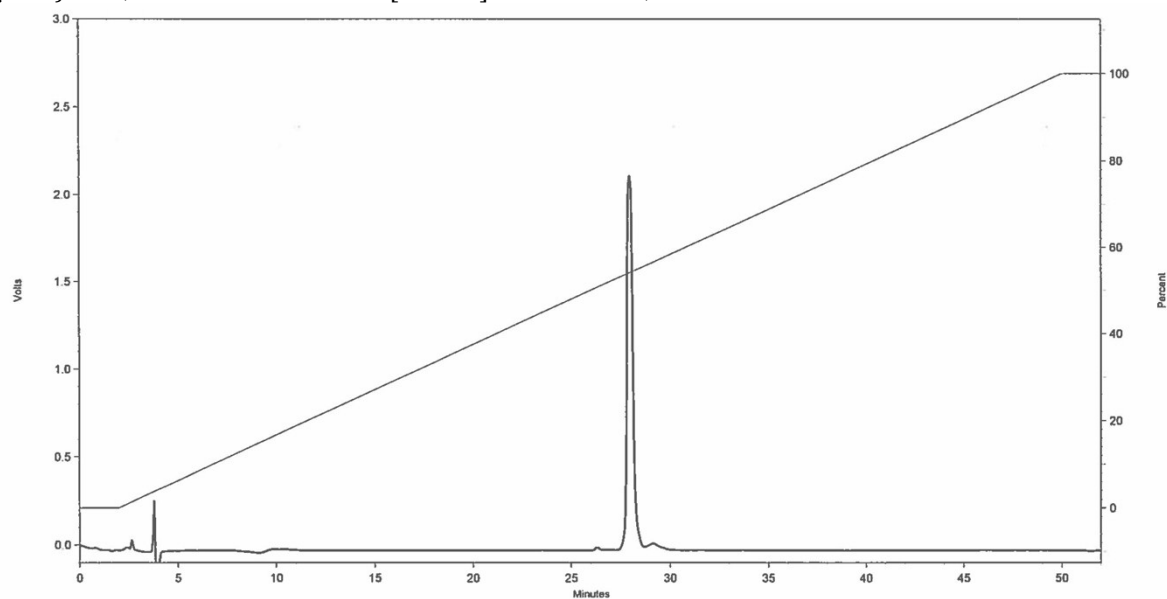
22d Peptide **19** (12.7 μmol scale) yield: 11.8 mg (6.9 μmol) 54%, Exact mass calculated $[\text{M} + \text{H}]^{+}$: 1708,9069; ESI-TOF MS found: 1708,9070. Rt = 30.91 min



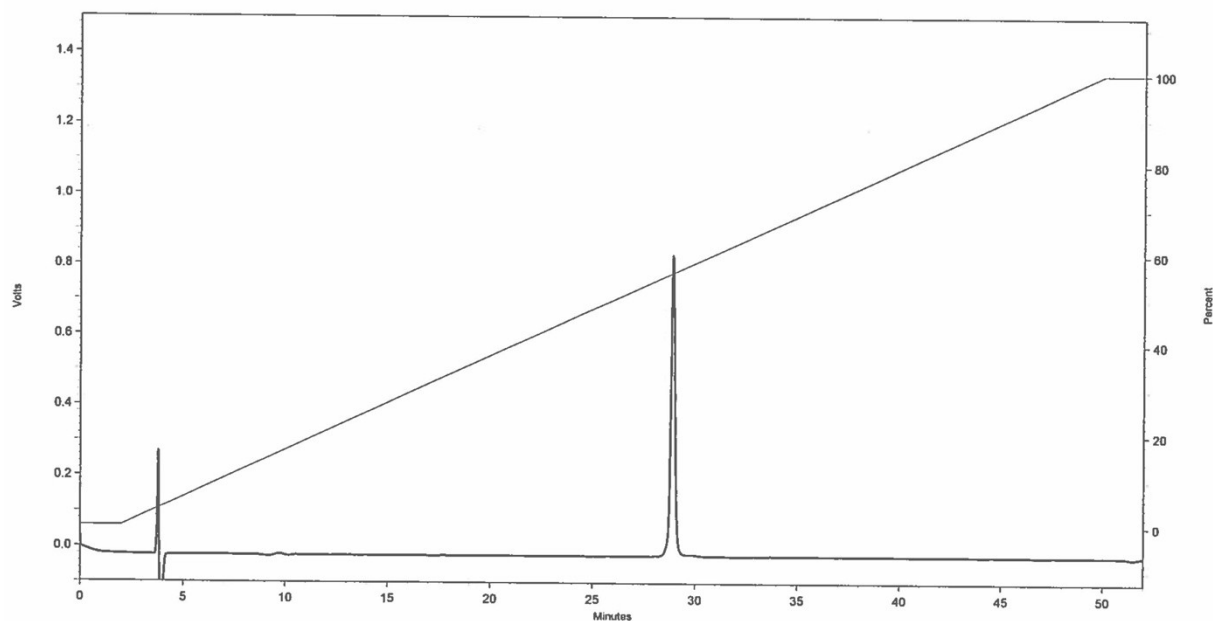
Constructs bearing two peptides and one protected alkyne (23a-d)



