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# Interfacing Native and Non Native Peptides: Using Affimers to Recognise α-Helix Mimicking Foldamers

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**Supporting information**

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## Contents

# General Experimental

**Solvents.** Unless stated otherwise, solvents and reagents were used as received from major suppliers without prior purification. Anhydrous $CH_3CN$, $CHCl_3$, $CH_2Cl_2$ and were obtained from the in-house solvent purification system from Innovative Technology Inc. PureSolv®. Anhydrous DMSO was obtained from major chemical suppliers equipped with a SureSeal or equivalent. Non-anhydrous solvents were of HPLC quality and provided by Fisher or Sigma-Aldrich. Water used for formation of aqueous solutions and quenching was deionised.

**Chromatograhy.** Purifications were performed with either silica gel 60 (0.043-0.063 mm VWR) using head bellows or by flash chromatography using an Isolera Four Biotage®. Ion-exchange columns were performed using Supleco Discovery SPE DSC-SAX columns. Analytical HPLC experiments were run in an Agilent 1290 Infinity Preparative system spectrometer.

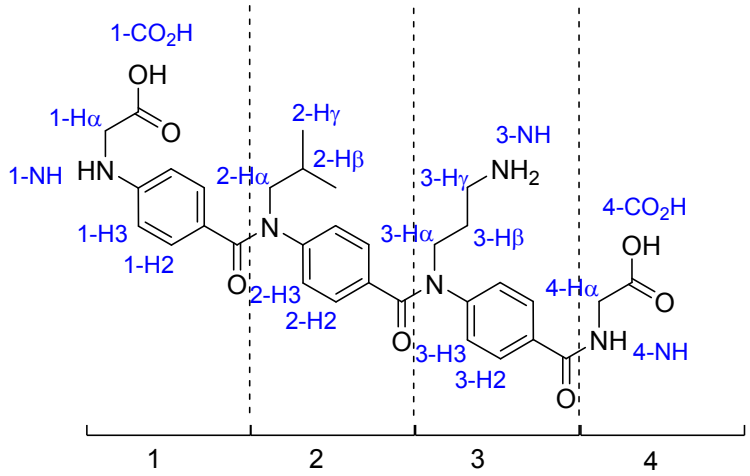
**NMR.** $^{1}H$ NMR spectra were recorded on Bruker DPX 300 (300 MHz) or Avance 500 (500 MHz) spectrometers and referenced to either residual non-deuterated solvent peaks or tetramethylsilane. $^{13}C$ NMR were recorded on a Bruker Avance 500 (125 MHz) and referenced to the solvent peak. Chemical shifts (δ) are expressed in part per million (ppm) and coupling constants are expressed in hertz (Hz). Assignments of spectra were assisted by the results of DEPT, COSY, NOESY, HMQC and HMBC experiments when appropriate.

**Mass.** HPLC LCMS were recorded on a Bruker HCT ultra under the conditions of electrospray ionisation (ESI). HPLC separation was performed on an Agilent 1200 series instrument equipped with a Phenomenex C18 column (50 x 2 mm) using $MeCN/H_2O$ as the eluent for positive ion spectra. HRMS were done with a Bruker Maxis impact mass spectrometer, using ESI. Values are reported as a ratio of mass to charge.

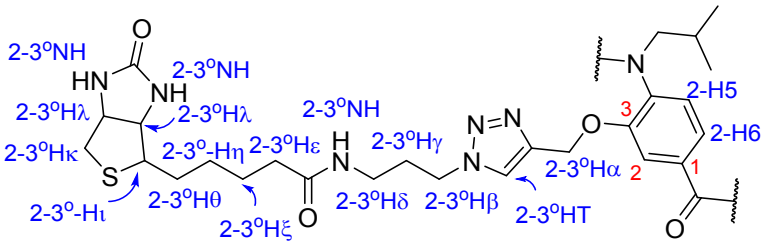
**IR.** Infrared spectra were recorded on a Perkin Elmer Fourier-Transfer spectrometer. Spectra were analysed neat and only structurally important absorptions are quoted. Absorption maxima ($\nu_{max}$) are quoted in wavenumbers ($cm^{-1}$).

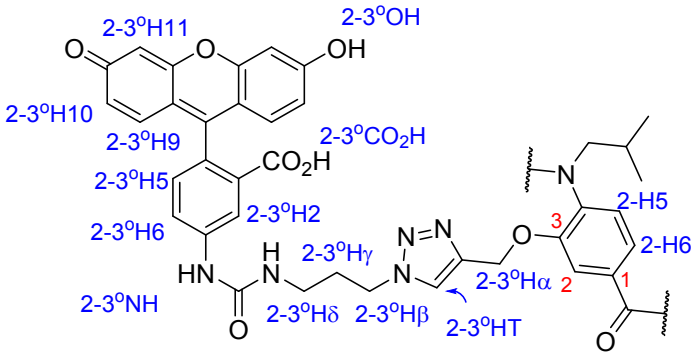
# Nomenclature

To simplify the NMR assignment of the trimers, the following nomenclature is used. The monomers constituting the trimers are considered separately, numbered 1 to 3 starting from the *N*-terminus, and the glycine is numbered 4. All the monomers are numbered following the same standard system: the carbon bearing the carboxylic acid is C1 and the one bearing the amine is C4. The carbon attached to the nitrogen is Cα, and the numbering of the aliphatic part of the chain continues with Cβ, etc. The numbering of the protons corresponds to the numbering of the carbons. For clarity purposes, the monomer number is added as a prefix to the proton number.



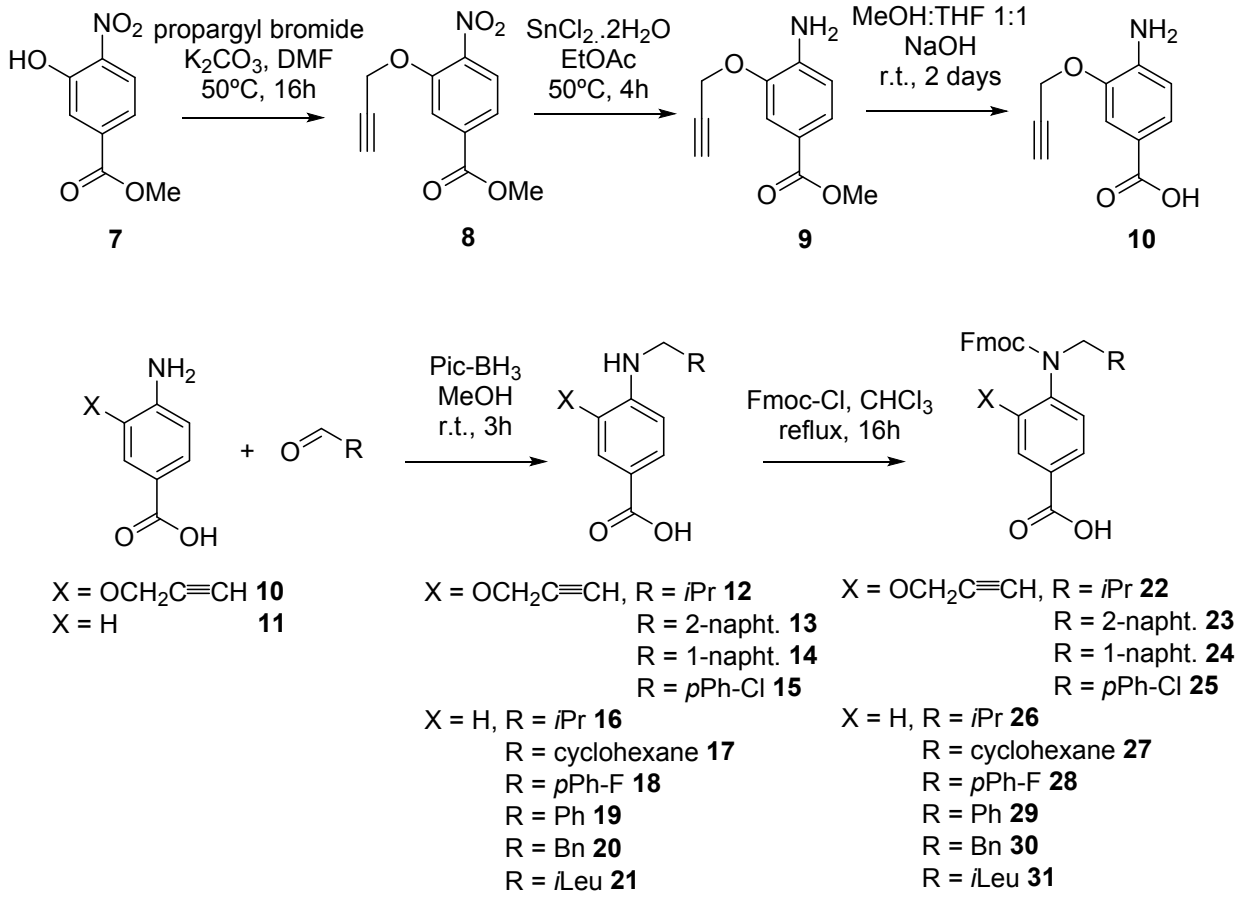

In the case of *O*-substituted compounds, a first number indicates the monomer bearing the side chain. A second number followed by $^{O}$ specifies the position of the functionalised oxygen. The aliphatic protons are numbered Hα, Hβ, etc. Triazole protons are indicated as HT.



# Monomer Synthesis

The general synthetic route used to obtain the monomers required for trimer synthesis are shown below. All building blocks were synthesized as described previously with the exception of building blocks **12** and **22**.[1, 2] These are described here.




*Scheme S1: Synthetic route used to prepare the monomers needed for trimer synthesis.*

**4-[(2-methylpropyl)amino]-3-(prop-2-yn-1-yloxy)benzoic acid 12**

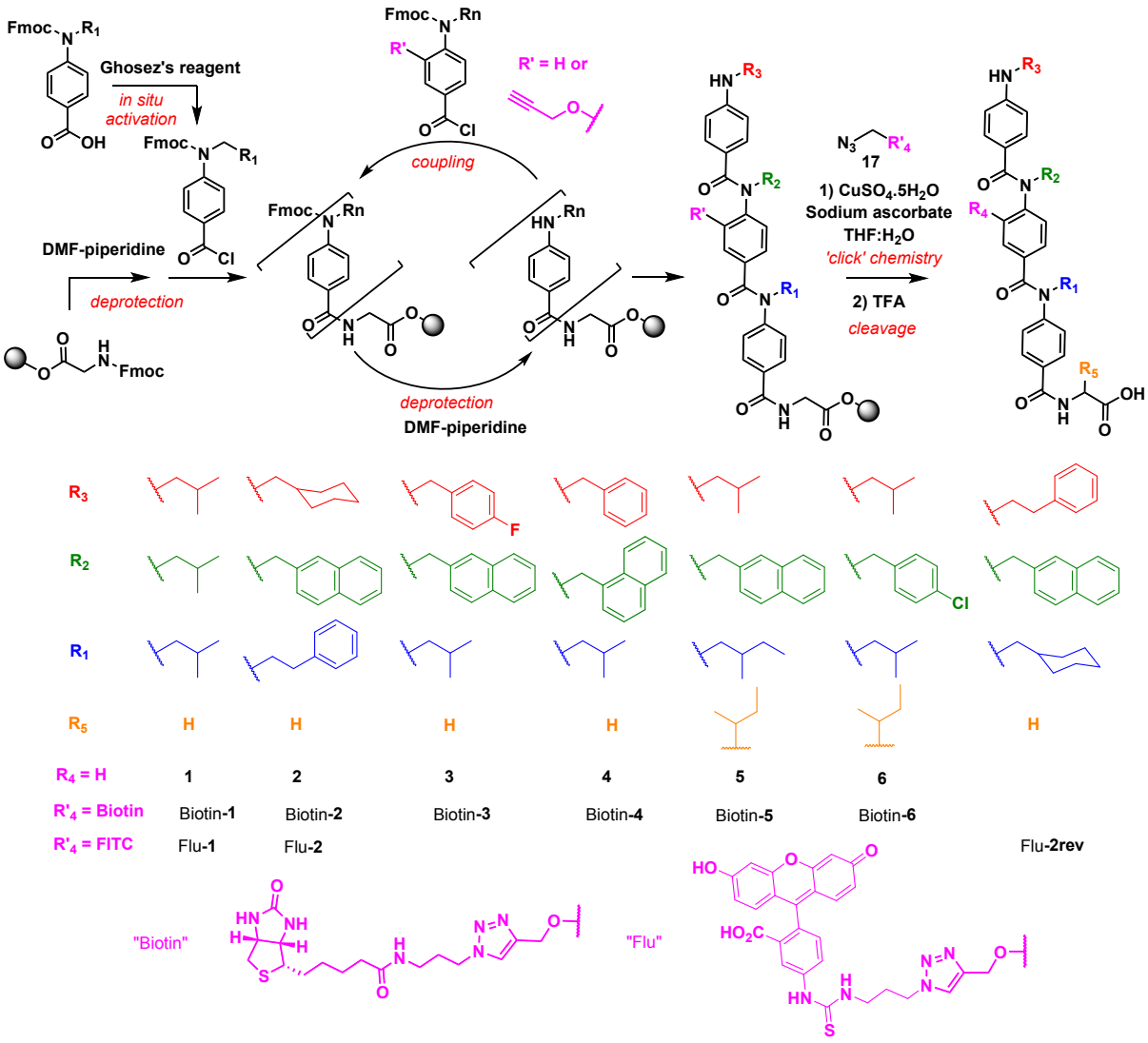
4-amino-3-(prop-2-yn-1-yloxy) benzoic acid **10** (3.92 g, 20.5 mmol), isobutyraldehyde (1.87 mL, 20.5 mmol) and picoline borane (2.63 g, 24.6 mmol) were stirred in methanol (100 mL) at room temperature for 3 hours. The solvent was evaporated, the obtained residue was dissolved in ethyl acetate (100 mL) and washed with a 1M aqueous solution of HCl (50 mL). The organic layer was dried over $MgSO_4$ and filtered, the filtrate concentrated under vacuum to give the desired product as a pale yellow solid (5.08 g, quant.). $\delta_H$ (500 MHz, DMSO-$d_6$) 12.08 (s, 1H, O*H*) 7.47 (dd, 1H, *J* = 8.1 Hz, 1.7 Hz, Ar-*H*), 7.41 (d, 1H, *J* = 1.7 Hz, Ar-*H*), 6.56 (d, 1H, *J* = 8.1 Hz, Ar-*H*), 5.59 (t, 1H, *J* = 5.9 Hz, N-*H*) 4.83 (d, 2H, *J* = 2.1 Hz, C$H_2$C≡CH), 3.57 (t, 1H, *J* = 2.1 Hz, $CH_2$C≡C*H*), 2.9 (t, 2H, *J* = 6.4 Hz, C$H_2$CH($CH_3$)$_2$), 1.81-1.90 (m, 1H, $CH_2$C*H*($CH_3$)$_2$), 0.9 (d, 6H, *J* = 6.4 Hz, $CH_2$CH(C$H_3$)$_2$). $\delta_C$ (125 MHz, DMSO-$d_6$) 167.4, 142.9, 142.8, 125.0, 116.2, 112.2, 107.8, 79.2, 78.4, 55.9, 49.8, 27.1, 20.2, 20.0. $\nu_{max}$/cm$^{-1}$ (solid state) = 3401 (NH), 3278 (CH alkyne), 2961 (COOH), 2122 (C≡C), 1659, 1410, 1280 (COOH). HRMS: Calcd. $[M+H]^+$ ($C_{14}H_{17}NO_3$) *m/z* = 248.1208. Found $[M+H]^+$ *m/z* = 248.1283.

**4-((((9H-fluoren-9-yl)methoxy)carbonyl)(isobutyl)amino)-3-(prop-2-yn-1-yloxy)benzoic acid 22**

Fluorenylmethyl chloroformate (6.24 g, 24.1 mmol) in chloroform (50 mL) was added to a solution of 4-[(2-methylpropyl)amino]-3-(prop-2-yn-1-yloxy)benzoic acid (4.97 g, 20.1 mol) in chloroform and the mixture was stirred and refluxed for 16 hours. The solvent was removed and the residue was purified by flash chromatography (eluent: ethyl acetate/hexane: 0/10 to 5/5). The collected oil was taken up in chloroform and precipitated with hexane, and the precipitate was isolated by centrifugation, dissolved in chloroform and evaporated under vacuum to get the desired product as a white foam (1.55 g, 16%). $\delta_H$ (500 MHz, DMSO-$d_6$) 13.09 (s, 1H, C(O)O*H*) 7.78 (s broad, 2H, Ar-*H*), 7.68 (s, 1H, Ar-*H*), 7.56 (d, 1H, *J* = 7.7 Hz, Ar-*H*), 7.34 (s broad, 2H, Ar-*H*), 7.21 (d, 2H, *J* = 8.1 Hz, Ar-*H*), 7.15 (s, 3H, Ar-*H*), 4.83 (s, 2H, C$H_2$C≡CH), 4.22 (s broad, 2H, C$H_2$(Fmoc)), 4.02 (s broad, 1H, C*H*(Fmoc)), 3.54 (s, 2H, C$H_2$CH($CH_3$)$_2$), 1.61 (s, 1H, $CH_2$C*H*($CH_3$)$_2$), 0.81 (s, 6H, $CH_2$CH(C$H_3$)$_2$). $\delta_c$ (125 MHz, DMSO-$d_6$) 166.6, 154.7, 152.7, 143.5, 140.6, 134.4, 130.7, 129.6, 127.4, 126.8, 124.9, 122.3, 119.9, 114.1, 79.2, 78.6, 66.7, 56.1, 55.9, 46.4, 26.8, 19.9. $\nu_{max}$/cm$^{-1}$ (solid state) = 2957, 1685, 1406, 1285 (COOH) HRMS: Calcd. [M+Na]$^+$ ($C_{14}H_{17}NO_3$) *m/z* = 492.1709. Found [M+Na]$^+$ *m/z* = 492.1787.

# Trimer synthesis

The trimers were prepared by Solid Phase Peptide Synthesis (SPPS) as described previously.[1, 2]




*Scheme S2: General SPPS scheme for the trimer synthesis and list of trimer prepared.*

### General Trimer Synthesis Procedure

Glycine loaded Wang resin (0.79 mmol.g$^{-1}$, 100-200 mesh; carrier: polystyrene, crosslinked with 1% DVB; 127 mg, 0.1 mmol) was swelled in anhydrous DMF (5 mL) 15 minutes prior to reaction. The appropriate monomers (0.5 mmol) were dissolved in anhydrous chloroform (5 mL each) and preactivated for coupling with Ghosez's reagent (20% in chloroform, 0.45 mmol) for 1 hour at room temperature. The reactions were carried out on a CEM Liberty® automated microwave assisted peptide synthesizer, using a double coupling method: two times 20 minutes under microwave heating at 60°C. Before each coupling, standard washing and deprotection (25% piperidine solution in DMF) cycles were carried out on the synthesizer. The samples on resin were washed with dichloromethane and ether. When required, cleavage off the resin was performed manually, using a 50% solution of TFA in dichloromethane (2 mL). TFA was removed by bubbling the solution with nitrogen and the solvent was evaporated under vacuum.

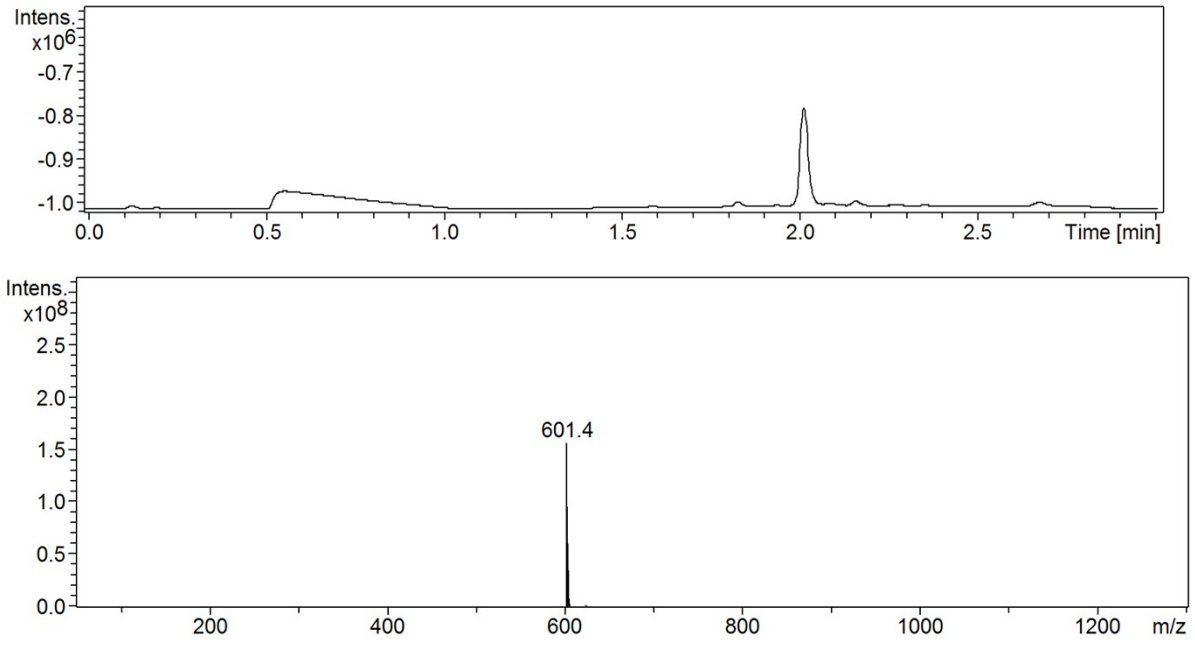
**General Click-Chemistry Procedure**

The trimer on resin was swelled in THF or DMF (1 mL). Azidobiotin or FITC-azide (0.1 mmol), water (1 mL), copper (II) sulphate (0.01 mmol) and sodium ascorbate (0.02 mmol) were added in that order. The reaction was carried on the spinner for 16 hours. Addition of more equivalents of reagents was sometimes required to observe the completion of the reaction. The resin was then washed with water, dichloromethane and ether and the trimer was cleaved off the resin using a 50% solution of TFA in dichloromethane (2 mL). TFA was removed by bubbling the solution with nitrogen and the solvent was evaporated under vacuum.