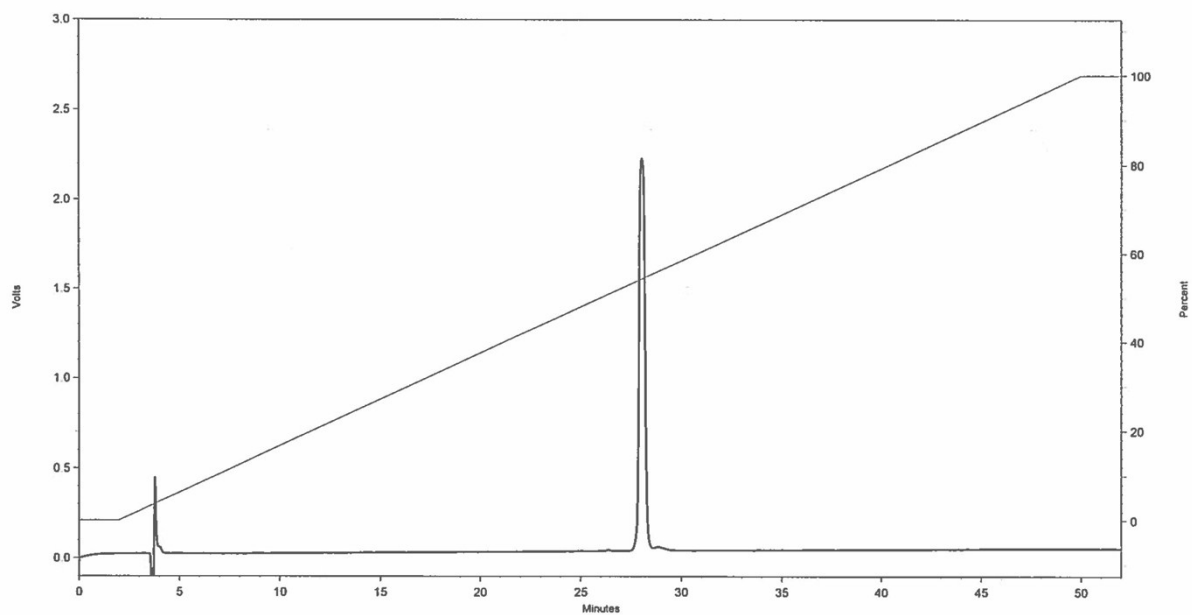
23a Peptide **18** (contains peptide **16** and **18** ligated in that order) (3.3 μmol scale) yield: 2.5 mg (2.6 μmol) 80%, Exact mass calculated $[M + 3H]^{3+}$: 1034.4829; ESI-TOF MS found: 1034.4842. Rt = 27.95 min