Trimers **2** to **6**, Biotin-**2** to Biotin-**6**, and Flu-**2** were previously described and characterised.[3]

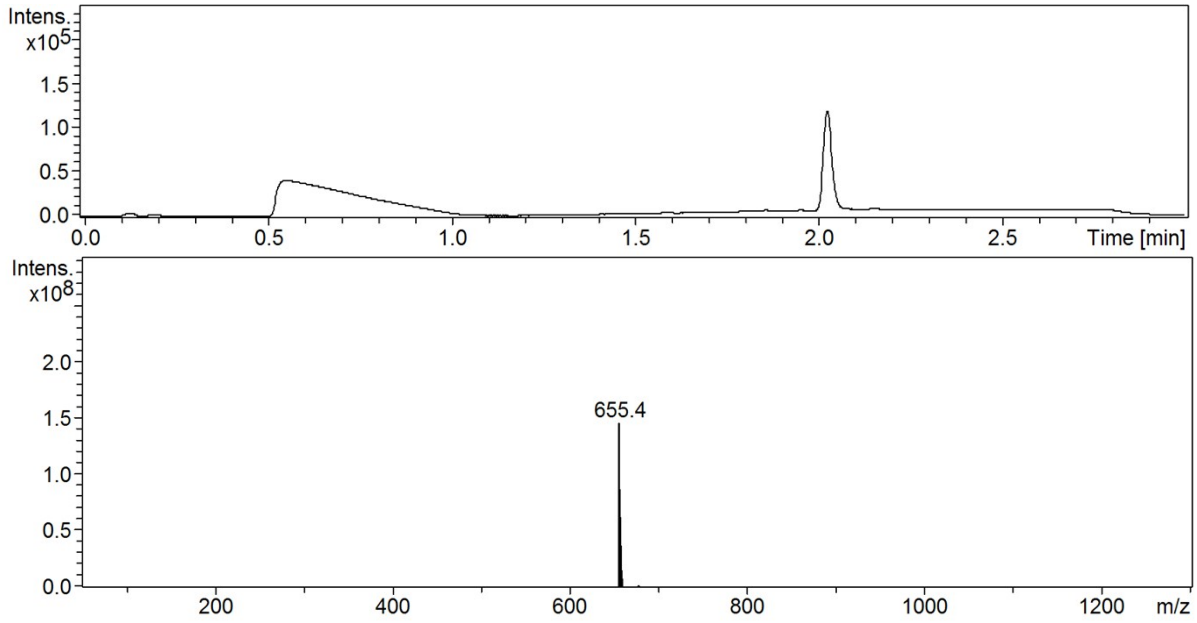
**(4-(N-isobutyl-4-(N-isobutyl-4-(isobutylamino)benzamido)-3-((1-(3-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)propyl)-1H-1,2,3-triazol-4-yl)methoxy)benzamido)benzoyl)glycine 1**

The monomer **26** (624 mg, 1.5 mmol) was used. The product was collected as a brown solid and used without further purification (57.9 mg, 96%). $\delta_H$ (500 MHz, DMSO-$d_6$) 8.82 (t, 1H, *J* = 5.9 Hz, 4-NH), 7.74 (d, 2H, *J* = 8.7 Hz, 3-H2), 7.19 (d, 2H, 8.5 Hz, 3-H3), 7.12 (d, 2H, *J* = 8.3 Hz, 2-H2), 6.93 (d, 2H, *J* = 8.5 Hz, 2-H3), 6.86 (d, 2H, *J* = 8.7 Hz, 1-H2), 6.27 (d, 2H, *J* = 8.7 Hz, 1-H3), 3.90 (d, 2H, *J* = 5.9 Hz, 4-Hα), 3.73 (d, 2H, *J* = 7.3 Hz, 3-Hα), 3.59 (d, 2H, *J* = 7.3 Hz, 2-Hα), 2.79 (d, 2H, *J* = 6.6 Hz, 1-Hα), 1.79-1.71 (m, 2H, 1-Hβ and 3-Hβ), 1.67-1.62 (m, 1H, 2-Hβ) 0.89 (d, 6H, J = 6.6 Hz, 1-Hγ), 0.85 (d, 6H, J = 6.6 Hz, 3-Hγ), 0.77 (d, 6H, J = 6.6 Hz, 2-Hγ). $\delta_C$ (125 MHz, DMSO-$d_6$) 171.7, 169.8, 169.7, 166.1, 150.8, 146.2, 145.9, 133.8, 131.7, 131.1, 129.2, 128.4, 127.7, 126.7, 122.0, 110.6, 56.4, 50.7, 41.6, 31.1, 27.8, 27.2, 27.1, 20.8, 20.5, 20.4. $[M+H]^+$ ($C_{35}H_{44}N_4O_5$) *m/z* = 601.3312. Found $[M+H]^+$ *m/z* = 601.3416.

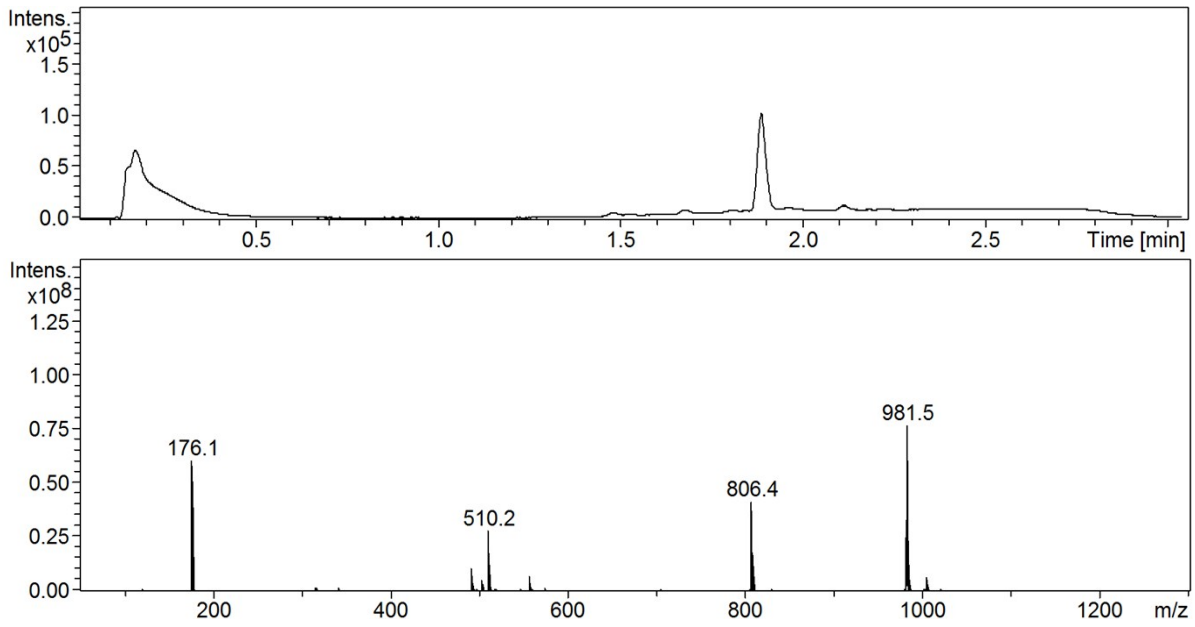


**(4-(N-isobutyl-4-(N-isobutyl-4-(isobutylamino)benzamido)-3-((1-(3-(4-(2-oxohexahy-dro-1H-thieno[3,4-d]imidazol-4-yl)butanamido)propyl)-1H-1,2,3-triazol-5-yl)methoxy)-benzamido)benzoyl)glycine Biotin-1**

The monomers **26** (415 mg, 1.0 mmol) and **22** (234 mg, 0.5 mmol) were used. A small amount of the sample was cleaved off the resin with TFA (50% in dichloromethane) to confirm the structure. $[M+H]^+$ ($C_{38}H_{46}N_4O_6$) *m/z* = 655.3417. Found $[M+H]^+$ *m/z* = 655.3502.




The biotin was clicked following the general Click-Chemistry procedure and the product was collected as a brown solid and used without further purification (59.9 mg, 60%). δH (500 MHz, DMSO-d6) 8.82 (t, 1H, J = 5.3 Hz, 4-NH), 7.98 (s, 1H, 2-3OT), 7.87 (t, 1H, J = 5.0 Hz, 4-CO2H), 7.70 (d, 2H, J = 8.1 Hz, 3-H2), 7.17 (d, 2H, J = 7.9 Hz, 3-H3), 7.06 (s, 1H, 2-H2), 6.90 (d, 1H, J = 7.7 Hz, 2-H5), 6.84 (d, 2H, J = 8.1 Hz, 1-H2), 6.67 (d, 1H, J = 7.7 Hz, 2-H6), 6.36 (d, 2H, J = 7.9 Hz, 1-H3), 4.93 (s broad, 1H, 2-3ONH), 4.83 (s broad, 1H, 2-3ONH), 4.36 (t broad, 2H, J = 6.2 Hz, 2-3OHβ), 4.29 (t broad, 1H, J = 6.2 Hz, 2-3OHλ), 4.11 (t broad, 1H, J = 6.2 Hz, 2-3OHλ), 3.89 (d, 2H, J = 5.3 Hz, 4-Hα), 3.69 (d, 2H, J = 6.6 Hz, 3-Hα), 3.55 (s broad, 2H, 2-3OHδ) 3.15 (s, 2H, 2-3OHα), 3.08 (d, 1H, J = 4.6 Hz, 2-3oHι), 3.05 (d, 2H, J = 5.3 Hz, 2-Hα), 2.81 (d, 2H, J = 6.6 Hz, 1-Hα), 2.77 (dd (under previous peak), 1H, J = 4.5 Hz, , 2-3OHκ), 2.53 (d, 1H, J = 4.9 Hz, 2-3OHκ), 2.07-2.05 (m, 2H, 2-3OHε), 1.95-1.91 (m, 2H, 2-3OHγ), 1.81-1.74 (m, 1H, 1-Hβ), 1.73-1.66 (m, 1H, 3-Hβ), 1.63-1.56 (m, 2H, 2-3OHθ) 1.55-1.47 (m, 4H, 2-Hβ and 2-3OHζ), 1.34-1.24 (m, 2H, 2-3OHη), 0.89 (d, 6H, J = 6.6 Hz, 1-Hγ), 0.84 (d, 6H, J = 6.4 Hz, 3-Hγ), 0.71 (d, 6H, J = 6.4 Hz, 2-Hγ). δc (125 MHz, DMSO-d6) 172.2, 171.1, 170.2, 169.8, 169.0, 165.8, 152.2, 148.1, 145.6, 142.0, 135.9, 133.6, 131.2, 129.4, 128.9, 127.8, 127.1, 124.4, 120.5, 113.5, 111.5, 61.5, 61.1, 59.6, 59.3, 55.3, 54.7, 51.2, 48.4, 47.3, 41.1, 35.6, 35.1, 30.0, 28.1, 27.9, 27.0, 26.7, 26.5, 25.1, 20.6, 20.1, 19.9. [M+H]+ ($C_{51}H_{68}N_{10}O_8S$) m/z = 981.4942. Found [M+H]+ m/z = 981.5024.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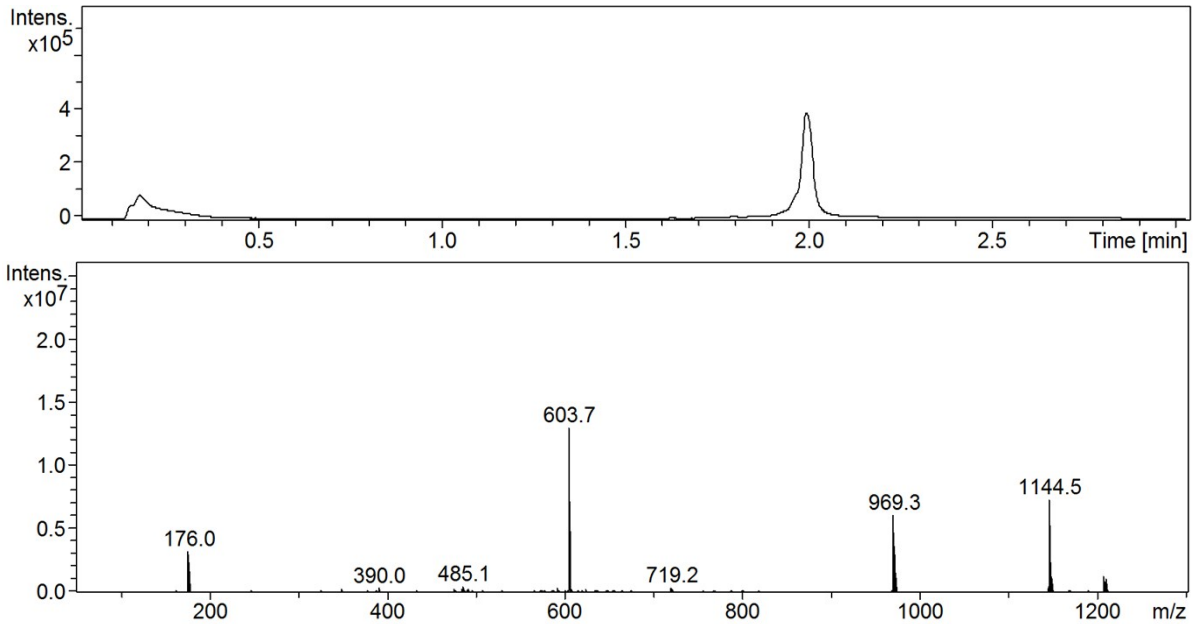
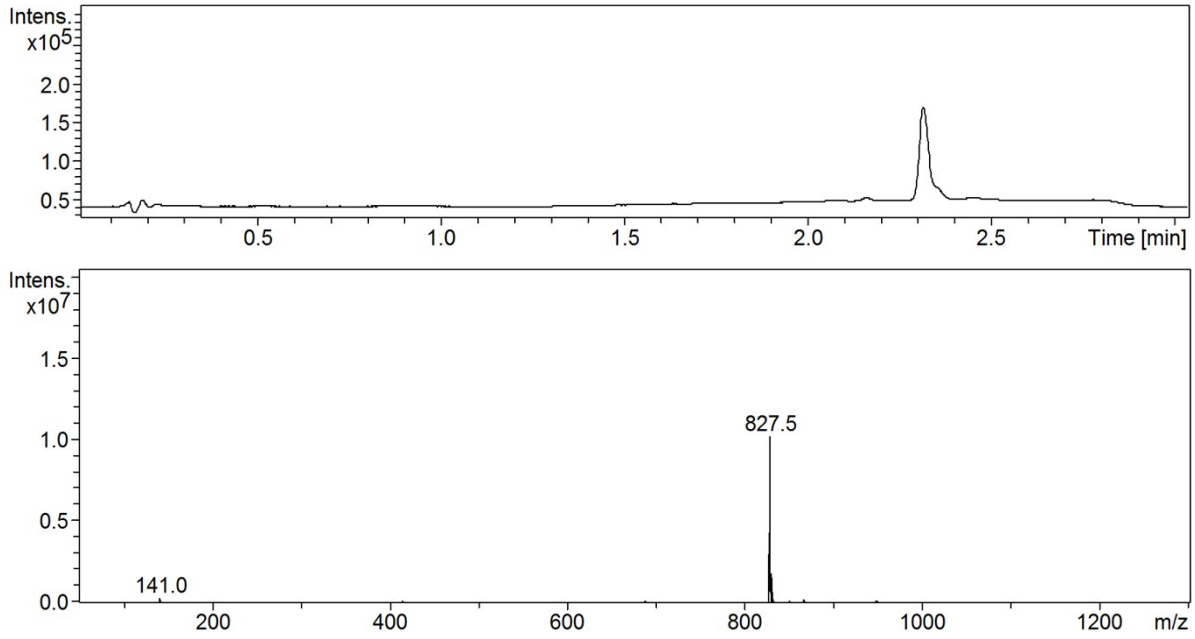
**5-(3-(3-(4-((5-((4-((carboxymethyl)carbamoyl)phenyl)(isobutyl)carbamoyl)-2-(N-isobutyl-4-(isobutylamino)benzamido)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)ureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid Flu-1**

The monomers **26** (415 mg, 1.0 mmol) and **22** (234 mg, 0.5 mmol) were used. A small amount of the sample was cleaved off the resin with TFA (50% in dichloromethane) to confirm the structure. $[M+H]^+$ ($C_{38}H_{46}N_4O_6$) *m/z* = 655.3417. Found $[M+H]^+$ *m/z* = 655.3502. The FITC was clicked following the general Click-Chemistry procedure and the product was purified by preparative HPLC (5-95 MeCN in $H_2O$), the fractions were evaporated to afford a dark red solid (3.5mg, 6%). δH (500 MHz, DMSO-d6) 8.81 (t, 1H, J = 5.8 Hz, 4-NH), 8.21 (s broad, 2-3OH2), 7.97 (s, 1H, , 2-3OOH), 7.75 (d, 1H (under previous peak), 2-3OH6), 7.73 (d, 2H, J = 8.5 Hz, 3-H2), 7.21 (d, 2H, J = 8.5 Hz, 3-H3), 7.16 (d, 1H, J = 8.3 Hz, 2-3OH5), 7.09 (s broad, 1H, 2-H2), 6.91 (d, 1H, J = 8.1 Hz, 2-H5), 6.81 (d, 2H, J = 8.7 Hz, 1-H2), 6.68 (d, 1H, J = 8.1 Hz, 2-H6), 6.65 (d, 2H, J = 2.3 Hz, 2-3OH10), 6.59 (t, 2H, J = 8.7 Hz, 2-3OH9), 6.54 (dd, 2H, J = 8.7 Hz, 2.3 Hz, 2-3OH11), 6.22 (d, 2H, J = 8.7 Hz, 1-H3), 5.82 (s broad, 1H, 1-NH), 4.93 (s broad, 1H, 2-3ONH), 4.87 (s broad, 1H, 2-3ONH), 4.47 (t, 2H, J = 7.0 Hz, 2-3OHδ), 3.89 (d, 2H, J = 6.0 Hz, 4-Hα), 3.71 (d, 2H, J = 7.0 Hz, 3-Hα), 3.55 (d, 2H, J = 5.3 Hz, 2-3OHβ), 2.77 (d, 2H, J = 6.6 Hz, 1-Hα), 2.15 (quint., 2H, J = 7.0 Hz, 2-3OHγ) 1.77-1.68 (m, 2H, 3-Hβ and 1-Hβ), 1.54-1.49 (m, 1H, 2-Hβ), 0.87 (d, 6H, J = 6.6 Hz, 1-Hγ), 0.84 (d, 6H, J = 6.8 Hz, 3-Hγ), 0.71 (d, 6H, J = 6.8 Hz, 2-Hγ). [M+H]+ ($C_{62}H_{65}N_9O_{11}S$) m/z = 1144.4558. Found [M+H]+ m/z = 1144.4598.