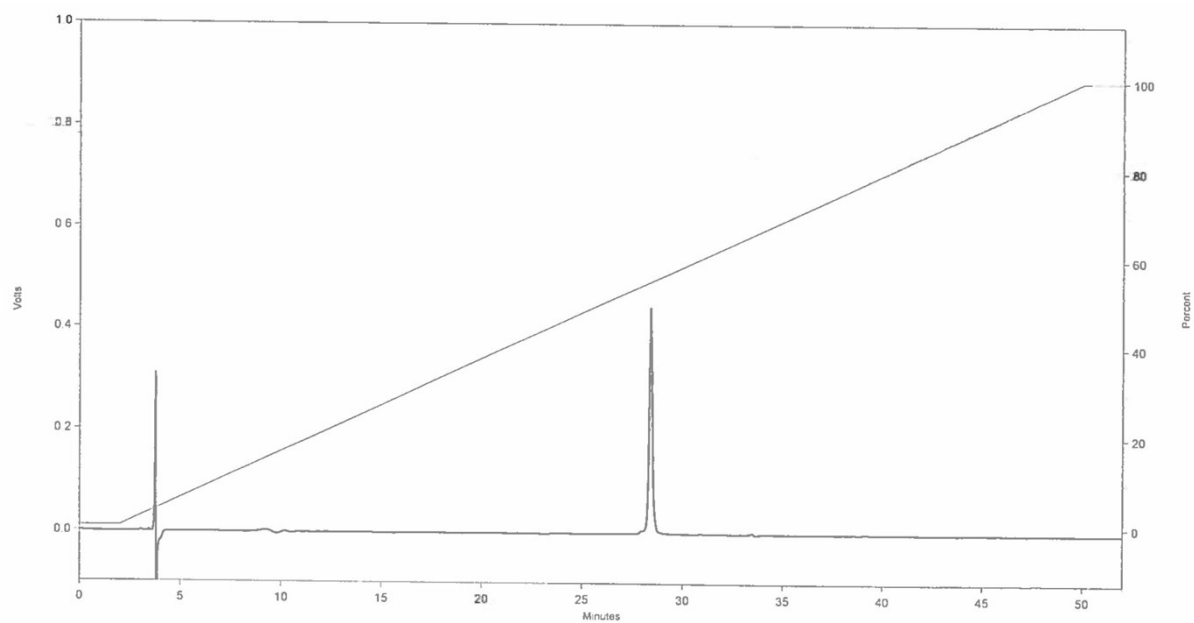
23b Peptide **18** (contains peptide **17** and **18** ligated in that order) (2.0 μmol scale) 4.5 mg (1.3 μmol) 63%, Exact mass calculated $[M + 2H]^{2+}$: 1744.8012; ESI-TOF MS found: 1744.7973. Rt = 28.89 min



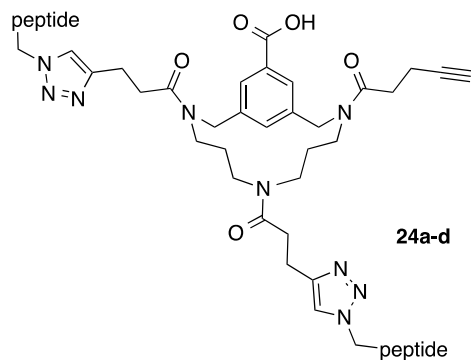
23c Peptide **16** (contains peptide **18** and **16** ligated in that order) (5.0 μmol scale) yield: 13 mg (4.2 μmol)
84%, Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 1551.2204; ESI-TOF MS found: 1551.2168. Rt = 28.03 min