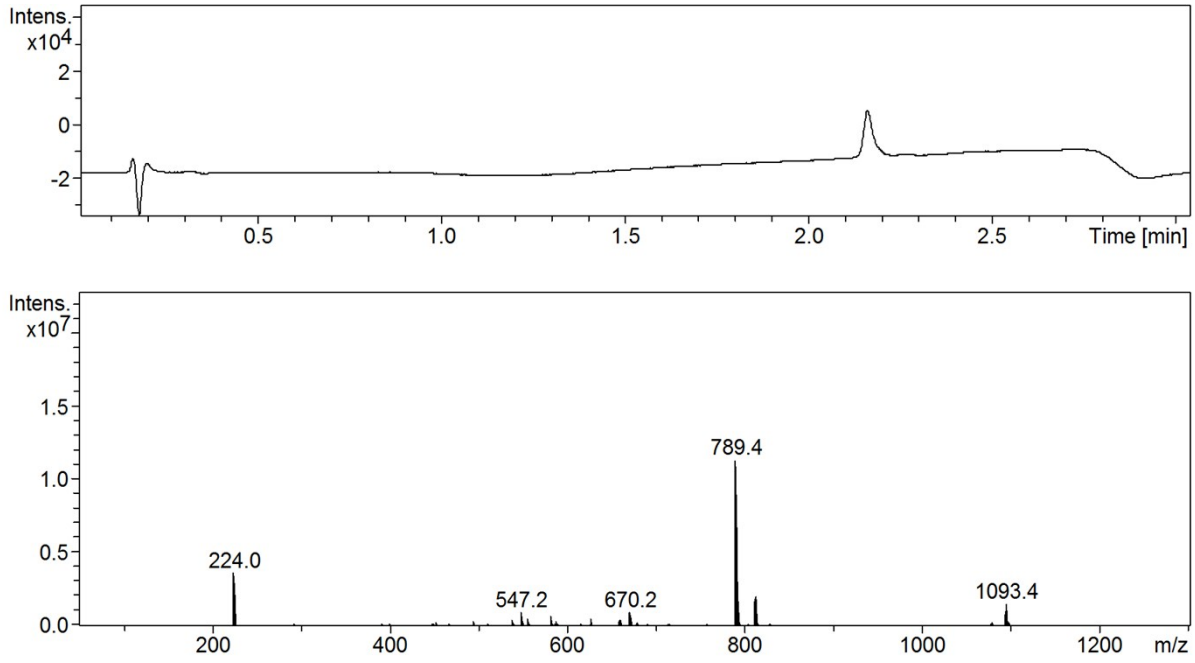
**5-(3-(3-(4-((5-((4-((carboxymethyl)carbamoyl)phenyl)(cyclohexylmethyl)carbamoyl)-2-(N-(naphthalen-2-ylmethyl)-4-(phenethylamino)benzamido)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)ureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid Flu-2rev**

The monomers **27** (232 mg, 0.5 mmol) and **24** (277 mg, 0.5 mmol) and **30** (228 mg, 0.5 mmol) were used. A small amount of the sample was cleaved off the resin with TFA (50% in dichloromethane) to confirm the structure. $[M+H]^+$ ($C_{52}H_{50}N_4O_6$) *m/z* = 827.3764. Found $[M+H]^+$ *m/z* = 827.3769.




The biotin was clicked following the general Click-Chemistry procedure and the product was purified by preparative HPLC (5-95 MeCN in $H_2O$), the fractions were evaporated to afford a dark red solid (3.5mg, 6%).

$\delta_H$ (500 MHz, DMSO-$d_6$) 10.1 (s, 2H, 2-3$^0$NH), 8.17 (s, 1H, 2-3$^0$H2), 7.81-7.80 (m, 1H, 2-3$^0$ArH), 7.77 (d, 3H, *J* = 8.3 Hz, 2-3$^0$ArH), 7.71 (d, 2H, *J* = 8.3 Hz, 2-3$^0$ArH), 7.64 (s, 1H, 1-NH), 7.45-7.41 (m, 2H, 1-Bn), 7.25 (d, 4H, *J* = 4.3 Hz, ArH), 7.23 (d broad, 1H, *J* = 8.1 Hz, 2-H5), 7.17-7.15 (m, 3H, 1-Bn), 7.09 (d, 1H, *J* = 8.3 Hz, 2-3$^0$H6), 6.93 (d broad, 2H, *J* = 8.1 Hz, 1-H2), 6.70 (d, 1H, *J* = 7.1 Hz, 2-Hnapht.), 6.66 (d, 2H, *J* = 2.3 Hz, 2-Hnapht.), 6.58 (d, 2H, *J* = 8.6 Hz, 2-Hnapht.), 6.55 (dd, 2H, *J* = 8.6 Hz, 2.3 Hz, 2-Hnapht.), 6.32 (d, 2H, *J* = 8.6 Hz, 1-H3), 5.98 (s broad, 1H, 1-NH), 4.43 (t, 2H, *J* = 6.4 Hz, 2-3$^0$Hγ), 3.93 (s broad, 2H), 3.71 (s broad, 2H), 3.25 (t broad, 2H, *J* = 7.3 Hz, 1-Hα), 3.09 (s, broad, 2H), 3.02 (t broad, 1H, *J* = 5.6), 2.81 (t, 2H, *J* = 7.3 Hz, 1-Hβ), 2.01 (t broad, 2H, *J* = 6.4 Hz, 2-3$^0$Hα), 1.67-1.63 (m, 3H), 1.63-1.58 (m, 3H, 3-Hβ,γ) 1.56-1.53 (m, 2H, 3-Hδ), 1.43 (s broad, 2H), 1.24 (s, 2H), 1.06 (s broad, 4H, 3-Hγ,δ), 0.90 (s broad, 1H), 0.87 (t, 2H, *J* = 7.3 Hz, 3-Hε).




The observed mass corresponds to fragmentation of Flu-2rev.

# Phage Display

**Phage Display**

The Affimer library was prepared by the BioScreening Technology Group in the School of Molecular and Cellular Biology.[4] The phage display procedure is represented in Figure S 1.

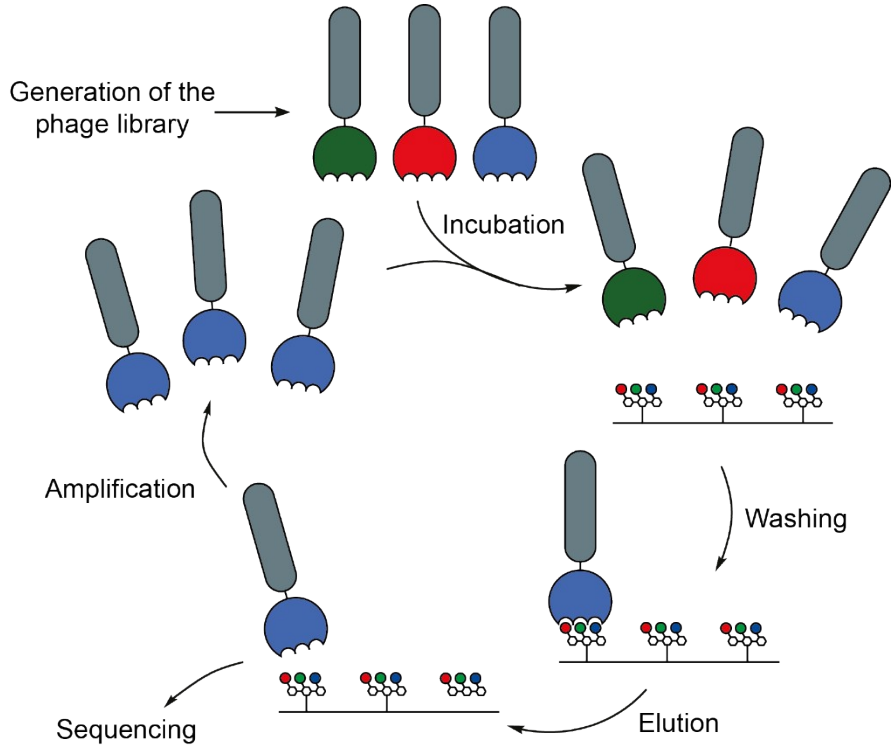



*Figure S1: Cartoon representation of a phage display panning round.*

Biotinylated trimers (5 µl, 1 mM in DMSO) were bound to streptavidin-coated wells (Pierce) for 1 h, then $10^{12}$ colony-forming units pre-panned phage were added for 2 hour with shaking (Heidolph VIBRAMAX 100). Panning wells were washed 6 times and eluted with glycine-HCl (0.2 mM, pH 2.2) for 10 min, neutralised with Tris-HCL (1M, pH 9.1), further eluted with triethylamine (100 mM) for 6 min, and neutralised with Tris-HCl (1M, pH 7). Eluted phage were used to infect ER2738 cells for 1 hour at 37°C and 90 rpm then plated onto LB agar plates with 100 mg/ml carbenicillin and grown overnight. Colonies were scraped into 5 ml of 2TYcarb (2TY containing 100 µg/ml carbenicillin) medium, inoculated in 25 ml of 2TYcarb and infected with ca. $1x10^9$ M13K07 helper phage. After 1 h at 90 rpm, kanamycin was added to 25 mg/ml for overnight at 25°C and 170 rpm. Phage were precipitated with 20% polyethylene glycol 8000, 0.3 M NaCl and resuspended in 1 ml of TE buffer (Tris 10 mM, pH 8.0, 1 mM EDTA). A 2 ml aliquot of phage suspension was used for the second round of selection using streptavidin magnetic beads (Invitrogen) labelled with 15 µl of the biotinylated target (1 mM in DMSO). The beads were washed and incubated with prepanned phage for 1 h then washed five times using a KingFisher robotic platform (ThermoFisher) and eluted and amplified as above. The third pan used Neutravidin High Binding Capacity plates (Pierce), as previously described for panning round one, and the fourth pan followed the procedure from panning round one. Phage were recovered from wells containing target protein and control wells to determine the level of amplification in target wells.

**Phage ELISA**

Individual ER2738 colonies were grown in 100 ml of 2TY with 100 mg/ml of carbenicillin in a 96-deep well plate at 37°C (1050 rpm) for 6 hours. A 25 ml aliquot of the culture was added to 200 ml of 2TYcarb and grown at 37°C (900 rpm) for 1 hour. Helper phage (10 µl of $10^{11}$/ml) were added, followed by kanamycin to 25 mg/ml overnight and incubated at 25°C, 750 rpm. Streptavidin-coated plates (96 Maxisorp Nunc-Immuno Plate (Thermo Scientific) incubated with Streptavidin (5 µg/ml) for 4 hours) were blocked with 2x casein blocking buffer (Sigma) overnight at 37°C. The plates were incubated with the biotinylated trimers for 1 hour, and 40 µl of phage containing growth medium was added and incubated for 1 hour. Following washing, phage were detected by a 1 : 1000 dilution of Anti-Fd-Bacteriophage-HRP (Seramun) for 1 hour, visualised with 3,3',5,5'-tetramethylbenzidine (TMB) (Seramun) and measured at 620 nm.

The reading of the phage ELISA plates is shown in Figure S2. The results yielded high numbers of selective binders for Biotin-**1** and Biotin-**2** and we decided to focus our work on those two trimers. Biotin-**3**, -**4**, -**5** and -**6** generated fewer binders although Biotin-**6** generated a number of selective affimers. The reasons for this are unclear but are likely related to the composition of side chains on these foldamers being unable to effectively present recognition handles for the affimers. Biotin-**3** to -**6** were initially chosen as they were good hits for p53-*h*DM2 inhibition,[3] so the number of affimers and selectivities do not relate to their ability to adopt an α-helix mimicking conformation.

For the Affimers raised against Biotin-**1** and Biotin-**2**, the plasmid DNA was extracted from selected hits using a QIAprep Spin Miniprep Kit (QIAGEN), and sequenced using a T7P primer by Beckman Coulter Genomics. The sequences are shown in Table S 1.

During the selection of Affimers for Biotin-**1** and -**2**, no pattern of amino acids stands out. Nevertheless, we can note that overall, non-polar or polar neutral amino acids tends to be predominant in the Affimer sequences, and polar positive amino acids outnumber polar negative amino acids. This can be explained by the presence of the carboxylic acid at the bottom of the foldamer, which is deprotonated under the conditions of the selection (pH 7).