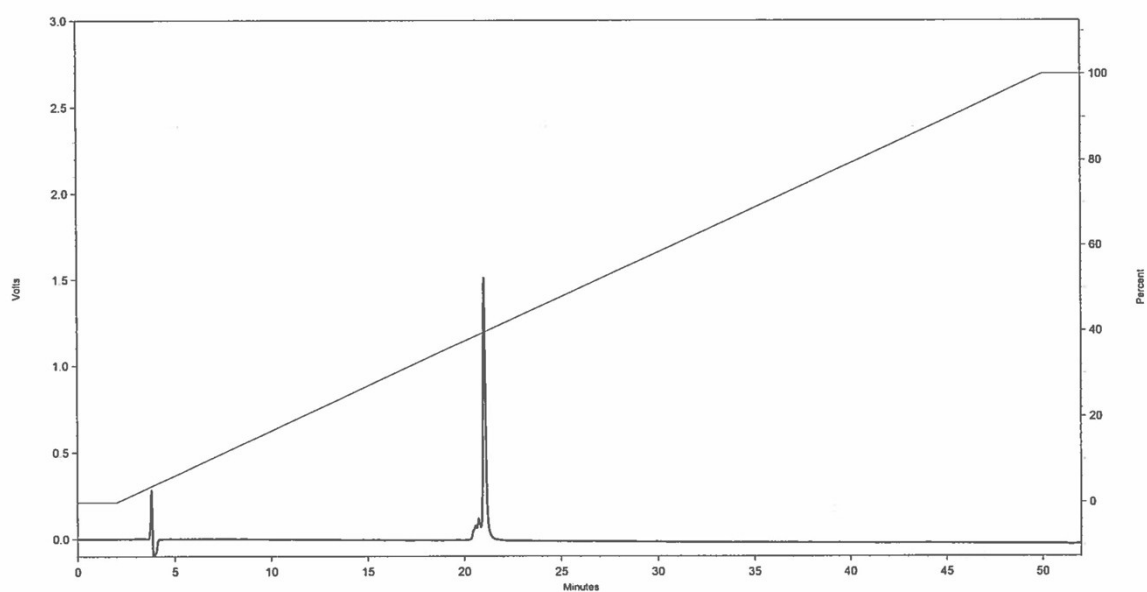
23d Peptide **21** (contains peptide **19** and **21** ligated in that order) (6.9 μmol scale) yield: 12 mg (4.2 μmol)
61%, Exact mass calculated $[\text{M} + 3\text{H}]^{3+}$: 957,8101; ESI-TOF MS found: 957,8010. Rt = 28.38 min



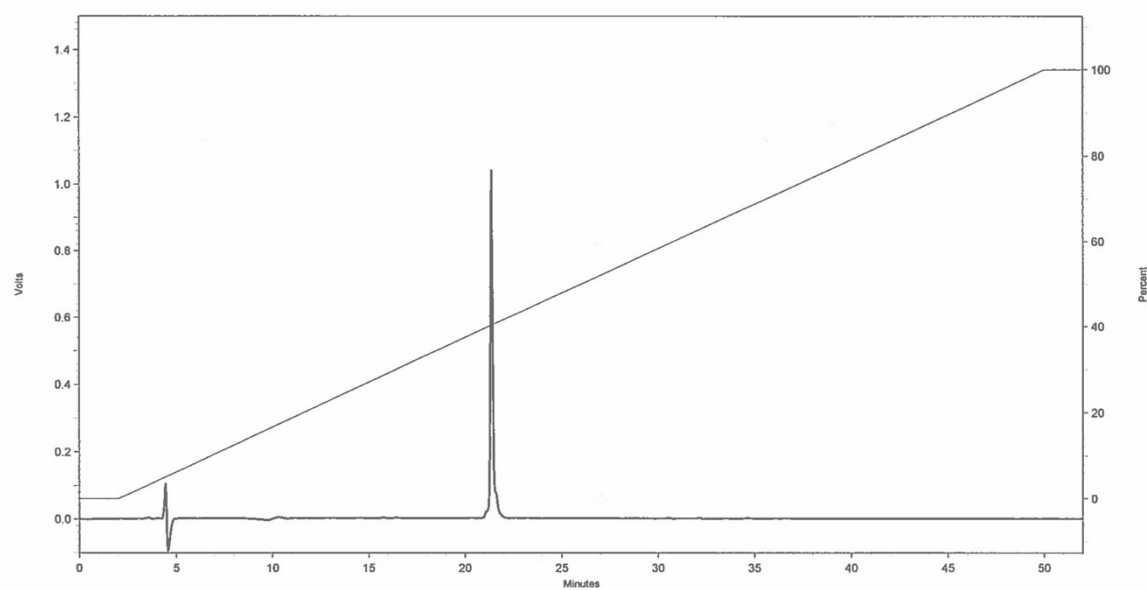
Constructs bearing two peptides and a free alkyne (24a-d)



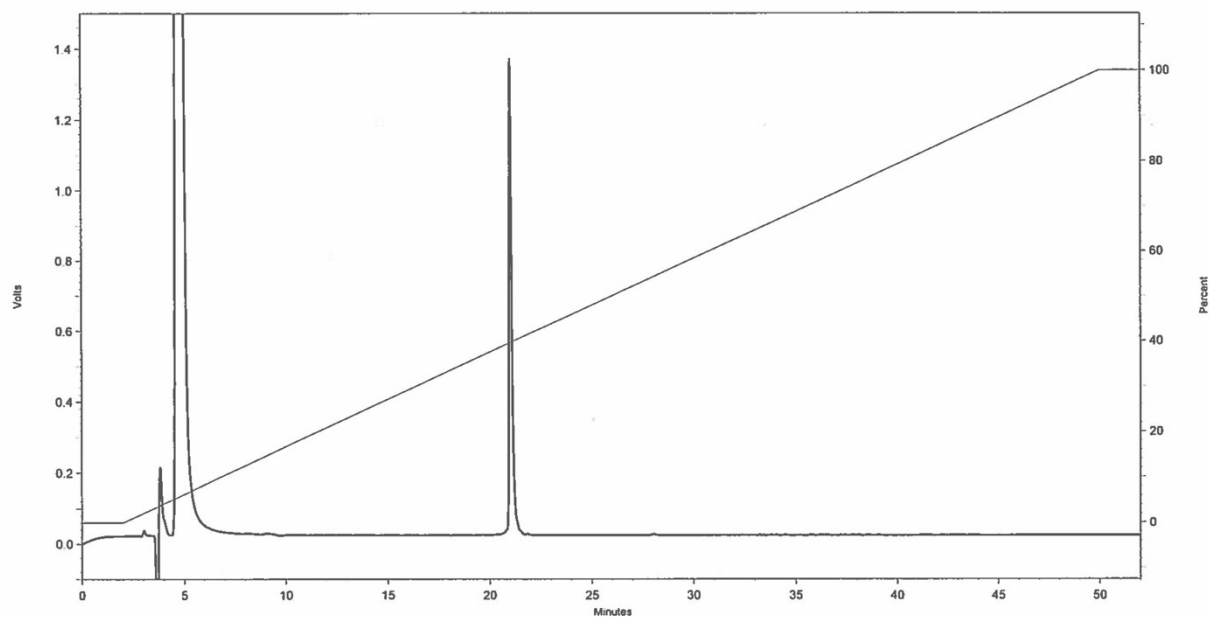
24a (contains peptide **16** and **18** ligated in that order) (2.8 μmol scale) yield: 7.3 mg (2.5 μmol) 87%,
Exact mass calculated $[M + 2H]^{2+}$: 1473.1537; ESI-TOF MS found: 1473.1494. Rt = 21.03 min