*Table S1: Summary of the Affimer sequences obtained from screening against Biotin-**1** and Biotin-**2**. Red: non-selective. Green: selective. The amino acids are color-coded according to the following principle: blue: polar positive, red: polar negative, green: polar neutral, grey: non-polar aliphatic, purple: non-polar aromatic, orange: proline and glycine.*

| Biotin-1 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AF | Sequence 1 | | | | | | | | | Sequence 2 | | | | | | | | | |
| 26, 27, 29 | P | H | R | N | S | L | V | S | D | Y | R | M | A | Y | G | F | S | W | |
| 28 | H | P | V | K | P | Q | Y | P | T | Y | K | R | W | G | I | Y | S | W | |
| 10, 19, 32, 47 | V | D | W | Y | G | P | V | Q | A | S | M | M | T | A | R | H | N | I | |
| 43 | H | S | Q | W | F | M | Y | P | G | P | A | T | V | D | E | I | M | A | |
| 46 | H | W | A | H | W | S | G | D | A | T | Y | M | E | E | M | N | L | N | |
| 5, 6, 39 | M | D | Q | P | W | W | G | H | I | E | N | E | G | W | P | H | L | W | |
| 7 | Q | A | W | V | H | V | H | Y | I | Y | N | G | P | A | Y | K | I | V | |
| 8, 14, 41 | Q | W | V | H | F | S | G | D | A | Q | P | P | Q | T | Y | N | M | R | |
| 17, 36 | Y | V | Q | N | E | D | Y | F | Y | Q | F | L | R | Y | F | P | H | H | Y |
| 20, 45, 48 | Y | Y | E | A | W | W | F | P | I | H | N | E | K | S | D | H | N | E | |
| 35 | R | V | Q | D | I | Y | P | T | Y | N | A | T | G | T | T | W | M | Q | |

| Biotin-2 | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AF | Sequence 1 | | | | | | | | | Sequence 2 | | | | | | | | | |
| 1 | T | V | S | T | W | G | G | D | F | A | N | I | K | Q | D | L | E | M | |
| 24 | S | V | Y | T | W | G | G | P | F | P | I | S | E | Q | R | F | L | P | |
| 4, 5, 25, 42 | F | G | P | R | Q | Y | F | H | H | N | G | V | P | H | M | S | M | L | |
| 13, 19, 23, 29, 30, 33-35 | S | G | Y | H | K | D | T | F | A | N | V | Y | Y | H | N | M | Q | I | |
| 6, 12 | R | G | I | R | E | W | T | H | H | P | S | Q | P | H | L | H | L | Y | |
| 11, 45 | P | D | K | S | Q | P | W | W | P | A | N | M | K | Q | L | Y | H | E | |
| 15 | N | G | P | G | R | H | H | R | H | P | T | Y | S | Q | W | Y | M | M | |
| 18 | W | N | N | V | G | S | T | L | M | Y | K | W | I | P | W | P | Y | I | |
| 46 | R | A | E | T | W | D | G | P | W | N | R | S | T | P | W | D | G | F | |

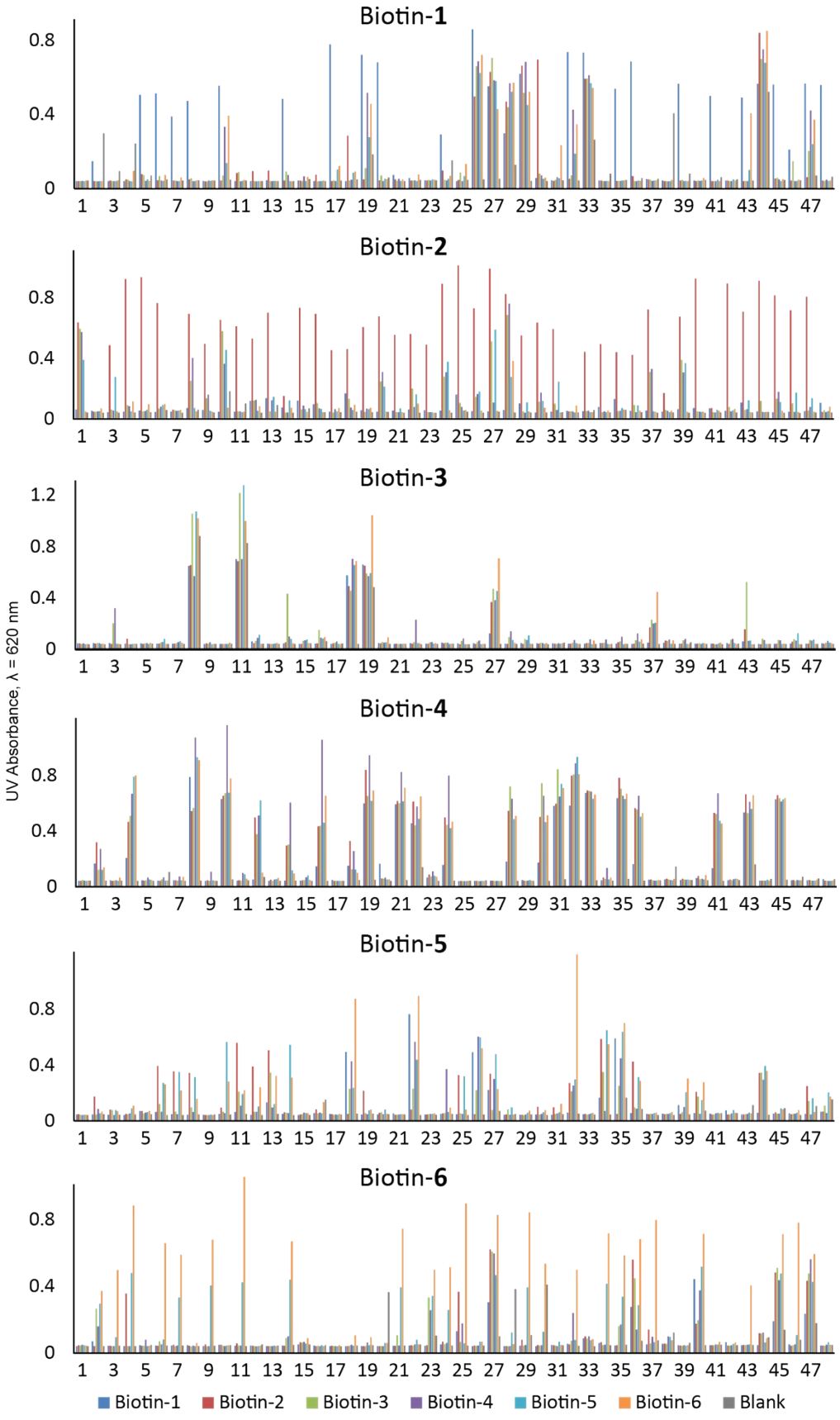



*Figure S2: Phage ELISA readings for trimers Biotin-**1** to Biotin-**6** at 620 nm.*

# Affimer production

The Affimers were prepared following the previously published procedure.[4] A 15% SDS-PAGE gel was ran to check their purity and the Affimers were also analysed by HRMS (Table S2).

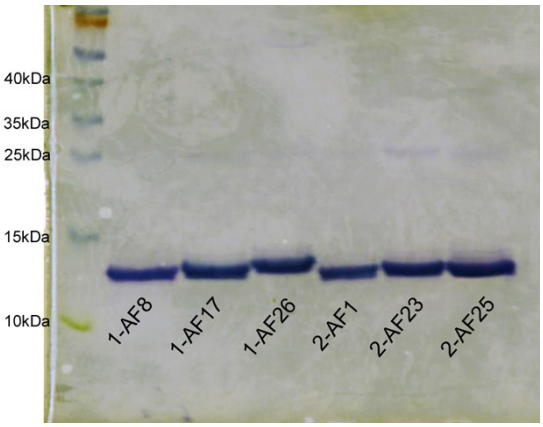



*Table S2: Deconvoluted HRMS values for the Affimers consistent with the cleavage of the N-terminal methionine.*

| Affimer | Expected mass | Measured mass |
|---|---|---|
| **1-AF8** | 12259.78 | 12259.0982 |
| **1-AF17** | 12727.33 | 12726.3642 |
| **1-AF26** | 12283.89 | 12283.2020 |
| **2-AF1** | 12109.68 | 12109.1284 |
| **2-AF23** | 12285.86 | 12285.1838 |
| **2-AF25** | 12252.94 | 12252.2021 |

# Biophysical Assays

**ELISA**

Streptavidin Coated (High Binding Capacity, Thermo Scientific) 8-well strips well plates and F96 Nunc-Immuno Plate (Thermo Scientific) were pre-blocked with 200 µl 2x Blocking Buffer (BB) and incubated overnight at 0°C. Both plates were washed with 300 µl of PBST. 30 µl of the biotinylated targets (30 µM in DMSO) was placed in each streptavidin-coated well. For negative control wells, 2x BB was used instead. The targets were incubated at room temperature on a platform shaker twice for 45 minutes, and washed with 300 µl of PBST between each incubation. The dilution was prepared in the F96 Nunc-Immuno Plate and incubated for 1 hour (**Direct titration:** The Affimer was serially diluted in filtered sterilised water. **Competition assay:** The competitor was serially diluted in filtered sterilised water with 5% DMSO, and the Affimer was added). The Streptavidin plate was washed with 300 µl of PBST, and the Affimer and/or competitor was transferred into it and incubated for 1 hour. The plate was then washed with 300 µl of PBST, and 50 µl of anti-6X his-tag HRP (abcam, diluted 5000 times in 2x BB from the commercial bottle) was added to the wells. The plate was incubated for 1 hour and washed 3 times with 300 µl of PBST. 50 µl of TMB (SeramunBlau® fast TMB/substrate solution) was added to the wells and allowed to develop for 15-30 minutes and the absorbance was measured at 620 nm on a Thermo Multiskan Ascent. Each experiment was run in triplicate and the normalised data was plotted to a sigmoidal fit in Origin 7 to determine the $EC_{50}$ or $IC_{50}$.

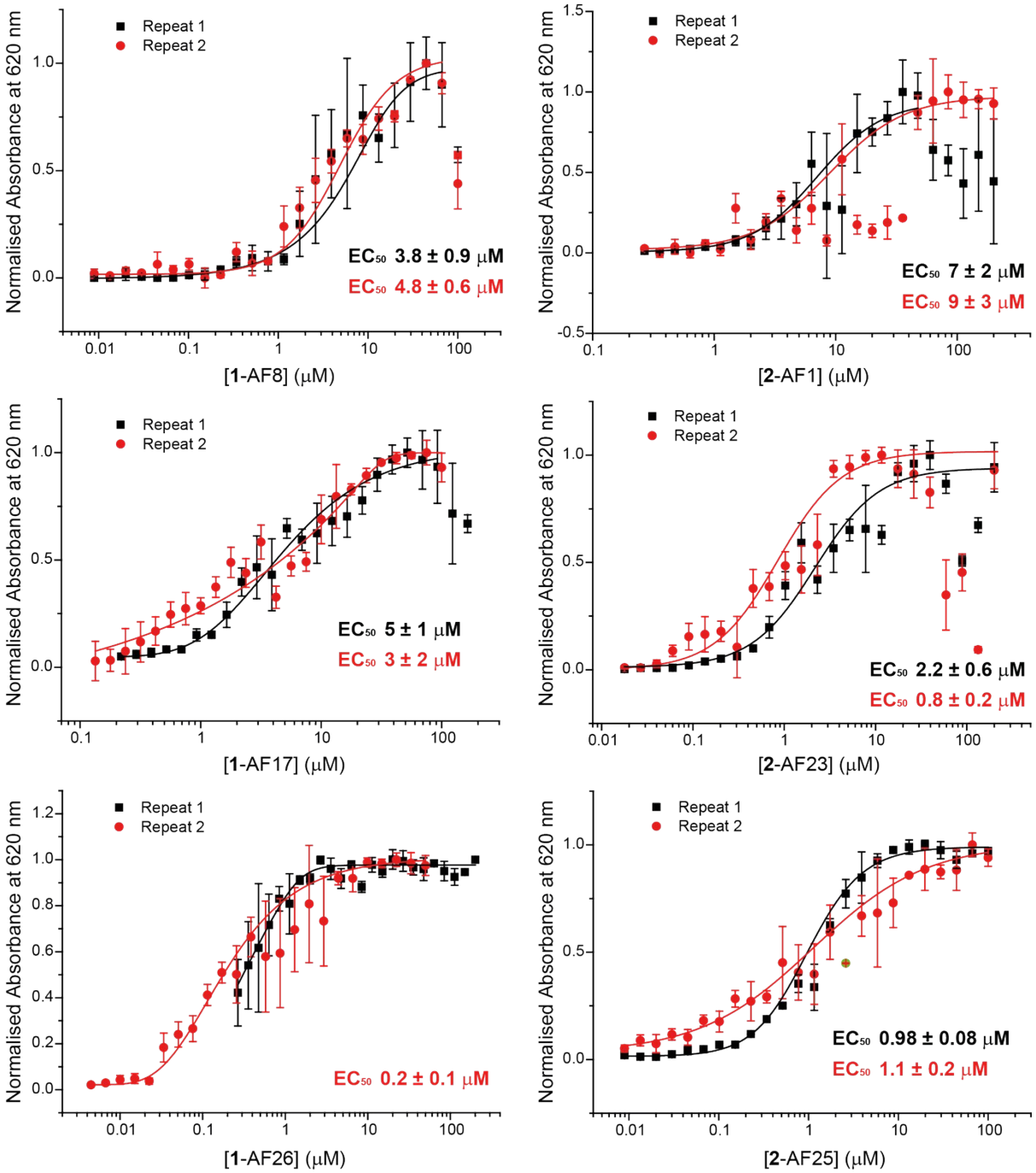



*Figure S3: Normalised readings from the direct titration ELISA assays.*

The direct titration results were used to determine the concentration to work at for the competitions: we used concentrations between the $EC_{50}$ and the top plateau in order to ensure the Affimers were binding to the immobilised foldamer without oversaturating the plate. The following concentrations were chosen: [1-AF8] = 10 μM, [1-AF17] = 10 μM, [1-AF26] = 0.3 μM, [2-AF1] = 20 μM, [2-AF23] = 2 μM and [2-AF25] = 5 μM. The results are shown as normalised average of 4 repeats, for which n = 3.

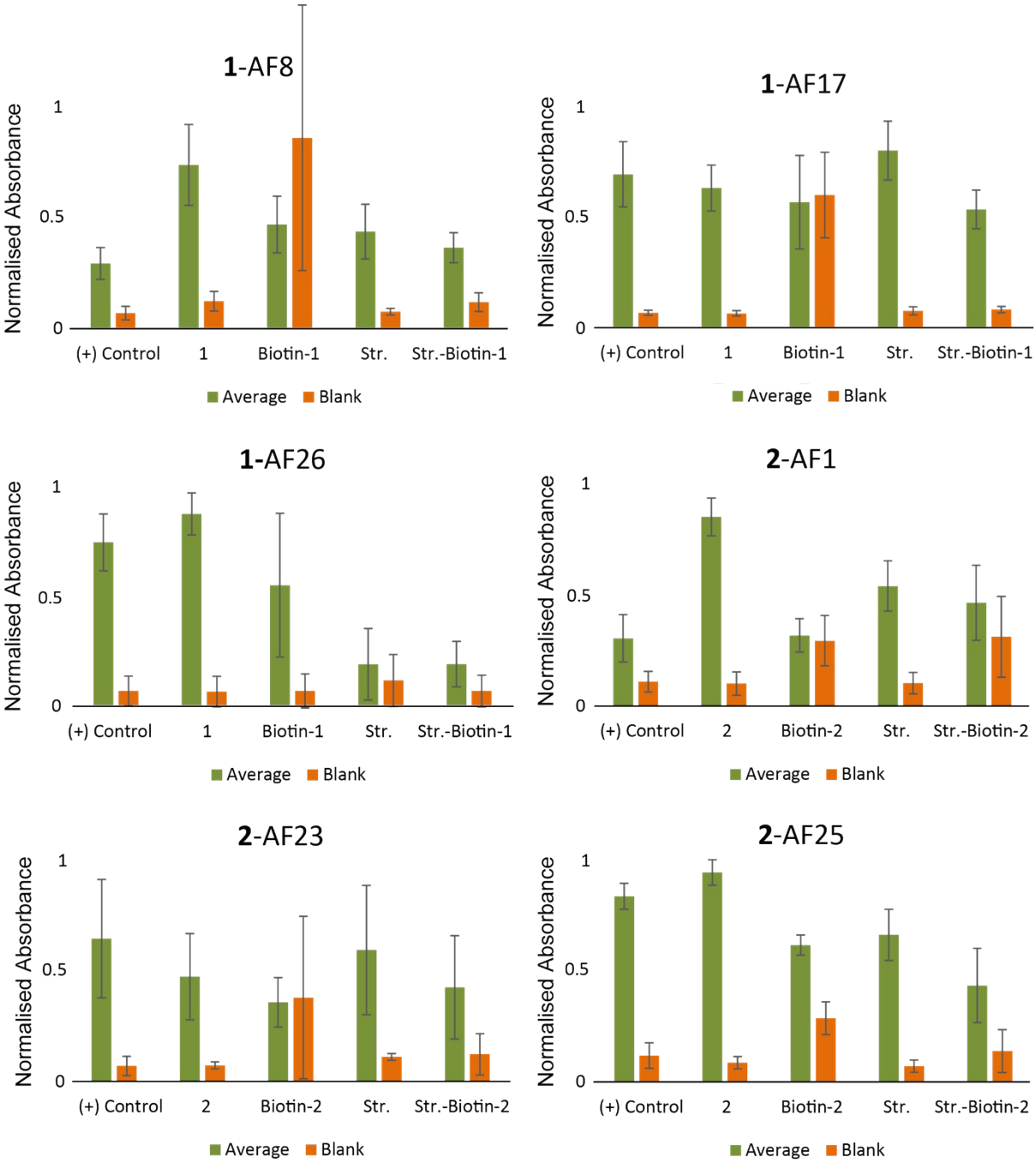



*Figure S4: Normalised readings from the single point competition ELISA assay at 1:1 ratio.*

For the single point assay at 1:10 and 1:100 ratio in Affimer:Competitor, we used the following concentrations: [1-AF8] = 10 μM, [1-AF17] = 5 μM, [1-AF26] = 0.3 μM, [2-AF1] = 20 μM, [2-AF23] = 5 μM and [2-AF25] = 10 μM.

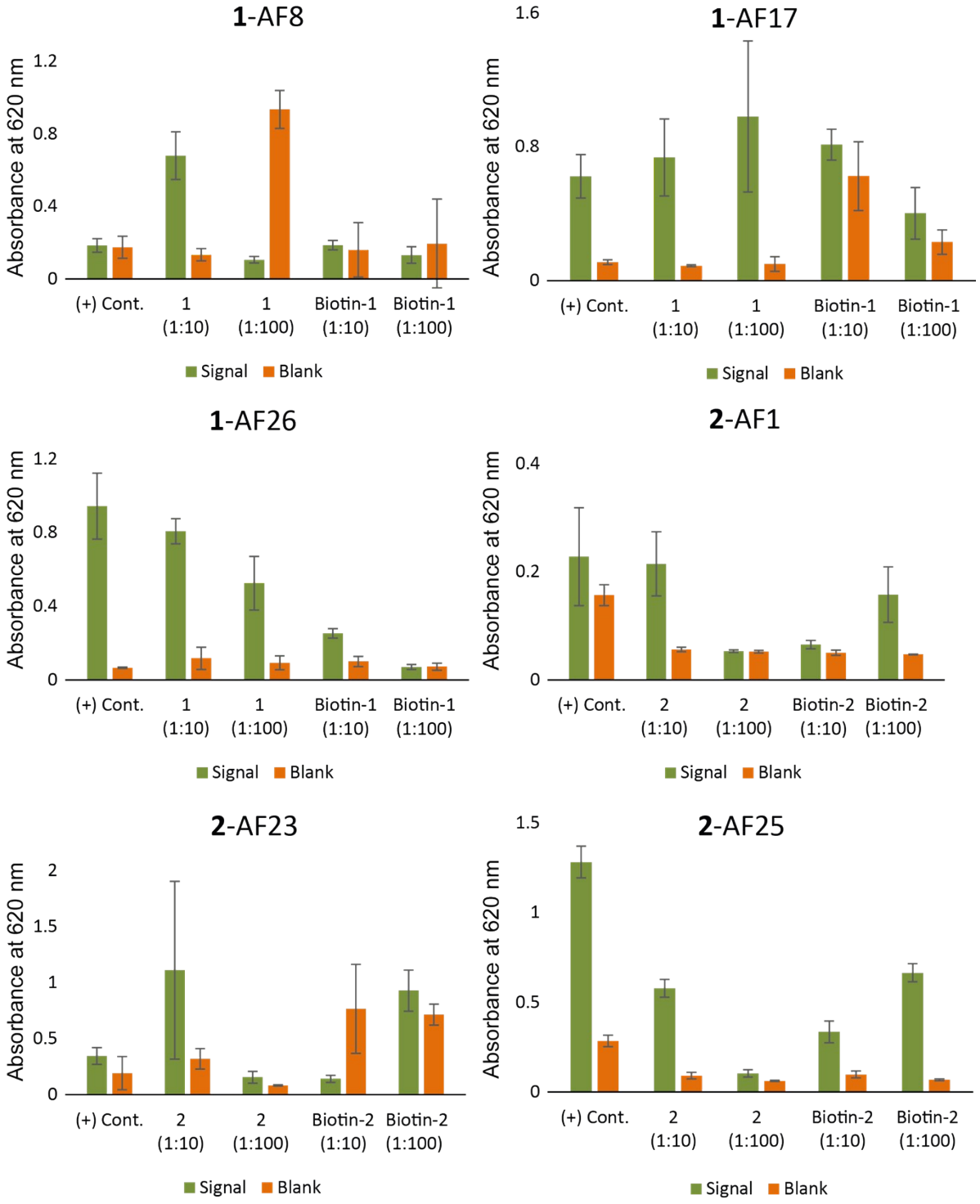



*Figure S5: Readings from the single point competition ELISA assays at 1:10 and 1:100 ratio, n = 3. (note: the observed increase of signal upon competition of Biotin-2 at the ratio 1:100 can be explained by precipitation of the trimer in the assay – attempts*

*to avoid this phenomenon by increasing the percentage of DMSO in the buffer were infructuous as the maximum quantity needed to be kept under 10% and ideally under 5%)*

For the serial dilution assay, the following concentrations in Affimers were used: [1-AF8] = 10 μM, [1-AF17] = 10 μM, [1-AF26] = 1 μM, [2-AF1] = 20 μM, [2-AF23] = 5 μM and [2-AF25] = 10 μM.