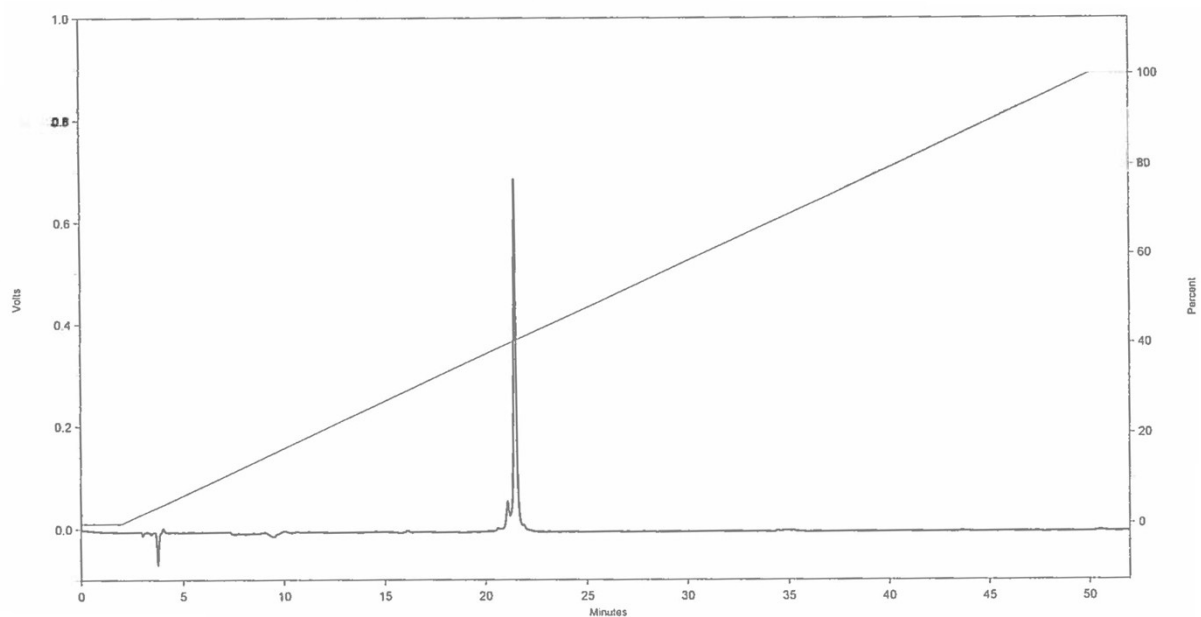
24b (contains peptide **17** and **18** ligated in that order) (1.2 μmol scale) yield: 3.0 mg (0.9 μmol) 78%,
Exact mass calculated $[M + 2H]^{2+}$: 1666.7345; ESI-TOF MS found: 1666.7269. Rt = 21.37 min



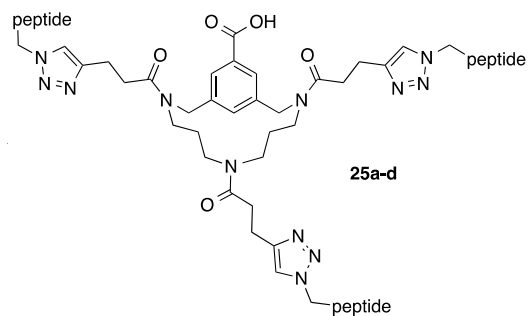
24c (contains peptide **18** and **16** ligated in that order) (3.6 μmol scale) yield: 4.6 mg (1.6 μmol) 43%,
Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 1473.1537; ESI-TOF MS found: 1473.1518. Rt = 21.03 min