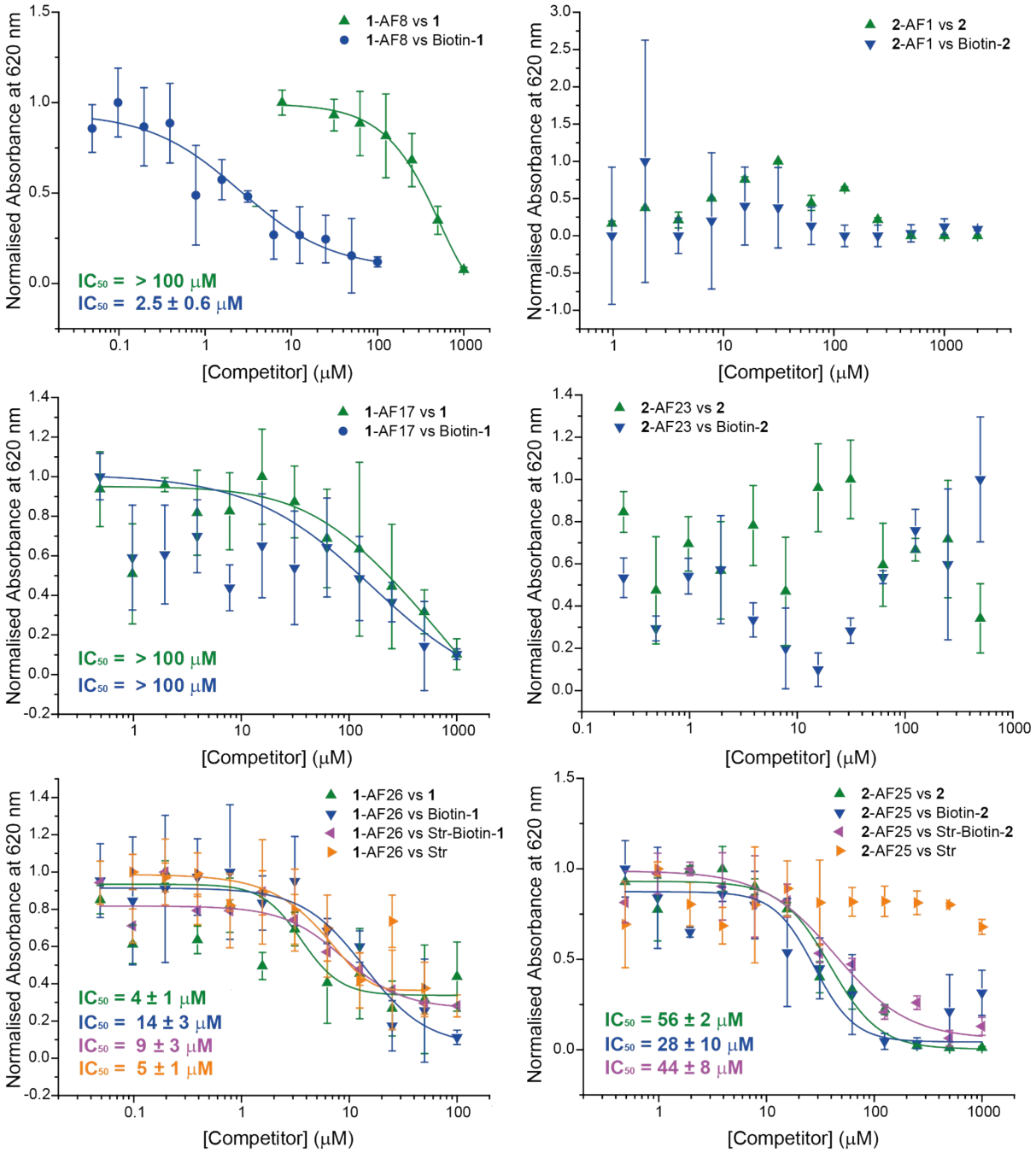



*Figure S6: Normalised readings from the competitive ELISA assays.*

**Fluorescence Anisotropy**

The buffer used were either Water (filtered sterilised water + 3% DMSO), Phosphate Buffer (40 mM sodium phosphate, 200 mM sodium chloride, 0.02 mg ml-1 bovine serum albumin, pH 7.50) or Tris-Triton buffer (50 mM Tris, 150 mM sodium chloride, 0.01% Triton, pH 7.40). The Affimer was serially diluted in a 96-well Optiplate and the Flu-trimer was added. The plates were allowed to incubate at room temperature for 45 minutes. Each experiment was run in triplicate and the fluorescence anisotropy measured using a Perkin Elmer EnVision™ 2103 MultiLabel plate reader, with excitation at 480 nm and emission at 535 nm (30 nM bandwidth) and the intensity (Eq. 1) was calculated for each point. This was used to calculate anisotropy (Eq. 2) and plotted to a sigmoidal fit in Origin 7 to determine the minimum and maximum anisotropies ($r_{min}$ and $r_{max}$). Using equation 3, the data for the anisotropy was converted to fraction bound and multiplied by the Flu-trimer concentration then fitted in origin 7 (Eq. 4) to give the dissociation constant $K_d$. From a plot of intensity (Eq. 1) against concentration of protein λ was calculated to be 1.

$$I = (2PG) + S \quad Equation\ 1$$

$$r = \frac{S - PG}{I} \quad Equation\ 2$$

$$L_b = \frac{(r - r_{min})}{\lambda(r_{max} - r) + r - r_{min}} \quad Equation\ 3$$

$$y = \frac{(k_1 + x + [FL] - \sqrt{k_1 + x + [FL]^2 - 4x[FL]}}{2} \quad Equation\ 4$$

r = anisotropy, I = total intensity, P = perpendicular intensity, S = parallel intensity, $L_b$ = fraction ligand bound, $\lambda = I_{bound}/I_{unbound} = 1$, [FL] = concentration of fluorescent ligand, $k_1 = K_d$ , $y = L_b$* Flu-trimer and x = [added titrant], G is an instrument factor set to 1.

For Flu-**2**rev against **2**-AF25, the value of anisotropy being around 0.25 suggested there could be some non-selective interaction between the compound and the plate. To address this question, we used a special non-stick plate (orange graph) and the new measurements were indeed around 0. Nevertheless, in all cases when we could not obtain a binding curve, low values in Affimer concentration lead to less accurate values in anisotropy that could not be interpreted due to the error.

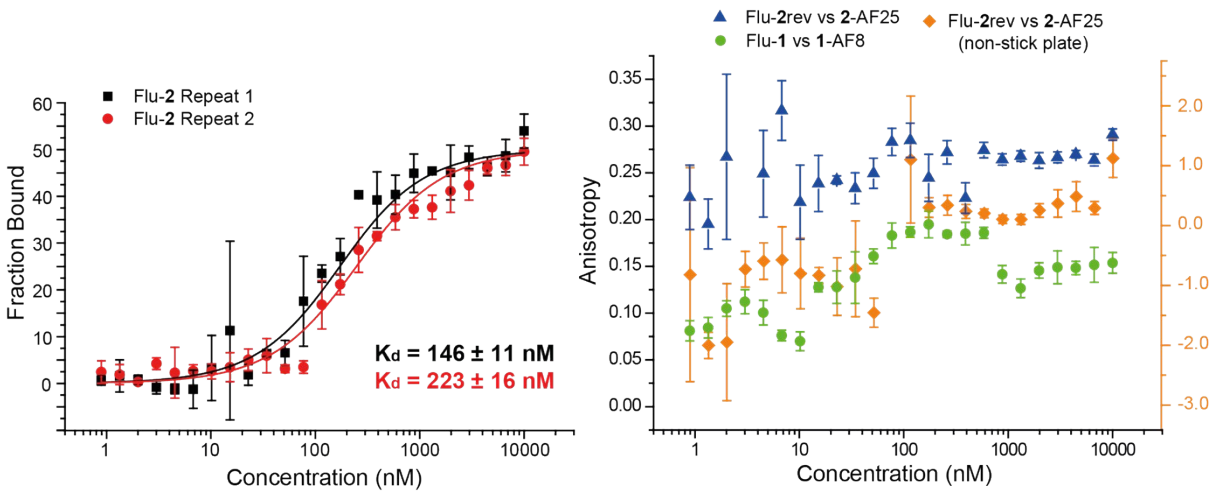



*Figure S7: Fluorescence Anisotropy direct titration. Left: Fraction bound of Flu-2 on 2-AF25. Right: Anisotropy for Flu-1 and 1-AF8 (green dots), Flu-2rev and 2-AF25 (blue triangles) and on a non-stick plate (orange squares).*

# References


1. K. Long, T. A. Edwards and A. J. Wilson, *Bioorg. Med. Chem.*, 2013, **21**, 4034–4040.
2. A. Barnard, K. Long, D. J. Yeo, J. A. Miles, V. Azzarito, G. M. Burslem, P. Prabhakaran, T. A. Edwards and A. J. Wilson, *Org. Biomol. Chem.*, 2014, **12**, 6794-6799.
3. A. Barnard, K. Long, H. L. Martin, J. A. Miles, T. A. Edwards, D. C. Tomlinson, A. Macdonald and A. J. Wilson, *Angew. Chem. Int. Ed.*, 2015, **54**, 2960-2965.
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