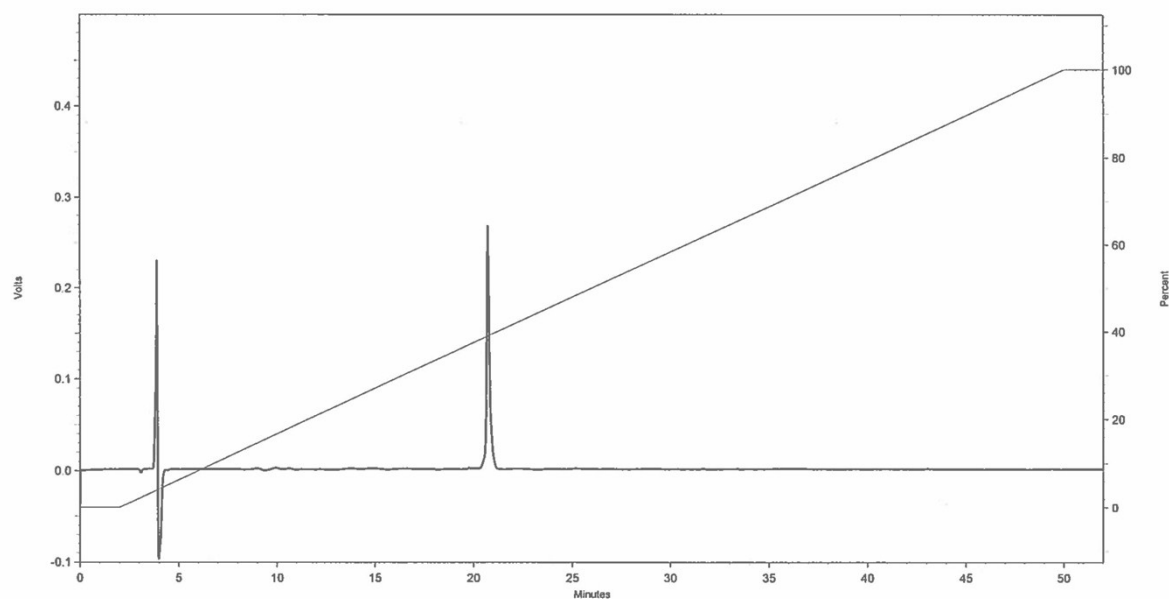
24d (contains peptide **19** and **21** ligated in that order) (4.2 μmol scale) yield: 9.7 mg (3.6 μmol) 85%,
Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 1358,1445; ESI-TOF MS found: 1358,1403. Rt = 21.47 min



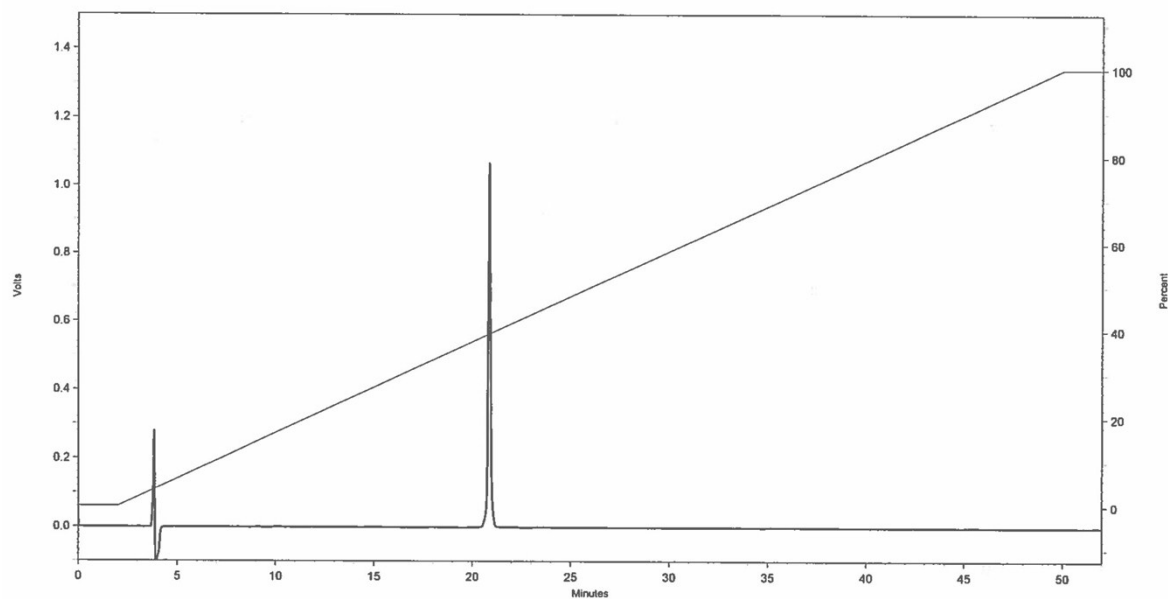
Final constructs bearing three peptides (25a-e)



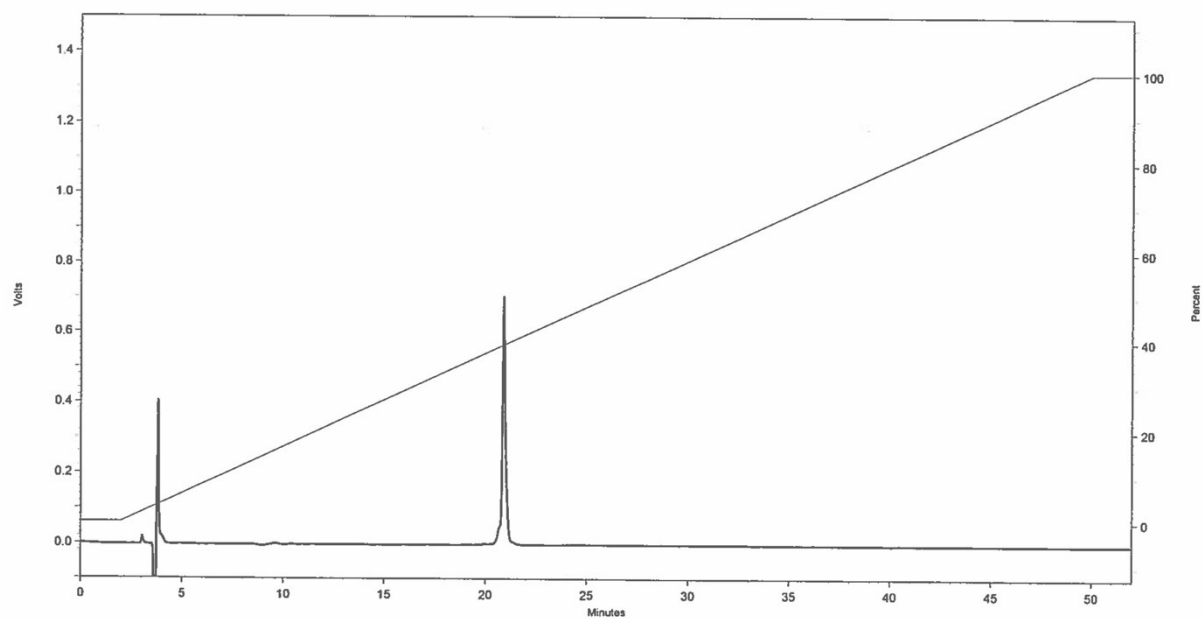
25a Peptide **17** (contains peptide **16**, **18** and **17** ligated in that order) (2.5 μmol scale) yield: 4.9 mg (1.1 μmol) 44%, Exact mass calculated $[M + 3H]^{3+}$: 1034.4829; ESI-TOF MS found: 1034.4842. Rt = 20.72 min



25b Peptide **16** (contains peptide **17**, **18** and **16** ligated in that order) (0.75 μmol scale) yield: 1.7 mg (0.38 μmol) 51%, Exact mass calculated $[M + 2H]^{2+}$: 1744.8012; ESI-TOF MS found: 1744.7973. Rt = 20.85 min



25c Peptide **17** (contains peptide **18**, **16** and **17** ligated in that order) (1.4 μmol scale) yield: 3.6 mg (0.8 μmol), 59% 4351 Exact mass calculated $[\text{M} + 2\text{H}]^{2+}$: 1551.2204; ESI-TOF MS found: 1551.2168. Rt = 20.89 min



25d Peptide **20** (contains peptide **19**, **21** and **20** ligated in that order) (1.5 μmol scale) yield: 2.2 mg (0.54 μmol) 36%, Exact mass calculated $[\text{M} + 3\text{H}]^{3+}$: 1379,6555; ESI-TOF MS found: 1379,6620. Rt = 23.41 min

